目　录

一、 实验室简介

1. 实验室概况

生物有机与分子工程教育部重点实验室是原国家教委 1993 年 12 月批准建立的专业实验室，于 1996 年正式对外开放，主要从事有机化学、生物有机化学和分子工程学的基础性与应用基础性研究。

实验室现有成员 25 人，其中教授 13 人，特聘研究员 5 人，副教授 3 人，副研究员 3 人，高级工程师 1 人。张礼和院士任学术委员会主任；陈鹏教授任实验室主任；赵美萍教授、罗佗平研究员任实验室副主任，樊新元副研究员任重点实验室学术秘书。

实验室的科研工作的基本定位是以有机化学为核心，积极开展有机化学的基础研究；同时，努力开展前沿交叉学科的研究，特别是生命科学相关的化学生物学方面的研究，以及材料科学相关的有机材料化学的研究。目前的研究方向主要包括：1）化学生物学；2）天然产物全合成；3）有机合成方法学；4）生物分离与分析。

实验室目前具有先进的研究条件和研究环境，大型仪器设备主要有 300 兆、400、500 兆超导核磁共振仪、HPLC 手性柱系统、旋光仪、圆二色光谱仪（CD 仪）、气质联用仪（GC-MS）、液质联用仪（LC-MS）、荧光分析仪、4200 真空探针台、基因扩增仪（PCR 仪）、电化学工作站（Autolab）、毛细管电泳色谱仪（Beckman CE）、制备 GPC、高效液相系统等。

2. 实验室学术委员会委员

主 任：张礼和

委 员（按姓氏排列）：邓宏魁、马大为、王剑波、王梅祥、周其林、周 翔、席真、俞飚、杨震

3. 实验室成员（按姓氏排列）

白玉、陈家华、陈鹏、陈兴、樊新元、甘良兵、贾桂芳、雷晓光、李娜、罗佗平、裴坚、王初、王剑波、王婕妤、王能东、魏俊年、席振峰、杨震、余志祥、张文雄、张新祥、赵美萍、周颖琳、邹鹏、朱戎

二、2020 年度工作总结报告

1. 概述

生物有机与分子工程教育部重点实验室在教育部、科技部、国家自然科学基金委以及北京大学的支持下，在 2020 年度，经过全体师生的共同努力，在科研、教学及其人才队伍建设等方面均取得了显著的成绩。

2020 年度在研的科研项目共计 70 项，总经费为 25761.9 万元。其中，新增科研经费 16 项，新增项目经费为 10098 万元，新增项目包括基础科学中心项目 1 项，国家自然科学基金委重大项目 1 项，重点项目 2 项，杰青 1 项，面上项目 3 项，国际合作项目 1 项等。

2020 年度实验室共发表论文 108 篇，包括 Nat. Chem. 1 篇，Nat. Chem. Biol. 1 篇 Nat. Commun. 2 篇，J. Am. Chem. Soc. 11 篇; Angew. Chem. Int. Ed. 8 篇; Anal. Chem. 3 篇; Chem. Commun. 3 篇，Acc. Chem. Res. 2 篇。本年度申请与授权发明专利共 14 项，其中新申请中国专利 5 项、国际专利 2 项，获得 6 项中国发明专利授权、1 项国际发明专利。

在人才队伍建设方面，2020 年度本实验室取得较为突出的成果。实验室成员陈鹏教授获得教育部自然科学奖一等奖；陈兴教授获得由腾讯基金会颁发的科学探索奖等；李娜教授获得北京高校“优质本科课程”奖；裴坚教授获得北京大学优秀教材奖、拔尖学生培养计划“优秀管理奖”；朱戎研究员获得黄廷方/信和青年杰出学者奖、青年教师教学基本功比赛理工科类二等奖和青年教师教学基本功比赛理工科类优秀教案奖等。王初研究员获得国家自然科学基金委杰出青年基金。2020 年度实验室新引进魏俊年副研究员。

在研究生培养方面，2020 年度本实验室共有 46 名研究生获得博士学位。目前实验室在读博士研究生 188 人，在读硕士研究生 20 人。本年度出站博士后 13 人，在站博士后 45 人。本科生教学方面，本实验室教师依然秉承以往的教学传统，积极吸纳大批优秀的本科生参与科研工作，使本科生的教育水平有了极大的提高。

2020 年度因新冠肺炎疫情的影响，重点实验室组织的大型学术会议相对较少。为在疫情期间实现国际学术交流不中断，丰富师生在线学习资源，实验室成员陈鹏教授课题组与芝加哥大学利用暑期时间共同举办了连续举行七周在线联合讲座。该系列讲座共有来自两校的 14 位学者相继围绕生物大分子的动态化学修饰、化学成像、细胞测量等主

题作了精彩的学术报告，总计 1200 多人次全程参加，每期报告的提问讨论环节都进行了热烈的线上互动，取得了很好的效果，促进了我校与国际化学生物学同行的学术交流与科研合作。在陈鹏教授与南京大学郭子建院士的共同组织下，于 2020 年 11 月 1 日在北京大学化学与分子工程学院 A204 学术报告厅成功举行了第一届“SFBC-ChemBIC”双边学术研讨会，会议吸引了 150 多位北京大学和南京大学化学生物学领域的资深科学家、中青年学者和学生参加。实验室成员朱戎研究员与芝加哥大学化学系联合组织了 5 场有机化学系列线上报告，邀请了 Viresh Rawal, 董广彬，Stuart Rowa，Scott Snyder, John Anderson 等世界知名化学家做学术报告，参与会议的研究生与本科生超过 500 人次，取得了良好的交流效果。此外，实验室成员贾桂芳研究员课题组成功举办了第二届全国化学生物学研究生论坛，朱戎研究员课题组举办了 PKU-POSTECH 双边线上论坛。与此同时，本实验室成员还积极参加国内线上或线下学术交流 39 人次。

2.科研工作一览表

（1）本年度在研科研项目

序号	项目负责人	项目名称	项目类别	批准号	执行时间	批准总经费（万元）
1	刘虎威	脂质组学分析系统的构建和应用	国家自然科学基金委科学仪器项目	21527809	2016.1－2020.12	563.5
2	刘虎威	表面等离子体共振-质谱联用研究人载脂蛋白与细胞膜之间的相互作用	国家自然科学基金面上项目	21775008	2018.1－2021.12	65
3	白玉	基于可裂解分子探针的糖蛋白/聚糖的质谱分析新方法研究	国家自然科学基金面上项目	21874003	2019.01-2022.12	66
4	白玉*	防治偏头痛的复方天然药物及其单药组分调节肠道菌群的相关代谢组学研究	北京市自然科学基金重点项目	Z170002	2018.01-2020.12	30
5	陈鹏	细胞命运调控的化学生物学研究	基金委创新群体项目	21521003	2016.01-2021.12	1200

6	樊新元	糖脂代谢网络蛋白的时空调控技术	基金委重大研究培育	91957101	2020.01-2022.12	78
7	陈兴	蛋白质糖基化的化学标记与功能调控	国家重点研发计划	2018YFA0507600	2018.5-2023.4	2671
8	陈兴	蛋白质 O-GlcNAc 糖基化修饰在胚胎干细胞中的功能研究	国家自然科学基金重大研究计划	91753206	2018.1-2021.12	300
9	陈兴	生物正交聚糖标记在肠道微生物组研究中的应用	国家自然科学基金面上项目	21672013	2017.1-2020.12	65
10	陈兴*	信号转导过程中蛋白质机器的活细胞标记与在体调控	国家重点研发计划	2016YFA0501500	2016.7-2021.6	270
11	甘良兵	基于富勒烯的非平面芳香体系研究	国家自然科学基金委面上项目	21672009	2017.1－2020.12	68
12	甘良兵	富勒烯环状配体及其金属配合物的合成与性能研究	国家自然科学基金委面上项目	21871015	2019.1－2022.12	66
13	贾桂芳	研发新型核糖核酸修饰鉴定与检测技术	国家重点研发计划	2019YFA0802201	2019.12-2024.11	170
14	贾桂芳*	基于蛋白化学合成的蛋白质动态可逆修饰样品制备技术	国家重点研发计划	2017YFA0505201	2017.07-2022.06	198
15	贾桂芳	化学生物学	国家自然科学基金优秀青年基金	21822702	2019.01-2021.12	130
16	贾桂芳*	核酸表观遗传的化学调控研究	国家自然科学基金重大国际合作研究项目	21820102008	2019.01-2023.12	100
17	贾桂芳	RNA修饰在结直肠癌发病与免疫微环境中的精准分析与功能解析	北京市基金委重点	Z200010	2020.10-2024.10	300
18	雷晓光	抗骨髓瘤药物靶点 DYRK2 激酶动态修饰蛋白酶体的特异性化学干预	基金委重大研究计划	91853202	2019.01-2022.12	280
19	雷晓光	基于生物兼容反应的蛋白质动态可逆修饰共价化学交联技术	国家科技部重点研发课题	2017YFA05052021	2017.07-2022.06	1084
20	雷晓光*	细胞命运调控的化学生物学研究	基金委创新研究群体	21521003	2016.01-2021.12	175
21	雷晓光	小分子探针导向的化学	基金委杰出青年	21625201	2017.01-2020.12	350

		生物学	科学基金			
22	雷晓光	源于泰国传统药用植物的、具有抗糖尿病活性天然产物的发现，合成与化学生物学研究	NSFC-TRF 项目(中泰)	21961142010	2020.01-2022.01	200
23	李娜	基于光学显微成像的纳米单颗粒计数方法基础与应用研究	国脚自然科学基金委面上	21974006	2020.1–2023.12	65
24	李娜*	基于长程共振能量转移的生物医学成像分析基础研究	国家自然科学基金 委重点项目	21535006	2016.1–2020.12	72
25	罗佗平*	干细胞与再生生物学	国家自然科学基金委创新研究群体科学基金	31521004	2016.1-2021.1	160
26	罗佗平	基于自由基加成/碎裂化策略的天然产物合成及其生物作用机制研究	面上项目	21672011	2017.1-2020.12	65
27	罗佗平*	利用小分子化合物诱导体细胞重编程及其机制研究	科技部重大研究计划	2017YFA0104000	2017.7-2021.12	900
28	罗佗平	天然产物全合成和化学生物学研究	国家自然科学基金（优秀青年基金）	21822101	2019.01-2021.12	130
29	罗佗平	(-)-Vinigrol 的不对称全合成、分子探针的制备及其作用机制研究	国脚自然科学基面上项目	21977002	2020.1-2023.12	66
30	罗佗平	抗生素 Pleuromutilin 的高效化学合成	李革赵宁生命科学青年研究基金	无	2019.4-2020.3	25
31	裴坚	聚集体激发态可调控的新颖杂稠环功能分子体系的精准构建	国家自然科学基金重大项目	21790360	2018.1-2022.12	1695.8
32	王婕妤	高迁移率有机半导体纳米功能材料的设计合成	国家重点研发计划纳米科技重点专项子课题	2017YFA0204701	2017.7-2020.6	100
33	王婕妤	有机π共轭功能材料化学	国家自然科学基金优秀青年科学基金	21722201	2018.1-2020.12	150
34	王婕妤	新型氮杂硼杂稠环分子聚集态的可控制备与性能调控	国家自然科学基金重大项目子课题	21790363	2018.1-2022.12	150
35	王初*	信号转导过程中蛋白质机器的活细胞标记与载体调控	科技部国家重点研发计划专项	2016YFA0501500	2016.07-2021.6	270

36	王初	基于硒同位素印记的化学蛋白质组学	基金委面上项目	21778004	2018.01-2021.12	65
37	王初*	细胞命运调控的化学生物学研究	基金委创新群体项目	21521003	2019.01-2021.12（中期加入）	100
38	王初	化学生物学	基金委国家杰出青年基金项目	21925701	2020.01-2024.12	400
39	王初	细胞铁死亡中新型羰基化修饰的组学发现和修饰率的定量分析	基金委重大研究计划培育项目	91953109	2020.01-2022.12	60
40	王剑波	有机谐二硼、谐二硅以及谐二锡类化合物的合成以及反应研究	国家自然科学基金面上项目	21871010	2019.01-2022.12	66
41	王剑波	基于卡宾化学的手性中心构建	国家自然科学基金委重大研究计划“多层次手性物质的精准构筑”培育项目	91956104	2020.01-2022.12	75
42	张文雄	金属有机化学	国家自然科学基金委员会国家杰出青年科学基金	21725201	2018.01-2022.12	350
43	张文雄	新型稀土有机配合物的合成、结构及反应性	国家自然科学基金委员会重大项目	21890721	2019.01-2023.12	284
44	席振峰	新型双/多金属试剂（物种）	国家自然科学基金委员会重大项目	21690061	2017.01-2021.12	473.6
45	席振峰	空气主份转化化学	科学中心项目	21988101	2020.01-2024.12	8000
46	杨震	具有连续桥头双季碳的活性天然产物的合成和生物活性的研究	国自然基金-重点项目	21632002	2017.1-2021.12	300
47	陈家华	天然产物 Phainanoid F 的全合成研究	国自然基金-面上项目	21772004	2018.1-2021.12	65
48	杨震	深海动植物药物先导化合物的规模化全合成与优化	“深海关键技术与装备”重点专项	2018YFC0310905	2018.8-2021.12	647
49	杨震	Haperforin G 的不对称全合成	国自然基金-面上项目	21871012	2019.1-2022.12	66
50	杨震	重要活性天然产物的合成途径解析及异源表达	广东省重点领域研发计划	2020B0303070002	2020.3-2025.3	400

51	余志祥	乙烯基环丙烷作为三碳组分参与的[3+x+y]反应的方法学发展与应用	常规面上项目	21672008	2017.01-2020.12	66
52	余志祥	金属催化的不对称成环反应：反应发展和机理研究	重大研究计划	91856105	2019.01-2021.12	75
53	余志祥	金属催化环加成反应机理研究和反应发展	国家自然科学基金委重点项目	21933003	2020.01-2024.12	300
54	张新祥	针对癌症关键蛋白翻译后修饰的靶向蛋白质组学 CE-MS 方法研究	国家自然科学基金委面上项目	21775006	2018.01-2021.12	65
55	周颖琳	G-四聚体与电活性小分子相互作用的研究与应用	国家自然科学基金委面上项目	21675004	2017.1-2020.12	65
56	赵美萍	分步表面印迹法精确制备人工受体纳米材料及其应用	国家自然科学基金委面上项目	21775009	2018.1-2021.12	77
57	赵美萍	活细胞的细胞核内 DNA 损伤修复酶的原位荧光成像方法及活性调控分子机理研究	国家自然科学基金委面上项目	21974005	2020.1-2023.12	66
58	赵美萍	驱动基因多位点、低丰度突变的快速联合检测方法研究	北京市自然科学基金面上项目	7192096	2019.1-2021.12	20
59	赵美萍	dPCR 检测试剂盒项目	京东方科技集团股份有限公司技术开发项目	2020002228	2020.7-2021.1	25
60	赵美萍	重组特定 EPO 蛋白的制备技术	国家体育总局反兴奋剂中心技术开发项目	2020004727	2020.12-2021.7	7
61	朱戎	钴催化自由基极性交叉氢官能团化反应研究	国家自然科学基金委青年基金	21901011	2020.1-2022.12	26
62	朱戎	新型聚合调节分子的设计、合成及其在牙科树脂中的应用研究	北京大学临床医学+X 青年专项	无	2020.1-2020.12	30
63	朱戎	有机功能材料化学	千人计划青年人才项目	无	2019.1-2021.12	300
64	邹鹏	青年千人科研启动项目（第十二批）	青年千人（物理化学）	无	2016.06-2021.08	300
65	邹鹏	新型荧光膜电位探针的	面上项目	21673009	2017.01-2020.12	68

		发展及其在神经信号传导机制研究中的应用				
66	邹鹏*	超高时空分辨率的光电联用生物检测一体化装置	国家重大科研仪器研制项目	21727806	2018.01-2020.12	90
67	邹鹏*	着丝粒蛋白质机器调控细胞命运抉择的分子机制	重点研发计划-蛋白质机器	2017YFA0503600	2017.07-2022.06	240
68	邹鹏	蛋白质脂基化修饰的时空特异性检测	重大研究计划-培育项目	91753131	2018.01-2020.12	70
69	邹鹏	基于空间特异性蛋白质标记技术的神经突触新合成蛋白质研究	面上项目	5182011	2018.01-2020.12	20
70	邹鹏*	蛋白质糖基化的化学标记与功能调控	重点研发计划-蛋白质机器	2018YFA0507600	2018.05-2023.04	222

注：（1）阴影部分为 2020 年度新增基金项目。（2）项目参与人员请用“*”标注。

（2）本年度获奖情况

获奖人	获奖项目名称、等级	授 奖 单 位	获奖人排名
陈 鹏	教育部自然科学一等奖	教育部	1
陈 兴	科学探索奖	腾讯基金会	个人奖
陈 兴	陈嘉庚青年科学家	陈嘉庚基金会	个人奖
陈 兴	中国青年科技奖	中国科协	个人奖
李 娜	2020 年北京高校“优质本科课程”（重点）	北京市教育委员会	1
裴 坚	北京大学优秀教材	北京大学	1
裴 坚	基础学科拔尖学生培养计划“优秀管理奖”	基础学科拔尖学生培养计划工作组秘书处	1
朱 戎	Thieme Chemistry Journal Award	Editorial Boards of Thieme Chemistry Journals, Germany	个人奖
朱 戎	黄廷方/信和青年杰出学者奖	北京大学	个人奖
朱 戎	青年教师教学基本功比赛理工科类二等奖	北京大学	个人奖

朱 戎	青年教师教学基本功比赛理工科类优秀教案奖	北京大学	个人奖

（3）本年度申请及授权专利

申请(授权)号	申请(授权)日	专利权人	发明人	发明名称
PCT/CN2020/083482	2020 年 4 月 7 日（申请）	北京大学	白玉，徐姝婷，刘虎威	一种新型有机质谱流式细胞分析技术
CN 104513831 B	2020 年 12 月 1 日（授权）	北京大学	贾桂芳，何川，段洪超	一种促进植物生长的方法
202010324004.0	2020 年 4 月 22 日（申请）	北京大学	雷晓光，赵天湖，张健	一种荧光素偶联体及其合成方法与应用
ZL201611262823.7	2020 年 11 月 6 日（授权）	北京大学	雷晓光，亚历山大•琼斯，董梦秋，曹勇，谭惠	一种蛋白质化学交联剂及其制备方法与应用
ZL201711021980.3	2020 年 11 月 10 日（授权）	北京大学	雷晓光，张静，吴凡， 宋福行， 张立新	一种抗结核化合物及其合成方法与应用
PCT/CN2020/000145	2020 年 6 月 28 日（申请）	兰州大学	戴建业，王初，乔宁	一种同时激活氧化磷酸化通路和抑制糖酵解途径的激活剂及其应用
202010408603.0	2020 年 5 月 14 日（申请）	深圳湾实验室,北京大学深圳研究生院	朱振东，车超 ，蒋顶，邢琦，张家银，黎婷，林光，杨震	穿心莲内酯类化合物及其制备方法、药物组合物和其在抗肿瘤药物中的应用
202010491053.3	2020 年 6 月 3 日（申请）	深圳湾实验室,北京大学深圳研究生院	朱振东，许正双，黎婷，车超，张庆舟，陈思贵，陈煌灿，杨震	靶向 EB 病毒核膜抗原蛋白的小分子抑制剂、制备方法及其应用
202011142825.9	2020 年 10 月 23 日（申请）	北京大学深圳研究生院	杨震，程江群，黄俊，龚建贤，张伟滨	含卤原子手性中心的化合物及其制备方法、应用和含卤原子手性的天然产物的制备方法
202011433461.X	2020 年 10 月 9 日（申请）	深圳湾实验室,北京大学深圳研究生院	车超，朱振东，陈煌灿，黎婷，狄曼，向德虎，杨奇，杨震	喹唑啉类化合物及其制备方法、药物组合物及其应用
ZL201710393589.X	2020 年 4 月 21 日（授权）	北京大学深圳研究生院；深圳白泽生物科技有限公司	张庆舟，张伟滨，林光，龚建贤，杨震	4-羟基环孢素的制备方法

ZL201711348996.5	2020 年 6 月 12 日（授权）	北京大学	赵美萍，翟筠秋，李梦圆，曹翔剑	脱碱基核酸内切酶磁性分子印迹纳米颗粒及其制备方法和应用
ZL201710100553.8	2020 年 5 月 22 日（授权）	北京大学	赵美萍，董建桐，吴曈勃，徐磊，邹梦冰	基于 RNA 聚合酶的体外转录机器快速多重检测 NTPs 的方法及试剂盒与应用
ZL201711348996.5	2020 年 6 月 12 日（授权）	北京大学	赵美萍，翟筠秋，李梦圆，曹翔剑	脱碱基核酸内切酶磁性分子印迹纳米颗粒及其制备方法和应用

（4）国内外学术机构任职情况

姓 名	所任职务名称	聘任时间及期限	聘任机构名称
刘虎威	副主编	2013年11月-	J. Separation Science编辑部
刘虎威	副主编	2016年11月	J. Analysis and Testing编辑部
刘虎威	国际顾问编委	2008年1月-	Springer-Verlag，Analytical Bioanalytical Chemistry
刘虎威	副主编	2008年1月-	《分析仪器》编辑部
刘虎威	编　委	3 年 到 9 年不等	化学通报，分析测试学报，分析实验室，色谱，中国药学-英文版，现代科学仪器，分析科学学报，科学仪器与医疗，岩矿测试，食品安全质量检测学报等刊物编辑部
白　玉	编　委	2014 年 4 月/10 月-	《生命科学仪器》、《质谱学报》编辑部
白　玉	国际顾问编委	2016年11月-	Wiley-VCH, Separation Science Plus
白　玉	Young Career Board	2020年10月	ACS, Analytical Chemistry
白　玉	编　委	2018年1月-	韩国质谱学会《Mass Spectrometry letter》
白　玉	青年执行编委	2019年	《高等学校化学学报》编辑部
白　玉	青年编委	2017年1月-	《分析测试学报》编辑部
白　玉	编　委	2018年4月	《分析实验室》编辑部
陈　鹏	主　任	2020年1月	中国化学会化学生物学专业委员会
陈　鹏	副主编	2019年一至今	美国化学会《ACS Chemical Biology》编辑部

陈　鹏	编　委	2016年一至今	美国化学会《ACS Central Science》编辑部
陈　兴	顾问编委	2014年一至今	美国化学会《ACS Central Science》编辑部
陈　兴	顾问编委	2020-至今	欧洲化学《Analysis & Sensing》编辑部
陈　兴	副主任委员	2020-至今	中国生物化学与分子生物学会糖复合物专业分会
陈　兴	副会长	2020-至今	中国生物物理学会糖生物学分会
陈　兴	副主任委员	2019-至今	中国化学会糖化学专业委员会
甘良兵	International Advisory Board	2011年一至今	International Symposium on Novel Aromatic Compounds
雷晓光	执行主编	2017年一至今	Elsevier《Bioorgani and Medicinal Chemistry》
李　娜	光谱专业组仪器技术评议专家	2007.6一至今	中国分析测试协会
李　娜	光谱理事会理事	2008.6一至今	北京市分析测试协会
李　娜	常务编委	2015.3一至今	《光谱学与光谱分析》编辑部
李　娜	光谱仪器专业委员会委员	2017.8一至今	中国仪器仪表学会分析仪器分会
李　娜	副主编	2014.6一至今	《大学化学》编辑部
裴　坚	International Advisory Board	2012年1月-	International Conference on the Science and Technology of Synthetic Metals
裴　坚	Editorial Board Chair	2016年8月-	Asian Journal of Organic Chemistry
裴　坚	International Advisory Board	2016年8月-	Chemistry-An Asian Journal
裴　坚	理　事	2017年7月-	中国高等教育学会第七届理事会
裴　坚	副主任	2018-2022	中国化学会光化学专业委员会
裴　坚	秘书长	2018-2022	教育部大学化学课程指导委员会
裴　坚	委　员	2018-2022	中国化学会有机固体专业委员会
裴　坚	委　员	2018-2022	中国科学院光化学重点实验室学术委员会
王　初	Advisory Board Member	2020.11-2022.12	Chemical Sciences
王剑波	副主编	2007 年一至今	《有机化学》编辑部

王剑波	副主编	2009 年一至今	Journal of Physical Organic Chemistry
王剑波	编　委	2013 年一至今	Organic Letters
王剑波	编　委	2014年一至今	Tetrahedron/Tetrahedron Letters
王剑波	编　委	2018年一至今	Journal of Fluorine Chemistry
王剑波	编　委	2018年一至今	Chinese Journal of Chemistry
席振峰	副主编	2013年一	美国化学会《Org. Lett.》
席振峰	International Advisory Board Member	2013年一	IUPAC 国际金属有机化学会议
席振峰	主　任	2017年-	北京分子科学国家研究中心
席振峰	化学学科评审组长	2018年-	《中国科学》编辑部
张文雄	青年编委	2018年-	《中国化学》编辑部
张文雄	编　委	2019年-	《中国化学快报》编辑部
张文雄	第一届青年执行编委	2019年-	《高等学校化学学报》编辑部
张文雄	副主编	2020年-	《绿色合成与催化》编辑部
杨　震	副主任委员	2019 年-2022 年	中国化学会有机化学学科委员会
杨　震	期刊编委	2019 年-2020 年	中国化学会《有机化学》期刊
杨　震	实验室主任	2018 年-2022 年	省部共建肿瘤化学基因组学国家重点实验室
杨　震	学术委员会委员	2017 年-2021 年	青岛海洋科学与技术国家实验室海洋药物与生物制品功能实验室
杨　震	学术委员会委员	2017 年-2021 年	新药研究国家重点实验室（上海药物研究所）
杨　震	编　委	2017 年-2020 年	英国皇家化学会“Natural Product Reports”
赵美萍	顾问编委	2018年7月一	Society for Applied Spectroscopy《Applied Spectroscopy》
赵美萍	编　委	2013年1月一	《分析科学学报》编辑部
赵美萍	理事会成员	2015年一	国际分子印迹协会
赵美萍	标记免疫分析专业委员会常务委员	2016年6月一	中国分析测试协会

（5）本年度实验室成员参加国内外学术会议情况

序号	参加会议名称及报告题目	参加人	时间、地点
1	ACS Fall meeting （Online）, “Bioorthogonal Cleavage Reactions in Space and Time”	陈　鹏	2020.09 北京
2	2020 Symposium on Chemistry and Biomedicine Interfaces ：“Chemical Labeling and Functional Elucidation of Glycans”	Xing Chen	December 4-6, 2020，Beijing
3	Nano Health – In Vivo Biometrics and Precision Medicine Forum: “Click Expansion Microscopy: Making Super-Resolution Bioimaging Easier”	Xing Chen	October 28-29, 2020，Suzhou, China
4	Symposium on Computational Glycomics: “Metabolic Glycan-Assisted Profiling of Intact Glycopeptides”	Xing Chen	January 19, 2020, Beijing, China
5	2020全国植物生物学大会，表观转录修饰对植物生长发育调控功能研究	贾桂芳	2020.10.14深圳
6	2020香山科学会议-核酸生物化学与技术，表观转录修饰在植物生长发育和作物改良中的研究与应用	贾桂芳	2020.9.24上海
7	京津有机化学沙龙，“脱烯自由基化反应”	罗佗平	2020.10.04，北京
8	化学与生命科学交叉学术交流研讨会，“天然产物高效全合成及化学生物学研究”	罗佗平	2020.10.23-25，北京
9	太湖（马山）生命与健康论坛，“化学遗传学和药物研发”	罗佗平	2020.11.22，无锡
10	合成化学青年学者研讨会，“弗斯可林的合成化学研究”	罗佗平	2020.12.21-22，北京
11	2020中国化工学会科技创新大会，用于柔性电子的透明聚酰亚胺CPI材料	裴　坚	2020年9月14-16日，北京
12	高分子物理和化学黄金周暨第14届“冯新德高分子奖”报告会，共轭高分子的溶液聚集态结构调控	裴　坚	2020年11月23-27日，长春
13	2020 RSC Emerging Investigator Forum, BN-Embedded Tetrabenzopentacene with Remarkable Ambient Stability	王婕妤	2020年9月26-27日，苏州
14	The China/US 10+10 workshop, “Chemoproteomic Profiling of Functional Modifications by Reactive Metabolites”	王　初	2020.01.16, San Diego, USA
15	第三届兰州自主创新论坛会议，“抗脂肪肝中药分子的靶标鉴定和机理研究”	王　初	2020.09.19, 兰州
16	中国生物化学与分子生物学会 2020 年全国学术会议，“Chemistry-enabled Functional Proteomics”	王　初	2020.10.23, 在线
17	第十一届质谱网路会以 iCMS2020, “化学驱动的功能蛋白质组学”	王　初	2020.12.11，在线
18	2020 金属组学研讨会，”Predicting Metalloproteomes by Machine Learning”	王　初	2020.12.27，北京
19	2020功能配位化学前沿研讨会**邀请报告**，题目：稀土金属有机杂	张文雄	2020年11月

	环化学		27-29日，佛山，广东
20	第二届热带海洋资源利用与绿色化工研讨会**邀请报告**，题目：白磷直接合成有机膦的新方法	张文雄	2020年12月4-6日，海口，海南
21	2020 年中部合成化学研讨会**邀请报告**，题目：白磷直接合成有机膦的新方法	张文雄	2020年12月10-12日，长沙，湖南
22	2020 配位化学与无机功能材料学术研讨会**主题邀请报告**，题目：稀土金属有机杂环化学	张文雄	2020年12月20-23日，兰州，甘肃
23	国家绿色化学智库--2020 流动化学技术专题研讨会	杨震、龚建贤	2020.11 广东省深圳市人才研修院
24	2020年世界科学家峰会-绿色化学国际研讨会	余志祥	2020/10/17-18，浙江温州
25	Dialogues in Discovery: Chemical Biology Symposium，邀请报告 Phosphorothioated DNA-guided deoxyribonuclease I for ultra-sensitive detection of genetic alterations	赵美萍	Scripps Research 2020 年 1 月 15 日，美国圣地亚哥
26	10th 10+10 bilateral UC-PKU workshop，邀请报告 Phosphorothioated DNA-guided deoxyribonuclease I for ultra-sensitive detection of genetic alterations	赵美萍	UCSD，2020年1月16-18日，美国圣地亚哥
27	第 21 届全国分子光谱学学术会议暨 2020 年光谱年会，邀请报告：荧光分析法测定基因组 DNA 碱基的损伤和突变	赵美萍	2020 年 10 月 30 日-11 月 2 日，成都
28	《绿色合成与催化》新刊发布会和第二届编委会暨不对称催化与合成学术会议	朱　戎	2020.8.15-18 江西井冈山
29	京津有机化学沙龙	朱　戎	2020.10.5 北京
30	基金委合成化学青年研讨会 分叉催化：从小分子到活性聚合	朱　戎	2020.12.21-22 北京
31	北大/浦项科技大学双边论坛 Bifurcating Transition-Metal Catalysts	朱　戎	2020.11.18 北京
32	Profiling subcellular transcriptome with proximity-dependent RNA labeling，Scripps研究所（邀请报告）	邹　鹏	2020/01、美国圣地亚戈
33	Profiling subcellular transcriptome with proximity-dependent RNA labeling，加州大学圣地亚戈分校（邀请报告，Session Chair）	邹　鹏	2020/01、美国圣地亚戈
34	Recent developments in neuronal voltage imaging，脑智卓越中心专题学术研讨会系列（邀请报告）	邹　鹏	2020/06、北京，线上
35	Mapping spatial transcriptome with chromophore-assisted proximity-dependent RNA labeling，北京大学-芝加哥大学联合系列讲座（邀请报告）	邹　鹏	2020/07、北京，线上
36	Profiling subcellular transcriptome with chromophore-assisted proximity-labeling and sequencing (CAP-seq)，拜耳线上论坛（邀请	邹　鹏	2020/08、北京，线上

	报告）		
37	高时空分辨的亚细胞转录组标记技术 CAP-seq，北京分子科学国家研究中心学术交流会（邀请报告）	邹　鹏	2020/10、北京
38	神经活动的化学探针，第一届 SFBC-ChemBIC 双边研讨会（邀请报告）	邹　鹏	2020/11、北京
39	神经活动的化学探针，中国科学院自动化所（邀请报告）	邹　鹏	2020/11、北京

(6) 本年度人才培养情况：在站博士后、在读博士生、硕士生人数

毕业 博士研究生	在读 博士研究生	在读 硕士研究生	出站 博士后	在站 博士后
46	188	20	13	45

(7) 本年度实验室成员承担本科生、研究生课堂教学情况

任课教师	课程名称(主讲）	授课对象	课程类型	总学时	听课人数
白　玉	高等色谱分析	研究生	专业必修	30	30
白　玉	色谱分析	本科生	限选	30	11
白　玉	定量分析化学实验	本科生(生科院)	专业必修	60	86
陈　鹏	化学生物学导论	研究生（化院）	限选课	36	80
陈　鹏	化学生物学 Track	全部专业(前沿交叉）	选修	8	30
陈　兴	化学生物学 Seminar	研究生（化学学院）	必修	32	17
陈　兴	化学生物学基础 I	研究生（化学学院）	必修	48	32
陈　兴	化学生物学进展	全部专业(前沿交叉）	必修	4	31
陈　兴	博雅理学讲堂	全校学生	选修	2	420
甘良兵	有机化学 B	本科生（医学部）	必修	64	170
贾桂芳	化学生物学实验课	大三本科生	实验课	64	24
雷晓光	化学基础（整合科学）	本科生（元培学院）	专业必修	64	25
雷晓光	改变世界的药物分子	本科生（化学学院）	任选	16	36
李　娜	定量化学分析（英）(主讲）	本科生（化学学院、元培）	必修	32	124
李　娜	仪器分析阅读小班课(主讲）	本科生（化学学院）	必修	32	12
李　娜	中级分析化学阅读小班课（主讲）	本科生（化学学院）	必修	32	12
罗佗平	有机化学（一）	化学学院本科生（小班）	必修	48	14

罗佗平	立体化学	化学学院本科生/ 研究生	选修	32	41
罗佗平	有机化学（二）	化学学院本科生（小班）	必修	32	12
罗佗平	中级有机化学	化学学院本科生（小班）	必修	32	12
裴　坚	基础有机化学 II	本科生（化学学院）	必修	32	72
裴　坚	基础有机化学习题课	本科生（化学学院）	必修	16	61
裴　坚	中级有机化学	本科生（化学学院）	选修	32	122
王婕妤	有机化学实验(A)小班	本科生（化学学院）	必修	180	28
王婕妤	中级有机化学实验小班	本科生（化学学院）	选修	128	20
王　初	化学生物学基础（二）	研究生（化院）	限选课	48	32
王　初	化学生物学实验	本科生（化院）	限选课	48	16
王　初	定量生物技术	研究生（又院）	必修课	48	80
王　初	整合科学实验	本科生（元培）	限选课	32	2
王剑波	有机化学（一）	化学学院本科	专业必修	48	95
王剑波	物理有机化学	化学学院	专业任选	32	23
席振峰	有机化学	本科生（医学部）	必修	64	137
张文雄	合成化学-有机合成	研究生	限选	30	42
张文雄	金属有机化学	研究生	必修	30	36
余志祥	计算化学（2）	研究生	限选	32	65
余志祥	前沿文献阅读	研究生	必修	48	31
余志祥	理论有机化学	研究生	必修	32	20
张新祥	仪器分析	本科生（化学学院）	限选	32	48
周颖琳	仪器分析实验	本科生（化学学院）	限选	60	120
周颖琳	生化分析	本研	任选	32	51
赵美萍	定量分析化学	本科生（环科、城环、地空学院等）	必修	32	50
朱　戎	《有机化学 seminar》	研究生	专业必修	32	11
朱　戎	《高等有机化学》	研究生	专业选修	48	28
朱　戎	《今日化学》	本科生	专业必修	16	166
邹　鹏	生命化学基础	本科生	理论课	48	180
邹　鹏	化学生物学实验	本科生	实验课	48	24
邹　鹏	今日化学	本科生	理论课	16	140
邹　鹏	学术道德规范与科技写作	研究生	理论课	32	200

(8) 本年度实验室组织学术报告

1-举办“北京大学—芝加哥大学化学生物学联合在线讲座”

为在疫情期间实现国际学术交流不中断，丰富师生在线学习资源，我们与芝加哥大学利用暑期时间共同举办了在线联合讲座。其中，以“化学生物学”为主题的在线系列讲座由北京大学国际合作部主办、北京大学合成与功能生物分子中心、化学与分子工程学院承办，从 7 月 22 日第一期开始，至 9 月 2 日第七期结束，共连续举行七周。该系列讲座共有来自两校的 14 位学者相继围绕生物大分子的动态化学修饰、化学成像、细胞测量等主题作了精彩的学术报告。总计 1200 多人次全程参加，每期报告的提问讨论环节都进行了热烈的线上互动，取得了很好的效果，促进了我校与国际化学生物学同行的学术交流与科研合作。

2-举办第一届“SFBC-ChemBIC”双边学术研讨会

在我们与南京大学郭子建院士的共同组织下，第一届“SFBC-ChemBIC”双边学术研讨会于 2020 年 11 月 1 日在北京大学化学与分子工程学院 A204 学术报告厅成功举行。会议吸引了 150 多位北京大学和南京大学化学生物学领域的资深科学家、中青年学者和学生参加。会议由北京大学化学与分子工程学院院长陈兴教授致辞开幕，特邀嘉宾国家自然科学基金委化学科学四处副处长兼化学生物学项目主任张艳博士致辞。随后来自南京大学和北京大学的 20 多位教授学者相继报告了自己的最新研究成果、并与在场师生进行了热烈的交流讨论。最后南京大学化学和生物医药创新研究院院长郭子建院士致闭幕词。本次会议充分展示了两校化学生物学领域的最新成果和研究进展，讨论了化学生物学未来发展的新思路、新领域和新趋势，搭建了高水平的院校间合作交流平台，很好地促进了两校间化学生物学领域的学术交流与合作。

3-第二届全国化学生物学研究生论坛

为了进一步加强各高校院所间的学术交流，促进化学生物学各分支学科的发展，开阔研究生的科研视野，化学生物学系于 2020 年 10 月 16-17 日在线举办了“第二届化学生物学研究生前沿论坛”。会议为期两天，邀请了芝加哥大学的何川教授及北京大学的黄岩谊教授进行了学术报告、随后参会研究生也相继做了精彩的学术展示。会议吸引了近 200 人在线参与，很好地促进了学生间的交流合作。

4-PKU-University of Chicago Joint Lecture Series on Organic and Organometallic Chemistry (2020.9-2020.11)

本年度秋季，我们与芝加哥大学化学系联合组织了系列线上报告，共主持 5 场，报告人包括 Viresh Rawal, 董广彬，Stuart Rowa，Scott Snyder, John Anderson 等世界知名化学家，面向对有机化学感兴趣的研究生和本科生展开交流，超过 500 人次参与。

5-北大/浦项科技大学双边论坛(2020.11.18)

我们与吕华课题组一同组织了 PKU-POSTECH 双边线上论坛，共有来自双方 8 位教授，12 位学生以及博士后参与交流。

(9) 科学传播

①2020 年北大化学学院“全国优秀大学生夏令营”活动于 2020 年 7 月在北京大学举行，我们参加并就化学生物学专业的相关问题与全国优秀大学生夏令营学员进行了热烈交流。

②“王初课题组”微信公众号每周从国际顶级期刊中 Nature 系列、Science 系列、Cell 系列、J. Am. Chem. Soc.和 Angewandte Chemie 等杂志中选取化学生物学领域的最新研究文章，通过组内的成员进行阅读、理解与撰写，再由组内学生编辑们设计排版后发布，让读者们能直观快速的了解化学生物学领域的最新动态。截止到 2020 年 12 月 17 日，“王初课题组”公众号总关注数已经达到了 54652 人。在 2020 年 1 月 1 日到 12 月 17 日这一段时间内，据不完全统计，“王初课题组”公众号大概发布了 800 多篇原创的图文信息，总阅读数超过 270 万次，总阅读人数超过 168 万人次，为宣传和科普化学生物学研究贡献了力量。

(10) 本年度实验室发表论文目录

序号	论文题目	作 者	期刊及年卷页
1	High-Throughput Single-Cell Immunoassay in the Cellular Native Environment Using Online Desalting Dual-Spray Mass Spectrometry	Shuting Xu, Jinjuan Xue, Yu Bai*, Huwei Liu	*Anal. Chem.* **2020**, *92*, 15854-15861
2	In Situ Laser Scattering Electrospray Ionization Mass Spectrometry and Its Application in the Mechanism Study of Photoinduced Direct C-H Arylation of Heteroarenes	Wanpeng Ai, Qirong Yang, Yunpeng Gao, Xiaoyun Liu, Huwei Liu, and Yu Bai*	*Anal. Chem.* **2020**, *92*, 11967-11972
3	Tracing and elucidating visible-light mediated oxidation and C-H functionalization of amines using mass spectrometry	Wanpeng Ai, Yunpeng Gao, Jinjuan Xue, Xiaoyun Liu, Huwei Liu, Jianbo Wang, Yu Bai*	*Chem. Commun.*, **2020**, *56*, 2163-2166
4	A novel online two-dimensional supercritical fluid chromatography/reversed phase liquid chromatography-mass spectrometry method for lipid profiling	Li Yang, Honggang Nie, Fan Zhao, Shiyao Song, Ying Meng, Yu Bai, Huwei Liu*	*Anal. Bioanal. Chem.*, **2020**, *412*, 2225-2235
5	Myriocin and D-PDMP ameliorate atherosclerosis in ApoE(-/-) mice via reducing	Zemou Yu, Qing Peng, Songyue Li, Hongjun	*Clinical Science*, **2020**, *134*, 439-458

	lipid uptake and vascular inflammation	Hao, Jianwen Deng, Lingbing Meng, Zhiyuan Shen, Weiwei Yu, Nan. Ding, Yu Bai*, Yining Huang*	
6	Phospholipid imaging of zebrafish exposed to fipronil using atmospheric pressure matrix-assisted laser desorption ionization mass spectrometry	Wenjie Liu, Hongxiang Nie, Dapeng Liang, Yu Bai*, Huwei Liu	*Talanta,* **2020**, *209*, 120357.
7	Severe Acute Respiratory Syndrome Coronavirus‐2 Spike Protein Nanogel as a Pro‐Antigen Strategy with Enhanced Protective Immune Responses	Long Chen, Bo Liu, Peng Sun, Wenjun Wang, Shiqiang Luo, Wenyuan Zhang, Yuanfan Yang, Zihao Wang, Jian Lin*, Peng R. Chen*	*Small*, **2020**, *16*, 2004237
8	Cationic Lipid-based Intracellular Delivery of Bacterial Effectors for Rewiring Malignant Cell Signaling	Yang S, Tang Q, Chen L, Chang J, Zhao J, Jiang T, Wang M, Peng R. Chen*	*Angew. Chem. Int. Ed.* (**2020**), *59*, 18087-18094
9	Genetically encoded protein labeling and crosslinking in living Pseudomonas aeruginosa	Zheng H, Lin S, Chen P	*Bioorg Med Chem.* (**2020**), *28*, 115545
10	SFPQ Is an FTO-Binding Protein that Facilitates the Demethylation Substrate Preference	Song H, Wang Y, Wang R, Zhang X, Liu Y, Jia G*, Peng R. Chen*	*Cell Chem. Biol.* **2020**, *27*, 283-291
11	Chemoproteomic Profiling of O-GlcNAcylation in *Caenorhabditis elegans*	Wei Qin,Zhongyun, Xie Jingyang Wang,Guangshuo Ou ,Chu Wang*****,Xing Chen*****	*Biochemistry,***2020,** *59, 34,* 3129–3134
12	Protein S-Glyco-Modification through an Elimination–Addition Mechanism	Ke Qin**,** Hao Zhang**,**Zhenqi Zhao**,**Xing Chen*****	*J. Am. Chem. Soc.***2020,** *142, 20,* 9382–9388
13	Metabolic RNA labeling for probing RNA dynamics in bacteria	Liying Meng,Yilan Guo,Qi Tang,Rongbing Huang,Yuchen Xie,Xing Chen*****	*Nucleic Acids Res.***2020**,*48,22,*12566-12576
14	Synthesis of Open-Cage Fullerenes with a Long Tail	Hao Zhang, Jie Su, * and Liangbing Gan*	*Org. Materials* **2020**, *2*, 282-287.
15	Antibody-free enzyme-assisted chemical approach for detection of N^6-methyladenosine.	Ye Wang, Yu Xiao, Shunqing Dong, Qiong Yu, Guifang Jia*	*Nature Chemical Biology*, **2020**, *16*, 896-903
16	SFPQ is an FTO-binding protein that facilitates the demethylation substrate preference	Haiping Song, Ye Wang, Ruixiang Wang, Xiao Zhang, Yaping Liu, Guifang Jia*, Peng	*Cell Chemical Biology,* **2020**, *27*, 283-291

		R.Chen*	
17	RNA 化学修饰 m6A 的生物功能研究进展	唐乾, 张梧桐, 贾桂芳	*中国科学化学*, **2020**, *50*:1233-1249
18	Detection methods of epitranscriptomic mark N6-methyladenosine	Ye Wang, Guifang Jia	*Essays in Biochemistry*, **2020**, *64*：967-979
19	Chemical screening identifies ROCK1 as a regulator of migrasome formation	Puzhong Lu, Rui Liu, Di Lu, Yue Xu, Xueyi Yang, Zheng Jiang, Chun Yang, Li Yu*, Xiaoguang Lei * and Yang Chen*	*Cell Discovery*, **2020**, *6(1)*, 51
20	Chemoenzymatic Total Syntheses of Artonin I with an Intermolecular Diels–Alderase	Xiaojing Liu, Jun Yang, Lei Gao, Liyun Zhang, Xiaoguang Lei*	*Biotechnology Journal*, **2020**, *15(11)*, 2000119
21	Syntheses of Skeletally Diverse Tetracyclic Isodon Diterpenoid Scaffolds Guided by Dienyne Radical Cyclization Logic	Weilong Liu, Zongwei Yue, Zhen Wang, Houhua Li and Xiaoguang Lei*	*Organic Letters*, **2020**, *22(20)*, 7991–7996
22	Inhibition of PU.1 ameliorates metabolic dysfunction and non-alcoholic steatohepatitis	Qiongming Liu1, Junjie Yu, Liheng Wang, Yuliang Tang, Quan Zhou, Shuhui Ji , Yi Wang , Luis Santos , Rebecca A. Haeusler, Jianwen Que, Prashant Rajbhandari, Xiaoguang Lei, Luca Valenti, Utpal B. Pajvani*, Jun Qin*, Li Qiang, *	*Journal of Hepatology*, **2020**, *73(2)*, 361–370
23	FAD-dependent enzyme-catalysed intermolecular [4+2] cycloaddition in natural product biosynthesis	Lei Gao, Cong Su, Xiaoxia Du, Ruishan Wang, Shuming Chen, Yu Zhou, Chengwei Liu, Xiaojing Liu, Runze Tian, Liyun Zhang, Kebo Xie, She Chen, Qianqian Guo, Lanping Guo, Yoshio Hano, Manabu Shimazaki, Atsushi Minami, Hideaki Oikawa, Niu Huang, K. N. Houk, Luqi Huang*, Jungui Dai*, Xiaoguang Lei*	*Nature Chemistry*, **2020**, *12(7)*, 620–628
24	Chrysomycin A Derivatives for the Treatment of Multi-Drug-Resistant Tuberculosis	Fan Wu, Jing Zhang, Fuhang Song, Sanshan	*ACS Central Science*, **2020**, *6(6)*, 928–938

		Wang, Hui Guo, Qi Wei, Huanqin Dai, Xiangyin Chen, Xuekui Xia, Xueting Liu, LixinZhang, Jin-Quan Yu and Xiaoguang Lei*	
25	Identification of the AMA Synthase from the Aspergillomarasmine A Biosynthesis and Evaluation of Its Biocatalytic Potential	Qianqian Guo, Dongshan Wu, Lei Gao, Yingjie Bai, Yuan Liu, NianxinGuo, Xiaoxia Du, Jun Yang, Xiaoming Wang and Xiaoguang Lei*	*ACS Catalysis*, **2020**, *10(11)*,6291–6298
26	Late-Stage Diversification of Natural Products	Benke Hong, Tuoping Luo and Xiaoguang Lei*	*ACS Central Science*, **2020**, *6(5)*, 622–635
27	Dissecting Programmed Cell Death with Small Molecules	Yingjie Bai, Hiu C. Lam and Xiaoguang Lei*	*Accounts of Chemical Research*, **2020**, *53(5)*, 1034–1045
28	Computation-Guided Development of the click ortho-Quinone Methide Cycloaddition with Improved Kinetics	XiaoyunZhang, Shuo-Qing Zhang, Qiang Li, Fan Xiao, Zongwei Yue, Xin Hong* and Xiaoguang Lei*	*Organic Letters*, **2020**, *22(8)*, 2920–2924
29	An Arabidopsis Secondary Metabolite Directly Targets Expression of the Bacterial Type III Secretion System to Inhibit Bacterial Virulence	Wei Wang, Jing Yang, Jian Zhang, Yong-Xin Liu, CaipingTian, Baoyuan Qu, Chulei Gao, Peiyong Xin, ShujingCheng, Wenjing Zhang, Pei Miao, Lei Li, XiaojuanZhang, Jinfang Chu, JianruZuo, Jiayang Li, Yang Bai, Xiaoguang Lei* and Jian-Min Zhou*	*Cell Host and Microbe*, **2020**, *27(4)*, 601–613.
30	Evaluation of chemical cross-linkers for in-depth structural analysis of G protein-coupled receptors through cross-linking mass spectrometry	Lisha Xia, Ziliang Ma, Jiahui Tong, YuliangTang, Shanshan Li, Shanshan Qin, Ronghui Lou, Suwen Zhao, XiaoguangLei* and Wenqing Shui*	*Analytica Chimica Acta*, **2020**, *1102*, 53–62
31	Biomimetic Synthesis of Rhytidenone A and Mode of Action of Cytotoxic Rhytidenone F	Zongwei Yue, Hiu C. Lam, KaiqiChen, IttiponSiridechakorn,	*Angew. Chem. Int. Ed.*, **2020**, *59(10)*, *p*4115–4120

		Yaxi Liu, Khanitha Pudhom and Xiaoguang Lei*	
32	Styryllactones from Goniothalamus tamirensis	Pornphimol Meesakul, Wuttichai Jaidee, Christopher Richardson, Raymond J. Andersen, Brian O. Patrick, Anthony C. Willis, Chatchai Muanprasat, Jin Wang, Xiaoguang Lei, Sarinya Hadsadee, Siriporn Jungsuttiwong, Stephen G. Pyne* and Surat Laphookhieo*	*Phytochemistry,* **2020**, *171,* 112248
33	Protecting-Group-Free Syntheses of ent-Kaurane Diterpenoids: [3+2+1] Cycloaddition/Cycloalkenylation Approach	Jin Wang, Benke Hong, Dachao Hu, Yuichiro Kadonaga, Ruyao Tang and Xiaoguang Lei*	*Journal of the American Chemical Society,* **2020**, *142(5),* 2238–2243
34	Biosynthetic Intermediate Probes for Visualizing and Identifying the Biosynthetic Enzymes of Plant Metabolites	Lei Gao* and Xiaoguang Lei*	*ChemBioChem* **2020**, *21,* 1–4
35	New Strategies in the Efficient Total Syntheses of Polycyclic Natural Products	Weilong Liu, Benke Hong, Jin Wang and Xiaoguang Lei*	*Acc. Chem. Res.***2020**, *53, 11,* 2569–2586
36	Colocalized particle counting platform FOR zeptomole level multiplexed quantification	Guangyu Tao, Tiancheng Lai, Xiao Xu, Yurou Ma, Xi Wu, Xiaojing Pei, Feng Liu, Na Li*	*Anal. Chem.* **2020**, *92*, 3697−3706
37	Competitive aptasensor for the ultrasensitive multiplexed detection of cancer biomarkers by fluorescent nanoparticle counting	Xiaojing Pei, Xi Wu, Jie Xiong,Guohong Wang, Guangyu Tao, Yurou Ma, Na Li*	*Analyst* **2020**, *145*, 3612−3619.
38	Nanomaterial-based multiplex optical sensors (invited review)	Xiaojing Pei, Guangyu Tao, Xi Wu, Yurou Ma, Rongsheng Li, Na Li*	*Analyst* **2020**, *145, 4111−4123.*
39	Synthesis of 17-Deacetoxyl Chromodorolide B Based on a Gold-Catalyzed Alkoxycyclization Reaction	Chen Li, Tianfei Quan, Yibin Xue, Yuhui Cao, Si-Cong Chen, Tuoping Luo*	*Org. Lett.* **2020**, *22*, 1655−1658.
40	Total Synthesis of (−)-Batrachotoxinin A: A Local-Desymmetrization Approach	Yinliang Guo, Zhixian Guo, Jia-Tian Lu, Runting Fang, Si-Cong Chen, Tuoping Luo*	*J. Am. Chem. Soc.* **2020**, *142*, 3675−3679.

41	Elimination of Senescent Cells by β-Galactosidase-Targeted Prodrug Attenuates Inflammation and Restores Physical Function in Aged Mice	Yusheng Cai, Huanhuan Zhou, Yinhua Zhu, Qi Sun, Yin Ji, Anqi Xue, Yuting Wang, Wenhan Chen, Xiaojie Yu, Longteng Wang, Han Chen, Cheng Li, Tuoping Luo*****; Hongkui Deng*	*Cell Res.* **2020**, *30*, 574-589.
42	Structural Insights Into the Inhibition Mechanism of Human Sterol O-acyltransferase 1 by a Competitive Inhibitor	Chengcheng Guan, Yange Niu, Si-Cong Chen, Yunlu Kang, Jing-Xiang Wu, Koji Nishi, Catherine C. Y. Chang, Ta-Yuan Chang, Tuoing Luo, Lei Chen*	*Nat. Commun.* **2020**, *11*, 2478.
43	Rapid Construction of Fold-Line-Shaped BN-Embedded Polycyclic Aromatic Compounds through Diels–Alder Reaction	Peng-Fei Zhang, Fang-Dong Zhuang, Zehao Sun, Yang Lu, Jie-Yu Wang,* Jian Pei*	*The Journal of Organic Chemistry*, **2020**, *85*, 241-247
44	Conformation-Dependent Spin Relaxation Behaviors of 6-Oxoverdazyl Radical Single Crystals	Zi-Yuan Wang, Ya-Zhong Dai, Ze-Fan Yao, Bo-Wei Dong, Yang Lu, Li Ding, Shang-Da Jiang,* Jie-Yu Wang,* Jian Pei*	*Crystal Growth & Design*, **2020**, *20*, 2141-2146
45	Chemoproteomic profiling of itaconation by bioorthogonal probes in inflammatory macrophages.	Qin, W. #; Zhang, Y. #; Tang, H.; Liu, D.; Chen, Y.; Liu, Y.; Wang, C.*	*J.Am.Chem.Soc.* **2020**, *142 (25)*, 10894-10898
46	Chemical Proteomic Profiling of Protein 4'-Phosphopantetheinylation in Mammalian Cells.	Chen, N.; Liu, Y.; Li, Y.; Wang, C.*,	*Angew. Chem. Int. Ed.* **2020**, *59(37)*, 16069-16075
47	Profiling of post-translational modifications by chemical and computational proteomics.	Yang, F.; Wang, C.*	*Chem Commun (Camb).* **2020**, *56(88)*, 13506-13519
48	Experimental and Computational Studies on Rh(I)-Catalyzed Reaction of Siloxyvinylcyclopropanes and Diazoesters	Sheng Feng, Kang Wang, Yifan Ping and Jianbo Wang*	*J. Am. Chem. Soc.* **2020,** *142*, 21032-21039.
49	Cu(I)/Chiral Bisoxazoline-Catalyzed Enantioselective Sommelet-Hauser Rearrangement of Sulfonium Ylides	Shu-Sen Li and Jianbo Wang*	*J. Org. Chem.* **2020**, *85,* 12343-12358.
50	Palladium-Catalyzed Reductive Coupling of Aromatic Bromides and Trimethylsilyldiazomethane: Its Application to Methylation of Aromatic Compounds	Shuai Wang, Cheng Yang, Shuo Sun, Hanli Sun, and Jianbo Wang*	*Chin. J. Org. Chem.* **2020**, *40,* 3881-3888.

51	Difluoroketenimine: Generation from Difluorocarbene and Isocyanide, and Its [3+2] Cycloadditions with Alkenes or Alkynes	Rui Zhang, Zhikun Zhang, Kang Wang, and Jianbo Wang*	*J. Org. Chem.* **2020**, *85*, 9791–9800.
52	Transition-Metal-Catalyzed Cross-Coupling with Ketones or Aldehydes via N-Tosylhydrazones	Ying Xia* and Jianbo Wang*	*J. Am. Chem. Soc.* **2020,** *142*, 10592–10605.
53	Construction of Alkenyl-Functionalized Spirocarbocyclic Scaffolds from Alkyne-Containing Phenol-Based Biaryls via Sequential Iodine-Induced Cyclization/Dearomatization and Pd-Catalyzed Coupling of N-Tosylhydrazones	Anjia Liu, Kaiming Han, Xin-Xing Wu, Shufeng Chen,* Jianbo Wang*	*Chin. J. Chem.* **2020,** *38*, 1257-1262.
54	Palladium-Catalyzed Cascade Cyclization/Dearomatization/Arylation of Alkyne-Containing Phenol-Based Biaryls with Aryl Halides: An Entry to Diversely Functionalized Spirocyclohexadienones	Yunlong Bai, Anjia Liu, Xin-Xing Wu, Shufeng Chen*, and Jianbo Wang*	*J. Org. Chem.* **2020**, *85*, 6687-6696.
55	Ring-Opening Iodination and Bromination of Unstrained Cycloalkanols through ß-Scission of Alkoxy Radicals	Jiang-Ling Shi, Yuankai Wang, Zixuan Wang, Bowen Dou and Jianbo Wang*	*Chem. Commun.* **2020,** *56*, 5002-5005.
56	Synthesis of Arylboronic Pinacol Esters from Corresponding Arylamines	Fanyang Mo, Di Qiu and Jianbo Wang*	*Org. Synth.* **2020,** *97*, 1
57	Mono- and Bis-Titanium Complexes Bridged by 2-Butene Tetraanion: Synthesis and Structural Characterization	Chao Yu, Wang Ma, Wen-Xiong Zhang, and Zhenfeng Xi*	*Organometallics* **2020**, *39*, 793–796.
58	Inverse-Sandwich Cyclobutadiene Dinickel Complexes: Synthesis and Structural Characterization	Chao Yu, Botao Wu, Zhenqiang Yang, Hui Chen, Wen-Xiong Zhang, and Zhenfeng Xi*	*Bull. Chem. Soc. Jpn.* **2020**, *93*, 1314–1318.
59	Butadienyl Diiron Complexes: Nonplanar Metalla-Aromatics Involving σ-Type Orbital Overlap	Chao Yu, Mingdong Zhong, Yongliang Zhang, Junnian Wei, Wangyang Ma, Wen-Xiong Zhang, Shengfa Ye*,and Zhenfeng Xi*	*Angew. Chem., Int. Ed.* **2020**, *59*, 19048–19053.
60	Dinickelaferrocene: A Ferrocene Analogue with Two Aromatic Nickeloles Realized by Electron Back-Donation from Iron	Zhe Huang, Yu Zheng, Wen-Xiong Zhang, Shengfa Ye*, Liang Deng*, and Zhenfeng Xi*	*Angew. Chem. Int. Ed.* **2020**, *59*, 14394–14398.
61	Direct Transformation of Dinitrogen: Synthesis of *N*-Containing Organic Compounds via N−C Bond Formation	Ze-Jie Lv, Junnian Wei, Wen-Xiong Zhang, Ping Chen, Dehui Deng, Zhang-Jie Shi, , and	*Natl. Sci. Rev.* **2020**, *7*, 1564–1583.

		Zhenfeng Xi*	
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三、发表论文首页

analytical chemistry

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High-Throughput Single-Cell Immunoassay in the Cellular Native Environment Using Online Desalting Dual-Spray Mass Spectrometry

Shuting Xu, Jinjuan Xue, Yu Bai,* and Huwei Liu

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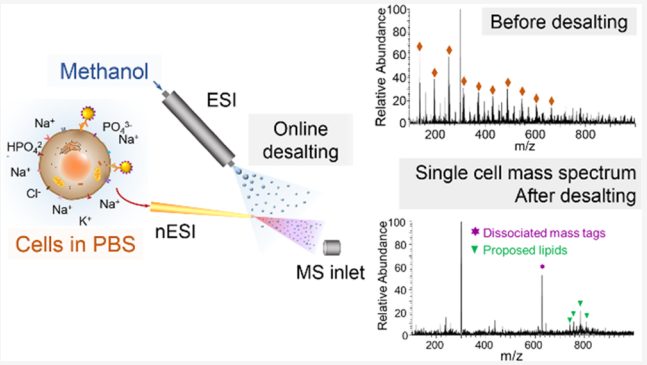
ABSTRACT: Single-cell mass spectrometry (MS) remains challenging in the analysis of cells in the native environment due to the severe ion suspension from nonvolatile salts. Synchronous desalting and ionization would be ideal to both ensure the native environment and remove the salt interference. Here, a novel dual-spray ionization technique combining electrospray and nano-electrospray ionization (ESI-nESI) was developed, enabling highly efficient online desalting during the ionization process. *In situ* detection of cell surface proteins from the intact cells in phosphate buffer saline (PBS) was achieved by dual ESI-nESI MS with the help of an MS-based immunoassay using rhodamine-based mass tags. These mass tags were confirmed to be highly competitive during desalting, which improved the protein detection sensitivity to a single-cell level. Through the combination of the single-cell immunoassay with ESI-nESI MS, the important surface protein markers, cancer antigen 125, in two cancer cell lines (OVCAR-3 and MCF-7) suspended in the PBS buffers were screened in a high-throughput cytometric mode, along with some proposed cellular endogenous lipids. The ESI-nESI MS system is promising for multidimensional organic mass cytometric analysis in the cellular native environment for clinical use and many basic biology researches.



Nonvolatile salts are essential in the cellular environment, participating in the structural maintenance and life function operation with biomolecules, especially proteins.[1] For example, sodium ions regulate the cell osmotic pressure, and carbonates and phosphates constitute the bodily buffer system. Phosphate buffer saline (PBS) with high concentration of nonvolatile salts is the most commonly used buffer, which serves to mimic the physiological environment to remain cell activity during *in situ* analysis using fluorescence techniques[2] like fluorescence-based flow cytometry (FCM),[3] electrochemical techniques,[4] or others.[5,6]

Mass spectrometry (MS) equipped with electrospray ionization (ESI) is one of the most popular techniques for biological analysis[7,8] and has ambitiously entered the field of single-cell analysis in recent years.[9−11] Comparable to FCM, label-free mass cytometry (named CyESI-MS)[12] and organic mass cytometry[13] were developed for high-throughput single-cell screening. These organic mass cytometry techniques take MS's shining advantages of high sensitivity, rich compositional and structural information, high throughput, and rapid detection, but it is difficult to maintain the biological activity during analysis. The dilemma is that the solvent commonly used for MS would probably cause the cell damage or protein degeneration, while the typical buffers with nonvolatile salts would bring destructive ion suspension and MS inlet contamination, which hampers the efficient detection of targets and harms the MS transmission system.[14,15] Since buffers are sometimes necessary to maintain the native and real state of cells, especially for the *in situ* biomolecule analysis based on intact cells, strategies are required to resolve the compatibility problems.

Various desalting strategies have been developed prior to MS detection; for example, microextraction,[16] ion-exchange chromatography,[17] and recrystallization[18] are performed offline in some cases for metabolite and protein detection. They usually produce the biological inactivation for cells after desalting and solvent exchange. More inspiringly, desalting has further been operated online based on microdialysis devices,[19,20] online electrophoresis,[21,22] and newly developed ionization methods with good salt tolerance.[23,24] Online desalting that keeps the physiological environment as long as possible would be ideal for cell screening. Among these online desalting methods, improved ESI-based ionization techniques that fundamentally confront the problem of ion suppression

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In Situ Laser Scattering Electrospray Ionization Mass Spectrometry and Its Application in the Mechanism Study of Photoinduced Direct C−H Arylation of Heteroarenes

Wanpeng Ai, Qirong Yang, Yunpeng Gao, Xiaoyun Liu, Huwei Liu, and Yu Bai*

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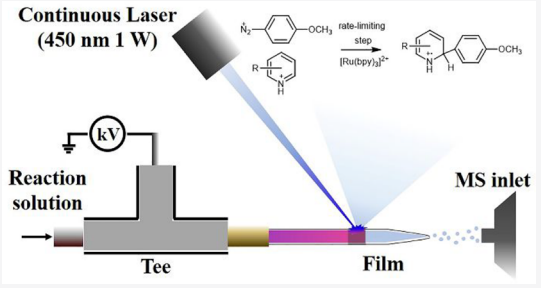
ABSTRACT: An in situ laser scattering electrospray ionization mass spectrometry (LS-ESI-MS) was developed, where the laser scattering was simply achieved through the laser radiation of the "media" modified on the capillary. The laser scattering extended the reaction window and powerfully promoted the reaction yield of the photoinduced organic reaction, which enables the trace intermediates to be efficiently tracked in real time. For instance, the key radical cation in the photoinduced direct C−H arylation of heteroarenes was captured inventively, which provided direct experimental evidence for the verification of the reaction mechanism. Together with the characterization of oxidative photocatalytic Ru(III) intermediate, the integral insight into the process of visible-light-mediated direct C−H arylation of heteroarenes was confirmed. This approach is facile, powerful, and promising in the mechanism study of organic reaction.



Mass spectrometry (MS), especially ambient MS, is a rapid, highly sensitive, and highly specific analytical approach.[1] Through combination with two soft ionization methods, electrospray ionization (ESI)[2−5] and desorption electrospray ionization (DESI),[6−13] it has been widely applied in the reaction monitoring by capturing and characterizing short-lived reaction intermediates as well as reaction mechanism study.[3,12] The application of MS in reaction monitoring mainly includes traditional online pressurized sample infusion (PSI) MS,[14,15] direct detection of short-time reaction using in situ DESI MS,[7−9,13] and microdroplets' acceleration[12,16](Figure 1a−c). However, online PSI MS faces the immediate reaction quenching induced by the addition of the diluent and the changes of pressure and temperature, and the long transmission line in PSI limits the detection of transient intermediates. Even the in situ DESI MS can directly capture the transient intermediate of some organic reaction, it remains challenging when the reaction rate-determining step takes long time (hours or days). In addition, the intermediates with low concentration is another challenge for their capturing and identification using direct in situ sampling technique. As an effective supplement, microdroplets' acceleration has been proposed and used in some simple system of long-time reactions, while Zare et al. proposed that only the diffusion-controlled reaction can be accelerated in the droplet medium.[17,18] Therefore, it is necessary to develop a novel MS method to characterize the short-lived intermediates and monitor the significant reaction that cannot be accelerated in microdroplets.

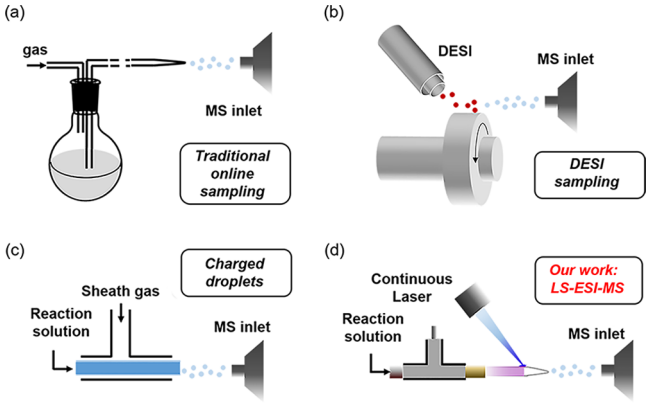


Figure 1. Comparison of (a) traditional online PSI MS; (b) direct detection of short-time reaction using in situ DESI MS; (c) microdroplets' acceleration; (d) our work: LS-ESI-MS.

Arylated heteroarenes widely exist in natural products, pharmaceuticals, agrochemicals, and organic materials with significant structural diversities and biological activities.[19−23] Compared with transition metal-catalyzed cross-coupling

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Tracing and elucidating visible-light mediated oxidation and C–H functionalization of amines using mass spectrometry†

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The co-existing mechanism of visible light mediated direct oxidation and C–H functionalization of amines was investigated by capturing all the intermediates using online mass spectrometry. The two-step dehydrogenation of amine involving a proton coupled electron transfer (PCET) process was revealed for the first time.

Cross-dehydrogenative coupling (CDC) reactions have emerged as a powerful tool for the efficient construction of C–C, C–N, C–O, and C–P bonds.[1–3] In particular, selective conversion of amines to amides through CDC is highly demanded in organic chemistry due to the ubiquity of amide bonds in various molecules, such as biological compounds and biopolymers.[4] Owing to the superiority of sustainability, and green and mild conditions, photoredox catalysis provides a promising replacement for the conventional organic synthesis.[5–10] Since Stephenson *et al.*[11] reported a photocatalytic aza-Henry reaction of *N*-aryltetrahydroisoquinolines (THIQ) with nitromethane through CDC in 2010, visible light mediated direct oxidation and functionalization of C–H bonds adjacent to nitrogen atoms have been diversely explored.[12–15]

In contrast to the rapid development of the photoredox oxidative transformations of amines, their mechanism investigations have lagged far behind. Efforts have been attempted using fluorescence quenching,[16] UV-vis absorption spectroscopy,[17,18] transient absorption spectroscopy,[17–19] electron spin resonance (ESR)[16,20] and NMR spectroscopy.[21] However, limited and indirect structural information of the transient intermediate, such as the radical cations, restricts the mechanism elucidation. Recently, electrospray ionization (ESI),[22–28] desorption electrospray ionization (DESI)[29–35] and low-temperature plasma (LTP)[36] as well as electron impact (EI) coupling with temporal analysis of products (TAP) reactor[37–39] mass spectrometry (MS) have been applied in the reaction mechanism study, providing a highly sensitive tool for gaining detailed structural information of reactive intermediates through *in situ* detection. Through combination with high power laser irradiance, the mechanisms of dehydrogenation and [3+2] annulation have been elucidated.[35,40] The proposed mechanism of photoredox transformation of C–H bonds adjacent to nitrogen atom involves the formation of amine radical cations by single-electron-transfer (SET) and the subsequent H-abstraction by a superoxide radical anion to form an iminium cation. Thereafter, two pathways have been reported to convert iminium into different products, one is nucleophilic addition leading to functionalization of the C–H bond adjacent to nitrogen; the other one is oxidation of the iminium cation resulting in the formation of amide products. Even though co-existing pathways were proposed (Scheme S1, ESI†),[12] no direct evidence for this mechanism of this significant photocatalytic reaction has been reported. To investigate the detailed mechanism of this process, ESI-MS assisted with a laser irradiation setup was applied in our work to explore the visible-light mediated oxidation and C–H functionalization of THIQ (Scheme 1). Two new peroxide addition intermediates were detected and characterized, which disclosed the cascade stepwise aerobic oxidation to obtain amide products for the first time.

A mixture of THIQ **1a** (100 μM) and $[Ru(II)(bpy)_3]Cl_2$ (5 μM, bpy = 2,2′-bipyridine) in methanol was injected into the capillary with a tapered tip as the ESI spray needle with the flow rate of 10 μL min^{-1}. A high power laser (450 nm, 1 W) was employed to irradiate the reaction mixture and trigger the photoredox catalysis reaction (Scheme 1 and Fig. S3, ESI†). For the clear demonstration of the laser catalyzed reaction and the origin of the intermediates, the laser beam was turned on and off sequentially and repetitively at 1 min intervals. When the laser was turned off, only an ion of *m*/*z* 210.1274 and ion clusters with the dominant peak at *m*/*z* 285.0546 were detected, corresponding to the protonated **1a** and $[Ru(bpy)_3]^{2+}$, respectively (Fig. 1a). It was noticed that a small peak of ions at *m*/*z* 208.1118 attributed to the THIQ iminium cation (2-phenyl-3,4-dihydroisoquinolin-2-ium, **2a**) was produced by in-source

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RESEARCH PAPER

A novel online two-dimensional supercritical fluid chromatography/reversed phase liquid chromatography–mass spectrometry method for lipid profiling

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Abstract

A novel online two-dimensional supercritical fluid chromatography/reversed-phase liquid chromatography–triple-quadrupole mass spectrometry (2D SFC/RPLC–QQQ MS) method based on a vacuum solvent evaporation interface was developed for lipid profiling in human plasma, in which lipid classes were separated by the first-dimension SFC and different lipid molecular species were further separated by the second-dimension RPLC. All separation condition parameters were carefully optimized, and their influence on the chromatographic behavior of lipids is discussed. Finally, the recoveries of 11 lipid standards were all more than 88% for the interface. Besides, the limit of detection for these lipid standards was on the order of nanograms per milliliter, and the relative standard deviations of the peak area and retention time ranged from 1.54% to 19.85% and from 0.00% to 0.10%, respectively. The final 2D SFC/RPLC–QQQ MS method allowed the identification of 370 endogenous lipid species from ten lipid classes, including diacylglycerol, triacylglycerol, ceramide, glucosylceramide, galactosylceramide, lactosylceramide, sphingomyelin, acylcarnitine, phosphatidylcholine, and lysophosphatidylethanolamine, in human plasma within 38 min, which was used for screening potential lipid biomarkers in breast cancer. The 2D SFC/RPLC–QQQ MS method is a potentially useful tool for in-depth studies focused on complex lipid metabolism and biomarker discovery.

Keywords Lipidomics · Two-dimensional · Supercritical fluid chromatography · Mass spectrometry

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Introduction

Lipids are hydrophobic or amphiphilic small molecules that play crucial roles in cellular energy storage, structure, and signaling [1]. According to their structures, they can be divided into eight categories, and then into different classes and molecular species [2]. As changes in lipid metabolism and function have important effects on cellular physiological functions and pathological disorders of living organisms, lipidomics has attracted more and more attention in recent years [3]. However, there are still challenges associated with the analytical techniques used in lipidomics because of the extreme structural and content diversity of lipids in real biological samples.

Currently, two main analytical strategies are used in lipidomics; namely, shotgun lipidomics and high-performance liquid chromatography (HPLC)–mass spectrometry (MS) [4]. In general, shotgun lipidomics is considered to be of high throughput, accurate, and

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Research Article

Myriocin and D-PDMP ameliorate atherosclerosis in ApoE$^{-/-}$ mice via reducing lipid uptake and vascular inflammation

Zemou Yu[1,*], Qing Peng[1,*], Songyue Li[2], Hongjun Hao[1], Jianwen Deng[1], Lingbing Meng[3], Zhiyuan Shen[1], Weiwei Yu[1], Ding Nan[1], Yu Bai[2] and Yining Huang[1]

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Sphingolipids have been implicated in the etiology of atherosclerosis. The commonly used sphingolipid inhibitors, myriocin (a ceramide inhibitor) and D-PDMP (D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol, a glycosphingolipid inhibitor), have shown therapeutic potential but their efficacy and their underlying mechanisms remain unclear. Here, apolipoprotein E-deficient (apoE$^{-/-}$) mice were fed a high-fat diet (HFD) and treated with a control, myriocin, D-PDMP, or atorvastatin for 12 weeks. We analyzed the effects of these drugs on the size and detailed composition of atherosclerotic plaques. Molecular biological approaches were used to explore how the inhibitors affect lipid metabolism and foam-cell formation. Treatment with myriocin or D-PDMP led to smaller and less vulnerable atherosclerotic lesions and was almost as effective as atorvastatin. Sphingolipid inhibitors down-regulated the expression of monocyte chemotactic protein 1 (MCP-1) and its receptor chemoattractant cytokine receptor 2 (CCR2), which play a key role in monocyte recruitment. They also decreased pro-inflammatory Ly-6c^{high} monocytes and influenced the uptake of modified LDL by down-regulating the expression of cluster of differentiation 36 (CD36) and lectin-like oxidized LDL (ox-LDL) receptor-1 (LOX-1). The inhibitors exhibited the advantage of maintaining normal glucose homeostasis compared with atorvastatin. These findings reveal for the first time that the modulation of sphingolipid synthesis can effectively alleviate atherosclerosis progression by preventing lipid uptake and reducing inflammatory responses in the arterial walls.

Introduction

Atherosclerosis is the fundamental pathological process underlying cardio-cerebral vascular diseases (CVDs), which are the leading cause of morbidity and mortality worldwide. Dyslipidemia is a major contributor to the pathogenesis of plaque formation in these diseases. Much effort has been directed to developing agents that reduce serum low-density lipoprotein cholesterol (LDL-C), and the most effective drugs developed so far being statins [1]. It has been recognized that a significant number of patients are either resistant or intolerant to statins, and that statin efficacy might diminish with patient age in the primary prevention of CVD [2]. Moreover, individuals using statins in clinical practice may be at a higher risk for hyperglycemia, insulin resistance (IR) and eventually type 2 diabetes [3]. In addition, there remains a residual CVD risk, despite these existing treatments being effective for governing plasma LDL-C concentration. These factors suggest that there is an urgent need for new lipid-modulating therapies or independent LDL-C-lowering therapies.

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Phospholipid imaging of zebrafish exposed to fipronil using atmospheric pressure matrix-assisted laser desorption ionization mass spectrometry

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ABSTRACT

Mass spectrometry imaging can effectively detect and reflect the information of molecular spatial distribution, and has been widely used for *in situ* analysis of endogenous or exogenous molecules in organisms. The present work applied the atmospheric pressure matrix-assisted laser desorption ionization mass spectrometry (AP-MALDI-MS) in the imaging of whole zebrafish slices exposed to fipronil. The chemical fingerprints in the range of *m/z* 600–950 showed significant differences in phospholipids between the fipronil exposed and untreated zebrafish groups. The major perturbed phospholipids were identified as PC(34:2), PC(34:1), PC(34:2) + Na, PC(36:4), PC(38:6), and PS(18:0/22:6), PI(18:0/20:4), PI(18:1/20:4) etc. Our results indicated that the exposure of fipronil obviously affected the phospholipid metabolism of zebrafish, especially of the fish eye region. Our work provides a new method or possibility for toxicological study and related metabolic analysis of pesticides in animals.

1. Introduction

Fipronil is an extensively used new generation of phenylpyrazole insecticides [1,2], which has been used on pests of a wide variety of food crops in agriculture controlling of veterinary pests. It has been designated by the United States Environmental Protection Agency (US EPA) as one of the alternatives to the organophosphates for termites and fire ant control [3]. Fipronil's mechanism of action is to block insect's chloride channels that are controlled by gamma-aminobutyric acid (GABA) in the neurons of the central nervous system. To assess the direct or indirect potential risks of fipronil, a variety of studies have been performed on different animals including Daphnia pulex, fish, rat, honey bees and dogs [4–6]. Toxicity studies of fipronil pesticide have provided sufficient evidences of its environmental contamination and effects on public health [7]. As an important component of the food chain, fish has been frequently used as the experimental model because the physiological changes when it exposed to low pesticide levels can provide a means to understand the levels of environmental pollution in biological terms. In our work, zebrafish were selected as experimental model to assess the environmental effects of fipronil.

A number of analytical techniques have been reported for the quantitation and quantification of pesticides in food, water and other matrices, including high performance liquid chromatography (HPLC) [8], gas chromatography (GC) [9], liquid/gas chromatography-mass spectrometry (LC/GC-MS) [10], electrochemistry [11], and enzyme-linked immunosorbent assays (ELISAs) [12] etc. For the fipronil and its metabolites, they can be frequently detected in surface water within the range of ng/L to μg/L using GC/LC-MS analysis [13,14]. Zhang et al. developed a LC-MS/MS method to determine residual fipronil in chicken egg and muscle [15]. The fipronil in peanut and soil were analyzed by LC-MS/MS combining with the pretreatment using quick, easy, cheap, effective, rugged and safe (QuEChERS) method [16].

Although the usage of pesticides are strictly controlled, it is still important to monitor bioaccumulation, assess biotransformation, and track the formation of any metabolites that may have detrimental effects [17]. Unfortunately, the effects of drugs/pesticides on various organs of animals could not be readily and directly obtained using above mentioned approaches. As a novel molecular imaging method, matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) [18] can analyse biological tissues directly, ranging from small drug compounds to very large proteins [19–21]. It presents significance in the measurement of drugs and organic compounds distributions in animals tissue sections [22], like rat, mouse, and rabbit tissues [23].

In our work, the direct analysis and imaging of phospholipid metabolism in fipronil exposed zebrafish organs using AP-MALDI-MS was

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Severe Acute Respiratory Syndrome Coronavirus-2 Spike Protein Nanogel as a Pro-Antigen Strategy with Enhanced Protective Immune Responses

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Prevention and intervention methods are urgently needed to curb the global pandemic of coronavirus disease-19 caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Herein, a general pro-antigen strategy for subunit vaccine development based on the reversibly formulated receptor binding domain of SARS-CoV-2 spike protein (S-RBD) is reported. Since the poor lymph node targeting and uptake of S-RBD by antigen-presenting cells prevent effective immune responses, S-RBD protein is formulated into a reversible nanogel (S-RBD-NG), which serves as a pro-antigen with enhanced lymph node targeting and dendritic cell and macrophage accumulation. Synchronized release of S-RBD monomers from the internalized S-RBD-NG pro-antigen triggers more potent immune responses in vivo. In addition, by optimizing the adjuvant used, the potency of S-RBD-NG is further improved, which may provide a generally applicable, safer, and more effective strategy for subunit vaccine development against SARS-CoV-2 as well as other viruses.

1. Introduction

The outbreak of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection has caused a pandemic of coronavirus disease-19 (COVID-19), posing a great threat to human life globally.[1] Till mid-June of 2020, more than 9 million individuals were tested positive for COVID-19, with a death toll over 470 000 worldwide.[2] Early efforts have focused on finding small-molecule drugs such as favipiravir, chloroquine, and remdesivir to treat SARS-CoV-2 infection.[3] Meanwhile, researchers have also reported the development of effective neutralizing antibodies using techniques such as single B cell sequencing.[4] Nevertheless, one of the most promising strategies for COVID-19 prevention relies on vaccine development. There have already been more than 100 vaccines under development, including whole virus vaccines (attenuated, inactivated, or recombinant virus), subunit vaccines, DNA, and RNA vaccines.[5] For example, an inactivated SARS-CoV-2 whole virus vaccine from China showed efficacy in mice, rats, and monkeys.[6] Another recombinant adenovirus vaccine clinical trial (NCT04313127) has posted its phase 1 results with neutralizing antibodies and reported specific T cell responses.[7] Whole virus vaccines are expensive, dangerous during production, and may cause severe vaccine-related diseases.[8] Alternatively, using subunit vaccines with virus antigen protein should be a safer, more effective, and economic strategy. Recombinant expression of the antigen in organisms such as *E. coli*, yeast, or mammalian cells can facilitate the large-scale production.

The receptor binding domain of SARS-CoV-2 spike protein (S-RBD) has been shown to mediate the entry of the virus into host cells via interacting with human angiotensin converting

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Cancer Therapy

Cationic Lipid-based Intracellular Delivery of Bacterial Effectors for Rewiring Malignant Cell Signaling

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Dedicated to Professor Youqi Tang on the occasion of his 100th birthday

Abstract: *The abundance of bacterial effectors have inspired us to explore their potential in rewiring malignant cell signaling. Their incapability for entering cells, however, hinders such application. Herein we developed a cationic lipid-based high throughput library screening platform for effective intracellular delivery of bacterial effectors. As the misregulated MAPK signaling is a hallmark of many types of cancer, we turned to the Shigella effector OspF which irreversibly inactivates ERK, the terminal component of MAPK cascade. We created a function-based screening assay to obtain AMPA-O16B lipid nanoparticles for effective OspF intracellular delivery, which inhibited the malignant MAPK signaling and tumor growth in vitro and in vivo. Furthermore, the optimized lipid nanoparticle formulation can deliver OspF to modulate the immunosuppressive responses in macrophages. Our work is a general strategy to explore the therapeutic potentials of naturally evolved bacterial effectors.*

Introduction

Bacterial pathogens have evolved a wide range of effector proteins to facilitate infections through rewiring host cell signaling.[1] Continuous evolution through host-pathogen competition has empowered these effectors with high specificity, and in many cases with unique mechanisms, towards certain cell signaling components.[1,2] This rich repertoire of bacterial effectors offers an attractive toolset for rewiring various signaling pathways, particularly inside malignant cells with a therapeutic potential. However, such applications are currently hindered, largely due to the absence of effective delivery strategies. Intracellular delivery of recombinant proteins has recently emerged as an exciting alternative strategy than traditional gene delivery and therapy, enabling direct utilization of proteins as therapeutic agents for various diseases.[3] Different non-viral nanocarriers have been developed to promote intracellular protein delivery and avoid the potential risks associated with viral delivery such as insert mutagenesis and high immunogenicity.[4] Among these methods, lipid-based delivery has drawn great attention due to their high delivery efficiency and excellent endosome escaping capability. Several lipid-based delivery strategies have also been approved by FDA for in vivo disease treatment.[5] In particular, modular characterization of the lipid nanoparticle components allows the utilization of library screening to find suitable lipids for a given protein and/or cell type as well as selective organ targeting.[6] We envisioned that the lipid-based delivery strategy could be employed to explore the potential of bacterial effectors as therapeutic agents to target and rewire the malignant cell signaling.

Misregulated kinase signaling cascade is a hallmark of many diseases. For example, abnormal mitogen-activated protein kinase (MAPK) signaling, derived from mutations of the components in the RAS-RAF-MEK-ERK cascade, contributes to a wide variety of cancers.[7] Furthermore, this conserved signaling pathway is also involved in the polarization of macrophages.[8] In tumor microenvironment (TME), tumor associated macrophages (TAMs) are educated and polarized to a pro-tumoral phenotype that is highly related to resistance and poor prognosis.[9] There are urgent needs to target these malignant cells for cancer treatment. Although small molecule inhibitors that target upstream components of the MAPK cascade have led to successful therapeutic interventions, the clinical benefits of these inhibitors are accompanied by rapid drug resistance.[10] As the terminal nodes of the MAPK cascade, ERK and p38 receive upstream signals and shuttle between cytoplasm and nucleus to phosphorylate more than 150 substrates in regulation of gene expression, cell proliferation as well as cellular responses.[11] Targeting these essential terminal components has attracted significant attentions recently as alternative targets to treat MAPK-driven cancers.[12] In particular, irreversible inhibitors that covalently interact and antagonize

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Genetically encoded protein labeling and crosslinking in living *Pseudomonas aeruginosa*

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ABSTRACT

Pseudomonas aeruginosa (PA) is a major human pathogen for hospital-acquired infections. We report the genetic code expansion of this opportunistic pathogen by using the pyrrolysyl-tRNA synthetase-tRNA system, which enabled the genetic and site-specific incorporation of unnatural amino acids bearing bioorthogonal handles or photo-affinity groups into proteins in PA. This strategy allowed us to conduct bioorthogonal labeling and imaging of flagella, as well as site-specific photo-affinity capturing of interactions between a Type III secretion effector and its chaperone inside living bacteria.

1. Introduction

Coupling the genetic code expansion strategy with bioorthognal reactions have significantly enhanced our ability for precise labeling and functional study of proteins of interest (POIs) under living conditions. In particular, the pyrrolysine (Pyl)-based system has recently emerged as a powerful tool for expanding the genetic code of both prokaryotic and eukaryotic cells.[1–7] The wild-type Pyl-tRNA synthetase (PylRS) and its mutants were shown to be directly used by the translation machinery of bacterial, yeast and mammalian cells to encode Pyl and diverse Pyl analogues at an in-frame amber mutation in response to $tRNA^{Pyl}_{CUA}$. This excellent orthogonality of the PylRS-tRNA pair has enabled the genetic code expansion of a fast-growing list of living species. Meanwhile, the bioorthogonal chemistry has been increasingly expanded in recent years, both in terms of reaction type and applications.[8–13] In addition, various photo-affinity reagents have been developed and unnatural amino acids (UAAs) bearing such moieties, either alone or in combination with bioorthogonal handles, allowed non-invasive photo-capturing of protein–protein interactions within living cells.[14–17] Together, the rapid growing toolbox of genetically encoded bioorthogonal chemistry has allowed precise protein labeling and capturing protein–protein interactions in diverse living species.

We have previously expanded the genetic code of enteric pathogens such as *Shigella flexneri* and *Salmonella typhimurium*,[18] as well as hepatitis viruses[19], which facilitated the study of pathogenesis and host-pathogen interactions of these infectious bacteria and viruses. To further leverage the exciting bioorthogonal chemistry toolbox to various pathogenic species, we decided to expand the genetic code of *Pseudomonas aeruginosa* (PA), a major human pathogen for hospital-acquired infections. PA is notoriously known for causing high fatality rates in patients with compromised immune systems when under the treatment of cancer, cystic fibrosis or burns.[20] As a successful opportunistic pathogen with intrinsic acquired drug resistance, PA employs an array of effector proteins to invade, survive and replicate within human host or hospital environment.[21] How PA coopts its endogenous proteins and signaling network to infect host cells and confer drug resistance remain unclear. Herein we decided to expand the genetic code of this Gram-negative pathogen by using the PylRS-tRNA system.

2. Results

2.1. Construction of the genetic code expansion system

We started by expanding the genetic code of PA in order to introduce unnatural functionalities into proteins in this bacterium. Although PA and *E. coli* are both Gram negative bacterial species, they

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SFPQ Is an FTO-Binding Protein that Facilitates the Demethylation Substrate Preference

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SUMMARY

The fat mass and obesity-associated protein (FTO) is the first identified demethylase of the internal RNA modification N^6-methyladenosine (m^6A), which also exhibits demethylation activity toward N^6,2′-*O*-dimethyladenosine (m^6A_m) and N^1-methyladenosine (m^1A). Demethylation of m^6A at specific sites on target transcripts is a key enzymatic function of FTO that modulates diverse physiological and/or pathological processes. However, how FTO selects target RNA and whether additional interaction proteins facilitate this process remain elusive. Herein, via the genetically encoded and site-specific photo-crosslinking strategy, we identified the major RNA-binding protein SFPQ as a direct interaction partner of FTO. Our study showed that FTO and SFPQ were located in close proximity throughout the transcriptome and that overexpression of SFPQ led to the demethylation of adjacent m^6As, likely through recruiting FTO to these specific RNA sites. These results uncovered a new layer of regulation mechanism that may assist FTO to gain substrate specificity.

INTRODUCTION

The fat mass and obesity-associated protein (FTO) has been attracting intense research interest for the last decade due to its critical roles in epitranscriptomics and human diseases (Jia et al., 2011; Zhao et al., 2014b). It belongs to the AlkB family of Fe(II)/α-ketoglutarate (αKG)-dependent dioxygenases (Gerken et al., 2007; Kurowski et al., 2003), which possesses demethylation activity toward N^6-methyladenosine (m^6A) in mRNA, small nuclear RNA (snRNA), and U6 RNA (Jia et al., 2011; Wei et al., 2018), N^6,2′-*O*-dimethyladenosine (m^6A_m) modification at the +1 position from the 5′ cap in mRNA and snRNA (Mauer et al., 2017, 2019; Wei et al., 2018), and N^1-methyladenosine (m^1A) modification in tRNA (Wei et al., 2018). Previous studies have demonstrated the role of FTO in multiple processes such as DNA-damage response (Xiang et al., 2017), virus infection (Gokhale et al., 2016), heat-shock response (Zhou et al., 2015), neuronal development (Yu et al., 2018), adipogenesis (Ben-Haim et al., 2015; Zhao et al., 2014a), and cancer cell development (Li et al., 2017; Su et al., 2018; Zhou et al., 2018), among others, by influencing the m^6A modification levels on target transcripts and thereby influencing RNA metabolism. The structure of FTO shows that FTO contains two domains: an N-terminal domain (residues 32–326, referred to as NTD) resembling the structure of AlkB domain for the demethylation function and a C-terminal domain (residues 327–498, referred to as CTD) folded with a β helix, although the biological function of CTD remains unknown (Han et al., 2010).

The structure of FTO coupled with m^6A-modified single-stranded DNA uncovers the molecular catalytic mechanism of FTO for the demethylation of multiple RNA substrates, revealing that FTO prefers the nucleobase N^6-methyladenine and that the sequence and tertiary structure of RNA affects the demethylation activity of FTO (Zhang et al., 2019). The subcellular localization of FTO affects its ability to approach different RNA substrates (Wei et al., 2018). However, despite intensive efforts, how FTO selects its substrate is not fully understood. It is also important to note that the other m^6A demethylase, ALKBH5, which shares high structural similarity with the NTD of FTO, plays crucial roles in spermatogenesis and is likely to recognize substrates different from FTO (Aik et al., 2014; Feng et al., 2014; Xu et al., 2014; Zheng et al., 2013). Given that the binding affinity of FTO or ALKBH5 toward RNA substrates is low (Baltz et al., 2012; Castello et al., 2012), we hypothesized that additional regulating mechanisms may exist that enabled FTO to recognize RNA substrates and that certain proteins may specifically interact with FTO or ALKBH5 to guide substrate recognition.

Until now, only a few FTO interaction proteins have been reported. For example, by using immunoprecipitation coupled with mass spectrometry (IP-MS), FTO was found to interact with Exportin 2 (XPO2) to shuttle from nucleus to cytoplasm (Gulati et al., 2014), where it further interacts with some components in the multi-tRNA synthetase complex to couple the cellular level of amino acids to the mammalian target of rapamycin complex 1 (mTORC1) signaling (Gulati et al., 2013). Through a yeast two-hybrid screening system, calcium/calmodulin-dependent protein kinase II (CaMKII) was identified as the interaction

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Chemoproteomic Profiling of O-GlcNAcylation in *Caenorhabditis elegans*

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Supporting Information

ABSTRACT: Genetic studies have revealed essential functions of O-linked *N*-acetylglucosamine (O-GlcNAc) modification in *Caenorhabditis elegans*. However, large-scale identification of O-GlcNAcylated proteins and mapping the modification sites in *C. elegans* remain relatively unexplored. By using a chemoproteomic strategy, we herein report the identification of 108 high-confidence O-GlcNAcylated proteins and 64 modification sites in *C. elegans*. Furthermore, quantitative proteomics upon altering O-GlcNAcylation show that the abundance of a large number of proteins are affected by O-GlcNAc. These proteins are involved in regulating reproduction and lifespan, which may correlate with the previously observed phenotypes in genetic studies. The data set in this study reveals the O-GlcNAc modification landscape in *C. elegans* and provides a valuable resource for dissecting the biological function of O-GlcNAcylation.

O-Linked *β*-*N*-acetylglucosamine modification (O-GlcNAcylation) is an abundant posttranslational modification of serine and threonine on thousands of nuclear and cytosolic proteins in mammalian cells.[1] To date, over 2000 O-GlcNAcylated proteins have been identified owing to recent MS-based proteomic profiling in various cell lines and tissues.[2−6] O-GlcNAcylated proteins are implicated in a wide range of biological processes, including transcription, translation, and metabolism. Intriguingly, only a pair of evolutionarily conserved enzymes, O-GlcNAc transferase (OGT) and O-GlcNAc hydrolase (OGA), regulate the addition and removal of O-GlcNAc.[7] Dysregulation of the O-GlcNAc cycling has been implicated in many human pathological processes, such as cancers and neurodegeneration.[8,9] Notably, genetic knockout of *OGT* or *OGA* is lethal in mice,[10] which underlines the importance of O-GlcNAc but imposes difficulties in genetic studies of O-GlcNAc biology in mammals. OGT and OGA are highly conserved in *C. elegans*. Interestingly, the null alleles of *ogt-1* and *oga-1* are viable and fertile, making *C. elegans* a convenient model organism for revealing the functional role of O-GlcNAc in lifespan, stress response, and dauer formation via genetic knockout.[11−15]

O-GlcNAcylation on a few proteins have been implicated in the observed phenotypes.[16,17] However, large-scale identification of O-GlcNAcylated proteins and mapping the modification sites in *C. elegans* remain relatively unexplored. By using the chemoenzymatic labeling method based on a mutant galactosyltransferase (Y289L GalT),[18] the Paik group identified 12 O-GlcNAcylated proteins.[12] The Berninsone group reported the identification of 20 O-GlcNAcylated proteins by performing metabolic glycan labeling in primary *C. elegans* embryonic cells using per-*O*-acetylated *N*-azidoacetylgalactosamine (Ac_4GalNAz) and *N*-azidoacetylglucosamine (Ac_4GlcNAz).[19] Of note, these O-GlcNAcylated protein candidates may need further validation, given the recently discovered artificial *S*-glycosylation induced by per-*O*-acetylation.[2,20] Moreover, large-scale profiling of the O-GlcNAcylation sites in *C. elegans* has not been achieved.

Aiming to gain a better insight into the *C. elgegans* O-GlcNAcylated proteome, we performed comprehensive profiling of O-GlcNAcylated proteins and modification sites. To quantitatively identify the O-GlcNAcylated proteins in *C. elegans*, we implemented the dimethyl labeling strategy into the chemoenzymatic labeling-based O-GlcNAc proteomics (Figure S1A). The lysates of N2 worms were incubated with Y289L GalT and uridine diphosphate *N*-azidoacetylgalactosamine (UDP-GalNAz). Y289L GalT recognizes terminal GlcNAc moieties, thus allowing for the transfer of a GalNAz moiety from UDP-GalNAz onto O-GlcNAc. After reacting the lysates with alkyne-Cy5 via Cu(I)-catalyzed azide−alkyne cycloaddition (CuAAC, i.e., click chemistry), in-gel fluorescence scanning exhibited significant labeling of endogenous O-GlcNAcylated proteins (Figure S1B). The labeling was almost completely abolished in the *ogt-1* knockout worms (ok-1474), while significantly increased in the *oga-1* knockout worms (ok-1207), supporting the labeling of *bona fide* O-GlcNAcylated proteins (Figure 1A and Figure S1C). The labeled O-GlcNAcylated proteins were then reacted with alkyne-biotin and enriched by streptavidin beads. Immunoblotting con-

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Protein S-Glyco-Modification through an Elimination−Addition Mechanism

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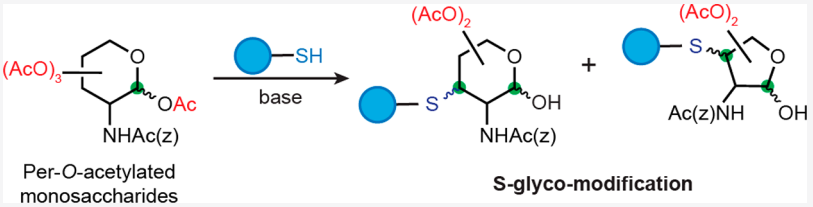

ABSTRACT: Per-*O*-acetylated unnatural monosaccharides containing a bioorthogonal group have been widely used for metabolic glycan labeling (MGL) in live cells for two decades, but it is only recently that we discovered the existence of an artificial "S-glycosylation" between protein cysteines and per-*O*-acetylated sugars. While efforts are being made to avoid this nonspecific reaction in MGL, the reaction mechanism remains unknown. Here, we present a detailed mechanistic investigation, which unveils the "S-glycosylation" being an atypical glycosylation termed S-glyco-modification. In alkaline protein microenvironments, per-*O*-acetylated monosaccharides undergo base-promoted β-elimination to form thiol-reactive α,β-unsaturated aldehydes, which then react with cysteine residues via Michael addition. This S-glyco-modification produces 3-thiolated sugars in hemiacetal form, rather than typical glycosides. The elimination−addition mechanism guides us to develop 1,6-di-*O*-propionyl-*N*-azidoacetylgalactosamine (1,6-Pr_2GalNAz) as an improved unnatural monosaccharide for MGL.

INTRODUCTION

Exploiting the long-known substrate promiscuity of enzymes involved in sialic acid biosynthesis and sialylation,[1] the Bertozzi group in 2000 developed an unnatural analog of *N*-acetylmannosamine (ManNAc), *N*-azidoacetylmannosamine (ManNAz), which could be metabolically converted to azido sialic acid (SiaNAz) and incorporated into sialoglycans in live cells.[2] Subsequently, the incorporated azide was conjugated with functional probes via Staudinger ligation, one of the very first bioorthogonal reactions. Since then, various unnatural monosaccharides containing a bioorthogonal functional group (e.g., azide or alkyne) have been developed for labeling sialoglycans as well as other types of glycans.[3−5] For example, *N*-azidoacetylgalactosamine (GalNAz) can be converted to UDP-GalNAz and UDP-*N*-azidoacetylglucosamine (GlcNAz) in live cells, which result in labeling of mucin-type O-linked glycans, N-linked glycans, and O-GlcNAc. 6-Alkynyl fucose (FucAl) metabolically labels fucosylated glycans. Furthermore, a series of new bioorthogonal reactions such as Cu(I)-catalyzed azide−alkyne cycloaddition (CuAAC or click chemistry) and strain-promoted azide−alkyne cycloaddition (SPAAC or copper-free click chemistry) are now available to choose from.[6,7] This strategy, termed metabolic glycan labeling (MGL), metabolic oligosaccharide engineering (MOE), or metabolic chemical reporter (MCR), has emerged as a widely used tool for tagging glycans with fluorophores for imaging and with enrichment probes for glycoproteomic profiling.

Experimentally, the hydrophilic nature of unnatural monosaccharides causes low efficiency for cellular uptake due to the barrier imposed by the hydrophobic plasma membrane. To improve cell entry, these unnatural monosaccharides are usually per-*O*-acetylated to increase hydrophobicity and membrane permeability. This simple and effective strategy exploits cytosolic nonspecific esterases to cleave the acetyl groups and produce unprotected free sugars inside the cells.[8] For the past two decades, MGL has been predominantly practiced with per-*O*-acetylated unnatural monosaccharides. Very recently, our group unexpectedly discovered that per-*O*-acetylated monosaccharides and their unnatural analogs could chemically react with the cysteine residues of various proteins in the cells.[9] This so-called artificial cysteine "S-glycosylation" occurs in parallel with esterase hydrolysis and metabolic incorporation, thus compromising the specificity of MGL at least to some extent (Figure 1a). One simple solution to this problem is to use unprotected sugars, but having to suffer from

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Metabolic RNA labeling for probing RNA dynamics in bacteria

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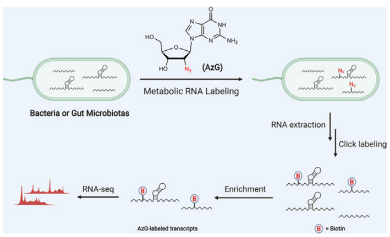
ABSTRACT

Metabolic labeling of RNAs with noncanonical nucleosides that are chemically active, followed by chemoselective conjugation with imaging probes or enrichment tags, has emerged as a powerful method for studying RNA transcription and degradation in eukaryotes. However, metabolic RNA labeling is not applicable for prokaryotes, in which the complexity and distinctness of gene regulation largely remain to be explored. Here, we report 2′-deoxy-2′-azidoguanosine (AzG) as a noncanonical nucleoside compatible with metabolic labeling of bacterial RNAs. With AzG, we develop AIR-seq (azidonucleoside-incorporated RNA sequencing), which enables genome-wide analysis of transcription upon heat stress in *Escherichia coli*. Furthermore, AIR-seq coupled with pulse-chase labeling allows for global analysis of bacterial RNA degradation. Finally, we demonstrate that RNAs of mouse gut microbiotas can be metabolically labeled with AzG in living animals. The AzG-enabled metabolic RNA labeling should find broad applications in studying RNA biology in various bacterial species.

GRAPHICAL ABSTRACT



INTRODUCTION

Cellular RNA levels result from the interplay of RNA transcription, processing and degradation (1–3). To dissect these tightly regulated processes, it is desirable to selectively analyze nascent RNAs in addition to measurements on total RNAs. Several complementary methods with nascent RNA-specificity have been developed and have greatly facilitated the understanding of gene regulation networks (4). One of the methods exploits chemically active nucleoside analogs (i.e. noncanonical nucleosides) that can serve as surrogates of natural nucleosides and be used for RNA synthesis in living cells (5). The noncanonical nucleoside-incorporated RNAs are then chemically conjugated with fluorophores for imaging or affinity tags for enrichment and sequencing. Since the noncanonical nucleosides can only be incorporated into newly transcribed RNAs, this method allows for studying transcription by separation of nascent RNAs from the pre-existing populations. Furthermore, by applying noncanonical nucleosides in pulse-chase labeling experiments, RNA degradation can be quantified and profiled by monitoring the decay of pulse-labeled RNAs (6–8). A variety of nucleoside analogs have been developed for metabolic RNA labeling in various eukaryotic cells (9–16). Among them, 4-thiouridine (4SU) and 5-ethynyluridine (EU) are two most widely used noncanonical nucleosides that can be conjugated via thiol coupling chemistry and click chemistry, respectively (9,10). Although the metabolic RNA labeling technique has been instrumental for studying RNA dynamics in eukaryotes, it is not applicable to bacteria.

For a long time, prokaryotic transcriptomes were generally considered to be much simpler. As a result, whole-transcriptome studies in bacteria lagged behind eukaryotes until recently (17). For the past two decades, transcriptomics has re-shaped our view on the complexity, dynamics, and regulatory mechanisms of bacterial transcriptomes (18). For example, bacterial mRNAs are now known to be generally regulated by hundreds of small noncoding RNAs

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Synthesis of Open-Cage Fullerenes with a Long Tail

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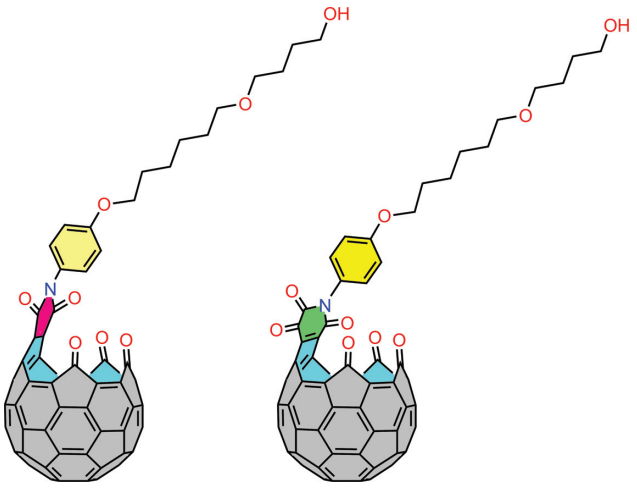

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Abstract To explore potential applications for open-cage fullerenes, we employed 4-((6-bromohexyl)oxy)aniline to react with an open-cage fullerene precursor which has an 11-membered orifice and prepared open-cage fullerenes with an 18-membered orifice. The bromo atom at the end of the hexyl chain in these open-cage compounds could be easily replaced by alkoxyl groups to further extend the linear chain. The results also show that the presence of the alkyl chain slightly changes the reactivity of the orifice-expansion reaction.

Key words fullerene, open-cage, amphiphilic

Introduction

Functionalization of fullerenes has played an important role in the exploration for their potential applications.[1] Addition of various functional groups on the spherical cage can modify their solubility,[2] redox potential,[3] absorption wavelength,[4] film formation ability,[5] and many other properties. For example, the well-known C_{60} derivative PCBM is a much better solar-cell component than pristine C_{60}.[3b] Open-cage fullerenes are a special type of fullerene derivatives with the fullerene skeleton carbon–carbon bonds selectively cleaved to form a hole. Due to the spherical nature of the fullerene cage, it is quite difficult to open the cage effectively. In spite of the difficulties, a few methods have been reported for the synthesis of open-cage fullerenes,[6] some of which have an orifice large enough to encapsulate small molecules such as water.[7]

Most reported results concerning open-cage fullerenes in the literature so far have focused on the methods to open the fullerene cage and encapsulation of small molecules into the cavity of the open-cage compounds. To explore their potential applications, it is usually required to attach a suitable functional group onto the open-cage fullerene for various purposes, for example, to improve the solubility in organic solvents or water.[2] We have been working on the preparation of open-cage fullerenes with different orifice sizes and functional groups on the rim of the orifice through the fullerene mixed peroxide chemistry.[8] Based on our previous method,[9] we report here a designed synthesis of open-cage fullerenes containing a long tail, which may be used for further assembly into Langmuir–Blodgett films or spherical aggregates in water.

Results and Discussion

In our previous studies, we have used aniline and some simple aniline derivatives such as 4-methyl, 4-isopropyl, and 4-bromoanilines[9a] to make open-cage fullerenes. It is almost impossible to add other functional groups through the aniline group once it is incorporated into the open-cage derivative. In a rare case, bromination of the attached aniline was observed for an open-cage derivative in moderate yield.[10] In the present work, we synthesized 4-((6-bromohexyl)oxy)aniline following a literature method[11] and tested its reactivity in open-cage fullerene procedures. The results are mainly analogous to those of simple anilines, but there are also minor differences.

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Antibody-free enzyme-assisted chemical approach for detection of N^6-methyladenosine

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The inert chemical property of RNA modification N^6-methyladenosine (m^6A) makes it very challenging to detect. Most m^6A sequencing methods rely on m^6A-antibody immunoprecipitation and cannot distinguish m^6A and $N^6,2'$-O-dimethyladenosine modification at the cap +1 position (cap m^6A_m). Although the two antibody-free methods (m^6A-REF-seq/MAZTER-seq and DART-seq) have been developed recently, they are dependent on m^6A sequence or cellular transfection. Here, we present an antibody-free, FTO-assisted chemical labeling method termed m^6A-SEAL for specific m^6A detection. We applied m^6A-SEAL to profile m^6A landscapes in humans and plants, which displayed the known m^6A distribution features in transcriptome. By doing a comparison with all available m^6A sequencing methods and specific m^6A sites validation by SELECT, we demonstrated that m^6A-SEAL has good sensitivity, specificity and reliability for transcriptome-wide detection of m^6A. Given its tagging ability and FTO's oxidation property, m^6A-SEAL enables many applications such as enrichment, imaging and sequencing to drive future functional studies of m^6A and other modifications.

N^6-methyladenosine (m^6A) is the most prevalent internal chemical modification in messenger RNA and long-noncoding RNA (lncRNA) in eukaryotes[1], and is the first identified reversible epitranscriptomic mark[2]. m^6A is installed by an m^6A writer complex—the core subunits have been identified as METTL3, METTL14 and WTAP in humans[3,4]—and is erased by the AlkB family dioxygenases (for example, the fat mass and obesity-associated protein (FTO) and ALKBH5 in humans)[2,5]. m^6A is read by m^6A-binding proteins, and such a reading has been shown to affect the RNA processing and metabolism that regulates cell physiology[6–11]. Moreover, aberrant m^6A methylation has been associated with various diseases[12–15]. Therefore, detection of m^6A can deepen our basic understanding of epitranscriptomic metabolism on cellular physiology and can potentially define new targets for fighting diseases.

Due to the inert reactivity of the methyl group of m^6A, available transcriptome-wide m^6A detection methods mostly rely on m^6A-antibody immunoprecipitation (m^6A-IP). m^6A-seq (also termed MeRIP-seq) was developed first and is currently the most widely used method for m^6A detection[16,17]: it combines m^6A-IP of fragment mRNAs with high-throughput sequencing to locate m^6A sites within RNA segments of approximately 200 nucleotides. Inspired by crosslinking immunoprecipitation RNA target identification methods (for example, iCLIP and PAR-CLIP), miCLIP (or m^6A-CLIP) and PA-m^6A-seq methods were developed that can map m^6A marks at higher resolution than m^6A-seq based on antibody crosslinking (via either ultraviolet or 4-thiouridine)[18–20]. Another antibody-based m^6A-LAIC-seq relies on m^6A-IP of full-length poly(A)$^+$ RNA and quantifies methylated transcripts versus non-methylated transcripts in the immunoprecipitated RNA population[21]. More recently, two antibody-free m^6A-seq methods have been developed. m^6A-REF-seq or MAZTER-seq uses RNA endoribonuclease MazF to draw single-base resolution m^6ACA map on transcriptome; but it can only identify ~16–25% of m^6A sites[22,23]. DART-seq expresses the m^6A-binding YTH domain fused with the cytidine deaminase APOBEC1 in cells to induce C-to-U deamination at the C nucleoside in m^6AC sequence, however, the quality in performance of DART-seq relies on the transfection efficiency, and the application of in vitro DART-seq is currently limited[24]. Notably, most of these methods are dependent on the specificity of anti-m^6A antibodies, thus emphasizing the strong desirability of developing transcriptome-wide and antibody-free methods to facilitate the routine drawing of m^6A atlases for many cell and even organismal contexts. However, it is very challenging to develop a chemical-assisted sequencing method for m^6A detection due to the inert chemical property of m^6A.

Here, we present a dithiothreitol (DTT)-mediated thiol-addition chemical reaction that transfers unstable N^6-hydroxymethyladenosine (hm^6A) to more stable N^6-dithiolsitolmethyladenosine (dm^6A). Combining this with FTO's enzymatic oxidation of m^6A in RNA to hm^6A, we developed an FTO-assisted m^6A selective chemical labeling method (termed m^6A-SEAL) for specific detection of transcriptome-wide m^6A. m^6A-SEAL identified 8,605 m^6A sites in human embryonic kidney 293T (HEK293T) and 12,297 m^6A sites in rice leaves, which displayed the known m^6A distribution features in the transcriptome. We have demonstrated that the current FTO oxidation condition used in m^6A-SEAL-seq is specific to detection of m^6A, but not cap m^6A_m. Through optimization of the FTO oxidation step, m^6A-SEAL can be used for specific identification of cap m^6A_m. Performing a comparison with all available m^6A sequencing methods, both metagene profiling analysis and single-base m^6A sites validation by SELECT showed that m^6A-SEAL has the lowest nonspecific and highest positive rate. Collectively, these results reveal that m^6A-SEAL is a reliable and robust method for transcriptome-wide detection of m^6A.

Results

FTO-assisted selective chemical labeling of m^6A in RNA. The inert chemical property of m^6A makes it very challenging to develop a chemical-assisted sequencing method for m^6A detection. To overcome

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Cell Chemical Biology
Article

SFPQ Is an FTO-Binding Protein that Facilitates the Demethylation Substrate Preference

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SUMMARY

The fat mass and obesity-associated protein (FTO) is the first identified demethylase of the internal RNA modification N^6-methyladenosine (m^6A), which also exhibits demethylation activity toward N^6,2′-*O*-dimethyladenosine (m^6A_m) and N^1-methyladenosine (m^1A). Demethylation of m^6A at specific sites on target transcripts is a key enzymatic function of FTO that modulates diverse physiological and/or pathological processes. However, how FTO selects target RNA and whether additional interaction proteins facilitate this process remain elusive. Herein, via the genetically encoded and site-specific photo-crosslinking strategy, we identified the major RNA-binding protein SFPQ as a direct interaction partner of FTO. Our study showed that FTO and SFPQ were located in close proximity throughout the transcriptome and that overexpression of SFPQ led to the demethylation of adjacent m^6As, likely through recruiting FTO to these specific RNA sites. These results uncovered a new layer of regulation mechanism that may assist FTO to gain substrate specificity.

INTRODUCTION

The fat mass and obesity-associated protein (FTO) has been attracting intense research interest for the last decade due to its critical roles in epitranscriptomics and human diseases (Jia et al., 2011; Zhao et al., 2014b). It belongs to the AlkB family of Fe(II)/α-ketoglutarate (αKG)-dependent dioxygenases (Gerken et al., 2007; Kurowski et al., 2003), which possesses demethylation activity toward N^6-methyladenosine (m^6A) in mRNA, small nuclear RNA (snRNA), and U6 RNA (Jia et al., 2011; Wei et al., 2018), N^6,2′-*O*-dimethyladenosine (m^6A_m) modification at the +1 position from the 5′ cap in mRNA and snRNA (Mauer et al., 2017, 2019; Wei et al., 2018), and N^1-methyladenosine (m^1A) modification in tRNA (Wei et al., 2018). Previous studies have demonstrated the role of FTO in multiple processes such as DNA-damage response (Xiang et al., 2017), virus infection (Gokhale et al., 2016), heat-shock response (Zhou et al., 2015), neuronal development (Yu et al., 2018), adipogenesis (Ben-Haim et al., 2015; Zhao et al., 2014a), and cancer cell development (Li et al., 2017; Su et al., 2018; Zhou et al., 2018), among others, by influencing the m^6A modification levels on target transcripts and thereby influencing RNA metabolism. The structure of FTO shows that FTO contains two domains: an N-terminal domain (residues 32–326, referred to as NTD) resembling the structure of AlkB domain for the demethylation function and a C-terminal domain (residues 327–498, referred to as CTD) folded with a β helix, although the biological function of CTD remains unknown (Han et al., 2010).

The structure of FTO coupled with m^6A-modified single-stranded DNA uncovers the molecular catalytic mechanism of FTO for the demethylation of multiple RNA substrates, revealing that FTO prefers the nucleobase N^6-methyladenine and that the sequence and tertiary structure of RNA affects the demethylation activity of FTO (Zhang et al., 2019). The subcellular localization of FTO affects its ability to approach different RNA substrates (Wei et al., 2018). However, despite intensive efforts, how FTO selects its substrate is not fully understood. It is also important to note that the other m^6A demethylase, ALKBH5, which shares high structural similarity with the NTD of FTO, plays crucial roles in spermatogenesis and is likely to recognize substrates different from FTO (Aik et al., 2014; Feng et al., 2014; Xu et al., 2014; Zheng et al., 2013). Given that the binding affinity of FTO or ALKBH5 toward RNA substrates is low (Baltz et al., 2012; Castello et al., 2012), we hypothesized that additional regulating mechanisms may exist that enabled FTO to recognize RNA substrates and that certain proteins may specifically interact with FTO or ALKBH5 to guide substrate recognition.

Until now, only a few FTO interaction proteins have been reported. For example, by using immunoprecipitation coupled with mass spectrometry (IP-MS), FTO was found to interact with Exportin 2 (XPO2) to shuttle from nucleus to cytoplasm (Gulati et al., 2014), where it further interacts with some components in the multi-tRNA synthetase complex to couple the cellular level of amino acids to the mammalian target of rapamycin complex 1 (mTORC1) signaling (Gulati et al., 2013). Through a yeast two-hybrid screening system, calcium/calmodulin-dependent protein kinase II (CaMKII) was identified as the interaction

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评 述 庆祝北京大学化学学科创立110周年专刊

RNA化学修饰m^{6}A的生物功能研究进展

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摘要 N^6-甲基腺嘌呤(m^{6}A)是真核生物mRNA上含量最高的化学修饰. m^{6}A动态可逆调控机制的发现大大推动了以m^{6}A为首的表观转录组学的发展. 多种m^{6}A高通量测序技术及检测方法加深了对m^{6}A的分布规律和调控机理的理解. 在动物或植物体内, m^{6}A可以被一系列甲基转移酶、去甲基酶和结合蛋白进行修饰、去修饰和识别. m^{6}A可以通过影响RNA加工代谢从而调控多种生物学功能. 在本文中, 我们总结介绍了近十年来m^{6}A检测技术、m^{6}A在动物和植物中的功能研究进展, 为后续m^{6}A相关领域的研究与发展提供方向与思考.

关键词 N^6-甲基腺嘌呤(m^{6}A), 表观转录组学, RNA化学修饰

1 引言

基因表达是一种多组分参与、多层次调控的过程. 根据中心法则, 储存在DNA中的遗传信息只有通过转录得到RNA才能最终翻译成蛋白质并行使生物功能[1]. 正因如此, DNA和蛋白质一直作为调控生命活动的重点研究对象. 除了序列中呈现的遗传信息, DNA和蛋白质上的化学修饰也可以影响基因的表达, 从而调控生命活动[2,3]. 与DNA类似, RNA上也存在着上百种化学修饰. 这些修饰广泛地存在于多种RNA中, 如信使RNA(messenger RNA, mRNA)、转运RNA(transfer RNA, tRNA)、核糖体RNA(ribosomal RNA, rRNA)、核内小RNA(small nuclear RNA, snRNA)等[4]. 这些化学修饰对RNA的功能具有重要的调控作用, 因此以研究这些转录后化学修饰的功能为主的表观转录组学成为近年来的研究热点.

N^6-甲基腺嘌呤(m^{6}A)是目前发现的真核生物mRNA上含量最多的化学修饰, 同时也广泛存在于多种细菌和RNA病毒中[5]. 早在20世纪70年代, 细胞中就已经鉴定到了m^{6}A, 但随着m^{6}A去甲基酶FTO的发现, m^{6}A动态可逆的调控机制受到越来越多的关注[6]. 随后通过多种针对m^{6}A的高通量测序技术, m^{6}A对RNA的调控机理越来越清晰. 目前调控m^{6}A的蛋白主要有三类: 称为“Writers”的m^{6}A甲基转移酶、称作“Easers”的去甲基酶和称作“Readers”的结合蛋白(图1, 表1). 本文主要介绍近10年来m^{6}A相关领域取得的研究进展, 主要包括m^{6}A的检测方法、m^{6}A在动物和在植物中的功能研究.

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Review Article

Detection methods of epitranscriptomic mark N^6-methyladenosine

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Research on N^6-methyladenosine (m^6A) in recent years has revealed the complex but elegant regulatory role of this RNA modification in multiple physiological processes. The advent of m^6A detection technologies is the basis for studying the function of this RNA modification. These technologies enable the detection of m^6A sites across transcriptome or at specific gene, thereby revealing the alternation and dynamic of RNA modification. However, non-specific signals that arise from the antibody-based methods and the low-resolution landscape have become the major drawback of classic m^6A detection methods. In this review, we summarize the current available methods and categorized them into three groups according to the utilization purpose, including measurement of total m^6A levels, detection m^6A locus in single gene, and m^6A sequencing. We hope this review helps researchers in epitranscriptomic field find an appropriate m^6A detection tool that suites their experimental design.

Introduction

Post-transcriptional modifications on eukaryotic messenger RNA involved in various cellular processes. Among those modifications, N^6-methyladenosine is the most prevalent and most studied one [1]. The fraction level of m^6A is controlled by the methyltransferases [2,3] and demethylases [4,5]. Recognized by reader proteins, m^6A on individual transcripts plays a critical role in the tuning of RNA processing and metabolism [6–10]. Therefore, the detection of m^6A abundance on RNA paves the way for the exploration of m^6A downstream functions.

In this mini-review, we summarized the current and emerging methods for measurement/detection m^6A at total level, specific locus of single gene, and whole transcriptomes. We will discuss both the advantages and limitations of established methods, while highlighting the potential new methods that improve the resolution, decrease the non-specificity of the existing method. As dozens of new techniques are emerging, for those methods that are widely used and proved to be powerful, we gave them emphasis in the corresponding text to make it convenient for readers to evaluate.

Measurement methods of total level of m^6A

Thin layer chromatography

Thin layer chromatography (TLC) is the most classical method to detect RNA modifications including m^6A. In this assay, RNA is first digested by RNase T1/A, then 5′-end is labeled by $\gamma^{32}P$ by T4-PNK. The radiolabeled nucleotide fragments are purified and completely digested by nuclease P1 to release the 5′-$\gamma^{32}P$ labeled nucleosides. Then, radiolabeled nucleosides are separated by cellulose glass plates and quantified by autoradiography [11]. Mapping of R_f values in different solvent systems for more than 100 modified RNA nucleosides laid a foundation for

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Chemical screening identifies ROCK1 as a regulator of migrasome formation

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Dear Editor,

Migrasomes are newly discovered cellular organelles, first described in 2015[1,2]. Migrasomes are vesicles with diameters of 0.5–3 μm which are generated during cell migration. Cellular contents such as cytosolic components are actively transported to migrasomes and eventually released extracellularly. Thus, migrasomes are proposed as a mechanism for cell–cell communications. Migrasomes are essential for organ morphogenesis during zebrafish embryonic development[3]. Moreover, it has been shown that migrasomes are detected in human serum[4]. Assembly of tetraspanin- and cholesterol-enriched membrane microdomains into micron-scale macrodomains are necessary and sufficient for migrasome formation[5]. In addition, integrins provide the adhesion force for retraction fiber tethering, which are pivotal in migrasome biogenesis process[6]. Pairing of integrins with specific ECM partners for proper adhesion is a determinant for migrasome formation. So far, the systematic studies on detailed regulatory mechanisms of migrasome biogenesis are still lacking.

We designed a chemical genetic screening to identify chemical compounds and their protein targets which interfered with migrasome formation. We used NRK cells stably expressing TSPAN4-GFP to generate migrasomes in 96-well plates and treated with compounds. A diagram of the workflow used for screening is shown in Fig. 1a. Image acquisition was achieved automatically. To assay migrasome generation, the number of cells and migrasomes was quantified and the average migrasome number per cell was calculated. It has been reported that fibronectin (FN) promotes migrasome formation[2]. Using our assay, we tested the effect of increasing the concentration of fibronectin. The average migrasome number per cell increased as the fibronectin concentration increased (Supplementary Fig. S1a). GLPG0187 is the inhibitor of integrin α5β1, which is essential for migrasome biogenesis. GLPG0187 inhibited migrasome biogenesis in a concentration-dependent manner without cytotoxicity (Supplementary Fig. S1b). Based on these results, we concluded that the assay was sufficiently robust and we proceeded with high-throughput screening. We performed the assay with 2240 compounds at a concentration of 10 μM in a 96-well plate format. We identified 507 compounds which had significant inhibitory effect on migrasome generation (Fig. 1b). Indeed, we found that 463 out of the 507 hits showed no or less retraction fibers indicating defect of cell migration (Fig. 1b, Supplementary Fig. S1c). This is a confirmation of the notion that migrasome formation is migration dependent[2]. We focused on the 12 candidates which show significant decreased migrasome number with relatively normal retraction fiber (Fig. 1b, Supplementary Fig. S1c). We performed secondary screening of the 12 candidates. SAR407899 showed stable inhibition of migrasome formation without cytotoxicity or impaired cell proliferation (Fig. 1c, d). The number of migrasomes/100 μm was also significantly reduced compared to DMSO-treated cells (Fig. 1e), which excluded the effect of retraction fiber and cell migration on migrasome formation.

In zebrafish embryos, generation of migrasomes has been observed during gastrulation. Migrasomes were shown to be essential for organ morphogenesis during embryonic development[3]. We thus tested the inhibitory

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Chemoenzymatic Total Syntheses of Artonin I with an Intermolecular Diels–Alderase

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Diels–Alder reaction is one of the most important transformations used in organic synthesis, with the ability to construct two new C–C bonds and up to four chiral centers simultaneously. However, the biggest synthetic challenge in Diels–Alder reaction lies in controlling its regio-, diastereo-, and enantioselectivity. Using Stille cross-coupling and enzymatic Diels–Alder reaction as the key steps, the first chemoenzymatic total synthesis of artonin I is achieved in 30% overall yield over only seven steps. This enzymatic Diels–Alder reaction catalyzed by MaDA is featured with excellent endo- and enantioselectivity and high catalytic efficiency ($k_{cat}/K_M = 362 \pm 54$ mM^{-1} s^{-1}). These successful chemoenzymatic total syntheses of artonin I and dideoxyartonin I demonstrated the remarkable potential of the intermolecular Diels–Alderase MaDA in biocatalysis.

1. Introduction

The root bark of Morus plant, known as a Traditional Chinese Medicine called "sang-bai-pi", has historically been used to treat inflammatory and respiratory diseases.[1] Over 90 different Diels–Alder type natural products have been isolated from Morus plant and some of them exhibit diverse biological activities[1,2] such as anti-HIV effect,[3,4] protein tyrosine phosphatase 1B (PTP1B) and α-glucosidase inhibitory activities.[5–7] There are mainly four different Diels–Alder type natural product skeletons,[8] which all belong to the Diels–Alder type cycloadducts between chalcones and four different types of dienes including benzofuran, chalcone, stilbene, and flavonoid. The representative natural products in this family such as chalcomoracin, kuwanon J, kuwanon Y, and artonin I (**1**) are shown in **Figure 1**, respectively.

Due to their structural complexity and promising biological activities, the Diels–Alder type natural products from Morus plant have attracted much attention from the synthetic community to achieve their total synthesis.[9] Several strategies such as thermal promotion,[10,11] Lewis acid catalysis,[12] and silver nanoparticle catalysis[13] have been successfully developed to promote the biomimetic Diels–Alder reaction, but these methodologies only can deliver the racemic natural products. The first asymmetric total synthesis of these natural products from Morus plants was reported by our group in 2014, in which chiral boron complex was used to promote the asymmetric Diels–Alder reaction to achieve biomimetic total syntheses of kuwanons I and J and brosimones A and B.[14] Using the same method, the asymmetric total syntheses of other Diels–Alder type natural products, such as kuwanons X and Y,[15] and panduratin A[16] were also reported by our group. Inspired by this methodology, Porco and co-worker have reported the first asymmetric total syntheses of sanggenons C and O.[17]

Artonin I (**1**) is a structurally complex Diels–Alder type flavonoid natural product isolated from *A. heterophyllus*[18] and the leaves of *Morus mesozygia Stapf*.[19] This natural product shows potent phosphodiesterase I inhibitory activity[19] and inhibits multidrug resistance in *Staphylococcus aureus*.[20] To our knowledge, no total synthesis of artonin I has been reported yet. Similar with the proposed biosynthetic pathway of other Diels–Alder type natural products in Morus plants,[21] the enzyme-catalyzed oxidation (or dehydrogenation) and Diels–Alder reaction were proposed as the final two key steps in the biosynthesis of artonin I by exogenous feeding of diene precursor in the *Morus alba* cell cultures.[18] Recently, we have identified the first standalone and intermolecular Diels–Alderase (MaDA) from the *M. alba* cell cultures, which catalyzes the endo- and enantio-specific Diels–Alder reaction to generate chalcomoracin and other related Diels–Alder type natural products,[22] demonstrating the synthetic utility of MaDA in the chemo-enzymatic synthesis of endo-selective Diels–Alder type natural products. Thus, we envision that the MaDA-catalyzed Diels–Alder reaction may provide an effective means to produce other Diels–Alder type natural products such as artonin I with high endo- and enantioselectivity, which has been proven to be challenging in chemical catalysis. Herein, we report the chemoenzymatic total

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Syntheses of Skeletally Diverse Tetracyclic *Isodon* Diterpenoid Scaffolds Guided by Dienyne Radical Cyclization Logic

Weilong Liu, Zongwei Yue, Zhen Wang, Houhua Li, and Xiaoguang Lei*

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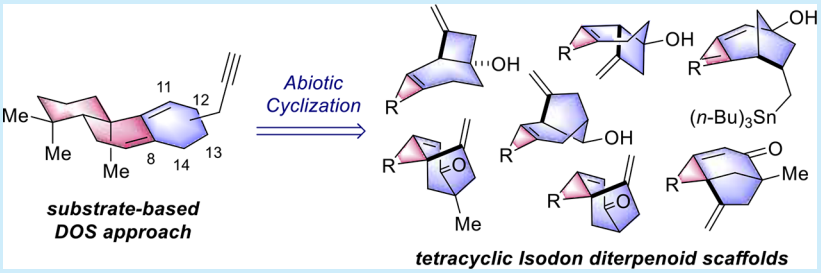

ABSTRACT: We report herein the diversity-oriented synthesis of various tetracyclic *Isodon* diterpenoid scaffolds guided by radical cyclization logic. Our substrate-based dienyne radical cyclization approach is distinctive from reagent-based rearrangement approaches that are generally applied in biosynthesis or previous synthetic studies. An unprecedented cyclization at C_{14} via 1,5-radical translocation/5-*exo*-trig cyclization is observed, which enriches our radical cyclization pattern. Furthermore, biological evaluations revealed that several new natural product-like compounds showed promising anticancer activities against various cancer cell lines.

Tetracyclic *Isodon* diterpenoids (C_{20}) are a large and unique class of natural products with more than 1200 members isolated to date, and their isolation, structural elucidation, (bio)synthesis, and biological evaluation have attracted considerable attention since the first investigations back in 1910.[1,2] Notably, as exemplified in Figure 1, they very often present distinguishable biological activities accordingly with intriguing structural diversity.[3] Thus, strategies that would allow direct access to a collection of tetracyclic *Isodon* diterpenoid scaffolds in a straightforward manner would be a great asset that currently remains elusive.[2,4]

Our group focuses on the total synthesis and biological evaluations of bioactive small molecules of *Isodon* diterpenoids.[2,3,5] As a consequence, a substrate-based regioselective dienyne radical cyclization debuted in our process of total synthesis (Figure 1, red). Based on our experimental evidence and DFT calculations, a jungermannenone-type skeleton was favored both kinetically and thermodynamically when C_{12} possesses alcohol,[2i,p] whereas geometry distortion dominates the regioselectivity to form *ent*-kaurene in the presence of ketone at the C_{12} position.[2t] On the basis of these findings, a sustained interest guided us in further exploration of the substrate-based dienyne radical cyclization. Herein, we report the diversity-oriented synthesis and preliminary biological evaluations of various tetracyclic *Isodon* diterpenoid scaffolds, relying upon a substrate-based dienyne radical cyclization.

Biogenetically, secondary metabolites that possess tetracyclic *Isodon* diterpenoid skeletons are originally derived from bicyclic

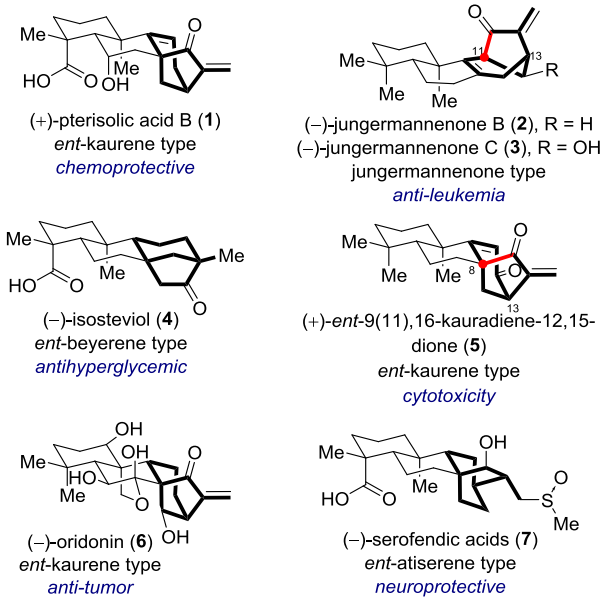

Figure 1. Representative biologically active tetracyclic *Isodon* diterpenoids.

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Inhibition of PU.1 ameliorates metabolic dysfunction and non-alcoholic steatohepatitis

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Background & Aims: Obesity is a well-established risk factor for type 2 diabetes (T2D) and non-alcoholic steatohepatitis (NASH), but the underlying mechanisms remain incompletely understood. Herein, we aimed to identify novel pathogenic factors (and possible therapeutic targets) underlying metabolic dysfunction in the liver.

Methods: We applied a tandem quantitative proteomics strategy to enrich and identify transcription factors (TFs) induced in the obese liver. We used flow cytometry of liver cells to analyze the source of the induced TFs. We employed conditional knockout mice, shRNA, and small-molecule inhibitors to test the metabolic consequences of the induction of identified TFs. Finally, we validated mouse data in patient liver biopsies.

Results: We identified PU.1/SPI1, the master hematopoietic regulator, as one of the most upregulated TFs in livers from diet-induced obese (DIO) and genetically obese (*db/db*) mice. Targeting PU.1 in the whole liver, but not hepatocytes alone, significantly improved glucose homeostasis and suppressed liver inflammation. Consistently, treatment with the PU.1 inhibitor DB1976 markedly reduced inflammation and improved glucose homeostasis and dyslipidemia in DIO mice, and strongly suppressed glucose intolerance, liver steatosis, inflammation, and fibrosis in a dietary NASH mouse model. Furthermore, hepatic *PU.1* expression was positively correlated with insulin resistance and inflammation in liver biopsies from patients.

Conclusions: These data suggest that the elevated hematopoietic factor PU.1 promotes liver metabolic dysfunction, and may be a useful therapeutic target for obesity, insulin resistance/T2D, and NASH.

Lay summary: Expression of the immune regulator PU.1 is increased in livers of obese mice and people. Blocking PU.1 improved glucose homeostasis, and reduced liver steatosis, inflammation and fibrosis in mouse models of non-alcoholic steatohepatitis. Inhibition of PU.1 is thus a potential therapeutic strategy for treating obesity-associated liver dysfunction and metabolic diseases.

Introduction

Obesity and the associated metabolic syndrome are significant worldwide public health concerns and account for tremendous costs for the affected individuals, families, healthcare systems, and society. In particular, obesity is associated with the development of insulin resistance, type 2 diabetes (T2D), liver and cardiovascular diseases, and cancer. Insulin responsive tissues, including adipose tissue, skeletal muscle, and liver, are profoundly affected by obesity both at biomolecular and functional levels. Insulin sensitivity in the liver is pivotal in the regulation of glucose and lipid metabolism.[1] Insulin suppresses liver glucose production by inhibiting glycogenolysis and gluconeogenesis and stimulating glycogen synthesis, glycolysis and lipogenesis.[2] In insulin-resistant state, when insulin fails to adjust lipid and carbohydrate metabolism, hyperglycemia and dyslipidemia ensue, and exacerbate the incidence of non-alcoholic fatty liver disease (NAFLD).[3] Indeed, obesity and associated insulin resistance have been established as risk factors of liver fat accumulation, which promotes a liver disease spectrum ranging from NAFLD to non-alcoholic steatohepatitis (NASH), with possible progression towards cirrhosis and even hepatocellular carcinoma (HCC).[4–6] In parallel, liver diseases also contribute to the

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FAD-dependent enzyme-catalysed intermolecular [4+2] cycloaddition in natural product biosynthesis

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The Diels–Alder reaction is one of the most powerful and widely used methods in synthetic chemistry for the stereospecific construction of carbon–carbon bonds. Despite the importance of Diels–Alder reactions in the biosynthesis of numerous secondary metabolites, no naturally occurring stand-alone Diels–Alderase has been demonstrated to catalyse intermolecular Diels–Alder transformations. Here we report a flavin adenine dinucleotide-dependent enzyme, *Morus alba* Diels–Alderase (MaDA), from *Morus* cell cultures, that catalyses an intermolecular [4+2] cycloaddition to produce the natural isoprenylated flavonoid chalcomoracin with a high efficiency and enantioselectivity. Density functional theory calculations and preliminary measurements of the kinetic isotope effects establish a concerted but asynchronous pericyclic pathway. Structure-guided mutagenesis and docking studies demonstrate the interactions of MaDA with the diene and dienophile to catalyse the [4+2] cycloaddition. MaDA exhibits a substrate promiscuity towards both dienes and dienophiles, which enables the expedient syntheses of structurally diverse natural products. We also report a biosynthetic intermediate probe (BIP)-based target identification strategy used to discover MaDA.

The Diels–Alder (D–A) reaction, a [4+2] cycloaddition between a conjugated diene and a dienophile to yield a cyclohexene skeleton, is one of the most powerful carbon–carbon bond forming reactions in synthetic chemistry[1]. Although putative Diels–Alderases, or [4+2] cyclases in the broader sense, have been predicted to be involved in the biosyntheses of many secondary metabolites[2,3], only a handful have been identified and characterized[4–7], with the majority being multifunctional enzymes[8–11]. The identification of the first stand-alone [4+2] cyclase, SpnF[12], led to the discovery of other intramolecular [4+2] cyclases from the biosynthetic pathways of spirotetronate[13,14] and/or spirotetramate[15] polyketides, decalin-containing polyketide–amino acid hybrids[16–20] and leporine[21], as well as intramolecular [6 + 4] cyclases from the biosynthetic pathway of streptoseomycin, a macrocyclic polyketide[22]. However, no stand-alone intermolecular [4+2] cyclases have been identified to date, even though intermolecular [4+2] cycloadditions have been catalysed by artificial enzymes generated via computational design[23,24] and immunological selection[25].

Methylcyclohexene motifs, accessible via D–A reactions, are common in *Moraceae* (mulberry) natural products with a role in Chinese traditional medicine[26,27]. These natural products display antimicrobial as well as diverse biological activities, such as the inhibition of hypoxia-inducible factor-1 and protein tyrosine phosphatase 1B[28–31]. Representative examples that incorporate diverse diene substituents are shown in Fig. 1a. Exogenous substrate feeding[32,33] and ^{13}C-labelling experiments[34] in *M. alba* cell cultures indicated the existence of an oxidase to furnish a reactive diene and a putative [4+2] pericyclase to catalyse the intermolecular [4+2] cycloaddition (Fig. 1b). We developed a chiral boron-mediated asymmetric D–A reaction for the enantioselective total syntheses of several natural products of this family[35]. A few racemic total syntheses are also reported[36,37]. Unfortunately, all the existing syntheses failed to achieve high levels of diastereo- and enantioselectivity, a problem that can be addressed chemoenzymatically if the relevant enzymes are isolated and characterized.

Historically, difficulties associated with identifying plant enzymes have slowed their discovery. Unlike in microbes, many biosynthetic genes for secondary metabolites in plants do not occur in gene clusters[38]. In addition, no stand-alone enzymes capable of catalysing intermolecular D–A reactions are known, which makes it difficult to perform homology-based or genome-context-based searches in modern biological databases. Both traditional activity-based purification and more modern transcriptomics-enabled methods are used to successfully identify elusive enzymes from plant natural product

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Research Article

Chrysomycin A Derivatives for the Treatment of Multi-Drug-Resistant Tuberculosis

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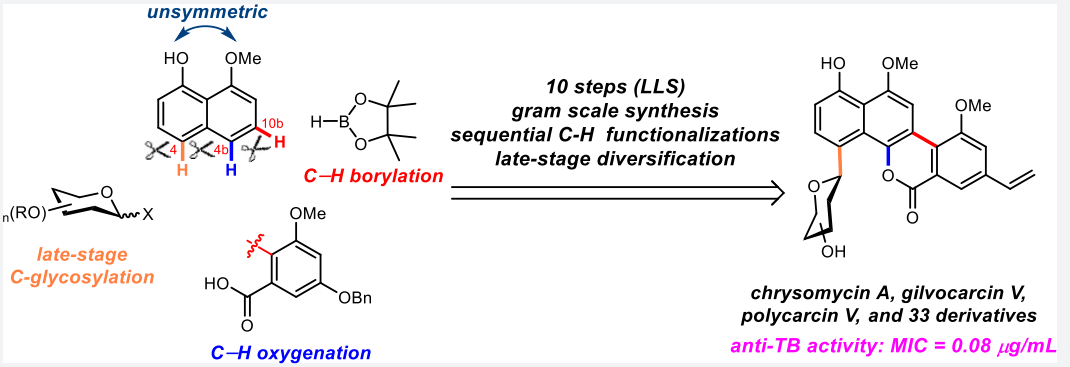

ABSTRACT: Tuberculosis (TB) is a life-threatening disease resulting in an estimated 10 million new infections and 1.8 million deaths annually, primarily in underdeveloped countries. The economic burden of TB has been estimated as approximately 12 billion USD annually in direct and indirect costs. Additionally, multi-drug-resistant (MDR) and extreme-drug-resistant (XTR) TB strains resulting in about 250 000 deaths annually are now widespread, increasing pressure on the identification of new anti-TB agents that operate by a novel mechanism of action. Chrysomycin A is a rare C-aryl glycoside first discovered over 60 years ago. In a recent high-throughput screen, we found that chrysomycin A has potent anti-TB activity, with minimum inhibitory concentration (MIC) = 0.4 μg/mL against MDR-TB strains. However, chrysomycin A is obtained in low yields from fermentation of *Streptomyces*, and the mechanism of action of this compound is unknown. To facilitate the mechanism of action and preclinical studies of chrysomycin A, we developed a 10-step, scalable synthesis of the isolate and its two natural congeners polycarcin V and gilvocarcin V. The synthetic sequence was enabled by the implementation of two sequential C−H functionalization steps as well as a late-stage C-glycosylation. In addition, >10 g of the advanced synthetic intermediate has been prepared, which greatly facilitated the synthesis of 33 new analogues to date. The structure−activity relationship was subsequently delineated, leading to the identification of derivatives with superior potency against MDR-TB (MIC = 0.08 μg/mL). The more potent derivatives contained a modified carbohydrate residue which suggests that further optimization is additionally possible. The chemistry we report here establishes a platform for the development of a novel class of anti-TB agents active against drug-resistant pathogens.

INTRODUCTION

Tuberculosis (TB) has become the number one life-threatening infectious disease, whose treatment is further complicated by the emergence of drug-resistant strains.[1] Over the past four decades, only two new drugs, bedaquiline[2] and delamanid,[3] have been approved by the FDA and EMA, respectively, for the treatment of MDR-TB. Considering the high attrition rate in clinical trials, more effective anti-TB drug candidates with distinct molecular scaffolds are in urgent need. Chrysomycin A (**1**) is an antitumor antibiotic first isolated from *Streptomyces* A-419 in 1955 as a mixture with chrysomycin B (**2**).[4] Recently, we rediscovered chrysomycin A (**1**) and its natural congeners from mining of a 10K actinobacteria genome sequences[5] and found that chrysomycin A showed promising antimicrobial activity against a number of Gram-positive strains and the MDR-TB strain with a minimum inhibitory concentration (MIC) of 0.4 μg/mL (see Tables S1 and S2). Kumar and co-workers also independently reported that chrysomycin A showed inhibitory activity against M. tb strains.[6] Congeneric chrysomycin C (**3**) was isolated from *Streptomyces sporoverrucosus* in 2013.[7] Chrysomycins A−C

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Identification of the AMA Synthase from the Aspergillomarasmine A Biosynthesis and Evaluation of Its Biocatalytic Potential

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ABSTRACT: The β-lactam antibiotic resistance has become a critical global health threat. One of the major reasons for drug resistance is the expression of β-lactamases especially metallo-β-lactamases such as New Delhi metallo-β-lactamase (NDM-1) by Gram-negative bacteria. The fungal natural product aspergillomarasmine A (AMA) was found to be a promising inhibitor of NDM-1 to potentiate currently used β-lactam antibiotics to overcome drug resistance both *in vitro* and *in vivo*. Although several chemical synthesis and chemoenzymatic synthesis approaches to access AMA have been reported, the biosynthesis of AMA was still elusive. Herein, we identified the key enzyme responsible for the biosynthesis of AMA in *Aspergillus oryzae*. AMA synthase is a PLP-dependent cysteine synthase homologous protein which utilizes *O*-acetyl-*L*-serine/*O*-phospho-*L*-serine and *L*-aspartic acid as its substrates. Remarkably, this enzyme catalyzes two consecutive C-N bond formations to produce AMA efficiently which may be attributed to the spacious substrate-binding pocket. PLP is covalently bound to Lys61 by an internal aldimine from the PLP *re* face, and the *si* face of PLP pyridine ring is accessible to the substrates to promote the nucleophilic addition of amino acids to the double bond of the external adiminine and ultimately to generate chiral C_α with *S* configuration. The catalytic mechanism was proposed based on molecular docking and biochemical experiments. In addition, we have further investigated the substrate scope of AMA synthase and identified a variant enzyme which shows promising potential in producing structurally diverse molecules containing C-N bond.

KEYWORDS: Antibiotic resistance • NDM-1• aspergillomarasmine A • biosynthesis • biocatalysis

INTRODUCTION

New Delhi metallo-β-lactamase-1 (NDM-1) was discovered as a key metallo-β-lactamase (MBL) in 2009.[1] Bacteria containing NDM-1 have spread worldwide, which hydrolyzes nearly all β-lactam antibiotics except aztreonam.[1-2] Unfortunately, no small molecule inhibitors of NDM-1 are currently available in clinic, which represents a significant unmet medical need for treatment of bacteria infectious disease.[2] In 2014, aspergillomarasmine A (AMA), a fungal natural product, has been found to inhibit the activity of NDM-1, and consequently this natural product showed promising activity to restore the sensitivity of multi-drug resistant Gram-negative bacteria towards meropenem both *in vitro* and *in vivo*.[3] AMA could efficiently remove Zn^{2+} from MBLs (NDM-1, VIM-2, IMP-7).[4] Therefore, AMA may become an excellent antibiotics adjuvant to overcome the clinical carbapenem resistance caused by MBL-containing Gram-negative bacteria.[5] AMA and related phytotoxins such as toxin A, aspergillomarasmine B (AMB) and lycomarasmine are produced by many filamentous fungi including *A. oryzae*, *A. flavus*, *A. versicolor*, *C. gloeosporioides*, *f. sp. Melonis* and *P. teres*., which were originally shown to cause plants to wilt.[6-13] AMA was first isolated from *A. oryzae* in 1965.[6] Later, AMA was further found to inhibit angiotensin-converting enzymes through its metal-chelating activity.[10, 12] As a phytotoxin, AMA was found to participate in the interaction with the host to promote fungi's growth. In addition, AMA was proposed to be the biosynthetic precursor of lycomarasmine that may serve as a siderophore.[14-16] Due to its intriguing structures and potential applications in combination therapy to address β-lactam antibiotics resistance, AMA and its analogues have attracted a lot of attention from the synthetic community. In 2016, our group reported the first total synthesis and structural reassignment of AMA using the reductive amination and late stage oxidation strategy.[17] Wright group also

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Outlook

Late-Stage Diversification of Natural Products

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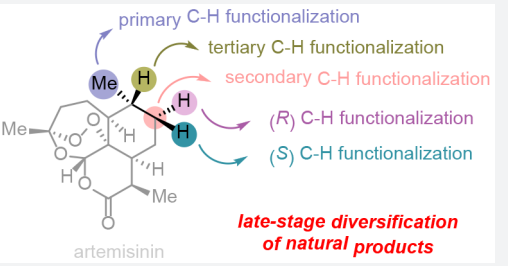
ABSTRACT: Late-stage diversification of natural products is an efficient way to generate natural product derivatives for drug discovery and chemical biology. Benefiting from the development of site-selective synthetic methodologies, late-stage diversification of natural products has achieved notable success. This outlook will outline selected examples of novel methodologies for site-selective transformations of reactive functional groups and inert C−H bonds that enable late-stage diversification of complex natural products. Accordingly, late-stage diversification provides an opportunity to rapidly access various derivatives for modifying lead compounds, identifying cellular targets, probing protein−protein interactions, and elucidating natural product biosynthetic relationships.



INTRODUCTION

Natural products play an important role in chemistry and drug discovery.[1,2] Over the years, chemists have sought to develop novel methods and strategies for their effective chemical synthesis[3] in order to elucidate their biological functions for novel therapeutic agents[4,5] and determine their biosynthesis for synthetic biology.[6] These studies have been motivated by the historic involvement of natural products such as penicillin (**1**), taxol (**2**), artemisinin (**3**), and vinblastine (**4**) in drug discovery (Figure 1). Over the last three decades, nearly 50% of newly approved small molecule drugs have been natural products or derivatives thereof.[5]

Natural product diversification is essential in drug discovery, particularly in the optimization of pharmacological properties and investigation of structure−activity relationships (SARs). Driven by the discovery of biologically relevant natural product-like derivatives, several strategies have been employed to prepare these molecules, including diverted total synthesis (DTS).[7,8] Since traditional synthesis of natural product derivatives from simple starting materials can be laborious and ineffective, an alternative approach is to derivatize natural products directly via selective reactions, which may shorten synthetic routes and provide a more effective means of producing these compounds. However, late-stage diversification of natural products has been underexplored owing to the synthetic challenge of performing selective functionalizations in the presence of the diverse functional groups often found in natural products.

Natural product diversification is essential in drug discovery, particularly in the optimization of pharmacological properties and investigation of structure-activity relationships (SARs).

Recently, a series of remarkable advances in synthetic organic methodologies, including developments in site-selective catalysts,[9,10] state-of-the-art C−H functionalization,[11,12] photochemistry,[13,14] electrochemistry,[15,16] and bio-

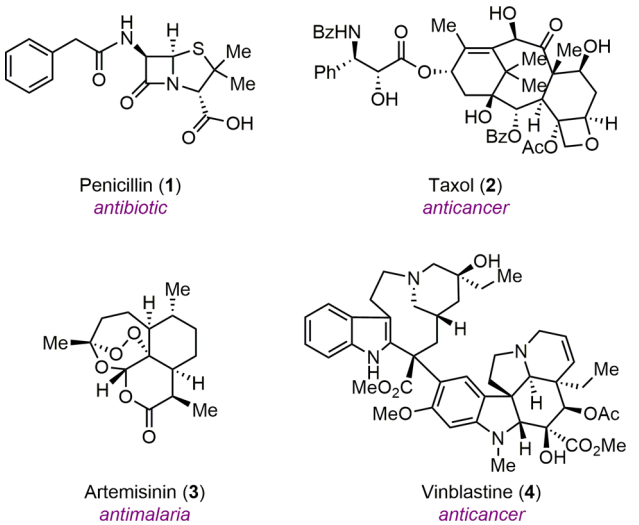


Figure 1. Natural products in human medicine.

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Dissecting Programmed Cell Death with Small Molecules

Yingjie Bai, Hiu C. Lam, and Xiaoguang Lei*

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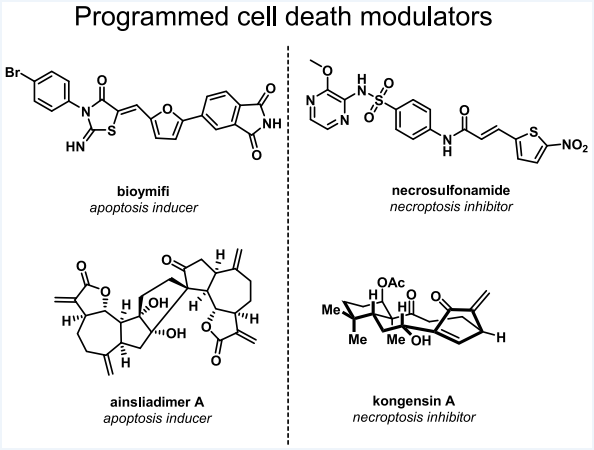
CONSPECTUS: Programmed cell death (PCD) is fundamentally an indispensable process in all cellular activities, including cell development, wound healing, and immune surveillance of tumors (Galluzzi, L. et al. *Cell Death Differ.* **2018**, *25*, 486−541). Malfunctioning of PCD has been shown to be closely related to human diseases such as acute pancreatitis, neurodegenerative diseases, and diverse types of cancers. To date, multiple PCD processes have been discovered and the corresponding regulatory pathways have been elucidated. For example, apoptosis and autophagy are two PCD mechanisms that have been well studied by sophisticated models and probe toolkits. However, limited genetic and chemical tools for other types of PCD hamper the elucidation of their molecular mechanisms. Our group has been studying PCD using both function-oriented synthesis and chemical biology strategies, including the development of diverse chemical probes based on novel PCD modulators. For instance, in the development of downstream programmed necrosis (or necroptosis) inhibitor necrosulfonamide, we used a chemical probe to unveil a functional protein that was not previously implicated in necroptosis, mixed lineage kinase domain-like protein (MLKL). In addition, high throughput screening and medicinal chemistry enabled the discovery of bioymifi, a small molecule agonist which selectively causes oligomerization of the death receptor 5 (DR5), to induce extrinsic apoptosis. Furthermore, we developed a biomimetic synthetic strategy based on diverse Diels−Alder reactions in the total syntheses of ainsliadimers A and B, ainsliatrimers A and B, and gonchnatiolides A−C, which are natural product inhibitors or activators for PCD. Using synthetic ainsliadimer A probe, we elucidated that ainsliadimer A inhibits the NF-κB pathway by covalently binding to Cys46 of IKKβ and triggers apoptosis of cancer cells. We have also revealed that IKKβ is allosterically inhibited by ainsliadimer A. In addition to total synthesis, we have developed a bioorthogonal click hetero-Diels−Alder cycloaddition of vinyl thioether and *o*-quinolinone quinone methide (TQ-ligation) to facilitate small molecule target identification. The combination of total synthesis and TQ-ligation enables subcellular imaging and identification of the cellular target of ainsliatrimer A to be PPARγ. In addition, TQ-ligation has been applied in the discovery of heat shock protein 90 (HSP90) as one of the functional target proteins for kongensin A. We also confirmed that kongensin A covalently attaches to Cys420 within HSP90 and demonstrated that kongensin A blocks the interaction between HSP90 and CDC37 and subsequently inhibits necroptosis. Our development of these diverse PCD modulators provides not only effective chemical tools for fundamental biomedical research, but also the foundation for drug discovery targeting important human diseases such as cancers and inflammation caused by malfunction of PCD.



INTRODUCTION

Programmed cell death (PCD) can be classified into apoptosis and nonapoptotic PCDs based on the nature of ubiquitous causes and outcome of cell death. Apoptosis, the prototype PCD, was first described by Kerr et al. in 1972,[1] characterized by its morphological changes, unique biochemistry, and lack of inflammation.[1−4] Besides apoptosis, typical nonapoptotic PCDs including autophagy,[5] necroptosis,[6] ferroptosis,[7] and pyroptosis,[8] have been discovered recently.[9,10] In addition to PCDs, regulated cell death (RCD) has emerged to extend the definition of PCDs to include the promiscuous cell death pathways.[9] In 2018, the Nomenclature Committee on Cell Death accepted MPT-driven necrosis, NETotic cell deaths,[11] parthanatos, entotic cell death, and mitotic catastrophe as RCD pathways.[9] Since the characterization of novel PCD-related signaling pathways has not yet been completed, space remains for component characterizations and development of small molecule modulators for PCDs.

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Computation-Guided Development of the "Click" *ortho*-Quinone Methide Cycloaddition with Improved Kinetics

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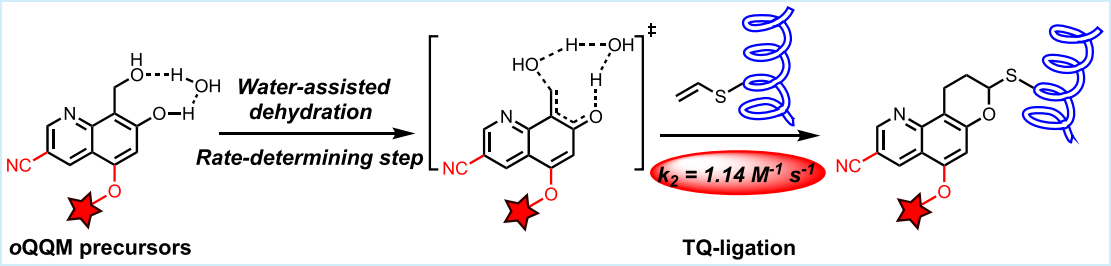

ABSTRACT: We report here a deep mechanistic study of the "click" *ortho*-quinone methide (*o*QM) cycloaddition between *ortho*-quinolinone quinone methide (*o*QQM) and thio-vinyl ether (TV), named as TQ-ligation. DFT calculations revealed the unexpected fact that dehydration of *o*QQM precursors is the rate-determining step of this transformation, and two highly reactive *o*QQM precursors were predicted. Guided by the calculations, a new "click" *o*QM cycloaddition which shows significantly improved kinetics and remarkable efficiency on protein labeling was developed.

Click chemistry is a powerful strategy that takes advantage of facile, selective, and high-yielding organic transformations to conjugate two substrates while producing few byproducts.[1] It has found wide applications in various fields, from bioconjugation[2] to materials science[3] and drug discovery.[4] Since the report of copper-catalyzed Huisgen 1,3-dipolar cycloaddition as a classic click reaction,[5] the development of new "click" transformations, such as sulfur-fluoride exchange (SuFEx) chemistry,[6] has received considerable interest to facilitate broad applications of click chemistry in many fields.

Recently, increasing attention has been focused on the cycloaddition reactions, which can easily fulfill most of the requirements of click chemistry along with the advantage of proceeding without potential toxic metal catalysts.[7] As an example, *ortho*-quinone methide (*o*QM) cycloaddition is an important class of facile hetero-Diels–Alder (HDA) reactions which have been widely used in the construction of complex natural products.[8,9] However, most of the *o*QM generation methods require thermal/UV activation or catalysts limiting their applications in biomedical research.

Understanding the fundamental mechanistic processes offers the opportunities to improve reactions kinetics and design new robust transformations. A notable example is a mechanism study of photodehydration, which suggested that a process of excited-state intramolecular proton transfer (ESIPT) from phenolic alcohol to the benzylic oxygen atom was involved.[10] Based on the ESIPT, the Popik group developed a robust photoclick reaction between 2-napthoquinone-3-methides and electron-rich polarized olefins in aqueous solution.[11] The rapid kinetics (4×10^4 M^{-1} s^{-1}) and mild reaction conditions allowed it to be successfully applied to high-resolution surface patterning,[12] but the potential phototoxicity limited the scope of its applications in biological systems. In 2013, we developed a UV-free and highly selective "click" *o*QM cycloaddition between *ortho*-quinolinone quinone methide (*o*QQM) and thio-vinyl ether (TV), named as TQ-ligation (Scheme 1a).[13] This reaction is biocompatible inside live cells with a relatively slow rate constant ($k_2 = 1.5 \times 10^{-3}$ M^{-1} s^{-1}). The rate constant of the ligation was then enhanced less than we expected by only around 18-fold ($k_2 = 2.8 \times 10^{-2}$ M^{-1} s^{-1}) by introducing electron-withdrawing groups to *o*QQMs (Scheme 1a).[14] This novel ligation has been further employed to live-cell imaging[13−15] and protein profiling of bioactive natural products.[16] However, a detailed mechanism has not been fully elucidated, especially which step might be the rate-determining step. This situation significantly hindered the rational design of a new reagent to improve the reaction kinetics. Herein, we report a systematic mechanistic investigation of *o*QM cycloaddition TQ-ligation. Unexpectedly, the dehydration of *o*QQM precursors is the rate-determining step based on

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Article

An *Arabidopsis* Secondary Metabolite Directly Targets Expression of the Bacterial Type III Secretion System to Inhibit Bacterial Virulence

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SUMMARY

Plants deploy a variety of secondary metabolites to fend off pathogen attack. Although defense compounds are generally considered toxic to microbes, the exact mechanisms are often unknown. Here, we show that the *Arabidopsis* defense compound sulforaphane (SFN) functions primarily by inhibiting *Pseudomonas syringae* type III secretion system (TTSS) genes, which are essential for pathogenesis. Plants lacking the aliphatic glucosinolate pathway, which do not accumulate SFN, were unable to attenuate TTSS gene expression and exhibited increased susceptibility to *P. syringae* strains that cannot detoxify SFN. Chemoproteomics analyses showed that SFN covalently modified the cysteine at position 209 of HrpS, a key transcription factor controlling TTSS gene expression. Site-directed mutagenesis and functional analyses further confirmed that Cys209 was responsible for bacterial sensitivity to SFN *in vitro* and sensitivity to plant defenses conferred by the aliphatic glucosinolate pathway. Collectively, these results illustrate a previously unknown mechanism by which plants disarm a pathogenic bacterium.

INTRODUCTION

Plants possess a large number of secondary metabolites, which serve as foot soldiers in the fight against pathogenic microbes and animal pests (Mithöfer and Boland, 2012; Piasecka et al., 2015; Rajniak et al., 2015). Some of these defense metabolites are produced constitutively in plants and are thus termed phytoanticipins, whereas others are synthesized upon pathogen attacks and are called phytoalexins (Paxton, 1981; VanEtten et al., 1994). Phytoanticipins are structurally diverse and include saponins, cyanogenic glucosides, glucosinolates, fatty acid derivatives, and terpenoids (Piasecka et al., 2015; Pedras and Yaya, 2015). Likewise, phytoalexins are also structurally diverse and different in plant species. At least 44 phytoalexins have been isolated from Brassicaceae alone, most of which are derived from (S)-tryptophan (Ahuja et al., 2012).

Although an increasing number of phytoaniticipins and phytoalexins has been discovered and their biosynthetic pathways elucidated, little is known about mechanisms by which these defense metabolites confer disease resistance. It is assumed that defense metabolites function through their antimicrobial activities. Indeed, high doses of the defense metabolites can inhibit or even kill microbes in *vitro* (Kliebenstein et al., 2005; Rogers et al., 1996; Sanchez-Vallet et al., 2010; Sellam et al., 2007b). For example, α-tomatine has been reported to inhibit mycelial growth of *Colletotrichum orbiculare*, *Septoria linicola*, and *Helminthosporium turcicum* at concentrations from 130 μM to 2 mM (Arneson and Durbin, 1968), and 500 μM brassinin can cause 50% growth inhibition of *Leptosphaeria maculans* (Pedras et al., 2017). However, it is not clear whether these concentrations tested *in vitro* correspond to the concentrations at the site of infection in plants. Camalexin has been reported to disrupt membrane integrity of *Pseudomonas syringae maculicola* ES4326 when applied at high concentration to the bacterium *in vitro*, but it does not play a role in disease resistance against the bacterium in plants (Rogers et al., 1996). Camalexin-

Evaluation of Chemical Cross-linkers for In-Depth Structural Analysis of G Protein-Coupled Receptors through Cross-Linking Mass Spectrometry

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Natural Products

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Biomimetic Synthesis of Rhytidenone A and Mode of Action of Cytotoxic Rhytidenone F

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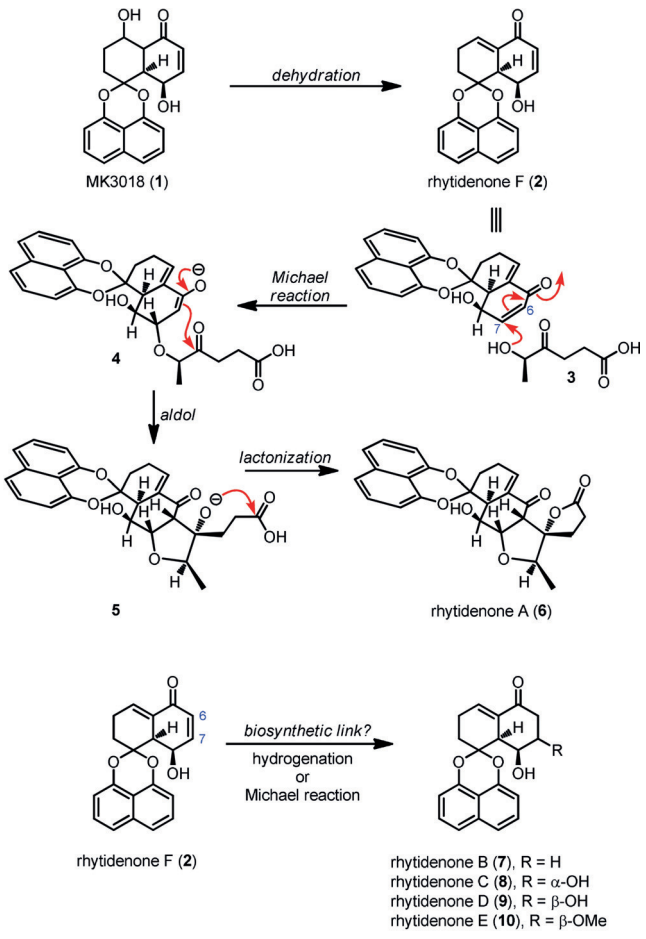
Abstract: *The rhytidenone family comprises spirobisnaphthalene natural products isolated from the mangrove endophytic fungus Rhytidhysteron rufulum AS21B. The biomimetic synthesis of rhytidenone A was achieved by a Michael reaction/aldol/lactonization cascade in a single step from the proposed biosynthetic precursor rhytidenone F. Moreover, the mode of action of the highly cytotoxic rhytidenone F was investigated. The pulldown assay coupled with mass spectrometry analysis revealed the target protein PA28γ is covalently attached to rhytidenone F at the Cys92 residue. The interactions of rhytidenone F with PA28γ lead to the accumulation of p53, which is an essential tumor suppressor in humans. Consequently, the Fas-dependent signaling pathway is activated to initiate cellular apoptosis. These studies have identified the first small-molecule inhibitor targeting PA28γ, suggesting rhytidenone F may serve as a promising natural product lead for future anticancer drug development.*

Introduction

p53 is an essential tumor suppressor in humans.[1] Loss or mutation of p53 is strongly associated with various cancers.[2] Extensive studies to develop drugs that could activate or restore the p53 pathway have now reached clinical trials.[3] Fundamentally, discovery of small molecules targeting p53 pathway with a new mode of action may further stimulate many exciting new approaches to cancer drug discovery. Spirobisnaphthalenes consist of two naphthalene-derived C10 units joined together through a spiroketal linkage, and belong to a novel family of natural products isolated from fungi.[4,5] They possess a variety of biological properties,[6] including antimicrobial,[7,8] antiplasmodial,[9] antileishmanial,[10] nematicidal,[11] and antitumor activities.[12] In 2014, Pudhom and coworkers identified a family of novel spirobisnaphthalene compounds from endophytic fungi *Rhytidhysteron rufulum* AS21B, namely rhytidenones A-F (Scheme 1).[13] Rhytidenone F (**2**) shows excellent cytotoxic activity against Ramos (Burkitt's lymphoma) and H1975 (non-small cell lung cancer) cells,[14] but the mode of action remains elusive. Moreover, rhytidenone A (**6**) bears the most complex structure in the rhytidenone family, containing six contiguous stereocenters



Scheme 1. Proposed biosynthetic connections of rhytidenones A–F and MK3018 from *Rhytidhysteron rufulum* AS21B.[13,15]

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Styryllactones from *Goniothalamus tamirensis*

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ABSTRACT

The phytochemical investigation of the twig and leaf extracts of *Goniothalamus tamirensis* led to the isolation and identification of 15 compounds including three rare previously undescribed styryllactones, goniotamirenones A-C, together with 12 known compounds. (*Z*)-6-Styryl-5,6-dihydro-2-pyranone and 5-(1-hydroxy-3-phenyl-allyl)-dihydro-furan-2-one are reported here for the first time as previously undescribed natural products. Their structures were elucidated by spectroscopic methods. Goniotamirenone A was synthesized *via* a [2 + 2] cycloaddition reaction of 6-styrrylpyran-2-one in quantitative yield. The absolute configurations of goniotamirenones B and C were identified from experimental and calculated ECD data, while the absolute configurations of (−)–5-acetoxygoniothalamin, (−)-isoaltholactone, parvistone E, and 5-(1-hydroxy-3-phenyl-allyl)-dihydro-furan-2-one were identified by single-crystal X-ray diffraction analysis using Cu Kα radiation. The absolute configurations of the other related compounds were determined from comparisons of their ECD spectra with relevant compounds reported in the literature. (−)–5-Acetoxygoniothalamin exhibited potent cytotoxicity against the colon cancer cell line (HCT116) with an IC_{50} value of 8.6 μM which was better than the standard control (doxorubicin, IC_{50} = 9.7 μM), while (*Z*)-6-styryl-5,6-dihydro-2-pyranone was less active with an IC_{50} value of 22.1 μM.

1. Introduction

One of the largest genera in the family Annonaceae is *Goniothalamus* which comprises over 160 species (Saunders and Chalermglin, 2008). These plants are distributed throughout tropical and subtropical countries. In Thailand, over 15 species have been found, mostly in the eastern, north-eastern and south-eastern regions (Soonthornchareonnon et al., 1999). Some species of *Goniothalamus* have been used in traditional medicines in Thailand. For example, the decoction of the stem bark of *G. laoticus* (Finet & Gagnep.) Bân has been used to reduce fever and as a tonic (Lekphrom et al., 2009), while *G. elegants* Ast has been used in the treatment of heart disease and diarrhea (Suchaichit et al., 2015). *G. tamirensis* Pierre ex Finet & Gagnep (syn. *G. marcanii* Craib) (Saunders and Chalermglin, 2008) is a small tree which is not known for its medcinal uses. Compounds reported from this plant have primarily been styryllactones (Ahmad et al., 1991; Fang et al., 1990; Goh et al., 1995) and alkaloids (Lekphrom et al., 2009; Tran et al., 2013). In this study, we report the isolation and identification of three rare and previously undescribed styryllactones (**1–3**) together with 12 known compounds (**4–15**) from the twig and leaf extracts of *G.*

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Communication

Protecting-Group-Free Syntheses of *ent*-Kaurane Diterpenoids: [3+2+1] Cycloaddition/Cycloalkenylation Approach

Jin Wang,[‖] Benke Hong,[‖] Dachao Hu, Yuichiro Kadonaga, Ruyao Tang, and Xiaoguang Lei*

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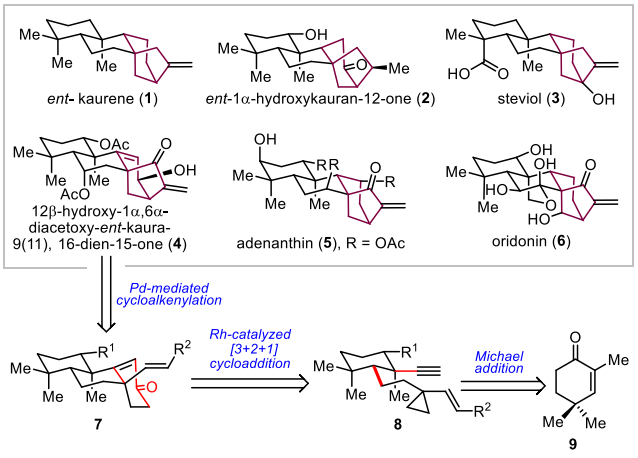
ABSTRACT: The Yu's Rh-catalyzed [3+2+1] cycloaddition followed by a Pd-mediated 5-*endo* cycloalkenylation is shown to be a general and powerful approach for efficient construction of the tetracyclic core structure of *ent*-kaurane diterpenoids. The utility of this strategy was further demonstrated by concise and protecting-group-free total syntheses of *ent*-1α-hydroxykauran-12-one, 12-oxo-9,11-dehydrokaurene, and 12α-hydroxy-9,11-dehydrokaurene.

The *ent*-kaurane diterpenoids are a unique family of tetracyclic natural products, and more than 1000 members have been isolated to date from different plants species, especially from the *Isodon* genus.[1] These natural products, including *ent*-kaurene (**1**),[2a] *ent*-1α-hydroxykauran-12-one (**2**),[2b] steviol (**3**),[2c] 12β-hydroxy-1α,6α-diacetoxy-*ent*-kaura-9(11),16-dien-15-one (**4**),[2d] adenanthin (**5**),[2e] and oridonin (**6**),[2f] feature a bicyclo[3.2.1]octene ring system with different oxidation states at the key tetracyclic skeleton (Scheme 1). It has been found that the *ent*-kaurane diterpenoids possess many promising biological activities, such as anticancer, antifungal, and antiviral activities.[1] Their intriguing structures as well as potential biological activities have attracted tremendous attention from the synthetic community, culminating in numerous elegant syntheses of their core structures and target molecules.[3−5] Most of the reported methods required multi-step reactions to construct the tetracyclic *ent*-kaurane skeleton, especially the [3.2.1] bicyclic moiety.[3]

Scheme 1. Representative *ent*-Kauranoids and Retrosynthetic Analysis of the Core Tetracyclic Skeleton



Herein, we report a concise and general synthetic approach to assemble the *ent*-kaurane diterpenoids skeleton. As demonstrated in Scheme 1, we envisaged that the [3.2.1] bicyclic motif of the tetracyclic skeleton of *ent*-kauranoids could be accessed from **7** via a palladium-mediated cycloalkenylation. The 6/6/6 tricyclic ring system of enone **7** could be efficiently accessed using the Rh-catalyzed [3+2+1] cycloaddition of 1-yne-vinylcyclopropane (VCP) precursor **8** and CO developed by Yu's group.[6] Using this two-step sequence, the *ent*-kauranoid skeleton bearing different functional groups could be constructed efficiently from various VCP precursors. If successful, this strategy could not only enable the total synthesis of *ent*-kaurane diterpenoids but also facilitate the efficient preparation of diverse natural product analogues for further biological study. The key precursor **8** might be derived from the commercially available enone **9** via Michael addition and alkynylation.

To explore the feasibility of the proposed Rh-catalyzed [3+2+1] cycloaddition/Pd-mediated alkenylation sequence, **8a** was chosen as a model substrate, which was accessed from commercial enone **9** through Michael addition[7] and alkynylation[8] in two steps. To our delight, the key Rh(I)-catalyzed [3+2+1] cycloaddition of **8a** and CO occurred smoothly with 10 mol% rhodium dimer catalyst under 0.2 atm CO atmosphere at 80 °C. The reaction afforded **7a** in 49% combined yield with a diastereomeric ratio of about 2.1:1 (Table 1, entry 1). Single-crystal X-ray diffraction analysis confirmed the desired stereochemistry of the major product (see Supporting Information). A brief screening of solvents indicated that toluene gave the best diastereomeric ratio (Table 1, entries 1, 3, and 4). Subsequent screenings revealed that higher CO pressure facilitated the formation of the desired diastereomer (Table 1, entry 5), while changing temperature did not improve the reaction (Table 1, entries 6 and 7). Upon

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Biosynthetic Intermediate Probes for Visualizing and Identifying the Biosynthetic Enzymes of Plant Metabolites

Lei Gao*[a] and Xiaoguang Lei*[a]

Plant metabolites play important roles in both plant physiology and drug discovery. Taking advantage of new emerging technologies such as next generation sequencing (NGS), whole genome assembly, bioinformatics, omics-based strategies have been demonstrated as popular and powerful ways to elucidate complex metabolic pathways in plants. In this viewpoint, biosynthetic intermediates probes have been proposed as the potentinal tools to study the plant natural product biosynthesis via chemical proteomics appoaches or transcriptome analysis.

Plant metabolites are known to participate in plant physiological activities such as regulating the growth and development of plants through plant hormones and response to microbial infection by phytoalexin.[1,2] On the other hands, plant natural products are an important source for drug discovery.[3,4] Thus, elucidating plant-specialized biosynthetic pathways will not only make a significant contribution to better understanding the endogenous roles of plant metabolites but also pave the way to manufacturing bioactive natural products by synthetic biology or biocatalysis.

Transcriptome analysis is one of the most practical and powerful strategies for elucidating the biosynthetic pathway of plant natural products,[5] especially in non-model plants.[6,7] However, when there are no available "bait genes" for co-expression analysis in the given pathway, candidate gene selection relies heavily on the assumption that the expression pattern of the candidate gene should be similar to the distribution pattern of the metabolite it produces. However, the distribution pattern of the metabolite revealed by metabolome analysis cannot always represent the true expression pattern of the candidate gene due to the diffusion and transport of the metabolite, thereby misleading transcriptome analysis to give unreliable candidate genes. On the other hand, when there are no reported enzymes sharing similar function with the gene of interest, candidate genes cannot be effectively narrowed down to a specified family of enzymes and the follow-up biochemical characterization will become risky and labor-intensive. Herein, we discuss how biosynthetic intermediate probes (BIPs) can be used to visualize and identify the biosynthetic enzymes of plant metabolites and offer us a complementary and alternative method to accelerate the process of identifying new biosynthetic enzymes of plant metabolites.

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Biosynthetic Intermediate Probes (BIPs) for Imaging the Enzymatic Activity

Using an exogenous biosynthetic intermediate as a probe that has similar activity but contains an extra small reporting group such as $^{2}D/^{13}C$ atoms or a methyl group, the spatiotemporal distribution of enzymatic activity in different tissues can be imaged accurately by mass spectrometric imaging (MSI)[8,9] of the enzymatic product at the tissue or single-cell level (Figure 1B).[10,11] Compared with directly visualizing the endogenous metabolites, this strategy can avoid the misleading distribution information that results from transport and diffusion of the metabolites.[12] On the other hand, we envision that the spatiotemporal distribution of the enzymatic activity in the biosynthetic pathway of plant metabolites can also be visualized using biosynthetic intermediate-based smart fluorescent probes, as smart fluorescent probes that emit fluorescence upon enzymatic transformation have been successfully used to detect and visualize enzymes[13,14] in disease such as β-galactosidase,[15] histone deacetylase,[16] caspase.[17] For example, monooxygenases catalyzing hydroxylation in a given biosynthetic pathway can be visualized by BIPs that release their fluorescent group after enzymatic transformation in self-immolative strategy[18] (Figure 1A).

Biosynthetic Intermediate Probes (BIPs) for Target Identification

Affinity-based probes are widely used in chemical proteomics approaches for target identification of bioactive molecules.[19] Recently, we successfully identified an intermolecular Diels-Alderase[20] in *Morus alba* by using a BIP with a photoaffinity

ACCOUNTS of chemical research

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New Strategies in the Efficient Total Syntheses of Polycyclic Natural Products

Published as part of the Accounts of Chemical Research special issue "Total Synthesis of Natural Products".

Weilong Liu,[§] Benke Hong,[§] Jin Wang,[§] and Xiaoguang Lei*

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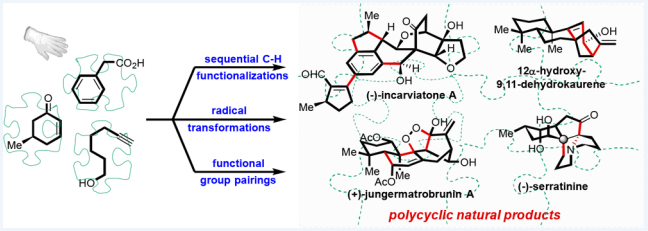
CONSPECTUS: Polycyclic natural products are an inexhaustible source of medicinal agents, and their complex molecular architecture renders challenging synthetic targets where innovative and effective approaches for their rapid construction are urgently required. The total synthesis of polycyclic natural products has witnessed exponential progression along with the emergence of new synthetic strategies and concepts, such as sequential C−H functionalizations, radical-based transformations, and functional group pairing strategies. Our group exerts continued interest in the construction of bioactive and structurally complex natural products as well as evaluation of the mode of action of these molecules. In this Account, we will showcase how these new synthetic strategies are employed and guide our total synthesis endeavors.



During the last two decades, a series of remarkable advances in C−H functionalization have led to the emergence of many new approaches to directly functionalize C−H bonds into useful functional groups. These selective transformations have provided a great opportunity for the step- and atom-economical construction of key fragments in complex molecule synthesis. We recently furnished the total syntheses for polycyclic natural products: incarviatone A, chrysomycin A, polycarcin V, and gilvocarcin V by employing a multiple C−H bond functionalization strategy. The polysubstituted benzene or naphthalene skeleton was constructed through sequential and site-selective C−H functionalizations from readily available simple starting materials, which reduced the number of steps and streamlined synthesis.

Recently, we have also completed the total syntheses for a number of skeletally diverse tetracyclic *Isodon* diterpenoids inspired by their biogenesis and radical-based retrosynthetic disconnections. Radical transformations are strategically and tactically utilized in our syntheses, and radical-based reactions, including organo-SOMO catalysis, Birch reduction, regioselective 1,6-dienyne reductive cyclization, visible-light-mediated Schenck ene reaction, and photoradical-mediated late-stage skeletal rearrangement, play significant roles in our synthetic endeavors. Protecting-group-free and scalable syntheses are also built into our work to achieve the "ideal" synthesis. Furthermore, our synthetic work reveals that late-stage skeletal rearrangement through a photo radical process is possible in a biological setting in complement with nature's carbocation chemistry in complex natural product biosynthesis.

Lycopodium alkaloids are a large family of structurally unique polycyclic natural products with impressive biological activities. Owing to their fascinating polycyclic architectures and diverse biological activities, these alkaloids have continued to serve as targets as well as inspirations for the synthetic community for decades. To access these bioactive natural products or natural product-like molecules for biological exploration and drug discovery, we applied a novel functional group pairing strategy to furnish the total syntheses for several *Lycopodium* alkaloids and obtained numerous skeletally diverse compounds with structural complexity comparable to natural products.

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analytical chemistry

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Colocalized Particle Counting Platform for Zeptomole Level Multiplexed Quantification

Guangyu Tao, Tiancheng Lai, Xiao Xu, Yurou Ma, Xi Wu, Xiaojing Pei, Feng Liu, and Na Li*

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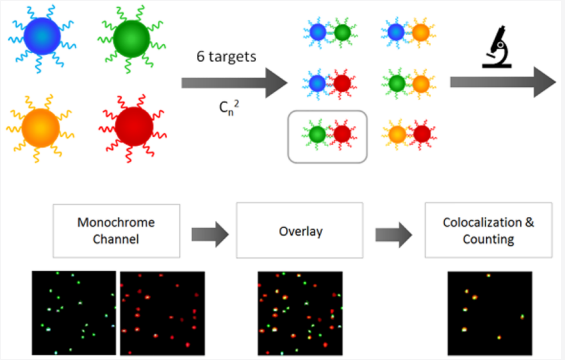
ABSTRACT: For multiplexed detection, it is important yet challenging to simultaneously meet the requirement of sensitivity, throughput, and implementation convenience for practical applications. Using the detection of DNAs and miRNAs for illustration, we present a colocalized particle counting platform that can realize the separation-free multiplexed detection of 6 nucleic acid targets with a zeptomole sensitivity and a dynamic range of up to 5 orders of magnitude. The presence of target induces the formation of a sandwich nanostructure via hybridization; thus, there is an occurrence of colocalization of two microbeads with two different colors. The sequence specific coding is realized by an arbitrary combination of two fluorescence channels with different emitting colors. The platform presents robustness in detecting multiple nucleic acid targets with a minimal cross talk and matrix effect as well as the ability to distinguish the specific miRNA from members of the same family. The results of simultaneous detection of 3 miRNAs in 3 different cell lines present straight consistency with that of the standard qRT-PCR. This platform can be adapted to other multiplexing designs such as the "turn-off" mode, in which the proportion of colocalized microbeads is decreased due to the strand-displacement reaction initiated by the specific target. This separation-free platform offers the possibility to achieve the on-site multiplexed detection with compatibility to different experimental designs and extensibility to other signal sources for enumeration.



The ability to detect multiple biomolecules in one single sample is of great importance in diagnostics and prognosis of human diseases, which allows the acquisition of high-density information with minimal assay time, sample volume, and cost.[1−4] In multiplexed detection, the signal readout is a central element that imparts the influence on the method sensitivity, the fairness of signal response to each analyte, the multiplexing capacity, and the instrumentation cost.[5−7] Among many available signal readouts, the optical measurements, such as fluorescence spectroscopy[8−13] and surface enhanced Raman scattering,[14−17] can easily realize the multiplexed detection using combinatorial coding by taking advantage of tunable wavelength, intensity, and fluorescence lifetime.[2,3,18−30] However, the need for accurate adjustment of the intensity makes it difficult to realize the intensity-involved coding strategy; the quantitative accuracy for each analyte in the sample, particularly low-abundant components, may be jeopardized, because emission efficiency of fluorescent or Raman probes may vary from probe to probe.[31,32] Therefore, for multiplexed detection, it is necessary to explore more signal readout modes and easy-to-implement encoding strategy with an ultrahigh and unbiased sensitivity toward each target of interest.

Colocalization is a frequently adopted technique for evaluating the association of two fluorophores by quantifying spatial overlaps from microscopic images of two channels.[33−36] So far, the quantification of biomolecules via colocalization has been limited to singleplexed detection.[37−39] The advantage of encoding via multicolor colocalization is that, with the combination of colocalization channels, n spectrum-separated fluorophores can be utilized to simultaneously detect $n(n-1)/2$ targets, which significantly increases the throughput compared with the spectrum-only encoding approach. As a proof of concept, multicolor colocalization based on quantum dot microscopic imaging has been proposed to study the potential for multiplexed detection using 100 pM triplex DNA targets,[40] yet no quantification and applications were further demonstrated. A universal platform, which makes the best use of the colocalization strategy, should greatly enhance the encoding capacity and sensitivity for multiplexed quantification.

Among many single particle techniques based on optical microscopic imaging,[41−46] single particle counting has been widely applied to biosensing purposes[47−55] as a simple and cost-effective approach. When serving as a sensing platform, single particle counting is capable of achieving high

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PAPER

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Competitive aptasensor for the ultrasensitive multiplexed detection of cancer biomarkers by fluorescent nanoparticle counting†

Xiaojing Pei,[a,b] Xi Wu,[a] Jie Xiong,[a] Guohong Wang,[c] Guangyu Tao,[a] Yurou Ma[a] and Na Li *[a]

Cancer biomarker quantification in human serum is of great importance for accurate patient diagnosis and informed clinical management. To date, ultrasensitive multiplexed detection of proteins without amplification is still a major challenge. Herein, we proposed a competitive aptasensor strategy for ultrasensitive multiplexed cancer biomarker detection by fluorescent nanoparticle (FNP) counting. The sequences are designed such that the binding abilities of linker DNA (L-DNA) with DNA-functionalized FNPs (DNA-FNPs) and aptamer are comparable. As long as one target binds with one molecule of aptamer, a signalling FNP forms a sandwich-structured nanocomposite, which was subsequently observed and enumerated with a fluorescence microscope. This 1 : 1 target-to-signal FNP production assured an improved sensitivity, benefiting from the reasonably good brightness and photostability of FNPs. For both singleplexed and multiplexed detection, this proposed strategy achieved an approximately 1000-fold improved limit of detection than the conventional method with the detection volume of 3.2 μL. Notably, the results for carcinoembryonic antigen (CEA) detection obtained directly from 9 human serum samples (colorectal/lung/healthy individuals) were consistent with that obtained by ELISA, showing potential application in clinical diagnosis.

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Introduction

Cancer is currently one of the most deadly diseases worldwide, and the quantitative analysis of cancer-associated biomarkers is vital for monitoring cancer progression and optimizing therapeutic interventions.[1,2] The sensitive and multiplexed detection of biomarkers in a single test during the onset of cancer can not only greatly reduce the analysis time and sample volume, but also help with more appropriate diagnostic decision making on monitoring the occurrence and development of cancer.[2,3] Based on antibody–antigen interactions, immunoassays have been the major approach to meet the growing demand for biomarker detection. However, the cost of antibody production and storage is high due to long immune response processes and undesirable stability, and the batch-to-batch reproducibility is unfavourable. The nucleic acid aptamers that recognize a variety of targets with high specificity and strong affinity by folding into distinct secondary or tertiary structures have been discovered and designed with the systematic evolution of ligands by exponential enrichment (SELEX).[4–6] Compared to the antibody recognition elements, aptamers can circumvent the aforementioned problems; in particular, aptamers can be rationally engineered without interfering with the binding abilities. Thus the cross-reaction can be effectively avoided, which is valuable for multiplexed assays.[5,7] To date, various aptameric biosensors based on the characterized signal readout mode such as plasmonic aptasensor,[8–10] fluorescent aptasensor,[11–18] electrochemical aptasensor,[19–21] and advanced microscopy[22–25] have been established. However, insufficient sensitivity is the most commonly cited weakness of the technology. Target or signal amplification strategies mostly suffered from the time-consuming procedures, expensive setup, large sample volumes, and limited multiplexing or quantification capabilities.[26–28] As a result, it remains a major challenge to develop a simple, ultrasensitive multiplexed aptasensor without amplification for the cancer biomarkers in human serum.

The automatic fluorescent nanoparticle (FNP) counting platform developed in our laboratory is an excellent approach

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Nanomaterial-based multiplex optical sensors

Xiaojing Pei,[a] Guangyu Tao,[b] Xi Wu,[b] Yurou Ma,[b] Rongsheng Li [b] and Na Li *[b]

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The drive for a simultaneous analysis of multiple targets with excellent accuracy and efficiency, which is often required in both basic biomedical research and clinical applications, demands the development of multiplexed bioassays with desired throughput. With the development of nanotechnologies, innovative multiplex optical bioassays have been achieved. Nanomaterials exhibit unique physical and chemical properties such as easily tunable size, large surface-to-volume ratio, excellent catalysis and the desired signal transduction mechanism, which makes them excellent candidates for the fabrication of novel optical nanoprobes. This mini review summarizes nanomaterial-based optical multiplex sensors from the last 5 years. Specific optical techniques covered in this review are fluorescence, surface-enhanced Raman scattering (SERS), localized surface plasmon resonance (LSPR), chemiluminescence (CL), and the multimodality with fundamentals and examples.

Introduction

Multiplexed analysis has attracted considerable interest in the biological and biomedical fields due to its high throughput and detection accuracy.[1–3] Compared to singleplexed assays, multiplexed detection allows the acquisition of high-density information with minimal assay time, sample volume and cost, thus being particularly favourable for a clinical diagnosis of fatal human diseases which generally involves a multitude of parameters for decision-making purposes.[4] Almost all types of signals, including optical, electrical, thermal, magnetic and mass, have been used for multiplexed detection. Through the light–matter interaction, optical signals such as spectral wavelength and intensity, and lifetime due to absorption, emission and scattering, can be harnessed for multiplexed encoding.

With the development of nanotechnology, innovative optical bioassays with high sensitivity and multiplexing capacity have been achieved (Fig. 1). Nanomaterials exhibit unique physical and chemical properties, which makes them excellent candidates for the fabrication of novel optical nanoprobes.[5–7] The great potential of such optical labels has paved the way for developing new biomolecule assays with unprecedented analytical performances, including sensitivity, cost-effectiveness and ease of use. Myriad multiplexed bioassay

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Guangyu Tao

Guangyu Tao is a Ph.D. candidate at the College of Chemistry and Molecular Engineering, at Peking University. He graduated with a bachelor's degree in 2016 from Peking University. His current research interest focuses on fluorescent nanoparticle based biosensing and imaging for multiplex nucleic acid detection.

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Synthesis of 17-Deacetoxyl Chromodorolide B Based on a Gold-Catalyzed Alkoxycyclization Reaction

Chen Li,[§] Tianfei Quan,[§] Yibin Xue, Yuhui Cao, Si-Cong Chen, and Tuoping Luo*

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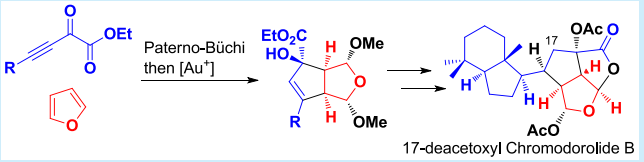
ABSTRACT: A novel strategy to construct the highly oxidized 3-oxabicyclo[3.3.0]octane skeleton was developed via a gold-catalyzed cascade cyclization with 2,7-dioxabicyclo[3.2.0]hept-3-ene as the substrate. We utilized this methodology as the key reaction to synthesize 17-deacetoxyl chromodorolide B.



Rearranged spongiane diterpenoids are small molecules isolated exclusively from sponges and marine shell-less mollusks (nudibranchs), which could play a key role as ecophysiological mediators and are of interest for potential applications given their wide range of bioactivities.[1] In particular, the Golgi-modifying properties of the rearranged spongian diterpenes norrisolide and macfarlandin E have been well established,[2] which inspired the development of a simplified analog (*t*-Bu-MacE) that led to similar phenotypes.[3] In comparison, (−)-chromodorolide B (Figure 1, **1**), one of

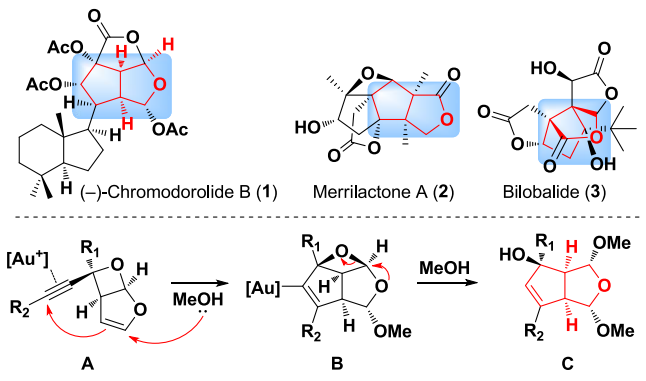


Figure 1. Representative natural products containing a 3-oxabicyclo[3.3.0]octane moiety and the hypothesized gold-catalyzed alkoxycyclization.

the most complex members within this family of natural products, was isolated from the tropical dorid nudibranch *Chromodoris cavae*, but the limited quantity of this natural product prevented in-depth biological investigation.[4] The only total synthesis of chromodorolide B to date was reported by the Overman group, which featured a bimolecular radical addition/cyclization/fragmentation cascade reaction to construct the key stereocenters.[5] A challenging structural feature of chomodorolide B is the presence of a 3-oxabicyclo[3.3.0]-octane skeletal motif, which is shared by a number of other natural products, such as merrilactone A (**2**)[6] and bilobalide (**3**).[7] To efficiently construct this unique subunit, we envisaged a gold-catalyzed alkoxycyclization: The activation of alkyne in substrate **A** by Au(I) catalysis would enable an intramolecular alkene attack (5-*endo-dig* cyclization),[8] followed by the nucleophilic trapping of the resulting oxocarbenium to obtain a highly strained tricycle, intermediate **B**;[9] the subsequent opening of the strained oxetane ring would ensue in situ to form the 3-oxabicyclo[3.3.0]octane skeleton, **C**. The bicyclic substrate (**A**) could, in turn, be easily prepared by alkynone and furan or furan derivatives via the Paternò−Büchi reaction.[10]

We commenced our work by evaluating the cascade reaction of oxetane **4a** (Figure 2, R^1 = −COOEt, R^2 = −^{i}Pr, R^3 = −H), the relative stereochemistry of which was determined by the X-ray of its derivative (Figure S1; see the Supporting Information (SI) for details). In the presence of 2 equiv of MeOH, the commonly used gold catalysis system (5 mol % Ph_3PAuCl/$AgSbF_6$) successfully converted **4a** to the desired product (**5a**) in dichloromethane (DCM) at room temperature; the structure of **5a** was confirmed by the X-ray diffraction (XRD) of its derivative (Figure S2). Even though the isolation yield was moderate (53%), the reaction was completed within 10 min, and elongation of the reaction time did not lead to an improved yield.

The substrate scope was preliminarily explored, indicating that desired products could be obtained in moderate yield (40−50%) for substrates with R^2 = −^{t}Bu, −Ph, or −Br (**5b**−**d**). In comparison, substrate **4e** with R^2 = −H afforded product **5e** in 34% yield, suggesting that other side reactions might be invoked in the presence of the terminal alkyne. Increasing the substitution at R^3 (−Me, **4f**) also led to deteriorated results

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Total Synthesis of (−)-Batrachotoxinin A: A Local-Desymmetrization Approach

Yinliang Guo, Zhixian Guo, Jia-Tian Lu, Runting Fang, Si-Cong Chen, and Tuoping Luo*

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ABSTRACT: An enantioselective total synthesis of (−)-batrachotoxinin A is accomplished based on a key photoredox coupling reaction and the subsequent local-desymmetrization operation. After the expedient assembly of the highly oxidized steroid skeleton, a delicate sequence of redox manipulations was carried out to deliver a late-stage intermediate on gram scale—and ultimately (−)-batrachotoxinin A in an efficient manner.

(−)-Batrachotoxin (Figure 1, **1**), a steroidal alkaloid with a high oxidation level, is one of the most toxic materials known to mankind.[1] First identified from the skin of *Phyllobates* poison-dart frogs in South America,[2] **1** has been widely used as an important tool for studying ion transport because it specifically stabilizes voltage-gated sodium (Na_V) channels in an active, open form.[3] Isolated together with **1**, (−)-batrachotoxinin A (**2**) is significantly less potent,[1] but it could be readily converted to **1** or other batrachotoxin analogs that serve different purposes for investigating Na_V's.[2,4] The complex structure and the uniqueness of **1** as a Na_V's agonist have made this family of natural products attractive synthetic targets for organic synthesis. As early as 1972, Imhof et al. reported a semi-synthesis of **2** starting from 11α-acetoxyprogesterone.[5] The first total synthesis of **2** was achieved by Kishi's group in a racemic manner,[6] whereas Du Bois and co-workers accomplished a 24-step synthesis of (−)-batrachotoxin (**1**) by employing an elegant radical cascade reaction.[7] In addition, Du Bois's group has revealed that analogs of (−)-batrachotoxin (**1**), including structural truncations, would be useful tools to interrogate the dynamic nature of Na_V's.[7,8] Other creative synthetic efforts have also been oriented toward this steroid skeleton, but further total synthesis studies were thwarted by the complicated ring system, contiguous stereogenic centers, and the challenging oxidation level.[9]

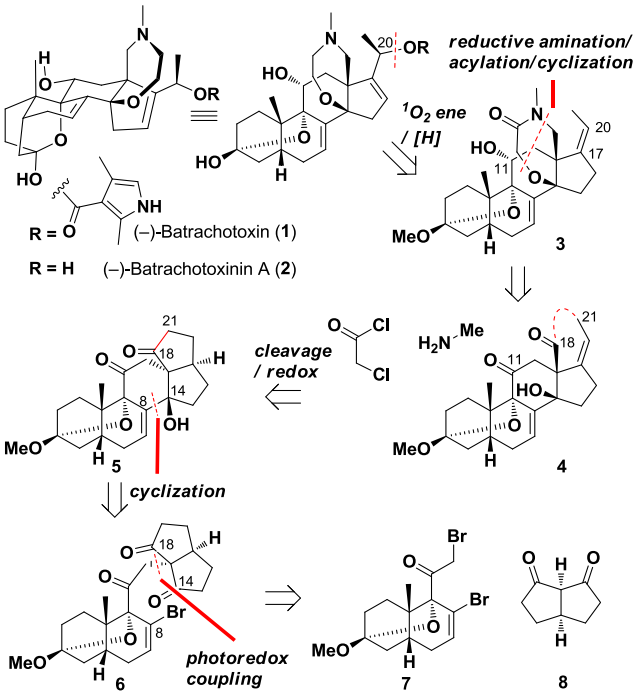

Figure 1. (−)-Batrachotoxin (**1**), (−)-batrachotoxinin A (**2**), and their retrosynthetic analyses.

The supply problem of batrachotoxin (**1**) due to the restricted natural sources,[10] together with our continued interests in highly oxidized steroids,[11] motivated us to develop a new and efficient route to (−)-**2**. We envisaged that the C20 hydroxyl group of **2** (batrachotoxin numbering, throughout) could be introduced using the singlet oxygen ene reaction of C17−C20 alkene in **3**. The seven-membered lactam ring would be traced back to aldehyde **4** by the well-established reductive amination/acylation/cyclization sequence,[5,7] while the C11 hydroxyl group might be constructed concomitantly during the reduction process. By connecting C18 and C21, we recognized a hidden symmetry of **4** that allowed us to propose a key synthetic intermediate, **5** (a shorter non-desymmetrization strategy was also attempted without success and is detailed in the Supporting Information (SI); see Scheme S1), which could be obtained from **6** by engaging the bromide to internally differentiate the two carbonyl groups (C14 and C18). This local-desymmetrization strategy significantly simplified the target structure,[12] and the precursor **6** would result from the coupling of bromide **7** and a known diketone, **8**.[13] Unlike previous tactics that generally involved a step-by-

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ARTICLE OPEN

Elimination of senescent cells by β-galactosidase-targeted prodrug attenuates inflammation and restores physical function in aged mice

Yusheng Cai[1], Huanhuan Zhou[1,2], Yinhua Zhu[3,4], Qi Sun[1], Yin Ji[1], Anqi Xue[1], Yuting Wang[1], Wenhan Chen[1], Xiaojie Yu[1], Longteng Wang[5], Han Chen[4], Cheng Li[6], Tuoping Luo[3,4] and Hongkui Deng[1,2]

Cellular senescence, a persistent state of cell cycle arrest, accumulates in aged organisms, contributes to tissue dysfunction, and drives age-related phenotypes. The clearance of senescent cells is expected to decrease chronic, low-grade inflammation and improve tissue repair capacity, thus attenuating the decline of physical function in aged organisms. However, selective and effective clearance of senescent cells of different cell types has proven challenging. Herein, we developed a prodrug strategy to design a new compound based on the increased activity of lysosomal β-galactosidase (β-gal), a primary characteristic of senescent cells. Our prodrug SSK1 is specifically activated by β-gal and eliminates mouse and human senescent cells independently of senescence inducers and cell types. In aged mice, our compound effectively cleared senescent cells in different tissues, decreased the senescence- and age-associated gene signatures, attenuated low-grade local and systemic inflammation, and restored physical function. Our results demonstrate that lysosomal β-gal can be effectively leveraged to selectively eliminate senescent cells, providing a novel strategy to develop anti-aging interventions.

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INTRODUCTION

Aging is the predominant risk for physiological degeneration, increased chronic morbidities, and age-specific mortality.[1,2] One major hallmark of aging is the chronic accumulation of cellular senescence, a permanent state of cell-cycle arrest in response to various damaging stimuli.[3,4] Cellular senescence impairs the ability of tissue regeneration and drives chronic low-grade inflammation, which exacerbates the aging process.[5,6] Importantly, transplantation of senescent cells into young mice was sufficient to drive age-related pathology and cause persistent physical dysfunction.[7] In contrast, deletion of senescent cells by a genetic approach attenuated age-related deterioration and extended the health-span in aged mice.[8,9] These studies demonstrated that senescence is one of the major drivers of aging and that clearing senescent cells is a promising approach to treat age-related diseases and improve physical function.[6,10]

Previous studies have shown that compounds termed 'senolytics' could kill senescent cells.[11–13] Reported senolytics target anti-apoptotic pathways, which are up-regulated to inhibit apoptosis in senescent cells.[11,14] These senolytics have been reported to eliminate certain types of senescent cells and have shown the potential to improve physiological function in several tissues.[7,12,15] However, senolytic drugs have significant limitations in killing senescent cells in terms of specificity and broad-spectrum activity because of the dynamic and highly heterogeneous nature of the senescence program, which leads to the varying sensitivity of different types of senescent cells to current senolytic drugs.[6,16,17] To overcome these challenges, it is highly demanded to develop a new strategy that permits selectively deleting senescent cells in a wide spectrum of cell types or tissues for anti-aging interventions.

To specifically target senescent cells, we focused on one primary characteristic of senescent cells — the increased activity of lysosomal β-galactosidase, exploited as senescence-associated β-galactosidase (SA-β-gal).[18,19] Notably, SA-β-gal in diverse types of senescent cells is one widely used marker for identifying senescence in vitro and in vivo, which is linked to the increased content of lysosomes.[20–23] Therefore, we hypothesized that lysosomal β-gal could be utilized for the design of a galactose-modified prodrug to target senescent cells in a broader spectrum. This prodrug could be processed into a cytotoxic compound by β-

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ARTICLE

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Structural insights into the inhibition mechanism of human sterol *O*-acyltransferase 1 by a competitive inhibitor

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Sterol *O*-acyltransferase 1 (SOAT1) is an endoplasmic reticulum (ER) resident, multi-transmembrane enzyme that belongs to the membrane-bound *O*-acyltransferase (MBOAT) family. It catalyzes the esterification of cholesterol to generate cholesteryl esters for cholesterol storage. SOAT1 is a target to treat several human diseases. However, its structure and mechanism remain elusive since its discovery. Here, we report the structure of human SOAT1 (hSOAT1) determined by cryo-EM. hSOAT1 is a tetramer consisted of a dimer of dimer. The structure of hSOAT1 dimer at 3.5 Å resolution reveals that a small molecule inhibitor CI-976 binds inside the catalytic chamber and blocks the accessibility of the active site residues H460, N421 and W420. Our results pave the way for future mechanistic study and rational drug design targeting hSOAT1 and other mammalian MBOAT family members.

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Rapid Construction of Fold-Line-Shaped BN-Embedded Polycyclic Aromatic Compounds through Diels−Alder Reaction

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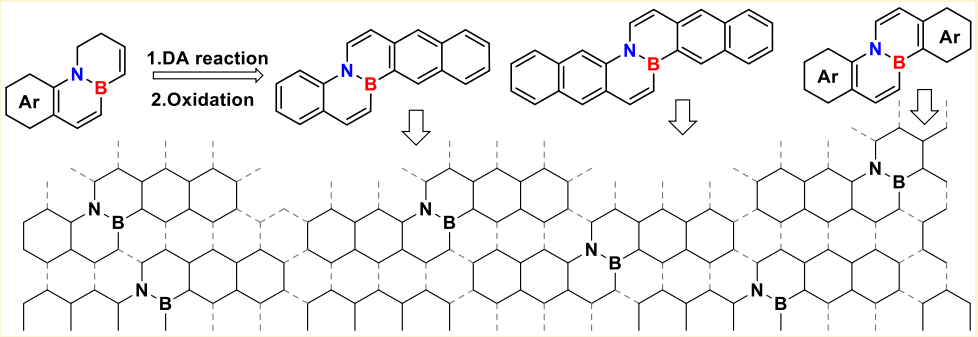
Supporting Information



ABSTRACT: The Diels−Alder reaction strategy that can rapidly extend the conjugated backbone was applied to facilely synthesize fold-line, coplanar BN-embedded polycyclic aromatic hydrocarbons from simple small BN compounds. The molecular structures and packing modes of these BN-embedded acenes were confirmed by single-crystal X-ray diffraction. Their electronic and photophysical properties were studied by using UV−vis, fluorescence spectroscopy, electrochemical cyclic voltammetry, and density functional theory calculations. These results demonstrate the efficiency and feasibility of this synthetic strategy.

INTRODUCTION

Graphene, due to its unique structure and properties, has become a hot topic in the research of next-generation nanoelectronics. However, the zero-bandgap nature of graphene greatly hinders its application in semiconducting electronic nanodevices.[1] Therefore, it is of great significance to develop techniques that are capable of controlling the structure of graphene and adjusting its band gap. Chemical doping of graphene with heteroatoms is a promising method.[2] In recent years, BN-doped graphene has received extensive attention due to its distinct electronic properties and potential applications as semiconductors.[3] In particular, BN-doped graphenes obtained from bottom-up synthetic methods have the advantage of precise structure and good dispersity of BN units in the graphene framework, which might regulate the charge transport properties with desired bandgaps.[4] In this context, the synthesis of BN-embedded polycyclic aromatic hydrocarbons (PAHs), which could be potentially assembled to large BN-doped graphene sheets, has been widely studied in the hope of unveiling the properties and potentials of well-defined, rigorously controlled BN-doped graphene materials.

In the past decades, research on the synthetic methods of azaborine compounds has received extensive attention, especially for large BN-embedded PAHs.[5] However, construction of BN-embedded aromatic hydrocarbons pioneered by Piers et al. is still challenging due to tedious stepwise synthetic routes or harsh reaction conditions, leading to low yields.[6] To resolve the issues, a method of tandem electrophilic boration was developed by Nakamura et al. to efficiently synthesize BN-embedded aromatic compounds. But the molar ratios of the catalysts should be carefully screened for different reaction substrates; otherwise, the yields would suffer severely.[7] Most recently, Cui et al. adopted a simple strategy to successfully synthesize a series of new BN-tetraphenes by cyclization of alkynes with BN-naphthalene derivatives via metal catalysis.[8] However, the rotatable phenyl groups in the products destroyed the planarity of the whole molecules, which may negatively impact their packing when deposited on substrate surface.[9] Therefore, a simple and efficient synthetic method for rapidly constructing large BN-embedded PAHs needs to be developed.

It is well-known that the Diels−Alder (DA) reaction is one of the most direct methods to rapidly generate six-membered rings, which can be used to synthesize various densely functionalized cyclic molecules by varying substrates.[10] The first example of Diels−Alder reactions of BN-fused aromatic

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Communication

Conformation-Dependent Spin Relaxation Behaviors of 6-Oxoverdazyl Radical Single Crystals

Zi-Yuan Wang, Ya-Zhong Dai, Ze-Fan Yao, Bo-Wei Dong, Yang Lu, Li Ding, Shang-Da Jiang,* Jie-Yu Wang,* and Jian Pei*

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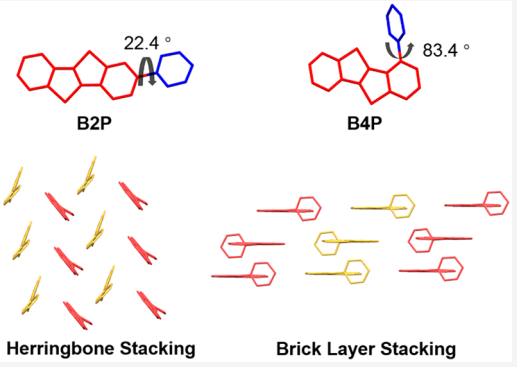
ABSTRACT: The molecular conformation and packing mode of organic radicals are vital to achieving distinctive electronic, spin relaxation, and magnetic properties. Herein two organic radicals, B2P and B4P, are developed by attaching a 6-oxoverdazyl radical at different peripheral positions of [1]benzothieno[3,2-*b*][1]benzothiophene (BTBT). B2P single crystals exhibit a typical herringbone packing mode, whereas B4P single crystals display a classic brick-layer stacking structure. Moreover, two crystalline phases, B4P-Y and B4P-R, are observed in B4P crystals owing to the rotational isomerization. Because of the distinct molecular conformation and packing structures, the spin relaxation behaviors of the two 6-oxoverdazyl radicals are significantly different, and B4P-Y with a distorted conformation exhibits a longer spin relaxation time.



Organic conjugated radicals have exhibited giant potential in organic electronics,[1−3] pure organic magnets,[4−7] and quantum information processing[8−10] due to their unique electronic and magnetic characteristics. In comparison with inorganic radicals, organic radicals have weaker spin−orbit coupling and hyperfine interactions, which are desirable for long-distance spin transport and electron spin manipulation. It is well known that the molecular conformation and packing modes of organic conjugated radicals in solid states play a decisive role in their electronic, spin relaxation, and magnetic properties. Very recently, a main-group element radical[6] displayed a 1D zigzag chain conformation and achieved antiferromagnetic interactions through self-assembly. A porous organic radical framework[11,12] exhibited ferromagnetic coupling because the tight $\pi-\pi$ packing in the monomer was destroyed. However, to date, distinct organic radical properties (electronic, spin relaxation, and magnetic) can be achieved only via designing totally different molecular structures, yet the molecular packing structures have hardly been investigated in detail. Hence, it is desirable to systematically explore the relationships between molecular crystal engineering and radical characteristics and realize the precise control of molecular stacking in these radicals.[13−15]

The 6-oxoverdazyl radical[16] shows high stability toward oxygen and moisture due to the delocalization of the spin density on the six-membered heterocycle.[17,18] The planar skeleton of the 6-oxoverdazyl radical, which has a small steric hindrance for stacking, makes it a good candidate to study the radical−radical interactions in solid states. Simultaneously, to tune the packing structure of the radicals, large conjugated skeletons with multiple intermolecular interactions could be connected to the radicals. [1]Benzothieno[3,2-*b*][1]-benzothiophene (BTBT) shows extremely good charge transport properties due to the strong intermolecular interactions including $\pi-\pi$ interactions and S···S interactions. Herein we introduce 6-oxoverdazyl radicals into the BTBT motif, along the long axis and minor axis of BTBT, respectively, affording two organic conjugated radicals, B2P and B4P (Figure 1). The large conjugated skeleton of BTBT as well as its different steric hindrances in B2P and B4P are expected to change the molecular conformation and tune the packing structures of these two radicals in single crystals. As a result, B2P molecules show a herringbone packing mode in single crystals, whereas B4P displays a face-to-face brick-layer packing structure. Interestingly, two crystalline rotational isomers, B4P-Y and B4P-R, are observed in B4P, which exhibit distinct spin relaxation properties and could be transformed by controlling the temperature. Although it is common in closed-shell systems,[19−21] there are few examples of temperature-dependent molecular conformation transformation in organic radicals. More spin density distribution on the BTBT in B2P and B4P-R single crystals could accelerate the spin relaxation process. As a result, B4P with a more distorted conformation

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Communication

Chemoproteomic Profiling of Itaconation by Bioorthogonal Probes in Inflammatory Macrophages

Wei Qin,[‖] Yanling Zhang,[‖] Huan Tang, Dongyang Liu, Ying Chen, Yuan Liu, and Chu Wang*

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ABSTRACT: Itaconate is an anti-inflammatory metabolite involved in pathogen−macrophage interactions, but the mechanisms underlying its effect are not fully understood. Competitive cysteine profiling has been performed to interrogate itaconate's reactivity in cell lysates, but methods for analyzing targets of itaconation directly in living macrophages are still lacking. In this work, we developed a specific bioorthogonal probe, itaconate−alkyne (ITalk), for quantitative and site-specific chemoproteomic profiling of itaconation in inflammatory macrophages. ITalk recapitulates the anti-inflammatory property of itaconate and enables biochemical evaluation and proteomic analysis of its direct targets. Our study delineates the widespread landscape of itaconate substrates, providing a versatile tool and comprehensive resource for investigating its function.

Itaconate plays important roles as an anti-inflammatory and antibacterial metabolite involved in macrophage stimulation.[1,2] The mitochondria-associated enzyme immune responsive gene 1 (IRG1) is highly expressed in lipopolysaccharide (LPS)-stimulated macrophages, generating massive itaconate from *cis*-aconitate.[3,4] Itaconate contains an electrophilic α,β-unsaturated carboxylic acid group, which can modify the cysteine residues of proteins via Michael addition, resulting in so-called "itaconation". Biochemical studies have shown that it can covalently modify cysteines in kelch-like ECH-associated protein 1 (KEAP1) and glutathione (GSH), which independently and partially exerts its anti-inflammatory effect.[5,6] We recently applied an S-glycosylation-based competitive cysteine profiling strategy to identify 260 itaconate-modified cysteines and found that itaconate can inhibit glycolysis by modifying and inhibiting key glycolytic enzymes, including fructose bisphosphate aldolase A (ALDOA) and L-lactate dehydrogenase A (LDHA).[7] Although this chemoproteomic method provides a global portrait of itaconate reactivity, its readout is indirect, and the assay can only be performed in cell lysates. It is therefore highly desired to develop tools for direct analysis of itaconation in living cells in a site-specific manner.

We reasoned that a bioorthogonal probe retaining the α,β-unsaturated carboxylic acid group would have similar thiol reactivity as itaconate and that the modified proteins could be then captured (Figure 1A). A cell-permeable esterified derivative of itaconate,[5] 4-octyl itaconate (OI) (Figure 1A), was previously shown to release itaconate intracellularly and exhibit the anti-inflammatory effect. However, the long carbon chain of OI is resistant to esterase hydrolysis to some extent and remains on the target proteins after modification, which provides an ideal site for introducing the bioorthogonal handle. On the basis of this rationale, an alkyne analogue of OI, itaconate−alkyne (ITalk), was designed and synthesized (Figures 1A, S1, and S11−S13).

The cysteine reactivity of ITalk was monitored by its reaction with GSH (Figure 1B). As expected, ITalk exhibits

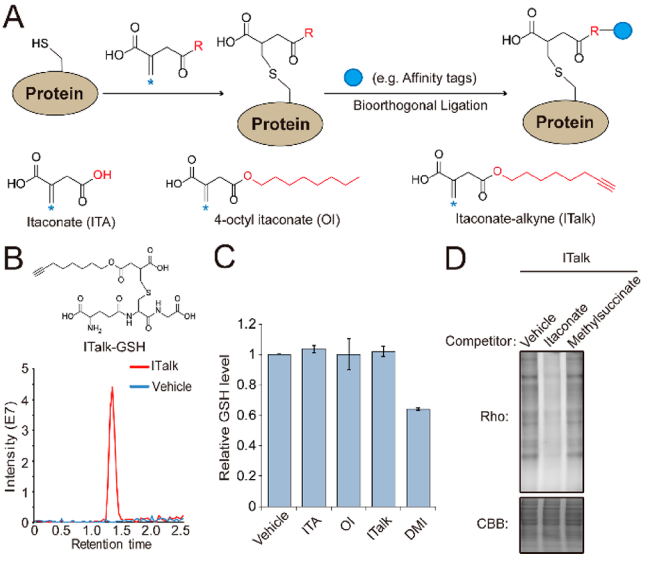

Figure 1. Design and evaluation of the itaconate−alkyne (ITalk) probe. (A) Design of ITalk to capture itaconate targets. The structures of itaconate (ITA), 4-octyl itaconate (OI), and ITalk are presented. (B) Confirmation of the adduct of ITalk with GSH by LC−MS. The extracted ion chromatographic trace of the ITalk−GSH adduct is shown. (C) Comparison of the cysteine reactivity of ITalk with those of ITA, OI, and dimethyl itaconate (DMI). (D) Competitive labeling of ITalk by itaconate. Raw264.7 cell lysates were treated with 1 mM itaconate and then labeled with 100 μM ITalk.

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Chemical Proteomic Profiling of Protein 4′-Phosphopantetheinylation in Mammalian Cells

*Nan Chen, Yuan Liu, Yuanpei Li, and Chu Wang**

Dedicated to Professor Youqi Tang on the occasion of his 100th birthday

Abstract: *Protein 4′-phosphopantetheinylation is an essential post-translational modification (PTM) in prokaryotes and eukaryotes. So far, only five protein substrates of this specific PTM have been discovered in mammalian cells. These proteins are known to perform important functions, including fatty acid biosynthesis and folate metabolism, as well as β-alanine activation. To explore existing and new substrates of 4′-phosphopantetheinylation in mammalian proteomes, we designed and synthesized a series of new pantetheine analogue probes, enabling effective metabolic labelling of 4′-phosphopantetheinylated proteins in HepG2 cells. In combination with a quantitative chemical proteomic platform, we enriched and identified all the currently known 4′-phosphopantetheinylated proteins with high confidence, and unambiguously determined their exact sites of modification. More encouragingly, we discovered, using targeted chemical proteomics, a potential 4′-phosphopantetheinylation site in the protein of mitochondrial dehydrogenase/reductase SDR family member 2 (DHRS2).*

Introduction

Co-enzyme A (CoA) participates in a large array of biochemical processes as a fundamental cofactor in essentially all living organisms.[1] Further to serving as an acyl carrier, CoA can also modulate protein structures and functions by post-translational modifications (PTMs), one of which is protein 4′-phosphopantetheinylation.[2] The modification was first discovered in 1965[3] and has been known to be mediated by a phosphopantetheinyl transferase (PPTase) with CoA as the substrate.[2] In prokaryotic species, protein 4′-phosphopantetheinylation has been extensively documented as an "acyl carrier prosthetic arm" for natural product chain elongation reactions in fatty acid synthase (FAS)-, polyketide synthase (PKS)-, and non-ribosomal peptide synthetase (NRPS)-mediated biosynthesis.[4] Mechanistically, all intermediates are tethered on the terminal thiol through a reactive thioester linkage and elongated by a series of enzymes to yield the final natural products with distinct structures and bioactivities.

In mammalian cells, only five protein substrates with 4′-phosphopantetheinylation have been reported (Supporting Information, Table S1). They play important functions, including fatty acid synthesis,[3b,5] folate metabolism,[6] and β-alanine activation.[7] Whether there are other novel 4′-phosphopantetheinylated substrates in mammalian proteomes remains unexplored.

Chemical biology tools have been developed to aid the functional study of protein 4′-phosphopantetheinylation. Burkart and colleagues pioneered in synthesizing unnatural pantetheine analogue probes to enable chemical labelling of acyl carrier proteins in bacteria.[8] They further demonstrated that the strategy could be exploited to capture transient protein–protein interactions with acyl carrier proteins for structural studies[9] and to discover novel carrier proteins from unsequenced prokaryotic organisms.[10] Unfortunately, when they tested the performance of these probes in human cells, they could only label one substrate protein, FAS, with a fluorescent analogue probe, likely due to the difference in pantetheine-importing mechanisms between eukaryotic and prokaryotic organisms.[10] Therefore, despite its functional essentiality in mammalian biology, there is still lack of efficient tools for comprehensive profiling of substrates of 4′-phosphopantetheinylation in mammalian cells to date.

Herein, we report the design and synthesis of a series of new pantetheine analogue probes that showed improved metabolic labelling in mammalian cells. By integrating these probes with quantitative chemical proteomics, we performed a global profiling of 4′-phosphopantetheinylated proteins in mammalian proteomes. Implementation of a targeted chemical proteomic strategy unambiguously identified the exact modification sites, which include all five known sites of 4′-phosphopantetheinylation and a potential site in a new substrate. Our study complements the toolbox of unnatural pantetheine probes and opens new opportunities for the functional study of protein 4′-phosphopantetheinylation in mammalian cells.

Results and Discussion

Establishment of a Quantitative Mass-Spectrometry (MS)-Based Platform for Chemoproteomic Profiling

In a previous study,[10] Burkart and colleagues tested the labelling of two pantetheine analogue probes in the human breast cancer SKBR3 cell line. They observed by in-gel

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FEATURE ARTICLE

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Profiling of post-translational modifications by chemical and computational proteomics

Fan Yang[a] and Chu Wang *[ab]

Post-translational modifications (PTMs) diversify the molecular structures of proteins and play essential roles in regulating their functions. Abnormal PTM status has been linked to a variety of developmental disorders and human diseases, highlighting the importance of studying PTMs in understanding physiological processes and discovering novel nodes and links with therapeutic intervention potential. Classical biochemical methods are suitable for studying PTMs on individual proteins; however, global profiling of PTMs in proteomes remains a challenging task. In this feature article, we start with a brief review of the traditional affinity-based strategies and shift the emphasis to summarizing recent progress in the development and application of chemical and computational proteomic strategies to delineate the global landscapes of functional PTMs. Finally, we discuss current challenges in PTM detection and provide future perspectives on how the field can be further advanced.

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Introduction

Protein post-translational modifications (PTMs) are covalent moieties introduced to the amino acid side chains or termini of proteins, either enzymatically or chemically, which can change the physicochemical properties of target proteins, and lead to structural changes, localizations, activities and binding partners.[1] Therefore, PTMs are fundamental tools to increase the chemical and biological diversities of the genome and play

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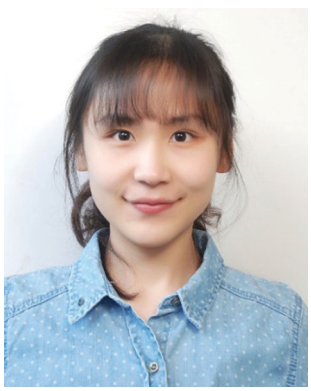

Fan Yang

Fan Yang was born in 1992 in Henan, China. She received her BS degree from Zhengzhou University in 2015. At present, she is pursuing her PhD at Peking University under the supervision of Prof. Chu Wang. Her current research interests focus on the development of quantitative chemical proteomics strategies for research in physiology, pharmacology and pathology.

Chu Wang

Chu Wang is currently a professor of chemical biology at Peking University, China. He obtained his PhD in 2007 from the University of Washington under the supervision of Professor David Baker. He then did his postdoc with Professor Benjamin Cravatt at The Scripps Research Institute. In 2014, he joined Peking University as a tenure-track assistant professor at the College of Chemistry and Molecular Engineering and is also a Principal Investigator affiliated with PKU-Tsinghua Center for Life Sciences. His research interest is in the development of chemical and computational proteomics methods to enable quantitative profiling of functional enzymes, post-translational modifications and protein–ligand interactions in proteomes.

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Article

Experimental and Computational Studies on Rh(I)-Catalyzed Reaction of Siloxyvinylcyclopropanes and Diazoesters

Sheng Feng, Kang Wang, Yifan Ping, and Jianbo Wang*

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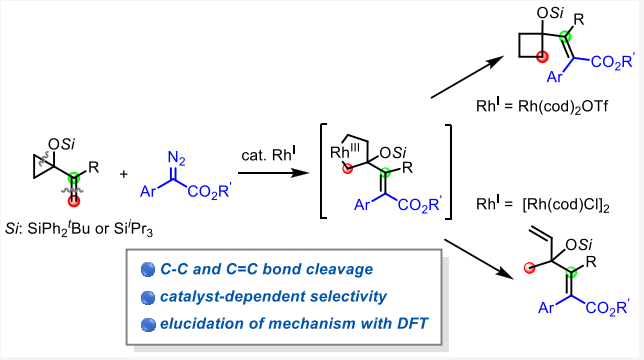
ABSTRACT: The Rh(I)-catalyzed reaction of siloxyvinylcyclopropanes and diazoesters leads to the formation of siloxyvinylcyclobutane and 1,4-diene derivatives. With $[Rh(cod)Cl]_2$ as the catalyst, the formation of 1,4-diene was favored over the formation of siloxyvinylcyclobutane. By changing the catalyst to $[Rh(cod)_2OTf]$, siloxyvinylcyclobutane derivatives are formed with excellent chemoselectivities and in moderate to good yields. The alkene products are also obtained as single *E* configured isomers. A detailed mechanism for this transformation is proposed on the basis of mechanistic experiments and DFT calculations. The effect of catalysts on the chemoselectivity of these reactions is also examined computationally.

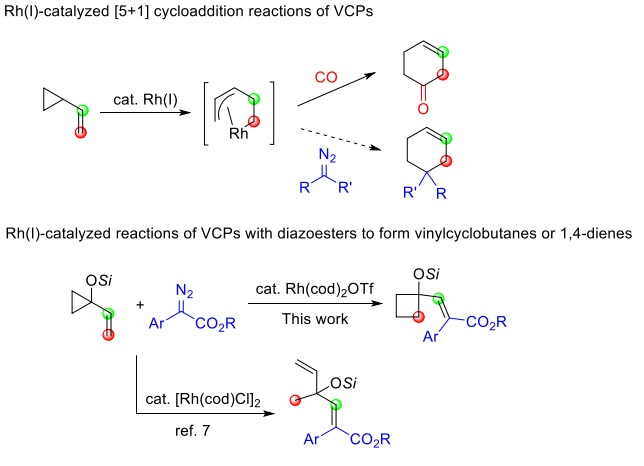


INTRODUCTION

Transition-metal-catalyzed cycloaddition reactions of vinylcyclopropanes (VCPs) and π-components have emerged as one of the efficient approaches for the synthesis of medium and large ring systems.[1] Since the first report of a Rh(I)-catalyzed intramolecular [5 + 2] reaction of β-yne-VCPs by Wender in 1995,[2] Rh(I)-catalyzed [5 + 2] cycloaddition reactions of VCPs with 2π-components such as alkynes, alkenes, and allenes have been actively studied for constructing various seven-membered rings.[3] On the other hand, Yu and co-workers have developed versatile methodology for constructing five-membered rings by using Rh(I)-catalyzed reactions of 1-ene/yne-VCPs as well as 2- and α-ene-VCPs through a [3 + 2] pathway.[4] In addition, they also introduced CO as a one-carbon coupling partner to achieve [5 + 2 + 1], [5 + 1], and [3 + 2 + 1] cycloaddition reactions of VCPs (Scheme 1).[5]

As part of our continuous interest in utilizing diazo compounds as a one-carbon synthon in transition-metal-catalyzed cross-coupling reactions,[6] and also inspired by the [5 + 1] reaction of VCPs with CO developed by Yu and co-workers,[5e] we envisioned that VCPs could potentially react with diazo compounds under Rh(I) catalysis to provide [5 + 1] products. In 2016, we reported the reaction between VCPs and diazoesters catalyzed by $[Rh(cod)Cl]_2$. Instead of the proposed [5 + 1] products, 1,4-dienes were formed in good yields.[7] During our further investigation, we discovered that when employing a cationic Rh(I) catalyst, $[Rh(cod)_2OTf]$, the reaction selectively afforded the vinylcyclobutane as the major product, along with the minor products of 1,4-dienes. Herein, we report methodology for the efficient synthesis of a variety of vinylcyclobutanes.[8] Furthermore, density functional theory (DFT) calculations were performed to study the mechanism of the reactions between VCPs and diazoesters catalyzed by $[Rh(cod)_2OTf]$ and $[Rh(cod)Cl]_2$, respectively, and shed light on the origin of the interesting catalyst-dependent chemoselectivity.

Scheme 1. Rh(I)-Catalyzed Reaction of Vinylcyclopropanes



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Cu(I)/Chiral Bisoxazoline-Catalyzed Enantioselective Sommelet–Hauser Rearrangement of Sulfonium Ylides

Shu-Sen Li and Jianbo Wang*

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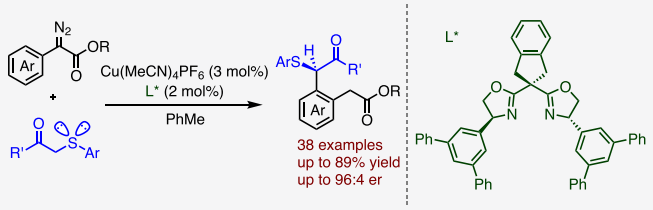
ABSTRACT: Catalytic asymmetric thia-Sommelet–Hauser rearrangement of sulfonium ylides remains a great challenge due to its multistep reaction mechanism involving metal carbene formation, proton transfer, and [2,3]-sigmatropic rearrangement. In particular, the key problem of such reactions is the differentiation of the enantiotopic lone pair electrons of sulfur, which generates the sulfonium ylide intermediate bearing chirality on the sulfur atom. With a modified chiral bisoxazoline ligand, we developed a Cu(I)-catalyzed asymmetric thia-Sommelet–Hauser rearrangement with good to excellent enantioselectivities. Mechanistic studies provide insights into the details of the reaction mechanism.



INTRODUCTION

The [2,3]-sigmatropic rearrangement reaction represents one of the important types of bond reorganizations in organic chemistry.[1] These reactions have found wide applications in organic synthesis, among which the [2,3]-sigmatropic rearrangement with sulfonium ylides is a powerful method to convert a C–S bond into C–C bond. The generation of sulfonium ylides with a catalytic carbene transfer process for this rearrangement, which is called Doyle–Kirmse reaction, further enhances the practicability of this transformation.[2,3] In a typical Doyle–Kirmse reaction, the α-diazocarbonyl compound is catalyzed by Rh(II) or Cu(I) complex to generate a metal carbene species, which reacts with allyl or propargyl sulfide to form an ylide intermediate for the subsequent [2,3]-sigmatropic rearrangement. Because a chiral center containing a C–S bond is generated in such process, the corresponding asymmetric catalysis has been explored, and significant progresses have been made recently.[4−6] For example, we have reported a highly enantioselective Doyle–Kirmse reaction by employing allyl or propargyl trifluoromethyl sulfides.[5a] The reaction served as an efficient method for the construction of C(sp^3)-SCF_3 bonds bearing chiral centers.

On the other hand, the thia-Sommelet–Hauser reaction is a unique type of [2,3]-sigmatropic rearrangement of sulfonium ylides, in which an unsaturated aromatic ring is involved.[7−11] While the classic thia-Sommelet–Hauser reaction is performed under stoichiometric conditions, we have previously developed a catalytic version of thia-Sommelet–Hauser reaction through Rh(II)-catalyzed carbene transfer (Scheme 1a).[10] We also attempted an asymmetric variant of this reaction; however, when Rh_2(*S*-DOSP)$_4$ was used as the chiral Rh(II) catalyst, the reaction showed no enantioselectivity.[10a] In connection to our continued interest in the asymmetric catalytic Doyle–Kirmse reaction,[4,5] we conceived to further explore the asymmetric catalytic thia-Sommelet–Hauser reaction. The challenge to achieve high enantioselectivity in this type of reaction lies in the fact the chiral metal catalyst is likely to dissociate from the ylide (Scheme 1b).[5a] Thus, the enantiocontrol must be in the step of ylide generation from chiral metal carbene intermediate **A**, in which the chiral metal carbene complex should differentiate the enantiotopic lone pair electrons of the sulfur to generate metal-complexed sulfonium ylide intermediate **B** (Scheme 1b, the chiral induction process). Dissociation of the chiral catalyst generates free sulfonium ylide **C**, which bears a chiral center on the sulfur. Subsequently, proton transfer occurs to form chiral ylide **D**, which undergoes [2,3]-sigmatropic rearrangement via a five-membered envelope transition state to afford the intermediate **E**, in which the chiral center is transferred from sulfur to the C(sp^3) carbon of the newly formed C(sp^2)–C(sp^3) bond. It is anticipated that catalytic asymmetric Sommelet–Hauser rearrangement is more arduous than the Doyle–Kirmse reaction because a 1,3-proton transfer process and a dearomatization rearrangement step are involved, which increases the possibility of racemization of the active ylide intermediates and thus decreases the efficiency of chirality transfer.

While our research was ongoing, Feng and coworkers reported an asymmetric catalytic thia-Sommelet–Hauser

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Chinese Journal of Organic Chemistry ARTICLE

钯催化芳香溴化物与三甲基硅基重氮甲烷的还原偶联及其在芳香化合物甲基化中的应用

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摘要 芳香化合物的甲基化反应是一类重要的转化. 其中, 由芳香卤化物出发的转化是在芳香体系中引入甲基基团的有效策略. 已有的方法多需要使用预先制备的甲基金属试剂或高毒性的甲基亲电试剂作为甲基化试剂. 本研究发展了一种利用三甲基硅基重氮化合物和芳香溴化物在钯催化下的还原偶联反应生成苄基硅化合物, 再经过脱硅质子化过程实现的甲基化方法. 此方法具有良好的官能团兼容性, 是一种具有潜在应用价值的对芳香卤化物进行甲基化的新方法. 同时, 该方法也可应用于在有机分子中引入硅甲基基团.

关键词 甲基化; 三甲基硅基重氮甲烷; 金属卡宾; 转移插入; 还原偶联

Palladium-Catalyzed Reductive Coupling of Aromatic Bromides and Trimethylsilyldiazomethane: Its Application to Methylation of Aromatic Compounds

Wang, Shuai Yang, Cheng Sun, Shuo Sun, Hanli Wang, Jianbo*
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Abstract The introduction of methyl group into aromatic compounds is a valuable transformation. A large number of known methods use organohalides as the starting materials. However, those methods require pre-synthesized methyl metal reagents or toxic methyl electrophiles. Herein, a palladium-catalyzed reductive coupling reaction between aryl bromides and trimethylsilyl- diazomethane is developed, and the following desilicification process can afford the methylated products. This transformation has broad functional group tolerance and allows methylation of (hetero)aryl halides in moderate to good yields. Thus, it has the potential to be an attractive approach for methylation of organic. In addition, this reductive coupling can also serve as an efficient way for the introduction of silylmethyl group.

Keywords methylation; trimethylsilyldiazomethane; metal carbene; migratory insertion; reductive coupling

1 Introduction

Methyl group (Me), as the smallest alkyl group, is ubiquitous in organic compounds, especially in a large number of bioactive molecules.[1] The introduction of methyl groups has been demonstrated to have significant influence on the bioactivity of molecules, which is very important for drug discovery and development.[2] The optimization of many properties by introducing various groups, which is referred as "magic methyl effect", can be attributed to the change of solubility, hydrophilicity, and molecular conformation.[3] For this reason, the development of efficient methylation methods has attracted considerable attentions in recent years.[4]

The traditional methylation methods involve nucleophilic substitution of *in situ* generated aryl lithium reagent and methyl electrophile, and Minisci-type reaction of electron-deficient arenes.[5] These methods commonly suffered from rigorous conditions and limited substrate scope. In the past decades, transition-metal-catalyzed cross-coupling reactions have been widely studied and have emerged as a powerful tool for the construction of C—C bonds. In recent

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Difluoroketenimine: Generation from Difluorocarbene and Isocyanide and Its [3 + 2] Cycloadditions with Alkenes or Alkynes

Rui Zhang, Zhikun Zhang, Kang Wang, and Jianbo Wang*

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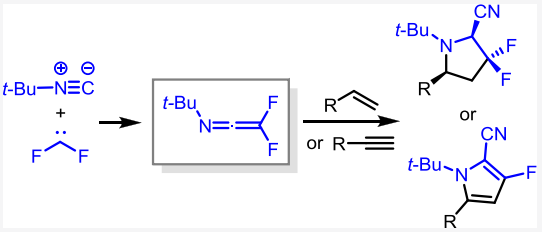
ABSTRACT: Ketenimines have been explored as useful building blocks for the synthesis of heteroatom-containing cyclic compounds through the cycloaddition with polar multiple bonds. Herein, we report the cycloaddition of difluoroketenimine with nonpolar multiple bonds, namely, the cycloaddition with alkenes or alkynes. The difluoroketenimine is generated from the coupling of *tert*-butyl isocyanide and difluorocarbene, which is formed in situ from (bromodifluoromethyl)trimethylsilane. The difluoroketenimine then reacts in situ with alkenes or alkynes to afford fluorinated pyrrolidines or pyrroles. DFT study suggests that a fluorinated cyclic (alkyl)(amino)-carbene is involved as the key intermediate in these reactions.



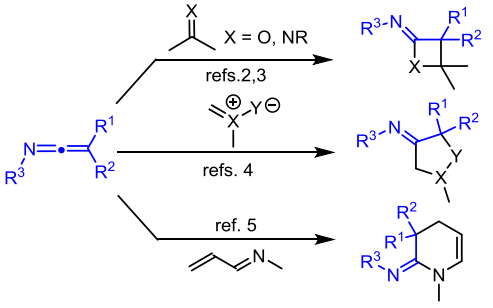
INTRODUCTION

Ketenimines are a type of unique building block in organic synthesis and generally served as a 2π species in cycloaddition reactions due to the high reactivity of their cumulated double bonds.[1] For instance, ketenimines can undergo formal [2 + 2] reactions with imines[2] and ketones,[3] [3 + 2] reactions with 1,3-dipolars,[4] and [4 + 2] reactions with azadienes[5] (Scheme 1a). Conjugated vinyl/phenyl-substituted ketenimines can also serve as 4π species in various [4 + 2] reactions.[6] Despite these known reaction modes, the cycloaddition reaction of ketenimines is still limited. In most cases, only the carbon−carbon bond in ketenimines is involved in the cycloaddition process[7] and another reaction component is restricted in polar multiple bonds, such as imines or ketones. So far, the cycloaddition reactions between ketenimines and nonpolar multiple bonds, especially those where both double bonds in ketenimines are involved, still remain challenging and unexplored.

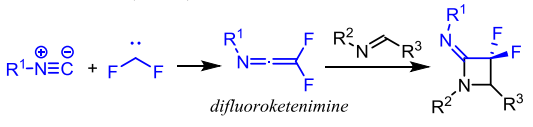
On the other hand, fluorine-containing molecules have attracted significant attention in recent years due to their importance in life science, drug industry, and materials science.[8] Consequently, various fluorinated reagents have been developed to introduce fluorine atoms into complex molecules.[9] We have recently reported the in situ generation of a new fluorinated intermediate, difluoroketenimine, via the reaction between difluorocarbene and isocyanides.[10] Difluoroketenimine can serve as the surrogate of the highly unstable intermediate difluoroketene[11] and undergoes the [2 + 2] reaction with imines to give α,α-difluoro-β-amino amides and α,α-difluoroazetidinimines (Scheme 1b). We also found that the introduction of fluorine atoms would significantly enhance ketenimines' reactivity in [2 + 2] cycloadditions. To further explore the intriguing chemistry of the newly discovered difluoroketenimine and its application in the synthesis of fluorine-containing molecules, we conceived to develop the [3 + 2] cycloaddition

Scheme 1. (a−c) Cylcoaddition Reactions of Ketenimines and Difluoroketenimines

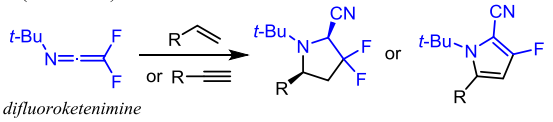
a) Reported cycloaddition reactions with ketenimines



b) Generation of difluoroketenimine and its [2+2] reaction with imines (ref.10).



c) Cycloaddition reaction of ketenimine with alkene or alkyne (this work).



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Perspective

Transition-Metal-Catalyzed Cross-Coupling with Ketones or Aldehydes via *N*-Tosylhydrazones

Ying Xia* and Jianbo Wang*

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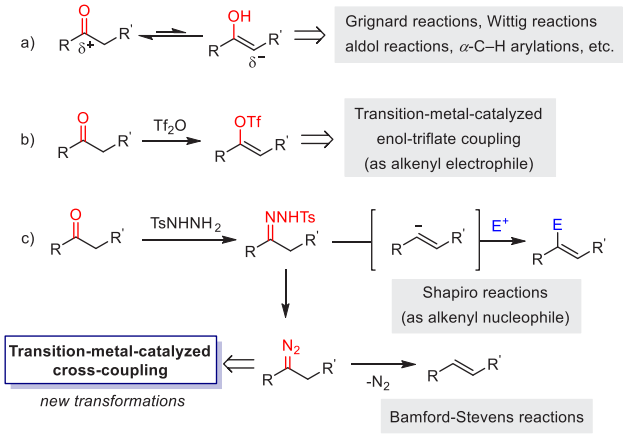
ABSTRACT: Ketones and aldehydes play central roles in organic synthesis. There are numerous broadly used reactions that are related to the carbonyl reactivity, such as Grignard reactions, Wittig reactions, aldol reactions, and so on. In addition, the formation of enol triflates is a classic protocol that enables the ketones to be applied in transition-metal-catalyzed cross-coupling reactions, in which case the ketones are considered as the precursors of alkenyl electrophiles in the C−C bond-forming transformations. In the past decade, a new type of ketone- or aldehyde-based C−C bond-forming transformations has emerged. In this type of reactions, the ketones or aldehydes are first converted to their corresponding *N*-tosylhydrazones, which are employed as reaction partners in various transition-metal-catalyzed carbene-based cross-coupling reactions. The *N*-tosylhydrazone-based carbene couplings significantly enhance the potential of ketones and aldehydes in modern organic synthesis. This Perspective aims to give an overview of carbene coupling reactions with *N*-tosylhydrazones from the viewpoint of exploring new potentials of ketones and aldehydes in organic synthesis.

INTRODUCTION

Ketones and aldehydes are fundamental building blocks in organic synthesis that can be employed in a variety of transformations. Principally, the reactivity of carbonyl functionality derives from the electrophilicity of the carbonyl carbon, as well as the acidity of the α-hydrogen. A series of widely used reactions are associated with these properties, such as Grignard reactions, Wittig reactions, aldol reactions, α-C−H arylations, and so on (Scheme 1a).[1,2] Moreover, the transformation of the carbonyl group into the corresponding enol triflates, which can serve as alkenyl electrophiles to participate in cross-coupling reactions, is another well-established protocol in organic synthesis (Scheme 1b).[3] *N*-Tosylhydrazones, which are readily derived from ketones or aldehydes via simple condensation, are also a type of useful synthon that provides unique routes to functionalized olefins via Shapiro reactions and Bamford−Stevens reactions (Scheme 1c).[4−6] In Bamford−Stevens reactions, the *N*-tosylhydrazone generates a diazo intermediate in situ in the presence of a base, followed by extrusion of dinitrogen gas to form the corresponding olefin.[4] This reaction has also served as an important method to prepare diazo compounds.[7−10] Shapiro reactions require two equivalents of strong base, usually alkyllithium, to generate an alkenyl anion, which can be trapped with an external electrophile to produce a functionalized olefin.[5,6] The feature of Shapiro reactions is that the ketone serves as an alkenyl nucleophile. The reaction has drawn much attention in organic synthesis.[11−14] For example, it was applied as one of the key steps in the total synthesis of Taxol.[15]

In addition to these classic reactions, the past decade has witnessed rapid development of *N*-tosylhydrazones in transition-metal-catalyzed cross-coupling reactions, which proceed through the in situ generated diazo intermediates (Scheme 1c). There have been several comprehensive reviews related to this rapidly growing area.[16−21] In this Perspective, C−C bond-forming carbene coupling reactions are used to highlight the new application of ketones and aldehydes in organic synthesis, which are mediated by their corresponding *N*-tosylhydrazones. The

Scheme 1. Transformations of Ketones for C−C Bond Formation



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Concise Report

Spirocarbocyclic compounds, Cyclization, Phenol-based biaryls, Dearomatization, Cross-coupling, *N*-Tosylhydrazones

CJC Chinese Journal of Chemistry 中国化学 An International Journal

Construction of Alkenyl-Functionalized Spirocarbocyclic Scaffolds from Alkyne-Containing Phenol-Based Biaryls *via* Sequential Iodine-Induced Cyclization/Dearomatization and Pd-Catalyzed Coupling of *N*-Tosylhydrazones

Anjia Liu,[a] Kaiming Han,[a] Xin-Xing Wu,[a] Shufeng Chen,*[,a] and Jianbo Wang*[,b]

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Summary of main observation and conclusion An efficient strategy for the formation of alkenyl-functionalized spirocarbocyclic scaffolds from alkyne-containing phenol-based biaryls *via* sequential iodine-induced cyclization/dearomatization and Pd-catalyzed coupling of *N*-tosylhydrazones is developed. The approach provides various spirocarbocyclic compounds in moderate to excellent yields with good functional tolerance. The results also demonstrate the feasibility for the direct cross-couplings of *N*-tosylhydrazones with sterically congested tetrasubstituted alkenyl halides.

Background and Originality Content

Spirocyclic molecular frameworks are important structural subunits, which could be widely found in chiral ligands,[1] functional materials,[2] pharmaceutically active molecules,[3] and bioactive natural products.[4] Among these, the spirocyclopentyl cyclohexadienones (Figure 1), containing a quaternary carbon center, have attracted considerable attention due to their unique structural characteristics and wide applications as useful building blocks in organic syntheses. Therefore, the development of an efficient and mild methodology for the construction of spirocyclopentyl cyclohexadienone core structures containing diverse functionalities continues to be an attractive area in the synthetic community.

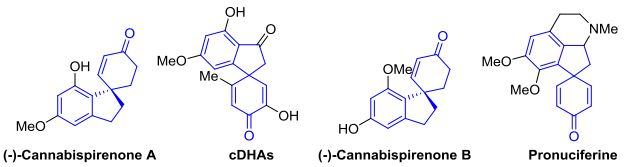
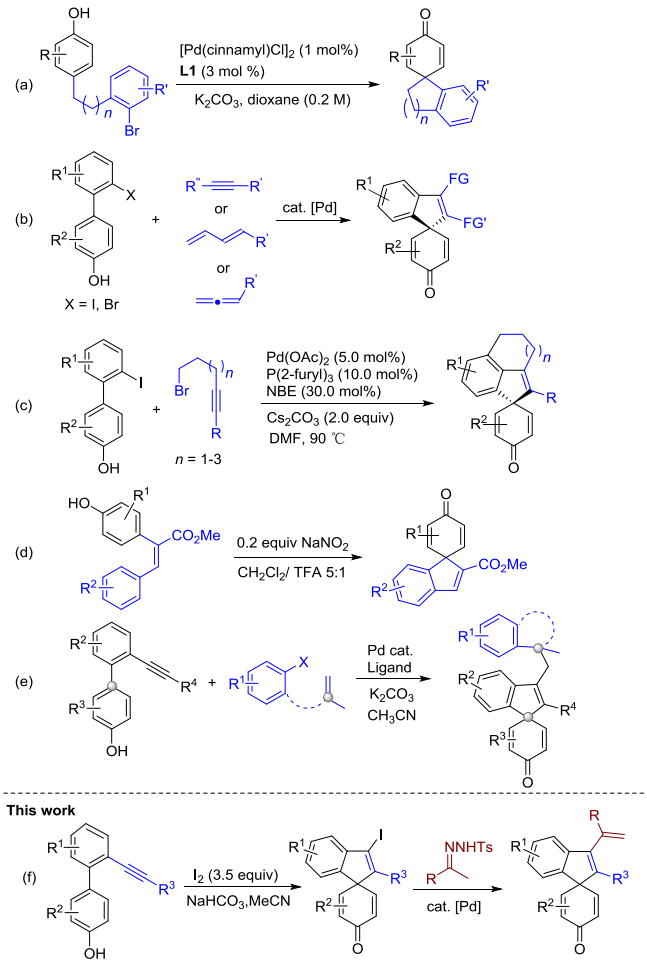


Figure 1 Representative examples of related natural products containing spirocyclopentyl cyclohexadienones.

Among the various methods for the preparation of these important and valuable scaffolds,[5] the direct dearomative spirocyclization of *para*-substituted phenol derivatives in an intra- or intermolecular manner is a straightforward and atom-efficient approach.[6] For example, in 2011, Buchwald and co-workers reported a palladium-catalyzed arylative dearomatization of phenols, to afford spirocyclopentyl cyclohexadienone products in moderate to good yields (Scheme 1a).[7] Later on, Luan and co-workers have successively developed several palladium-catalyzed intermolecular dearomatizing spiroannulation reactions of phenol-derived halogenated biaryls with alkynes,[8a] 1,3-dienes[8b] and allenes,[8c] leading to the formation of a series of highly functionalized spirocyclopentyl cyclohexadienone molecules in good yields (Scheme

Scheme 1 Synthesis of spirocyclopentyl cyclohexadienone scaffolds



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Palladium-Catalyzed Cascade Cyclization/Dearomatization/Arylation of Alkyne-Containing Phenol-Based Biaryls with Aryl Halides: An Entry to Diversely Functionalized Spirocyclohexadienones

Yunlong Bai, Anjia Liu, Xin-Xing Wu, Shufeng Chen,* and Jianbo Wang*

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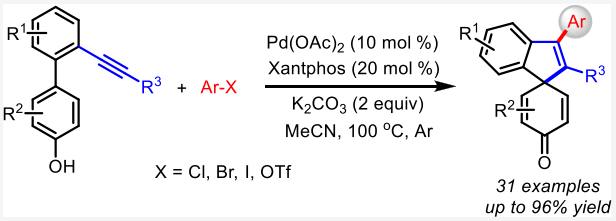
ABSTRACT: A convenient method for the synthesis of aryl-functionalized spirocyclohexadienone scaffolds from alkyne-containing phenol-based biaryls with aryl halides via palladium-catalyzed cyclization/dearomatization/arylation is developed. The approach provides a series of spirocyclohexadienone molecules in moderate to high yields. The reaction occurs chemoselectively through dearomative C-arylation rather than common O-arylation of phenols.



INTRODUCTION

Spirocyclohexadienones are recognized as an important class of bioactive molecules because of their broad distribution in biologically interesting natural products and pharmaceuticals (Figure 1).[1] Moreover, they are also versatile intermediates

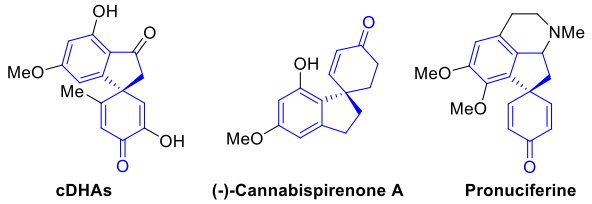


Figure 1. Representative examples of related natural products containing the spirocyclohexadienone skeleton.

for complex molecule syntheses due to their unique structural characteristic with the spirocyclic skeleton and the dienone functionality.[2] Therefore, the development of an innovative method for the construction of diverse functionalized spirocyclohexadienone molecules continues to be in high demand in the organic synthetic community.

Phenol and derivatives are readily available and widely used as starting materials in organic synthesis.[3] Among the various transformations of phenols, transition-metal-catalyzed dearomatization reactions have been proven to be one of the most straightforward methodologies for the facile construction of the valuable spirocyclohexadienone structures.[4] In dearomatization chemistry, pioneering examples have been demonstrated by the groups of Hamada,[5] You,[6] Buchwald,[7] and Feringa[8] independently, where several transition-metal-catalyzed dearomative spirocyclizations were achieved from diversely functionalized phenols with a tethered electrophilic moiety in an intramolecular manner. Besides, the intermolecular elegant strategies have also been explored.[9] For instance, Luan,[9a,e] Gulias,[9f] Lam,[9g] and You[9h] independently reported a series of transition-metal-catalyzed [3 + 2] spiroannulations of substituted phenols with various unsaturated compounds under oxidative reaction conditions, leading to the formation of various diversely functionalized spirocyclohexadienone molecules in good yields. Recently, we have reported a cascade Heck cyclization/phenol dearomatization process catalyzed by palladium from well-designed alkyne-containing phenol-based biaryls, where a series of indolone-, azaindoline-, dihydrobenzofuran-, dihydrobenzopyran-, and hydroquinoline-containing spirocyclohexadienone derivatives were synthesized in a single synthetic sequence (Scheme 1a).[10]

Despite significant achievements made in the synthesis of spirocyclohexadienone scaffolds by the strategy of dearomatization of phenols, highly efficient, chemoselective, and diverse functionalized synthetic routes are still in great demand. Inspired by those previous studies and our continuing interest in the area of transition-metal-catalyzed cascade reactions,[10,11] herein we describe a palladium-catalyzed cascade cyclization/dearomatization/arylation process for the rapid assembly of aryl-functionalized spirocyclohexadienone scaffolds from alkyne-containing phenol-based

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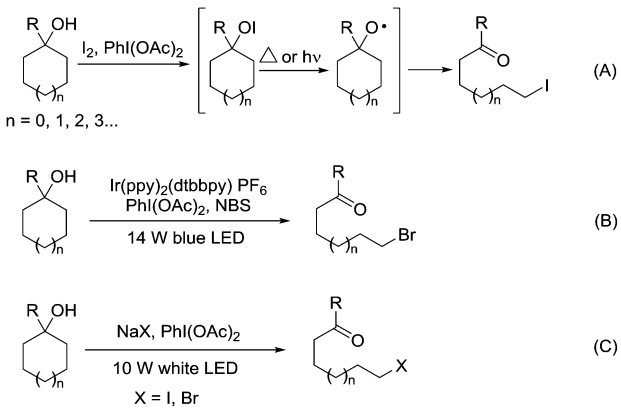
Ring-opening iodination and bromination of unstrained cycloalkanols through β-scission of alkoxy radicals†

Jiang-Ling Shi, Yuankai Wang, Zixuan Wang, Bowen Dou and Jianbo Wang *

Ring-opening iodination or bromination of unstrained cycloalkanols with NaI or NaBr and $PhI(OAc)_2$ under visible light irradiation is developed. In this protocol the concentration of I_2 is modulated through the generation of triiodide (I_3^-), thus significantly avoiding undesired side reactions. The reaction is under mild conditions and has a wide substrate scope, thus providing a practically useful method for accessing ω-iodo or ω-bromoketones.

The β-scission of alkoxy radicals has been extensively studied as a unique strategy for C(sp^3)–C(sp^3) bond cleavage, among which the ring-opening halogenation of cycloalkanols provides a straightforward approach toward distally halo-substituted ketones.[1] In this context, the ring-opening halogenation of strained cycloalkanols (3- and 4-membered rings) through β-scission of alkoxy radicals is well studied,[2] but the corresponding reaction of unstrained cycloalkanols has recently attracted attention (Scheme 1A).[3] Traditional methods for generating alkoxy radicals involve the conversion of alcohols into the corresponding hypoiodites with I_2/$PhI(OAc)_2$ (Suárez reagent), I_2/$Pb(OAc)_2$ or I_2/HgO, followed by photolysis or thermolysis.[4,5] However, these methods suffer from some drawbacks. In particular, the iodine (I_2) and the *in situ* generated reactive species acetyl hypoiodite (AcOI) are prone to homolysis to generate radical species which may lead to undesired side reactions. Besides, substrates containing electron-rich aromatic rings or reactive C–H bonds are not tolerated under these conditions.

Recently, new methods for generating alkoxy radicals based on photoredox catalytic processes have emerged.[3c,6,7] In particular, Zhu and co-workers explored an [$Ir(ppy)_2(dtbbpy)$]PF_6, NBS and $PhI(OAc)_2$ system for visible light-promoted ring-opening functionalization of unstrained cycloalkanols (Scheme 1B).[3c] Regardless of the significant improvement of the reaction conditions, an expensive transition-metal catalyst is required, which may limit its wide application. Thus, it is still highly desirable to develop mild and economical procedures for this important transformation.



Scheme 1 The ring-opening halogenation of cycloalkanols.

In 2016, Nagib and co-workers reported a δ-amination of secondary C–H bonds (Hofmann–Loffler–Freytag reaction) by using a NaI–$PhI(OAc)_2$ reaction system under visible light irradiation.[8] The highlight of this method is the *in situ* requisition of I_2, which is derived from sodium iodide, and I^- to yield a triiodide (I_3^-). This procedure limits the concentration of I_2 and reactive intermediate AcOI, thus significantly preventing undesired side reactions. Inspired by Nagib's work, we have conceived that the same protocol may be applied to ring-opening iodination and bromination of unstrained cycloalkanols. Herein we report our study along this line (Scheme 1C).

At the outset of the study, 1-phenylcyclohexan-1-ol (0.2 mmol) in 1 mL MeCN was irradiated with a 10 W white LED in the presence of NaI (4.0 equiv.) and $PhI(OAc)_2$ (4.0 equiv.) at room temperature for 16 h (Table 1, entry 1). The expected ring-opening iodization product **1** could be generated in 34% yield. Optimization experiments indicated that the loading of NaI and $PhI(OAc)_2$ could be reduced, while the yield of **1** was significantly increased (entries 2 and 3). We reasoned that this might

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Organic Syntheses

Discussion Addendum for:

Synthesis of Arylboronic Pinacol Esters from Corresponding Arylamines

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The wide applications of Suzuki-Miyaura cross-coupling reaction in constructing C–C bond have created an increasing demand for arylboronic acids or arylboronates.[2] The traditional synthetic route of arylboronic acids relied on the trapping of organolithium or Grignard reagents with a trialkyl borate, followed by acidic hydrolysis.[3] In 1995, Miyaura et al. developed the palladium-catalyzed borylation of arylhalides with bis(pinacolato)diboron, representing another important approach toward arylboronates.[4] Direct borylation via aromatic C−H bond activation has been extensively explored in the recent decades.[5] Following our report in 2010,[6] a series of similar Sandmeyer-type borylation reactions under modified conditions appeared in

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Communication

Mono- and Bis-Titanium Complexes Bridged by 2-Butene Tetraanion: Synthesis and Structural Characterization

Chao Yu, Wangyang Ma, Wen-Xiong Zhang, and Zhenfeng Xi*

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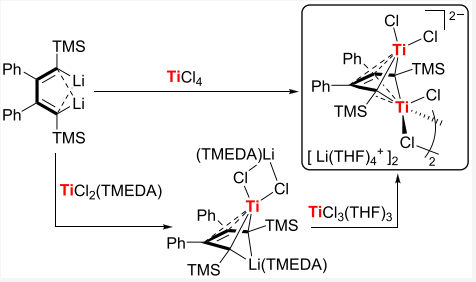
ABSTRACT: An unprecedented class of organo-dimetallic compounds, mono-titanium (Ti/Li) and bis-titanium (Ti/Ti) complexes bridged by 2-butene tetraanionic ligands, were obtained from the reaction of dilithio reagents **1** with $TiCl_2(TMEDA)$ or $TiCl_4$, respectively. The Ti/Li complex could be transformed to the Ti/Ti complex when treated with $TiCl_3(THF)_3$. Mechanistic study demonstrated that the dilithio reagents **1** acted as both reductant and oxidant in the reaction process.



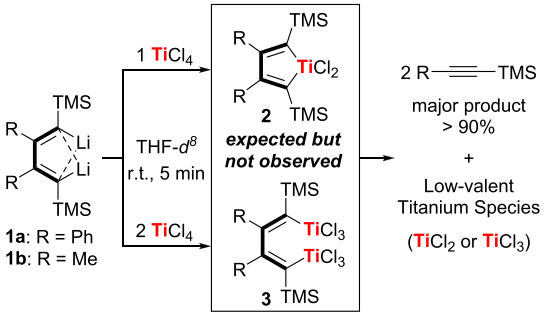
Organo-dimetallic compounds, in which two identical or different metals are bridged by an organic skeleton, are of significant interest because of their unique structures and wide synthetic applications.[1] However, efficient synthetic methods and structural diversity of organo-dimetallic compounds are very much limited in the literature.[1a,b,2−4]

1,4-Dilithio-1,3-butadienes (dilithio reagents for short) have been proven to be synthetically useful.[1a,b] Various metallacyclic compounds including organo-dimetallic compounds have been synthesized from the reaction of dilithio reagents **1** with metal salts, mainly via a transmetalation process.[1,3h−j] However, as shown in Scheme 1a, when $TiCl_4$ was reacted with **1**, the expected titanacyclopentadienes **2** and 1,4-bis($TiCl_3$)-1,3-butadienes **3** were not formed. Instead, alkynes were generated in high yields, obviously via a $TiCl_4$-mediated oxidative β,β'-C−C bond cleavage[1c,4c,5] of the 1,3-butadienyl skeleton in **1**. This interesting observation prompted us to investigate the reaction mechanism. Our study demonstrated that the molar ratio of the reactants played a key role in this reaction. Mono-titanium (Ti/Li) and bis-titanium (Ti/Ti) complexes bridged by 2-butene tetraanions could be synthesized from the reaction of **1** with $TiCl_2(TMEDA)$ or $TiCl_4$, respectively. In the formation process of bis-titanium complexes, low-valent titanium species were in situ generated and the dilithio reagents **1** acted as both reductant and oxidant.

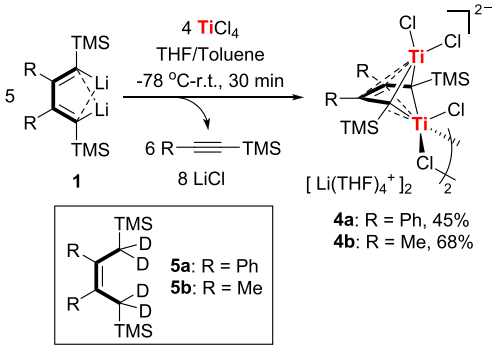
As given in Scheme 1a, when dilithio reagent **1** was treated with 1 or 2 equiv of $TiCl_4$ in THF-d^8, the alkyne was the only major product. In this reaction process, the dilithio reagent **1** might behave as a reductant and was oxidized by $TiCl_4$ to form alkynes along with low-valent titanium species such as $TiCl_2$[6] or $TiCl_3$. Apart from these major products mentioned above, single crystals of bis-titanium complex **4a** were also found as a minor byproduct, as determined by X-ray structural analysis (vide infra).

Scheme 1. Reactions of Dilithio Reagents 1 with $TiCl_4$ in Different Molar Ratios

a) Reactions of Dilithio Reagents 1 with $TiCl_4$ in Ratio of 1:1 or 1:2.



b) Reactions of Dilithio Reagents 1 with $TiCl_4$ in Ratio of 5:4.



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Selected Paper

Article for Frontiers of Molecular Science

Inverse-Sandwich Cyclobutadiene Dinickel Complexes: Synthesis and Structural Characterization#

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Abstract

Synthesis and structural characterization of inverse-sandwich complexes, whose planar conjugated ligand is a cyclobutadiene, have attracted much attention for their unique chemical bonding modes and synthetic methods. In this article, a new class of inverse sandwich cyclobutadiene dinickel complexes, the first case of inverse sandwich cyclobutadiene complexes with transition metals, were synthesized from the reaction of dilithio reagents with $NiBr_2$. Magnetometry and DFT calculations have revealed that the two nickel centers have antiferromagnetic coupling with each other and there exists electron back donation from the Ni(3d) orbitals to the π^* orbital of the cyclobutadiene ligand. Besides, the inverse sandwich cyclobutadiene dinickel complex could be reversed, being transformed to its corresponding normal sandwich complex, the bis(cyclobutadiene) nickel complex.

Keywords: Inverse-sandwich complex | Cyclobutadiene dinickel complex | Synthetic method

1. Introduction

Sandwich complexes usually refer to those organometallic complexes composed of one metal center and two planar conjugated ligands located in parallel on both sides of the metal center, while inverse-sandwich complexes[1–30] consist of one planar conjugated ligand and two metal centers binding on both sides of the ligand plane. Compared with sandwich complexes, the metal centers of inverse-sandwich complexes are relatively more exposed, thus resulting in potential instability. As a matter of fact, the synthesis of inverse-sandwich complexes is more difficult than those stable sandwich complexes, especially for cyclobutadiene complexes with a strained ring.

So far, in the literature there are only a very limited number of reports on the synthesis and structural characterization of inverse-sandwich cyclobutadiene complexes,[1–6] mainly due to the difficulty in the synthesis of cyclobutadiene ligands or the in situ construction of four-membered rings during the synthesis of metal complexes. The inverse-sandwich cyclobutadiene complexes of s-block metals ($\mathbf{M_1}$ = Li, Na, K, Scheme 1)[1–5] are mainly obtained by using reactive alkali metal to reduce cyclobutadiene derivatives or their complexes, while similar complexes of an f-block metal ($\mathbf{M_2}$ = U, Scheme 1)[6] have been

Metalla-Aromatics Hot Paper

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Butadienyl Diiron Complexes: Nonplanar Metalla-Aromatics Involving σ-Type Orbital Overlap

Chao Yu, Mingdong Zhong, Yongliang Zhang, Junnian Wei, Wangyang Ma, Wen-Xiong Zhang, Shengfa Ye, and Zhenfeng Xi**

Abstract: *A new class of nonplanar metalla-aromatics, diiron complexes bridged by a 1,3-butadienyl dianionic ligand, were synthesized in high yields from dilithio reagents and two equivalents of $FeBr_2$. The complexes consist of two antiferromagnetically coupled high-spin Fe^{II} centers, as revealed by magnetometry, Mössbauer spectroscopy, and DFT calculations. Furthermore, experimental (X-ray structural analysis) and theoretical analyses (NICS, ICSS, AICD, MOs) suggest that the complexes are aromatic. Remarkably, this nonplanar metalla-aromaticity is achieved by an uncommon σ-type overlap between the ligand p and metal d orbitals, in sharp contrast to the intensively studied planar aromatic systems featuring delocalized π-type bonding. Specifically, the σ-type interaction between the two Fe $3d_{xz}$ orbitals and the butadienyl π orbital results in the formation of a six-electron conjugated system and hence enables the aromatic character.*

Aromaticity is one of the essential foundational concepts in chemistry.[1] It can be closely related to the extent of orbital overlap, electron delocalization and molecular geometry.[2] The degree of electron delocalization depends on the overlap mode between different orbitals, thus resulting in the stable geometry of a molecule. As well-known in Hückel theory and aromaticity, the side-by-side overlap between several p orbitals (π-type orbital overlap, Scheme 1 a-Type I) makes phenyl rings or other similar conjugated organic cyclic compounds tend to exhibit planar structures. In addition to p orbitals, other types of orbitals, such as d orbitals in common transition metallacycles[3,4] and f orbitals in U and Th metallacycles,[5] may also overlap with p orbitals to realize electron delocalization. However, because d orbitals have different shapes and orientations, when they participate in a cyclic conjugated system and overlap with p orbitals, these orbitals may adopt disparate space positions (π-type, Scheme 1 a-Type II; σ-type, Scheme 1 a-Type III and Type IV) in order to maximize the degree of orbital overlap and achieve the utmost electron delocalization.[6] As a result, the most stable molecular geometry is not necessarily a planar structure. Based on the above analysis, it is reasonable that certain nonplanar cyclic compounds could feature aromaticity. More importantly, strong electron delocalization caused by appropriate orbital overlap in a conjugated ring should be deemed as a determinant that dictates whether a system is aromatic or not.

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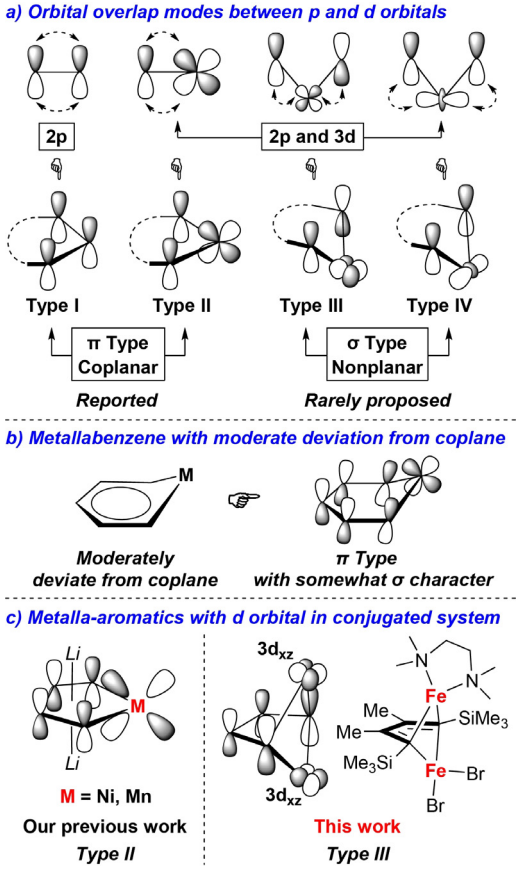

Scheme 1. Different overlap modes between p and d orbitals.

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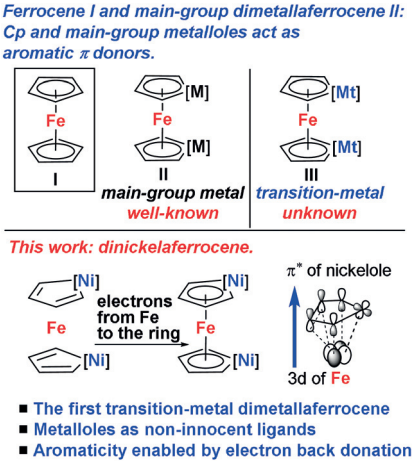
Sandwich Complexes

Dinickelaferrocene: A Ferrocene Analogue with Two Aromatic Nickeloles Realized by Electron Back-Donation from Iron

Zhe Huang, Yu Zheng, Wen-Xiong Zhang, Shengfa Ye, Liang Deng,* and Zhenfeng Xi**

Dedicated to Professor Tamotsu Takahashi on the occasion of his retirement

Abstract: *The first example of ferrocene analogues with two transition-metal metallole ligands of the general formula $(\eta^5\text{-}C_4R_4M)_2Fe$ in a sandwich structure are reported. Specifically, dinickelaferrocene* **2***, a type of dimetallametallocene, is efficiently synthesized from the reaction of dilithionickelole* **1** *with $FeBr_2$ or $FeCl_2$, presumably via a redox process, and is subjected to detailed experimental (single-crystal X-ray structural analysis, ICP-OES, magnetometry, ^{57}Fe Mössbauer, XPS) and theoretical (MOs, CDA, NICS, ICSS, and AICD) characterizations. Unlike ferrocene and its Cp ligands, the aromaticity of dinickelaferrocene and its nickelole ligands is accomplished by electron back-donation from the Fe 3d orbitals to the π* orbitals of nickelole. Taken together, this work describes a new class of metallaferrocene sandwich complexes and provides a novel approach to effect aromaticity that will contribute to further development of metallocene chemistry.*



Scheme 1. Essential differences between transition-metal dimetallaferrocene and ferrocene or main-group dimetallaferrocene.

Because of the great importance of ferrocene (Scheme 1, **I**) in chemistry and chemical industry,[1,2] chemical research on ferrocene analogues has been a major topic for decades,[3] resulting in the discovery of organometallic compounds of novel structures and various applications. Among all types of ferrocene analogues, dimetallaferrocene (Scheme 1, **II, III**) of the general formula $[(\eta^5\text{-}C_4R_4M)_2Fe]$ is of particular interest.[4,5] However, when M in **II** are transition metals, ferrocene analogues containing transition-metal metallole ligands (Scheme 1, **III**) have yet to be realized, except a theoretically proposed dinickelaferrocene reported in 2014.[5] Even for more general dimetallametallocenes with two transition-metal metallole ligands few examples are known.[6] In contrast, metallole ligands with main-group metals, such as Sb, Bi, Ge, Sn, which are generally 6π aromatic systems similar to Cp ligands in ferrocene, have often been used to prepare their corresponding metallametallocenes, for instance **II**.[4,7] The lack of metallametallocenes with transition-metal metallole ligands is likely due to, besides the shortage of synthetic methods, the essential difference in aromaticity between main-group-metal metalloles and transition-metal metalloles, which ultimately can be traced back to their intrinsically distinct bonding modes.[4–9]

A straightforward way to synthesize dimetallaferrocene is to react iron salts, such as $FeBr_2$, with their corresponding anionic or dianionic metalloles.[4] Dilithionickelole **1** (Scheme 2), an aromatic dianionic nickelole, was recently realized in high yields in our research group from the reaction of 1,4-dilithio-1,3-diene with $Ni(cod)_2$ complex.[8] Herein we report the synthesis of dinickelaferrocenes **2** from **1** and iron salts (Scheme 2), and their structural and spectroscopic characterizations. Dinickelaferrocenes **2** represent the first dimetallaferrocene bearing two transition-metal metallole ligands. In contrast to Cp and main-group metallole ligands (Scheme 1), the nickelole ligand here acts as a non-innocent ligand that accepts a remarkable electron density from the Fe atom to achieve an aromatic structure.

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REVIEW

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CHEMISTRY

Direct transformation of dinitrogen: synthesis of *N*-containing organic compounds via N—C bond formation

Ze-Jie Lv[1], Junnian Wei[1], Wen-Xiong Zhang[1], Ping Chen[2], Dehui Deng[2], Zhang-Jie Shi[3] and Zhenfeng Xi[1,*]

ABSTRACT

N-containing organic compounds are of vital importance to lives. Practical synthesis of valuable *N*-containing organic compounds directly from dinitrogen (N_2), not through ammonia (NH_3), is a holy-grail in chemistry and chemical industry. An essential step for this transformation is the functionalization of the activated N_2 units/ligands to generate N—C bonds. Pioneering works of transition metal-mediated direct conversion of N_2 into organic compounds via N—C bond formation at metal-dinitrogen [N_2-M] complexes have generated diversified coordination modes and laid the foundation of understanding for the N—C bond formation mechanism. This review summarizes those major achievements and is organized by the coordination modes of the [N_2-M] complexes (end-on, side-on, end-on-side-on, etc.) that are involved in the N—C bond formation steps, and each part is arranged in terms of reaction types (*N*-alkylation, *N*-acylation, cycloaddition, insertion, etc.) between [N_2-M] complexes and carbon-based substrates. Additionally, earlier works on one-pot synthesis of organic compounds from N_2 via ill-defined intermediates are also briefed. Although almost all of the syntheses of *N*-containing organic compounds via direct transformation of N_2 so far in the literature are realized in homogeneous stoichiometric thermochemical reaction systems and are discussed here in detail, the sporadically reported syntheses involving photochemical, electrochemical, heterogeneous thermo-catalytic reactions, if any, are also mentioned. This review aims to provide readers with an in-depth understanding of the state-of-the-art and perspectives of future research particularly in direct catalytic and efficient conversion of N_2 into *N*-containing organic compounds under mild conditions, and to stimulate more research efforts to tackle this long-standing and grand scientific challenge.

Keywords: dinitrogen transformation, metal-dinitrogen complex, N—C bond formation, *N*-containing organic compounds

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INTRODUCTION

As the most abundant constituent in Earth's atmosphere (atm), dinitrogen (N_2) is the main nitrogen source of *N*-containing compounds on the Earth. Therefore, N_2 fixation and activation are essential both for nature and humans. Nevertheless, the high bond dissociation energy (942 kJ/mol) and large highest occupied molecular orbital (HOMO)—lowest unoccupied molecular orbital (LUMO) gap (10.82 eV) make N_2 exhibit extremely low reactivity and be regarded as an inert gas. Currently, the N_2 fixation and conversion in nature and industry mainly rely on two pathways, in which ammonia (NH_3) is the product [1]. In nature, nitrogenase metalloenzymes employ iron-sulfur clusters as the key cofactor (FeMo, FeV or FeFe cofactor) and water as the proton source to transfer N_2 into NH_3 at ambient temperature and pressure [2]. This biosynthetic NH_3 is a versatile precursor for the synthesis of *N*-containing organic compounds, such as amino acids and nucleic acids. Although the precise biological N_2 reduction mechanism is still controversial, spectroscopic and computational studies suggested the presence of an interstitial carbon atom at the center of the FeMo and FeV cofactors [3–5].

Critical Review

Frustrated Lewis pairs, Metal-free catalysis, Hydrogenation, Hydrosilylation, Reduction of CO_2

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Frustrated Lewis Pairs: Discovery and Overviews in Catalysis

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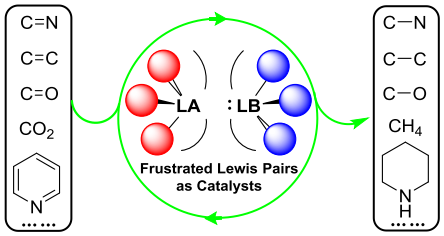

Frustrated Lewis Pairs (FLPs) are derived from simple combinations of Lewis acids (electron acceptors) and Lewis bases (electron donors), in which steric demands prevent from forming classical Lewis acid-base adducts. Since 2006, FLP chemistry has emerged as a novel strategy for the design and application of main-group chemistry and development of new metal-free catalytic processes. This strategy has been applied to stoichiometric reactivity and then extended to catalysis. In this review, we briefly summarize the representative discoveries and developments of FLP chemistry in the field of catalysis, including hydrogenation, hydrosilylation, reduction of CO_2, transformations of alkynes to organic derivatives, C—H bond borylation and polymerization.

Nan Li (left) was born in Henan, China, in 1988. She received her BS degree from Xinyang University in 2012. She obtained her PhD degree in 2017, supervised by Professor Bing-Tao Guan at Nankai University. Following her graduate research, she moved to the college of chemistry at Peking University as a Postdoctoral Fellow in the laboratory of Professor Zhenfeng Xi and Professor Wen-Xiong Zhang (2017—2019).

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Emerging Topic

Tetravalent, Rare-earth, Terbium, Praseodymium, Oxidizing agent

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Molecular Complexes of Emerging Tetravalent Rare-Earth Metals

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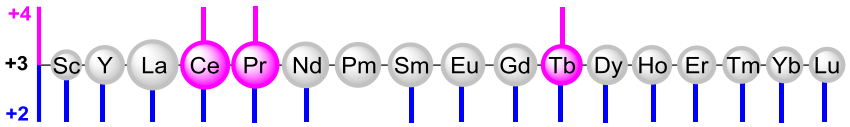

Exploring new oxidation state of elements is an important and challenging issue. Rare-earth elements all exhibit the stable +3 oxidation state, while the +2 oxidation state is accessible except radioactive Pm. However, until 2019, only cerium has the +4 oxidation state in molecular complexes. Since 2019, four Tb(IV) and Pr(IV) molecular complexes have been successful prepared and will open a new avenue for the development of tetravalent rare-earth chemistry. This emerging topic briefly describes the recent advances and prospects of the tetravalent rare-earth chemistry.

Background

Exploring new oxidation state of rare-earth metals (scandium, yttrium and lanthanides) is an important and challenging research subject for inorganic and organometallic chemists. Rare-earth metals all exhibit the stable +3 oxidation state,[1] while it is more than a century of exploration before the +2 oxidation state is accessible for all rare-earth elements except radioactive promethium.[2] Besides, cerium has the +4 oxidation state in molecular complexes, in which its electronic configuration adopts the noble gas xenon ($4f^0$).[3] The coordination and organometallic chemistry of cerium(IV) molecular complexes (*e.g.*, Cp_3CeO^tPr) started in 1976. Then it took almost 13 years that another cerium(IV) complex (Cp_3CeO^tBu) was reported. After that, more and more cerium(IV) complexes bearing *O*-, *N*-, and *C*-donor ligands were synthesized.[4] Until 2019, cerium was the only rare-earth metal for which the +4 oxidation state could be accessed in molecular complexes. Compared with Ce(IV), the synthesis of other tetravalent rare-earth molecular complexes remains a challenge due to their higher reduction potentials (*e.g.*, Tb(IV)/Tb(III): +3.3 V; Pr(IV)/Pr(III): +3.4 V; Nd(IV)/Nd(III): +5.4 V; Dy(IV)/Dy(III): +5.6 V vs. NHE) than that of Ce(IV)/Ce(III) (+1.61 V vs NHE).[5] A very important turning point came in 2019 when Mazzanti *et al*. reported the synthesis and characterization of the first Tb(IV) molecular complex. Since then, four examples of Tb(IV) and Pr(IV) molecular complexes have been reported.[6-9] In this emerging topic, we will for the first time cover the field of rare-earth (except Ce) chemistry contributed to the +4 oxidation state. Meanwhile, we hope that this topic will open a new avenue for the development of tetravalent rare-earth organometallic chemistry.

Recent Advances

Molecular complexes of Ce(IV), were generally prepared by the oxidation of the suitable trivalent precursors. Thus, besides the supporting ligands, the match between the reduction potentials of RE(IV)/RE(III) and the ability of oxidizing agents plays a vital role in obtaining the well-defined molecular complexes. On the basis of the calculated redox potential of 3.3 V for Tb(IV)/Tb(III), the most accessible +4 oxidation state after Ce(IV) should be the terbium ion. While Tb(IV) has only existed in metal oxides or fluorides and in concentrated aqueous carbonate solutions, the Tb(IV) molecular complexes were unsuccessful before 2019. With reference to the successful preparation of tetravalent $[Ce(OSi(O^tBu)_3)_4]$ from trivalent $[KCe(OSi(O^tBu)_3)_4]$, in 2019, Mazzanti *et al*. synthesized the fisrt Tb(IV) molecular complex **2** bearing the bulky tris(tertbutoxy)siloxide ligand by the oxidation of the Tb(III) precursor **1** using an oxidizing agent $[N(C_6H_4Br)_3][SbCl_6]$ (Scheme 1**a**).[6] X-ray analysis of **2** revealed that the Tb ion is five-coordinate and surrounded by three κ^1-$OSi(O^tBu)_3$ and one κ^2:O,O-$OSi(O^tBu)_3$ ligands. Meanwhile, the electron paramagnetic resonance and magnetometry unambiguously confirm the presence of Tb(IV). The isolation of Tb(IV) complex is very important, because it adds a new paramagnetic property to the periodic table, so that the crystal field effect in the tetravalent lanthanides can be compared with the transition metals and trivalent lanthanides.

Later, Mazzanti *et al*. continued their work on tetravalent rare-earth chemistry and reported another molecular complex of Tb(IV) **4** from Ph_3SiO-substitued trivalent precursor **3** (Scheme 1**b**).[7] This six-coordinate complex shows high stability and the MeCN ligands can be replaced by phosphinoxide ligands (Et_3PO and Ph_3PO). Unlike **2**, the supporting ligands ($OSiPh_3$) **4** do not saturate the coordination sphere but can also stabilize this complex. DFT studies suggest that this stability can be explained by a strong π(O-Tb) interaction, which is stronger than that in the Tb(IV) complex **2**.

Concomitant with Mazzanti's work, La Pierre *et al*. also reported Tb(IV) molecular complex bearing the bulky σ- and π-donor imidophosphorane ligand.[8] Complex **5** was oxidized rapidly with mild oxidant AgI to give a stable Tb(IV) molecular complex **6** (Scheme 1c). X-ray diffraction study demonstrates that **6** has an S_4

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Metallacycles | *Very Important Paper*

VIP Cyclic Bis-alkylidene Complexes of Titanium and Zirconium: Synthesis, Characterization, and Reaction

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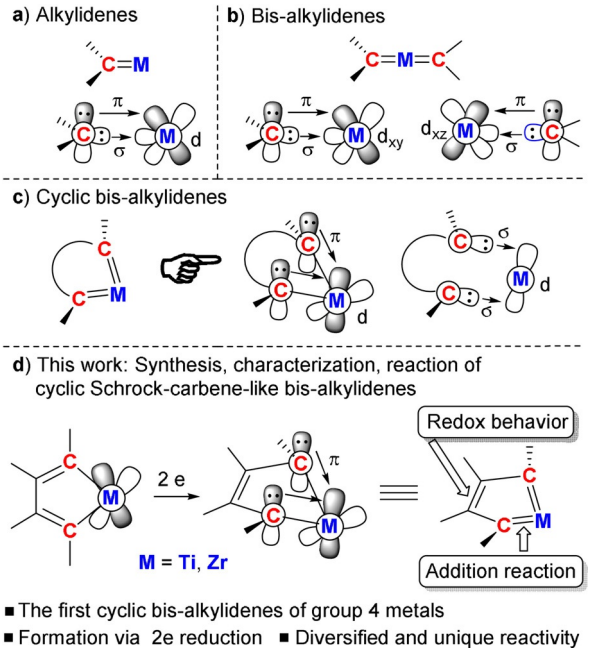
Dedicated to Prof. Pierre H. Dixneuf for his great contribution to the good friendship between the Chinese and French chemistry communities.

Abstract: Transition-metal alkylidenes have exhibited wide applications in organometallic chemistry and synthetic organic chemistry, however, cyclic Schrock-carbene-like bis-alkylidenes of group 4 metals with a four-electron donor from an alkylidene have not been reported. Herein, the synthesis and characterization of five-membered cyclic bis-alkylidenes of titanium (**4a**,**b**) and zirconium (**5a**,**b**) are reported, as the first well-defined group 4 metallacyclopentatrienes, by two-electron reduction of their corresponding titana- and zirconacyclopentadienes. DFT analyses of **4a** show a four-electron donor (σ-donation and π-donation) from an alkylidene carbon to the metal center. The reaction of **4a** with *N*,*N*′-diisopropylcarbodiimide (DIC) leads to the [2+2]-cycloaddition product **6**. Compound **4a** reacted with CO, affording the oxycyclopentadienyl titanium complex **7**. These reactivities demonstrate the multiple metal–carbon bond character. The reactions of **4a** or **5a** with cyclooctatetraene (COT) or azobenzene afforded sandwich titanium complex **8** or diphenylhydrazine-coordinated zirconacyclopentadiene **9**, respectively, which exhibit two-electron reductive ability.

Introduction

Transition-metal carbenes and alkylidenes consisting of a M=CR_2 unit have exhibited wide applications in organometallic chemistry and synthetic organic chemistry.[1] Fischer carbenes have the typical characteristics of σ-donation to the metal center, and π-back-donation from the metal center,[2] whereas Schrock carbenes or alkylidenes have the radical recombination of two triplet fragments, where there is no π-back bond.[3] In contrast to well-known Fischer and Schrock carbenes, a special type of carbenes (Scheme 1a, hereafter Schrock-carbene-like alkylidenes) can be described by a four-electron donor (σ-donation and π-donation) from the carbene carbon to the metal center, however, these are less explored.

Examples of Schrock-carbene-like alkylidenes are mainly formed from group 3 and 4 metals.[4–7] They are usually unstable and very difficult to access owing to the high negative charges on the carbene carbon atom, and the lack of d electrons and high d energy level of the metal center. These alkylidene carbons are generally stabilized by supporting ligands with strongly stabilizing moieties, such as P^V or S^{VI} substituents at two α-positions of the carbon atoms, which delocalize the



Scheme 1. Schrock-carbene-like alkylidene structures.

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Phosphorus Compounds | *Hot Paper*

Fragmentation Mechanism of White Phosphorus: A Theoretical Insight into Multiple Cleavage/Formation of P–P and P–C Bonds

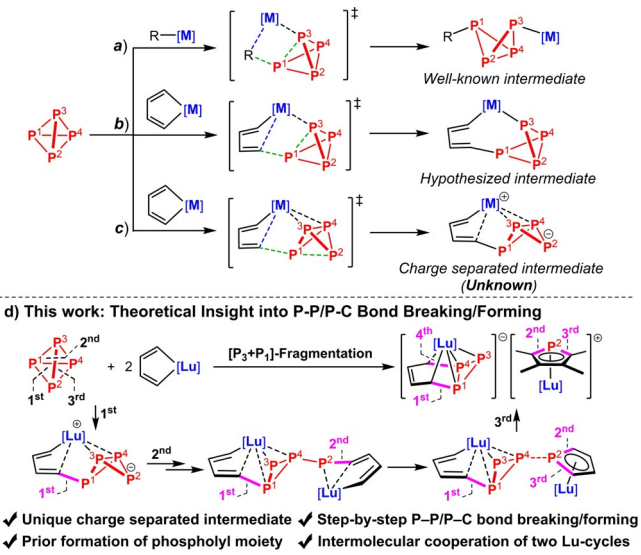
Gen Luo,[a, b] Shanshan Du,[c] Pan Wang,[b] Fan Liu,[b] Wen-Xiong Zhang,*[c] and Yi Luo*[b]

Abstract: Molecular-level understanding of metal-mediated white phosphorus (P_4) activation is meaningful but challenging because of its direct relevance to the conversion of P_4 into useful organophosphorus compounds as well as the complicated and unforeseeable cleavage process of P–P bonds. The related study, however, has still rarely been achieved to date. Here, a theoretical insight into the step-by-step process of three P–P bond cleavage/four P–C bond formation for $[P_3+P_1]$-fragmentation of P_4 mediated by lutetacyclopentadienes is reported. The unique charge-separated intermediate and the intermolecular cooperation between two lutetacyclopentadienes play a vital role in the subsequent P–P/P–C bond breaking/forming. It is found that, although the first P–C formation is involved in the assembly of the *cyclo*-P_3 $[R_4C_4P_3]^-$ unit, the construction of the aromatic five-membered P_1 heterocycle $[R_4C_4P]^-$ is completed prior to the *cyclo*-P_3 formation. The reaction mechanism has been carefully elucidated by analyses of the geometric structure, frontier molecular orbitals, bond index, and natural charge, which greatly broaden and enrich the general knowledge of the direct functionalization of P_4.

Introduction

Metal-mediated functionalization of white phosphorus (P_4) to phosphorus-based chemicals has been of great interest in recent years as such a process could avoid undesired multiple synthetic steps, toxic reagents, and waste hydrochloric acid.[1,2] In the past decades, numerous complexes with various phosphorus and polyphosphorus ligands derived from P_4 have been isolated and characterized.[3–6] However, in most cases, the experimental results are unpredictable and confusing.[7] Hence, to understand the versatile reaction patterns of P_4 at the molecular level, theoretical studies on P_4 activation are clearly necessary, helpful, and meaningful.[8] As far as we are aware, detailed mechanistic study is extremely lacking although several P_4 activation pathways have been proposed and some key intermediates have been isolated.[9]

The nucleophilic activation of P_4 is a convenient method to construct P–C bonds.[10] Despite the fact that the nucleophilic activation of P_4 will often result in a complicated mixture,[11] the first activation step is assumed to the cleavage of one P–P bond via σ-bond metathesis providing a butterfly-type bicyclo[1.1.0]-tetraphosphabutane unit (butterfly-P_4, Scheme 1 a).[1c] Based on the general knowledge for the nucleophilic activation of P_4, cyclic organometallic reagents such as metallacyclopentadienes should undergo the similar activation step to afford a metal-phosphorus-containing cyclic intermediate (Scheme 1 b).



Scheme 1. The patterns of nucleophilic activation of P_4 and this work elucidating the $[P_3+P_1]$-fragmentation mechanism of P_4.

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Carbodiimide-based synthesis of N-heterocycles: moving from two classical reactive sites to chemical bond breaking/forming reaction

Yang Wang, *[ab] Wen-Xiong Zhang *[c] and Zhenfeng Xi [c]

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Carbodiimides are a unique class of heterocumulene compounds that display distinctive chemical properties. The rich chemistry of carbodiimides has drawn increasing attention from chemists in recent years and has made them exceedingly useful compounds in modern organic chemistry, especially in the synthesis of N-heterocycles. This review has outlined the extensive application of carbodiimides in the synthesis of N-heterocycles from the 1980s to today. A wide range of reactions for the synthesis of various types of N-heterocyclic systems (three-, four-, five-, six-, seven-, larger-membered and fused heterocycles) have been developed on the basis of carbodiimides and their derivatives.

1. Introduction

Heterocyclic compounds, both naturally derived and synthetically produced, constitute a wide variety of biologically active and industrially important compounds. More than 67% of compounds contain heterocycles in the Comprehensive Medicinal Chemistry (CMC) database.[1] Therefore, the synthesis and application of heterocyclic compounds have always been in the forefront of attention and have rapidly grown in the past few decades. Among these, N-heterocycles constitute an important branch, showing superior pharmaceutical effects compared to non-nitrogen heterocycles. N-Heterocycles, as

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Wen-Xiong Zhang

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Aromaticity **Hot Paper**

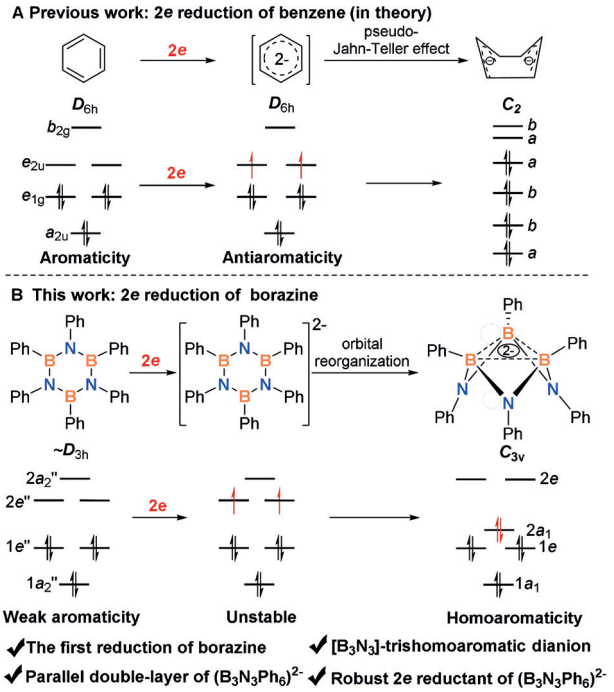
Trishomoaromatic ($B_3N_3Ph_6$) Dianion: Characterization and Two-Electron Reduction

Nan Li[+], Botao Wu[+], Chao Yu, Tianyu Li, Wen-Xiong Zhang, and Zhenfeng Xi**

Dedicated to Professor Youqi Tang on the occasion of his 100th birthday

Abstract: *Benzene, a common aromatic compound, can be converted into an unstable antiaromatic 8π-electron intermediate through two-electron reduction. However, as an isoelectronic equivalent of benzene, borazine ($B_3N_3Ph_6$), having weak aromaticity, undergoes a totally different two-electron reduction to afford $(B_3N_3R_6)^{2-}$ homoaromatic compounds. Reported here is the synthesis of homoaromatic $(B_3N_3Ph_6)^{2-}$ by the reduction of $B_3N_3Ph_6$ with either potassium or rubidium in the presence of 18-crown-6 ether. Theoretical investigations illustrate that two electrons delocalize over the three boron atoms in $(B_3N_3Ph_6)^{2-}$, which is formed by the geometric and orbital reorganization and exhibits (π,σ)-mixed homoaromaticity. Moreover, $(B_3N_3Ph_6)^{2-}$ can act as a robust 2e reductant for unsaturated compounds, such as anthracene, chalcone, and tanshinones. This 2e reduction is of high efficiency and selectivity, proceeds under mild reaction conditions, and can regenerate neutral borazine.*

Aromaticity is an important and fascinating concept in organic chemistry.[1,2] Benzene is a common aromatic compound with a planar D_{6h} structure, which can be converted into dearomatized 1,4-cyclohexadiene by a classic Birch reduction. Theoretically, benzene could turn into an unstable antiaromatic intermediate (6C, 8π-electron) through a two-electron reduction. This reduction may lead to rapid distortion of this intermediate based on a pseudo-Jahn–Teller effect, and eventually form a C_2-symmetric dearomatized dianion (Scheme 1 A).[3] To maintain aromaticity of benzene, four-electron reduction is required.[4] Borazine, as an isoelectronic equivalent of benzene, has a planar D_{3h} structure and equalized B–N bond lengths (1.44 Å).[5,6] Despite bearing similar electronic structure, the aromaticity of borazine has a long and controversial history. To date, it is widely accepted that borazine shows weak aromaticity because its limited π-electron delocalization caused by the electronegativity difference between boron and nitrogen atoms.[5] This structural feature provides many unique properties for borazine, which is remarkably different from benzene. Although the reductions of arenes have been well studied, the reductions of borazine and its derivatives still remain a mystery. Herein, for the first time, we report the two-electron reduction of borazine ($B_3N_3Ph_6$) by either potassium or rubidium in the presence of 18-crown-6 (18C6) to yield the $(B_3N_3Ph_6)^{2-}$. Single-crystal X-ray diffraction analysis reveals that it has a C_{3v}-symmetric parallel double-layer structure (Scheme 1 B). Theoretical investigations illustrate that two electrons delocalize over the three boron atoms in $(B_3N_3Ph_6)^{2-}$ and exhibit (π,σ)-mixed homoaromaticity. These results clearly show that borazine can accept two electrons to its two degenerate LUMOs (2*e*″) to give an unstable planar intermediate which undergoes geometric and orbital reorganization to give a parallel double-layer $(B_3N_3Ph_6)^{2-}$ structure (Scheme 1 B). In addition, $(B_3N_3Ph_6)^{2-}$ can act as a robust 2*e* reductant for unsaturated compounds.



Scheme 1. The 2*e* reductions of benzene and borazine.

As illustrated in Scheme 2, the phenyl-substituted borazine **1** was synthesized according to the literature procedure.[7] Treatment of **1** with potassium in THF at room temperature for 6 hours gave a dark-red solution. The expected ionic

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Article

2-Butene Tetraanion Bridged Dinuclear Samarium(III) Complexes via Sm(II)-Mediated Reduction of Electron-Rich Olefins

Yu Zheng,[§] Chang-Su Cao,[§] Wangyang Ma,[§] Tianyang Chen, Botao Wu, Chao Yu, Zhe Huang, Jianhao Yin, Han-Shi Hu,* Jun Li, Wen-Xiong Zhang,* and Zhenfeng Xi

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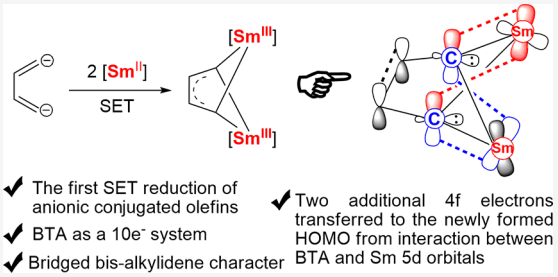
ABSTRACT: While reduction reactions are ubiquitous in chemistry, it is very challenging to further reduce electron-rich compounds, especially the anionic ones. In this work, the reduction of 1,3-butadienyl dianion, the anionic conjugated olefin, has been realized by divalent rare-earth metal compounds (SmI_2), resulting in the formation of novel 2-butene tetraanion bridged disamarium(III) complexes. Density functional theory (DFT) analyses reveal two features: (i) the single electron transfer (SET) from 4f atomic orbitals (AOs) of each Sm center to the antibonding π*-orbitals of 1,3-butadienyl dianion is feasible and the new HOMO formed by the bonding interaction between Sm 5d orbitals (AOs) and the π*-orbitals of 1,3-butadienyl dianion can accept favorably $2e^-$ from 4f AOs of Sm(II); (ii) the 2-butene tetraanionic ligand serves as a unique $10e^-$ donating system, in which $4e^-$ act as two σ-donation bonding interactions while the rest $6e^-$ as three π-donation bonding interactions. The disamarium(III) complexes represent a unique class of the bridged bis-alkylidene rare-earth organometallic complexes. The ligand-based reductive reactivity of 2-butene tetraanion bridged disamarium(III) complexes demonstrates that 2-butene tetraanionic ligand serves as a $3e^-$ reductant toward cyclooctatetraene (COT) to provide doubly COT-supported disamarabutadiene complexes. The reaction of the disamarium(III) complexes with Cp*Li produces the doubly Cp*-coordinated Sm(III) complexes via salt metathesis. In addition, the reaction with $Mo(CO)_6$ affords the oxycyclopentadienyl dinuclear complex via CO insertion.



INTRODUCTION

Among various reduction reactions, the reduction of olefins by divalent rare-earth metal complexes is a fundamental process in rare-earth organometallic chemistry.[1,2] For example, samarium diiodide (SmI_2) as a ubiquitous reagent in organic synthesis can serve as a powerful class of single electron transfer (SET) agent.[1] Typically, SmI_2-mediated reduction of conjugated olefins is initiated by SET from the central Sm(II) to the substrate affording the radical intermediate (Scheme 1a).[3] When R is an electron-donating group, the addition of an electron into an electron-rich C═C double bond is not favored.[4] In contrast, it is more difficult and challenging for an anionic conjugated olefin, which acts as a highly electron-rich species, to accept the additional electron from the Sm(II) center via an SET process (Scheme 1b). This type of reduction of an anionic conjugated olefin by divalent rare-earth metal complexes has not been reported so far.

Recent studies in our laboratory have revealed that the antibonding π*-orbitals of 1,3-butadienyl dianions can accept two d-electrons from transition metals, producing a series of mononuclear metalloaromatic complexes.[5] In these processes, the π*-orbitals have higher energy level than the d-orbitals of transition metals, permitting the overlap between these two orbitals.[5e,f] We envisioned that such π*-orbitals of 1,3-butadienyl dianions might become eligible to accept electrons from 4f orbitals under suitable conditions. Herein we report for the first time 1,3-butadienyl dianions of 1,4-dilithio-1,3-butadienes **1** can gain two electrons from two molecules of $SmI_2(THF)_2$ via an SET process to yield the first 2-butene tetraanion (abbreviated as BTA) bridged disamarium(III) complexes **2**. Through two SET processes, the LUMO π*-orbital of 1,3-butadienyl dianions receives two electrons from each Sm 4f atomic orbitals (AOs) because this π*-orbital interacts with Sm 5d AOs to form the HOMO of BTA. With the two additional electrons on the new HOMO, the generated BTA has ten electrons in total available for donating to metal centers (Scheme 1c). Unique and fascinating reactivities of **2** including ligand-based reduction, salt metathesis, and CO insertion are unraveled by the reactions with cyclooctatetraene (COT), Cp*Li, and $Mo(CO)_6$, respectively.

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双锂试剂的发现与发展: 意料之外情理之中

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摘要 本文介绍了本研究室自1998年至今科学研究工作的几个重要历程/事件, 尤其是双锂试剂(1,4-双锂-1,3-丁二烯衍生物的简称)的"意外"发现过程和进一步发展过程. 首先, 我们"意外"地发现了过渡金属与路易斯酸的"协同效应", 为了深入研究该"协同效应"作用机制, 我们"意外"地发现了双锂有机化合物的"协同效应". 进一步地, 我们将双锂有机化合物发展成双锂有机合成试剂, 并通过转金属反应发展了其他双金属有机合成试剂. 而我们近年来最主要的研究课题"双锂试剂作为氧化还原活性化合物"也是"意外"发现与深入坚持的结果, 充分体现了我们一直坚持的"深·新·信"科学研究方法和理念. 以上所述所有所谓"意外"发现都是意料之外情理之中的.

关键词 双锂试剂, 金属有机试剂, 机理研究, 协同效应, 意外发现与坚持

1 引言

1998年春, 我从日本北海道大学触媒化学研究中心高桥保(Tamotsu Takahashi)教授研究室结束助理教授工作回国, 到北京大学化学学院任教. 彼时, 北京大学化学学院正在推行PI (principal investigator)制, 也就是学术小组制. 我很荣幸作为北京大学化学学院的独立PI开始了自己的科学研究工作. 正如大多数年轻PI在开始自己的独立学术生涯时所面临的问题一样, 我也很犹豫自己的独立科学研究工作做什么. 当时, 我只知道自己希望开展不同于我博士和博士后期间所从事的研究工作, 希望开展创新性显著的、有自己特色的研究工作, 借用现在经常听到的说法, 希望开展受人尊重的研究工作, 不跟风、不追逐潮流, 但是, 也无能力、无必要完全脱离过去的教育背景和工作经历. 因此, 我希望在自己过去工作积累的基础上找到一个切入点, 逐步推进和深入, 慢慢形成自己的研究特色.

基于以上思考, 我决定从二茂锆杂环戊二烯衍生物**1** (图1)的进一步应用研究开始[1~3]. 其理由是: 一、该类化合物理论上很有用, 但是实际上很难用. 该类化合物由四个碳的丁二烯骨架组成, 同时含有两根碳–金属(C–Zr)键, 具有多个反应位点, 理论上可能作为活性金属有机化合物用于多种有机和金属有机化合物的合成[4~7]. 但是, 实际上该类化合物的反应活性极低, 与实验室常见常用的高活性有机化合物包括醛酮等羰基化合物均不发生反应, 所以被认为是一个死胡同. 二、我在博士和博士后期间合成了多种该类化合物, 掌握了其合成方法[2]. 基于低价二茂锆化学, 锆杂环戊二烯衍生物**1**可以从分子间两分子相同或者不同的炔烃(包括原位产生的高活性苯炔)高效合成, 也可以从分子内双炔合成, 而分子内双炔可以具有不同的有机桥联骨架和不同的取代基[1~7].

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Outlook of nitrogen fixation by carbene

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ABSTRACT

From the first N_2-metal complex discovered in the 1960s to the borylene N_2 complex reported very recently, chemists have been trying to activate N_2 under milder conditions by investigating many approaches or strategies. Among them, carbene species has been considered a good choice to construct N–C bond to obtain N-containing organic compounds directly. Since the first glimpse of the N_2 exchange of diazomethane, chemists have obtained a deeper understanding of the rebound reaction between carbene and N_2. This review mainly summarizes the progress in the field of N_2 fixation by using carbene species, both experimentally and theoretically. We believe this review will provide readers with an in-depth understanding of the state-of-the-art and perspectives of future research particularly in the use of carbene species for activation and transformation of N_2 into N–C containing organic compounds.

1. Introduction

Nitrogen is an essential element in maintaining human living and civilization. Dinitrogen (N_2) is the richest and "cheapest" source of nitrogen, which is inexhaustible. However, it is exceptionally stable and thus difficult to utilize. Accordingly, there is no doubt that realizing activation and direct transformation of N_2 under mild conditions is a grand scientific problem that people need to solve.

Nature has already developed the means to realize N_2 fixation under mild conditions through the nitrogenases [1,2], whereas industry has been relied on the Haber-Bosch process to transfer N_2 to ammonia (NH_3) for the production of fertilizer in the presence of a transition-metal catalyst [3,4]. Moreover, with the discovery of the first metal-N_2 complex in 1965 [5], many catalysts for N_2 fixation were synthesized based on metallic reducing agents [6,7]. Over 50 years of development, chemists have concluded that the peculiar ability of certain transition metal complexes to bind N_2 gets benefit from their advantageous combination of occupied and unoccupied d orbitals, which are of reasonable energy and symmetry to synergically back-donate to and accept electron density from N_2 (Fig. 1A). Up until recently, H. Branuschweig et al. demonstrated that modification of the electronic environment of the B atom, which is stabilized by CAAC ligand [CAAC = 1-(2,6-di*iso*propylphenyl)-3,3,5,5-tetramethylpyrrolidin-2-ylidene], could enable N_2 binding and reduction at a B center (Fig. 1B) [8,9]. Beyond metallic catalysts, the non-metallic process of N_2 fixation affords another way to solve the grand scientific problem.

Carbenes as highly active species, play an important role in the field of transition metal catalysis and main-group chemistry [10,11]. The electronic structures of carbene are shown in Fig. 2. In 1932, Mulliken recognized that methylene should have two, low-lying electronic states [12]. One of these states is the triplet (**A**-σ $[1]\pi^1$ in Fig. 2). In the **A**-σ $[1]\pi^1$ state, two nonbonding electrons separately occupy the hybridized σ orbital and the carbon 2p-π atomic orbital (AO) with the spins of the two electrons are parallel. Another low-lying state is the singlet (**B**-σ $[2]\pi^0$ in Fig. 2). In the **B**-σ $[2]\pi^0$ state, both nonbonding electrons occupy the σ orbital; whereas, the π orbital consists of pure carbon 2p-π AO. Each one of them could be the ground state with selectively stabilized by substituents of carbene [13]. Except for these two stable states, there are two high energy unstable electronic states: the open-shell **C**-σ $[1]\pi^1$ state, which has the same orbital occupancy as the **A**-σ $[1]\pi^1$ state with antiparallel spins of the two nonbonding electrons; the highest energy **D**-$\sigma^0\pi^2$ state configuration, both nonbonding electrons occupy the lower energy π orbital, whereas, the σ orbital consists of pure carbon σ MO. In the general system, such as all alkyl substituted carbene, both of them (**C**-σ $[1]\pi^1$ state and **D**-$\sigma^0\pi^2$ state) are computed to be 33–59 kcal/mol higher than that of the stable electronic configuration (**A**-σ $[1]\pi^1$ or **B**-σ $[2]\pi^0$) [14]. Compared with the compounds mentioned above, **D**-$\sigma^0\pi^2$ carbene also has unoccupied *sp* [2] orbital and occupied *p* orbital [15]. It is

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Asymmetric Total Synthesis of Pre-schisanartanin C

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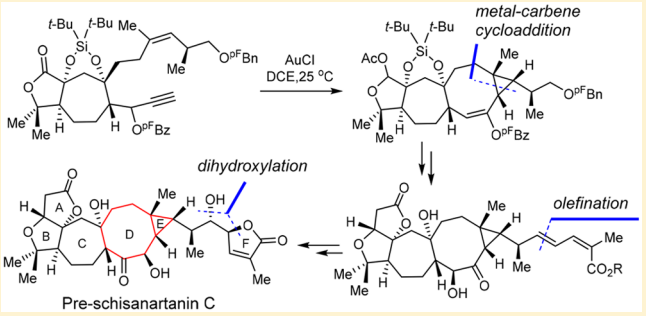
Supporting Information

ABSTRACT: Pre-schisanartanin C belongs to the family of *Schisandra* nortriterpenoids with potent antihepatitis, antitumor, and anti-HIV activities. This paper presents the enantioselective total synthesis of pre-schisanartanin C (**1**). An important step in the total synthesis of **1** is gold-catalyzed intramolecular cyclopropanation of a 1,8-enyne substrate bearing a secondary ester group at the propargylic position to prepare a bicyclo[6.1.0]nonane core. Additional highlights include (i) an asymmetric Diels−Alder reaction to install the initial C5 stereogenic center of **1** and (ii) a sequential Pd-catalyzed Stille coupling, regio- and stereoselective Sharpless asymmetric dihydroxylation, and a subsequent intramolecular lactonization to construct the side chain of **1**. The developed chemistry paves the way for the total syntheses of other family members bearing highly rigid bicyclo[6.1.0]nonane cores.



INTRODUCTION

Pre-schisanartanin C (Figure 1, **1**), which is a typical *Schisandra* nortriterpenoid,[1] was isolated from the medicinal plant *Schisandra propinqua* var. *propinqua* by Sun and coworkers in 2010.[2] The structure and relative configuration of **1** were determined using NMR spectroscopy; however, its absolute configuration is still unknown.

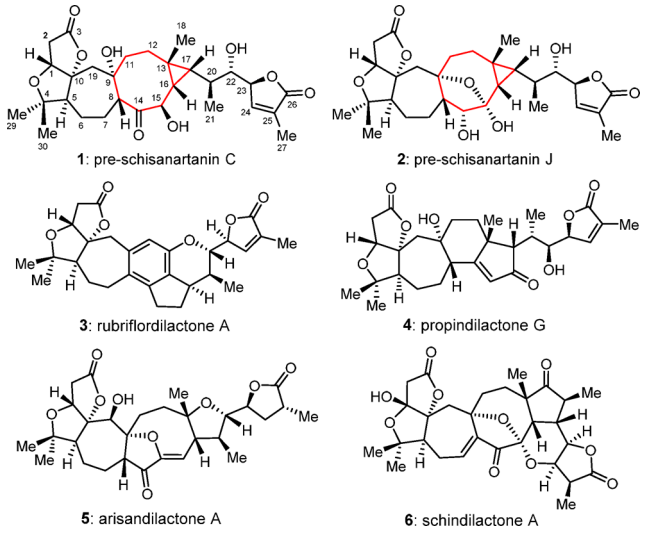


Figure 1. Selected naturally occurring nortriterpenoids (**1**−**6**).

Schisandra nortriterpenoids have recently attracted considerable attention because they possess potent antihepatitis, antitumor, and anti-HIV activities.[1] The total syntheses of several family members, including **3**−**6** (Figure 1), have been reported.[3] Compared to **3**−**6**, the most intriguing parts of **1** are its rare[4] and highly substituted bicyclo[6.1.0]nonane core bearing an all-carbon quaternary stereogenic center[5] at C13 and a highly labile α-hydroxy ketone motif nestled in the central medium-sized cyclooctane ring.[6] Hence, the synthesis of pre-schisanartanin C (**1**) presents challenges to existing synthetic methods and strategies.. Herein we present the total synthesis of **1** including the planning and experimentation that enabled us to reach this goal.

RESULTS AND DISCUSSION

To provide a proper setting, we need to briefly recapitulate our initially proposed synthetic strategy. From perspective of the total synthesis of **1**, we initially recognized the need to address two compelling synthetic challenges: (i) stereoselective generation of a highly functionalized bicyclo[6.1.0]nonane domain (i. e., DE rings) and (ii) stereoselective incorporation

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Communication

Asymmetric Total Synthesis of (−)-Spirochensilide A

Xin-Ting Liang, Jia-Hua Chen,* and Zhen Yang*

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ABSTRACT: An asymmetric total synthesis of (−)-spirochensilide A has been achieved for the first time. The synthesis features a semipinacol rearrangement reaction to stereoselectively construct the two-vicinal quaternary chiral centers at C8 and C10, a tungsten-mediated cyclopropene-based Pauson−Khand reaction to install the C13 quaternary chiral center, and a furan-based oxidative cyclization to stereoselectively form the spiroketal motif.

Spirochensilide A (**1**, Figure 1)[1] is a member of an emerging and biologically important class of natural products with a unique spirocyclic core[2,3] and has been isolated by Gao and co-workers from *Abies chensiensis*, which is an endemic Chinese plant.[4] The crude extracts and metabolites of the *Abies* species have been found to possess various bioactivities, including antitumor, antimicrobial, antiulcerogenic, anti-inflammatory, antihypertensive, antitussive, and central nervous system activities.[5] Biologically, **1** showed a moderate inhibitory effect on the NO production with 30% inhibition at the concentration of 12.5 μg/mL, indicating **1** could be a useful probe for study of inflammatory diseases.[6]

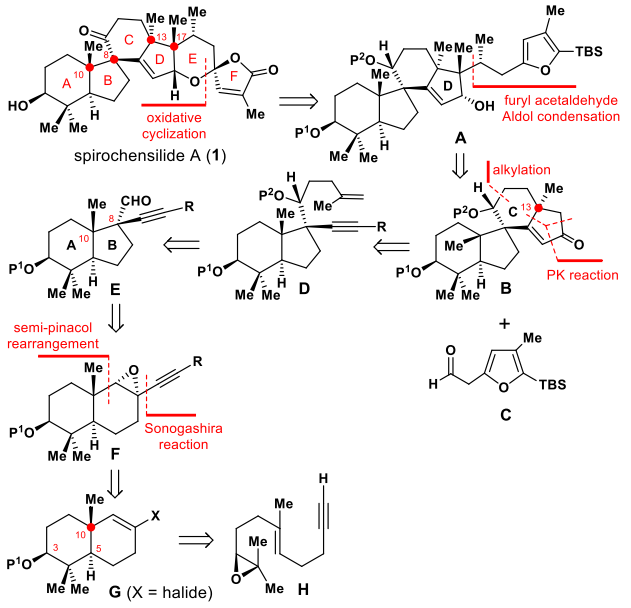

Figure 1. Retrosynthetic analysis of spirochensilide A (**1**).

The structure of **1** was determined on the basis of NMR spectroscopic data and single-crystal X-ray diffraction analysis. The structure contains two pairs of vicinal all-carbon quaternary chiral centers[7] (C8/C10 and C13/17), an unusual spiro[4.5]ring system (BC ring), and an anomeric spiroketal (EF ring).[8] Natural products bearing both quaternary chiral centers and spirocycles can impose conformational constraints to reduce the conformational entropy penalty upon binding to a protein target in a favorable geometry.[9]

Herein, we report our effort on the development of an approach for the asymmetric total synthesis of spirochensilide A (**1**). The synthesis features a semipinacol rearrangement and a tungsten-mediated cyclopropene-based Pauson−Khand (PK) reaction as key steps.

Figure 1 illustrates our retrosynthetic analysis. We envisioned that the anomeric spiroketal of **1** could be derived from furyl alcohol **A** via an intramolecular oxidative cyclization.[10] **A** was expected to be constructed from ketones **B** and **C** via a furyl acetaldehyde aldol condensation[11] as a key step. To construct the cyclopentenone bearing an all-carbon quaternary chiral center in intermediate **B**, we intended to employ the PK reaction[12] of enyne **D** because this reaction has been successfully applied in our total synthesis of the nontriterpenoid propindilactone G.[13] Enyne **D** was expected to be derived from aldehyde **E** with a pair of vicinal quaternary chiral centers at C8 and C10, which was envisioned to be derived from epoxide **F** through a semipinacol rearrangement.[14] **F** could be prepared via a sequential Pd-catalyzed Sonogashira reaction and epoxidation from vinyl halide **G**, which in turn could be prepared via a biomimetic cyclization of the functionalized isoprenoid polyene **H**.[15]

Our synthesis began by exploring the chemistry for an enantioselective preparation of enyne **8** (Scheme 1). We rationalized that a Lewis acid induced cyclization[16] of polyenoid **2** could enantioselectively afford halogenated decalin[18] **3** bearing three stereogenic centers at C3, C5, and C10 via a concerted cyclization process.[17] The selectivity results from the chair-like transition state were achieved via a

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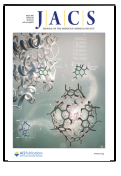

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Communication

Total Synthesis of (+)-Haperforin G

Wei Zhang, Zhenyu Zhang, Jun-Chen Tang, Jin-Teng Che, Hao-Yu Zhang, Jia-Hua Chen,* and Zhen Yang*

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ABSTRACT: A concise chemical synthesis of (+)-haperforin G in 20 steps from commercially available starting materials is achieved with the integration of the Co-catalyzed intramolecular Pauson−Khand reaction for the stereoselective construction of cyclopentanone bearing an all-carbon quaternary stereogenic center at the bridge-head position and the light-initiated photocatalysis for convergent and asymmetric cross-coupling of the unstabilized C(sp^3)-radical with an enone. The developed chemistry paves the way to synthesizing structurally diverse analogs of haperforin G (**6**).

A trace compound, (+)-haperforin G (**6**) (37 mg/25 kg), isolated from *Harrisonia perforata*,[1] is a newly discovered member of a biologically important class of limonoid tetranortriterpenoid natural products (Figure 1).[2] Haperforin

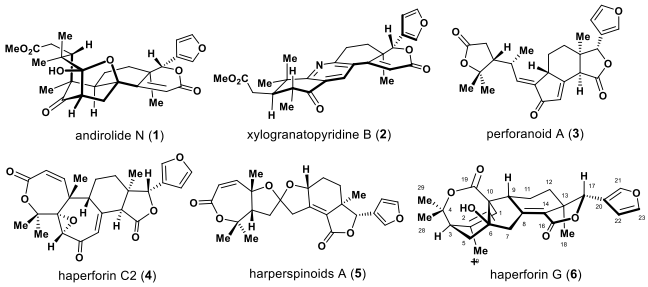

Figure 1. Haperforin G and selected limonoids.

G has captured the attention of the biomedical community, because it is a potent inhibitor of human 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) (IC_{50} 0.58 μM). It has, therefore, become an attractive new chemical entity for the treatment of a number of diseases involving metabolic disorders, such as Alzheimer's disease, vascular inflammation, cardiovascular disease, and glaucoma.[3]

Given the biological importance of haperforin G and the scarcity of its natural source, the development of a concise approach toward its asymmetric total synthesis and derivatizations will provide ample material to probe biological functions of **6** in 20 steps, which include a concise Co-catalyzed Pauson−Khand (PK) reaction[4] and a convergent photoredox catalysis[5] as key steps.

The structure of **6** contains a novel limonoid 6/5/6 tricyclic carbon skeleton bearing six stereogenic centers (including two all-carbon quaternary stereogenic centers), two lactones, and a 3-substituted furan ring. The most challenging aspect of synthesizing **6** is the realization of the enantioselective incorporation of its two all-carbon quaternary carbons (C10 and C13)[6] and one tertiary chiral alcohol (C6) and regioselective installations of trisubstituted (C1 and C2) and tetrasubstituted (C8 and C14) double bonds.

Figure 2 highlights our retrosynthetic analysis of **6**. With the aim of divergently accessing its other family members, we

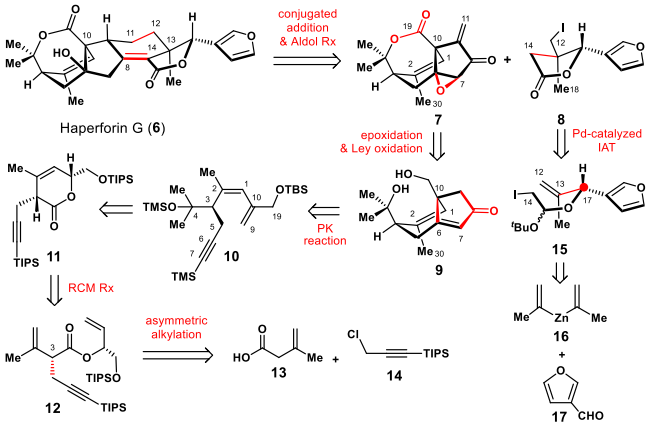

Figure 2. Retrosynthetic analysis of haperforin G.

designed a convergent strategy wherein two fragments (enone **7** and iodide **8**) would be joined through the C11−C12 bond via a visible-light photoredox catalyzed[7] alkyl radical coupling reaction followed by an intramolecular aldol reaction and dehydration, to assemble the polycyclic core. This would make use of the stereocenters in the two fragments **7** and **8** to influence the stereochemical outcome of the newly created ones. However, the implementation of such a convergent strategy in the total synthesis of haperforin G requires the

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CCS Chemistry

COMMUNICATION

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Synthesis of 4-*Desmethyl*-Rippertenol and 7-*Epi*-Rippertenol via Photoinduced Cyclization of Dienones

Zi-Chun Zhang[1†], Dan-Dan Zhao[1†], Zhong-Chao Zhang[1], Xin-Yu Tan[1], Jian-Xian Gong[1*], Jun-Kai Fu[2*] & Zhen Yang[1,3,4*]

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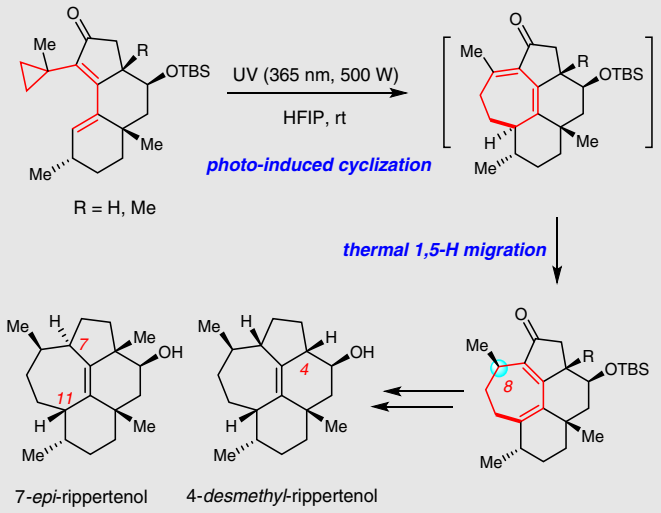
The synthesis of cycloheptanoid-based fused polycyclic frameworks is a challenge for organic chemists due to unfavorable entropic factors and ring strains. Herein, a concise synthesis of 4-*desmethyl*-rippertenol and 7-*epi*-rippertenol bearing a unique, [6,6,5,7]-fused tetracyclic framework is reported. The route features a novel photoinduced intramolecular cyclization of α-cyclopropyl dienone followed by an unexpected thermal 1,5-hydrogen migration, which provides efficient access to the fused seven-membered ring system in a stereochemically well-defined manner. Further density functional theory (DFT) calculations disclose that the stereoselectivity of this photoinduced process is mainly attributed to transition state conformation and steric effects.



Keywords: seven-membered ring, photoinduced cyclization, dienone, rippertenol, 1,5-hydrogen migration

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catalysts

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Review

Evolution of Pauson-Khand Reaction: Strategic Applications in Total Syntheses of Architecturally Complex Natural Products (2016–2020)

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Abstract: Metal-mediated cyclizations are important transformations in a natural product total synthesis. The Pauson-Khand reaction, particularly powerful for establishing cyclopentenone-containing structures, is distinguished as one of the most attractive annulation processes routinely employed in synthesis campaigns. This review covers Co, Rh, and Pd catalyzed Pauson-Khand reaction and summarizes its strategic applications in total syntheses of structurally complex natural products in the last five years. Additionally, the hetero-Pauson-Khand reaction in the synthesis of heterocycles will also be discussed. Focusing on the panorama of organic synthesis, this review highlights the strategically developed Pauson-Khand reaction in fulfilling total synthetic tasks and its synthetic attractiveness is aimed to be illustrated.

Keywords: metal-mediated reactions; Pauson-Khand reaction; cyclopentenones; natural products total syntheses

1. Introduction

The metal-mediated reaction plays an important role in constructing complex organic molecules [1–3]. The Pauson-Khand reaction (PKR), an effective set of annulation protocol defined in 1973 [4] for the construction of cyclopentenone-containing moieties, stands as a promising method to permit efficient cyclic frameworks. Its efficient and atom-economic elaboration to substituted cyclopentenones renders this process highly prized in the construction of architecturally complex natural products. Since reported more than 40 years ago [5–12], it has been developed with different metal catalytic systems, including Co [13–17], Rh [18–25], Ru [26–30], Ti [31–34], Ir [35–37], Ni [38], Mo [39,40], Fe [41]; and other metals could promote the PKR to build the heterocycle frameworks [42–44]. By identifying reactivity patterns for diverse PKR precursors in the prominent synthetic application, we aim to elevate this powerful reaction to a method of choice in the synthetic designation of complex biologically active entities.

1.1. Classic PK Reaction Catalyzed by Co

In 1973, I.U. Khand and P.L. Pauson found that the generation of enyne/$Co_2(CO)_6$ complex with olefin as substrates could lead to the formation of cyclopentenone. Moving forward, P.L. Pauson

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Photoredox-Catalyzed Isomerization of Highly Substituted Allylic Alcohols by C–H Bond Activation

Kai Guo[+], Zhongchao Zhang[+], Anding Li[+], Yuanhe Li, Jun Huang, and Zhen Yang**

Dedicated to Professor Henry N. C. Wong on the occasion of his 70th birthday

Abstract: *Photoredox-catalyzed isomerization of γ-carbonyl-substituted allylic alcohols to their corresponding carbonyl compounds was achieved for the first time by C–H bond activation. This catalytic redox-neutral process resulted in the synthesis of 1,4-dicarbonyl compounds. Notably, allylic alcohols bearing tetrasubstituted olefins can also be transformed into their corresponding carbonyl compounds. Density functional theory calculations show that the carbonyl group at the γ-position of allylic alcohols are beneficial to the formation of their corresponding allylic alcohol radicals with high vertical electron affinity, which contributes to the completion of the photoredox catalytic cycle.*

Introduction

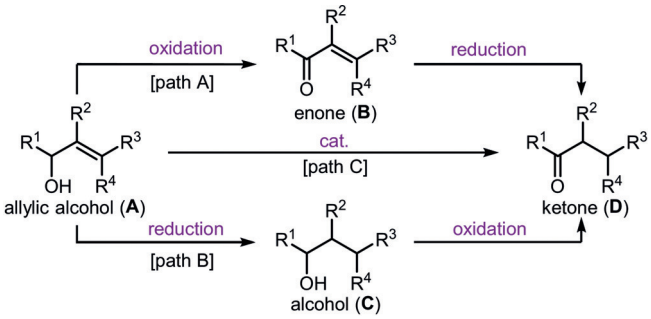
The development of chemical reactions for the isomerization of allylic alcohols to the corresponding carbonyl compounds is a fertile field in organic synthesis.[1] Conventional methods for this transformation usually follow a two-step sequential process (Scheme 1, path A or B), featuring an oxidation/reduction or reduction/oxidation pathway, which is uneconomical in terms of both atoms and steps.[1,2]

Considerable progress has been made in the past decades in metal-catalyzed catalytic isomerization of allylic alcohols to the corresponding carbonyl compounds in one-pot redox-neutral processes[3–8] (Scheme 1, path C).[9] However, the isomerization becomes more difficult as the number of substituent groups increases[1b] on the double bond, especially for secondary allylic substituted alcohols. This trend is explained by the fact that it is difficult for transition-metal complexes to bind and react with heavily substituted olefins, and not surprisingly, a limited number of methods[6d,7d] have been reported for the isomerization of allylic alcohols with a tetrasubstituted double bond.

Recently, photoredox-catalyzed bond activation as a redox-neutral and atom- and step-economical strategy has come to the forefront in organic synthesis.[10] We became interested in light-induced isomerization of highly substituted allylic alcohols to their corresponding carbonyl compounds through a process of C–H bond activation in the presence of hydrogen-atom-transfer (HAT) catalysts.[11]

We envisaged that the proposed isomerization could be initiated by single-electron oxidation (oxidative SET process) of a HAT catalyst in the presence of an excited photoredox catalyst (PRC*). The resultant radical cation species ($HAT^{\bullet+}$) could abstract the α-H atom from the corresponding allylic alcohol (**A**) to afford an allylic alcohol radical species (**F**), which could then undergo single-electron reduction (reductive SET process) to furnish an allylic alcohol anion (**G**), followed by protonation and tautomerization to give a ketone (**D**) (Scheme 2a).

However, in reality, the proposed reductive SET process of the allylic alcohol radical (**F**) is difficult,[10b] and therefore, prevents the formation of the allylic alcohol anion (**G**); as a result, the proposed photoredox catalysis could not occur. To better understand this issue, we conducted a computational experiment.[12] Our results indicated that the tendency for the reduction of allylic alcohol radicals (**F**) will increase as the vertical electron affinities[13] (EA_{vert}) of substituents at the γ-substitution position of allylic alcohols are increased. That is, allylic alcohols substituted with γ-carbonyl groups (-CONHMe, -CO_2Me, and -COMe) might have a stronger tendency to participate in the reductive SET process than allylic alcohols bearing γ-substituents such as -OMe, -Me, and -H groups (Scheme 2b).



Scheme 1. Approaches to the isomerization of allylic alcohols.

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Communication

Asymmetric Total Synthesis of (+)-Waihoensene

Yongzheng Qu, Zheyuan Wang, Zhongchao Zhang, Wendou Zhang, Jun Huang,* and Zhen Yang*

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ABSTRACT: The asymmetric total synthesis of (+)-waihoensene, which has a *cis*-fused [6,5,5,5] tetracyclic core bearing an angular triquinane, a *cis*-fused six-membered ring, and four contiguous quaternary carbon atoms, was achieved through a sequence of chemical reactions in a stereochemically well-defined manner. The total synthesis features the following: (1) Cu-catalyzed asymmetric conjugated 1,4-addition; (2) diastereoselective Conia-ene type reaction; (3) diastereoselective intramolecular Pauson−Khand reaction; (4) Ni-catalyzed diastereoselective conjugated 1,4-addition; and (5) radical-initiated intramolecular hydrogen atom transfer (HAT). Control experiments and density functional theory calculations support the proposed HAT process.

Polyquinanes constitute an important class of carbocyclic frameworks containing fused 5-membered rings (Figure 1) and are found in various natural products, such as terpenoids and steroids.[1] In 1997, Weavers and co-workers isolated (+)-waihoensene (**1**) from the New Zealand podocarp, *Podocarpus totara var. waihoensis*.[2] Structurally, **1** contains a highly congested and *cis*-fused tetracyclic core decorated with six contiguous stereogenic centers; among them, four are contiguous all-carbon quaternary carbon atoms (C3a, C5a, C9a, and C11a). Thus, **1** was widely regarded as a challenging target for total synthesis.[3]

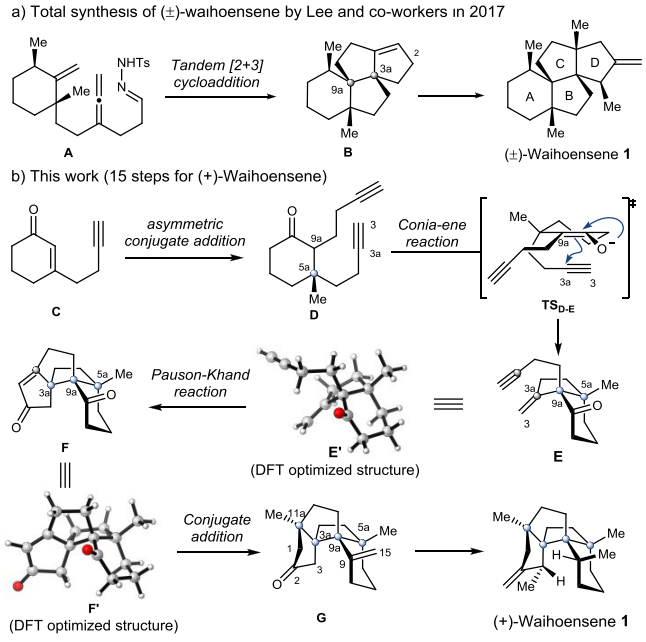

Figure 1. Total synthesis of (+)-waihoensene (**1**).

Given its structural complexity, **1** has been a focus of the synthetic community for many years.[4] In 2017, Lee and co-workers published an impressive synthesis of (±)-waihoensene for the first time in 18 steps, featuring a tandem [2 + 3] cycloaddition to construct the BCD tricyclic ring with two contiguous quaternary stereogenic centers[5] (Figure 1a).

We recently became interested in the total synthesis of complex natural products bearing all-carbon quaternary stereogenic centers,[6] and the total synthesis of **1** was a particular challenge involving such a structure.[7] Herein, we report our recent contribution for the asymmetric total synthesis of **1** in 15 steps with 3.8% overall yield. The cornerstone of our plan is the recognition of structural and stereochemical relationships between substrates **D**, **E**, and **F** and their corresponding products **E**, **F**, and **G**, which allowed us to construct the key intermediates **E**, **F**, and **G** in a highly diastereoselective manner.

As shown in Figure 1b, the initial chiral quaternary stereogenic center at C5a in diyne **D** could be installed via a Cu-catalyzed asymmetric conjugate addition[8] of enone **C**. From inspection of the favorable transition state $\mathbf{TS}_{\mathbf{D-E}}$ and the 3D structure of enyne **E** (see **E′** in Figure 1b), two of the three requisite quaternary stereogenic centers at C9a and C3a in the core of angular triquinane[9] **F** could be constructed diastereoselectively by a sequence of Conia-ene type reaction[10] of diyne **D** and intramolecular Pauson−Khand reaction[11] of enyne **E**. According to the results reported in Lee's total synthesis of waihoensene (**1**),[5] one of the four requisite quaternary stereogenic centers at C11a in intermediate **G** could be build up via a cuprate-mediated conjugated addition reaction. Thus, the asymmetric total synthesis of (+)-waihoensene (**1**) could be accomplished upon diastereoselective saturation of its C9−C15 double bond in **G**.

The total synthesis began with developing an asymmetric approach for enantioselective synthesis of diyne **7**. Initially, we

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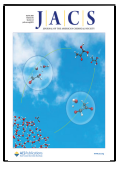

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Protecting-Group-Free Total Syntheses of (±)-Norascyronones A and B

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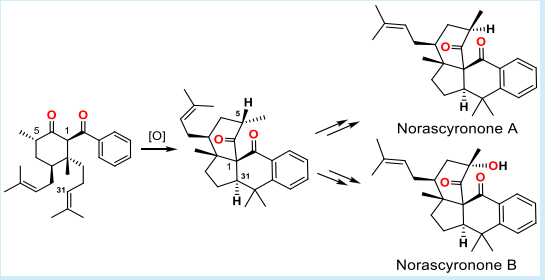
ABSTRACT: Protecting-group-free total syntheses of natural products norascyronone A and norascyronone B were accomplished in eight steps from the commercially available starting material 1-bromo-4-methoxy-2-methylbenzene. The key step was a Mn/Cu-mediated oxidative cascade annulation reaction that formed the tetracyclic core of the target molecules bearing vicinal bridge-head all-carbon quaternary chiral centers. Our investigation indicated that the C5 stereogenic center of norascyronone C plays a critical role in the proposed biomimetic oxidative reaction for B-ring formation.



Norascyronones A (**2**) and B (**3**) (Figure 1) were recently isolated from *Hypericum ascyron*. Both compounds

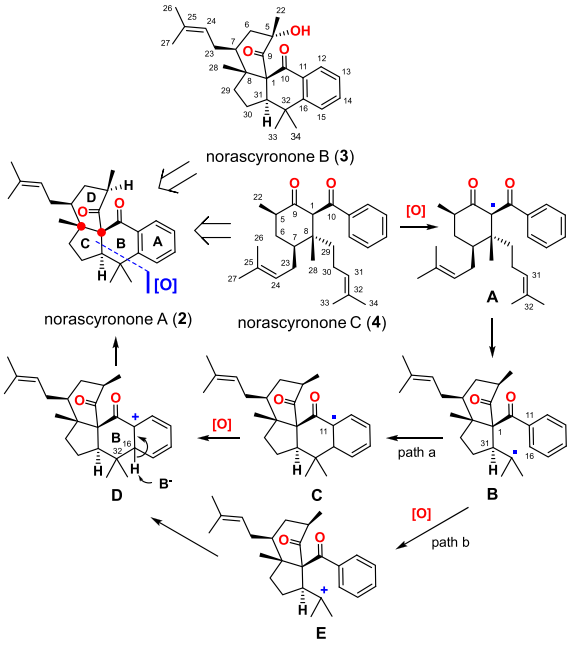


Figure 1. Proposed synthetic transformations.

contain a congested 6/6/5/6 polycyclic core bearing five stereogenic centers, two of which are all-carbon quaternary carbons (C1 and C8).[1] Their absolute configurations have been determined from X-ray diffraction data for **2** and experimental and calculated electronic circular dichroism spectra of **3**. Biologically, both compounds show cytotoxicities against the SK-BR-3 cell line (IC_{50} values of 4.3 and 7.8 μM, respectively).[1]

In the biosynthetic pathway proposed when these compounds were isolated,[1] **2** and **3** are postulated to be derived from norascyronone C (**4**) via an oxidative 1,3-dicarbonyl radical-initiated cascade cyclization through intermediates **A**−**D** (Figure 1). Compound **4** undergoes an oxidative 5-*exo*-*trig* radical cyclization to afford radical **B** through intermediate **A**. Further cyclization of **B** at C16 of the phenyl ring yields resonance-stabilized radical **C** (path a), which undergoes further oxidation to carbonium ion **D** and then aromatization via proton loss to give **2**. From a chemistry perspective, intermediate **B**, bearing a stable tertiary radical, might also undergo further oxidation to form stabilized carbocation **E**, as proposed by George and Lee,[2] which might trigger an intramolecular Friedel−Crafts reaction to form carbonium ion **D**, followed by aromatization to give **2**.

Among various oxidative transformations of enolized carbonyl moieties mediated by metal ions (such as Mn(III), Cu(II), Fe(III), and Ce(IV)),[3] Mn(III)-based oxidative radical cyclizations of carbonyl compounds with unactivated olefins[4] have attracted much interest because they allow unconventional and efficient access to a range of molecules[5] that cannot be easily synthesized using other methods.

As part of our continuing interest in the development of diastereoselective syntheses of complex natural products bearing vicinal bridged all-carbon quaternary chiral centers,[6] we were intrigued by the possibility of constructing the

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Asymmetric Total Synthesis of (−)-Guignardones A and B

Zhiming Yan, Chunbo Zhao, Jianxian Gong,* and Zhen Yang*

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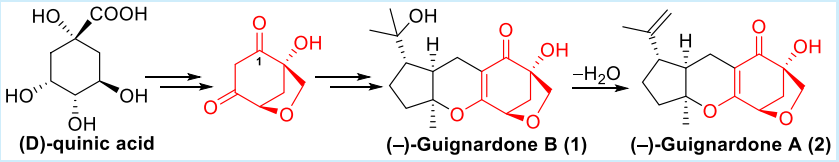

ABSTRACT: The asymmetric total synthesis of (−)-guignardones A (**2**) and B (**1**) has been accomplished. The highly oxidized 6-oxabicyclo[3.2.1]octane core was constructed from D-quinic acid via substitution/desulfurization reaction with thiophenol to forge the bridged ring scaffold, and a Pummerer rearrangement and 1,4-addition/elimination sequence was employed to install the β-carbonyl group at the congested C-1 position. A late-stage Knoevenagel condensation−6π-electrocyclization and directed hydrogenation formed (−)-guignardone B (**1**), which was subjected to dehydration to furnish (−)-guignardone A (**2**).

The 6-oxabicyclo[3.2.1] octane framework was first found in the natural products guignardones A−C, isolated by Tan and co-workers from the cultures of *Guignardia mangiferae* IFB-GLP-4, which are associated with the normal *Ilex cornuta* leaves, in 2010.[1] (Figure 1) Other members of this family of

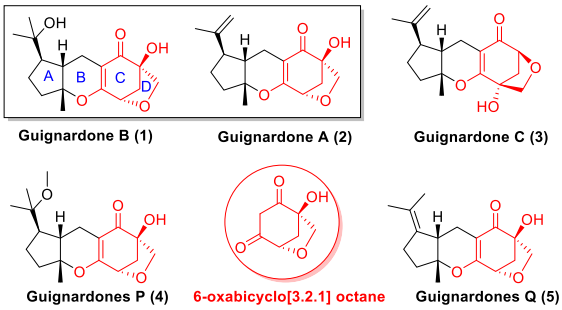

Figure 1. 6-Oxabicyclo[3.2.1] octane core fragment and structures of guignardones A (**2**) and B (**1**) and related natural products (**3**−**5**).

natural products were isolated[2] and found to possess the 6-oxabicyclo[3.2.1]octane core in common, which contains an oxygen on the bridged carbon center. More than 12 types of these tetracyclic meroterpenes have been isolated until now, and some of them exhibited interesting bioactivities, such as antibacterial[2a,d] and TLR3-regulating activities,[2b] cytotoxicity aginst MCF-7 cell lines,[2e] and inhibitory activity for *Candida albicans*. Guignardone B (**1**) exhibited the most potent inhibition of the growth of *Candida albicans* with an MIC value of 0.05 μM.[2d]

Structurally, guignardones A (**2**) and B (**1**) feature tricycloalternarenes (TCAs)[3] with an additional bridging tetrahydrofuran ring D, which possesses a highly oxidized 6-oxabicyclo[3.2.1]octane bearing a 1,3-diketone and a bridgehead hydroxyl group. The relatively uncommon structure of these tetracyclic meroterpenes attracted our interest for conducting the total synthesis of **2** and **1**. Recently, Ito and co-workers reported the first asymmetric total synthesis of tricyclic guignardones H (**6**) and I (**7**) by employing Knoevenagel condensation−6π-electrocyclization with the unsaturated aldehyde **8** and a novel 1,3-cyclohexanedione **9**, followed by a chemo- and stereoselective directed hydrogenation as the key steps and realized the construction of the A/B/C ring of guignardones (Figure 2a).[4] However, tetracyclic

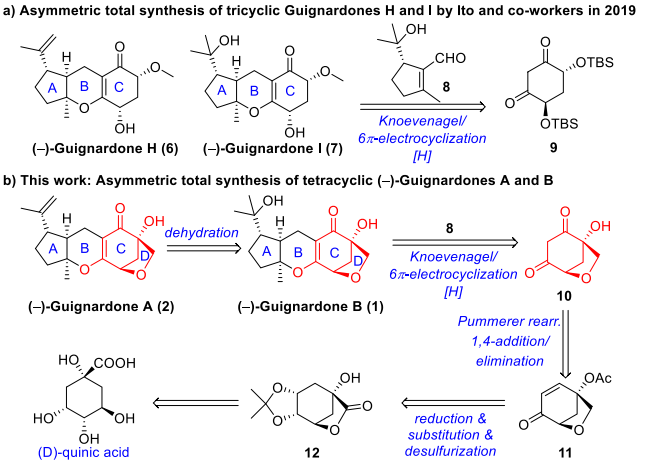

Figure 2. Total synthesis of guignardones.

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Concise gram-scale synthesis of Euphorikanin A skeleton through a domino ring-closing metathesis strategy†

Linlin Shi, ‡[a] Yingdong He,‡[a] Jianxian Gong *[a] and Zhen Yang *[ab]

Euphorikanin A is a diterpenoid possessing a highly congested and unprecedented 5/6/7/3-fused tetracyclic ring skeleton. To access the challenging chemical structure of Euphorikanins, an efficient total synthetic approach is described. The stereoselective synthesis of the core structure of Euphorikanin A has been achieved from a simple dienyne building block, and a domino ring-closing metathesis (RCM) strategy was used for the gram-scale synthesis of the highly strained Euphorikanin A core. This paves the way for the synthesis of structurally diverse Euphorikanins.

Natural products and small molecule drugs are vital to drug discovery and development, and play irreplaceable roles in the recognition and interaction with specific targets in organisms. In comparison to the typical acyclic or cyclic natural products, some natural products comprising strained ring systems have been extensively studied by chemists the world over, owing to the abilities of these molecules to recognize and bind to biological targets more tightly and selectively. Because of their high rigidity, these complex natural products can effectively suppress the entropy loss upon binding to the target.[1] While these complex natural products comprising highly strained frameworks have strong potential therapeutic relevance, their effective design and synthesis has always presented a challenging prospect to chemists, and continues to be an intriguing area of research.

The spurge family (*Euphorbiaceae*) encompasses several varieties of dicotyledon plants, which are famous for their wide distribution, great variability, and important biological activities.[2] The genus *Euphorbia* is the largest genus of *Euphorbiaceae*. Since the establishment of this of the genus by Linn,[3] more than 2000 species of the genus have been discovered across the world and over 80 species have been discovered in mainland China.[4] The plants of *Euphorbia* have been found to consist of flavonoids, diterpenoids, triterpenoids, sesquiterpenoids, phenolic acids, and other constituents.[5] There have also been several intense efforts towards the syntheses of the *Euphorbias*.[6] In 2016, Qiu and co-workers reported the discovery and identification of three novel biogenetically related diterpenoids, named pepluacetal (**1**), pepluanol A (**2**), and B (**3**), from the plants of *Euphorbia peplus*.[7] Compounds **1–3** exhibit the unprecedented 5/4/7/3, 5/6/7/3, and 5/5/8/3 ring systems, respectively, and displayed effective inhibitory activity at the kv1.3 potassium channel.[8] Further, a related natural product, Euphorikanin A (**4**), was also isolated from the roots of *Euphorbia kansui* in 2016 (Fig. 1).[7] This natural product presents a novel diterpenoid carbon skeleton possessing a highly strained 5/6/7/3-membered ring-fused tetracyclic ring architecture. In addition, Euphorikanin A comprises an unprecedented carbon skeleton in the natural kingdom.[9] It is noteworthy that compound **4** exhibited moderate cytotoxic activities with IC_{50}

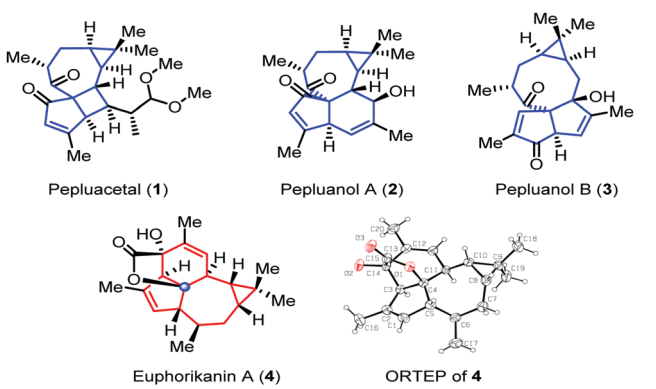

Fig. 1 Selected diterpenoids with tetracyclic carbon skeletons from *Euphorbia* peplus.

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† Electronic supplementary information (ESI) available: Experimental procedures and analytical data of the products. CCDC 1963179 (**15**). For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c9cc08830j

‡ These authors contributed equally to this work.

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Symmetric C···H···C Hydrogen Bonds Predicted by Quantum Chemical Calculations

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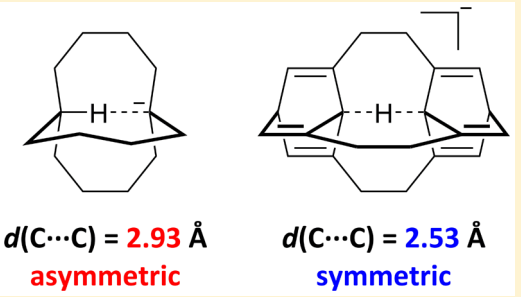
Supporting Information

ABSTRACT: The symmetry of hydrogen bonds is a fundamental question regarding hydrogen bonding interactions. Although asymmetric C−H···C hydrogen bonds are known in the literature, no symmetric C···H···C hydrogen bonds have been reported. Herein, we propose the theoretical possibility of symmetric C···H···C hydrogen bonds on the basis of quantum chemical calculations. Several bridged carbanions with intramolecular symmetric C···H···C hydrogen bonds were designed computationally. The key to this design is to shorten the C···C distance to ca. 2.5 Å, which is predicted to be necessary for a single-well C···H···C hydrogen bond.



INTRODUCTION

The importance of hydrogen bonds[1] in natural science has been demonstrated by an enormous number of experimental and theoretical studies.[2] A frequently discussed topic related to the nature of hydrogen bonding is the symmetry of hydrogen bonds.[3] If the electron donors are identical, the hydrogen bond is either symmetric (X···H···X) or asymmetric (X−H···X). These two kinds of hydrogen bonds can be described by single- and double-well potentials, respectively (Figure 1a). Some representative examples of symmetric hydrogen bonds are given in Figure 1b (X = N,[4] O,[5] and F[6]).

Besides nitrogen, oxygen, and fluorine, carbon is also known to be more electronegative than hydrogen.[7] Thus, not only a C−H bond can act as the hydrogen bond donor but also a carbon atom may serve as the hydrogen bond acceptor.[2b,c] Even C−H···C,[2b,8] C−H···π[2b,c] and π···H$^+$···π[9] hydrogen bonds are known in the literature. To the best of our knowledge, all the reported C−H···C hydrogen bonds are asymmetric and no symmetric C···H···C hydrogen bonds have been reported, which is in sharp contrast to the other elements (N, O, and F) in the same period (row) of the periodic table. Therefore, it is important to answer the question of whether symmetric C···H···C hydrogen bonds exist or not, which may shed more light on the nature of hydrogen bonding.

Here, we propose the theoretical possibility of symmetric C···H···C hydrogen bonds. Several bridged carbanions with intramolecular symmetric C···H···C hydrogen bonds were predicted by quantum chemical calculations. To the best of our knowledge, these carbanions are the first examples of symmetric C···H···C hydrogen bonds.

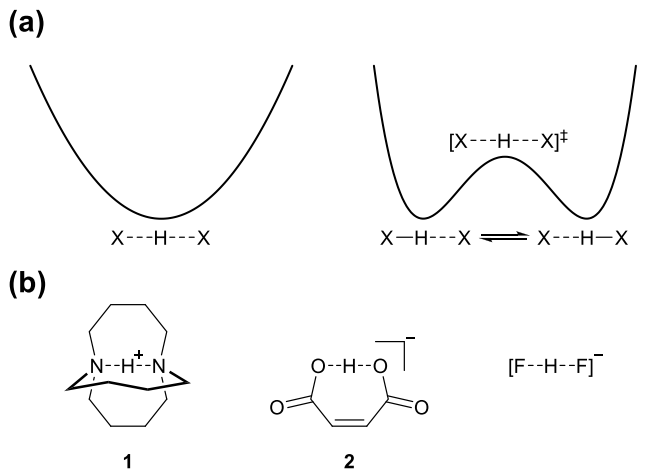


Figure 1. (a) Single- and double-well potentials for hydrogen bonding. (b) Representative examples of symmetric hydrogen bonds.

COMPUTATIONAL METHODS

All quantum chemical calculations were performed with Gaussian 09.[10] For ab initio calculations, all electrons were included in the correlation calculations. For density functional theory (DFT) calculations, pruned integration grids with 99 radial shells and 590 angular points per shell were used. Potential energy surface scans were carried out at either the CCSD(T)/aug-cc-pVTZ//MP2/aug-cc-pVTZ level[11] or the ωB97XD/6-311+G(d,p) level.[12] Geometry optimizations of the stationary points were carried out at the ωB97XD/6-311+G(d,p) level. We chose this level of theory based on our previous ab initio benchmark study on carbon-to-carbon proton transfers.[13] Unscaled harmonic frequency calculations at the same level were performed to validate each structure as either a minimum or a transition state and to evaluate its zero-point energy and thermal corrections at 298 K and 1 atm. Quasiharmonic corrections were

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Article

Mechanistic Study on Gold-Catalyzed Cycloisomerization of Dienediynes Involving Aliphatic C−H Functionalization and Inspiration for Developing a New Strategy to Access Polycarbocycles

Yi Wang, Pei-Jun Cai, and Zhi-Xiang Yu*

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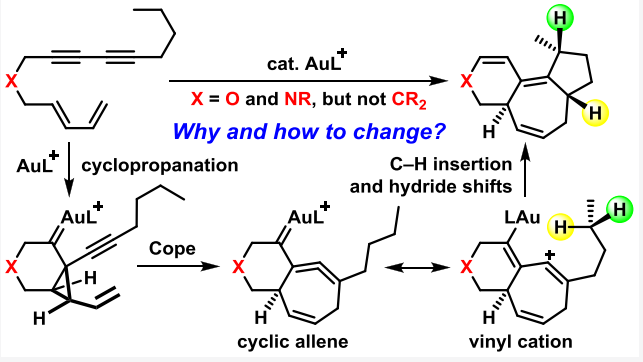
ABSTRACT: Previously, we developed a gold-catalyzed cycloisomerization of dienediynes to synthesize the fused 6,7,5-tricyclic compounds. This reaction involves aliphatic C−H functionalization under mild conditions with high regio- and diastereoselectivities. Herein, we present a combined density functional theory (DFT) and experimental study to understand its mechanism. The reaction starts with a 6-*endo*-dig cyclization to generate a *cis*-1-alkynyl-2-alkenylcyclopropane. Then, a Cope rearrangement takes place to give a seven-membered-ring allene intermediate, whose central carbon atom possesses vinyl cation character and thus is highly reactive toward aliphatic C−H insertion. After the C−H insertion, two successive [1,2]-hydride shifts then occur to give the tricyclic product and to complete the catalytic cycle. Notably, steric effect induced by the bulky ligand is found to be important for the diastereocontrol in the C−H insertion step. DFT calculations suggested that the malonate-tethered substrate utilized in our previous work may undergo an undesired 5-*exo*-dig cyclization under gold catalysis, which could be the reason why the desired fused 6,7,5-tricarbocyclic product was not generated. These mechanistic insights then guided us to design substrates with a shortened carbon tether in the present work to inhibit the *exo*-dig cyclization so that the tandem cyclopropanation/Cope rearrangement/C−H functionalization could occur to construct polycarbocycles containing a seven-membered ring. This prediction was supported by new experiments, providing a new strategy to access fused 5,7,5-tricyclic and 5,7,6,6-tetracyclic carbocycles. In addition, how the substituents affect the chemoselectivity was also investigated.



INTRODUCTION

Over the past decades, gold catalysis has become a convenient tool for the construction of molecular complexity under mild conditions.[1] Among the gold-catalyzed transformations, cycloisomerization of unsaturated hydrocarbons, such as enynes,[2] dienynes,[3] trienynes,[4] diynes,[5] allenes,[6] allenenes,[7] allenynes,[7] and allenedienes,[8] has attracted extensive attentions due to its capability of generating various types of valuable cyclic compounds from simple acyclic starting materials. Notably, these reactions have been widely applied in the synthesis of natural products and pharmaceuticals.[9]

Previously, we developed a gold-catalyzed tricyclization of dienediynes to construct fused 6,7,5-tricyclic compounds in a diastereoselective manner (Scheme 1).[10] For instance, under the catalysis of [(MeCN)Au(JohnPhos)]SbF_6 (**A**; JohnPhos = 2-(di-*tert*-butylphosphino)biphenyl), Echavarren's catalyst,[11] the cycloisomerization of oxygen-tethered dienediyne **1** proceeded smoothly at room temperature to furnish the tricyclic product **2** in 74% yield with >20:1 diastereomeric ratio (dr). In such a transformation, three stereogenic centers, three C−C bonds, and three rings are simultaneously constructed with high efficiency. It is also noteworthy that this reaction merges gold-catalyzed formal (4 + 3) cycloaddition[4,8] with C−H functionalization, which is currently one of the most prevailing research frontiers in chemistry.[12,13] The catalytic reaction of nitrogen-tethered dienediynes **3** and **5** also worked very well. One limitation of our method is that malonate-tethered substrate **7** did not give the desired product **8** under the standard conditions. In this case, other reaction products could not be identified and only the starting material was partially recovered.[14]

A catalytic cycle was previously proposed by us (Figure 1).[10] We suggested that the reaction starts with the generation of gold−substrate complex **B**, which undergoes an intramolecular cyclopropanation to form *cis*-1-alkynyl-2-alkenylcyclopropane **C**.[2] Then, a Cope rearrangement takes place, leading to cyclic allene **D**,[15] which triggers an aliphatic C−H insertion to furnish tricyclic intermediate **E**. Finally, two successive [1,2]-hydride shifts occur to complete the catalytic cycle.[16] To support or disprove this

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Transient-axial-chirality controlled asymmetric rhodium-carbene C(sp^2)-H functionalization for the synthesis of chiral fluorenes

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In catalytic asymmetric reactions, the formation of chiral molecules generally relies on a direct chirality transfer (point or axial chirality) from a chiral catalyst to products in the stereo-determining step. Herein, we disclose a transient-axial-chirality transfer strategy to achieve asymmetric reaction. This method relies on transferring point chirality from the catalyst to a dirhodium carbene intermediate with axial chirality, namely a transient-axial-chirality since this species is an intermediate of the reaction. The transient chirality is then transferred to the final product by C(sp^2)-H functionalization reaction with exceptionally high enantioselectivity. We also generalize this strategy for the asymmetric cascade reaction involving dual carbene/alkyne metathesis (CAM), a transition-metal-catalyzed method to access chiral 9-aryl fluorene frameworks in high yields with up to 99% *ee*. Detailed DFT calculations shed light on the mode of the transient-axial-chirality transfer and the detailed mechanism of the CAM reaction.

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Research Article

Lewis Base-Catalyzed Amino-Acylation of Arylallenes via C−N Bond Cleavage: Reaction Development and Mechanistic Studies

Zheng-Bing Zhang, Yusheng Yang, Zhi-Xiang Yu,* and Ji-Bao Xia*

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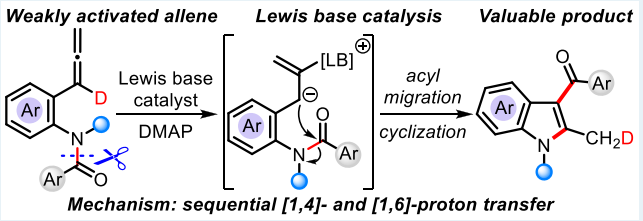
ABSTRACT: Lewis base-catalyzed transformations of allenes have received much attention over the last decades. However, this type of reaction has so far been limited to activated allenes bearing an electron-withdrawing group. On the other hand, cleavage of an amide C−N bond to forge other chemical bonds has been widely reported but restricted to low atom economy due to the waste of the amine moiety of amides. We initiated a project of metal-catalyzed amino-acylation of allenes via cleavage of amide C−N bonds. Surprisingly, an amino-acylation of weakly activated aryl allenes was discovered via Lewis base catalysis, providing 2-methyl-3-aroylindole products, "privileged structures" in drug discovery. This is a unique example of Lewis base catalysis of weakly activated allenes, which was not reported yet. Extensive experimental and computational studies have been conducted to provide insight into the reaction mechanism. The nucleophilic addition of Lewis base catalyst to aryl allene is the rate-limiting step. A challenging [1,3]-proton transfer is realized by nitrogen anion intermediate assisted sequential [1,4]- and [1,6]-proton transfer in the reaction pathway.

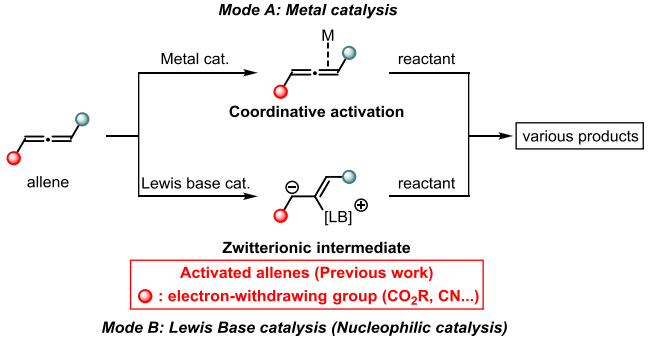


KEYWORDS: *Lewis base catalysis, C−N bond cleavage, proton transfer, weakly activated allenes, 3-aroylindoles*

INTRODUCTION

The allenes are three-carbon functional groups possessing a 1,2-diene moiety and serve as valuable synthetic precursors for the construction of highly complex target molecules of biological and industrial importance.[1] Coordinative activation of the cumulated double bonds with metal catalyst is one of the most popular reaction modes for transformation of allenes, which facilitate the attack of nucleophiles to form a new C−C or C−heteroatom bond in an inter- or intramolecular fashion (Scheme 1, mode A).[2] Transiton-metal catalyst, such as Pd, Rh, Ir, or Ru, have been widely used in the conversion of allenes by coordinative activation, mostly via a π-allyl metal intermediate.[2a,d,3] Because of their soft and carbophilic character, the gold or platinum catalysts have also been widely used for the selective activation of allenes in cyclization reactions.[4] Another important mode for allene activation is Lewis base catalysis, also named nucleophilic catalysis (Scheme 1, mode B).[5] This type of reaction starts from a nucleophilic addition of allene with a Lewis base catalyst, such as phosphine, to generate a zwitterionic intermediate.[6,7] Countless catalytic transformations of allenes have been reported affording useful products via Lewis base catalysis. However, all of these reactions are limited to activated allenes bearing an electron-withdrawing group, for example, allenyl esters. To date, there is no example of catalytic transformations of nonactivated or weakly activated allenes via Lewis base catalysis, such as aryl allenes or alkyl allenes. This may be due to the high activation barrier of nucleophilic addition of the central carbon atom of nonactivated or weakly activated allene with Lewis base catalyst, which kinetically disfavors the formation of a zwitterionic intermediate.[8] To the best of our knowledge, only one stoichiometric addition reaction of weakly

Scheme 1. Two General Activation Modes for the Transformation of Allenes



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Synergy of activating substrate and introducing C–H···O interaction to achieve Rh_2(II)-catalyzed asymmetric cycloisomerization of 1,*n*-enynes

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We report the first Rh_2(II)-catalyzed asymmetric cycloisomerization of activated enynes to provide cyclopropane-fused tetrahydropyridines in good yields and excellent enantioselectivities under mild conditions. The activated group, CHZ (Z is electron-withdrawing group (EWG)), in the enyne substrates exerts two synergetic roles, one is to activate alkyne for the cyclopropanation reaction; the other is to introduce the C–H···O interaction between substrate and catalyst (reducing the energy barrier of the reaction). This double-mode activation was supported by both density functional theory (DFT) calculations and experimental tests. This strategy was also extended to other CH_2Z (Z can be OH, OMe, F) as activating groups that made the CH_2 more acidic so that the substrates could also form increased C–H···O interaction with the catalyst.

asymmetric cycloisomerization, chiral cyclopropane, dirhodium catalysis, carbene

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1 Introduction

Chiral cyclopropane-annulated bicyclic system represents a kind of unique and important motif, which has been found in many natural products and bioactive molecules [1]. Among the reported synthetic methods towards these bicyclic scaffolds, transition metal-catalyzed asymmetric cycloisomerization of 1,*n*-enynes is one of the most efficient methods [2]. The widely used chiral catalysts for the asymmetric transformations are based on Au and Pt complexes [3,4]. Chiral dirhodium(II) complexes, structurally well-defined paddlewheel compounds with Rh_2^{4+} motif, have been known to catalyze carbene-transfer and nitrene-transfer reactions. Their structural uniqueness and excellent stereoselection in catalytic chemical reactions place them among the most important asymmetric catalysts employed for chemical transformations, especially in carbene chemistry by the decomposition of diazo compounds [5]. However, they were rarely used to activate the carbon-carbon triple bond. This is mainly because the Rh_2(II) unit has too low alkynophilicity to activate an alkyne (Scheme 1(a)) [6]. In our preliminary reaction condition screening, we found that dirhodium(II) nonfluorocarboxylate complexes $Rh_2(O_2CR)_4$ displayed very low reactivity and asymmetric induction (Scheme 1(b)). These negative results further implied the low alkynophili-

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Remote γ-C(sp^3)−H Alkylation of Aliphatic Carboxamides via an Unexpected Regiodetermining Pd Migration Process: Reaction Development and Mechanistic Study

Ya Li,[§] Pan Zhang,[§] Yue-Jin Liu, Zhi-Xiang Yu,* and Bing-Feng Shi*

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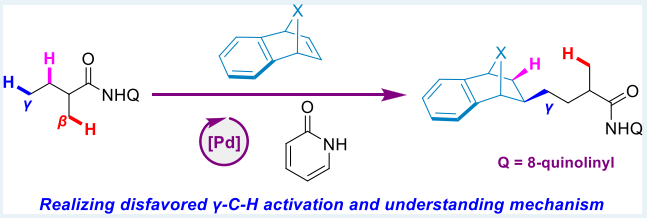
ABSTRACT: In the presence of accessible β-C−H bonds, γ-C−H activation of saturated aliphatic carboxamides remains unresolved because β-C−H activation is kinetically favored. This is almost a dogma in the development of C−H activation reactions. Here we report a strategy to change this dogma, as we have found that a Pd-catalyzed, ligand-enabled remote γ-alkylation of saturated aliphatic carboxamides can be realized in the presence of more accessible β-C−H bonds by using strained bicyclic alkenes as the coupling partners. Density functional theory calculations and experiments suggested that the realization of the present reaction is due to a change in the regiodetermining step from commonly encountered irreversible C−H activations, which are reversible here, to an unexpected Pd migration process, which is regiodetermining. This is a new strategy to achieve γ-C−H activation, compared with the previous strategy, making γ-C−H activation both the turnover- and regiodetermining step.



Realizing disfavored γ-C-H activation and understanding mechanism

KEYWORDS: *C−H activation, site-selective, remote selectivity, ligand promotion, 1,4-Pd migration*

INTRODUCTION

Pd-catalyzed aliphatic C−H functionalization has become a powerful synthetic tool for rapidly increasing molecular complexity and diversity.[1−13] To be synthetically useful, strategies that selectively cleave one single C−H bond among many chemically similar ones present in organic molecules must be developed.[14−17] Directing groups (DGs) are typically used to achieve this goal by positioning the palladium catalyst near a target C−H bond and enabling the selective cleavage via cyclopalladation.[18] Among various functionalities capable of assisting C−H activation, carboxylic acids and their derivatives are attractive because of their ubiquity and synthetic versatility.[18] Nevertheless, most carboxyl-directed aliphatic C−H activation reactions proceed through a kinetically favored five-membered palladacycle, thus enabling functionalization at the β-position (Scheme 1a). The direct functionalization of aliphatic C−H bonds at the remote γ-position remains a formidable challenge because of the difficulty of forming the kinetically less favored six-membered palladacycle. To date, only scattered examples of γ-C(sp^3)−H functionalizations have been reported. Corey,[19] Chatani,[20] Chen,[21,22] Shi,[23] and Maiti[24−27] have elegantly demonstrated γ-C(sp^3)−H functionalizations assisted by strong bidentate amide DGs.[6−9,28] The Yu group pioneered Pd(II)-catalyzed γ-C−H olefination, carbonylation, and arylation using the combination of a quinoline-based ligand and a weakly coordinating amide DG.[29,30] Recently, Maiti and our group also independently reported *N*-protected amino acid assisted γ-C(sp^3)−H arylation of free aliphatic acid.[31,32] It should be noted that *these reactions are limited to carboxamides without primary or secondary β-C−H bonds* (Scheme 1b). Further advances in this field should concentrate on selective functionalization of γ-methyl C−H bonds of aliphatic carboxylic acids and their derivatives in the presence of β-methyl and/or β-methylene C−H bonds. We think that this can be realized by the use of two strategies.

It is believed that the inherent β-selectivity is governed kinetically because β-C−H activation is more geometrically accessible than γ-C−H activation. The C−H activation steps are followed by fast β-C−Pd bond transformation and functionalization. Consequently, C−H activation becomes both the rate-determining and regiodetermining step. Therefore, strategy I for realizing γ-C−H activation in the presence of β-C−H bonds is to make γ-C−H activation more favorable than β-C−H activation by designing new DGs. Yu and co-workers designed new strained DGs that were assembled to generate the geometrically favored six-membered palladacycles in order to realize strategy I for remote C−H functionalizations of amines and alcohols.[33,34] Those DGs are not applicable to aliphatic carboxylic acids and their derivatives.

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Cycloaddition

Mechanism and Regioselectivity of Intramolecular [2+2] Cycloaddition of Ene–Ketenes: A DFT Study

Xing Fan,[a] Pan Zhang,[a] Yi Wang,[a] and Zhi-Xiang Yu*[a]

Abstract: Intramolecular [2+2] cycloaddition of ene–ketenes gives either fused-ring (via normal [2+2] cycloaddition) or bridged-ring (via cross-[2+2] cycloaddition) cyclobutanones. For example, terminal ene–ketenes give the fused-ring cycloadducts, whereas dimethyl-substituted ene–ketenes furnish bridged-ring cycloadducts. For monomethyl-substituted ene–ketenes, both [2+2] cycloadducts are generated. However, there are no systematic theoretical studies on such regiochemistry in the literature. Herein, we report our DFT study on the mechanism and regioselectivity of these intramolecular [2+2] cycloadditions. DFT calculations reveal that both normal and cross-[2+2] cycloadditions are concerted processes. The normal [2+2] cycloaddition transition state is forming an internal carbocation while the cross-[2+2] cycloaddition transition state is generating an external carbocation (see Scheme 1 of the paper). On the basis of the relative stability of these carbocations, which is affected by both the tether and the substituent(s) on the alkene, a regiochemistry prediction model is proposed to understand and predict the reaction outcome.

Introduction

[2+2] cycloaddition of ketenes and alkenes is one of the most powerful reactions for cyclobutanone synthesis.[1] This reaction, which is one of the so-called Staudinger ketene cycloadditions, has been widely used in the total synthesis of natural products.[2] In addition, the resulting cyclobutanone products and their derivatives (for example, cyclobutanols) have been used as substrates in transition-metal-catalyzed C–C bond activation reactions.[3] The [2+2] cycloaddition of ketenes with alkenes, which is close to the symmetry-forbidden [2+2] cycloaddition of two alkenes in terms of the reaction format, was regarded as an exception to the Woodward–Hoffmann rules.[4] Due to this, intensive studies on the mechanism of [2+2] cycloaddition of ketenes and alkenes have been carried out.[4] Now it is accepted that this [2+2] cycloaddition is concerted and starts from the interaction between alkene's HOMO and ketene's LUMO (Figure 1). The secondary orbital interaction between ketene's π(C=C) orbital and alkene's LUMO has been used to explain why a perpendicular transition state is required for the intermolecular [2+2] cycloaddition. The proposed orbital interaction for this symmetry-allowed reaction is $[_{\pi}2_s + (_{\pi}2_s + _{\pi}2_s)]$.[4c,4d] Charge separation model was also proposed to account for the regioselectivity.[4f] Recently, Lewis-acid-catalyzed intermolecular [2+2] cycloaddition of ketenes and alkenes have also been developed[1d] and its mechanism has been investigated.[4h]

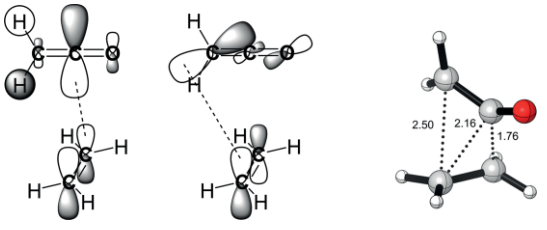

Figure 1. The main (left) and secondary (middle) orbital interactions between ketene and alkene and the [2+2] cycloaddition transition state (right).

Some examples for intramolecular [2+2] cycloaddition of ketenes (and keteniminium salts, which are not discussed in this work) with alkenes are shown in Table 1.[5] Two types of intramolecular [2+2] cycloadducts could be obtained, depending on the substitution pattern of the ene–ketene substrates, which were in situ generated from acyl chlorides. One type is the normal [2+2] cycloaddition that gives bicyclic 5/4 products (bicyclic 6/4 products can also be formed by using elongated substrates). The other type is the cross-[2+2] cycloaddition, giving rise to the bridged-ring products. Similar intramolecular [2+2] cycloaddition of ene–ketenimines, which were in situ generated from 1,6-enynes, was also reported.[6] Other intramolecular [2+2] cycloadditions of ketenes have been developed, including the intramolecular [2+2] cycloaddition of in situ generated ketenes from alkynyl ethers and cyclobutenones[7] and the intramolecular [2+2] cycloaddition of ketenes with allenes.[8]

Previous theoretical studies have been focused on the intermolecular [2+2] cycloaddition of ketenes with alkenes. However, for intramolecular [2+2] cycloaddition of ene–ketenes,[9] no systematic theoretical rationale for the regioselectivity has been reported, which is now disclosed in this paper.

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Chinese Journal of Organic Chemistry REVIEW

过渡金属催化环加成反应合成八元碳环研究进展(2010～2020)

王路宁 余志祥*
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生物有机与分子工程教育部重点实验室 北京 100871)

摘要 八元碳环结构广泛存在于天然产物、药物分子(如紫杉醇和瑞他帕林)和香料分子等功能分子中. 许多含有八元碳环的天然产物如 vinigrol 和 ophiobolin(蛇孢假壳素)都显示出较好的生物活性. 因此, 合成这些含有八元碳环的分子将会为药物化学、化学生物学、香料化学、材料化学和其它科学的发展提供分子基础. 八元碳环的合成一直是有机合成化学中的挑战之一. 为了迎接这一挑战, 许多卓有成就的化学家发展出了许多金属催化的环加成反应以合成八元碳环, 从而为高效、原子经济性和步骤经济性地合成目标分子提供帮助. 在 2010 年时, 我们曾经对利用过渡金属催化的环加成反应制备八元碳环这一前沿方向进行过总结. 本综述总结了 2010～2020 年期间该领域的最新进展, 内容包括新型环加成反应的发展、环加成反应的应用以及机理研究, 希望可以为关注此领域的有机化学家提供一定的启发和有益的指引, 同时鼓励更多合成化学家利用这些环加成反应进行各种功能分子的合成, 并发展出更多用于八元碳环合成的高效方法.

关键词 八元碳环; 过渡金属催化; 环加成; 全合成

Transition-Metal-Catalyzed Cycloadditions for the Synthesis of Eight-Membered Carbocycles: An Update from 2010 to 2020

Wang, Lu-Ning Yu, Zhi-Xiang*
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Abstract Eight-membered carbocycles are widely found in natural products with significant biological activities and other molecules ranging from perfumes to potential materials. Therefore, accessing these eight-membered carbocycle embedded molecules is important for drug discovery, biological investigation, fragrance industry, material development and many other fields. However, the synthesis of eight-membered carbocycles is still posing challenges to synthetic chemists. Hence, tremendous efforts have been endeavored by many leading chemists to discover and develop new reactions in order to synthesize eight-membered carbocycles. Among these reactions, transition-metal-catalyzed cycloadditions of [m+n], [m+n+o], [m+n+o+p] have evolved as powerful tools to achieve this aim. This topic has been reviewed in 2010. Summarized here are many new developments in this field and applications of the previously developed reactions in natural product synthesis since then.

Keywords eight-membered carbocycles; transition metal catalysis; cycloaddition; total synthesis

Molecules containing eight-membered carbocycles exist widely in natural products.[1] Many of these natural products have significant biological activities and some of them have been developed as useful drugs, such as anticancer drug taxol[2] (from *yew*) and antibacterial drug retapamulin[3] (a derivative from *pleuromutilin*). Thus, obtaining these natural products and their analogs for further investigation in medicinal chemistry, chemical biology and other related areas has become paramount. Considering that isolation of these molecules from natural resources usually affords very limited amounts of compounds, which prevents both direct extensive biological studies of these natural products and further derivatization of these molecules to obtain their analogs, chemical synthesis in most cases is the only way to achieve this goal. In addition, chemical synthesis can in principle provide "flexible" derivatized products compared

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analytical chemistry

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Ultrasensitive Multiplex Detection of Single Nucleotide Polymorphisms Based on Short-Chain Hybridization Combined with Online Preconcentration of Capillary Electrophoresis

Qian-Yu Zhou, Li-Juan Wang, Ying Liu, Xin-Ying Zhong, Jia-Hui Dong, Ying-Lin Zhou,* and Xin-Xiang Zhang

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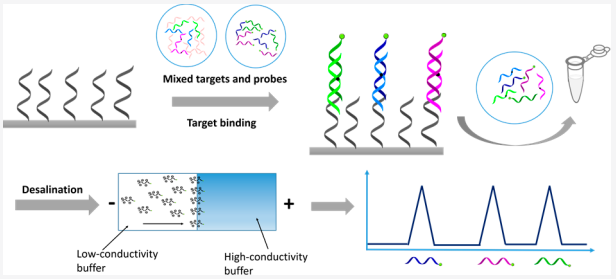
ABSTRACT: Reliable multiple single nucleotide polymorphisms (SNPs) detection at low abundance is of great significance for disease diagnosis and biomedical research. Herein, we have developed a novel and simple method for multiple SNPs detection combining solid-phase capture by specific hybridization with online preconcentration of capillary gel electrophoresis-laser-induced fluorescence (CGE-LIF). The method presents an excellent performance due to its favorable traits: the solid-phase short-chain hybridization ensures the high specificity of SNP detection; the effective separation ability of CGE can easily achieve multiplex detection; the simple online preconcentration significantly improves the detection sensitivity of fluorescent probe by nearly 100-fold. For a single SNP target, the assay achieves a limit of detection as low as 0.01−0.02% for three different *NRAS* mutations in the same codon. For multiple SNP targets, as low as 0.05% abundance can be easily realized. Our method is simple, efficient, ultrasensitive, and universal for multiple SNPs detection without complex enzymatic or chemical ligation reaction, which shows great potential in early clinical diagnosis.



Single nucleotide polymorphisms (SNPs) are the most common forms of base mutations in the human genome.[1] The detection of SNP is of great importance for disease diagnosis, drug discovery and biomedical research.[2,3] Generally, one type of disease is always connected to several mutation types and is associated with different mutation sites of the same mutation gene.[4] Colon cancer is closely related to not only multiple genes such as *KRAS*, *NRAS*, and *BRAF* genes but also same codon12 mutations in *KRAS* gene including 12ASP, 12VAL, 12ALA, and so on.[5] Simultaneous multiple SNPs detection is of great significance in improving the accuracy of disease diagnosis and providing the targeted therapy for individuals. Tumor-specific mutation gene as a biomarker for the early diagnosis and treatment of cancer is usually found at low abundance in samples.[6−8] It is necessary to develop a simple analytical technique that can achieve multiple SNPs detection at low abundance.

Many efforts have been dedicated to realizing the multiple SNPs detection. Novel sequencing assays, such as next-generation sequencing using the DNA barcodes, have a limit of detection (LOD) of 0.1−1% for multiple gene mutations.[9−11] However, these assays are limited due to expensive instruments and consuming time. For PCR approaches, which can combine different fluorophores for multiplex DNA melting analysis, the LODs for mutant alleles are about 1−3%,[12−14] but the complex analysis of the melting data and identification of the allele of each target are required. Compared with conventional PCR, digital PCR (dPCR) and droplet digital PCR (ddPCR) can achieve lower LODs in the range from 0.006−0.06%.[15−18] However, these methods suffer from time-consuming steps and high costs. Moreover, an additional microfluidic device for droplet formation limits the widespread use of ddPCR. Fluorescence-based techniques can also be used for multiplex detection due to the availability of different fluorophores,[19,20] but the spectral overlapping of different fluorescent groups and the limited number of fluorescent reporters make high-throughput detection difficult. Meanwhile, capillary electrophoresis (CE) has been widely used for the multiplex detection of nucleic acids.[21−24] The capability of CE for multiplex detection depends on its ability to separate products with different sizes. Multiplex ligation-dependent probe amplification (MLPA) is a reliable and efficient technique for the detection of multiple gene mutations.[25,26] Specific probes with unique lengths were designed for different targets so that the PCR products of the ligated probes could be separated by CE. Another CE-based technique for multiple

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Synthesis of a pH-Responsive Functional Covalent Organic Framework via Facile and Rapid One-Step Postsynthetic Modification and Its Application in Highly Efficient N^1-Methyladenosine Extraction

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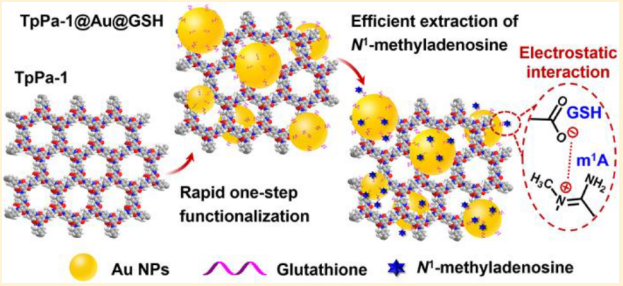
Supporting Information

ABSTRACT: A facile and rapid postsynthetic modification strategy for functionalization of covalent organic framework (COF) was developed to synthesize a tailor-made pH-responsive COF called TpPa-1@Au@GSH for highly efficient extraction of N^1-methyladenosine (m^1A). Glutathione (GSH) was judiciously designed as the functional group for extracting and releasing m^1A by pH variations. With the aid of gold nanoparticles (Au NPs) as linkers, GSH was successfully introduced to the robust substrate TpPa-1 in only one step spending only 1 h. Owing to the several-to-one immobilization of GSH on Au NPs and the large surface area of TpPa-1, this functional COF was constructed with abundant m^1A binding sites. TpPa-1@Au@GSH showed excellent selectivity for m^1A extraction by capturing m^1A from a mixture of 14 nucleoside analogues followed by mass spectrometry analysis. It was proved to have ultrafast adsorption ability (only 1 min incubation time), high binding capacity (5 mg g^{-1}, m^1A/TpPa-1@Au@GSH), good reusability (at least 5 times), and good storage stability (at least 8 months at room temperature). Great performance was also achieved in extracting m^1A from both animal and plant biological samples. The adsorption mechanism was demonstrated to be based on the electrostatic interaction. This work proposed a new approach for m^1A extraction, demonstrated the high potential of COFs in biological sample pretreatment, and offered an effective and versatile route for functionalization of COFs.



N^1-Methyladenosine (m^1A), a prevalent RNA post-transcriptional modification bearing a positively charged base, has been found to be dynamic and reversible in mRNA[1,2] and participate in many significant biological events.[3−5] These exciting achievements attract increasing interest from scientific researchers and encourage them to make more attempts to discover other biological functions of m^1A and its role in clinical diagnoses. On account of the particular chemical structure, m^1A can undergo Dimroth rearrangement to m^6A in alkaline environment,[6] which emphasizes the importance of condition control during the measurement of m^1A.

To date, mass spectrometry (MS) has been universally used for nucleoside detection due to its great detection sensitivity and qualitative ability.[7,8] Before MS analyses, removing the impurities, which may cause MS ionization source contamination and signal suppression of target compounds, is quite necessary. Several materials have been developed for nucleoside isolation and purification. The most often used ones are boronate affinity materials,[9,10] which can capture and release *cis*-diol-containing targets by changing pH from basic to acidic, and polymeric reverse-phase materials,[11,12] which extract nucleosides based on the hydrophobicity and hydrophilicity. But these aforementioned materials are not as suitable for highly efficient extraction of m^1A because of the ionic characteristic of m^1A and its instability in alkaline conditions. New approaches based on other mechanisms should be proposed for m^1A extraction.

Covalent organic frameworks (COFs) are an emerging class of crystalline porous materials, whose backbones are entirely made up of light elements.[13,14] Diverse organic building blocks with rigid conformation are linked by strong covalent bonds and precisely integrated into periodic networks. Owing to their intriguing properties, including low mass density, large surface area, high thermal and chemical stability, highly ordered pore structures, tunable pore size, and very flexible molecular design, COFs are well-known for their applications in gas adsorption and storage,[15−17] catalysis,[18,19] optoelectronics,[20,21] separation,[22,23] etc. With the aim of making COFs appropriate for further advanced functions and enhancing their application efficiency, functionalization of COFs has raised great concern and developed rapidly. Until now, taking advantage of the highly flexible designability, various functional COFs have

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5hmC-MIQuant: Ultrasensitive Quantitative Detection of 5-Hydroxymethylcytosine in Low-Input Cell-Free DNA Samples

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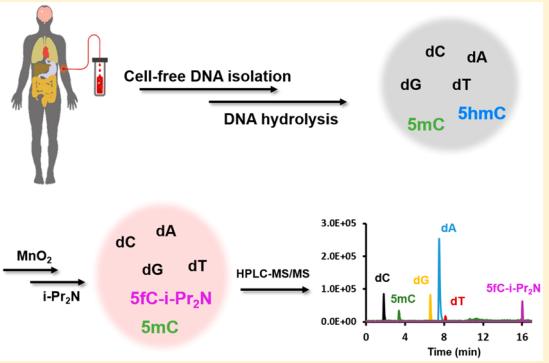
Supporting Information

ABSTRACT: Cell-free DNA (cfDNA)-based biomarkers such as mutation and methylation offer promising noninvasive strategies for disease diagnosis and prognosis. However, besides high-throughput sequencing, there has been no alternative approach to date to detect the epigenetic marks, such as 5-hydroxymethylcytosine (5hmC), in cfDNA. Here, we described a MnO_2 oxidation and hydrazine-*s*-triazine reagent (i-Pr_2N) labeling based method named 5hmC-MIQuant that achieved ultrasensitive high-performance liquid chromatography−tandem mass spectrometry (HPLC−MS/MS) quantification of 5hmC in low-input DNA samples. This strategy improved the detection sensitivity of 5hmC by 178 times, and the limit of detection was as low as 14 amol. With simple preparation steps, 5hmC-MIQuant could quantify the 5hmC level in as little as 340 pg genomic DNA (equivalent to 57 copies of diploid genome). cfDNA samples from human plasma were successfully analyzed using 5hmC-MIQuant. This method is promising for the identification of 5hmC function in precious samples and the 5hmC-based noninvasive disease diagnosis.



For decades, increasing attention has been paid to noninvasive liquid biopsy, which is based on the detection of circulating cell-free DNA (cfDNA) in the human plasma.[1−3] Characteristics of cfDNA, like concentration and tumor-specific gene mutations, are demonstrated to have the potential of clinical utility in noninvasive cancer diagnostics,[4] treatment monitoring,[5] and prenatal medicine.[6] Recently, epigenetic alterations of cfDNA give new impetus to the cfDNA-based analysis in both the research and clinical setting.[1,7] DNA cytosine methylation is a well-established epigenetic mark that affects a broad range of biological processes.[8] Plenty of works have already revealed the 5-methylcytosine (5mC) signature in cfDNA of different healthy conditions using high-throughput sequencing.[9−11] Besides 5mC, 5-hydroxymethylcytosine (5hmC) is also identified to be a stable epigenetic mark associated with gene regulation and cellular development.[12−14] Unlike 5mC, the distribution of 5hmC in mammals is tissue-specific,[15,16] and different 5hmC signatures are often observed in many cancer types compared with the healthy control.[17] In 2017, the hydroxymethylome in cfDNA of several types of cancer was first reported using a sensitive high-throughput sequencing method.[18,19] According to their results, compared with 5mC, 5hmC displayed distinct features in several cancer types and was an excellent marker for solid tumors.

Although scientists achieved an exciting beginning in the exploration of 5hmC in cfDNA, large scales of clinical samples are yet to be analyzed to fully understand the cancer-associated 5hmC pattern in cfDNA and to demonstrate the potential of 5hmC in cfDNA for cancer diagnostics. Therefore, there is an enormous demand for novel techniques with high sensitivity, low cost, and fast speed that can detect 5hmC in only a few nanograms of cfDNA.

High-performance liquid chromatography−tandem mass spectrometry (HPLC−MS/MS) is the most widely used method for global quantification of cytosine modifications.[20−22] However, due to the sensitivity of the instrument and the low abundance of 5hmC in the human genome (10−100-fold <5mC), micrograms of DNA sample are usually required in this analysis in order to get an accurate 5hmC level.[15,23] Introducing an easily ionizable moiety into 5hmC can improve its MS detection sensitivity. Shahal et al. utilized the enzyme β-glucosyltransferase (βGT) to convert 5hmC to 6-azide-β-glucosyl-5hmC, resulting in an 8-fold increase of the HPLC−MS/MS detection sensitivity.[24] Yuan and co-workers found that chemical reagents with a bromoacetonyl moiety could react with the N3 and N4 positions of cytosine.[25] Yuan and co-workers also used an oxidation−derivatization MS analysis strategy to quantify 5hmC in both DNA and RNA.[26] Using these reagents, they greatly increased the detection sensitivity of 5hmC by HPLC−MS/MS. However, the 5hmC level in limited DNA samples, such as cfDNA (less than 10 ng), still cannot be achieved by existing HPLC−MS/MS methods.

Recently, our group used a series of novel hydrazine-based reagents to specifically react with 5fC and realized the most

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Ultrasensitive detection of microRNA based on a homogeneous label-free electrochemical platform using G-triplex/methylene blue as a signal generator

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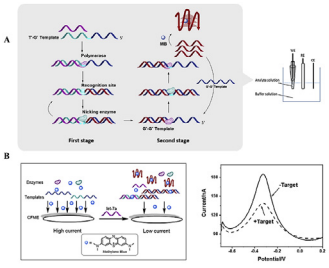
HIGHLIGHTS

- A homogeneous electrochemical biosensor for miRNA detection has been fabricated with G-triplex DNA/methylene blue.
- An efficient two-stage amplification system was employed to improve the detection performances.
- A detection limit of 0.45 fM with single-base recognition can be realized.
- Target microRNA in cancer cell-derived RNA samples was directly quantified.

GRAPHICAL ABSTRACT



The strategy of G3-EXPAR for miRNA detection

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ABSTRACT

The electrochemical methods for microRNA (miRNA) detection have received increasing attention because high portability and affordability of electrochemical biosensors may facilitate point-of-care quantitative detection of miRNAs. Among these biosensors, the homogenous label-free electrochemical biosensors for miRNAs are rarely reported due to the lack of a universal and efficient signal read-out-mode. A newly discovered G-triplex, 5′-CTGGGAGGGAGGGA-3' (denoted as G3), can specifically bind with methylene blue (MB), leading to a significant decrease of the diffusion current of MB. By using miRNAs as a driving force, a two-stage isothermal exponential amplification reaction was proposed to generate G3 through miRNAs. The generated G3 can combine with MB and produce observable current changes, which depend on the concentration of miRNAs. Therefore, a novel homogeneous label-free electrochemical biosensor for miRNA detection was successfully constructed. By choosing let-7a, the down-regulation of which is possibly associated with the over-expression of RAS and HMGA2 oncogenes, as a model, we discovered that this biosensor demonstrated excellent analytical performance in detecting let-7a, with an ultralow limit of detection (0.45 fM) and high specificity (discriminating one nucleotide variation). Moreover, the proposed biosensor was successfully applied in monitoring the expression levels of the low-abundant miRNAs in the human lung adenocarcinoma cell lines. This assay successfully verified the feasibility of G-triplex/MB as an efficient and sensitive probe for immobilization-free and label-free electrochemical detection of nucleic acids, which would greatly promote the rapid development of homogeneous label-free electrochemical biosensors.

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Snake venom characteristic peptides: novel fingerprints for species identification by sheathless capillary electrophoresis-electrospray ionization-mass spectrometry†

Ying Liu,‡[a] Xiao-Hui Zhang,‡[b] Yue Yu,[a] Hong-Xu Chen, *[c] Ying-Lin Zhou *[a] and Xin-Xiang Zhang*[a]

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Snake venom is a complex mixture mainly consisting of proteins and peptides which varies with different species. These variations lead to different toxic mechanisms and different anti-venom serums for treatment and the determination of their use as drugs. Hence, it is important to develop a sensitive and reliable method to identify the species of snakes from venoms. Herein, we present a novel strategy based on the sheathless capillary electrophoresis-electrospray ionization-mass spectrometry (CESI-MS) system to characterize snake venom proteins. Through the determination of peptides, we found the characteristic peptides of 8 different snakes with high sensitivity (1 μg mL^{-1}) and high selectivity, which provided a reliable method for the species identification and purity detection of snake venom samples.

Introduction

Venomous snakes are widely found almost everywhere in the world, and they cause extensive public health problems.1 200 000–5 500 000 bites, at least 421 000 envenomings, and 20 000 deaths were estimated globally every year.[1] Snake venom is a complex mixture of proteins (including peptides and main components), amino acids, nucleotides, and metal elements binding with proteins.[2] The composition of snake venom is not always constant, even if the same component has different distributions among different individuals, species, genera, and families.[3,4] These variations lead to different mechanisms of poisoning, such as neurotoxicity, hematotoxicity, and cytotoxic pathologies, and to different anti-venom serums for treatment.[5] In addition, people use different kinds of snake venoms to develop new drugs for the treatment of many diseases, such as haemostatic disorders, hypertension, thrombosis and cancer.[6–9] Therefore, it is of great importance to identify the species of snakes from venoms for the treatment, medicolegal examination of snakebites and quality control of pharmaceuticals.

Species identification strategies have been developed for decades. Enzyme-linked immunosorbent assay (ELISA) is a widely used species identification method, which uses a snake venom antibody for immune recognition. Its significant advantage is being simple and fast, but the problem is that there are large cross-reactions between different species of snakes. To overcome this problem, strict screening of species-specific antibodies is required.[10,11] Another most common strategy is two-dimensional gel electrophoresis (2D-GE), which separates components by the isoelectric point and apparent molecular weight of proteins. Through staining with the certain protein and post-translational modification (PTM) antibody, the trains of spots show different species.[12,13] Furthermore, in-gel digestion and MS detection can be used to obtain more protein information. However, abundant biologically active proteins with small molecular weights and extreme isoelectric points are easily lost, resulting in the loss of information, and it is difficult to use this method for quantification.[14]

With the development of chromatography, and mass spectrometry technology and software, shotgun proteomics has become another widely used strategy. Shotgun proteomics usually uses the combination of high-performance liquid chromatography (HPLC)/capillary electrophoresis (CE) and mass spectrometry/mass spectrometry (MS/MS).[15] Compared with ELISA and 2D-GE, shotgun proteomics can obtain high-throughput, high-sensitivity and high-resolution peptide mass spectrum information. Combined with the database, the

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‡These authors contributed equally to this work.

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High-throughput ultra-sensitive discrimination of single nucleotide polymorphism *via* click chemical ligation†

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Single nucleotide polymorphisms (SNPs) have been proven to be important biomarkers for disease diagnosis, prognosis and disease pathogenesis. Here, taking the advantages of a self-assembled oligonucleotide sandwich structure and robust chemical reactions, we have developed a simple, high-throughput and effective colorimetric analytical technique termed CuAAC-based ligation-assisted assays (CuAAC-LA) for SNP detection using a DNA-BIND 96-well plate. With the 5′-azide and 3′-alkyne groups labelled on two oligonucleotide probes, the target DNA can direct a Cu(I)-catalyzed alkyne–azide cycloaddition (CuAAC) click reaction. Since the small difference in duplex stability caused by a single-nucleotide mismatch was amplified by the steric effects of these reactive groups for the ligation reaction of an unstable duplex, CuAAC-LA exhibited an ultra-sensitive discrimination ability for a mutant type target in the presence of large amounts of wild type targets. As low as 0.05% SNP could be clearly detected, which was better than most previously reported methods by various DNA ligases, indicating that a simple and rapid synthetic method *i.e.*, the DNA template-directed click reaction held the potential to replace the ligase for SNP detection.

Introduction

Recently, there have been increasing number of studies focused on the identification and detection of single nucleotide polymorphisms (SNPs) associated with the disease diagnosis, prognosis and disease pathogenesis.[1,2] A variety of technologies have been developed for SNP genotyping.[3–9] Among these methods, ligation-based techniques[10–14] have been considered as promising and powerful detection assays. The most commonly used ligation-based strategies for the detection of SNPs involve the use of DNA ligase enzymes, which are sensitive to the mismatched bases at the ligation site. Although these methods can achieve the sensitive detection of SNPs, the complexity of the operation, demand for a strict environment and high cost of DNA ligase enzymes hinder their wide use for clinical diagnosis.

Over the past decade, numerous non-enzymatic template-directed chemical reactions[15–18] have been developed. In contrast to the enzymatic methods, these non-enzymatic methods have the advantages of simple operations, common environments and low cost without sample preparation or target isolation.[19,20] Based on the high specificity and programmability of these template-directed chemical reactions, the non-enzymatic methods have found valuable applications in many aspects. They have been used as a powerful tool to translate the instructions of nucleic acids into the direction of controlled organic synthesis, as well as small molecules and some useful materials.[21–23] The DNA-templated synthesis also makes the libraries for the drug discovery based on the DNA-encoded chemistry.[24,25] In the latter case, templated reactions can be used for sensing DNA or RNA *in vitro*.[19,26] Furthermore, the cellular nucleic acids (miRNA, rRNA, and mRNA) can also be detected *via* the templated reactions in living cells, circulating exosomes, and tissues,[20,27–29] while enzymatic methods cannot work in intact cells due to the difficulty of delivering enzymes into cells.

Based on the above, the non-enzymatic template-directed chemical reactions show the promise to replace the DNA ligase enzymes for some applications. Most templated strand ligations, such as nucleophilic substitution, condensation, and cycloaddition, show different levels of biocompatibility, ortho-

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DNAzyme-powered nucleic acid release from solid supports†

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Here, we demonstrate use of a Mg^{2+}-dependent, site-specific DNA enzyme (DNAzyme) to cleave oligos from polyacrylamide gel beads, which is suitable for use in drop-based assays. We show that cleavage efficiency is improved by use of a tandem-repeat cleavage site. We further demonstrate that DNAzyme-released oligos function as primers in reverse transcription of cell-released mRNA.

Numerous molecular-biology techniques require that nucleic acids be captured onto a solid support to enable enrichment or modification, followed by release from the support for subsequent manipulations.[1–3] In a classic example, nucleic acids are captured through specific base-pairing, followed by release using a low-salt elution buffer, as is done to purify polyadenylated RNA through capture onto immobilized polyT-DNA.[4] Alternatively, captured nucleic acids can be released by a restriction endonuclease.[5] In addition, proteases may be used, as in the case of modified DNA.[6] However, the required elution buffers or enzymes may be incompatible with downstream applications and these methods are not suitable if buffer exchange after nucleic acid release is not possible, such as, for example, in methods that use microfluidic droplets for high-throughput compartmentalized assays.[7,8] These issues can be partially overcome through use of a photo-labile linker.[9] In the single cell RNA sequencing (scRNAseq) method inDrop,[10] hydrogel beads decorated with barcoding oligos are encapsulated into nanoliter-sized droplets along with single cells, lysis buffer and reverse transcription reagents. The oligos anneal to mRNA released from the cells and reverse transcription generates barcoded cDNA. During development of this method, it was found that reverse transcription efficiency was dramatically increased by intra-drop release of the oligos from the gels, accomplished through UV-light mediated cleavage of a 1-(2-nitrophenyl)ethyl ester used to link barcoding oligos to the hydrogel beads.[10,11] As a high-throughput single cell mRNA sequencing method based on droplets, inDrop has become a very useful tool to realize scRNAseq without crosstalk between cells by addition of a barcode region with the polyT-primer.[12,13] UV light-triggered primer release is simple and efficient. However, this method suffers from high synthesis costs and the need to protect the beads from light prior to droplet encapsulation. Also, in some applications, UV light treatment may damage molecules of interest.[14,15] Thus, an improved method to cleave nucleic acids from solid support, such as hydrogel beads, would be of great value, particularly for drop-based assays.

In this paper, we present a new method to cleave barcode molecules from solid substrates in a manner that is suitable for use in drop-based assays. We use the inDrop system as our prototypic research target, and we introduce a Mg^{2+}-dependent DNAzyme to release oligos from hydrogel beads to capture cell-released mRNAs.[16,17] DNAzymes, also called DNA enzymes, are DNA oligonucleotides with site-specific DNA-cleavage activity, usually obtained through *in vitro* selection techniques.[18,19] These oligos often require a co-factor, such as metal ions, amino acids or proteins, for cleavage activity,[20–22] and DNAzyme-based biosensors have been developed.[23] This Mg^{2+}-triggered release process can continue as long as DNAzyme strands and Mg^{2+} ions exist in the reaction system. We design and test a tandem dual Mg^{2+}-dependent DNAzyme structure, which shows improved DNA cleavage efficiency. Critically, use of these DNAzymes does not interfere with conversion of captured mRNA into cDNA, or with subsequent PCR amplification. Mg^{2+} ions are compatible with most biological reactions and Mg^{2+}-triggered oligo are released under mild reaction conditions. This DNAzyme-based system is a promising and economical alternative to UV-triggered release

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Dissolvable Polyacrylamide Beads for High-Throughput Droplet DNA Barcoding

Yongcheng Wang, Ting Cao, Jina Ko, Yinan Shen, Will Zong, Kuanwei Sheng, Wenjian Cao, Sijie Sun, Liheng Cai, Ying-Lin Zhou, Xin-Xiang Zhang, Chenghang Zong, Ralph Weissleder,* and David Weitz**

Droplet-based single cell sequencing technologies, such as inDrop, Drop-seq, and 10X Genomics, are catalyzing a revolution in the understanding of biology. Barcoding beads are key components for these technologies. What is limiting today are barcoding beads that are easy to fabricate, can efficiently deliver primers into drops, and thus achieve high detection efficiency. Here, this work reports an approach to fabricate dissolvable polyacrylamide beads, by crosslinking acrylamide with disulfide bridges that can be cleaved with dithiothreitol. The beads can be rapidly dissolved in drops and release DNA barcode primers. The dissolvable beads are easy to synthesize, and the primer cost for the beads is significantly lower than that for the previous barcoding beads. Furthermore, the dissolvable beads can be loaded into drops with >95% loading efficiency of a single bead per drop and the dissolution of beads does not influence reverse transcription or the polymerase chain reaction (PCR) in drops. Based on this approach, the dissolvable beads are used for single cell RNA and protein analysis.

Droplet microfluidics have proven critical for next-generation single-cell analysis.[1] Coupled with high-throughput sequencing, droplet-based single cell sequencing technologies have been developed to analyze RNA,[2,3] DNA,[4,5] and even proteins[6,7] in single cells. To track single cells, barcodes need to be introduced, which is mainly achieved by adding synthetic DNA barcode primers to beads. For these applications, the beads are mostly made of biocompatible polymers, for example, polyacrylamide beads in inDrop,[2] hydroxylated methacrylic polymer beads used in Drop-seq,[3] and polyacrylamide beads used in 10X Genomics.[8]

Each of these chemically different beads has their own advantages and shortcomings. The inDrop polyacrylamide bead can be closely packed in a microfluidic device channel to achieve more than 95% loading of single bead per drop.[2] However, in the inDrop system, UV light is necessary to release primers from the bead, which somewhat complicates bead fabrication and makes it less cost effective.[9] Another shortcoming is that UV light may introduce damage to DNA or RNA and skew the results.[10,11] The Drop-seq system does not release primers from the bead and the reaction efficiency is low as reactions happen only near the surface of beads.[3] In the 10X Genomics platform, gel beads can be efficiently delivered into drops, but the comparably high cost and lack of flexibility in designing

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Review

Fluorescence imaging of intracellular nucleases—A review

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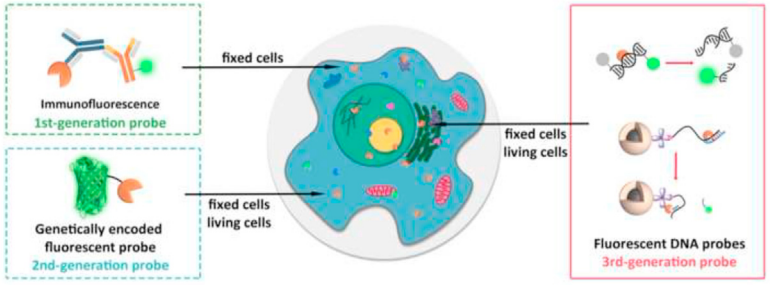
HIGHLIGHTS

- The principle of different fluorescent probes for tracking intracellular nucleases.
- The advantages and limitations of available methodology for cellular DNA delivery.
- Visualization of intracellular distribution and translocation of various nucleases.
- Monitoring of the interactions between nucleases and other proteins within cells.

GRAPHICAL ABSTRACT



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ABSTRACT

Nucleases play crucial roles in maintaining genomic integrity. Visualization of intracellular distribution and translocation of nucleases are of great importance for understanding the in-vivo physiological functions of these enzymes and their roles in DNA repair and other cellular signaling pathways. Here we review the recently developed approaches for fluorescence imaging of nucleases in various eukaryotic cells. We mainly focused on the immunofluorescence techniques, the genetically encoded fluorescent probes and the chemically synthesized fluorescent DNA-substrate probes that enabled in-situ visualization of the subcellular localization of nucleases and their interactions with other protein/DNA molecules within cells. The targeted nucleases included important endonucleases, 3′ exonucleases and 5′ exonucleases that were involved in the DNA damage repair pathways and the intracellular DNA degradation. The advantages and limitations of the available tools were summarized and discussed.

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Construction of Specific and Reversible Nanoreceptors for Proteins via Sequential Surface-Imprinting Strategy

Muhua Zhao, Shan Huang, Huaisyuan Xie, Jiayu Wang, Xiaoli Zhao, Mengyuan Li,* and Meiping Zhao*

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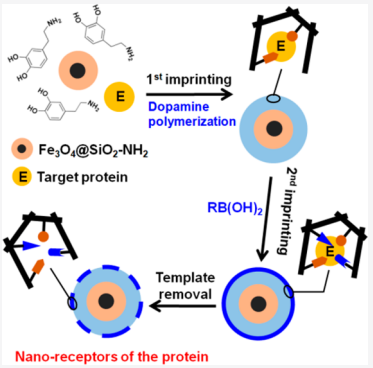
ABSTRACT: Molecular recognition of proteins is critical for study and manipulation of protein-related biological processes. However, design and synthesis of abiotic receptors for precise recognition of proteins still remains a challenging task. Herein, we developed a universal sequential surface-imprinting strategy that integrated two different types of imprinting reactions to construct artificial protein receptors with high selectivity. Employing dopamine self-polymerization and boronate/diol complexation as the first-step and second-step imprinting reactions, respectively, we synthesized surface-imprinted magnetic nanocomposites against two different enzyme proteins: deoxyribonuclease I (DNase I) and apurinic/apyrimidinic endonuclease/redox effector factor 1 (APE1). The obtained nanocomposites both showed strong and specific binding toward their respective template proteins. Moreover, the bound enzymes could be totally recovered with high activity under mild buffer conditions. These antibody-like specific and reversible binding properties enabled effective purification and enrichment of the low-abundance target proteins from complex serum samples. Compared to existing one-pot or one-step imprinting methods, the proposed sequential surface-imprinting approach offers a more flexible combination of different functional monomers and greatly enhances the performance and biocompatibility of the imprinted materials. The generality and simplicity of the sequential imprinting strategy would make it an appealing and competitive method to prepare artificial protein receptors.



Molecular recognition of proteins is critical for study and manipulation of protein-related biological processes, sensing of proteins in living cells, and design of inhibitors for protein-based disease therapy.[1,2] Over the years, biomolecules, including antibodies, aptamers, and small synthetic molecules, have been developed for selective recognition of proteins.[2,3] However, they show disadvantages of high cost, low stability, and inability to cross the cell membrane to get access to endogenous proteins.[3−5] Thus, it is of urgent need to develop universal and simple methods for synthesis of abiotic materials that are comparable to antibodies for recognition of proteins.

Molecular imprinting represents an effective approach for preparing tailor-made polymer materials with a specific affinity toward target molecules.[6,7] Compared with antibodies, molecularly imprinted polymers (MIPs) exhibit the merits of physical robustness, low cost, and high stability, thus showing wide applications in separation, chem-/biosensing, and enzyme mimicking.[8−10] To date, imprinting of small molecules has achieved substantial advancements; however, synthesis of protein-imprinted materials with high affinity and selectivity still remains a formidable task because of the structural complexity and conformational flexibility of proteins and limited availability of water-soluble monomers.[11,12] Researchers have pursued several strategies in order to obtain highly selective protein-imprinted polymers. For example, Takeuchi et al. developed a postimprinting strategy to allow site-directed modification of the imprinted cavity to endow it with desirable functions;[13,14] Haupt and co-workers reported the design and synthesis of an MIP-based enzyme inhibitor by using a strong anchoring monomer with a polymerizable moiety;[15] Shea and his colleagues prepared MIP nanoparticles with antibody-like binding affinity and selectivity for melittin via a functional monomer-optimization strategy during the molecular imprinting process;[16] and Liu and others combined molecular imprinting with the boronate-affinity techniques to achieve specific glycoproteins recognition.[17,18] Very recently, we developed a bionanocomposite with high specificity for the important DNA repair enzyme via surface molecular imprinting by employing avidin as a bioaffinity ligand and dopamine as a functional monomer.[19]

Despite the progress made, the commonly used one-step polymerization approach only allows the combination of a limited number of functional monomers that prefer similar polymerization conditions and have no cross-reactions between each other. On the other hand, because of the flexibility of the protein's conformation, the initial interactions

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A target-driven DNA-based molecular machine for rapid and homogeneous detection of arginine-vasopressin†

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Rapid detection of physiological changes of neuropeptides is of great importance as they are involved in a wide range of physiological processes and behaviors. Abnormalities in their expression level are correlated with various neurological diseases. However, current methods such as radioimmunoassay, enzyme-linked immunosorbent assays and liquid chromatography tandem mass spectrometry relied on cumbersome operation steps and could not rapidly provide the information of their concentration fluctuations. Thus motivated, we developed a target-driven DNA-based molecular machine that could be triggered only in the presence of a specific target neuropeptide. Using arginine-vasopressin (AVP) as a model neuropeptide, we integrated the DNA-based molecular machine with fluorescence signal transduction and amplification technology. The assay was rapid and homogeneous, which offered a linear range of 75–700 pM and a limit-of-detection as low as 75 pM. It holds great potential for further applications in real-time monitoring of the variations of the AVP level in biological samples.

Introduction

Neuropeptides are a class of bioactive signaling molecules that have been implicated in the regulation of a wide range of physiological processes and behaviors, such as feeding and reproduction, adaptation to external factors (*e.g.* temperature fluctuation) and internal factors (*e.g.* depression, anxiety), memory and pair bonding, autism spectrum disorder, *etc*.[1–8] Real-time analysis of physiological changes of neuropeptides is of great importance as abnormalities in their expression level are correlated with various neurological diseases.[4–8] Current methods mainly include radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), and liquid chromatography tandem mass spectrometry (LC-MS/MS).[9–13] RIA is very sensitive but requires hazardous radioactive reagents and cumbersome washing and centrifugation steps. Neither RIA nor LC-MS/MS could provide information regarding concentration fluctuations due to rapid degradation of neuropeptides and long working time. Therefore, it remains a formidable task to develop a simple and homogeneous method for rapid and real-time detection of neuropeptides in biological samples.

With its unique structural motif and specific recognition ability, ease of synthesis, and biocompatibility, DNA has been used as attractive building blocks for designing and engineering a variety of programmable molecular machines.[14–20] Such DNA-based molecular machines were usually activated by a specific molecular input or by an environmental stimuli (*e.g.*, pH, temperature) to report an output signal, release a cargo, or perform other useful functions, providing unprecedented opportunities for various applications ranging from targeted drug transport, molecular sensing and programmable chemical synthesis.[14–16] In particular, fluorescent DNA-based molecular machines for sensing have emerged rapidly because of their design flexibility for signal transduction and amplification and that fluorescence readout can be recorded in real time and *in situ*.[17–23] Despite the impressive progress being made, current DNA-based molecular machines are mainly triggered by a restricted class of molecular inputs, which are limited to oligonucleotides (DNAs and RNAs), more seldom, to proteins and small molecules.[21–23] In particular, a DNA-based molecular machine that can be adapted to provide a signal output in the presence of a specific peptide has not yet been reported to date.

In this work, we designed and synthesized a target-driven DNA-based molecular machine that can be triggered only in the presence of a specific target neuropeptide. Employing arginine-vasopressin (AVP) as a model neuropeptide, we integrated the DNA-based molecular machine with fluorescence signal

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Radical Philicity Inversion in Co- and Fe-Catalyzed Hydrogen-Atom-Transfer-Initiated Cyclizations of Unsaturated Acylsilanes

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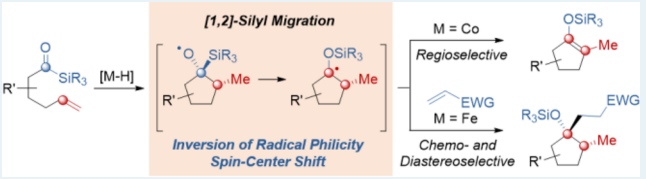
Supporting Information

ABSTRACT: [1,2]-Radical Brook rearrangement (RBR) has been identified as a viable pathway in M−H (M = Co, Fe) catalyzed hydrogen-atom-transfer reactions involving unsaturated acylsilanes. Guided by the same concept, we have explored two transformations, namely, a Co-catalyzed cycloisomerization reaction and a Fe-catalyzed cyclization/Giese addition reaction. Both reactions involve the generation of a versatile α-siloxy radical intermediate via concomitant philicity inversion and radical translocation, which is mechanistically distinct from coupling reactions involving fragmentation/reduction pathways. Synthesis of cyclic silyl enol ethers and sterically congested cyclopentanol derivatives have been thus achieved with high regio- and diastereo-selectivity.



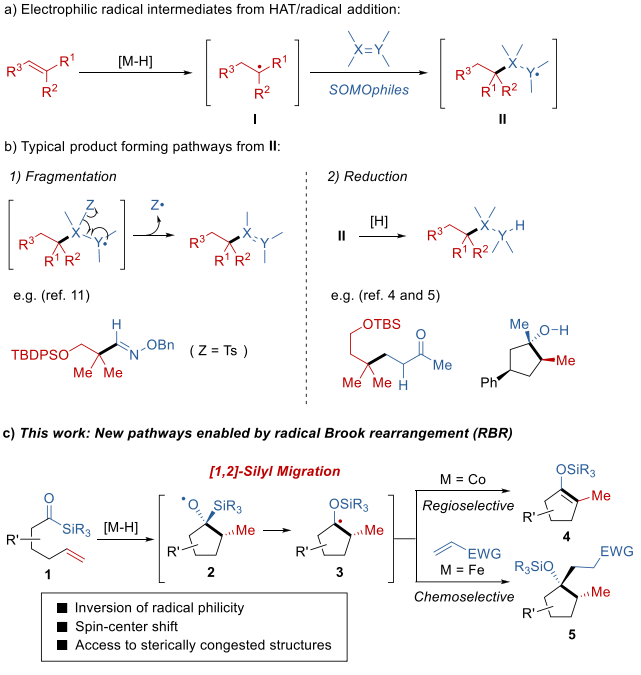
KEYWORDS: *hydrogen atom transfer, radicals, acylsilanes, cobalt, iron, Brook rearrangement*

Radical addition to unsaturated systems typically involves an early transition state.[1] It thus enables the facile construction of sterically congested structures that are otherwise difficult to access.[2] This has been demonstrated by the recent advances in the first-row transition-metal-catalyzed hydrogen-atom-transfer (HAT) reactions of alkenes.[3] In particular, the nucleophilic nature of the alkyl radical intermediates (**I**) derived from HAT allows highly chemoselective addition to various polarized SOMOphiles (Scheme 1a), including electron-deficient alkenes/alkynes,[4] ketones,[5] hydrazones,[6] imines,[7] nitriles,[8] diazo compounds,[9] heteroarenes,[10] and so on.[11]

Such addition produces an electrophilic radical species (**II**), whose fate typically falls into two scenarios: fragmentation and reduction (Scheme 1b). For example, Carreira has achieved cyanation, aldoximation, and azidation via the fragmentation pathway.[8a,9c,11a,b] Boger has developed a broad spectrum of Fe-mediated hydrofunctionalization reactions.[11e] The reduction pathway is more frequently encountered, such as in Baran's HAT-initiated Giese additions.[4a,b] Very recently, the group of Pronin developed a rapid access to complex terpenoid motifs through reductive radical-polar crossover cascades.[12] Reductive cyclizations involving alkenes and less-activated SOMOphiles such as ketones, imines, and nitriles have been reported by Bonjoch and Bradshaw,[5a] Bode,[7b] Ma,[8b] and Turner,[8c] respectively. Notably, for carbonyl addition, the reduction process traps the thermodynamically disfavored oxygen-centered radicals, thereby pushing the reaction forward.

We became interested in identifying a new pathway that enables further functionalization of **II**. Specifically, we were inspired by the elegant studies by Tsai and more recently Smith on the [1,2]-radical Brook rearrangement (RBR) and related processes, in the context of classical free radical

Scheme 1. Searching for New Pathways via Electron-Deficient Radicals Derived from HAT/Radical Addition Sequence



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Dual Cobalt and Photoredox Catalysis Enabled Intermolecular Oxidative Hydrofunctionalization

Han-Li Sun, Fan Yang, Wei-Ting Ye, Jun-Jie Wang, and Rong Zhu*

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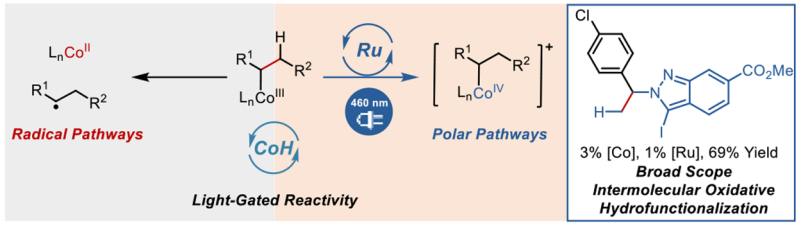

ABSTRACT: A general protocol has been developed for the Markovnikov-selective intermolecular hydrofunctionalization based on visible-light-mediated Co/Ru dual catalysis. The key feature involves the photochemical oxidation of an organocobalt(III) intermediate derived from hydrogen atom transfer, which is supported by electrochemical analysis, quenching studies, and stoichiometric experiments. This redox process enables the efficient branch-selective alkylation of pharmaceutically important nucleophiles (phenols, sulfonamides, and various N-heterocycles) using a wide range of alkenes including moderately electron-deficient ones. Moreover, light-gated polar functionalization via organocobalt species was demonstrated.

KEYWORDS: *hydrogen atom transfer, radicals, cobalt, photoredox catalysis, hydrofunctionalization*

The Brønsted acid-mediated intermolecular Markovnikov addition has been known for more than a hundred years.[1] On paper, it offers a straightforward and modular approach to many pharmaceutically relevant structures such as branched-alkyl-substituted phenols and N-heterocycles, without resorting to preactivated coupling partners or subsequent multistep conversions (Scheme 1a).[2] Not surprisingly, such transformations in practice are often complicated by the thermodynamically disfavored protonation process and high-energy carbocations involved.[3] To this, chemists have been searching for catalytic alternatives mimicking this process in a more controlled fashion.[4] Guided by the rich literature on the first-row transition metal,[5] in particular the cobalt-catalyzed hydroelementations via hydrogen atom transfer (HAT),[6] we recently introduced an I(III)-promoted intermolecular oxidative hydrofunctionalization reaction catalyzed by a cobalt salen complex.[7] Highly chemo- and regioselective additions to unactivated terminal olefins and electron-rich styrenes have been realized with a number of heteroatom-based nucleophiles, which includes carboxylic acids, phenols, and sulfonamides. More recently, benzotriazoles have also been added as viable reaction partners by Yahata and Akai.[8] A key mechanistic hypothesis of this transformation involves the oxidation of an organocobalt(III) intermediate **A** into a Co(IV) species **B**, an organometallic radical cation that displays polar reactivity (Scheme 1b).[9] Our studies suggested that a Co(III)−X complex (X = counterion/anionic ligand) **C** likely functions as the oxidant for this process, whose reduction potential lies close to the oxidation potential of **A**.

As part of the continuing effort exploring the oxidative functionalization of organocobalt complexes in catalysis by a mechanistic-driven approach, it came to our attention that such reversible electron transfer between cobalt species could be a major limiting factor. When either the oxidation of **A** or the following nucleophilic trapping of **B** is sluggish, a significant increase in the concentration of **A** is anticipated, which would eventually lead to traditional radical-based reactions via Co−C bond homolysis. For instance, the resulting radical **D** once escaped from a solvent cage could dimerize, abstract a halogen atom, or react with trace dioxygen. It is thus predicted that the reaction efficiency would be largely limited by the electronic property of the alkyl group in **A** and the nucleophilicity of the reaction partner.[10] In other words, the outcome would be highly substrate-dependent. Indeed, we observed contrasting behaviors of *p*-methoxystyrene (**2a**) and *p*-chlorostyrene (**2b**) under the standard reaction conditions (Scheme 1c). The former produced Markovnikov adduct in

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Salen钴配合物催化自由基反应的研究进展

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摘要 平面型N/O四配位钴配合物参与的自由基反应研究近年来取得了许多新进展, 双水杨醛缩乙二胺类(Salen)钴配合物作为其中重要的代表, 在小分子和高分子合成中展现出丰富全面的反应性质. 本文总结了近10年来这一领域的重要进展, 包括以下方面: (1) 基于氢原子转移的Co(II/III)催化反应; (2) 基于Co(IV)的氧化型氢官能团化反应; (3) 基于Co(III)卡宾自由基的环丙烷化反应; (4) 基于Co(I)的还原偶联反应; (5) Co介导的自由基聚合, 并重点介绍这些反应之间的机理联系、新型催化模式的发展以及合成应用.

关键词 自由基, 钴催化, Salen, 氢原子转移, 活性聚合

1 引言

对第一过渡系金属有机化学的探索起步很早, 但是在催化反应研究及合成应用中, 第二、三过渡系金属后来居上, 并在很长一段时间内占据了主要地位. 部分原因在于第一过渡系金属价态多变、半径和电负性较小、碳–金属键较弱, 这些特点导致极其复杂的反应模式, 其中很多过程涉及自由基物种. 有趣的是, 进入21世纪以来, 随着自由基化学、光化学、电化学等领域的进一步发展, 第一过渡系金属的上述反应特点及其相对低成本和低毒性, 使得相关研究重新回到了合成化学的舞台中心[1,2].

作为代表, 由维生素B_{12}衍生而来的平面型N/O四配位钴催化剂参与的自由基反应研究在近年来取得了许多新进展, 有力地促进了小分子和高分子合成领域的进步. Shenvi等[3]系统回顾了锰、铁、钴催化的自由基氢官能团化反应的发展历程, 并对氢原子转移(hydrogen atom transfer, HAT)过程的机理研究作了总结. Detrembleur等[4]详细介绍了有机钴配合物作为碳中心自由基的来源, 在小分子合成与可控聚合中的应用. Komeyama等[5]从金属中心价态出发, 分类介绍了本领域中催化研究的一些新亮点. 最近, 吴骊珠、佟振合等[6]以及Kojima和Matsunaga[7]分别对光致氧化还原结合钴催化自由基反应这一新兴领域进行了评述.

这些反应涉及的经典催化剂包括卟啉、双丁二酮肟、双乙酰丙酮、双水杨醛缩乙二胺(Salen)等与钴形成的配合物(图1). 它们共同的特点是利用较为刚性的骨架, 固定钴中心周围的平面四配位环境, 相较非平面结构, 反键性质的$d_{x^2-y^2}$轨道的能量升高, 获得额外的稳定化能, 并使得反应主要发生在高活性的d_{z^2}方向, 基于钴中心价态的不同, 可展现出亲自由基、亲核、

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Current Opinion in Chemical Biology

The evolving capabilities of enzyme-mediated proximity labeling

Ying Zhou[1] and Peng Zou[1,2]

Abstract

The subcellular organization of proteins and RNA molecules is crucial for their proper functions. Over the past decade, both ligase-mediated and peroxidase-mediated proximity labeling (PL) techniques have been developed to map biomolecules at near-nanometer spatial resolution and subminute temporal resolution. These methods are shedding light on the spatial arrangement of proteome and transcriptome in their native context. Here, we review the recent evolution and applications of PL techniques, compare and contrast the two classes of methods, and highlight emerging trends and future opportunities.

Addresses

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Keywords

Proximity labeling, APEX, BioID, Peroxidase, Biotin ligase.

Introduction

Eukaryotic cells are elaborately divided into subcellular compartments that feature distinct biochemical characteristics. The spatial organization of proteins and RNAs in these subcellular regions is intimately linked to their biological functions, including signal transduction [1,2], localized protein synthesis [3,4], regulation of chromatin structure [5], etc. While most famously observed at membrane-bound organelles (either in the interior [6] or on the surface [7]), the subcellular targeting of proteins and RNAs have also been discovered in membrane-less compartments, such as highly dynamic liquid-like condensates that form via liquid–liquid phase separation (LLPS) [8]. For example, stalled translation-initiation causes mRNAs and mRNA-binding proteins (RBPs) to assemble into stress granules (SGs), in a process of protecting cells from oxidative damages or other cellular stress [9].

The subcellular proteome and transcriptome have been traditionally investigated by co-immunoprecipitation (co-IP) and biochemical fractionation. However, both methods require prior cell lysis, which is prone to losing low-affinity and transient protein–protein interactions. In addition, co-IP is limited by the availability of high-quality antibodies against the bait, while biochemical fractionation often suffers from incomplete purification. For example, the transcriptomic profiling of isolated mitochondria has identified abundant contaminations from the cytoplasm [10]. Furthermore, not all subcellular structures are amenable to fractionation [11]. Over the past decade, enzyme-mediated proximity labeling (PL) techniques have emerged as powerful tools for locating proteins and RNAs in live cells. In these methods, an engineered enzyme is expressed at a specific subcellular locale, where it catalyzes the *in situ* synthesis of a highly reactive small-molecule intermediate, which subsequently diffuses away and reacts with proteins and/or RNAs to form a covalent label (Figure 1a). Due to its limited lifetime, the local density, and hence the labeling efficiency of the intermediate drops off as a function of the distance from the enzyme. Thus, all else being equal, proteins/RNAs proximal to the enzyme are more likely to be labeled than distal ones. Compared with biochemical fractionation, PL could access information from subcellular compartments that are impossible to purify or highly dynamic (e.g. signaling complexes, LLPS, etc.). PL also complements co-IP studies because it is capable of mapping distant protein–protein interactions, with an 'action contour map' that spans over several 'interaction layers' (Figure 1a).

In this review, we highlight several emerging trends of PL technology development and new avenues of its applications. As discussed below, enzyme-mediated PL is now moving rapidly from membrane-enclosed compartments to open subcellular space, from protein-centered profiling to RNA/DNA-centered analysis, and from cell culture to animal. As this is not intended as a

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Brief Communication

A Clickable APEX Probe for Proximity-Dependent Proteomic Profiling in Yeast

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SUMMARY

The engineered ascorbate peroxidase (APEX) is a powerful tool for the proximity-dependent labeling of proteins and RNAs in live cells. Although widely use in mammalian cells, APEX applications in microorganisms have been hampered by the poor labeling efficiency of its biotin-phenol (BP) substrate. In this study, we sought to address this challenge by designing and screening a panel of alkyne-functionalized substrates. Our best probe, Alk-Ph, substantially improves APEX-labeling efficiency in intact yeast cells, as it is more cell wall-permeant than BP. Through a combination of protein-centric and peptide-centric chemoproteomic experiments, we have identified 165 proteins with a specificity of 94% in the yeast mitochondrial matrix. In addition, we have demonstrated that Alk-Ph is useful for proximity-dependent RNA labeling in yeast, thus expanding the scope of APEX-seq. We envision that this improved APEX-labeling strategy would set the stage for the large-scale mapping of spatial proteome and transcriptome in yeast.

INTRODUCTION

Eukaryotic cells are highly compartmentalized. Understanding the spatial organization of the cellular proteome is thus crucial for elucidating the molecular mechanism of cellular physiology (Beck et al., 2011; Kim and Roux, 2016). Over the past decade, proximity-dependent protein-labeling reactions, including ascorbate peroxidase (APEX) (Rhee et al., 2013)/APEX2 (Lam et al., 2015), and BioID (Choi-Rhee et al., 2004)/TurboID (Branon et al., 2018), have emerged as powerful techniques for profiling the molecular inventories of various important subcellular compartments (Kim and Roux, 2016). Among these, APEX is an engineered peroxidase that catalyzes the one-electron oxidation of biotin-conjugated phenol (BP) substrate into a phenoxyl free radical, which rapidly reacts with nearby proteins to form a covalent linkage with electron-rich amino acid side chains (Rhee et al., 2013). Due to the high reactivity and the short lifetime of phenoxyl radicals, APEX-mediated proximity labeling is characterized with fast labeling kinetics (<1 min) (Mortensen and Skibsted, 1997) and a small labeling radius (~10 nm) (Bendayan, 2001; Mayer and Bendayan, 1997). Since its invention in 2013, APEX labeling has complemented classic fractionation-based methods to provide a comprehensive proteomic map of the mitochondria (Hung et al., 2014, 2017; Rhee et al., 2013), signaling complexes (Paek et al., 2017), RNA granules (Markmiller et al., 2018), etc., in the live cell context. Notably, a majority of APEX applications are in metazoan cells, with only a few examples in microorganisms, such as yeast cells (Hwang and Espenshade, 2016; Santin et al., 2018; Singer-Krüger et al., 2020).

Yeast is a powerful model organism for studying eukaryotic cell biology (Duina et al., 2014; Pan, 2011). However, APEX labeling has not been successful in yeast due to the poor cellular permeability of its BP substrate (Hwang and Espenshade, 2016; Singer-Krüger et al., 2020). To facilitate probe penetration, the yeast cell wall had to be partially destroyed via the action of zymolase (Hwang and Espenshade, 2016) or freeze-thaw cycle (Singer-Krüger et al., 2020). We reasoned that replacing the biotin moiety with a small clickable handle, such as an alkynyl group, could improve the membrane permeability of APEX substrate. Owing to its promiscuity in substrate recognition, APEX is known to turn over alkyne-conjugated phenol (Rhee et al., 2013), a feature that motivated us to design a strategy to solve the problem of APEX labeling in yeast. As outlined in Figure 1A, following APEX-mediated labeling and cell lysis, the alkynyl handle could then be derivatized with biotin-conjugated azide using click chemistry reaction for visualization and enrichment.

frontiers in Aging Neuroscience

ORIGINAL RESEARCH
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Exosome α-Synuclein Release in Plasma May be Associated With Postoperative Delirium in Hip Fracture Patients

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Background: Little is known about the underlying mechanisms of the similarities in the core features of postoperative delirium (POD) and α-synuclein (α-syn)-related cognitive disorders. We herein investigated associations between fluctuated levels of exosomal α-syn in the plasma and POD presentation in geriatric hip fracture patients.

Methods: We conducted an observational, prospective, and 1:1 matched (on age older than 65, hip fracture diagnosis, American Society of Anesthesiologist' (ASA) physical status, duration of surgery, and intraoperative bleeding) case-control study: POD cases and non-POD controls were selected from the overall cohort by using Confusion Assessment Method (CAM). Delirium severity was measured by the Memorial Delirium Assessment Scale (MDAS). Plasma exosome levels of α-syn were examined preoperatively and at the time that POD was diagnosed, by using an established immunocapture technology based on a putative brain-cell-specific marker. Circulating concentrations of interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were also determined. The relationship between α-syn levels and POD risk, as well as the association between α-syn and MDAS scores and plasma cytokines, were assessed.

Results: POD incidence was 8.4% (17/202). Postoperative α-syn were either elevated or lowered. As primary outcome variables, the change of α-syn in POD patients was significantly higher than non-POD ones (21.0 ± 29.3 pg.ml^{-1} vs.1.9 ± 20.0, $P = 0.047$). The α-syn alteration was positively correlated to MDAS ($r = 0.436$, $P = 0.010$) and the change of IL-6 ($r = 0.383$, $P = 0.025$).

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Abbreviations: α-syn, α-synuclein; ASA, American Society of Anesthesiologists'; BMI, Body Mass Index; BSA, Bovine Serum Albumin; CAM, Confusion Assessment Method; CI, Confidence Intervals; CNS, Central Nervous System; CSF, Cerebrospinal Fluid; IL-1β, Interleukin-1β; IL-6, Interleukin-6; IQR, Inter Quartile Range; L_1CAM, L_1-Cell Adhesion Molecular; MDAS, Memorial Delirium Assessment Scale; MMSE, Mini-Mental State Examination; NRS, Numerical Rating Scale; OR, Odds Ratios; PBS, Phosphate Buffered Saline; PCA, Patient Control Analgesia; PD, Parkinson's disease; POD, Postoperative delirium; TICS, Telephone Interview for Cognitive Status; TNF-α, Tumor Necrosis Factor-α.

DNA Labeling Hot Paper

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Chromophore-Assisted Proximity Labeling of DNA Reveals Chromosomal Organization in Living Cells

*Tao Ding[+], Liyuan Zhu[+], Yuxin Fang, Yangluorong Liu, Wei Tang, and Peng Zou**

Dedicated to the 100th Birthday of Professor Youqi Tang

Abstract: *The spatial arrangement of chromosome within the nucleus is linked to genome function and gene expression regulation. Existing genome-wide mapping methods often rely on chemically crosslinking DNA with protein baits, which raises concerns of artifacts being introduced during cell fixation. By genetically targeting a photosensitizer protein to specific subnuclear locations, we achieved blue-light-activated labeling of local DNA with a bioorthogonal functional handle for affinity purification and sequence identification through next-generation sequencing. When applied to the nuclear lamina in human embryonic kidney 293T cells, it revealed lamina-associated domains (LADs) that cover 37.6 % of the genome. These LADs overlap with heterochromatin hallmarks and are depleted with CpG islands. This simple labeling method avoids the harsh treatment of chemical crosslinking and is generally applicable to the genome-wide high-resolution mapping of the spatial chromosome organization in living cells.*

In eukaryotic cells, the three-dimensional chromosome architecture has profound effects on gene functions.[1,IIIIo2] The formation of local chromatin structures is crucially involved in a diverse array of biological processes, ranging from gene activation[3,4] to replication timing.[5,6] For example, genomic regions associated with the nuclear lamina, a fibrillar network at the periphery of the nucleus, are mainly composed of silent genes. Moreover, dysregulated chromatin structures have been implicated in many diseases,[7] and manipulation of 3D genome has been shown to alter gene expression levels and cellular functions.[8,9]

Conventional approaches for studying DNA-protein interactions and the spatial arrangement of chromatin, such as chromatin immunoprecipitation-sequencing (ChIP-seq), often require formaldehyde-mediated chemical crosslinking, which may introduce bias.[10] In addition, successful immunoprecipitation depends critically on the availability of specific antibodies against the bait protein. Alternatively, various proximity labeling techniques have been developed to profile DNA-protein contacts at specific subcellular locations. For example, by employing enzyme-mediated DNA methylation near a bait protein, DamID (DNA adenine methyltransferase identification) has been widely used to investigate protein-DNA interactions in vivo.[11,12] Recently, an antibody-targeted peroxidase-mediated labeling strategy, termed TSA-seq, was developed to biotinylate proximal DNA with phenoxyl free radicals, which has revealed chromatin organization near the nuclear lamina and nuclear speckle in fixed cells.[13] Each of these methods has its own merits and weaknesses. For example, TSA-seq requires cellular fixation and membrane permeabilization to allow the intracellular delivery of antibody-peroxidase conjugate, while DamID-catalyzed DNA methylation is restricted to labeling adenine within the palindromic tetrad sequence GATC.[11]

Herein, we present a conceptually novel DNA proximity labeling technique that offers both high spatial and high temporal resolutions in live cells. Our method is built upon the photosensitized DNA oxidative damage. Among all four DNA nucleobases, guanine has the lowest redox potential and is readily oxidized by singlet oxygen ($^1O_2$) to yield a range of products, including spiroiminodihydantoin (Sp), imidazolone (Iz), oxazolone (Oz), etc.[14] We chose miniSOG, an engineered flavoprotein originally derived from *A. thaliana* phototropin 2, as the photosensitizer because it can be genetically targeted to specific subnuclear locations in the form of protein fusion.[15] Upon blue light illumination, miniSOG generates $^1O_2$ via type II photoreaction.[16]

While this chromophore-assisted proximity labeling strategy has been previously implemented by our group and others to profile the spatial organization of RNA,[17–19] it cannot be easily extended to labeling DNA because of the following unique challenges associated with chromatin: 1) substantially lower copy number of DNA (2 in diploid cells) compared to RNA (often >100 per cell); 2) lower reactivity of double-stranded DNA (dsDNA) versus single-stranded RNA;[17] and 3) shielding of DNA by nucleosome and higher order

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Protocol

Protocol for Proximity-Dependent Proteomic Profiling in Yeast Cells by APEX and Alk-Ph Probe

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SUMMARY

Alk-Ph is a clickable APEX2 substrate developed for spatially restricted protein/RNA labeling in intact yeast cells. Alk-Ph is more water soluble and cell wall permeable than biotin-phenol substrate, allowing more efficient profiling of the subcellular proteome in microorganisms. We describe the protocol for Alk-Ph probe synthesis, APEX2 expression, and protein/RNA labeling in yeast and the workflow for quantitative proteomic experiments and data analysis. Using the yeast mitochondria as an example, we provide guidelines to achieve high-resolution mapping of subcellular yeast proteome and transcriptome.

For complete details on the use and execution of this protocol, please refer to Li et al. (2020).

BEFORE YOU BEGIN

Traditional physical or biochemical tools to isolate specific subcellular organelles for proteomic studies have inherent limitations. They are often prone to contamination and could not easily access transient or weak protein-protein interaction. The peroxidase-mediated proximity labeling technique has been developed and widely used to determine the protein inventories of distinct subcellular compartments. As an engineered ascorbate peroxidase, APEX2 oxidizes biotin-phenol in the presence of H_2O_2 to generate short-lived phenoxyl free radical that covalently links to nearby electron-rich amino acid residues, such as tyrosine. Biotin-phenol based APEX labeling has been used to map the proteome at various subcellular compartments, including both membrane-bound organelles (e.g., mitochondria) (Hung et al., 2017; Hung et al., 2014; Rhee et al., 2013) and membraneless organelles (e.g., stress granules) (Markmiller et al., 2018). APEX techniques have also been applied *in vivo*, including *Drosophila* (Chen et al., 2015) and *C.elegans* (Reinke et al., 2017).

Despite the wide use of APEX in mammalian cell culture, its application in microorganisms, such as yeast, has been limited due to poor cell wall permeability of biotin-phenol (Hwang and Espenshade, 2016; Singer-Krüger et al., 2020). Zymolase digestion (Hwang and Espenshade, 2016) or freeze-thaw cycles (Singer-Krüger et al., 2020) were used to compromise the cell wall to facilitate probe penetration. Recently, we designed and synthesized a clickable APEX substrate, Alkyne-Phenol (Alk-Ph), by replacing the biotin moiety to a small clickable alkynyl group (Li et al., 2020), which exhibited higher solubility and improved membrane permeability than biotin-phenol. APEX2-mediated proximity-