



The Yang Research Group
Precise Synthesis Lab at Tongji University



Group Topic

铁酶催化简介

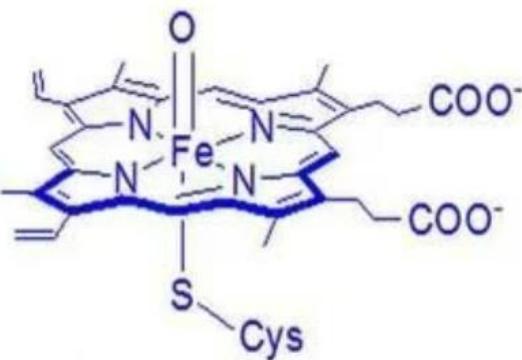
Reporter: Canxiang Liu

Date: 2025.11.1

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- 细胞色素 P450 (cytochrome P450, CYP) 酶以血红素卟啉铁为中心
- 能够代谢多种异源物质，与环氧化、羟基化、脱烷基化、脱氨、脱硫、脱卤化、亚砜氧化和 N-氧化物还原等多种反应相关。
- CYP 的结构解析对于研究和预测药物代谢非常重要，人类 CYP 是药物的靶标并参与多种药物相互作用，其结构解析大大简化了药物的设计和临床研究。
- CYP 的表达和功能受到许多因素的影响，包括 CYP 的诱导、抑制和遗传多态性等。这些因素以及药物之间的相互作用导致
- CYP 的诱导或抑制是药物相互作用的主要机制。



酶是由生物体产生的、具有高效和专一催化功能的蛋白质或RNA。它们是生命体内的“分子机器”，驱动着新陈代谢、DNA复制、信号传导等所有生物过程。



高效率

酶能将反应速度提高
数百万甚至数万亿倍



高选择性

区域选择性、立体选
择性、化学选择性



反应条件温和

通常在常温、常压、
近中性pH的水溶液中
进行，与传统化学工
业的高温高压相比，
更加绿色、环保、节
能。

局限性：

天然功能有限：自然界的酶是进化来服务于生物体自身需求的，它们通常无法催化非天然的、工业生产所需的化学反应。

稳定性差：许多酶在高温、有机溶剂或极端pH下容易失活。

难以重复使用：游离酶不易从反应体系中分离回收。

挑战与目标：如何克服天然酶的局限，将其强大的催化能力应用于更广阔的工业和科研领域。



Frances H. Arnold

Professional experience

Nobel Prize in Chemistry(2018).

1986 - present Professor, California Institute of Technology;

Ph.D. University of California, Berkeley (1985);

B.S. Princeton University (1979);

Research

Frances H. Arnold教授因开创“酶的定向进化”方法而闻名，该方法通过模拟自然进化在实验室中优化和设计酶，用于催化非天然反应及生产可持续化学品与药物。



贾知军

Professional experience

2020-至今 四川大学，研究员；

2018-2020 美国加州理工学院，博士后，师从Frances H. Arnold教授；

2017-2018 德国马克斯·普朗克分子生理所，博士后，师从Herbert Waldmann教授；

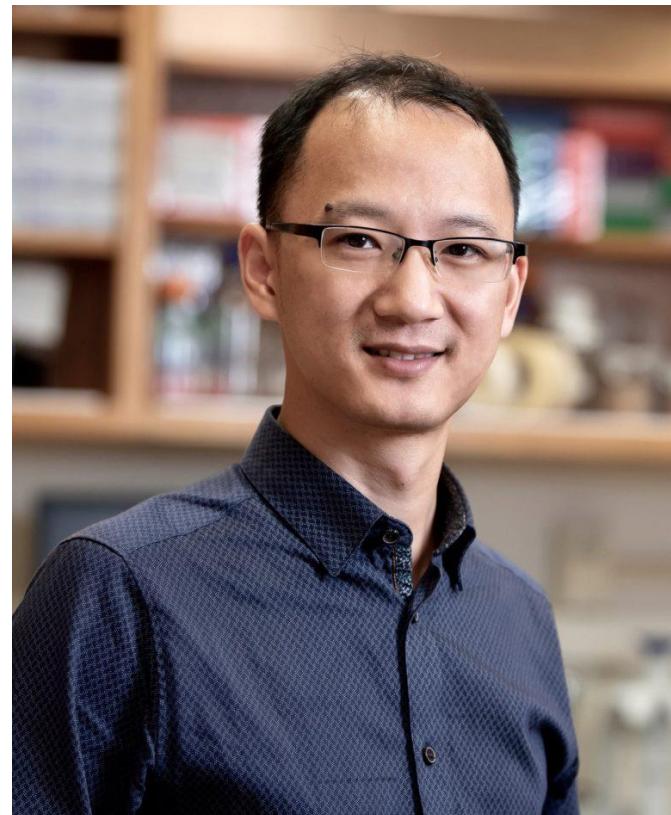
2012-2016 德国多特蒙德工业大学化学生物学专业，博士，师从Herbert Waldmann教授；

2009-2012 四川大学华西药学院药物化学专业，硕士，师从陈应春教授；

2005-2009 四川大学生命科学院生物技术基地班，本科；

Research

综合利用定向进化和从头设计策略，创造非天然的酶功能，从而发展手性药物绿色制造的新方法，以及儿童疾病预防、诊断和治疗的新手段。



Xiongyi Huang

Professional experience

2019 – 至今 Assistant Professor, Johns Hopkins University;

2016 - 2019 Postdoc, California Institute of Technology; Advisor: Prof. Frances Arnold;

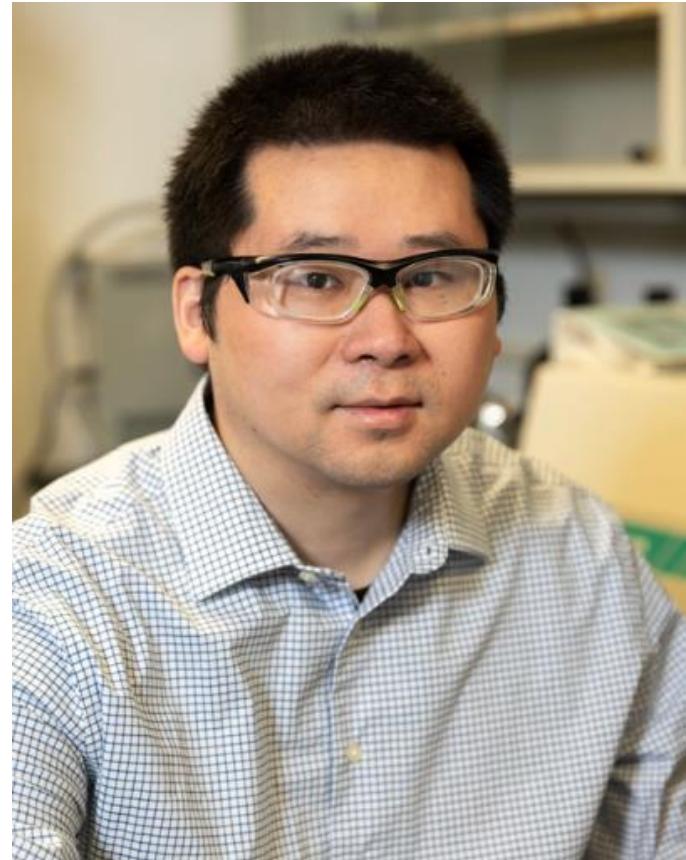
2011 - 2016 Ph.D., Princeton University; Advisor: Prof. John T. Groves;

2006 - 2010 B.S., University of Science and Technology of China; Advisor: Prof. Yao Fu

Research

主要涉及酶催化、化学生物学、有机和生物无机化学。致力于通过定向进化和机理研究，改造和设计酶，使其能够催化自然界中不存在的反应，从而拓展生物催化的边界。

Professional experience

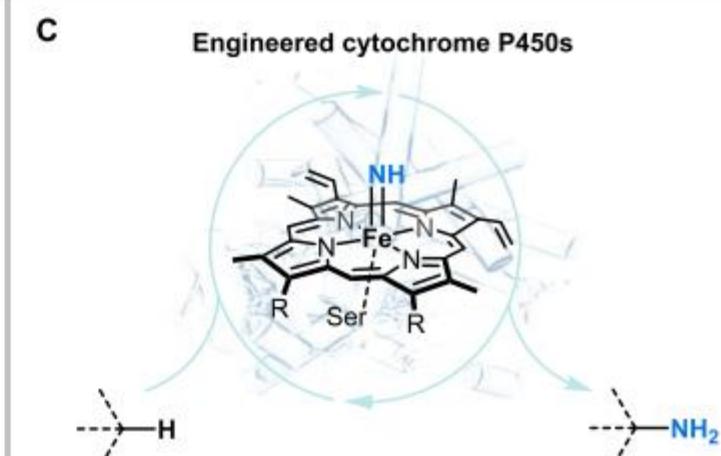
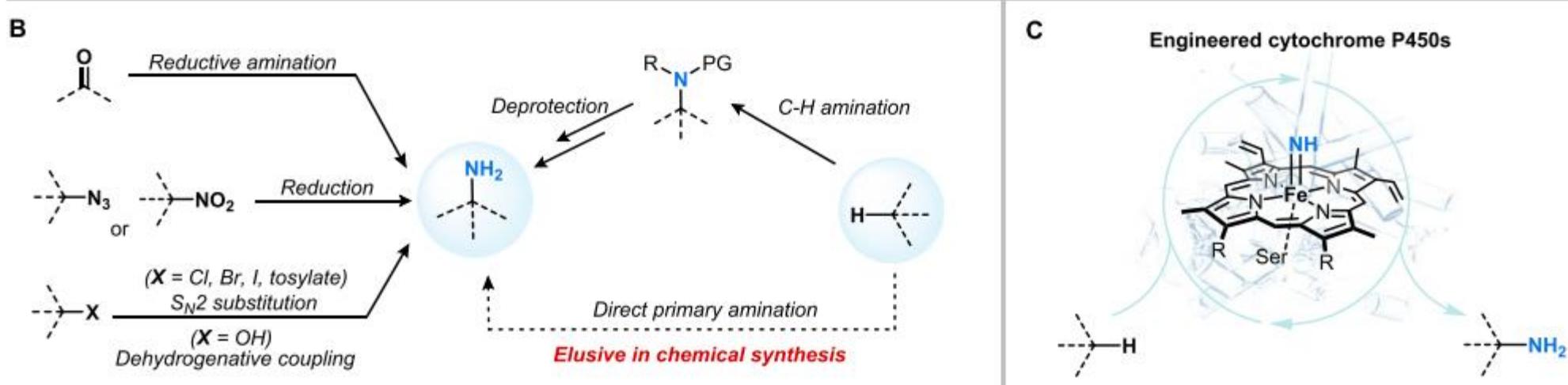
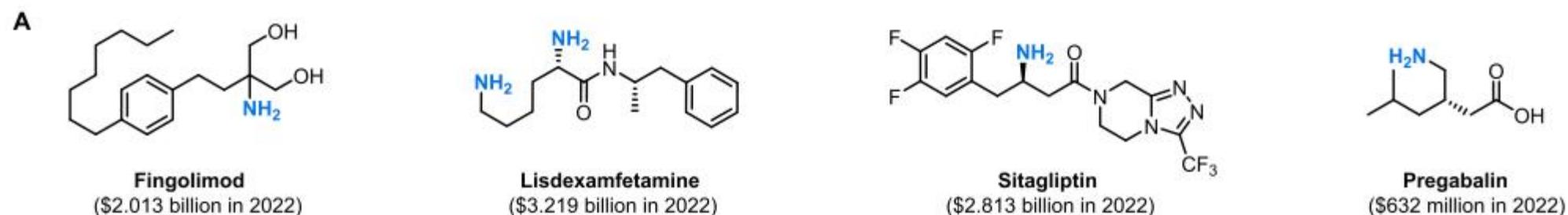


2020 - present Associate Professor, University of California Santa Barbara;
NIH Postdoctoral Fellow, California Institute of Technology (2020). Advisor: Prof. Frances Arnold
Miller Postdoctoral Fellow, University of California, Berkeley (2018). Advisor: Prof. Jeffrey Long
Ph.D. Organic Chemistry, Massachusetts Institute of Technology (2016). Advisor: Prof. Stephen Buchwald
B.S. Chemistry, Peking University (2011). Advisor: Prof. Jianbo Wang;

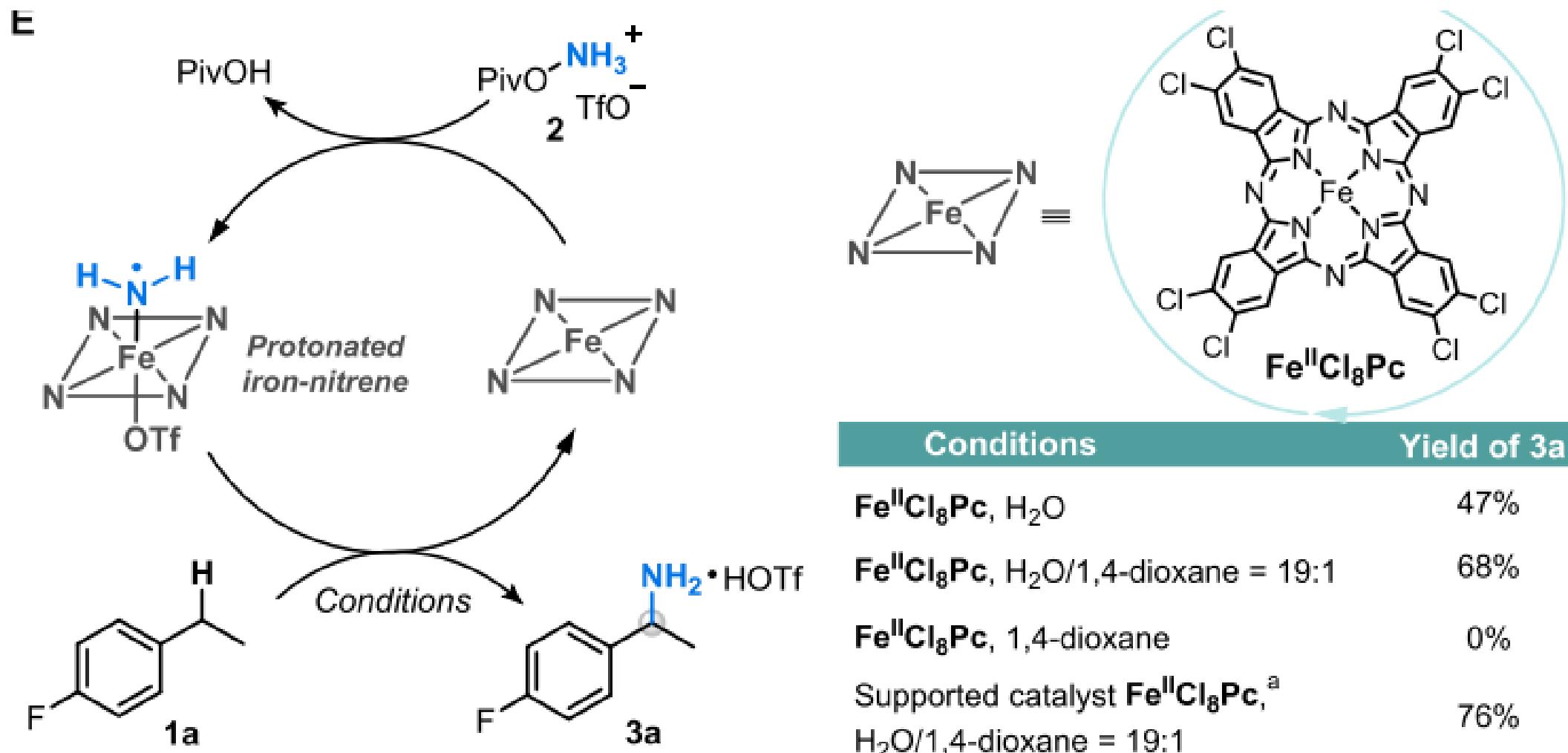
Research

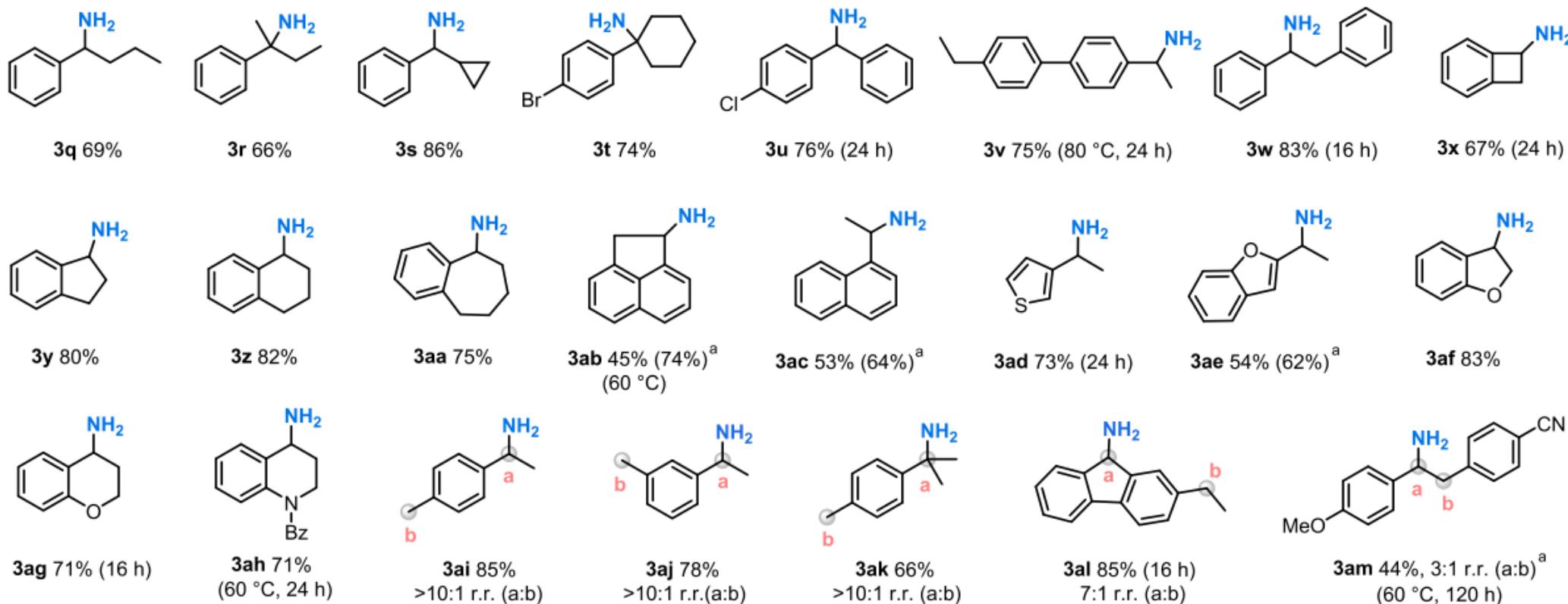
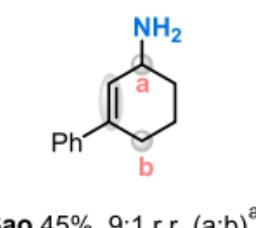
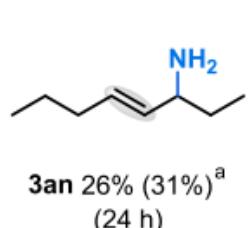
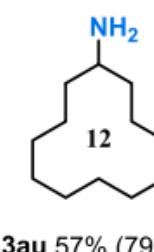
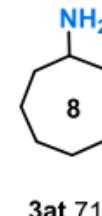
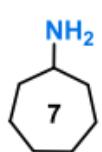
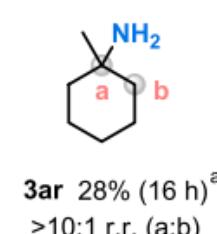
专注于通过定向进化等手段改造酶，以催化自然界中不存在的、具有高立体选择性的非天然反应（如自由基环化反应）。其研究整合了有机合成、蛋白质工程与计算模拟，并探索光生物催化等新型催化模式。

Yangyang

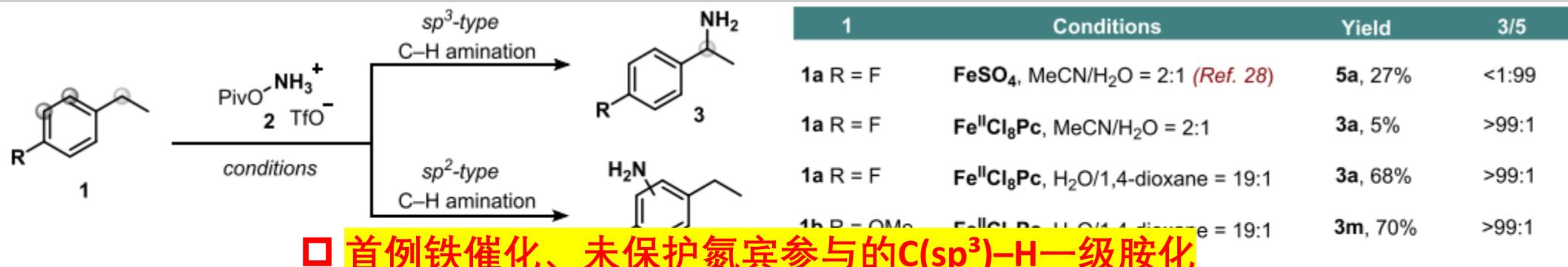


- 一级胺在药物中广泛存在，并可作为引入其他官能团的关键前体。
- 传统合成方法依赖“预官能团化”策略（如还原胺化、叠氮还原、取代反应等），步骤繁琐，原子经济性差。
- 直接C(sp³) - H胺化是最理想的策略，但目前大多只能引入受保护的胺基。
- 近年来酶催化方法有所突破，但仅适用于简单分子，复杂分子的后期胺化仍是挑战。

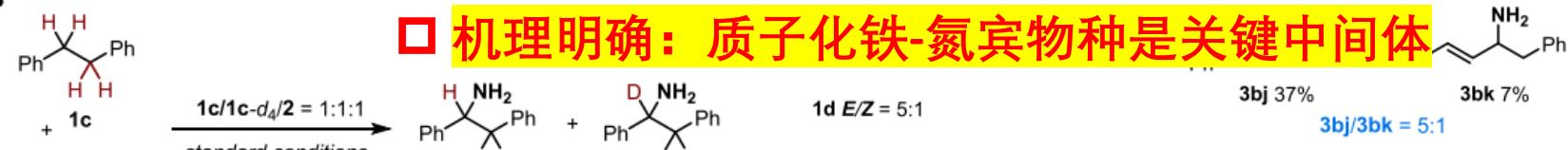


(b) Allylic C(sp³)-H Bonds(c) Unactivated C(sp³)-H Bonds

A

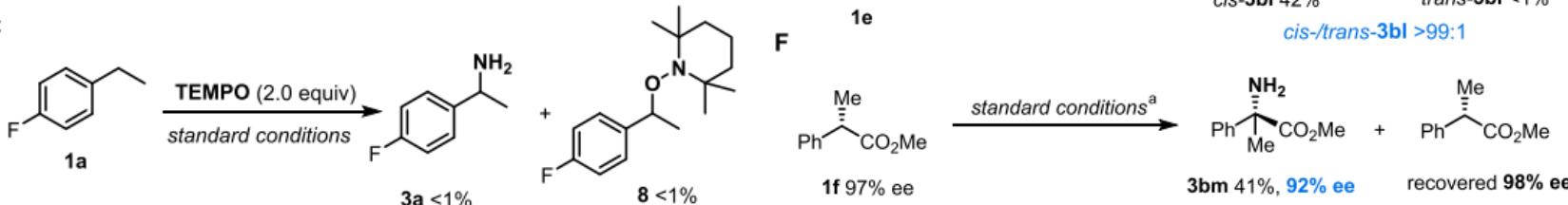


B



- 催化剂与溶剂决定化学选择性：Fe^{II}Cl₈Pc + 高水含量 → 优先C(sp³)-H胺化；FeSO₄ + 有机溶剂 → 优先C(sp²)-H胺化。
- 动力学同位素效应：KIE = 2.0，表明C-H断裂是速率决定步骤。
- 自由基捕获实验：TEMPO抑制反应，并检测到TEMPO加合物，支持自由基机制。
- 立体化学研究：从顺式十氢萘得到高立体保持产物，说明自由基寿命极短 (~100 ps)，快速“反弹”形成C-N键。

C



c) This work: new-to-nature enzymatic trifluoromethylazidation of alkenes

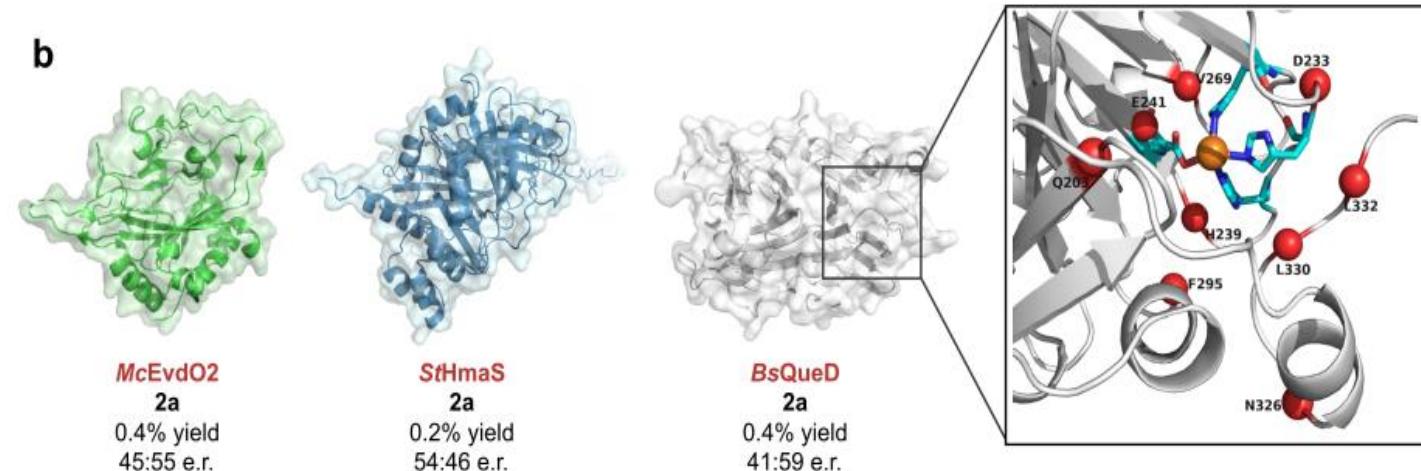
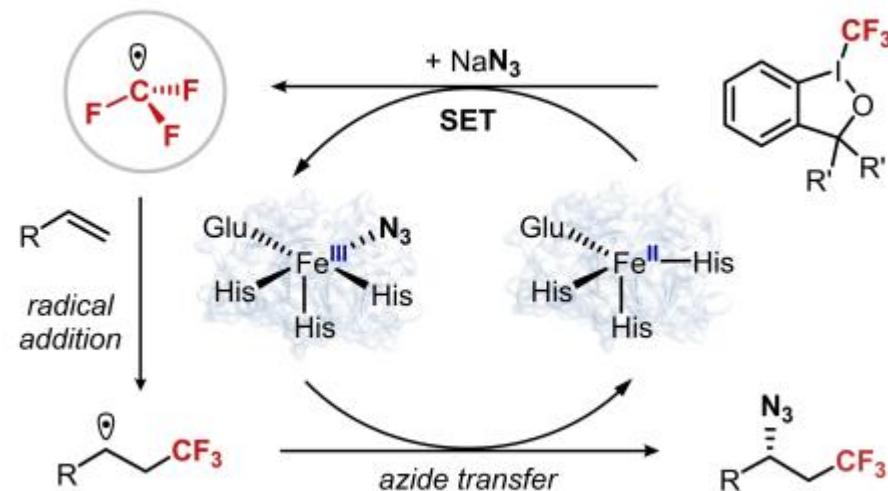
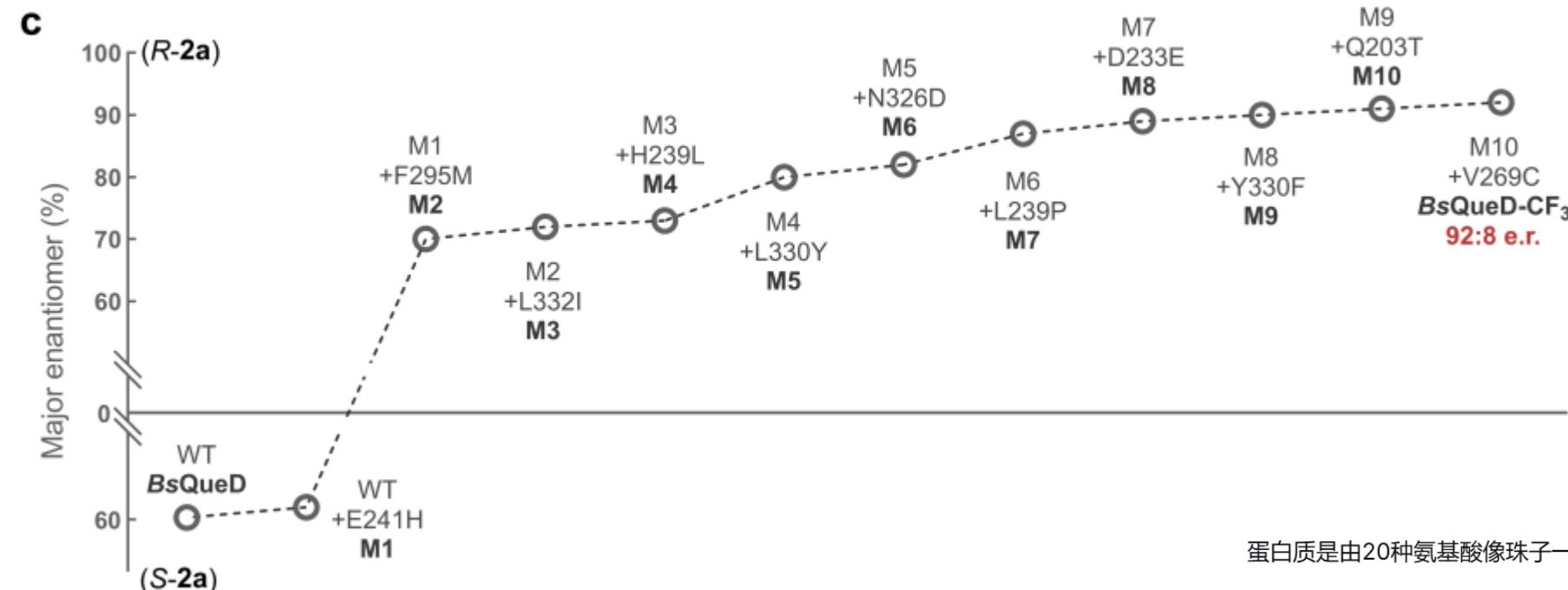


Figure 1. Trifluoromethylazidation of alkenes.

- BsQueD活性中心含有两个铁原子，并采用了一种相对罕见的3-组氨酸-1-羧酸盐配位环境
- 初始活性与选择性最佳
- 蛋白结构与可塑性已知



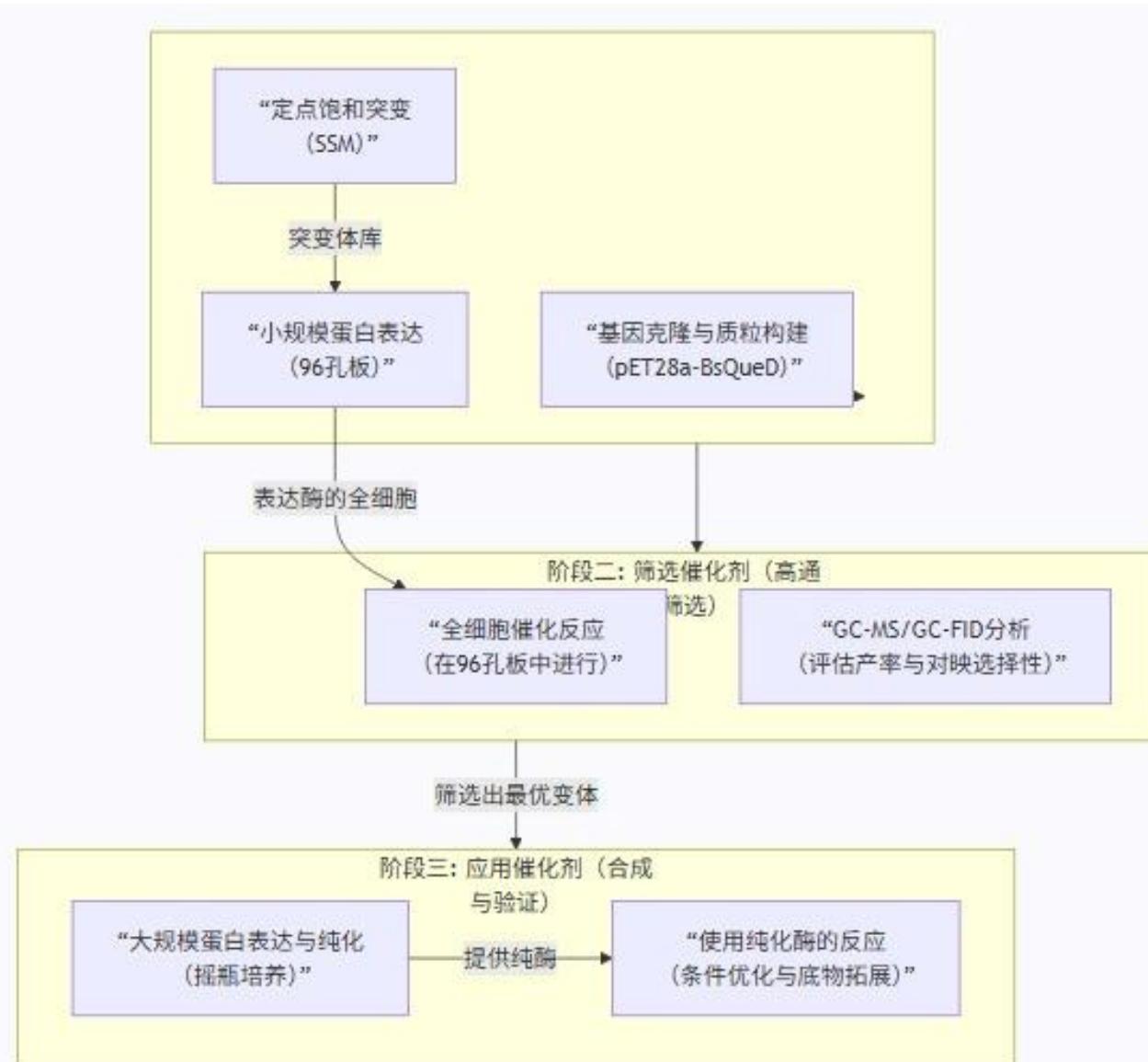
蛋白质是由20种氨基酸像珠子一样串起来的长链。每个氨基酸都有名字和缩写。

- **F** 代表 苯丙氨酸
- **M** 代表 甲硫氨酸
- **L** 代表 亮氨酸
- **P** 代表 脯氨酸

2. 以 F295M 为例进行分解

- **F**：在蛋白质的第295号位置上，原本是一个苯丙氨酸。
- **295**：这是氨基酸在序列中的位置编号。
- **M**：通过基因突变，研究人员将这个位置的苯丙氨酸**替换了**甲硫氨酸。

第一个字母：野生型（原始、天然的）氨基酸的缩写。
 数字：该氨基酸在蛋白质序列中的位置。
 第二个字母：突变型（替换上去的）氨基酸的缩写。



阶段一：准备催化剂 – 制造“酶工厂”

基因克隆：将目的酶的基因插入到表达载体（如pET-28a (+)）中。这个载体就像一个“生产指令”，告诉大肠杆菌如何生产我们想要的酶。

定点饱和突变：这是定向进化的核心。使用一种称为“22-密码子技巧”的方法，在特定位点（如E241）上产生所有可能的氨基酸替换，构建一个突变体库。

蛋白表达（96孔板）：在深孔板中大规模培养转化后的大肠杆菌，并用IPTG诱导蛋白表达。最终获得含有不同突变体酶的全细胞催化剂。

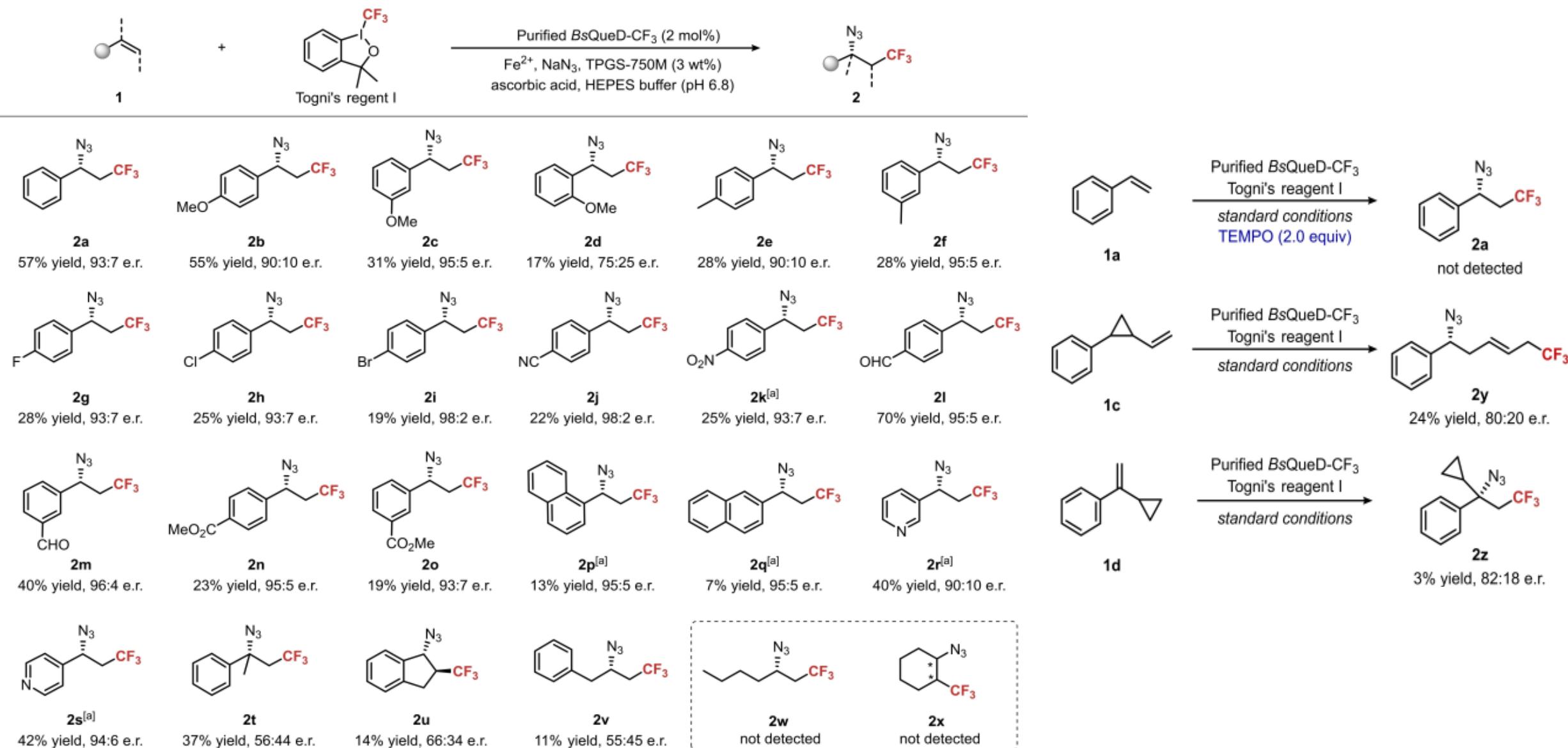
阶段二：筛选催化剂 – 大海捞针，找到最佳

- ✓ 反应筛选（96孔板，全细胞形式）：这是高通量筛选的核心步骤。
- ✓ 反应环境：在厌氧手套箱中进行，这至关重要，因为氧气会抑制或干扰依赖Fe(II)的自由基反应。
- ✓ 反应组分：细胞悬浮液：作为生物催化剂。
- ✓ 抗坏血酸&Fe(NH₄)₂(SO₄)₂：用于维持铁中心在Fe(II)活性状态，辅助Togni试剂的活化。
- ✓ NaN₃：叠氮源；苯乙烯&Togni试剂：底物和CF₃自由基前体。
- ✓ 萃取与分析：反应后，用有机溶剂萃取产物，并通过GC-MS（定量）和GC-FID（分析对映选择性）进行快速分析。这一步能从成千上万个突变体中快速找出产率高、对映选择性好的“明星酶”。

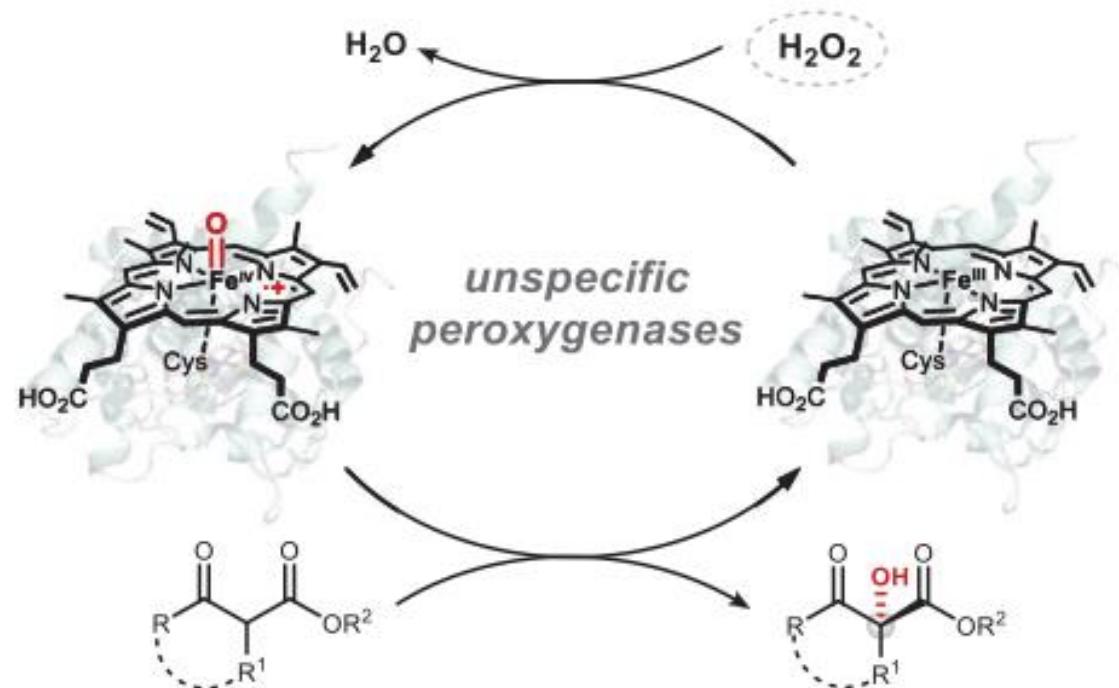
阶段三：应用催化剂 – 合成与验证

- ✓ 蛋白表达与纯化（摇瓶）：对筛选出的最佳变体（如BsQueD-CF₃）进行大规模培养和纯化。
- ✓ 分析反应（使用纯化酶）：这是进行条件优化和底物拓展时使用的标准方法。

注：使用纯化的酶代替全细胞，并加入了表面活性剂TPGS-750M。这使得反应体系更干净、更可控，排除了细胞代谢的干扰。TPGS-750M能形成胶束，提高疏水性底物（如苯乙烯和Togni's试剂）在水相中的溶解度，从而显著提高产率（从~5%提升至57%）。



b) This work: biocatalytic enantioselective α -hydroxylation of β -ketoesters



Scheme 1. Overview of enantioselective α -hydroxylation of β -ketoesters.

选择性氧官能团化 是有机合成中引入含氧官能团的关键反应，传统化学方法往往条件苛刻、选择性差。

生物催化 因其高效、高选择性和温和条件成为理想替代方案。

细胞色素P450 是研究最广泛的生物催化剂，但其依赖昂贵辅酶、稳定性差，限制了工业应用。

非特异性过氧合酶：

使用廉价 H_2O_2 作为氧化剂，无需辅酶；

是真菌分泌酶，通常更稳定；

催化潜力巨大，但天然多样性有限，工程化难度大。

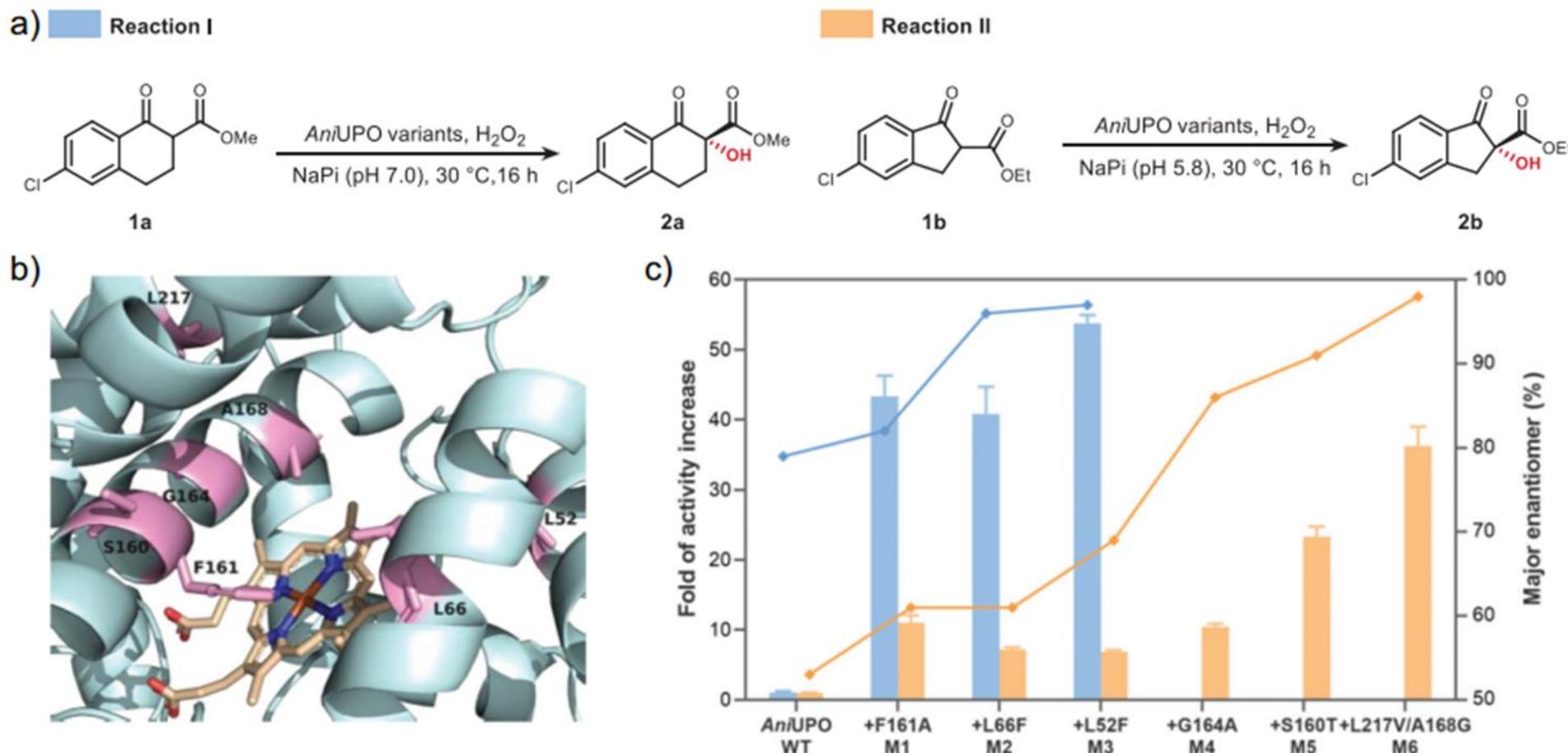
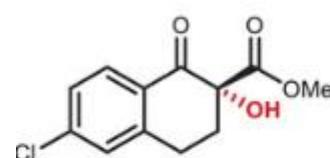
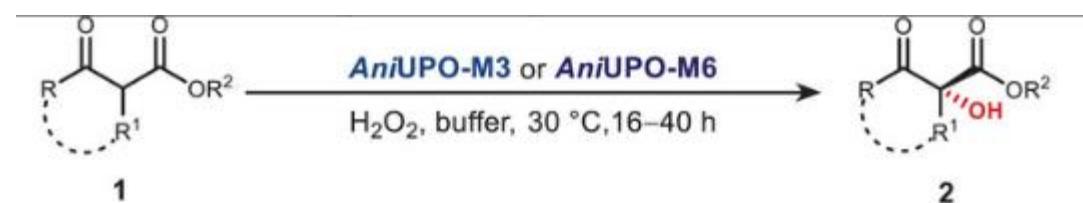
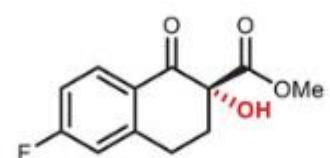


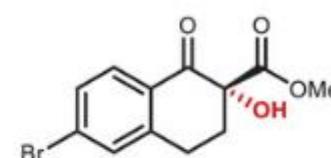
Figure 1. Engineering AniUPO for enantioselective α -hydroxylation of β -ketoesters. a) Model Reactions I and II. b) Alphafold-model of AniUPO with mutated residues marked in pink. c) Mutation trajectory of α -hydroxylation activity for Reaction I (blue) and Reaction II (orange). For each variant, bars indicate fold of activity increase (relative activity) for the reactions, whereas blocks indicate the ratio of major enantiomer of product (R)-2a (blue) and (R)-2b (orange). Error bars represent the standard deviation from three independent replicates ($n = 3$). Refer to Supporting Information for experimental details.



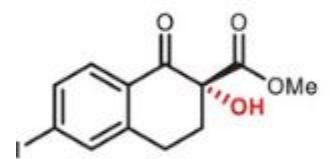
2a
80% yield (**M3**)
1330 TTN, >99:1 er



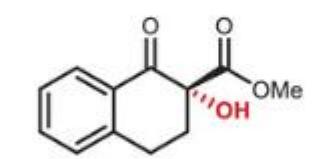
2c
92% yield (**M6**)
3940 TTN, >99:1 er



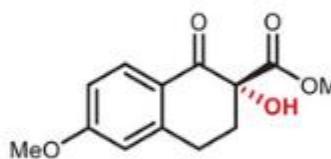
2d
85% yield (**M3**)
1410 TTN, >99:1 er



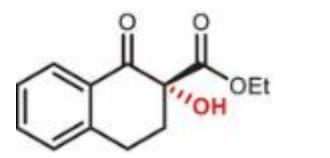
2e
61% yield (**M6**)
2630 TTN, >99:1 er



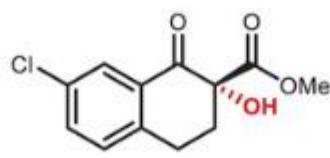
2f
81% yield (**M6**)
3470 TTN, 97:3 er



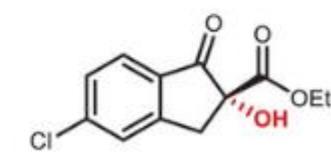
2g
96% yield (**M6**)
4100 TTN, >99:1 er



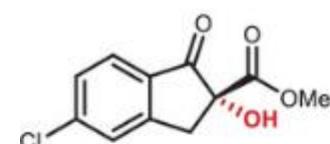
2h
79% yield (**M6**)
3390 TTN, 95:5 er



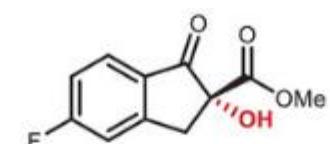
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77% yield (**M6**)
3280 TTN, 96:4 er



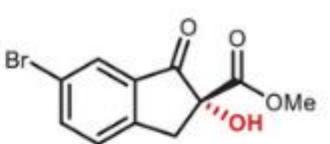
2b
97% yield (**M6**)
4140 TTN, 97:3 er



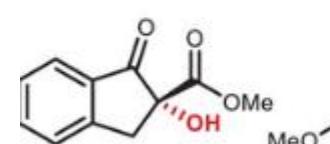
2j
59% yield (**M6**)
2500 TTN, 92:8 er



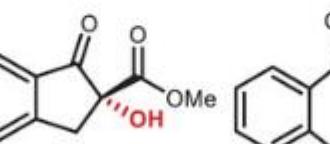
2k
71% yield (**M6**)^[a]
1780 TTN, 91:9 er



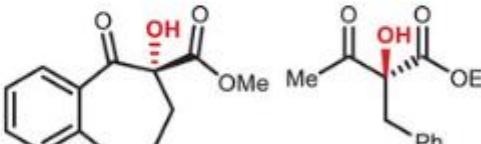
2l
48% yield (**M6**)^[b]
400 TTN, 80:20 er



2m
58% yield (**M6**)^[b]
480 TTN, 80:20 er

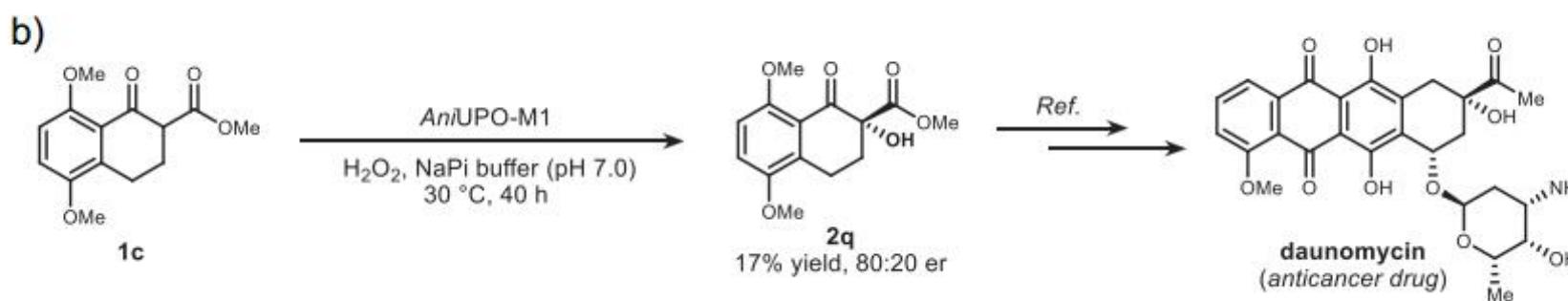
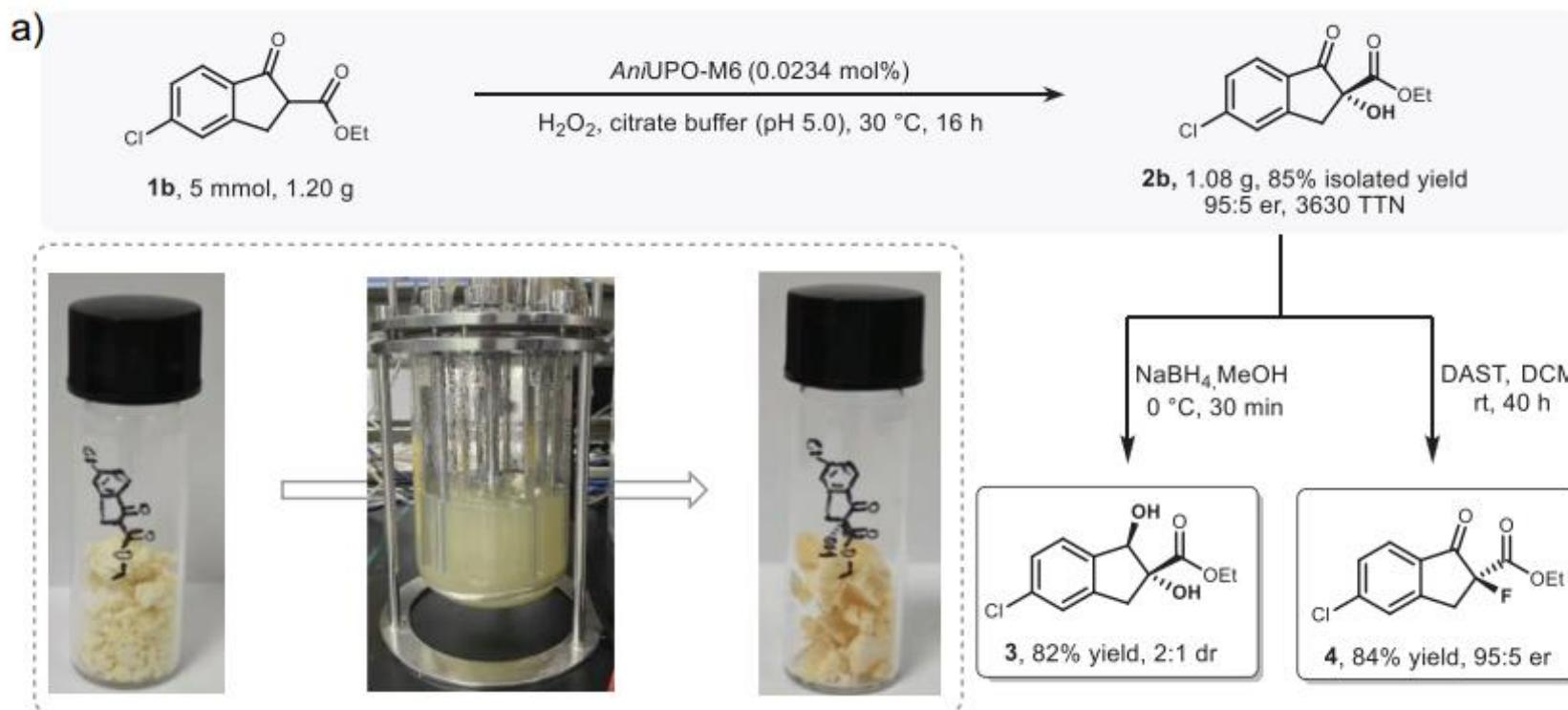


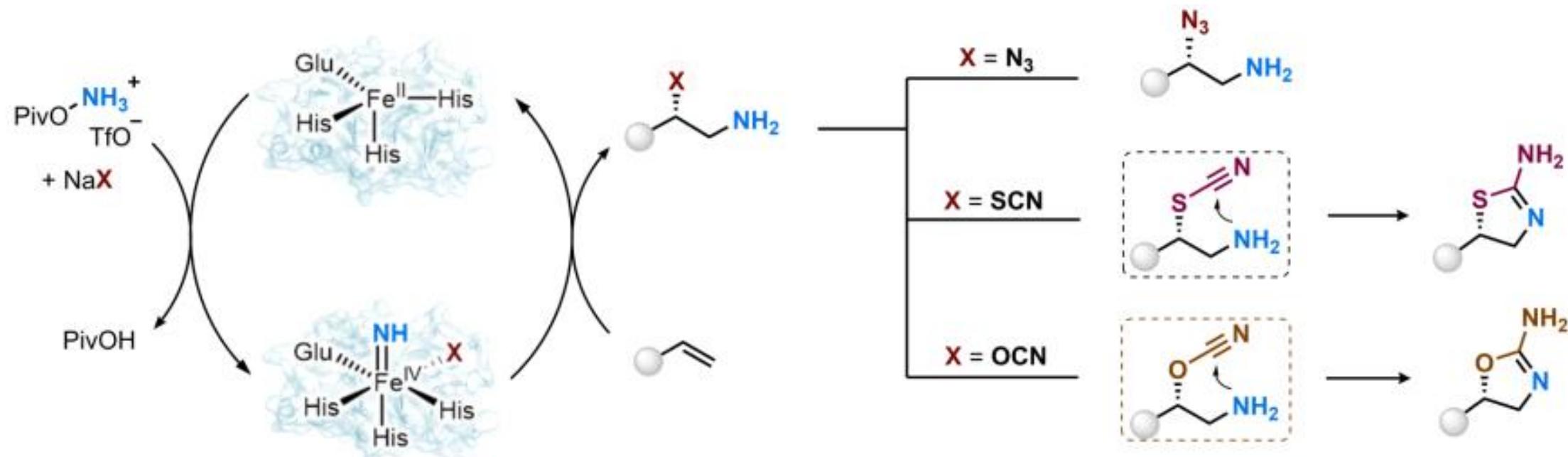
2n
56% yield (**M6**)
2380 TTN, 99:1 er



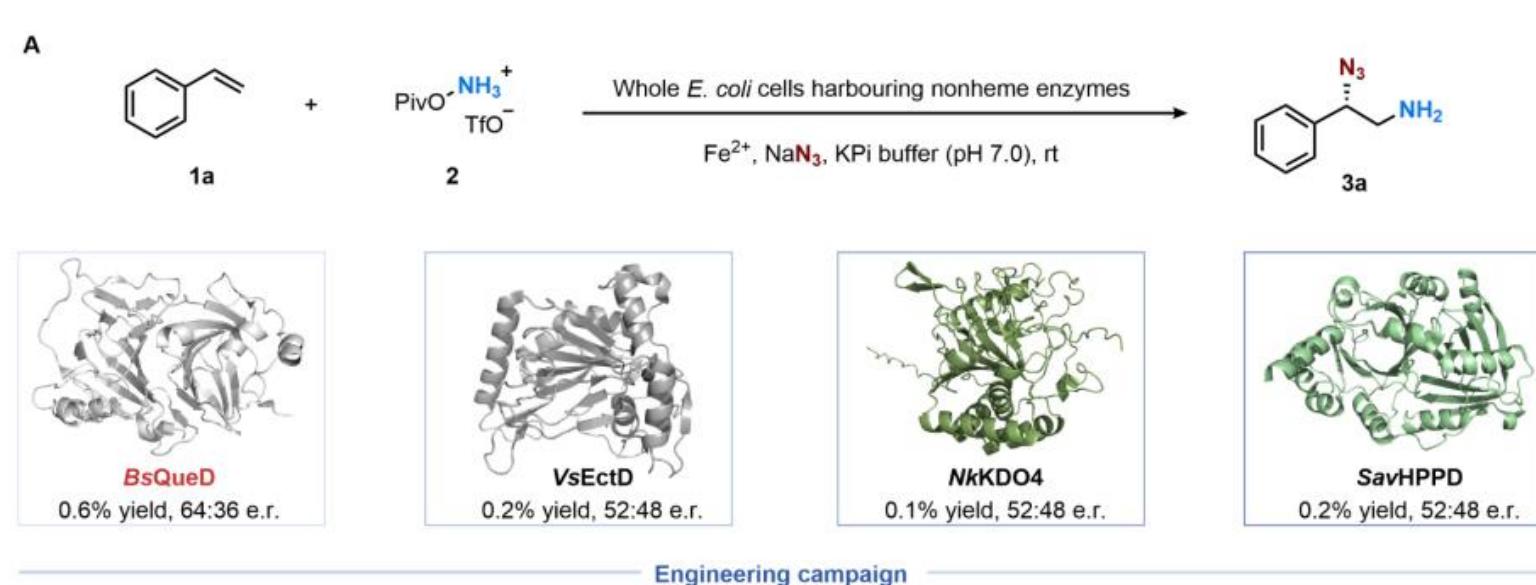
2o
51% yield (**M3**)^[c]
250 TTN, 92:8 er

2p
10% yield (**M3**)
170 TTN, 57:43 er

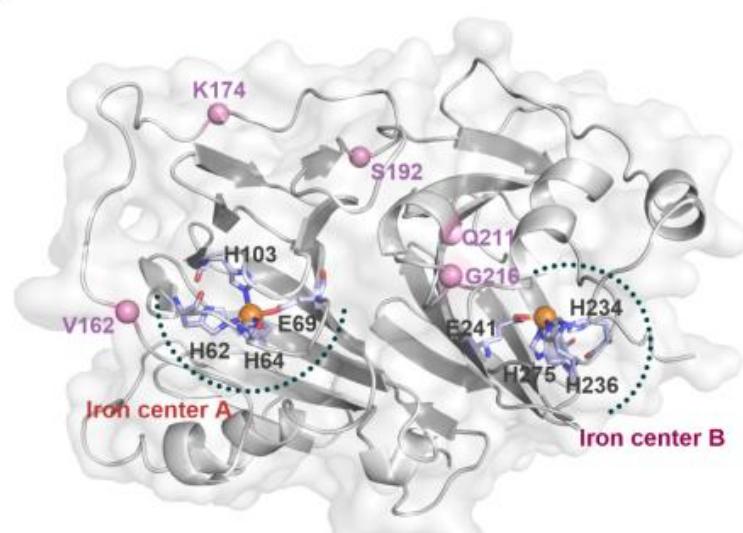




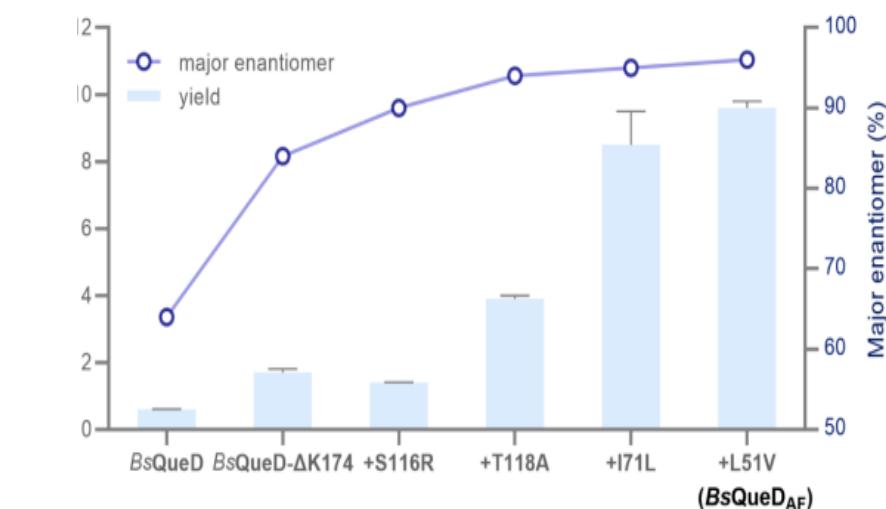
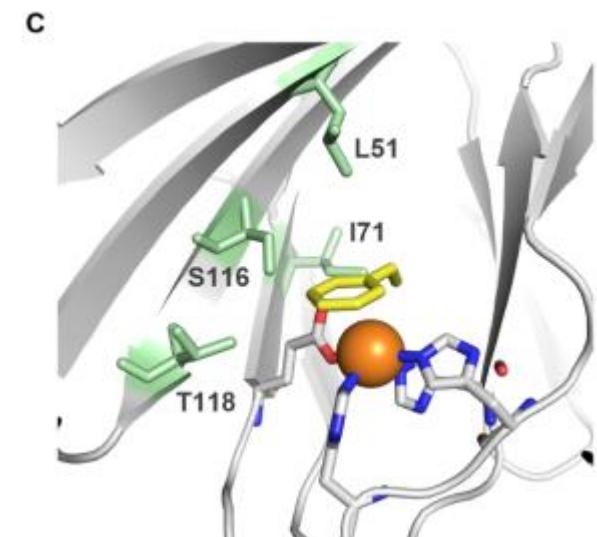
工程化改造了一种非血红素铁酶——来自枯草芽孢杆菌的槲皮素2,3-双加氧酶，以高效、高对映选择性地催化三种不同的烯烃氨基官能团化反应。通过定向进化，开发出一种优化的BsQueD变体，能够生产多种手性2-叠氮胺、2-氨基噻唑啉和2-氨基噁唑啉，最高产率达72%，对映体比率高达99:1。

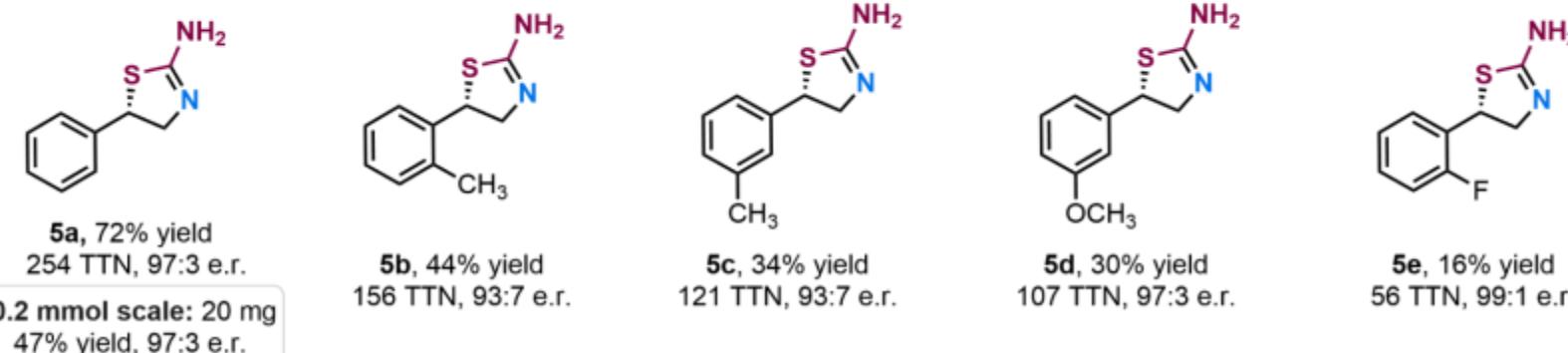
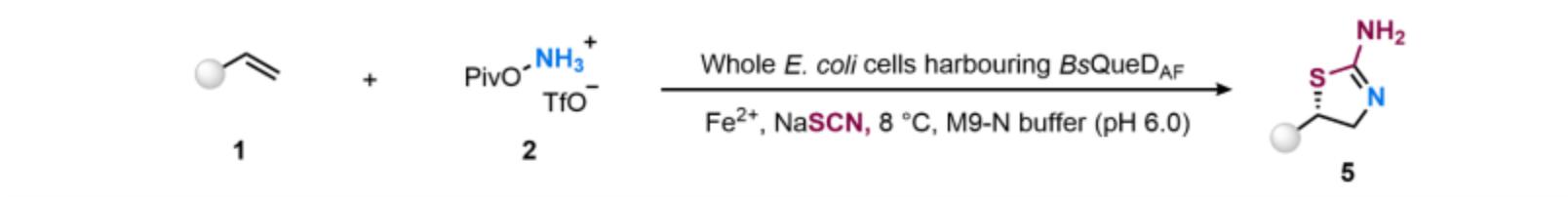
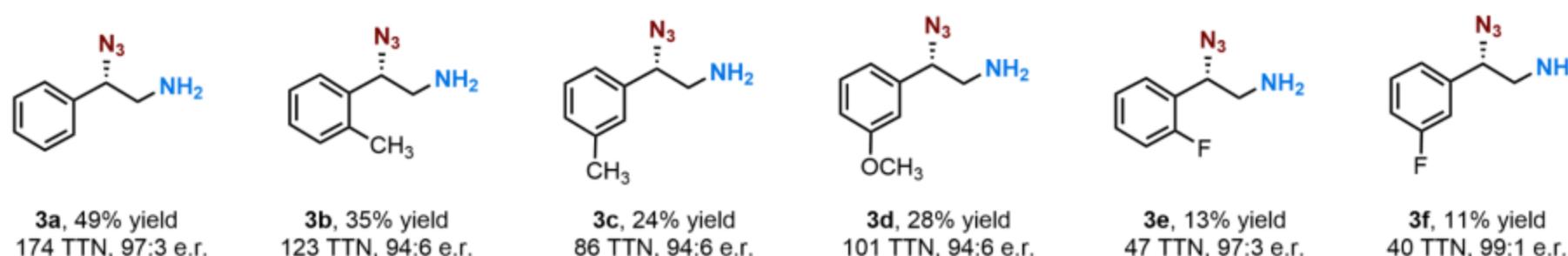
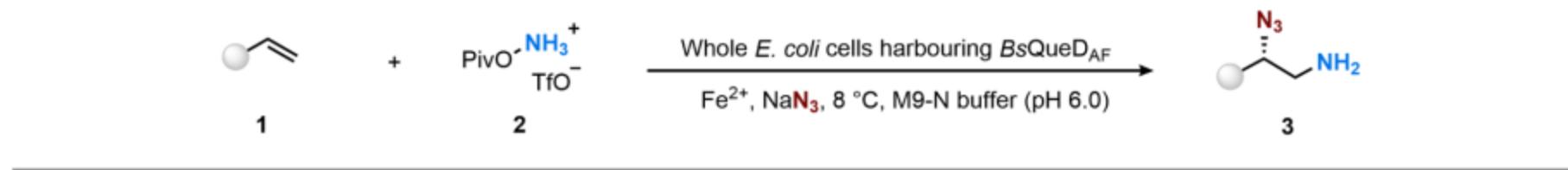


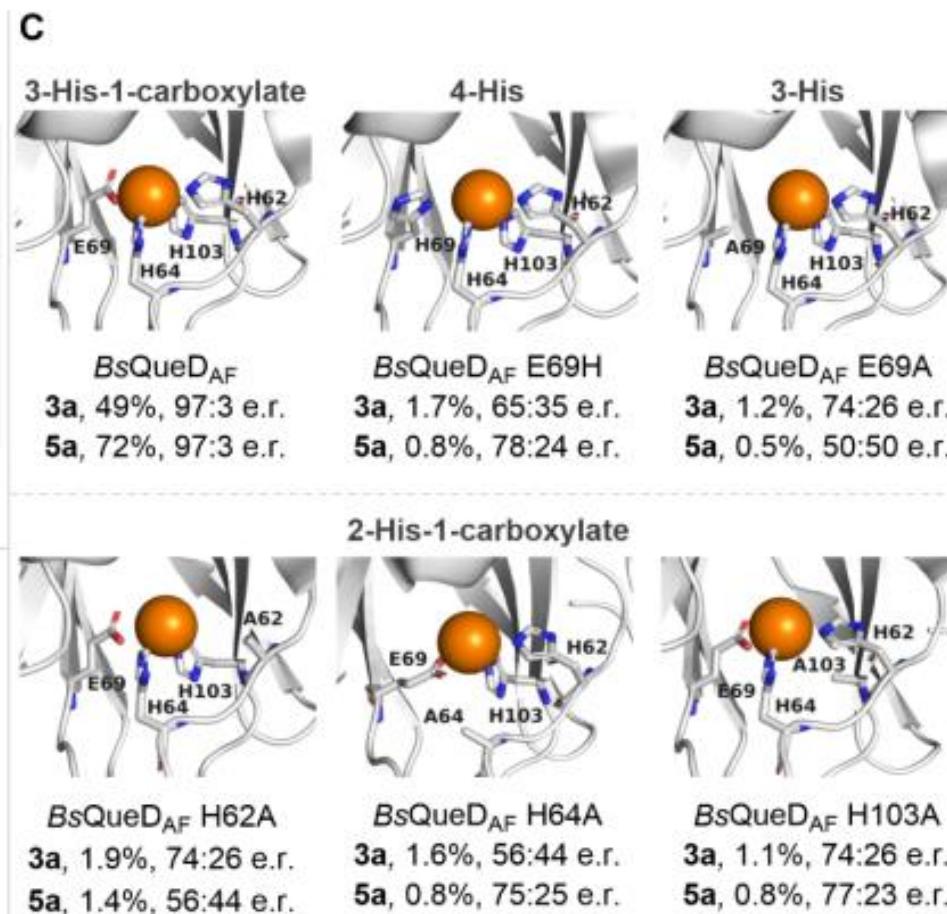
Engineering campaign



| BsQueD variants | Yield | e.r. |
|--|-------|-------|
| Wt | 0.6% | 64:36 |
| <i>BsQueD</i> H62A/H64A/H103A (silencing iron center A) | 0.4% | 48:52 |
| <i>BsQueD</i> H234A/H236A/H275A (silencing iron center B) | 0.7% | 82:18 |
| Protein truncation at G216 site | 0.7% | 82:18 |
| Protein truncation at Q211 site | 0.7% | 82:18 |
| Protein truncation at S192 site | 0.6% | 80:20 |
| Protein truncation at K174 site | 1.7% | 84:16 |
| Protein truncation at V162 site | 0.4% | 62:38 |







铁离子由3个组氨酸和一个谷氨酸残基配位
研究方法：系统地改变了这些直接与铁离子配位的氨基酸残基，然后测试这些突变体酶的催化性能

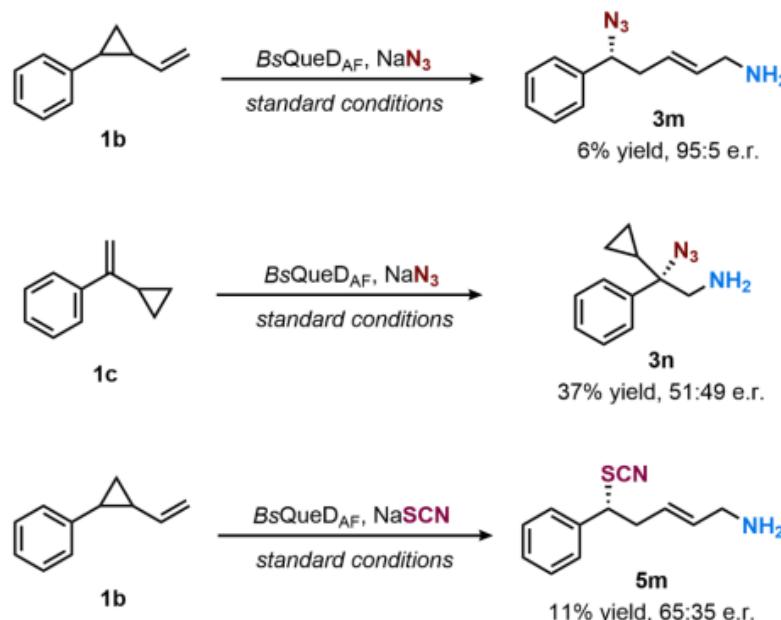
具体突变：

- 将谷氨酸突变为组氨酸 或 丙氨酸，从而将配位环境分别变为 4-组氨酸 或 3-组氨酸（缺少了羧酸根配体）。
- 将三个组氨酸中的每一个分别突变为丙氨酸，构建了三种不同的 2-组氨酸-1-羧酸 配位结构。

结论：铁的初级配位层是酶催化功能的结构性基石，其完整性不容破坏。

| BsQueD variants | Yield of 3a (e.r.) | Yield of 5a (e.r.) |
|--|--------------------|--------------------|
| BsQueD _{AF} R116A | 5% (76:24) | 6.5% (86:14) |
| BsQueD _{AF} L71A | 8% (85:15) | 24% (94:6) |
| BsQueD _{AF} V51A | 10% (85:15) | 9% (88:12) |
| BsQueD S116R/T118A/I71L/L51V (BsQueD-RALV) | 13% (96:4) | 12% (96:4) |
| BsQueD-RALV H234A/H236A/H275A | 12% (95:5) | 10% (95:5) |

B

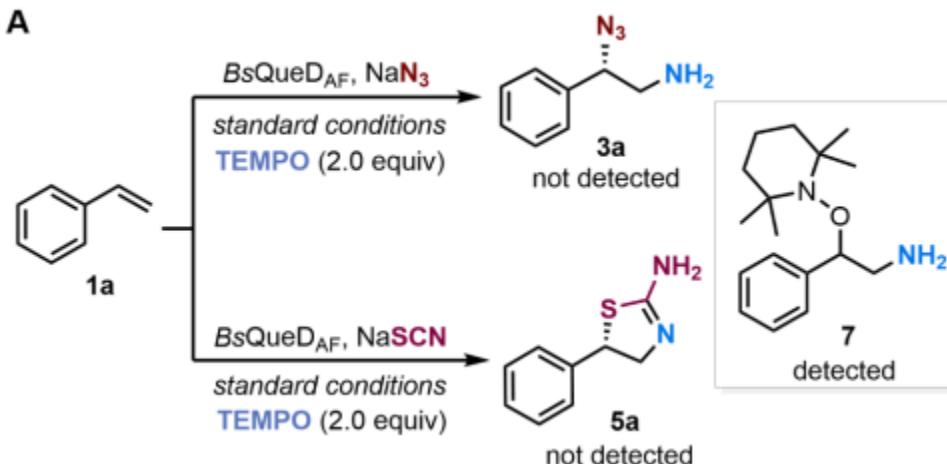


BsQueD-AF包含四个关键突变：S116R, T118A, T71L, L51V 和一个结构域截短 Δ K174

- 将 R116 变回 丙氨酸。
- 将 L71 和 V51 分别变回 丙氨酸。
- 将 截短的结构域恢复，构建 BsQueD-RALV 变体（即包含所有有益突变，但蛋白质是全长结构）。

结论：定向进化中发现的有益突变通过不同的机制发挥作用：有的（如S116R）直接参与手性识别；有的（如T71L, L51V）主要通过微调活性口袋的几何结构来提升活性；还有的（如 Δ K174）通过去除结构性干扰来优化整体性能。

A



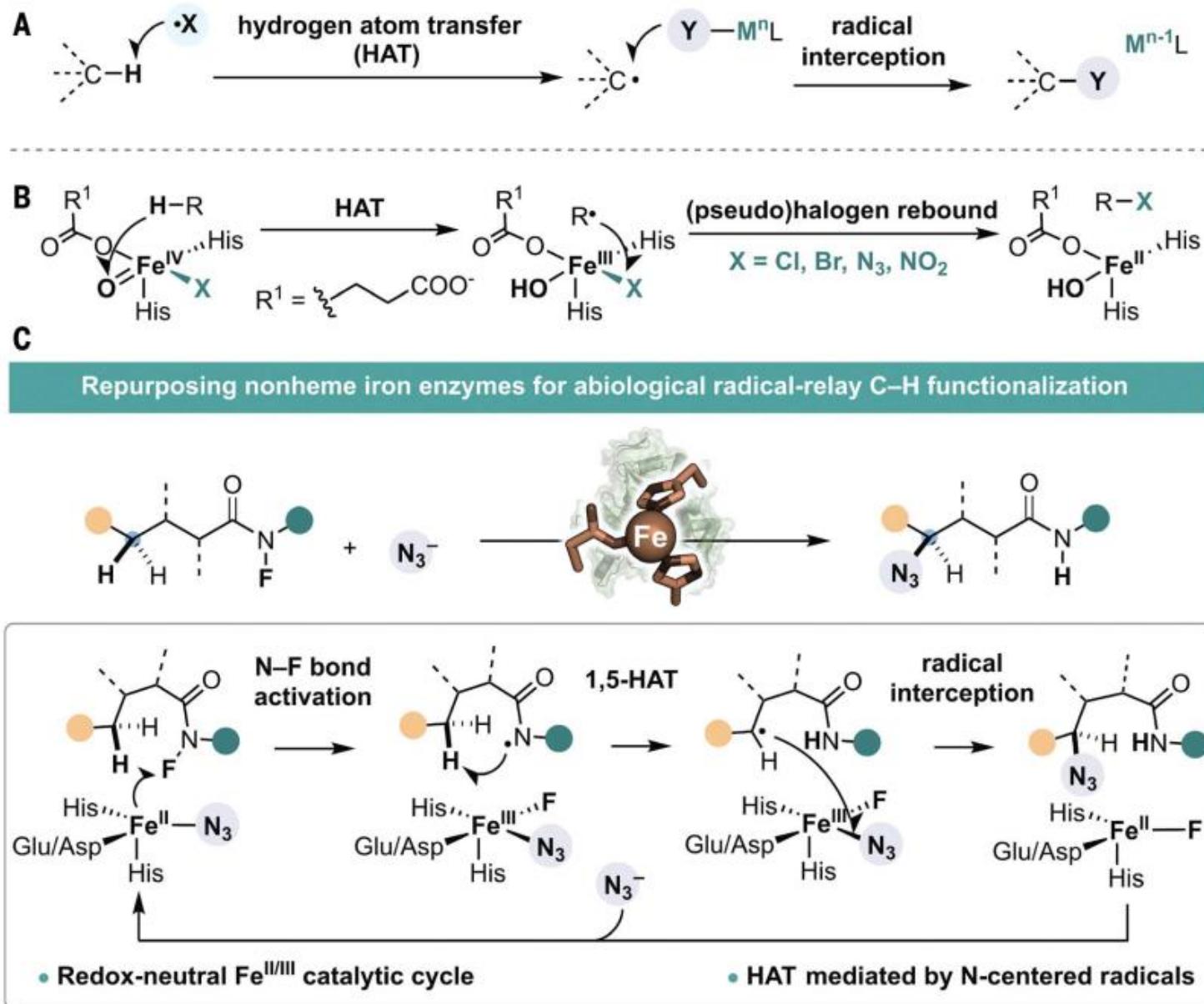
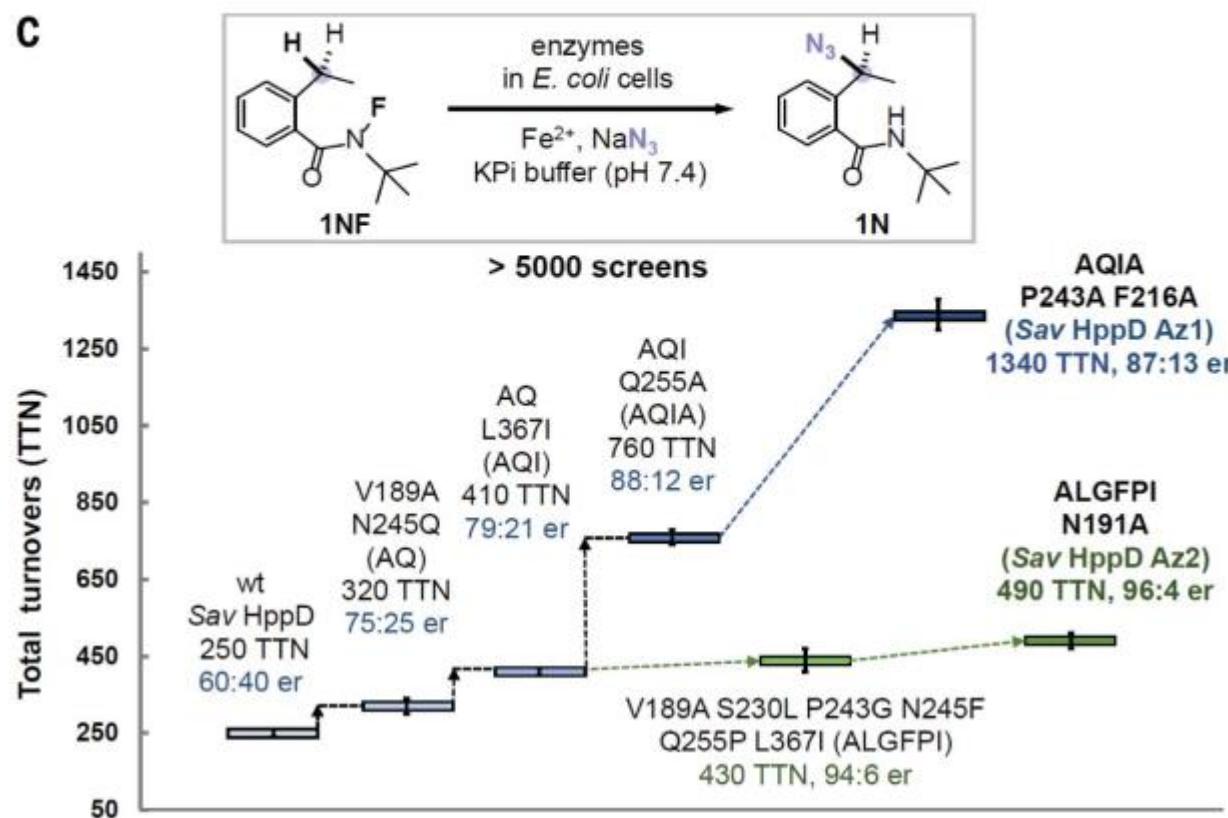
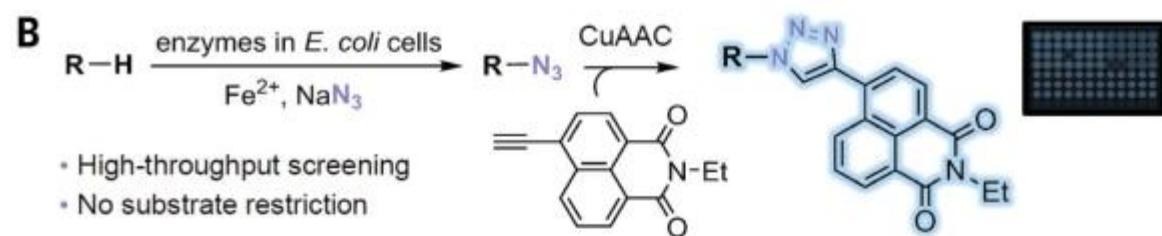


Fig. 1. Conceptualization of enzymatic C–H functionalization via metal-catalyzed radical relay. (A) Radical-relay C–H functionalization involves an initial hydrogen atom transfer (HAT) mediated by a heteroatom-centered radical ($X\cdot$) followed by trapping of the carbon-centered radical with a redox-active metal complex. **(B)** Mechanism employed by natural nonheme iron enzymes for $C(sp^3)$ -H halogenation/azidation. **(C)** Integration of radical relay chemistry into nonheme iron enzymes enables unnatural C–H functionalization reactions.



目标产物是有机叠氮化物，浓度很低，在复杂的细胞裂解液或全细胞反应体系中很难直接、快速地检测。

1. 酶催化反应：待测的酶变体催化底物，在产物分子上引入一个叠氮基团。
2. 点击化学反应：向反应体系中加入：
 - 一种带有炔基的荧光染料分子
 - 铜催化剂
 - 进行铜催化的叠氮-炔环加成反应。
 - 叠氮化物和炔烃快速、高效、选择性地反应，生成一个带有荧光标签的三唑化合物。
3. 荧光检测：使用酶标仪测量整个反应体系的荧光强度。
 - 荧光强度越强，说明生成的三唑化合物越多，进而说明初始的酶催化反应生成的叠氮化物越多。

对15个关键残基进行突变，筛选超过5000个克隆。

获得两个关键变体：

- **Az1**：高活性 (TTN 1340)，中等对映选择性 (87:13 e.r.)
- **Az2**：高对映选择性 (96:4 e.r.)，中等活性 (TTN 490)



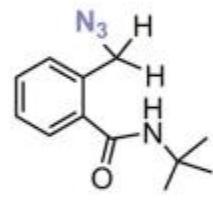
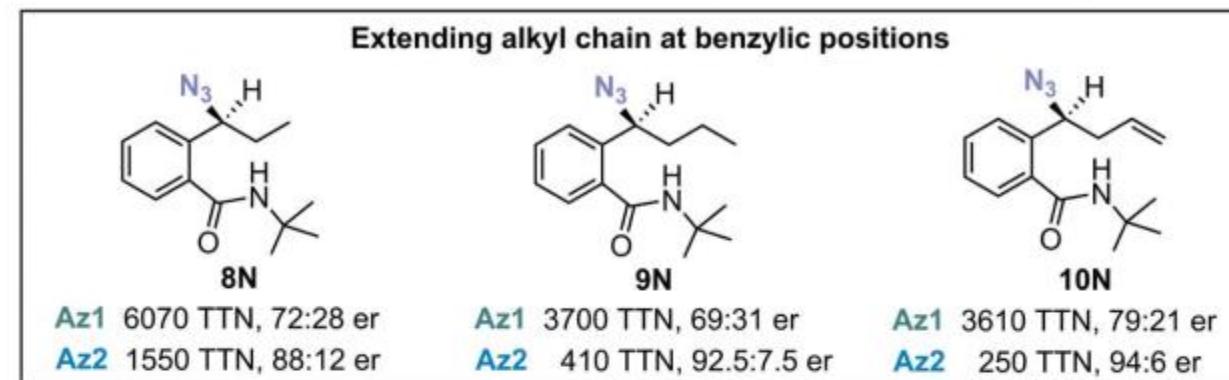
Az1 1530 TTN, 87:13 er

Az2 1470 TTN, 95:5 er



Az1 5300 TTN, 56:44 er

Az2 630 TTN, 94:6 er



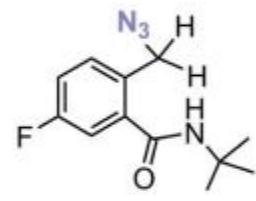
Az1 3180 TTN



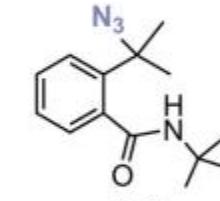
Az1 360 TTN



Az1 820 TTN



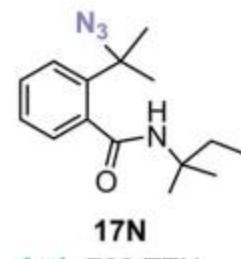
Az1 2980 TTN



Az1 3830 TTN

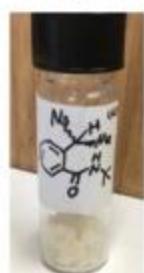


Az1 3030 TTN

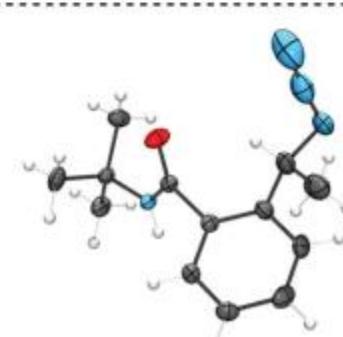
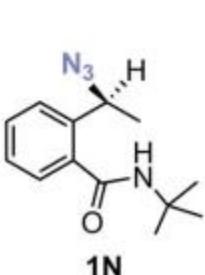


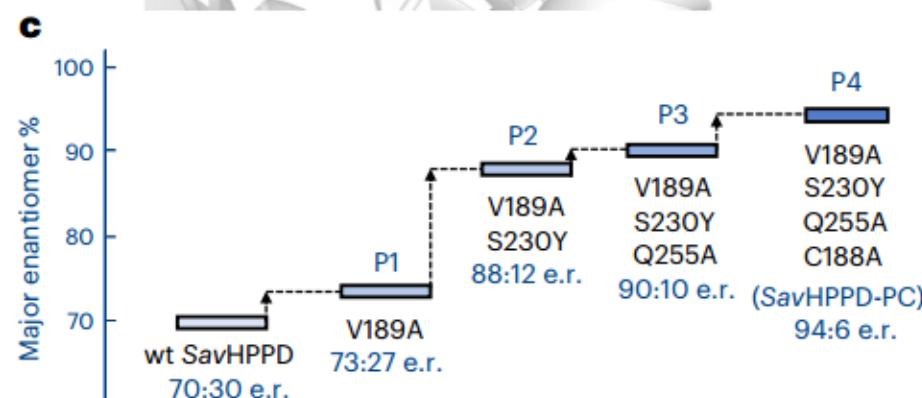
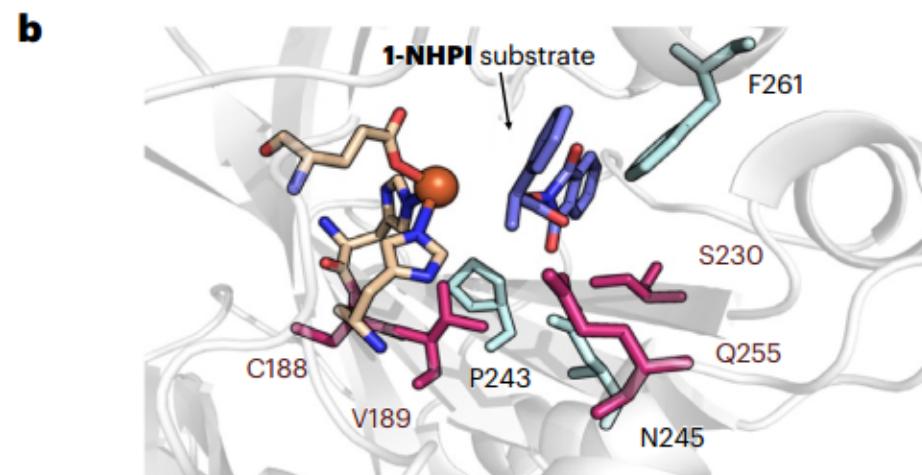
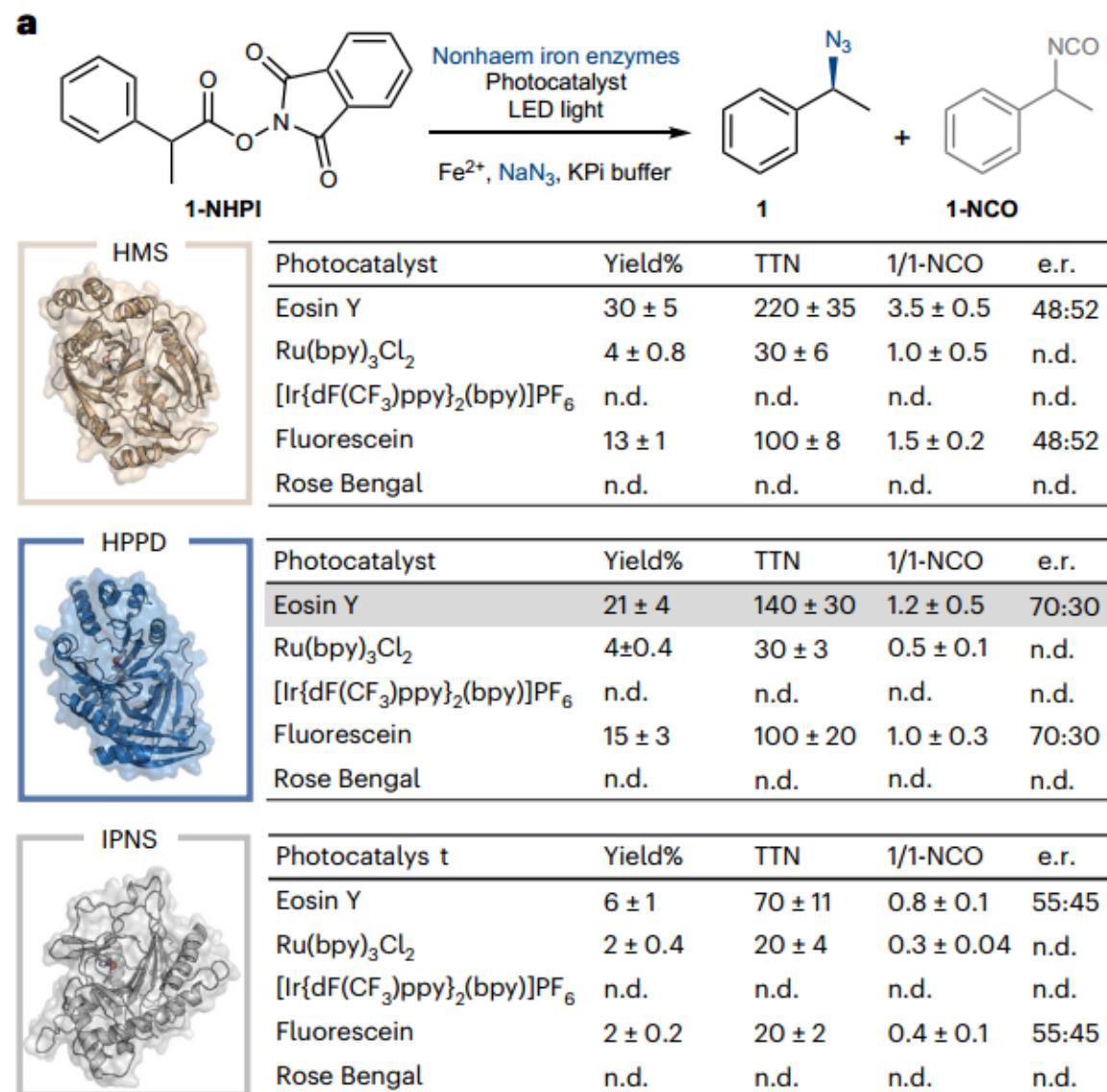
Az1 760 TTN

B Preparative scale synthesis

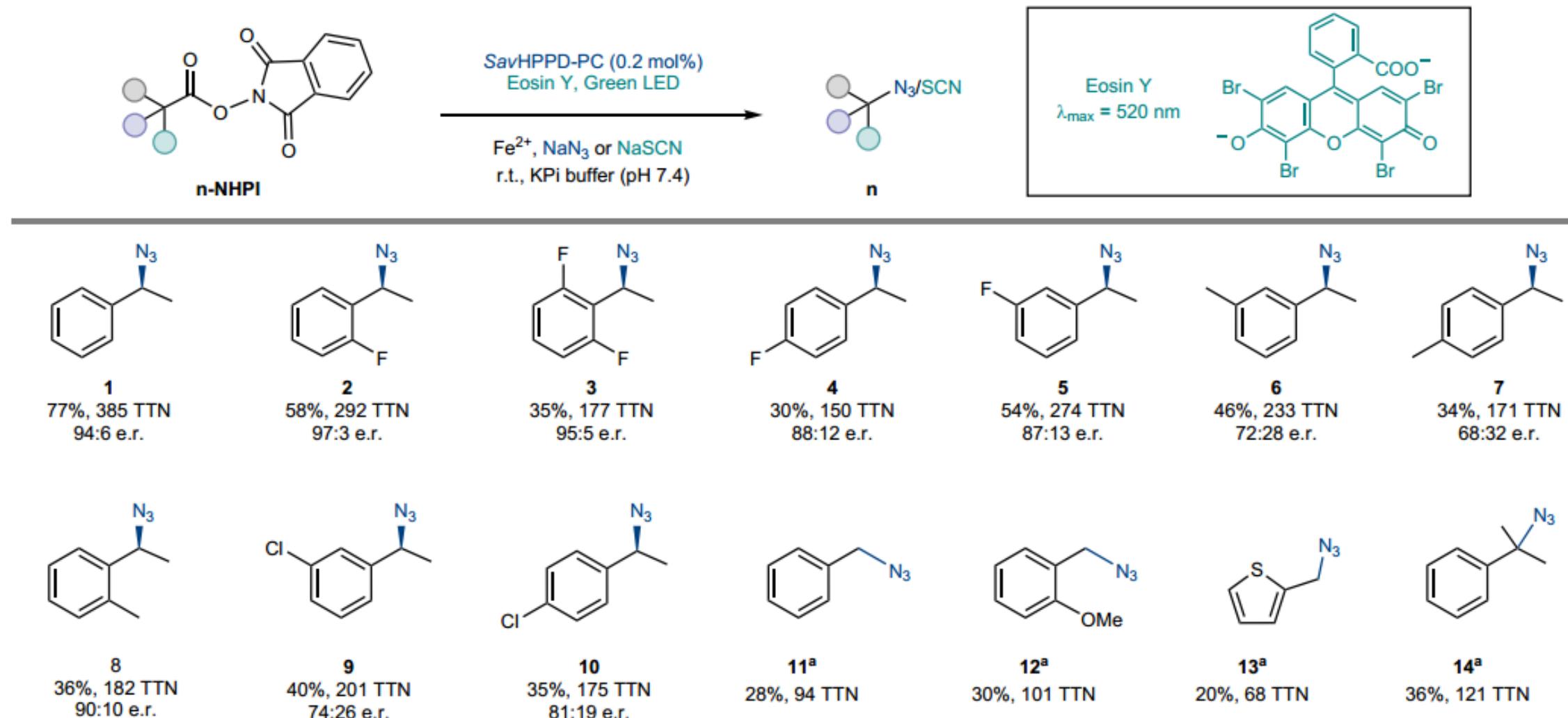


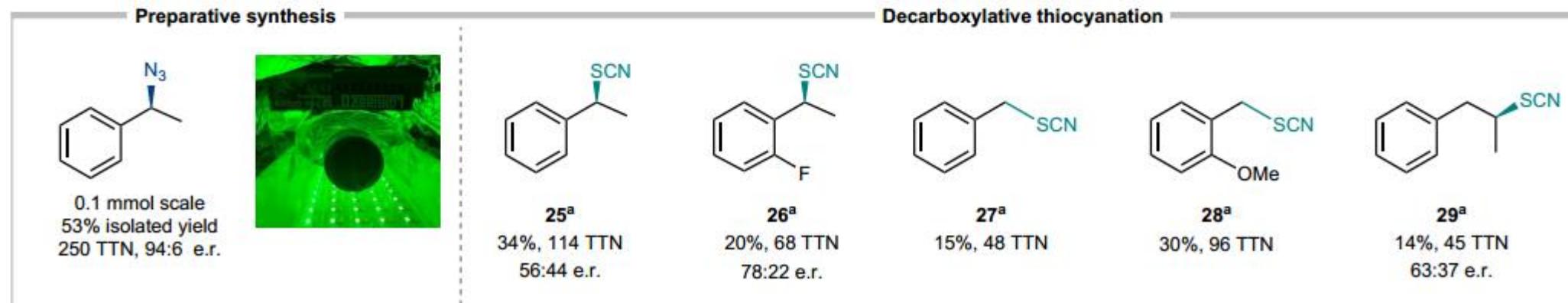
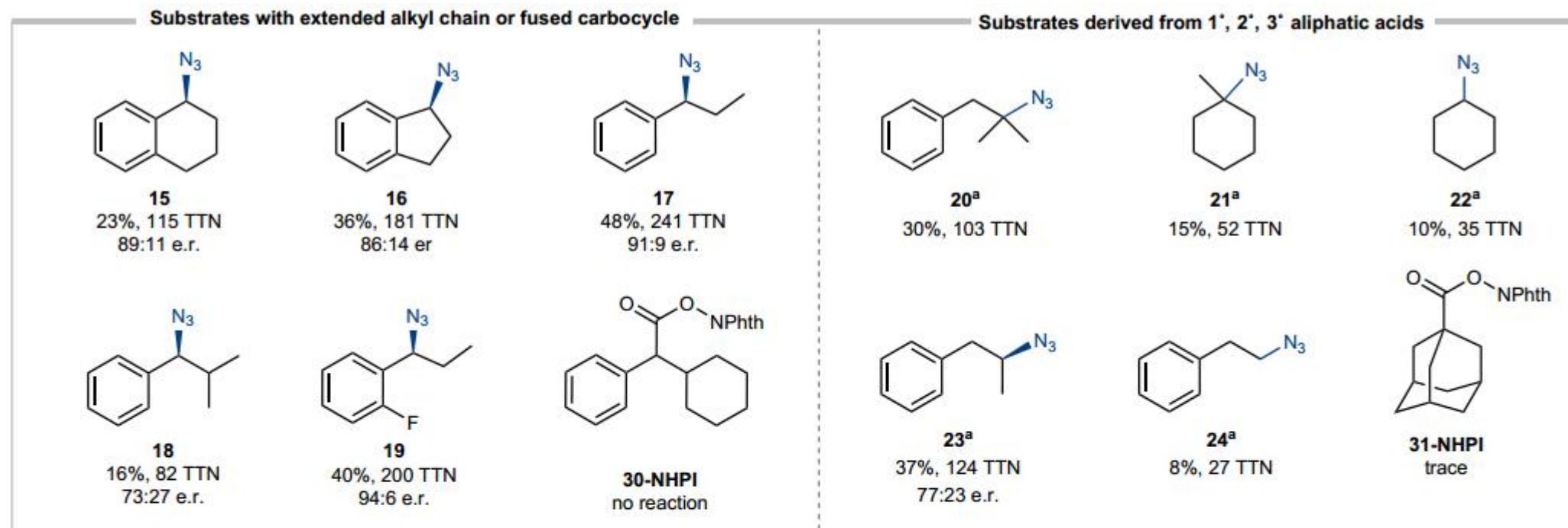
Az1
120 mg scale
65% isolated yield
3650 TTN
87:13 er

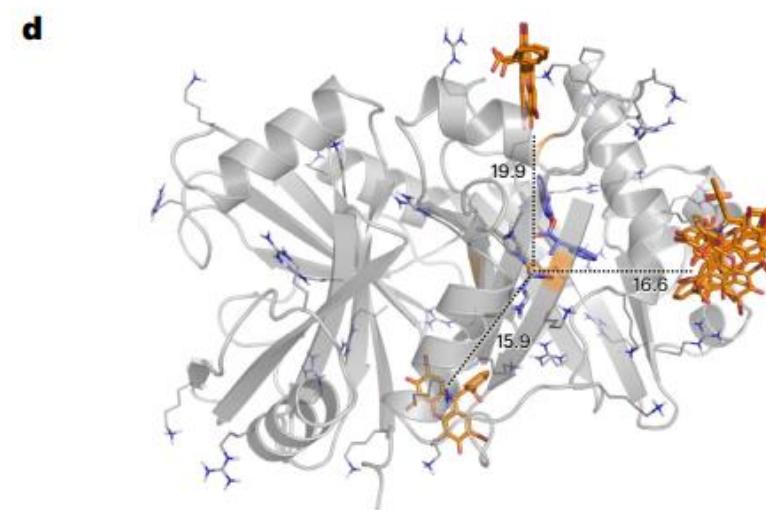
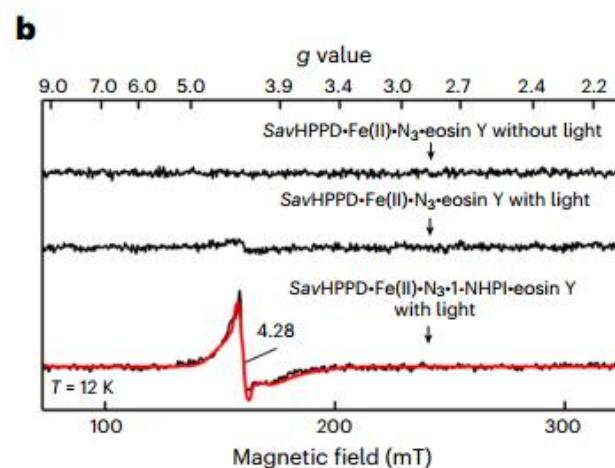
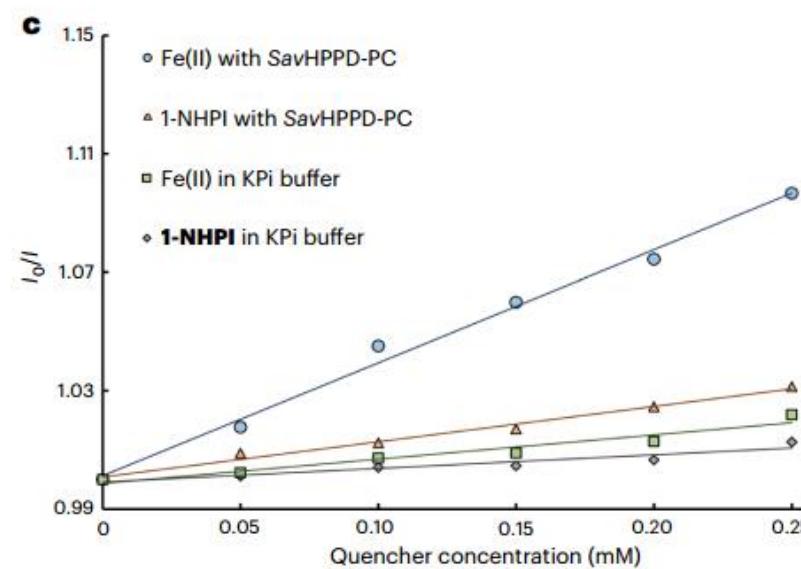
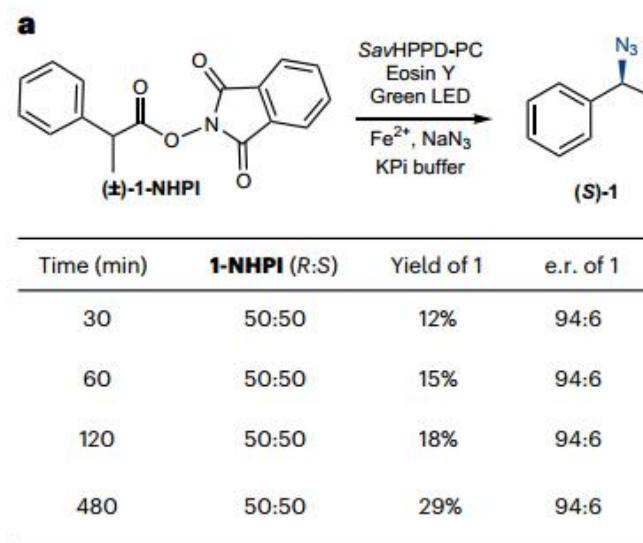




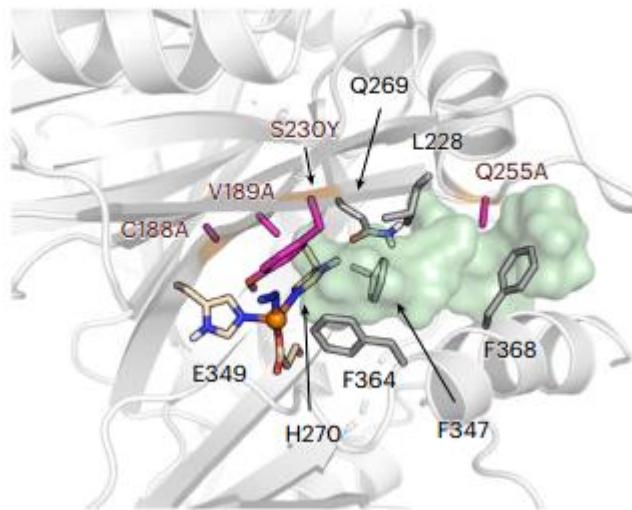
| Mutant | Yield% | TTN | 1/1-NCO | e.r. |
|--------|--------|----------|-----------|-------|
| Wt | 22 ± 3 | 170 ± 20 | 1.5 ± 0.2 | 70:30 |
| P1 | 40 ± 2 | 240 ± 10 | 2.5 ± 0.4 | 73:27 |
| P2 | 39 ± 1 | 260 ± 10 | 4.0 ± 0.2 | 88:12 |
| P3 | 34 ± 2 | 300 ± 20 | 4.3 ± 0.3 | 90:10 |
| P4 | 26 ± 4 | 200 ± 30 | 2.3 ± 0.3 | 94:6 |





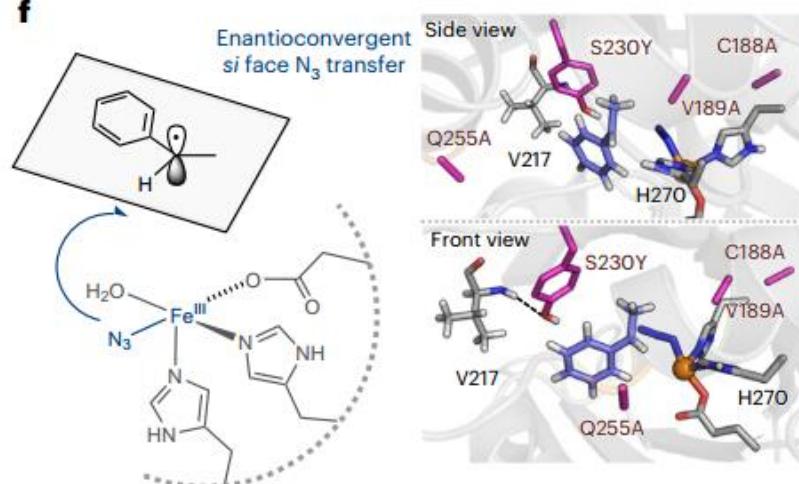


e



引入的突变，特别是 **V189A**（缬氨酸→丙氨酸，侧链变小）和 **Q255A**（谷氨酰胺→丙氨酸，侧链显著变小），移除了活性位点中庞大的侧链，显著扩大了活性位点的疏水空腔。这个新创造的空间能够更好地容纳体积较大的非天然底物以及铁结合的叠氮离子

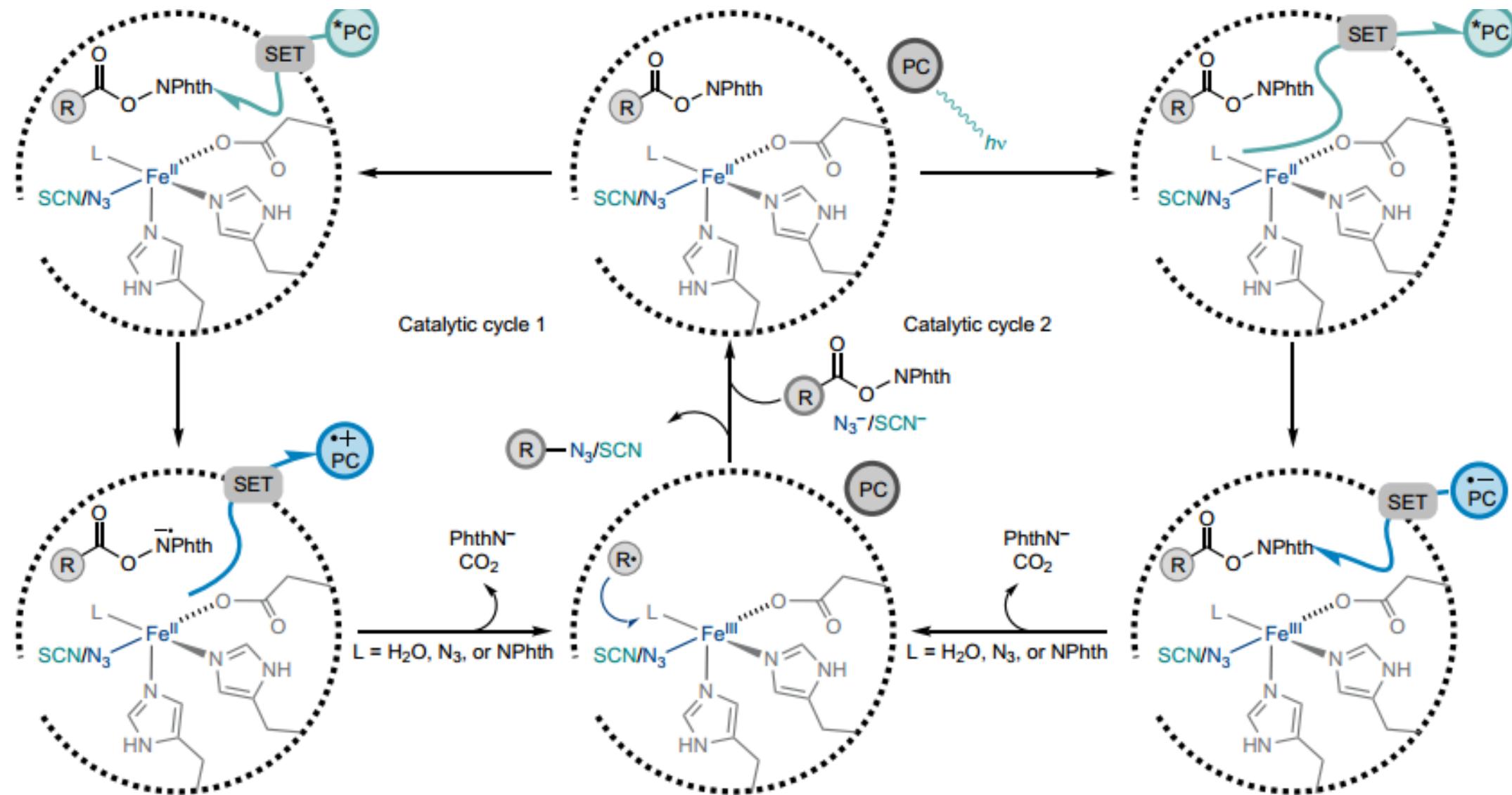
f

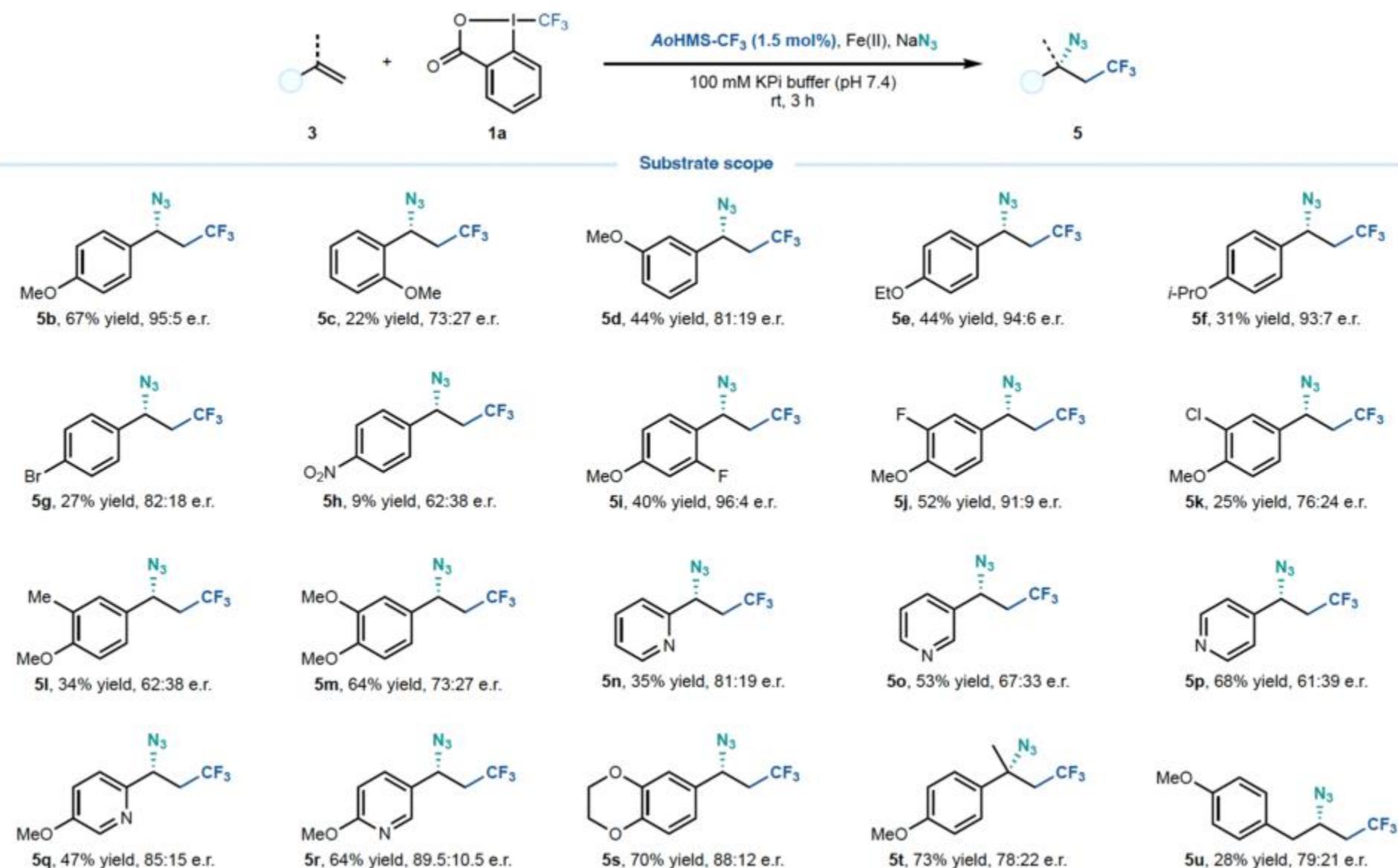


分析了底物自由基在活性位点中的稳定构象，并观察其与周围残基的相互作用。

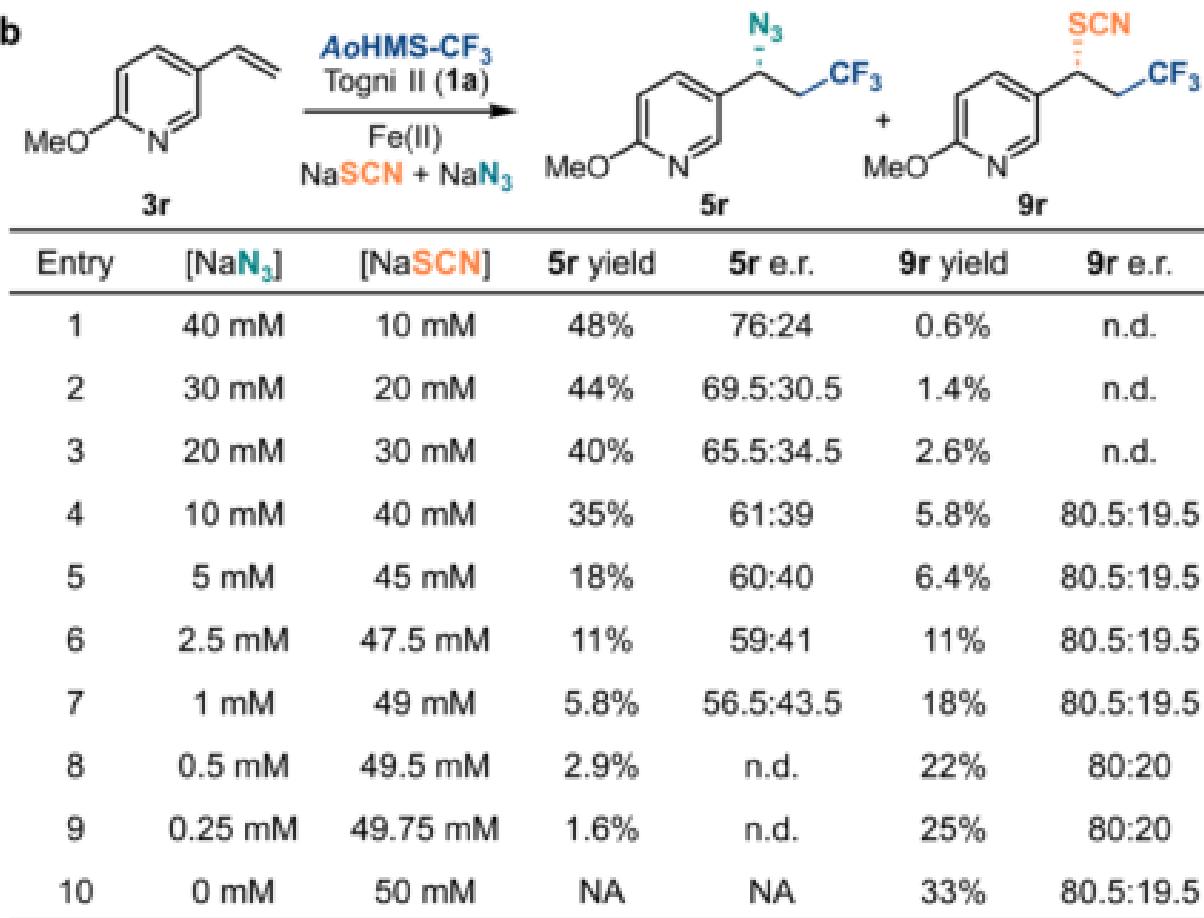
形成疏水口袋与引导叠氮离子： **S230Y** 突变（丝氨酸→酪氨酸，引入大芳香环）与 **P243** 和 **F359** 一起，形成了一个疏水口袋。这个口袋能够容纳并引导叠氮离子，使其位于 **H270** 残基的反式位置。这与天然非血红素铁卤化酶中观察到的“卤化物结合口袋”非常相似。

控制底物自由基的取向：有助于将苄基的苯基部分对准铁中心，这些结构排列使底物自由基的 **si** 面朝向铁结合的叠氮化物，导致最终产物的“**S**”绝对构型





b



阴离子竞争实验：

硫氰根竞争：在叠氮化和硫氰化竞争实验中，即使SCN⁻大大过量，叠氮化产物仍占主导，表明酶对N₃⁻转移有内在偏好。

选择性扰动：高浓度SCN⁻存在下，叠氮化产物的对映选择性显著下降。表明SCN⁻结合到铁中心后，可能改变了Fe(III)-N₃中间体的配位环境，从而影响了决定对映选择性的自由基转移步骤。

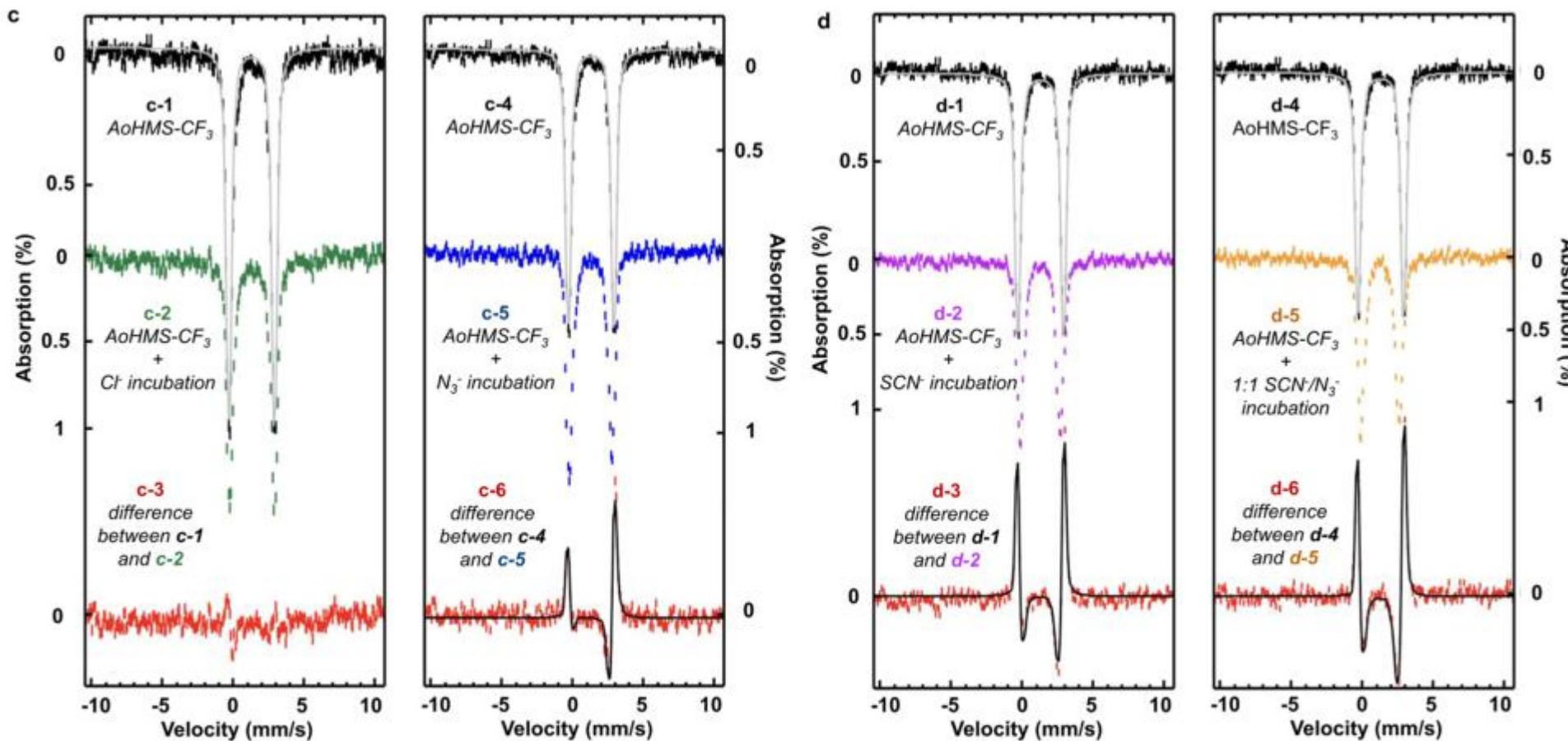
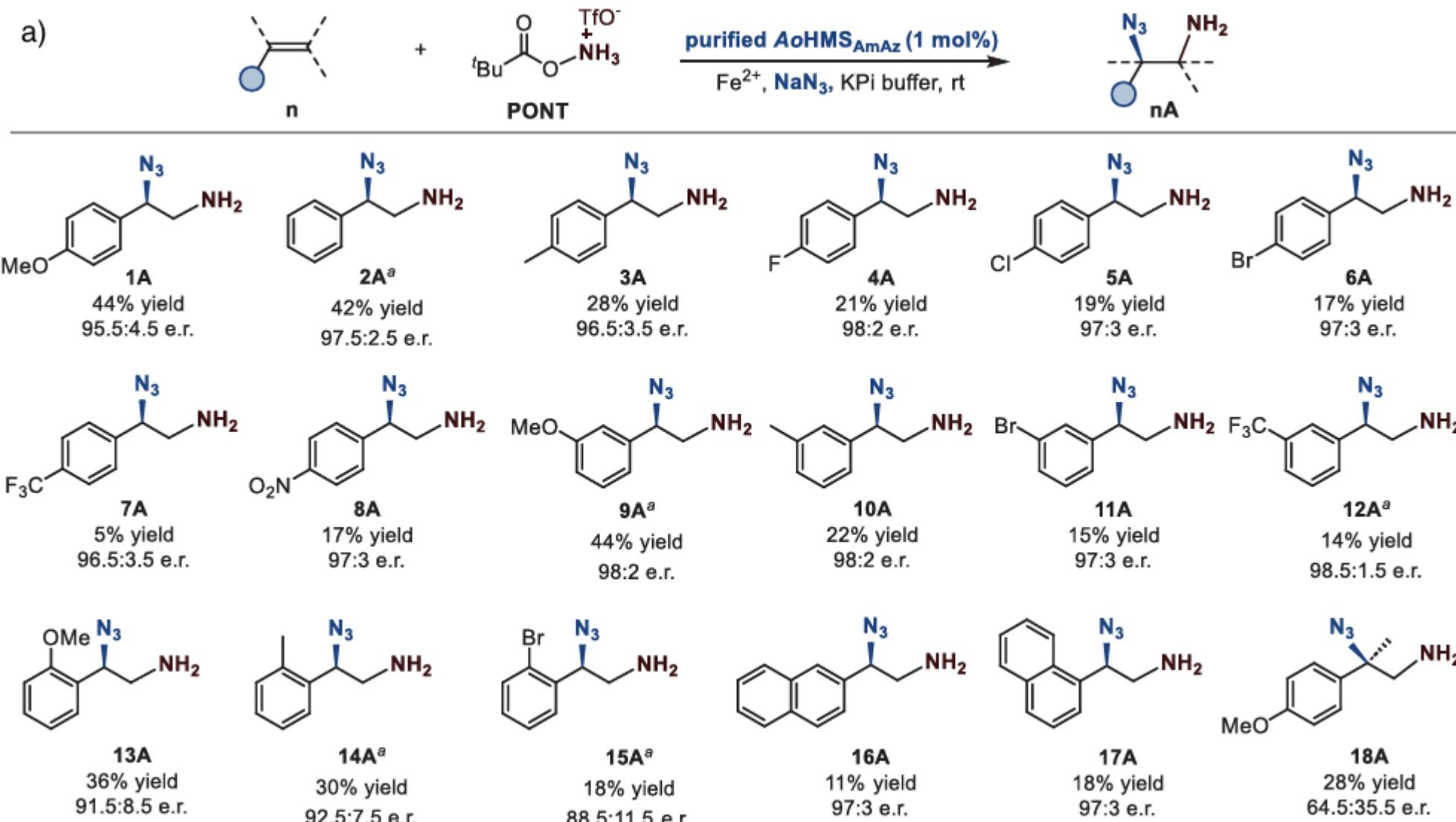
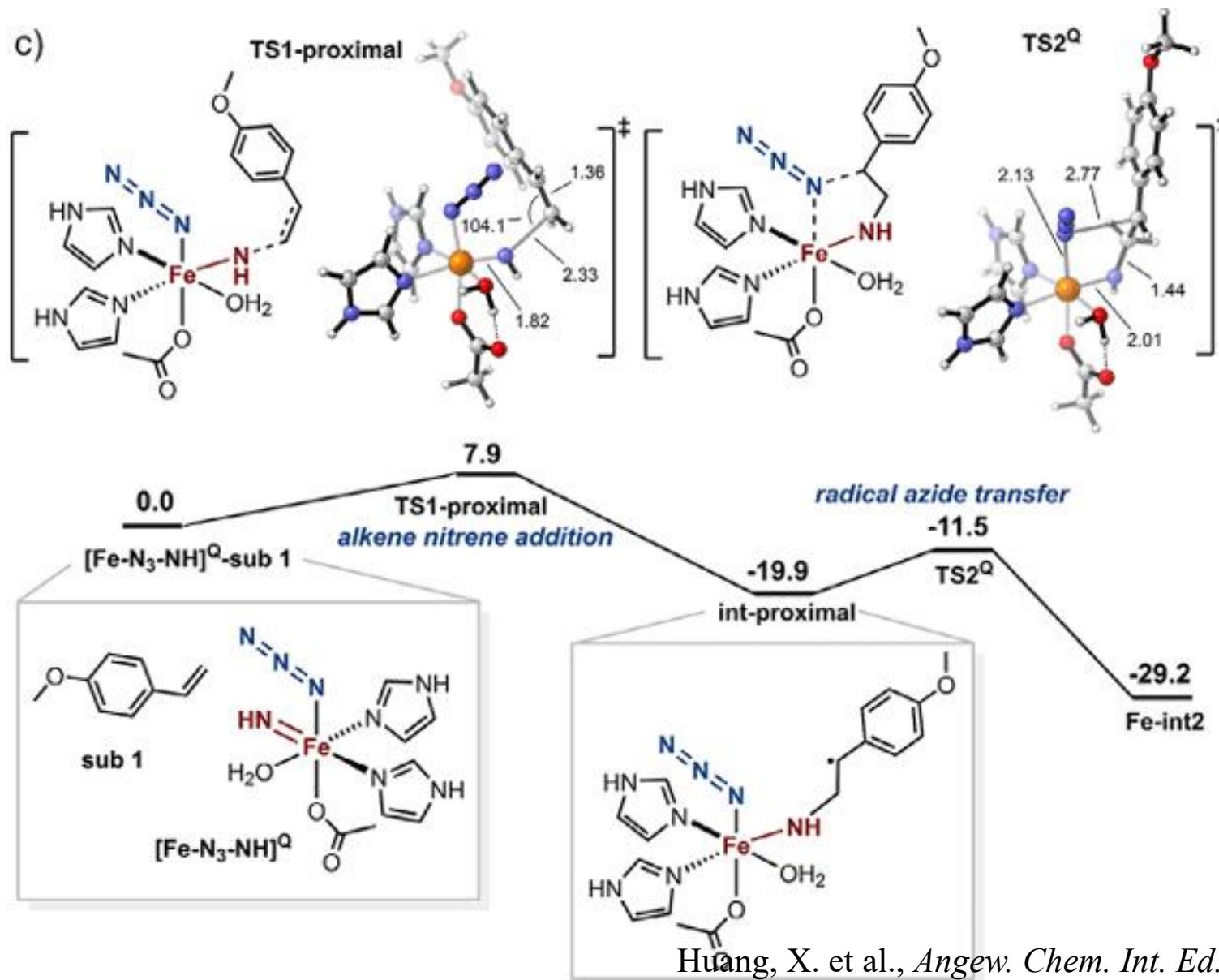
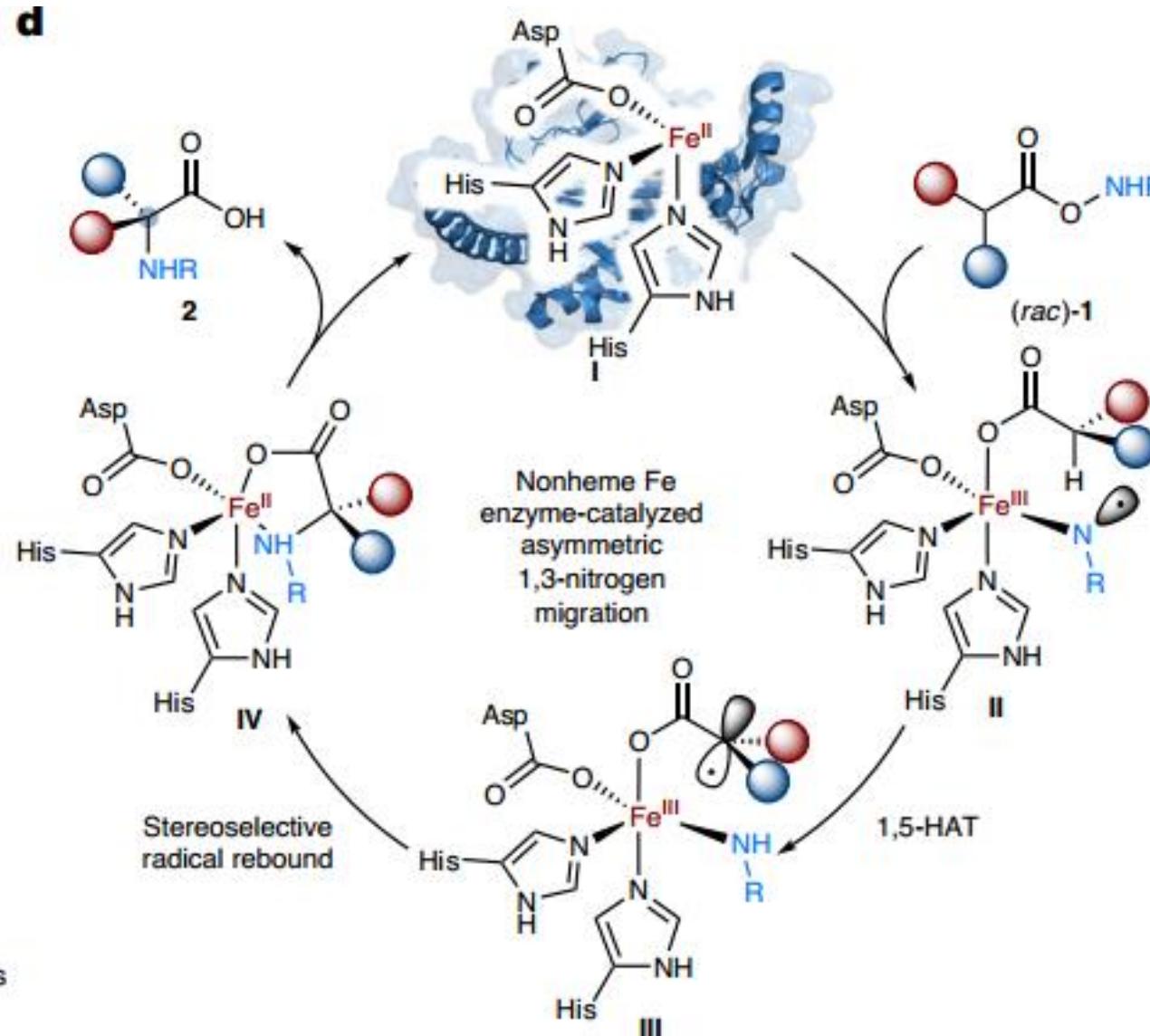


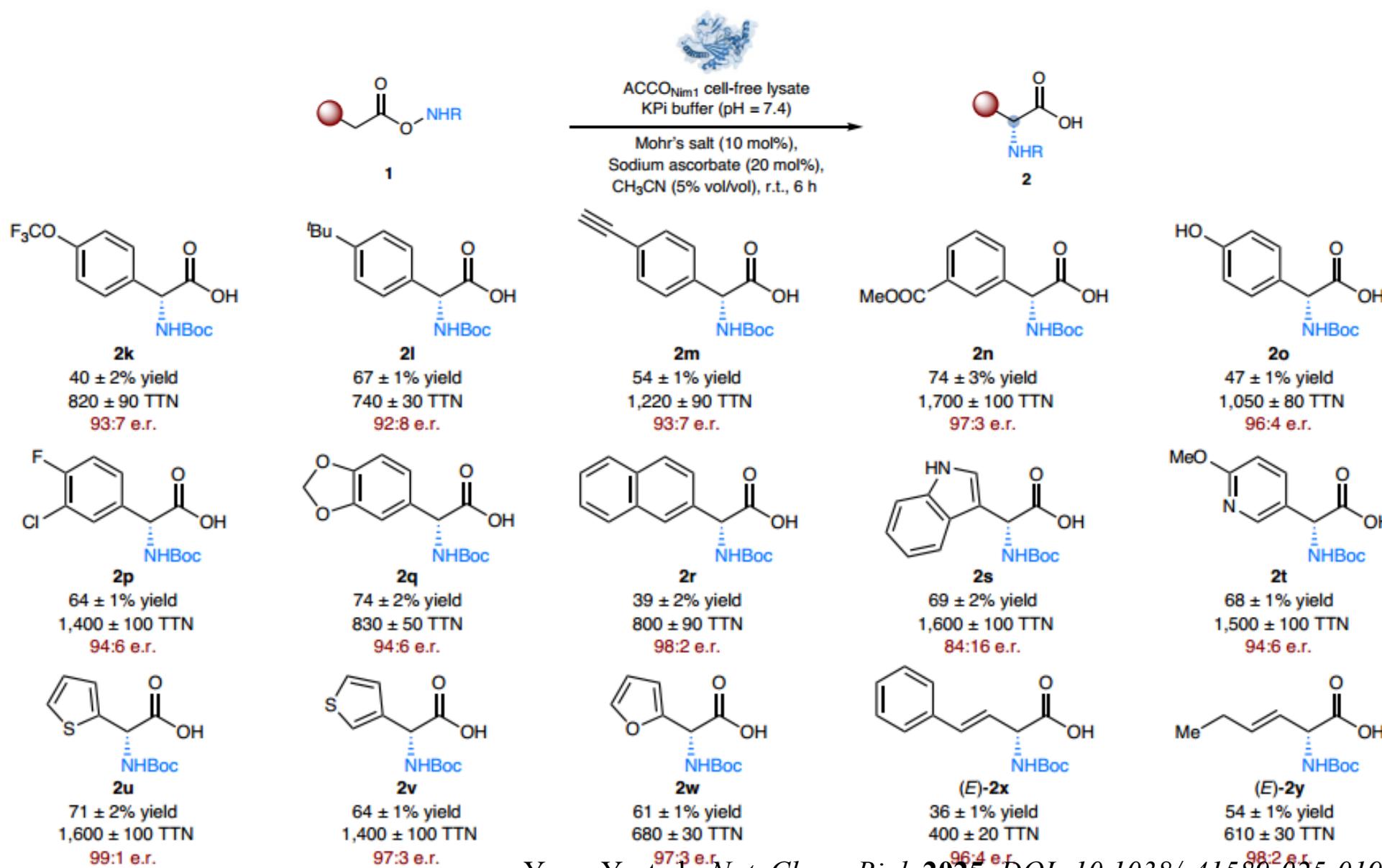
Figure 4. Mechanistic Studies. (a) N_3^- /SCN competition experiment. (b) Experimental studies of the CF_3 radical generation and azide transfer steps. n.d. e.r. not determined due to low activity. (c) 4.2 K Mössbauer spectra of ferrous-loaded AoHMS-CF_3 and the incubation with chloride or azide anions. (d) 4.2 K Mössbauer spectra of ferrous-loaded AoHMS-CF_3 and incubation with thiocyanate and/or azide anions. For experimental details see Figures S6 and S7.

- N_3^- 和 SCN^- 能与酶的 Fe(II) 中心结合, 引起光谱变化, 而 Cl^- 则不能。
- 当 N_3^- 和 SCN^- 同时存在时, 产生了与单一阴离子存在时不同的光谱信号, 表明两者共同存在时对铁中心的电子结构产生了独特的扰动, 为竞争实验中观察到的对映选择性扰动提供了支持。

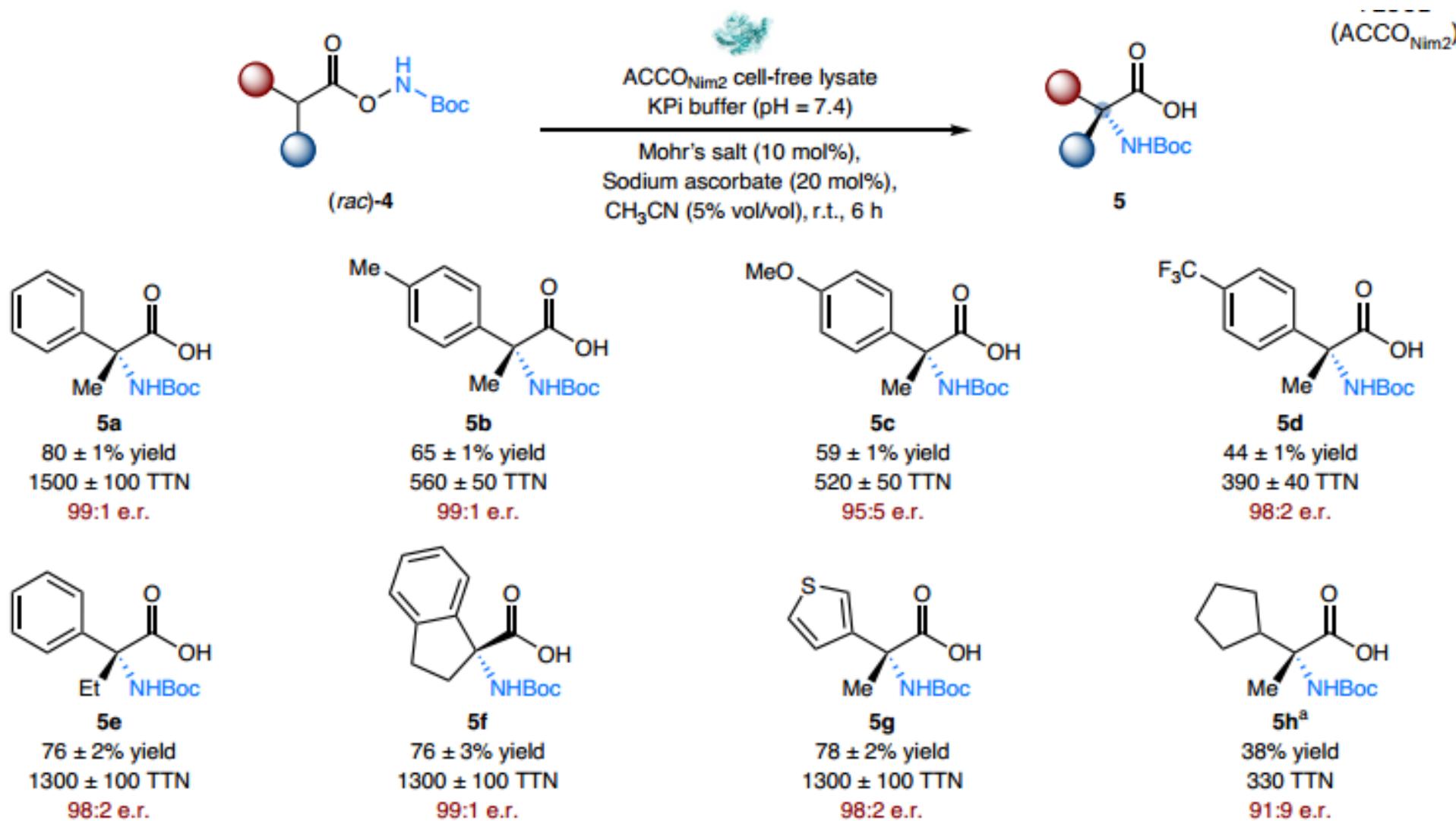


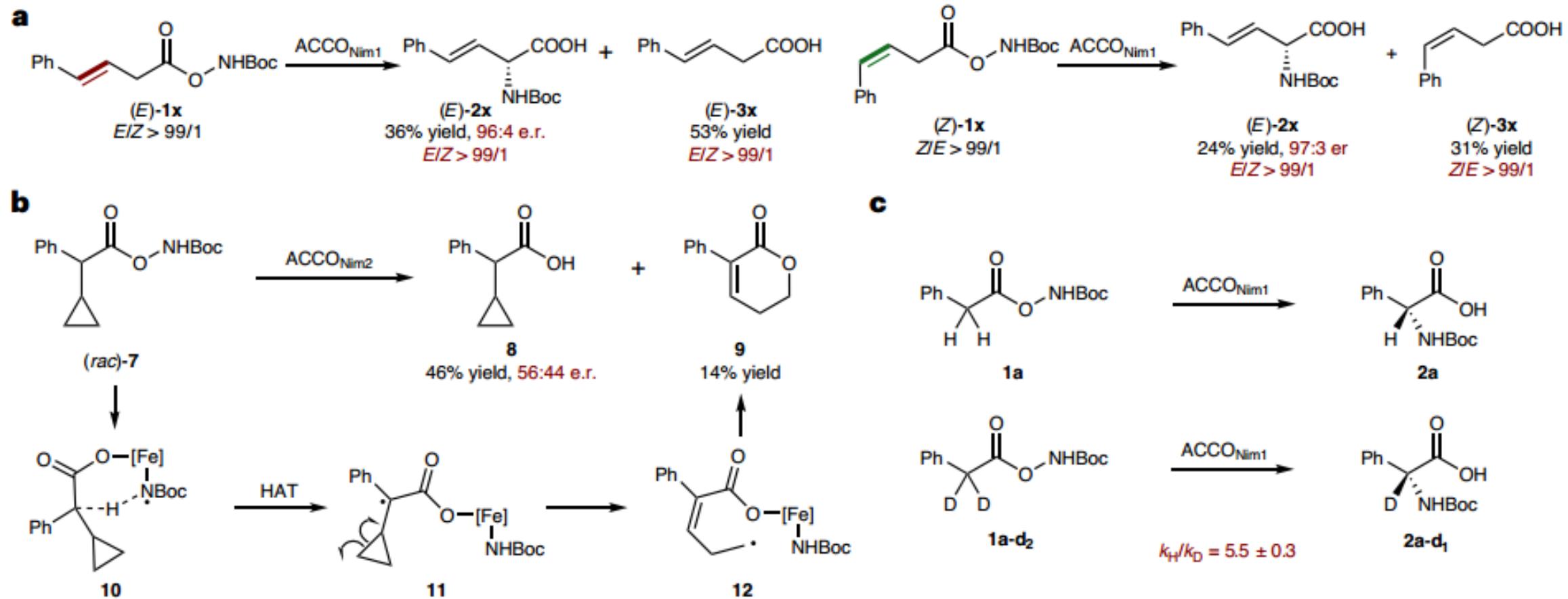


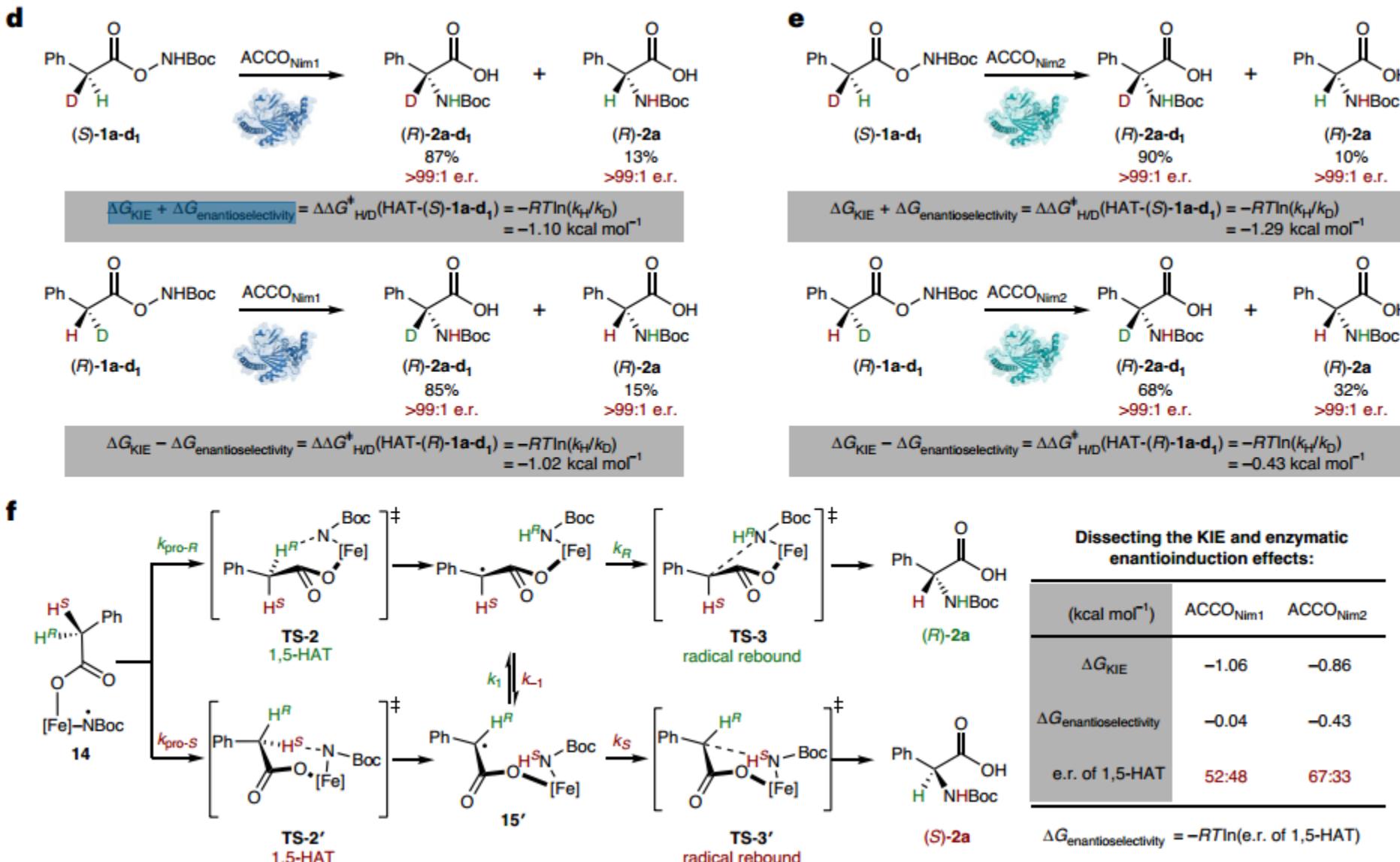




b







$\Delta G_{\text{enantioselectivity}}$ (对映选择性自由能差) :

这代表了酶在氢原子转移步骤中，对于底物上前手性碳上的两个对映异位氢 (H^R 和 H^S) 的内在偏好程度。想象一个前手性亚甲基 ($-\text{CH}_2-$)，酶要从中抽取一个氢。

如果酶活性中心的环境完全对称，那么它 H^R 和 H^S 的能垒是一样的，此时 $\Delta G_{\text{enantioselectivity}} = 0$ 。

如果酶的活性中心更“偏爱”抽取其中一个氢 (比如 H^R)，那么抽取这个氢的能垒就会更低，此时 $\Delta G_{\text{enantioselectivity}}$ 就是一个负值 (因为 $\Delta G = -RT \ln(K)$ ，能垒低则 K 大， ΔG 为负)。

ΔG_{KIE} (动力学同位素效应自由能差) :

这代表了固有的、纯粹的动力学同位素效应。它源于化学键断裂的本身难度：断裂一个C-H键 vs 断裂一个更强的C-D键的内在能垒差。这是一个物理化学本质属性，理论上对分子中任何相同的化学键都应该一样。

这是一个基准值。它告诉我们，如果没有酶的对映选择性干扰，单纯从化学物理角度看，抽取H比抽取D要快多少 (体现为能垒降低多少)。

$\Delta G_{KIE} + \Delta G_{enantioselectivity}$:

假设酶偏爱抽取 Pro-R H。

在 (S)-1a-d₁ 这个分子中， Pro-R 位点上是氘， Pro-S 位点上是氢。

现在酶要发生HAT，它面临两个选择：

从Pro-S位点抽H: 这符合它的对映选择性偏好吗？不，它偏爱的是Pro-R位点。所以这不符合偏好，需要克服 $\Delta G_{enantioselectivity}$ 的惩罚。

从Pro-R位点抽D: 这符合它的对映选择性偏好，但需要克服巨大的KIE能垒 ΔG_{KIE} 。

能垒分析：

路径1（抽H，但位置不对）的能垒 = $\Delta G_{KIE} + \Delta g_{enantioselectivity}$

ΔG_{KIE} ：代表固有的KIE能垒。

$+\Delta G_{enantioselectivity}$ ：因为违反了酶的对映选择性偏好。

实际上，这个公式计算的是“抽H路径”相对于“抽D路径”的能垒差。在这种情况下，两种效应都使得抽H变得困难，所以它们相加，导致观测到的表观KIE值较大。

$\Delta G_{KIE} - \Delta G_{enantioselectivity}$:

假设酶偏爱抽取 Pro-R H。

在 (S)-1a-d₁ 这个分子中， Pro-R 位点上是氢， Pro-S 位点上是氘。

现在酶要发生HAT，它面临两个选择：

从Pro-R位点抽H：这既符合对映选择性偏好，又避免了KIE惩罚（因为抽的是H）。这是最理想的路径。

从Pro-S位点抽D：这既不符合对映选择性偏好，又有KIE惩罚。

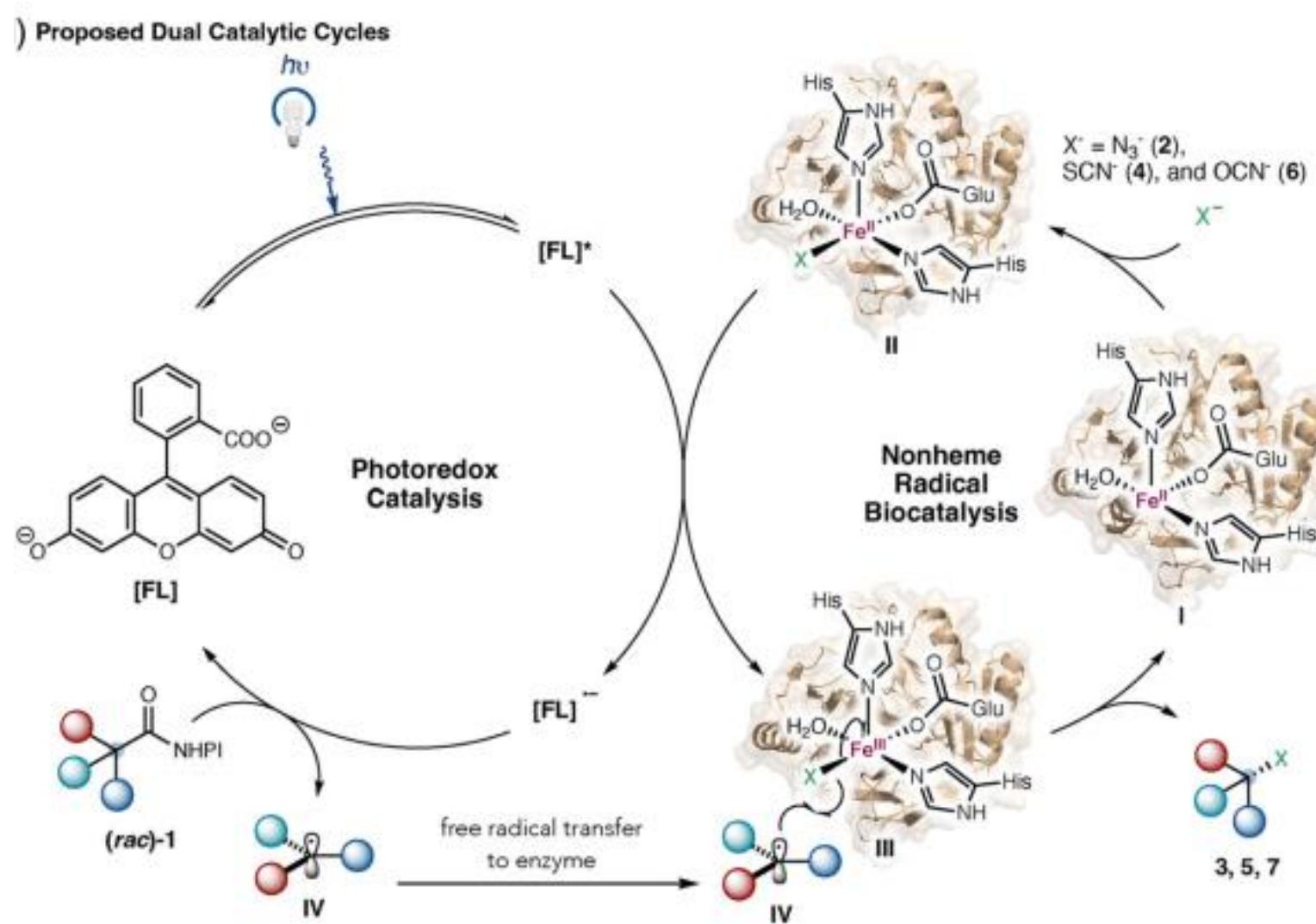
能垒分析：

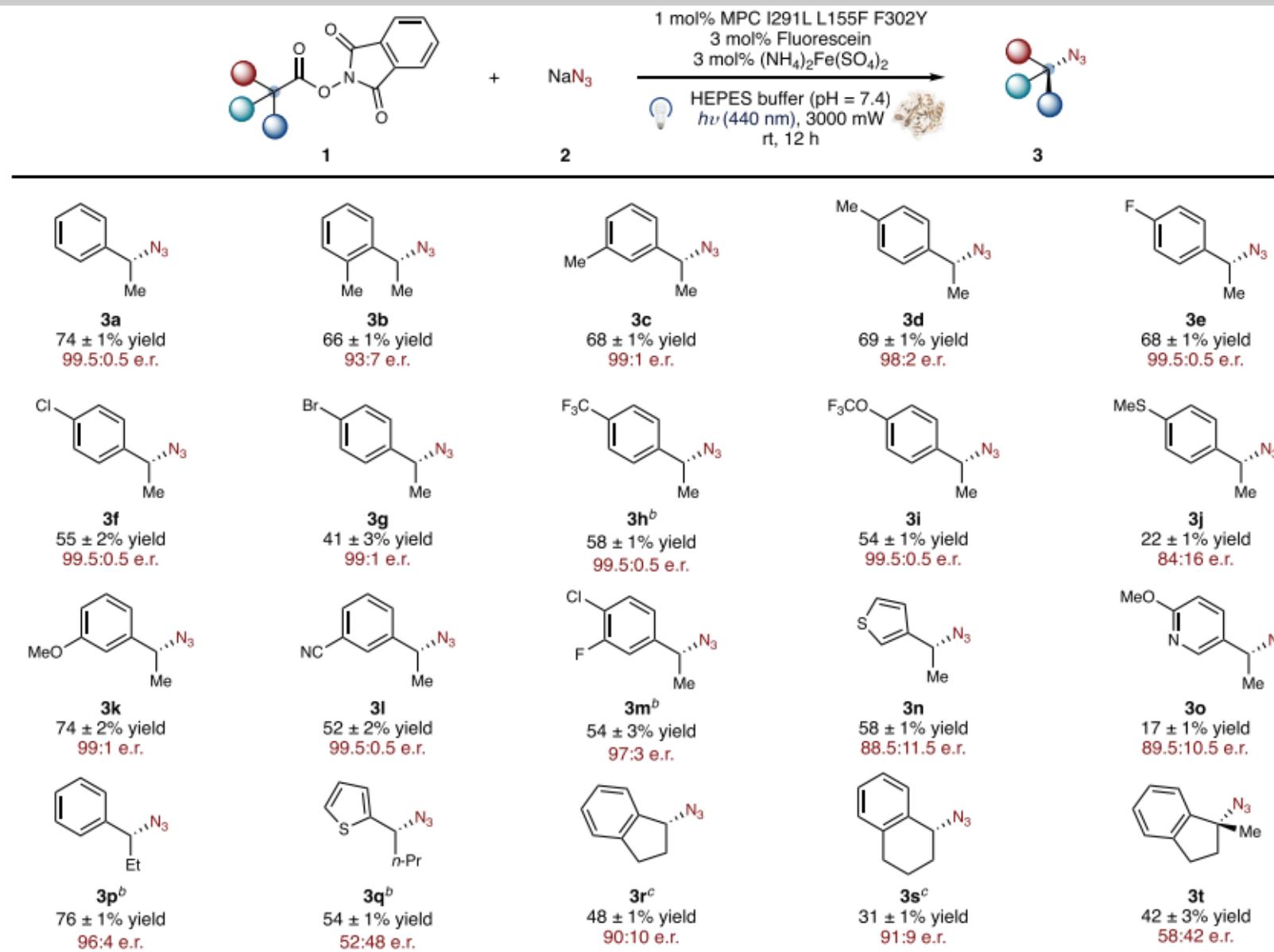
路径1（抽H，位置正确）的能垒 = $\Delta G_{KIE} - \Delta G_{enantioselectivity}$

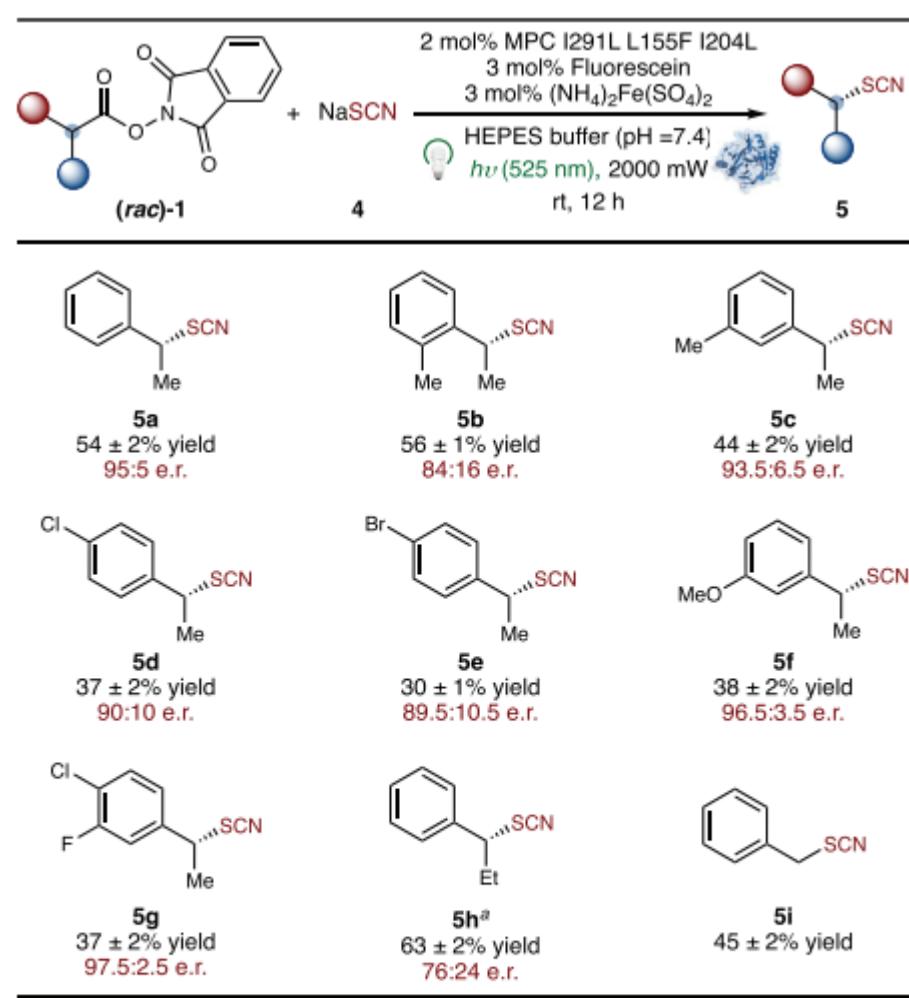
ΔG_{KIE} ：代表固有的KIE能垒。

$-\Delta G_{enantioselectivity}$ ：因为符合酶的对映选择性偏好，所以总能垒减去了这一部分。酶的偏好帮助克服了一部分固有的KIE能垒。

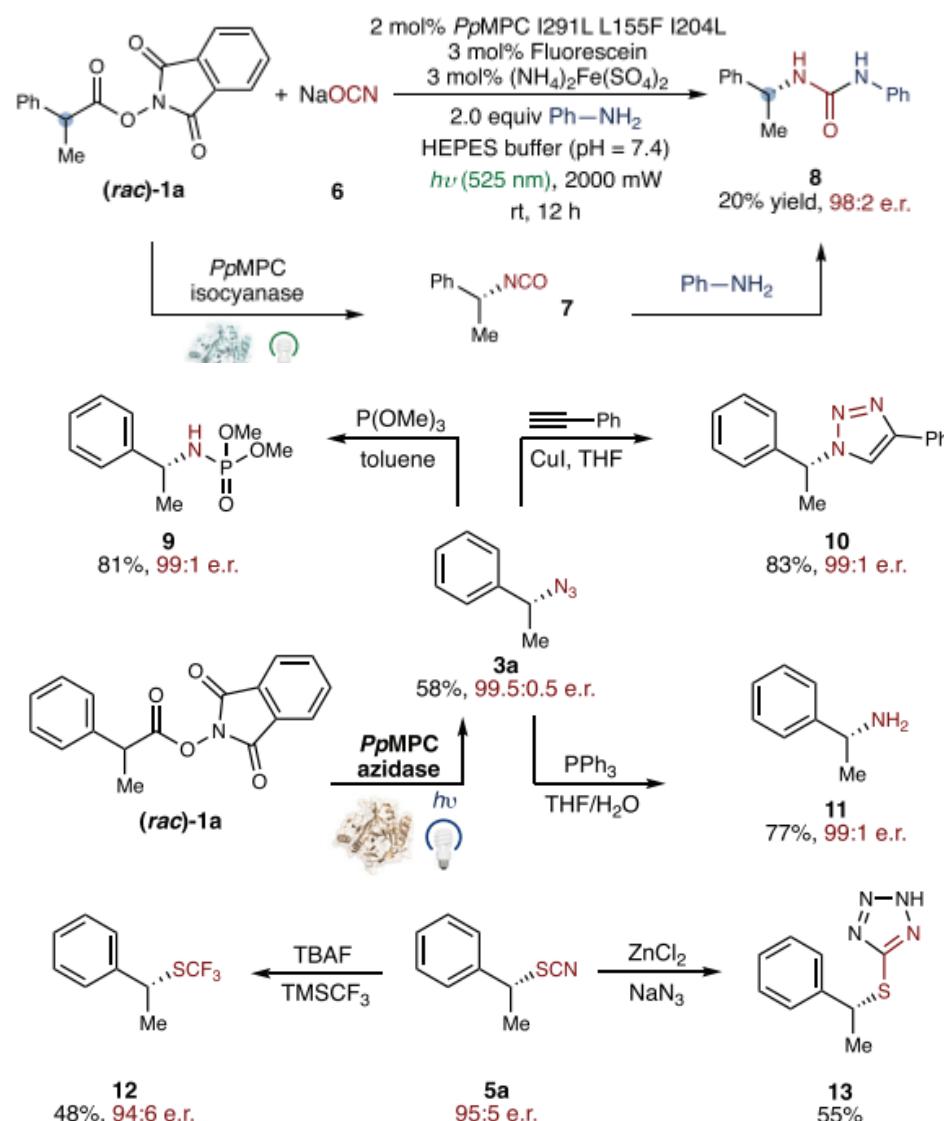
在这种情况下，对映选择性效应部分抵消了KIE效应，导致观测到的表观KIE值较小。。

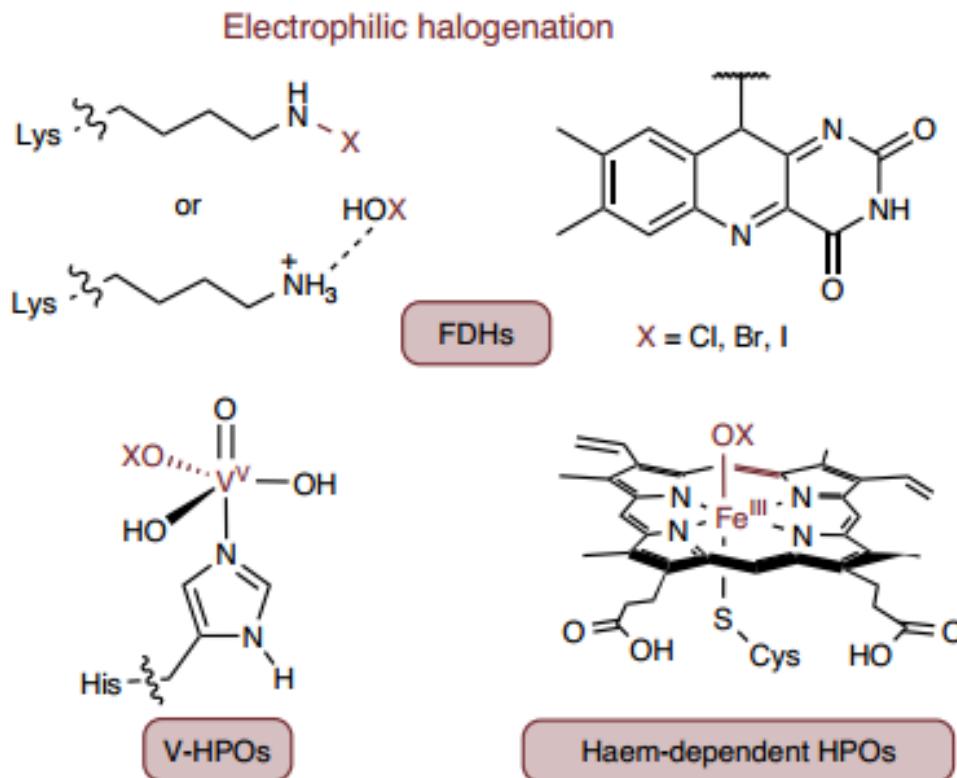




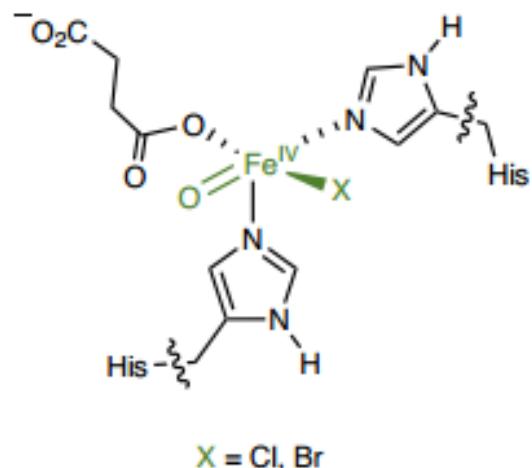


^a) Reaction conditions: **1** (6.67 mM), **4** (66.7 mM, 10.0 equiv), 2.0 mol% PpMPC I291L L155F I204L (133.4 mM), 3 mol% fluorescein sodium salt (0.20 mM), 3 mol% $(\text{NH}_4)_2\text{Fe}(\text{II})(\text{SO}_4)_2$ (0.20 mM), $h\nu$ (525 nm), 10 mM HEPES buffer, pH = 7.4, rt, 6 h. The red sphere is an aryl substituent.

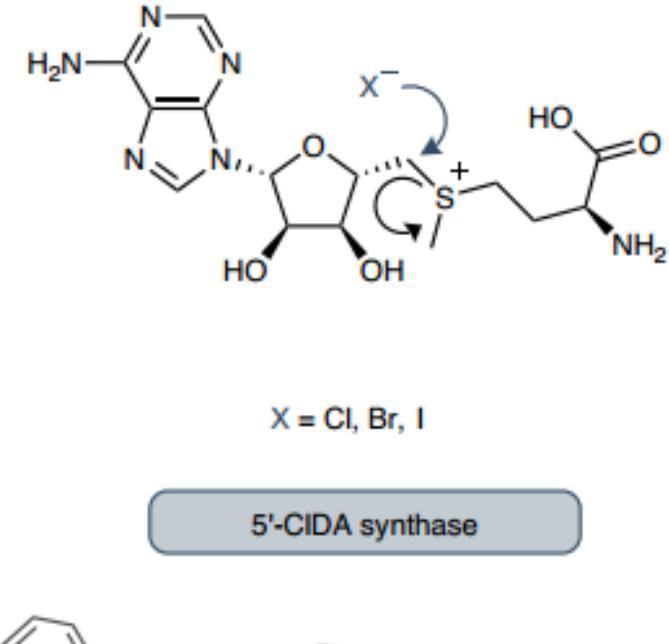


a

Radical halogenation

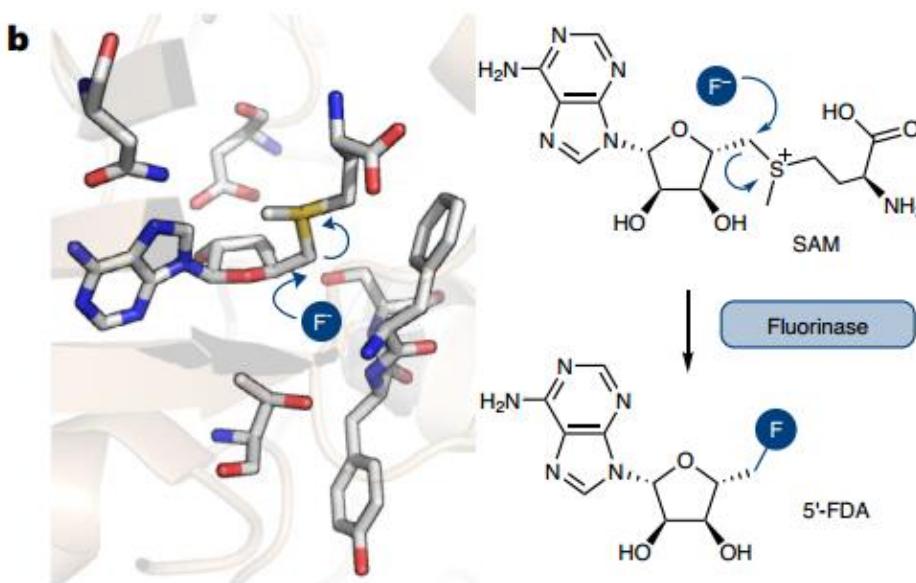


Nucleophilic halogenation

**c**

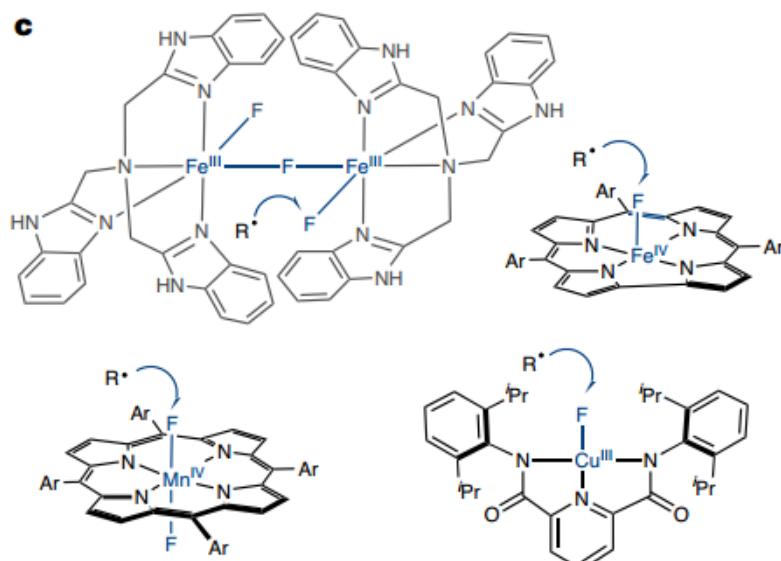
| 机制类型 | 代表酶 | 关键中间体/特点 |
|-------|---|--|
| 亲电卤化 | 黄素依赖卤化酶 (FDHs) 钒依赖卤过氧化物酶 (V-HPOs) 血红素依赖卤过氧化物酶 (Haem-HPOs) | 使用 O_2 或 H_2O_2 将卤离子 (Cl^- , Br^- , I^-) 氧化成次卤酸 (HOX)，作为亲电试剂。酶中的赖氨酸 (Lys) 通过形成卤胺中间体或氢键来协助反应。 |
| 自由基卤化 | 非血红素铁卤化酶 (NHFe halogenases) | 形成 $Fe(IV)=O-X^-$ 的高价铁-氧-卤素中间体。该中间体可以抽取底物的氢原子，生成碳自由基，随后发生卤原子回弹，形成 C-X 键。 |
| 亲核卤化 | 5'-氯代脱氧腺苷合成酶 (5'-ClDA synthase) | 酶直接活化氯离子 (Cl^-) 作为亲核试剂，取代 SAM (S-腺苷甲硫氨酸) 分子中的离去基团，形成 C-Cl 键。 |

自然界有多种成熟的酶机制来构建C-Cl、C-Br、C-I键，但这些机制几乎不适用于C-F键的构建，因为氟离子的氧化极其困难，且其水合作用强，难以作为亲核试剂。



目前已知的唯一天然C-F键形成酶——**氟化酶**

氟化酶能够去溶剂化并结合氟离子 (F^-)，并通过精巧的氢键网络将其精准定位到底物SAM (S-腺苷甲硫氨酸) 的碳中心。 F^- 亲核进攻该碳中心，取代离去基团，生成产物 **5'-氟代脱氧腺苷 (5'-FDA)**。



人工设计的金属-氟络合物用于C-F键形成 (非酶体系)

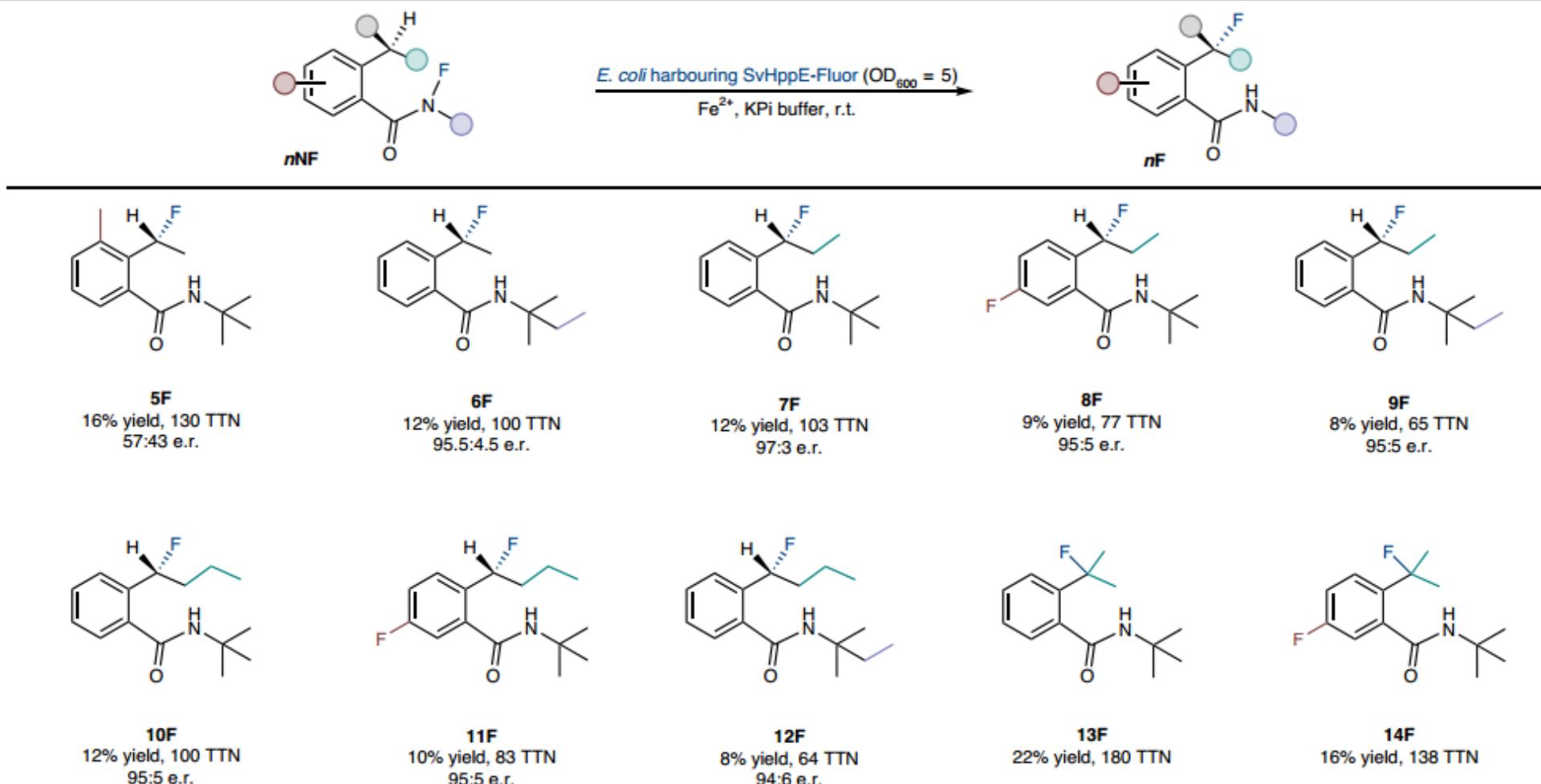


Fig. 3 | Reaction scope and determination of absolute configuration of products. Fluorination product **1F** was determined to be *R* through X-ray crystallography. The absolute configurations of all other fluorination products were inferred by analogy. Experiments were performed at analytical scale using suspensions of *E. coli* cells expressing *SvHppE* variants ($OD_{600} = 5$), 10 mM substrate **nNF**, 2.5 mM Fe^{2+} in KPi buffer (pH 7.4) at room temperature under

anaerobic conditions for 24 hours. TTNs were determined by dividing the quantity of the resulting product (calculated using the GC-MS calibration curve) by the concentration of the enzyme catalyst expressed in *E. coli*. The protein concentration of $OD_{600} 5$ whole-cell suspension was determined to be 12 μ M using protocols described in sections E and F of the Supplementary Information.

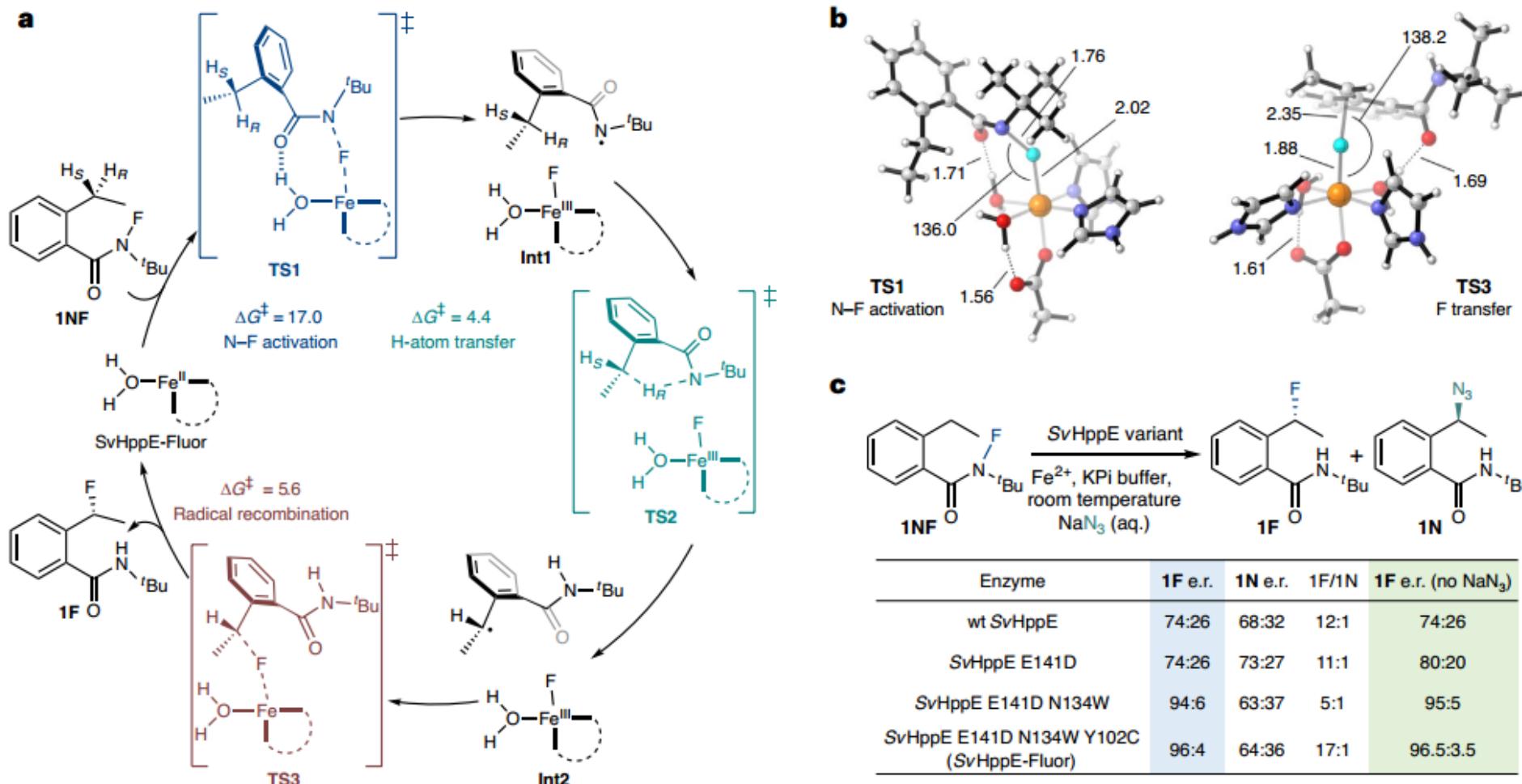
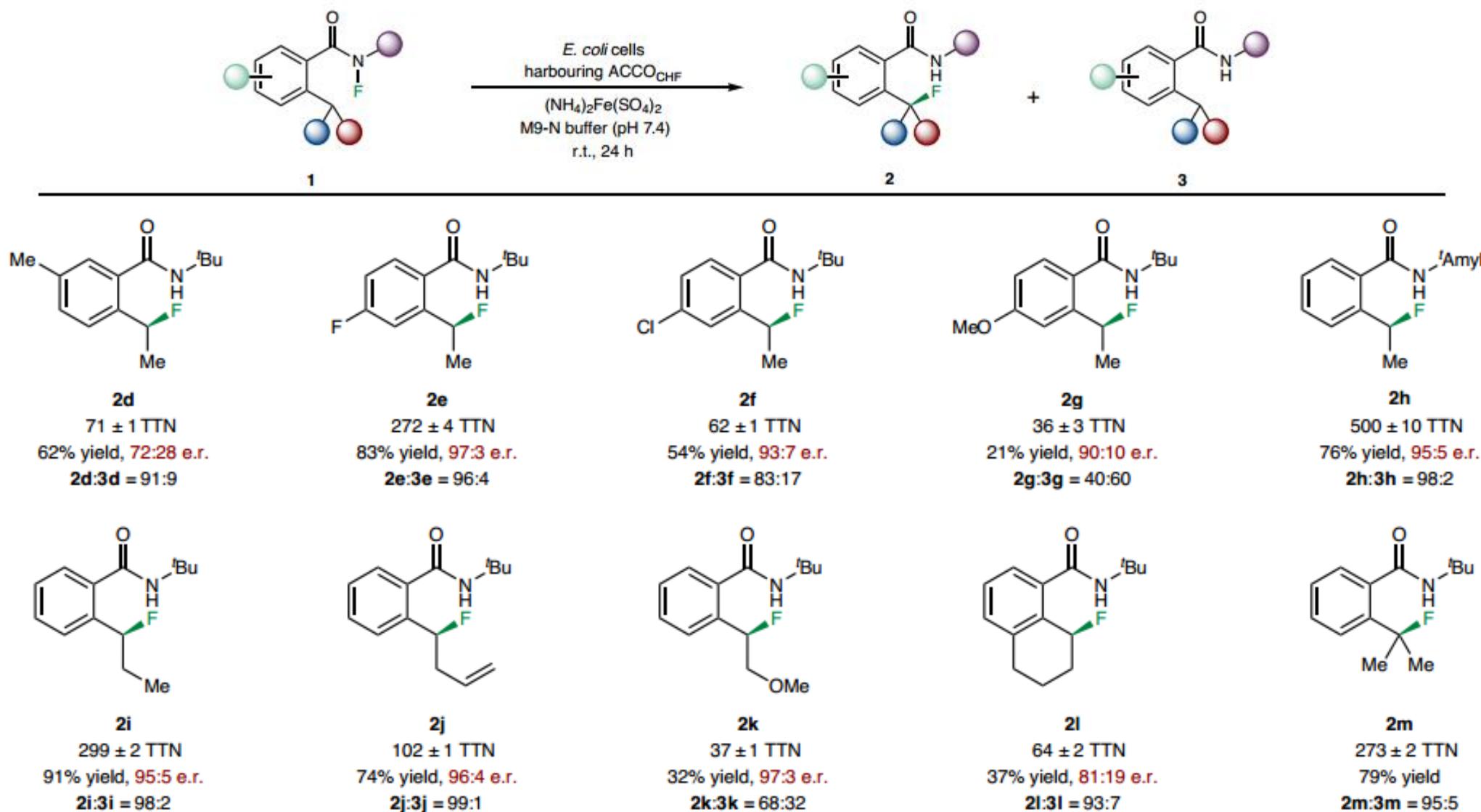


Fig. 4 | Mechanistic studies. **a**, DFT calculations using a truncated model to explore the intrinsic energy barriers of each elementary step of the reaction. Energies are given in kcal mol^{-1} . **b**, Key structural parameters of optimized transition states for N-F bond activation and fluorine transfer steps. Distances

and angles are given in angstroms (\AA) and degrees ($^\circ$), respectively. The transition state structures were rendered using CYLview. **c**, Impact of NaN_3 on reaction outcomes of SvHppE and its variants.



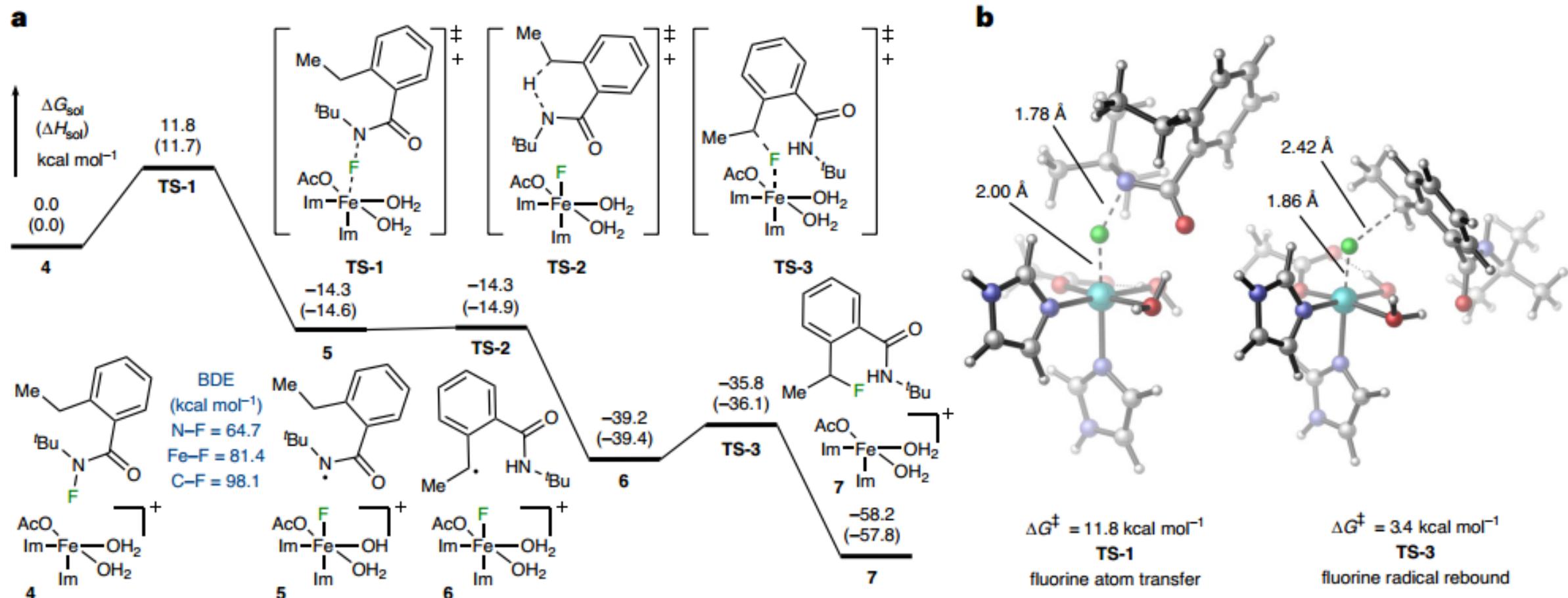
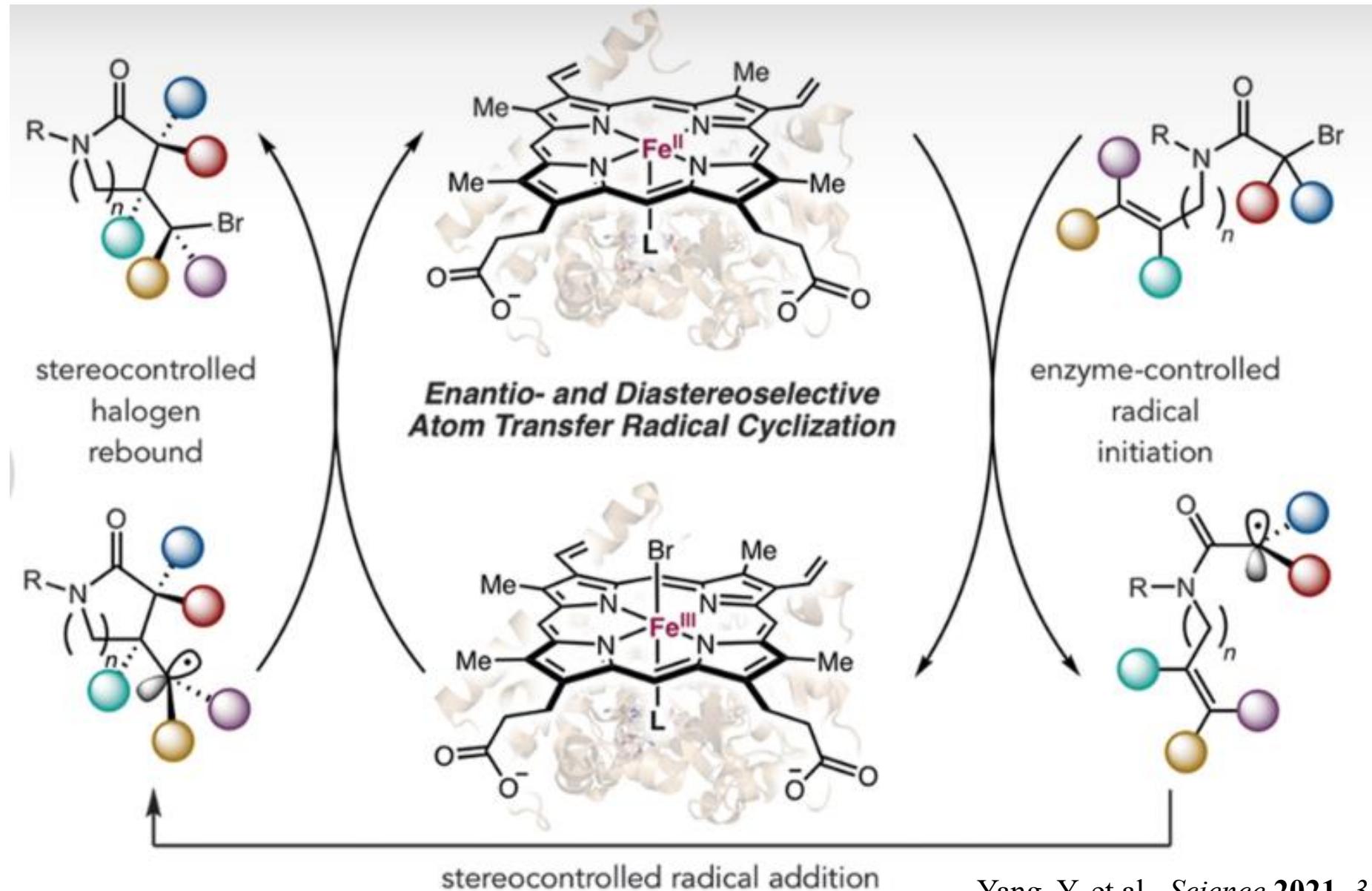
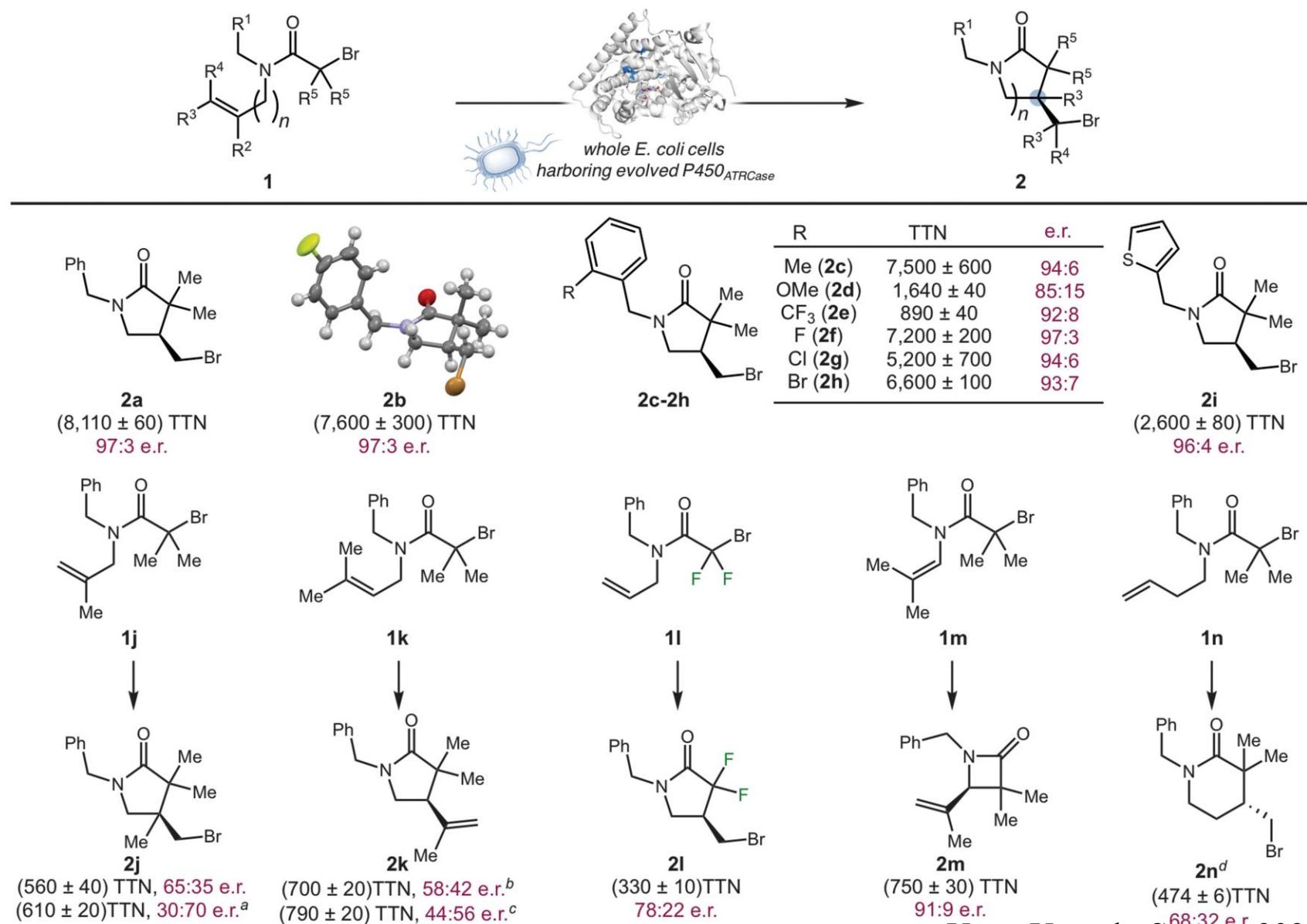


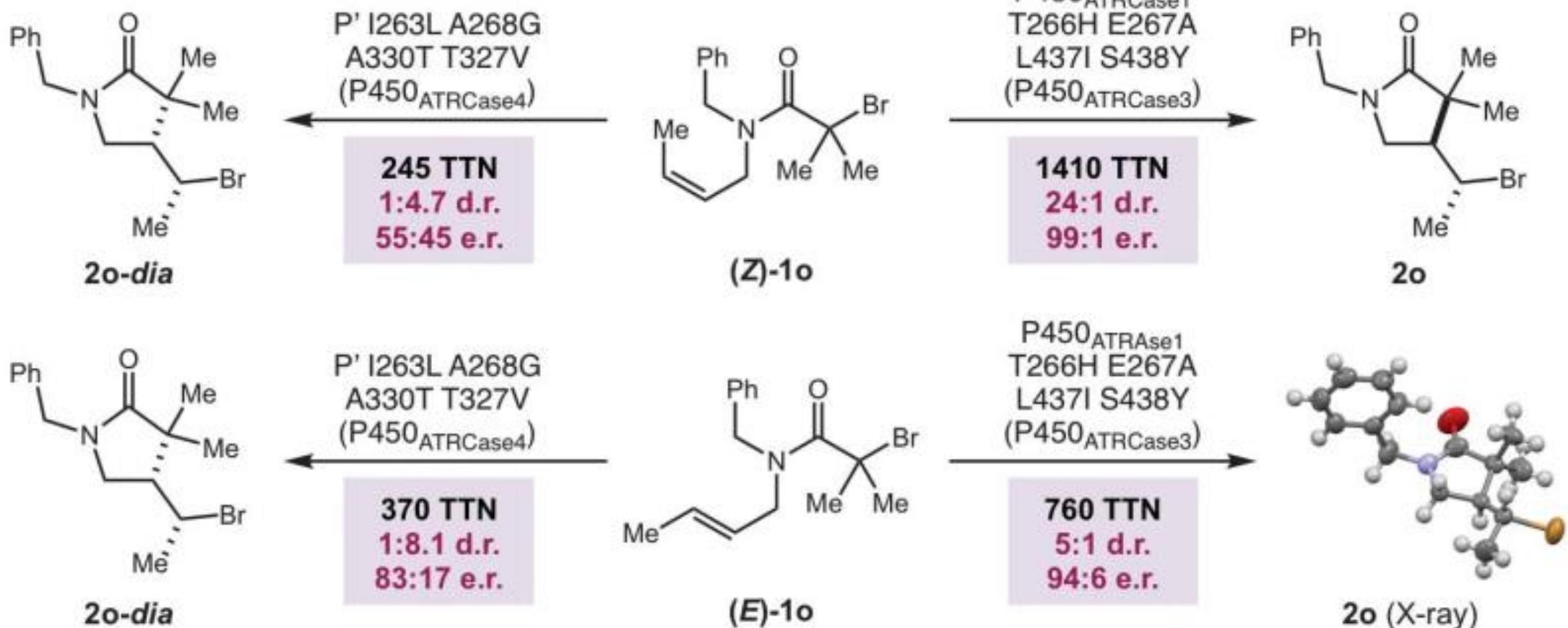
Fig. 5 | Computational studies using a truncated active site model. a, DFT-computed reaction energy profile of C–H fluorination of *N*-fluoroamide **1** using a truncated model based on the active site of ACCO enzyme at the (U)B3LYP-D3(BJ)/def2-TZVP/SMD//(U)B3LYP-D3(BJ)/6-31 G(d)-SDD(Fe) level of theory.

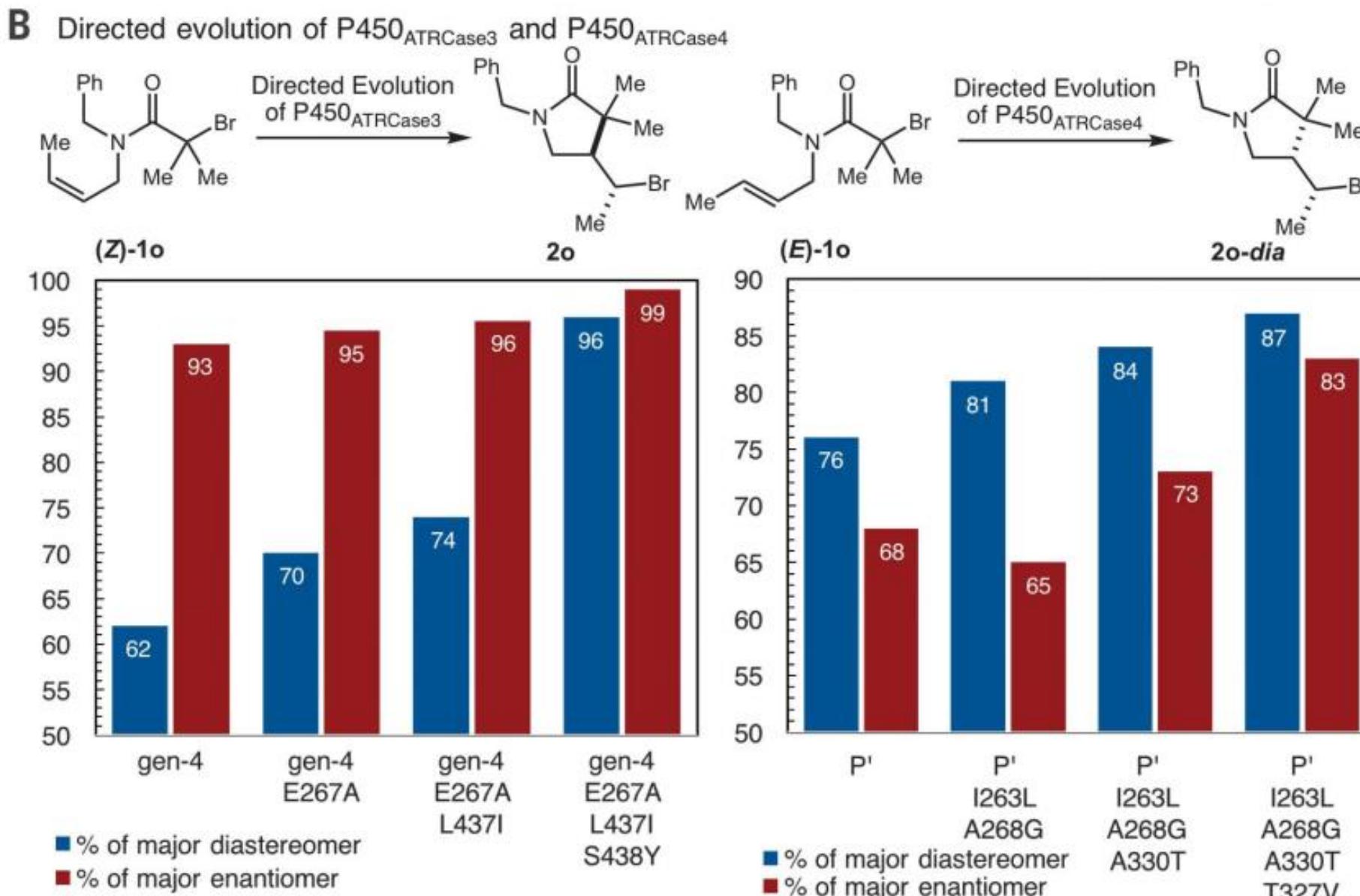
Energies are with respect to **4**. Im and AcO groups are models for aspartate and histidine residues, respectively. **b**, Optimized geometries of the fluorine atom transfer (**TS-1**) and fluorine radical rebound (**TS-3**) transition states.



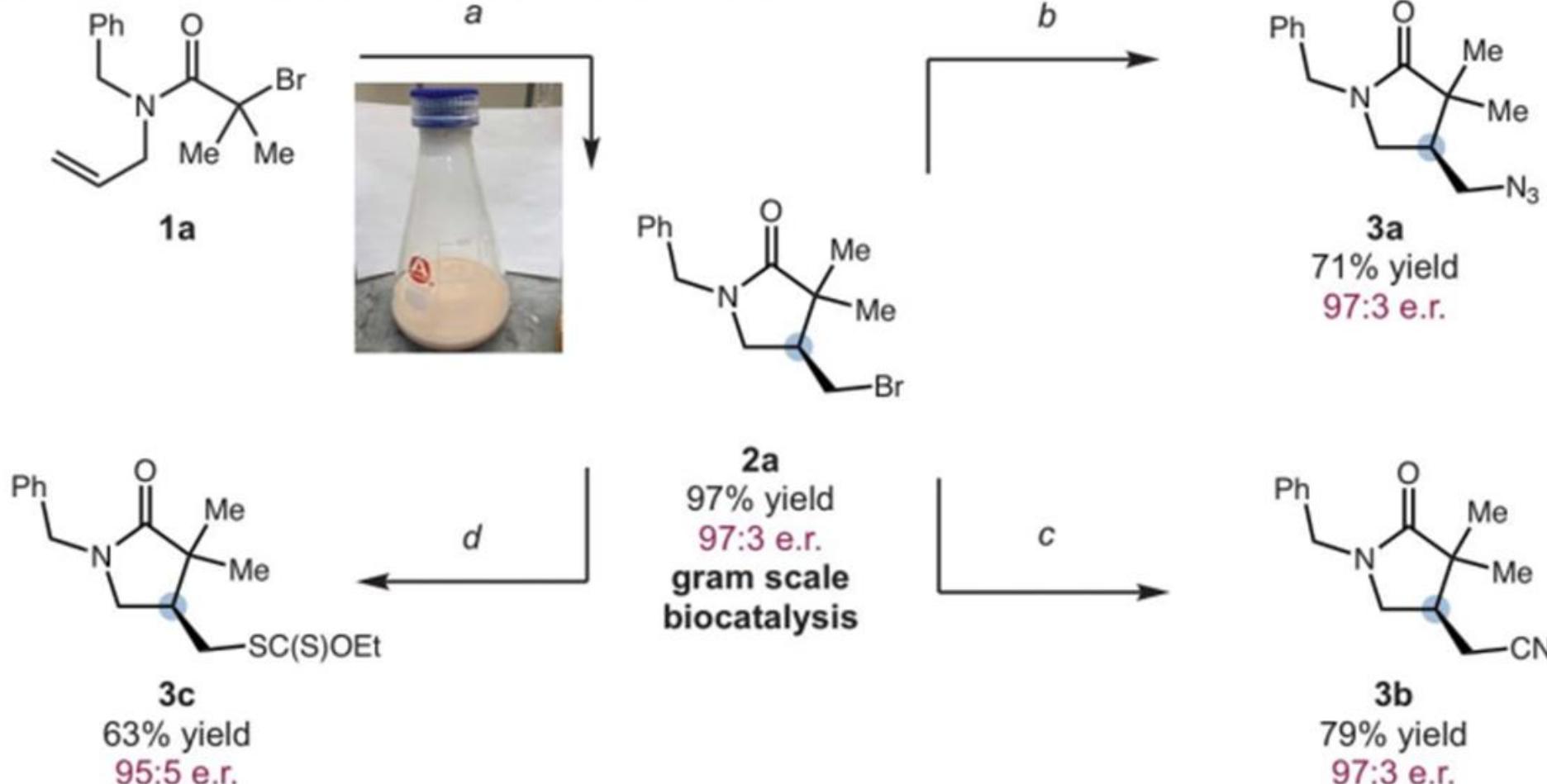


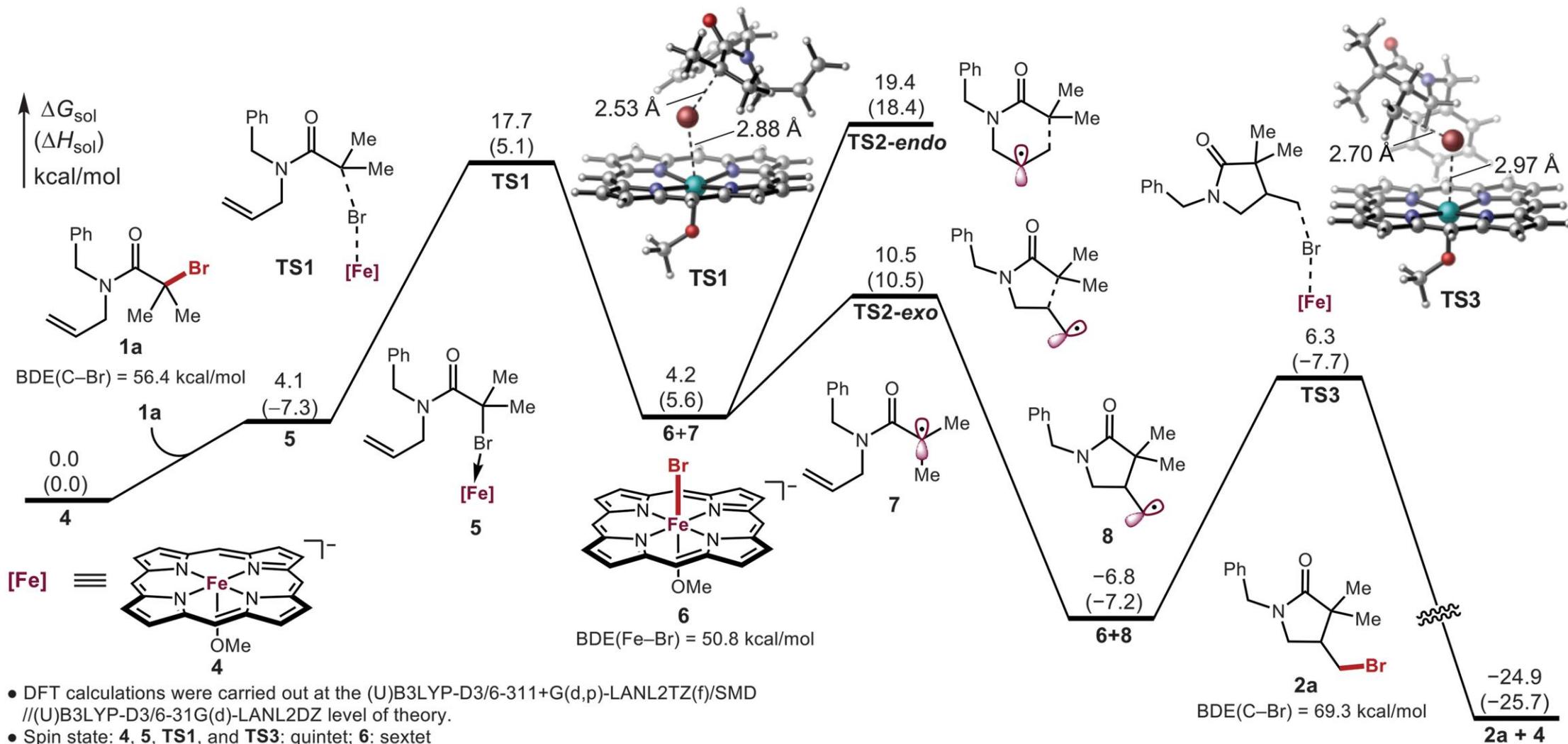
A Diastereodivergent ATRC with $P450_{ATRCase3}$ and $P450_{ATRCase4}$





C Transformation of enantioenriched ATRC product





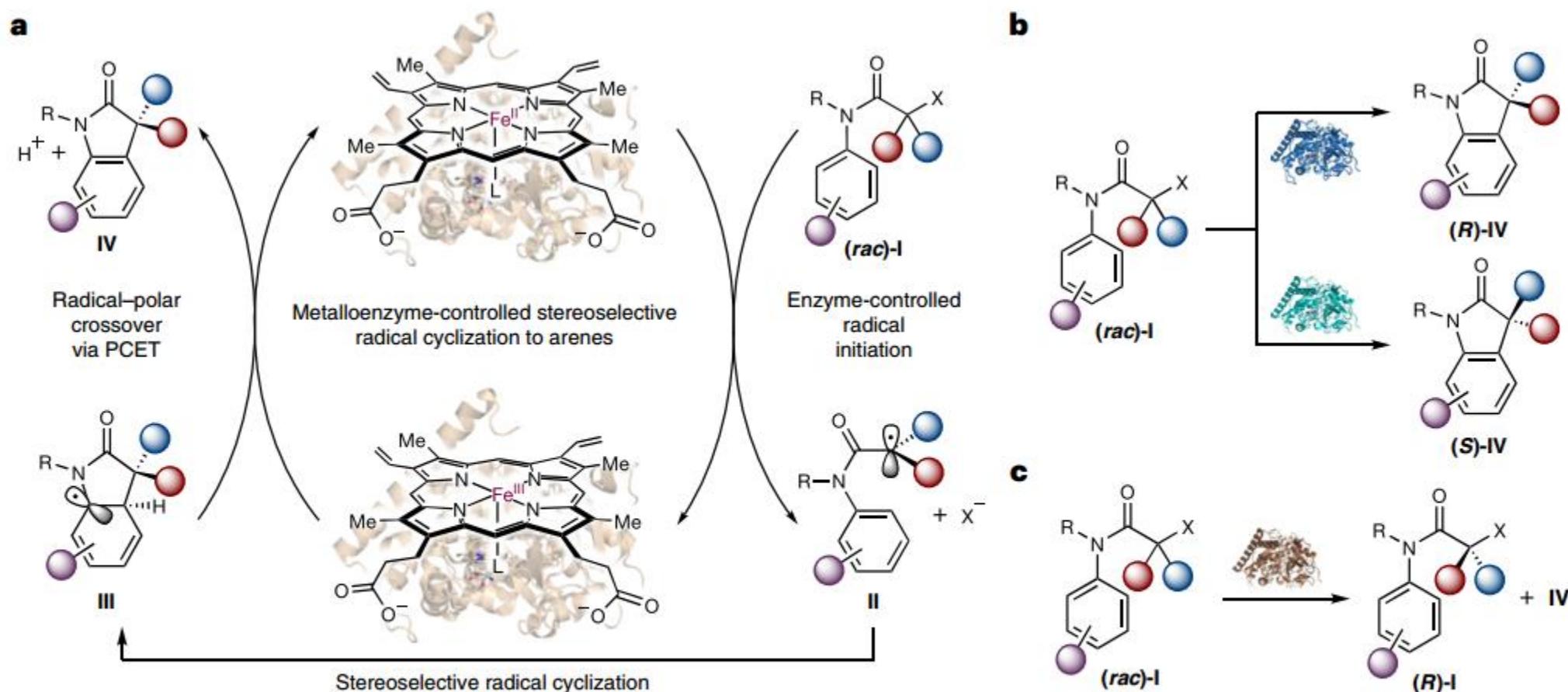
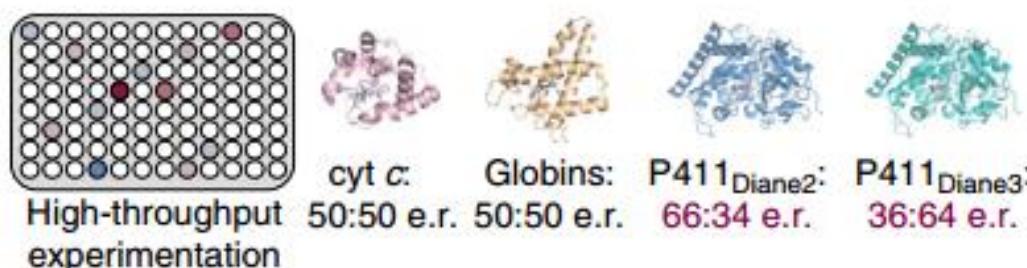
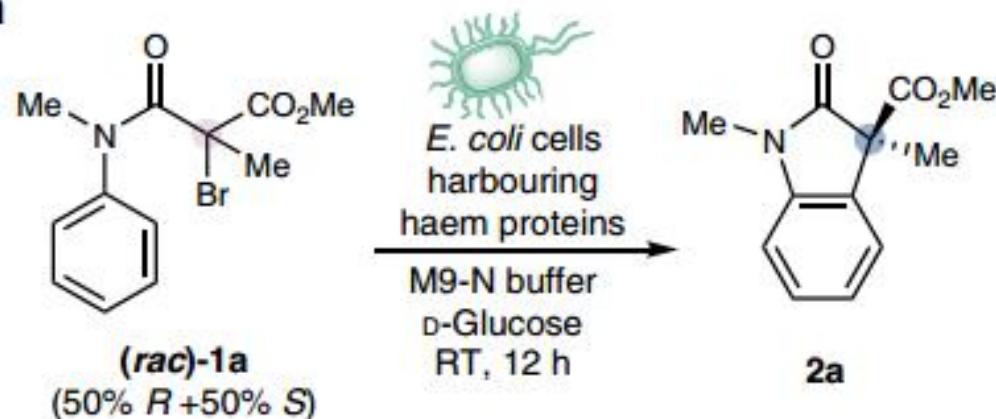
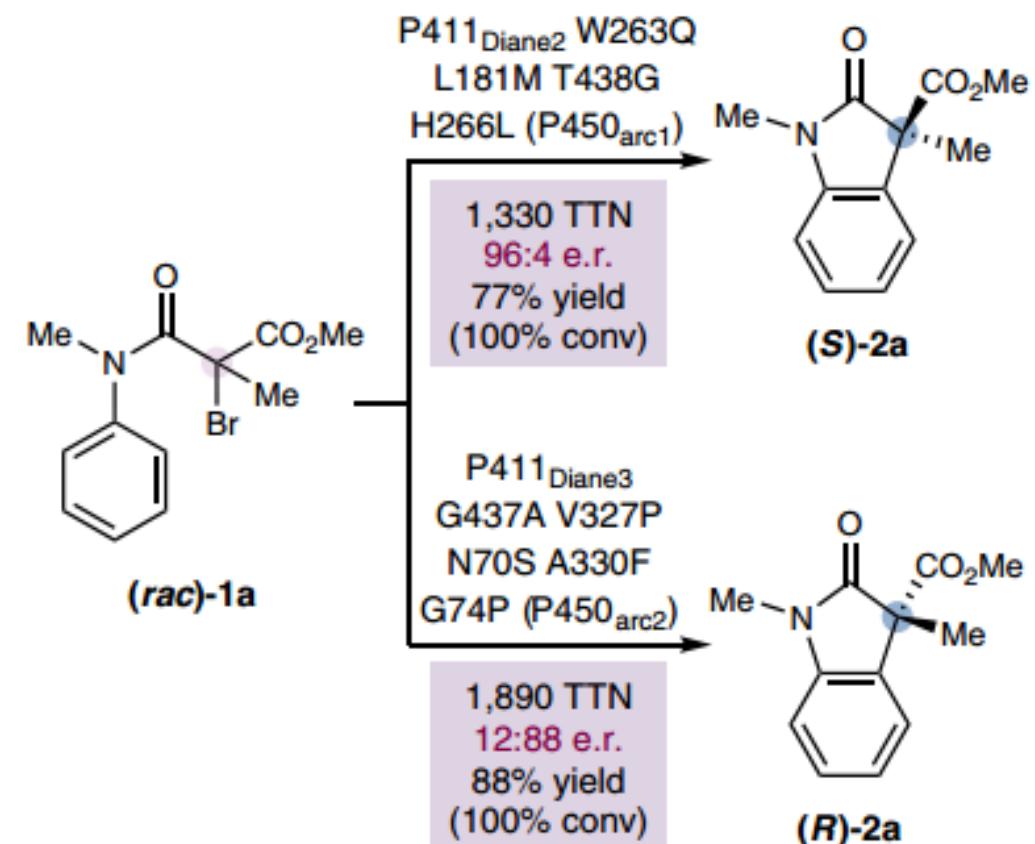
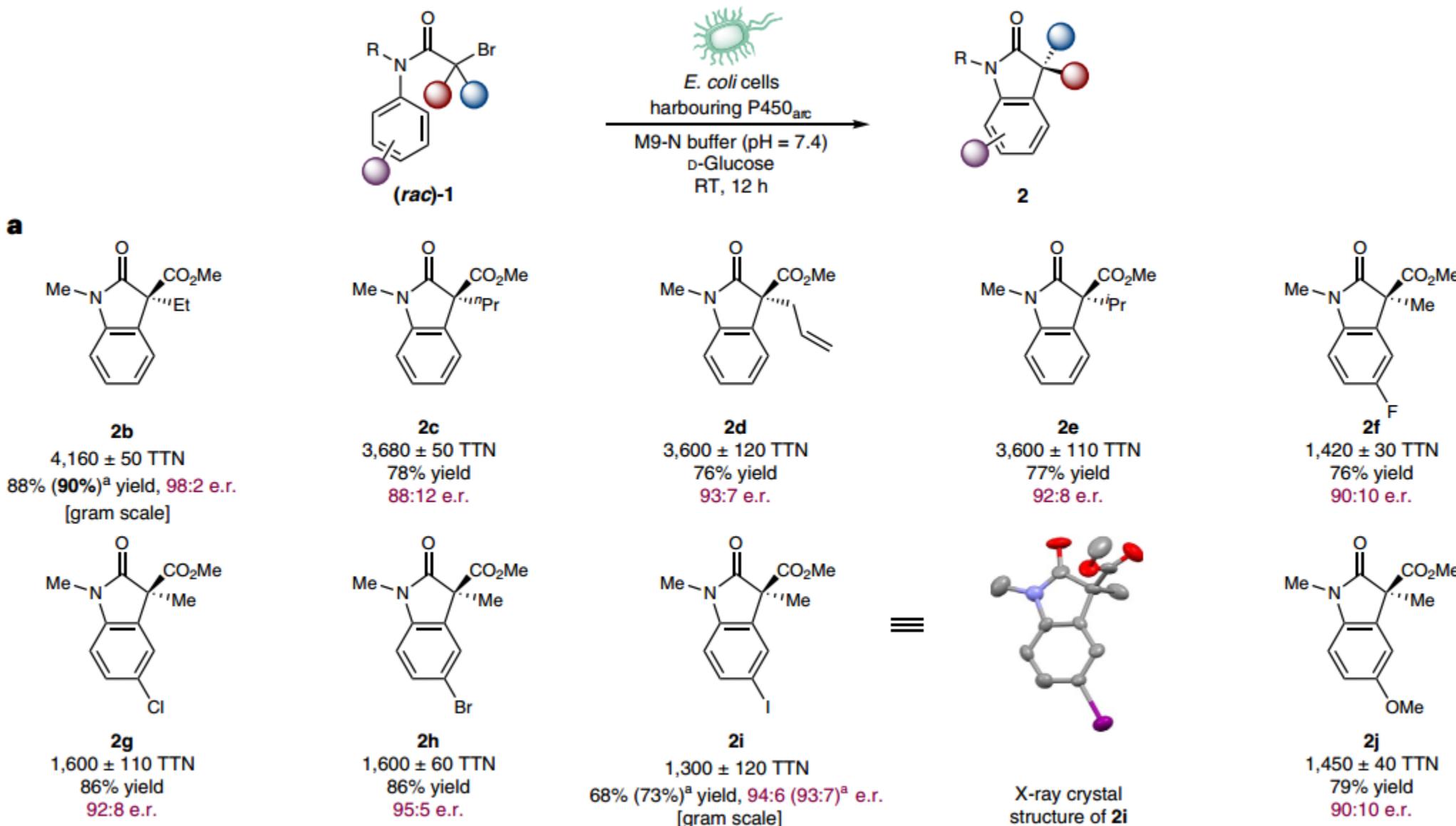


Fig. 1 | A metalloenzyme platform for stereoselective radical cyclization.

a, Proposed catalytic cycle with a haemoprotein catalyst. **b**, Biocatalytic enantioconvergent radical cyclization leading to either enantiomer of the products. **c**, Biocatalytic kinetic resolution to prepare enantioenriched tertiary

alkyl halides. L is an Fe-binding amino acid residue, which is serine in this work. X is either Br or Cl. PCET, proton-coupled electron transfer. Coloured spheres are generic substituents of the molecule.

a**b**



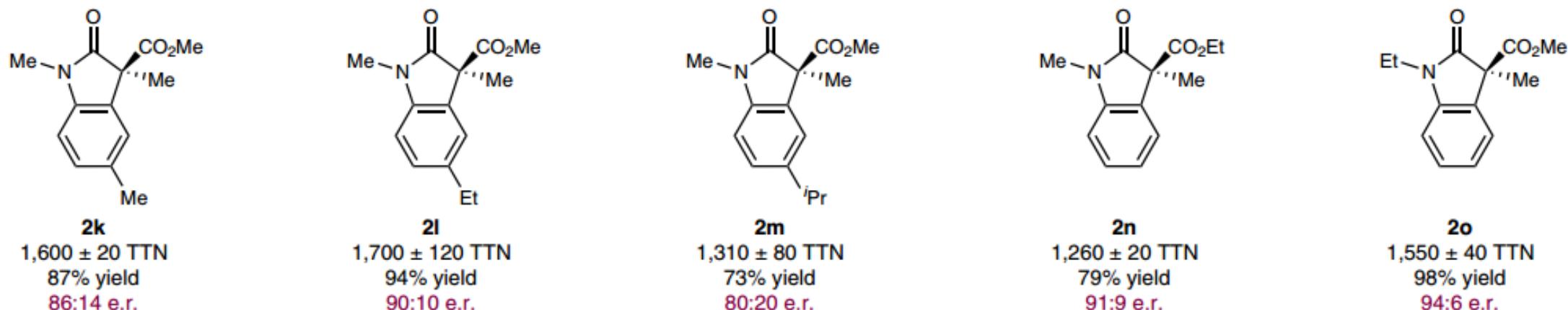
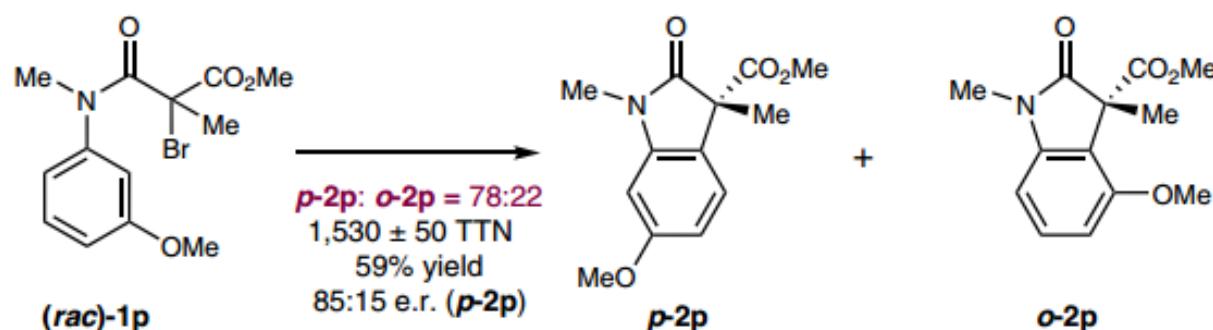
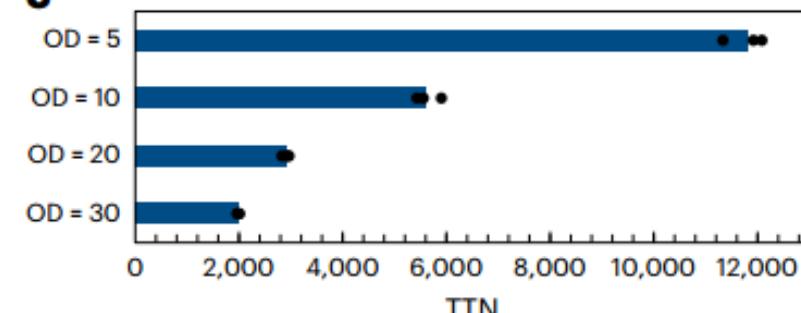
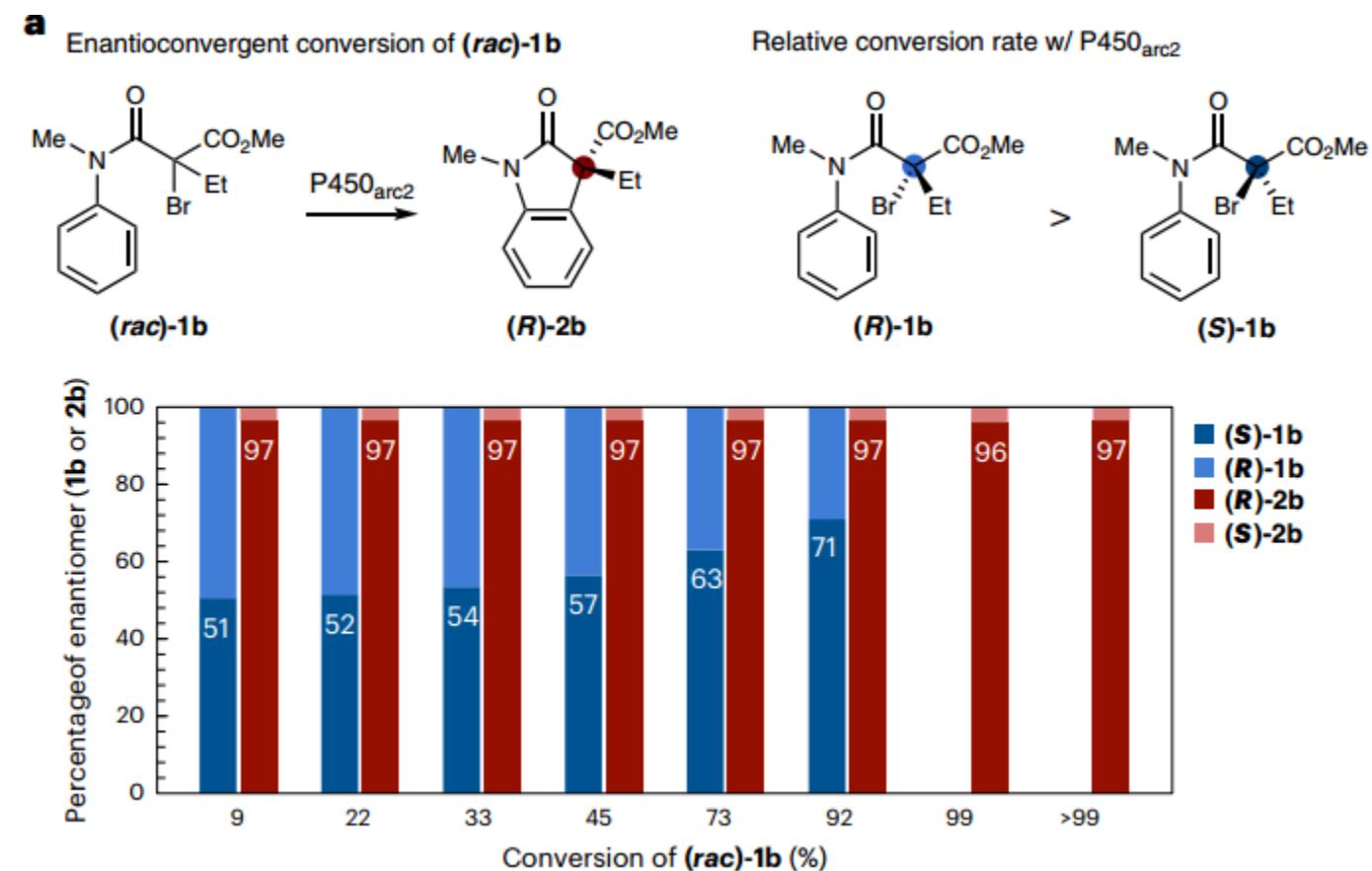
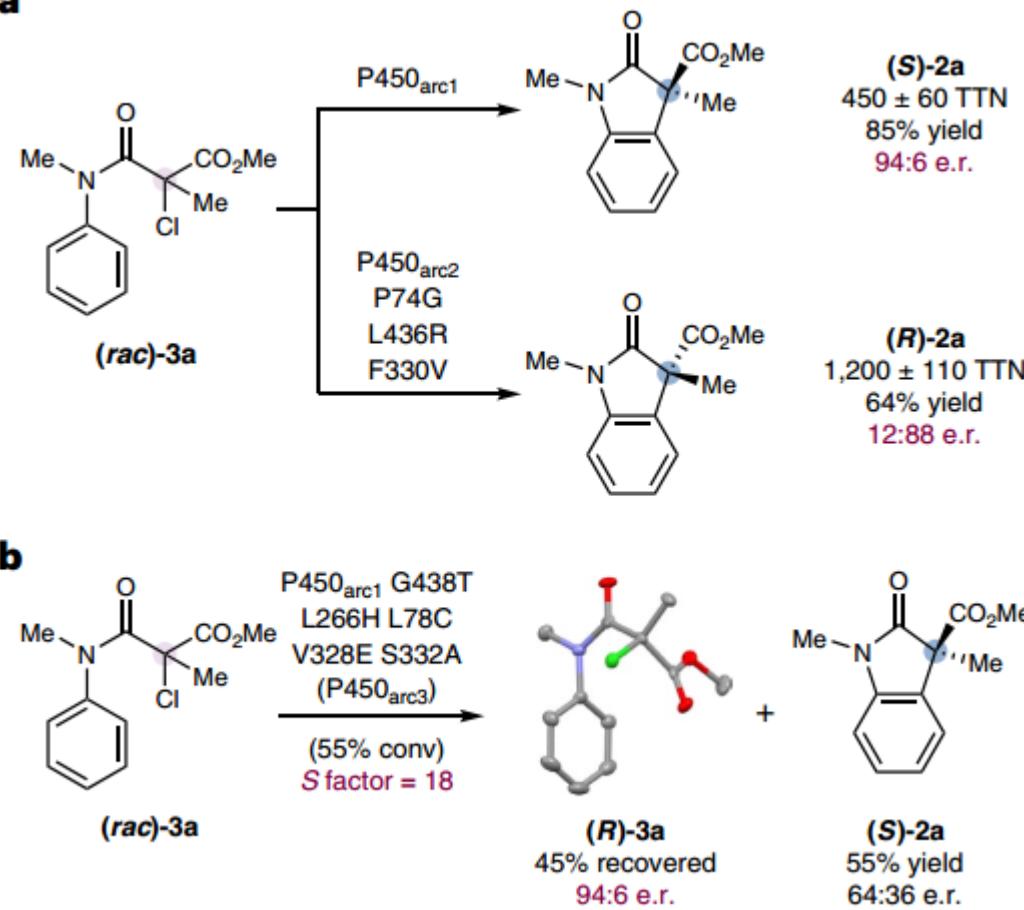
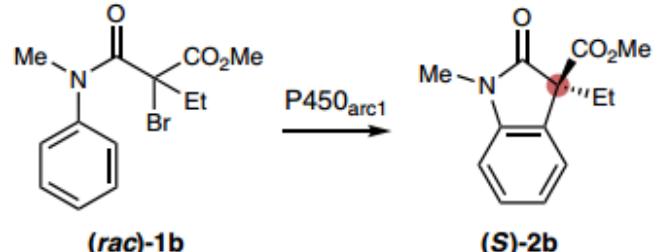
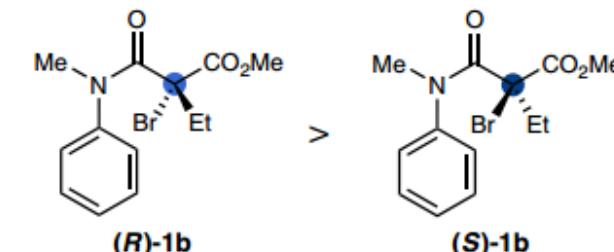
**b****c**

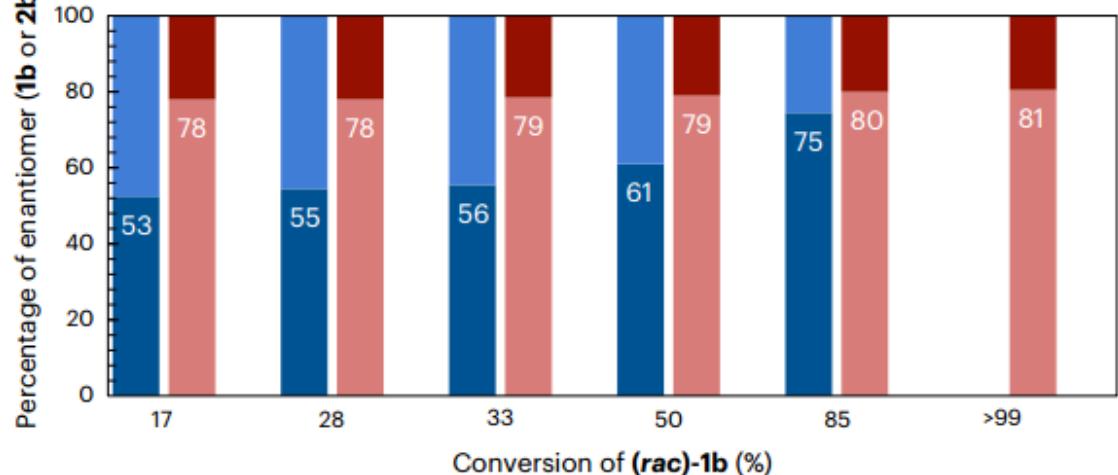
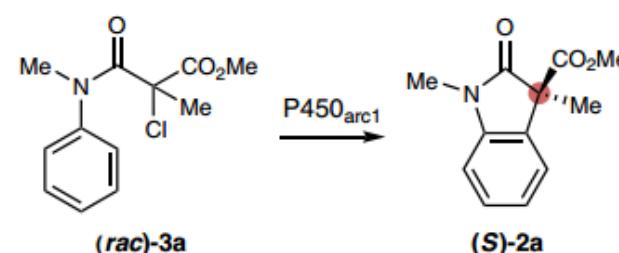
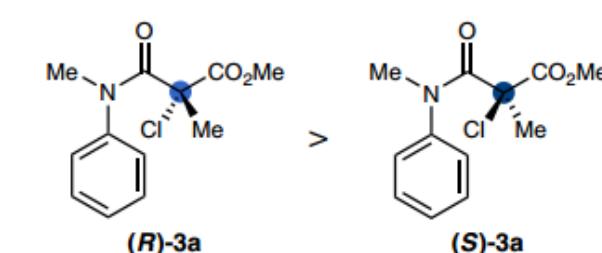
Fig. 3 | Substrate scope of P450_{arc}-catalysed enantioconvergent radical cyclization. a, Substrate scope of α -bromoesters ($OD_{600} = 15–35$ unless otherwise noted; Supplementary Methods). All the reactions were performed in triplicate

and averaged results and standard deviations are provided. **b**, Biocatalytic site-selective radical cyclization of *meta*-substituted substrate **1p**. **c**, Whole-cell radical cyclization of **1b** with high TTNs. ^aGram-scale reaction.

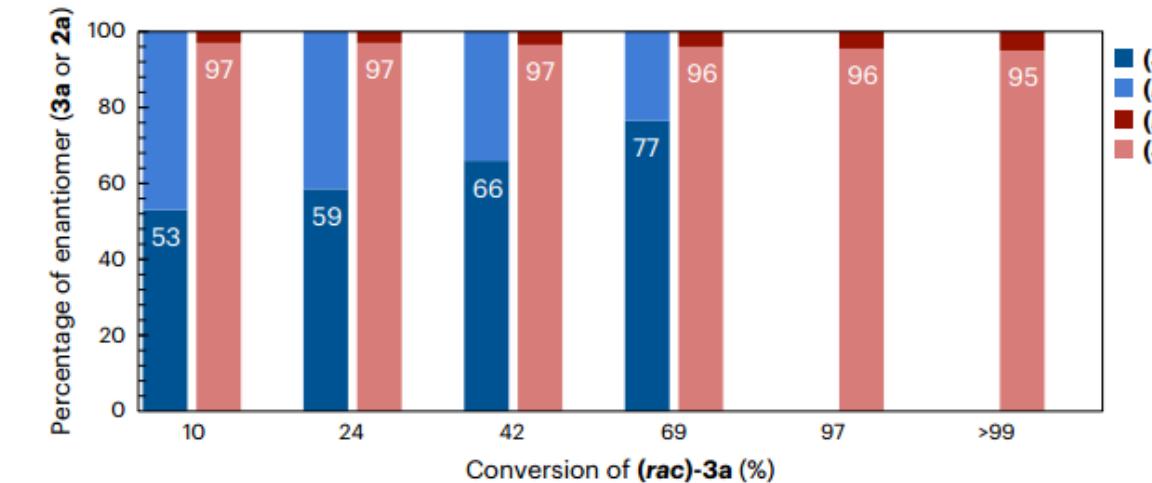


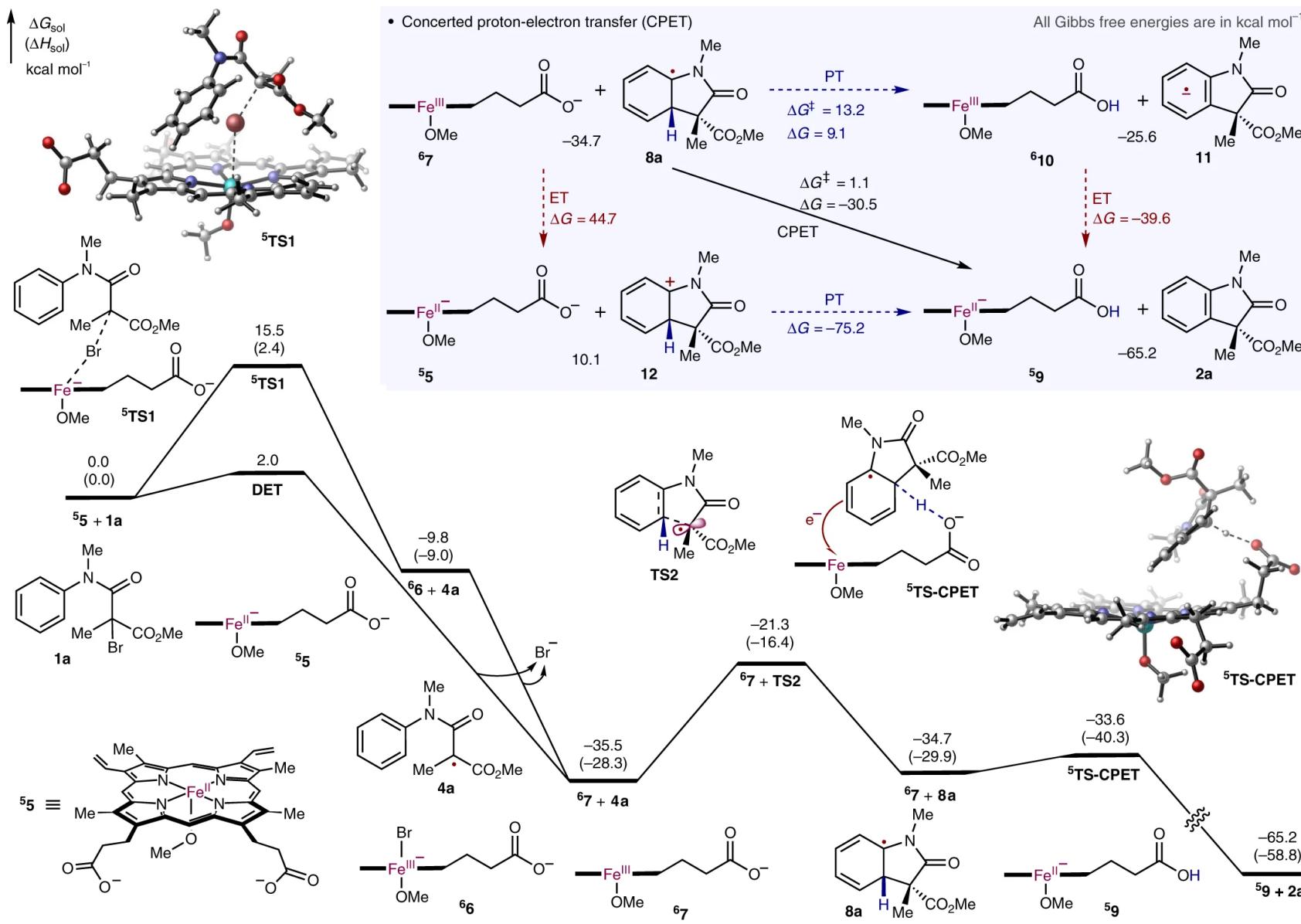
bEnantioconvergent conversion of *(rac)*-1bRelative conversion rate w/ P450_{arc1}

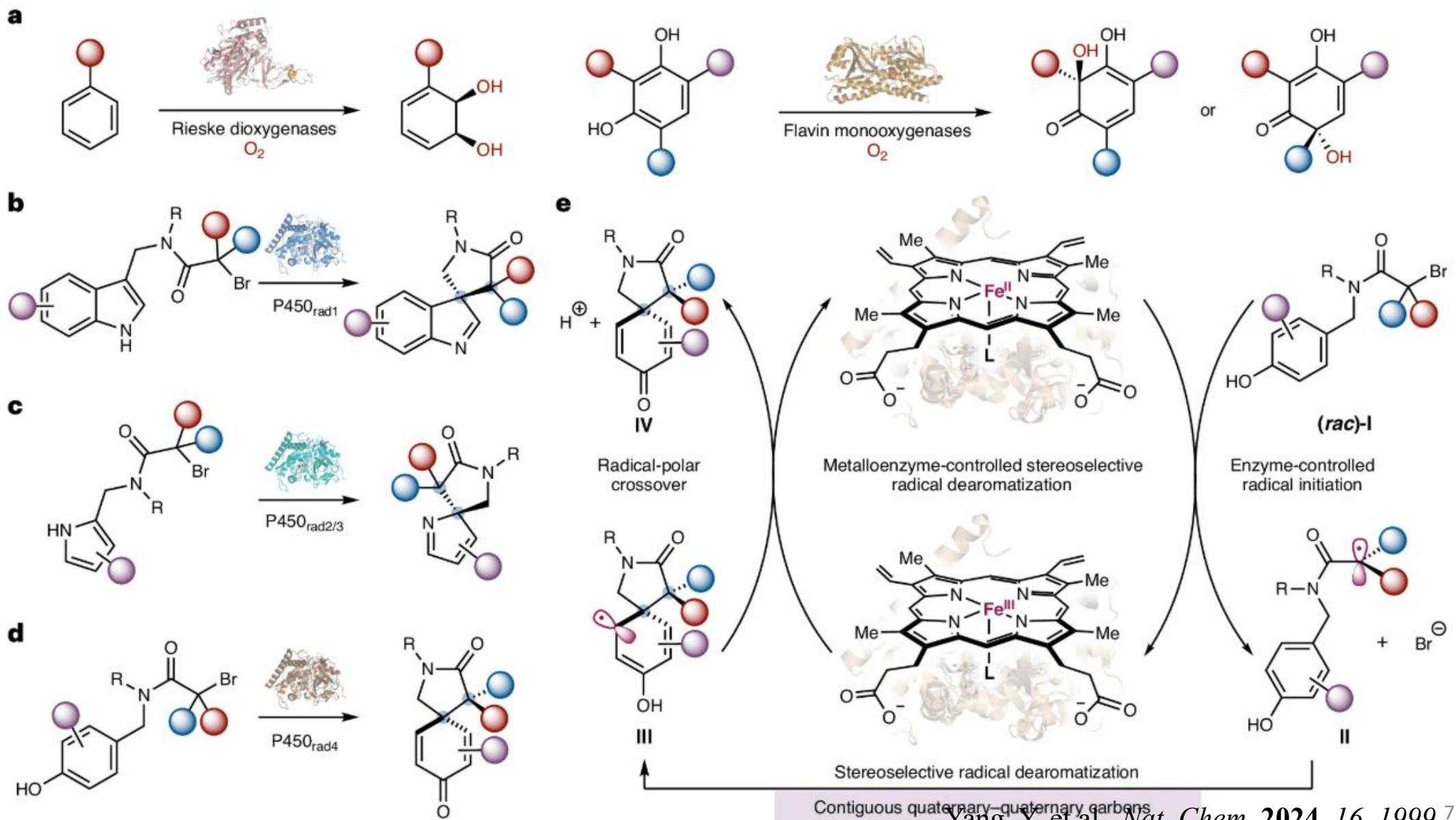
Percentage of enantiomer (1b or 2b)

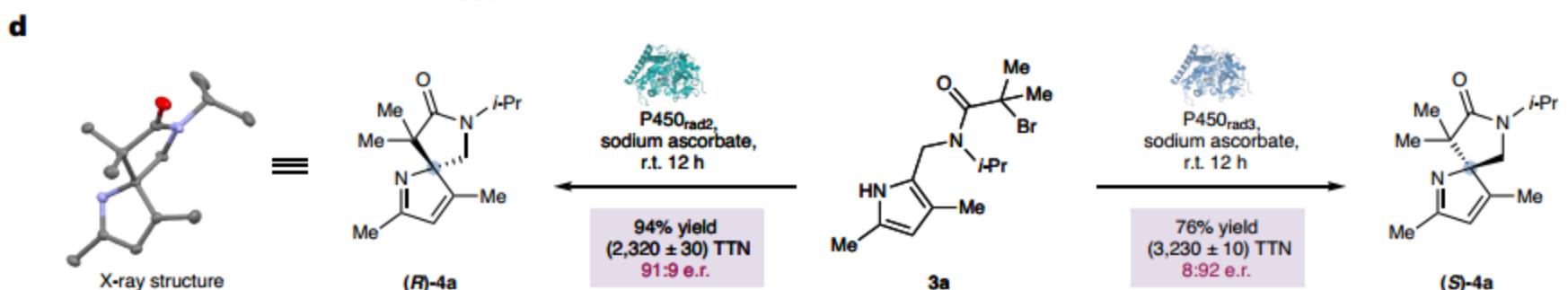
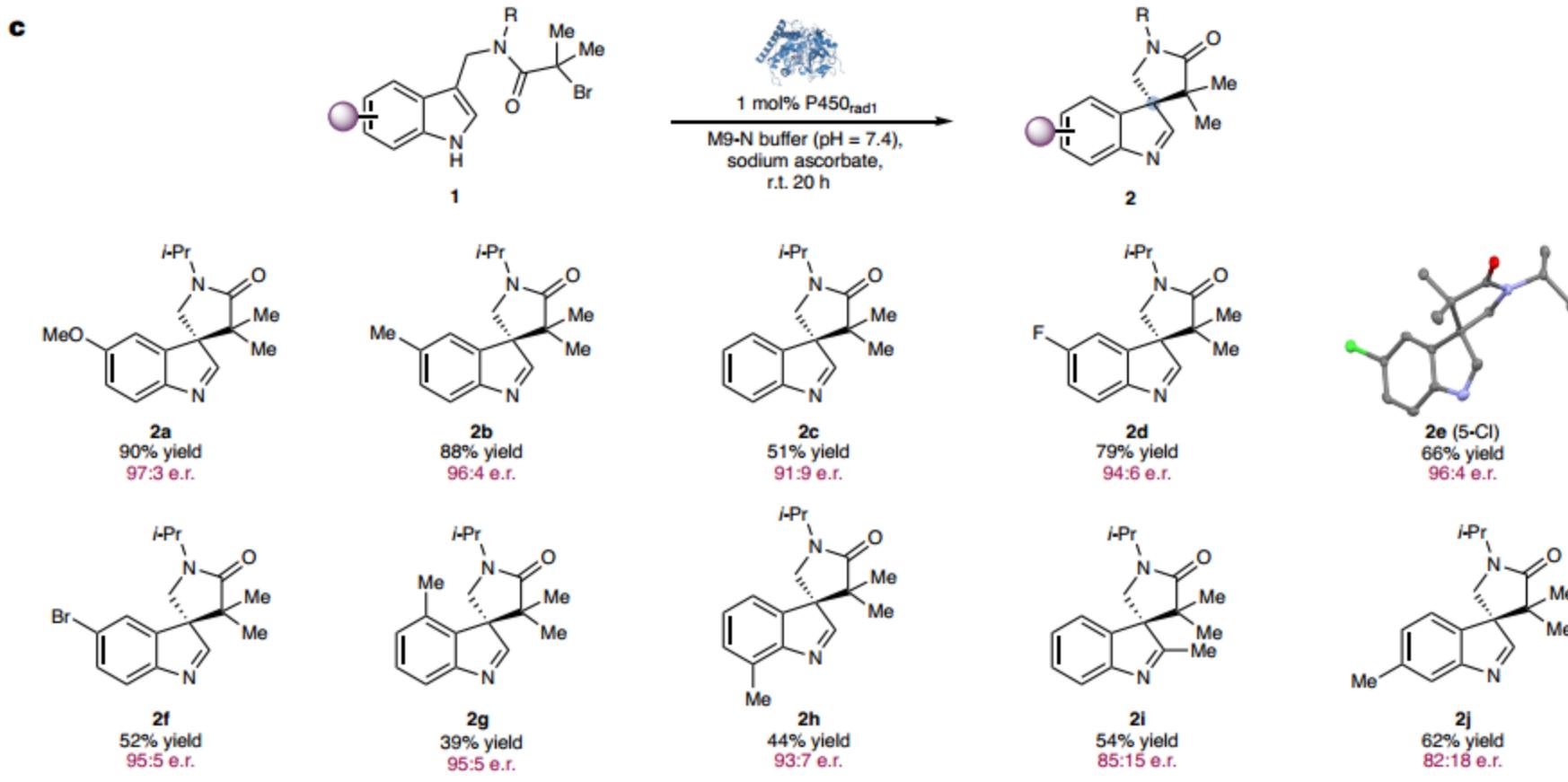
**c**Enantioconvergent conversion of *(rac)*-3aRelative conversion rate w/ P450_{arc1}

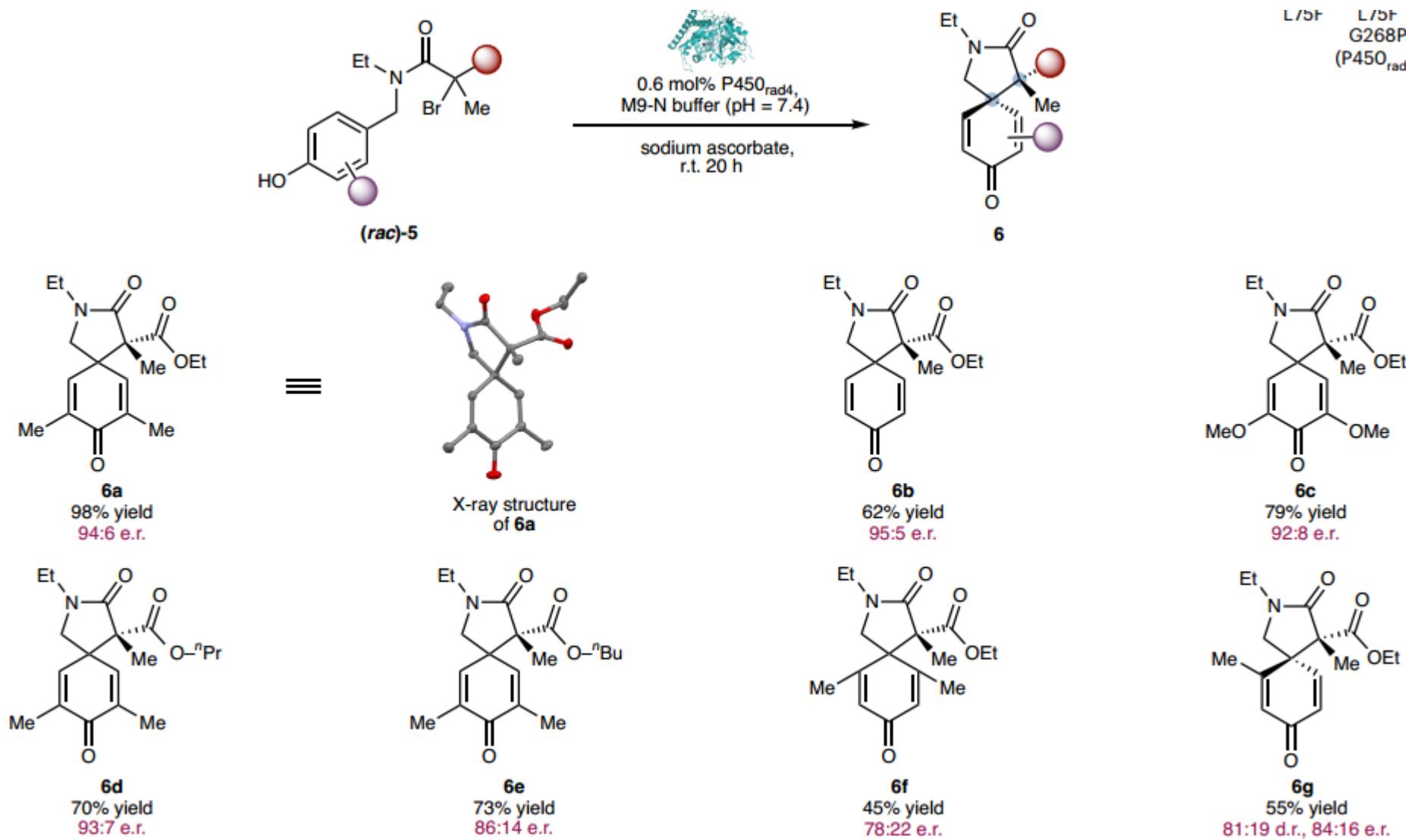
Percentage of enantiomer (3a or 2a)



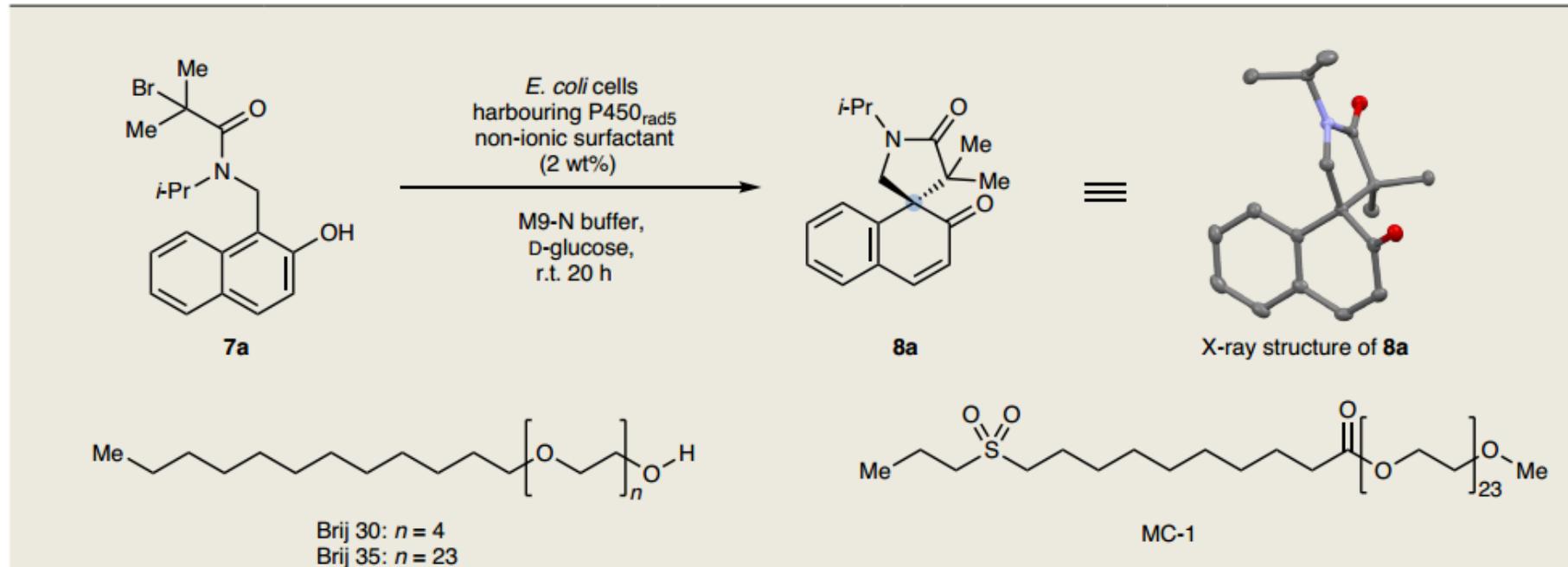




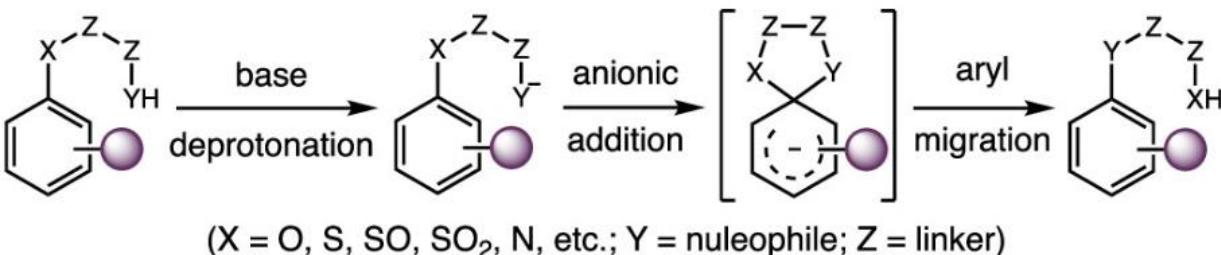




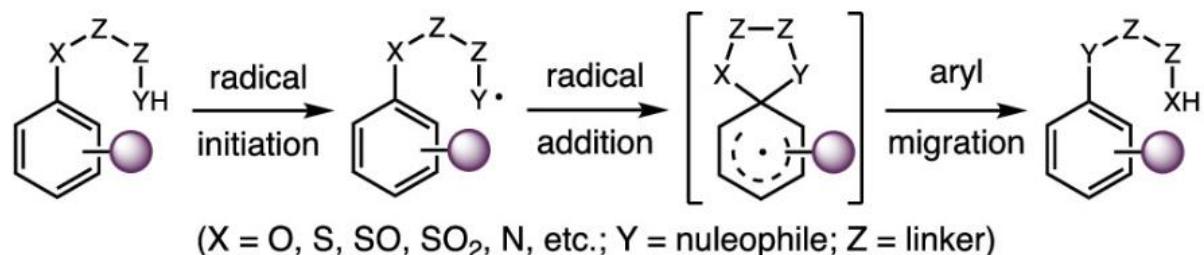
| Entry ^a | Surfactant | e.r. | Yield (%) | Total turnover number |
|--------------------|------------------|-------------|-------------|-----------------------|
| 1 ^b | - | 91:9 | 21±3 | 650±80 |
| 2 | Brij 30 | 62:38 | 83±2 | 1,890±40 |
| 3 | Brij 35 | 68:32 | 85±1 | 1,930±30 |
| 4 | MC-1 | 84:16 | 21±1 | 470±20 |
| 5 | Nok | 91:9 | 57±4 | 1,300±90 |
| 6 | TPGS-750-M | 89:11 | 58±4 | 1,330±90 |
| 7 | TPGS-1000 | 92:8 | 71±5 | 1,630±120 |
| 8 ^c | TPGS-1000 | 97:3 | 84±4 | 1,510±80 |
| 9 ^d | - | 97:3 | 33±3 | 600±60 |



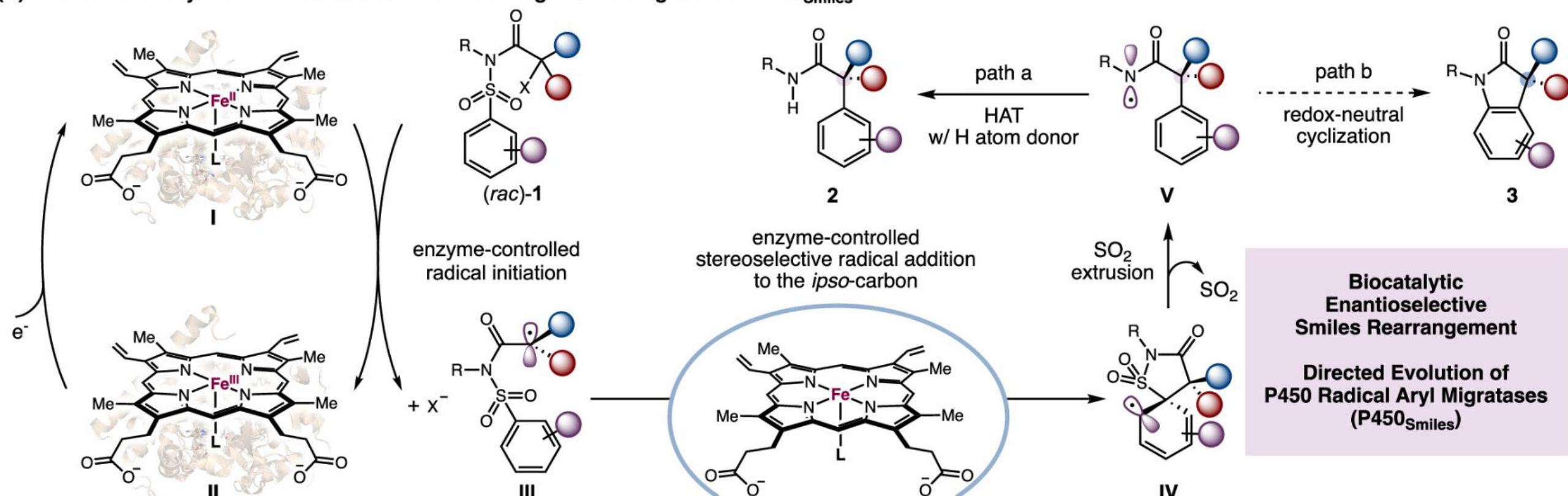
(A) Classic Smiles rearrangement: anionic reaction modes

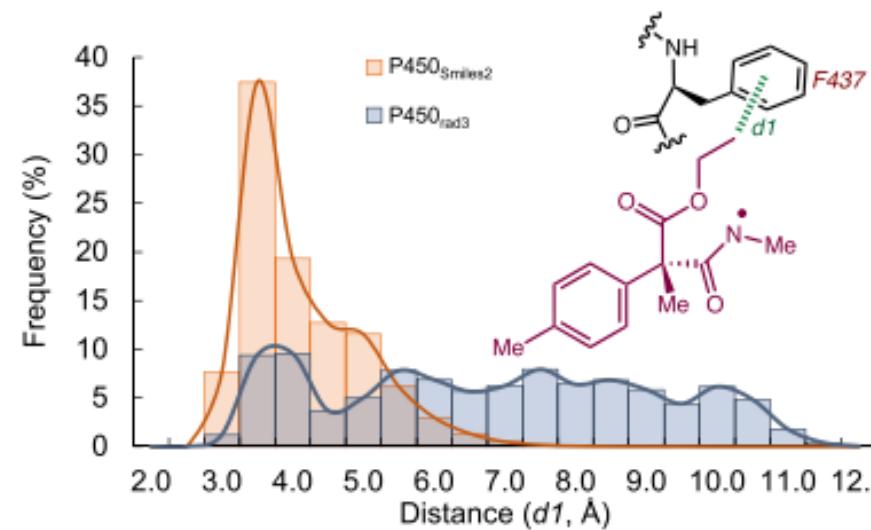
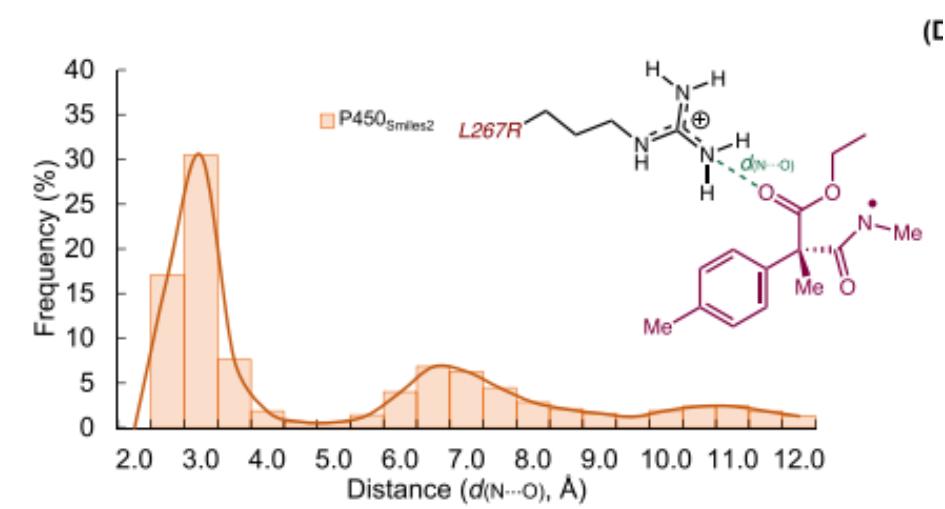
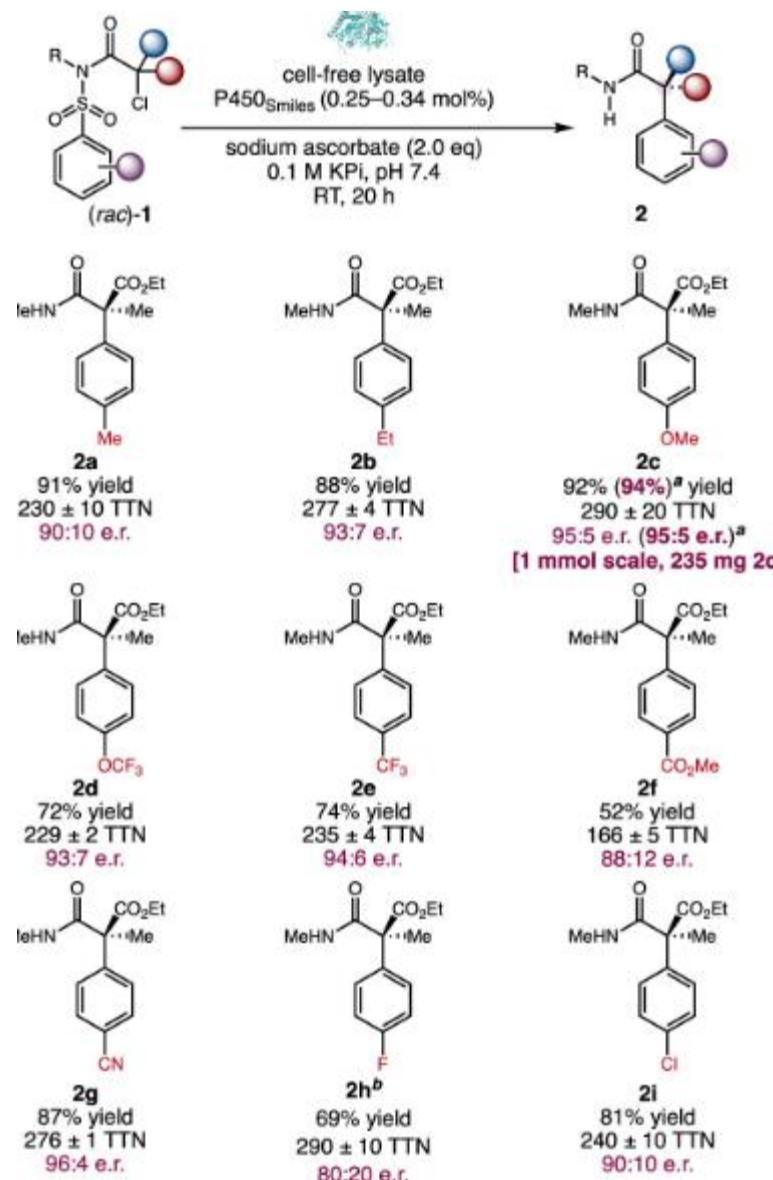


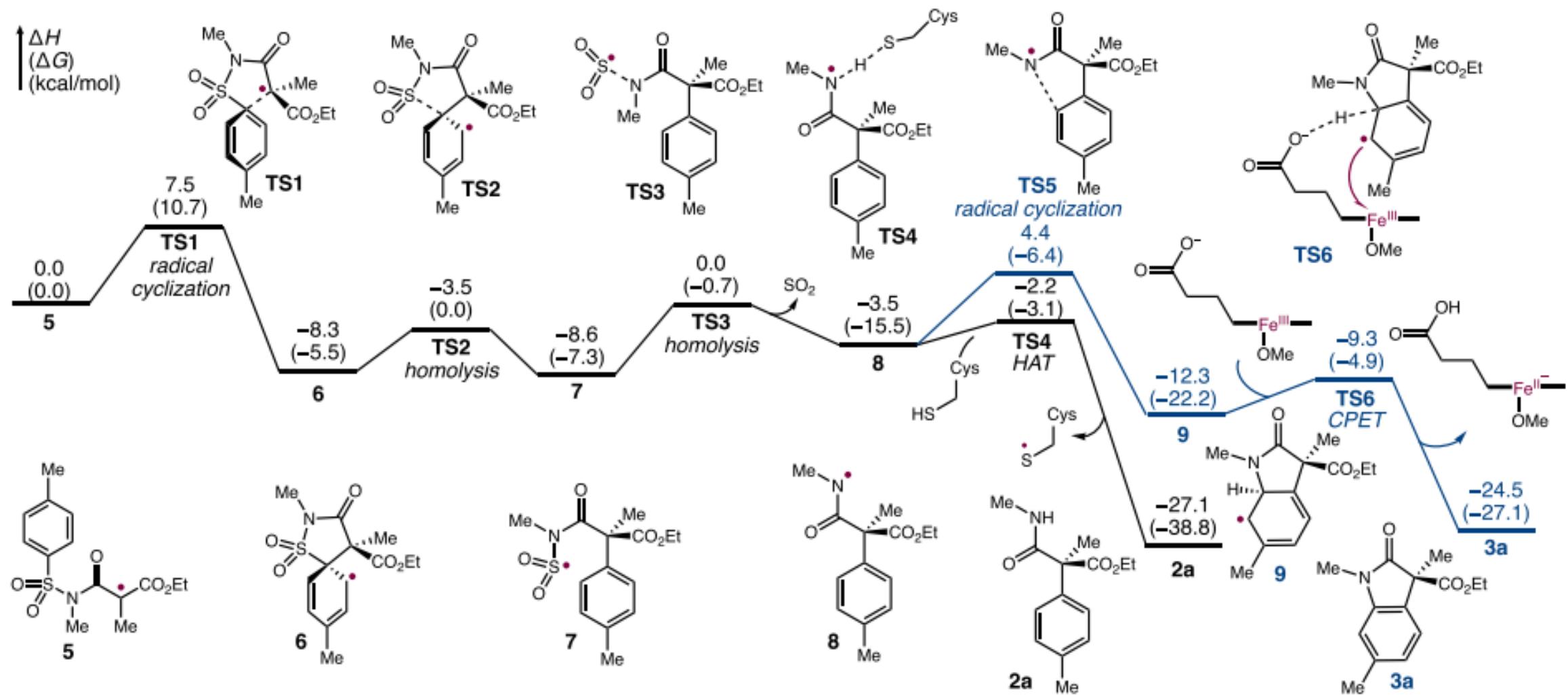
(B) Classic Smiles rearrangement: radical reaction modes



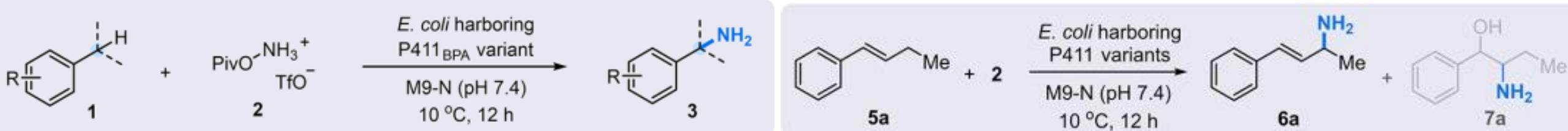
(C) This work: catalytic enantioselective Smiles rearrangement using evolved P450_{Smiles}





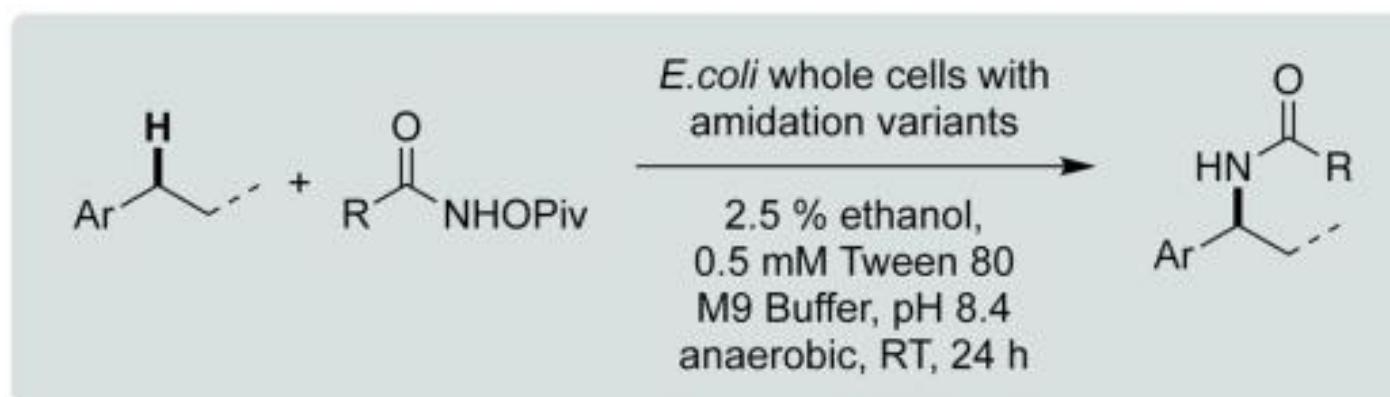


酶催化的芳位和烯丙位C(sp³)–H键的一级胺化反应



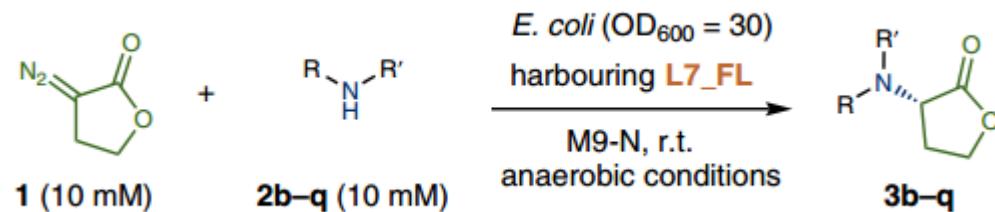
Arnold, F. et al., *J. Am. Chem. Soc.* **2020**, *142*, 10279.

用于合成高对映体富集酰胺的生物催化分子间C–H键官能团化



Arnold, F et al., *Angew. Chem. Int. Ed.* **2021**, *60*, 24864.

双功能酶催化对映选择性碳氮键形成



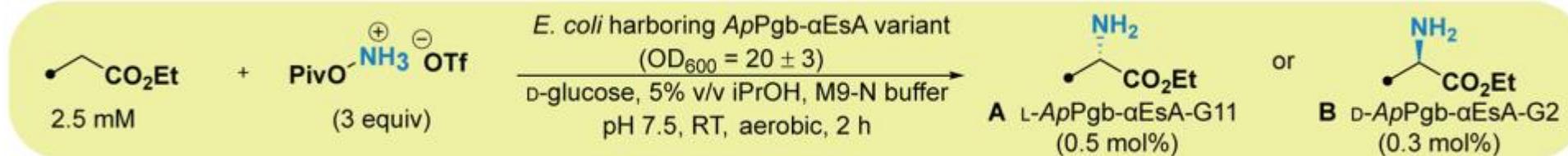
Arnold, F. et al., *Nat. Chem.* 2011, 13, 1166.

酶催化实现1-芳基-2-烷基炔的丙炔位对映选择性伯胺化



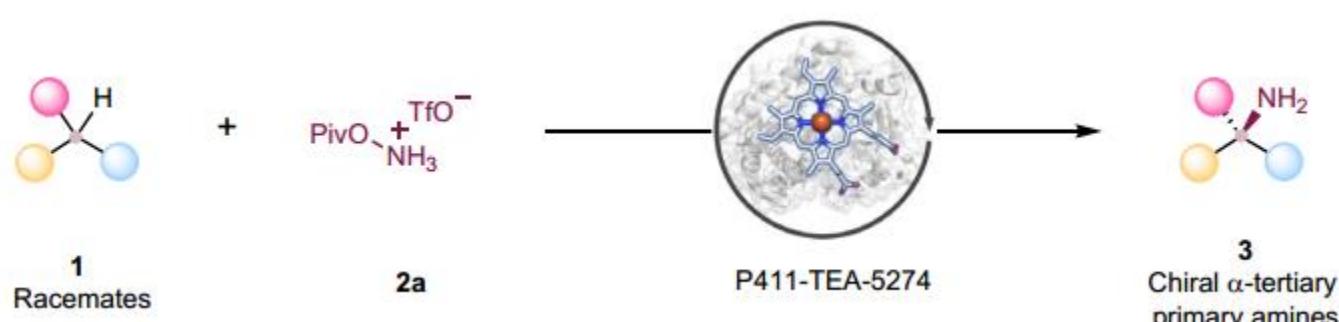
Arnold, F et al., *J. Am. Chem. Soc.* 2022, 144, 80. 80

生物催化氮宾C-H插入法合成 α -氨基酸酯



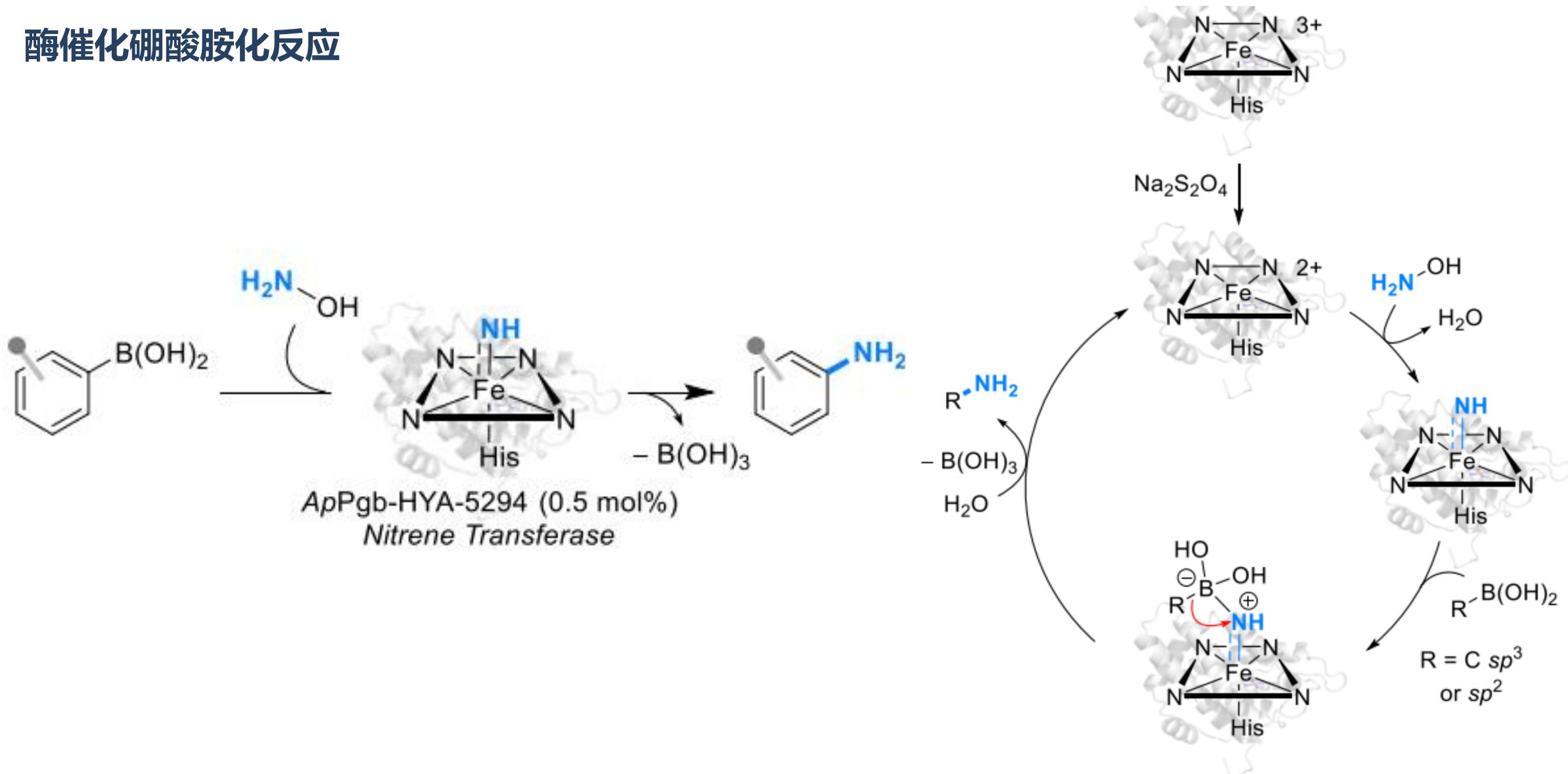
Arnold, F. et al., *J. Am. Chem. Soc.* **2024**, 146, 27267.

生物催化实现三级C-H键的高对映选择性伯胺化

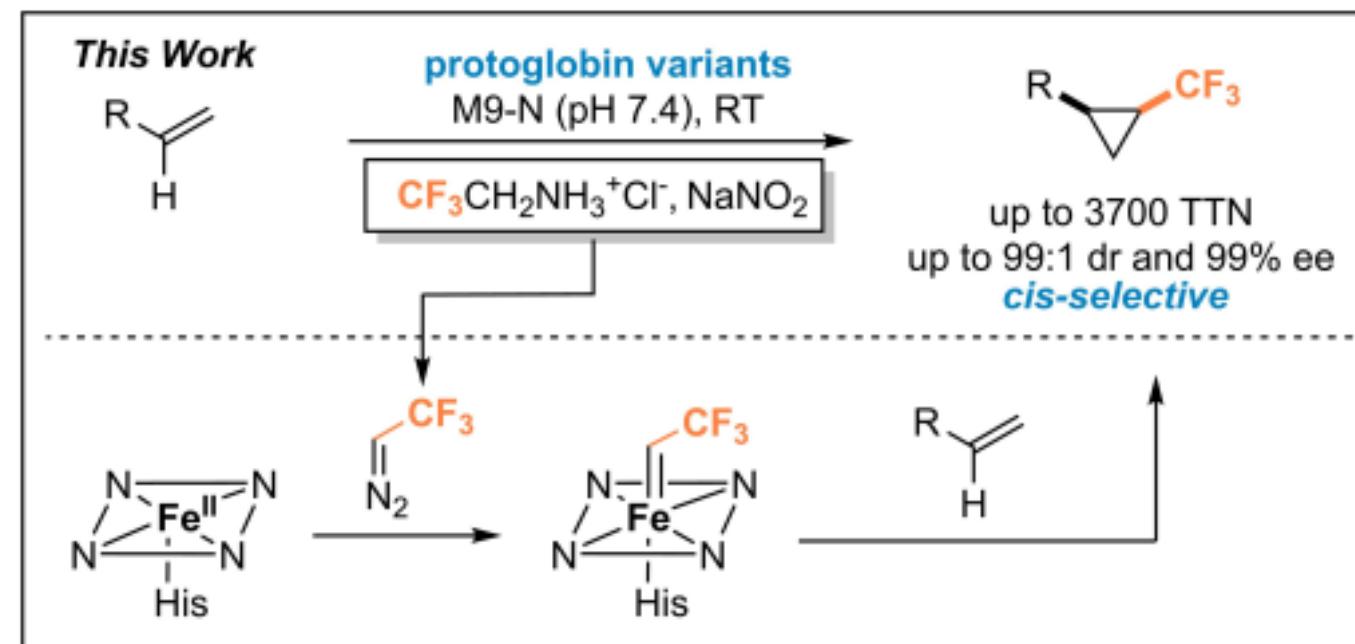
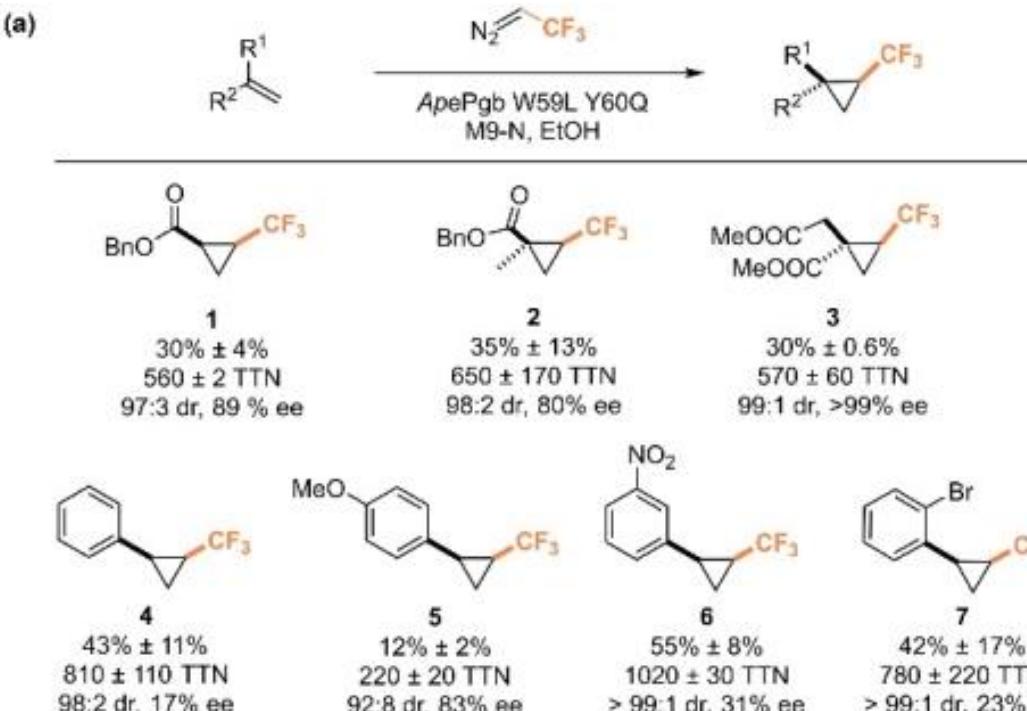


Arnold, F et al., *Nat. Catal.* **2024**, 7, 585. 81

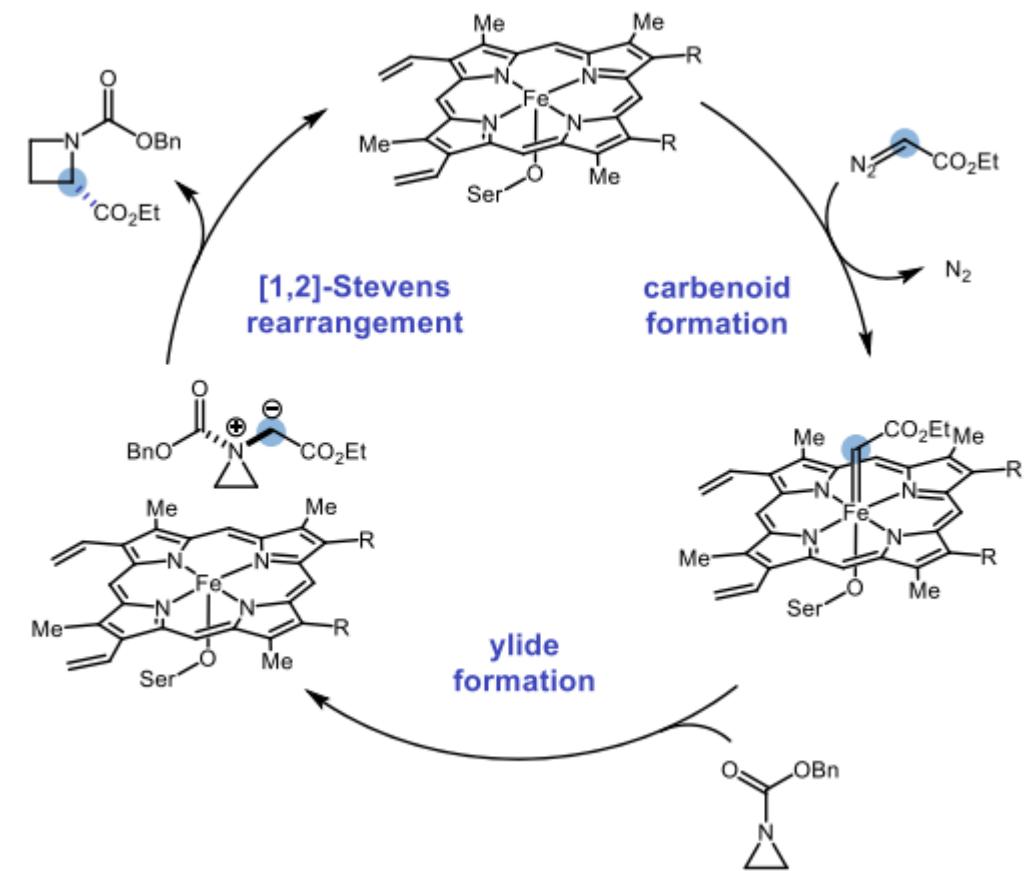
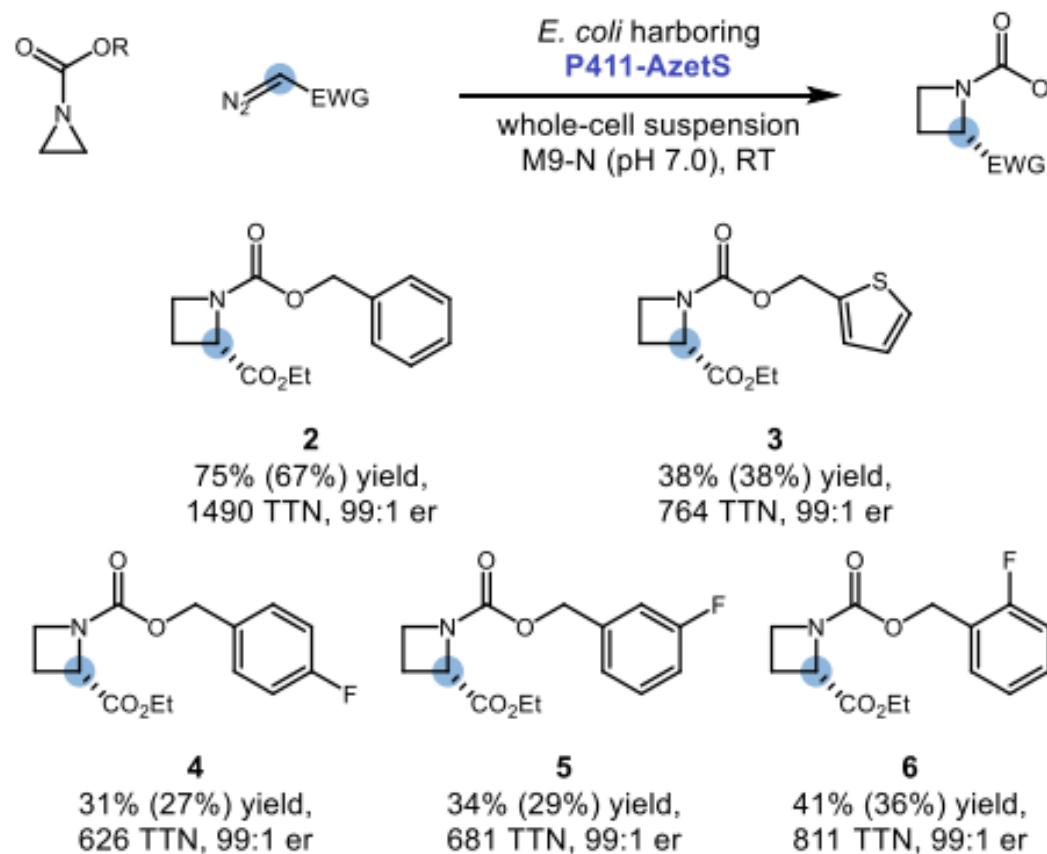
酶催化硼酸胺化反应



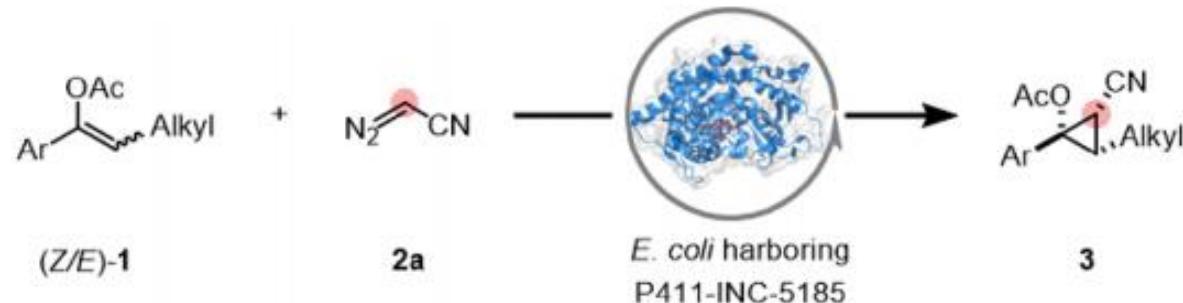
原球蛋白催化卡宾转移生成顺式三氟甲基取代环丙烷



通过高对映选择性1,2-Stevens重排实现氮丙啶到氮杂环丁烷的生物催化单碳扩环

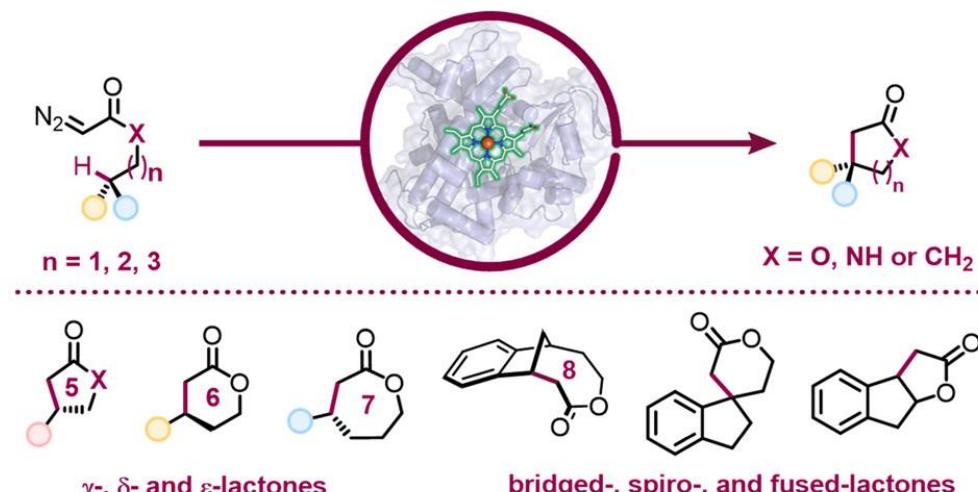


从(Z/E)-异构体混合的三取代烯醇乙酸酯出发，高选择性地合成具有对映和双对映富集的1,2,3-多取代环丙烷



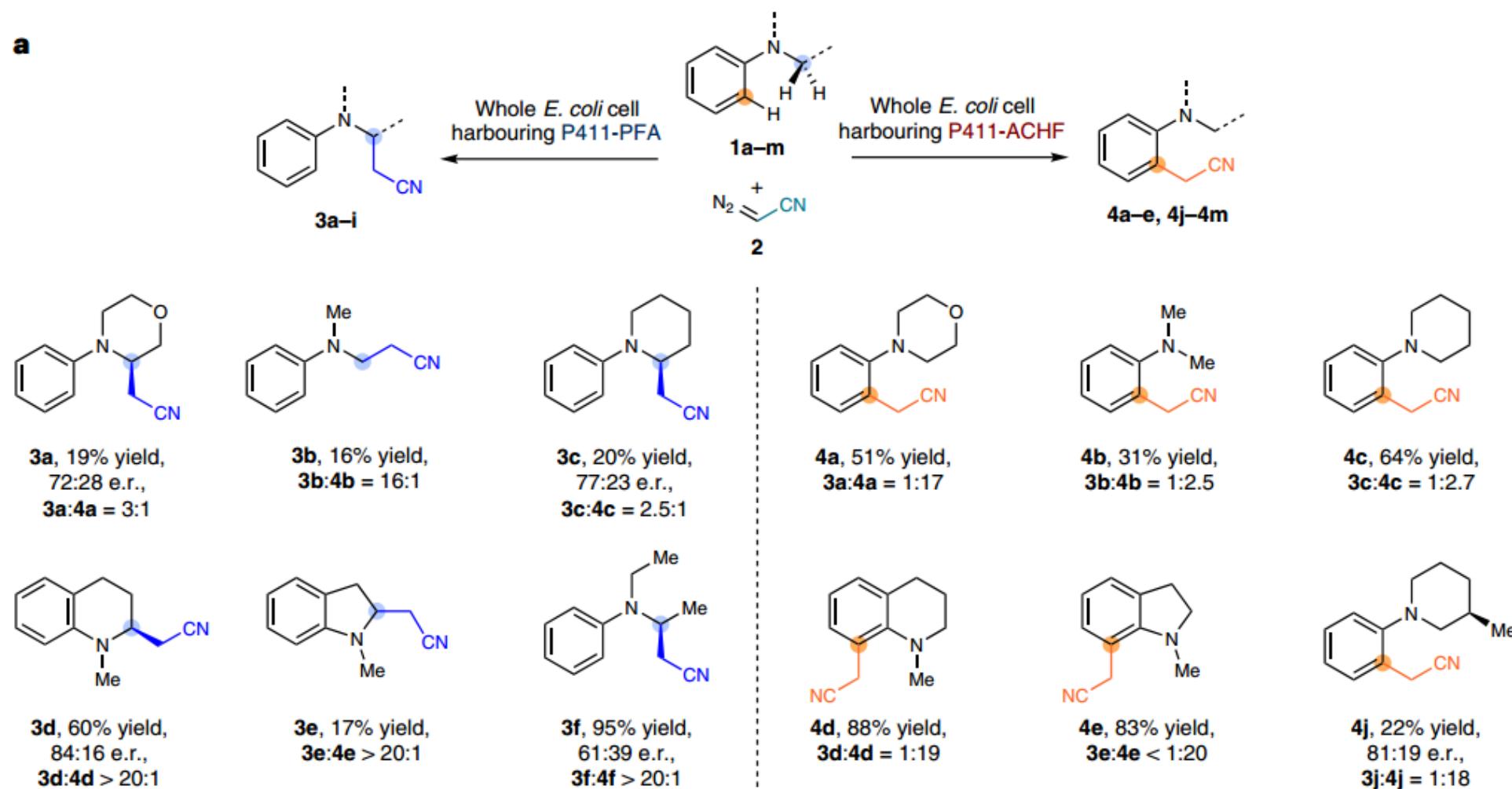
Arnold, F et al., *J. Am. Chem. Soc.* **2023**, *145*, 16176.

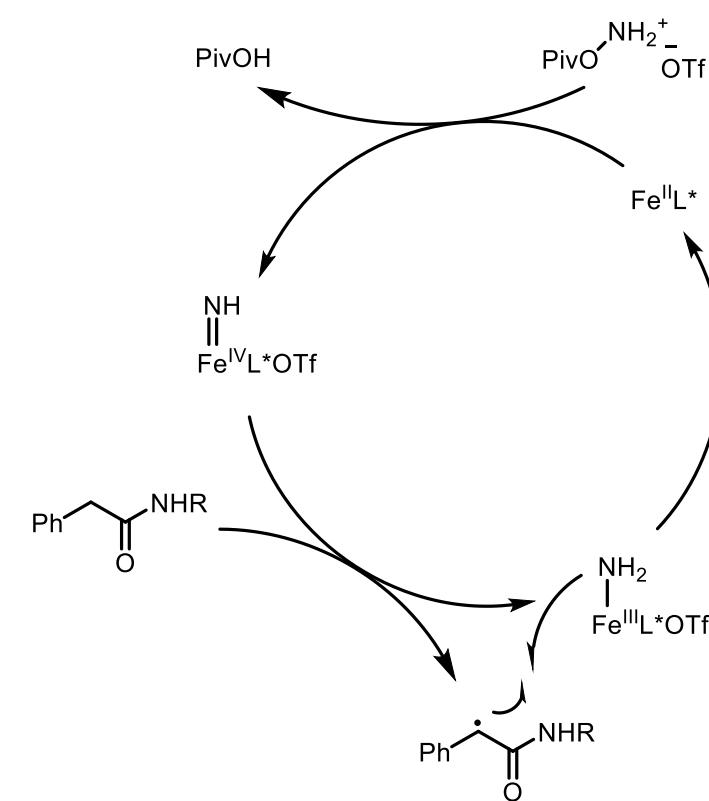
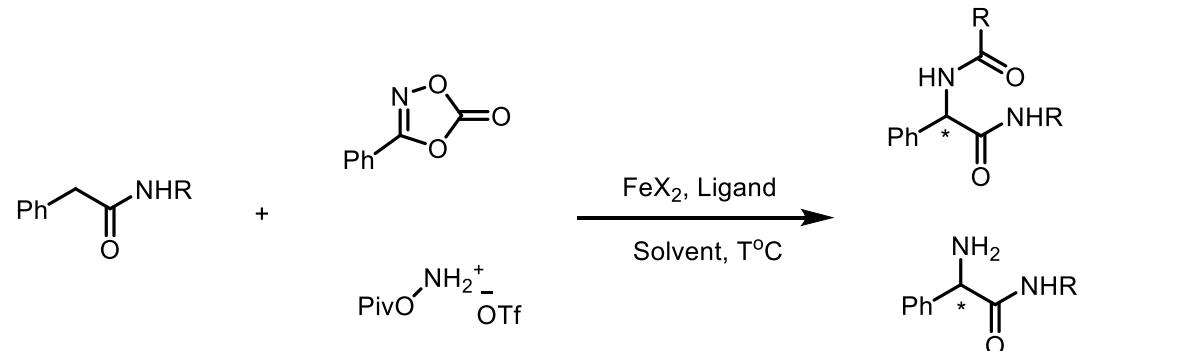
酶催化分子内C-H键环化构建种类多样的内酯化合物



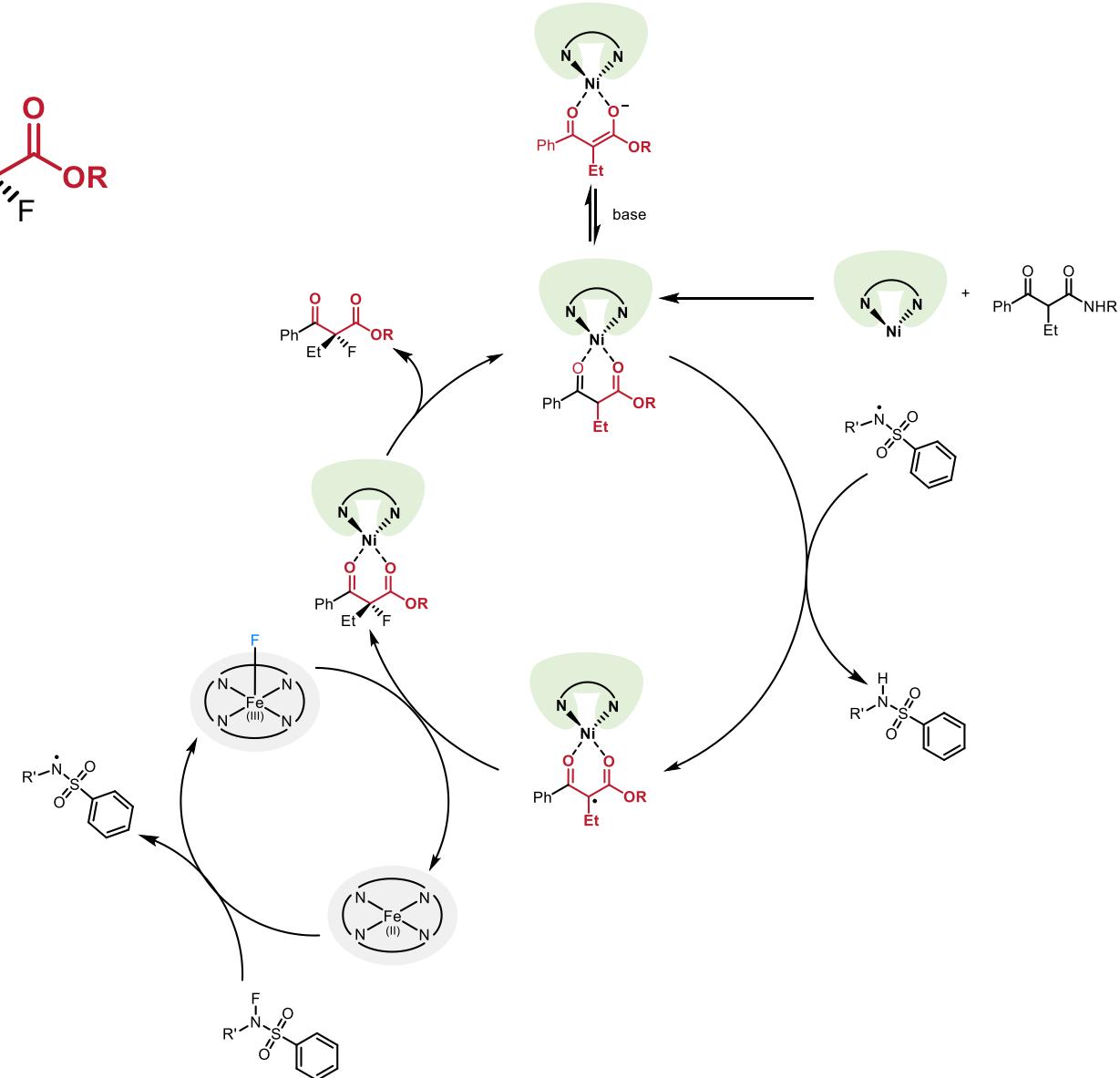
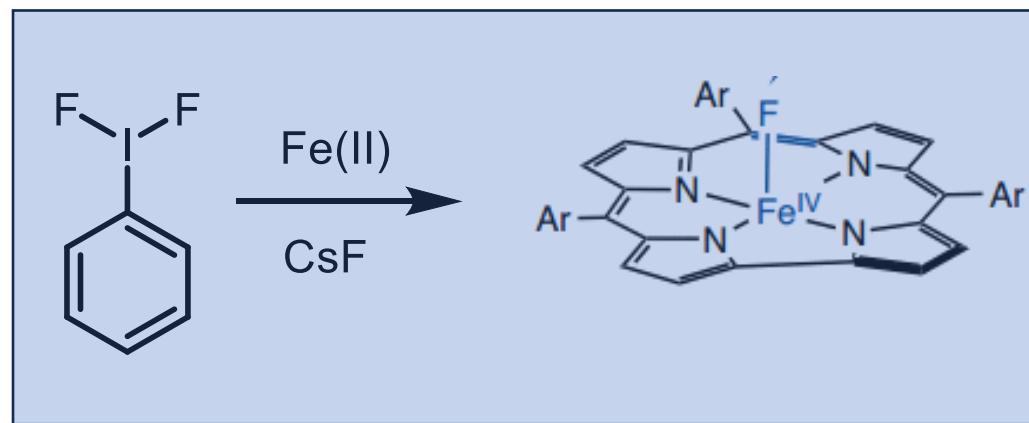
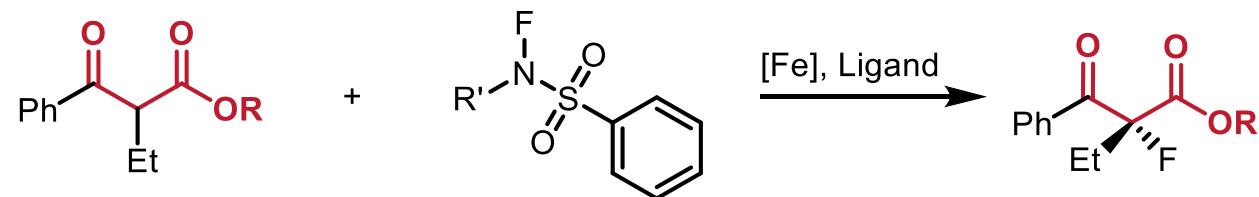
Arnold, F et al., *J. Am. Chem. Soc.* **2024**, *146*, 1580.

使用工程化卡宾转移酶实现化学发散的 C(sp³)–H 和 C(sp²)–H 氰甲基化





第五部分:Proposal C-F键的不对称构建



Thanks