Nature-inspired radical pyridoxal-mediated C–C bond formation

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ABSTRACT: Pyridoxal-5'-phosphate (PLP) and derivatives of this cofactor enable a plethora of reactions in both enzyme-mediated and free-in-solution transformations. With few exceptions in each category, such chemistry has predominantly involved two-electron processes. This sometimes poses a significant challenge for using PLP to build tetrasubstituted carbon centers, especially when the reaction is reversible. The ability to access radical pathways is paramount to broadening the scope of reactions catalyzed by this coenzyme. In this study, we demonstrate the ability to access a radical PLP-based intermediate and engage this radical intermediate in a number of C–C bond forming reactions. By selecting an appropriate oxidant, single-electron oxidation of the quinonoid intermediate can be achieved, which can subsequently be applied to C–C bond forming reactions. Through this radical reaction pathway, we synthesized a series of α-tertiary amino acids and esters to investigate the substrate scope and identify non-productive reaction pathways. Beyond the amino acid model system, we demonstrate that other classes of amine substrates can be applied in this reaction and that a range of small molecule reagents can serve as a coupling partner to the semiquinone radical. We anticipate that this versatile semiquinone radical species will be central to the development of a range of novel reactions.

Pyridoxal-5'-phosphate (PLP) is a highly versatile cofactor, both in the diversity of reactions mediated and the protein scaffolds that accommodate this cofactor.[1-5] Through the formation of a covalent intermediate with amine substrates, commonly amino acids, a key quinonoid intermediate can be formed (see **1**, Figure 1A).[1] Depending on its protonation state, this intermediate can exhibit either nucleophilic or electrophilic reactivity at various positions through two-electron processes. As a result, PLP-dependent enzymes catalyze a range of reactions, including transamination, Claisen condensation, Aldol and Mannich reactions, β-elimination, β-substitutions, γ-substitutions, decarboxylation, and more.[1, 6] This platform for various reactions has attracted significant attention from chemists. Primarily focusing on functionalization α to the amino group via nucleophilic attack pathways, organocatalysis with PLP and its derivatives offers a diverse array of reactions, including transamination, Aldol and Mannich reactions, Michael addition, and allylation reactions among others (Figure 1A).[2-5]

However, developing PLP-dependent chemistry to address challenging bond formations, such as the construction of tetrasubstituted carbon at the α-position of amino acids (Figure 1C, **ATAA)**, presents a significant challenge.[6-14] Two major hurdles toward achieving this transformation are the limited range of electrophilic reagents available for α-functionalization[15] and the reversibility of many PLP-mediated reactions,[16] which hampers the formation of sterically encumbered bonds (Figure 1C). Additionally, the diversity of amino acid side chains bring high demands for functional group tolerance. To develop reactions that avoid these problems and expand the chemistry accessible through PLP-mediated reactions, it is important to consider alternative approaches.

Toward this goal, one strategy involves increasing the energy of reactants to inhibit the reversibility of a reaction. For example, radical reactions, in particular radical–radical coupling reactions, can be a productive approach for building sterically hindered bonds.[17-18] In addition, the orthogonal functional group compatibility to two-electron processes also render radical reactions an attractive alternative approach. The development of radical reactions has relied on various approaches[19-28] to control selectivity including transition metal catalysis,[29-35] organocatalysis,[36-44] and enzyme catalysis.[45-50] These approaches, combined with the high reactivity of radicals, have enabled the construction of challenging chemical bonds with high selectivity. However, radical reactions involving PLP, either in nature or organic catalysis, remain largely unexplored until very recently.[6, 51-53] One classic example is lysine-2,3-aminomutase which facilitates a 1,2-amino shift reaction with [4Fe4S] cluster, SAM and PLP cofactor work together.[6, 51-52] Recently, Liu, Yang and coworkers reported an elegant enzymatic method which involves a radical pathway.[53] This method allows for the β-functionalization of α-amino acid substrates bearing a β-hydroxyl group, which can form a α,β-unsaturated intermediate (see **2**). This PLP-bound intermediate can engage with a non-enzymatically generated radical, such as a benzyl radical, to form a C–C bond at the β-position of the amino acid substrate.[54] In contrast to this method which relies on radical addition to a closed-shell PLP-bound intermediate, nature has devised an approach for generation of a PLP radical intermediate (see **3**). A class of PLP enzymes discovered by the Silvaggi and Ryan groups use molecular oxygen to oxidize the quinonoid intermediate (**1**) to a radical intermediate **3** (Figure 1B).[55-59] However, the rapid combination of **3** and superoxide renders the resulting α-radical difficult to capture in unnatural C–C bond forming reactions (Figure 1B). Therefore, exploring ways to access radical PLP intermediates which can be engaged in subsequent bond formation is a ripe area for development.

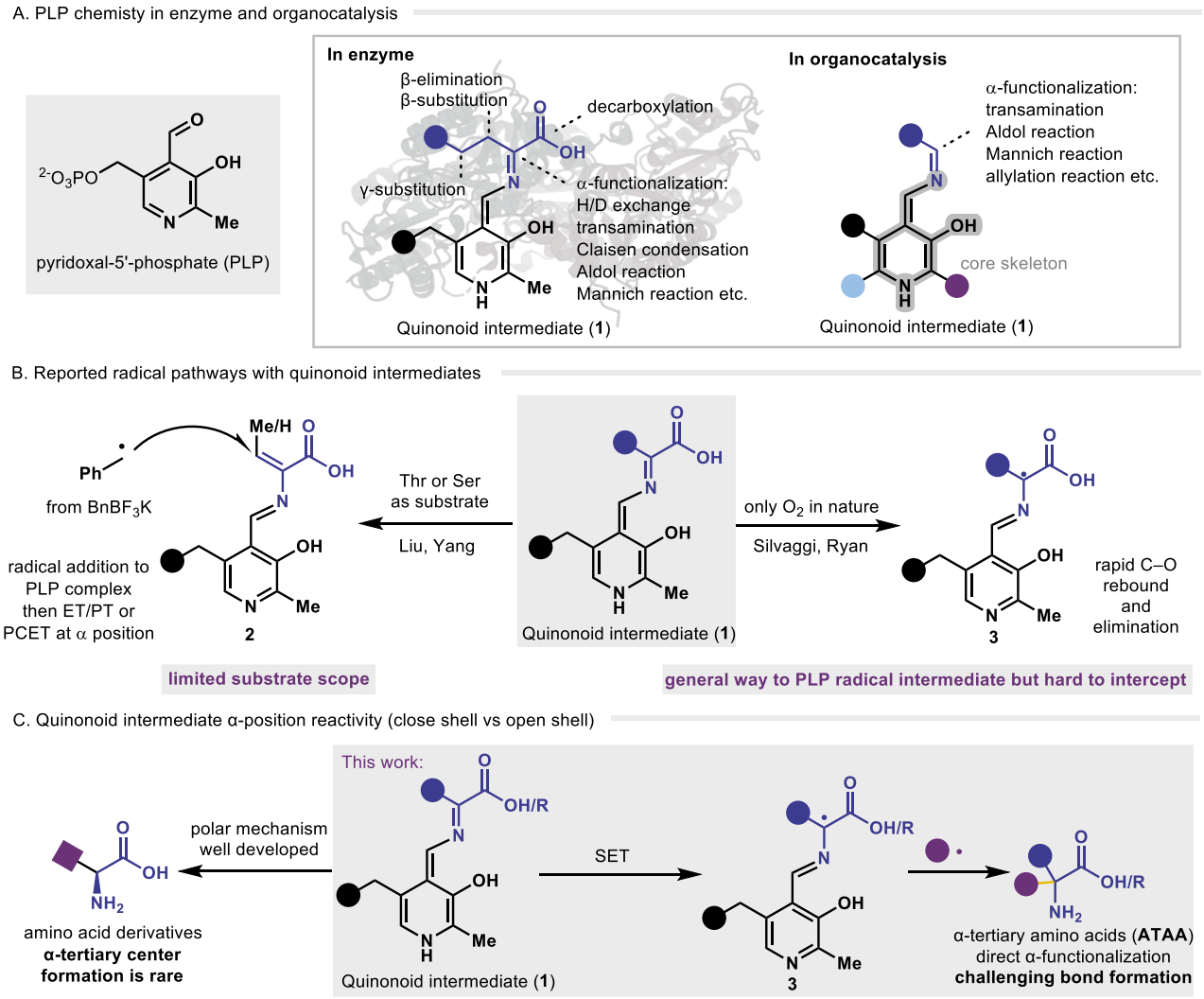
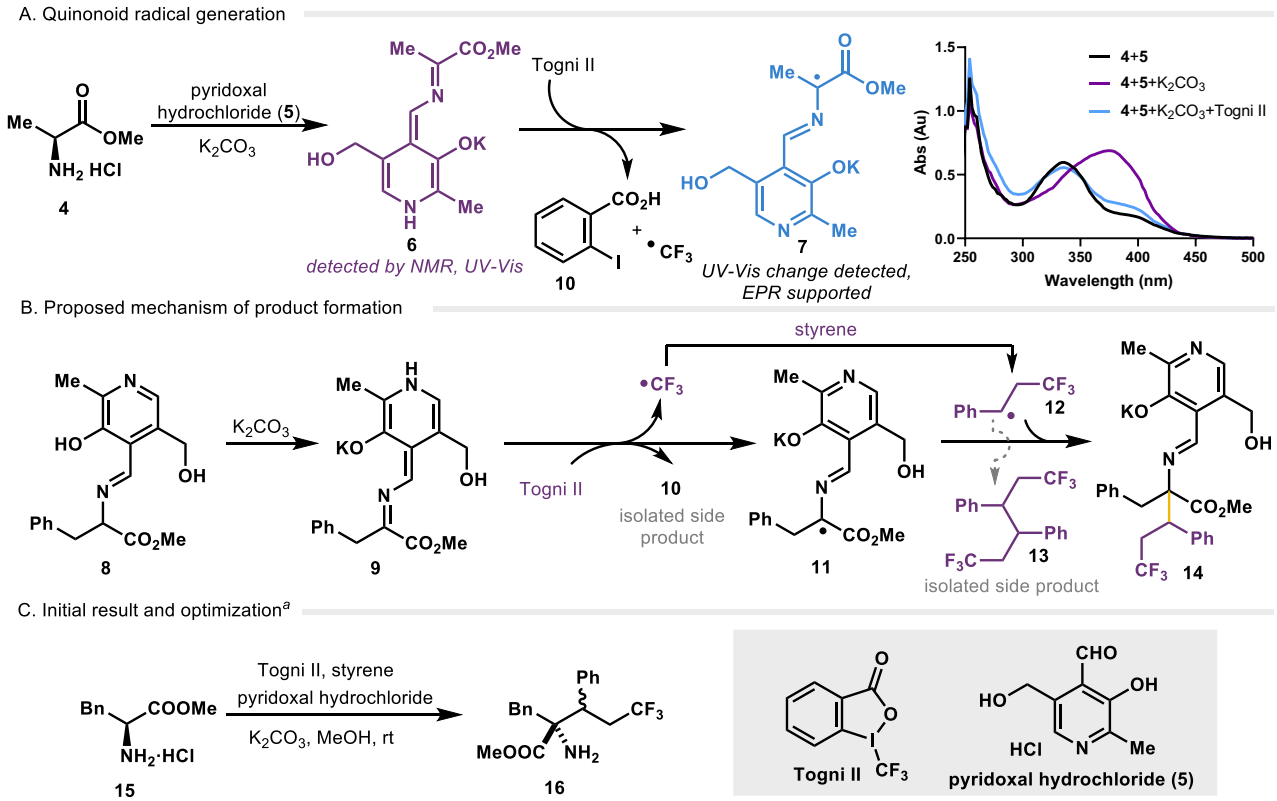


Figure 1. PLP chemistry in a radical pathway background and synthetic application. A. Different reaction types of PLP chemistry in enzymatic systems and organocatalysis. B. Reported examples of PLP radical chemistry and their limitations. C. Reactivity of the quinonoid intermediate α-position and development of PLP radical pathway for C–C coupling reactions.

The first key question in achieving a platform for PLP-based radical chemistry lies in devising approaches for single-electron oxidation of the quinonoid intermediate (**1**). Typically, this intermediate acts as a nucleophilic species.[1, 6] From the perspective of organic chemistry, a one-electron oxidation should also be feasible from this electron-rich intermediate. By introducing a suitable oxidizing agent, we envisioned generating a radical such as **3**, which would be capable of participating in subsequent radical reactions. However, this hypothesis is based on some fundamental assumptions. For example, in the case of amino acid substrates, the quinonoid intermediate should be more easily oxidized compared to amino, carboxyl and side chain functional groups. As O_2 has been reported to be a successful oxidant,[55-59] this finding suggests the theoretical feasibility of oxidizing the quinonoid intermediate, by judicious selection of oxidizing agents. Herein, we report the initial validation for the generation and reactivity of semiquinone radical intermediate through the use of an exogenous oxidant and explore the potential of this intermediate in the construction of C–C bonds (Figure 1C).

Considering the complexity of developing the envisioned transformation in an enzymatic system, we first attempted forming the quinonoid intermediate with free pyridoxal cofactor in solution. We first sought conditions for quinonoid formation using L-Ala-OMe hydrochloride (**4**) as a model substrate. When commercially available pyridoxal hydrochloride (**5**) and **4** were mixed in methanol-d_4, the characteristic signals of both compounds remained unchanged, indicating no reaction had occurred. However, upon addition of potassium carbonate, the characteristic doublet-to-singlet transformation of the alanine methyl group was observed by ^{1}H NMR within five minutes, supporting the formation of a quinonoid intermediate (**6**, Fig. 2A and Supporting Figs. S2-S3), which was further confirmed by UV-Vis spectroscopy (Fig. 2A, see new peak at 375 nm). When various oxidizing agents were added to mixtures of pyridoxal and L-Ala-OMe in the presence of K_2CO_3, we found that Togni II can efficiently oxidize **6** to a semiquinone radical intermediate (**7**), liberating a trifluoromethyl radical. Based on rapid screening using UV-Vis spectroscopy, peak at 375 nm corresponding to quinonoid decreased (light blue spectrum, Fig. 2A). The single electron transfer process was further supported by electron paramagnetic resonance (EPR) spectroscopic experiments. A radical signal was trapped by mixing **6** with togni II (without styrene) in 1:1 methanol:



C. Initial result and optimization[a]

entry	pyridoxal hydrochloride (equiv)	styrene (equiv)	yield (%)[b]	dr
1	0.2	0.75	23	1:1
2	1.0	0.75	38	1:1
3	1.0	1.5	49	1:1

Figure 2. Quinonoid radical generation and initial results and proposed mechanism of the subsequent coupling. A. ^{1}H NMR and UV-Vis spectroscopy study of quinonoid radical generation. B. Proposed reaction mechanism of the Togni II and styrene system. C. Initial results and brief condition optimization of the Togni II and styrene system. [a] Reaction conditions: 0.1 mmol of substrate **15**, 0.15 mmol of Togni II, 0.02-0.1 mmol of **5**, 0.075-0.15 mmol of styrene, 0.25 mmol of K_2CO_3 in MeOH (2.0 mL), 25 °C, 24h. [b] yield was determined ^{19}F NMR with $PhCF_3$ as internal standard.

water at low temperatures (90 K), frozen in liquid nitrogen within 30 sec, and measured by continuous wave X-band EPR. Due to the expected high reactivity nature of the CF_3 radical and the lack of any ^{19}F (I=1/2) hyperfine splittings (expected to be >100G),[60] the radical is assigned as the structure represented by **7** (see Supporting Fig. S4)

With this initial evidence for the semiquinone radical generation, we anticipated that the presumed radical intermediate could be intercepted in a C–C bond forming event. For ease of monitoring the reaction by UV-Vis detection, we chose to use L-Phe-OMe as the standard substrate. We envisioned that the trifluoromethyl radical generated during the oxidation could be trapped by styrene to afford a stabilized radical **12**, which could serve as a suitable coupling partner for **11** (Fig. 2B).[61] When styrene, L-Phe-OMe hydrochloride (**15**), Togni II, potassium carbonate, and 20 mol % pyridoxal hydrochloride (**5**) were combined in methanol, **16**(X-ray structure of one compound **16** isomer supported**)** was detected in 23% yield and 1:1 dr by ^{19}F NMR spectroscopy. After further optimization, product **16** could be obtained in a 49% NMR yield in the same 1:1 diastereomeric ratio (Fig. 2C, see Supporting Table S1 for further details). In these initial experiments, we observed that the reaction yield increased with an increase in the amount of pyridoxal hydrochloride, suggesting that turnover of the pyridoxal-bound product **14** could be a limitation in this reaction. This problem is likely specific to the non-enzymatic reaction, as the product can be displaced in an analogous enzyme-mediated reaction by the conserved lysine residue, which can reform the internal aldimine, formally turning over the catalyst.[62] This finding aligns with previous observations in reactions involving free pyridoxal derivative catalysis.[63]

Interestingly, even with the use of stoichiometric pyridoxal hydrochloride, the reaction yield plateaued at 49%, although the yield should theoretically approach 100% (Fig. 2C, entry 3). This result prompted us to investigate the mass balance of this reaction to gain a better understanding of potential unproductive pathways. Toward this end, we designed a reaction using 2,6-dibromo-L-Tyr-OMe (**17**). By leveraging the unique isotope ratio of bromine, we could identify which products originated from the brominated amino acid substrate. Using mass spectrometry analysis, we found that, apart from excess Togni II reagent, the main side product did not contain the brominated substrate but was derived from pyridoxal (**5**). We identified **19** the main side product of the reaction. Additionally, we detected trace amounts of direct trifluoromethylation of the aromatic ring of **17**, which could arise through precedented mechanisms.[64-65]

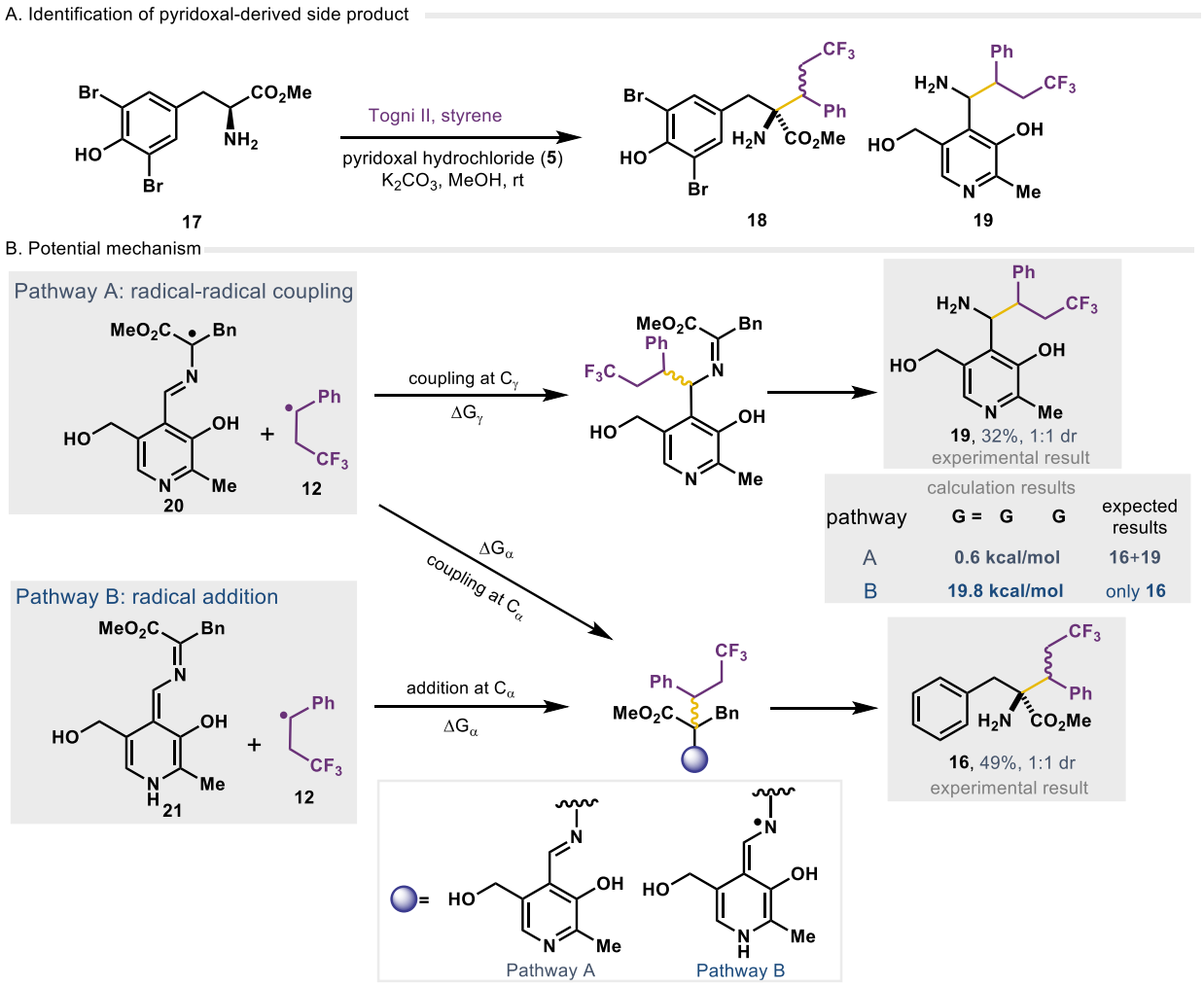


Figure 3. Identification of second product formed and computational investigation of two potential mechanisms. A. Identification of the pyridoxal-derived side product **19**. B. Computational investigation of two potential mechanisms and a comparison of the experimental amounts of **16** and **19** compared to the ratio of **16** and **19** predicted to be produced through pathway A and pathway B. For pathway B, possibility of addition at Cγ is not shown since it is highly disfavored compared to the addition at Cα.

The formation of **19** along with the anticipated product **16** led us to reconsider the mechanistic possibilities for the reaction. In particular, we used quantum chemical simulations to provide insight into the observed yields of **16** (49% NMR yield) and **19** (32% NMR yield). A radical-radical coupling pathway (Pathway **A** , see Fig. 3B) starting from **20** and radical addition to the quinonoid intermediate **21** (Pathway **B,** Fig. 3B) were simulated. DFT calculations suggest that pathway **A** should give rise to an almost equal distribution of products as the coupling to the α-position and γ-position have similar reaction energies (ΔΔG = 0.6 kcal/mol). Additionally, the spin density distribution of radical **20** (see Fig. S6) at the α-position (approximately 48%) and γ-position (approximately 36%) suggest the two centers would be favorable points for bond making. On the contrary, pathway **B** has very different reaction energies (ΔΔG = 19.8 kcal/mol) for the same two possibilities. This can be traced to higher delocalization of the radical character in one product compared to other (see Fig. S7). With this understanding, we sought to explore the compatibility of substrates and other potential coupling reactions of this semiquinone radical intermediate.

Using the optimized reaction conditions, we found that a plethora of functional groups were tolerated in this transformation. Specifically, the majority of amino esters tested were tolerated, affording product in yields up to 52% and diastereomeric ratios ranging from 1:1 to 1.5:1 (Figure 5). However, when the β-position of the substrate was sterically hindered, such as L-Ile-OMe or L-Thr-OMe, the reaction yield decreased significantly, with only trace amounts of product (see **30** and **31**) detectable by LCMS suggesting that this radical-radical coupling reaction is sensitive to the steric properties of the β-position. Additionally, amino esters with side chains containing carboxylic acid or amide groups, such as L-Asp-OMe, L-Asn-OMe, L-Glu-OMe, and L-Gln-OMe, were not compatible with the reaction, likely due to the presence of additional acidic hydrogens that could hinder the formation or rapid quenching of the quinonoid intermediate. This hypothesis is supported as diesters (see **44** and **45**), provided a higher yield of product compared to the corresponding acids (**36** and **37**). Additionally, when L-Glu dimethyl ester was used as a substrate, an intriguing cyclic pyroglutamate product (**46**) was isolated. Notably, electron- rich aromatic side chains led to the formation of the Pictet–Spengler product after α-functionalization (see **42** and **43**).

Furthermore, we observed that substrates containing thiol groups, such as cysteine, did not undergo the desired reaction,

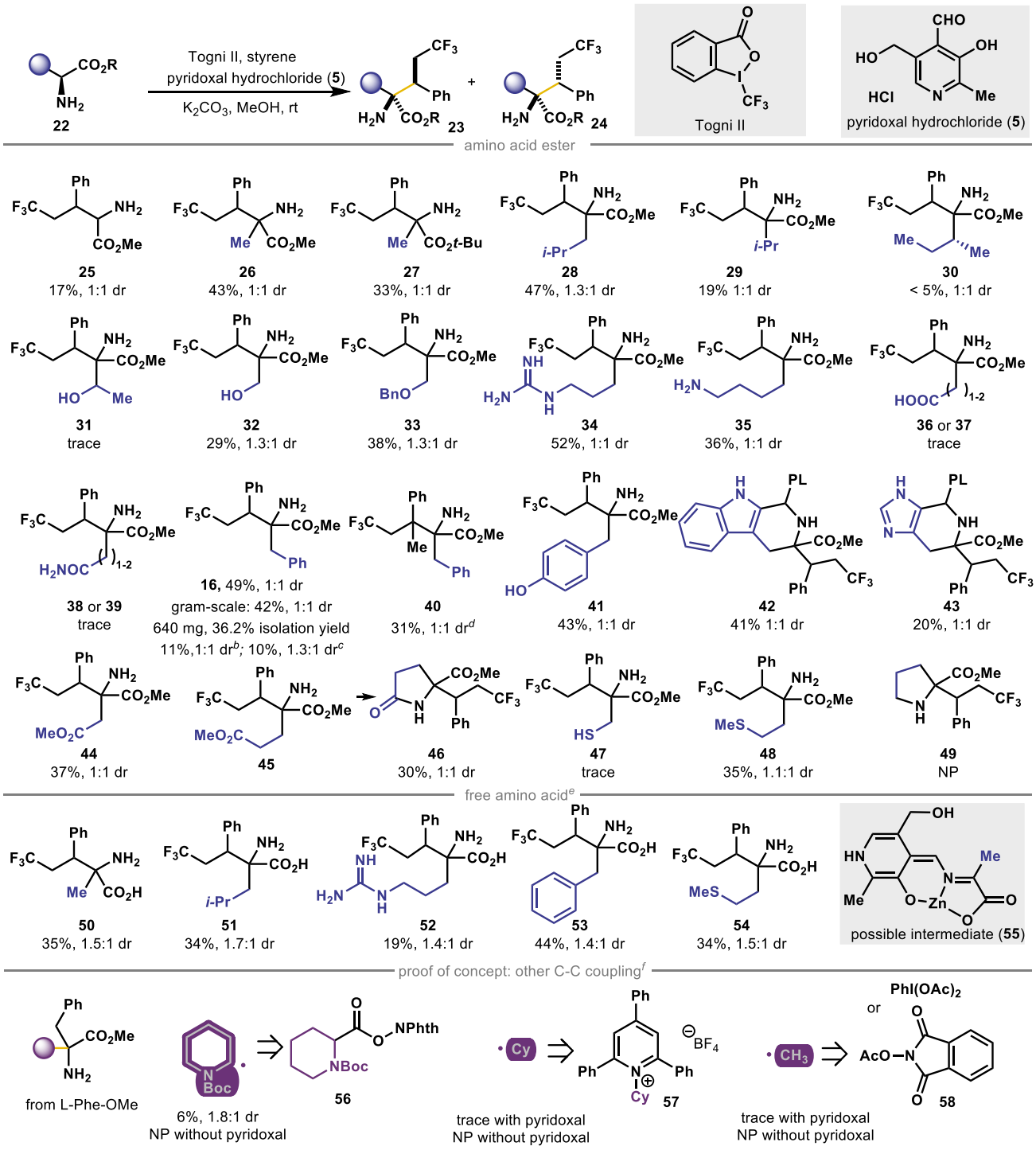


Figure 4. Substrate scope and other potential radical coupling partners.[a] [a]Reaction conditions: 0.5 mmol of substrate, 0.75 mmol of Togni II, 0.5 mmol of **5**, 0.75 mmol of styrene, 1.25 mmol of K_2CO_3, 0.5 mmol of $ZnCl_2$ (only when amino acid used as substrate) in MeOH (10.0 mL), 25 °C. yield was determined by ^{19}F NMR or ^1H NMR with $PhCF_3$ and CH_2Br_2 as internal standard, respectively. [b]Reaction with Umemoto reagent. [c]Reaction with *t*-BuOOH and Langlois reagent. [d]50 °C work up after add 1N HCl [e]$ZnCl_2$ (1.0 equiv) was added. [f]Reaction conditions: 0.5 mmol of L-Phe-OMe hydrochloride, 0.75 mmol of "R·" precursor reagent (**56-58**), 0.5 mmol of **5**, 1.25 mmol of K_2CO_3 in DMSO(10.0 mL), 25 °C.

which was expected due to their common role as hydrogen atom transfer reagents that can quench radical intermediates[66] or directly reacted with Togni II reagent.[67] The successful reaction of methionine supports this hypothesis, which afforded product **48** in 35% yield, further expanding the functional group tolerance to include thioethers. In addition, α-methylstyrene was tested in place of styrene to afford a 31% yield of product **40,** which further support this method as productive approach for building sterically hindered bonds. Overall, the substrate scope investigation suggests that once the quinonoid intermediate can be formed, this radical pathway is useful in building sterically

congested bonds such as α-tertiary C–C bonds while tolerating a range of functional groups present in natural amino acids.

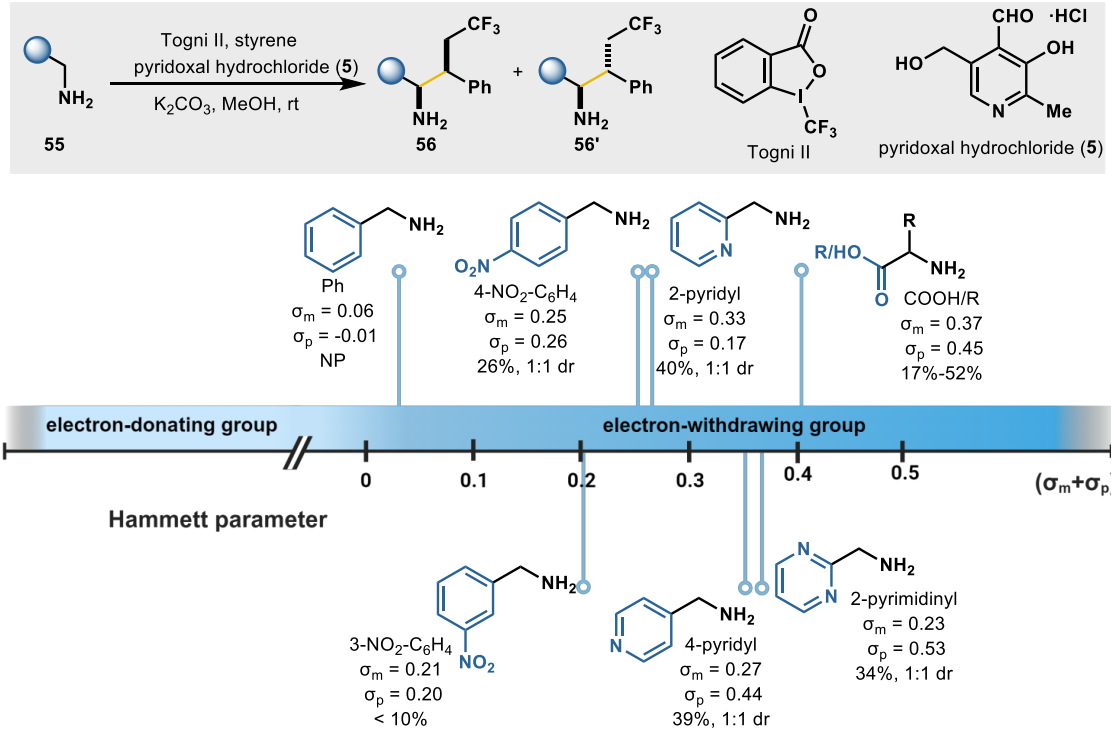


Figure 5. Evaluation of amine substrates containing different electron-withdrawing substituents.

Using L-Phe-OMe as the substrate, a gram-scale reaction was conducted to afford **16** in a 42% NMR yield and 36% isolated yield (640 mg, 1:1 dr). Additional experiments with the same substrate, revealed that two alternative CF_3 radical precursors were compatible in this reaction (Figure 4, product **16**). For example, the Umemoto reagent[68] afforded the product in the same diastereomeric ratio and lower yield. Similarly, the use of *t*-BuOOH and Langlois reagent[69] in DMSO, a 10% yield was observed. However, compared to reactions with Togni II, the reaction with *t*-BuOOH and Langlois reagent gave an altered diastereomeric ratio (1.3:1), which might suggest a different reaction mechanism. Interestingly, in this system, the product can even be detected when potassium phosphate buffer at pH 8.0 is used without additional base. As a buffer-compatible reaction system, it may be well-suited to be adapted as a biocatalytic reaction upon identification of appropriate PLP enzymes.

Next, we determined if substrates with a free carboxylate group could also be tolerated. Upon reviewing the literature, we were encouraged by reports showing that the addition of Zn^{2+} could effectively mimic enzyme-catalyzed systems, mitigating the influence of acidic hydrogens on quinonoid intermediate formation.[70-71] Gratifyingly, adding one equivalent of $ZnCl_2$ to reactions with amino acid substrates led to productive product formation. On this basis, we examined several canonical amino acids, including those with alkyl side chains (L-Ala, l-Leu), alkaline side chains (L-Arg), aromatic side chains (L-Phe), and heteroatom-containing side chains (L-Met), all of which were compatible (**50**-**54**,19%-44% yield, 1.4-1.7:1 dr). Interestingly, the addition of Zn^{2+} resulted in a change of diastereoselectivity from the typical 1:1 ratio observed for amino ester substrates. This is likely related to the formation of a more conformationally rigid metal complex upon coordination of the substrate with Zn^{2+} ions (see **55**), thereby altering the selectivity of the subsequent radical coupling step.

Next, we investigated other carbon radical sources to engage in the coupling with semiquinone radical **11**. During our investigation of various oxidizing agents, we discovered that other carbon radical precursors were also compatible. For instance, we found that the nitrogen α-radical formed form *N*-hydroxyphthalimide (NHPI)[72] reagent **56** successfully coupled with the semiquinone radical to afford the target product in a 6% NMR yield with 1.8:1 dr. More interestingly, the cyclohexyl radical derived from Katritzky salt **57**[73] and methyl radicals derived from NHPI reagent **58** or $PhI(OAc)_2$ were all compatible. Although their reactivity was much lower compared to the optimized Togni II system, LC-MS comparisons with synthetic standards confirmed the generation of the anticipated products. Importantly, in all three cases, product formation was dependent on the inclusion of pyridoxal in the reaction, supporting the role of the pyridoxal radical intermediate in these C–C bond forming reactions.

Finally, we asked the question if this transformation could be applied to other classes of amine substrates. After testing several amine substrates shown in Figure 5 we found a striking correlation between the Hammett parameter of the substrate substituent and reaction outcome.[74] When the σ-value of the substituent was greater than 0.2, the reaction proceeded, generally affording the product in up to 52% yield. The higher σ-value correlates with the electron-withdrawing ability of the substituent. This matches with the lower p*K*a of the α-C–H of the amine substrates, facilitating the formation of the quinonoid intermediate. Further Hammett analysis found the the ρ-value is 2.667, which indicates that the rate determine step is possibly the first quinonoid formation step (see Fig. S5). With a $\rho > 1$, this

suggests that the substituent with varying electronic properites is directly attached to the carbon undergoing deprotonation. This significantly broadens the applicability of this radical chemistry, both in enzymatic system and organocatalysis system and allows for the prediction of substrate feasibility.

In summary, our research focused on the discovery of a new radical pathway in PLP-mediated chemistry. We explored the potential of semiquinone radical intermediates derived from amine substrates and PLP in radical C–C coupling reactions. Using various oxidizing agents and reagents, we identified that the Togni II reagent and styrene system give direct α-C–H functionalization of α-amino ester substrates. With the addition of zinc chloride, we further expanded the substrate scope, rendering this reaction compatible with free amino acids. Further, it was demonstrated that other primary amines substrates, which contained a sufficiently strong electron-withdrawing groups ($\sigma > 0.2$), are also compatible with this chemistry. Other carbon radical precursors, such as methyl radicals, were also shown to be compatible with this system, further demonstrating the potential utility of this strategy. In addition, an alternative set of oxidants enabled the reaction under aqueous buffered conditions, setting the stage for the development of biocatalytic versions of the reactions described herein with the opportunity to control stereoselectivity, minimize byproduct formation, and achieve superior chemoselectivity.

ASSOCIATED CONTENT

The Supporting Information is available free of charge via the Internet at http://pubs.acs.org.
Experimental procedures, characterization of products, and NMR spectra (PDF)
Crystallographic information file for one compound **16** isomer, CCDC 2345376 (XLSX)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

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ABBREVIATIONS

PLP, Pyridoxal-5'-phosphate; ATAA, α-tertiary amino acid; NHPI, *N*-hydroxyphthalimide

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15. Electrophilic reagents need to be no backgroud reaction with amine and carboxyl group.

16. Most of PLP-mediated α-functionalization reactions such as transamination, racemization (protonation), Adol reaction, Mannich reaction, and Claisen condensation are often considered reversible.

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