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Facile one-pot synthesis of tetrahydroisoquinolines from amino acids via hypochlorite-mediated decarboxylation and Pictet–Spengler condensation

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ABSTRACT

A convenient method for oxidative decarboxylation of α-amino acids is presented. The aldehyde products may be isolated or converted to tetrahydroisoquinolines by addition of dopamine via Pictet–Spengler reaction. Racemic products are generated by phosphate buffer >300 mM to maximize regioselectivity. (*S*)-Enantiomer products are generated by norcoclaurine synthase reaction in maleic acid buffer to minimize chemical background reaction.

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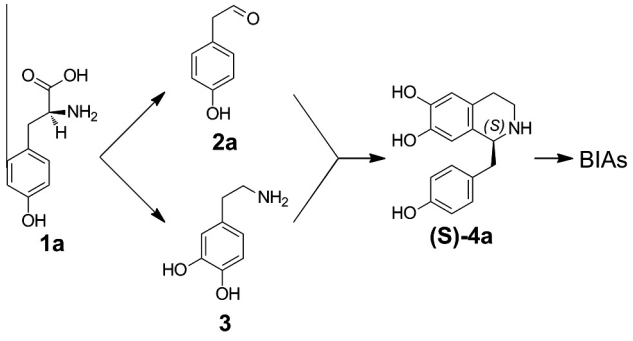
Introduction

The tetrahydroisoquinoline moiety is found in numerous natural products and synthetic compounds with pharmacological activity. In particular, this group is found in the benzoisoquinoline alkaloids (BIAs) produced by 20% of all flowering plants.[1] Biosynthetically, BIAs are derived from the conversion of tyrosine (**1a**) to 4-hydroxyphenyl-acetaldehyde (4-HPAA, **2a**) and dopamine (**3**). These latter two compounds are condensed to the tetrahydroisoquinoline compound (*S*)-norcoclaurine ((***S***)-**4a**) by a Pictet–Spengler reaction (Scheme 1). (*S*)-Norcoclaurine is further transformed into about 2500 identified BIAs, including the analgesic morphine, the antitussive noscapine, the muscle relaxant tubocurarine, the antioxidant magnoflorine, and the vasodilator papaverine.[1]

Here we report optimized conditions for the generation of halogenated 4-HPAA and norcoclaurine from amino acids in aqueous media. This biomimetic process uses low toxicity regents and solvent to produce 4-HPAA analogs and halogenated alkaloid intermediates from amino acids. We also present conditions for enantioselective enzymatic synthesis of halogenated (*S*)-norcoclaurine analogs.



Scheme 1. Biosynthesis of the BIA common precursor, (*S*)-norcoclaurine (**4a**), from L-tyrosine (**1a**).

Without chromatography, our preparations are free of any chemical species that would be toxic to cells. This is notable, because it is our objective to synthesize novel BIAs with potential pharmacological utility by employing precursor-directed biosynthesis (PDB), the method of generating novel natural products in a producing organism that incorporates a precursor molecule into its biosynthetic pathways.[2,3] Our preparations of 4-HPAA and norcoclaurine analogs are suitable for direct application to PDB studies. Those results will be described elsewhere.

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Results and discussion

Sodium hypochlorite oxidation of tyrosine to 4-HPAA

We initially evaluated multiple approaches to the synthesis of **2a** and halogenated variants **2b–d** (numbering in Table 1). We attempted oxidation of 4-(2-hydroxyethyl)phenol by pyridinium dichromate[4], Dess–Martin periodinane, Swern oxidation, and Parikh–Doering oxidation.[5] All methods gave poor yields and significant side products in our hands.[6] Parikh–Doering oxidation was the only method to give yields >30%, but produced significant side products that must be removed by column chromatography (Supporting Table S1 and Fig. S1).

We next turned our attention to oxidative decarboxylation of α-amino acids. We selected sodium hypochlorite (NaOCl) over chloramine-T, the most common reagent for this reaction,[7] because it is inexpensive and the only byproducts are biologically compatible salts, thus allowing the products to be used directly for biological studies. In humans, the enzyme myeloperoxidase generates hypochlorite ion and is known to transform L-tyrosine (**1a**) into 4-HPAA (**2a**) in the enzyme active site.[8] The same transformation was observed in pH 7.0 phosphate buffer with hypochlorous acid, but not with hypobromous acid.[9]

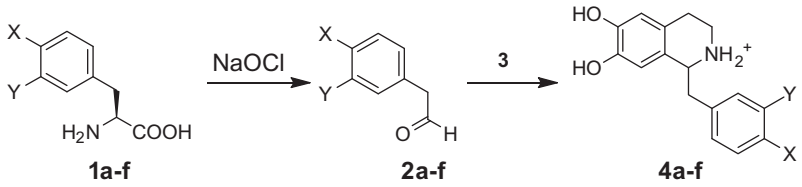
We found no report that describes the general application of NaOCl oxidative decarboxylation in organic synthesis. The reaction is prone to many side reactions such as nitrile formation,[10] aromatic chlorination,[11,12] and formation of unidentified products[13] when pH, concentration, and rate of addition are not controlled. The first high-yield procedure was reported by Langheld[14] who used an apparatus that mixed amino acids with NaOCl at ice-cold temperature and then dropped the reaction into a tube of glass wool with a jet of steam passing through to simultaneously catalyze rapid decomposition of the amino acid while removing the volatile products. The aldehydes were isolated as nitrophenylhydrazone derivatives. Tryptophan and 5-methoxy-tryptophan have been converted into 2-(indol-3-yl)acetaldehyde and 2-(5-methoxy-indol-3-yl)acetaldehyde using a two-phase reaction with benzene, requiring multiple additions of reagents and column chromatography to remove side products.[15,16] Hazen's method[9] of mixing NaOCl and tyrosine at ice bath temperature followed by warming to 37 °C, was used to synthesize 4-HPAA (**2a**) for enzymatic (*S*)-norcoclaurine synthesis.[17] However, the aldehyde product itself was not isolated.

We found that slow addition of one equivalent of NaOCl solution at ⩽0.2 mM of over 5–10 min with vigorous stirring gave clean, quantitative conversion of 1.0 mM tyrosine to 4-HPAA in 10 mM phosphate buffer after 1–2 h at 37–45 °C (see Fig. 1).[18] Cold NaOCl addition[9] had no noticeable effect on the overall reaction. Control of reaction stoichiometry is crucial. Two equivalents of NaOCl yielded significant concentrations of the corresponding nitrile. This side product was identified by matching HPLC-UV, ^{1}H NMR, and GC–MS data to those of authentic 4-hydroxyphenylacetonitrile. Therefore, we recommend measuring the active hypochlorite concentration of the NaOCl solution (see Supplemental material for details).[19] Solid $Ca(OCl)_2$ is not a suitable source of hypochlorite for this reaction as it produced significant side products. The rate of reagent addition is also critical. Rapid addition of NaOCl resulted in a yellow reaction mixture with several unidentified minor side products apparent by HPLC, including the corresponding nitrile. Finally, the initial concentration of tyrosine was also critical for minimization of side-products. Supplemental Figure S2 shows the results of oxidation at six different concentrations of tyrosine ranging from 1.0 to 8.0 mM. We observed that concentrations >4.0 mM yielded significant side products.

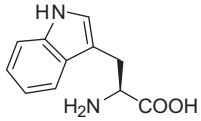
Three intermediates were observed by HPLC-UV during the course of the reaction (Fig. 1). Initial attempts to characterize these species by GC–MS from extraction of the reaction mixture have been inconclusive. All species present in solution have nearly identical UV spectra. Intermediate 1 appears immediately after the addition of NaOCl and maintains the greatest intensity (Supplemental Fig. S3). An iminium intermediate species has been previously isolated from oxidative decarboxylation of α-*N*-diphenylglycine.[20] By analogy, we propose that intermediate 1 is the iminium ion that precedes hydrolysis in Scheme 2.[13] A detailed characterization of this reaction is in progress.

When the observed turnover of tyrosine to 4-HPAA (Fig. S3) was fit to a two-step irreversible kinetic model, the rate constant for reactant disappearance was 16.7 ± 0.6 M^{-1} s^{-1} and rate constant

Table 1



1a-f 2a-f 4a-f

X	Y	Amino acid	Oxidation product	Conversion[a] (isolated yield)	Pictet–Spengler product	Conversion in phosphate[a] (isolated yield)	Relative rate with NCS[c]
OH	H	**1a**	**2a**	>99% (71%)	**4a**	>98% (81%)	100%
OH	Cl	**1b**	**2b**	>99% (83%)	**4b**	>96% (83%)	24 ± 4%
OH	Br	**1c**	**2c**	>99% (82%)	**4c**	>95% (82%)	40 ± 8%
OH	I	**1d**	**2d**	>99% (77%)	**4d**	>95% (70%)	43 ± 4%
H	H	**1e**	**2e**	>99% (61%)	**4e**	>98% (58%)	185 ± 16%
		**1f**	**2f**	61%[b] (N/A)	**4f**	>98% (38%)	N/A

[a] Estimated from HPLC peak area at 225 nm for the product and starting material peaks just before extraction.
[b] For this reaction yield estimate, side product peaks were assumed to have the same extinction coefficient as the product.
[c] Initial rates were measured at 1.0 mM concentration of both the aldehyde and dopamine substrates at 23 °C in 50 mM BES at pH 7.0. The reported rates are referenced to the rate of **2a** reaction. Error represents the standard deviation of three trials.

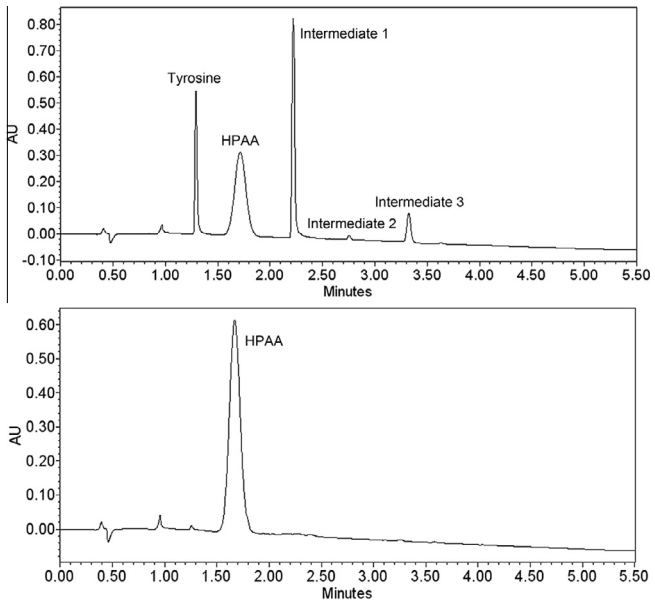
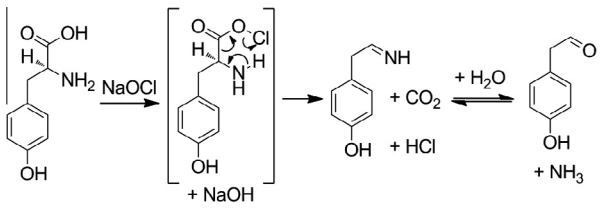


Figure 1. HPLC-UV chromatogram at 225 nm for a reaction of 1.0 mM tyrosine and 1.0 mM NaOCl at 37 °C approximately 9 min (top) and 75 min (bottom) after the start of reaction.



Scheme 2.

for aldehyde appearance was $1.9 \pm 0.5 \times 10^{-3}\ s^{-1}$. This rate for tyrosine disappearance is significantly slower than the reported rate of hypochlorite reaction with amines at pH 7.0 ($k_{obs} = 10^4\ M^{-1}\ s^{-1}$).[21] Therefore, chlorination is not likely to be rate limiting for amino acid disappearance. We propose that either decarboxylation or imine hydrolysis are rate-limiting. Both amino acid disappearance and aldehyde appearance were independent of phosphate concentration from 4 to 40 mM. Thus, phosphate ion has no apparent catalytic role in the reaction.

Although buffer does not catalyze the reaction, it does apparently play a role in side reactions. We observed inconsistent results and significant side product formation when the reaction was conducted with less than 3 molar equivalents of buffer. Similarly, side products were generated when buffer concentration exceeded 100 mM. Maintaining the reaction pH just below the pK_a of hypochlorous acid (7.53) also appears to minimize side product formation. As a result, we recommend a low buffer concentration of at least four molar equivalents.

The aldehyde products were isolated as oils by extraction into dichloromethane and in vacuo evaporation in an unheated water bath. The products were found to be highly pure when analyzed by ^1H NMR, GC–MS, and HPLC. Extraction into solvents with a higher boiling point, for example, ethyl acetate, resulted in significant decomposition due to the heat required for evaporation. Isolated aldehyde oils may be stored at −20 °C. Unpurified aqueous solutions of the aldehydes may also be stored at −80 °C and thawed when needed. We have observed no degradation after six months.

Sodium hypochlorite oxidation of other amino acids

Table 1 summarizes the results of our optimized NaOCl oxidation procedure on several other amino acids. Reaction of halogenated tyrosine analogs and phenylalanine in the presence of one equivalent of NaOCl in water at 37 °C consistently yielded nearly quantitative conversion of the corresponding halogenated 4-HPAA analogs between 1.5 and 2 h as monitored by either HPLC or GC–MS analysis of an ether extract (1 mL of reaction extracted into 1 mL of diethyl ether[18]). Tryptophan, with a second reactive nitrogen atom, required two equivalents of NaOCl for a maximum conversion of 60–70% based on HPLC and GC–MS. It was not possible to improve the product yield as additional NaOCl generated significant side products. Since we aimed to avoid column chromatography in this investigation, purification of aldehyde **2f** was not attempted. We conclude that NaOCl oxidative decarboxylation should be generally useful for any amino acid lacking functional groups that are sensitive to hypochlorite.

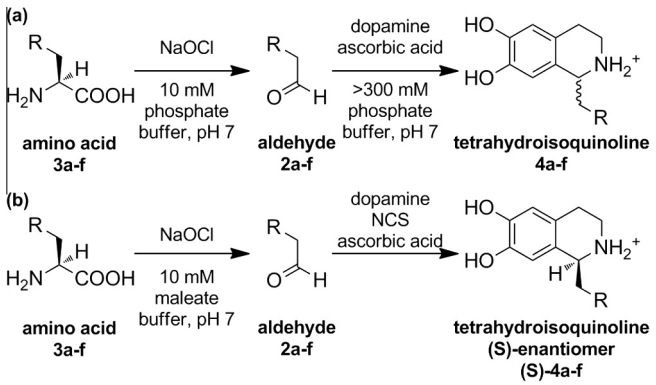
Chemical synthesis of tetrahydroisoquinolines

Scheme 3a illustrates our one-pot biomimetic procedure for tetrahydroisoquinoline synthesis from amino acids. An amino acid was suspended in phosphate buffer (10 mM, pH 7) at 37 °C and oxidized by slow addition of 0.1 M NaOCl. After 2 h, solid ascorbic acid was added (0.2–1.0 equiv gave similar results), followed by an increase in buffer concentration to 200–350 mM and the addition of 1.3 M equivalents of solid dopamine hydrochloride. After 1–2 h of further stirring at 37 °C, conversion of aldehydes **2a–f** to products **4a–f** was complete as monitored by HPLC.

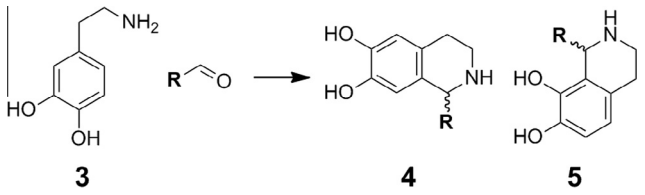
Pesnot et al. found that phosphate ion uniquely catalyzes this Pictet–Spengler reaction.[22] In addition, we have observed that phosphate ion uniquely catalyzes the formation of the target tetrahydroisoquinoline **4** over the other possible regioisomer **5** (Scheme 4 and Fig. S4). When the reaction of **2a** was carried-out in 10 mM phosphate buffer side product **5a** was significant (Fig. 2). By increasing the concentration of phosphate buffer, formation of the side product was reduced to <1%. A detailed mechanistic study of phosphate catalysis of this Pictet–Spengler reaction will be published elsewhere.

The reducing agent ascorbic acid performs the dual role of quenching the excess NaOCl oxidant that may oxidize the catechol group and slowing the oxidation of the catechol group by atmospheric oxygen. Without ascorbic acid, the Pictet–Spengler reactions often turned brown and developed a dark precipitate.

The use of limiting aldehyde greatly simplifies purification. Excess dopamine ensures complete conversion of aldehyde. In contrast, Pesnot et al. reported 75–77% conversion to product with limiting dopamine. We found that tetrahydroisoquinolines (**4**)



Scheme 3.



Scheme 4.

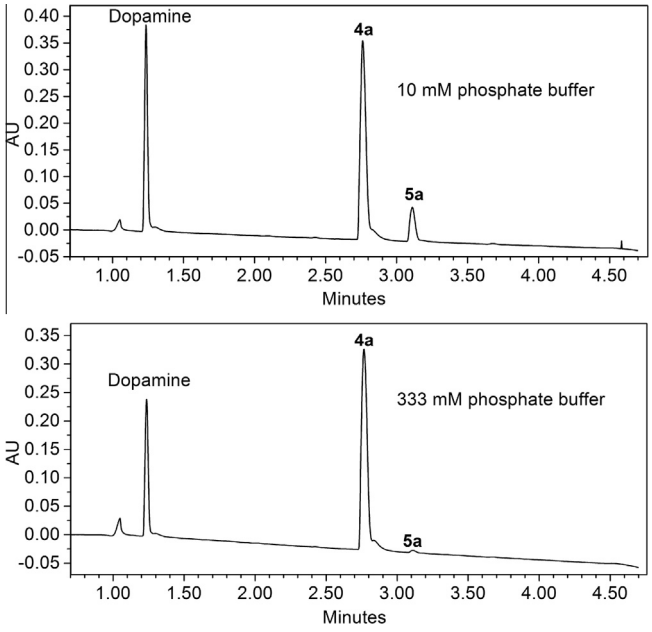


Figure 2. HPLC-UV chromatogram at 225 nm for Pictet–Spengler condensation of 4-HPAA (**2a**) with dopamine at 37 °C. At 10 mM and 333 mM phosphate buffer, **5a** is 13.9% and 0.8% of the total area of the product peaks, respectively.

and aldehydes (**2**) consistently partitioned into the same organic solvent. With limiting dopamine, chromatographic separation from the aldehyde is necessary.[22] We isolated all products **4a**–**f**, free of dopamine, by ethyl acetate extraction (Table 1).[23] The previously reported solid phase extraction of norcoclaurine (**4a**) with activated charcoal[17] does not appear to be general as it failed to give good recovery for products **4b**–**f**, despite attempts at optimization.[6]

Enzymatic synthesis of (*S*)-tetrahydroisoquinolines

The enzyme norcoclaurine synthase (NCS) reacts with dopamine and 4-HPAA (**2a**) to stereoselectively generate (*S*)-norcoclaurine ((***S***)-**4a**). Two recent reports have suggested that NCS has significant promiscuity for aldehyde substrates.[24,25] No studies to date have examined halogenated versions of the natural phenol substrate, which we propose to be the most likely compounds to be accepted by native biosynthetic pathways in PDB. Our aqueous oxidation approach generates such substrates for NCS and avoids the requirement for purification by chromatography.

Phosphate buffer has been previously used for enzymatic (*S*)-norcoclaurine synthesis. However, rapid phosphate catalysis generates trace amounts of racemic product.[17] Therefore, we screened alternative buffers for slower background catalysis at the optimum pH (7.0) for *Thalictrum flavum* NCS enzyme.[26,27] Other than phosphate, the majority of suitable buffers contain a reactive nitrogen group, which we found to be incompatible with NaOCl oxidation. Of particular note, Tris buffer is incompatible with Pictet–Spengler chemistry because it forms 1,3-oxazolidine products with aldehydes[28] (see Supporting Fig. S5 and Scheme S1). This side reaction provides a likely explanation for the previously observed slow and incomplete NCS conversion of dopamine to tetrahydroisoquinolines in Tris buffer.[24] To avoid reaction of either hypochlorite ion or aldehyde with an amine buffer, we selected maleic acid ($pK_a = 6.23$[29]). Maleic acid produced no side products during NaOCl oxidation, catalyzed minimal background Pictet–Spengler reaction, and had no inhibitory effect on NCS. The maleic acid modification of our one pot procedure (Scheme 3b), produced single enantiomer tetrahydroisoquinoline analogs (Supporting Fig. S6) as observed by HPLC with a chiral stationary phase. Additionally, we screened imidazole, HEPES, ACES, MOPSO, Bicine, Tris, Bis–Tris, BES, ADA, and maleic acid as potential buffers for quantitative NCS kinetics experiments (Supporting Table S2). We conclude that only BES ($pK_a = 7.17$[30]) and ADA ($pK_a = 6.62$[30]) exhibited background catalysis <1% of phosphate, with no side products, and no inhibition of NCS. We recommend BES buffer for future mechanistic studies on this enzyme. The relative initial rates for the generation of (***S***)-**4a**–**e** from isolated **2a**–**e** samples in BES buffer are shown in Table 1. All substrates were accepted, confirming the apparent promiscuity of NCS for aldehyde substrates and underscoring its utility as a synthetic catalyst.

Summary

We have demonstrated optimized biomimetic conditions for the facile conversion of a variety of α-amino acids to aldehydes using NaOCl. In one pot, the aldehydes may be transformed into either racemic tetrahydroisoquinolines by reaction in a high concentration of phosphate buffer or (*S*)-enantiomer tetrahydroisoquinolines with norcoclaurine synthase in maleic acid buffer. The products from either procedure may be obtained in high purity from extraction.

Acknowledgments

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Supplementary data

Supplementary data (experimental procedures, figures, and spectra characterization) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.07.043. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Hagel, J. M.; Facchini, P. J. *Plant Cell Physiol.* **2013**, *54*, 647.
2. Kirschning, A.; Taft, F.; Knobloch, T. *Org. Biomol. Chem.* **2007**, *5*, 3245.
3. Kennedy, J. *Nat. Prod. Rep.* **2008**, *25*, 25.
4. Guiso, M.; Bianco, A.; Marra, C.; Cavarischia, C. *Eur. J. Org. Chem.* **2003**, *2003*, 3407.
5. Parikh, J. R.; Doering, W. v. E. *J. Am. Chem. Soc.* **1967**, *89*, 5505.
6. Crowe, S. In *Synthesis and Evaluation of Unnatural HPAA, Nocroclaurine, and Tyramine Analogs;* Maresh, J. J. Ed.; DePaul University, Chicago, IL, 2013; Vol. Masters Thesis. pp 98
7. Dakin, H. D. *Biochem. J.* **1917**, *11*, 79.
8. Hazen, S. L.; Hsu, F. F.; Heinecke, J. W. *J. Biol. Chem.* **1996**, *271*, 1861.
9. Hazen, S. L.; Gaut, J. P.; Hsu, F. F.; Crowley, J. R.; d'Avignon, A.; Heinecke, J. W. *J. Biol. Chem.* **1997**, *272*, 16990.
10. Wright, N. C. *Biochem. J.* **1936**, *30*, 1661.

11. Pereira, W. E.; Hoyano, Y.; Summons, R. E.; Bacon, V. A.; Duffield, A. M. *Biochim. Biophys. Acta* **1973**, *313*, 170.
12. Fu, S.; Wang, H.; Davies, M.; Dean, R. *J. Biol. Chem.* **2000**, *275*, 10851.
13. van Tamelen, E. E.; Haarstad, V. B.; Orvis, R. L. *Tetrahedron* **1968**, *24*, 687.
14. Langheld, K. *Ber. Dtsch. Chem. Ges.* **1909**, *42*, 2360.
15. Schlecht, M. F.; Tsarouhtsis, D.; Lipovac, M. N.; Debler, E. A. *J. Med. Chem.* **1990**, *33*, 386.
16. Capuano, B.; Crosby, I. T.; Lloyd, E. J.; Podloucka, A.; Taylor, D. A. *Aust. J. Chem.* **2003**, *56*, 875.
17. Bonamore, A.; Rovardi, I.; Gasparrini, F.; Baiocco, P.; Barba, M.; Molinaro, C.; Botta, B.; Boffi, A.; Macone, A. *Green Chem.* **2010**, *12*, 1623.
18. *General procedure for sodium hypochlorite oxidation*: To a vigorously stirred solution of amino acid (1 mmol) in 500 mL of sodium phosphate buffer (10 mM, pH 7.0) was added a solution of NaOCl (1 equiv as a 0.1 M aqueous solution) slowly over 10 min via syringe pump in a 37 °C warm room or water bath. The resulting solution was stirred vigorously at this temperature until the reaction was judged to be complete by HPLC (1–2 h). In general, the reaction may be stopped after 2 h if monitoring is inconvenient. The solution was then extracted into dichloromethane (4 × 100 mL), washed with brine (2 × 50 mL), dried over $MgSO_4$, and the solvent removed under reduced pressure in an unheated water bath to afford the desired aldehyde.
19. Harris, D. C. *Quantitative Chemical Analysis*; W.H. Freeman and Co.: New York, 2010.
20. Spenser, I. D.; Crawhall, J. C.; Smyth, D. G. *Chem. Ind.* **1956**, 796.
21. Matte, D.; Solastiouk, B.; Merlin, A.; Deglise, X. *Can. J. Chem.* **1989**, *67*, 786.
22. Pesnot, T.; Gershater, M. C.; Ward, J. M.; Hailes, H. C. *Chem. Commun.* **2011**, 3242.
23. *General procedure for the synthesis of racemic tetrahydroisoquinolines from α-amino acids*: Each 1 mmol of amino acid was suspended with 2.0 mL of 1.0 M phosphate buffer at pH 6.9 (this concentrated buffer gives pH 7.0 upon dilution) in 200 mL of purified water at 37 °C. A solution of 1 molar equivalent of sodium hypochlorite (e.g. 700 μL of 9.16% NaOCl) was dissolved in 10 mL of purified water. NaOCl was added dropwise over 10 min via syringe pump with vigorous stirring using a magnetic stir bar. This solution was allowed to stir at 37 °C for 1.5–2 h and was quenched by the addition of ascorbic acid (88 mg, 0.5 mmol). Additional phosphate catalyst, 100 mL of 1.0 M phosphate buffer at pH 6.9, was then added to the solution followed by dopamine hydrochloride (291 mg, 1.5 mmol). The reaction was monitored until all of the aldehyde was consumed as monitored by HPLC. If monitoring is not convenient, reactions are generally complete after 2 h. The reaction was then extracted into ethyl acetate (6 × 50 mL), the combined organic layers were washed brine (2 × 50 mL), dried with $MgSO_4$, and the solvent was removed under reduced pressure to yield the product as a solid.
24. Ruff, B. M.; Bräse, S.; O'Connor, S. E. *Tetrahedron Lett.* **2012**, *53*, 1071.
25. Pesnot, T.; Gershater, M. C.; Ward, J. M.; Hailes, H. C. *Adv. Synth. Catal.* **2012**, *354*, 2997.
26. Samanani, N.; Liscombe, D. K.; Facchini, P. J. *Plant J.* **2004**, *40*, 302.
27. Luk, L. Y. P.; Bunn, S.; Liscombe, D. K.; Facchini, P. J.; Tanner, M. E. *Biochemistry* **2007**, *46*, 10153.
28. Bubb, W. A.; Berthon, H. A.; Kuchel, P. W. *Bioorg. Chem.* **1995**, *23*, 119.
29. German, W. L.; Vogel, A. I.; Jeffery, G. H. *Philos. Mag.* **1936**, *22*, 790 (1798–1977).
30. Good, N. E.; Winget, G. D.; Winter, W.; Connolly, T. N.; Izawa, S.; Singh, R. M. M. *Biochemistry* **1966**, *5*, 467.