Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 4 October 2012, Revised 2 November 2012, Accepted 9 November 2012 Published online 21 December 2012 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.3004

Chirally deuterated benzyl chlorides from benzyl alcohols via hexachloroacetone/polymer-supported triphenylphosphine: synthesis of protected (2*S*, 3*S*)-[3-^{2}H, ^{15}N]-tyrosine

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Chirally deuterated benzyl chlorides were prepared using novel, general hexachloroacetone/polymer-supported triphenylphosphine treatment of chirally deuterated benzyl alcohols. Doubly labeled protected tyrosine was obtained in 62% yield with 86% de at the α-carbon and 82% de at the β-carbon. Key in the synthesis was the alkylation of ^{15}N-labeled (−)-8-phenylmenthylhippurate with *R*-(−)-4-triisopropylsilyloxybenzyl-α-*d* chloride.

Keywords: chiral; benzylic; deuteration; chlorides; alcohols; tyrosine

Introduction

Stable isotope labeled amino acids are valuable research tools that have many uses in the fields of mechanistic enzymology and biomolecular nuclear magnetic resonance (NMR) spectroscopy. These compounds are often employed to study biosynthesis, the stereochemical course of enzyme-mediated processes,[1] and protein structure by NMR methods.[2] Alkylation of glycine equivalents is a well precedented route to α-amino acid derivatives.[3] In earlier work,[4] we used (*S*)-benzyl-α-*d* mesylate to alkylate ^{15}N-labeled (−)-8-phenylmenthyl hippurate[3t] to give a (2*S*,3*R*)-phenylalanine derivative. It was also shown that (*R*)-benzyl-α-*d* chloride could be used as the electrophilic species to generate the (2*S*,3*S*)-epimer. This method allows synthetic access to all stereoisomers at C2 and C3 by varying the chirality of the ester-protecting group and the electrophile. Availability of labeled glycine in multiple isotopomeric forms also allows the incorporation of different labeling patterns into the final product.

The preparation of tyrosine is a logical extension of this work. However, a key consideration is the impact of the *para*-hydroxy group on the alkylation and deprotection reactions. We now report novel hexachloroacetone (HCA)/polymer-supported triphenylphosphine (psTPP) chlorination conditions to produce an enantiotopically deuterated benzylic electrophile and use it in the stereoselective synthesis of a protected doubly labeled tyrosine.

Results and discussion

Shown in Scheme 1 is the synthesis of (*S*)-4-triisopropylsilyloxybenzyl-α-*d* alcohol (**1b**) that served as the precursor for the electrophilic species in the tyrosine synthesis. Alternative phenol-protecting groups investigated included the methyl, benzyl, and *tert*-butyldimethylsilyl ethers, but triisopropylsilyl displayed the best combination of stability and reactivity as the electrophile. Thus, aldehyde **2** was treated with morpholine perchlorate and KCN to give the morpholinonitrile derivative, which was then deprotonated and treated with D_2O to effect ^{1}H–^{2}H exchange.[5] Deuteration proceeded with high efficiency as determined by ^{1}H NMR spectroscopy (relative integration of the residual methine proton vs. the morpholine methylene protons; reconfirmed by integration of proton resonances of aldehydes **3** and **4**), and acid hydrolysis of the morpholinonitrile returned 4-benzyloxybenzaldehyde-α-*d* (**3**). Benzyl group removal (trimethylsilyl iodide) and reprotection as the triisopropylsilyl ether gave 4-triisopropylsilyloxybenzaldehyde-α-*d* (**4**). Finally, Alpine Borane® reduction of **4** to give **1b** introduced asymmetry with approximately 99% ee as determined by NMR analysis of the (−)-camphanate ester.[6]

To demonstrate the versatility of the strategy, preparation of the β-position epimer of tyrosine, relative to the phenylalanine synthesis,[4] was planned. This would require the use of the chloride derived from **1b** as the electrophile.[4] Several halogenation conditions using alkoxyphosphonium intermediates to ensure inversion of the benzylic center during alcohol to halide conversion were investigated.[7] Unfortunately, these reactions also produced by-products, which interfered with or prohibited isolation of the labile halides, as others have also documented.[8,9]

The chlorination of alcohols using HCA (**5**) and soluble triphenylphosphine (TPP) was first reported by Magid and co-workers.[10] They detailed the regioselective conversion of

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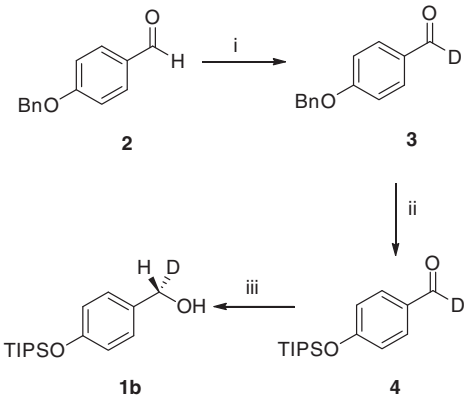
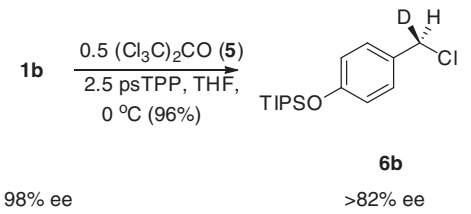


Scheme 1. Synthesis of **1b**. *Reagents and conditions*: (a) i, morpholine, perchloric acid, KCN, 90 °C (quant.); ii, NaH, D_2O, THF (86%, 99% ^2H); iii, 2 N HCl (92%; 99% ^2H); (b) i, trimethylsilyl iodide, CH_2Cl_2 (59%); ii, triisopropylsilyl chloride, NEt_3, THF; (c) (*R*)-Alpine Borane® (79% from unprotected phenol; 99% ee).

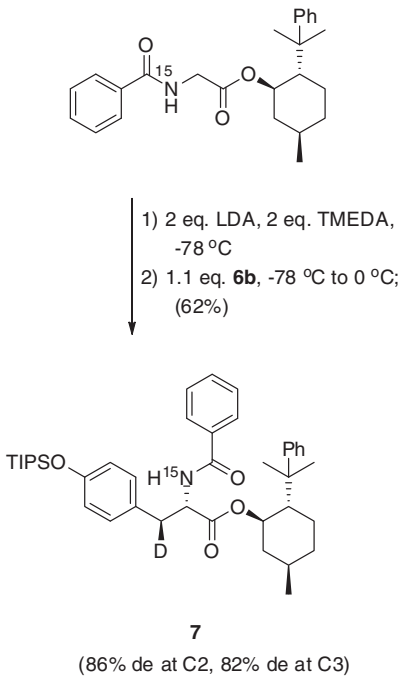
allylic alcohols into chlorides with inversion of stereochemistry under very mild conditions with excellent yields. Villeneuve and Chan also reported these conditions to be an acid-free procedure to prepare acid chlorides.[11]

In our work, model chlorinations with HCA and soluble TPP still suffered from purification difficulties as mentioned previously. Therefore, a source of TPP allowing unreacted phosphine and by-products to be removed by filtration was employed. Shown in Scheme 2 are the conditions used for the synthesis of (*R*)-4-triisopropylsilyloxybenzyl-α-*d* chloride (**6b**). The stoichiometry is based on earlier findings.[11] Chloride **6b** was obtained with excellent apparent stereoselectivity and in high yield from treatment of **1b** with 2.5 eq. of psTPP and 0.5 eq. of HCA in THF at 0 °C. Because optically pure **6b** is not known, the optical purity estimate is based on analysis of the alkylation product of **6b** in the labeled protected tyrosine synthesis (Scheme 3). Although this is an indirect stereochemical analysis of the chloride, it establishes the lower limit of its optical purity at 82% ee. The filterable source of phosphine greatly facilitated product isolation, and to our knowledge, this represents the first use of psTPP for conversion of benzyl alcohols to chlorides.[12]

Because we were interested in the production of chiral benzylic chlorides, we investigated the effect of the *para*-substituent on the stereoselectivity of the reaction. A small number of enantiotopically deuterated alcohols were prepared as in Scheme 1 and are shown in Table 1. In the case of the 4-nitro analog, the propensity of the deuterated morpholinonitrile to undergo ^1H–^2H exchange during hydrolysis required an alternative synthesis. Thus, we reduced methyl 4-nitrobenzoate with $NaBD_4$ and oxidized to the dueterio aldehyde analogous to **4** and then converted to the benzyl alcohol as in Scheme 1. The enantioselectively deuterated



Scheme 2. Synthesis of **6b** using hexachloroacetone/polymer-supported triphenylphosphine conditions.

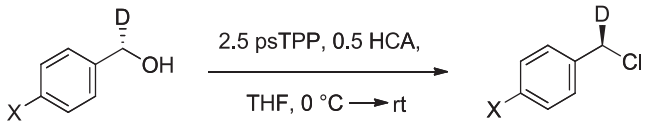


Scheme 3. Synthesis of doubly labeled protected tyrosine **7**.

chlorides were then prepared as in Scheme 2. Optical rotations of the alcohols and chlorides were recorded and the optical purities of the alcohols were determined using NMR analysis of the (−)-camphanate esters.[6] As shown in Table 1, yields of the chlorination reaction are essentially quantitative except in the case of the unsubstituted benzyl alcohol (**1a**). The specific rotation observed for **6a** here is comparable to that reported for optically pure material.[13] Optical rotation data for the remaining chlorides **6** (as well as **1c–e**) have not been reported. If desired, the optical purity of these chlorides could be indirectly determined using the alkylation strategy similar to **6b**. It should be noted that the chlorination of the nitro compound (**1e**) proceeded noticeably faster than with the other benzyl alcohols.

With **6b** in hand, alkylation of ^{15}N-labeled (−)-8-phenylmenthylhippurate to provide protected tyrosine **7** proceeded as for phenylalanine (Scheme 3).[4] These conditions afforded doubly labeled **7** in 62% yield with 86% de at the α-carbon and 82% at the β-carbon as determined by ^1H NMR spectroscopy and HPLC. We saw an even larger decrease in β-position de in our phenylalanine synthesis from chirally deuterated benzyl mesylate[4] further suggesting **6b** has high ee. Figure 1 shows the amide proton region of the 400-MHz ^1H NMR spectrum of crude **7**. The pair of doublets (~90 Hz ^{15}N–H coupling and 7.5 Hz three bond coupling to the β-proton) centered at 6.27 ppm is the amide proton resonance of the (2*S*)-product, whereas the doublet centered at 5.90 ppm is the (2*R*)-product (the triplet pair centered at 6.03 ppm is unreacted starting material showing coupling to the additional α-proton). As for protected phenylalanine,[4] the HPLC chromatogram (Figure 2) shows the (2*S*)-diastereomer ($t_R = 21$ min) elutes after the (2*R*)-diastereomer ($t_R = 16$ min) with starting material at $t_R = 8$ min. Integration of these bands is consistent with the NMR data and supports the assigned α-position de. Integration of the benzylic proton region of the ^1H NMR spectrum of **7** (Figure 3) was used to estimate the de at the β-position.

Table 1. Chlorination studies using hexachloroacetone/polymer-supported triphenylphosphine conditions



Entry	X	ee of (*S*) – **1**[a]	$[\alpha]_D^{21}$ of (*S*) – **1**	Chloride yield	$[\alpha]_D^{21}$ of (*R*) – **6**
a	H	96%	+1.34° (neat)	64%	−1.52° (*c* 6.9, THF)[b]
b	OTIPS	98%	+0.30° (neat)	96%	−0.39° (*c* 15.4, THF)
c	OCH_3	92%	+0.91° (neat)	97%	−0.18° (*c* 16.6, THF)
d	OBn	95%	+0.59° (*c* 5.1, THF)	quant.	−0.37° (*c* 13.6, THF)
e	NO_2	95%	+2.46° (*c* 6.1, THF)	quant.	−1.31° (*c* 6.9, THF)

[a]estimated by ^{1}H NMR analysis of (−)-camphanate derivative.
[b]$[\alpha]_D^{25} = -1.53°$ reported for specific rotation of optically pure material.[13]

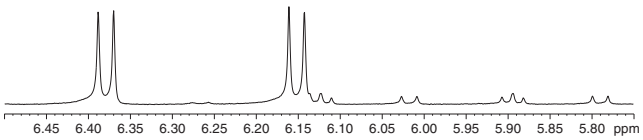


Figure 1. Amide proton region of ^{1}H NMR spectrum (400 MHz) of **7** showing an approximate 86% de at the α-carbon.

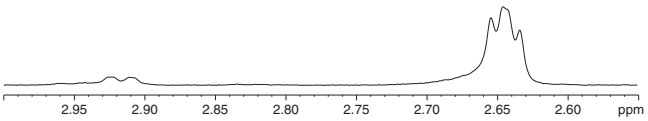


Figure 3. Benzylic proton region of ^{1}H NMR spectrum (400 MHz) of **7** showing an approximate 82% de at C3.

Desilylation of **7** gave **8** in high yield (Scheme 4) with no impact on stereochemistry as evidenced by ^{1}H NMR and HPLC analysis. However, final deprotection of **8** in conc. HCl at 70 °C gave tyrosine·HCl (**9**) in 59% yield but with an approximate 73% de at the α-carbon. This slight epimerization was seen in our phenylalanine synthesis[4] and has been seen by others.[14–16] Inspection of the benzylic region of the ^{1}H NMR spectrum of **9** showed that the product now also has about 44% de at the β-position. Although this decrease will not likely hinder use of **9**, it is surprising and may result via formation of a quinone methide species known to form in many processes[17] and implicated in epimerization of chiral benzylic centers in natural products under similar conditions.[18] Regardless of mechanism, ^{1}H NMR studies of intermediates during the hydrolysis of **8** showed that the ester is cleaved quickly with no β-position epimerization, but the longer reaction times needed for benzamide hydrolysis permit decrease of stereochemical integrity. Because the benzamide group participates in ensuring the high stereoselectivity of α-position alkylation,[3r–t] perhaps a more readily removed benzamide-protecting group would improve stereochemical outcome. To this end, we modeled hydrolysis rates by colorimetric (ninhydrin) assay of liberated glycine from 6M HCl treatment of ethyl hippurate and its *para*-nitro and 3,5-dinitro analogs. As expected, the rate of hydrolysis increases with degree of nitration, and the 3,5-dinitro analog hydrolyzes at least 3.5–5 times faster than the unsubstituted benzamide, which our studies show may prove useful for the current synthesis.

Conclusions

In summary, our previous method for preparation of ^{15}N-labeled *L*-phenylalanine with chiral β-deuteration has been successfully extended to protected tyrosine. Some degradation of β-position stereochemical integrity occurred during benzamide group hydrolysis to give tyrosine, and we offer a solution for this problem. The labeled tyrosine should still be useful for a variety

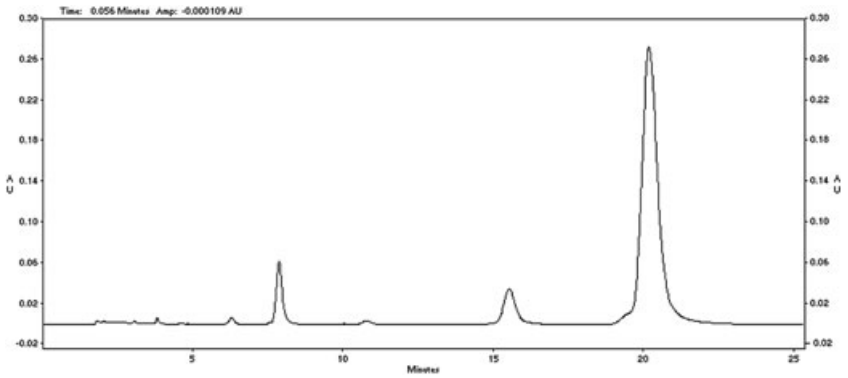
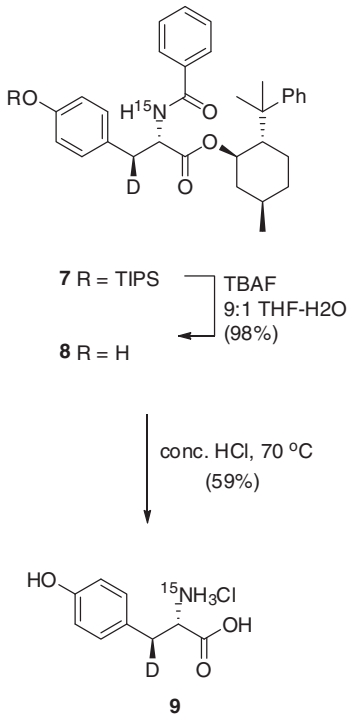


Figure 2. Chromatogram of crude **7** showing retention times of starting material, (2*R*)-, and (2*S*)-diastereomer, left to right.



Scheme 4. Deprotection of doubly labeled tyrosine **7**.

of purposes and methods to enhance hydrolysis rate should diminish this problem. More importantly, in accomplishing this synthesis, a new solid phase-assisted method for the preparation of chirally deuterated benzyl chlorides has been developed. The process is high yielding, proceeds with apparent excellent stereochemical fidelity, and allows convenient work-up and purification.

Experimental section

All reactions were carried out under a dry argon atmosphere unless otherwise noted and in dry glassware when necessary. ^{15}N-Glycine and D_2O were from Cambridge Isotope Laboratories (Cambridge Isotope Laboratories Inc., Andover, MA, USA). All other reagents and solvents were from Sigma-Aldrich (Sigma-Aldrich Corp., St. Louis, MO, USA) and were used as obtained unless otherwise indicated. Analytical thin-layer chromatography (TLC) and column chromatography materials were from EM Separations (EM Separations, Gibbstown, NJ, USA), whereas preparative TLC plates were from Analtech (Analtech, Inc., Newark, DE, USA). Optical rotations were obtained using a Perkin-Elmer 241 polarimeter (Perkin-Elmer Inc., Waltham, MA, USA). Analytical HPLC analysis was performed on a Beckman Ultrasphere ODS column (Beckman Coulter Inc., Brea, CA, United States) (4.6 × 250 mm) using a Beckman System Gold 127 solvent module and 166 UV detection module. NMR spectra were recorded on a Bruker DRX400 instrument (Bruker Corp., Billerica, MA, USA) operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C measurements, respectively. A Bruker DRX800 instrument in The Ohio State University Campus Chemical Instrument Center was used for measuring 800-MHz (^{1}H) spectra. Mass spectra were recorded at The Ohio State University Campus Chemical Instrument Center using a Micromass QTOF spectrometer.

General procedure for synthesis of deuterated benzyl alcohols

The procedure used for the synthesis of the deuterated aldehydes and their resulting stereoselectively deuterated benzyl alcohols consisted of four steps. The procedure that follows was used for the preparation of benzaldehyde-α-*d*/(*S*)-(+)-benzyl-α-*d* alcohol (**1a**) and is representative of the other deuterated electrophile precursors. The reaction times and temperatures, as well as the method of purification, varied slightly depending on the starting aldehyde.

A flask was charged with morpholine (20 mL) and cooled to 0 °C. Perchloric acid (70%, 9.5 mL; 0.11 mol) was added dropwise to the stirred solution followed by careful addition of benzaldehyde (0.11 mol). The mixture became a yellow solid, which dissolved on warming to 70 °C. After 4 h at 70 °C, the mixture was cooled to room temperature and an aqueous solution of KCN (0.12 mol, minimal water) was added. The mixture was warmed to 90 °C for 1 h and then cooled to room temperature and poured onto ice with stirring. The resulting precipitate was washed with water, recrystallized from absolute ethanol, and dried under vacuum at 40 °C for 12 h to give 18.8 g (91%) of α-phenyl-4-morpholineacetonitrile: mp 67–68 °C (lit. 68–70 °C); [19] ^{1}H NMR ($CDCl_3$) δ 2.27–2.38 (m, 4H), 3.45–3.57 (m, 4H), 4.65 (s, 1H), 7.15–7.22 (m, 3H), 7.34–7.36 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 49.79, 62.31, 66.55, 115.03, 127.80, 128.61, 128.79, 132.55; HRMS-ES (*m/z*): $[M + Na]^+$ calculated for 225.1004, found 225.0991.

A dry, argon flushed flask was charged with α-phenyl-4-morpholineacetonitrile (50 mmol) and THF (50 mL). Sodium hydride (95%; 99 mmol) was carefully added, and the pink mixture was heated to 40 °C for 1 h. The mixture was cooled to 0 °C, and deuterium oxide (1.0 mol) was carefully added dropwise as the pink color faded over 30 min of stirring. The solution was acidified (pH 1–2) by addition of freshly distilled thionyl chloride and poured onto ice with stirring. The resulting precipitate was washed with water and dried to give white solid α-phenyl-4-morpholinoeacetonitrile-α-*d* (9.91 g, 99%; 99% ^{2}H incorporation as determined by ^{1}H NMR): mp 67–68 °C (lit. 69–70 °C)[19]; ^{1}H NMR ($CDCl_3$) δ 2.42 (t, 4H, *J* = 4.4 Hz), 3.51–3.60 (m, 4H), 7.15–7.28 (m, 3H), 7.38–7.40 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 49.75, 61.85 (t, *J* = 22.1 Hz), 66.60, 115.00, 127.82, 128.74, 132.46; HRMS-ES (*m/z*): $[M + Na]^+$ calculated for 226.1082, found 226.1070.

A flask was charged with α-phenyl-4-morpholineacetonitrile-α-*d* (25 mmol) and 2M HCl (60 mL). The suspension was refluxed for 12 h, and the resulting two-phase mixture was cooled to room temperature and extracted with ether. The combined organic phases were washed with saturated $NaHCO_3$, water and brine, dried over $MgSO_4$, filtered, and concentrated to the oil benzaldehyde-α-*d* (2.5 g, 93%; 98% ^{2}H incorporation as determined by ^{1}H NMR), which was used as obtained: ^{1}H NMR ($CDCl_3$) δ 7.39–7.68 (m, 3H), 7.87 (d, 2H, *J* = 4.9 Hz), 10.01 (s, residual CHO).

*(S)-(+)-benzyl-α-d alcohol (**1a**)*

A THF solution of 0.5M *R*-Alpine-Borane® (70 mL, 35 mmol) was added to benzaldehyde-α-*d* (2.3 g, 21.5 mmol). The mixture was stirred at room temperature for 12 h then refluxed for 1.5 h. After cooling to room temperature, acetaldehyde (5 mL) was added and the mixture was stirred for 30 min, and then solvent was removed. Some of the pinene by-products were then removed under high vacuum at 50 °C for 5 h. The resulting orange oil was dissolved in ether (75 mL and, cooled to 0 °C, and ethanolamine (2.1 g, 35 mmol) was added and the mixture was stirred for 30 min at 0 °C. The resulting white precipitate was filtered and washed with ether. The combined ether filtrate was concentrated, and the resulting oil was dissolved in 10% aqueous methanol and washed several times with heptane. Isopropanol was added to the methanolic fraction, and the solution was concentrated under reduced pressure. The resulting yellow oil was chromatographed (silica gel; 5% ethyl acetate/hexane) to give 2.0 g (87%; >96% ee) of **1a** as a colorless oil: $[\alpha]_D^{21} + 1.34^o$ (neat)[20]; ^{1}H NMR ($CDCl_3$) δ 1.90 (s, 1H), 4.64 (t, 1H, *J* = 1.8 Hz), 7.26–7.37 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 64.20 (t, *J* = 22.1 Hz), 126.79, 126.23, 128.21, 140.64; HRMS-EI (70 eV) *m/z*: calculated 109.0637, found 109.0569.

*(S)-(+)-4-triisopropylsilyloxybenzyl-α-d alcohol (**1b**)*

$[\alpha]_D^{21} + 0.30^o$ (neat); ^{1}H NMR ($CDCl_3$) δ 1.08 (d, 18H, *J* = 7.04 Hz), 1.23 (m, 3H), 4.57 (br t, 1H), 6.85 (d, 2H, *J* = 8.6 Hz), 7.20 (d, 2H, *J* = 8.6 Hz); HRMS-ES (*m/z*): $[M + Na]^+$ calculated for 304.1835, found 304.1837.

*(S)-(+)-4-methoxybenzyl-α-d alcohol (**1c**)*

$[\alpha]_D^{21} + 0.91^o$ (neat); ^{1}H NMR ($CDCl_3$) δ 3.79 (s, 3H), 4.58 (br t, 1H), 6.87 (d, 2H, *J* = 8.6 Hz), 7.27 (d, 2H, *J* = 8.6 Hz); ^{13}C NMR ($CDCl_3$) δ 55.26, 64.50

(t, J = 22.0 Hz), 113.89, 128.63, 133.09, 159.12; HRMS-ES (*m/z*): $[M + Na]^+$ calculated for 162.0641, found 162.0645.

*(S)-(+)-4-benzyloxybenzyl-α-d alcohol (**1d**)*

$[\alpha]_D^{21}$ + 0.59° (*c* 5.1, THF); ^{1}H NMR ($CDCl_3$) δ 2.07 (br s, 1H), 4.55 (br s, 1H), 5.06 (s, 2H), 6.96 (d, 2H, J = 8.8 Hz), 7.27 (d, 2H, J = 8.8 Hz), 7.31–7.45 (m, 5H); ^{13}C NMR (CDCl3) δ 22.1 (t, J = 22.1 Hz), 69.90, 114.79, 127.32, 127.84, 128.46, 128.52, 133.26, 136.84, 158.23; HRMS-ES (*m/z*): $[M + Na]^+$ calculated for 238.0954, found 238.0946.

*(S)-(+)-4-nitrobenzyl-α-d alcohol (**1e**)*

$[\alpha]_D^{21}$ + 2.46° (*c* 6.1, THF); ^{1}H NMR ($CDCl_3$) δ 4.81 (s, 1H), 7.52 (d, 2H, J = 8.8 Hz), 8.21 (d, 2H, J = 8.8 Hz); ^{13}C NMR ($CDCl_3$/acetone-d_6) δ 62.24 (t, J = 23 Hz), 123.03, 126.79, 146.78, 150.19; HRMS-ES (*m/z*): $[M + Na]^+$ calculated for 177.0386, found 177.0378.

General procedure for stereochemical analysis of deuterated benzyl alcohols

The procedure used for the preparation of diastereomeric derivatives of labeled benzyl alcohols was adapted from Williams and co-workers.[6] The procedure that follows was used for the preparation of the (−)-camphanate ester of (*S*)-(+)-benzyl-α-*d* alcohol (**1a**) and is representative of the other deuterated alcohols. A solution of the alcohol in a small amount of toluene/pyridine was added to a solution of (−)-camphanic chloride (2 equivalents) in toluene at 0 °C. The reaction mixture was stirred at 0 °C for 30 min then 5 h at 25 °C. The reaction mixture was concentrated under reduced pressure, purified by preparative TLC, and analyzed by NMR spectroscopy. For the (−)-camphanate ester of **1a**: ^{1}H NMR (800 MHz; $CDCl_3$) δ 5.35 (br s, major), 5.39 (br s, minor), major/minor >98:2.

General procedure for synthesis of deuterated benzyl chlorides

Polymer-supported triphenylphosphine was washed with THF and dried under high vacuum at 100 °C for 12 h prior to use. To a dry flask under argon was added TPP resin (~3 mmol/g, 1.89 mmol) and dry THF (25 mL). The suspension was cooled to 0 °C, and HCA (0.5 equivalents) was added. After it was stirred at 0 °C for 20 min, a solution of the benzyl-α-*d* alcohol (1.0 equivalent) in dry THF was added dropwise. The reaction mixture was stirred for 1 h at 0 °C then warmed to room temprerature. When TLC of the mixture showed no remaining starting material, the resin was removed by filtration and washed with CH_2Cl_2, and the combined solvents were removed under reduced pressure. The product chlorides were then purified using silica gel chromatography with yields shown in Table 1.

*(R)-(−)-benzyl-α-d chloride (**6a**)*

$[\alpha]_D^{21}$ − 1.52° (*c* 6.9, THF); ^{1}H NMR ($CDCl_3$) δ 4.53 (s, 1H), 7.28–7.36 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 45.76 (t, J = 23 Hz), 128.18, 128.28, 137.29; HRMS-EI (70 eV) *m/z*: calculated 127.02980, found 127.03019.

*(R)-(−)-4-triisopropylsilyloxybenzyl-α-d chloride (**6b**)*

$[\alpha]_D^{21}$ − 0.39° (*c* 15.4, THF); ^{1}H NMR ($CDCl_3$) δ 1.09 (d, 18H, J = 7.4 Hz), 1.25 (m, 3H), 4.63 (br t, 1H), 6.89 (d, 2H, J = 8.5 Hz), 7.31 (d, 2H, J = 8.5 Hz); ^{13}C NMR ($CDCl_3$) δ 13.27, 18.15, 46.45 (t, J = 23.2 Hz), 120.65, 131.07, 131.50, 156.93; HRMS-ES (*m/z*): $[M + Na]^+$ calculated for 322.1480, found 322.1470.

*(R)-(−)-4-methoxybenzyl-α-d chloride (**6c**)*

$[\alpha]_D^{21}$ − 0.18° (*c* 16.6, THF); ^{1}H NMR ($CDCl_3$) δ 3.79 (s, 3H), 4.53 (br t, 1H, J = 1.6 Hz), 6.87 (d, 2H, J = 8.8 Hz), 7.30 (d, 2H, J = 8.8 Hz); ^{13}C NMR (acetone-d_6) δ 45.89 (t, J = 23.2 Hz), 55.15, 113.99, 129.91, 159.52, 175.42; HRMS-EI (70 eV) *m/z*: calculated 157.04037, found 157.04137.

*(R)-(−)-4-benzyloxybenzyl-α-d chloride (**6d**)*

$[\alpha]_D^{21}$ − 0.37° (*c* 13.6, THF); ^{1}H NMR ($CDCl_3$) δ 4.53 (br s, 1H), 5.50 (s, 2H), 6.93 (d, 2H, J = 8.6 Hz), 7.28–7.42 (m, 7H); ^{13}C NMR ($CDCl_3$) δ 45.64 (t, J = 23.0 Hz), 62.71, 66.15, 69.47, 75.46, 114.78, 127.39, 127.66. 128.29, 130.10, 137.13, 158.81; HRMS-ES (*m/z*): $[M + Na]^+$ calculated for 256.0615, found 256.0617.

*(R)-(−)-4-nitrobenzyl-α-d chloride (**6e**)*

$[\alpha]_D^{21}$ − 1.31° (*c* 6.9, THF); ^{1}H NMR (acetone-d_6) δ 4.81 (s, 1H), 7.71 (d, 2H, J = 8.6 Hz), 8.21 (d, 2H, J = 8.6 Hz); ^{13}C NMR (acetone-d_6) δ 43.98 (t, J = 23 Hz), 123.54, 129.58, 144.82, 147.57; HRMS-EI (70 eV) *m/z*: calculated 172.01488, found 172.01506.

^{15}N-(−)-8-phenylmenthylhippurate

A mixture of ^{15}N-hippuric acid (prepared from ^{15}N-glycine and benzoyl chloride; 2.0 g, 11.2 mmol), (−)-8-phenylmenthol (prepared by the procedure of Ort[21]; 0.75 g, 3.2 mmol), and *p*-toluenesulfonic acid (100 mg) was refluxed under Dean–Stark conditions. Upon disappearance of the alcohol by TLC, the reaction was cooled to room temperature, saturated $NaHCO_3$ was added (15 mL) and the mixture was stirred for 15 min. The phases were separated, and the organic phase was washed with water and brine, concentrated, and purified by silica gel flash column chromatography to yield a white solid (1.2 g, 93%): ^{1}H NMR ($CDCl_3$) δ 0.87 (d, 3H, J = 6.5 Hz), 0.89–1.02 (m), 1.52–2.11 (m), 3.41 (dd, 1H, J = 18.4, 5.7 Hz), 3.58 (dd, 1H, J = 18.4, 4.6 Hz), 4.91 (dd, 1H, J = 10.7, 4.6 Hz), 6.05 (dt, 1H, J = 91.4, 5.0 Hz), 7.12–7.73 (m, 10H).

*(2S, 3S)-N-benzoyl-O-triisopropylsilyl-[3-^{2}H, ^{15}N]-tyrosine-(−)-8-phenylmenthyl ester (**7**)*

Under dry conditions, a 0.5M solution of LDA was prepared by the addition of a solution of *n*-BuLi (1 equivalent) in hexanes to a solution of diisopropylamine (1 equivalent) in dry THF at −78 °C. The solution was stirred for 10 min, and then tetramethylethylenediamine (1 equivalent) was added. ^{15}N-(−)-8-Phenylmenthylhippurate (0.5 equivalent) was dissolved in dry THF (0.25M solution) and added slowly. The mixture was stirred at −78 °C for 20 min; after which, it was warmed to 0 °C, and electrophile **1b** (slight excess) was added slowly with further stirring for 2 h. The reaction was quenched with 1M HCl, warmed to room temperature, diluted with ethyl acetate, and the phases were separated. The ethyl acetate layer was dried ($MgSO_4$) and evaporated, and the residue was purified by silica gel preparative TLC (15% ethyl acetate/hexane) to give 32 mg (62%) of **7** as a white solid: $[\alpha]_D^{21}$ + 11.2° (*c* 13.5, THF); ^{1}H NMR ($CDCl_3$) δ 0.85–1.30 (m), 0.88 (d, 3H, J = 6.6 Hz), 1.04 (s), 1.06 (s), 1.65–1.86 (m, 5H), 2.06 (dt, 1H, J = 11.8, 3.5 Hz), 2.65 (br t, 1H, J = 3.7 Hz), 4.17 (dt, 1H, J = 2.4, 1.3 Hz), 4.78 (dt, 1H J = 10.7, 4.2 Hz), 6.27 (dd, 1H, J = 91.4, 7.5 Hz), 6.71 (d, 2H, J = 8.3 Hz), 6.85 (d, 2H, J = 8.3 Hz), 7.24–7.26 (m, 4H), 7.66 (d, 2H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 12.28, 14.12, 17.83, 20.93, 21.62, 23.82, 26.37, 28.81, 31.24, 34.41, 35.99 (t, J = 18.9 Hz), 39.40, 41.54, 50.33, 53.15, 53.27, 60.28, 76.06, 119.80, 125.19, 125.34, 126.92, 128.02, 128.43, 130.37, 131.42, 134.29, 134.37, 151.23, 154.80, 166.31, 166.47, 170.91; HPLC (95% CH_3OH/H_2O): t_R = 20.2 min (major), 15.6 min (minor).

*(2S, 3S)-N-benzoyl-[3-^{2}H, ^{15}N]-tyrosine-(−)-8-phenylmenthyl ester (**8**)*

To a stirred solution of 14 mg (0.024 mmol) of **7** in THF-H_2O (9:1) was added 13 mg (2 equivalent) of TBAF·H_2O. After 3 h of stirring, TLC showed consumption of starting material, and the solution was diluted with water and extracted with ethyl acetate. The ethyl acetate layer was washed with brine, dried ($MgSO_4$), concentrated, and purified by preparative silica gel TLC (40% ethyl acetate/hexane) to give 10 mg (98%) of **8** as a white solid: ^{1}H NMR ($CDCl_3$) δ 0.89–1.29 (m), 0.90 (d, 3H, J = 6.4 Hz), 1.02 (s), 1.20 (s), 1.47 (br s, 1H), 1.70 (d, 1H, J = 12.7 Hz), 1.84 (dt, 2H, J = 13.8, 3.1 Hz), 2.12 (dt, 1H, J = 11.6, 3.1 Hz), 2.65 (m, 1H), 4.20 (t, 1H, J = 6.1 Hz), 4.86 (dt, 1H, J = 11.0, 4.2 Hz), 6.44 (dd, 1H, J = 91.2, 7.9 Hz), 6.70 (d, 2H, J = 8.8 Hz), 6.88 (d, 2H, J = 8.8 Hz), 7.17–7.30 (m, 4H), 7.37–7.51 (m, 4H), 7.68 (d, 2H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 12.24, 14.07, 17.62, 20.95, 21.69, 23.37, 26.28, 29.12, 31.19, 34.35, 36.10 (m), 39.34, 41.48, 50.23, 53.36, 53.49, 60.45, 76.19, 115.37, 125.16, 125.35, 126.94, 128.01, 128.53, 130.35, 131.66, 133.83, 133.91, 151.51, 155.49, 167.03, 167.19, 170.97; HPLC (85% CH_3OH/H_2O): t_R = 8.4 min (major), 7.4 min (minor).

*(2S, 3S)-[3-^{2}H, ^{15}N]-tyrosine hydrochloride (**9**)*

A suspension of 40 mg (0.08 μmol) of **8** in concentrated HCl (10 mL) was warmed to 70 °C for 12 h. The cooled solution was then washed with CH_2Cl_2, and the aqueous phase was evaporated to give 10.3 mg (59%) of **9** as a white solid: ^{1}H NMR (D_2O) δ 2.2 (br dd, 1H, J = 7.5, 2.2 Hz), 3.10 (residual ^{1}H, br t, J = 3.4 Hz), 4.08 (d, 1H, J = 7.5 Hz), 6.75 (d, 2H, J = 8.3 Hz), 7.05 (d, 2H, J = 8.3 Hz); ^{13}C NMR (D_2O/CD_3OD) δ 35.25 (t, J = 19.6 Hz), 55.13, 116.56, 126.40, 131.47, 155.87, 172.43; HRMS-ES (*m/z*): $[M]^+$ calculated for 184.0958, found 184.0951.

Acknowledgements

We gratefully acknowledge financial support of this work by the National Science Foundation (NSF MCB9723642).

Conflict of Interest

The authors did not report any conflict of interest.

References

[1] (a) D. W. Young, *Top. Stereochem.* **1994**, *21*, 381–465; (b) J. C. Vederas, *Nat. Prod. Rep.* **1987**, *4*, 277–337; (c) J. Mann, Secondary Metabolism, 2nd ed.; Oxford University Press: New York, **1987**.

[2] (a) K. Wuthrich *Nat. Struct. Biol.* **1998**, *5*, 492–495; (b) G. M. Clore, A. M. Gronenborn *Nat. Struct. Biol.* **1997**, *4*, 849–853; (c) A. M. Gronenborn, G. M. Clore, *Crit. Rev. Biochem. Mol. Biol.* **1995**, *30*, 351–385; (d) C. H. Arrowsmith, Y. S. Wu, *Prog. Nucl. Magn. Reson. Spectrosc.* **1998**, *32*, 277–286.

[3] (a) R. O. Duthaler, *Tetrahedron* **1994**, *50*, 1539–1650; (b) R. M. Williams, J. A. Hendrix, *Chem. Rev.* **1992**, *92*, 889–917; (c) R. M. Williams, Synthesis of Optically Active α-Amino Acids; Pergamon: Oxford, **1989**; (d) M. J. O'Donnell, W. D. Bennett, *Tetrahedron* **1988**, *44*, 5389–5401; (e) S. Caddick, N. J. Parr, M. C. Pritchard, *Tetrahedron* **2001**, *57*, 6615–6626; (f) V. A. Soloshonok, X. Tang, V. J. Hruby, *Tetrahedron* **2001**, *57*, 6375–6382; (g) D. B. Berkowitz, J. M. Mcfadden, M. K. Sloss, *J. Org. Chem.* **2000**, *65*, 2907–2918; (h) R. Chinchilla, P. Mazon, C. Najera, *Tetrahedron: Asymmetry* **2000**, *11*, 3277–3281; (i) A. Reyes, E. Juaristi, *Tetrahedron: Asymmetry* **2000**, *11*, 1411–1423; (j) A. G. Myers, P. Schnider, S. Kwon; D. W. Kung, *J. Org. Chem.* **1999**, *64*, 3322–3327; (k) A. G. Myers, J. L. Gleason, T. Yoon, D. W. Kung, *J. Am. Chem. Soc.* **1997**, *119*, 656–673; (l) D. B. Berkowitz, M. K. Smith, *J. Org. Chem.* **1995**, *60*, 1233–1238; (m) C. Alvarez-Ibarra, A. G. Csakye, R. Maroto, M. L. Quiroga, *J. Org. Chem.* **1995**, *60*, 7934–7940; (n) H. Yu, S. Takigawa, H. Koshima, *Tetrahedron* **2004**, *60*, 8405–8410; (o) A. Viso, R. Fernandez de la Pradilla, In Amino Acids, Peptides and Proteins in Organic Chemistry, Vol. 1, (Ed.: A. B. Hughes), Wiley-VCH: Weinheim, **2009**; Chapter 9: (p) C. M. Reid, A. Sutherland, In Amino Acids, Peptides and Proteins in Organic Chemistry, Vol. 1, (Ed.: A. B. Hughes), Wiley-VCH: Weinheim, **2009**; Chapter 11; (q) D. J. Ager, In Amino Acids, Peptides and Proteins in Organic Chemistry, Vol. 1, (Ed.: A. B. Hughes), Wiley-VCH: Weinheim, **2009**; Chapter 12; (r) J M. McIntosh, S. Peters, *Synthesis* **1999**, 635–638; (s) J. M. McIntosh, E. J. Kiser, Z. Tian, *Can. J. Chem.* **1998**, *76*, 147–151; (t) J. M. McIntosh; R. Thangarasa, N. K. Foley, D. J. Ager, D. E. Froen, R. C. Klix, *Tetrahedron* **1994**, *50*, 1967–1974.

[4] D. W. Barnett, M. J. Panigot; R. W. Curley, Jr. *Tetrahedron: Asymmetry* **2002**, *13*, 1893–1900.

[5] R. W. Curley, Jr., M. J. Panigot, A. P. Hansen, S. W. Fesik, *J. Biomol. NMR* **1994**, *4*, 335–340.

[6] R. M. Williams, D. Zhai, P. J. Sinclair, *J. Org. Chem.* **1986**, *51*, 5021–5022.

[7] O. Mitsunobu, *Synthesis* **1981**, 1–28.

[8] Pollastri, M. P.; Sagal, J. F.; Chang, G. *Tetrahedron Lett.* **2001**, *42*, 2459–2460.

[9] A. B. Charette, M. K. Janes, A. A. Boezio, *J. Org. Chem.* **2001**, *66*, 2178–2180.

[10] R. M. Magid, O. S. Fruchey, W. L. Johnson, T. G. Allen, *J. Org. Chem.* **1979**, *44*, 359–363.

[11] G. B. Villeneuve, T. H. Chan, *Tetrahedron Lett.* **1997**, *38*, 6489–6492.

[12] S. M. Mershon, A. L. Anding, J. S. Chapman, M. Clagett-Dame, L. A. Stonerock, R. W. Curley, Jr. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 836–840.

[13] Y. Becker, J. K. Stille, *J. Am. Chem. Soc.* **1978**, *100*, 838–844.

[14] J. Csapo, Z. Csapo-Kiss, L. Wagner, T. Talos, T. G. Martin, S. Folestad, A. Tivesten, S. Nemethy, *Anal. Chim. Acta* **1997**, *339*, 99–107.

[15] A. D'aniello, L. Petrucelli, C. Gardner, G. Fisher, *Anal. Biochem.* **1993**, *213*, 290–296.

[16] R. Liardon, R. Jost, *Int. J. Pept. Res.* **1981**, *18*, 500–505.

[17] H. Amouri, J. L. Bras, *Acc. Chem. Res.* **2002**, *35*, 501–510.

[18] E. Kiehlmann, E. P. M. Li, *J. Nat. Prod.* **1995**, *58*, 450–455.

[19] D. J. Bennett, G. W. Kirby, V. A. Moss, *J. Chem. Soc, C* **1970**, 2049–2051.

[20] M. M. Midland, S. Greer, A. Tramontano, S. A. Zderic, *J. Am. Chem. Soc.* **1979**, *101*, 2353–2355.

[21] O. Ort, *Org. Synth.* **1987**, *65*, 203–214.