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Stereoselective route to ^{15}N-labeled-β-deuterated amino acids: synthesis of (2*S*,3*R*)-[3-^{2}H,^{15}N]-phenylalanine

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Abstract—(2*S*,3*R*)-[3-^{2}H,^{15}N]-Phenylalanine hydrochloride was synthesized utilizing ^{15}N-labeled 8-phenylmenthylhippurate as a chiral glycine equivalent. The key step in the synthesis was the alkylation of the glycine template with (*S*)-(+)-benzyl-α-d-mesylate. The doubly labeled alkylation product was obtained in 89% yield with 92% de at the α-carbon and 74% de at the β-carbon. The nature of the electrophile employed in the alkylation step significantly affects the stereochemical outcome at the β-carbon. Hydrolysis of the alkylation product under acidic conditions followed by recrystallization from isopropanol yielded the title compound as the hydrochloride salt. Analysis of the (−)-camphanamide derivative of the final product by NMR spectroscopy and HPLC revealed a 76% de at the α-carbon and a 72% de at the β-carbon. The synthetic strategy described represents a simple yet versatile route to chirally deuterated β-methylene unit containing amino acids.

1. Introduction

Isotopically labeled amino acids are valuable biochemical tools that are used in many different areas of research. They are frequently employed in mechanistic enzymology to study biosynthetic pathways and the stereochemical course of enzyme-mediated processes.[1–3] Another field that relies extensively on these materials is protein structure determination using NMR methods, which can be greatly facilitated by the incorporation of deuterium labeled amino acids.[4–8] Stereospecific ^{1}H NMR assignments of prochiral protons can be obtained using chiral deuteration, and these assignments significantly ease obtaining coupling constant information and distance constraints, and thus aid conformational analysis of the molecules under study. Previous work from our laboratory has led to the synthesis of (2*R*)-[2-^{2}H,^{15}N]-glycine for NMR studies of FK506 binding protein.[9,10] Once incorporated into the protein, stereospecific NMR assignments of the glycine α-protons of FK506 binding protein bound to the immunosuppressant ascomycin were obtained using ^{15}N-edited TOCSY (TOtally Correlated SpectroscopY) experiments. As an extension of this previous work, we are currently interested in developing a general route to ^{15}N-labeled amino acids that are stereoselectively deuterated at the β-carbon. These materials will permit the stereospecific NMR assignments of the prochiral β-methylene protons, which will aid conformational studies of amino acid residue side-chains and conformational analysis of receptor-bound peptide ligands.[11–17] This report details our efforts on the synthesis of a doubly labeled phenylalanine derivative.

Several synthetic routes to diasterotopically β-deuterated phenylalanine derivatives are known. These routes can generally be characterized as methods that utilize reduction of dehydroamino acids or halogenated derivatives,[18–23] or methods that rely on enzymatic steps to introduce chirality and/or resolve stereoisomers.[24,25] The method reported herein relies on the alkylation of a labeled glycine equivalent with an enantiotopically deuterated electrophile.

Alkylation of glycine equivalents is well precedented and represents a common route to α-amino acid derivatives.[26–38] Excellent stereochemical control of the nascent carbon–carbon bond can be achieved with the use of the appropriate chiral protecting groups, establishing the desired stereochemistry at the α-carbon. If an enantiotopically deuterated electrophile were employed in the alkylation reaction, it would be possible to introduce asymmetry at the β-carbon during construction of the C2–C3 bond, assuming an S_N2

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reaction mechanism (Fig. 1). This methodology has several features that make it an attractive approach to these materials. The availability of labeled glycine in multiple isotopomeric forms allows for the incorporation of different labeling patterns into the final product that may be beneficial to certain NMR experiments. In addition, the strategy is versatile, allowing synthetic access to all stereoisomers at C2 and C3 by varying the chirality of the protecting groups and electrophile. Finally, the method should be general enough to allow for the synthesis of other β-methylene unit containing amino acids.

2. Results and discussion

As demonstrated by McIntosh and co-workers, *N*-benzoyl glycinate (hippurate) esters undergo *C*-alkylation stereoselectively when esterified with the appropriate chiral alcohol.[39–41] With (–)-8-phenylmenthol serving as the chiral discriminator, formation of the C2–C3 bond proceeds with high stereoselectivity to give the precursor to the L-amino acid.[41] Under these same reaction conditions, the C-alkylation of a diastereomeric lithium enolate derived from ^{15}N-labeled 8-phenylmenthylhippurate with a chiral benzylic electrophile would yield a

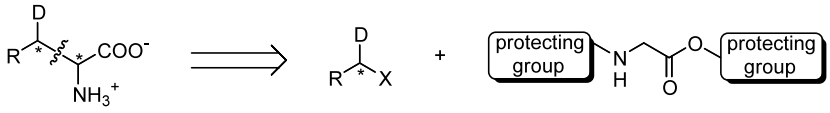


Figure 1. Retrosynthesis of stereoselectively β-deuterated α-amino acids by alkylation of a glycine equivalent. The protecting groups of the glycine equivalent control the stereochemistry at the α-carbon. The use of an enantiotopically deuterated electrophile introduces asymmetry at the β-carbon provided an S_N2 mechanism.

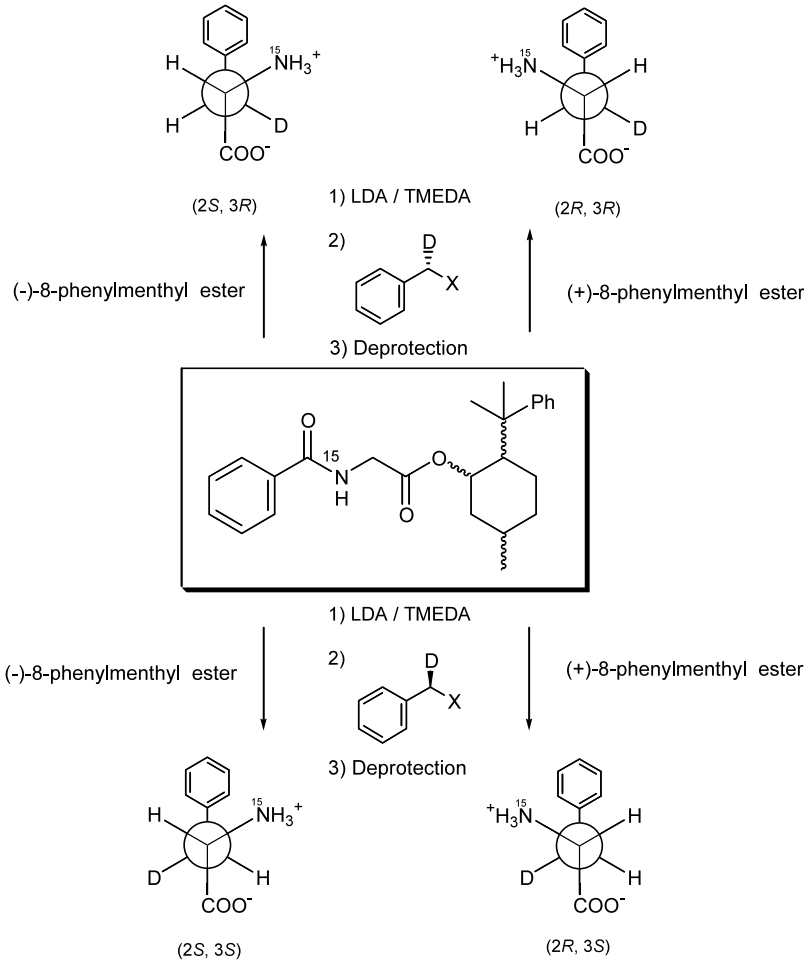


Figure 2. Synthesis of stereoselectively β-deuterated ^{15}N-labeled phenylalanines using 8-phenylmenthylhippurates as chiral glycine equivalents.

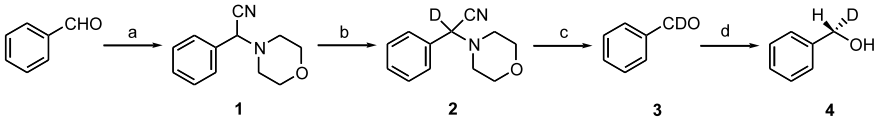
doubly labeled phenylalanine derivative (Fig. 2). The use of the enantiomeric 8-phenylmenthyl alcohols would allow the generation of both 2-position epimers. Additionally, the chirality of the electrophile would impact the stereochemistry at the benzylic position, permitting both 3-position epimers to be synthesized. However, incorporating and maintaining chirality at the sensitive benzylic position throughout the synthesis was a concern. Therefore, the synthesis of the chirally deuterated electrophile and its properties in the ensuing alkylation chemistry were of paramount importance.

Initial efforts focused on investigating electrophile reactivity in hopes of optimizing the yield and stereoselectivity of the alkylation reaction. Model reactions were performed using several unlabelled benzylic electrophiles and (−)-8-phenylmenthylhippurate. This model study established benzyl bromide as the most reactive electrophile of those investigated, alkylating the chiral enolate at −78°C with high stereoselectivity. Benzyl mesylate and benzyl chloride each alkylated the chiral substrate at −42 and −15°C, respectively, with equally good stereoselectivity at the α-carbon. In all cases, the alkylation products were obtained in approximately 90–92% de with the (2*S*)-epimer being preferred as expected.[41] Benzyl tosylate did not undergo the alkylation even at room temperature, perhaps due to increased steric congestion. These results suggested that the two halides and mesylate were candidates for further studies as deuterated electrophiles.

Scheme 1 depicts the synthesis of (*S*)-(+)-benzyl-α-*d* alcohol **4**, which served as the precursor for deuterated electrophile synthesis. This strategy, which was based on the generation of a deuterated aldehyde[42] and its subsequent asymmetric reduction with a chiral borane reagent,[43] was the method of choice for several reasons. The chemistry proceeded exceptionally well on a large scale with excellent deuterium incorporation and represented a simple way to obtain these materials in a cost effective manner. Treating benzaldehyde with morpholine perchlorate and KCN gave morpholineacetonitrile **1**. Proton-deuteron exchange was accomplished by reaction of **1** with NaH and D_2O to yield the deuterated analog **2** with 99% ^2H incorporation as determined by ^1H NMR spectroscopy. Acid hydrolysis gave **3** which was then reduced asymmetrically with (*R*)-Alpine Borane® to yield **4** with greater than 96% ee as determined by ^1H NMR analysis of the (−)-camphanate ester.[44] It was critical to avoid degradation of the benzylic stereogenic center in the subsequent conversion to an electrophilic species. Therefore, sulfonate esters and halides obtained via stereospecific processes[45,46] were investigated as potential electrophiles.

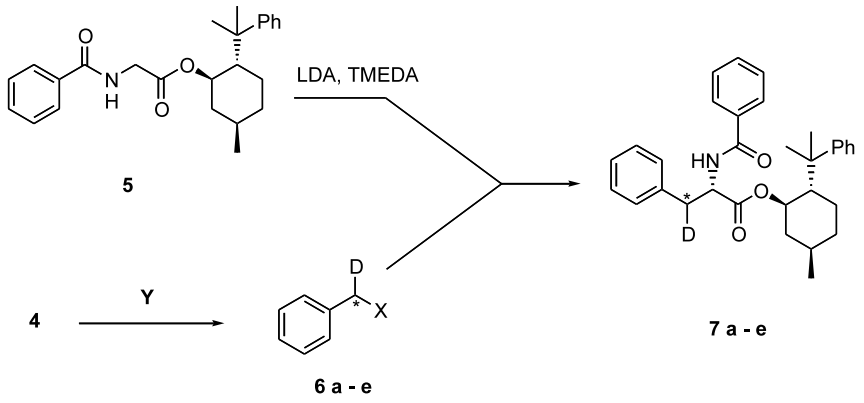
It was believed that the bromide would be the preferred electrophile due to its greater reactivity. This would result in a higher chemical yield of the amino acid derivative and, perhaps, enhanced stereoselectivity at C3. However, use of any of the halides for the alkylation reaction would require two formal inversions of configuration at the benzylic center, one for the functional group interconversion from **4** and one for the alkylation reaction. The use of the mesylate derivative would have the apparent advantage of only requiring one formal inversion during the process. However, the impact, if any, of its attenuated reactivity in the alkylation reaction on C3 stereochemistry in the product was unknown. Deuterated electrophiles **6a–d** (Table 1) were prepared from **4** by halogenation reactions known to proceed with inversion of configuration[47–49] and **6e** was synthesized by methyl sulfonate ester formation. The labeled electrophiles were used to alkylate (−)-8-phenylmenthylhippurate **5** and the products **7** were analyzed by ^1H NMR spectroscopy to investigate C2 and C3 stereochemistry.

The first electrophile investigated was the labeled benzyl bromide **6a**, prepared using triphenylphosphine and carbon tetrabromide.[48] Unfortunately, product **7a** showed a 1:1 ratio of stereoisomers at the benzylic position as assessed by NMR spectroscopy (Table 1, Fig. 3b). Likewise the use of **6b**, the labeled bromide prepared using modified Mitsunobu conditions,[49] resulted in similarly poor selectivity in the formation of **7b**. However, **7c** obtained from the chloride prepared using triphenylphosphine and carbon tetrachloride, showed a slight stereoselectivity (3*R*/3*S* = 2:3). Investigating other chlorination conditions to perhaps enhance this modest selectivity, the modified Mitsunobu conditions were employed to generate **6d**, which resulted in a selectivity of approximately 9:1 in favor of the (3*S*)-epimer of **7d** (Table 1, Fig. 3c). It was expected that mesylate **6e** would yield the (3*R*)-epimer of **7** as the major product since **6e** was of opposite configuration from **6d**. Indeed, **7e** showed a similar degree of selectivity (approximately 9:1) in favor of the (3*R*)-epimer (Table 1, Fig. 3d). These results were very encouraging and demonstrated that a high degree of stereoselectivity could be introduced at the β-carbon while constructing the C2–C3 bond to give the desired α-carbon stereoisomer. The racemization observed in **7a**/**b** synthesized from the bromides may be due to the inherent enhanced reactivity of the electrophile. It is feasible to suggest that the labeled benzyl bromide was reactive enough to undergo bromide displacement reactions that ultimately resulted in racemization of the electrophile. Examination of the literature revealed that other researchers have observed similar findings using the triphenylphosphine and carbon tetrabromide bromina-



Scheme 1. Synthesis of **4**. *Reagents and conditions*: (a) Morpholine, perchloric acid, KCN, (91%); (b) NaH, D_2O, (99%, 99% ^2H incorporation); (c) 2N HCl, (93%); (d) (*R*)-Alpine Borane®, (87, 96% ee).

Table 1. Evaluation of deuterated electrophiles



E^+	Y	X	Configuration of **6a–e**	Alkylation reaction temp. (°C)	Product	(3*R*):(3*S*) in **7a–e**
6a	Ph_3P/CBr_4	Br	*R*	−78	**7a**	1:1
6b	Mitsunobu	Br	*R*	−78	**7b**	1:1
6c	Ph_3P/CCl_4	Cl	*R*	−15	**7c**	2:3
6d	Mitsunobu	Cl	*R*	−15	**7d**	1:9
6e	$DMAP/MsCl/NEt_3$	OMs	*S*	−42	**7e**	9:1

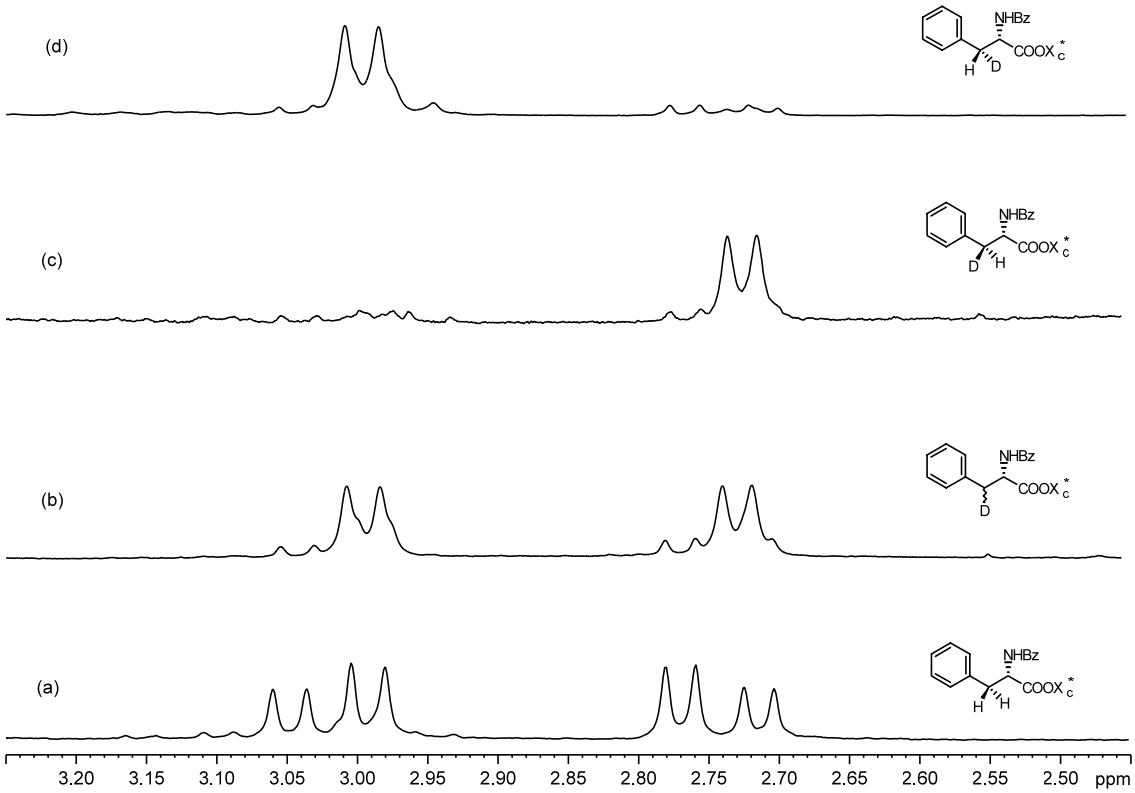
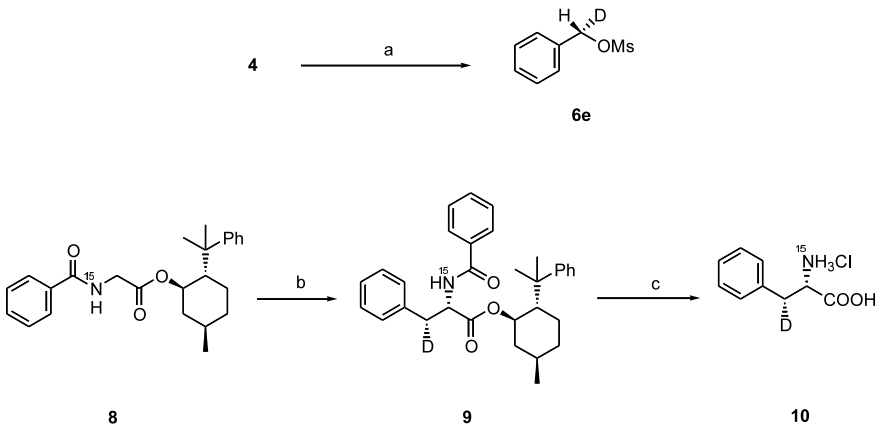


Figure 3. Benzylic region of ^1H NMR spectra of **7** (400 MHz) $X_c^* = (-)$-8-phenylmenthyl. (a) Undeuterated compound from authentic L-phenylalanine. (b) Compounds **7a** and **7b**, showing a 1:1 ratio of stereoisomers at β-carbon. (c) Compound **7d**, showing (3*R*/3*S*) of approximately 1:9. (d) Compound **7e**, showing (3*R*/3*S*) of approximately 9:1.

tion of optically active alcohols.[50] The attenuated reactivity of the chlorides and mesylate may have been sufficient enough to prevent this racemization, which ultimately led to the observed stereoselectivity. Based on these results, the mesylate was selected as the electrophile for the synthesis of the doubly labeled material, however, these results clearly indicate that the labeled chloride could be used to generate the 3-epimer.

Shown in Scheme 2 are the key steps in the synthesis of (2*S*,3*R*)-[3-^{2}H,^{15}N]-phenylalanine hydrochloride **10**. ^{15}N-(−)-8-Phenylmenthylhippurate **8** was obtained from



Scheme 2. Synthesis of **10**. *Reagents and conditions*: (a) DMAP, MsCl, NEt_3, THF, 0°C, (86%); (b) i. 2 equiv. LDA, 2 equiv. TMEDA, THF, −78°C, ii. 1.1 equiv. **6e**, −42°C, 12 h, (89%); (c) 6N HCl, reflux in sealed bottle, 36 h, (86%).

the benzoylation of ^{15}N-glycine followed by esterification with (−)-8-phenylmenthol.[51] Mesylate **6e** was obtained from **4** in 86% yield using DMAP catalyzed conditions. Treatment of **8** with 2 equiv. of LDA and TMEDA followed by the addition of **6e** afforded the doubly labeled alkylation product **9** in 89% yield with 92% de at C2 and 74% de at C3.

In early model reactions with unlabelled compounds, the (2*R*)-epimer of the alkylation product was obtained in diastereomerically pure form from the alkylation product mixture by crystallization from hexanes/ethyl acetate and was used as a standard for HPLC analyses. Thus, fractional crystallization represents a way to obtain further enriched mixtures of the (2*S*)-epimer of **9**.

Acid hydrolysis afforded **10**, which was purified by recrystallization from an isopropanol/water mixture (Fig. 4). The hydrolysis of the hindered benzamide of **9** proved to be problematic requiring long reaction times under reflux conditions in a closed vessel. Stereochemical analysis of the final product **10** revealed only partial degradation of the enantiomeric purity at the α-carbon relative to **9**. Analysis of the (−)-camphanamide methyl ester[52] of **10** by HPLC showed 76% de at the 2-position. Deuterium NMR studies of the (−)-camphanamide derivative (free acid) of **10** revealed a 72% de at the 3-position which is consistent with **9**. The loss of stereochemical integrity at the α-carbon during the hydrolysis was attributed to the harsh conditions employed in the deprotection.

3. Conclusions

A simple yet versatile strategy for the chemical synthesis of ^{15}N-labeled stereoselectively β-deuterated amino acids has been presented. These labeled compounds are valuable research tools that find use in NMR or in mechanistic enzymology studies. The utility of the method was demonstrated by the synthesis of **10**. The key step in the synthesis was the alkylation of an isotopically labeled glycine equivalent with an enantiotopically deuterated electrophile. Several deuterated benzylic electrophiles were evaluated in the alkylation reaction and it was determined that **6d**/**e** resulted in higher stereoselectivities at C3 in **7** than did **6a**/**b**. These results suggest that the alkylation reaction proceeds predominantly by an S_N2 type mechanism since much of the optical activity present in **4** is ultimately transferred into the alkylation products. These results also demonstrate that asymmetry can be introduced at C3 during the stereoselective construction of the C2–C3 bond.

The versatility of the strategy allows for the synthesis of all stereoisomers at the α and β carbons as well as for the incorporation of different isotopic labeling patterns through the use of isotopically labeled glycine equivalents. Efforts are underway to further optimize and extend this strategy to other β-methylene unit containing amino acids. The current deprotection protocol resulted in partial racemization at the α-stereocenter. It should be possible to avoid this stereochemical degradation by the use of alternative deprotection condi-

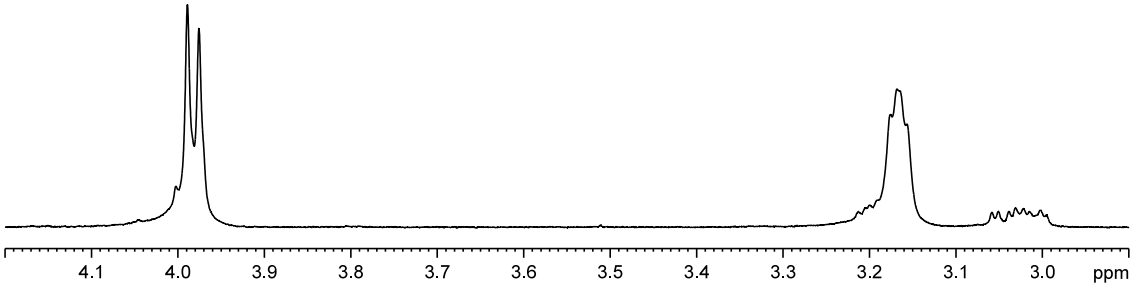


Figure 4. Partial ^{1}H NMR (400 MHz) spectrum of **10**.

tions. In addition, alternative glycine equivalents are being investigated in an effort to further enhance the stereoselectivity of the alkylation reaction.

4. Experimental

4.1. General methods

Cambridge Isotope Laboratories supplied ^{15}N-glycine and D_2O. All moisture sensitive reactions were performed using oven or flame-dried glassware cooled under argon and were maintained under an argon atmosphere. Anhydrous THF was obtained by distillation from sodium/potassium benzophenone ketyl immediately prior to use. Lithium diisopropylamide concentrations were determined by titration against diphenylacetic acid. Thin layer chromatography was performed using silica gel 60 F_{254} aluminum-backed plates. Column chromatography was performed using silica gel 60 (70–230 mesh). Melting points were determined using a Thomas–Hoover capillary apparatus and are uncorrected. Analytical scale HPLC analyses were performed using a Beckman Ultrasphere ODS column (4.6×250 mm) using UV detection. Semi-preparative HPLC was performed using a Zorbax ODS column (9.4×250 mm) with UV detection. Infrared spectra were acquired as films on AgCl plates. Nuclear magnetic resonance spectra were acquired at 400 MHz (^{1}H) and 100 MHz (^{13}C) unless otherwise indicated and referenced to non-deuterated solvent resonances. ^{15}N NMR spectra were recorded at 40.5 MHz and referenced externally to a ^{15}N-glycine sample in D_2O (31.5 ppm). Deuterium NMR spectra were recorded at 92 MHz. Mass spectrometry was performed using a Micromass QTOF spectrometer.

4.2. α-Phenyl-4-morpholineacetonitrile, 1[42]

Perchloric acid (70%; 9.5 mL, 0.11 mol) was added dropwise to a stirred solution of morpholine (20 mL) at 0°C followed by the addition of benzaldehyde (11.5 g, 0.11 mol). The mixture was heated to 70°C for 4 h after which time, a solution of KCN (7.8 g, 0.12 mol; 5 mL H_2O) was added. After heating to 90°C for 1 h, the contents were poured onto ice with stirring. The resulting light yellow precipitate was collected, washed with water, and recrystallized from absolute ethanol to yield white needles (18.8 g, 91%): mp 67–68°C (lit. 68–70°C);[53] IR (cm^{-1}): 1117 (s), 2857 (m), 1453 (m), 1007 (m), 866 (m), 701 (m), 2227 (w); ^{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): $[M+Na]^+$ calcd for $C_{12}H_{14}N_2ONa$ 225.1004; found 225.0991.

4.3. α-Phenyl-4-morpholineacetonitrile-α-*d*, 2[54]

Sodium hydride (95%; 2.5 g, 99 mmol) was carefully added to a THF solution of **1** (10 g, 50 mmol; dried under high vacuum at 40°C for 12 h) and the mixture was heated to 40°C for 1 h. The reaction mixture was cooled to 0°C and D_2O (15 mL, 0.83 mol) was carefully added. The mixture was then stirred for 30 min at 0°C, acidified (pH 1–2) by the addition of freshly distilled $SOCl_2$, and poured onto ice with stirring. The resulting white precipitate was collected, washed with water, and dried to yield a white crystalline solid (9.91 g, 99%; 99% ^{2}H incorporation determined by ^{1}H NMR): mp 67–68°C (lit. 69–70°C);[42] IR (cm^{-1}): 1118 (s), 2864 (m), 1453 (m), 1015 (m), 862 (m), 700 (m), 2229 (w); ^{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, 128.97, 132.46; HRMS-ES (m/z): $[M+Na]^+$ calcd for $C_{12}H_{13}DN_2ONa$ 226.1082; found 226.1070.

4.4. Benzaldehyde-formyl-*d*, 3

A mixture of **2** (5 g, 25 mmol) and 2N HCl (60 mL) was heated under reflux for 12 h under an argon atmosphere after which the resulting two phase mixture was cooled to room temperature and extracted with diethyl ether. The combined organic phases were then washed with saturated $NaHCO_3$, water, and brine. The organic solution was dried over $MgSO_4$, filtered, and concentrated under reduced pressure at ambient temperature to yield a slightly yellow oil that was used without further purification (2.45 g, 93%; 98% ^{2}H incorporation determined by ^{1}H NMR): ^{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).

4.5. (*S*)-(+)-Benzyl-α-*d* alcohol, 4[43]

A THF solution of *R*-Alpine-Borane® (0.5 M, 70 mL; 35 mmol) was added to **3** (2.3 g, 21.5 mmol). The mixture was stirred at room temperature for 12 h and then heated under reflux for 1.5 h. After cooling to room temperature, acetaldehyde (5 mL) was added and the mixture was stirred for 30 min after which the solvent was removed. Some of the pinene by-products were then removed using a rotary evaporator under high vacuum at 50°C for 5 h. The remaining orange oil was dissolved in diethyl ether (75 mL) and cooled to 0°C. Ethanolamine (2.1 g, 35 mmol) was added and the mixture was stirred for 30 min at 0°C during which time a white solid precipitated from the mixture. The mixture was filtered and washed with diethyl ether. The filtrate was concentrated and the resulting oil was dissolved in 10% aq. MeOH and washed several times with heptane. Isopropanol was added to the methanolic fraction and the solution was concentrated on a rotary evaporator. The resulting yellow oil was chromatographed on silica gel (95:5 hexanes/ethyl acetate) to yield 2.0 g of colorless oil (87, >96% ee): α_D^{21} +1.2° neat);[43] IR (cm^{-1}): 3349 (s), 2924 (s), 1452 (s), 1406 (s), 1320 (s), 1043 (s), 1024 (s), 723 (s), 697 (s), 3029 (m), 1205 (m), 2137 (w), 1949 (w); ^{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, 127.23, 128.21, 140.64; HRMS-EI (70 eV) m/z: M^+ calcd for C_7H_7DO 109.0637; found 109.0569.

4.6. Stereochemical analysis of 4

The enantiomeric purity of **4** was determined using ^{1}H NMR spectroscopy of the (−)-camphanate ester derivative.[44] Briefly, **4** was treated with excess (1*S*)-(−)-camphanic chloride and K_2CO_3 in toluene. The product was purified using preparative TLC and analyzed by NMR: ^{1}H NMR (800 MHz, $CDCl_3$, δ): 5.35 (broad s, major), 5.39 (broad s, minor), major/minor >98:2.

4.7. (*S*)-(+)-Benzyl-α-*d* mesylate, 6e

Methanesulfonyl chloride (0.66 mL, 8.5 mmol) was added to a THF solution of **4** (750 mg, 6.87 mmol), DMAP (84 mg, 0.69 mmol), and NEt_3 (1.43 mL, 10.3 mmol) at 0°C. The reaction mixture was stirred at 0°C for 1 h and then diluted with water and extracted with ethyl acetate. The solvent was evaporated and the crude product was chromatographed on silica gel (1:1 hexanes/ethyl acetate) to yield 1.1 g of colorless oil (86%): $[\alpha]_D^{21}$ +0.5 (*c* 12.8, ethyl acetate); ^{1}H NMR (250 MHz, $CDCl_3$, δ): 2.87 (s, 3H), 5.21 (t, 1H, $J=1.7$ Hz), 7.35–7.45 (m, 5H); ^{13}C NMR (62.9 MHz, $CDCl_3$, δ): 38.39, 71.23 (t, $J=23.1$ Hz), 128.90, 129.42; HRMS-ES (*m*/*z*): $[M+Na]^+$ calcd for $C_8H_9DSO_3Na$ 210.0327; found 210.0319.

4.8. (2*S*,3*R*)-*N*-Benzoyl-[3-^{2}H,^{15}N]-phenylalanine-(−)-8-phenylmenthyl ester, 9

Diisopropylamine (0.43 mL, 3.05 mmol) was added to a THF solution of 2.5 M *n*-BuLi (1.22 mL, 3.1 mmol) at −78°C with stirring. After 15 min, TMEDA (0.46 mL, 3.1 mmol) was added and the mixture was allowed to warm to room temperature. After cooling back to −78°C, a THF solution of **8** (600 mg, 1.52 mmol) was added via cannula. The resulting yellow solution was stirred at −78°C for 1 h and then warmed to −42°C. A THF solution of **6e** (300 mg, 1.60 mmol) was then added via cannula and the reaction mixture was stirred at −42°C for 12 h. The reaction was quenched at −42°C with 1N HCl (10 mL) and allowed to warm to room temperature. After stirring for 30 min, the phases were separated and the aqueous phase was extracted with ethyl acetate. The organic phases were combined, washed with water, diluted with isopropanol, and concentrated to give an orange oil. HPLC and ^{1}H NMR analysis of the crude product revealed 92% de at the 2-position with the (2*S*)-epimer as the major product and 74% de at the 3-position. The crude product was partially purified on silica gel (85:15 hexanes/ethyl acetate) yielding **9** as a mixture of the (2*S*)- and (2*R*)-epimers (660 mg, 89%). A portion of the mixture was then further purified using semi-preparative HPLC (80% MeOH/H_2O) to yield 83 mg of a white solid which was used for all analytical measurements: $[\alpha]_D^{21}$ −12.1 (*c* 4.1, ethyl acetate); HPLC (85% MeOH/H_2O, $\lambda=254$ nm) $t_{R(2S,3R/S)}=20.0$ min, $t_{R(2R,3R/S)}=15.8$ min; IR (cm^{-1}): 2955 (s), 2924 (s), 1729 (s), 1666 (s), 1479 (s), 700 (s), 3360 (m), 3060 (m), 1602 (m), 1497 (m), 1368 (m), 1210 (m); ^{1}H NMR ($CDCl_3$, δ): 0.81–1.02 (m, 5H), 1.03–1.31 (m, 8H), 1.68 (d, $J=12.5$ Hz, 1H), 1.83 (dt, $J=13.8$ Hz, 3.4 Hz, 2H), 2.09 (dt, $J=11.3$ Hz, 3.4 Hz, 1H), 2.70–2.79 (m, residual CH), 3.00 (d, $J=5.8$ Hz, 1H), 4.23 (dd, $J=7.5$ Hz, 6.5 Hz, 1H), 4.81 (dt, $J=10.8$ Hz, 4.3 Hz, 1H), 6.29 (dd, $J=91.1$ Hz, 7.5 Hz, 1H), 7.00–7.69 (m, 15H); ^{13}C NMR ($CDCl_3$, δ): 21.73, 23.89, 26.45, 28.85, 29.31, 31.29, 34.48, 36.91 (t, $J=19.2$ Hz), 39.49, 41.58, 50.41, 53.16, 53.29, 76.21, 125.25, 125.41, 126.66, 126.98, 128.08, 128.23, 128.51, 129.53, 131.49, 134.34, 134.43, 136.20, 151.54, 166.52 (d, $J=16.2$ Hz), 170. 83; ^{15}N NMR ($CHCl_3$, δ): 112.01 (d, $J=91.1$ Hz); HRMS-ES (*m*/*z*): $[M+Na]^+$ calcd for $C_{32}H_{36}D^{15}NO_3Na$ 508.2828; found 508.2806.

4.9. Stereochemical analysis of 9

Retention times for HPLC analyses were determined using phenylalanine standards prepared from authentic L- and D,L-phenylalanine. The amino acids were benzoylated and the crude products esterified with (−)-8-phenylmenthol.[51] Enantiomerically pure (2*R*)-epimer was obtained from fractional crystallization of the racemate mixture containing solution.

4.10. (2*S*,3*R*)-[3-^{2}H, ^{15}N]-Phenylalanine hydrochloride, 10

Chromatographed **9** was heated with 6N HCl under reflux in a sealed vessel for 36 h after which the reaction mixture was extracted with ethyl acetate. TLC analysis of the organic phases revealed unreacted **9**, which was isolated and re-submitted to the hydrolysis conditions. This process was repeated until the organic phase was devoid of starting material. The aqueous phases were pooled, diluted with isopropanol, and concentrated to give crude **10** which was recrystallized from isopropanol/water (86%): mp 190–200°C dec.; $[\alpha]_D^{20}$ −10.75 (*c* 5.0, H_2O); ^{1}H NMR (D_2O, δ): 2.98–3.04 (m, residual 1H), 3.14 (broad triplet, 1H), 3.96 (d, $J=5.2$ Hz, 1H), 7.16–7.29 (m, 5H); ^{13}C NMR (D_2O/MeOH, δ): 36.34 (t, $J=19.6$ Hz), 55.92 (d, $J=7.7$ Hz), 128.47, 129.80, 130.00, 135.22, 173.52; ^{15}N NMR (D_2O, δ): 39.80; HRMS-ES (*m*/*z*): $[M-Cl]^+$ calcd for $C_9H_{11}D^{15}NO_2$ 168.0901; found 168.0897.

4.11. Stereochemical analysis of 10

The enantiomeric purity of **10** was assessed using the (−)-camphanamide methyl ester derivative.[52] HPLC analysis of the derivatized material showed a 76% de at the 2-position, and deuterium NMR studies revealed a 72% de at the 3-position.

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