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Improved synthesis of (*R*)-glycine-*d*-^{15}N

Joel R. Walker and Robert W. Curley, Jr.*

Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA

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Abstract—Previously, we have synthesized the title glycine to permit assignment of the prochiral α-protons of glycine residues in the NMR study of the protein FKBP. A key, and low yielding step in this synthesis occurs in the ruthenium tetraoxide mediated degradation of *N*-*t*-Boc-*p*-methoxybenzyl amine to *N*-*t*-Boc-glycine. Efforts to improve this key step by exploring different substrates and *N*-protecting groups were successful to render this synthesis amenable for the large scale production of (*R*)-glycine-*d*-^{15}N.

1. Introduction

Stable isotope labeled amino acids are useful tools for protein structure elucidation and mechanistic enzymology studies. For processes involving metabolism of amino acids, these labeled analogs can aid in the elucidation of biochemical pathways.[1] For protein structural studies, these incorporated labeled residues can be selectively analyzed using isotope edited NMR experiments.[2,3] Deuterium can be incorporated to observe the absence of signal, thus simplifying proton resonance assignments.[4] Also, when a deuteron is stereospecifically substituted for a proton, interpretations of structural elements within a protein (or of a ligand binding in that area) can be achieved.[5,6]

Glycine is an amino acid that plays an important role in defining protein secondary structure and is involved in many metabolic processes. A stereospecifically deuterated analog has been used to elucidate many enzyme mechanisms and biosynthetic pathways.[7–12] Also, stereospecifically labeled glycine can aid in examining the conformation of proteins or large molecules.[13] Glycine doubly labeled with ^{2}H and ^{15}N is even more useful. For example, (*R*)-glycine-*d*-^{15}N has been previously synthesized by our group for use in structural studies of the FK-506 binding protein.[14] Uniformly labeled ^{15}N and (*R*)-glycine-*d*-^{15}N FKBP were prepared and used in ^{15}N-edited TOCSY (TOtally Correlated SpectroscopY) experiments in order to assign the resonances of the prochiral methylene protons of glycine residues.

Several published methods exist to produce singly labeled, stereospecifically deuterated glycines. Some involve utilizing the naturally occurring chiral pool such as other amino acid derivatives,[15–17] carbohydrates,[18,19] or natural products[20] as starting materials. Other procedures include a combination of chemical and enzymatic methods to incorporate deuterium and chirality, respectively.[21–26] It has also been shown that cobalt complexes of amino acids can be used to synthesize chiral glycine and other labeled amino acids.[27,28] The synthetic route that we previously used to synthesize (*R*)-glycine-*d*-^{15}N was adapted from Woodard and co-workers.[29] This route can be used to obtain either enantiomer of glycine-*d* through the use of a chiral reducing reagent. A key step in the synthesis requires a ruthenium tetraoxide oxidative degradation of a *para*-methoxyphenyl ring to the carboxylic acid functionality of the eventual glycine. However, in our hands, the reported oxidation conditions yielded variable and low quantities of product and numerous by-products. Because the oxidation proceeded poorly and both isotope labels had already been incorporated prior to this step, we have investigated this process and now report a much improved procedure for this synthesis.

2. Results and discussion

2.1. Previous work

Due to an interest in applying doubly labeled glycine to other protein structural problems, additional title compound was required. The details of the synthetic route are shown in Scheme 1. Starting with *p*-anisaldehyde (**1**), incorporation of deuterium was achieved by using the Umpolung technique to give **3** with >97% deuterium incorporation. Following hydrolysis, stereochemistry is introduced by reduction of **4** using a chiral reducing agent, Alpine–Borane,®[30] to produce the (*S*)-alcohol **5** (76% yield from **1**). Analysis of enantiomeric purity of the alcohol-*d* was made possible by derivatization to Mosher's ester,[31,32] which showed 96% de by ^{1}H NMR analysis of the benzylic

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* Corresponding author. Tel.: +1-614-292-7628; fax: +1-614-292-2435; e-mail: curley.1@osu.edu

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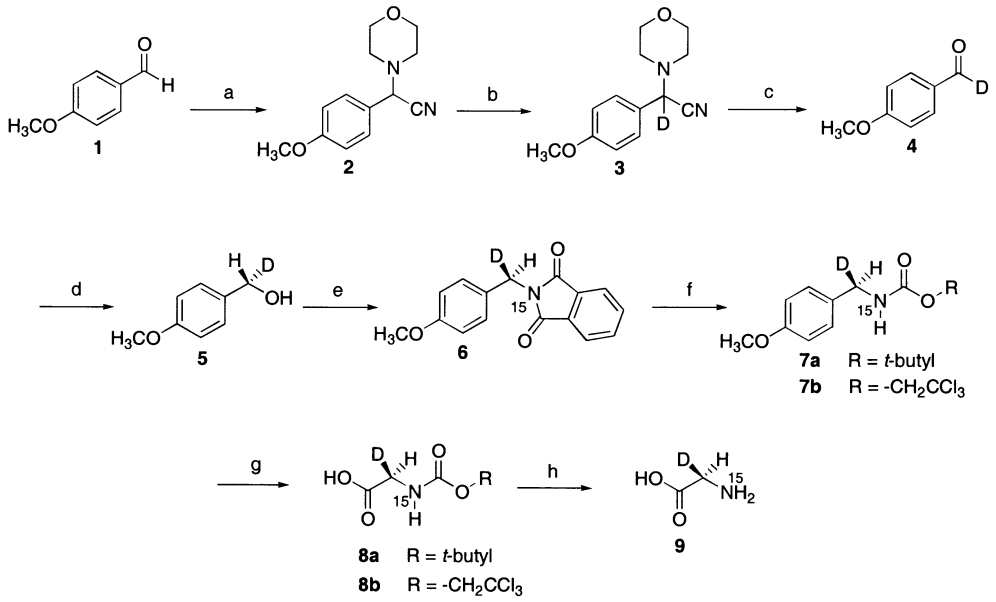


Scheme 1. *Reagents and conditions*: (a) (i) morpholine, $HClO_4$; (ii) KCN, 90°C, 96%; (b) (i) NaH, THF; (ii) D_2O, 93%; (c) 2N HCl, Δ, 95%; (d) *R*-Alpine–Borane®, THF, 90%; (e) Ph_3P, DEAD, ^{15}N-phthalimide, THF, 60%; (f) (i) $NaBH_4$, AcOH, PrOH, Δ; (ii) for **7a**, $(CH_3COOC)_2O$, Na_2CO_3, dioxane, H_2O, 88%; for **7b**, 2,2,2-trichloroethyl chloroformate, Na_2CO_3, dioxane, H_2O, 88%; (g) for **8a**, $RuCl_3{\cdot}H_2O$, $NaIO_4$, CH_3CN, CCl_4, H_2O, 10%; for **8b**, $RuCl_3{\cdot}H_2O$, H_5IO_6, CH_3CN, CCl_4, H_2O, 62%; (h) from **8a**, (i) trifluoroacetic acid, CH_2Cl_2; (ii) DOWEX 50W, 50%, from **8b**; (i) Zn, AcOH, H_2O; (ii) AG 1, HCl (iii) AG 50W, NH_4OH, 73%.

methylene proton region. Fig. 1a shows the coupling pattern of three materials: the doubly protonated benzylic contaminant, the *R*-diastereomer, and the major *S*-diastereomer. Incorporation of ^{15}N was accomplished with ^{15}N-phthalimide in modest yield (60%) to give the conjugate **6**. The amino-protecting group was exchanged to the *t*-butyl carbamate, which is stable to subsequent oxidation chemistry, to give **7a**.

The protected benzylamine **7a** was subjected to a ruthenium tetraoxide oxidation. The oxidation conditions that were used mimicked those described previously.[29,33] In summary, the ruthenium source was ruthenium trichloride hydrate (2.2 mol%), the stoichiometric re-oxidant was sodium periodate (18 equiv.), and the solvent mixture of CH_3CN/CCl_4/H_2O (2:2:3) were used to obtain the carboxylic acid product in good yields. Unfortunately in our hands, the doubly labeled glycinate was never obtained in greater than 10% yield due to extensive benzylic oxidation and, thus, investigations toward improving the oxidation reaction were initiated.

2.2. Ruthenium tetraoxide oxidation

The oxidation of organic compounds with ruthenium tetraoxide (RuO_4) to give a carboxylic acid functionality is commonly employed.[34] Some common aromatic systems that serve as carboxylic acid precursors are a furan ring and an unsubstituted phenyl ring. In our case, the furan ring is not useful due to its instability in strongly acidic conditions used in the formation of the morpholinonitrile compounds. A phenyl ring possessing a homobenzylic heteroatom is also not desirable because of its susceptibility to benzylic oxidation.[33,35] Since our reassessment of the utility of the *para*-methoxyphenyl ring was disappointing, we looked at other types of aromatic systems, namely the 1- and 2-naphthyl rings, which were claimed to be superior to other systems in the oxidation reaction.[36]

2.3. Aromatic oxidation study

In order to investigate the behavior of 1- and 2-substituted naphthyl rings in the ruthenium oxidation, unlabeled *N*-protected naphthyl amines **11**–**12** were synthesized (Scheme 2). For comparison, unlabeled *N*-*t*-Boc-(4-methoxybenzyl) amine **13** was also synthesized from the analogous benzylamine.

Various oxidation conditions were used to determine the optimal ring system and reaction environment. Three catalytic ruthenium reagents were investigated at different temperatures using sodium periodate as the ruthenium re-oxidant; *cis*-$[Ru(bpy)_2Cl]{\cdot}2H_2O$ (bpy=2,2′-bipyridine), $RuO_2{\cdot}2H_2O$, and $RuCl_3{\cdot}2H_2O$. The bipyridine ruthenium complex is a thermally stable ruthenium complex, which enabled refluxing reactions condtions.[37] Ruthenium dioxide hydrate was claimed to be superior to the chloride form for the oxidation of aromatic rings to carboxylic acids.[38] These reagents were compared to the original ruthenium form used, ruthenium chloride hydrate. The reactions were semi-quantitatively monitored for formation of product, *N*-*t*-Boc-glycine **14** (Scheme 2), by examination of ^{1}H NMR spectra for extent of reaction and side product formation.

Table 1 is a summary of the results of the initial oxidation condition study (entries 1–12) with various substrates. Entries 1–4 show experiments with the bipyridine ruthenium complex at different temperatures and it was found to be unfavorable for any of the molecules **11**–**13**. The experiments with ruthenium chloride (entries 5–8) show similar results to the ruthenium dioxide (entries 9–12) at different temperatures. As far as determining which ring

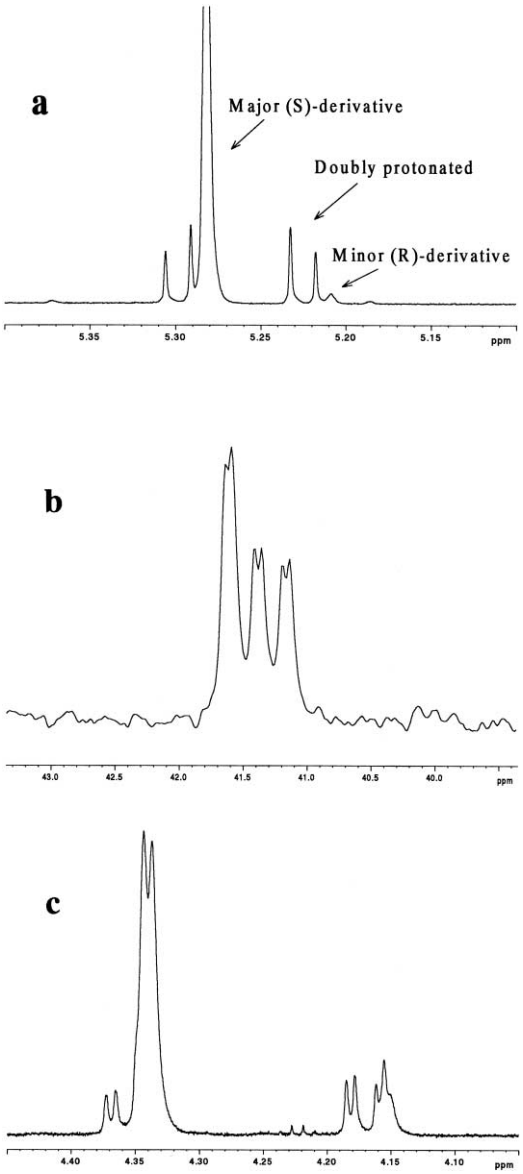


Figure 1. (a) Partial ^{1}H (800 MHz) spectrum of the benzylic region of the (*R*)-(−)-MTPA ester of (*S*)-**5**. (b) Partial ^{13}C spectrum of the methylene region of (*R*)-glycine-*d*-^{15}N. (c) Partial ^{1}H (800 MHz) spectrum of the methylene region of (1*S*)-(−)-camphanamide of (*R*)-glycine-*d*-^{15}N.

Table 1. Ruthenium oxidation of aromatic substrates

Entry	Compound	Oxidation conditions[a]	Results[b]
1	**11**	**1a**	No reaction
2	**11**	**1b**	− − −[c]
3	**12a**	**1b**	−
4	**13**	**1b**	+[d]
5	**11**	**2a**	− −
6	**12a**	**2a**	+
7	**12a**	**2b**	+
8	**13**	**2b**	+ +
9	**11**	**3a**	−
10	**12a**	**3a**	+
11	**12a**	**3b**	+ +
12	**13**	**3b**	+
13	**13**	**4**	− −
14	**12b**	**4**	−
15	**7b**	**4**	+ + + +

[a] Oxidation conditions: (**1a**) *cis*-$[Ru(bpy)_2Cl]\cdot 2H_2O$, $NaIO_4$, CH_3CN, H_2O, 72 h, 35–40°C; (**1b**) *cis*-$[Ru(bpy)_2Cl]\cdot 2H_2O$, $NaIO_4$, CH_3CN, H_2O, 46 h, 80°C; (**2a**) $RuCl_3\cdot H_2O$, $NaIO_4$, CH_3CN, CCl_4, H_2O, 72 h, rt; (**2b**) $RuCl_3\cdot H_2O$, $NaIO_4$, CH_3CN, CCl_4, H_2O, 68 h, 40–45°C; (**3a**) $RuO_2\cdot H_2O$, $NaIO_4$, $NaHCO_3$, CH_3CN, CCl_4, H_2O, 72 h, rt; (**3b**) $RuO_2\cdot H_2O$, $NaIO_4$, $NaHCO_3$, CH_3CN, CCl_4, H_2O, 68 h, 40–45°C; (**4**) $RuCl_3\cdot H_2O$, H_5IO_6, CH_3CN, CCl_4, H_2O, 2 h, rt.

[b] The reactions were semiquantitatively monitored by weight of crude product, examination of ^{1}H NMR for the formation of product, *N*-*t*-Boc-glycine **14**, and by the complexity of the product mixture.

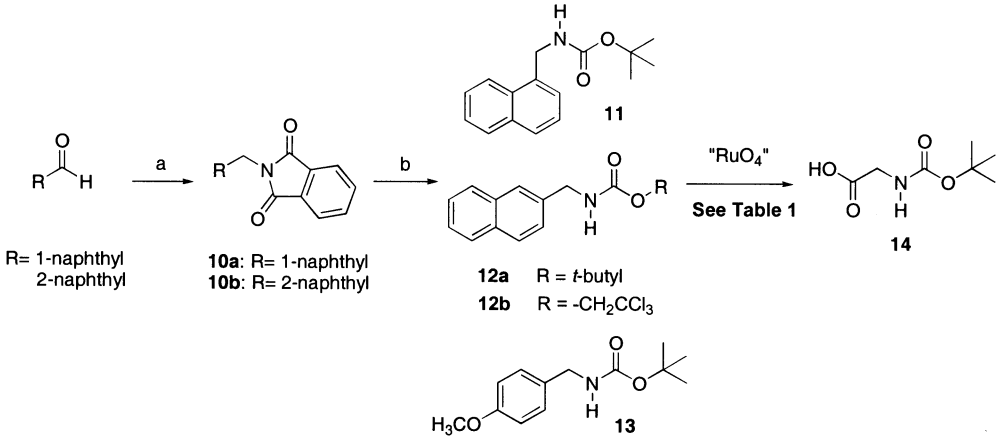
[c] Minus signs indicate increasing number of by-products and decreasing product.

[d] Plus signs indicate increasing amount of product with decreasing by-protucts.

system was the optimal carboxylic acid precursor, the 1-naphthyl group was found to be poor (entries 2, 5 and 9) while the *p*-methoxyphenyl and 2-naphthyl rings were comparable in either RuO_2 or $RuCl_3$/sodium periodate conditions (entries 6–8 and 10–12). However, overall the results from this study revealed that none of the substrates **11**, **12a**, and **13** or their oxidation conditions gave the desired improvement to make this a generally applicable method for the production of doubly labeled glycine.

2.4. Acidic oxidation condition

Another published oxidation condition uses periodic acid instead of sodium periodate as the stoichiometric re-oxidant.[39]



Scheme 2. *Reagents and conditions*: (a) (i) $NaBH_4$, EtOH, H_2O; (ii) Ph_3P, DEAD, phthalimide, THF; 44% (**10a**), 55% (**10b**); (b) (i) $NaBH_4$, AcOH, PrOH, Δ; (ii) $(CH_3COOC)_2O$ or 2,2,2-trichloroethyl chloroforamate, Na_2CO_3, dioxane, H_2O; 83% (**11**), 80% (**12a**), 70% (**12b**).

This method is claimed to give the greatest yields and shortest reaction times for the oxidation of aromatic rings.[40] Entries 13–15 in Table 1 show the results of the ruthenium oxidation using H_5IO_6 as the re-oxidant. These conditions were previously unexplored due to the instability of the *t*-Boc protecting group in acidic conditions. Subjecting the *N*-*t*-Boc protected aryl amines to these conditions resulted in deprotection and the oxidation of the benzylic position to give the corresponding aryl amide (entry 13). Therefore, in order to use these conditions, the acid stable 2,2,2-trichloroethyl carbamate[41] protecting group was employed. Unlabeled *N*-(2-naphthyl methylamine)- and *N*-(*p*-methoxyphenyl methylamine)-2,2,2-trichloroethyl carbamates were synthesized and subjected to the acidic oxidation conditions to determine the optimal substrate. The 2-naphthyl analog (**12b**, entry 14) yielded a typical complex mixture as seen with the sodium periodate conditions. However, the *p*-methoxyphenyl analog (unlabeled **7b**, entry 15) yielded the product and very few by-products, a substantial improvement from the neutral, sodium periodate conditions.

Due to the apparent success using the unlabeled *p*-methoxyphenyl analog in the periodic acid method, the synthesis of (*R*)-glycine-*d*-^{15}N was repeated as shown in Scheme 1. The same chemistry as previously employed facilitated the incorporation of both ^{2}H and ^{15}N. Through the use of the acid stable amine protecting group, **7b** was synthesized and subjected to the acidic oxidation conditions to give **8b** in good yield (62% after chromatography). The cleavage of the trichlorocarbamate was performed with Zn dust in acetic acid and water.[42] Purification of the final product was performed with anion exchange followed by cation exchange chromatography in tandem to give **9** in 73% yield. The overall yield of the title compound is 18.2%, as opposed to 10.2% we originally reported.[14] Spectroscopic and physical characteristics confirmed the production of (*R*)-glycine-*d*-^{15}N. For example, the ^{15}N–^{13}C and ^{2}H–^{13}C one bond coupling can be seen in Fig. 1b. Chirality was demonstrated by analysis with circular dichroism spectropolarimetry. Analysis of enantiomeric purity of the chiral glycine was conducted by derivatization of the amine with (1*S*)-(−)-camphanic chloride.[43] ^{1}H NMR spectroscopy was used to determine the de which was 82% by integration of the glycine methylene proton region (Fig. 1c). This was in agreement with ^{2}H NMR analysis of the same derivative and is comparable to that previously obtained.[14]

3. Conclusions

Poor yields in the ruthenium oxidation led us to investigate the optimal conditions and optimal aromatic ring carboxylic acid precursor for the production of labeled glycine. The overall conclusions from the semi-quantitative ruthenium oxidation condition study was that the use of periodate salts for the stoichiometric re-oxidant is not favored for the oxidative degradation, regardless of the aromatic ring substrate. Subsequently, the optimal oxidation condition was found to be with periodic acid as the stoichiometric re-oxidant and the optimal substrate was the *N*-(*p*-methoxyphenyl methylamine)-2,2,2-trichloroethyl carbamate (**7b**). Since the oxidation reaction is a key step, optimization was paramount for the applicability of this synthesis. The presented synthesis of the doubly labeled glycine has been substantially improved and further characterized from our initial efforts and is applicable to large scale production with minimal cost for the incorporation of isotopes.

4. Experimental

4.1. General

Anhydrous THF and CH_2Cl_2 were obtained by distillation from sodium benzophenone ketyl and calcium hydride, respectively. Sigma–Aldrich supplied starting materials and reagents. Cambridge Isotopes Laboratories supplied isotope labeled reagents. TLC was performed on Merck silica gel 60 F_{254} aluminum plates. Column chromatography was performed with Merck silica gel 60 and reverse phase flash chromatography with Merck Lichroprep® RP-18. Ion exchange chromatography was performed using Bio-Rad AG 1 X8 and AG 50W X8 200–400 mesh. Double deionized, demineralized water was used in the resin preparation and elution. Melting points were determined using a Thomas–Hoover capillary apparatus and are uncorrected. Circular dichroism was performed uncalibrated on a JASCO J-500A spectropolarimeter and reported in molar ellipticity (degrees deciliter mol^{-1} decimeter^{-1}). Infrared spectra were recorded as films on silver chloride plates using a Nicolet Protégé 460. NMR spectra were recorded on a Bruker DRX 400 in $CDCl_3$, D_2O or acetone-d_6 unless otherwise noted and referenced to residual protio solvent. Standard ^{15}N-glycine in D_2O was used as an external reference for ^{15}N NMR (31.5 ppm). Analysis of the MTPA ester and the camphanamide derivatives were recorded on a Bruker DRX 800 in $CDCl_3$. Mass spectra were recorded on a Micromass QTOF electrospray mass spectrometer.

4.1.1. α-(*p*-Methoxyphenyl)-4-morpholine acetonitrile (2). To a 250 mL flask chilled to −15°C, was added 60 mL of morpholine and 28.4 mL (0.33 mol) of perchloric acid (70%) dropwise and stirred for 15 min. The flask was removed from the cold bath and 40.8 g (0.3 mol) of *p*-anisaldehyde was added. The mixture was heated to 60°C for 2 h. An aqueous solution of 21.45 g (0.33 mol) of potassium cyanide was then added in portions and the reaction heated to 90°C for 2 h. The mixture was cooled to ~40°C and then poured onto ice while stirring. Yellowish solid formed which was washed with ample water via vacuum filtration. Recrystallization (ethanol/water) yielded 67.2 g of white needles (96% yield), mp 79–80°C (lit. mp 81–82°C).[44] IR (cm^{-1}) 2965 (m), 1506 (s), 1255 (s), 1117 (s); ^{1}H NMR ($CDCl_3$) δ 2.53–2.57 (m, 4H), 3.69–3.71 (m, 4H), 3.80 (s, 3H, CH_3O), 4.73 (s, 1H (α-H), 6.90 (d, 2H, J=8.8 Hz), 7.41 (d, 2H, J=8.8 Hz); ^{13}C NMR ($CDCl_3$) δ 49.86, 55.34, 61.79, 66.61, 114.12, 115.35, 124.25, 129.31, 160.10; HRMS (ES) calcd for $C_{13}H_{16}N_2O_2$ (M+Na) 255.1109, found 255.1120.

4.1.2. α-(*p*-Methoxyphenyl)-4-morpholine acetonitrile-α-*d* (3). Under an argon atmosphere, a dry three-neck 250 mL flask equipped with a reflux condenser was charged with 22.4 g (0.097 mol) of **2** into 75 mL of dry THF. Under an argon envolope, 4.9 g (0.58 mol) of sodium hydride

(95%) was weighed and added to the reaction mixture in ~1.2 g portions. The gray solution was heated to 50°C for 6 h. After cooling the solution to 0°C, 17.3 mL (10 equiv.) of D_2O was carefully added dropwise and allowed to stir for 30 min. Thionyl chloride was then added dropwise until the mixture was slightly acidic (pH<5). The entire solution was poured onto ice and stirred until white solid precipitated. The solid was dried via vacuum filtration to give 20.9 g (93% yield), mp 79–80°C (lit. mp 81–82°C).[44] IR (cm^{-1}) 2959 (m), 1511 (s), 1250 (s), 1111 (s), 839 (m); ^1H NMR ($CDCl_3$) δ 2.54 (br, 4H), 3.67–3.71 (m, 4H), 3.80 (s, 3H, CH_3O), 6.90 (d, 2H, J=8.8 Hz), 7.41 (d, 2H, J=8.8 Hz); ^{13}C NMR ($CDCl_3$) δ 49.80, 55.34, 61.50 (t, J=22.6 Hz), 66.63, 114.11, 115.38, 124.29, 129.29, 160.09; HRMS (ES) calcd for $C_{13}H_{15}DN_2O_2$ (M+H) 234.1353, found 234.1354.

4.1.3. 4-Methoxybenzaldehyde-formyl-*d* (4). To a 1 L flask equipped with a reflux condenser was added 62.5 g (0.27 mol) of **3** along with 600 mL of 2 M HCl. The suspension was refluxed for 14 h. The mixture was allowed to cool and extracted with CH_2Cl_2. The organic layers were combined and washed with conc. $NaHCO_3$, water, and dried ($MgSO_4$). Evaporation of the solvent yielded 34.7 g of an orange oil (95%). IR (cm^{-1}) 3016 (w), 2843 (m), 1685 (s), 1603 (s), 1271 (s), 1168 (s); ^1H NMR ($CDCl_3$) δ 3.86 (s, 3H, CH_3O), 6.98 (d, 2H, J=8.3 Hz), 7.82 (d, 2H, J=8.7 Hz); ^{13}C NMR ($CDCl_3$) δ 55.52, 114.27, 129.81, 131.92, 164.58, 190.49 (t, J=26.7 Hz); HRMS (ES) calcd for $C_8H_7DO_2$ (M+H) 138.0665, found 138.0667.

4.1.4. (α*S*)-4-Methoxy-benzenemethan-*d*-ol (5). Under an argon atmosphere, a dry 1 L flask equipped with a reflux condenser was charged with 500 mL (0.25 mol) of 0.5 M THF solution of *R*-Alpine–Borane® along with 20.5 g (0.15 mol) of aldehyde **4**. The solution was stirred for 20 h followed by reflux for 1.5 h. After cooling to rt, 23 mL of acetaldehyde was added and the mixture stirred for 1 h. Rotary evaporation removed the solvent and pinene is partially removed by vacuum pump at 50°C for 3 h. The resultant orange oil was dissolved in 250 mL of ether and cooled to 0°C. Then 15.25 g (0.25 mol) of aminoethanol was added and left to stir at rt for 1 h. The white precipitate was removed by vacuum filtration and washed with ether. The organic fractions were combined, washed with water, and dried ($MgSO_4$). Evaporation of the solvent yielded an orange oil which was further purified by partitioning between 10% aqueous methanol and octane. The alcohol was isolated from the methanol layer by vacuum distillation or silica gel chromatography (95:5 followed by 9:1 hexanes/ethyl acetate) to yield 18.8 g of yellow oil (90%). IR (cm^{-1}) 3348 (br), 2936 (w), 1608 (m), 1511 (s), 1247 (s), 1033 (s), 804 (m); ^1H NMR ($CDCl_3$) δ 3.79 (s, 3H, CH_3O), 4.58 (br m, 1H, α-H), 6.87 (d, 2H, J=8.7 Hz), 7.27 (d, 2H, J=8.4 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) calcd for $C_8H_9DO_2$ (M+Na) 162.0641, found 162.0645.

4.1.5. (*R*)-2-[(Methoxyphenyl) methyl-*d*]-1*H*-isoindole-1,3(2*H*)-dione-2-^{15}N (6). Under an argon atmosphere, a dry three-neck 500 mL flask equipped with a reflux condenser was charged with 5.78 g (41.6 mmol) of alcohol **5**, 11.96 g (45.7 mmol) of triphenylphosphine, and 6.76 g (45.7 mmol) of ^{15}N-phthalimide stirred in 300 mL of dry THF. The solution was chilled to 0°C and 7.95 g (45.7 mmol) of diethyl azodicarboxylate was added dropwise. The solution stirred at 0°C for 4 h and then at rt for 18 h. The solvent was evaporated to give a yellow solid. The resultant mixture was dissolved and passed through a silica gel filter using 3:2 hexanes/ethyl acetate. The eluent was subjected to silica gel chromatography using 4:1 hexanes/acetone and the product recrystallized using acetone/H_2O to give 6.71 g (60%) of white solid, mp 129–131°C (lit. mp 133–134°C).[29] IR (cm^{-1}) 3045 (w), 2967 (w), 1705 (s), 1515 (m), 1243 (m), 707 (m); ^1H NMR ($CDCl_3$) δ 3.75 (s, 3H, CH_3O), 4.74 (br m, 1H, α-H), 6.82 (d, 2H, J=8.7 Hz), 7.37 (d, 2H, J=6.7 Hz), 7.66–7.68 (m, 2H), 7.80–7.82 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 40.75 (dt, J=8.8, 22.7 Hz), 55.21, 113.95, 123.23, 128.59, 130.12, 132.09, 132.16, 133.88, 159.18, 168.01 (d, J=12.7 Hz); HRMS (ES) calcd for $C_{16}H_{12}D^{15}NO_3$ (M+Na) 292.0826, found 292.0830.

4.1.6. (*R*)-2,2,2-Trichloroethyl ester [(4-methoxyphenyl) methyl-*d*]-carbamic acid-^{15}N (7b). In a 500 mL flask equipped with a reflux condenser was added 3.45 g (12.8 mmol) of **6** stirred in *n*-propanol (140 mL) and water (22 mL). Sodium borohydride (2.43 g, 64.3 mmol) was added in portions and left to stir at rt for 24 h. Carefully, 14 mL of glacial acetic acid was added dropwise and the mixture heated to 80–90°C for 5 h. After cooling to rt, the solvent was removed by rotary evaporation and dissolved in 50 mL of dioxane/water (2:1). The solution was chilled to 0°C and 5.66 g (53.4 mmol) of sodium carbonate was added to give a basic solution (pH~8). 2,2,2-Trichloroethyl chloroformate (4.08 g, 19.3 mmol), dissolved in 50 mL of dioxane/water (2:1), was added in portions to the reaction which was left to stir on ice and warm to rt overnight. The solvent was removed and the remaining aqueous suspension acidified with 1 M HCl to pH 2. The mixture was extracted with ether and dried ($MgSO_4$). The organic fraction was concentrated and acetic acid removed by rotary evaporation using toluene azeotrope. Silica gel chromatography using 3:2 hexanes/ethyl acetate gave an oil which was crystallized with ether/hexanes yielding 3.55 g (88%), mp 63–64°C (unlabeled lit. mp 61–62°C).[45] IR (cm^{-1}) 3328 (br), 3002 (w), 2951 (w), 1713 (s), 1511 (s), 1239 (s), 816 (m), 715 (m); ^1H NMR ($CDCl_3$) δ 3.78 (s, 3H, CH_3O), 4.31 (br m, 1H, α-H), 4.73 (s, 2H, CH_2), 5.18 (dd, 1H, J=5.8, 91.8 Hz, ^{15}N–H), 6.86 (d, 2H, J=14.5 Hz), 7.21 (d, 2H, J=11.4 Hz); ^{13}C NMR ($CDCl_3$) δ 44.22–44.72 (m), 55.28, 74.54, 95.58, 114.10, 128.96, 129.79, 154.54 (d, J=27.7 Hz), 159.16; HRMS (ES) calcd for $C_{11}H_{11}D^{15}NO_3Cl_3$ (M+Na) 335.9814, found 335.9821.

4.1.7. (*R*)-*t*-Butyl ester [(4-methoxyphenyl) methyl-*d*]-carbamic acid-^{15}N (7a). This compound was obtained by the same procedure as that for the synthesis of **7b** using di-*tert*-butyl dicarbonate instead of 2,2,2-trichloroethyl chloroformate to give **7a**, a colorless oil, 88% yield. ^1H NMR ($CDCl_3$) δ 1.44 (s, 9H), 3.77 (s, 3H, CH_3O), 4.21 (br, 1H, α-H), 4.76 (dd, 1H, J=5.4, 90.4 Hz, ^{15}N–H), 6.84 (d, 2H, J=8.67 Hz), 7.18 (d, 2H, J=8.5 Hz); HRMS (EI) calcd for $C_{13}H_{18}D^{15}NO_3$ 239.1398, found 239.1402.

4.1.8. (*R*)-*N*-[(2,2,2-Trichloroethyoxy) carbonyl]-glycine-*d*-^{15}N (8b). In a water bath, a rt 50 mL flask was charged with 2.45 g (7.78 mmol) of **7b** along with carbon tetrachloride

(15.7 mL), acetonitrile (15.7 mL), and water (23.5 mL). Periodic acid (24.86 g, 0.109 mol) was added and stirred for 10 min. Ruthenium chloride hydrate (35.5 mg, 0.171 mmol) was added and the mixture stirred at rt for 2 h. The solution immediately turned black then changed to orange. The solution was placed in an ice bath, diluted with ether, and stirred for 10 min. The reaction mixture was extracted with ether and the organic fractions were combined, washed with brine, dried ($MgSO_4$), and concentrated to an orange oil. The product was isolated with step-gradient reverse-phase flash chromatography using 4:1 and 1:1 water/methanol to give 1.22 g (62%) of white solid, mp 123–124°C (unlabeled lit. mp 123–125°C).[46] IR (cm^{-1}) 3320 (br), 2955 (m), 1720 (s), 1511 (m), 1227 (m), 815 (m); ^1H NMR ($CDCl_3$) δ 4.06–4.09 (br m, 1H, α-H), 4.74 (s, 2H, CH_2), 5.43 and 5.82 (2dd, 1H, J=5.6, 93.5 Hz, ^{15}N–H rotamers); ^{13}C NMR (DMK-d_6) δ 42.34–42.91 (m), 74.92, 96.72, 155.76 (d, J=28.6 Hz), 171.15; ^{15}N NMR ($CDCl_3$) 75.1 and 78.1 (^{15}N rotamers); HRMS (ES) calcd for $C_5H_5D^{15}NO_4Cl_3$ (M+Na) 273.9293, found 273.9297.

4.1.9. (*R*)-*N*-[(*t*-Butoxy) carbonyl]-glycine-*d*-^{15}N (8a). To a 50 mL flask was added 1.0 g (4.18 mmol) of **7a** dissolved in carbon tetrachloride (4 mL), acetonitrile (4 mL), and water (6 mL). Sodium periodate (16.0 g, 75.2 mmol) was added and stirred for 10 min. Ruthenium chloride hydrate (19 mg, 0.09 mmol) was added and the mixture stirred at rt for 72 h. The mixture was filtered and the solid washed with ethyl acetate. The organic filtrate was washed with water, dried ($MgSO_4$) and concentrated. The resultant oil was redissolved in chloroform, filtered, and concentrated to give an orange oil, crude yield, 10%. ^1H NMR ($CDCl_3$) δ 1.44 (s, 9H), 3.90 (br, 1H, α-H), 5.01 (br d, 1H, J=97.7 Hz, ^{15}N–H).

4.1.10. (*R*)-Glycine-*d*-^{15}N (9). To a 50 mL flask was added 0.56 g (2.2 mmol) of **8b** dissolved in 7.4 mL of glacial acetic acid. The solution was diluted with 8.6 mL of water and then 1.21 g (18.6 mmol) of zinc dust was added which caused violent bubbling. The solution was stirred for ~2 min after which 6.1 mL of water was added and stirring continued for 10 min. The suspension was vacuum filtered and the aqueous phase washed with CH_2Cl_2. Water was removed by lyophilization and acetic acid by rotary evaporation using toluene azeotrope. The resultant solid was applied to anion exchange resin AG 1 in the OH^- form. The resin was washed with water until neutral and then glycine was eluted with 1 M HCl. Concentration of the eluent gave a colored gel that was taken up in water and applied to cation exchange resin AG 50W in the H^+ form. The resin was washed until neutral and the glycine eluted with 1 M NH_4OH. The eluent was degassed, concentrated, and lyophilized to yield 125 mg (73%) of white solid, mp 229–231°C (dec.), (deuterated lit. dec. 234°C).[15] CD $\theta^{25}_{208(max)}$=+9.56 (*c* 2.0 in H_2O);[22] ^1H NMR (D_2O) δ 3.36 (br, 1H, α-H); ^{13}C NMR (D_2O, CH_3NO_2 ref.) δ 41.38 (dt, J=5.4, 22.3 Hz), 173.10; ^{15}N NMR (D_2O) 30.3; HRMS (ES) calcd for $C_2H_4D^{15}NO_2$ (M+Na) 100.0251, found 100.0254.

4.1.11. 2-[(1-Naphthyl) methyl]-1*H*-isoindole-1,3(2*H*)-dione (10a). To a 500 mL flask was added 3.34 g (21.4 mmol) of 1-naphthaldehyde dissolved in 55 mL of ethanol and 17 mL of water. Sodium borohydride (0.89 g, 23.5 mmol) was added in portions and the mixture stirred at rt overnight. The reaction was quenched with 1 M HCl until bubble formation ceased. The solution was extracted with ether and the organic fractions were washed with brine, dried ($MgSO_4$), and concentrated to give 3.35 g (99%) of a yellow oil. The alcohol was taken to the next step without further purification. The title compound was obtained by the same procedure as that for the synthesis of **6** using unlabeled reagents to yield a white solid **10a**, 2.7 g (44%). ^1H NMR ($CDCl_3$) δ 5.32 (s, 2H, CH_2), 7.34–7.88 (m, 10H), 8.36 (d, 1H, J=12.5 Hz).

4.1.12. 2-[(2-Naphthyl) methyl]-1*H*-isoindole-1,3(2*H*)-dione (10b). This compound was obtained by the same procedure as that for the synthesis of **10a** using 2-naphthaldehyde (5.28 g) instead of 1-naphthaldehyde as the starting material to give a white solid **10b**, 5.38 g (55%). ^1H NMR ($CDCl_3$) δ 4.98 (s, 2H, CH_2), 7.42–7.88 (m, 11H).

4.1.13. *t*-Butyl ester [(1-naphthyl) methyl]-carbamic acid (11). Starting with 2.0 g (7.0 mmol) of **10a**, the title compound was obtained by the same procedure as that for the synthesis of **7a** to give a clear solid **11**, 1.47 g (83%). ^1H NMR ($CDCl_3$) δ 1.52 (s, 9H), 4.75 (br, 3H, N–H, CH_2), 7.34–8.1 (m, 7H).

4.1.14. *t*-Butyl ester [(2-naphthyl) methyl]-carbamic acid (12a). Starting with 2.0 g (7.0 mmol) of **10b**, the title compound was obtained by the same procedure as that for the synthesis of **7a** to give a clear solid **12a**, 1.43 g (80%). ^1H NMR ($CDCl_3$) δ 1.46 (s, 9H), 4.46 (d, 2H, J=5.8 Hz, CH_2), 4.91 (br, 1H, N–H), 7.37–7.81 (m, 7H).

4.1.15. 2,2,2-Trichloroethyl ester [(2-naphthyl) methyl]-carbamic acid (12b). Starting with 2.0 g (7.0 mmol) of **10b**, the title compound was obtained by the same procedure as that for the synthesis of **7b** to give a yellow solid **12b**, 1.64 (70%). ^1H NMR ($CDCl_3$) δ 4.55 (d, 2H, J=6.0 Hz, α-CH_2), 4.77 (s, 2H, CH_2), 5.41 (d, 1H, J=6.0 Hz, N–H), 7.38–7.82 (m, 7H).

4.1.16. Preparation of the MTPA ester of (α*S*)-4-methoxybenzenemethan-*d*-ol. To a dry 50 mL flask equipped with an argon atmosphere was added 47 mg (0.34 mmol) of **5** and 58 mg (0.47 mmol) of 4-dimethylaminopyridine dissolved in 6 mL dry methylene chloride. (*R*)-(−)-α-methoxy-α-trifluoromethylphenylacetyl chloride (100 mg, 0.4 mmol) was added and the mixture stirred for 3 h at rt. The solvent was removed by rotary evaporation and the product isolated by preparative TLC using a 3:1 hexanes/ethyl acetate mobile phase to give a yellowish oil, 98mg (90%). The %ee of **5** was determined by ^1H NMR (800 MHz, $CDCl_3$) analysis of the MTPA ester: (*R*)-MTPA ester of (*S*)-**5**, δ 5.28 (98%); (*R*)-MTPA ester of (*R*)-**5**, δ 5.21 (2%).

4.1.17. Preparation of the camphanate amide of (*R*)-glycine-*d*-^{15}N. To a 25 mL flask chilled on ice was added 7 mg (0.09 mmol) of **9** dissolved in 2.2 mL of 0.1 M NaOH. (1*S*)-(−)-camphanoyl chloride (19 mg, 0.1 mmol) dissolved in 2 mL of toluene was added and the mixture stirred for 3 h. The basic solution was washed with methylene chloride and then acidified with 1 M HCl. The aqueous layer was

extracted with methylene chloride once and then with diethyl ether three times. The ether layers were combined, dried ($MgSO_4$), and concentrated to give a white solid, 13 mg (56%). The %ee of **9** was determined by ^1H NMR (800 MHz, $CDCl_3$) analysis of the derivative: (*S*)-camphanamide of (*R*)-**9**, δ 4.34 (br d, 91%, J=4.9 Hz); (*S*)-camphanamide of (*S*)-**9**, δ 4.15 (br d, 9%, J=4.9 Hz); ^2H NMR (600 MHz, $CHCl_3$) (*S*)-camphanamide of (*R*)-**9**, δ 4.12 (br, 90%); (*S*)-camphanamide of (*S*)-**9**, δ 4.28 (br, 10%).

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