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Enantioselective Syntheses of α-Fmoc-Pbf-[2-^{13}C]-L-arginine and Fmoc-[1,3-$^{13}C_2$]-L-proline and Incorporation into the Neurotensin Receptor 1 Ligand, NT_{8-13}

Chuanjun Song,[†] Satita Tapaneeyakorn,[‡] Annabel C. Murphy,[†] Craig Butts,[†] Anthony Watts,[‡] and Christine L. Willis*,[†]

[†]*School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, United Kingdom, and* [‡]*Biomembrane Structure Unit, Department of Biochemistry, University of Oxford, Oxford OX1 3QU, United Kingdom*

chris.willis@bristol.ac.uk

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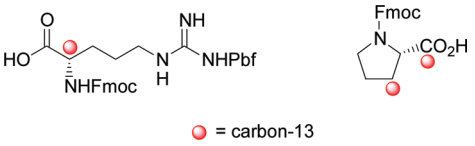

Enantioselective syntheses of selectively labeled, orthogonally protected [2-^{13}C]-L-arginine and [1,3-$^{13}C_2$]-L-proline are described from the commercially available precursors [2-^{13}C]bromoacetic acid and potassium [^{13}C]cyanide. Interestingly the enhanced signal assigned to C-2 in the ^{13}C NMR spectrum of α-Fmoc-Pbf-[2-^{13}C]-L-arginine was very broad at room temperature. The two Fmoc-labeled amino acids were used to prepare [2-^{13}C]-Arg9 and [1,3-$^{13}C_2$]-Pro10 labeled ligand (NT_{8-13}) by manual Fmoc-SPSS.

Introduction

Isotopically labeled amino acids are important for a range of studies at the chemistry−biology interface. A key application is incorporation into ligands, peptides, and proteins for determining their 3D structures by NMR spectroscopy.[1,2] However, often the required selectively labeled amino acid which would lead to the most structural information is not commercially available. In contrast, use of uniformly labeled amino acids which can be purchased may result in spectra that are too complex to be assigned with confidence due to unwanted dipolar couplings from neighboring spin sites. A particular challenge is to resolve the conformation of ligands on their sites of action in large ($M_r \gg 30$K) targets, such as membrane receptors, including G protein-coupled receptors (GPCRs). Solid state NMR (SSNMR) can address this challenge with the prerequisite that site-specific labeling is required. Thus the development of efficient methods for the selective labeling of amino acids is an important goal.

A typical example is neurotensin (NT), a tridecapeptide (Glu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg^8-Arg^9-Pro^{10}-Tyr-Ile-Leu^{13}) that is the ligand of neurotensin receptor 1 (NTS1). NT acts as a neurotransmitter in the central nervous system and as a local hormone at the periphery. Only the six C-terminal amino acids of NT (NT_{8-13}) are required for ligand binding.[3] The membrane receptor, NTS1, belongs to the family of GPCRs and is a potential target for the treatment of pain, stress, schizophrenia, Parkinson's disease, Alzheimer's disease, and colon cancer.[4] Solving the conformation of such a ligand when bound to its target can be very useful for the design of new synthetic agonists, antagonists, and reverse agonists of putative therapeutic use. Furthermore, NMR gives electronic and conformational details of value in drug design.[1,5] To gain further structural information, we required site-specific carbon-13 labeling of the peptide NT_{8-13} at Arg9 and Pro10 in the [2-^{13}C] and [1,3-$^{13}C_2$] sites, respectively. For the peptide synthesis a supply of the analogous protected amino acids was needed. The enantioselective syntheses of orthogonally protected [2-^{13}C]-L-arginine and [1,3-$^{13}C_2$]-L-proline are now described

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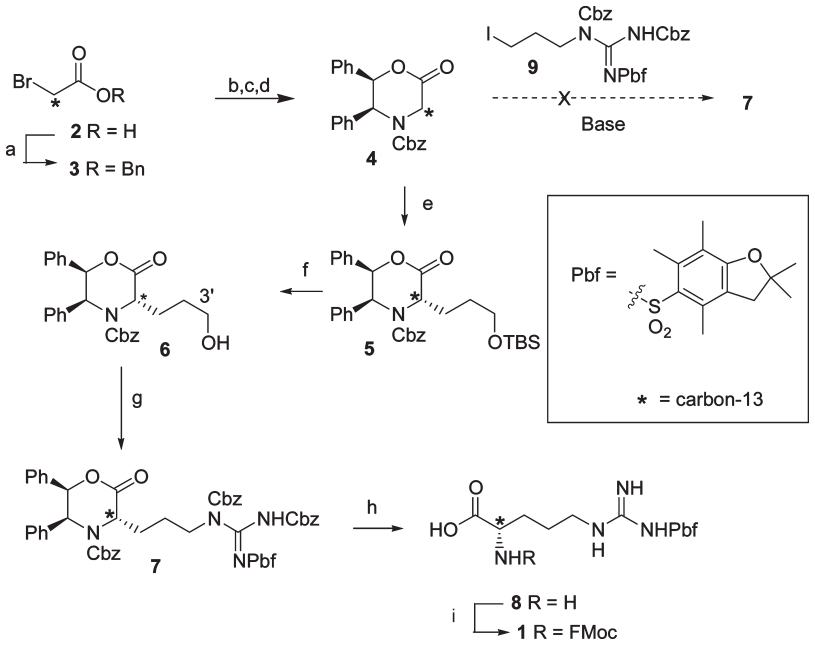
SCHEME 1. Preparation of α-Fmoc-Pbf-[2-^{13}C]-L-arginine[a]



[a]Reagents and conditions: (a) BnOH, DCC, DMAP, DCM, rt, 4 h, 100%; (b) (1*R*,2*S*)-2-amino-1,2-diphenylethanol, Et_3N, THF, rt, 2.5 h; (c) Cbz-Cl, $NaHCO_3$, H_2O/DCM, 0 °C, 2.5 h; (d) *p*-TsOH.H_2O, toluene, Dean−Stark, reflux, 60% over the 3 steps; (e) 3-(*tert*-butyldimethylsilyloxy)-1-iodopropane, LiHMDS, HMPA, THF, −78 °C to rt, 6 h, 63%; (f) $KHSO_4$, THF/MeOH/H_2O, rt, 3 h, 78%; (g) **12**, DIAD, PPh_3, 80 °C, 16 h; (h) H_2, Pd/C, MeOH/THF, 2 days, 18% over the 2 steps; (i) Fmoc-ONSu, Na_2CO_3, dioxane/H_2O, 0 °C to rt, 2.5 h, then citric acid, 75%.

as well as their incorporation into [2-^{13}C]-Arg9- and [1,3-$^{13}C_2$]-Pro10-labeled NT_{8-13}.

Results and Discussion

Synthesis of α-Fmoc-Pbf-[2-^{13}C]-L-arginine. The first synthetic target was orthogonally protected [2-^{13}C]-L-arginine.[6] Commonly used protecting groups for the highly basic guanidine function of arginine are arylsulfonyl derivatives, including PMC (2,2,5,7,8-pentamethylchroman-6-sulfonyl)[7] and analogous Pbf (2,2,4,6,7-pentamethyldihydrobenzo-5-sulfonyl) groups[8] which are removed with TFA. Literature precedents for the synthesis of arginine are rare[6,9] and to our knowledge there are no reports of the preparation of L-arginine selectively labeled at C-2. Valuable approaches to unlabeled arginine include the use of aspartic acid and 4-guanidinobutanoic acid as starting materials, but these routes are not readily adapted for the incorporation of carbon-13 at C-2.[10] Thus we designed an enantioselective approach to Fmoc-Pbf-[2-^{13}C]-L-arginine **1** from commercially available [2-^{13}C]bromoacetic acid **2** using Williams' oxazinone as a chiral glycinate equivalent[11] to generate the required stereogenic center.

First [^{13}C]oxazinone **4** was prepared in 3 steps and 60% overall yield starting from [2-^{13}C]bromoacetic acid **2** (Scheme 1).[11a] With use of unlabeled oxazinone, it has been shown that alkylations may be achieved with a range of electrophiles to generate anti-α-monosubstituted oxazines in good yields and excellent stereocontrol (>95% de).[11] Thus we proposed that a direct approach for the synthesis of the protected arginine **7** (Scheme 1) would be via alkylation of **4** with iodide **9**. However, it was found that on treatment of oxazine **4** with iodide **9** under a variety of conditions (including with either NaHMDS or LiHMDS in THF/HMPA commonly used for unactivated alkyl halides[11]) the results were disappointing as either no reaction or decomposition occurred. An alternative strategy to **7** was to alkylate the oxazine with an electrophile that could be readily manipulated to give a good leaving group at C-3′ for displacement by a protected guanidine. To this end, protected guanidine **12** was prepared as shown in Scheme 2. Initial CBz protection of guanidine hydrochloride **10**[12] was

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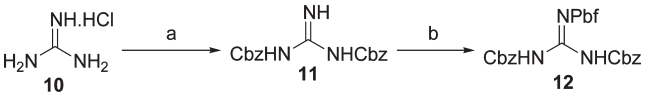
followed by treatment of the diCBz derivative **11** with PbfCl to give **12**.

Following investigations, a successful approach to our target was developed with 3-(*tert*-butyldimethylsilyloxy)-1-iodopropane as the electrophile in the key alkylation step as shown in Scheme 1. First the alkylation conditions were optimized by using unlabeled diphenyloxazinone **4**. LiHMDS was added dropwise to a solution of **4** and 3-(*tert*-butyldimethylsilyloxy)-1-iodopropane in THF/HMPA (10:1 mixture) at −78 °C giving the novel unlabeled silyl ether **5** as the sole product. The reaction was repeated with [^{13}C]-oxazinone **4** giving **5** in 63% yield. Deprotection of the silyl group of **5** with potassium bisulfate[13] gave alcohol **6**, the substrate required for introduction of the guanidine group.

SCHEME 2. Preparation of Protected Guanidine 12[a]



[a]Reagents and conditions: (i) BnOCOCl, NaOH, DCM/H_2O, 0 °C, 19 h, 94%; (b) LiHMDS, THF, 0 °C, 2 h then Pbf-Cl, −78 °C to rt, 20 h, 42%.

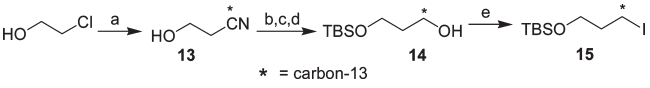
Various approaches were investigated to effect an S_N2 displacement of a derivative of alcohol **6** with a good leaving group (including a tosylate and iodide) at C-3′ with protected guanidine **12**. The most reliable method proved to be a Mitsunobu reaction of **12** with alcohol **6** to generate the protected arginine **7** (Scheme 1). The crude product was subjected to a palladium-mediated hydrogenation to give Pbf-arginine **8**. Marfey's analysis[14] of α-amino acid **8** confirmed that the L-enantiomer had been prepared with excellent stereocontrol.

For the peptide synthesis it was necessary to protect the α-amino group of **8** as the Fmoc carbamate, which proceeded smoothly[15] to furnish the target, protected [2-^{13}C]-L-arginine **1** in 75% yield. On analysis of the ^{13}C NMR spectrum of **1** in $CDCl_3$ at room temperature we were surprised that no significantly enhanced signal due to C-2 was apparent although there was a broad signal at the expected chemical shift δ 54 ppm. Hence the ^{1}H NMR and ^{13}C NMR spectra of **1** were compared with the analogous commercial unlabeled α-Fmoc-Pbf-L-arginine. The broad resonance at δ 54 was confidently assigned to C-2 by analysis of multiplicity-edited HSQC, DEPT, and COSY spectra. In the unlabeled material, this crucial resonance (δ 54) could only be observed in the ^{13}C NMR spectrum following extended acquisition and with application of a strong apodization function. However, warming the NMR sample to 40 °C caused a sharpening of all of the broad resonances in the carbon spectrum, confirming a dynamic origin to the behavior. The ^{13}C NMR spectrum of protected [2-^{13}C]-L-arginine **1** was run in DMSO at 25 °C and while the enhanced signal at δ54 was broad, on warming to 80 °C a significant sharpening of the signal was apparent. Recently Martin and Liskamp have reported the synthesis of a series of Fmoc N^G-substituted Pbf L-arginine analogues.[16] Interestingly the ^{13}C NMR spectra of these acids tended to show a broadened signal assigned to C-2.

Synthesis of Fmoc-[1,3-$^{13}C_2$]-L-proline. With orthogonally protected [2-^{13}C]-L-arginine **1** in hand, we next turned our attention to the synthesis of Fmoc-[1,3-$^{13}C_2$]-L-proline **22** for which we again used a Williams' oxazinone as a chiral glycinate equivalent (Scheme 4). In this case the electrophile was [1-^{13}C]-3-(*tert*-butyldimethylsilyloxy)-1-iodopropane **15**, which was prepared as shown in Scheme 3. Reaction of chloroethanol with potassium [^{13}C]cyanide gave the known[17] [^{13}C]hydroxynitrile **13** which, following protection as the silyl ether, was reduced to give [1-^{13}C]-3-(*tert*-butyldimethylsilyloxy)propan-1-ol **14**. Finally the alcohol was converted to iodide **15** with I_2, PPh_3, and imidazole.

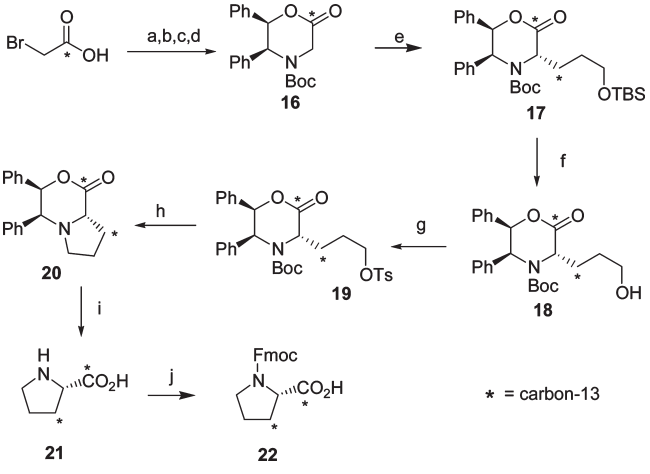
Boc-protected oxazinone **16**, prepared from [1-^{13}C]-bromoacetic acid, was alkylated with iodide **15**, using LiHMDS as the base in a solvent mixture of THF/HMPA, giving **17** in 48% yield and excellent stereocontrol

SCHEME 3. Preparation of [1-^{13}C]-3-(*tert*-Butyldimethylsilyloxy)-1-iodopropane[a]



[a]Reagents and conditions: (a) K^{13}CN, NaI, H_2O/EtOH, 80 °C, 22 h, 90%; (b) TBSCl, DMAP, Et_3N, DCM, rt, 6 h; (c) DIBAL-H, THF, rt, 3 h, then Rochelle salt, 36% over the 2 steps; (d) $NaBH_4$, EtOH, 0 °C, 0.5 h, then rt, 1 h, 89%; (e) I_2, PPh_3, imidazole, DCM, 0 °C, 2 h, then rt, 1 h, 76%.

SCHEME 4. Preparation of Fmoc-[1,3-$^{13}C_2$]-L-proline[a]



[a]Reagents and conditions: (a) BnOH, DCC, DMAP, DCM, 0 °C, 3 h, then rt, 1 h, 89%; (b) (1*R*,2*S*)-2-amino-1,2-diphenylethanol, Et_3N, THF, rt, 2 h; (c) Boc_2O, toluene, reflux, 18.5 h; (d) *p*-TsOH·H_2O, toluene, Dean−Stark, reflux 1 h, 59% over the 3 steps; (e) **15**, LiHMDS, HMPA, THF, −78 °C to rt, 3.5 h, 48%; (f) TBAF, THF, rt, 16.5 h, 57%; (g) TsCl, DMAP, DCM, rt, 41 h, 67%; (h) TFA, DCM, rt, 2 h, then $NaHCO_3$ (aq), 63%; (i) H_2, Pd/C, THF/EtOH, 21.5 h, 78%; (j) Fmoc-Cl, Na_2CO_3, H_2O/dioxane, 0 °C to rt, 17 h, 85%.

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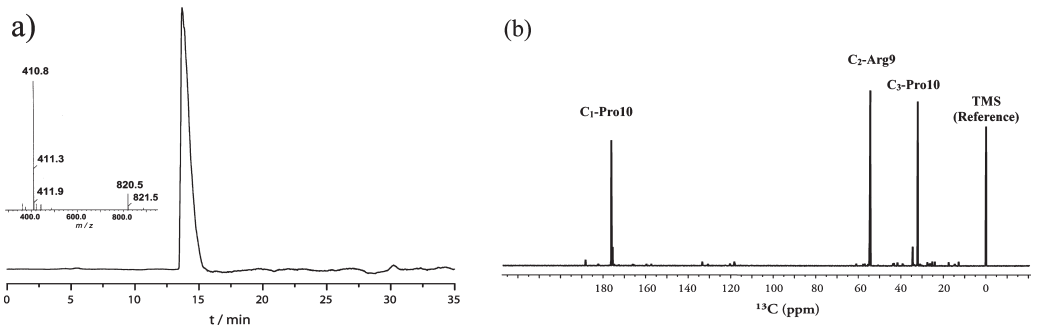

FIGURE 1. (a) HPLC and ESI mass spectrum (insert) of purified [2-^{13}C]-Arg9- and [1,3-$^{13}C_2$]-Pro10-labeled NT_{8-13}. Mass observed: 820.5 Da (M + H) and 410.8 Da (double positive charge). (b) ^{13}C NMR spectrum of labeled ligand: δC_1−Pro10 175.5, 176.2 ppm; δC_2−Arg9 54.5 ppm; δC_3−Pro10 32.1, 34.4 ppm.

(Scheme 4). Deprotection of silyl ether **17** with TBAF gave the novel alcohol **18** labeled at the requisite sites for the synthesis of the target Fmoc-[1,3-$^{13}C_2$]-L-proline **22** (Scheme 4). Activation of the alcohol as the tosylate **19**, then removal of the Boc protecting group with TFA and a base workup furnished the heterocyclic product **20** in 63% yield. It was apparent from the NMR data that a single diastereomer was present with enhanced signals in the ^{13}C NMR spectrum at δ_C 172.6 (C-2) and 29.7 (C-9). The optical rotation, $[\alpha]_D$ 135.6 (*c* 2.0, CH_2Cl_2), of **20** was in accord with the literature value[18] for the unlabeled opposite enantiomer, $[\alpha]_D$ −130.4 (*c* 2.6, CH_2Cl_2). Hydrogenolysis of **20** to [1,3-$^{13}C_2$]-L-proline **21**, followed by Fmoc protection gave Fmoc-[1,3-$^{13}C_2$]-L-proline **22** in good yield, ready for synthesis of the peptide. The ^{13}C NMR spectrum ($CDCl_3$) of **22** showed the expected enhanced signals with no apparent broadening. The melting point (115−116 °C) and optical rotation ($[\alpha]_D$ −35.8, *c* 1.0, DMF) were in accord with literature values for unlabeled Fmoc L-proline, mp 114−115 °C, $[\alpha]_D$ −33.4 (*c* 1.0, DMF).[19]

Synthesis of the Labeled Neurotensin Receptor 1 Ligand, NT_{8-13}. The two protected labeled amino acids **1** and **22** were used to synthesize [2-^{13}C]-Arg9- and [1,3-$^{13}C_2$]-Pro10-labeled ligand NT_{8-13} by manual Fmoc-SPSS. Synthesis was performed on a 0.1 mmol scale with a Fmoc-Leu-Novasyn TGA resin with no excess of amino acids and the activating mixture was HBTU/HOBt/DIEA (1:1:1.5). The Fmoc group was deprotected with 20% piperidine and the peptide was cleaved from the resin with trifluoroacetic acid (TFA). As shown in Figure 1a, purification by preparative RP-HPLC purification gave the peptide in a purity of >95% and 27% yield. The ^{13}C- NMR spectrum (Figure 1b), MS, and ^{1}H NMR spectrum of labeled ligand were used to authenticate product purity and labeled positions. Detailed NMR studies with the labeled peptide are in hand and will be reported in due course.

Conclusions

[2-^{13}C]-Arg9- and [1,3-$^{13}C_2$]-Pro10-labeled NT_{8-13} have been designed as the target to facilitate the determination of the conformation of the ligand bound to the purified neurotensin receptor 1 by solid state NMR methods. α-Fmoc-Pbf-[2-^{13}C]-L-arginine **1** and Fmoc-[1,3-$^{13}C_2$]-L-proline **22** were required to assemble the peptide and their enantioselective syntheses are described from selectively labeled oxazonines **4** and **16**. Readily available [2-^{13}C]-bromoacetic acid and potassium [^{13}C]cyanide were used as the sources of isotopic labels. The signal assigned to C-2 in the ^{13}C NMR spectrum of α-Fmoc-Pbf-[2-^{13}C]-L-arginine was broad. The two protected labeled amino acids were used in the synthesis of [2-^{13}C]-Arg9- and [1,3-$^{13}C_2$]-Pro10-labeled ligand (NT_{8-13}) by manual Fmoc-SPSS giving the required product in 27% yield. The synthetic strategies described herein may be readily adapted for the selective labeling of further sites in both protected arginine and proline and hence have widespread application both in biological NMR and metabolic studies.

Experimental Section

General experimental details are as previously described.[20]

[3-^{13}C](5*S*,6*R*)-4-Benzyloxycarbonyl-5,6-diphenylmorpholin-2-one, 4. Benzyl [2-^{13}C]bromoacetate **3** (6.54 g, 28.4 mmol) in THF (10 mL) was added dropwise to a solution of (1*R*,2*S*)-2-amino-1,2-diphenylethanol (6.37 g, 29.9 mmol) and triethylamine (3.45 g, 4.8 mL, 34.1 mmol) in dry THF (100 mL) over 1 h. After addition, the mixture was stirred at ambient temperature for 3 h. A precipitate formed, which was filtered, then the filter cake was rinsed with THF (30 mL). The filtrate was concentrated. The solid residue was dissolved in DCM (50 mL) and washed with water (50 mL). The separated aqueous phase was extracted with DCM (2 × 50 mL). Saturated aqueous sodium hydrogen carbonate (100 mL) was added to the combined organic extracts. The resulting mixture was cooled in ice. Benzyl chloroformate (5.33 g, 4.5 mL, 31.3 mmol) was added dropwise over 20 min to this vigorously stirred mixture. The reaction was stirred at 0 °C for an additional 2 h, then the layers were separated. The aqueous layer was extracted with DCM (2 × 50 mL). The combined organic extracts were washed with brine (50 mL), then dried ($MgSO_4$), filtered, and evaporated. The residue was dissolved in toluene (200 mL). *p*-Toluenesulfonic acid monohydrate (0.54 g, 2.8 mmol) was added. The resulting mixture was heated to reflux with a Dean−Stark apparatus until ~150 mL of toluene was separated. The reaction was then cooled to ambient temperature. The resulting solid was collected by filtration and rinsed with toluene−hexane (1:3, 40 mL), then hexane (20 mL). The mother liquor and washes were combined and evaporated. The residue was recrystallized from toluene to

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provide a second crop of product. The combined solid was dissolved in DCM (100 mL) and washed with saturated aqueous sodium bicarbonate (50 mL). The separated aqueous phase was extracted with DCM (50 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated to give [3-^{13}C]-morpholine carbamate **4** (6.62 g, 60%) as a colorless solid, which showed a mixture of two rotamers; $[\alpha]^{24}_D$ −63.0 (*c* 1.0, DCM) {lit.[11a] $[\alpha]_D^{22}$ −68.7} for the unlabeled (*c* 1.0, DCM); mp 204 °C (lit.[11a] mp 206−207 °C for the unlabeled); ν_{max}/cm^{-1} 1749 (CO) and 1695 (CO); δ_H (400 MHz, $CDCl_3$) 7.39−6.65 (15H, m, Ar−H), 5.88 (1H, m, 6-H), and 5.41−4.18 (5H, m, 5-H, 3-H_2 and CH_2Ph); δ_C (100 MHz, $CDCl_3$) 45.3 (C-3, enhanced signal); *m*/*z* (ESI) 411 (M^+ + Na, 100%) [found (M^+ + Na) 411.1399, $C_{23}{}^{13}CH_{21}NNaO_4$ requires 411.1396].

[3-^{13}C]-(3*S*,5*S*,6*R*)-4-Benzyloxycarbonyl-3-[3′-(*tert*-butyldimethylsilyloxy)prop-1′-yl]-5,6-diphenylmorpholin-2-one, 5. First the reaction was optimized by using unlabeled material. Lithium bis(trimethylsilyl)amide (1.0 M solution in THF, 7.7 mL, 7.7 mmol) was added dropwise to a solution of unlabeled morpholine carbamate **4** (2.0 g, 5.2 mmol) and 3-(*tert*-butyldimethylsilyloxy)-1-iodopropane (2.3 g, 7.7 mmol) in HMPA (6 mL) and dry THF (60 mL) at −78 °C under nitrogen. The resulting mixture was stirred at −78 °C for 0.5 h, before being allowed to warm to ambient temperature and left for 5 h. Ethyl acetate (50 mL) was added, and the mixture was washed with saturated aqueous ammonium chloride (50 mL). The separated aqueous phase was extracted with ethyl acetate (2 × 50 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated in vacuo. The residue was purified by column chromatography (10%, then 20% ethyl acetate in petroleum ether) to give unlabeled carbamate **5** (1.7 g, 58%) as a colorless solid, which showed a mixture of two rotamers; $[\alpha]^{24}_D$ −26.5 (*c* 12.3, DCM); mp 128−130 °C; ν_{max}/cm^{-1} 1751 (CO) and 1698 (CO); δ_H (400 MHz, $CDCl_3$) 7.38−6.55 (15H, m, Ar−H), 5.97 (1H, d, *J* = 2.9 Hz, 6-H), [5.27 (0.36H, d, *J* = 2.9 Hz) and 5.15 (0.64H, d, *J* = 2.9 Hz), 5-H], [5.19 (0.36H, d, *J* = 12.3 Hz) and 5.01 (0.64H, d, *J* = 12.3 Hz), *H*HPh], [5.11 (0.36H, d, *J* = 12.3 Hz) and 4.89 (0.64H, d, *J* = 12.3 Hz), H*H*Ph], [5.05 (0.64H, dd, *J* = 10.0 and 5.3 Hz) and 4.92 (0.36H, dd, *J* = 10.0 and 5.3 Hz), 3-H], 3.78−3.62 (2H, m, 3′-H_2), 2.35−1.69 (4H, m, 1′-H_2 and 2′-H_2), 0.91 and 0.90 (9H, s, 3 × CH_3), 0.09, 0.08, and 0.05 (6H, s, 2 × CH_3); δ_C (100 MHz, $CDCl_3$) 168.9 and 168.7 (C-2), 154.3 and 153.9 (CO), 135.7, 135.6, 134.1 (all C), 128.6, 128.4, 128.2, 128.1, 127.9, 127.8, 127.5, 127.4, 126.5, 126.4 (all CH), 79.2 and 78.8 (C-6), 68.0 and 67.6 (CH_2Ph), 62.3 and 62.2 (C-3′), 61.1 and 60.8 (C-5), 57.5 and 57.2 (C-3), 32.2 and 31.4 (C-1′), 29.2 and 29.1 (C-2′), 26.0 and 25.9 (3 × CH_3), 18.3 (C), and −5.3 (2 × CH_3); *m*/*z* (ESI) 582 (M^+ + Na, 100%) and 560 (M^+ + H, 15) [found (M^+ + Na) 582.2654, $C_{33}H_{41}NNaO_5Si$ requires 582.2646].

The above reaction was repeated with [^{13}C]morpholine carbamate **4** (5.91 g, 15.2 mmol) giving [^{13}C]carbamate **5** (5.37 g, 63%) as a colorless solid, which showed a mixture of two rotamers; δ_H (400 MHz, $CDCl_3$) 7.40−6.56 (15H, m, Ar−H), 5.99 (1H, d, *J* = 2.9 Hz, 6-H), [5.28 (0.35H, d, *J* = 2.9 Hz) and 5.17 (0.65H, d, *J* = 2.9 Hz), 5-H], [5.21 (0.35H, d, *J* = 12.2 Hz) and 5.02 (0.65H, d, *J* = 12.2 Hz), *H*HPh], [5.13 (0.35H, d, *J* = 12.2 Hz) and 4.92 (0.65H, d, *J* = 12.2 Hz), H*H*Ph], [5.06 (0.65H, ddd, *J* = 148.8, 9.6, and 5.5 Hz) and 4.93 (0.35H, ddd, *J* = 148.8, 9.6, and 5.5 Hz), 3-H], 3.78−3.62 (2H, m, 3′-H_2), 2.37−1.70 (4H, m, 1′-H_2 and 2′-H_2), 0.93 and 0.91 (9H, s, 3 × CH_3), 0.10, 0.09, and 0.07 (6H, s, 2 × CH_3); δ_C (100 MHz, $CDCl_3$) 57.4 and 57.2 (C-3, enhanced signal); *m*/*z* (ESI) 583 (M^+ + Na, 100%), 561 (M^+ + H, 10), and 455 (40) [found (M^+ + Na) 583.2702, $C_{32}{}^{13}CH_{41}NNaO_5Si$ requires 583.2680].

[3-^{13}C]-(3*S*,5*S*,6*R*)-4-Benzyloxycarbonyl-5,6-diphenyl-3-(3′-hydroxyprop-1′-yl)morpholin-2-one, 6. First the reaction was optimized with use of unlabeled material. Potassium hydrogensulfate (11 mg, 0.08 mmol) was added to a mixture of unlabeled silyl ether **5** (45 mg, 0.08 mmol) in methanol (10 mL) and water (2 mL). The resulting mixture was stirred at ambient temperature for 5 h. The methanol was evaporated and the residue was partitioned between ethyl acetate (20 mL) and water (20 mL). The separated aqueous phase was extracted with ethyl acetate (20 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated in vacuo. The residue was purified by column chromatography (50% ethyl acetate in petroleum ether) to give unlabeled carbamate **6** (31 mg, 87%) as a colorless solid, which showed a mixture of two rotamers; $[\alpha]^{24}_D$ −40.0 (*c* 5.3, DCM); mp 160 °C; ν_{max}/cm^{-1} 3389 (OH), 1749 (CO), and 1699 (CO); δ_H (400 MHz, $CDCl_3$) 7.38−6.53 (15H, m, Ar−H), 5.97 (1H, d, *J* = 2.8 Hz, 6-H), [5.24 (0.3H, d, *J* = 2.8 Hz) and 5.14 (0.7H, d, *J* = 2.8 Hz), 5-H], [5.21 (0.3H, d, *J* = 12.3 Hz), 5.04−5.01 (1.7H, m), 4.92 (0.3H, dd, *J* = 10.5 and 4.2 Hz) and 4.87 (0.7H, d, *J* = 12.3 Hz), 3-H and CH_2Ph], 3.85−3.60 (2H, m, 3′-H_2), 2.88 (1H, s, OH), and 2.39−1.69 (4H, m, 1′-H_2 and 2′-H_2); δ_C (100 MHz, $CDCl_3$) 168.8 and 168.7 (C-2), 154.3 and 153.7 (CO), 135.6, 135.4, 135.3, 134.8, 133.9 (all C), 128.5, 128.4, 128.3, 128.1, 128.0, 127.8, 127.7, 127.4, 127.3, 127.2, 126.3, 126.2 (all CH), 79.1 and 78.9 (C-6), 67.9 and 67.6 (CH_2Ph), 61.5 and 61.0 (C-3′), 60.8 and 60.6 (C-5), 57.0 and 56.2 (C-3), 31.9 and 31.0 (C-1′), 28.7 and 28.4 (C-2′); *m*/*z* (ESI) 468 (M^+ + Na, 100%) [found (M^+ + Na) 468.1779, $C_{27}H_{27}NNaO_5$ requires 468.1781].

The above reaction was repeated with [^{13}C]silyl ether **5** (5.36 g, 9.6 mmol) to give [^{13}C]carbamate **6** (3.33 g, 78%) as a colorless solid, which showed a mixture of two rotamers; δ_H (400 MHz, $CDCl_3$) 7.38−6.54 (15H, m, Ar−H), 5.96 (1H, m, 6-H), 5.27−4.73 (4H, m, 5-H, 3-H, and CH_2Ph), 3.93−3.63 (2H, m, 3′-H_2), and 2.39−1.75 (4H, m, 1′-H_2 and 2′-H_2); δ_C (100 MHz, $CDCl_3$) 57.1 and 56.0 (C-3, enhanced signal); *m*/*z* (ESI) 469 (M^+ + Na, 100%) [found (M^+ + Na) 469.1815, $C_{26}{}^{13}CH_{27}NNaO_5$ requires 469.1815].

***N*,*N*′-Dibenzyloxycarbonyl-*N*″-(2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)guanidine, 12.** Lithium bis(trimethylsilyl)-amide (1.0 M solution in THF, 18.3 mL, 18.3 mmol) was added to an ice−salt bath cooled solution of dibenzyloxycarbonylguanidine **11** (3.0 g, 9.2 mmol) in dry THF (70 mL). The resulting mixture was stirred for 2 h, then cooled to −78 °C. 2,2,4,6,7-Pentamethyldihydrobenzofuran-5-sulfonyl chloride (2.7 g, 9.2 mmol) was added. The resulting mixture was allowed to warm to ambient temperature and left for 20 h. Water (100 mL) was added, and the bulk of THF was evaporated. The residue was extracted with DCM (3 × 100 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated in vacuo. The residue was purified by column chromatography (DCM) to give the protected guanidine **12** (2.2 g, 42%) as a colorless solid; mp 138−140 °C; ν_{max}/cm^{-1} 3237 (NH), 3171 (NH), 1784 (CO), and 1734 (CO); δ_H (400 MHz, $CDCl_3$) 10.37 (2H, s, 2 × NH), 7.33 (10H, s, Ar−H), 5.16 (4H, s, 2 × CH_2Ph), 2.93 (2H, s, 3-H_2), 2.61 (3H, s, CH_3), 2.53 (3H, s, CH_3), 2.09 (3H, s, CH_3), and 1.45 (6H, s, 2 × CH_3); δ_C (100 MHz, $CDCl_3$) 159.7 (CO), 144.6, 139.7, 134.3, 133.4, 129.9 (all C), 128.7, 128.5, 128.4 (all CH), 124.8, 117.7 (both C), 86.6 (C-2), 68.5 (CH_2Ph), 42.9 (C-3), 28.4 (2 × CH_3), 19.0, 17.6, and 12.3 (all CH_3); *m*/*z* (ESI) 602 (M^+ + Na, 100%) and 580 (M^+ + H, 40) [found (M^+ + Na) 602.1952, $C_{30}H_{33}N_3NaO_7S$ requires 602.1931].

[3-^{13}C]-(3*S*,5*S*,6*R*)-4-Benzyloxycarbonyl-3-{3′-[*N*,*N*′-dibenzyloxycarbonyl-*N*″-(2″,2″,4″, 6″,7″-pentamethyldihydrobenzofuran-5″-sulfonyl)guanidino]prop-1′-yl}-5,6-diphenylmorpholin-2-one,7. First the reaction was investigated with use of unlabeled material. Diisopropyl azodicarboxylate (81 mg, 0.08 mL, 0.40 mmol) was added dropwise to a solution of the unlabeled alcohol **6** (100 mg, 0.22 mmol), protected guanidine **12** (261 mg, 0.45 mmol), and triphenylphosphine (105 mg, 0.40 mmol) in dry THF (20 mL) at 0 °C under nitrogen. After addition, the resulting mixture was heated to 80 °C for 29 h and cooled. Water

(20 mL) was added and the separated aqueous phase was extracted with ethyl acetate (2 × 50 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated in vacuo. The residue was purified by column chromatography (20%, then 30% ethyl acetate in petroleum ether) to give unlabeled masked arginine **7** (80 mg, 36%) as a sticky oil, which showed a mixture of rotamers; $[\alpha]_D^{23}$ −4.0 (*c* 1.5, DCM); ν_{max}/cm^{-1} 1759 (CO) and 1707 (CO); δ_H (400 MHz, $CDCl_3$) 7.37−6.51 (25H, m, Ar−H), 5.99 (1H, m, 6-H), 5.21−4.73 (8H, m, 3-H, 5-H, and 3 × CH_2Ph), 3.98−3.73 (2H, m, 3′-H_2), 2.97−2.86 (2H, m, 3″-H_2), 2.53 and 2.49 (3H, s, CH_3), 2.46 and 2.43 (3H, s, CH_3), 2.31−1.74 (4H, m, 1′-H_2 and 2′-H_2), 2.07 and 2.06 (3H, s, CH_3), 1.46 and 1.45 (6H, s, 2 × CH_3); δ_C (100 MHz, $CDCl_3$) 168.4 and 168.3, 160.1, 154.1 and 153.7, 150.1 and 149.7, 139.6 and 139.5, 135.8, 135.6, 135.0, 134.7 and 134.6, 134.4, 134.3, 133.4, 129.0 (all C), 128.6, 128.5, 128.4, 128.2, 128.0, 127.8, 127.7, 127.6, 127.5, 127.3, 126.6, 126.5 (all CH), 125.3 and 125.2 (C), 118.1 (C), 87.0 (C-2″), 78.9 and 78.6 (C-6), 69.1 and 69.0 (CH_2Ph), 68.4 and 68.3 (CH_2Ph), 67.8 and 67.4 (CH_2Ph), 61.0 and 60.7 (C-5), 57.1 and 56.9 (C-3), 48.4 and 48.3 (C-3′), 42.9 (C-3″), 32.4 and 31.3 (C-1′), 28.5 (2 × CH_3), 25.3 and 25.1 (C-2′), 19.1, 17.9, and 12.4 (all CH_3); *m*/*z* (ESI) 1029 (M^+ + Na, 100%) and 1007 (M^+ + H, 40) [found (M^+ + Na) 1029.3743, $C_{57}H_{58}N_4NaO_{11}S$ requires 1029.3715].

The above reaction was repeated with [^{13}C]alcohol **6** (3.33 g, 7.46 mmol) and protected guanidine **12** (4.39 g, 7.57 mmol) to give an inseparable mixture of protected [^{13}C]arginine **7** and hydrazodicarboxylate, which was used directly for the next step without further purification; δ_C (100 MHz, $CDCl_3$) 57.3 and 57.0 (C-3, enhanced signal); *m*/*z* (ESI) 1030 (M^+ + Na, 19%), 879 (15), 603 (75), and 313 (100) [found (M^+ + Na) 1030.3745, $C_{56}{}^{13}CH_{58}N_4NaO_{11}S$ requires 1030.3749].

[2-^{13}C]-(*S*)-*N*^{5}-(2′,2′,4′,6′,7′-Pentamethyldihydrobenzofuran-5′-sulfonyl)arginine, 8. To a solution of the mixture of diisopropyl hydrazodicarboxylate and the [^{13}C]-masked arginine **7** in methanol (10 mL) and THF (10 mL) was added Pd/C (10%). The resulting mixture was hydrogenated at 50 psi for 19 h, then at atmospheric pressure for 2 days. The catalyst was filtered off. The filtrate was concentrated in vacuo. The residue was partitioned between ethyl acetate (50 mL) and water (100 mL). The separated aqueous phase was freeze-dried to give [^{13}C]arginine (Pbf)-OH **8** (0.58 g, 18% over 2 steps) as a colorless solid; $[\alpha]^{24}{}_D$ −40.0 (*c* 0.3, H_2O); mp 156−162 °C (lit.[21] mp 154−157 °C for the unlabeled); ν_{max}/cm^{-1} 3333, 1571, and 1546; δ_H (400 MHz, DMSO-d_6) 7.88−6.97 (5H, m, 5 × NH), 3.03 (2H, m, 5-H_2), 2.96 (2H, s, 3′-H_2), 2.48 (3H, s, CH_3), 2.42 (3H, s, CH_3), 2.00 (3H, s, CH_3), 1.71−1.43 (4H, m, 3-H_2 and 4-H_2), and 1.40 (6H, s, 2 × CH_3) (2-H was obscured by the water peak); δ_C (100 MHz, DMSO-d_6) 53.8 (C-2, enhanced signal); *m*/*z* (ESI) 450 (M^+ + Na, 34%), 442 (100) and 428 (M^+ + H, 30) [found (M^+ + H) 428.2040, $C_{18}{}^{13}CH_{31}N_4O_5S$ requires 428.2043].

[2-^{13}C]-(*S*)-*N*^{2}-(9′-Fluorenylmethoxycarbonyl)-*N*^{5}-(2″,2″,4″,6″,7″-pentamethyldihydrobenzofuran-5″-sulfonyl)arginine, 1. Fmoc-ONSu (54 mg, 0.16 mmol) was added to a mixture of L-arginine (Pbf)-OH **8** (70 mg, 0.16 mmol) and sodium carbonate (42 mg, 0.40 mmol) in water (4 mL) and dioxane (2 mL) at 0 °C. After addition, the resulting mixture was allowed to warm to ambient temperature and left for 2.5 h. Brine (100 mL) was added and washed with diethyl ether (3 × 50 mL). The separated aqueous phase was acidified with saturated citric acid (20 mL), then extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were washed with water (3 × 50 mL), then brine (100 mL), and then dried ($MgSO_4$), filtered, and evaporated in vacuo. The residue was precipitated by the addition of diethyl ether to give Fmoc-[^{13}C]arginine (Pbf)-OH **1** (78 mg, 75%) as a colorless solid; $[\alpha]^{24}{}_D$ −2.4 (*c* 0.7, DCM) and $[\alpha]_D$ −6.0 (*c* 1.0, DMF), $[\alpha]_D$ −5.5 (*c* 1.0, DMF) for an unlabeled commercial sample, mp 134−136 °C (lit.[21] mp 98−101 °C for the unlabeled and unlabeled commercial sample mp 132 °C); ν_{max}/cm^{-1} 3332 (NH), 1703 (CO), 1616 and 1548; δ_H (400 MHz, $CDCl_3$) 7.72−7.19 (8H, m, Ar−H), 6.56−6.33 (3H, m), 4.55−4.12 (4H, m, CH_2O, 2-H and 9′-H), 3.25 (2H, m, 5-H_2), 2.88 (2H, s, 3″-H_2), 2.56 (3H, s, CH_3), 2.49 (3H, s, CH_3), 2.06 (3H, s, CH_3), 1.99−1.68 (4H, m, 3-H_2 and 4-H_2) and 1.42 (6H, s, 2 × CH_3); δ_C (100 MHz, $CDCl_3$) 143.6, 141.2, 127.6, 127.0, 125.2, 119.9, 67.1 (CH_2O), 54.3 and 53.8 (C-2, enhanced signal), 47.0 (C-9′), 43.1 (C-3″), 40.8 (C-5), 29.2 (C-3), 28.5 (CH_3), 24.8 (C-4), 22.9, 19.3, 17.9, and 12.4 (all CH_3); *m*/*z* (ESI) 672 (M^+ + Na, 100%) and 650 (M^+ + H, 90) [found (M^+ + Na) 672.2563, $C_{33}{}^{13}CH_{40}N_4NaO_7S$ requires 672.2543; found: (M^+ + H) 650.2735, $C_{33}{}^{13}CH_{41}N_4NaO_7S$ requires 650.2724].

(3*S*,5*S*,6*R*)-4-(*tert*-Butoxycarbonyl)-3-[3′-(*tert*-butyldimethylsilyloxy)prop-1′-yl]-5,6-diphenylmorpholin-2-one, 17. First the reaction was optimized with unlabeled material. To a solution of unlabeled morpholine carbamate **16** (0.80 g, 2.25 mmol) and iodide **15** (1.35 g, 4.50 mmol) in dry THF (20 mL) and HMPA (2 mL) at −78 °C under nitrogen was added lithium bis(trimethylsilyl)amide (1.0 M solution in THF, 3.4 mL, 3.38 mmol). The resulting mixture was stirred at −78 °C for 10 min, before being allowed to warm to rt and left for a further 2.5 h. Ethyl acetate (50 mL) was added and washed with saturated aqueous ammonium chloride (50 mL). The separated aqueous layer was extracted with ethyl acetate (2 × 50 mL). The combined organic extracts were washed with brine (2 × 50 mL), then dried ($MgSO_4$), filtered, and evaporated in vacuo. The residue was purified by column chromatography (5% ethyl acetate in petroleum ether) to give unlabeled carbamate **17** (0.50 g, 42%) as a colorless solid, which showed a mixture of two rotamers; $[\alpha]^{23}{}_D$ −35.6 (*c* 1.7, DCM); mp 122−124 °C; ν_{max}/cm^{-1} 1746 (CO) and 1702 (CO); δ_H (400 MHz, $CDCl_3$) 7.26−6.56 (10H, m, Ar−H), 5.96 (1H, d, *J* = 2.9 Hz, 6-H), [5.23 (0.5H, d, *J* = 2.9 Hz) and 5.02 (0.5H, d, *J* = 2.9 Hz), 5-H], [5.05 (0.5H, dd, *J* = 9.3 and 6.0 Hz) and 4.82 (0.5H, dd, *J* = 10.2 and 4.4 Hz), 3-H], 3.78−3.64 (2H, m, 3′-H_2), 2.40−1.78 (4H, m, 1′-H_2 and 2′-H_2), 1.46 and 1.10 (9H, s, 3 × CH_3), 0.92 and 0.91 (9H, s, 3 × CH_3), 0.08 and 0.07 (6H, s, 2 × CH_3); δ_C (100 MHz, $CDCl_3$) 169.4 and 169.3 (C-2), 153.6 and 152.9 (CO), 136.5 and 135.3 (C), 134.3 (C), 128.5, 128.1, 128.0, 127.8, 127.6, 127.5, 127.4, 127.3, 126.5, 126.4 (all CH), 81.5 and 81.0 (C), 79.4 and 78.8 (C-6), 62.3 and 62.2 (C-3′), 61.5 and 60.3 (C-5), 57.7 and 56.4 (C-3), 32.4 and 31.6 (C-1′), 29.4 and 29.2 (C-2′), 28.2 and 27.7 (3 × CH_3), 25.9 (3 × CH_3), 18.3 and 18.2 (C), and −5.3 (2 × CH_3); *m*/*z* (ESI) 548 (M^+ + Na, 100%) [found (M^+ + Na) 548.2811, $C_{30}H_{43}NNaO_5Si$ requires 548.2803].

The above reaction was repeated with [^{13}C]morpholine carbamate **16** (1.80 g, 5.08 mmol) and [^{13}C]iodide **15** to give [$^{13}C_2$]carbamate **17** (1.30 g, 48%) as a colorless solid, which showed a mixture of two rotamers; δ_H (400 MHz, $CDCl_3$) 7.27−6.56 (10H, m, Ar−H), 5.96 (1H, d, *J* = 2.9 Hz, 6-H), [5.23 (0.4H, d, *J* = 2.9 Hz), 5.07−5.01 (1.2H, m) and 4.82 (0.4H, m), 5-H and 3-H], 3.77−3.65 (2H, m, 3′-H_2), 2.57−1.80 (4H, m, 1′-H_2 and 2′-H_2), 1.46 and 1.09 (9H, s, 3 × CH_3), 0.92 and 0.90 (9H, s, 3 × CH_3), 0.08 and 0.07 (6H, s, 2 × CH_3); δ_C (100 MHz, $CDCl_3$) 169.4 and 169.3 (C-2, enhanced signal), 32.4 and 31.6 (C-1′, enhanced signal); *m*/*z* (ESI) 550 (M^+ + Na, 100%), 528 (20), and 472 (10) [found (M^+ + Na) 550.2877, $C_{28}{}^{13}C_2H_{43}NNaO_5Si$ requires 550.2870].

(3*S*,5*S*,6*R*)-4-(*tert*-Butoxycarbonyl)-5,6-diphenyl-3-(3′-hydroxyprop-1′-yl)morpholin-2-one, 18. First the reaction was otpimised with use of unlabeled material. Tetrabutylammonium fluoride (1.0 M solution in THF, 1.7 mL, 1.70 mmol) was added to a solution of unlabeled silyl ether **17** (92 mg, 0.17 mmol) in

(21) Du, X.; Zhang, P.; Zhu, Y.; Guo, C. *Hua gong shi kan* **2004**, *18*, 28–29.

DCM (10 mL). The resulting mixture was stirred at rt for 6 days. The solvent was evaporated under reduced pressure. The residue was purified by column chromatography (30%, then 50% ethyl acetate in petroleum ether) to give unlabeled carbamate **18** (43 mg, 60%) as a colorless solid, which showed a mixture of two rotamers; $[\alpha]^{23}_{D}$ −49.1 (*c* 2.2, DCM); ν_{max}/cm^{-1} 3277 (OH), 1745 (CO) and 1698 (CO); δ_H (400 MHz, $CDCl_3$) 7.27−6.56 (10H, m, Ar−H), [5.96 (0.25H, d, *J* = 3.0 Hz) and 5.94 (0.75H, d, *J* = 3.0 Hz), 6-H], [5.23 (0.25H, d, *J* = 3.0 Hz) and 5.01 (0.75H, d, *J* = 3.0 Hz), 5-H], [5.05 (0.75H, dd, *J* = 10.4 and 4.4 Hz) and 4.89 (0.25H, dd, *J* = 10.4 and 4.4 Hz), 3-H], 3.94−3.77 (2H, m, 3′-H_2), 2.39−1.85 (4H, m, 1′-H_2 and 2′-H_2), 1.46 and 1.11 (9H, s, 3 × CH_3); δ_C (100 MHz, $CDCl_3$) 169.6 and 169.0 (C-2), 154.8 and 153.8 (CO), 136.2, 134.2 (both C), 128.6, 128.1, 127.8, 127.7, 127.6, 127.4, 127.3, 126.5, 126.4 (all CH), 81.5 (C), 79.1 (C-6), 61.4 (C-5), 61.2 (C-3′), 55.2 (C-3) 31.5 (C-1′), 28.3 (C-2′), and 27.8 (3 × CH_3); *m*/*z* (ESI) 434 (M^+ + Na, 100%) [found (M^+ + Na) 434.1944, $C_{24}H_{29}NNaO_5$ requires 434.1938].

The above reaction was repeated with [$^{13}C_2$]silyl ether **17** (1.45 g, 2.75 mmol) to give [$^{13}C_2$]carbamate **18** (0.65 g, 57%) as a colorless solid, which showed a mixture of two rotamers; δ_H (400 MHz, $CDCl_3$) 7.28−6.58 (10H, m, Ar−H), [5.99 (0.2H, d, *J* = 2.9 Hz) and 5.96 (0.8H, d, *J* = 2.9 Hz), 6-H], [5.23 (0.2H, d, *J* = 2.9 Hz) and 5.01 (0.8H, d, *J* = 2.9 Hz), 5-H], 5.10−4.87 (1H, m, 3-H), 3.95−3.78 (2H, m, 3′-H_2), 2.54−1.85 (4H, m, 1′-H_2 and 2′-H_2), 1.46 and 1.11 (9H, s, 3 × CH_3); δ_C (100 MHz, $CDCl_3$) 169.4 and 169.2 (C-2, enhanced signal), 32.2 and 31.4 (C-1′, enhanced signal); *m*/*z* (ESI) 436 (M^+ + Na, 100%) [found (M^+ + Na) 436.1995, $C_{22}{}^{13}C_2H_{29}NNaO_5$ requires 436.2005].

(3*S*,5*S*,6*R*)-4-(*tert*-Butoxycarbonyl)-5,6-diphenyl-3-[3′-(4″-methylphenylsulfonyloxy)prop-1′-yl]morpholin-2-one, 19. First the reaction was optimized with use of unlabeled material. A mixture of unlabeled alcohol **18** (43 mg, 0.10 mmol), DMAP (28 mg, 0.23 mmol), and tosyl chloride (40 mg, 0.21 mmol) in dry DCM (5 mL) was stirred at rt for 27 h. Water (20 mL) was added, and the separated aqueous phase was extracted with DCM (2 × 50 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated in vacuo. The residue was purified by column chromatography (20%, then 30% ethyl acetate in petroleum ether) to give unlabeled carbamate **19** (49 mg, 83%) as a colorless solid, which showed a mixture of two rotamers; $[\alpha]^{23}_{D}$ −28.0 (*c* 0.5, DCM); mp 185−186 °C; ν_{max}/cm^{-1} 1747 (CO) and 1701 (CO); δ_H (400 MHz, $CDCl_3$) 7.81−6.53 (14H, m, Ar−H), 5.89 (1H, d, *J* = 2.9 Hz, 6-H), [5.21 (0.3H, d, *J* = 2.9 Hz) and 4.99 (0.7H, d, *J* = 2.9 Hz), 5-H], [4.94 (0.7H, m) and 4.75 (0.3H, m), 3-H], 4.18−4.10 (2H, m, 3′-H_2), 2.44 and 2.42 (3H, s, CH_3), 2.26−1.95 (4H, m, 1′-H_2 and 2′-H_2), 1.44 and 1.09 (9H, s, 3 × CH_3); δ_C (100 MH_Z, $CDCl_3$) 169.0 (C-2), 157.3 (CO), 144.9 and 142.0 (C), 136.3 and 135.1 (C), 134.1 and 133.0 (C), 129.9, 128.6, 128.1, 128.1, 127.9, 127.8, 127.7, 127.6, 127.4, 127.3, 126.5, 126.4 (all CH), 81.3 (C), 78.9 (C-6), 69.8 (C-3′), 61.4 (C-5), 56.0 (C-3), 31.2 (C-1′), 28.2 and 27.8 (3 × CH_3), 25.6 (C-2′), and 21.6 (CH_3); *m*/*z* (ESI) 588 (M^+ + Na, 100%) [found (M^+ + Na) 588.2038, $C_{31}H_{35}NNaO_7S$ requires 588.2026].

The reaction was repeated with [$^{13}C_2$]alcohol **18** (619 mg, 1.5 mmol) to give [$^{13}C_2$]carbamate **19** (566 mg, 67%) as a colorless solid, which showed a mixture of two rotamers; δ_H (400 MHz, $CDCl_3$) 7.84−6.55 (14H, m, Ar−H), 5.91 (1H, d, *J* = 2.8 Hz, 6-H), [5.23 (0.3H, d, *J* = 2.8 Hz) and 5.02 (0.7H, d, *J* = 2.8 Hz), 5-H], 4.87 (1H, m, 3-H), 4.22−4.12 (2H, m, 3′-H_2), 2.46 and 2.45 (3H, s, CH_3), 2.43−1.96 (4H, m, 1′-H_2 and 2′-H_2), 1.46 and 1.11 (9H, s, 3 × CH_3); δ_C (100 MH_Z, $CDCl_3$) 169.0 and 168.9 (C-2, enhanced signal), 31.7 and 31.3 (C-1′, enhanced signal); *m*/*z* (ESI) 590 (M^+ + Na, 100%) [found (M^+ + Na) 590.2090, $C_{29}{}^{13}C_2H_{35}NNaO_7S$ requires 590.2094].

(1*S*,4*R*,5*S*)-6-Aza-4,5-diphenyl-2-oxo-3-oxabicyclo[4.3.0]-nonane, 20. First the reaction was optimized with use of unlabeled material. A mixture of unlabeled carbamate **19** (49 mg, 0.09 mmol) and trifluoroacetic acid (1 mL) in DCM (10 mL) was stirred at rt for 1.5 h. Saturated aqueous sodium hydrogen carbonate (50 mL) was added and the mixture was stirred for a further 5 min. The layers were separated and the aqueous layer was extracted with DCM (2 × 50 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated in vacuo. The residue was purified by column chromatography (20% ethyl acetate in petroleum ether) to give the unlabeled bicyclic compound **20** (23 mg, 91%) as a colorless oil; $[\alpha]^{23}_{D}$ 135.6 (*c* 2.0, DCM) {lit.[18] $[\alpha]^{25}_{D}$ −130.4 for the opposite enantiomer (*c* 2.6, DCM)}; δ_H (400 MHz, $CDCl_3$) 7.26−6.96 (10H, m, Ar−H), 5.60 (1H, d, *J* = 3.8 Hz, 4-H), 4.30 (1H, d, *J* = 3.8 Hz, 5-H), 4.17 (1H, dd, *J* = 9.5 and 7.8 Hz, 1-H), 3.16 (1H, ddd, *J* = 10.3, 7.1, and 3.4 Hz, 7-*H*H), 2.58 (1H, app. td, *J* = 9.7 and 6.4 Hz, 7-H*H*), 2.42 (1H, dddd, *J* = 12.7, 10.1, 7.8, and 2.7 Hz, 9-*H*H), 2.21 (1H, dddd, *J* = 12.7, 10.6, 9.5, and 7.7 Hz, 9-H*H*), and 1.92−1.73 (2H, m 8-H_2); δ_C (100 MH_Z, $CDCl_3$) 172.9 (C-2), 136.9, 135.2 (both C), 128.7, 128.4, 128.3, 128.0, 127.9, 127.8 (all CH), 84.0 (C-4), 66.6 (C-5), 60.3 (C-1), 55.0 (C-7), 29.9 (C-9), and 23.7 (C-8); *m*/*z* (EI) 293 (M^+, 4%), 180 (2), 159 (50), and 58 (100) [found (M^+) 293.1414, $C_{19}H_{19}NO_2$ requires 293.1416].

The reaction was repeated with [$^{13}C_2$]carbamate **19** (545 mg, 0.96 mmol) to give the [$^{13}C_2$]bicyclic compound **20** (178 mg, 63%) as a colorless oil; δ_H (400 MHz, $CDCl_3$) 7.25−6.95 (10H, m, Ar−H), 5.59 (1H, dd, *J* = 4.9 and 3.9 Hz, 4-H), 4.27 (1H, d, *J* = 3.9 Hz, 5-H), 4.15 (1H, ddt, *J* = 9.5, 7.8, and 4.6 Hz, 1-H), 3.12 (1H, m, 7-*H*H), 2.55 (1H, ddt, *J* = 9.3, 6.6, and 4.4 Hz, 7-H*H*), and 2.40−1.70 (4H, m, 8-H_2 and 9-H_2); δ_C (100 MH_Z, $CDCl_3$) 172.6 (C-2, enhanced signal) and 29.7 (C-9, enhanced signal); *m*/*z* (EI) 295 (M^+, 3%), 180 (35), and 160 (100) [found (M^+) 295.1481, $C_{17}{}^{13}C_2H_{19}NO_2$ requires 295.1483].

[1,3-$^{13}C_2$]-L-Proline, 21. A mixture of the [$^{13}C_2$]bicyclic compound **20** (179 mg, 0.60 mmol), palladium chloride (53 mg, 0.30 mmol), THF (2.5 mL), and ethanol (4.5 mL) was hydrogenated at 50 psi for 21.5 h. The catalyst was filtered through a pad of Celite. The filtrate was evaporated in vacuo. The residue was partitioned between water (20 mL) and diethyl ether (20 mL). The separated aqueous phase was washed with diethyl ether (20 mL) and then evaporated in vacuo. The residue was purified by column on Dowex ion-exchange resin (50WX8-100), flushing with 1 M aqueous ammonia to give [1,3-$^{13}C_2$]-L-proline **21** (55 mg, 78%) as a colorless solid; mp 206−208 °C; δ_H (400 MHz, D_2O) 4.32 (1H, m, 2-H), 3.40−3.26 (2H, m, 5-H_2), and 2.57−1.86 (4H, m, 3-H_2 and 4-H_2); δ_C (100 MHz, D_2O) 172.2 (C-1, enhanced signal) and 28.4 (C-3, enhanced signal); *m*/*z* (EI) 117 (M^+, 40%) and 91 (100) [found (M^+) 117.0699, $C_3{}^{13}C_2H_9NO_2$ requires 117.0700].

[1,3-$^{13}C_2$] *N*-(9′-Fluorenylmethoxycarbonyl)-L-proline, 22. 9-Fluorenylmethyl chloroformate (115 mg, 0.45 mmol) was added portionwise to a solution of [1,3-$^{13}C_2$]-L-proline **21** (55 mg, 0.47 mmol) and potassium carbonate (162 mg, 1.18 mmol) in water (10 mL) and dioxane (3 mL) at 0 °C. After addition, the resulting mixture was stirred for 17 h without further cooling. The mixture was diluted with water (20 mL) and extracted with diethyl ether (2 × 20 mL). The separated aqueous phase was acidified with 1 M hydrochloric acid, and then extracted with DCM (3 × 20 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated in vacuo to give Fmoc-[1,3-$^{13}C_2$]-L-proline **22** (129 mg, 81%) as a colorless solid, which showed a mixture of two rotamers; mp 115−116 °C (lit[19] mp 114−115), $[\alpha]^{25}_{D}$ −35.8 (*c* 1.0, DMF) {lit.[19] $[\alpha]^{25}_{D}$ −33.4 for the unlabeled (*c* 1.0, DMF)}; δ_H (400 MHz, $CDCl_3$) 7.77−7.27 (8H, m, Ar−H), 4.50−4.13 (4H, m, 2-H, 9′-H, and 10′-H_2), 3.63−3.45 (2H, m, 5-H_2), and 2.46−1.90 (4H, m, 3-H_2 and 4-H_2); δ_C (100 MHz, $CDCl_3$) 177.6 and 175.6 (C-1, enhanced signal), 31.0 and 29.1 (C-3, enhanced signal); *m*/*z* (ESI) 362 (M^+ + Na, 100%)

[found (M^+ + Na) 362.1264, $C_{18}{}^{13}C_2H_{19}NNaO_4$ requires 362.1273].

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Supporting Information Available: ^{1}H- and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.