Synthesis of enantiopure α-deuteriated Boc-L-amino acids

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An alternative scheme for the synthesis of enantiopure α-deuteriated amino acids from a common intermediate is presented. Methyl bis(methylsulfanyl)methylene[2,2-$^2H_2$]glycinate was prepared in a mixture of MeOD and D_2O with a catalytic amount of Na_2CO_3 and attached to (2*R*)-bornane-10,2-sultam. Alkylation of the corresponding enolate provided intermediates which after careful purification were first deprotected on nitrogen and then cleaved from the auxiliary to give deuteriated α-amino acids of very high purity (> 99% ee and > 98% D). These were directly converted into the corresponding Boc-L-[2-^2H]-Ala, -Leu, -Phe and -(*O*-Bzl)Tyr derivatives, suitable for application in peptide synthesis. Boc-[2,2-$^2H_2$]Gly was also prepared.

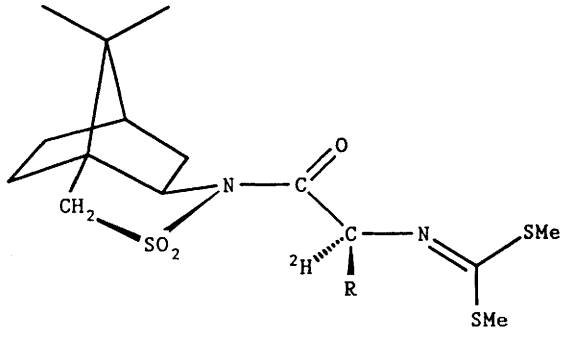
Asymmetric transformations are presently receiving a great deal of attention for the synthesis of enantiomerically pure α-amino acids.[1] In particular, several methods have been introduced that involve the application of chiral auxiliaries.[2] Most of such reagents, *e.g.*, bis(lactim ethers),[2a] *N*-isothiocyanoacetyloxazolidinones,[2b] *N*-protected imidazolidinones[2c] and diphenyltetrahydrooxazinones,[2d] camphor-derived sultams[2e] and metal complexes of multifunctional Schiff bases[2f] constitute chiral glycine equivalents that allow direct insertion of side-chains. These and other methods have provided convenient access to many non-proteinogenic amino acids.

We have for some time been interested in isotopic labelling of α-amino acids and, especially, the insertion of stable isotopes in the backbone[3a] with the aim of using suitable derivatives of these in the synthesis of peptides for structural studies.[3b] In this context we first prepared a set of ^{13}C and/or ^{15}N-labelled glycine isotopomers.[3c,d] For the incorporation of the ^{15}N and ^{13}C nuclei into the backbones of chiral amino acids, we tried Schöllkopf's bis(lactim ether) and Oppolzer's camphor sultam procedures.[2a,e] Although both methods worked well in this context, in our hands the latter provided products with the highest optical purity. This method has, therefore, now been applied for the selective introduction of deuterium in the C_α-position.

Asymmetric synthesis of specifically α-deuteriated amino acids has been described previously. Seebach *et al.*[4a–c] reported that proper quenching of enolates of imidazolidinones lead to diastereospecific incorporation of deuterium in this position, from which such labelled L- or D-amino acids with high optical purity could be isolated. Lastra and Hegedus[4d] showed that photolysis of chiral chromium carbene complexes in MeOD gave rise to α-deuteriated amino acid derivatives with ee = 95%. Recently Gani *et al.*[4e] demonstrated the convenience of Schöllkopf's bis(lactim ether) method for the same purpose and obtained α-deuteriated amino acids with > 95% D and ee of the order of 95%. Their paper also encouraged us to communicate our own results on the same topic, based on highly stereoselective enolate C_α alkylation of *N*-protected glycine attached to (2*R*)-bornane-10,2-sultam.

Results

Our final approach to α-deuteriated amino acids started from $(MeS)_2C{=}Gly\text{-}OMe$,[5] the two α-protons of which were exchanged in MeOD–D_2O in the presence of Na_2CO_3 to give $(MeS)_2C{=}[2,2\text{-}^2H_2]Gly\text{-}OMe$ **1a**. This intermediate was coupled with commercial (2*R*)-bornane-10,2-sultam as previously described[3a,6] to give compound **2**.



2 R = ^2H
3a R = Me
3b R = Bu^i
3c R = $PhCH_2$
3d R = 4-$(PhCH_2O)C_6H_4CH_2$

Carefully purified compound **2** was subsequently alkylated as its enolate with various alkyl iodides to afford **3a–d** in 69–85% yield. The crude products generally contain a few percent of the unwanted diastereoisomers, but these can normally be removed by crystallization and/or column chromatography before cleavage from the auxiliary, which is performed in two steps. Since we are primarily interested in applying our isotope-labelled amino acids in peptide synthesis, we prefer not to isolate them as such, or as salts thereof, but instead directly convert them into protected derivatives. Therefore, in this work, we chose to make directly the corresponding Boc-derivatives.

Boc–L–NH–[2–^2H]CR–CO_2H **4**

a R = Me
b R = Bu^i
c R = $PhCH_2$
d R = 4-$(PhCH_2O)C_6H_4CH_2$

The optical purity of all chiral amino acid derivatives was determined by two independent methods.[7] As expected,[3a,6] all of them were of excellent optical purity (> 99% ee except for tyrosine). Their deuterium content was also very high (> 98%). The yields for all Boc-L-amino acids were 60–85% starting from their sultam precursors, except for Boc-(*O*-Bzl)-L-[2-^2H]-tyrosine (42%).

Discussion

Many methods have been used earlier for the preparation of deuteriated amino acids.[8] In this work we chose an asymmetric approach based on a bis-deuteriated precursor; such a course was also recently adopted by Gani *et al.*[4e] In their procedure this was of the bis-lactim type, whereas we used a camphor sultam auxiliary. As a consequence, half of the initial amount of deuterium present in the precursors is lost in both procedures.

We accomplished deuterium labelling by exchange of the two methylene protons in methyl bis(methylsulfanyl)methylene glycinate in MeOD–D_2O with Na_2CO_3 as a catalyst. Similar conditions were recently reported to work for α-deuteriation of aldehydes.[9] Three cycles were required to give the labelled isotopomer **1a** with >98% D_2 in 80% yield together with 15% of the corresponding *N*,*N*-bisprotected glycine with a somewhat lower deuterium content (~94%). Alternatively, this product could be transformed back to the ester or, after deprotection on nitrogen, converted into Boc-[2,2-$^2H_2$]glycine. The presence of two α-deuterium atoms in **1a** was confirmed by the absence of a proton signal at 4.22 ppm in its ^1H NMR spectrum, by splitting of the signal of the α-carbon to a quintet in the ^{13}C spectrum ($^1J_{C,D}$ 20.8 Hz) and also by ^2H NMR spectroscopy. Mass spectrometry indicated 98.5% di- and 1.5% mono-deuteriation and the absence of nondeuteriated material.

Coupling of **1a** to the sultam auxiliary to give **2** was accomplished according to an established method[3a,6,11] and occurred without loss of deuterium, as demonstrated by ^1H and ^{13}C NMR spectroscopy ($^1J_{C,D}$ 20.2 Hz). However, attempts at chromatographic purification of **2** resulted in pronounced H/D scrambling. It should also be mentioned that quenching of the coupling reaction with MeOH and H_2O caused back exchange up to 25%, as a consequence of which we used MeOD and D_2O instead for this purpose. It also seemed important to use dry EtOH for the recrystallization of **2**.

After crystallization of **2** *ca.* 50% of the excess of **1a** used in the coupling step could be recovered and it was processed to **1b** as described in the experimental part. The product was carefully analysed and characterized by ^1H, ^2H and ^{13}C NMR spectroscopy ($^1J_{C,D}$ 20.8 Hz).

All alkylation products **3a–d** were checked for the absence of α-protons in their ^1H NMR spectra and exhibited triplets ($^1J_{C,D}$ ~20 Hz) for the α-carbons. Before proceeding to the next step the optical purity (ee) of the products after cleavage was determined and was found to be >99% except for Tyr(Bzl). Compound **3d** resisted crystallization and was therefore purified by column chromatography. A repetition of this step resulted in an increase from 97.7 to 98.3%, confirming previous experiences.[3a] In this context we should mention that no detectable scrambling took place in the chromatographic purification of **3a–d** in contrast to that of **2**.

Consecutive treatment of the sultam derivatives **3a–d** with acid and base[3a] furnished the corresponding free amino acids mixed with salt which were directly converted into their Boc derivatives **4a–d** without loss of deuterium as judged from their ^1H NMR spectra. The α-carbons invariably appeared as triplets ($^1J_{C,D}$ ~20 Hz), thus indicating the presence of one α-deuteron. In addition, significant upfield shifts were noticed for all α-carbons upon deuteriation in the range of 0.4–0.7 ppm (for **3a–d**), reaching 1.0 ppm in **2**, in which two deuterons are present. For the Boc-derivatives it is somewhat smaller (0.2–0.4 ppm, 0.5 ppm for Boc-[2,2-$^2H_2$]Gly). In the corresponding FTIR spectra the effect of α-deuteriation is less significant except for Boc-Gly, in which two α-deuterium nuclei are also present (see Experimental section).

From the present work and the experiences previously gained in the synthesis of backbone-labelled amino acids,[3a] it appears most likely that α-deuteriation of ^{15}N- and/or ^{13}C-labelled glycine isotopomers[3c,d] will provide suitable precursors for preparation of correspondingly labelled chiral species.

Experimental

Materials

(2*R*)-Bornane-10,2-sultam was obtained from Oxford Asymmetry Ltd., MeOD (99% and 99.5% D) from Cambridge Isotope Laboratory and D_2O (99.8% D) from Glaser, Basel. Generally all other chemicals used were of best analytical quality.

General methods

Mps were recorded on a Gallenkamp apparatus and are uncorrected. Distilled acetone (after being dried over K_2CO_3), $CHCl_3$ (Na_2SO_4), THF and toluene (both Na) were kept over activated molecular sieves (4 A). TLC analyses were performed on 0.25 mm thick pre-coated silica plates (Merck DC-Fertigplatten, Kieselgel 60 F254), eluted with (A) CH_2Cl_2–Me_2CO–HOAc (40:10:1) and (B) light petroleum (bp 40–65 °C)–diethyl ether (2:1). All compounds gave one spot unless otherwise stated. Spots were visualized by inspection under UV light and/or treatment with Cl_2–dicarboxidine[10] or starch solution. Column chromatography was carried out on Merck Kieselgel 60 (70–230 mesh), with light petroleum (bp 40–65 °C)–diethyl ether (1:1) as eluent. Optical rotations were measured with a Perkin-Elmer 241 polarimeter, with $[\alpha]_D$ values reported in units of 10^{-1} deg cm^2 g^{-1}, and IR spectra (in KBr) with a Mattson Polaris FTIR spectrometer. ^1H, ^2H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a JEOL JNM-EX 270 spectrometer unless otherwise noted. All shifts are given in ppm and coupling constants in Hz (estimated error ±0.5 Hz). Assignments were made by comparison of chemical shifts and peak multiplicities.

Methyl bis(methylsulfanyl)methylene[2,2-$^2H_2$]glycinate 1a

Unlabelled $(MeS)_2C$=Gly-OMe was dissolved in a mixture of MeOD and D_2O (1:1; 2 cm^3 each for 5–6 mmol of substrate) in the presence of catalytic amounts of Na_2CO_3 (17 mol%). After three cycles (24 h, with removal of the solvents on the evaporator after each), the remaining material was partitioned between EtOAc and water. After two more extractions of the aqueous phase with EtOAc, the combined organic phase and extracts were dried (Na_2SO_4) and evaporated to afford the deuteriated ester in 80% yield with $^2H_2$ and ^2H contents of >98.5 and <1.5%, respectively, as determined by MS. No ^1H signal was detected at 4.22 ppm. The ^{13}C spectrum exhibited a quintet for the α-carbon characteristic for two α-deuterium atoms with $^1J_{C,D}$ ~20 Hz; δ_H 2.45 (s, 3 H, MeS), 2.57 (s, 3 H, MeS) and 3.76 (s, 3 H, MeO); δ_D 4.22 (br s); δ_C 14.4 (SMe), 14.7 (SMe), 51.8 (MeO), 53.2 [quintet, $^1J_{C,D}$ 20.8 (53.9 for the ^1H isotopomer)], 163.3 (C=N) and 170.5 (CO).

On acidification with 1 mol dm^{-3} aqueous $KHSO_4$ and extraction with EtOAc, the last aqueous phase provided, in addition, some *N*-protected glycine as an oil (15%) which gradually solidified.

(2*R*)-*N*-{Bis(methylsulfanyl)methylene[2,2-$^2H_2$]glycyl}bornane-10,2-sultam 2

Coupling of **1a** to (2*R*)-bornane-10,2-sultam was accomplished essentially as described previously.[3a,11] Yield 81% after crystallization from EtOH with >98% D (^1H NMR); mp 104–104.5 °C; $[\alpha]_D^{26}$ −110.3 (*c* 0.3 in $CHCl_3$); δ_H 0.97 (s, 3 H, Me), 1.17 (s, 3 H, Me), 1.30–1.47 (m, 2 H), 1.82–1.95 (m, 3 H), 2.07 (dd, 1 H, *J* 7.75, 14.02), 2.15–2.26 (m, 1 H), 2.40 (s, 3 H, SMe), 2.51 (s, 3 H, SMe), 3.42 (d, 1 H, $^2J_{H,H}$ 13.53), 3.49 (d, 1 H, $^2J_{H,H}$ 13.86) and 3.89 (dd, 1 H, $J_{H,H}$ 5.12, 7.42); δ_D 4.64 (br s); δ_C 14.3 (SMe), 14.7 (SMe), 19.6, 20.4, 26.1, 32.4, 38.0, 44.3, 47.4, 48.6, 52.4, 54.5 [quintet, CD_2, $^1J_{C,D}$ 20.2 (55.5 for the ^1H isotopomer)], 64.8, 163.9 (C=N) and 167.7 (CO); ν_{max}(FTIR)/cm^{-1} 1710 (CO) and 1576 (C=N) (1712 and 1577 for the ^1H isotopomer).

(2*R*,2′*S*)-*N*-{Bis(methylsulfanyl)methylene[2′-^{2}H]alanyl}bornane-10,2-sultam 3a

Alkylation of **2** to give **3a–d** was effected in the presence of HMPA according to Oppolzer *et al.*[11] with 2.5 g (6.6 mmol) of **2**, except for tyrosine (2.0 g, 5.29 mmol). Yield 85.5% after crystallization from Et_2O–hexane; mp 116–117 °C; $[\alpha]_D^{26}$ −68.7 (*c* 0.33 in $CHCl_3$); δ_H 0.98 (s, 3 H, Me), 1.17 (s, 3 H, Me), 1.24–1.43 (m, 2 H), 1.49 (s, 3 H, Me), 1.80–1.9 (m, 3 H), 2.07 (s, 1 H), 2.09 (s, 1 H), 2.43 (s, 3 H, SMe), 2.56 (s, 3 H, SMe), 3.45 (d, 1 H, $^2J_{H,H}$ 13.86), 3.53 (d, 1 H, $^2J_{H,H}$ 13.86), 3.92 (t, 1 H, $J_{H,H}$ 6.27), >99% D; δ_C 14.5 (SMe), 14.9 (SMe), 19.61, 19.64 (α-Me), 20.5, 26.2, 32.5, 38.1, 44.3, 47.5, 48.3, 52.8 and 60.0 [t, $^1J_{C,D}$ 20.8 (60.7 for the ^{1}H isotopomer)], 64.9, 161.1 (C=N) and 172.1 (CO); ν_{max}(FTIR)/cm^{-1} 1712 (CO) and 1579 (C=N) (1714 and 1578 for the ^{1}H isotopomer); ee, after cleavage: 99.0% (HPLC) and 99.0% (GC).

(2*R*,2′*S*)-*N*-{Bis(methylsulfanyl)methylene[2′-^{2}H]leucyl}bornane-10,2-sultam 3b

Yield 90% after crystallization from Et_2O–hexane; mp 125.5–126.5 °C; $[\alpha]_D^{26}$ −80.5 (*c* 0.2 in $CHCl_3$); δ_H 0.92 (d, 3 H, $^3J_{H,H}$ 6.6), 0.94 (d, 3 H, $^3J_{H,H}$ 6.6), 0.98 (s, 3 H, Me), 1.17 (s, 3 H, Me), 1.30–1.45 (m, 2 H), 1.60–1.95 (m, 6 H), 2.06 (s, 1 H), 2.08 (s, 1 H), 2.42 (s, 3 H, SMe), 2.56 (s, 3 H, SMe), 3.44 (d, $^2J_{H,H}$ 13.85), 3.51 (d, $^2J_{H,H}$ 13.86), 3.92 (t, $J_{H,H}$ 6.27), >97% D; δ_C 14.7 (SMe), 15.1 (SMe), 19.7, 20.5, 21.7, 22.9, 25.1, 26.3, 32.6, 38.2, 43.0, 44.4, 47.6, 48.2, 52.9, 63.3 [t, $^1J_{C,D}$ 20.8 (63.8 for the ^{1}H isotopomer)], 65.1, 161.2 (C=N) and 171.8 (CO); ν_{max}(FTIR)/cm^{-1} 1700 (CO) and 1572 (C=N) (1702 and 1572, respectively, for the ^{1}H isotopomer); ee, after cleavage: 99.9% (HPLC) and 99.7% (GC).

(2*R*,2′*S*)-*N*-{Bis(methylsulfanyl)methylene[2′-^{2}H]phenylalanyl}bornane-10,2-sultam 3c

Yield 76% after crystallization from Et_2O–hexane; mp 133.5–134.5 °C; $[\alpha]_D^{26}$ −116 (*c* 0.36 in $CHCl_3$); δ_H 0.87 (s, 3 H, Me), 0.91 (s, 3 H, Me), 1.26–1.40 (m, 2 H), 1.76–1.94 (m, 4 H), 2.02 (dd, 1 H, *J* 7.59, 13.86), 2.41 (s, 3 H, SMe), 2.45 (s, 3 H, SMe), 3.04 (d, 1 H, $^2J_{H,H}$ 12.87), 3.31 (d, 1 H, $^2J_{H,H}$ 12.54), 3.39 (d, 1 H, $^2J_{H,H}$ 13.86), 3.45 (d, 1 H, $^2J_{H,H}$ 13.86), 3.86 (dd, 1 H, $^1J_{H,H}$ 4.78, 7.75) and 7.12–7.31 (m, 5 H); δ_C 14.8 (SMe), 15.3 (SMe), 19.7, 20.4, 26.3, 32.6, 38.1, 40.0 (β-C), 44.4, 47.5, 48.2, 52.9, 65.0, 66.2 [t, $^1J_{C,D}$ 21.4 (66.8 for the ^{1}H isotopomer)], 126.4, 127.9, 129.8, 136.9 (all Ar), 162.5 (C=N) and 170.7 (CO); ν_{max}(FTIR)/cm^{-1} 1702 (CO) and 1572 (C=N); ee, after cleavage: 99.5% (HPLC) and 99.7% (GC).

(2*R*,2′*S*)-*O*-Benzyl-*N*-{bis(methylsulfanyl)methylene[2′-^{2}H]-tyrosyl}bornane-10,2-sultam 3d

Oil, which could not be crystallized; yield 69% after chromatography on silica twice; δ_H 0.86 (s, 3 H, Me), 0.91 (s, 3 H, Me), 1.25–1.43 (m, 2 H), 1.77–1.90 (m, 4 H), 2.01 (dd, 1 H, *J* 7.92, 13.86), 2.41 (s, 3 H, SMe), 2.46 (s, 3 H, SMe), 2.99 (d, 1 H, $^2J_{H,H}$ 13.19), 3.25 (d, 1 H, $^2J_{H,H}$ 12.87), 3.38 (d, 1 H, $^2J_{H,H}$ 13.86), 3.45 (d, 1 H, $^2J_{H,H}$ 13.85), 3.86 (dd, 1 H, *J* 4.78, 7.42), 5.02 (s, 2 H, Bzl CH_2O), 6.85 (d, 2 H, $^3J_{H,H}$ 8.58, ArH), 7.20 (d, 2 H, $^3J_{H,H}$ 8.58, ArH), 7.28–7.44 (m, 5 H); δ_C 14.9 (SMe), 15.4 (SMe), 19.8, 20.4, 26.3, 32.7, 38.2, 39.3 (β-C), 44.5, 47.5, 48.2, 53.0, 65.1, 66.5 [t, $^1J_{C,D}$ 21.4 (66.9 for the ^{1}H isotopomer)], 69.8 (Ph-CH_2O), 114.4, 127.4, 127.8, 128.5, 129.4, 130.9, 137.1, 157.5 (all Ar), 162.6 (C=N) and 170.9 (CO); ee, after cleavage and catalytic hydrogenation of once and twice chromatographed product, 97.7% and 98.2%, (GC), respectively.

Boc-L-[2-^{2}H]alanine 4a

Compounds **4a–d** were all obtained from **3a–d** by liberation of their amino groups, cleavage from the sultam auxiliary and reaction of the amino acid salts with Boc_2O, as described in detail previously.[3a] Yield 64% after crystallization from EtOAc–hexane; mp 79.5–80.5 °C; $[\alpha]_D^{26}$ −21.6 (*c* 1.0 in HOAc); δ_H 1.43 (s, 3 H, α-Me), 1.45 (s, 9 H, Boc), 5.23 (s, NH, *E*), 6.88 (s, NH, *Z*) and 11.47 (br s, 1 H, CO_2H); δ_D 4.28 (br s); δ_C 18.2 (α-Me), 28.2 (Boc), 48.8 [t, $^1J_{C,D}$ 19.6, *E* (49.0 for the ^{1}H isotopomer)], 49.8 [t, $^1J_{C,D}$ 22.0, *Z* (50.1 for the ^{1}H isotopomer)], 80.1 (Boc, *E*), 81.6 (Boc, *Z*), 155.4 (CO-N, *E*), 156.9 (CO-N, *Z*), 177.3 (CO_2H, *Z*), 177.8 (CO_2H, *E*); ν_{max}(FTIR)/cm^{-1} 1742 (CO_2H), 1691 (CO-N) and 1515 (amide II) (1740, 1692 and 1517, respectively, for the ^{1}H isotopomer); ee: 99.0% (HPLC) and 99.0% (GC).

Boc-L-[2-^{2}H]leucine 4b

Yield 85% after crystallization from EtOH–H_2O (1:2); mp 82.5–83.5 °C; $[\alpha]_D^{26}$ −24.7 (*c* 1.01 in HOAc); δ_H 0.96 (d, 6 H, Me_2C, $^3J_{H,H}$ 6.27), 1.45 (s, 9 H, Boc), 1.50–1.85 (m, 3 H, $CHCH_2$), 4.99 (s, ~0.7 H, NH, *E*), 6.21 (s, ~0.3 H, NH, *Z*), 7.50 (br s, 1 H, CO_2H); δ_C 21.7 (Me_2C), 22.8 (Me_2C), 24.7 (Me_2CH), 28.2 (Boc), 41.3 (CH_2), 51.6 [t, CD, $^1J_{C,D}$ 20.2, *E* (51.9 for the ^{1}H isotopomer)], 52.8 [t, CD, *Z* (53.1 for the ^{1}H isotopomer)], 80.1 (Boc, *E*), 81.6 (Boc, *Z*), 155.7 (CO-N, *E*), 156.7 (CO-N, *Z*), 177.7 (CO_2H, *Z*), 178.0 (CO_2H, *E*); ν_{max}(FTIR)/cm^{-1} 1714 (CO_2H), 1676 (CO-N) and 1542 (amide II) (1718, 1676 and 1541, respectively, for the ^{1}H isotopomer); ee: 99.9% (HPLC) and 99.7% (GC).

Boc-L-[2-^{2}H]phenylalanine 4c

This compound was isolated as its dicyclohexylamine salt in 65% yield from **3c**; mp 217–219 °C; $[\alpha]_D^{26}$ 38.8 (*c* 0.1 in $CHCl_3$); δ_H 1.41 (s, 9 H, Boc), 2.9 (m, 2 H, $+NH_2$), 3.07 (d, 1 H, β-H_a $^2J_{H,H}$ 13.53), 3.20 (d, 1 H, β-H_b, $^2J_{H,H}$ 13.52), 5.17 (br s, ~0.3 H, NH, *Z*), 5.27 (br s, ~0.7 H, NH, *E*), 7.10–7.21 (m, 5 H, ArH), in addition several signals 1.1–2.0, partially hidden, belonging to dicyclohexylamine; δ_C 24.8, 25.1, 28.2 (Boc, *Z*), 28.4 (Boc, *E*), 29.0, 29.1, 38.4 (β-C, *E*), 40.2 (β-C, *Z*), 52.4, 56.0 [t, CD, $^1J_{C,D}$ 19.0, *E* (56.3 for the ^{1}H isotopomer)], 57.1 [t, CD, *Z* (57.5 for the ^{1}H isotopomer)], 78.5 (Boc, *E*), 79.0 (Boc, *Z*), 125.9, 127.7, 129.8, 138.5 (all Ar), 155.1 (CO-N), 175.4 (CO_2H); ν_{max}(FTIR)/cm^{-1} 1707, 1567 and 1393 (1708, 1572 and 1398 for the ^{1}H isotopomer); ee: 99.5% (HPLC) and 99.3% (GC).

Boc-(*O*-benzyl)-L-[2-^{2}H]tyrosine 4d

The compound was first obtained as an oil in 42% yield; it was subsequently converted into the dicyclohexylamine salt; mp 107–109 °C; $[\alpha]_D^{26}$ 24.72 (*c* 0.55 in MeOH); δ_H 1.34 (s, 9 H, Boc), 2.84 (m, 2 H, $+NH_2$), 2.95 (d, 1 H, β-H_a, $^2J_{H,H}$ 13.52), 3.08 (d, 1 H, β-H_b, $^2J_{H,H}$ 13.53), 4.92 (s, 2 H, Bzl-CH_2O), 5.10 (br s, NH, *Z*), 5.22 (br s, NH, *E*), 6.75 (d, 2 H, ArH, $^3J_{H,H}$ 8.58), 7.05 (d, 2 H, ArH, $^3J_{H,H}$ 8.58), 7.19–7.35 (m, 6 H, ArH), in addition several signals 1.1–1.9, partially hidden, belonging to dicyclohexylamine; δ_C 24.7, 25.1, 28.2 (Boc, *Z*), 28.4 (Boc, *E*), 29.0, 29.1, 52.4 (β-C), 56.1 (m, C-D, *E*), 57.2 (m, C-D, *Z*), 69.8 (Bzl CH_2O), 78.3 (Boc, *E*), 78.5 (Boc, *Z*), 114.8, 127.3, 127.8, 128.4, 130.7, 137.1 (all Ar), 155.1, 157.1 (CO-N), 175.5 (CO_2H); ν_{max}(FTIR)/cm^{-1} 2937, 2857, 1706, 1577, 1511 and 1391.

Boc-[2,2-$^2H_2$]glycine 1b

This compound was obtained from **1a** by cleavage of its amino protecting group with 1 mol dm^{-3} HCl followed by subsequent reaction of the HCl salt with Boc_2O. Saponification of the ester group with NaOH afforded **1b** containing >99% ^{2}H; yield 70%, after crystallization from EtOAc–hexane; mp 87.5–88.5 °C; δ_H 1.45 (s, 9 H, Boc), 5.33 (br s, NH, *E*), 6.75 (br s, NH, *Z*) and 11.79 (br s, 1 H, CO_2H); δ_D 3.90 (br s); δ_C 28.2 (Boc), 41.6 [complex quintet, $^1J_{C,D}$ 20.8, *E* (42.1 for the ^{1}H isotopomer)], 42.7 [complex quintet, *Z* (43.2 for the ^{1}H isotopomer)], 80.3 (Boc, *E*), 81.8 (Boc, *Z*), 156.1 (CO-N, *E*), 157.4 (CO-N, *Z*), 174.0 (CO_2H, *Z*) and 174.5 (CO_2H, *E*); ν_{max}(FTIR)/cm^{-1} 1751 (CO_2H), 1681 (CO-N) and 1523 (amide II) (1748, 1670 and 1536, respectively, for the ^{1}H isotopomer).

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