Synthesis of Amino Acid Derivatives Substituted in the Backbone with Stable Isotopes for Application in Peptide Synthesis

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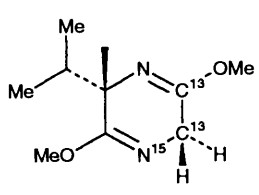
Starting from the corresponding precursors, **1** and **2**, the two well known procedures for asymmetric synthesis of amino acids, those of Schöllkopf and Oppolzer, were compared for the preparation of [1,2-$^{13}C_2$,^{15}N]-labelled (substituted) Boc-leucine enantiomers. Although both gave the final products in comparable overall yields, analysis of optical purity by two independent chromatographic methods revealed that the two procedures differed considerably in this respect. The enantiomeric excess found was 97.2–97.4 and 99.7%, respectively. As a consequence, the latter method was preferred for the synthesis of a number of additional backbone-labelled Boc-derivatives of the three proteinogenic amino acids alanine, phenylalanine and tyrosine, including such deuteriated in the amino acid side-chain. These derivatives exemplify precursors suitable for chemical synthesis of specifically backbone-labelled peptides and should allow greater exploitation of the properties of the ^{13}C and, especially, the ^{15}N nuclei in structural studies.

Contemporary strides in asymmetric synthesis are clearly reflected in a revived interest in amino acid synthesis.[1] As proteinogenic amino acids of high quality are already available industrially,[2] most of these efforts have been directed towards the synthesis of either artificial or naturally occurring, non-proteinogenic ones. Recent research has resulted in improved availability of α,α-disubstituted,[3] N-substituted[4] and unsaturated amino acids,[3a,5] *etc.*, in addition to more common or spectacular ones of either L- or D-configuration. Also, deuteriated amino acids have been prepared.[4a,6] Several methods of wide applicability and great beauty have been developed in the course of this work.[7]

For some time we have been interested in the synthesis of ^{15}N-labelled† (substituted) proteinogenic amino acid derivatives for application in peptide synthesis.[8] For the initial step we chose a classical route starting from an amino acid and giving rise to the corresponding hydroxy acid.[9] The isotope was inserted with the aid of an imidodicarbonate.[10] For the formation of the new C–N bond we first adopted Mitsunobu,[11] but later switched to triflate[4b] methodology. Both of these gave rise to N-protected ^{15}N-labelled amino acid derivatives of excellent sterical purity suitable for their purpose. The same labelled imidodicarbonate was also used to make the whole set of Boc-[^{13}C,^{15}N]glycines.[1,2]‡

As a continuation of the work described in the previous paragraph, this paper describes asymmetric synthesis of a number of novel selectively (backbone) [1,2-$^{13}C_2$,^{15}N]-labelled amino acid derivatives from [$^{13}C_2$,^{15}N]glycine with the aim of simultaneously exploring reliable routes to such derivatives. Initially two procedures, Schöllkopf's and Oppolzer's, were compared for the synthesis of enantiomeric leucines. The latter method was subsequently applied also for the synthesis of a number of additional backbone-labelled amino acids. All products were carefully characterized with respect to their enantiomeric purity. The results obtained and experiences gained from this work are reported below.



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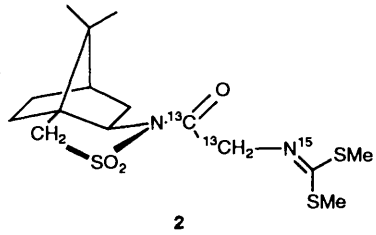

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Results

Although resolution of racemates *via* the formation of diastereoisomeric salts is still a relevant method for preparation of chiral amino acids, enantioselective synthesis is preferable for several reasons. An attractive way to accomplish the latter is to treat the prochiral precursor, in this case isotope-labelled glycine, with a suitable auxiliary to induce discrimination between the diastereotopic hydrogens of the intermediary in the subsequent alkylation step. The auxiliary is then cleaved off and, if possible, regenerated. As mentioned at the outset, several methods are nowadays available for asymmetric synthesis of amino acids.[7] Three of them[7a,b,d] should be suitable for the synthesis of backbone-labelled amino acids starting from labelled glycines.[12] Considering the need for resolution of the heterocyclic precursor(s) in one of these methods,[7b] we decided to confine our comparison to the remaining two, those of Schöllkopf[7a] and Oppolzer.[7d] In the former, glycine is incorporated into a cyclic dipeptide and converted into the corresponding bislactim ether, whereas in the latter, after initial derivatization of the amino function, it is attached to a chiral sultam auxiliary. Consequently, our initial efforts centred about the synthesis of the two required triply labelled precursors **1** and **2** shown below.

Synthesis of Triply Labelled Bislactim Ether **1** *and Sultam* **2**.—The precursor **1**, required in the preparation of Boc-D-Leu below, was obtained by the following three-step sequence (Scheme 1):

† According to IUPAC definitions, compounds such as those made in this paper should be described as isotopically *substituted* rather than *labelled* (*Nomenclature of Organic Chemistry*, Pergamon Press, Oxford, 1979, p. 514).

‡ Abbreviations for amino acids and protecting groups are in agreement with the 1983 Recommendations of the IUPAC–IUB Joint Commission on Biochemical Nomenclature (*Eur. J. Biochem.*, 1984, **138**, 9).

Z-L-Val + [1,2-$^{13}C_2$,^{15}N]Gly-OEt $\xrightarrow{i}$ **3**

3 $\xrightarrow{ii}$ **4**

4 $\xrightarrow{iii}$ cyclo-(L-Val-[1,2-$^{13}C_2$,^{15}N]Gly-) **1** **5**

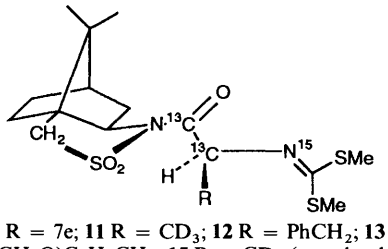
Scheme 1 *Reagents:* i, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoro boramide (TBTU)[13]; ii, H_2/Pd, cyclization; iii, Me_3OBF_4 [5a]

The cyclization reaction was very slow. Crude compound **1** was used as such in the subsequent alkylation step. The total yield of compound **1** was 74% calculated from labelled glycine.

From the labelled ester **3** mentioned above the required intermediate $(MeS)_2C$ = [1,2-$^{13}C_2$,^{15}N]Gly-OEt **6** was made in high yield on a smaller scale than that described by Hoppe and Beckmann[14] for the non-labelled methyl ester. It was purified by fast flash chromatography instead of distillation. Some decomposition of compound **6** was thereby noticed but at high flow rate it became negligible. Compound **6** was treated with commercial (2*R*)-bornane-10,2-sultam by a procedure adapted from Oppolzer *et al.*[15] It was first purified by column chromatography and was then crystallized to give pure sultam **2** in 68% yield. The total yield of **2** was 52% calculated from labelled glycine.

*Synthesis of Backbone-Labelled Boc-*D*-Leu with Schöllkopf's Reagent and Boc-*L*-Leu with Oppolzer's Method.*—Compound **1** was isobutylated and hydrolysed as described previously.[5a] The resulting mixture of L-Val-OMe·HCl and D-[1,2-$^{13}C_2$,^{15}N]Leu-OMe·HCl was separated by chromatography instead of distillation and the fraction corresponding to the latter compound was treated directly with an excess of Boc_2O. Finally, saponification furnished Boc-D-[1,2-$^{13}C_2$,^{15}N]Leu **7**, which was characterized with respect to optical purity after cleavage to the free amino acid. The total yield of **7** was 31% calculated from labelled glycine.

Compound **2** was treated with isobutyl iodide at low temperature and the crude product was first chromatographed and then crystallized to give pure (2*R*,2′*S*)-*N*-{bis(methylsulfanyl)methylene-[1,2-$^{13}C_2$,^{15}N]leucyl}bornane-10,2-sultam **9**. Careful purification of this doubly protected intermediate is presumably an essential step in order to obtain the final free amino acid or derivative thereof in the highest possible optical purity. Treatment first with acid to split off the N-protecting group followed by alkaline hydrolysis to cleave off the auxiliary gave a crude product containing additional salt. This material was directly treated with an excess of Boc_2O to give Boc-L-[1,2-$^{13}C_2$,^{15}N]Leu **8**, the enantiomer of compound **7**. The optical purity of **8** was established after removal of its Boc-group. The total yield of compound **8** was 34% calculated from labelled glycine.



9 R = Bu^i; **10** R = 7e; **11** R = CD_3; **12** R = $PhCH_2$; **13** R = $PhCD_2$; **14** R = 4-$(PhCH_2O)C_6H_4CH_2$; **15** R = CD_3 (no other labels)

Results from Determinations of the Optical Purity of Isomers **7** *and* **8**.—Small samples of isomers **7** and **8** were deprotected and assayed for their optical purity by HPLC using the Flec-method[16a] and by GLC on chiral capillary columns.[16b,c] Both methods allow careful quantification even of trace amounts of enantiomeric impurities. In non-labelled commercial proteinogenic amino acids the major component is nowadays generally present in better than 99.5% enantiomeric excess (%ee). Our present goal is to achieve synthesis of the corresponding isotope-labelled Boc-amino acids in at least 99%ee.

Starting from L-valine (99.7%ee by HPLC and 99.5 by GLC), compound **7** was obtained in 97.4 and 97.2%ee, respectively. In previous work using the Schöllkopf method products were generally characterized by ^{1}NMR spectroscopy, which is less sensitive and accurate for this purpose. This value is under all circumstances inferior to that obtained for compound **8**, in which case both methods indicated 99.7%ee, in close agreement with the value reported by Oppolzer *et al.*[15a] for non-labelled L-leucine (> 99.8%ee).

Additional Backbone-labelled Amino Acid Derivatives Made.—Since, in our hands, Oppolzer's method gave a leucine derivative of higher optical purity than that obtained with Schöllkopf's, we decided to use it to make a number of additional backbone-labelled L-amino acids, including a few deuteriated in the side chain. Since our ultimate goal is to apply our labelled derivatives in solid-phase peptide synthesis, we chose to prepare them as Boc-derivatives:

(*S*)-Boc-^{15}NH-^{13}CHR-$^{13}CO_2$H

16 R = Me; **17** R = CD_3; **18** R = $PhCH_2$; **19** R = $PhCD_2$; **20** R = 4-$(PhCH_2O)C_6H_4CH_2$; **21** R = CD_3 (no other labels)

This paper therefore also describes the synthesis of a number of corresponding sultam derivatives **10**–**15**. These were obtained from sultam **2** (except **15**) in 72–87% yield. From the latter compounds, the Boc-amino acids **16**-**21** could be prepared in pure form in 59–89% yield and 99.0–99.8%ee.

Discussion

Amino acids selectively labelled with stable isotopes complement uniformly labelled ones currently prepared by hydrolysis of biomass. Their purification is generally accomplished by well established, but time-consuming, chromatographic methods.[17] Biosynthetic incorporation of such isotopomers into proteins greatly assists in the assignment and interpretation of NMR spectra of these macromolecules.[18] For up-to-date reviews of this field, including appraisals of isotope labelling in this context, see ref. 19.

To our knowledge, extensive isotope-labelling of amino acids by asymmetric synthesis of the type described in this paper has not previously been described, although the methodology for such work has been available for several years.[7] Our approach relies heavily on precursors developed recently for this purpose.[10,12] Initially, we have restricted the applications to backbone-labelling with additional deuteriation in position 3 in a few cases. The products are primarily intended for application in solid-phase synthesis,[20] as a consequence of which the corresponding Boc-derivatives have been prepared.

Schöllkopf *et al.*[3a,5] developed a very attractive procedure for asymmetric synthesis of amino acids utilizing alkylation of bislactims derived from cyclic dipeptides.[1b] As the required intermediate **1** could easily be made from a labelled precursor already prepared in our laboratory,[12] we decided to explore the Schöllkopf method for the synthesis of the Boc-D-leucine isotopomer **7**.

Oppolzer *et al.*[15a] more recently devised an alternative scheme for asymmetric synthesis of amino acids employing a bornane sultam as a chiral auxiliary. By attaching an N,N-

bisprotected glycine to this complement, a suitable enolate, most probably further stabilized by chelation, can be generated for alkylation. At the outset of this work few details regarding the experimental procedure were available. Nevertheless, the incredible stereospecificity reported immediately attracted our attention, as a result of which the intermediate **2** was prepared and used to obtain the Boc-L-leucine isotopomer **8**.

In our hands, both methods investigated gave rise to backbone-labelled Boc-leucines in about the same yield (31 and 34%, calculated from glycine). However, with respect to the optical purity of the products, there was a significant difference between them and the amount of enantiomeric impurity was about 10 times higher in D-form **7** than in L-form **8**. High-quality L-valine was used to make the cyclic dipeptide, so there seems to be no trivial explanation for the increased amount of enantiomer in this case. On the other hand, this difference does not necessarily reflect a higher stereospecificity in the alkylation step in the Oppolzer compared with the Schöllkopf method. In fact, analysis of the crude, alkylated sultams indicated purities only in the range of 94.7–98.4% diastereoisomeric excess [15a] but these values rose to above 99% after chromatography and/or crystallization. A preliminary attempt to chromatograph the crude, alkylated bislactim was accompanied by severe loss of material. Further attempts to remove diastereoisomeric impurities were not undertaken in this context.

Therefore, we decided to apply the Oppolzer method for the synthesis of three labelled Boc-L-alanines **16**, **17** and **21**. As the corresponding sultams **10**, **11** and **15** could be crystallized easily,[15a] we abandoned chromatographic purification even in these cases without reduction of the enantiomeric purity. However, in two cases when we used a slightly smaller excess of alkylating agent CD_3I, we noticed that the final products were contaminated by Boc-glycine, which therefore had to be removed by chromatography. With this experience in mind we decided in the subsequent work both to chromatograph and to crystallize the alkylated sultams, whenever possible.

Two additional Boc-L-phenylalanines, **18** and **19**, as well as Boc-(*O*-benzyl)-L-tyrosine **20** were also prepared. The crude, alkylated sultams **12**, **13** and **14** were purified by column chromatography, and in the first two cases also by crystallization before cleavage from the auxiliary. On the other hand, sultam **14** resisted crystallization and was therefore cleaved as it was. This fact is obviously reflected in the lower optical purity of our labelled tyrosine [1st experiment, single sultam chromatography: crude product 97.2%ee (GLC)] and supports the idea that small amounts of enantiomer are actually formed but are removed by chromatography/crystallization of the sultam [2nd experiment, dual sultam chromatography: crude product 98.1%ee (GLC)].

In addition to its general convenience and high stereoselectivity,[15a] Oppolzer's method features a low number of synthetic steps for the conversion of glycine into products and the yields in the individual steps are high, which makes it ideal for the present application. Furthermore, in work on a somewhat larger scale it should be worthwhile to recover part of the labelled glycine lost in its derivatization and attachment to the sultam.

In conclusion, this paper demonstrates that selectively isotope-labelled amino acid derivatives of high steric purity with ^{15}N and ^{13}C in the backbone and with or without additional labelling in the side chain can be prepared in quantities adequate for further synthetic manipulations. This work therefore opens up new possibilities to accomplish also synthesis of backbone-labelled peptides for structural studies of folding in solution. Work is therefore now in progress dealing with the incorporation of these isotopomers into peptides. Further efforts along the same lines as in this paper are also expected to provide useful derivatives of proteinogenic amino acids with functionalized side chains.

Experimental

General Methods.—M.p.s were recorded on a Gallenkamp apparatus and are uncorrected. Acetone, $CHCl_3$, CH_2Cl_2, dimethylformamide (DMF), MeCN, tetrahydrofuran (THF) and toluene were dried for several days over activated molecular sieves (4 Å), in the case of toluene and THF after prior distillation in the presence of sodium wire and $LiAlH_4$, respectively, for THF under argon. TLC analyses were performed on 0.25 mm thick precoated silica plates (Merck DC-Fertigplatten, Kieselgel 60 F_{254}), eluted with (A) PhMe–MeCN, (B) $CHCl_3$–EtOH–water (100:50:4), (C) CH_2Cl_2–Me_2CO–HOAc (40:10:1), (D) light petroleum (40–60 °C)–diethyl ether (2:1), (E) hexane–diethyl ether (1:1) and (F) CH_2Cl_2–MeOH–HOAc (18:2:1). All compounds gave one spot unless otherwise stated. Spots were visualized by inspection under UV light and/or Cl_2-dicarboxidine.[21] Column chromatography was carried out on Merck Kieselgel 60 (70–230 mesh). 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 FT IR spectrometer. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a JEOL JNM-EX 270 spectrometer, and ^{15}N spectra, also in $CDCl_3$ on a JEOL FX 90Q instrument. All shifts are given in ppm, the ^{15}N ones using $\delta HCO^{15}NH_2$ = 113.2 [$Cr(acac)_3$ 0.1 mol dm^{-3}, when used] as reference, and coupling constants in Hz (estimated error ±0.5 Hz). Assignments were made by comparison of chemical shifts and peak multiplicities. For simplicity, only relevant resonances have been included. In selected cases only major features of the spectra are briefly outlined.

(2*R*)-Bornane-10,2-sultam was from Oxford Asymmetry Ltd, UK, C^2H_3I from CIL and PhC^2H_2Br from Isotec, both USA. Most chemicals were of the best analytical quality. The analytical HPLC cartridge column, Spherisorb C_8, 5 μm, 4.6 × 150 mm, originated from Phase Separations Ltd, UK and the semipreparative Vydac C_{18} column, 10 μm, 20 × 250 mm, from The Separations Group, USA. The chiral GLC columns (Chirasil-L-Val and -D-Val) were from Chrompack, The Netherlands. (+)- and (−)-1-(fluoren-9-yl)ethyl chloromethanoate (Flec) were purchased from EKA Nobel, Sweden.

Determination of Chiral Purity of Amino Acids.—The chiral purity, expressed as %ee, of all amino acids made, was determined by both HPLC and capillary GLC. Analyses according to the former method were performed as described in ref. 16*a*, except that a 5 μm cartridge was used; tyrosine was detected by fluorescence (excitation/emission at 260/310 nm, Shimadzu, RF-551) and other amino acids by UV at 265 nm at 0.02 absorbance units full scale (AUFS). An automatic integrator was used. For every enantiomeric pair of amino acids a standard curve was prepared covering the range studied (down to 0.1–0.2%ee). Correction factors have so far never deviated by more than 5% from unity. The minor peak was clearly visible even in the low-content region. L-Amino acids were analysed with (+)-Flec, and D-leucine with (−)-Flec reagent, so that the minor peak always eluted first. Capillary GLC, using trifluoroacetylamino acid isopropyl esters, was performed on commercial Chirasil columns [16b] essentially as described earlier.[16c] Trifluoroacetylation was accomplished with trifluoroacetic acid anhydride (TFAA) in EtOAc under reflux. Nitrogen was used as carrier gas. The GLC set-up included an autoinjector and an automatic integrator. Standard curves were prepared as above (down to 0.1–0.2%ee). All labelled Boc-amino acids were checked for their optical purity after removal of the Boc groups with 50% TFA/CH_2Cl_2 for 1 h at room temperature. No particular problems were encountered in the determinations of optical purity of labelled amino acids and no increase in bandwidth was noted due to the presence of additional isotopomers.

*Ethyl Benzyloxycarbonyl-*L*-valyl-*[1,2-$^{13}C_2$,^{15}N]*glycinate* **4**. —Z-L-Val (4.49 g, 17.9 mmol) was dissolved in MeCN (140 cm^3) and finely ground labelled glycine ester **3** (2.55 g, 17.9 mmol), obtained by deprotection of $Boc_2{}^{15}N{}^{13}CH_2{}^{13}CO_2Et$[12b] with 4 mol dm^{-3} HCl in 1,4-dioxane, was added. The stirred, ice-cooled slurry was treated dropwise with NEt_3 (1.81 g, 17.9 mmol) followed (i) by solid TBTU (6.32 g, 19.7 mmol) in portions and (ii) by more NEt_3 (1.99 g, 19.7 mmol). After 1 h in ice and 13 h at ambient temperature the turbid mixture was partitioned between EtOAc (800 cm^3) and 1 mol dm^{-3} aq. $KHSO_4$ (400 cm^3), the extract washed successively with aq. $KHSO_4$, 1 mol dm^{-3} aq. $NaHCO_3$ and saturated aq. NaCl (3 times each) and dried (Na_2SO_4). Evaporation gave a solid (5.81 g, 96%); pure by TLC (A); fluffy needles with m.p. 170–170.5 °C [from heptane–EtOAc (3:2; 60 cm^3 g^{-1})] (lit.,[22] 167–168 °C); $[\alpha]_D^{25}$ −11.3 (*c* 1, $CHCl_3$) [lit.,[22] $[\alpha]_D^{25}$ −12.9° (*c* 2, $CHCl_3$)]; δ_H 0.94 and 0.98 (6 H, 2 d, *J* 6.8, Val Me_2), 1.27 (3 H, t, *J* 7.1, CH_2Me), 2.16 (1 H, m, *J* ~7, Pr^i), 4.02 [2 H, dm, ^1J(^{1}H, ^{13}C) 141, $^{13}CH_2$], 4.09 (1 H, dd, Val α-H), 4.20 (2 H, dq, J_H ~7.2, ^3J(^{1}H, ^{13}C) 3.0, CH_2Me], 5.09 and 5.12 (2 H, ABq, J_{gem} 12.2, $PhCH_2$), 5.44 (~1 H, br d, Val NH), 6.58 [1 H, pert. d, J(^{1}H, ^{15}N) 92.7, Gly^{15}NH] and 7.35 (5 H, s, Ph); δ_C 14.1 [d, ^3J(^{13}C, ^{13}C) 2.4, CH_2Me], 17.7 and 19.2 (Val Me), 31.1 ($CHMe_2$), 41.2 [dd, ^1J(^{13}C, ^{13}C) 61.1, ^1J(^{15}N, ^{13}C) 13.4, $^{13}CH_2$], 60.2 [d, ^2J(^{13}C, ^{13}C) 8.6, CH_2Me], 61.5 (Val C-α), 67.0 $PhCH_2$), 128.0, 128.2, 128.5 and 136.2 (Ar), 156.4 Bzl-O-*C*O), 169.6 [d, ^1J(^{13}C, ^{13}C) 62.3, Gly^{13}CO] and 171.6 [d, ^1J(^{13}C,^{15}N) 14.7, Val CO]; δ_N 105.5 [d, ^1J(^{15}N,^{13}C) 13.2, Gly^{15}N]; ν_{max}/(cm^{-1}) 1716 and 1705 (^{13}CO), 1690 (urethane CO), ~1650 (amide I) and ~1540 (amide II) (1758, 1748, 1690, 1654, 1563 and 1540 for non-labelled reference).

Cyclo-(L-*valyl-*[1,2-$^{13}C_2$,^{15}N])*glycine* **5**.—Compound **4** (2.27 g, 6.69 mmol) in absolute EtOH (45 cm^3) was hydrogenolysed for 2 h over Pd (5% on C, ~0.3 g), when TLC (B) indicated complete reaction. The catalyst was filtered off and the filtrate was evaporated to give an oil, which was redissolved in toluene, filtered and again taken to dryness. The residual product, after drying at ~0.01 mmHg, consisted of essentially pure (TLC, B) L-Val-[1,2-$^{13}C_2$,^{15}N]Gly-OEt, weighed 1.27 g (93%). It was dissolved in toluene (15 cm^3) and the solution was refluxed for 7 days (TLC). The resulting jelly was chilled to 0 °C, and the still gelatinous precipitate was collected by filtration, rinsed first with cold toluene and then with dry diethyl ether, and dried (~0.01 mmHg) to give a product (837 mg). From the combined filtrate and washings an additional crop (91 mg) could be obtained after further reaction for 7 days (total yield 94%). An analytical specimen was obtained by recrystallization from water (10 cm^3 g^{-1}, decolourizing carbon); pure by TLC (B); m.p. 264–266 °C (decomp.) [lit.,[5a] (^{14}N-analogue) 254 °C]; $[\alpha]_D^{25}$ 30.7 (*c* 0.9, water) {lit.,[5a] (^{14}N-analogue) $[\alpha]_D^{20}$ 20.2 (*c* 0.9, water)}; δ_H[$(CD_3)_2SO$] 0.85 and 0.93 (6 H, 2 d, J_H 6.9, $CHMe_2$), 2.11 (1 H, m, $CHMe_2$), 3.54 (1 H, pert. sign., Val α-H), 3.62 (H^α) and 3.82 (H^β) [2 H, AB syst., J_{gem} 17.7, further split by coupling to $^{13}C_2$: ^1J($^1H^\alpha$, ^{13}C) 142, ^1J($^1H^\beta$, ^{13}C) 141 and coupling of H^α to vicinal NH: ^3J(^{1}H, ^{1}H) 3.1, additional coupling to $^{13}C_1$: ^2J($^1H^\beta$, ^{13}C 5.6), $^{13}CH^\alpha H^\beta$], 8.03 [~1 H, d, ^1J(^{1}H, ^{15}N) ~90, ^{15}NH] and 8.21 (~1 H, br s, ^{14}NH); δ_C[$(CD_3)_2SO$] 17.0 and 18.4 ($CHMe_2$), 32.1 ($CHMe_2$), 44.0 [dd, ^1J(^{13}C, ^{13}C) 50.1, ^1J(^{15}N, ^{13}C) 8.5, $^{13}CH_2$], 59.7 [d, ^2J(^{15}N, ^{13}C) 6.1, Val C-α], 166.0 [d, ^1J(^{13}C, ^{13}C) 50.1, ^{13}C] and 167.1 [dd, J(^{13}C, ^{15}N) 14.6, ^2J(^{13}C, ^{13}C) 4.2, Val CO]; δ_N[$(CD_3)_2SO$] 107.6 [dd, J(^{15}N, ^{13}C) 8.5, ^2J(^{15}N, ^{13}C) 0.8, ^{15}NH]; ν_{max}/(cm^{-1}) 2 broad peaks centred at ~1660 and ~1635 (amides) (broad peak centred at ~1672 for non-labelled reference).

*Boc-*D-[1,2-$^{13}C_2$,^{15}N]*leucine* **7**.—A. *Methylation of Compound* **5**. Crude compound **5** was suspended in CH_2Cl_2 and methylated with Me_3OBF_4 as previously described.[5a] The yield of the crude pyrazine **1** was 82%, essentially pure by TLC (A,B). ^{1}H and ^{13}C NMR spectra indicated the presence of the desired product; δ_N 217.3 [d, ^1J(^{15}N, ^{13}C) 0.9, ^{15}N].

B. *Alkylation step.* Compound **1** was dissolved in THF and isobutylated at −78 °C with Bu^iI/BuLi according to the established procedure.[5a] After conventional work-up, the isobutyl analogue was obtained in 96% yield as a light brown oil. ^{1}H and ^{13}C NMR spectra indicated only minor amounts of impurities; δ_N 231.6 [d, ^1J(^{15}N, ^{13}C) 1.0, ^{15}N].

C. *Acid hydrolysis. Preparative LC.* The above crude isobutyl derivative was hydrolysed with dil. HCl. The resulting mixture of L-Val-OMe·HCl and D-[1,2-$^{13}C_2$,^{15}N]Leu-OMe·HCl (amino acid analysis: Val 1.02, Leu 0.98, Gly 0.03) was separated by semi-preparative ion-pair chromatography. An aliquot (~300 mg) of the mixture, dissolved in starting buffer (1 cm^3), was applied each time. Using 0.1 mol dm^{-3} phosphate buffer, pH 3.00, containing also D-camphor-10-sulfonic acid (0.075 mol dm^{-3})/EtOH (90:10), L-Val-OMe·HCl was eluted first (detection at 216 nm). The D-[1,2-$^{13}C_2$,^{15}N]Leu-OMe·HCl could be obtained with a gradient up to 30:70 of buffer mixture. The resulting eluate (350 cm^3) was concentrated to ~20 cm^3 and partitioned between CH_2Cl_2 (50 cm^3) and 30% K_2CO_3/saturated aq. NaCl (1:2; 150 cm^3), the aq. phase was backwashed (4 times) with CH_2Cl_2 and the combined extracts were washed with brine and dried (Na_2SO_4). TLC (B) showed one spot.

D. *Boc-*D-[1,2-$^{13}C_2$,^{15}N]*Leu-OMe.* The CH_2Cl_2 extract was concentrated to ~5 cm^3 and treated with an excess of Boc_2O–*N*-methylmorpholine overnight. Removal of the solvent left a yellow oil, which was partitioned between diethyl ether and 0.2 mol dm^{-3} citric acid. The organic extract was washed and dried as usual, whereupon evaporation furnished the crude Boc-derivative in essentially quantitative yield as calculated from the crude hydrolysis mixture. ^{1}H and ^{13}C NMR spectra showed only minor amounts of Boc_2O and other impurities; δ_N 89.3 [d, ^1J(^{15}N, ^{13}C) 12.8, Leu ^{15}N].

E. *Preparation of compound* **7**. The crude methyl ester was dissolved in 1,4-dioxane (15 cm^3 g^{-1}) and treated with 1.00 mol dm^{-3} aq. NaOH (1.00 mol equiv. as calculated from the weight of crude methyl ester). After 3 h two volumes of water were added, most of the dioxane was stripped off and the remaining turbid mixture was partitioned between diethyl ether (30 cm^3 g^{-1}) and 1 mol dm^{-3} aq. $KHSO_4$ (15 cm^3 g^{-1}). The aq. phase was extracted twice with diethyl ether and the extracts were washed with brine, dried (Na_2SO_4), and taken to complete dryness. The residual oil was crystallized from EtOH–water (1:3; 45 cm^3 g^{-1}). After seeding and chilling, the title compound precipitated as shiny flakes. The overall yield was 62% as calculated from the crude mixture of Me esters before chromatography; TLC (C) showed one spot; ^{1}H NMR spectroscopy revealed only traces (<1%) of remaining Me ester; m.p. 84–86 °C (softens at ~80 °C [lit.,[8b] (^{15}N analogue) 68–72 °C]; $[\alpha]_D^{25}$ 25.1 (*c* 2.04, HOAc) [lit.,[8b] $[\alpha]_D$ 24.0 (*c* 1.99, HOAc)]; δ_H 0.96 (6 H, d, J_H 6.4, $CHMe_2$), 1.45 (9 H, s, Boc), 1.46–1.76 ~3H, complex, CH_2CH), 4.14 [~0.3 H, pert. dm, ^1J(^{1}H, ^{13}C) 144.0, ^{13}CH, *Z*], 4.33 [~0.7 H, pert. dm, ^1J(^{1}H, ^{13}C) 142.2, ^{13}CH, *E*], 4.98 [~0.7 H, dd, ^1J(^{1}H, ^{15}N) 90.6, ^{15}NH, *E*], 6.41 [~0.3 H, pert. dd, ^1J(^{1}H, ^{15}N) 92.4, ^{15}NH, *Z*] and 11.42 (~1 H, br s, CO_2H); δ_C 21.8 [d, ^3J(^{13}C, ^{13}C) 2.4] and 22.8 [d, ^3J(^{13}C,^{13}C) 3.1] ($CHMe_2$), 24.7 (perturbed d, ^2J(^{13}C, ^{13}C) ~11, $CHMe_2$], 28.3 (Boc), 41.5 [d, ^1J(^{13}C, ^{13}C) 34.2, CH_2CH], 52.0 [dd, ^1J(^{13}C, ^{13}C) 58.6, ^1J(^{13}C, ^{15}N) 12.8, ^{13}CH, *E*], 53.1 [dd, ^1J(^{13}C, ^{13}C) 59.2, ^1J(^{13}C, ^{15}N) 11.0, ^{13}CH, *Z*], 80.1 (CMe_3, *E*), 81.7 (CMe_3, *Z*), 155.7 [d, ^1J(^{13}C, ^{15}N) 25.6, Boc CO, *E*], 156.9 [d, ^1J(^{13}C, ^{15}N) 25.6, Boc CO, *Z*], 178.1 [d, ^1J(^{13}C, ^{13}C) 58.6, CO_2H, *Z*] and 178.5 [d, ^1J(^{13}C, ^{13}C) 59.2, CO_2H, *E*]; δ_N 88.6 [d, ^1J(^{15}N, ^{13}C)

12.6, ^{15}NH, *E*] and 91.7 [d, $^{1}J(^{15}N,^{13}C)$ 10.3, ^{15}N, *Z*]; 97.4 (HPLC) and 97.2%ee (GLC).

Ethyl Bis(methylsulfanyl)methylene[1,2-$^{13}C_2$,^{15}N]*glycinate* **6** [$(MeS)_2C{=}^{15}N{-}^{13}CH_2{-}^{13}CO_2Et$].—This compound was prepared according to Hoppe and Beckmann with minor modification in the final purification step.[14] Starting from compound **3** (2.14 g, 15 mmol), compound **6** (2.77 g, 87%) was obtained as a slightly yellow oil. The final distillation was replaced by fast flash chromatography using light petroleum–Et_2O (3:1 v/v); flow rate ~10 cm^3 min^{-1}, when the decomposition was negligible; TLC (D); δ_H 1.30 (3 H, t, J_H 7.1, *Me*CH_2), 2.45 (3 H, s, MeS), 2.57 (3 H, s, MeS), 4.23 [2 H, dd, $^{1}J(^{1}H,^{13}C)$ 136.1, $^{2}J(^{1}H,^{13}C)$ 6.7, $^{13}CH_2$] and 4.23 [2 H, dq, J_H 7.2, $^{3}J(^{1}H,^{13}C)$ 3.1, *C*H_2Me]; δ_C 14.2 (s, *Me*CH_2), 14.6 (d, $^{3}J(^{13}C,^{15}N)$ 4.9, MeS), 14.8 (s, MeS), 54.2 [d, $^{1}J(^{13}C,^{13}C)$ 65.9, $^{13}CH_2$], 60.9 (s, *C*H_2Me), 163.1 [d, $^{1}J(^{13}C,^{15}N)$ 2.4, $(MeS)_2C{=}$] and 170.2 [dd, $^{1}J(^{13}C,^{13}C)$ 65.3, $^{2}J(^{13}C,^{15}N)$ 3.1, ^{13}CO]; δ_N 277.4 [d, $^{2}J(^{15}N,^{13}C)$ 3.4, ^{15}N, Cr(acac)$_3$]; ν_{max}/(cm^{-1}) 1705 (^{13}CO) and 1557 (C=^{15}N) (1733 and 1575 for non-labelled reference).

(2R)-N-{*Bis(methylsulfanyl)methylene*[1′,2′-$^{13}C_2$,^{15}N]*glycyl*}*bornane*-10,2-*sultam* **2**.—This procedure was adapted from ref.[15a] To a suspension of sultam (1.08 g, 5 mmol) in toluene (10 cm^3) was added dropwise Me_3Al (2 mol dm^{-3} in hexane; 3 cm^3, 1.2 mol equiv.) at room temp., resulting in a clear, refluxing solution. After this had been stirred for 30 min, a solution of glycine ester **6** (1.20 g, 5.71 mmol*) in toluene (4 cm^3) was added dropwise, followed by heating of the mixture at 55–58 °C for 24 h, when, after cooling in ice, the reaction was quenched by addition of MeOH (5 cm^3), followed after 30 min by water (4 cm^3). After being stirred for 1 h, the reaction mixture was filtered through a pad of Celite (subsequently washed twice with EtOAc) and the combined solution was dried ($MgSO_4$). After evaporation, the resulting oil was chromatographed [light petroleum–Et_2O (1:2, v/v); flow rate 10–15 cm^3 min^{-1}] and was finally crystallized from EtOH to give compound **2** (1.30 g, 68%, 60% calc. on labelled **6**); m.p. 106–108 °C [lit.,[15a] 107–109 °C (non-labelled); $[\alpha]_D^{27}$ −111.4 (*c* 0.22, $CHCl_3$) [lit.,[15b] $[\alpha]_D$ −115.6° (*c* 3.27, $CHCl_3$) (non-labelled)]; TLC (E); ^{1}H and ^{13}C NMR spectra indicated the presence of compound **2**; δ_H 4.65 and 4.69 [2 H, ABq, J_{gem} 17.9, split by coupling to $^{13}C_2$: $^{1}J(^{1}H,^{13}C)$ 136.6 and additionally to $^{13}C_1$: $^{2}J(^{1}H,^{13}C$ 5.4, $^{13}CH_2$]; δ_C 14.7 [d, $^{3}J(^{13}C,^{15}N)$ 6.1, MeS], 15.0 (s, MeS), 55.4 [d, $^{1}J(^{13}C,^{13}C)$ 56.1, $^{13}CH_2$], 164.3 [d, $^{1}J(^{13}C,^{15}N)$ 3.6, $(MeS)_2C{=}$] and 168.2 [dd, $^{1}J(^{13}C,^{13}C)$ 55.0, $^{2}J(^{13}C,^{15}N)$ 3.6, ^{13}CO]; δ_N 275.1 [d, $^{2}J(^{13}C,^{15}N)$, 2.7, ^{15}N, Cr(acac)$_3$]; ν_{max}/(cm^{-1}) 1669 (^{13}CO) and 1553 (C=^{15}N) (1712 and 1577 for non-labelled reference).

(2R,2′S)-N-{*Bis(methylsulfanyl)methylene*[1′,2′-$^{13}C_2$,^{15}N]-*leucyl*}*bornane*-10,2-*sultam* **9**.—This experiment was initiated at −78 °C with careful temperature control. To a stirred solution of sultam **2** (0.95 g, 2.5 mmol) in THF (20 cm^3) was added dropwise BuLi (1.6 mol dm^{-3} in hexane; 1.8 cm^3, 1.1 mol equiv.) followed, after 1 h, by hexamethylphosphoric triamide (HMPA) (3.1 cm^3, 17.5 mmol) and, 10 min later, by Bu^{i}I (1.43 cm^3, 12.5 mmol). After 1 h the temperature was allowed to rise to −30 °C and after an additional 16 h to ambient, when the reaction was quenched with brine (5 cm^3). The aqueous phase was extracted 3 times with EtOAc and the combined extracts washed with brine and dried ($MgSO_4$). After evaporation, the crude semisolid product was chromatographed [hexane–Et_2O (1:1, v/v); flow rate 10–15 cm^3 min^{-1}] and then was crystallized from EtOH with small amounts of hexane to yield compound **9** (0.77 g, 70%); TLC (E); m.p. 124–125 °C [lit.,[15a] 125–127 °C (non-labelled)]; $[\alpha]_D^{27}$ −80.7 (*c* 0.20, $CHCl_3$) [lit.,[15b] $[\alpha]_D$ −82.4° (*c* 1.37, $CHCl_3$) (non-labelled)]; ^{1}H and ^{13}C NMR spectra indicated compound **9**; δ_H 4.99 [1 H, 2 complex m, split by coupling to $^{13}C_2$: $^{1}J(^{13}C,^{1}H)$ 136.4, additional coupling to βCH_2, $^{13}C_1$ and ^{15}N, ^{13}CH]; δ_C 14.8 [d, $^{3}J(^{13}C,^{15}N)$ 4.9, MeS], 15.2 (s, MeS), 25.3 [d, $^{2}J(^{13}C,^{13}C)$ 2.5, Me_2CH], 43.2 [d, $^{1}J(^{13}C,^{13}C)$ 34.1, $^{13}CCH_2$], 63.7 [d, $^{1}J(^{13}C,^{13}C)$ 55.0, ^{13}CH], 161.4 [d, $^{1}J(^{13}C,^{15}N)$ 2.5, $(MeS)_2C{=}$], 172.1 [d, $^{1}J(^{13}C,^{13}C)$ 54.9, ^{13}CO]; δ_N 291.1 [s, ^{15}N, Cr(acac)$_3$]; ν_{max}/(cm^{-1}) 1663 (^{13}CO) and 1548 (C=^{15}N) (1702 and 1572 for non-labelled reference).

Other experiments indicated that no reaction took place below −55 °C and that racemization could be detected above −10 °C.

Cleavage of Compound **9** *for Determination of the Optical Purity of* L-[1,2-$^{13}C_2$,^{15}N]*Leucine*.—This procedure was adapted from that of Oppolzer *et al.*[23] A sample of compound **9** (20 mg, 45.8 μmol) was stirred in a mixture of 1 mol dm^{-3} HCl and THF (2.5 cm^3 each) for 4 h, when the solvents were stripped off and the residue was dried overnight (P_2O_5, KOH). Subsequent hydrolysis with 1 mol dm^{-3} aq. LiOH (0.5 cm^3) and THF (2 cm^3) for 16 h, acidification with 1 mol dm^{-3} HCl, and evaporation followed by drying (P_2O_5, KOH) furnished the sample used for analysis: 99.7 (HPLC) and 99.6%ee (GLC).

Boc-L-[1,2-$^{13}C_2$,^{15}N]*leucine* **8**.—*Cleavage of the bis(methylsulfanyl)methylene group from compound* **9**. To a stirred solution of compound **9** (0.61 g, 1.4 mmol) in THF (10 cm^3) was added 1 mol dm^{-3} HCl (10 cm^3) and the reaction was allowed to take place for 4 h. Part of the solvent was stripped off and the remaining mixture was washed twice with Et_2O which was backwashed twice with 1 mol dm^{-3} HCl. The combined aqueous phases were evaporated completely and the product was dried carefully (P_2O_5 + KOH) (0.51 g, quant.).

B. *Cleavage of leucine from sultam.* The above intermediate (0.34 g, 0.95 mmol) was stirred overnight with 1 mol dm^{-3} aq. LiOH (4 cm^3) and THF (16 cm^3). Most of the THF was evaporated off and water (20 cm^3) was added; the aqueous phase was first extracted 3 times with CH_2Cl_2 to recover the sultam (extracts backwashed with water twice) and then was acidified with 1 mol dm^{-3} HCl and evaporated to dryness to give leucine·HCl (130 mg). TLC [BuOH–HOAc-water (4:1:1, v:v:v)] indicated pure leucine.

C. *Preparation of compound* **8**. The free leucine·HCl was converted into the corresponding crystalline Boc-derivative **8** with Boc_2O in 92% yield; TLC (F); m.p. 74–76 °C, [lit.,[24] 78–81 °C]; $[\alpha]_D^{27}$ −24.1 (*c* 1.01, HOAc) {lit.,[8b] $[\alpha]_D$ −24° (*c* 1.99, HOAc)}; ν_{max}/(cm^{-1}) 1697 (CO_2H CO), 1651 (urethane CO) and 1528 (amide II) (1718, 1676 and 1541 for non-labelled reference, respectively); 99.7%ee (both HPLC and GLC).

(2R,2′S)-N-{*Bis(methylsulfanyl)methylene*[1′,2′-$^{13}C_2$,^{15}N]-*alanyl*}*bornane*-10,2-*sultam* **10**.—This experiment was performed similarly to that described for the homologue **9**. To a solution of compound **2** (1.33 g, 3.5 mmol) in THF (25 cm^3) were added BuLi (1.6 mol dm^{-3} in hexane; 2.5 cm^3, 1.1 mol equiv.) and MeI (0.7 cm^3, 10.5 mmol) followed by HMPA (1.9 cm^3, 10.5 mmol). Typical work-up and purification yielded title product (1.03 g, 75%); TLC (E); m.p. 116.5–118 °C [lit.,[15a] 119–120 °C (non-labelled)]; $[\alpha]_D^{27}$ −69.3 (*c* 0.18, $CHCl_3$) {lit.,[15b] $[\alpha]_D$ −70.1° (*c* 1.34, $CHCl_3$) (non-labelled)}; ^{1}H and ^{13}C NMR spectra indicated compound **10**; δ_H 1.51 (3 H, complex m, Me^{13}C), 5.02 [1 H, ddq, J_H 6.8, split by coupling to $^{13}C_2$: $^{1}J(^{1}H,$

* Up to 50% of the excess of ester **6** used could be recovered in the chromatography experiment. As this compound is unstable in contact with silica, it is essential to use a high flow rate in the purification step.

^{13}C) 137.3, additional coupling to $^{13}C_1$: $^2J(^1H, ^{13}C)$ 2.8 and to ^{15}N: $^2J(^1H, ^{15}N)$ 1.5, ^{13}CH]; δ_C 14.8 [d, $^3J(^{13}C, ^{15}N)$ 4.8, MeS], 15.1 (s, MeS), 19.9 [d, $^1J(^{13}C, ^{13}C)$ 35.4, *Me*^{13}C], 60.6 [d, $^1J(^{13}C, ^{13}C)$ 55.5, ^{13}CH], 161.8 [s, (MeS)$_2C$=] and 172.3 [dd, $^1J(^{13}C, ^{13}C)$ 55.5, $^2J(^{13}C, ^{15}N)$ 3.1, ^{13}CO]; δ_N 291.6 [d, $^2J(^{13}C, ^{15}N)$ 2.7, ^{15}N, Cr(acac)$_3$]; ν_{max}/(cm^{-1}) 1671 (^{13}CO) and 1555 (C=^{15}N) (1714 and 1578 for the non-labelled reference).

Boc-L-[1,2-$^{13}C_2$,^{15}N]*alanine* **16**.—This compound was obtained using the method as described for compound **8**, from compound **10** (0.98 g, 2.5 mmol). After the usual work-up procedure, the product (83%) was purified *via* its dicyclohexylammonium (DCHA) salt and, after liberation, was recrystallized from EtOAc–hexane to yield a powder (59%); TLC (F); m.p. 83–83.5 °C [lit.,[25] 81–82 °C (non-labelled)]; $[\alpha]_D^{26}$ −25.1 (*c* 1.0, HOAc) {lit.,[25] $[\alpha]_D^{25}$ −24° (*c* 1.0, HOAc)}; δ_H 1.41–1.48 (partly obscured, *Me*CH) and 1.45 (together 12 H, s, Boc), 4.16 [~0.3 H, pert. dq, $^1J(^1H, ^{13}C)$ 142.3, α-H, *Z*], 4.35 [~0.7 H, pert. dq, $^1J(^1H, ^{13}C)$ 143.9, α-H *E*], 5.16 [~0.7 H, dd, $^1J(^1H, ^{15}N)$ 91.1, ^{15}NH, *E*], 6.93 [~0.3 H, pert. d, $^1J(^1H, ^{15}N)$ 93.3, ^{15}NH, *Z*] and 11.33 (~1 H, br s, $^{13}CO_2H$); δ_C 18.4 [d, $^1J(^{13}C, ^{13}C)$ 34.8, C-β], 28.3 (Boc Me), 49.1 [dd, $^1J(^{13}C, ^{13}C)$ 59.2, $^1J(^{13}C, ^{15}N)$ 12.8, C-α, *E*], 50.2 [dd, $^1J(^{13}C, ^{13}C)$ 59.2, $^1J(^{13}C, ^{15}N)$ 11.6, C-α, *Z*], 80.2 (*C*Me$_3$, *E*), 81.6 (*C*Me$_3$, *Z*), 155.4 [d, $^1J(^{13}C, ^{15}N)$ 25.6, Boc CO, *E*], 156.9 [d, $^1J(^{13}C, ^{15}N)$ 25.6, Boc CO, *Z*], 177.3 [d $^1J(^{13}C, ^{13}C)$ 58.6, $^{13}CO_2H$, *Z*] and 178.0 [d, $^1J(^{13}C, ^{13}C)$ 58.6, $^{13}CO_2H$, *E*]; δ_N 90.8 [d, $^1J(^{15}N, ^{13}C)$ 13.2, ^{15}NH, *E*] and 94.1 [d, $^1J(^{15}N, ^{13}C)$ 11.7, ^{15}NH, *Z*]; ν_{max}/(cm^{-1}) 3375 (NH), 1692 (CO_2H CO), 1681 (urethane CO) and 1503 (amide II) (3384, 1740, 1692 and 1517 for non-labelled reference, respectively); 99.4 (HPLC) and 99.8%ee (GLC); content of Boc-Gly in crude product as shown by amino acid analysis: <0.1% (after removal of Boc).

(2R,2'S)-N-{*Bis*(*methylsulfanyl*)*methylene*[1',2'-$^{13}C_2$,3',3',3'-$^2H_3$,^{15}N]*alanyl*}*bornane*-10,2-*sultam* **11**.—This experiment was performed, starting from compound **2** (0.86 g, 2.27 mmol), as detailed for compound **9** but using a smaller excess of alkylating agent as in the preparation of compound **15** (see later). Yield of compound **11** was 0.77 g (85%); m.p. 117–118 °C [lit. as above]; $[\alpha]_D^{27}$ −68.1 (*c* 0.20, $CHCl_3$) [lit. as above]; ^1H and ^{13}C spectra indicated compound **11**; δ_H 5.01 [d, $^1J(^1H, ^{13}C)$ 136.8, ^{13}CH]; δ_C 14.8 [d, $^3J(^{13}C, ^{15}N)$ 4.9, MeS], 15.1 (s, MeS), 18.8–20.1 (complex, CD_3), 60.5 [d, $^1J(^{13}C, ^{13}C)$ 54.9, ^{13}CH], 161.5 [s, (MeS)$_2$C=], 172.4 [dd, $^1J(^{13}C, ^{13}C)$ 55.5, $^2J(^{13}C, ^{15}N)$ 3.0, ^{13}CO]; δ_N 291.7 [d, $^2J(^{13}C, ^{15}N)$ 2.9, ^{15}N, Cr(acac)$_3$]; ν_{max}/(cm^{-1}) 1671 (^{13}CO), and 1555 (C=^{15}N) (1714 and 1578 for the non-labelled reference); 99.5%ee (HPLC and GLC).

Boc-L-[1,2-$^{13}C_2$,3,3,3-$^2H_3$,^{15}N]*alanine* **17**.—This compound was obtained in a similar manner to compounds **8** and **16** from compound **11** (0.66 g, 1.66 mmol) in 77% yield; minor amounts of Boc-Gly (4.2% as shown by amino acid analysis) could be removed by chromatography on silica with CH_2Cl_2–Me_2CO–HOAc (90:10:1); total yield 59%; tiny crystals (from EtOAc–hexane); m.p. 83.5–84 °C [lit. as for **16**]; $[\alpha]_D^{26}$ −25.3 (*c* 1.0, HOAc) [lit. as for **16**]; the ^1H spectrum was similar to that of compound **16** except that the obscured signal (δ 1.41–1.48) was absent; the ^{13}C spectrum was virtually identical with that of compound **16** except that the doublet (δ_C 18.4) was replaced by a complex signal centred at δ_C 18.2 (CD_3); other signals shifted <0.2 ppm; the ^{15}N spectrum was identical with that of compound **16**; ν_{max}/(cm^{-1}) 3375 (NH), 1692 (CO_2H CO), 1682 (urethane CO), 1502 (amide II) (3384, 1740, 1692 and 1517 for non-labelled reference, respectively); 99.8%ee (HPLC) and 99.9%ee (GLC).

Benzyl Iodide.—A solution of benzyl bromide (13.6 g, 80 mmol) in acetone (40 cm^3) was treated with NaI (32 g, 2.5 mol equiv./140 cm^3 of acetone) according to an earlier procedure.[26] The excess of iodide was removed by successive washes with 2.5% aq. $Na_2S_2O_3$ (3 × 100 cm^3) and brine, and dried (Na_2SO_4). Evaporation to dryness afforded a brown solid, which was recrystallized from diethyl ether at −20 °C to give a faintly coloured cotton-like product (12 g, 69%); m.p. 23–24 °C [lit.,[27] 23–25 °C]; δ_H 4.49 (2 H, s, CH_2) and 7.22–7.39 (5 H, m, Ph).

(2R,2'1S)-N-{*Bis*(*methylsulfanyl*)*methylene*[1',2'-$^{13}C_2$,^{15}N]-*phenylalanyl*}*bornane*-10,2-*sultam* **12**.—This compound was prepared similarly to compound **9** from compound **2** (1.40 g, 3.7 mmol) in THF (19.5 cm^3) and BuLi (2.7 cm^3, 4.32 mmol) at −78 °C followed by addition of a solution of $PhCH_2I$ (4.10 g, 18.8 mmol) in HMPA (4.7 cm^3, 26.3 mmol). The resulting crude product obtained after chromatography (1.65 g, 95%) was recrystallized from hexane to give crystals (1.5 g, 87%); m.p. 129–130 °C; $[\alpha]_D^{25}$ −116.5 (*c* 0.35, $CHCl_3$); δ_H 3.05–3.31 (m, β-H), 5.24 [br d, $^1J(^1H, ^{13}C)$ 138.8, α-H] and 7.14–7.31 (5 H, m, Ph); δ_C 40.0 (Ph*C*H$_2$), 66.7 (m, C-α), 126.5, 128.1, 129.9 and 137.1 (Ar) and 170 (m, CO); δ_N 290.3 (d, *J* 2.7); ν_{max}/(cm^{-1}) 1664 (^{13}CO) and 1550 (C=^{15}N).

Boc-L-[1,2-$^{13}C_2$,^{15}N]*phenylalanine* **18**.—Compound **12** (1.317 g, 2.8 mmol) was fully deprotected as described for compound **8** by subsequent reactions with 1 mol dm^{-3} HCl (20 cm^3) and 1 mol dm^{-3} aq. LiOH (10 cm^3) to afford Phe•HCl (~0.47 g, 93%). The hydrochloride salt was converted into its Boc derivative (0.65 g, 92%) as an oily product; δ_H 1.30 (~2 H, s, Boc Me, *Z*), 1.41 (~7 H, s, Boc Me, *E*), 2.90–3.17 (complex m, β-H$_2$), 4.38 [~0.3 H, br d, $^1J(^1H, ^{13}C)$ 144, ^{13}Cα-H, *Z*], 4.60 [~0.7 H, br d, $^1J(^1H, ^{13}C)$ 135, ^{13}Cα-H, *E*], 5.01 [~0.7 H, br dd, $^1J(^1H, ^{15}N)$ 91.7, ^{15}NH, *E*), 6.35 [~0.3 H, br d, $^1J(^1H, ^{15}N)$ 91.4, ^{15}NH, *E*], 7.20–7.30 (~5 H, complex m, Ph) and 10.73 (~1 H, br s, $^{13}CO_2H$); δ_C 28.0 (Boc Me, *Z*), 28.3 (Boc Me, *E*), 37.8 [d, $^1J(^{13}C, ^{13}C)$ 34.1, C-β, *E*], 38.9 [d, $^1J(^{13}C, ^{13}C)$ 30.5, C-β, *Z*], 54.3 [dd, $^1J(^{13}C, ^{13}C)$ 59.2, $^1J(^{13}C, ^{15}N)$ 13.4, C-α, *E*], 56.1 [dd, $^1J(^{13}C, ^{13}C)$ 59.8, $^1J(^{13}C, ^{15}N)$ 11.0, C-α, *Z*], 80.3 (*C*Me$_3$, *E*), 81.5 (*C*Me$_3$, *Z*), 127.1, 128.6, 129.4 and 135.9 (Ar), 155.4 [d, $^1J(^{13}C, ^{15}N)$ 26.8, Boc CO, *E*], 156.3 [d, $^1J(^{13}C, ^{15}N)$ 25.6, Boc CO, *Z*], 176.7 [d, $^1J(^{13}C, ^{13}C)$ 59.9, $^{13}CO_2H$, *Z*] and 177.1 [d, $^1J(^{13}C, ^{13}C)$ 58.6, $^{13}CO_2H$, *E*]; δ_N 86.2 [d, $^1J(^{15}N, ^{13}C)$ 13.4, ^{15}NH, *E*] and 90.1 [d, $^1J(^{15}N, ^{13}C)$ ~12, ^{15}NH, *Z*]; ν_{max}/ (oil, neat)/cm^{-1} 1688 and 1682 (CO) (1711 for non-labelled reference).

Part of this compound was converted into its DCHA salt in 84% yield; m.p. 210–212 °C; $[\alpha]_D^{25}$ +28.6 (*c* 0.10, $CHCl_3$) and then the free amino acid was regenerated from this salt; 99.2 (HPLC) and 99.1%ee (GLC).

(2R,2'S)-N-{*Bis*(*methylsulfanyl*)*methylene*[1',2'-$^{13}C_2$,3',3'-$^2H_2$,^{15}N]*phenylalanyl*}*bornane*-10,2-*sultam* **13**.—This compound was prepared as described above starting from compound **2** (0.95 g, 2.5 mmol/13 cm^3 THF), BuLi (1.8 cm^3, 1.1 mol equiv.) at −78 °C and PhC^2H_2I (0.90 g, 1.7 mol equiv.) in HMPA (2.3 cm^3, 13 mmol, 5.2 mol equiv.) in 72% yield (0.86 g); m.p. 129.5–130 °C (hexane–diethyl ether); $[\alpha]_D^{25}$ −145.7 (*c* 0.14, $CHCl_3$); δ_H 5.24 (d, *J* 138.7, α-H), 7.25–7.28 (5 H, m, PhC^2H_2), β-H not detected; δ_C see compound **12** but C-β (δ_C 39.9) is not detectable in this compound; δ_N 289.7 (d, *J* 2.7); ν_{max}/(cm^{-1}) 1664 (CO) and 1549 (C=^{15}N).

Boc-L-[1,2-$^{13}C_2$,3,3-$^2H_2$,^{15}N]*phenylalanine* **19**.—Compound **13** (0.80 g, 1.98 mmol) was fully deprotected as described for compound **8** and the HCl salt thus obtained (0.30 g, 93%) was converted into its Boc derivative (0.35 g, 64%) as an oily product; $[\alpha]_D^{25}$ +28.4 (*c* 0.10, $CHCl_3$); the ^1H spectrum of

compound **19** resembled that of isotopomer **18** but the complex signal (δ 2.90–3.17) was not present; the ^{13}C spectrum did not exhibit the distinct doublets at δ_C 37.7/38.9; instead a weak complex signal was barely visible in the same region; the ^{15}N spectrum was virtually identical with that of compound **18**; ν_{max}(neat)/(cm^{-1}) 1689 (CO); 99.1 (HPLC) and 99.0%ee (GLC).

p-*Benzyloxybenzyl Iodide*.—This compound was prepared from the corresponding chloride (5.0 g, 21.5 mmol, Fluka) in acetone (50 cm^3) with NaI (13 g, 86.6 mmol, 4 mol equiv.) in acetone (55 cm^3) in 86% yield according to Burawoy and Spinner;[28] m.p. 87–88 °C [from EtOAc–light petroleum (1:9)]; δ_H 4.47 (2 H, s, CH_2I), 5.05 (2 H, s, CH_2O), 6.89 and 7.31 (4 H, ABq, *J* 8.0, ArH), 7.36–7.64 (5 H, m, *Ph*CH_2O).

(2R,2'S)-O-*Benzyl*-N-{*bis*(*methylsulfanyl*)*methylene*[1',2'-$^{13}C_2$,^{15}N]*tyrosyl*}*bornane*-10,2-*sultam* **14**.—Compound **14** was prepared similarly to compound **9** from compound **2** (1.40 g, 3.7 mmol) in THF (20 cm^3), BuLi (2.7 cm^3, 4.32 mmol) and a solution of *p*-benzyloxybenzyl iodide (5.10 g, 15 mmol) in HMPA (6.5 cm^3, 36.3 mmol). After chromatographic purification the desired product (1.85 g, 87%) was isolated as a semisolid; $[\alpha]_D^{25}$ −207 (*c* 0.10, $CHCl_3$); δ_H see compound **12** and 5.02 and 5.07 (s, *E*/*Z*, PhCH_2O), 5.23 (dm, *J* 138.7, α-H) and 6.83–7.40 (9 H, m, ArH); δ_C see compound **12** and 66.9 (m, C-α), 70.1 (s, PhCH_2O) and 171.0 (m, CO); δ_N 290.3 (d, *J* 2.7); ν_{max}/(cm^{-1}) 1662 (CO) and 1549 (C=^{15}N).

Boc-(O-*benzyl*)-L-[1,2-$^{13}C_2$,^{15}N]*tyrosine* **20**.—Compound **14** (1.25 g, 2.17 mmol) was deprotected as above and *O*-Bzl-L-Tyr·HCl (0.49 g, 90%, 98.1/97.2%ee (GLC; see Discussion section) was transformed as usual into its Boc derivative (0.58 g, 89%). This product was further recrystallized from light petroleum–diethyl ether (15:1); m.p. 108.5–109 °C [lit.,[25] 109–111 °C (non-labelled)]; $[\alpha]_D^{26}$ +16.2 (*c* 0.50, MeOH) {lit.,[25] $[\alpha]_D$+16.6° (*c* 1, MeOH; non-labelled)}; δ_H 1.23 and 1.37 (s, 2 × Boc Me, *Z* and *E*), 2.80–3.32 (2 H, m, β-H), 4.27 [d, ^1J(^{1}H, ^{13}C) 147.5, α-H, *Z*] and 4.48 [d, ^1J(^{1}H, ^{13}C) 136.6, α-H, *E*], 6.91 and 6.95 (4 H, ABq, *J* 8.1, ArH) and 7.23–7.35 (5 H, m, *Ph*CH_2); δ_C 28.0 and 28.3 (s, Boc Me, *Z* and *E*), 36.6 (s, C-β, *Z*), 37.1 (s, C-β, *E*), 54.3 [dd, ^1J(^{13}C, ^{13}C) 58.6, ^1J(^{13}C, ^{15}N) 13.4, C-α, *E*], 56.1 [dd, ^1J(^{13}C, ^{13}C) 58.6, ^1J(^{13}C, ^{15}N) 11.0, C-α, *Z*], 69.9 (s, PhCH_2O), 80.3 (s, *C*Me_3), 114.9, 127.5, 127.9, 128.5, 130.4, 136.9 and 157.9 (Ar), 170.0 [d, J(^{13}C, ^{13}C) 58.6, CO_2H, *Z*] and 176.5 [d, ^1J(^{13}C, ^{13}C) 58.6, CO_2H, *E*]; δ_N 90.1 (d, *J* 12) and 86.0 (d, *J* 13.4); ν_{max}/(cm^{-1}) 1688 (CO) (1712 for non-labelled reference); 99.2/97.2 (HPLC) and 99.0/97.1%ee (GLC).

(2R,2'S)-N-{*Bis*(*methylsulfanyl*)*methylene*[3',3',3'-$^2H_3$]-*alanyl*}*bornane*-10,2-*sultam* **15**.—This experiment was performed similarly to that described for compound **9** using the non-labelled precursor corresponding to sultam **2** (1.72 g, 4.57 mmol) but a smaller excess of alkylating agent as for sultam **10**. Typical work-up procedure yielded product (1.50 g, 86%; TLC in hexane–Et_2O (1:1; v/v) gave one spot; m.p. 116.5–117.5 °C; $[\alpha]_D^{27}$ −68.3 (*c* 0.25, $CHCl_3$) [lit. as for **10**]; ^{1}H and ^{13}C spectra indicated compound **15**; δ_H 5.01 (1 H, s, α-H); δ_C 18.9–20.1 (m, CD_3); IR data as for the corresponding non-labelled analogue with slight losses in intensity in the CH region; 98.7 (HPLC) and 99.0%ee (GLC).

Boc-L-[3,3,3-$^2H_3$]*alanine* **21**.—The title compound was obtained as described for compound **8** and **16** from compound **15** (1.30 g, 3.3 mmol) in 78% yield; minor amounts of Boc-Gly (2.8%) could be removed as under compound **17**; total yield 69%; m.p. 83.5–84 °C; $[\alpha]_D^{26}$ −24.8 (*c* 1.0, HOAc) [lit. as for **16**]; the ^{1}H spectrum of compound **21** resembled that of Boc-L-Ala with the exception that the doublet (δ 1.44, α-Me) was missing, the signals pertaining to α-H were now broad doublets; in the ^{13}C spectrum, the α-Me singlet appeared as a multiplet (δ_C 17.9, CD_3); IR data as for the corresponding non-labelled analogue; 99.8 (HPLC) and 99.9%ee (GLC).

Acknowledgements

The generous advice of Professor W. Oppolzer, University of Geneva, is gratefully acknowledged. This research is part of a programme supported by the Swedish Natural Science Research Council and the National Board for Industrial and Technical Development. Additional support for the purchase of isotopes was received from the Carl Trygger, H. Ax:son Johnson and M. Bergvall Foundations. L. L. acknowledges a leave of absence from the University of Gdansk and a scholarship from the Swedish Institute. B. N. acknowledges a leave of absence from the University of Yaoundé and a scholarship from the International Science Programs.

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Paper 4/00713A
Received 4*th February* 1994
Accepted 16*th May* 1994