Synthesis and spectroscopic characterization of derivatives of proteinogenic amino acids, simultaneously labelled with ^{13}C, ^{15}N and ^{2}H in the backbone

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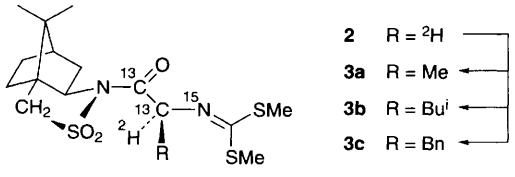
As typical examples of derivatives of proteinogenic α-amino acids, simultaneously labelled with the stable isotopes ^{13}C, ^{2}H and ^{15}N in the backbone, Boc-L-[1,2-$^{13}C_2$, 2-^{2}H, ^{15}N]amino acids are synthesized in enantiopure form and spectroscopically characterized.

In continuation of our work[1] directed towards the synthesis of enantiopure L-amino acid derivatives containing the stable isotopes ^{15}N and/or ^{13}C or ^{2}H for use in peptide synthesis,[2] we now report the preparation of additional isotopomers simultaneously containing all three of these nuclei. To our knowledge such compounds have not been reported previously.

The synthetic scheme underlying the present work is based on that for the corresponding α-deuteriated isotopomers recently reported.[1c] Consequently, we shall only describe it very briefly here. The synthesis started from ethyl bis(methylsulfanyl)methylene[1,2-$^{13}C_2$, ^{15}N]glycinate,[1b] but instead of coupling directly to the chiral auxiliary for asymmetric synthesis as reported,[1b] the substance was first α-deuteriated in MeOD/D_2O with base catalysis to give ethyl bis(methylsulfanyl)methylene[1,2-$^{13}C_2$,2,2,-$^{2}H_2$, ^{15}N]glycine, $(MeS)_2C$ = ^{15}N-$^{13}C^{2}H_2$-^{13}CO-OEt **1** which was then coupled to the chiral auxiliary, providing (*R*)-*N*-{bis(methylsulfanyl)methylene [1′,2′-$^{13}C_2$,2′,2′-$^{2}H_2$, ^{15}N]glycyl}bornane-10,2-sultam **2**. Subsequent alkylation of the enolate with MeI, Bu^{i}I and BnI, crystallization and chromatography on silica provided pure **3a–c** with (2′*S*) configuration which were cleaved off from the sultam in two steps, in accordance with Oppolzer's general procedure, to give the crude, free, labelled amino acids.[3] Finally, these were *N*-protected with Boc_2O to give the corresponding derivatives **4a–d**, suitable for future synthetic work (**4a** and **4c** were isolated as DCHA salts).

Boc-L-[1,2-$^{13}C_2$, 2-^{2}H, ^{15}N]R
R = Ala **4a**, R = Leu **4b** and R = Phe **4c**
Boc-[1,2-$^{13}C_2$, 2,2-$^{2}H_2$, ^{15}N]Gly **4d**

Compounds **4a–d** were carefully characterized by mp, optical rotation and TLC and also by ^{1}H, ^{2}H, ^{13}C and ^{15}N NMR and FTIR spectroscopy. The ^{2}H content was determined by integration of the residual proton signals in the ^{1}H NMR spectra and was ≥98% except for **4b** (~95%). The optical purity of **4a–c** (after deprotection) was confirmed by at least one chromatographic method (≥99.5% ee),[1b,c,4] again confirming the unique efficiency of the original method.[3] Although the alkylation of **2** takes place with loss of one ^{2}H nucleus, this is not an unreasonable price to pay for the convenience of the procedure and, in our opinion, even more so for the excellent optical purity of the product. With all $^{13}C/^{15}N$ glycine isotopomers already previously available,[5] it appears that the way is now open to further $^{13}C/^{2}H/^{15}N$ backbone-labelled amino acids.



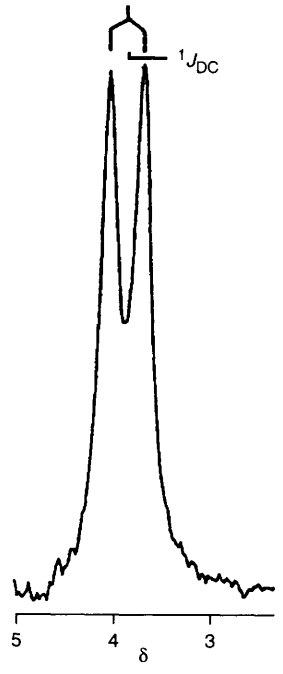


Fig. 1 61.25 MHz ^{2}H NMR spectrum of **4a** (as DCHA salt) in $CDCl_3$

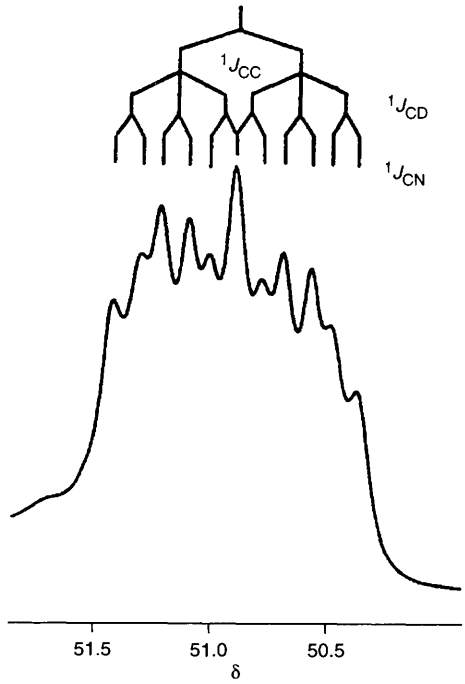


Fig. 2 100.4 MHz ^{13}C NMR spectrum of **4a** (as DCHA salt) in $CDCl_3$

In their ^2H NMR spectra, compounds **4a–d** exhibit typical broad doublets at δ 3.9–4.3 with $^1J_{DC}$ 20–21 Hz, as illustrated for **4a** in Fig. 1. The presence of the ^2H nucleus in this compound is also prominently reflected in the ^{13}C NMR spectrum as shown in Fig. 2. $^{13}C^{\alpha}$ couples to all three neighbouring nuclei, $^{13}C'$, $^2H^{\alpha}$ and ^{15}N, and in this case the signal is almost completely resolved and shows 11 of the expected 12 resonances. From this spectrum all of the three coupling constants involved can be derived easily: $^1J_{CC}$ 53, $^1J_{CD}$ 20 and $^1J_{CN}$ 12 Hz. A small shift to a higher field, −0.2 to −0.5 for the $^{13}C^{\alpha}$ atom also seems to accompany deuteriation. Incomplete deuteriation in **4a–c** can be detected by the appearance of two signals in the ^1H NMR spectrum at δ ~ 4.1–4.6 (for **4d** at higher field), Δδ ~ 0.2 ppm, for the *E* (major) and *Z* (minor) conformers.[6] Otherwise, the ^1H spectra exhibit clean windows in the region mentioned. On the other hand, we have not yet been able to detect any effect of α-deuteriation in the ^{15}N NMR spectra of the new compounds, except for small shifts to higher field, Δδ −1.5 for both conformers of **4d** and Δδ −1.1 (*E*) and −0.7 (*Z*) for **4b**. The typical spectrum of **4d** is shown in Fig. 3. The two nitrogen signals appear as a doublet of doublets with $^1J_{NH}$ 92 and $^1J_{NC}$ 14 Hz for the major *E* conformer and $^1J_{NH}$ 92 and $^1J_{NC}$ 12 Hz for the minor *Z* conformer.

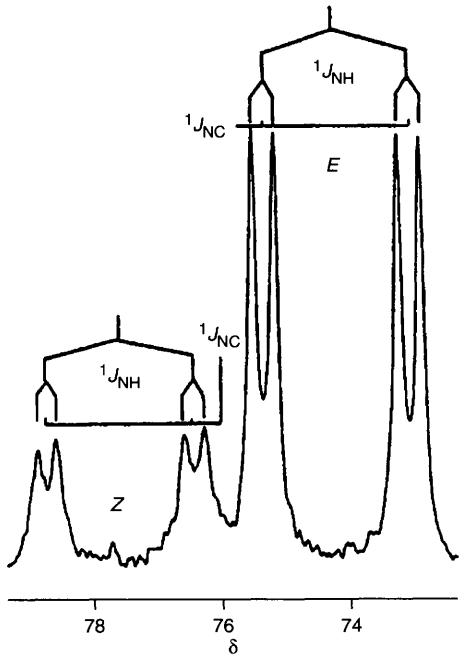


Fig. 3 40.4 MHz ^{15}N NMR spectrum of **4d** in $CDCl_3$

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