Synthesis of ^{13}C- and ^{15}N-labelled *tert*-Butoxycarbonyl (Boc) Glycines and Glycine Amides as Precursors and Simple Models of Backbone-labelled Peptides

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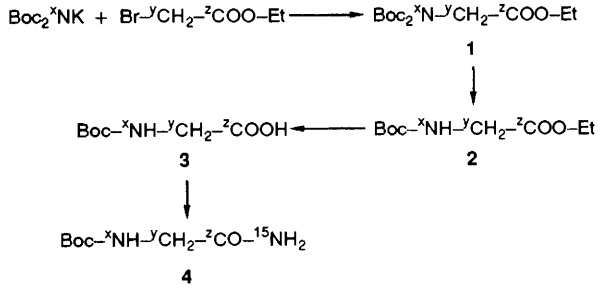
Boc-derivatives of the six ^{13}C-labelled glycines with or without ^{15}N have been prepared by a direct approach from the corresponding bromoacetates and three of them converted into ^{15}N-labelled amides by an efficient procedure to give isotopomers with 2–4 adjacent ^{13}C and ^{15}N nuclei; their coupling constants have been recorded.

With the double aim of providing suitably labelled derivatives of glycine for direct incorporation into synthetic peptides as well as convenient precursors for synthesis of backbone-labelled α-amino acids in general using various recently developed techniques,[1] we have prepared the complete set of six ^{13}C-containing Boc-protected glycines **3** with or without an additional ^{15}N-label (Scheme 1).[2] Furthermore, to study the corresponding spin-coupling systems, three of them were converted into ^{15}N-labelled amides with 4 (**4a**), 3 (**4b**) and 2 (**4d**) vicinally labelled nuclei by a facile procedure. These substances constitute simple models of backbone-labelled peptides.

Our synthetic sequence started from the potassium salt[3] of labelled[4a] or unlabelled[4b] di-*tert*-butyl imidodicarbonate[4c] and three alternative ^{13}C-labelled ethyl bromoacetates to give the fully protected glycine derivative **1**, from which one Boc group was cleaved off selectively with a slight excess of trifluoroacetic acid (TFA).[5] The product **2** was subsequently saponified, giving directly Boc-glycine **3**.[6] All three steps took place in essentially quantitative yield, which is of particular importance in this type of work. Enriched **3d** (90%) and highly enriched **3f** have been prepared previously[7] for application in NMR spectroscopy.

The three labelled Boc-glycines, **3a**, **3b** and **3d**, were converted into the corresponding amides by activation with carbonyl diimidazole[8] and subsequent addition of $^{15}NH_4Cl$ in the presence of base. This new procedure can be performed with only a modest excess of $^{15}NH_4Cl$ and therefore avoids waste of expensive isotope. A few other methods with potential application in this context have been described.[9]



Scheme 1 Compounds made (x/y/z): **1a–f**, **2a–f**, **3a–f**, **4a**, **4b**, **4d**, (**a**, 15/13/13; **b**, 14/13/13; **c**, 15/12/13; **d**, 14/12/13; **e**, 15/13/12; **f**, 14/13/12)

Table 1 Chemical shifts of ^{1}H, ^{13}C and ^{15}N nuclei (Bruker AMX, 500 MHz) in conformers of **3** and **4**[a]

Compound	*T*/K	Bu^t			C=O	NH		CH_2		CO(OH) (NH_2)		
		^{1}H	^{13}C		^{13}C	^{1}H	^{15}N	^{1}H	^{13}C	^{13}C	^{1}H	^{15}N
3-(*E*)	296	1.43	28.22	80.37	156.01	5.24	75.7	3.95	42.14	174.68	11.68	—
3-(*Z*)	296	1.44	28.14	81.79	157.37	6.83	79.1	3.88	43.30	173.98	11.68	—
4-(*E*)	276	1.40	28.17	79.94	156.17	5.88	77.4	3.76	43.47	173.06	6.76(*Z*) 6.84(*E*)	102.9
4-(*Z*)	276	1.40	28.06	80.62	155.94	6.15	80.1	3.67	44.99	173.56	6.81	102.2

[a] Measured at 500 MHz for ^{1}H, 125.7 MHz for ^{13}C and 50.7 MHz for ^{15}N and reported in δ relative Me_4Si (^{1}H, ^{13}C) or NH_3 (^{15}N) [in the latter case with $MeNO_2$ (1 : 1 in $CDCl_3$) as external reference, $\delta_{MeNO_2} = 379.6$].

Table 2 Absolute values of spin–spin coupling constants in conformers of **3** and **4** (in Hz)

Compound	$^{3}J_{1HNC^{1}H}$	$^{1}J_{^{13}C^{1}H}$	$^{1}J_{O^{13}C^{15}N}$	$^{1}J_{H^{15}N^{13}C}$	$^{1}J_{^{13}C^{13}CO}$	$^{1}J_{^{15}N^{1}H}$	$^{2}J_{CCON}$	$^{1}J_{CNH_2}$
3-(*E*)	5.5	139.9	26.3	13.9	58.9	92.5/—	—	—
3-(*Z*)	4.8	140.8	26.0	12.5	58.9	93.7/—	—	—
4-(*E*)	5.7	138.7	26.2	12.8	51.3	92.5/89.7	8.0	15.6
4-(*Z*)	5.4	139.5	25.3	10.5	52.5	93.3/[a]	6.0	16.3

[a] Overlaps with its conformer.

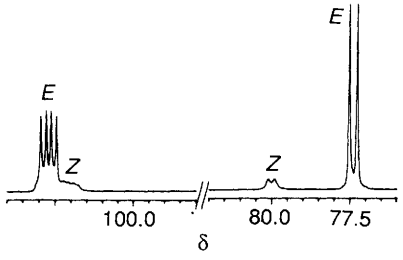


Fig. 1 ^{15}N NMR spectrum of Boc–^{15}NH–$^{13}CH_2$–^{13}CO–$^{15}NH_2$ **4a** in $CDCl_3$ (0.5 mol dm^{-3}) at 50.7 MHz (276 K)

Various NMR studies on **3** in $CDCl_3$, including such involving ^{15}N, confirmed the presence of *Z* and *E* conformers,[10] the ^{1}H, ^{13}C and ^{15}N chemical shifts of which are presented in Table 1. All numerical values for the two first nuclei are in excellent agreement with those given previously.[10] The corresponding coupling constants are given in Table 2. The two tables also document these data for **4**. As far as the ^{13}C shifts are concerned, they are also in agreement with those published earlier.[11] However, instead of discussing the shift and coupling parameters in detail we should like to focus on a few observations made in **4** with relevance to backbone-labelling of peptides. Thus, amide **4d** gave a ^{15}N doublet around δ 102, whereas this was split into a double doublet (dd) in **4b**. No further splitting due to coupling of the amide nitrogen with the carbamate nitrogen of **4a** was observed (Fig. 1, *Z*-conformer also visible). The signal pattern of the *E*-conformer could be observed easily even with a 90 MHz instrument. In the ^{13}C NMR spectra of **4**, the corresponding observations were made with amides showing up as a doublet (**4d**) and dd (**4a**,**b**) and CH_2 as a dd (**4b**), further resolved in the case of **4a**.

The isotopic purity of all labelled precursors was 99% according to the manufacturers' specifications and our own NMR data. The appropriate labelling of all products was confirmed by high resolution fast atom bombardment mass spectrometry (Finnigan MAT90 instrument). The exact masses of the molecular ions as well as the (M − 56) and (M − 100) fragments agreed satisfactorily with the theoretical values. The NMR spectra did not indicate any loss in isotopic purity.

To summarize, isotopomers of **3** together allow a multitude of specific labellings of glycine in synthetic peptides for subsequent identification by NMR. Besides, used as precursors,[1] they should, in principle, provide access to most backbone-labelled α-amino acids.

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