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Synthesis and Spectroscopic Properties of ^{13}C- and ^{15}N-Labelled *tert*-Butoxycarbonylglycines

Leif Grehn,[a] Tönis Pehk[b] and Ulf Ragnarsson[a]

[a]Department of Biochemistry, University of Uppsala, Biomedical Center, P.O. Box 576, S-751 23 Uppsala, Sweden and [b]Institute of Chemical Physics and Biophysics, Estonian Academy of Sciences, Ravala 10, EE-200001 Tallinn, Estonia

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Three ^{13}C-labelled bromoacetates have been reacted both with ^{15}N-labelled and with unlabelled di-*tert*-butyl imidodicarbonate and the intermediates selectively deprotected to give the corresponding isotopomeric Boc-glycines for application in peptide synthesis. The products were characterized by ^{1}H, ^{13}C and ^{15}N NMR and FT-IR spectroscopy. Isotope effects on chemical shifts and IR spectra for this set of compounds are tabulated. In addition, from three isotopomers ^{15}N-labelled amides were also prepared.

Owing to the low natural abundance of the ^{13}C and ^{15}N nuclei, amino acids, enriched in one or, preferably, both of them would no doubt be an asset in peptide synthesis aimed at structural studies by NMR spectroscopy. Although attempts in this direction are on record in the literature, the shortage of suitably labelled amino acid derivatives has so far precluded wider application of this technique. As part of a programme directed towards the preparation of ^{15}N-labelled[1–3] and $^{13}C,^{15}N$-doubly labelled Boc-amino acids for this purpose, we recently made the whole set of ^{13}C-labelled Boc-glycines (Boc = *tert*-butoxycarbonyl).[4] Their synthesis and spectroscopic properties are detailed in this paper.

Synthesis of labelled Boc-glycines. Our synthetic approach to ^{13}C-labelled Boc-glycines is outlined below.

$$\mathrm{Boc_2{}^{x}NK + Br\text{–}{}^{y}CH_2\text{–}{}^{z}CO\text{–}OEt}$$

$$\rightarrow \mathrm{Boc_2{}^{x}N\text{–}{}^{y}CH_2\text{–}{}^{z}CO\text{–}OEt}$$

1

$$\rightarrow \mathrm{Boc\text{–}{}^{x}NH\text{–}{}^{y}CH_2\text{–}{}^{z}CO\text{–}OEt}$$

2

$$\rightarrow \mathrm{Boc\text{–}{}^{x}NH\text{–}{}^{y}CH_2\text{–}{}^{z}COOH}$$

3

$$\rightarrow \mathrm{Boc\text{–}{}^{x}NH\text{–}{}^{y}CH_2\text{–}{}^{z}CO\text{–}{}^{15}NH_2}$$

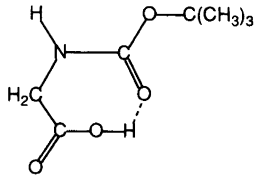
4

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).

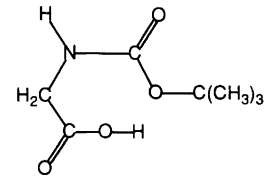
The potassium salts[5] of labelled[6] or unlabelled[7] di-*tert*-butyl imidodicarbonate[8] were the sources of both the amino group nitrogen and the protecting group in this procedure. The salts were reacted with three alternative ^{13}C-labelled ethyl bromoacetates to give the fully protected glycine derivative **1**. In compounds of this type, one Boc group is particularly labile to acid. Consequently, it could be cleaved off selectively with a slight molar excess of trifluoroacetic acid (TFA).[9] The product **2** was subsequently saponified, giving *directly* Boc-glycine **3**.[10,11] Typical procedures for the preparation of compounds **1–3** are detailed in the Experimental. Note that all three steps can be carried out in nearly quantitative yield, thus minimizing the loss of precious isotope-labelled material. Enriched **3d** and **3f** (90 %) have been prepared previously for application in NMR spectroscopy.[12,13]

In three cases (**3a**, **3b**, and **3d**), the Boc-glycines were converted into the corresponding ^{15}N-labelled amides. Such amidations are normally carried out with a large excess of ammonia, but for obvious reasons we had to choose an alternative procedure. As the literature does not abound with amidation methods requiring only a modest excess of ammonia[14,15] and our $^{15}NH_3$ was present as ammonium chloride, we chose to employ *N,N'*-carbonyldiimidazole (CDI)[16] for this purpose. In this way, pure products were obtained in about 80% yield. The three amides had 4 (**4a**), 3 (**4b**) and 2 (**4d**) vicinally labelled nuclei.

NMR spectroscopy. The chemical shifts of the ^{1}H, ^{13}C and ^{15}N nuclei and the corresponding $^{1}H\text{–}^{1}H$, $^{1}H\text{–}^{13}C$, $^{13}C\text{–}^{15}N$, $^{13}C\text{–}^{13}C$ and $^{1}H\text{–}^{15}N$ coupling constants have already been reported in the previous communication.[4] In the solid state, Boc-glycine is known to adopt the *Z* (*trans*) conformation,[17,18] but in $CDCl_3$ solution the *E*



3Z



3E

Fig. 1.

and *Z* conformers are both observed at room temperature (*E*/*Z* ~2:1 for **3** and ~6:1 for **4**, assignments for **3** as previously reported[19]) and upon increasing the temperature the equilibrium is shifted in favour of the *E* form (Fig. 1). The equilibrium in **3** is strongly temperature dependent. Below ~280 K the *Z* conformer prevails, the ratio reaching 5:95 at 223 K.[19] In **4**, however, the equilibrium was not affected upon cooling the solution down to 243 K. The cause of the dramatically different temperature dependence of this equilibrium in **3** and **4** must therefore be connected with the carboxy group in **3**. Various alternatives for hydrogen bonds in **3** (*Z*) and **3** (*E*) have been discussed in detail on the basis of their ^{1}H NMR spectra.[19] The authors concluded that for the *E* conformer only the dimerization characteristic of carboxylic acids is important. For the *Z* conformer an intramolecular hydrogen bond between the carboxy proton and the urethane carbonyl (Fig. 1) offers possibilities for intermolecular association which is strongly favoured at low temperatures. Such an aggregation could be confirmed by comparison of the spin-lattice relaxation times of the ^{13}C nuclei of **3** (*Z*) and **3** (*E*) at 276 K. Aggregation leads to a larger mean molecular volume which is associated with longer correlation times τ_c^{eff} giving rise to shorter spin-lattice relaxation times T_1. As shown in Table 1, all corresponding carbon atoms of the *Z* conformer indeed have lower T_1 values than those of the *E* conformer pointing to stronger association for the former.

Table 1. ^{13}C spin-lattice relaxation times (s) of *E* and *Z* conformers of **3** at 276 K.

	Carbon atom				
Conformer	CH_3	$C(Me)_3$	C=O	CH_2	COOH
E	0.64	4.59	3.84	0.33	2.19
Z	0.55	4.35	3.60	0.29	1.89

This set of isotopomers also offers excellent opportunities for studying the dependence of the NMR parameters on the conformation and isotopic substitution. Such a comparison shows that the *Z* conformation is characterized by low-field shifts of the ^{15}N nucleus, NH proton and α-carbon as well as the quaternary carbon of the *tert*-butyl group. On the other hand, the CH_2 protons in the *Z* conformation show small high-field shifts.[4]

Spin-spin coupling constants provide additional information for the analysis of the urethane conformation. The $^1J_{^{15}N^1H}$ coupling is larger for the *Z* conformation, as it is at the peptide bond.[20] The same regularity is observed for the $^1J_{^{13}C^1H}$. The *Z* conformer is characterized by smaller values of the two coupling constants $^3J_{^1HNC^1H}$ and $^1J_{O^{13}C^{15}N}$.

Owing to the high purity of the isotopomers, the presence of unlabelled species was difficult to detect even by ^{1}H NMR spectroscopy. In order to measure isotope effects on the chemical shifts in the urethane conformers, mixtures of labelled and unlabelled **3** were used (Table 2).

No major differences were observed between the *E* and *Z* conformers. The values in Table 2 are within the normal ranges tabulated in Ref. 21. The largest isotope effect is observed for ^{15}N originating from ^{13}C. This type of effect has not been reported earlier.[21] For the methylene carbon of **3a**, good additivity of two one-bond effects was observed (22 ppb for both the *E* and *Z* conformers). The regularities in the NMR parameters of the ^{1}H, ^{13}C and ^{15}N nuclei of the *E* and *Z* conformers thus provide a solid basis for conformational analysis.

Table 2. High-field isotope effects on chemical shifts (in ppb) in labelled Boc-glycines **3**.[a]

		High field effects					
		on ^{1}H		on ^{13}C		on ^{15}N	
Label	Conformer	one-bond	two-bond	one-bond	two-bond	one-bond	two-bond
$^{13}CH_2$	*E*	2.2	0.4	1.6	[b]	24	—
	Z	2.6	0.4	1.6	[b]	22	—
^{13}COOH	*E*	—	0.4	11	—	—	[b]
	Z	—	0.6	11	—	—	[b]
^{15}N	*E*	0.4	[b]	12	[b]	8[c]	—
	Z	0.2	[b]	11	[b]	9[c]	—

[a] Measured with mixtures of labelled and unlabelled material. [b] Too small to measure. [c] One-bond effect on ^{13}C of Boc.

FT-IR spectroscopy. The isotopomers **3a–f** were characterized by FT-IR spectroscopy. Spectra were also recorded for the unlabelled (**3U**) and ^{15}N-labelled (**3N**) compounds, all in KBr. For **3U** significant bands of comparable intensity appeared at 3407, 3343, 3116 (broad), 2979 and 2941 cm^{-1}. Stronger bands were visible at 1748, 1669, 1536, 1410, 1215, 1198 and 1166 (intensity order 1198 ~ 1669 > 1215 ~ 1536 > 1166 ~ 1748 > 1410) with additional significant ones at 1368, 1301, 1281, 1257, 1057, 959, 859 and 585 cm^{-1}. Data on those shifted by 7 cm^{-1} or more to lower frequency upon labelling are collected in Table 3. The spectrum of **3U** was previously recorded by Toniolo *et al.* under similar conditions.[17]

Of all the bands discussed in this context, the carboxy carbonyl stretching vibration band at 1748 cm^{-1} gave rise to the largest shift upon ^{13}C-labelling and instead appeared at 1706 cm^{-1}. This shift is in good agreement with that calculated (1709 cm^{-1}) using Hooke's law. The second major peak in this region, at 1669 cm^{-1}, was split in isotopomers **3a–d**, owing to a small shift in its position by 5–7 cm^{-1}. Toniolo *et al.* recorded a shoulder at 1680 cm^{-1} and assigned this and a band at 1664 cm^{-1} to strongly but differently hydrogen-bonded carbonyls of urethane type. In isotopomers **3a–d** the otherwise hidden band appeared at 1680 ± 1 cm^{-1}.

The 'amide II' band which appeared at 1536 cm^{-1}, if originating from coupling between N–H bending and C–N stretching,[22] should be sensitive to ^{15}N labelling and, as can be seen from Table 1, this was the case. In four such labelled compounds it was shifted to 1523–1524 cm^{-1}, whereas in the four unlabelled ones it remained constant. Throughout the series of compounds studied, this band was easily recognized, owing to its strength and the fact that it appeared to be the only one in the 1455–1660 cm^{-1} range between the dual band mentioned in the preceding paragraph and another one of medium intensity appearing at 1453–1454 cm^{-1} and assigned to CH_2 hydrogen bending vibrations.[22] Compared with the 'amide II' band, the opposite picture upon labelling was shown by the somewhat less intense 1410 cm^{-1} band as no shift could be discerned upon introduction of ^{15}N. The presence of ^{13}C in the carboxy or methylene group or both resulted in shifts of 13, 5 or 17 cm^{-1}, respectively. An assignment of this band in fair agreement with these new facts and with the literature is a coupled carboxy C–OH stretching and OH in-plane bending vibration, although the effect of vicinal ^{13}C labelling is not easily explained in this case.

The 1368, 1257 and 1166 cm^{-1} bands appear at the same positions in all compounds studied, and those at 1301 and 1281 cm^{-1} are shifted by a maximum of 2–4 cm^{-1}, and are not further discussed in this context. On the other hand, the strong bands at 1215 and 1198 cm^{-1} undergo clear shifts of 14–16 cm^{-1} in isotopomers containing $^{13}COOH$, whereas no other effects of labelling are observed. This (double) band therefore qualifies as the strong twin of that at 1410 cm^{-1}, known to show up anywhere within the range 1320–1210 cm^{-1},[22] which only in long-chain fatty acids has been reported to appear as a doublet.

In primary amines, a C–N stretching vibration involving primary α-carbons has been reported at 1079 ± 11 cm^{-1}.[22] Considering the significant effects of both ^{15}N and $^{13}CH_2$, and the smaller effect of $^{13}COOH$ labelling in the case of the 1057 cm^{-1} band, a similar assignment would seem fully justified. All shifts are essentially additive and so they are for the 959 cm^{-1} band, although the effect of $^{13}CH_2$ now predominates over $^{13}COOH$ labelling. A strong carboxy OH out-of-plane deformation vibration has been reported previously to appear in this region,[22] but such an assignment would, under the present circumstances, be less attractive than one directly involving both carbon atoms.

In the NH-region there were two bands, at 3407 and 3343 cm^{-1} the latter of which was invariably slightly stronger, and which were sensitive to isotope labelling. They were both shifted by 7 cm^{-1} in ^{15}N-labelled species. Their positions deviated considerably from those in $CDCl_3$, in which case the 3445 and 3300 cm^{-1} bands were assigned to the *E*-NH and *Z*-NH conformers, respectively.[23] These bands are often attributed to free and bonded NH.[22]

Table 3. Selected FT-IR data for compounds **3a–f** and **3N**.[a]

Cpd.	**3U** 141212	**3N** *151*212	**3a** *151313*	**3b** 14*1313*	**3c** *151213*	**3d** 1412*13*	**3e** *151*312	**3f** 14*1*312
	3407	3400	3399	3408	3400	3410	3399	3410
	3343	3336	3335	3344	3336	3345	3335	3344
	1748	1748	1705	1705	1706	1706	1748	1748
	1669	1667	1662	1664	1663	1664	1666	1668
	1536	1524	1523	1538	1523	1536	1524	1536
	1410	1410	1393	1393	1397	1397	1404	1405
	1215	1215	1200	1201	1200	1201	1215	1215
	1198	1197	1182	1183	1182	1185	1197	1197
	1057	1051	1044	1049	1048	1054	1046	1052
	959	958	945	945	954	955	949	950

[a] The positions of all bands are given in cm^{-1}.

Experimental

All m.p.s. were recorded with a Gallenkamp apparatus and are uncorrected. All solvents used as reaction media were of analytical grade and were dried for several days over molecular sieves (4A). TLC analyses were performed on 0.25 mm thick precoated silica plates (Merck DC-Fertigplatten, Kieselgel 60 F_{254}) and the mobile phases used were toluene–MeCN (2:1) (A), light petroleum (b.p. 40–65°C)–Et_2O (2:1) (B) and CH_2Cl_2–acetone–HOAc (40:10:1) (C). TLC spots were visualized by exposure to Cl_2 followed by dicarboxidine spray. The NMR studies were performed on JEOL JNM-EX 270 (Uppsala) and Bruker AMX-500 (Tallinn) instruments with 0.5 M solutions of isotopomers **3** and **4** in $CDCl_3$. Isotope effects on chemical shifts were measured on mixtures of labelled and unlabelled compounds. FT-IR absorbance spectra were taken in KBr at 4 cm^{-1} resolution on a computer-operated Mattson Polaris instrument, equipped with software for conversion to % transmission and unbiased determination of positions of bands and their intensities. Most of the data presented thus originates from automatic printouts of material, but the existence of every single relevant peak presented was corroborated by proper manipulation.

Typical procedure for the synthesis of Boc_2–^{x}N–$^{y}CH_2$–^{z}CO–*OEt* isotopomers: *(15/13/13)*, **1a**. To finely ground, dry $Boc_2{}^{15}NK$ (5.12 g, 20 mmol), suspended in dry DMF (30 ml) at 0°C, was added Br–$^{13}CH_2$–^{13}CO–OEt (3.41 g, 20.2 mmol, obtained from Icon Services, USA) in DMF (20 ml) dropwise (15 min) with stirring (15 min at 0°C, 1 h at room temp. and overnight at 40°C). The DMF was stripped off (oil-pump), the residue partitioned between ether (400 ml) and 1 M $KHSO_4$ (200 ml). The ether extract was washed with $KHSO_4$, 1 M $NaHCO_3$, and brine (3 × 100 ml each) and dried ($MgSO_4$). Evaporation left an oil (6.10 g, 100%) containing <1% $Boc_2{}^{15}NH$ [^{1}H NMR, TLC (A, B)].

Conversion into Boc–^{x}NH–$^{y}CH_2$–^{z}CO–*OEt: (15/13/13)*, **2a**. Compound **1a** (6.10 g) in CH_2Cl_2 (150 ml) was treated dropwise (15 min) with TFA (1.5 equiv.) in CH_2Cl_2 (60 ml) under argon with stirring. After 3 h, evaporation left a yellow oil which was partitioned between ether (400 ml) and 1 M $KHSO_4$ (200 ml) and washed and dried as for **1a**. Evaporation left a yellow oil (4.07 g, 99%), containing starting materials in only trace amounts [^{1}H NMR, TLC (A, B)].

Hydrolysis to Boc–^{x}NH–$^{y}CH_2$–$^{z}COOH$*: (15/13/13)* **3a**. To compound **2a** (4.07 g) in dioxane (50 ml) was slowly added 1.0 M NaOH (20 ml) with stirring. The initially turbid mixture became clear after being stirred for 3 h, after which it was diluted with water (225 ml) and concentrated to ~200 ml under reduced pressure. The slightly alkaline solution was extracted with ether (3 × 75 ml), acidified (with 1 M $KHSO_4$) and extracted exhaustively with ether. The extract was washed twice with brine and dried (Na_2SO_4). Evaporation left a viscous oil which soon solidified (3.42 g, 97%); m.p. 88–88.5°C (from EtOAc-hexane) [lit. 85–89°C,[10] 88.5–89°C[11] (unlabelled)]. TLC (C) gave one spot.

Amide formation, Boc–^{x}NH–$^{y}CH_2$–^{z}CO–$^{15}NH_2$*: (15/13/13)*, **4a**. A solution of recrystallized **3a** (535 mg, 3 mmol) in DMF (3 ml) at −30°C was treated dropwise with carbonyldiimidazole (730 mg, 4.50 mmol) in additional DMF (6 ml) with stirring (10 min + 1 h at −10°C). $^{15}NH_4Cl$ (327 mg, 6.00 mmol) was added at −30°C, followed by a solution of Et_3N (606 mg, 6.00 mmol) in DMF (3 ml), whereupon the turbid reaction mixture was allowed to warm to room temp. and left overnight. The DMF was stripped off with an oil-pump and the semisolid residue was partitioned between EtOAc (20 ml) and 1 M $NaHCO_3$–brine 1:2, (20 ml). The EtOAc phase was washed with 1 M $NaHCO_3$–sat. NaCl, 1 M $KHSO_3$–sat. NaCl and sat. NaCl (3 × 10 ml) and dried (Na_2SO_4). Additional material was recovered by exhaustive extraction of all aq. phases with EtOAc, which was washed and dried as before. Evaporation of the combined EtOAc extract left a viscous colorless oil which was dissolved in dry ether. At −20°C, white crystals (410 mg, 77%) were deposited and recrystallized from ether to give material with m.p. 94.5–95°C [lit.[12] 99–100°C (unlabelled)]. TLC (B, C) gave one spot.

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