On the Stabilization of the *Syn*-Rotamer of Amino Acid Carbamate Derivatives by Hydrogen Bonding

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The *syn*-rotamers of carbamate derivatives of α-amino acids are shown to be stabilized by the formation of H-bond complexes with carboxylic acid moieties in solution. This effect is, as expected, concentration and temperature dependent. Thermodynamic parameters (both $\Delta G°$ and $\Delta G^{‡}$) for *N*-Boc-alanine were determined by NMR in the ±60 °C range.

Introduction

Carbamates are known to exist as *syn*- and *anti*-rotamers (Figure 1). The latter are more stable by *ca.* 1 kcal/mol,[1] as supported by detection of about 90% *anti*-rotamers in the NMR spectra. The *syn*-rotamers that exist in small quantities at room temperature are usually difficult to identify, since their signals broaden because of the rotation, causing their lines to blend into the baseline (a "hidden partner"[2]) .

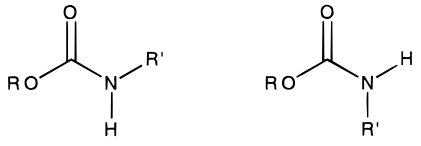

Figure 1. Rotamers of carbamates.

Results and Discussion

In the course of our investigations, several *N*-Boc derivatives of unnatural amino acids such as *N*-Boc-L-vinylglycine[3] (**1a**) were prepared. The ^{1}H-NMR spectrum of **1a** reveals that instead of the expected pattern for a Boc-carbamate, where the NH proton appears at *ca.* 5 ppm, two different Boc-carbamates are present. One of them displays the normal profile (NH peak at 5.20 ppm), while the other exhibits a rather extraordinary NH* at 7.10 ppm. All remaining features of the molecule are duplicated.

The formation of two carbamate products, wherein one of them shows a low-field NH proton, was unexpected. At first, it was considered that the double bond in vinylglycine caused the abnormal behavior of the Boc-derivative. In order to verify this assumption, the NMR spectra of several *N*-Boc-amino acids (Figure 2) and esters were taken.

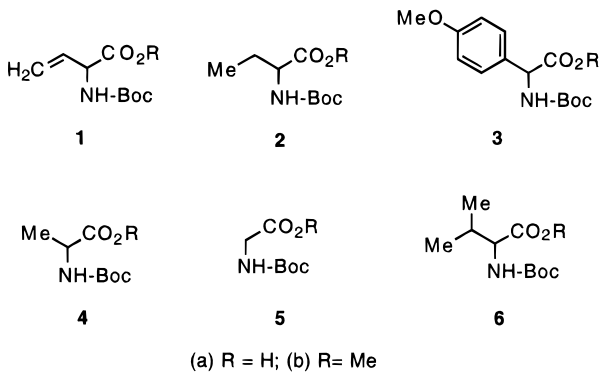

Figure 2. Selected *N*-Boc-amino acid probes for ^{1}H-NMR.

The experiments were conducted with a variety of commercially available *N*-Boc-amino acids (**2a**, **4a**−**6a**), *N*-Boc-(4-methoxyphenyl)glycine[4] (**3a**), and their corresponding methyl esters (prepared by reaction of the acids with trimethylsilyl diazomethane)[5] (Table 1). In order to monitor the correlation between unsaturation and new species formation, experiments using *N*-Boc-α-aminobutyric acid (**2a**), the saturated analog of *N*-Boc-vinylglycine (**1a**), and *N*-Boc-(4-methoxyphenyl)glycine (**3a**) were also performed.

All of the *N*-Boc-amino acids studied form this new species along with the regular-pattern product. For **3a** the new species is the major one, while in all other probes, the new species is the minor product. However, all of the corresponding *N*-Boc-amino acid esters give the expected "normal" spectra, consistent with previous reports of only a single species observed at room temperature.[1]

The identification of the "unusual" species as the *syn*-rotamer of the unsaturated *N*-Boc-amino acids was confirmed by the strongly upfield position of the Boc methyl resonances (1.23 ppm and 1.28 ppm vs. *ca.* 1.45 ppm, Table 1), attributed to shielding by the aryl or vinyl moieties, possible only for the *syn*-rotamer (Figure 3).

The "special" pattern of *N*-Boc-amino acids is also observed in their ^{13}C-NMR spectra (Table 2), while the corresponding esters once again exhibited only one product. ^{13}C-NMR spectra revealed duplication not only around the CH−NH bonds, but also in more remote parts of the molecules, such as the carbonyls of both the acids and carbamates.

In order to search for the spectral features attributable to the ester *syn*-rotamers, low-temperature ^{1}H-NMR spectra were obtained. Indeed, at −54.3 °C, an extra set of small peaks, which broadened and disappeared above *ca.* 10 °C, was observed (Table 3). Noteworthy is the fact that the chemical shifts for the *anti*- and *syn*-NH hydrogens in these esters are very similar.

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(2) Hassner, A.; Maurya, R.; Friedman, O.; Gottlieb, H. E.; Padwa, A.; Austin, D. *J. Org. Chem.* **1993**, *58*, 4539−4546.
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Table 1. ^{1}H-NMR Chemical Shifts for *N*-Boc-Amino Acids and Esters

N-Boc probe	CH	CH*	NH	NH*	Boc (Me_3C)
1a	4.90	4.70	5.10	7.10	1.45 (major) + 1.28
2a	4.30	4.10	5.10	6.28	1.45
2b	4.25	–	5.05	–	1.43
3a	5.26	5.06	5.48	7.80	1.42 + 1.23 (major)
3b	5.30	–	5.56	–	1.48
4a	4.34	4.18	5.10	6.73	1.45
4b	4.32	–	5.10	–	1.45
5a	3.98 (CH_2)	3.95 (CH_2)	5.10	6.78	1.43
5b	3.92 (CH_2)	–	5.06	–	1.45
6a	4.25	4.05	5.05	6.16	1.43
6b	4.21	–	5.02	–	1.44
4a (DMSO-d_6)	3.92	3.83	7.10	6.74	1.38 + 1.35 (minor)

Table 2. ^{13}C-NMR Chemical Shifts for *N*-Boc-Amino Acids and Esters

N-Boc probe	CH	CMe_3	Me_3	OCO–NH	RCOO
2a	54.47 + 55.77*	80.12	28.29	155.58	177.24
2b	54.50	79.73	28.25	155.30	173.18
3a	56.90 + 58.20*	80.31 + 81.53*	27.99* + 28.23	154.94 + 156.75*	173.82* + 175.25
3b	56.98	79.99	28.24	154.73	171.77
4a	49.16 + 50.27*	80.24 + 81.83*	28.31	155.47 + 156.72*	177.45
4b	49.08	79.66	28.19	154.98	173.66
5a	42.19 + 43.32*	80.38 + 81.77*	28.23	156.04 + 157.24*	173.95* + 174.38
5b	42.20	79.88	28.20	155.62	170.72
6a	58.43 + 60.11*	80.00 + 81.58*	28.25	155.82 + 157.06*	176.73
6b	58.53	79.68	28.26	155.57	172.79

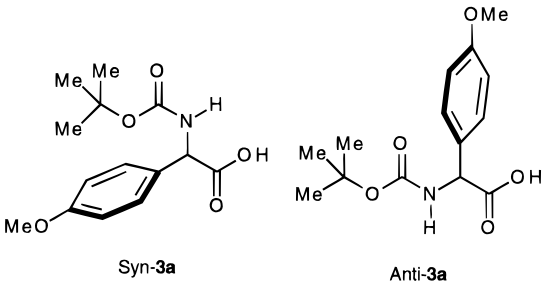
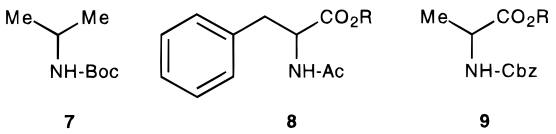


Figure 3. Rotamers of (4-methoxyphenyl)glycine **3a**.



(a) R = H; (b) R= Me

Figure 4. Selected probes for ^{1}H-NMR.

Table 3. ^{1}H-NMR Spectra for Carbamate Esters at –54.3 °C

probe	rotamer	NH	CH	*K*, *anti*/*syn*
4b	anti	5.24	4.34	12
	syn	5.13	4.18	
9b	anti	5.55	4.41	8
	syn	5.48	4.29	
3b	anti	5.69	5.24	6
	syn	5.62	5.07	

In order to explore the generality of these observations, several related compounds were prepared (Figure 4), whose relevant room temperature NMR data are presented in Table 4. *N*-Boc-isopropylamine (**7**), which lacks an acid moiety, shows a single rotamer, as does an amide probe, *N*-acetamidophenylalanine (**8a**). However, *N*-Cbz-alanine (**9a**) gives a similar ^{1}H-NMR spectrum to those of the *N*-Boc-carbamates, showing a single rotamer for the ester and two for the acid. In all cases, the minor rotamer had the same low-field NH signal. Thus, the presence of both an acid and a carbamate (not only a Boc-carbamate) were found to be necessary and sufficient for this phenomenon to occur.

Table 4. ^{1}H-NMR Chemical Shifts for Boc, Cbz, and Amide Probes

probe	CH	NH
4a	4.34 + 4.18*	5.10 + 6.73*
4b	4.32	5.10
9a	4.42 + 4.34*	5.39 + 6.88*
9b	4.40	5.34
8a	4.81	5.98
8b	4.82	5.90
7	3.75	4.37

Table 5. Concentration Dependence of the NH* Chemical Shift

probe	conc (M)	NH	NH*	*K*, *anti*/*syn*
4a	0.0079	5.02	6.46	12
	0.0311	5.07	6.77	2.5
	0.1619	5.20	6.84	1.33
4b	0.0205	5.04		
	0.0829	5.05		
	0.4023	5.12		
9a	0.0179	5.28	6.36	19
	0.0785	5.38	6.63	7.5
	0.3729	5.49	6.90	2
6b	0.0144	5.03		
	0.0772	5.03		
	0.3557	5.06		

Aggregation Studies. The low-field absorption of the *syn*-NH is strongly reminiscent of the deshielded OH resonances detected in H-bonded dimers of carboxylic acids. To explore this possibility, concentration-dependent ^{1}H-NMR spectra of two carbamoylated amino acids **4a** and **9a** and two *N*-Boc-esters **4b** and **6b** were obtained (Table 5 and Figure 5). Indeed, the chemical shifts of the low-field *syn*-rotamer NH protons are two to three times more concentration-dependent than the NH's of the corresponding *anti*-rotamers. Moreover, these experiments indicate that the proportion of the *syn*-rotamers increases with concentration, supporting the existence of an aggregation process.

Decreasing temperature offers another method for stabilizing oligomerization. Thus, spectra of three carbamoylated amino acids **3a**, **4a**, and **9a** were taken over

Table 6. Temperature-Dependent Chemical Shift Changes for NH and NH*

	3a			4a			9a		
T, °C	NH	NH*	*K*, *anti/syn*	NH	NH*	*K*, *anti/syn*	NH	NH*	*K*, *anti/syn*
−54.3				5.21	7.91	0.09	5.55	8.17	0.56
−33.1	5.63	8.38	0.05	5.16	7.82	0.15	5.50	8.09	0.33
−11.9	5.57	8.28	0.17	5.16	7.55	0.59	5.45	7.78	0.82
+9.3	5.51	8.06	0.37	5.13	7.09	1.20	5.40	7.24	
+22.1	5.48	7.81	0.57	5.10	6.75	1.75	5.36	6.79	2.73
+35.2	5.46	7.53	1.22	5.07	6.43	2.20	5.33	6.48	2.58
+47.7	5.44	7.12		5.06	6.08		5.30		
+62.2	5.47	6.72		5.13			5.33		

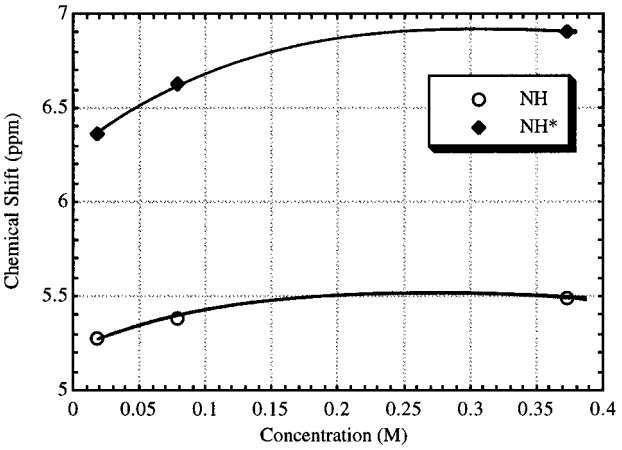


Figure 5. Concentration-dependent chemical shifts of NH and NH* in *N*-Cbz-alanine (**9a**).

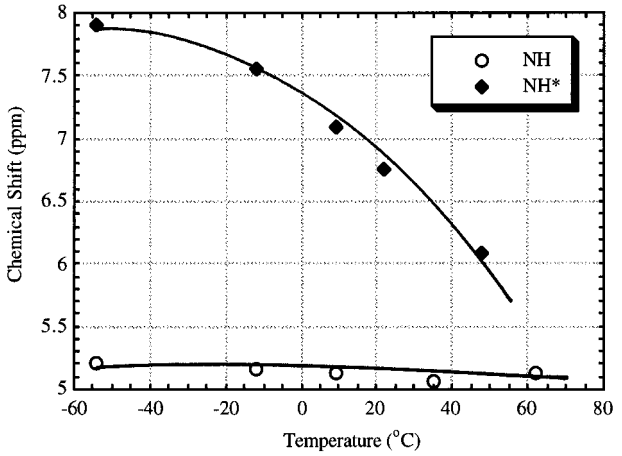


Figure 6. Temperature-dependent chemical shift changes for *N*-Boc-alanine (**4a**).

a wide range of temperatures (Table 6). In all cases as the temperature increases, the favored rotamer switches from *syn* to *anti*. Furthermore, the temperature coefficient for the chemical shifts of the NH hydrogens was at least 1 order of magnitude larger for the *syn* than the for the *anti* species (*ca.* 2×10^{-2} vs *ca.* 1.5×10^{-3} ppm/°C, respectively, Figure 6). The CH chemical shifts were largely temperature-independent.

The above data, strongly support the notion that *syn*-rotamers of *N*-carbamoylated amino acids form intermolecularly H-bonded species. Furthermore, the OH of the carboxylic acids must be involved in this process, since the corresponding esters do not behave similarly. To explain this phenomenon, we suggest the formation of a dimer as shown in Figure 7. It seems irrelevant whether R″ represents a *syn*- or an *anti*-rotamer, or, for that matter, any other substituent. In order to test this hypothesis, increasing amounts of acetic acid were added

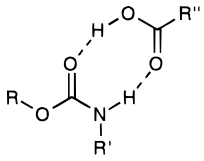


Figure 7. Possible dimer between a *syn*-carbamate and an acid group.

Table 7. Acetic Acid-Influenced Formation of Dimers

probe	AcOH added (μL)	NH	NH*	*K*, *anti/syn*
4b	0	5.08		
	1.5	5.08	5.51	5.82
	3	5.09	5.74	5.71
	9	5.13	6.13	3.79
	30	5.21	6.47	2.85
4a	0	5.08	6.76	2.10
	1.5	5.08	6.51	2.75
	3	5.08	6.42	3.04
	9	5.10	6.39	3.00
	30	5.17	6.55	2.29
	50	5.22	6.63	1.98

to a solution of a carbamoylated amino acid ester (Table 7). Indeed, from the first addition, the signals of the *syn*-rotamer appeared, and both its concentration and the downfield shift of its NH increased as a function of the amount of added acid. In contrast, addition of acetic acid to a solution of the corresponding carbamoylated amino acid affected neither the *anti/syn* ratio nor the NH chemical shifts.

In order to further confirm this hypothesis, the ^{1}H-spectrum of **4a** was obtained in DMSO-d_6. This solvent is known to be a good H-bond acceptor and would be expected to stabilize both the *syn*- and the *anti*-rotamers to a similar extent. Indeed (Table 1, last entry), under these conditions, the chemical shifts for both NH protons are quite close and strongly indicative of H-bonding. Also, the *anti/syn* equilibrium constant ($K = 6.2$) is much higher than for the $CDCl_3$ solution at the same temperature ($K = 1.8$, Table 6) and in line with the "normal" values observed for the methyl ester (e. g. $K = 10$ for **4b** in $CDCl_3$ at room temperature).

Quantitative Measurements. In order to obtain more accurate measurements of both the equilibrium constants and the rate of interconversion of the rotamers, temperature dependent NMR spectra of *N*-Boc-alanine **4a** over a wide range of temperatures were measured. Full lineshape analyses were performed, fitting the experimental spectra to lines calculated with specific rate constants[6] (Figure 8 and Table 8).

Conclusions

The switch in isomer preference mentioned above, which occurs at *ca.* 0 °C, is reflected in $\Delta H°$ and $\Delta S°$ of

(6) The computer program was based on Sutherland, I. O. *Annual Reports in NMR Spectroscopy*; Mooney, E. F., Ed.; Academic Press: London, 1971; Vol. 4, p 80.

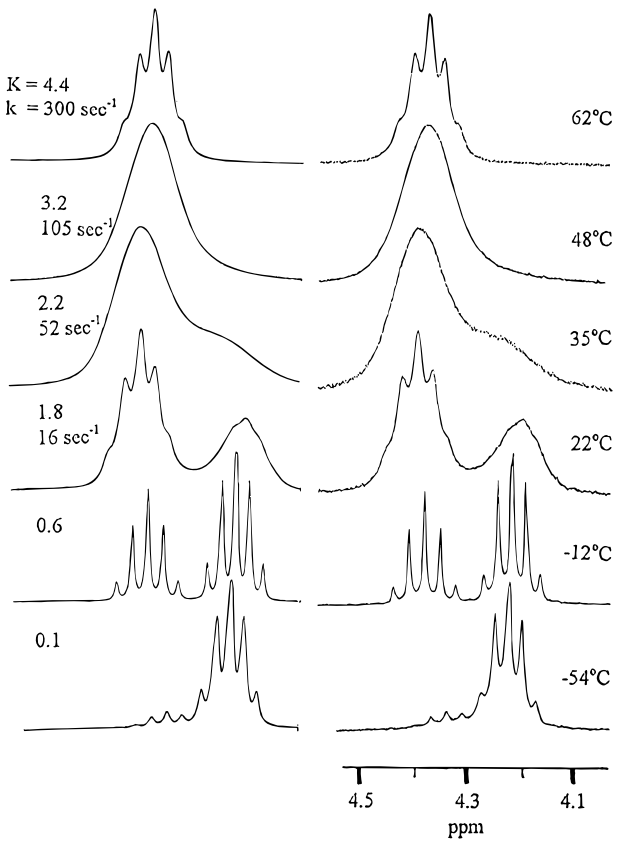


Figure 8. Calculated (left) and experimental (right) ^{1}H-NMR spectra of the α-H of **4a** as a function of temperature.

Table 8. Equilibrium Constants and Exchange Rate for the Rotamers of *N*-Boc-Alanine (4a)[a]

T, K	*K*, *anti*/*syn*	Δ*G*° cal/mol	*k*, s^{-1} anti → syn	Δ*G*‡, kcal/mol
218.9	0.09	1061	–	–
240.4	0.15	907	–	–
261.3	0.59	277	–	–
282.5	1.20	−103	5.5	15.5 ± 0.1
295.3	1.75	−330	16.0	15.6 ± 0.1
308.4	2.20	−486	52.0	15.6 ± 0.2
320.9	3.17	−740	105.0	15.8 ± 0.2
335.4	4.37	−989	300.0	15.9 ± 0.2

[a] Δ*H*° = 4.8 ± 0.3 kcal/mol; Δ*S*° = 17 ± 1 cal/mol K.

the same sign, the latter being quite large, consistent with an aggregation phenomenon. In the esters, Δ*S*° is very small. Thus the value of Δ*G*° in *N*-Boc-alanine methyl ester **4b** (Table 3), 1.1 kcal/mol for the *anti*/*syn* pair, is to a good approximation, the value of Δ*H*° as well, reflecting the natural steric preference for the *anti*-rotamer. If the *syn*- and *anti*-rotamers of the corresponding acid have a similar enthalpy difference, then the stabilization induced to the *syn*-rotamer of the acid by the H-bond is approximately 6 kcal/mol (4.8+1.1), in excellent agreement with other estimates of H-bonding energies.[8] The energy barrier for the rotation process is *ca.* 16 kcal/mol (Table 8). Surprisingly, few quantitative measurements of rotation barriers in carbamates have been reported.[1,7] Usually, Δ*S*‡ for rotation of amides and similar compounds is close to zero. Here, the data seem to indicate a finite negative Δ*S*‡, which may imply that the H-bond is already partially formed in the transition state.

(7) Oki, M. *Applications of Dynamic NMR Spectroscopy to Organic Chemistry*; VCH: Deerfield Beach, FL, 1985; pp 54−60.

(8) Maskill H. *The Physical Basis of Organic Chemistry*; Oxford University Press: Oxford, 1985; p 148.

Experimental Section

The NMR spectra were all recorded on a Bruker AM-300, at 300.1 (^{1}H) and 75.5 (^{13}C) MHz. All spectra were taken in $CDCl_3$ solutions, at 24 ± 2 °C, using TMS as the internal reference, unless otherwise indicated. The probe temperatures were measured with a calibrated Eurotherm 840/T digital thermometer and are believed to be accurate to 0.5 K. For the complete line shape analysis (Figure 8 and Table 8) a modified version of a program written by R. E. D. McClung, University of Alberta, Edmonton, Canada T6J 262, was used. Mass spectra were obtained on a Varian Mat 731 spectrometer (CI = chemical ionization). Most protected amino acids were purchased from Sigma and were used without further purification.

***N*-(*tert*-Butyloxycarbonyl)-L-vinylglycine (1a).** Di-*tert*-butyl dicarbonate (0.24 g, 1.1 mmol) was added to a solution of L-vinylglycine (1 mmol) and NaOH (0.08 g, 2 mmol) in distilled H_2O (2 mL) and *tert*-BuOH (4 mL). The mixture was stirred at room temperature overnight and was then diluted with H_2O, extracted with hexane (3×), acidified to pH = 2 (1 N $KHSO_4$), and extracted with EtOAc (3×). The combined EtOAc layers were dried over $MgSO_4$, filtered, and evaporated to give the desired *N*-protected product as a light yellow oil (65% yield) (*note*: the product existed in the form of two distinguishable rotamers): ^{1}H-NMR ($CDCl_3$) δ 1.45 (s, 9H), 4.69 and 4.91 (two m, 1H), 5.10 and 7.10 (two brd, *J* = 7 Hz, 1H), 5.30 (d, *J* = 10.5 Hz, 1H), 5.39 (d, *J* = 17.2, 1H, CH_2), 5.90 (m, 1H); ^{13}C-NMR ($CDCl_3$) δ 28.25, 55.68, 57.10,), 80.55, 81., 117.80, 132.31, 156.00, 173.77, 174.42; MS (CI/*i*-Bu) *m*/*e* 200 (M − 1$^+$, 3), 146 ($MH^+ - C_4H_8$, 23), 102 ($MH^+ - C_5H_8O_2$, 100).

General Procedure for the Preparation of Methyl Esters Using Trimethylsilyl Diazomethane.[5] A 2 M solution of TMS diazomethane in hexane (0.65 mL, 1.3 mmol) was added dropwise to a solution of *N*-protected amino acid (1 mmol) in hexane (5 mL) and dry MeOH (2 mL). The mixture was stirred at room temperature overnight and became cloudy. The solvent was evaporated to dryness, and the residue dissolved in $CHCl_3$ was washed with 5% $NaHCO_3$. The aqueous layer was extracted with $CHCl_3$ (2×), and the combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give the desired ester. *Note*: Although it is not required to wash the product with 5% $NaHCO_3$,[5] we found this necessary, in order to obtain a clean product, without a trace of the starting acid.

Methyl (2*S*)-2-[(*tert*-Butyloxycarbonyl)amino]butyrate (2b). Obtained as a colorless oil (57% yield): ^{1}H NMR ($CDCl_3$) δ 0.91 (t, *J* = 7.5 Hz, 3H), 1.43 (s, 9H,), 1.55−1.90 (m, 2H), 3.73 (s, 3H), 4.25 (q, *J* = 6.8 Hz, 1H), 5.05 (d, *J* = 6.2 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 9.55, 25.85, 28.25, 52.07, 54.50, 79.73, 155.30, 173.18; MS (CI/*i*-Bu) *m*/*e* 218 (MH^+, 15), 203 (MH^+ − Me, 3), 186 (MH^+ − MeOH, 15), 162 ($MH^+ - C_4H_8$, 100), 118 ($MH^+ - C_5H_8O_2$, 30).

***N*-(*tert*-Butyloxycarbonyl)-L-(*p*-methoxy)phenylglycine Methyl Ester (3b).** Obtained as white crystals (61% yield): ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 9H), 3.77 (s, 3H), 3.85 (s, 3H), 5.31 (d, *J* = 7.5 Hz, 1H), 5.56 (d, *J* = 5.8 Hz, 1H), 6.93 (m, 2H), 7.33 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 28.24, 52.50, 55.20, 56.98, 79.99, 114.20, 128.28, 128.91, 154.73, 159.58, 171.77; MS (CI/*i*-Bu) *m*/*e* 295 (M^+, 3), 239 ($M^+ - C_4H_8$, 8), 179 ($M^+ - C_5H_8O_2$, 76).

***N*-(*tert*-Butyloxycarbonyl)-L-alanine Methyl Ester (4b).** Obtained as white crystals (50% yield): ^{1}H NMR ($CDCl_3$) δ 1.38 (d, *J* = 7.2 Hz, 3H), 1.45 (s, 9H), 3.75 (s, 3H), 4.32 (quintet, *J* = 7.2 Hz, 1H), 5.09 (brs, 1H); ^{13}C NMR ($CDCl_3$) δ 18.43, 28.19, 49.08, 52.10, 79.66, 154.98, 173.66; MS (EI) m/e 203 (M^+, 8), 147 ($M^+ - C_4H_8$, 64), 143 ($M^+ - C_2H_4O_2$, 8), 103 ($M^+ - C_5H_8O_2$, 34).

***N*-(*tert*-Butyloxycarbonyl)-L-glycine Methyl Ester (5b).** Obtained as white crystals (47% yield): ^{1}H NMR ($CDCl_3$) δ

1.46 (s, 9H), 3.76 (s, 3H), 3.92 (d, J = 5.6 Hz, 2H), 5.05 (brs, 1H); ^{13}C NMR ($CDCl_3$) δ 28.20, 42.20, 52.06, 79.88, 155.62, 170.72; MS (CI/*i*-Bu) *m/e* 190 (MH^+, 16), 134 ($MH^+ - C_4H_8$, 100), 90 ($MH^+ - C_5H_8O_2$, 84).

***N*-(*tert*-Butyloxycarbonyl)-L-valine Methyl Ester (6b).** Obtained as white crystals (59% yield): ^{1}H NMR ($CDCl_3$) δ 0.88 (d, J = 6.9 Hz, 3H), 0.95 (d, J = 6.8 Hz, 3H), 1.44 (s, 9H), 2.10 (octet, J = 6.7 Hz, 1H), 3.73 (s, 3H), 4.21 (dd, J = 9.1, 4.8 Hz, 1H), 5.02 (d, J = 7.8 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 17.59, 18.91, 28.26, 31.27, 51.91, 58.53, 79.68, 155.57, 172.79; MS (CI/*i*-Bu) *m/e* 232 (MH^+, 21), 176 ($MH^+ - C_4H_8$, 70), 132 ($MH^+ - C_5H_8O_2$, 54), 116 ($MH^+ - C_5H_8O_2$ − Me, 18), 72 ($MH^+ - C_5H_8O_2 - MeCO_2H$, 100).

***N*-(Benzyloxycarbonyl)-L-alanine Methyl Ester (9b).** Obtained as a yellow oil (52% yield): ^{1}H NMR ($CDCl_3$) δ 1.41 (d, J = 7.2 Hz, 3H), 3.75 (s, 3H), 4.40 (quintet, J = 7.3 Hz, 1H), 5.12 (s, 2H), 5.34 (br d, J = 6 Hz, 1H), 8.06 (s, 5H); ^{13}C NMR ($CDCl_3$) δ 18.54, 49.56, 52.30, 66.83, 127.97, 128.03, 128.40, 136.22, 155.52, 173.32; MS (EI) *m/e* 237 (M^+, 26), 177 ($M^+ - C_2H_4O_2$, 2), 107 ($PhCH_2^+$, 15), 91 ($ArCH_2$, 100).

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