FULL PAPER

DOI: 10.1002/ejoc.200((will be filled in by the editorial staff))

Synthesis of fluorescent alanines by a rhodium catalysed conjugate addition of arylboronic acids to dehydroalanine derivatives

Paula M.T. Ferreira,[a]* Luís S. Monteiro,[a] Goreti Pereira,[a] Elisabete M.S. Castanheira,[b] and Christopher G. Frost[c]*

Keywords: dehydroalanines / rhodium-catalysis / arylboronic acids / conjugate addition / fluorescent marker

Several β-arylalanine derivatives containing fluorescent groups were prepared in good yields using a rhodium catalysed conjugate addition of arylboronic acids to *N*,*N*-diprotected and *N*-monoprotected dehydroalanines. The best conditions for these reactions require the use of an excess of aryl boronic acid (4 equiv.), $[Rh(COD)_2]BF_4$ as catalyst and CsF as base in dioxane:H_2O (10:1) at 110 ºC. These conditions were also applied to several dipeptides with dehydroalanine residues.

The photophysical properties of some of the β-arylalanines were studied in three solvents of different polarity. Due to the absence of the α,β-double bond, the absorption and fluorescence emission of the new compounds are dominated by the photophysical properties of the polycyclic aromatic fluorophores (naphthalene, phenanthrene and pyrene). Considering the relatively high fluorescence quantum yield of these compounds, some of them may be useful as fluorescent markers for peptides and proteins.

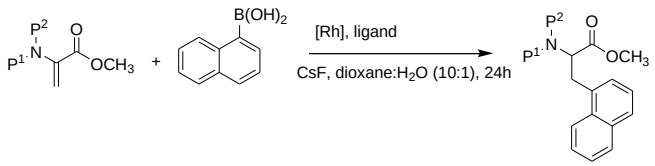
[a] Centre of Chemistry, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
Fax: +351253604382
E-mail: pmf@quimica.uminho.pt
[b] Centre of Physics, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[c] Department of Chemistry, University of Bath, UK, BA2 /AY
Fax: +441225386231
E-mail: c.g.frost@bath.ac.uk
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.xxxxxxxxx.

Introduction

Transition-metal catalysed conjugate addition of organometallics to activated alkenes constitutes an important methodology for the construction of new C-C bonds. In particular the rhodium catalysed conjugate addition of organoboron compounds to α,β-unsaturated carbonyl compounds has been an important tool in organic synthesis.[1] This type of reaction was applied to the synthesis of new non-proteinogenic amino acids using α,β-dehydroamino acid derivatives as substrates to afford different enantioselectivities that depended on the ligand.[2] In recent years we have been interested in the synthesis of α,β-dehydroamino acid derivatives and their application as substrates in several types of reactions to obtain new amino acids.[3] Thus, an efficient synthetic procedure was developed that allowed the preparation of *N*,*N*-diacyldehydroamino acid derivatives from the corresponding β-hydroxyamino acids. Owing to the high reaction yields and to the simple work-up procedures we were able to prepare these compounds in large amounts and to use them successfully as substrates in Michael addition reactions. Herein, we describe the synthesis of fluorescent alanines from serine derivatives by a sequential dehydration and a rhodium catalysed conjugate addition of arylboronic acids.

Results and Discussion

Several fluorescent alanine derivatives were prepared using a rhodium catalysed conjugate addition of arylboronic acids to dehydroalanines. This reaction was initially screened using several reaction conditions and several types of substrates. Thus, *N*,*N*-diprotected and *N*-monoprotected dehydroalanines were synthesized from the corresponding serine derivatives using a methodology previously developed in our research group.[3e] These compounds were then reacted with (naphthalen-1-yl)boronic acid under different conditions (Scheme 1, Table 1).



Scheme 1. Synthesis of β-naphthylalanine derivatives.

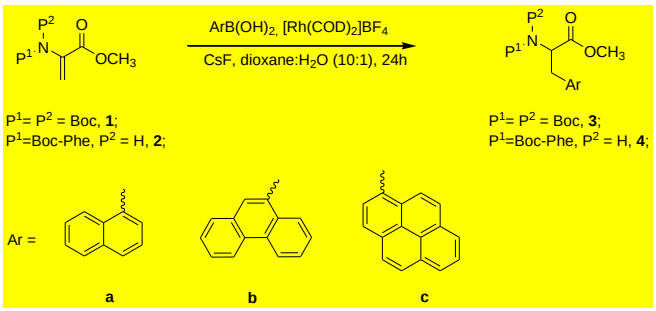
Table 1. Results obtained in the synthesis of β-naphthylalanine derivatives using different reaction conditions.

Entry	P^1	P^2	Catalyst	Ligand	Comp.	Yield/%
1	Boc	Boc	$[Rh(acac)(C_2H_4)_2]$	(*S*)-BINAP	--	--[a]
2	Z(NO_2)	H	$[Rh(acac)(C_2H_4)_2]$	(*S*)-BINAP	--	--[a]
3	Boc-Phe	H	$[Rh(acac)(C_2H_4)_2]$	(*S*)-BINAP	**4a**	40
4	Boc	Boc	$[Rh(COD)_2]BF_4$	---	**3a**	70
5	Z(NO_2)	Boc	$[Rh(COD)_2]BF_4$	---	**5**	60
6	Tos	Boc	$[Rh(COD)_2]BF_4$	---	--	--[a]
7	Z(NO_2)	H	$[Rh(COD)_2]BF_4$	---	**6**	85
8	Boc-Phe	H	$[Rh(COD)_2]BF_4$	---	**4a**	50
9	Boc_2-Ala	Boc	$[Rh(COD)_2]BF_4$	---	**8**	17
10	Boc-Ala	H	$[Rh(COD)_2]BF_4$	---	**7**	72

[a] no product isolated.

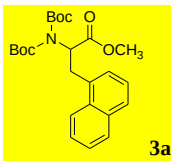
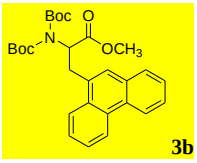
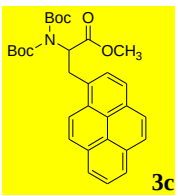
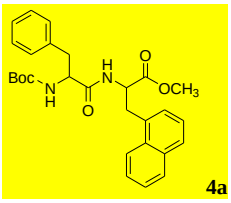
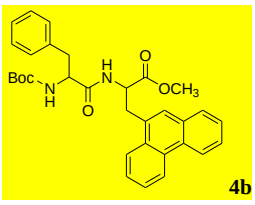
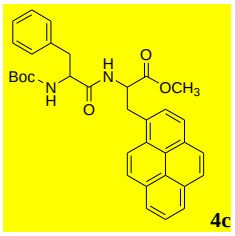
From the results obtained, it is possible to conclude that the catalytic system $[Rh(COD)_2]BF_4$/(*S*)-BINAP failed to give the β-naphthylalanines when the substrates were the methyl esters of *N*,*N*-diprotected and *N*-monoprotected dehydroalanines. In the case of the methyl ester of the dipeptide *N*-*tert*-butoxycarbonyl

phenylalanyldehydroalanine (Boc-Phe-ΔAla-OMe) it was possible to isolate the corresponding β-naphthylalanine derivative in 40% yield. In view of these results it was decided to change the catalyst to $[Rh(COD)_2]BF_4$. The β-naphthylalanines were obtained in good yields using the methyl esters of *N*,*N*-diacyl and *N*-acyldehydroalanines as substrates, except when one of the protecting groups was the *p*-toluenesulfonyl group. *N*,*N*-Diacyldehydrodipeptides were found to be poor acceptors in this type of reaction. In view of these results several β-arylalanines were prepared using as substrates the methyl ester of *N*,*N*-*tert*-butoxycarbonyldehydroalanine (Boc_2-ΔAla-OMe) and Boc-Phe-ΔAla-OMe, and several arylboronic acids with $[Rh(COD)_2]BF_4$ as catalyst (Scheme 2, Table 2).



Scheme 2. Synthesis of β-arylalanine derivatives.

Table 2. Yields obtained in the synthesis of several β-arylalanine derivatives.

Substrate	Product	Yield / %
1	 **3a**	70
1	 **3b**	69
1	 **3c**	67
2	 **4a**	50
2	 **4b**	54
2	 **4c**	34

The absorption and fluorescence properties of the β-arylalanine derivatives **3a-c** and **4a-c** were studied in three solvents of different polarity (cyclohexane, acetonitrile and ethanol). The absorption (λ_{abs}) and emission maximum wavelengths (λ_{em}), molar absorption coefficients (ε) and fluorescence quantum yields (Φ_F) are presented in Tables 3 and 4. The normalized absorption and fluorescence spectra of compounds **3a-c** and **4a-c** are shown in Figures 1 and 2.

All compounds present relatively high molar absorption coefficients at the lowest energy maximum ($\varepsilon \geq 3.4\times10^3$ M^{-1} cm^{-1}) in all solvents studied (Tables 3 and 4), pointing to $\pi\rightarrow\pi^*$ transitions. The ε values are significantly lower for compounds with a naphthyl group (**3a**, **4a**) and a phenanthrenyl group (**3b**, **4b**). In fact, pyrene has a much higher molar absorption coefficient (ε=54000 M^{-1} cm^{-1} at 335 nm[4]) than naphthalene (ca. 6000 M^{-1} cm^{-1} at 275 nm[4]) and phenanthrene (ε ~14000 M^{-1} cm^{-1} at 292 nm[5]), justifying this behaviour. A methyl ester group is present in all compounds and it is known that many carbonyl compounds present a low-lying $n\rightarrow\pi^*$ state. The $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ electronic transitions can be nearby in energy, resulting in state-mixing.[6] The relatively high values of the molar absorption coefficient can be explained by a predominance of $\pi\rightarrow\pi^*$ character in these compounds (Tables 3 and 4).

Emission spectra of compounds **3a-c** and **4a-c** also resemble those of the pure fluorophores.[4] In fact, the absence of an α,β-double bond in these compounds does not allow conjugation between the aromatic moieties (in **4a-c**) and the carbonyl groups (in all compounds), as previously observed for other β,β-diarylalanine compounds.[8]

Table 3. Maximum absorption (λ_{abs}) and emission wavelengths (λ_{em}), molar absorption coefficients (ε) and fluorescence quantum yields (Φ_F) for compounds **3a**, **3b** and **3c** in cyclohexane (CyHx), acetonitrile (ACN) and ethanol (EtOH).

Solvent	λ_{abs} (nm) ($\varepsilon/10^4$ M^{-1} cm^{-1})			λ_{em} (nm)			Φ_F [a]		
	3a	**3b**	**3c**	**3a**	**3b**	**3c**	**3a**	**3b**	**3c**
CyHx	292 (0.41), 283 (0.57), 273 (0.49), 225 (6.27)	298 (0.53), 286 (0.47), 275 (0.67), 254 (3.10), 249 (2.68) *sh*, 209 (2.19)	343 (3.08), 327 (2.07), 313 (0.83), 276 (3.06), 266 (1.76), 256 (0.80), 243 (4.83), 236 (3.03) *sh*	317, 327, 340, 352, 371 *sh*	352, 359 *sh*, 370, 390, 411 *sh*	376, 383, 387, 393, 397, 404 *sh*, 422 *sh*	0.13	0.14	0.64
ACN	292 (0.44), 282 (0.64), 273 (0.56), 224 (6.22)	298 (0.59), 286 (0.54), 275 (0.73), 253 (3.44), 248 (2.97) *sh*, 211 (2.00)	342 (3.75), 326 (2.56), 313 (1.06), 276 (4.25), 265 (2.87), 253 (3.79), 243 (8.04), 235 (4.99) *sh*	319, 325, 340, 353, 372 *sh*	352, 359 *sh*, 370, 389, 411 *sh*	376, 383, 387, 393, 397, 404 *sh*, 424 *sh*	0.10	0.08	0.61
EtOH	292 (0.52), 282 (0.68), 272 (0.59), 224 (6.56)	297 (0.79), 285 (0.79), 275 (1.01), 253 (3.74), 248 (3.25) *sh*, 211 (2.59)	342 (2.96), 326 (2.01), 313 (0.86), 276 (3.14), 265 (1.90), 255 (1.21), 242 (5.07), 235 (3.20) *sh*	318, 326, 340, 351, 372 *sh*	352, 359 *sh*, 369, 389, 412 *sh*	376, 383, 387, 393, 397, 405 *sh*, 422 *sh*	0.19	0.15	0.57

[a] relative to naphthalene in cyclohexane (Φ_F = 0.23 at 25 ºC)[4] for **3a** and **3b** and relative to anthracene in ethanol (Φ_F = 0.27 at 25 ºC)[7] for **3c**; *sh*: shoulder.

Table 4. Maximum absorption (λ_{abs}) and emission wavelengths (λ_{em}), molar absorption coefficients (ε) and fluorescence quantum yields (Φ_F) for compounds **4a**, **4b** and **4c** in cyclohexane (CyHx), acetonitrile (ACN) and ethanol (EtOH).

Solvent	λ_{abs} (nm) ($\varepsilon/10^4$ $M^{-1}cm^{-1}$)			λ_{em}(nm)			Φ_F [a]		
	4a	**4b**	**4c**	**4a**	**4b**	**4c**	**4a**	**4b**	**4c**
CyHx	293 (0.34), 283 (0.53), 274 (0.49), 226 (5.86)	299 (0.96), 288 (0.87), 277 (1.12), 255 (4.92), 249 (4.10) *sh*, 221 (2.04) *sh*, 211 (2.92)	344 (3.52), 327 (2.37), 313 (0.94), 277 (3.41), 266 (1.95), 256 (0.85), 244 (5.10), 236 (3.13) *sh*	317, 326, 340, 351 *sh*, 374 *sh*	352, 361 *sh*, 370, 389, 410 *sh*	376, 383, 388, 393, 397, 421 *sh*	0.12	0.13	0.48
ACN	291 (0.39), 283 (0.57), 273 (0.48), 224 (6.35)	298 (0.90), 286 (0.81), 276 (1.02), 254 (4.84), 249 (4.20) *sh*, 221 (2.28) *sh* 211 (3.35)	343 (3.50), 327 (2.41), 313 (0.99), 276 (3.52), 265 (2.02), 256 (0.95), 243 (5.58), 235 (3.57) *sh*	319, 325, 340, 354 *sh*, 372 *sh*	351, 360 *sh*, 369, 389, 409 *sh*	376, 382, 387, 393, 398, 419 *sh*	0.06	0.06	0.47
EtOH	291 (0.50), 282 (0.71), 273 (0.61), 225 (7.07)	298 (1.02), 286 (0.94), 276 (1.19), 254 (5.25), 248 (4.44) *sh*, 222 (2.66) *sh*, 211 (3.91)	343 (3.27), 327 (2.30), 313 (1.00), 276 (3.42), 265 (2.07), 256 (1.06), 243 (5.33), 235 (3.46) *sh*	319, 326, 340, 352 *sh*, 375 *sh*	351, 357 *sh*, 369, 389, 409 *sh*	376, 382, 387, 393, 397, 420 *sh*	0.13	0.10	0.43

[a] relative to naphthalene in cyclohexane (Φ_F=0.23 at 25 ºC)[4] for **4a** and **4b** and relative to anthracene in ethanol (Φ_F=0.27 at 25 ºC)[7] for **4c**; *sh*: shoulder.

All compounds present reasonable to high fluorescence quantum yields in all solvents studied (Tables 3 and 4). The Φ_F values are similar to those of the pure polycyclic aromatic hydrocarbons (PAHs) at room temperature (Φ_F values are 0.23 in cyclohexane and 0.21 in ethanol for naphthalene; 0.13 for phenanthrene and 0.65 for pyrene in both ethanol and cyclohexane[4,9]). A decrease in Φ_F values relative to the PAHs was expected, considering the flexibility of the chain linked to the fluorescent moieties in the new compounds, which favours the non-radiative deactivation pathways. As expected, compounds **4a-c** exhibit lower fluorescence quantum yields than the corresponding analogues in the **3a-c** series, indicating a larger deactivation by non-radiative transitions, as would also expected. This effect is especially significant for compound **4c**, due to the extremely long excited state lifetime of the pyrene moiety (450 ns for pyrene in cyclohexane and 410 ns in ethanol[9]).

It is well known that the ratio of intensities between the first and the third pyrene vibronic bands, I_1/I_3, is solvent dependent (the pyrene polarity scale), rising with the increase of solvent polarity.[10] Similarly, compounds **3c** and **4c** exhibit a solvent sensitive I_1/I_3 ratio (Table 5), following the observed behaviour in the pyrene fluorescence spectrum, despite a lower degree of sensitivity. In fact, the groups in close proximity to the pyrenyl moiety in compounds **3c** and **4c** influence the local polarity, and thus the relative intensities of the vibronic bands in the emission spectra.

The generally high fluorescence quantum yields of these compounds make them good candidates to be used as fluorescence markers. In particular, compounds **3c** and **4c** may be useful as probes for peptides and proteins, since both compounds can be excited without simultaneous excitation of other aromatic amino acids (phenylalanine, tyrosine and tryptophan) that absorb light at wavelengths lower than 300 nm.[11, 12] The solvent sensitivity of the vibrational structure of their emission spectrum make compounds **3c** and **4c** also promising as polarity probes.

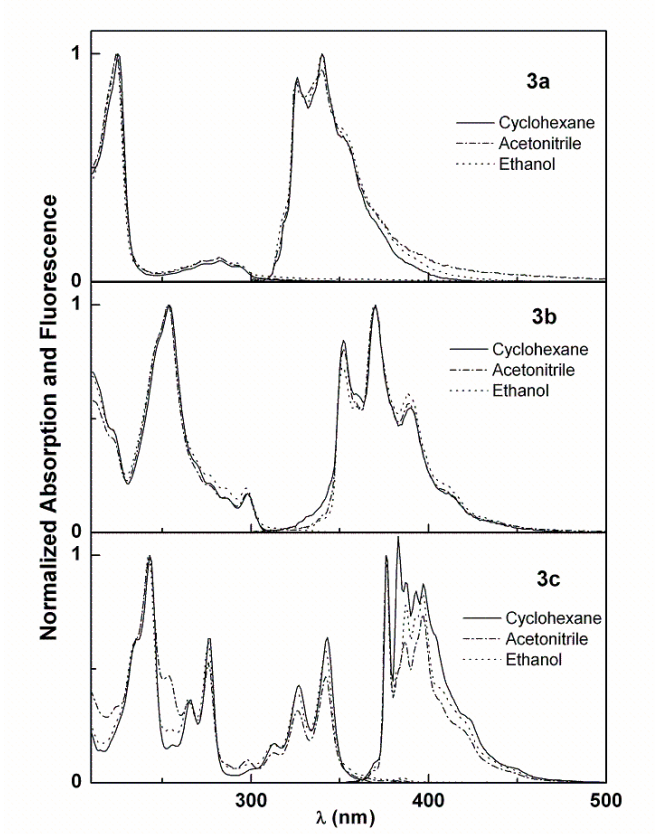


Figure 1. Normalized absorption and fluorescence spectra of solutions of compounds **3a-c** (10^{-5} M for absorption and 10^{-6} M for emission) in cyclohexane, acetonitrile and ethanol (λ_{exc}=270 nm for **3a** and **3b** and λ_{exc}=345 nm for **3c**).

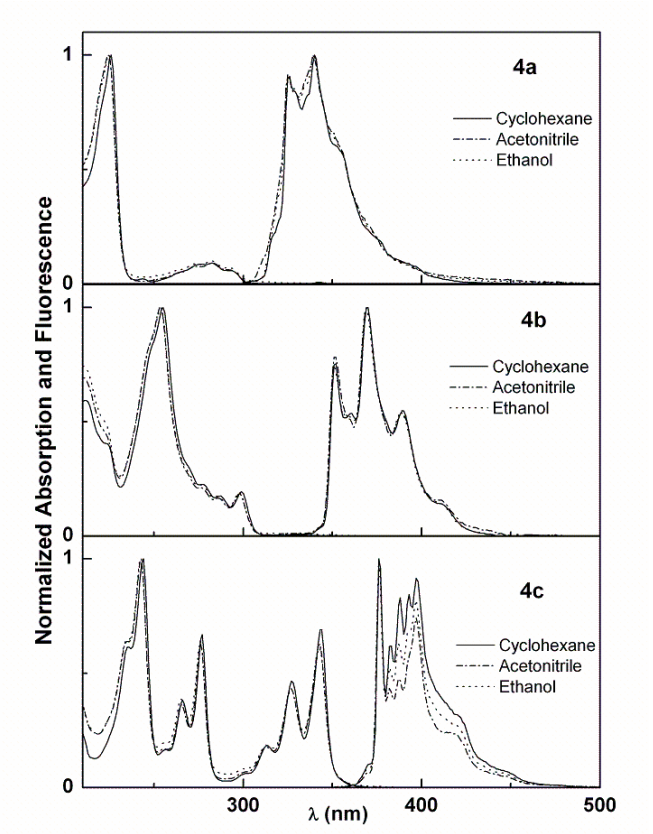


Figure 2. Normalized absorption and fluorescence spectra of solutions of compounds **4a-c** (10^{-5} M for absorption and 10^{-6} M for emission) in cyclohexane, acetonitrile and ethanol (λ_{exc}=270 nm for **4a** and **4b** and λ_{exc}=345 nm for **4c**).

Table 5. Intensity ratio between the first and third vibronic bands, I_1/I_3, of the fluorescence spectrum for compounds **3c** and **4c**. Values for pyrene are also shown for comparison.

Solvent	I_1/I_3		
	3c	**4c**	**Pyrene**[10]
CyHx	0.92	1.18	0.58
EtOH	1.28	1.56	1.18
ACN	1.61	2.08	1.79

Conclusions

Several fluorescent β-arylalanines were prepared in good to high yields using a rhodium catalysed conjugate addition of aryl boronic acids to *N,N*-diprotected dehydroalanine derivatives. This reaction was also applied successfully to dipeptides with a dehydroalanine residue. The photophysical properties of these compounds were studied in solvents with different polarities. The results show that all compounds prepared, due to the generally high fluorescence quantum yields, can be used as fluorescent markers. The β-pyrenylalanine derivatives may be used as probes for peptides and proteins since these compounds can be excited without simultaneous excitation of other aromatic amino acids.

Experimental Section

Melting points (°C) were determined in a a Buchi 535 melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV300 spectrometer at 300 and 75.4 MHz, respectively or on a Bruker AVANCE 400 spectrometer at 400 and 100.6 MHz, respectively. ^{1}H-^{1}H spin-spin decoupling and DEPT θ 45° were used. Chemical shifts are given in ppm and coupling constants in Hz. MS and HRMS data were recorded using a Bruker ESI-TOF or ESI-QTOF Mass Spectrometry Service at the University of Bath.

The reactions were monitored by thin layer chromatography (TLC). Column chromatography was performed on Macherey-Nagel silica gel 230-400 mesh. Petroleum ether refers to the boiling range 40-60 °C. When solvent gradient was used, the increase of polarity was made from neat petroleum ether to mixtures of diethyl ether/petroleum ether, increasing 10% of diethyl ether each time until the isolation of the product.

Spectroscopic measurements: The solutions were prepared using spectroscopic grade solvents. All measurements were performed at 25.0±0.5 °C. Absorption spectra were recorded in a Shimadzu UV-3101PC UV-Vis-NIR spectrophotometer. Fluorescence measurements were performed using a Fluorolog 3 spectrofluorimeter, equipped with double monochromators in both excitation and emission and a temperature controlled cuvette holder. Fluorescence spectra were corrected for the instrumental response of the system. For fluorescence quantum yield determination, the solutions were previously bubbled for 30 minutes with ultrapure nitrogen. The fluorescence quantum yields (Φ_s) were determined using the standard method (equation 1)[13, 14]

$$\Phi_S = \frac{A_r F_S n_S^2}{A_S F_r n_r^2} \Phi_r \quad (1)$$

where *A* is the absorbance at the excitation wavelength, *F* the integrated emission area and *n* the refraction index of the solvents used. Subscripts refer to the reference (r) or sample (s) compound. The excitation wavelengths of the samples were chosen to ensure that there is a linear relationship between the intensity of emitted light and the concentration of the absorbing/emitting species (A ≤ 0.05).

Synthesis of dehydroalanine derivatives: The synthesis of these compounds was described elsewhere.[3a, 3e]

General procedure for the rhodium-catalyzed addition of arylboronic acids to dehydroalanine derivatives

An oven dried Shlenk tube was charged with dehydroalanine derivative (0.2 mmol), boronic acid (4 equiv., 0.8 mmol), $[Rh(COD_2)]BF_4$ (5mol%, 0,01 mmol), CsF (3 equiv., 0.6 mmol), anhydrous 1,4-dioxane (2 mL) and water (0.2 mL). The reaction mixture was heated to 110ºC for 24 hours under an atmosphere of nitrogen. The reaction was cooled to room temperature and the solvent evaporated. The resulting residue was dissolved in ethyl acetate (20 mL) and washed with water (2x20 mL) and brine (2x20 mL). The organic layer was dried over MgSO4 and concentrated in vacuo. The resulting residue was purified by column chromatography.

Synthesis of methyl 2-[bis(*tert*-butoxycarbonyl)amino]-3-(naphthalen-1-yl)propanoate **3a**:

The general procedure described above was followed with compound **1** (0.2 mmol, 60.5 mg) and 1-naphthaleneboronic acid (0.8 mmol, 138 mg) to give compound **3a** (60.2 mg, 70%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.12 (s, 18H, CH_3), 3.48-3.56 (dd, 1 H, *J* = 11.1 and 3.3 Hz, CH_2), 3.73 (s, 3 H, OCH_3), 3.96-4.02 (dd, 1 H, *J* = 3.6 and 10.8 Hz, CH_2), 5.18-5.23 (dd, 1 H, *J* = 3.9 and 6.9 Hz, CH), 7.21 (d, 1 H, *J* = 9.6 Hz, HAr), 7.31 (t, 1 H, *J* = 8.1 Hz, HAr), 7.37-7.47 (m, 2 H, HAr), 7.67 (d, 1 H, *J* = 8.1 Hz, HAr),7.77 (d, 1 H, *J* = 6.9 Hz, HAr), 7.96 (d, 1 H, *J* = 7.8 Hz, HAr) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 27.60 (CH_3), 33.07 (CH_2), 52.42 (OCH_3), 59.10 (CH), 82.89 [$C(CH_3)_3$], 123.40 (CH), 125.57 (CH), 125.61 (CH), 126.21 (CH), 127.51 (CH), 128.14 (CH), 128.86 (CH), 132.14 (C), 133.87 (C), 133.91 (C), 151.27 (C=O), 170.95 (C=O) ppm. HMRS (ESI): calcd. for $C_{24}H_{31}NNaO_6$ 452.20491; found 452.2032.

Synthesis of methyl 2-[bis(*tert*-butoxycarbonyl)amino]-3-(phenanthren-9-yl)propanoate **3b**:

The general procedure described above was followed with compound **1** (0.2 mmol, 60.5 mg) and 9-phenanthraneboronic acid (0.8 mmol, 177 mg)to give compound **3b** (66.0 mg, 69%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.07 (s, 18H, CH_3), 3.51-3.59 (dd, 1 H, *J* = 10.8 and 3.6 Hz, CH_2), 3.75 (s, 3 H, OCH_3), 4.03-4.09 (dd, 1 H, *J* = 3.6 and 10.8 Hz, CH_2), 5.23-5.28 (dd, 1 H, *J* = 3.9 and 6.9 Hz, CH), 7.47-7.59 (m, 5 H, HAr), 7.72 (d, 1 H, *J* = 7.5 Hz, HAr), 8.00-8.04 (m, 1 H, HAr), 8.57 (d, 1 H, *J* = 7.8 Hz, HAr), 8.64-8.67 (m, 1 H, HAr) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 27.54 (CH_3), 33.56 (CH_2), 52.48 (OCH_3), 58.63 (CH), 82.88 [$C(CH_3)_3$], 122.33 (CH), 123.33 (CH), 123.98 (CH), 126.37 (CH), 126-39 (CH), 126.66 (CH), 126.90 (CH), 128.28 (CH), 128.79 (CH), 130.04 (C), 130.74 (C), 131.06 (C), 131.61 (C), 131.98 (C), 151.47 (C=O), 170.97 (C=O) ppm. HMRS (ESI): calcd. for $C_{28}H_{33}NNaO_6$ 502.22056; found 502.2245.

Synthesis of methyl 2-[bis(*tert*-butoxycarbonyl)amino]-3-(pyren-1-yl)propanoate **3c**:

The general procedure described above was followed with compound **1** (0.2 mmol, 60.5 mg) and 1-pyreneboronic acid (0.8 mmol, 197 mg) to give compound **3c** (67.0 mg, 67%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.02 (s, 18H, CH_3), 3.76 (s, 3 H, OCH_3), 3.84-3.92 (dd, 1 H, *J* = 10.5 and 3.6 Hz, CH_2), 4.16-4.22 (dd, 1 H, *J* = 4.2 and 10.2 Hz, CH_2), 5.29-5.34 (dd, 1 H, *J* = 4.2 and 6.6 Hz, CH), 7.78 (d, 1 H, *J* = 7.8 Hz, HAr), 7.89-7.95 (m, 3 H, HAr), 8.03 (d, 2 H, *J* = 8.1 Hz, HAr), 8.10 (d, 2 H, *J* = 7.8 Hz, HAr), 8.22 (d, 1 H, *J* = 9.3 Hz, HAr) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 27.50 (CH_3), 33.42 (CH_2), 52.46 (OCH_3), 59.85 (CH), 82.87 [$C(CH_3)_3$], 123.13 (CH), 124.84 (CH), 124.90 (CH), 125.00 (C), 125.03 (CH), 125.84 (CH), 126.98 (CH), 127.44 (CH), 127.68 (CH), 128.63 (CH), 129.52 (C), 130.46 (C), 130.85 (C), 131.30 (C), 131.88 (C), 151.54 (C=O), 170.93 (C=O) ppm. HMRS (ESI): calcd. for $C_{30}H_{33}NNaO_6$ 526.22056; found 526.2205.

Synthesis of methyl 2-{*tert*-butoxycarbonyl[(4-nitrobenzyloxy)carbonyl]amino)-3-(naphthalen-1-yl}propanoate **5**:

The general procedure described above was followed with methyl 2-{*tert*-butoxycarbonyl[(4-nitrobenzyloxy)carbonyl]amino}acrylate (0.2 mmol, 76 mg) and 1-naphthaleneboronic acid (0.8 mmol, 138 mg) to give compound **5** (61.3 mg, 60%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.17 (s, 9H, CH_3), 3.48-3.57 (dd, 1 H, *J* = 11.1 and 3.6 Hz, CH_2), 3.72 (s, 3 H, OCH_3), 3.98-4.04 (dd, 1 H, *J* = 3.9 and 10.5 Hz, CH_2), 4.93 (q, 2 H, *J* = 13.5 Hz, CH_2), 5.26-5.31 (m, 1 H, CH), 7.12-7.15 (m, 3 H, HAr), 7.26 (t, 1 H, *J* = 7.2 Hz, HAr), 7.38-7.44 (m, 2 H, HAr), 7.65 (d, 1 H, *J* = 8.1 Hz, HAr), 7.75-7.79 (m, 1 H, HAr), 7.90-7.94 (m, 1 H, HAr), 8.05 (d, 2 H, *J* = 6.9 Hz, HAr) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 27.58 (CH_3), 32.80 (CH_2), 52.66 (OCH_3), 59.49 (CH), 66.89 (CH_2), 84.00 [$C(CH_3)_3$], 123.16 (CH), 123.67 (CH), 125.46 (CH), 125.76 (CH), 126.30 (CH), 127.71 (CH), 128.05 (CH), 128.97 (CH), 131.91 (C), 133.37 (C), 133.82 (C), 142.37 (C), 150.40 (C=O), 153.20 (C=O), 170.29 (C=O) ppm.HMRS (ESI): calcd. for $C_{27}H_{28}N_2NaO_8$ 531.17434; found 531.1750.

Synthesis of methyl 3-(naphthalen-1-yl)-2-[(4-nitrobenzyloxy)carbonylamino]propanoate **6**:

The general procedure described above was followed with methyl 2-[(4-nitrobenzyloxy)carbonylamino]acrylate (0.2 mmol, 56 mg) and 1-naphthaleneboronic acid (0.8 mmol, 138 mg) to give compound **6** (69.5 mg, 85%) as a white solid. M.p. 140.0-141.0 ºC (from ethyl acetate/ n-hexane). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.38-3.45 (dd, 1 H, *J* = 6.9 and 7.2 Hz, CH_2), 3.54-3.60 (m, 4 H, OCH_3 and CH_2), 4.72 (d, 1 H, *J* = 7.8 Hz, CH), 5.07 (s, 2 H, CH_2), 5.29 (d, 1 H, *J* = 8.1 Hz, NH), 7.14-7.19 (m, 2 H, HAr), 7.28-7.35 (m, 2 H, HAr), 7.42-7.46 (m, 2 H, HAr), 7.71 (d, 1 H, *J* = 8.4 Hz, HAr), 7.78-7.82 (m, 1 H, HAr), 7.95-8.01 (m, 1 H, HAr), 8.12 (d, 2 H, *J* = 8.7 Hz, HAr) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 35.58 (CH_2), 54.48 (OCH_3), 54.74 (CH), 65.34 (CH_2), 123.28 (CH), 123.75 (CH), 125.28 (CH), 125.88 (CH), 126.45 (CH), 127.52 (CH), 128.00 (CH), 128.16 (CH), 128.99 (CH), 132.00 (C), 132.08 (C), 133.92 (C), 143.67 (C), 147.58 (C), 155.15 (C=O), 172.20 (C=O) ppm. HMRS (ESI): calcd. for $C_{22}H_{20}N_2NaO_6$ 431.12191; found 431.1220.

Synthesis of methyl 2-[2-(*tert*-butoxycarbonylamino)-3-phenylpropanamido]-3-(naphthalen-1-yl)propanoate **4a**:

The general procedure described above was followed with compound **2** (0.2 mmol, 70.0 mg) and 1-naphthaleneboronic acid (0.8 mmol, 138 mg) to give compound **4a** (48.0 mg, 50%) as a white solid. M.p. 139.0-140.0 ºC (from ethyl acetate/ n-hexane). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.30 (s, 9H, CH_3), 2.87-2.98 (m, 2 H, CH_2), 3.37-3.49 (m, 5 H, CH_2 and OCH_3), 4.25 (br. s, 1 H, CH), 4.79-4.86 (m, 2 H, CH and NH), 6.23-6.39 (dd, 1 H, *J* = 7.5 Hz, NH), 7.00-7.30 (m, 7 H, HAr), 7.40-7.51 (m, 2 H, HAr), 7.68 (d, 1 H, *J* = 8.4 Hz, HAr), 7.78 (d, 1 H, *J* = 8.7 Hz, HAr), 7.98 (t, 1 H, *J* = 8.7 Hz, HAr) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 28.24 (CH_3), 35.41 and 35.46 (CH_2), 38.37 (CH_2), 52.24 (OCH_3), 53.08 and 53.26 (CH), 55.64 (CH), 80.23 [$C(CH_3)_3$], 123.37 and 123.50 (CH), 125.28 (CH), 125.82 and 125.86 (CH), 126.38 and 126.48 (CH), 126.98 (CH), 127.41 and 127.48 (CH), 128.04 and 128.09 (CH), 128.64 and 128.67 (CH), 128.92 (CH), 129.31 and 129.37 (CH), 131.98 and 132.04 (C), 132.08 and 132.12 (C), 133.90 (C), 136.49 (C), 155.28 (C=O), 170.83 and 170.96 (C=O), 171.67 and 171.97 (C=O) ppm. HMRS (ESI): calcd. for $C_{28}H_{32}N_2NaO_5$ 499.22089; found 499.2251.

Synthesis of methyl 2-[2-(*tert*-butoxycarbonylamino)-3-phenylpropanamido]-3-(phenanthren-9-yl)propanoate **4b**:

The general procedure described above was followed with compound **2** (0.2 mmol, 70.0 mg) and 9- phenanthraneboronic acid (0.8 mmol, 177 mg) to give compound **4b** (57.0 mg, 54%).[2c]

Synthesis of methyl 2-[2-(*tert*-butoxycarbonylamino)-3-phenylpropanamido]-3-(pyren-1-yl)propanoate **4c**:

The general procedure described above was followed with compound **2** (0.2 mmol, 70.0 mg) and 1-pyreneboronic acid (0.8 mmol, 197 mg) to give compound **4c** (37.6 mg, 34%) as a white solid. M.p. 185.0-186.0 ºC (from ethyl acetate/ n-hexane). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.15 and 1.18 (s, 9 H, CH_3), 2.82-2.87 (m, 2 H, CH_2), 3.36 and 3.41 (s, 3 H, OCH_3), 3.60-3.63 (m, 2 H, CH_2), 4.17 (br. s, 1 H, CH), 4.68-4.91 (m, 2 H, CH and NH), 6.22-6.37 (dd, 1 H, *J* = 7.8 Hz, NH), 6.92-7.03 (m, 4 H, HAr), 7.47-7.51 (dd, 1 H, *J* = 2.4 and 5.4 Hz, HAr), 7.83-7.92 (m, 5 H, HAr), 7.97-8.14 (m, 4 H, HAr) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 28.15 and 28.20 (CH_3), 35.64 and 35.79 (CH_2), 38.36 (CH_2), 52.31 and 52.34 (OCH_3), 53.73 and 53.82 (CH), 55.75 (CH), 80.22 [$C(CH_3)_3$], 122.83 and 123.02 (CH), 124.75 (CH), 124.84 (C), 125.08 (C), 125.15 (CH), 125.30 (CH), 126.04 (CH), 126.92 (CH), 127.31 (CH), 127.44 (CH), 127.91 (CH), 127.44 and 128.04 (CH), 128.60 (CH), 129.27 and 129.32 (CH), 129.41 and 129.52 (C), 129.70 and 129.79 (C), 130.69 and 130.73 (C), 130.76 and 130.79 (C), 131.36 (C), 136.48 (C), 155.28 (C=O), 155.25 (C=O), 170.90 and 171.04 (C=O), 171.59 and 171.89 (C=O) ppm. HMRS (ESI): calcd. for $C_{34}H_{34}N_2NaO_5$ 573.23654; found 573.2374.

Synthesis methyl 2-[2-(*tert*-butoxycarbonylamino)propanamido]-3-(naphthalen-1-yl)propanoate **7**:

The general procedure described above was followed with compound methyl 2-[2-(*tert*-butoxycarbonylamino)propanamido]acrylate (0.2 mmol, 54.5 mg) and 1-naphthaleneboronic acid (0.8 mmol, 138 mg) to give compound **7** (57.3 mg, 72%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.15-1.21 (m, 3 H, CH_3), 1.35 (s, 9H, CH_3), 3.46-3.54 (m, 5 H, CH_2 and OCH_3), 4.05 (q, 1 H, *J* = 7.2 Hz, CH), 4.73 (br. s, 1 H, NH), 4.85-4.94 (m, 1 H, CH), 6.54-6.65 (dd, 1 H, *J* = 6.9 Hz, NH), 7.16-7.19 (m, 1 H, HAr), 7.31 (t, 1 H, *J* = 8.1 Hz, HAr), 7.38-751 (m, 2 H, HAr), 7.70 (d, 1 H, *J* = 8.1 Hz, HAr), 7.78 (d, 1 H, *J* = 8.4 Hz, HAr), 8.00-8.05 (dd, 1 H, *J* = 3.0 and 5.4 Hz, HAr) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 18.17 (CH_3), 28.28 and 28.30 (CH_3), 35.31 and 35.46 (CH_2), 52.31 (OCH_3), 53.08 and 53.20 (CH), 60.44 (CH), 80.24 [$C(CH_3)_3$], 123.43 and 123.58 (CH), 125.26 and 125.29 (CH), 125.82 (CH), 126.36 and 126.44 (CH), 127.49 (CH), 128.02 and 128.05 (CH), 128.90 (CH), 132.05 and 132.16 (C), 132.20 and 132.26 (C), 13387 (C), 155.45 (C=O), 171.98 and 172.14 (C=O), 172.26 and 171.32 (C=O) ppm. HMRS (ESI): calcd. for $C_{22}H_{29}N_2O_5$ 401.20765; found 401.2097.

Synthesis methyl 2-[2-bis(*tert*-butoxycarbonyl)amino-*N*-(*tert*-butoxycarbonyl)propanamido]-3-(naphthalen-1-yl)propanoate **8**:

The general procedure described above was followed with compound methyl 2-2-[bis(*tert*-butoxycarbonyl)amino]-*N*-(*tert*-butoxycarbonyl)propanamido]acrylate (0.2 mmol, 95 mg) and 1-naphthaleneboronic acid (0.8 mmol, 138 mg) to give compound **8** (20.0 mg, 17%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.40-1.44 (m, 30 H, CH_3), 3.29-3.37 (dd, 1 H, *J* = 5.7 and 8.7 Hz, CH_2), 3.57 (s, 3 H, OCH_3), 3.66 (t, 1 H, *J* = 10.2 Hz, CH), 3.92-3.99 (dd, 1 H, *J* = 7.5 and 6.9 Hz, CH_2), 5.48-5.56 (m, 1 H, CH), 7.29-7.51 (m, 4 H, HAr), 7.66 (d, 1 H, *J* = 8.1 Hz, HAr), 7.77 (d, 1 H, *J* = 6.9 Hz, HAr), 8.11 (d, 1 H, *J* = 8.1 Hz, HAr) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 15.85 (CH_3), 27.65 and 28.04 (CH_3), 33.55 (CH_2), 52.19 (OCH_3), 56.98 (CH), 57.66 (CH), 82.60 and 83.85 [$C(CH_3)_3$], 124.00 (CH), 125.13 and 125.32 (CH), 125.52 (CH), 125.90 (CH), 126.16 (CH), 127.40 and 127.62 (CH), 128.49 and 128.72 (CH), 132.03 (C), 133.81 (C), 134.37 (C), 151.67 and 152.59 (C=O), 170.85 (C=O), 173.52 (C=O) ppm. HMRS (ESI): calcd. for $C_{32}H_{44}N_2NaO_9$ 623.29445; found 623.2946.

Supporting Information (see footnote on the first page of this article): … . ((Copies of the ^{1}H NMR and ^{13}C NMR spectra are REQUIRED for all key intermediates and final products; additional information as needed.))

Acknowledgments

Thanks are due to the Foundation for Science and Technology (FCT, Portugal), QREN and FEDER/EU for financial support through the research centers, CQ/UM [PEst-C/QUI/UI0686/2011 (FCOMP-01-0124-FEDER-022716)]) and CFUM [PEst-C/FIS/UI0607/2011 (F-COMP-01-0124-FEDER-022711)], and project PTDC/QUI/81238/2006 (cofinanced by FEDER/COMPETE, ref. FCOMP-01-0124-FEDER-007467). G. Pereira (SFRH/BD/38766/2007) acknowledges her PhD grant to FCT, POPH-QREN, FSE.

[1] a) K. Yoshida, T. Hayashi in *Modern Rhodium-Catalysed Organic reactions* (Eds.: P. A. Evans) Wiley-VCH:Weinheim, Germany, **2005**, Chapter 3; b) H. J. Edwards, J. D. Hargrave, S. D. Penrose, C. G. Frost, *Chem. Soc. Rev.* **2010**, *39*, 2093-2105; c) J. C. Allen, G. Kociok-Köhn, C. G. Frost, *Org. Biomol.Chem.* **2012**, *10*, 32-35.

[2] a) C. J. Chapman, C. G. Frost, *Adv. Synth. Catal.* **2003**, *345*, 353-355; b) C. J. Chapman, K. J. Wadsworth, C. G. Frost *J. Organometallic Chem.* **2003**, *680*, 206-211; c) C. J. Chapman, J. D. Hargrave, G. Bish, C. G. Frost *Tetrahedron* **2008**, *64*, 9528-9539; d) J. D. Hargrave, G. Bish, G.K. Kociok-Köhn, C.G. Frost, *Org. Biomol. Chem.* **2010**, *8*, 5120-5125; e) D. Ray, A. M. Nyong, A. Natarajan, *Tetrahedron Lett.* **2010**, *51*, 2655-2656.

[3] a) P. M. T. Ferreira, H. L. S. Maia, L. S. Monteiro, J. Sacramento *J. Chem. Soc., Perkin Trans. 1* **1999**, 3697-3703; b) P. M. T. Ferreira, H. L. S. Maia, L. S. Monteiro, J. Sacramento *J. Chem. Soc., Perkin Trans. 1* **2001**, 3167-3174; c) P. M. T. Ferreira, H. L. S. Maia, L. S. Monteiro *Eur. J. Org. Chem.* **2003**, 2635-2644; d) A. S. Abreu, P. M. T. Ferreira, L. S. Monteiro, M-J. R. P. Queiroz, I. C. F. R. Ferreira, R. C. Calhelha, L. M. Estevinho, *Tetrahedron* **2004**, *60*, 11821-11828; e) P. M. T. Ferreira, L. S. Monteiro, G. Pereira, L. Ribeiro, J. Sacramento, L. Silva *Eur. J. Org. Chem.* **2007**, 5934-5949.

[4] I. B. Berlman, *Handbook of Fluorescence Spectra of Aromatic Molecules*, Academic Press, London, **1971**.

[5] Y. Nakamura, T. Tsuihiji, T. Mita, T. Minowa, S. Tobita, H. Shizuka, J. Nishimura, *J. Am. Chem. Soc.* **1996**, *118*, 1006-1012.

[6] N .J. Turro, J. C. Scaiano, V. Ramamurthy, in *Modern Molecular Photochemistry of Organic Molecules*, University Science Books, Sausalito (California), **2009**.

[7] W. H. Melhuish, *J. Phys. Chem.* **1961**, *65*, 229-235; b) W. R. Dawson, M. W. Windsor, *J. Phys. Chem.* **1968**, *72*, 3251-3260.

[8] P. M. T. Ferreira, L. S. Monteiro, E. M. S. Castanheira, G. Pereira, C. Lopes, H. Vilaça, *Tetrahedron* **2011**, *67*, 193-200.

[9] B. Valeur, *Molecular Fluorescence: Principles and Applications*, Wiley-VCH, Weinheim, **2001**.

[10] D. C. Dong, M. A. Winnik, *Can. J. Chem.* **1984**, *62*, 2560-2565.

[11] J. R. Lakowicz, Principles of Fluorescence Spectroscopy, 2nd Ed., Kluwer Academic/Plenum Publishers, New York, **1999**.

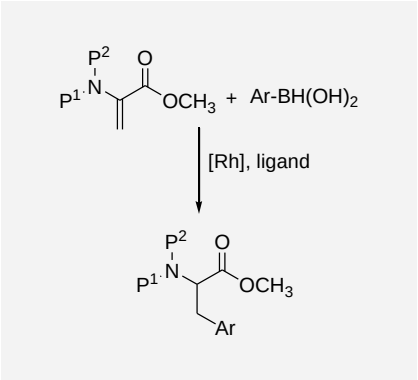
[12] M. R. Eftink, *Intrinsic fluorescence of proteins* in: *Topics in fluorescence spectroscopy* (Ed.: J. R. Lakowicz), Kluwer Academic/Plenum Publishers, New York, **2000**, pp. 1-13.

[13] G. A. Crosby, J. N. Demas, *J. Phys. Chem.* **1971**, *75*, 991-1024.

[14] S. Fery-Forgues, D. Lavabre, *J. Chem. Educ.* **1999**, *76*, 1260-1264.

Received: ((will be filled in by the editorial staff))
Published online: ((will be filled in by the editorial staff))

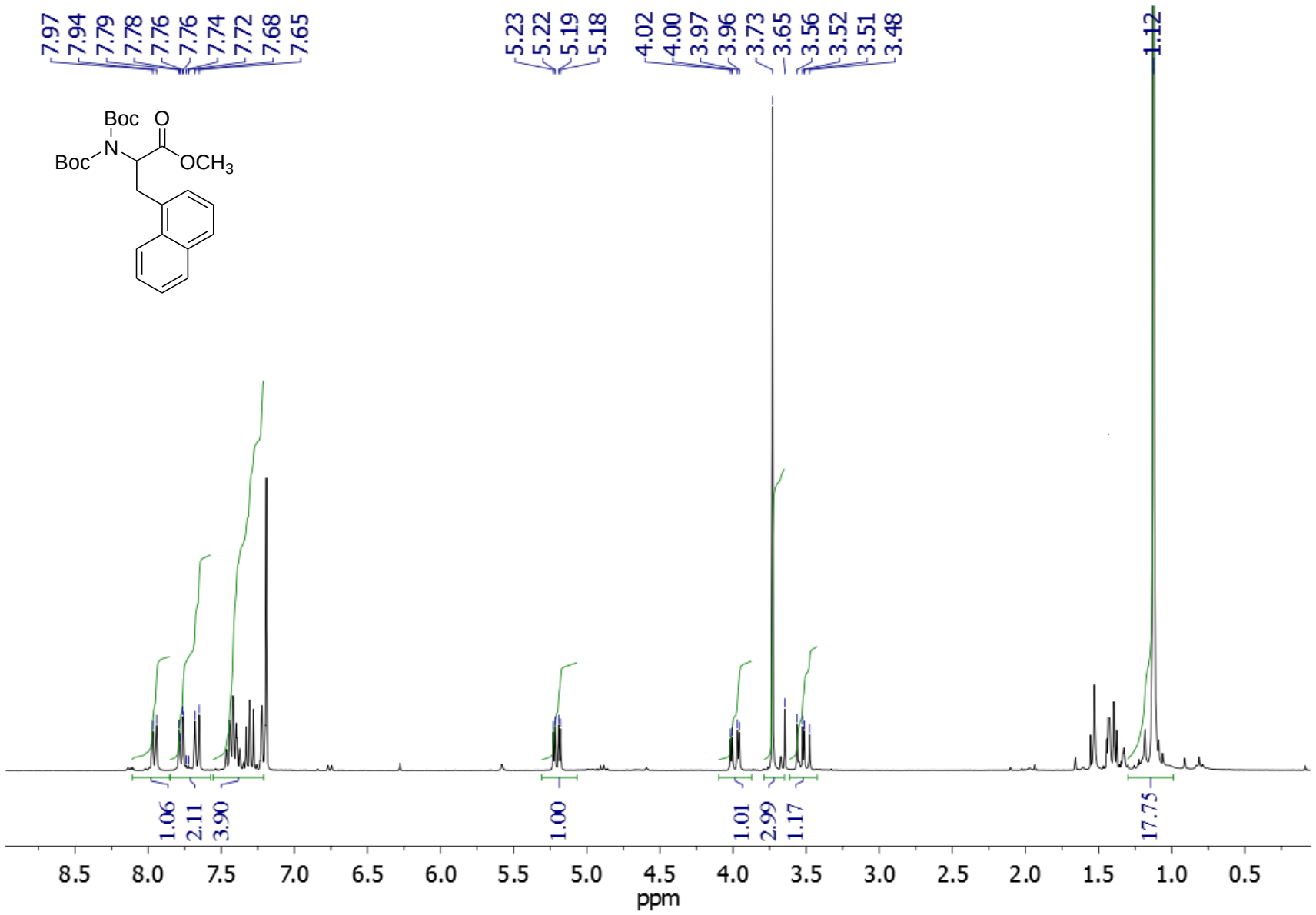
Several fluorescent β-arylalanine derivatives were prepared in good yields using a rhodium catalysed conjugate addition of arylboronic acids to *N*-protected dehydroalanines. The photophysical properties of some of the β-arylalanines were studied in three solvents of different polarity and considering the relatively high fluorescence quantum yield of these compounds, some of them may be useful as fluorescent markers.

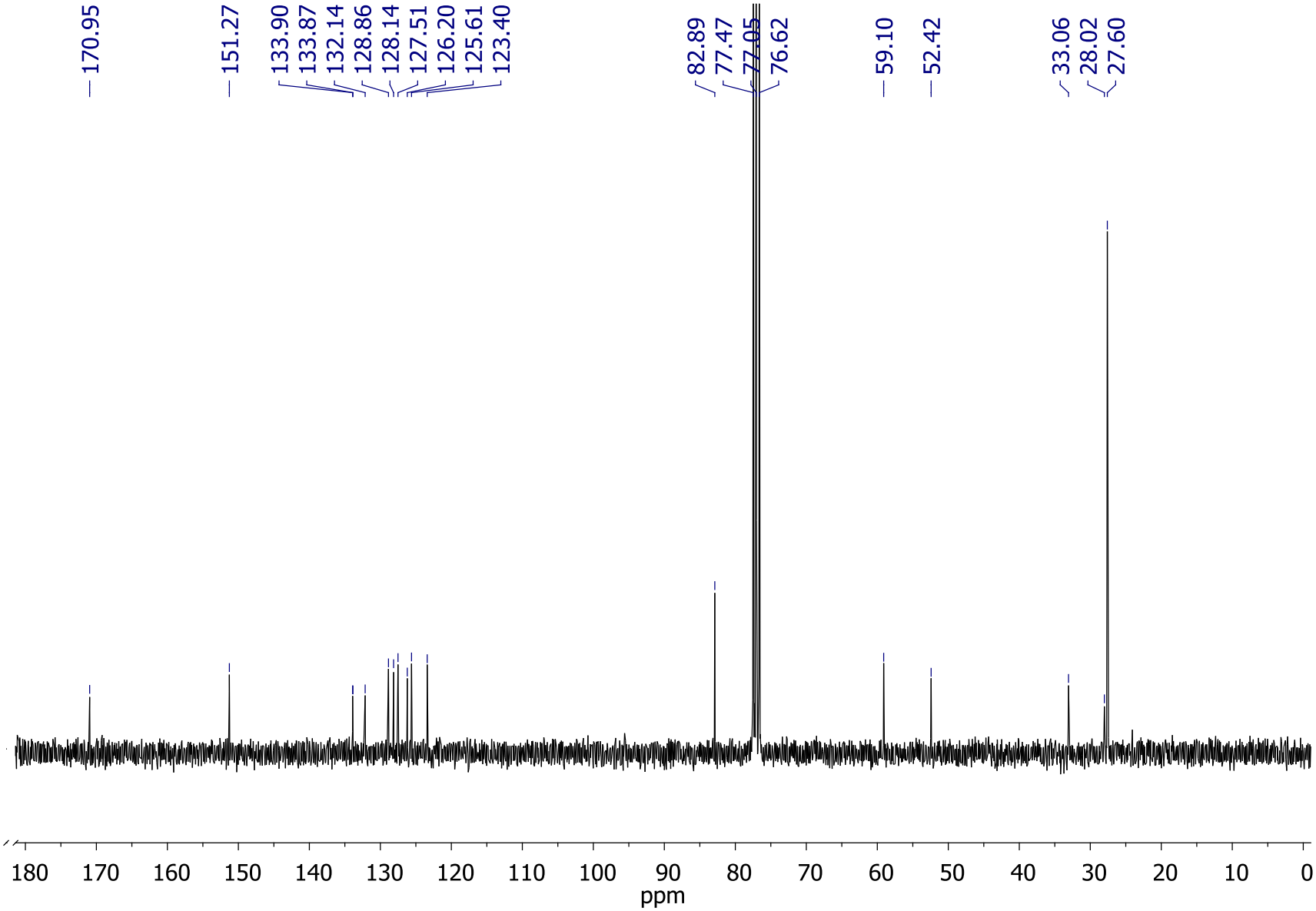


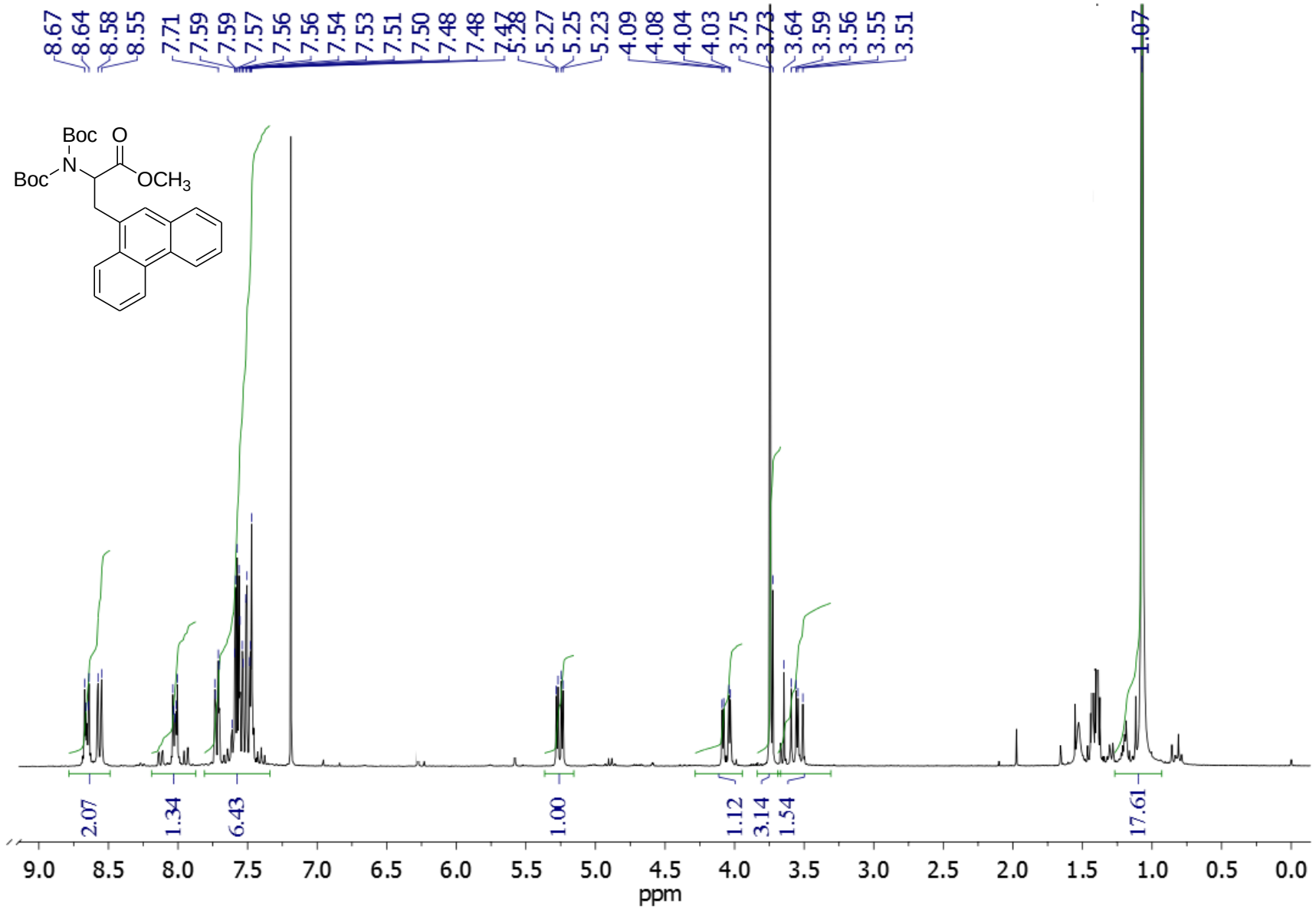
P.M.T. Ferreira,* L.S. Monteiro, G. Pereira, E.M.S. Castanheira and C.G. Frost*……..Page No. – Page No.

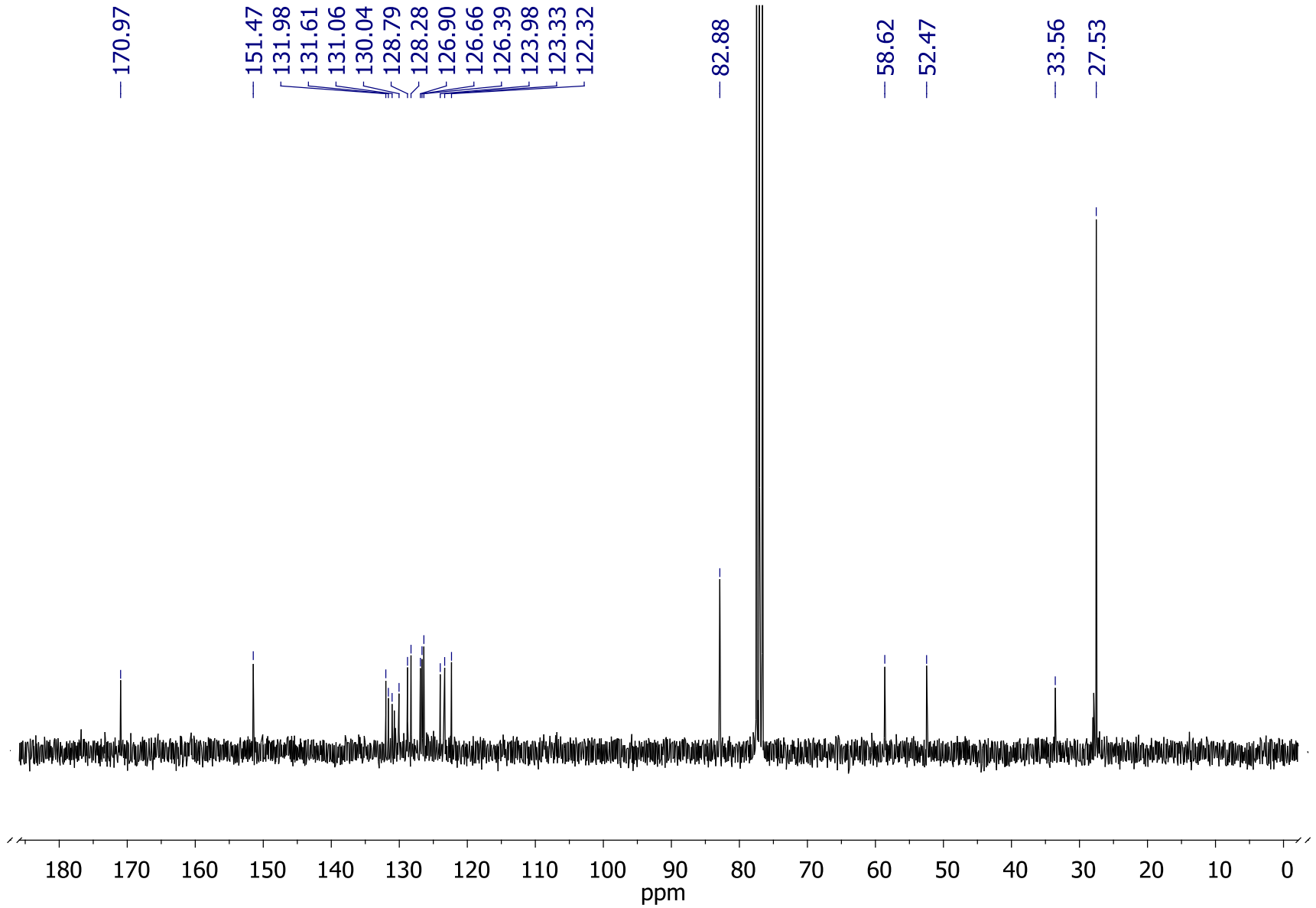
Synthesis of fluorescent alanines by a rhodium catalysed conjugate addition of arylboronic acids to dehydroalanine derivatives

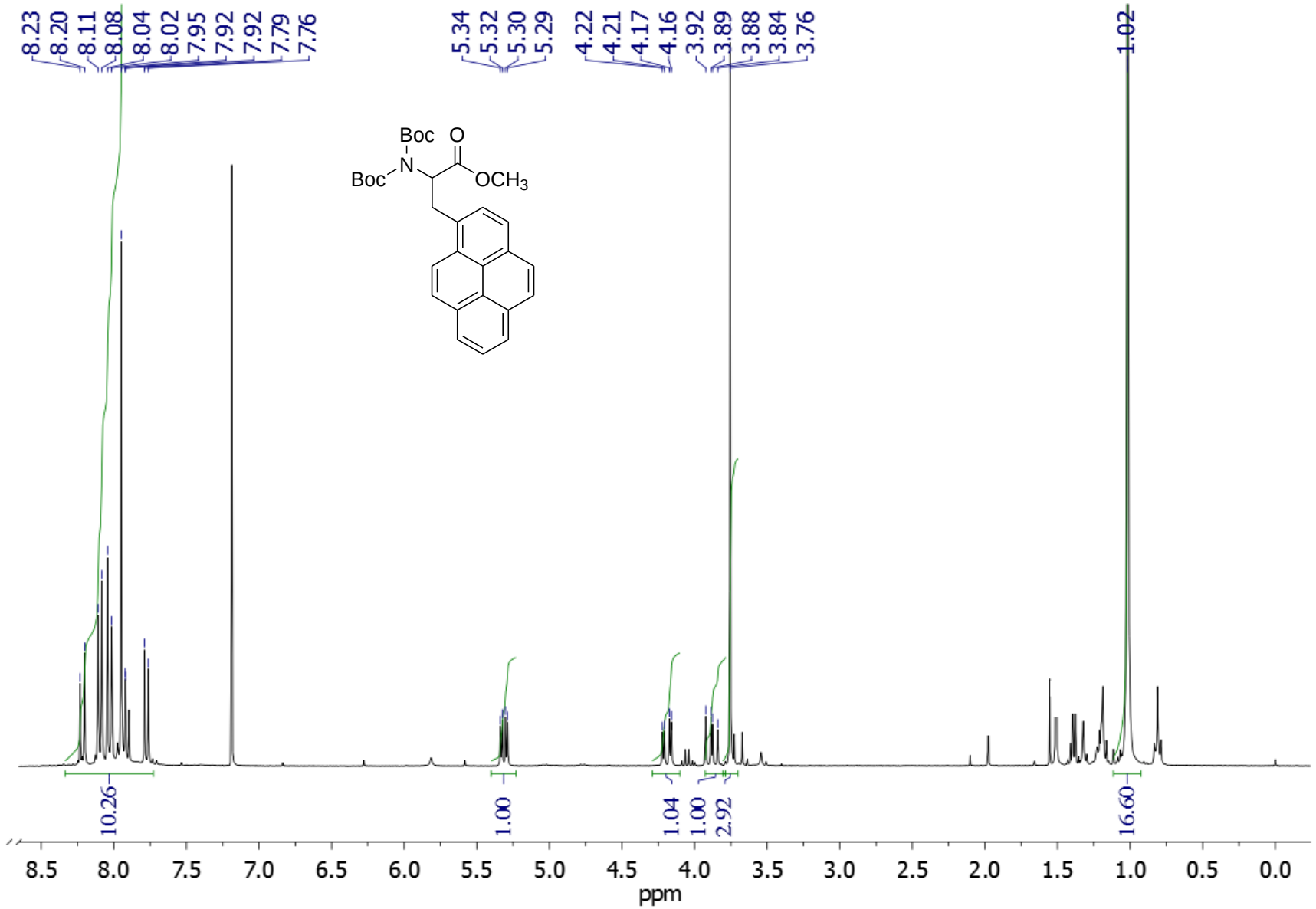
Keywords: dehydroalanines / rhodium-catalysis / arylboronic acids / conjugate addition / fluorescent marker

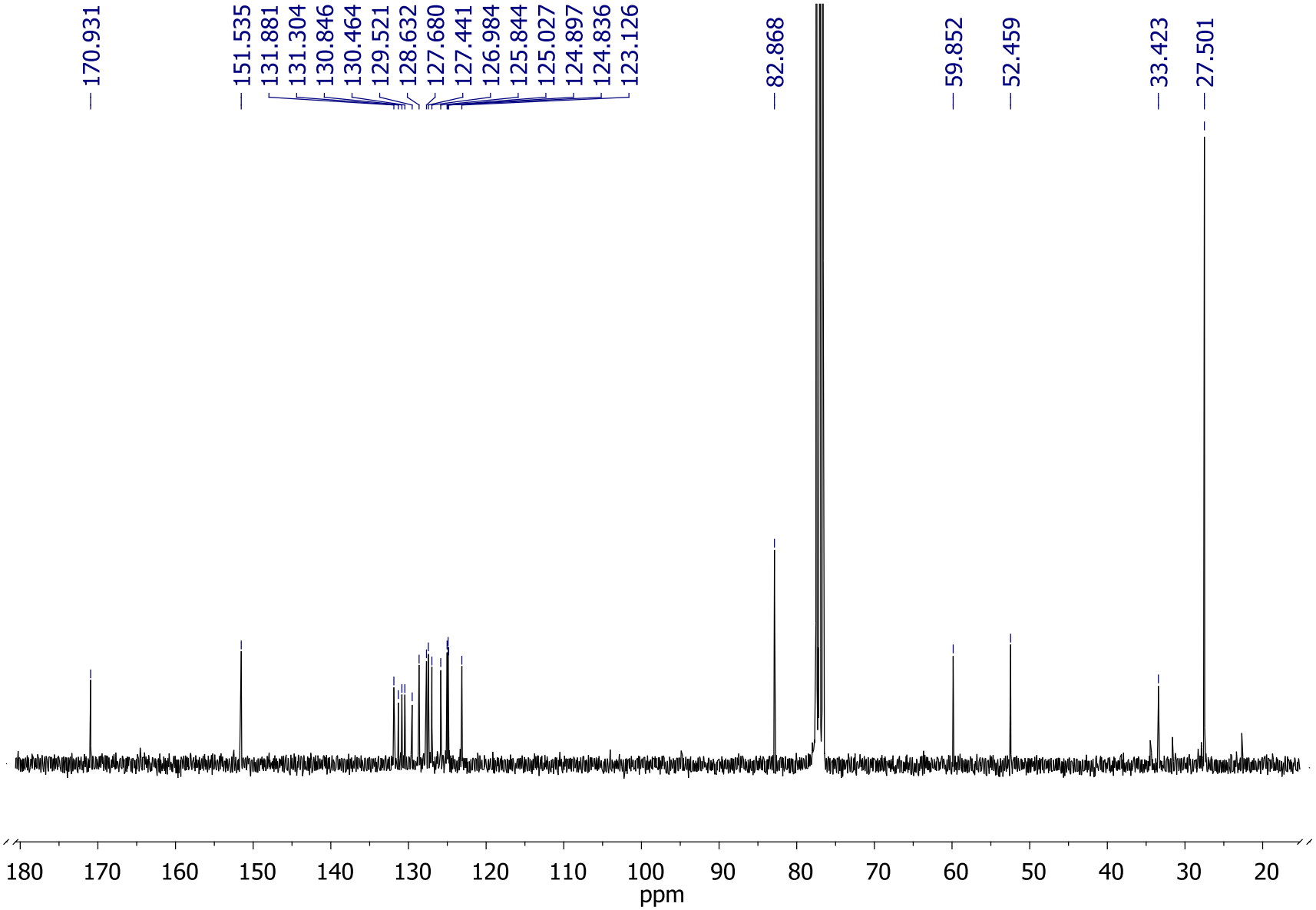

7.97
7.94
7.79
7.78
7.76
7.76
7.74
7.72
7.68
7.65
5.23
5.22
5.19
5.18
4.02
4.00
3.97
3.96
3.73
3.65
3.56
3.52
3.51
3.48
1.12
Boc
O
N
Boc
OCH3
1.06
2.11
3.90
1.00
1.01
2.99
1.17
17.75
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

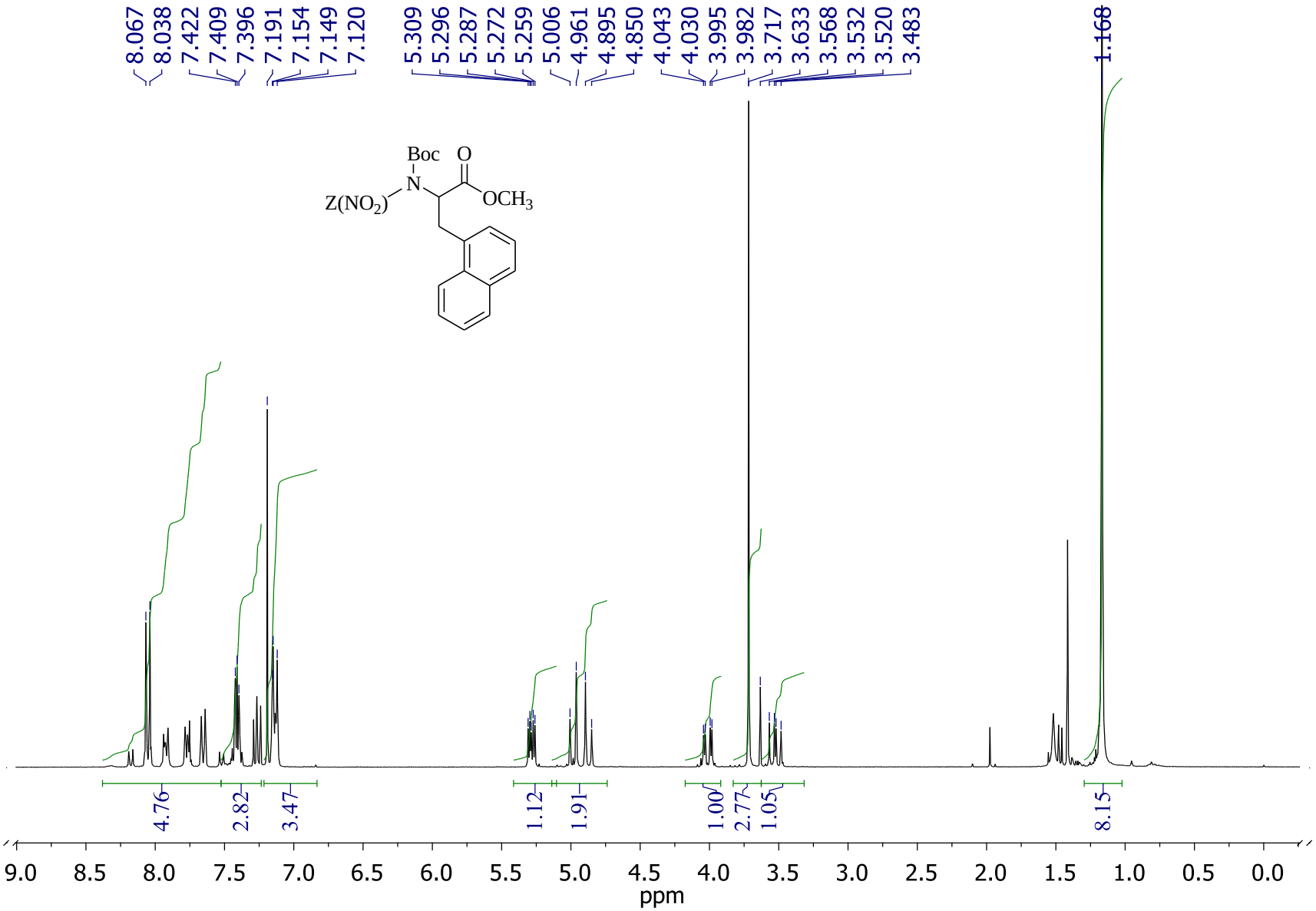

170.95
151.27
133.90
133.87
132.14
128.86
128.14
127.51
126.20
125.61
123.40
82.89
77.47
77.05
76.62
59.10
52.42
33.06
28.02
27.60
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

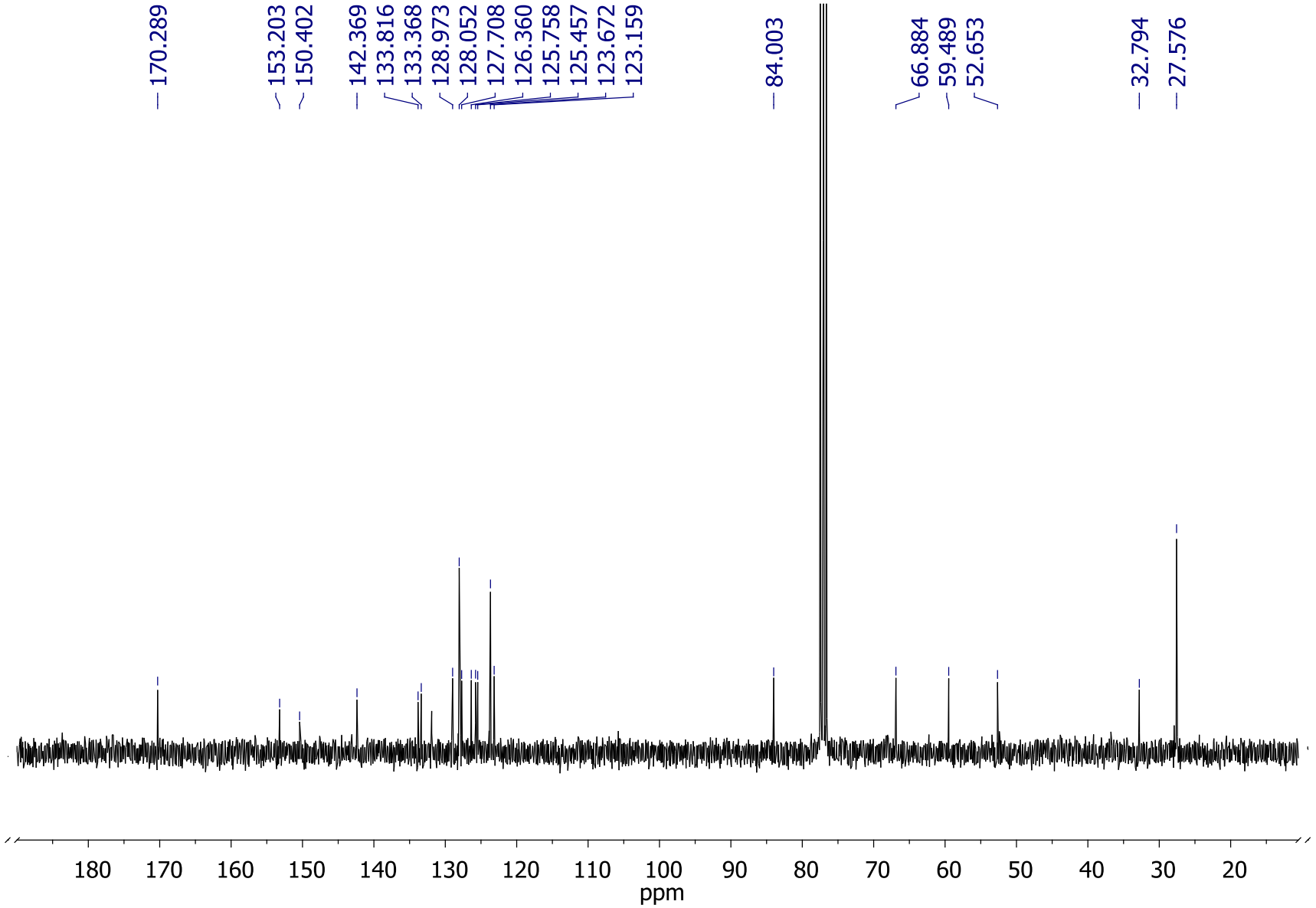

8.67
8.64
8.58
8.55
7.71
7.59
7.59
7.57
7.56
7.56
7.54
7.53
7.51
7.50
7.48
7.48
7.47
5.28
5.27
5.25
5.23
4.09
4.08
4.04
4.03
3.75
3.73
3.64
3.59
3.56
3.55
3.51
1.07
Boc
O
N
Boc
OCH3
2.07
1.34
6.43
1.00
1.12
3.14
1.54
17.61
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm

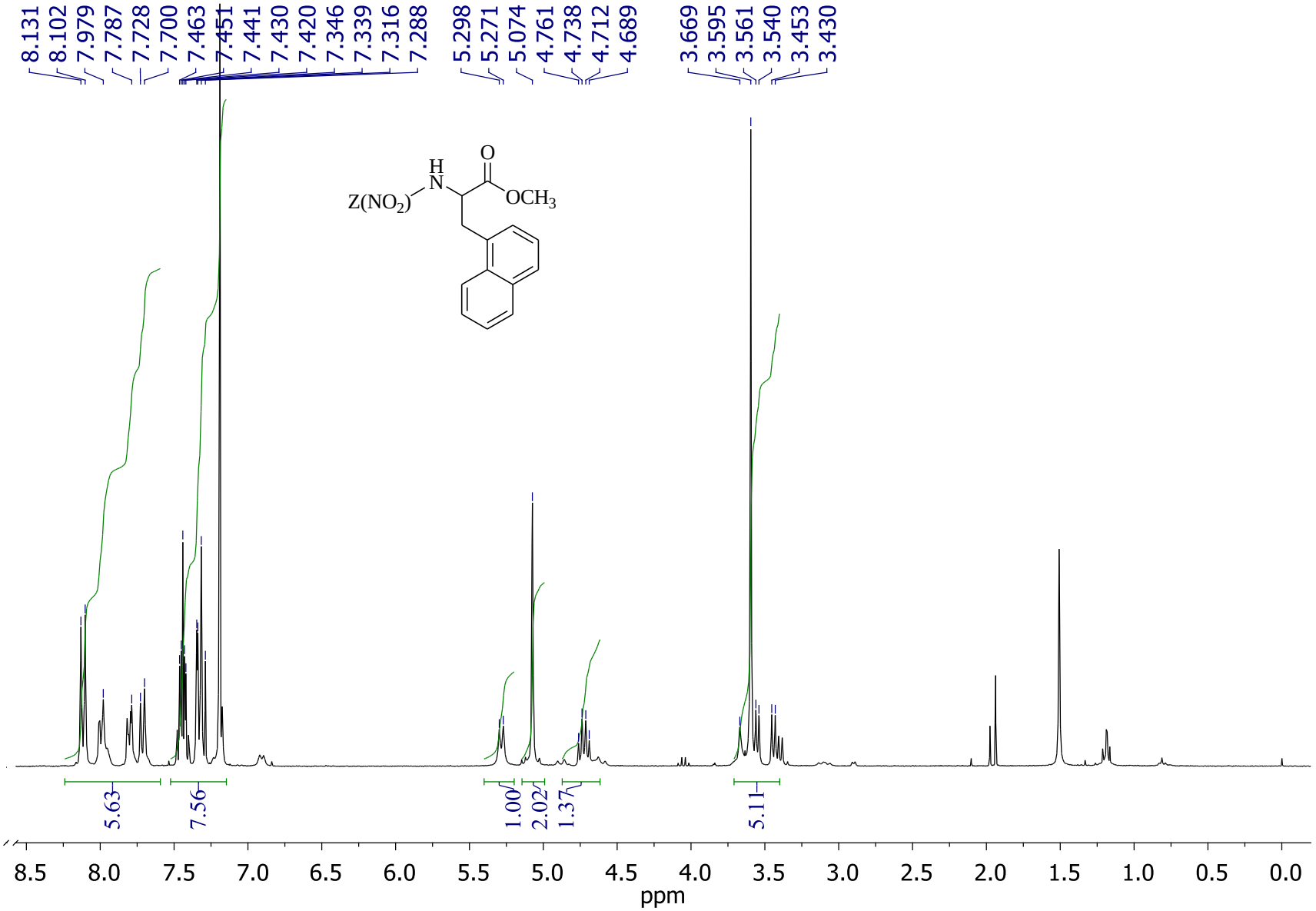

170.97
151.47
131.98
131.61
131.06
130.04
128.79
128.28
126.90
126.66
126.39
123.98
123.33
122.32
82.88
58.62
52.47
33.56
27.53
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

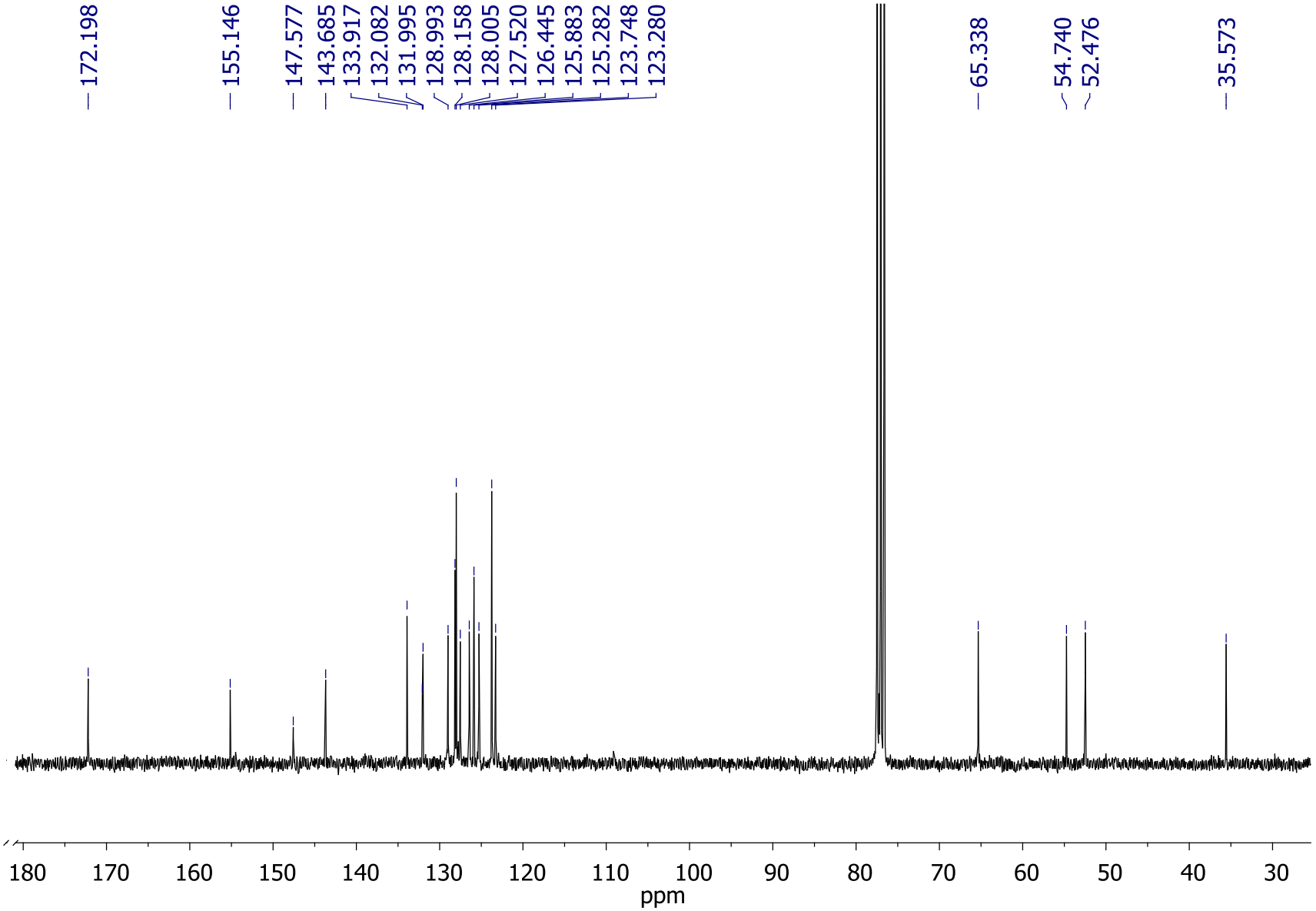

8.23
8.20
8.11
8.08
8.04
8.02
7.95
7.92
7.92
7.79
7.76
5.34
5.32
5.30
5.29
4.22
4.21
4.17
4.16
3.92
3.89
3.88
3.84
3.76
1.02
Boc
O
N
Boc
OCH3
10.26
1.00
1.04
1.00
2.92
16.60
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm

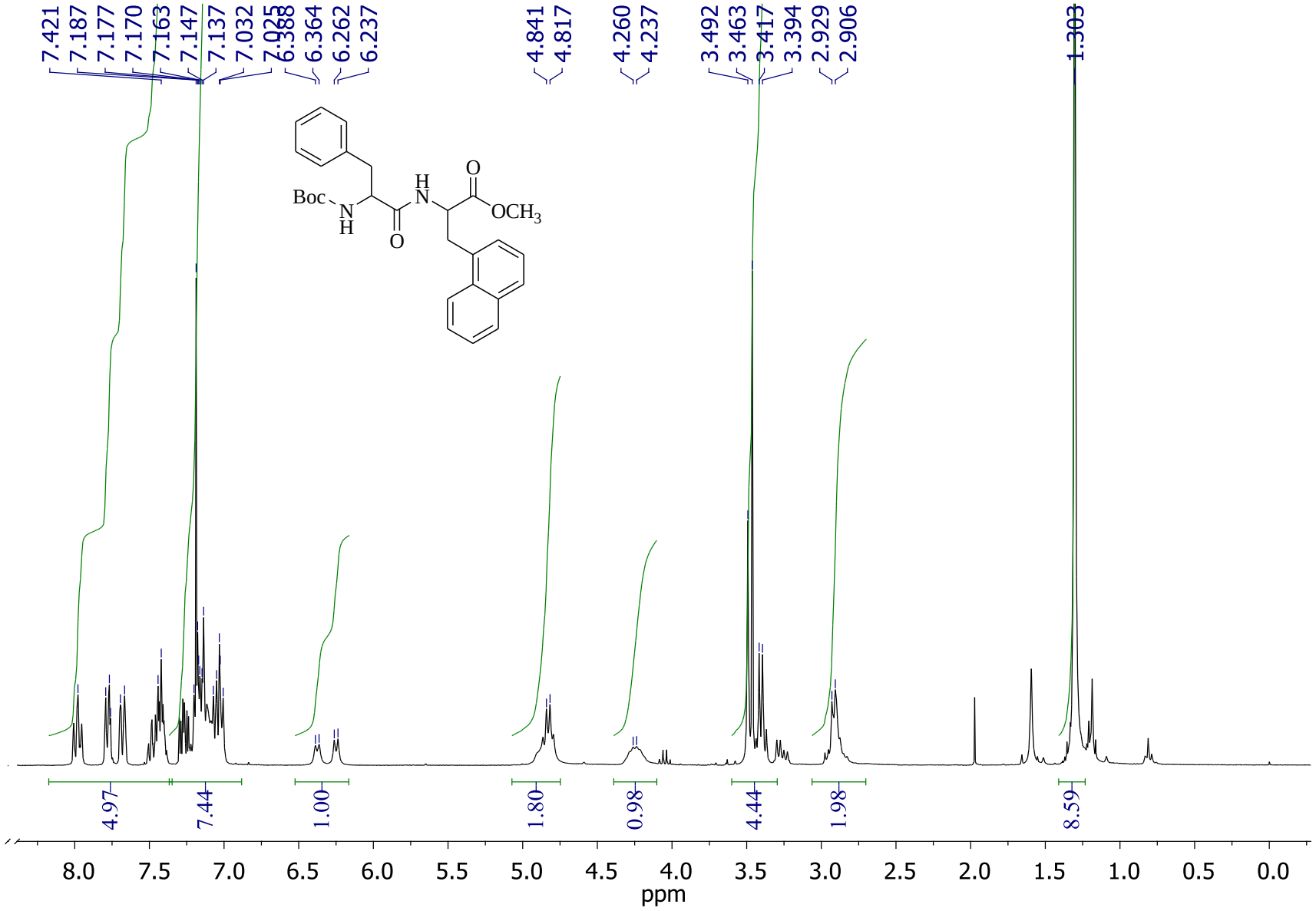

170.931
151.535
131.881
131.304
130.846
130.464
129.521
128.632
127.680
127.441
126.984
125.844
125.027
124.897
124.836
123.126
82.868
59.852
52.459
33.423
27.501
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

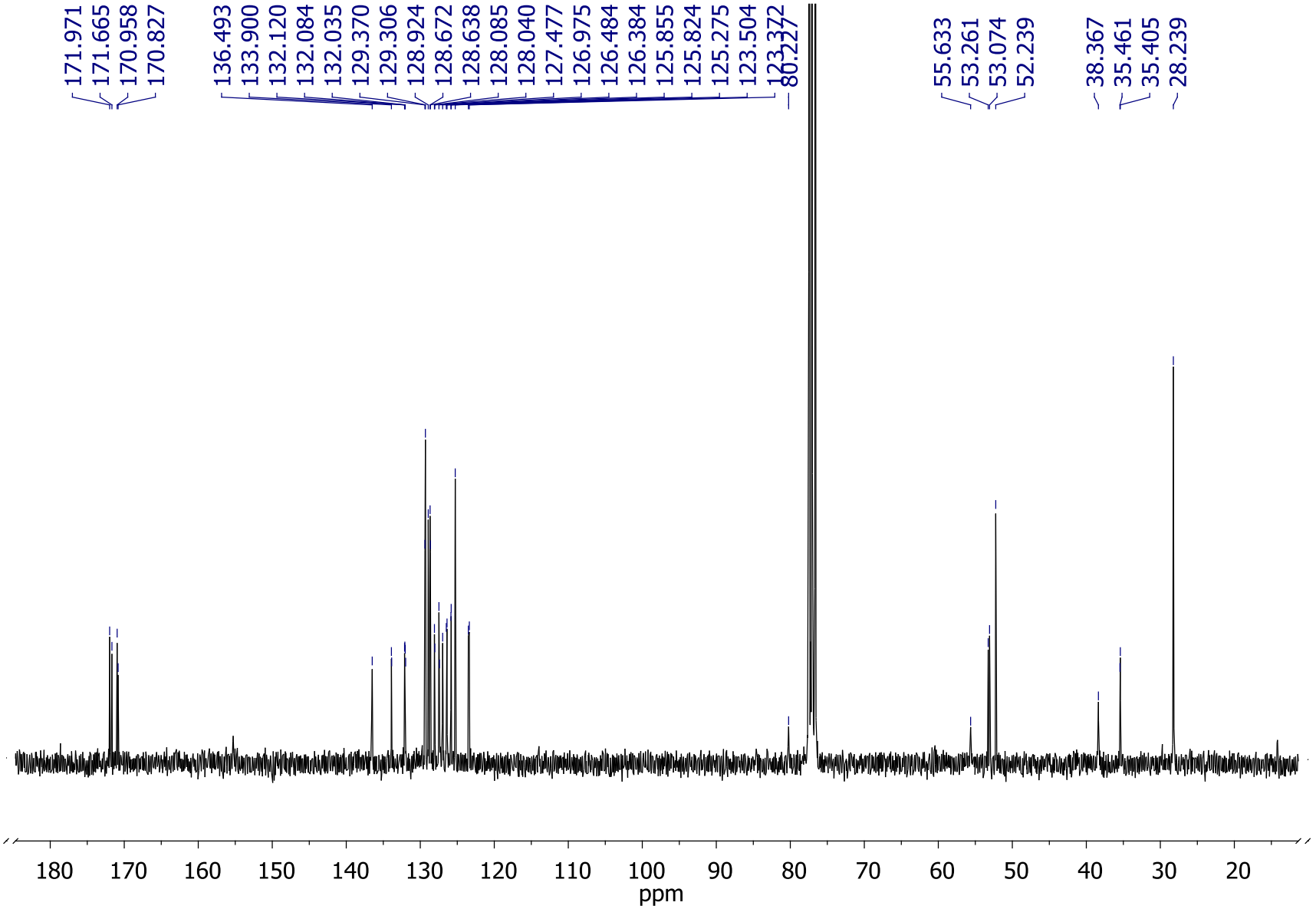

8.067
8.038
7.422
7.409
7.396
7.191
7.154
7.149
7.120
5.309
5.296
5.287
5.272
5.259
5.006
4.961
4.895
4.850
4.043
4.030
3.995
3.982
3.717
3.633
3.568
3.532
3.520
3.483
1.168
Boc
O
N
Z(NO2)
OCH3
4.76
2.82
3.47
1.12
1.91
1.00
2.77
1.05
8.15
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm

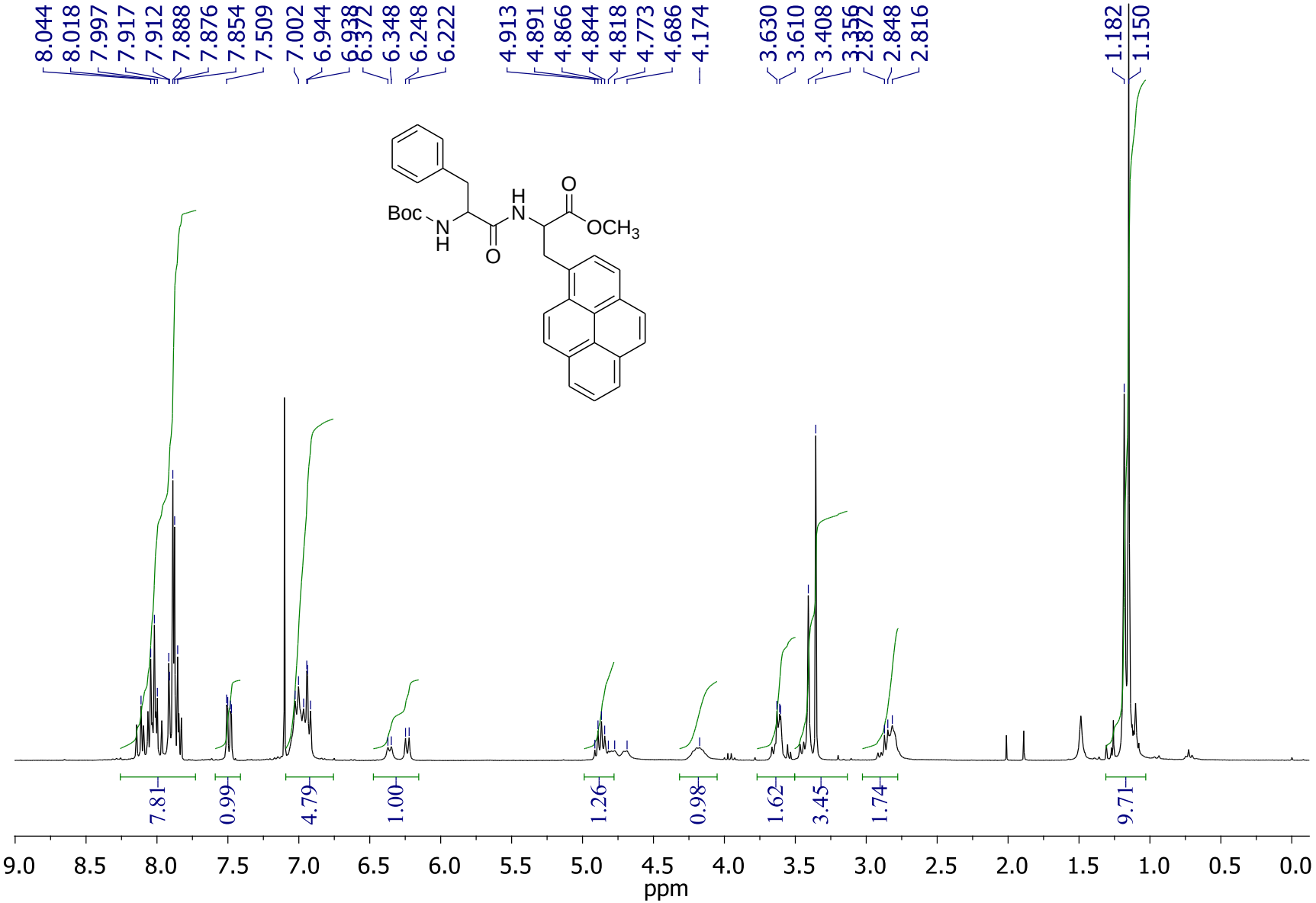

170.289
153.203
150.402
142.369
133.816
133.368
128.973
128.052
127.708
126.360
125.758
125.457
123.672
123.159
84.003
66.884
59.489
52.653
32.794
27.576
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

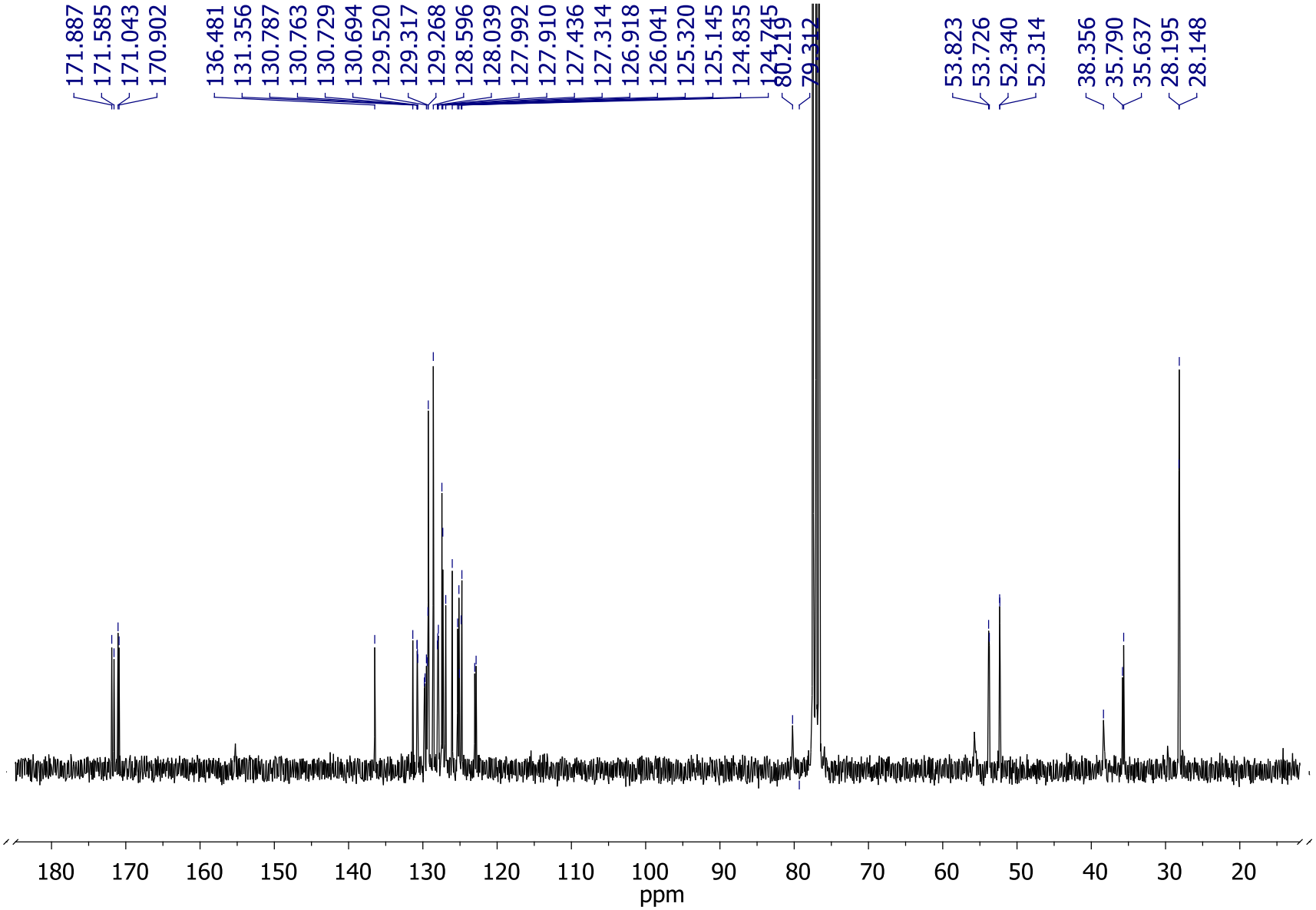

8.131
8.102
7.979
7.787
7.728
7.700
7.463
7.451
7.441
7.430
7.420
7.346
7.339
7.316
7.288
5.298
5.271
5.074
4.761
4.738
4.712
4.689
3.669
3.595
3.561
3.540
3.453
3.430
H
N
O
Z(NO2)
OCH3
5.63
7.56
1.00
2.02
1.37
5.11
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm

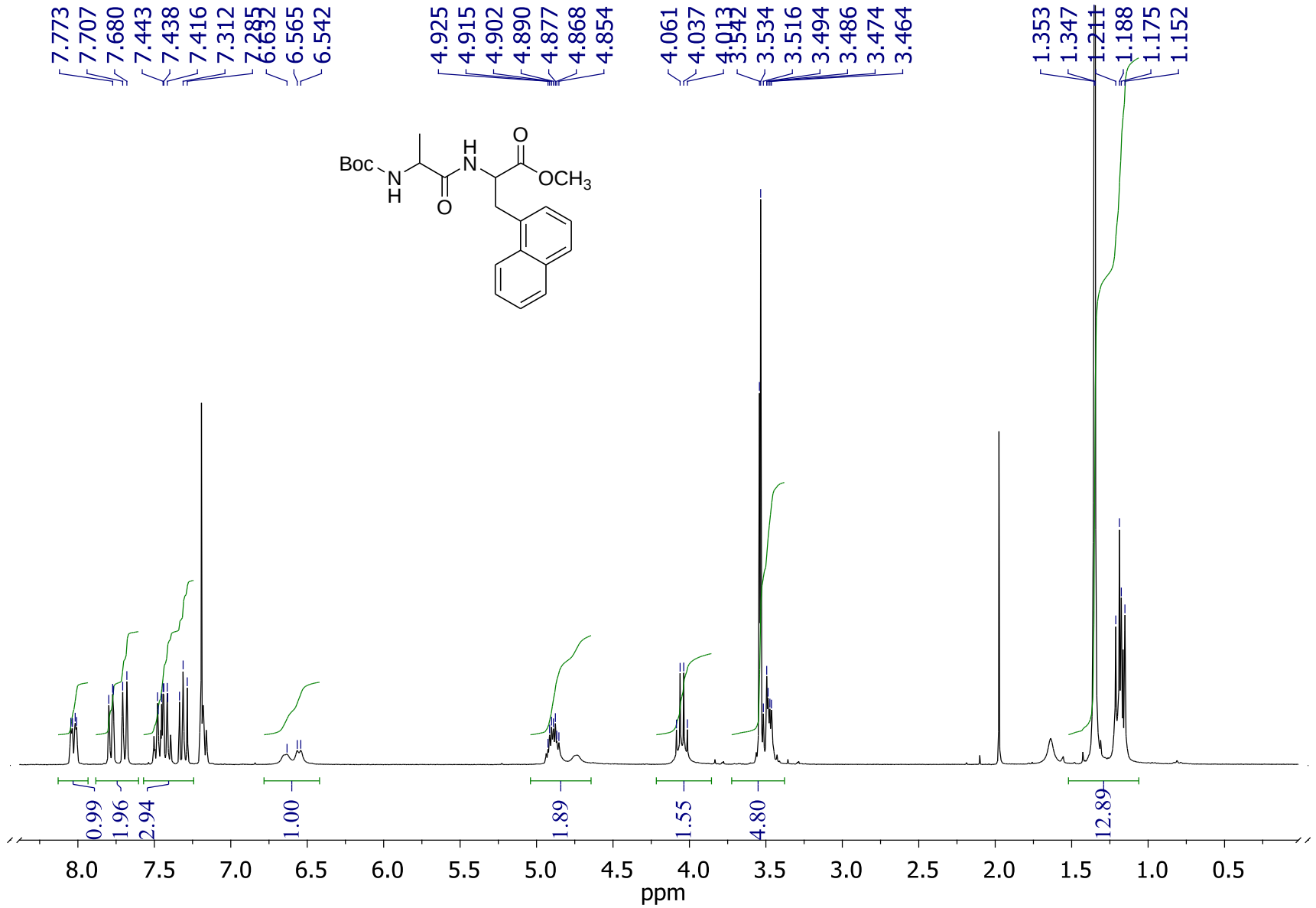

172.198
155.146
147.577
143.685
133.917
132.082
131.995
128.993
128.158
128.005
127.520
126.445
125.883
125.282
123.748
123.280
65.338
54.740
52.476
35.573
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm

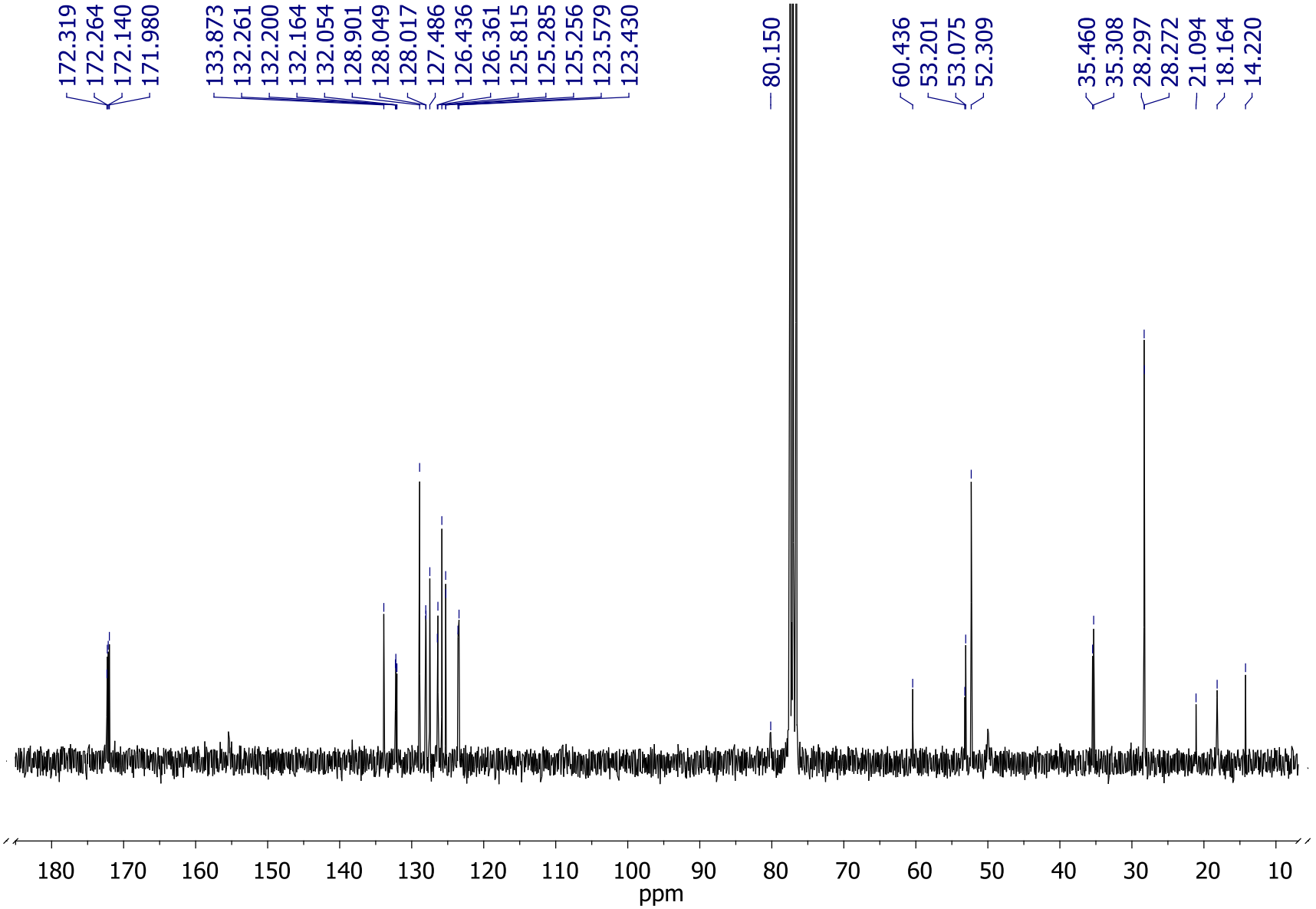

7.421
7.187
7.177
7.170
7.163
7.147
7.137
7.032
7.025
6.388
6.364
6.262
6.237
4.841
4.817
4.260
4.237
3.492
3.463
3.417
3.394
2.929
2.906
1.303
Boc
N
H
H
N
O
O
OCH3
4.97
7.44
1.00
1.80
0.98
4.44
1.98
8.59
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm

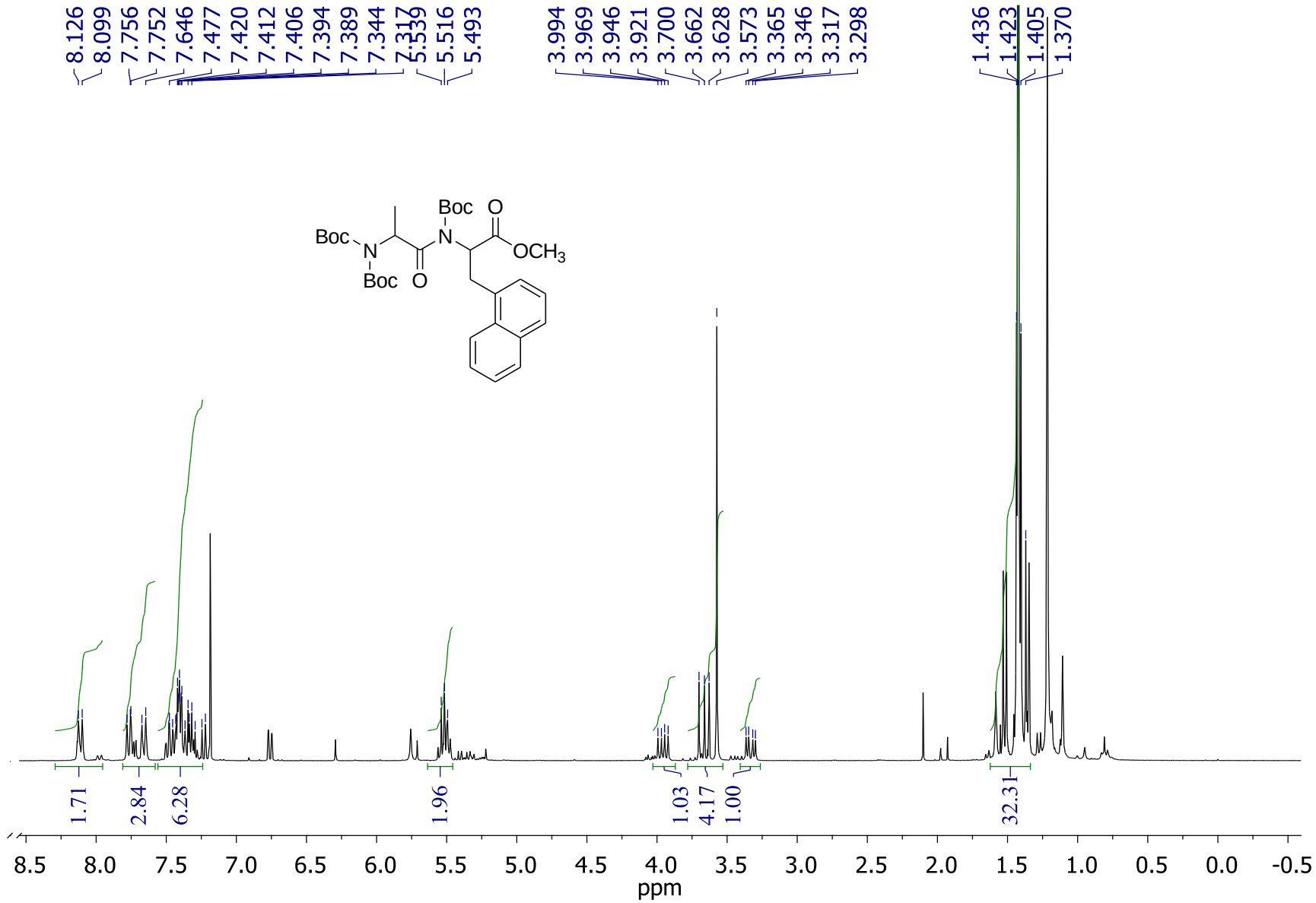

171.971
171.665
170.958
170.827
136.493
133.900
132.120
132.084
132.035
129.370
129.306
128.924
128.672
128.638
128.085
128.040
127.477
126.975
126.484
126.384
125.855
125.824
125.275
123.504
123.372
80.227
55.633
53.261
53.074
52.239
38.367
35.461
35.405
28.239
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

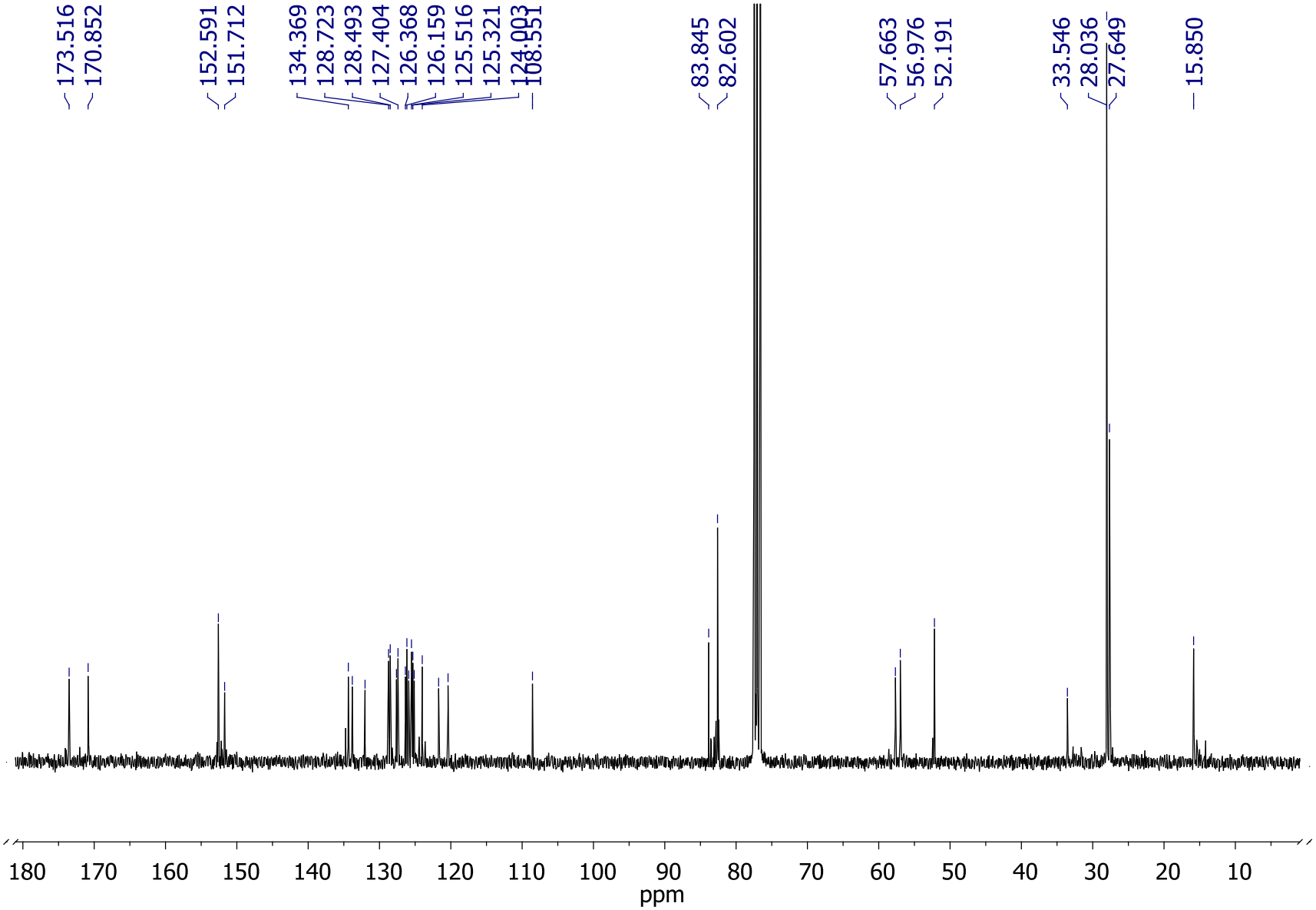

8.044
8.018
7.997
7.917
7.912
7.888
7.876
7.854
7.509
7.002
6.944
6.938
6.372
6.348
6.248
6.222
4.913
4.891
4.866
4.844
4.818
4.773
4.686
4.174
3.630
3.610
3.408
3.356
2.872
2.848
2.816
1.182
1.150
Boc
N
H
O
H
N
O
OCH3
7.81
0.99
4.79
1.00
1.26
0.98
1.62
3.45
1.74
9.71
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm


171.887
171.585
171.043
170.902
136.481
131.356
130.787
130.763
130.729
130.694
129.520
129.317
129.268
128.596
128.039
127.992
127.910
127.436
127.314
126.918
126.041
125.320
125.145
124.835
124.745
80.219
79.312
53.823
53.726
52.340
52.314
38.356
35.790
35.637
28.195
28.148
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm


7.773
7.707
7.680
7.443
7.438
7.416
7.312
7.285
6.632
6.565
6.542
4.925
4.915
4.902
4.890
4.877
4.868
4.854
4.061
4.037
4.013
3.542
3.534
3.516
3.494
3.486
3.474
3.464
1.353
1.347
1.211
1.188
1.175
1.152
Boc
N
H
H
N
O
O
OCH3
0.99
1.96
2.94
1.00
1.89
1.55
4.80
12.89
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm


172.319
172.264
172.140
171.980
133.873
132.261
132.200
132.164
132.054
128.901
128.049
128.017
127.486
126.436
126.361
125.815
125.285
125.256
123.579
123.430
80.150
60.436
53.201
53.075
52.309
35.460
35.308
28.297
28.272
21.094
18.164
14.220
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


8.126
8.099
7.756
7.752
7.646
7.477
7.420
7.412
7.406
7.394
7.389
7.344
7.317
5.539
5.516
5.493
3.994
3.969
3.946
3.921
3.700
3.662
3.628
3.573
3.365
3.346
3.317
3.298
1.436
1.423
1.405
1.370
Boc
N
Boc
O
Boc
O
N
OCH3
1.71
2.84
6.28
1.96
1.03
4.17
1.00
32.31
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
ppm


173.516
170.852
152.591
151.712
134.369
128.723
128.493
127.404
126.368
126.159
125.516
125.321
124.003
108.551
83.845
82.602
57.663
56.976
52.191
33.546
28.036
27.649
15.850
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm