

Supporting Information

Mechanochemical Synthesis of Amides with Uronium-Based Coupling Reagents, a Method for Hexa-amidation of Biotin[6]juril

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General information

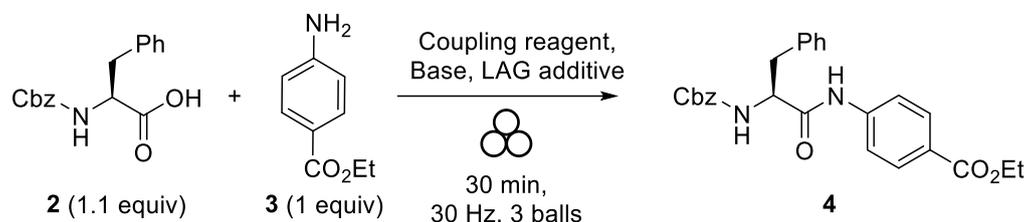
All reagents were purchased from commercial suppliers (Fluorochem, Sigma-Aldrich and Alfa Aesar) and used without further purification. Inorganic salts (e.g. Na_2CO_3 , K_2HPO_4) were dried prior to use by heating under reduced pressure. Mechanochemical experiments were carried out in a FTS-1000 shaker mill at 30 Hz frequency by using 14 mL ZrO_2 -coated grinding jar with 7 mm ZrO_2 milling balls.

Silica gel 40 – 100 μm was used for column chromatography; silica gel 60 F₂₅₄ plates were used for TLC. ^1H NMR (400 MHz), ^{13}C NMR (101 MHz), ^{19}F (376 MHz) and ^{31}P (162 MHz) spectra were recorded on Bruker Avance III spectrometer. Chemical shifts were referenced to residual protio solvent peaks and solvent resonances (δ ^1H 7.26 and δ ^{13}C 77.16 measured in CDCl_3 ; δ ^1H 1.73 and δ ^{13}C 67.57 measured in $\text{THF-}d_8$) as internal standards for ^1H NMR and ^{13}C NMR spectra, respectively. All chemical shifts are reported in ppm units. For ^{31}P NMR spectra, 85% aq. H_3PO_4 was used as an external reference with δ ^{31}P 0.00. For ^{19}F NMR spectra, CFCl_3 was used as an external reference δ ^{19}F 0.00. FT-IR spectra were recorded on a Bruker Tensor 27 FT spectrometer. HPLC determination of enantiomeric excess was performed with Agilent Technologies 1200 series chromatograph using Chiralpak AD-H column (250 x 4.6 mm) and OD-H column (250 x 4.6 mm). Specific rotations were measured using an Anton Paar MCP 500 polarimeter. HRMS data was obtained on Agilent Technologies 6540 UHD Accurate-Mass Q-TOF/LC-MS Spectrometer using AJS-ESI method in positive ion detection mode. HPLC separation and analysis were carried out on Agilent 1200 Series HPLC system equipped with a multiple wavelength detector (MWD) and a single quadrupole mass detector (MSD). Melting points were determined with Stuart SMP40 apparatus.

1. Optimization Studies

1.1. Amide coupling of Cbz-protected L-phenylalanine **2** and benzocaine **3**

Amide coupling of Cbz-protected L-phenylalanine (**2**) and ethyl 4-aminobenzoate (benzocaine **3**) was selected as a model process.



General procedure: Benzocaine **3** (0.182 mmol, 1 equiv.), Cbz-protected L-phenylalanine **2** (0.200 mmol, 1.1 equiv.), Coupling reagent (0.200 mmol, 1.1 equiv.) and Base (0.55–1.82 mmol, 3–10 equiv.) were placed into a 14 mL ZrO₂-coated jar charged with 3×7 mm ZrO₂ balls. Then LAG additive was added to the jar, which was then set to mill at 30 Hz for 10–60 minutes. The resulting crude reaction mixture was analysed by ¹H NMR using CDCl₃ as a solvent, after separation of insoluble inorganic material.

Table S1. Optimization studies on the amide coupling of compounds **2** and **3** (extended data set)

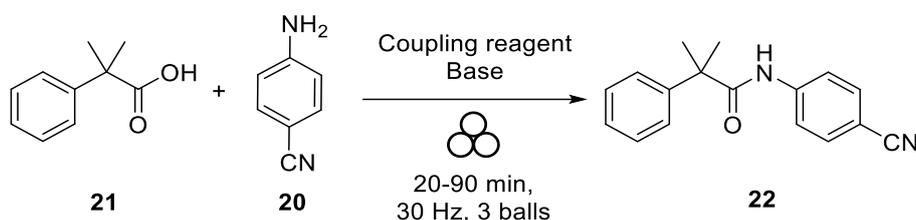
Entry	Coupling agent	Base	LAG (μl/mg)	Conversion by ¹ H NMR, % ^a		
				10 min	30 min	1 hr
1	Effect of LAG					
1.1	COMU (1.1 equiv.)	Na ₂ CO ₃ (10 equiv.)	EtOAc, η = 0	38	44	-
1.2	COMU (1.1 equiv.)	Na ₂ CO ₃ (10 equiv.)	EtOAc, η = 0.14	62	75	-
1.3	COMU (1.1 equiv.)	Na ₂ CO ₃ (2 equiv.)	EtOAc, η = 0.24	-	72	-
1.4	COMU (1.1 equiv.)	Na ₂ CO ₃ (10 equiv.)	EtOAc, η = 0.40	63	63	63
1.5	COMU (1.1 equiv.)	Na ₂ CO ₃ (10 equiv.)	EtOAc, η = 0.82	-	63	-
2	Effect of Base					
2.1	COMU (1.1 equiv.)	DIPEA (2 equiv.) ^b	DIPEA, η = 0.11	-	72	-
2.2	COMU (1.1 equiv.)	Na ₂ CO ₃ (10 equiv.)	EtOAc, η = 0.14	62	75	-
2.3	COMU (1.1 equiv.)	K ₂ CO ₃ (7.6 equiv.)	EtOAc, η = 0.40	41	41	41
2.4	COMU (1.1 equiv.)	K ₃ PO ₄ (5 equiv.)	EtOAc, η = 0.14	-	72	-
2.5	COMU (1.1 equiv.)	K ₂ HPO ₄ (6 equiv.)	EtOAc, η = 0.14	-	96	-
2.6	COMU (1.1 equiv.)	K₂HPO₄ (3 equiv.)	EtOAc, η = 0.19	93	96	96
2.7	COMU (1.1 equiv.)	KH ₂ PO ₄ (6 equiv.)	EtOAc, η = 0.15	-	18	-
2.8	COMU (1.1 equiv.)	Na ₂ HPO ₄ (3 equiv.)	EtOAc, η = 0.19	-	33	-
2.9	COMU (1.1 equiv.)	Na ₂ HPO ₄ (3.7 equiv.)	EtOAc, η = 0.19	-	37	-
2.10	COMU (1.1 equiv.)	K ₄ P ₂ O ₇ (3 equiv.)	EtOAc, η = 0.19	-	88	-
2.11	COMU (1.1 equiv.)	Na ₄ P ₂ O ₇ (3 equiv.)	EtOAc, η = 0.19	-	44	-
3	Effect of Coupling Reagent					
3.1	COMU (1.1 equiv.)	K ₂ HPO ₄ (3 equiv.)	EtOAc, η = 0.19	93	96	96

3.2	TCFH (1.1 equiv.)	K ₂ HPO ₄ (3 equiv.)	EtOAc, $\eta = 0.19$	97	97	-
3.3	HATU (1.1 equiv.)	K ₂ HPO ₄ (3 equiv.)	EtOAc, $\eta = 0.19$	-	67	-
3.4	EDC (1.1 equiv.)	K ₂ HPO ₄ (3 equiv.)	EtOAc, $\eta = 0.19$	-	63	-
3.5	EDC	-	MeNO ₂ , $\eta = 0.25$	-	87 ^c	-
3.6	EDC	DMAP (2 equiv.)	MeNO ₂ , $\eta = 0.25$	-	32 ^c	-

^a Conversion of amine **3** (as limiting reagent) into amide **4**, determined by ¹H NMR (characteristic signals of aromatic protons from starting benzocaine **3** at δ 6.63 and product **4** at δ 7.93 were integrated and recalculated into conversion). ^b NaCl (18 equiv.) was used as grinding auxiliary. ^c Following the published procedure.¹

1.2. Challenging Amide Bond Formation

1.2.1. Amide coupling of 4-aminobenzonitrile **20** with 2-methyl-2-phenylpropanoic acid **21**.



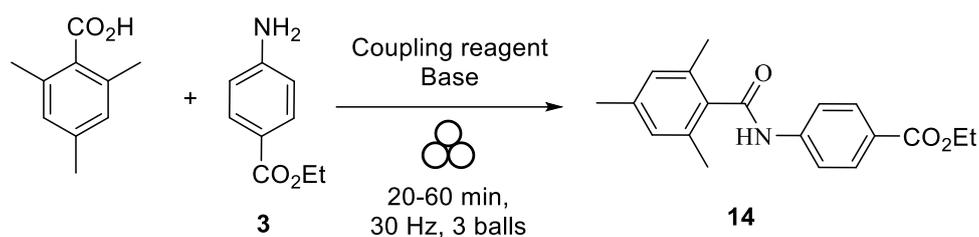
General procedure: 4-aminobenzonitrile **20** (0.200 mmol, 1 equiv.), 2-methyl-2-phenylpropanoic acid **21** (0.200 mmol, 1 equiv.), Coupling reagent (0.220–0.260 mmol, 1.1–1.3 equiv.) and Base (0.600–1.200 mmol, 3–6 equiv.) were placed into a 14 mL ZrO₂-coated jar charged with 3×7 mm ZrO₂ balls. Then LAG additive was added into the jar if required (Table S2, entries 1–4), which was then set to mill at 30 Hz for 1 hour. The resulting crude reaction mixture was analysed by ¹H NMR using CDCl₃ as a solvent, after separation of insoluble inorganic material.

Table S2. Optimization studies on the amide coupling of compounds **20** and **21**

Entry	Coupling agent	Base	LAG (μ l/mg)	Conversion by ¹ H NMR, % ^b		
				20-30 min	1 hr	1.5 hrs
1	EDC (1 equiv.) ^a	-	MeNO ₂ , $\eta = 0.25$	-	9	9
2	COMU (1.1 equiv.)	K ₂ HPO ₄ (3 equiv.)	EtOAc, $\eta = 0.19$	6.5	10	-
3	TCFH (1.1 equiv.)	K ₂ HPO ₄ (3 equiv.)	EtOAc, $\eta = 0.19$	8.2	22	-
4	TCFH (1.1 equiv.)	K ₂ HPO ₄ (6 equiv.)	EtOAc, $\eta = 0.13$	-	24	-
5	TCFH (1.1 equiv.)	NMI (3 equiv.)	NMI, $\eta = 0.40$	74	79	-
6	TCFH (1.3 equiv.)	NMI (3 equiv.)	NMI, $\eta = 0.37$	-	84	88
7	TCFH (1.3 equiv.)	NMI (3 equiv.) ^c	NMI, $\eta = 0.35$	-	80	82

^a Following the published procedure. ^b Conversion of **20** into **22**, determined by ¹H NMR (characteristic signals of aromatic protons from starting 4-aminobenzonitrile **20** at δ 6.64 and product **22** at δ 7.54/ δ 7.48 were integrated and recalculated into conversion). ^c 1.3 equiv. of amine **20** was used.

1.2.2. Amide coupling of mesitoic acid with benzocaine **3**.



General procedure: Benzocaine **3** (0.400–0.440 mmol, 1–1.1 equiv.), mesitoic acid (0.400 mmol, 1 equiv.), Coupling reagent (0.440–0.520 mmol, 1.1–1.3 equiv.) and Base (1.201 mmol, 3 equiv.) were placed into a 14 mL ZrO₂-coated jar charged with 3×7 mm ZrO₂ balls. Then LAG additive was added to the jar if required (Table S3, entries 1–4), which was then set to mill at 30 Hz for 20–60 minutes. The resulting crude reaction mixture was analysed by ¹H NMR using CDCl₃ as a solvent, after separation of insoluble inorganic material.

Table S3. Optimization studies on the amide coupling of mesitoic acid with benzocaine **3**

Entry	Coupling agent	Base	LAG (μl/mg)	Conversion by ¹ H NMR, % ^a		
				20 min	40 min	60 min
1	COMU (1.1 equiv.)	K ₂ HPO ₄ (3 equiv.)	EtOAc, η = 0.19	22	22	24
2	TCFH (1.1 equiv.)	K ₂ HPO ₄ (3 equiv.)	EtOAc, η = 0.19	90	92	-
3	TCFH (1.3 equiv.)	K ₂ HPO ₄ (3 equiv.)	EtOAc, η = 0.19	95	-	99
4	TCFH (1.3 equiv.)	K ₂ HPO ₄ (3 equiv.) ^b	EtOAc, η = 0.19	91	-	93
5	TCFH (1.3 equiv.)	NMI (3 equiv.) ^b	NMI, η = 0.36	18	-	-
6	TCFH (1.3 equiv.)	NMI (3 equiv.) ^b	NMI, η = 0.38	18	-	18

^aConversion of **3** into **14** was determined by ¹H NMR (characteristic signals of aromatic protons from starting benzocaine **3** at δ 6.64 and product **14** at δ 7.54-7.48 were integrated and recalculated into conversion).

^b1.1 equiv. of amine **3** was used.

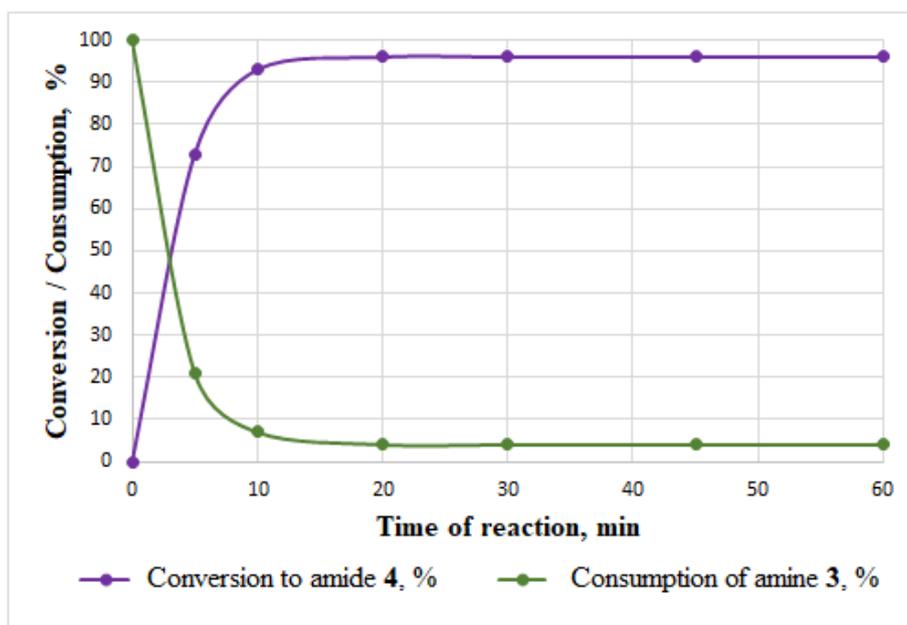
2. Kinetics

2.1. Mechanochemistry

Benzocaine **3** (0.182 mmol, 1 equiv., 30 mg), Cbz-protected L-phenylalanine **2** (0.200 mmol, 1.1 equiv., 60 mg), COMU (0.200 mmol, 1.1 equiv., 86 mg) and K₂HPO₄ (0.545 mmol, 3 equiv., 95 mg) were placed into a 14 mL ZrO₂-coated jar charged with 3×7 mm ZrO₂ balls. Then ethyl acetate as a LAG was added into the jar, which was then set to mill at 30 Hz. Samples of the crude reaction mixture were taken in 5, 10, 20, 30, 45 and 60 minutes. Conversion of **3** into **4** was determined by ¹H NMR spectra (characteristic signals of aromatic protons from starting benzocaine **3** at δ 6.63 and product **4** at δ 7.93 were integrated and recalculated into conversion) (Table S4, Figure S1).

Table S4. Conversion data, mechanochemistry

Time of reaction, min	Conversion by ¹ H NMR, %
5	73
10	93
20	96
30	96
45	96
60	96

**Figure S1.** Accumulation of amide **4** (violet) and consumption of starting amine **3** (green) over time.

2.2. Kinetics in solution

A solution of benzocaine **3** (0.088 mmol, 1 equiv., 14.5 mg), Cbz-protected L-phenylalanine **2** (0.097 mmol, 1.1 equiv., 28.9 mg) and DIPEA (0.176 mmol, 2 equiv., 30.5 μ l) in DMF-*d*₇ (0.5 mL) was transferred into the NMR tube. Then a solution of COMU (0.097 mmol, 1.1 equiv., 41.4 mg) in DMF-*d*₇ (0.2 mL) was added and the reaction progress was monitored by ¹H NMR. Conversion of **3** into **4** was determined (characteristic signals of aromatic protons from starting benzocaine **3** at δ 7.73/ δ 6.72 and product **4** at δ 7.99/ δ 7.87 were integrated and recalculated into conversion) (Table S5, Figure S2).

Table S5. Conversion data in the solution of DMF-*d*₇

Time of reaction, hrs	Conversion by ¹ H NMR, %
0.083	4.3
0.250	13.6
0.417	21.4
0.583	26.7

0.750	31.1
0.917	36.3
1.083	39.6
1.250	43.6
1.417	46.2
1.583	48.4
2.583	56.2
3.583	60.5
4.583	63.3
5.583	65.4
18.583	69.6
28.583	70.2
42.583	70.9
62.583	71.1
66.583	71.1

Since COMU is hydrolytically unstable in DMF solution,^{2,3} one more experiment was carried out to monitor decomposition of the coupling reagent. A solution of COMU (41.4 mg) in DMF-*d*₇ (0.7 mL) was transferred into the NMR tube and degradation of the reagent was monitored by ¹H NMR by following the growing signals of *N,N*-dimethylmorpholine-4-carboxamide product (characteristic signal at δ 2.80, see Table S6 and Figure S2).

Table S6. Degradation of COMU in DMF-*d*₇ over time

Time , hrs	COMU degradation by ¹H NMR, %
0.333	96.6
0.833	92.0
1.833	88.5
2.833	85.0
6.500	78.3
7.833	77.4
11.833	72.4
16.833	71.7
21.833	71.7

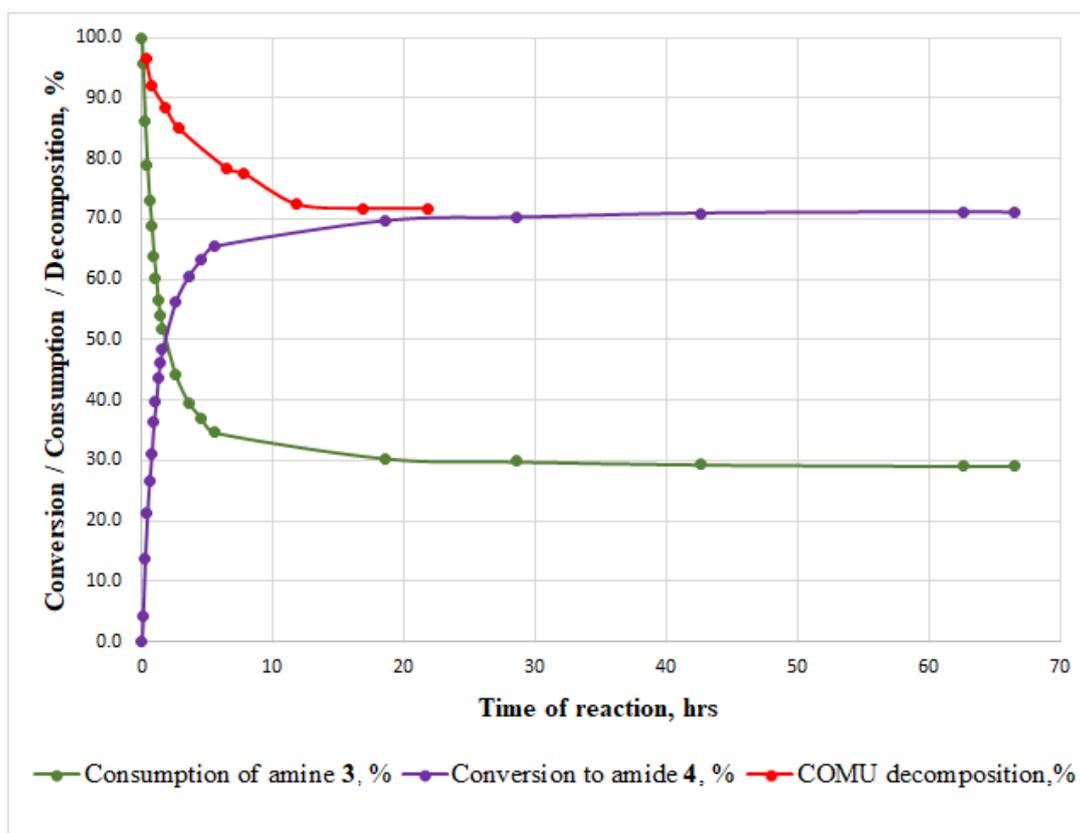
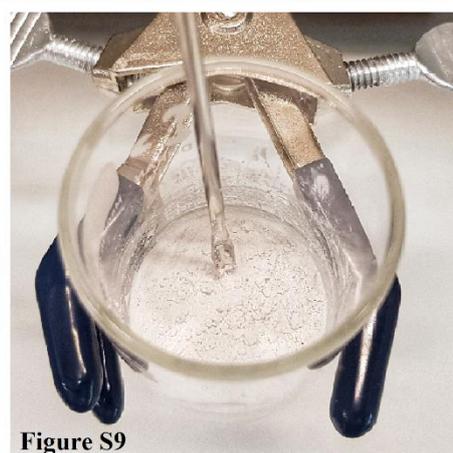
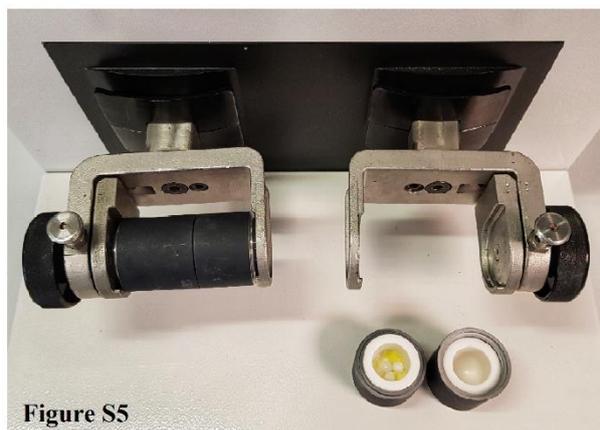


Figure S2. Accumulation of amide **4** (violet), consumption of starting amine **3** (green) and decomposition of COMU (red) over time.

3. Optimized procedure for the amide coupling of Cbz-protected L-phenylalanine **2** and benzocaine **3**

Benzocaine **3** (0.363 mmol, 60.0 mg, 1 equiv.), Cbz-protected L-phenylalanine **2** (0.363 mmol, 108.7 mg, 1 equiv.), COMU (0.400 mmol, 171.0 mg, 1.1 equiv.) and K_2HPO_4 (1.090 mmol, 190.0 mg, 3 equiv.) were placed into a 14 mL ZrO_2 -coated jar charged with 3×7 mm ZrO_2 balls (Figure S3). Then 100 μ l of ethyl acetate (LAG, $\eta = 0.19 \mu$ l mg^{-1}) was added into the jar (Figure S4), which was then set to mill at 30 Hz for 20 minutes (Figure S5). The resulting crude reaction mixture (Figure S6) was transferred to the filter (Figure S7) and washed with distilled water ($3-5 \times 10$ mL; Figures S8, S9). The filtered product was then dried in air to afford amide **4** in 96% yield. 1H NMR and HPLC of the crude product were recorded (Figures S10 and S11), evidencing high purity of the obtained product (98.6% purity based on HPLC peak area). See page S11 below for the full set of characterization data.



Figures S3–S9. General procedure for the amide coupling of Cbz-protected L-phenylalanine **2** and benzocaine **3**.

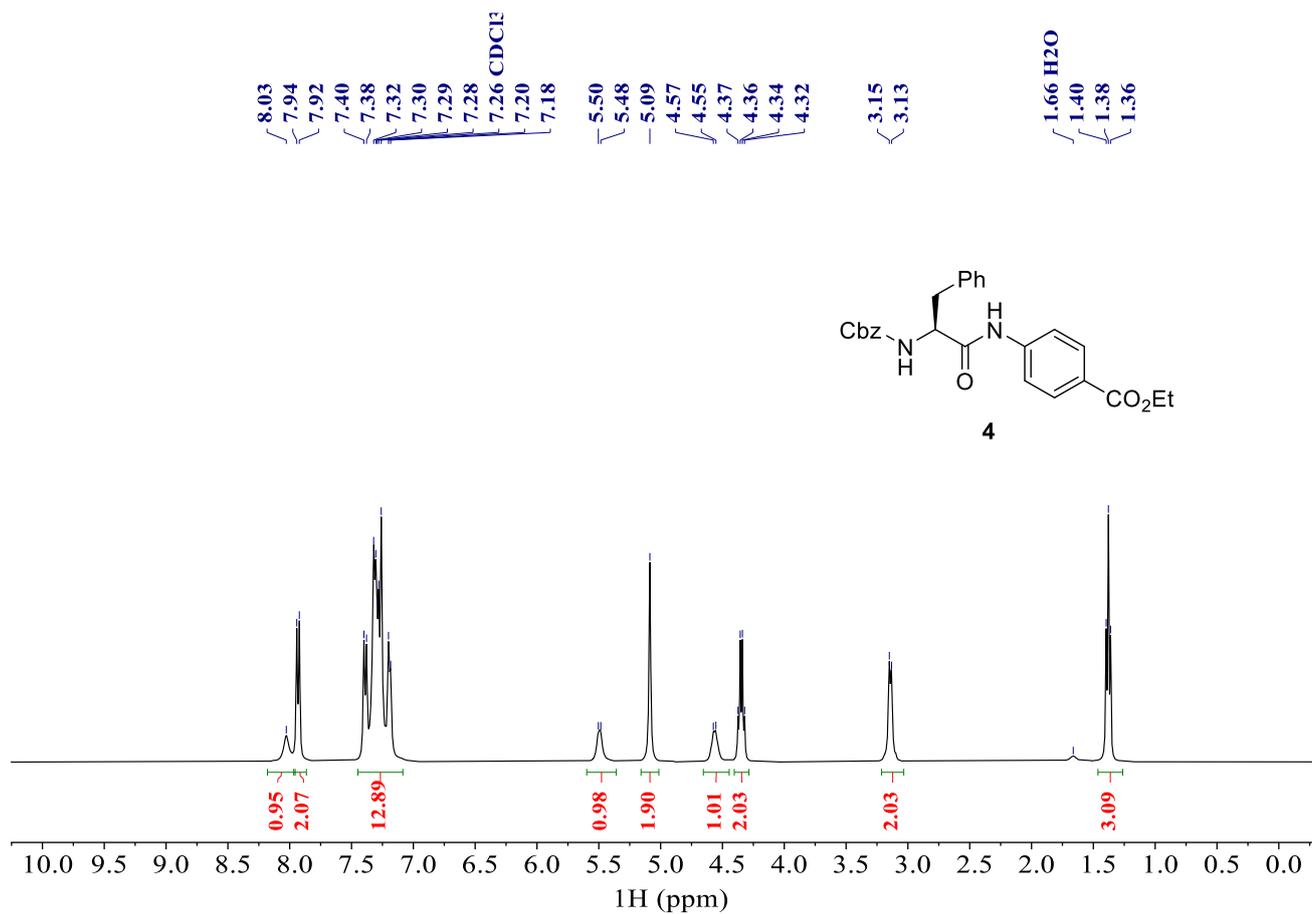


Figure S10. ¹H NMR of the crude product **4** after washing with distilled water.

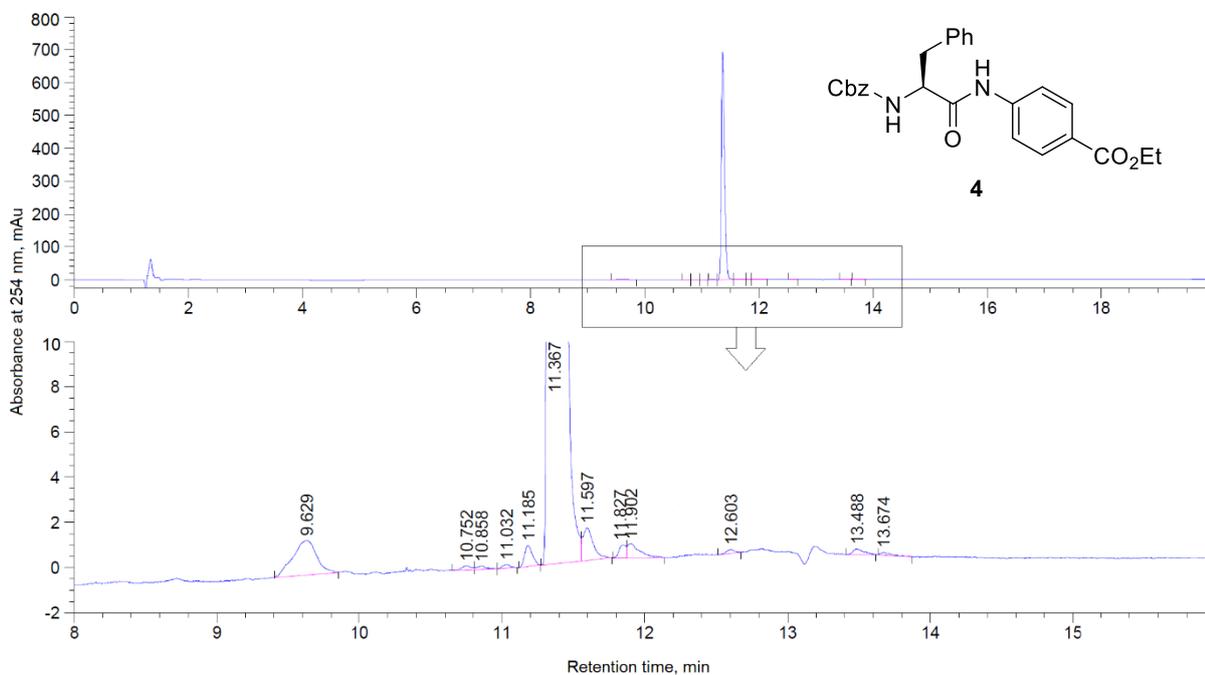


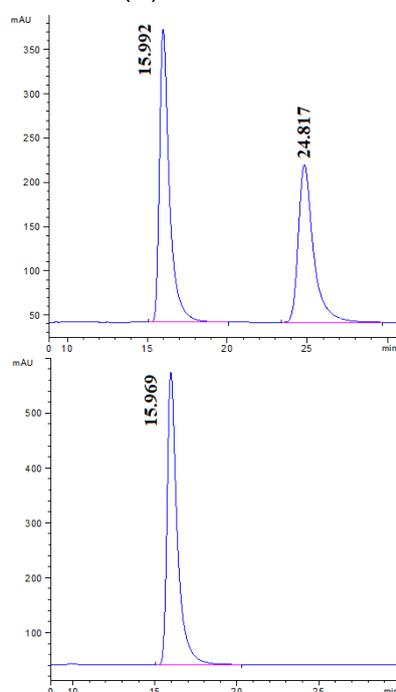
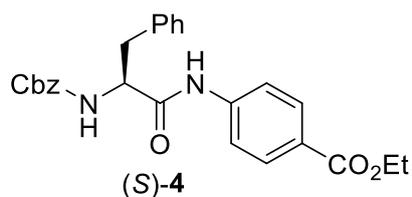
Figure S11. HPLC chromatogram of amide **4** after washing with distilled water, HPLC purity 98.6% (based on peak area percentage). MN Nucleoshell RP18 column, 150 mm×3.0 mm, 2.7 μm, eluents A (H₂O) and B (CH₃CN) in a 10-minute gradient mode from A:B 90:10 to A:B 10:90, injection volume 2 μl.

4. Experimental protocols and characterization of products

4.1. Synthesis of amides with COMU/K₂HPO₄

General procedure A. Amine (1 equiv.), acid (1 equiv.), COMU (1.1 equiv.) and K₂HPO₄ (3 equiv.) were placed into a 14 mL ZrO₂-coated jar charged with 3×7 mm ZrO₂ balls. Then ethyl acetate ($\eta = 0.19 \mu\text{l mg}^{-1}$) was added into the jar, which was then set to mill at 30 Hz for 20 minutes. The resulting crude reaction mixture was transferred to the glass filter and washed with distilled water (3–5×10 mL). The obtained solid amide product was then dried in air.

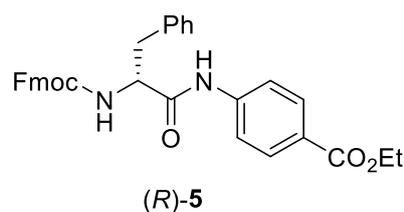
Ethyl (*S*)-4-(2-(((benzyloxy)carbonyl)amino)-3-phenylpropanamido) benzoate **4**.



Prepared by following **general procedure A** from ethyl 4-aminobenzoate (0.363 mmol, 60.0 mg, 1 equiv.), Cbz-protected L-phenylalanine (0.363 mmol, 108.7 mg, 1 equiv.), COMU (0.400 mmol, 171.0 mg, 1.1 equiv.), K₂HPO₄ (1.090 mmol, 190.0 mg, 3 equiv.) and ethyl acetate ($\eta = 0.19 \mu\text{l mg}^{-1}$). Amide (*S*)-**4** was obtained as white solid (156 mg, 96%). Analytically pure sample was prepared by recrystallization (petroleum ether/ethyl acetate = 3:1). (*R*)-**4** enantiomer was obtained analogously from Cbz-protected D-phenylalanine. $R_f = 0.48$ (2:1 petroleum ether/ ethyl acetate, UV 254 nm). mp = 160.7–161.2 °C. ¹H NMR (CDCl₃, 400 MHz): δ 8.03 (s, 1H), 7.93 (d, $J = 8.3$ Hz, 2H), 7.44 – 7.14 (m, 12H), 5.59–5.40 (m, 1H), 5.09 (s, 2H), 4.66 – 4.48 (m, 1H), 4.35 (q, $J = 7.0$ Hz, 2H), 3.14 (d, $J = 6.6$ Hz, 2H), 1.38 (t, $J = 7.0$ Hz, 3H). ¹³C NMR (CDCl₃, 100.6 MHz): δ 169.64, 166.17, 156.54, 141.35, 136.23, 135.93, 130.79, 129.40, 129.05, 128.75, 128.52, 128.15, 127.44, 126.39, 119.18, 67.58, 61.02, 57.34, 38.46, 14.47. $[\alpha]_D^{25} = -11.4$ (c 0.33, CHCl₃). IR (KBr): $\nu = 3325, 3285, 3032, 2986, 1712, 1695, 1667, 1524, 1281, 1259, 1177, 769, 742 \text{ cm}^{-1}$. HRMS (AJS-ESI) calcd. for C₂₆H₂₆N₂O₅Na⁺ [M+Na]⁺ 469.1734, found m/z 469.1737. Enantiomeric excess was determined by HPLC [AD-H column (7:3 *n*-hexane/2-propanol), flow rate 0.8

mL·min⁻¹, detection at 210 nm]: major (*S*)-**4** $t_R = 16.0$ min, minor (*R*)-**4** $t_R = 24.8$ min (see HPLC signals provided). No (*R*)-**4** enantiomer was detected.

Ethyl (*R*)-4-(2-(((9H-fluoren-9-yl) methoxy) carbonyl) amino)-3-phenylpropanamido) benzoate **5**.

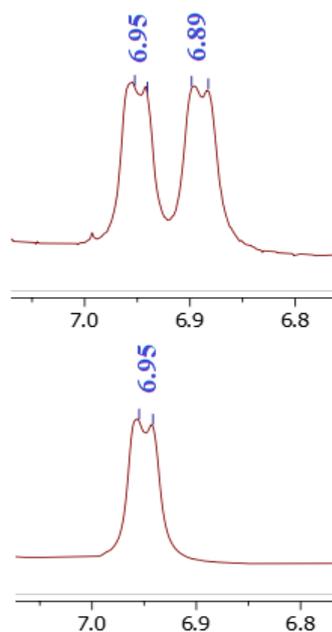
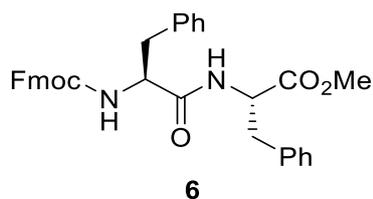


Prepared following **general procedure A** from ethyl 4-aminobenzoate (0.400 mmol, 66.0 mg, 1 equiv.), Fmoc-D-phenylalanine (0.400 mmol, 155.0 mg, 1 equiv.), COMU (0.440 mmol, 189.0 mg, 1.1 equiv.), K₂HPO₄ (1.200 mmol, 209.0 mg, 3 equiv.) and ethyl acetate ($\eta = 0.19 \mu\text{l mg}^{-1}$). Amide **5** was obtained as white solid (203 mg, 95% yield).

Analytically pure sample was prepared by recrystallization (ethyl acetate). $R_f = 0.24$ (2:1 petroleum ether/ ethyl acetate, UV 254 nm). mp = 209.1–212.8 °C. ¹H NMR (400 MHz, THF-

d_8): δ 9.42 (s, 1H), 7.97 – 7.87 (m, 2H), 7.77 (d, $J = 7.5$ Hz, 2H), 7.68 – 7.57 (m, 4H), 7.34 (t, $J = 7.5$ Hz, 2H), 7.30 – 7.10 (m, 8H), 4.48 (q, $J = 7.6$ Hz, 1H), 4.42 – 4.22 (m, 4H), 4.18 (t, $J = 7.0$ Hz, 1H), 3.16 (dd, $J = 13.5, 7.3$ Hz, 1H), 2.97 (dd, $J = 13.5, 7.3$ Hz, 1H), 1.34 (t, $J = 7.1$ Hz, 3H). ^{13}C NMR (100.6 MHz, THF- d_8): δ 171.25, 166.13, 157.10, 145.24, 144.18, 142.29, 142.27, 138.54, 131.08, 130.24, 129.07, 128.34, 127.78, 127.33, 126.08, 126.00, 120.63, 119.46, 67.16 (overlapped with solvent peak), 61.11, 58.22, 48.27, 39.39, 14.73. $[\alpha]_{\text{D}}^{25} = -36.7$ (c 0.43, DMSO). IR (KBr): $\nu = 3331, 3296, 1711, 1687, 1669, 1596, 1525, 1450, 1256, 1176, 1105, 1034$ cm^{-1} . HRMS (AJS-ESI) calcd. for $\text{C}_{33}\text{H}_{31}\text{N}_2\text{O}_5^+$ $[\text{M}+\text{H}]^+$ 535.2227, found m/z 535.2220.

Methyl (((9H-fluoren-9-yl) methoxy) carbonyl)-L-phenyl-alanyl-L-phenylalaninate **6**.

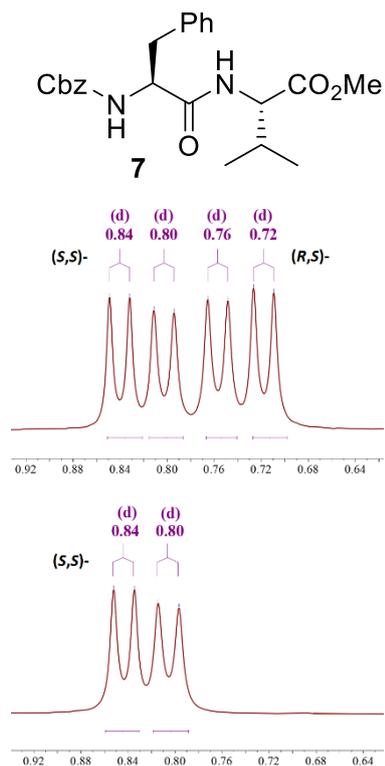


^1H NMR fragment for (*S,S*)- and (*R,S*)-**6**

Prepared following **general procedure A** from L-phenylalanine Me-ester hydrochloride (0.400 mmol, 86.3 mg, 1 equiv.), Fmoc-L-phenylalanine (0.400 mmol, 155.0 mg, 1 equiv.), COMU (0.440 mmol, 189.0 mg, 1.1 equiv.), K_2HPO_4 (1.200 mmol, 209.0 mg, 3 equiv.) and ethyl acetate ($\eta = 0.19$ $\mu\text{l mg}^{-1}$). Amide **6** was obtained as white solid (220 mg, 94%). Analytically pure sample was prepared by recrystallization (petroleum ether/ethyl acetate = 2:1). (*R,S*)-**6** diastereomer was obtained analogously from Fmoc-D-phenylalanine. $R_f = 0.40$ (2:1 petroleum ether/ethyl acetate, UV 254 nm). mp = 174.1–174.7 $^\circ\text{C}$; lit. mp = 171–173 $^\circ\text{C}$.⁴ ^1H NMR (CDCl_3 , 400 MHz): δ 7.76 (d, $J = 7.5$ Hz, 2H), 7.52 (t, $J = 6.4$ Hz, 2H), 7.40 (t, $J = 7.4$ Hz, 2H), 7.34 – 7.22 (m, 5H), 7.22 – 7.10 (m, 5H), 7.00 – 6.90 (m, 2H), 6.30 – 6.16 (m, 1H), 5.35 – 5.23 (m, 1H), 4.77 (q, $J = 6.1$ Hz, 1H), 4.47 – 4.35 (m, 2H), 4.33 – 4.23 (m, 1H), 4.17 (t, $J = 6.9$ Hz, 1H), 3.66 (s, 3H), 3.18 – 2.90 (m, 4H). ^{13}C NMR (CDCl_3 , 100.6 MHz): δ 171.40, 170.41, 155.92, 143.86, 143.83, 141.42, 136.32, 135.62, 129.52, 129.29, 128.85, 128.68, 127.88, 127.28, 127.24, 127.21, 125.24, 125.14, 120.13, 67.27, 56.08, 53.45, 52.46, 47.21, 38.48, 38.01. $[\alpha]_{\text{D}}^{25} = +22.8$ (c 0.37, CHCl_3). IR (KBr): $\nu = 3301, 3030, 2951, 1737, 1698, 1653, 1536, 1445, 1288, 1257, 1034, 740$ cm^{-1} . HRMS (AJS-ESI) calcd. for $\text{C}_{34}\text{H}_{33}\text{N}_2\text{O}_5^+$ $[\text{M}+\text{H}]^+$ 549.2384, found m/z 549.2382. The diastereomeric ratio was determined by NMR: major (*S,S*)-**6** doublet at 6.95 ppm, minor (*R,S*)-**6** doublet at 6.89 ppm (see ^1H NMR spectra fragment provided). Spectral data are in agreement with previously reported.^{4,5}

No (*R,S*)-**6** diastereomer was detected.

Methyl ((benzyloxy)carbonyl)-L-phenylalanyl-L-valinate **7**.

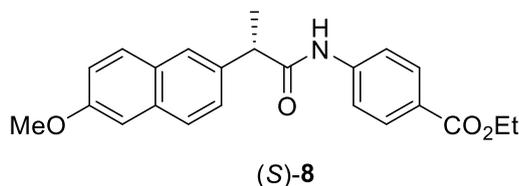
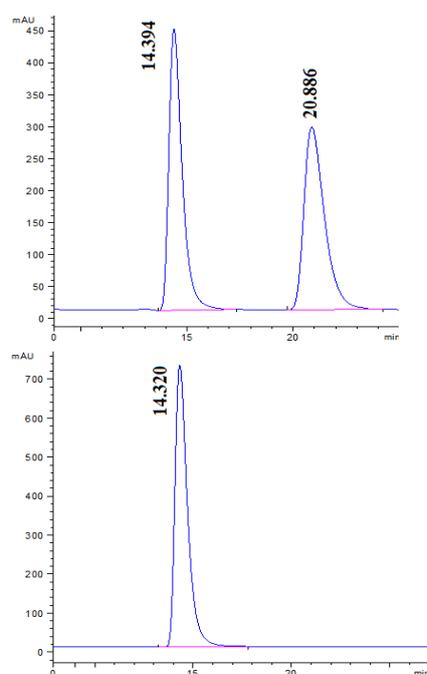


¹H NMR fragment for (*S,S*)- and (*R,S*)-**7**

ppm, minor (*R,S*)-**7** two doublets at 0.72 and 0.76 ppm (see ¹H NMR fragments provided). Spectral data are in agreement with previously reported.^{4,6} No (*R,S*)-**7** diastereomer was detected.

Prepared following **general procedure A** from L-valine Me-ester hydrochloride (0.400 mmol, 119.7 mg, 1 equiv.), Cbz-L-phenylalanine (0.400 mmol, 119.7 mg, 1 equiv.), COMU (0.440 mmol, 189.0 mg, 1.1 equiv.), K₂HPO₄ (1.200 mmol, 209.0 mg, 3 equiv.) and ethyl acetate ($\eta = 0.19 \mu\text{l mg}^{-1}$). Amide **7** was obtained as white solid (158 mg, 96%). Analytically pure sample was prepared by recrystallization (petroleum ether/ ethyl acetate = 3:1). (*R,S*)-**7** diastereomer was obtained analogously from Cbz-D-phenylalanine. $R_f = 0.43$ (2:1 petroleum ether/ethyl acetate, UV 254 nm). mp = 114.2–115.5 °C; lit. mp = 103–105 °C.⁴ ¹H NMR (CDCl₃, 400 MHz): δ 7.45 – 7.13 (m, 10H), 6.46 – 6.17 (m, 1H), 5.47 – 5.29 (m, 1H), 5.16 – 5.00 (m, 2H), 4.55 – 4.39 (m, 2H), 3.69 (s, 3H), 3.15 – 3.00 (m, 2H), 2.16 – 1.96 (m, 1H), 0.84 (d, $J = 6.8$ Hz, 3H), 0.80 (d, $J = 6.8$ Hz, 3H). ¹³C NMR (CDCl₃, 100.6 MHz): δ 171.84, 170.93, 156.08, 136.37, 136.24, 129.45, 128.83, 128.66, 128.33, 128.16, 127.16, 67.23, 57.44, 56.36, 52.26, 38.43, 31.33, 18.91, 17.89. $[\alpha]_D^{25} = -16.3$ (c 0.56, MeOH). IR (KBr): $\nu = 3333, 3299, 3086, 3034, 2957, 2928, 1742, 1683, 1656, 1529, 1438, 1286, 1243, 1134, 1024, 737 \text{ cm}^{-1}$. HRMS (AJS-ESI) calcd. for C₂₃H₂₉N₂O₅⁺ [M+H]⁺ 413.2071, found m/z 413.2070. The diastereomeric ratio was determined by NMR: major (*S,S*)-**7** two doublets at 0.80 and 0.84

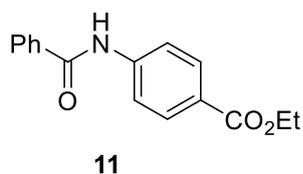
Ethyl (*S*)-4-(2-(6-methoxynaphthalen-2-yl) propanamido) benzoate **8**.



Prepared following **general procedure A** from (*S*)-2-(6-Methoxy-2-naphthyl) propionic acid (0.400 mmol, 92.1 mg, 1 equiv.), ethyl 4-aminobenzoate (0.400 mmol, 66.0 mg, 1 equiv.), COMU (0.440 mmol, 189.0 mg, 1.1 equiv.), K₂HPO₄ (1.200 mmol, 209.0 mg, 3 equiv.) and ethyl acetate ($\eta = 0.19 \mu\text{l mg}^{-1}$). Amide **8** was obtained as pale-coloured solid (130 mg, 86%). Analytically pure sample was prepared by recrystallization (petroleum ether/ethyl acetate = 3:1). Racemic mixture was obtained analogously from racemic 2-(6-Methoxy-2-naphthyl) propionic acid. $R_f = 0.48$ (2:1 petroleum ether/ethyl acetate, UV 254 nm). mp = 166.2 – 167.5 °C. ¹H NMR (CDCl₃, 400 MHz): δ 7.93 (d, $J = 8.7$ Hz, 2H), 7.79 – 7.67 (m, 3H), 7.49 (d, $J = 8.7$ Hz, 2H), 7.45 – 7.34 (m, 2H), 7.21 – 7.10 (m, 2H), 4.32 (q, $J = 7.1$ Hz, 2H), 3.92 (s, 3H), 3.86 (q, $J = 7.1$ Hz, 1H), 1.66 (d, $J = 7.1$ Hz, 3H), 1.36 (t, $J = 7.1$ Hz, 3H). ¹³C NMR (CDCl₃, 100.6 MHz): δ

172.83, 166.24, 158.08, 142.09, 135.68, 134.08, 130.78, 129.36, 129.13, 128.12, 126.51, 126.10, 125.95, 119.56, 118.77, 105.81, 60.97, 55.48, 48.31, 18.64, 14.44. $[\alpha]_D^{25} = +112.9$ (c 0.43, CHCl_3). IR (KBr): $\nu = 3303, 2985, 1714, 1657, 1606, 1531, 1276, 1107, 1028, 767 \text{ cm}^{-1}$. HRMS (AJS-ESI) calcd. for $\text{C}_{23}\text{H}_{24}\text{NO}_4^+$ $[\text{M}+\text{H}]^+$ 378.1700, found m/z 378.1701. Enantiomeric excess was determined by HPLC [OD-H column (9:1 *n*-hexane/2-propanol), flow rate 1 $\text{mL}\cdot\text{min}^{-1}$, detection at 210 nm]: major (*S*)-**8** $t_R = 14.3$ min, minor (*R*)-**8** $t_R = 20.9$ min (see HPLC signals provided). No (*R*)-**8** enantiomer was detected.

Ethyl 4-benzamidobenzoate **11**.



Prepared following **general procedure A** from ethyl 4-aminobenzoate (0.400 mmol, 66.0 mg, 1 equiv.), benzoic acid (0.400 mmol, 49.0 mg, 1 equiv.), COMU (0.440 mmol, 189.0 mg, 1.1 equiv.), K_2HPO_4 (1.200 mmol, 209.0 mg, 3 equiv.) and ethyl acetate ($\eta = 0.19 \mu\text{l mg}^{-1}$). Amide **11** was obtained as white solid (100 mg, 93%). Analytically pure sample was prepared by recrystallization (ethyl acetate). $R_f = 0.30$ (8:2 petroleum ether/ethyl acetate, UV 254 nm). mp = 149.4–152.5 °C; lit. mp = 146 °C,¹ 147–148 °C.² $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 8.13 – 7.97 (m, 3H), 7.96 – 7.81 (m, 2H), 7.80 – 7.69 (m, 2H), 7.68 – 7.54 (m, 1H), 7.53 – 7.43 (m, 2H), 4.37 (q, $J = 7.1$ Hz, 2H), 1.40 (t, $J = 7.1$ Hz, 3H). $^{13}\text{C NMR}$ (100.6 MHz, CDCl_3): δ 166.25, 165.93, 142.18, 134.69, 132.35, 131.00, 129.04, 127.21, 126.35, 119.28, 61.06, 14.50. IR (KBr): $\nu = 3332, 2986, 1705, 1657, 1595, 1529, 1410, 1279, 1107 \text{ cm}^{-1}$. HRMS (AJS-ESI) calcd. for $\text{C}_{16}\text{H}_{16}\text{NO}_3^+$ $[\text{M}+\text{H}]^+$ 270.1125, found m/z 270.1119. Spectral data are in agreement with previously reported.⁸⁻¹⁰

5.2. Synthesis of amides with TCFH/ K_2HPO_4

General procedure B. Amine (1 equiv.), acid (1 equiv), TCFH (1.1-1.3 equiv.) and K_2HPO_4 (3 equiv.) were placed into a 14 mL ZrO_2 -coated jar charged with 3×7 mm ZrO_2 balls. Then ethyl acetate ($\eta = 0.19 \mu\text{l mg}^{-1}$) was added to the jar, which was then set to mill at 30 Hz for 20-60 minutes.

Work-up procedure I. The resulting crude reaction mixture was transferred to the glass filter and washed with distilled water ($3-5 \times 10$ mL). The filtered product was then dried in air and, if required, further purified by silica gel chromatography with petroleum ether/ethyl acetate as eluent to give the desired amide product.

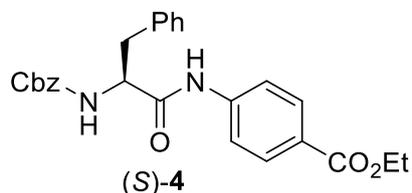
Work-up procedure II. The resulting crude reaction mixture was diluted with ethyl acetate, filtered, and concentrated under reduced pressure before purification by silica gel chromatography with petroleum ether/ethyl acetate as eluent to give the desired product.

Work-up procedure III. The resulting crude reaction mixture was diluted with ethyl acetate (15 mL) and washed with water (3×10 mL). The organic layer was separated, dried over Na_2SO_4 , filtered, and concentrated under reduced pressure before purification by silica gel chromatography with petroleum ether/ethyl acetate as eluent to give the desired product.

Comment: Since the crude amide product **10** appeared as oil after the water treatment, the previously applied isolation protocol (filtration of solid product) became unsuitable. Similar problem was noticed for a few other amides (e.g. **12** and **15**). For such cases, a modified isolation protocols were developed (procedures II and III), involving the initial treatment of the

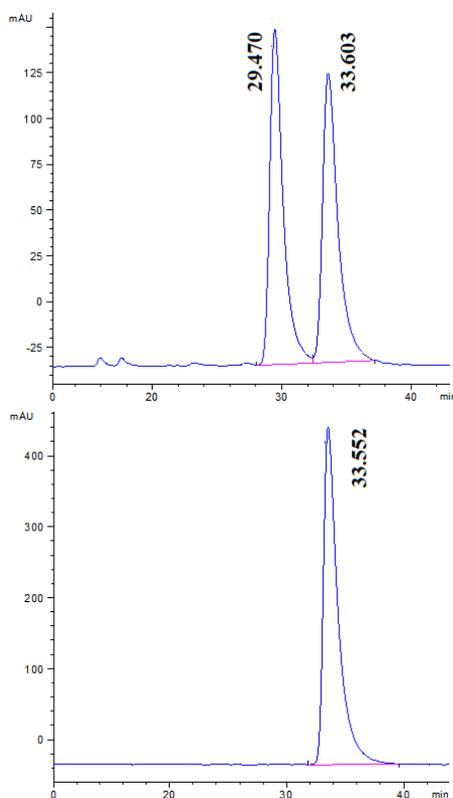
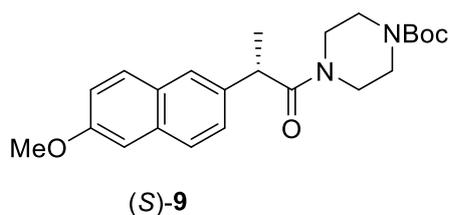
crude reaction mixtures with either ethyl acetate or ethyl acetate-water, followed by silica gel column chromatography. We found that use of TCFH is more advantageous than COMU in these cases, since TCFH produces single and easily removable tetramethylurea by-product. Short column chromatography had to be applied in some cases to remove trace impurities (procedure I), e.g. compound **14** was purified by column chromatography due to the contamination of mesitoic anhydride.

Ethyl (*S*)-4-(2-(((benzyloxy)carbonyl)amino)-3 phenylpropanamido) benzoate **4**.



Prepared by following **general procedure B** and **work-up procedure I** from ethyl 4-aminobenzoate (0.363 mmol, 60.0 mg, 1 equiv.), Cbz-protected L-phenylalanine (0.363 mmol, 108.7 mg, 1 equiv.), TCFH (0.400 mmol, 112.0 mg, 1.1 equiv.), K₂HPO₄ (1.090 mmol, 190.0 mg, 3 equiv.) and ethyl acetate ($\eta = 0.19 \mu\text{l mg}^{-1}$). Amide **4** was obtained as white solid (150 mg, 92%) after filtration and washing with water (3×10 mL). Enantiomeric excess was determined by HPLC [AD-H column (7:3 n-hexane/2-propanol), flow rate 0.8 mL·min⁻¹, detection at 210 nm]: major (*S*)-**4** $t_R = 16.0$ min, minor (*R*)-**4** $t_R = 24.8$ min (see HPLC signals provided). No (*R*)-**4** enantiomer was detected. See above for full characterization data.

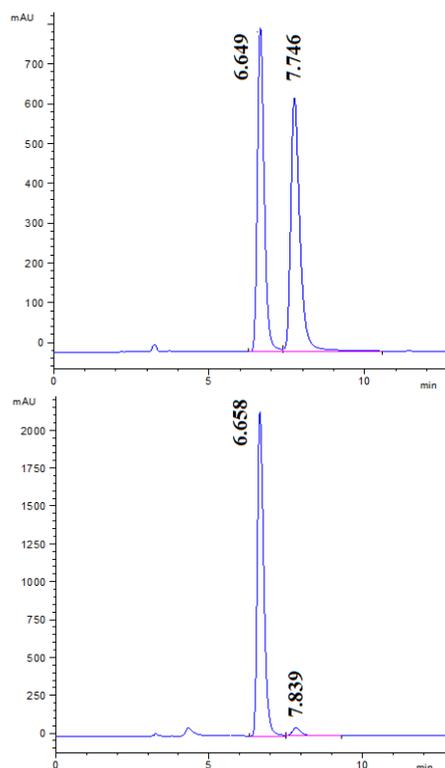
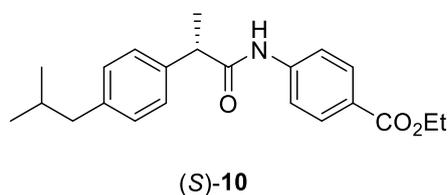
tert-Butyl (*S*)-4-(2-(6-methoxynaphthalen-2-yl) propanoyl) piperazine-1-carboxylate **9**.



Prepared following **general procedure B** and **work-up procedure I** from (*S*)-2-(6-methoxy-2-naphthyl) propionic acid (0.400 mmol, 92.1 mg, 1 equiv.), *N*-Boc-piperazine (0.400 mmol, 75.0 mg, 1 equiv.), TCFH (0.440 mmol, 123.5 mg, 1.1 equiv.), K₂HPO₄ (1.200 mmol, 209.0 mg, 3 equiv.) and ethyl acetate ($\eta = 0.19 \mu\text{l mg}^{-1}$), milling time – 20 minutes. Amide **9** was purified by silica gel chromatography with petroleum ether/ethyl acetate (7:3) as eluent and obtained as white solid (138 mg, 87 %). Analytically pure sample was prepared by recrystallization (petroleum ether/ethyl acetate = 3:1). Racemic mixture was obtained analogously from racemic 2-(6-Methoxy-2-naphthyl) propionic acid. $R_f = 0.43$ (1:1 petroleum ether/ethyl acetate, UV 254 nm). mp = 131.3–132.3 °C. ¹H NMR (CDCl₃, 400 MHz): δ 7.73 – 7.65 (m, 2H), 7.61 – 7.57 (m, 1H), 7.33 (dd, $J = 8.5, 1.9$ Hz, 1H), 7.14 (dd, $J = 9.0, 2.5$ Hz, 1H), 7.11 (d, $J = 2.5$ Hz, 1H), 3.97 (q, $J = 6.8$ Hz, 1H), 3.91 (s, 3H), 3.87 – 3.77 (m, 1H), 3.54 – 3.39 (m, 2H), 3.39 – 3.23 (m, 3H), 3.23 – 3.11 (m, 1H), 2.72 – 2.62 (m, 1H), 1.51 (d, $J = 6.8$ Hz, 3H), 1.39 (s, 9H). ¹³C NMR (CDCl₃, 100.6 MHz): δ 172.44, 157.81, 154.53, 137.08, 133.61, 129.25, 129.22, 127.85, 126.03, 125.59, 119.32, 105.76, 80.26, 55.47, 45.47, 43.53, 41.96, 28.45, 20.88. $[\alpha]_D^{25} = +19.1$ (c 0.33, CHCl₃). IR (KBr): $\nu = 3442, 3002, 2978, 2934, 2853, 1693, 1638, 1606, 1432, 1239, 1164, 858$ cm⁻¹. HRMS (AJS-ESI) calcd. for C₂₃H₃₀N₂O₄Na⁺ [M+Na]⁺ 421.2098, found m/z 421.2099.

Enantiomeric excess was determined by HPLC [OD-H column (95:5 *n*-hexane/2-propanol), flow rate 1 mL·min⁻¹, detection at 210 nm]: minor (*R*)-**9** t_R = 29.5 min, major (*S*)-**9** t_R = 33.6 min (see HPLC signals). No (*R*)-**9** enantiomer was observed.

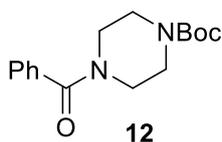
Ethyl (*S*)-4-(2-(4-isobutylphenyl) propanamido) benzoate **10**.



Prepared following **general procedure B** and **work-up procedure III** from (*S*)-2-(4-(2-methylpropyl) phenyl) propanoic acid (0.400 mmol, 82.5 mg, 1 equiv., 98% *ee*), ethyl 4-aminobenzoate (0.400 mmol, 66.0 mg, 1 equiv.), TCFH (0.440 mmol, 123.5 mg, 1.1 equiv.), K₂HPO₄ (1.200 mmol, 209.0 mg, 3 equiv.) and ethyl acetate (η = 0.19 μ l mg⁻¹), milling time – 20 minutes. Amide **10** was purified by silica gel chromatography with petroleum ether/ethyl acetate (9:1) as eluent and obtained as white solid (99 mg, 70%). Analytically pure sample was prepared by recrystallization (petroleum ether/ethyl acetate = 3:1). Racemic mixture was obtained analogously from racemic 2-(4-(2-methylpropyl) phenyl) propanoic acid. R_f = 0.56 (3:1 petroleum ether/thyl acetate, UV 254 nm). mp = 70.8–76.7 °C. ¹H NMR (CDCl₃, 400 MHz): δ 7.95 (d, J = 8.8 Hz, 2H), 7.48 (d, J = 8.8 Hz, 2H), 7.26 (d, J = 8.1 Hz, 2H), 7.17 (d, J = 8.1 Hz, 2H), 7.14 (s, 1H), 4.34 (q, J = 7.1 Hz, 2H), 3.71 (q, J = 7.1 Hz, 1H), 2.48 (d, J = 7.2 Hz, 2H), 1.93 – 1.80 (m, 1H), 1.60 (d, J = 7.2 Hz, 3H), 1.37 (t, J = 7.1 Hz, 3H), 0.91 (d, J = 6.6 Hz, 6H). ¹³C NMR (CDCl₃, 100.6 MHz): δ 172.90, 166.25, 142.11, 141.48, 137.79, 130.82, 130.14, 127.56, 125.97, 118.72, 60.98, 48.06, 45.14, 30.31, 22.51, 18.59, 14.47. $[\alpha]_D^{25}$ = +66.1 (*c* 0.39, CHCl₃). IR (KBr): ν = 3339, 2959, 2868, 1709, 1669, 1520, 1285, 1175, 767 cm⁻¹. HRMS (AJS-ESI) calcd. for C₂₂H₂₈NO₃⁺ [M+H]⁺ 354.2064, found m/z 354.2063.

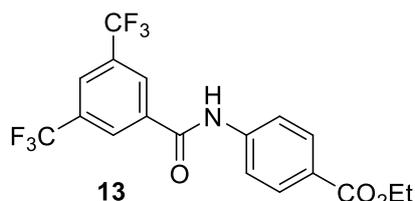
Enantiomeric excess (*ee* = 93%) was determined by HPLC [OD-H column (9:1 *n*-hexane/2-propanol), flow rate 1 mL·min⁻¹, detection at 210 nm]: major (*S*)-**10** t_R = 6.8 min, minor (*R*)-**10** t_R = 7.8 min (see HPLC signals).

tert-Butyl 4-benzoylpiperazine-1-carboxylate **12**.



Prepared following **general procedure B** and **work-up procedure II** from *N*-Boc-piperazine (0.40 mmol, 75.0 mg, 1.0 equiv.), benzoic acid (0.40 mmol, 49.0 mg, 1 equiv.), TCFH (0.44 mmol, 124.0 mg, 1.1 equiv.), K₂HPO₄ (1.21 mmol, 210.0 mg, 3 equiv.) and ethyl acetate (η = 0.19 μ l mg⁻¹), milling time – 20 minutes. Amide **12** was purified by silica gel chromatography with petroleum ether/ethyl acetate (7:3) as eluent and obtained as white solid (102 mg, 87%). R_f = 0.21 (7:3 petroleum ether/ethyl acetate, UV 254 nm). mp = 107.8–108.7 °C. ¹H NMR (400 MHz, CDCl₃) δ 7.52 – 7.29 (m, 5H), 3.91 – 3.60 (br m, 2H), 3.60–3.17 (br m, 6H), 1.46 (s, 9H). ¹³C NMR (100.6 MHz, CDCl₃) δ 170.72, 154.67, 135.58, 130.03, 128.70, 127.15, 80.47, 47.62 (br), 43.90 (br), 42.24 (br), 28.49. IR (KBr): ν = 1694, 1624, 1434, 1245 cm⁻¹. HRMS (AJS-ESI) calcd. for C₁₆H₂₃N₂O₃⁺ [M+H]⁺ 291.1703, found m/z 291.1697.

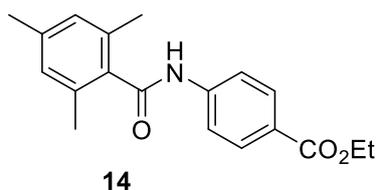
Ethyl 4-(3,5-bis(trifluoromethyl)benzamido)benzoate **13**.



Prepared following **general procedure B** and **work-up procedure I** from ethyl 4-aminobenzoate (0.40 mmol, 66.0 mg, 1.0 equiv.), 3,5-bis(trifluoromethyl)benzoic acid (0.40 mmol, 103.0 mg, 1.0 equiv.), TCFH (0.52 mmol, 146.0 mg, 1.3 equiv.), K_2HPO_4 (1.20 mmol, 209.0 mg, 3 equiv.) and ethyl acetate ($\eta = 0.19 \mu\text{l mg}^{-1}$), milling time – 20 minutes.

Amide **13** was purified by silica gel chromatography with petroleum ether/ethyl acetate (8:2) as eluent and obtained as white solid (155 mg, 96%). Analytically pure sample was prepared by recrystallization (petroleum ether/ethyl acetate = 1:1). $R_f = 0.62$ (8:2 petroleum ether/ethyl acetate, UV 254 nm). mp = 183.0–184.3 °C. $^1\text{H NMR}$ (400 MHz, $CDCl_3$): δ 8.36 – 8.31 (m, 2H), 8.22 (s, 1H), 8.12 – 8.03 (m, 3H), 7.79 – 7.70 (m, 2H), 4.38 (q, $J = 7.1$ Hz, 2H), 1.40 (t, $J = 7.1$ Hz, 3H). $^{13}\text{C NMR}$ (100.6 MHz, $CDCl_3$) δ 166.18, 163.05, 141.28, 136.79, 132.69 (q, $J = 34.1$ Hz), 131.06, 127.63 (d, $J = 3.1$ Hz), 127.21, δ 125.76 (dt, $J = 7.4, 3.6$ Hz), 122.92 (q, $J = 273.2$ Hz), 119.78, 61.26, 14.47. $^{19}\text{F NMR}$ ($CDCl_3$, 376 MHz): $\delta = -62.9$. IR (KBr): $\nu = 3300, 1708, 1669, 1601, 1544, 1281, 1148 \text{ cm}^{-1}$. HRMS (AJS-ESI) calcd. for $C_{18}H_{14}F_6NO_3^+$ $[M+H]^+$ 406.0872, found m/z 406.0865.

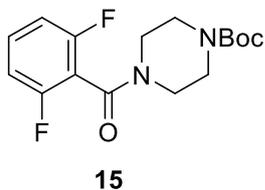
Ethyl 4-(2,4,6-trimethylbenzamido)benzoate **14**.



Prepared following **general procedure B** and **work-up procedure I** from ethyl 4-aminobenzoate (0.40 mmol, 66.0 mg, 1.0 equiv.), 2,4,6-trimethylbenzoic acid (0.40 mmol, 66.0 mg, 1.0 equiv.), TCFH (0.52 mmol, 146.0 mg, 1.3 equiv.), K_2HPO_4 (1.20 mmol, 209.0 mg, 3equiv.) and ethyl acetate ($\eta = 0.19 \mu\text{l mg}^{-1}$), milling time – 1 hour. Amide **14** was

purified by silica gel chromatography with petroleum ether/ethyl acetate (8:2) as eluent and obtained as white solid (113 mg, 89%). Analytically pure sample was prepared by recrystallization (ethyl acetate). $R_f = 0.47$ (8:2 petroleum ether/ethyl acetate, UV 254 nm). mp = 231.1–232.5 °C. $^1\text{H NMR}$ (400 MHz, $CDCl_3$): δ 8.10 – 7.97 (m, 2H), 7.72 – 7.65 (m, 2H), 7.56 (s, 1H), 6.89 (s, 2H), 4.36 (q, $J = 7.1$ Hz, 2H), 2.34 (s, 6H), 2.31 (s, 3H), 1.40 (t, $J = 7.1$ Hz, 3H). $^{13}\text{C NMR}$ (100.6 MHz, $CDCl_3$): δ 169.13, 166.21, 142.04, 139.35, 134.71, 134.41, 131.03, 128.58, 126.38, 118.88, 61.05, 21.28, 19.34, 14.50. IR (KBr): $\nu = 3239, 1713, 1653, 1597, 1537, 1403, 1281 \text{ cm}^{-1}$. HRMS (AJS-ESI) calcd. for $C_{19}H_{22}NO_3^+$ $[M+H]^+$ 312.1594, found m/z 312.1590.

tert-Butyl 4-(2,6-difluorobenzoyl)piperazine-1-carboxylate **15**.

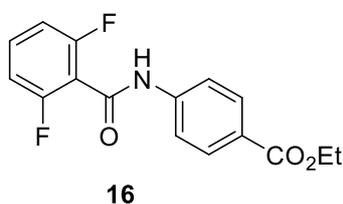


Prepared following **general procedure B** and **work-up procedure II** from *N*-Boc-piperazine (0.40 mmol, 75.0 mg, 1.0 equiv.), benzoic acid (0.40 mmol, 64.0 mg, 1.0 equiv.), TCFH (0.52 mmol, 147.0 mg, 1.3 equiv.), K_2HPO_4 (1.21 mmol, 210.0 mg, 3 equiv.) and ethyl acetate ($\eta = 0.19 \mu\text{l mg}^{-1}$), milling time – 40 minutes. Amide **15** was purified

by silica gel chromatography with petroleum ether/ethyl acetate (7:3) as eluent and obtained as white solid (120 mg, 91%). $R_f = 0.36$ (7:3 petroleum ether/ethyl acetate, UV 254 nm). mp = 105.1–106.6 °C. $^1\text{H NMR}$ (400 MHz, $CDCl_3$): δ 7.41 – 7.30 (m, 1H), 7.00 – 6.89 (m, 2H), 3.82 – 3.74 (m, 2H), 3.56 – 3.48 (m, 2H), 3.48 – 3.36 (m, 2H), 3.32 – 3.25 (m, 2H), 1.45 (s, 9H). $^{13}\text{C NMR}$ (100.6 MHz, $CDCl_3$): δ 160.35, 158.99 (dd, $J = 250.4, 7.8$ Hz), 154.55, 131.40 (t, $J = 9.8$ Hz), 113.56 (t, $J = 22.9$ Hz), 112.49 – 111.50 (m), 80.55,

46.82, 43.72 (br), 41.96, 28.47. ^{19}F NMR (CDCl_3 , 376 MHz): $\delta = -111.9$. IR (KBr): $\nu = 1690$, 1633, 1471, 1421, 1248 cm^{-1} . HRMS (AJS-ESI) calcd. for $\text{C}_{16}\text{H}_{20}\text{F}_2\text{N}_2\text{O}_3\text{Na}^+$ $[\text{M}+\text{Na}]^+$ 349.1334, found m/z 349.1332.

Ethyl 4-(2,6-difluorobenzamido)benzoate **16**.



Prepared following **general procedure B** and **work-up procedure I** from ethyl 4-aminobenzoate (0.40 mmol, 66.0 mg, 1.0 equiv.), 2,6-difluorobenzoic acid (0.40 mmol, 63.0 mg, 1.0 equiv.), TCFH (0.52 mmol, 146.0 mg, 1.3 equiv.), K_2HPO_4 (1.20 mmol, 210.0 mg, 3 equiv.) and ethyl acetate ($\eta = 0.19 \mu\text{l mg}^{-1}$), milling time – 1 hour. Amide **16** was purified by silica gel chromatography with petroleum ether/ethyl acetate (7:3) as eluent and obtained as white solid (109 mg, 88%). Analytically pure sample was prepared by recrystallization (petroleum ether/ethyl acetate = 1:2). $R_f = 0.40$ (7:3 petroleum ether/ethyl acetate, UV 254 nm). mp = 169.4–170.1 $^\circ\text{C}$. ^1H NMR (400 MHz, CDCl_3): δ 8.08 – 8.01 (m, 2H), 7.98 (s, 1H), 7.75 – 7.67 (m, 2H), 7.46 – 7.37 (m, 1H), 7.04 – 6.93 (m, 2H), 4.36 (q, $J = 7.1$ Hz, 2H), 1.39 (t, $J = 7.1$ Hz, 3H). ^{13}C NMR (100.6 MHz, CDCl_3): δ 166.19, 160.15 (dd, $J = 253.4$, 6.6 Hz), 158.64, 141.50, 132.61 (t, $J = 10.4$ Hz), 130.96, 126.80, 119.36, 114.14 (t, $J = 19.3$ Hz), 112.67 – 112.10 (m), 61.12, 14.48. ^{19}F NMR (CDCl_3 , 376 MHz): $\delta = -111.6$. IR (KBr): $\nu = 3315$, 1693, 1596, 1531, 1291 cm^{-1} . HRMS (AJS-ESI) calcd. for $\text{C}_{16}\text{H}_{14}\text{F}_2\text{NO}_3^+$ $[\text{M}+\text{H}]^+$ 306.0936, found m/z 306.0933.

5.3. Challenging Amide Bond Formation with TCFH/NMI

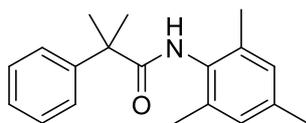
General procedure C. Amine (1 equiv.), acid (1 equiv.), TCFH (1.3 equiv.) and *N*-methyl imidazole (3 equiv.) were placed into a 14 mL ZrO_2 -coated jar charged with 3×7 mm ZrO_2 balls and milled at 30 Hz for 1.5 hours. The resulting crude reaction mixture was transferred to the glass filter and washed with distilled water (3–5 \times 10 ml). The solid product was then dissolved in ethyl acetate (15 mL), filtered through the layer of silica gel and concentrated under reduced pressure. The obtained solid amide product was then dried in air.

N-(4-Cyanophenyl)-2-methyl-2-phenylpropanamide **22**.



Prepared following **general procedure C** from 4-aminobenzonitrile (0.40 mmol, 47.3 mg, 1.0 equiv.), 2-methyl-2-phenylpropanoic acid (0.40 mmol, 65.7 mg, 1.0 equiv.), TCFH (0.52 mmol, 146.0 mg, 1.3 equiv.) and *N*-methyl imidazole (1.20 mmol, 96 μl , 3 equiv.). Amide **22** was obtained as pale-cream colored solid (97 mg, 92%). Analytically pure sample was prepared by recrystallization (petroleum ether/ethyl acetate=2:1). $R_f = 0.47$ (3:1 PE/EA, UV 254 nm). mp = 143.1–143.7 $^\circ\text{C}$; lit. mp = 140–141 $^\circ\text{C}$.¹¹ ^1H NMR (CDCl_3 , 400 MHz): δ 7.54 (d, $J = 8.9$ Hz, 2H), 7.48 (d, $J = 8.9$ Hz, 2H), 7.42 (d, $J = 4.4$ Hz, 4H), 7.38 – 7.31 (m, 1H), 6.95 (br s, 1H), 1.67 (s, 6H). ^{13}C NMR (CDCl_3 , 100.6 MHz): δ 176.08, 143.92, 142.07, 133.26, 129.35, 127.87, 126.52, 119.50, 118.92, 107.09, 48.50, 27.00. IR (KBr): $\nu = 3295$, 3000, 2966, 2919, 1650, 1516, 1260, 1168, 852, 698 cm^{-1} . HRMS (AJS-ESI) calcd. for $\text{C}_{17}\text{H}_{17}\text{N}_2\text{O}^+$ $[\text{M}+\text{H}]^+$ 265.1335, found m/z 265.1337. Spectral data are in agreement with previously reported.^{11,12}

N-Mesityl-2-methyl-2-phenylpropanamide **23**.



23

Prepared following **general procedure C** from 2,4,6-trimethylaniline (0.40 mmol, 57.3 μ l, 1.0 equiv.), 2-methyl-2-phenylpropanoic acid (0.40 mmol, 65.7 mg, 1.0 equiv.), TCFH (0.52 mmol, 146.0 mg, 1.3 equiv.) and *N*-methyl imidazole (1.20 mmol, 96 μ l, 3 equiv.). Amide **23** was obtained as white solid (104 mg, 92%). Analytically pure sample was prepared by recrystallization (petroleum ether/ethyl acetate=2:1). R_f = 0.51 (4:1 PE/EA, UV 254 nm). mp = 156.3–156.9 $^{\circ}$ C. $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 7.57–7.52 (m, 2H), 7.46–7.39 (m, 2H), 7.36–7.29 (m, 1H), 6.81 (s, 2H), 6.25 (br s, 1H), 2.22 (s, 3H), 2.01 (s, 6H), 1.71 (s, 6H). $^{13}\text{C NMR}$ (CDCl_3 , 100.6 MHz): δ 175.68, 145.19, 136.71, 135.07, 131.34, 128.97, 128.94, 127.43, 126.74, 47.60, 27.11, 21.00, 18.32. IR (KBr): ν = 3347, 2982, 2224, 1699, 1588, 1513, 1309, 1135, 699 cm^{-1} . HRMS (AJS-ESI) calcd. for $\text{C}_{19}\text{H}_{24}\text{NO}^+$ $[\text{M}+\text{H}]^+$ 282.1852, found m/z 282.1851.

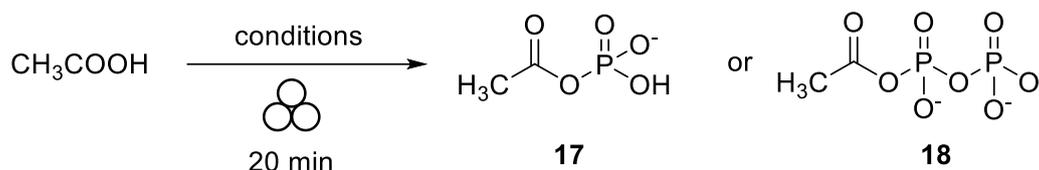
5. Mechanistic studies

5.1. Generation of acyl phosphates

Generation and NMR detection of acetyl phosphates: A 14-mL milling jar was loaded with a potassium phosphate salt (274 mg, 1.56 mmol, 3 equiv.), coupling reagent (247 mg, 0.58 mmol, 1.1 equiv.), acetic acid (30 μ l, 0.52 mmol, 1 equiv.) and milled for 20 min. The residue was dissolved in D₂O, NMR spectra were immediately recorded.

For the reactions with K₂HPO₄ and K₃PO₄, ³¹P NMR spectra indicated formation of acetyl phosphate **17** along with formation of minor amounts of inorganic pyrophosphate (Table S7, entries 1, 2 and 3). In the case of K₄P₂O₇, the formation of acyl pyrophosphate **18** was observed. For the entry 1, addition of morpholine in D₂O solution caused disappearance of acetyl phosphate signal.

Table S7. Mechanochemical generation of acetyl phosphates from acetic acid and phosphate salts.^a



Entry	Coupling reagent	Phosphate salt	Phosphorus-containing products	Yield, % ^b
1	COMU	K ₂ HPO ₄	acetyl phosphate 17	60
			HP ₂ O ₇ ³⁻	10
2	COMU	K ₃ PO ₄	acetyl phosphate 17 ^c	15
			P ₂ O ₇ ⁴⁻	7
3	COMU	K ₄ P ₂ O ₇	acetyl pyrophosphate 18	50
4	TCFH	K ₂ HPO ₄	acetyl phosphate 17	30
			HP ₂ O ₇ ³⁻	10

^a Reaction conditions: Acetic acid (0.524 mmol, 1 equiv.), phosphate salt (3 equiv.), coupling reagent (1.1 equiv.), 20 min milling time. ^b NMR yields, calculated relative to PF₆⁻ signal in ³¹P NMR. Integration of ¹H NMR spectra gives virtually the same yield for acetyl phosphate products. ^c Likely as dipotassium salt

Acetyl phosphate 17. ³¹P NMR (D₂O, 162 MHz): $\delta = -2.1$ ppm. ¹³C NMR (D₂O, 100.6 MHz): $\delta = 168.1$ (d, $J_{\text{CP}} = 8.0$ Hz, CO), 17.9 (d, $J_{\text{CP}} = 4.7$ Hz, CH₃). ¹H NMR (D₂O, 400 MHz): $\delta = 2.27$ (d, $J_{\text{HP}} = 1.0$ Hz, CH₃).

Acetyl pyrophosphate 18. ³¹P NMR (D₂O, 162 MHz): $\delta = -5.0$ (d, $J_{\text{PP}} = 21.7$ Hz, AcOPO₃PO₃), -17.9 (d, $J_{\text{PP}} = 21.7$ Hz, AcOPO₃PO₃). ¹³C NMR (D₂O, 100.6 MHz): $\delta = 166.7$ (d, $J_{\text{CP}} = 8.8$ Hz, CO), 18.0 (d, $J_{\text{CP}} = 6.3$ Hz, CH₃). ¹H NMR (D₂O, 400 MHz): $\delta = 2.37$ (d, $J_{\text{HP}} = 1.1$ Hz, CH₃).

Inorganic phosphates: ^{31}P NMR (D_2O , 162 MHz): $\delta = 2.8$ (K_3PO_4), 0.7 (K_2HPO_4), -6.5 ($\text{K}_4\text{P}_2\text{O}_7$), -8.5 ($\text{K}_3\text{HP}_2\text{O}_7$; generated in solution by mixing $\text{K}_4\text{P}_2\text{O}_7$ and HCl in 1:1 ratio).

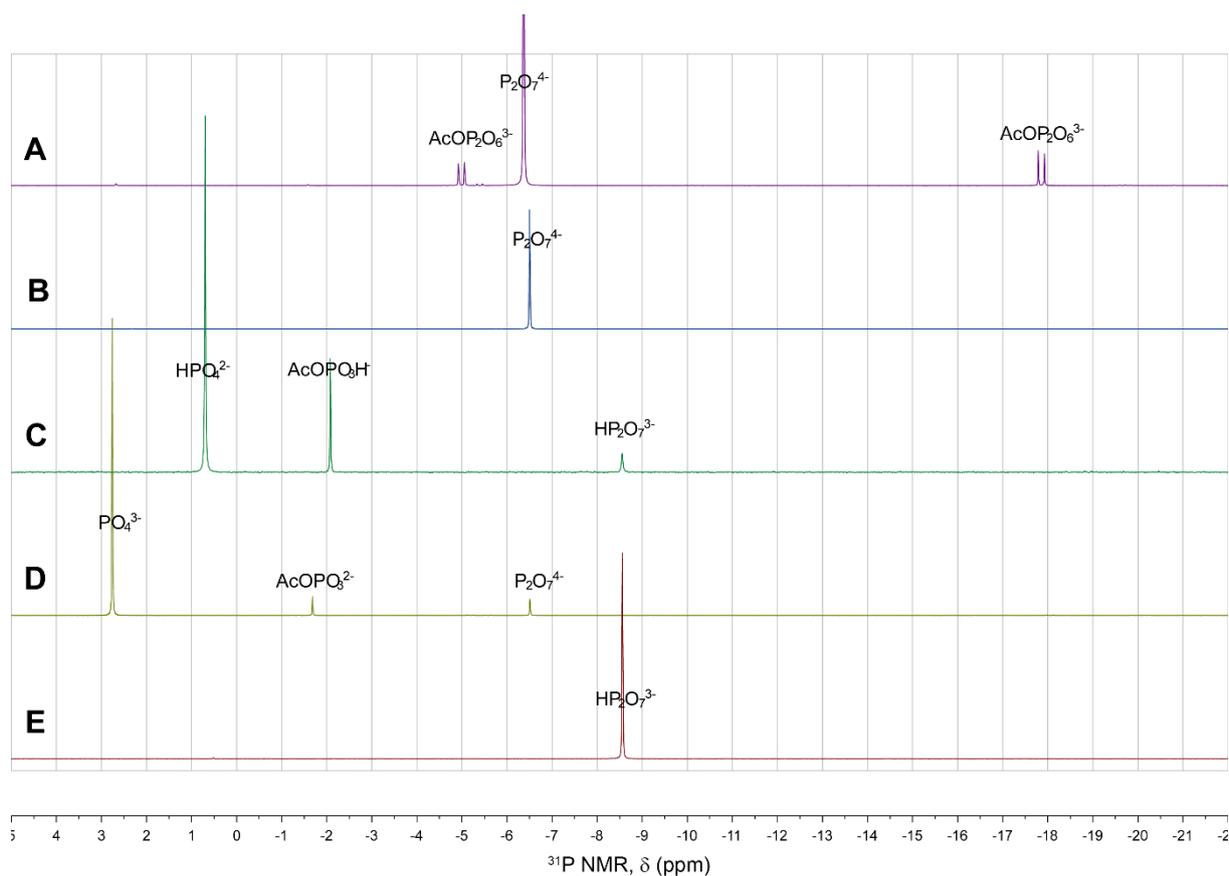


Figure S12. ^{31}P NMR spectra of the reaction mixtures and reference compounds: **(A)** COMU / acetic acid / $\text{K}_4\text{P}_2\text{O}_7$; **(B)** $\text{K}_4\text{P}_2\text{O}_7$ (reference); **(C)** COMU / acetic acid / K_2HPO_4 ; **(D)** COMU / acetic acid / K_3PO_4 ; **(E)** $\text{K}_3\text{HP}_2\text{O}_7$ (reference, generated from $\text{K}_4\text{P}_2\text{O}_7$ + HCl).

Mechanochemical amidation of acetic acid with morpholine and COMU / K_2HPO_4 system:

A 14-mL milling jar was loaded with K_2HPO_4 (274 mg, 1.57 mmol, 3 equiv.), COMU (247 mg, 0.58 mmol, 1.1 equiv.), acetic acid (30 μl , 0.52 mmol, 1 equiv.) and milled for 20 min. A sample of the reaction mixture was dissolved in D_2O , ^{31}P NMR spectrum was immediately recorded (Sample 1, Figure S13). Then morpholine (46 μl , 0.52 mmol, 1 equiv.) was added and reaction mixture was milled for additional 20 min. The residue was dissolved in D_2O , ^{31}P NMR spectrum was immediately recorded (Sample 2).

Control experiment: A 14-mL milling jar was loaded with K_2HPO_4 (274 mg, 1.57 mmol, 3 equiv.), COMU (247 mg, 0.58 mmol, 1.1 equiv.), acetic acid (30 μl , 0.52 mmol, 1 equiv.), morpholine (46 μl , 0.52 mmol, 1 equiv.) and milled for 40 min. The residue was dissolved in D_2O , ^{31}P NMR spectrum was immediately recorded (Sample 3, see Figure S13).

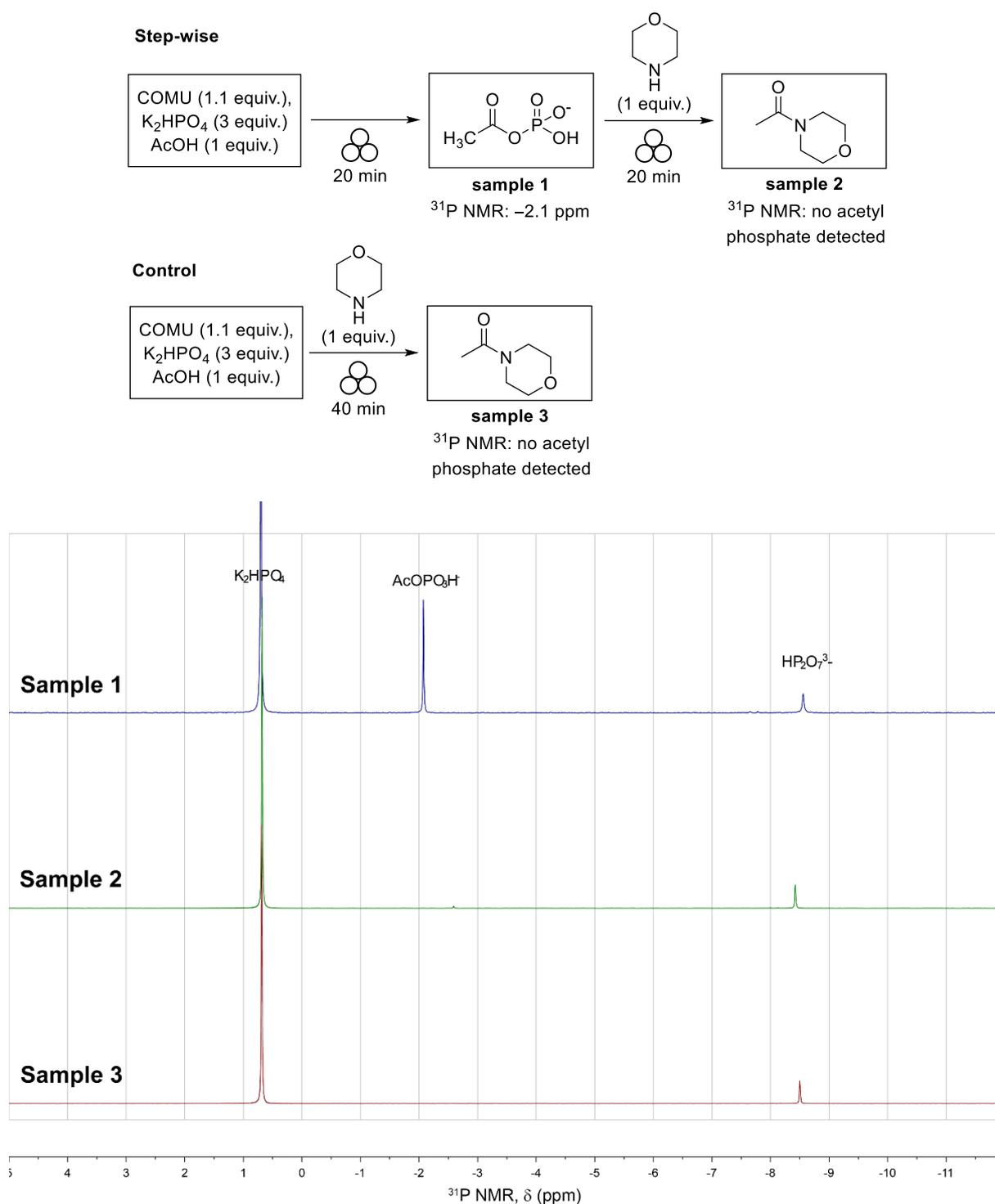


Figure S13. Reaction schemes of mechanochemical amidation of acetic acid with morpholine and COMU/K₂HPO₄ system and the corresponding ³¹P NMR spectra.

Generation of acyl phosphate from Cbz-Phe-OH: A 14-mL milling jar was loaded with K₂HPO₄ (380 mg, 2.18 mmol, 6 equiv.), COMU (171 mg, 0.40 mmol, 1.1 equiv.), Cbz-Phe-OH (108.7 mg, 0.36 mmol, 1 equiv.), ethylacetate (90 μ l) and milled for 20 min. The residue was dissolved in D₂O / DMSO mixture (ratio 2.7:1), and ³¹P NMR spectrum was immediately recorded evidencing formation of the corresponding acyl phosphate **19** in 50% yield ($\delta = -7.6$ ppm, see Figure S14). The signal of **19** disappears after addition of benzylamine.

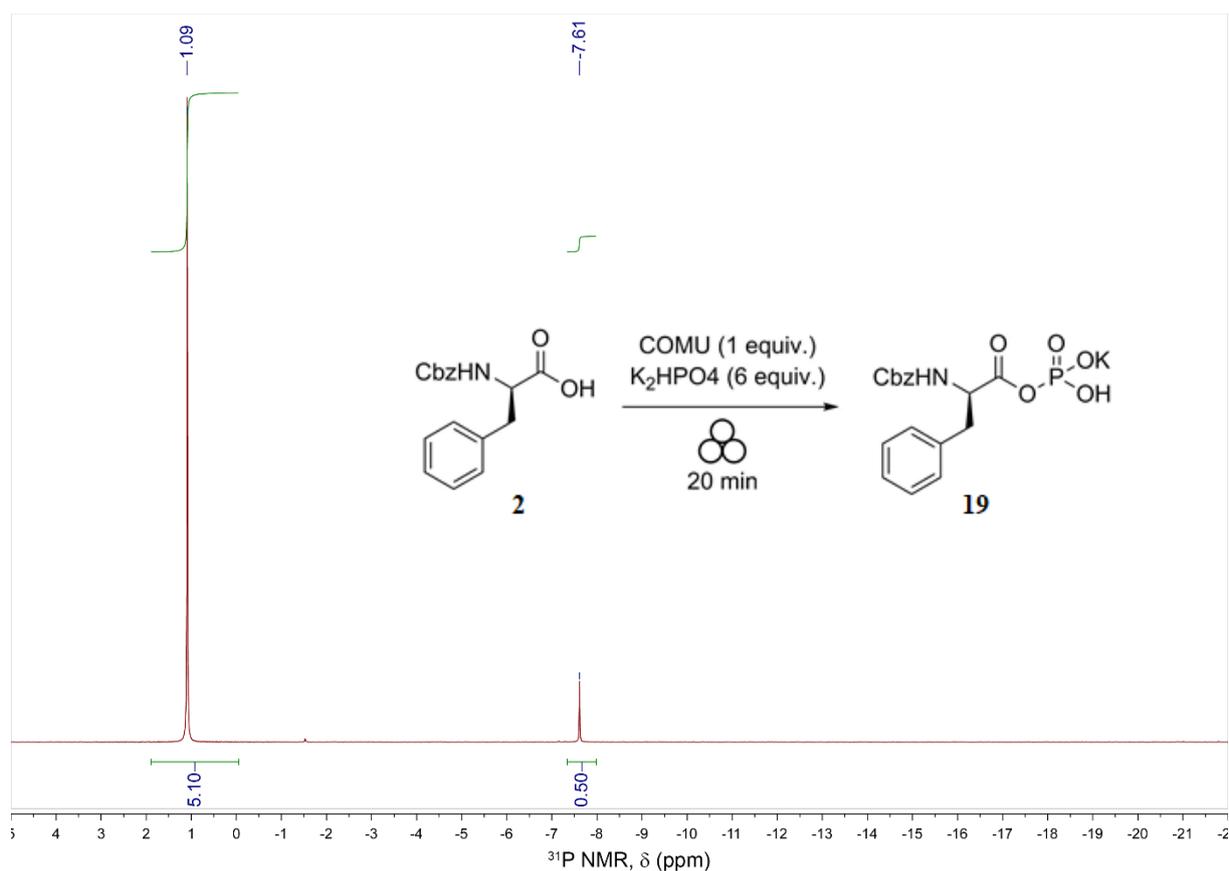
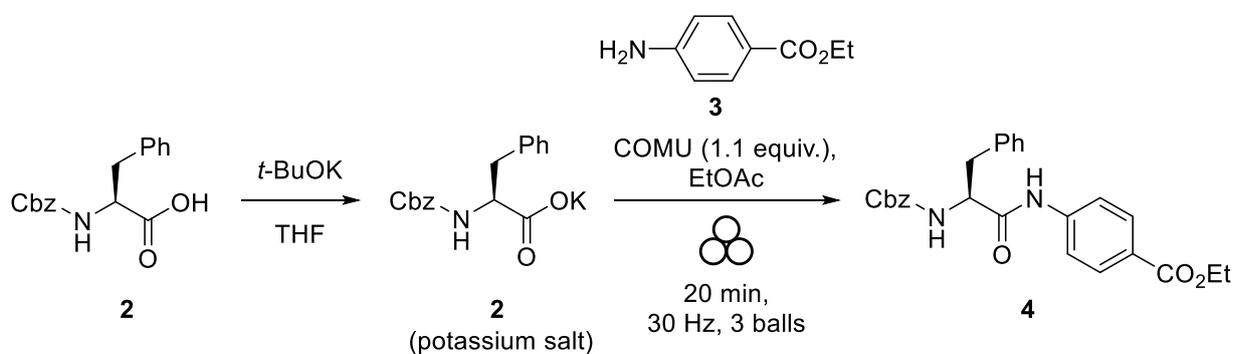


Figure S14. ^{31}P NMR (D_2O / DMSO) spectrum of the crude reaction mixture, showing generation of acyl phosphate from acid **2**.

Reaction of Cbz-Phe-OH potassium salt with benzocaine **3**.



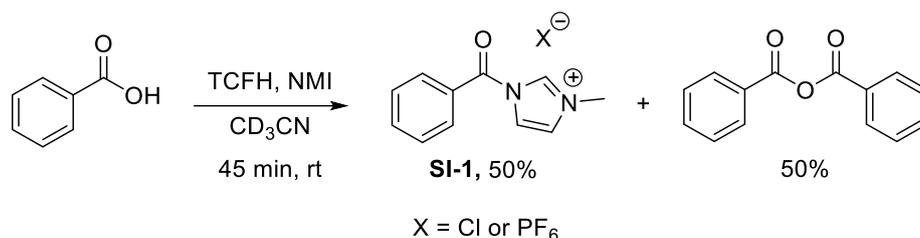
Preparation of Cbz-Phe-OH potassium salt: A solution of $t\text{-BuOK}$ (112 mg, 1 mmol, 1 equiv.) in anhydrous THF (1.5 mL) was added dropwise to the solution of Cbz-Phe-OH (300 mg, 1 mmol, 1 equiv.) in anhydrous THF (3 mL). Then the solvent was evaporated, and the residue was dried for 1–2 hours at 60°C under reduced pressure. The obtained potassium salt was transferred to the glass filter, washed with diethyl ether (3×10 ml) and dried under reduced pressure to afford 270 mg of salt as a white solid (80% yield).

Reaction of Cbz-Phe-OH potassium salt with benzocaine **3.** Benzocaine **3** (0.200 mmol, 33.7 mg, 1 equiv.), Cbz-protected L-phenylalanine **2** (0.220 mmol, 74.2 mg, 1.1 equiv.), COMU (0.220 mmol, 94.0 mg, 1.1 equiv.) were placed into a 14 mL ZrO_2 -coated jar charged with 3×7 mm ZrO_2 balls. Then ethylacetate ($37 \mu\text{l}$, $\eta = 0.19 \mu\text{l mg}^{-1}$) was added to the jar, which was then set to mill at 30 Hz for 20 minutes. ^1H NMR spectra of the crude

reaction mixture was recorded using CDCl_3 as a solvent, after separation of insoluble inorganic material. Conversion (80%) was determined by ^1H NMR. Conversion in the same reaction with K_2HPO_4 was 96% (see above). Thus, K_2HPO_4 mostly acts as a base performing deprotonation of **2**, but also contributes at least 16% to the formation of amide **4** via acyl phosphate **19**.

5.2. Generation and reactivity of *N*-benzoyl imidazoliums

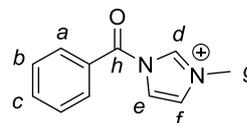
Reaction of benzoic acid with TCFH and *N*-methylimidazole (NMI)



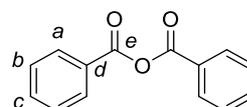
Following the published protocol,¹¹ to a solution of TCFH (93.5 mg, 0.33 mmol, 1.0 equiv.) in CD_3CN (0.6 mL) was added a solution of NMI (54.7 mg, 0.66 mmol, 2.0 equiv.) in the same solvent (0.2 mL). The mixture was aged for 30 min and added to a suspension of benzoic acid (36.6 mg, 0.3 mmol, 0.9 equiv.) in CD_3CN (0.2 mL). Clear solution was immediately obtained. ^1H NMR of the crude reaction mixture was recorded after 45 min, evidencing formation of *N*-benzoyl methylimidazolium derivative **SI-1** (50%) and benzoic anhydride (50%). Signal assignment was performed with an aid of HSQC and HMBC correlation spectra.

NMR data:

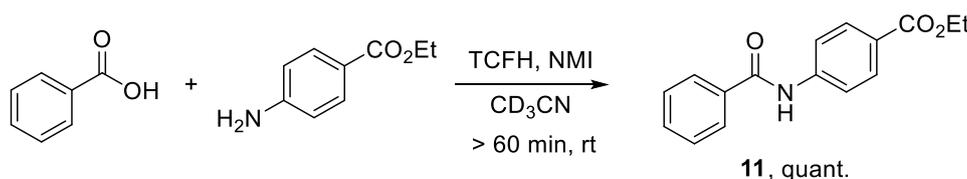
^1H NMR (CD_3CN , 400 MHz): δ 9.41 (s, 1H, H-*d*), 7.94–7.90 (m, 3H, H-*a* and *f*), 7.82 (m, 1H, H-*c*), 7.66–7.62 (m, 3H, H-*b* and *e*), 4.01 (s, 3H, H-*g*). ^{13}C NMR (CD_3CN , 100.6 MHz): δ 164.4 (CO-*h*), 139.6 (CH-*d*), 136.2 (CH-*c*), 131.6 (CH-*a*), 130.3 (CH-*b*), 125.4 (CH-*e*), 121.6 (CH-*f*), 37.5 (CH_3 -*g*).



^1H NMR (CD_3CN , 400 MHz): δ 8.14 (m, 4H, H-*a*), 7.75 (m, 2H, H-*c*), 7.58 (m, 4H, H-*b*). ^{13}C NMR (CD_3CN , 100.6 MHz): δ 163.5 (CO-*e*), 135.7 (CH-*c*), 131.2 (CH-*a*), 130.0 (CH-*b*), 129.5 (C-*d*).



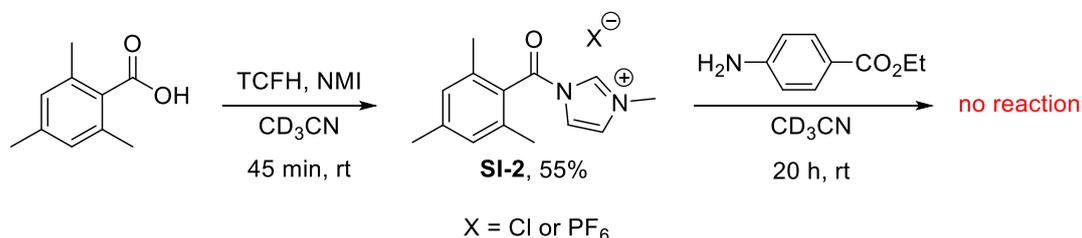
Amidation of benzoic acid with ethyl 4-aminobenzoate promoted by TCFH and NMI



Following the published protocol,¹¹ to a solution of benzoic acid (16.5 mg, 0.135 mmol, 0.9 equiv.), ethyl 4-aminobenzoate (24.8 mg, 0.15 mmol, 1 equiv.) and NMI (43 mg, 0.53 mmol,

3.5 equiv.) in CD₃CN (0.6 mL) was added TCFH (42 mg, 0.15 mmol, 1.0 equiv.). Progress of the reaction was monitored by ¹H NMR, evidencing fast formation of the amide product: 96% yield of **11** was achieved already in 12 min, and total consumption of starting material was observed within 60 min.

Reaction of 2,4,6-trimethylbenzoic acid with TCFH and *N*-methylimidazole

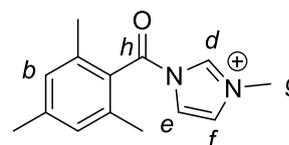


Following the published protocol,¹¹ to a solution of TCFH (93.5 mg, 0.33 mmol, 1.0 equiv.) in CD₃CN (0.6 mL) was added a solution of *N*-methylimidazole (54.7 mg, 0.66 mmol, 2.0 equiv.) in the same solvent (0.2 mL). The mixture was aged for 30 min and added to a suspension of 2,4,6-trimethylbenzoic acid (49.2 mg, 0.3 mmol, 0.9 equiv.) in CD₃CN (0.2 mL). Clear solution was immediately obtained. ¹H NMR of the crude reaction mixture was recorded after 45 min, evidencing formation of *N*-acyl imidazolium derivative **SI-2** (55%, based on characteristic singlet signal of aromatic protons at δ 7.06 ppm), along with two other mesityl-containing components (30% + 15%, singlet signals at δ 6.94 and 6.84 ppm respectively), which likely could be attributed to mesitoic anhydride and starting acid. Signal assignment for **SI-2** was performed with an aid of HSQC and HMBC correlation spectra (see NMR data below).

Addition of ethyl 4-aminobenzoate (49.5 mg, 0.3 mmol, 1.0 equiv.) to the obtained solution did not lead to the formation of the corresponding amide product even after 20 h, thus demonstrating inertness of **SI-2**.

Key characteristic NMR signals for **SI-2**:

¹H NMR (CD₃CN, 400 MHz): δ 9.16 (s, 1H, H-*d*), 7.73 (br s, 1H, H-*f*), 7.62 (t, *J* = 1.9 Hz, 1H, H-*e*), 7.06 (s, 2H, H-*b*), 3.94 (s, 3H, H-*g*). ¹³C NMR (CD₃CN, 100.6 MHz): δ 166.2 (CO-*h*), 139.0 (CH-*d*), 129.9 (CH-*b*), 126.5 (CH-*e*), 119.8 (CH-*f*), 37.7 (CH₃-*g*).

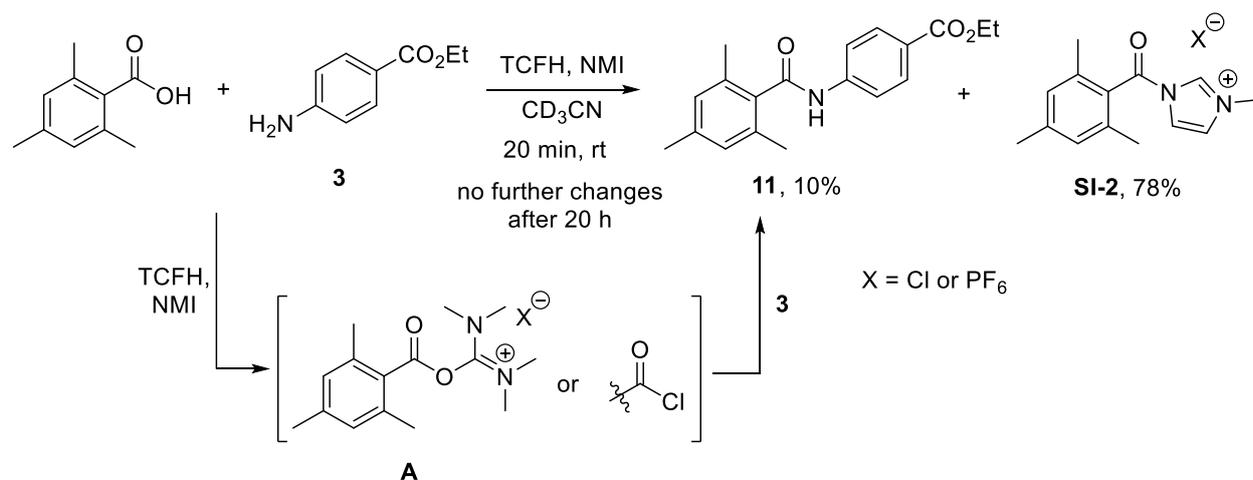


HRMS (AJESI), *m/z*: [M]⁺ calcd. for C₁₄H₁₇N₂O⁺ 229.1335; found 229.1329.

Amidation of 2,4,6-trimethylbenzoic acid with ethyl 4-aminobenzoate promoted by TCFH and NMI

Following the published protocol,¹¹ to a solution of mesitoic acid (24.6 mg, 0.15 mmol, 1 equiv.), ethyl 4-aminobenzoate (24.8 mg, 0.15 mmol, 1 equiv.) and NMI (43 mg, 0.53 mmol, 3.5 equiv.) in CD₃CN (0.6 mL) was added TCFH (42 mg, 0.15 mmol, 1.0 equiv.). Progress of the reaction was monitored by ¹H NMR. The first ¹H NMR was run after 20 min, and no further changes were observed after 1 h and 20 h reaction time. Only 10% of ethyl 4-aminobenzoate

was converted into the corresponding amide, while nearly 80% of mesitoic acid transformed into the inert imidazolium derivative **SI-2**.



Since imidazolium derivative **SI-2** is inert towards ethyl 4-aminobenzoate, minor amount of amide **11** could be formed via the corresponding uronium intermediate **A**, although generation of the corresponding chloroanhydride cannot be also excluded.

Inertness of carbonyl group in **SI-2** towards nucleophilic attack in this case can be explained by steric shielding introduced by the nearby imidazolium and mesityl moieties. According to MM2 simulation in Chem3D program, in the lowest energy conformer of **SI-2** plane of the imidazolium ring is tilted (OCNC dihedral angle = 130° , Figure S15) in respect to the plane of 2,4,6-trimethylbenzoyl moiety. As a result, the carbonyl group in **SI-2** is efficiently blocked against attack of a nucleophile approaching along the Bürgi–Dunitz trajectory.

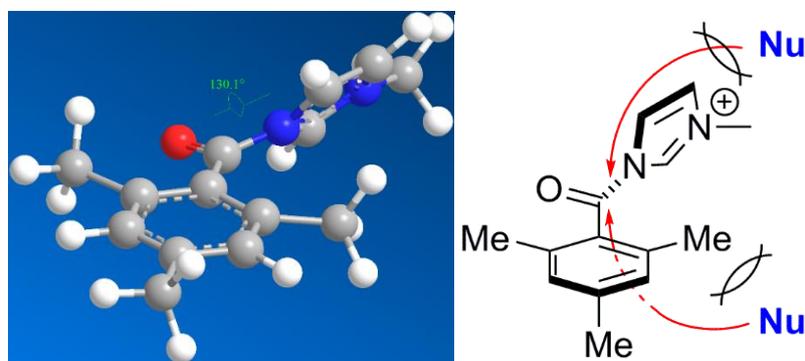
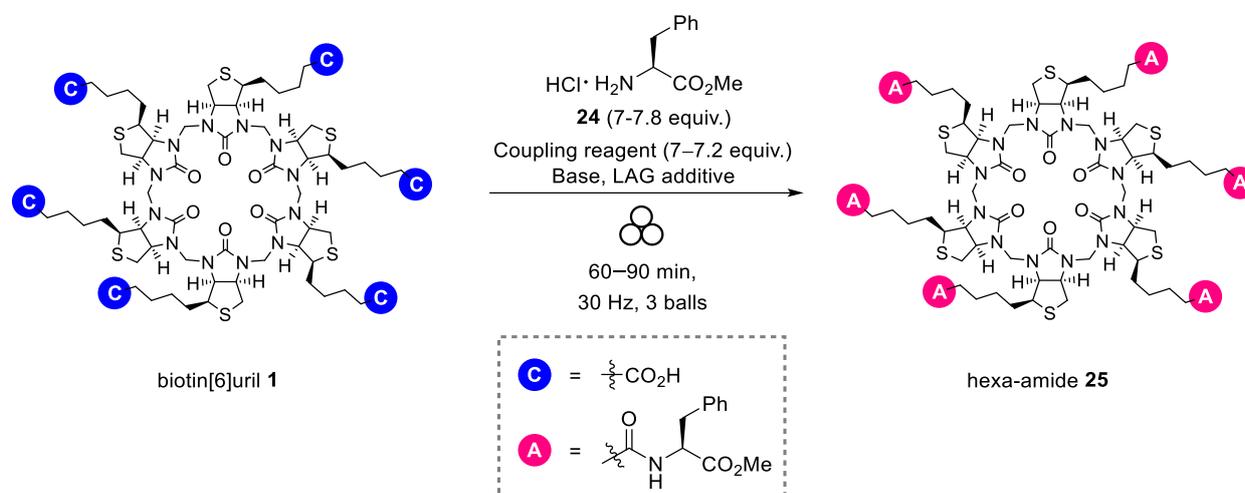


Figure S15. The lowest energy conformer of imidazolium intermediate **SI-2** with sterically shielded carbonyl group.

6. Functionalization of Biotin[6]uril

Amide coupling of biotin[6]uril **1** and L-phenylalanine methyl ester hydrochloride **24** was selected as a model process.



General procedure: Biotin[6]uril **1** (0.033–0.046 mmol, 1 equiv.), L-phenylalanine methyl ester hydrochloride **24** (0.231–0.356 mmol, 7–7.8 equiv.), Coupling reagent (0.231–0.331 mmol, 7–7.2 equiv.) and Base (0.594–0.966 mmol, 18–21 equiv.) were placed into a 14 mL ZrO₂-coated jar charged with 3×7 mm ZrO₂ balls. Then LAG additive was added to the jar, which was then set to mill at 30 Hz for 60–90 minutes. The resulting crude reaction mixture was analysed using HPLC/HRMS analysis.

General conditions for HRMS characterization of products and intermediates

The samples were introduced into a mass spectrometer by direct injection of test solutions (injection volume 2 μl). Acetonitrile with 0.05% formic acid additive was used as an eluent, the flow rate was set at either 0.1 ml/min or 0.2 mL/min. For analysis, ca. 1 mM solution of the sample was prepared in a suitable solvent or solvents mixture.

General conditions for HPLC analysis

Macherey-Nagel Nucleoshell RP18 column (150 mm×3.0 mm, 2.7 μm) was employed in the measurements. Eluents A (water / 0.1% formic acid) and B (acetonitrile / 0.1% formic acid) were used in a 10-minute gradient mode from A:B 85:15 (v/v) to A:B 10:90 (v/v) with the flow rate of 0.5 mL/min. The column temperature was set at 30 °C, injection volume at either 5 or 10 μl and detection wavelength at 254 nm.

MSD was involved in some measurements with the purpose of additional identification of the sample components by ESI-MS. The ionization source conditions were kept consistent throughout the measurements with following parameters: positive polarity, capillary voltage 2000 V, charging electrode voltage 2000 V, drying gas temperature 300 °C, drying gas flow 5 L/min, nebulizer pressure 60 psig, vaporizer temperature 150 °C.

Sample preparation included sampling ~5 mg of the crude reaction mixture, dissolving in DMSO (1 mL), followed by centrifuging the solution and analyzing the clear supernatant. For

purity determination, ~2 mg of product was dissolved in 1 mL of DMSO or methanol/chloroform mixture.

Distribution of products was calculated by using the corresponding HPLC peak areas. Only identified peaks were taken into account, namely: biotin[6]juril, mono-, di-, tri-, tetra-, penta-, hexa-amides. Relative peak areas (S_{rel} , %) were calculated as follows:

$$S_{rel}(\%) = \frac{S_i}{\sum S_i} \cdot 100,$$

where S_i – area of an individual peak, mAu·s; $\sum S_i$ – sum of areas of all peaks considered, mAu·s.

HPLC-HRMS conditions for identification of different L-phenylalanine-biotin[6]juril amides

Test solution from **Entry 2** (see Table S9 below) was used for identification of products. HPLC-UV chromatogram of the sample (Figure S18) contained a series of peaks, which were assumed to be mono-, di-, tri-, tetra-, penta- and hexa-amide in detectable amounts, and then confirmed by MS measurements (Figure S16 and Table S8).

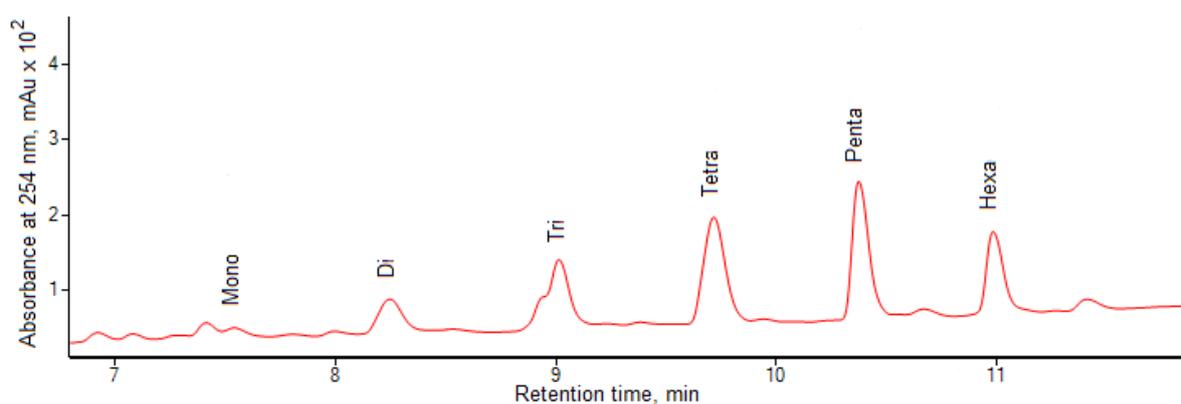


Figure S16. HPLC chromatogram with MS-identified peaks of various amide products.

Table S8. Calculated and found m/z values for different amide products.

Amide	Ion	Formula	m/z calc	m/z found
Mono-	$[M+H]^+$	$C_{76}H_{109}N_{13}O_{19}S_6$	1698.6203	1698.6227
Di-	$[M+2H]^{2+}$	$C_{86}H_{120}N_{14}O_{20}S_6$	930.3558	930.3553
Tri-	$[M+2H]^{2+}$	$C_{96}H_{131}N_{15}O_{21}S_6$	1010.8979	1010.8999
Tetra-	$[M+2H]^{2+}$	$C_{106}H_{142}N_{16}O_{22}S_6$	1091.4399	1091.4388
Penta-	$[M+2H]^{2+}$	$C_{116}H_{153}N_{17}O_{23}S_6$	1171.9819	1171.9843
Hexa-amide	$[M+2H]^{2+}$	$C_{126}H_{164}N_{18}O_{24}S_6$	1252.5240	1252.5244

HPLC chromatograms of the crude reaction mixtures are shown below (Figures S17–S28). Summary of quantitative analysis results is presented in Table S9.

Optimization experiments (see Table S9)

Entry 1: 14-mL milling jar was loaded with biotin[6]juril **1** (0.046 mmol, 70 mg, 1 equiv.), L-phenylalanine methyl ester hydrochloride **24** (0.319 mmol, 69 mg, 7 equiv.), COMU (0.319 mmol, 137 mg, 7 equiv.), K_2HPO_4 (0.820 mmol, 143 mg, 18 equiv.), ethyl acetate ($\eta = 0.19$ $\mu\text{L}/\text{mg}$) and milled for 90 min. The resulting crude reaction mixture was analysed by HPLC/HRMS (Entry 1, Table S9).

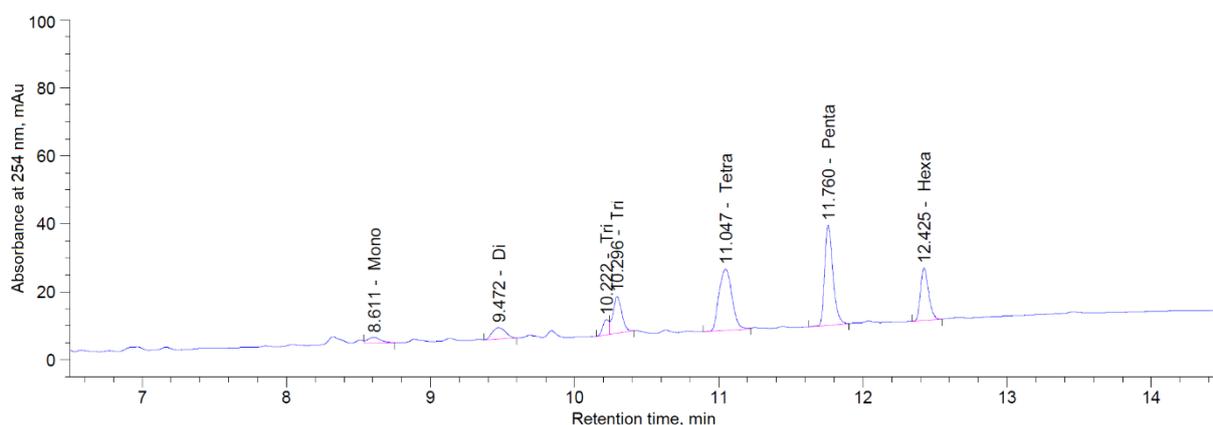


Figure S17. HPLC chromatogram of the crude reaction mixture, obtained with COMU/ K_2HPO_4 after 90 min of milling with ethyl acetate as a LAG (Entry 1, Table S9).

Entry 2: A 14-mL milling jar was loaded with biotin[6]uril **1** (0.046 mmol, 70 mg, 1 equiv.), L-phenylalanine methyl ester hydrochloride **24** (0.319 mmol, 69 mg, 7 equiv.), TCFH (0.319 mmol, 90 mg, 7 equiv.), K_2HPO_4 (0.820 mmol, 143 mg, 18 equiv.), ethyl acetate ($\eta = 0.19 \mu\text{L}/\text{mg}$) and milled for 90 min. The resulting crude reaction mixture was analysed by HPLC/HRMS (Entry 2, Table S9).

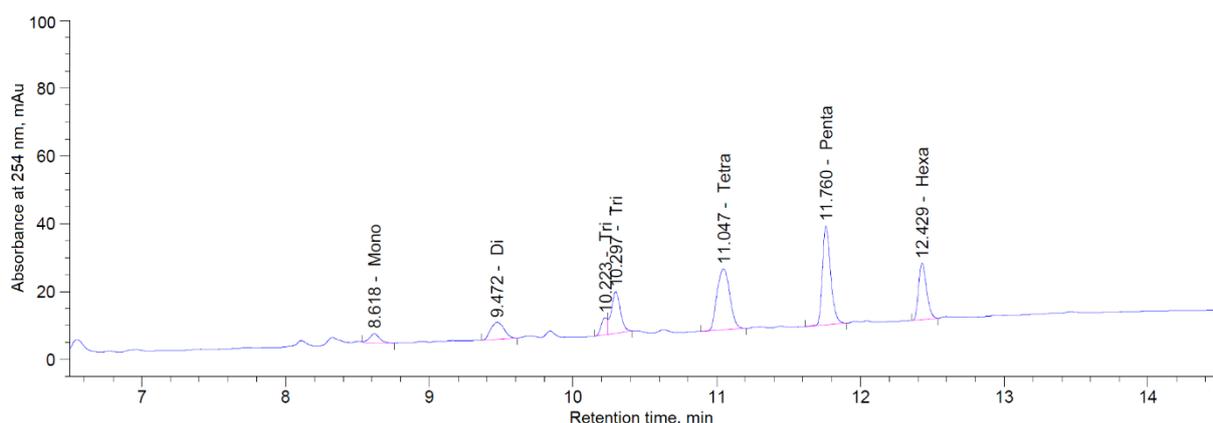


Figure S18. HPLC chromatogram of the crude reaction mixture, obtained with TCFH/ K_2HPO_4 after 90 min of milling with ethyl acetate as a LAG (Entry 2, Table S9).

Entry 3: Following the published protocol,¹ a 14-mL milling jar was loaded with biotin[6]uril **1** (0.046 mmol, 70 mg, 1 equiv.), L-phenylalanine methyl ester hydrochloride **24** (0.319 mmol, 69 mg, 7 equiv.), EDC·HCl (0.319 mmol, 62.4 mg, 7 equiv.), DMAP (0.547 mmol, 67 mg, 12 equiv.), $MeNO_2$ ($\eta = 0.25 \mu\text{L}/\text{mg}$) and milled for 90 min. The resulting crude reaction mixture was analysed by HPLC/HRMS (Entry 3, Table S9).

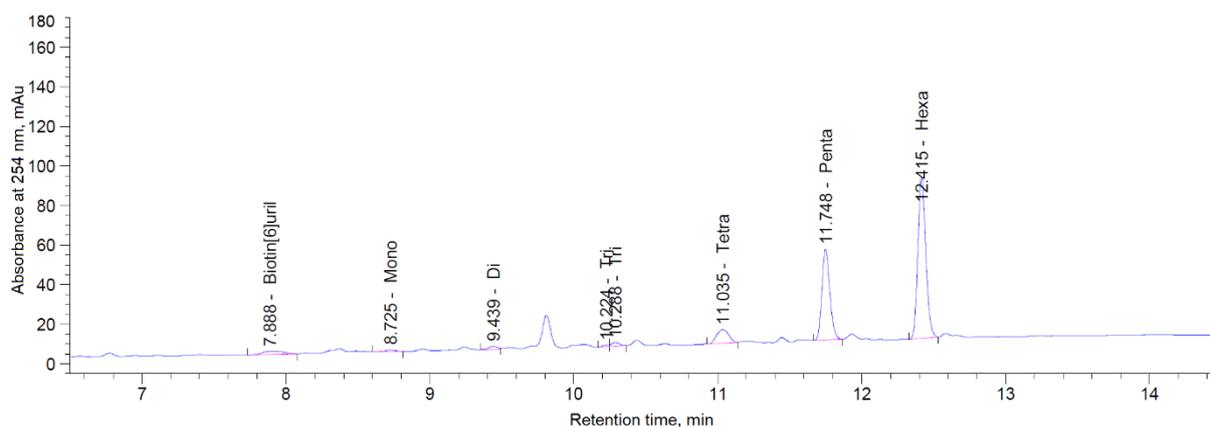


Figure S19. HPLC chromatogram of the crude reaction mixture, obtained with EDC/DMAP after 90 min of milling with MeNO₂ as a LAG (Entry 3, Table S9).

Entry 4: A 14-mL milling jar was loaded with biotin[6]juril **1** (0.046 mmol, 70 mg, 1 equiv.), L-phenylalanine methyl ester hydrochloride **24** (0.319 mmol, 69 mg, 7 equiv.), TCFH (0.319 mmol, 90 mg, 7 equiv.), *N*-methyl imidazole (0.820 mmol, 66 μ l, 18 equiv., $\eta = 0.29 \mu\text{L}/\text{mg}$) and milled for 90 min. The resulting crude reaction mixture was analysed by HPLC/HRMS (Entry 4, Table S9).

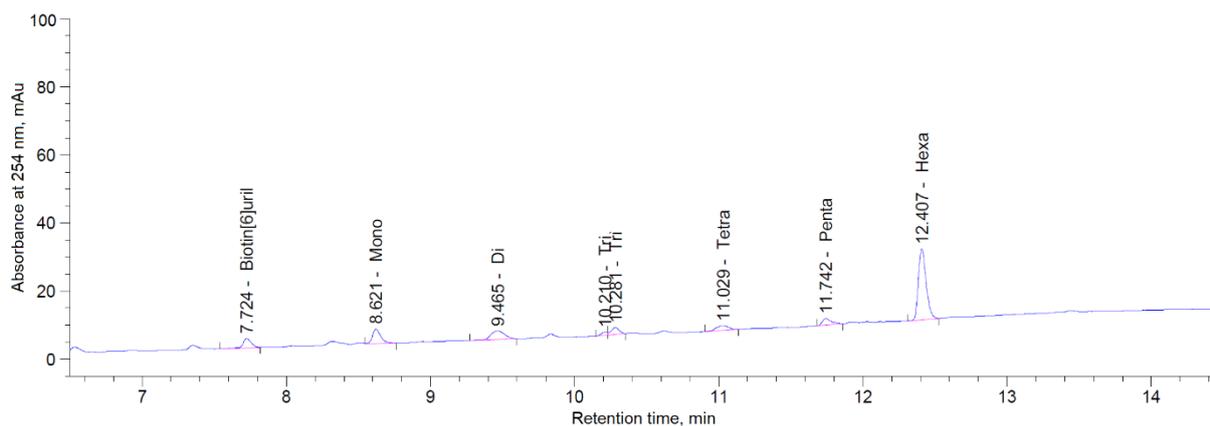


Figure S20. HPLC chromatogram of the crude reaction mixture, obtained with TCFH/NMI after 90 min of milling with NMI as a LAG (Entry 4, Table S9).

Entry 5. A 14-mL milling jar was loaded with biotin[6]juril **1** (0.033 mmol, 50 mg, 1 equiv.), L-phenylalanine methyl ester hydrochloride **24** (0.254 mmol, 55 mg, 7.8 equiv.), TCFH (0.234 mmol, 66 mg, 7.2 equiv.), *N*-methyl imidazole (0.684 mmol, 55 μ l, 21 equiv., $\eta = 0.32 \mu\text{L}/\text{mg}$) and milled for 60 min. The resulting crude reaction mixture was analysed by HPLC/HRMS (Entry 5, Table S9).

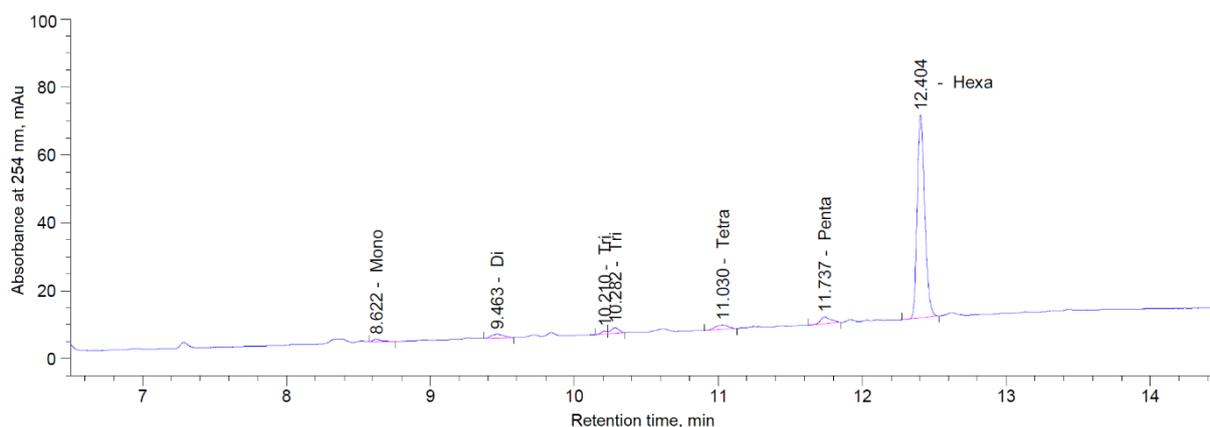


Figure S21. HPLC chromatogram of the crude reaction mixture, obtained with TCFH/NMI after 60 min of milling with NMI as a LAG (Entry 5, Table S9).

Entry 6: A 14-mL milling jar was loaded with biotin[6]uril **1** (0.033 mmol, 50 mg, 1 equiv.), L-phenylalanine methyl ester hydrochloride **24** (0.254 mmol, 55 mg, 7.8 equiv.), TCFH (0.234 mmol, 66 mg, 7.2 equiv.), *N*-methyl imidazole (0.684 mmol, 55 μ l, 21 equiv., $\eta = 0.16 \mu\text{L/mg}$), NaCl (3.092 mmol, 180 mg, 95 equiv) and milled for 60 min. The resulting crude reaction mixture was analysed by HPLC/HRMS (Entry 6, Table S9).

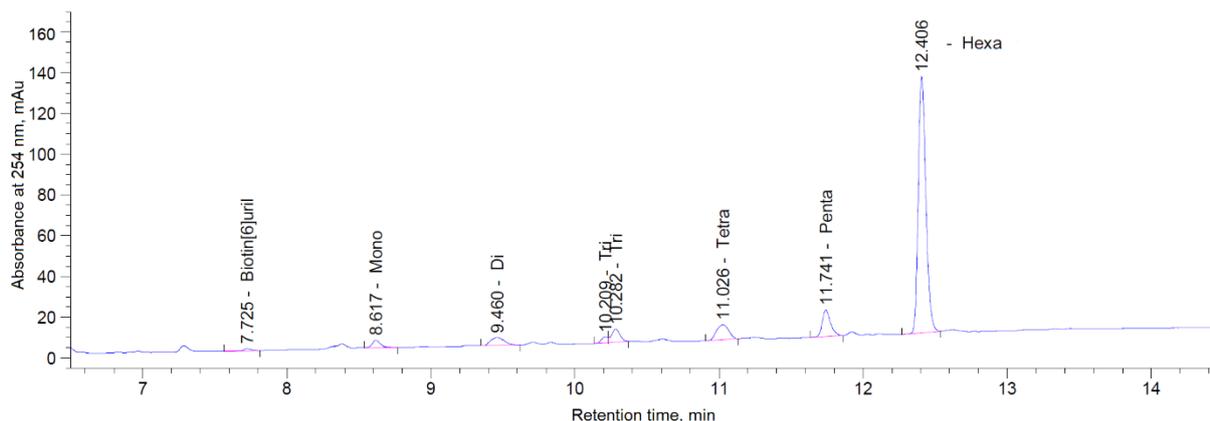


Figure S22. HPLC chromatogram of the crude reaction mixture, obtained with TCFH/NMI after 60 min of milling with NMI as a LAG and NaCl as a grinding additive (Entry 6, Table S9).

Entry 7: A 14-mL milling jar was loaded with biotin[6]uril **1** (0.033 mmol, 50 mg, 1 equiv), L-phenylalanine methyl ester hydrochloride **24** (0.254 mmol, 55 mg, 7.8 equiv), TCFH (0.234 mmol, 66 mg, 7.2 equiv), *N*-methyl imidazole (0.684 mmol, 55 μ l, 21 equiv), DMF (35 μ l, $\eta = 0.53 \mu\text{L/mg}$) and milled for 60 min. The resulting crude reaction mixture was analysed by HPLC/HRMS (Entry 7, Table S9).

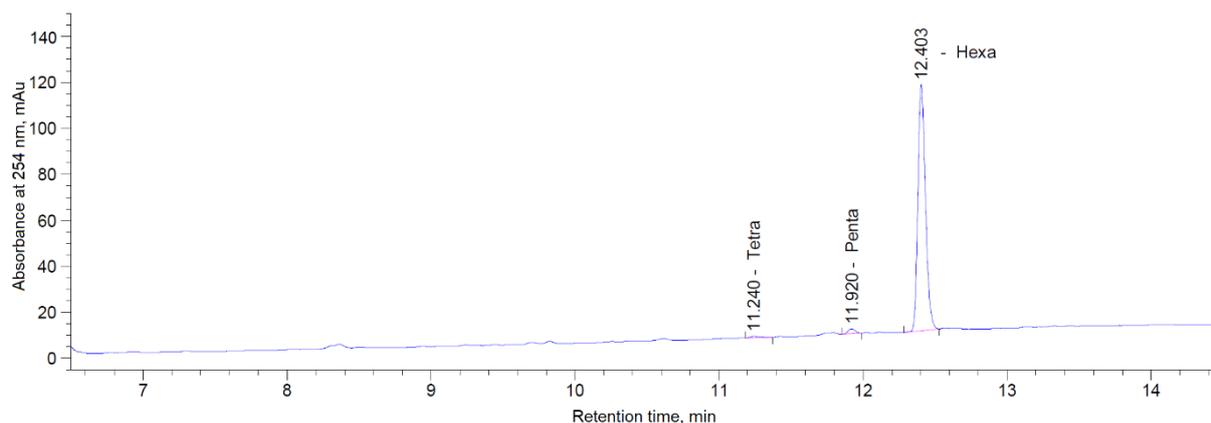


Figure S23. HPLC chromatogram of the crude reaction mixture, obtained with TCFH/NMI after 60 min of milling with DMF as a LAG (Entry 7, Table S9).

Entry 8: A 14-mL milling jar was loaded with biotin[6]juril **1** (0.033 mmol, 50 mg, 1 equiv), L-phenylalanine methyl ester hydrochloride **24** (0.254 mmol, 55 mg, 7.8 equiv), TCFH (0.234 mmol, 66 mg, 7.2 equiv), *N*-methyl imidazole (0.684 mmol, 55 μ l, 21 equiv), heptane (55 μ l, $\eta = 0.64 \mu\text{L/mg}$) and milled for 60 min. The resulting crude reaction mixture was analysed by HPLC/HRMS (Entry 8, Table S9).

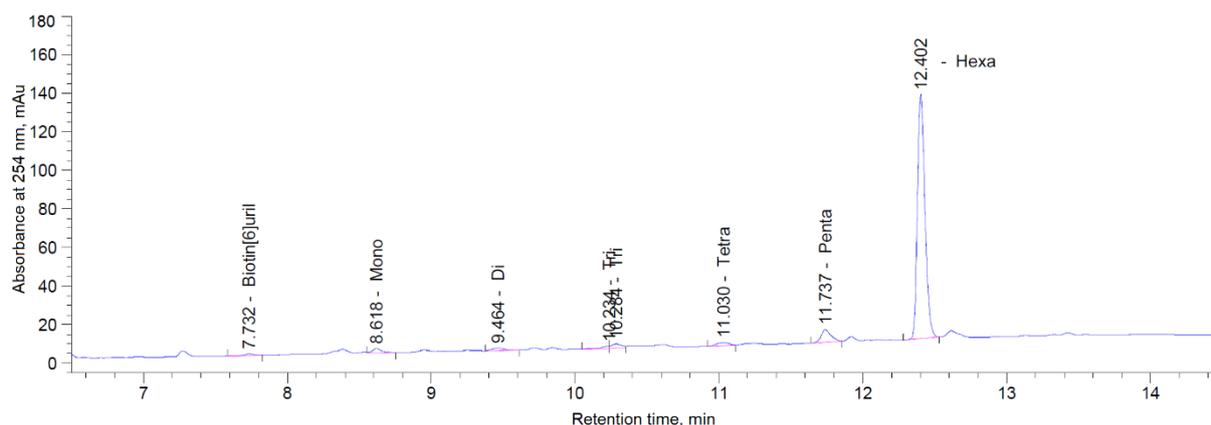


Figure S24. HPLC chromatogram of the crude reaction mixture, obtained with TCFH/NMI after 60 min of milling with heptane as a LAG (Entry 8, Table S9).

Entry 9: A 14-mL milling jar was loaded with biotin[6]juril **1** (0.033 mmol, 50 mg, 1 equiv), L-phenylalanine methyl ester hydrochloride **24** (0.254 mmol, 55 mg, 7.8 equiv), TCFH (0.234 mmol, 66 mg, 7.2 equiv), *N*-methyl imidazole (0.684 mmol, 55 μ l, 21 equiv), ethyl acetate (55 μ l, $\eta = 0.64 \mu\text{L/mg}$) and milled for 60 min. The resulting crude reaction mixture was analysed by HPLC/HRMS (Entry 9, Table S9).

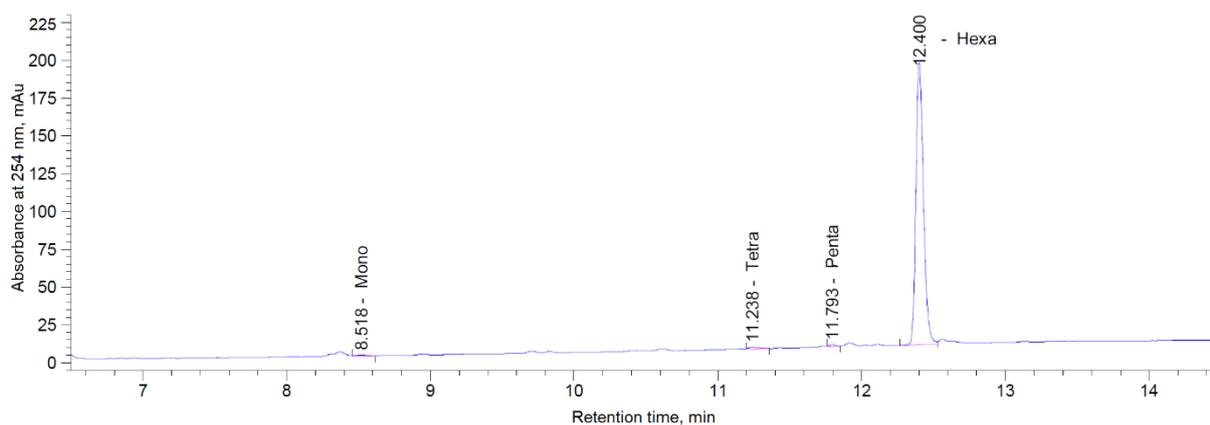


Figure S25. HPLC chromatogram of the crude reaction mixture, obtained with TCFH/NMI after 60 min of milling with ethyl acetate as a LAG (Entry 9, Table S9).

Entry 10: A slurry of biotin[6]uril **1** (0.033 mmol, 50 mg, 1 equiv), L-phenylalanine methyl ester hydrochloride **24** (0.254 mmol, 55 mg, 7.8 equiv), TCFH (0.234 mmol, 66 mg, 7.2 equiv), *N*-methyl imidazole (0.684 mmol, 55 μ l, 21 equiv) and ethyl acetate (55 μ l, $\eta = 0.64 \mu\text{L}/\text{mg}$) was stirred for 60 min. The resulting crude reaction mixture was analysed by HPLC/HRMS (Entry 10, Table S9).

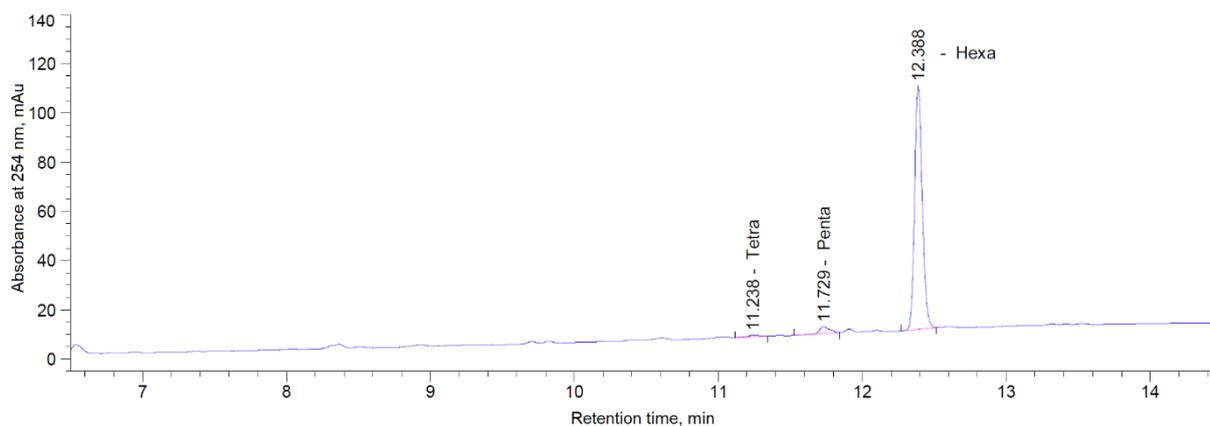


Figure S26. HPLC chromatogram of the crude reaction mixture, obtained with TCFH/NMI after 60 min of slurry stirring with ethyl acetate as a LAG (Entry 10, Table S9).

Entry 11: A solution of biotin[6]uril **1** (0.046 mmol, 70 mg, 1 equiv), L-phenylalanine methyl ester hydrochloride **24** (0.356 mmol, 77 mg, 7.8 equiv), HATU (0.319 mmol, 121 mg, 7 equiv), DIPEA (0.319 mmol, 56 μ l, 7 equiv) in anhydrous DMF (0.5 mL, $\eta = 2.1 \mu\text{L}/\text{mg}$) was stirred for 60 min. The resulting reaction mixture was analysed by HPLC/HRMS (Entry 11, Table S9).

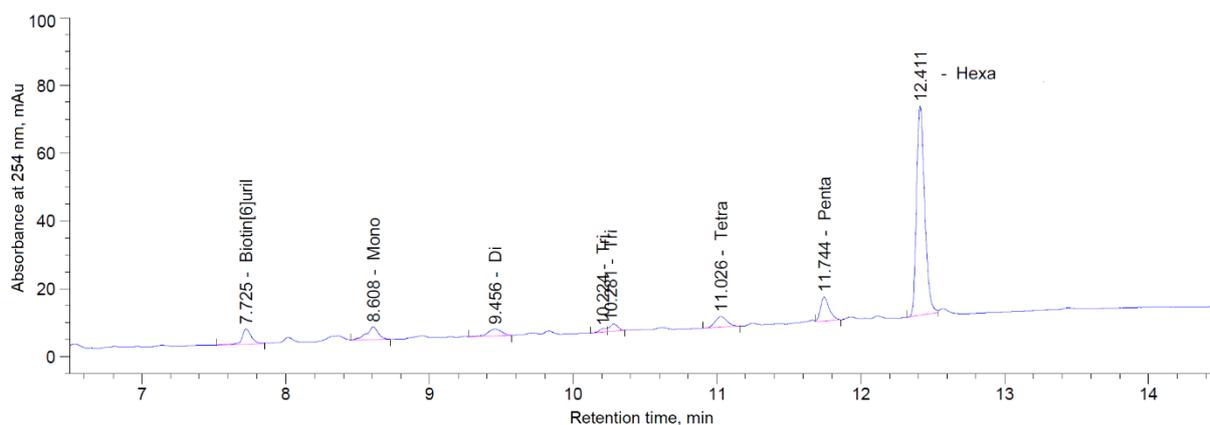


Figure S27. HPLC chromatogram of the crude reaction mixture, obtained with TCFH/NMI after 60 min of stirring in DMF solution (Entry 11, Table S9).

Entry 12: A solution of biotin[6]uril **1** (0.046 mmol, 70 mg, 1 equiv), L-phenylalanine methyl ester hydrochloride **24** (0.356 mmol, 77 mg, 7.8 equiv), TCFH (0.328 mmol, 92 mg, 7.2 equiv), *N*-methyl imidazole (0.957 mmol, 77 μ L, 21 equiv) in anhydrous DMF (0.5 mL, η = 2.4 μ L/mg) was stirred for 60 min. The resulting reaction mixture was analysed by HPLC/HRMS (Entry 12, Table S9).

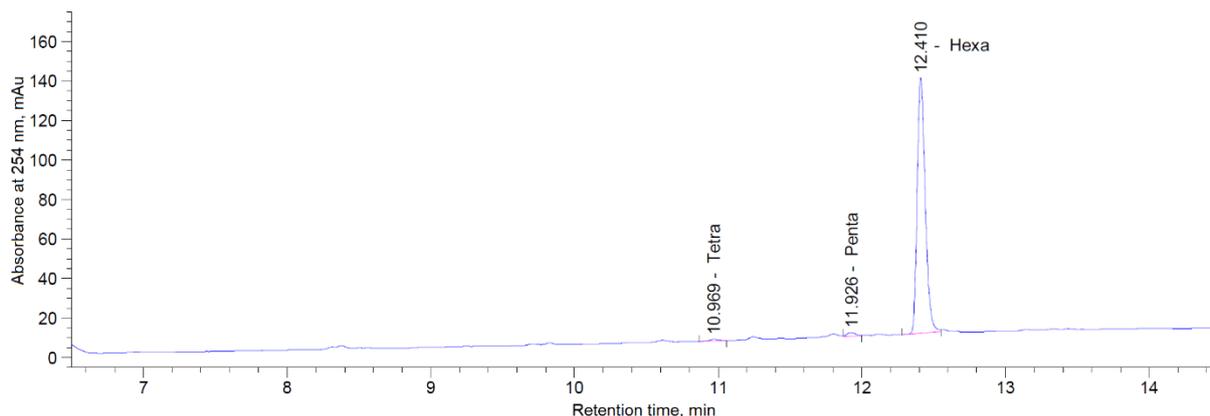


Figure S28. HPLC chromatogram of the crude reaction mixture, obtained with TCFH/NMI after 60 min of stirring in DMF solution (Entry 12, Table S9).

Table S9. Amide coupling of biotin[6]uril with L-phenylalanine methyl ester, distribution of products according to HPLC.

Entry	Reaction conditions ^a	Liquid chemicals (additives, solvents)	η , $\mu\text{L}/\text{mg}$	Time, min	<i>S_{rel}</i> , %							
					Biotin-[6]uril	Mono-	Di-	Tri-	Tetra-	Penta-	Hexa-amide	
1	COMU / K ₂ HPO ₄	EtOAc	0.19	90	-	2.7	5.5	16	30	31	16	
2	TCFH / K ₂ HPO ₄	EtOAc	0.19	90	-	3.3	8.0	17	28	29	16	
3	EDC / DMAP ^c	CH ₃ NO ₂	0.25	90	2.8	0.7	1.1	1.7	7.1	31	55	
4	TCFH / NMI	NMI	0.29	90	7.7	11	11	6.9	5.6	5.7	52	
5	Ball milling	TCFH / NMI ^d	NMI	0.32	60	-	1.1	2.7	3.3	3.1	3.9	86
6		TCFH / NMI ^d / NaCl ^e	NMI	0.16	60	0.6	2.4	4.0	5.4	6.8	8.3	73
7		TCFH / NMI ^d	NMI, DMF	0.53	60	-	-	-	-	0.9	1.8	97
8		TCFH / NMI ^d	NMI, heptane	0.64	60	0.8	1.8	1.5	4.3	1.8	5.6	84
9		TCFH / NMI^d	NMI, EtOAc	0.64	60	-	0.5	-	-	0.8	0.6	98
10	Slurry stirring	TCFH / NMI ^d	NMI, EtOAc	0.64	60	-	-	-	-	0.9	3.8	95
11	Solution (DMF)	HATU, DIPEA	DIPEA, DMF	2.1	60	5.5	5.9	3.7	3.3	5.0	8.3	68
12		TCFH, NMI ^d	NMI, DMF	2.4	60	-	-	-	-	0.6	1.5	98

^a Reaction conditions: biotin[6]uril (50–70 mg, 0.03–0.05 mmol), **24** (7 equiv.), coupling reagent (7 equiv.), base (18 equiv.), unless other specified.

^b HPLC area percentage of hexa-amide **25**, relative to other amides. ^c 12 equiv. of DMAP was used. ^d With 7.2 equiv. of TCFH, 7.8 equiv. of L-phenylalanine **24** and 21 equiv. of NMI. ^e NaCl was used as grinding additive.

Synthesis of hexa-amide **25**: effect of milling time.

Prolonged milling times (60–90 min) are required to complete the reaction, especially at higher loadings. Representative examples are shown below.

Experiment 1: Entry 4 from Table S9 (Samples were taken in 60 and 90 minutes).

Experiment 2: Entry 9 from Table S9, 2×scale-up (Samples were taken in 30 and 90 minutes).

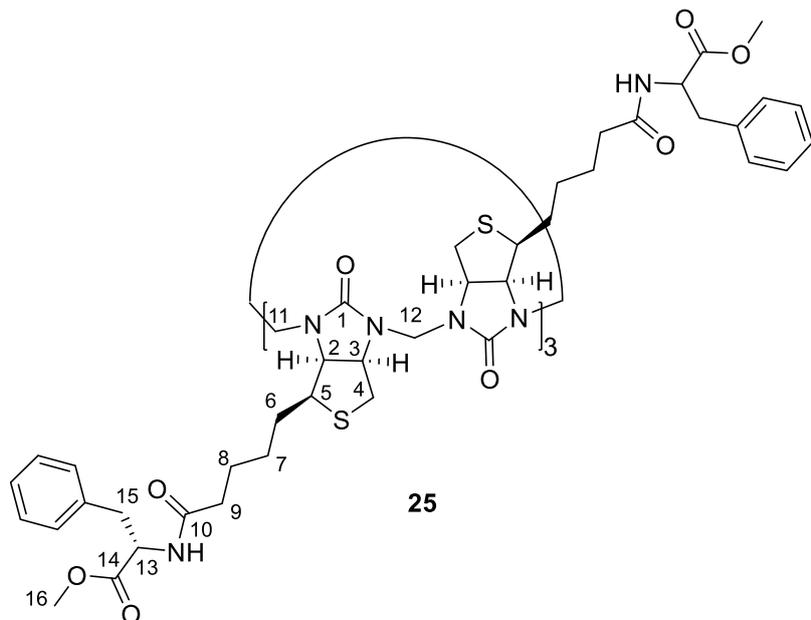
Table S10. HPLC conversion (based on area percentage) after 30 min, 60 and 90 min.

Entry	Reaction conditions	Time, min	<i>S_{rel}</i> , %						
			Biotin-[6]juril	Mono-	Di-	Tri-	Tetra-	Penta-	Hexa-amide
1	Ball milling TCFH/NMI	60	13	21	11	6.2	3.6	4.1	41
		90	7.7	11	11	6.8	5.6	5.7	52
2	Ball milling TCFH/NMI, EtOAc^a	30	-	-	1.7	2.0	2.9	4.6	89
		90	-	-	-	-	1.2	2	97

^a 2×scale-up of Entry 9 from Table S9

Optimized procedure for the synthesis of hexa-amide **25**.

Biotin[6]juril **1** (0.098 mmol, 150 mg, 1 equiv.), L-phenylalanine methyl ester hydrochloride **24** (0.762 mmol, 164.3 mg, 7.8 equiv.), TCFH (0.703 mmol, 197.3 mg, 7.2 equiv.) and *N*-methyl imidazole (2.051 mmol, 165 μL, 21 equiv.) were placed into a 14 mL ZrO₂-coated jar charged with 3×7 mm ZrO₂ balls. Then ethyl acetate (165 μL) as a LAG additive was added to the jar, which was then set to mill at 30 Hz for 90 minutes. The resulting crude reaction mixture was transferred with water (40 mL) to the beaker with a magnetic stir bar and was vigorously stirred for 90 minutes. Then the solid product was filtered, washed with water (3×5 mL), dried in air (249 mg of the crude product was obtained; see below the results of ¹H NMR and HPLC analysis, Figure S29A and Figure S30). The crude product was dissolved in chloroform (15–20 mL), filtered through 5 mm layer of Celite® and evaporated under the reduced pressure to afford a solid residue (225 mg; see below the results of ¹H NMR and HPLC analysis, Figures S29B and S31). To remove traces of reagent-derived impurities (NMI, tetramethylurea), the residue was dissolved in ethyl acetate (7–10 mL) under heating, filtered and solid **25** was precipitated by addition of *n*-hexane (15–20 mL), to give 196 mg of pure hexa-amide **25** as white solid (80 % yield, see the results of ¹H NMR and HPLC analysis below, Figures S29C and S32).



$R_f = 0.43$ (10:1 $\text{CHCl}_3/\text{MeOH}$, UV 254 nm). $^1\text{H NMR}$ (CDCl_3 , 400 MHz): δ 7.32 – 7.20 (m, 3H, *m*, *p*-Ph), 7.12 – 7.07 (m, 2H, *o*-Ph), 6.15 (d, $J = 8.0$ Hz, 1H, NH), 4.90 (dt, $J = 8.0, 6.0$ Hz, 1H, CH(13)), 4.86 (br s, 1H, $\text{CH}_2(11)$), 4.73 (br s, 1H, $\text{CH}_2(12)$), 4.07 – 3.93 (m, 1H, CH(2)), 3.93 – 3.80 (m, 1H, CH(3)), 3.71 (s, 3H, $\text{CH}_3(16)$), 3.41 – 3.25 (m, 1H, CH(5)), 3.14 (dd, $J = 13.9, 6.0$ Hz, 1H, $\text{CH}_2(15a)$), 3.12 – 3.04 (m, 1H, $\text{CH}_2(4b)$), 3.06 (dd, $J = 13.9, 6.0$ Hz, 1H, $\text{CH}_2(15b)$), 2.93 (dd, $J = 12.5, 4.9$ Hz, 1H, $\text{CH}_2(4a)$), 2.33 – 2.07 (m, 2H, $\text{CH}_2(9)$), 1.82 – 1.65 (m, 2H, $\text{CH}_2(8a)+\text{CH}_2(6a)$), 1.65 – 1.48 (m, 2H, $\text{CH}_2(8b)+\text{CH}_2(7a)$), 1.48 – 1.26 (m, 2H, $\text{CH}_2(6b)+\text{CH}_2(7b)$). $^{13}\text{C NMR}$ (CDCl_3 , 100.6 MHz): δ 172.42 (C10), 172.25 (C14), 160.47 (C1), 136.00 (*i*-Ph), 129.30 (*o*-Ph), 128.65 (*m*-Ph), 127.19 (*p*-Ph), 62.02 (C2), 59.75 (C3), 53.55 (C5), 53.02 (C13), 52.43 (C16), 51.44 (C11), 46.96 (C12), 38.03 (C15), 35.92 (C9), 34.73 (C4), 27.97 (C7), 27.50 (C6), 24.79 (C8). $[\alpha]_D^{25} = -67.3$ (c 0.62, MeOH). IR (KBr): $\nu = 3319, 2935, 1745, 1702, 1539, 1443, 1373, 1211, 701 \text{ cm}^{-1}$. HRMS (AJS-ESI) calcd. for $\text{C}_{126}\text{H}_{164}\text{N}_{18}\text{O}_{24}\text{S}_6^{2+}$ $[\text{M}+2\text{H}]^{2+}$ 1252.5240, found m/z 1252.5244.

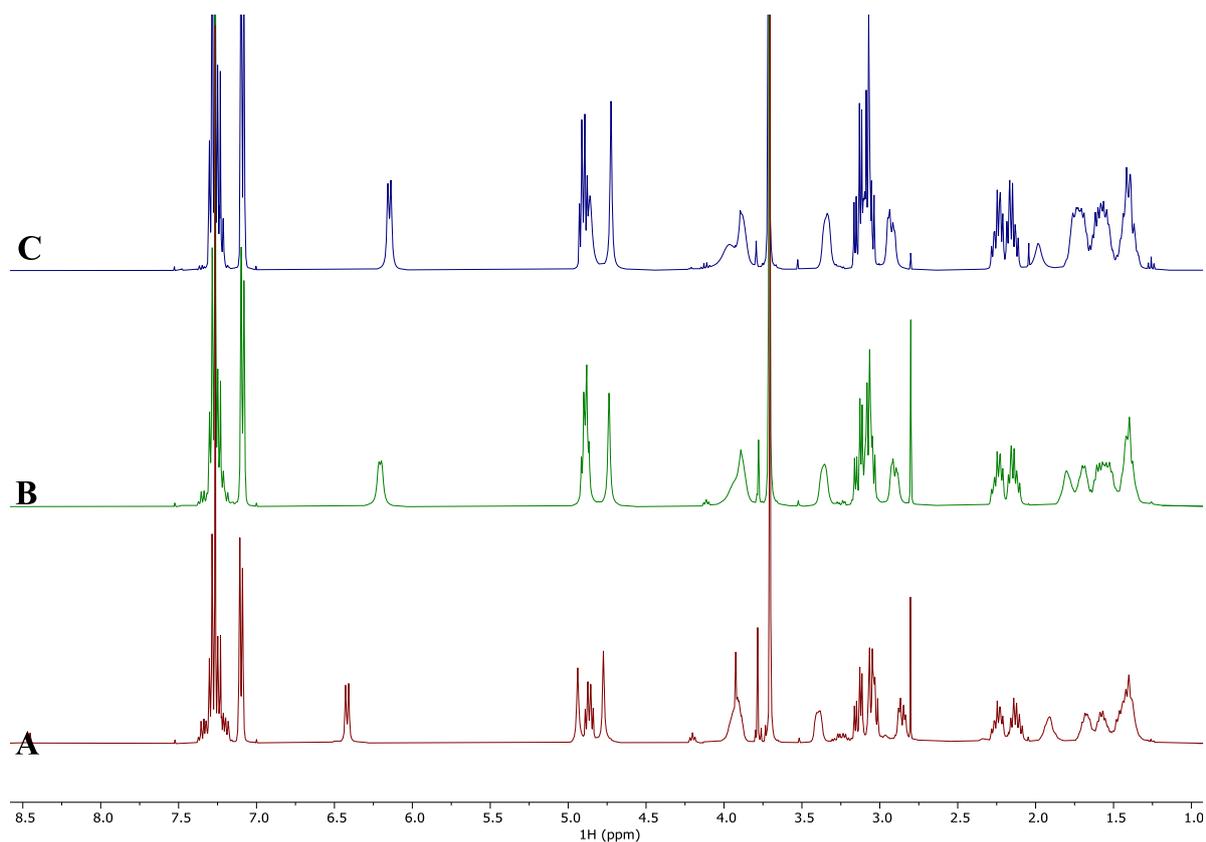


Figure S29. ^1H NMR spectra of hexa-amide **25**, **A** – after washing with water, **B** – after filtration through Celite[®] and **C** – after precipitation with *n*-hexane from ethyl acetate solution.

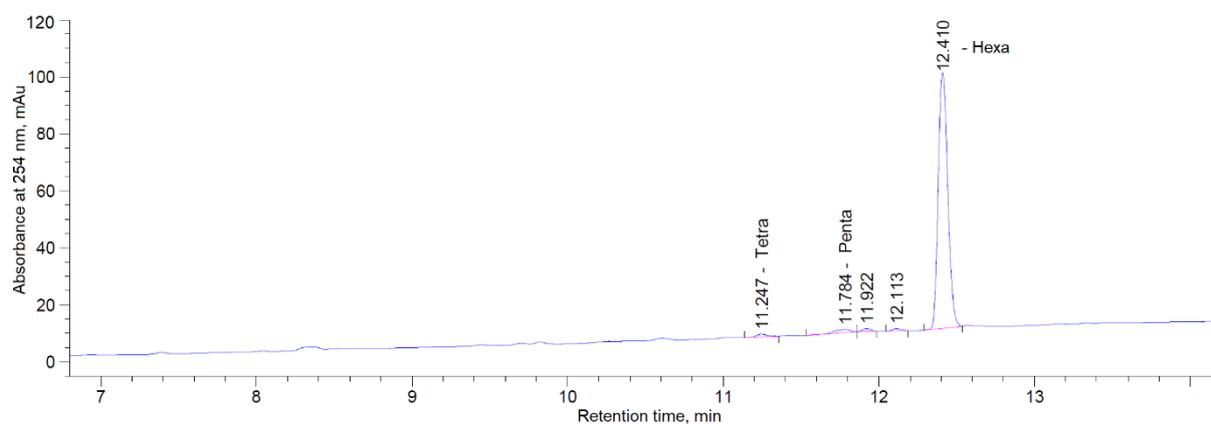


Figure S30. HPLC chromatogram of hexa-amide **25** after washing with water.

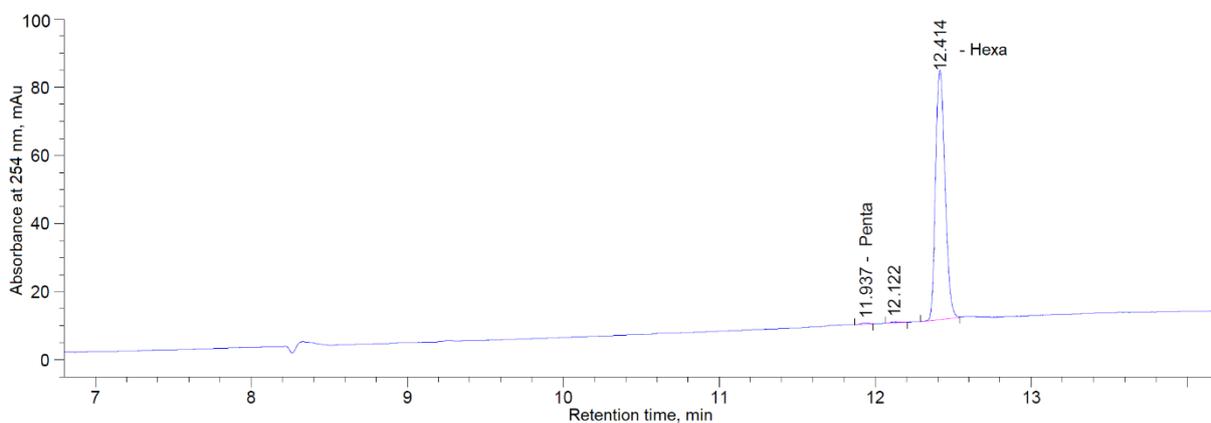


Figure S31. HPLC chromatogram of hexa-amide **25** after filtration through Celite[®]

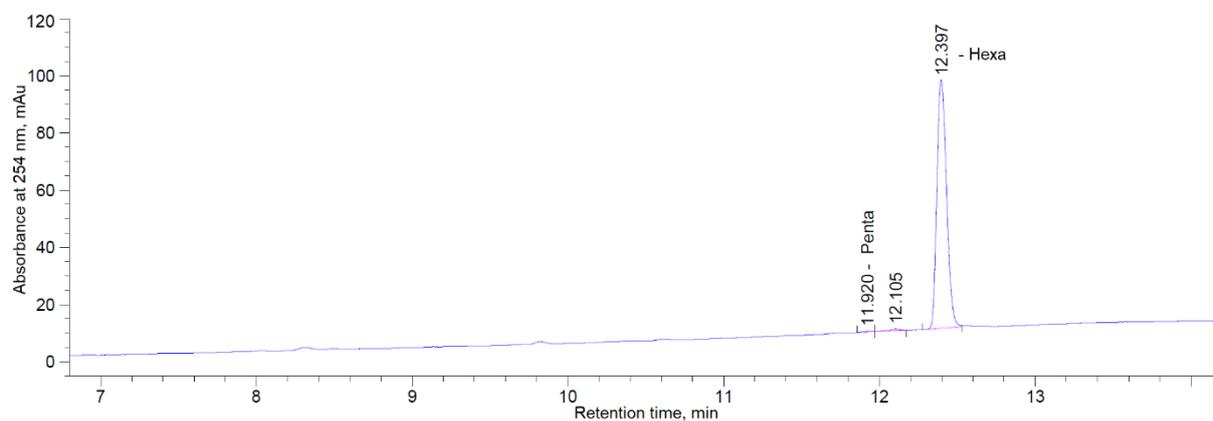


Figure S32. HPLC chromatogram of hexa-amide **25** after precipitation with *n*-hexane from ethyl acetate solution.

Table S11. HPLC purity of hexa-amide product **25**

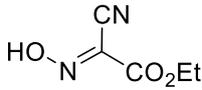
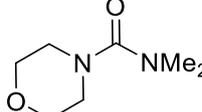
Entry	Purification technique	HPLC purity ^a , %
1	Washing with water	95
2	Filtration through Celite®	99
3	Purification with ethyl acetate/ <i>n</i> -hexane	99

^a Based on HPLC area percentage relative to all other peaks

7. Green Chemistry Metrics

7.1. Safety considerations

Table S12. Hazards of the coupling reagents and by-products derived from them.

Compound	Hazard statements	Meaning	Thermal stability
COMU	H315 H319 H335	Causes skin irritation Causes eye irritation May cause respiratory irritation	Decomposes , thermal onset 127 °C, decomposition energy is −736 J/g ¹³
Oxyrna 	H315 H319 H302, H312, H332	Causes skin irritation Causes eye irritation Acute toxicity	n/a
<i>N,N</i>-dimethylmorpholine-4-carboxamide 	H315 H319 H335 H302	Causes skin irritation Causes eye irritation May cause respiratory irritation Acute toxicity	n/a
TCFH	H315 H319 H335	Causes skin irritation Causes eye irritation May cause respiratory irritation	Probably highly stable (see comments below)
Tetramethylurea	H302 H360	Acute toxicity May damage fertility or the unborn child	n/a

Comments: DMF as being flammable (H226), acute toxic (H312, 332) and reproductive toxin (H360) can be undoubtedly tagged as the major hazard contributor for the solution-based process. COMU and by-products derived from it (Oxyrna, *N,N*-dimethylmorpholine-4-carboxamide) poses skin, eye and respiratory irritation and acute oral toxicity as potential threats, which, however, cannot be considered as severe hazards. On the other hand, COMU contains energetic N–O bond and was listed among the least preferred coupling reagents in the recent study.¹³ It possesses quite large exothermic decomposition energy (−736 J/g) with thermal onset at 127 °C.

To the best of our knowledge, thermal stability of TCFH was not evaluated, however its fluorine analog, *N,N,N',N'*-tetramethylfluoroformamidinium hexafluorophosphate (TFFH) was listed among the most preferred coupling reagents with high thermal decomposition onset 361 °C and low thermal decomposition energy (−209 J/g).¹³

No known risks can be attributed to potassium phosphates, nevertheless KPF₆ by-product possesses acute toxicity (LD₅₀ = 460 mg/kg for rat).¹⁴

7.2.1. COMU/K₂HPO₄, mechanochemistry

Critical elements											
Supply remaining	Flag colour	Note element									
5-50 years	Red Flag										
50-500 years	Amber Flag	+									
+500 years	Green Flag										

Remaining years until depletion of known reserves (based on current rate of extraction)																																																																																																																																																																																																																																						
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Energy (First Pass)		Tick			Tick
Reaction run between 0 to 70°C	Green Flag	+	Reaction run at reflux	Red Flag	
Reaction run between -20 to 0 or 70 to 140°C	Amber Flag		Reaction run 5°C or more below the solvent boiling point	Green Flag	+
Reaction run below -20 or above 140°C	Red Flag				

Batch/flow		Tick	Work Up		List
Flow	Green Flag		quenching	Green Flag	+
Batch	Amber Flag	+	filtration		
			centrifugation		
			crystallisation		
			Low temperature distillation/evaporation/ sublimation		
			solvent exchange, quenching into aqueous solvent	Amber Flag	
			chromatography/ion exchange	Red Flag	
			high temperature multiple recrystallisation		

Health & safety				List substances and H-codes	List substances and H-codes	List substances and H-codes
	Red Flag	Amber Flag	Green Flag			COMU
Highly explosive	H200, H201, H202, H203	H205, H220, H224	If no red or amber flagged H codes present then green flag			Oxyma
Explosive thermal runaway	H230, H240, H250	H241				N,N-dimethylmorpholine-4-carboxamide
Toxic	H300, H310, H330	H301, H311, H331,				
Long Term toxicity	H340, H350, H360, H370, H372	H341, H351, H361, H371, H373				
Environmental implications	H400, H410, H411, H420	H401, H412				
Use of chemicals of environmental concern			List substances of very high concern			
Chemical identified as Substances of Very High Concern by ChemSec which are utilised	Red Flag					

7.2.2. TCFH/K₂HPO₄, mechanochemistry

Critical elements			Remaining years until depletion of known reserves (based on current rate of extraction)																																																																																																																																																																																																																																																																																																			
Supply remaining	Flag colour	Note element	<table border="1"> <tr> <td>1</td><td>H</td><td colspan="14"></td><td>2</td><td>He</td> </tr> <tr> <td>3</td><td>Li</td><td>4</td><td>Be</td><td colspan="10"></td><td>5</td><td>B</td><td>6</td><td>C</td><td>7</td><td>N</td><td>8</td><td>O</td><td>9</td><td>F</td><td>10</td><td>Ne</td> </tr> <tr> <td>11</td><td>Na</td><td>12</td><td>Mg</td><td colspan="10"></td><td>13</td><td>Al</td><td>14</td><td>Si</td><td>15</td><td>P</td><td>16</td><td>S</td><td>17</td><td>Cl</td><td>18</td><td>Ar</td> </tr> <tr> <td>19</td><td>K</td><td>20</td><td>Ca</td><td>21</td><td>Sc</td><td>22</td><td>Ti</td><td>23</td><td>V</td><td>24</td><td>Cr</td><td>25</td><td>Mn</td><td>26</td><td>Fe</td><td>27</td><td>Co</td><td>28</td><td>Ni</td><td>29</td><td>Cu</td><td>30</td><td>Zn</td><td>31</td><td>Ga</td><td>32</td><td>Ge</td><td>33</td><td>As</td><td>34</td><td>Se</td><td>35</td><td>Br</td><td>36</td><td>Kr</td> </tr> <tr> <td>37</td><td>Rb</td><td>38</td><td>Sr</td><td>39</td><td>Y</td><td>40</td><td>Zr</td><td>41</td><td>Nb</td><td>42</td><td>Mo</td><td>43</td><td>Tc</td><td>44</td><td>Ru</td><td>45</td><td>Rh</td><td>46</td><td>Pd</td><td>47</td><td>Ag</td><td>48</td><td>Cd</td><td>49</td><td>In</td><td>50</td><td>Sn</td><td>51</td><td>Sb</td><td>52</td><td>Te</td><td>53</td><td>I</td><td>54</td><td>Xe</td> </tr> <tr> <td>55</td><td>Cs</td><td>56</td><td>Ba</td><td>57</td><td>La*</td><td>58</td><td>Hf</td><td>59</td><td>Ta</td><td>60</td><td>W</td><td>61</td><td>Re</td><td>62</td><td>Os</td><td>63</td><td>Ir</td><td>64</td><td>Pt</td><td>65</td><td>Au</td><td>66</td><td>Hg</td><td>67</td><td>Tl</td><td>68</td><td>Pb</td><td>69</td><td>Bi</td><td>70</td><td>Po</td><td>71</td><td>At</td><td>72</td><td>Rn</td> </tr> <tr> <td>87</td><td>Fr</td><td>88</td><td>Ra</td><td>89</td><td>Ac†</td><td>90</td><td>Rf</td><td>91</td><td>Db</td><td>92</td><td>Sg</td><td>93</td><td>Bh</td><td>94</td><td>Hs</td><td>95</td><td>Mt</td><td>96</td><td>Ds</td><td>97</td><td>Rg</td><td>98</td><td>Uub</td><td>99</td><td>Uut</td><td>100</td><td>Uuq</td><td>101</td><td>Uup</td><td>102</td><td>Lv</td><td>103</td><td>Uus</td><td>104</td><td>Uuo</td> </tr> <tr> <td colspan="3">Lanthanides*</td> <td>58</td><td>Ce</td><td>59</td><td>Pr</td><td>60</td><td>Nd</td><td>61</td><td>Pm</td><td>62</td><td>Sm</td><td>63</td><td>Eu</td><td>64</td><td>Gd</td><td>65</td><td>Tb</td><td>66</td><td>Dy</td><td>67</td><td>Ho</td><td>68</td><td>Er</td><td>69</td><td>Tm</td><td>70</td><td>Yb</td><td>71</td><td>Lu</td> </tr> <tr> <td colspan="3">Actinides†</td> <td>88</td><td>Th</td><td>89</td><td>Pa</td><td>90</td><td>U</td><td>91</td><td>Np</td><td>92</td><td>Pu</td><td>93</td><td>Am</td><td>94</td><td>Cm</td><td>95</td><td>Bk</td><td>96</td><td>Cf</td><td>97</td><td>Es</td><td>98</td><td>Fm</td><td>99</td><td>Md</td><td>100</td><td>No</td><td>101</td><td>Lr</td> </tr> </table>																1	H															2	He	3	Li	4	Be											5	B	6	C	7	N	8	O	9	F	10	Ne	11	Na	12	Mg											13	Al	14	Si	15	P	16	S	17	Cl	18	Ar	19	K	20	Ca	21	Sc	22	Ti	23	V	24	Cr	25	Mn	26	Fe	27	Co	28	Ni	29	Cu	30	Zn	31	Ga	32	Ge	33	As	34	Se	35	Br	36	Kr	37	Rb	38	Sr	39	Y	40	Zr	41	Nb	42	Mo	43	Tc	44	Ru	45	Rh	46	Pd	47	Ag	48	Cd	49	In	50	Sn	51	Sb	52	Te	53	I	54	Xe	55	Cs	56	Ba	57	La*	58	Hf	59	Ta	60	W	61	Re	62	Os	63	Ir	64	Pt	65	Au	66	Hg	67	Tl	68	Pb	69	Bi	70	Po	71	At	72	Rn	87	Fr	88	Ra	89	Ac†	90	Rf	91	Db	92	Sg	93	Bh	94	Hs	95	Mt	96	Ds	97	Rg	98	Uub	99	Uut	100	Uuq	101	Uup	102	Lv	103	Uus	104	Uuo	Lanthanides*			58	Ce	59	Pr	60	Nd	61	Pm	62	Sm	63	Eu	64	Gd	65	Tb	66	Dy	67	Ho	68	Er	69	Tm	70	Yb	71	Lu	Actinides†			88	Th	89	Pa	90	U	91	Np	92	Pu	93	Am	94	Cm	95	Bk	96	Cf	97	Es	98	Fm	99	Md	100	No	101	Lr
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Reaction run below -20 or above 140°C	Red Flag		Green Flag																																																																																																																																																																																																																																																																																																			
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Health & safety			List substances and H-codes				List substances and H-codes				List substances and H-codes																																																																																																																																																																																																																																																																																											
	Red Flag	Amber Flag	Green Flag	Tetramethylurea								TCFH																																																																																																																																																																																																																																																																																										
Highly explosive	H200, H201, H202, H203	H205, H220, H224	If no red or amber flagged H codes present then green flag																																																																																																																																																																																																																																																																																																			
Explosive thermal runaway	H230, H240, H250	H241																																																																																																																																																																																																																																																																																																				
Toxic	H300, H310, H330	H301, H311, H331,																																																																																																																																																																																																																																																																																																				
Long Term toxicity	H340, H350, H360, H370, H372	H341, H351, H361, H371, H373																																																																																																																																																																																																																																																																																																				
Environmental implications	H400, H410, H411, H420	H401, H412																																																																																																																																																																																																																																																																																																				
Use of chemicals of environmental concern			List substances of very high concern																																																																																																																																																																																																																																																																																																			
Chemical identified as Substances of Very High Concern by ChemSec which are utilised			Red Flag	Tetramethylurea																																																																																																																																																																																																																																																																																																		

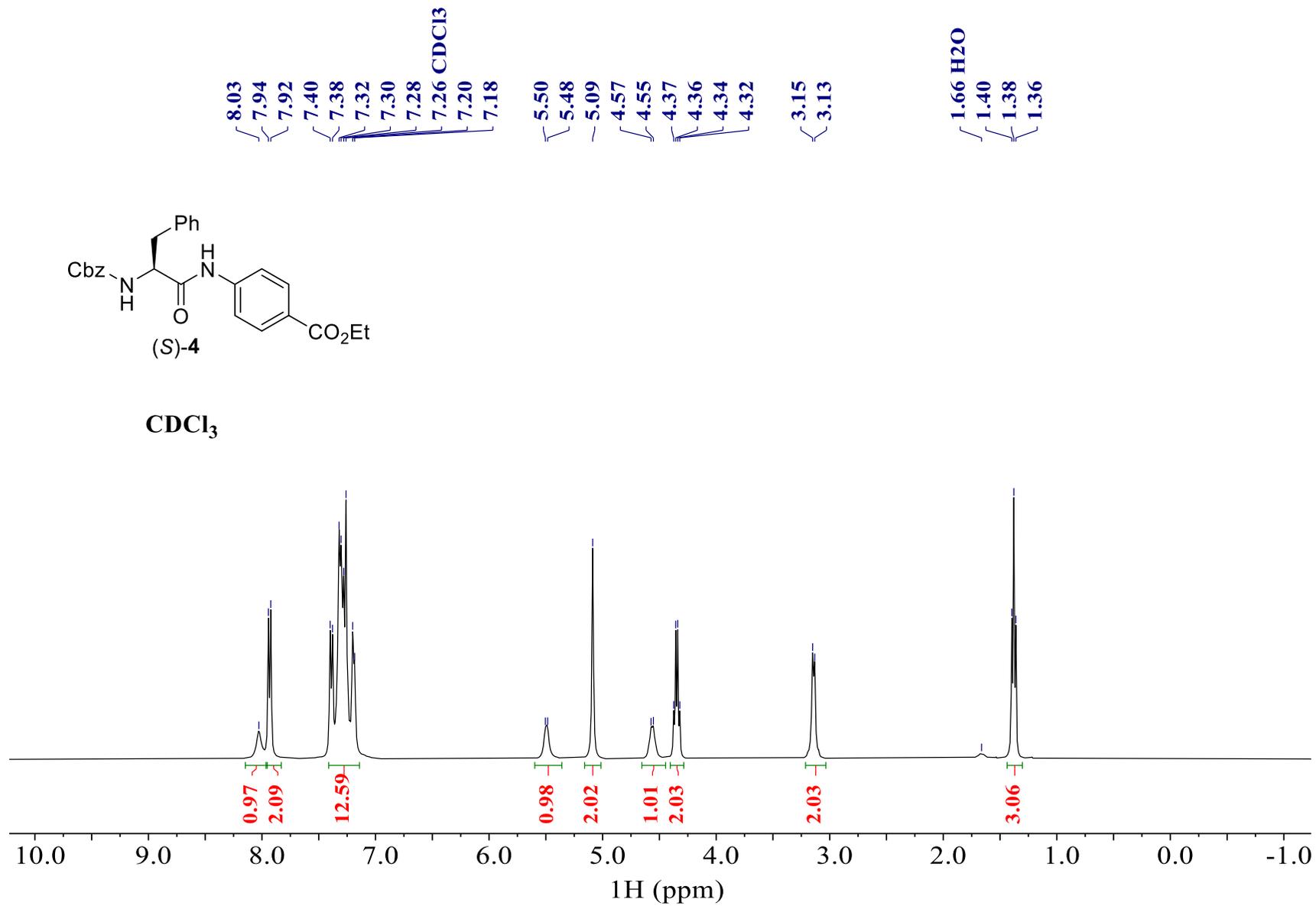
7.2.3. COMU/DIPEA, in solution (following the published procedure¹⁵)

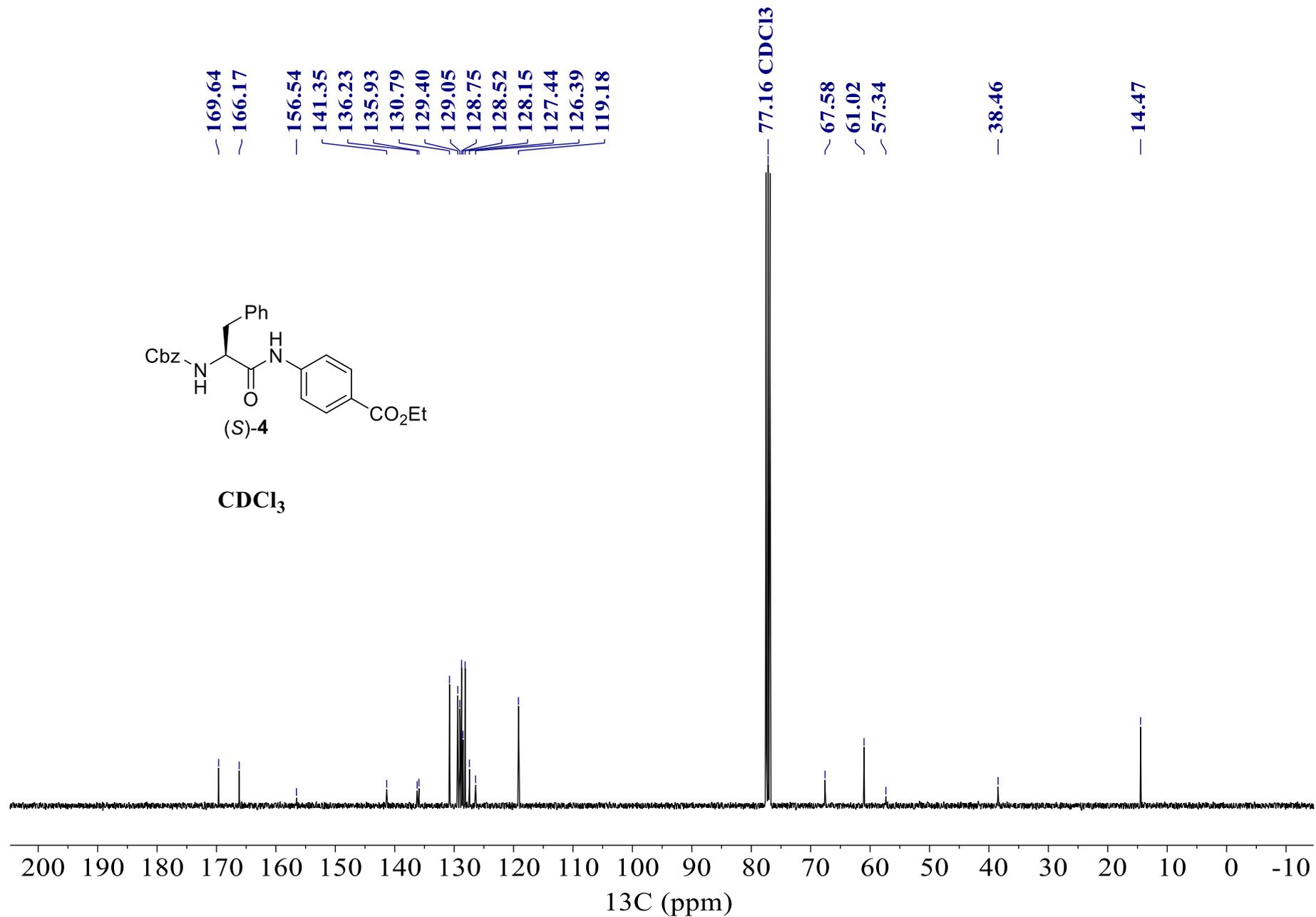
Critical elements			Remaining years until depletion of known reserves (based on current rate of extraction)												
Supply remaining	Flag colour	Note element													
5-50 years	Red Flag														
50-500 years	Amber Flag	+													
+500 years	Green Flag														
Energy (First Pass)	Tick	Tick	Reaction run between 0 to 70°C										Reaction run at reflux		
Reaction run between -20 to 0 or 70 to 140°C	Green Flag	+											Red Flag		
Reaction run below -20 or above 140°C	Amber Flag												Reaction run 5°C or more below the solvent boiling point		
	Red Flag												Green Flag		
													+		
Batch/flow	Tick	Tick	Work Up										List		
Flow	Green Flag		quenching										Green Flag		
Batch	Amber Flag		filtration										Green Flag		
			centrifugation										Green Flag		
			crystallisation										Green Flag		
			Low temperature distillation/evaporation/ sublimation										Green Flag		
			solvent exchange, quenching into aqueous solvent										Amber Flag		
			chromatography/ion exchange										Red Flag		
			high temperature										Red Flag		
			multiple recrystallisation										Red Flag		
Health & safety	Red Flag	Amber Flag	Green Flag	List substances and H-codes			List substances and H-codes			List substances and H-codes					
Highly explosive	H200, H201, H202, H203	H205, H220, H224	If no red or amber flagged H codes present then green flag	DMF			DIPEA			COMU					
Explosive thermal runaway	H230, H240, H250	H241								Oxyma					
Toxic	H300, H310, H330	H301, H311, H331,								N,N - dimethylmorpholine-4-carboxamide					
Long Term toxicity	H340, H350, H360, H370, H372	H341, H351, H361, H371, H373													
Environmental implications	H400, H410, H411, H420	H401, H412													
Use of chemicals of environmental concern				List substances of very high concern											
Chemical identified as Substances of Very High Concern by ChemSec which are utilised				Red Flag	DMF										

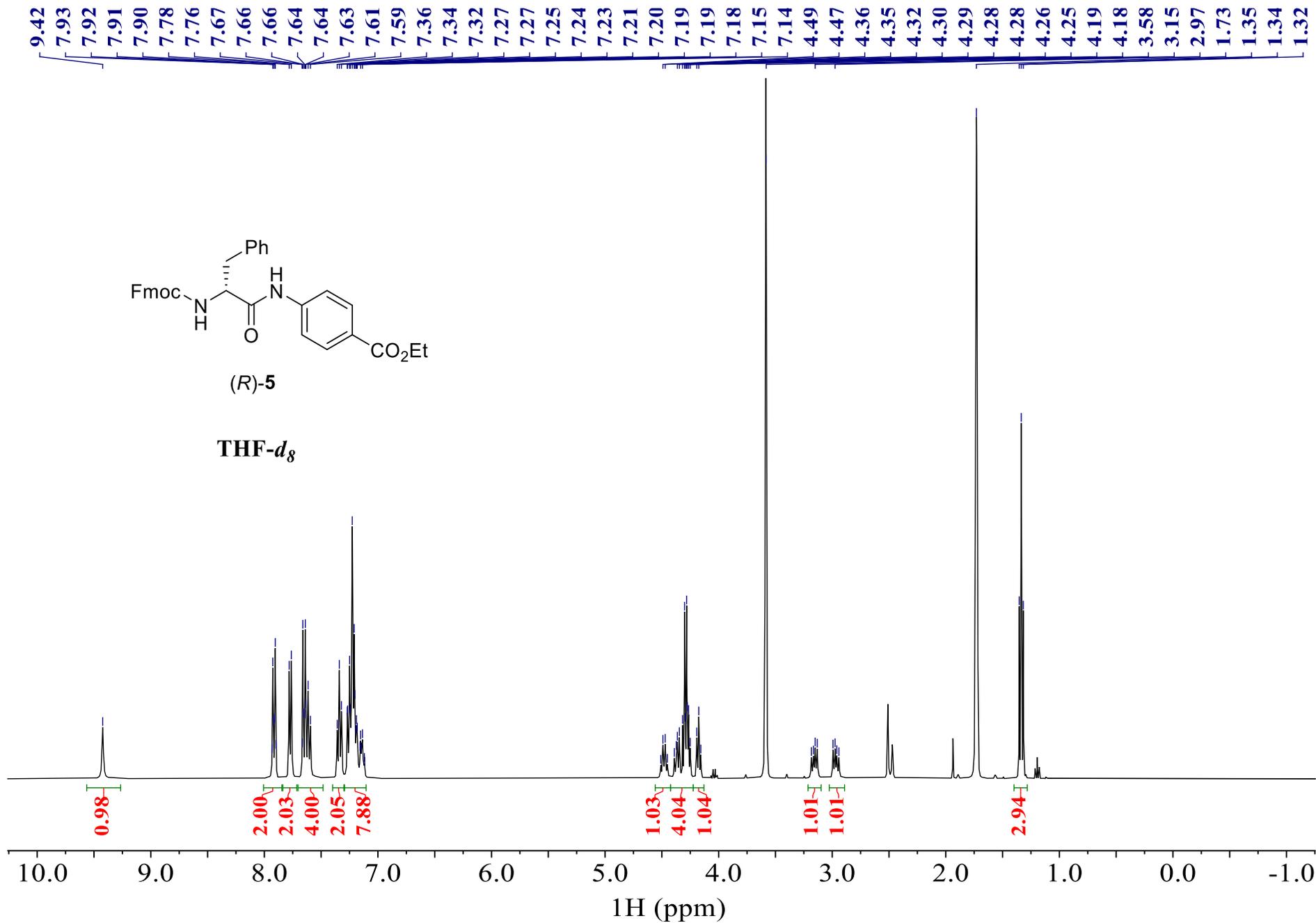
8. References

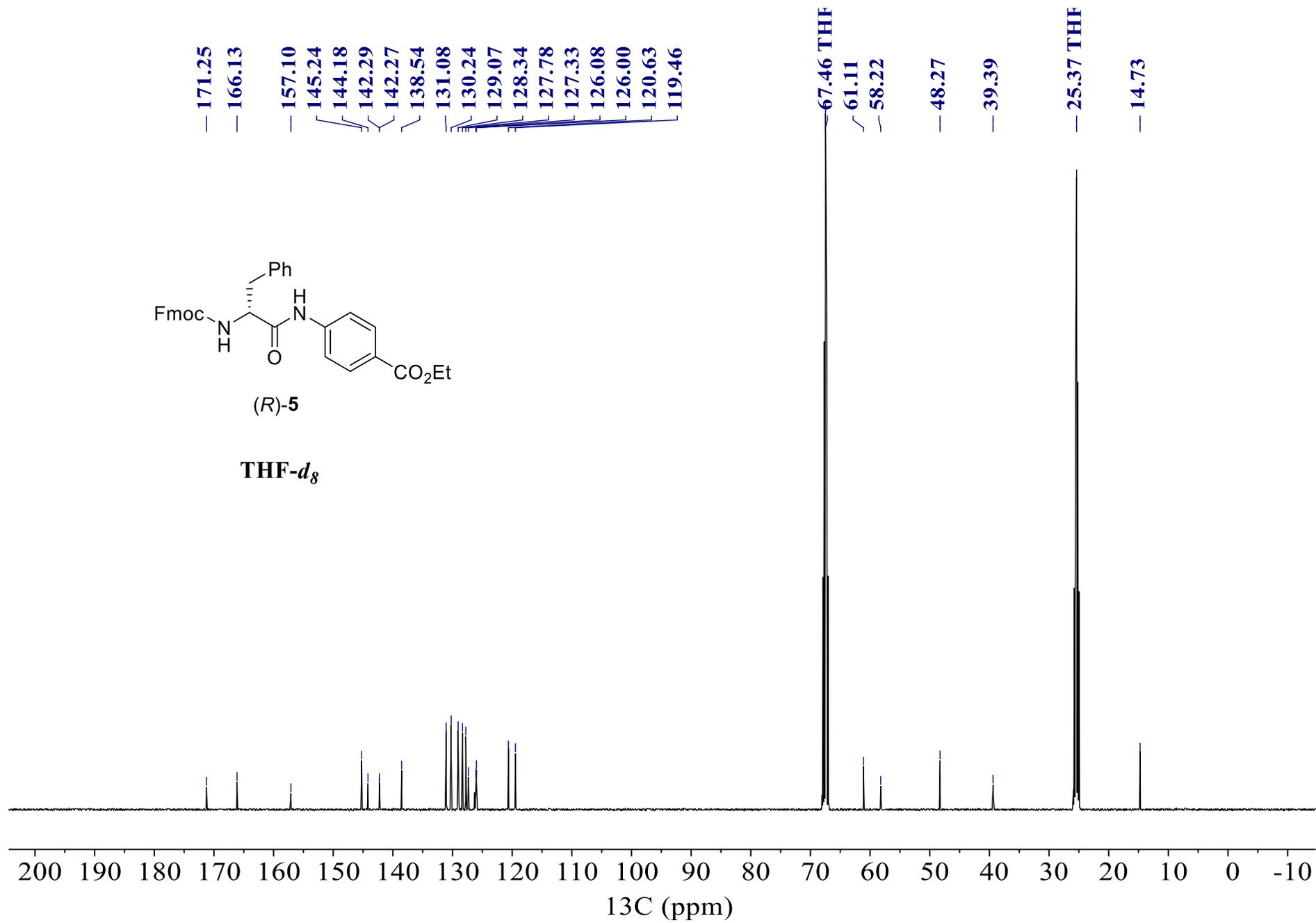
- (1) Štrukil, V.; Bartolec, B.; Portada, T.; Đilović, I.; Halasz, I.; Margetić, D. *Chem. Commun.* **2012**, 99, 12100–12102.
- (2) Subirós-Funosas, R.; Nieto-Rodríguez, L.; Jensen, K. J.; Albericio, F. *J. Pept. Sci.* **2013**, 19, 408–414.
- (3) Kumar, A.; Jad, Y. E.; de la Torre, B. G.; El-Faham, A.; Albericio, F. *J. Pept. Sci.* **2017**, 23, 763–768.
- (4) Chandra, J.; Manne, S. R.; Mondal, S.; Mandal, B. *ACS Omega* **2018**, 3, 6, 6120–6133.
- (5) Prabhu, G.; Dr. Nagendra, G.; Sagar, N. R.; Pal, R.; Prof. Dr. Guru Row, T. N.; Prof. Dr. Sureshbabu, V.V. *Asian J. Org. Chem.* **2016**, 5, 127–137.
- (6) Erapalapati, V.; Hale, U. A.; Madhavan, N. *Tetrahedron Letters* **2019**, 60, 15131.
- (7) Khalifa, M.A.; Ismail, M. F.; Eisa, S. I.; Ammar, Y. *Der Pharma Chemica* **2012**, 4, 1552–1566.
- (8) Amir, M.; Khan, M. S. Y.; Zaman, M. S. *IJC-B.* **2004**, 43B, 2189–2194.
- (9) Inamoto, K.; Shiraiishi, M.; Hiroya, K.; Doi, T. *Synthesis* **2010**, 18, 3087–3090.
- (10) Correa, A.; Elmore, S.; Bolm, C. *Chem. Eur. J.* **2008**, 14, 3527–3529.
- (11) Beutner, G. L.; Young, I. S.; Davies, M. L.; Hickey, M. R.; Park, H.; Stevens, J. M.; Ye, Q. *Org. Lett.* **2018**, 20, 4218–4222.
- (12) Larrivéé-Aboussafy, C.; Jones, B.P.; Price, K.E.; Hardink, M. A.; McLaughlin, R.A.; Lillie, B. M.; Hawkins, J. M.; Vaidyanathan, R. *Org. Lett.* **2010**, 12, 324–327.
- (13) Sperry, J. B.; Minter, C. J.; Tao, J.; Johnson, R.; Duzguner, R.; Hawksworth, M.; Oke, S.; Richardson, P. F.; Barnhart, R.; Bill, D. R.; et al. *Org. Process Res. Dev.* **2018**, 22, 1262–1275.
- (14) Hodge, H. C.; Smith, F. A. In *Fluorine chemistry*, vol. 4, ed. by Simons, J. H; Academic Press, New York, 1965, p. 39.
- (15) El-Fahama, A., Albericio, F. *J. Pept. Sci.* **2010**, 16, 6–9.

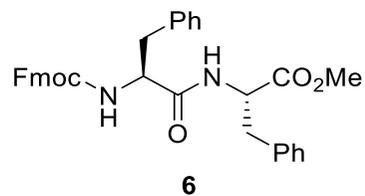
9. ^1H and ^{13}C NMR spectra



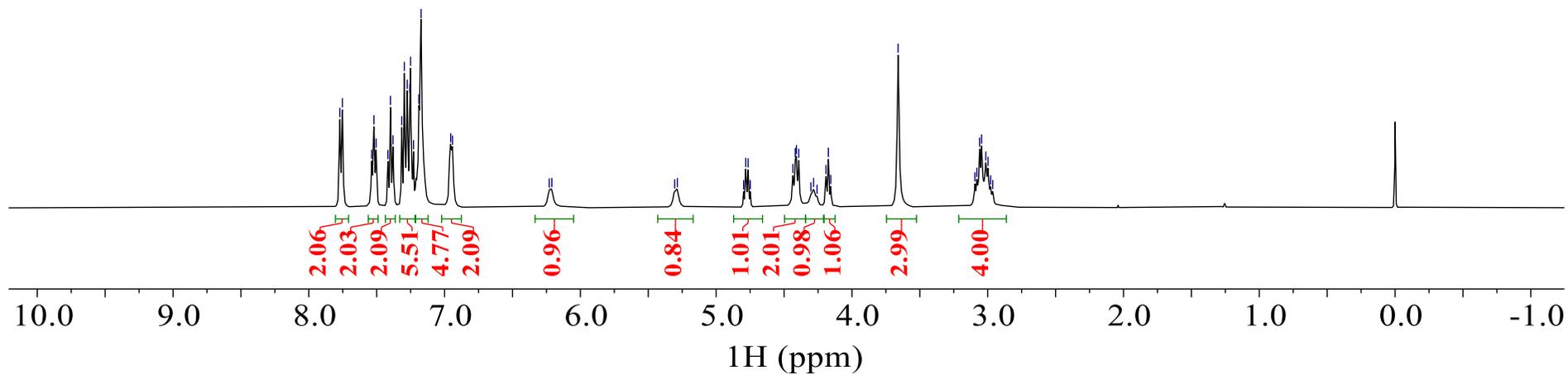






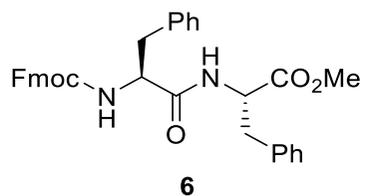


CDCl₃

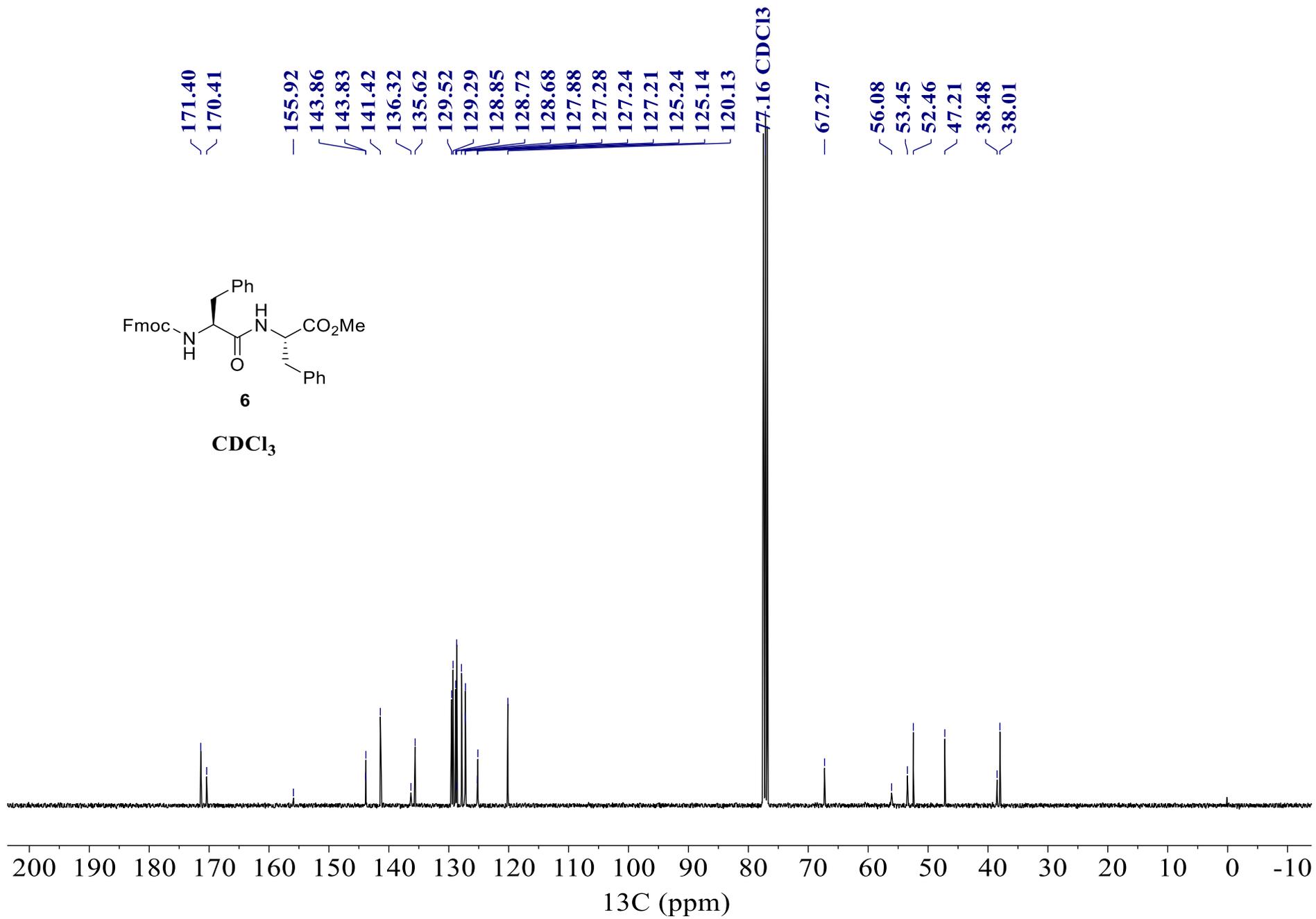


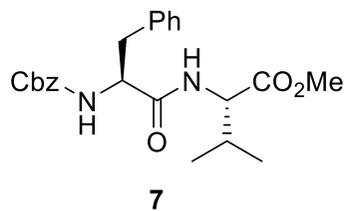
7.77
7.75
7.52
7.50
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7.38
7.32
7.30
7.28
7.26
7.25
7.23
7.19
7.17
6.96
6.94
6.23
6.21

5.31
5.29
4.80
4.78
4.76
4.75
4.44
4.42
4.41
4.39
4.30
4.28
4.26
4.19
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4.16
3.66
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3.04
3.01
3.00
2.98
2.96

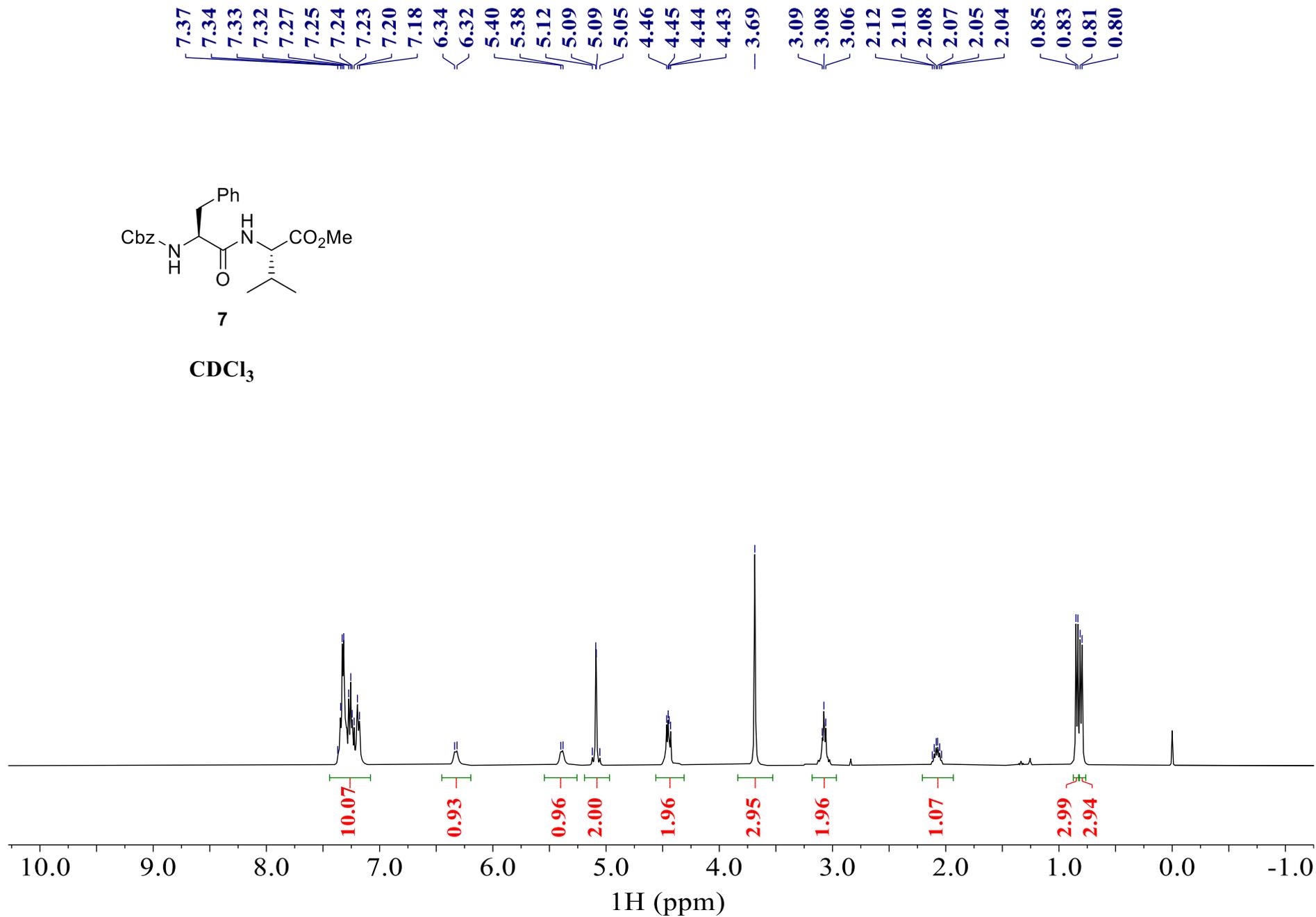


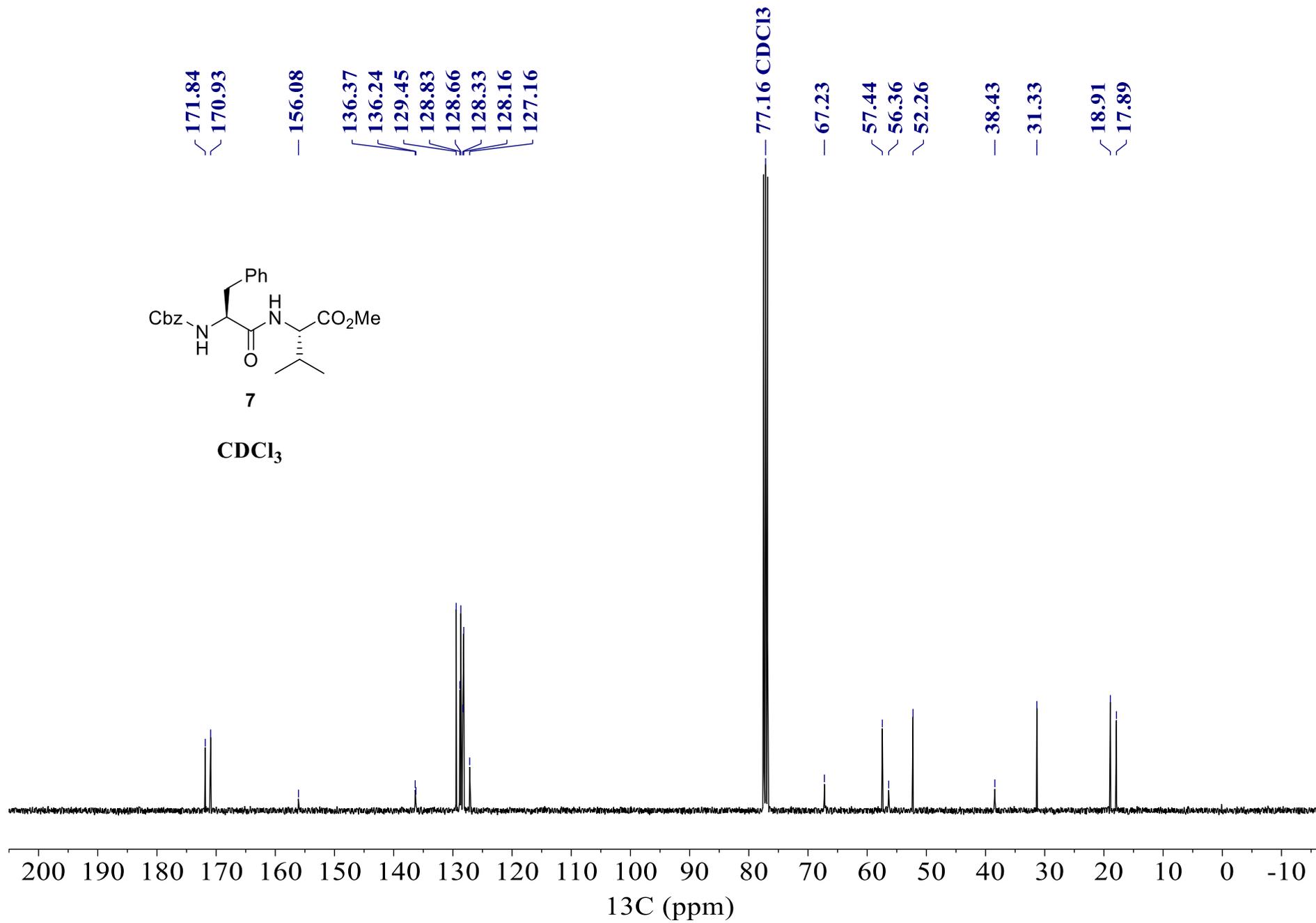
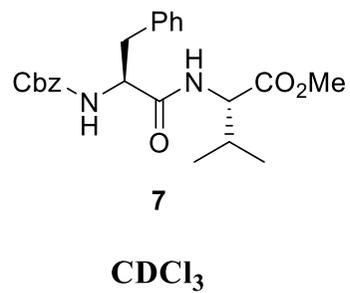
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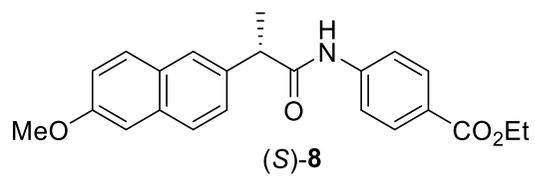




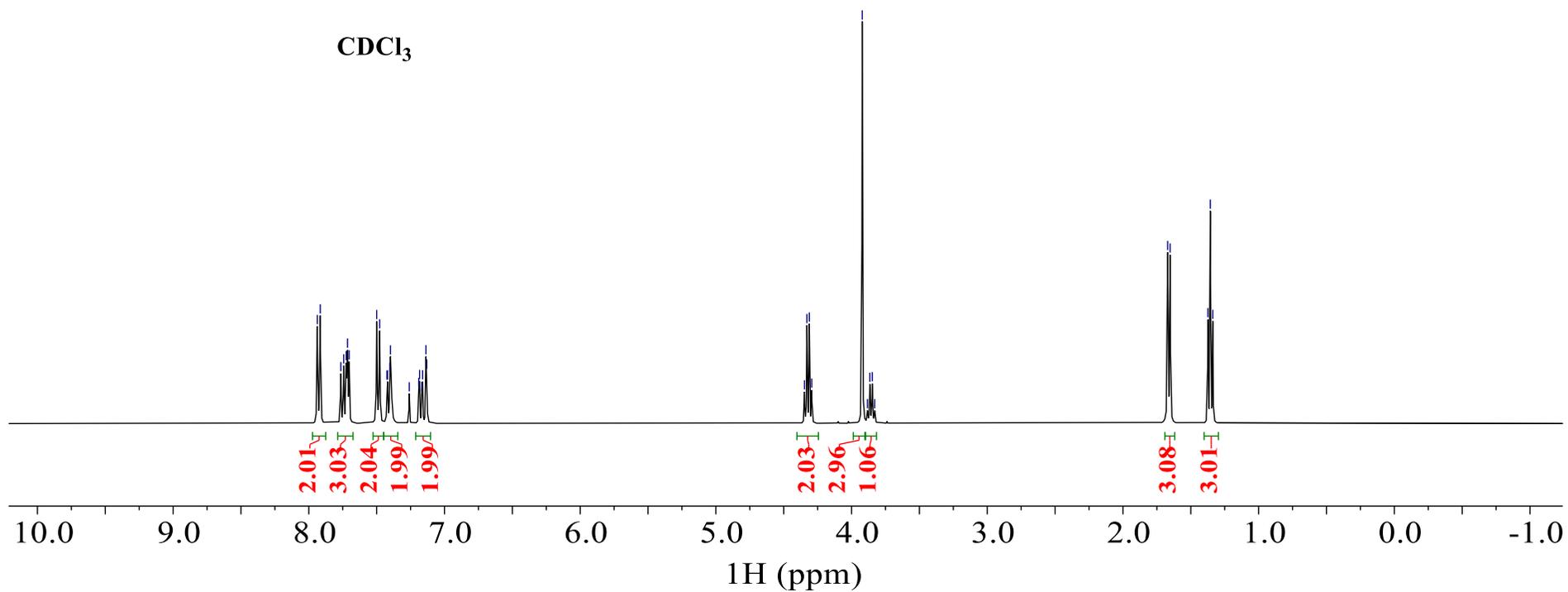
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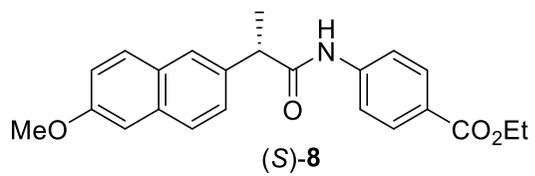




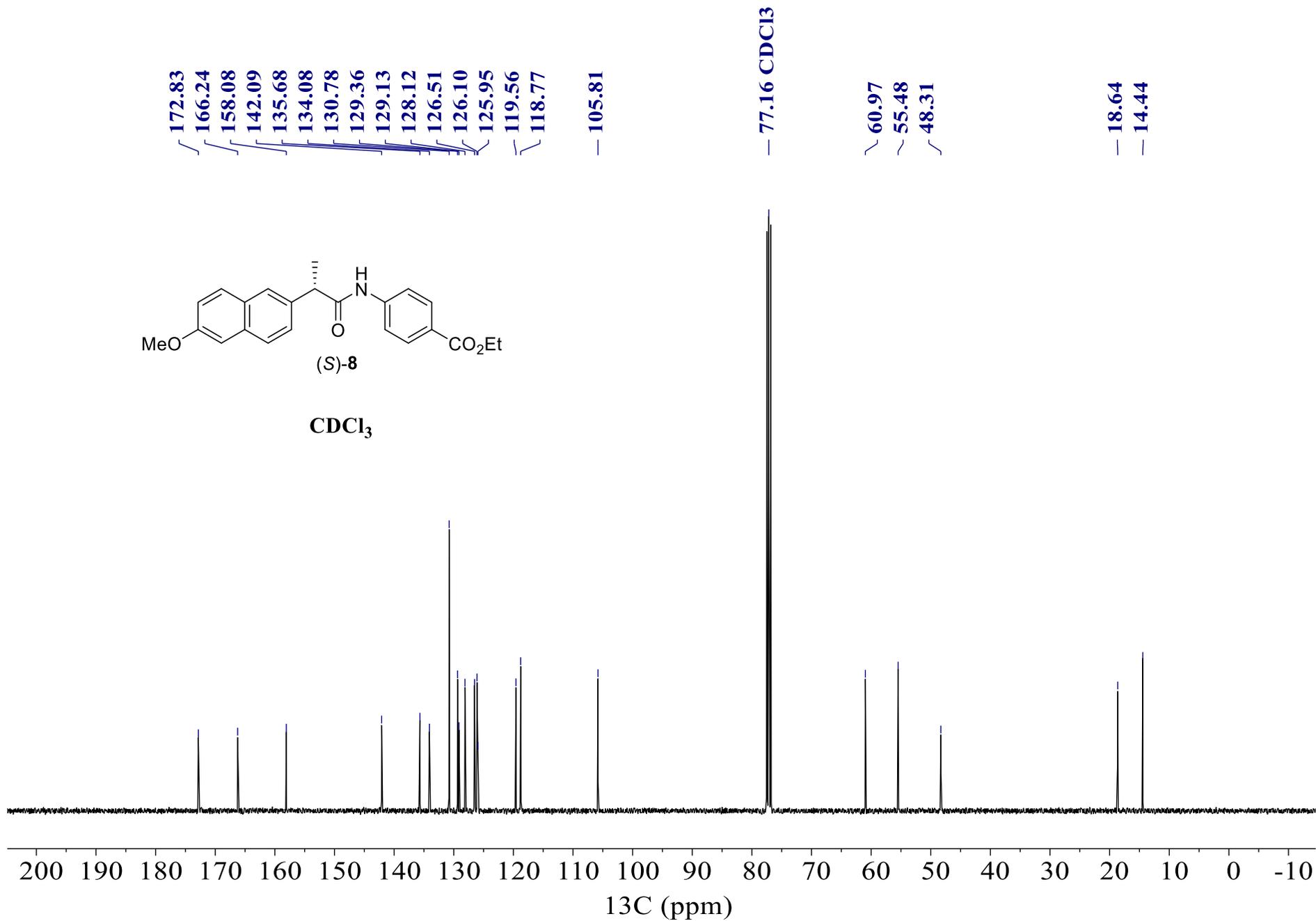


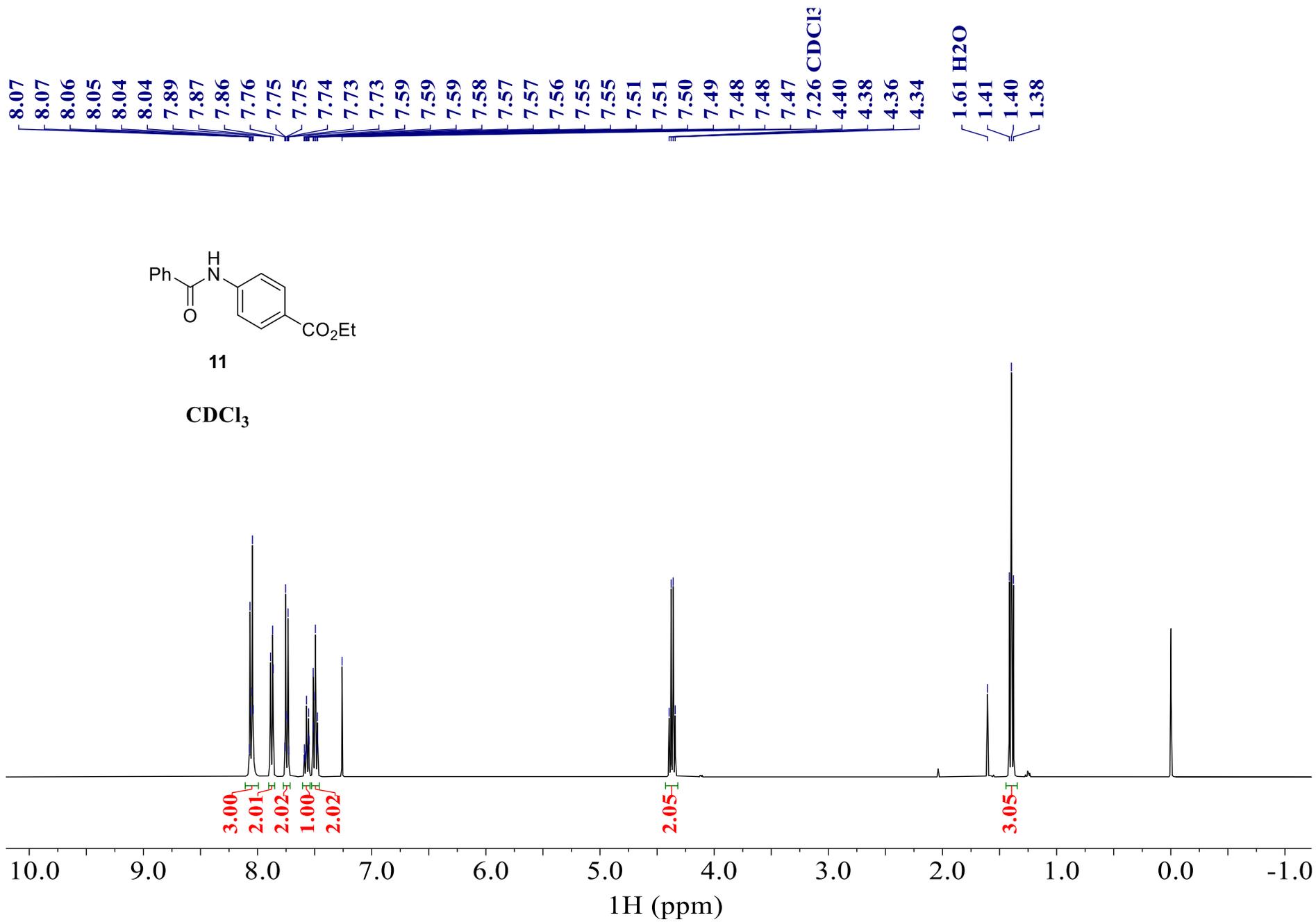
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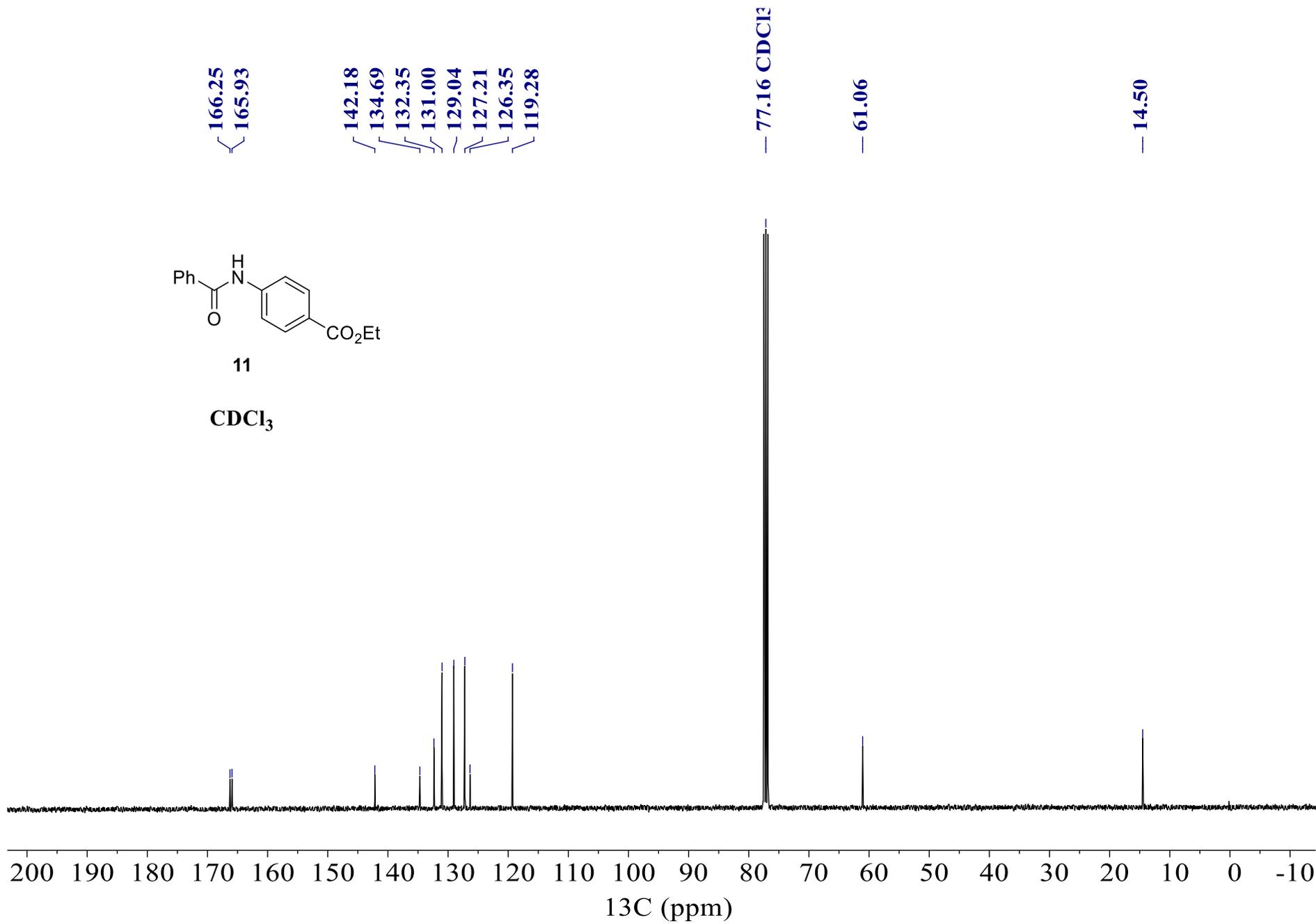


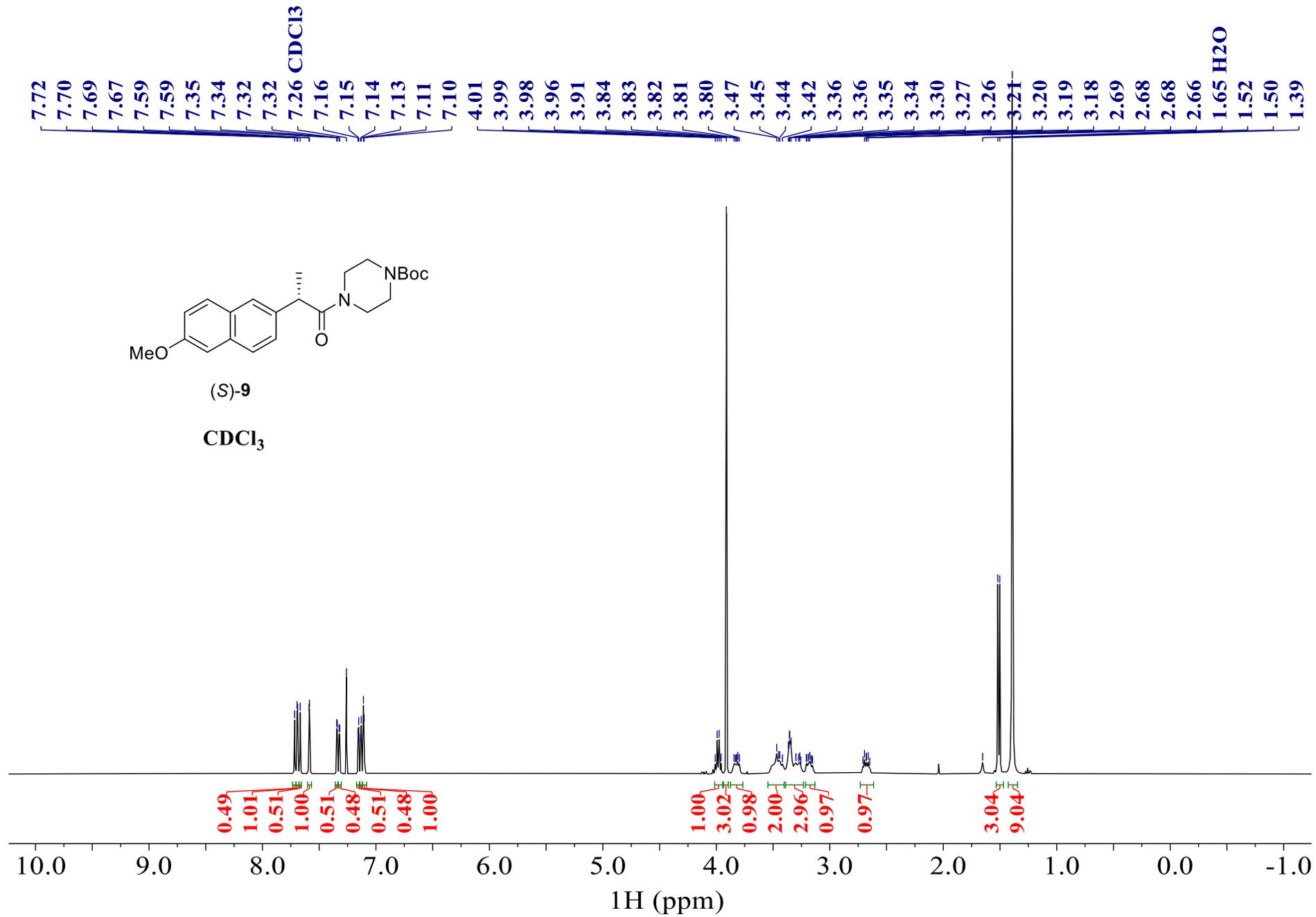


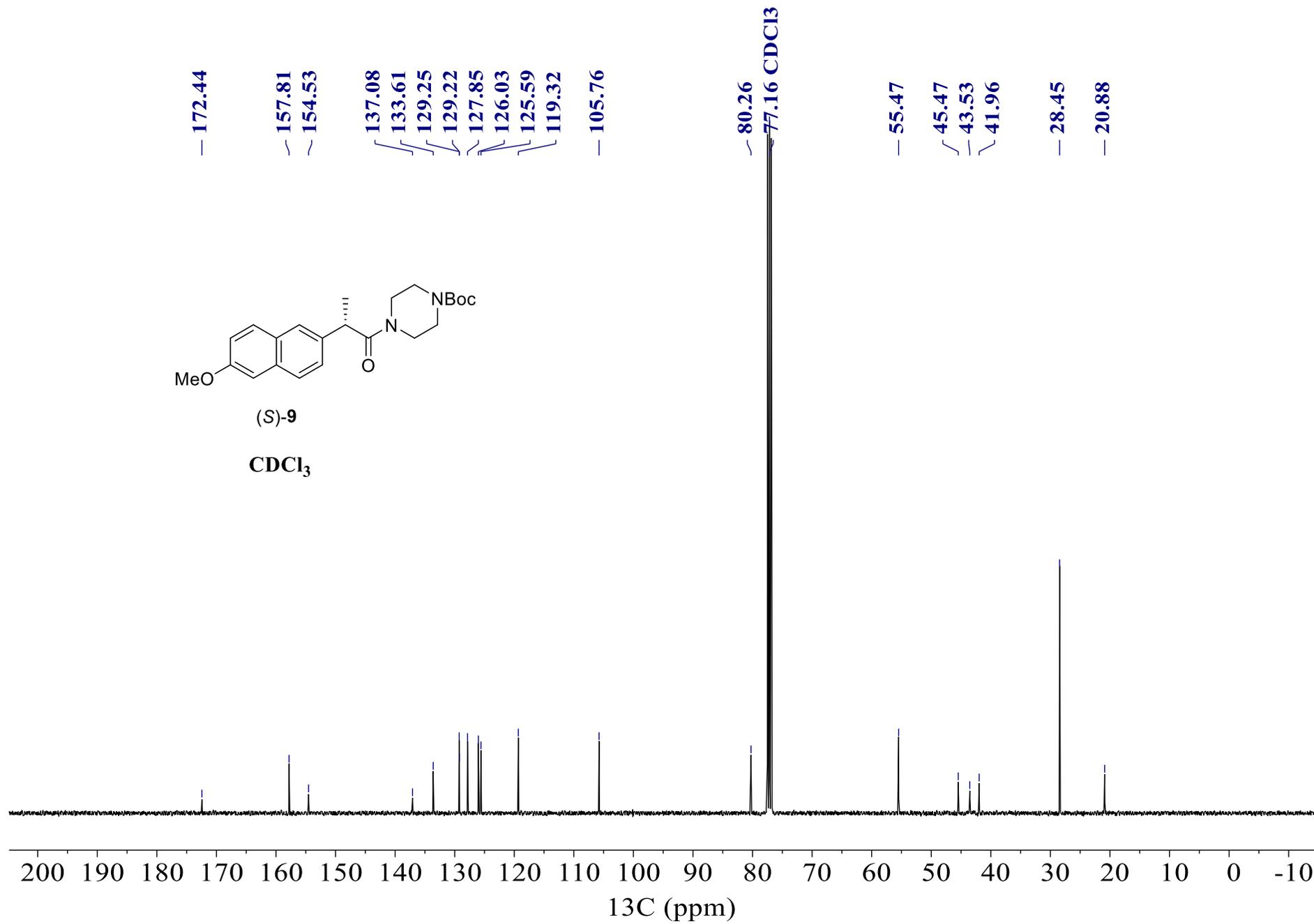
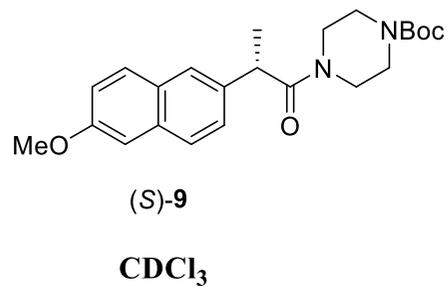
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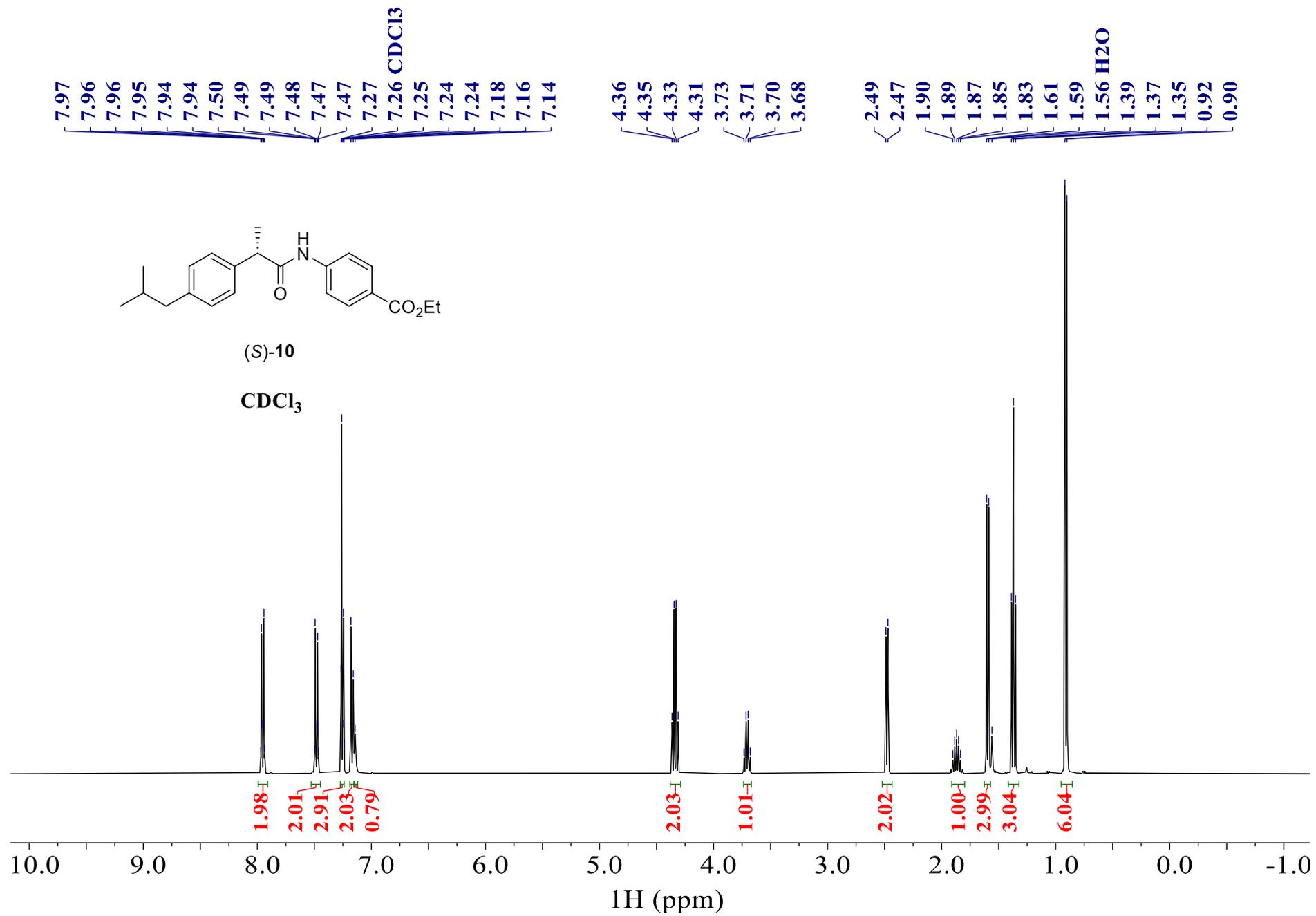


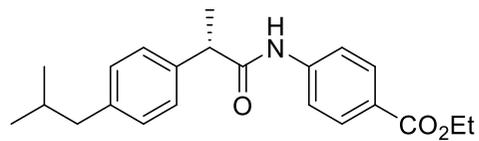












(S)-10

CDCl₃

— 172.90

— 166.25

142.11

141.48

137.79

130.82

130.14

127.56

125.97

118.72

— 77.16 CDCl₃

— 60.98

— 48.06

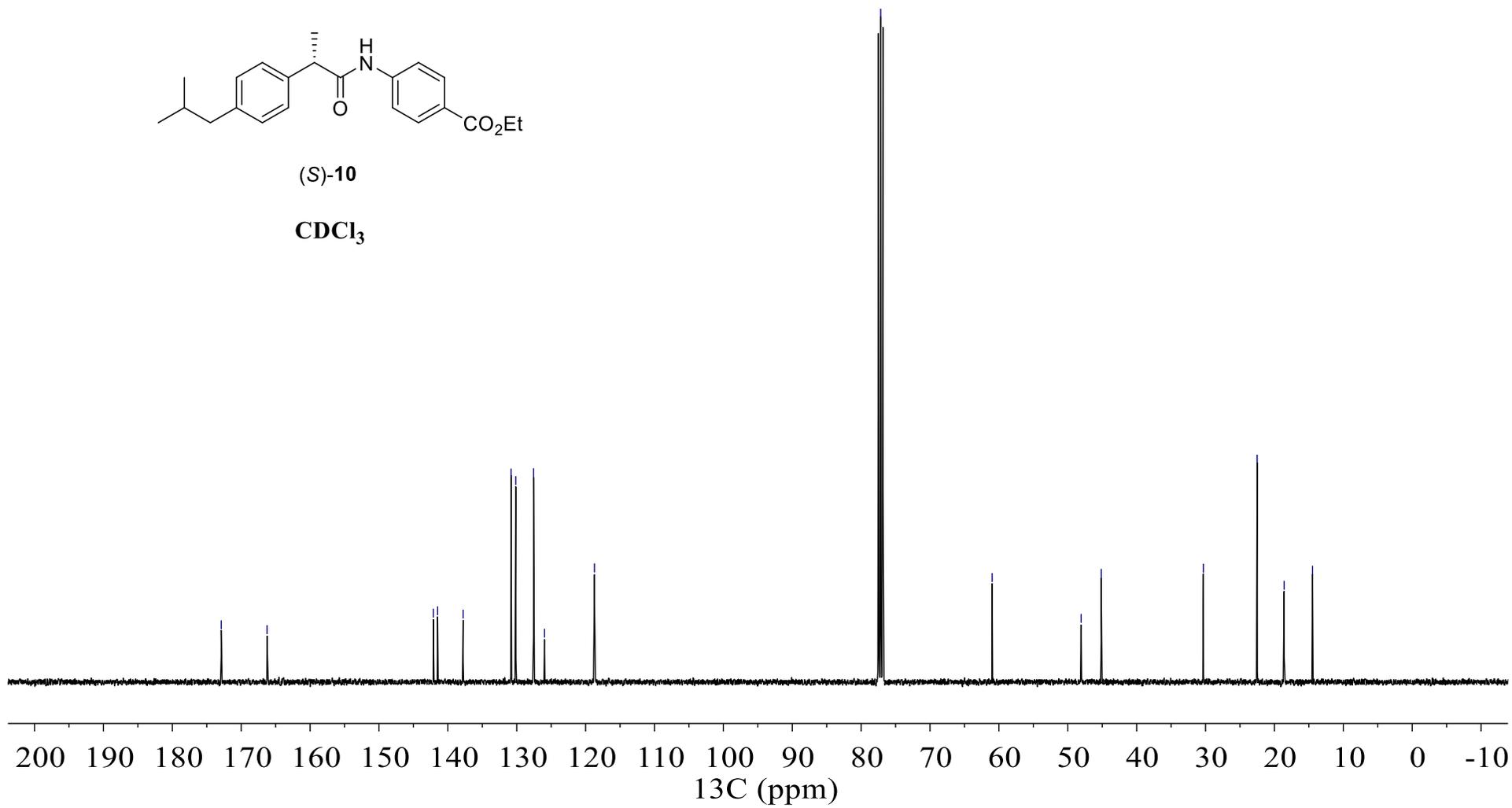
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— 30.31

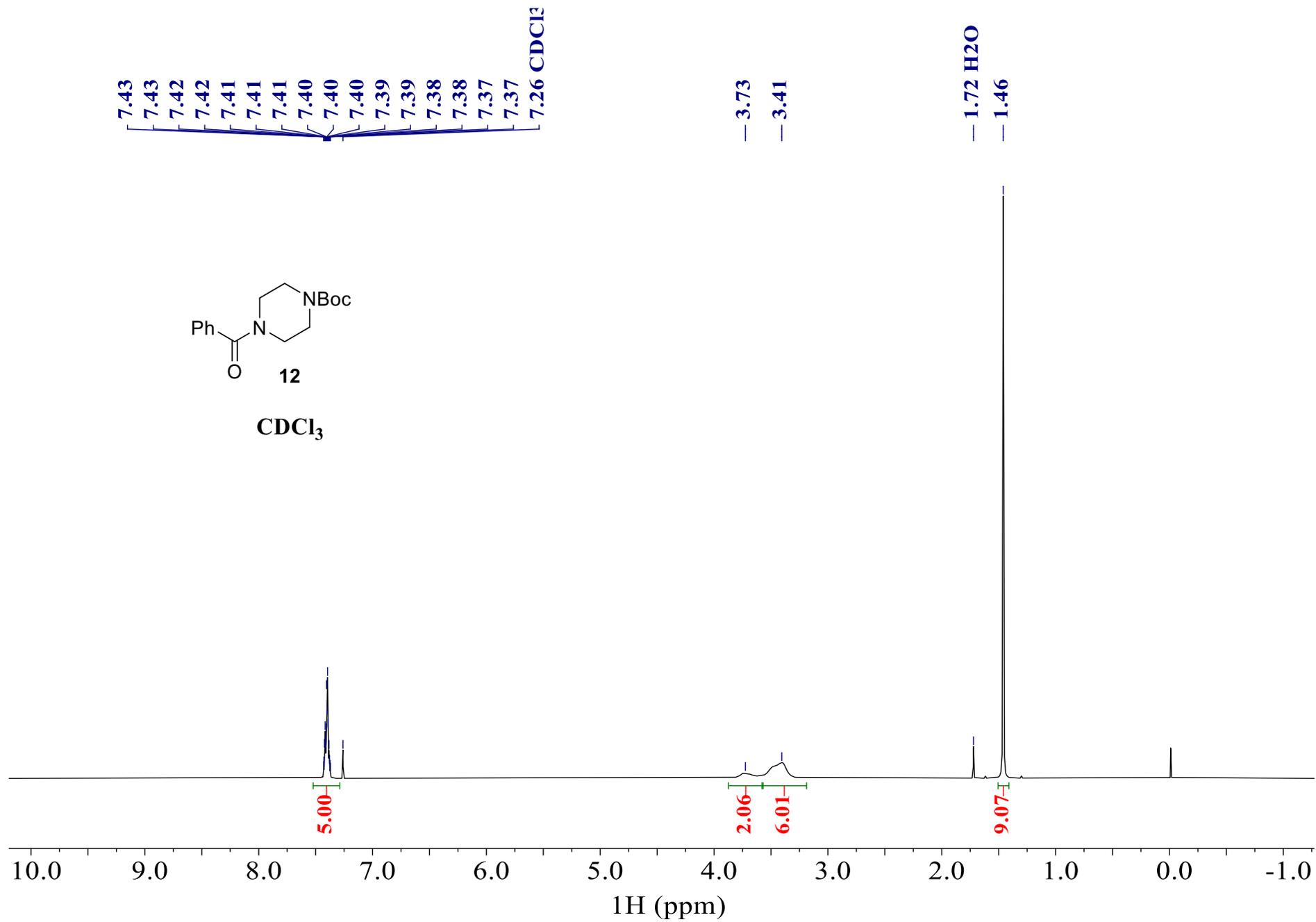
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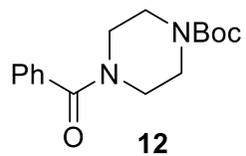
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— 14.47

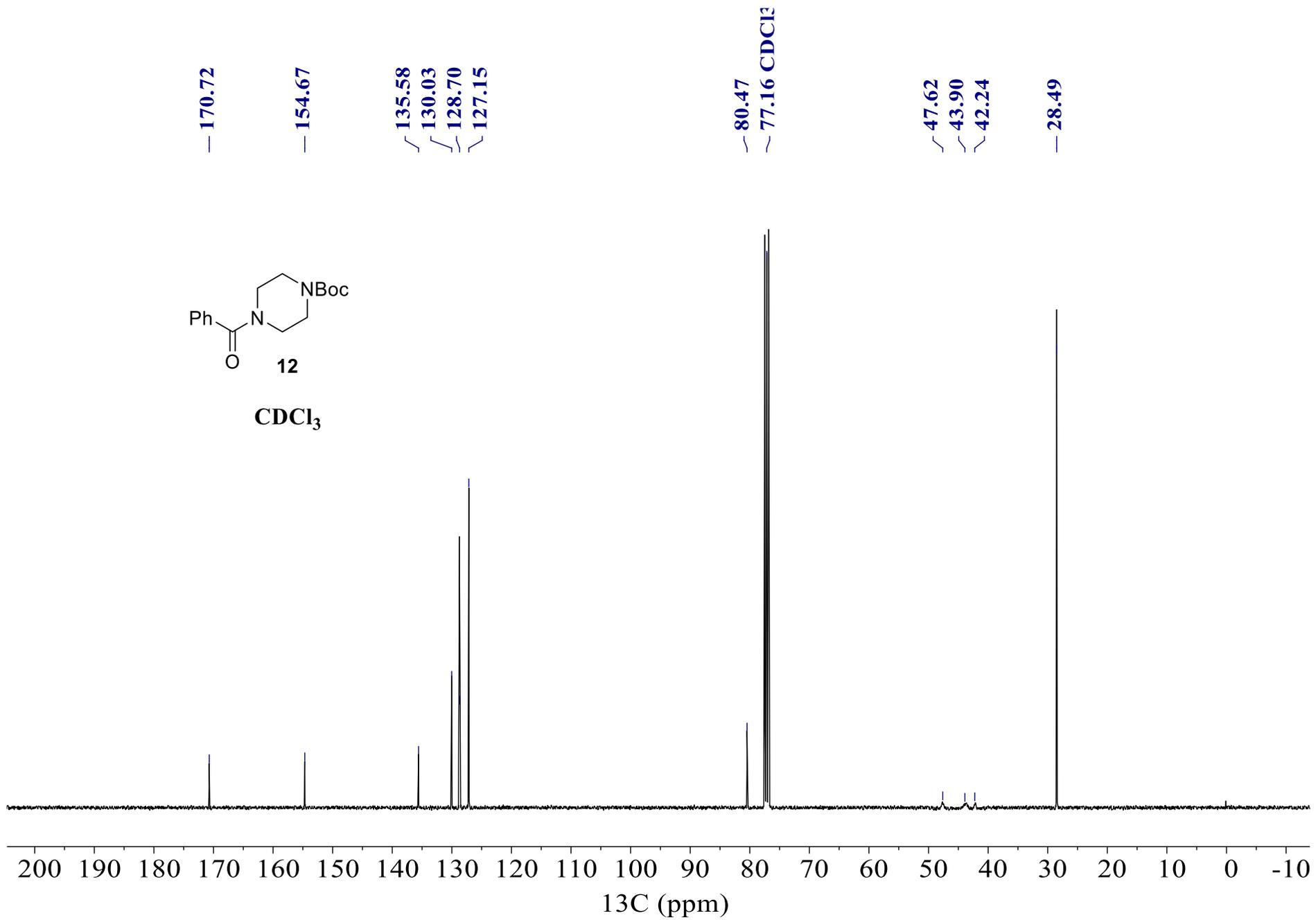


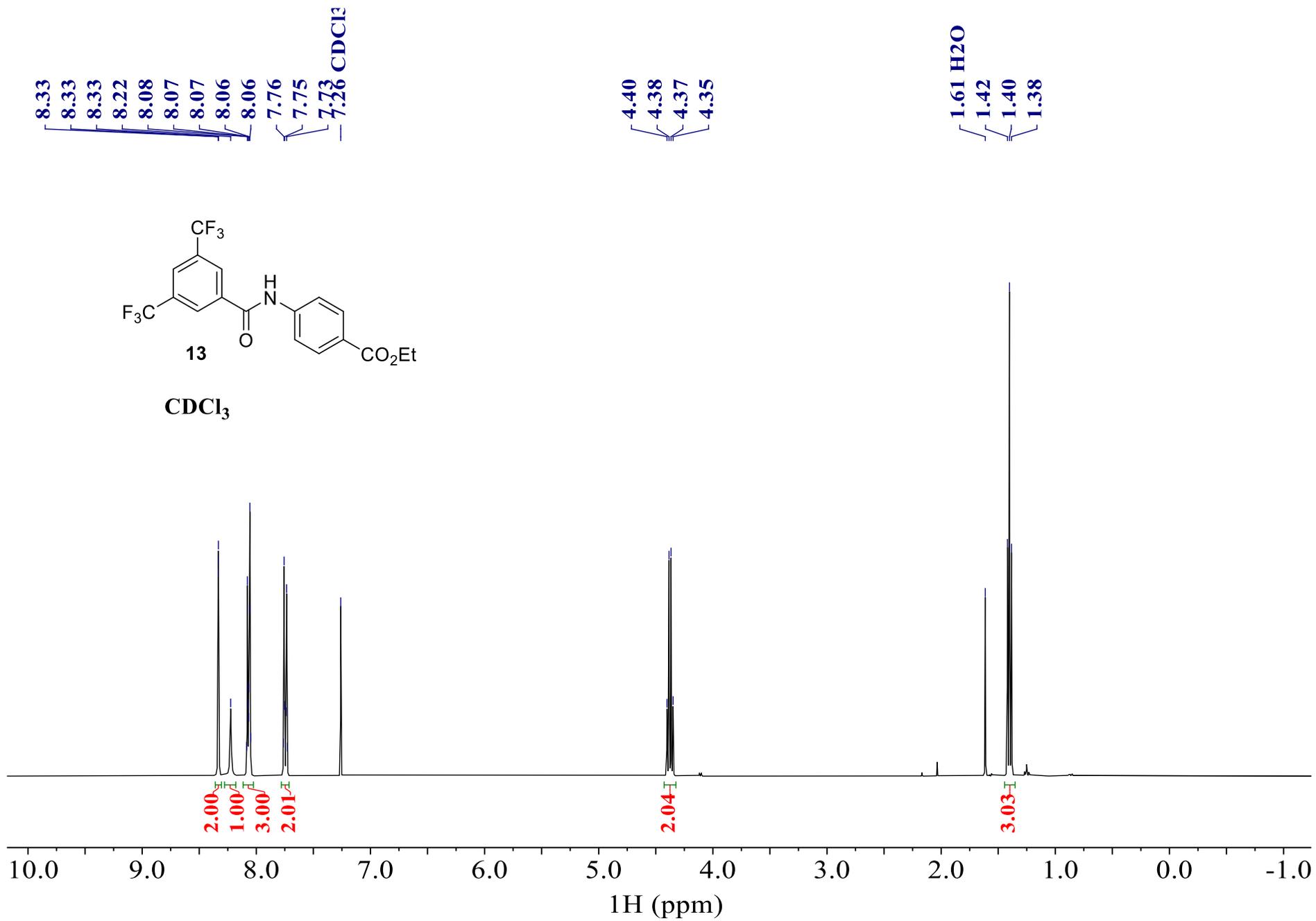
S63

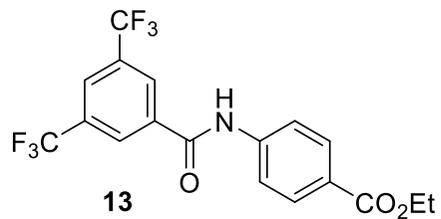




CDCl_3





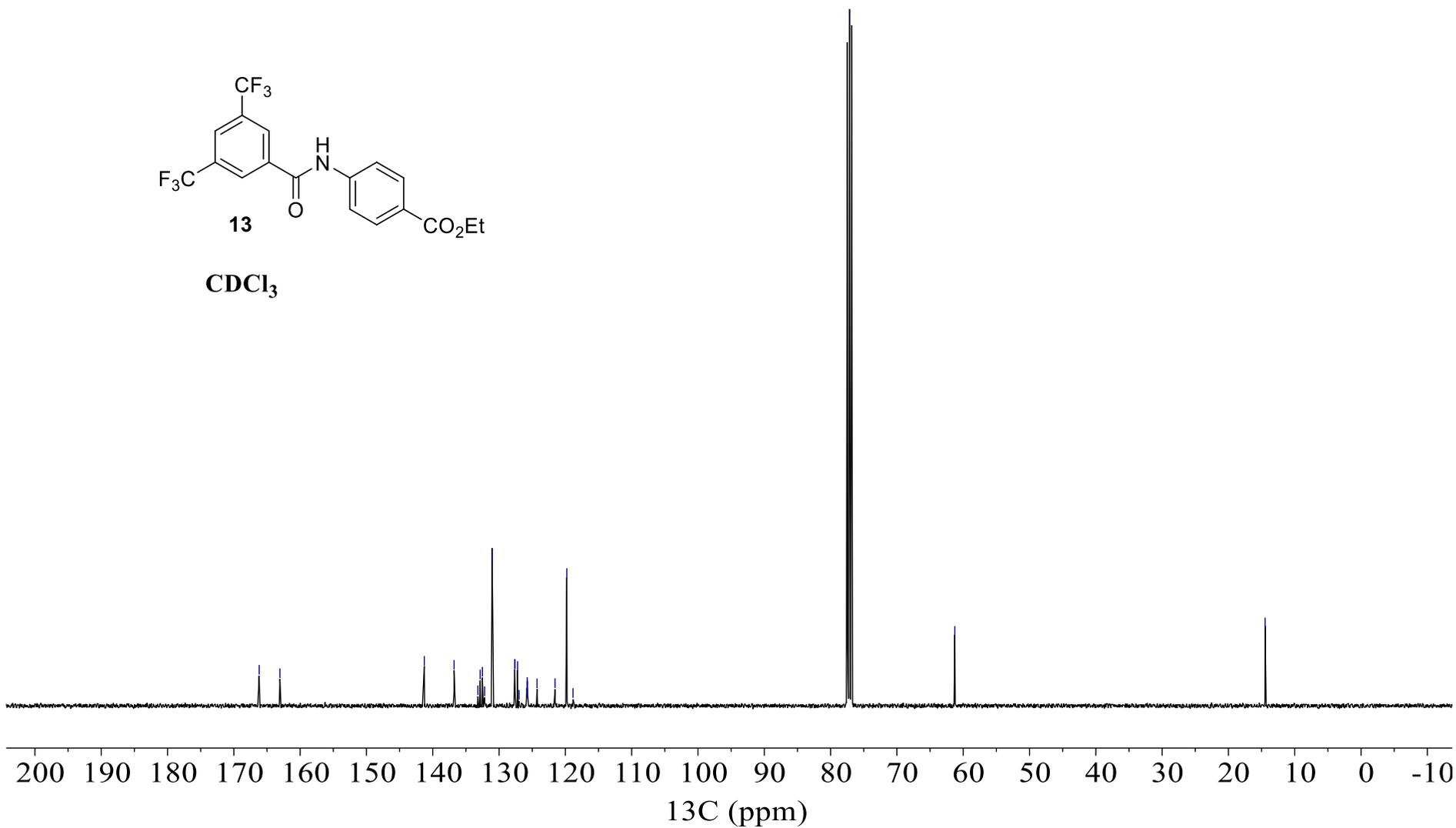


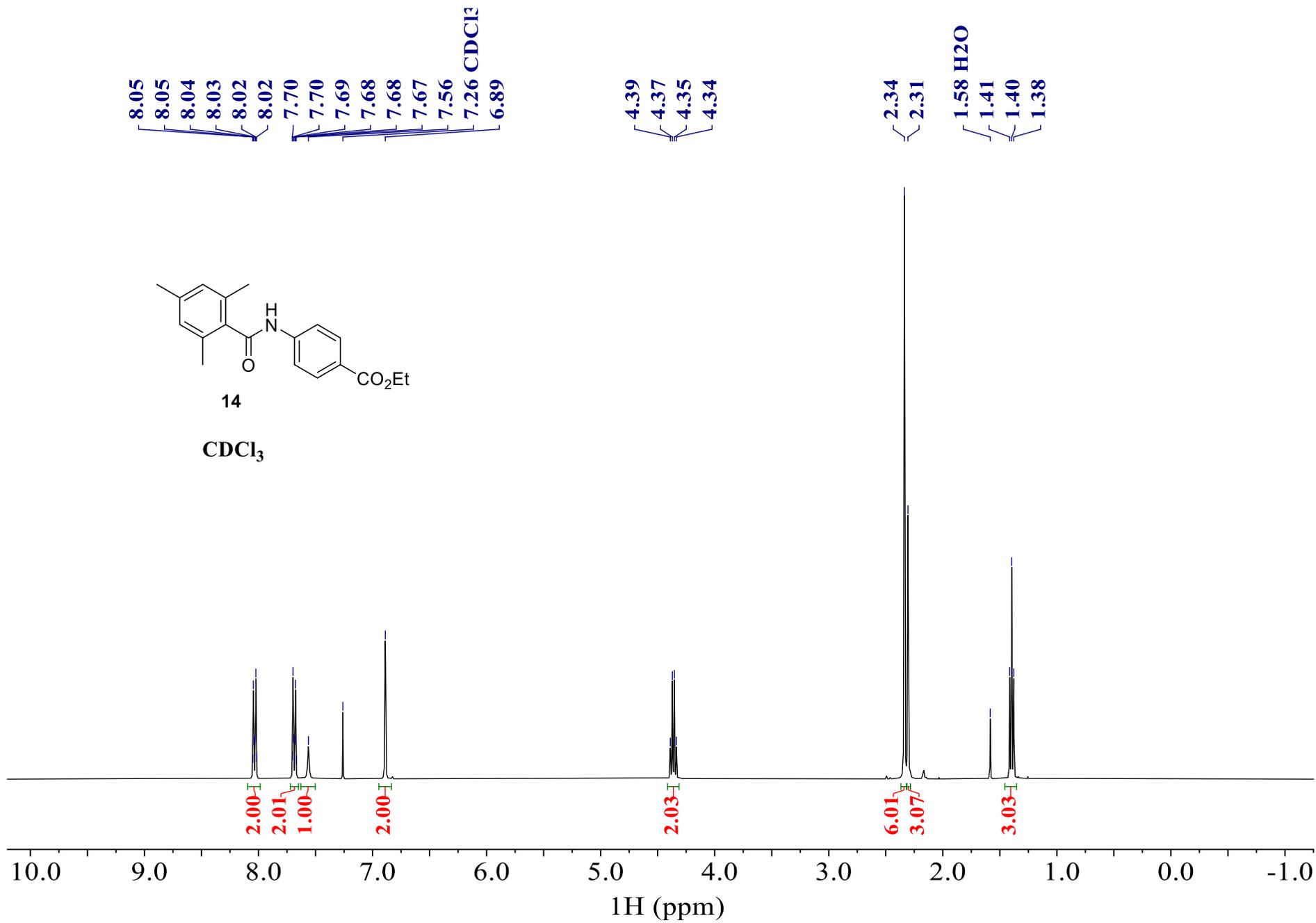
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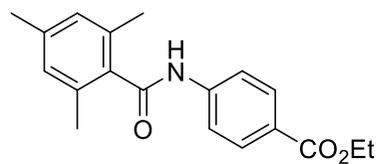
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~ 163.05

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133.20
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125.83
125.79
125.75
125.72
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124.27
121.56
119.78
118.85
77.16 CDCl₃
— 61.26

— 14.47







14

CDCl₃

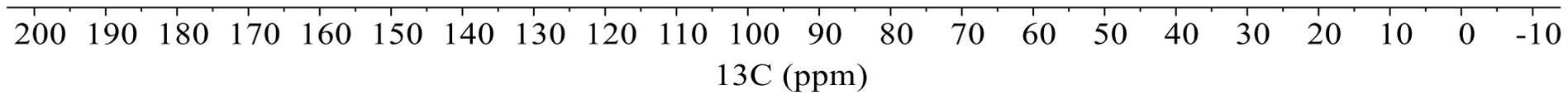
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~ 166.21

~ 142.04
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77.16 CDCl₃

— 61.05

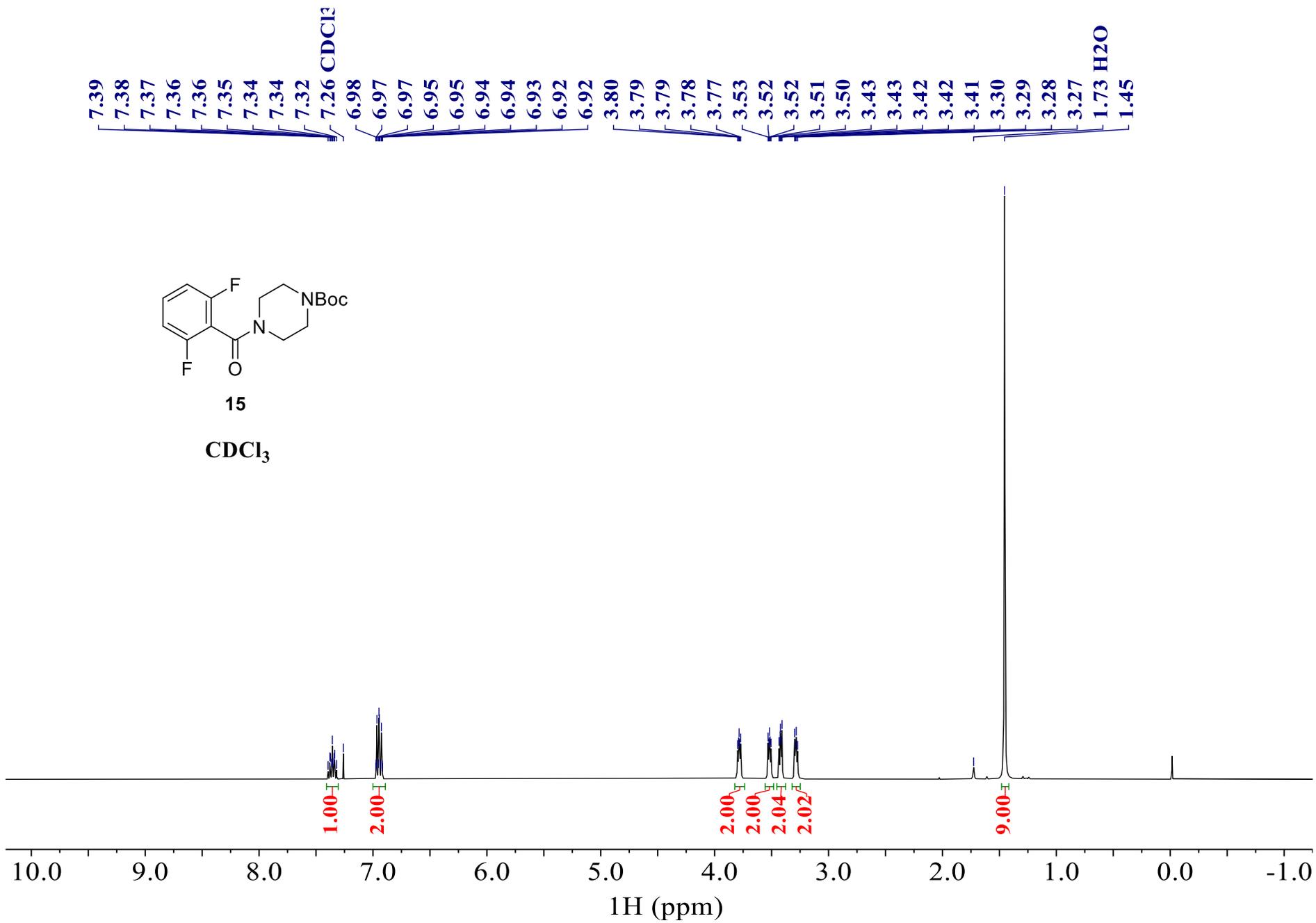
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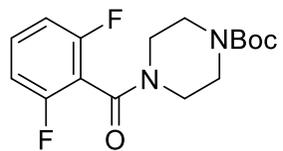


15

CDCl₃



S70



15

CDCl₃

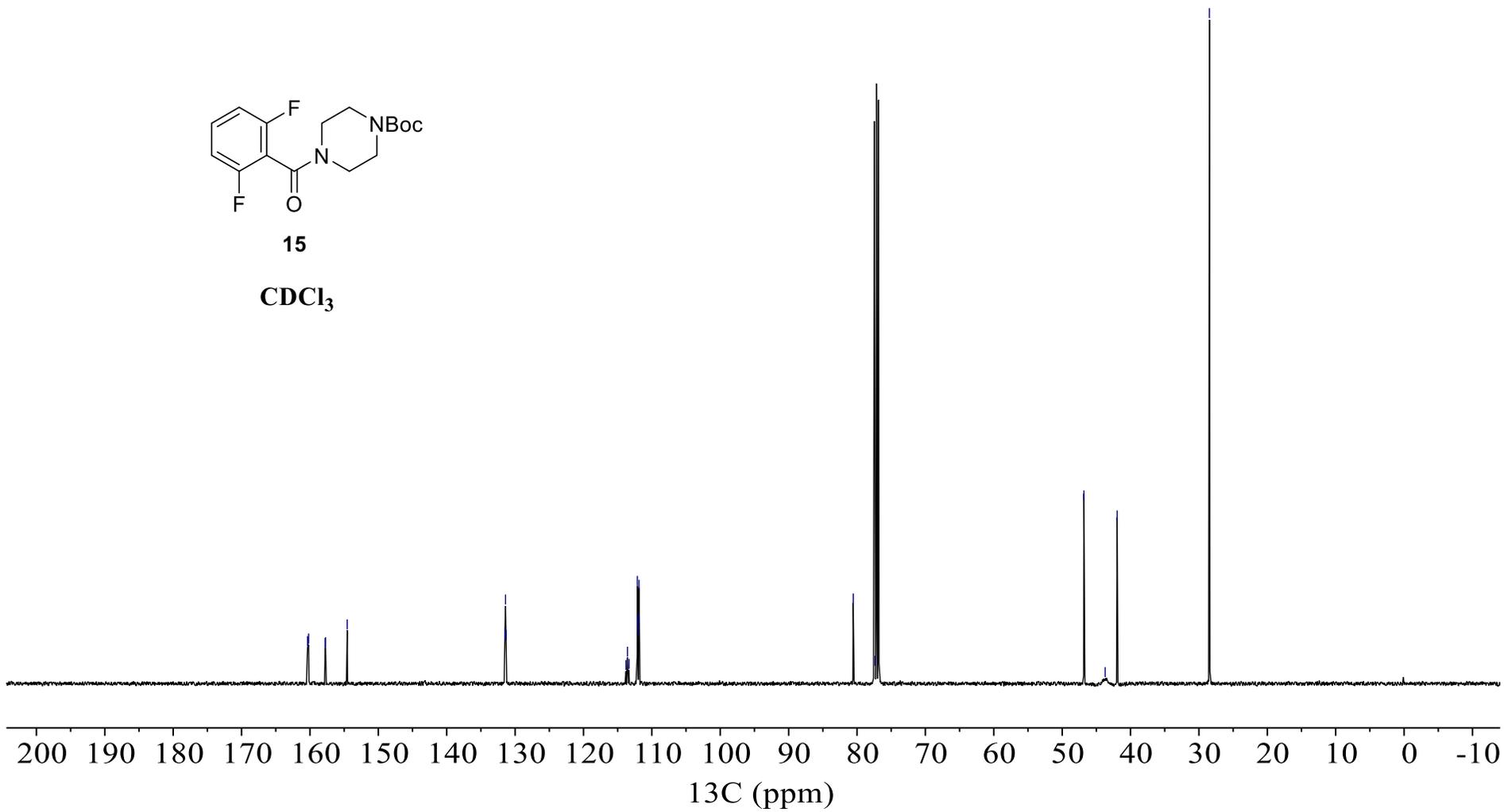
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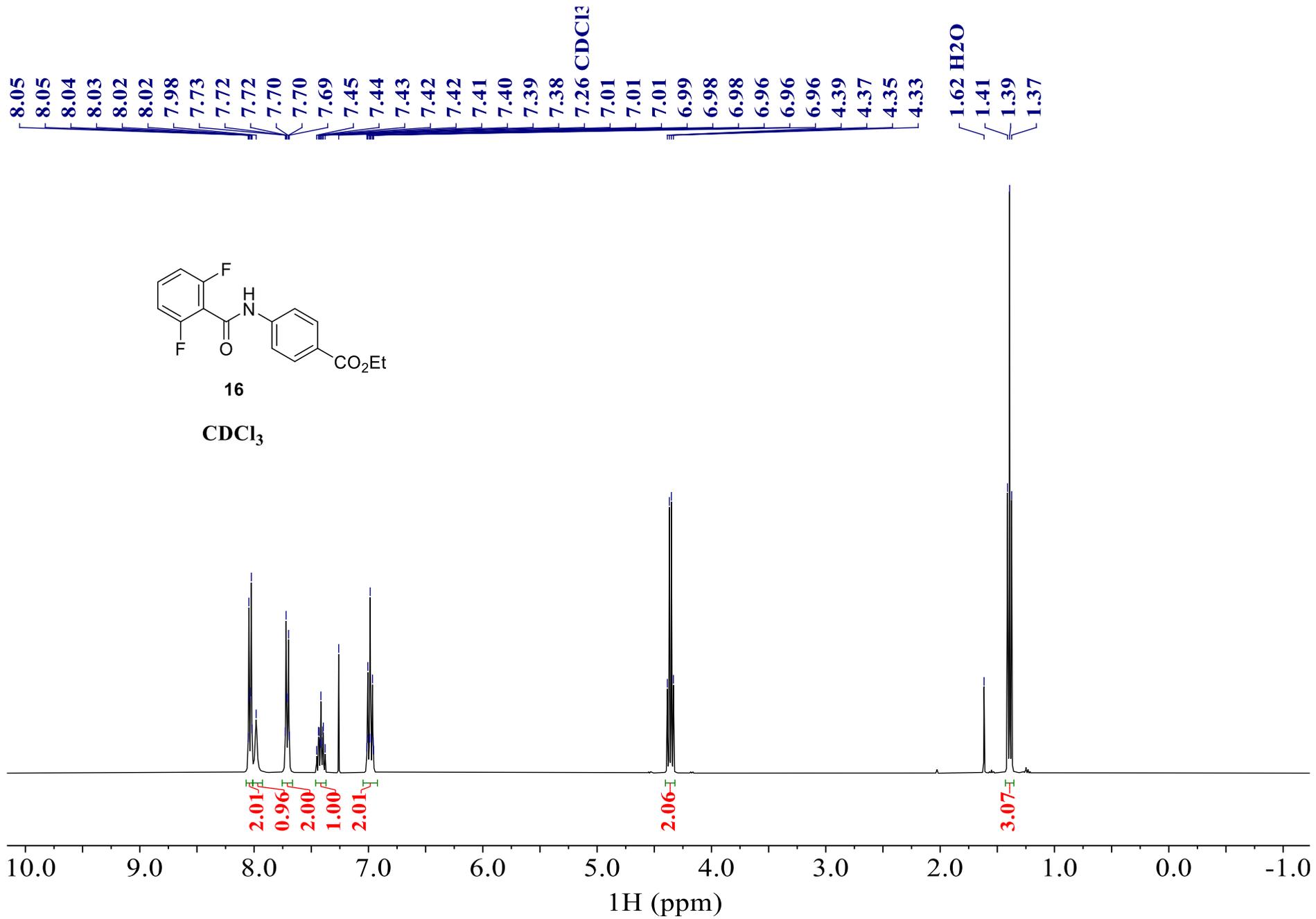
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80.55
77.36 CDCl₃

46.82
43.72
41.96

28.47

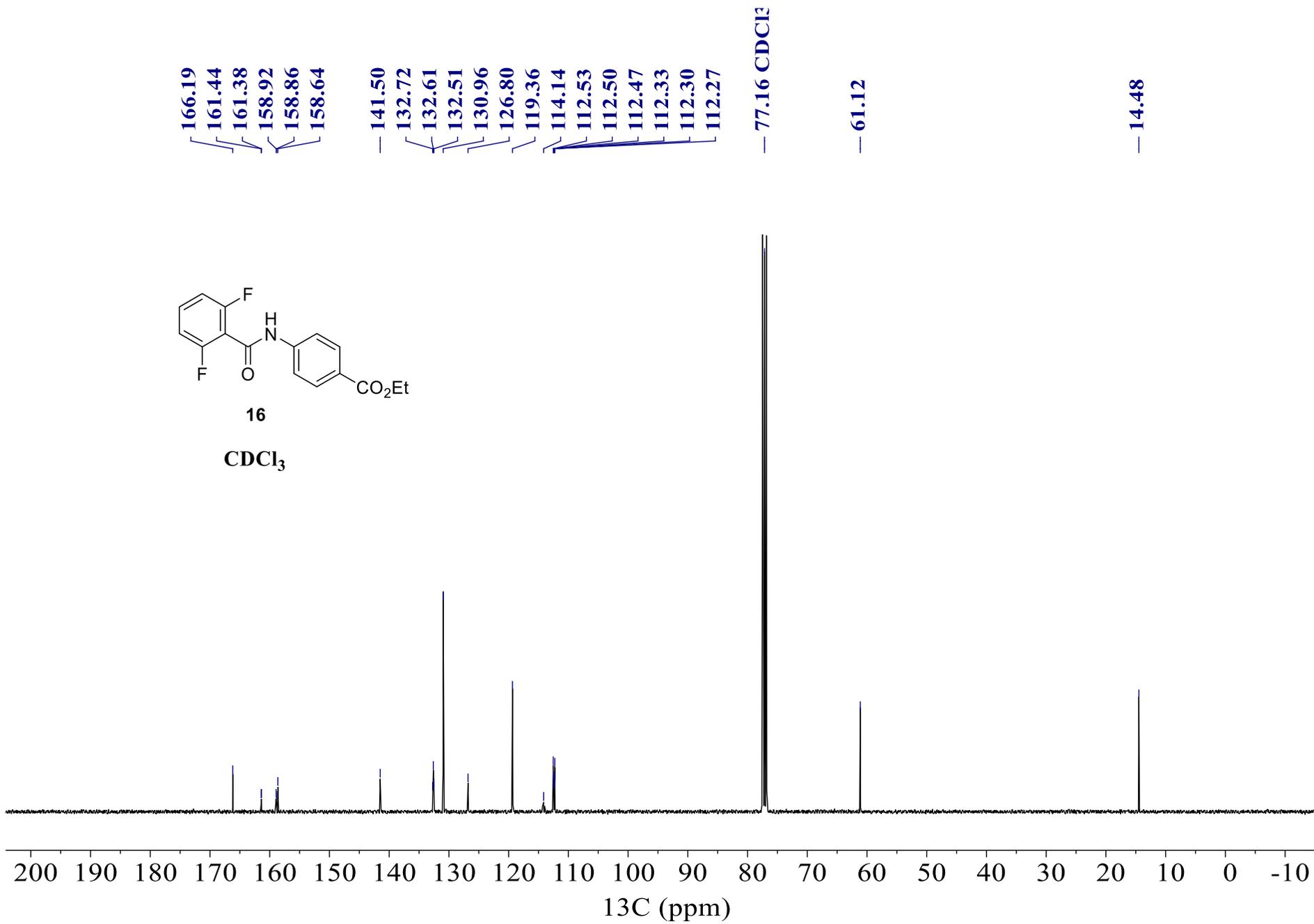


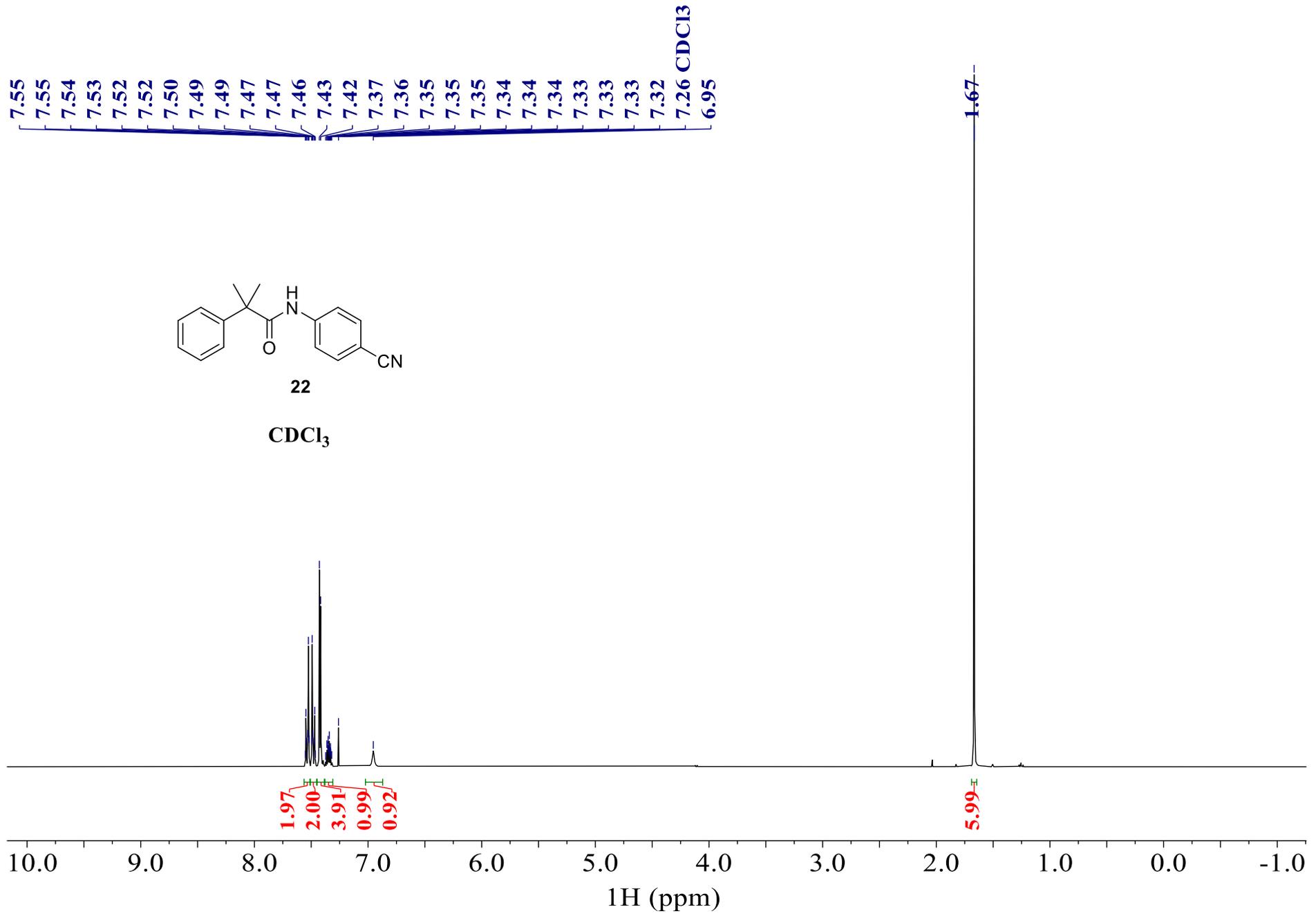


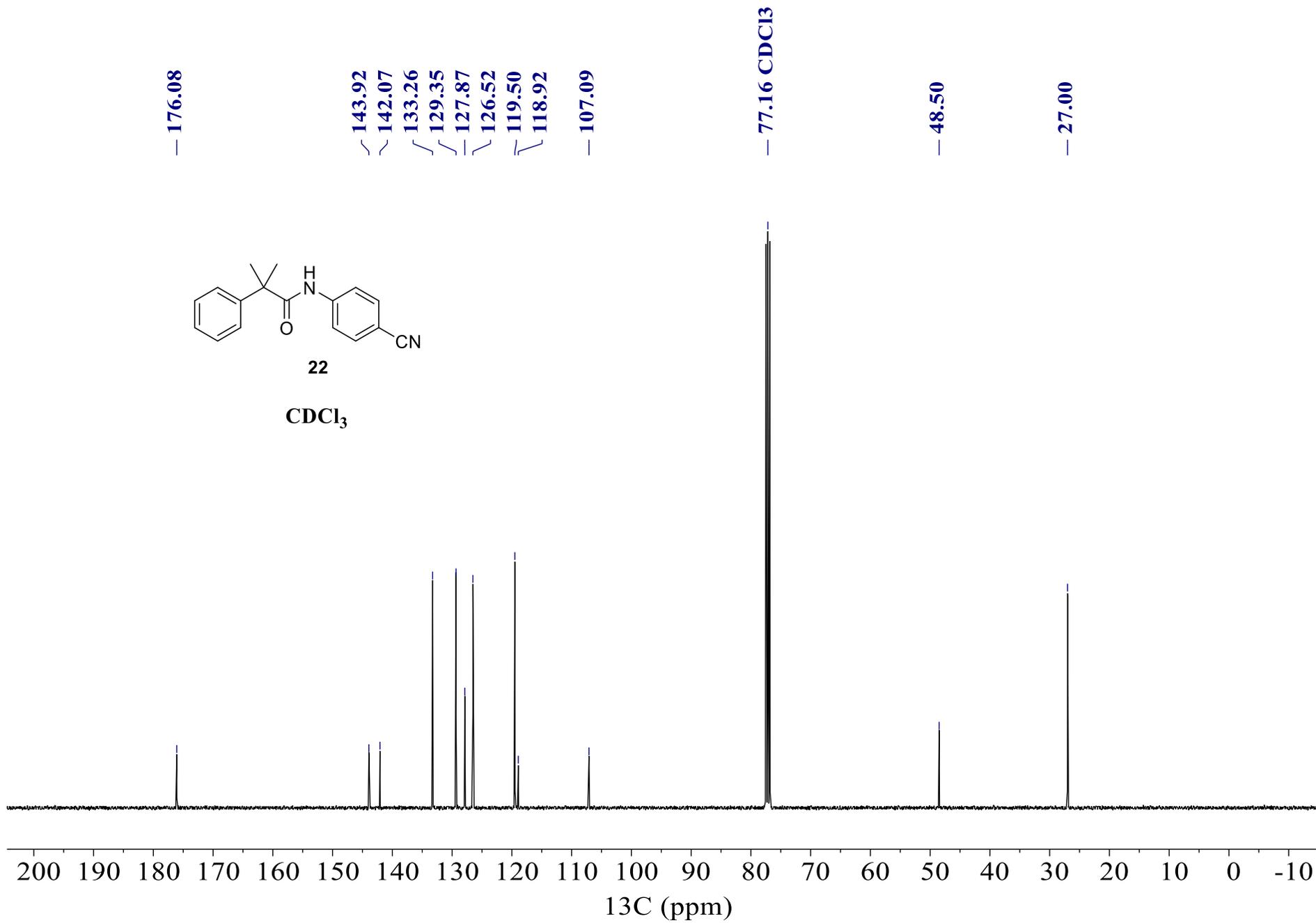


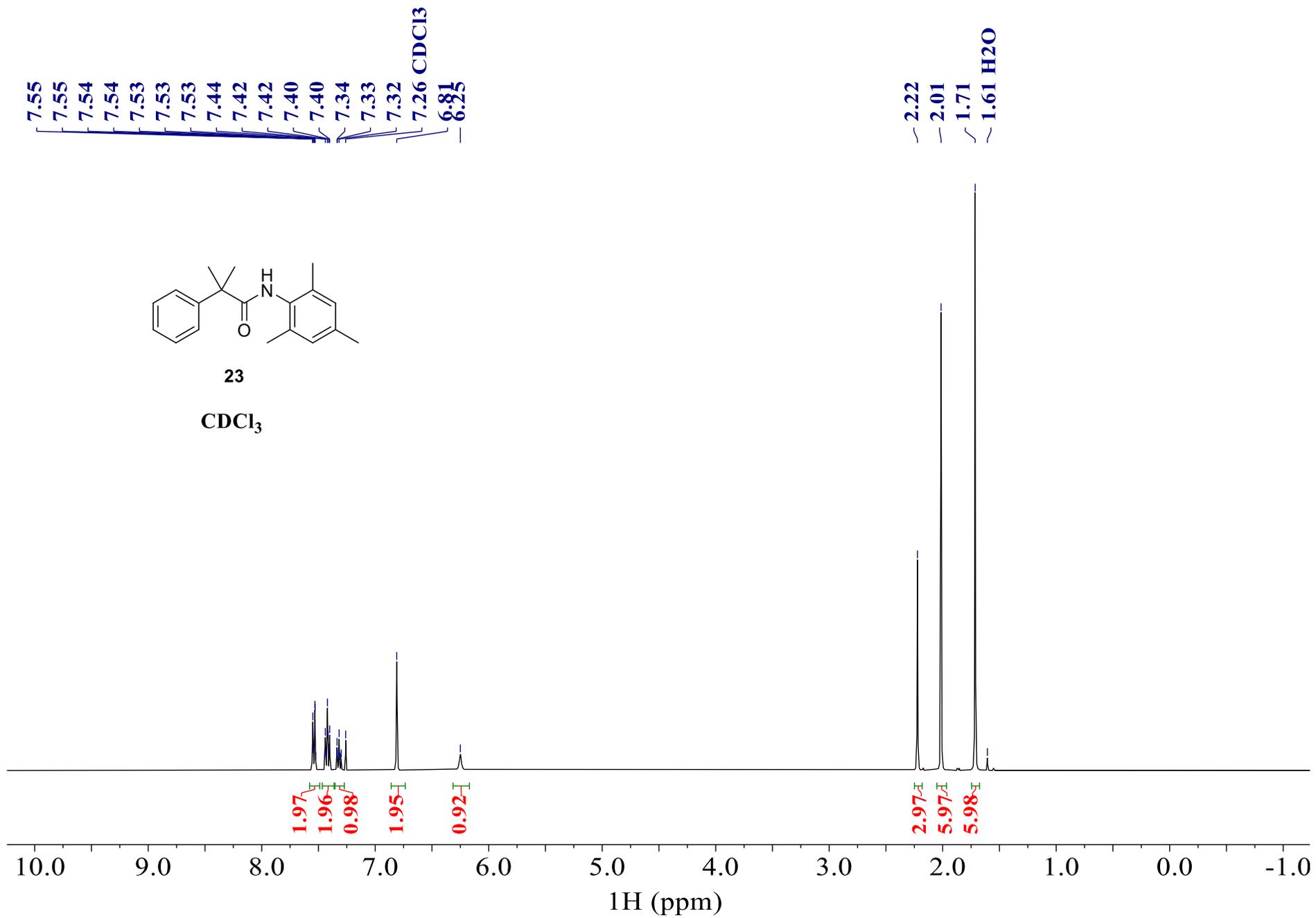
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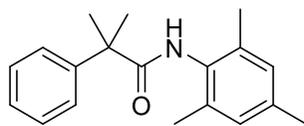
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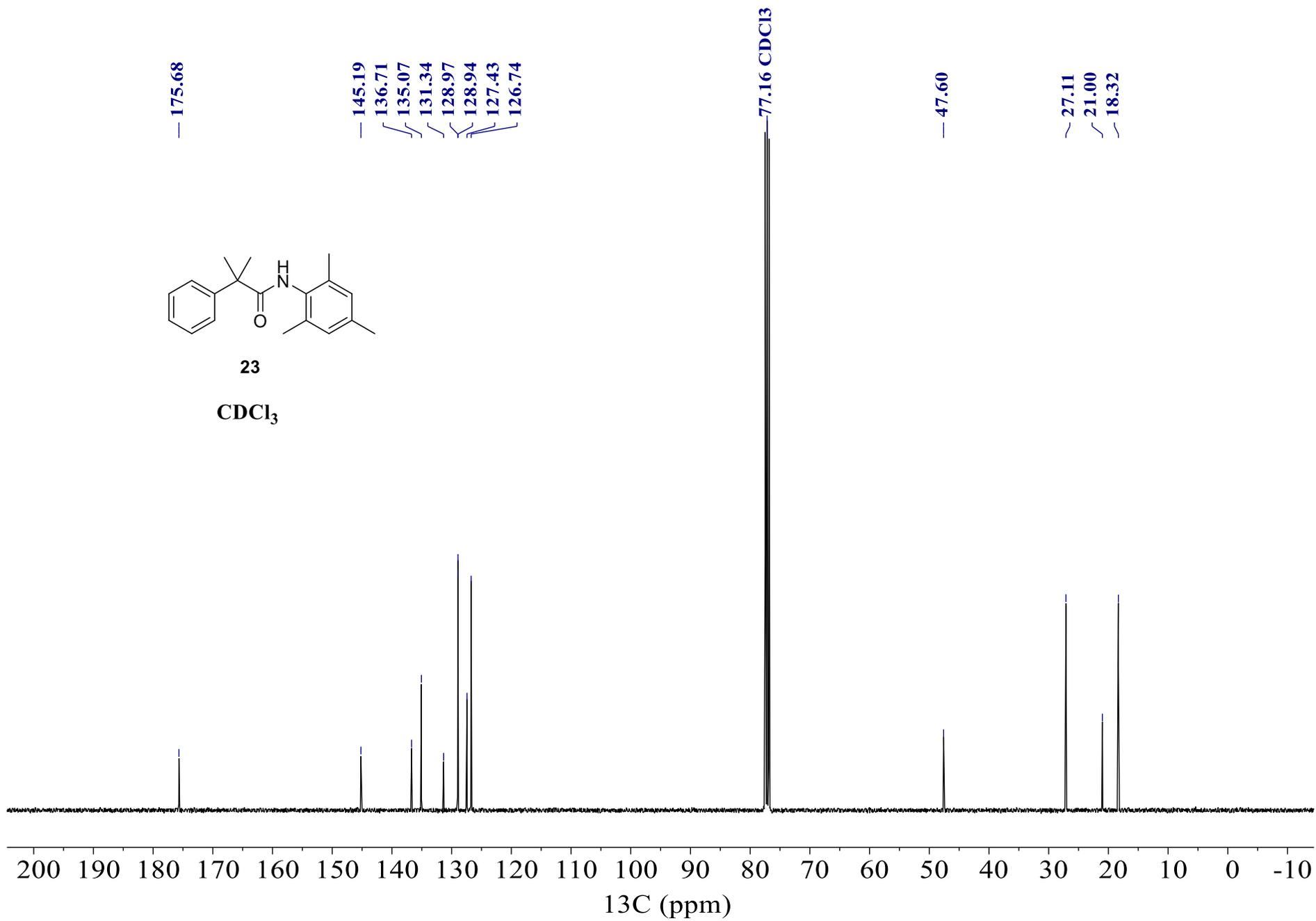


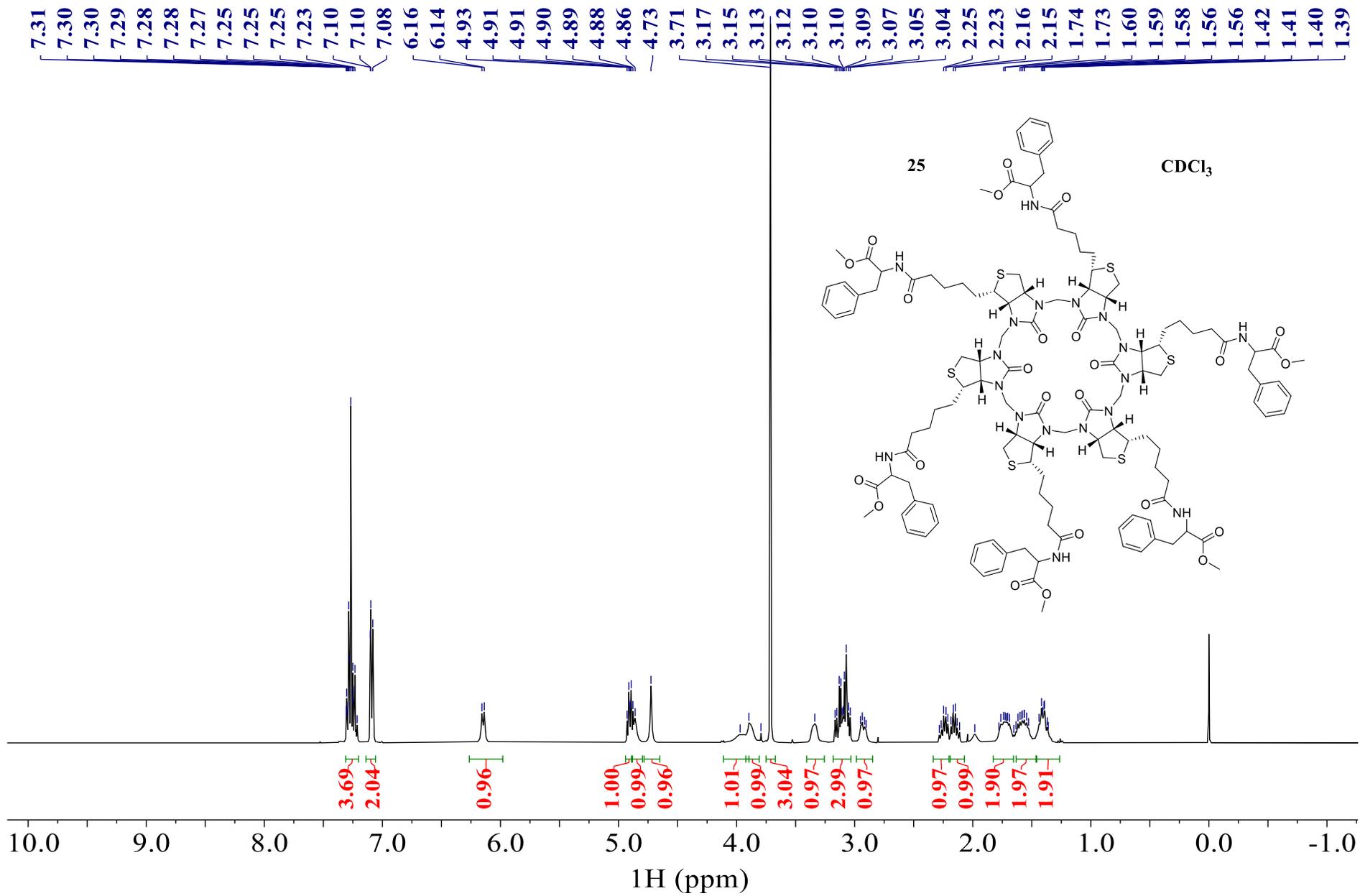


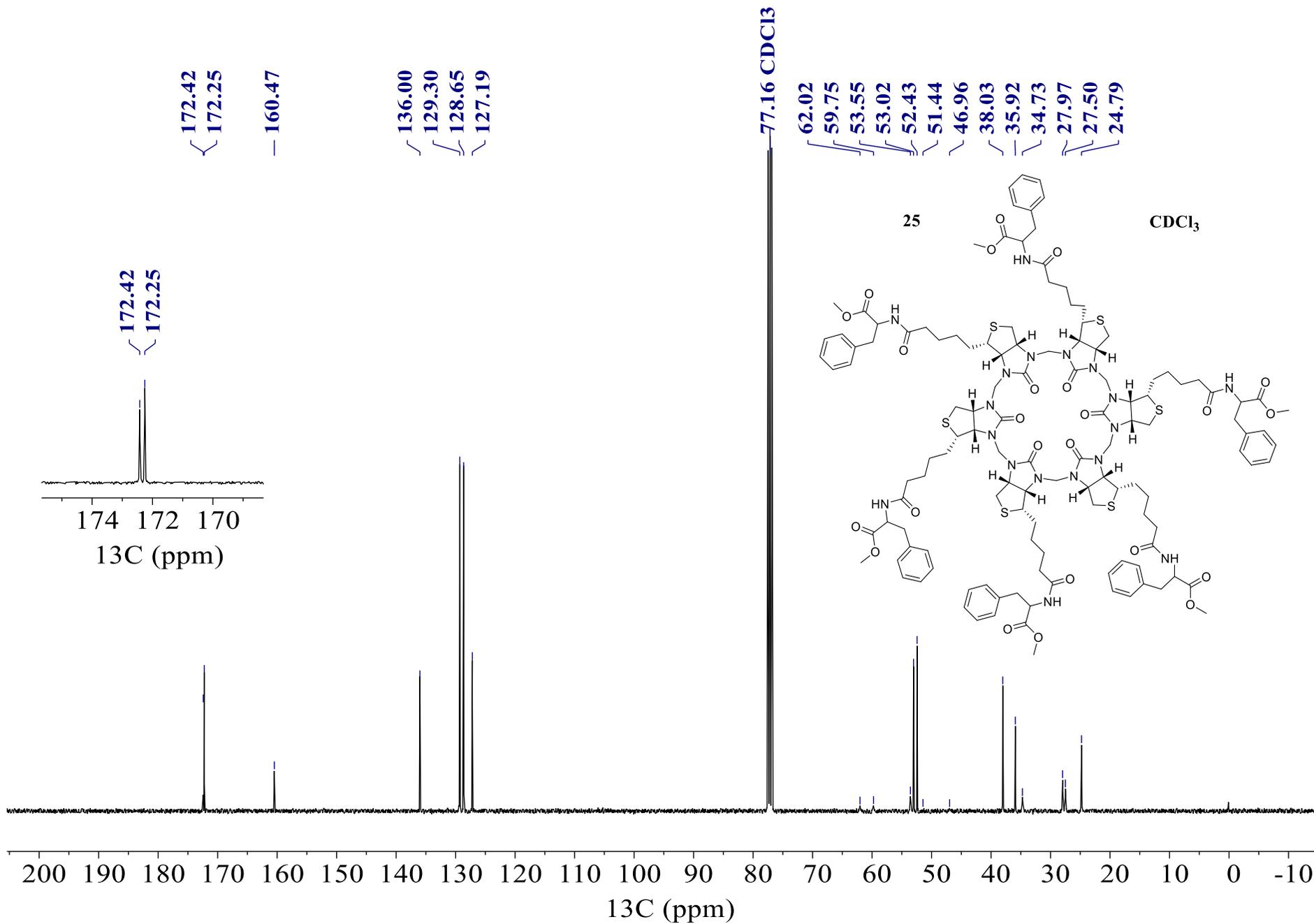


23

CDCl₃







2D-NMR HSQC of 25 in CDCl₃

