

ELECTRONIC SUPPLEMENTARY INFORMATION (ESI).

Modified siRNAs for the Study of the PAZ Domain

Álvaro Somoza,^{*a} Montserrat Terrazas,^{b,c} Ramón Eritja^{*b,c}

^a Instituto Madrileño de Estudios Avanzados en Nanociencia (IMDEA-Nanociencia), E-28049, Madrid, Spain. ^b Instituto de Química Avanzada de Cataluña-Consejo Superior de Investigaciones Científicas (IQAC-CSIC), E-08034, Barcelona, Spain.

^c Institut de Recerca Biomèdica, CIBER-BBN, C/ Baldori Reixac 15, E-08028, Barcelona, Spain.

Correspondence should be addressed to: A.S. (alvaro.somoza@imdea.org) or R.E. (ramon.eritja@irbbarcelona.org).

CONTENTS:

GENERAL PROCEDURES	2
General experimental methods	
Procedure A: Amide Formation	
Procedure B: DMTr Protection	
Procedure C: CPG Functionalization	
 CHARACTERIZATION OF ORGANIC COMPOUNDS	 3
RNA SYNTHESIS AND PURIFICATION METHODS	9
OLIGONUCLEOTIDE CHARACTERIZATION	10
THERMAL STABILITY OF siRNAs	10
STABILITY OF siRNAs IN 50% HUMAN SERUM	11
RNA INTERFERENCE METHODS	12
AUTODOCK-VINA DOCKING	13
NMR SPECTRA	14

General Procedures:

General experimental methods: Reagents were purchased from Aldrich and used without further purification. Thin layer chromatography was carried out using Silica Gel 60 F254 plates. Column chromatography was performed using Silica Gel (60 Å, 230 x 400 mesh). All NMR spectra were recorded on Varian Mercury 400 MHz (Unitat d'RMN, Serveis Científicotècnics, Parc Científic de Barcelona) instrument as solutions in the deuterated solvent indicated, and the chemical shifts are reported in parts per million (ppm). Coupling constants are reported in hertz (Hz). HRMS were performed on a LC/MSD-TOF (Agilent technologies) high resolution mass spectrometer by Servei d'Espectrometria de Masses (Universitat de Barcelona).

Procedure A: Amide Formation :

To a solution of the corresponding acid (1.2 equiv) in DMF (0.5 M) at room temperature, *N*-hydroxybenzotriazole (1.1 equiv) and diisopropylcarbodiimide (1.1 equiv) were added. After stirring the mixture for 5 minutes D- or L- Threoninol (1 equiv) was added. The resulting mixture was stirred at room temperature for 24 h and then quenched by the addition of methanol. The solvent was evaporated under vacuum and the residue purified by flash chromatography.

Procedure B: DMTr Protection:

To a solution of the corresponding diol (1 equiv) in pyridine (0.2 M) at 0° C, diisopropylethylamine (1.5 equiv), 4,4'-dimethoxyltritylchloride (1.2 equiv) and dimethylaminopyridine (catalytic amount) were added. After 15 min the mixture was allowed to reach room temperature, then it was stirred for 24 h and finally the reaction was quenched with methanol. The solvent was evaporated under vacuum and the residue purified by flash chromatography.

Procedure C: CPG Functionalization (Two Steps):¹

I

To a solution of the corresponding alcohol (1 equiv) in dichloromethane (0.2 M) succinic anhydride (1.3 equiv), diisopropylethylamine (1.4 equiv) and dimethylaminopyridine (catalytic amount) were added. The solution was stirred for 24 h at room temperature. Then, the solution was washed with a solution of sodium dihydrogen phosphate (1%). The organic layer was dried over Magnesium sulfate, filtered and evaporated to yield a yellowish oil.

II

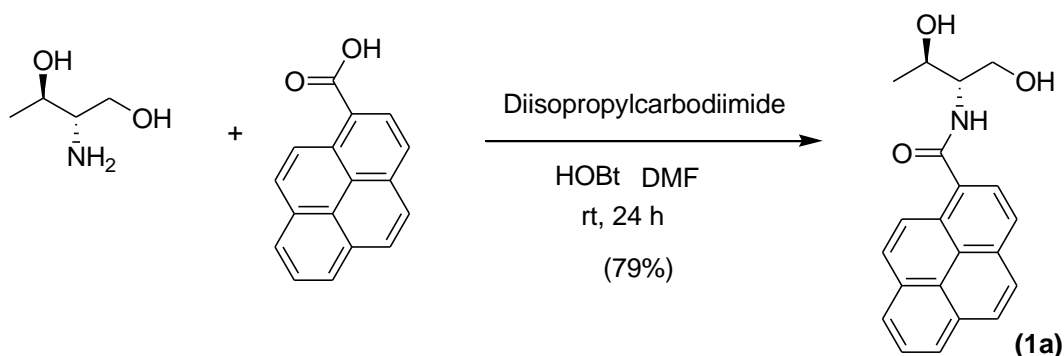
To a solution of DMAP (1.5 equiv) in acetonitrile (60 mM) the product obtained in step I was added (1 equiv). After mixing it well (vortex), the solution was added to a solution of 2,2'-dithiobis-(5-nitropyridine) (1.5 equiv). The solution was mixed well and added to triphenyl phosphine (1.5 equiv). The mixture was vortexed till all reagents were dissolved giving rise to a reddish solution, which was added to 200

¹ Gupta, K. C.; Kumar, P.; Bhatia, D.; Sharma, A. K. Nucleosides and Nucleotides, **1995**, *14*, 829-832.

mg of CPG (500 Å). After 1 h at room temperature the solution was removed and the CPG washed with methanol (3 X 20 mL) and, dry acetonitrile (3 x 20 mL). Once the CPG was dry, 2 mL of a 1:1 mixture of the capping reagents utilized on oligonucleotide synthesis (Cap Mix A: Acetic anhydride/Py/THF; Cap Mix B: 1-Methylimidazole /THF; from GlenResearch) was added. After 15 min at room temperature, the CPG was washed with dry acetonitrile (6 x 20 mL) and dried. The CPG loading was calculated by detritylation of a sample as follows: 10 mg of CPG were treated with 5 mL of a detritylation solution (3mL of perchloric acid and 2 mL of ethanol) for 30 min. Then 200 uL of the mixture were dissolved in 800 uL of the detritylation solution and absorbance was measured at 498. Functionalization (F) was determined by Lambert-Beer law. $F = (ABS \times V) / (\epsilon \times gr) = M/ gr$.

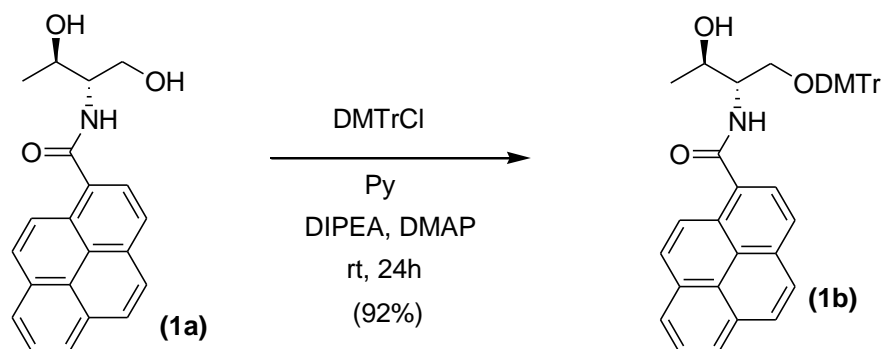
CHARACTERIZATION OF ORGANIC COMPOUNDS

N-[(2*S*, 3*R*)-1,3-dihydroxybutan-2-yl]pyrene-1-carboxamide (**1a**).



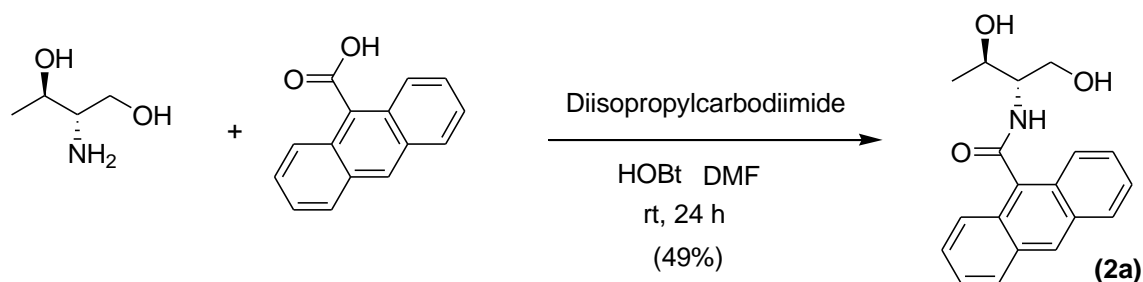
Compound **1a** was obtained following procedure A. After flash chromatography (cyclohexane 4 / CH₂Cl₂ 4 / MeOH 0.5) 302 mg (0.906 mMol) were obtained as a white solid (79%). ¹H NMR (400 MHz, CD₃OD): 8.53 (d, *J* = 9.3 Hz, 1H), 8.25 (t, *J* = 7.5 Hz, 3H), 8.21-8.02 (m, 5H), 4.28 (dt, *J* = 6.2 and 3.6 Hz, 1H), 4.18 (dq, *J* = 6.4 and 3.6 Hz, 1H), 3.87 (System ABX, *J*_{AB} = 11.1 Hz, *J*_{AX} = 5.8 Hz, and *J*_{BX} = 6.6 Hz, 2H), 1.38 (d, *J* = 6.4 Hz, 3H). ¹³C NMR (100 MHz, CD₃OD): 173.4, 133.9, 132.8, 132.6, 132.1, 129.7, 129.6, 129.5, 128.3, 127.6, 126.9, 126.8, 126.1, 125.8, 125.61, 125.59, 125.51, 67.5, 63.0, 58.5, 20.8. HRMS *m/z*: Calc for C₂₁H₂₀NO₃ (M + H⁺) 334.1437, found 334.1449.

***N*-[*(2S, 3R)*-1-(bis(4-methoxyphenyl)(phenyl)methoxy)-3-hydroxybutan-2-yl]-pyrene-1-carboxamide (**1b**).**



Compound **1b** was obtained following procedure B. After flash chromatography (cyclohexane 2 / EtOAc 1) 133 mg (0.209 mMol) were obtained as a white solid and 24 mg of the starting material were recovered. (70% yield, or 92% yield based on the recovered starting material). ^1H NMR (400 Mz, CDCl_3): 8.62 (d, $J = 9.3$ Hz, 1H), 8.21 (d, $J = 7.7$ Hz, 2H), 8.12 (s, 3H), 8.09 (d, $J = 2.1$ Hz, 1H), 8.04 (t, $J = 7.7$ Hz, 2H), 7.45 (d, $J = 7.6$ Hz, 2H), 7.34 (d, $J = 8.7$ Hz, 4H), 7.28 (t, $J = 7.7$ Hz, 2H), 7.20 (t, $J = 7.2$ Hz, 1H), 6.89 (d, $J = 8.7$ Hz, 1H), 6.81 (dd, $J = 8.9$ and 2.8 Hz, 4H), 4.38-4.32 (m, 1H), 4.28-4.22 (m, 1H), 3.73 (s, 3H), 3.72 (s, 3H), 3.63 (ABX System, $J_{AB} = 9.6$ Hz, $J_{AX} = 3.7$ Hz, $J_{BX} = 3.6$ Hz, 2H), 1.35 (d, $J = 6.3$ Hz, 3H). ^{13}C NMR (100 MHz, CDCl_3): 170.2, 158.6, 158.5, 144.3, 135.4, 135.2, 132.6, 131.1, 130.7, 130.6, 129.9 (4C), 128.7, 128.6, 128.5, 128.0 (2C), 127.9 (2C), 127.0, 126.9, 126.3, 125.8, 125.7, 124.7, 124.5, 124.5, 124.3, 124.2, 113.3 (4C), 86.9, 69.0, 65.6, 55.1 (2C), 54.3, 20.3. HRMS m/z : Calc for $\text{C}_{42}\text{H}_{37}\text{NO}_5\text{Na}$ ($M + \text{Na}^+$) 658.2563, found 658.2563.

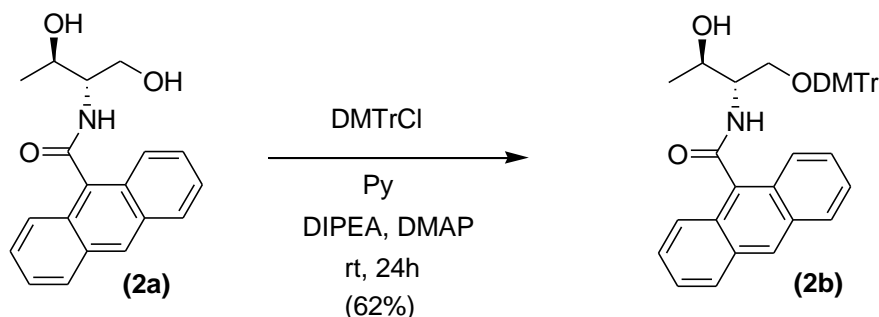
***N*-[*(2S, 3R)*-1,3-dihydroxybutan-2-yl]anthracene-9-carboxamide (**2a**).**



Compound **2a** was obtained following procedure A. After flash chromatography (cyclohexane 6 / CH_2Cl_2 3 / MeOH 0.5) 273 mg (1.052 mMol) were obtained as a yellowish solid (49%). ^1H NMR (400 MHz, CD_3OD): 8.56 (s, 1H), 8.27-8.15 (m, 2H), 8.06 (d, $J = 8.3$ Hz, 2H), 7.57-7.47 (m, 4H), 4.40 (ddd, $J = 6.7$, 5.6 and 3.9 Hz, 1H), 4.15 (dq, $J = 6.4$ and 3.8 Hz, 1H), 3.87 (ABX System: $J_{AB} = 11.0$ Hz, $J_{AX} = 5.6$ Hz, $J_{BX} = 6.9$ Hz, 2H), 3.35 (s, 1H), 1.39 (d, $J = 6.4$ Hz, 3H). ^{13}C NMR (100 MHz, D_3OD): 172.7, 133.5, 132.7, 129.6 (2C), 129.3, 129.2 (2C), 127.7 (2C), 126.6 (4C),

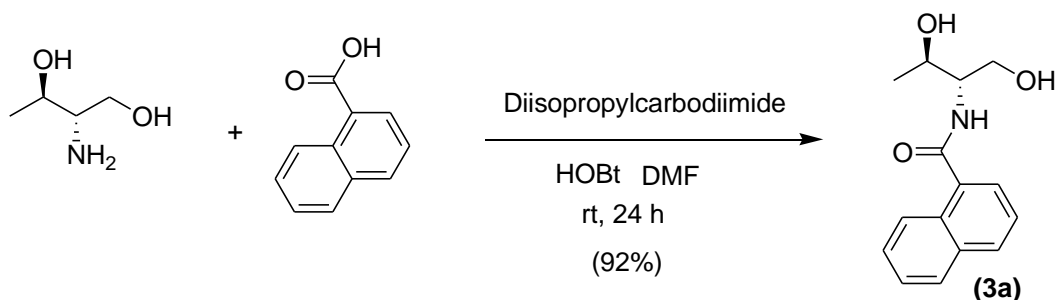
126.4, 67.4, 63.0, 58.8, 20.9. HRMS m/z : Calc for $C_{19}H_{19}NNaO_3$ ($M + Na$)⁺ 332.1257, found 332.1258.

***N*-[*(2S, 3R)*-1-(bis(4-methoxyphenyl)(phenyl)methoxy)-3-hydroxybutan-2-yl]-anthracene-9-carboxamide (**2b**).**



Compound **2b** was obtained following procedure B. After flash chromatography (cyclohexane 3 / EtOAc 1) 130 mg (0.213 mMol) were obtained as a white solid (62%). ¹H NMR (400 Mz, CDCl₃): 8.48 (s, 1H), 8.11 (bs, 1H), 8.01 (d, $J = 9.2$ Hz, 2H), 7.47 (t, $J = 8.2$ Hz, 2H), 7.41 (d, $J = 7.2$ Hz, 2H), 7.31 (d, $J = 8.8$ Hz, 4H), 7.26 (t, $J = 7.4$ Hz, 2H), 7.21 (d, $J = 7.0$ Hz, 1H), 6.80 (d, $J = 8.8$ Hz, 4H), 6.73 (d, $J = 8.8$ Hz, 1H), 4.49 (td, $J = 7.3$ and 3.9 Hz, 1H), 4.16 (m, 1H), 3.77 (s, 3H), 3.76 (s, 3H), 3.62 (ABX System, $J_{AB} = 9.6$ Hz, $J_{AX} = 3.6$ Hz, $J_{BX} = 4.4$ Hz, 2H), 3.07 (s, 1H), 1.35 (d, $J = 6.3$ Hz, 3H). ¹³C NMR (100 MHz, CDCl₃): 169.9, 158.6 (2C), 144.2 (2C), 135.4, 135.1, 131.7, 131.1 (2C), 130.0 (3C), 129.9 (3C), 128.5 (2C), 128.3, 128.1, 128.0 (2C), 127.9 (2C), 127.0, 126.7 (2C), 125.4, 125.2, 113.3 (2C), 113.2 (2C), 86.9, 68.8, 65.5, 55.2 (2C), 54.6, 20.6. HRMS m/z : Calc for $C_{40}H_{37}NNaO_5$ ($M + Na$)⁺ 634.2564, found 634.2570.

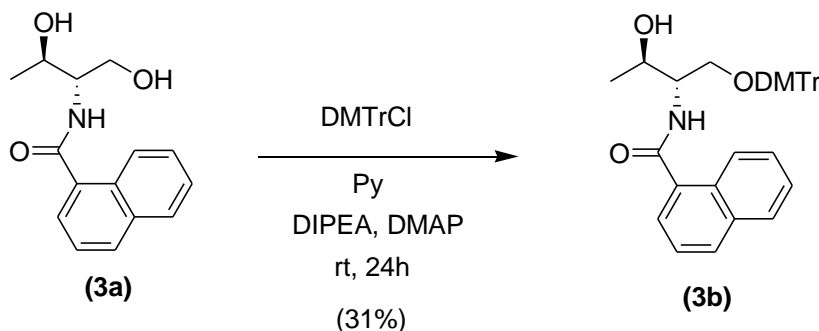
***N*-[*(2S, 3R)*-1,3-dihydroxybutan-2-yl]-1-naphthamide (**3a**).**



Compound **3a** was obtained following procedure A. After flash chromatography (cyclohexane 6 / CH₂Cl₂ 3 / MeOH 0.5) 273 mg (1.052 mMol) were obtained as a yellowish solid (92%). ¹H NMR (400 MHz, CD₃OD): 8.26 (d, $J = 9.1$ Hz, 1H), 7.95 (d, $J = 8.3$ Hz, 1H), 7.90 (d, $J = 7.3$ Hz, 1H), 7.69 (d, $J = 7.0$ Hz, 1H), 7.57-7.47 (m, 3H), 4.21-4.16 (m, 1H), 4.15-4.09 (m, 1H), 3.79 (System ABX; $J_{AB} = 11.1$ Hz, $J_{AX} = 5.8$ Hz, $J_{BX} = 6.5$ Hz, 2H), 1.30 (d, $J = 6.4$ Hz, 3H). ¹³C NMR (100 MHz, CD₃OD):

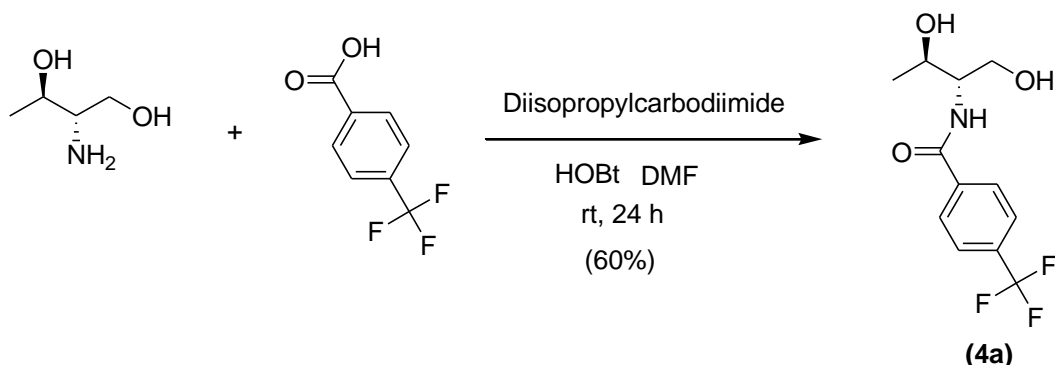
173.1, 136.1, 135.3, 131.7, 131.6, 129.6, 128.2, 127.6, 126.6, 126.5, 126.1, 67.6, 63.1, 58.4, 20.9. HRMS m/z : Calc for $C_{15}H_{18}NO_3$ ($M + H^+$) 260.1281, found 260.1291.

***N*-[*(2S, 3R)*-1-(bis(4-methoxyphenyl)(phenyl)methoxy)-3-hydroxybutan-2-yl]-1-naphthamide (**3b**).**



Compound **3b** was obtained following procedure B. After flash chromatography (cyclohexane 3 / EtOAc 1) 146 mg (0.259 mMol) were obtained as a white solid (31%). ^1H NMR (400 Mz, CDCl_3): 8.37-8.32 (m, 1H), 7.95 (d, $J = 8.2$ Hz, 1H), 7.92-7.87 (m, 1H), 7.66 (dd, $J = 7.0$ and 1.1 Hz, 1H), 7.56-7.52 (m, 2H), 7.48 (dd, $J = 8.2$ and 7.1 Hz, 1H), 7.40 (d, $J = 7.2$ Hz, 2H), 7.31 (d, $J = 8.9$ Hz, 4H), 7.26 (t, $J = 7.3$ Hz, 2H), 7.20 (t, $J = 7.1$ Hz, 1H), 6.80 (dd, $J = 8.8$ and 3.7 Hz, 4H), 6.71 (d, $J = 8.8$ Hz, 1H), 4.28-4.24 (m, 1H), 4.23-4.18 (m, 1H), 3.76 (s, 3H), 3.75 (s, 3H), 3.56 (System ABX, $J_{AB} = 9.7$ Hz, $J_{AX} = 3.8$ Hz, $J_{BX} = 3.6$ Hz, 2H), 3.17 (s, 1H), 1.29 (d, $J = 6.34$ Hz, 3H). ^{13}C NMR (100 MHz, CDCl_3): 169.8, 158.7, 158.6, 144.2, 135.4, 135.2, 134.4, 133.7, 130.7, 130.1, 129.9 (4C), 128.3, 128.0 (2C), 127.9 (2C), 127.2, 127.0, 126.4, 125.5, 124.9, 124.7, 113.3 (4C), 86.9, 69.1, 65.7, 55.2 (2C), 53.9, 20.2. HRMS m/z : Calc for $C_{36}H_{35}NNaO_5$ ($M + Na$) $^+$ 584.2407, found 584.2402.

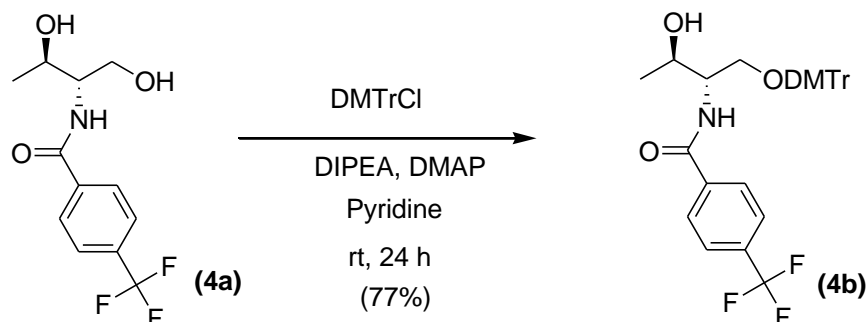
***N*-[*(2S, 3R)*-1,3-dihydroxybutan-2-yl]-4-(trifluoromethyl)benzamide (**4a**).**



Compound **4a** was obtained following procedure A. After flash chromatography (cyclohexane 4 / CH_2Cl_2 4/ MeOH 0.5) 157 mg (0.566mMol) were obtained as a white solid (60%). ^1H NMR (400 MHz, CD_3OD): 8.03 (d, $J = 8.2$ Hz, 2H), 7.78 (d, $J = 8.2$ Hz, 2H), 4.14-4.04 (m, 2H), 3.75 (System ABX: $J_{AB} = 11.2$ Hz, $J_{AX} = 5.6$ Hz, $J_{BX} = 6.2$ Hz, 2H), 1.22 (d, $J = 6.2$ Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD): 169.4, 139.7,

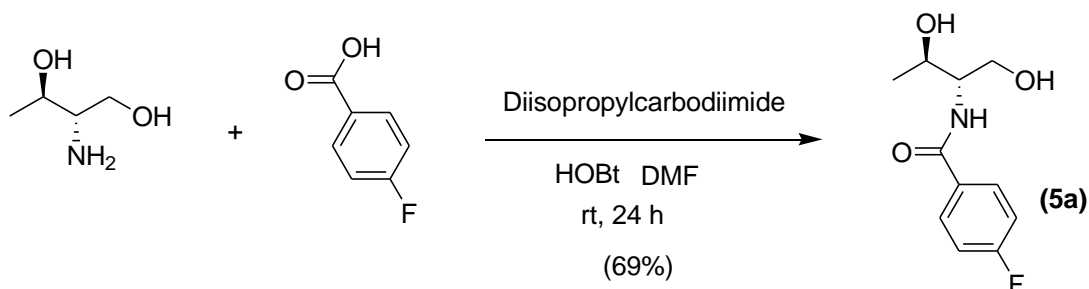
134.1 (q, $J_{F-C} = 32.4$ Hz, 1C), 129.3 (2C), 126.5 (q, $J_{F-C} = 3.8$ Hz, 2C), 124.0, 67.3, 62.8, 58.4, 20.6. ^{19}F NMR (376 MHz, CD_3OD): -64.86. HRMS m/z : Calc for $\text{C}_{12}\text{H}_{15}\text{NO}_5\text{F}_3$ ($\text{M} + \text{H}^+$) 278.0998, found 278.0999.

***N*-[*(2S, 3R)*-1-(bis(4-methoxyphenyl)(phenyl)methoxy)-3-hydroxybutan-2-yl]-4-(trifluoromethyl)benzamide (**4b**).**



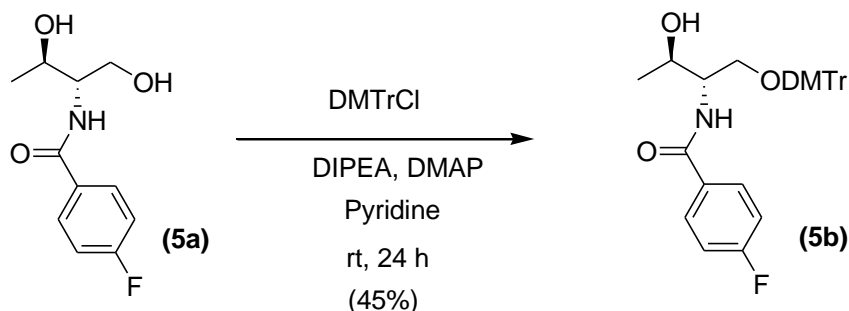
Compound **4b** was obtained following procedure B. After flash chromatography (cyclohexane 3 / EtOAc 1) 580 mg (0.828 mMol) were obtained as a white solid (77%). ^1H NMR (400 Mz, CDCl_3): 7.89 (d, $J = 8.1$ Hz, 2H), 7.73 (d, $J = 8.2$ Hz, 2H), 7.38 (d, $J = 7.2$ Hz, 2H), 7.30-7.18 (m, 4H), 6.79 (dd, $J = 8.8$ and 6.8 Hz, 2H), 4.28-4.20 (m, 1H), 4.15-4.10 (m, 1H), 3.76 (s, 3H), 3.75 (s, 3H), 3.49 (System ABX: $J_{AB} = 9.8$ Hz, $J_{AX} = 4.2$ Hz, $J_{BX} = 3.4$ Hz, 2H), 3.07 (s, 1H), 1.21 (d, $J = 6.4$ Hz, 3H). ^{13}C NMR (100 MHz, CDCl_3): 166.3, 158.6 (2C), 144.2, 137.6, 135.3, 135.2, 133.3 (q, $J_{F-C} = 32.8$ Hz, 1C), 129.9 (2C), 129.8 (2C), 128.0 (2C), 127.8 (2C), 127.4 (2C), 127.0, 125.7 (q, $J_{F-C} = 3.8$ Hz, 2C), 124.9, 113.3 (2C), 113.2 (2C), 86.9, 68.5, 65.2, 55.1(2C), 54.0, 20.1. ^{19}F NMR (376 MHz, CDCl_3): -63.36. HRMS m/z : Calc for $\text{C}_{33}\text{H}_{32}\text{F}_3\text{NNaO}_5$ ($\text{M} + \text{Na}$) $^+$ 602.2125, found 602.2126.

***N*-[*(2S, 3R)*-1,3-dihydroxybutan-2-yl]-4-fluorobenzamide (**5a**).**



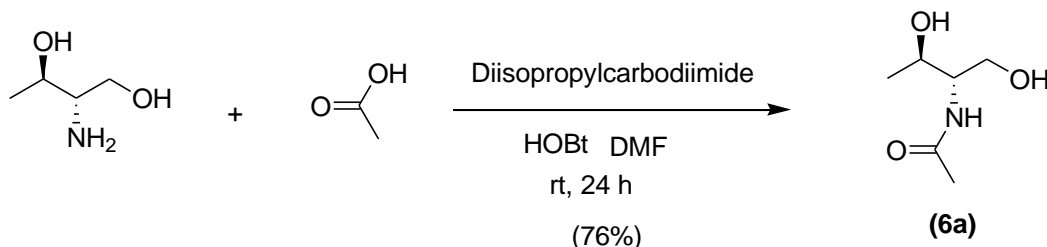
Compound **5a** was obtained following procedure A. After flash chromatography (cyclohexane 5 / CH_2Cl_2 4 / MeOH 0.5) 180 mg (0.792 mMol) were obtained as a white solid (69%). ^1H NMR (400 MHz, CD_3OD): 7.92 (dd, $J = 8.8$ and 5.4 Hz, 2H), 7.20 (t, $J = 8.7$ Hz, 2H), 4.13-4.02 (m, 2H), 3.73 (System ABX, $J_{AB} = 11.2$ Hz, $J_{AX} = 5.9$ Hz, $J_{BX} = 6.3$ Hz, 2H), 1.21 (d, $J = 6.4$ Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD): 169.5, 166.2 (d, $J_{F-C} = 250.5$ Hz, 1C), 132.1, 131.0 (d, $J_{F-C} = 8.9$ Hz, 2C), 116.4 (d, $J_{F-C} = 22.1$ Hz, 2C), 67.3, 62.8, 58.2, 20.6. ^{19}F NMR (376 MHz, CD_3OD): -111.2. HRMS m/z : Calc for $\text{C}_{11}\text{H}_{15}\text{NO}_3\text{F}$ ($\text{M} + \text{H}^+$) 228.1030, found 228.1035.

***N*-[*(2S, 3R)*-1-(bis(4-methoxyphenyl)(phenyl)methoxy)-3-hydroxybutan-2-yl]-4-fluorobenzamide (**5b**).**



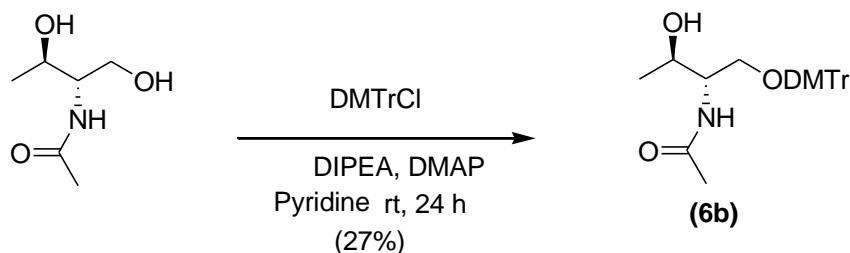
Compound **5b** was obtained following procedure B. After flash chromatography (cyclohexane 3 / EtOAc 1) 313 mg (0.591 mMol) were obtained as a white solid (45%). ^1H NMR (400 Mz, CDCl_3): 7.81 (dd, $J = 8.8$ and 5.3 Hz, 2H), 7.37 (d, $J = 7.1$ Hz, 2H), 7.30-7.18 (m, 7H), 7.14 (t, $J = 8.6$ Hz, 2H), 6.79 (dd, $J = 8.9$ and 6.8 Hz, 4H), 4.21 (d, $J = 6.3$ Hz, 1H), 4.15-4.05 (m, 1H), 3.77 (s, 3H), 3.76 (s, 3H), 3.56 (dd, $J = 9.7$ and 4.2 Hz, 1H), 3.38 (dd, $J = 9.7$ and 3.4 Hz, 1H), 3.13 (s, 1H), 1.20 (d, $J = 6.37$ Hz, 3H). ^{13}C NMR (100 MHz, CDCl_3): 166.6, 164 (d, $J_{\text{F-C}} = 252$ Hz, 1C), 158.6 (2C), 144.2, 135.4, 135.2, 130.5 (d, $J_{\text{F-C}} = 3.2$ Hz, 1C), 129.9 (2C), 129.8 (2C), 129.3 (d, $J_{\text{F-C}} = 8.9$ Hz, 2C), 128.0 (2C), 127.8 (2C), 127.0, 115.6 (d, $J_{\text{F-C}} = 21.8$ Hz, 2C), 113.3 (4C), 86.9, 68.9, 65.5, 55.2 (2C), 53.9, 20.0. ^{19}F NMR (376 MHz, CDCl_3): -108.44. HRMS m/z : Calc for $\text{C}_{32}\text{H}_{32}\text{NO}_5\text{FNa}$ ($M + \text{Na}^+$) 552.2156, found 552.2143.

***N*-[*(2S, 3R)*-1,3-dihydroxybutan-2-yl]-acetamide (**6a**).**



Compound **6a** was obtained following procedure A. After flash chromatography (cyclohexane 4 / CH_2Cl_2 4 / MeOH 0.5) 159 mg (1.08 mMol) were obtained as a colorless oil (76%). ^1H NMR (400 MHz, CD_3OD): 4.04-3.94 (m, 1H), 3.84-3.76 (m, 1H), 3.61 (System ABX, $J_{\text{AB}} = 11.0$ Hz, $J_{\text{AX}} = 5.9$ Hz, $J_{\text{BX}} = 6.3$ Hz, 2H), 2.00 (s, 3H), 1.14 (d, $J = 6.4$ Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD): 173.8, 67.2, 62.8, 57.6, 22.7, 20.4. HRMS m/z : Calc for $\text{C}_6\text{H}_{11}\text{NO}_2$ ($M - \text{H}_2\text{O}$) 129.0790, found 129.1524.

***N*-[*(2S, 3R)*-1-(bis(4-methoxyphenyl)(phenyl)methoxy)-3-hydroxybutan-2-yl]-acetamide (**6b**).**



Compound **6b** was obtained following procedure B. After flash chromatography (cyclohexane 1 / EtOAc 3) 85 mg (0.189 mMol) were obtained as a yellowish solid. ^1H NMR (400 Mz, CDCl_3): 7.38 (d, $J = 7.3$ Hz, 2H), 7.27 (d, $J = 8.8$ Hz, 6H), 7.22 (t, $J = 7.2$ Hz, 1H), 6.83 (d, $J = 8.74$ Hz, 4H), 6.05 (d, $J = 8.7$ Hz, 1H), 4.15-4.06 (m, 1H), 3.94-3.88 (m, 1H), 3.79 (s, 6H), 3.35 (System ABX, $J_{AB} = 9.7$ Hz, $J_{AX} = 4.4$ Hz, $J_{BX} = 3.5$ Hz, 2H), 3.02 (s, 1H), 2.02 (s, 3H), 1.13 (d, $J = 6.4$ Hz, 3H). ^{13}C NMR (100 MHz, CDCl_3): 170.4, 158.7 (2C), 144.3, 135.5, 135.3, 129.9 (2C), 129.8 (2C), 128.0 (2C), 127.9 (2C), 127.0, 113.3 (4C), 86.9, 68.7, 65.4, 55.2 (2C), 53.4, 23.3, 19.9. HRMS m/z : Calc for $\text{C}_{27}\text{H}_{31}\text{NNaO}_5$ ($M + \text{Na}$) $^+$ 472.2100, found: 472.2096.

RNA synthesis and purification methods:

RNA oligonucleotides were synthesized in a ABI 394 DNA / RNA Synthesizer using 2'-TBDMS phosphoramidites (Sigma-Aldrich). RNA oligonucleotides were synthesized on the 1 μmol scale. After solid-phase synthesis, the solid support was transferred to a screw-cap glass vial and incubated at 55 $^\circ\text{C}$ for 1 h with 1.5 mL of NH_3 solution (33%) and 0.5 mL of ethanol. After the vial was cooled briefly on ice, the supernatant was transferred by pipet into 2 mL Eppendorf tubes; the solid support and vial were rinsed with 50% ethanol (2×0.25 mL). The combined solutions were evaporated to dryness using an evaporating centrifuge. The residue was dissolved in a total volume of 0.33 mL of TBAF (1M) in THF and rocked at room temperature for 12 h. Then, 0.33 mL of Et_3NHAcO (1 M) and 0.33 mL of water were added to the solution, and the oligonucleotide was desalted on a NAP-10 column using water as the eluent and evaporated to dryness. The oligonucleotides were purified by HPLC (DMT-ON). The pure fractions were combined and evaporated to dryness. The residues were treated with 0.4 mL of AcOH solution (3%) and incubated at room temperature for 1 h. The deprotected oligonucleotides were purified by HPLC (DMT-OFF). The pure fractions were combined and

evaporated to reach a volume of 0.5 mL and desalted using a NAP-5 column and water as eluent.

Oligonucleotide Characterization.

All synthesized oligonucleotides were characterized by MALDI-TOF mass spectrometry:

Oligonucleotide	Sequence	MW calculated	MW found
WT GS	5-UCGAAGUAUCCGCGUACGTT-3'	6646.2	6646.2
Py GS	5-UCGAAGUAUCCGCGUACGT- Py -3'	6736.1	6731.1
Anthra GS	5-UCGAAGUAUCCGCGUACGT- Anthra -3'	6712.1	6707.8
Naph GS	5-UCGAAGUAUCCGCGUACGT- Naph -3'	6662.1	6657.4
CF3 GS	5-UCGAAGUAUCCGCGUACGT- CF3 -3'	6680.1	6673.3
F GS	5-UCGAAGUAUCCGCGUACGT- F -3'	6630.1	6625.6
Me GS	5-UCGAAGUAUCCGCGUACGT- Me -3'	6550.1	6550.9
WT PS	5'-CGUACGCGGAAUACUUCGATT-3'	6669.2	6669.4
Py PS	5'-CGUACGCGGAAUACUUCGAT- Py -3'	6759.1	6753.5
Anthra PS	5'-CGUACGCGGAAUACUUCGAT- Anthra -3'	6735.1	6735.3
Naph PS	5'-CGUACGCGGAAUACUUCGAT- Naph -3'	6685.1	6686.4
CF3 PS	5'-CGUACGCGGAAUACUUCGAT- CF3 -3'	6703.1	6707.4
F PS	5'-CGUACGCGGAAUACUUCGAT- F -3'	6653.1	6656.6
Me PS	5'-CGUACGCGGAAUACUUCGAT- Me -3'	6573.1	6576.9

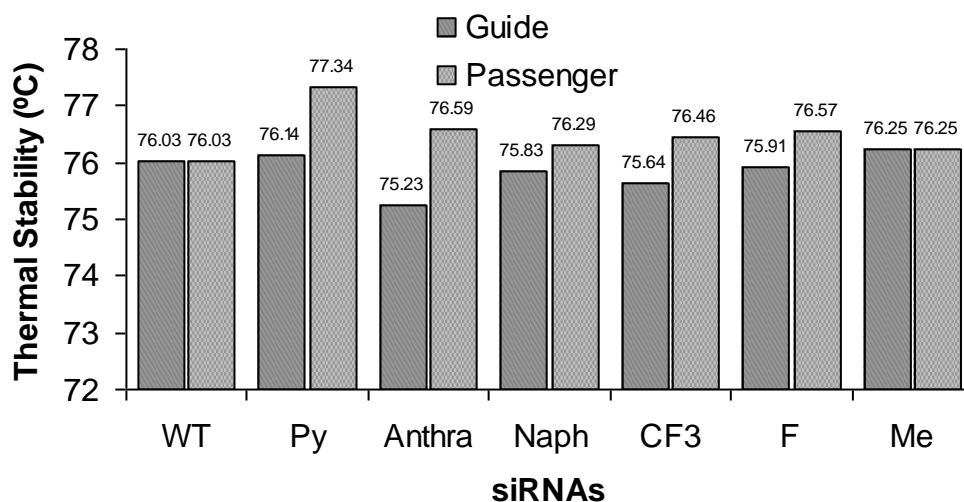
GS: Guide Strand; PS: Passenger Strand.

Thermal Stability of siRNAs

For annealing of siRNAs, a solution of guide and passenger strand at 20 μ M in annealing buffer (100mM potassium acetate, 30mM HEPES-KOH at pH 7.4, 2mM magnesium acetate) were incubated for 1 min at 92 °C and cooled down slowly to room temperature.

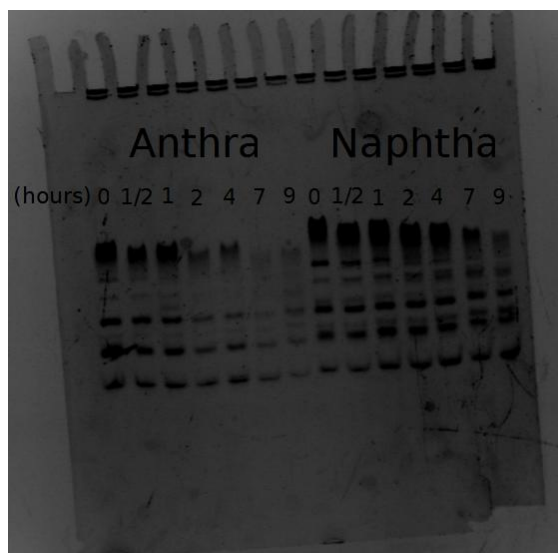
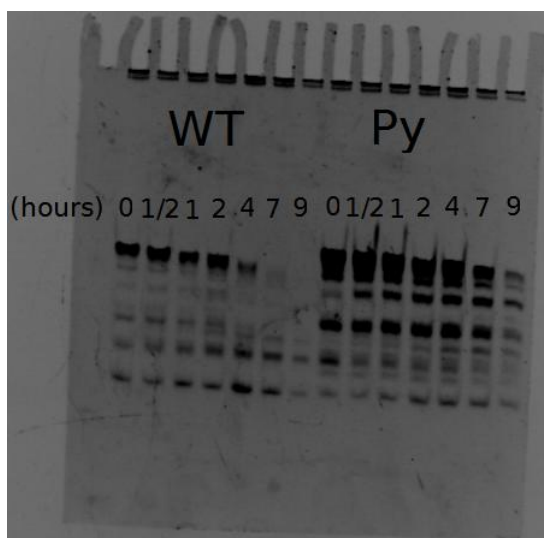
SiRNAs (0.5 μ M each) were dissolved in buffer (50 mM potassium acetate, 1 mM magnesium acetate, 15 mM HEPES-KOH at pH 7.4). Experiments were performed in Teflon-stoppered 1 cm path length quartz cells on a JASCO V-650 spectrophotometer equipped with thermoprogrammer. The denaturation experiments were done at a rate of 1 °C/min to 90 °C, monitoring absorbance at 260 nm. In all cases the complexes displayed sharp, apparently two-state transitions. The data were analyzed by the denaturation curve processing program,

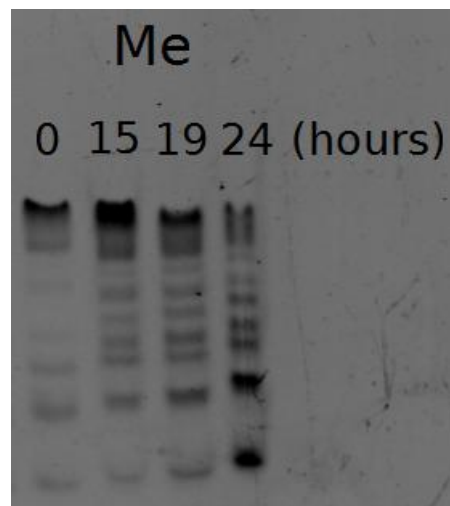
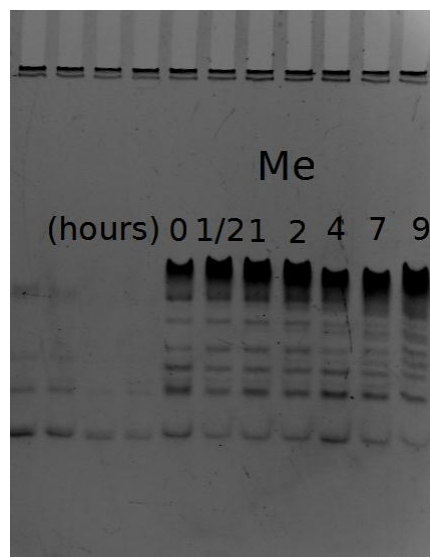
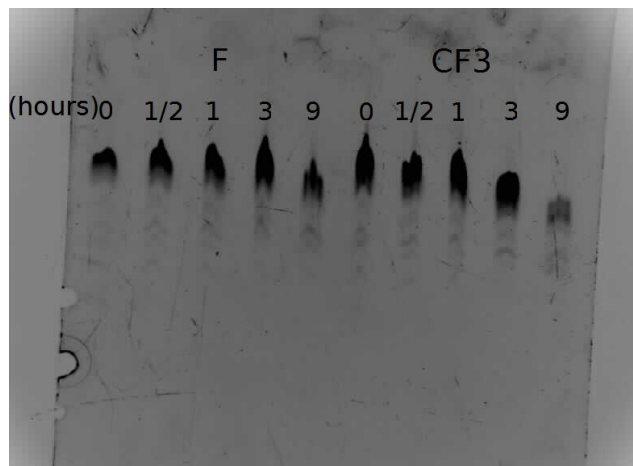
MeltWin v. 3.0. Melting temperatures (T_m) were determined by computer-fit of the first derivative of absorbance with respect to $1/T$.



Stability of siRNAs in 50% human serum

Unmodified or modified double-stranded siRNA samples (20 μ M; 24 μ L) were incubated in human serum (24 μ L) at 37 °C. At appropriate periods (0, 0.5, 1, 2, 4, 7 and 9 hours), 6 μ L aliquots of the reaction mixture were added to 54 μ L of a 1% sodium dodecyl sulphate aqueous solution and the mixtures were heated-denatured for 5 min at 90 °C. siRNAs were isolated by hot phenol extraction followed by ethanol precipitation. In the case of F and CF3 the hot phenol extraction was skipped due to problems with the extraction. After re-suspension in 20 μ L of loading buffer (90% formamide, 10% 1X TBE), the samples were run on a denaturing 14% polyacrylamide gel containing 20% formamide. RNA bands were visualized with the SYBR Green II reagent (Sigma-Aldrich) according to the manufacturer's instructions.





RNA interference methods

SH-SY5Y and Hela cells were grown at 37 °C, 5% CO₂ in Dulbecco's modified Eagles's medium (DMEM, GIBCO) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 µg/mL streptomycin. The cells were maintained in exponential growth. The cells were plated in 24-well plates (0.5 mL medium/well) to reach about 50% confluence at transfection. The cells were grown for 24 h and the culture medium was changed to OPTIMEM 1 (GIBCO), 0.5 mL/well. Two luciferase plasmids, firefly luciferase (pGL2) and *Renilla* luciferase (pRL-CMV) from Promega, were used as reporter and control, respectively. Co-transfection of plasmids and siRNAs was carried out with Lipofectamine 2000 (Invitrogen) as

described by the manufacturer for adherent cell lines. Per well, 1.0 µg pGL2, 0.1 µg pRL-CMV and the required amount of siRNAs, formulated into liposomes, were applied. The final volume was 500 µL per well. The cells were harvested 24 h after transfection, and lysed by using passive lysis buffer (PLB), 100 µL per well, according to the instructions of the Dual-Luciferase Reporter Assay System (Promega, USA). The luciferase activities of the samples were measured using a MicroLuma^{Plus} LB 96V (Berthold Technologies) with a delay time of 2 s and an integrate time of 10 s. The volumes used were: 20 µL of sample and 30 µL of each reagent (luciferase assay reagent II and Stop & Glo Reagent). The inhibitory effects generated by siRNAs were expressed as normalized ratios between the activities of the reporter GL2 (*Photinus pyralis*) luciferase gene and the control RL (*Renilla reniformis*) luciferase gene.

Autodock-vina docking.²

The protein (PDB: 1si3) and the ligands (pyrene, anthracene, naphthalene, trifluoromethyl benzene and fluorobenzene) were prepared for the docking using autodock tools (ADT).³ The following parameters were employed in the docking:

Search Space:

Center_x = 16.837.

Center_y = 77.117.

Center_z: -13.227.

Size_x: 12 angstroms.

Size_y: 12 angstroms.

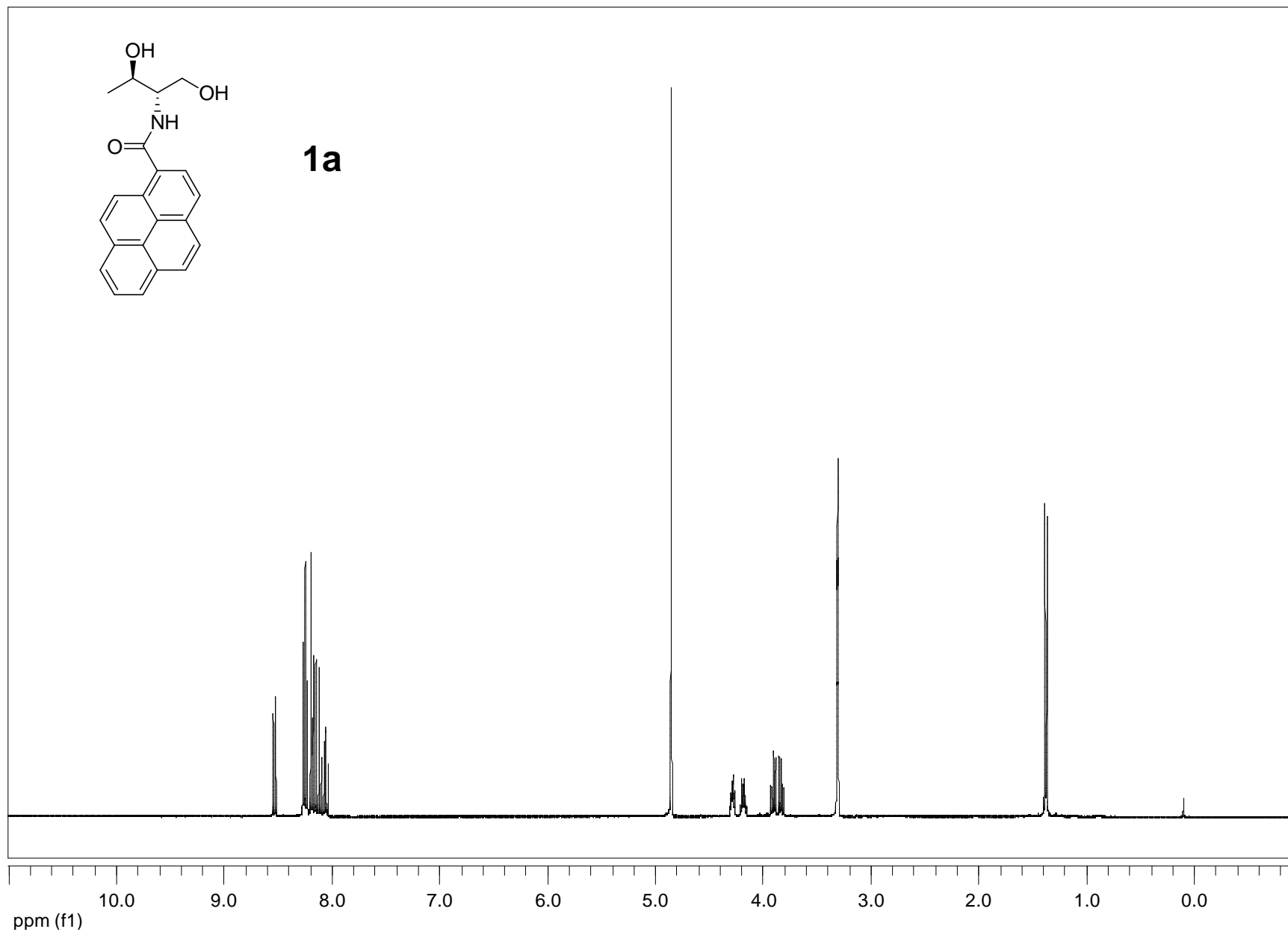
Size_z: 12 angstroms.

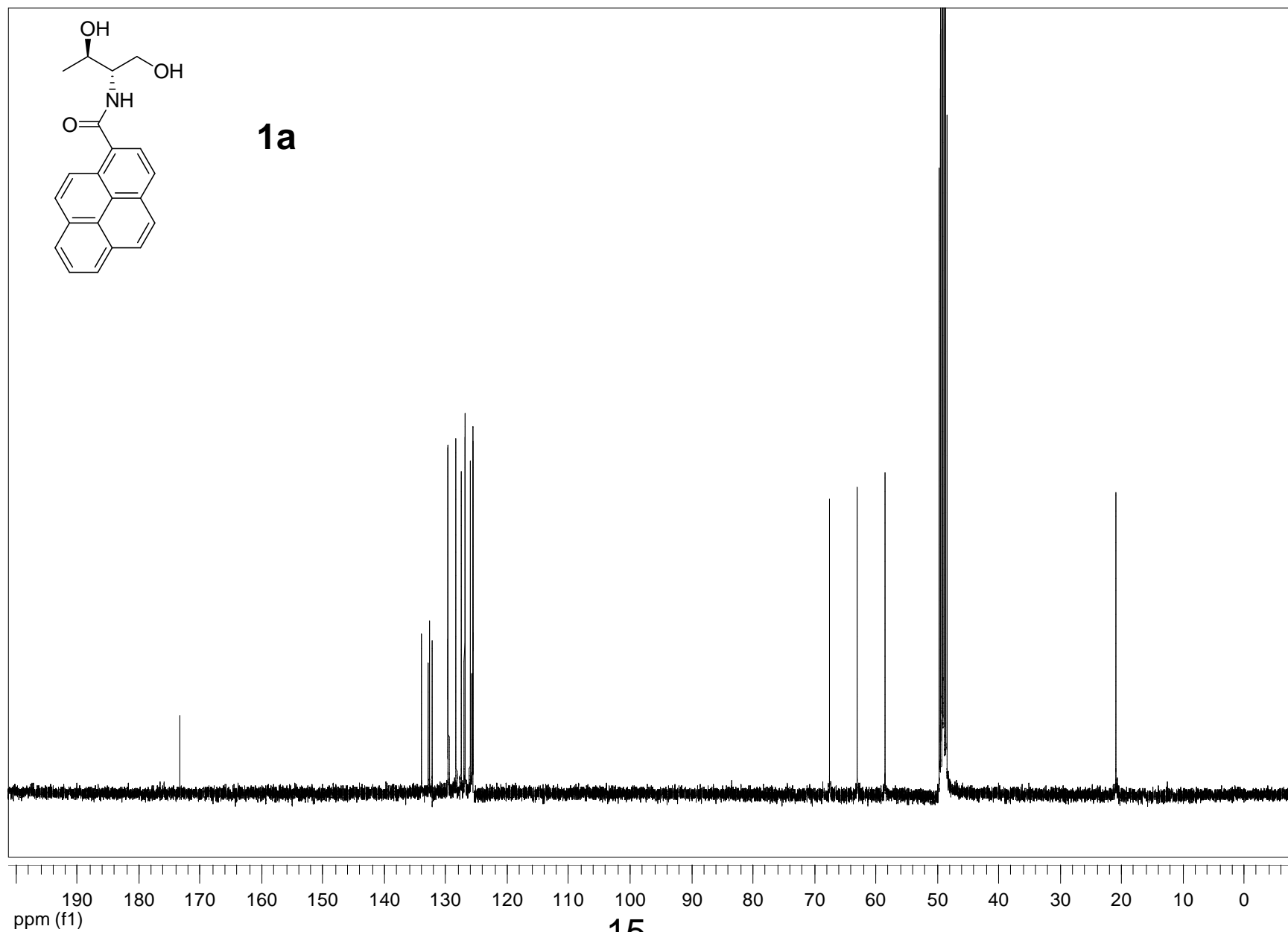
Exhaustiveness: 300.

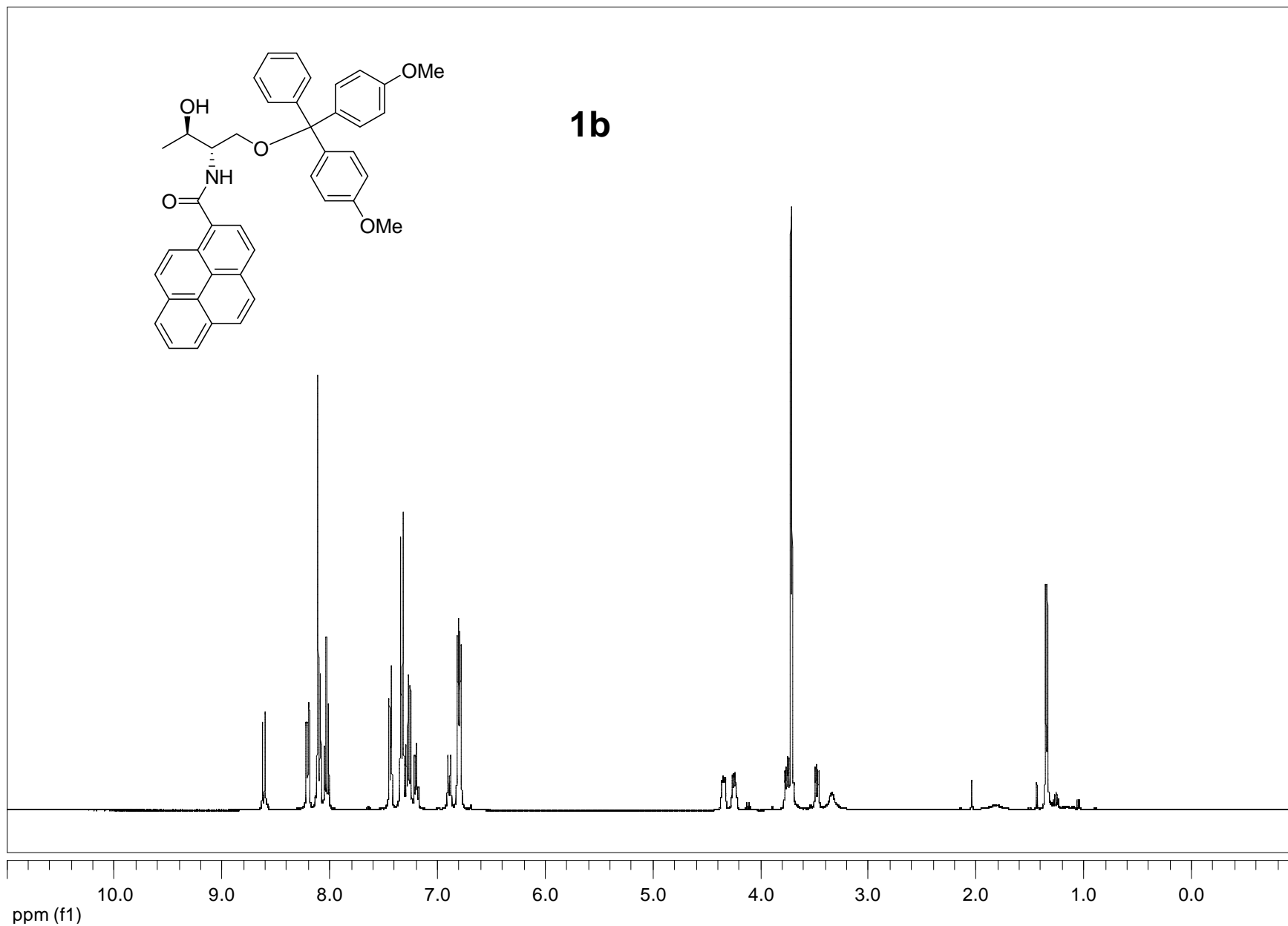
² O. Trott, A. J. Olson *J. Comput. Chem.* **2009**, DOI: 10.1002/jcc.21334.

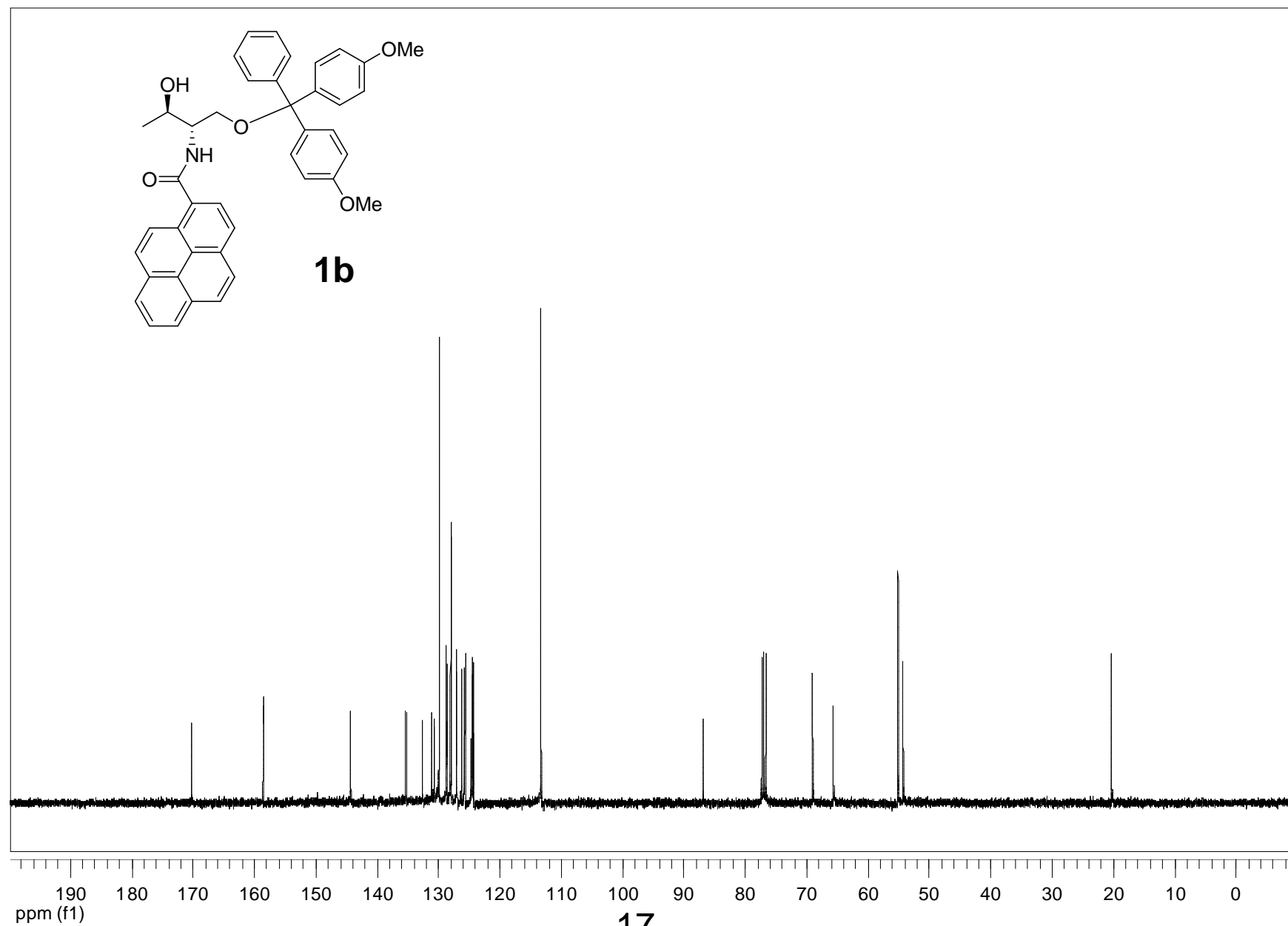
³ M. F. Sanner. *J. Mol Graphics Mod.* **1999**, 17, 57-61.

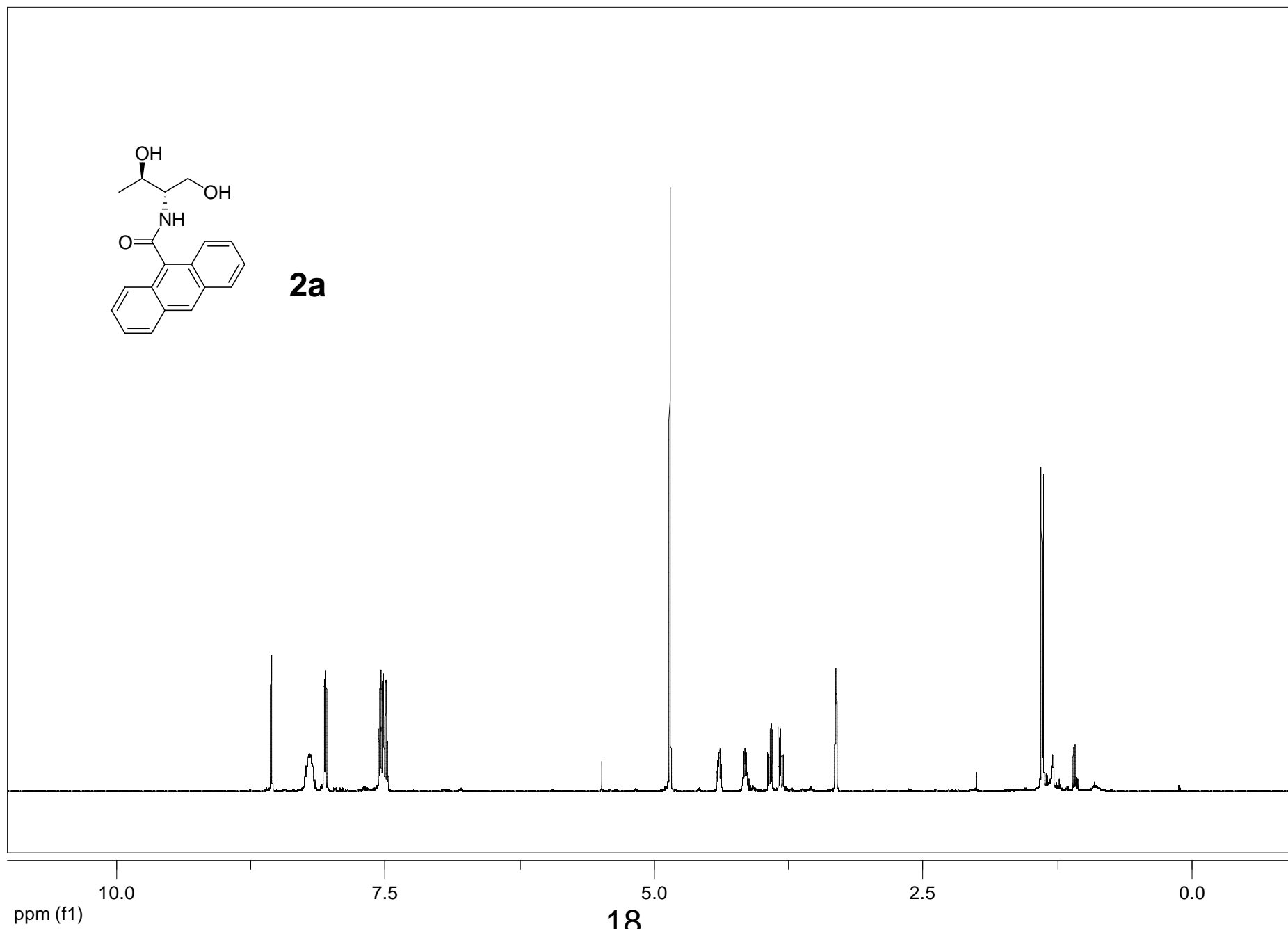
NMR SPECTRA

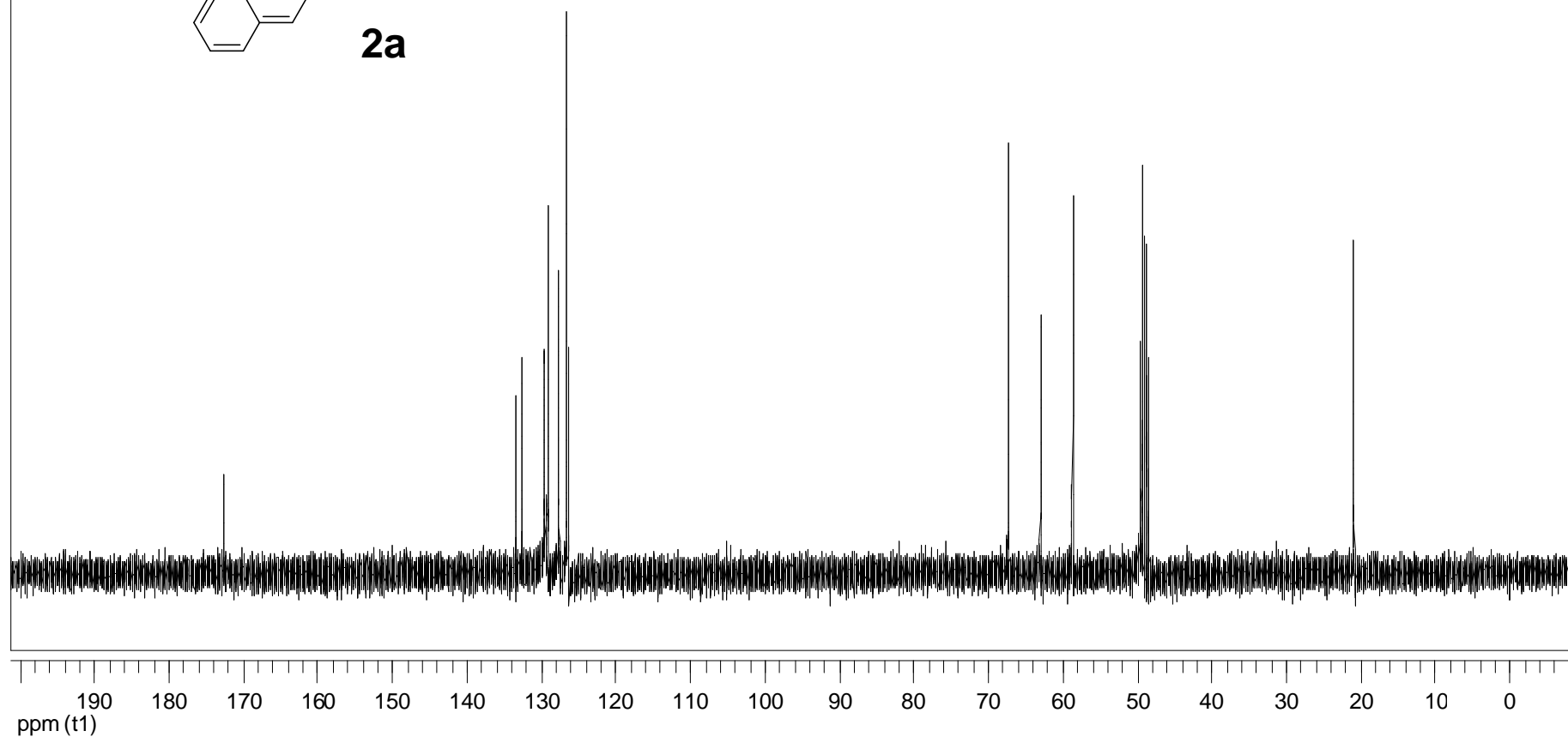
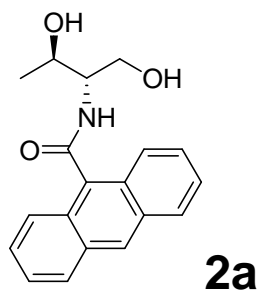


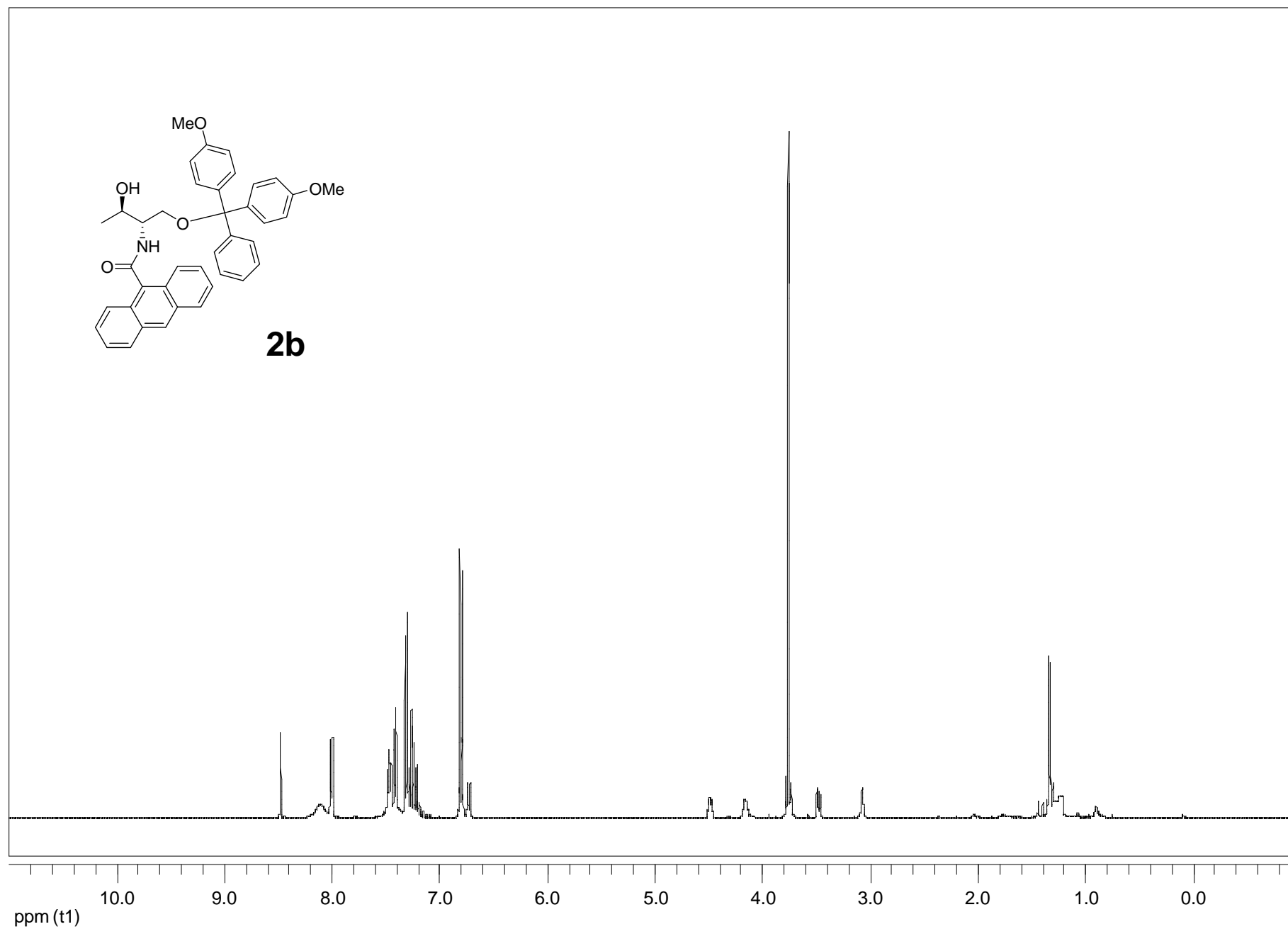


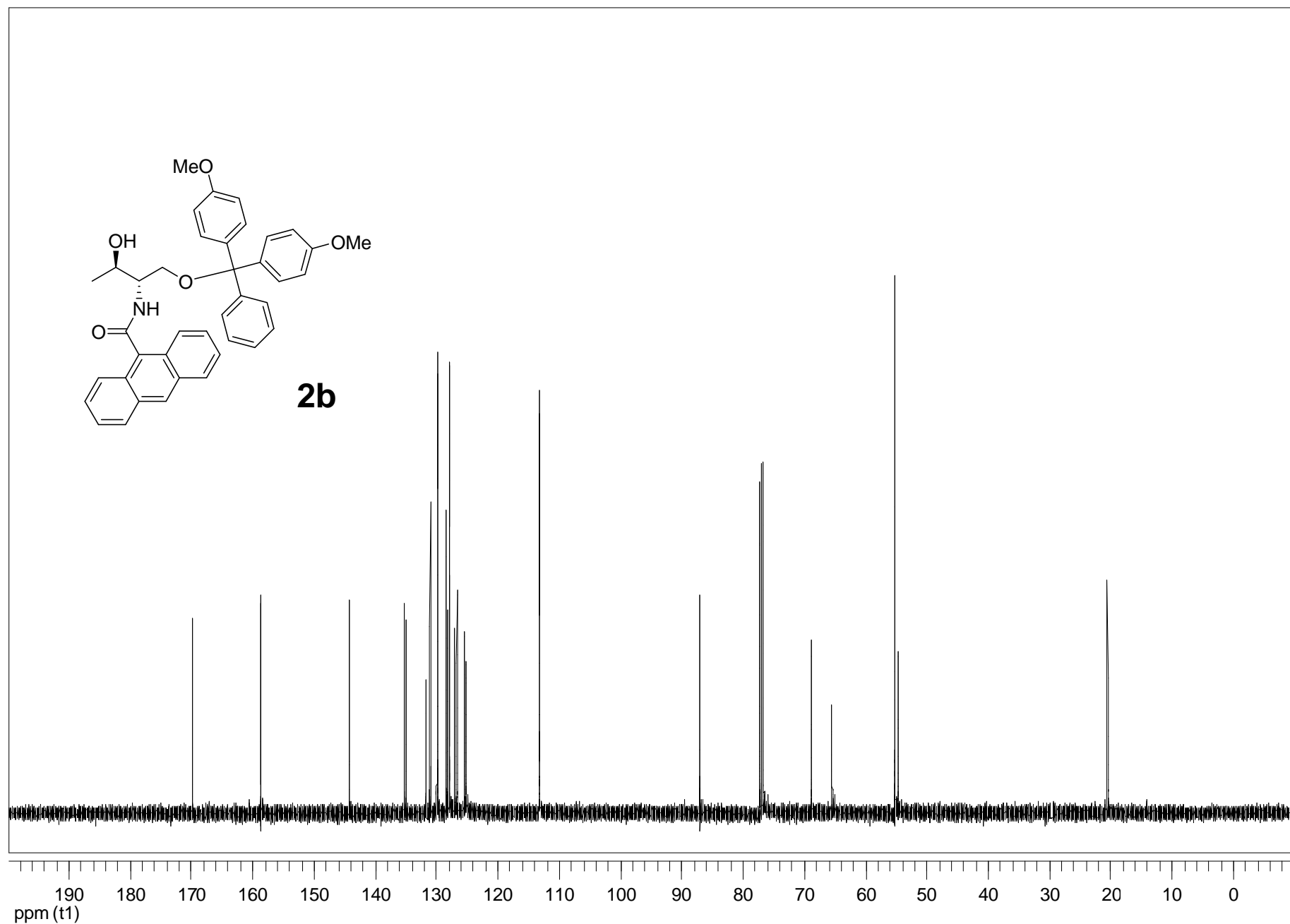


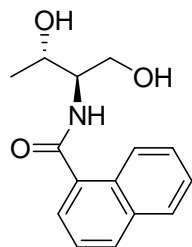




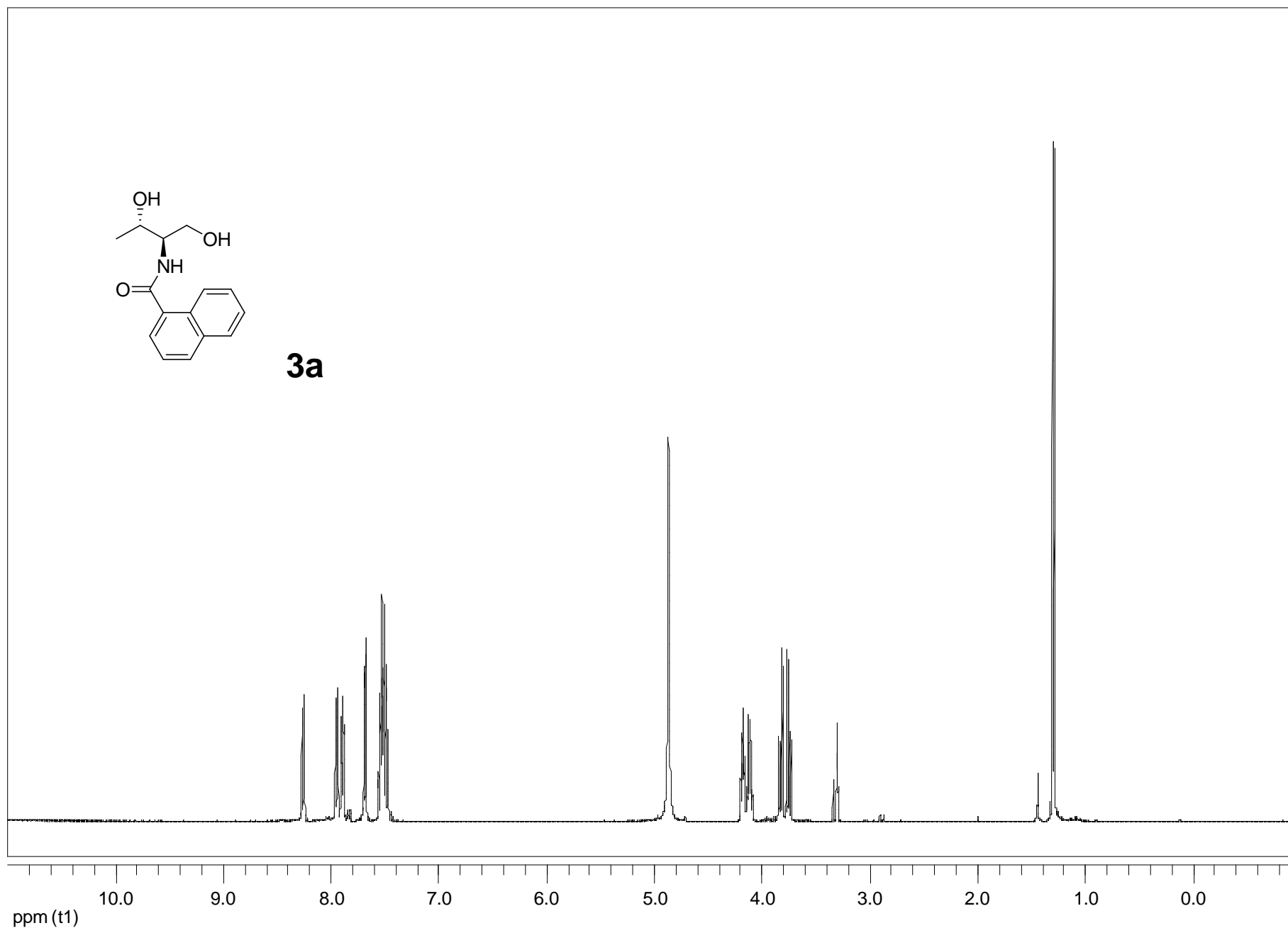




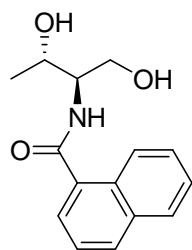




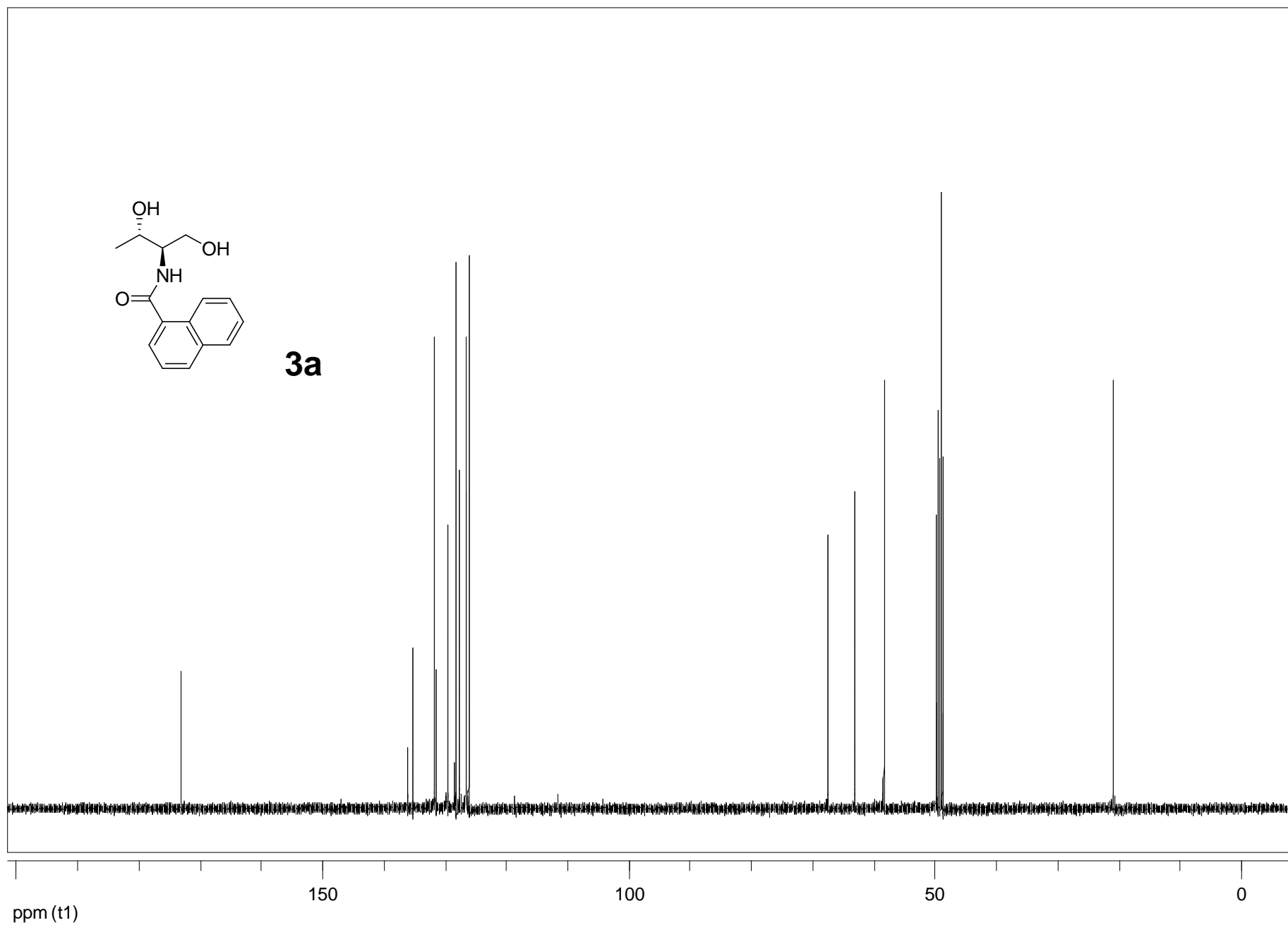
3a



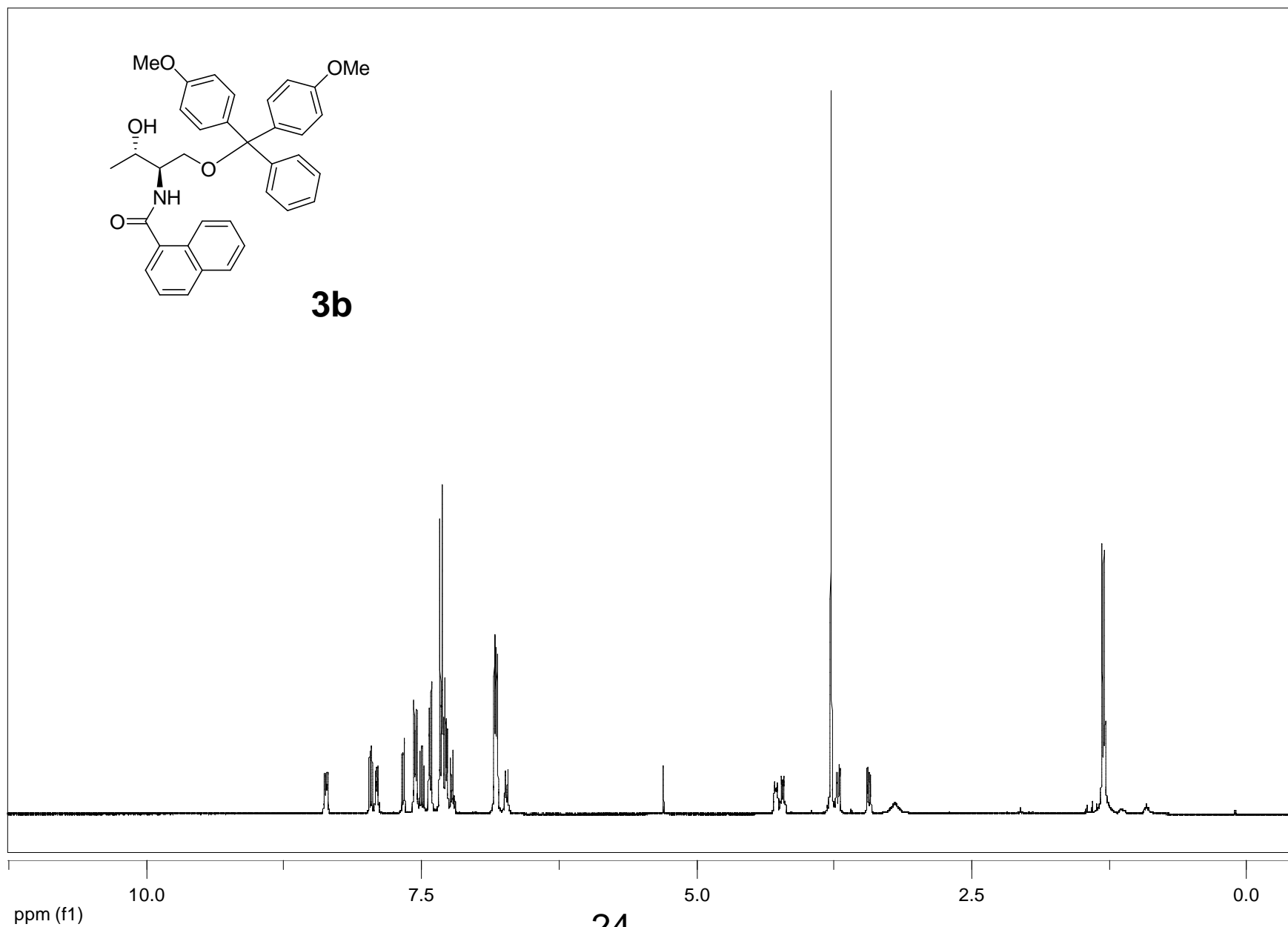
22

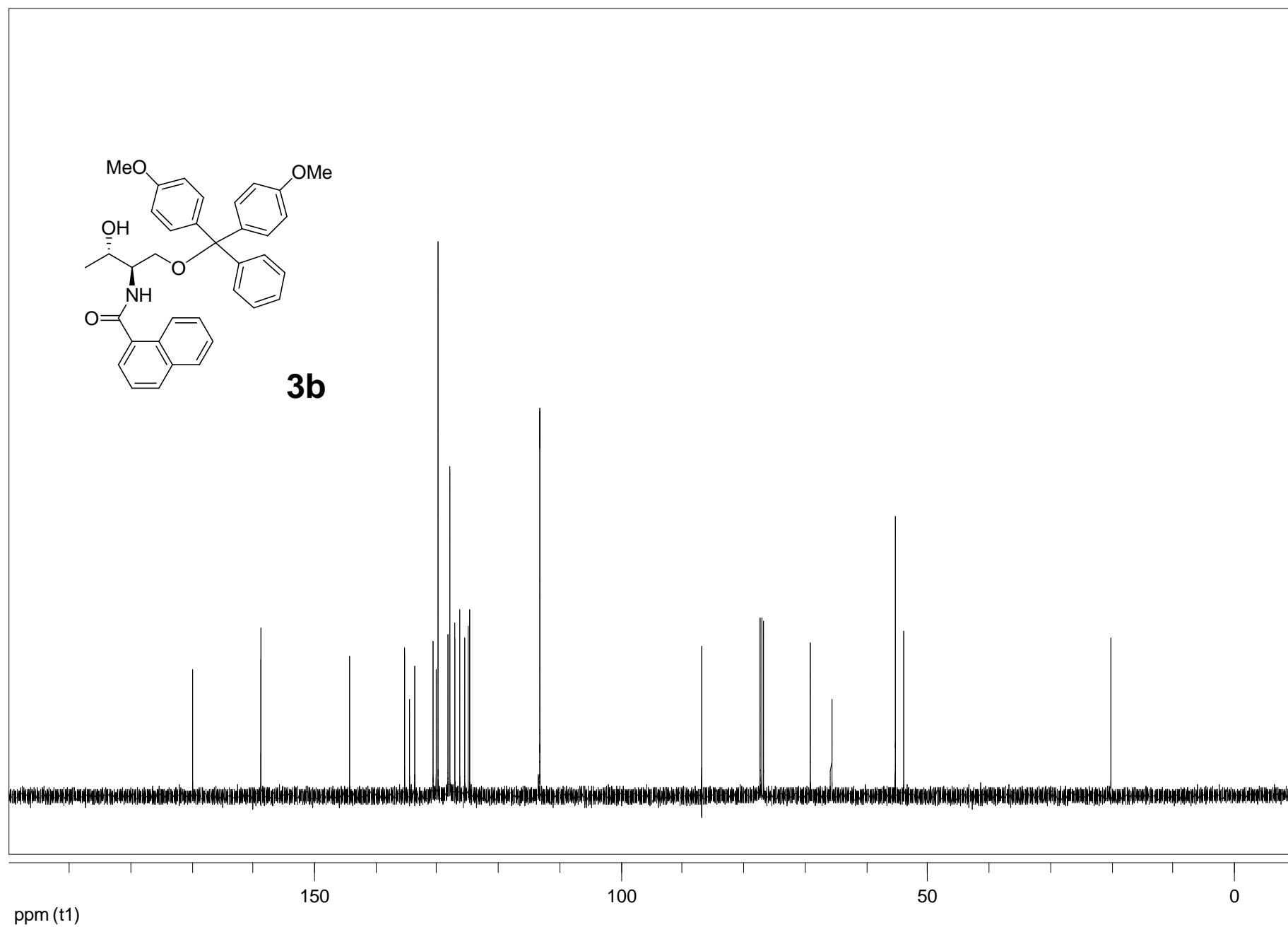


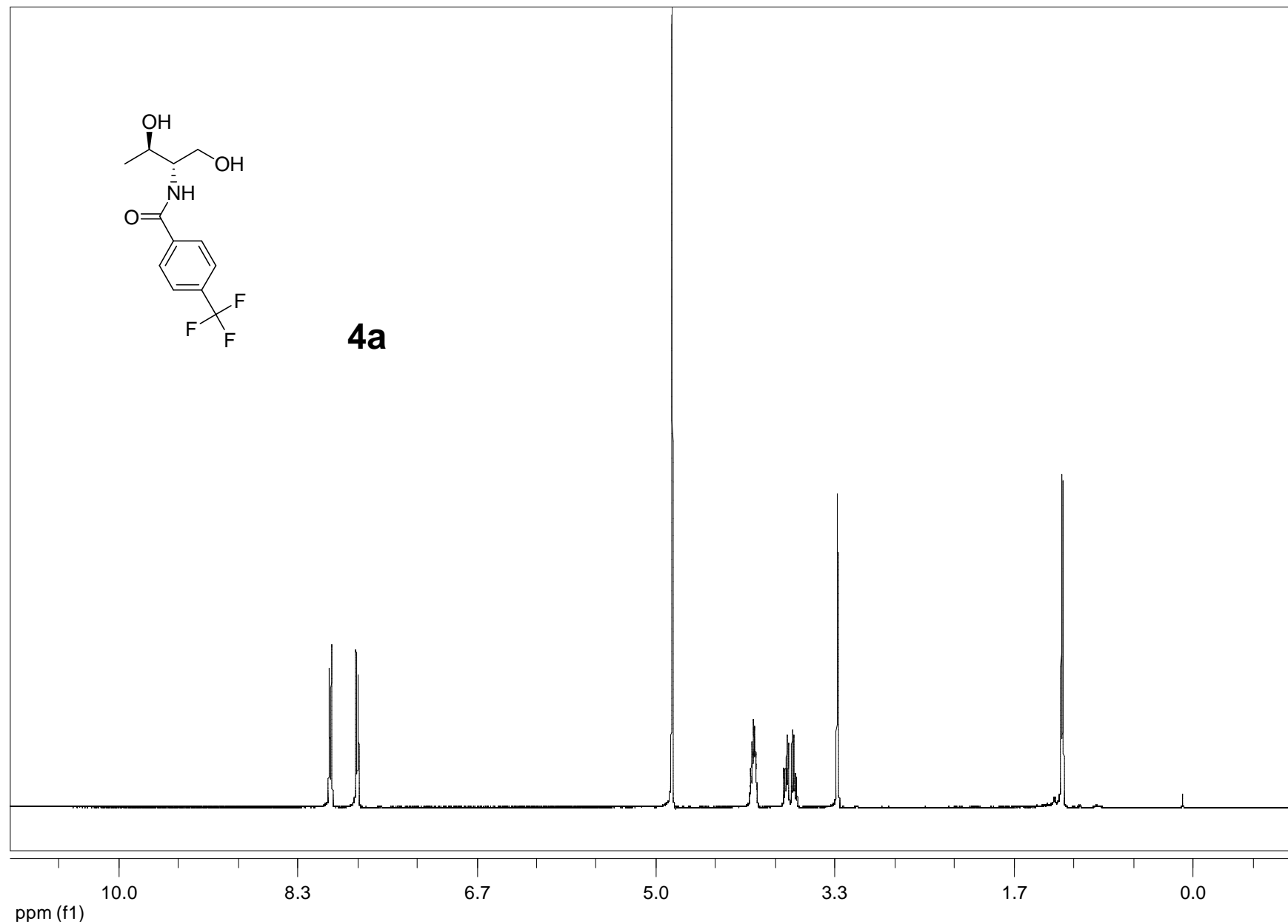
3a

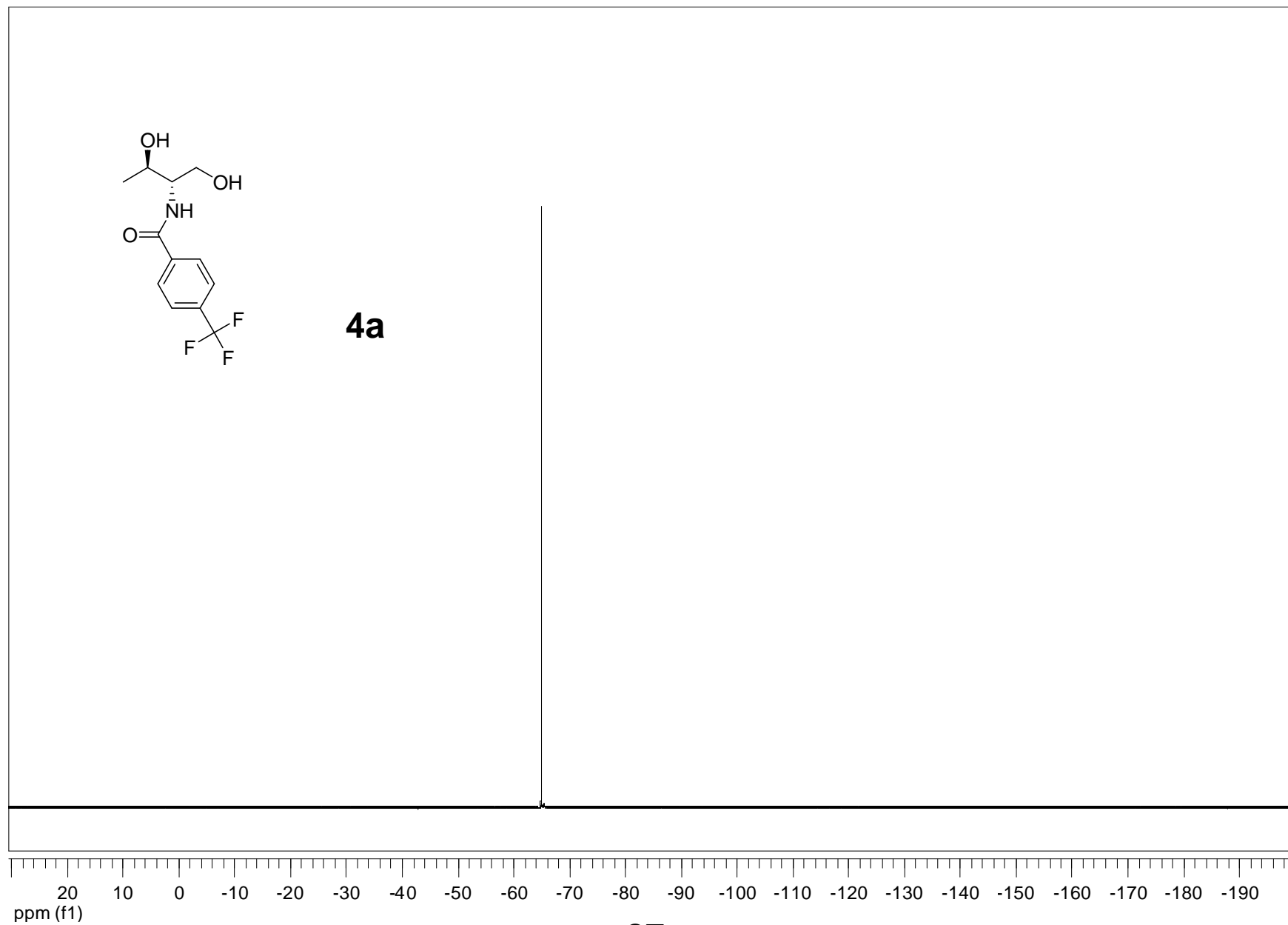


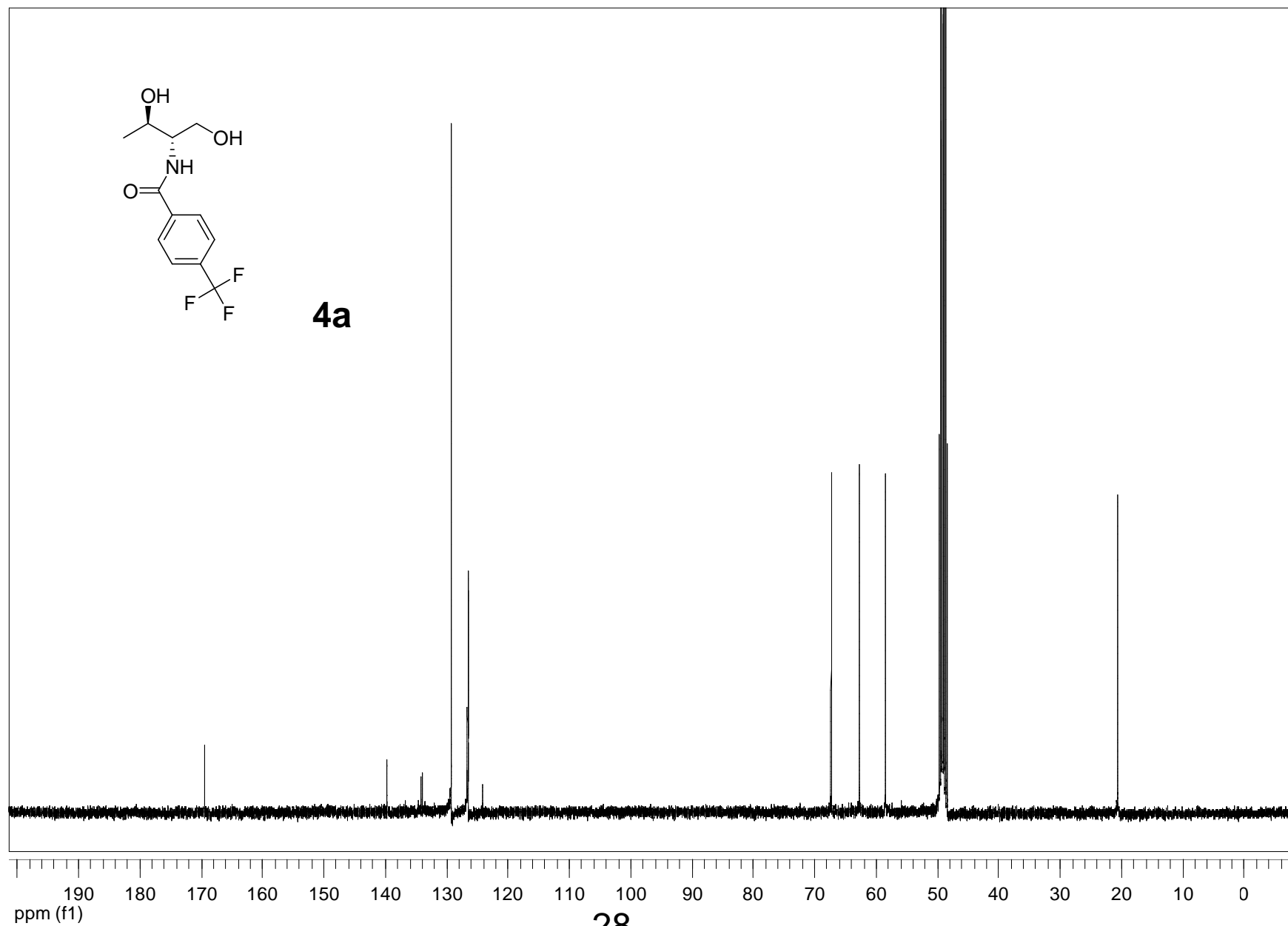
23

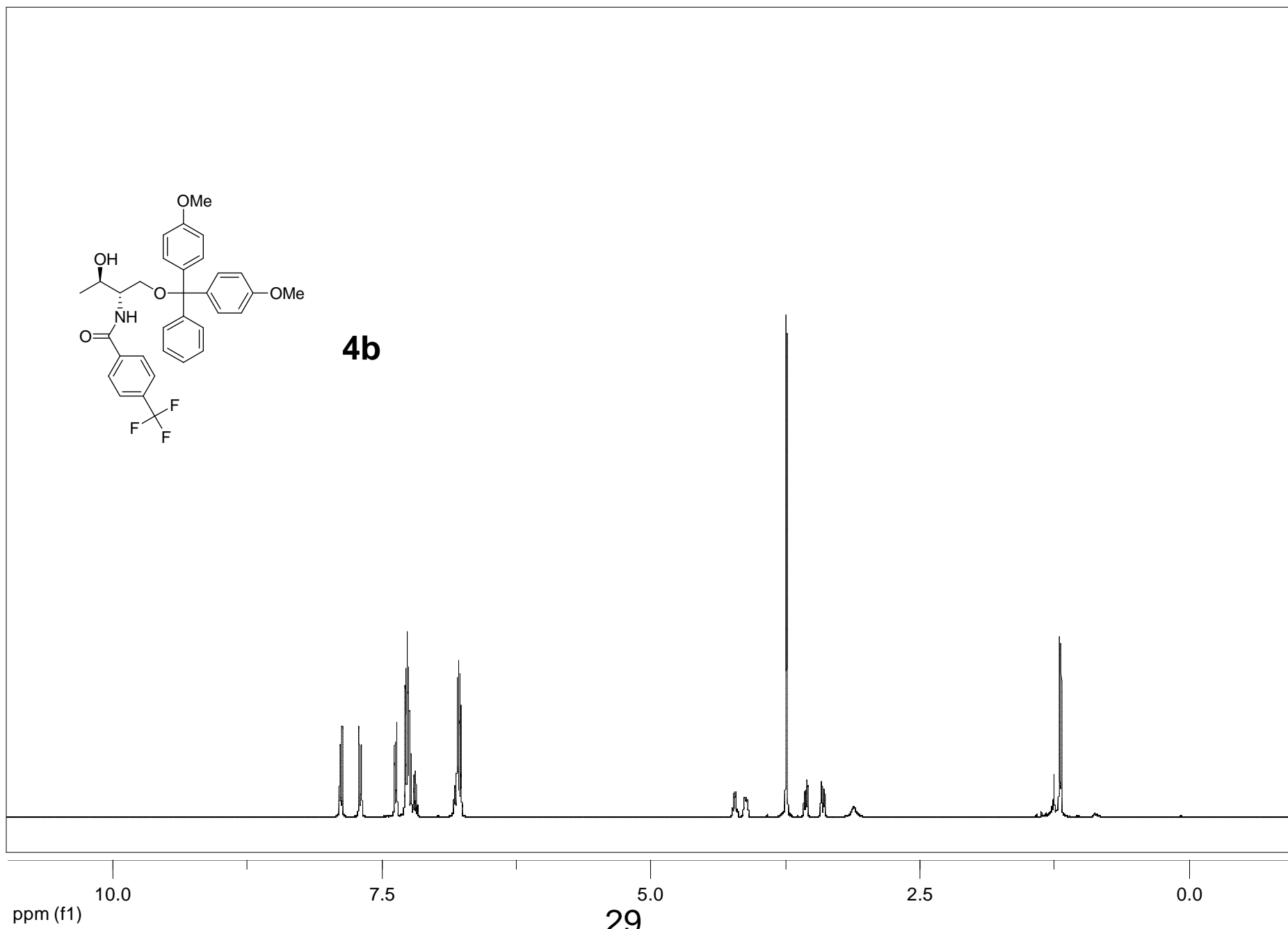


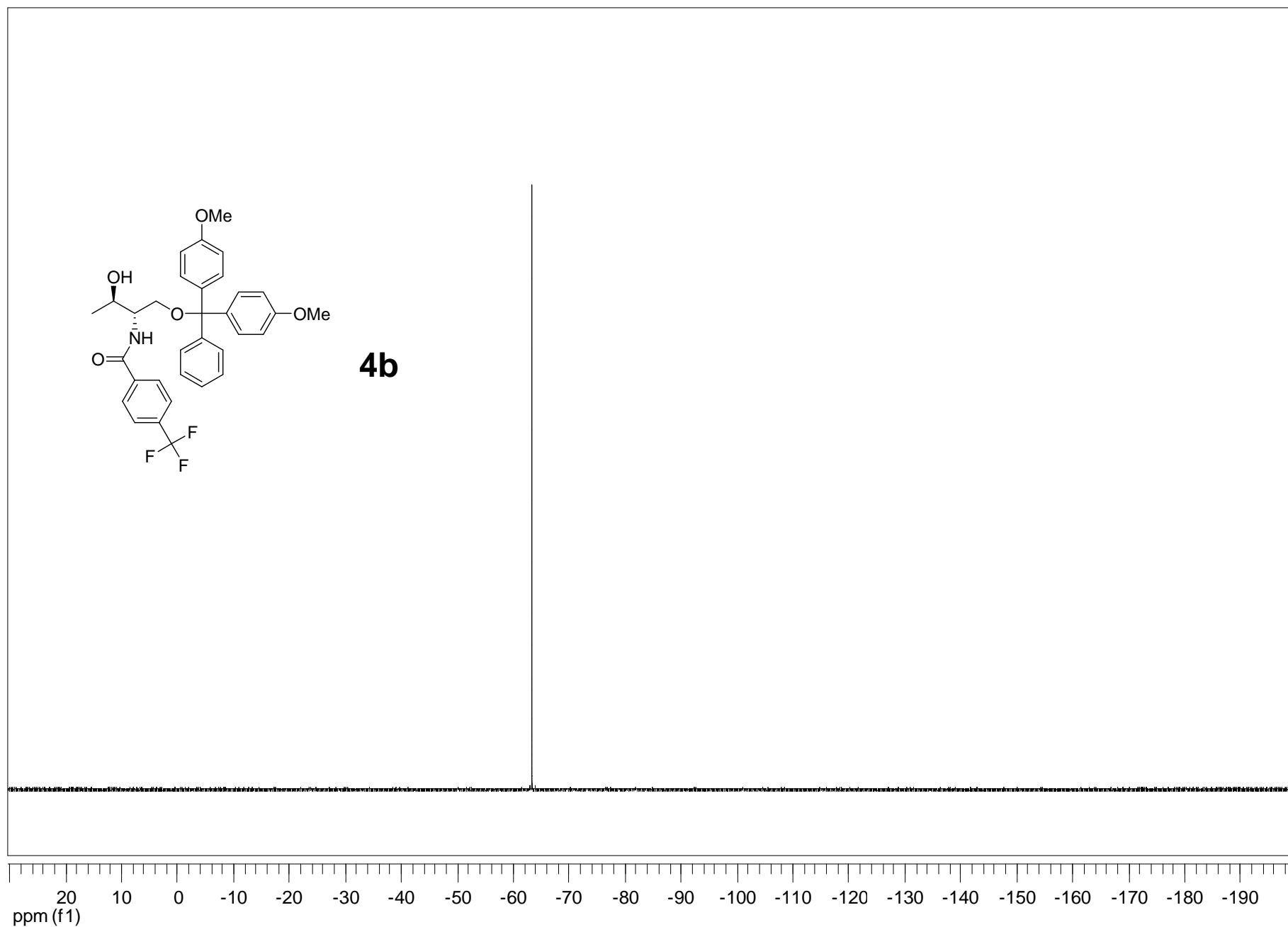


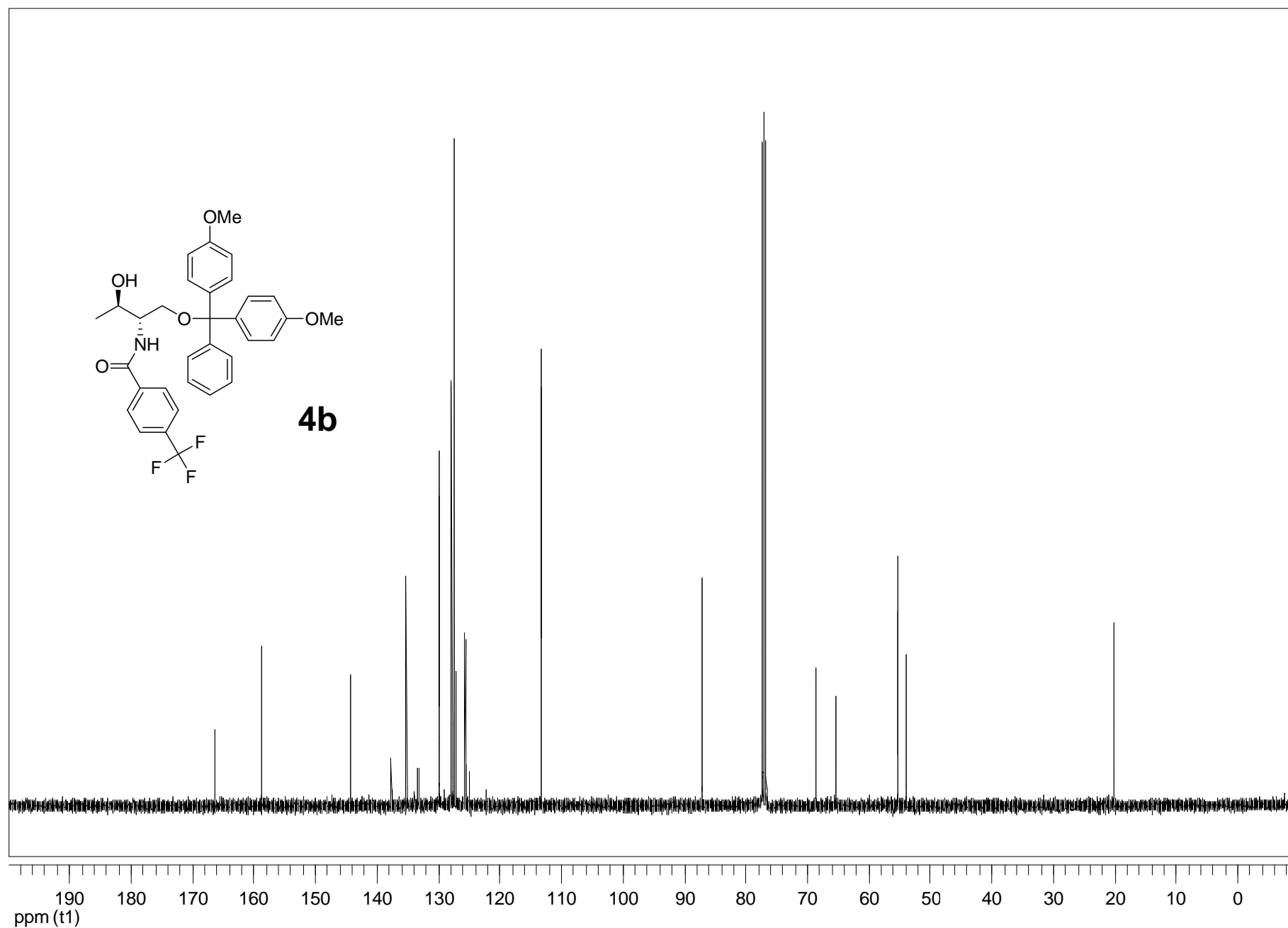


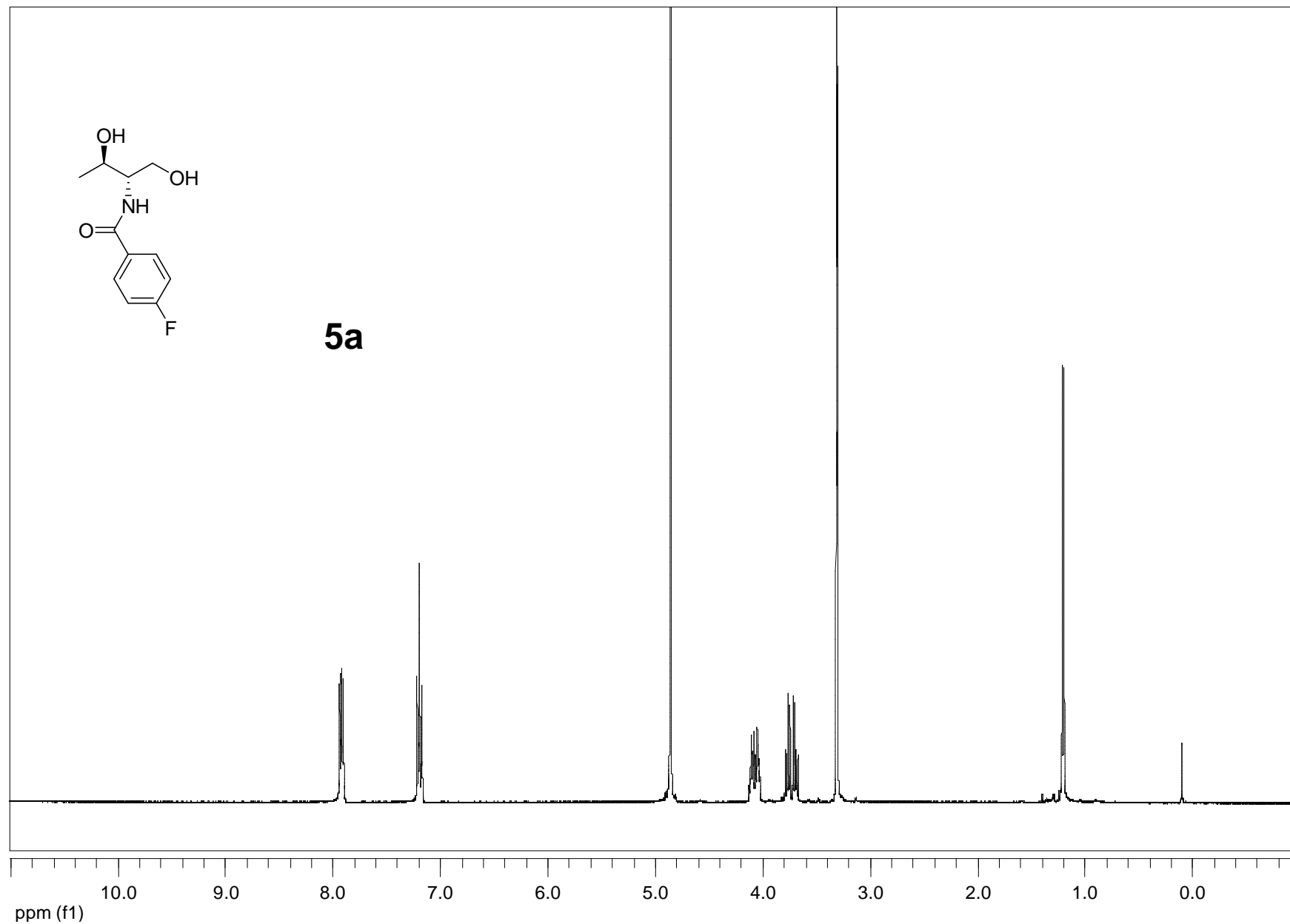


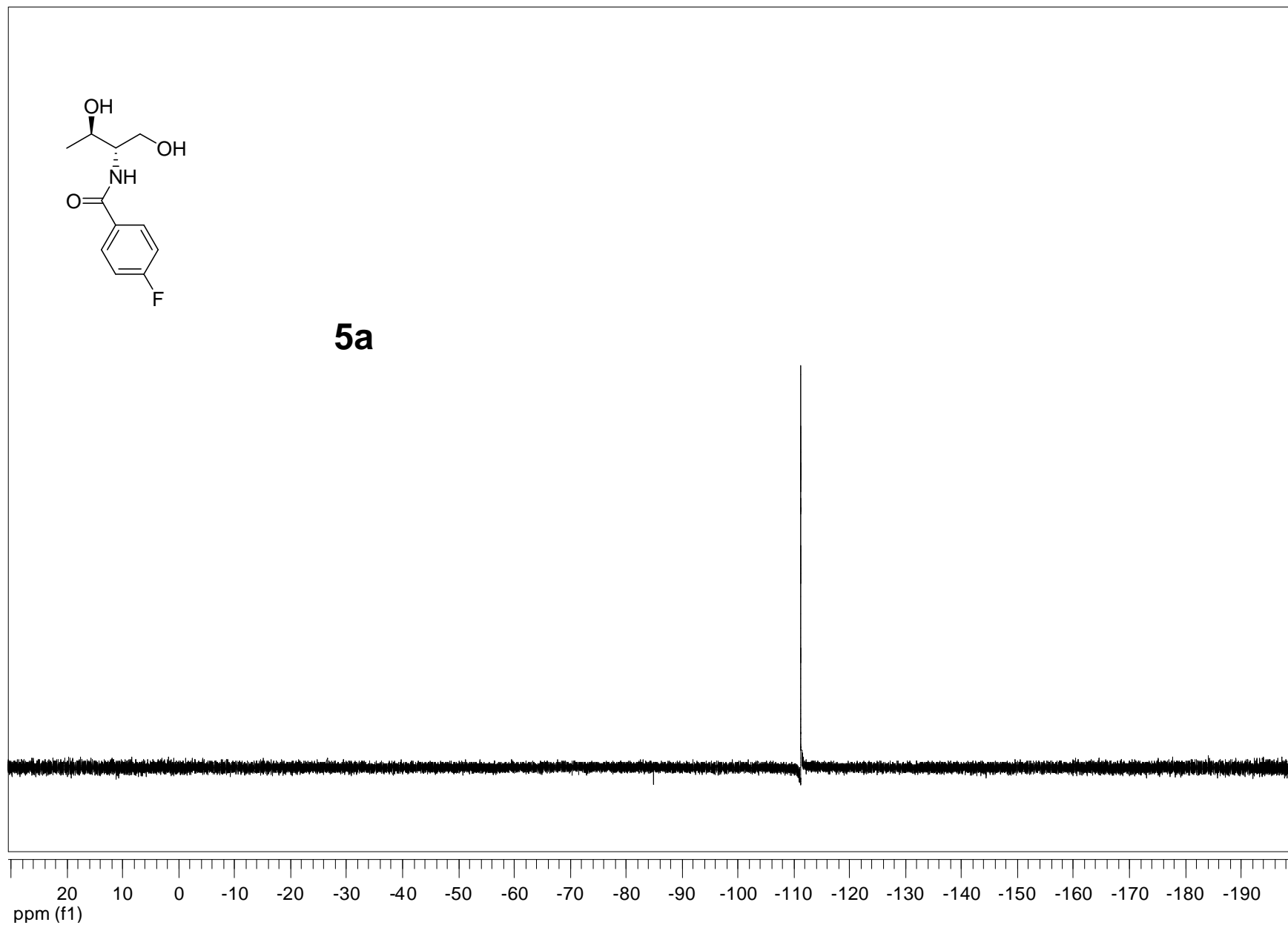


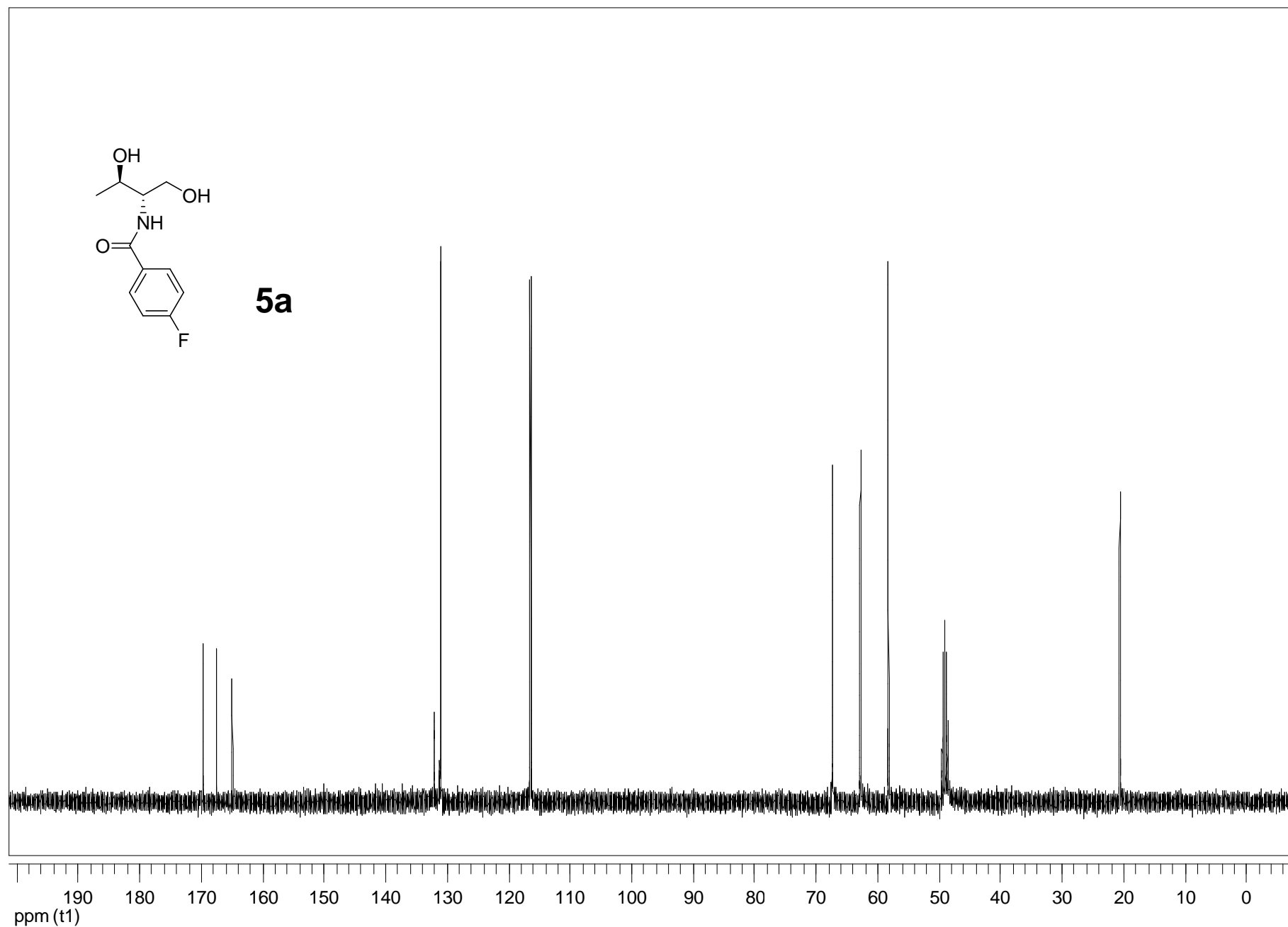


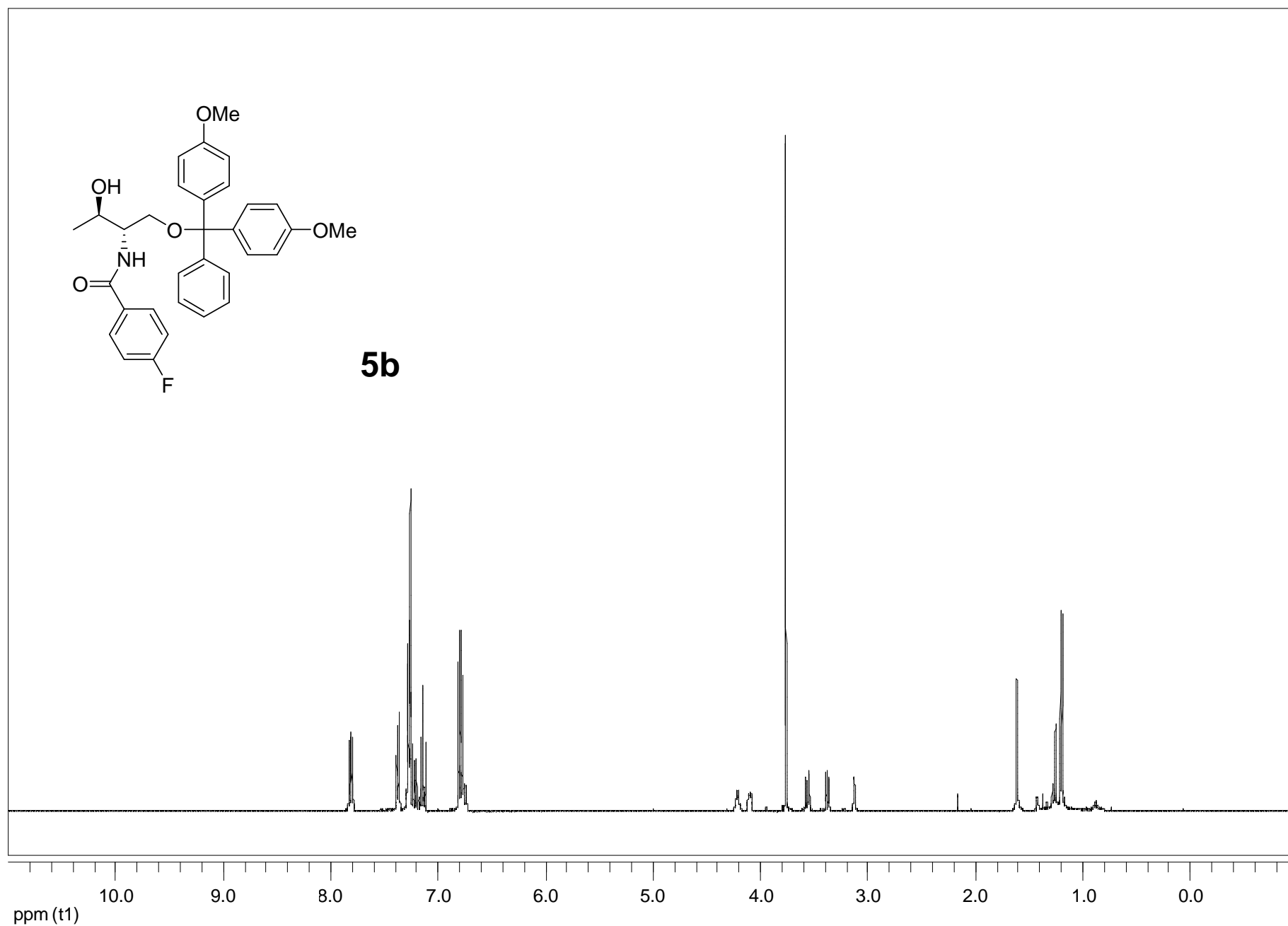


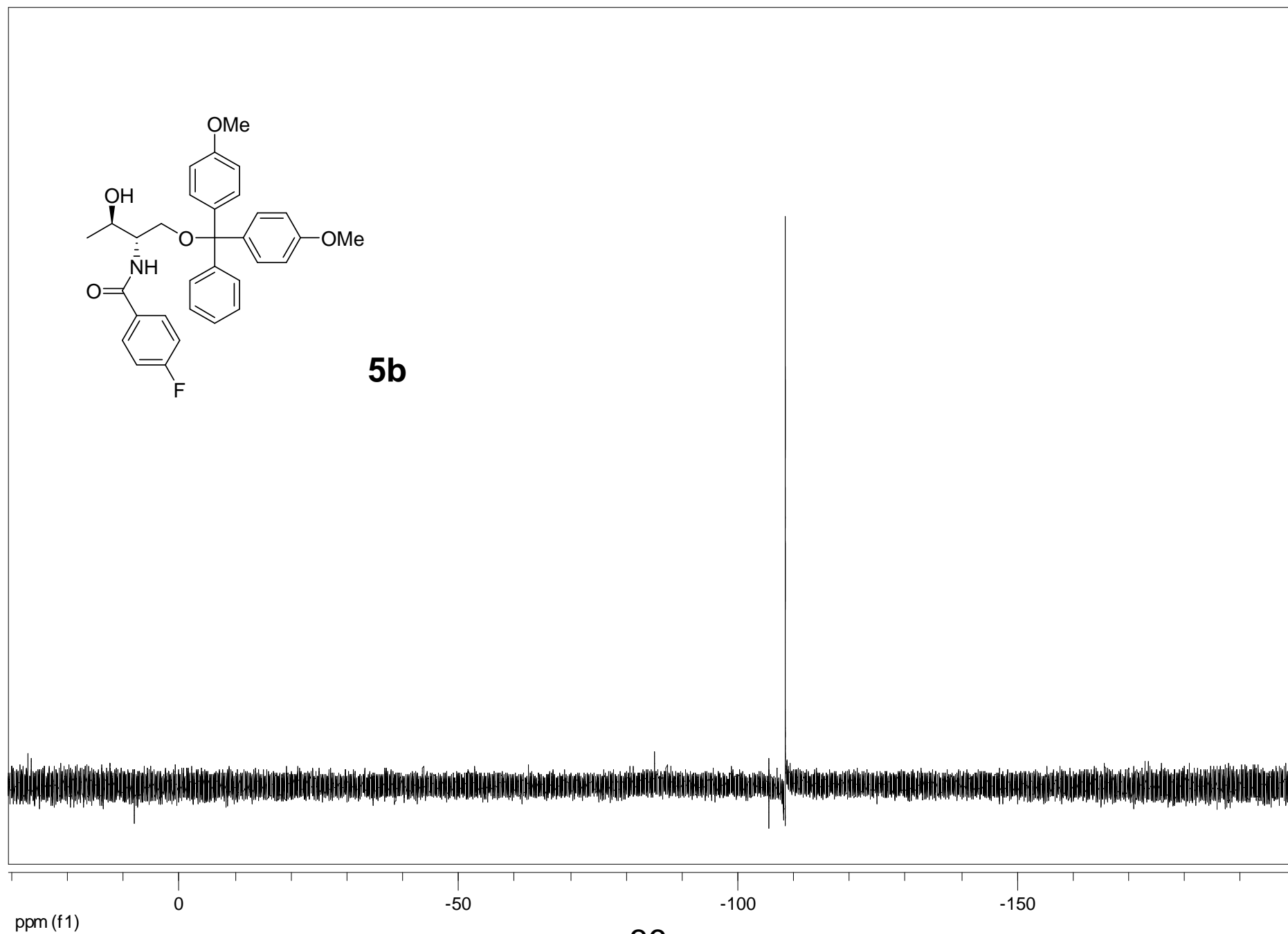


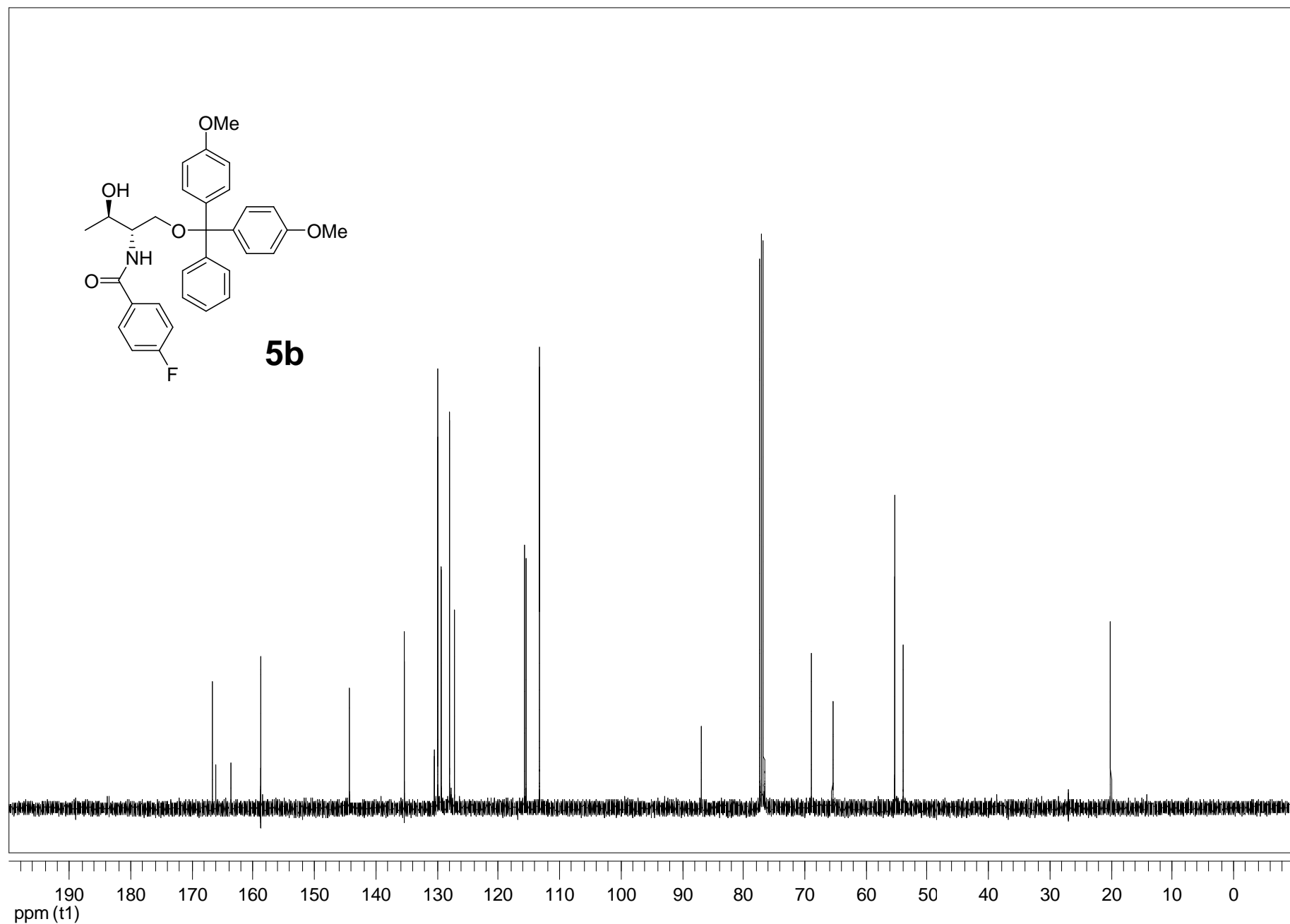


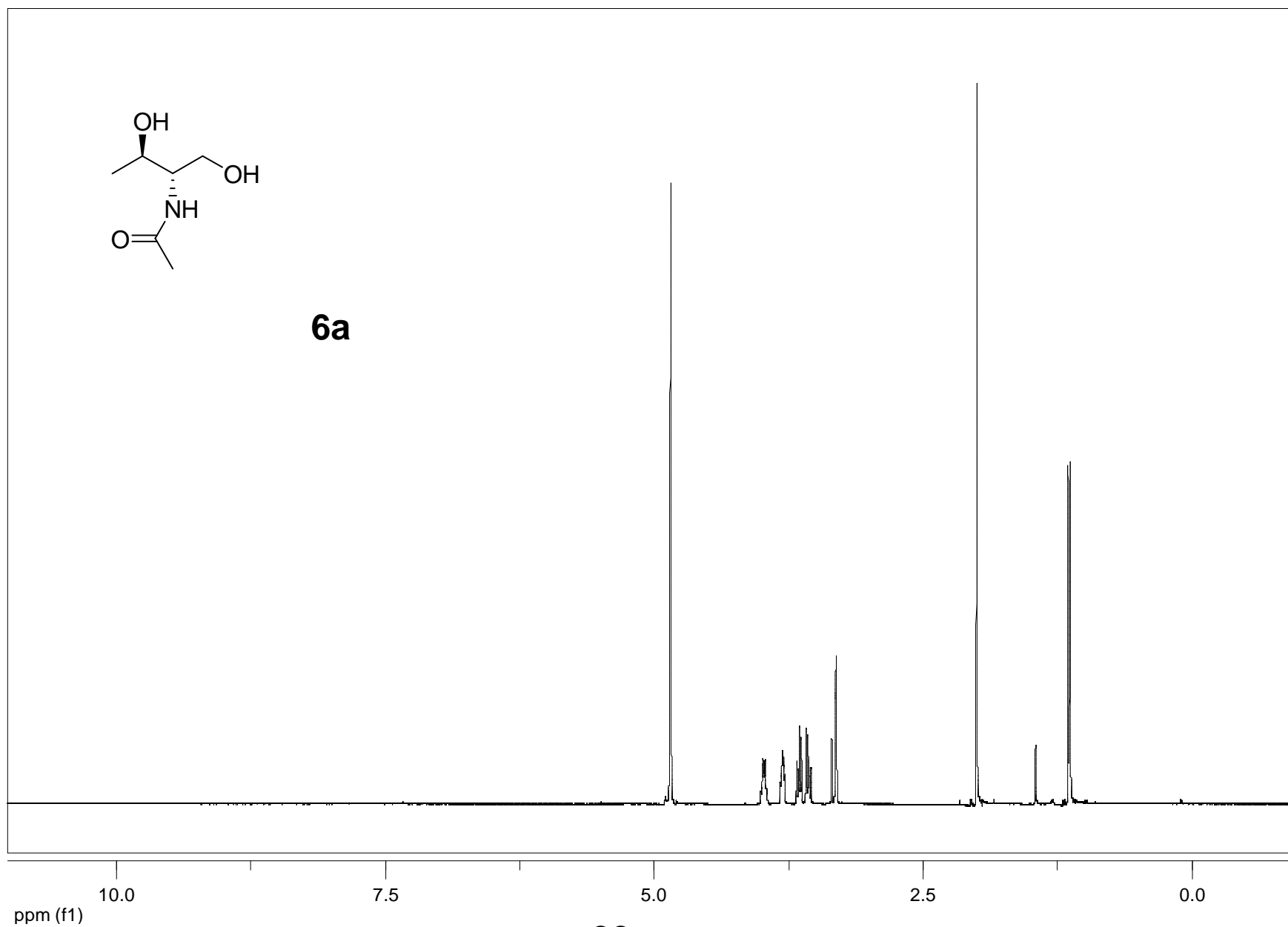


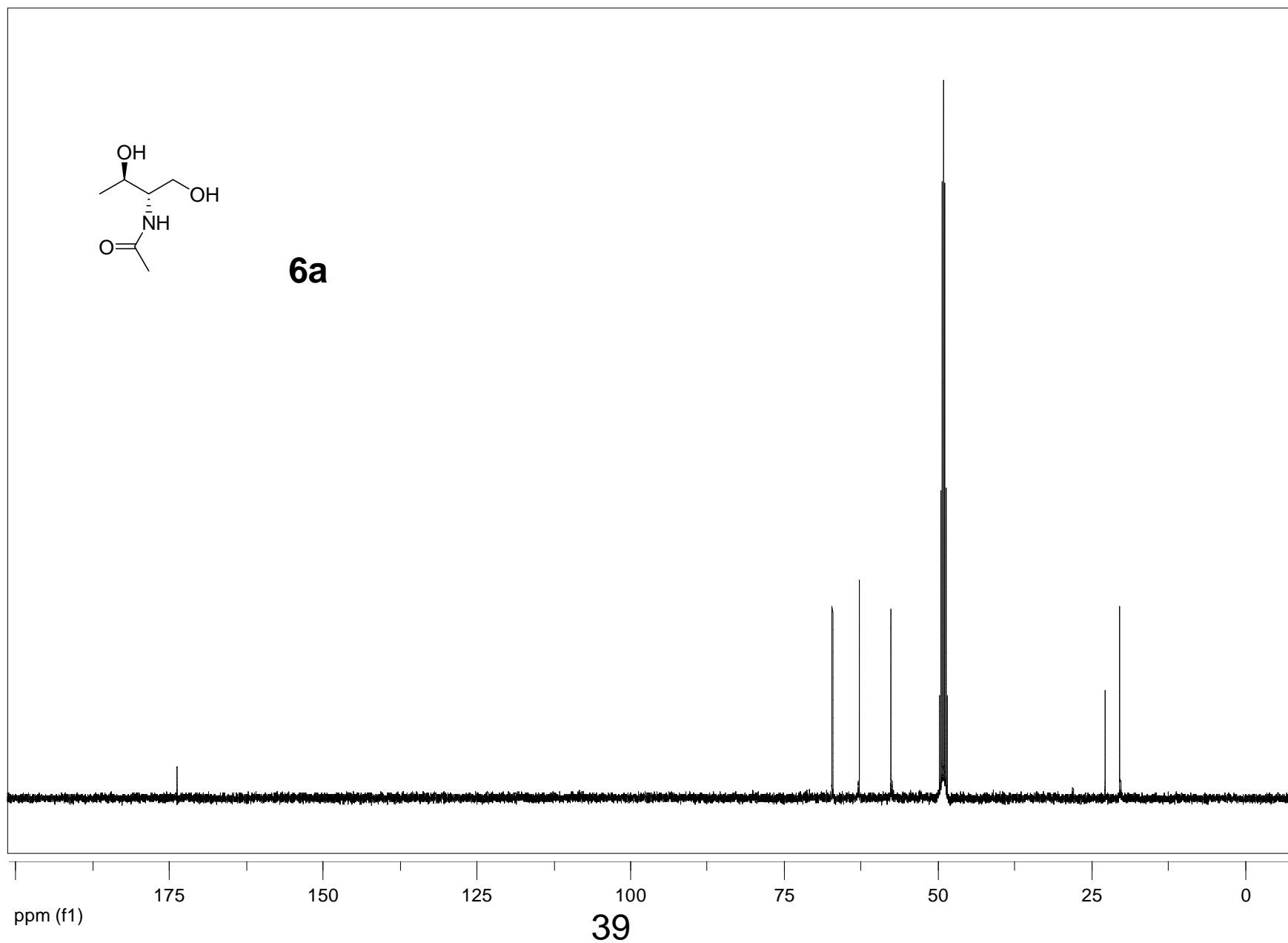


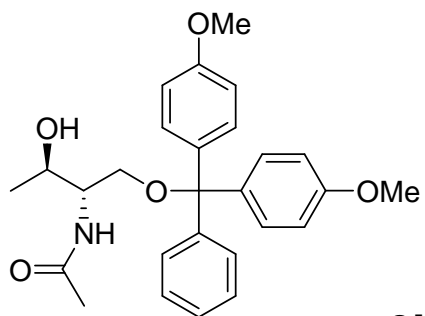












6b

