Electronic Supplementary Material (ESI) for Chemical Communications

Rigid lanthanide binding tag for NMR 3D structure determination of carbohydrates.

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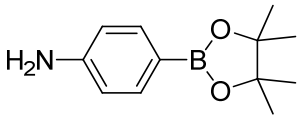
General comments:

^{1}H NMR and ^{13}C NMR routine spectra were adquired on Bruker AM-300, Bruker AV. 500 and Bruker AV. 600 spectrometers. HSQC-coupled experiment was adquired in a Bruker AVIII. 700 spectometer. Chemical shifts (TM) are in parts per million relative to tetramethylsilane at 0.00 ppm. IR spectra were determined by a FT-IR Perkin-Elmer 2000 spectrometer. TLC analyses were performed on commercial aluminium sheets bearing 0.25 mm layer of Merck Silica gel 60F254. Silica gel Acros Organics 0.035-0.070 mm, 60 Å was used for column chromatography. Silica gel Varian Bondesil-C18, 40UM was used for reverse phase column chromatography and as eluents: A= Water (mili-Q):TFA (0.001%) and B= Methanol:TFA (0.001%). Elemental analyses were carried out at the Elemental Analysis Center of the Complutense University of Madrid, using a Perkin Elmer 2400 CHN. Electrospray ionisation mass spectrometry (ESI-MS) analyse was obtained on an Esquire 3000 (Bruker) spectrometer. Dioxane was refluxed over Na/Benzophenone, CH_3CN over calcium hydride and Et_3N over KOH. All reactives were bought to Aldrich and Acros Organics.

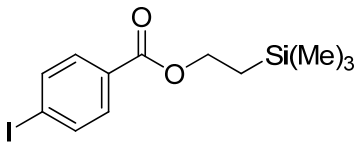
(*S*)-2,3-Bis(bis(2-*tert*-butoxy-2-oxoethyl)amino)propanoic acid, **4**[1] and 1-β-aminoquitobiose[2] were synthesized following the literature.



Preparation of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (1):

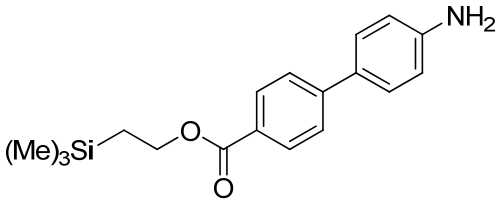
Over a solution of 4-iodoaniline (1.5 g, 6.85 mmol), Pd(dppf)Cl_2*DCM (168.0 mg, 0.20 mmol) and 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (1.49 mL, 10.27 mmol) in 30 mL of anhydrous dioxane and under Argon atmosphere, distilled Et_3N (2.86 mL, 20.5 mmol) was added and the reaction was heated at 90 ºC. After stirring for 1.5h, the crude was

cooled to r.t., filtered through Celite and the solvent was eliminated under reduced pressure. The residue was purified by performing silica gel chromatography (the silica gel was previously washed with a solution of Hex:Et_3N 5% in order to remove the acid traces, and then gently with Hex:AcOEt 6:1 to remove the Et_3N. Chromatography was performed in Hex:AcOEt 6:1, Rf= 0.60 in Hex:AcOEt 1:1) affording **1** (1.2 g, 5.48 mmol, 80%) as a light brown solid, Mp: 164-169 ºC.
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (d, J = 8.8 Hz, 2H, Ar), 6.67 (d, J = 8.8 Hz, 2H, Ar), 3.84 (bs, 2H, NH_2), 1.33 (s, 12H, 4xCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.3, 136.3, 117.2, 114.0, 83.2, 24.7; IR (KBr) 3450, 3358, 2976, 1629, 1603; Anal. Calcd for $C_{12}H_{18}BNO_2$: C, 65.79; H, 8.28; N, 6.39. Found: C, 65.83; H, 8.41; N, 6.32.



Preparation of 2-(trimethylsilyl)ethyl 4-iodobenzoate (2):

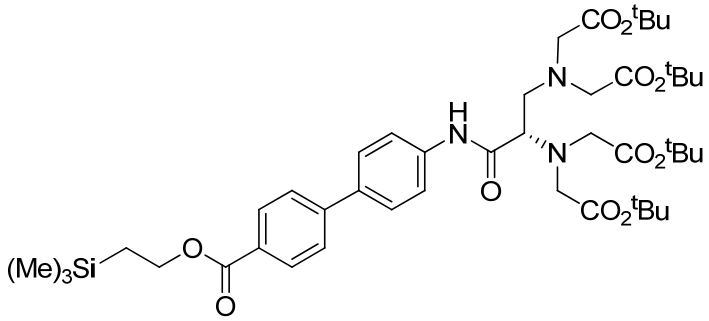
4-Iodobenzoic acid (3.0 g, 12.1 mmol), 2-(trimethylsilyl)ethanol (1.89 mL, 13.3 mmol) and dicyclohexylcarbodiimide (2.74 g, 13.3 mmol) were solved in 61 mL of anhydrous CH_3CN under Argon atmosphere. *N*,*N*-dimethylpyridin-4-amine (30.0 mg, 0.24 mmol) was added and the reaction was stirred for 24h. The white suspension was filtered and removed, and the solvent was evaporated under reduced pressure. The oily residue was purified by performing silica gel chromatography (Hex:AcOEt 49:1, Rf= 0.55 in Hex:AcOEt 20:1) affording **2** (3.19 g, 9.16 mmol, 76%) as a colorless oil.
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (d, J = 8.3 Hz, 2H, Ar), 7.75 (d, J = 8.3 Hz, 2H, Ar), 4.41 (t, J = 8.3 Hz, 2H, OCH_2), 1.13 (t, J = 8.3 Hz, 2H, CH_2Si), 0.08 (s, 9H, 3xCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.1, 137.5, 130.9, 130.0, 100.5, 63.5, 17.3, -1.5; IR (neat) 2953, 1720, 1587; Anal. Calcd for $C_{12}H_{17}IO_2Si$: C, 41.39; H, 4.92. Found: C, 41.47; H, 4.79.



Preparation of 2-(trimethylsilyl)ethyl 4'-aminobiphenyl-4-carboxylate (3):

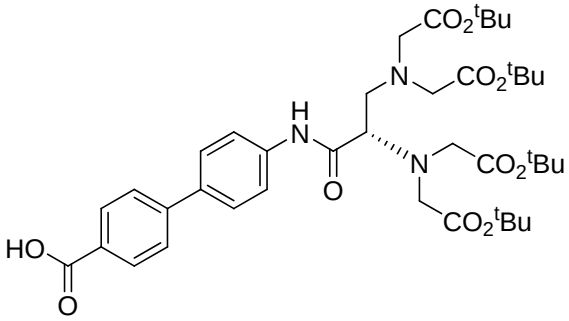
Compounds **1** (0.9 g, 4.11 mmol), **2** (1.72 g, 4.93 mmol), Pd(dppf)Cl_2*DCM (160.0 mg, 0.21 mmol) and K_3PO_4 (4.36 g, 20.55 mmol) where suspended in a mixture of 18 mL of dioxane and 1.8 mL of water, and the mixture was heated at 95 ºC. After 18h the crude was filtered through Celite and the solvent was eliminated under reduced pressure. The residue was purified by performing silica gel chromatography (Hex:AcOEt 2:1, Rf= 0.79 in Hex:AcOEt 1:1) affording **3** (1.07 g, 3.41 mmol, 75%) as a white solid, Mp: 101-104 ºC.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (d, J = 8.3 Hz, 2H, Ar), 7.60 (d, J = 8.3 Hz, 2H, Ar), 7.48 (d, J = 8.3 Hz, Ar), 6.78 (d, J = 8.3 Hz, 2H, Ar), 4.44 (t, J = 8.3 Hz, 2H, OCH_2), 3.82 (bs, 2H, NH_2), 1.16 (t, J = 8.3 Hz, 2H, CH_2Si), 0.10 (s, 9H, 3xCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.7, 146.7, 145.2, 129.8, 129.5, 128.0, 127.9, 125.7, 115.1, 62.9, 17.2, -1.6; IR (KBr) 3433, 3345, 2954, 1693, 1633, 1595; Anal. Calcd for $C_{18}H_{23}NO_2Si$: C, 68.97; H, 7.40; N, 4.47. Found: C, 69.08; H, 7.29; N, 4.57.



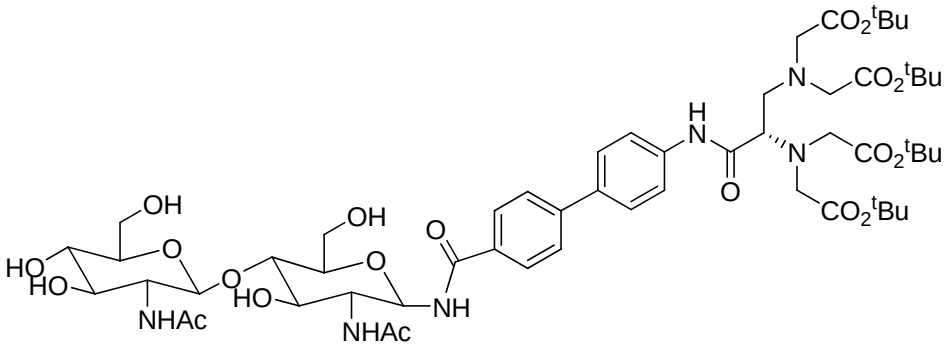
Preparation of 2-(trimethylsilyl)ethyl (*S*)-4'-[2,3-bis(bis(2-*tert*-butoxy-2-oxoethyl)amino)propanamido]biphenyl-4-carboxylate:

Compounds **4** (1.97 g, 3.51 mmol), **3** (1.0 g, 3.19 mmol), HATU (1.33 g, 3.51 mmol) and DIPEA (0.57 mL, 3.51 mmol) were solved in a mixture of 12.8 mL of DMSO and 6 mL of DCM, and the reaction was stirred at r.t. After 24h, 50 mL of AcOEt were added and the crude was washed twice with 15 mL of phosphate buffer (0.1M, pH 2.0) and 15 mL of brine. The organic phase was dried (molecular sieves 0.4 nm) and concentrated under reduced pressure. The residue was purified by performing silica gel chromatography (Hex:AcOEt 20:1 to 9:1, Rf= 0.52 in Hex:AcOEt 4:1) affording (2.15 g, 2.51 mmol, 79%) as a yellow oil. $[\alpha]^{26}_{D}$ = -18.4 (c = 0.25, DCM).
^{1}H NMR (300 MHz, $CDCl_3$) δ 10.73 (s, 1H, NHCO), 8.09 (d, J = 8.8 Hz, 2H, Ar), 7.81 (d, J = 8.8 Hz, 2H, Ar), 7.65 (d, J = 8.8 Hz, 2H, Ar), 7.60 (d, J = 8.8 Hz, 2H, Ar), 4.45 (t, J = 8.3 Hz, 2H, OCH_2), 3.78-3.73 (m, 1H, (*S*)-CH), 3.65-3.59 (m, 6H, 3xNCH_2CO_2), 3.48-3.36 (m, 3H, NCH_2CO_2 & (*S*)-CHC$\underline{H}_2$N), 3.01 (dd, J_1 = 14.2 Hz, J_2 = 7.8 Hz, 1H, (*S*)-CHC$\underline{H}_2$N), 1.47 (s, 36H, 12xCH_3), 1.16 (t, J = 8.3 Hz, 2H, CH_2Si), 0.10 (s, 6H 3xCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.5, 171.3, 170.5, 166.4, 144.8, 138.8, 134.6, 129.8, 128.7, 127.3, 126.3, 119.5, 81.1, 80.8, 64.7, 62.9, 56.3, 54.1, 54.0, 27.9, 27.9, 17.2, -1.6; IR (neat) 3260, 2970, 1715, 1595, 1560 cm^{-1}; Anal. Calcd for $C_{45}H_{69}N_3O_{11}Si$: C, 63.13; H, 8.12; N, 4.91. Found: C, 63.02; H, 8.18; N, 4.81.



Preparation of (*S*)-4'-(2,3-bis(bis(2-*tert*-butoxy-2-oxoethyl)amino)propanamido)biphenyl-4-carboxylic acid (5):

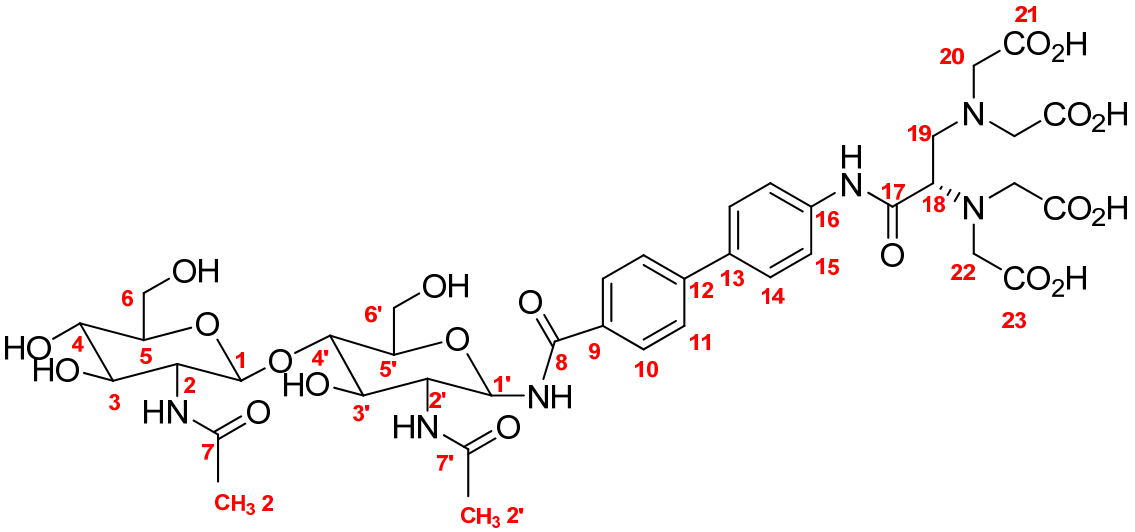
Over a solution of 2-(trimethylsilyl)ethyl (*S*)-4'-[2,3-bis(bis(2-*tert*-butoxy-2-oxoethyl)amino)propanamido]biphenyl-4-carboxylate (1.8 g, 2.10 mmol) in 10.5 mL of DMF, TBAF (1 M in THF, 8.41 mL, 8.41 mmol) was added and the mixture was stirred for 24h. The reaction was cooled to 4 ºC, 20 mL of HCl 1 M were added and the crude was extracted with DCM (3x15 mL). The organic phase was dried (molecular sieves 0.4 nm) and solvent was evaporated under vacuo. The residue was purified by performing silica gel chromatography (Hex:AcOEt 4:1 to 1:1, Rf= 0.70 in AcOEt) affording **5** (1.4 g, 1.84 mmol, 88%) as a yellow solid. Mp: 57-61 ºC, $[\alpha]^{28}_{D}$ = -18.07 (c = 0.218, DCM). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.76 (s, 1H, NHCO), 10.30 (bs, 1H, CO_2H), 8.14 (d, *J* = 8.3 Hz, 2H, Ar), 7.81 (d, *J* = 8.8 Hz, 2H, Ar), 7.66 (d, *J* = 8.3 Hz, 2H, Ar), 7.59 (d, *J* = 8.8 Hz, 2H, Ar), 3.80-3.76 (m, 1H, (*S*)-CH), 3.65-3.59 (m, 6H, 3xNCH_2CO_2), 3.48-3.36 (m, 3H, NCH_2CO_2 & (*S*)-CHC*H*$_2$N), 3.02 (dd, J_1 = 13.9 Hz, J_2 = 7.5 Hz, 1H, (*S*)-CHC*H*$_2$N), 1.45 (s, 36H, 12xCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.7, 171.6, 171.3, 170.7, 145.8, 139.0, 134.8, 130.7, 127.8, 127.6, 126.6, 119.8, 81.4, 81.2, 64.9, 56.4, 54.3, 54.2, 28.1, 28.1; IR (KBr) 3265, 2980, 1732, 1689, 1605, 1521 cm^{-1}; Anal. Calcd for $C_{40}H_{57}N_3O_{11}$: C, 63.56; H, 7.60; N, 5.56. Found: C, 63.38; H, 7.53; N, 5.35.



Preparation of *tert*-butyl 2,2',2'',2'''-{{(2S)-3-{{4'-{[2-acetamido-4-*O*-(2-acetamido-2-desoxy-β-D-glucopyranosil)-2-desoxy-β-D-glucopyranosil]carbamoil}biphenyl-4-yl}amino}-3-oxopropane-1,2-diyl}dinitrilo}tetraacetate:

A solution of **5** (603 mg, 0.79 mmol), HATU (362 mg, 0.95 mmol) and DIPEA (0.157 mL, 0.95 mmol) were preincubated in a solution of 4 mL of DMSO and 2 mL of DCM, and after 10 min 1-β-aminoquitobiose (225 mg, 0.58 mmol) was added and the reaction mixture was stirred for 24 h. The crude was carefully powdered (to avoid foams) over 30 mL of water covered by 20 mL of AcOEt, and the organic layer was extracted. The aqueous layer was carefully washed three times with 15 mL of AcOEt, all the organic layers were joined together, dried (molecular sieve 0.4 nm) and concentrated under reduced pressure. The residue was purified by performing silica gel chromatography (CH_3Cl:EtOH 6:1, Rf= 0.82 in CH_3Cl:EtOH 1:1) affording (836.0 g, 0.72 mmol, 90%) as a white solid. Mp: 230-236 ºC with decomposition.
^{1}H NMR (500 MHz, CD_3OD) δ 7.90 (d, *J* = 5.0 Hz, 2H, Ar), 7.78 (d, *J* = 5.1 Hz, 2H, Ar), 7.74 (d, *J* = 5.1 Hz, 2H, Ar), 7.67 (d, *J* = 5.2 Hz, 2H, Ar), 5.17 (d, *J* = 5.9 Hz, 1H, OCHN β-AChb), 5.17 (bs, 1H, OCHO β-AChb), 4.03 (t, *J* = 10.1 Hz, 1H, β-AChb), 3.96 (dd, J_1 = 7.0 Hz, J_2 = 1.0 Hz, 1H, β-AChb), 3.86 (d, *J* = 6.5 Hz, 1H, β-AChb),

3.79-3.64 (m, 10H, 3xβ-AChb, (*S*)-CH & 3xNCH_2CO_2), 3.55-3.47 (m, 6H, 4xβ-AChb & NCH_2CO_2), 3.44-3.40 (m, 1H, β-AChb), 3.40-3.33 (m, 1H, β-AChb), 3.26 (dd, J_1 = 8.4 Hz, J_2 = 3.3 Hz, 1H, (*S*)-CHC$\underline{H}_2$N), 3.07 (dd, J_1 = 8.4 Hz, J_2 = 4.7 Hz, 1H, (*S*)-CHC$\underline{H}_2$N), 1.98 (s, 3H, COCH_3), 2.05 (s, 3H, COCH_3), 1.49 (s, 36H, 12xCH_3); ^{13}C NMR (125 MHz, CD_3OD) δ 173.8, 172.9, 172.6, 171.9, 171.6, 169.0, 144.6, 138.8, 135.6, 132.0, 128.2, 127.5, 126.8, 120.3, 102.3, 81.8, 81.6, 80.5, 80.2, 77.1, 77.1, 74.8, 73.2, 71.0, 65.1, 61.6, 60.6, 56.5, 56.4, 54.6, 27.5, 27.4, 22.1, 21.7; IR (KBr) 3302, 2978, 2930, 1735, 1663, 1607, 1537 cm^{-1}; Anal. Calcd for $C_{56}H_{84}N_6O_{20}$: C, 57.92; H, 7.29; N, 7.24. Found: C, 57.78; H, 7.33; N, 7.37.



Preparation of 2,2',2'',2'''-{{(2S)-3-{{4'-{[2-acetamido-4-*O*-(2-acetamido-2-desoxy-β-D-glucopyranosil)-2-desoxy-β-D-glucopyranosil]carbamoil}biphenyl-4-yl}amino}-3-oxopropane-1,2-diyl}dinitrilo}tetraacetic acid (6):

tert-butyl 2,2',2'',2'''-{{(2S)-3-{{4'-{[2-acetamido-4-*O*-(2-acetamido-2-desoxy-β-D-glucopyranosil)-2-desoxy-β-D-glucopyranosil]carbamoil}biphenyl-4-yl}amino}-3-oxopropane-1,2-diyl}dinitrilo}tetraacetate (610 mg, 0.52 mmol) was dissolved in a deprotection mixture (TFA/DCM/^{i}Pr$_3$SiH, 5:5:1.8, v/v, 5.7 mL). After 24h, toluene (5 mL) was added, and the solvent was removed by evaporation under reduced pressure. Pure product was obtained by precipitation from EtOH:Acetone followed by a reverse phase silica gel chromatography (A to A:B 4:1). The combined fractions showing fluorescence in UV (λ=254 nm) were lyophilized affording **6** (354 mg, 73%) as a white solid. Mp: 178-182 ºC with decomposition.

^{1}H NMR (600 MHz, D_2O) δ 7.54 (d, J = 7.4 Hz, 2H, H-10), 7.33 (s, 2H, H-11), 7.28 (s, 4H, H-14 & 15), 5.11 (d, J = 9.6 Hz, 1H, H-1'), 4.51 (d, J = 8.3 Hz, 1H, H-1), 4.07 (s, 4H, H-22), 4.00 (bs, 1H, H-18), 3.88 (t, J = 10.1 Hz, 1H, H-2'), 3.81 (d, J = 11.6 Hz, 1H, H-6a), 3.75-3.72 (m, 2H, 6'a & H-3'), 3.66-3.53 (m, 9H, H-2, 19a, 6b, 20, 4', 6'b, 5' & 19b), 3.47 (t, J = 9.4 Hz, 1H, H-3), 3.39-3.37 (m, 2H, H-4 & 5), 1.98 (s, 3H, CH_3-2), 1.80 (s, 3H, CH_3-2'); ^{13}C NMR (125 MHz, D_2O) δ 175.8 (2xC, C-23), 175.3 (C-7), 175.0 (C-7'), 170.3 (C-8), 169.5 (2xC, C-21), 168.6 (C-17), 143.9 (C-12), 137.0 (C-16), 135.9 (C-13), 131.2 (C-9), 128.3 (2xC, C-10), 127.9 (2xC, C-14), 127.0 (2xC, C-11), 121.42 (2xC, C-15), 101.9 (C-1), 79.9 (C-1'), 79.6 (C-4'), 76.6 (C-5'), 76.3 (C-5), 73.9 (C-3), 73.0 (C-3'), 70.1 (C-4), 61.1 (C-18), 61.0 (C-6), 60.5 (C-6'), 56.8 (2xC, C-22),

56.0 (C-2), 55.1 (C-19), 54.3 (C-2'), 52.5 (2xC, C-20), 22.6 (C-CH_3-2), 22.4 (C- CH_3-2'); IR (KBr) 3356, 2978, 3094, 2931, 1732, 1661, 1539 cm^{-1}; Anal. Calcd for $C_{40}H_{52}N_6O_{20}$: C, 51.28; H, 5.59; N, 8.97. Found: C, 51.39; H, 5.41; N, 8.66. ESI-MS (H_2O, positive mode) calculated for $C_{40}H_{52}N_6NaO_{20}$ $[M+Na]^+$: 959.31. Found: 959.34

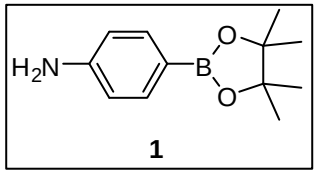
Abbreviations:

β-AChb: 1–β-Aminochitobiose
DCM: Dichloromethane
DIPEA: *N*ethyldiisopropylamine
DMSO: dimethyl sulfoxide
HATU: *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-b]pyridine-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide
HSQC: Heteronuclear Single Quantum Correlation
NMR: nuclear magnetic resonance
R.t.: Room temperature
TBAF: Tetra-n-butylammonium floride
TFA: Trifluoroacetic acid
TLC: Thin Layer Chromatography

References:

[1] Leonov, A.; Voigt, B.; Rodriguez-Castañeda, F.; Sakhaii, P.; Griesinger, C. *Chem. Eur. J.* **2005**, *11*, 3342–3348.
[2] For the synthesis of heptaacetylated 1-β-aminochitobiose see: Spinola, M. S.; Jeanloz, R. W. *J. Biol. Chem.* **1970**, *245*, 4158-4162. For deacetylation see: Wagner, M.; Dziadek, S.; Kunz, H. *Chem. Eur. J.* **2003**, *9*, 6018-6030.

NMR Spectra



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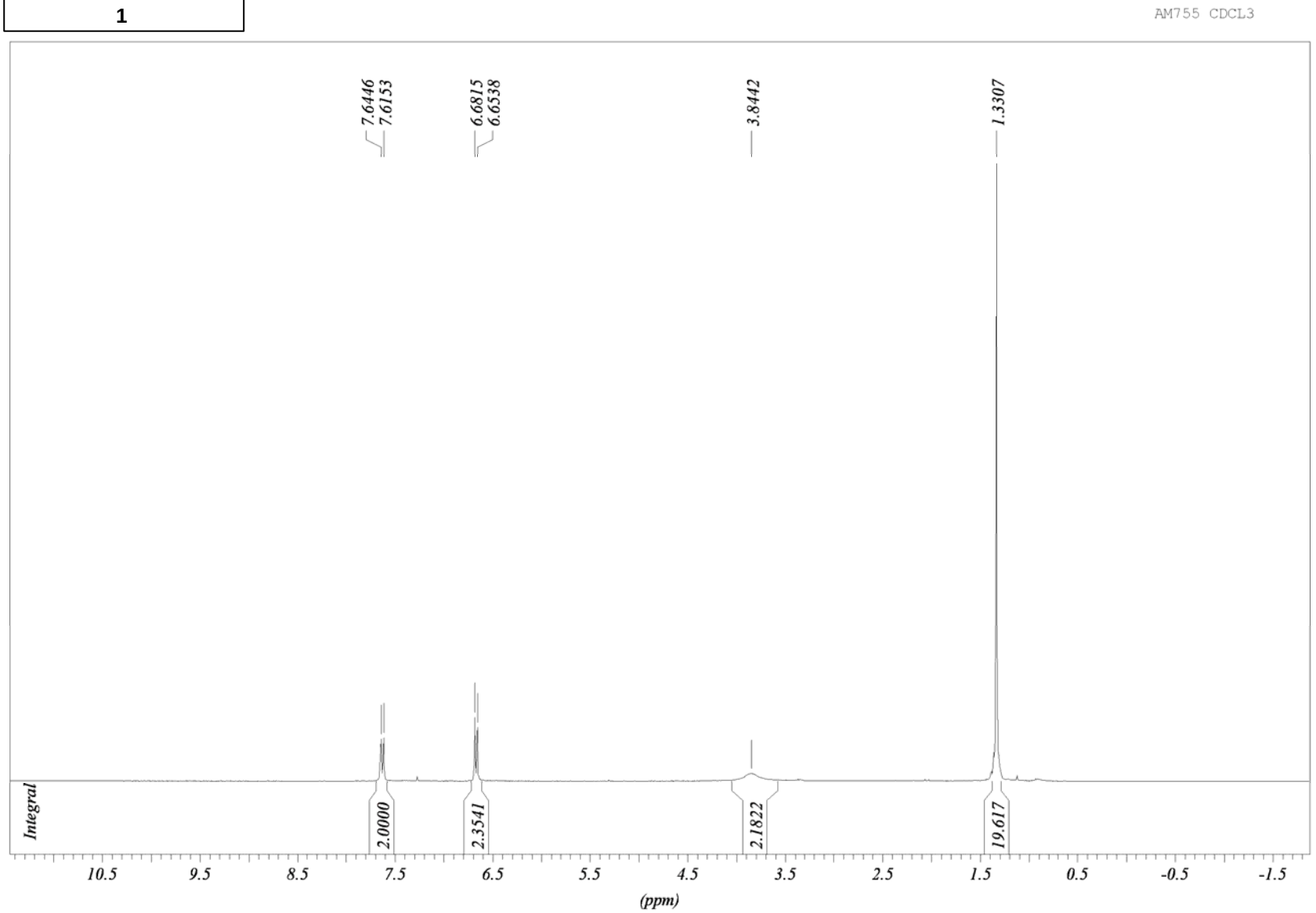

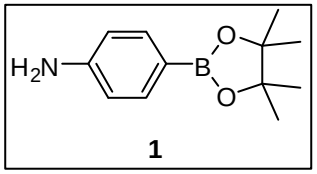

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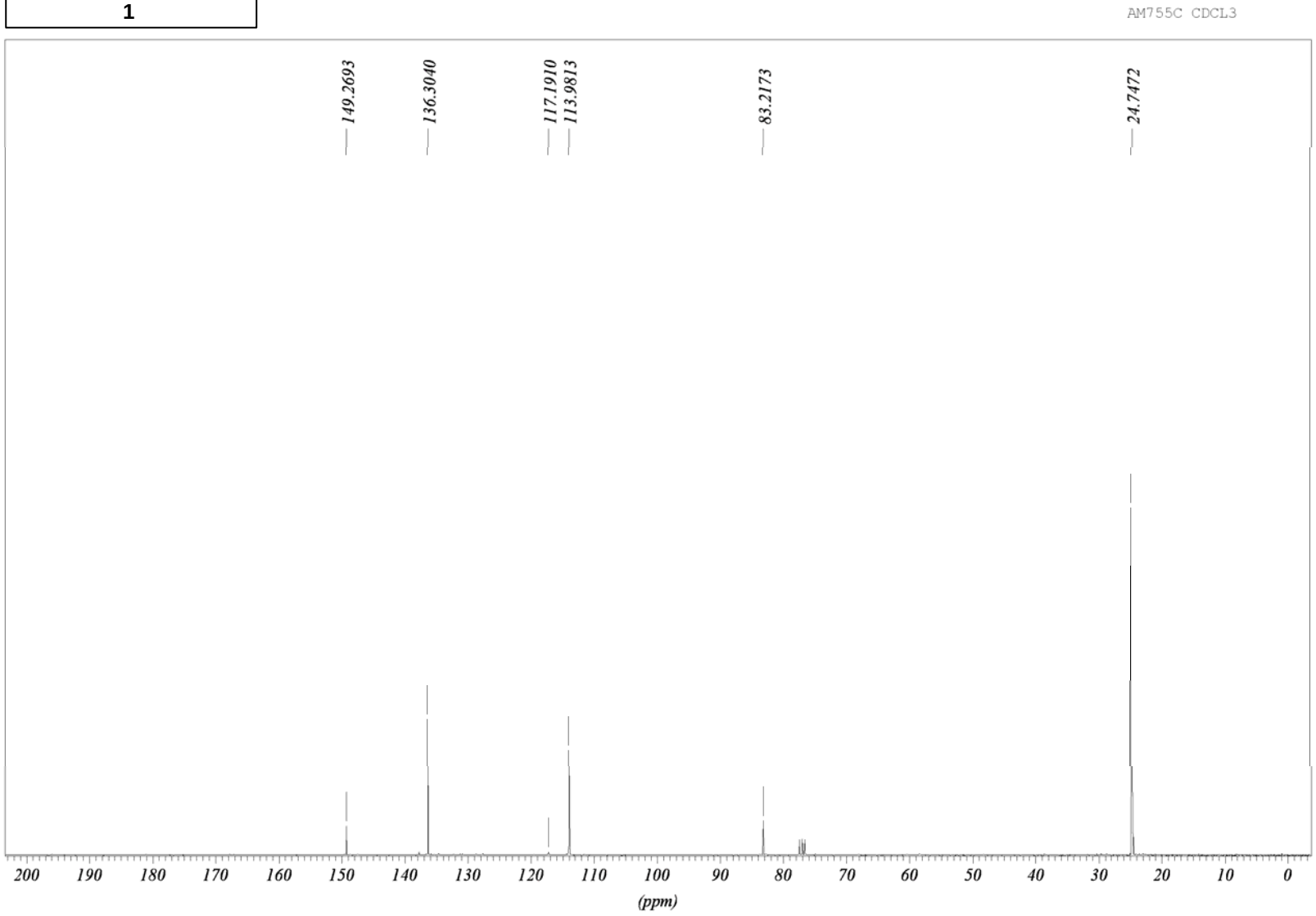

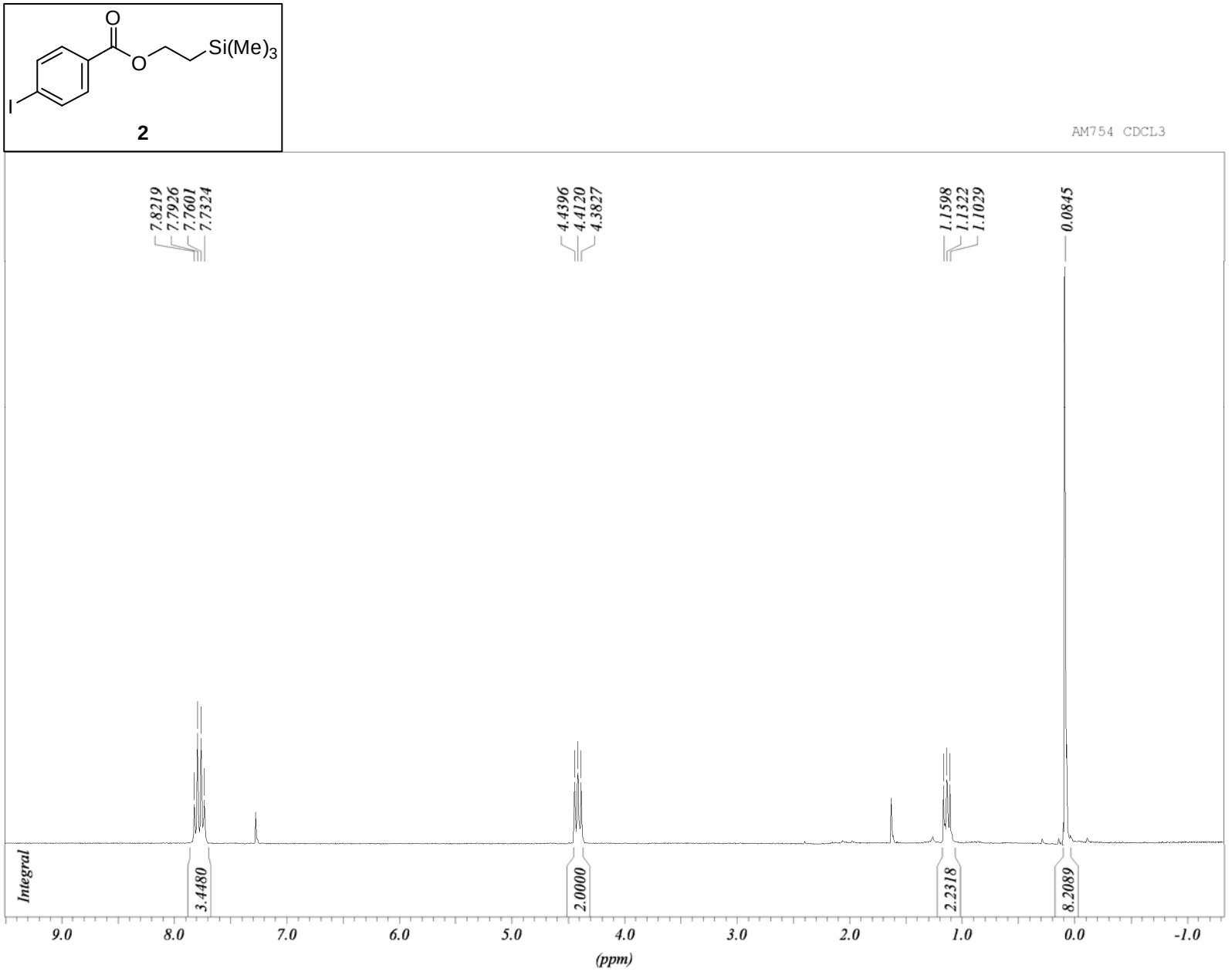

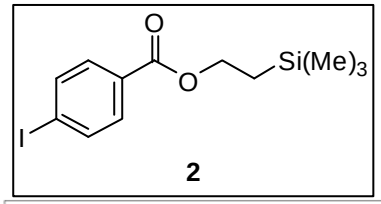

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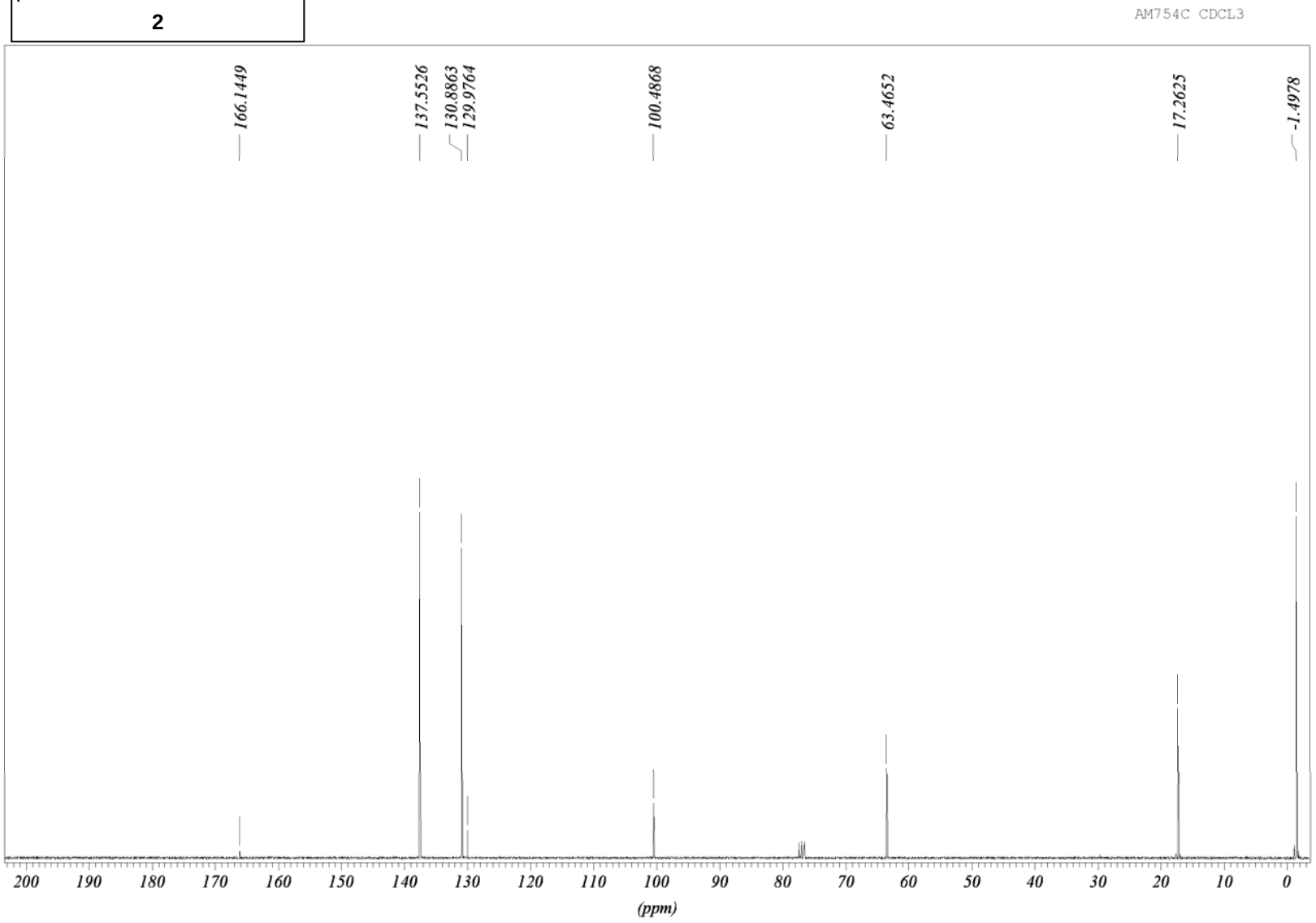

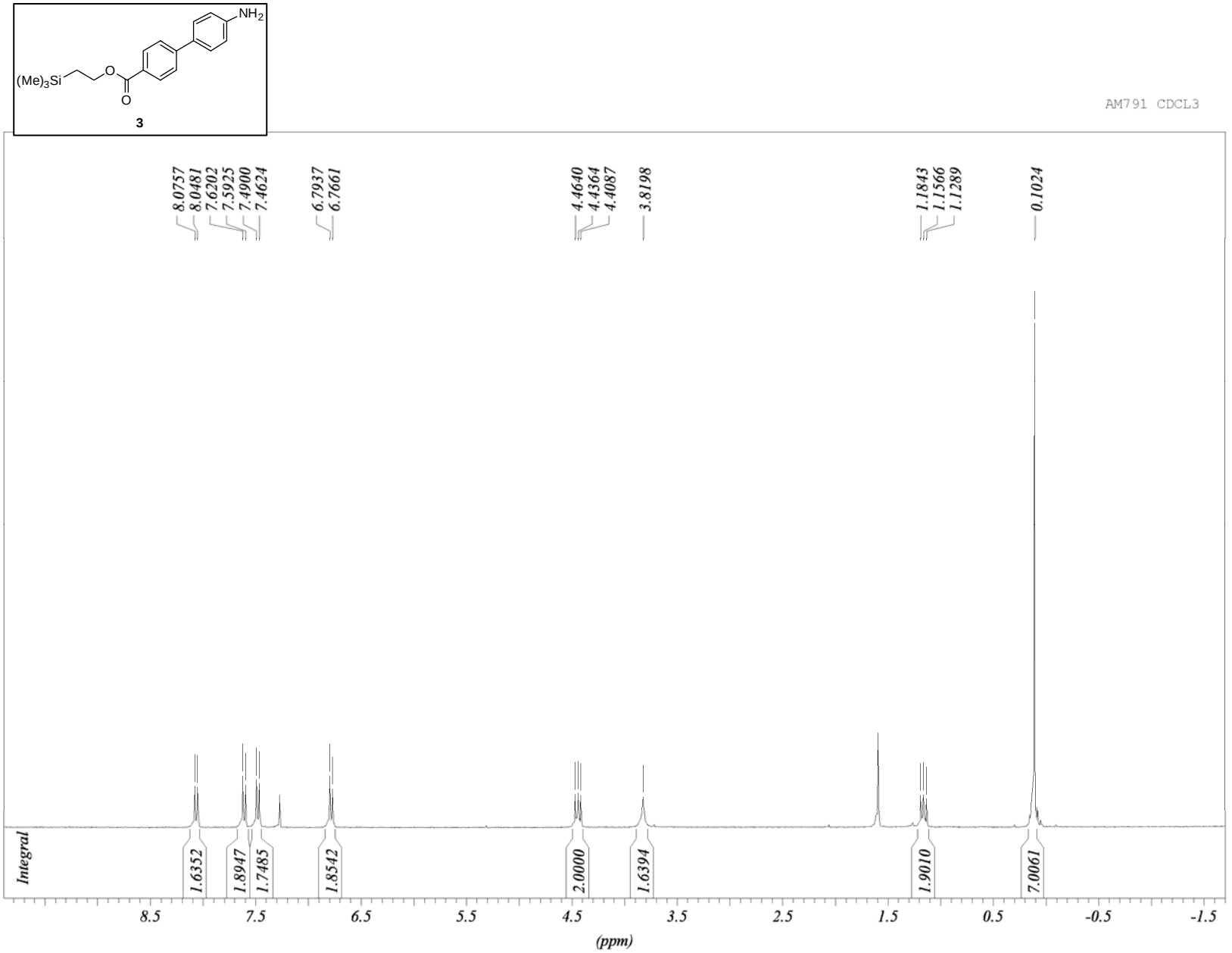

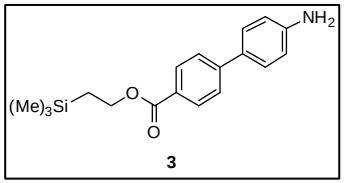

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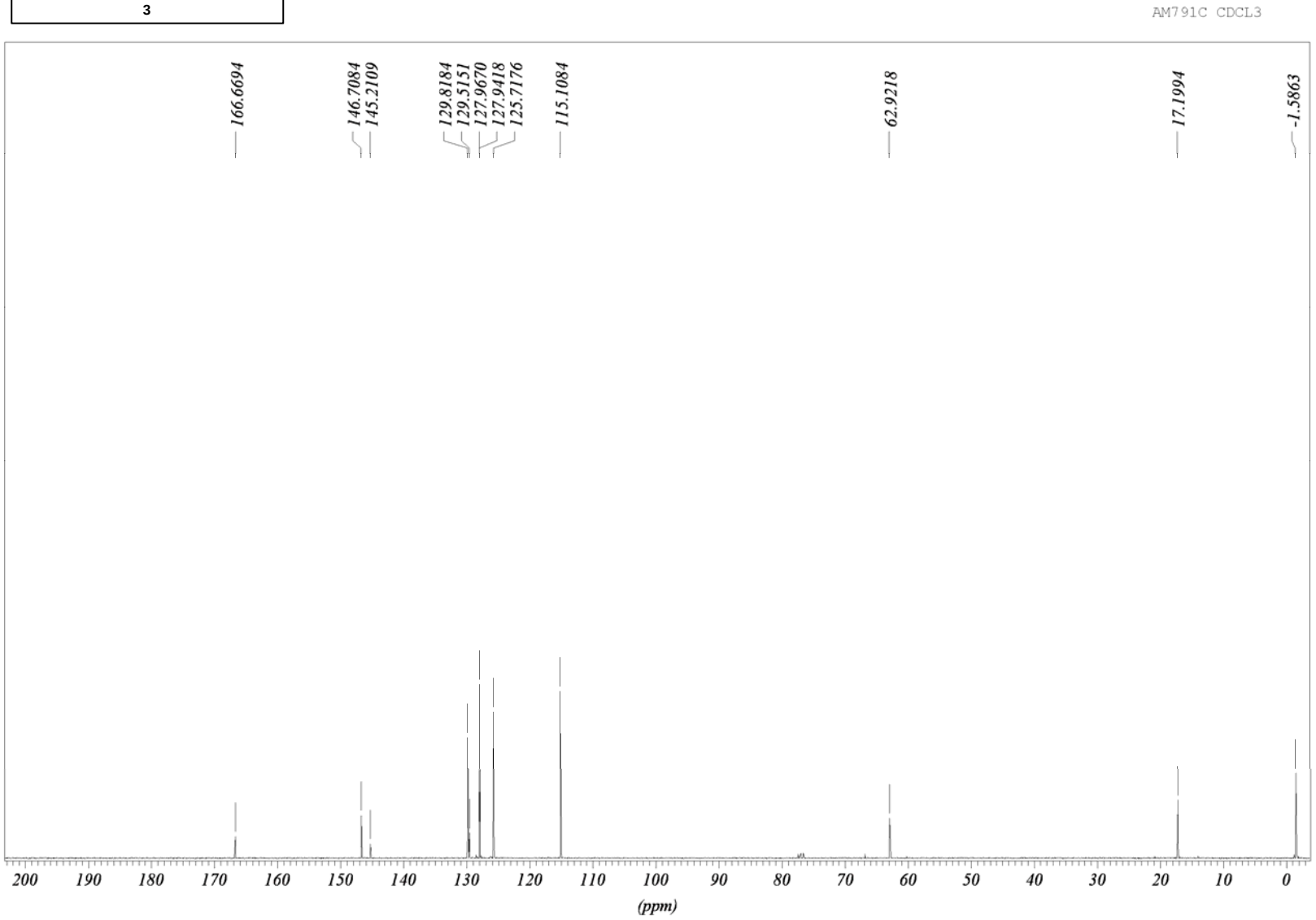

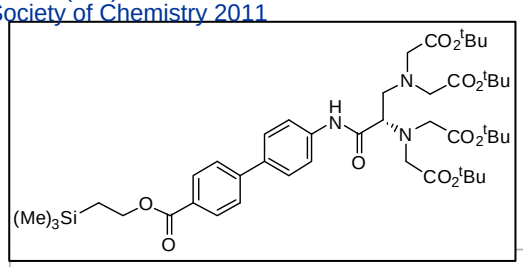

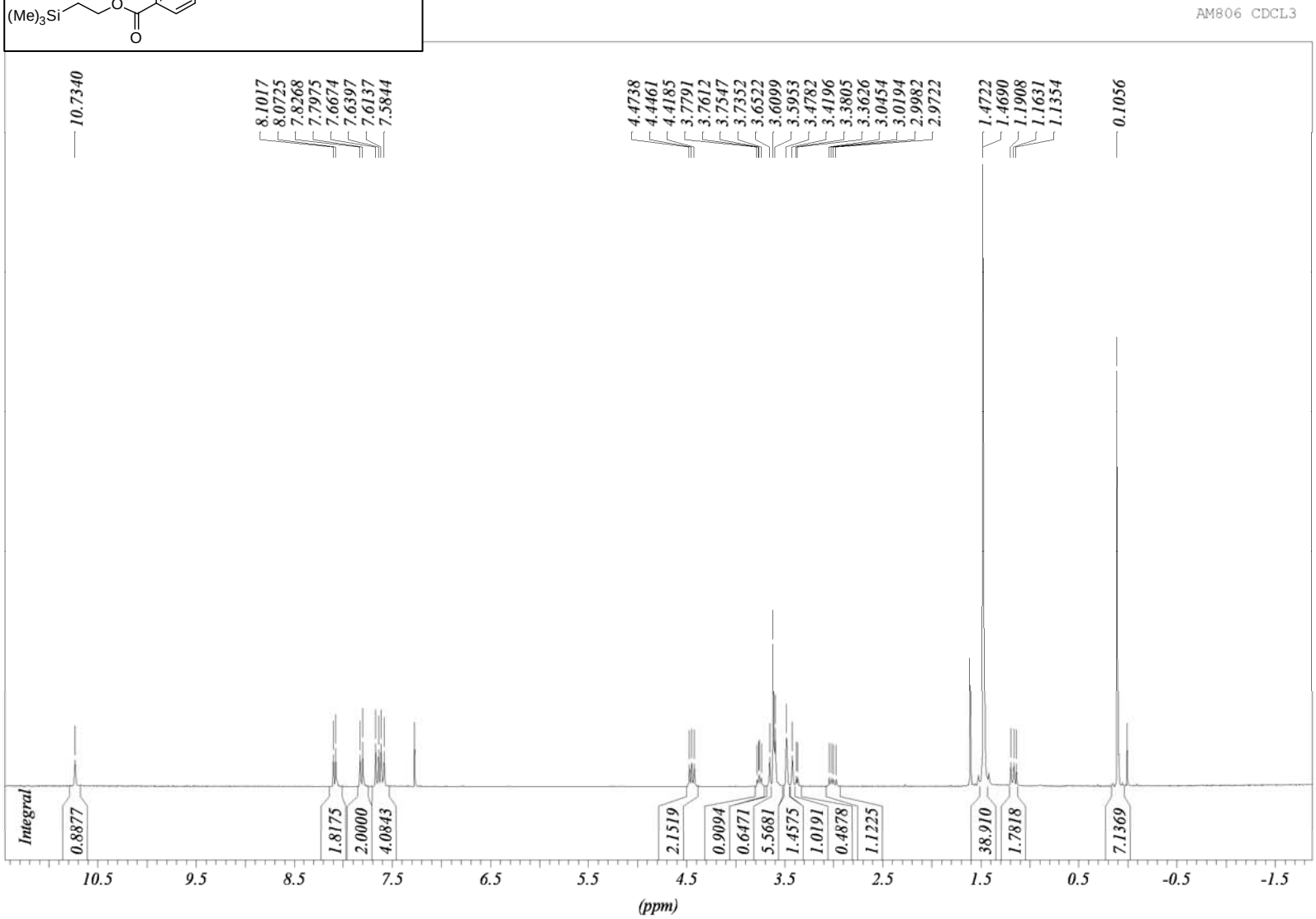

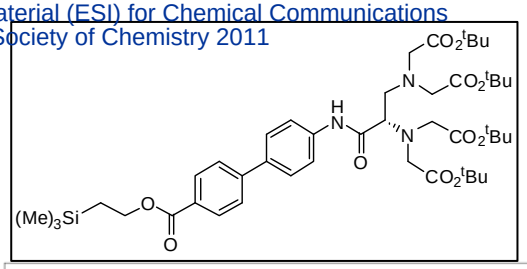

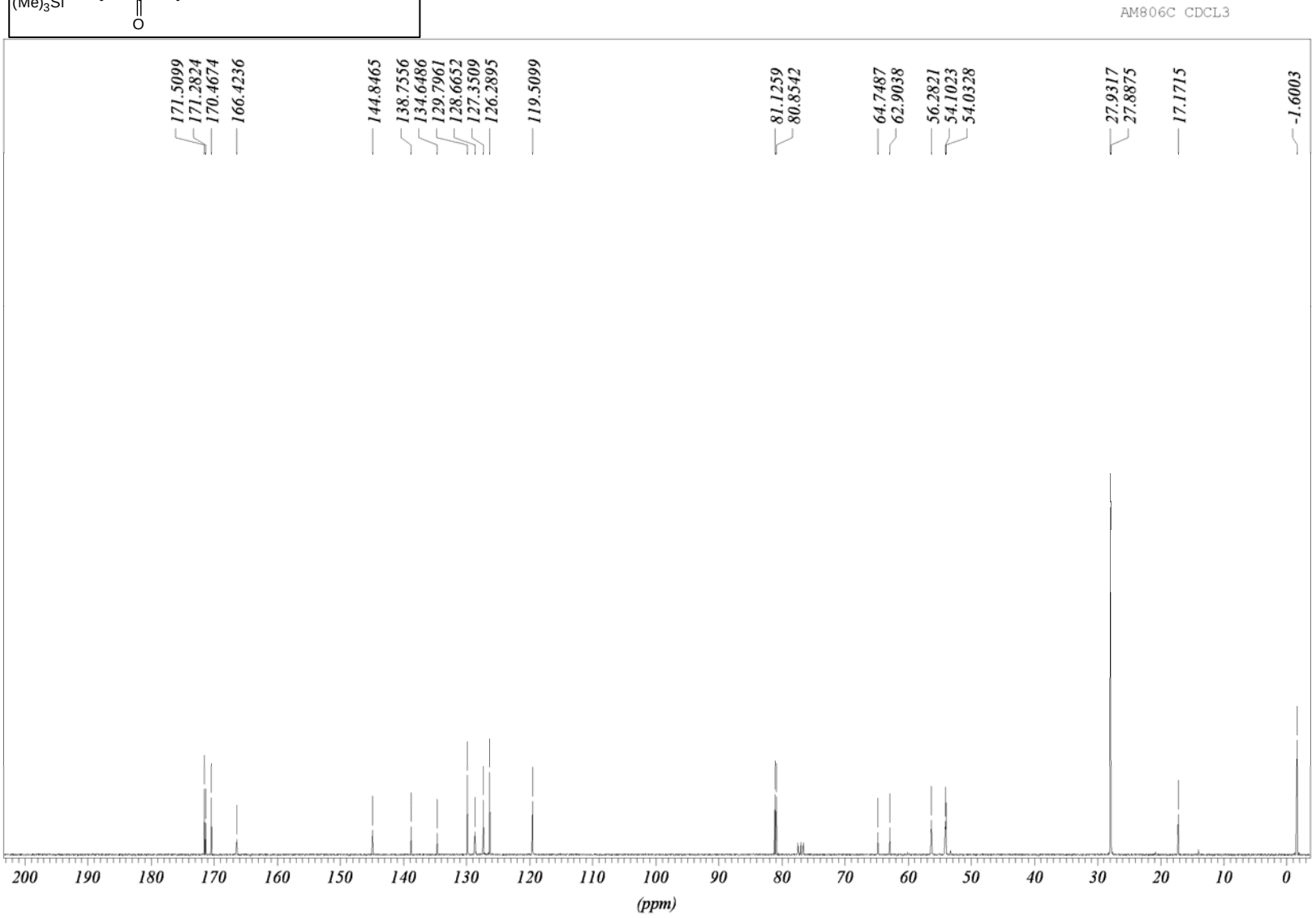

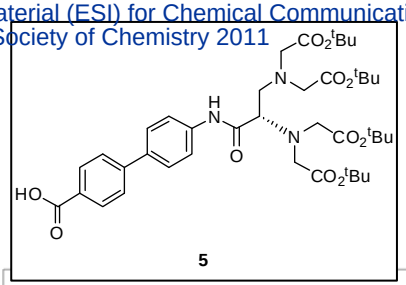

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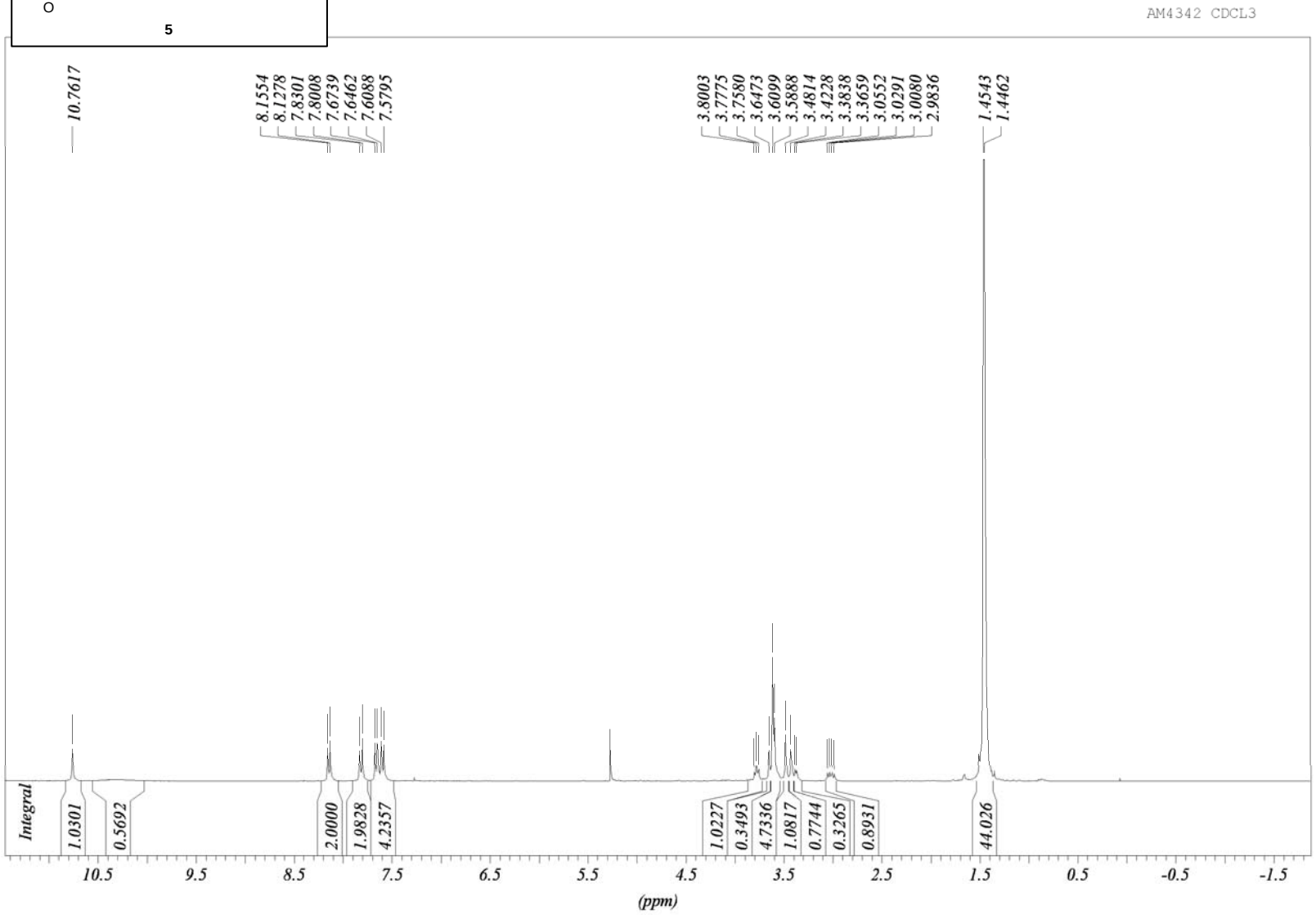

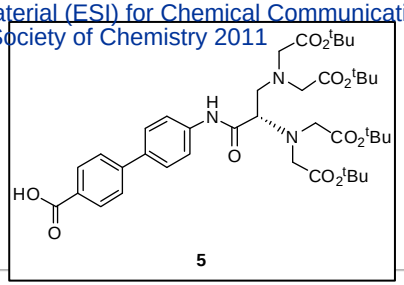

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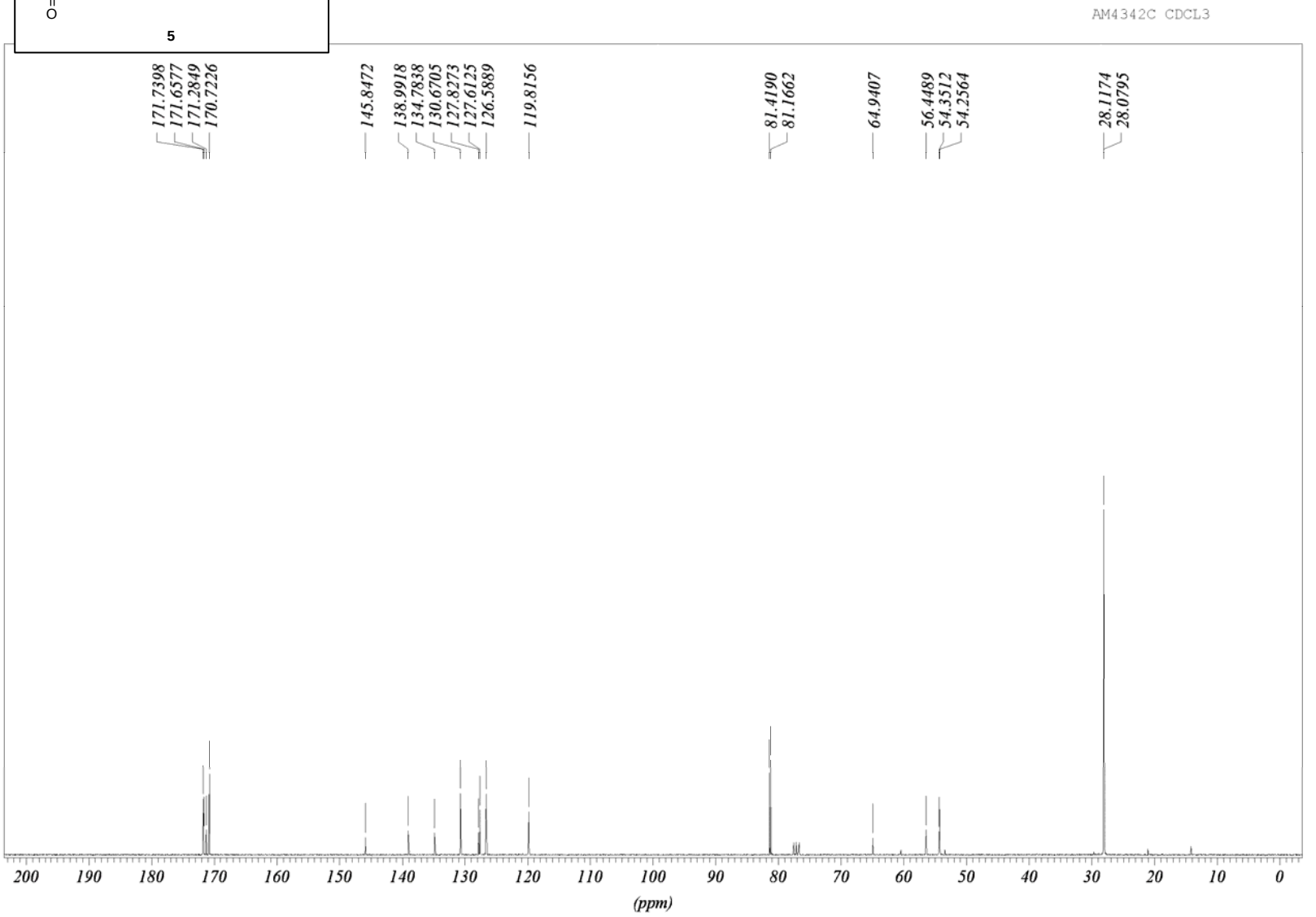

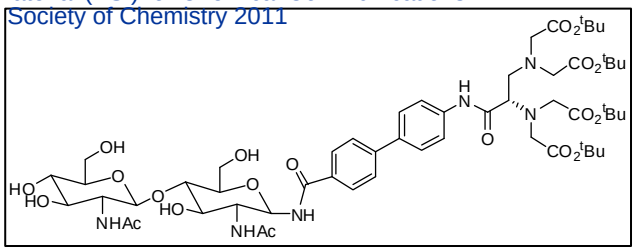

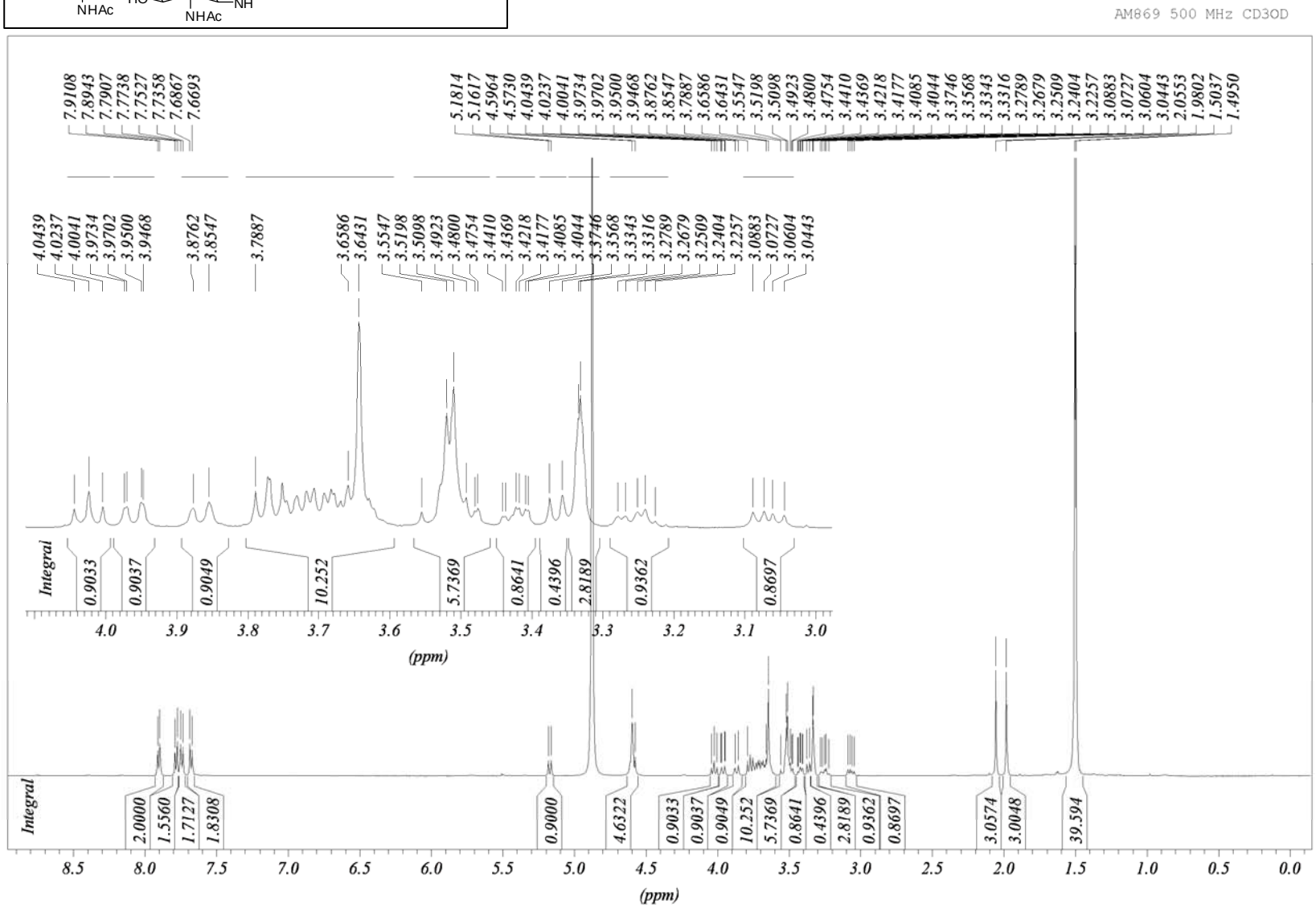

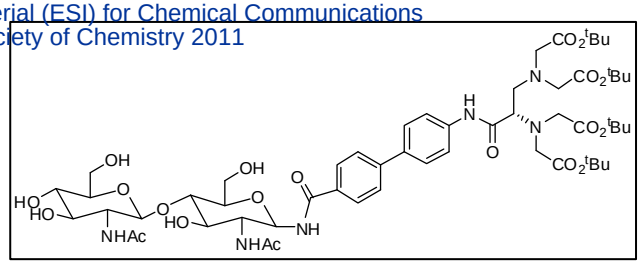

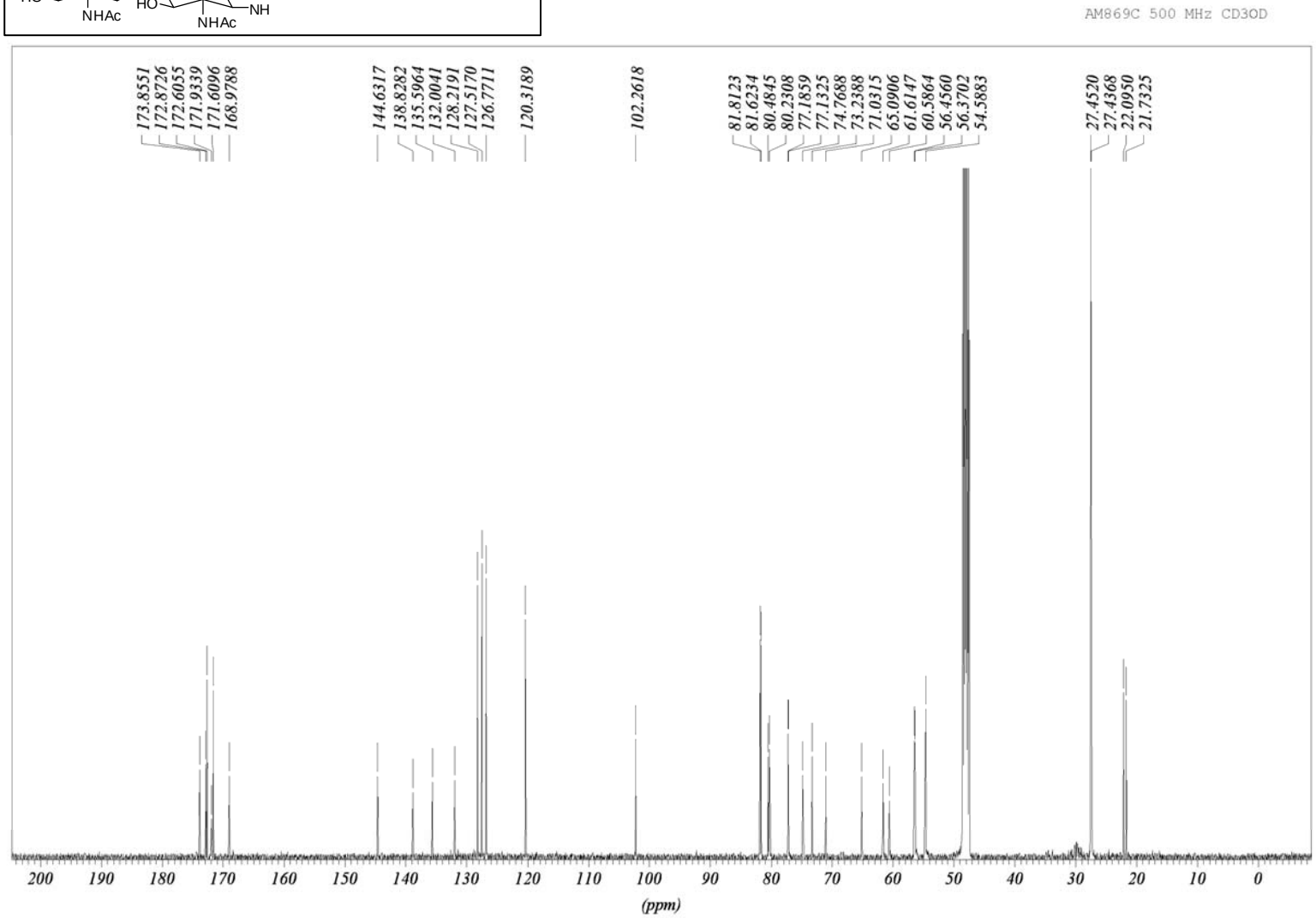

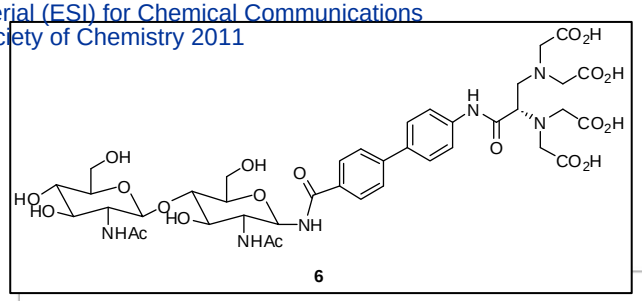

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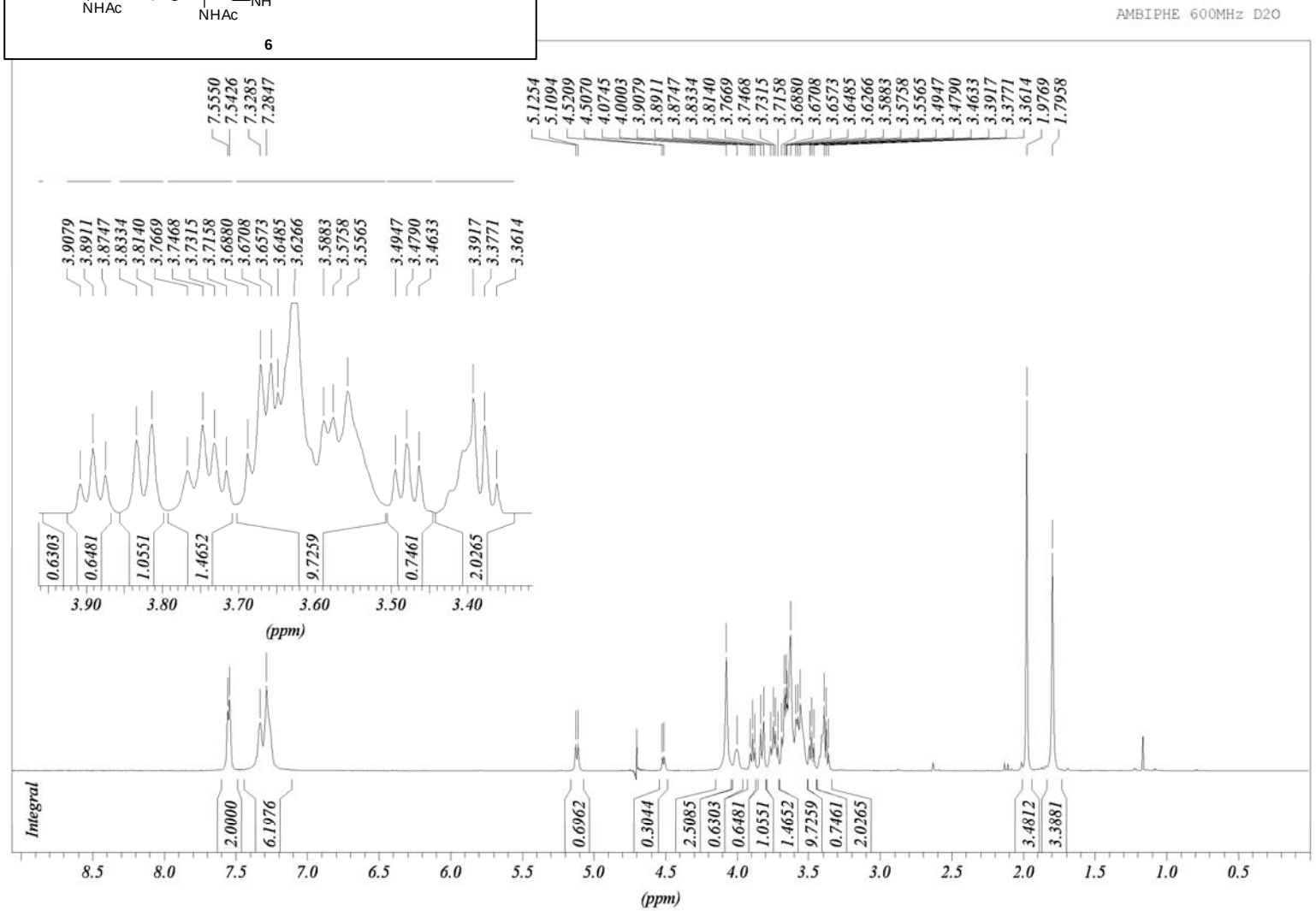

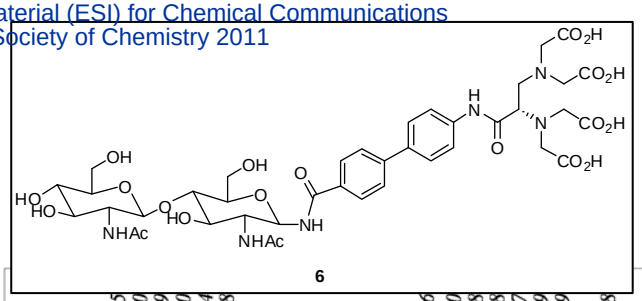

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