

Synthesis of fluorescent probes based on the pyochelin siderophore scaffold

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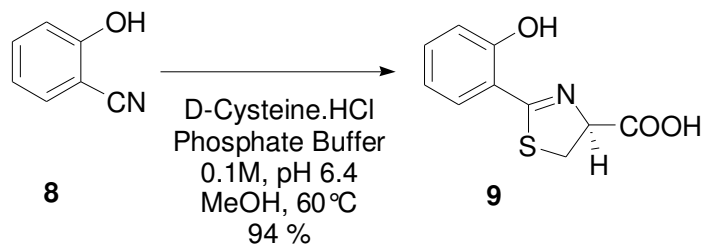
I. Chemistry

A. General informations and procedures

All reactions were carried out under argon. Solvents used were of analytical grade purity (>99.9%). THF and toluene were purchased extra-dry. Amines were distilled and stored on KOH before use. All chemicals were obtained from commercial suppliers and were used as received, unless otherwise stated. All reactions were monitored by thin-layer chromatography (TLC) using Merck precoated silica gel 60F²⁵⁴ (0.25 mm). Column chromatography purifications were performed using Merck kieselgel 60 (63-200 μ m). In some case the silica should be demetallated prior to use. For this purpose commercial silica was suspended and stirred 10 h in 1N aqueous HCl. After filtration and several rinsing with MilliQ water, the silica gel was dried under reduced pressure and then in oven for at least 72 h. NMR spectra were recorded either on a Bruker Avance 300 (¹H: 300 MHz, ¹³C: 75 MHz) or on Bruker Avance 400 (¹H: 400 MHz, ¹³C: 100 MHz), using the residual non-deuterated solvent as reference. The chemical shifts (δ) and coupling constants (*J*) are expressed in ppm and hertz respectively. Mass spectra were recorded in the *Service Commun d'Analyse* (SCA) de la *Faculté de Pharmacie de l'Université de Strasbourg* and were measured after calibration in ES-TOF experiments on a Bruker Daltonic MicroTOF mass spectrometer.

B. Experimental procedures

2-(2-Hydroxy-phenyl)-4,5-dihydro-thiazole-4-carboxylic acid 9



To a solution of 2-hydroxybenzonitrile **8** (2.0 g, 16.8 mmol) in methanol (75 mL) were added D-Cysteine hydrochloride (5.9 g, 33.6 mmol) and phosphate buffer 0.1M pH 6.4 (75 mL). The solution was adjusted to pH 6.4 by addition of solid K₂CO₃ and the reaction mixture was stirred at 60 °C for 12 h (overnight). The mixture was concentrated under reduced pressure, and the yellow crude material was diluted with water (100 mL). The solution was adjusted to pH 2.0 by addition of solid citric acid. After extraction with CH₂Cl₂ (3 x 100 mL), the organic layers were collected, dried over Na₂SO₄ and filtered. The

solvent was removed under reduced pressure to give the expected thiazoline compound **9** isolated as a yellow powder (3.5 g, 15.8 mmol, yield : 94 %).

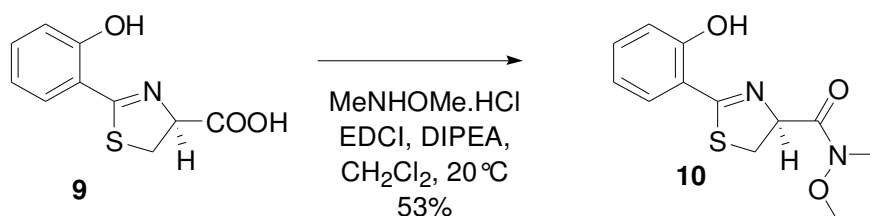
R_f : 0.15 (CH₂Cl₂/MeOH 9/1)

¹H NMR (300 MHz, (CD₃)₂CO): δ 7.43 (m, 2H), 7.03 (d, *J* = 8.5 Hz, 1H), 6.89 (t, *J* = 7.5 Hz, 1H), 5.42 (t, *J* = 8.12 Hz, 1H), 3.69 (m, 2H).

¹³C NMR (75 MHz, (CD₃)₂CO): δ 175.1, 174.7, 159.1, 133.8, 130.8, 119.1, 117.4, 115.8, 76.3, 33.6.

MS: *m/z* (ESI) 223 [M+H]⁺

2-(2-Hydroxy-phenyl)-4,5-dihydro-thiazole-4-carboxylic acid methoxy-methyl-amide **10**



To a solution of thiazoline **9** (1.00 g, 4.48 mmol) in CH₂Cl₂ (60 mL) was added successively, EDCI (0.90 g, 4.70 mmol) and a solution of *N,O*-dimethylhydroxylamine hydrochloride (0.44 g, 4.48 mmol) and DIPEA (0.780 mL, 4.48 mmol) in CH₂Cl₂ (60 mL). The solution was stirred 12 hours (overnight) at 20°C. The mixture was washed with a 0.5 N aqueous solution of HCl and the resulting organic phase was dried over Na₂SO₄ and finally filtered. The solvent was removed under reduced pressure to give brownish oily residue. This crude material was purified by chromatography on silica gel column (cyclohexane/Et₂O : 8/2) to give the expected carbamate **10** isolated as a white solid (0.63 g, 2.37 mmol, yield : 53 %).

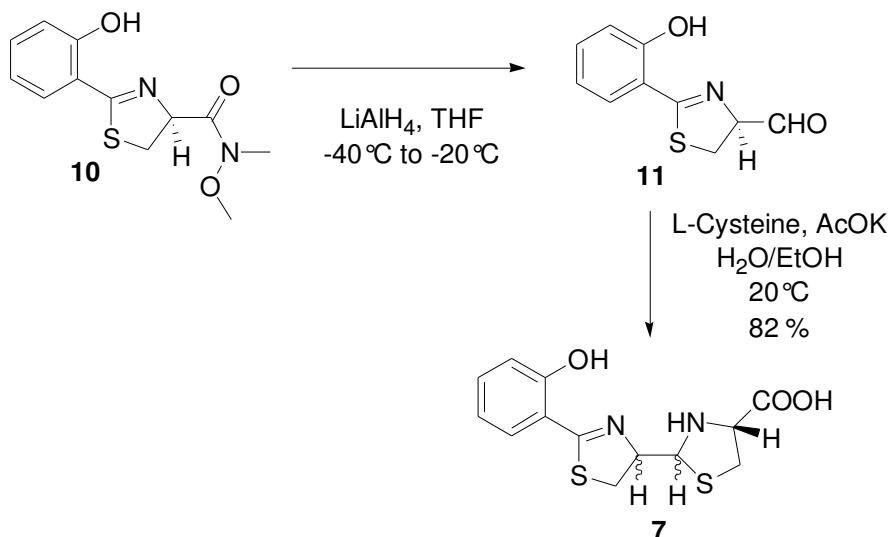
R_f : 0.68 (CH₂Cl₂/MeOH 9/1)

¹H NMR (400 MHz, CDCl₃): δ 7.42 (dt, *J* = 7.8 Hz, 1.6 Hz, 1H), 7.34 (dd, *J* = 7.3 Hz, 1.4 Hz, 1H), 6.98 (d, *J* = 8.3 Hz, 1H), 6.88 (t, *J* = 7.7 Hz, 1H), 5.70 (t, *J* = 8.8 Hz, 1H), 3.81 (s, 3H), 3.48 (dd, *J* = 11.0 Hz, 9.2 Hz, 2H), 3.30 (s, 3H).

¹³C NMR (100 MHz, CDCl₃): δ 174.3, 159.2, 133.5, 130.6, 119.0, 117.2, 116.2, 74.7, 61.9, 32.9, 32.6.

MS *m/z* (ESI) 267.2 [M+H]⁺

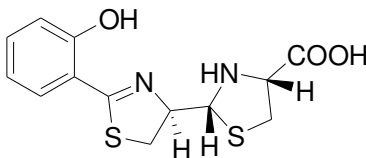
2-(2-Hydroxy-phenyl)-4,5-dihydro-thiazole-4-carbaldehyde **11** and *nor*-pyochelin **7**



To a solution of Weinreb amide **10** (250 mg, 0.95 mmol) in THF (18 mL), at -40°C , LiAlH_4 .THF 1 M (1.00 mL, 1.05 mmol) was added dropwise. The temperature was allowed to rise up to -30°C in 30 min. The reaction mixture was then hydrolyzed by successive additions of aqueous solutions of saturated NH_4Cl (9.5 mL) and of a 1 M solution of KHSO_4 (3.75 mL). The mixture was vigorously stirred and allowed to warm up to the room temperature until two phases were formed. After partition and extraction with CH_2Cl_2 (2×30 mL), the organic layers were collected, dried over Na_2SO_4 and filtered before being evaporated under reduced pressure. The crude aldehyde **11**, very sensitive, was directly used for subsequent reaction without any further purification.

To a solution of aldehyde **11** (197 mg, 0.95 mmol) in a mixture of ethanol (36 mL) and water (12 mL) were successively added L-Cysteine hydrochloride (328 mg, 2.09 mmol) and potassium acetate (652 mg, 6.64 mmol). This suspension was gently stirred 12 h (overnight) at 20°C in the dark. Ethanol was removed under reduced pressure and the crude was diluted in water. The aqueous layer was acidified to pH 5.0 by addition of solid citric acid before being extracted with ethyl acetate (2×60 mL). The organic layers were collected, dried over Na_2SO_4 , filtered and evaporated to dryness under reduced pressure. The expected *nor*-pyochelin **7** was isolated as a yellow oil. This residue, was then dissolved in a minimum amount of CH_2Cl_2 and cyclohexane was added till a turbidity appear in the solution. Evaporation of the solvents under reduced pressure leads to the *nor*-pyochelin **7** (241 mg, 0.78 mmol, yield : 82 %) under the form of a yellow powder. This powder is a mixture of the three inseparable diastereoisomers **7a** ($4'R, 2''R, 4''R$), **7b** ($4'R, 2''S, 4''R$), **7c** ($4'S, 2''S, 4''R$) in 60/30/10 proportions.

7a/b/c MS: m/z (ESI) 311.4 [$M+H^+$].

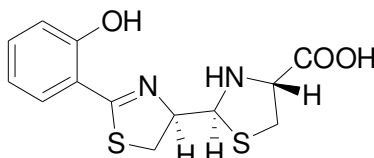


7a

R_f : 0.33 (*n*BuOH/H₂O/MeOH/Cyclohexane 2/2/1/1)

¹H NMR (400 MHz, (CD₃)₂CO): δ 7.46-7.38 (m, 2H), 6.99-6.91 (m, 2H), 5.04-5.00 (m, 1H), 4.58 (d, J = 3.6 Hz, 1H), 3.73 (dd, J = 11.2 Hz, 8.4 Hz, 1H), 3.67-3.55 (m, 2H), 3.49-3.28 (m, 2H).

¹³C NMR (100 MHz, (CD₃)₂CO): δ 174.2, 172.4, 159.9, 134.3, 131.4, 119.9, 117.8, 82.8, 72.4, 68.0, 55.0, 38.0, 35.1.

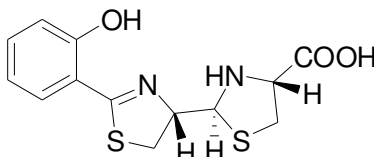


7b

R_f : 0.31 (*n*BuOH/H₂O/MeOH/Cyclohexane 2/2/1/1)

¹H NMR (400 MHz, (CD₃)₂CO): δ 7.46-7.38 (m, 2H), 6.99-6.91 (m, 2H), 4.86 (q, J = 4.0 Hz, 1H), 4.56 (d, J = 3.6 Hz, 1H), 3.73 (t, J = 8.0 Hz, 1H), 3.67-3.55 (m, 2H), 3.06-2.97 (m, 2H).

¹³C NMR (100 MHz, (CD₃)₂CO): δ 174.2, 172.7, 159.9, 134.3, 131.4, 119.9, 117.1, 83.1, 72.7, 64.9, 55.0, 38.1, 34.2.



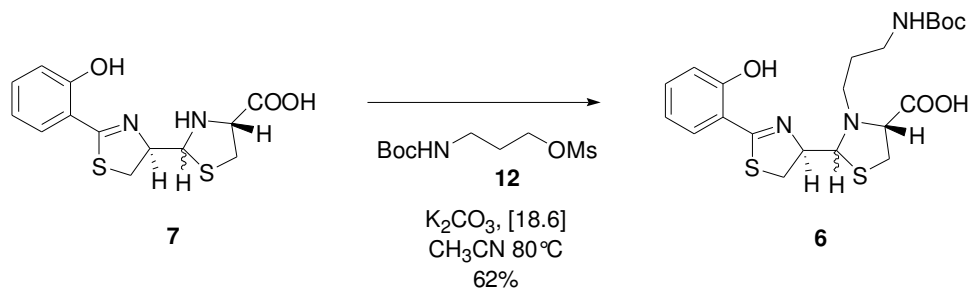
7c

R_f : 0.28 (*n*BuOH/H₂O/MeOH/Cyclohexane 2/2/1/1)

¹H NMR (400 MHz, (CD₃)₂CO): δ 7.46-7.38 (m, 2H), 6.99-6.91 (m, 2H), 5.02 (dt, J = 8.8 Hz, 5.2 Hz, 1H), 5.00 (d, J = 8.4 Hz, 1H), 4.00 (dd, J = 8.8 Hz, 6.8 Hz, 1H), 3.67-3.55 (m, 2H), 3.49-3.28 (m, 2H).

¹³C NMR (100 MHz, (CD₃)₂CO): δ 174.2, 173.4, 159.9, 134.3, 131.4, 119.9, 117.1, 78.0, 73.3, 66.0, 55.0, 38.5, 35.6.

Functionalized pyochelin **6**



To a solution of *nor*-pyochelin **7** (158 mg, 0.51 mmol) in CH_3CN (10 mL) were added 3-*tert*-butoxycarbonylamino-propyl ester **12**¹ (194 mg, 0.77 mmol), potassium carbonate (106 mg, 0.77 mmol) and crown-ether [18.6] (13 mg, 0.051 mmol). The mixture was stirred for 8 h at 75°C . The solvent was evaporated under reduced pressure and the crude was diluted in CH_2Cl_2 . The organic layer was washed successively with H_2O , a 0.5 N aqueous solution of HCl and brine. The organic layer was dried over Na_2SO_4 , filtered and evaporated under reduced pressure. This crude material was purified by chromatography on demetallated silica gel column (CH_2Cl_2 /acetone 95/5) leading to the expected functionalized pyochelin **6** (148 mg, 0.317 mmol, yield : 62 %) isolated under the form of a yellow oil.

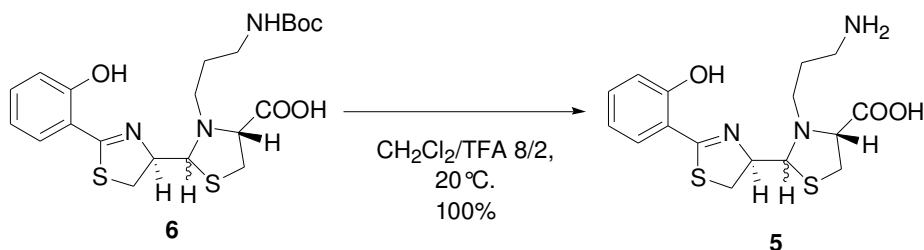
R_f : 0.64 ($\text{CH}_2\text{Cl}_2/\text{EtOH}$)

$^1\text{H NMR}$ (400 MHz, $(\text{CD}_3)_2\text{CO}$): δ 12.5 (s, 1H), 7.45-7.38 (m, 2H), 6.03 (bs, 1H), 5.98-6.90 (m, 2H), 5.19 (td, $J = 9.2$ Hz, 5.6 Hz, 1H), 4.90-4.87 (m, 3H), 3.51-3.46 (m, 1H), 3.36-3.29 (m, 2H), 3.24-3.20 (m, 2H), 3.09-3.02 (m, 2H), 1.74-1.68 (m, 2H), 1.26 (s, 9H).

$^{13}\text{C NMR}$ (100 MHz, $(\text{CD}_3)_2\text{CO}$): δ 173.5, 171.9, 160.2, 157.3, 153.6, 134.2, 131.4, 119.8, 117.8, 83.1, 78.6, 72.6, 65.2, 63.6, 38.5, 35.5, 28.7.

MS: m/z (ESI) 468.1 $[\text{M}+\text{H}^+]$.

Deprotected functionalized pyochelin **5**



¹ Brouwer, A.J.; Liskamp, R.M.J. *Eur. J. Org. Chem.* **2005**, 487-495.

The pyochelin analog **7** (148 mg, 0.31 mmol) was dissolved in a mixture of CH₂Cl₂/TFA 8/2 (3 mL) and stirred at 20°C for 4 h. The mixture was evaporated to dryness under reduced pressure and dissolved in fresh CH₂Cl₂. The organic layer was washed successively with water and brine before being dried over Na₂SO₄, filtered and evaporated under reduced pressure. The resulting yellow oil was triturated in Et₂O to give the free amine **6** (116 mg, 0.31 mmol, yield : 100 %) isolated by filtration as a yellow solid.

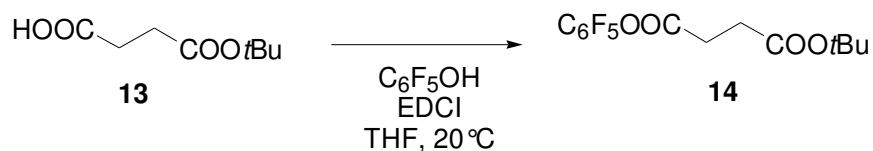
R_f: 0.16 (CH₂Cl₂/EtOH)

¹H NMR (400 MHz, (CD₃)₂CO): δ 7.40-7.27 (m, 2H), 6.99-6.79 (m, 2H), 5.05-4.99 (m, 1H), 4.82-4.77 (m, 3H), 4.32-4.13 (m, 2H), 3.65-3.58 (m, 1H), 3.54-3.07 (m, 4H), 1.71-1.62 (m, 2H).

¹³C NMR (100 MHz, (CD₃)₂CO): δ 173.7, 159.1, 156.2, 155.6, 133.7, 130.8, 119.4, 117.4, 72.2, 66.6, 59.9, 36.7, 34.9, 32.2, 29.9.

MS: *m/z* (ESI) 368.1 [M+H⁺].

Succinic acid *tert*-butyl pentafluorophenyl ester **14**



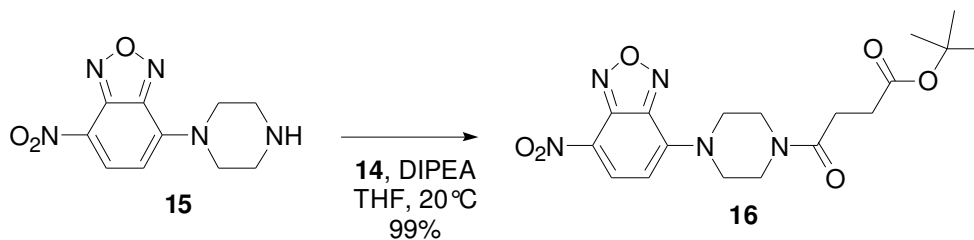
To a suspension of succinate derivative **13** (790 mg, 4.54 mmol) and pentafluorophenol (750 mg, 4.07 mmol) in THF (50 mL) was added EDCI (990 mg, 5.16 mmol). The mixture was stirred 12 hours (overnight) at 20°C. The solvent was removed under reduced pressure and the crude material was purified by chromatography on a silica gel column (cyclohexane/Et₂O: 1/1) to give the expected pentafluorophenylester **14** (1041 mg, 4.36 mmol, yield : 75%) isolated as a white solid.

¹H NMR (CDCl₃, 400MHz): δ (ppm) = 2.92 (t, *J* = 6.4 Hz, 2H), 2.66 (t, *J* = 6.4 Hz, 2H), 1.44 (s, 9H).

¹³C NMR (CDCl₃, 100MHz): δ (ppm) = 145.2, 144.9, 144.7, 136.2, 120.4, 103.2, 51.3, 45.8.

¹⁹F NMR (CDCl₃, 400 MHz): δ -152.5 - -152.6 (m, 2F), -158.1 (t, *J* = 23.2 Hz, 1F), -162.4 - -162.5 (m, 2F).

4-[4-(7-Nitro-benzo[1,2,5]oxadiazol-4-yl)-piperazin-1-yl]-4-oxo-butyric acid *tert*-butyl ester **16**



To a solution of piperazinyl-NBD **15**² (250 mg, 1.0 mmol) in THF (5 mL) were added successively the diester **14** (300 mg, 1.2 mmol) and DIPEA (2.0 mL). The mixture was stirred at 20°C for 1 h. The mixture was then diluted with CH₂Cl₂ (100 mL) and washed with an aqueous solution saturated in NaHCO₃. The organic layer was dried over Na₂SO₄, filtered and adsorbed onto silica gel before being purified by chromatography through a silica gel column (CH₂Cl₂/acetone 98/2). The expected NBD derivative **16** (405 mg, 1.0 mmol, yield : 99%) was obtained as a red powder.

R_f : 0.56 (CH₂Cl₂/EtOH 9/1)

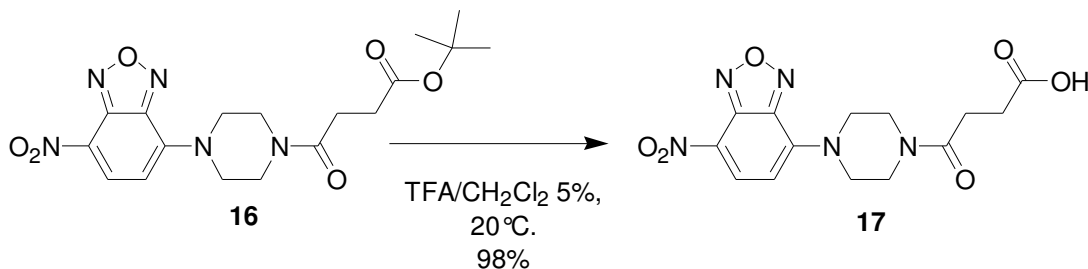
¹H NMR (CDCl₃, 400MHz): δ 8.30 (d, *J* = 8.8 Hz, 1H), 6.23 (d, *J* = 8.8 Hz, 1H), 4.24 (t, *J* = 4.0 Hz, 2H), 4.02 (t, *J* = 4.0 Hz, 2H), 3.91 (t, *J* = 4.0 Hz, 2H), 3.91 (t, *J* = 4.0 Hz, 2H), 2.60 (s, 4H), 1.40 (s, 9H).

¹³C NMR (CDCl₃, 100MHz): δ 172.4, 170.8, 145.0, 144.8, 144.7, 135.1, 124.0, 102.7, 80.9, 48.9, 44.5, 40.8, 30.4, 28.2, 28.0.

MS: *m/z* (ESI) 406.1 [M+H⁺].

HRMS: C₁₈H₂₃N₅O₆: calcd 405.16697, found 405.16493.

4-[4-(7-Nitro-benzo[1,2,5]oxadiazol-4-yl)-piperazin-1-yl]-4-oxo-butyric acid **17**



² Nudelman, R.; Ardon, O.; Hadar, Y.; Chen, Y.; Libman, J.; Shanzer, A. *J. Med. Chem.* **1998**, *41*, 1671-1678.

Compound **16** (405 mg, 1.00 mmol) and Na₂SO₄ (200 mg) were suspended in a 10% TFA/CH₂Cl₂ solution (10 mL). The mixture was stirred at 20°C for 1 h. The solvents were removed under reduced pressure leading to the expected carboxylic acid **17** (403 mg, 0.98 mmol, yield : 98 %) isolated as a red solid.

R_f : 0.10 (CH₂Cl₂/EtOH 95/5)

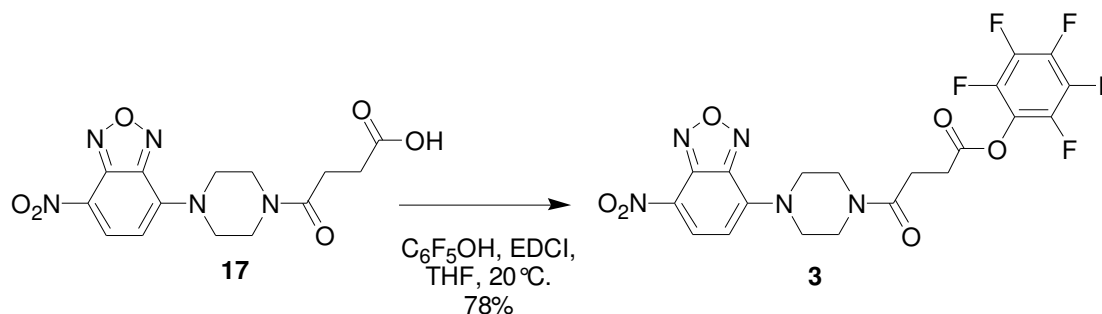
¹H NMR (400 MHz, DMSO-*d*₆): δ 8.45 (d, *J* = 8.8 Hz, 1H), 6.57 (d, *J* = 8.8 Hz, 1H), 4.23 (sl, 2H), 4.12 (s, 2H), 3.83 (t, *J* = 4.0 Hz, 2H), 3.75 (t, *J* = 4.0 Hz, 2H), 2.61 (t, *J* = 4.0 Hz, 2H), 2.47 (t, *J* = 4.0 Hz, 2H).

¹³C NMR (100 MHz, DMSO-*d*₆): δ 173.9, 170.2, 145.4, 144.7, 136.2, 121.1, 103.0, 48.9, 48.6, 43.3, 28.8, 27.5.

MS: *m/z* (ESI) 350.1 [M+H⁺].

HRMS: C₁₄H₁₅N₅O₆: calcd 349.10291, found 349.10223.

4-[4-(7-Nitro-benzo[1,2,5]oxadiazol-4-yl)-piperazin-1-yl]-4-oxo-butyric acid pentafluorophenyl ester
3



To a suspension of compound **17** (403 mg, 0.98 mmol) in CH₂Cl₂ (10 mL) were successively added pentafluorophenol (312 mg, 1.90 mmol) and EDCI (561 mg, 2.94 mmol). The mixture was stirred at 40°C for 8 h. The mixture was washed successively with a 0.5 N aqueous solution of HCl, water and brine. The organic layer was dried over Na₂SO₄, filtered and evaporated under reduced pressure. The crude residue was purified by chromatography on a silica gel column (CH₂Cl₂/EtOH 98/2) to give the expected pentafluorophenylester **3** (394 mg, 0.76 mmol, yield : 78 %) isolated as a red powder.

R_f : 0.52 (CH₂Cl₂/EtOH 95/5)

¹H NMR (CDCl₃, 400 MHz): δ 8.39 (d, *J* = 8.8 Hz, 1H), 6.28 (d, *J* = 8.8 Hz, 1H), 4.28 (t, *J* = 4.0 Hz, 2H), 4.00 (t, *J* = 4.0 Hz, 2H), 3.95 (t, *J* = 4.0 Hz, 2H), 3.85 (t, *J* = 4.0 Hz, 2H), 3.08 (t, *J* = 4.0 Hz, 2H), 2.82 (t, *J* = 4.0 Hz, 2H).

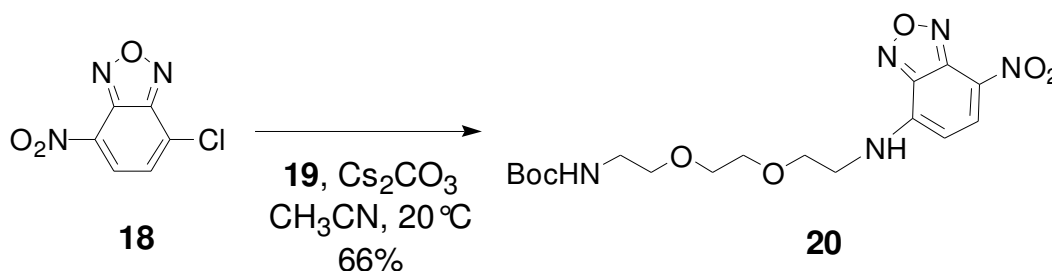
¹³C NMR (CDCl₃, 100 MHz): δ 169.5, 169.3, 145.0, 144.9, 144.8, 135.0, 124.8, 102.8, 48.9, 44.7, 40.9, 28.7, 28.0.

¹⁹F NMR (CDCl₃, 400 MHz): δ -152.3 - -152.4 (m, 2F), -157.8 (t, J = 23.2 Hz, 1F), -162.2 - -162.3 (m, 2F).

MS: m/z (ESI) 516.09 [M+H⁺].

HRMS: C₂₀H₁₄F₅N₅O₆: calcd 515.08746, found 515.08642.

(2-{2-[2-(7-Nitro-benzo[1,2,5]oxadiazol-4-ylamino)-ethoxy]-ethoxy}-ethyl)-carbamic acid *tert*-butyl ester **20**



To a solution of {2-[2-(2-amino-ethoxy)-ethoxy]-ethyl}-carbamic acid *tert*-butyl ester **19**³ (248 mg, 1.0 mmol) in CH₃CN (50 mL) was added NBD chloride **18** (199 mg, 1.0 mmol). After addition of Cs₂CO₃ (651 mg, 2.0 mmol), the mixture was stirred at 40 °C for 30 min. The solvent was removed under reduced pressure and the crude was diluted in a mixture of water (100 mL) and CH₂Cl₂ (100 mL). After partition, the organic layer was collected, dried over MgSO₄ and filtered. The filtrate was adsorbed onto silica gel before being filtered through a silica gel column (CH₂Cl₂/acetone 98/2) to give the expected NBD derivative **20** (271 mg, 0.66 mmoles, yield : 66 %) isolated as a red oil.

R_f : 0.56 (CH₂Cl₂/EtOH 95/5)

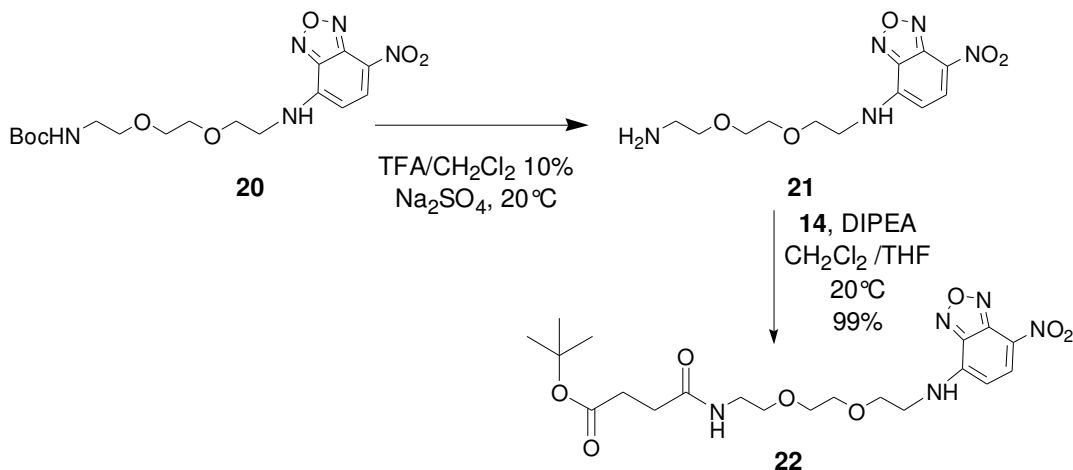
¹H NMR (400 MHz, CD₃COOD): δ 8.53 (d, J = 8.0 Hz, 1H), 6.54 (d, J = 8.0 Hz, 1H), 3.90-3.86 (m, 4H), 3.67-3.59 (m, 4H), 3.50 (t, J = 6.0 Hz, 2H), 3.21 (q, J = 6.0 Hz, 2H), 1.38 (s, 9H).

¹³C NMR (100 MHz, CD₃COOD): δ 156.7, 146.0, 145.6, 145.2, 137.9, 100.1, 78.7, 71.3, 71.1, 70.8, 69.4, 44.4, 41.1, 28.6.

MS m/z (ESI) 412.14 [M+H]⁺.

³ Schneider, R.; Schmitt, F.; Frochot, C.; Fort, Y.; Lourette, N.; Guillemin, F.; Mueller, J.-F.; Barberi-Heyob, M. *Bioorg. Med. Chem.* **2005**, *13*, 2799-2808.

N-(2-{2-[2-(7-Nitro-benzo[1,2,5]oxadiazol-4-ylamino)-ethoxy]-ethoxy}-ethyl)-succinamic acid *tert*-butyl ester **22**



To a solution of compound **20** (271 mg, 0.66 mmol) in CH₂Cl₂ (8.5 mL) were added successively Na₂SO₄ (200 mg) and TFA (2.5 mL). The mixture was then stirred at 20°C and the release of isobutene was checked. After 1h, the reaction mixture was filtered and the desiccant washed several times with EtOH. The solvents were evaporated under reduced pressure to give the amine **21** as a red oil. This compound was used as it in the next synthetic step.

To a solution of amine **21** (205.3 mg, 0.66 mmol) in a mixture of CH₂Cl₂ (50 mL) and THF (25 mL) were added successively the succinic diester **14** (300 mg, 1.20 mmol) and DIPEA (2.0 mL). After 2 min of sonication, the reaction mixture was stirred at 20°C for 1 h. The mixture was then diluted with CH₂Cl₂ (100 mL) and washed with a saturated aqueous solution of NaHCO₃. The organic layer was collected, dried over Na₂SO₄ and filtered. The filtrate was adsorbed onto silica gel before being filtered through a silica gel column (Et₂O/THF 90/10 then 50/50) to give the expected NBD derivative **22** (300 mg, 0.66 mmol, yield : 99 %) isolated as a red oil.

R_f : 0.43 (CH₂Cl₂/EtOH 95/5)

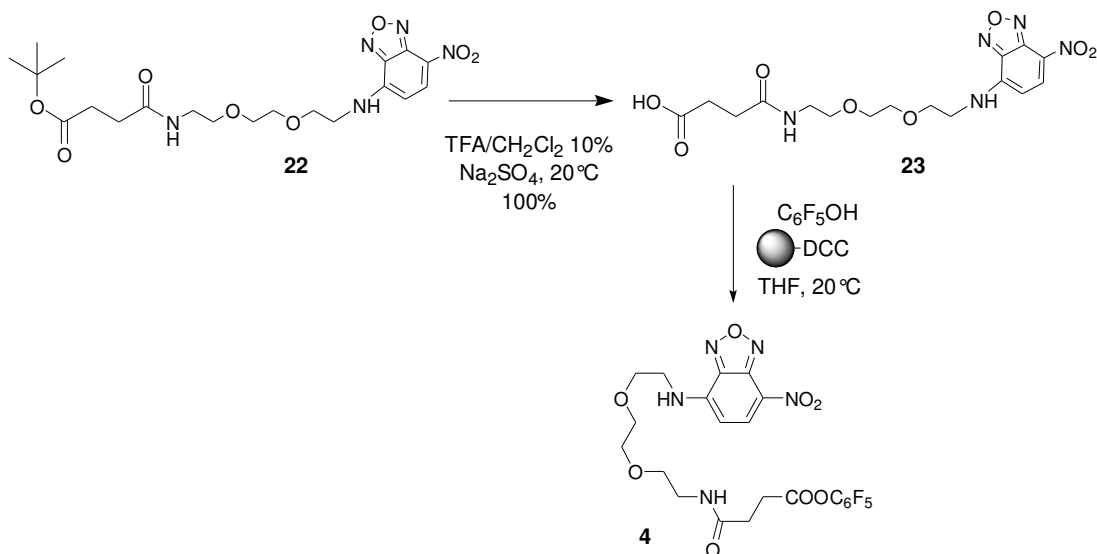
¹H NMR (400 MHz, CDCl₃): δ 8.43 (d, *J* = 8.4 Hz, 1H), 6.16 (d, *J* = 8.6 Hz, 1H), 3.84 (t, *J* = 4.8 Hz, 2H), 3.70-3.62 (m, 6H), 3.56 (t, *J* = 5.2 Hz, 2H), 3.45 (q, *J* = 6.4 Hz, 2H), 2.55 (t, *J* = 6.4 Hz, 2H), 2.41 (t, *J* = 6.4 Hz, 2H), 1.39 (s, 9H).

¹³C NMR (100 MHz, CDCl₃): δ 172.6, 172.1, 144.5, 144.3, 144.1, 136.6, 124.0, 99.0, 81.0, 70.4, 68.3, 44.0, 39.4, 31.4, 31.0, 29.9, 28.0.

MS : *m/z* (ESI) 468.2 [M+H⁺].

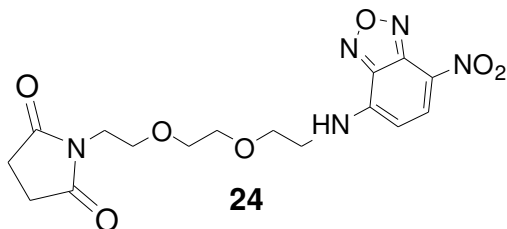
HRMS : C₂₀H₂₉N₅O₈ : calcd 467.20217, found 467.20161.

N-(2-{2-[2-(7-Nitro-benzo[1,2,5]oxadiazol-4-ylamino)-ethoxy]-ethoxy}-ethyl)-succinamic acid pentafluorophenyl ester **4**



To a solution of *tert*-butylester **22** (557 mg, 1.19 mmol) in CH₂Cl₂ (9.0 mL) were successively added Na₂SO₄ (200 mg) and TFA (1.0 mL). The mixture was stirred at 20 °C and the release of isobutene was checked. After 3 h, the reaction mixture was filtered and desiccant was washed several times with EtOH. The solvents were evaporated under reduced pressure to give the expected amine **23** as a red oil. This compound was used as it in the next synthetic step.

To a solution of acid **23** (490 mg, 1.19 mmol) in DMF (10 mL) were added successively pentafluorophenol (219 mg, 2.38 mmol) and PS-DCC (940 mg, 1.78 mmol). The suspension was stirred at 20 °C for 5 h. The mixture was filtered and the resin was washed several times with acetone. Acetone was removed under reduced pressure and the solution of compound **4** in DMF was directly used in the next synthetic step. Indeed, the pentafluorophenylester **4** is very reactive and the purification of compound **4** on a silica gel column (CH₂Cl₂/EtOH 9/1), lead quantitatively to the succinamide derivative **24** (388 mg, 0.987 mmol, yield : 83%).

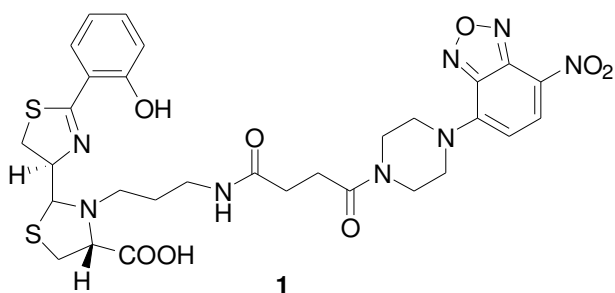


MS : *m/z* (ESI) 394.1 [M+H⁺]

HRMS : C₁₆H₁₉N₅O₇ : calcd 393.12848, found 393.12845.

Probe 1

To a solution of pentafluorophenyl ester derivate **3** (773 mg, 1.5 mmol) in CH₂Cl₂ (15 mL) were added successively the functionalized pyochelin **5** (367 mg, 1.0 mmol) and DIPEA (258 mg, 348 μ L, 2.0 mmol). The mixture was stirred 12 h (overnight) at 20°C. The mixture was washed successively with a 0.5 N aqueous solution of HCl, water and brine. The organic layers was collected, dried over Na₂SO₄, filtered and evaporated under reduced pressure. The crude residue was purified by chromatography on demetallated silica gel column (CH₂Cl₂/EtOH 99/1 to 98/2) to give the expected probe **1** (286 mg, 0.41 mmol, yield : 41%) isolated as a red solid.



R_f: 0.42 (CH₂Cl₂/EtOH 95/5)

¹H NMR (400 MHz, CDCl₃): δ 8.35 (d, J = 8.4 Hz, 1H), 7.35-7.24 (m, 2H), 6.93-6.80 (m, 2H), 6.23 (d, J = 8.6 Hz, 1H), 5.02-4.95 (m, 1H), 4.81-4.76 (m, 1H), 4.23-4.19 (m, 4H), 4.03 (bs, 2H), 3.90-3.84 (m, 4H), 3.64 (q, J = 6.8 Hz, 1H), 3.35-3.28 (m, 4H), 3.07 (q, J = 7.2 Hz, 2H), 2.72 (t, J = 5.6 Hz, 2H), 2.55 (t, J = 6.4 Hz, 2H), 1.90-1.83 (m, 2H).

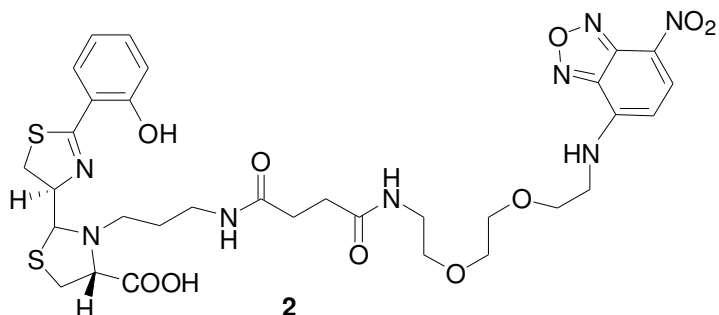
¹³C NMR (100 MHz, CDCl₃): δ 173.5, 172.6, 171.2, 159.2, 145.0, 144.9, 144.7, 135.1, 133.5, 130.8, 124.3, 119.1, 117.3, 116.3, 102.8, 81.7, 72.3, 71.2, 64.5, 63.3, 53.9, 48.8, 44.5, 42.1, 40.9, 37.8, 36.3, 34.7, 29.9, 28.7, 27.1, 18.2, 12.1.

HRMS: C₃₀H₃₄N₈O₈S₂: calcd 698.19465, found 698.19410.

Probe 2

To a solution of pentafluorophenyl ester derivate **4** (687 mg, 1.19 mmol) in DMF (15 mL) were added successively the functionalized pyochelin **5** (367 mg, 1.0 mmol) and DIPEA (258 mg, 348 μ L, 2.0 mmol). The mixture was stirred 12 h (overnight) at 20°C. The solvent was removed under reduced pressure and the oily residue was dissolved in CH₂Cl₂. This organic layer was washed successively with a 0.5 N aqueous solution of HCl, water and brine, dried over Na₂SO₄, filtered and the solvent were evaporated under reduced

pressure. The crude residue was purified on silica gel column (CH₂Cl₂/EtOH 99/1 to 98/2) to give the expected probe **2** (561 mg, 0.738 mmol, yield : 62%) isolated as a red solid.



R_f: 0.45 (CH₂Cl₂/EtOH 95/5)

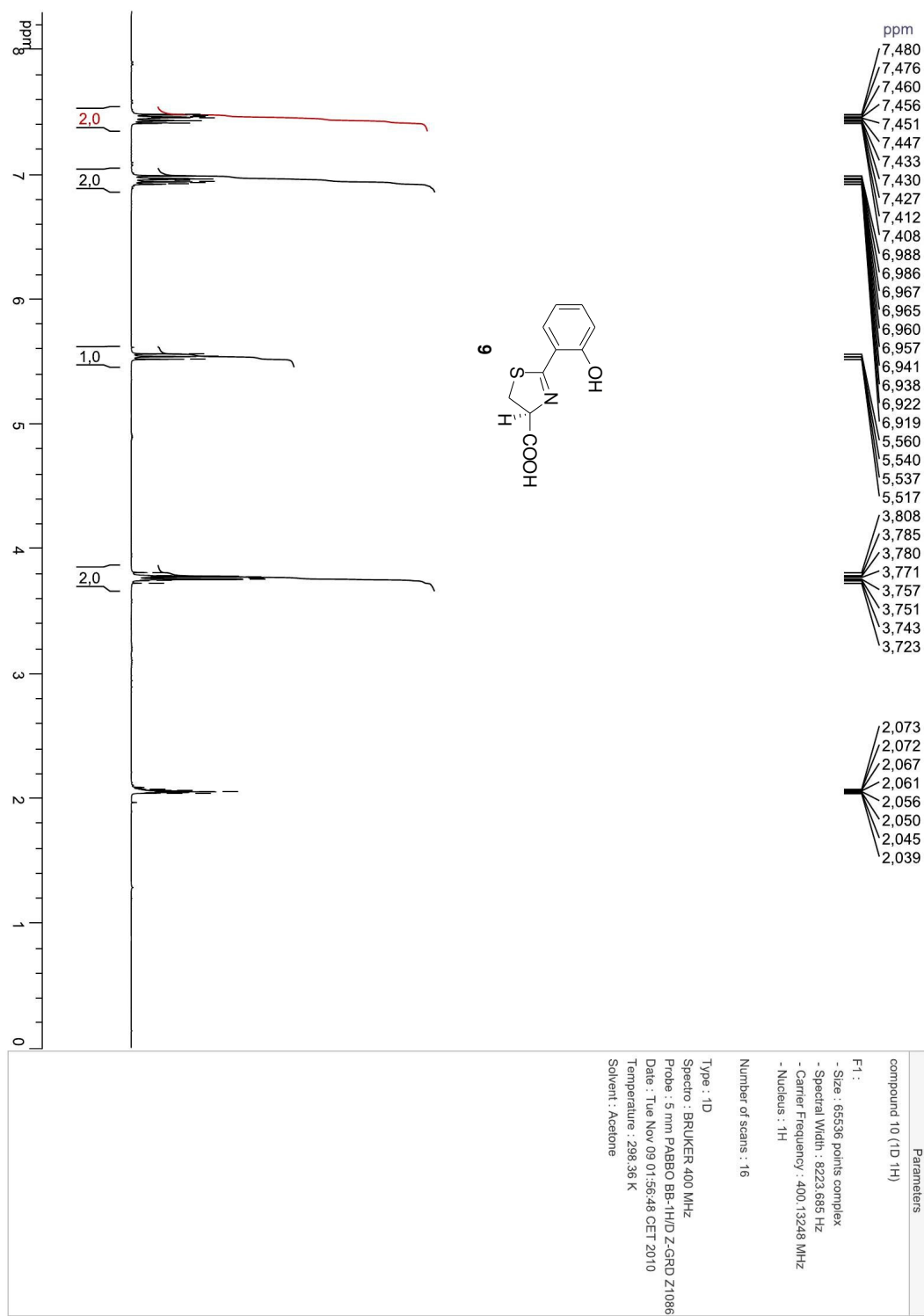
¹H NMR (400 MHz, CD₃COOD): δ 8.51 (d, *J* = 8.4 Hz, 1H), 7.44-7.38 (m, 2H), 6.97-6.89 (m, 2H), 6.52 (d, *J* = 8.6 Hz, 1H), 5.20-5.13 (m, 1H), 4.89-4.85 (m, 1H), 4.21-4.18 (m, 2H), 3.88-3.80 (m, 5H), 3.65-3.50 (m, 8H), 3.39-3.27 (m, 8H), 2.56 (sl, 4H).

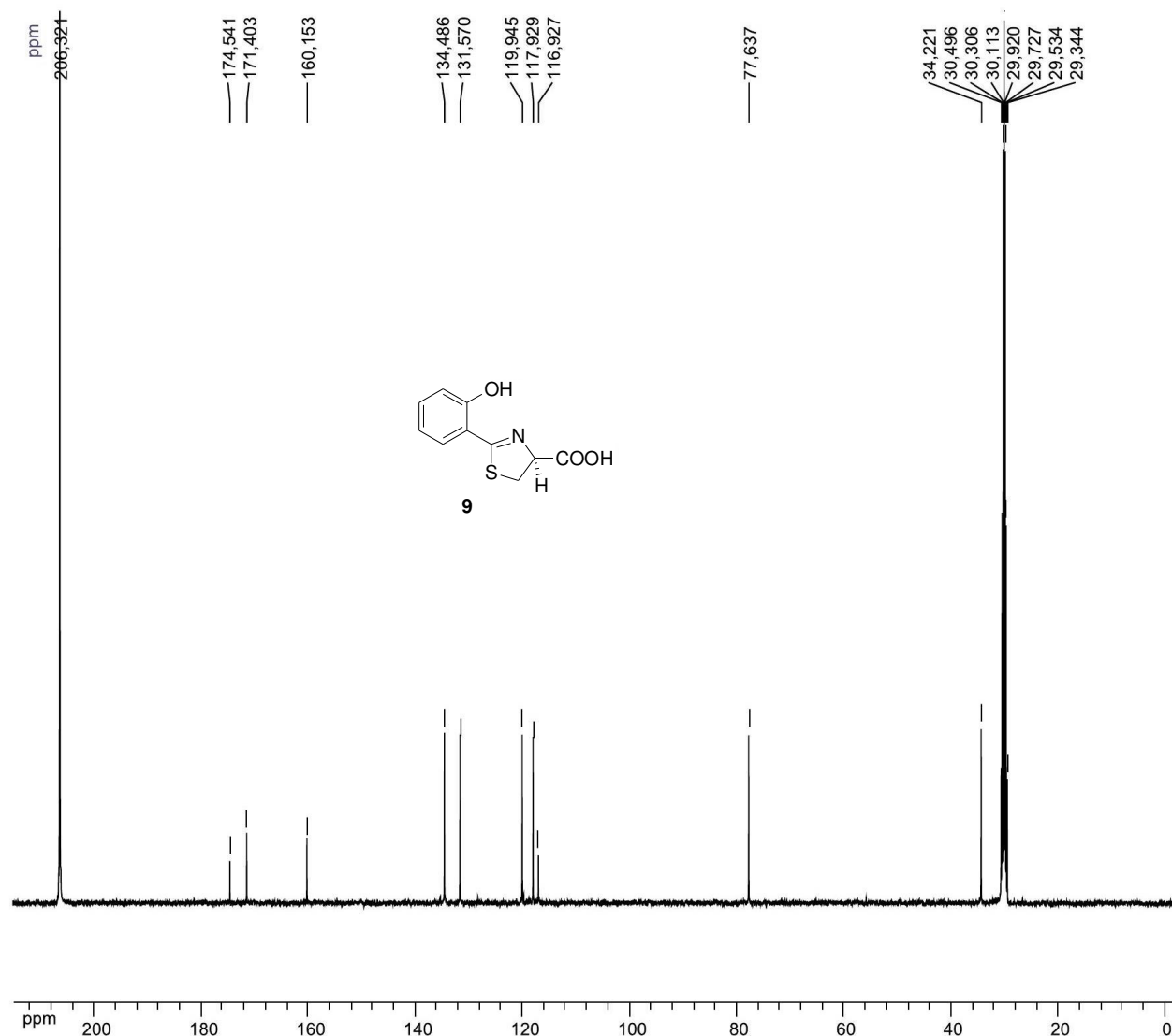
¹³C NMR (100 MHz, CD₃COOD): δ 171.7, 162.0, 161.3, 142.9, 137.9, 134.2, 131.4, 119.9, 119.1, 117.8, 116.2, 83.1, 79.3, 72.7, 71.1, 70.3, 69.4, 54.9, 43.1, 40.1, 38.5, 36.8, 35.5, 32.0, 27.6, 18.6, 17.4, 12.6.

MS : *m/z* (ESI) 761.2 [M+H⁺].

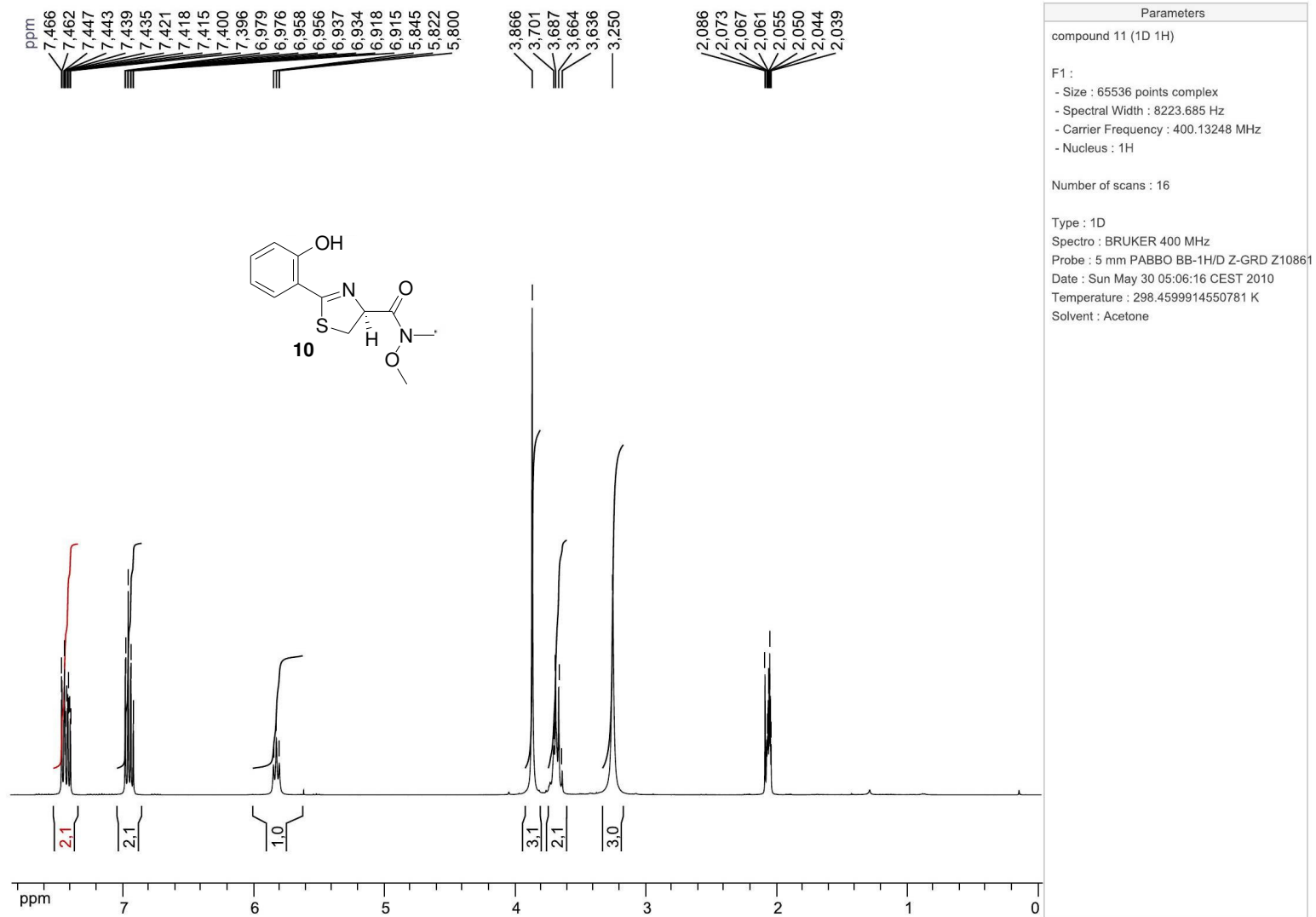
HRMS: C₃₂H₄₀N₈O₁₀S₂: calcd 760.23144, found 760.23088.

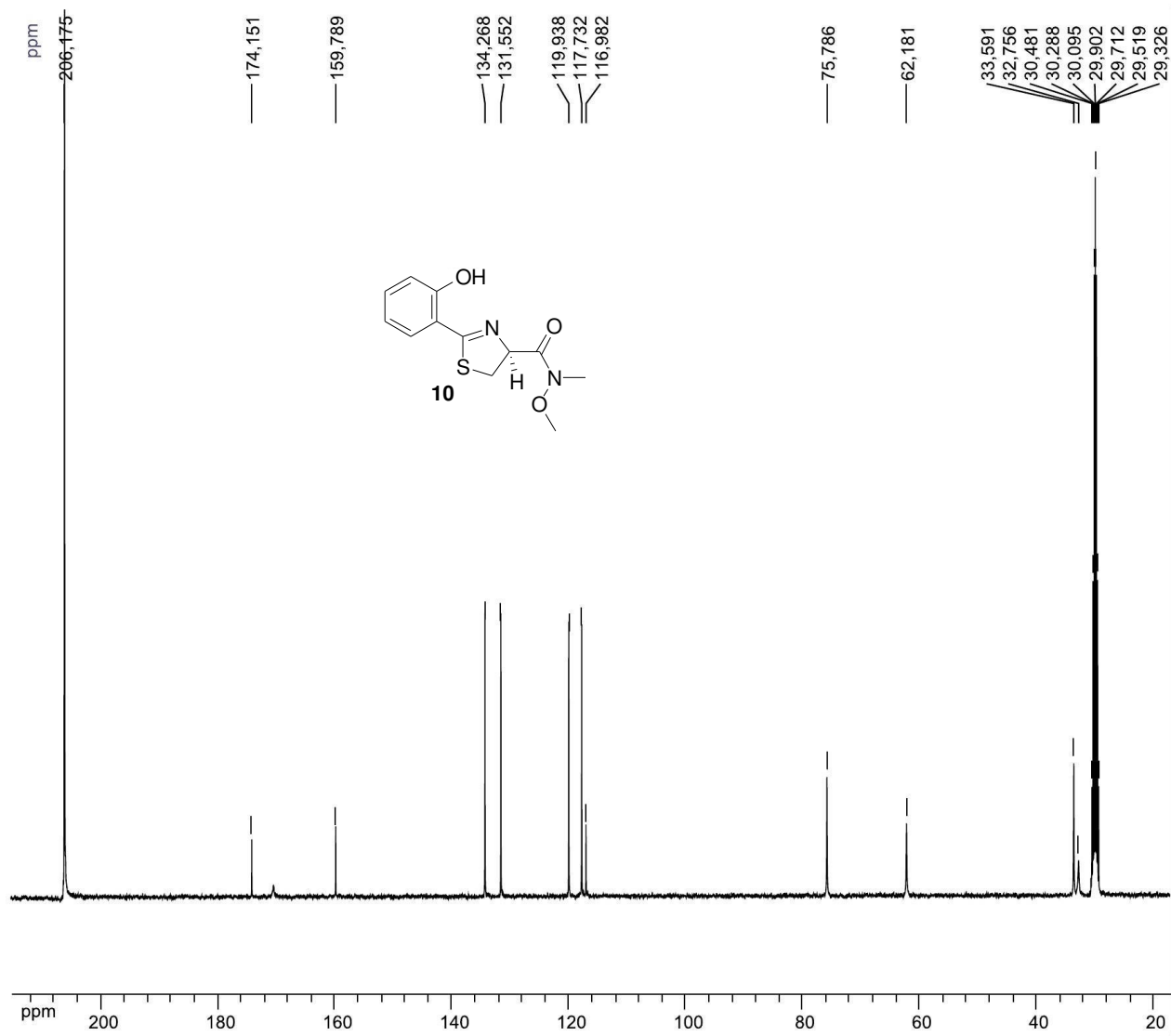
C. NMR Spectra



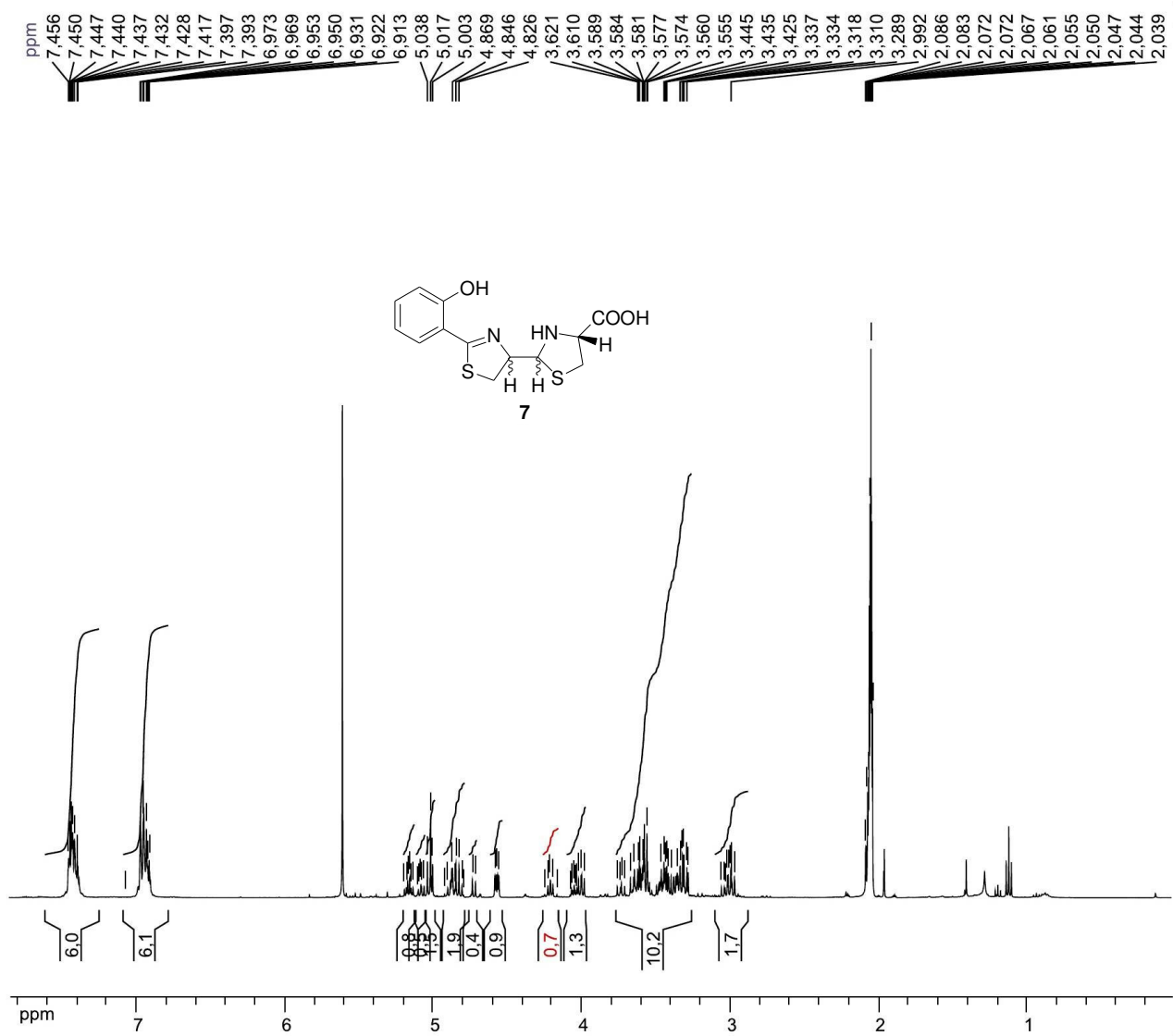


Parameters
compound 10 (1D 13C)
F1 :
- Size : 65536 points complex
- Spectral Width : 24038.46 Hz
- Carrier Frequency : 100.62283 MHz
- Nucleus : 13C
Number of scans : 512
Type : 1D
Spectro : BRUKER 400 MHz
Probe : 5 mm PABBO BB-1H/D Z-GRD Z10861
Date : Tue Nov 09 02:27:36 CET 2010
Temperature : 298.76 K
Solvent : Acetone

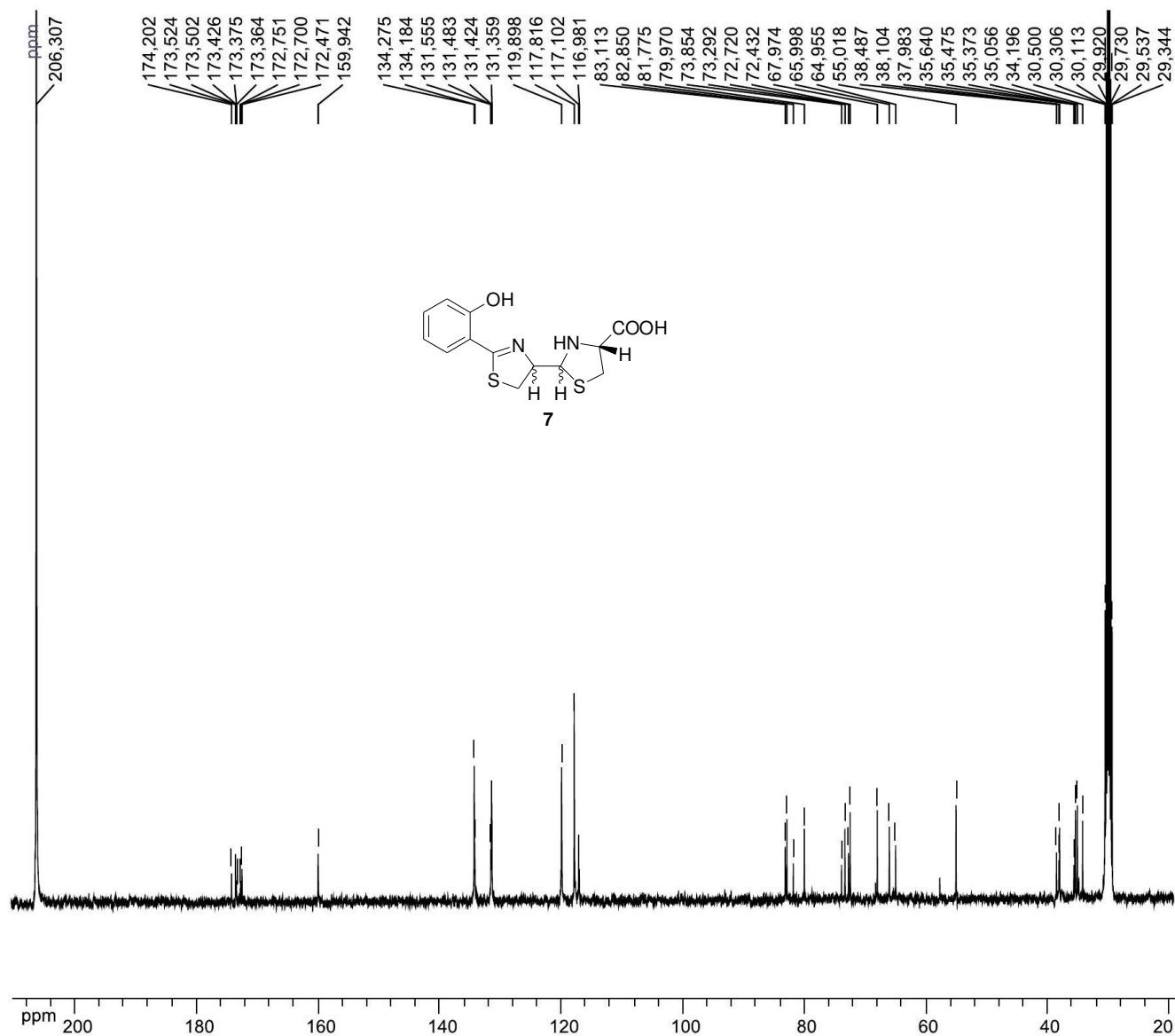




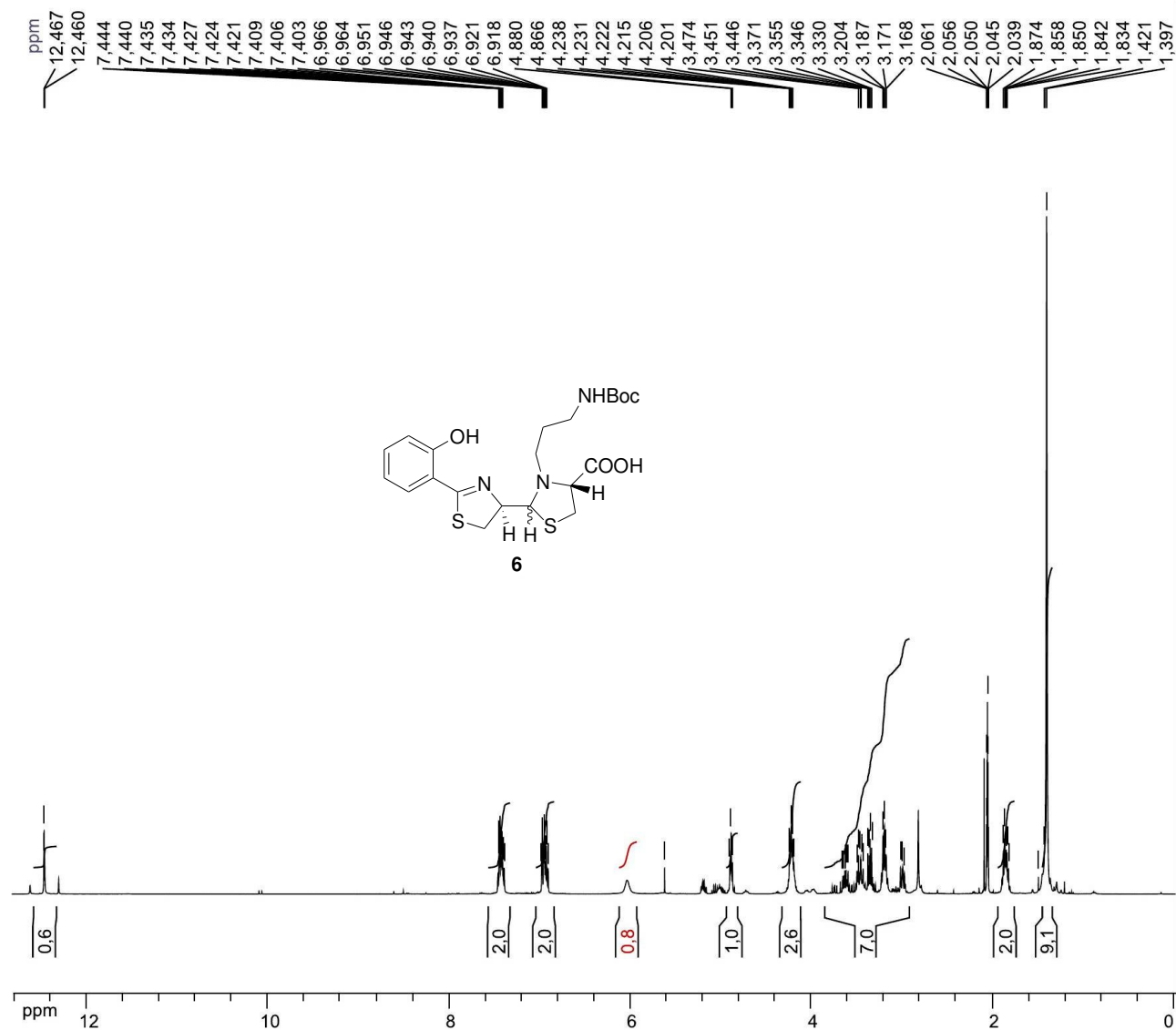
Parameters	
amide de weinreb 2 (1D 13C)	
F1 :	
- Size :	65536 points complex
- Spectral Width :	24038.46 Hz
- Carrier Frequency :	100.62283 MHz
- Nucleus :	13C
Number of scans : 512	
Type : 1D	
Spectro : BRUKER 400 MHz	
Probe : 5 mm PABBO BB-1H/D Z-GRD Z10861	
Date : Sun May 30 05:36:59 CEST 2010	
Temperature : 299.26 K	
Solvent : Acetone	



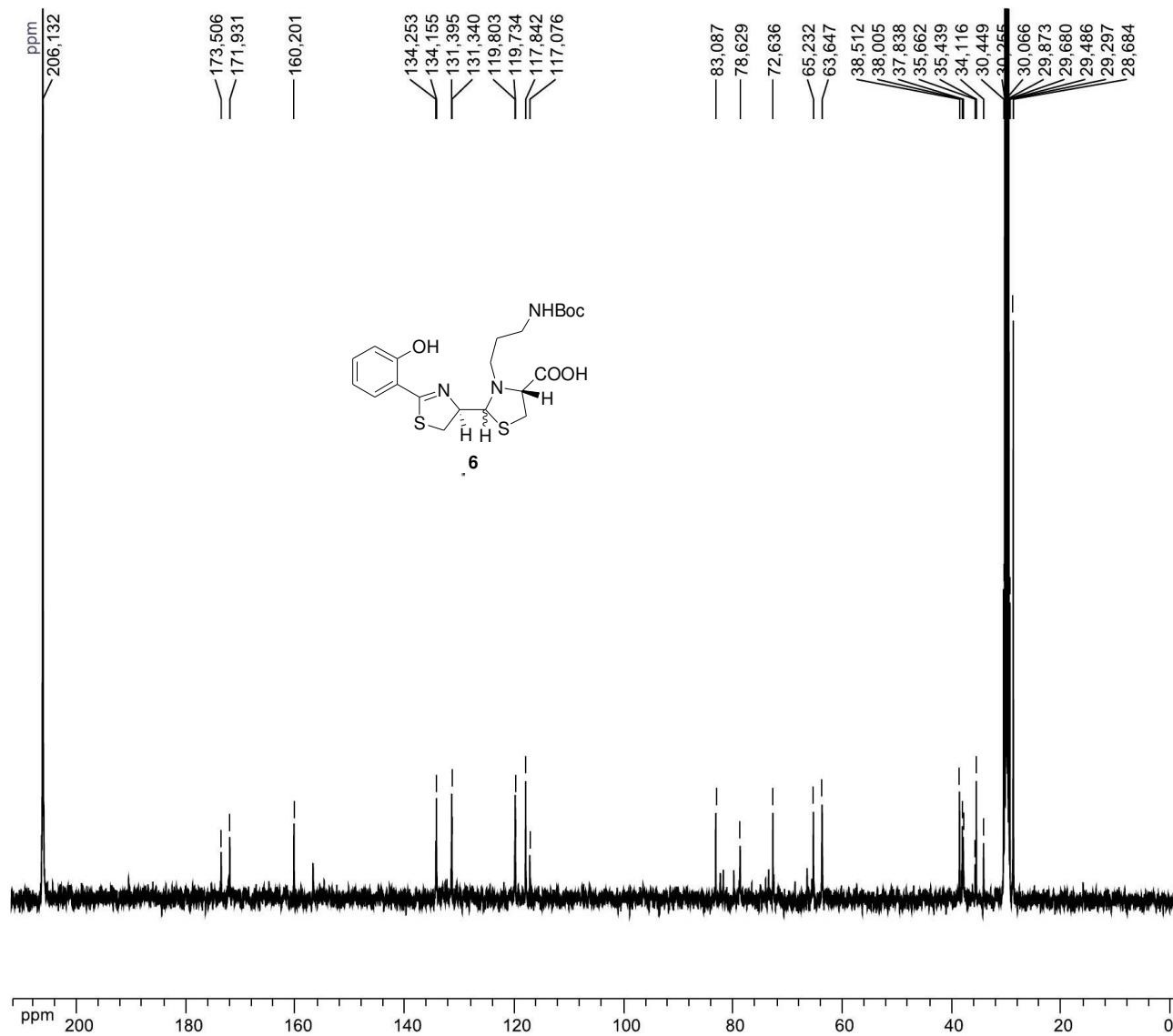
Parameters
compound 8 (1D 1H) (1D 1H)
F1 :
- Size : 65536 points complex
- Spectral Width : 8223.685 Hz
- Carrier Frequency : 400.13248 MHz
- Nucleus : 1H
Number of scans : 16
Type : 1D
Spectro : BRUKER 400 MHz
Probe : 5 mm PABBO BB-1H/D Z-GRD Z10861
Date : Sat May 29 20:08:00 CEST 2010
Temperature : 298.260009765625 K
Solvent : Acetone



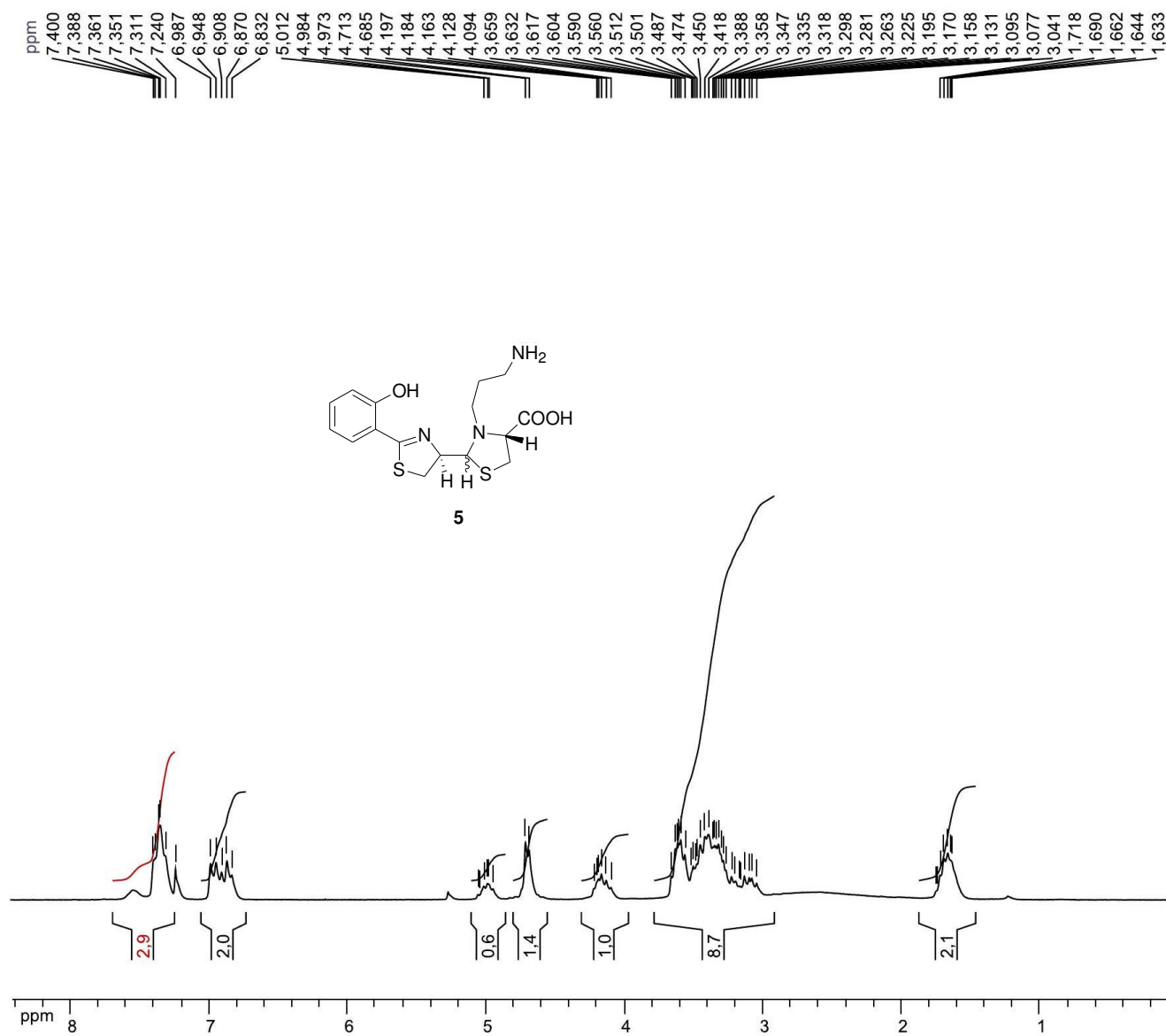
Parameters	
compound 8 (1D 13C) (1D 13C)	
F1 :	
- Size :	65536 points complex
- Spectral Width :	24038.46 Hz
- Carrier Frequency :	100.62283 MHz
- Nucleus :	13C
Number of scans : 512	
Type : 1D	
Spectro : BRUKER 400 MHz	
Probe : 5 mm PABBO BB-1H/D Z-GRD Z10861	
Date : Sat May 29 20:38:38 CEST 2010	
Temperature : 299.05999755859375 K	
Solvent : Acetone	



Parameters
compound 7 (1D 1H)
F1 :
- Size : 65536 points complex
- Spectral Width : 8223.685 Hz
- Carrier Frequency : 400.13248 MHz
- Nucleus : 1H
Number of scans : 16
Type : 1D
Spectro : BRUKER 400 MHz
Probe : 5 mm PABBO BB-1H/D Z-GRD Z10861
Date : Tue May 05 06:57:37 CEST 2009
Temperature : 300.1600036621094 K
Solvent : Acetone



Parameters
compound7 C13 (1D 13C)
F1 :
- Size : 65536 points complex
- Spectral Width : 24038.46 Hz
- Carrier Frequency : 100.62283 MHz
- Nucleus : ¹³ C
Number of scans : 128
Type : 1D
Spectro : BRUKER 400 MHz
Probe : 5 mm PABBO BB-1H/D Z-GRD Z10861
Date : Tue May 05 07:06:18 CEST 2009
Temperature : 300.16 K
Solvent : Acetone



Parameters

compound 6 (1D 1H) (1D 1H)

F1 :

- Size : 16384 points complex
- Spectral Width : 4006.4102 Hz
- Carrier Frequency : 200.13124 MHz
- Nucleus : 1H

Number of scans : 16

Type : 1D

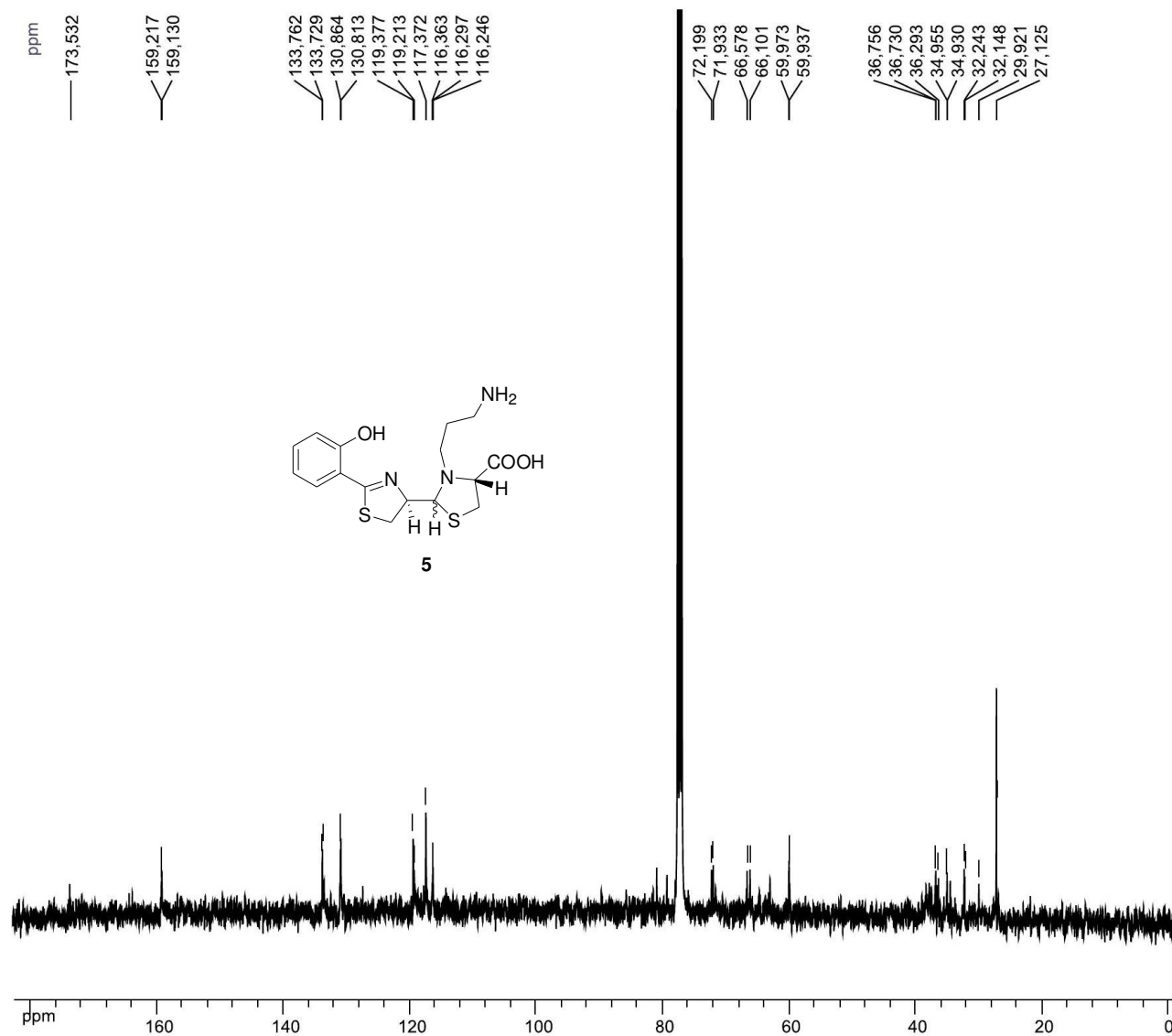
Spectro : BRUKER 200 MHz

Probe : 5 mm Multinuclear inverse Z-grad

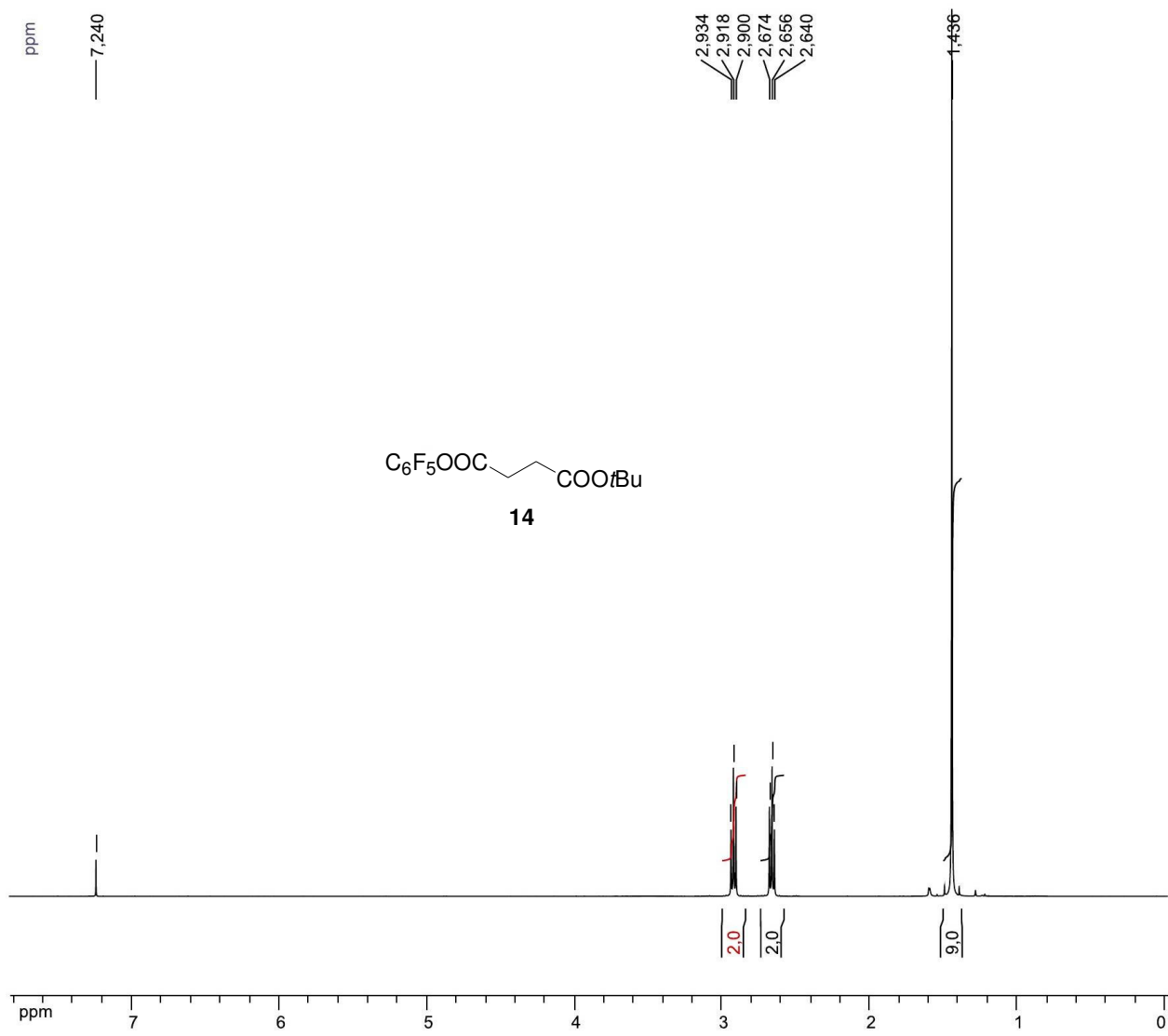
Date : Wed Oct 01 15:15:00 CEST 2008

Temperature : 300.0 K

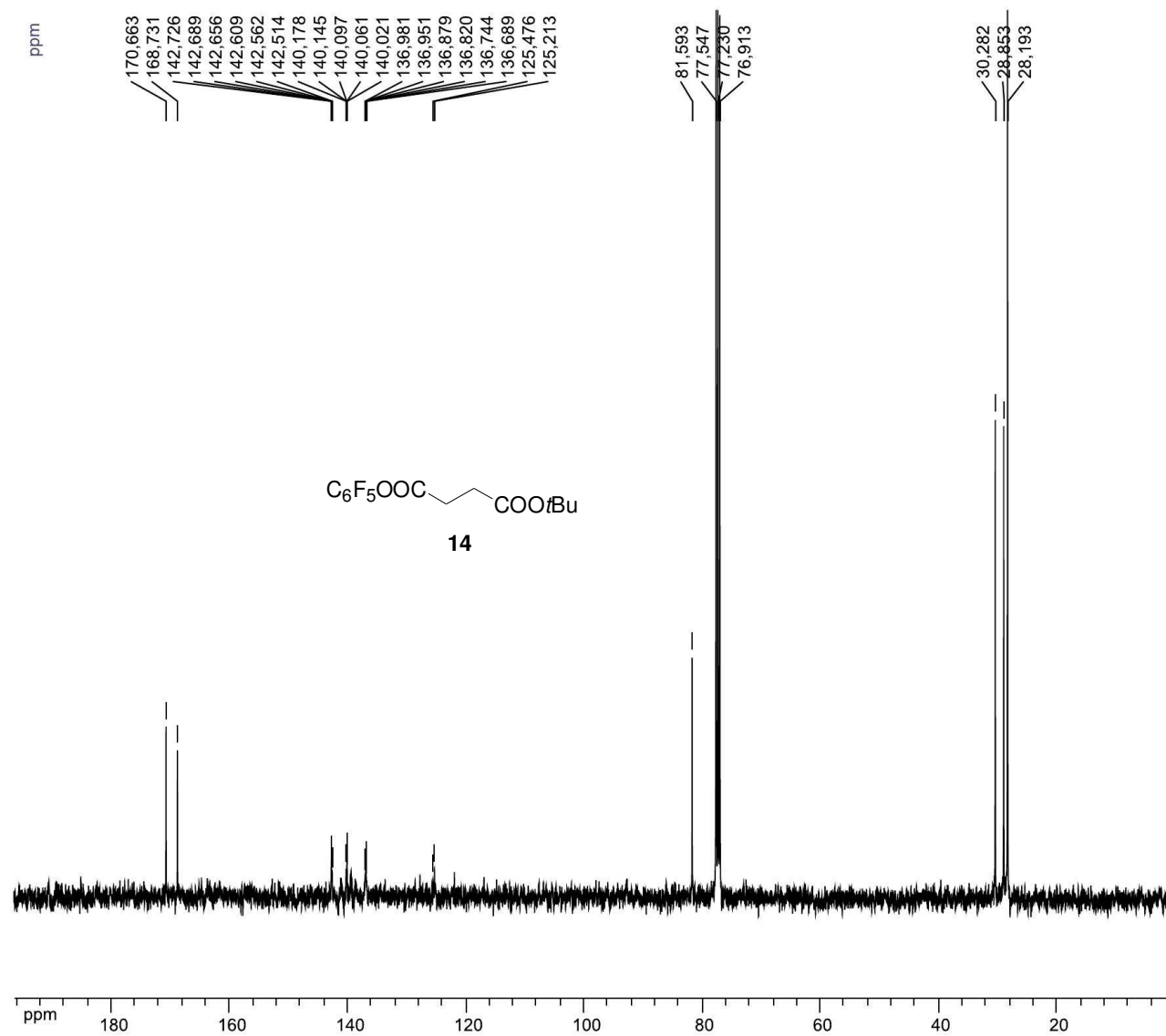
Solvent : CDCl₃



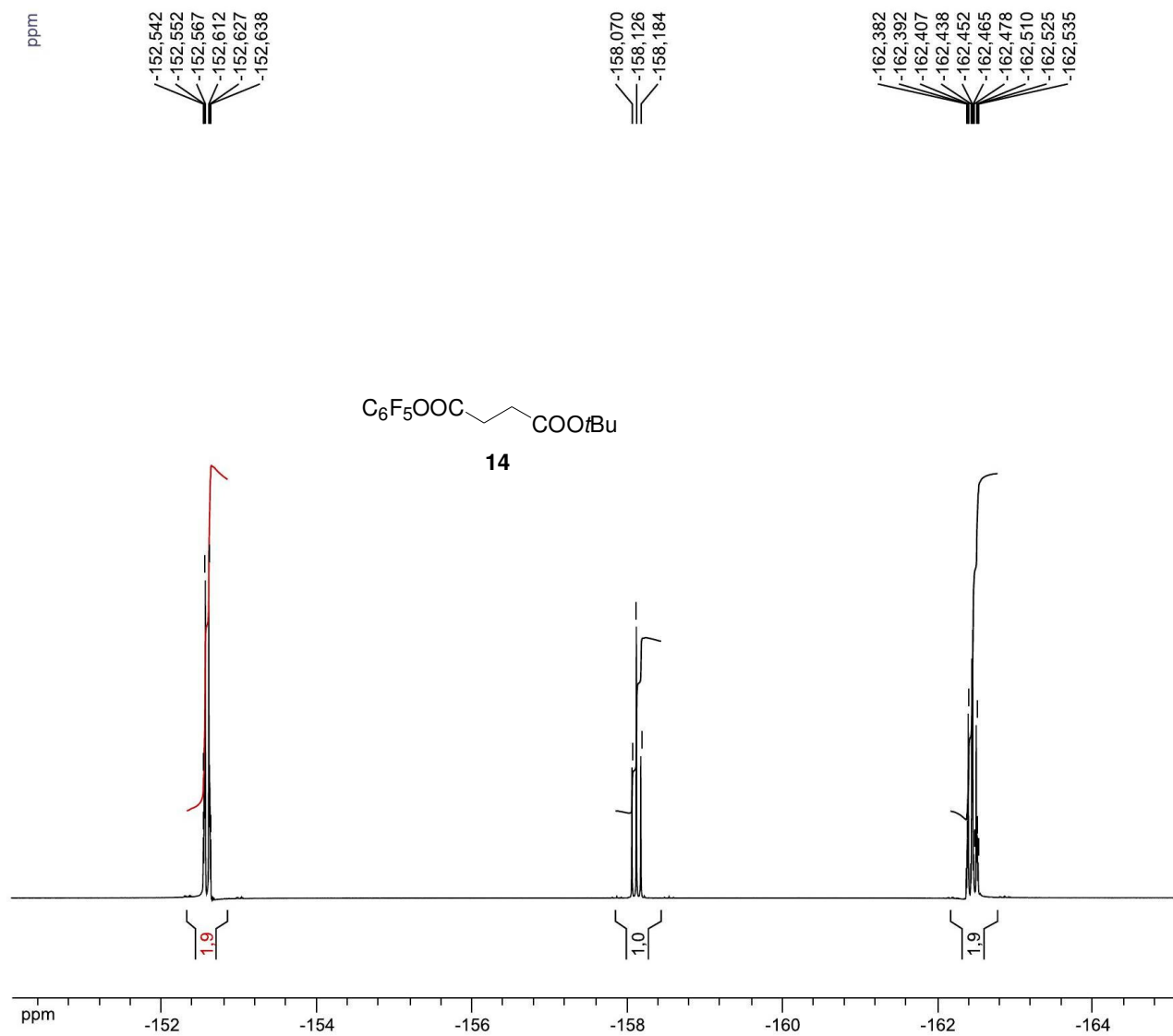
Parameters
compound 6 (1D 13C) (1D 13C)
F1 :
- Size : 65536 points complex
- Spectral Width : 24038.46 Hz
- Carrier Frequency : 100.62283 MHz
- Nucleus : 13C
Number of scans : 1024
Type : 1D
Spectro : BRUKER 400 MHz
Probe : 5 mm PABBO BB-1H/D Z-GRD Z10861
Date : Wed May 13 04:39:27 CEST 2009
Temperature : 300.1600036621094 K
Solvent : DMSO



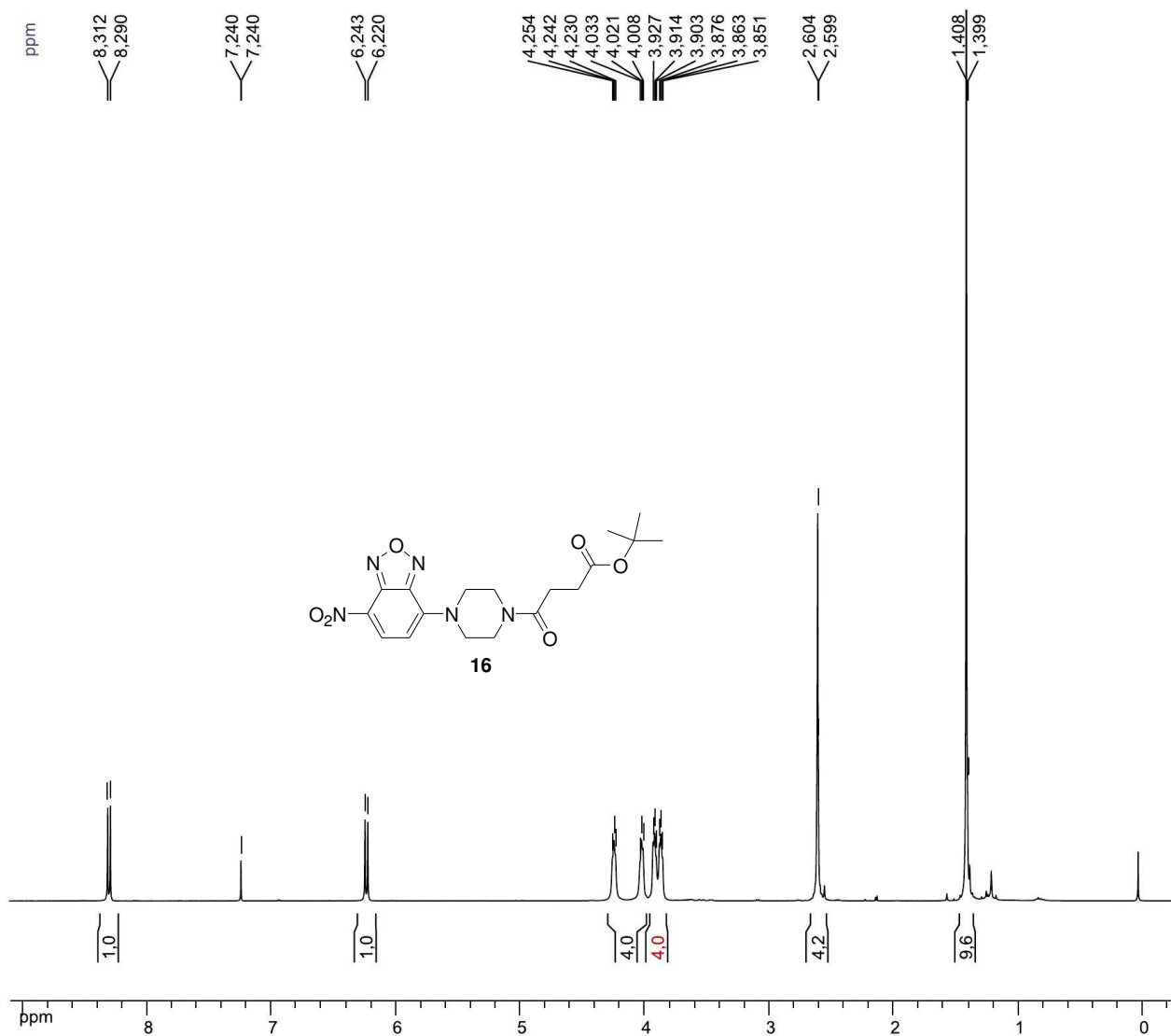
Parameters
compound 15 (1D 1H)
F1 :
- Size : 65536 points complex
- Spectral Width : 8223.685 Hz
- Carrier Frequency : 400.13248 MHz
- Nucleus : 1H
Number of scans : 16
Type : 1D
Spectro : BRUKER 400 MHz
Probe : 5 mm PABBO BB-1H/D Z-GRD Z10861
Date : Tue Apr 21 06:08:00 CEST 2009
Temperature : 298.9599914550781 K
Solvent : CDCl3



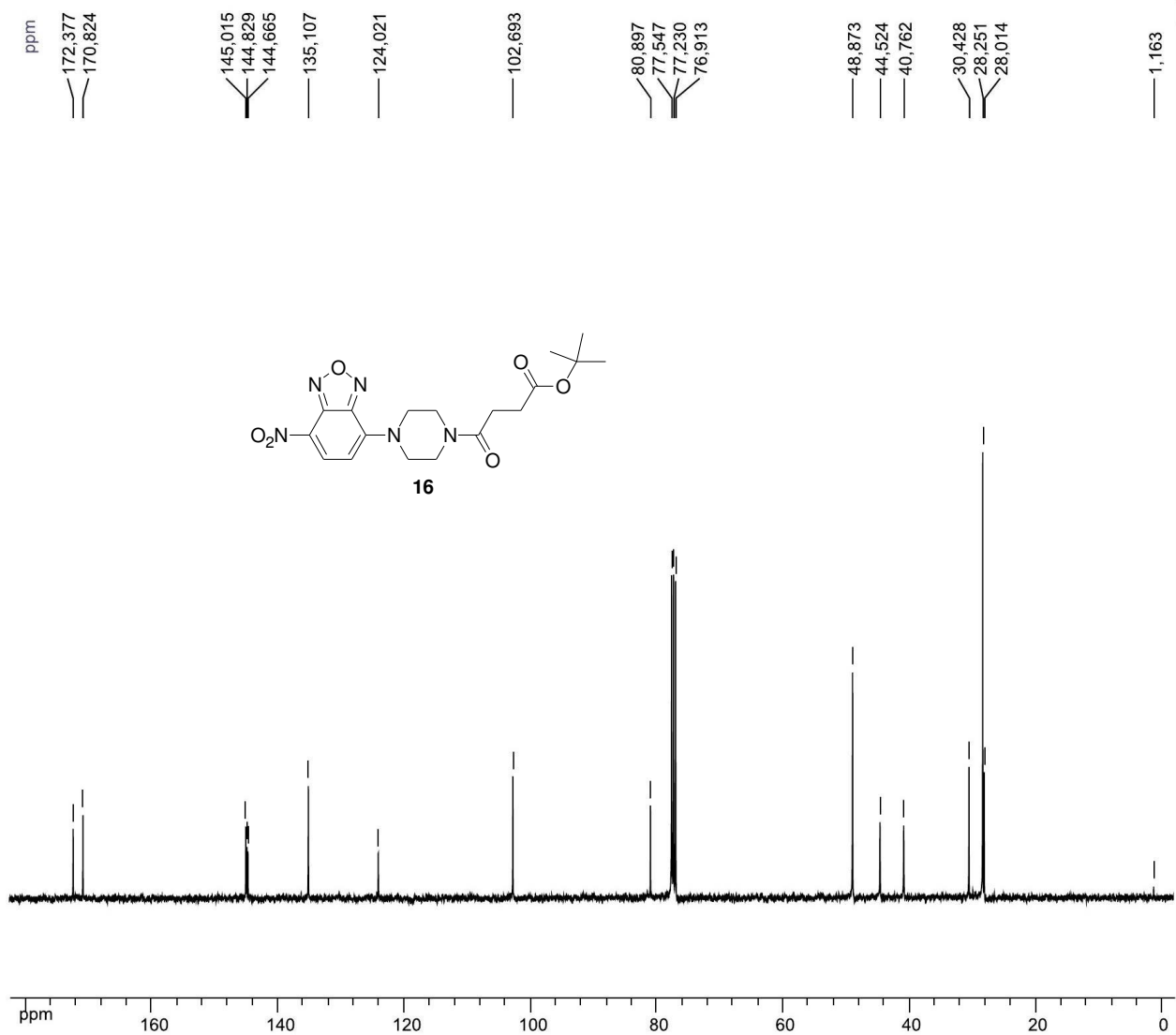
Parameters
compound 15 (1D 13C)
F1 :
- Size : 65536 points complex
- Spectral Width : 24038.46 Hz
- Carrier Frequency : 100.62283 MHz
- Nucleus : 13C
Number of scans : 128
Type : 1D
Spectro : BRUKER 400 MHz
Probe : 5 mm PABBO BB-1H/D Z-GRD Z10861
Date : Tue Apr 21 06:16:37 CEST 2009
Temperature : 299.96 K
Solvent : CDCl3



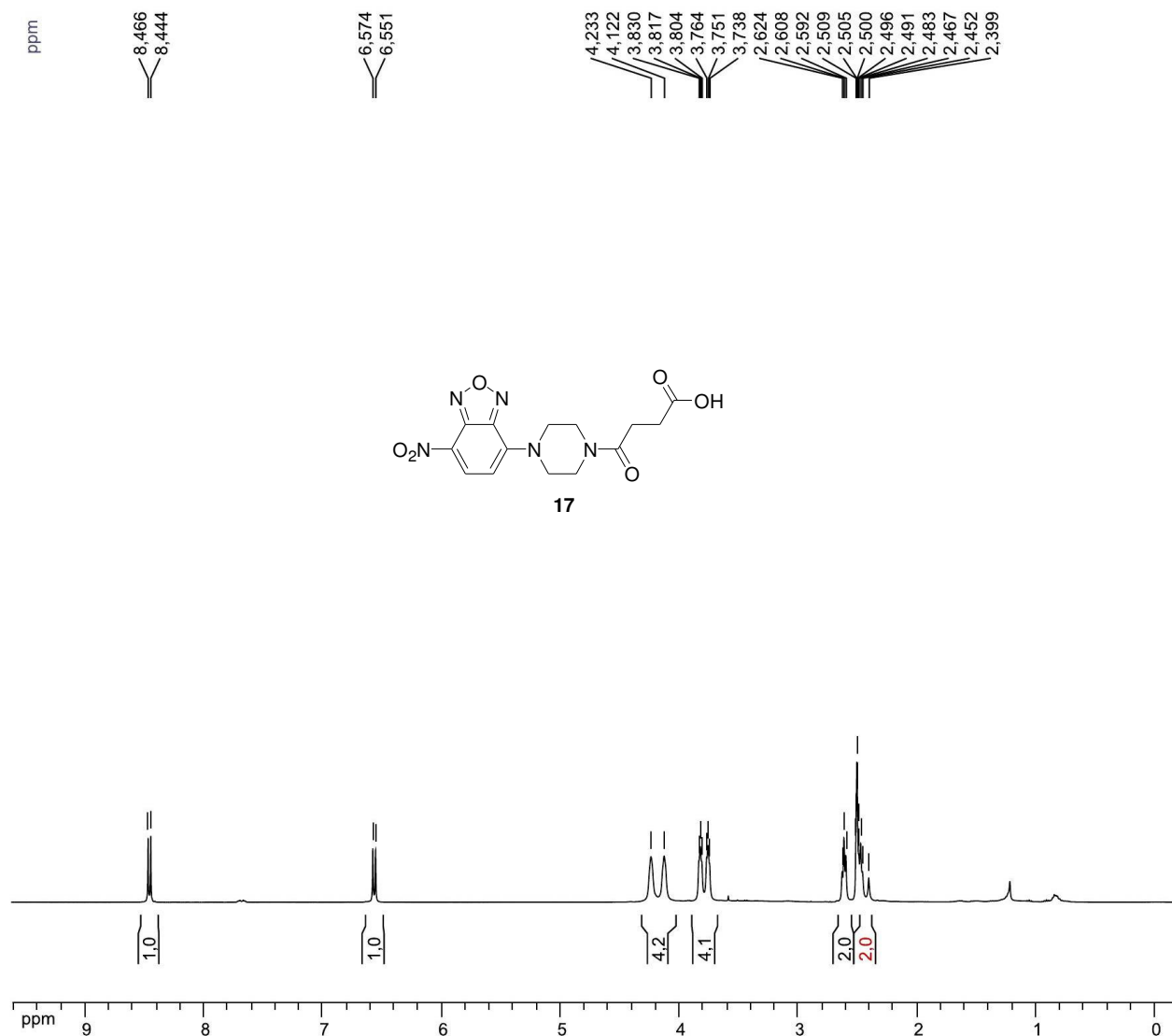
Parameters
compound 15 (1D 19F)
F1 :
- Size : 131072 points complex
- Spectral Width : 89285.71 Hz
- Carrier Frequency : 376.46072 MHz
- Nucleus : 19F
Number of scans : 16
Type : 1D
Spectro : BRUKER 400 MHz
Probe : 5 mm PABBO BB-1H/D Z-GRD Z10861
Date : Tue Apr 21 06:26:20 CEST 2009
Temperature : 299.36 K
Solvent : CDCl3



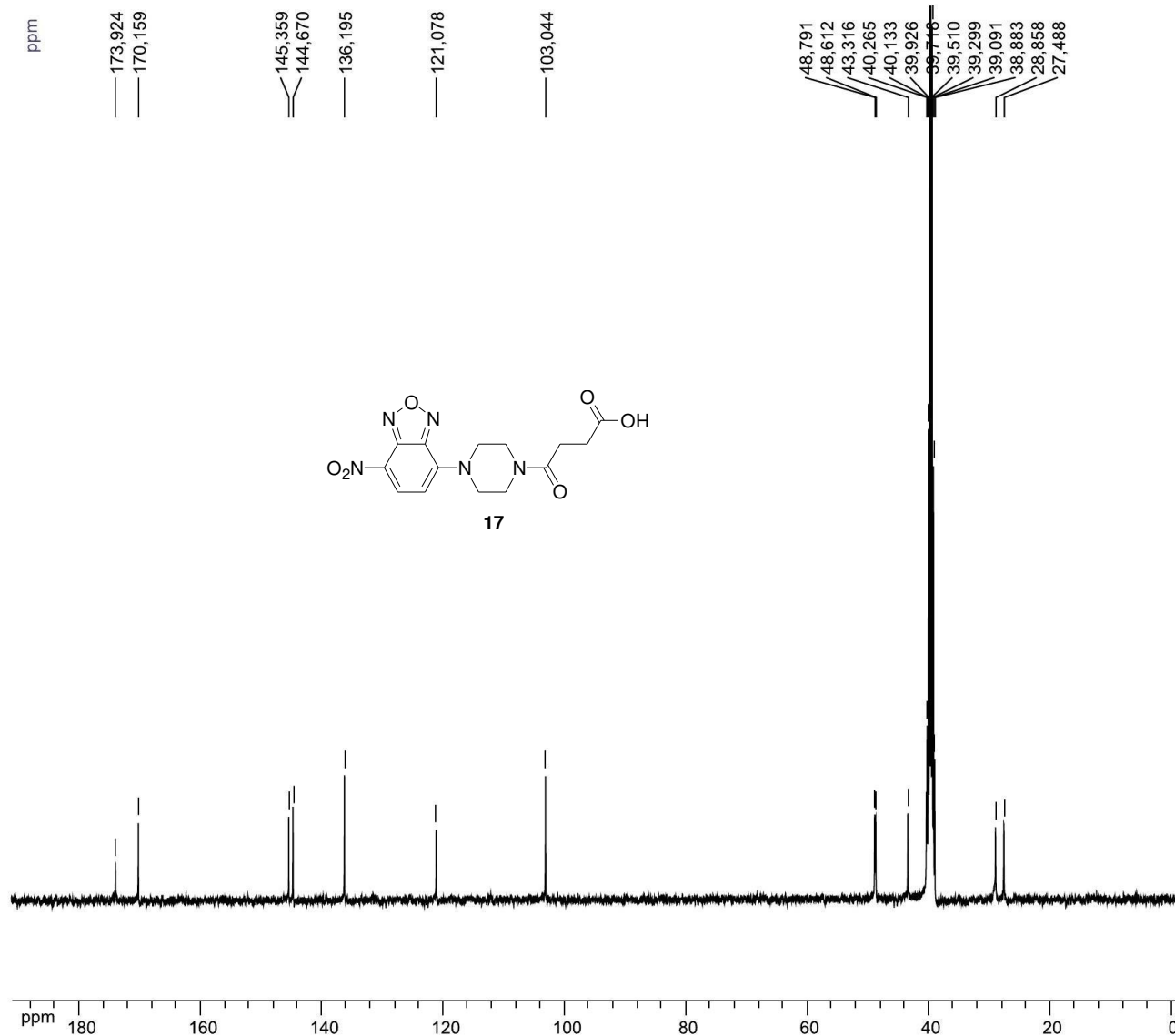
Parameters
compound 17 (1D 1H)
F1 :
- Size : 65536 points complex
- Spectral Width : 8223.685 Hz
- Carrier Frequency : 400.13248 MHz
- Nucleus : 1H
Number of scans : 16
Type : 1D
Spectro : BRUKER 400 MHz
Probe : 5 mm PABBO BB-1H/D Z-GRD Z10861
Date : Wed May 26 14:08:02 CEST 2010
Temperature : 298.16 K
Solvent : CDCl3



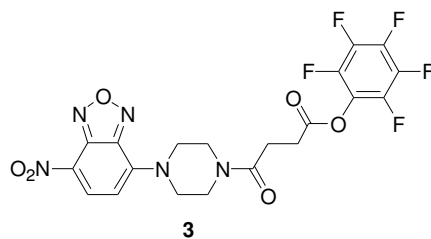
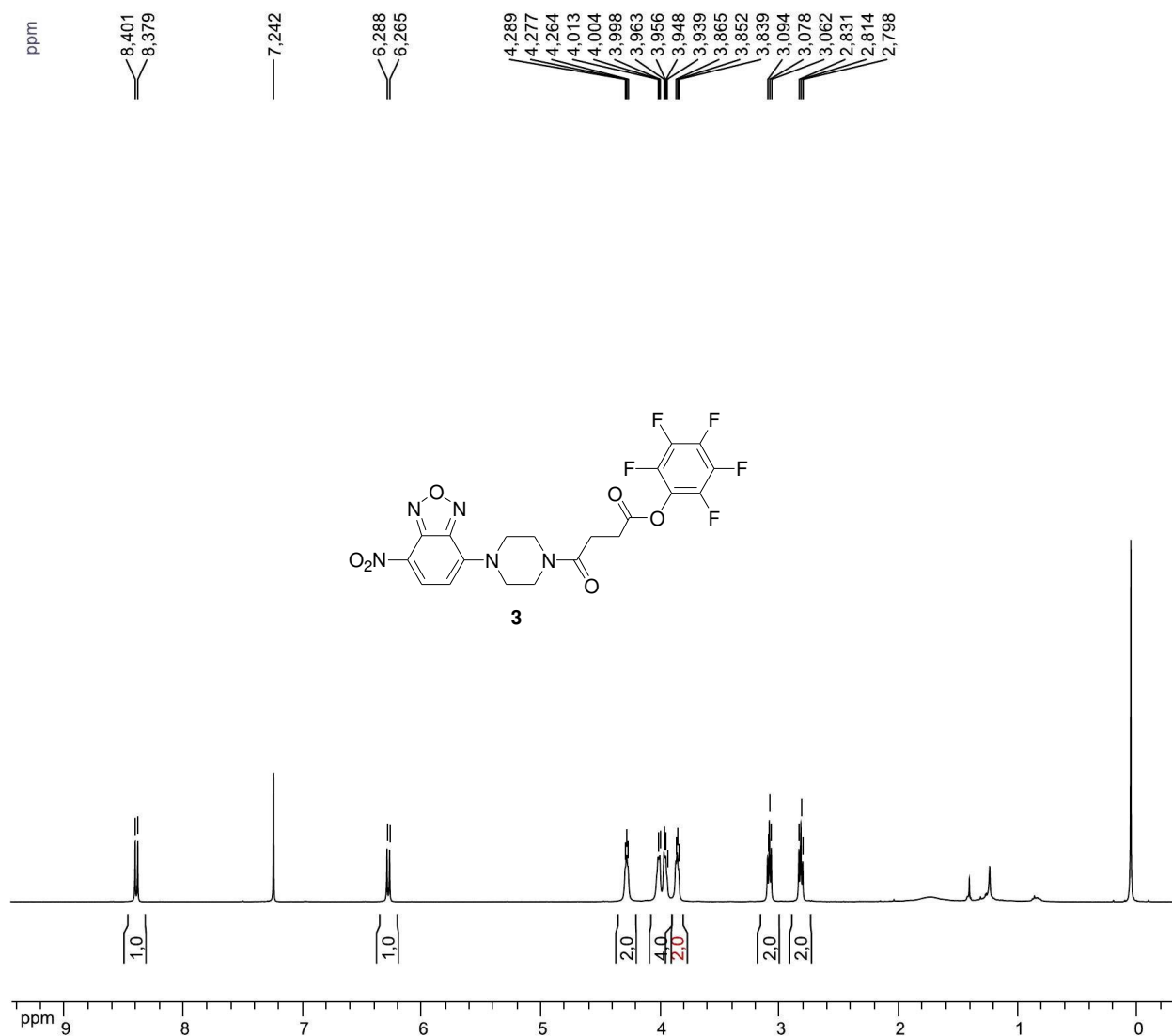
Parameters	
compound 17 (1D 13C)	
F1 :	
- Size :	65536 points complex
- Spectral Width :	24038.46 Hz
- Carrier Frequency :	100.62283 MHz
- Nucleus :	13C
Number of scans : 256	
Type : 1D	
Spectro : BRUKER 400 MHz	
Probe : 5 mm PABBO BB-1H/D Z-GRD Z10861	
Date : Wed May 26 14:24:00 CEST 2010	
Temperature : 299.06 K	
Solvent : CDCl3	



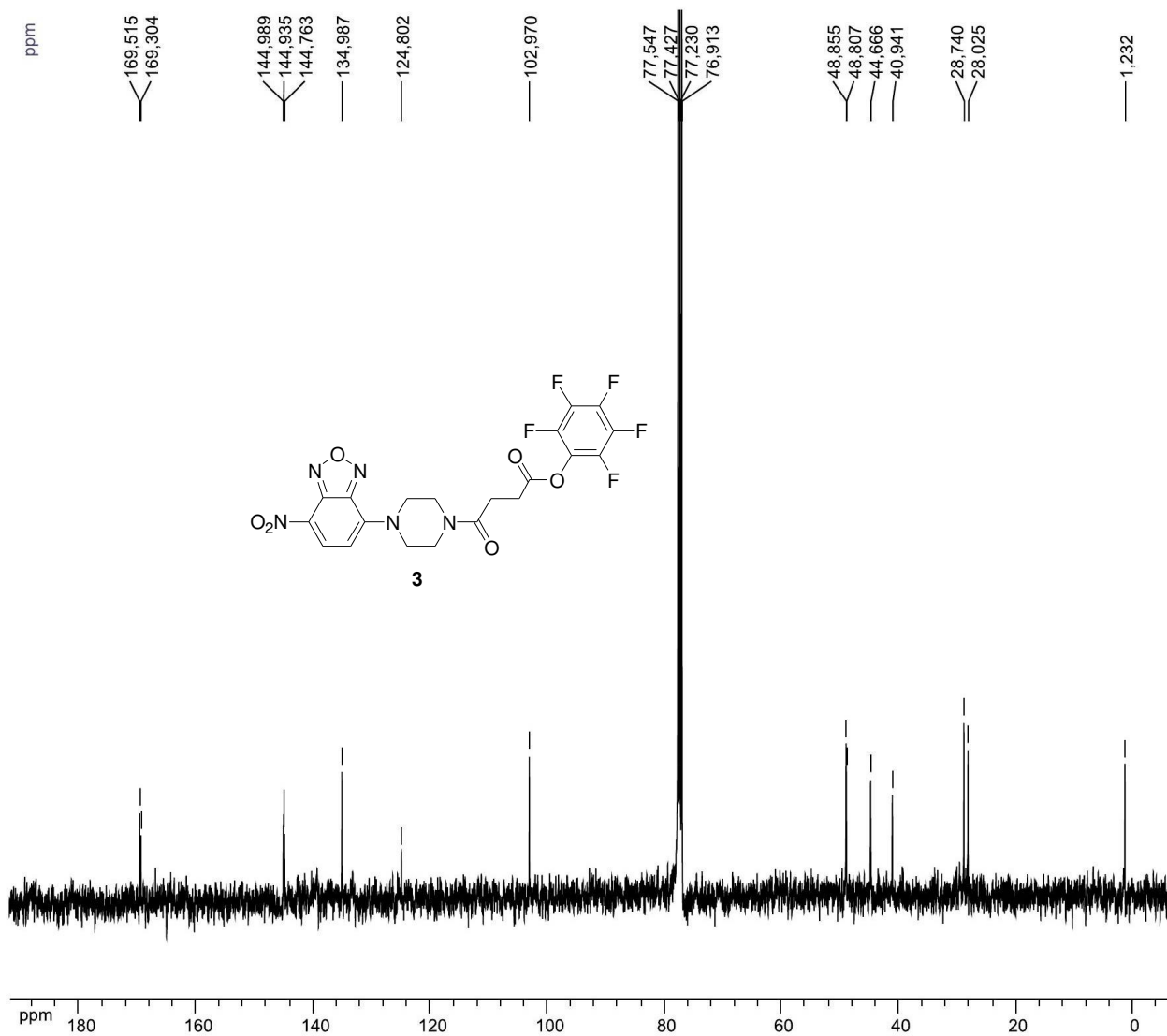
Parameters
compound 18 (1D 1H)
F1 :
- Size : 65536 points complex
- Spectral Width : 8223.685 Hz
- Carrier Frequency : 400.13248 MHz
- Nucleus : ¹ H
Number of scans : 16
Type : 1D
Spectro : BRUKER 400 MHz
Probe : 5 mm PABBO BB-1H/D Z-GRD Z10861
Date : Wed May 26 14:50:49 CEST 2010
Temperature : 298.16 K
Solvent : DMSO



Parameters
compound 18 (1D 13C)
F1 :
- Size : 65536 points complex
- Spectral Width : 24038.46 Hz
- Carrier Frequency : 100.62283 MHz
- Nucleus : 13C
Number of scans : 256
Type : 1D
Spectro : BRUKER 400 MHz
Probe : 5 mm PABBO BB-1H/D Z-GRD Z10861
Date : Wed May 26 15:06:44 CEST 2010
Temperature : 299.16 K
Solvent : DMSO

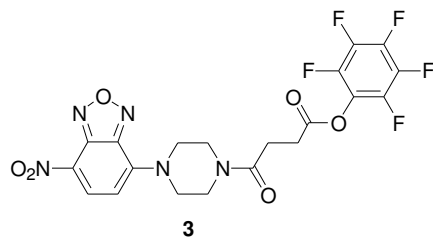
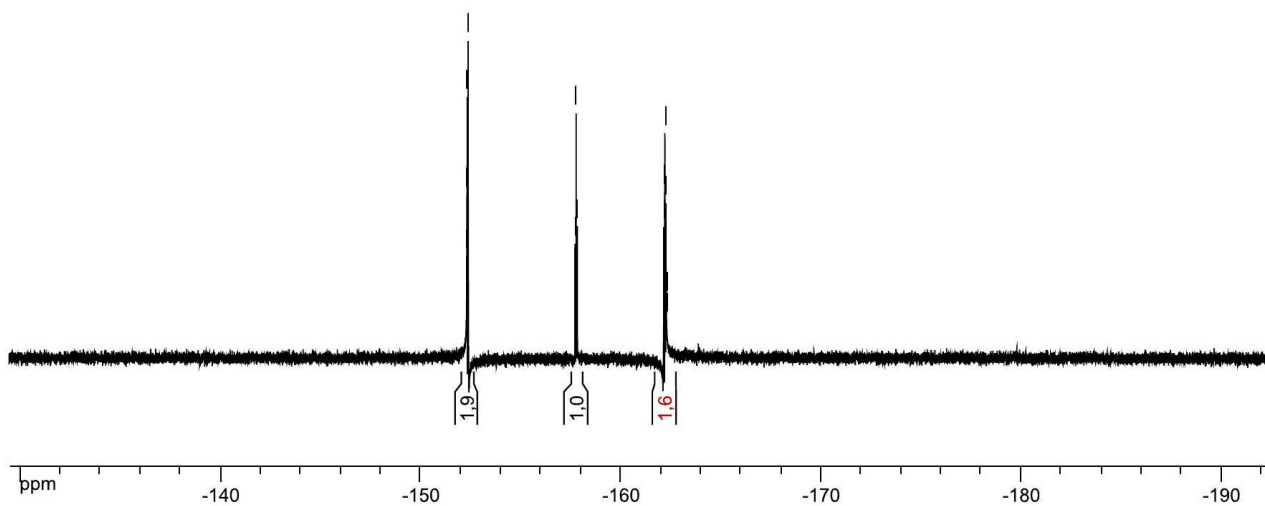


Parameters
compound 4 (1D 1H)
F1 :
- Size : 65536 points complex
- Spectral Width : 8223.685 Hz
- Carrier Frequency : 400.13248 MHz
- Nucleus : 1H
Number of scans : 16
Type : 1D
Spectro : BRUKER 400 MHz
Probe : 5 mm PABBO BB-1H/D Z-GRD Z10861
Date : Wed May 26 16:36:24 CEST 2010
Temperature : 297.76 K
Solvent : CDCl3



Parameters	
compound 4 (1D 13C)	
F1 :	
- Size :	65536 points complex
- Spectral Width :	24038.46 Hz
- Carrier Frequency :	100.62283 MHz
- Nucleus :	13C
Number of scans : 256	
Type : 1D	
Spectro : BRUKER 400 MHz	
Probe : 5 mm PABBO BB-1H/D Z-GRD Z10861	
Date : Wed May 26 16:52:24 CEST 2010	
Temperature : 298.76 K	
Solvent : CDCl3	

ppm



Parameters

compound 4 (1D 19F)

F1 :

- Size : 131072 points complex
- Spectral Width : 89285.71 Hz
- Carrier Frequency : 376.46072 MHz
- Nucleus : 19F

Number of scans : 16

Type : 1D

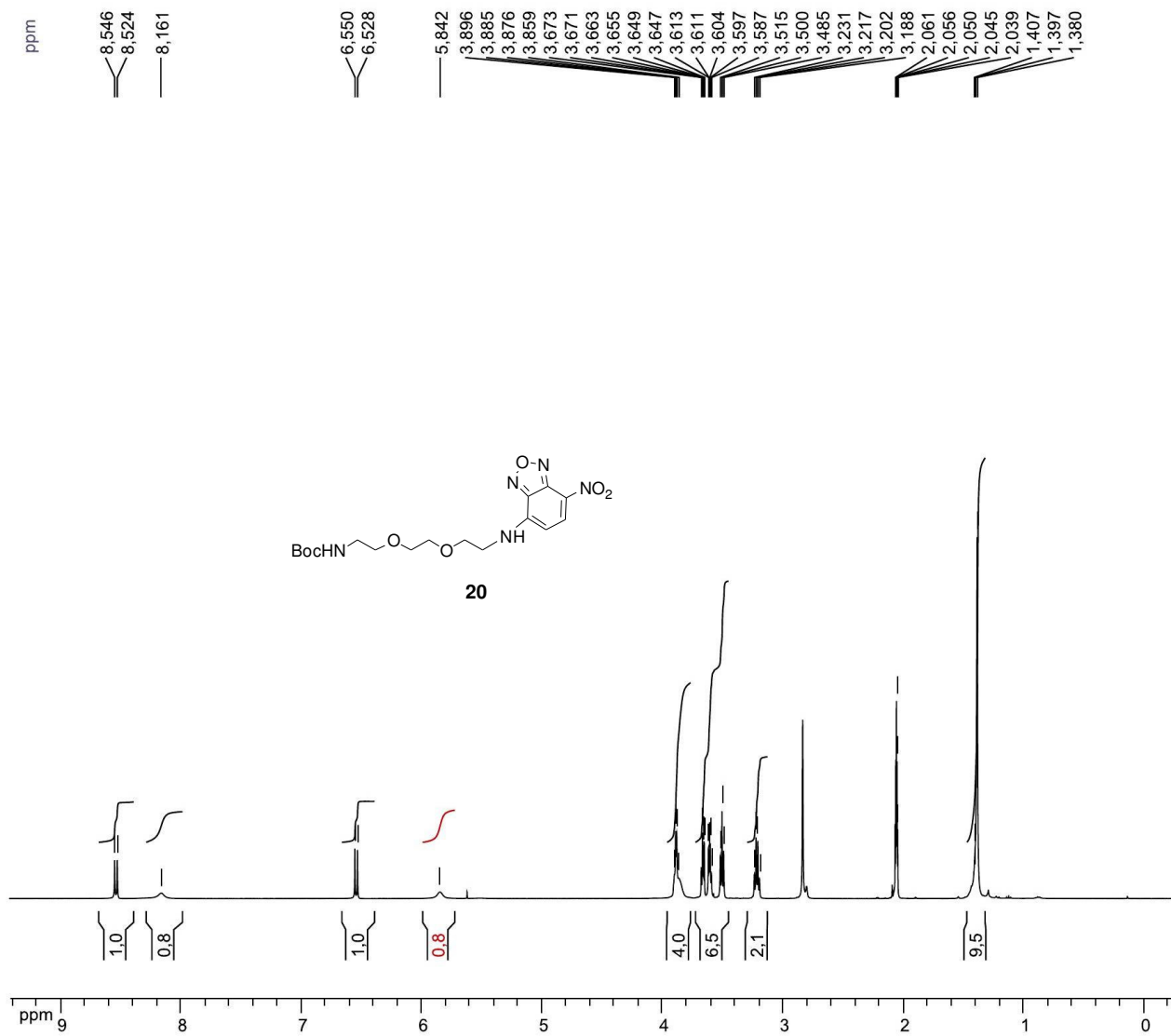
Spectro : BRUKER 400 MHz

Probe : 5 mm PABBO BB-1H/D Z-GRD Z10861

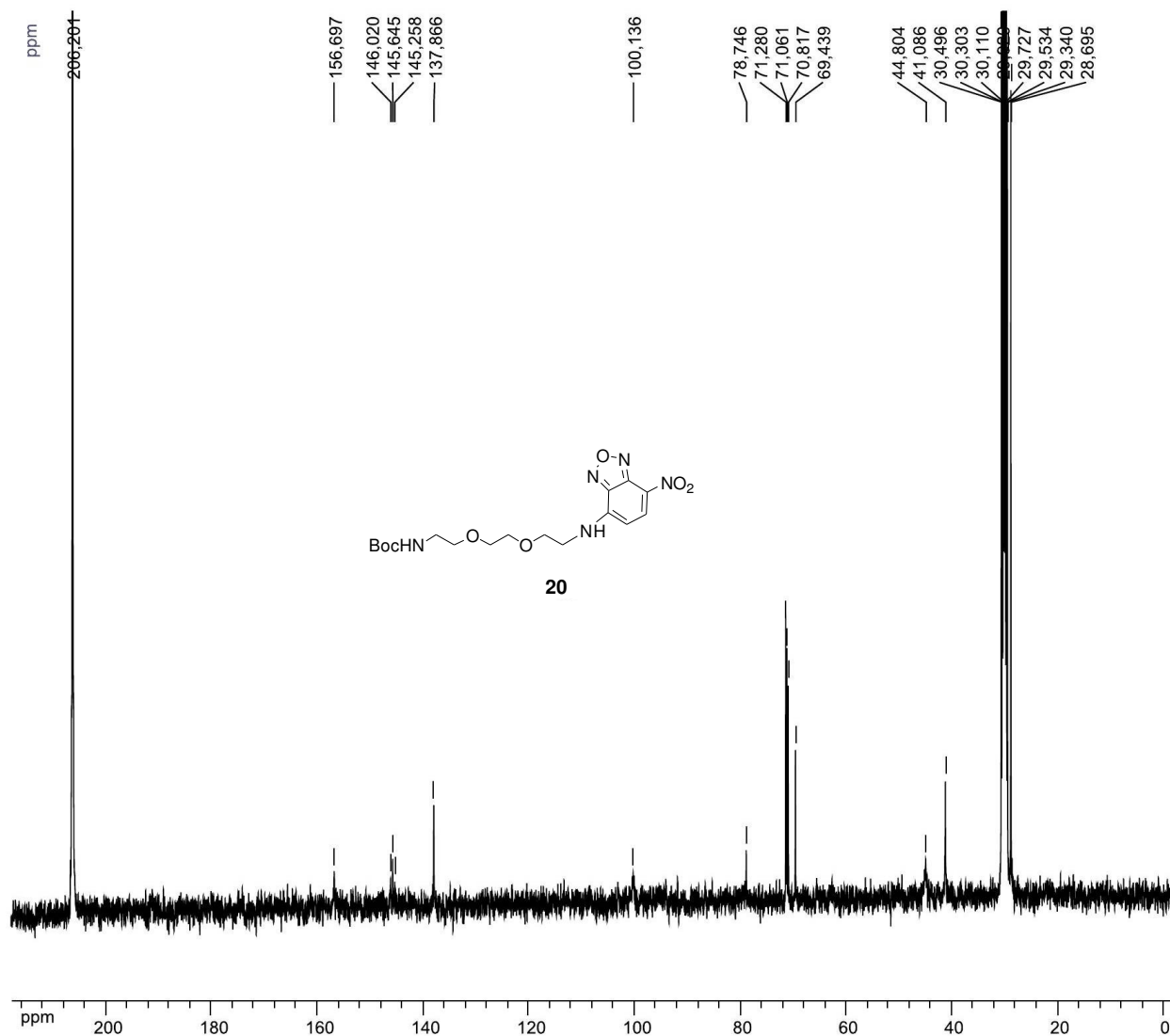
Date : Wed May 26 17:01:36 CEST 2010

Temperature : 298.26 K

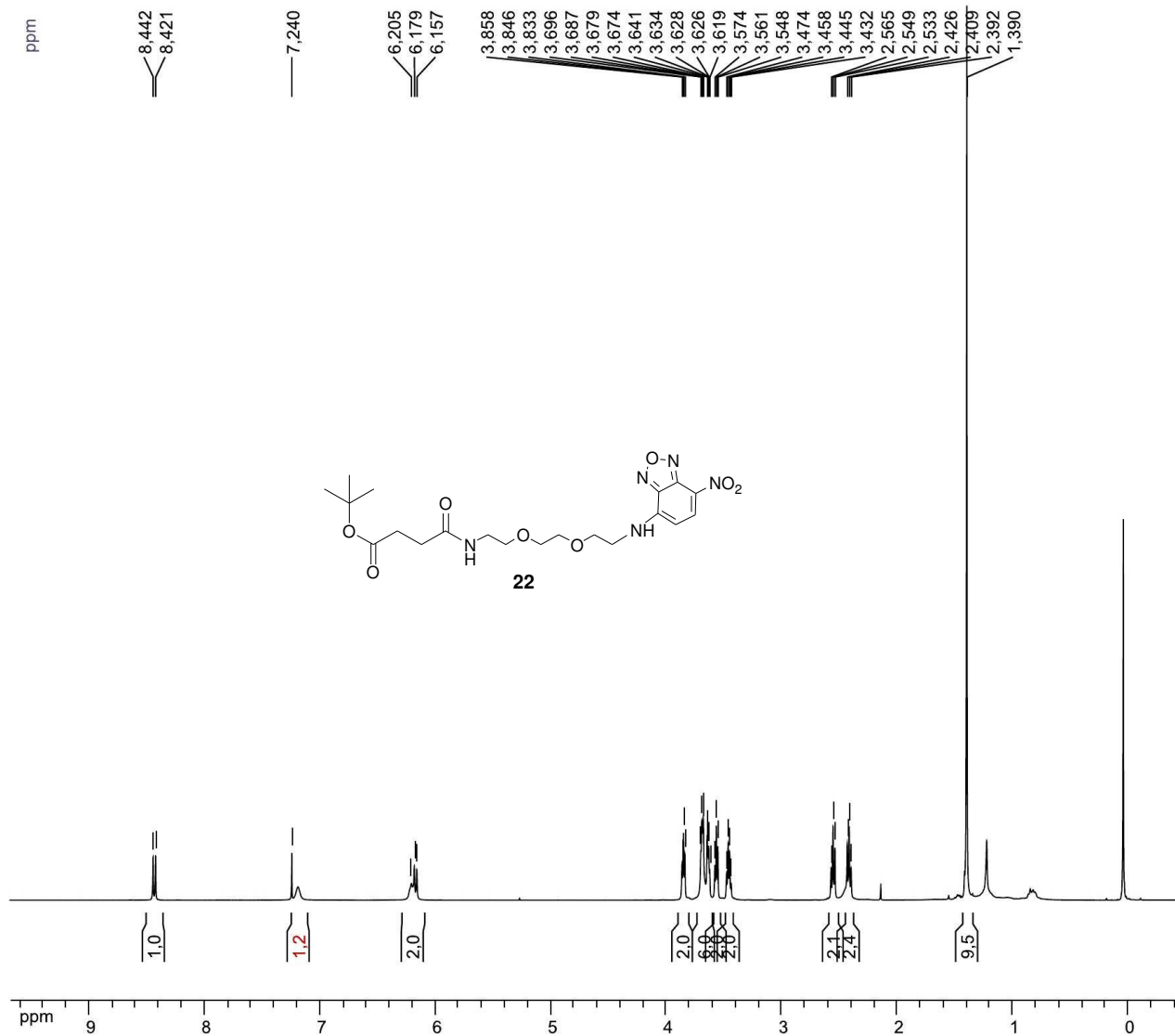
Solvent : CDCl3



Parameters
compound 21 (1D 1H)
F1 :
- Size : 65536 points complex
- Spectral Width : 8223.685 Hz
- Carrier Frequency : 400.13248 MHz
- Nucleus : 1H
Number of scans : 16
Type : 1D
Spectro : BRUKER 400 MHz
Probe : 5 mm PABBO BB-1H/D Z-GRD Z10861
Date : Sun May 30 01:23:44 CEST 2010
Temperature : 298.16 K
Solvent : Acetone



Parameters
compound 21 (1D 13C)
F1 :
- Size : 65536 points complex
- Spectral Width : 24038.46 Hz
- Carrier Frequency : 100.62283 MHz
- Nucleus : 13C
Number of scans : 512
Type : 1D
Spectro : BRUKER 400 MHz
Probe : 5 mm PABBO BB-1H/D Z-GRD Z10861
Date : Sun May 30 01:07:27 CEST 2010
Temperature : 298.96 K
Solvent : Acetone



Parameters

compound 23 (1D 1H)

F1 :

- Size : 65536 points complex
- Spectral Width : 8223.685 Hz
- Carrier Frequency : 400.13248 MHz
- Nucleus : 1H

Number of scans : 16

Type : 1D

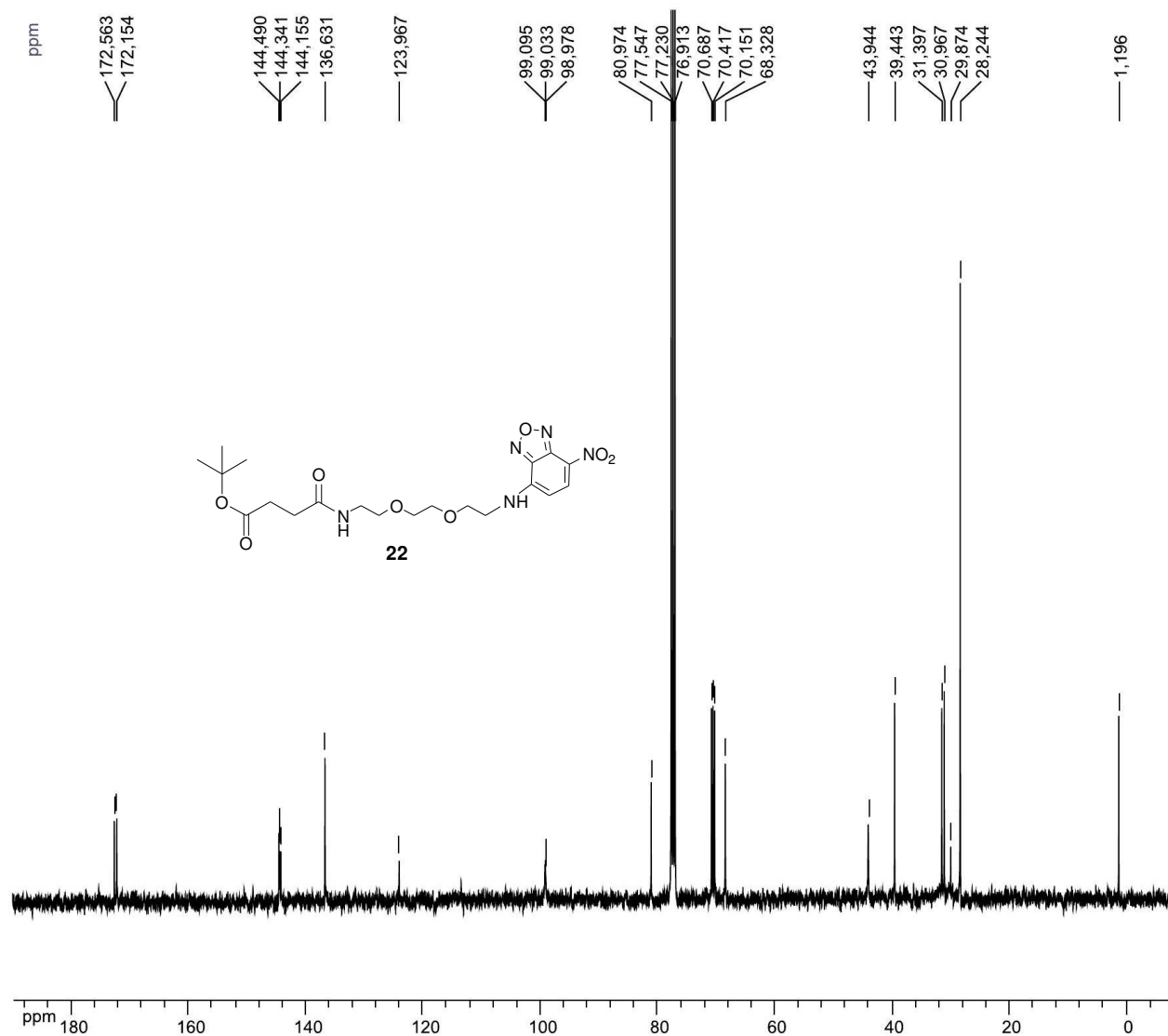
Spectro : BRUKER 400 MHz

Probe : 5 mm PABBO BB-1H/D Z-GRD Z10861

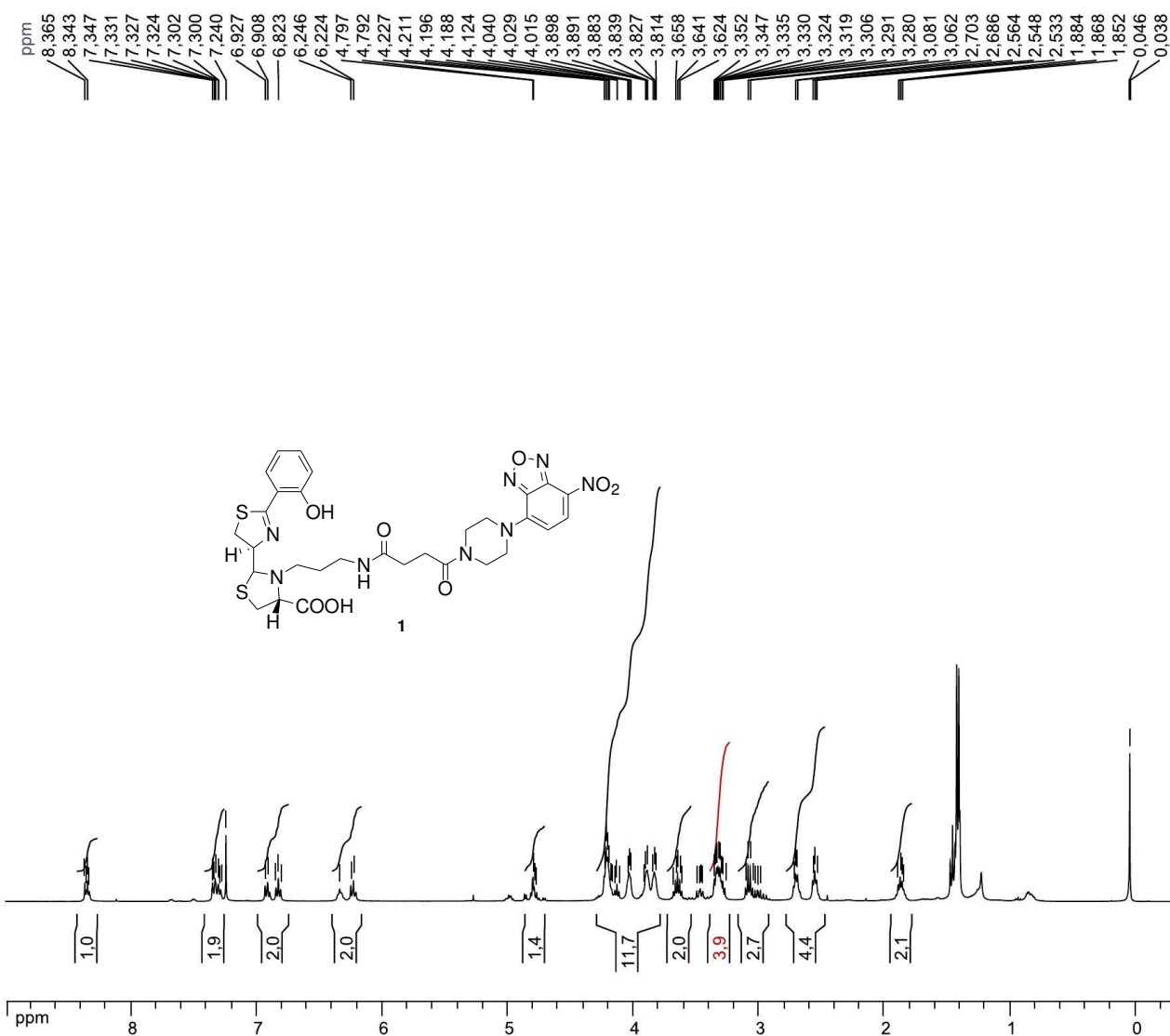
Date : Wed May 26 17:22:27 CEST 2010

Temperature : 297.66 K

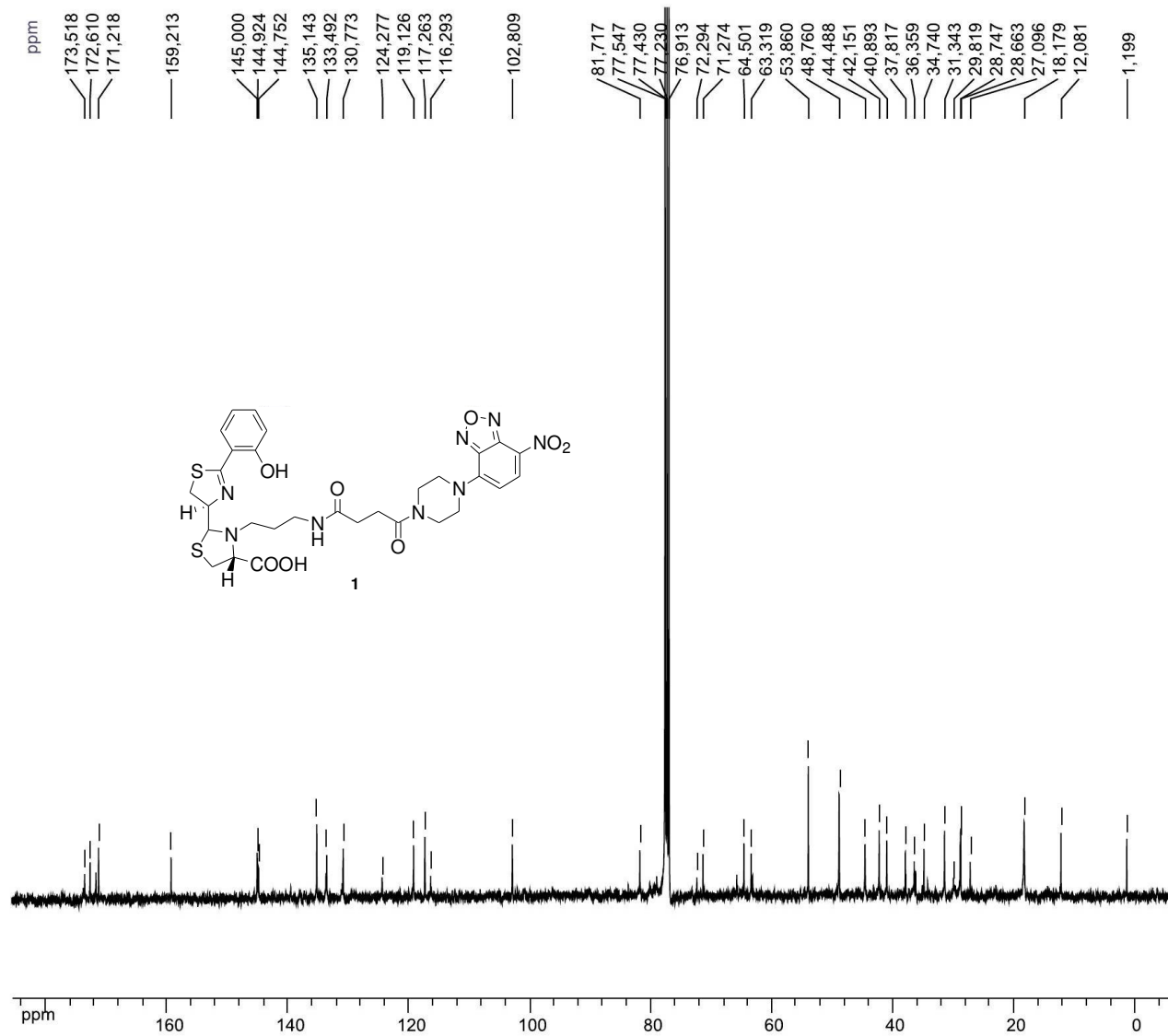
Solvent : CDCl3



Parameters	
compound 23 (1D 13C)	
F1 :	
- Size :	65536 points complex
- Spectral Width :	24038.46 Hz
- Carrier Frequency :	100.62283 MHz
- Nucleus :	13C
Number of scans : 256	
Type : 1D	
Spectro : BRUKER 400 MHz	
Probe : 5 mm PABBO BB-1H/D Z-GRD Z10861	
Date : Wed May 26 17:38:19 CEST 2010	
Temperature : 298.66 K	
Solvent : CDCl3	

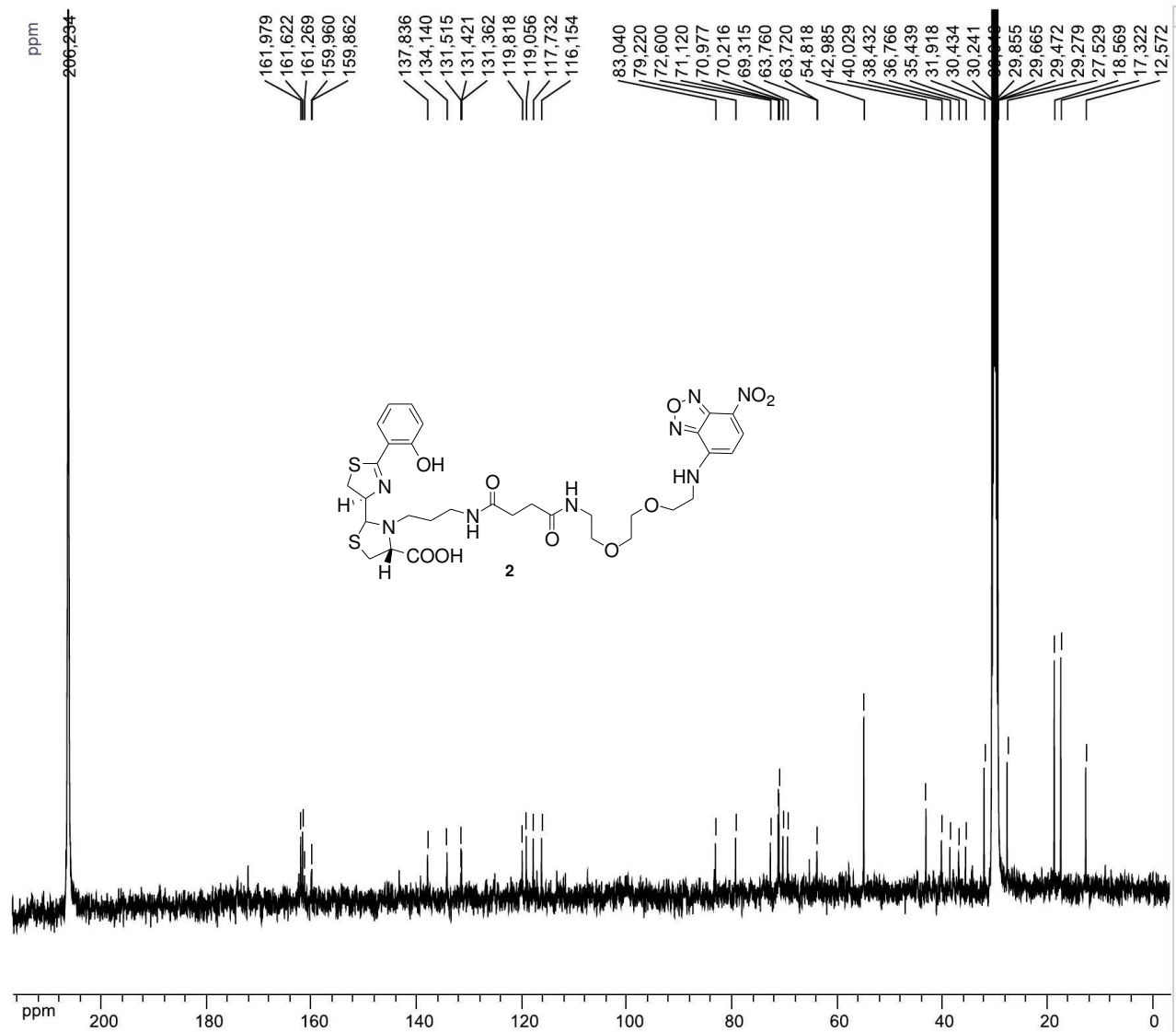


Parameters
compound1 (1D 1H)
F1 :
- Size : 65536 points complex
- Spectral Width : 8223.685 Hz
- Carrier Frequency : 400.13248 MHz
- Nucleus : 1H
Number of scans : 16
Type : 1D
Spectro : BRUKER 400 MHz
Probe : 5 mm PABBO BB-1H/D Z-GRD Z10861
Date : Sat May 01 10:57:20 CEST 2010
Temperature : 297.96 K
Solvent : CDCl3



Parameters
compound 1 (1D 13C)
F1 :
- Size : 65536 points complex
- Spectral Width : 24038.46 Hz
- Carrier Frequency : 100.62283 MHz
- Nucleus : 13C
Number of scans : 1024
Type : 1D
Spectro : BRUKER 400 MHz
Probe : 5 mm PABBO BB-1H/D Z-GRD Z10861
Date : Sat May 01 10:40:52 CEST 2010
Temperature : 298.66 K
Solvent : CDCl3

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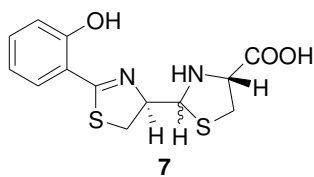
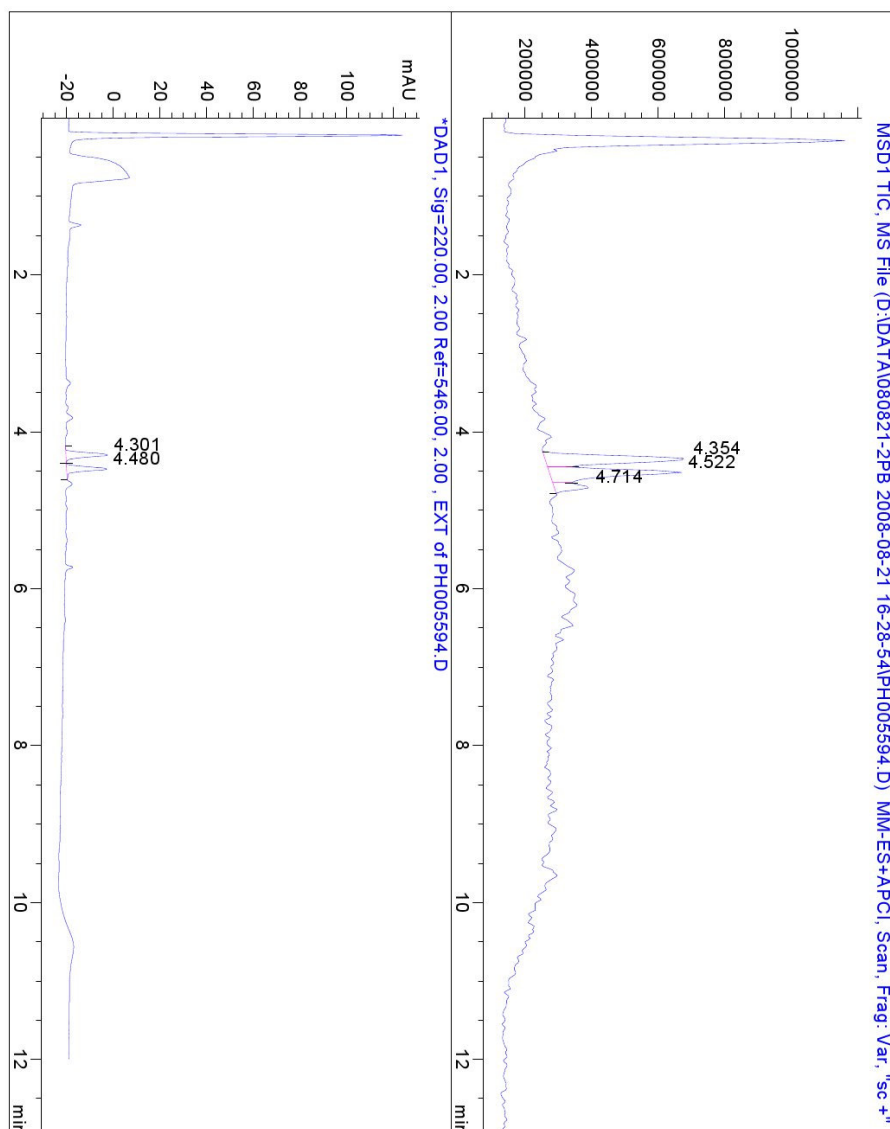


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- Spectral Width : 24038.46 Hz
- Carrier Frequency : 100.62283 MHz
- Nucleus : 13C
Number of scans : 1024
Type : 1D
Spectro : BRUKER 400 MHz
Probe : 5 mm PABBO BB-1H/D Z-GRD Z10861
Date : Sat May 01 05:42:32 CEST 2010
Temperature : 298.56 K
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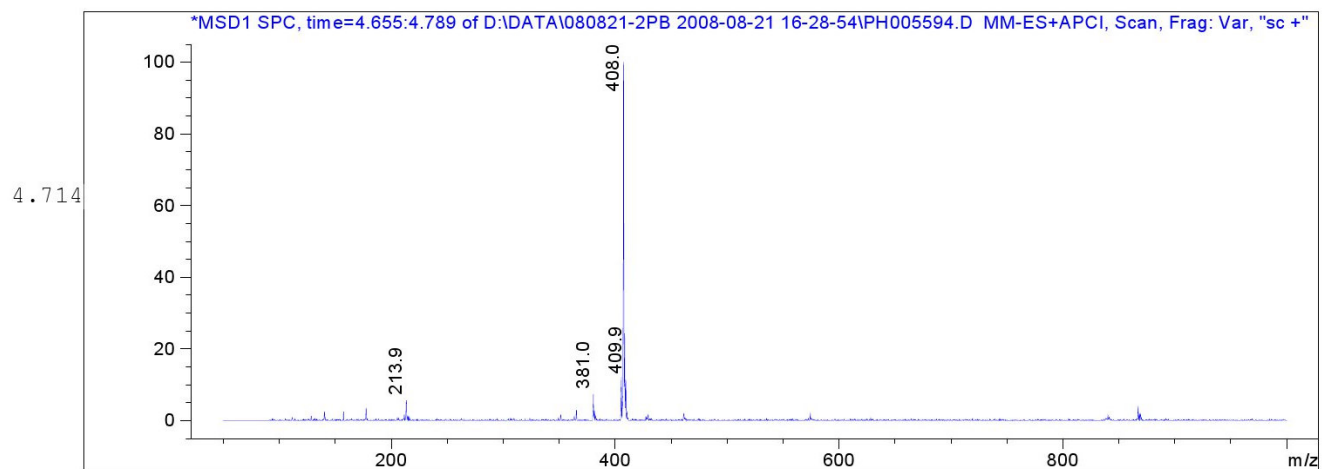
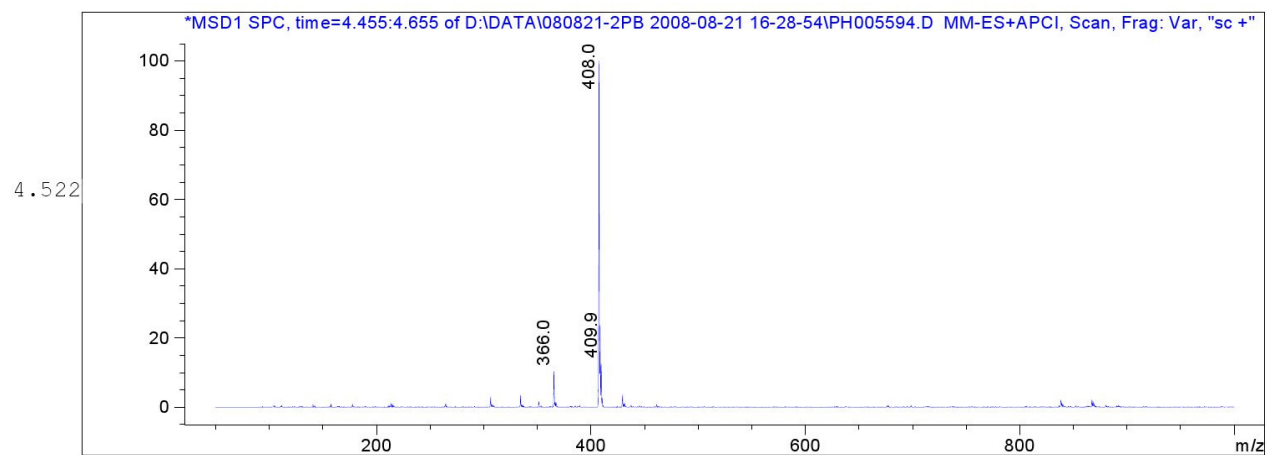
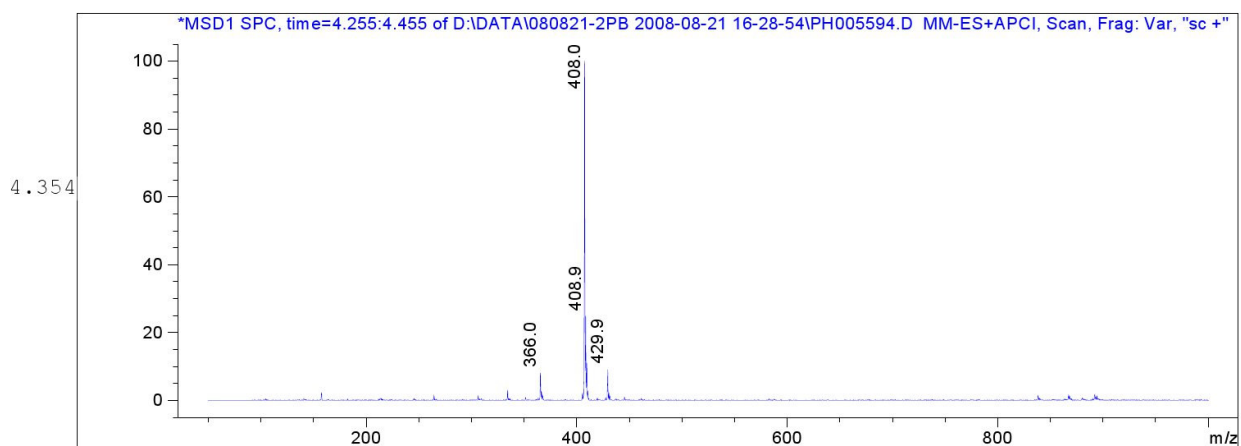
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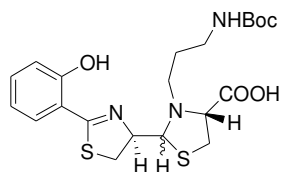
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D. Mass Spectra

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4.480	17.138	63.089	48
4.522	3.985e5	2.377e6	43
4.714	1.053e5	5.244e5	9





6

#	RT [min]	Area
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2	1.5	5169302960
n.a.	1.5	n.a.

Compound Mass Spectrum Report

Analysis Info

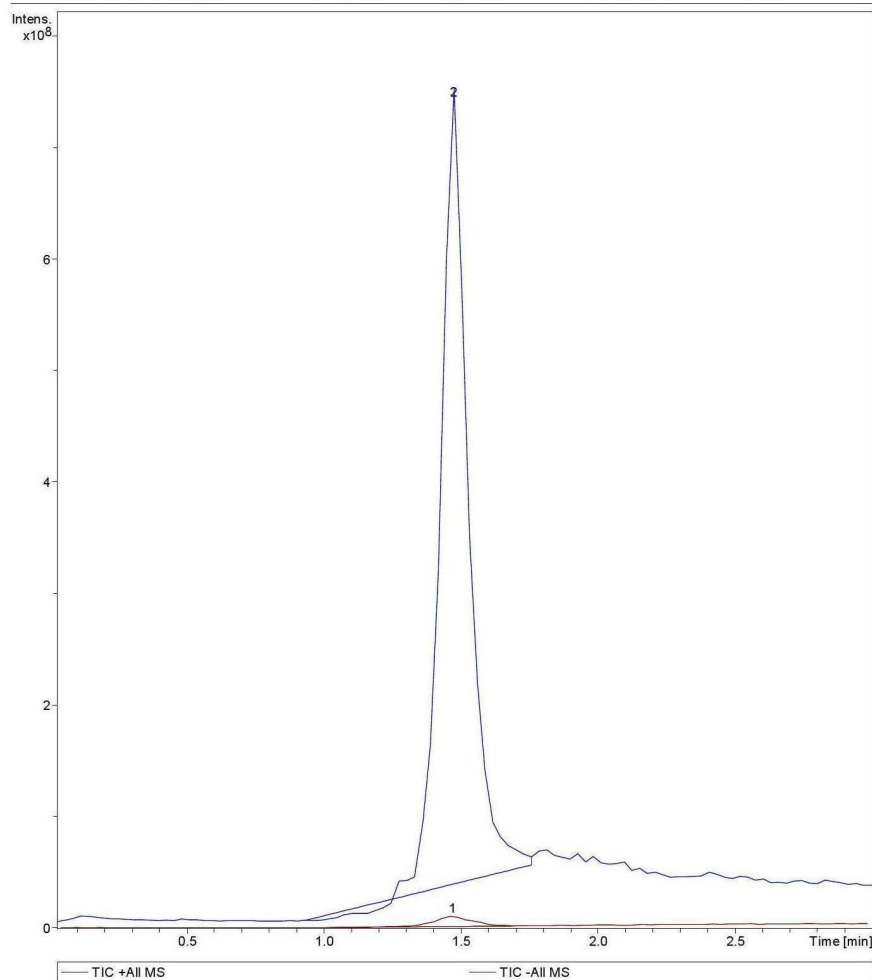
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 Sample Name SN237
 Comment

Acquisition Date 4/20/2009 11:16:13 AM

Operator PB
 Instrument HCTultra

Acquisition Parameter

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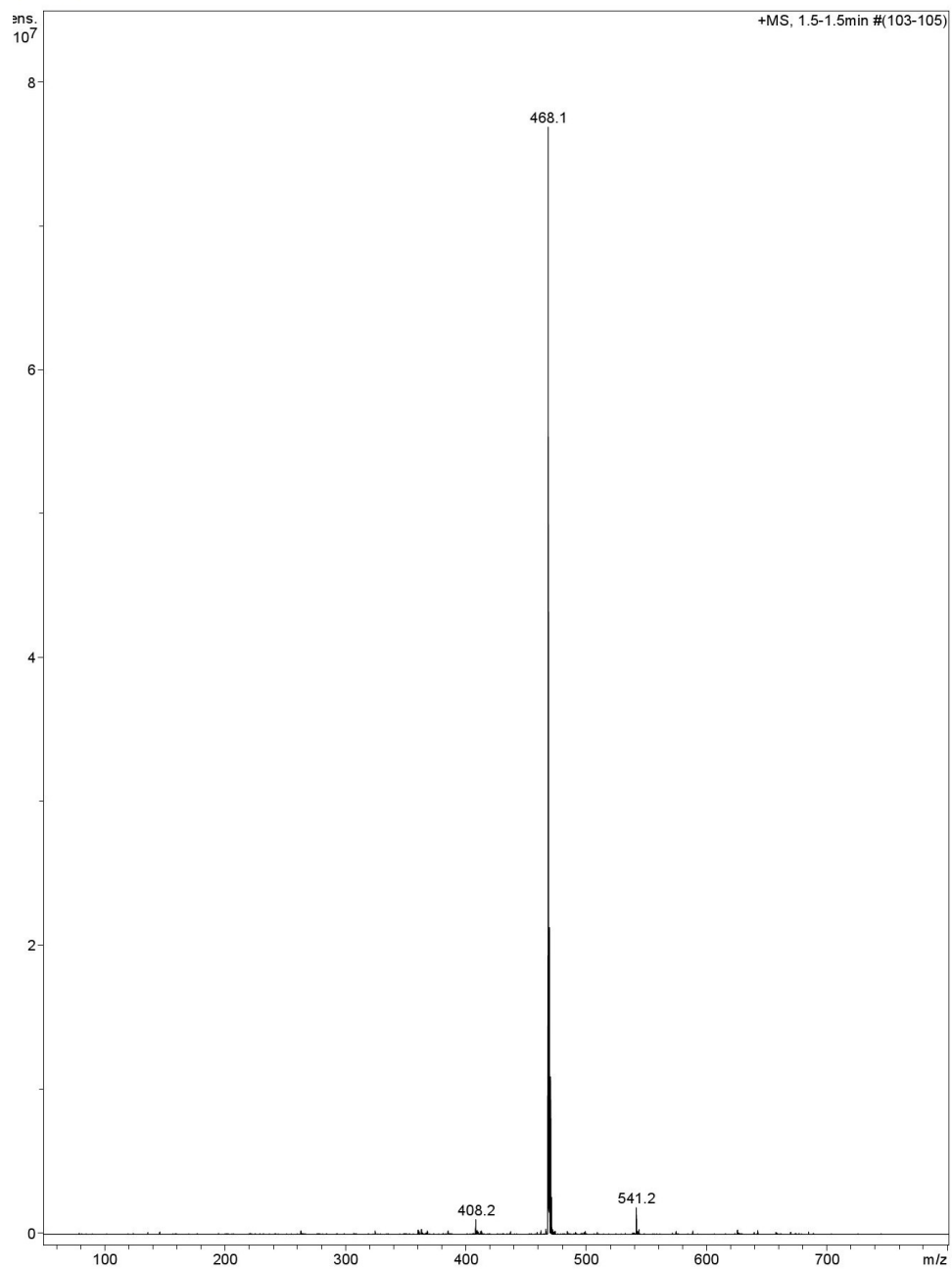


Compound Mass Spectrum Report

MS, 1.5-1.5min
103-105)

Analysis Nam HCT004991.D

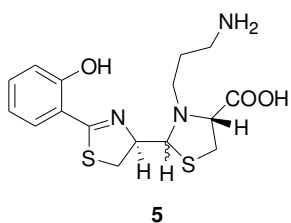
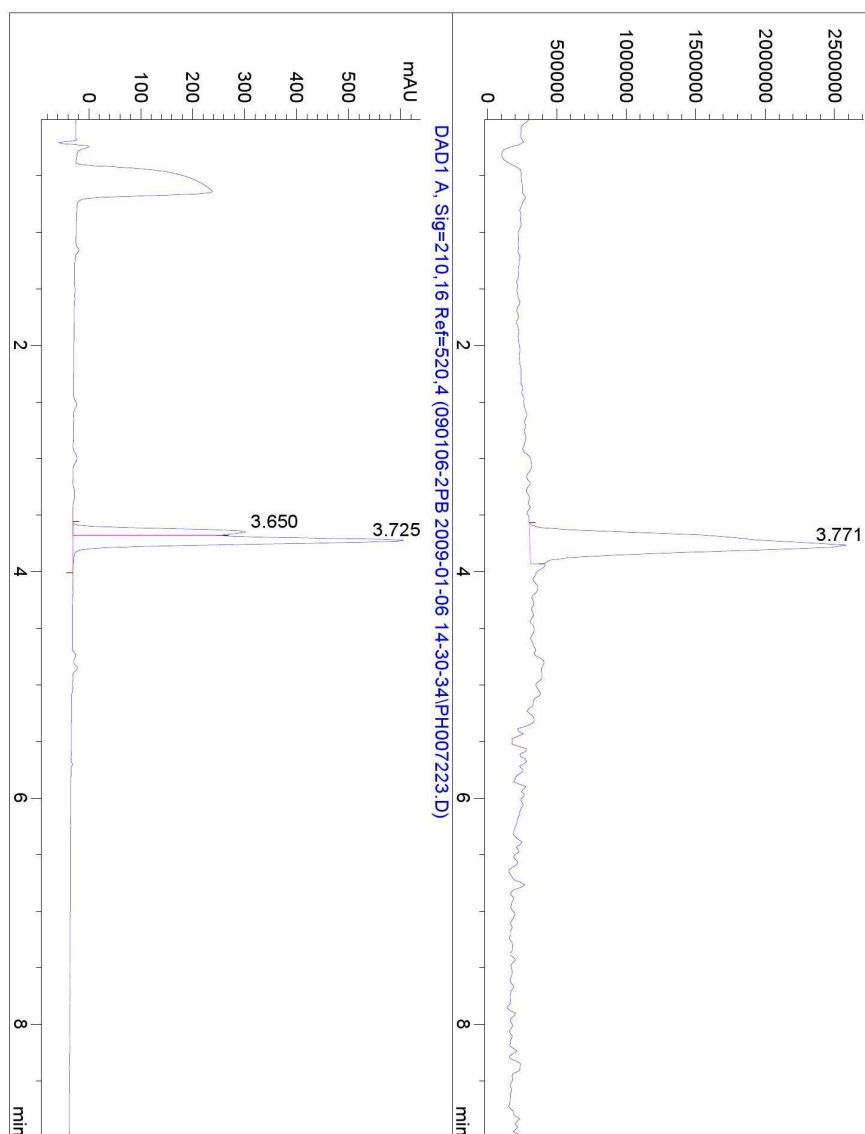
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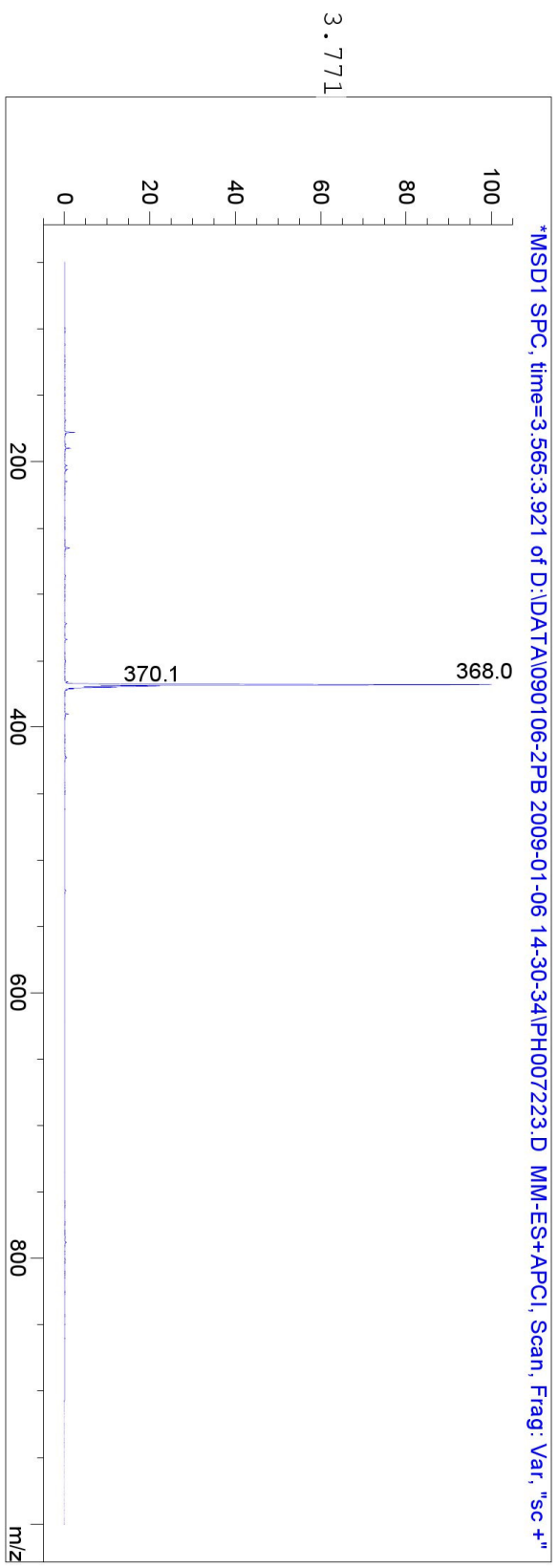
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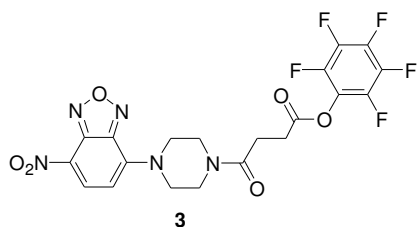
\\DATA\\090106-2PB 2009-01-06 14-30-34\\HYPERSTIGOLD98.M

MSD1 TIC, MS File (D:\\DATA\\090106-2PB 2009-01-06 14-30-34\\PH007223.D) MM-ES+APCI, Scan, Frag: Var, "Sc +"



Meas. R	Height	Area	Area %
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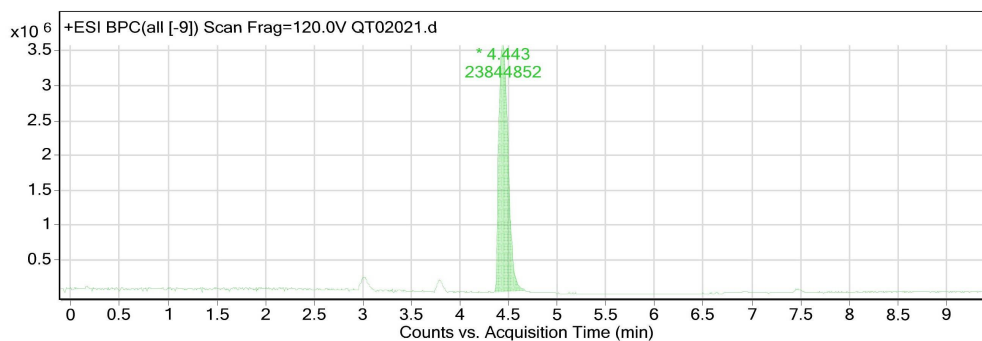


Qualitative Analysis Report

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Sample Type Sample **Position** P1-C4
Instrument Name SCA Pharma Stbg QToF **User Name** PW
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Comment
Formula

User Chromatograms

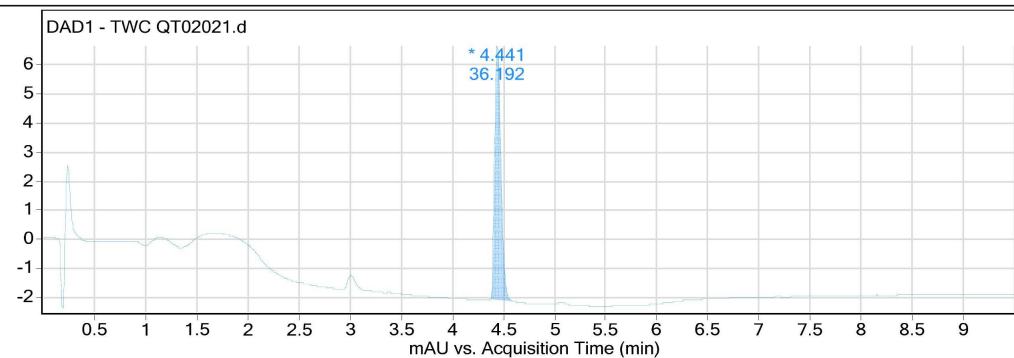
Fragmentor Voltage 120 **Collision Energy** 0 **Ionization Mode** ESI



Integration Peak List

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Qualitative Analysis Report

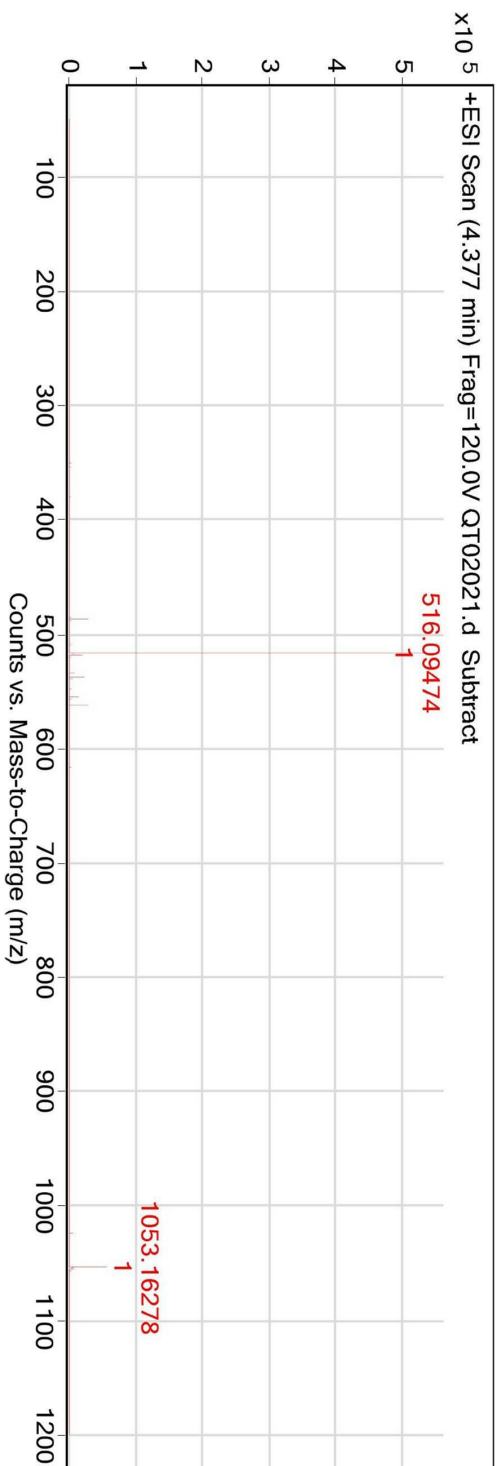


Integration Peak List

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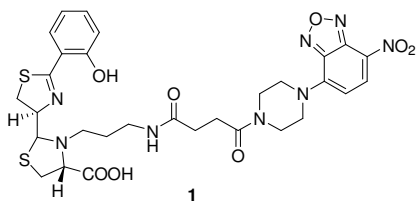
User Spectra

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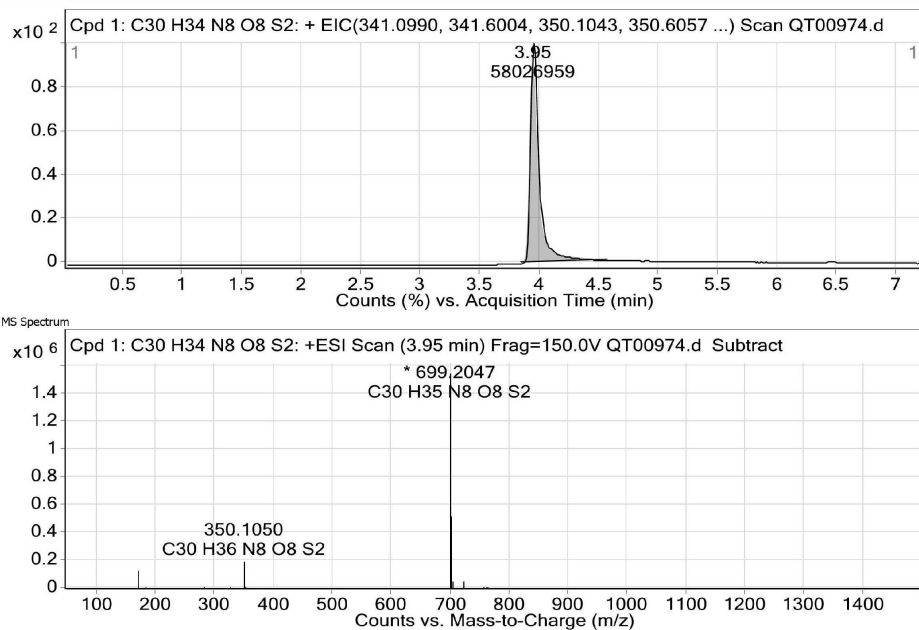


Peak List

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486.11935		28912		
516.09474	1	541223	C20 H15 F5 N5 O6	(M+H)+
517.09697	1	124622	C20 H15 F5 N5 O6	(M+H)+
561.15127		27882		
1053.16278	1	57547		
1054.16654	1	28115		



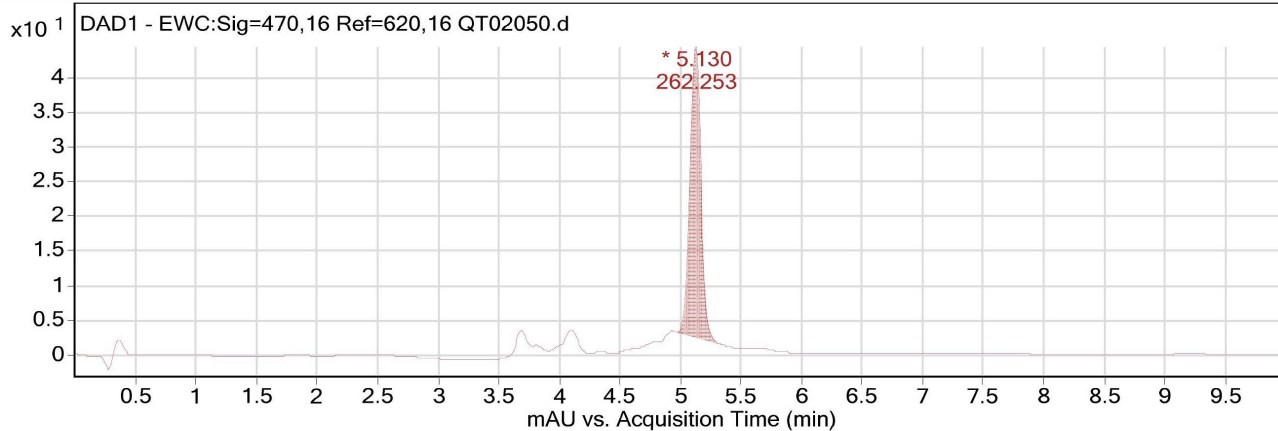
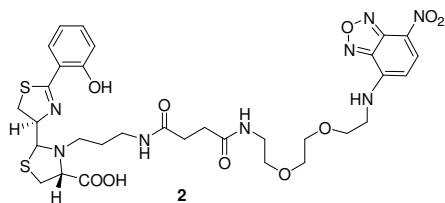
Qualitative Compound Report



MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
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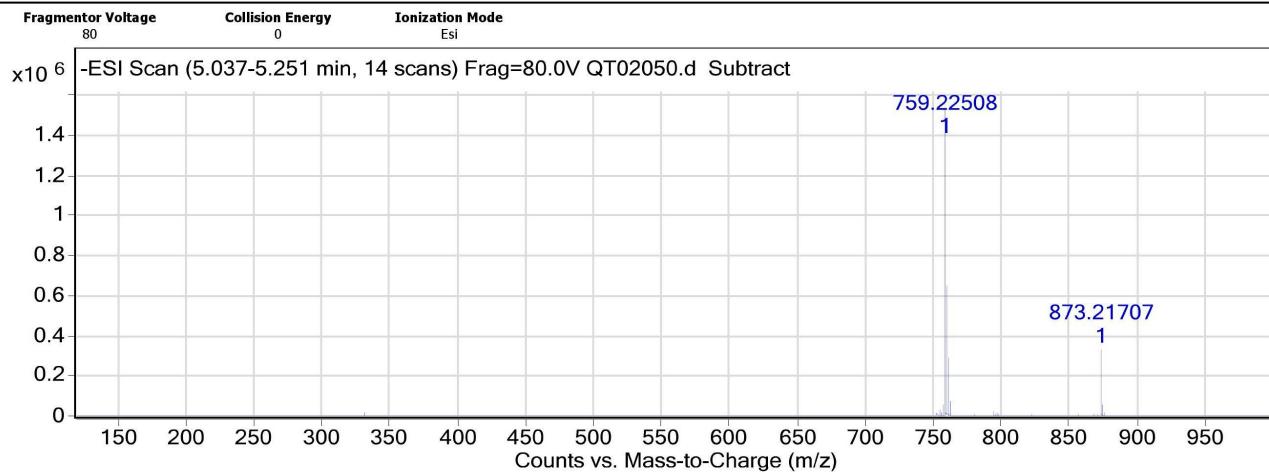
350,105	350,1043	1,91	2	193058	C30 H36 N8 O8 S2	(M+2H)+2
350,6062	350,6057	1,55	2	68527	C30 H36 N8 O8 S2	(M+2H)+2
351,1054	351,1045	2,51	2	26986	C30 H36 N8 O8 S2	(M+2H)+2
351,6065	351,605	4,15	2	9045	C30 H36 N8 O8 S2	(M+2H)+2
699,2047	699,2014	4,75		1543573	C30 H35 N8 O8 S2	(M+H)+
699,4241				47895		
699,5486				8894		
699,7741				3938		
700,2061	700,2041	2,9		517880	C30 H35 N8 O8 S2	(M+H)+
700,3223				14669		
700,4418				15099		
700,5444				5118		
701,2038	701,2018	2,86		209217	C30 H35 N8 O8 S2	(M+H)+
701,4392				5165		
702,2036	702,2027	1,29		51941	C30 H35 N8 O8 S2	(M+H)+
703,2027	703,2023	0,61		12311	C30 H35 N8 O8 S2	(M+H)+
721,185	721,1833	2,36	1	51459	C30 H34 N8 Na O8 S2	(M+Na)+
722,1856	722,1861	-0,62	1	20762	C30 H34 N8 Na O8 S2	(M+Na)+
723,1841	723,1837	0,54	1	8711	C30 H34 N8 Na O8 S2	(M+Na)+
737,1583	737,1573	1,37	1	2991	C30 H34 K N8 O8 S2	(M+K)+



Integration Peak List

Peak	Start	RT	End	Height	Area	Area %
1	4.97	5.13	5.33	41.89	262.253	100

User Spectra



Peak List

m/z	z	Abund	Formula	Ion
759.22508	1	1547706	C32 H39 N8 O10 S2	(M-H)-
760.22726	1	648102	C32 H39 N8 O10 S2	(M-H)-
761.22467	1	298405	C32 H39 N8 O10 S2	(M-H)-
873.21707	1	332804	C34 H40 F3 N8 O12 S2	(M+CF3COO)-
874.21923	1	133415	C34 H40 F3 N8 O12 S2	(M+CF3COO)-

II. Fluorescent properties of probes 1 and 2

A. General informations and procedures

Fluorescence emission spectra were measured with a PTI (Photon Technology International TimeMaster; Bioritech) spectrofluorimeter and absorption spectra were recorded SPECORD 205 (Analytikjena) spectrophotometer. Samples were contained in 10 mm path length quartz cuvette. Upon excitation at 470 nm, the emission spectra were integrated over the range 480 nm to 650 nm. For titration experiments, a FeCl_3 solution in 0.5 N HCl or a $\text{Fe}(\text{NO}_3)_3$ solution in H_2O was used for the addition of iron equivalents in aqueous buffer (150 mM Tris-HCl, pH 8.0) or CH_3CN buffer, respectively. For each titration point, FeCl_3 or $\text{Fe}(\text{NO}_3)_3$ was added to HCl 0.5 N or H_2O , respectively, to maintain a final volume of 30 μL . A fluorescent probe solution was prepared at the desired concentration from a 10 mM solution in DMSO in the appropriate solvent (150 mM Tris-HCl, pH 8.0 or CH_3CN at a constant pH). 1 mL aliquots of this solution were then distributed to each titration point. Samples were incubated at 4°C overnight before fluorescence measurements. Association constants were determined by non linear regression using *Kaleida graph* software (Energy Software).

B. Results

1. Spectroscopic behavior in aqueous solution

For the titration of a 15 μM solution of probe **1** in Tris buffer, a series of 0 - 0.1 - 0.3 - 0.5 - 0.7 - 1 - 2 iron(III) equivalents were used. Under these conditions, the maximum fluorescence enhancement (290%) for probe **1** was reached after addition of one equivalent of iron(III). (Figure 1A). For the titration of a 2 μM solution of probe **2** in Tris buffer, a serie of 0 - 0.5 - 1 - 2 - 3 - 5 - 10 iron equivalents were added to a 2 μM solution (Figure 1B). In these conditions, the highest level of fluorescence (320%) was observed after addition of five equivalents of iron(III) (Figure 1B). In addition, the influence of iron(III) on the fluorescence of the two NBD-spacer arm building blocks **16** and **22** was tested by adding 0 and 5 iron equivalents on a 5 μM or 2 μM solution of the fluorescent compound **16** or **22** (Figure 1C and 1D), respectively. In these experiments, no significant effect on the fluorescence was observed.

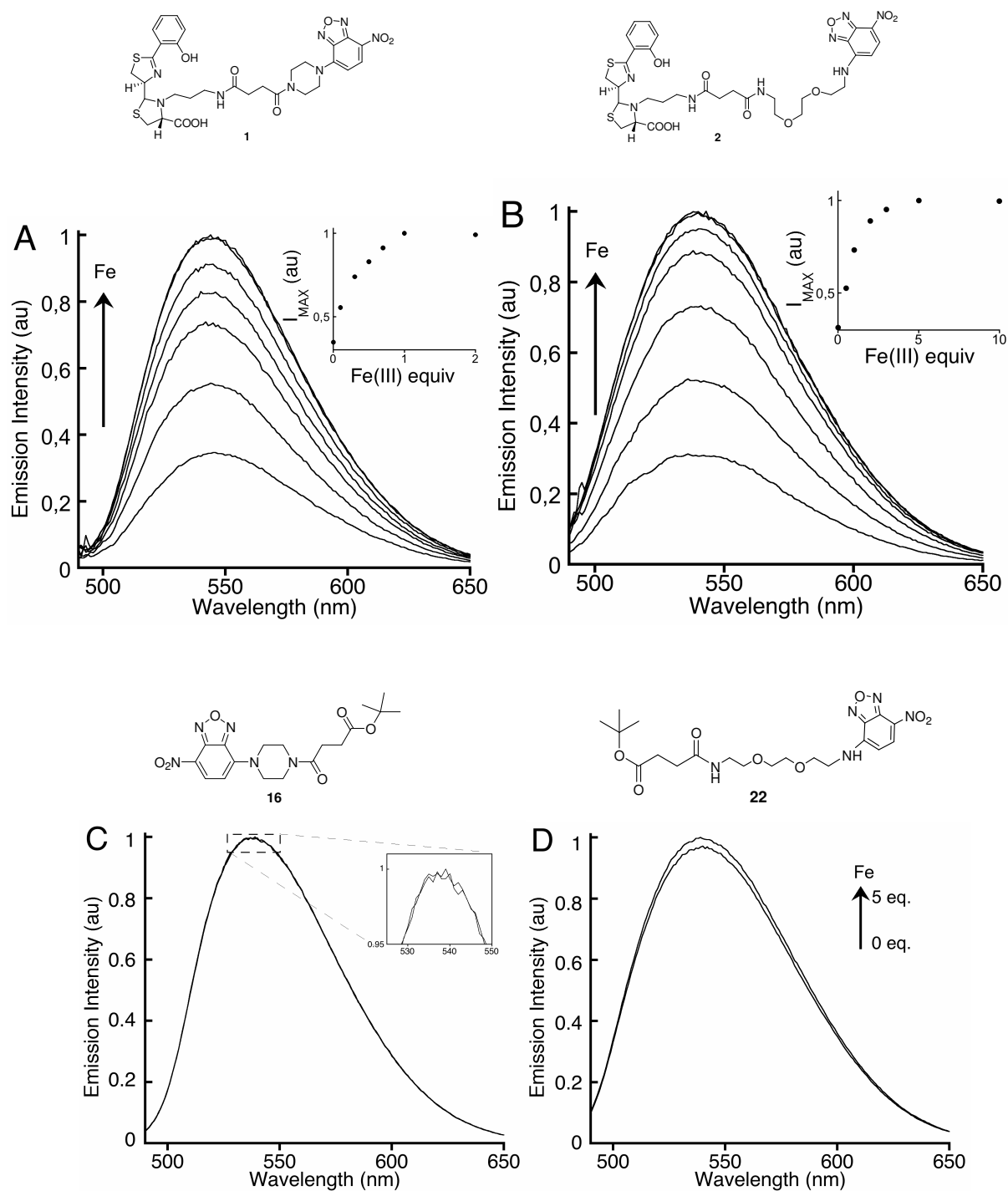


Figure 1. Corrected emission spectra of probe **1** and **2** and compounds **16** and **22** in the presence of increasing Fe^{3+} equivalents. $\lambda_{\text{exc}} = 470$ nm in aqueous solution (Tris-HCl buffer, pH 8.0). (A) probe **1** (15 μM), (B) probe **2** (2 μM) Insets : Intensity plots at maximal emission wavelength $\lambda_{\text{em}}^{\text{max}} = 545$ nm. For (C) compound **16** (5 μM) and (D) compound **22** (2 μM), 5 Fe^{3+} equivalents were added.

Iron(III) usually induces a quenching of a chelator fluorescence, especially in aqueous media. However, the intrinsic fluorescence of our probes **1** and **2** dissolved in Tris buffer (pH 8.0) was strikingly enhanced in the presence of iron(III). To assess the relative brightness of the different compounds, fluorescence quantum yields (Φ_F) were determined. In Tris buffer (150 mM final, pH 8.0), a series of 25 - 20 - 15 - 10 - 6 μM solutions of probe **1**, with or without 1 iron equivalent, and 6 - 4.8 - 3.6 - 2.4 - 1.2 μM solutions of compound **16** were prepared and absorbance and fluorescence spectra were recorded. The same procedure was performed for probe **2** and compound **22** with concentrations of 20 - 16 - 12 - 6 - 3 μM and 15 - 12 - 9 - 6 - 3 μM , respectively, and a 25 - 20 - 15 - 10 - 6 μM solutions of probe **2** complemented with 5 iron equivalents. Plots of integrated fluorescence intensities against the absorbance at the excitation wavelength (470 nm) and the linear regression are given in Figure 2. Quantum yields values were standardized to the most fluorescent sample set at 100 for each couple probe **1**/compound **16** and probe **2**/compound **22**. Calculated values are reported in Table 1.

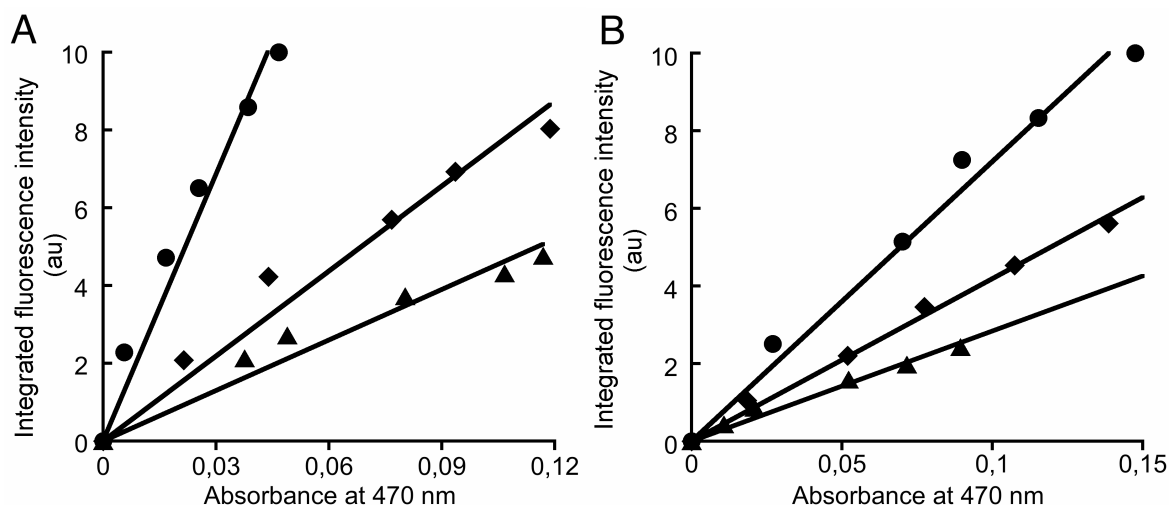


Figure 2. Fluorescence quantum yields determinations in aqueous solution (Tris-HCl buffer, pH 8.0). Superimposition of experimental values indicated by markers and linear regression represented as solid lines. (A) probe **1** (▲), probe **1** + 1 iron equivalent (◆) and compound **16** (●), (B) probe **2** (▲), probe **2** + 5 iron equivalents (◆) and compound **22** (●).

Fluorescent probe	Φ_F
Probe 1	19 (0.943)
Probe 1 + Fe	32 (0.962)
Compound 16	100 ^a (0.961)
Probe 2	39 (0.974)
Probe 2 + Fe	58 (0.992)
Compound 22	100 ^a (0.981)

Table 1. Fluorescence quantum yields Φ_F of the fluorescent probes and their iron complexes, compounds **16** and **22** in aqueous buffer. R square values are indicated in brackets.

^a Sample used as standard (set to 100).

The comparison between the fluorescence quantum yields in aqueous buffer (Figure 4A, 4B and Table 2) of the probes relative to their corresponding NBD-building blocks clearly shows a higher brightness without the pyochelin moiety. The changes in quantum yields of the probes can be interpreted as follows. In the free probe, the pyochelin moiety can interact with the NBD, which causes some quenching. Upon complexation, the pyochelin fluorescence is quenched, which leads to an increase in quantum yield.

2. Stoichiometry and association constant

The stoichiometry of the complexes was assessed by the Method of Continuous Variation. (Likussar W., *Anal. Chem.* **1971**, 43, 1265). The job's plot indicates a 2:1 stoichiometry (Probe **1**:Fe) (Figure 3A) and a binding constant of $\log K = 10,74$. For the Probe **2** : Fe complex, the stoichiometry is 3:1 (Figure 3B) and the binding constant is $\log K = 19,41$.

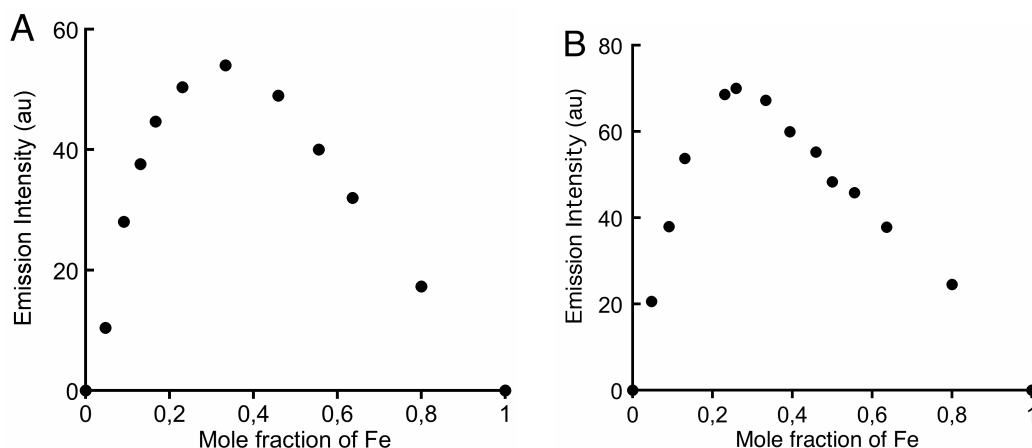
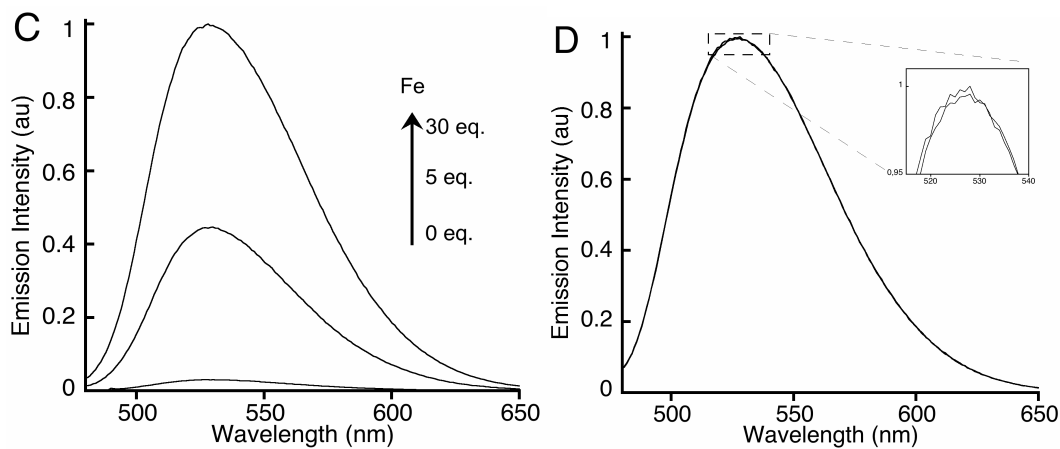
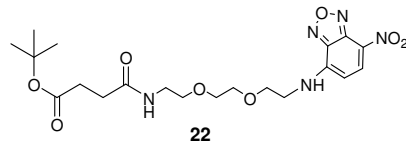
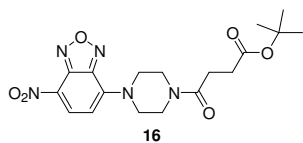
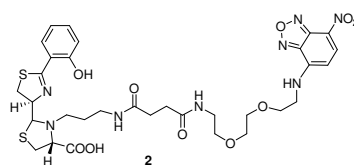


Figure 3. Job's plot according to the Method of Continuous Variations. (A) probe **1** [the total concentration of probe **1** and Fe(III) is 15 μ M]. (B) probe **2** [the total concentration of probe **2** and Fe(III) is 2 μ M].

1. Spectroscopic behavior in acetonitrile

In order to check if these fluorescence enhancements are solvent dependent, the same experiments were performed in CH_3CN at a constant pH. In CH_3CN , a 1 μ M solution of probe **1** was titrated by 0 - 1 - 2 - 3 - 5 - 10 - 20 iron(III) equivalents. In these conditions iron induces a fluorescence quenching (Figure 4A). On the opposite, in the same solvent, titration of a 0.5 μ M solution of probe **2** by 0 - 0.1 - 0.3 - 0.5 - 1 - 1.5 iron(III) equivalents showed only a small fluorescence enhancement as in aqueous media (Figure 1B). The maximal fluorescence enhancement (130%) was reached after addition of one equivalent of iron(III) (Figure 4B). Nevertheless, the variation in fluorescence intensities is very low as compared to the one observed in aqueous media and does not allow reasonable further data treatment. In addition, the influence of iron(III) on the fluorescence of NBD-spacer arm building blocks **16** and **22** was tested by adding 0 and 5 iron equivalents on a 1 μ M compound **16** solution (Figure 4C) or 0.5 μ M solution of compound **22** (Figure 4D). No significant effect on the fluorescence was observed, except for compound **16**. In this case a fluorescence enhancement was observed upon addition of 5 iron equivalents, additional titration points were recorded but no saturation was observed even for 30 iron equivalents added (Figure 4C).



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To gain insights into the atypical behaviors of probe **1** and compound **16**, we measured their fluorescence quantum yields in presence or absence of iron. Φ_F were determined for probe **1** and compound **16** with or without 10 iron equivalents using a 10 - 8 - 6 - 4 - 2 μM and a 8 - 6.4 - 4.8 - 3.2 - 2.4 μM concentration ranges, respectively. Plots of integrated fluorescence intensities against the absorbance at the excitation wavelength (470 nm) and the linear regression are given in Figure 5. Quantum yields values were standardized to the most fluorescent sample set at 100 (compound **16** + Fe). Calculated values are reported in Table 2.

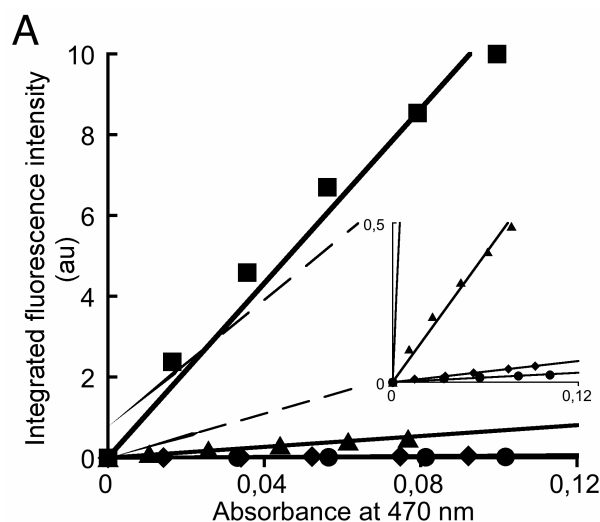


Figure 5. Fluorescence quantum yields determinations in CH_3CN . Superimposition of experimental values indicated by markers and linear regression represented as solid lines. (A) probe **1** (▲), probe **1** + 10 iron equivalents (◆), compound **16** (●) and compound **16** + 10 iron equivalents (■); a zoomed view is included to emphasize the differences between the 3 first data sets.

Fluorescent probe	Φ_F
Probe 1	6 (0.981)
Probe 1 + Fe	0.5 (0.997)
Compound 16	0.2 (0.939)
Compound 16 + Fe	100 ^a (0.973)

Table 2. Fluorescence quantum yields (Φ_F) of the compounds **1** and **16** and their iron complexes in CH_3CN . R square values are indicated in brackets. ^a Sample used as standard (set to 100).

In contrast with aqueous media, the brightness of probe **1** alone is higher than the one of its corresponding NBD-spacer arm building block. It may indicate that, in this case, the interaction between the pyochelin moiety and the NBD induces a fluorescence enhancement. Upon iron addition, the fluorescence decreases due to pyochelin fluorescence quenching. However, in this condition, compound **16** interacts with iron present in the media. Further work is required to find a model that can fulfill these characteristics.

III. Fluorescence microscopy experiments

A. General informations and procedures

All strains of *Pseudomonas aeruginosa* used in this study are deficient in pyoverdine (Pvd) biosynthesis and are derived from wild type strain PAO1. Strain PAD07 is also deficient in pyochelin (Pch) synthesis and strain DH51 does not express FptA, the pyochelin outer membrane receptor (Table 3).

Strains	Phenotype	Reference
PAD07	PAO1 Δ pvdA:: Ω Tc Δ pchD:: Ω Sm/Sp	4
DH51	PAO1 Δ pvd Δ fptA:: Ω Tc	5

Table 3. *Pseudomonas aeruginosa* strains used in this study.

For strain PAD07, 50 μ g/mL tetracycline and 100 μ g/mL streptomycin were added. For DH51 strain, was added 50 μ g/mL tetracycline. Bacteria were grown overnight at 37°C in LB medium, collected into a pellet by centrifugation, washed and resuspended in succinate medium at a dilution of 1 : 2 (composition: 6.0 g/L K₂HPO₄, 3.0 g/L KH₂PO₄, 1.0 g/L (NH₄)₂SO₄, 0.2 g/L MgSO₄·7H₂O, 4.0 g/L succinic acid, with the pH adjusted to 7.0 by adding NaOH). After a 24 h culture at 30°C, cells were inoculated in succinate medium without antibiotic at a dilution of 1:10 and grown for an additional 24 h. Cells were finally grown for 3 h from a starting optical density of 0.1 in succinate medium at 30°C. For the preparation of the microscopy samples, cells were pelleted, washed in succinate medium and resuspended at an optical density of 0.1. Iron complexes of fluorescent probes were tested. The probes were first incubated with iron at a ratio of 1 : 1 for 15 min at room temperature before addition to the cells. The iron complexes of the fluorescent probes were then added to 1 mL aliquots of cells to obtain a 1 μ M final concentration. The mixtures were then incubated at 30°C for 15 min. Cells were pelleted, washed with succinate medium, and then mounted on S750-agarose-coated slides, as previously described⁶. Images of fluorescence microscopy were acquired on a Nikon 50i (objective: CFI Achroplan 100X A ON 1.25 DT 0.18) microscope equipped with a 12-bit digital DS-Fi1 camera. Images were captured with NIS-Elements imaging software. For NBD conjugates fluorescence acquisition, the following filter set was used: exc. 472 \pm 30 nm / em. 520 \pm 30 nm / Dichroic 502-730 nm.

⁴ Takase, H.; Nitanai, H.; Hoshino, K.; Otani, T. *Infect. Immun.* **2000**, 68, 1834-1839.

⁵ Mislin, G. L.; Hoegy, F.; Cobessi, D.; Poole, K.; Rognan, D.; Schalk, I. J. *J. Mol. Biol.* **2006**, 357, 1437-1448.

⁶ Yeterian, E.; Martin, L. W.; Guillon, L.; Journet, L.; Lamont, I. L.; Schalk, I. J. *Amino Acids* **2010**, 38, 1447-1459.

B. Results

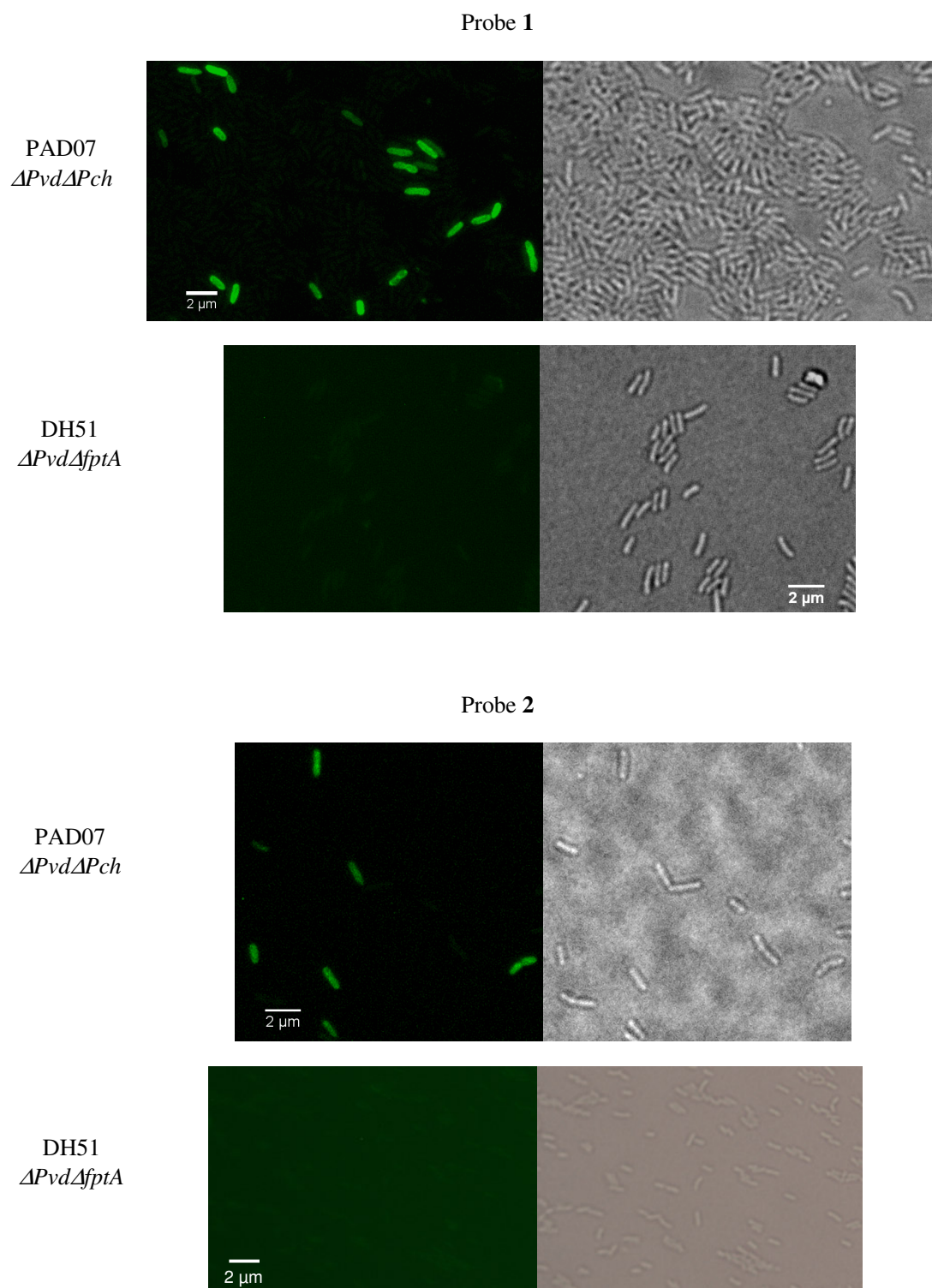


Figure 6. Epifluorescence microscopy of *P. aeruginosa* PAD07 and DH51 mutants. The cells were incubated with 1 μ M of the iron complexes of the fluorescent probes **1** and **2** for 15 min at 30°C and observed by fluorescence microscopy. For each pair of images, the picture on the left was taken using the following filter set : exc. 472 ± 30 nm / em. 520 ± 30 nm / Dichroic 502-730 nm. The picture on the right was taken by light microscopy. Scale bar, 2 μ m.

These experiments showed that the *P. aeruginosa* strain PAD07 was labeled by the probes **1** and **2** when, on the opposite, the DH51 strain was not labeled (Figure 6). DH51 strain did not express the specific outer membrane receptor of the pyochelin siderophore, proving that this receptor is crucial in the recognition of the probes **1** and **2**. As a control we repeated the same experiences using NBD-building blocks **16** and **22** as fluorescent probes. In these conditions, we observed no fluorescent labeling even on the PAD07 strain (Figure 7), suggesting that the FptA receptor specifically recognizes our probes **1** and **2** thank to the pyochelin moiety.

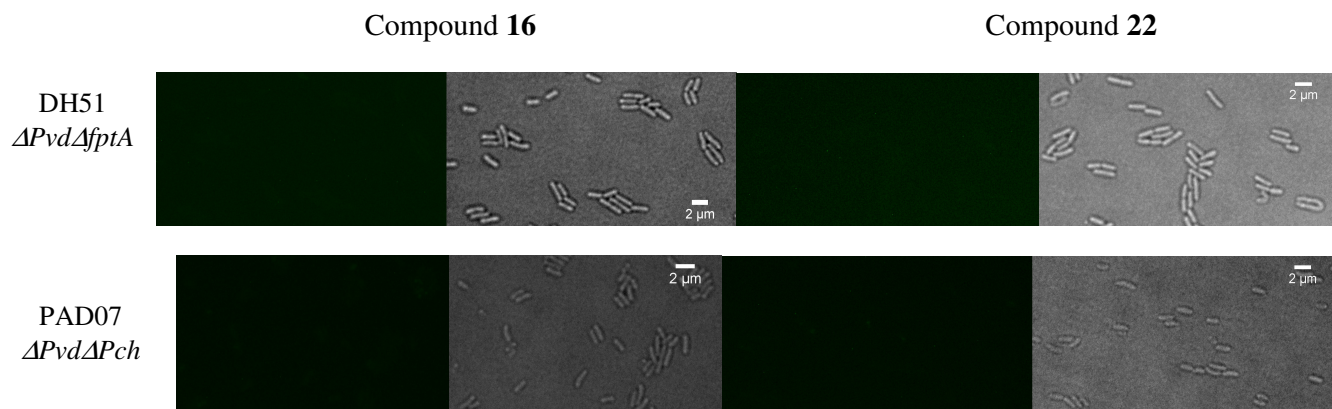


Figure 7. Epifluorescence microscopy of *P. aeruginosa* PAD07 and DH51 mutants. The cells were incubated with 1 μ M of the NBD-spacer arm building blocks **16** and **22**, in presence of iron(III), for 15 min at 30°C and observed by fluorescence microscopy. For each pair of images, the picture on the left was taken using the following filter set : exc. 472 \pm 30 nm / em. 520 \pm 30 nm / Dichroic 502-730 nm. The picture on the right was taken by light microscopy. Scale bar, 2 μ m.