Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications.

Supporting Information

Synthesis of Cyclohexapeptides as Antimalarial and Anti-trypanosomal Agents

Table of Contents

General Experimental Methods: Optical rotations were measured using a Kruss Optronic GmbH P8000 polarimeter with a 0.5 mL cell. IR spectra were recorded on a Shimadzu FTIR 8101A spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Advance DPX- 400. Chemical shifts are related to TMS as an internal standard. High resolution mass spectra (HRMS) were obtained on a micro Q-TOF (ESI) (Bruker Daltonics) and LTQ-FT Ultra (NanoESI) (Thermo Scientific). Flash column chromatography was carried out with Silica gel 60 (J.T. Baker, 40 μm average particle diameter). All reactions and chromatographic separations were monitored by TLC, conducted on 0.25 mm Silica gel plastic sheets (Macherey/Nagel, Polygram_ SIL G/UV 254). TLC plates were analyzed under 254 nm UV light, iodine vapor, p-hydroxybenzaldehyde spray or ninhydrin spray. Yields are reported for chromatographically and spectroscopically (^{1}H and ^{13}C NMR) pure compounds. Analytical HPLC was performed on a Waters 2695 Separations Module equipped with a Waters XBridge C18 column (75 x 4.6 mm, 2.5 μm particle size) and Waters 996 photodiode array detector or on a Shimadzu (LC-10AT Pump) equipped with a Waters μBondapak™ C18 column (150 x 3.9 mm, 10 μm) and a SPD20Aprominence UV/Vis detector. All solvents were purified according to literature procedures.[1] All reactions were carried out in dry, freshly distilled solvents under anhydrous conditions unless otherwise stated.

SPPS general procedure 1: The synthesis was done in a plastic syringe equipped with teflon filters attached to a vacuum manifold to enable rapid removal of reagents and solvents. The 2-chlorotritylchloride resin (loading =1.0 mmol/g; 100 mg) was washed with CH_2Cl_2 (5 × 30 sec), DMF (5 × 30 sec) and again with CH_2Cl_2 (5 × 30 sec). A solution of Fmoc-AA-OH (1 mmol/g of resin) in minimal CH_2Cl_2 and DIPEA (7 eq.) was gently shaken for 1 h, then an extra 3 eq. of DIPEA was added and shaking was continued for 5 min. MeOH (0.08 mL/ g of resin) was then added in order to cap unreacted functional groups on the resin; the mixture was then shaken for 20-30 min. The resin was filtered, and washed with CH_2Cl_2 and DMF (5 × 30 sec. each). The N-terminus was deprotected using 20% piperidine in DMF (2 × 5 min and 1 x 10 min). The resin was then washed with DMF, CH_2Cl_2 and DMF (5 × 30 sec. each). Fmoc-AA-OH (3 eq.) was pre-activated by vigorous shaking for 4 min in the presence of DIC (3 eq.) and HOAt (3 eq.) in minimal DMF,

[1] Perrin, D. D. ; Armarego, W. L. F. "Purification of Laboratory Chemicals", 3th Ed. Pergamon Press, Oxford, 1988.

and then poured onto the resin and the resulting mixture was gently shaken for 1-2 h. After the coupling was completed, the resin was washed with CH_2Cl_2 and DMF (5 × 30 sec. each). Deprotection and coupling cycles were repeated with the appropriate amino acids to provide the desired compound.

The peptide was cleaved from the resin by treatment with 1% TFA in CH_2Cl_2 for 2-3 minutes at room temperature followed by filtration and collection of the filtrate in water. The treatment was repeated 4-5 times. Solvents were removed *in vacuo* and the crude peptide was washed twice with CH_2Cl_2, cooled with N_2 liquid and lyophilized to render the desired hexapeptide.

SPPS general procedure 2: The synthesis was done in a plastic syringe equipped with Teflon filters attached to a vacuum manifold to enable rapid removal of reagents and solvents. The 2-chlorotritylchloride resin (100-300 mesh, 1.43 mmol/g) was swelled in CH_2Cl_2 (3 × 30 sec). A solution of the first protected aminoacid (Fmoc-Gly-OH, 2.0 eq) in CH_2Cl_2 and DIPEA (3.0 eq) was gently shaken for 10 min, then an extra 7.0 eq. of DIPEA was added and shaking was continued for 45 min. MeOH (0.8 mL/ g of resin) was then added in order to cap unreacted functional groups on the resin; the mixture was then shaken for 10 min. The resin was filtered, and then washed with CH_2Cl_2 (x3) and DMF (x3). The N-terminus was deprotected using 20% piperidine in DMF (2 × 5 min and 1 x 10 min). The resin was then washed with DMF (x3). A solution of Fmoc-AA-OH (3 eq.) and DIPEA (6 eq.) in DMF was added to the resin, followed by a solution of HBTU or HCTU (2.9 eq) in DMF. The mixture was stirred for 90 min. After the coupling was completed, the resin was washed with DMF (×3). Deprotection and coupling cycles were repeated with the appropriate amino acids to provide the desired compound. The peptide was cleaved from the resin by treatment with 1% TFA in CH_2Cl_2 for 2-3 minutes at room temperature followed by filtration and collection of the filtrate in MeOH. The treatment was repeated three times and then the resin washed with CH_2Cl_2 (x5). Solvents were removed *in vacuo* to obtain the crude hexapeptide.

cyclo-[L-Val-L-Ser-D-Ile-L-Cys(Trt)-L-Ala-L-Thr(^{t}Bu)-] ***(16)***

The trifluoroacetate salt of H_2N-D-Ile-L-Cys(Trt)-L-Ala-L-Thr(^{t}Bu)-L-Val-L-Ser-OH was obtained following the general SPPS procedure 1. Lyophilization yielded 825 mg (96% yield) of the hexapeptide as a white solid. The purity (91%) was determined by HPLC (linear gradient: 70 to 100% acetonitrile (0.036% TFA) in H_2O (0.045% TFA) over 8 min; flow rate = 1.0 mL/min; tR = 2.78 min). mp: 215-217 ºC. α_D = +

3.04 (c 0.67, MeOH/$CHCl_3$ (1:1)). HRMS calculated for $C_{47}H_{67}N_6O_9S_1$ ($[M+H]^+$) 891.46963 observed 891.46848

Macrocyclization reaction was performed following the general procedure (dilution 5 mM, 4 days), starting from the trifluoroacetate salt of the amino acid H_2N-D-Ile-L-Cys(Trt)-L-Ala-L-Thr(O^{t}Bu)-L-Val-L-Ser-OH (100 mg, 0.099 mmol). Further purification by flash chromatography, the desired macrocycle was obtained (48 mg, 0.055 mmol) in 55% yield.

White solid, mp: 108-109 ºC, Rf = 0.45 (AcOEt:EP, 3:2). α_D = - 20.5 (c 1.3, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.90 – 1.00 (m, 9H), 1.05 (d, *J* = 6.8 Hz, 3H), 1.10 (d, *J* = 6.5 Hz, 3H), 1.15 - 1.21 (m, 2H), 1.32 (s, 9H), 1.35 -1.41 (m, 1H), 1.56 (d, *J* = 7.1 Hz, 3H), 2.18 - 2.23 (m, 1H), 2.29 (dd, J_1 = 12.8 Hz, J_2 = 6.3 Hz, 1H), 2.34 - 2.41 (m, 1H), 2.75 (dd, J_1 = 12.8 Hz, J_2 = 5.2 Hz, 1H), 3.47 (dd, J_1 = 9.9 Hz, J_2 = 3.5 Hz, 1H), 3.72 (t, *J* = 7.2 Hz, 1H), 3.97 (t, *J* = 4.12 Hz, $1H_{15}$), 3.99 (dd, J_1 = 9.9 Hz, J_2 = 1.90 Hz, 1H), 4.21 (m, 2H), 4.39 - 4.44 (m, 1H), 4.47 (dd, J_1 = 6.5, J_2 = 4.6 Hz, 1H), 4.83 (dd, J_1 = 9.7 Hz, J_2 = 3.6 Hz, 1H), 6.62 (d, *J* = 7.9 Hz, 1H), 6.94 (d, *J* = 7.8 Hz, 1H), 7.03 (d, *J* = 7.2 Hz, 1H), 7.12 (d, *J* = 4.9 Hz, 1H), 7.23 - 7.37 (m, 15H), 7.54 (d, *J* = 9.6 Hz, 1H), 8.16 (d, *J* = 3.5 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 11.9, 14.8, 15.4, 17.5, 17.7, 19.8, 26.6, 28.3 (3C), 29.1, 29.7, 31.8, 51.5, 52.1, 54.7, 55.6, 57.5, 61.8, 62.8, 65.5, 67.4, 76.3, 127.1 (3C), 127.3 (2C), 127.9 (6C), 128.2 (2C), 129.6 (2C), 144.3 (2C), 146.9, 170.1, 170.4, 170.5, 170.9, 172.3, 172.5. IR ν(cm^{-1}) 1080, 1150, 1265, 1385, 1447, 1516, 1628, 1655, 2878, 2932, 2970, 3059, 3306, 3406. HRMS calculated for $C_{47}H_{64}N_6NaO_8S$ ($[M+Na]^+$) 895.4404 observed 895.4396.

*cyclo-[Gly-L-Cys(Trt)-L-Ile-L-Cys(Trt)-L-Phe-L-Ser(^{t}Bu)-] (**17**)*

The trifluoroacetate salt of H_2N-L-Cys(Trt)-L-Ile-L-Cys(Trt)-L-Phe-L-Ser(^{t}Bu)-Gly-OH was obtained following the general SPPS procedure 2. Yielded 350 mg (91% yield) of the hexapeptide as a white solid. The purity (90 %) was determined by HPLC (linear gradient: 8 to 100% acetonitrile in H2O/ 0.1% TFA over 20 min; flow rate = 1.0 mL/min; tR = 13.97 min). mp= decompose above 200 °C. $[\alpha]_D^{25}$= -145.7 (c 0.35, MeOH/H_2O) HRMS m/z calc. for $C_{68}H_{76}N_6O_8S_2$ ($[M+H]^+$) 1169.5244, found 1169.5115. Macrocyclization reaction was performed following the general procedure (dilution 5mM, 3 days), starting from the trifluoroacetate salt of the amino acid H_2N-L-Cys(Trt)-L-Ile-L-Cys(Trt)-L-Phe-L-Ser(Ot-Bu)-L-Gly-OH (300

mg, 0.23 mmol). Further purification by flash chromatography, the desired macrocycle was obtained (129 mg, 0.112 mmol) in 48% yield.

White solid, mp: 123-129 °C, Rf= 0.4 (EtOAc:EP, 3:1). $[\alpha]_D^{25}$= -25.9 (c 1.35, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.87 (t, *J*=6.7 Hz, 3H), 0.88 (d, *J*=6.7 Hz, 3H), 1.07 (m, 1H), 1.11 (s, 9H), 1.48 (m, 1H), 2.00 (m, 1H), 2.55 (m, 1H), 2.59 (m, 1H), 2.70 (m, 1H), 2.80 (m, 1H), 3.01 (m, 1H), 3.17 (m, 1H), 3.36 (m, 1H), 3.43 (m, 1H), 3.55 (m, 1H), 3.62 (m, 1H), 3.87 (t, *J*=7.2 Hz, 1H), 4.04 (m, 1H), 4.06 (m, 1H), 4.28 (m, 2H), 6.79 (d, *J*=5.4 Hz, 1H), 6.94 (m, 1H), 7.09-7.18 (m, 6H), 7.18-7.25 (m, 7H), 7.25-7.34 (m, 14H), 7.35-7.40 (m, 6H), 7.40-7.46 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ(ppm):11.07, 15.71, 25.16, 27.43 (3C), 32.26, 32.46, 35.58, 36.52, 38.64, 43.63, 53.37, 54.08, 54.68, 56.07, 60.31, 67.31 (2C), 82.04, 126.94 (2C), 126.97 (3C), 127.26, 127.34, 127.93 (2C), 127.96 (3C), 128.12 (8C), 128.30, 128.66 (2C), 129.36 (2C), 129.48 (5C), 129.54 (5C), 144.21 (3C), 144.25 (3C), 146.90, 170.05, 170.16, 170.62, 170.69, 171.32 (2C). IR ν(cm^{-1}) 1034, 1157, 1236, 1265, 1365,1531, 1645, 2931, 2970, 3059, 3296. HRMS m/z calc. for $C_{68}H_{74}N_6O_7S_2$ ([M+Na]$^+$) 1173,4958, found 1173.4912.

cyclo-[Gly-L-Cys(Trt)-L-Ile-L-Cys(Trt)-L-Ile-L-Cys(Trt)] ***(18)***

The trifluoroacetate salt of H_2N-L-Cys(Trt)-L-Ile-L-Cys(Trt)-L-Ile-L-Cys(Trt)-Gly-OH was obtained following the general SPPS procedure 2. Yielded 410 mg (94% yield) of the hexapeptide as a white solid. The purity (80 %) was determined by HPLC (linear gradient: 8 to 100% acetonitrile in H2O/ 0.1% TFA over 20 min; flow rate = 1.0 mL/min; tR = 14.52 min m.p. = 182-190. $[\alpha]_D^{25}$= -204.0 (c 0.25, MeOH/H_2O). HRMS m/z calc. for $C_{80}H_{84}N_6O_7S_3$ ([M+Na]$^+$) 1359.5462, found 1359.5309.

Macrocyclization reaction was performed following the general procedure (dilution 5mM, 3 days), starting from the trifluoroacetate salt of the amino acid H_2N-L-Cys(Trt)-L-Ile-L-Cys(Trt)-L-Ile-L-Cys(Trt)-Gly-OH (330 mg, 0.227 mmol). Further purification by flash chromatography, the desired macrocycle was obtained (192 mg, 0.145 mmol) in 66% yield.

White solid, mp: 145-147 °C, Rf= 0.5 (EtOAc)., $[\alpha]_D^{25}$= -58.4 (c 0.5, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.83-0.89 (m, 9H), 0.91 (d, *J*=6.8 Hz, 3H), 0.99-1.20 (m, 2H), 1.39-1.60 (m, 2H), 1.90-2.10 (m, 2H), 2.52-2.65 (m, 2H), 2.65-2.79 (m, 4H), 3.21-3.30 (m, 1H), 3.51-3.63 (m, 1H), 3.69-3.83 (m, 2H), 3.89-4.03 (m,

3H), 6.78 (d, *J*=6.3 Hz, 1H), 6.85-6.96 (m, 1H), 7.01 (d, *J*=7.5 Hz, 1H), 7.04-7.16 (m, 2H), 7.18-7.25 (m, 10H), 7.25-7.33 (m, 18H), 7.38-7.46 (m, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ(ppm):10.78, 11.44, 15.74, 15.87, 24.69, 25.07, 31.93 (2C), 34.86, 35.44,36.51, 43.88, 53.32, 53.49, 53.98, 59.06, 59.6, 67.03, 67.21(2C), 126.85 (3C), 126.92 (3C), 126.99 (3C), 127.93 (2C), 127.95 (2C), 128.02 (6C), 128.10 (4C), 128.15 (4C), 129.38 (6C), 129.49 (6C), 129.56 (6C), 144.07 (3C), 144.30 (3C), 144.53 (3C), 165.27, 170.63, 170.76, 170.88, 170.97, 171.43. IR ν(cm^{-1}) 1033, 1082, 1265, 1445, 1526, 1651, 2931, 2965, 3059, 3385, 3400.HRMS m/z calc. for $C_{80}H_{82}N_6O_6S_3$ ([M+Na]$^+$) 1341,5456, found 1341.5231.

cyclo-[Gly-L-Cys(Trt)-L-Met-L-Cys(Trt)-L-Ile-L-Thr(^{t}Bu)-] ***(19)***

The trifluoroacetate salt of H_2N-L-Cys(Trt)-L-Met-L-Cys(Trt)-L-Ile-L-Thr(Ot-Bu)-Gly-OH was obtained following the general SPPS procedure 2. Yielded 480 mg (87% yield) of the hexapeptide as a white solid. The purity (94%) was determined by (linear gradient: 8 to 100% acetonitrile in H2O/ 0.1% TFA over 20 min; flow rate = 1.0 mL/min; tR = 15.09 min mp= decompose above 200 °C. $[\alpha]_D^{25}$= -73.6 (c 0.5, MeOH/H_2O). HRMS m/z calc. for $C_{65}H_{78}N_6O_8S_3$ ([M+H]$^+$) 1167.5121, found 1167.5096.

Macrocyclization reaction was performed following the general procedure (dilution 5mM, 3 days), starting from the trifluoroacetate salt of the amino acid H_2N-L-Cys(Trt)-L-Met-L-Cys(Trt)-L-Ile-L-Thr(Ot-Bu)-L-Gly-OH (340 mg, 0.265 mmol). Further purification by flash chromatography, the desired macrocycle was obtained (165 mg, 0.144 mmol) in 54% yield.

White solid (54%), mp= 127-129 °C, Rf= 0.5 (EtOAc:EP, 3:1)., $[\alpha]_D^{25}$= -28.0 (c 1.35, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.85-0.95 (m, 6H), 1.02 (d, *J*=6.3 Hz, 3H), 1.05-1.19 (m, 1H), 1.23 (s, 9H), 1.39-1.51 (m,1H), 1.75-1.86 (m, 1H), 1.80 (s, 3H), 1.89-1.99 (m, 1H), 1.89-2.09 (m, 2H), 2.14-2.24 (m, 1H), 2.48-2.63 (m, 3H), 2.63-2.72 (m, 1H), 2.96-3.06 (m, 1H), 3.17-3.26 (m, 1H), 3.39-3.49 (m, 1H), 3.53-3.71 (m, 2H), 3.94 (t, *J*=6.1 Hz, 1H), 4.02-4.13 (m, 2H), 4.20-4.27 (m, 1H), 4.46-4.55 (m, 1H), 6.42 (s, 1H), 6.69-6.78 (m, 1H), 6.78-6.88 (m, 1H), 7.20-7.27 (m, 7H), 7.27-7.35 (m, 14H), 7.40 (m, 11H), 7.55 (d, *J*=8.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ(ppm):11.30, 14.20, 15.39, 15.84, 19.16, 21.04, 22.66, 25.29, 28.26, 29.81, 30.37, 32.18(2C), 35.59, 44.07, 53.01, 53.57, 55.15, 58.69, 60.56, 67.33, 67.43, 75.05, 127.00 (6C), 128.15 (12C), 129.47 (12C), 144.21 (3C), 144.30 (3C), 165.76, 169.68, 170.36, 171.12, 171.46, 172.03. IR ν(cm^{-1}) 1082,

1148, 1192, 1236, 1234, 1383, 1445, 1512, 1647, 2931, 2970, 3059, 3287. HRMS m/z calc. for $C_{65}H_{76}N_6O_7S_3$ ($[M+Na]^+$) 1171.4836, found 1171.4790.

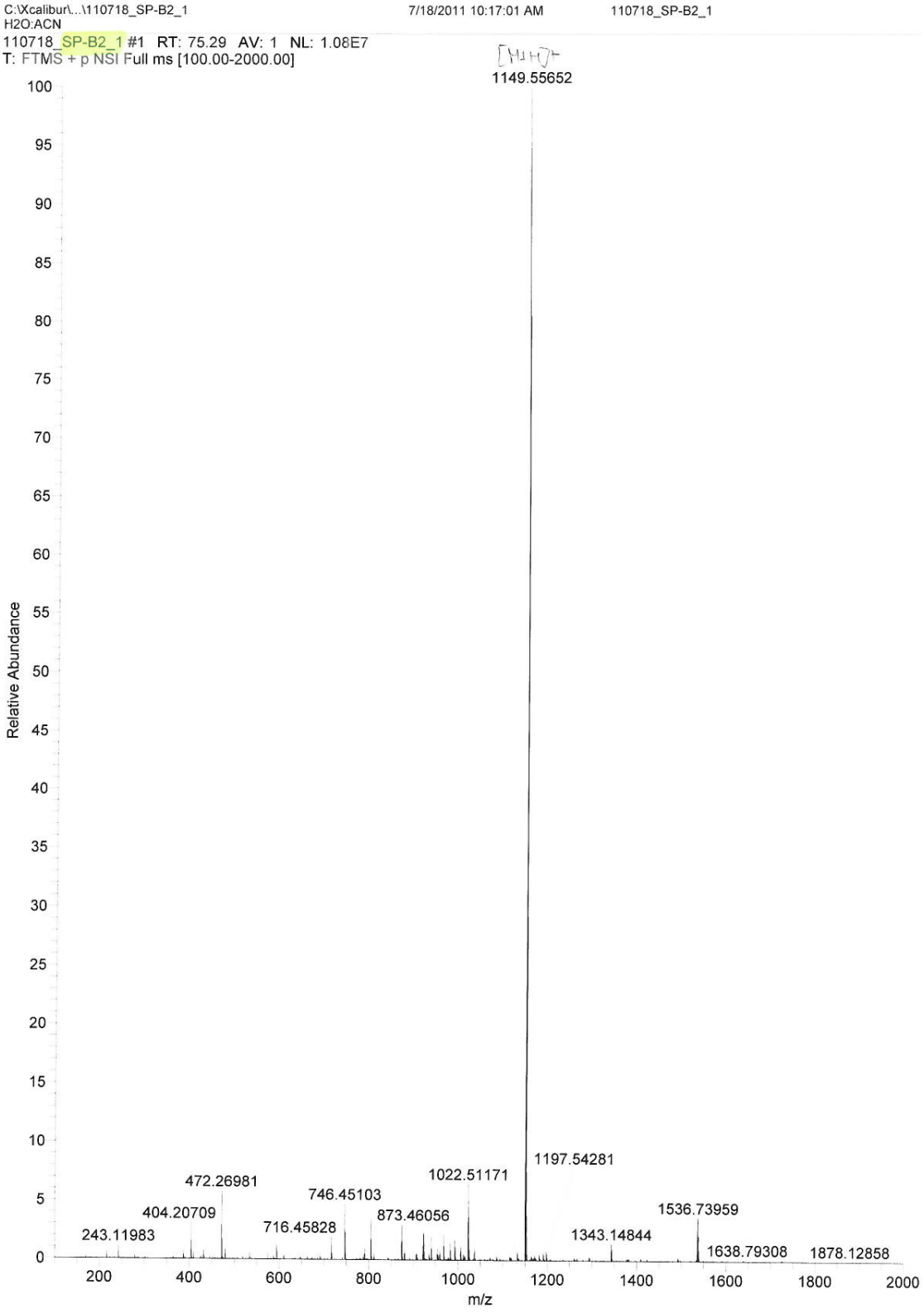
*cyclo-[Gly-Thz-L-Met-L-Cys-L-Phe-L-Thr(^{t}Bu)] (**21**):*

The trifluoroacetate salt of H_2N-L-Met-L-Cys-L-Phe-L-Thr(^{t}Bu)- Gly- Thz- OH was obtained following the general SPPS procedure 1. Lyophilization yielded 372 mg (98% yield) of the hexapeptide as a pale yellow solid. The purity (90 %) was determined by HPLC (linear gradient: 40 to 90% acetonitrile (0.036% TFA) in H_2O (0.045% TFA) over 8 min; flow rate = 1.0 mL/min; t_R = 5.19 min). mp: 89-91 ºC. α_D = - 5.24 (c 0.67, MeOH:CH_2Cl_2, (1:1)). HRMS calculated for $C_{49}H_{59}N_6O_7S_3$ ($[M+H]^+$) 939.3629 observed 939.36019.

Macrocyclization reaction was performed following the general procedure (dilution 1 mM, 5 days) starting from the trifluoroacetate salt of the amino acid H_2N-L-Met-L-Cys-L-Phe-L-Thr(^{t}Bu)-Gly-Thz- OH (100 mg, 0.095 mmol). Further purification by flash chromatography, the desired macrocycle was obtained (35 mg, 0.038 mmol) in 40% yield.

White yellow (35 mg, 40%), mp: 107-109 ºC, Rf = 0.5 (AcOEt:EP, 4:1). α_D = - 42.8 (c 1.0 g/100 mL, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.13 (d, J = 6.5 Hz, 3H), 1.22-1.24 (m, 9H), 1.82 -1.93 (m, 1H), 2.09 (s, 3H), 2.14 - 2.23 (m, 1H), 2.28 (dd, J_1 = 14.1 Hz, J_2 = 5.4 Hz, 1H), 2.54 (t, J = 7.6 Hz, 1H), 2.58-2.66 (m, 1H), 2.87 (dd, J_1 = 14.1 Hz, J_2 = 8.5 Hz, 1H), 3.26 (dd, J_1 = 14.1 Hz, J_2 = 10.7 Hz, 1H), 3.42 (dd, J_1 = 14.1 Hz, J_2 = 4.3 Hz, 1H), 3.96-4.03 (m, 1H), 4.21-4.29 (m, 1H), 4.40 (dd, J_1 = 16.6 Hz, J_2 = 4.7 Hz, 1H), 4.43-4.52 (m, 2H), 4.88 (dd, J_1 = 16.6 Hz, J_2 = 6.3 Hz, 1H), 6.4 (bs, 1H), 6.60 (bs, 1H), 6.99 - 7.02 (m, 3H), 7.05 - 7.10 (m, 2H), 7.23-7.30 (m, 9H), 7.31-7.37 (m, 7H) 7.87 (s, 1H), 7.94 (bs, 1H), 8.49 (bs, 1H).^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 15.5, 19.1, 28.2 (3C), 30.1, 31.9, 32.5, 35.0, 41.1, 52.2, 54.3, 57.8, 58.4, 66.7, 67.9, 75.6, 122.9, 126.9, 127.1 (3C), 128.2 (6C), 128.8 (2C), 129.1 (2C), 129.5 (6C), 137.4, 143.9 (3C), 149.3, 160.7, 164.9, 169.3, 170.5, 170.7, 171.0. IR ν(cm^{-1}) 1032, 1082, 1155, 1186, 1265, 1370, 1379, 1445, 1491, 1508, 1528, 1545, 1655, 1676, 2849, 2918, 3055, 3345. HRMS calculated $C_{49}H_{56}N_6NaO_6S_2$ ($[M+Na]^+$) 943.3221 observed 943.3279.

HRMS - Hexapeptide precursor of 15



HPLC - Hexapeptide precursor of 15

Empower2 software

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SAMPLE INFORMATION

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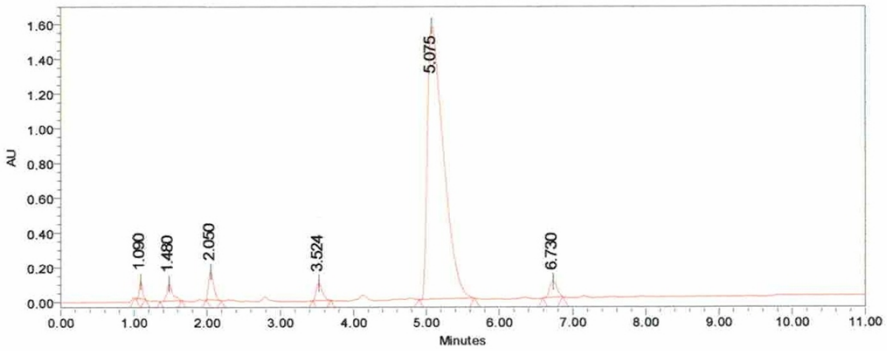

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Page: 1 of 2

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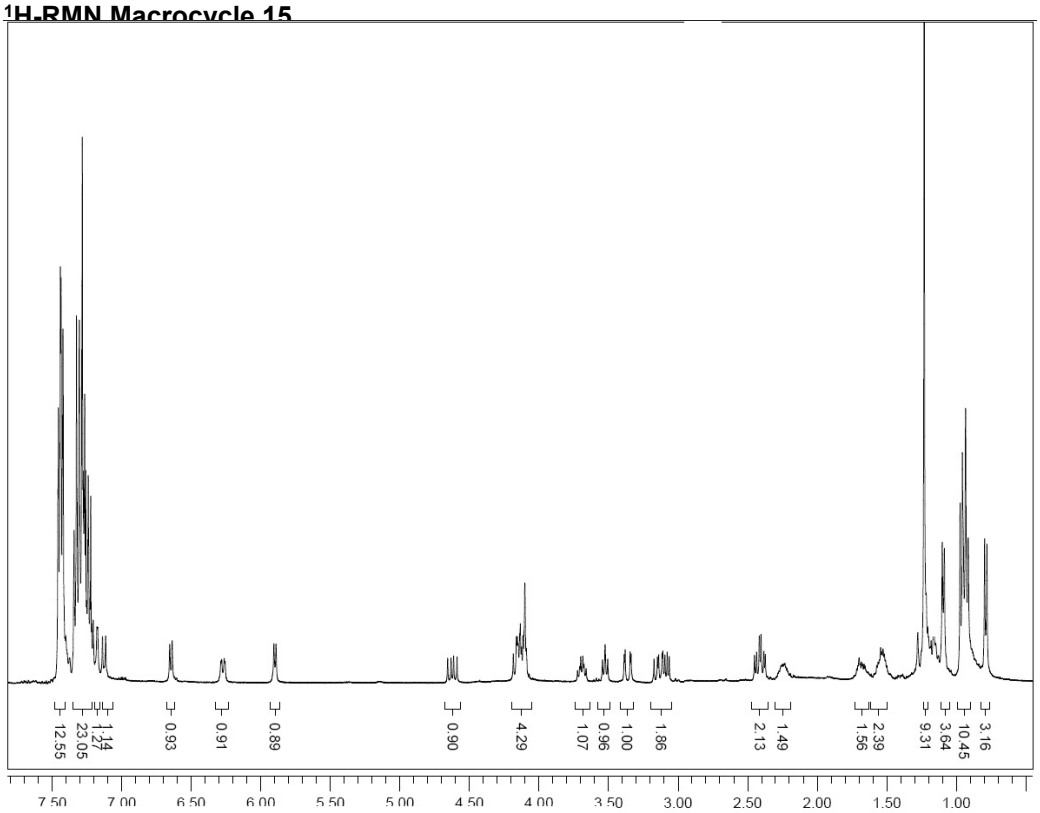

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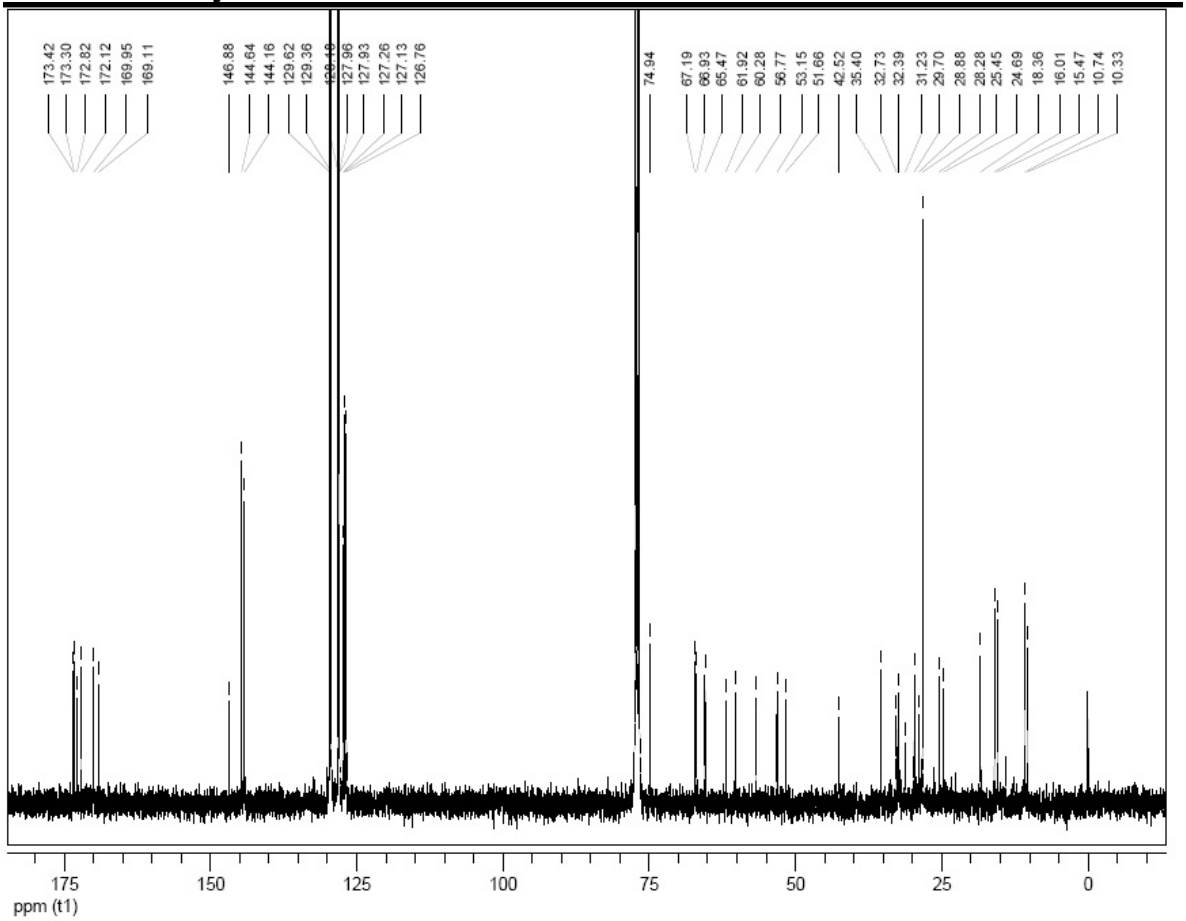

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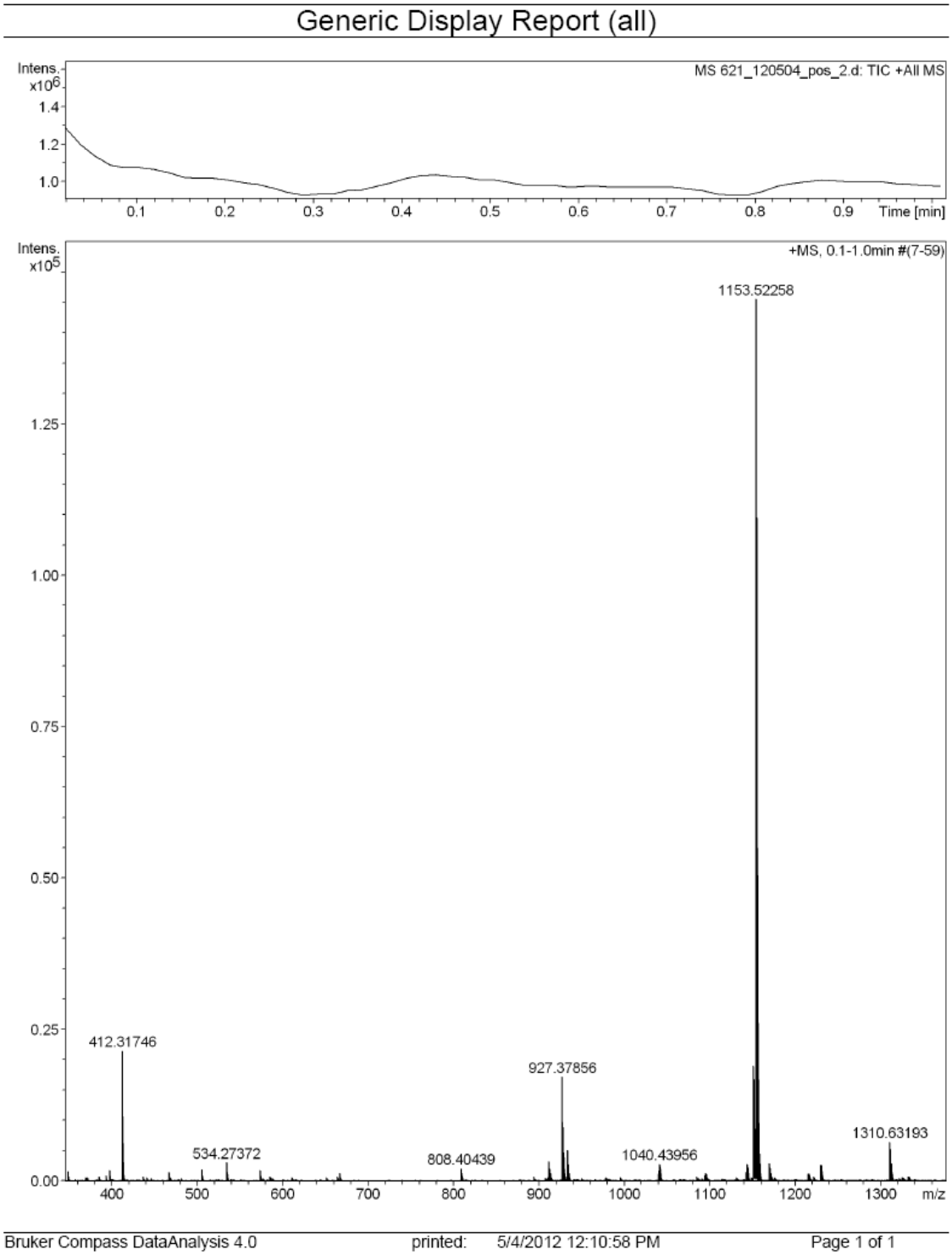

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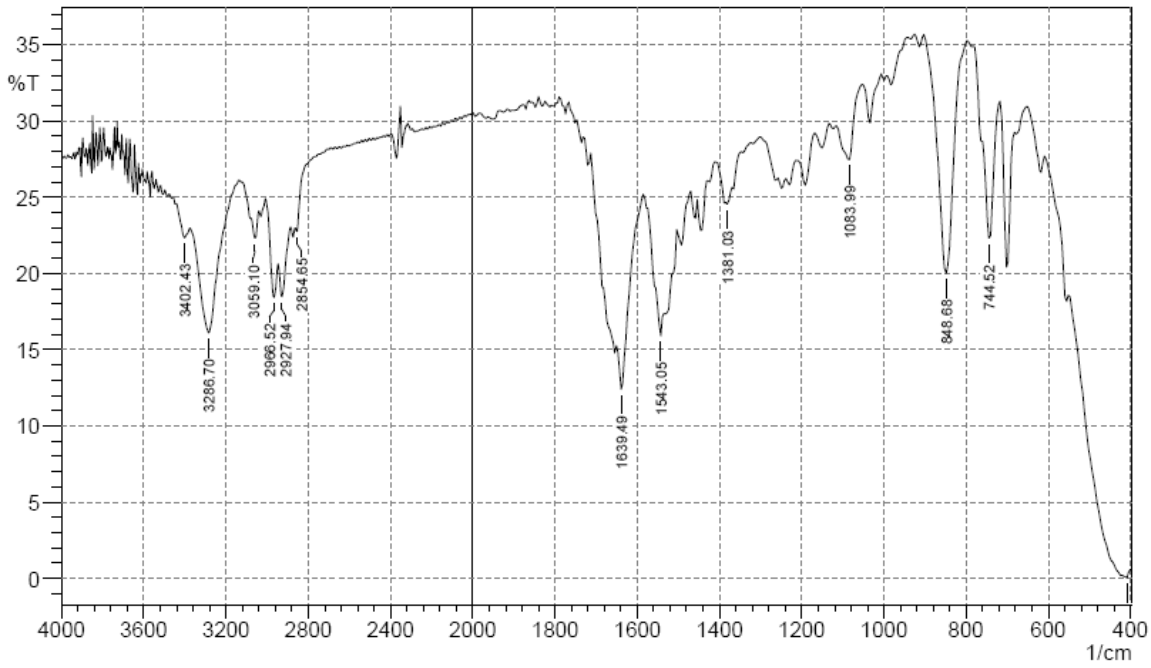
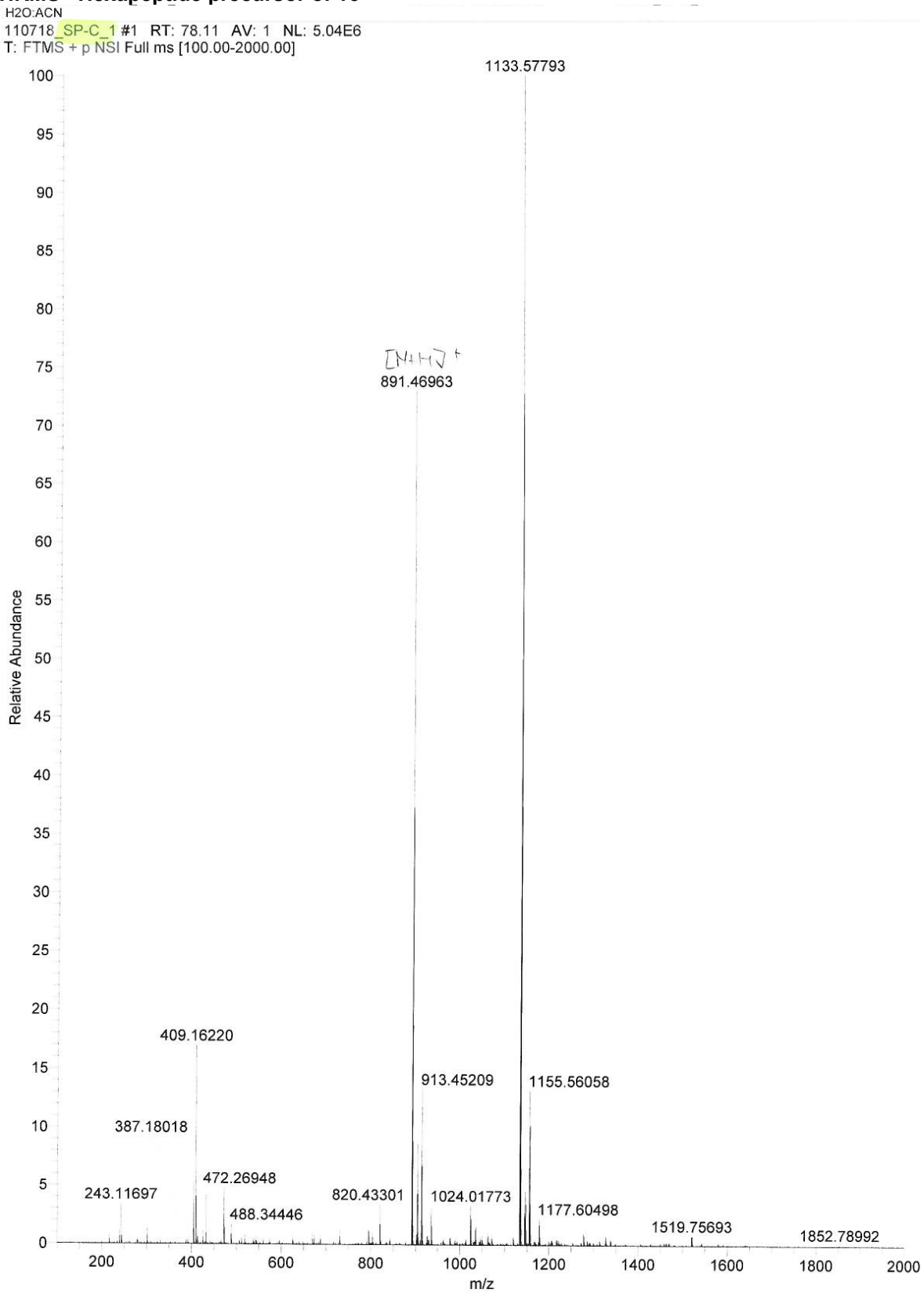


HRMS –Hexapeptide precursor of 16



HPLC – Hexapeptide precursor of 16

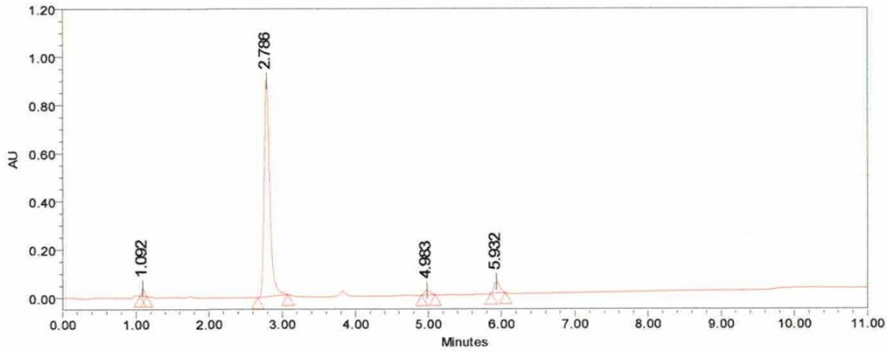
Empower 2 software

Default

SAMPLE INFORMATION

Sample Name:	SP_C g70100	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	120711
Vial:	79	Acq. Method Set:	A70100t8Tamb
Injection #:	1	Processing Method:	Default
Injection Volume:	5.00 ul	Channel Name:	220.0nm
Run Time:	11.0 Minutes	Proc. Chnl. Descr.:	PDA 220.0 nm
Date Acquired:	7/12/2011 2:05:11 PM CEST		
Date Processed:	7/12/2011 2:20:35 PM CEST		

Auto-Scaled Chromatogram



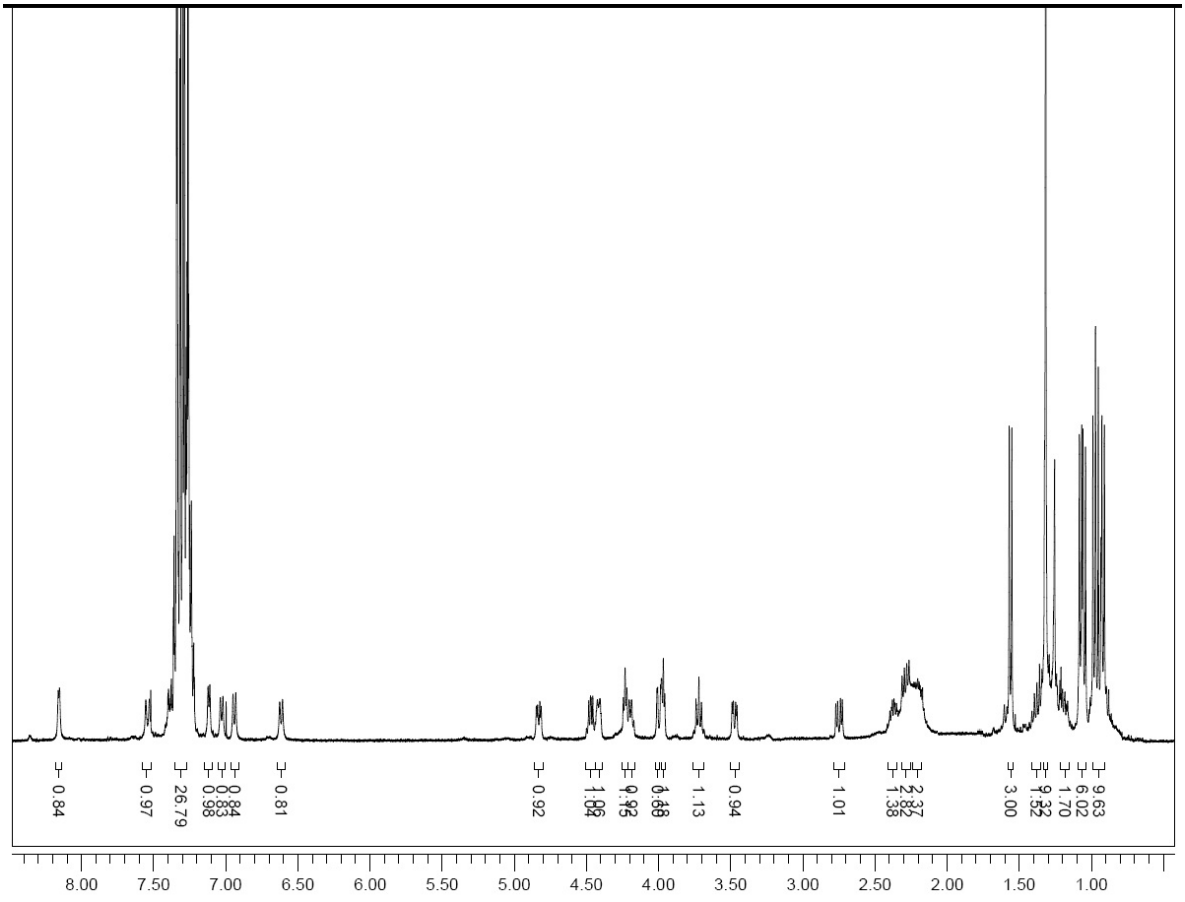
Peak Results

	RT	Area	Height	Total Area	% Area
1	1.092	89903	31302	5292937	1.70
2	2.786	4821112	897164	5292937	91.09
3	4.983	111093	20574	5292937	2.10
4	5.932	270829	47927	5292937	5.12

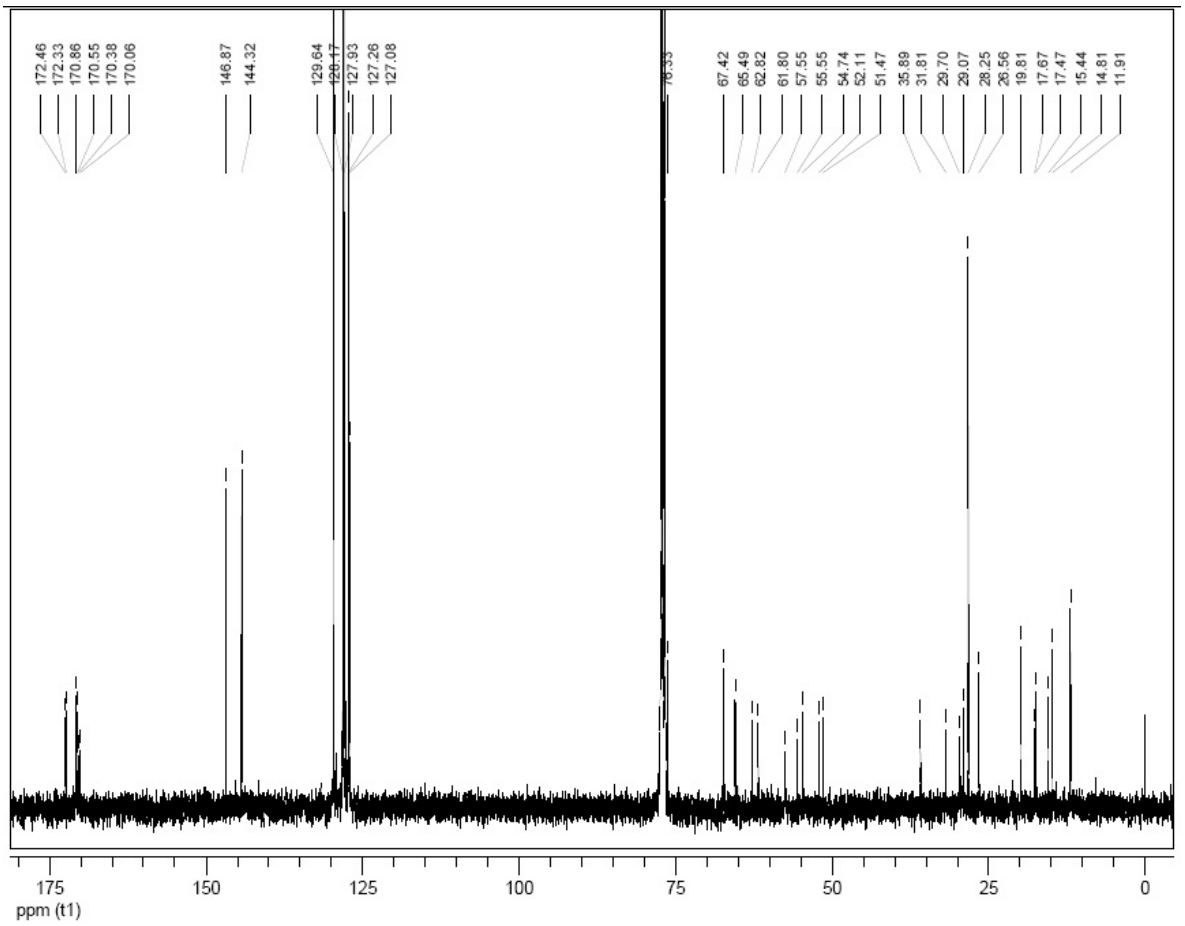
Reported by User: System
Report Method: Default
Report Method ID: 1624
Page: 1 of 2

Project Name: Juliol_2011
Date Printed:
7/12/2011
2:22:03 PM Europe/Madrid

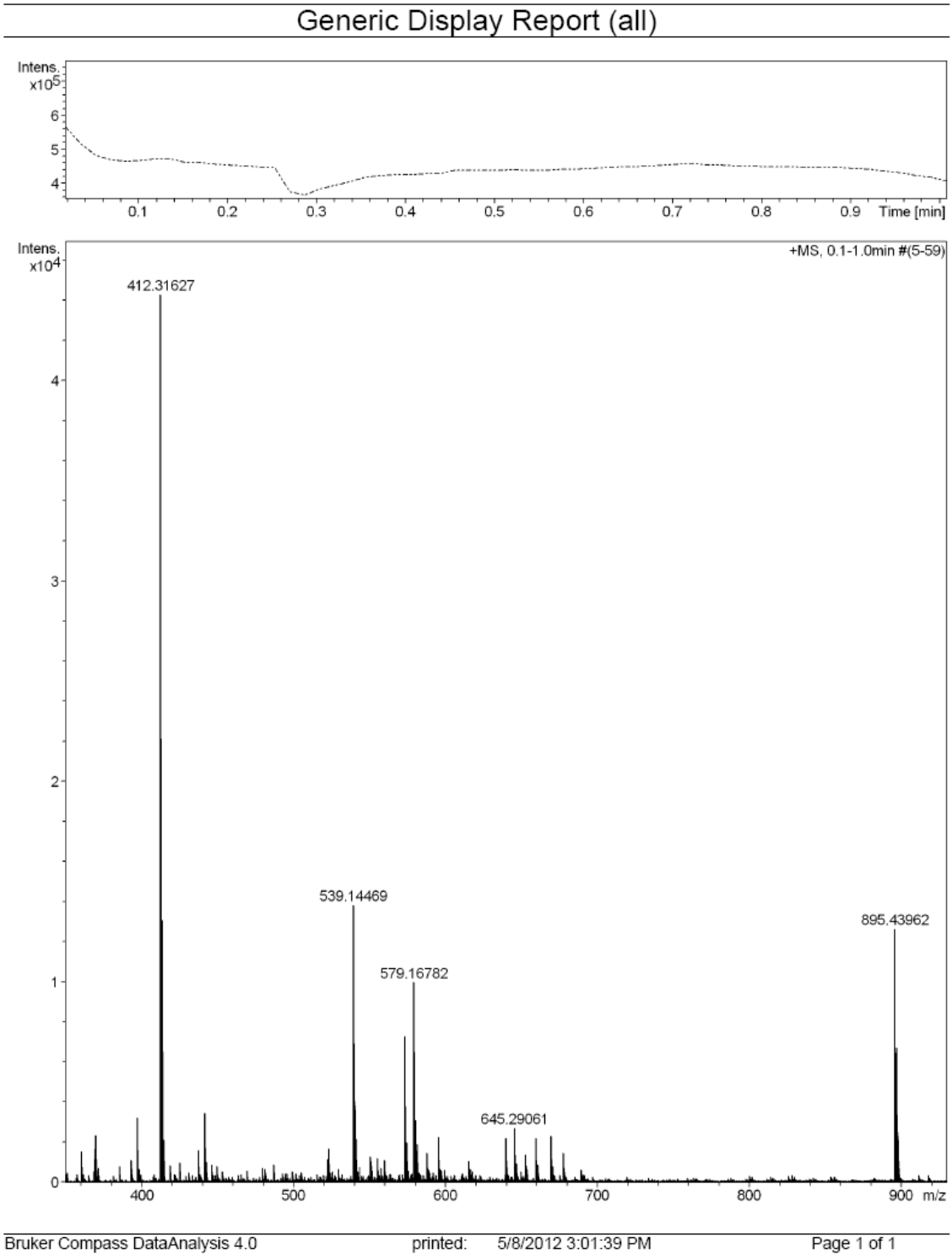
^{1}H-NMR Macrocycle 16



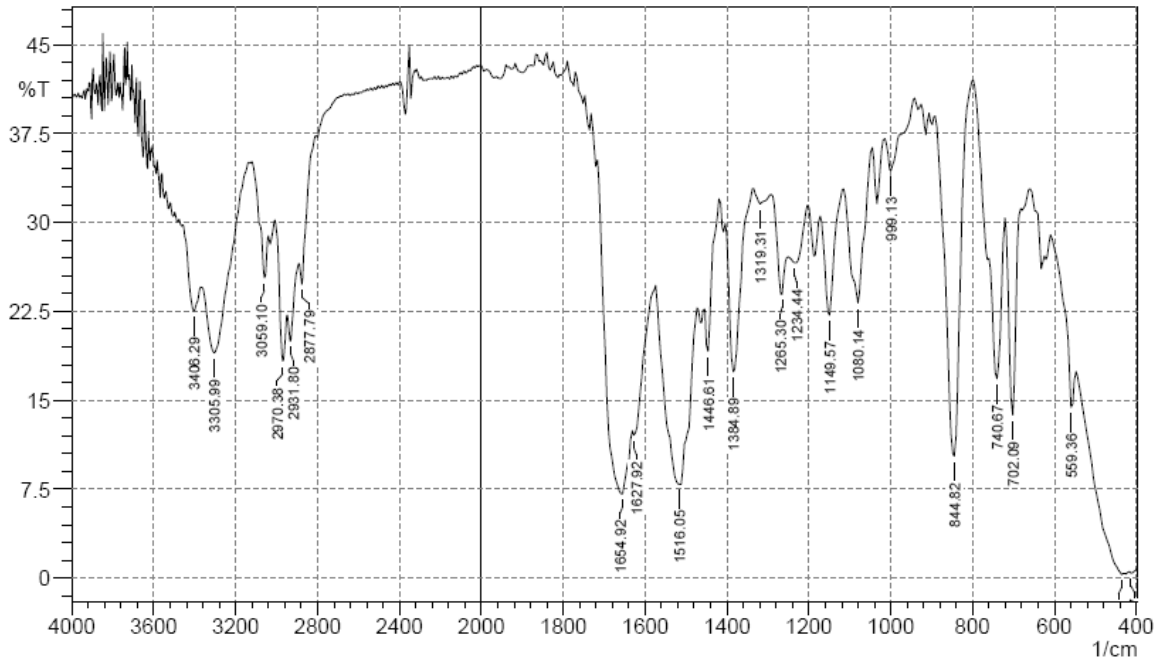
^{13}C-NMR Macrocycle 16



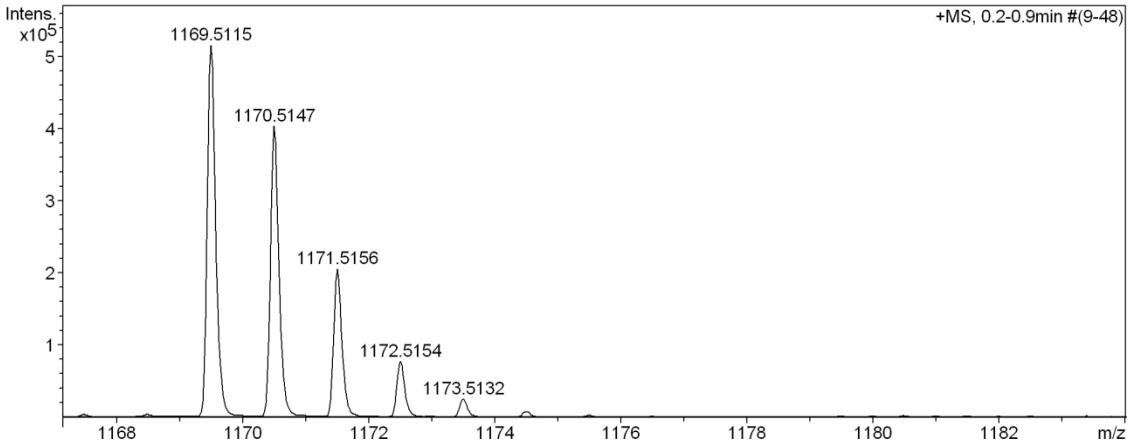
HRMS – Macrocycle 16



IR – Macrocycle 16



HRMS Hexapeptide precursosr of 17



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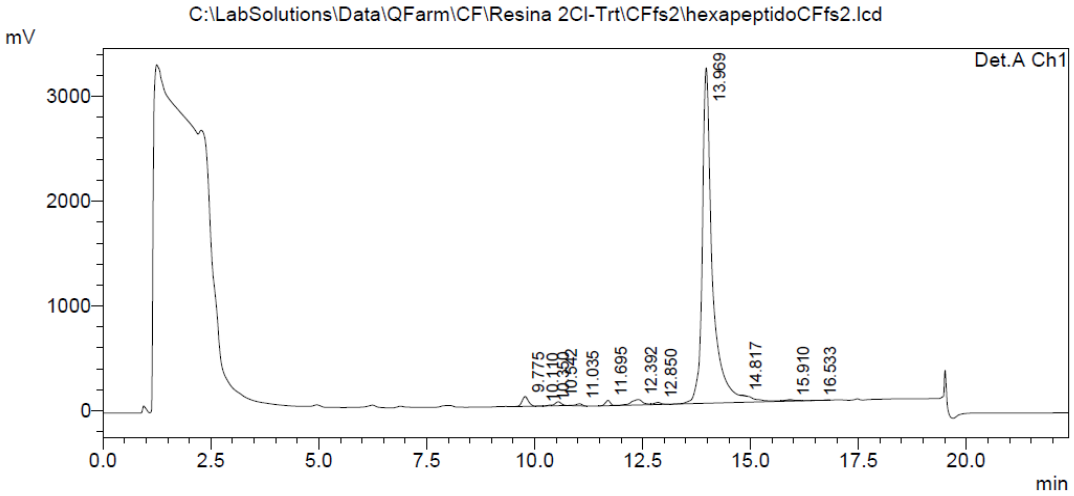


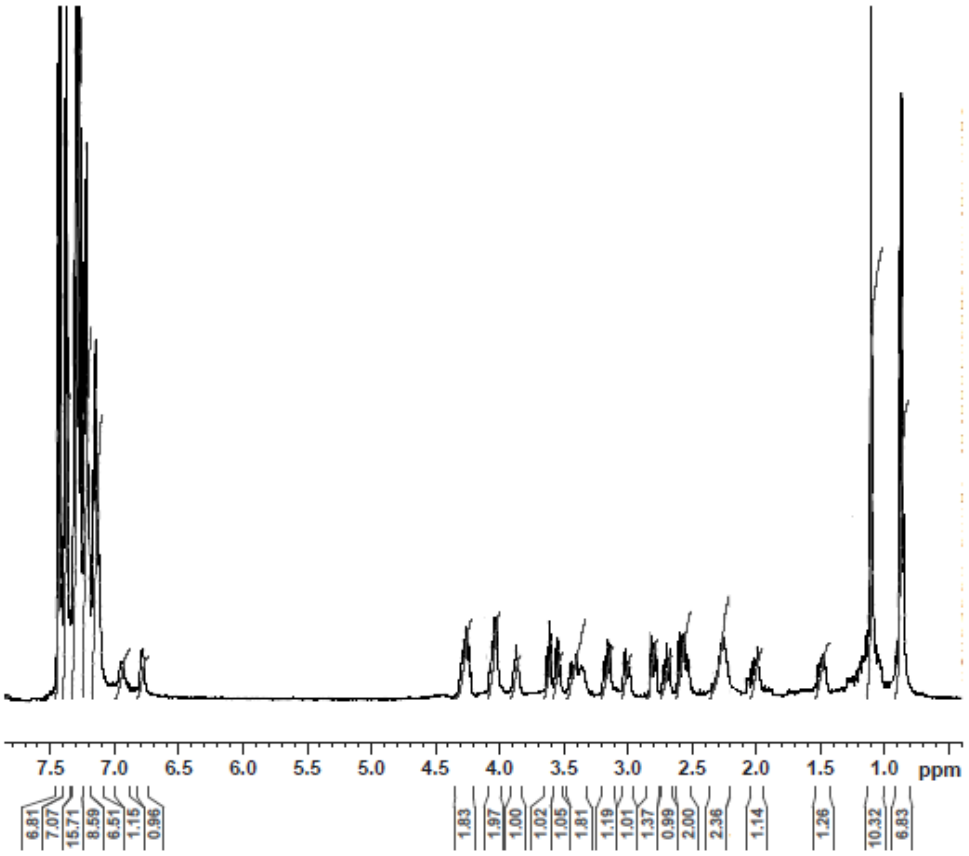
1 Det.A Ch1/220nm

PeakTable

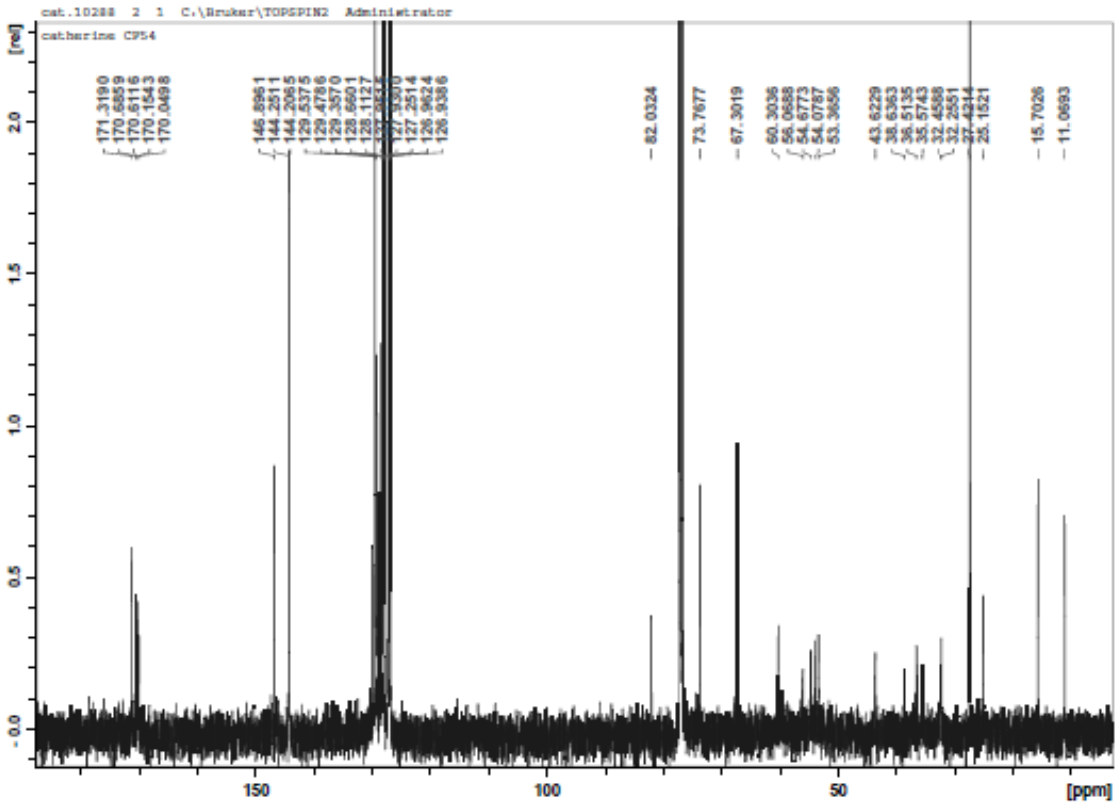
Detector A Ch1 220nm

Peak#	Name	Ret. Time	Area	Height	Area %	Height %
1		9.775	961601	93636	1.699	2.678
2		10.110	12094	1733	0.021	0.050
3		10.350	50826	7114	0.090	0.204
4		10.542	369030	37209	0.652	1.064
5		11.035	197095	19535	0.348	0.559
6		11.695	409603	50851	0.724	1.455
7		12.392	970179	51566	1.714	1.475
8		12.850	226428	20465	0.400	0.585
9		13.969	53265459	3199643	94.087	91.526
10		14.817	16515	3240	0.029	0.093
11		15.910	124001	10000	0.219	0.286
12		16.533	10124	906	0.018	0.026
Total			56612955	3495898	100.000	100.000

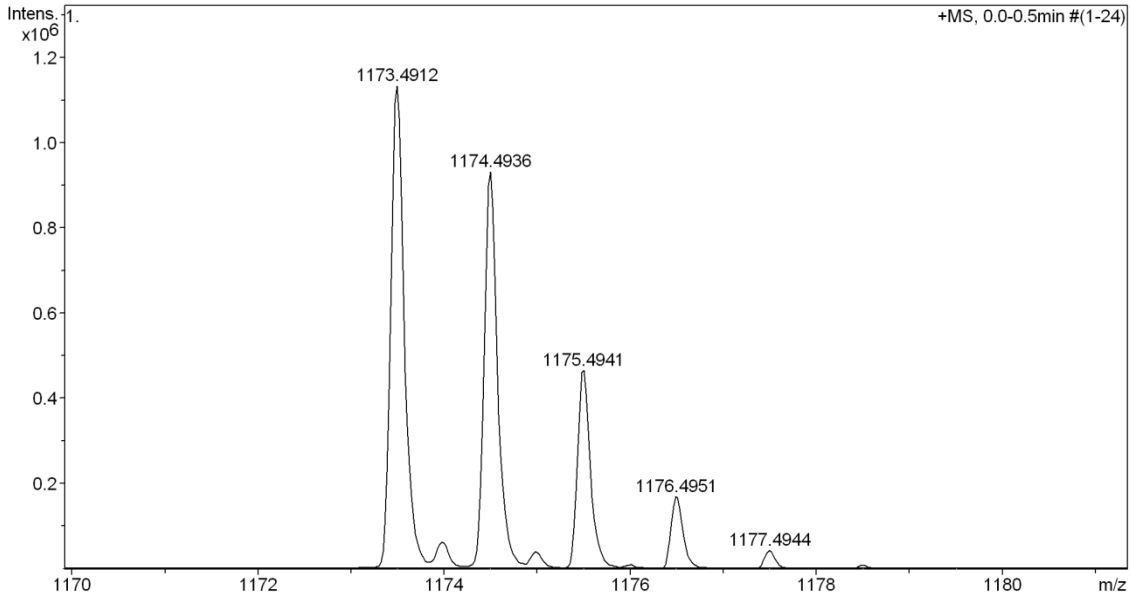
^{1}H-NMR Macrocycle 17



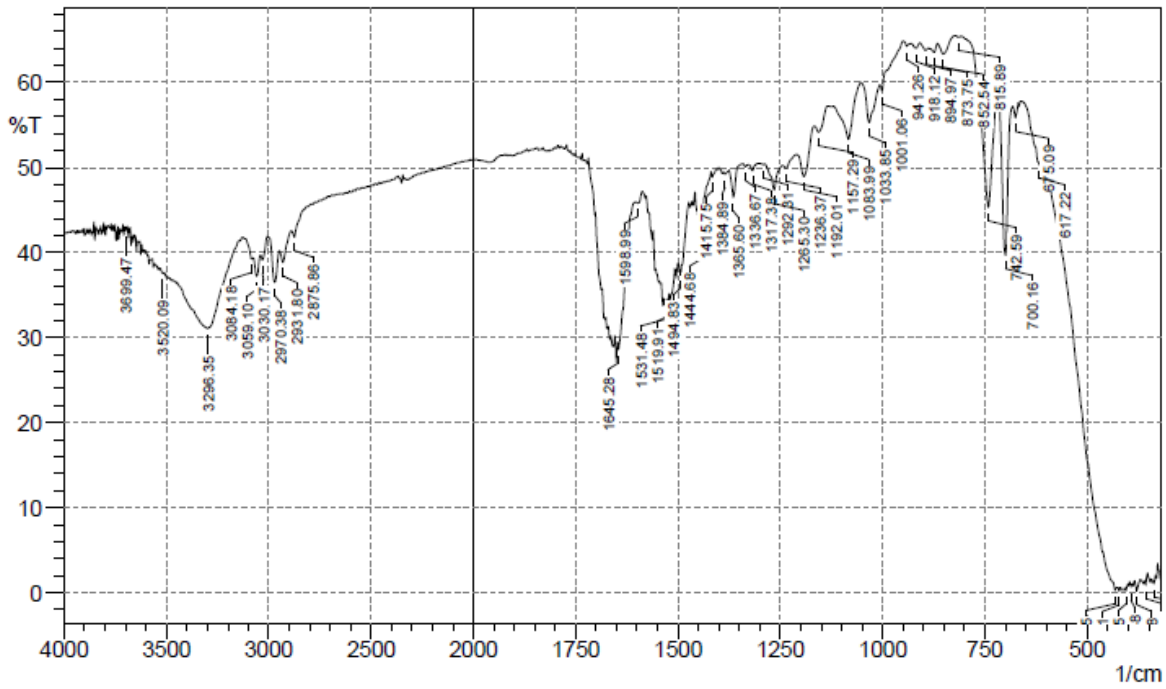
^{13}C-NMR Macrocycle 17



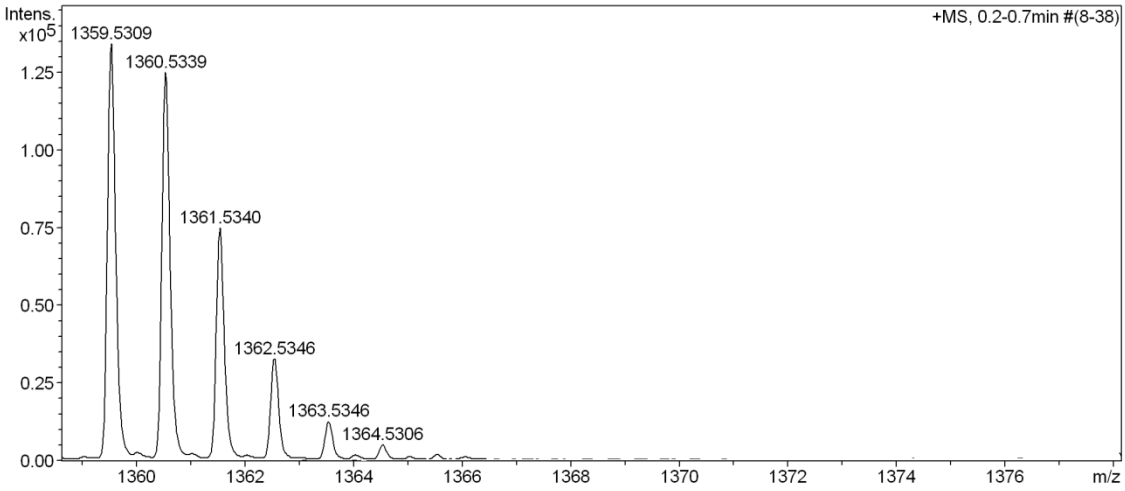
HRMS Macrocycle 17



IR Macrocycle 17



HRMS Hexapeptide precursor of 18



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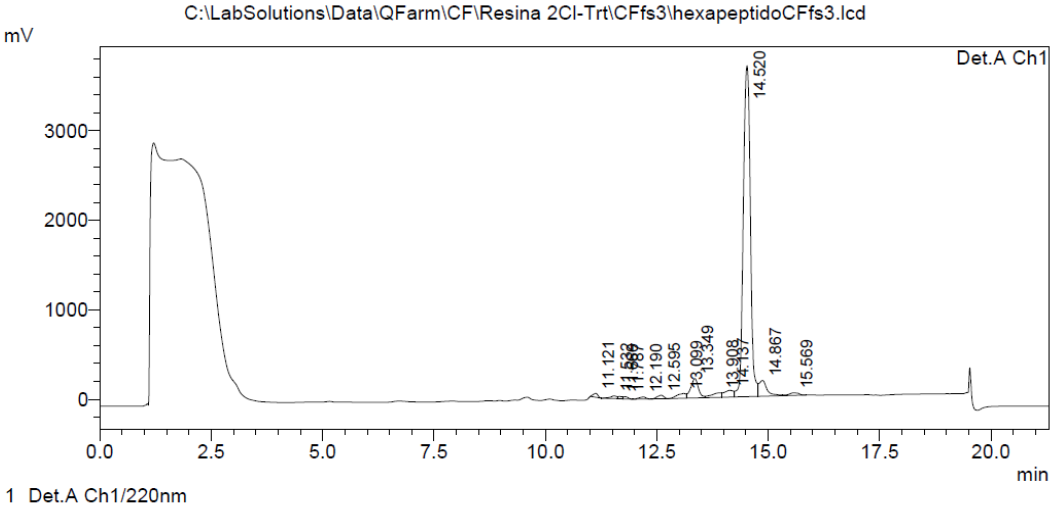


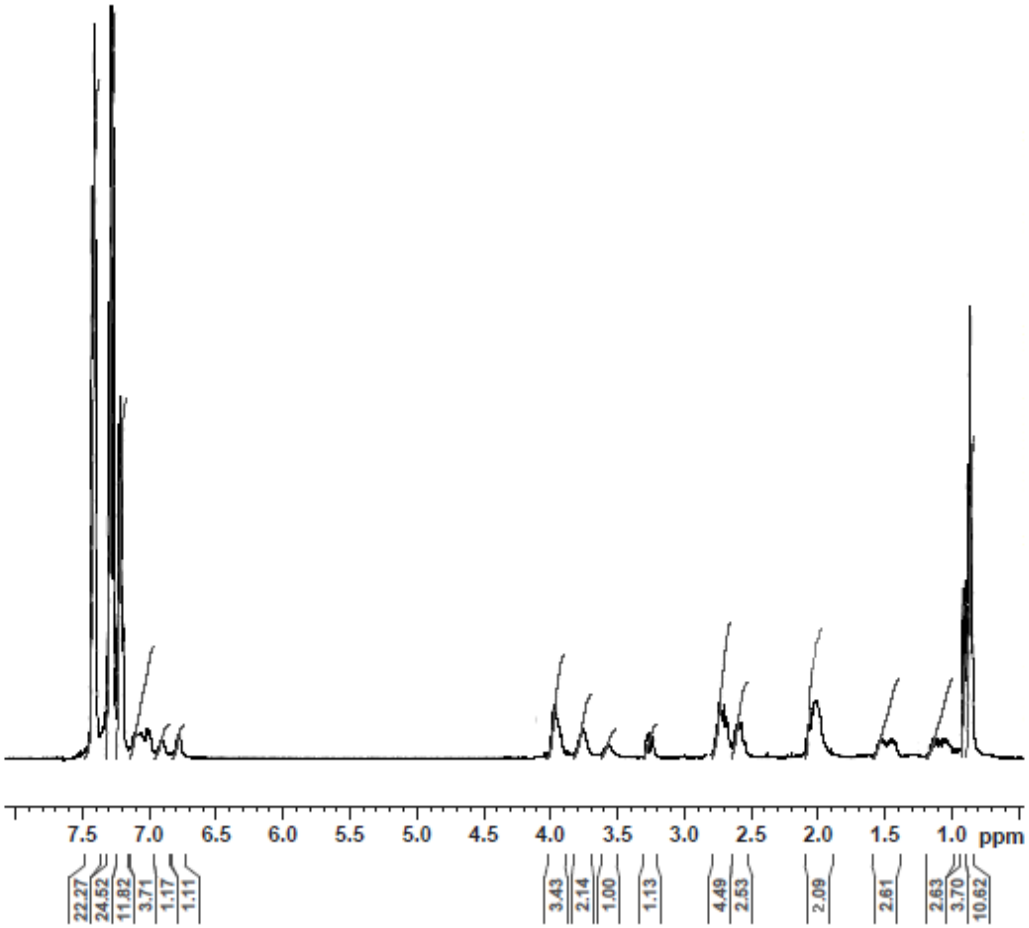
1 Det.A Ch1/220nm

PeakTable

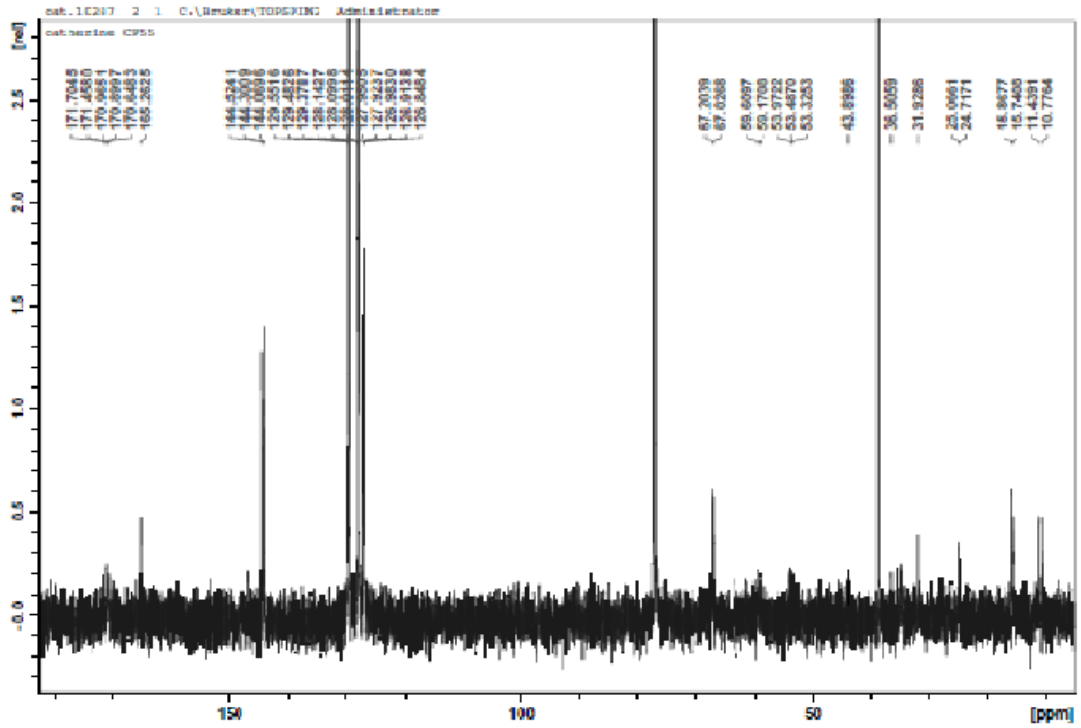
Detector A Ch1 220nm

Peak#	Name	Ret. Time	Area	Height	Area %	Height %
1		11.121	310905	42465	0.619	0.946
2		11.532	309360	30393	0.615	0.677
3		11.660	176765	24772	0.352	0.552
4		11.787	172274	24313	0.343	0.542
5		12.190	248240	23380	0.494	0.521
6		12.595	404787	39841	0.805	0.888
7		13.099	759445	53533	1.511	1.193
8		13.349	2560018	217768	5.093	4.853
9		13.908	795801	50060	1.583	1.116
10		14.137	1097192	75267	2.183	1.677
11		14.520	40779357	3699081	81.130	82.432
12		14.867	2186197	176302	4.349	3.929
13		15.569	463876	30262	0.923	0.674
Total			50264218	4487439	100.000	100.000

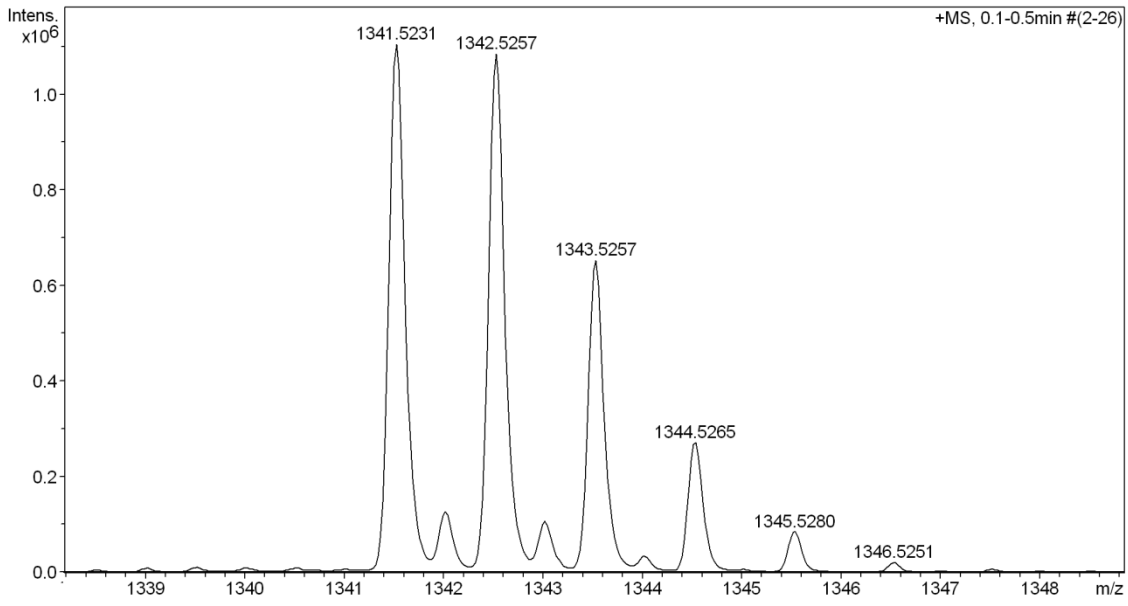
^{1}H-NMR Macrocycle 18



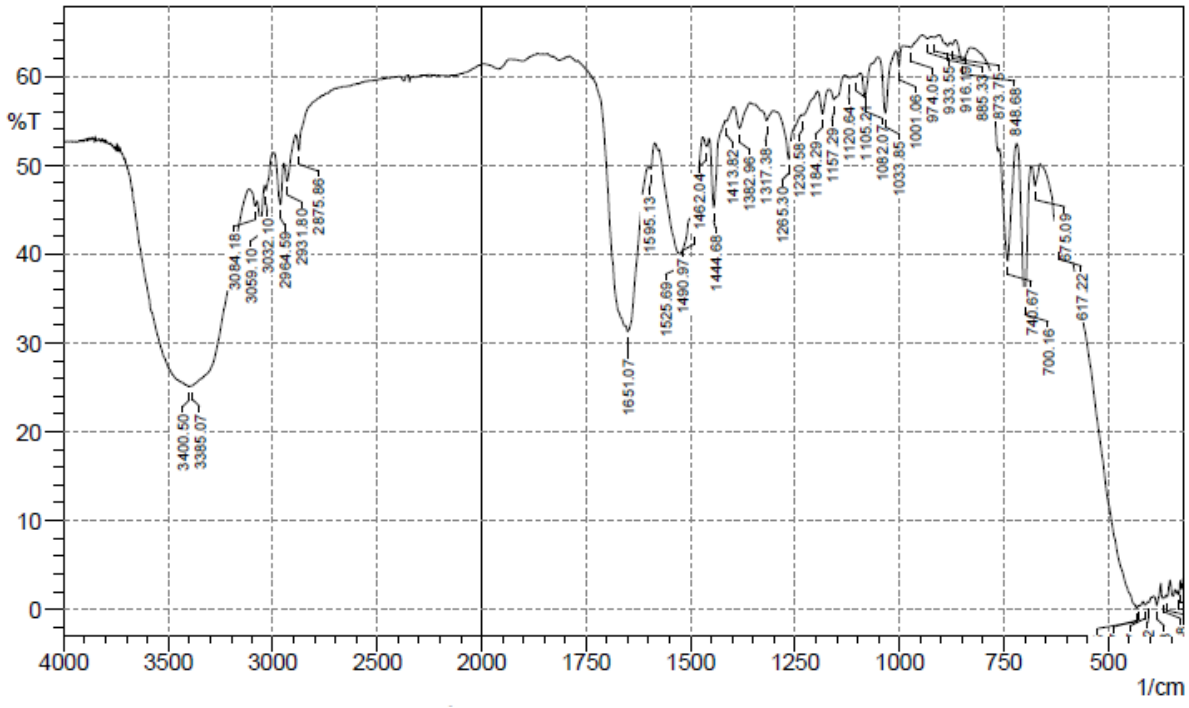
^{13}C-NMR Macrocycle 18



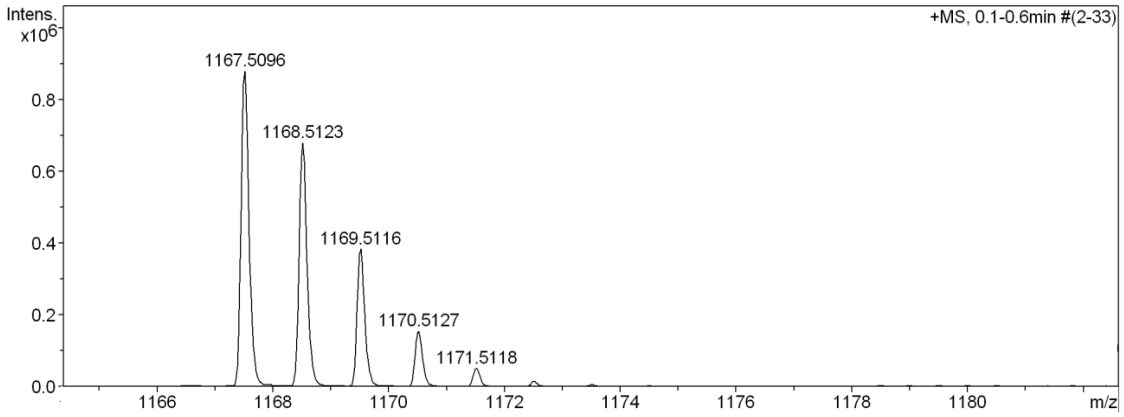
HRMS Macrocycle 18



IR Macrocycle 18



HRMS Hexapeptide precursor of 19



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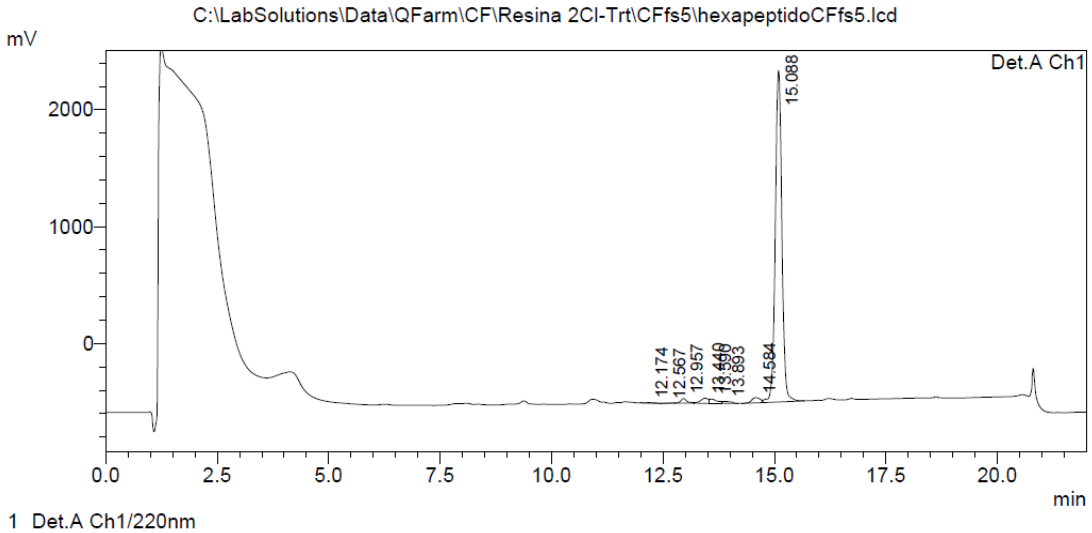


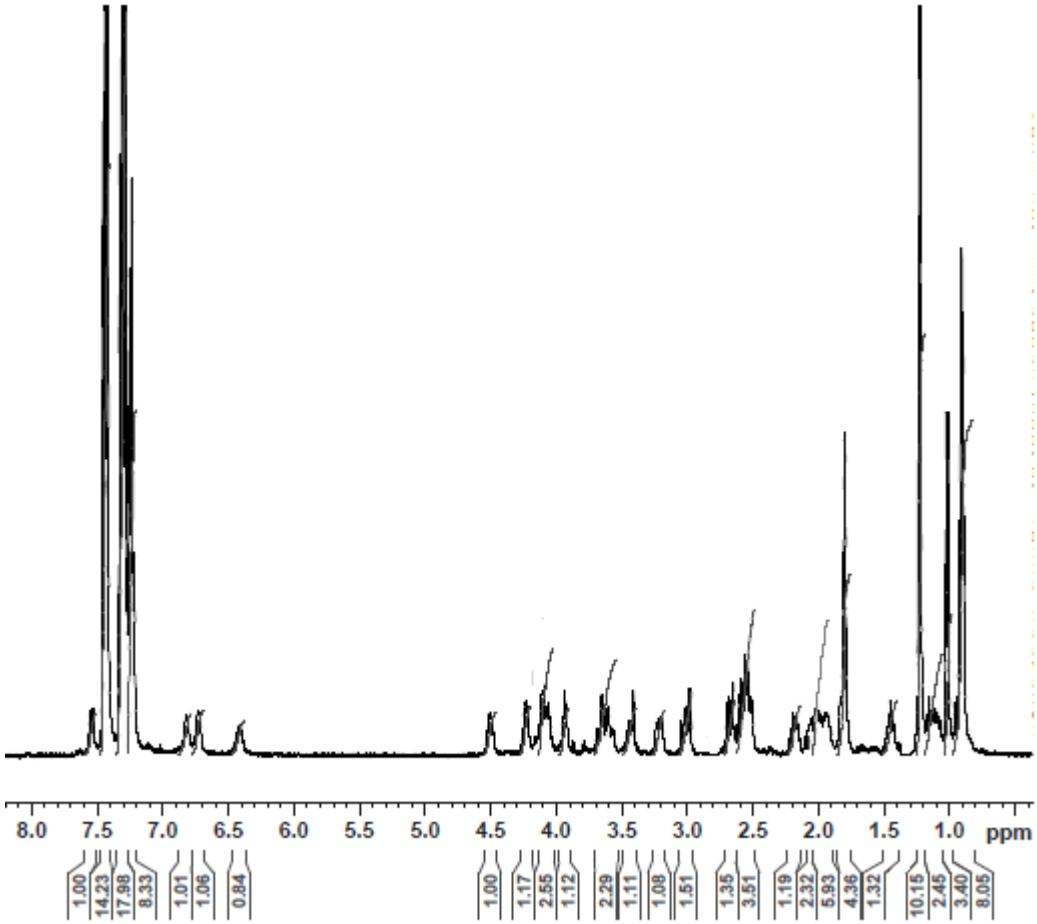
1 Det.A Ch1/220nm

PeakTable

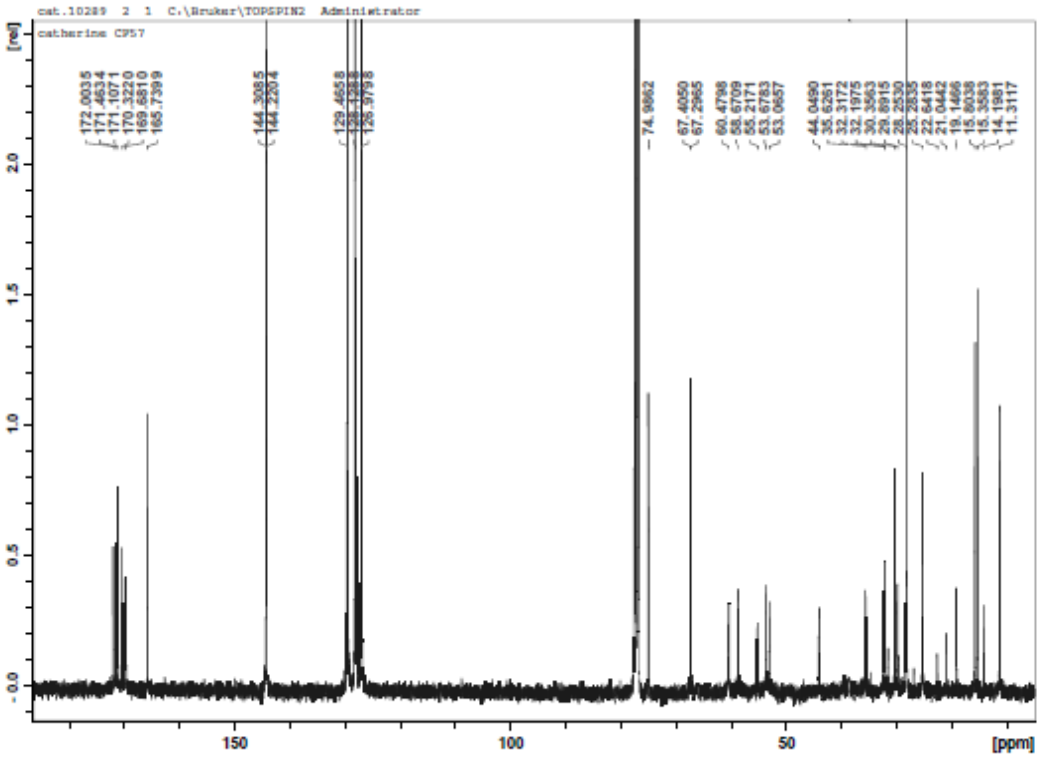
Detector A Ch1 220nm

Peak#	Name	Ret. Time	Area	Height	Area %	Height %
1		12.174	56053	3618	0.179	0.120
2		12.567	10761	1424	0.034	0.047
3		12.957	374190	39441	1.197	1.305
4		13.440	536313	43166	1.716	1.429
5		13.590	421540	36186	1.349	1.198
6		13.893	223347	16737	0.715	0.554
7		14.584	587317	45111	1.880	1.493
8		15.088	29038431	2835939	92.929	93.855
Total			31247952	3021622	100.000	100.000

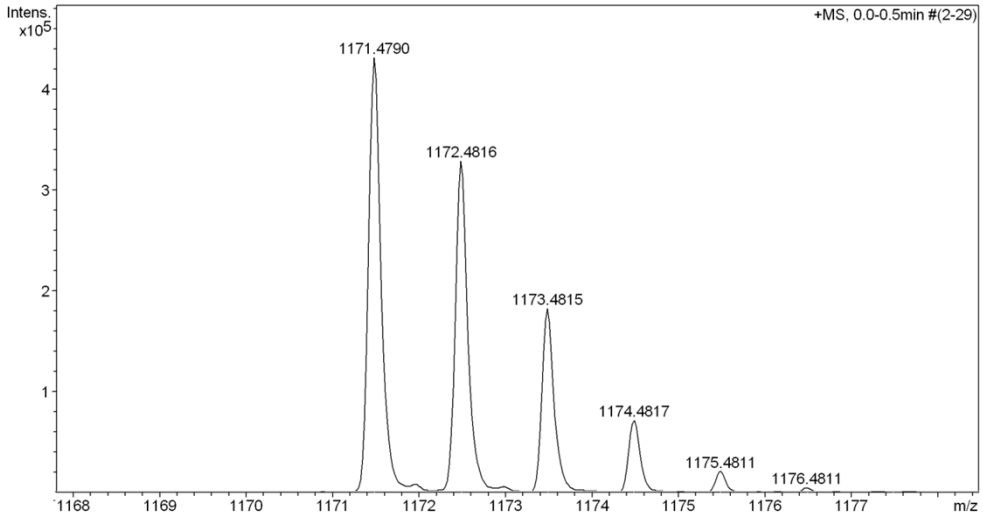
^{1}H-NMR Macrocycle 19



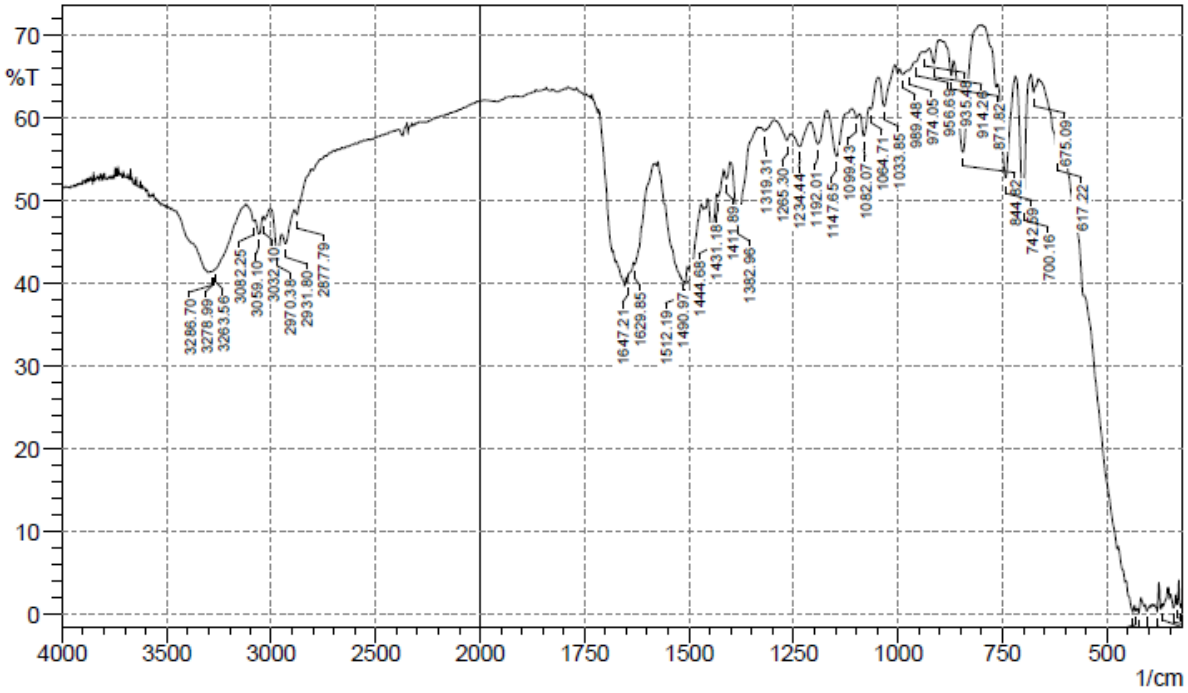
^{13}C-NMR Macrocycle 19



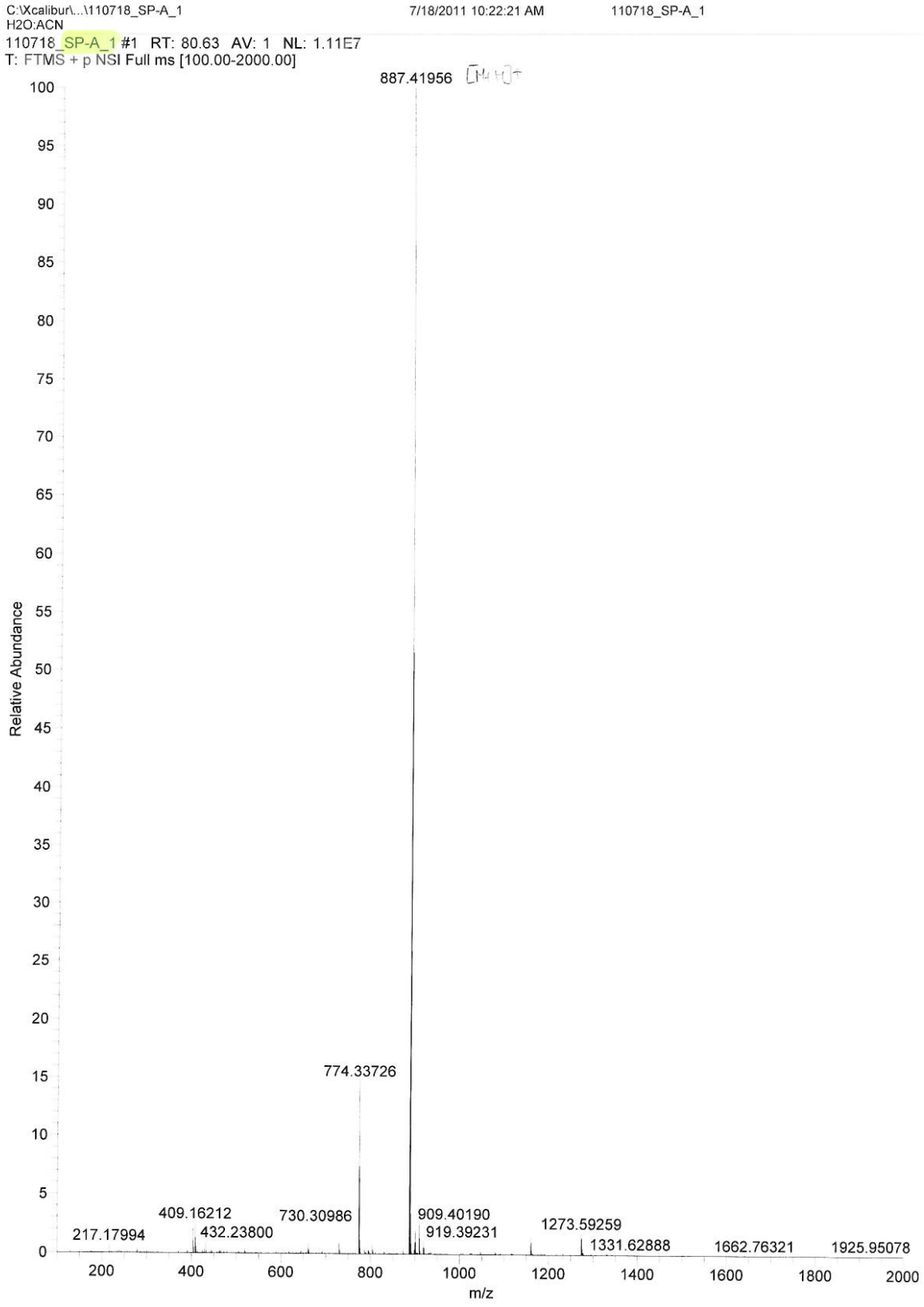
HRMS Macrocycle 19



IR Macrocycle 19



HRMS –Hexapeptide precursor of 20



HPLC – Hexapeptide precursor of 20

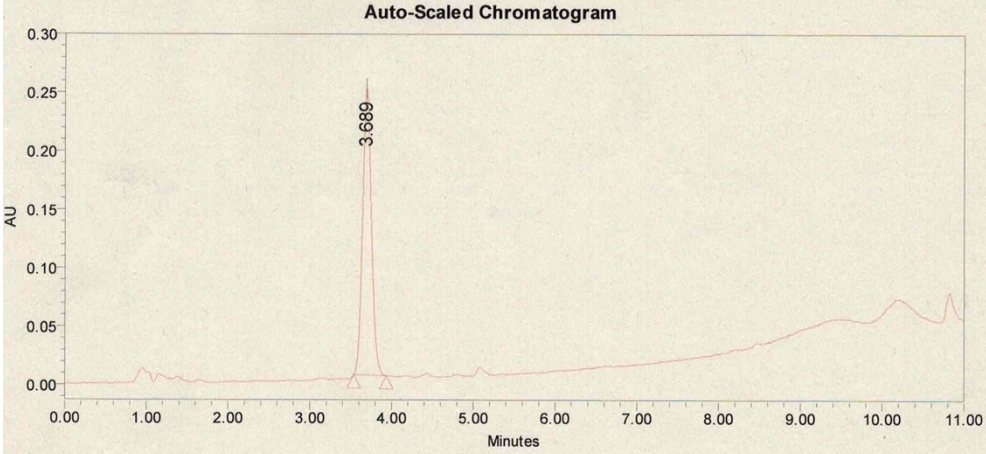
Empower 2 software

Default

SAMPLE INFORMATION

Sample Name:	SP_A g50100 escalado final	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	11072011
Vial:	76	Acq. Method Set:	A50100t8Tamb
Injection #:	1	Processing Method:	STE
Injection Volume:	20.00 ul	Channel Name:	220.0nm
Run Time:	11.0 Minutes	Proc. Chnl. Descr.:	PDA 220.0 nm
Date Acquired:	7/11/2011 3:12:32 PM CEST		
Date Processed:	7/11/2011 4:02:12 PM CEST		

Auto-Scaled Chromatogram



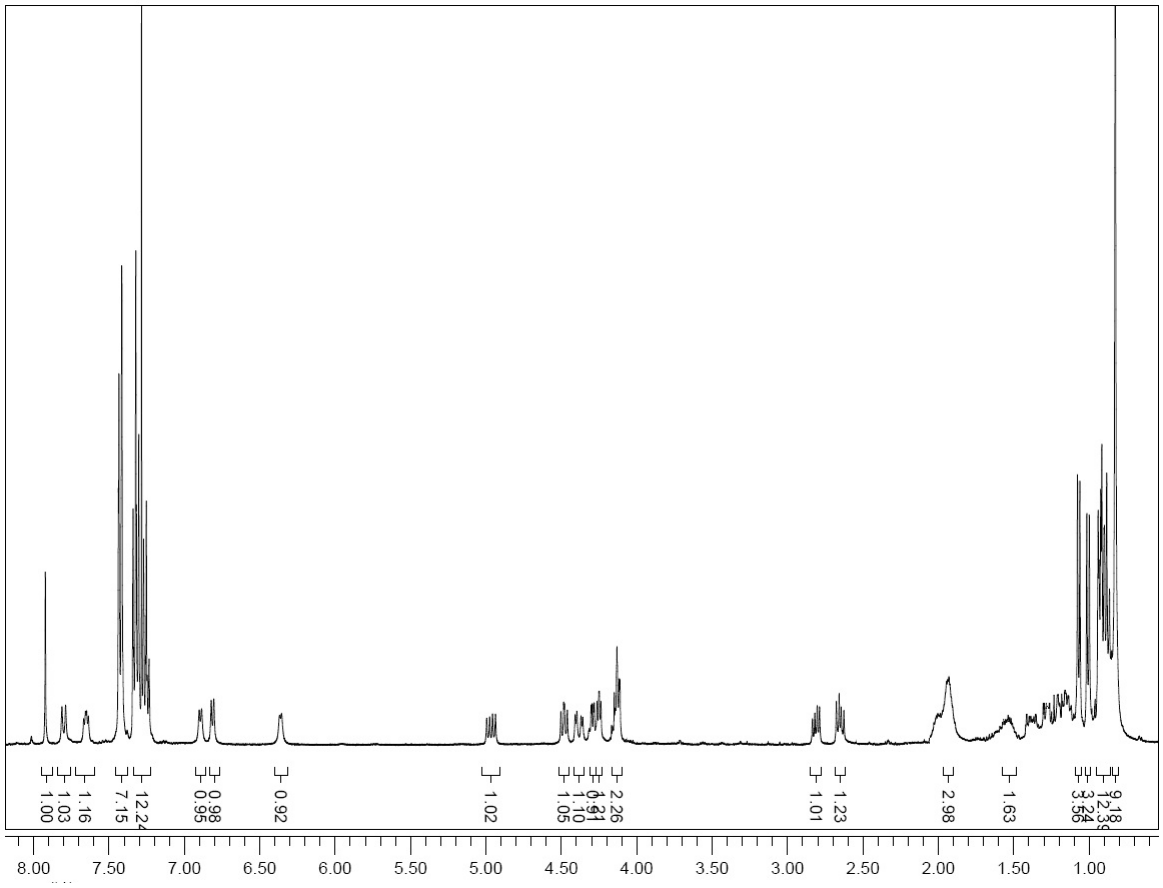
Peak Results

	RT	Area	Height	Total Area	% Area
1	3.689	1887481	244889	1887481	100.00

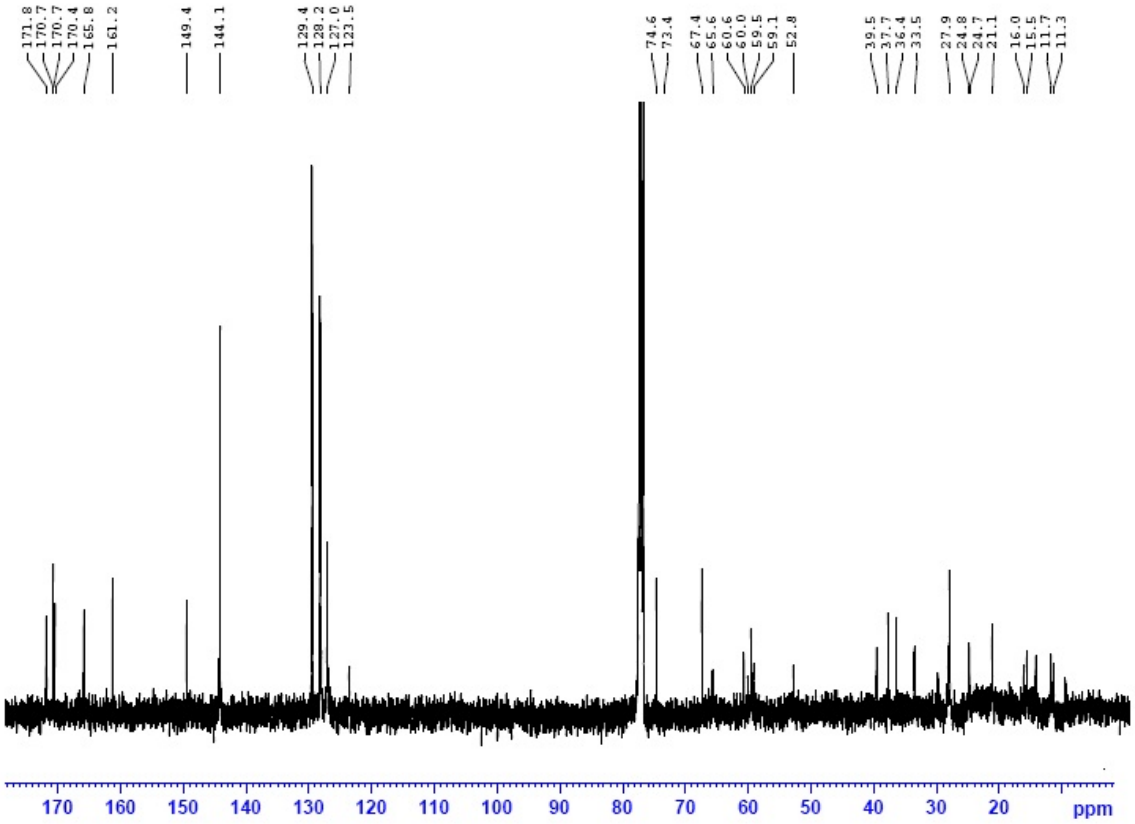
Reported by User: System
Report Method: Default
Report Method ID: 1309
Page: 1 of 2

Project Name: Juliol_2011
Date Printed:
7/11/2011
5:05:11 PM Europe/Madrid

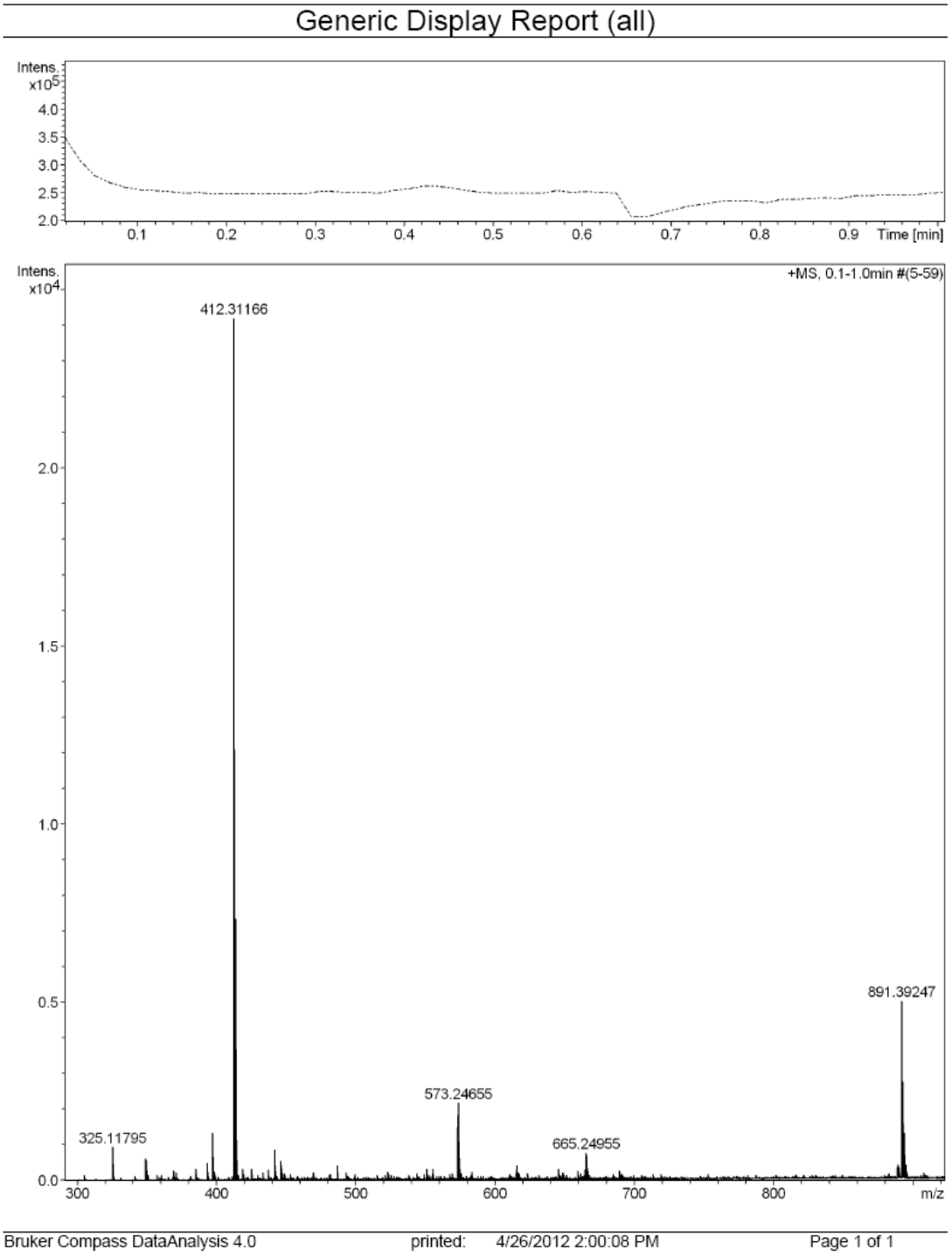
^{1}H-NMR Macrocycle 20



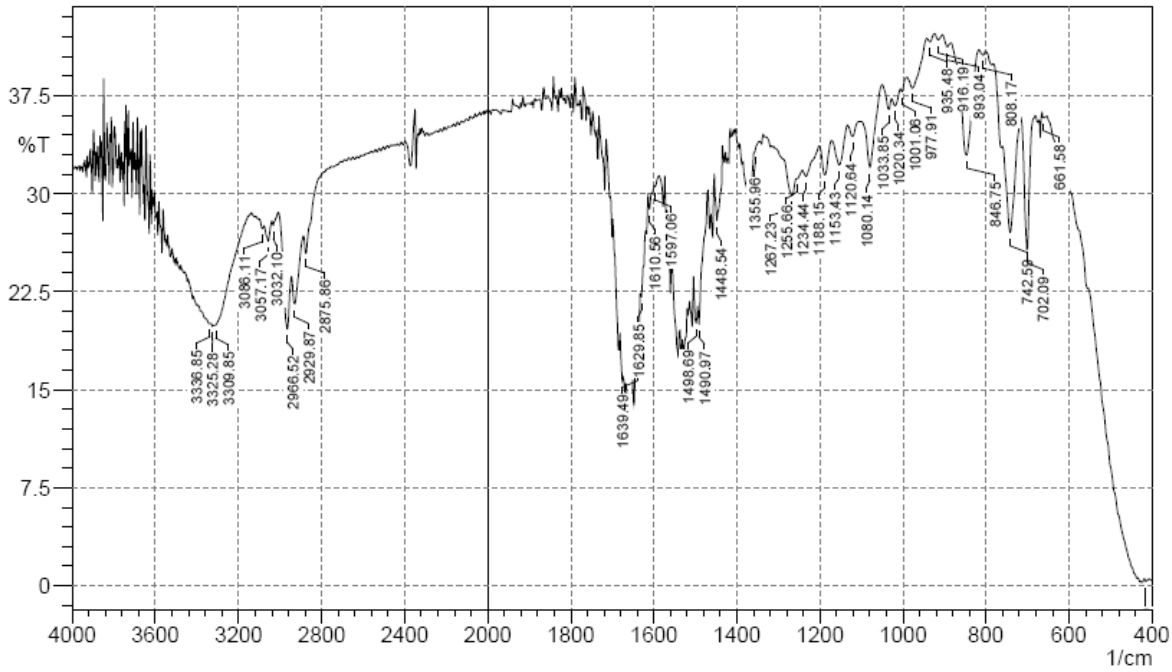
^{13}C-NMR Macrocycle 20



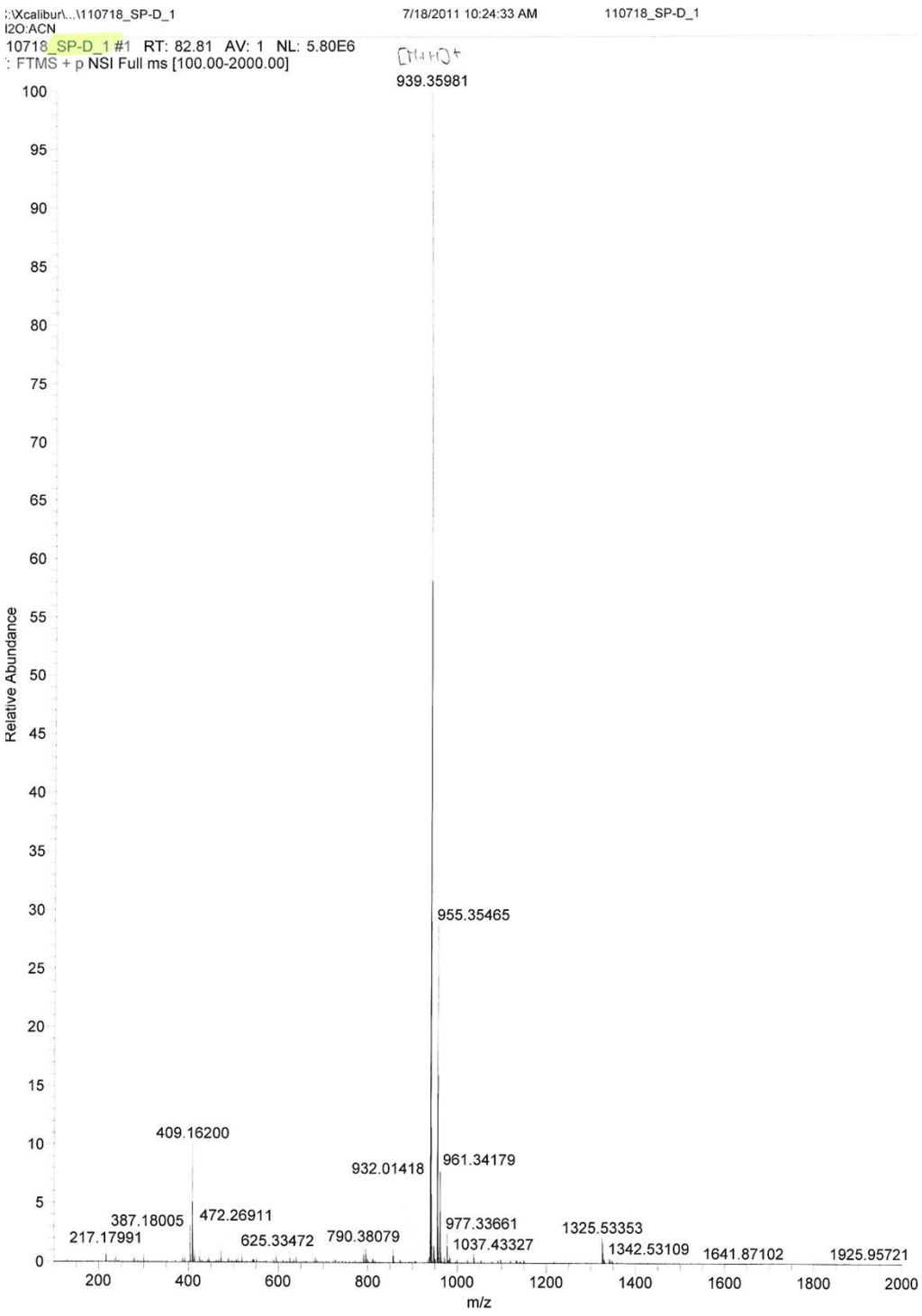
HRMS – Macrocycle 20



IR – Macrocycle 20



HRMS - Hexapeptide precursor 2o



HPLC – Hexapeptide precursor of 21

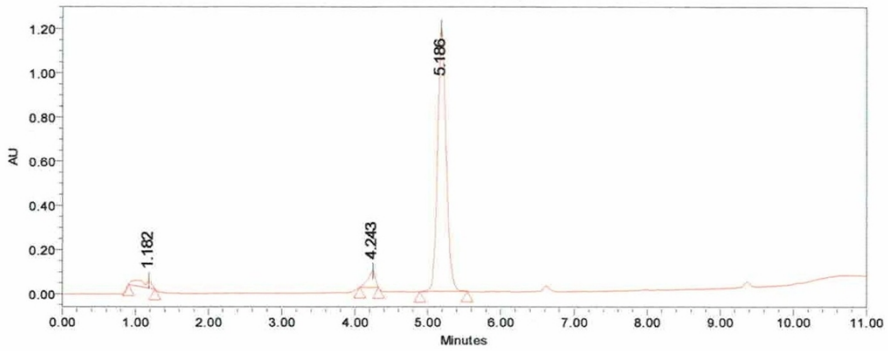
Empower 2 software

Default

SAMPLE INFORMATION

Sample Name:	SP_D g4090	Acquired By:	System
Sample Type:	Unknow n	Sample Set Name:	110711
Vial:	82	Acq. Method Set:	A4090t8Tamb
Injection #:	1	Processing Method:	ste
Injection Volume:	30.00 ul	Channel Name:	220.0nm
Run Time:	11.0 Minutes	Proc. Chnl. Descr.:	PDA 220.0 nm
Date Acquired:	7/11/2011 6:43:06 PM CEST		
Date Processed:	7/12/2011 12:23:13 PM CEST		

Auto-Scaled Chromatogram



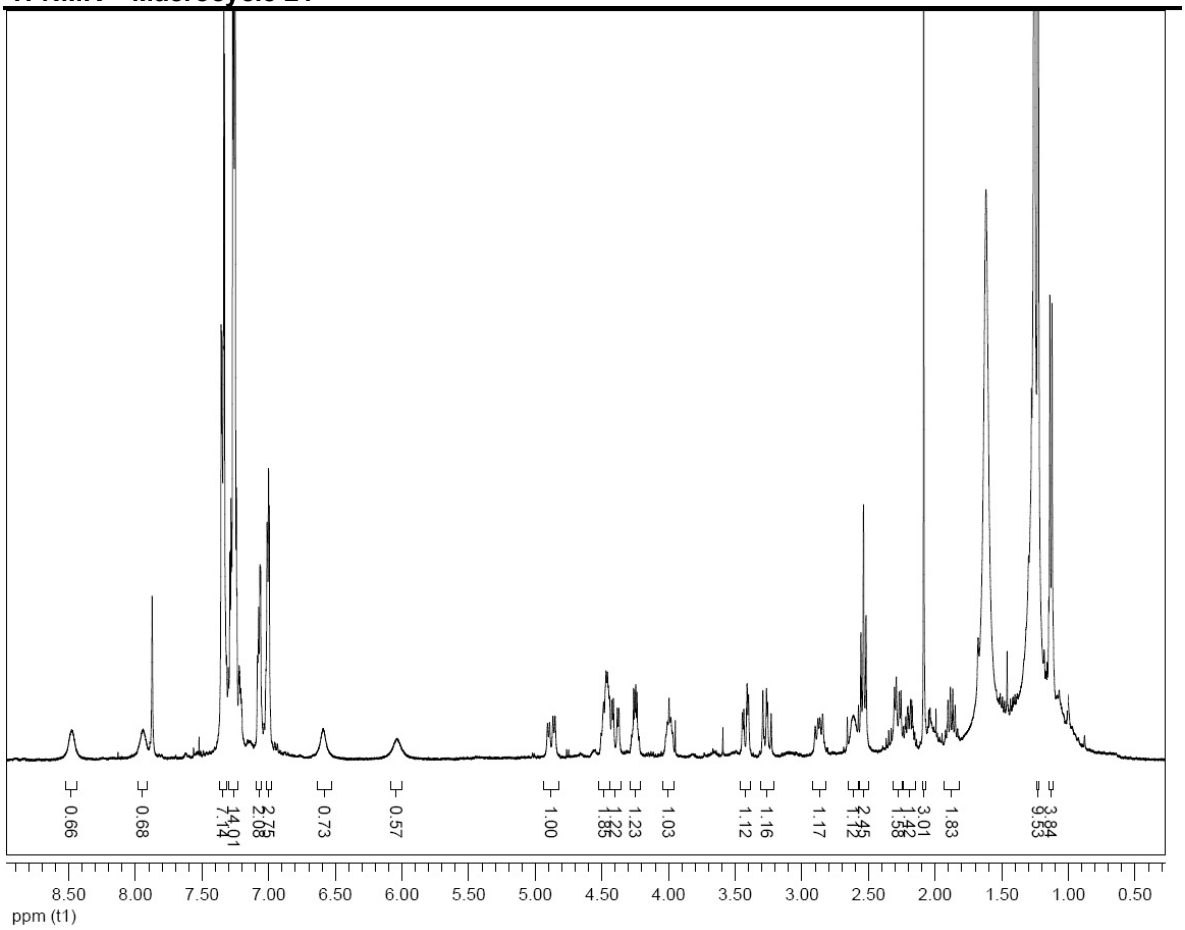
Peak Results

	RT	Area	Height	Total Area	% Area
1	1.182	441480	35089	10738355	4.11
2	4.243	540237	76398	10738355	5.03
3	5.186	9756638	1189291	10738355	90.86

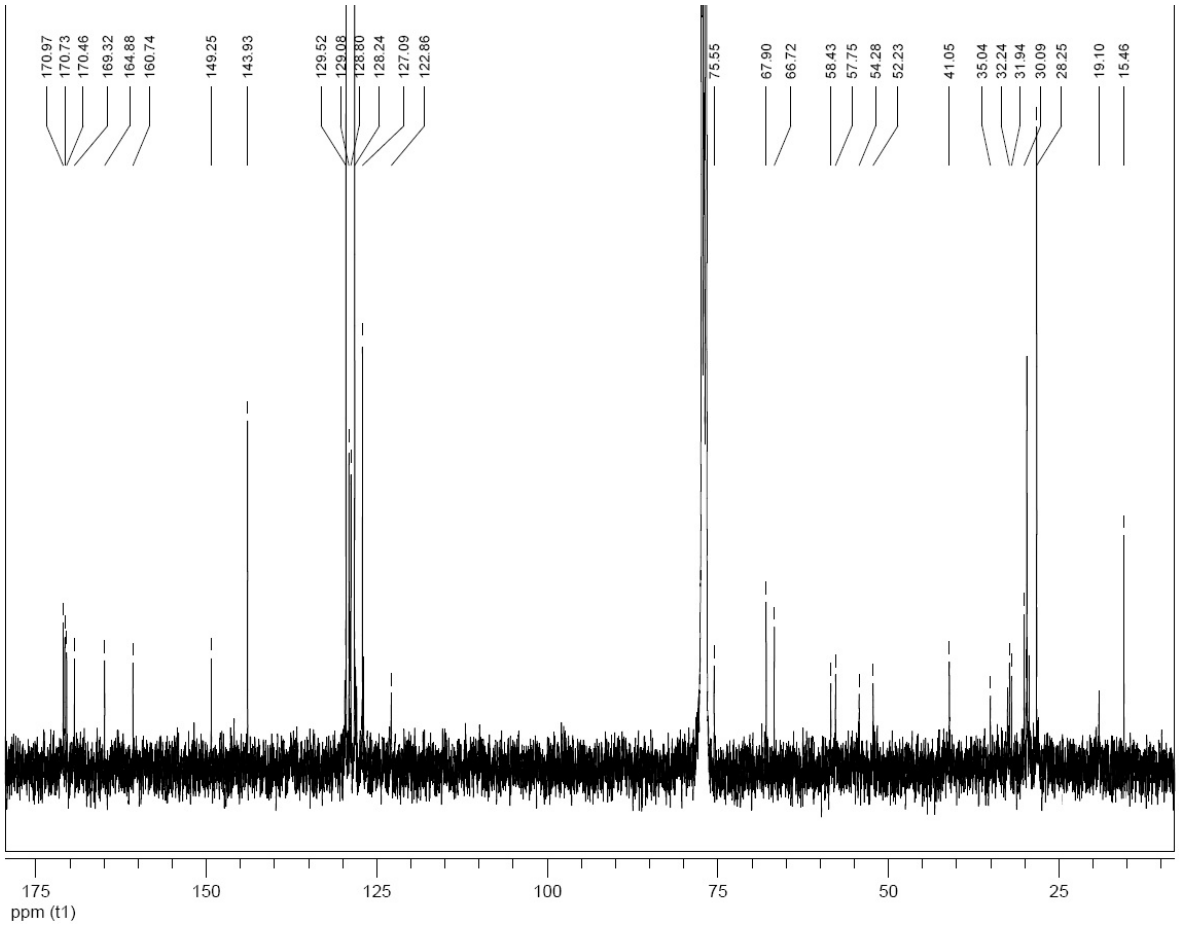
Reported by User: System
Report Method: Default
Report Method ID: 1567
Page: 1 of 2

Project Name: Juliol_2011
Date Printed:
7/12/2011
12:47:56 PM Europe/Madrid

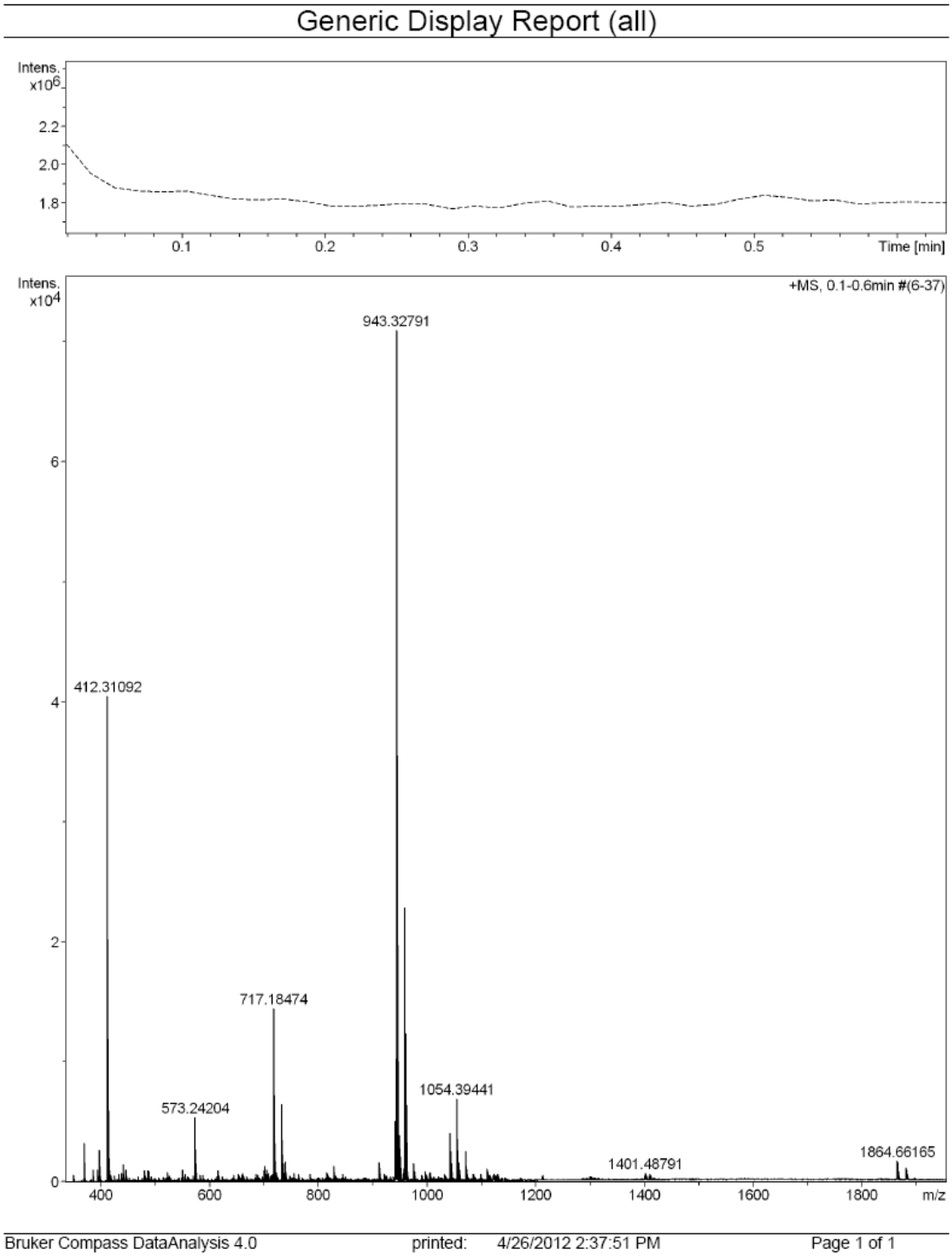
^{1}H-NMR – Macrocycle 21



^{13}C-NMR – Macrocycle 21



HRMS – Macrocycle 21



IR – Macrocycle 21