Supporting Information

Solid-Phase Polyamine Synthesis Using Piperazine and Piperidine Building Blocks

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General Methods

Unless otherwise stated, starting materials were obtained from commercial suppliers. (*S*)-*O*-(*tert*-Butyl)tyrosine and trityl resins (100-200 mesh, 1% DVB) were obtained from Novabiochem (Läufelingen, Switzerland). Tetrahydrofuran (THF) was distilled under N_2 from sodium/benzophenone immediately before use. Dry dichloromethane was distilled from P_2O_5 and kept over 4 Å molecular sieves. Water for reversed-phase high-performance liquid chromatography (HPLC) was filtered through a 0.22 µm pore filter. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400.14 MHz and 100.62 MHz, respectively, on a Bruker Avance 400 spectrometer, or at 600.13 and 150.92 MHz, respectively, on a Bruker Avance 600 spectrometer, using $CDCl_3$ or CD_3OD as solvent and TMS as internal standard Coupling constants (*J* values) are given in hertz (Hz), and multiplicities of ^{1}H NMR signals are reported as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad signal, and signals corresponding to the minor rotamers of the final products are marked with an asterisk (*). Vacuum liquid chromatography (VLC) was performed using Merck silica gel 60H, particle size < 45 µm (90%). Preparative HPLC system consisted of a Waters model 590 pump, a Waters Lambda-Max model 481 spectrophotometric detector and a 25 × 2.12 cm Phenomenex Luna 5 C18(2) column, 5 µm. The chromatograph operated isocratically at a flow-rate of 14 mL/min, using water/acetonitrile/TFA 82:18:0.1 as mobile phase. High-resolution mass (HRMS) measurements for exact mass determination were performed on a Bruker APEX III Fourier transform mass spectrometer equipped with a 7 tesla superconducting magnet and the external electrospray ion source (Apollo source). The spectra were externally calibrated with a capillary skimmer dissociation spectrum of LHRH (luteinizing hormone releasing hormone). The samples were introduced into the electrospray ion source using a 250 µL syringe, with a syringe pump flow 2 µL/min.

N-Teoc-4-[*O*-(2-nitrobenzenesulphonyl)ethanol]piperidine (7)

2-(4-Piperidyl)ethanol (1.176 g, 9.10 mmol) was dissolved in MeOH (15 mL) and 2-trimethylsilyl ethyl *p*-nitrophenylcarbonate (2.60 g, 9.10 mmol) in CH_2Cl_2 (15 mL) was added and the mixture was stirred at room temperature for 16 h. The methanol was removed in vacuo and EtOAc (10 mL) was added. The organic phase was washed with water (4 × 100 mL) and brine (2 × 10 mL), dried (Na_2SO_4), filtered, concentrated and dried in vacuo. This gave 2.15 g (87%) of the crude product as a yellow syrup. The crude product (2.09 g, 7.64 mmol) was dissolved in CH_2Cl_2 (20 mL) and treated with TEA (2.65 mL, 19.1 mmol, 2.5 eq) and NsCl (4.23 g, 19.1 mmol, 2.5 eq) in CH_2Cl_2 (10 mL). After stirring for 0.5 h at 0°C and 2 h at room temperature, the mixture was concentrated and the residue was loaded onto a VLC-column (6 × 6 cm). Elution with hexane/EtOAc (10:1, 6:1, 5:1 and 4:1) afforded 2.90 g (82%) of **7** as a colourless syrup. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.13 (ddd, *J* = 7.8 Hz, *J* = 1.4 Hz, *J* = 0.4 Hz, 2H), 7.84-7.75 (m, 3H), 4.34 (t, *J* = 6.0 Hz, 2H), 4.17-4.10 (m, 4H), 2.70 (br s, 2H), 1.71-1.64 (m, 5H), 1.09 (m, 2H), 0.99 (m, 2H), 0.03 (br s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 155.5, 148.5, 134.9, 132.2, 131.3, 129.5, 124.8, 69.9, 63.4, 43.8 (2 C), 35.3 (2 C), 32.1, 31.6, 17.8, -1.4 (3 C). HRMS: $C_{19}H_{30}N_2O_7SSiNa$ requires MNA^+ at m/z 481.14352; found, 481.14333 (ΔM: 0.42 ppm).

N-Aminoethyl-*N'*-Teoc piperazine (8)

N-(2-Aminoethyl)piperazine (0.50 mL, 3.81 mmol) was dissolved in MeOH (25 mL) and stirred at -50°C under N_2. Ethyl trifluoroacetate (0.50 mL, 4.19 mmol, 1.1 eq) in MeOH (2 mL) was added dropwise during 0.5 h, the temperature was allowed to reach 0°C, and the mixture was stirred for additional 0.5 h. A solution of 2-trimethylsilyl ethyl *p*-nitrophenyl carbonate (1.41 g, 4.95 mmol, 1.3 eq) in CH_2Cl_2 (10 mL) was added and the mixture was stirred for 16 h at room temperature. Water (2 mL) and concentrated NaOH (2 mL) were added and stirring was continued for 3 h. The methanol was removed in vacuo and EtOAc (150 mL) was added. The organic phase was washed with water (4 × 100 mL) and brine (2 × 100 mL), dried (Na_2SO_4), filtered, concentrated and dried in vacuo. The crude product was loaded directly onto a trityl chloride resin. ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.6, 63.5, 60.7 (2 C), 53.0 (2 C), 43.6, 38.5, 17.7, -1.4 (3 C).

Preparation of Resin 9

Trityl chloride resin (160 mg, 0.216 mmol) was suspended in CH_2Cl_2 (2 mL), compound **8** (300 mg, 1.10 mmol, 5 eq) in CH_2Cl_2 (2 mL) was added and the resin was shaken for 16 h. The resin was then drained, washed with CH_2Cl_2, DMF and CH_2Cl_2 (3 × 5 mL of each), and treated with $Bu_4NF \bullet 3\ H_2O$ (341 mg, 1.10 mmol, 5 eq) in DMF (3 mL) for 2 h. Washing with CH_2Cl_2, DMF and CH_2Cl_2 (3 × 5 mL of each) and drying in vacuo afforded resin **9**.

Compound 2

Solid-Phase Sulphonate Coupling and Removal of the Teoc Group

Compound **7** (297 mg, 0.648 mmol, 3 eq) in THF (2 mL) and DIEA (113 µL, 0.648, 3 eq) were added to the resin **9** (0.216 mmol) suspended in CH_2Cl_2 (2 mL) and the mixture was shaken at 50°C for 16 h. After cooling to room temperature the resin was drained and washed with CH_2Cl_2, DMF and CH_2Cl_2 (3 × 5 mL of each). The Teoc group was then removed by treatment with $Bu_4NF \bullet 3\ H_2O$ (341 mg, 1.10 mmol, 5 eq) in DMF (2 mL) for 30 min at 50°C. Washing with CH_2Cl_2, DMF and CH_2Cl_2 (3 × 5 mL of each) followed by drying in vacuo gave resin **10**.

Attachment of Tyrosine and Butyryl Residues

Resin **10** was suspended in DMF (1 mL). DIEA (98 µL, 0.56 mmol, 2.6 eq), HODhbt (35 mg, 0.216 mmol, 1 eq) in DMF (1 mL) and (*S*)-Fmoc-Tyr(Bu^t)-OPfp (405 mg, 0.648 mmol, 3 eq) in DMF (2 mL) were added succesively and the mixture was shaken at room temperature under N_2 for 5 h. The resulting resin was treated with piperidine in DMF (20% v/v, 2 mL) for 10 min at room temperature, and washed with DMF (3 × 5 mL), then treated with piperidine in DMF (20% v/v, 2 mL) for 10 min again, and washed with DMF, CH_2Cl_2 and DMF (3 × 5 mL each). After removal of the Fmoc group the resin was suspended in DMF (1 mL) and DIEA (98 µL, 0.56 mmol, 2.6 eq), HODhbt (35 mg, 0.216 mmol, 1 eq) in DMF (1 mL) and butanoic acid Pfp ester (165 mg, 0.648 mmol, 3 eq) in DMF (2 mL) were added succesively. The resulting mixture was shaken at room temperature under N_2 for 16 h. The resin was drained and washed with CH_2Cl_2, DMF and CH_2Cl_2 (3 × 5 mL of each) to give resin **11**.

Deprotection and Cleavage from the Resin

The resin was treated with CH_2Cl_2-TFA (50:50, v/v; 3 mL) at room temperature for 1 h. The drained solvent was combined with washings [MeOH (2 × 5 mL) and CH_2Cl_2 (2 × 5 mL)], evaporated in vacuo and purified by RP-18 VLC (H_2O-MeCN-TFA (95:5:0.1, 90:10:0.1, 85:15:0.1 and 80:20:0.1), followed by reversed phase HPLC [H_2O-MeCN-TFA (82:18:0.1)] to give 50 mg (28%) of the final product (**2**) as a clear syrup. ^{1}H NMR (400 MHz, CD_3OD): δ 7.06/7.01* (each, d, *J* = 8.4 Hz, 2 H, Ar-H), 6.73/6.69* (each, d, *J* = 8.4 Hz, 2 H, Ar-H), 4.98 (m, 1 H, H-α), 4.44 (br d, *J* = 12.3 Hz, 1 H, $C\mathit{H_2}NCO_{eq1}$), 3.96/3.93* (each, br d, *J* = 12.7 Hz, 1 H, $C\mathit{H_2}NCO_{eq2}$), 3.67-2.38 (20 H), 2.17/2.15* (t, *J* = 7.5 Hz, 2 H, CH_2CO), 1.71-1.08 (7 H), 1.60/1.58* (m, 2 H, $\mathit{CH_2}CH_2CO$), 0.91/0.88* (t, *J* = 7.5 Hz, 3 H, CH_3). ^{13}C NMR (100 MHz, CD_3OD): δ 175.5 (CO), 171.8 (CO), 157.6*/157.5 (Ar-C_{para}), 131.9/131.4* (2 C), 128.9/128.8* (Ar-C_{ipso}), 116.6/116.2* (2 C), 55.8*/55.7 ($\mathit{C}H_2N(CH_2)_2$), 54.5 ($\mathit{C}H_2N(CH_2)_2$), 53.0 (2 C), 51.8*/51.4 (C-α), 50.6 (2 C), 47.1*/46.8 ($\mathit{C}H_2NCO$), 43.4/43.3* ($\mathit{C}H_2NCO$), 39.0*/38.6 (C-β), 38.5 ($\mathit{C}H_2CO$), 37.3 (CH_2NH_2), 34.9*/34.4 (CH), 33.1*/32.3, 32.4*/32.3, 31.0, 20.2 ($\mathit{C}H_2CH_3$), 13.9 (CH_3). ESI-MS: 474.4 (M + 1). HRMS: $C_{26}H_{44}N_5O_3$ requires M + 1 at m/z 474.34387; found, 474.34373 (ΔM: 0.30 ppm).

Compound 3

Piperazine bound to trityl resin was treated as described above for **2**. The crude was purified by RP-18 VLC (H_2O-MeCN-TFA (95:5:0.1, 90:10:0.1, 85:15:0.1 and 80:20:0.1), followed by reversed phase HPLC [H_2O-MeCN-TFA (82:18:0.1)] to give 50 mg (60%) of the final product (**3**) as a clear syrup. ^{1}H NMR (400 MHz, CD_3OD): δ 7.06/7.01* (each, d, *J* = 8.4 Hz, 2 H, Ar-H), 6.75/6.69* (each, d, *J* = 8.4 Hz, 2 H, Ar-H), 4.98 (m, 1 H, H-α), 4.43 (br d, *J* = 12.3 Hz, 1 H, $C\mathit{H_2}NCO_{eq1}$), 3.91/3.89* (2 br d, *J* = 12.7 Hz, 1 H, $C\mathit{H_2}NCO_{eq2}$), 3.48-3.30 (8 H), 3.13-2.78 (6 H), 2.59*/2.50 (each, br t, *J* = 12.3 Hz, *J* = 12.7 Hz, 2 H, $C\mathit{H_2}NCO_{ax}$), 2.18/2.15* (each, t, *J* = 7.4 Hz, 2 H, $C\mathit{H_2}CO$), 1.71-1.08 (7 H), 1.60/1.58* (m, 2 H, $C\mathit{H_2}CH_2CO$), 0.91/0.88* (each, t, *J* = 7.4 Hz, 3 H, $C\mathit{H_3}$). ^{13}C NMR (100 MHz, CD_3OD): δ 175.5 (CO), 171.8 (CO), 157.6*/157.5 (Ar-C_{para}), 131.9/131.4* (2 C), 128.8/128.7* (Ar-C_{ipso}), 116.6/116.2* (2 C), 56.1*/56.0 ($\mathit{C}H_2N(CH_2)_2$), 51.8*/51.4 (C-α), 50.0 (2 C), 47.1*/46.9 ($\mathit{C}H_2NCO$), 43.5/43.4* ($\mathit{C}H_2NCO$), 42.8 (2 C), 39.0*/38.6 (C-β), 38.5 ($\mathit{C}H_2CO$), 34.9*/34.4 (CH), 33.3*/32.4, 32.5*/32.3, 31.7, 20.2 ($\mathit{C}H_2CH_3$), 13.9 (CH_3). HRMS: $C_{24}H_{39}N_4O_3$ requires M + 1 at m/z 431.30167 found, 431.30161 (ΔM: 0.14 ppm).

Compound 4

Solid-Phase Sulphonate Coupling and Removal of the Teoc Group

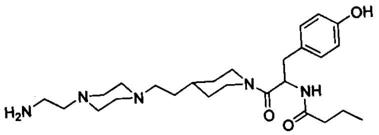
Compound **7** (205 mg, 0.446 mmol, 3 eq) in THF (2 mL) and DIEA (78 µL, 0.446, 3 eq) were added to trityl resin bound piperazine (0.149 mmol) suspended in toluene (2 mL) and shaken at 50°C for 16 h. After cooling to room temperature the resin was washed with CH_2Cl_2, DMF and CH_2Cl_2 (3 × 5 mL of each), and the procedure was repeated, shaking for 2 h. The Teoc group was then removed by treatment with Bu_4NF • 3 H_2O (235 mg, 0.745 mmol, 5 eq) in DMF (2 mL) for 30 min at 50°C. Washing with CH_2Cl_2, DMF and CH_2Cl_2 (3 × 5 mL of each) followed by drying in vacuo gave resin **12**. Coupling and Teoc removal as described above was repeated to give resin **13**.

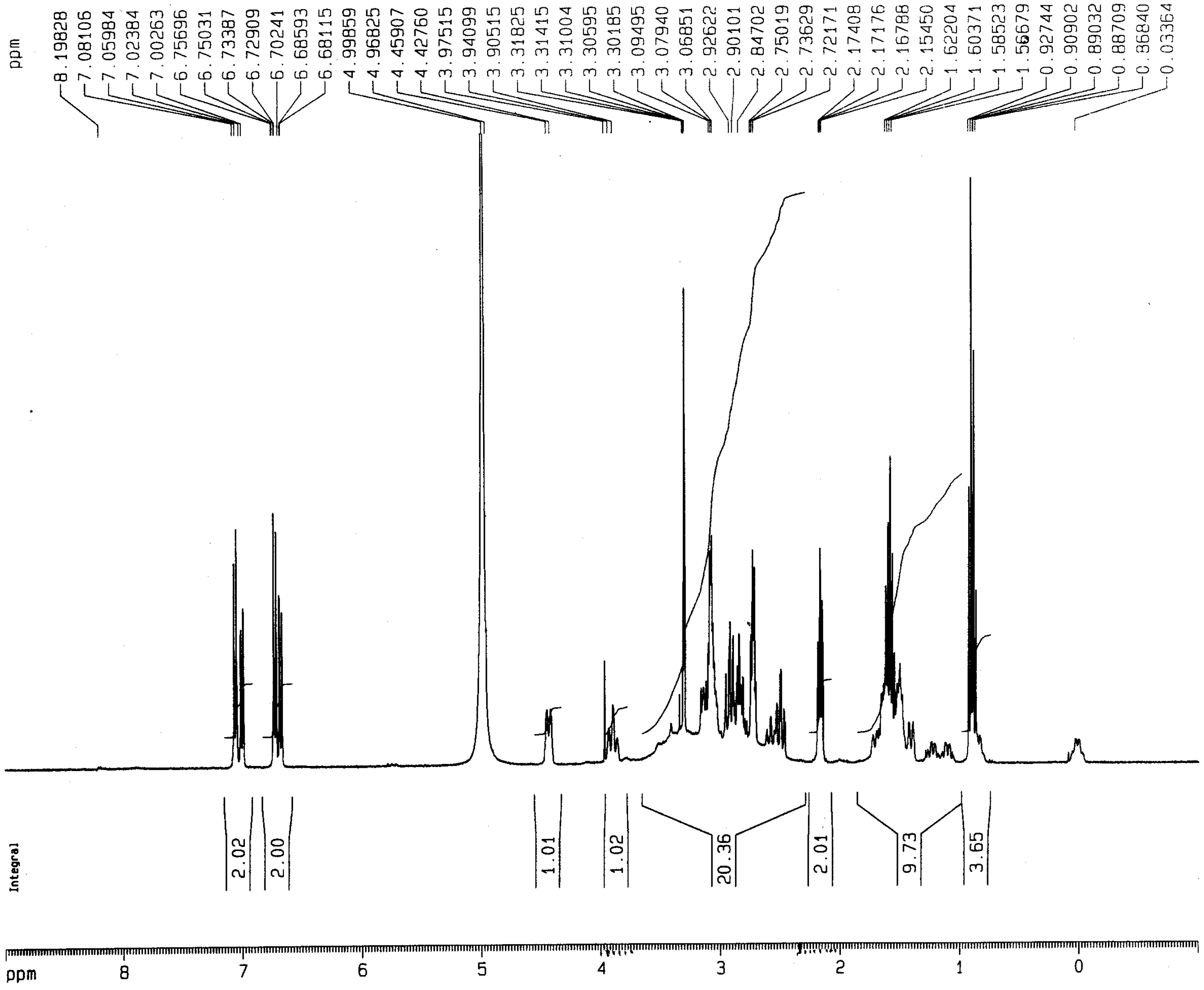
Attachment of Tyrosine and Butyryl Residues

Resin **13** was suspended in DMF (1 mL). DIEA (67 µL, 0.387 mmol, 2.6 eq), HODhbt (24 mg, 0.149 mmol, 1 eq) in DMF (1 mL) and (*S*)-Fmoc-Tyr(Bu^t)-OPfp (279 mg, 0.446 mmol, 3 eq) in DMF (2 mL) were added succesively and the mixture was shaken at room temperature under N_2 for 3 h. The resulting resin was treated with piperidine in DMF (20% v/v, 2 mL) for 10 min at room temperature, and washed with DMF (3 × 5 mL), then treated with piperidine in DMF (20% v/v, 2 mL) for 10 min again, and washed with DMF, CH_2Cl_2 and DMF (3 × 5 mL each). After removal of the Fmoc group the resin was suspended in DMF (1 mL) and DIEA (67 µL, 0.387 mmol, 2.6 eq), HODhbt (24 mg, 0.149 mmol, 1 eq) in DMF (1 mL) and butanoic acid Pfp ester (113 mg, 0.446 mmol, 3 eq) in DMF (2 mL) were added succesively. The resulting mixture was shaken at room temperature under N_2 for 16 h. The resin was drained and washed with CH_2Cl_2, DMF and CH_2Cl_2 (3 × 5 mL of each) to give resin **14**.

Deprotection and Cleavage from the Resin

Resin **14** was treated with CH_2Cl_2-TFA (50:50, v/v; 3 mL) at room temperature for 1 h. The drained solvent was combined with washings [MeOH (2 × 5 mL) and CH_2Cl_2 (2 × 5 mL)], evaporated in vacuo and purified by RP-18 VLC (H_2O-MeCN-TFA (95:5:0.1, 90:10:0.1, 85:15:0.1 and 80:20:0.1), followed by reversed phase HPLC [H_2O-MeCN-TFA (82:18:0.1)] to give 34 mg (26%) of the final product (**4**) as a clear syrup. ^1H NMR (400 MHz, CD_3OD): δ 7.07/7.01* (each, d, *J* = 8.4 Hz, 2 H, Ar-H), 6.73/6.69* (each, d, *J* = 8.4 Hz, 2 H, Ar-H), 4.99 (m, 1 H, H-α), 4.44 (br d, *J* = 12.3 Hz, 1 H, $C\mathit{H}_2NCO_{eq1}$), 3.92/3.89* (each, br d, *J* = 12.7 Hz, 1 H, $C\mathit{H}_2NCO_{eq2}$), 3.57 (br s, 8 H), 3.30-2.78 (10 H), 2.59*/2.50 (each, br t, *J* = 12.3 Hz, *J* = 12.7 Hz, 2 H, $C\mathit{H}_2NCO_{ax}$), 2.18/2.15* (each, t, *J* = 7.3 Hz, 2 H, CH_2CO), 2.09-1.12 (14 H), 1.62/1.59* (m, 2 H, CH_2CH_2CO), 0.91/0.89* (each, t, *J* = 7.3 Hz, 3 H, CH_3). ^{13}C NMR (100 MHz, CD_3OD): δ 175.6 (CO), 171.8 (CO), 157.5 (Ar-C_{para}), 131.1/130.6* (2 C), 128.8/128.7* (Ar-C_{ipso}), 116.6/116.2* (2 C), 56.2/56.0*, 55.8, 53.9*/53.7, 53.6, 51.8*/51.4 (C-α), 49.8 (2 C), 47.1/46.8* ($\mathit{C}H_2NCO$), 43.4/43.3* ($\mathit{C}H_2NCO$), 42.3/42.1* (2 C), 39.0*/38.6 (C-β), 38.5 ($\mathit{C}H_2CO$), 34.9/34.8* (CH), 34.5/34.3* (CH), 33.1 (CH_2), 32.4*/32.3 (CH_2), 32.1 (CH_2), 31.2*/31.1 (CH_2), 30.3 (2 C), 20.2 ($\mathit{C}H_2CH_3$), 13.9 (CH_3). ESI-MS: 542.5 (M + 1). HRMS: $C_{31}H_{52}N_5O_3$ requires M + 1 at m/z 542.40647; found, 542.40640 (ΔM: 0.13 ppm).





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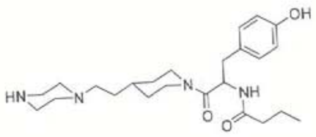

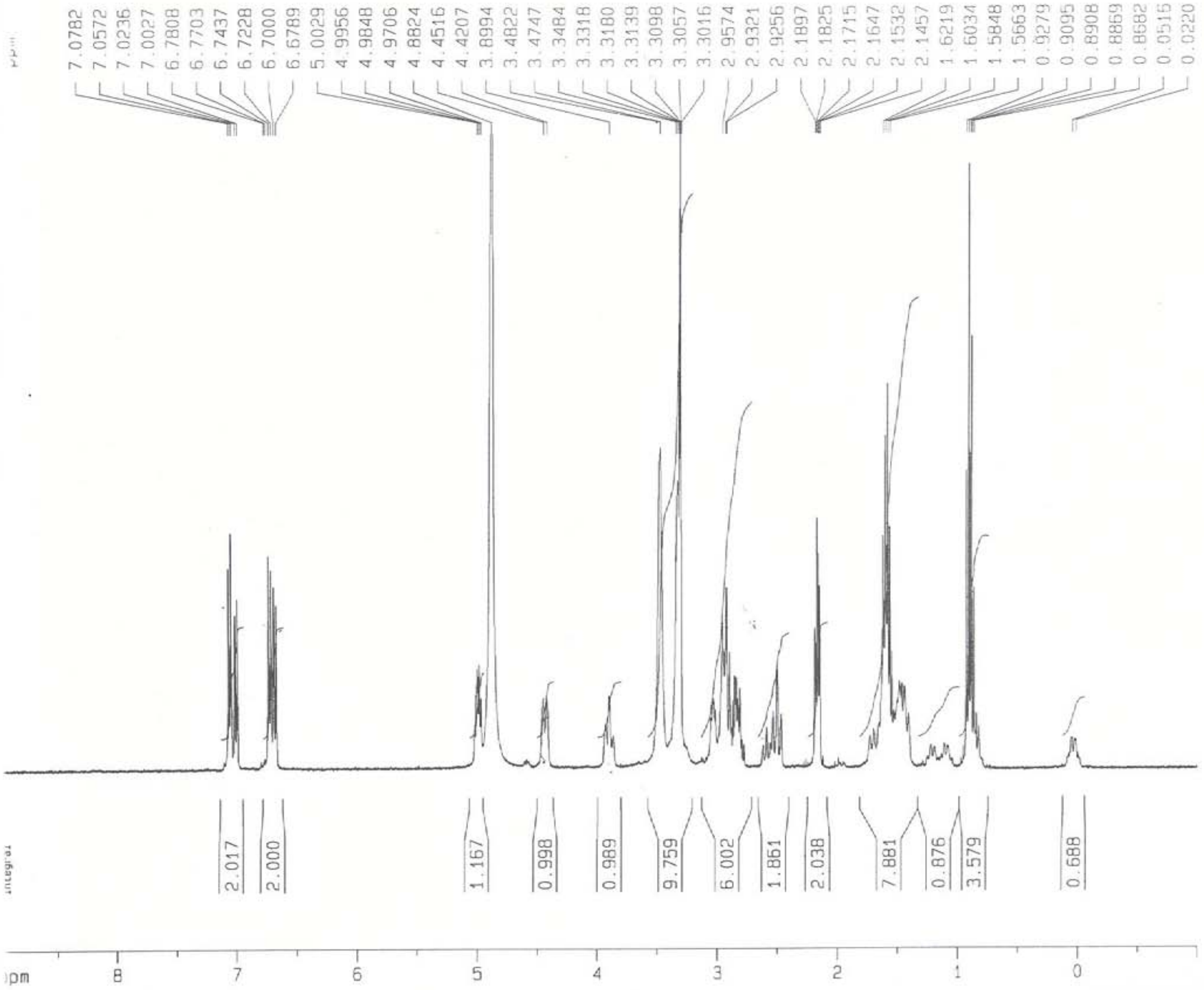

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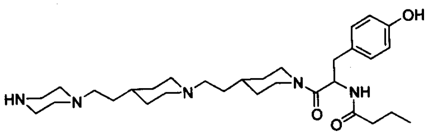

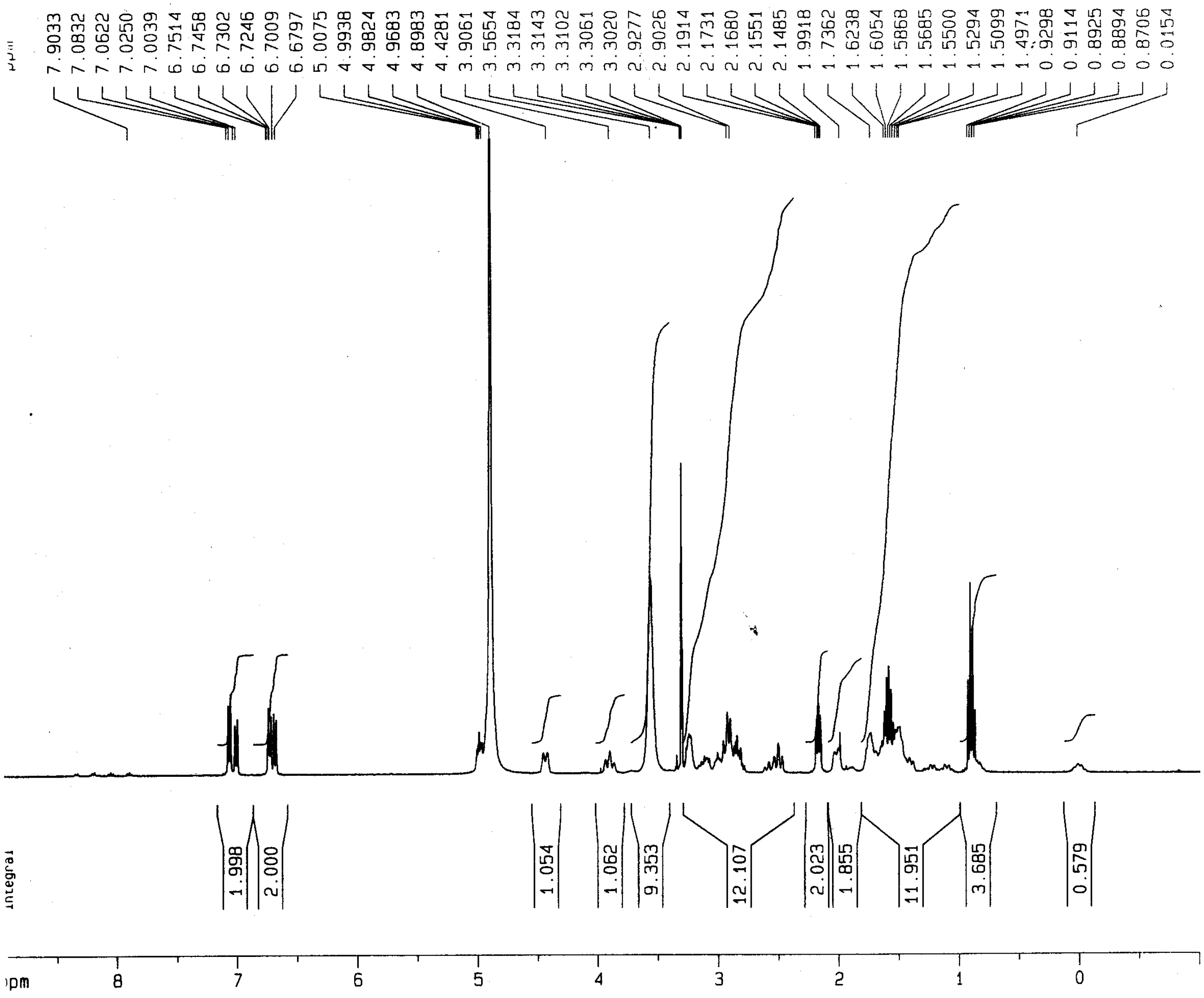

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Compound Chromatogram Report - MS

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Method: Copy of peptid1.MS
Sample Name: [redacted]
Analysis Info:

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Operator: NAJ

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Acq. Date: 11-07-03 09:53:45

Acquisition Parameter:

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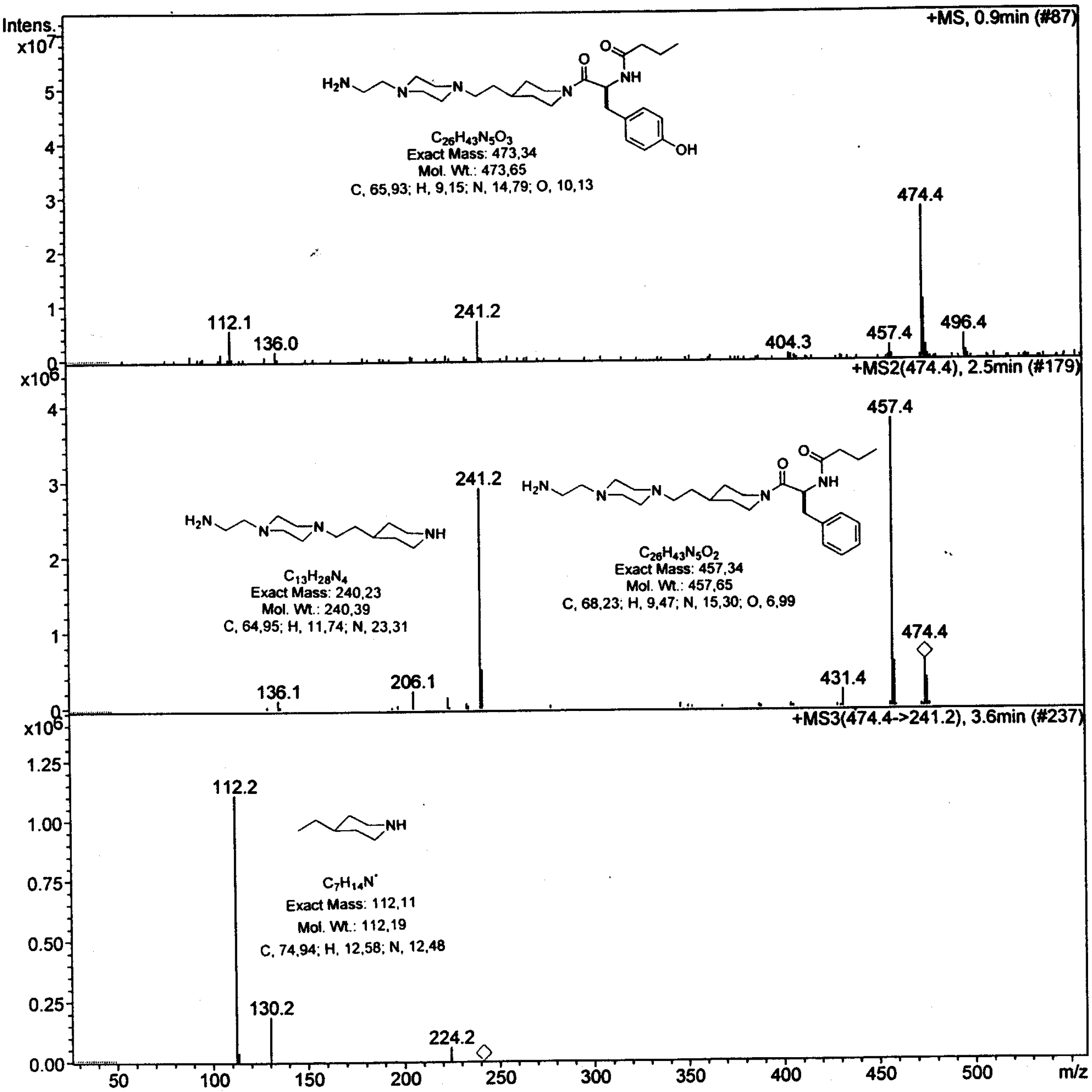

Compound Chromatogram Report - MS

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Analysis Info:					

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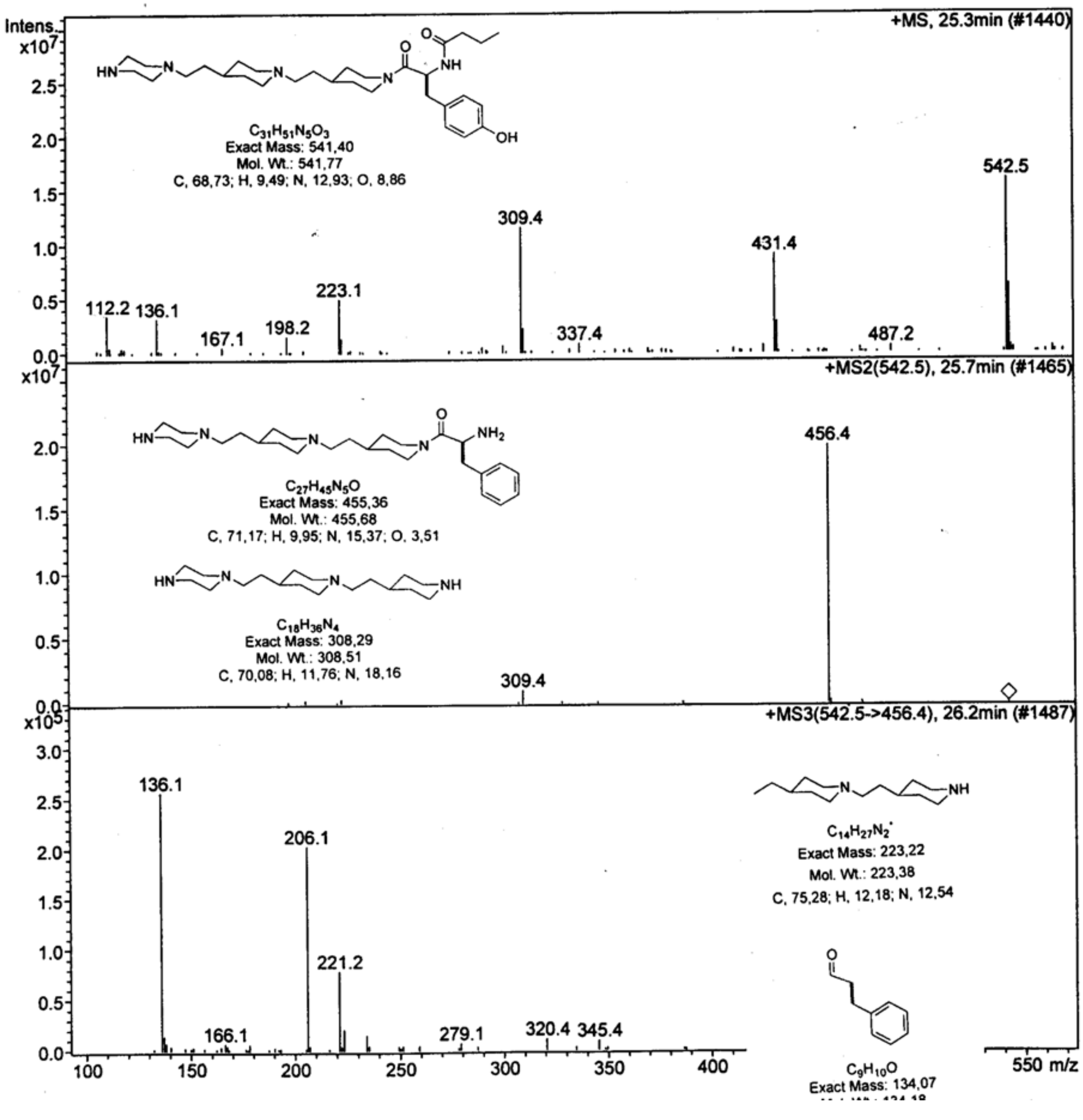
Nebulizer (Set)	15.00 psi	Capillary Exit	146.0 Volt	ICC Target	20000
Dry Gas (Set)	4.00 l/min	Cap Exit Offset	86.8 Volt	Charge Control	on



Sample CAO 2.38

Chemical formula $[M+H]^{+\cdot}$: $[C_{26}H_{44}N_5O_3]^+$

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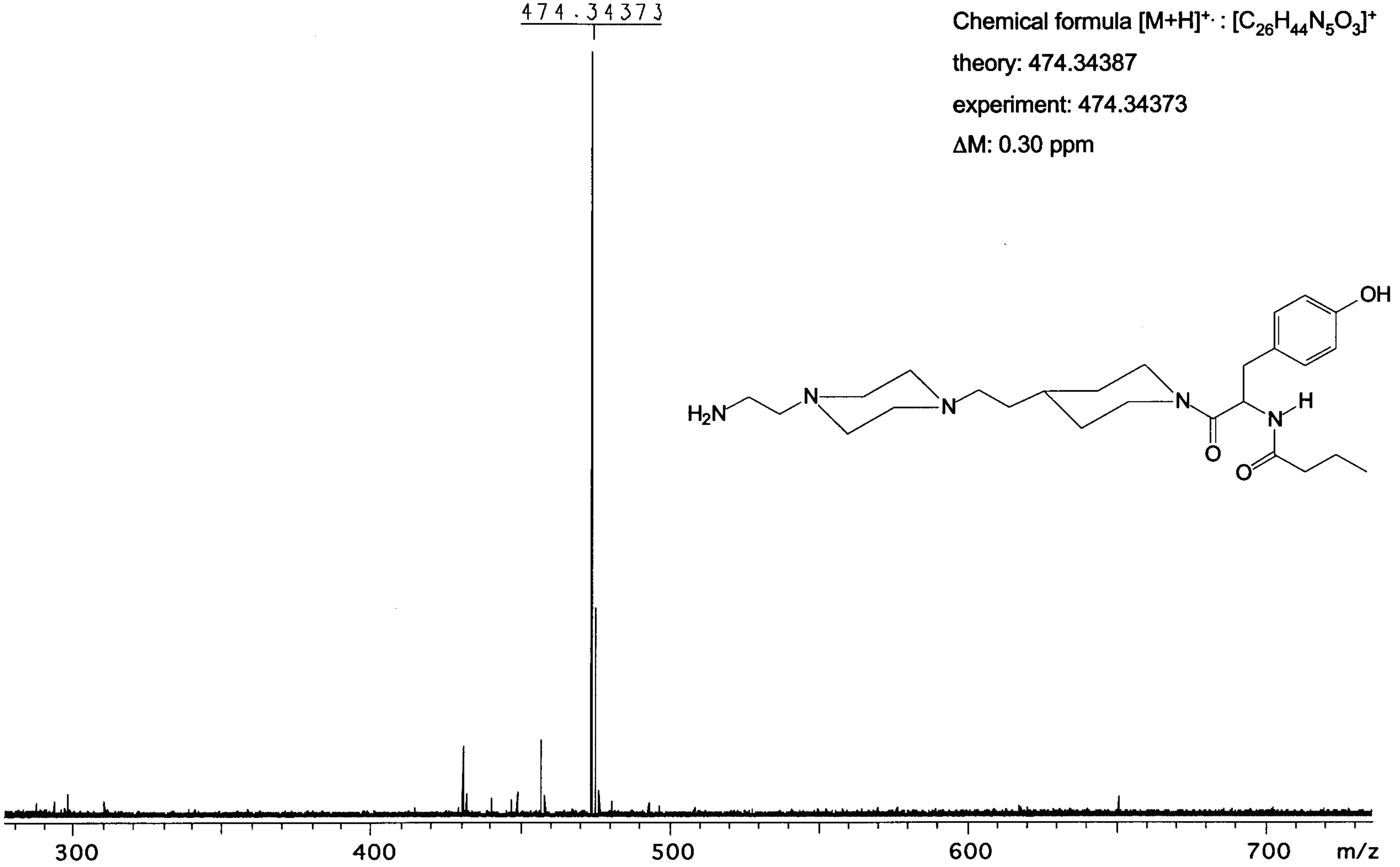
experiment: 474.34373

ΔM: 0.30 ppm



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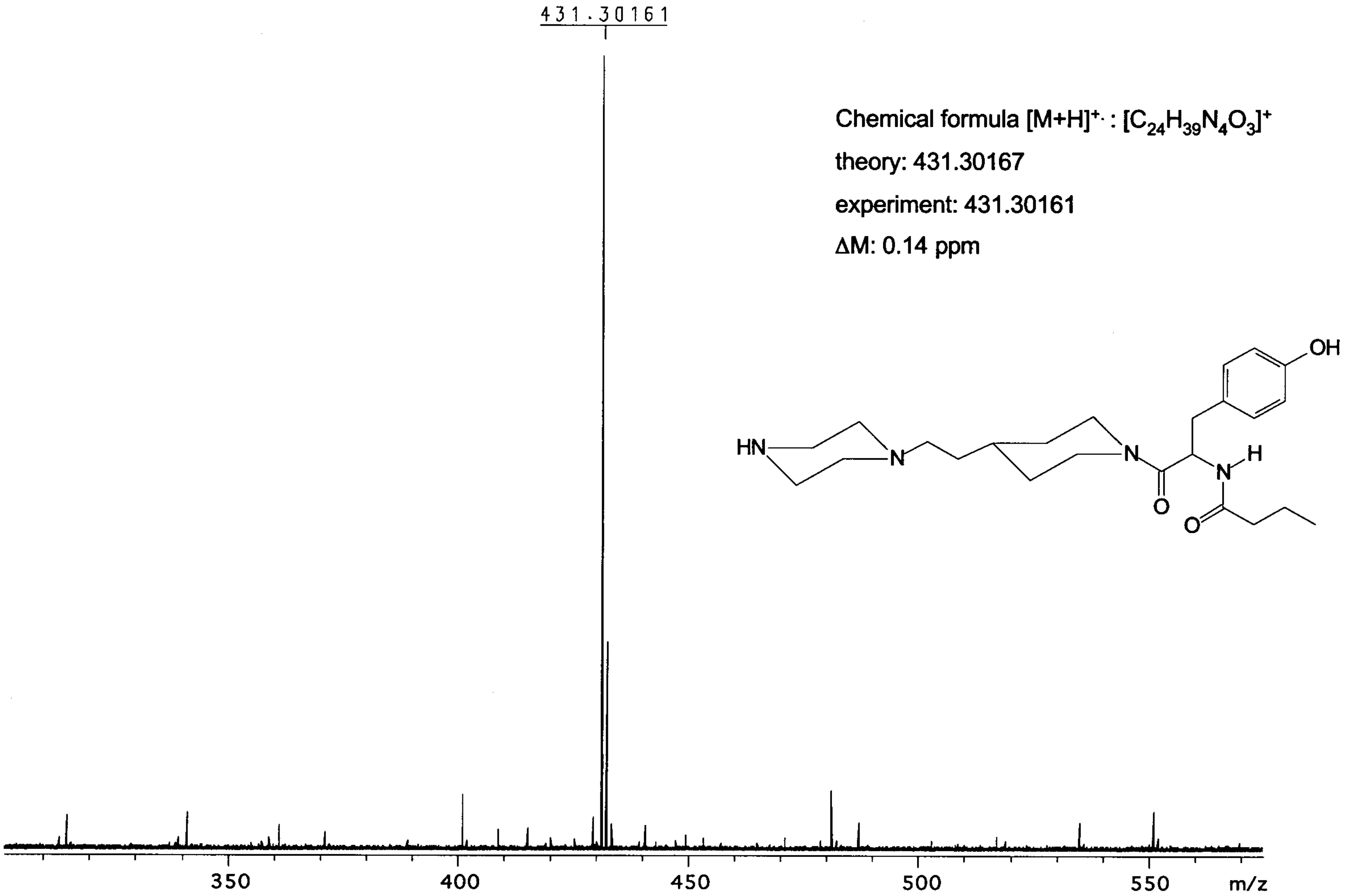


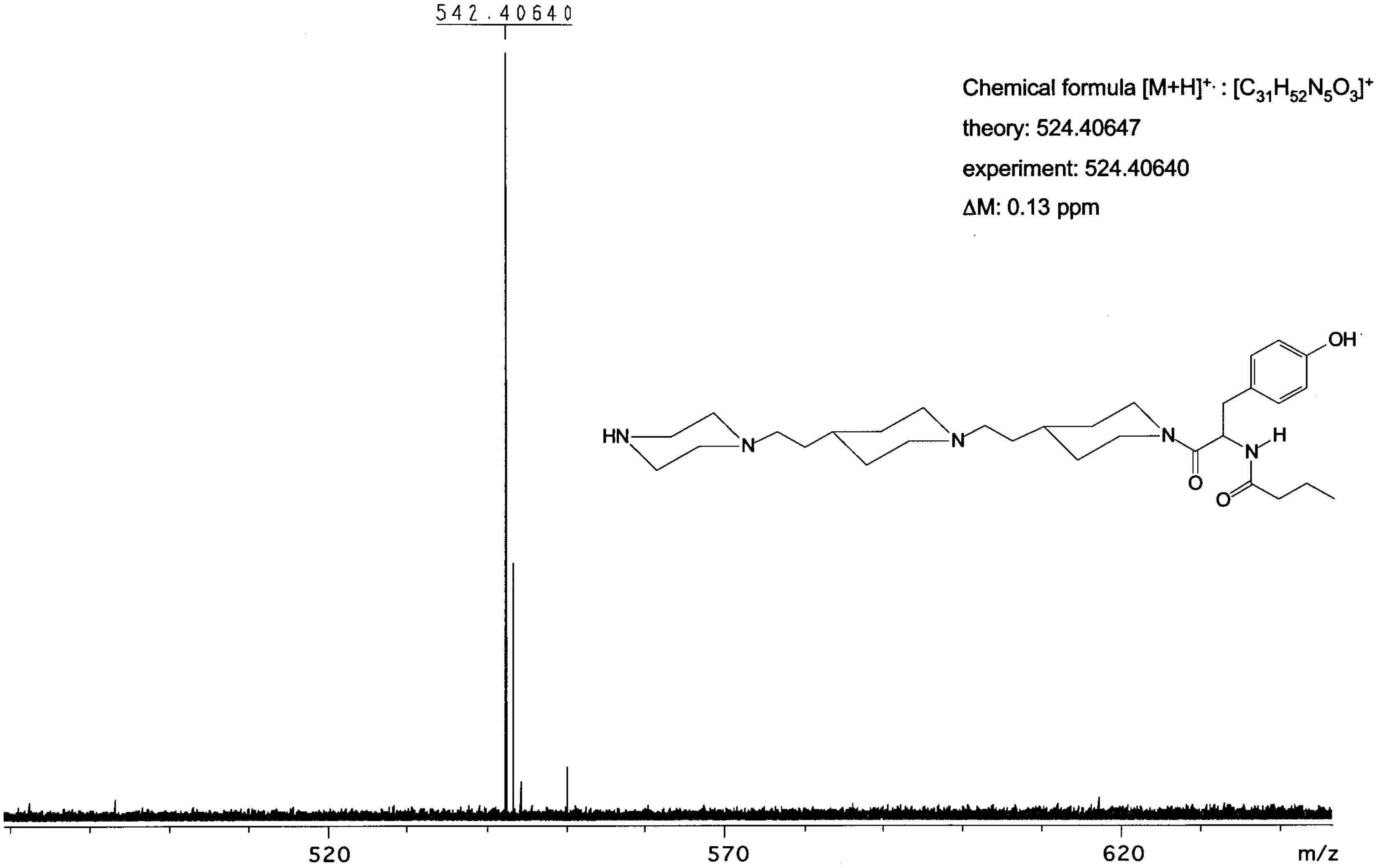
Chemical formula $[M+H]^{+\cdot}$: $[C_{24}H_{39}N_4O_3]^+$

theory: 431.30167

experiment: 431.30161

ΔM: 0.14 ppm

Sample CAO 2.53



Chemical formula [M+H]$^{+\cdot}$: $[C_{31}H_{52}N_5O_3]^+$

theory: 524.40647

experiment: 524.40640

ΔM: 0.13 ppm