Supporting Information

Synthesis of DOTA-conjugated multivalent cyclic-RGD peptide dendrimers via 1,3-dipolar cycloaddition and their biological evaluation: implications for tumor targeting and tumor imaging purposes[†]

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Experimental Section

Instruments and methods

Peptides were synthesized on an ABI 433A automatic Peptide Synthesizer using the FastMoc solid phase peptide synthesis protocols. Microwave-assisted reactions were carried out in a Biotage microwave reactor. Analytical HPLC runs were carried out on a Shimadzu HPLC system and preparative HPLC runs were performed on a Gilson HPLC workstation. Analytical HPLC runs were performed on Alltech Prosphere C4 or C8 and Adsorbosphere XL C18 columns (250 × 4.6 mm, pore size 300Å, particle size: 5 μm) or on a Merck LiChroCART CN column (250 × 4.6 mm, pore size 100Å, particle size: 5 μm) at a flow rate of 1.0 mL/min using a linear gradient of buffer B (0 – 100% in 25 min) in buffer A (buffer A: 0.1% TFA in H_2O, buffer B: 0.1 % TFA in CH_3CN/H_2O 95:5 v/v). Preparative HPLC runs were performed on an Alltech Prosphere C4 or C8 column (250 × 22 mm, pore size 300Å, particle size: 10 μm), and semi-prep HPLC runs were performed on an Alltech Adsorbosphere XL C18 column (250 × 10 mm, pore size 300Å, particle size: 10 μm) or on a Merck LiChroCART CN column (250 × 10 mm, pore size 100Å, particle size: 10 μm) at a flow rate of 10.0 mL/min (semi-prep HPLC: 4.0 mL/min) using a linear gradient of buffer B (0 – 100% in 50 min) in buffer A (buffer A: 0.1% TFA in H_2O, buffer B: 0.1 % TFA in CH_3CN/H_2O 95:5 v/v). Liquid chromatography electrospray ionization mass spectrometry was measured on a Shimadzu LCMS-QP8000 single quadrupole bench-top mass spectrometer operating in a positive ionization mode. LC/MS(MS) runs were performed on a Finnigan LCQ Deca XP MAX LC/MS equipped with a Shimadzu 10A VP analytical HPLC system. The samples were dissolved in 10% formic acid in CH_3CN/H_2O 1:1 v/v and analyzed using a Phenomenex Gemini C18 column (150 × 4.6 mm, particle size: 3 μm, pore size: 110Å) at a flow rate of 1.0 mL/min using a linear gradient of 100% buffer A (0.1% TFA in H_2O/CH_3CN 95:5 v/v) to 100% buffer B (0.1% TFA in CH_3CN/H_2O 95:5 v/v) in 50 min. MALDI-TOF analysis was performed on a Kratos Axima CFR apparatus with bradykinin(1-7) (monoisotopic $[M + H]^+$ 757.399), human ACTH(18-39) (monoisotopic $[M + H]^+$ 2465.198) and bovine insulin oxidized B chain (monoisotopic $[M + H]^+$ 3494.651) as external references and α-cyano-4-hydroxycinnamic acid or sinapinic acid as matrices. ^{1}H NMR spectra were recorded on a Varian G-300 (300 MHz) spectrometer and chemical shifts are given in ppm (δ) relative to TMS. ^{13}C NMR spectra were recorded on a Varian G-300 (75.5 MHz) spectrometer and chemical shifts are given in ppm relative to $CDCl_3$ (77.0 ppm). The ^{13}C NMR spectra were recorded using the attached proton test (APT) sequence. ^{1}H NMR spectra in H_2O/D_2O 9:1 v/v were recorded on a Varian Inova-500 (500 MHz) spectrometer and chemical shifts are given in ppm (δ) relative to 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (0.00 ppm). Peak assignments are based on DQF-COSY, TOCSY (mixing times: 20 or 60 ms) and ROESY (mixing times: 150 or 250 ms) spectra. HSQC and HMBC spectra were measured on a Varian Inova-500 spectrometer and chemical shifts are given in ppm (δ) relative to 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (0.00 ppm). Fourier transform infrared spectra (FTIR) were measured on a Bio-Rad FTS-25 spectrophotometer. Melting points were measured on a Büchi Schmelzpunktbestimmungsapparat and are uncorrected. Elemental analyses were done by Kolbe Mikroanalytisches Labor (Mülheim/Ruhr, Germany). R_f values were determined by thin layer chromatography (TLC) on Merck precoated silica gel 60F254 plates. Spots were visualized by UV-quenching, ninhydrin or Cl_2/TDM.[1] The 2-chlorotrityl chloride resin (Hecheng Science & Technology Company) was used in all solid phase syntheses. The coupling reagents 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and benzotriazol-1-yloxy-tris-(dimethylamino)phosphonium hexafluorophosphate (BOP) were obtained from Biosolve. *N*-hydroxybenzotriazole (HOBt) was from Advanced ChemTech and N^α-9-fluorenylmethyloxycarbonyl (Fmoc) amino acids were obtained from MultiSynTech. The side-chain protecting groups were chosen as *tert*-butyl for aspartic acid, *tert*-butyloxycarbonyl (Boc) for lysine and 2,2,4,6,7-pentamethyl-dihydrobenzofuran-5-sulfonyl (Pbf) for arginine. Peptide-grade *tert*-butanol (^{t}BuOH), dichloromethane, *N,N*-dimethylformamide (DMF), 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) *tert*-butyl methylether (MTBE), *N*-methylpyrrolidone (NMP), and trifluoroacetic acid (TFA) and HPLC-grade acetronitrile were purchased from Biosolve. 2-(4,7,10-tris(2-*tert*-butoxy-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl) acetic acid (DOTA(O^tBu)$_3$) was purchased from Macrocyclics. Piperidine, *N,N*-diisopropylethylamine (DIPEA), $CuSO_4$ and sodium ascorbate were obtained from Acros Organics. Triisopropylsilane (TIS) and HPLC-grade TFA were obtained from Merck. Triflic anhydride and propargylbromide were purchased from Aldrich.

Syntheses

3-(prop-2-ynyloxy) methyl benzoate (2): 3-hydroxymethylbenzoate[2] (2.35 g, 15.5 mmol) was dissolved in dry DMF (25 mL) and anhydrous K_2CO_3 (2.63 g, 19.8 mmol, 1.3 equiv) was added. To this suspension, a solution of propargylbromide in toluene (2 mL, 17.9 mmol, 1.15 equiv) was added dropwise. The reaction mixture was stirred for 16 h at room temperature. Then, DMF was removed by evaporation and the residue was redissolved in EtOAc (75 mL) and the organic phase was washed with H_2O (3 × 25 mL), 1N $KHSO_4$ (3 × 25 mL) and brine (3 × 25 mL), dried (Na_2SO_4) and evaporated *in vacuo*. Propargyl ether **2** was obtained as a pale brownish waxy solid in 95% yield (2.80 g). R_f(EtOAc/hexane 4:1 v/v): 0.69; ^{1}H-NMR ($CDCl_3$) δ 2.56 (t (J 2.47 Hz), 2H, CH), 3.90 (s, 3H, OCH_3), 4.72 (d (J 2.47 Hz), 4H, ~O-CH_2), 7.16 (m, 1H, arom H), 7.35 (d (J 7.81 Hz), 1H, arom H), 7.62 (s, 1H, arom H), 7.66 (d (J 7.81 Hz), 1H, arom H); ^{13}C-NMR ($CDCl_3$) δ 52.0, 55.8, 76.0, 75.8, 115.1, 120.0, 122.7, 131.4, 157.3; MS analysis: calcd for $C_{11}H_{10}O_3$ 190.20, found ES-MS 191.38 $[M + H]^+$.

3,5-bis-(prop-2-ynyloxy) methyl benzoate (3): This compound was synthesized as described for **2** on a 130 mmol scale (3,5-dihydroxymethylbenzoate[2], 21.4 g) in dry DMF (250 mL) in the presence of anhydrous K_2CO_3 (45 g, 330 mmol, 2.5 equiv) and a solution of propargylbromide in toluene (35 mL, 314 mmol, 2.5 equiv). Diallyl ether **3** was obtained as off-white crystals in 81% yield (25.2 g). M.p.: 110 °C; R_f(EtOAc/hexane 4:1 v/v): 0.76; R_f(CH_2Cl_2/MeOH 98:2 v/v): 0.87; R_f($CHCl_3$/MeOH/AcOH 95:20:3 v/v): 0.83; ^{1}H-NMR ($CDCl_3$) δ 2.55 (t (*J* 2.47 Hz), 2H, CH), 3.91 (s, 3H, OCH_3), 4.72 (d (*J* 2.47 Hz), 4H, ~O-CH_2), 6.81 (t (*J* 2.20 Hz), 1H, arom H4), 7.29 (d (*J* 2.20 Hz), 2H, arom H2/H6); ^{13}C-NMR ($CDCl_3$) δ 52.4, 56.0, 76.0, 77.9, 107.5, 108.8, 132.0, 157.8, 158.4; MS analysis: calcd for $C_{14}H_{12}O_4$ 244.24, found ES-MS 244.99 $[M + H]^+$; Elemental analysis: calcd for $C_{14}H_{12}O_4$ C 68.83, H 4.95, found C 68.76, H 4.95.

3-(prop-2-ynyloxy) benzoic acid (4): Compound **2** (1.0 g, 5.24 mmol) was dissolved in dioxane/MeOH (50 mL, 14:5 v/v) and 4N NaOH (2 mL, 1.5 equiv) was added in one portion.[3] The obtained reaction mixture was stirred for 5 h at room temperature. Then, the reaction mixture was neutralized by the addition of 1N HCl and the solvents were removed by evaporation. The residue was redissolved in EtOAc (50 mL) and the organic phase was washed with 1N $KHSO_4$ (3 × 20 mL) and brine (3 × 20 mL), dried (Na_2SO_4) and evaporated *in vacuo*. The residual solid was obtained in 97% yield (900 mg) and used without further purification in the next synthesis step. M.p.: 126-131 °C; R_f(EtOAc/hexane 7:3 v/v): 0.65; ^{1}H NMR ($CDCl_3$) δ: 2.55 (s, 1H, CH), 4.77 (s, 2H, ~O-CH_2), 7.25 (m, 1H, arom H), 7.42 (t, 1H, arom H5), 7.72 (m, 1H, arom H), 7.77 (d, 1H, arom H); ^{13}C NMR ($CDCl_3$) δ: 56.0, 76.0, 78.0, 115.6, 121.2, 123.5, 129.6, 130.6, 157.5, 171.9; Elemental analysis: calcd for $C_{10}H_8O_3$ C 68.18, H 4.58, found C 67.87, H 4.70.

3,5-bis-(prop-2-ynyloxy) benzoic acid (5): Methyl ester **3** (5.66 g, 23.2 mmol) was saponified as described for compound **4**. The acid **5** was obtained in 96% yield (5.13 g) and used without further purification in the next synthesis step. M.p.: 171-174 °C; R_f(CH_2Cl_2/MeOH 9:1 v/v): 0.26; ^{1}H-NMR (DMSO-d_6) δ: 3.59 (broad s, 2H, CH), 4.85 (d (*J* 2.20 Hz), 4H, ~O-CH_2), 6.86 (t (*J* 2.47 Hz), 1H, arom

H4), 7.17 (d (*J* 2.47 Hz), 2H, arom H2/H6); ^{13}C-NMR (DMSO-d$_6$) δ: 55.8, 78.6, 78.9, 107.0, 108.4, 132.9, 158.2, 166.8; MS analysis: calcd for $C_{14}H_{12}O_4$ 230.22, found ES-MS 231.01 $[M + H]^+$; Elemental analysis: calcd for $C_{13}H_{10}O_4$ C 67.82, H 4.38, found C 67.56, H 4.11.

3,5-bis-(2-(3,5-bis(prop-2-ynyloxy)benzamido)ethoxy methyl benzoate (7): To a solution of 3,5-bis-(2-*tert*-butyloxycarbonylamino-ethoxy) methyl benzoate[2] (**6**; 2.27 g, 5.0 mmol) in CH_2Cl_2 (25 mL), TFA (25 mL) was added to remove the Boc functionalities. After 1 h of stirring at room temperature, the volatiles were removed by evaporation and the residue was coevaporated with CH_2Cl_2 to remove any residual TFA. The obtained solid was used without further purification. Acid **5** (2.53 g, 11 mmol, 2.2 equiv) was dissolved in CH_2Cl_2 (100 mL) and the TFA-salt (dissolved in 50 mL CH_2Cl_2) followed by DIPEA (3.53 mL, 25 mmol, 5 equiv) were added. Finally, BOP (4.86 g, 11 mmol) was added and the obtained reaction mixture was stirred for 16 h at room temperature. Then, the solvent was removed *in vacuo* and the residue was redissolved in EtOAc (150 mL) and this solution was subsequently washed with H_2O (3 × 75 mL), 1N $KHSO_4$ (3 × 75 mL), H_2O (3 × 75 mL), 5% $NaHCO_3$ (3 × 75 mL) and brine (3 × 75 mL), dried (Na_2SO_4) and evaporated to dryness. The residue was crystallized from MeOH and was obtained as a white solid in 75% yield (2.54 g). M.p.: 113-124 °C; R_f(EtOAc/hexane 4:1 v/v): 0.49; R_f(CH_2Cl_2/MeOH 98:2 v/v): 0.13; R_f($CHCl_3$/MeOH/AcOH 95:20:3 v/v): 0.80; ^{1}H NMR (DMSO-d$_6$) δ: 3.58 (s, 4H, CH), 3.65 (m, 4H, ~CH_2~), 3.84 (s, 3H, OCH_3), 4.19 (m, 4H, ~CH_2~), 4.85 (s, 8H, ~O-CH_2), 6.80 (s, 2H, arom H4'), 6.87 (s, 1H, arom H4), 7.12 (s, 2H, arom H2/H6), (s, 4H, arom H2'/H6'), 8.67 (m, 2H, NH amide); ^{13}C-NMR ($CDCl_3$) δ: 40.4, 53.2, 56.9, 57.6, 77.0, 78.8, 106.3, 107.1, 107.6, 109.0 132.9, 137.4, 159.6, 160.3, 167.4, 168.1; MS analysis: calcd for $C_{38}H_{34}N_2O_{10}$ 678.22, found ES-MS 679.40 $[M + H]^+$, 701.45 $[M + Na]^+$; MALDI-TOF 679.298 $[M + H]^+$, 701.245 $[M + Na]^+$; Elemental analysis: calcd for $C_{38}H_{34}N_2O_{10}$ C 67.25, H 5.05, N 4.13 found C 66.92, H 5.09, N 4.10.

3,5-bis-(2-(3,5-bis(prop-2-ynyloxy)benzamido)ethoxy benzoic acid (8): Methyl ester **7** (1.36 g, 2 mmol) was saponified as described for compound **4**. Acid **8** was obtained as a white powder with nearly quantitative yield (1.33 g). M.p.: 163-168 °C; R_f(CH_2Cl_2/MeOH 9:1 v/v): 0.23; ^{1}H NMR (DMSO-d_6) δ: 3.58 (s, 4H, CH), 3.65 (m, 4H, ~CH_2~), 4.19 (m, 4H, ~CH_2~), 4.85 (s, 8H, ~O-CH_2), 6.80 (s, 2H, arom H4'), 6.83 (s, 1H, arom H4), 7.11 (s, 2H, arom H2/H6), 7.15 (s, 4H, arom H2'/H6'), 8.66 (m, 2H, NH amide); ^{13}C-NMR (DMSO-d_6) δ: 38.9, 55.8, 66.3, 78.4, 78.9, 105.0, 105.8, 106.8, 107.8, 132.9, 136.3, 158.2, 159.6, 165.8, 166.9; MS analysis: calcd for $C_{37}H_{32}N_2O_{10}$ 664.66, found ES-MS 665.75 $[M + H]^+$, 687.60 $[M + Na]^+$; Elemental analysis: calcd for $C_{37}H_{32}N_2O_{10}$ C 66.86, H 4.85, N 4.21 found C 66.76, H 4.72, N 4.11.

2-*tert*-butyloxycarbonylamino ethylamine (10): To a solution of 1,2-diaminoethane (13.4 mL, 200 mmol) in dioxane (100 mL) a solution of Boc_2O (5.46 g, 25 mmol) in dioxane (100 mL) was added dropwise over a period of 2 h. After the addition was complete, the obtained reaction mixture was stirred for 16 h at room temperature. Then, the solvent was removed by evaporation and the residue was suspended in H_2O (100 mL) and the white precipitate (bis-substitution product) was removed by filtration. The aqueous solution was extracted with CH_2Cl_2 (3 × 50 mL), and the collected organic layers were washed with brine (1 × 50 mL), dried ($MgSO_4$) and evaporated to dryness. Compound **10** was obtained as a yellowish oil with 56% yield (2.25 g). R_f(CH_2Cl_2/MeOH 94:6 v/v): 0.24 ^{1}H NMR ($CDCl_3$) δ: 1.45 (broad s, 11H, $(CH_3)_3$ Boc/~NH_2), 2.79 (m, 2H, ~CH_2-NH_2), 3.16 (m, 2H, ~NH-CH_2~), 5.32 (m, 1H, NH urethane); ^{13}C-NMR ($CDCl_3$) δ 28.2, 41.6, 43.1, 78.9, 156.2; MS analysis: calcd for $C_7H_{16}N_2O_2$ 160.12, found ES-MS 161.15 $[M + H]^+$.

H-Asp(O^{t}Bu)-D-Phe-Lys(Boc)-Arg(Pbf)-Gly-*O*-2-chlorotrityl resin (17): The 2-chlorotrityl chloride resin was treated with $SOCl_2$/CH_2Cl_2 (1:1 v/v; 2 × 6 mL, 10 min) to convert it completely into the chloride form prior loading of the first amino acid. To remove any residual $SOCl_2$, the resin was extensively washed with CH_2Cl_2 (6 × 10 mL, 10 min). Then, Fmoc-Gly-OH (430 mg, 1.44 mmol) was dissolved in CH_2Cl_2 (10 mL) and DIPEA (510 µL, 2.88 mmol) followed by the 2-chlorotrityl chloride resin (360 mg (1 mmol/g), 0.36 mmol) were added and the obtained slurry was gently swirled for 2 h at room temperature. The resin was subsequently washed with CH_2Cl_2/MeOH/DIPEA (17:2:1 v/v/v; 3 × 10 mL, 10 min) to cap any remaining linked tritylchloride, followed by CH_2Cl_2 (3 × 10 mL, 2 min), DMF (3 × 10 mL, 2 min) and CH_2Cl_2 (3 × 10 mL, 2 min). The loading of the resin, as calculated from an Fmoc determination, was 64% (0.64 mmol/g). The linear peptide sequence H-Asp(O^{t}Bu)-D-Phe-Lys(Boc)-Arg(Pbf)-Gly-OH was synthesized according to the FastMoc solid phase peptide synthesis protocols[4] and the final Fmoc-group was removed to obtain peptide resin **17**.

cyclo**(Arg-Gly-Asp-D-Phe-Lys) (18)**: Peptide resin **17** was treated twice with HFIP/CH_2Cl_2 (32 mL, 1:4 v/v) for 45 min each to cleave the protected peptide acid from the resin.[5] After this, the resin was washed with CH_2Cl_2 (3 × 20 mL, 10 min) and all fractions were collected and evaporated to dryness. The crude protected peptide acid (MS analysis: calcd for $C_{49}H_{75}N_9O_{13}S$, 1030.24, found ES-MS 1031.55 $[M + H]^+$) was obtained with 85% yield (202 mg, 0.20 mmol). This crude peptide was dissolved in CH_2Cl_2 (30 mL) and HOBt (30 mg, 0.20 mmol), BOP (41 mg, 0.20 mmol) followed by DIPEA (80 µL, 0.45 mmol, 2.25 equiv) were added and the obtained reaction mixture was stirred for 16 h at room temperature. Subsequently, the solvent was partly removed by evaporation and the oily residue was dissolved in $CHCl_3$ (50 mL) and washed with 1N $KHSO_4$ (3 × 20 mL), H_2O (3 × 20 mL) and brine (3 × 20 mL). After the final wash steps, the solvent was removed *in vacuo* a white solid was obtained. This compound was dissolved in TFA/H_2O/TIS (10 mL; 95:2.5:2.5 v/v/v) and stirred for 3 h to remove the side chain protecting groups. The crude cyclic peptide was isolated by precipitation with MTBE/hexane 1:1 v/v at –20°C. After centrifugation, the pellet was dissolved in *tert*-BuOH/H_2O 1:1

v/v, lyophilized and subsequently purified by HPLC (C8). Cyclic peptide **18** was obtained in 42% (51 mg) yield. *R*t: 9.97 min (C4); *R*t: 15.26 min (CN); MS analysis: calcd for $C_{27}H_{41}N_9O_7$, 603.31, found ES-MS 604.60 $[M + H]^+$.

General procedure for the microwave-assisted click reaction:[6] the alkyne (1 equiv) and the azide (1.3 equiv per arm) were dissolved in DMF/H_2O. To this solution, $CuSO_4.5H_2O$ (0.05 equiv) and Na-ascorbate (0.50 equiv) were added. The reaction mixture was placed in a microwave reactor and irradiated during 10 – 30 min at 100°C. The cycloaddition was monitored on TLC and LC-MS for completion of the reaction.

monovalent *cyclo*[RGDfK] peptide dendrimer (20): Alkyne **2** (1.3 mg, 6.6 μmol) and azido peptide **19** (5.4 mg, 7.3 μmol, 1.1 equiv) were dissolved in DMF (500 μL) and 0.05 M Na-ascorbate (66 μL, 3.3 μmol, 0.50 equiv) followed by 6 mM $CuSO_4.5H_2O$ (55 μL, 0.33 μmol, 0.05 equiv) were added. The reaction mixture was placed in the microwave reactor and irradiated for 30 min at 100°C. Then, the solvents were removed under reduced pressure and the residue was dissolved in *tert*-BuOH/H_2O 1:1 v/v and lyophilized and subsequently purified by semi-prep HPLC (C18). Yield: 3.1 mg (57%). *R*t: 18.9 min (CN); MS analysis: calcd for $C_{38}H_{49}N_{11}O_{10}$, 819.366, found ES-MS 820.60 $[M + H]^+$, found MALDI-TOF 820.641 $[M + H]^+$, 842.620 $[M + Na]^+$.

divalent *cyclo*[RGDfK] peptide dendrimer (21): For this synthesis, alkyne **3** (0.8 mg, 3.3 μmol) and azido peptide **19** (6.3 mg, 8.5 μmol, 1.3 equiv) were used. After purification by semi-prep HPLC compound **21** was obtained with 40% yield (1.9 mg). *R*t: 19.0 min (C4); *R*t: 20.30 min (CN); MS analysis: calcd for $C_{68}H_{90}N_{22}O_{18}$, 1502.680, found MALDI-TOF 1503.647 $[M + H]^+$.

tetravalent *cyclo*[RGDfK] peptide dendrimer (22): For this synthesis, alkyne **7** (1.10 mg, 1.6 μmol) and azido peptide **19** (6.3 mg, 8.5 μmol, 1.3 equiv) were used. After purification by semi-prep HPLC compound **22** was obtained with 14% yield (0.7 mg). R_t: 21.2 min (CN); MS analysis: calcd for $C_{146}H_{190}N_{46}O_{38}$, 3195.435 ($M_{ave}$), found MALDI-TOF 3196.573 $[M + H]_{ave}^{+}$.

References and notes

1 E. von Arx, M. Faupel and M. J. Brugger, *J. Chromatogr.*, 1976, **120**, 224.

2 A. J. Brouwer, S. J. E. Mulders and R. M. J. Liskamp, *Eur. J. Org. Chem.*, 2001, 1903.

3 Tesser's base is a mixture of dioxane, methanol and aqueous NaOH, see: G. I. Tesser and I. C. Balvert-Geers, *Int. J. Peptide Protein Res.*, 1975, **7**, 295.

4 C. G. Fields, D. H. Lloyd, R. L. Macdonald, K. M. Otteson and R. L. Noble, *Peptide Res.*, 1991, **4**, 95.

5 R. Bollhagen, M. Schmiedberger, K. Barlos and E. Grell, *J. Chem. Soc., Chem. Commun.*, 1994, 2559.

6 (*a*) D. T. S. Rijkers, G. W. van Esse, R. Merkx, A. J. Brouwer, H. J. F. Jacobs, R. J. Pieters and R. M. J. Liskamp, *Chem. Commun.*, 2005, 4581; (*b*) J. A. F. Joosten, N. T. H. Tholen, F. Ait El Maate, A. J. Brouwer, G. W. van Esse, D. T. S. Rijkers, R. M. J. Liskamp and R. J. Pieters, *Eur. J. Org. Chem.*, 2005, 3182; (*c*) R. M. J. Liskamp, D. T. S. Rijkers, R. J. Pieters, A. J. Brouwer and J. A. F. Joosten, International Patent Application: P73572PC00, 2005, Dendrimers multivalently substituted with active groups.

HPLC Report Medicinal Chemistry

sample ID: **cRGDmono10**
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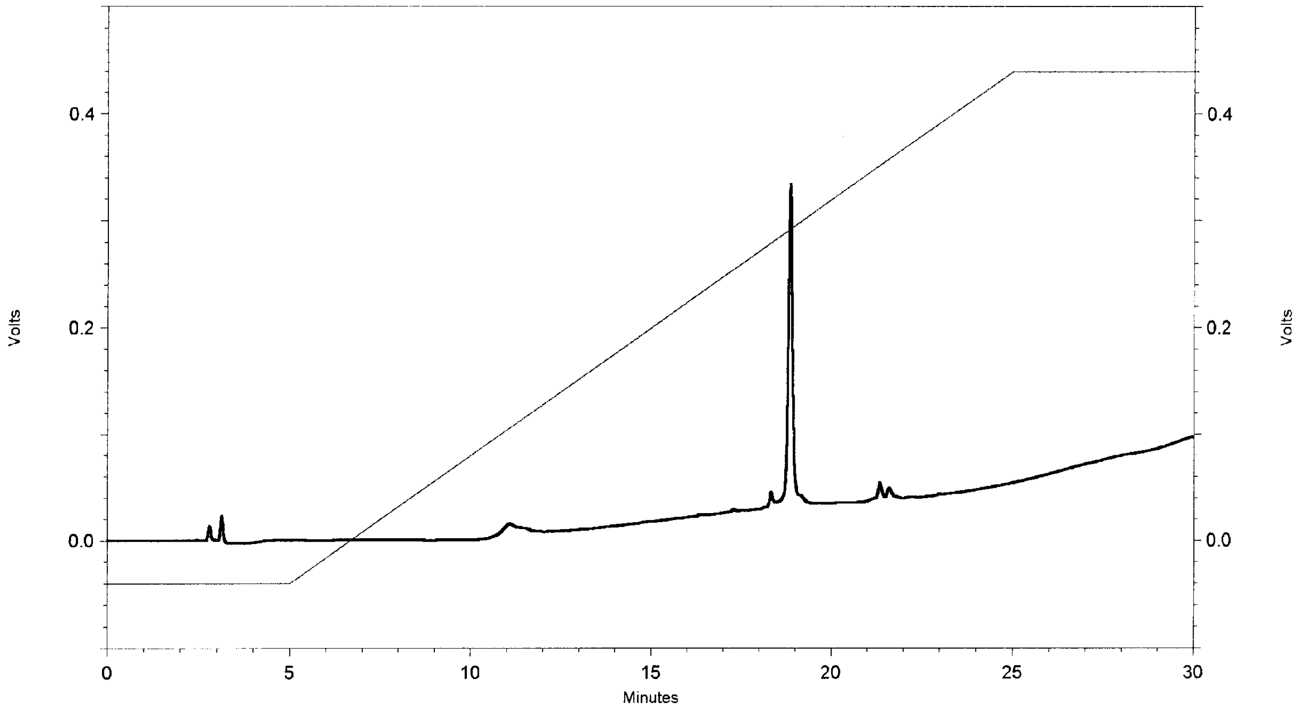
Detectors: SPD-10Avp UV-Vis



Peak table for UV-VIS detector

SPD-10Avp - 1
(220nm)

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Totals				
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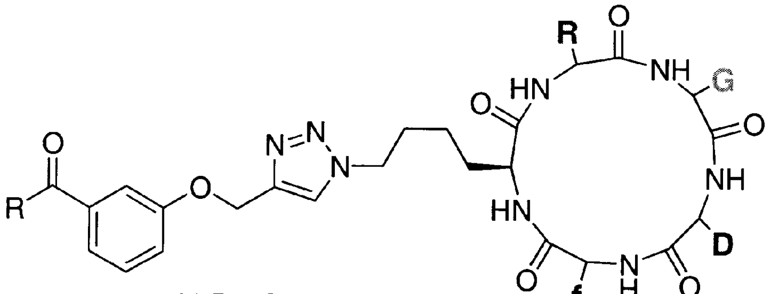

20 R = OMe

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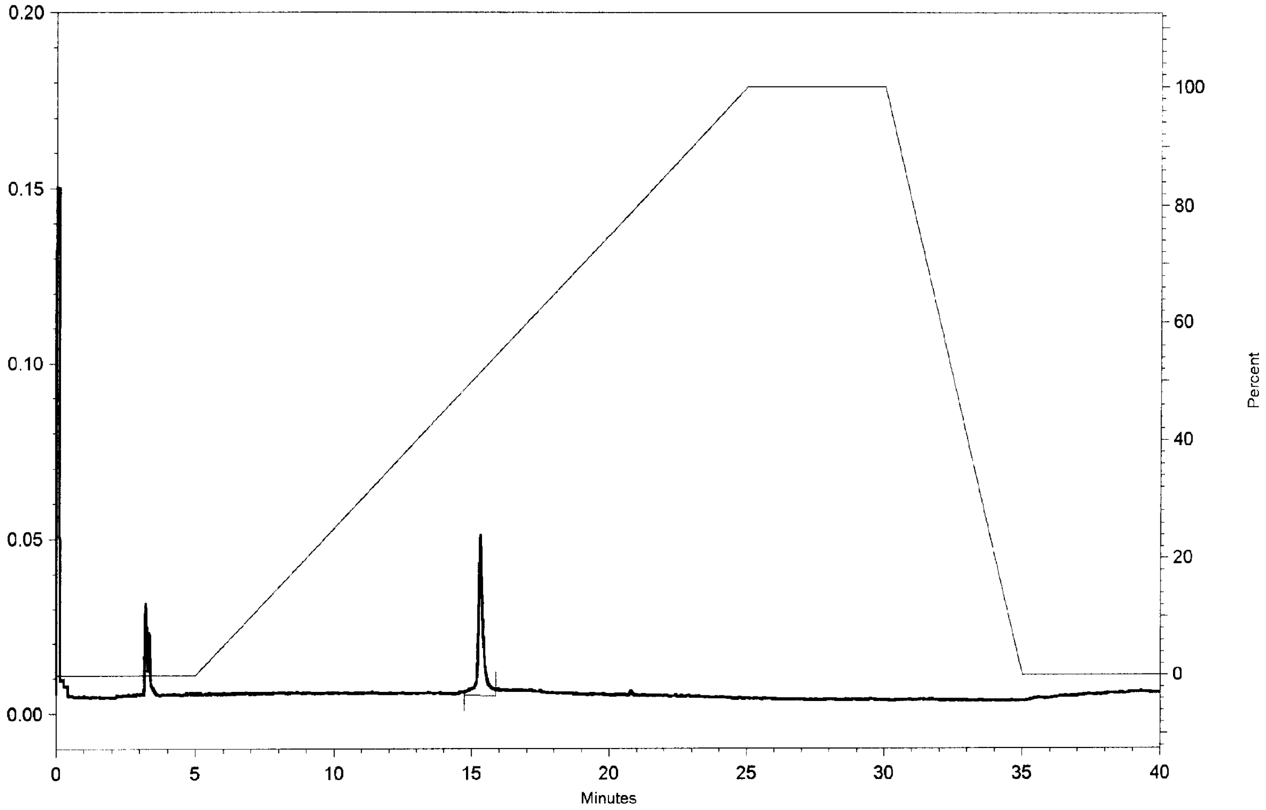
Detectors: PL-ELS1000



Peak table for PL-ELSD detector

PL-ELS1000

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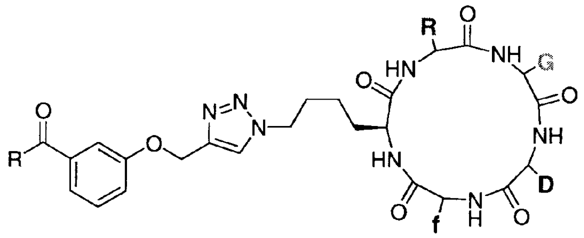
Totals				
			559228	100.00



23 R = DOTA-EtNH

einde

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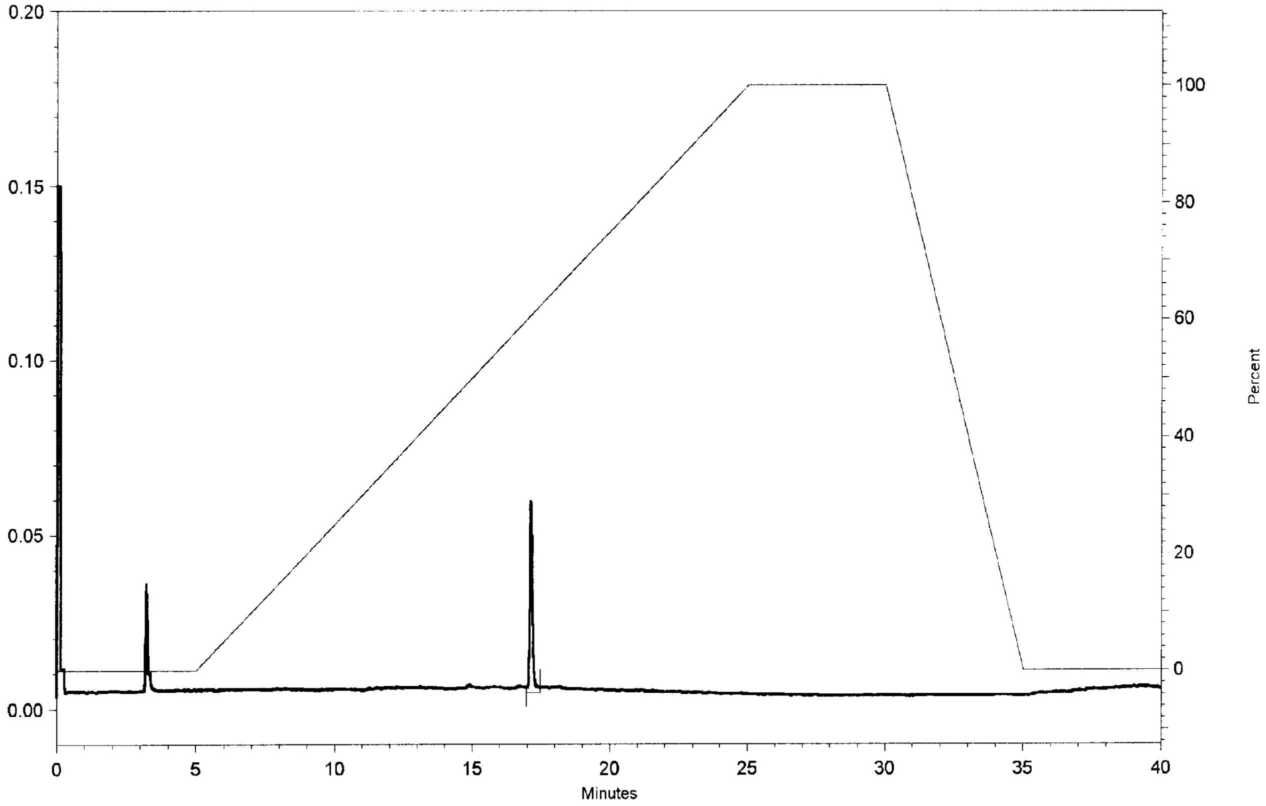
sample ID: **AYR052-3**
filename: C:\Users\Anneloes\Data\060419\Ayr052-3
Method file: C:\Users\Anneloes\Methods\ELSD.met
Detectors: PL-ELS1000

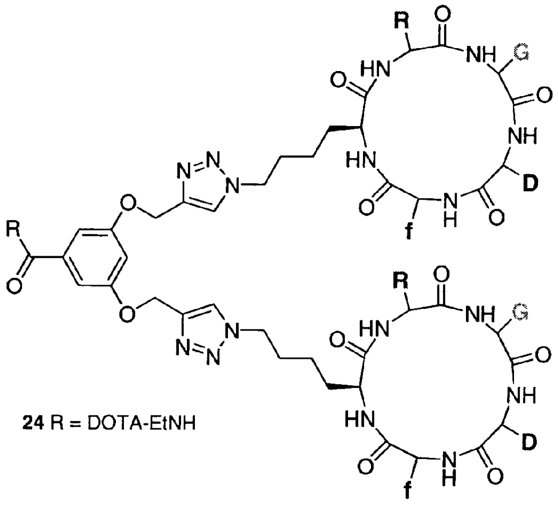


Peak table for PL-ELSD detector

PL-ELS1000

Pk #	Name	Retention Time	Area	Area Percent
1		17.125	378852	100.00

Totals			378852	100.00

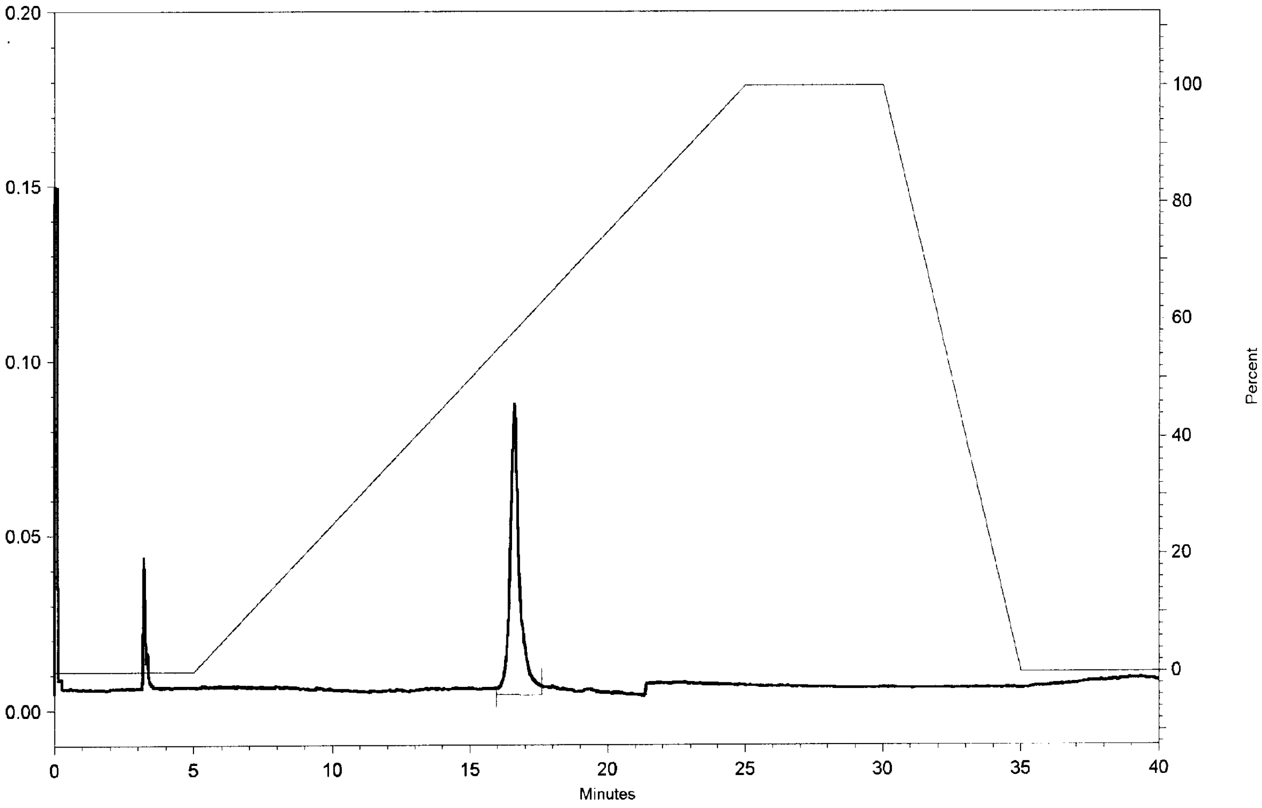


24 R = DOTA-EtNH

einde

HPLC Report Medicinal Chemistry

sample ID: **AYR065-1-1**
filename: C:\Users\Anneloes\Data\060419\AYR065-1-1
Method file: C:\Users\Anneloes\Methods\ELSD.met
Detectors: PL-ELS1000

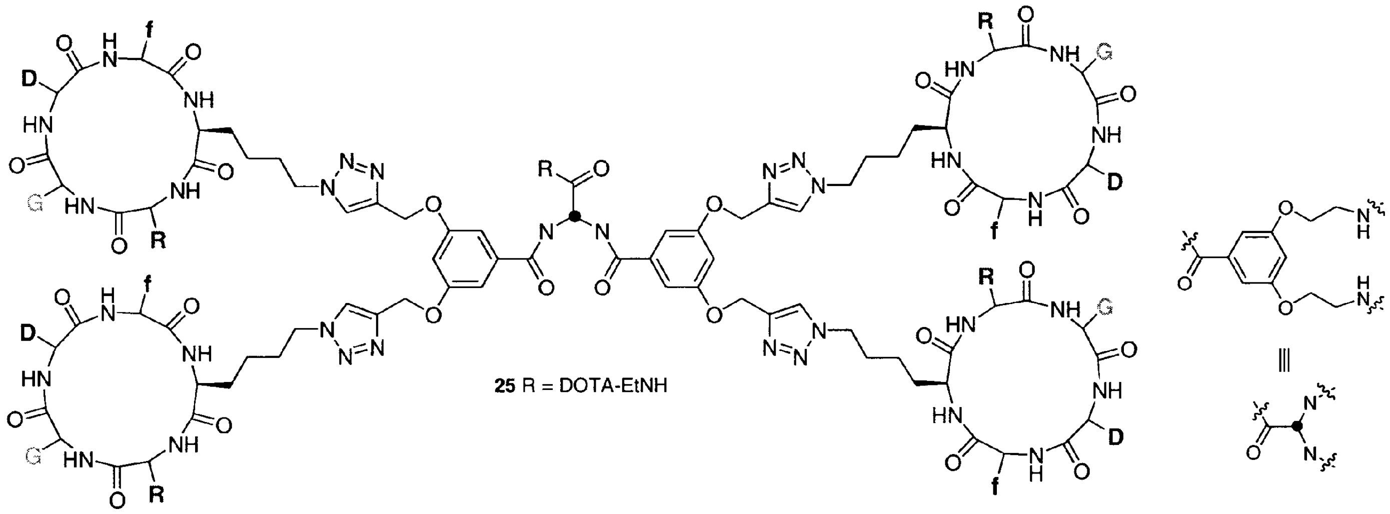


Peak table for PL-ELSD detector

PL-ELS1000

Pk #	Name	Retention Time	Area	Area Percent
1		16.608	1968319	100.00

Totals			1968319	100.00



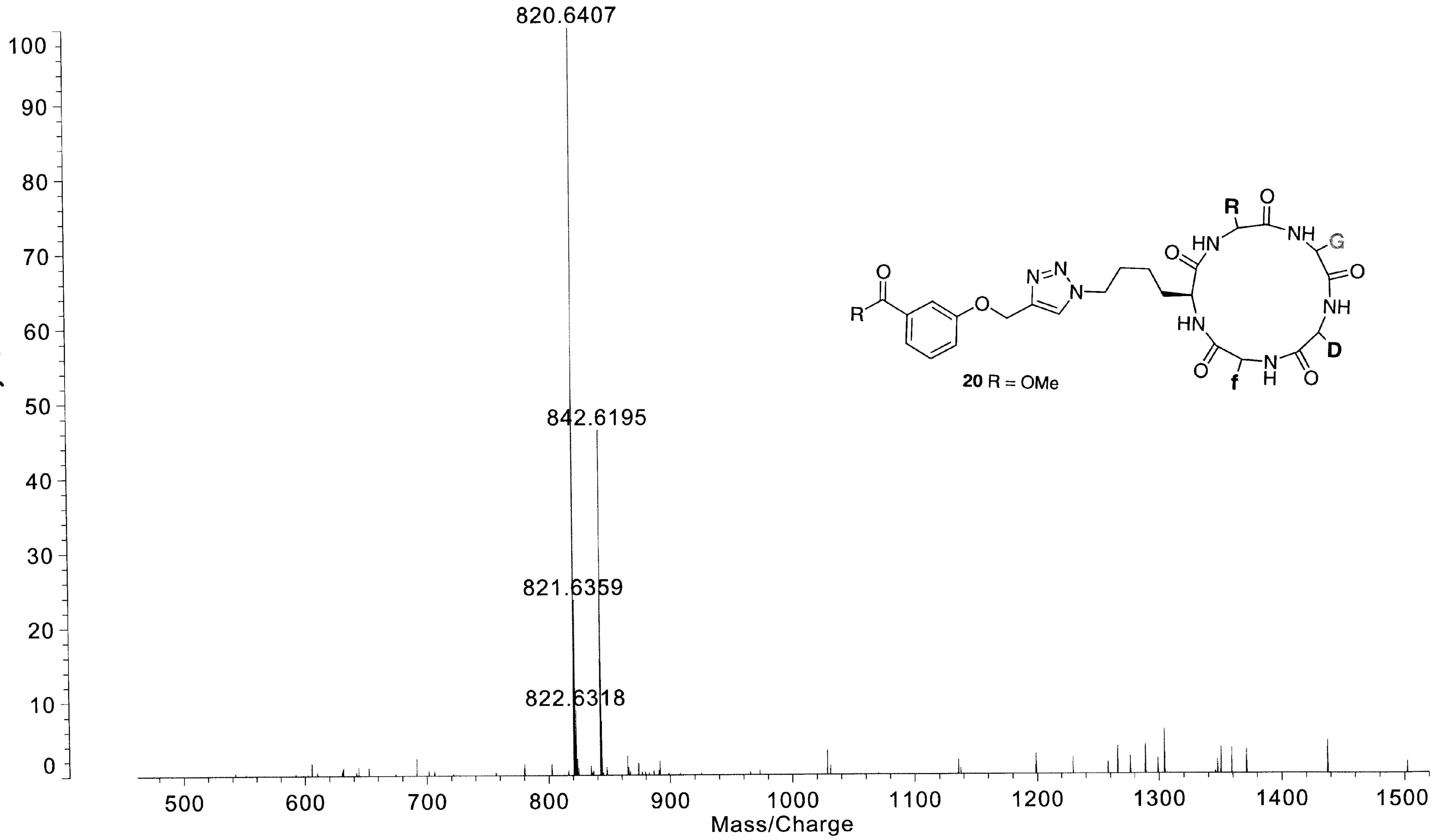
25 R = DOTA-EtNH

einde

515

Data: G1mono_cyclischRGD0001.M1 3 May 2005 14:33 Cal: DR_ACTH_050503 3 May 2005 13:42
Kratos PC Axima CFR V2.2.1: Mode reflectron, Power: 45, P.Ext. @ 819 (bin 89)

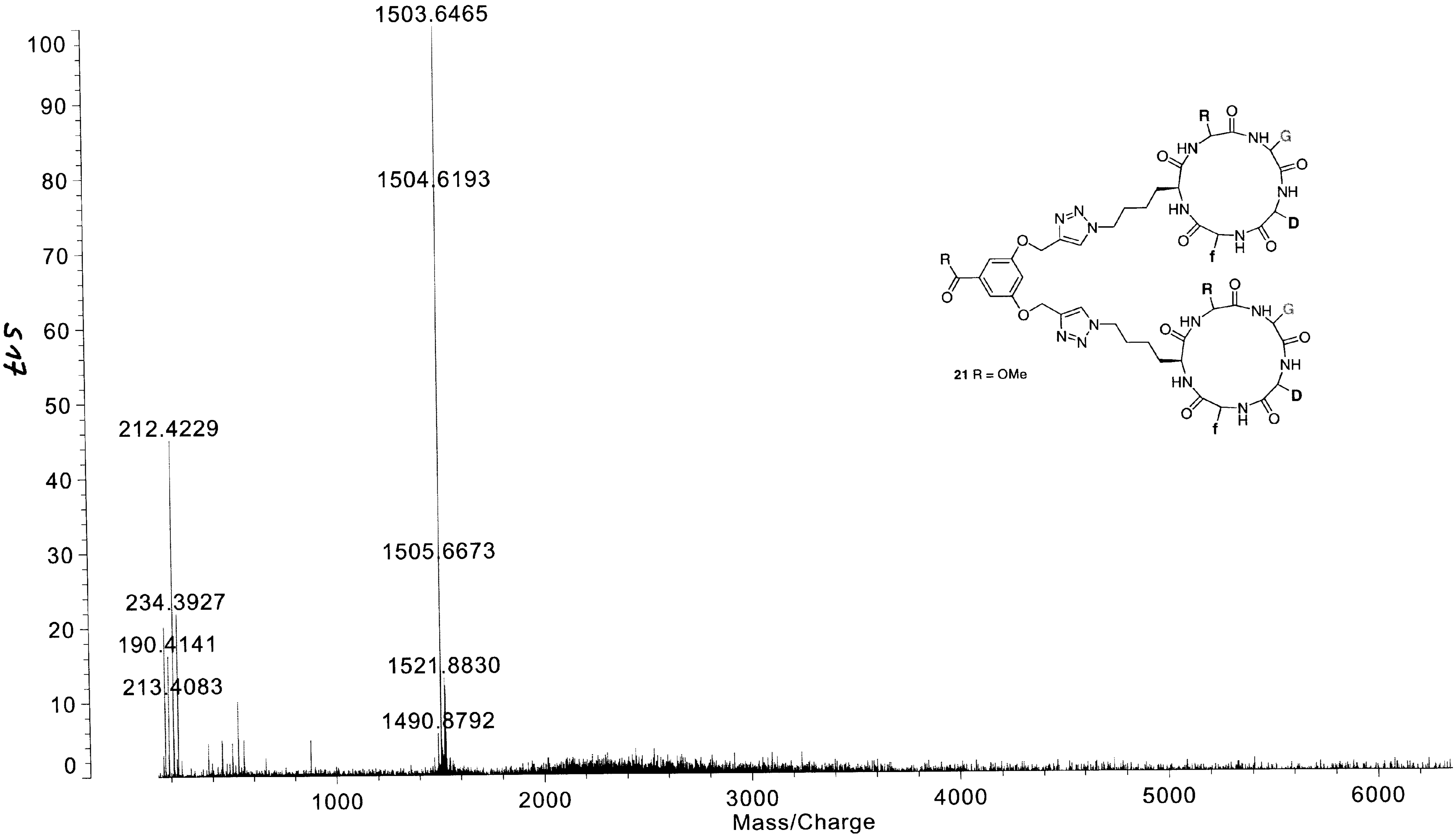
%Int. 5.0 mV[sum= 1288 mV] Profiles 1-260: (260 Tagged) Smooth Av 2 -Baseline 25



20 R = OMe

Data: 1G_cyclischRGD_fr_9-100001.M6 3 May 2005 15:03 Cal: DR_ACTH_050503 3 May 2005 13:42
Kratos PC Axima CFR V2.2.1: Mode reflectron, Power: 45, P.Ext. @ 1502 (bin 120)

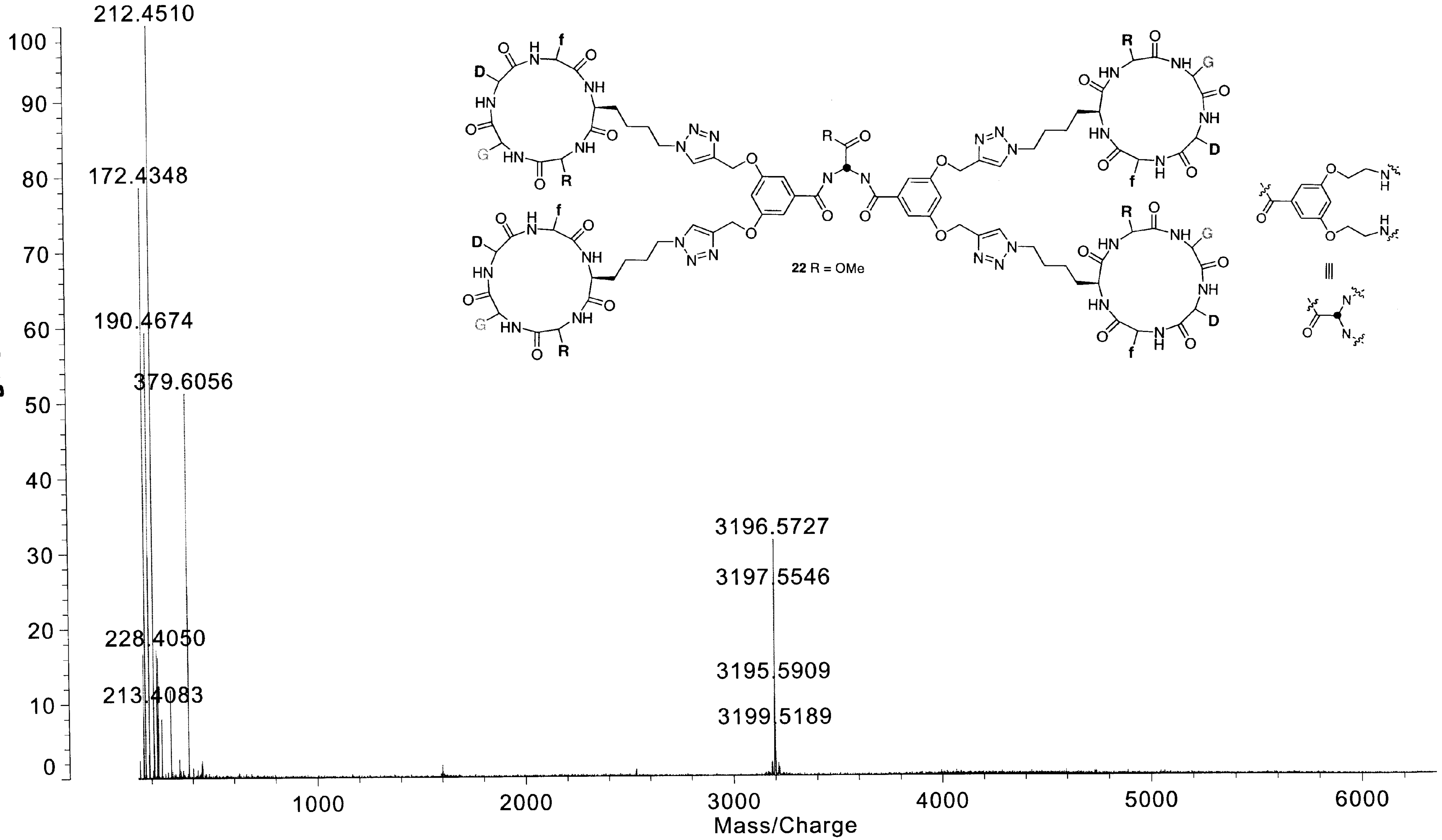
%Int. 83 mV[sum= 23863 mV] Profiles 1-287: (287 Tagged) Unsmoothed -Baseline 25



21 R = OMe

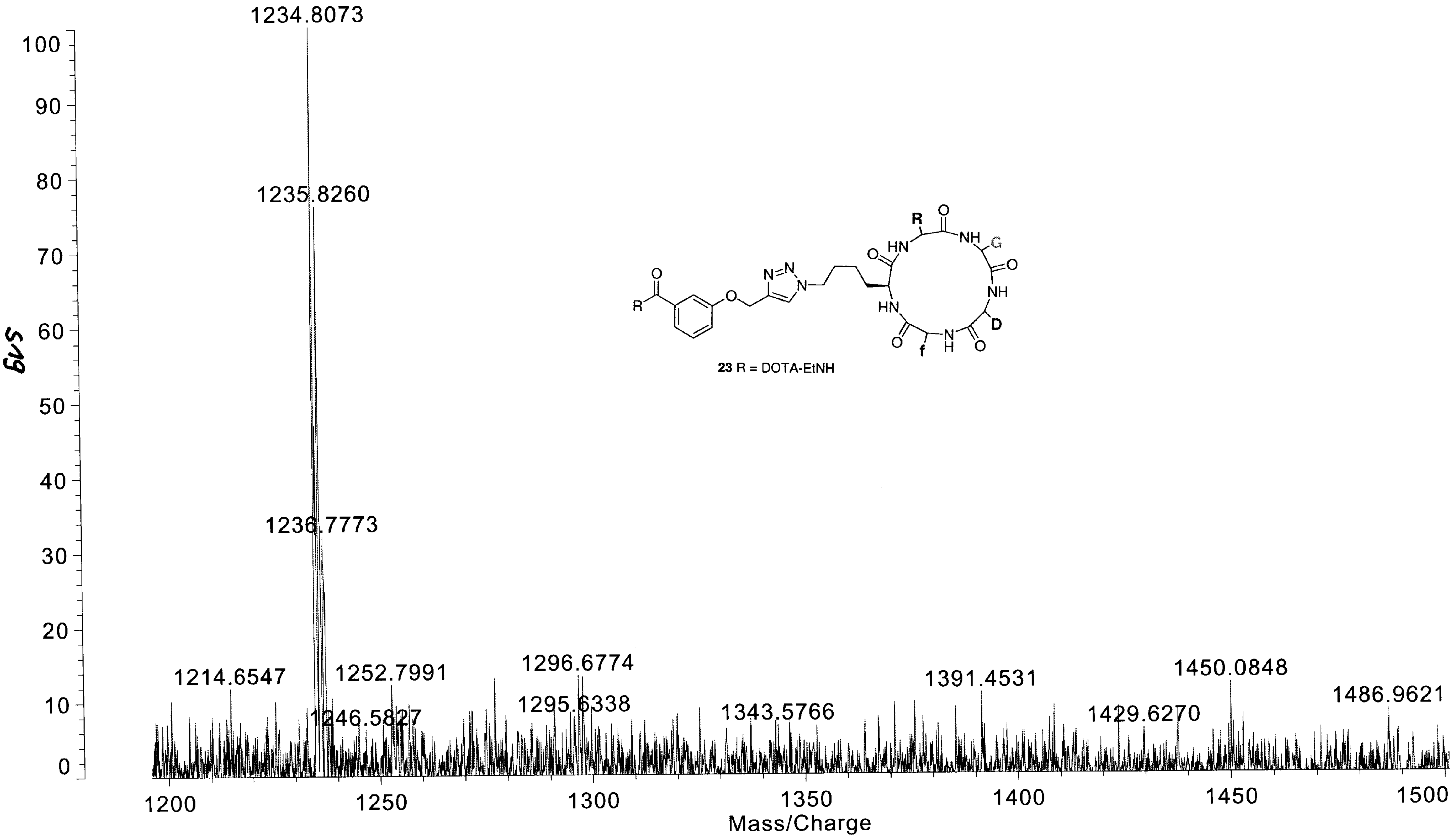
Data: 2G_cyclischRGD_fr_17_B0001.N5 3 May 2005 15:40 Cal: DR_ACTH_050503 3 May 2005 13:42
Kratos PC Axima CFR V2.2.1: Mode reflectron, Power: 43, P.Ext. @ 3195 (bin 176)

%Int. 91 mV[sum= 32332 mV] Profiles 1-356: (356 Tagged) Smooth Av 2 -Baseline 25



Data: AYR041_20001.K5 19 Apr 2006 13:24 Cal: 06419P14R_2 19 Apr 2006 13:20
Kratos PC Axima CFR V2.2.1: Mode reflectron, Power: 65, P.Ext. @ 1233 (bin 109)

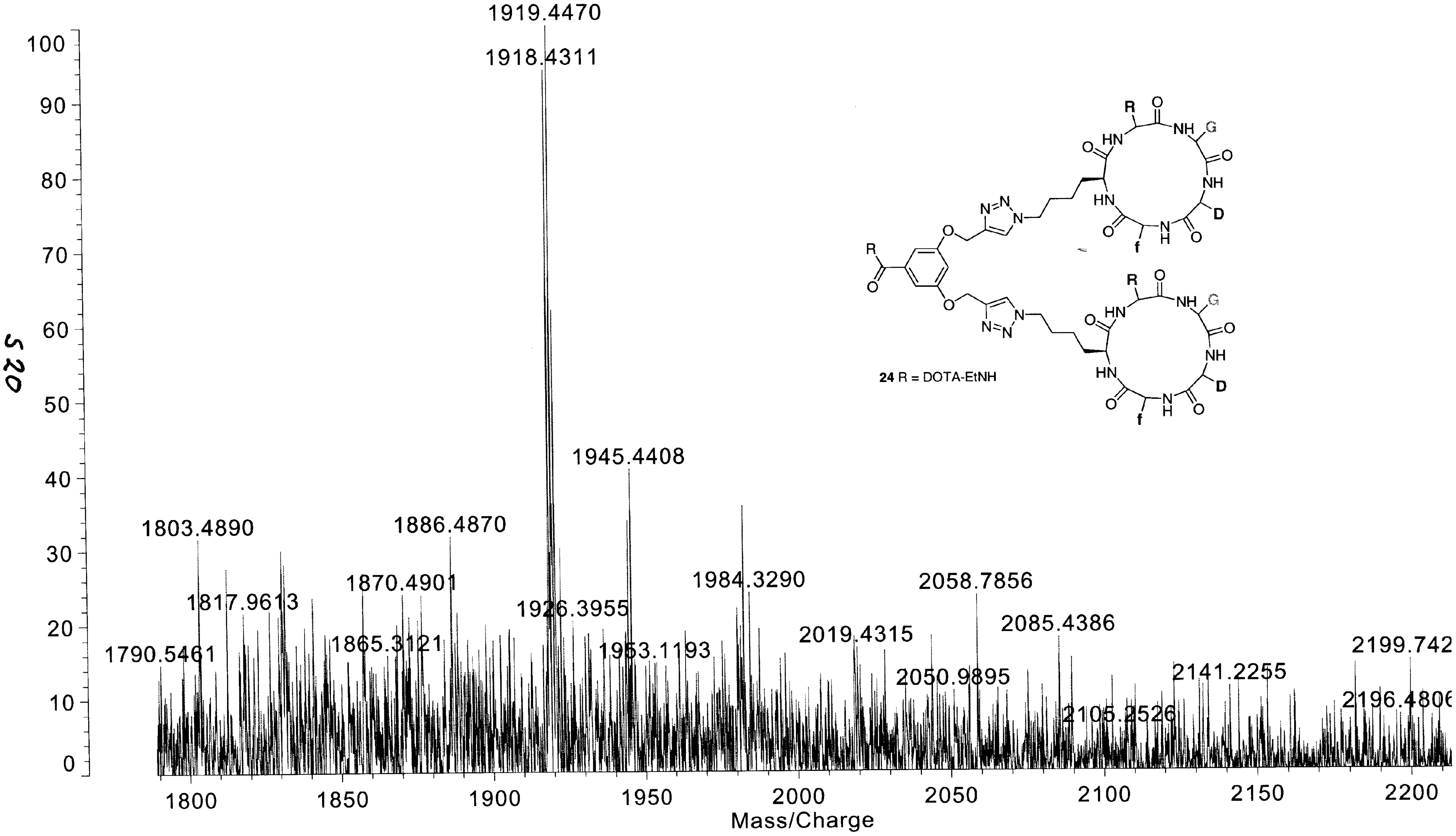
%Int. 114 mV[sum= 4000 mV] Profiles 1-35: (35 Tagged) Unsmoothed -Baseline 25



23 R = DOTA-EtNH

Data: AYR052_10001.K8 19 Apr 2006 13:27 Cal: 06419P14R_2 19 Apr 2006 13:20
Kratos PC Axima CFR V2.2.1: Mode reflectron, Power: 70, P.Ext. @ 1916 (bin 136)

%Int. 117 mV[sum= 2340 mV] Profiles 240-259: (20 Tagged) Unsmoothed -Baseline 25



24 R = DOTA-EtNH

Data: AYR065_1_10001.K15 19 Apr 2006 13:36 Cal: 06419lo 19 Apr 2006 13:34
Kratos PC Axima CFR V2.2.1: Mode reflectron, Power: 75, P.Ext. @ 3609 (bin 187)

%Int. 16 mV[sum= 3545 mV] Profiles 1-220: (220 Tagged) Unsmoothed -Baseline 25