SUPPORTING INFORMATION

Polycationic Sulfonamides for the Sequestration of Endotoxin

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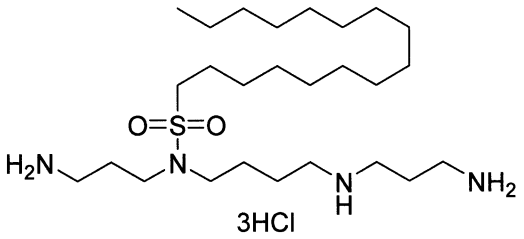
General experimental methods: The sources of all chemical reagents and starting materials were of the highest grade available and were used without further purification. Drying of organic layers was performed using $MgSO_4$. LC/MS analyzes were performed using a Hewlett-Packard 1050 system. Detection was by a Finnigan AQA operating in ESI^+ mode (m/z range 140 to 1600 amu). Gradient elution from 2 to 7 min. at 0.2 mL/min. was performed using 2% to 100% CH_3CN in H_2O (both with 0.05% TFA) using a Waters XTerra MS C_{18} 2.1 x 150 mm (3.5 μm) column. ^1H NMR spectra were recorded at 300 MHz on a Bruker AV300 spectrometer at the University of Washington, Seattle. ^1H NMR signals were generally multiples unless otherwise noted as s = singlet, d = doublet, t = triplet or m = multiplet. Chemical shifts are relative to external 3-(trimethylsilyl)-1-propanesulfonic acid, sodium salt.



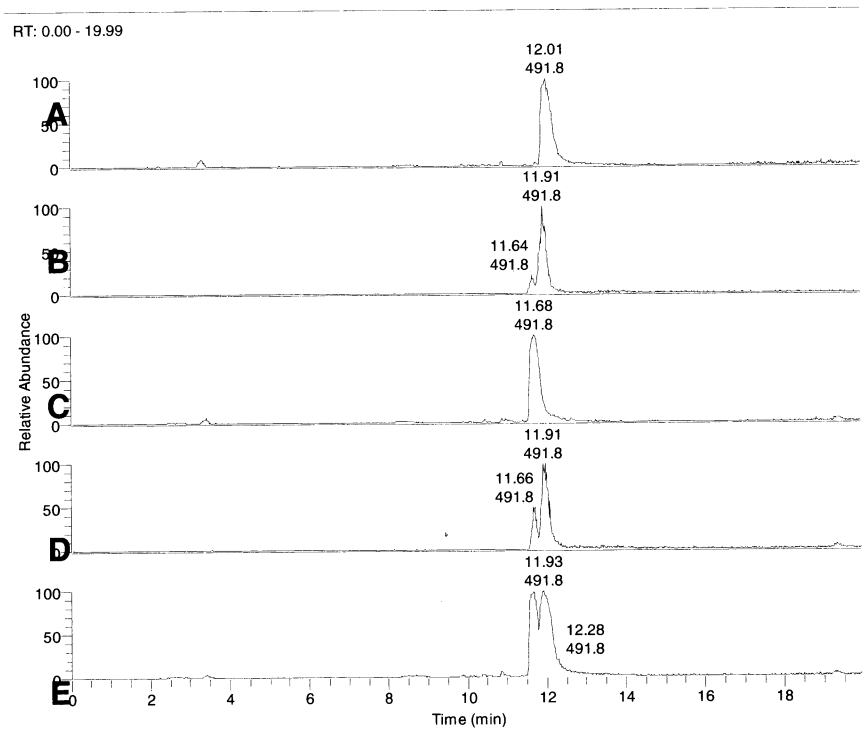
22

Regiospecific synthetic method to internally substituted spermine analogs: *N^4-Hexadecylsulfonyl-1,14-diamino-5,10-diazabuta-decane tris(hydrochloride) salt* **22** – To 0.105 g (0.17 mmole) of N^1, N^{14}-bistrifluoroacetylspermine diTFA salt[1] dissolved in 5 mL of dry THF was added 0.075 mL (0.54 mmole, 3.2 equiv) of Et_3N followed by 0.18 g (0.54 mmole, 3.2 equiv) of hexadecylsulfonyl chloride as a solid. After 3 hrs reaction time TLC analysis (90:8:2 CH_2Cl_2/MeOH/concd NH_4OH) showed the formation of

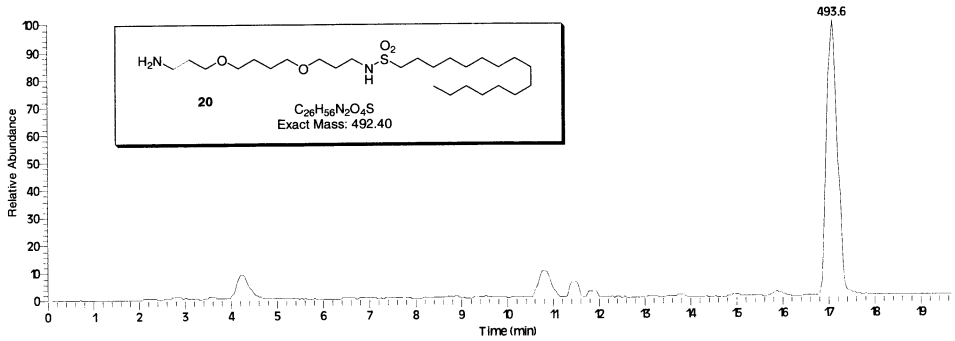
mono- and di-sulfonamide derivatives. Some starting material was also observed. Workup was by evaporation of THF and dilution in 15 mL each of CH_2Cl_2 and H_2O. The organic layer was removed and washed with brine, dried and evaporated to give the crude reaction product. This was dissolved in 10 mL MeOH and treated with 2 mL of 1N NaOH followed by overnight stirring. The reaction mixture was diluted with 5 mL each of CH_2Cl_2 and brine. The organic layer was removed, dried and evaporated to give the crude reaction mixture. Column chromatography using 9:1 CH_2Cl_2/MeOH containing a step-wise gradient of 1%, 2% then 2.5% concd NH_4OH gave pure mono- and di-internally sulfonylamidated spermine analogs. Following conversion to its tri-HCl salt form (MeOH/3N HCl then evaporation) received 15.8 mg (15% yield) **22** as a white solid. LC/MS analysis showed a single peak with desired m/z value of 491 amu at 11.7 min (see chromatograms below).

Comparison of internally and externally (made directly versus regiospecific method) spermine sulfonamides by LC/MS: Samples of 1D produced via the regiospecific route (1D) or the direct route (1D*) together with sample of **22** above were prepared in 1% TFA at 0.1 mM concentration and analyzed by LC/MS. The following chromatograms showed these species were distinct. It is interesting to note that 1D produced by the direct route contains a minor amount of the internally substituted analog as a side product. A) Cmpd **1D**; B) Cmpd **1D***; C) Sample **22**; D) 1:3 mix of sample **22** and **1D**; E) 1:1 mix of sample **22** and **1D**.

Figure 1: LC/MS Chromatograms of regioisomers.



LC/MS Chromatogram of Table 2 Analogs 20-32:



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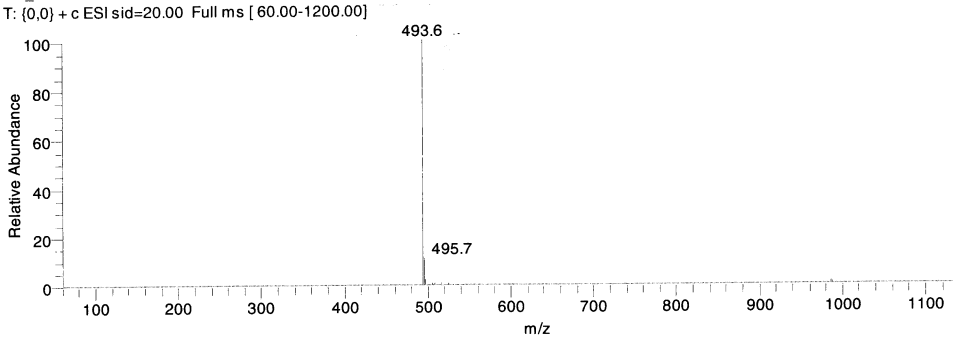

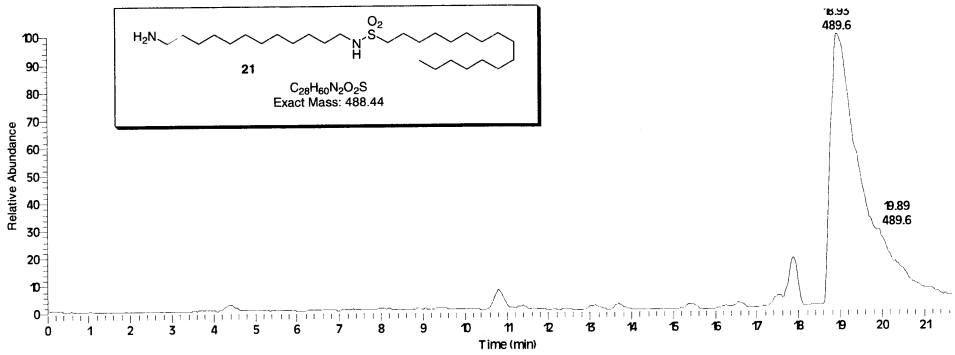

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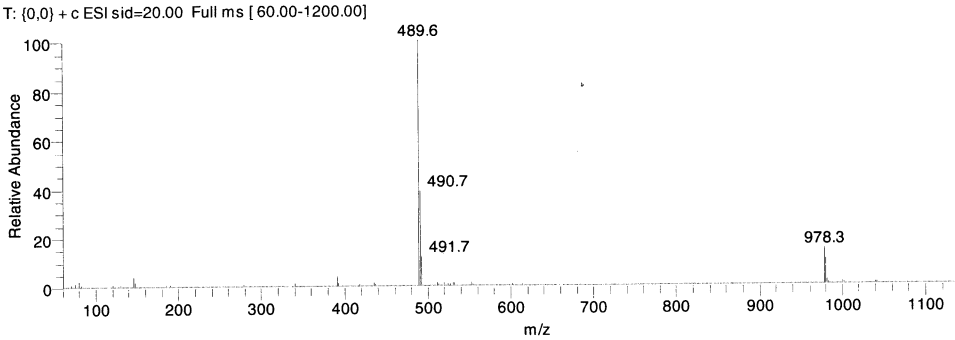

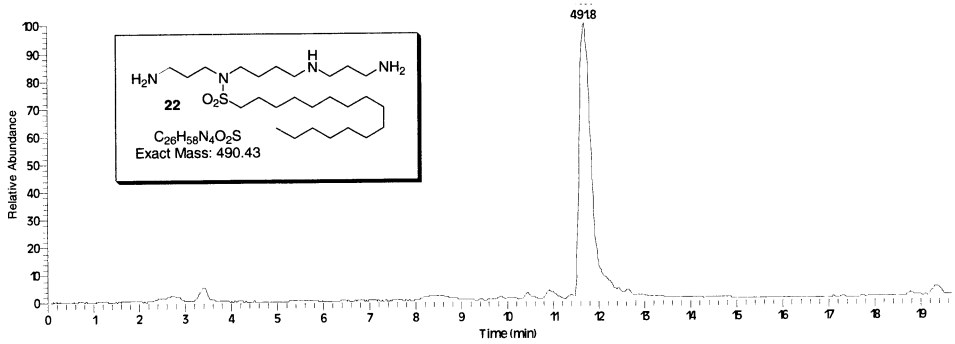

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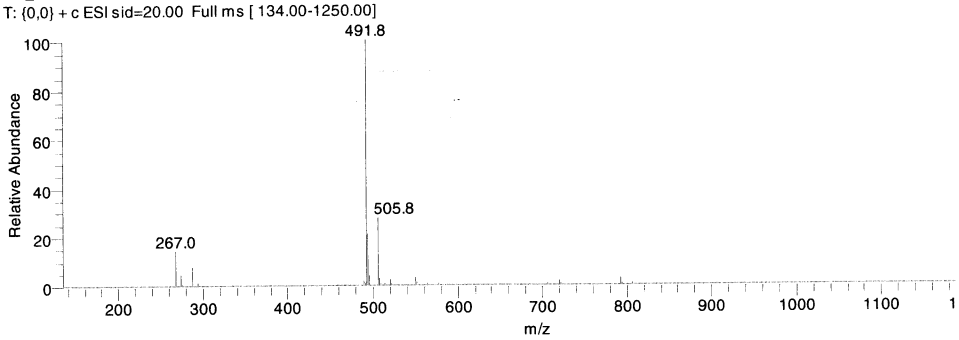

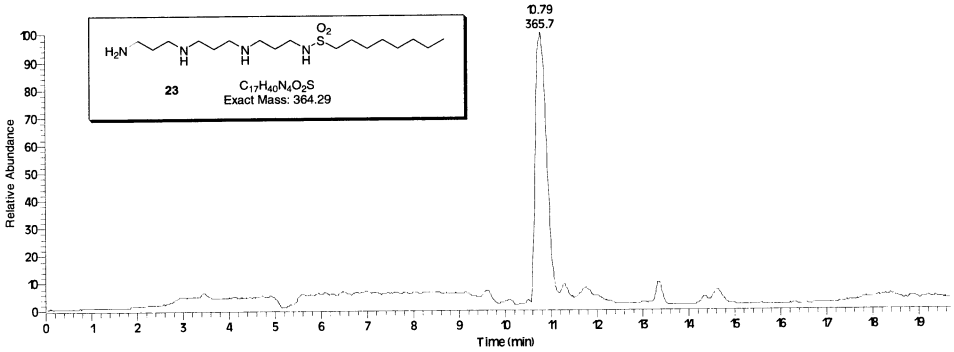

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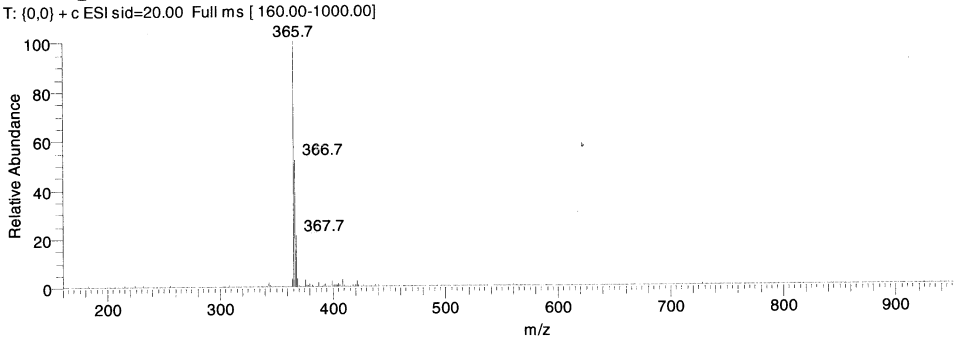

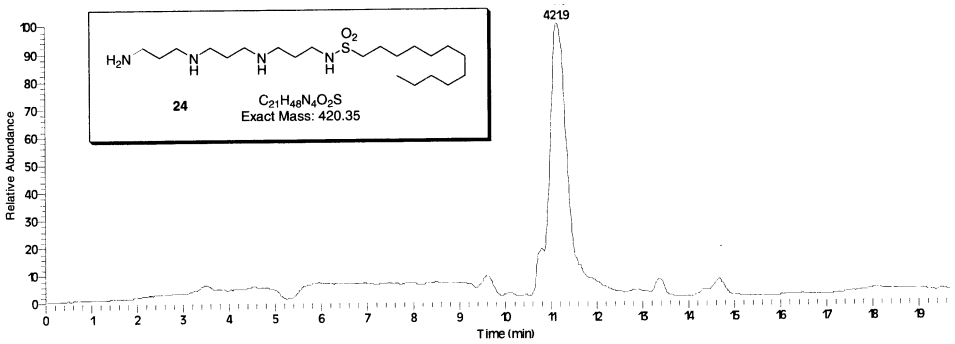

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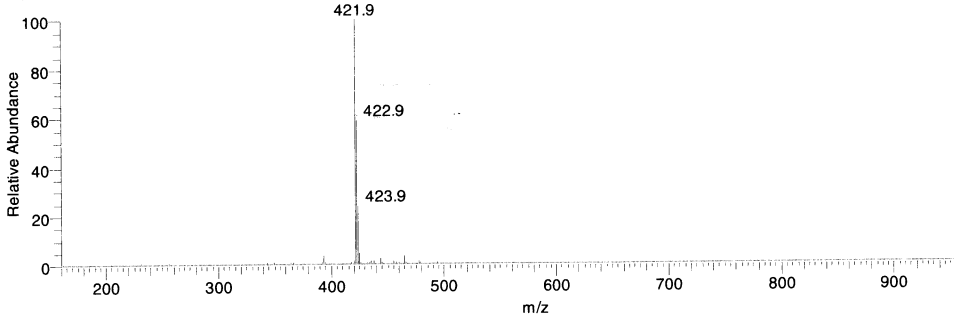

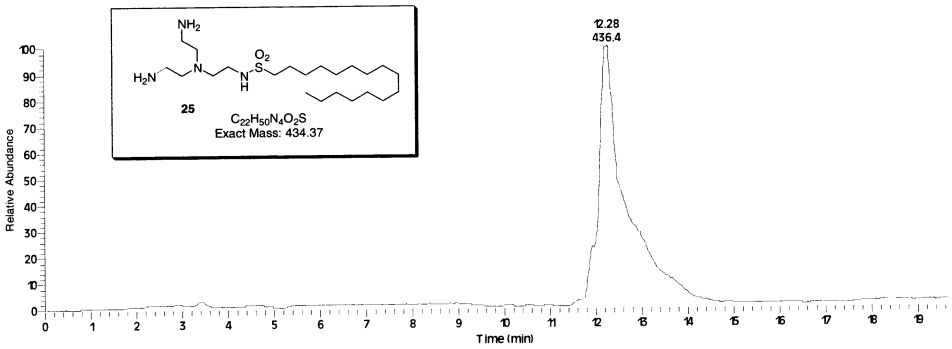

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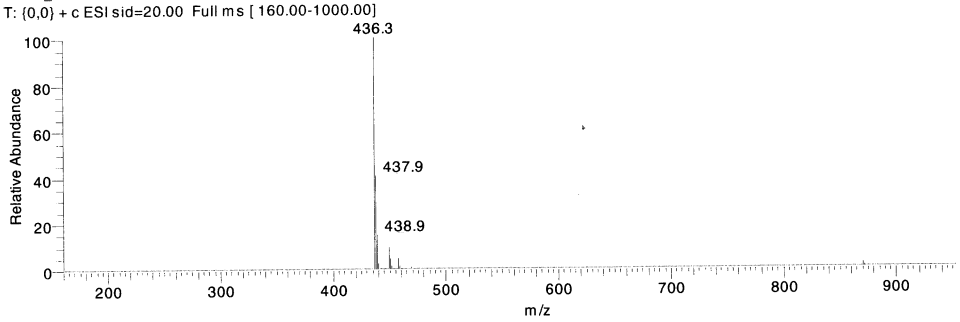

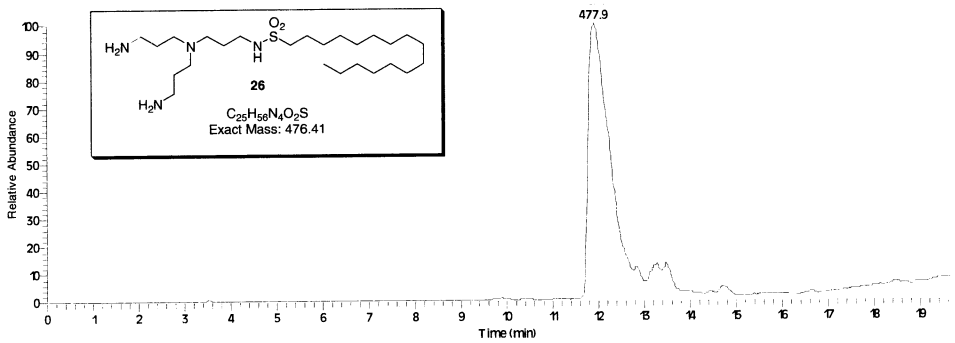

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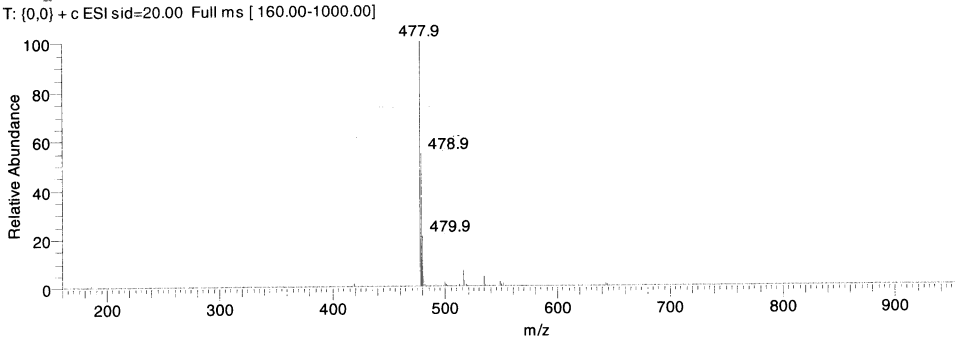

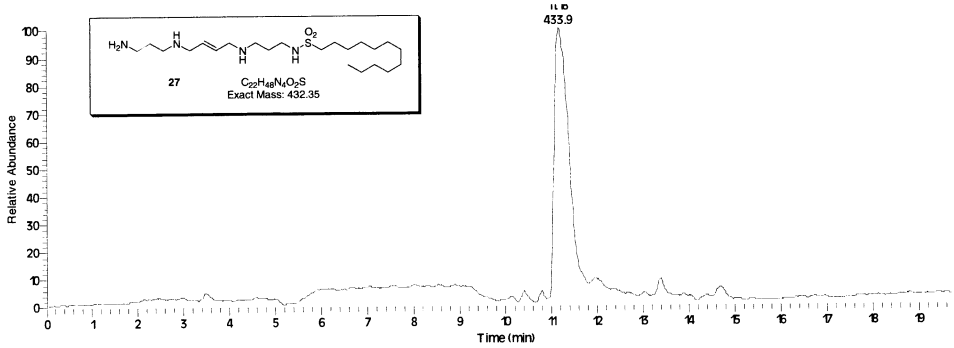

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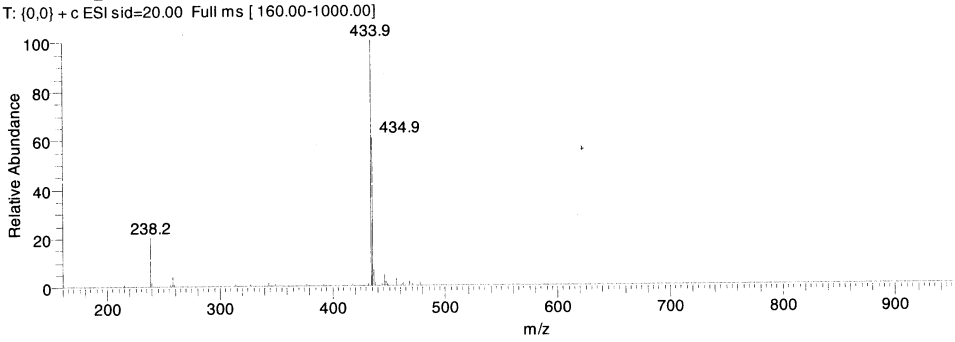

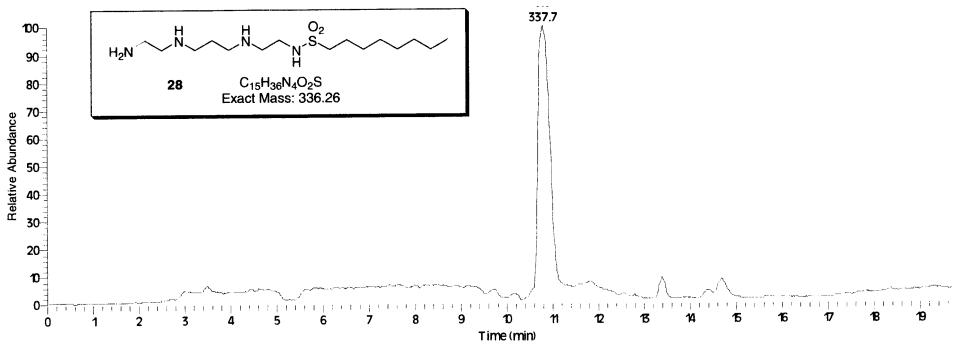

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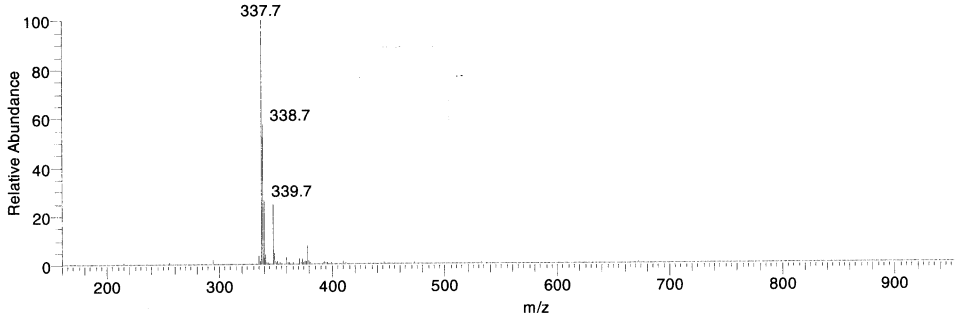

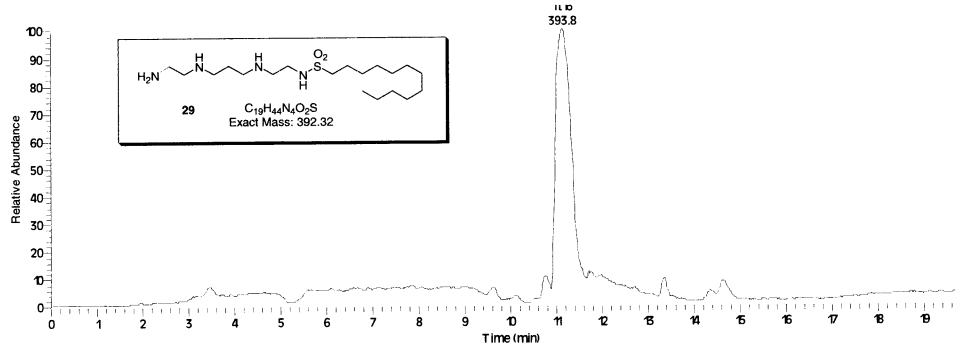

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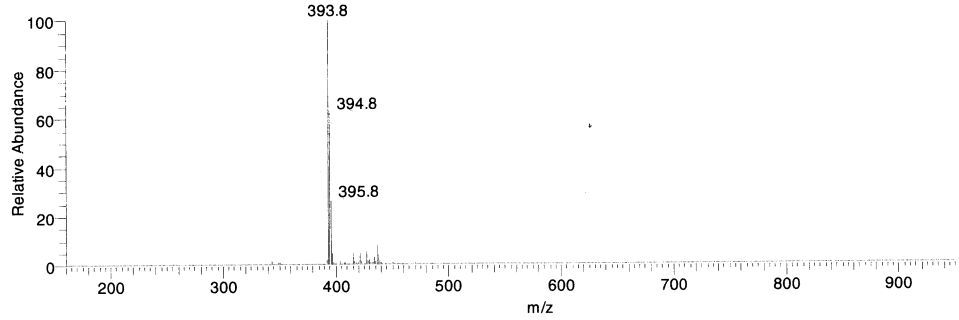

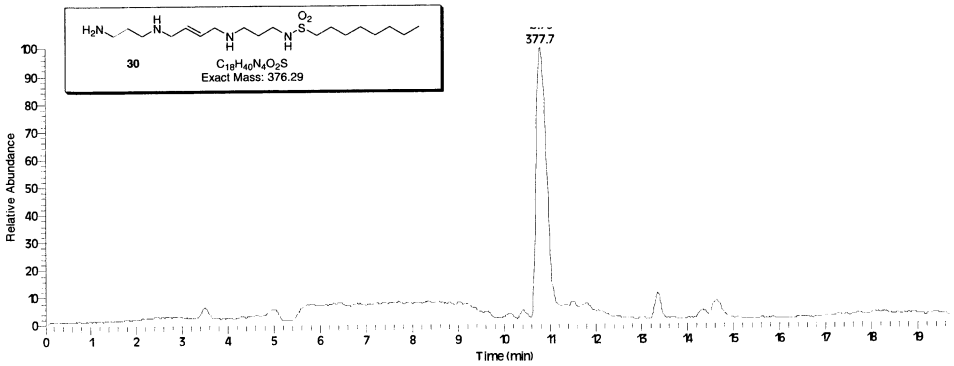

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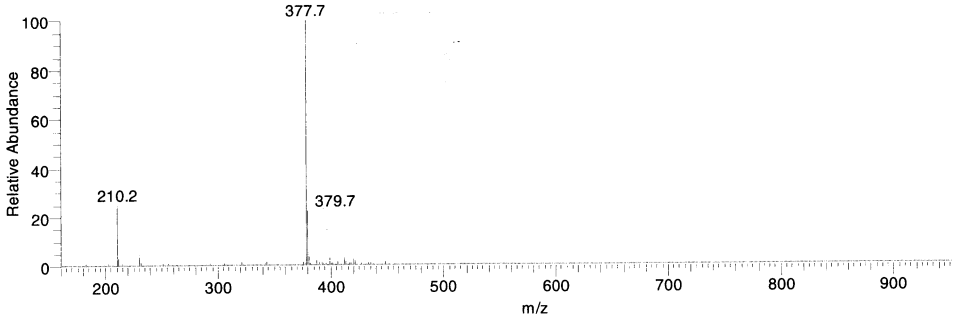

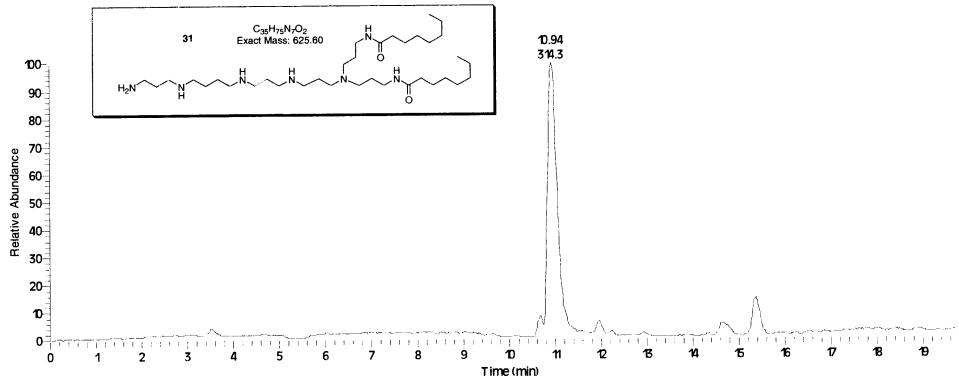

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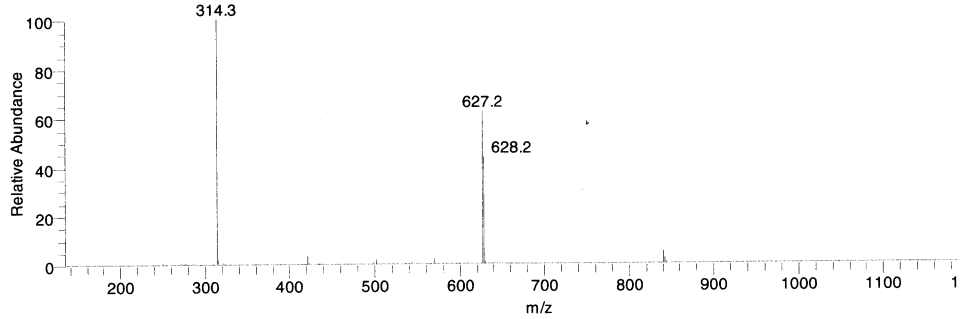

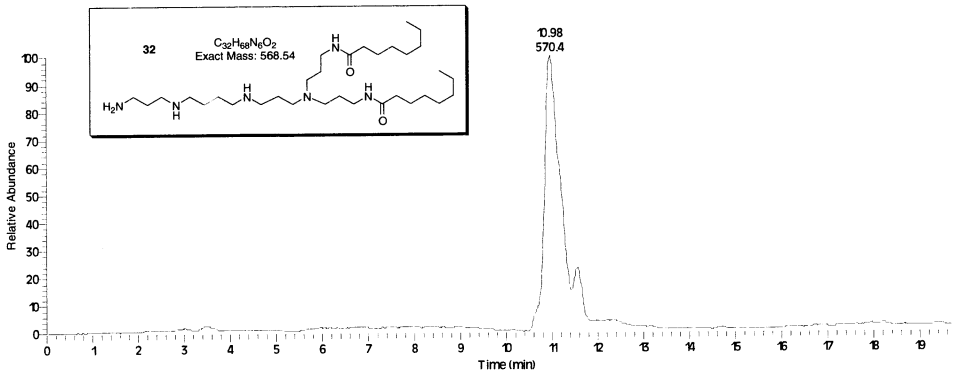

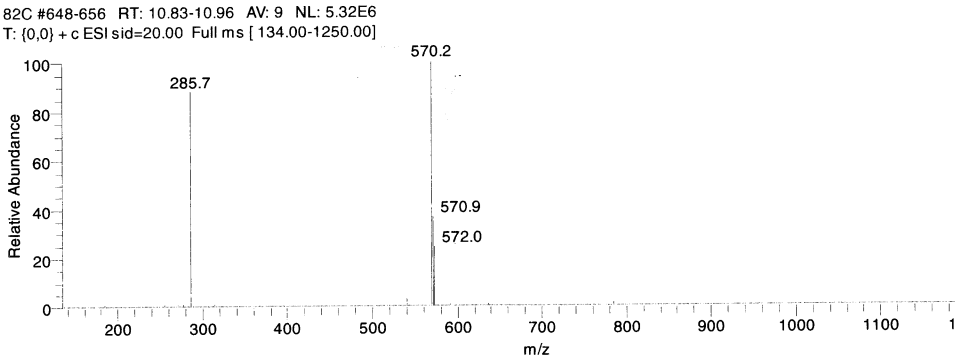

Reference List

1. O'Sullivan, M. C. and Dalrymple, D. M. A one-step procedure for the selective trifluoroacetylation of primary amino groups of polyamines. *Tetrahedron Letters* **1995,** *36*, 3451-3452.