Experimental Procedures for [G-2]- and [G-3]-Dendrimers and Complexes

General Procedures

CH_2Cl_2 distilled from CaH_2. THF distilled from Na/benzophenone. NEt_3 distilled from CaH_2. Gel filtration chromatography was performed with Biogel P-6 gel fine (column 2.5 cm x 98 cm) eluting with water at 0.7 mL min^{-1}. Detection was performed with a Knauer Differential Refractomer.

[G-2]-ol and [G-3]-ol prepared according to (a) Jayaraman, M.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1998**, *120*, 12996-12997. (b) Grayson, S. M.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2000**, *122*, 10335-10344.

[G-2]-azide
To a stirred solution of [G-2]-ol (2.154g, 3.50 mmol) and Et_3N (1.22 mL, 8.75 mmol) in CH_2Cl_2 at 0 ^oC under an Ar atmosphere was added dropwise methanesulfonylchloride (0.60 mL, 7.74 mmol). The reaction mixture was stirred at 0 ^oC for 1 h, then H_2O was added (20 mL) and the mixture stirred vigorously for 10 min. The mixture was transferred to a separatory funnel and CH_2Cl_2 was then added (100 mL). The organic layer was then washed with 1 M HCl (100 mL), saturated $NaHCO_3$ solution (100 mL) and H_2O (100 mL). The organic layer was dried ($MgSO_4$), filtered and evaporated to dryness to afford the crude mesylate (2.48 g), which was used immediately. ^1H NMR (300 MHz, $CDCl_3$): δ = 2.23 – 2.27 (1H, m), 2.79 (3H, s), 3.47 – 3.68 (14H, m), 4.31 (2H, d, J = 5.5 Hz), 4.48 (8H, s), 7.24 – 7.35 (20H, m).^{13}C NMR (75 MHz, $CDCl_3$): δ = 36.7, 40.4, 67.7, 68.7, 70.35, 70.37, 73.6, 78.8, 127.9, 128.6, 138.4.

A suspension of crude mesylate and NaN_3 in DMF (20 mL) was stirred at 60 ^oC under an Ar atmosphere for 14 h. The solvent was then removed and the residue partioned between H_2O (100 mL) and CH_2Cl_2 (100 mL). The aqueous phase was further extracted with CH_2Cl_2 (2 x 100 mL), and the organics combined, dried ($MgSO_4$), filtered and evaporated to dryness. The product was purified by column chromatography (SiO_2: EtOAc/Hexanes 15:85) to afford the [G-2]-azide as a clear oil (1.89 g, 84%). ^1H NMR (300 MHz, $CDCl_3$): δ = 2.10 -2.18 (1H, m), 3.46 (2H, d, J = 6.0 Hz), 3.53 – 3.68 (14H, m), 4.53 (8H, s), 7.26 – 7.37 (20H, m).^{13}C NMR (75 MHz, $CDCl_3$): δ = 40.7, 50.4, 68.8, 70.3, 73.6, 78.7, 127.82, 127.84, 128.6, 138.5. ES MS $[M]^+$ (calcd 662.3206) found 662.3237.

[G-2]-amine, 2
A suspension of [G-2]-azide (1.89 g), 10% $Pd(OH)_2/C$ and $N_2H_4.H_2O$ in THF/EtOH was stirred at 90 ^oC and the progress of the reaction monitored by ES^+. Further 10% $Pd(OH)_2/C$ and $N_2H_4.H_2O$ were added as required. Upon completion, the reaction was filtered through a celite pad and the filtrate evaporated to dryness to afford the crude [G-2]-amine as a slightly yellow oil (767 mg, 100%). The [G-2]-amine was further purified by gel filtration chromatography. ^1H NMR (300 MHz,D_2O): δ = 2.08 – 2.16 (1H, m), 2.94 (2H, d, J = 6.0 Hz), 3.37 – 3.64 (14H, m).^{13}C NMR (75 MHz, D_2O): δ = 38.3, 40.8, 60.6, 69.3, 81.1. ES MS $[M + H]^+$ (calcd 254.1604) found 254.1597.

[G-3]-azide
A solution of [G-2]-ol (2.677 g, 2.06 mmol) and NEt_3 in CH_2Cl_2 (80 mL) was cooled to 0 ^oC, and methanesulfonyl chloride added dropwise. The reaction mixture was stirred at 0 ^oC for 45 min, then H_2O was added (20 mL) and the mixture stirred vigorously for 10 min. The mixture was transferred to a separatory funnel and CH_2Cl_2 was then added (100 mL). The organic layer was then washed with 1 M HCl (50 mL), saturated $NaHCO_3$ solution (50 mL) and H_2O (50 mL). The organic layer was dried ($MgSO_4$), filtered and evaporated to dryness to afford the crude mesylate (2.934 g), which was used immediately without further purification. ^1H NMR (300 MHz, $CDCl_3$): δ = 2.12-2.22 (3H, m), 3.31-3.64 (36H, m), 4.21 (2H, d, J = 5.0 Hz), 4.51 (16H, s), 7.26-7.35 (40H, m).^{13}C NMR (75 MHz, $CDCl_3$): δ = 36.9, 39.8, 41.1, 68.5, 68.8, 69.7, 70.3, 73.5, 78.6, 127.7, 127.8, 128.58, 128.65, 138.6.

A suspension of crude mesylate and NaN_3 (1.34 g, 20.6 mmol) in DMF (10 mL) was stirred at 60 ^oC under an argon atmosphere for 14 h. The solvent was then removed and the residue partitioned between H_2O (100 mL) and CH_2Cl_2 (100 mL). The aqueous phase was further extracted with CH_2Cl_2 (2 x 100 mL), and the organics combined, dried ($MgSO_4$), filtered and evaporated to dryness. The product was purified by column chromatography (SiO_2: EtOAc/Hexanes 20:80) to afford desired azide as a clear oil (2.14 g, 78%). ^1H NMR (300 MHz, $CDCl_3$): δ = 2.01-2.06 (1H, m), 2.13-2.17 (2H, m), 3.23-3.63 (38H, m), 4.50 (16H, s), 7.23-7.33 (40H, m).^{13}C NMR (75 MHz, $CDCl_3$): δ = 41.1, 49.4, 60.6, 68.8, 69.60, 69.61, 70.2, 73.5, 78.6, 127.75, 127.80, 128.6, 138.6. ES MS $[M + H]^+$ (calcd 1346.9659) found 1346.9859.

[G-3]-amine, 4
To a suspension of [G-3]-azide (270 mg) and 10% $Pd(OH)_2/C$ (224 mg) in THF:EtOH (20 mL) was added 1 drop 1 M HCl. The resulting mixture was shaken under a H_2 atmosphere (45 psi) for 4 d, and then filtered through a celite pad. The filtrate was evaporate to dryness to afford the crude amine as a clear oil (158 mg). The [G-3]-amine was further purified by gel filtration chromatography. ^1H NMR (300 MHz, D_2O): δ = 1.89-2.03 (2H, m), 2.06-2.08 (1H, m), 2.92 (2H, d, J = 6.0 Hz), 3.29-3.53 (36H, m).^{13}C NMR (75 MHz, D_2O): δ = 37.0, 39.9, 40.5, 60.7, 68.4, 69.5, 70.1, 81.0. ES MS $[M + H]^+$ (calcd 578.3388) found 578.3394.

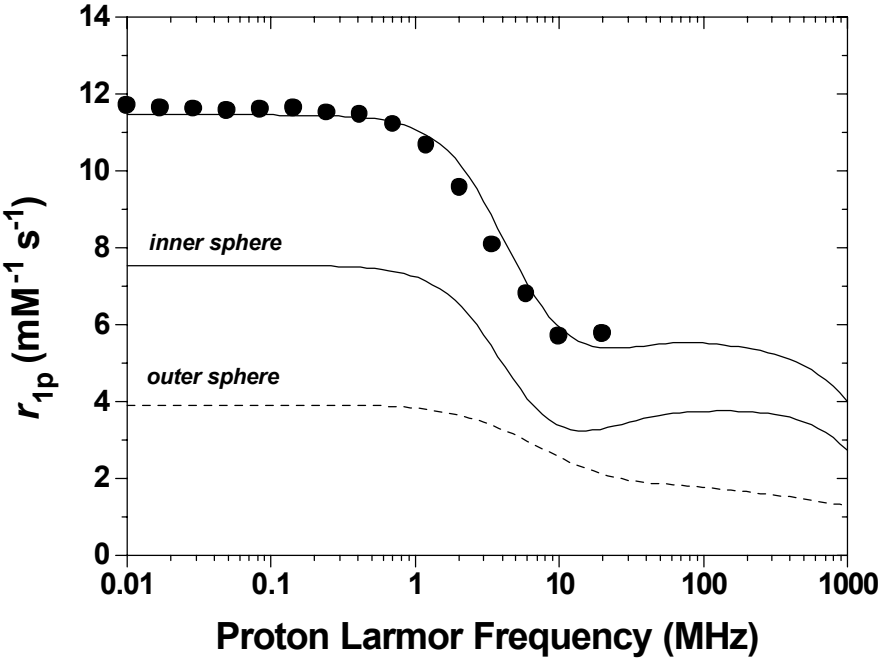
[G-2]-Dendrimer , 6b
To a solution of GdgDOTA (20 mg) and HOBt (41 mg) in 1:1 H_2O/Dioxane (1.0 mL) was added EDC (60 mg) and the reaction allowed to stir for 15 min. A solution of [G-2]-amine (70 mg) in H_2O (260 µL) was added to the reaction, and the mixture allowed to stir at room temperature for 4 d, during which time further EDC (60 mg) was added to the reaction. The reaction was then freeze-dried, the residue suspended in H_2O (0.5 mL) and centrifuged (1500 rev/min) for 5 min. The liquid was carefully removed and freeze-dried to afford the crude dendrimer as a pale brown oil (239 mg). Purification by gel-filtration chromatography afforded the pure [G-2]-dendrimer (20 mg, 48%). MALDI-TOF MS $[M + H]^+$ = 1788.7

[G-3]-Dendrimer, 6d

To a suspension of GdgDOTA (23 mg) in DMF (0.5 mL) was added *O*-benzotriazol-1-yl-*N*, *N*, *N'*, *N'*-tetramethyluronium tetrafluoroborate (51 mg) and the mixture allowed to stir for 2 h. A solution of [G-3]-amine (91 mg) in DMF (0.5 mL) was added to the reaction, and the mixture allowed to stir at room temperature for 5 d, during which time further *O*-benzotriazol-1-yl-*N*, *N*, *N'*, *N'*-tetramethyluronium tetrafluoroborate (47 mg) was added to the reaction. The reaction was then filtered through a syringe filter and evaporated to dryness. Purification by gel-filtration chromatography afforded the [G-3]-dendrimer (25 mg, 30%) i.e. the fully substituted tetraamide (major product) and the under-substituted triamide (10%, minor product). MALDI-TOF MS $[M + H]^+ = 3083.5$ (tetraamide product).

6a

NMRD (25°C)



$\Delta^2 = 5.6\times10^{19}$

$\tau_V = 10$ ps

$\tau_R = 100$ ps

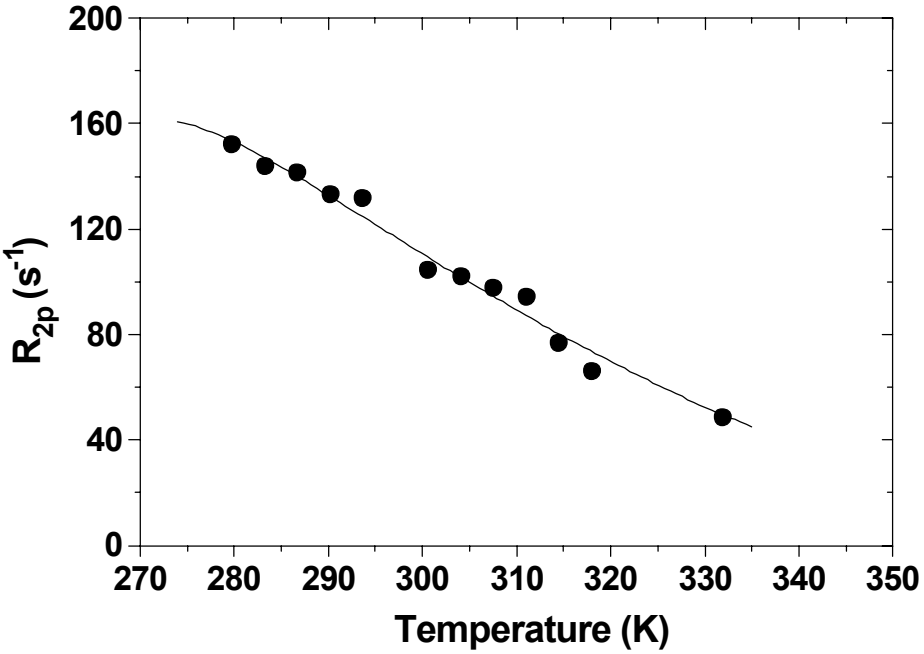
$r = 3.0$ Å

$q = 1$

$a = 4.0$ Å

$D = 2.24\times10^{-5}$ cm^2 s^{-1}

VT ^{17}O NMR (17 mM, 2.1 Tesla)



$\Delta^2 = 5.6\times10^{19}$

$\tau_V = 10$ ps

$\Delta H_V = 8.2$ *k*J/mol

$\tau_M = 42$ ns

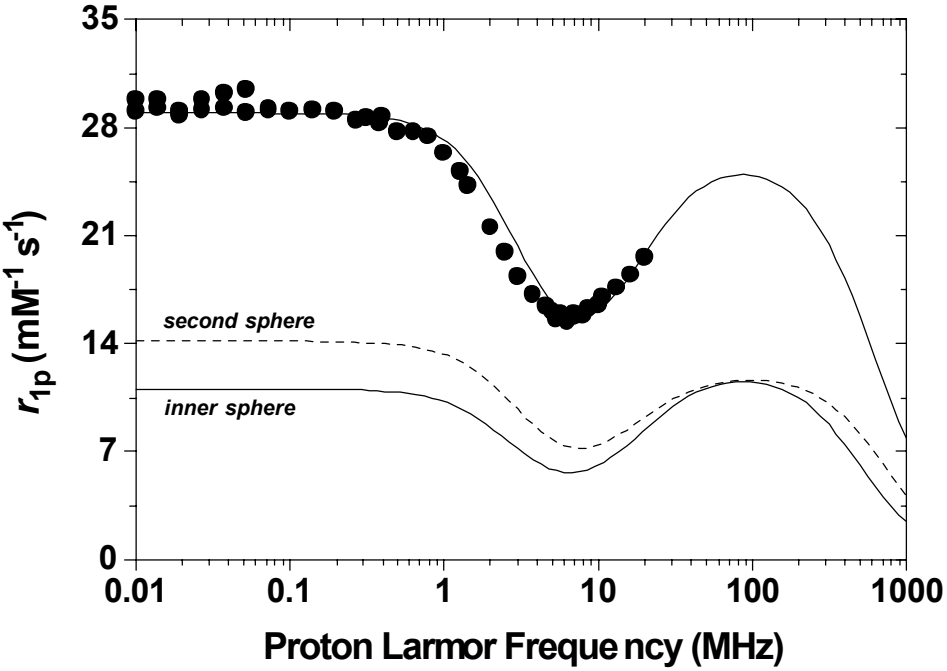
$\Delta H_M = 51.0$ *k*J/mol

$q = 1$

$A/\hbar = -3.8\times10^6$ rad s^{-1}

6d

NMRD (25°C)



$\Delta^2 = 4.4\times10^{19}$

$\tau_V = 15.8$ ps

$\tau_R = 330$ ps

$r = 3.0$ Å

$q = 1$

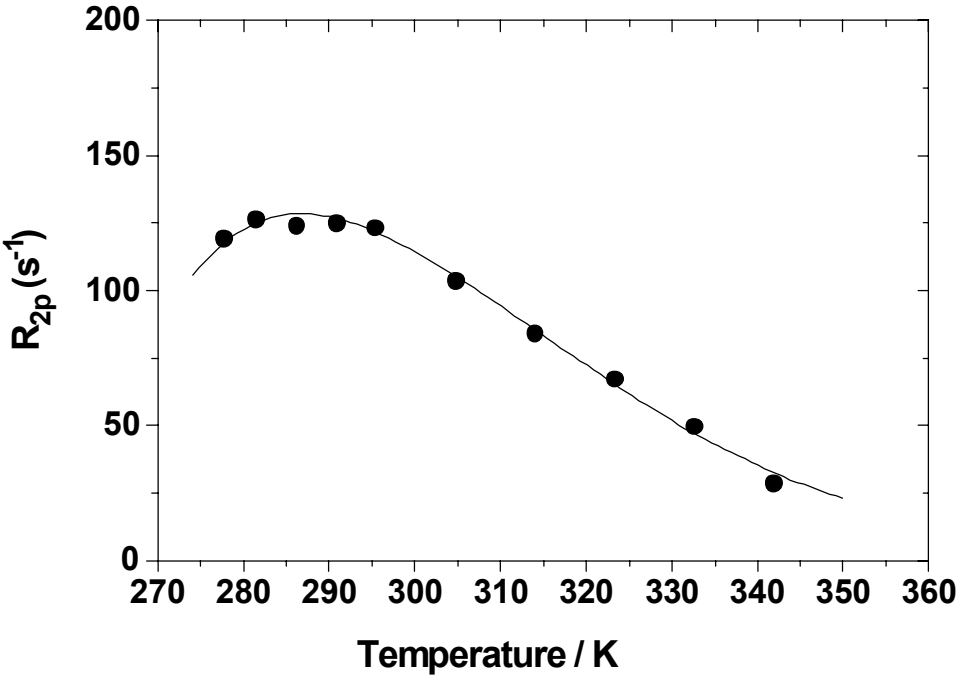
$a = 4.0$ Å

$D = 2.24\times10^{-5}$ cm^2 s^{-1}

$q' = 8$

$r' = 4.0$ Å

VT ^{17}O NMR (10 mM; 2.1 Tesla)



$\Delta^2 = 4.4\times10^{19}$

$\tau_V = 16$ ps

$\Delta H_V = 6.0$ *k*J/mol

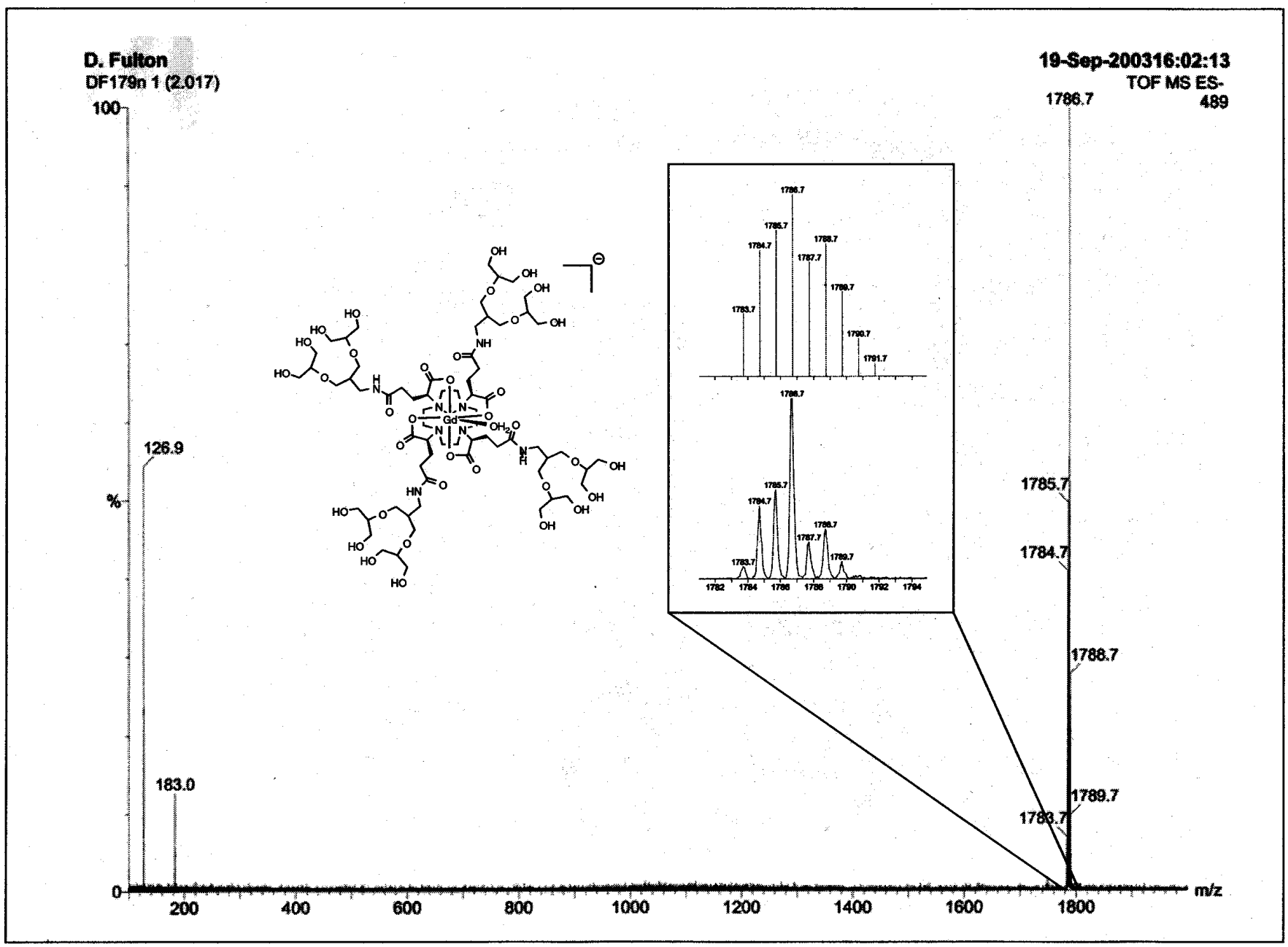
$\tau_M = 85$ ns

$\Delta H_M = 59.0$ *k*J/mol

$q = 1$

$A/\hbar = -3.8\times10^6$ rad s^{-1}

Electrospray Mass Spectrum Of [G-2]-Dendrimer



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20-Mar-2002
12:06:31

KsGdgDOTADE1 23 (2.364)

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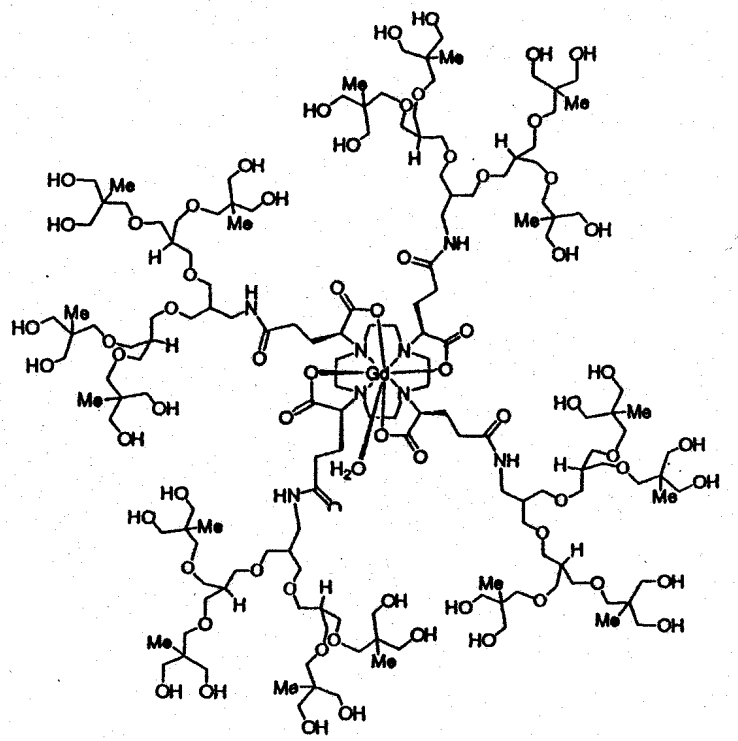

coupling reaction

13-Feb-2004
10:33:07

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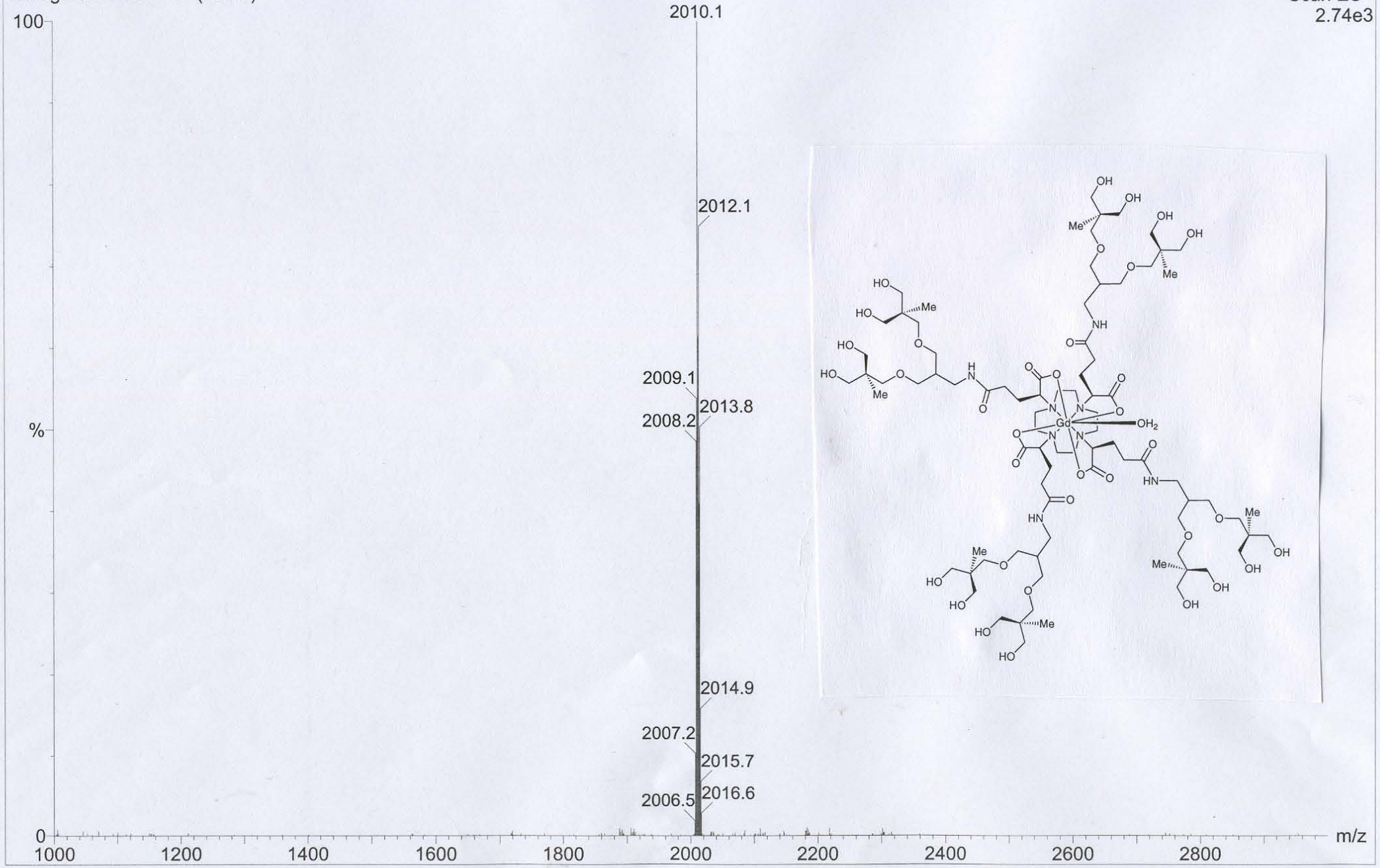

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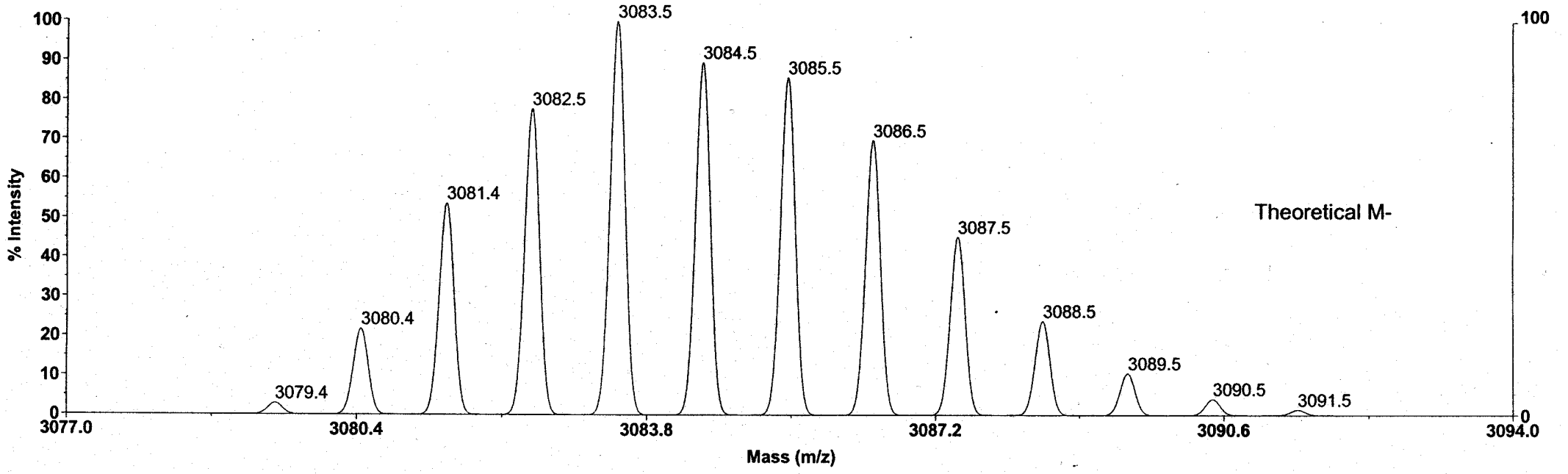
ISO:C124H236N8O68Gd



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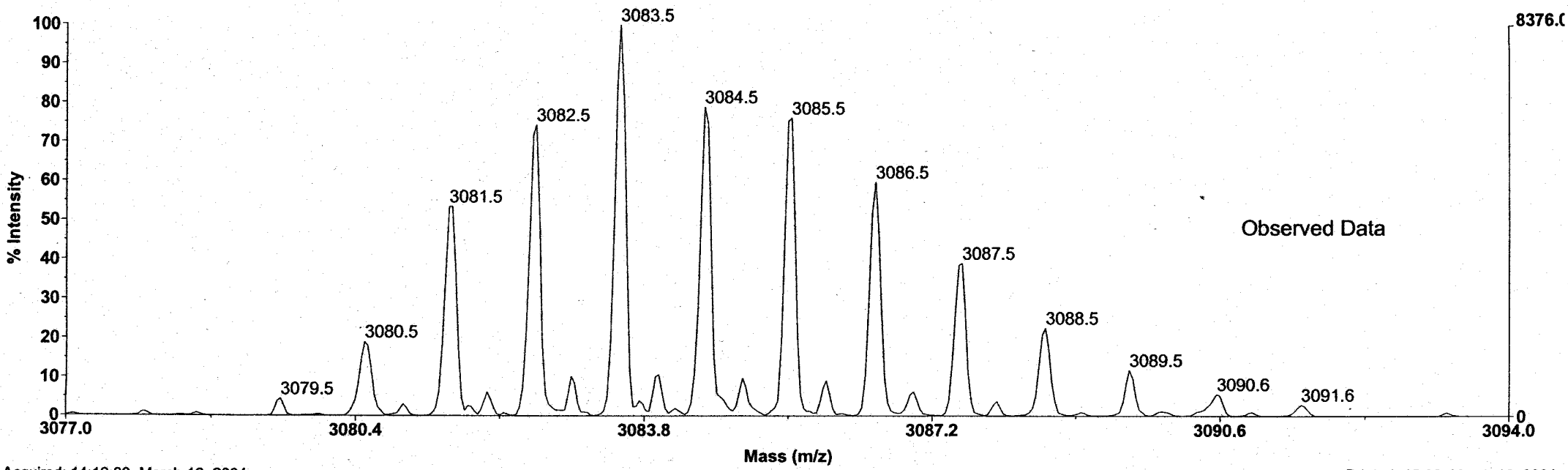

Acquired: 14:19:00, March 16, 2004
Dr Fulton DF/1/74 MW=3081?? NegRef [1:49] (THAP;1:1H2O:MeCN,9:1DAC:H2O)

Printed: 15:02, March 16, 2004

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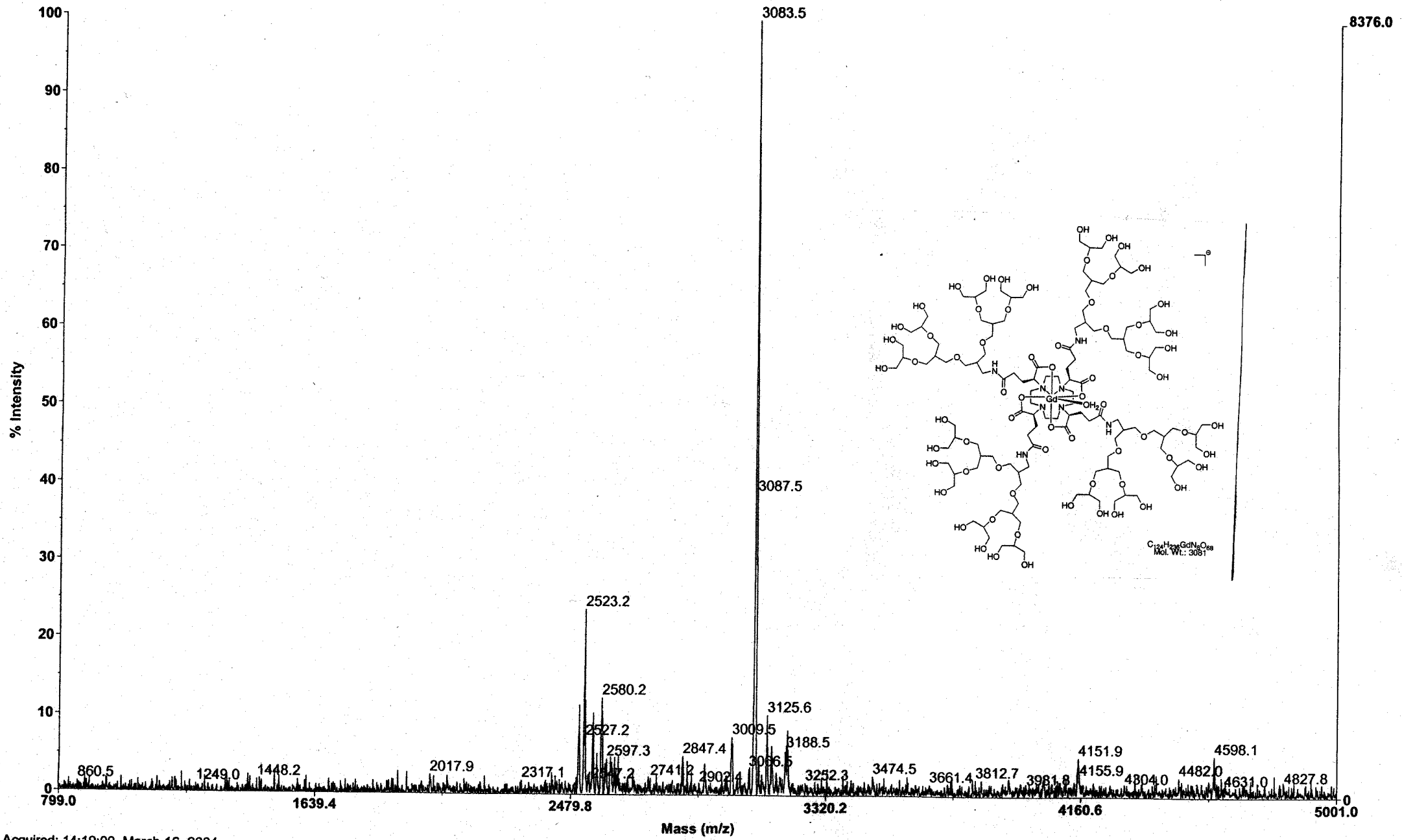
<<DUR12PAR(neg)_0001>> Voyager Spec #1=>AdvBC(64,0.5,0.1)=>NF0.7[BP = 3083.5, 8376]



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Printed: 14:58, March 16, 2004