Supporting Information

Synthesis of a new class of ribose functionalized dinucleotide cap analogues for biophysical studies on interaction of cap-binding proteins with the 5' end of mRNA.

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Synthesis of N^2,N^2-7-trimethylguanosine 5'-diphosphate ($m_3^{2,2,7}$GDP) from N^2,N^2-dimethylguanosine

1. N^2,N^2-dimethylguanosine 5'-monophosphate ($m_2^{2,2}$GMP)

Phosphorus trichloride oxide (201 μL) in trimethyl phosphate (4.65 mL) was cooled to 4°C and N^2,N^2-dimethylguanosine (150 mg, 0.48 mmol) was added to the solution. The reaction mixture was stirred at 4°C. After 3h 1M TEAB was added to maintain pH as neutral. The product was separated from the reaction mixture by chromatography on DEAE–Sephadex using a 0 – 0.8 M gradient of TEAB. $m_2^{2,2}$GMP was obtained as a colourless crystals (177 mg, 0.36 mmol, TEA salt, 75%); *m/z*: calcd for $C_{12}H_{18}N_5O_8P_1$: 391.08928, found: 392.0969 $[M+H]^+$.

2. N^2,N^2-7-trimethylguanosine 5'-monophosphate ($m_3^{2,2,7}$GMP)

Methyl iodide (0.21 mL, 3.4 mmol) was added to a suspension of N^2,N^2-dimethylguanosine 5'-monophosphate (141 mg, 0.28 mmol, TEA salt) in anhydrous dimethylsulfoxide (1.2 mL) and stirred at RT for 2h. The reaction mixture was poured into water (10 mL) and extracted three times with diethyl ether. Aqueous phase was concentrated and applied on DEAE–Sephadex. The product was eluted using a 0 – 0.8 M gradient of TEAB. $m_3^{2,2,7}$GMP was obtained as a colourless crystals (85 mg, 0.17 mmol, TEA salt, 61%): *m/z*: calcd for $C_{13}H_{21}N_5O_8P_1$: 406.1127, found: 406.1160.

3. N^2,N^2-7-trimethylguanosine 5'-diphosphate ($m_3^{2,2,7}$GDP)

N^2,N^2-7-trimethylguanosine 5'-monophosphate (90 mg, 0.18 mmol, TEA salt), imidazole (242 mg, 3.6 mmol), 2,2'-dithiodipyridine (80 mg, 0.36 mmol) and triethylamine (25 μL) were mixed in anhydrous DMF (0.9 mL). After 20 min triphenylphosphine (95 mg, 0.36 mmol) was added and the mixture was stirred for 6–8 h at RT. The product was precipitated from the reaction mixture with a solution of anhydrous sodium perchlorate (87 mg, 0.7 mmol) in dry acetone (1.3 mL). After cooling at 4°C, the precipitate was filtered, washed repeatedly with cold, dry acetone, and dried overnight in vacuum over P_4O_{10}. N^2,N^2-7-trimethylguanosine 5'-monophosphate imidazolide was obtained as a white powder. N^2,N^2-7-trimethylguanosine 5'-diphosphate imidazolide (142 mg, 0.2 mmol, Na salt) and $ZnCl_2$ (270 mg, 2 mmol) were stirred in anhydrous DMF (4 mL) with triethylammonium phosphate (600 mg, 1.5 mmol) The reaction mixture was poured into a solution of EDTA (95 mg, 0.25 mmol) in water (1.5 mL) and neutralized to pH 7 by addition of 1M TEAB. The product was separated from the reaction mixture by chromatography on DEAE–Sephadex using a 0 – 1.0 M gradient of TEAB. N^2,N^2-7-trimethylguanosine 5'-diphosphate was obtained as a colourless crystals (97 mg, 0.14 mmol, TEA salt, 78%). *m/z*: calcd for $C_{13}H_{22}N_5O_{11}P_2$: 486.0790, found: 486.0721; ^{1}H NMR (D_2O, 500MHz): δ 6.153 (d, 1H, $J_{1',2'}$=3.4, H-1'), 4.750 (dd, 1H, $J_{2',3'}$=5.0, H-2'), 4.501 (dd, 1H, $J_{3',4'}$=5.2, H-3'), 4.396 (m, 1H, $J_{4,5'}$=2.3, $J_{4,5''}$=2.2, H-4'), 4.195 (m, 1H, $J_{5',5''}$=11.8, $J_{5',P}$=5.0, H-5'), 4.061 (m, 1H, $J_{5'',P}$=5.8, H5''), 4.120 (s, 3H; 7-CH_3), 3.190 (s, 6H; $(CH_3)_2$).

Synthesis of 7-methylguanosine 5'-diphosphate (m^7GDP) from guanosine 5'-diphosphate

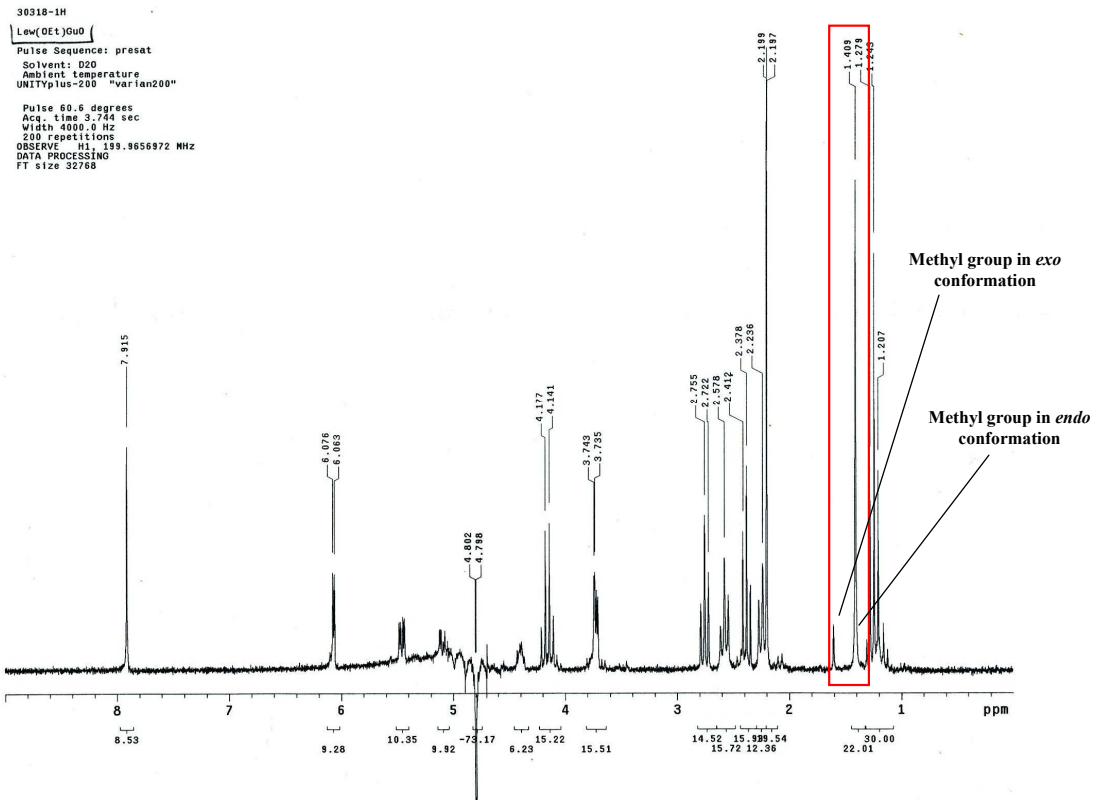
4. 7-methylguanosine 5'-diphosphate (m^7GDP)

Methyl iodide (0.16 mL, 2.5 mmol) was added to a suspension of guanosine 5'-diphosphate (322 mg, 0.5 mmol, TEA salt) in anhydrous dimethylsulfoxide (4 mL) and stirred at RT for 2h. The reaction mixture was poured into water and extracted three times with diethyl ether. Aqueous phase was purified on DEAE–Sephadex using a 0 – 0.8 M gradient of TEAB. m^7GDP was obtained as a colourless crystals (231 mg, 0.35 mmol, TEA salt, 70%). *m/z*: calcd for $C_{11}H_{18}N_5O_{11}P_2$: 458.047, found: 458.050; ^{1}H NMR (D_2O, 500MHz): δ 6.059 (d, 1H $J_{1',2'}$=3.0, H-1'), 4.662 (dd, 1H, $J_{2',3'}$=4.8, H-2'), 4.475 (dd, 1H, $J_{3',4'}$=5.8, H-3'), 4.389 (m,

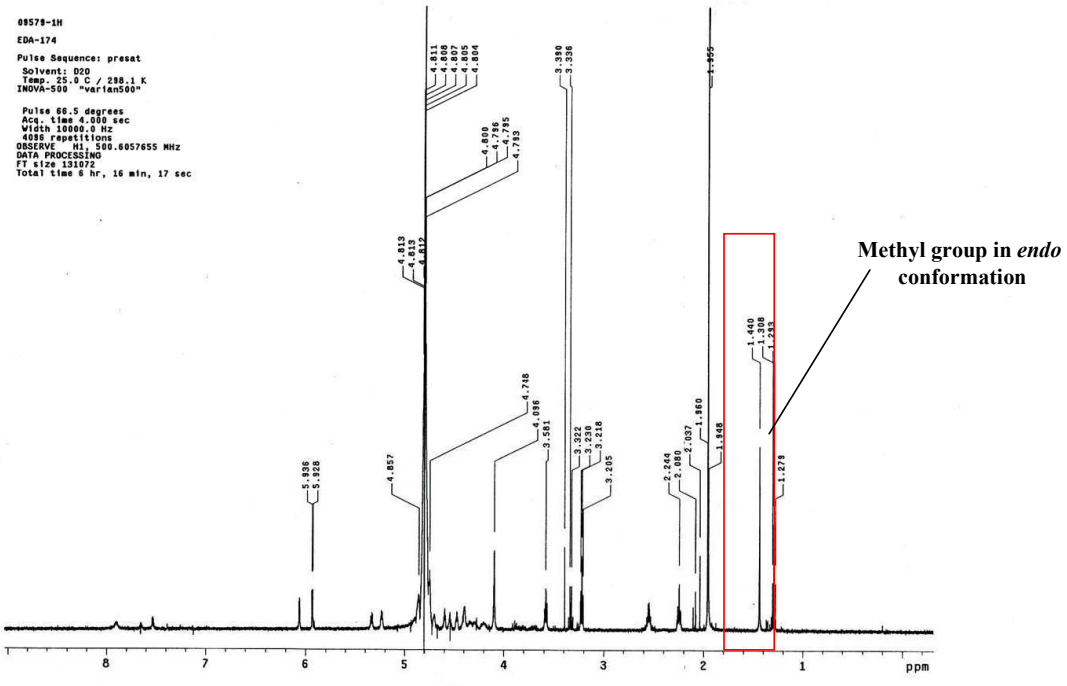
1H, $J_{4,5'}$=2.2, $J_{4,5''}$=1.8 H-4'), 4.353 (m, 1H $J_{5',5''}$=11.6, $J_{5',P}$=4.6, H-5'), 4.262 (m, 1H $J_{5'',P}$=5.5, H5''), 4.122 (s, 3H; 7-CH_3).

2',3'-O-[1-[2-(ethoxycarbonyl)ethyl]ethylidene]guanosine Lew(OEt)Guo

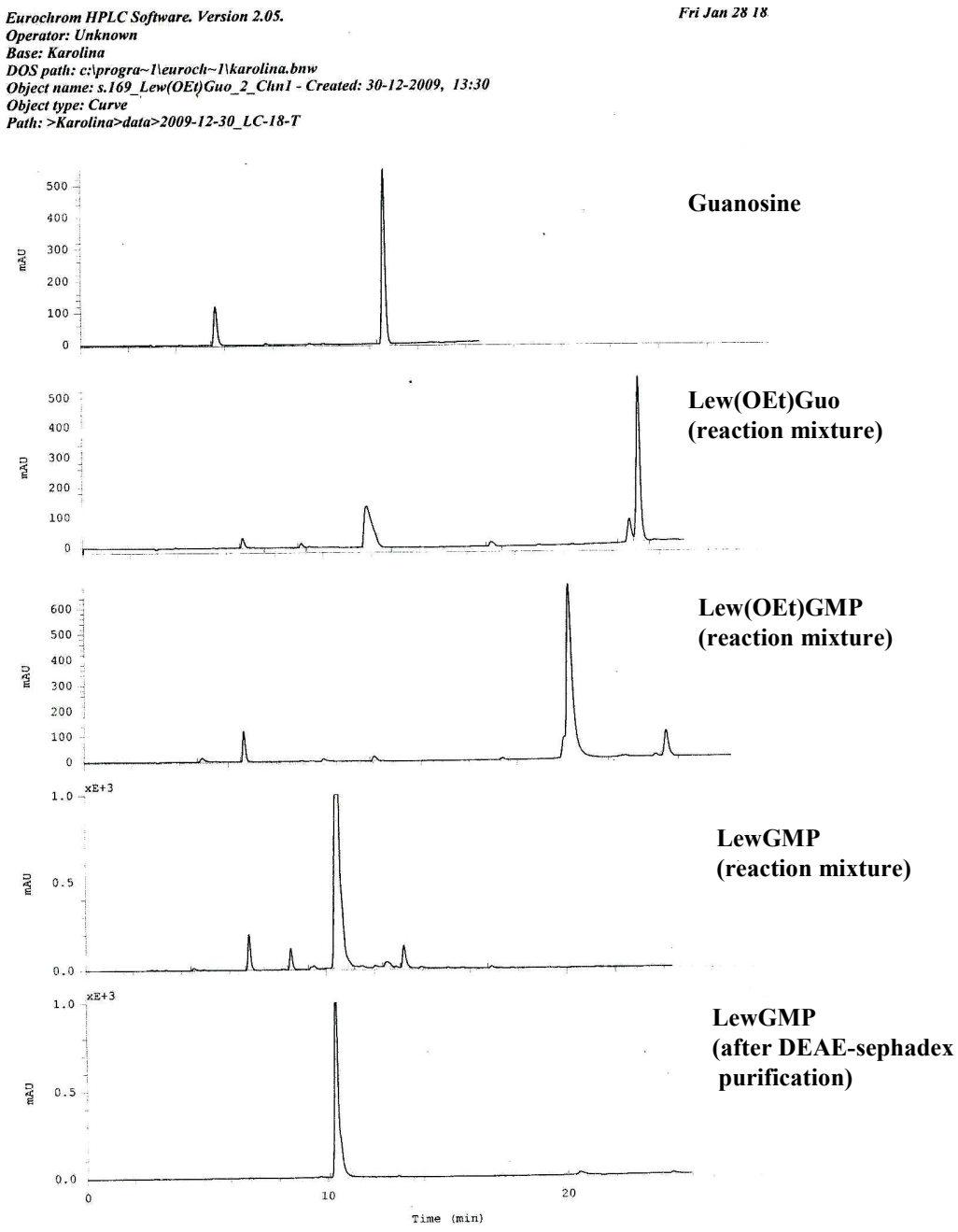
^{1}H NMR spectra of obtained Lew(OEt)Guo, two diastereoisomers were formed (10:1)



Exemplary ^{1}H NMR spectra ($m^7GpppG_{Lew-EDA}$) showing that the final product does not contain signal of methyl group in *exo* orientation

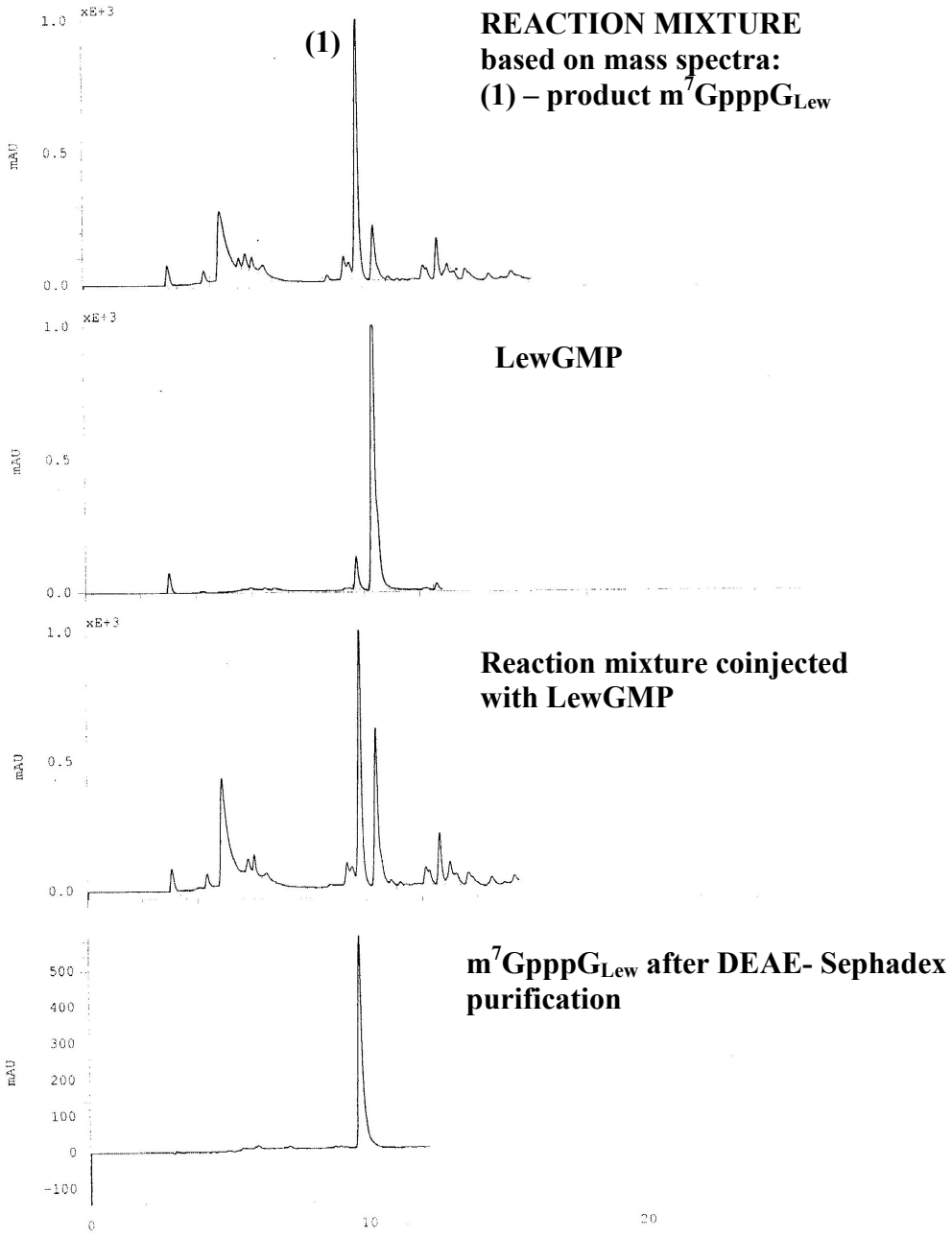


Synthesis of 2',3'-O-[1-(2-carboxyethyl)ethylidene]guanosine 5'-monophosphate (LewGMP) from guanosine (HPLC chromatograms)



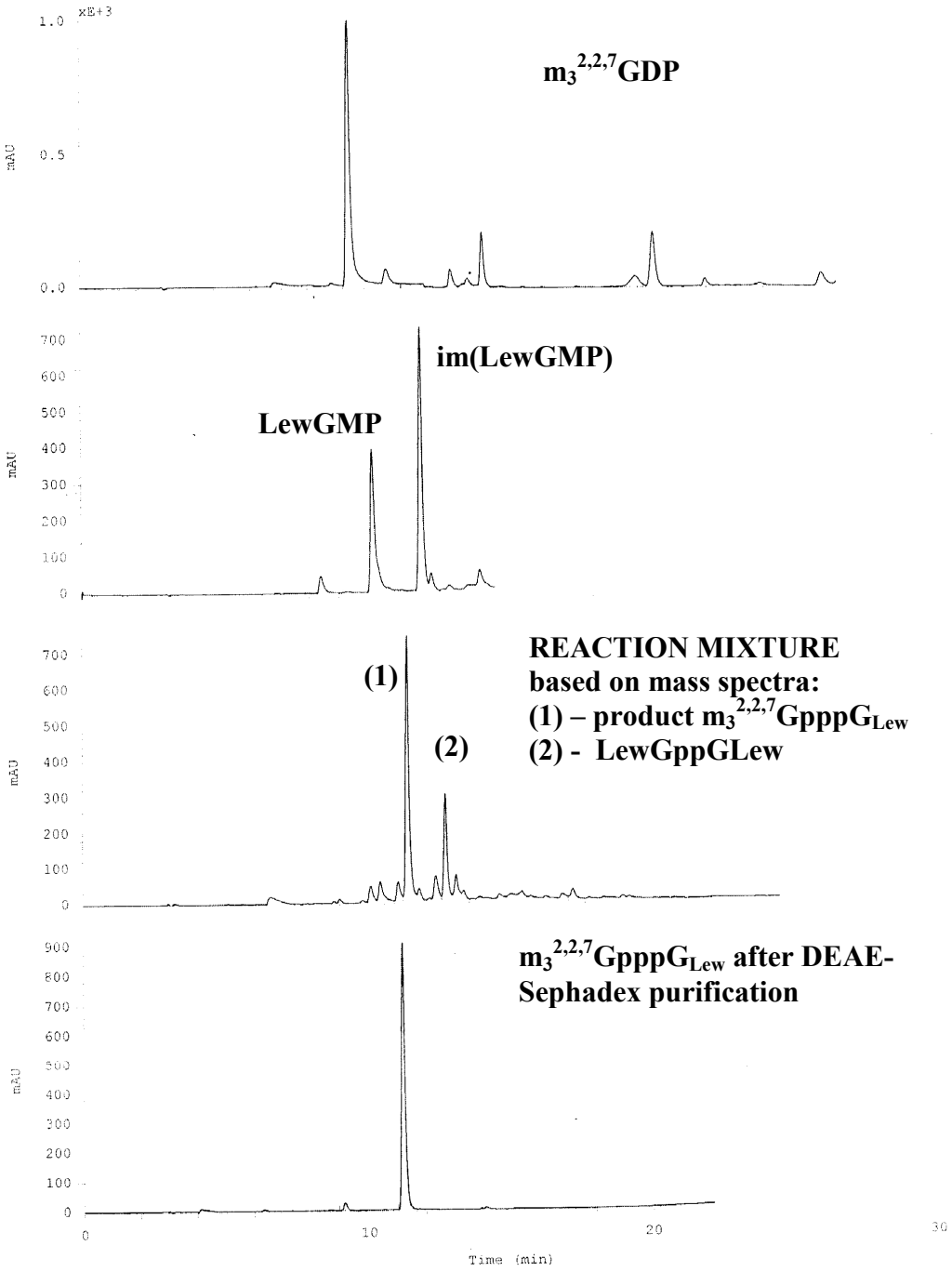
Supelcosil LC-18-T RP column (4.6 X 250 mm, flow rate 1.0 mL/min) with a linear gradient of methanol from 0 to 50% (v/v) in 0.05 M ammonium acetate (pH 5.9) over 20 min. UV detection was performed at 254 nm.

m^7GpppG_{Lew} synthesis



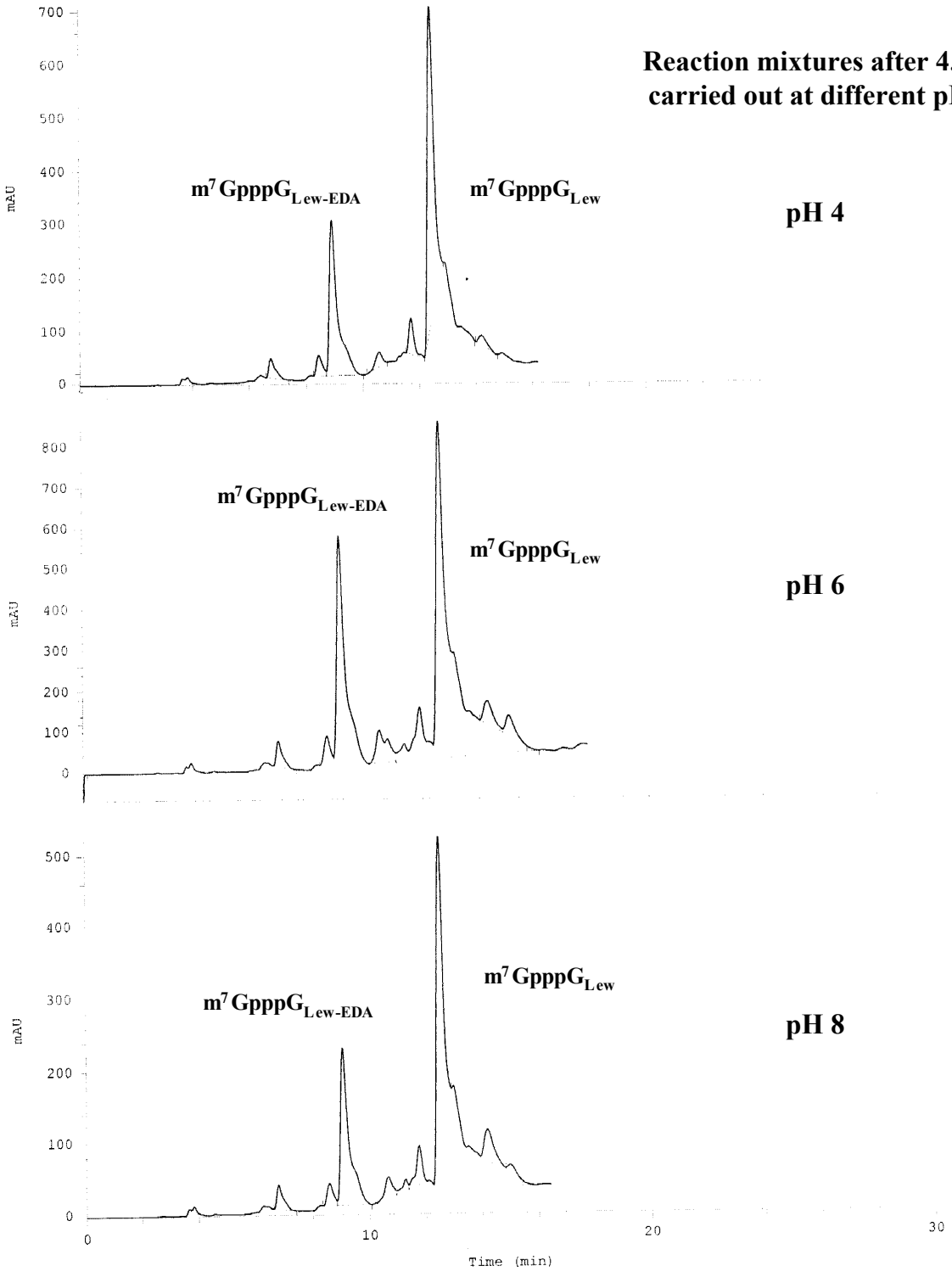
Supelcosil LC-18-T RP column (4.6 X 250 mm, flow rate 1.0 mL/min) with a linear gradient of methanol from 0 to 50% (v/v) in 0.05 M ammonium acetate (pH 5.9) over 20 min. UV detection was performed at 254 nm.

$m_3^{2,2,7}GpppG_{Lew}$ synthesis



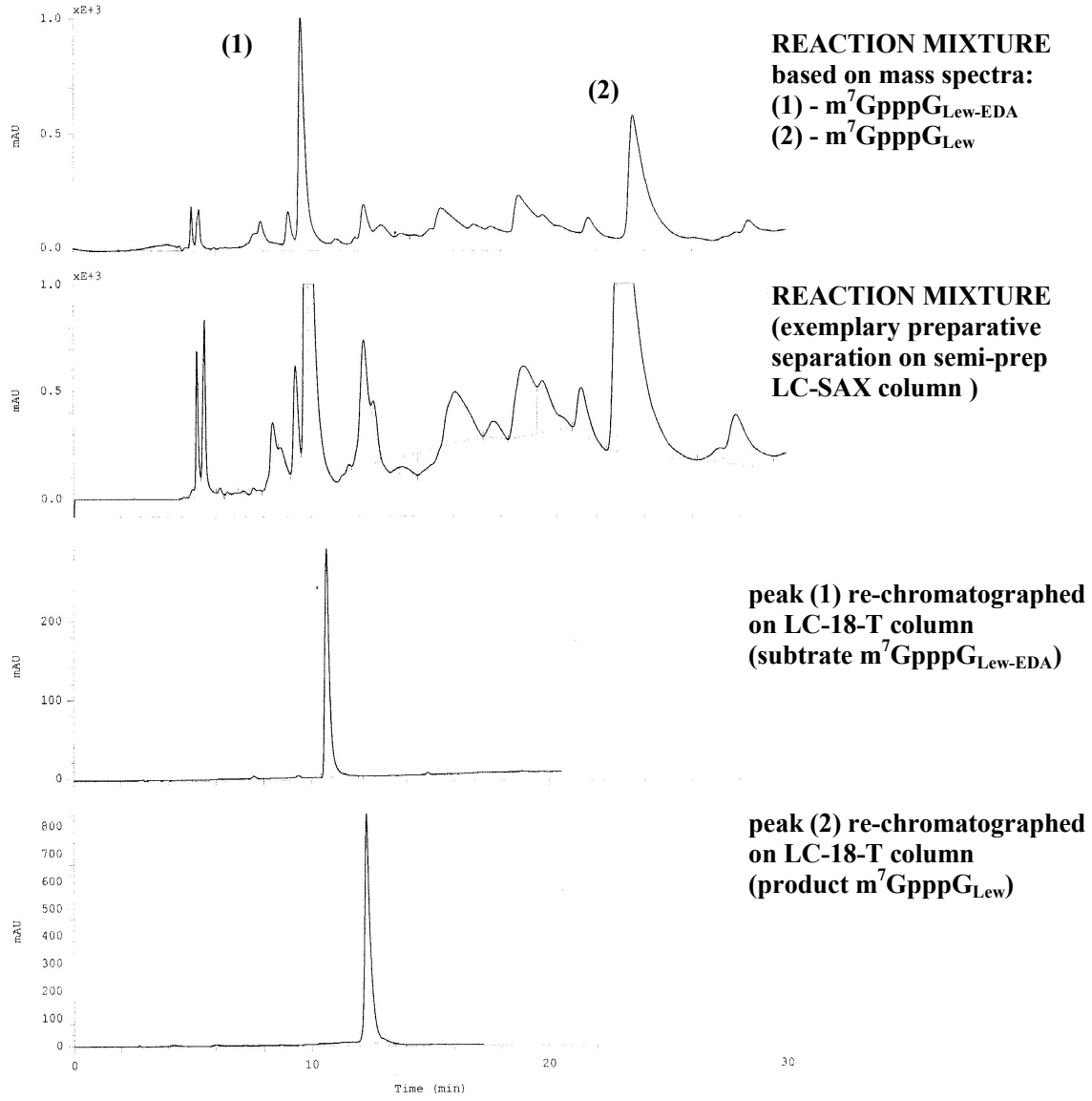
Supelcosil LC-18-T RP column (4.6 X 250 mm, flow rate 1.0 mL/min) with a linear gradient of methanol from 0 to 50% (v/v) in 0.05 M ammonium acetate (pH 5.9) over 20 min. UV detection was performed at 254 nm.

HPLC chromatograms showing reaction progress of coupling m^7GpppG_{Lew} with EDA carried out at different pHs



Supelcosil LC-SAX1 column (4.6 X 250 mm, flow rate 1.0 mL/min) with a linear gradient of 0.006 M KH_2PO_4 containing 0.01 M acetic acid (pH 4) to 0.6 M KH_2PO_4 (pH 5) over 20 min. UV detection was performed at 254 nm.

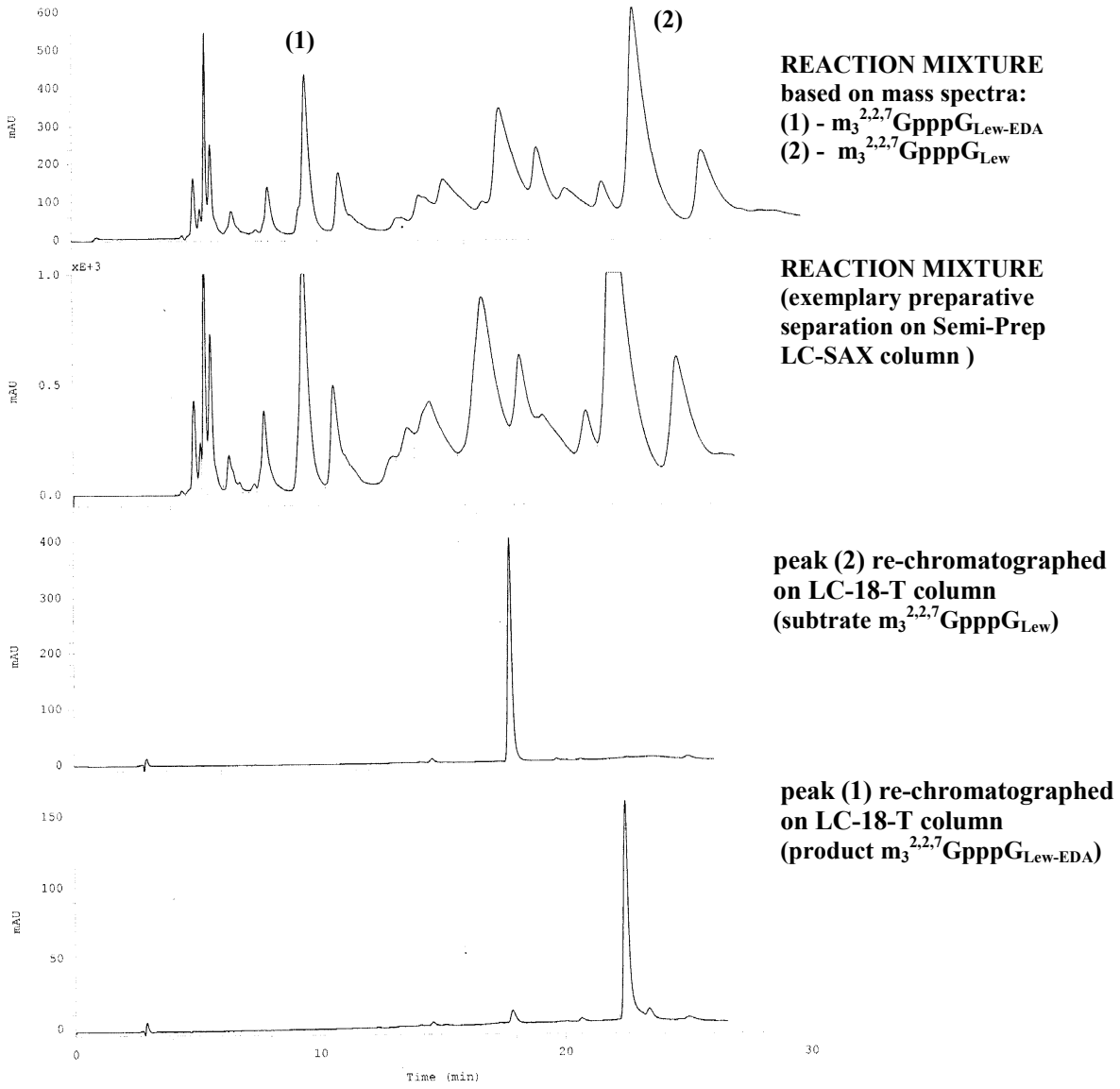
Purification of $m^7GpppG_{Lew\text{-}EDA}$



Chromatogram 1 and 2 Waters Spherisorb SAX column (10.0 X 250 mm, flow rate 3.0 mL/min) with a linear gradient of 0.006 M KH_2PO_4 containing 0.01 M acetic acid (pH 4) to 0.6 M KH_2PO_4 (pH 5) over 20 min. UV detection was performed at 254 nm.

Chromatogram 3 and 4 Supelcosil LC-18-T RP column (4.6 X 250 mm, flow rate 1.0 mL/min) with a linear gradient of methanol from 0 to 50% (v/v) in 0.05 M ammonium acetate (pH 5.9) over 20 min. UV detection was performed at 254 nm.

Purification of $m_3^{2,2,7}GpppG_{Lew\text{-}EDA}$



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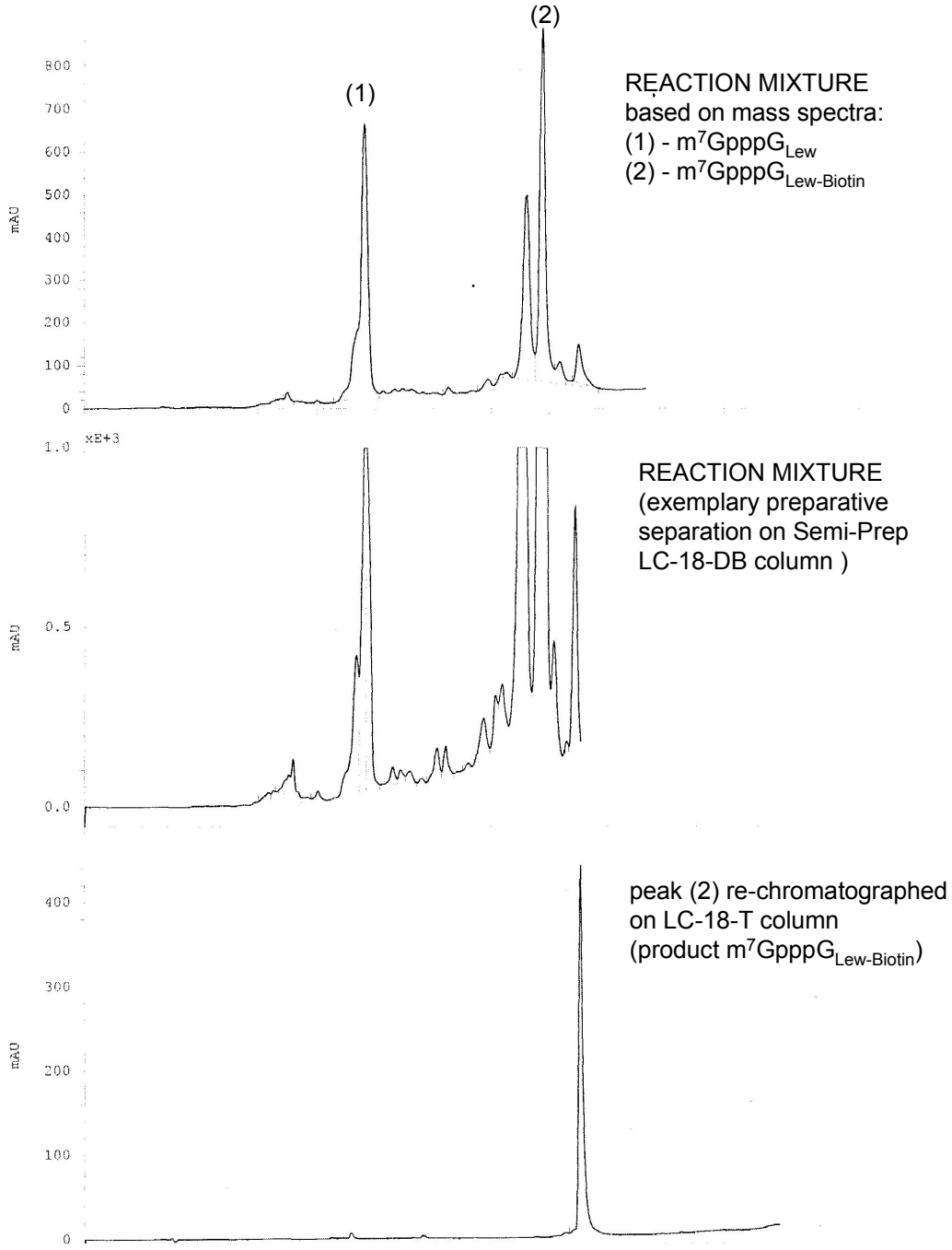
Chromatogram 3 and 4 Supelcosil LC-18-T RP column (4.6 X 250 mm, flow rate 1.0 mL/min) with a linear gradient of methanol from 0 to 33% (v/v) in 0.05 M ammonium acetate (pH 5.9) over 20 min. UV detection was performed at 254 nm.

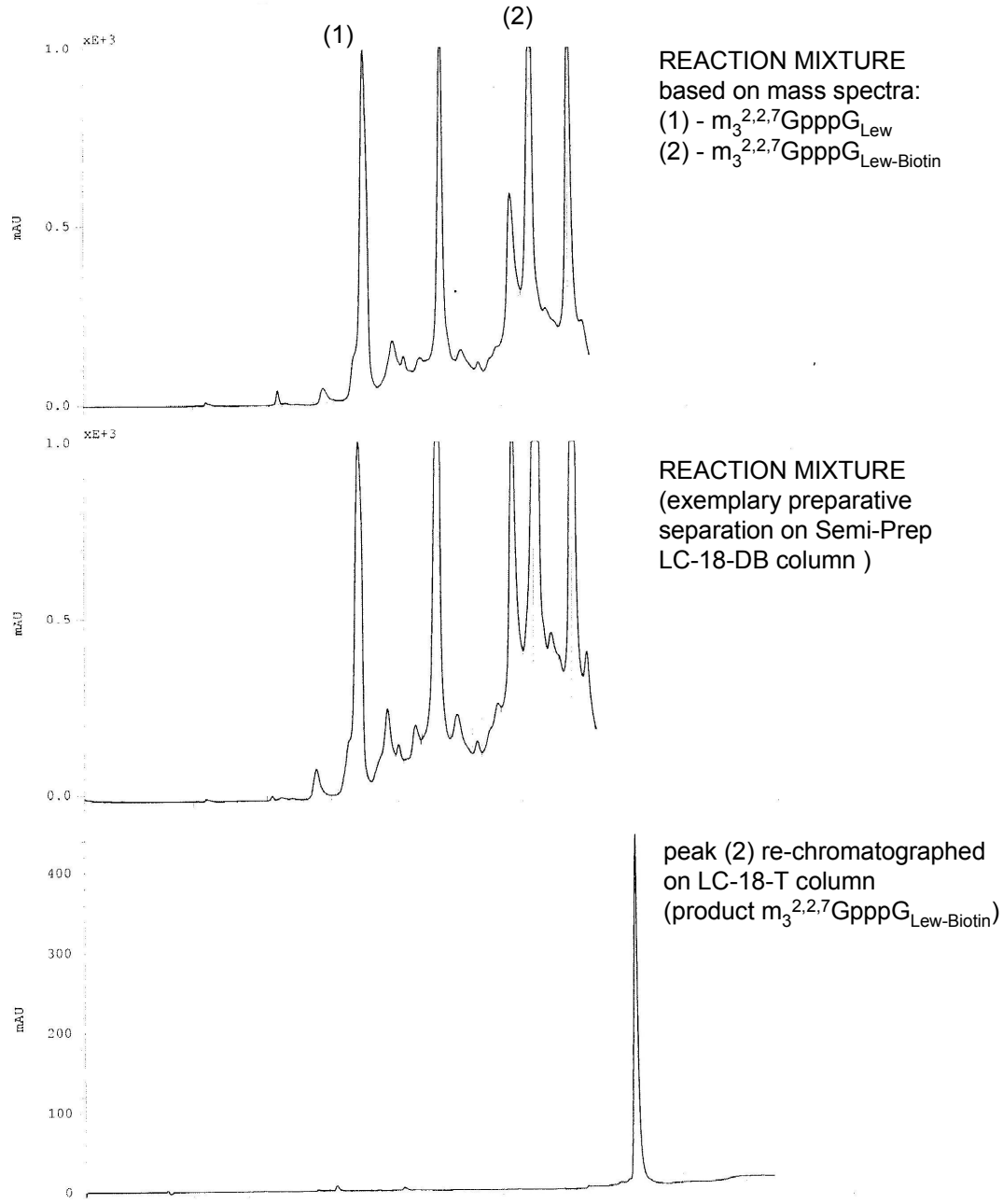
Purification of $m^7GpppG_{Lew\text{-}biotin}$



Chromatogram 1 and 2 Supelcosil LC-18-DB column (10 X 250 mm, flow rate 3.0 mL/min) with a linear gradient of methanol from 0 to 50% (v/v) in 0.05 M ammonium acetate (pH 5.9) over 20 min. UV detection was performed at 254 nm

Chromatogram 3 and 4 Supelcosil LC-18-T RP column (4.6 X 250 mm, flow rate 1.0 mL/min) with a linear gradient of methanol from 0 to 50% (v/v) in 0.05 M ammonium acetate (pH 5.9) over 20 min. UV detection was performed at 254 nm

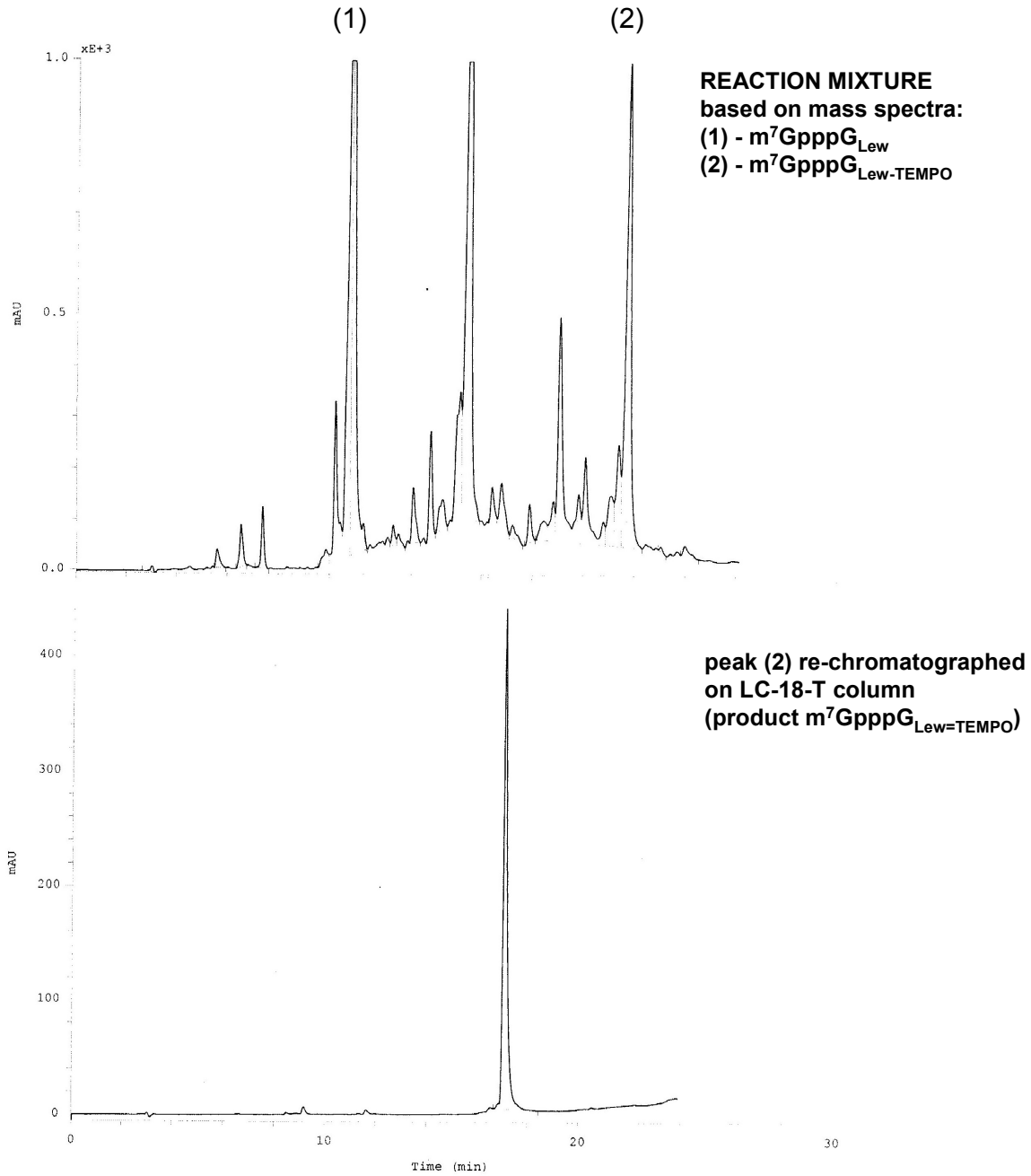
Purification of $m_3^{2,2,7}GpppG_{Lew\text{-}biotin}$



Chromatogram 1 and 2 Supelcosil LC-18-DB column (10 X 250 mm, flow rate 3.0 mL/min) with a linear gradient of methanol from 0 to 50% (v/v) in 0.05 M ammonium acetate (pH 5.9) over 20 min. UV detection was performed at 254 nm

Chromatogram 3 and 4 Supelcosil LC-18-T RP column (4.6 X 250 mm, flow rate 1.0 mL/min) with a linear gradient of methanol from 0 to 50% (v/v) in 0.05 M ammonium acetate (pH 5.9) over 20 min. UV detection was performed at 254 nm.

Purification of $m^7GpppG_{Lew\text{-}TEMPO}$

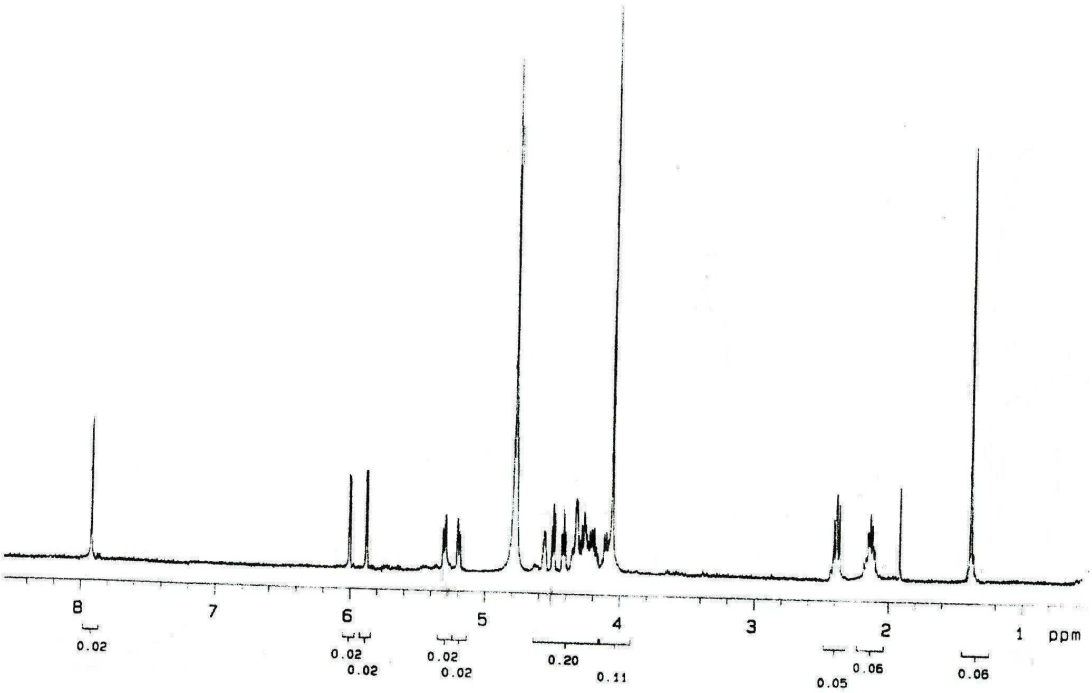


Chromatogram 1 Supelcosil LC-18-DB column (10 X 250 mm, flow rate 3.0 mL/min) with a linear gradient of methanol from 0 to 50% (v/v) in 0.05 M ammonium acetate (pH 5.9) over 20 min. UV detection was performed at 254 nm

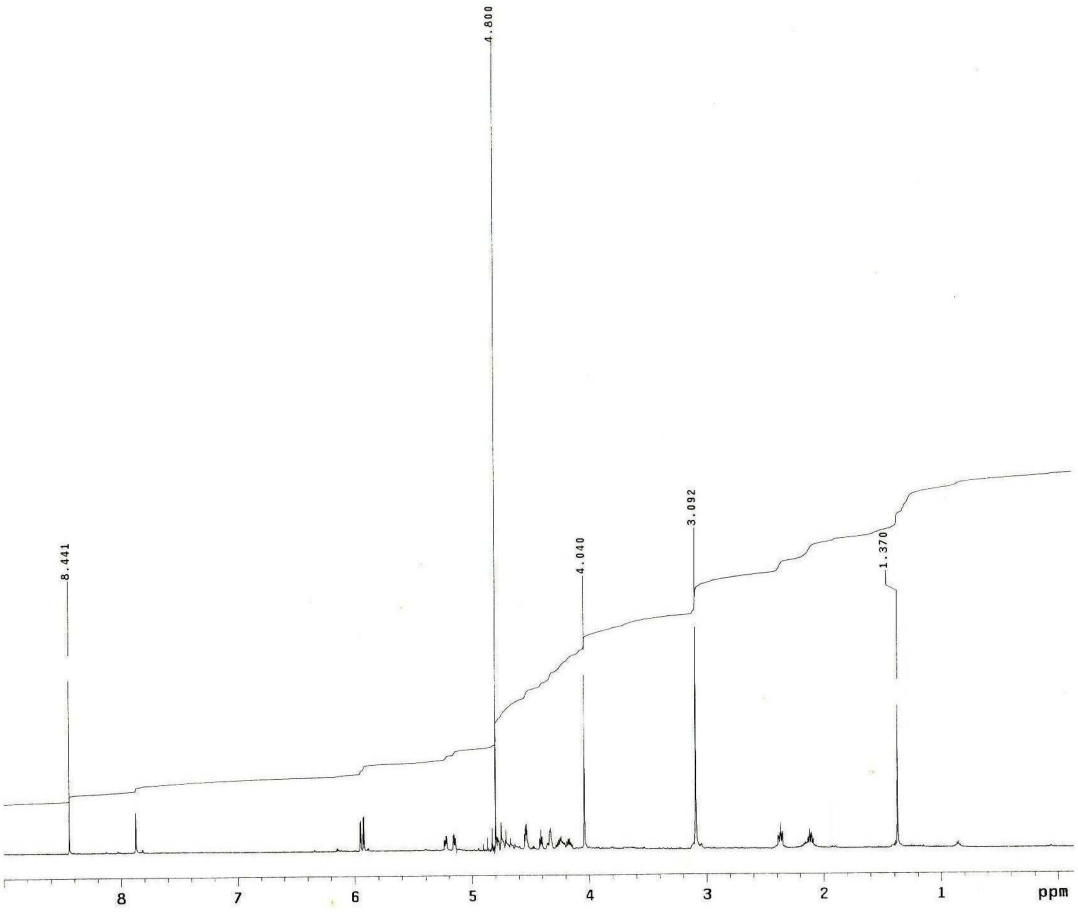
Chromatogram 2 Supelcosil LC-18-T RP column (4.6 X 250 mm, flow rate 1.0 mL/min) with a linear gradient of methanol from 0 to 50% (v/v) in 0.05 M ammonium acetate (pH 5.9) over 20 min. UV detection was performed at 254 nm.

^{1}H NMR SPECTRA

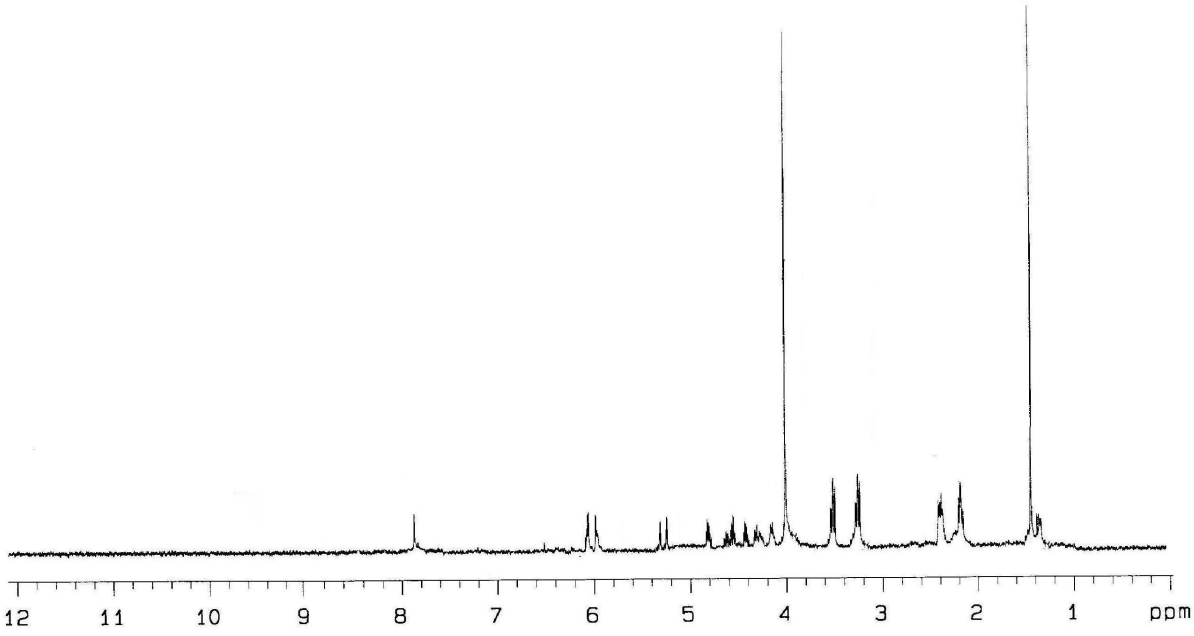
$m^{7}GpppG_{Lew}$



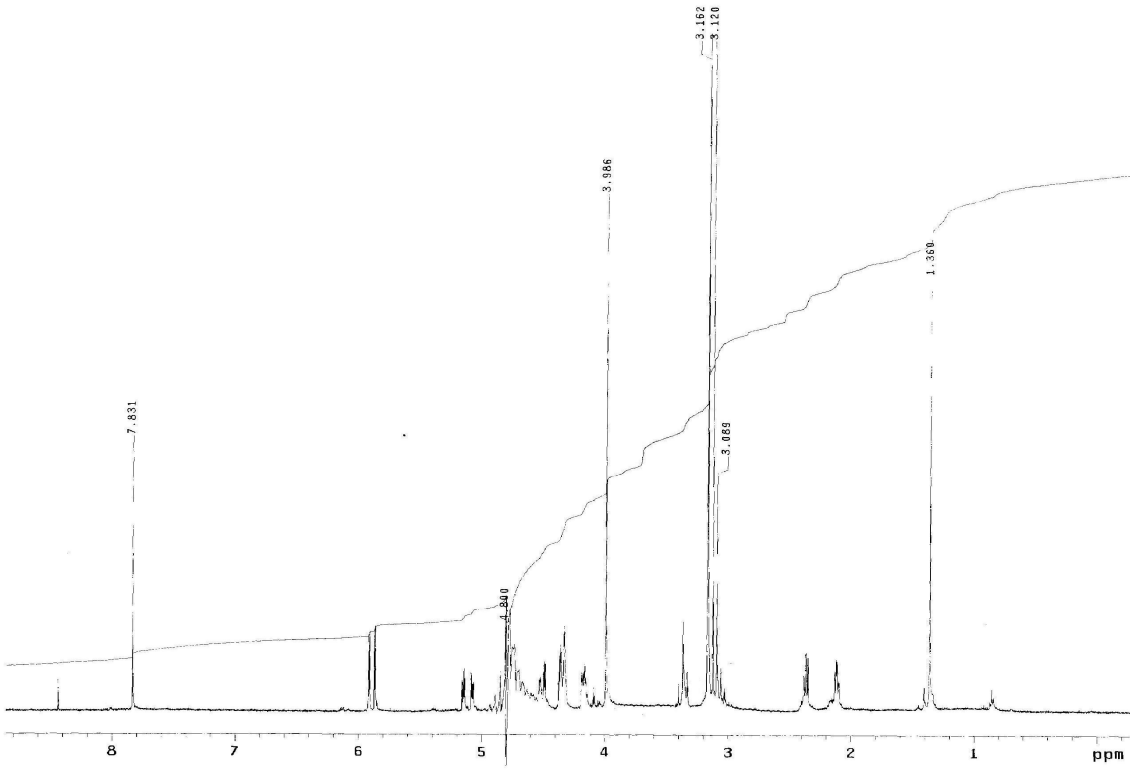
$m_3^{2,2,7}GpppG_{Lew}$



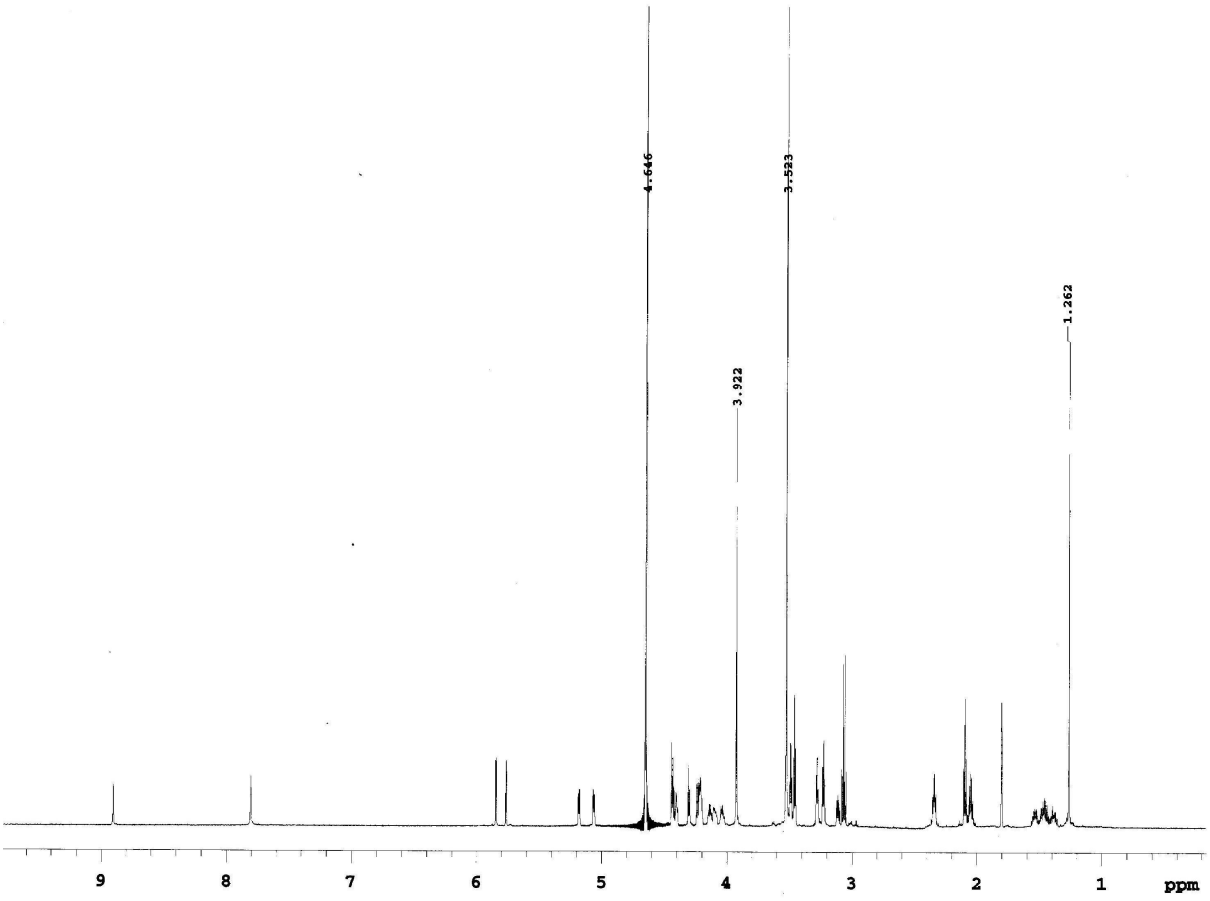
$m^7GpppG_{Lew\text{-}EDA}$



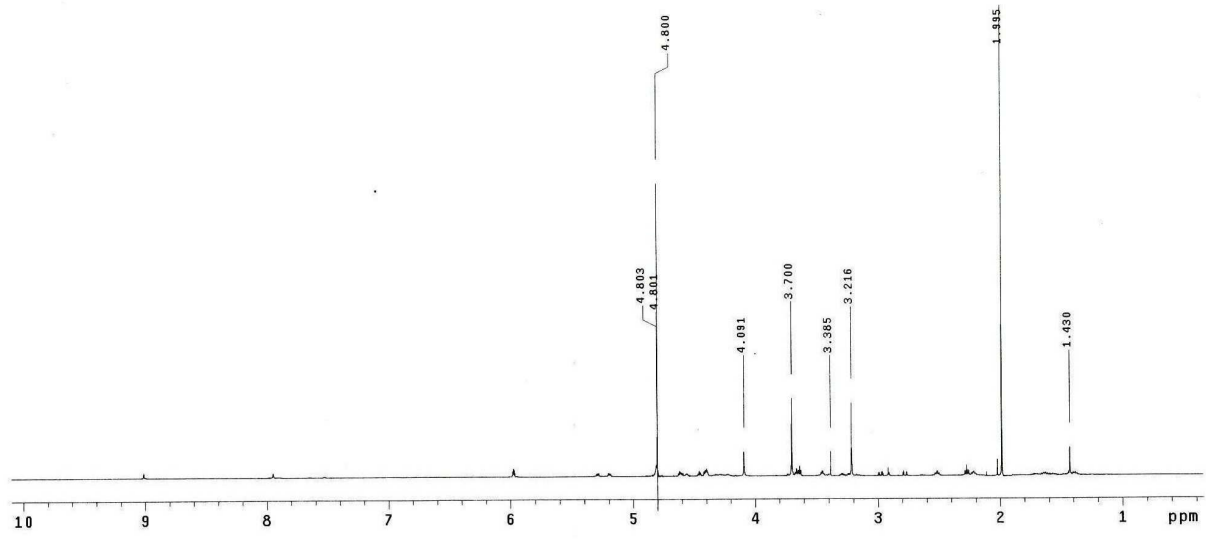
$m_3{}^{2,2,7}GpppG_{Lew\text{-}EDA}$



$m^7GpppG_{Lew\text{-}biotin}$



$m_3{}^{2,2,7}GpppG_{Lew\text{-}biotin}$



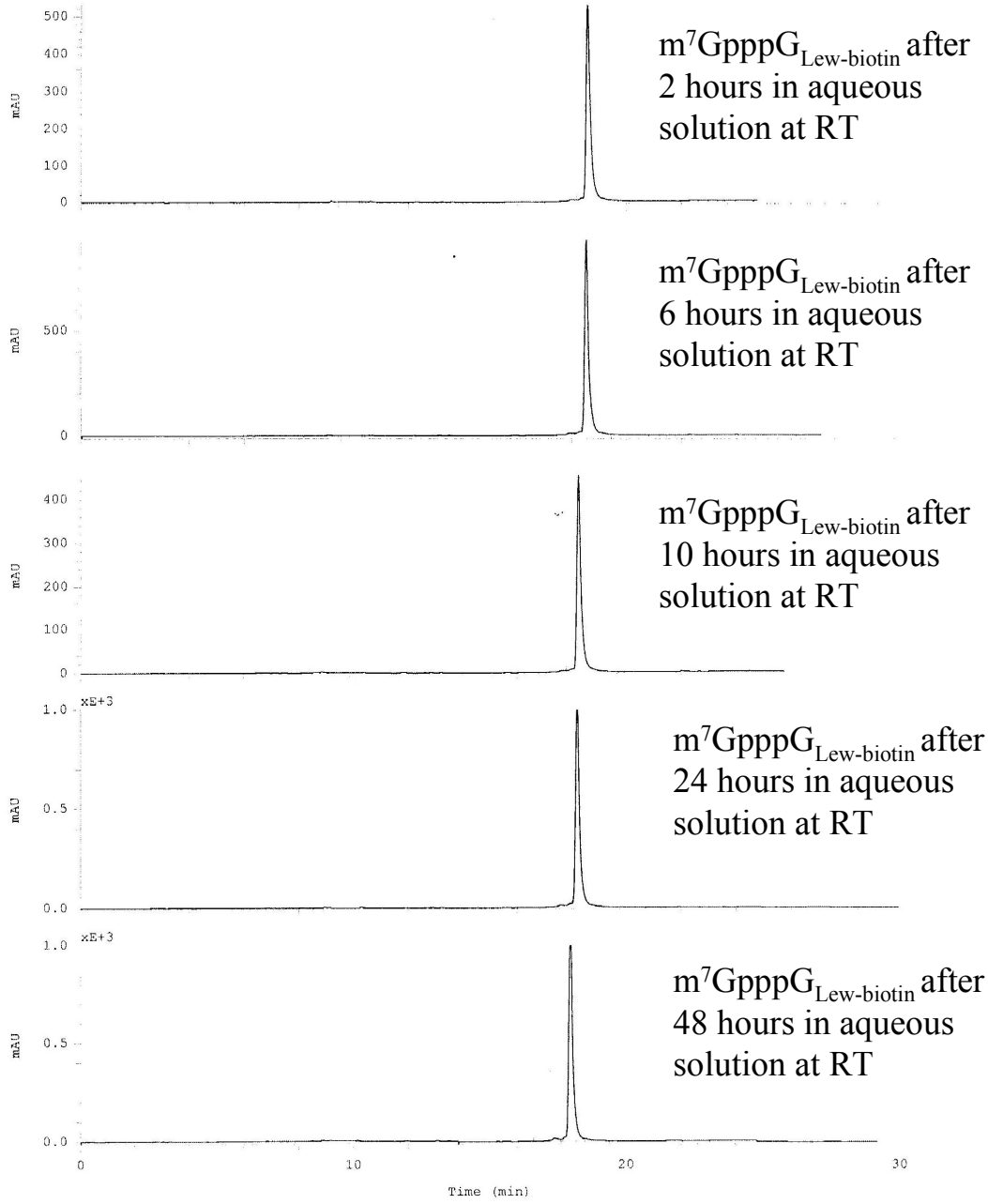
Electron Spin Resonance (ESR) Measurements

The conventional field-swept ESR spectra from aqueous solutions of the compound 9a were obtained with an X-band (9.75 GHz) EMX EPR spectrometer (Bruker, USA), equipped with a standard TE_{102} rectangular resonator, ER4102ST-O. Aliquots of *ca.* 7 μL of samples (height of ~25 mm) of 9a at 200 μM were transferred into 0.6 mm i.d. quartz capillary tubes (Composite Metal Services Ltd., UK) and sealed on both ends with Cha-Seal™ tube sealing compound (Medex International, USA). ESR spectra were acquired at room temperature at microwave power, 2 mW; modulation amplitude, 0.2 G_{pp}; modulation frequency, 100 kHz; time constant, 20.5 ms; integration time, 41 ms; scan width from 70 to 220 G; receiver gain, $4 \cdot 10^4$. Two scans per one EPR trace were accumulated. The spectra were calibrated according to a weak pitch spectrum (g = 2.0028).

Synthesis of $m^7GpppG_{Lew\text{-}EDA}$

1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) (75 mg, 0.4 mmol), N-hydroxysuccinimide (NHS) (11.5 mg, 0.1 mmol) and compound **5a** (360 mg, 0.3 mmol, TEA salt) were dissolved in 1mL of 0.1M MES buffer pH 6 and stirred at RT. After 20 minutes ethylenediamine (0.4 mmol) was added and reaction mixture was stirred additional 4.5 h. The product was separated from the reaction mixture by chromatography on DEAE–Sephadex using a 0 – 1.0 M gradient of TEAB. $m^7GpppG_{Lew\text{-}EDA}$ was obtained as a colourless crystals (67 mg, 0,05 mmol, 18%)

Stability of $m^7GpppG_{Lew\text{-}EDA}$ in aqueous solution at RT



Supelcosil LC-18-T RP column (4.6 X 250 mm, flow rate 1.0 mL/min) with a linear gradient of methanol from 0 to 50% (v/v) in 0.05 M ammonium acetate (pH 5.9) over 20 min. UV detection was performed at 254 nm.