Synthesis of Nucleoside α-Thiotriphosphates via an Oxathiaphospholane Approach

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SUPPORTING INFORMATION

General procedure for nucleoside 5'-*O*-(2-thio-1,3,2-oxathiaphospholanes) (**2a-h**): Protected nucleoside (3 mmol) was dried by co-evaporation with dry pyridine (2 x 30 mL) and re-dissolved in this solvent (30 mL). Into this solution sulfur (0.48 g, 15 mmol) and 2-chloro-2-thio-1,3,2-oxathiaphospholane (0.47 g, 3.3 mmol) were added and resulting mixture was left with stirring for 16 h. Pyridine was evaporated and the residue was dissolved in toluene (30 mL) and evaporated again. Then the residue was dissolved in acetonitrile and precipitated solid was filter off. The filtrate was concentrated and obtained crude product was purified by means of silica-gel chromatography using chloroform supplemented with methanol (0-5%) as an eluent. Appropriate fractions (TLC assay) were combined and concentrated to dryness to give product as a solid foam. The yields and physico-chemical characteristics of obtained **2a-h** are presented in Table 1 and in Chart 1.

General procedure for nucleoside 5'-*O*-(α-thiotriphosphates) (**1a-h**): Oxathiaphospholane **2a-h** (0.3 mmol) was dissolved in dry 30 mM solution of **3**[1] (10 mL) and resulting solution was additionally dry by keeping it with molecular sieves 3A (0.6 g) for 3 h. Then DBU (50 μL, 0.33 mmol; stored over CaH_2) was added. After 2 h reaction mixture was analyzed by ^{31}P NMR (Fig. 1) and then concentrated and re-dissolved in a mixture of conc. ammonia (7.5 mL) and acetonitrile (1.5 mL). For protected nucleoside α-thiotriphosphate **5e** deprotection took 2 h at room temperature; 6 h for **5f** and **5g**, respectively). Complete removal of protecting groups from **5a**, **5b**, **5c**, **5d**, and **5h** required heating for 6 h at bath temperature 55°C. Obtained crude products were purified on DEAE -Sephadex A-25 (150 mL) using a linear gradient of TEAB buffer pH 7.5 (3 L). Appropriate fractions were combined and concentrated to dryness to give desired product as a solid. Compounds **1d** and **1h** were additionally purified by RP-HPLC chromatography. The yields and physico-chemical characteristics of obtained **1a-h** are presented in Table 2 and in Fig. 2-4.

[1] Tris(tetra-*n*-butylammonium pyrophosphate) obtained from sodium pyrophosphate and tetra-*n*-butylammonium hydroxide using Poulter's method (Davisson, V. J.; Woodside, A. B.; Neal, T. R.; Stremler, K. E.; Muehlbacher, M.; Poulter, C. D. *J. Org. Chem.* **1986**, *51*, 4768-4779). Acetonitrile solution of this compound was dried with molecular sieves 3A (6-8 h) until water content (cuolometric assay) was below 50 ppm.

Adenosine 5'-*O*-triphosphate (6)

Solution of ATPαS (**1f**, 0.1 mmol) in water (1 mL) was mixed with 0.1 M solution of Oxone® buffered with sodium acetate until pH 7.0 was reached (1.5 mL, 0.15 mmol), and left for 16 h. Product was isolated by ion-exchange chromatography on DEAE-Sephadex A-25 (50 mL) using a linear gradient (0.1-0.7 M) of TEAB buffer pH 7.5. Appropriate fractions were combined and concentrated to dryness to give desired product as a solid: (0.063 mmol, 63% yield); t_R (min) (conditions as above), 9.52; δ_{31P} (D_2O): -6,08 (d, *J* 20.4 Hz), -11.15 (d, *J* 19.4 Hz), -21.89 (m); MS-MALDI TOF *m/z* 506 (M-H).

In a similar way, using 1 M solution of hydrogen peroxide (1.5 mL, 1.5 mmol), compound **1f** was converted into ATP with 65% yield.

Thymidine 5'-*O*-(α-thiodiphosphate) (7)

Starting from **2a** and using a general procedure as above, with replacement of **3** with bis(tetra-*n*-butylammonium) hydrogen phosphate[2] dTDPαS was obtained with 24% yield: t_R (min) (conditions as above): 7.64, 8.02; δ_{31P} (D_2O): 41.58 (d, *J* 30.1 Hz), 41.12 (d, *J* 30.1 Hz), -8.34 (d, *J* 30.1 Hz); MS-MALDI TOF *m/z* 474 (M+Na).

2',3'-*O,O*-Diacetyladenosine 5'-*O*-(2-seleno-1,3,2-oxathiaphospholane) (8)

Starting from 2',3'-*O,O*-diacetyladenosine and following a general procedure for nucleoside 5'-*O*-(2-thio-1,3,2-oxathiaphospholanes) elemental selenium was used instead of sulfur, and the title compound was obtained as a solid foam with 72% yield: TLC (chloroform/ethanol 9:1), R_f 0.48; δ_{1H} (CD_3Cl): 8.38 (s, 1H,), 8.13 (s, 1H), 6.30 (d, *J* 6.3 Hz, 1H), 5.75-5.89 (m, 2H), 5.59-5.67 (m, 1H), 4.34-4.65 (m, 4H), 3.39-3.67 (m, 2H), 2.16 (s, 3H), 2.06 (s, 3H); δ_{31P} (CD_3Cl): 100.00 (J_{PSe} 963 Hz), 99.93 (J_{PSe} 963 Hz); MS-FAB (+VE) *m/z* 538.0 (M+H, 1Se).

Adenosine 5'-*O*-(α-selenotriphosphate) (9)

Starting from **8** and using a general procedure as for preparation of nucleoside 5'-*O*-(α-thiotriphosphates), ATPαSe was obtained with 31% yield: t_R (min) (conditions as above): 9.19, 9.75; δ_{31P} (D_2O): 32.38 (d, J_{PP} 33.6, J_{PSe} 790 Hz), 32.31 (d, J_{PP} 35.6, J_{PSe} 791 Hz), -6.62

[2] This compound was obtained from sodium dihydrogen phosphate and tetra-*n*-butylammonium hydroxide using the same procedure as described for **3** (see previous Note). Concentration of bis(tetra-*n*-butylammonium) hydrogen phosphate after treatment with molecular sieves was established by means ^{31}P NMR assay using weight amount of HMPT as an internal standard.

(d, *J* 21.1 Hz), -23.69 (dd,33.6, 21.1 Hz), -24.16 (dd, 35.6, 21.1 Hz); MS-MALDI TOF *m/z* 576 (M, 1Se).

Adenosine 5'-*O*-(α-selenodiphosphate) (10)

Starting from **8** and using a general procedure as above, with an employment of bis(tetra-*n*-butylammonium) hydrogen phosphate, ADPαSe was obtained with 23% yield: t_R (min) (conditions as above): 7.64, 8.02; δ_{31P} (D_2O): 29.92 (d, *J* 37.1 Hz), -7.00 (d, *J* 37.1 Hz); MS-MALDI TOF *m/z* 490 (M, 1Se).

Table 1. Yields and Physico-chemical characteristics of Oxathiaphospholanes **2a-h**

compound	yield (%)	TLC[a] R_f	^{31}P-NMR[b] δ (ppm)	FAB (+VE) (M+H)	FAB (-VE) (M-H)
2a	80	0.48	105.51 105.27	423.2	421.3
2b	84	0.51	106.23 106.08	536.0	533.9
2c	79	0.55	106.30 106.12	511.9	510.1
2d	81	0.38	107.59 107.51	518.3	516.3
2e	75	0.45	106.33 106.15	467.2	465.2
2f	80	0.38	106.48	490.2	488.1
2g	78	0.41	106.58 106.51	508.1	506.1
2h	73	0.43	107.189 107.044	610.1	608.1

[a] developing system: chloroform/ethanol 9:1
[b] spectra run in CD_3Cl

Chart 1. ^{1}H NMR Spectra of Oxathiaphospholanes **2a**-**h**

2a, ^{1}H-NMR ($CDCl_3$) δ 8.51 (d, 1H, J=7.3Hz), 7.49 (d, 1H, J=20Hz), 6.36-6.40 (m, 1H), 5.29 (t, 1H, J=7.0Hz), 4.32-4.59 (m, 4H), 4.22 (s, 1H), 3.49-3.69 (m, 2H), 2.41-2.47 (m, 1H), 2.13-2.20 (m, 1H,) 2.11 (s, 3H), 1.98 (s, 3H)

2b, ^{1}H-NMR ($CDCl_3$) δ 9.13 (s, 1H), 8.80 (s, 1H), 8.36-8.40 (m, 1H), 8.02 (d, 2H, 7.6Hz), 7.60-7.61 (m, 1H), 7.53-7.54 (m, 2H), 6.53-6.60 (m, 1H), 5.47-5.52 (m, 1H), 4.33-4.56 (m, 5H), 3.46-3.54 (m, 2H), 2.85-2.93 (m, 1H), 2.67-2.72 (m, 1H), 2.15 (s, 3H)

2c, ^{1}H-NMR ($CDCl_3$) δ 8.21-8.27 (m, 1H), 7.89 (d, 2H, J=7.6Hz), 7.50-7.62 (m, 4H), 6.30-6.36 (m, 1H), 5.26-5.32 (m, 1H), 4.34-4.55 (m, 5H), 3.48-3.59 (m, 2H), 2.78-2.84 (m, 1H), 2.10-2.16 (m, 4H), 1.64-2.09 (m, 1H)

2d, ^{1}H-NMR ($CDCl_3$) δ 12.07(d, 1H, J=10.7Hz), 8.73-8.84 (m, 1H), 7.80 (d, 1H, J=3.1Hz), 6.16-6.22 (m, 1H), 5.51-5.57 (m, 1H), 4.26-4.58 (m, 5H), 3.43-3.55 (m, 2H), 3.09-3.26 (m, 1H), 2.66-2.73 (m, 1H), 2.43-2.51 (m, 1H), 2.13 (s, 3H), 1.27 (d, 6H, J=6.8Hz)

2e, ^{1}H- NMR (d_6-DMSO) δ 11.45 (d, 1H, J=2Hz), 7.64-7.70 (m, 1H), 5.88-5.91 (m, 1H), 5.66-5.71 (m, 1H), 5.26-5.42 (m, 2H), 4.62-4.30 (m, 2H), 4.27-4.20 (m, 2H), 3.52-3.75 (m, 2H), 2.06 (s, 1H), 2.05 (s, 3H), 2.01 (s, 3H),

2f, ^{1}H-: NMR (d_6-DMSO) δ 8.28 (s, 1H), 8.14 (s, 1H), 7.36-7.40 (m, 2H), 6.18 (d, 1H, J=5.6Hz), 5.93-6.00 (m, 1H), 5.56-5.61 (m, 1H), 4.23-4.57 (m, 4H), 3.46-3.66 (m, 2H), 2.56-2.81 (m, 1H), 2.09 (s, 3H), 2.05 (s, 3H),

2g, ^{1}H-NMR (d_6-DMSO) δ 10.99 (s, 1H), 8.10 (d, 1H, J=7.4Hz), 7.22 (d, 1H, J=7.5Hz), 5.92-6.83 (m, 1H), 5.47-5.50 (m, 1H), 5.36-5.39 (m, 1H), 4.53-4.60 (m, 1H), 4.33-4.43 (m, 3H), 4.24-4.32 (m, 1H), 3.61-3.74 (m, 2H), 2.11 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H)

2h, ^{1}H-NMR ($CDCl_3$) δ 12.22 (d, 1H, J=22Hz), 9.28 (d, 1H, J=2.4Hz), 8.06 (dd, 2H, J=7.6Hz, J=2.4Hz), 7.78(d, 1H, J=5.2Hz), 7.64-7.67 (m, 1H), 7.53-7.57 (m, 2H), 6.16-6.18 (m, 1H), 5.95-6.08 (m, 1H), 4.20-4.43 (m, 6H), 3.42-3.47 (m, 1H), 3.33-3.38 (m, 1H), 2.12-2.30 (m, 6H)

Table 2. The Yields and Physico-chemical Characteristics of Nucleoside α-Thiotriphoshates **1a-h**

compound	t_R[a] (min.)	MS MALDI	^{31}P-NMR[b] δ (ppm)	$J_{P\alpha-P\beta}$ (Hz)	$J_{P\beta-P\gamma}$ (Hz)
1a	7.16	497	Sp 44.07(d), -23.07 (m), -9.51(d)	32.4	17.7
(dTTPαS)	7.55		Rp 43.68(d), -23.07(m), -9.51 (d)	30.0	17.7
1b	8.07	506	Sp 45.08(d), -20.53 (m), -5.03(d)	26.7	17.0
(dATPαS)	8.30		Rp 44.75(d), - 20.65(m), -5.03 (d)	25.9	17.0
1c	5.85	482	Sp 44.37(d), -22.14 (m), -7.80 (d)	26.5	18.4
(dCTPαS**)**	6.23		Rp 44.05(d), -22.42 (m), -7.80(d)	26.6	18.4
1d	7.20	522	Sp 44.78(d), -21.53 (m), -7.55(d)	24.1	16.4
(dGTPαS)	7.39		Rp 44.52(d), -21.80(m), -7.55(d)	24.8	16.4
1e	7.36	499	Sp 43.29(d), -23.17(m), -8.44(d)	27.4	19.6
(UTPαS)	7.82		Rp 42.95(d), -23.64(m), -8.44(d)	27.8	19.6
1f	8.02	522	Sp 43.67(d), -23.80 (m), -10.56(d)	27.2	19.7
(ATPαS)	8.45		Rp 43.32(d), -24.25(m), -10.56(d)	27.9	19.7
1g	7.35	498	Sp 43.60(d), -23.65(m),-10.39(d)	27.5	19.5
(CTPαS)	7.75		Rp 43.25(d),-24.07 (m),-10.39(d)	27.9	20.4
1h	6.95	538	Sp 43.77(d), -22.08(m), -5,24(d)	31.1	20.4
(GTPαS)	7.40		Rp 43.48(d), -22.20 (m),-5,24(d)	29.8	20.4

[a] HPLC in 0.1 M TEAB, pH 7.5 with 0→60% acetonitrile in 20 min.; [b] spectra run in D_2O

Fig. 1. ^{31}P NMR Spectra of Reaction Mixture of Oxathiaphospholane **2a** with "wet" pyrophosphate **3** in a presence of DBU.

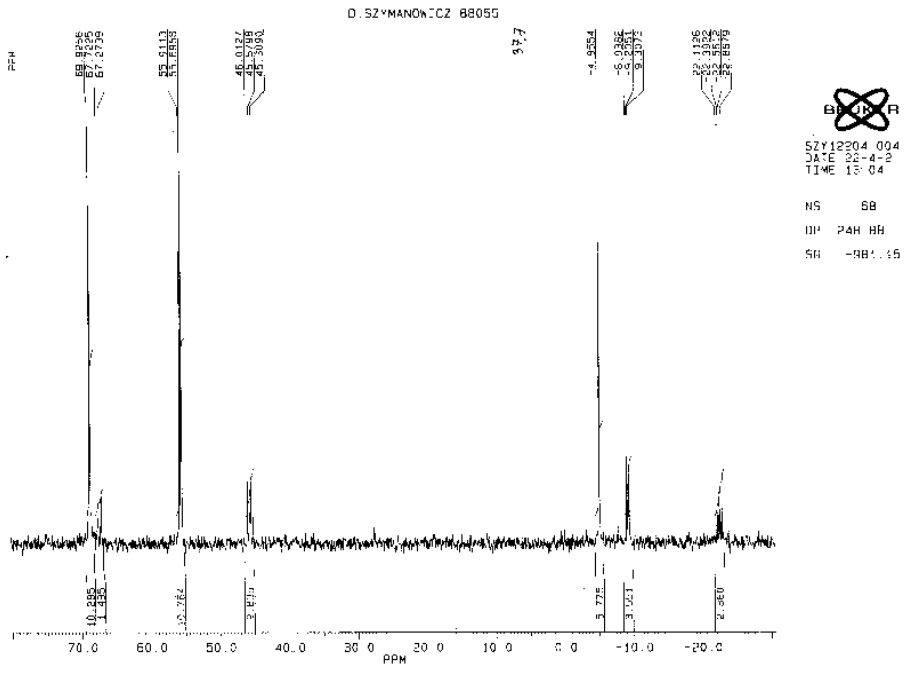

Fig. 2. HPLC Trace of **1f** (ATPαS)

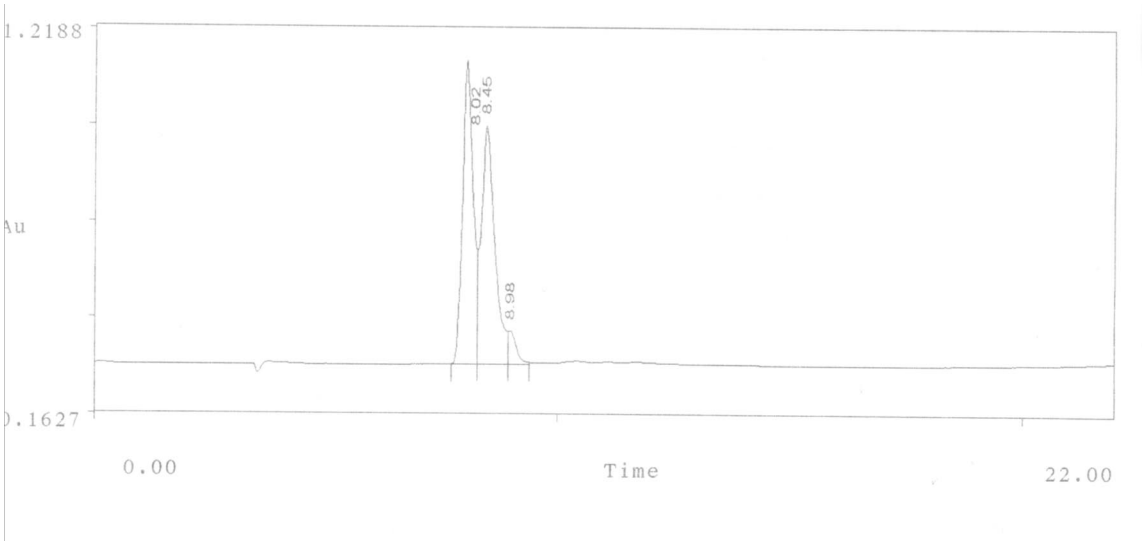

Fig. 3. ^{31}P NMR Spectra of **1f** (ATPαS)

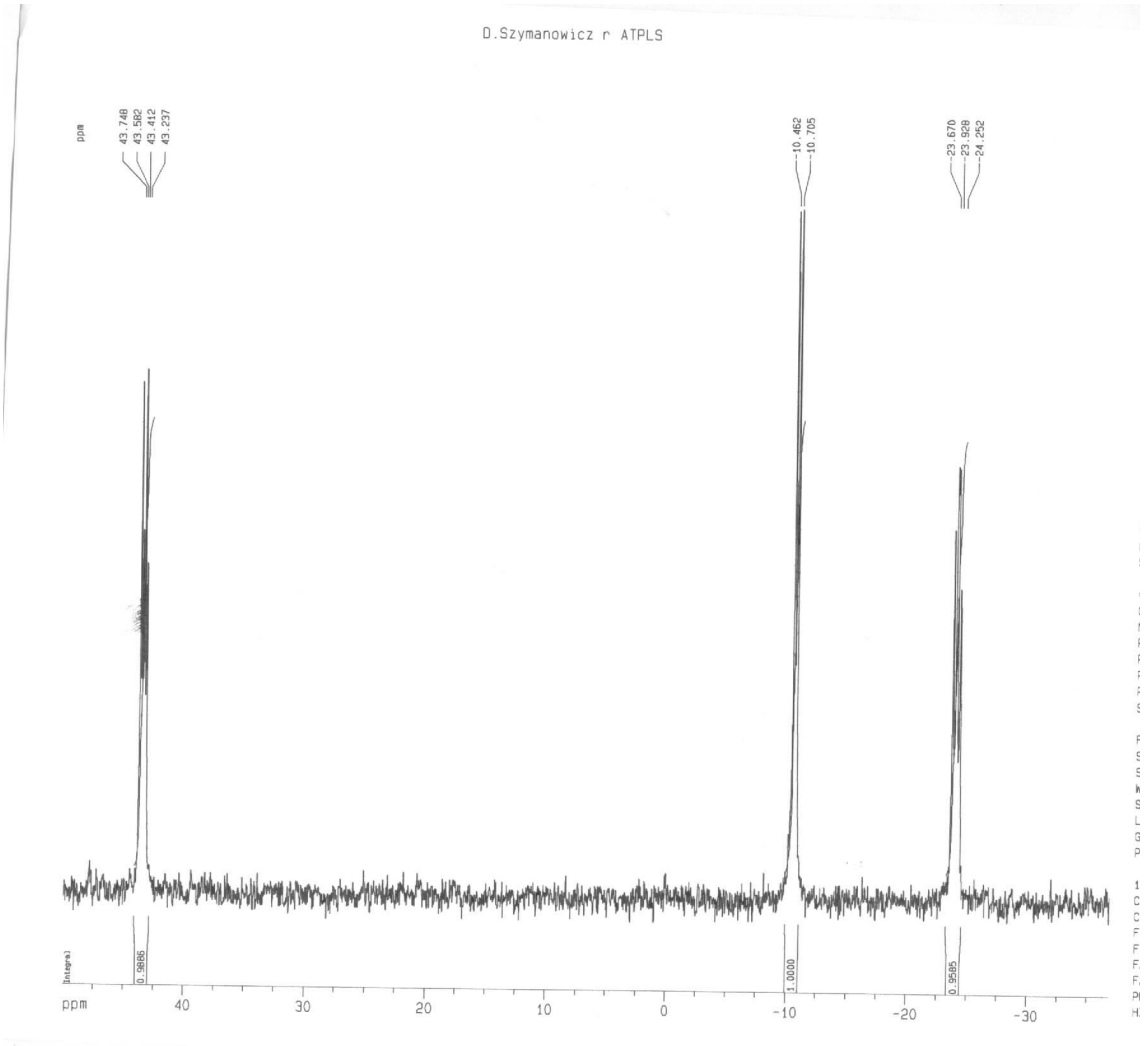

Fig. 4. MALDI-TOF MS Spectra of **1f** (ATPαS)

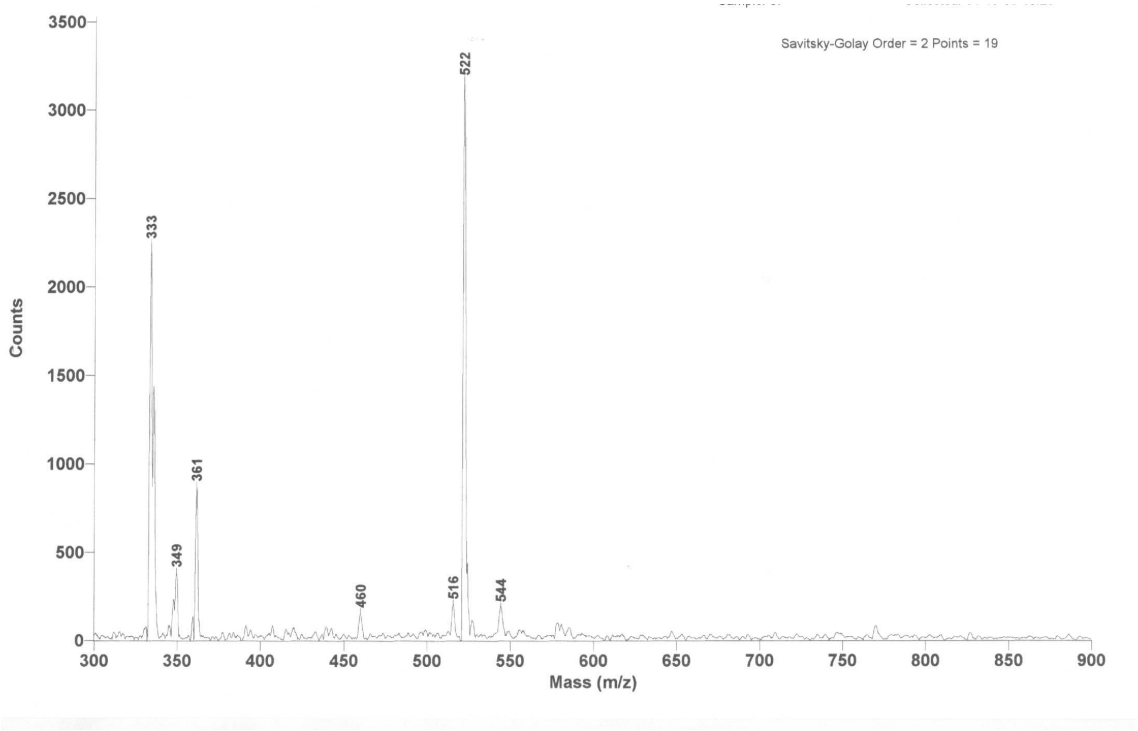

Fig. 5. MALDI-TOF MS Spectra of “dimer” obtained from **4** by ammoniacal deacetylation.