Synthesis of Novel Tetrathiafulvalene System Containing Redox-active Ribonucleoside and Oligoribo-nucleotide

Ojars Neilands* [a], Vilnis Liepinsh [a] and Baiba Turovska [b].

[a] *Department of Organic Chemistry, Riga Technical University, 14 Azenes Str., LV-1048, Riga, Latvia*

[b] *Latvian Institute of Organic Synthesis, 21 Aizkraukles Str., LV-1006, Riga, Latvia*

neilands@ktf.rtu.lv

Supporting Information.

UV spectra were obtained using Hitachi 557 and Unicam SP 1800 spectrometers. NMR spectra were recorded on a Bruker Avance DRX-400 or EOL GSX-270 spectrometer at 20 ^{o}C. Chemical shifts are given in ppm using residual solvent signal as an internal standart.

Cyclic voltammetry (potentiol scan rate 100mV/s) was performed using EG&G PAR 170 model potentiostat. Working electrodes - stationary glassy carbon and platinum disks of 2 mm diameters. Reference electrode was saturated calomel electrode (SCE), Pt used as a counter electrode. Measurements was performed in dry MeCN and in 50% MeCN / 50% H_2O. Anhydrous tetrabutylammonium perchlorate was used as a supporting electrolyte in the both cases.

Pyridine, toluene, dichloroethane and dichloromethane were dried over 4Å molecular sieves. Acetonitrile was dried over 3Å molecular sieves. Triethylamine was dried by refluxing with CaH_2, followed by distillation and was stored over CaH_2. Triethylammonium bicarbonate (TEAB) buffer (2.0M, pH ca. 7.5) was prepared by passing CO_2 (g) through a mixture of triethylamine and water until saturation. Ethanolic ammonia solution was prepared by saturating ethanol with NH_3 (g) at 0^{o}C. Imidazole, triphenylphosphine were purchased from Lancaster, hexamethyldisilazane, trimethylsilyl chloride and 4-methoxytrityl chloride were purchased from Fluka, o-chlorobenzoyl chloride and trimethylsilyl triflate were from Aldrich and were used without any further purification.

TLC analysis was done on Merck silica gel 60 F_{254} precoated plates using iodine camera or UV-light as visualization tools. Silica gel (35-70 μ) from Amicon Europe was used for column chromatography, and the columns were run in the flash mode.

Synthesis of **3**

1 (3g, 11.32 mmol) was refluxed with 25 ml of HMDS and 0.2 ml of TMS-Cl for 22h. The mixture was evaporated to dryness, coevaporated with dry toluene (3×50 ml) and dried in vacuum for 1h. The crystalline product was dissolved in MeCN (70 ml), tetra-O-acetyl ribose **2** was added (3.06g, 9.62 mmol) and solution was cooled to -50 ^{o}C in acetone-dry ice bath. Trimethylsilyl triflate (2.36 ml, 13 mmol) was added during 10 min. via septum with vigorous stirring. Mixture was warmed up to RT in 2h, stirred at RT for 37h, poured into saturated $NaHCO_3$ (aqueous) (200 ml) and stirred for 1 h. The resulting mixture was filtered, the precipitate was washed with chloroform and the aqueous layer was

extracted with chloroform (400 ml). Organic layer was separated, dried over Na_2SO_4 evaporated and purified by silica gel column chromatography using gradient of methanol (0-1.5%) in dichloromethane as eluent. Product was obtained as red amorphous foam. Yield: 3.3g, 65%. R_f = 0.39 (PhMe-EtOAc 1:1). ^{1}H NMR ($CDCl_3$, 400.13 MHz) d: 6.56 (d, *J* = 3.34 Hz, 1H, H1'), 5.77 (m, 1H, H2'), 5.47 (dd, *J* = 8.4 Hz, *J* = 4.42 Hz, 1H, H3'), 4.66 (m, 1H, H4'), 4.48 and 4.26 (ABX system, $J_{H5'-H5''}$ = 12.6 Hz, 2H, H5' and H5''), 2.17 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.03 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100.62 MHz) d: 200.13 (C=Se), 170.97, 170.33, 169.33 ($COCH_3$), 153.86 (C4), 152.77 (C2), 149.31 (C6), 119.60 (C5), 87.35 (C1'), 78.75 (C4'), 71.36 (C2'), 70.16 (C3'), 62.59 (C5'), 21.23, 20.90, 20.81 (CH_3).

Synthesis of **5**

Fully protected nucleoside **3** (1.2g, 2.29 mmol) and selone **4** (1.2g, 5.73 mmol) were dissolved in dry toluene (20 ml). Solution of triphenylphosphine (3.0g, 11.47 mmol) in toluene (15 ml) was added to this solution and the reaction mixture was stirred at RT for 3h. Solution was filtered through silica gel, silica gel was washed with EtOAC and the filtrate was evaporated to dryness. Residue was purified by silica gel column chromatography using gradient of ethyl acetate (0-35%) in toluene as eluent. Product was obtained as a red amorphous foam. Yield: 0.33g, 25%. R_f = 0.40 (PhMe-EtOAc 1:1). ^{1}H NMR ($CDCl_3$, 400.13 MHz) d: 9.69 (bs, 1H, NH), 6.50 (d, *J* = 3.65 Hz, 1H, H1'), 5.73 (t, 1H, H2'), 5.40 (dd, 1H, H3'), 4.73 (m, 1H, H4'), 4.47 and 4.24 (2m, 2H, H5' and H5''), 2.17 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 1.98 (s, 6H, 2×Me).

Synthesis of **6**

Compound **5** (310 mg, 0.54 mmol) was dissolved in saturated ethanolic ammonia (2.5 ml) and saturated aqueous ammonia was added (3 ml). Mixture was left at RT for 6h, then evaporated to dryness, residue was washed with EtOAc (50 ml), filtered and precipitate was dissolved in hot ethanol (100 ml), filtered and evaporated to small volume (ca 4 ml). Solution was left at +4^{o}C overnight, cristalline product was filtered, washed with Et_2O (10 ml), ethanol (1 ml), Et_2O (5 ml) and dried. Product was obtained as a light red cristalline solid. Yield: 130 mg, 54%. Compound **6** is soluble in DMFA, acetic acid and diluted acetic acid (till 50 %), tetrahydrofurane, can be crystallised from dimethoxyethane, ethanol, 50% acetic acid, tetrahydrofurane by dilution with hexane, R_f = 0.71 (DCM-MeOH 4:1). By heating gradual decomposition above 200^0 C has been observed.

^{1}H NMR (DMSO-d_6, 270 MHz) d: 11.84 (bs, 1H, NH), 5.70 (d, *J* = 5.77 Hz, 1H, H1'), 5.30 (d, *J* = 5.77 Hz, 1H, OH), 5.05 (d, *J* = 5.49 Hz, 1H, OH), 4.81 (t, *J* = 5.77 Hz, 1H, OH), 4.31 (m, 1H, H2'), 4.01 (m, 1H, H3'), 3.81 (m, 1H, H4'), 3.65 (m, 2H, H5' and H5''), 1.98 (s, 6H, 2×CH_3). ^{13}C NMR (DMSO-d_6, 67.9 MHz) d: 155.31 (C4), 149.59 (C2), 145.84 (C6), 122.91 and 122.72 (C-Me), 108.26 and 99.58 (C=C), 116.97 (C5), 91.83 (C1'), 85.70 (C4'), 70.89 (C2'), 69.27 (C3'), 61.28 (C5'), 13.34 (2×Me).

UV in MeCN, λ_{max} nm (ε, Lmol^{-1}cm^{-1}): 196(18800), 216(14600), 288(13600), 324(11600), 400(2000).

In MeCN / H_2O (1 : 1): 192(16200), 219(13400), 292(13600), 324(12000), 400-410, broad maximum (2000).

CVA , glassy carbon electrode, reference SCE, supporting electrolyte tetrabutylammonium perchlorate, scan rate 100mV/s, E_{pa} / E_{pc} (V):

in MeCN: E^1 0.54 / 0.46, E^2 0.87 / 0.79; in MeCN / H_2O (1 : 1): E^1 0.45 / 0.34, E^2 0.73 / 0.60.

Found,% : C 40.10; H 3.70; N 5.90; S 28.25. $C_{15}H_{16}N_2O_6S_4$. Calculated,% : C 40.16; H 3.60; N 6.24; S 28.59.

Synthesis of **7**

Nucleoside **6** (100 mg, 0.223 mmol) was coevaporated with dry pyridine (5 ml) and dissolved in dry Py (2 ml). Solution was cooled to 0 ^{o}C in melting ice bath and monomethoxytrityl chloride was added with stirring (72.3 mg, 0.234 mmol). Ice bath was removed and mixture was stirred at RT for 22h. Methanol (0.5 ml) was added and the mixture was evaporated to dryness. Chloroform was added (100 ml) and solution was washed with aqueous saturated $NaHCO_3$ (20 ml). Organic layer was separated, dried over Na_2SO_4 and purified by silica gel column chromatography using gradient of methanol (0-1.5%) in chloroform containing 0.2% of triethylamine as eluent. Product was obtained as a red amorphous foam. Yield: 64 mg, 40%. ^{1}H NMR ($CDCl_3$, 400.13 MHz) d: 7.47-7.17 and 6.82 (m, 14H, Ar), 5.71 (d, *J* = 3.48 Hz, 1H, H1'), 4.64 (m, 1H, H2'), 4.27 (m, 1H, H3'), 4.10 (m, 1H, H4'), 3.77 (s, 3H, OMe), 3.54-4.47 (m, 2H, H5' and H5''), 1.95 and 1.92 (2s, 6H, 2×Me). ^{13}C NMR ($CDCl_3$, 100.62 MHz) d: 158.99 (Ar), 155.78 (C4), 150.63 (C2), 149.1 (C6), 144.60, 130.89, 128.91, 128.49, 128.21, 127.31 (Ar), 123.59 and 122.92 (CMe), 118.84 (C5), 113.57 (Ar), 110.04 and 99.09 (C=C), 95.41 (C1'), 87.19 (CPh_3), 84.62 (C4'), 73.08 (C2'), 71.05 (C3'), 64.30 (C5'), 55.61 (OMe), 14.07 and 13.99 (Me).

Synthesis of **8**

Nucleoside **7** (105 mg, 0.146 mmol) was coevaporated with dry pyridine (5 ml) and dissolved in dry CH_2Cl_2/pyridine (19:1). The reaction mixture was cooled to -78 ^{o}C (acetone-dry ice), and a solution of *o*-chlorobenzoyl chloride (20.87 µl, 0.16 mmol) in CH_2Cl_2 (1 ml) was added with stirring during 15 min. The mixture was stirred at -78 ^{o}C for 45 min. (TLC) and then added dropwise to a stirred and cooled (-78 ^{o}C) mixture of imidazole (0.116g, 1.7 mmol), PCl_3 (48.5 µl, 0.53 mmol), and triethylamine (0.243 ml, 1.75 mmol) in dry CH_2Cl_2 (15 ml). The reaction mixture was stirred at -78 ^{o}C for 30min. and then poured onto and extracted with 1.0 M TEAB (aqueous) pH 7.5 (30 ml). The organic layer was separated, dried over Na_2SO_4, evaporated and purified by silica gel column chromatography using gradient of methanol (0-7%) in chloroform containing 0.1% of triethylamine as eluent. Solvent was evaporated, the residue dissolved in $CHCl_3$ (3 ml) and precipitated from petroleum ether (100 ml). Precipitate was filtered off and dried. Product was obtained in a form of orange solid, which gradually decomposes by heating above 200^{0} C. Yield: 87 mg, 58%. R_f = 0.36 (DCM-MeOH 4:1). ^{1}H NMR ($CDCl_3$, 400.13 MHz) d: 10.34 (bs, 1H, NH), 8.21, 7.90, 7.61-7.0 (m, 16H, Ar), 6.82 (d, *J* = 645.63 Hz, 1H, PH), 6.77 (m, 2H, Ar), 6.01 (s, 1H, H1'), 5.57 (m, 1H, H2'), 5.34 (m, 1H, H3'), 4.23 (m, 1H, H4'), 3.71 (s, 3H, OMe), 3.60 and 3.44 (2m, 2H, H5' and H5''), 2.98 (q, *J* = 7.24 Hz, 6H, CH_2N), 1.92 (s, 6H, 2×Me), 1.25 (t, 9H, NCH_2CH_3). ^{31}P NMR ($CDCl_3$, 162.0 MHz) d: 2.83.
Found,%; C 55.95; H 5.20; N 3.85; S 12.20. $C_{48}H_{51}ClN_3O_{10}S_4P$. Calculated,%: C 56.27; H 5.02; N 4.10; S 12.52.

Synthesis and purification of oligonucleotide **9**

Solid phase synthesis using H-phosphonate approach was done on Pharmacia Gene Assembler Plus solid support oligonucleotide synthesiser using 2'-O-*o*-ClBz protected monomeric building blocks and modified polystyrene solid support (Perkin Elmer). Synthesis was done on 50 mg of solid support using 30 mM solution of H-phosphonates and 90 mM solution of pivaloyl chloride as coupling agent. After synthesis the support was oxidized with 15 mg of S_8 in pyridine (1 ml) at RT for 16h. Support was washed with pyridine (10 ml), acetonitrile (2×2 ml), ether (2×2 ml) and dried. Deprotection and cleavage from the support of the oligonucleotide was done with 30% NH_3 (aqueous)-EtOH (3:1, 1.2 ml) at RT for 8h. Support was filtered off, washed with water-ethanol (3:1, 5 ml) and the solution was liophilized. The residue was dissolved in 30% MeCN in water (0.5 ml), passed through C-18 disposable cartridge (Waters Sep-Pak®), cartridge was washed with 30% MeCN (3×0.5 ml) and the combined solution was filtered through a disposable syringe filter (Millipore Millex®-GV 0.22µm membrane filter) prior to HPLC analysis. Oligonucleotide was analyzed and purified on a RP JASCO HPLC system equipped with Hypersil ODS 5µ (250×10) reversed phase semipreparative column using a linear

gradient of 0-50% MeCN in 100 mM triethylammonium acetate (pH 6.5) for 40 min. at 30 ^{o}C. The oligonucleotide was collected, liophilized, dissolved in water and liophilized again (3×). Product was obtained as a mixture of phosphothioate diastereomers with t_r = 23.7-27.6 min. in a form of an yellow-orange solid.

UV in MeCN / H_2O (1 : 1), $c = 5.10^{-5}$ molL^{-1}, λ_{max} nm, (ε,Lmol^{-1}cm^{-1}): 214(71800), 265(60600), broad max. 305 - 335(15900), 418(2700).

CVA in MeCN / H_2O (1 : 1), glassy carbon electrode, reference SCE, supporting electrolyte tetrabutyl-ammonium perchlorate, scan rate 100 mV/s, $c = 5.10^{-5}$ molL^{-1}, E_{pa} / E_{pc} (V): E^1 0.29 / 0.16. E^2 can not be observed due to intensive oxidation peak beginning from 0.4 V, probably oxidation of phosphothioate anion (see fig.1).

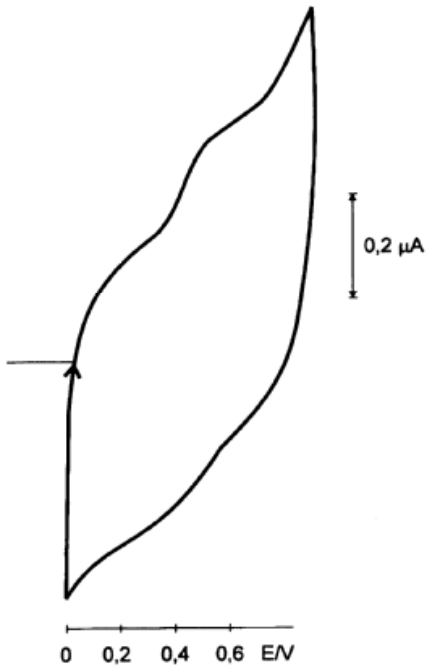

Figure 1. CVA of oligonucleotide **9**.