ORGANIC LETTERS

1999
Vol. 1, No. 13
2065−2067

Synthesis of Novel Tetrathiafulvalene System Containing Redox-Active Ribonucleoside and Oligoribonucleotide

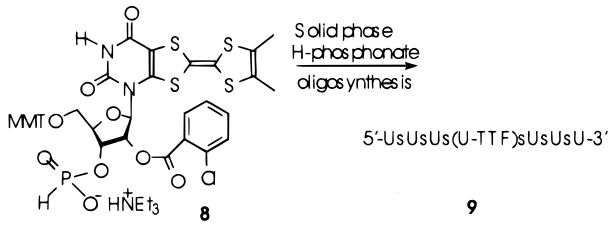
Ojars Neilands,*,† Vilnis Liepinsh,† and Baiba Turovska‡

Department of Organic Chemistry, Riga Technical University, 14 Azenes Street, LV-1048, Riga, Latvia, and Latvian Institute of Organic Synthesis, 21 Aizkraukles Street, LV-1006, Riga, Latvia

neilands@ktf.rtu.lv

Received September 24, 1999

ABSTRACT



RNA oligosynthesis block 8 was synthesized from selone 1 by successive silylation, acetylribosylation, coupling with selone 4, desacetylation, and treatment of obtained ribonucleoside 6 with monomethoxytrityl chloride, followed by *o*-chlorobenzoylation and phosphonylation. Solid-phase oligosynthesis is being performed, and redox-active heptaribonucleotide phosphothioate 9 is obtained which has a lower oxidation potential than ribonucleoside 6.

Redox-active nucleoside and nucleic base derivatives capable of forming intermolecular complementary hydrogen bonds (CHB) during recent years have received much attention due to the recognition and detection possibility of nucleic acids and their components. Bäuerle[1] has investigated conducting polythiophenes functionalized with uracil or adenine derivatives. As was demonstrated in voltammetric and spectroelectrochemical experiments, successive addition of complementary bases to polymers leads to specific modulation of the electrochemical and optical properties due to specific formation of CHB. Garnier[2] used functionalized polypyrroles and prepared a polypyrrole electrode functionalized with an oligodesoxyribonucleoside and showed that the cyclic voltammogram is significantly modified upon addition of a complementary oligodesoxyribonucleoside target. Letsinger[3a] and Ihara[3b] have synthesized oligonucleotides linked with a ferrocene moiety. An electrochemical gene sensor system has been developed using a redox-active ferrocene-modified oligonucleotide anchored on a gold electrode by phosphothioate units on its 5′-terminus. We have aimed to investigate the use of dioxopyrimidotetrathiafulvalenes containing directly fused uracil and tetrathiafulvalene systems for design of oligonucleotides useful for nucleic acids and their component recognition. This hybrid molecule is not only a strong electron donor capable of forming stable cation radical salts but also a target for CHB formation; therefore the close location of the CHB center to the redox-active moiety can promote a change in redox-properties and absorption spectra. We were able to observe a change in oxidation potentials and UV absorption spectra for dimethyl[1-butyl-2,4-dioxo-(1*H*,3*H*-pyrimido]tetrathiafulvalene in the presence of 2,6-di(acetylamino)pyridine.[4] The goal of our investigation is the design of oligonucleotides containing a tetrathiafulvalene moiety.

† Riga Technical University.
‡ Latvian Institute of Organic Synthesis.

(1) (a) Emge, A.; Bäuerle, P. *Synth. Met.* **1997**, *84*, 213. (b) Bäuerle, P.; Emge, A. *Adv. Mater.* **1998**, *3*, 324.

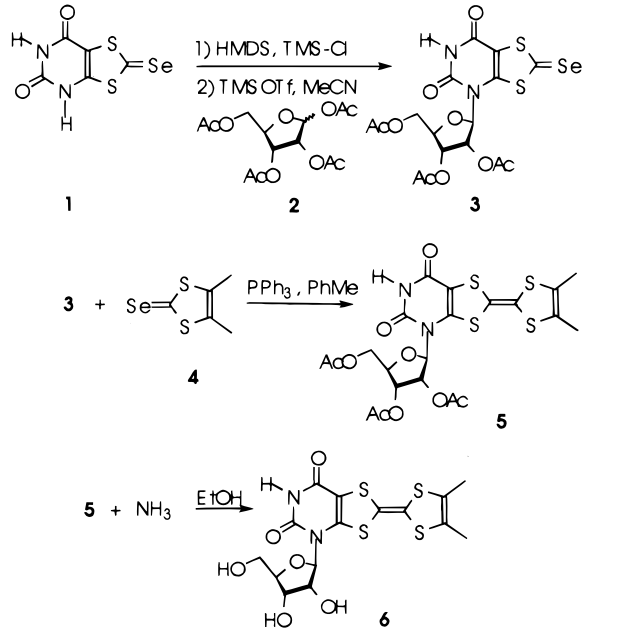
(2) Garnier, F.; Korri-Youssoufi, H.; Srivastava, P.; Mandrand, B.; Delair, Th. *Synth. Met.* **1999**, *100*, 89.

(3) (a) Mucic, C. R.; Herrlein, M. K.; Mirkin, C. A.; Letsinger, R. L. *Chem. Commun.* **1996**, 555. (b) Ihara, T.; Nakayama, M.; Murata, M.; Nakano, K.; Maeda, M. *Chem. Commun.* **1997**, 1609.

10.1021/ol9910868 CCC: $18.00
Published on Web 12/02/1999

The key compound for oligoribonucleotide synthesis is ribonucleoside **6**.[5] In this Letter we describe the synthesis and properties of compound **6**, transformation of **6** to RNA oligosynthesis building block **8**, and preparation of the first heptaribonucleotide phosphothioate **9** bearing the tetrathiafulvalene moiety. The starting compound **1** has been synthesized from barbituric acid.[6] The acetylribosylation of **1** has been performed by trimethylsilylation and interaction with tetraacetylribose in the presence of trimethylsilyl triflate (Scheme 1). The acetylribosyl product **3** was purified by

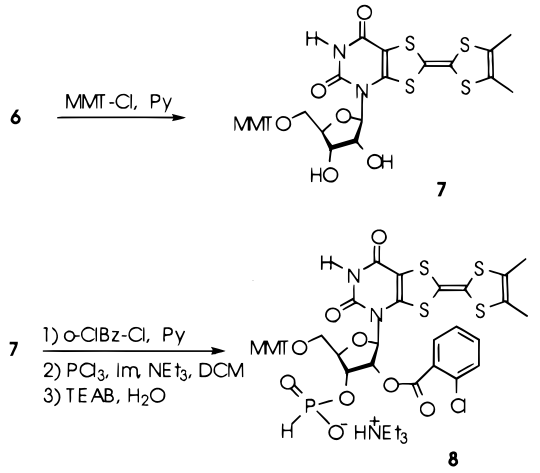
Scheme 1



column chromatography and reacted with selone **4** in the presence of triphenylphosphine.[7] Deprotection of purified compound **5** by an ethanolic ammonia solution gave ribonucleoside **6** as light red crystals in 9% isolated yield (calculated on **1**). The structure of compound **6**[8] has been confirmed by NMR spectra.[8] In UV spectra (MeCN/H_2O 1:1) a broad absorption maximum was observed at 400−410 nm (ϵ = 2000) which is characteristic of dioxopyrimidotetrathiafulvalenes.[4] Cyclic voltammetric investigation confirmed two stages of reversible redox behavior in MeCN solution independently of the electrode used, $E_{pa}^1 = 0.54$, $E_{pa}^2 = 0.88$ V (vs SCE), $\Delta E_p = 0.08$ V. In MeCN/H_2O (1:1) solution, only the first redox couple can be registered, $E_{ox}^1 = 0.44$ V, and the separation of cathodic/anodic peaks is increased until 0.15 V.

RNA synthesis building block **8**[9] has been synthesized from nucleoside **6** (Scheme 2) by monomethoxytritylation

Scheme 2



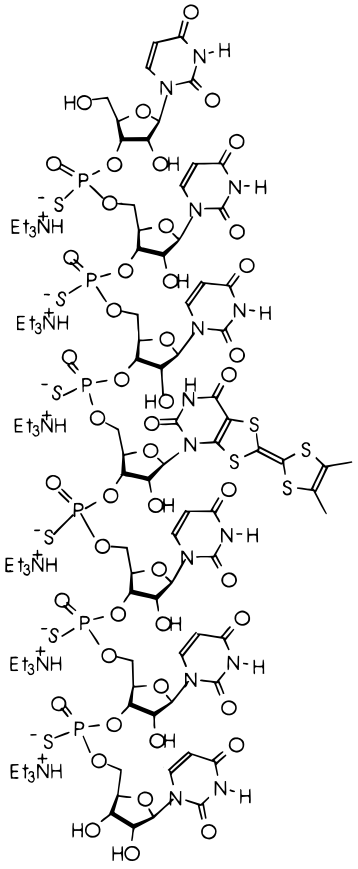
in position 5′ and successive *o*-chlorobenzoylation and phosphonylation[10] in the same reacton mixture[11] using the PCl_3/imidazole reagent[12] of 5′ O-protected nucleoside **7**.

(4) Goldenberg, L.; Neilands, O. *J. Electroanal. Chem.* **1999**, *463*, 212.
(5) Neilands, O.; Liepinsh, V. *Chimia* **1997**, 392.
(6) Neiland, O. Ya.; Khodorkovsky, V. Yu.; Tilika, V. Zh. *Khim. Geterots. Soedin*. **1992**, 1667 (Engl. Ed. *Chem. Heterocycl. Comp*. **1992**, 1432).
(7) Neiland, O. Ya.; Tilika, V..Zh.; Edzhina, A. S. *Khim. Geterots. Soedin.* **1994**, 1285 (Engl. Ed. *Chem. Heterocycl. Comp.* **1994**, 1116).
(8) **Synthesis of 6: 1** (3 g, 11.32 mmol) was refluxed with 25 mL of HMDS and 0.2 mL of TMS-Cl for 22 h. The mixture was evaporated to dryness, coevaporated with dry toluene (3 × 50 mL), and dried in a vacuum for 1 h. The crystalline product was dissolved in MeCN (70 mL), tetra-*O*-acetylribose **2** was added (3.06 g, 9.62 mmol), and the solution was cooled to −50 °C in an acetone−dry ice bath. Trimethylsilyl triflate (2.36 mL, 13 mmol) was added during 10 min via septum with vigorous stirring. Mixture was warmed to rt in 2 h, stirred at rt for 37 h, 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). After silica gel column chromatography using a gradient of methanol (0−1.5%) in dichloromethane as eluent, product **3** was obtained as a red amorphous foam and characterized by NMR. Yield: 3.3 g, 65%. **3** (1.2 g, 2.29 mmol) and selone **4** (1.2 g, 5.73 mmol) were dissolved in dry toluene (20 mL). A solution of triphenylphosphine (3.0 g, 11.47 mmol) in toluene (15 mL) was added and stirred at rt for 3 h. The solution was filtered through silica gel, evaporated, and purified by silica gel chromatography using gradient of toluene (0−35%) in ethyl acetate as eluent. Product **5** was obtained as a red foam. Yield: 0.33 g, 25%. **5** (0.31 g, 0.54 mmol) was dissolved in saturated ethanolic ammonia (2.5 mL), and saturated aqueous ammonia was added (3 mL). The mixture was left at rt for 6 h, evaporated to dryness, washed with EtOAc, and filtered, and the precipitate was dissolved in hot ethanol (100 mL), filtered, evaporated to a small volume (ca 4 mL), and left at +4 °C overnight. Compound **6** was obtained as a red crystalline solid. Yield: 0.13 g, 54%. ^{1}H NMR (DMSO-d_6, 270 MHz) δ: 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) δ: 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).
(9) **Synthesis of 8: 6** was alkylated in dry pyridine at 0 °C by monomethoxytrityl chloride and purified by silica gel column chromatography using chloroform−methanol (0−1.5%) containing 0.2% Et_3N as eluent. Product **7** was obtained as a red amorphous foam in a yield of 40%. Nucleoside **7** underwent acylation by *o*-chlorobenzoyl chloride in a CH_2Cl_2/pyridine solution at −78 °C and without isolation of the acyl product was phosphonylated by PCl_3 in the presence of imidazole and triethylamine at −78 °C. The reaction mixture was poured onto and extracted with 1.0 M aqueous triethylammonium bicarbonate, pH 7.5. The organic layer was dried and evaporated, and the product was purified by silica gel chromatography using chloroform−methanol (0−7%) containing 0.1% Et_3N as eluent and the reprecipitated from a chloroform solution by light petroleum ether. The yield of orange solid **8** was 58%. ^{1}H NMR ($CDCl_3$, 400.13 MHz) δ: 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) δ: 2.83.

The obtained building block **8** was used in an automated solid-phase H-phosphonate method for RNA synthesis[13] and 2′-*O*-*o*-ClBz protection[10] using a modified Pharmacia Gene Assembler Plus oligonucleotide synthesizer.

Our first attempts involved standard oxidation conditions with 2% iodine solution in wet pyridine after the final coupling step in order to get phosphate internucleotidic bonds. These attempts, however, were not successful due to instability and breakage of the tetrathiafulvalene system in these oxidative reaction conditions. Therefore, we decided to oxidize the internucleotidic bonds with elemental sulfur in pyridine[14] in order to obtain the desired oligonucleotide phosphothioate as a mixture of diastereoisomers (chirality of P atoms). The synthesized sequence was 5′-UsUsUs(U-TTF)sUsUsU-3′ **9** (Scheme 3). After the deprotection of the oligonucleotide and cleavage from the polystyrene support with an ethanolic ammonia solution, the crude oligonucleotide phosphothioate was purified by reversed phase HPLC. Pure heptaribonucleotide **9** was obtained in the form of a yellow solid as a mixture of diastereomers, unsoluble in MeCN or CH_2Cl_2. In UV spectra (MeCN/H_2O 1:1) absorption at 418 nm ($\epsilon = 2700$) has been observed. The spectra confirmed the molecular composition of oligoribonucleotide **9**, containing dioxopyrimido-TTF and U moieties. In MeCN/H_2O solution CVA measurements on a glassy carbon electrode showed that **9** is redox-active: the first quasi-reversible oxidation takes place at $E_{pa}^1 = 0.29$ V; the second redox-stage cannot be registered in this media.

Scheme 3



9

It should be noted that in the UV spectra of **9** the absorption maximum at 418 nm is bathochromic shifted in comparison with that of ribonucleotide **6** (410 nm), but the E_{pa} of **9** is lower than that of **6** (0.29 and 0.44 V). This phenomenon could be explained by intramolecular interaction in this unusual molecule **9** that reduces the electron acceptor ability of the uracil fragment.

To the best of our knowledge this is the first case where the tetrathiafulvalene system has been incorporated into an oligoribonucleotide in a manner that preserves the system's potential to form complementary hydrogen bonds. The investigation of oligonucleotide **9** properties in the presence of complementary nucleobases and its binding on the gold surface is in progress.

Acknowledgment. We thank Prof. Roger Strömberg of Karolinska Institute (Stockholm) for help with the oligonucleotide synthesizer and HPLC instruments.

Supporting Information Available: Experimental procedures for compounds **3**, **5**, **6**, **7**, **8**, and **9** and physical measurements. This information is available free of charge via the Internet at http://pubs.acs.org.

OL9910868

(10) Rozners, E.; Strömberg, R.; Bizdena, E. *Nucleosides Nucleotides* **1995**, *14*, 855. (b) Rozners, E.; Renhofa, R.; Petrova, M.; Popelis, J.; Kumpins, V.; Bizdena, E. *Nucleosides Nucleotides* **1992**, *11*, 1579.

(11) Rozners, E.; Strömberg, R. *J. Org. Chem.* **1997**, *62*, 1846.

(12) Garegg, P.; Regberg, T.; Stawinski, J.; Strömberg, R. *Chem. Script.* **1986**, *26*, 59. (b) Stawinski, J.; Strömberg, R.; Thelin, M.; Westman, E. *Nucleic Acids Res.* **1988**, *16*, 9285.

(13) (a) Garegg, P. J.; Lindh, I.; Regberg, T.; Stawinski, J.; Strömberg, R.; Henrichson, C. *Tetrahedron Lett.* **1986**, *27*, 4055. (b) Westman, E.; Sigurdson, S.; Stawinski, J.; Strömberg, R. *Nucleic Acids Res. Symp. Ser. No. 31* **1994**, 25.

(14) Stec, W. J.; Zon, G.; Egan, W.; Stec, B. *J. Am. Chem. Soc.* **1984**, *106*, 6077.