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Oxathiaphospholane Approach to the Synthesis of Oligodeoxyribonucleotides Containing Stereodefined Internucleotide Phosphoroselenoate Function†

Piotr Guga, Anna Maciaszek, and Wojciech J.Stec*

Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Department of Bioorganic Chemistry, Sienkiewicza 112, 90-363 Łódź, Poland

wjstec@bio.cbmm.lodz.pl

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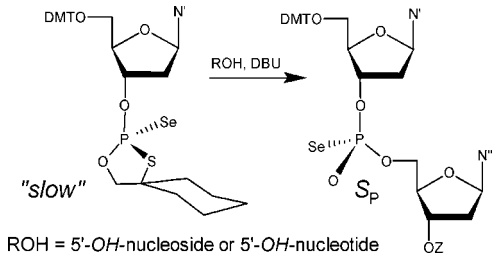
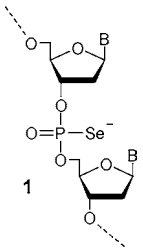
ABSTRACT



5′-*O*-DMT-deoxyribonucleoside-3′-*O*-(2-selena-4,4-pentamethylene-1,3,2-oxathiaphospholane) monomers, derivatives of dA, dC, dG, and T, can be resolved into pure P-diastereomers by silica gel column chromatography. They have been used for DBU-promoted, either solution- or solid-phase synthesis of P-stereodefined phosphoroselenoate analogues of oligodeoxyribonucleotides. Fast- and slow-eluting monomers are precursors of phosphoroselenoate internucleotide linkage of R_P and S_P absolute configuration, respectively.

The role of selenium in biological systems is of increasing interest.[1] Analogues of proteins and oligonucleotides, with sulfur or oxygen atoms replaced with selenium, have been evaluated as research tools for studies of interactions with metal ions, and selenium-labeled biopolymers can be effectively analyzed by X-ray crystallography because of multiwavelength anomalous dispersion (MAD).[2] Here we report on the stereocontrolled chemical synthesis of phosphoroselenoate oligonucleotides with internucleotide function(s) of predetermined sense of P-chirality. To date, a phase transfer approach was developed to introduce the selenium functionality in nucleosides at 5′-positions.[3] Alternatively, a SeMe group can be incorporated at the C2′-ribo position of nucleoside, although this modification enforces the C3′-endo structure.[4,5] Labeling of oligonucleotides with a selenium atom at internucleotide functions, yielding phosphoroselenoate analogues of DNA (PSe-Oligo, **1**), basically does not affect conformation of the sugar ring.



Diribonucleotide phosphoroselenoates were synthesized in solution by Ogilvie in 1980.[6] Solid-phase syntheses of PSe-Oligos were performed using a phosphoramidite or H-phosphonate approach with "selenation" of the P^{III} interme-

† Dedicated to Professor Przemyslaw Mastalerz on the occasion of his 80th birthday.

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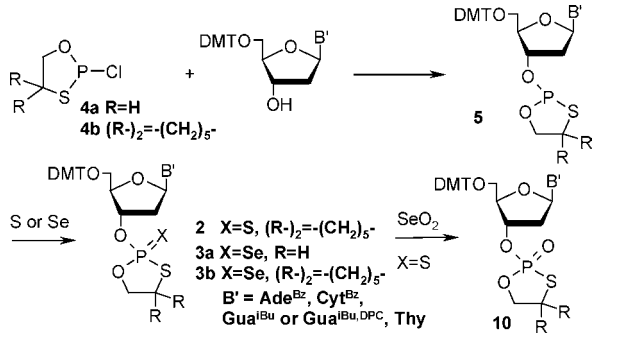
diate.[7] In all instances, the products consist of a mixture of P-diastereoisomers.[8] Diastereomerically pure dinucleoside phosphoroselenoates[9] and DNA hexamers containing a single internucleotide phosphoroselenoate linkage[10] were obtained by HPLC resolution of diastereomeric mixtures. Recently, enzymatic synthesis of stereodefined PSe-Oligo has been published, based on the use of both S_P and R_P diastereomers of TTPαSe and DNA polymerase.[11] It has been found that stereorandomal PSe-Oligos have a diminished hybridization capability[7b] to complementary DNA and RNA templates, as compared with both the unmodified and phosphorothioate oligomers. Nonetheless, selenium-labeled biopolymers are useful probes for their structural and functional analysis. We synthesized P-stereodefined phosphoroselenoate oligodeoxyribonucleotides by modification of our method for synthesis of oligo(nucleoside phosphorothioate)s,[12,13] which employs diastereomerically pure 5′-*O*-DMT-nucleoside-3′-*O*-(2-thio-4,4-pentamethylene-1,3,2-oxathiaphospholane) monomers **2** (X = S, Scheme 1). The mechanism of the condensation

Scheme 1



step (Scheme 1S, Supporting Information) suggested that the synthesis of pure P-diastereomers of nucleoside-3′-*O*-(2-selena-1,3,2-oxathiaphospholane) **3** (X = Se) should allow for preparation of P-stereodefined PSe-Oligos. Earlier, the 2-selena-1,3,2-oxathiaphospholane derivative of 5′-*O*-DMT-thymidine (**3a**, B′ = Thy, R = H) allowed for the synthesis of thymidyl dinucleoside phosphoroselenoate.[14] Within the present work, two sets of deoxyribonucleoside monomers **3a** (see Table 1S, Supporting Information) and **3b** (B = Ade^{Bz}, Cyt^{Bz}, Gua^{iBu,DPC}, and Thy; Table 1) were synthesized as depicted in Scheme 1. Either mercaptoethanol or (1-sulfanylcyclohexyl)-methanol[15] were reacted with PCl_3 to obtain the phospitylating reagents **4a** or **4b**, respectively.[16] The details of conversion **4b** → **5** → **3b** are provided in Supporting Information.

Table 1. Yield and Chromatographic and Spectroscopic Properties of Separated Monomers **3b**

nucleobase	yield[a] [%]	FAB-MS[b] *m/z*	yield [%]	R_f[c]	δ ^{31}P NMR [ppm][d]	$^1J_{P,Se}$ [Hz]
Cyt^{Bz}	56	886.5	31	0.64	99.61	946
			31	0.62	100.04	945
Ade^{Bz}	59	910,5	20	0.65	99.06	945
			40	0.63	99.73	945
Thy	60	797,4	23	0.59	99.25	944
			31	0.57	100.05	944
Gua^{iBu,DPC}	45	1087.8	20	0.53	99.90	947
			30	0.51	100.44	947

[a] Yield of isolated mixture of both diastereomers, calculated over starting 5′-*O*-DMT-*N*-protected nucleosides. [b] Calculated value (for ^{80}Se) *m/z* 887, 911, 798, 1088, respectively. Technical parameters: Cs^+, 13 keV, matrix—3-nitrobenzyl alcohol, negative ions mode. [c] HP TLC plates; ethyl acetate/butyl acetate 2:1 v/v (dA^{Bz}, dC^{Bz}) or butyl acetate:benzene 1:1 v/v (T, dG^{iBu,DPC}) were used to elute the silica gel columns and to develop the plates. [d] 200 MHz (for ^{1}H), CD_3CN.

In the ^{31}P NMR spectra recorded for **3a** and **3b**, the resonances in the range of 99—100 ppm accompanied by satellite doublets resulting from the direct ^{31}P—^{77}Se spin—spin coupling ($^1J_{P,Se}$ = 945—954 Hz) were found. Unfortunately, attempts at chromatographic separation of P-diastereomers of the monomers **3a** (B′ = Ade^{Bz}, Cyt^{Bz}, Gua^{iBu}, and Thy) on a silica gel column have failed. The "*spiro*" monomers **3b** (B′ = Cyt^{Bz}, Ade^{Bz}, Thy) were much more useful as we were able to separate amounts of 400—500 mg on a single silica gel column (see Table 1). The guanosyl monomer **3b** (B′ = Gua^{iBu}) was resolved onto pure diastereomers only after protection at the O^6-site with diphenylcarbamoyl chloride (66% yield). The slow-eluting monomer **3b** (B′ = Ade^{Bz}, 95% diastereomeric purity) was used for condensation with 3′-*O*-Ac-thymidine (5 equiv) in the presence of DBU (1.05 equiv) in dry pyridine. The ^{31}P NMR spectrum recorded after 2 h showed the presence of resonances at 50.78 [95%, $^1J_{P,Se}$ = 814 Hz] and 50.30 ppm

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(5%), indicating virtually quantitative conversion into dinucleoside phosphoroselenoate with stereoselectivity close to 100% (Figure 1S, Supporting Information). After deprotection, the $A_{PSe}T$ dinucleotide was isolated (RP-HPLC) and its molecular weight was confirmed with MALDI-TOF MS (*m*/*z* 618).

Correlation of chromatographic mobility of *fast*- and *slow*-**3b** with the respective R_P and S_P absolute configuration of the resulting internucleotide phosphoroselenoate bond in $d(N_{PSe}T)$ (N = dA, dC, T, or dG) has been achieved enzymatically using Rp-specific snake-venom phosphodiesterase (svPDE) and Sp-specific nuclease P1 (nP1)[9] (Figure 2S, Supporting Information).

The *fast*-**3b** (B′ = Ade^{Bz}, 20 mg) was used for elongation at the 5′-end of d(AGCGGTCGGC) (**6**) (synthesized by phosphoramidite approach using the DBU-resistant sarcosinyl-succinoyl linker[16] to yield 5′-*O*-DMT-$d(A_{PSe}$AGCGGTCGGC) (**7**) of R_P absolute configuration. The HPLC analysis of the DMT-tagged **6** (ca. 10% of the support was taken out from the synthetic column to ensure accurate analysis of the "core" oligomer to be elongated) and resulting **7** (Figure 3S, Supporting Information) showed that the condensation process furnished PSe-Oligo in ca. 90% yield. Subsequent HPLC purification followed by MALDI-TOF MS analysis confirmed the identity of the product. The molecular ion at *m*/*z* 3746 was accompanied by a signal at *m*/*z* 3444, attributed to the detritylated (due to acidity of the matrix used) oligomer (Figure 1A).

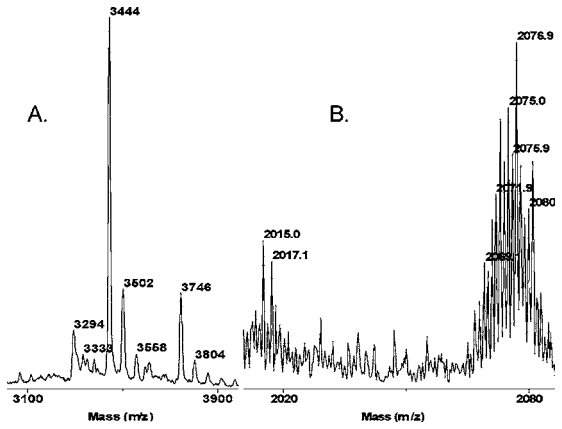

Figure 1. (A) MALDI-TOF MS analysis of **7** obtained from **6** using *fast*-**3b** (B′ = Ade^{Bz}), after HPLC (DMT-on) purification. (B) MALDI-TOF MS analysis of **8a,** after HPLC purification.

The [All-R_P]- and [All-S_P]-PSe-d(TCTCAG) hexamers (**8a**,**b**) possessing phosphoroselenoate linkages of R_P or S_P absolute configuration at each internucleotide position, respectively, were synthesized on solid support (0.5 μmol scale, 10 mg of the appropriate monomer **3b** per coupling) using a protocol adapted from the synthesis of phosphorothioate analogues of DNA.[12] Since model studies showed that routine detritylation of the DMT-ON isolated oligomer with 50% acetic acid resulted in massive loss of selenium from the internucleotide bonds, the oligomers were detritylated on the support under anhydrous conditions. After cleaveage from the support and deprotection with concentrated NH_4OH at 55 °C for 16 h, they were finally isolated by RP-HPLC (Figure 4S). The products were collected when absorption exceeded 20% of the height of main peaks, yielding 6.5 and 5.5 OD units of **8a** and **8b**, respectively, which were analyzed using MALDI-TOF MS. The molecular ions centered at *m*/*z* 2077 confirmed their identity (Figure 1B). The signals around *m*/*z* 2015 (ca. 18% compared to the desired product) has been attributed to the molecules with one out of five selenium atoms along the chain, randomly replaced with oxygen.

Another selenium-labeled oligomer of the sequence (S_P)-$d(AAC_{PSe}TGC)$ (**9**) was obtained by synthesis of d(TGC) by phosphoramidite approach (at 1-μmol scale), followed by elongation with *slow*-**3b** (B′ = Cyt^{Bz}, 20 mg) and two consecutive couplings with 5′-*O*-DMT-deoxyadenosine-3′-*O*-(2-oxo-4,4-pentamethylene-1,3,2-oxathiaphospholane) (**10**, B′ = Ade^{Bz}, Scheme 1). The monomer **10** was prepared in almost quantitative yield from its 2-thio precursor **2** (X = S, unresolved mixture of diastereomers) upon treatment with 5 molar equiv of SeO_2 in dry acetonitrile.[16] The product **9** was detritylated on the support, deprotected, and isolated by RP-HPLC as described above, yielding 7.5 OD units. Its identity was confirmed by MALDI-TOF MS (Figure 5S).

For assignment of melting temperatures of complexes of stereodefined PSe-Oligos with complementary DNA, RNA, and 2′-OMe-RNA templates, two stereoregular dodecamers [All-R_P]- and [All-S_P]-PSe-dA_{12} (R_P- and S_P-**10**, respectively)-were synthesized, isolated in the amounts of 15 and 19 OD units, respectively, and analyzed by MALDI-TOF MS (Figure 6S) and PAGE. Obtained melting data (collected in Table 2) show interesting stereodependence of melting temperatures on the type of the template used, while relevant melting curves have good S-shape indicating high cooperativity of the transition (Figure 7S).

Table 2. Melting Temperatures[a] for Complexes of Stereodefined [All-R_P]-, [All-S_P]-PSe-dA_{12} and dA_{12} with Complementary DNA, RNA, and 2′-OMe-RNA Templates

	template		
form of dA_{12}	T_{12}[b]	U_{12}[b]	(2′-OMe)U_{12}[c]
[All-R_p]-PSe	22.2	29.5	58.1[d]
[All-S_p]-PSe	32.5	19.1	28.3
PO	35.4	23.6	34.7

[a] Buffer: 10 mM TRIS-Cl pH7.4, 100 mM NaCl, 10 mM $MgCl_2$. dA_{12} oligonucleotide concentration, 2 μmol.Temperature gradient, 0.2 °C/min. [b] PSe- or PO-dA_{12}/template molar ratio, 1:1. [c] PSe- or PO-dA_{12}/template molar ratio, 1:2. [d] Similar unusually high thermal stability (T_m = 53.3 °C) was observed for phosphorothioate [All-R_p]-PS-dA_{12}/(2′-OMe)U_{12}; manuscript in preparation.

In summary, a novel, efficient, and stereocontrolled method for the solid-support synthesis of phosphoroselenoate analogues of oligodeoxyribonucleotides has been developed. It allows for the synthesis of oligomers with any combination

of internucleotide phosphoroselenoate linkages of R_P or S_P absolute configuration, as well as unmodified phosphate bonds, which in general cannot be achieved using enzymatic methods.

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Supporting Information Available: Table containing spectroscopic properties of monomers **3a**. Text giving experimental details. ^{13}C NMR and FAB MS characteristics of monomers **3b**. Scheme illustrating the mechanism of condensation. ^{31}P NMR spectrum of crude 5′-*O*-DMT-d($A^{Bz}_{PSe}T$)-3′-*O*-Ac. Chromatograms for **6**, **7**, and **8a**,**b** and enzymic digestion of d($A_{PSe}T$), MALDI-TOF MS spectrum for **9** and S_P-**10**, melting curve for the complex of R_P-**10**/(2′-OMe)U_{12}, ^1H and ^{13}C NMR spectra for monomers **3b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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