Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 8, pp. 1033-1036, 1994

0960-894X/94 $6.00+0.00

0960-894X(94)E0096-W

A NEW CLASS OF DINUCLEOTIDE ANALOGUES. THE SYNTHESIS OF 3'-O-THYMIDYLYL(5'-DEOXY-5'-SELENE-THYMIDYLYL)-Se-PHOSPHOROSELENOLATE, ITS O-METHYL ESTER AND METHANEPHOSPHONATE DERIVATIVES.

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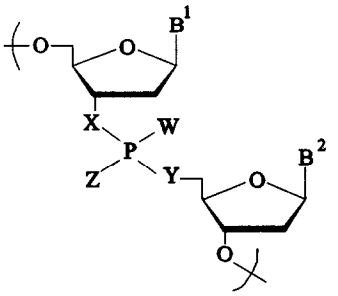
Abstract: Title compounds **12**, **13** and **14** have been prepared *via* alkylation of 5'-O-protected nucleoside 3'-O-(O-alkylphosphoroselenoates) (**5**, **6**) and -3'-O-(methanephosphonoselenoates) (**7**). After deprotection, 5'-deoxy-5'-selene dinucleoside Se-phosphates and Se-phosphonates have been obtained with good yields. Their chemical and antiviral (HIV-1) properties have also been examined.

Elemental replacement of one or both of the "non-bridging" oxygens (W,Z) surrounding the phosphorus atom of an internucleotide phosphate junction leads to oligonucleotide analogues such as **1**, that have unaltered nucleoside components.[1] In contrast, replacement of " bridging "(X and/or Y) oxygens results in constructs, that give 3'-and/or 5'-modified nucleosides[2] after exhaustive chemical or enzymatic hydrolysis.

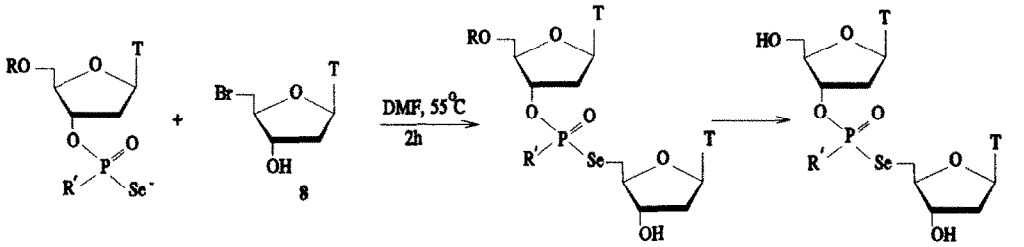


1	X=O	Y=O	Z=O	W=S
2	X=O	Y=Se	Z=W=O	
3	X=O	Y=Se	$Z=CH_3$	W=O
4	X=O	Y=Se	$Z=CH_3O$	W=O

The synthesis of oligonucleotide analogues such as **2**, **3** and **4** was tempting, since we expected these analogues to display increased stability against nucleases. Thus, such oligonucleotides might find potential application in antisense technology,[1] or might be useful as research tools for molecular and cell biology.[3] Aside from these applications, constructs such as **2**, **3** and **4** could be used as prodrugs for the transport of nucleoside analogues which are potential antimetabolites.[4]

In this report we have described the synthesis of 5'-DMT-O-thymidine 3'-O-[O-2-cyanoethyl phosphoroselenoate] (**5**, $NHEt_3^+$)[5], 5'-Pixyl-O-thymidine 3'-O-[O-methyl phosphoroselenoate] (**6**, $NHEt_3^+$)[6], and 5'-Pixyl-O-thymidine 3'-O-[methanephosphonoselenoate] (**7**, Na^+)[7], and their subsequent alkylation[8] with 5'-deoxy-5'-bromothymidine (**8**)[9]. Such reactions led, respectively, to 3'-O-(5'-DMT-O-thymidylyl)-(5'-deoxy-5'-

selenethymidylyl)-(3',5')-O-(2-cyanoethyl)phosphoroselenolate (**9**), 3'-O-(5'-Pixyl-O-thymidylyl)-(5'-deoxy-5'-selenethymidylyl)-(3',5')-O-methyl phosphoroselenolate (**10**) and 3'-O-(5'-Pixyl-O-thymidylyl)(5'-deoxy-5'-selenethymidylyl)-(3',5')-methanephosphonoselenolate (**11**). These compounds (**9**, **10** and **11**) exist as a mixture of two P-diastereomers, due to the stereogenicity of the phosphorus atom. Treatment of a diastereomeric mixture of **9** with 80% acetic acid (5'-O-deprotection) and, subsequently, with triethylamine/pyridine (phosphoroselenolate deprotection) gave P-achiral 3'-(O-thymidylyl)-(5'-deoxy-5'-selenethymidylyl)-(3',5')-phosphoroselenolate (**12** represented by general structure **2**). 5'-O-deprotection of each separated diastereomer of compound **10** led to the diastereomerically pure 3'-(O-thymidylyl)-(5'-deoxy-5'-selenethymidylyl)-(3',5')-O-methyl phosphoroselenolate (**13**, represented by general formula **4**).
5'-O-deprotection of **11** led to diastereomeric mixture of 3'-(O-thymidylyl)-(5'-deoxy-5'-selenethymidylyl)-(3',5')-methanephosphonoselenolate (**14**, represented by general formula **3**).



5 R=DMT R'=$NCCH_2CH_2O$-
6 R=Pixyl R'=CH_3O-
7 R=Pixyl R'=CH_3-

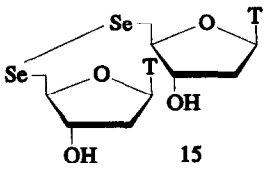
9 R=DMT R'=$NCCH_2CH_2O$-
10 R=Pixyl R'=CH_3O-
11 R=Pixyl R'=CH_3-

12 R'= O^-
13 R'= CH_3O-
14 R'= CH_3-

Compounds **10** and **11**, precursors of **13** and **14**, respectively, were isolated by means of silica gel chromatography. Compound **10** was separated by this same technique into diastereomerically pure P-stereoisomers ("fast"-eluted **10** and "slow"-eluted **10**).

All new compounds, including **10**, **11**, **12**, **13** and **14**, were characterized by mass spectrometry and ^{31}P NMR.[10] Both techniques confirmed the chemical composition and the structure of the carbon-phosphoro(no)selenolate backbone of these modified dinucleotides. The bridging position of the selenium atom in **10**, **11**, **12**, **13** and **14** is confirmed by the presence of "side bands" in the ^{31}P NMR spectra, resulting from one-bond spin-coupling interactions between ^{31}P- and ^{77}Se-nuclei. The absolute value of the $^1J_{P\text{-}Se}$ parameter unambiguously supports the presence of the 3'-O-thymidylyl-5'-deoxy-5'-selenethymidylyl-(3',5')-phosphoro-(no)selenolate structural motif.[11] Since the preparative procedure for **12** required the treatment of its precursor **9** with triethylamine/pyridine, fully deprotected **12** (triethylammonium salt) was converted into the sodium salt *via* ion-exchange chromatography (Dowex 50W); its analytical purity was confirmed by HPLC (ODS-Hypersil, gradient 0-20% MeCN in 0.1 M. TEAB, pH=7.0, R_t=16.08 min.). The compound **12** (sodium salt) and its O-methyl esters **13** are well-soluble in water. However, the solubility of both diastereomers of **14** is relatively

low. Because of this, DMSO solutions of **13** and **14** were used for testing of their anti-HIV activity (*vide infra*). The compound **12** is stable in buffered water solutions (pH 7.5) at 4°C for several days unless heavy metal cations or oxidants are present.[12] In the case of compounds **13** and **14** slow decomposition was observed within the days, even if their aqueous solutions were kept at 4°C.



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A freshly prepared solution of **12** (sodium salt) is slowly degraded in the presence of snake venom phosphodiesterase (SVPD) or nuclease P1.[13] Spleen phosphodiesterase (bovine) also causes slow degradation of **12** (HPLC assay). In all enzymatic processes there was an isolated by-product containing selenium, which was identified as di(5'-deoxydithymidyl) diselenide (**15**), by comparison with a genuine sample.[14] Since the hydrolytic cleavage of P-Se bond in compounds **12**, **13** and **14** was expected to give 5'-deoxy-5'-selenethymidine (scission of the P-O bond should lead to thymidine 5'-phosphoroselenolate, thymidine 5'-O-(O-methyl phosphoroselenolate) and thymidine 5'-O-methanephosphonoselenolate, respectively), it was tempting to check the antiviral activity of **12**, **13** and **14**. Compounds **12** (sodium salt, 1 mM concentration), **13**-"fast" and **13**-"slow" (2.6 mM for "fast"- and 2.5 mM for "slow"-eluted diastereomers, respectively, in DMSO) and **14** (2.4 mM DMSO solution) were tested for antiretroviral activity against HIV-1 in the HIV cytopathic effect inhibition assay using $CD4^+$ ATH8 cells as the target cells.[15] None of these compounds showed detectable antiviral activity at 5 and 25 μM concentrations in this assay system. Compound **12** was moderately toxic at 25 μM, while compounds **13** and **14**-"fast" were substantially toxic, bringing about cellular growth suppression by 60-80%. However, no cells survived the exposure to 25 μM solution of compound **13**-"slow". In light of the profound cytotoxicity of oligo(nucleoside phosphoroselenoate)s,[16] this result was not completely unexpected, despite the well documented fact that phosphoroselenoic anion participates in the biosynthesis of selenecysteine.[17] Although biological applications of the compounds presented in this communication are rather doubtful, it remains interesting to elucidate which structural motif of compounds **12**, **13**, and **14** is responsible for the observed cytotoxicity.

Acknowledgments: This project was financially assisted by State Committee for Scientific Research, grant no. 0034-P2/92/03, and supported in part by Genta Inc., San Diego, CA.

Notes and References:

(1) *Antisense Research and Applications*; Ed. Crooke, S.T. and Lebleu, B., CRC Press Boca Raton, 1993.
(2) Cosstick, R., and Vyle, J.S., *Nucleic Acids Research*, 1990, **18**, 829.
(3) Akhtar, S., Shoji, Y., and Juliano, R.L., 1991, in *Gene Regulation. Biology of Antisense RNA and DNA*, vol.I, Erickson, R.P., and Izant, J.G., eds.(Raven Press, New York) pp.133-146.
(4) Witczak, Z., J.,*Nucleosides & Nucleotides*,1983,**2**, 295.

(5) Compounnd **5** was obtained by reaction of 5'-DMT-O-thymidine 3'-O-[O-2-cyanoethyl- N,N-diisopropylphosphoramidite] with 3-hydroxypropionitrile in the presence of tetrazole, followed by oxidation of transiently formed 5'-DMT-O-thymidine 3'-O-di(2-cyanoethyl)phosphite with elemental selenium, with subsequent base-catalyzed elimination of acrylonitrile:[31] NMR δ: 52.93, 52.64, $^1J_{P\text{-}Se}$=829 Hz ($CDCl_3$).

(6) Compound **6** was obtained by reaction of 5'-Pixyl-O-thymidine 3'-[O-2-cyanoethyl N,N-diisopropylphosphoramidite] [^{31}P NMR, δ: 149.35, 148.85 ($CDCl_3$)] with methanol in the presence of tetrazole, followed oxidation of intermediary phosphite with elemental selenium, and elimination of acrylonitrile by means of triethylamine/pyridine; ^{31}P NMR, δ: 52.57 and 52.32, $^1J_{P\text{-}Se}$ 900 Hz ($CDCl_3$).

(7) Compound **7** was obtained by reaction of 5'-Pixyl-O-thymidine with $MePCl_2$ (-40°C)(1 equiv.) in THF solution in the presence of triethylamine (2 equiv.), followed by aniline (2 equiv.) and elemental selenium (2.5 equiv). Resultant 5'-Pixyl-O-thymidine 3'-O-(methanephosphonoanilidoselenolate) [^{31}P NMR, δ: 76.57 and 76.36, $^1J_{P\text{-}Se}$=812 Hz ($CDCl_3$), MS: 712, 714 (M-1)$^-$] was isolated as the mixture of two diastereomers in the ratio 1:1. Its treatment with NaH/CO_2 (Stec, W.J., *Acc. Chem. Res.*, 1983, **16**, 411) in DMF solution gave **7**; ^{31}P NMR, δ: 69.64 and 69.43, $^1J_{P\text{-}Se}$=718 Hz ($CDCl_3$).

(8) Alkylation of **5**, **6** and **7** with **8** was performed in DMF solution at 55°C. Reactions were essentially completed within 2 hrs., as estimated by disappearance of substrates (TLC control).

(9) Verheyden, J.P.H., Moffatt, J.G., *J. Org. Chem.*, 1972, **37**, 2289.

(10) **9** : ^{31}P NMR, δ: 21.27 and 21.45 ($CDCl_3$), $^1J_{P\text{-}Se}$=514 and 516 Hz,resp.; MS FAB: 962, 964 (M-1)$^-$.
10: ^{31}P NMR, δ: 26.40 and 26.54 ($CDCl_3$). Separation of diastereomers on Kieselgel 60 with eluent gradient 0-4% MeOH/ $CHCl_3$. "Fast"-eluted **10**: δ 26.40,$^1J_{P\text{-}Se}$=434 Hz ($CDCl_3$). "Slow"-eluted **10**: δ 26.54, $^1J_{P\text{-}Se}$=438 Hz($CDCl_3$). MS FAB:725,727(M-Px+Cs^{2+})(LSIMSCs^{2+},13keV).
11: ^{31}P NMR, δ: 49.71 and 49.93 $^1J_{P\text{-}Se}$=417 and 422 Hz($CDCl_3$), MS FAB: 861, 863 (M-1)$^-$;
12: ^{31}P NMR, δ: 19.87 (DMSO-d_6), $^1J_{P\text{-}Se}$=700 Hz, MS FAB: 609, 611 (M-1)$^-$, UV λ_{min} 240, λ_{max} 266. **13**: ^{31}P NMR, δ: 26.93 (for "Fast"-eluted) and 27.43 (for "Slow"-eluted).
MS FAB: 619, 621 (M-1)$^-$ **14**: ^{31}P NMR, δ: 50.29 and 50.47 ($CDCl_3$), $^1J_{P\text{-}Se}$=420 Hz.
MS FAB: 603, 605(M-1)$^-$.

(11) Stec W.J., Okruszek A., Uznanski B., Michalski J., *Phosphorus*, 1972, **2**, 97.

(12) Cations of heavy metals (Hg^{2+} or Ag^+ but not Zn^{2+}) accelerated decomposition of **12** in water.

(13) Enzymatic digestions were performed at 37°C: SVPD - 0.1M TRIS-HCl, 15 mM $MgCl_2$, pH=8.5; Spleen Bovine PD - 0.05M AcONa, pH=5.0, and Nuclease P1 - 0.1M TRIS-HCl, 1mM $ZnCl_2$, pH=6.5. The enzyme concentrations were approx. 10 times higher than usually used for degradation of phosphorothioate oligonucleotides.

(14) Compound **15** [MS FAB: 609, 611 (M-1)$^-$; UV λ_{min} 239 nm, λ_{max} 265 nm] was prepared in reaction of 5'-deoxy 5'-bromothymidine with a mixture of KOH/hydrazine and elemental selenium in water, in analogy to: Jakiwczyk O.M., Kristoff E.M., McPhee D.J., *Synth. Communications*, 1993, **23**, 195.

(15) Mitsuya, H., Weinhold, K.J., Furman, P.A., St.Clair, M.H., Lehrman, S.N., Gallo, R.C., Bolognesi, D., Barry, D.W., and Broder, S., *Proc. Natl. Acad. Sci. USA*, 1985, **82**, 7096.

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(17) Veres, Z., Tsai, L., Scholz, T.D., Politino, M., Balaban, R.S., Stadtman, T.C., *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 975.

(*Received* 30 *September* 1993)