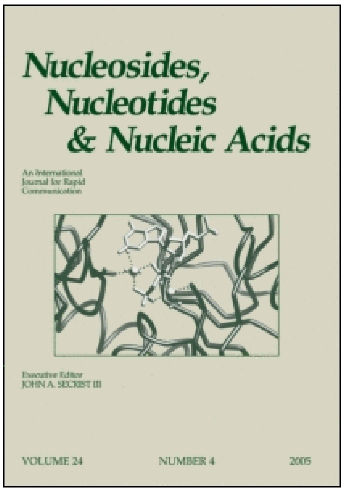

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Preparation of Nucleoside H-Phosphonoselenoate Monoesters Via the Phosphinate Approach

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PREPARATION OF NUCLEOSIDE H-PHOSPHONOSELENOATE MONOESTERS VIA THE PHOSPHINATE APPROACH

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□ *An efficient entry to nucleoside 3′-H-phosphonoselenoate monoesters via phosphinate intermediates was developed. It involves a reaction of suitably protected nucleosides with triethylammonium phosphinate in the presence of pivaloyl chloride, followed by selenization of the intermediate nucleoside phosphinates with triphenylphosphine selenide, to produce the corresponding nucleoside H-phosphonoselenoates in 86–92% yields.*

Keywords H-Phosphonoselenoate monoesters; H-Phosphinates; H-Phosphonate approach; Triphenylphosphine selenide; Triphenyl phosphoroselenoate

INTRODUCTION

As part of a program on the development of synthetic methods based on H-phosphonate chemistry,[1] we recently embarked on investigations of nucleoside H-phosphonoselenoates,[2,3] as novel analogues of H-phosphonate monoesters that may be useful synthetic intermediates for the preparation of biologically important nucleotide analogues. Although toxicity of selenium may pose some limitation on therapeutic applications of selenophosphate derivatives,[4–6] oligonucleotides containing selenium are finding growing applications in X-ray studies on oligonucleotide-protein complexes due to multiwavelength anomalous dispersion (MAD) of selenium.[7–9] In this respect, nucleoside H-phosphonoselenoate monoesters may provide alternative substrates for the preparation of selenium-labelled biopolymers.

In honor and celebration of the life and career of John A. Montgomery.

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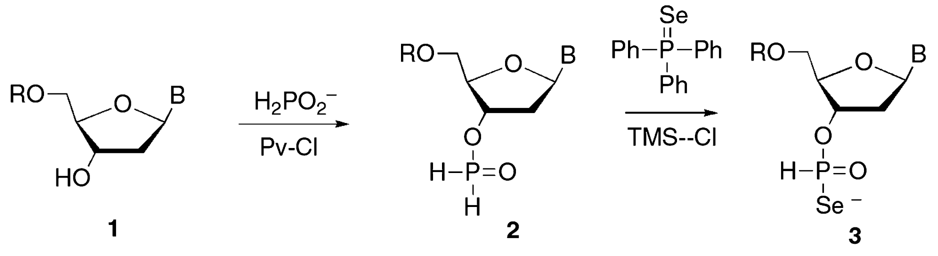
A unique feature of H-phosphonoselenoates is that being P(III) derivatives, they already bear selenium, and thus their use in the synthesis of phosphorus compounds should permit preparation of selenophosphate analogues with multiple modifications at the phosphorus center, e.g., phosphorodiselenoates, phosphorothioselenoates, phosphoroselenofluoridates. Such analogues are usually difficult to prepare via selenization of the corresponding P(III) precursors, which is a common procedure for the synthesis of selenophosphate derivates.[4,5,10–12]

RESULTS AND DISCUSSION

As a possible synthetic method for the preparation of nucleoside H-phosphonoselenoates **3** we considered a phosphinate approach, analogously to that described previously for the corresponding thio analogues.[13] This approach consists of in situ generation of a nucleoside phosphinate intermediate of type **2**, which upon oxidation with elemental sulfur affords the corresponding H-phosphonothioate monoesters in high yields.

Using analogous reaction conditions to those previously developed for the thio analogues (1 equiv. of triethylammonium phosphinate, 1.5 equiv. of nucleoside **1a**, and 1 equiv. of pivaloyl chloride in pyridine),[13] the corresponding phosphinate intermediates **2a** was produced almost quantitatively, as it was apparent from a complete disappearance of a triplet from $H_2PO_2^-$ at $\delta_P = 2.85$ ppm in the ^{31}P NMR spectrum ($^1J_{PH} = 517$ Hz) and formation of a triplet of doublets due to **2a** at $\delta_P = 14.22$ ppm ($^1J_{PH} = 572$ Hz and $^3J_{PH} = 9.8$ Hz). Unfortunately, in contradistinction to sulfurization, a selenization of this type of an intermediate with elemental selenium (3 equiv.) turned out to be a rather difficult task, partly due to lower reactivity/solubility of selenium under the reaction conditions, and partly because of more pronounced instability of the H-phosphonoselenoate monoesters **3** formed. Although initially the reaction appeared to be clean (after ca. 30 min, only formation of the desired H-phosphonoselenoate **3a**; two signals at $\delta_P = 48.2$ and 47.2 ppm with characteristic satellite bands due to phosphorus-selenium coupling, $^1J_{PSe} = 713$ Hz), after 4 h ^{31}P NMR spectroscopy revealed formation of several side products that amounted to ca 60% of the phosphorus-containing compounds (various oxidized products, e.g., phosphonic acid, H-phosphonate mono- and diesters, symmetrical phosphoroselenoate diesters [$\delta_P = 53.5$ ppm, $^1J_{PSe} = 820$ Hz] and symmetrical phosphorodiselenoate diesters [$\delta_P = 87.8$ ppm, $^1J_{PSe} = 759$ Hz]).[14] However, when the analogous reaction was carried out with ethanol as a hydroxylic component, the selenization was complete within 5 min and the reaction was much cleaner (ca. 90% of ethyl H-phosphonoselenoate formed; $\delta_P = 50.6$ ppm, $^1J_{PH} = 575$ Hz and $^3J_{PH} = 10.0$ Hz, dt; $^1J_{PSe} = 720$ Hz).



R = 4,4'-dimethoxytrityl; TPPSe = triphenylphosphine selenide
1a-3a, B = thymin-1-yl
1b-3b, B = N^4-benzoylcytosin-1-yl
1c-3c, B = N^6-benzoyladenin-9-yl
1d-3d, B = N^2-isobutylguanin-9-yl

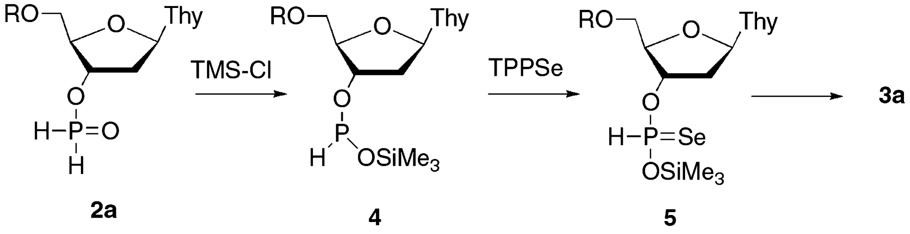
SCHEME 1

Attempted selenization of nucleoside phosphinate intermediates **2** with various selenium-transferring reagents soluble in organic solvents (e.g., 3*H*-benzothiaselenol-3-one,[15] potassium selenocyanate[16]) failed to produce appreciable amounts of the desired H-phosphonoselenoates **3**. Instead, the major products of these reactions were symmetrical dinucleoside phosphoroselenoate diesters, and nucleoside H-phosphonate monoesters and diesters.

Fortunately, triphenylphosphine selenide (TPPSe)[17] in combination with trimethylsilyl chloride (TMS-Cl) turned out to be an excellent source of electrophilic selenium for our purpose by providing the desired reactivity together with high solubility in organic solvents. According to the developed protocol, the syntheses of nucleoside H-phosphonoselenoates **3** were carried out as “one pot” reactions in chloroform-pyridine (3:1, v/v) using TPPSe (2 equiv.) and TMS-Cl (3 equiv.) for the selenization step. The method appeared to be rather general and consistently afforded nucleoside H-phosphonoselenoates **3** in high yields and of purity >98% (^{1}H NMR spectroscopy).

TMS-Cl was found to be an indispensable reaction component and its beneficial effect on this reaction was probably due to securing anhydrous reaction conditions and thus preventing hydrolysis of the phosphinate intermediates by spurious water, on one hand, and by facilitating selenization of intermediates **2** via formation of tervalent silyl species, on the other one.

The latter possibility was substantiated by monitoring selenization of phosphinate intermediate **2a** by ^{31}P NMR spectroscopy (Scheme 2). It was found that in the presence of TMS-Cl, the generated in situ thymidine 3′-phosphinate intermediate **2a** ($\delta_P = 14.22$ ppm, $^1J_{\mathrm{PH}} = 572$ Hz, t) was immediately converted into a compound resonating at $\delta_P = 149.95$ and



2a **4** **5**

R = 4,4'-dimethoxytrityl; TPPSe = triphenylphosphine selenide

SCHEME 2

148.51 ppm, ${}^{1}J_{\mathrm{PH}} = 356$ and 349 Hz, respectively). These signals were tentatively assigned on the basis of the chemical shift values and the splitting pattern in the phosphorus-hydrogen coupled spectra, to two diastereomers of one-phosphorus spin system of silyl phosphonite **4**. The addition of TPPSe to such reaction mixture resulted in rapid selenization of **4** to afford presumably trimethylsilyl H-phosphonoselenoate **5** ($\delta_P = 55.35$ and 56.22 ppm; ${}^{1}J_{\mathrm{PH}} = 642$ Hz, ${}^{3}J_{\mathrm{PH}} = 12.8$ Hz and ${}^{1}J_{\mathrm{PH}} = 639$ Hz, ${}^{3}J_{\mathrm{PH}} = 12.4$ Hz, respectively; dt). This upon hydrolysis provided thymidine 3′-H-phosphonoselenoate **3a** ($\delta_P = 48.2$ and 47.2 ppm).

Somewhat unexpectedly, the attempted replacement of TPPSe by a less reactive selenium-transferring reagent, triphenyl phosphoroselenoate[18] (TPOPSe), did not result in the desired silyl phosphoroselenoate **5** formation. ^{31}P NMR spectroscopy indicated that rapid decomposition of the silyl phosphonite intermediate **4** under the reaction conditions was faster than the selenization reaction. We assumed that the silylated intermediate **4** should be more stable in the absence of chloride anions and used instead of TMS-Cl, *O*,*N*-bis(trimethylsilyl)acetamide (BSA) for its generation. Indeed, ^{31}P NMR spectroscopy revealed that this time the key intermediate **4** was stable for at least 30 min under the reaction conditions and could be efficiently selenized with TPOPSe. When applied to preparative syntheses [the reaction in CH_3CN/pyridine (3:1, v/v); for the selenization step, 3 equiv. BSA and 3 equiv. TPOPSe, 20 min), this alternative protocol afforded nucleoside H-phosphonoselenoates **3** in comparable yields to those obtained using TPPSe/TMS-Cl as a selenium-transferring system.

In conclusion, a reaction of suitably protected nucleosides with triethylammonium phosphinate promoted by pivaloyl chloride, followed by selenization of the generated nucleoside phosphinate intermediates with TPPSe in the presence of TMS-Cl, provides a convenient and general method for the preparation of nucleoside H-phosphonoselenoate monoesters in high yields, starting from inexpensive, commercial available materials. Alternatively, for the selenization step, BSA/TPOPSe reagent system can also be used. Nucleoside H-phosphonoselenoates **3** as solids can be stored for months at −20°C without noticeable decomposition.

EXPERIMENTAL

^{1}H- and ^{31}P NMR spectra were recorded on a Varian-300 FT spectrometer. ^{1}H NMR spectra were referenced to the internal TMS signal and for ^{31}P NMR spectra 2% H_3PO_4 in D_2O was used as an external standard (coaxial inner tube). TLC was carried out on Merck silica gel 60 F_{254} or Kieselgel 60 F_{254} silanized precoated plates using chloroform/methanol 9:1 (v/v) (system A). Pyridine was refluxed with CaH_2 overnight and then distilled and stored over molecular sieves (4Å) or CaH_2. Pivaloyl chloride (Aldrich) and 50% aq. phosphinic acid (BDH) were commercial grade. Suitably protected deoxyribonucleosides were prepared by standard methods.[19] Anhydrous triethylammonium phosphinate was prepared by neutralization of 50% aq. phosphinic acid with triethylamine, followed by repeated evaporation of added pyridine.

General Procedure for the Preparation of 5′-*O*-Dimethyxytritylnucleoside 3′-H-Phosphonoselenoate Monoesters, Triethyl Ammonium Salts 3

A suitably protected deoxynucleoside (0.75 mmol, 1.5 equiv.) and triethyl ammonium phosphinate (0.5 mmol, 1 equiv.) were rendered anhydrous by evaporation of added pyridine (2 × 10 mL) and then dissolved in chloroform-pyridine (10 mL, 3:1 v/v). This mixture was treated with pivaloyl chloride (0.75 mmol, 1.5 equiv.) for 10 min, and then triphenylphosphine selenide (1 mmol, 2 equiv.) and trimethyl silyl chloride (1.5 mmol, 3 equiv.) were added. After 15 min the reaction mixture was partitioned between sat. aq. $NaHCO_3$ (50 mL) and dichloromethane (150 mL), the organic layer was dried with anhydrous Na_2SO_4, and the solvents evaporated. The residue was purified by silica gel chromatography using EtOAc to wash out the excess nucleoside, and then CH_2Cl_2 containing 5% methanol and 0.5% triethylamine to elute the product. Nucleoside 3′-H-phosphonoselenoate monoesters **3** of purity >98% (^{1}H NMR spectroscopy) were obtained as white foams.

5′-*O*-Dimethoxytrityl-thymidine 3′-*H*-Phosphonoselenoate Triethylammonium Salt (3a). 621 mg Yield 80%. $R_f = 0.30$. HRMS $[M\text{–}TEA]^+$ found: 673.1225; $C_{31}H_{34}N_2O_8PSe$ requires: 673.1218. ^{31}P NMR ($CDCl_3$, δ in ppm, mixture of diastereomers) 48.1 ($^1J_{PH} = 564$ Hz, $^3J_{PH} = 12.2$ Hz, dd; $^1J_{PSe} = 713$ Hz) and 47.1 ($^1J_{PH} = 569$ Hz, $^3J_{PH} = 12.8$ Hz, dd; $^1J_{PSe} = 713$ Hz). ^{1}H NMR ($CDCl_3$, δ in ppm, mixture of diastereomers, selected signals) 8.69 and 8.62 (2d, $^1J_{PH} = 565$ and 569 Hz, 1H, P-H), 6.46 (m, 1H, H1′), 5.42

(m, 1H, H3′), 4.37 and 4.27 (2q, 1H, H4′), 3.58, 3.48 and 3.37 (3dd, 2H, H5′), 2.69, 2.59 and 3.39 (3m, 2H, H2′), 1.38 and 1.36 (2d, J = 1 Hz, 3H, C5-CH$_3$). ^{13}C NMR (CDCl$_3$, δ in ppm, mixture of diastereomers, selected signals) 85.88 and 85.01 (2d, J = 6 Hz, C4′), 84.88 and 84.81 (C1′), 76.74 and 76.13 (2d, J = 5 Hz, C3′), 63.82 and 63.65 (C5′), 40.08 and 39.50 (2d, J = 4 Hz, C2′), 11.75 and 11.69 (2 × C5-CH$_3$).

5′-*O*-Dimethoxytrityl-*N*^{4}-benzoylcytidine 3′-H-Phosphonoselenoate Triethylammonium Salt (3b). 791 mg Yield 92%. R$_f$ = 0.28. HRMS [M–TEA]$^+$ found: 762.1499; $C_{37}H_{37}N_3O_8PSe$ requires: 762.1483. ^{31}P NMR (CDCl$_3$, δ in ppm, mixture of diastereomers) 46.0 ($^1J_{PH}$ = 562 Hz, $^3J_{PH}$ = 12.0 Hz, dd; $^1J_{PSe}$ = 717 Hz) and 44.8 ($^1J_{PH}$ = 573 Hz, $^3J_{PH}$ = 12.0 Hz, dd; $^1J_{PSe}$ = 714 Hz). ^{1}H NMR (CDCl$_3$, δ in ppm, mixture of diastereomers, selected signals) 8.67 and 8.61 (2d, $^1J_{PH}$ = 562 and 573 Hz, 1H, P-H), 6.3 (m, 1H, H1′), 5.41 and 5.24 (2m, 1H, H3′), 4.47 and 4.36 (3m, 1H, H4′), 3.56-3.40 (m, 2H, H5′), 2.3 (m, 2H, H2′). ^{13}C NMR (CDCl$_3$, δ in ppm, mixture of diastereomers, selected signals) 87.61 (C1′), 86.35 and 85.67 (C4′), 75.88 and 74.83 (C3′), 63.13 and 62.95 (C5′), 41.60 and 40.98 (C2′).

5′-*O*-Dimethoxytrityl-N^6-benzoyladenosine 3′-*H*-Phosphonoselenoate Triethyl Ammonium Salt (3c). 710 mg Yield 86%. R$_f$ = 0.37. HRMS [M–TEA]$^+$ found: 786.1588; $C_{38}H_{37}N_5O_7PSe$ requires: 786.1596. ^{31}P NMR (CDCl$_3$, δ in ppm, mixture of diastereomers) 48.6 ($^1J_{PH}$ = 568 Hz, $^3J_{PH}$ = 12.5 Hz dd; $^1J_{PSe}$ = 715.9 Hz) and 48.7 ($^1J_{PH}$ = 567 Hz, $^3J_{PH}$ = 12.5 Hz, dd; $^1J_{PSe}$ = 714.7 Hz). ^{1}H NMR (CDCl$_3$, δ in ppm, mixture of diastereomers, selected signals) 8.7 and 8.65 (2d, $^1J_{PH}$ = 567 and 568 Hz, 1H, PH), 8.7 (s, 1H, H2), 8.16 (s, 1H, H8), 6.6 (m, 1H, H1′), 5.41 and 5.44 (2m, 1H, H3′), 4.43 and 4.52 (2m, 1H, H4′), 3.41 (m, 2H, H5′), 2.94-2.74 (m, 2H, H2′). ^{13}C NMR (CDCl$_3$, δ in ppm, mixture of diastereomers, selected signals) 86.16 and 85.66 (C4′), 85.01 (C1′), 75.94 and 76.51 (C3′), 63.77 (C5′), 40.04 and 39.76 (C2′).

5′-*O*-Dimethoxytrityl-*N*^{2}-isobutyrylguanosine 3′-*H*-Phosphonoselenoate Triethyl Ammonium Salt (3d). 788 mg Yield 91%. R$_f$ = 0.35. [M–TEA]$^+$ found: 768.1715; $C_{35}H_{39}N_5O_8PSe$ requires: 768.1701. ^{31}P NMR (CDCl$_3$, δ in ppm, mixture of diastereomers) 45.5 ($^1J_{PH}$ = 572 Hz, $^3J_{PH}$ = 14.1 Hz, dd; $^1J_{PSe}$ = 703.7 Hz) and 45.0 ($^1J_{PH}$ = 578 Hz, $^3J_{PH}$ = 12.6 Hz, dd; $^1J_{PSe}$ = 707.1 Hz). ^{1}H NMR (CDCl$_3$, δ in ppm, mixture of diastereomers, selected signals) 8.58 and 8.55 ($^1J_{PH}$ = 572 and 578 Hz), 7.80 and 7.79 (H8), 6.2 (m, 1H, H1′), 5.7 (m, 1H, H3′), 4.3 and 4.2 (2m, 1H, H4′), 3.22–3.4 (m, 2H, H5′), 2.6 (m, 2H, H2′), 1.14 and 1.09 (2d, J = 6.7 and 6.7 Hz, CH3 of iBu). ^{13}C NMR (CDCl$_3$, δ in ppm, mixture of diastereomers, selected signals) 85.21 and 84.48 (C4′), 84.62 and 84.34 (C1′), 75.11 and 74.38 (C3′), 63.62 and 63.23 (C5′), 38.74 and 38.52 (C2′).

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