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Facile Synthesis of Oligonucleotide Phosphoroselenoates

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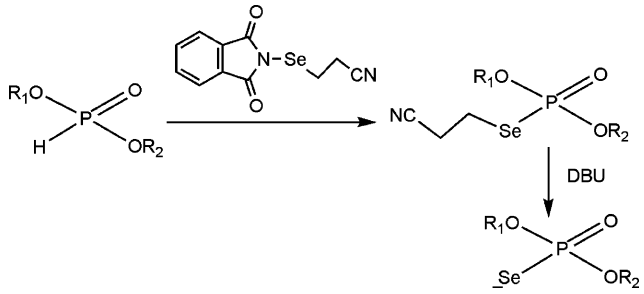
ABSTRACT



***Se*-(2-Cyanoethyl)phthalimide was synthesized from di-(2-cyanoethyl) diselenide. This reagent was found to be an efficient selenium transfer reagent in the synthesis of selenophosphates. Thus, nucleotide H-phosphonate diesters that are formed in situ through the H-phosphonate chemistry undergo quantitative reaction with *Se*-(2-cyanoethyl)phthalamide. The resulting *Se*-(2-cyanoethyl) oligonucleotide phosphoroselenoate triesters are subsequently deprotected to give oligonucleotide phosphoroselenoate diesters in excellent yields.**

Selenium derivatization has proved to be a useful approach in solving the phase problem in X-ray crystallography.[1] One approach of selenium derivatization is to replace a nonbridging oxygen atom of the internucleotide linkages with selenium.[1e,g,i] In the chemical synthesis of oligonucleotide selenophosphate diesters, a general approach involves the oxidative transfer of selenium to P(III) centers, such as phosphite triesters[2] and H-phosphonate diesters.[3] However, these transformations are usually not efficient due to the relatively low reactivity of selenium toward P(III).

A few years ago, Reese and co-workers[4] demonstrated the synthesis of sulfur-transfer reagents and their application in oligonucleotide synthesis using the modified H-phosphonate approach.[5] Their approach has been successfully used in oligonucleotide synthesis, both of natural phosphates and phosphorothioates.[6] We therefore embarked on the investigation of a selenium transfer reagent that is efficient for the synthesis of oligonucleotide phosphoroselenoates via the modified H-phosphonate approach.

We first tested the reaction between *Se*-(2-cyanoethyl)-diselenide **1**, which is readily prepared using the literature procedure,[7] with diethylphosphite in pyridine (Scheme 1). Consumption of diethylphosphite was followed by ^{31}P NMR. After the solution was stirred overnight at room temperature,

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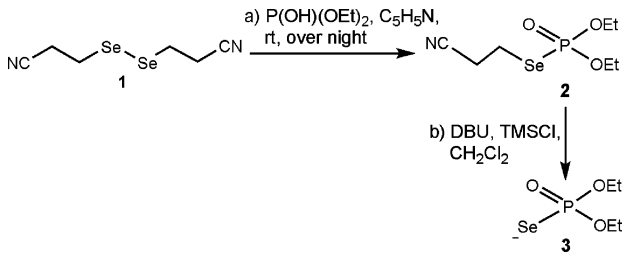
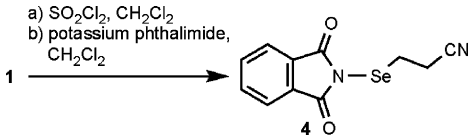
Scheme 1. Reaction of Diethylphosphite with *Se*-(2-Cyanoethyl)diselenide **1**



Se-(2-cyanoethyl)diethylphosphoselenoate triester **2** was isolated by column chromatography. Treatment of this triester **2** with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) gave diethylphosphoroselenoate diester **3** as a single product (Scheme 1).

Although selenium transfer took place with *Se*-(2-cyanoethyl)diselenide **1**, it is not efficient enough to be a useful reagent in this regard. Therefore a more efficient selenium transfer reagent was sought. In the past, *N*-phenylselenophthalimide and *N*-phenylselenosuccinimide have been successfully synthesized.[8] However, synthesis of their *Se*-alkyl analogues has been challenging due to the instability of these compounds. With modification of the literature method,[9] we were able to synthesize *Se*-(2-cyanoethyl)phthalimide **4** from *Se*-(2-cyanoethyl)diselenide **1** (Scheme 2). Thus, 2-cyano-

Scheme 2. Preparation of *Se*-Cyanoethylphthalimide **4**

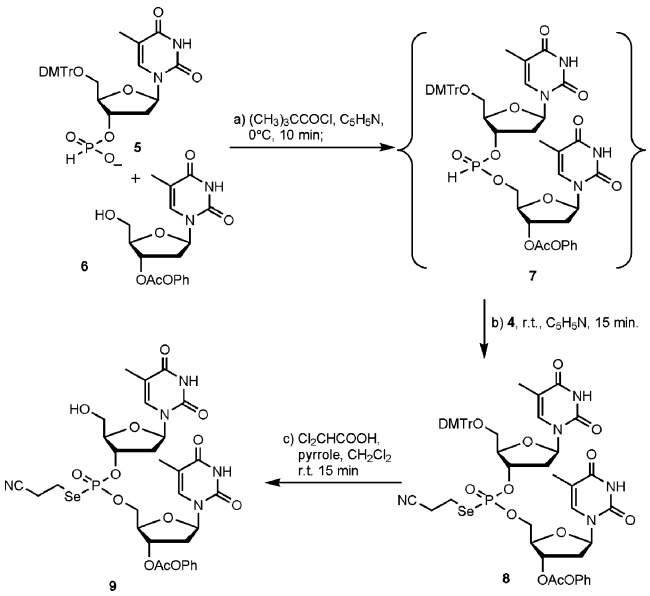


ethyl selenyl chloride, which was generated by treating di-(2-cyanoethyl) diselenide **1** with sulfuryl chloride prior to use, was allowed to react with potassium phthalimide to give *Se*-(2-cyanoethyl)phthalimide **4** as a colorless solid in 65% yield. This material appeared to be very sensitive to oxygen and moisture. However, when stored at −10 °C in an atmosphere of inert gas, such as nitrogen, it is stable over 1 month. It is noted that when preparing NMR solutions of this compound, the procedure should be carried out in a glove box, using deuterated chloroform dried and deoxygenated by conventional procedures.

We then tested the ability of *Se*-(2-cyanoethyl)phthalimide **4** to react with H-phosphonate diesters **7** in the modified H-phosphonate approach (Scheme 3). The coupling reaction and selenium transfer reaction were followed by ^{31}P NMR (Figure 1). Thus, coupling of 5′-*O*-DMTr-thymidine H-

(8) Nicolaou, K. C.; Claremon, D. A.; Barnette, W. E.; Seitz, S. P. *J. Am. Chem. Soc.* **1979**, *101*, 3704.
(9) Kirby, G. W.; Trethewey, A. N. *J. Chem. Soc., Chem. Commun.* **1986**, 1152.

Scheme 3. Synthesis of *Se*-(2-Cyanoethyl) Phosphoroselenoate Triester



phosphonate **5** with 3′-*O*-phenoxyacetylthymidine **6** was effected by pivaloyl chloride to form dinucleotide H-phosphonate diester **7** (7.99 and 9.56 ppm, panel b, Figure 1), which reacted in situ with *Se*-(2-cyanoethyl)phthalimide

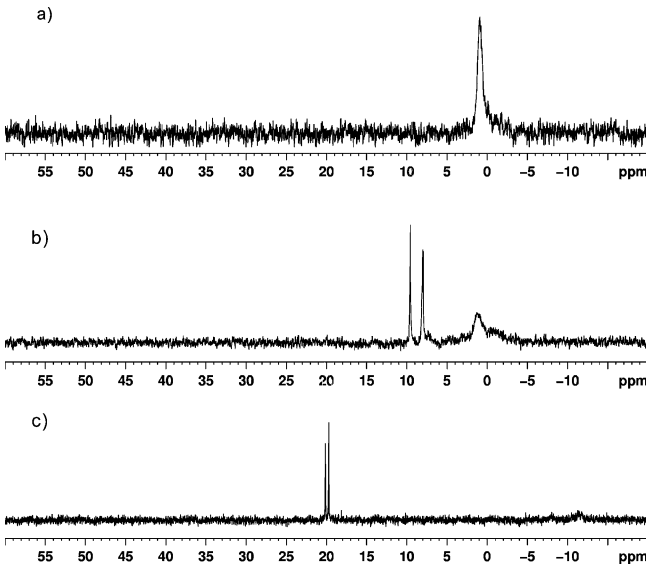


Figure 1. ^{31}P NMR spectra of the reaction described in Scheme 3: (a) H-phosphonate **5**; (b) **5** and **6** in the presence of pivaloyl chloride (step a); and (c) after addition of selenium transfer reagent **1** (step b).

4 to form *Se*-(2-cyanoethyl)phosphoroselenoate triester **8** (19.71 and 20.74 ppm, panel c, Figure 1). The reaction was found to be complete in less than 2 min. On a preparative scale (1 mmol), *Se*-(2-cyanoethyl)phosphoroselenoate triester

8 was isolated in excellent yields (95%). The DMTr-protecting group was readily removed by treatment with dichloroacetic acid in the presence of pyrrole. These phosphoroselenoate triesters are stable compounds when stored at 4 °C.

The *Se*-(2-cyanoethyl)phosphoroselenoate triester **9** was characterized by NMR spectroscopy. The ^{31}P NMR spectrum indicated a mixture of two diastereoisomers, split by coupling with adjacent selenium with a coupling constant of 490 Hz

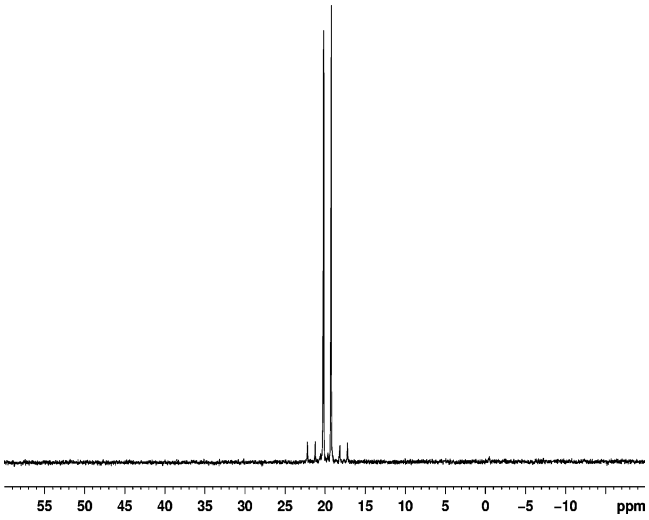


Figure 2. ^{31}P NMR spectrum of *Se*-(2-cyanoethyl) phosphoroselenoate triester **9**.

(Figure 2). The purity of the triester **9** is shown by the reverse-phase HPLC profile (Figure 3).

Removal of the *Se*-cyanoethyl group from the *Se*-(2-cyanoethyl)phosphoroselenoate triester **9** was effected with DBU in the presence of trimethylchlorosilane (Scheme 4). The phenoxyacetyl protecting group was completely removed by incubation with concentrated aqueous ammonium hydroxide for 5 min at room temperature.[10]

After precipitation from diethyl ether containing 1% mercaptoethanol, phosphoroselenoate diester **10** was obtained as the only product as is indicated by the ^{31}P NMR spectrum in Figure 4. Coupling constants of 777 Hz were observed.

These phosphoroselenoate diesters, when dissolved in solution, showed extreme sensitivity toward molecular oxygen. Attempts to precipitate the product from organic solvents was generally accompanied by loss of selenium, which is indicated by the appearance of a new signal at around 0 ppm. The extent of loss of selenium seemed to be related to the capacity of the organic solvent to dissolve molecular oxygen. Thus, when diethyl ether, which is one of the best organic solvents for dissolving molecular oxygen,[11] was used to precipitate the product, up to 50%

(10) Reese, C. B.; Stewart, J. C. M. *Tetrahedron Lett.* **1968**, 4273.
(11) Che, Y.; Tokuchi, K.; Ohsaka, T. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 651.

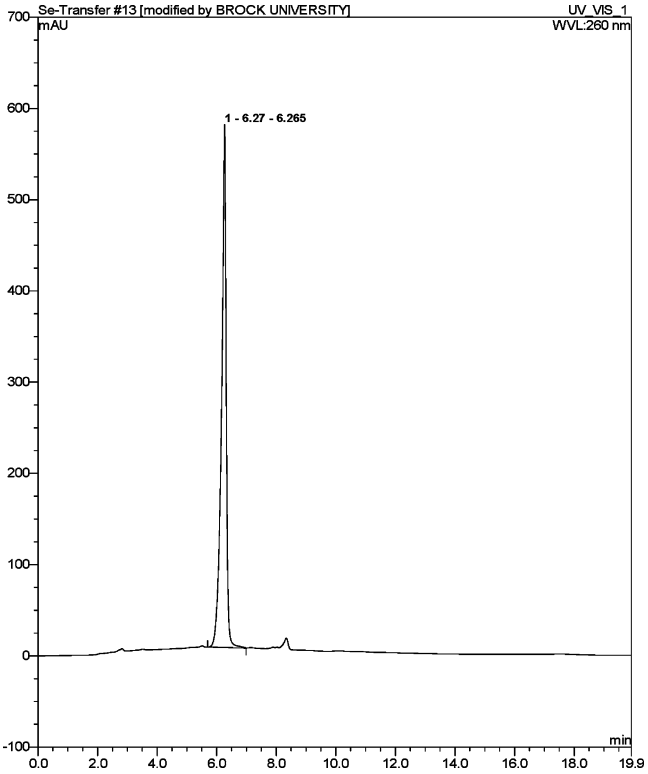
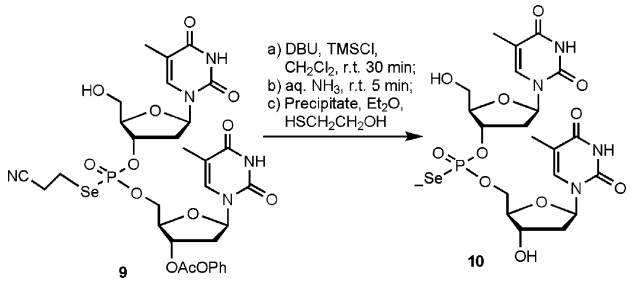


Figure 3. C_{18} HPLC profile of triester **9** (see the Supporting Information for the eluting program).

loss of selenium was observed. When ethyl acetate, which is a moderate solvent for oxygen, was used instead, around 23% loss of selenium was recorded. However, when 1%

Scheme 4. Unblocking of Dinucleotide *Se*-(2-Cyanoethyl) Phosphoroselenoate Triester **9**



mercaptoethanol was added to diethyl ether, loss of selenium was completely suppressed, presumably because mercaptoethanol functioned as an oxygen scavenger.

The choice of protecting group for the hydroxyl of the sugar ring and exocyclic amino group of base residues seemed to be critical. Thus, when a levulinyl group was used to protect the 3′-hydroxyl of thymidine, a concomitant loss of 5% of selenium was observed during incubation with aqueous ammonium hydroxide over 45 min. However, because phenoxyacetyl can be completely removed in 5 min

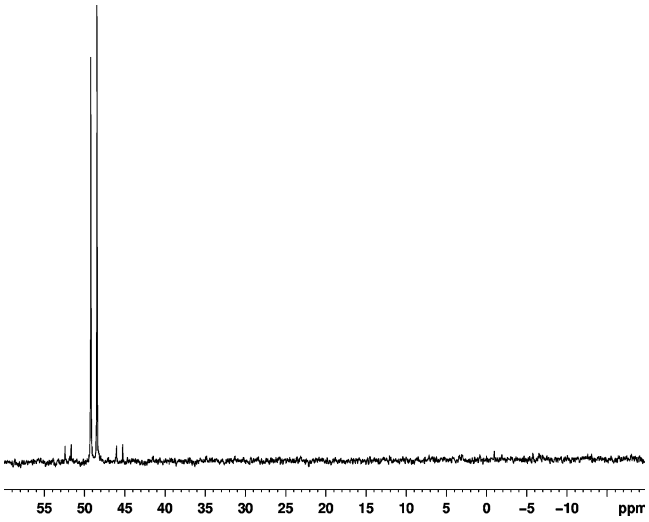
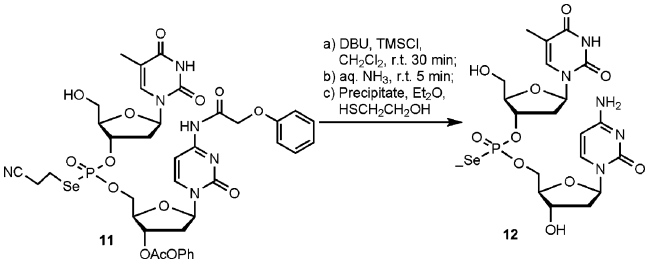


Figure 4. ^{31}P NMR spectrum of phosphoroselenoate diester **10**.

under the same conditions,[10] exposure of the protected substrate to oxygen is minimized so that loss of selenium can be suppressed. This protecting group strategy was extended to the synthesis of Tp(Se)C dimer phosphoroselenoate diester **12** (Scheme 5) successfully.

Scheme 5. Unblocking of Tp(Se)C Dimer Phosphoroselenoate **11**



In summary, we have demonstrated the synthesis of *Se*-(2-cyanoethyl)phthalimide and its use in the synthesis of phosphoroselenoate tri- and diesters. The purity of these dinucleotide phosphoroselenoates **10** and **12** is illustrated by the reverse-phase HPLC profiles as shown in Figure 5. This approach may prove useful in the future preparation of oligonucleotide phosphoroselenoates.

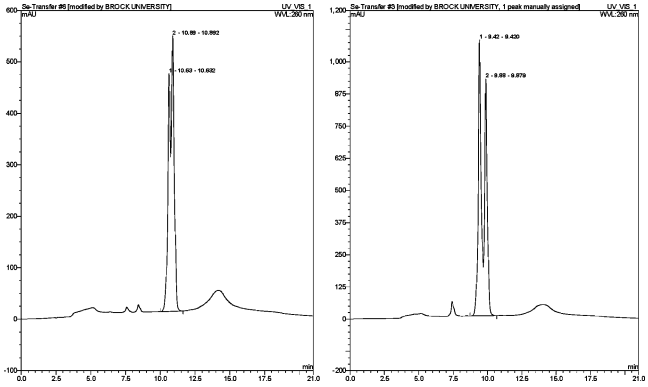


Figure 5. C18 HPLC profiles on **10** (left) and **12** (right). See the Supporting Information for the eluting program.

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Supporting Information Available: Experimental procedures and spectroscopic data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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