Nucleoside H-Phosphonates XX. Efficient Method for the Preparation of Nucleoside H-Phosphonoselenoate Monoesters

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Abstract: The preparation of an H-phosphonoselenoyl group transferring reagent, 9-fluorenemethyl H-phosphonoselenoate, and its application to the synthesis of separate diastereomers of nucleoside 3′-H-phosphonoselenoate monoesters, are described.

Key words: nucleotides, phosphorylation, selenium, protecting groups, elimination

Introduction

A close resemblance of selenium to biologically important element sulfur, constitutes a strong rationale for incorporation of selenium into potential medicinal agents. The toxic nature of most selenium compounds may pose a serious obstacle in drug development but does not *per se* rule out this type of modification for drug use.[1] Kindled by hopes of finding novel, useful properties, selenium has been incorporated into various biologically important compounds, e.g. carbohydrates,[2] lipids,[3,4] nucleosides,[5] oligonucleotides.[6,7]

Selenophosphates are usually prepared via selenization of suitable P(III) precursors, e.g. phosphite triesters,[6,8] H-phosphonate[3,7] and H-phosphonothioate[7] diesters, although derivatives accessible by these routes are usually limited to the corresponding selenophosphate di- and triesters, and selenothiophosphate diesters. Recently, we started to explore a new type of synthetic intermediates, H-phosphonoselenoate monoesters[9,10] that can provide access to new selenophosphate derivatives with double modifications at the phosphorus centre.

In this paper we report on the synthesis and application of a dedicated H-phosphonoselenoyl group-transferring reagent, namely, 9-fluorenemethyl H-phosphonoselenoate, that permits an easy preparation of nucleoside H-phosphonoselenoate monoesters either as diastereomeric mixtures or as separate P-diastereomers. This latter possibility can be potentially exploited in a stereospecific synthesis of P-chiral selenophosphate derivatives.

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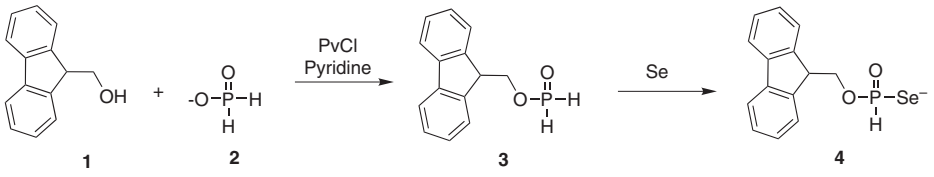
Results and Discussion

Since nucleoside H-phosphonoselenoate monoesters are chiral at the phosphorus centre, access to the separate R_P and S_P diastereomers is required to exploit in full their synthetic potential. Unfortunately, the presence of a negative charge in an H-phosphonoselenoate moiety usually overshadows possible chromatographic differences between isomeric compounds and effectively prevents separation of their P-diastereomers on silica gel.[9] To overcome this problem, we designed a dedicated H-phosphonoselenoyl group transferring reagent, 9-fluorenemethyl H-phosphonoselenoate **4** (Scheme 1), which upon reaction with a hydroxylic component (e.g. a nucleoside) can produce uncharged H-phosphonoselenoate diesters of type **6** (Scheme 2). These are much more easy to separate into diastereomers and after the removal of the fluorenemethyl group, R_P and S_P diastereomers of H-phosphonoselenoate monoesters **7** can be obtained. The fluorenemethyl group in H-phosphonoselenoate diesters **6** acts as a lipophilic handle that facilitates separation of these compounds by a silica gel column chromatography, and since it is removable via a β-elimination mechanism, its deprotection does not affect stereochemical integrity of the phosphorus center. The added value of this approach is that, if separate diastereomers of H-phosphonoselenoates **7** are not required the synthesis can be simplified by subjecting H-phosphonoselenoate diesters **6** to deprotection directly after condensation to afford diastereomeric mixture of the corresponding H-phosphonoselenoate monoesters.

Synthesis of H-Phosphonoselenoyl Group Transferring Reagent 4

9-Fluorenemethyl H-phosphonoselenoate **4**[10] was prepared using standard phosphinate approach previously developed for the synthesis of H-phosphonothioate monoesters.[11] Thus treatment of 9-fluorenemethanol (**1**) with triethylammonium phosphinate (**2**) (^{31}P NMR: δ = 2.85, $^1J_{P,H}$ = 517 Hz, t) in pyridine in the presence of pivaloyl chloride, followed by selenization of the produced phosphinate intermediate **3** (^{31}P NMR: δ = 14.22, $^1J_{P,H}$ = 572 Hz, $^3J_{P,H}$ = 9.8 Hz, tt) with elemental selenium for two hours, produced 9-fluorenemethyl H-phosphonoselenoate **4** as the major product (>90%, ^{31}P NMR: δ = 50.41, $^1J_{P,H}$ = 570 Hz, $^3J_{P,H}$ = 9.1 Hz, dt; $^1J_{P,Se}$ = 685.2 Hz) (Scheme 1). Since reagent **4** in the form of triethylam-



PvCl = pivaloyl chloride

Scheme 1

monium salt was a sticky oil and difficult to handle, it was converted into a *S*-(*p*-chlorobenzyl)isothiuronium salt (88% overall yield). This solid salt was stable, non-hygroscopic and could be used directly for the condensations without necessity of changing the cationic part.

Synthesis of Nucleoside 3′-H-Phosphonoselenoate Monoesters 7

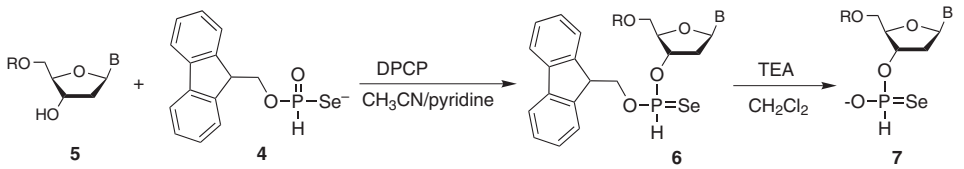
First, reaction conditions for condensation of H-phosphonoselenoate **4** with a hydroxylic component **5** to produce 9-fluorenemethyl derivatives **6** were investigated. Among the condensing agents tried [pivaloyl chloride, 2-chloro-2-oxo-5,5-dimethyl-1,3,2-dioxaphosphinane, diphenyl phosphorochloridate (DPCP)],[12] the best in terms of chemoselectivity, purity of the produced compounds and the shortest reaction time, turned out to be DPCP. With this reagent (2.5 equiv), a model reaction of nucleoside **5a** with H-phosphonoselenoate **4** (1.1 equiv) in acetonitrile containing pyridine (5 equiv) was complete within 10 minutes affording the expected H-phosphonoselenoate **6a** as the sole nucleotidic material [^{31}P NMR: δ = 77.29 ($^{1}J_{P,Se}$ = 870 Hz) and 76.43 ($^{1}J_{PSe}$ = 871 Hz)]. These reaction conditions worked well also for other nucleosides **5** investigated (Scheme 2).

Due to some instability of nucleoside H-phosphonoselenoate diesters **6** in aqueous basic media, the work-up of the reaction mixtures after condensations was critical. Optimal conditions found consisted of quenching the reaction mixtures with water containing pyridine, followed by the dilution with toluene–ethyl acetate, and passing the mixture through silica gel pad. This procedure provided fluorenemethyl nucleoside H-phosphonate **6** of purity >98% (^{1}H NMR spectroscopy) and in consistently high yields.

Removal of a fluorenemethyl group from H-phosphonoselenoates **6** to produce the corresponding H-phosphonoselenoate monoesters **7** was rather straightforward and could be effected quantitatively by treatment of **6** in dichloromethane with triethylamine (TEA) for 1 h. Progress of this reaction can be conveniently monitored by TLC analysis (decrease in mobility on a silica gel upon conversion to **7**) or by ^{31}P NMR spectroscopy. In the latter method, the removal of the fluorenemethyl group from **6** is accompanied by a huge change in the chemical shift from that of ca. 70 ppm for compounds of type **6** to ca. 45 ppm for compounds **7**. Other diagnostic parameters that vary significantly with structure are one-bond phosphorus–selenium ($^{1}J_{P,Se}$) and phosphorus–hydrogen ($^{1}J_{P,H}$) coupling constants (see experimental section).

If separated diastereomers of nucleoside H-phosphonoselenoate monoester **7** would be required, fluorenemethyl derivatives **6** could be separated into R_P and S_P diastereomers via silica gel chromatography prior to the deprotection step. As expected, the removal of the fluorenemethyl group from **6** was completely stereospecific and occurred most likely with retention of configuration.

Although the absolute configurations at the phosphorus centre in compounds **6** and **7** are yet remain to be determined, there seems to be a relationship between the configuration and the observed ^{31}P NMR chemical shift or the mobility on silica gel, as reported previously for other P-chiral nucleotide analogues.[13] For the compounds investigated, chromatographically faster moving diastereomers of **6** always resonated at higher field in the ^{31}P NMR spectra, while those of the lower chromatographic mobility, gave signals at lower field. For H-phosphonoselenoate monoesters **7**, the diastereomers showed almost identical mobility on silica gel, but they differed in the ^{31}P NMR shift values. Since P-diastereomers of **7** resonating at high



5a, **6a**, **7a**, B = thymin-1-yl
5b, **6b**, **7b** B = N^4-benzoylcytosin-1-yl
5c, **6c**, **7c**, B = N^6-benzoyladenin-9-yl
5d, **6d**, **7d**, B = N^2-isobutylguanin-9-yl

R = 4,4'-dimethoxytrityl
DPCP = diphenyl chlorophosphate
TEA = triethylamine

Scheme 2

field were formed 'high field' (or 'fast') diastereomers of **6**, while those at low field, were formed from 'low field' (or 'slow') **6**, these established a configurational relationship between diastereomeric compounds **6** and **7**.

Conclusion

In conclusion, we have developed a dedicated reagent, 9-fluorenemethyl H-phosphonoselenoate **4**, for transferring an H-phosphonoselenoate moiety into hydroxylic components, and used it for a simple preparation in high yields of the separate R_P and S_P diastereomers of nucleoside H-phosphonoselenoate monoesters **7**. The reagent is stable, easy to prepare, and has a fluorenemethyl group as a lipophilic handle that facilitates separation into diastereomers of the intermediate produced, 9-fluorenemethyl nucleoside H-phosphonoselenoate diesters **6**. The fluorenemethyl group can be removed via β-elimination to keeps the integrity of the configuration at the phosphorus intact. In addition, 9-fluorenemethyl nucleoside H-phosphonoselenoates **6**, which are accessible in diastereomerically pure forms, can be considered as potentially useful intermediates on their own for the preparation of various P-chiral phosphoroselenoate monoester analogues.

^{1}H and ^{31}P NMR spectra were recorded on a Varian Unity 400 BB VT spectrometer. The ^{31}P NMR experiments were carried out at 25 °C in 5 mm tubes using 0.1 M concentrations of phosphorus-containing compounds in appropriate solvents (0.6 mL), and the spectra were referenced to 2% H_3PO_4 in D_2O (external standard). TLC analyses were carried out on Merck silica gel 60 F_{254} precoated plates using the following solvent systems: (A) $CHCl_3$–MeOH (9:1); (B) toluene–EtOAc (1:1). Pyridine (LabScan Ltd.) and anhyd MeCN (LabScan Ltd.) were stored over molecular sieves 4Å. The starting materials for the synthesis, suitably protected nucleoside **5** were prepared by modifications of published procedures.[14] Anhyd triethylammonium phosphinate (**2**) was prepared by neutralization of 50% aq phosphinic acid with Et_3N, followed by repeated evaporation of added pyridine.

The assignments of signals in the ^{31}P NMR spectra to particular products or intermediates were done on the basis of their chemical shifts, multiplicity of the signals in ^{1}H-coupled and ^{1}H-decoupled spectra, by spiking the reaction mixtures with appropriate species and, if possible, by isolation of a compound in question from reaction mixtures. The assignment of proton and carbon resonances of **4**, **6** and **7** was done on the basis of known or expected chemical shifts in conjunction with ^{1}H-^{1}H, ^{1}H-^{13}C, and DEPT correlated NMR spectroscopy.

Fluorenemethyl H-Phosphonoselenoate (*S*-Chlorobenzyl)isothiuronium Salt (4)

Fluorenylmethanol (1.0 g, 5.1 mmol, 1.5 equiv) and triethylammonium phosphinate (568 mg, 3.4 mmol, 1 equiv) were rendered anhydrous by coevaporation of added pyridine and then dissolved in pyridine (15 mL). The solution was cooled to 0 °C, pivaloyl chloride (628 μL 5.1 mmol, 1.5 equiv), followed by elemental Se (805 mg, 10.2 mmol, 3 equiv) were added, and the reaction mixture was allowed to attain r.t. After stirring for 3 h, the solution was filtered through Celite onto H_2O (200 μL), the filtrate was concentrated and then dissolved in $CHCl_3$ (100 mL). This solution was washed with H_2O (3 × 50 mL; to the last extraction 200 μL of Et_3N was added to improve the separation of layers), the organic phase was dried (Na_2SO_4), filtered and then concentrated. The residue was co-evaporated with toluene (100 mL) to remove pyridine. The residue was added on top of a silica gel column that had been equilibrated with CH_2Cl_2 containing 1% MeOH and 0.2% Et_3N, and the product was eluted with CH_2Cl_2 containing 1% MeOH. This afforded fluorenemethyl H-phosphonoselenoate **4** (1.32 g, triethylammonium salt) in 96% yield as a sticky, colorless oil. The residue was dissolved in Et_2O (100 mL), and H_2O (100 mL) together with *S*-(4-chlorobenzyl)isothiuronium chloride (1.1 equiv) was added. The H_2O phase was extracted with Et_2O (2 × 100 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated to yield the fluorenemethyl H-phosphonoselenoate *S*-(4-chlorobenzyl)isothiuronium salt as a white foam in 88% overall yield (1.51 g). The foam can be dissolved in minimal amount of $CHCl_3$ and precipitated from pentane to give the product as a white powder (1.32 g) in 77% overall yield; R_f 0.42 (System A).

^{1}H NMR ($CDCl_3$): δ = 8.53 (br, 4 H, NH), 8.49 (d, *J* = 570 Hz, PH), 7.68 (d, *J* = 7.32 Hz, 2 H, H-4 and H-5), 7.56 (dd, *J* = 7.32, 2.54, 2 H, H-1, H-8), 7.33 (t, *J* = 7.32 Hz, 2 H, H-3 and H-6), 7.21 (m, 2 H, H-2 and H-7), 7.15–7.07 (q, 4 H, *J* = 8.42 Hz, ArH of chlorobenzyl), 4.29–4.24 (m, 1 H, H-9), 4.17–4.14 (m, 4 H, 2 CH_2).

^{13}C NMR ($CDCl_3$): δ = 169.94 (C of thiourea), 144.10, 144.02, 141.44 and 141.36 (4 C of fluorenyl), 134.63 (*ipso* C of chlorobenzyl), 131.31 (*para* C of chlorobenzyl), 130.50 and 129.38 (4 CH of chlorobenzyl), 127.85 (C-3 and C-6), 127.26 and 127.20 (C-1 and C-2), 125.36 and 125.31 (C-1 and C-8), 120.06 (C-4 and C-5), 67.41 (C-9), 48.20 (CH_2 at C-9), 35.40 (CH_2).

^{31}P NMR ($CDCl_3$): δ = 44.64 ($^1J_{P,H}$ = 570 Hz, $^1J_{P,Se}$ = 685.2 Hz, $^3J_{P,H}$ = 9.1 Hz).

HRMS: *m/z* calcd for $C_{22}H_{23}ClN_2O_2PSSe$ [M + H]$^+$: 525.0072; found: 525.0066.

Anal. Calcd for $C_{22}H_{22}ClN_2O_2PSSe$: C, 50.44; H, 4.23; N, 5.35. Found: C, 50.14; H, 4.10; N, 5.42.

5′-*O*-Dimethoxytritylnucleoside Fluorenemethyl 3′-H-Phosphonoselenoate Diesters 6; General Procedure

To a solution of 5′-*O*-dimethoxytritylnucleoside **5** (272 mg, 0.5 mmol) and fluorenemethyl H-phosphonoselenoate *S*-(4-chlorobenzyl)isothiuronium salt (**4**; 278 mg, 0.55 mmol, 1.1 equiv) in MeCN (5 mL) containing pyridine (201 μL, 2.5 mmol, 5 equiv), was added diphenyl chlorophosphate (DPCP, 259 μL, 1.25 mmol, 2.5 equiv). After 10 min, the reaction mixture was quenched with H_2O (2 mL) and pyridine (806 μL, 20 equiv), stirred for 10 min and then diluted with EtOAc (50 mL) and toluene (50 mL). The insoluble material was removed and the organic layer was dried (Na_2SO_4). After concentration, the residue was passed through a silica gel pad (3 × 3 cm) and washed with toluene–EtOAc (1:1, 200 mL). This gave the desired products **6** as a mixture of diastereoisomers of purity >98% (^{1}H NMR spectroscopy). Chromatography of the diastereomeric mixture of **6** on a silica gel column using toluene–EtOAc (2:1) as an eluent, afforded separate diastereomers of **6**, designated as 'fast' and 'slow', according to their chromatographic mobility.

5′-*O*-Dimethoxytritylthymidine Fluorenemethyl 3′-H-Phosphonoselenoate Diester (6a)

White solid (1:1 mixture of diastereomers); yield: 357 mg (84%); R_f 0.62 and 0.57 (System B).

HRMS: *m/z* calcd for $C_{45}H_{44}N_2O_8PSe$ [M + H]$^+$: 851.2000; found: 851.2010.

Fast Moving Diastereoisomer of 6a

R_f 0.62 (System B).

^{1}H NMR ($CDCl_3$): δ = 9.19 (s, 1 H, NH), 8.20 (d, *J* = 650 Hz, 1 H, PH), 7.76 (dd, *J* = 7.32, 3.66 Hz, 2 H, H-4 and H-5 of fluorenyl), 7.63 (dd, *J* = 7.01, 2.13 Hz, 2 H, H-1 and H-8 of fluorenyl), 7.45–

7.20 (m, 13 H, ArH), 6.84 (d, *J* = 8.84 Hz, 4 H, ArH *ortho* to OMe) 6.38 (t, *J* = 7.62 Hz, 1 H, H-1′), 5.36 (m, 1 H, H-3′), 4.58–4.40 (m, 2 H, CH_2), 4.25 (t, *J* = 6.40 Hz, 1 H, H-9 of fluorenyl), 4.14 (m, 1 H, H-4′), 3.78 (s, 6 H, 2 CH_3O), 3.41 (m, 2 H, H-5′), 2.3–2.2 (m, 2 H, H-2′), 1.47 (s, 3 H, CH_3 at C-5).

^{13}C NMR ($CDCl_3$): δ = 164.02 (C-4), 159.02 [2 C of dimethoxytrityl (DMT)], 150.62 (C-2), 144.47 (2 C of DMT), 143,16 and 143.03 (2 C of fluorenyl), 135.49, 135.44 and 135.37 (2 C of DMT, C-6), 130.31, 128.39, 128.31, 127.55, 127.51 and 127.44 (13 ArCH), 125.26 (C-1 and C-8 of fluorenyl), 120.45 and 120.42 (C-4 and C-5 of fluorenyl), 113.62 (4 C of DMT), 111.85 (C-5), 87.50 (C of DMT), 85.12 (d, *J* = 5.44 Hz, C-4′), 84.57 (C-1′), 77.79 (C-3′), 69.42 (d, *J* = 8.00 Hz, CH_2 of fluorenyl), 63.29 (C-5′), 55.51 (2 CH_3O), 48.03 (d, *J* = 7.73 Hz, CH of fluorenyl), 39.25 (d, *J* = 4.00 Hz, C-2′), 12.00 (CH_3 at C-5).

^{31}P NMR ($CDCl_3$): δ = 70.89 ($^1J_{P,Se}$ = 870.8 Hz, $^1J_{P,H}$ = 649.5 Hz).

Slow Moving Diastereoisomer of 6a

R_f 0.57 (System B).

^{1}H NMR ($CDCl_3$): δ = 9.49 (s, 1 H, NH), 8.31 (d, *J* = 647 Hz, 1 H, PH), 7.90–7.10 (m, 17 ArH and H-6), 6.85 (d, *J* = 8.84 Hz, 4-H, ArH *ortho* to OMe), 6.47–6.42 (m, 1 H, H-1′), 5.53–5.47 (m, 1 H, H-3′), 4.53–4.43 (m, 2 H, CH_2 of fluorenyl), 4.27–4.15 (m, 1 H, CH of fluorenyl), 4.04 (br, 1 H, H-4′), 3.78 (s, 6 H, 2 CH_3O), 3.40–3.30 (m, 2 H, H-5′), 2.56–2.27 (m, 2 H, H-2′), 1.50 (s, 3 H, CH_3 at C-5).

^{13}C NMR ($CDCl_3$): δ = 164.14 (C-4), 159.03 (2 C of DMT), 150.80 (C-2), 144.40 (2 C of DMT), 143.14 and 142.98 (2 C of fluorenyl), 141.63 and 141.58 (2 C of fluorenyl), 135.50, 135.41 and 135.34 (2 C of DMT and C-6), 130.29, 128.32 and 127.50 (13 ArCH), 125.37 and 125.16 (C-1 and C-8 of fluorenyl), 120.39 (C-4 and C-5 fluorenyl), 113.65 (4 C of DMT), 111.95 (C-5), 87.50 (C of DMT), 85.05 (C-4′), 84.69 (C-1′), 78.37 (C-3′), 69.27 (d, *J* = 6.87 Hz, CH_2 of fluorenyl), 63.19 (C-5′), 55.51 (2 CH_3O), 47.95 (d, *J* = 8.02 Hz, CH of fluorenyl), 39.41 (d, *J* = 5.73, C-2′), 12.06 (CH_3 at C-5).

^{31}P NMR ($CDCl_3$): δ = 71.66 ($^1J_{P,Se}$ = 871.2 Hz, $^1J_{P,H}$ = 647.0 Hz).

5′-*O*-Dimethoxytrityl-*N*^{4}-benzoylcytidine Fluorenemethyl 3′-H-Phosphonoselenoate Diester (6b)

White solid (1:1 mixture of diastereomers); yield: 436 mg (90%); R_f 0.45 and 0.37 (System B).

HRMS: *m*/*z* calcd for $C_{51}H_{47}N_3O_8PSe$ [M + H]$^+$: 940.2266; found: 940.2276.

Fast Moving Diastereoisomer of 6b

R_f 0.45 (System B).

^{1}H NMR ($CDCl_3$): δ = 8.87 (br, 1 H, NH), 8.16 (d, *J* = 649 Hz, 1 H, PH), 8.14 (d, *J* = 7.42 Hz, ArH), 7.89 (d, *J* = 7.42 Hz, 1 H, ArH), 7.73 (t, *J* = 7.42 Hz, 1 H, ArH), 7.61 (d, *J* = 7.42 Hz, 1 H, ArH), 7.58–7.11 (m, 12 H, ArH, H-5 and H-6), 6.84 (d, *J* = 8.79 Hz, 4 H, *ortho* to OMe), 6.28–6.21 (m, 1 H, H-1′), 5.27–5.21 (m, 1 H, H-3′), 4.57–4.39 (m, 2 H, H-4′ and CH of fluorenyl), 3.76 (s, 6 H, 2 CH_3O), 3.43–3.41 (m, 2 H, H-5′), 2.66–2.60 (m, 1 H, H-2′), 2.19–2.08 (m, 1 H, H-2′).

^{13}C NMR ($CDCl_3$): δ = 162.56 (C=O of Bz, C-4 and C-2), 158.99 (2 C of DMT), 144.57 (ArC), 144.21 and 144.19 (2 C of DMT), 143.15 and 143.00 (2 C of fluorenyl), 141.61 and 141.58 (2 C of fluorenyl), 135.39, 135.26 and 135.24 (2 C of DMT and ArC), 133.40 (ArCH), 130.36, 130.30, 129.29, 129.24, 128.33, 127.89 and 127.51 (ArCH, C-6 and C-5), 125.44 and 125.25 (C-1 and C-8 of fluorenyl), 120.42 (ArCH), 113.64 (4 C of DMT), 87.49 (C-1′), 85.78 (C-4′), 69.43 (CH_2 of fluorenyl), 62.64 (C-5′), 55.49 (2 CH_3O), 48.05 (d, *J* = 8.43 Hz, CH of fluorenyl), 40.73 (C-2′).

^{31}P NMR: ($CDCl_3$): δ = 74.57 ($^1J_{P,Se}$ = 872.44 Hz, $^1J_{P,H}$ = 648.9 Hz).

Slow Moving Diastereoisomer of 6b

R_f 0.37 (System B).

^{1}H NMR ($CDCl_3$): δ = 8.69 (br, 1 H, NH), 8.26 (d, *J* = 644 Hz, 1 H, PH), 8.07 (d, *J* = 7.42 Hz, 1 H, ArH), 7.89 (d, *J* = 7.23 Hz, 2 H, ArH), 7.69 (d, *J* = 7.62 Hz, 2 H, ArH), 7.55 (t, 2 H, *J* = 7.42 Hz, 2 H, ArH), 7.51–7.10 (m, 12 H, ArH, H-5 and H-6), 6.80–6.77 (m, 4 H, *ortho* to OMe), 6.26 (t, *J* = 6.44 Hz, 1 H, H-1′), 5.90–5.32 (m, 1 H, H-3′), 4.81–4.40 (m, 1 H, CH_2 of fluorenyl), 4.22–4.12 (m, 3 H, H-4′, CH and CH_2 of fluorenyl), 3.73 (s, 3 H, CH_3O), 3.72 (CH_3O), 3.36–3.31 (m, 2 H, H-5′), 2.83–2.78 (m, 1 H, H-2′), 2.25–2.17 (m, 1 H, H-2′).

^{13}C NMR ($CDCl_3$): δ = 162.39 (C=O of Bz, C-4 and C-2), 158.94 (2 C of DMT), 144.42 (ArC), 144.07 (2 C of DMT), 143.10 and 142.93 (2 C of fluorenyl), 141.55 (2 C of DMT), 135.31 and 135.12 (2 C of DMT), 133.41 (ArCH), 130.36, 130.19, 129.26, 128.28, 127.75 and 127.46 (ArCH, C-5 and C-6), 125.35 and 125.14 (C-1 and C-8 of fluorenyl), 120.33 (ArCH), 113.59 (4 C of DMT), 87.43 (C-1′), 85.72 (C-4′), 69.21 (CH_2 of fluorenyl), 62.58 (C-5′), 55.44 (2 CH_3O), 47.91 (d, *J* = 7.67 Hz, CH of fluorenyl), 40.72 (C-2′).

^{31}P NMR ($CDCl_3$): δ = 75.137 ($^1J_{P,Se}$ = 872.4 Hz, $^1J_{P,H}$ = 644 Hz).

5′-*O*-Dimethoxytrityl-*N*^{6}-benzoyladenosine Fluorenemethyl 3′-H-Phosphonoselenoate Diester (6c)

White solid (1:1 mixture of diastereomers); yield: 433 mg (90%); R_f 0.43 and 0.38 (System B).

HRMS: *m*/*z* calcd for $C_{52}H_{47}N_5O_7PSe$ [M + H]$^+$: 964.2378; found: 964.2389.

Fast Moving Diastereoisomer of 6c

R_f 0.43 (System B).

^{1}H NMR ($CDCl_3$): δ = 9.10 (s, 1 H, NH), 8.71 (s, 1 H, H-2), 8.28 (d, *J* = 648 Hz, 1 H, PH), 8.11 (s, 1 H, H-8), 8.03 (d, *J* = 7.32 Hz, 2 H, ArH), 7.71 (d, *J* = 6.71 Hz, 1 H, ArH), 7.67–7.48 (m, 5 H, ArH), 7.40–7.12 (m, 14 H, ArH), 6.79 (d, *J* = 8.84 Hz, 4 H, *ortho* to OMe), 6.32 (dd, *J* = 8.38, 5.64 Hz, 1 H, H-1′), 5.40–5.33 (m, 1 H, H-3′), 4.62–4.47 (m, 2 H, H-4' and CH of fluorenyl), 3.76 (s, 6 H, 2 CH_3O), 3.39–3.37 (m, 2 H, H-5′), 2.85–2.74 (m, 1 H, H-2′), 2.33–2.24 (m, 1 H, H-2′).

^{13}C NMR ($CDCl_3$): δ = 164.83 (C=O of Bz), 158.84 (2 C of DMT and C-6), 152.87 (C-2), 151.81 (C-6), 149.78 (C-4), 144.56 (2 C of DMT), 143.23 and 142.99 (2 C of fluorenyl), 141.72 (C-8), 141.57 (2 C of DMT), 135.69 and 135.64 (2 C of DMT), 133.87, 133.04, 130.28, 129.12, 128.34, 128.28, 128.18, 128.10, 127.51 and 127.23 (ArCH), 125.22 (C-1 and C-8 of fluorenyl), 120.41 and 120.34 (ArCH), 113.49 (4 C of DMT), 87.05 (C of DMT), 85.38 (d, *J* = 6.58 Hz, C-4′), 84.66 (C-1′), 77.91 (d, *J* = 5.44 Hz, C-3′), 69.20 (d, *J* = 6.30 Hz, CH_2 of fluorenyl), 63.20 (C-5′), 55.47 (2 CH_3O), 48.06 (d, *J* = 7.73 Hz, CH of fluorenyl), 38.88 (C-2′).

^{31}P NMR ($CDCl_3$): δ = 71.07 ($^1J_{P,Se}$ = 872.5 Hz, $^1J_{P,H}$ = 648 Hz).

Slow Moving Diastereoisomer of 6c

R_f 0.38 (System B).

^{1}H NMR ($CDCl_3$): δ = 9.07 (s, 1 H, NH), 8.70 (s, 1 H, H-2), 8.30 (d, *J* = 644 Hz, 1 H, PH), 8.14 (s, 1 H, H-8), 8.02 (d, *J* = 7.01 Hz, 2 H, ArH), 7.72 (dd, *J* = 7.32, 2.74 Hz, 2 H, ArH), 7.70–7.47 (m, 5 H, ArH), 7.41–7.16 (m, 14 H, ArH), 6.76 (d, *J* = 8.84 Hz, 4 H, *ortho* to OMe), 6.44 (dd, *J* = 8.38, 5.64 Hz, 1 H, H-1′), 5.53–5.46 (m, 1 H, H-3′), 4.60–4.50 (m, 1 H, CH_2 of fluorenyl), 4.33–4.15 (m, 3 H, H-4′, CH_2 and CH of fluorenyl), 3.74 (s, 6 H, 2 CH_3O), 3.41–3.30 (m, 2 H, H-5′), 3.00–2.90 (m, 1 H, H-2′), 2.68–2.60 (m, 1 H, H-2′).

^{13}C NMR ($CDCl_3$): δ = 164.81 (C=O), 158.84 (2 C of DMT), 152.87 (C-2), 151.78 (C-6), 149.79 (C-4), 144.48 (2 C of DMT), 143.23 and 143.02 (2 C of fluorenyl), 141.59 (2 C of DMT), 135.62 (2 C of DMT), 133.86, 133.04, 130.23, 129.11, 128.33, 128.18, 128.09,

127.49 and 127.25 (ArCH), 125.34 and 125.13 (C-1 and C-8 of fluorenyl), 120.37 (ArCH), 113.49 (4 C of DMT), 87.09 (C of DMT), 85.42 (C-4′), 84.83 (C-1′), 78.50 (d, J = 6.01 Hz, C-3′), 69.29 (d, J = 6.59 Hz, CH_2 of fluorenyl), 63.07 (C-5′), 55.46 (2 CH_3O), 48.00 (d, J = 7.73 Hz, CH of fluorenyl), 38.96 (C-2′).

^{31}P NMR ($CDCl_3$): δ = 71.05 ($^1J_{P,Se}$ = 875.05 Hz, $^1J_{P,H}$ = 644.9 Hz).

5′-*O*-Dimethoxytrityl-*N*^{2}-isobutyrylguanosine Fluorenemethyl 3′-H-Phosphonoselenoate Diester (6d)

White solid (1:1 mixture of diastereomers); yield: 378 mg (80%); R_f 0.09 and 0.08 (System B).

HRMS: *m*/*z* calcd for $C_{49}H_{49}N_5O_8PSe$ $[M + H]^+$: 946.2484; found: 946.2475.

Fast Moving Diastereoisomer of 6d

R_f 0.09 (System B).

^{1}H NMR ($CDCl_3$): δ = 11.94 (s, 1 H, NH), 8.21 (d, J = 650 Hz, 1 H, PH), 7.99 (s, 1 H, H-8), 7.74–7.57 (m, 4 H, ArH), 7.42–7.12 (m, 13 H, ArH), 6.79–6.73 (m, 4 H, *ortho* to OMe), 5.96 (t, J = 6.56 Hz, 1 H, H-1′), 5.66–5.57 (m, 1 H, H-3′), 4.57–4.42 (m, 2 H, CH_2 of fluorenyl), 4.14–4.10 (m, 1 H, H-4′), 3.75 (s, 6 H, 2 CH_3O), 3.35 (dd, J = 10.82, 3.20 Hz, 1 H, H-5′), 3.20 (dd, J = 10.67, 3.66 Hz, 1 H, H-5′), 2.86–2.76 (m, 1 H, H-2′), 2.18–2.10 (m, 1 H, H-2′), 2.08–1.97 [m, 1 H, CH of IBu (isobutylguanin)], 1.06 (d, J = 7.01 Hz, 3 H, CH_3 of Ibu), 0.93 (d, J = 7.01 Hz, 3 H, CH_3 of Ibu).

^{13}C NMR ($CDCl_3$): δ = 178.40 (C=O), 158.90 (2 C of DMT), 155.61 (C-6), 147.86 (C-3), 147.28 (C-4), 144.81 (2 C of DMT), 143.11 and 142.95 (2 C of fluorenyl), 141.70 (2 C of DMT), 138.45 (C-8), 135.95 and 135.68 (2 C of DMT), 130.22, 129.26, 128.28, 128.19, 127.47, 127.28 and 127.17 (ArCH), 120.43 and 120.37 (ArCH), 113.44 (4 C of DMT), 86.70 (C of DMT), 84.30 (C-1′ and C-4′), 69, 32 (CH_2 of fluorenyl), 62.77 (C-5′), 55.47 (2 CH_2O), 48.00 (d, J = 8.00 Hz, CH of fluorenyl), 38.04 (C-2′), 36.57 (CH of Ibu), 19.98 and 18.89 (2 CH_3 of Ibu).

^{31}P NMR ($CDCl_3$): δ = 71.59 ($^1J_{P,Se}$ = 869.08 Hz, $^1J_{P,H}$ = 649.6 Hz).

Slow Moving Diastereoisomer of 6d

R_f 0.08 (System B).

^{1}H NMR ($CDCl_3$): δ = 11.98 (s, 1 H, NH), 8.24 (d, J = 645 Hz, 1 H, PH), 8.23 (s, 1 H, H-8), 7.75–7.13 (m, 17 H, ArH), 6.78–6.71 (m, 4 H, *ortho* to OMe), 6.09 (dd, J = 7.62, 5.79 Hz, 1 H, H-1′), 5.61–5.53 (m, 1 H, H-3′), 4.53–4.43 (m, 1 H, CH_2 of fluorenyl), 4.25–4.09 (m, 3 H, H-4′, CH_2 and CH of fluorenyl), 3.72 (s, 3 H, OCH_3), 3.71 (s, 3 H, OCH_3), 3.34 (dd, J = 10.67, 3.05 Hz, 1 H, H-5′), 3.18 (dd, J = 10.52, 3.81 Hz, 1 H, H-5′), 3.06–2.96 (m, 1 H, H-2′), 2.53–2.44 (m, 1 H, H-2′), 2.20–2.03 (m, 1 H, CH of Ibu), 1.06 (d, J = 6.71 Hz, 3 H, CH_3 of Ibu), 0.94 (d, J = 6.71 Hz, 3 H, CH_3 of Ibu).

^{13}C NMR ($CDCl_3$): δ = 178.62 (C=O), 158.91 (2 C of DMT), 155.67 (C-6), 148.07 (C-3), 147.45 (C-4), 144.75 (2 C of DMT), 143.05 and 142.96 (2 C fluorenyl), 141.58 (2 C of DMT), 138.19 (C-8), 135.88 and 135.67 (2 C of DMT), 130.18, 129.27, 128.37, 128.32, 128.207, 127.46, 127.32, 127.28 and 125.04 (ArCH), 120.39 (ArCH), 113.48 (4 C of DMT), 86.74 (C of DMT), 84.77 (d, J = 4.58 Hz, C-4′), 84.57 (C-1′), 69.25 (d, J = 6.30 Hz, CH_2 of fluorenyl), 62.93 (C-5′), 55.47 (2 CH_3), 47.94 (d, J = 7.73 Hz, CH of fluorenyl), 38.67 (C-2′), 36.50 (CH of Ibu), 18.99 (CH_3 of Ibu), 18.90 (CH_3 of Ibu).

^{31}P NMR ($CDCl_3$): δ = 71.43 ($^1J_{P,Se}$ = 871.64 Hz, $^1J_{P,H}$ = 645 Hz).

5′-*O*-Dimethoxytritylnucleoside 3′-H-Phosphonoselenoate, Triethylammonium Salts 7; General Procedure

Separated diastereomers of nucleoside fluorenemethyl 3′-H-phosphonoseleonate diesters **6** (425 mg, 0.5 mmol) were dissolved in CH_2Cl_2 (15 mL) and treated with Et_3N (695 μL, 5 mmol, 10 equiv) for 1 h. Nucleoside 3′-H-phophonoselenoate monoesters **7** were isolated by precipitation from pentane–Et_2O (2:1) as white solids of purity >98% (^{1}H NMR spectroscopy).

5′-*O*-Dimethoxytritylthymidine 3′-H-Phosphonoselenoate, Triethylammonium Salt (7a)

7a Fast

From faster moving diastereoisomer of **6a**; white solid; yield: 71 mg (74%); R_f 0.30 (System A).

^{1}H NMR ($CDCl_3$): δ = 11.56 (br, 1 H, NH), 9.38 (s, 1 H, NH), 8.61 (d, J = 570.1 Hz, 1 H, PH), 7.59 (d, J = 0.92 Hz, 1 H, H-6), 7.42–7.16 (m, 10 ArH), 6.83–6.79 (m, 4 ArH *ortho* to OMe), 6.44 (dd, J = 8.84, 5.83 Hz, 1 H, H-1′), 5.48–5.41 (m, 1 H, H-3′), 4.25–4.24 (m, 1 H, H-4′), 3.76 (s, 6 H, 2 CH_3O), 3.46 (dd, J = 10.52, 2.59 Hz, 1 H, H-5′), 3.36 (dd, J = 10.52, 2.59 Hz, 1 H, H-5′), 3.16–3.06 (m, 6 H, 3 CH_2 of Et_3N), 2.70–2.34 (m, 2 H, H-2′), 1.37–1.28 (m, 12 H, 3 CH_3 of Et_3N and CH_3 at C-5).

^{13}C NMR ($CDCl_3$): δ = 164.24 (C-4), 158.89 (2 C of DMT), 150.80 (C-2), 144.62 (2 C of DMT), 135.96, 135.75 and 135.57 (2 C of DMT and C-6), 130.37, 128.42, 128.23 and 127.26 (8 C of DMT), 113.54 (4 C of DMT), 111.46 (C-5), 87.27 (C of DMT), 85.11 (C-4′), 84.86 (C-1′), 76.23 (d, J = 4.87 Hz, C-3′), 63.93 (C-5′), 55.48 (2 CH_3O), 45.95 (3 CH_2 of Et_3N), 40.12 (C-2′), 11.86 (CH_3 at C-5), 8.87 (3 CH_3 of Et_3N).

^{31}P NMR ($CDCl_3$): δ = 44.785 (dd, $^1J_{P,Se}$ = 714.8 Hz, $^1J_{P,H}$ = 570.3 Hz, $^3J_{P,H}$ = 12.57 Hz).

Anal. Calcd for $C_{37}H_{48}N_3O_8PSe$: C, 57.51; H, 6.26; N, 5.44. Found: C, 57.33; H, 6.08; N, 5.51.

7a Slow

From slower moving diastereoisomer of **6a**; white solid; yield: 80 mg (98%); R_f 0.30 (System A).

^{1}H NMR ($CDCl_3$): δ = 11.57 (br, 1 H, NH), 9.28 (br, 1 H, NH), 8.69 (d, J = 564.7 Hz, 1 H, PH), 7.60 (br, 1 H, H-6), 7.42–7.14 (m, 10 ArH), 6.83–6.80 (m, 4 H, *ortho* to OMe), 6.47 (dd, J = 8.54, 5.49 Hz, 1 H, H-1′), 5.41–5.35 (m, 1 H, H-3′), 4.36 (br, 1 H, H-4′), 3.76 (s, 6 H, 2 CH_3O), 3.57–3.34 (m, 2 H, H-5′), 3.12–3.05 (m, 6 H, 3 CH_2 of Et_3N), 2.60–2.31 (m, 2 H, H-2′), 1.38–1.27 (m, 12 H, 3 CH_3 of Et_3N and CH_3 at C-5).

^{13}C NMR ($CDCl_3$): δ = 164.21 (C-4), 158.88 (2 C of DMT), 150.76 (C-2), 144.67 (2 C of DMT), 135.97, 135.80 and 135.61 (2 C of DMT and C-6), 130.39, 128.55, 128.44, 128.23 and 127.26 (8 C of DMT), 113.55 (4 C of DMT), 111.42 (C-5), 87.28 (C of DMT), 85.93 (d, J = 5.44 Hz, C-4′), 84.94 (C-1′), 63.76 (C-5′), 55.49 (2 CH_3O), 45.93 (3 CH_2 of Et_3N), 39.58 (d, J = 3.48 Hz, C-2′), 11.82 (CH_3 at C-5), 8.87 (3 CH_3 of Et_3N).

^{31}P NMR ($CDCl_3$): δ = 45.69 (dd, $^1J_{P,Se}$ = 714.8 Hz, $^1J_{P,H}$ = 564.7 Hz, $^3J_{P,H}$ = 12.6 Hz).

Anal. Calcd for $C_{37}H_{48}N_3O_8PSe$: C, 57.51; H, 6.26; N, 5.44. Found: C, 57.18; H, 6.15; N, 5.60.

5′-*O*-Dimethoxytrityl-*N*^{4}-benzoylcytidine 3′-H-Phosphonoselenoate, Triethylammonium Salt (7b)

7b Fast

From faster moving diastereoisomer of **6b**; white solid; yield: 65 mg (92%); R_f 0.28 (System A).

^{1}H NMR ($CDCl_3$): δ = 8.60 (d, J = 573 Hz, 1 H, PH), 8.21 (d, J = 7.62 Hz, 1 H, ArH), 7.88 (d, J = 7.62 Hz, 2 H, ArH), 7.61–7.10 (m, 13 H, 11 ArH, H-5 and H-6), 6.87–6.83 (m, 4 H, *ortho* to OMe), 6.30 (t, J = 6.10 Hz, 1 H, H-1′), 5.45–5.37 (m, 1 H, H-4′), 3.78 (s, 6 H, 2 CH_3O), 3.54–3.49 (m, 2 H, H-5′), 3.10–3.02 (m, 6 H, 3 CH_2 of Et_3N), 2.97–2.91 (m, 1 H, H-2′), 2.41–2.29 (m, 1 H, H-2′), 1.33–1.25 (m, 9 H, 3 CH_3 of Et_3N).

^{13}C NMR ($CDCl_3$): δ = 162.27 (C=O of Bz, C-2 and C-4), 158.85 (2 C of DMT), 144.96 and 144.40 (2 C of DMT), 135.71 and 135.50 (2 C of DMT), 133.29 (ArCH), 130.41, 130.34, 129.22, 128.46, 128.25, 127.79 and 127.26 (ArCH), 113.56 (4 C of DMT), 87.54 (C-1′), 85.67 (C-4′), 74.83 (C-3′), 63.11 (C-5′), 55.46 (2 CH_3O), 45.89 (3 CH_2 of Et_3N), 41.58 (C-2′), 8.92 (3 CH_3 of Et_3N).

^{31}P NMR ($CDCl_3$): δ = 44.76 (d, $^1J_{P,Se}$ = 713.5 Hz, $^1J_{P,H}$ = 573 Hz, $^3J_{P,H}$ = 11.93 Hz).

Anal. Calcd for $C_{43}H_{51}N_4O_8PSe$: C, 59.93; H, 5.96; N, 6.50. Found: C, 59.71; H, 5.76; N, 6.81.

7b Slow

From slower moving diastereoisomer of **6b**; white solid; yield: 44 mg (79%); R_f 0.28 (System A).

^{1}H NMR ($CDCl_3$): δ = 8.75 (d, *J* = 562 Hz, 1 H, PH), 8.21 (d, *J* = 7.32 Hz, 1 H, ArH), 7.88 (d, *J* = 7.01 Hz, 2 H, ArH), 7.62–7.17 (m, 13 H, 11 ArH, H-5 and H-6), 6.87–6.83 (m, 4 H, *ortho* to OMe), 6.31 (t, *J* = 6.10 Hz, 1 H, H-1′), 5.28–5.20 (m, 1 H, H-3′), 4.48–4.45 (m, 1 H, H-4′), 3.78 (s, 6 H, 2 CH_3O), 3.56–3.42 (m, 2 H, H-5′), 3.11–3.02 (m, 6 H, 3 CH_2 of Et_3N), 2.92–2.84 (m, 1 H, H-2′), 2.37–2.27 (m, 1 H, H-2′), 1.31–1.25 (m, 9 H, CH_3 of Et_3N).

^{13}C NMR ($CDCl_3$): δ = 162.28 (C=O of Bz, C-2 and C-4), 158.85 (2 C of DMT), 144.95 and 144.41 (2 C of DMT), 135.72 and 135.50 (2 C of DMT), 133.30 (ArCH), 130.42, 130.34, 129.22, 128.46, 128.25, 127.81 and 127.26 (ArCH), 113.57 (4 C of DMT), 87.62 (C-1′), 86.35 (d, *J* = 6.30 Hz, C-4′), 75.88 (C-3′), 62.92 (C-5′), 55.47 (2 CH_3O), 45.88 (3 CH_2 of Et_3N), 40.95 (C-2′), 8.95 (3 CH_3 of Et_3N).

^{31}P NMR ($CDCl_3$): δ = 45.99 ($^1J_{P,Se}$ = 717.3 Hz, $^1J_{P,H}$ = 562 Hz, $^3J_{P,H}$ = 12.15 Hz).

Anal. Calcd for $C_{43}H_{51}N_4O_8PSe$: C, 59.93; H, 5.96; N, 6.50. Found: C, 59.63; H, 5.70; N, 6.72.

5′-*O*-Dimethoxytrityl-*N*^{6}-benzoyladenosine H-Phosphonoselenoate, Triethylammonium Salt (7c)

7c Fast

From faster moving diastereoisomer of **6c**; white solid; yield: 82 mg (88%); R_f 0.37 (System A).

^{1}H NMR ($CDCl_3$): δ = 9.04 (br, 1 H, NH), 8.67 (s, 1 H, H-2), 8.62 (d, *J* = 568 Hz, 1 H, PH), 8.14 (s, 1 H, H-8), 7.96 (d, *J* = 7.62 Hz, 2 H, ArH), 7.54 (t, *J* = 7.32 Hz, 1 H, ArH), 7.45 (t, *J* = 7.52 Hz, 2 H, ArH), 7.36 (d, *J* = 7.22 Hz, 2 H, ArH), 7.27–7.11 (m, 7 H, ArH), 6.73 (d, *J* = 8.79 Hz, 4 H, *ortho* to OMe), 6.56–6.51 (m, 1 H, H-1′), 5.41–5.36 (m, 1 H, H-3′), 4.39–4.38 (m, 1 H, H-4′), 3.71 (s, 6 H, 2 CH_3O), 3.40 (dd, *J* = 10.25, 4.39 Hz, 1 H, H-5′), 3.34 (dd, *J* = 10.25, 3.81 Hz, 1 H, H-5′), 3.11–3.05 (m, 6 H, 3 CH_2 of Et_3N), 2.95–2.83 (m, 1 H, H-2′), 1.30–1.26 (m, 9 H, 3 CH_3 of Et_3N).

^{13}C NMR ($CDCl_3$): δ = 164.79 (C=O), 158.69 (2 C of DMT), 152.76 (C-2), 151.76 (C-6), 149.55 (C-4), 144.73 (2 C of DMT), 141.65 (2 C of DMT), 135.88 (2 C of DMT), 132.92, 132.92, 130.30, 129.04, 128.39, 128.06 and 127.04 (ArCH), 113.37 (4 C of DMT), 86.78 (C of DMT), 85.64 (C-4′), 84.63 (C-1′), 75.99 (C-3′), 63.78 (C-5′), 55.42 (2 CH_3O), 45.82 (3 CH_2 of Et_3N), 40.00 (C-2′), 8.84 (3 CH_3 of Et_3N).

^{31}P NMR ($CDCl_3$): δ = 48.61 (d, $^1J_{P,Se}$ = 715.9 Hz, $^1J_{P,H}$ = 568 Hz, $^3J_{P,H}$ = 12.48 Hz).

Anal. Calcd for $C_{44}H_{51}N_6O_7PSe$: C, 59.66; H, 5.80; N, 9.49. Found: C, 59.38; H, 5.48; N, 9.73.

7c Slow

From slower moving diastereoisomer of **6c**; white solid; yield: 80 mg (96%); R_f 0.37 (System A).

^{1}H NMR ($CDCl_3$): δ = 9.04 (s, 1 H, NH), 8.66 (s, 1 H, H-2), 8.66 (d, *J* = 567 Hz, 1 H, PH), 8.14 (s, 1 H, H-8), 7.97–7.94 (m, 2 H, ArH), 7.53 (t, *J* = 7.32 Hz, 1 H, ArH), 7.45 (t, *J* = 7.41 Hz, 2 H, ArH), 7.36 (d, *J* = 7.81 Hz, 2 H, ArH), 7.27–7.10 (m, 7 H, ArH), 6.73 (d, *J* = 8.79 Hz, 4 H, *ortho* to OMe), 6.56–6.52 (m, 1 H, H-1′), 5.42–5.35 (m, 1 H, H-3′), 4.52–4.48 (m, 1 H, H-4′), 3.70 (s, 6 H, 2 CH_3O), 3.45 (dd, *J* = 10.25, 4.2 Hz, 1 H, H-5′), 3.35 (dd, *J* = 10.25, 3.42 Hz, 1 H, H-5′), 3.09–3.02 (m, 6 H, 3 CH_2 of Et_3N), 2.92–2.84 (m, 1 H, H-2′), 2.78–2.72 (m, 1 H, H-2′), 1.29–1.19 (m, 9 H, 3 CH_3 of Et_3N).

^{13}C NMR ($CDCl_3$): δ = 164.80 (C=O), 158.68 (2 C of DMT), 152.76 (C-2), 151.77 (C-6, 149.55 (C-4), 144.73 (2 C of DMT), 141.65 (2 C of DMT), 135.87 and 135.82 (2 C of DMT), 133.92, 132.92, 130.32 129.64 128.39, 128.05 and 127.04 (ArCH), 113.37 (4 C of DMT), 86.79 (C of DMT), 86.29 (C-4′), 84.98 (C-1′), 76.49 (C-3′), 63.71 (C-5′), 55.41 (2 CH_3O), 45.79 (3 CH_2 of Et_3N), 39.73 (C-2′), 8.81 (3 CH_3 of Et_3N).

^{31}P NMR ($CDCl_3$): δ = 48.66 ($^1J_{P,Se}$ = 714.7 Hz, $^1J_{P,H}$ = 567 Hz, $^3J_{P,H}$ = 12.48 Hz).

Anal. Calcd for $C_{44}H_{51}N_6O_7PSe$: C, 59.66; H, 5.80; N, 9.49. Found: C, 59.47; H, 5.55; N, 9.65.

5′-*O*-Dimethoxytrityl-*N*^{2}-isobutyrylguanosine H-Phosphonoselenoate, Triethylammonium Salt (7d)

7d Fast

From faster moving diastereoisomer of **6d**; white solid; yield: 21 mg, (74%); R_f 0.35 (System A).

^{1}H NMR ($CDCl_3$): δ = 11.98 (s, 1 H, NH), 8.54 (d, *J* = 572 Hz, 1 H, PH), 7.77 (s, 1 H, H-8), 7.37–7.13 (m, 9 H, ArH), 6.75–6.71 (m, 4 H, *ortho* to OMe), 6.13 (dd, *J* = 7.01, 4.88 Hz, 1 H, H-1′), 5.87–5.75 (m, 1 H, H-3′), 4.23–4.18 (m, 1 H, H-4′), 3.74 (s, 6 H, OCH_3), 3.36 (dd, *J* = 10.67, 3.05 Hz, 1 H, H-5′), 3.22 (dd, *J* = 10.67, 4.12 Hz, 1 H, H-5′), 3.14–3.06 (m, 7 H, H-2′ and 3 CH_2 of Et_3N), 2.69–2.59 (m, 1 H, H-2′), 2.55–2.45 (m, 1 H, CH of Ibu), 1.31–1.27 (m, 9 H, 3 CH_3 of Et_3N), 1.14 (d, *J* = 7.01 Hz, 3 H, CH_3 of Ibu), 1.08 (d, *J* = 6.71, 3 H, CH_3 of Ibu).

^{13}C NMR ($CDCl_3$): δ = 179.31 (C=O), 158.67 (2 C of DMT), 156.01 (C-6), 147.87 (C-3), 147.45 (C-4), 144.89 (2 C of DMT), 138.97 (C-8), 136.10 and 135.98 (2 C of DMT), 130.26, 128.39, 127.97 and 127.00 (8 C of DMT), 113.27 (4 C of DMT), 86.44 (C of DMT), 84.39 (C-4′), 74.31 (C-3′), 62.99 (C-5′), 55.44 (2 CH_3O), 45.96 (3 CH_2 of Et_3N), 38.50 (C-2′), 36.39 (CH of Ibu), 19.20 and 19.11 (CH_3 of Ibu), 8.89 (3 CH_3 of Et_3N).

^{31}P NMR ($CDCl_3$): δ = 45.50 ($^1J_{P,Se}$ = 703.7 Hz, $^1J_{P,H}$ = 572 Hz, $^3J_{P,H}$ = 14.07 Hz).

Anal. Calcd for $C_{41}H_{53}N_6O_8PSe$: C, 56.74; H, 6.16; N, 9.68. Found: C, 56.39; H, 6.03; N, 9.88.

7d Slow

From slower moving diastereoisomer of **6d**; white solid; yield: 41 mg (76%); R_f 0.35 (System A).

^{1}H NMR ($CDCl_3$): δ = 8.50 (d, *J* = 578 Hz, 1 H, PH), 7.78 (s, 1 H, H-8), 7.40–7.13 (m, 9 H, ArH), 6.75–6.71 (m, 4 H, *ortho* to OMe), 6.18–6.13 (m, 1 H, H-1′), 5.84–5.74 (m, 1 H, H-3′), 4.36–4.31 (m, 1 H, H-4′), 3.74 (s, 6 H, 2 CH_3O), 3.51–3.35 (m, 2 H, H-5′), 3.09–3.00 (m, 7 H, H-2′ and 3 CH_2 of Et_3N), 2.65–2.46 (m, 2 H, H-2′ and CH of Ibu), 1.34–1.19 (m, 9 H, 3 CH_3 of Et_3N), 0.90–0.84 (m, 6 H, 2 CH_3 of Ibu).

^{13}C NMR ($CDCl_3$): δ = 179.41 (C=O), 158.67 (2 C of DMT), 156.02 (C-6), 147.95 (C-3), 147.55 (C-4), 144.94 (2 C of DMT), 138.78 (C-8), 136.08 (2 C of DMT), 130.36, 128.48, 127.94 and 126.95 (8 C of DMT), 113.24 (4 C of DMT), 86.45 (C of DMT), 85.13 (C-4′), 84.64 (C-1′), 75.08 (C-3′), 63.61 (C-5′), 55.40 (2 CH_3O), 45.95 (3 CH_3 of Et_3N), 38.22 (C-2′), 36.34 (CH of Ibu), 19.21 and 19.07 (CH_3 of Ibu), 8.99 (3 CH_3 of Et_3N).

^{31}P NMR ($CDCl_3$): δ = 44.97 ($^1J_{P,Se}$ = 707.1 Hz, $^1J_{P,H}$ = 578.4 Hz, $^3J_{P,H}$ = 12.57 Hz).

Anal. Calcd for $C_{41}H_{53}N_6O_8PSe$: C, 56.74; H, 6.16; N, 9.68. Found: C, 56.48; H, 6.09; N, 9.77.

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