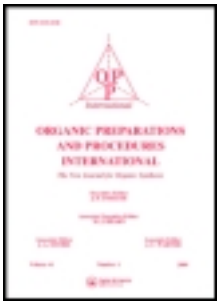

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SYNTHESIS OF HAPTEN PHOSPHORAMIDITES

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SYNTHESIS OF HAPTEN PHOSPHORAMIDITES

Submitted by (3/29/01) M. Adamczyk,* D. D. Johnson, P. G. Mattingly and R. E. Reddy

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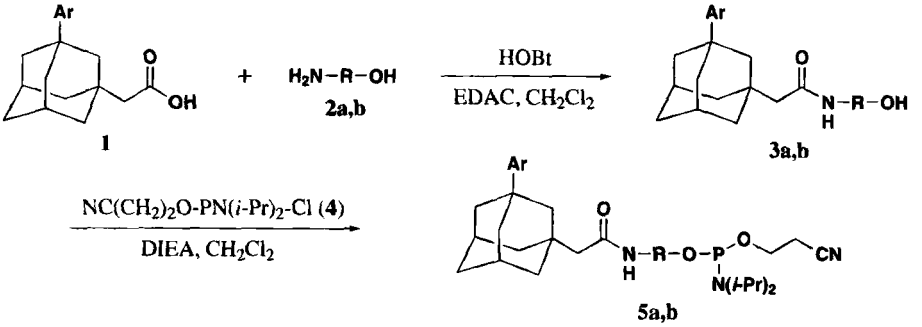
Oligonucleotide probes containing non-radioactive hapten reporter groups are used in amplified nucleic acid testing (NAT) assays that identify sequences of clinical interest in patient samples.[1,2] Structurally diverse haptens and other reporter groups have been efficiently introduced into oligonucleotide probes on automated synthesizers using phosphoramidite chemistry.[3,4] In 1996, we described the synthesis of phosphoramidite probes containing haptens such as adamantane, carbazole and dansyl, in a 1,3-diol framework.[5] These bifunctional reagents were suitable for incorporation at either the 3′ or 5′ end of the oligonucleotide or within the sequence. This flexibility is extraneous if 5′ labeling is all that is required. This paper describes the synthesis of monofunctional phosphoramidites based on the same haptens (adamantane **5a,b**, carbazole **9a,b**, dansyl **12a,b**) but designed exclusively for 5′ labeling. Phosphoramidites with both C-6 aliphatic and solubility-enhancing oxygenated tethers are reported.

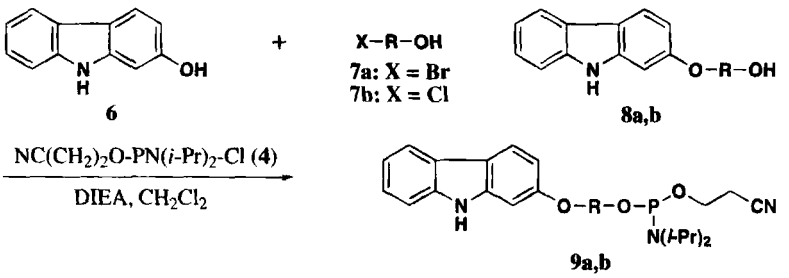
Thus, 2-[3-(4-nitrophenyl)-1-adamantyl]acetic acid (**1**) was coupled with 6-amino-1-hexanol (**2a**) or 2-[(2-aminoethoxy)ethoxy]ethanol (**2b**)[6-8] using HOBt and EDAC in dichloromethane to afford **3a** and **3b** in 80 and 81% yield respectively (*Scheme 1*). The hydroxyl group in **3a** and **3b** was then converted to the 2-(cyanoethyl)-*N,N*-diisopropylphosphoramidite functionality by treatment with (2-cyanoethyl)-*N,N*-diisopropylchlorophosphoramidite (**4**) and *N,N*-diisopropylethylamine (DIEA) to afford the phosphoramidites **5a** and **5b** in 41 and 61% yield, respectively.



Ar = p-$NO_2C_6H_4$ a) R = $(CH_2)_6$ b) R = $(CH_2CH_2O)_2(CH_2)_2$

Scheme 1

The phosphoramidites **9a** and **9b** were prepared in two steps from 2-hydroxycarbazole (**6**) (*Scheme 2*). Thus, alkylation of **6** with 6-bromohexanol (**7a**) or 2-[(2-chloroethoxy)ethoxy]ethanol (**7b**) using anhydrous potassium carbonate and methylethyl ketone (MEK) afforded the hydroxy compounds **8a** and **8b** in 63 and 79% yield, respectively. The compounds **8a** and **8b** were treated with (2-cyanoethyl)-*N,N*-diisopropylchlorophosphoramidite (**4**) and diisopropylethylamine (DIEA) in dichloromethane to afford phosphoramidites **9a** and **9b** in 47 and 55% yield, respectively.

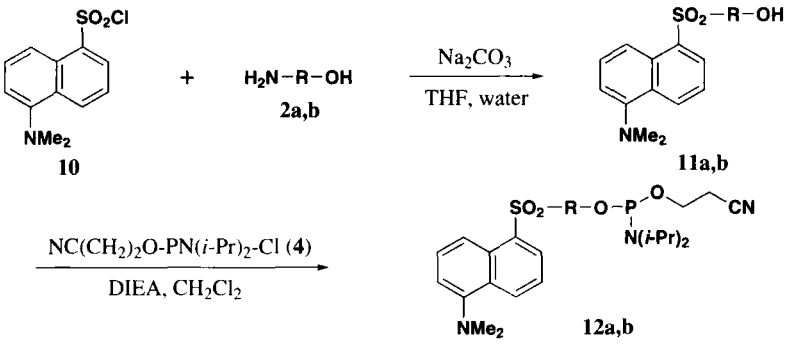


a) R = $(CH_2)_6$ b) R = $(CH_2CH_2O)_2(CH_2)_2$

Scheme 2

Similarly, dansyl chloride (**10**) was treated with amines **2a** or **2b** using excess of sodium carbonate in THF-water to afford **11a** and **11b** in 83-97% yield. Finally, the hydroxy group of **11a** and **11b** was converted to the 2-(cyanoethyl)-*N,N*-diisopropylphosphoramidite derivative by treatment with (2-cyanoethyl)-*N,N*-diisopropylchlorophosphoramidite (**4**) and diisopropylethylamine (DIEA) to afford phosphoramidites **12a** and **12b** in 50 and 79% yield, respectively (*Scheme 3*).

In summary, six hapten phosphoramidites **5a,b; 9ab** and **12a,b** were prepared in good overall yield for use in amplified hybridization assays for detection of nucleic acids.



a) R = $(CH_2)_6$ b) R = $(CH_2CH_2O)_2(CH_2)_2$

Scheme 3

EXPERIMENTAL SECTION

^1H and ^{13}C NMR spectra were recorded on a Varian Gemini spectrometer (300 MHz); the chemical shifts (δ) are reported in ppm relative to TMS; and coupling constants (*J*) are reported in Hz. ^{31}P NMR spectra were recorded using 10% H_3PO_4 in a sealed capillary as an internal standard; the chemical shifts are reported in ppm relative to H_3PO_4. Electrospray ionization mass spectrometry (ESI-MS) was carried out on a Perkin-Elmer (Norwalk, CT) Sciex API 100 Benchtop system employing a Turbo Ionspray ion source; HRMS were obtained on Nermang 3010 MS-50 or JEOL SX102-A mass spectrometers. Thin layer chromatography was performed on pre-coated Whatman MK6F silica gel 60 Å plates (layer thickness: 250 μm), which were pre-run in an appropriate solvent containing Et_3N; visualized with UV light and/or using a 0.2% ninhydrin in EtOH. Column chromatography was performed on silica gel, Merck grade 60 (230–400 mesh). The silica gel was loaded in to the column as a slurry made with 4% Et_3N in hexanes for purification of probes (**5a,b**; **9a,b** and **12a,b**). THF was freshly distilled from a purple solution of sodium and benzophenone under nitrogen. Methylene chloride was freshly distilled from CaH_2 under nitrogen. All reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI) or Sigma Chemical Co. (St. Louis, MO) and used without purification. All the solvents employed were of HPLC grade purchased from EM Science (Gibbstown, NJ) and used as received. Analytical reverse phase (RP) HPLC was performed using a Waters, RCM, Novapak, C18, 6.0 μm, 8 × 100 mm column or Waters, RCM, Symmetry, C18, 7.0 μm, 8 × 100 mm column (solvents ratio v/v reported). Melting points were recorded in open capillary tubes on an Electrothermal Melting Point Apparatus and were uncorrected. IUPAC names were obtained using the ACD/ILab Web service version 3.5 at http://www.acdlabs.com/ilab/.

2-[3-(4-Nitrophenyl)-1-adamantyl]acetic acid (**1**) was prepared from a commercially available 1-adamantylacetic acid according to our published procedure.[5] 0.1 M Aqueous triethylammonium acetate used for analytical reversed phase HPLC was prepared by diluting 200 mL of 2.0 M aqueous triethylammonium acetate to 4.0 L volume with water. The 2.0 M aqueous triethylammonium acetate was prepared by mixing glacial acetic acid (461.4 mL, 8.0 mole, 1.0 equiv.) followed by triethylamine (1115.04 mL, 8.0 mol, 1.0 equiv.) in water (2.0 L). The total volume was made up to 4.0 L with additional water and the mixture was stirred for 30 min and the pH (7.72) was adjusted to 6.83 using glacial acetic acid (about 10 mL).

2-[(2-Aminoethoxy)ethoxy]ethanol Hydrochloride (2b).- Sodium azide (7.31 g, 112.5 mmol, 1.5 equiv.) was added in one portion to a solution of 2-[(2-chloroethoxy)ethoxy]ethanol (**7b**, 12.6 g, 75.0 mmol,) in DMF (150 mL) at room temperature under nitrogen. The mixture was gently heated at 90° (bath temperature) for 24 h and concentrated on a rotary evaporator under vacuum. The residue was diluted with water (100 mL) and CH_2Cl_2 (400 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (100 mL). The combined organic layers were washed with water (75 mL), dried ($MgSO_4$) and concentrated on a rotary evaporator. The crude product was purified by silica gel column chromatography (80% EtOAc in hexanes) to afford 8.28 g (63%) of the corresponding 2-[(2-azidoethoxy)ethoxy]ethanol[7,8] as a pale yellow viscous oil. R_f: 0.28 (80% EtOAc in hexanes). Analytical RP HPLC (Waters, Symmetry): MeCN: 0.1% aq. trifluoroacetic acid/20:80, 2.0 mL/min at 225 nm, t_R: 2.95 min, 97.5%; ^{1}H NMR ($CDCl_3$): δ 3.77–3.61 (m, 10 H), 3.41 (t, 2 H, J = 5.0 Hz), 2.32 (t, 1 H, OH, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$): δ 72.5, 70.6, 70.4, 70.1, 61.8, 50.6; ESI-MS (*m/z*): 176 $(M + H)^+$, 193 $(M + NH_4)^+$, 198 $(M + Na)^+$; HRMS (FAB, *m/z*): calcd for $C_6H_{13}N_3O_3$: 176.1035 $(M + H)^+$; observed, 176.1031.

The 10% Pd/C (0.307 g) was added to 2-[(2-azidoethoxy)ethoxy]ethanol (3.07 g, 17.54 mmol) prepared above in a mixture of MeOH (86 mL) and $CHCl_3$ (18 mL) and hydrogenated (25 psi) at room temperature for 16 h. An additional amount of 10% Pd/C (0.154 g) was added and the hydrogenation was continued at 50 psi pressure for 24 h. The mixture was filtered and the filtrate was concentrated on a rotary evaporator. The residue was dried using a vacuum pump (0.5 mm/Hg) for 24 h to afford 3.33 g (99%) of 2-[(2-aminoethoxy)ethoxy]ethanol (**2b**) isolated as its hydrochloride salt (the HCl salt of **2b** was isolated as pale red viscous oil).[6] ^{1}H NMR ($CDCl_3$): δ 8.14 (br s, 2 H), 3.89–3.63 (m, 10 H), 3.26 (t, 2 H, J = 5.1 Hz), 2.25 (br s, 1 H, OH); ^{13}C NMR ($CDCl_3$): δ 72.3, 70.0, 69.9, 66.7, 60.9, 39.7; ESI-MS (*m/z*): 150 $(M + H)^+$, 172 $(M + Na)^+$.

***N*-(6-Hydroxyhexyl)-2-[3-(4-nitrophenyl)-1-adamantyl]acetamide (3a).**- *N*-Hydroxybenzotriazole (HOBt, 7.72 g, 57.1 mmol, 1.2 equiv.) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC•HCl, 10.95 g, 57.1 mmol, 1.2 equiv.) were added sequentially to a mixture of 2-[3-(4-nitrophenyl)-1-adamantyl]acetic acid (**1**, 15.0 g, 47.6 mmol) and 6-amino-1-hexanol (**2a**, 5.58 g, 47.6 mmol, 1.0 equiv.) dissolved in CH_2Cl_2 (250 mL) at room temperature under nitrogen. The mixture was stirred under nitrogen for 2 h and concentrated on a rotary evaporator. The residue was purified by silica gel column chromatography (5% MeOH in CH_2Cl_2) to afford 15.76 g (80%) of **3a** as a white solid, mp. 117–118°. R_f: 0.25 (5% MeOH in CH_2Cl_2). Analytical RP HPLC (Waters, Novapak): MeCN:0.1% aq. trifluoroacetic acid/60:40, 2.0 mL/min, 215 nm, t_R: 3.08 min, 96%; ^{1}H NMR ($CDCl_3$): δ 8.18–8.12 (m, 2 H), 7.52–7.48 (m, 2 H), 5.47 (distorted t, 1 H), 3.62 (t, 2 H, J = 6.0 Hz), 3.24 (q, 2 H, J = 13.8, 6.9 Hz), 2.26–2.20 (m, 2 H), 2.02 (s, 2 H), 1.92–1.78 (m, 6 H), 1.76–1.65 (m, 8 H), 1.64–1.45 (m, 4 H, 1.44–1.30 (m, 4 H); ^{13}C NMR ($CDCl_3$): δ 171.0, 158.0, 145.7, 125.9, 123.2, 62.1, 50.9, 47.4, 41.8, 41.3, 39.1, 37.7, 35.4, 33.5, 32.2, 29.3, 28.8, 26.4, 25.1; ESI-MS (*m/z*): 415 $(M + H)^+$, 437 $(M + Na)^+$; HRMS (FAB, *m/z*): calcd for $C_{24}H_{35}N_2O_4$: 415.2597 $(M + H)^+$; observed, 415.2594.

Anal. Calcd for $C_{24}H_{34}N_2O_4$: C, 69.54; H, 8.27; N, 6.76. Found: C, 69.65; H, 8.27; N, 6.77

***N*-{2-[2-(2-Hydroxyethoxy)ethoxy]ethyl}-2-[3-(4-nitrophenyl)-1-adamantyl]acetamide (3b).**- Triethylamine (3.34 mL, 24.0 mmol, 4.0 equiv.), HOBt (1.22 g, 9.0, mmol, 1.5 equiv.) and EDAC HCl (1.73 g, 9.0 mmol, 1.5 equiv.) were added sequentially to a mixture of 2-[3-(4-nitrophenyl)-1-adamantyl]acetic acid (**1**, 1.89 g, 6.0 mmol) and 2-[(2-aminoethoxy)ethoxy]ethanol hydrochloride (**2b**, 1.11 g, 6.0 mmol, 1.0 equiv.) dissolved in CH_2Cl_2 (60 mL) at room temperature under nitrogen. The mixture was stirred for 18 h and concentrated on a rotary evaporator. The residue was purified by silica gel column chromatography (EtOAc to 5% MeOH in EtOAc) to afford 2.18 g (81%) of **3b** as a pale yellow viscous oil. R_f: 0.14 (4% MeOH in EtOAc). Analytical RP HPLC (Waters, Symmetry): MeCN:0.1% aq. trifluoroacetic acid/40:60, 2.0 mL/min, 225 nm, t_R: 10.95 min, >99%; ^{1}H NMR ($CDCl_3$): δ 8.18–8.13 (m, 2 H), 7.57–7.48 (m, 2 H), 6.05 (distorted t, 1 H), 3.77–3.72 (m, 2 H), 3.67–3.58 (m, 6 H), 3.57–3.54 (m, 2 H), 3.50–3.43 (m, 2 H), 2.44 (br s, 1 H), 2.28–2.20 (m, 2 H), 2.05 (s, 2 H), 1.92 –1.78 (m, 6 H), 1.74–1.56 (m, 8 H); ^{13}C NMR ($CDCl_3$): δ 170.7, 158.0, 146.0, 126.0, 123.4, 72.4, 70.3, 70.1, 61.8, 51.0, 47.5, 42.0, 41.5, 39.0, 37.8, 35.6, 33.6, 29.0; ESI-MS (*m/z*): 447 $(M + H)^+$, 464 $(M + NH_4)^+$, 469 $(M + Na)^+$, 447 $(2 \times M + H)^+$; HRMS (FAB, *m/z*): calcd for $C_{24}H_{35}N_2O_6$: 447.2495 $(M + H)^+$; observed, 447.2486.

2-Cyanoethyl-6-({[3-(4-nitrophenyl)-1-adamantyl]acetyl}amino)hexyldiisopropylamidophosphite (5a).- *N,N*-Diisopropylethylamine (DIEA, 19.0 mL, 109.0 mmol, 4.1 equiv.) and (2-cyanoethyl)-*N,N*-diisopropylchlorophosphoramidite (**4**, 8.3 mL, 37.0 mmol, 1.4 equiv.) were added to a solution of **3a** (11.0 g, 26.5 mmol) in CH_2Cl_2 (220 mL) at room temperature under nitrogen. After stirring the mixture for 1 h, MeOH (1.1 mL, 26.5 mmol, 1.0 equiv.) was added, and the mixture was stirred for an additional 30 min. The mixture was then concentrated on a rotary evaporator, dissolved in a mixture of CH_2Cl_2:Et_3N (98:2 ratio, 20 mL) and purified by silica gel column chromatography (EtOAc/hexanes/Et_3N, 60:40:2). The product was concentrated, azeotroped with toluene (5 × 50 mL) and CH_2Cl_2 (5 × 50 mL) and dried under vacuum (0.5 mm/Hg) for 48 h to afford 9.89 g (61%) of probe **5a** as a pale yellow viscous oil. R_f: 0.48 (EtOAc/hexanes/Et_3N, 70:30:2). Analytical RP HPLC (Waters, Novapak): MeCN:0.1 M aq. triethylammonium acetate/80:20, 2.0 mL/min at 215 nm, t_R: 5.95 min, 91%; ^{1}H NMR (CD_3CN): δ 8.19–8.14 (m, 2 H), 7.62–7.58 (m, 2 H), 6.30 (dist t, 1 H), 3.82–3.68 (m, 2 H), 3.67–3.50 (m, 4 H), 3.10 (q, 2 H, J = 12.6, 6.6 Hz), 2.63 (t, 2 H, J = 6.0 Hz), 2.20–2.14 (m, 2 H), 1.95 (s, 2 H, merged with CD_3CN), 1.90–1.62 (m, 12 H), 1.58–1.50 (m, 2 H), 1.48–1.26 (m, 6 H)1.16 (d, 6 H, J = 3.6 Hz), 1.14 (d, 6 H, J = 3.6 Hz); ^{13}C NMR (CD_3CN): δ 171.0, 159.5, 146.9, 127.1, 124.2, 119.5, 64.4, 64.1, 59.3, 59.1, 55.3, 51.3, 48.3, 43.8, 43.6, 42.6, 42.2, 39.6, 38.6, 36.3, 34.3, 31.9, 31.8, 30.3, 30.1, 27.3, 26.3, 24.9, 24.8, 21.1, 21.0; ^{31}P NMR (CD_3CN): δ 147.6, 147.5; ESI-MS (*m/z*): 615 $(M + H)^+$; HRMS (FAB, *m/z*): calcd for $C_{33}H_{52}N_4O_5P$: 615.3675 $(M + H)^+$; observed, 615.3687.

Anal. Calcd for $C_{33}H_{51}N_4O_5P$: C, 64.47; H, 8.36; N, 9.11; P, 5.04

Found: C, 64.48; H, 8.33; N, 9.16; P, 4.85

2-Cyanoethyl-2-{2-[2-({[3-(4-nitrophenyl)-1-adamantyl]acetyl}amino)ethoxy]ethoxy}ethyl diiso-

propylamidophosphite (5b).- *N,N*-Diisopropylethylamine (DIEA, 5.47 mL, 31.48 mmol, 4.0 equiv.) and (2-cyanoethyl)-*N,N*-diisopropylchlorophosphoramidite (**4**, 2.45 mL, 11.02 mmol, 1.4 equiv.) were added to a solution of **3b** (3.51 g, 7.87 mmol) in CH_2Cl_2 (78 mL) at room temperature under nitrogen. After stirring the mixture for 1 h, MeOH (0.318 mL, 7.87 mmol, 1.0 equiv.) was added, and the mixture stirred for an additional 30 min. The mixture was then concentrated on a rotary evaporator, dissolved in a mixture of CH_2Cl_2:Et_3N (96:4 ratio, 20 mL) and purified by silica gel column chromatography (EtOAc/hexanes/Et_3N, 48:48:4 to 68:28:4). The product was concentrated to give 3.02 g, which was dissolved in a mixture of CH_2Cl_2/Et_3N (96:4, 20 mL) and further purified by silica gel column chromatography (EtOAc/hexanes/Et_3N, 48:48:4 to 58:38:4). The column fractions containing the product were combined, concentrated on a rotary evaporator, azeotroped with toluene (5 × 25 mL) and CH_2Cl_2 (5 × 25 mL) and dried under vacuum (0.5 mm/Hg) for 48 h to afford 2.08 g (41%) of probe **5b** as a pale yellow viscous oil. R_f 0.43 (EtOAc/hexanes/Et_3N, 68:28:4). Analytical RP HPLC (Waters, Symmetry): MeCN/0.1 M aq. triethylammonium acetate (80:20), 2.0 mL/min at 254 nm, t_R: 5.25 min, 93%; ^{1}H NMR (CD_3CN): δ 8.18–8.12 (m, 2 H), 7.61–7.56 (m, 2 H), 6.36 (disorted t, 1 H), 3.82–3.48 (m, 10 H), 3.45 (t, 2 H, *J* = 5.4 Hz), 3.26 (q, 2 H, *J* = 11.1, 5.4 Hz), 2.66–2.61 (m, 2 H), 2.20–2.13 (m, 2 H), 1.96 (s, 2 H), 1.90 –1.73 (m, 6 H), 1.72–1.58 (m, 8 H), 1.16 (d, 6 H, *J* = 2.4 Hz), 1.14 (d, 6 H, *J* = 2.7 Hz); ^{13}C NMR (CD_3CN): δ 171.3, 159.8, 147.2, 127.3, 124.5, 119.8, 72.2, 72.1, 71.3, 71.1, 70.6, 64.0, 63.7, 59.7, 59.5, 51.4, 48.5, 44.1, 43.9, 42.8, 42.3, 39.8, 38.9, 36.5, 34.5, 30.3, 25.2, 25.0, 21.2, 21.1; ^{31}P NMR (CD_3CN): δ 148.6, 148.5; ESI-MS (*m/z*): 647 $(M + H)^+$, 664 $(M + NH_4)^+$, 1310 $(2 \times M + NH_4)^+$; HRMS (FAB, *m/z*): calcd for $C_{33}H_{52}N_4O_7P$: 647.3574 $(M + H)^+$; observed, 647.3575.

Anal. Calcd for $C_{33}H_{51}N_4O_7P$: C, 61.28; H, 7.95; N, 8.66; P, 4.79

Found: C, 61.19; H, 7.83; N, 8.49; P, 4.66

6-(9*H*-Carbazol-2-yloxy)-1-hexanol (8a).- 6-Bromo-1-hexanol (**7a**, 45.0 g, 249.0 mmol, 1.5 equiv.) and anhydrous K_2CO_3 (68.0 g, 492.0 mmol, 3.0 equiv.) were added to a solution of 2-hydroxycarbazole (**6**, 30.0 g, 164.0 mmol) in methylethylketone (MEK, 300 mL) and refluxed the mixture under nitrogen for 17 h. An additional amount of anhydrous K_2CO_3 (22.7g, 164.0, 10 equiv.) was added to the reaction mixture and the reflux was continued for 5 h. The warm reaction mixture was then filtered and the filtrate concentrated on a rotary evaporator. The resulting solid was triturated in EtOAc (300 mL) and filtered. The solid was washed with EtOAc (3 × 100 mL) and dried under vacuum (0.5 mm/Hg) for 24 h to afford 29.5 (63%) g of **8a** as a white solid, mp: 174–175°. R_f 0.06 (EtOAc/hexanes/Et_3N, 30:68:2). Analytical RP HPLC (Waters, Novapak): MeCN/0.1% aq. trifluoroacetic acid (60:40), 2.0 mL/min, 215 nm, t_R: 3.44 min, >99%; ^{1}H NMR (DMSO-d_6): δ 7.97 (d, 1 H, *J* = 8.0 Hz), 7.94 (d, 1 H, *J* = 8.7 Hz), 7.41 (d, 1 H, *J* = 8.1 Hz), 7.30–7.25 (m, 1 H), 7.24–7.07 (m, 1 H), 6.94 (d, 1 H, *J* = 2.1 Hz), 6.75 (dd, 1 H, *J* = 8.7, 2.1 Hz), 4.36 (t, 1 H, *J* = 5.1 Hz), 4.03 (t, 2 H, *J* = 6.6 Hz), 3.41 (q, 2 H, *J* = 11.4, 6.0 Hz), 3.34 (s, 1 H), 1.78–1.70 (m, 2 H), 1.52–1.30 (m, 6 H); ^{13}C NMR (DMSO-d_6): δ 157.9, 141.1, 139.8, 124.1, 122.7, 120.9, 119.2, 118.5, 116.2, 110.6, 108.1, 95.1, 67.6, 60.7, 32.6, 29.0, 25.6, 25.4; ESI-MS (*m/z*): 284 $(M + H)^+$; HRMS (FAB, *m/z*): calcd for

$C_{18}H_{21}NO_2$: 283.1572 $(M)^+$; observed, 283.1585.

Anal. Calcd for $C_{18}H_{21}NO_2$: C, 76.30; H, 7.47; N, 4.94. Found: C, 76.38; H, 7.49; N, 4.92

2-{2-[2-(9*H*-Carbazol-2-yloxy)ethoxy]ethoxy}ethanol (8b).- 2-[(2-Chloroethoxy)ethoxy]ethanol (**7b**, 3.26 mL, 22.5 mmol, 1.5 equiv.), anhydrous K_2CO_3 (6.21 g, 45.0 mmol, 3.0 equiv.) and sodium iodide (1.12 g, 7.5 mmol, .0.5 equiv.) were added sequentially to a solution of 2-hydroxycarbazole (**6**, 2.745 g, 15.0 mmol) in methylethylketone (MEK, 30 mL) and refluxed under nitrogen for 18 h. The hot reaction mixture was filtered and the filtrate was evaporated to dryness on a rotary evaporator. The resulting crude compound was purified by silica gel column chromatography (2–3% MeOH in EtOAc) to afford 3.73 (79%) g of **8b** as a white solid, mp: 121–124°. R_f: 0.39 (2% MeOH in EtOAc). Analytical RP HPLC (Waters, Symmetry): MeCN/0.1% aq. trifluoroacetic acid (50:50), 2.0 mL/min, 225 nm, t_R: 3.63 min, >99%; ^{1}H NMR (CD_3OD): δ 7.91 (d, 1 H, J = 7.8 Hz), 7.88 (d, 1 H, J = 8.7 Hz), 7.36 (d, 1 H, J = 8.1 Hz), 7.28–7.23 (m, 1 H), 7.22–7.06 (m, 1 H), 6.97 (d, 1 H, J = 2.1 Hz), 6.79 (dd, 1 H, J = 8.4, 2.1 Hz), 4.22–4.19 (m, 2 H), 3.90–3.87 (m, 2 H), 3.76–3.73 (m, 2 H), 3.69–3.64 (m, 4 H), 3.59–3.55 (m, 2 H); ^{13}C NMR (CD_3OD): δ 159.4, 142.7, 141.5, 125.3, 124.5, 121.6, 120.1, 119.6, 118.5, 111.4, 109.2, 96.5, 73.7, 71.8, 71.4, 71.0, 69.0, 62.2; ESI-MS (*m/z*): 316 $(M+H)^+$, 333 $(M + NH_4)^+$, 338 $(M + Na)^+$; HRMS (FAB, *m/z*): calcd for $C_{18}H_{21}NO_4$: 315.1471 $(M)^+$; observed, 315.1471.

Anal. Calcd for $C_{18}H_{21}NO_4$: C, 68.55; H, 6.71; N, 4.44. Found: C, 68.58; H, 6.84; N, 4.34

6-(9*H*-Carbazol-2-yloxy)hexyl-2-cyanoethyldiisopropylamidophosphite (9a).- *N,N*-Diisopropylethylamine (30.2 mL, 173.0 mmol, 4.0 equiv.) and (2-cyanoethyl)-*N,N*-diisopropylchlorophosphoramidite (**4**, 13.5 mL, 60.6 mmol, 1.4 equiv.) were added to a solution of **8a** (12.3 g, 43.4 mmol) in CH_2Cl_2 (350 mL) at room temperature under nitrogen. After stirring the mixture for 1 h, MeOH (1.76 mL, 43.4 mmol, 1.0 equiv.) was added, and stirred for an additional 30 min. The mixture was then concentrated on a rotary evaporator and the residue dissolved in a mixture of CH_2Cl_2/EtOAc/hexanes/Et_3N (40:10:50:4, 50 mL). The crude compound was purified by silica gel column chromatography (CH_2Cl_2/EtOAc/hexanes/Et_3N, 15:15:70:4 to 20:20:60:4). The column fractions containing the product were combined and concentrated on a rotary evaporator. The residue was dissolved (CH_2Cl_2/Et_3N, 96/4 ratio, 20 mL) and further purified by silica gel column chromatography [CH_2Cl_2/EtOAc/hexanes/Et_3N (15:15:70:4 to 20:20:60:4)]. The product was azeotroped with toluene (5 × 50 mL), CH_2Cl_2 (5 × 50 mL) and dried under vacuum (0.5 mm/Hg) for 48 h to afford 9.93 (47%) g of probe (**9a**) as a pale yellow gummy material. R_f: 0.32 (CH_2Cl_2/EtOAc/hexanes/Et_3N, 20:20:60:4). Analytical RP HPLC (Waters, Novapak): MeCN/0.1 M aq. triethylammonium acteate (80:20), 2.0 mL/min, 215 nm, t_R: 6.97 min, 94.3%; ^{1}H NMR (CD_3CN): δ 9.20 (br s, 1 H), 7.96 (dd, 1 H, J = 7.8, 0.6 Hz), 7.91 (d, 1 H, J = 8.7 Hz), 7.43 (d, 1 H, J = 8.1 Hz), 7.34–7.28 (m, 1 H), 7.18–7.12 (m, 1 H), 6.99 (d, 1 H, J = 2.4 Hz), 6.79 (dd, 1 H, J = 8.4, 2.1 Hz), 4.05 (t, 2 H, J = 6.3 Hz), 3.82–3.34 (m, 6 H), 2.62 (t, 2 H, J = 5.7 Hz), 1.86–1.76 (m, 2 H), 1.68–1.58 (m, 2 H), 1.56–1.44 (m, 4 H), 1.16 (d, 12 H, J = 6.6 Hz); ^{13}C NMR (CD_3CN): δ 159.5, 142.2, 140.8, 125.3, 124.1, 121.7, 120.1, 120.0, 119.7, 117.5, 111.5, 109.5, 96.2, 69.0, 64.4, 64.1, 59.3, 59.1, 43.8, 43.7, 31.9, 31.8, 30.0, 26.5, 24.9, 24.8, 21.1,

21.0; ^{31}P NMR (CD_3CN): δ 147.6, 147.5; ESI-MS (*m/z*): 484 $(M + H)^+$, 969 $(2 \times M + H)^+$; HRMS (FAB, *m/z*): calcd for $C_{27}H_{38}N_3O_3P$: 483.2651 $(M)^+$; observed, 483.2644.

Anal. Calcd for $C_{27}H_{38}N_3O_3P$: C, 67.06; H, 7.92; N, 8.69. Found: C, 67.17; H, 7.96; N, 8.70

2-{2-[2-(9*H*-Carbazol-2-yloxy)ethoxy]ethoxy}ethyl-2-cyanoethyldiisopropylamidophosphite (9b).- *N,N*-Diisopropylethylamine (44.16 mL, 254.0 mmol, 4.0 equiv.) and (2-cyanoethyl)-*N,N*-diisopropylchlorophosphoramidite (**4**, 19.76 mL, 88.9 mmol, 1.4 equiv.) were added to a solution of **8b** (20.0 g, 63.5 mmol) in CH_2Cl_2 (825 mL) at room temperature under nitrogen. After stirring the mixture for 1 h, MeOH (2.6 mL, 63.5 mmol, 1.0 equiv.) was added, and the mixture stirred for an additional 30 min. The mixture was then concentrated on a rotary evaporator to about 75 mL volume and purified by silica gel column chromatography (CH_2Cl_2/EtOAc/hexanes/Et_3N, 15:15:66:4 to 20:40:36:4). The product was azeotroped with toluene (5 × 125 mL) and CH_2Cl_2 (5 × 125 mL) and dried under vacuum (0.5 mm/Hg) for 48 h to afford 17.9 g (55%) of probe (**9b**) as a pale yellow gummy material. R_f: 0.37 (CH_2Cl_2/EtOAc/hexanes/Et_3N, 20:40:36:4); Analytical RP HPLC (Waters, Symmetry): MeCN/0.1 M aq. triethylammonium acteate (75:25), 2.0 mL/min, 225 nm, t_R: 6.64 min, 94%; ^{1}H NMR (CD_3CN): δ 9.22 (br s, 1 H), 7.95 (dd, 1 H, *J* = 7.8, 0.6 Hz), 7.92 (d, 1 H, *J* = 9.0 Hz), 7.44 (d, 1 H, *J* = 8.1 Hz), 7.33–7.28 (m, 1 H), 7.18–7.12 (m, 1 H), 7.00 (d, 1 H, *J* = 2.4 Hz), 6.80 (dd, 1 H, *J* = 8.4, 2.1 Hz), 4.19–4.16 (m, 2 H, *J* = 6.3 Hz), 3.84–3.80 (m, 14 H), 2.64–2.60 (m, 2 H), 1.14 (d, 12 H, *J* = 6.9 Hz); ^{13}C NMR (CD_3CN): δ 159.2, 142.2, 140.9, 125.4, 124.0, 121.8, 120.2, 120.1, 119.7, 117.7, 111.6, 109.5, 96.4, 72.0, 71.9, 71.4, 71.3, 70.4, 68.8, 63.8, 63.6, 59.6, 59.3, 43.9, 43.7, 24.9, 24.8, 21.0, 20.9; ^{31}P NMR (CD_3CN): δ 148.6, 148.5; ESI-MS (*m/z*): 516 $(M + H)^+$; HRMS (FAB, *m/z*): calcd for $C_{27}H_{39}N_3O_5P$: 516.2627 $(M + H)^+$; observed, 516.2623.

Anal. Calcd for $C_{27}H_{38}N_3O_5P$: C, 62.90; H, 7.43; N, 8.15; P, 6.01

Found: C, 62.87; H, 7.28; N, 8.13; P, 5.84

5-(Dimethylamino)-*N*-(6-hydroxyhexyl)-1-naphthalenesulfonamide (11a): Na_2CO_3 (9.41 g, 88.8 mmol, 4.0 equiv.) and dansyl chloride (**10**, 6.00 g, 22.2 mmol, 1.0 equiv.) were added to a mixture of 6-amino-1-hexanol (**2a**, 2.60 g, 22.2 mmol, 1.0 equiv.) dissolved in THF (130 mL) and water (100 mL) at room temperature. An additional amount of THF (130 mL) was added and the resulting yellow mixture was stirred for 30 min. The mixture was diluted with EtOAc (240 mL) washed with 20% aq. NaCl solution (2 × 240 mL), dried (Na_2SO_4) and concentrated on a rotary evaporator. The crude compound was purified by silica gel column chromatography (70% EtOAc in hexanes) to afford 7.52 g (97%) of **11a** as a pale yellow-green gummy material. R_f: 0.27 (70% EtOAc in hexanes). Analytical RP HPLC (Waters, Novapak): MeCN/0.1% aq. trifluoroacetic acid (30:70), 2.0 mL/min, 215 nm, t_R: 3.67 min, >99%; ^{1}H NMR ($CDCl_3$): δ 8.54 (d, 1 H, *J* = 8.7 Hz), 8.29 (d, 1 H, *J* = 8.4 Hz), 8.25 (dd, 1 H, *J* = 7.2, 1.2 Hz), 7.60–7.50 (m, 2 H), 7.19 (dd, 1 H, *J* = 7.8, 0.9 Hz), 4.65 (t, 1 H, *J* = 6.3 Hz), 3.51 (q, 2 H, *J* = 11.4, 6.3 Hz), 2.92–2.86 (m, 2 H), 2.90 (s, 6 H), 1.44–1.26 (m, 5 H), 1.22–1.12 (m, 4 H); ^{13}C NMR ($CDCl_3$): δ 151.7, 134.8, 130.2, 129.7, 129.5, 129.4, 128.2, 123.1, 118.8, 115.1, 62.4, 45.3, 43.0, 32.2, 29.3, 25.9, 24.9; ESI-MS (*m/z*): 383 $(M + H)^+$, 400 $(M + NH_4)^+$, 782 $(2 \times M + NH_4)^+$; HRMS (FAB, *m/z*): calcd for $C_{18}H_{26}N_2O_3S$: 350.1664 $(M)^+$; observed, 350.1664.

Anal. Calcd for $C_{18}H_{26}N_2O_5S$: C, 61.69; H, 7.48; N, 7.99; S, 9.15
Found: C, 61.55; H, 7.72; N, 7.75; S, 8.89

5-(Dimethylamino)-*N*-{2-[2-(2-hydroxyethoxy)ethoxy]ethyl}-1-naphthalenesulfonamide (11b): Na_2CO_3 (2.54 g, 24.0 mmol, 4.0 equiv.) and dansyl chloride (**10**, 1.614 g, 6.0 mmol, 1.0 equiv.) were added to a mixture of 2-[(2-aminoethoxy)ethoxy]ethanol hydrochloride (**2b**, 1.11 g, 6.0 mmol, 1.0 equiv.) dissolved in THF (36 mL) and water (27 mL) at room temperature. An additional amount of THF (36 mL) was added and the resulting yellow mixture was stirred for 1.5 h. The mixture was diluted with EtOAc (360 mL) washed with brine (3 × 50 mL), dried (Na_2SO_4) and concentrated on a rotary evaporator. The crude compound was purified by silica gel column chromatography (80% EtOAc in hexanes to 1% MeOH in EtOAc) to afford 1.91 g (83%) of **11b** as a pale yellow-green gummy material. R_f: 0.34 (1% MeOH in EtOAc). Analytical RP HPLC (Waters, Symmetry): MeCN/0.1% aq. trifluoroacetic acid (20:80), 2.0 mL/min, 225 nm, t_R: 6.03 min, >99%; ^{1}H NMR ($CDCl_3$): δ 8.53 (d, 1 H, J = 8.7 Hz), 8.32 (d, 1 H, J = 8.4 Hz), 8.24 (d, 1 H, J = 7.2 Hz), 7.60–7.49 (m, 2 H), 7.19 (d, 1 H, J = 7.8 Hz), 5.74 (dist t, 1 H), 3.80–3.72 (m, 2 H), 3.62–3.48 (m, 4 H), 3.48–3.38 (m, 4 H), 3.16–3.06 (m, 2 H), 2,89 (s, 6 H); ^{13}C NMR ($CDCl_3$): δ 151.9, 135.0, 130.4, 129.9, 129.7, 129.4, 128.3, 123.2, 119.0, 115.2, 72.6, 70.3, 70.2, 69.4, 61.7, 45.4, 43.0; ESI-MS (*m/z*): 383 $(M + H)^+$, 400 $(M + NH_4)^+$, 782 $(2 \times M + NH_4)^+$; HRMS (FAB, *m/z*): calcd for $C_{18}H_{26}N_2O_5S$: 382.1562 $(M + H)^+$; observed, 382.1567.

2-Cyanoethyl-6-({[5-(dimethylamino)-1-naphthyl]sulfonyl}amino)hexyldiisopropylamidophosphite (12a).- *N,N*-Diisopropylethylamine (14.3 mL, 82.0 mmol, 4.0 equiv.) and (2-cyanoethyl)-*N,N*-diisopropylchlorophosphoramidite (**4**, 6.40 mL, 28.7 mmol, 1.4 equiv.) were added to a solution of **11a** (7.18 g, 20.5 mmol) in CH_2Cl_2 (200 mL) at room temperature under nitrogen. After stirring the mixture for 1 h, MeOH (0.83 mL, 20.5 mmol, 1.0 equiv.) was added, and the reaction stirred for an additional 30 min. The mixture was then concentrated on a rotary evaporator and the residue dissolved in 2% Et_3N in CH_2Cl_2 (15 mL). Purification of the crude product by silica gel column chromatography (EtOAc/hexanes/Et_3N, 30:70:2) gave the product which was azeotroped with toluene (5 × 50 mL), CH_2Cl_2 (5 × 50 mL) and dried under vacuum (0.5 mm/Hg) for 48 h to afford 5.65 g (50%) of probe (**12a**) as a pale green viscous oil. R_f: 0.26 (EtOAc/hexanes/Et_3N, 40:60:2). Analytical RP HPLC (Waters, Novapak): MeCN/ 0.1 M aq. triethylammonium acteate (80:20), 2.0 mL/min, 215 nm, t_R: 4.89 min, 93%; ^{1}H NMR (CD_3CN): δ 8.54 (d, 1 H, J = 8.4 Hz), 8.28 (d, 1 H, J = 9.0 Hz), 8.17 (dd, 1 H, J = 7.2, 1.2 Hz), 7.60 (t, 1 H, J = 7.5 Hz), 7.58 (t, 1 H, J = 7.2 Hz), 7.26 (d, 1 H, J = 6.9 Hz), 5.75 (dist t, 1 H), 3.78–3.68 (m, 2 H), 3.62–3.42 (m, 4 H), 2.86 (s, 6 H), 2.86–2.75 (m, 2 H), 2.61 (t, 2 H, J = 5.7 Hz), 1.38–1.02 (m, 6 H), 1.15 (d, 6 H, J = 6.9 Hz), 1.12 (d, 6 H, J = 6.9 Hz); ^{13}C NMR (CD_3CN): δ 152.9, 136.6, 130.9, 130.6, 130.4, 130.0,129.0, 124.3, 120.0, 119.5, 116.1, 64.2, 64.0, 59.3, 59.0, 55.2, 45.7, 43.7, 43.6, 43.5, 31.6, 31.5, 29.9, 26.7, 26.0, 24.9, 24.8, 21.0, 21.9; ^{31}P NMR (CD_3CN): δ 147.5, 147.4; ESI-MS (*m/z*): 552 $(M + H)^+$; HRMS (FAB, *m/z*): calcd for $C_{27}H_{44}N_4O_4PS$: 551.2821 $(M + H)^+$; observed, 551.2802.

Anal. Calcd for $C_{27}H_{43}N_4O_4PS$: C, 58.89; H, 7.87; N, 10.17; S, 5.82
Found: C, 58.77; H, 7.88; N, 10.12; S, 5.98

2-Cyanoethyl-2-{2-[2-({[5-(dimethylamino)-1-naphthyl]sulfonyl}amino)ethoxy]ethoxy}ethyldiisopropylamidophosphite (12b): *N,N*-Diisopropylethylamine (3.31 mL, 19.06 mmol, 4.0 equiv.) and (2-cyanoethyl)-*N,N*-diisopropylchlorophosphoramidite (**4**, 1.66 mL, 6.67 mmol, 1.4 equiv.) were added to a solution of **11b** (1.82 g, 4.764 mmol) in CH_2Cl_2 (48 mL) at room temperature under nitrogen. After stirring the mixture for 1 h, MeOH (0.19 mL, 4.764 mmol, 1.0 equiv.) was added, and the reaction stirred for an additional 30 min. The mixture was concentrated on a rotary evaporator and the residue was dissolved in 4% Et_3N in CH_2Cl_2 (15 mL). Purification of the crude product by silica gel column chromatography (EtOAc/hexanes/Et_3N, 20:76:4 to 48:48:4) gave the product, which was azeotroped with toluene (5 × 25 mL), CH_2Cl_2 (5 × 25 mL). The product was dried under vaccum (0.5 mm/Hg) for 48 h to afford 2.18 g (79%) of probe (**12b**) as a pale green viscous oil. R_f: 0.39 (EtOAc/hexanes/Et_3N, 48:48:4); Analytical RP HPLC (Waters, Symmetry): MeCN/0.1 M aq. triethylammonium acteate (80:20), 2.0 mL/min, 254 nm, t_R: 4.52 min, 92%; ^1H NMR (CD_3CN): δ 8.54 (dt, 1 H, *J* = 8.4, 1.2, 1.2 Hz), 8.26 (dt, 1 H, *J* = 8.7, 0.9, 0.9 Hz), 8.18 (dd, 1 H, *J* = 7.5, 1.5 Hz), 7.60 (t, 1 H, *J* = 7.5 Hz), 7.57 (t, 1 H, *J* = 7.2 Hz), 7.25 (dd, 1 H, *J* = 7.5, 0.6 Hz), 5.87 (br s, 1 H), 3.82–3.52 (m, 6 H), 3.48 (t, 2 H, *J* = 4.8 Hz), 3.35–3.32 (m, 2 H), 3.27–3.20 (m, 4 H), 3.01–2.95 (m, 2 H), 2.85 (s, 6 H), 2.64–2.59 (m, 2 H), 1.15 (d, 6 H, *J* = 5.7 Hz), 1.13 (d, 6 H, *J* = 5.7 Hz); ^{13}C NMR (CD_3CN): δ 153.0, 136.6, 131.0, 130.7, 130.4, 130.0, 129.1, 124.1, 119.9, 119.7, 116.2, 71.9, 71.8, 70.9, 70.8, 69.7, 63.7, 63.5, 59.5, 59.3, 45.7, 43.9, 43.8, 43.7, 24.9, 24.8, 21.0, 20.9; ^{31}P NMR (CD_3CN): δ 148.6, 148.5; ESI-MS (*m/z*): 583 $(M + H)^+$, 605 $(M + Na)^+$, 1164 $(2 \times M + H)^+$, 1187 $(2 \times M + Na)^+$; HRMS (FAB, *m/z*): calcd for $C_{27}H_{44}N_4O_6PS$: 583.2719 $(M + H)^+$; observed, 583.2729.

Anal. Calcd for $C_{27}H_{43}N_4O_6PS$: C, 55.65; H, 7.44; N, 9.62; P, 5.32

Found: C, 55.40; H, 7.28; N, 9.47; P, 5.19

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