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Synthesis of hapten–phosphoramidites from 2′-deoxyuridine

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Abstract—A total of 11 novel phosphoramidites, **3a–d**, **4a–d** and **14a–c** were prepared from 2′-deoxyuridine functionalized at 5 and 6-position of the pyrimidine ring with hapten reporter groups, e.g. adamantane, carbazole, dansyl and dabsyl, suitable for use in immunodetection nucleic acid testing assays.

1. Introduction

Molecular diagnostics is the fastest growing area in the field of clinical analysis.[1] The completion of the Human Genome Mapping Project (HGMP)[2,3] has accelerated identification of new nucleic acid markers useful in the diagnosis of disease states and monitoring the therapy for those ailments. Concurrently, nucleic acid testing (NAT) has progressed from a laborious manual process using radiolabeled reagents to highly automated high throughput screening (HTS). At the heart of the current technology for detection of specific nucleic acid sequences, is the preparation of probes bearing reporter groups.[4–6] The reporter groups employed are as varied as the assay formats.[7] Beacon[8–11] and Taqman[12] formats require dual-labeled fluorophore/quencher probes, while hybridization protection assays make use of chemiluminescent probes.[13] In the Abbott LCx system (Fig. 1) the reporter groups are haptens,[14] i.e. low molecular weight compounds that bind to specific antibodies. In that system oligonucleotide probes complementary to a target sequence are differentially labeled with haptens $\mathbf{H}_a$ and $\mathbf{H}_b$. The ligase chain reaction (LCR)[15–17] joins the probes, which then become viable targets in the next round of amplification. At the completion of target amplification, the ligated probes are captured using anti-hapten $\mathbf{H}_a$ antibody-coated microparticles and detected with an anti-hapten $\mathbf{H}_b$ antibody-alkaline phosphatase conjugate using 4-methylumbilliferone phosphate (MUP) substrate.

Many strategies have been reported for labeling oligonucleotides probes, but they can be broadly grouped into either post-synthetic or integrated approaches. In the former, specific linker functional groups are incorporated into the probes that can be further elaborated with the reporter group after isolation and purification. Most often nucleoside phosphoramidites bearing the linkers are used.[18] Examples include phosphoramidites of 5-(trifluoroacetylamidoalkyl)-,[19–25] 5-(alkylthio)-,[26] 5-(alkyldiol)-,[27] 5-(carboxymethyl)-,[28,29] deoxythymidine, and N^4-(trifluoroacetylamidoalkyl)deoxycytidine.[21,30,31] Alternatively, the linker can be introduced on the phosphate backbone of the probe via H-phosphonate chemistry,[4,5,32] or non-selectively via transamination of cytosine residues.[33,34] The integrated methodology introduces the reporter group during the synthesis of the probe. This can be done enzymatically using 5-substituted dUTP[35–38] or N^4-substituted 5-methyl dCTP,[39] but more control is achieved with automated synthesis using phosphoramidites. Recent examples of modified-nucleoside phosphoramidites in this category include 5-substituted deoxythymidine bearing photolabels,[40] spin-labels,[41] thymine,[42] and metal chelators.[43]

We have detailed the synthesis of hapten-bearing, non-nucleoside phosphoramidites useful for the preparation of 5′-[44–48] and 3′-haptenated probes.[44,46–48] A preliminary communication on hapten phosphoramidites based on 6-substituted-2′-deoxyuridine has recently appeared.[49] Here we present the full report for the preparation and characterization of 6-haptenated-2′-deoxyuridine phosphoramidites. Additionally, novel 5-haptenated-2′-deoxyuridine phosphoramidites are also described.

2. Results and discussion

2.1. 5-[(2*E*)-*N*-(Alkyl)prop-2-enamidyl]-2′-deoxyuridine hapten phosphoramidites

Introduction of reporter groups at the C-5 position of 2′-deoxyuridine has been often cited as the preferred regiochemistry to minimize unfavorable steric interactions

Keywords: 2′-deoxyuridine; haptens; phosphoramidites.

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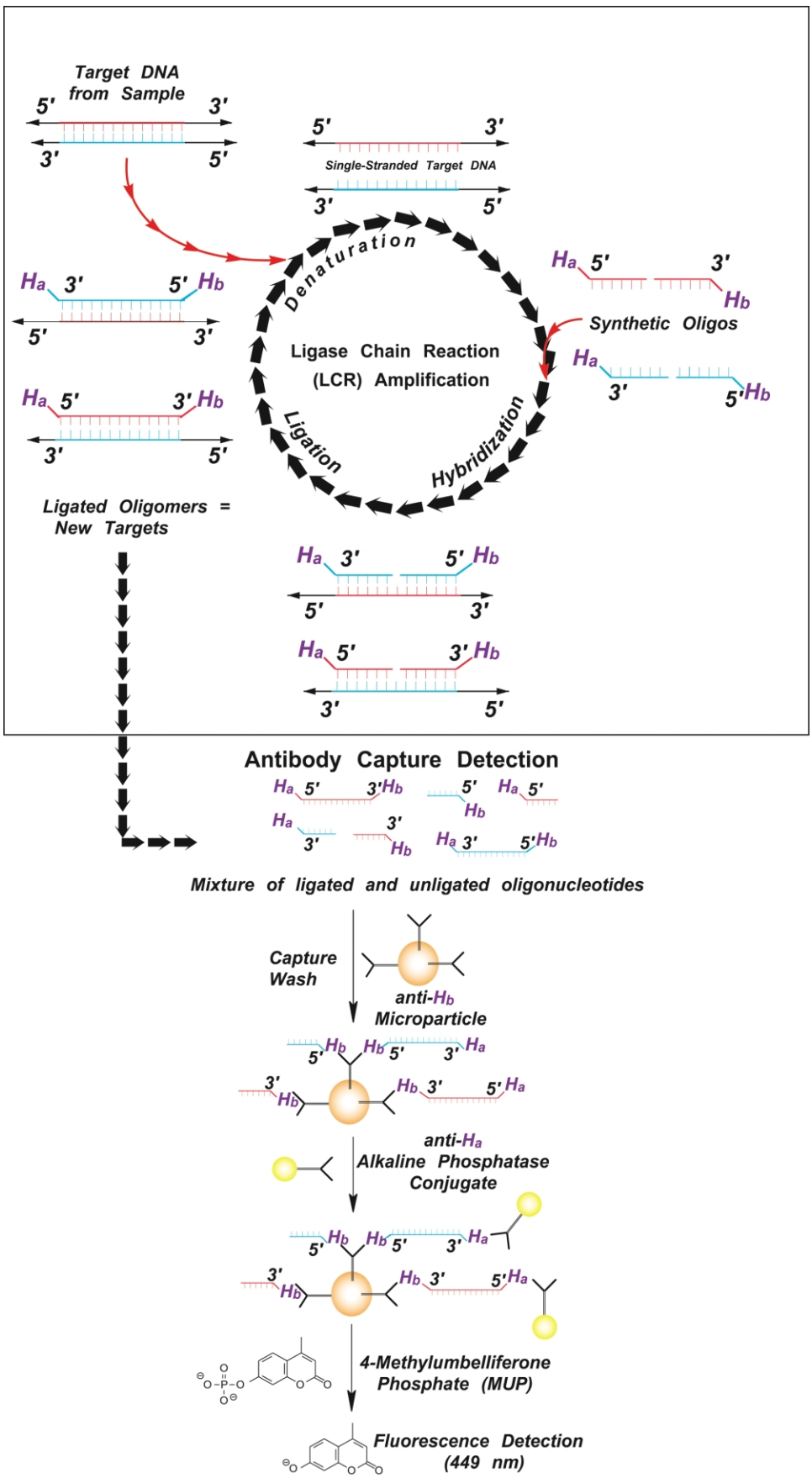
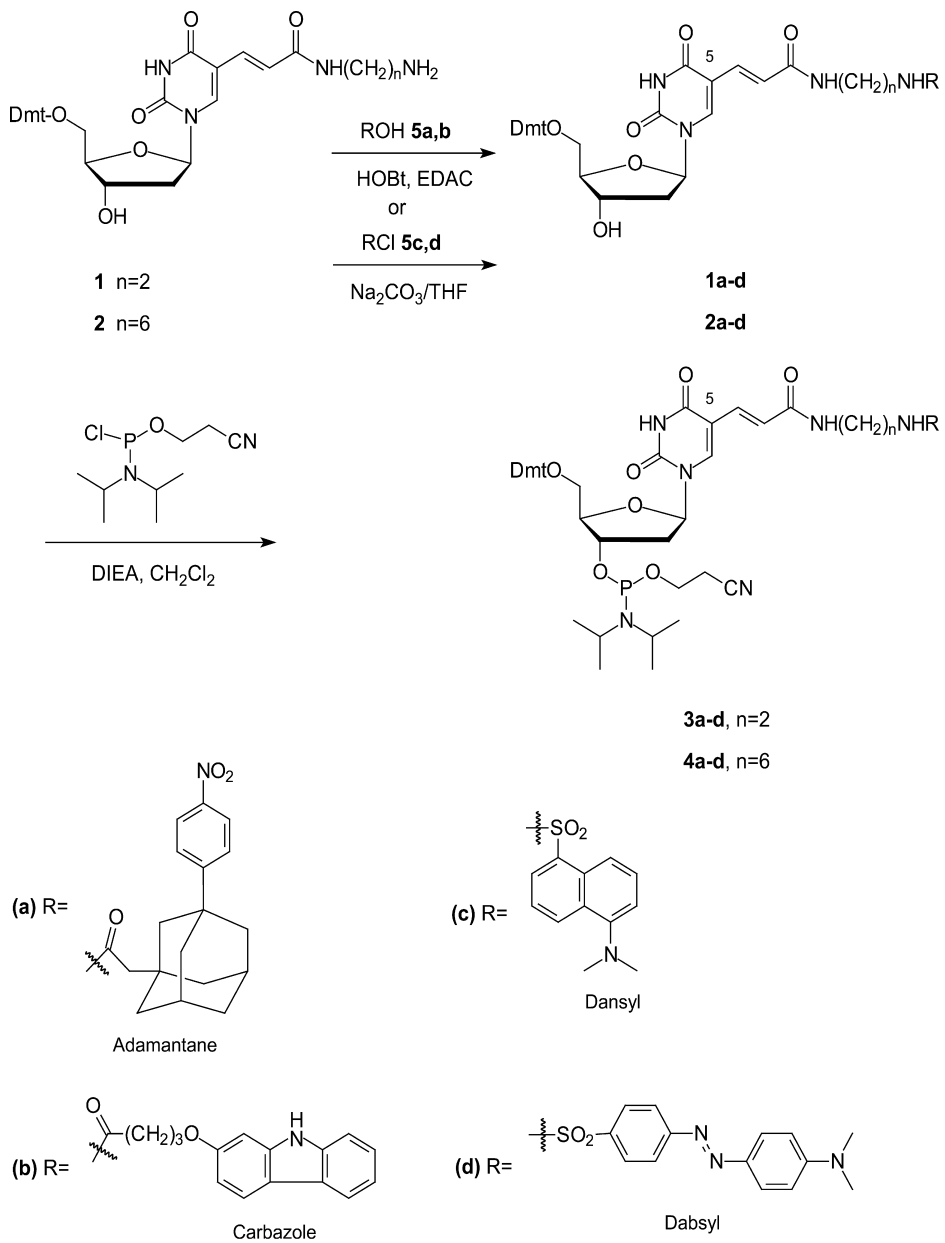


Figure 1. NAT Signal amplification and antibody capture detection.

and perturbation of Watson–Crick base-pairing[18] during hybridization of probes containing modified bases. However, the exact nature of the substituent can have a positive or negative effect on the stability of the duplex formed.[25] The commercial availability of 5-substituted-2′-deoxyuridine derivatives **1** and **2** (Scheme 1) has added to their popularity. To introduce the required modifications to enable various immunodetection formats, **1** and **2** were coupled (HOBt, EDAC) with our desired haptens, 2-[3-(4-nitrophenyl)-1-adamantyl]acetic acid (**5a**)[48] and 4-(9*H*-carbazol-2-yloxy)butanoic acid (**5b**),[48] as shown to produce intermediates **1a**,**b** and **2a**,**b**, respectively in good to excellent yield and purity. The dansyl (**5c**) and dabsyl (**5b**) moieties are dual reporter groups. Both can function as haptens, while dansyl is also a fluorescent label and dabsyl is an efficient fluorescence quencher. The dansyl **1c**, **2c** and dabsyl **1d**, **2d** derivatives were prepared by sulfonylation of amines **1** and **2** with the corresponding sulfonyl chlorides in THF/aqueous sodium carbonate in good yield. Conversion to 3′-position phosphoramidites **3a–d**, **4a–d** was accomplished by treatment with 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite in THF in the presence of diisopropylethylamine in 41–77% yield. In most cases flash silica chromatography was sufficient to provide phosphoramidites in greater than 95% purity. Dansyl (**3c** and **4c**) and dabsyl phosphoramidites (**4d**) required additional purification by preparative reverse phase HPLC to reach that level of purity.

2.2. 6-[(2*E*)-*N*-(Alkyl)prop-2-enamidyl]-2′-deoxyuridine hapten phosphoramidites

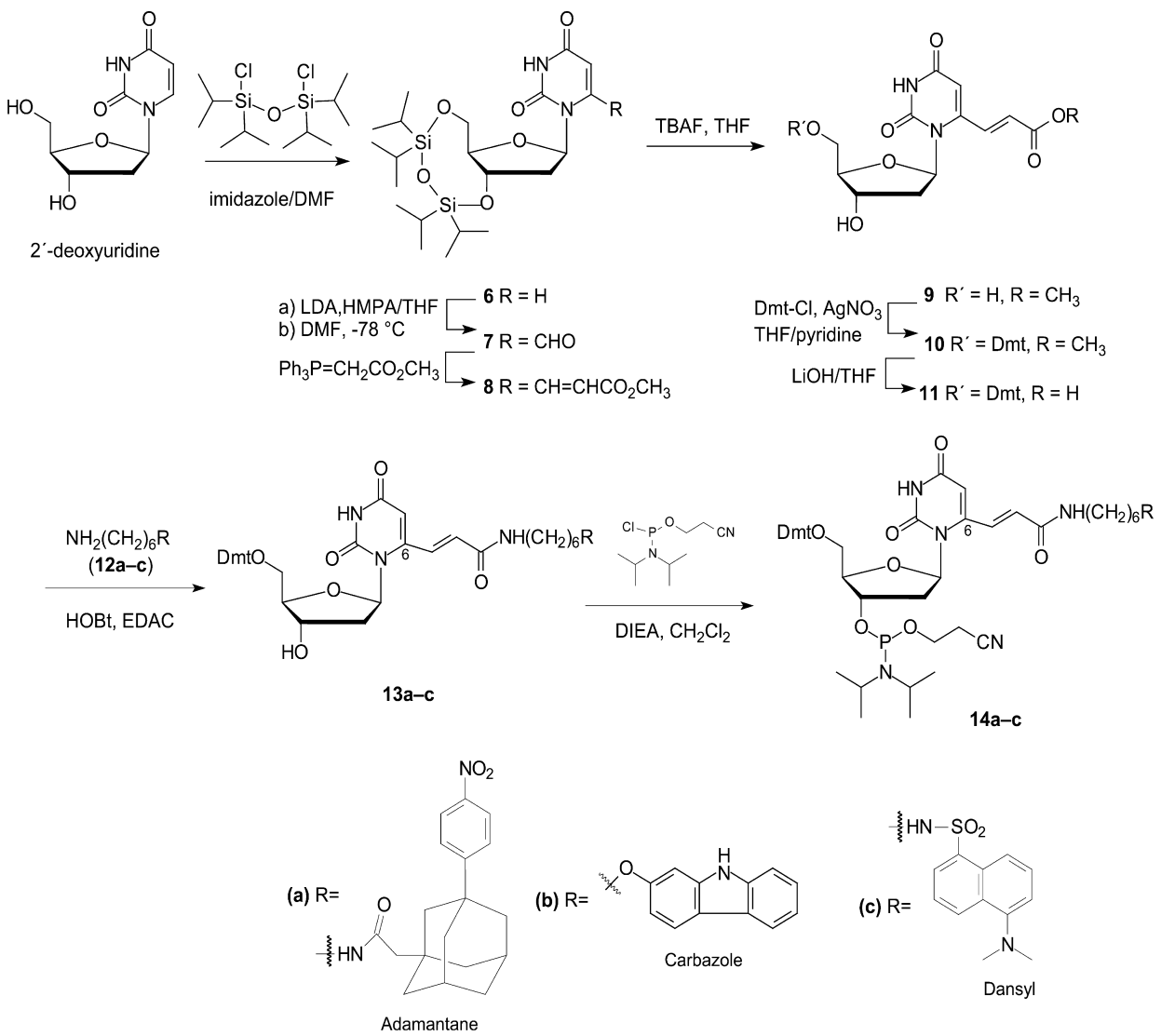
2′-Deoxyuridine phosphoramidites labeled at position C-6 have only recently been described.[49] Unlike the C-5 analogues, the stability of hybridized probes containing the modified bases might be even more sensitive to the



Scheme 1.

substituent. The work of Sanghvi, et al.[50] indicated that even introduction of a methyl group at that position destabilized duplexed oligos. However, it appears that any destabilization due to base modification can be accommodated in the overall probe design, for example, by limiting the placement of the modified base to the 3′ or 5′ termini or adjusting the sequence length to achieve the desired hybridization stringency. Our strategy for synthesis of phosphoramidites **14a–c** from 2′-deoxyuridine first involved the introduction of a handle [e.g. ester group in compound **8**] at position-6, which could be conjugated to the haptenic group via suitable linking arm. Thus, the 3′- and 5′-hydroxyl groups in 2′-deoxyuridine (Scheme 2) were protected with the 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl (TIPDS)[7] group to give **6**, which was then treated with LDA and DMF in the presence of HMPA.[51,52] The crude reaction mixture was treated with acetic acid at −78°C to give the aldehyde (**7**) in 52% yield after purification by silica gel column chromatography. The aldehyde **7** was then subjected to the Wittig reaction with methyl(triphenylphosphoranylidene)acetate in benzene to afford the unsaturated ester **8** in almost quantitative yield as the *E*-isomer (>98%). The next step was to exchange the silyl protective group at the 5′-position of **8** to the 4,4′-dimethoxytrityl group (DMT), which is compatible with automated oligonucleotide synthesis. Thus, the TIPDS protective group in **8** was hydrolyzed by treatment with tetra-*n*-butylammonium fluoride in THF and the crude compound was purified by silica gel column chromatography to afford diol (+)-**9** in excellent yield (98%). The 5′-hydroxyl group in **9** was protected as the DMT ether by treatment with 4,4′-dimethoxytrityl chloride in the presence of silver nitrate and pyridine. The crude compound was purified by silica gel column chromatography (ethyl acetate/triethylamine/methanol, 97:2:1) to afford ester **10**.

The next step in the synthesis of probes **14a–c** was to conjugate ester **10** to the hapten reporter groups via a linking arm (Scheme 2). Thus, **10** was subjected to hydrolysis by lithium hydroxide in aqueous THF and the resulting crude acid (**11**) was conjugated with amines **12a–c** using HOBt and EDAC in anhydrous DMF. The conjugates **13a–c** were isolated in moderate yield and high purity after purification by silica gel column chromatography. Finally, **13a–c** were



Scheme 2.

treated with 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite in THF in the presence of diisopropylethylamine to afford the probes **14a**–**c** in 37–52% yield as mixtures of diastereomers following reverse phase HPLC purification.

In summary, 11 new oligonucleotide building blocks, phosphoramidites **3a**–**d**, **4a**–**d** and **14a**–**c**, were prepared from 2′-deoxyuridine modified with an amino-terminated linker from either the 5 or 6-position. When paired with a complementary antibody, probes bearing adamantyl, carbazole, dansyl, and dabsyl haptens serve as powerful immunodetection reagents employed in the Abbott LCx. Moreover, other haptens can be likewise incorporated to further expand the utility of 5- and 6-substituted 2′-deoxyuridine phosphoramidites, i.e, multianalyte detection, incorporation of internal controls, etc. The fluorescent properties of probes built from dansyl phosphoramidites **3c**, **4c**, **14c** and the quenching ability of dabsyl phosphoramidite **3d**, **4d**-derived probes are attractive for construction of a variety of donor-quencher based homogeneous NAT formats such as Beacon[8–11] and Taqman.[12]

3. Experimental

3.1. General methods and materials

The ^{1}H, ^{13}C and ^{31}P NMR were recorded on a Varian Gemini spectrometer (300 MHz) and the chemical shifts for ^{1}H and ^{13}C (δ) were reported in ppm relative to TMS and coupling constants (J) were reported in Hz; ^{31}P chemical shifts are relative to phosphoric acid. Electrospray ionization mass spectrometry (ESI-MS) was carried out on a Perkin–Elmer (Norwalk, CT) Sciex API 100 Benchtop system employing the Turbo Ionspray ion source. High-resolution mass spectra (HRMS) were obtained on a Nermang 3010 MS-50 or JEOL SX102-A mass spectrometers. Column chromatography was performed on silica gel, Merck grade 60 (230–400 mesh). THF was freshly distilled from a solution of sodium benzophenone ketyl. Dichloromethane 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, except where noted. 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 (Milford, MA) μBondapak RCM C18 10μ (8×100 mm) column (solvent ratio v/v reported) unless otherwise stated. Preparative reverse phase (RP) HPLC was performed using a Waters μBondapak RCM C18 10μ (40×100 mm) column (solvent ratio v/v reported) unless otherwise stated. Optical rotations were measured on Autopol III polarimeter from Rudolph Research (Flanders, NJ). Melting points were recorded in open capillary tubes on an Electrothermal Melting Point Apparatus (Barnstead International, Dubuque, IA) and were uncorrected. IUPAC names were obtained using the ACD/ILab Web service version 3.5 at http://www.acdlabs.com/ilab/.

(*E*)-*N*-(2-Aminoethyl)-3-[1-((4*S*,5*R*)-5-{[bis(4-methoxyphenyl) (phenyl)methoxy]methyl}-4-hydroxy-tetrahydro-2-furanyl)-2,4-dioxo-1,2,3,4-tetrahydro-5-pyrimidinyl]-2-propenamide (**1**) and (*E*)-*N*-(6-amino-hexyl)-3-[1-((4*S*,5*R*)-5-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-hydroxytetrahydro-2-furanyl)-2,4-dioxo-1,2,3,4-tetrahydro-5-pyrimidinyl]-2-propenamide (**2**) were purchased from Berry & Associates, Inc. (Dexter, MI). 2-[3-(4-Nitrophenyl)-1-adamantyl]acetic acid (**5a**), 4-(9*H*-carbazol-2-yloxy)butanoic acid (**5b**) were prepared according to our published procedure.[48] 2-Cyanoethyl *N*,*N*-diisopropylchlorophosphoramiditewas purchased from ChemGenes, Inc. (Ashland, MA).

3.2. Preparation of 5-haptenated-5′-*O*-dimethoxytrityl-2′-deoxyuridine 1a,b and 2a,b

Hapten carboxylic acid (**5a** or **b**, 3 mmol), *N*-hydroxybenzotriazole (HOBt, 0.61 g, 4.5 mmol, 150 mol%), and triethylamine (1.7 mL, 12 mmol, 400 mol%) were sequentially added to a suspension of the 5-aminoalkyl substituted 2′-deoxyuridine derivative (**1** or **2**, 3 mmol) in dichloromethane or dimethylformamide (30 mL). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC, 0.86 g, 4.5 mmol, 150 mol%) was added and the resulting solution was stirred at ambient temperature for 18 h. The solvent was then removed on a rotary evaporator under reduced pressure. The residue was purified by silica gel chromatography (300 g), eluting with ethyl acetate/methanol/triethylamine (v:v:v shown). The material was concentrated on a rotary evaporator under reduced pressure and then the residue was repeatedly co-evaporated with toluene (5×50 mL) followed by dichloromethane (5×50 mL). Analytical reversed-phase HPLC [acetonitrile/0.1 M aq triethylammonium acetate, v:v (shown). 2.0 mL/min, 225 nm].

3.2.1. (+)-(*E*)-3-[1-((4*S*,5*R*)-5-{[Bis(4-methoxyphenyl)-(phenyl)methoxy]methyl}-4-hydroxytetrahydro-2-furanyl)-2,4-dioxo-1,2,3,4-tetrahydro-5-pyrimidinyl]-*N*-[2-({2-[3-(4-nitrophenyl)-1-adamantyl]acetyl} amino)-ethyl]-2-propenamide (1a). Chromatography (3–6% MeOH in ethyl acetate containing 2% Et_3N). Yield (84% as a colorless glassy material). Mp 145–55°C. Analytical RP HPLC [75:25] 2.90 min, 95.2%. $[\alpha]_D^{23}=+5.6$ (*c* 1.03, MeOH). ^{1}H NMR ($CDCl_3$) δ 8.10–8.04 (m, 2H), 7.87 (s, 1H), 7.45–7.02 (m, 11H), 6.83 (d, 4H, J=6.0 Hz), 6.78–6.70 (m, 1H), 6.55 (dist t, 1H), 6.30 (t, 1H, J=4.0 Hz), 4.53–4.47 (m, 1H), 4.22–4.16 (m, 1H) 3.75 (s, 3H), 3.74 (s, 3H), 3.52–3.34 (m, 6H), 2.66–2.56 (m, 1H), 2.28–2.16 (m, 1H), 2.12 (br s, 2H), 2.01 (s, 2H), 1.88–1.67 (m, 8H), 1.66–1.45 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 171.7, 167.5, 161.8, 158.7, 158.6, 158.0, 149.4, 145.8, 144.4, 141.2, 135.5, 135.4, 132.1, 130.0, 129.9, 128.0, 127.9, 127.1, 125.9, 123.4, 122.4, 113.4, 110.2, 86.8, 86.7, 86.2, 72.4, 64.4, 63.7, 60.4, 55.2, 51.1, 47.3, 41.9, 41.6, 41.4, 40.3, 38.7, 37.7, 35.5, 33.6, 30.6, 28.9, 21.0, 19.1, 14.2, 13.7. ESI-MS (*m*/*z*) 941 $(M+H)^+$, 958 $(M+NH_4)^+$, 1898 $(2M+NH_4)^+$. HRMS (FAB, *m*/*z*) calcd for $C_{53}H_{57}N_5O_{11}Na$, 962.3949 $(M+Na)^+$; observed, 962.3947.

3.2.2. (−)-(*E*)-3-[1-((4*S*,5*R*)-5-{[Bis(4-methoxyphenyl)-(phenyl)methoxy]methyl}-4-hydroxytetrahydro-2-furanyl)-2,4-dioxo-1,2,3,4-tetrahydro-5-pyrimidinyl]-*N*-(2-{[4-(9*H*-carbazol-2-yloxy)butanoyl]amino} ethyl)-2-propenamide (1b). Chromatography (88:10:2). Yield (63%

colorless glassy material. Mp 164–66°C. Analytical RP HPLC [50:50] 7.96 min, 97%. $[\alpha]_D^{23}=-3.39$ (*c* 0.765, MeOH). ^{1}H NMR ($CDCl_3$) δ 8.59 (br s, 1H), 7.86–7.73 (m, 3H), 7.36–7.05 (m, 11H), 6.80–6.62 (m, 9H), 6.15–6.06 (m, 1H), 4.42–4.37 (m, 1H), 4.15–4.08 (m, 1H), 3.86–3.79 (m, 2H), 3.65 (s, 6H), 3.41–3.22 (m, 6H), 2.58–2.42 (m, 1H), 2.36–2.24 (m, 2H), 2.18–1.97 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 173.8, 167.6, 162.2, 158.5, 158.0, 149.4, 144.4, 140.9, 139.6, 135.5, 135.4, 129.9, 129.0, 128.2, 127.9, 127.0, 125.2, 124.4, 123.1, 120.8, 119.2, 116.8, 113.3, 110.6, 109.8, 108.4, 95.4, 86.6, 86.3, 77.2, 67.2, 63.3, 55.1, 52.3, 45.8, 32.8, 25.2, 10.2, 7.1. ESI-MS (*m/z*) 894 $(M+H)^+$, 995 $(M+Et_3NH)^+$.

3.2.3. (+)-(*E*)-3-[1-((4*S*,5*R*)-5-{[Bis(4-methoxyphenyl)-(phenyl)methoxy]methyl}-4-hydroxytetrahydro-2-furanyl)-2,4-dioxo-1,2,3,4-tetrahydro-5-pyrimidinyl]-*N*-[6-({2-[3-(4-nitrophenyl)-1-adamantyl]acetyl} amino)-hexyl]-2-propenamide (2a). Chromatography (3–6% MeOH in ethyl acetate containing 2% Et_3N). Yield (86%, colorless glassy material). Mp 125–35°C. Analytical RP HPLC [75:25] 3.82 min, >99%. $[\alpha]_D^{23}=+7.9$ (*c* 0.9, MeOH). ^{1}H NMR ($CDCl_3$) δ 8.14–8.11 (m, 2H), 7.90 (s, 1H), 7.50–7.10 (m, 11H), 6.82 (d, 4H, *J*=8.4 Hz), 6.63 (d, 1H, *J*=15.3 Hz), 6.33 (t, 1H, *J*=6.6 Hz), 5.74 (t, 1H, *J*=5.7 Hz), 5.24 (t, 1H, *J*=5.44 Hz), 4.53–4.47 (m, 1H), 4.10–4.08 (m, 1H), 3.76 (s, 6H), 3.52–3.04 (m, 6H), 2.66–2.56 (m, 1H), 2.56–2.46 (m, 1H), 2.32–1.56 (m, 12), 1.52–1.18 (m, 14H). ^{13}C NMR ($CDCl_3$) δ 171.2, 166.3, 161.9, 159.1, 158.5, 149.5, 146.3, 144.9, 140.5, 135.9, 135.8, 132.1, 130.4, 130.3, 128.5, 128.4, 127.6, 126.4, 123.8, 122.5, 113.8, 110.8, 87.1, 86.6, 85.9, 72.4, 64.0, 60.8, 55.7, 51.7, 48.0, 46.5, 42.4, 42.0, 41.6, 39.6, 39.4, 38.2, 36.1, 34.1, 29.8, 29.7, 29.4, 26.6, 26.4, 21.5, 19.5, 14.6, 14.1. ESI-MS (*m/z*) 997 $(M+H)^+$, 1014 $(M+NH_4)^+$, 2010 $(2M+NH_4)^+$. HRMS (FAB, *m/z*) calcd for $C_{57}H_{65}N_5O_{11}Na$, 1018.4574 $(M+Na)^+$; observed, 1018.4573.

3.2.4. (+)-(*E*)-3-[1-((4*S*,5*R*)-5-{[Bis(4-methoxyphenyl)-(phenyl)methoxy]methyl}-4-hydroxytetrahydro-2-furanyl)-2,4-dioxo-1,2,3,4-tetrahydro-5-pyrimidinyl]-*N*-(6-{[4-(9*H*-carbazol-2-yloxy)butanoyl]amino} hexyl)-2-propenamide (2b). Chromatography (88:10:2). Yield (72% colorless glassy material). Mp 144–46°C. Analytical RP HPLC [50:50] 10.7 min, 99%. $[\alpha]_D^{23}=+6.0$ (*c* 1.0, MeOH). ^{1}H NMR ($CDCl_3$) δ 9.06 (s, 1H), 7.91–7.82 (m, 3H), 7.38–7.19 (m, 11H), 6.83–6.72 (m, 7H), 6.27 (t, 1H, *J*=6.3 Hz), 6.05–5.99 (m, 1H), 5.60–5.55 (m, 1H), 4.48–4.42 (m, 1H), 4.13–4.07 (m, 1H), 3.98–3.92 (m, 1H), 3.67 (s, 6H), 3.37–3.28 (m, 2H), 3.18–2.94 (m, 3H), 2.51–2.32 (m, 3H), 2.22–2.01 (m, 3H), 1.31–0.96 (m, 10H). ^{13}C NMR ($CDCl_3$) δ 172.8, 166.3, 161.9, 158.6, 158.1, 149.3, 144.4, 141.0, 140.5, 139.7, 135.5, 131.9, 129.9, 128.8, 129.0, 128.0, 127.9, 127.1, 124.4, 123.3, 122.1, 120.8, 119.2, 119.1, 117.0, 113.3, 110.6, 110.3, 108.4, 95.3, 86.6, 86.2, 85.6, 77.2, 71.8, 66.9, 63.6, 55.2, 41.0, 39.2, 33.0, 29.2, 26.0, 25.3. ESI-MS (*m/z*) 950 $(M+H)^+$, 972 $(M+Na)^+$. HRMS (FAB, *m/z*) calcd for $C_{55}H_{59}N_5O_{10}Na$, 972.4154 $(M+Na)^+$; observed, 972.4149.

3.3. Preparation of 5-haptenated-5′-dimethoxytrityl-2′-deoxyuridine 1c,d and 2c,d

Hapten sulfonyl chloride (**5c** or **d**, 3 mmol) and sodium carbonate (1.27 g, 12 mmol, 400 mol%) were sequentially added to a suspension of the 5-aminoalkyl substituted 2′-deoxyuridine derivative (**1** or **2**, 3 mmol) in aqueous tetrahydrofuran (40%, 32 mL). An additional amount of tetrahydrofuran (18 mL) was added and the reaction mixture was stirred for 30 min. Saturated aq sodium chloride solution (36 mL) was added to the reaction mixture then extracted with ethyl acetate (300 mL). The organic layer was separated and washed with brine (3×50 mL), dried over anhydrous sodium sulfate and concentrated on a rotary evaporator.

The residue was purified by silica gel chromatography (300 g), eluting with ethyl acetate/methanol/triethylamine (v:v:v shown). The eluted material was evaporated on a rotary evaporator under reduced pressure then the residue was repeatedly co-evaporated with toluene (5×50 mL) and dichloromethane (5×50 mL). Analytical reversed-phase HPLC [acetonitrile/0.1 M aq triethylammonium acetate, v:v (shown), 2.0 mL/min].

3.3.1. (+)-(*E*)-3-[1-((4*S*,5*R*)-5-{[Bis(4-methoxyphenyl)-(phenyl)methoxy]methyl}-4-hydroxytetrahydro-2-furanyl)-2,4-dioxo-1,2,3,4-tetrahydro-5-pyrimidinyl]-*N*-[2-({[5-(dimethylamino)-1-naphthyl]sulfonyl} amino)-ethyl]-2-propenamide (1c). Chromatography (2–4% MeOH in ethyl acetate containing 2% of Et_3N). Yield (58%, pale yellow powder). Mp 145–49°C. Analytical RP HPLC [75:25] 2.48 min, >99%. $[\alpha]_D^{23}=+11.8$ (*c* 0.87, acetonitrile/MeOH 9:1). ^{1}H NMR ($CDCl_3$+5 drops of CD_3OD) δ 8.53 (d, 1H, *J*=8.7 Hz), 8.27 (d, 1H, 8.4 Hz), 8.20 (dd, 1H, *J*=7.2, 1.2 Hz), 7.88 (s, 1H), 7.57–7.17 (m, 14H), 6.91 (d, 1H, *J*=15.3 Hz), 6.81 (d, 4H, *J*=8.7 Hz), 6.61 (dd, 1H, *J*=15.3, 2.1 Hz), 6.36–6.32 (m, 1H), 6.27 (t, 1H, *J*=7.2 Hz), 4.44–4.39 (m, 1H), 4.14–4.09 (m, 1H), 3.75 (s, 3H), 3.74 (s, 3H), 3.44–3.16 (m, 6H), 2.87 (s, 6H), 2.52–2.44 (m, 1H), 2.28–2.18 (m, 1H). ^{13}C NMR ($CDCl_3$+5 drops of CD_3OD) δ 166.8, 162.0, 158.3, 158.2, 151.5, 149.3, 144.1, 140.7, 135.3, 135.2, 134.3, 132.1, 130.0, 129.7, 129.6, 129.2, 128.9, 128.0, 127.7, 127.6, 126.8, 122.8, 121.1, 118.6, 115.0, 113.0, 109.7, 86.4, 86.3, 85.6, 71.1, 63.3, 54.8, 44.9, 42.1, 40.8, 39.0. ESI-MS (*m/z*) 876 $(M+H)^+$, 898 $(M+Na)^+$, 1752 $(2M+H)^+$. HRMS (FAB, *m/z*) calcd for $C_{47}H_{49}N_5O_{10}SNa$, 898.3092 $(M+Na)^+$; observed, 898.3090.

3.3.2. (+)-(*E*)-3-[1-((4*S*,5*R*)-5-{[Bis(4-methoxyphenyl)-(phenyl)methoxy]methyl}-4-hydroxytetrahydro-2-furanyl)-2,4-dioxo-1,2,3,4-tetrahydro-5-pyrimidinyl]-*N*-(2-{[(4-{(*E*)-2-[4-(dimethylamino)phenyl] diazenyl}phenyl)sulfonyl]amino}ethyl)-2-propenamide (1d). Chromatography (94:4:2). Yield (90%). Mp 182–84°C. Analytical RP HPLC [55:45, 254 nm] 7.16 min, 98%. $[\alpha]_D^{23}=+107.0$ (*c* 0.86, MeOH). ^{1}H NMR ($CDCl_3$) δ 8.39–8.20 (m, 2H), 7.98–7.76 (m, 6H), 7.41–7.05 (m, 12H), 6.82–6.63 (m, 5H), 6.18–6.06 (m, 1H), 4.43–4.05 (m, 3H), 3.66 (s, 6H), 3.42–3.20 (m, 3H), 3.08 (s, 6H), 3.04–2.86 (m, 3H), 2.64–2.51 (m, 2H), 2.25–2.12 (m, 2H). ^{13}C NMR (CD_3CN) δ 167.8, 162.4, 159.6, 156.2, 154.4, 150.1, 145.9, 144.1, 142.9, 140.8, 136.8, 136.7, 133.8, 130.9, 130.9, 129.0, 128.9, 128.9, 127.9, 126.3, 123.3, 122.2, 114.1, 112.6, 110.5, 87.2, 87.1, 86.2, 71.8, 64.5, 55.9, 44.3, 41.0, 40.5, 39.8. ESI-MS (*m/z*) 930 $(M+H)^+$, 1860 $(2M+H)^+$. HRMS (FAB, *m/z*) calcd for $C_{49}H_{52}N_7O_{10}S$, 930.3491 $(M+H)^+$; observed, 930.3499.

3.3.3. (+)-(*E*)-3-[1-((4*S*,5*R*)-5-{[Bis(4-methoxyphenyl)-(phenyl)methoxy]methyl}-4-hydroxytetrahydro-2-furanyl)-2,4-dioxo-1,2,3,4-tetrahydro-5-pyrimidinyl]-*N*-[6-({[5-(dimethylamino)-1-naphthyl]sulfonyl} amino)-hexyl]-2-propenamide (2c). Chromatography (96:2:2). Yield (94%). Mp 115–16°C. Analytical RP HPLC [60:40, 254 nm] 6.4 min, 99%. $[\alpha]_D^{23}$=+4.21 (*c* 0.83, MeOH). ^{1}H NMR ($CDCl_3$) δ 8.49 (d, 1H, *J*=8.5 Hz), 8.30 (d, 1H, *J*=8.8 Hz), 8.19 (d, 1H, *J*=7.1 Hz), 7.91 (s, 1H), 7.51–7.44 (m, 2H), 7.41–7.17 (m, 2H), 7.30–7.10 (m, 11H), 6.81–6.71 (m, 4H), 6.32 (t, 1H, *J*=6.6 Hz), 5.69–5.58 (m, 1H), 4.51–4.45 (m, 1H), 4.2–4.08 (m, 1H), 3.69 (s, 6H), 3.45–3.24 (m, 2H), 3.08–2.90 (m, 3H), 2.84 (s, 6H), 2.58–2.47 (m, 1H), 2.33–2.21 (m, 1H), 1.42–0.98 (m, 8H). ^{13}C NMR ($CDCl_3$) δ 166.1, 162.1, 158.5, 158.5, 151.8, 149.5, 144.5, 140.6, 135.5, 135.5, 135.0, 131.8, 130.2, 129.9, 129.8, 129.7, 129.6, 129.3, 128.2, 127.9, 127.0, 123.1, 122.0, 118.9, 115.1, 113.3, 110.3, 86.5, 86.2, 85.5, 71.7, 63.6, 55.2, 45.3, 43.0, 40.1, 39.0, 29.1, 28.9, 25.8, 25.7. ESI-MS (*m*/*z*) 933 $(M+H)^+$, 1033 $(M+Et_3NH)^+$. HRMS (FAB, *m*/*z*) calcd for $C_{51}H_{57}N_5O_{10}SNa$, 954.3718 $(M+Na)^+$; observed, 954.3721.

3.3.4. (+)-(*E*)-3-[1-((4*S*,5*R*)-5-{[Bis(4-methoxyphenyl)-(phenyl)methoxy]methyl}-4-hydroxytetrahydro-2-furanyl)-2,4-dioxo-1,2,3,4-tetrahydro-5-pyrimidinyl]-*N*-(6-{[(4-{(*E*)-2-[4-(dimethylamino)phenyl] diazenyl}phenyl)sulfonyl]amino}hexyl)-2-propenamide (2d). Chromatography (94:4:2). Yield (91%). Analytical RP HPLC [55:45, 254 nm] 13.03 min, 99%. $[\alpha]_D^{23}$=+5.6 (*c* 0.75, MeOH). ^{1}H NMR (CD_3CN) δ 7.92–7.80 (m, 6H), 7.73 (s, 1H), 7.43–7.38 (m, 2H), 7.32–7.17 (m, 8H), 6.89–6.77 (m, 8H), 6.23–6.14 (m, 2H), 5.67 (t, 1H, *J*=6.0 Hz), 4.35–4.31 (m, 1H), 3.98–3.94 (m, 1H), 3.71 (s, 6H), 3.27–3.23 (m, 2H), 3.14–3.05 (m, 2H), 3.06 (s, 6H), 2.89–2.83 (m, 2H), 2.34–2.17 (m, 3H), 1.43–1.27 (m, 4H), 1.26–1.12 (m, 4H). ^{13}C NMR (CD_3CN) δ 166.7, 162.5, 159.6, 156.2, 154.4, 150.2, 145.9, 144.1, 142.4, 141.3, 136.8, 136.7, 133.0, 130.9, 129.0, 128.9, 128.9, 127.9, 126.4, 123.2, 122.9, 114.1, 112.5, 110.7, 87.2, 87.1, 86.3, 71.8, 64.6, 55.8, 43.8, 41.0, 40.5, 39.7, 30.0, 29.9, 26.8, 26.6. ESI-MS (*m*/*z*) 986 $(M+H)^+$. HRMS (FAB, *m*/*z*) calcd for $C_{53}H_{60}N_7O_{10}S$, 986.4117 $(M+H)^+$; observed, 986.4125.

3.4. Preparation of 5-haptenated-5′-*O*-dimethoxytrityl-2′-deoxyuridine phosphoramidites 3a–d and 4a–d

Diisopropylethylamine (1.4 mL, 8 mmol, 400 mol%) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.67 mL, 3 mmol, 150 mol%) were added sequentially to a solution of 5-haptenated-5′-dimethoxytrityl-2′-deoxyuridine (**1a–d**, **2a–d**, 2 mmol) dissolved in dichloromethane (25 mL) at room temperature under nitrogen. After stirring the mixture for 1 h, dry methanol (0.08 mL, 2 mmol, 100 mol%) was added, and stirred the reaction for an additional 30 min. The reaction mixture was diluted with 6% triethylamine in ethyl acetate (265 mL) then washed sequentially with 10% aq sodium carbonate (3×40 mL) and brine (3×40 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated on a rotary evaporator. The residue was purified by silica gel chromatography (300 g), eluting with ethyl acetate/methanol/triethylamine (v:v:v shown). The eluted material was evaporated under reduced pressure then the residue was repeatedly co-evaporated with toluene (5×50 mL) and dichloromethane (5×50 mL). Analytical reversed-phase HPLC [acetonitrile/0.1 M aq triethylammonium acetate, v:v (shown), 2.0 mL/min].

3.4.1. 2-{[Bis(4-methoxyphenyl)(phenyl)methoxy]-methyl}-5-[5-((1*E*)-3-{[2-({[3-(4-nitrophenyl)-1-adamantyl]acetyl}amino)ethyl]amino}-3-oxoprop-1-enyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]tetrahydrofuran-3-yl 2-cyanoethyl diisopropylamidophosphite (3a). Chromatography (3–4% MeOH containing 2% Et_3N). Yield (74%, mixture of diastereomers). Analytical RP HPLC [60:40, 254 nm] 11.34 and 13.88 min, 95%. ^{1}H NMR (CD_3CN) δ 8.22–8.14 (m, 2H), 7.84–6.60 (m, 14H), 6.78–6.70 (m, 2H), 6.32–6.24 (m, 1H), 4.66–4.56 (m, 1H), 4.28–4.18 (m, 1H), 3.92–3.56 (m, 6H), 3.84 (s, 6H), 3.48–3.28 (m, 6H), 2.76–2.42 (m, 8H), 2.30–1.14 (m, 25H). ^{31}P NMR (CD_3CN) δ 148.62, 148.58. ESI-MS (*m*/*z*) 1058 $(M+NH_4)^+$. HRMS (FAB, *m*/*z*) calcd for $C_{62}H_{74}N_7O_{12}PNa$, 1162.5025 $(M+Na)^+$; observed, 1162.5041. Anal. calcd for $C_{62}H_{74}N_7O_{12}P$: C, 65.31%. H, 6.54%. N, 8.60%. P, 2.72%. Found: C, 65.81%. H, 6.65%. N, 8.13%. P, 3.04%.

3.4.2. 2-{[Bis(4-methoxyphenyl)(phenyl)methoxy]-methyl}-5-[5-((1*E*)-3-{[2-(9*H*-carbazol-2-yloxy)ethyl]-amino}-3-oxoprop-1-enyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]tetrahydrofuran-3-yl 2-cyanoethyl diisopropylamidophosphite (3b). Chromatography (93:5:2). Yield (77% as a mixture of diastereomers). Analytical RP HPLC [60:40, 254 nm] 12.29 and 14.20 min, 99%. ^{1}H NMR ($CDCl_3$) δ 8.61 (d, 1H, *J*=2.2 Hz), 7.89 (d, 1H, *J*=7.4 Hz), 7.81 (d, 1H, *J*=8.5 Hz), 7.73 (d, 1H, *J*=15.6 Hz), 7.46–7.14 (m, 11H), 7.11–7.00 (m, 1H), 6.87–6.61 (m, 8H), 6.17 (t, 1H, *J*=5.7 Hz), 6.08–5.98 (m, 1H), 4.56–4.47 (m, 1H), 4.23–4.15 (m, 1H), 3.93 (t, 2H, *J*=6.0 Hz), 3.73 (s, 3H), 3.73 (s, 3H), 3.68–3.51 (m, 4H), 3.47–3.34 (m, 1H), 3.33–3.24 (m, 5H), 2.57 (t, 1H, *J*=6.3 Hz), 2.41 (t, 1H, *J*=6.3 Hz), 2.37–2.32 (m, 2H), 2.24–2.04 (m, 1H), 1.92–1.61 (m, 2H), 1.98–1.04 (m, 12H). ^{31}P NMR ($CDCl_3$) δ 150.17, 149.95. ESI-MS (*m*/*z*) 1094 $(M+H)^+$, 1111 $(M+NH_4)^+$; HRMS (FAB, *m*/*z*) calcd for $C_{60}H_{68}N_7O_{11}PNa$, 1116.4607 $(M+Na)^+$; observed,1116.4604. Anal. calcd for $C_{60}H_{68}N_7O_{11}P$: C, 65.86%. H, 6.26%. N, 8.96%. P, 2.83%. Found: C, 66.43%. H, 6.31%. N, 8.47%. P, 3.08%.

3.4.3. 2-{[Bis(4-methoxyphenyl)(phenyl)methoxy]-methyl}-5-[5-((1*E*)-3-{[2-({[5-(dimethylamino)-1-naphthyl]sulfonyl}amino)ethyl]amino}-3-oxoprop-1-enyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]tetrahydrofuran-3-yl 2-cyanoethyl diisopropylamidophosphite (3c). Chromatography (2–3% MeOH in ethyl acetate containing 2% Et_3N). This material was further purified by preparative RP HPLC [acetonitrile/H_2O 76:24, 45 mL/min, 225 nm]. The product was collected, concentrated on rotary evaporator (<35°C) and azeotroped with toluene (5×40 mL) followed by dichloromethane (5×40 mL). Yield (58%, mixture of diastereomers, pale yellow solid). Analytical RP HPLC [acetonitrile/water 85:15, 225 nm] 4.04 and 4.80 min, 98.3%. ^{1}H NMR (CD_3CN) δ 8.22–8.14 (m, 1H), 7.84–6.60 (m, 18H), 6.78–6.70 (m, 3H), 4.60–4.56 (m, 1H), 4.20–4.08 (m, 1H),

3.80–3.10 (m, 5H), 3.84 (s, 6H), 2.80 (s, 6H), 2.92–2.20 (m, 10H), 1.28–1.05 (m, 12H). ^{31}P NMR (CD_3CN) δ 148.49. ESI-MS (*m/z*) 1076 $(M+H)^+$. Anal. calcd for $C_{56}H_{66}N_7O_{11}PS$: C, 62.50%. H, 6.18%. N, 9.11%. P, 2.88%. Found: C, 63.87%. H, 6.23%. N, 8.47%. P, 2.77%.

3.4.4. 2-{[Bis(4-methoxyphenyl)(phenyl)methoxy]-methyl}-5-[5-{(1*E*)-3-[(2-{[(4-{(*E*)-[4-(dimethylamino)-phenyl]diazenyl}phenyl)sulfonyl]amino}ethyl)amino]-3-oxoprop-1-enyl}-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]tetrahydrofuran-3-yl 2-cyanoethyl diisopropylamidophosphite (3d). Chromatography (96:2:2). Yield (75%). Analytical RP HPLC [70:30, 254 nm] 6.9 and 7.72 min, 99%. ^{1}H NMR (CD_3CN) δ 9.10 (br s, 1H), 7.88–7.79 (m, 6H), 7.69 (d, 1H, *J*=7.9 Hz), 7.44–7.38 (m, 2H), 7.33–7.15 (m, 8H), 6.95–6.74 (m, 8H), 6.36 (q, 1H, *J*=5.7 Hz), 6.13 (q, 1H, *J*=6.8 Hz), 6.09–6.03 (m, 1H), 4.55–4.44 (m, 1H), 4.15–4.06 (m, 1H), 3.81–3.70 (m, 7H), 3.67–3.48 (m, 3H), 3.33–3.15 (m, 4H), 3.07 (s, 3H), 3.07 (s, 3H), 3.03–2.96 (m, 2H), 2.62 (t, 1H, *J*=5.8 Hz), 2.49 (t, 1H, *J*=6.0 Hz), 2.45–2.36 (m, 1H), 2.32–2.23 (m, 1H), 1.16–1.12 (m, 9H), 1.02 (d, 3H, *J*=6.8 Hz). ^{31}P NMR (CD_3CN) δ 148.86. ESI-MS (*m/z*) 1129 $(M+H)^+$. HRMS (FAB, *m/z*) calcd for $C_{58}H_{69}N_9O_{11}PS$ $(M+H)^+$ 1130.4569; observed 1130.4569. Anal. calcd for $C_{58}H_{69}N_9O_{11}PS$: C, 61.63%. H, 6.06%. N, 11.15%. P, 2.74%. S, 2.84%. Found: C, 62.16%. H, 6.28%. N, 10.94%. P, 2.48%. S, 2.63%.

3.4.5. 2-{[Bis(4-methoxyphenyl)(phenyl)methoxy]-methyl}-5-[5-((1*E*)-3-{[6-({[3-(4-nitrophenyl)-1-adamantyl]acetyl}amino)hexyl]amino}-3-oxoprop-1-enyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]tetrahydrofuran-3-yl 2-cyanoethyl diisopropylamidophosphite (4a). Chromatography (3–4% MeOH in ethyl acetate containing 2% Et_3N). Yield (64%, mixture of diastereomers). Analytical RP HPLC [85:15, 225 nm] 5.76 and 6.87 min, 95%. ^{1}H NMR (CD_3CN) δ 8.22–8.14 (m, 2H), 7.84–6.60 (m, 14H), 6.78–6.70 (m, 2H), 6.32–6.24 (m, 1H), 4.66–4.56 (m, 1H), 4.28–4.18 (m, 1H), 3.92–3.56 (m, 6H), 3.84 (s, 6H), 3.48–3.28 (m, 6H), 2.76–2.42 (m, 8H), 2.30–1.14 (m, 32H). ^{31}P NMR (CD_3CN) δ 148.71. ESI-MS (*m/z*) 1197 $(M+H)^+$. HRMS (FAB, *m/z*) calcd for $C_{66}H_{82}N_7O_{12}PNa$, 1218.5651 $(M+Na)^+$; observed, 1218.5667. Anal. calcd for $C_{66}H_{82}N_7O_{12}P$: C, 66.26%. H, 6.91%. N, 8.20%. P, 2.59%. Found: C, 66.95%. H, 7.01%. N, 7.79%. P, 2.97%.

3.4.6. 2-{[Bis(4-methoxyphenyl)(phenyl)methoxy]-methyl}-5-[5-((1*E*)-3-{[6-(9*H*-carbazol-2-yloxy)hexyl]-amino}-3-oxoprop-1-enyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]tetrahydrofuran-3-yl 2-cyanoethyl diisopropylamidophosphite (4b). Chromatography (96:2:2). Yield (67%). Analytical RP HPLC [60:40, 254 nm] 16.53 and 19.37 min, 99%. ^{1}H NMR ($CDCl_3$) δ 9.45 (s, 1H), 7.94 (d, 1H, *J*=7.7 Hz), 7.89 (d, 1H, *J*=8.8 Hz), 7.75 (d, 1H, *J*=8.2 Hz), 7.44–7.39 (m, 3H), 7.33–7.10 (m, 9H), 7.40–6.75 (m, 8H), 6.46–6.38 (m, 1H), 6.27–6.14 (m, 2H), 4.54–4.45 (m, 1H), 4.37–4.08 (m, 1H), 4.03 (t, 2H, *J*=6.0 Hz), 3.72 (s, 6H), 3.67–3.49 (m, 3H), 3.38–3.22 (m, 2H), 3.16–3.06 (m, 4H), 2.64–2.46 (m, 1H), 2.36–2.27 (m, 2H), 2.18–2.13 (m, 1H), 2.09–2.01 (m, 1H), 1.97–1.92 (m, 2H), 1.43–1.32 (m, 5H), 1.28–1.18 (m, 5H), 1.16–1.11 (m, 9H), 1.02 (d, 3H, *J*=6.6 Hz). ^{31}P NMR ($CDCl_3$) δ 148.73, 148.76. ESI-MS (*m/z*) 1150 $(M+H)^+$, 1168 $(M+NH_4)^+$. HRMS (FAB, *m/z*) calcd for $C_{64}H_{76}N_7O_{11}PNa$ $(M+Na)^+$ 1172.5233; observed 1172.5244. Anal. calcd for $C_{64}H_{76}N_7O_{11}P$: C, 66.83%. H, 6.66%. N, 8.52%. P, 2.69%. Found: C, 67.35%. H, 6.72%. N, 7.87%. P, 2.47%.

3.4.7. 2-{[Bis(4-methoxyphenyl)(phenyl)methoxy]-methyl}-5-[5-((1*E*)-3-{[6-({[5-(dimethylamino)-1-naphthyl]sulfonyl}amino)hexyl]amino}-3-oxoprop-1-enyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]tetrahydrofuran-3-yl 2-cyanoethyl diisopropylamidophosphite (4c). Chromatography (96:2:2). The material was further purified by preparative RP HPLC [acetonitrile/water 78:22, 45 mL/min, 254 nm]. After lyophilization the yield was 41%. Analytical RP HPLC [67:33, 254 nm] 9.76 and 11.24 min, 95%. ^{1}H NMR (CD_3CN) δ 9.21 (br s, 1H), 8.52–8.49 (m, 1H), 8.25 (d, 1H, *J*=8.5 Hz), 8.16–8.13 (m, 1H), 7.79–7.75 (m, 1H), 7.61–7.52 (m, 2H), 7.44–7.39 (m, 2H), 7.33–7.15 (m, 10H), 7.07–6.95 (m, 1H), 6.90–6.83 (m, 4H), 6.22–6.06 (m, 2H), 5.82 (t, 1H, *J*=5.8 Hz), 4.55–4.46 (m, 1H), 4.17–4.08 (m, 1H), 3.72 (s, 3H), 3.71 (s, 3H), 3.67–3.48 (m, 4H), 3.39–3.23 (m, 2H), 3.03–2.95 (m, 2H), 2.82 (s, 6H), 2.81–2.75 (m, 2H), 2.64–2.46 (m, 1H), 2.44–2.28 (m, 2H), 1.26–0.93 (m, 20H). ^{31}P NMR ($CDCl_3$) δ 149.31. ESI-MS (*m/z*) 1132 $(M+H)^+$. HRMS (FAB, *m/z*) calcd for $C_{60}H_{74}N_7O_{11}PSNa$ $(M+Na)^+$ 1154.4797; observed 1154.4806. Anal. calcd for $C_{60}H_{74}N_7O_{11}PS$: C, 63.64%. H, 6.59%. N, 8.66%. P, 2.74%. S, 2.83%. Found: C, 63.68%. H, 6.61%. N, 8.33%. P, 2.46%. S, 2.60%.

3.4.8. 2-{[Bis(4-methoxyphenyl)(phenyl)methoxy]-methyl}-5-[5-{(1*E*)-3-[(6-{[(4-{(*E*)-[4-(dimethylamino)-phenyl]diazenyl}phenyl)sulfonyl]amino}hexyl)amino]-3-oxoprop-1-enyl}-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]tetrahydrofuran-3-yl 2-cyanoethyl diisopropylamidophosphite (4d). Chromatography (97:1:2). The material was further purified by preparative RP HPLC (acetonitrile/water 80:20, 45 mL/min at 254 nm). After lyophilization the yield was 69%. Analytical RP HPLC [acetonitrile/water 65:35, 254 nm] 17.32 and 20.23 min, 95%. ^{1}H NMR (CD_3CN) δ 7.92–7.74 (m, 7H), 7.44–7.38 (m, 2H), 7.32–7.16 (m, 8H), 7.50–6.97 (m, 1H), 6.87–6.78 (m, 7H), 6.21–6.09 (m, 2H), 5.72–5.65 (m, 1H), 4.54–4.45 (m, 1H), 4.16–4.06 (m, 1H), 3.72 (s, 3H), 3.71 (s, 3H), 3.68–3.46 (m, 4H), 3.38–3.22 (m, 2H), 3.12–3.06 (m, 1H), 3.06 (s, 6H), 2.89–2.81 (m, 2H), 2.61 (t, 1H, *J*=6.0 Hz), 2.48 (t, 1H, *J*=5.8 Hz), 2.45–2.27 (m, 2H), 1.42–1.25 (m, 4H), 1.25–1.09 (m, 13H), 1.01 (d, 3H, *J*=6.8 Hz). ^{31}P NMR (CD_3CN) δ 148.79. ESI-MS (*m/z*) 1185 $(M+H)^+$. HRMS (FAB, *m/z*) calcd for $C_{62}H_{76}N_9O_{11}PSNa$ $(M+Na)^+$ 1208.5015; observed 1208.5030. Anal. calcd for $C_{62}H_{76}N_9O_{11}PS$: C, 62.77%. H, 6.46%. N, 10.63%. P, 2.61%. S, 2.70%. Found: C, 62.42%. H, 6.56%. N, 10.53%. P, 2.49%. S, 2.60%.

3.4.9. 2′-Deoxyuridine-3′,5′-(1,1,3,3-tetraisopropyl)disiloxane (6). Imidazole (17.5 g, 256.5 mmol, 450 mol%) and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (20.0 g, 62.72 mmol, 110 mol%) were added to a solution of 2′-deoxyuridine (13.0 g, 57.01 mmol) in DMF (80 mL) at room temperature under nitrogen. After stirring for 23 h, the mixture was poured into water (1.1 L) and extracted with ethyl acetate (0.8 L). The organic layer was washed with

water (0.6 L), brine (0.4 L), dried over anhydrous sodium sulfate and concentrated on a rotary evaporator. The crude product was purified by silica gel column chromatography (40% ethyl acetate in hexane) to afford of **6** (27.0 g, quantitative). Analytical RP HPLC [acetonitrile/0.05% aq trifluoroacetic acid 80:20, 2.0 mL/min at 254 nm] 11.14 min, 99%. $[\alpha]_D^{23}=+34.5$ (*c* 1.5, $CHCl_3$). ^1H NMR ($CDCl_3$) δ 9.85 (br s, 1H), 7.79 (d, 1H, *J*=8.2 Hz), 6.06 (dd, 1H, *J*=6.8, 1.4 Hz), 5.71 (dd, 1H, *J*=8.2, 1.6 Hz), 4.48–4.39 (m, 1H), 4.14 (dd, 1H, *J*=13.2, 1.2 Hz), 4.01 (dd, 1H, *J*=13.2, 2.7 Hz), 3.76 (td, 1H, *J*=8.5, 2.7, 2.2 Hz), 2.57–2.46 (m, 1H), 2.30–2.23 (m, 1H), 1.12–1.07 (m, 6H), 1.06–0.95 (m, 10H). ^{13}C NMR ($CDCl_3$) δ 163.8, 150.2, 139.6, 101.7, 85.0, 84.2, 66.9, 59.9, 39.8, 17.4, 17.3, 17.3, 17.2, 17.0, 16.9, 16.7, 13.3, 12.9, 12.8, 12.3. ESI-MS (*m*/*z*) 471 $(M+H)^+$, 488 $(M+NH_4)^+$, 941 $(2M+H)^+$.

3.4.10. 6-Formyl-2′-deoxyuridine-3′,5′-(1,1,3,3-tetraisopropyl)disiloxane (7). A mixture of 2′-deoxyuridine-3′,5′-(1,1,3,3-tetraisopropyl)disiloxane (**6**, 10.0 g, 21.27 mmol) in THF (100 mL) and HMPA (22.0 mL, 127.6 mmol, 600 mol%) was added to via canula to a solution of LDA (1 M solution in cyclohexane, 42.5 mL, 63.83 mmol, 300 mol%) in THF (100 mL) at −78°C under nitrogen. After 30 min, DMF (18.1 mL, 233.9 mmol, 1100 mol%) was added at −78°C and the stirring was continued for an additional 2 h. Acetic acid (12.1 mL, 212.7 mmol, 1000 mol%) was added to the reaction at −78°C and the mixture was poured into water (350 mL). The mixture was then extracted with ethyl acetate (450 mL). The organic layer was washed with saturated aq sodium bicarbonate solution (400 mL), water (500 mL), brine (500 mL), dried over anhydrous sodium sulfate and concentrated on a rotary evaporator. The crude product was purified by silica gel column chromatography (30–40% ethyl acetate in hexanes) to obtain aldehyde **7** (5.5 g, 52%, colorless glass). ^1H NMR ($CDCl_3$) δ 9.72 (s, 1H), 8.89 (brs, 1H), 6.36 (dd, 1H, *J*=8.5, 4.9 Hz), 6.09 (s, 1H), 4.82–4.74 (m, 1H), 4.04–3.93 (m, 2H), 3.83–3.78 (d, 1H), 2.72–2.63 (m, 1H), 2.54–2.45 (m, 1H), 1.15–0.92 (m, 16H). ^{13}C NMR ($CDCl_3$) δ 184.3, 161.6, 149.3, 148.3, 109.3, 85.8, 85.0, 71.8, 62.2, 40.1, 17.5, 17.4, 17.3, 17.2, 17.1, 16.9, 16.8, 13.3, 13.1, 12.8, 12.5. ESI-MS (*m*/*z*) 516 $(M+NH_4)^+$, 1014 $(2M+NH_4)^+$.

3.4.11. (+)-6-[2-(Methoxycarbonyl)ethenyl]-2′-deoxyuridine-3′,5′-(1,1,3,3-tetraisopropyl)disiloxane (8). Methyl (triphenylphosphoranylidene)acetate (7.37 g, 22.1 mmol, 100 mol%) was added to a solution of aldehyde **7** (11.0 g, 22.1 mmol) in benzene (100 mL) at room temperature under nitrogen. The mixture was stirred for 2 h then concentrated on a rotary evaporator. Purification by silica gel column chromatography (30–40% ethyl acetate in hexanes) to afforded **8** (11.8 g, 96%, pale yellow solid). Mp 113–114°C. Analytical RP HPLC [acetonitrile/0.05% aq trifluoroacetic acid 80:20, 2.0 mL/min, 254 nm] 16.31 min, 98%. $[\alpha]_D^{23}=+26.7$ (*c* 0.86, $CHCl_3$). ^1H NMR ($CDCl_3$) δ 8.15 (br s, 1H), 7.53 (d, 1H, *J*=15.6 Hz), 6.39 (d, 1H, *J*=15.6 Hz), 5.93–5.88 (m, 1H), 5.75 (s, 1H), 4.91–4.84 (m, 1H), 4.07–3.96 (m, 2H), 3.83 (s, 3H), 3.82–3.77 (m, 1H), 2.82–2.75 (m, 1H), 2.38–2.28 (m, 1H), 1.30–1.00 (m, 16H). ^{13}C NMR ($CDCl_3$) δ 165.1, 162.2, 150.9, 149.5, 135.8, 126.7, 102.9, 85.6, 85.2, 73.3, 63.9, 52.3, 39.4, 23.4, 20.1, 17.5, 17, 4, 17.3, 17.2, 17.1, 16.9, 13.2, 12.6, 12.5. ESI-MS (*m*/*z*) 572 $(M+NH_4)^+$, 1126 $(2M+NH_4)^+$. HRMS (FAB, *m*/*z*) calcd for $C_{25}H_{43}N_2O_8Si_2$ $(M+H)^+$ 555.2558; observed 555.2537.

3.4.12. (+)-6-[2-(Methoxycarbonyl)ethenyl]-2′-deoxyuridine (9). Tetrabutylammonium fluoride (1.0 M solution in THF, 42.6 mL, 42.6 mmol, 200 mol%) was added to a solution of olefinic ester **8** (11.8 g, 21.3 mmol) in THF (500 mL) at room temperature under nitrogen. The mixture was stirred for 40 min then concentrated on a rotary evaporator. Purification by silica gel column chromatography (5% MeOH in ethyl acetate) afforded compound **9** (6.5 g, 98%). Mp 267–8°C. Analytical RP HPLC [acetonitrile/0.05% aq trifluoroacetic acid 8:92, 1.0 mL/min, 225 nm] 12.65 min, 98%. $[\alpha]_D^{23}=+44.3$ (*c* 0.91, MeOH). ^1H NMR ($CDCl_3$+0.1 mL of CD_3OD) δ 7.59 (d, 1H, *J*=15.6 Hz), 6.41 (d, 1H, *J*=15.6 Hz), 6.00 (t, 1H, *J*=7.1 Hz), 5.78 (d, 1H, *J*=0.6 Hz), 4.59–4.54 (m, 1H), 3.96–3.87 (m, 2H), 3.84 (s, 3H), 3.75 (dd, 1H, *J*=12.1, 3.5 Hz), 2.86–2.76 (m, 1H), 2.18–2.10 (m, 1H). ^{13}C NMR ($CDCl_3$+0.1 mL of CD_3OD) δ 165.5, 162.6, 151.0, 150.6, 135.7, 126.8, 103.3, 87.3, 87.2, 70.6, 62.0, 52.4, 38.1. ESI-MS (*m*/*z*) 311 $(M-H)^-$, 623 $(2M-H)^-$. HRMS (FAB, *m*/*z*) calcd for $C_{13}H_{17}N_2O_7$ $(M+H)^+$ 313.1036; observed 313.1029.

3.4.13. (+)-5′-*O*-(4,4′-Dimethoxytrityl)-6-[2-(methoxycarbonyl)ethenyl]-2′-deoxyuridine (10). Compound (**9** (6.5 g, 20.8 mmol) was dissolved in pyridine (120 mL) and the mixture was concentrated on a rotary evaporator. The residue was dissolved in THF (350 mL). Pyridine (8.4 mL, 104.1 mmol, 500 mol%) and silver nitrate (3.54 g, 20.8 mmol, 100 mol%) were added to the mixture. After stirring for 5 h at room temperature, the mixture was filtered through Celite into a saturated aq sodium bicarbonate solution (300 mL) and the filter cake was washed with 5% triethylamine in chloroform (50 mL). The filtrate was then extracted with 5% triethylamine in chloroform (500 mL). The organic layer was dried over anhydrous sodium sulfate then concentrated under reduced pressure. Purification by silica gel column chromatography (ethyl acetate/triethylamine/methanol 97:2:1) to afforded DMT–ether **10** (9.8 g, 76% as a colorless solid). Mp 100–1°C. Analytical RP HPLC [acetonitrile/water 50:50, 2.0 mL/min, 254 nm] 7.42 min, 99%. $[\alpha]_D^{23}=+18.1$ (*c* 0.585, MeOH). ^1H NMR (CD_3CN) δ 7.45–7.22 (m, 10H), 6.86–6.81 (m, 4H), 6.41 (d, 1H, *J*=15.6 Hz), 6.23–6.18 (m, 1H), 5.81 (d, 1H, *J*=0.6 Hz), 4.24–4.16 (m, 1H), 3.88–3.81 (m, 1H), 3.75 (s, 3H), 3.74 (s, 3H), 3.63 (s, 3H), 3.38–3.32 (m, 1H), 3.23–3.18 (m, 1H), 2.65–2.50 (m, 1H), 2.14–2.04 (m, 1H). ^{13}C NMR (CD_3CN) δ 166.2, 163.0, 159.6, 151.4, 151.3, 146.2, 137.0, 136.9, 136.8, 131.0, 130.9, 129.3, 129.0, 128.7, 127.7, 127.1, 103.9, 86.0, 71.8, 65.4, 55.8, 52.7, 39.1. ESI-MS (*m*/*z*) 637 $(M+Na)^+$. HRMS (FAB, *m*/*z*) calcd for $C_{34}H_{34}N_2O_9$ $(M)^+$ 614.2264; observed 614.2257.

3.4.14. *N*-(6-Aminohexyl)-2-[3-(4-nitrophenyl)-1-adamantyl]acetamide (12a). *tert*-Butyl 6-aminohexylcarbamate (2.4 g, 11.11 mmol), 3-(4-nitrophenyl)-adamantaneacetic acid (**5a**, 3.50 g, 11.11 mmol, 100 mol%), *N*-hydroxybenzotriazole (2.55 g, 16.66 mmol, 150 mol%) and triethylamine (6.20 mL, 44.44 mmol, 400 mol%) were dissolved in dichloromethane (40 mL) at room temperature under nitrogen. To this mixture EDAC (2.13 g, 16.66 mmol, 150 mol%)

was added. The reaction mixture was stirred for 5 h at room temperature then was diluted with chloroform (50 mL); washed with water (50 mL), saturated aq sodium bicarbonate (50 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated on a rotary evaporator. The crude product was purified by silica gel column chromatography (60% ethyl acetate in hexanes) to afford *N*-Boc-**12a** (4.88 g, 85%). Mp 85–6°C. Analytical RP HPLC [acetonitrile/0.05% aq trifluroacetic acid 70:30, 2.0 mL/min, 254 nm] 6.53 min, 99%. ^{1}H NMR ($CDCl_3$) δ 8.17–8.12 (m, 2H), 7.53–7.47 (m, 2H), 5.79 (br s, 1H), 4.60 (br s, 1H), 3.24 (d, 1H, J=6.8 Hz), 3.20 (d, 1H, J=6.8 Hz), 3.11 (d, 1H, J=6.3 Hz), 3.06 (d, 1H, J=6.3 Hz), 2.25–2.19 (m, 2H), 2.03 (br s, 1H), 1.90–1.85 (m, 4H), 1.82 (br s, 1H), 1.73–1.66 (m, 8H), 1.53–1.42 (m, 13H), 1.37–1.29 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 170.5, 158.0, 156.0, 145.8, 129.9, 123.3, 79.0, 51.0, 47.5, 41.9, 41.4, 40.0, 38.9, 37.7, 35.5, 33.5, 29.9, 29.4, 28.9, 28.3, 26.1, 25.8. ESI-MS (m/z) 514 $(M+H)^+$, 531 $(M+NH_4)^+$, 536 $(M+Na)^+$. HRMS (FAB, m/z) calcd for $C_{29}H_{44}N_3O_5$ $(M+H)^+$ 514.3281; observed 514.3279.

N-Boc-**12a** (0.163 g, 0.317 mmol) was dissolved in 1,4-dioxane (10 mL) then treated with hydrogen chloride in ether (1 M, 5.0 mL) for 45 h at ambient temperature. The reaction mixture was concentrated to dryness on a rotary evaporator to give **12a** hydrochloride (0.140 g). Analytical RP HPLC [acetonitrile/0.05% aq trifluoroacetic acid 40:60, 2.0 mL/min at 254 nm] 3.21 min, 98%. ^{1}H NMR (CD_3OD) δ 8.19–8.14 (m, 2H), 7.65–7.58 (m, 2H), 3.81 (t, 2H, J=7.1 Hz), 2.89 (t, 2H), J=7.7 Hz), 2.19 (brs, 1H), 2.06 (brs, 1H), 1.96–1.50 (m, 18H), 1.44–1.35 (m, 4H). ESI-MS (m/z) 414 $(M+H)^+$; HRMS (FAB, m/z) calcd for $C_{24}H_{36}N_3O_3$ $(M+H)^+$ 414.2757; observed 414.2751.

3.4.15. 6-(9*H*-Carbazol-2-yloxy)hexylamine (12b). *p*-Toluenesulfonyl chloride (3.75 g, 19.77 mmol, 110 mol%) was added to a solution of 6-(*tert*-butoxycarbonylamino)hexanol (3.90 g, 17.97 mmol) in dichloromethane (20 mL) and pyridine (4.30 mL, 53.91 mmol, 300 mol%). The mixture was stirred for 20 h at ambient temperature. The reaction mixture was diluted with dichloromethane (50 mL), washed with water (100 mL), 0.5N aq HCl (75 mL), saturated aq sodium bicarbonate (75 mL), dried over anhydrous sodium sulfate and concentrated on a rotary evaporator. The crude product was purified by silica gel column chromatography (30% ethyl acetate in hexane) to obtain 6-(*p*-toluenesulfonyloxy)-*N*-(*tert*-butoxycarbonyl)hexylamine (4.8 g, 72%). Analytical RP HPLC [acetonitrile/0.05% aq trifluoroacetic acid 50:50, 2.0 mL/min at 225 nm] 6.71 min, 99%. ^{1}H NMR ($CDCl_3$) δ 7.82–7.75 (m, 2H), 7.37–7.33 (m, 2H), 4.51 (br s, 1H), 4.01 (t, 2H, J=6.5 Hz), 3.06 (q, 2H, J=6.6 Hz), 2.45 (s, 3H), 1.68–1.60 (m, 2H), 1.48–1.38 (m, 2H), 1.43 (s, 9H), 1.36–1.22 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 155.9, 144.6, 133.1, 129.8, 127.8, 70.4, 40.3, 29.8, 28.7, 28.4, 26.0, 25.0, 21.6. ESI-MS (m/z) 372 $(M+H)^+$, 760 $(2M+NH_4)^+$.

A mixture of 2-hydroxycarbazole (2.20 g, 12.02 mmol), 6-(*p*-toluenesulfonyloxy)-*N*-(*tert*-butoxycarbonyl)hexylamine (4.80 g, 12.93 mmol, 107 mol%), sodium iodide (1.80 g, 12.02 mmol, 100 mol%) and anhydrous potassium carbonate (2.50 g, 18.03 mmol, 150 mol%) in 2-butanone (50 mL) was heated at reflux for 30 h. The reaction mixture was concentrated and the residue was partitioned between ethyl acetate (150 mL) and water (100 mL). The organic layer was separated, washed with brine (75 mL), dried over anhydrous sodium sulfate and concentrated on a rotary evaporator. The crude product was purified by silica gel column chromatography (30% ethyl acetate in hexane) to afford 2-*O*-[[6-(*tert*-butoxycarbonylamino)hexyl]oxy]carbazole (3.0 g 61%, pale yellow solid). Mp 129–30°C. Analytical RP HPLC [acetonitrile/0.05% aq trifluoroacetic acid 65:35, 2.0 mL/min at 225 nm] 11.49 min, 99%. ^{1}H NMR ($CDCl_3$) δ 8.11 (br s, 1H), 7.95 (d, 1H, J=7.4 Hz), 7.91 (d, 1H, J=8.5 Hz), 7.38–7.30 (m, 2H), 7.23–7.16 (m, 1H), 6.86–6.81 (m, 2H), 4.55 (br s, 1H), 4.00 (t, 2H, J=6.5 Hz), 3.12 (q, 2H, J=6.3 Hz), 1.85–1.76 (m, 2H), 1.56–1.33 (m, 15H). ^{13}C NMR ($CDCl_3$) δ 158.4, 156.0, 140.8, 139.5, 124.4, 123.5, 120.9, 119.4, 117.0, 110.3, 108.7, 95.3, 79.1, 68.1, 40.5, 30.0, 29.1, 28.4, 26.5, 25.7. ESI-MS (m/z) 383 $(M+H)^+$, 405 $(M+Na)^+$, 792 $(2M+NH_4)^+$. HRMS (FAB, m/z) calcd for $C_{23}H_{30}N_2O_3$ $(M)^+$ 382.2256; observed 382.2250.

2-*O*-[[6-(*tert*-Butoxycarbonylamino)hexyl]oxy]carbazole (1.21 g, 3.17 mmol) in 1,4-dioxane (10 mL) was treated with hydrogen chloride in 1,4-dioxane (4.0 M, 10 mL) for 1 h at ambient temperature. The reaction mixture was concentrated to dryness on a rotary evaporator to give of the **12b** hydrochloride (1.02 g) Analytical RP HPLC [acetonitrile/0.05% aq trifluoroacetic acid 30:70, 2.0 mL/min at 225 nm] 11.98 min, 99%. ^{1}H NMR (CD_3OD) δ 7.93–7.88 (m, 1H), 7.86 (d, 1H, J=8.8 Hz), 7.42–7.38 (m, 1H), 7.31–7.25 (m, 1H), 7.14–7.08 (m, 1H), 6.97 (d, 1H, J=2.2 Hz), 6.75 (dd, 1H, J=8.5, 2.2 Hz), 4.04 (t, 2H, J=6.3 Hz), 2.93 (t, 2H, J=7.7 Hz), 1.86–1.77 (m, 2H), 1.75–1.65 (m, 2H), 1.60–1.42 (m, 4H). ^{13}C NMR (CD_3OD). δ 159.4, 142.6, 141.3, 125.3, 124.3, 121.6, 120.0, 119.8, 118.2, 111.4, 109.2, 96.4, 69.3, 40.6, 30.0, 28.3, 27.1, 26.6. ESI-MS (m/z) 283 $(M+H)^+$; HRMS (FAB, m/z) calcd for $C_{18}H_{23}N_2O$ $(M+H)^+$ 283.1810; observed 283.1804.

3.4.16. *N*-(6-Aminohexyl)-5-(dimethylamino)naphthalene-1-sulfonamide (12c). *tert*-Butyl 6-aminohexylcarbamate (2.0 g, 9.26 mmol) and sodium carbonate (3.92 g, 37.03 mmol, 400 mol%) were dissolved in a mixture of THF/water (4:3, 30 mL). Dansyl chloride (2.49 g, 9.26 mmol, 100 mol%) in THF (17 mL) was added. After stirring the mixture for 30 min at ambient temperature, 25% aq sodium chloride (50 mL) was added and the mixture was extracted with ethyl acetate (50 mL). The organic layer was washed with 25% aq sodium chloride (50 mL), dried over anhydrous sodium sulfate and concentrated on a rotary evaporator. The crude product was purified by silica gel column chromatography (30–40% ethyl acetate in hexane) to afford *N*-Boc-**12c** (3.9 g, 94%, viscous oil). Analytical RP HPLC [acetonitrile/0.05% aq trifluoroacetic acid 50:50, 2.0 mL/min at 254 nm] 10.38 min, 99%. ^{1}H NMR ($CDCl_3$) δ 8.53 (d, 1H, J=8.5 Hz), 8.31 (d, 1H, J=8.5 Hz), 8.26–8.23 (m, 1H), 7.58–7.49 (m, 2H), 7.18 (d, 1H, J=7.8 Hz), 4.99 (t, 1H, J=6.0 Hz), 4.52 (br s, 1H), 3.01–2.92 (m, 2H), 2.91–2.84 (m, 8H), 1.43 (s, 9H), 1.37–1.29 (m, 2H), 1.28–1.21 (m, 2H), 1.17–1.04 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 156.1, 151.9, 134.8, 130.2, 129.8, 129.6, 129.5, 128.3, 123.1, 118.7, 115.1, 79.2, 45.3, 42.9, 40.1, 29.7, 29.3, 28.3, 25.8, 25.8. ESI-MS (m/z) 450 $(M+H)^+$.

HRMS (FAB, *m/z*) calcd for $C_{23}H_{35}N_3O_4S$ $(M)^+$ 448.2270; observed 448.2240.

N-Boc-**12c** (0.095 g, 0.2117 mmol) was dissolved in methanol (4.0 mL) then treated with 6N aq HCl (5 mL) for 30 min at 40°C. The mixture was concentrated to dryness to give of the **12c** hydrochloride (0.085 g). Analytical RP HPLC [acetonitrile/water 20:80, 2.0 mL/min at 254 nm] 4.99 min, 98%. ^{1}H NMR (CD_3OD) δ 8.96 (d, 1H, J=8.8 Hz), 8.64 (d, 1H, J=8.8 Hz), 8.40–8.36 (m, 1H), 8.17 (d, 1H, J=7.7 Hz), 7.93 (d, 1H, J=8.5 Hz), 7.87 (d, 1H, J=8.5 Hz), 3.52 (s, 6H), 2.90–2.82 (m, 4H), 1.60–1.51 (m, 2H), 1.48–1.38 (m, 2H), 1.32–1.23 (m, 4H). ^{13}C NMR (CD_3OD) δ 140.2, 131.1, 131.1, 130.7, 128.9, 128.6, 128.1, 127.1, 126.6, 120.7, 43.7, 40.6, 30.5, 28.4, 26.9, 26.8. ESI-MS (*m/z*) 350 $(M+H)^+$, 699 $(2M+H)^+$. HRMS (FAB, *m/z*) calcd for $C_{18}H_{28}N_3O_2S$ $(M+H)^+$ 350.1902; observed 350.1913.

3.5. Preparation of 6-haptenated-[(2*E*)-*N*-(alkyl)prop-2-enamidyl]-2′-deoxyuridines 13a–c

A solution of LiOH monohydrate (0.34 g, 8.06 mmol, 330 mol%) in water (75 mL) was added to a solution of ester (+)-**10** (1.5 g, 2.44 mmol) in THF (90 mL) and the mixture was stirred for 2 h at ambient temperature. Reaction was concentrated to dryness and the resulting crude lithium salt (**11**) was dissolved in DMF (40 mL) and a solution of HCl salt of amine **12a–c** (120 mol%) and triethylamine (2.37 mL, 17.08 mmol, 700 mol%) in DMF (40 mL) was added followed by *N*-hydroxybenzotriazole (HOBt, 0.49 g, 3.7 mmol, 150 mol%) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC, 0.71 g, 0.37 mmol, 150 mol%). The reaction mixture was stirred for 18–40 h at ambient temperature. Solid sodium bicarbonate (2.0 g) was added to the reaction and DMF was removed under reduced pressure. The residue was partitioned between ethyl acetate (300 mL) and 10% aqueous sodium bicarbonate solution (250 mL). The organic layer was dried over anhydrous sodium sulfate, concentrated on a rotary evaporator and purified by silica gel column chromatography (ethyl acetate/methanol/triethylamine 93:5:2) to afford conjugate conjugates **13a–c**.

3.5.1. (2*E*)-3-[3-(5-{[Bis(4-methoxyphenyl)(phenyl)-methoxy]methyl}-4-hydroxytetrahydrofuran-2-yl)-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl]-*N*-[6-({[3-(4-nitrophenyl)-1-adamantyl]acetyl}amino)hexyl]acrylamide (13a). (53%). Mp 109–11°C. Analytical RP HPLC [acetonitrile/water 60:40, 2.0 mL/min at 254 nm] 13.44 min, 96%. $[\alpha]_D^{23}$=+1.5 (*c* 0.975, MeOH). ^{1}H NMR (CD_3CN+0.1 mL of CD_3OD) δ 8.14–8.09 (m, 2H), 7.57–7.52 (m, 2H), 7.44–7.16 (m, 10H), 6.86–6.78 (m, 4H), 6.73–6.68 (m, 1H), 6.43 (d, 1H, J=15.1 Hz), 6.12–6.08 (m, 1H), 5.70 (d, 1H, J=0.6 Hz), 4.30–4.23 (m, 1H), 3.86–3.78 (m, 1H), 3.73 (s, 3H), 3.72 (s, 3H), 3.39–3.32 (m, 1H), 3.20–3.04 (m, 6H), 2.81–2.70 (m, 2H), 2.68–2.56 (m, 1H), 2.16–2.02 (m, 3H), 1.86–1.80 (m, 4H), 1.75–1.72 (m, 2H), 1.69–1.60 (m, 6H), 1.48–1.37 (m, 4H), 1.33–1.25 (m, 4H). ^{13}C NMR (CD_3CN+0.1 mL of CD_3OD) δ 172.1, 172.0, 164.8, 163.7, 159.6, 153.2, 151.4, 146.4, 137.3, 137.2, 132.2, 131.2, 131.1, 129.2, 128.8, 127.8, 127.2, 124.3, 114.0, 102.9, 87.0, 86.6, 86.4, 71.9, 65.7, 55.9, 51.4, 42.7, 42.3, 40.0, 39.6, 39.0, 38.8, 36.4, 35.9, 34.4, 30.2, 29.9, 27.1, 27.0. ESI-MS (*m/z*) 1013 $(M+NH_4)^+$. HRMS (FAB, *m/z*) calcd for $C_{57}H_{65}N_5O_{11}Na$ $(M+Na)^+$ 1018.4571; observed 1018.4573.

3.5.2. (2*E*)-3-[3-(5-{[Bis(4-methoxyphenyl)(phenyl)-methoxy]methyl}-4-hydroxytetrahydrofuran-2-yl)-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl]-*N*-[6-({[5-(dimethylamino)-1-naphthyl]sulfonyl}amino)hexyl]-acrylamide (13b). (25%). Mp 113–4°C. Analytical RP HPLC [acetonitrile/water 60:40, 2.0 mL/min at 225 nm] 13.68 min, 96%. $[\alpha]_D^{23}$=+3.63 (*c* 0.55, MeOH). ^{1}H NMR ($CDCl_3$+few drops of CD_3OD) δ 8.92 (br s, 1H), 7.95–7.88 (m, 2H), 7.44–7.13 (m, 13H), 6.89–6.75 (m, 6H), 6.25 (d, 1H, J=15.1 Hz), 5.99 (dd, 1H, J=8.5, 5.2 Hz), 5.62 (s, 1H), 4.49–4.42 (m, 1H), 4.03 (t, 2H, J=6.3 Hz), 3.91–3.84 (m, 1H), 3.74 (s, 6H), 3.48–3.42 (m, 1H), 3.38–3.24 (m, 3H), 2.82–2.70 (m, 1H), 2.16–2.06 (m, 1H), 1.87–1.78 (m, 2H), 1.62–1.36 (m, 6H). ^{13}C NMR ($CDCl_3$+0.1 mL of CD_3OD) δ 163.7, 163.0, 158.3, 152.2, 149.7, 144.7, 140.9, 140.8, 139.6, 139.5, 136.0, 135.9, 131.4, 130.0, 128.2, 127.6, 126.6, 124.3, 123.2, 120.8, 119.2, 119.1, 117.0, 112.9, 110.3, 108.3, 101.7, 95.4, 86.3, 85.9, 85.1, 71.7, 68.0, 64.4, 55.1, 39.5, 37.7, 29.6, 28.9, 26.2, 25.5. ESI-MS (*m/z*) 882 $(M+NH_4)^+$, 887 $(M+Na)^+$; negative ion mode, 863 $(M-H)^-$, 899 $(M-Cl)^-$. HRMS (FAB, *m/z*) calcd for $C_{51}H_{52}N_4O_9Na$ $(M+Na)^+$ 887.3626; observed, 887.3628.

3.5.3. (2*E*)-3-[3-(5-{[Bis(4-methoxyphenyl)(phenyl)-methoxy]methyl}-4-hydroxytetrahydrofuran-2-yl)-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl]-*N*-[6-(9*H*-carbazol-2-yloxy)hexyl]acrylamide (13c). (53%, as a pale yellow solid). Mp 127–8°C. Analytical RP HPLC [acetonitrile/0.05% aq trifluoroacetic acid 60:40, 2.0 mL/min, 254 nm] 8.88 min, 98%. $[\alpha]_D^{23}$=−1.1 (*c* 0.64, MeOH). ^{1}H NMR (CD_3CN) δ 8.51 (d, 1H, J=8.5 Hz), 8.27 (d, 1H, J=8.5 Hz), 8.16 (dd, 1H, J=7.1, 1.1 Hz), 7.60–7.52 (m, 2H), 7.43–7.17 (m, 11H), 6.82–6.77 (m, 4H), 6.46 (t, 1H, J=5.5 Hz), 6.41 (d, 1H, J=15.4 Hz), 6.11 (dd, 1H, J=8.5, 4.9 Hz), 5.88 (br s, 1H), 5.70 (s, 1H), 4.32–4.23 (m, 1H), 3.86–3.78 (m, 1H), 3.72 (s, 3H), 3.71 (s, 3H), 3.39–3.32 (m, 1H), 3.21–3.03 (m, 3H), 2.82 (br s, 7H), 2.73–2.56 (m, 2H), 2.13–2.03 (m, 1H), 1.30–1.19 (m, 4H), 1.12–1.02 (m, 4H). ^{13}C NMR (CD_3CN) δ 164.3, 163.2, 159.5, 153.0, 151.2, 146.2, 137.2, 137.1, 132.0, 131.2, 131.0, 130.9, 130.6, 130.4, 130.0, 129.3, 129.0, 128.7, 127.7, 124.4, 120.0, 116.2, 102.9, 86.8, 86.5, 86.2, 71.9, 65.5, 55.8, 45.7, 43.6, 39.9, 38.9, 29.9, 29.7, 26.7, 26.5. ESI-MS (*m/z*) 932 $(M+H)^+$. HRMS (FAB, *m/z*) calcd for $C_{51}H_{57}N_5O_{10}SNa$ $(M+Na)^+$ 954.3717; observed 954.3718.

3.6. Preparation of 6-haptenated-5′-*O*-dimethoxytrityl-2′-deoxyuridine phosphoramidites 14a–c

Diisopropylethylamine (0.73 mL, 4.22 mmol, 400 mol%) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.35 mL, 1.58 mmol, 150 mol%) were sequentially added to a solution of conjugate **13a–c** (1.055 mmol) in dichloromethane (15 mL) at room temperature under nitrogen. After stirring for 1 h, dry methanol (0.055 mL, 1.37 mmol, 130 mol%) was added and stirring was continued for an additional 10 min. Triethylamine (3.7 mL, 26.37 mmol, 2500 mol%) and ethyl acetate

(100 mL) were then added. The solution was washed with 10% aq sodium carbonate (80 mL), brine (50 mL), dried over anhydrous sodium sulfate and concentrated on a rotary evaporator. The crude product was purified by silica gel column chromatography (ethyl acetate/methanol/triethylamine 96:2:2) to afford 0.86 g of material, which was further purified by preparative RP HPLC (acetonitrile/water v:v shown, 45 mL/min, 225 nm). The fractions were concentrated on a rotary evaporator to afford **14a–c**.

3.6.1. 2-{[Bis(4-methoxyphenyl)(phenyl)methoxy]-methyl}-5-[6-((1*E*)-3-{[6-({[3-(4-nitrophenyl)-1-adamantyl]acetyl}amino)hexyl]amino}-3-oxoprop-1-enyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]tetrahydrofuran-3-yl 2-cyanoethyl diisopropylamidophosphite (14a). Preparative RP-HPLC (76:24). Yield (54%). Analytical RP HPLC [acetonitrile/water 76:24, 2.0 mL/min, 225 nm] 11.59 and 14.60 min, 96%. ^{1}H NMR (CD_3CN) δ 9.15 (br s, 1H), 8.14–8.10 (m, 2H), 7.56–7.52 (m, 2H), 7.45–7.40 (m, 2H), 7.34–7.15 (m, 8H), 6.88–6.78 (m, 5H), 6.50–6.45 (m, 1H), 6.40 (br t, 1H), 6.14–6.06 (m, 1H), 5.69 (d, 1H, J=1.4 Hz), 4.56–4.36 (m, 1H), 4.08–3.96 (m, 1H), 3.75–3.62 (m, 8H), 3.56–3.28 (m, 5H), 3.23–3.08 (m, 5H), 2.75–2.65 (m, 1H), 2.58 (t, 1H, J=6.0 Hz), 2.45 (t, 1H), J=6.0 Hz), 2.31–2.18 (m, 1H), 2.17–2.11 (m, 2H), 1.89–1.81 (m, 4H), 1.75 (br s, 1H), 1.69–1.62 (m, 6H), 1.48–1.38 (m, 4H), 1.36–1.27 (m, 4H), 1.14–1.05 (m, 9H), 0.92 (d, 3H, J=6.8 Hz). ^{31}P NMR (CD_3CN) δ 148.63, 148.55. ESI-MS (m/z) 1197 $(M+H)^+$, 1218 $(M+Na)^+$; negative ion mode, 1195 $(M-H)^-$. HRMS (FAB, m/z) calcd for $C_{66}H_{82}N_7O_{12}PNa$ $(M+Na)^+$ 1218.5651; observed 1218.5652. Anal. calcd for $C_{66}H_{82}N_7O_{12}P$: C, 66.26; H, 6.91; N, 8.20; P, 2.59. Found: C, 66.41; H, 6.99; N, 8.17; P, 2.46.

3.6.2. 2-{[Bis(4-methoxyphenyl)(phenyl)methoxy]-methyl}-5-[6-((1*E*)-3-{[6-({[5-(dimethylamino)-1-naphthyl]sulfonyl}amino)hexyl]amino}-3-oxoprop-1-enyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]tetrahydrofuran-3-yl 2-cyanoethyl diisopropylamidophosphite (14b). Preparative RP HPLC (77:23). Yield (42%). Analytical RP HPLC [acetonitrile/water 75:25, 2.0 mL/min, 225 nm] 14.47 and 18.14 min, 98%. ^{1}H NMR (CD_3CN) δ 9.28 (br s, 1H), 7.93 (d, 1H, J=7.7 Hz), 7.89 (d, 1H, J=8.5 Hz), 7.42–7.11 (m, 13H), 6.96 (d, 1H, J=2.2 Hz), 6.84–6.72 (m, 6H), 6.45–6.39 (m, 1H), 6.17–6.07 (m, 1H), 5.70 (d, 1H, J=2.5 Hz), 4.55–4.34 (m, 1H), 4.03 (t, 2H, J=6.5 Hz), 4.0–3.92 (m, 1H), 3.74–3.62 (m, 7H), 3.55–3.31 (m, 5H), 3.27–3.18 (m, 3H), 2.73–2.63 (m, 1H), 2.57 (t, 1H, J=6.3 Hz), 2.44 (t, 1H, J=6.0 Hz), 2.30–2.22 (m, 1H), 1.83–1.74 (m, 2H), 1.56–1.35 (m, 6H), 1.12–1.03 (m, 9H), 0.92 (d, 3H, J=6.6 Hz). ^{31}P NMR (CD_3CN) δ 148.66, 148.59. ESI-MS (m/z) 1065 $(M+H)^+$; negative ion mode, 1063 $(M-H)^-$. HRMS (FAB, m/z) calcd for $C_{60}H_{69}N_6O_{10}PNa$ $(M+Na)^+$ 1087.4705; observed 1087.4791. Anal. calcd for $C_{60}H_{69}N_6O_{10}P$: C, 67.65; H, 6.53; N, 7.89; P, 2.91. Found: C, 67.48; H, 6.49; N, 7.60; P, 2.80.

3.6.3. 2-{[Bis(4-methoxyphenyl)(phenyl)methoxy]-methyl}-5-[6-((1*E*)-3-{[6-(9*H*-carbazol-2-yloxy)hexyl]-amino}-3-oxoprop-1-enyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]tetrahydrofuran-3-yl 2-cyanoethyl diisopropylamidophosphite (14c). Preparative RP HPLC (72:28). Yield (37%). Analytical RP HPLC [acetonitrile/water 72:28, 2.0 mL/min, 225 nm] 13.98 and 17.76 min, 96%. ^{1}H NMR (CD_3CN) δ 8.97 (br s, 1H), 8.51 (d, 1H, J=8.5 Hz), 8.27 (d, 1H, J=8.5 Hz), 8.18–8.15 (m, 1H), 7.62–7.56 (m, 2H), 7.45–7.40 (m, 2H), 7.34–7.16 (m, 9H), 6.84–6.77 (m, 4H), 6.59 (br q, 1H, J=5.5 Hz), 6.44–6.39 (m, 1H), 6.16–6.07 (m, 1H), 5.81 (t, 1H, J=6.0 Hz), 5.70–5.69 (m, 1H), 4.55–4.36 (m, 1H), 4.01–3.93 (m, 1H), 3.75–3.51 (m, 6H), 3.52–3.60 (m, 2H), 3.56–3.24 (m, 4H), 3.31–3.03 (m, 2H), 2.85–2.77 (m, 8H), 2.74–2.64 (m, 1H), 2.57 (t, 1H, J=6.0 Hz), 2.45 (t, 1H, J=6.0 Hz), 2.31–2.16 (m, 1H), 1.30–1.20 (m, 4H), 1.12–1.03 (m, 13H), 0.92 (d, 3H, J=6.6 Hz). ^{31}P NMR (CD_3CN) δ 148.64, 148.55. ESI-MS (m/z) 1132 $(M+H)^+$; negative ion mode, 1130 $(M-H)^-$. HRMS (FAB, m/z) calcd for $C_{60}H_{74}N_7O_{11}PSNa$ $(M+Na)^+$ 1154.4797; observed 1154.4806. Anal. calcd for $C_{60}H_{74}N_7O_{11}PS$: C, 63.64; H, 6.59; N, 8.66; P, 2.74; S, 2.83. Found: C, 63.56; H, 6.84; N, 8.56.

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