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Hapten-phosphoramidites based on 6-[(2*E*)-*N*-(hexyl)prop-2-enamidyl]-2′-deoxyuridine

Maciej Adamczyk,* Srinivasa Rao Akireddy, Phillip G. Mattingly and Rajarathnam E. Reddy

Dept. 9NM, Bldg AP20, Diagnostics Division, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064-6016, USA

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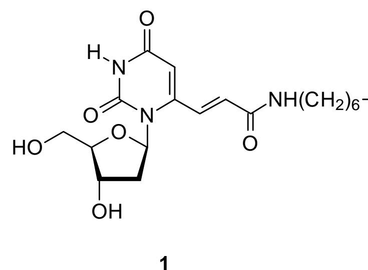
Abstract—Novel hapten-phosphoramidites **11a–c** were prepared from 2′-deoxyuridine (**2**) by functionalization at the 6-position and subsequent conjugation with adamantane, carbazole and dansyl reporter groups in good overall yield. © 2001 Elsevier Science Ltd. All rights reserved.

The inclusion of reporter groups in synthetic oligonucleotides is essential for the detection, quantification and identification of nucleic acid target sequences as a part of medical diagnoses or characterization of biotechnological processes.[1–3] Such reporter molecules are a diverse group that include fluorescent[4] and chemiluminescent species[5] that can be directly measured, and other low molecular weight compounds that facilitate indirect detection. The latter category includes species with high affinity receptors (biotin/avidin)[6–9] and an assortment of haptens (molecules that can be measured by immunoassays).[10] The reporter groups are conveniently introduced by solid-phase phosphoramidite chemistry using both nucleoside[6,9,11–14] and non-nucleoside scaffolds.[2,15] The nucleoside scaffold approach first described by Ruth[16,17] utilized an amino-containing linker at the 5-position of deoxyuridine to which the reporter group is attached. However, functionalization of deoxyuridine at the 6-position for this purpose has not been exploited. An explanation may lie in the report that 6-methyl-2′-deoxyuridine lowers the melt temperature (T_m) of some antisense oligonucleotides by varying degrees depending on the overall sequence, placement within the sequence and frequency of substitution.[18,19] The authors believed that 6-Me-dU adopted a *syn* conformation which was unfavorable for Watson–Crick hydrogen bonding. In light of our success incorporating reporter groups into oligonucleotide probes using *non-nucleoside scaffolds*[4,10] which also lack Watson–Crick hydrogen bonding, and using them in hybridization assays, we proceeded to prepare a series of hapten-phosphoramidites (**11a–c**) with a common scaffold, 6-[(2*E*)-*N*-(hexyl)prop-2-enamidyl)]-2′-deoxyuridine (**1**).



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Thus, 3′- and 5′-hydroxyl groups of 2′-deoxyuridine (**2**) (Scheme 1) were protected with 1,1,3,3-tetraisopropyl disioxane-1,3-diyl (TIPDS)[20,21] group to give **3**, which was then deprotonated at the 6-position using lithium diisopropylamide and reacted with DMF in the presence of HMPA.[13] The crude reaction mixture was treated with acetic acid at −78°C to give the aldehyde (**4**) in 52% yield after purification by silica-gel column chromatography (unreacted starting material **3**, could be recovered and recycled). The aldehyde **4** was then subjected to a Wittig reaction with methyl (triphenylphosphoranylidene)acetate in benzene to afford unsaturated ester **5** in almost quantitative yield as a *E*-isomer (>98%). The next step was to exchange the silyl protective group at the 5′-position of **5** to a 4,4′-dimethoxytrityl group (DMT), which is compatible for automated oligonucleotide synthesis. Thus, TIPDS protective group in **5** was hydrolyzed by treatment with tetra-*n*-butylammonium fluoride in THF and the crude product was purified by silica-gel column chromatography to afford diol (+)-**6** in excellent yield (98%). The 5′-hydroxyl group in **6** was protected as DMT ether by treatment with 4,4′-dimethoxytrityl chloride in the presence of silver nitrate and pyridine. The crude com-

* Corresponding author. E-mail: maciej.adamczyk@abbott.com

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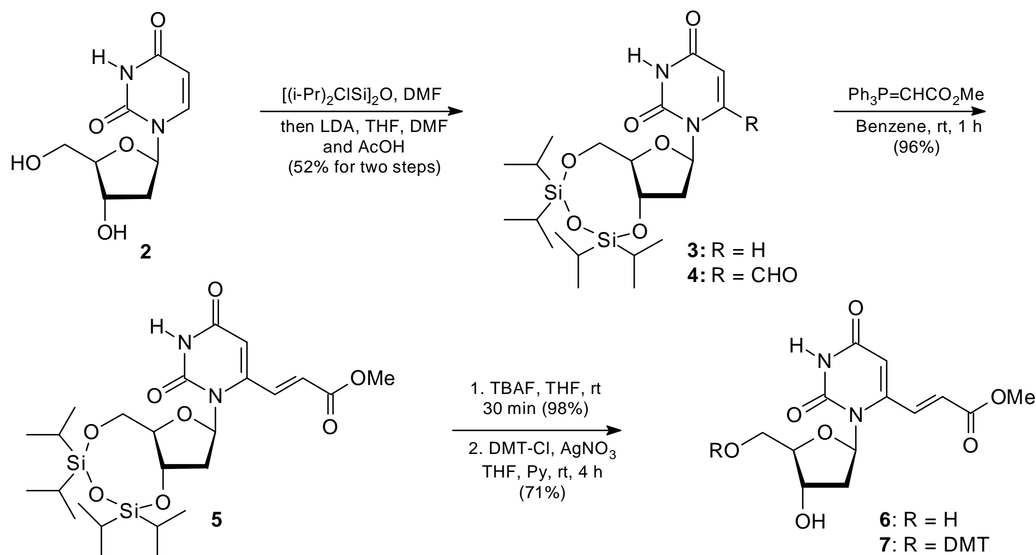
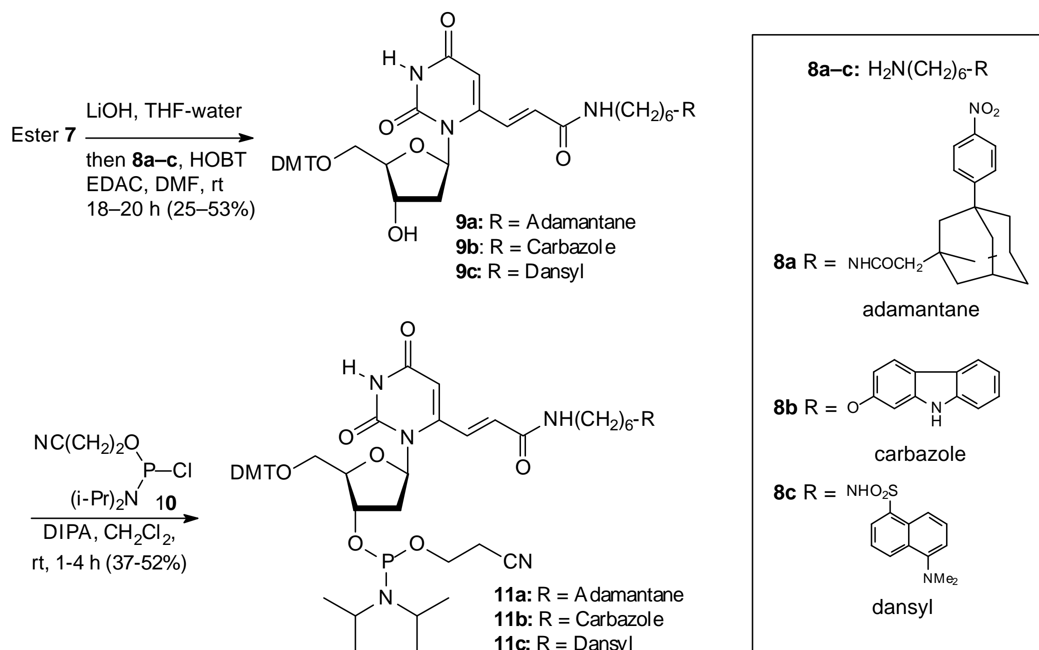


Scheme 1.

pound was purified by silica-gel column chromatography using EtOAc:Et_3N:MeOH in 97:2:1 ratio to afford ester **7** in 76% yield as a colorless solid (mp: 100–101°C). The ester **7** has the required protective group (DMT) at 5′-hydroxyl group and free 3′-hydroxy functionality for introduction of 2-(cyanoethyl)-*N*,*N*-diisopropylphosphoramidite, which are needed for incorporation of modified nucleotide building block into oligonucleotide via solid-phase synthesis.

The next step in synthesis of modified nucleotide building blocks **11a**–**c** was to conjugate ester **7** to the hapten reporter groups. Accordingly, adamantane, carbazole and dansyl reporter groups were coupled to a C-6 linking arm containing a terminal amino functionality to provide **8a**–**c**.[22] The ester **7** was then subjected to hydrolysis (Scheme 2) with lithium hydroxide in THF–water and the resulting crude acid was conjugated with amines **8a**–**c** using HOBt and EDAC in anhydrous DMF. The conjugates **9a**–**c** were purified by silica-gel column chromatography in 25–53% yield. Finally, **9a**–**c** were treated with (2-cyanoethyl)-*N*,*N*-diisopropylchlorophosphoramidite (**10**) in THF in the presence of 4.0 equiv. of diisopropylethylamine and the crude product was purified by preparative reversed-phase HPLC[23] to afford the phosphoramidites **11a**–**c** in 37–52% yield[24] as a mixture of diastereomers.



Scheme 2.

Adamantane, carbazole and dansyl haptens (**8a**–**c**) form highly antigenic protein conjugates that elicit selective, high affinity antibodies.[25–27] The distinctive structure of the haptens ensures minimal cross-reactivity with potentially interfering substances when they are used in nucleic acid testing (NAT). The requisite hapten-phosphoramidites (**11a**–**c**) necessary for NAT were prepared on a 6-[(2*E*)-*N*-(6-hexyl)prop-2-enamidyl)]-2′-deoxyuridine scaffold in good overall yield from 2′-deoxyuridine.

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22. The amine **8a** was prepared from 3-(4-nitrophenyl)adamantaneacetic[10] and 2-amino-6-(*tert*-butoxycarbonyl)aminohexane in two steps (HOBt, Et_3N, EDAC in CH_2Cl_2 then 6.0 M aq HCl); amine **8b** was prepared from 2-hydroxy-carbazole and 6-(*p*-toluenesulfonyloxy)-*N*-(*tert*-butoxycarbonyl)aminohexane in two steps (anhydrous K_2CO_3, NaI, methyl ethyl ketone then 4.0 M aq HCl); amine **8c** was prepared from dansyl chloride and 2-amino-6-(*tert*-butoxycarbonyl)-aminohexane in two steps (Na_2CO_3 THF–water then 6.0 M aq HCl).
23. Preparative reversed-phase (RP) HPLC was carried out using Waters column (Symmetry, C18, 7.0 μm, 10×100 mm; MeCN:water/75:25, 45.0 mL/min at 225 nm). Analytical reversed phase (RP) HPLC was carried out using Waters column (Symmetry, C18, 7.0 μm, 10×100 mm).
24. Selected data for DNA Probes **11a**–**c**: Probe **11a**: Analytical RP HPLC: MeCN:water/76:24, 2.0 mL/min at 225 nm, t_R: 11.59 and 14.60 min, 96%; ^1H NMR (CD_3CN): 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, 8 H), 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, 8 H), 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): 148.63, 148.55; ESI–MS (*m*/*z*): 1197 $(M+H)^+$, 1218 $(M+Na)^+$. Probe **11b**: Analytical RP HPLC: MeCN:water/75:25, 2.0 mL/min at 225 nm, t_R: 14.47 and 18.14 min, 98%; ^1H NMR (CD_3CN): 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): 148.66, 148.59; ESI–MS (*m*/*z*): 1065 $(M+H)^+$. Probe **11c**: Analytical RP HPLC: MeCN:water/72:28, 2.0 mL/min at 225 nm, t_R: 13.98 and 17.76 min, 96%; ^1H NMR (CD_3CN): 8.97 (brs, 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): 148.64, 148.55; ESI–MS (*m*/*z*): 1132 $(M+H)^+$.
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