Synthesis of Uridine and 2'-Deoxyuridine Mono- and Tri-phosphates Alkylated in Position 5 by Glycosides of α-D-Mannose and *N*-Acetyl-β-D-glucosamine: DNA and RNA Monomers with Tethered Lectin Targets

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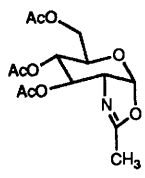
A simple synthesis of deoxyuridine and uridine 5'-mono- and 5'-tri-phosphates, C-5 alkylated by glycosides of 2-acetamido-2-deoxy-β-D-glucopyranose and α-D-mannopyranose, is reported. The glycosides (**5**) and (**7**) have been obtained respectively, by condensation of 3,4,6-tri-*O*-acetyl-1,2-dideoxy-1,2-dihydro-2'-methyl-α-D-glucopyranoso[2,1-*d*]oxazole (**2**), and tetra-*O*-benzoyl-α-D-mannopyranosyl bromide (**3**) with 6-(*N*-acryloylamino)hexan-1-ol (**1**) as aglycone. These two glycosides were then coupled to the C-5 position of dUMP and UMP in the presence of Li_2PdCl_4 to give compounds (**15**)–(**18**) in 30–45% yield. Their structure and those of the by-products (**13**), (**14**), (**22**), and (**23**) were determined by mass and NMR spectroscopies. The same reaction was successfully applied to the synthesis of triphosphate analogues (**19**)–(**21**) in 14–19% yield.

Several alternative methods to the use of radioisotopes have been recently developed for labelling acid probes.[1–3] Biotin used as a label allows the detection of DNA in the sub-picogram range. However, the use of an avidin (or steptavidin)–enzyme complex as a detection system is prone to give spurious results.[4]

The choice of C-5 in the pyrimidine ring as the attachment site of the label is widely made, due to the low interference with base-pairing and thus with hybridization of the probe. Several non-radioactive labels have been coupled to deoxyuridine and then incorporated into DNA either chemically[4–6] or enzymatically through the corresponding triphosphate.[1,7,8]

We now propose the use of carbohydrates as reporter labels. Lectins are plant storage proteins able to bind carbohydrates specifically and reversibly in a non-covalent fashion.[9] They should therefore bind to nucleic acids containing modified target carbohydrates.[10] In this paper, we describe the preparation of C-5-substituted deoxyuridines and uridines as 5'-mono- and -tri-phosphate derivatives according to Ward's methodology.[1] The preparation of these compounds should be also possible by phosphorylation of the corresponding suitably protected nucleoside derivatives. We chose to avoid this route in order to eliminate several additional reactions and purification steps. In a preliminary study, we have derivatized the pyrimidine moiety with two monosaccharides, D-mannose and 2-acetamido-2-deoxy-D-glucopyranose, using an alkyl tether.

$HO[CH_2]_6NHCOCH{=}CH_2$



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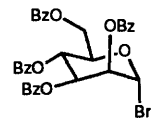

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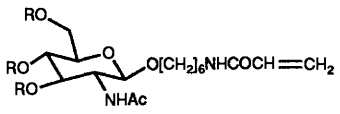
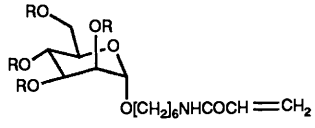


(4) R = Ac
(5) R = H



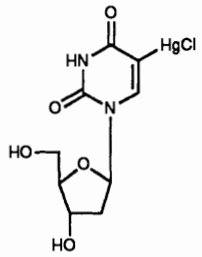
(6) R = Bz
(7) R = H

Results

The synthetic route is based on the common palladium-catalysed addition of alkenes to C-5-mercuriated uridines and deoxyuridines (modification of the Heck reaction).[11–13] As previously reported, we observed that the use of a conjugated olefin is essential in order to obtain efficient condensation with C-5-mercuriated pyrimidine derivatives. Condensation of a simple olefin ending with a sugar on a C-5-mercuriated deoxyuridine was inefficient (data not shown).[8]

In a first step, we synthesized the two olefinic glycosides (**5**) and (**7**). Reaction of 6-aminohexan-1-ol with a small excess of acryloyl chloride, followed by *O*- and *N*-deacylation with sodium methoxide, gave 6-(*N*-acryloylamino)hexan-1-ol (**1**) in 53% isolated yield. Condensation of the hydroxy acrylamide (**1**) with 3,4,6-tri-*O*-acetyl-1,2-dideoxy-1,2-dihydro-2'-methyl-α-D-glucopyranoso[2,1-*d*]oxazole (**2**),[14] or with tetra-*O*-benzoyl-α-D-mannopyranosyl bromide (**3**)[15] afforded the protected glycosides (**4**) and (**6**), in 73 and 58% yield respectively. Treatment of compounds (**4**) and (**6**) with sodium methoxide gave the corresponding deprotected glycosides (**5**) and (**7**), in 86 and 94% yield respectively.

Condensation of C-5-mercuriated deoxyuridine (**8**)[16,17] with glycosides (**5**) or (**7**) (2 mol equiv. with regard to the mercuriated nucleoside), catalysed by lithium tetrachloropalladate (Li_2PdCl_4),[18] gave as major products the expected C-5-substituted deoxyuridines (**9**) and (**10**), respectively. Purification of crude reaction materials was accomplished by chromatography on a Sephadex G-10 column, followed by high-



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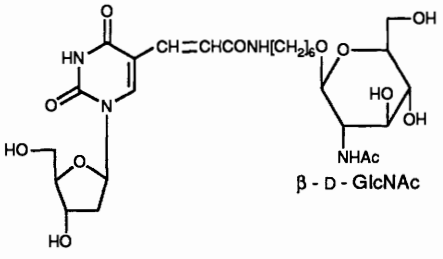
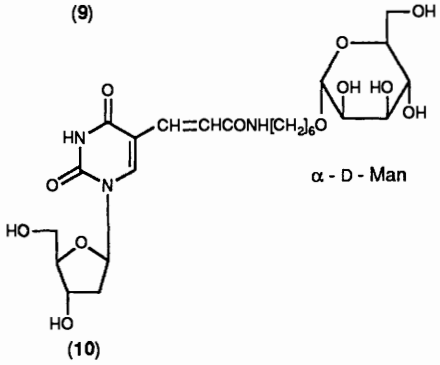


β - D - GlcNAc

(9)

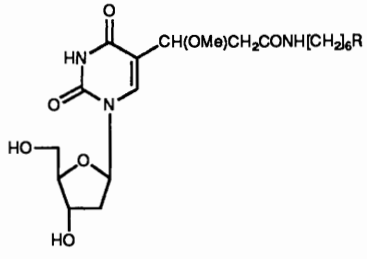


α - D - Man

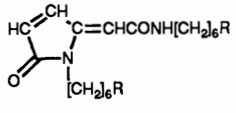
(10)

performance liquid chromatography (hplc) on a Nucleosil 5-C18 reverse-phase silica gel column. We thus isolated compound (**9**) in 17% yield, and (**10**) in 25% yield, and starting materials (**5**) and (**7**) could also be recovered. Using glycoside (**7**) (5 mol equiv.), we similarly obtained compound (**10**) in 68% yield. The ^{1}H NMR spectra of the nucleosides (**9**) and (**10**) indicated the presence of the *E*-stereoisomer (*J* coupling constants of 16 Hz for the olefin protons and a C-6 pyrimidine singlet). The UV spectra (pH 7.5) showed two characteristic absorptions at 268 and 300 nm.

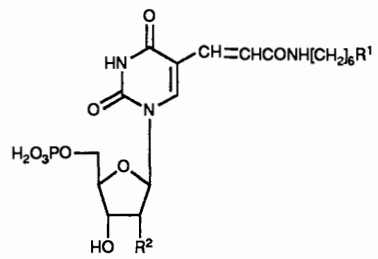
In addition to the nucleoside (**9**) or (**10**), we detected the formation of two by-products (**11**) or (**12**), and (**13**) or (**14**), in modest yield. In the case of the condensation of compound (**7**) with C-5-mercuriated deoxyuridine (**8**), these by-products were identified by their UV, NMR, and mass spectra, as the palladium-catalysed adduct of methanol to the C-5 vinylic bond (**12**), and the product from the self-condensation of the glycoside (**14**), in 12 and 4.5% yield respectively.



(**11**) R = β - D - GlcNAc
(**12**) R = α - D - Man



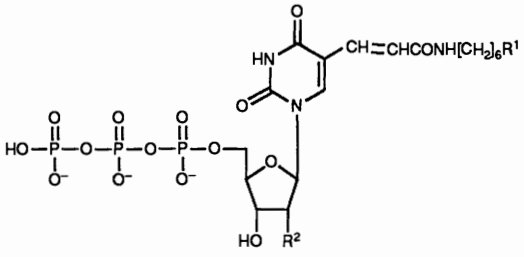
(**13**) R = β - D - GlcNAc
(**14**) R = α - D - Man



(**15**) R^1 = β - D - GlcNAc, R^2 = H
(**16**) R^1 = α - D - Man, R^2 = H
(**17**) R^1 = β - D - GlcNAc, R^2 = OH
(**18**) R^1 = α - D - Man, R^2 = OH

We extended this reaction to the synthesis of substituted monophosphate (dUMP, UMP), and triphosphate (dUTP, UTP) nucleosides, and used for the condensation 5 mol equiv. of the olefin with regard to the mercuriated nucleoside. The crude reaction mixtures were purified by chromatography on a DEAE-cellulose column, followed by hplc with a Nucleosil 5-C18 reverse-phase silica gel column.

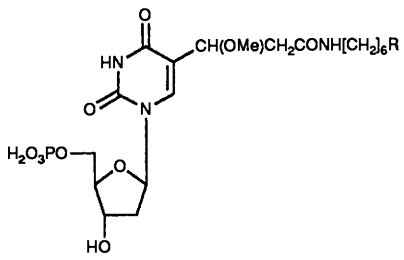
Condensation of mercuriated dUMP and UMP with olefin (**5**) or (**7**) afforded the corresponding 5-substituted 5′-monophosphate nucleosides (**15**)–(**18**) in 30–45% yield. The ^{1}H NMR spectra of compounds (**15**)–(**18**) indicated a mixture of *E* (*J* 16–18 Hz) and *Z* (*J* 8–11 Hz) isomers, which could not be separated by hplc. The UV spectra (pH 7.5) showed two characteristic absorptions at 260 and 300 nm. The ^{31}P NMR spectra exhibited a signal at +1.2 ppm (in D_2O) for the deoxyuridine derivatives (**15**) and (**16**), and a signal at +1.8 and +2.7 ppm (in D_2O) for the uridine derivatives (**17**) and (**18**), respectively. As in the nucleoside series, two by-products from palladium-catalysed addition of methanol to the C-5 vinylic bond, and from the self-condensation of glycosides, were again observed. The structure of these by-products in the deoxyribose series, (**22**) and (**23**) and (**13**) and (**14**) respectively, was inferred on the basis of NMR evidence (see Experimental section). As an example, the ^{13}C NMR spectrum of compound (**13**) recorded at 150 MHz is detailed.



(**19**) R^1 = β - D - GlcNAc, R^2 = H
(**20**) R^1 = α - D - Man, R^2 = H
(**21**) R^1 = β - D - GlcNAc, R^2 = OH

Likewise, condensation of mercuriated dUTP (or UTP) with glycosides (**5**) and (**7**) produced, mainly, the corresponding 5-substituted 5′-triphosphate nucleosides (**19**)–(**21**), in 12–19% yield after hplc purification. The ^{31}P NMR spectra of the latter compounds presented three characteristic signals at −23.2 ppm (t), −11.5 ppm (d), and −10.9 ppm (d) with *J* 21 ± 1 Hz.

The enzymatic incorporation of the 5′-substituted dUTP (**19**) and (**21**) into DNA is under study. We are also investigating the extension of our synthetic scheme to more complex carbohydrates like chitobiose and chitotriose, in order to

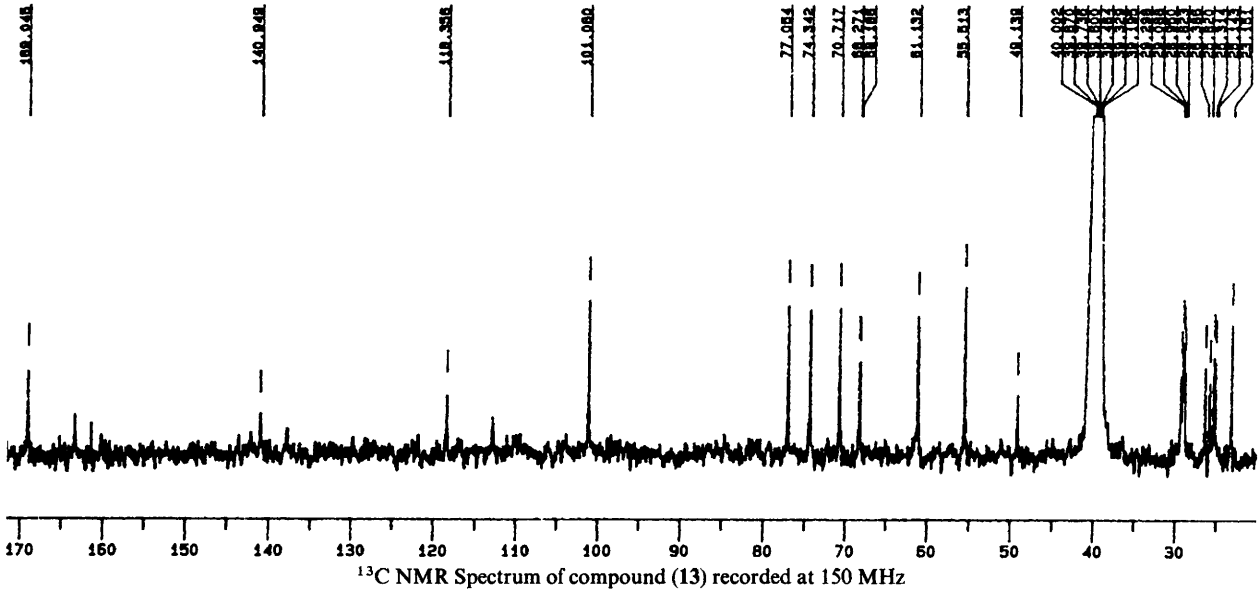


(22) R = β - D - GlcNAc
(23) R = α - D - Man

increase the binding affinity and specificity by appropriate lectins.

of each nucleoside was checked by hplc on a Nucleosil 5-C18 column by using a gradient of 5–50% of A in B during 20 min at a flow rate of 0.5 ml min^{-1} (retention times are given in the Experimental section), on a Perkin-Elmer Series 4 hplc instrument coupled to a Hewlett Packard 1040 A diode-array detector. 2′-Deoxyuridine and uridine were obtained from Pharma Waldhof; 2′-deoxyuridine 5′-monophosphate disodium salt, 2′-deoxyuridine and uridine 5′-triphosphate disodium salts were purchased from Sigma, and uridine 5′-monophosphate disodium salt from Aldrich.

6-(N-*Acryloylamino*)*hexan*-1-*ol* (**1**).—To a stirred solution of 6-aminohexan-1-ol (4.69 g, 40 mmol) in a mixture of dry dichloromethane (100 ml) and distilled triethylamine (20 ml) at 0 °C was added dropwise a solution of acryloyl chloride (12 ml) in dichloromethane (10 ml). The solution was stirred at 0 °C for 1 h, and then for 3 h at room temperature. At this stage TLC



^{13}C NMR Spectrum of compound (**13**) recorded at 150 MHz

Experimental

M.p.s were taken on a Kofler hot-plate. TLC was performed on silica gel $60F_{254}$ (Merck). All compounds were located by spraying with 10% sulphuric acid and heating, or with anisaldehyde–H_2SO_4–EtOH (1:1:18, v:v:v) and charring at 110 °C for few minutes. Phosphorus-containing compounds were revealed by spraying with Dittmer and Lester reagent.[19] ^1H NMR spectra were obtained on a Bruker WH-90, an MSL-300, or a Jeol GSX-270 instrument. Assignments of ^1H NMR spectral peaks were achieved by using ^1H–^1H-shift-correlated 2D-NMR spectroscopy (COSY). ^{31}P NMR spectra were recorded on a Bruker MSL-300 instrument operating at 121 MHz, or on a Jeol GSX-270 at 109.25 MHz, with H_3PO_4 as external reference. ^{13}C NMR spectra were recorded on a Bruker MSL-300 instrument operating at 75.47 MHz or on a Jeol GSX-270 instrument at 67.80 MHz. Complete assignments of ^{13}C NMR resonances were performed by using ^{13}C–^1H-shift-correlated 2D-NMR spectroscopy. Mass spectra were recorded on a NERMAG R10–10C apparatus for chemical ionization (CI), and on a VG 70–250 double-focusing instrument (VG instruments, Le Chesnay, France) equipped with a fast-atom bombardment gun (Ion Tech., UK) for fast-atom bombardment (FAB). The gun was operated with xenon at 8 kV and 1 mA. Caesium iodide or glycerol were used for calibration, glycerol as the matrix. Purification by hplc was accomplished on a Nucleosil 5-C18 column, by using a gradient of acetonitrile (A) in a 0.05M-triethylammonium acetate buffer (pH 7.5) (B). Purity [CH_2Cl_2–MeOH (95:5)] revealed that most of the amino alcohol had reacted affording a mixture of the di- and tri-acryloylated compounds. Addition of methanol followed by evaporation to dryness resulted in a gum which could be used directly for the saponification step.

The crude mixture of acryoylated products (18.7 g) was treated with a 1% solution of sodium methoxide in methanol (100 ml). After completion of the reaction visualized by TLC [CH_2Cl_2–MeOH (90:10)], the solution was neutralized with Dowex 50W resin (H^+). Filtration and evaporation to dryness afforded a syrup (6.9 g), which was purified by column chromatography on silica gel with dichloromethane enriched with methanol as eluant to give the title compound (**1**) (3.6 g, 53%), m.p. 49–51 °C (from methanol); R_f 0.40 [CH_2Cl_2–MeOH (90:10)]; δ_H(90 MHz; DMSO) 1.36 (8 H, m, 4 × CH_2), 3.15 (2 H, q, *J* 5.7 Hz, NCH_2), 3.42 (2 H, m, OCH_2), 4.34 (1 H, m, OH), 5.58 (1 H, dd, *J* 4.5 and 10 Hz, =C*H*H *cis*), 6.03 (1 H, dd, *J* 4.5 and 18 Hz, =CH*H trans*), 6.20 (1 H, dd, *J* 10 and 18 Hz, =CH), and 8.02 (1 H, m, NH); *m*/*z* (CI, NH_3) 189 (*M* + 18), 172 (*M* + H^+) (Found: C, 63.0; H, 10.0; N, 8.1. Calc. for $C_9H_{17}NO_2$: C, 63.12; H, 10.00; N, 8.18%).

6-(N-*Acryloylamino*)*hexyl* 2-*Acetamido*-2-*deoxy*-3,4,6-*tri*-O-*acetyl*-β-D-*glucopyranoside* (**4**).—A solution of 3,4,6-tri-*O*-acetyl-1,2-dideoxy-1,2-dihydro-2′-methyl-α-D-glucopyranoso-[2,1-*d*]oxazole (**2**)[14] (5.10 g, 15.5 mmol) in a mixture of anhydrous toluene (60 ml) and anhydrous nitromethane (60 ml)

was stirred at 70 °C with toluene-*p*-sulphonic acid (25 mg) and anhydrous hydroxy acrylamide (**1**) (2.91 g, 17 mmol). TLC examination [toluene–diethyl ether–methanol (7:7:1)] showed the reaction to be complete after 14 h. The reaction mixture was diluted with dichloromethane (200 ml) and washed in turn with ice–water, saturated aqueous sodium hydrogen carbonate and ice–water, and dried (Na_2SO_4). Solvents were removed under reduced pressure and the residue was purified on silica gel (same solvent as for tlc) to afford the 6-(N-*acryloylamino*)*hexylglycoside* (**4**) (4.5 g, 58%), δ_H(90 MHz; $CDCl_3$) 1.40 (8 H, m, 4 × CH_2), 1.95 (3 H, s, *Me*CONH), 2.02 and 2.08 (9 H, 2 s, 3 × AcO), 3.35 (2 H, m, NCH_2), 3.81 (2 H, m, OCH_2), 4.68 (1 H, d, $J_{1,2}$ 8 Hz, 1-H), 5.65 (1 H, dd, *J* 3.5 and 8 Hz, =C*H*H *cis*), 6.07 and 6.29 (2 H, each dd, *J* 16 and 8 Hz, =CH*H trans* and =CH), 6.43 (1 H, m, N*H*CH_2), and 6.70 (1 H, d, *J* 9.9 Hz, AcN*H*); *m/z* (CI, NH_3) 501 ($M + H^+$) (Found: C, 55.0; H, 7.2; N, 5.5. $C_{23}H_{26}N_2O_{10}$ requires C, 55.20; H, 7.25; N, 5.60%).

6-(N-*Acryloylamino*)*hexyl* 2-*Acetamido*-2-*deoxy*-β-D-*glucopyranoside* (**5**).—The glucosaminide (**4**) (0.39 g, 0.78 mmol) was treated with a catalytic quantity of sodium methoxide in methanol (10 ml) at room temperature for 20 min. The solution was neutralized with Dowex 50W resin (H^+) and then filtered and evaporated to give the title compound (**5**) (0.25 g, 86%); δ_H(250 MHz; CD_3OD) 1.36 (4 H, m, 2 × CH_2), 1.53 (4 H, m, 2 × CH_2), 1.96 (3 H, s, *Me*CONH), 3.25 (2 H, m, NCH_2), 3.30–3.90 (8 H, m, OCH_2, 2-, 3-, 4-, 5-H, and 6-H_2), 4.37 (1 H, d, $J_{1,2}$ 8 Hz, 1-H), 5.55 (1 H, dd, *J* 4 and 9 Hz, =C*H*H *cis*), 6.07 (1 H, dd, *J* 4 and 17.5 Hz, =CH*H trans*), and 6.24 (1 H, dd, *J* 9 and 17.5 Hz, =CH); *m/z* (CI, NH_3) 375 ($M + H^+$) (Found: C, 54.3; H, 8.2; N, 7.2. Calc. for $C_{17}H_{30}N_2O_7$: C, 54.52; H, 8.07; N, 7.48%)..

6-(N-*Acryloylamino*)*hexyl* 2,3,4,6-*Tetra*-O-*benzoyl*-α-D-*mannopyranoside* (**6**).—A solution of hydroxy acrylamide (**1**) (0.51 g, 3 mmol) in a mixture of nitromethane (15 ml) and toluene (15 ml) containing mercury(II) cyanide (1.13 g, 4.5 mmol), mercury(II) bromide (1.62 g, 4.5 mmol), and molecular sieves (3 Å and 4 Å) was stirred for 2 h at room temperature and was then treated with 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide (**3**)[15] (2.96 g, 4.5 mmol) for 20 h. At this stage TLC [EtOAc–hexane (2:1)] revealed that the bromo sugar (R_f 0.94) had all reacted, and that the glycoside (**6**) (R_f 0.46) had been formed. The reaction mixture was diluted with toluene, and solids were filtered off through Celite. The filtrate was washed with an ice-cold solution of sodium hydrogen carbonate, then with ice–water, and was then dried (Na_2SO_4). Solvents were removed under reduced pressure, and the residue was purified by column chromatography on silica gel with EtOAc–hexane (1:2) as eluant to afford the glycoside (**6**) (1.65 g, 73%), R_f 0.46 [EtOAc–hexane (2:1)]; δ_H(90 MHz; $CDCl_3$) 1.47 (8 H, m, 4 × CH_2), 5.08 (1 H, d, $J_{1,2}$ 8 Hz, 1-H), 5.60 (1 H, dd, *J* 3 and 8 Hz, =C*H*H *cis*), 6.08 (1 H, dd, *J* 8 and 16.5 Hz, =CH*H trans*), 6.34 (1 H, dd, *J* 3 and 8 Hz, =CH), and 7.24–8.16 (21 H, m, 4 × COPh and NH); *m/z* (CI, NH_3) 750 ($M + H^+$) (Found: C, 68.9; H, 5.8; N, 1.8. Calc. for $C_{43}H_{43}NO_{11}$: C, 68.93; H, 5.78; N, 1.87%).

6-(N-*Acryloylamino*)*hexyl* α-D-*Mannopyranoside* (**7**).—The tetra-*O*-benzoylmannoside (**6**) (1.45 g, 1.93 mmol) was treated with a catalytic quantity of sodium methoxide in methanol (30 ml) at room temperature for 20 h. The solution was neutralized with Dowex 50W resin (H^+) and was then filtered and evaporated to give an oil, which was triturated with hexane (30 ml × 3). The residue was recrystallized from methanol to give compound (**7**) (0.6 g, 94%), m.p. 85–87 °C; δ_H(300 MHz; DMSO) 1.30 (4 H, m, 2 × CH_2), 1.44 (2 H, m, CH_2CH_2N), 1.52 (2 H, m, CH_2CH_2O), 3.12 (2 H, m, NCH_2), 3.35 (2 H, m, OCH and 4-H), 3.42 (2 H, m, 5- and 6-H), 3.44 (1 H, m, 3-H), 3.57 (1 H, s, 2-H), 3.62 (1 H, m, OCH), 3.68 (1 H, m, 6-H), 4.58 (1 H, d, $J_{1,2}$ 0.5 Hz, 1-H), 5.58 (1 H, dd, *J* 10 and 2.6 Hz, =C*H*H *cis*), 6.08 (1 H, dd, *J* 17 and 2.6 Hz, =CH*H trans*), and 6.21 (1 H, dd, *J* 17 and 10 Hz, =CH); *m/z* (CI, NH_3) 334 ($M + H^+$).

General Procedure for Condensation of 6-(N-*Acryloylamino*)*hexyl Glycosides* (**5**) *and* (**7**) *with Nucleoside and Nucleotide Mercuriated Analogues*.—To a solution of a 5-mercuriated derivative[16,17] (0.1 mmol) and a glycoside acrylamide (0.2* or 0.5 mmol) in methanol–water–acetonitrile (3:1:2.7 v/v/v) was added 0.1M-methanolic lithium palladium chloride (1 ml). The mixture was stirred overnight at room temperature. Methanol (5 ml) was then added and hydrogen sulphide was passed through the solution for a few minutes, followed by compressed air to remove excess of H_2S. The precipitated metal sulphides were removed by filtration through Celite. After abundant washings with water–methanol, the filtrate was evaporated to dryness.

In the case of condensation of glycoside (**5**) or (**7**) with deoxyuridine (**8**), the crude material was applied to a Sephadex G-10 column (eluted with water). The first eluted fractions contained unchanged glycoside (**5**) or (**7**), and the two secondary products (**11**) or (**13**), and (**12**) or (**14**). The last eluted component was identified as the C-5-substituted deoxyuridine (**9**) or (**10**) by its UV spectrum (λ 268 and 300 nm). Further purification by hplc on Nucleosil 5-C18 reverse-phase silica gel (gradient 5–30% of A in B during 20 min at a flow rate of 0.5 ml min^{-1}) afforded product (**9**) (10 mg, 17% yield)* and product (**10**) (14 mg, 25% yield)*. When glycoside (**7**) (5 mol equiv.) was used for the condensation with 5-mercuriated deoxyuridine, we isolated, after purification by hplc, compound (**10**) (38 mg 68% yield.

Compound (**9**): 10 mg (17%); t_R 11.65 min; δ_H(250 MHz; CD_3OD) 1.20 (4 H, m, 13- and 14-H_2), 1.40 (4 H, m, 12- and 15-H_2), 1.80 (3 H, s, *Me*CONH), 2.15 (2 H, m, 2′-H_2), 3.80–4.00 (3 H, m, 4′-H and 5′-H_2), 4.20 (1 H, d, $J_{1'',2''}$ 7.5 Hz, 1″-H), 4.25 (1 H, m, 3′-H), 6.08 (1 H, t, $J_{1',2'}$ 7 Hz, 1′-H), 6.90 (1 H, d, $J_{7,8}$ 15 Hz, 7-H), 7.05 (1 H, d, $J_{7,8}$ 15 Hz, 8-H), and 8.23 (1 H, s, 6-H); *m/z* (CI, NH_3) 601 ($M + H^+$), 558 ($M + H - COCH_3$), and 485 ($B + 1$).

Compound (**10**): 38 mg (68%); t_R 11.34 min; δ_H(250 MHz; CD_3CN) 1.23 (4 H, m, 13- and 14-H_2), 1.42 (4 H, m, 12- and 15-H_2), 2.16 (2 H, m, 2′-H_2), 3.10 (2 H, t, *J* 7 Hz, 11-H_2), 3.25–3.80 (8 H, m, 16-H_2, 2″-, 3″-, 4″-, 5″-H, and 6″-H_2), 3.80–3.95 (3 H, m, 4′- and 5′-H_2), 4.26 (1 H, m, 3′-H), 4.62 (1 H, d, $J_{1'',2''}$ 1 Hz, 1″-H), 6.06 (1 H, t, $J_{1',2'}$ 7 Hz, 1′-H), 6.86 (1 H, d, $J_{7,8}$ 15 Hz, 7-H), 7.06 (1 H, d, $J_{7,8}$ 15 Hz, 8-H), and 8.13 (1 H, s, 6-H); δ_C(75.47 MHz; CD_3OD) 27.04, 27.79, 30.38, and 30.49 (C-12, -13, -14, and -15), 40.46 and 41.79 (C-11 and -2′), 62.54 and 62.96 (C-5′ and -6″), 68.43 and 68.66 (C-16 and -4″), 71.89, 72.28, and 72.65 (C-3′, -2″, and -3″), 74.62 (C-5″), 86.95 (C-4′), 89.15 (C-1′), 101.54 (C-1″), 110.96 (C-5), 122.28 (C-7), 134.12 (C-8), 143.93 (C-6), 151.23 (C-2), 163.81 (C-4), and 169.15 (C-9); *m/z* (FAB^+) 560.53 ($M + H$).

Compound (**12**): 7 mg (12%); t_R 9.90 min; δ_H(300 MHz; DMSO) 1.28 (4 H, m, 13- and 14-H_2), 1.38 (2 H, m, 12-H_2), 1.48 (2 H, m, 15-H_2), 2.12 (2 H, m, 2′-H_2), 2.40 (2 H, m, 8-H_2), 3.02 (2 H, m, 11-H_2), 3.14 (3 H, s, MeO), 3.25–3.35 (2 H, m, 16- and 4″-H), 3.35–3.42 (3 H, m, 3″-, 5″-, and 6″-H), 3.43–3.66 (5 H, m, 16-H, 5′-H_2, 2″-, and 6″-H), 3.82 (1 H, m, 4′-H), 4.26 (1 H, m, 3′-H), 4.48 (1 H, m, 7-H), 4.59 (1 H, s, 1″-H), 6.18 (1 H, t, $J_{1',2'}$ 6.5 Hz, 1′-H), and 7.84 and 7.85 (1 H, each s, 6-H); *m/z* (CI, NH_3) 582 ($M + H^+$).

Compound (**14**): 3 mg (4.5%); t_R 12.56 min; δ_H(500 MHz; DMSO) 1.33 (8 H, m, 3-, 4-, 17-, and 18-H_2), 1.51 (6 H, m, 2-, 5-, and 19-H_2), 1.65 (2 H, t, 16-H_2), 3.20 (2 H, m, 6-H_2), 3.29–3.46 (8 H, m, 3′-, 3″-, 4′-, and 4″-H, 5′-, 5″-, 6′-, and 6″-H), 3.43 (2 H, m, 1- and 20-H), 3.58 (2 H, m, 2′- and 2″-H), 3.60 (4 H, m, 1-, 20-, 6′-,

and 6″-H), 3.91 (2 H, m, 15-H_2), 4.48 (2 H, t, 6′- and 6″-OH), 4.61 (2 H, s, 1′- and 1″-H), 4.62 (2 H, m, 4′- and 4″-OH), 4.73 (4 H, m, 2′-, 2″-, 3′-, and 3″-OH), 6.40 (1 H, d, *J* 11.6 Hz, CH=C*H*CO), 7.86 (1 H, dd, *J* 3 and 11.6 Hz, C*H*=CHCO), 8.27 (1 H, t, CH_2NHCO), and 8.34 (1 H, d, COC*H*=C); *m/z* (Cl, NH_3) 663 (*M* + H^+).

In the case of mono- and tri-phosphate compounds, the purification of the crude material was performed on a DEAE-cellulose column washed with water (until unchanged glycoside was eluted), then with a gradient of 0.05–0.25M-triethylammonium hydrogencarbonate. Appropriate fractions [tlc in propan-2-ol-NH_4OH–water (7:2:1), detection at 306 nm] were pooled, lyophilysed, and further purified by hplc on Nucleosil 5-C18 reverse-phase silica gel (gradient 5–25% of A in B during 15 min at a flow rate of 0.5 ml min^{-1}). Thus, condensation of dUMP with compound (**5**) gave products (**15**) (26 mg, 30%), (**22**) (45 mg, 55%), and (**13**) (18 mg), condensation of dUMP with compound (**7**) gave products (**16**) (28 mg, 41%), (**23**) (25 mg, 16%), and (**14**) (6 mg); condensation of UMP with (**5**), and (**7**), afforded products (**17**) (26 mg, 36%), and (**18**) (30 mg, 45%), respectively.

Compound (**15**): 26 mg; t_R 8.79 min; δ_H(90 MHz: D_2O) 1.27 (8 H, m, 12-, 13-, 14-, and 15-H_2), 1.92 (3 H, s, *Me*CONH), 2.29 (2 H, m, 2′-H_2), 6.15 (0.17 H, d, $J_{7,8}$ 8 Hz, 7-H, *cis*-form), 6.22 (1 H, m, 1′-H), 6.34 (0.17 H, d, $J_{7,8}$ 8 Hz 8-H *cis*-form), 6.81 (0.83 H, d, $J_{7,8}$ 17 Hz, 7-H *trans*-form), 7.14 (0.83 H, d, $J_{7,8}$ 17 Hz, 8-H *trans*-form), 7.94 (0.17 H, s, 6-H *cis*-form), and 8.14 (0.83 H, s, 6-H *trans*-form); δ_P(121.5 MHz; D_2O) +0.87; *m/z* (FAB^+) 883.87 (*M* + 2Et_3N + H), 782.86 (*M* + Et_3N + H), 681.53 (*M* + H), 584.37, and 478.47.

Compound (**22**): 45 mg; t_R 7.76 min; δ_H(90 MHz: D_2O) 1.27 (8 H, m, 12-, 13-, 14-, and 15-H_2), 1.99 (3 H, s, *Me*CONH), 2.34 (2 H, m, 2′-H_2), 2.74 (2 H, m, 8-CH_2) 3.26 (3 H, s, MeO), 6.25 (1 H, t, $J_{1',2'}$ 7.2 Hz, 1′-H), and 7.85 and 7.79 (1 H, each s, 6-H); δ_P(121.5 MHz; D_2O) +2.62 and +2.78 (1:1); *m/z* (FAB^+) 814.87 (*M* + Et_3N + H), 713.71 (*M* + H), 611.42, and 478.27.

Compound (**13**): 18 mg; t_R 12.41 min; δ_H(500 MHz: DMSO) 1.29 (8 H, m, 3-, 4-, 17-, and 18-H_2), 1.47 (6 H, m, 2-, 5-, and 19-H_2), 1.63 (2 H, m, 16-H_2), 1.79 and 1.80 (6 H, each s, 2 × NHCO*Me*), 3.06 (2 H, m, 3′- and 3″-H), 3.20 (2 H, m, 6-H_2), 3.36 (6 H, m, 2′-, 2″-, 4′, 4″-, 5′-, and 5″-H), 3.40 (2 H, m, 1- and 20-H), 3.44 (2 H, m, 6′- and 6″-H), 3.68 (2 H, m, 6′- and 6″-H), 3.72 (2 H, m, 1- and 20-CH), 3.91 (2 H, t, 15-H_2), 4.26 (2 H, d, *J* 8.5 Hz, 1′- and 1″-H), 4.55 (2 H, m, 6′- and 6″-OH), 4.94 (2 H, m, 4′-and 4″-OH), 5.82 (2 H, m, 3′- and 3″-OH), 6.40 (1 H, d, *J* 9.5 Hz, CH=C*H*CO), 7.70 (2 H, each d, N*H*Ac), 7.85 (1 H, dd, *J* 2 and 9.5 Hz, C*H*=CHCO), 8.22 (1 H, t, CH_2NHCO), and 8.34 (1 H, d, *J* 2 Hz, COC*H*=C); δ_C(150 MHz; DMSO) 23.15 (CH_3), 25.14, 25.31, 25.82, and 26.38 (C-3, -4, -17, and -18), 28.82, 28.95, 29.08, and 29.29 (C-2, -5, -16, and -19), 49.10 (C-6 and -15), 55.51 (C-2′ and -2″), 61.13 (C-6′ and -6″), 68.18 and 68.27 (C-1 and -20), 70.71 (C-4′ and -4″), 74.34 (C-3′ and -3″), 77.05 (C-5′ and -5″), 101.08 (C-1″ and -1″), 112.80, 118.35, 137.74, and 140.95 (ethylenic carbons), 161.34 and 163.33 (C-8 and -13), and 169.04 (NH*C*OMe); *m/z* (FAB^+) 745.71 (*M* + H), 704 and 543.

Compound (**16**): 28 mg; t_R 9.70 min; δ_H(300 MHz; D_2O), 1.19 (4 H, m, 13- and 14-H_2), 1.42 (4 H, m, 12- and 15-H_2), 2.23 (2 H, m, 2′-H_2), 3.05 (0.4 H, t, *J* 6.8 Hz, 11-H_2 *cis*-form), 3.10 (1.6 H, t, *J* 6.7 Hz, 11-H_2 *trans*-form), 3.30–3.75 (8 H, m, 16-H_2, 4″-, 5″-, 3″-, and 2″-H and 6″-H_2), 3.86 (1.6 H, m, 5′-H_2 *trans*), 3.90 (0.4 H, m, 5′-H_2*cis*), 4.05 (0.8 H, m, 4′-H *trans*), 4.08 (0.2 H, m, 4′-H *cis*), 4.37 (0.2 H, m, 3′-H *cis*), 4.42 (0.8 H, m, 3′-H *trans*), 4.67 (1 H, s, 1″-H), 5.96 (0.2 H, d, $J_{7,8}$ 12 Hz, 7-H *cis*-form), 6.14 (0.2 H, t, $J_{1',2'}$ 7 Hz, 1′-H *cis*-form), 6.16 (0.8 H, t, $J_{1',2'}$ 7 Hz, 1′-H *trans*-form), 6.37 (0.2 H, d, $J_{7,8}$ 12 Hz, 8-H *cis*-form), 6.78 (0.8 H, d, $J_{7,8}$ 16 Hz, 7-H *trans*-form), 7.08 (0.8 H, $J_{7,8}$ 16 Hz, 8-H *trans*-form), 7.87 (0.2 H, s, 6-H *cis*-form), and 8.11 (0.8 H, s, 6-H *trans*-form); δ_P(36.5 MHz; D_2O) +1.23; δ_C(75.47 MHz; D_2O) 27.04, 27.83, 30.29, and 30.36 (C-12, -13, -14, and -15), 41.40 and 41.48 (C-11 and -2′), 62.88 (C-6″), 66.32 (C-5′), 68.75 and 69.79 (C-16 and -4″), 72.08, 72.60, and 73.07 (C-3′, -2″, and -3″), 74.69 (C-5″), 87.00, 87.87, 88.22, and 88.33 (C-1′ and -4′ *trans*- and *cis*-form), 101.64 (C-1″), 112.33 and 112.90 (C-5 *trans* and *cis*), 122.89 and 128.14 (C-7), 129.57 and 133.45 (C-8), 142.42 and 143.42 (C-6), 152.76 (C-2), 165.00 and 166.00 (C-4), and 170.61 and 171.23 (C-9); *m/z* (FAB^+) 640 (*M* + H).

Compound (**23**): 25 mg; t_R 8.51 min; δ_H(500 MHz; DMSO) 1.28 (4 H, m, 13- and 14-H_2), 1.36 (2 H, m, 12-H_2), 1.50 (2 H, m, 15-H_2), 2.06 (2 H, m, 2′-H_2), 2.38 and 2.52 (1 H, each m, 8-H_2), 3.00 (2 H, m, 11-H_2), 3.13 (3 H, s, MeO), 3.25–3.40 (3 H, m, 16-, 3″-, and 4″-H), 3.40–3.50 (2 H, m, 5″- and 6″-H), 3.55–3.67 (3 H, m, 16-, 2″-, and 6″-H), 3.78–3.88 (3 H, m, 4′-H and 5′-H_2), 4.31 and 4.37 (1 H, each m, 3′-H), 4.59 (1 H, s, 1″-H), 4.68 (1 H, t, 7-H), 6.22 and 6.23 (1 H, each t, 1′-H), and 7.80 and 7.89 (1 H, each s, 6-H); *m/z* (CI, NH_3) 672 (*M* + H^+).

Compound (**14**): 6 mg; t_R 12.95 min; *m/z* (CI, NH_3) 663 (*M* + H); spectral data are given above.

Compound (**17**): 26 mg; t_R 7.75 min; δ_H(270 MHz; DMSO) 1.23 (4 H, m, 13- and 14-H_2), 1.40 (4 H, m, 12- and 15-H_2), 1.79 (3 H, s, MeCON), 3.07 (5 H, m, 11-H_2, 3″-, 5″-, and 6″-H), 3.37 (3 H, m, 2″-, 4″-, and 6″-H), 3.65 (2 H, m, 16-H_2), 3.94 (3 H, m, 4′-and 5′-H), 4.08 (2 H, m, 2′- and 3′-H), 4.20 and 4.36 (0.4 H, and 0.6 H, each d, $J_{1',2'}$ 10 Hz, 1″-H *cis*- and *trans*-form), 5.77 (1 H, d, $J_{1',2'}$ 3 Hz, 1′-H *cis*), 5.78 (1 H, d, $J_{1',2'}$ 3.5 Hz, 1′-H *trans*), 5.96 (0.4 H, d, $J_{7,8}$ 13 Hz, 7-H *cis*-form), 6.47 (0.4 H, d, $J_{7,8}$ 13 Hz, 8-H *cis*-form), 6.98 (0.6 H, d, $J_{7,8}$ 16.2 Hz, 7-H *trans*-form), 7.16 (0.6 H, d, $J_{7,8}$ 16.2 Hz, 8-H *trans*-form), 7.96 (1 H, d, N*H*Ac), 8.42 and 8.98 (1 H, each s, 6-H *trans*- and *cis*-form), and 9.19 (1 H, m, $NHCH_2$); δ_P(109 MHz; DMSO) −1.41 (121.5 MHz; D_2O) +1.79; δ_C(67.8 MHz; DMSO) 23.72 (*Me*CONH), 25.84 and 26.89 (C-13 and -14), 29.60 and 29.80 (C-12 and -15), 40.00 (C-11 and -16), 56.17 (C-2″), 61.81 (C-6″), 68.93 and 69.08 (C-3′ *trans*- and *cis*-form), 70.19 (C-5′), 71.14 and 71.37 (C-4″), 74.67 and 74.83 (C-2′), 75.25 (C-3″), 77.44 (C-5″), 84.48 and 84.63 (C-4′), 88.68 and 89.01 (C-1′), 101.57 and 101.83 (C-1″), 110.00 and 110.31 (C-5), 122.33 and 124.16 (C-7), 129.95 (C-8), 138.60 and 142.79 (C-6), 150.64 and 150.85 (C-2), 162.85 and 163.07 (C-4), 166.46 and 166.63 (C-9), and 170.60 (CH_3CONH); *m/z* (FAB^+) 697 (*M* + H).

Compound (**18**): 30 mg; t_R 8.06 min; δ_H(270 MHz; DMSO) 1.27 (4 H, m, 13- and 14-H_2), 1.40 (2 H, m, 12-H_2), 1.47 (2 H, m, 15-H_2), 3.06 (2 H, t, 11-H_2), 4.57 (1 H, d, $J_{1',2'}$ 0.5 Hz, 1″-H), 5.77 (0.2 H, d, $J_{1',2'}$ 4.5 Hz, 1′-H *cis*-form), 5.78 (0.8 H, d, $J_{1',2'}$ 3.6 Hz, 1′-H *trans*-form), 5.92 (0.2 H, d, $J_{7,8}$ 12 Hz, 7-H *cis*-form), 6.46 (0.2 H, d, $J_{7,8}$ 12 Hz, 8-H *cis*-form), 6.96 (0.8 H, d, $J_{7,8}$ 16 Hz, 7-H *trans*-form), 7.15 (0.8 H, d, $J_{7,8}$ 16 Hz, 8-H *trans*-form), 8.34 (0.8 H, s, 6-H *trans*-form), and 8.89 (0.2 H, s, 6-H *cis*-form); δ_P(109 MHz; DMSO) −1.51; (121.5 MHz; D_2O) + 2.75; *m/z* (FAB^+) 758 (*M* + Et_3N + H) and 656 (*M* + H).

Condensation of dUTP or UTP with glycoside (**5**) afforded, after purification by hplc compounds (**19**) and (**21**) in 12 and 16% yield respectively; likewise, reaction of dUTP with glycoside (**7**) gave compound (**20**) in 19% yield.

Compound (**19**): 14 mg; t_R 7.40 min; δ_P(36.5 MHz; D_2O) −10.90 (d, P_γ), −11.56 (d, P_α), and −23.28 (t, P_β), *J* 21 ± 1 Hz; *m/z* (FAB^+) 1 151 (*M* + Et_3N + H).

Compound (**20**): 22 mg; t_R 7.90 min; δ_H(300 MHz; D_2O) 1.23 (4 H, m, 13- and 14-H_2), 1.44 (4 H, m, 12- and 15-H_2), 2.27 (2 H, t, 2′-H_2), 4.10 (3 H, m, 4′- and 5′-H_2), 4.50 (1 H, m, 3′-H), 4.69 (1 H, s, 1″-H), 6.19 (1 H, t, *J* 6.9 Hz, 1′-H), 6.74 (1 H, d, *J* 16 Hz, 7-H), 7.13 (1 H, d, *J* 16 Hz, 8-H), and 8.10 (1 H, s, 6-H); δ_P(121.5 MHz; D_2O) −10.00 (d, P_γ), −10.78 (d, P_α), and −22.47 (t, P_β), *J* 21 Hz; *m/z* (FAB^+) 1 109.51 (*M* + 3Et_3N + H), 1 008.41 (*M* + 2Et_3N + H), and 901.64 (*M* + Et_3N + H).

Compound (**21**): 15 mg; t_R 7.85 min; δ_P(36.5 MHz; D_2O) −10.87 (d, P_γ), −11.50 (d, P_α), and −23.27 (t, P_β), *J* 21 Hz; *m/z* (FAB^+) 925 (*M* + Et_3N + H)

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