Synthesis of a D-lactosyl cluster–nucleoside conjugate†

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The synthesis of a nucleoside–oligolactoside conjugate, expected to provide site-specific drug delivery to the human liver, is described.

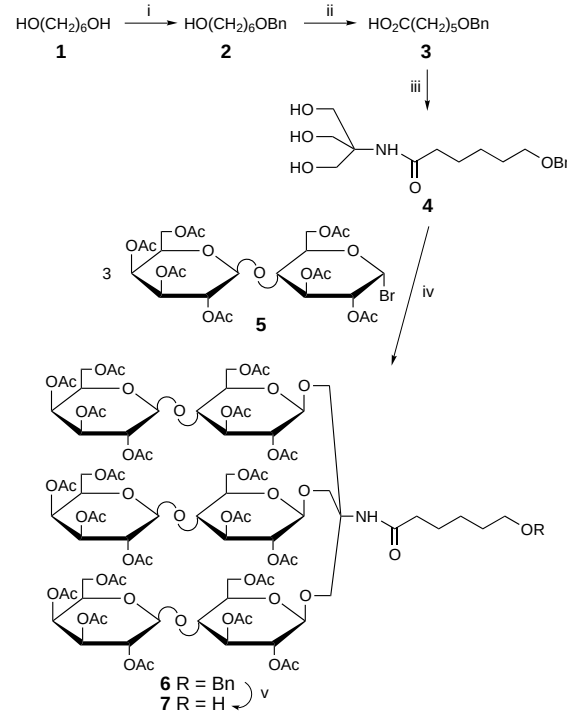
The hepatitis B virus (HBV) represents a major health concern, afflicting 350 million people worldwide.[1] While the virus itself is debilitating, it is not always fatal. However, liver cancer, which often arises from HBV infection, is responsible for upwards of 1 million deaths annually.[2] The mode of action of the HBV is thought to be similar to that of the HIV virus, in that it involves reverse transcription in the cytoplasm.[3] Thus, many nucleosides known to inhibit HBV reverse transcriptase, with their unwanted side-effects, have been examined as a means of controlling this deadly affliction.

The mammalian hepatocyte plasma membrane expresses the asialoglycoprotein receptor (ASGP-R),[4] a unique integral membrane receptor exhibiting specificity for terminal, nonreducing, β-D-galactopyranosyl or 2-acetamido-2-deoxy-β-D-galactopyranosyl residues. This specific binding to liver cells has been examined with a variety of oligogalactosides[5] and oligolactosides.[6] The biological evaluation of the binding of a host of molecules containing various numbers of terminal D-galactopyranosyl residues has demonstrated that, as the number of these residues increased, so did binding.[5b,6] Previous studies in this laboratory have involved adding one, two or three D-lactose units to a glycerol backbone.[7] Thus, attempts were made to extend this type of molecule, with its *hepatophilicity*, by the addition of an extra functional group, through which a hepatotoxic nucleoside could be appended. Ideally, by directing such a nucleoside directly to the liver, the devastating side-effects of HBV treatment would be obviated.

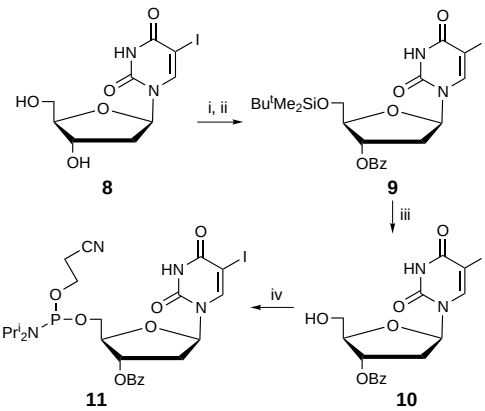
Previous work with D-galactose has utilized the well-known buffer tris(hydroxymethyl)methylamine (TRIS) as a backbone.[5] In the present work TRIS was modified such that, after addition of three D-lactose units, a cytotoxic nucleoside could be readily introduced. The nucleoside chosen was 2′-deoxy-5-iodouridine (dIU), known to inhibit HBV.[8]

To synthesize the acetylated *delivery vehicle* **7** (Scheme 1), a suitably modified backbone was required. Beginning with hexane-1,6-diol, a single benzyl group was introduced, followed by oxidation of the remaining hydroxy group to the corresponding carboxylic acid. Addition to the amino group of TRIS was performed using the well-known peptide-coupling agent 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ).[9] Glycosylation of the three primary hydroxy groups of **7** with 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-α-D-lactosyl bromide, prepared by the method of Kartha and Jennings,[10] was achieved in 69% yield using the Helferich modification[11] of the Königs–Knorr reaction. Removal of the benzyl group was readily accomplished in 85% yield by catalytic hydrogenolysis. The confirmation of the structure of this oligolactoside was accomplished using Fourier-transform, proton chemical-shift correlation spectroscopy (COSY)[12] and heteronuclear correlation spectroscopy (HETCOR).[13] The β-D configuration for the glycosidic linkage between the lactose units and the backbone was revealed by the observation of a characteristic coupling constant[14] ($J_{1,2}$ 7.9 Hz). It should be noted, however, that identification of trace amounts of material having α-D linkages would have been precluded by the detection limit of the NMR experiment.

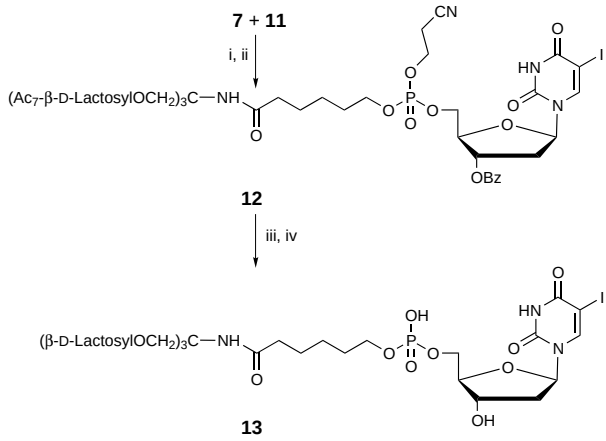
The nucleoside selected, namely dIU, required protection of the hydroxy group at C-3′ prior to attachment to the delivery moiety. Thus, the selectivity of the Bu^tMe_2Si group for primary hydroxy groups[15] allowed for an efficient two-step, one-pot procedure to form **9** (Scheme 2) in a high yield. The Bu^tMe_3Si



Scheme 1 *Reagents*: i, BnBr (0.7 equiv.), NaH, THF; ii, Jones reagent; iii, $(HOCH_2)_3CNH_2$, EEDQ, EtOH; iv, **5** (3 equiv.), $Hg(CN)_2$, 1 : 1 $MeNO_2$–C_6H_6; v, H_2, 10% Pd/C, HCO_2H, MeOH



Scheme 2 *Reagents and conditions*: Bu^tMe_2SiCl, pyridine, room temp.; ii, BzCl, 0 °C, pyridine; iii, 1% I_2 in MeOH; iv, β-CDCP, Pr^i_2NEt, MeCN, 0 °C



Scheme 3 *Reagents*: i, 1*H*-tetrazole, MeCN; ii, I_2, THF, H_2O, pyridine; iii, LiOH, MeOH; iv, Amberlite IR-120 (H^+)

group was readily removed by treatment with a solution of I_2 in MeOH at reflux temperature[16] to afford **10**, ready for coupling.

Many possibilities exist for the joining of the nucleoside and the carrier molecule, however, most of these were quickly discounted on the basis of possible reactivity *in vivo*; the use of a phosphoric monoester was selected. A well-established modification of the standard phosphite assembly method[17] was employed, with the reagent β-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (β-CDCP)[18] as the coupling agent. Compound **10** afforded an unstable intermediate, presumably **11**, which, on coupling to the carrier molecule **7** in the presence of 1*H*-tetrazole followed by oxidation of the product with iodine, afforded **12** (Scheme 3) in 12% yield.‡ Simultaneous cleavage of the acetic and benzoic esters, along with elimination of the β-cyanoethyl group, afforded the desired conjugate **13**.§

To conclude, we have demonstrated the synthesis of an easily prepared vehicle for the targeting of drugs directly to the mammalian liver. The advantages of site-specific, drug delivery are clear. Thus, the potential for conjugates such as **13**, in which a vehicle known to have a high specificity for hepatocytes is coupled to a hepatotoxic nucleoside, is tremendous. Full experimental details of this and related compounds, along with biological evaluation of the binding of these compounds to the ASGP-R and inhibition studies with HBV, will be reported in due course.

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Footnotes and References

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† Synthesis and binding of D-galactose-terminated ligands to human and rabbit asialoglycoprotein receptor. Part VII. For Part VI, see ref. 6.

‡ Subsequent couplings with other nucleosides have been achieved in yields approaching 80%.

§ All products were identified by ^1H, ^{13}C, and, where applicable, ^{31}P NMR spectroscopy, as well as mass spectrometry (except for **9** and **10**). Also, novel compounds **3**, **4**, **6**, **7**, **9** and **13** gave satisfactory elemental analyses.

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