Recl. Trav. Chim. Pays-Bas **113**, 337–338 (1994) 0165–0513/94/06337–02$0.700

Synthesis of 5-(β-D-glucopyranosyloxymethyl)-2'-deoxyuridine and derivatives thereof. A modified d-nucleoside from the DNA of *Trypanosoma brucei*

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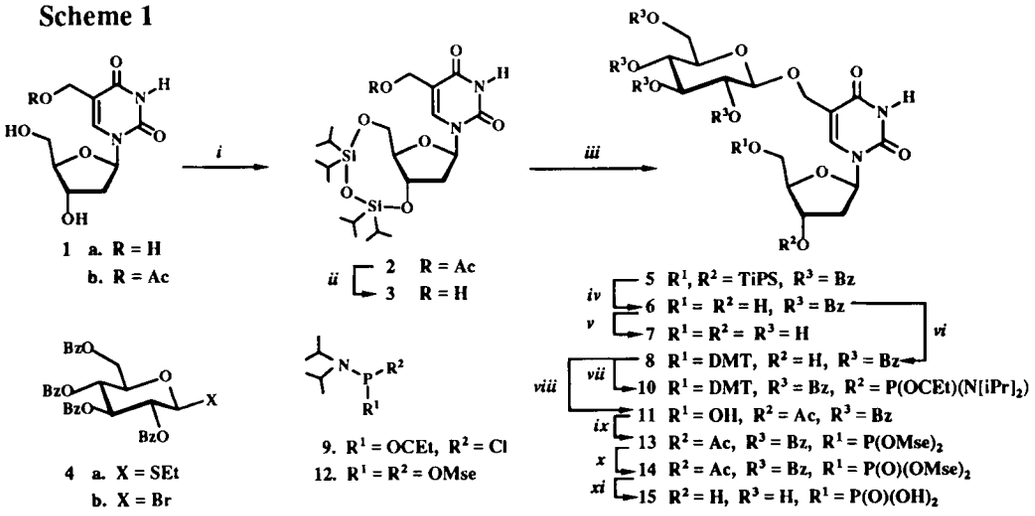
(Received April 27, 1994)

Abstract. An appropriately protected 3'-phosphoramidite and 5'-phosphate derivative of the modified 2'-deoxyuridine were obtained by β-glycosylation of a properly protected 5-hydroxymethyl-2'-deoxyuridine, and subsequent elaboration of the resulting intermediates using standard protocols devised in nucleic acids chemistry.

Trypanosoma brucei is a unicellular parasitic eukaryote transmitted by tsetse flies and causing sleeping sickness in mammals. The parasite can evade the immune response[1] of its mammalian host by antigenic variation of its surface coat, which is composed of only one protein species, the variant specific glycoprotein (VSG). The trypanosome genome contains numerous VSG genes that can be expressed one at a time in telomeric expression sites. Changing coat can occur either by replacing the VSG gene in the active site or by activating another expression site and turning the previously active off. How expression sites are switched on and off is still unclear. One of the mechanisms proposed[2] involves a specific telomeric DNA modification. Recently, Gommers-Ampt *et al*[3]. showed that 5-(β-D-glucopyranosyloxymethyl)-2'-deoxyuridine (*i.e.* compound **7**) is a characteristic modified d-nucleoside of the DNA from *T. brucei*.

In order to gain a deeper insight in the biological function and conformational behaviour of DNA fragments containing at predetermined positions the rare d-nucleoside **7**, we here present the first synthesis of the suitably protected phosphoramidite building unit **10** as well as the 5'-phosphate derivative **13**. Prior to the preparation of the target molecules **10** and **13**, we synthesized first, as outlined in Scheme 1, the naturally occurring compound **7**. To this end, we examined whether the primary and secondary hydroxyl groups in the ribofuranosyl moiety of known 5-hydroxymethyl-2'-deoxyuridine[4] (**1a**) could be silylated selectively with 1,3-dichlorotetraisopropyldisiloxane[5] ($TiPSCl_2$). However, work up and purification of the reaction mixture, resulting after treatment of **1a** with a slight excess of $TiPSCl_2$ in pyridine, gave the 3',5'-TiPS-derivative **3** in 30% yield. On the other hand, the fully protected compound **2** could be isolated in an excellent yield by silylation of the 5-O-acetyl derivative **1b**[6] under the same conditions. Zemplén deacetylation of **2** proceeded smoothly to give the nucleosyl acceptor **3**. Initially, the formation of the β-glycosidic linkage was elaborated by glycosylation of the allylic alcohol in **3** with the glycosyl donor ethyl 2,3,4,6-tetra-O-benzoyl-1-thio-β-D-glucopyranoside **4a**. Unfortunately, condensation of **4a** with **3** in the presence of N-iodo-succinimide and catalytic triflic acid[7] led to an intractable mixture of products. However, glycosylation of **3** with the glycosyl bromide **4b** under Helferich conditions[8] resulted in the isolation of the expected β-linked product **5**. Desilylation of **5** followed by debenzoylation of **6** afforded, after gel filtration, homogeneous **7**, the analytical data[9] of which are in excellent accord with those reported[3] for the naturally occurring minor d-nucleoside. Having the glucosylated and partially protected d-uridine **6** in hand, the introduction of the 5'-O-dimethoxytrityl protecting group and the 3'-O-cyanoethyl-phosphoramidite function was readily accomplished by the following standard two-step protocol[10]. Thus, tritylation of **6** with 4,4'-dimethoxytrityl chloride (DMT-Cl), and subsequent phosphitylation of **8** with reagent **9** gave the homogeneous target building unit **10** (^{31}P NMR δ 149.2 & 149.13 ppm) in 49% overall yield. Furthermore, acetylation of **8** followed by detritylation[11] led to compound **11**, the primary hydroxyl of which was phosphitylated with the new phosphitylating reagent **12**[12]

Scheme 1



Reagents and conditions:

i) $TiPSCl_2$, pyridine (90%); *ii*) KOtBu, MeOH, 30 min (60%); *iii*) $Hg(CN)_2$ - $Hg(Br)_2$, CH_3CN, 2 h (51%); *iv*) TBAF, pyridine-HCl, THF, 30 min (78%); *v*) KOtBu, MeOH, 16 h (91%); *vi*) DMT-Cl, pyridine, 1.5 h (71%); *vii*) **9**, DiPEA, CH_2Cl_2, 30 min (70%); *viii*) Ac_2O, pyridine, DMAP, then pyrrole, CF_3COOH, CH_2Cl_2, 5 min. (97%); *ix*) **12**, (2 eq), CH_2Cl_2, 1H tetrazole, 1 h; *x*) tBuOOH, 5 min (85%); *xi*) MeOH/dioxane/4N NaOH (5 / 14 / 1, v / v / v), 16 h (73%).

in the presence of 1H-tetrazole. *In-situ* oxidation of the resulting phosphitetriester intermediate **13** with *tert*-butylhydroperoxide[13] afforded the corresponding phosphotriester derivative **14** (^{31}P NMR δ -1.8 ppm) in 85% yield based on **8**. Removal of the benzoyl (Bz), acetyl and methylsulfonylethyl (Mse) groups from **8** was effected in one step with sodium hydroxide[14] to give, after purification by gel filtration and further processing, homogeneous[9] **15** (Na^+-salt, ^{31}P NMR δ 0.83 ppm) in 60% yield.

In conclusion, the results presented in this paper may open the way to the assembly of DNA, bearing at specific locations the ß-glucosylated d-nucleoside **7**, by either a solid-phase or enzymatic synthesis using the 3'-phosphoramidite **10** or 5'-phosphate **15**, respectively, as the incoming building units.

Acknowledgement: We wish to thank Prof. P. Borst for his stimulating advice and the Netherlands Foundation for Chemical Research (SON) with the financial aid from the Netherlands Organization for the advancement of Pure Research (NWO) for financial support.

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9. Satisfactory mass spectrometric data were obtained for compounds **7**, **10** and **15**. Relevant ^1H and ^{13}C NMR data (δ-values in ppm) for compounds **7** and **15**.
   7: ^1H NMR 300 MHz (D_2O): 8.02 (s, 1H, H-6 dU); 6.27 (t, 1H, H-1' dU, J 6.6 Hz); 4.63 (d, 1H, J 12.3 Hz, H-7, dU); 4.50 (d, 1H, $J_{1',2'}$ 7.8 Hz, H-1', dU); 3.27 (dd, $J_{2',3'}$, 9.2 Hz, H-2', Glu); 2.39 (m, 2H, H-2'and H-2'', dU).^{13}C NMR (D_2O): 102.2 (C-1', Glu); 87.4 and 86.2 (C-4' and C-1', dU); 64.9, 61.8 and 61.4 (C-6', Glu, C-5'and C-7, dU).
   15: ^1H NMR 400 MHz (D_2O): 8.08 (s, 1H, H-6, dU); 6.33 (t, 1H, $J_{1',2'}$ 6.8 and $J_{1',2''}$ 7.0 Hz, H-1', dU); 4.64 (d, 1H, J 12.1 Hz, H-7, dU); 4.54 (d, 1H, $J_{1'2'}$ 8.0 Hz, H-1', Glu); 2.39 (dd, 2H, H-2'and H-2'', dU). ^{13}C (D_2O): 102.45 (C-1', Glu); 86.57 and 86.49 (C-4', dU, J_{pc} 8.5 Hz); 86.2 (C-1', dU).
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