16. Nucleosides and Nucleotides

Part 25[1])

Synthesis of a Protected 1-(2'-Deoxy-β-D-ribofuranosyl)-1*H*-benzimidazole 3'-Phosphate

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1-(2'-Deoxy-5'-*O*-dimethoxytrityl-β-D-ribofuranosyl)-1*H*-benzimidazole 3'-[(*p*-chlorophenyl) (2-cyanoethyl) phosphate] (**6**) has been synthesized from 1-(β-D-ribofuranosyl)-1*H*-benzimidazole (**3b**) using regiospecific 2'-deoxygenation. The latter compound was obtained by glycosylation of benzimidazole with the D-ribose derivative **2** leading exclusively of the β-D-anomer.

Introduction. – For our continuing efforts towards the study of the specificity of DNA polymerase [2], we required an oligonucleotide sequence with a β-D-deoxyribonucleoside in which an unnatural base could be incorporated. The 1-(2'-deoxy-β-D-ribofuranosyl)-1*H*-benzimidazole (Drb) was chosen for this purpose since it has been shown that Drb are simulating purine-nucleoside analogs [3]. For example, the inhibition of influenza-B virus by 5,6-dichloro-1-(2'-deoxy-β-D-ribofuranosyl)-1*H*-benzimidazole is reversed by adenosine [4]. It has been suggested that this compound interferes with preliminary synthesis of ribonucleic acid.

Results. – Because of the biochemical interest of Drb, a number of syntheses of this compound have been reported, all starting from 2-deoxy-D-ribose derivatives giving quite low yields of the β-D-anomer [5]. To overcome this problem, a strategy based on glycosylation of benzimidazole with D-ribose derivatives followed by regiospecific 2'-deoxygenation was adopted. It was anticipated that the presence of a suitable protected 2'-hydroxy group could lead to stereochemical control giving only the β-D-anomer. The resulting nucleoside could then be readily transformed to the corresponding nucleotide **6** needed for the synthesis of the sequence.

It is known that fusion between benzimidazole and tetra-*O*-acetyl-D-ribose [6] or the reaction between silylated benzimidazole and tri-*O*-benzoyl-D-ribofuranosyl bromide [7] both yielded mixtures enriched in the β-D-anomer. In order to avoid this problem, the condensation of chloromercurio-benzimidazole **1** with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride (**2**) was studied *(Scheme)*. Analogous glycosylations giving exclusively the β-D-anomers had already been reported [8].

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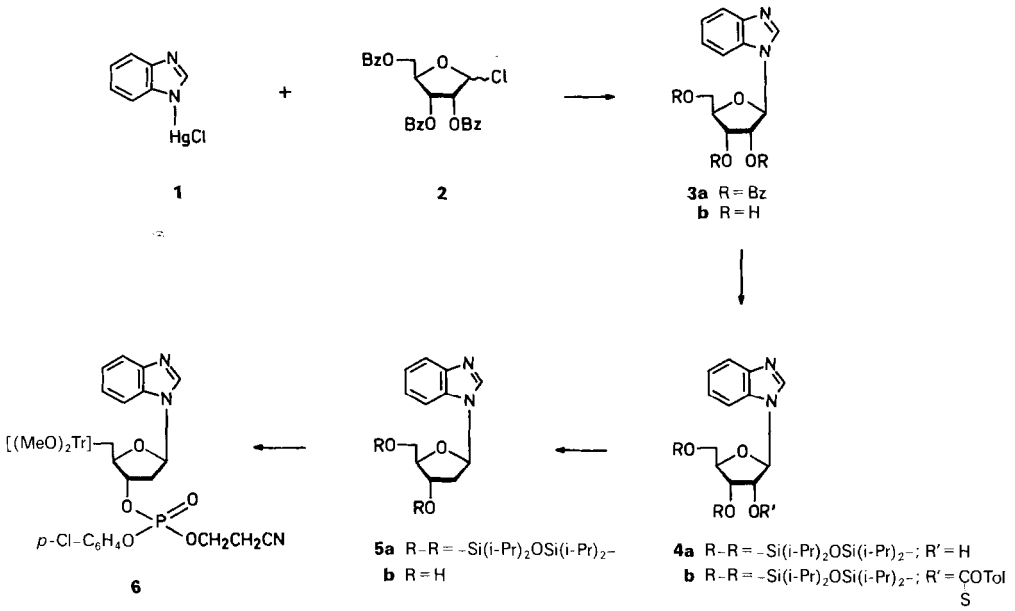
Scheme



In our case, the reaction proceeded with good yield in refluxing toluene to give only the anomer **3a**. The ^{1}H-NMR spectrum of this compound showed a *d* at 6.42 ppm (J = 5.4) representing the anomeric proton at C(1'). This signal corresponds to the upfield resonance for H–C(1') in the spectrum of the α-D/β-D mixture prepared according to [7]. Thus, the configuration at the glycosidic bond of **3a** is established as β-D.

Debenzoylation of the nucleoside **3a** with NaOMe afforded **3b**, which was then subjected to the four-step procedure for transforming a ribonucleoside to the 2'-deoxy analog [9]. Selective protection of the 5'- and 3'-hydroxy functions was achieved with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ([(i-Pr)$_2$SiCl]$_2$O) in pyridine yielding **4a**. The remaining 2'-hydroxy group was first transformed to a thiocarbonate leading to **4b** which was homolytically deoxygenated with Bu_3SnH to give **5a**. Removal of the protecting groups with Bu_4NF in THF afforded the 2'-deoxy compound **5b**. The deoxygenation sequence proceeded with an overall yield of 43%. Confirmation of the β-D-configuration of **5b** was again obtained by its ^{1}H-NMR. The signal for H–C(1') appeared as a pseudo-*t* at 6.35 ppm. Two signals exchangeable with D_2O, a *t* and a *d*, assured the presence of a primary and a secondary alcohol.

The desired fully protected deoxynucleoside 3'-phosphate was obtained from **5b** in two steps with 65% overall yield. First, the 5'-hydroxy function was protected as a (MeO)$_2$Tr ether making the 3'-OH available for phosphorylation. This latter operation was effected with the monofunctional (*p*-chlorophenyl) (2-cyanoethyl) phosphochloridate [10] to give the desired protected nucleoside **6**.

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Experimental Part

General. All reactions were routinely run in a flame-dried *(in vacuo)* round-bottom flask under dry Ar. Toluene was dried over Na. Other solvents and reagents were reagent-grade and stored over 4-Å molecular sieves. TLC: precoated silica-gel plates *(60 GF 254 Merck)*. Column chromatography: silica gel *C-560* from *Chemische Fabrik Uetikon*. M.p.: *Will-Wetghar* apparatus; uncorrected. Optical rotations: *Perkin-Elmer-141* polarimeter; cell length, 10 cm, 1 ml capacity; Na light. ^{1}H-NMR spectra: *Varian EM-390*, chemical shifts in ppm downfield from tetramethylsilane (TMS) as internal standard, coupling constants J in Hz. The elemental analyses were performed by the Microanalytical Laboratory of our Institute.

*1-(2',3',5'-Tri-O-benzoyl-β-*D*-ribofuranosyl)-1*H*-benzimidazole* (**3a**). To a stirred suspension of 7.7 g (22 mmol) of 1-chloromercurio-1*H*-benzimidazole (**1**) [8] in 100 ml of toluene, a soln. of 10.45 g (20 mmol) of tetra-*O*-benzoyl-D-ribofuranosyl chloride [8] in 30 ml of toluene was added. The heterogeneous mixture was refluxed for 2 h under continuous stirring and then allowed to cool. After filtration of the insoluble material, the filtrate was first washed with a 30% aq. KI soln., then with H_2O. The org. layer was dried over $MgSO_4$ and concentrated to a sirup. Chromatography over silica gel using $CHCl_3$ afforded 6.1 g (55%) of **3a** as a white foam. $[\alpha]_D^{20} = -66.1°$ ($c = 1.0$, CH_2Cl_2). ^{1}H-NMR ($CDCl_3$): 8.3–7.1 (*m*, 5 arom. H); 6.43 (*d*, $J(1',2') = 5.4$, H–C(1')); 6.2–5.9 (*m*, H–C(2'), H–C(3')); 5.0–4.7 (*m*, H–C(4'), 2 H–C(5')).

*1-[3',5'-O-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-β-*D*-ribofuranosyl]-1*H*-benzimidazole* (**4a**). To a soln. of 0.50 g (0.90 mmol) of NaOMe in 150 ml of MeOH were added 5.0 g (8.9 mmol) of **3a**. After refluxing for 15 min, the soln. was allowed to cool. AcOH (5 ml) was added, and the solvents were removed under reduced pressure. H_2O (40 ml) was added to the crude product, and the resulting soln. was evaporated. This operation was repeated twice to ensure the complete removal of the methyl benzoate. TLC (CH_2Cl_2/MeOH 85:15) indicated that the material thus obtained was mainly **3b**. After careful drying, it was used without further purification for the next step. To a soln. of **3b** in pyridine (30 ml), 2.8 g (2.8 ml, 8.9 mmol) of $[(i\text{-}Pr)_2SiCl]_2O$ were added dropwise, and the soln. was allowed to stand overnight at r.t. After removal of the solvent, the residue was partitioned between Et_2O and H_2O. The org. layer was dried over $MgSO_4$, filtered, evaporated, and the crude material obtained by chromatography over silica gel (CH_2Cl_2/Et_2O 2:1) to give 2.6 g (62%) of **4a**. M.p. 128°. $[\alpha]_D^{20} = -3.1°$ ($c = 5.0$, MeOH). ^{1}H-NMR ($CDCl_3$): 8.21 (*s*, H–C(2)); 7.9–7.2 (*m*, 4 arom. H); 6.00 (*d*, $J(1',2') = 2$, H–C(1')); 4.7–4.4 (*m*, H–C(2')); 4.3–4.1 (*m*, H–C(3'), H–C(4'), 2 H–C(5')); 3.17 (*m*, OH); 1.1–0.9 (*m*, 4 Me_2CH). Anal. calc. for $C_{24}H_{40}N_2O_5Si$ (464.64): C 58.50, H 8.13, N 5.69; found: C 58.02, H 8.18, N 5.68.

*1-[2'-Deoxy-3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-*D*-ribofuranosyl]-1*H*-benzimidazole* (**5a**). A soln. of 2.0 g (4.0 mmol) of **4a**, 1.0 g (80 mmol) of 4-(dimethylamino)pyridine and 0.8 g (0.7 ml, 4.4 mmol) of *p*-tolyl chlorothioformate in CH_3CN (50 ml) was stirred for 2 h at r.t. Then, TLC (CH_2Cl_2/Et_2O 4:1) indicated that the reaction was complete. The solvent was evaporated and the crude material partitioned between Et_2O and H_2O. The org. layer was washed with cold 1N HCl, dried over $MgSO_4$, and evaporated. To the yellow oil (**4b**), degassed toluene (50 ml) was added followed by 1.2 g (1.0 ml, 4.0 mmol) of Bu_3SnH and 0.15 g of 2,2'-azobis(2-methylpropanenitrile). The soln. was refluxed for 2 h and then allowed to cool. Evaporation of toluene followed by silica-gel chromatography of the resulting oil (CH_2Cl_2/Et_2O 4:1) afforded 1.6 g (85%) of **5a**. Oil; $[\alpha]_D^{20} = -0.7°$ ($c = 3.0$, CH_2Cl_2). ^{1}H-NMR ($CDCl_3$): 8.12 (*s*, H–C(2)); 7.9–7.7 (*m*, 1 arom. H); 7.6–7.1 (*m*, 3 arom. H); 6.22 (*t*, $J(1',2') = 6$, H–C(1')); 5.77 (*t*, $J(2',3') = J(3',4') = 7$, H–C(3')); 4.2–3.8 (*m*, H–C(4'), 2 H–C(5')); 2.66 (*dd*, 2 H–C(2')); 1.1–0.9 (*m*, 4 Me_2CH). Anal. calc. for $C_{24}H_{40}N_2O_4Si_2$ (476.70): C 60.50, H 8.40, N 5.88; found: C 60.39, H 8.42, N 5.89.

*1-(2'-Deoxy-β-*D*-ribofuranosyl)-1*H*-benzimidazole* (**5b**). To a THF soln. of 2.0 g (6.3 mmol) of Bu_4NF, 1.5 g (3.1 mmol) of **5a** were added. After stirring for 15 min, the solvent was evaporated and H_2O was added. The resulting soln. was extracted with Et_2O, and the H_2O phase was collected. Evaporation followed by silica-gel chromatography (CH_2Cl_2/MeOH 9:1) gave 0.56 g (80%) of **5b**. M.p. 152° ([5]: 154°). $[\alpha]_D^{20} = -33.1°$ ($c = 3.0$, MeOH; [5]: $[\alpha]_D = -30.5°$). ^{1}H-NMR ((D$_6$)DMSO): 8.45 (*s*, H–C(2)); 7.7–7.6 (*m*, 2 arom. H); 7.3–7.1 (*m*, 2 arom. H); 6.40, 6.31 (*dd*, $J(1',2') = 6.1$, $J(1',2') = 6.3$, H–C(1')); 5.32 (*d*, $J = 4.2$, OH); 4.94 (*t*, $J = 5.2$, OH); 4.4–4.3 (*m*, H–C(3')); 3.9–3.8 (*m*, H–C(4')); 3.6–3.5 (*m*, 2 H–C(5')); 2.6–2.3 (*m*, 2 H–C(2')).

*1-(2'-Deoxy-5'-O-dimethoxytrityl-β-*D*-ribofuranosyl)-1*H*-benzimidazole 3'-[(*p*-Chlorophenyl) (2-Cyanoethyl) Phosphate]* (**6**). A soln. of 0.5 g (1.9 mmol) of **5b** in pyridine (5 ml) was evaporated to dryness to ensure the removal of traces of H_2O. This operation was repeated twice, and then pyridine (3 ml) as well as 0.8 g (2.3 mmol) of dimethoxytrityl chloride were added. Stirring was immediately started, and after 2 h, MeOH (5 ml) was added. After 30 min, the solvents were evaporated, and the residue was partitioned between H_2O and Et_2O. The org. layer was dried over $MgSO_4$ and concentrated to a sirup. The presumed 5'-*O*-dimethoxytrityl-deoxynucleoside was co-evaporated twice with pyridine (5 ml). The rotatory evaporator was flushed with Ar. Then, dioxane (10 ml), 1.3

g (1.2 ml, 5.0 mmol) of (*p*-chlorophenyl) (2-cyanoethyl) phosphochloridate as well as 1.5 ml (6.0 mmol) of *N*-methylimidazole were added. The soln. was stirred for 1 h and then hydrolyzed with H_2O/pyridine 1:1 (5 ml) for 15 min. After evaporation of dioxane, a 4% aq. $NaHCO_3$ soln. was added, and the product was extracted with CH_2Cl_2. The org. layer was dried ($MgSO_4$), concentrated, and chromatographed over silica gel (CH_2Cl_2/MeOH 9:1) to afford 0.8 g (61%) of **6** as a foam. $[\alpha]_D^{20} = -21.3°$ ($c = 1.0$, CH_2Cl_2). ^{1}H-NMR ($CDCl_3$): 8.45 (*s*, H–C(2)); 7.7–7.1 (*m*, 2 arom. H); 6.37 (*t'*, $J(1',2') = J(1',2'') = 6.2$, H–C(1')); 4.4–4.2 (*m*, H–C(3'), OCH_2CH$_2$CN); 3.9–3.8 (*m*, H–C(4')); 3.80 (*s*, 2 CH_3O); 3.6–3.5 (*m*, 2 H–C(5')); 2.7–2.3 (*m*, 2 H–C(2'), OCH_2CH$_2$CN). Anal. calc. for $C_{42}H_{39}ClN_3O_7P$ (764.20): C 66.06, H 5.11, N 5.50; found: C 66.16, H 5.08, N 5.51.

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