The Synthesis of Phosphorylated 3′-Amino-3′-deoxythymidine and 5′-Amino-5′-deoxythymidine

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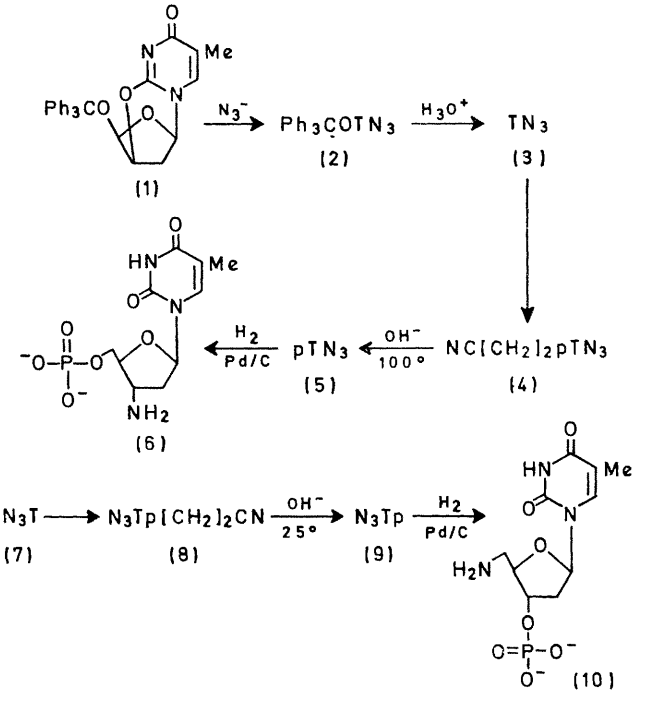
Summary 3′-Amino-3′-deoxythymidine 5′-phosphate (**6**) and 5′-amino-5′-deoxythymidine 3′-phosphate (**10**) were prepared as analogues of the corresponding naturally occurring nucleotides.

No naturally occurring pentose or deoxypentose nucleotides in which an amino-group replaces one of the hydroxy-groups of the carbohydrate moiety have been prepared, although a number of related nucleosides have been synthesized[1,2] or found as components of antibiotics.[3] We report the first preparation of two such aminodeoxypentose nucleotides,† 3′-amino-3′-deoxythymidine 5′-phosphate (**6**) and 5′-amino-5′-deoxythymidine 3′-phosphate (**10**).

The key concept underlying the success of these syntheses was the use of stable azidodeoxynucleosides, instead of amino- or protected amino-deoxynucleosides, in the phosphorylation reaction sequences.‡ The resulting azido-nucleotides were converted into the desired aminopentose nucleotides by mild catalytic reduction in excellent yields. The azido-group was stable to 1 N-NaOH at 100° and mild acid hydrolysis conditions used in the removal of protecting groups.

5′-*O*-Trityl-2,3′-anhydrothymidine (**1**), available by a three-step reaction sequence[1] from thymidine, was allowed to react with sodium azide in a dimethylformamide–water mixture (9 : 1, v/v) heated under reflux for 11 hr. to give compound (**2**). The course of the reaction was monitored by silica gel t.l.c. using ethyl acetate as the developing solvent. Compound (**2**) was purified by column chromatography over silica gel using benzene–chloroform mixtures and chloroform as the eluting solvent, or by silica gel preparative t.l.c. using diethyl ether as the developing solvent. The purified product (70% yield) crystallized only with difficulty [m.p. 104—105°;§ λ_{max} (MeCN) 267 nm (ε 10,800)] and was used in the next reaction without crystallization. Compound (**2**) was hydrolysed in 80% acetic acid at 50° for 2 hr. and at room temperature for 16 hr. to give 3′-azido-3′-deoxythymidine (**3**) in 80% yield. Compound (**3**) was crystallized from isopropyl alcohol–n-pentane mixtures [m.p. 105—106°, softens at 100°; λ_{max} (H_2O) 267 nm (ε 10,200)] or hot water [m.p. 120—122°; λ_{max} (H_2O) 267 nm (ε 10,000); mixed m.p. with lower-melting form, 120—122°]. Catalytic reduction of compound (**3**) gave the known 3′-amino-3′-deoxythymidine, prepared by a different route.[1a] Compound (**3**) was phosphorylated with β-cyanoethyl phosphate[6] and dicyclohexylcarbodi-imide to give 3′-azido-3′-deoxythymidine 5′-(β-cyanoethyl phosphate) sodium salt (**4**) in essentially quantitative yield. A small sample was purified for characterization by preparative paper chromatography using n-butanol–acetic acid–water (5 : 2 : 3, v/v) and fractional precipitation from methanol–ethanol–isopropyl alcohol mixtures [λ_{max} (H_2O) 267 nm (ε 10,200)]. Compound (**4**) was allowed to react with 1N-NaOH at 100° for 1·5 hr. to cleave the β-cyanoethyl ester group to afford 3′-azido-3′-deoxythymidine 5′-phosphate disodium salt (**5**). Compound (**5**) was purified by large-scale preparative paper chromatography using n-butanol–acetic acid–water (5 : 2 : 3, v/v) and fractional precipitation from methanol–isopropyl alcohol mixtures [λ_{max} (H_2O) 267 nm (ε 10,200), 40% yield]. Mild reduction of the azido-group of compound (**5**) in the presence of 10% Pd/C gave 3′-amino-3′-deoxythymidine 5′-phosphate disodium salt (**6**) [λ_{max} (H_2O) 267 nm (ε 9400)] in 90% yield.



5′-Azido-5′-deoxythymidine (**7**), available *via* a six-step reaction sequence from thymidine,[2] was phosphorylated with β-cyanoethyl phosphate[6] and dicyclohexylcarbodi-imide to give 5′-azido-5′-deoxythymidine 3′-(β-cyanoethyl phosphate) (**8**), which was not purified. Compound (**8**) was allowed to stand in 1 N-NaOH at room temperature for 45 min. to cleave the β-cyanoethyl group to afford 5′-azido-5′-deoxythymidine 3′-phosphate (**9**). Compound (**9**) was purified by preparative paper chromatography using ethanol–aqueous 1% NH_4OAc (5 : 2, v/v) and fractional

† Two nucleotides containing an aminohexose sugar are known.[4]
‡ This approach has been used in the synthesis of D-ribofuranosylamine 5′-phosphate.[5]
§ All new compounds for which physical constants have been recorded have acceptable C, H, N, O or C, H, N, P analyses.

precipitation from water–methanol mixtures [λ_{max} (H_2O) 266 nm (ϵ 10,200), 55%]. Compound (**9**) was converted into 5′-amino-5′-deoxythymidine 3′-phosphate dilithium salt (**10**) [λ_{max} 266 nm (ϵ 9600), 90%] by catalytic reduction in aqueous solution in the presence of 10% Pd/C.

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