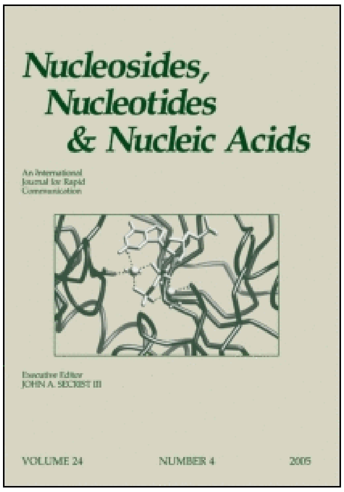

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O^6-PROTECTION AND OTHER TRANSFORMATIONS AT GUANOSINE AND INOSINE LACTAM SITES WITH APPLICATION OF RELATED PYRIDINIUM SALTS

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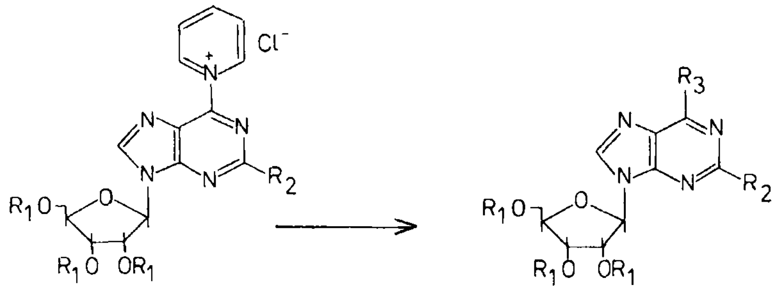
Abstract: Nucleophilic displacement reactions of guanosine- and inosine-derived pyridinium salts will be discussed in a view of their preparative applications in nucleoside and oligonucleotide chemistry.

Recently we have reported[1-4] on the formation of nucleobase-derived pyridinium salts as ionic side-products generated during oligonucleotide synthesis by phosphotriester method. This new type of nucleoside derivatives show interesting chemical properties[1-3,5,6], some of them also of preparative value. Easy accessible pyridinium salt I[1] undergoes a selective nucleophilic displacement at C-6 with thiols, azide and methoxide ion[6]. Various amines and hydroxide ion react with I at α-carbons of pyridinium ring with formation of 6-aminopurine derivatives (Zincke reaction). In this paper we would like to present results concerning the reactivity of inosine and guanosine-derived pyridinium salts I and II towards nucleophiles especially with respect to the problem of O^6-protection in oligonucleotide synthesis. Selected structural data for the compounds discussed are given in the references.

Guanosine-derived pyridinium salt II reacts with sodium ethoxide (2 eqv.) in ethanol with formation of 6-ethoxy-2-aminopurine riboside IV isolated with 30% yield as crystalline solid. III undergoes total deprotection within 17 hrs needed for the reaction to be completed. Due to opening of the pyridinium ring of II, 2,6-diaminopurineriboside is formed as the major product isolated with 55% yield. Our attempts to achieve displacement with sodium isopropoxide were not successful. Salt II reacts



I R_1=Ac, R_2=H
II R_1=Ac, R_2=NHib

III R_1=Ac, R_2=NHib, R_3=OCH_2CH_3
IV R_1=H, R_2=NH_2, R_3=OCH_2CH_3
V R_1=H, R_2=R_3=NH_2
VI R_1=Ac, R_2=NHib, R_3=SCH_2CH_3
VII R_1=Ac, R_2=NHib, R_3=SCH_2CH_2OH
VIII R_1=Ac, R_2=NHib, R_3=N_3
IX R_1=Ac, R_2=NHAc, R_3=N_3
X R_1=Ac, R_2=H, R_3=OPhCl-3
XI R_1=R_2=H, R_3=OPhCl-3
XII R_1=Ac, R_2=NHib, R_3=OPhCl-3
XIII R_1=H, R_2=NHib, R_3=OPhCl-3
XIV R_1=Ac, R_2=NHib, R_3=$OPhNO_2$-2
XV R_1=Ac, R_2=NH_2, R_3=SPh

with thiols exclusively at C-6, however with much lower rate than inosine salt I[6]. II treated over 5 days with ethanethiol (10 eqv.) in water-dioxan is transformed to 6-ethylthio derivative VI with only 50% yield. Overnight reaction of II with 2-hydroxyethanethiol (5 eqv.) in aqueous solution is virtually quantitative (tlc); VII was isolated by silica gel chromatography with 85% yield. The influence of the solvent on reactions of I and II with thiols is under studies. Reaction of II with sodium azide (1.5 eqv.) in DMF, instead of clean transformation to 6-azido derivative VIII, leads to complex mixture. After peracetylation IX was isolated with low yield.

Reactivity of I and II toward phenolate ions was studied in a view of the introduction of O^6-aryl protection[7,8] for inosine and guanosine lactam systems. In above context the case of inosine is of special interest since, in contrary to guanosine[9], inosine undergoes N^1-arenesulphonation (see[1]) making the introduction of O^6-aryl protection via O^6-arenesulphonyl intermediates not available. The following procedure has been designed for efficient transformation of I into O^6-(3-chloro)phenyl derivative X. In two-phase system composed of readily available aqueous solution of I[1] and chloroform, 3-chlorophenol (1.1-1.2 eqv.) and $(ipr)_2EtN$ (1 eqv.) were added and mixture vigorously stirred overnight in absence of light. Chloroform layer was separated and fractionated on silica gel in chloroform-methanol to give X with 85% yield. Treatment of X with 10% solution of triethylamine in methanol leads to deacetylated XI with 80% yield.

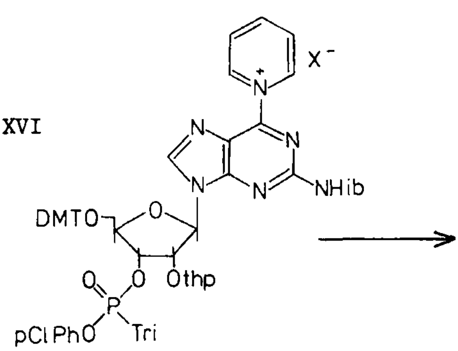
Crystalline salt II[2] reacts with 3-chlorophenol (1.1 eqv.) and $(ipr)_2EtN$ (1.5 eqv.) in acetonitrile with formation of XII with 92% isolated yield. Deacetylation of XII in a similar manner leads to crystalline XIII with 90% yield. In one-flask procedure 2',3',5'-tri-O-acetyl-N^2-isobutyrylguanosine was treated with 4-chlorophenylphosphorodichloridate (1.5 eqv.) and 1,2,4-triazole (3 eqv.) in pyridine as described[2] to give II. Mixture was treated with water, neutralized with $(ipr)_2EtN$ (5 eqv.) and subjected to reaction with 3-chlorophenolate in two-phase system as above; XII was isolated with 70% yield.

All O^6-(3-chloro)phenyl derivatives could be easy detected on tlc due to their pale-yellow fluorescence.

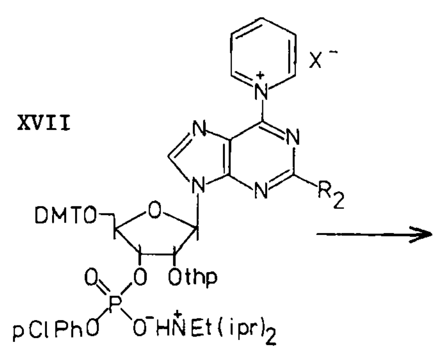
Transformation of II into O^6-(2-nitro)phenyl derivative XIV was achieved in two-phase system with 75% yield. Reaction of II with potassium thiophenolate (2 eqv.) in aqueous solution leads to 6-phenylthio-2-aminopurine riboside XV (50% yield) due to removal of protective groups under those conditions.

The following, multi-step, one-flask procedure for transformation of 5'-O-dimethoxytrityl-2'-O-tetrahydropyranyl-N^2-isobutyrylguanosine into its 3'-phosphodiester XVIIIa or 3'-triester XIXa might be of interest for all having "oldfashioned" 3'-OH guanosine components on the stock. Pyridine solution of 3'-OH component[10] was treated with dioxane solution of

XVI

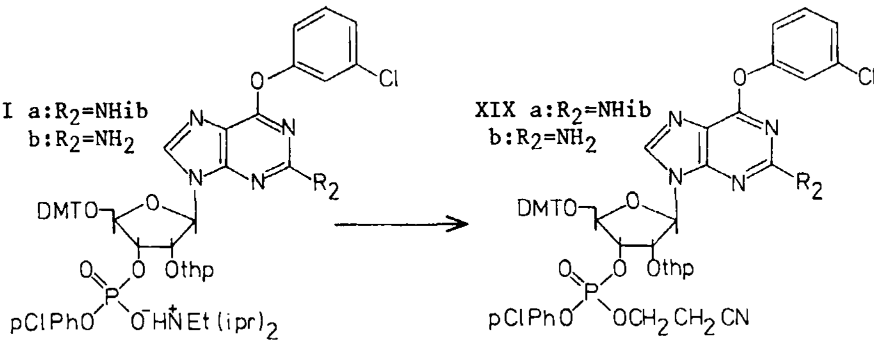


XVII



XVIII a:R_2=NHib
b:R_2=NH_2

XIX a:R_2=NHib
b:R_2=NH_2



4-chlorophenylphosphoro-di(1,2,4-triazolide) (3 eqv.), mixture concentrated and left over 3 days in order to obtain XVI (for recent data concerning reactivity of di(1,2,4-triazolide)see[4]). XVI gives positive Zincke reaction test[2] and shows phosphorylation activity toward methanol. Mixture was evaporated, treated with water to obtain XVII and neutralized with $(ipr)_2EtN$ (5 eqv.). After 1 hr chloroform was added and two-phase system treated with 3-chlorophenol and amine as above. After 24 hrs tlc analysis revealed the presence of two spots being both trityl-positive and pale-yellow fluorescent. Chloroform layer was subjected to:
(i) fractionation on RP-silica gel in acetone gradient in water containing 0.5% of Et_3N to give 3′-phosphodiesters XVIIIa and XVIIIb with 48 and 26% yield respectively or procedure (ii). In the latter, chloroform was evaporated, residue taken to pyridine and subjected to condensation with 2-cyanoethanol (2.5 eqv.) in the presence of TPS-Cl (2.5 eqv.) and 1-MeIm (5 eqv.) to give fully protected 3′-phosphotriesters XIXa and XIXb with 33 and 13% yield respectively.

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IV. mp. 108-110°C, λ_{max}(MeOH) 212 nm (ϵ 23300), 247 (10300), 284 (10100); δ^1H(DMSO-d_6) 8.11 (1H,s), 6.40 (2H,s), 5.81 (1H,d,J=5.86 Hz), 3.61 (2H,m), 1.37 (3H,t,J=7.08 Hz).

V. mp. 239-241°C, λ_{max}(MeOH) 255 nm (ϵ 9330), 280 (10000); ^{1}H(DMSO-d_6) 7.91 (1H,s), 6.79 (2H,s), 5.75 (2H,s), 5.71 (1H,d,J=5.85 Hz).

VI. δ^1H($CDCl_3$) 8.71 (1H,s), 8.10 (1H,s), 6.20 (1H,d,J=5.37 Hz), 3.39 (2H, q,J=7.32 Hz), 1.45 (3H,t,J=7.32 Hz), δ^{13}C($CDCl_3$) 23.35, 14.79 ($-SCH_2CH_3$).

VII. λ_{max}(MeOH) 226 nm (ϵ 10800), 249 (23100), 294 (15700), 303 (15000), δ^1H($CDCl_3$) 8.44 (1H,s), 7.96 (1H,s), 6.09-5.77 (3H,m), 4.47 (3H,m), 3.98 (2H,t), 3.54 (2H,t), 2.75 (1H,h,J=6.83), 2.15, 2.09, 2.06 (9H,s), 1.28 (6H,d,J=6.83); δ^{13}C($CDCl_3$) 62.85, 31.75 ($-S\cdot CH_2\cdot CH_2OH$).

IX. λ_{max}(MeOH) 203 nm (ϵ 19700), 227 (18100), 242 (25000), 295 (13400), 261 (14100)sh, δ^1H($CDCl_3$) 8.38 (1H,s), 8.03 (1H,s), 6.85 (1H,d,J= 4.40), 5.91 (1H,t), 5.73 (1H,m), 4.45 (3H,m), 2.52 (3H,s), 2.15, 2.10, 2.09 (9H,s).

XI. λ_{max}(MeOH) 206 nm (ϵ 26200), 254 (13600); δ^1H(DMSO-d_6) 8.79 (1H,s), 8.52 (1H,s) 7.63-7.23 (4H,m), 6.06 (1H,d,J=5.6 Hz), 5.55 (1H,d,J=5.8 Hz), 5.25 (1H,d,J=4.8 Hz), 5.13 (1H,t), 4.64 (1H,q), 4.22 (1H,m), 4.01 (1H,m), 3.66 (2H,m).

XIII. mp. 177-181°C, λ_{max}(MeOH) 209 nm (ϵ 24500), 274 (15700), δ^1H(DMSO-d_6) 10.48 (1H,s), 8.72 (1H,s), 7.76-7.41 (4H,m), 6.08 (1H,d,J=5.9 Hz), 5.68 (1H,d,J=5.8 Hz), 5.35 (1H,d,J=4.6 Hz), 5.11 (1H,t,J=5.4 Hz), 4.78 (1H,m), 4.35 (1H,m) 4.10 (1H,m), 3.77 (2H,m), 2.99 (1H,h, J=6.8 Hz), 1.12 (6H,d,J=6.8 Hz).

XIV. λ_{max}(MeOH) 222 nm (ϵ 28800), 261 (20700); δ^1H($CDCl_3$) 8.20-7.39 (5H,m), 8.07 (1H,s), 6.14 (1H,d,J=4.4 Hz), 5.96-5.85 (2H,m), 4.44 (3H,m), 2.88 (1H,h,J=6.8 Hz), 2.15, 2.10 (9H,s), 1.02 (6H,d,J=6.84).

XV. λ_{max}(MeOH) 213 nm (ϵ 21200), 247 (14700), 314 (13200); δ^1H($CDCl_3$) 7.77 (1H,s), 7.67-7.36 (5H,m), 6.04-5.90 (2H,m), 5.75 (1H,m), 4.90 (2H,br), 4.40 (3H,m), 2.12, 2.08 (9H,s).

XVIIIa. δ^{31}P (Py, external 85% H_3PO_4), -6.35 ppm; δ^1H($CDCl_3$), 8.06 (1H,s), 7.30-6.68 (m, 4-Cl-Ph, 3-Cl-Ph, DMTr), 6.20 (1H,d,5 Hz), 3.75 (6H, s), 0.99 (6H,d,J=6.8 Hz). The latter signal is not present in XVIIIb.

XIXa. δ^{31}P (Py, external 85% H_3PO_4) -7.88, 8.21, 9.50 ppm; δ^1H($CDCl_3$) 8.03 (1H,s), 7.30-6.73 (4-Cl-Ph, 3-Cl-Ph, DMTr), 6.15 (1H,d,6 Hz), 3.75 (6H,s), 4.23 (2H,m), 2.66 (2H,m), 1.1 (6H,d,J=6.8 Hz). The latter signal is not present in XIXb. XIXa is converted to XVIIIa by Et_3N/Py treatment as indicated by ^{31}P NMR and tlc.