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CHEMO AND REGIOSELECTIVE ACYLATION OF DEOXYRIBONUCLEOSIDES BY MEANS OF N,N-BIS-(2-OXO-OXAZOLIDIN-3-YL) PHOSPHORODIAMMIDIC CHLORIDE (BOPDC)

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Summary: High yields of 5´-acyl-deoxyribonucleosides can be obtained by direct acylation of the substrates with carboxylic acid after activation with N,N-bis-(2-oxo-oxazolidin-3-yl) phosphorodiammidic chloride (BOPDC). In the same experimental conditions a chemoselective benzoylation of unprotected deoxyadenosine can be carried out. Nucleotide building blocks have been prepared from the base labile 5´-protected nucleosides thus obtained.

Introduction

In the synthesis of oligonucleotides by means of any of the available procedures[1], large use is made of acid labile groups for the protection of the 5´ hydroxyl function of deoxyribonucleoside units. Trytil derivatives such as chloro-di(p-methoxyphenyl)-phenylmethane (DMT-Cl)[2], 9-chloro-9-phenylxanthene[3] and the like, can be regioselective introduced on the primary hydroxyl function of nucleosides owing to sterical hindrances of the reagents employed. The final deblocking of oligonucleotide chains often requires a temporary modification of the 5´-terminal protecting group from acid to base labile masking function.[4] Regioselective benzoylation of deoxynucleosides would allow the obtainment of building blocks to be used as terminal unit in oligonucleotide synthesis. The results obtained in the functionalization of deoxythymidine with the mixed anhydride likely formed, *in situ*, from MTMB and MTMT acid and N,N-bis-(2-oxo-oxazolidin-3yl) phosphorodiammidic chloride (BOPDC, 1)[5] suggested that the latter could be the reagent of choice for the activation of benzoic acid in the obtainment of the target 5´-benzoyl deoxynucleosides. BOPDC originally introduced for the synthesis of esters and amides[6], proved to be extremely useful in the formation of the peptide bond. [7,8]

Results and Discussions

The effectiveness of BOPDC as activating agent of carboxylic acid in the regioselective 5´-esterification of deoxynucleosides has been first checked on deoxythymidine (dT, 3a). The reaction of dT with acetic and benzoic acid, in the experimental conditions reported in table I, afforded 80±5 % estimated yields (TLC) of 5´-acyl-deoxythymidine; in similar conditions, dT was converted into 5´-benzoyl-deoxythymidine (5´-BzdT, 4a) with 60±5 % estimated yields by reaction

with benzoic anhydride or benzoyl chloride (Table I)

Table I. Acylation of deoxythymidine (0.5 mmol, TLC experiments)

Reagent (mmol.)	BOPDC (mmol.)	t(1/2) (hrs.)	5'-Ac-dT (yield)
PhCOOH (0.75)	1.5	1	80±5%
CH_3COOH (0.75)	1.5	1	80±5%
$(PhCO)_2O$ (0.6)	-	1.5	60±5%
PhCOCl (0.6)	-	0.5	60±5%

Benzoylation of deoxynucleosides was then performed on preparative scale by reaction of PhCOOH/BOPDC (1:2) with N^6-benzoyl-deoxyadenosine (dA^{Bz}, 3b), N^4-benzoyl-deoxycytidine (dC^{Bz}, 3c), N^2-isobutyryl-deoxyguanosine (dG^{ib}, 3d) and deoxythymidine itself, in pyridine solution at room temperature. Good isolated yields (Table II) of the 5'-benzoyl derivatives 4a-d were obtained after work-up and short column chromatography. Structural assignment has been performed by ^1H NMR and fast-atom-bombardment mass spectrometry (FABMS)[9,10]. In particular NMR spectra clearly showed the disappearance of the resonance signal due to the 5'-OH group of the starting molecule, while FABMS gave the expected $(M+H)^+$ species and diagnostic fragment ions.

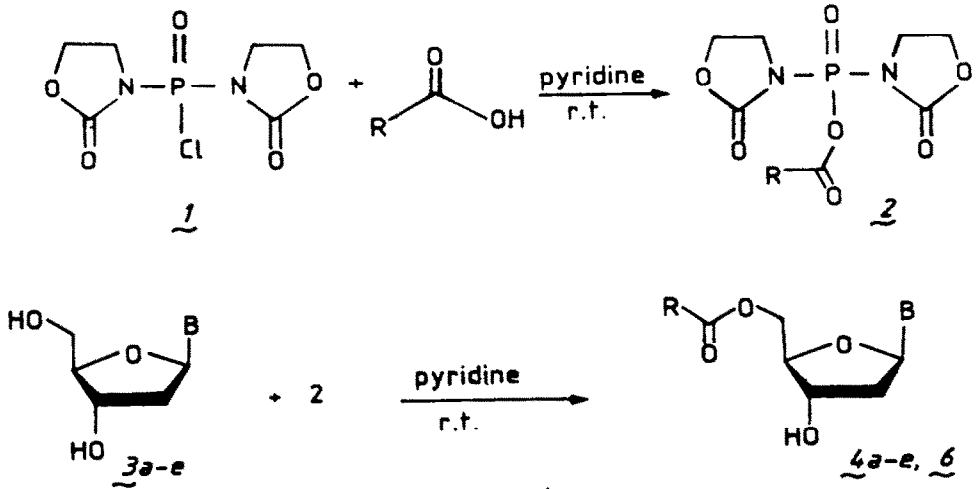
Table II. Acylation of deoxyribonucleosides with PhCOOH/BOPDC

Substrate	Reagent	Products (isol. yields %)
dT	PhCOOH	4a (70)
dA^{Bz}	"	4b (75)
dC^{Bz}	"	4c (70)
dG^{ib}	"	4d (67)
dA	"	4e (76)
dT	$C_6H_3Cl_2COOH$	6 (75)

The comparison of these results with the data reported in Table I clearly suggests that the mechanism of activation of BOPDC can not procede via the formation of symmetric anhydrides such as $(PhCO)_2O$. The latter, in fact, did not show marked differences in its reactivity toward both the hydroxyl groups of the reacting nucleosides. Further evidences which support the given interpretation have been obtained by allowing dT (3a) to react with 2,6-dichlorobenzoic acid (5), in the same experimental conditions as above. As expected compound 5 reacted slower than benzoic acid with the same substrate **3a** but it gave rise to similar yields of 5'-(2,6-dichlorobenzoyl)-deoxythymidine (5'-DCBz-dT, 6, table II). It must be pointed out that 2,6-dichlorobenzoylchloride (7) is a good activating agent for the acylation of deoxynucleosides[5] and that this remarkable reactivity has to be associated with the good leaving group properties of 2,6-dichlorobenzoate and with the presence of the chlorine atoms "ortho" to the carboxyl group which prevent the incoming nucleophile to approach, eventually, the carboxyl carbon linked to the aromatic ring. The reactivity data observed on going from acetic acid (table I) to 2,6-dichlorobenzoic acid (table II) towards the same substrate **3a** suggest that the regioselectivity experienced in the acylation of nucleosides with BOPDC should be determined by the phosphorodiammidic counterpart of mixed anhydride **2** (Scheme 1), which has been proposed as intermediate in the acylation reactions via BOPDC.[11]

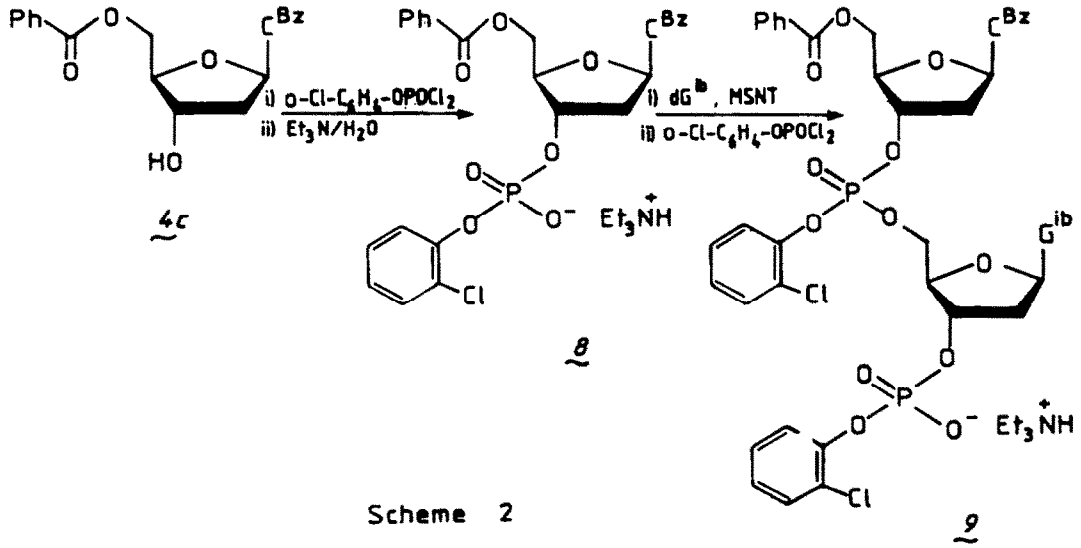
5'acyl-deoxynucleosides can be, therefore, obtained with similar yields and degree of regioselectivity than the 5'-acid labile analogs. It has been,

therefore, evaluated the possibility of preparing building blocks to be employed in oligonucleotide synthesis. The diesterophosphate **9** has been prepared according to scheme 2, with good isolated yields and its structure has been determined by FABMS (see experimental). The PxdNp terminal unit employed in the synthesis of oligonucleotides by the "filtration" method[4] could therefore be replaced by nucleotides of type structure **8** or **9** (scheme 2), thus avoiding the final acid treatment of the oligomer.



Scheme 1

In the experiments discussed so far, the pyrimidine and purine bases of the examined nucleosides have been protected in the usual way[1], except for deoxythimidine where the thymine base have been left unprotected.

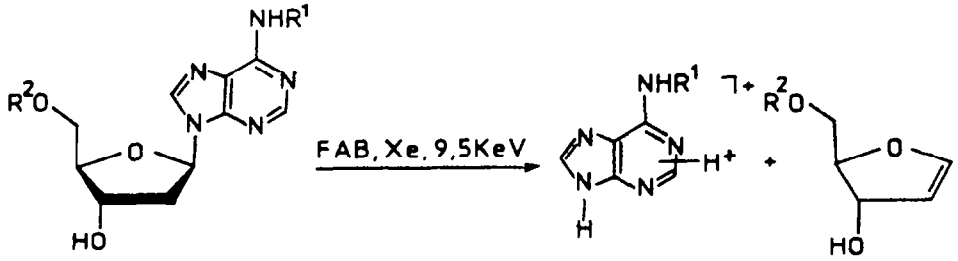


Scheme 2

The possibility of carrying out chemoselective benzoylation by using the proposed activating agent have been checked with 2´-deoxyadenosine (dA, **3e**). The nucleoside **3e**, treated with PhCOOH/BOPDC in the usual conditions, afforded 76% yield of a colourless solid (m.p. 188-190) which showed on SiO_2 TLC plate the same Rf than

N^6-benzoyldeoxyadenosine (dA^{Bz}, 3d). Although melting points and spectroscopic (IR, NMR) data of the isolated compound differ from that of **3d**, unambigous structural information have been obtained by FABMS. The spectrum of the positive ions desorbed from glycerol solution of m.p. 188-190 gave, besides the $(M+H)^+$ species, a fragment ion (base peak) at m/z 136 corresponding to protonated adenine (10, scheme 3). The FAB spectrum of **3d**, in the same experimental conditions, was characterized by the presence of a base peak at m/z 240, corresponding to protonated benzoyladenine (11, scheme 3). The same trend was also observed in the FAB-MS/MS[11-12] spectra obtained from the isobaric $(M+H)^+$ ions produced from both samples. Direct benzoylation of **3e** in the presence of BOPDC gave rise, therefore, to 5′-benzoyldeoxyadenosine (4e), whose conjugated acid afforded, in the gas phase, the protonated adenine at m/z 136, according to the mechanism reported in scheme 3. When the excess of benzoic acid and activating agent **1** was increased with respect to the usual conditions (see experimental) and the reaction time was prolongued, 3e gave appreciable amounts of **4e** togheter with 3′,5′-dibenzoyladenosine (12) and 3′,5′,N^6-tribenzoyladenosine (13), obtained with 25 and 21 % isolated yields respectively.

The benzoylation of deoxyadenosine can be, therefore, kinetically controlled in the direction of producing the chemo and regioselective product **4e**. The observed chemoselectivity can be interpreted assuming that the adeninic base residue is involved, in the reaction environment, in prototropic equilibria which prevent to some extent the benzoylation of the base.



3b, 4e *10-11*

3b) R^1 = PhCO; R^2 = H

4e) R^1 = H; R^2 = PhCO

10) m/z 240; R^1 = PhCO

11) m/z 136; R^1 = H

Scheme 3

Conclusions

Regioselective acylation of deoxyribonucleosides can be carried out with commercially available carboxylic acids after activation, in pyridine solution, with BOPDC. The isolated yields of the 5′-acyl derivatives **4a-e** and **6** are comparable with those obtained in the protection of the same substrate with classic acid labile groups. The nucleosides **4** can be further elaborated to give nucleotides **8** and **9** which can be employed as 5′-terminal units in oligodeoxyribonucleotide synthesis by the phosphotriester approach. Finally, when unprotected deoxyadenosine is used, the process can be controlled in the direction of producing high isolated yields of 5′-benzoyldeoxyadenosine. The results above

presented extend the range of applicability of BOPDC in the formation of the ester functionality.

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Experimental

Base protected deoxyribonucleosides **3b-d** were prepared by literature procedures[1], BOPDC was synthesized as described.[6] Merck Kieselgel 60H without gypsum was used for short column chromatography and Merck SiO_2 precoated plates for TLC experiments. ^1H NMR spectra have been obtained from a WM 300 Bruker spectrometer or, when indicated, from a XL 100 Varian spectrometer, DMSO-d_6 was used as solvent and the chemical shifts are reported in (TMS=0). FABMS spectra have been obtained from a Vacuum Generators (VG) ZAB 2F mass spectrometer equipped with a M-SCAN stearable gun. Melting points have been determined by a kofler hot-stage and are uncorrected. Satisfactory elemental analysis have been obtained for the new compounds.

Synthesis of Deoxyribonucleosides **4a-e** and **6**: General Procedure.

A solution of 1.5 mmol of the appropriate nucleoside **3a-e** and 2.25 mmol of benzoic acid in 30 ml of dry pyridine was evaporated three times to half volume. To the final solution (15 ml) 4.5 mmol of BOPDC were added and the reaction monitored by TLC (chloroform:methanol/9:1, v.v.). After 95% ca. of conversion of the substrates **3a-e**, the solution was partitioned between chloroform (30 ml) and 1M potassium carbonate (5 ml). The organic layer was washed with water (3x5 ml) and evaporated to dryness by vacuum azeotropic distillation with toluene (2x15 ml) and ethanol (2x15 ml). The glass residue was purified by short column chromatography (chloroform:methanol/97.5:2.5, v.v).

5′-benzoyldeoxythymidine **4a**: m.p. 167 C, 70% yield, 5 hrs. ^1H NMR: 11.33 (1H, s, 3-H), 8.02 (2H, d, o-Ar, J=7.30 Hz), 7.69 (1H, t, p-Ar, J=7.22 Hz), 7.55 (2H, t, m-Ar, J=7.29 Hz), 7.40 (1H, s, 6-H), 6.23 (1H, t, 1′-H, J=6.40 Hz), 5.52 (1H, s(broad), 3′-OH), 4.56 (1H, m, 3′-H), 4.45 (2H, m, 5′-H), 4.07 (1H, m, 4′-H), 2.16-2.33 (2H, m, 2′-H), 1.61 (3H, s, CH_3); FABMS (m/z, %) : 347 [21, $(M+H)^+$], 221 (11), 127 (48), 105 (57), 81 (100, b.peak).

5′,N^6-dibenzoyldeoxyadenosine **4b**: m.p. 88-89 C, 75% yield, 6 hrs. ^1H MMR : 11.23 (1H, s, NH), 8.73 (1H, s, 8-H), 8.67 (1H, s, 2-H), 8.06 (2H, d, o-Ar, J=7.4 Hz), 7.93 (2H, d, o-Ar, J=7.5 Hz), 7.68-7.49 (6H, m, p-Ar + m-Ar), 6.54 (1H, t, 1′-H, J=6.5 Hz), 5.64 (1H, d, 3′-OH, J=4.09), 4.71 (1H, m, 3′-H), 4.45-4.62 (2H, m, 5′-H), 4.22 (1H, m, 4′-H), 3.01-3.38 (1H, m, 2′-H), 2.45-2.50 (1H, m, 2′-H); FABMS (m/z, %) : 460 [4, $(M+H)^+$], 240 (42), 136 (10), 105 (100, b. peak), 81 (29), 77 (19).

5′,N^4-dibenzoyldeoxycytidine **4c**: m.p. 182-183 C, 70%, 5 hrs. ^1H NMR: 11.17 (1H, s, 4-H), 8.16 (1H, d, J=8.2Hz, 6-H), 7.51-8.12 (10H, m, Ar), 7.27 (1H, d, 8.2 Hz, 5-H), 6.20 (1H, t, J=6.1 Hz, 1′-H), 5.49(1H, d, J= 4.1 Hz, 3′-OH), 4.21-4.55 (4H, m, 3′-H + 4′-H + 5′-H), 2.43 (1H, m, 2′-H), 2.24 (1H, m, 2′-H); FABMS (m/z, %): 436 [3, $(M+H)^+$], 216 (44), 112 (11), 105 (100, b. peak), 81 (41).

5′-benzoyl-N^2-isobutyryldeoxyguanosine **4d**: m.p. 120-121 C, 67% yield, 2 hrs. ^1H NMR: 12.09 (1H, s, 2-H), 11.64 (1H, s, 1-H), 8.22 (1H, s, 8-H), 7.92 (2H, d, J= 7.1 Hz, o-Ar), 7.67 (1H, t, J= 7.0 Hz, p-Ar), 7.52 (2H, t, J= 7.0 Hz, m-Ar), 6.27 (1H, t, J= 6.6 Hz, 1′-H), 5.57 (1H, d, J= 3.9 Hz, 3′-OH), 4.35-4.65 (3H, m, 3′-H + 5′-H), 4.16 (1H, m, 4′-H), 2.77 (2H, m, 2′-H), 2.43 (1H, m, C-H^{ib}), 1.12 (6H, d, J= 6.9 Hz, CH_3); FABMS (m/z, %): 442 [10, $(M+H)^+$], 222 (100, b. peak), 178 (2), 152 (20), 135 (5), 105 (46), 81 (19).

5′-benzoyldeoxyadenosine **4e**: m.p. 188-190 C, 76% yield, 3 hrs. ^1H NMR: 8.35 (1H, s, 8-H), 8.19 (1H, s, 2-H), 7.95 (2H, d, J= 7.34 Hz, o-Ar), 7.67 (1H, t, J= 7.20 Hz, p-Ar), 7.52 (2H, t, J= 7.30, m-Ar), 7.37 (2H, s, NH_2), 6.44 (1H, t, J= 6.5 Hz, 1′-H), 5.65 (1H, m, 3′-OH), 4.71 (1H, m, 3′-H), 4.45-4.62 (2H, m. 5′-H), 4.21 (1H, m, 4′-H), 2.96-3.05 (1H, m, 2′-H), 2.41-2.48 (1H, m, 2′-H). FABMS (m/z, %): 356 [33, $(M+H)^+$], 162 (12), 136 (100, b. peak), 105 (61), 81 (45).

5′-(2,6-dichlorobenzoyl)-deoxythymidine **6**: m.p. 225-228 C, 75%, 48 hrs. ^1H NMR (100 MHz): 11.47 (1H, s, 3-H), 7.66 (3H, m, Ar), 7.45 (1H, m, 6-H), 6.30 (1H, t, J= 6.2 Hz, 1′-H), 5.56 (1H, d, J= 4.0 Hz, 3′-OH), 4.63 (2H, d, J= 5.2 Hz, 5′-H), 4.39 (1H, m, 3′-H), 4.12 (1H, m, 4′-H), 2.22 (2H, m. 2′-H), 1.66 (3H, s, CH_3). FABMS[14] (m/z, %): 415* [8, $(M+H)^+$], 381*(3), 242 (100, b. peak), 173* (19), 142 (41), 127 (52), 81 (60).

Synthesis of dinucleotide phosphate 9: The synthesis of the building block 9 has been performed according to scheme 3, starting from 0.5 mmol of 4c and following the published general procedure.[15] Compound 9 has been obtained as pale yellow solid with 89.5% yield. FABMS[14] (m/z, %): 1133 [100, b. peak, $(M-H)^-$], 716 (25, $pG^{ib}p$), 624 (26, $BzC^{Bz}p$).

Synthesis of deoxyribonucleosides 12 and 13: The procedure above described was applied to the benzoylation of 0.5g (1.86 mmol) of deoxyadenosine (3e) in the presence of 0.5g of benzoic acid (4.1 mmol) and 1.56 g (6.1 mmol) of BOPDC suspendend in 15 ml of dry pyridine. After 72 hrs. the mixture was partitioned between chloroform (30 ml) and (2x5 ml) of 1M potassium carbonate. After the usual work-up the glass residue was purified by short column chromatography to give:

3'-5'-dibenzoyldeoxyadenosine 12: m.p. 75-78 C, 24.6% yield. ^1H NMR: 8.34 (1H, s, 8-H), 8.14 (1H, s, 2-H), 8.09-7.48 (10H, m, Ar), 7.26 (2H, s, NH_2), 6.55 (1H, t, J= 6.5 Hz, 1'-H), 5.87 (1H, m, 3'-H), 4.59-4.71 (3H, m, 5'-H + 4'-H), 3.36- 3.43 (1H, m, 2'-H), 2.75-2.81 (1H, m. 2'-H); FABMS (m/z, %): 460 [8, $(M+H)^+$], 136 (100, b. peak), 105 (85), 81 (90).

3'-5'-N^6-tribenzoyladenosine 13: m.p. 77-78 C, 21% yield. ^1H NMR: 11.14 (1H, s, NH), 8.69 (2H, s, 8-H + 2-H), 8.30-7.49 (15H, m, Ar), 6.69 (1H, t, J= 6.6 Hz, 1'-H), 5.91 (1H, m, 3'-H), 4.61- 4.73 (3H, m, 4'-H+ 5'-H), 3.44-3.48 (1H, m, 2'-H), 2.90--2.85 (1H, m, 2'-H); FABMS (m/z, %): 564 [1.5 $(M+H)^+$], 240 (21), 105 (100, b. peak), 81 (40), 77 (20).

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