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Synthesis of Novel Carbazoyl Linked Pyrimidine-Pyrimidine and Pyrimidine-Purine Dinucleotide Analogues.

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Abstract: The synthesis of two backbone modified dinucleotide analogues is described in which the natural phosphodiester linkage is replaced by a 3'-5' carbazoyl linkage. In both cases the bridge was formed through a coupling reaction between an appropriate 3'-carbazoyl nucleoside analogue and an aldehyde nucleoside derivative. It is noteworthy that starting nucleosides **4** could be common materials to obtain the 3'-carbazoyl nucleoside derivatives **2**, by means of a simple, previously-described chemoenzymatic procedure, and the aldehyde nucleoside **3**, by an oxidation reaction.

Modulation of gene expression by antisense technologies requires the development of modified oligonucleotides possessing enhanced cellular uptake, resistance toward degradation by nucleases, and appropriate hybridization to target natural oligonucleotides. Consequently, these modified oligonucleotides are now being actively investigated as a new generation of pharmaceuticals.[1] In the last decade, a great deal of effort has been directed toward the synthesis of analogues with an altered phosphodiester linkage,[1,2] to improve the stability of duplex and triplex formation, to enhance the cellular uptake and to decrease the rate of degradation of oligonucleotides by nucleases which cleave the phosphodiester linkage. One of the most important modifications is the complete substitution of the phosphate internucleoside bridge. Thus, several properties of the natural oligonucleotides have been improved for the potential therapeutic application of the antisense strategy. To design alternative phosphate-free backbones, a general criteria should be considered:[2] (a) *conformational flexibility*, given by 5 to 7 rotatable bonds; (b) *charge*, neutral or positive is preferred; (c) *hydration*, the internucleoside linkage should be strongly hydrated to present a hydrophilic face to aqueous solution; (d) *chemical stability*, regarding general base catalysis; and (e) *synthesis*, the synthetic chemistry should be readily adaptable to modern solid phase synthetic methods.

To our knowledge there has been no investigation of backbone linkages that contain carbazoyl groups and yet this type of linkage appears to have properties that make it an attractive surrogate for the phosphodiester linkage, being non-ionic, hydrolytically stable, non-chiral, and meeting all the previously-mentioned criteria related to the development of alternative phosphodiester backbones.

We offer here the synthesis of the carbazoyl linked pyrimidine-pyrimidine and pyrimidine-purine dinucleotide analogues **1**. Retrosynthetic analysis of desired dimers **1** indicated that 3'-carbazoyl nucleoside derivatives **2** and aldehyde nucleoside analogues **3** would serve as key building blocks (Chart 1).

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3'-Carbazoyl nucleosides **2** were synthesized from the corresponding nucleosides **4** (B= T or A) through a regioselective enzymatic alkoxycarbonylation process followed by reaction with hydrazine.[3] We take advantage of the ability of enzymes in discerning one of several reactive positions to obtain the corresponding 3'-carbamates of nucleosides in a direct and regioselective manner. We used the two-step procedure due to the fact that certain carbamates have been shown to be good inhibitors of many serine hydrolases.[4] This method involves the regioselective synthesis of an alkoxycarbonylated nucleoside **5** and further reaction with hydrazine giving place to carbazoyl nucleosides **2**. The enzymatic process was carried out using acetone *O*-[(vinyloxy)carbonyl]oxime (VCO) as alkoxycarbonylating reagent, at 30 °C in tetrahydrofuran (THF), with *Pseudomonas cepacia* lipase (PSL) as biocatalyst. Then, reaction with hydrazine (80% in water) at room temperature yielded 3'-carbazoyl 2'-deoxyribonucleoside derivatives **2** quantitatively (B= T, A) (Scheme 1).

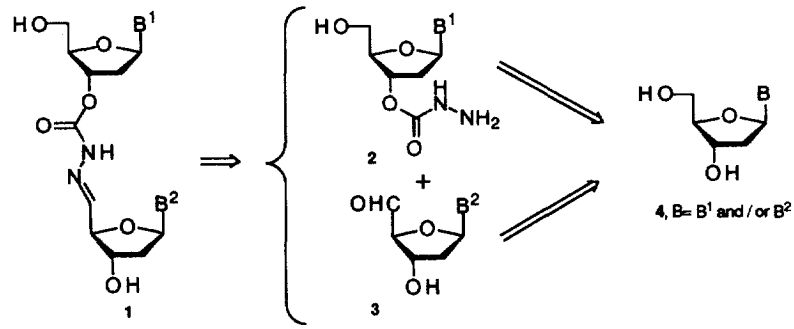
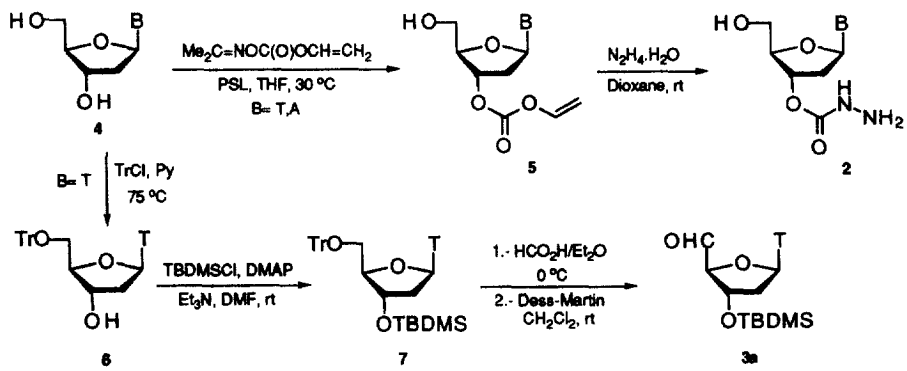


Chart 1

The synthesis of the appropriate protected aldehyde **3** is shown in Scheme 1. We focused our attention on the preparation of aldehyde **3a** which possess a *t*-butyldimethylsilyl protecting group in the 3'-position that will be easily removable at the end of the synthesis.



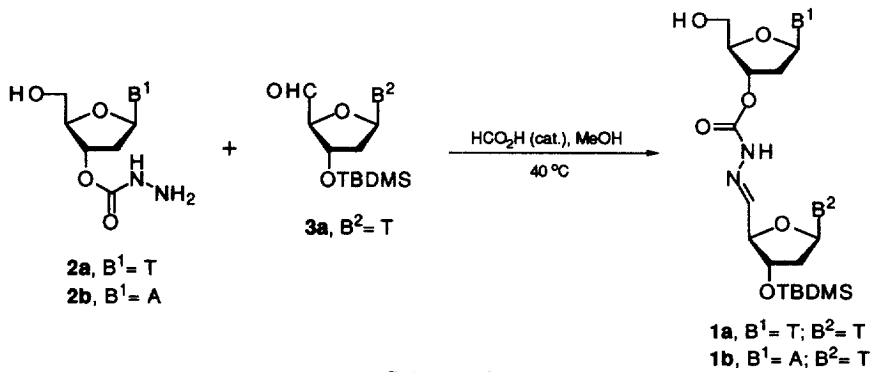
PSL: *Pseudomonas cepacia* lipase

Scheme 1

Thus, thymidine **4a** (B= T) was selectively protected at the 5'-hydroxyl group (primary *vs* secondary) with trityl chloride in pyridine at 75 °C through a high yielding (80%) procedure, given rise to trityl derivative

6 in 20 h. The latter reacted in 24 h with *t*-butyldimethylsilylchloride at room temperature to form silylated compound **7** (82%). Deprotection of the 5'-hydroxyl in **7** with acetic acid using THF as cosolvent at 55 °C gave the 3'-*t*-butyldimethylsilyl protected 5'-alcohol with 40% yield, another 40% being the compound which results from the deprotection of 3'-hydroxyl group, too. We subsequently optimized the reaction conditions, changing the reagents until we obtained 99% yield of only the 5'-alcohol after 2 h of reaction using the mixture formic acid / diethyl ether (3:2) at 0 °C.[5] The procedure continued with Dess-Martin oxidation[6] in methylene chloride at room temperature, and aldehyde **3a** was isolated quantitatively with high degree of purity after 5 min of reaction.

Once we had obtained 3'-carbazoyl nucleosides **2a** (B^1= T) and **2b** (B^1= A), and aldehyde **3a**, we attempted to synthesize the dimers **1**. To study the coupling conditions, we previously used benzaldehyde as a model for **3a**, using **2a** as carbazoyl nucleoside. The best reaction conditions were when MeOH was used as solvent at room temperature in 2 h with *p*-toluenesulfonic acid as catalyst gave an 88% yield of the coupled product.



2a, B^1= T
2b, B^1= A

3a, B^2= T

1a, B^1= T; B^2= T
1b, B^1= A; B^2= T

Scheme 2

When the procedure was applied to nucleoside **2a** (B^1= T) and aldehyde **3a**, it was necessary to change the catalyst to formic acid (pKa= 3.77) from *p*-toluenesulfonic acid (pKa= -6.50), since the latter was too acidic and provoked deprotection of silyl group. In these conditions, it was necessary to increase the temperature to 40 °C to obtain 70% of coupled product. An increment of temperature (60 °C) provoked aldehyde decomposition. Similar conditions led to dimer **1b** starting from 3'-carbazoyl nucleoside **2b** (B^1= A) and nucleoside aldehyde **3a**. Thus, in 48 h at 40 °C it was possible to obtain the coupled product **1b** in good yield (71%).

The above mentioned structures were determined by means of their spectroscopic data. The structural assignment of the compounds described in this paper is based on the analysis of their ^{1}H- and ^{13}C-NMR spectra. Additional DEPT experiments and the correct assignment were confirmed by ^{1}H-^{13}C heteronuclear correlation experiments.[7]

In summary, 3'-carbazoyl nucleoside derivatives **2a**, **2b** readily available through chemoenzymatic procedure, have been coupled with aldehyde **3a** in very mild reaction conditions given place to 3'-5' carbazoyl linked pyrimidine-pyrimidine and pyrimidine-purine dinucleotide analogues **1a** and **1b**. These are described

for the first time as phosphate-free backbone-modified dinucleotide analogues. These dimers with carbazoyl bridges may represent a new class of potential therapeutic agents. The methodology developed here should therefore allow the synthesis of carbazoyl linked oligonucleotides. Currently, we are engaged in the improvement of the synthesis of dimers of this type which contains purine-purine as bases.

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7. Select data for compound **1a**: . M.p.: 130 °C (syrup), 210 °C (liquid); $[\alpha]_D^{22}$ = -10.3° (c 0.64, MeOH); IR (KBr): ν 1692 cm^{-1}; (Note: assignations designed with * originally belonged to compound **2a** and all others to compound **3a**). ^{1}H-NMR (MeOH-d_4, 400 MHz): δ 0.37 (s, 6H, 2 × Me), 1.12 (s, 9H, ^{t}Bu), 2.10 (s, 3H, H_7*), 2.15 (s, 3H, H_7), 2.50 (m, 2H, $H_{2'}$), 2.61 (m, 2H, $H_{2'}$*), 4.05 (m, 2H, $H_{5'}$*), 4.34 (m, 1H, $H_{4'}$*), 4.65 (m, 1H, $H_{4'}$), 4.85 (m, 1H, $H_{3'}$), 5.55 (d, 1H, $H_{3'}$*), 6.50 (m, 1H, $H_{1'}$), 6.50 (m, 1H, $H_{1'}$*), 7.63 (m, 1H, N=CH), 8.10 (br s, 1H, H_6), and 8.10 (br s, 1H, H_6*); ^{13}C-NMR (MeOH-d_4, 100.6 MHz): δ 4.41 (2 × Me), 12.66, 12.80 (C_7, C_7*), 19.14 (CMe_3), 26.52 (CMe_3), 38.87 ($C_{2'}$), 40.39 ($C_{2'}$*), 63.32 ($C_{5'}$*), 75.99 ($C_{3'}$), 77.95 ($C_{3'}$*), 86.31 ($C_{1'}$*), 87.16, 87.22 ($C_{4'}$,$C_{4'}$*), 88.05 ($C_{1'}$), 112.21 (C_5, C_5*), 138.11 (C_6), 139.14 (C_6*), 146.96 (C=N), 152.61, 152.69 (C_2, C_2*), 155.38 (*C=O), and 166.63, 166.77 (C_4, C_4*); Anal. Calcd. (%) for $C_{27}H_{40}N_6O_{10}Si$: C, 50.93; H, 6.33; N, 13.20. Found: C, 51.0; H, 6.4; N, 13.0; MS (FAB$^+$, NBA): *m/z* 637 (M^++1, 3%), 325 (7), 312 (26), and 225 (18).