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Synthesis of C-4 substituted pyrimidines exhibiting various H-bonding patterns

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Abstract

A series of pyrimidine nucleosides modified at the C-4 position by different donor/acceptor groups has been synthesized. Since binding properties rely on the conformation of the exocyclic moiety, their structure has been determined by 2D NMR experiments and X-ray crystallography. © 1999 Published by Elsevier Science Ltd. All rights reserved.

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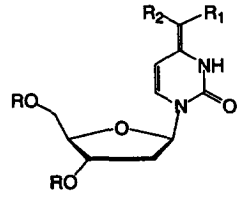
Among the many known nucleoside analogs, various pyrimidine nucleosides modified at the four position have been prepared. 4-*N*-Substituted pyrimidines are of interest as naturally occuring constituents of tRNA,[1] 4-*C*-substituted pyrimidine analogs have been studied as potential cytidine deaminase inhibitors[2] and 4-*O*-alkylated thymine derivatives have been synthesized in order to understand their role in the carcinogenic and mutagenic properties of DNA-alkylating *N*-nitroso compounds.[3]

We are interested in nonnatural pyrimidine nucleosides with an extended conjugation system and an enlarged set of hydrogen-bond donor and acceptor groups (Fig. 1). Indeed, such compounds could possess new binding properties towards proteins and nucleic acids and ultimately modulate gene expression, e.g. through triple helix formation[4] when incorporated into oligonucleotides.



R_1 = C(NH)CH_3, $CONH_2$, C(NH)NH_2

Figure 1. General structure of the modified nucleosides

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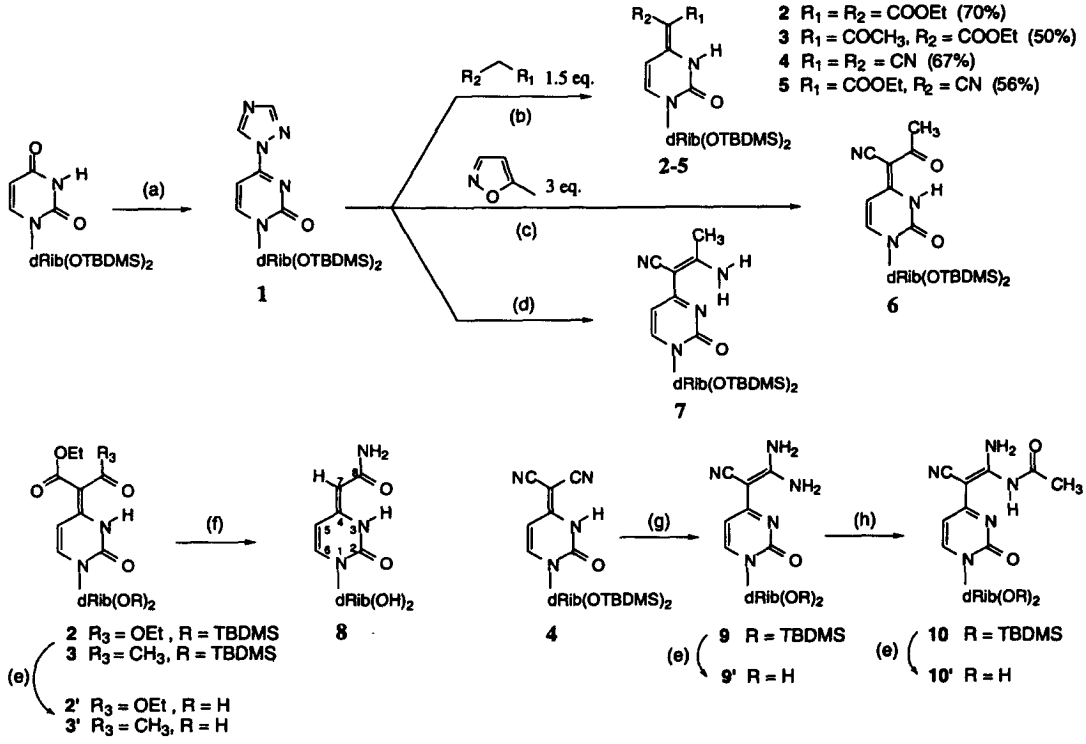
followed by transformation of ester and cyano groups to the desired H-bond-forming residues. Ester and cyano groups were used as precursors of amide and amidine functions, respectively.

Reaction of **1** with the sodium salts of diethylmalonate, ethylacetoacetate, malononitrile or ethylcyanoacetate in THF at 0°C, followed by aqueous work-up (saturated NH_4Cl solution) and silica gel chromatography afforded the Knoevenagel condensation products **2–5** in 50–70% yield. 2′-Deoxyuridine was used instead of thymidine in order to avoid methyl group steric hindrance. Ring-opening of 5-methylisoxazole[7] by *t*-BuOK followed by condensation with **1** in *t*-BuOH at 30°C gave compound **6** (Scheme 1).



Scheme 1. Reagents and conditions: (a) 1,2,4-triazole (15 equiv.), $POCl_3$ (3.5 equiv.), Et_3N (17 equiv.), CH_3CN, 92%; (b) NaH (3 equiv.), THF, 0°C; (c) *t*-BuOK (5 equiv.), *t*-BuOH, 30°C, 61%; (d) Na (10 equiv.), CH_3CN, 0°C, 55%; (e) TBAF (2.5 equiv.), THF, 25°C, 90%; (f) conc. aqueous NH_3, 25°C, 70%; (g) MeAl(Cl)NH_2 (3.4 equiv.), toluene, 80°C, 24 h, 75%; (h) CH_3COCl (2 equiv.), NEt_3 (3 equiv.), 4-DMAP (0.1 equiv.), CH_2Cl_2, 66%

After deprotection of the silyl ethers of compounds **2** and **3** by TBAF in THF, the resulting products **2′** and **3′** were reacted with concentrated aqueous ammonia at room temperature in order to afford the corresponding bis-amide and keto-amide, respectively. Instead, **2′** and **3′** both underwent basic cleavage followed by ammonolysis to give the mono-amide **8**. Compounds **2′** and **3′** indeed are β-tricarbonyl-like and therefore are very sensitive to basic conditions. Attempts to obtain the mono-nitrile from compounds **4–6** following the same method were unsuccessful. Direct reaction of **1** with acetonitrile gave compound **7** instead of the mononitrile derivative, as acetonitrile first dimerized in the presence of Na.

For conversion of the nitrile function of **4** into an amidine group, the Pinner method (HCl/MeOH, NH_4Cl)[8] and the reaction with sodamide ($NaNH_2$)[9] were tested without success. We then used an aluminium reagent, which was described by Garigipati for direct transformation of nitriles to amidines.[10] This reagent was used later to synthesize amidines from sterically hindered or from nonactivated nitriles that were inert under standard Pinner method.[11] Reaction of compound **4** with methylchloroaluminium

amide in anhydrous toluene at 80°C, followed by acidic hydrolysis (HCl 5%) gave **9** with 75% yield. Even with a large excess of reagent and prolonged reaction times, only one of the nitrile functions could be brought to react. Likewise, reaction of **5** and **6** with this reagent did not lead to the corresponding amidines. We expect this to be due to the mechanism of action of the aluminium complex which could proceed via interaction of the complex with N3-H.

2D NMR experiments were performed to determine the orientation of the exocyclic groups. The results obtained are consistent with the structures as represented in Scheme 1. A strong NOESY correlation peak was observed between N3-H and the ketone methyl group, as well as between H-5 and the ester CH_2 group for compound **3**. Thus, the ketone group appeared to be on the same side as N3-H in compound **3**. Likewise, for compound **8**, a strong NOESY correlation peak was observed between H-5 and H-7, indicating that the amide group was on the side of N3-H. This isomeric preference presumably results from an intramolecular hydrogen bond between N3-H and the heteroatom at C-8. The very large N3-H chemical shifts in **5** and **6** (δ=ca. 12 ppm) also agree well with the existence of an intramolecular hydrogen bond. In the case of **7**, COSY and ^{15}N–^{1}H HSQC experiments revealed that the exocyclic nitrogen is bearing two hydrogens and that the conformation is also locked by an intramolecular hydrogen bond, giving rise to a normal and to a high field NH ^{1}H NMR peak.

For compound **9**, it was not possible to observe NOESY correlations as all NH were exchanging. This compound was of utmost interest as a candidate for bidentate H-binding to guanine in triple helix context.[4] The solid state structure of an amidine derivative was therefore investigated by X-ray crystallography.[12] Suitable monocrystals could only be obtained from compound **10′**, the monoacetyl derivative of **9**. Analysis of the crystallographic data revealed that the upper conjugated part of the nucleoside is planar and that the amidine extends on the same side as N3 (Fig. 2). Insofar as a general conclusion can be drawn from the structure of the acetyl derivative, bond lengths analysis led us to assume that amidine derivatives are best described by the tautomer drawn in Scheme 1. As observed for compound **7** (see NMR section), the conformation of **10′** is locked by an intramolecular hydrogen bond between N3 and the exocyclic NH group.

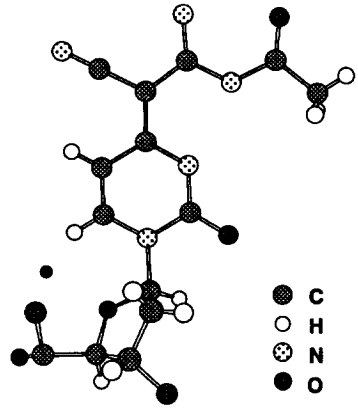


Figure 2. X-Ray structure of **10′**

The nonnatural nucleosides[13] **8** and **9′** have UV-absorption bands at 320 nm (ε=24 ml/μmol) and 335 nm (ε=35 ml/μmol) respectively, i.e. far away those of the natural nucleosides. The pKa value of the amidine group of **9′** was determined spectroscopically to be 3.2, hence no protonation should occur at physiological pH.

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12. X-Ray data for **10′**: A yellow crystal of **10′** was obtained upon slow liquid–liquid diffusion of H_2O into a DMSO solution of **10′**. $C_{14}H_{18}N_5O_5$, M=335.33, monoclinic, a=5.4046(2), b=11.8158(5), c=12.4770(4) Å, V=788.99(9) Å^3, space group P 1 2_1 1, Z=2, Dc=1.48 g cm^{-3}, μ (Mo-Kα)=0.118 mm^{-1}. Crystal dimensions: 0.20×0.14×0.10 mm. Data were measured at 173 K on a Kappa CCD diffractometer with graphite monochromated Mo-Kα radiation. The structure was solved by direct methods using OpenMoleN 2.2 and refined anisotropically using absorption corrected data to give R=0.057, Rw=0.078 for 1806 independent observed reflections [$|Fo|>3\sigma(|Fo|)$]. The 5′-OH group was disordered with two positions O(4) and O(5). Atomic coordinates, bond lengths and angles have been deposited at the Cambridge Crystallographic Data Centre.
13. The final compounds **8** and **9′** gave satisfactory ^{1}H and ^{13}C NMR spectra, mass spectra and elemental analyses.