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Regioselective Synthesis of β-N1- and β-N3-Alloxazine Nucleosides

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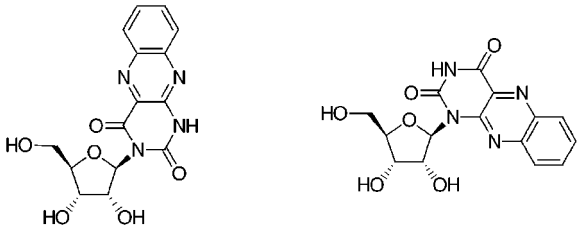
ABSTRACT



A regio- and stereoselective glycosylation of ribose tetraester with persilylated alloxazine to give either β-N1 or β-N3 nucleosides is described. The N3 product is potentially of interest as a fluorescent nucleoside and is predicted to have the hydrogen-bonding characteristics of thymidine.

The fluorescence properties of many organic fluorophores are sensitive to their chemical environment, making such reagents of tremendous interest as probes for biochemical processes. Fluorescent nucleoside analogues such as 2-aminopurine (**1**) have been widely used to study protein−DNA interactions (Figure 1).[1] Of particular interest is the development of fluorescent nucleosides that maintain the hydrogen-bonding properties of natural nucleoside bases. One example is pteridine-based nucleoside **2** developed by Pfleiderer[2] which has the advantage of possessing the hydrogen-bonding properties of guanosine as well as high fluorescence.[3] The related alloxazine nucleosides **3** and **4a** have also been prepared and found to have low fluorescence quantum yields relative to quinine sulfate.[3a]

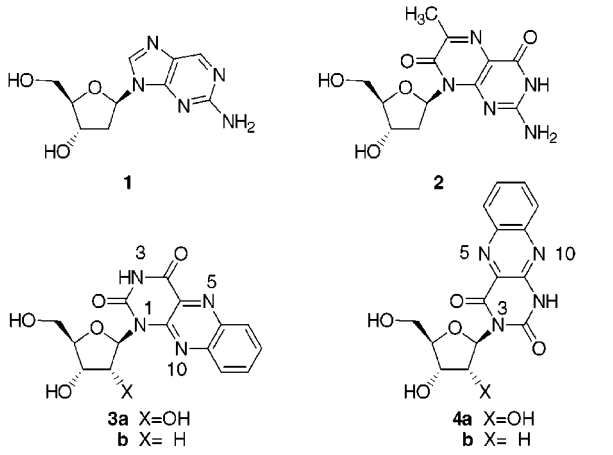


Figure 1. Fluorescent nucleosides.

In addition to their fluorescence properties, we are interested in N3-alloxazine nucleosides (**4**) as potentially more hydrophobic analogues of thymidine. We predict that nucleoside **4b** will maintain the hydrogen-bonding profile of thymidine since the N10 nitrogen of **4b** can serve as a hydrogen bond acceptor (Figure 2). Matteucci had shown phenoxazine nucleoside **5** to be a more hydrophobic analogue of cytidine.[4] Oligonucleotides containing **5** showed enhanced stabilization of 2−5 °C per modification as measured by T_m, when hybridized to a complementary strand of RNA. The

(1) Millar, D. P. *Curr. Opin Struct. Biol.* **1996**, *6*, 322.
(2) Pfleiderer, W. In *Chemistry of Nucleosides and Nucleotides*; Townsend, L. B., Ed.; Plenum Press: New York, 1994; Vol. 3, pp 145−261.
(3) (a) Hawkins, M. E.; Pfleiderer, W.; Belis, F. M.; Porter, D.; Knutson, J. R. *Anal. Biochem.* **1997**, *244*, 86. (b) Hawkins, M. E.; Pfleiderer, W.; Mazumder, A.; Pommier, Y. G.; Balis, F. M. *Nucleic Acids Res.* **1995**, *23*, 2872, (c) Jungmann, O.; Pfleiderer, W. *Tetrahedron Lett.* **1996**, *37*, 8355.
(4) Wagner, R. W.; Matteucci, M. D.; Lewis, J. G.; Gutierrez, A. J.; Molds, C.; Froehler, B. C. *Science* **1993**, *260*, 1510.

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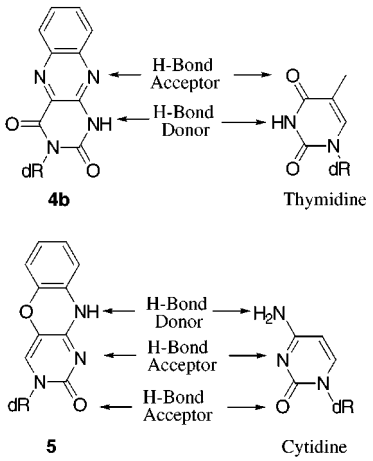


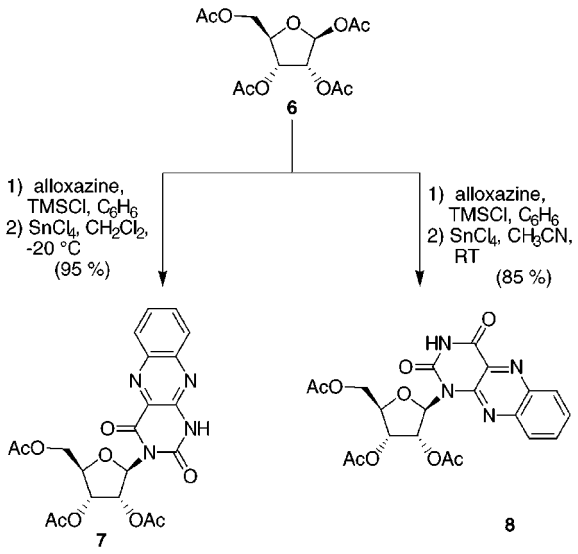
Figure 2. Hydrophobic nucleoside analogues.

more dramatic stabilization came when several phenoxazine nucleotides were clustered, which allowed for maximum hydrophobic stacking of the tricyclic ring system upon hybridization.

Alloxazine ribonucleosides have been previously prepared by the glycosylation of ribose tetraesters with alloxazine to give predominately the β-N1 product **8**, with β-N3 (**7**) and the N1,N3-diribosyl products as minor components.[5] Pfleiderer has also studied the glycosylation of 3,5-di-*O*-*p*-toluoyl-α-D-2-deoxyribosyl chloride with a number of persilylated lumazine derivatives.[6] The N1 regioisomer was formed exclusively; however a mixture of anomers was usually obtained. Interestingly, the glycosylation of persilylated alloxazine was regio- and stereoselective, giving **3b** as the only product after deprotection. We report here glycosylation conditions to give the β-N1 or β-N3 ribonucleosides regioselectively and the subsequent conversion of the β-N3 isomer to the 2′-deoxynucleoside.

In analogy with the glycosylation of guanine,[7,8] we reasoned that glycosylation at N3 may be the kinetic product, which is converted to the thermodynamically favored N1 product via the N1,N3-bis-glycosylated intermediate, which has been observed.[5] Using 1,2,3,5-tetraacetylribose (**6**) as the donor, Vorbrüggen glycosylation[9] with silylated alloxazine at room temperature in acetonitrile using tin tetrachloride as a Lewis acid gave a mixture of N1 and N3 glycosylation products. Prolonged reaction times favored the N1 product (**8**), consistent with our initial hypothesis; after 2 h, **8** was the exclusive product (Scheme 1). Glycosylation for 15 min

Scheme 1



at −20 °C in dichloromethane gave exclusively the N3 product **7**. The structures of the β-N1 and β-N3 products were unambiguously determined by long-range $^{1}H-^{13}C$ heteronuclear correlated 2-D NMR (HMBC). For the N3 product (**7**, Figure 3), the anomeric proton (H1′, δ = 6.53

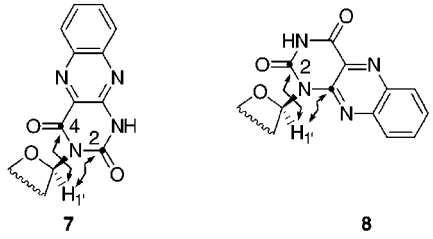


Figure 3. Structure determination of the β-N3 and β-N1 regioisomers by $^{1}H-^{13}C$ heteronuclear correlation.

ppm) showed three-bond coupling to both carbonyl carbons of the base (C2, C4, δ = 148.8, 159.2 ppm); for the N1 product (**8**), the anomeric proton (H1′, δ = 6.80 ppm) showed three-bond coupling to only one carbonyl carbon (C2, δ = 149.1 ppm) and to the 10a bridgehead carbon (δ = 144.7 ppm).[5,6b,10]

We have reported a streamlined synthesis of β-2′-deoxyribonucleosides via the glycosylation of **9** followed by selective deoxygenation of the 2′-*m*-(trifluoromethyl)benzoate via a photoinduced electron-transfer (PET) mechanism using carbazoles as the photosensitizer.[11] Glycosylation of **9** with persilylated alloxazine gave a 1:1 mixture of the β-N3 and β-N1 products in 70% combined yield (Scheme 2); these

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(6) (a) Maurinsch, Y.; Pfleiderer, W. *Nucleosides Nucleotides* **1996**, *15*, 431. (b) Rösler, A.; Pfleiderer, W. *Helv. Chim. Acta* **1997**, *80*, 1869.
(7) (a) Dudycz, L. W.; Wright, G. E. *Nucleosides Nucleotides* **1984**, *3*, 33. (b) Wright, G. E.; Dudycz, L. W. *J. Med. Chem.* **1985**, *27*, 175. (c) Garner, P.; Ramakanth, S. *J. Org. Chem.* **1988**, *53*, 1294.
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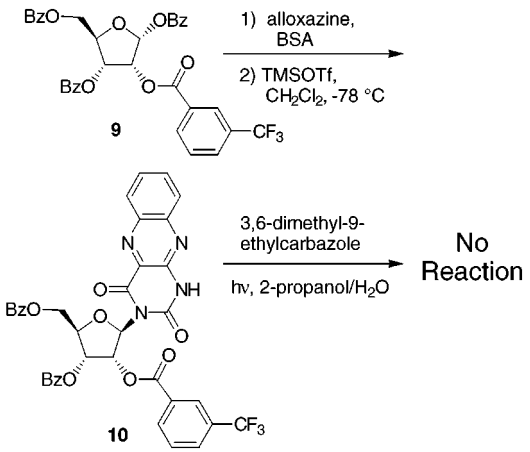
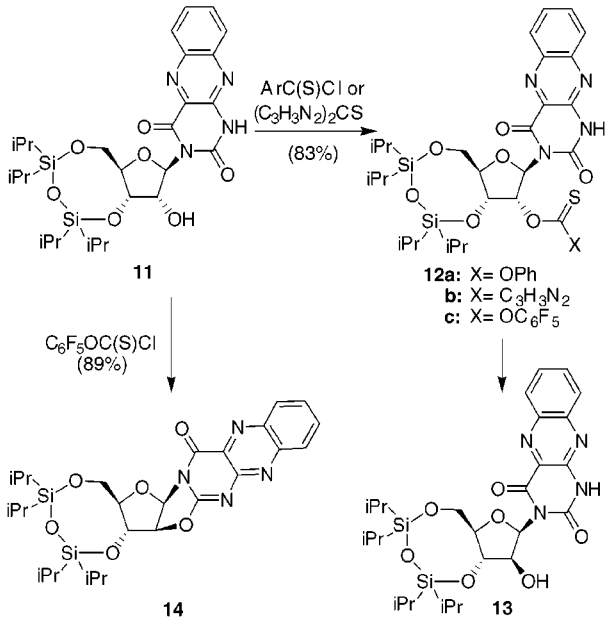
(11) (a) Park, M.; Rizzo, C. J. *J. Org. Chem.* **1996**, *61*, 6092. (b) Wang, Z.; Rizzo, C. J. *Tetrahedron Lett.* **1997**, *38*, 8177. (c) Prudhomme, D. R.; Wang, Z.; Rizzo, C. J. *J. Org. Chem.* **1997**, *62*, 8257.

Scheme 2



conditions were not optimized. The desired regioisomer **10** was easily separated by flash chromatography; however, PET deoxygenation of **10** provided none of the desired 2′-deoxynucleoside, returning starting material instead. It is possible that the alloxazine ring of **10** may quench the carbazole excited state, preventing electron transfer to the *m*-(trifluoromethyl)benzoyl group, or may be acting as the electron acceptor. We then attempted to achieve 2′-deoxygenation of the alloxazine ribonucleoside by a more conventional route as developed by Robins (Scheme 3).[12]

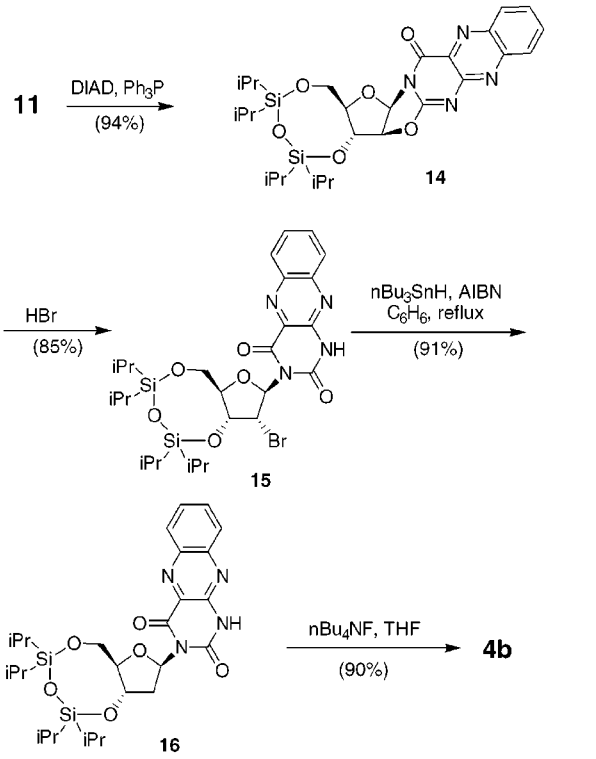
Scheme 3



Methanolysis of **7** (MeONa, MeOH) followed by simultaneous protection of the 5′- and 3′-hydroxyl groups (($^{i}Pr_2$-SiCl)$_2$O, pyridine) gave **11** in 90% overall yield.[13] The free 2′-hydroxyl group was converted to the corresponding phenoxy thionocarbonate or thiocarbonylimidazolide under standard conditions.[12,14] Unfortunately, subsequent tin hydride reduction of **12** proceeded poorly, giving **4b** in 30−35% yield. To our surprise, the major product was the corresponding protected arabinonucleoside **13**. Presumably, heating **12a** or **12b** caused displacement of the 2′-thionocarbonyl group by the C2-carbonyl of the base, to give the 2,2′-anhydronucleoside **14** which is subsequently hydrolyzed to **13**. Thus **13** is probably formed by a thermal process, perhaps catalyzed by trace acid or base, and not a radical reaction. When **11** was treated with pentafluorophenyl chlorothionoformate,[15] we obtained none of the expected product **12c** (Scheme 3). Instead, we obtained an 89% yield of **14**. It is known that 2,2′-anhydropyrimidines such as **14** are obtained upon activation of the 2′-hydroxyl group,[16] although we were somewhat surprised to obtain this product under these conditions. This sequence involving 2,2′-anhydronucleosides is a know method for the conversion of ribonucleoside to arabinonucleosides.[16]

When alcohol **11** was reacted under Mitsunobu conditions (DIAD, Ph_3P, toluene), the same 2,2′-anhydronucleoside (**14**) was obtained in 94% yield (Scheme 4). A variety of

Scheme 4



nucleophiles have been shown to open 2,2′-anhydropyrimidines to give the corresponding α-2′-derivatives.[16] Treatment of **14** with HBr afforded the 2′-bromide (**15**) in 85% yield.

(12) Robins, M. J.; Wilson, J. S.; Hansske, F. *J. Am. Chem. Soc.* **1983**, *105*, 4059.

(13) Markiewicz, W. T. *J. Chem. Res., Synop.* **1979**, 24.

Typically **14** was not purified. Reduction with tributyltin hydride and desilylation proceeded in 82% overall yield for two steps to give the desired β-2′-deoxynucleoside **4b**.[16b] The 2′-debromination could also be accomplished photochemically in 44% yield, using 3,6-dimethyl-9-ethylcarbazole as a photosensitizer in 2-propanol, thus avoiding the use of toxic tin hydride. The photodebromination, however, is somewhat slower, requiring 3 h for complete reaction as compared to 30−60 min for the tin hydride mediated reaction. The dehalogenation does not require photosensitizer, although the reaction is faster and proceeds with less side reactions when it is included. The synthesis of **4b** was accomplished in seven steps from commercially available **6** and proceeded in better than 50% overall yield.

We have measured the fluorescence properties of **4b** and **16**. Using quinine sulfate as a standard ($Q = 0.51$), the fluorescence quantum yield of **4b** in water (excitation, 332 nm; emission, 455 nm) was determined to be 0.06, which is larger than values previously determined for alloxazine ribonucleosides **3a** and **4a**.[3a] For **16**, the fluorescence quantum yield was 0.02 when measured in acetonitrile (excitation, 321 nm; emission, 427 nm). This solvent dependence may indicate that the fluorescence properties of **4b** will change in a more hydrophobic environment.

In conclusion, we have developed conditions for the regiocontrolled glycosylation of alloxazine and ribose tetraacetate to give either the N1- or N3-ribonucleoside. The N3 product was taken on to the corresponding 2′-deoxynucleoside **4b** via an efficient sequence. This fluorescent nucleoside analogue is predicted to possess the same hydrogen-bonding properties as thymidine and may be of interest for antisense or antigene applications or as a fluorescent probe. Conversion of **4b** to the corresponding phosphoramidite required for solid-phase DNA synthesis and subsequent studies of oligonucleotides containing **4b** will be the focus of future work in our laboratory.

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Supporting Information Available: Experimental procedures for the preparation of **7**, **8**, **4a**, **11**, **14**, and **15** and their ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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