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A Set of Nonpolar Thymidine Nucleoside Analogues with Gradually Increasing Size

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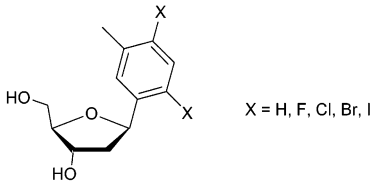
ABSTRACT



We describe a series of nonpolar nucleoside analogues having similar shapes and gradually increasing size. The structure of the nucleobase thymine was mimicked with toluene derivatives, replacing O2/O4 with hydrogen, fluorine, chlorine, bromine, and iodine. Glycosidic bonds were formed by reactions of lithiated 2,4-dihalotoluenes with a deoxyribonolactone derivative. Structural analysis by NMR showed similar conformations across the series. The compounds are useful for study of the biological recognition of nucleotides and nucleic acids.

The biological effects of nucleosides and nucleotides range widely, from their incorporation into DNA (and their resulting interactions with many DNA-recognizing proteins and enzymes) to their involvement in many other aspects of cellular metabolism. Understanding the recognition of nucleotides by enzymes is important not only because of these biological roles but also because such enzymes are often medicinal targets. In an effort to separate steric and electronic effects in nucleotide recognition, we have designed nonpolar nucleoside isosteres,[1] which maintain the size and shape of natural nucleosides as closely as possible but lack polar hydrogen bonding groups such as the ones responsible for Watson−Crick hydrogen bonds. Such compounds have been useful in underlining the importance of hydrogen bonding in some biochemical activities[2] and in revealing that some biological processes such as DNA replication can fare surprisingly well without hydrogen bonding groups.[3]

To probe steric effects in a systematic way, it would be desirable to have a set of molecules of gradually increasing size, to act as "molecular rulers" for active site size and deformability.[4] Here we describe the preparation and structures of such a series of compounds. We took thymidine as a starting point and designed a set of compounds with smaller (the dihydrogen case, **1**) or increasingly larger substituents (dihalogen deoxyribosides **2**−**5**). Computer models suggested that progression from hydrogen and moving down the halogen series would produce bond lengths at the 2,4-positions (corresponding to oxygens of thymidine, **6**) ranging from 1.1 to 2.0 Å (Figure 1). Group radii also would increase gradually along the series as well.

We adopted a generalized strategy for preparing the first four of the five *C*-glycosides. Our approach involved

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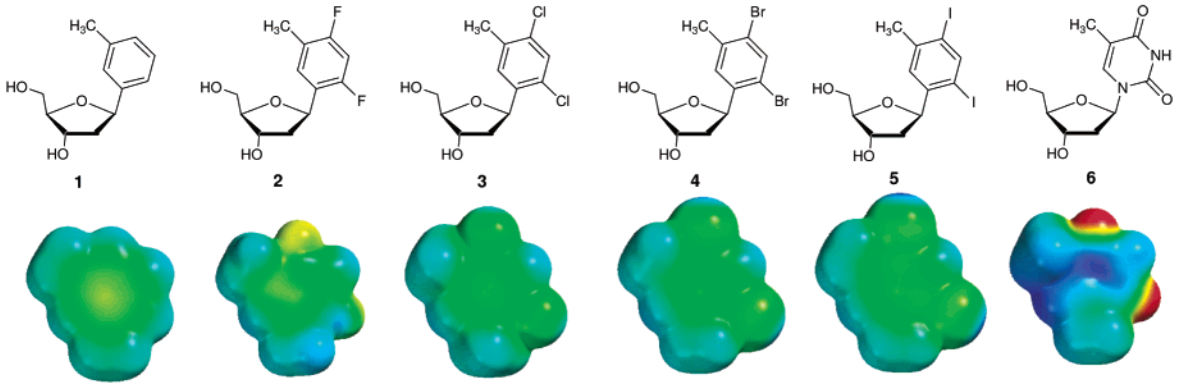
A)



B)

bond length	C-H (**1**)	C-F (**2**)	C-Cl (**3**)	C-Br (**4**)	C-I (**5**)	C-O (**6**)
calculated (Å)[a]	1.10	1.34	1.69	1.87	1.97	1.22
crystal structure (Å)[b]	--	1.37 (C2-F, C4-F)	1.75 (C2-Cl), 1.74 (C4-Cl)	--	--	1.21 (C2-O), 1.23 (C4-O)

[a] with methyl replacing deoxyribose; [b] from free nucleoside.

Figure 1. Structures of the thymidine analogues, designed to have gradually increasing steric demand. (A) Space-filling models of the analogues with methyl groups at the point of attachment to deoxyribose, with calculated electrostatic potentials mapped on the van der Waals surfaces (electrostatic scale: −50 to 30). (B) PM3-calculated bond lengths for the 2,4-substituents, which range in size from H to I (Spartan '02, Wavefunction, Inc., Ivine, CA). Calculated thymine bond lengths are shown for comparison. Also shown are corresponding bond lengths from crystal structures of three of the compounds (**2**, **3**, **6**). Calculated bond lengths are shown with methyl replacing the deoxyribose. Crystal structure bond lengths shown are given for the free nucleoside.

reactions of selectively lithiated dihalotoluenes, or toluene itself in the case of **1**, with the known deoxyribonolactone derivative utilized by Woski.[5] In practice, the lithiated arenes reacted moderately well with the lactone, yielding the *C*-glycosides with yields ranging from 29% (for **4**) to 35% (for **5**). The acidic H3 between two fluorides of **2a** hampered the lithiation at the bromide, so we replaced the lithiation step with Grignard for **2b**. In the four coupling reactions the desired β-isomer was the major product; the minor α-isomers were obtained in yields less than 10%. The desired compounds were easily separated by silica gel column, and the β-orientation was confirmed by NOE measurements involving the 2′-protons and their vicinal neighbors (Supporting Information). Deprotection of the siloxane 3′,5′-protecting group was carried out smoothly with tetrabutylammonium fluoride, giving the first four compounds of the series in yields of 90−97%. The 2,4-substitutions were confirmed (relative to other possible isomers) by HMBC and NOE measurements (Supporting Information), and mass spectra confirmed that the 2,4-halogens in **2**−**4** remained intact.

The diiodide **5** required a different approach and was constructed from the dibromonucleoside **4**. To avoid the problem of selective lithiation in the presence of two other iodo groups, we prepared this final compound (the largest of the series) by replacing the bromo groups of deoxynucleoside **4** with iodines using the copper-catalyzed strategy described by Buchwald.[6] Although the procedure had not been previously described for multiple bromo groups, we applied it with modest success in this case, obtaining the diiodide in 22% yield. PM3 calculations of the aryl substituents of this series were used to estimate bond lengths of the base mimics. Of particular interest are the bond lengths of the varied 2,4-substituents, which vary over a 1.0 Å range (Figure 1). Maps of the electrostatic charges over the van der Waals surfaces are also shown for comparison (Figure 1). Overall, the new dichloro, dibromo, and diiodo com-

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Scheme 1

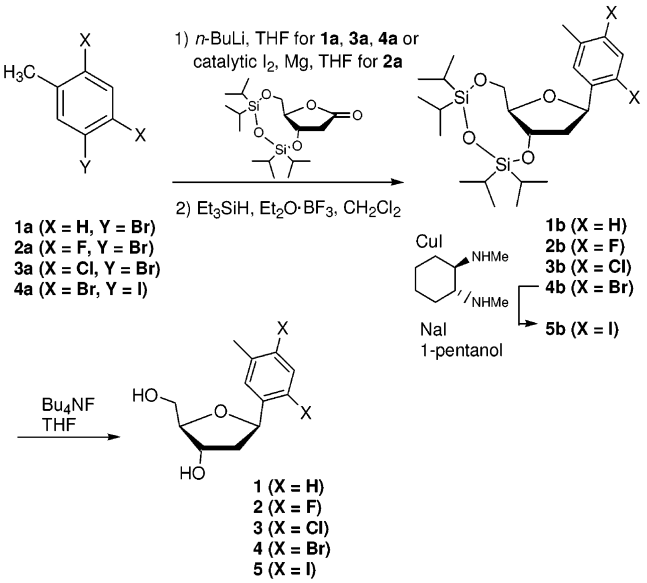


Table 1. Nucleoside Conformations for **1**−**5** Determined by ^{1}H NMR Measurements in D_2O

	coupling constants					
	H1′−H2′	H1′−H2″	H2′−H3′	H2″−H3′	H3′−H4′	H2′−H2″
1	10.68	5.49	5.92	1.68	2.24	13.71
2[b]	10.37	5.80	5.79	~1.0	2.44	13.58
3	10.26	5.62	6.01	1.85	2.18	13.70
4	10.23	5.76	6.08	1.95	2.47	13.76
5	10.14	5.73	5.75	1.53	2.33	13.59

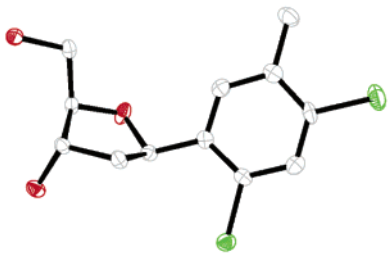
	summed *J* values[a]			
	Σ 1′	Σ 2′	Σ 2″	Σ 3′
1	16.17	30.31	20.88	9.84
2[b]	16.17	29.74	20.88	9.73
3	15.88	29.97	21.17	10.04
4	15.99	30.07	21.47	10.50
5	15.87	29.48	20.85	9.61

[a] $\Sigma 1' = J1'2' + J1'2''$; $\Sigma 2' = J1'2' + J2'3' + J2'2''$; $\Sigma 2' = J1'2'' + J2''3' + J2'2''$; $\Sigma 3' = J2'3' + J2''3' + J3'4'$. [b] Data for **2** from ref 7a.

pounds appear to have quite low polarity. The surface potentials vary by a relatively small amount, but they suggest that the three largest cases have negative charge density at the center of the ring similar to that of the dihydrogen case, with a small increase for the largest case (the diiodide). Only the difluoro case has less negative charge density, presumably because of the high electronegativity of fluorine. Overall, the shapes are similar to that of the parent thymine.

We were able to obtain crystals of the dichlorotoluene deoxyribose derivative from chloroform, which afforded a solid-state X-ray structure (Figure 2A). The structure shows

A)



B)

	dihedral angles [°]					
	C6-C2′	O4′-C3′	C1′-C4′	C2′-C5′	C(N)1-C3′	C3′-C1′
6	-79.5	+28.4	-37.2	-87.3	+147.8	-16.8
2	-97.6	+32.2	-36.7	-91.5	+153.2	-9.0
3	-90.1	+31.7	-32.5	-95.5	+152.4	-2.4

Figure 2. Solid-state X-ray crystal structure of dichlorotoluene deoxyglycoside **3**. (A) ORTEP drawing of **3**, showing β-anomeric configuration, C-3′-exo (S type) conformation of deoxyribose, and anti glycosidic orientation. (B) Comparison of dihedral angles in the solid-state structures of **3**, **2**, and thymidine (**6**). Data for **2** are from ref 7a, and data for thymidine (**6**) are from ref 7b.

a C2′-*endo* sugar conformation, falling into the "S" family of conformers, and an anti glycosidic orientation is present. Comparison of this structure to that of the difluorotoluene analogue[7a] shows a similar structure and conformation (Figure 2B). The C−Cl bond lengths in the crystal structure are 1.74 and 1.75 Å, which is, of course, substantially longer than the corresponding C−F bonds in the difluorotoluene deoxyriboside crystal structure (1.34 Å) (Figure 1B).[7a] Overall, then, the dichloro analogue adopts a structure nearly the same as the reported crystal structure of thymidine.[7b]

Because crystal packing forces may alter conformations in relatively flexible five-membered rings, the structures of these isosteres in solution were of greater interest than the solid-state structures. We carried out an analysis of ring coupling constants to assign deoxyribose conformations for the series of five compounds.[8] The data are shown in Table 1. All five compounds are quite similar, and all are assigned as S-type sugars. Thus, the conformations fall into a relatively narrow range, and this range is not greatly different from the conformational preference of thymidine itself (which is reported as 70% S).[7a] We conclude that varying bond lengths (at the 2-position on the benzene ring in particular) does not markedly affect the ring conformation.

We also examined bond rotameric preferences for the glycosidic bonds, which was evaluated by NOE experiments focused on the C-1′ proton. A *syn*-glycoside generally shows large NOE enhancements at the 6-position of the "nucleobase" due to the proximity of these two protons. However, natural thymidine shows only a small enhancement due to its relatively strong anti orientational preference, and our

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experiments showed similarly small NOEs to the C6-protons of the four dihalo compounds in this series.

Overall, the results show that this new series of five compounds is readily prepared and that all five adopt conformations very close to that of thymidine. Thus, we expect these nucleoside analogues to be broadly useful as steric probes of enzyme active sites that normally process thymidine nucleosides or nucleotides. In addition, when incorporated into DNA they may well be useful as steric probes of protein−DNA recognition in general. Experiments directed along these lines are underway.

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Supporting Information Available: Experimental details of nucleoside synthesis and characterization, details of NMR structural methods, and details of the crystal structure of nucleoside **3** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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