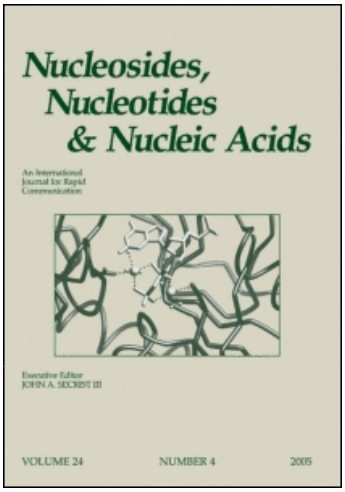

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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C. Thibaudeau[a]; N. Nishizono[b]; Y. Sumita[b]; A. Matsuda[b]; J. Chattopadhyaya[a]
[a] Department of Bioorganic Chemistry, Biomedical Center, University of Uppsala, Uppsala, Sweden [b] Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan

To cite this Article Thibaudeau, C. , Nishizono, N. , Sumita, Y. , Matsuda, A. and Chattopadhyaya, J.(1999) 'Determination of the Group Electronegativity of CF_3 Group in 3'-*O*-CF_3-Thymidine by ^{1}-NMR', Nucleosides, Nucleotides and Nucleic Acids, 18: 4, 1035 — 1053
To link to this Article: DOI: 10.1080/15257779908041642
URL: http://dx.doi.org/10.1080/15257779908041642

NUCLEOSIDES & NUCLEOTIDES, 18(4&5), 1035–1053 (1999)

DETERMINATION OF THE GROUP ELECTRONEGATIVITY OF CF_3 GROUP IN 3'-*O*-CF_3-THYMIDINE BY ^{1}H-NMR

C. Thibaudeau,[1] N. Nishizono,[2] Y. Sumita,[2] A. Matsuda[2] and J. Chattopadhyaya[1]*

[1]Department of Bioorganic Chemistry, Box 581, Biomedical Center, University of Uppsala, S-751 23 Uppsala, Sweden.
[2]Graduate School of Pharmaceutical Sciences, Hokkaido University, a Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan

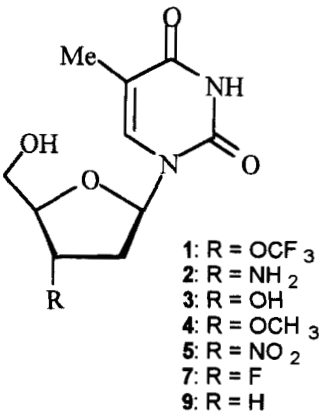
Phone: +46 18 471 4577. Fax: +46 18 55 44 95. E-mail: jyoti@bioorgchem.uu.se. http://bioorgchem.boc.uu.se

ABSTRACT: The interplay of enthalpy of the gauche effect ($\Delta H°_{GE}$) of the [X3'-C3'-C4'-O4'] fragment in various 3'-substituted (X) 2',3'-dideoxythymidine derivatives **1** - **7** and the inherent anomeric effect drives the two-state North⇄South equilibrium in the constituent sugar moiety. The group electronegativity of 3'-OCF_3 substituent in Marriott's, Inamoto's and Mullay's scales has been determined from simple calibration graphs correlating the group electronegativity of various 3'-substituents (X) in 2',3'-dideoxythymidine derivatives **1** - **7** with the experimental strength ($\Delta H°_{GE}$) of the [X3'-C3'-C4'-O4'] gauche effect. $\Delta H°_{GE}$ has been experimentally determined from pseudorotational analyses of temperature-dependent $^3J_{HH}$ coupling constants, and can be used as an unambiguous tool for direct experimental estimation of the group electronegativity of a specific substituent covalently attached to 3'-carbon of 2',3'-dideoxythymidine, which can be compared, in turn, with the theoretical estimation carried out according to Marriott's or Inamoto's procedure. Inconsistency found between theoretical values in Marriott's and Inamoto's scales, on the one hand, and between our experimental estimate and the theoretical value in Marriott's scale, on the other, have been solved by refining the electronegativity scale using our experimental data for **1** - **7**.

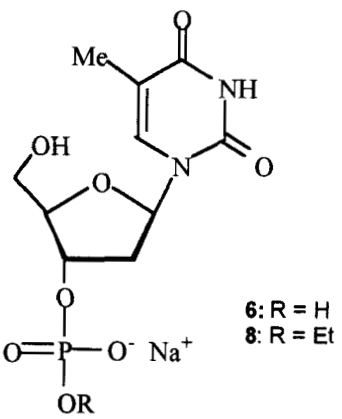
Introduction

The conformation of the sugar moiety in modified nucleosides can be predisposed by tailoring intramolecular stereoelectronic gauche and anomeric effects[1]. It has been found that the anomeric effect (orbital mixing between non-bonded lonepair of O4' and the σ*

orbital of the C1'-N glycosyl bond: n(O4') ➡ $\sigma^*_{(C\text{-}N)}$ interactions) in natural nucleosides and in the analogous C1'-modified nucleosides is dictated by the intrinsic electronic character of the constituent nucleobase[1a,c,k,p,r,s-v], driving the sugar conformation in a specific manner. Similarly, the electronic nature of a 2' or 3' substituent steers the conformation of the pentose sugar by the gauche effect[1i,j]. The interplay of the relative strengths of the gauche and anomeric effects finally dictates[1] the outcome of the overall sugar conformation. Each of these gauche and anomeric effects has both stereoelectronic and steric components[1] in the enthalpy part of the drive of the two-state dynamic North [N, C2'-*exo*-C3'-*endo*] ⇄ South [S, C2'-*endo*-C3'-*exo*] pseudorotational equilibrium of the sugar moiety. It has been shown in particular[1i] that the strength



1: R = OCF_3
2: R = NH_2
3: R = OH
4: R = OCH_3
5: R = NO_2
7: R = F
9: R = H



6: R = H
8: R = Et

($\text{Æ}H^\circ_{GE}$) of the [X3'-C3'-C4'-O4'] gauche effect in 3'-substituted-2',3'-dideoxythymidine **2** - **7** derivatives depends linearly on the group electronegativity of 3'-substituent expressed in Mullay's[2a], Marriott's[2b] and Inamoto's[2c-f] electronegativity scales. In that work[1i], we had elaborated the substituent effect of both 3'-F and 3'-OMe substituents along with others (such as OH, NO_2, NH_2, OPO_3H^-) using the 3'-H of 2',3'-dideoxythymidine as the reference point. Keeping in mind the role of fluorine atom as strongly electron-withdrawing, and the fact that its atomic size is between that of a proton and a hydroxyl group, it is important to experimentally and theoretically investigate how the group electronegativity of 3'-OCF_3 substituent will change vis-a-vis 3'-OCH_3 or 3'-F substituent! Clearly, this will have an impact on our ability to engineer the sugar moiety in nucleosides

and nucleotides into preferred puckering mode by simply weakening or strengthening gauche interactions in conjunction with the modulation by the strength of the anomeric effect[1].

Furthermore, introduction of a fluorine atom at a sugar carbon in nucleosides alters their biological activities towards various cellular, pathogenic and tumor-specific enzymes in various ways[3,4]. The replacement of a proton or a hydroxy group by a fluorine atom causes only a minor change in the steric effect of the functionality, but such a substitution has profound effects on the chemical properties as well as on the stereoelectronic properties[3,4]. This can be exemplified by the following observations: (i) The stability of a hydrolyzable function is strengthened (as the glycosyl bond in 2'-deoxynucleosides) when fluorine group is present in the proximity (in the α , β or even in γ position), resisting enzymatic or acidic hydrolysis[4]. (ii) Fluorine atom is strongly electron-withdrawing, hence its gauche effect at C2'-F drives the conformation of 2'-deoxysugar moiety in oligodeoxynucleotides, for example, in a way that resembles RNA more than DNA[5]. (iii) The stronger gauche effect of the fluorine substituent due to its high electronegativity has a profound stereoelectronic effect on the stereochemical orientation of the neighbouring groups, thereby fluorine substituent governs the overall conformation of the sugar ring[5-8].

Thus the usefulness of fluorine substitution for a proton or a hydroxyl group in bioorganic chemsitry makes it important to define its effect upon conformation and the conformational equilibria in solution for the sugar-fluorinated nucleosides and nucleotides as well as in saccharides, in general, in a dependable manner using NMR methods to correlate the structure-activity relationships, both in the presence and in the absence of target enzymes.

Result & Discussion

(A) Determination of $\text{Æ}H^{\circ}_{GE}$ in 3'-substituted-2',3'-dideoxythymidines 1 - 7.

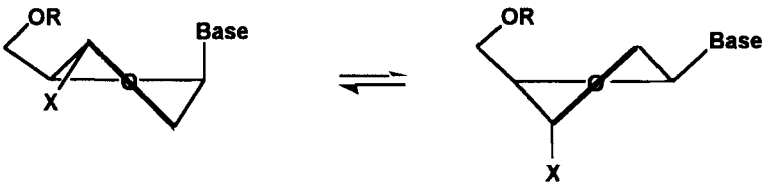
The thermodynamics of the two-state N$\rightleftarrows$S pseudorotational equilibrium in **1** have been initially calculated basing upon pseudorotational analyses of vicinal $^3J_{HH}$ coupling constants using our published methodology[1]. A theoretical estimate for the group electronegativity of OCF_3 was earlier obtained in Inamoto's ι scale[2f] [$\iota_{Inamoto,theor.}(OCF_3)$ = 2.79]. It has been also derived in this work from *ab initio*

calculations using a procedure prescribed by Marriott[2b] [*i.e.* Marriott's scale[2b]: $\chi_{Marriott,theor.}(OCF_3) = 0.48$, *vide infra*]. Using our simple calibration graphs[1i] of $ÆH°_{GE}$ as a function of ι or χ, we have independently verified the validity of these theoretical values for 3'-OCF_3 in 3'-*O*-trifluoromethylthmidine (**1**). The comparison of our experimental estimates [$\iota_{Inamoto,exp.}(OCF_3) = 2.64 \pm 0.10$; $\chi_{Marriott,exp.}(OCF_3) = 0.38 \pm 0.05$] with the theoretical values both in Inamoto's and Marriott's scales reveals clear discrepancies: (i) Our $\chi_{Marriott,exp.}(OCF_3)$ value is reduced by -0.1 in comparison with the theoretical value [$\chi_{Marriott,theor.}(OCF_3)$]; (ii) The comparison of the theoretical values in Inamoto's scale [$\iota_{Inamoto,theor.}(OCF_3)$] and in Marriott's scale [$\chi_{Marriott,theor.}(OCF_3)$] shows that in the former scale the electronegativity of OCF_3 is rather similar to that of OH and OMe which is rather in good agreement with our experimental findings [$\iota_{Inamoto,exp.}(OCF_3) = 2.64 \pm 0.10$], whereas in the latter scale [$\chi_{Marriott,theor.}(OCF_3) = 0.48$] the electronegativity is much higher, and surprisingly closer to that of fluorine [$\chi_{Marriott,theor.}(F) = 0.52$]. These observations have prompted us to refine Marriott's electronegativity scale specifically for 3'-substituted-2',3'-dideoxythymidine nucleosides **1** - **7**.

We have performed this by conformational analysis of 3'-*O*-trifluoromethylthmidine (**1**) and by comparing the thermodynamics of its N ⇄ S pseudorotational equilibrium with the other analogs **2** - **7** in our correlation plot of group electronegativity as a function of $ÆH°_{GE}$ [1i].

(B) Conformational Analyses of 3'-*O*- OCF_3-thmidine (1) by ^{1}H-NMR spectroscopy.

The stabilization of *gauche* with respect to *trans* orientations of [X-C-C-Y] fragments (where X and Y are strongly electronegative elements) is known as the *gauche effect*[9]. The origin of the gauche effect is still a matter of debate. Several hypotheses have been so far put forward on basis of mainly theoretical calculations: (i) σ ➡ σ* orbital interactions have been advocated[10,11]; (ii) The preferential gauche arrangement of 1,2-difluoroethane has been explained by the fact that in this rotamer, the C-C bond paths are bent in opposite directions, whereas in the energetically disfavoured *trans* form bending occurs in the same direction for both bond paths, leading to a reduced overlap which itself results into a weaker C-C bond, as shown by Wiberg *et al.*[12] (iii) Alternatively, a



North sugar (C_3-*endo*-C_2-*exo*)
Phase angle (P) = 0° ≤ P ≤ 36°;
Puckering amplitude (ψ_m) = 38.6° ± 3°

South sugar (C_2-*endo*-C_3-*exo*)
Phase angle (P) = 144° ≤ P ≤ 180°;
Puckering amplitude (ψ_m) = 38.6° ± 3°

Base = thymin-1-yl (T);
X = OCF_3 (**1**), NH_2 (**2**), OH (**3**), OCH_3 (**4**), NO_2 (**5**), OPO_3H- (**6**) and F (**7**)

Scheme 1: The two-state N ⇌ S pseudorotational equilibrium in 3'-substituted-2',3'-dideoxythymidine derivatives **1** - **7**

combination of through space and through bond interactions[13-15] has also been suggested, and (iv) the important role played by H-bonding interactions has been also pointed out[16].

Nucleos(t)ides in aqueous solution are involved in a two-state dynamic N⇄S pseudorotational equilibrium (Scheme 1)[1,17-19]. Through an approach consisting of pairwise comparisons, we have shown[1] that the thermodynamics (*i.e.* the enthalpy contribution ΔH° to the free energy ΔG°, as determined using our published methodology based on pseudorotational analyses of temperature-dependent vicinal $^3J_{HH}$ coupling constants) of the two-state N ⇄ S equilibria in a series of α- and β -D/L- *C*- and *N*-nucleosides can be correlated with the electronic and chemical nature of the substituents attached to C1', C2' and C3' atoms of the constituent sugar moieties. Our works[1] showed that ΔH° of the N⇄S equilibrium in nucleos(t)ides is controlled by the interplay of a various stereoelectronic and steric effects (*i.e.* anomeric effect of the nucleobase [O4'-C1'-N] and the counteracting steric effect, gauche effects of [O3'-C3'-C4'-O4'], [O2'-C2'-C1'-N], [O2'-C2'-C1'-O4'] and [O5'-C5'-C4'-O4']). The validity of our two-state N⇄S equilibrium model and the accuracy of our thermodynamics as derived from the procedure described above[1] are experimentally evidenced[1p,s-v] by the fact that the pD values at the inflection points of the plots of the pD-dependent ΔH°, ΔS° and resulting ΔG° of the N⇄S pseudorotational equilibrium correspond to the pK_a values available in the

litterature[20] for the constituent nucleobases of 2',3'-dideoxynucleosides[1t], 2'-deoxynucleosides[1p,u] and *C*-[1s,v] and *N*- ribonucleosides[1p].

(C) Determination of the Group Electronegativity of 3'-OCF_3 Using Simple Calibration Graphs.

In the case of 3'-substituted-2',3'-dideoxythymidine derivatives, we have shown that the strength ($ÆH°_{GE}$) of the [X3'-C3'-C4'-O4'] gauche effect can be easily estimated using simple calibration graphs[1i] to give the electronegativity of the substituent; alternatively, if the electronegativity of a given substituent is known, it is possible to predict the strength of the gauche effect, which can be, in turn, proven by our experimental procedure[1i]. This means that a validation of the theoretical group electronegativity in the Marriott's [$\chi_{Marriott,theor.}(X)$][2b], Mullay's [$\chi_{Mullay,theor.}(X)$][2a] or Inamoto's [$\iota_{Inamoto,theor.}(X)$][2c-f] scale is important to fine tune the scale for future use in a dependable manner.

The overall strength of the gauche effect in [X3'-C3'-C4'-O4'] fragment in any of 3'-substituted dideoxythymidine derivatives **1** - **7** is the overall result of two counteracting contributions: (i) The purely stereoelectronic tendency of O3'-C3' to adopt a gauche orientation relative to the C4'-O4' bond. This stabilizing gauche over trans interaction is maximally achieved when the sugar adopts an S-type conformation, because only in this situation the H3'-C3' and C4'-O4' bonds are in antiperiplanar orientation; (ii) The counteracting tendency of X3' to reduce its steric interactions with the rest of the molecule by adopting a pseudoequatorial orientation, which is expected to drive the two-state pseudorotational equilibrium toward N-type sugars.

However, the steric effect does not seem to play a preponderant role here. This is evident from the following observations: (i) ÆH° values of the two-state N⇄S equilibrium in thymidine 3'-ethylphosphate (**8**) and in thymidine 3'-monophosphate (**6**) are the same (-2.6 kJ/mol)[1g]. This suggests that although the steric bulk of 3'-OPO_3Et^- in **8** is much larger than that of 3'-OPO_3H^- in **6**, there is no measurable increase in the contribution of the steric effect of 3'-substituent to the overall ÆH° value in **8** compared to **6**. The change in the bulk of the 3'-substituent from 3'-OPO_3H^- in **6** to 3'-OPO_3Et^- in **8** is only reflected in the modulation[1g] of -TÆS°contribution to ÆG° of the two-state N⇄S

equilibrium in the latter (-TÆS° = -1.9 kJ/mol; ÆG° = 0.6 kJ/mol) compared to the former (-TÆS° = -4.3 kJ/mol; ÆG° = 1.3 kJ/mol). (ii) The ÆH° values (-2.6 kJ/mol) for **6** and **8** are slightly larger[1g] than ÆH° for thymidine itself (-1.8 kJ/mol), which is consistent with the fact that 3'-phosphate and 3'-ethylphosphate are expected to be slightly more electronegative than the 3'-hydroxyl. If the steric effect of the 3'-substituent played a significant role in the drive of the pseudorotational equilibrium in **6** and **8**, then their ÆH° values should be less negative than that of the 3'-OH, which is not the case. (iii) Our published[1i] experimental estimate for the group electronegativity of 3'-OPO_3H^- in **6** expressed in Marriott's scale (0.44 ± 0.1, determined using the corresponding correlation plot shown in Fig. 1) is nearly identical to the theoretical value (0.48, *i.e.* calculated through *ab initio* calculations according to the original Marriott's methodology described above).

The electronegativity scale of Marriott[2b] is based on *ab initio* calculations with Gaussian program[21] (at HF/6-31G* level). The actual electronegativity value of X in H-X according to Marriott's scale corresponds to the atomic electron population on the hydrogen in H-X, as determined from a Mulliken population analysis of the geometrically optimized H-X structure. Mullay's[2a] group electronegativities are based on modified Slater effective nuclear charges, effective principal quantum numbers, fractional p characters, assuming charge conservation and electronegativity equalization within each bond in the group. Inamoto's[2f] ι scale is an inductive substituent parameter scale, which is derived from the corresponding group electronegativities[2c-f] showing a strong correlation with trans HH couplings in monosubstituted ethene fragments.

The two-state N⇄S pseudorotational equilibrium in 3'-OCF_3-2',3'-dideoxythymidine **1** (ÆH° = 0.0 kJ/mol; $-TÆS^{298}$ = -2.2 kJ/mol; $ÆG^{298}$ = -2.3 kJ/mol) is completely driven by the entropy (-TÆS°) contribution (See the experimental section for the detail of the pseudorotational analyses; Table 1 for experimental $^3J_{HH}$ coupling constants; Table 2 for the thermodynamics of the pseudorotational equilibrium in **1**). The fact that the ÆH° value is zero means that the anomeric effect of the base (in conjunction with the almost negligible effect of 4'-CH_2OH group) and the counteracting gauche effect of [3'-OCF_3-C3'-C4'-O4'] fragment cancel each other. The subtraction of ÆH° value of the N⇄S equilibrium in 3'-OCF_3-2',3'-dideoxythymidine (**1**) from that found previously[1i]

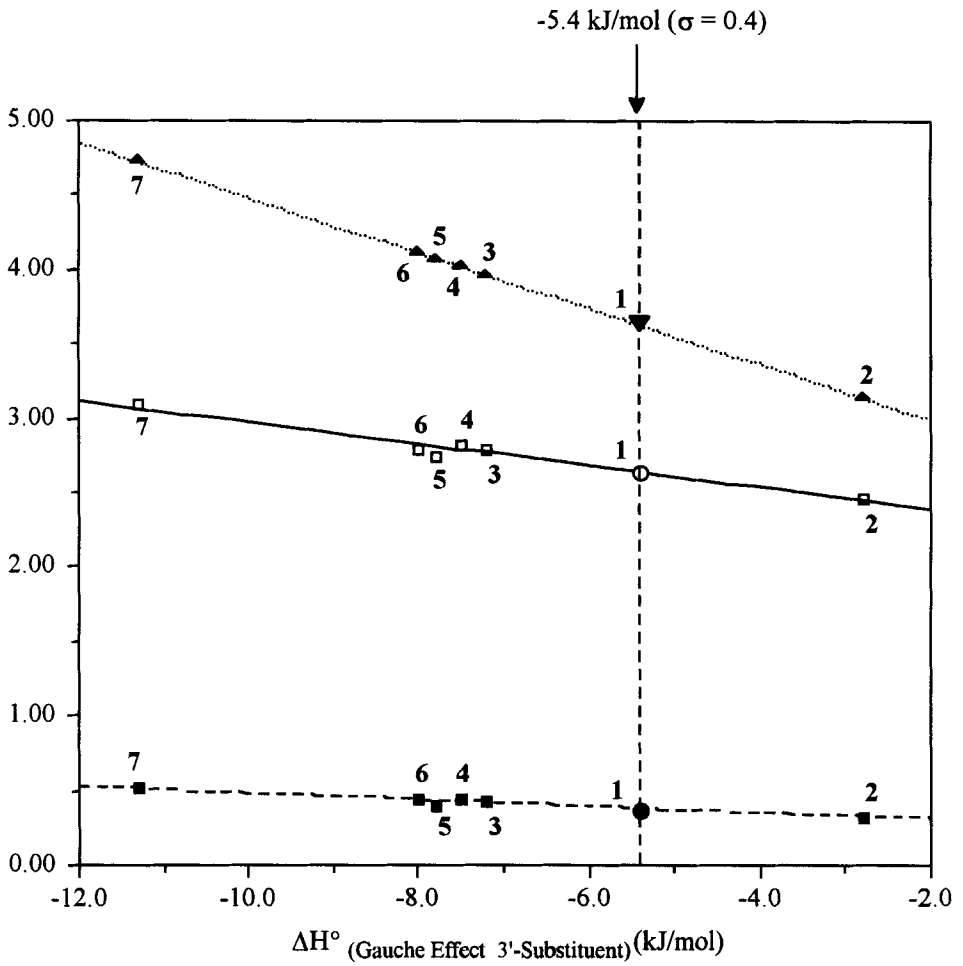


Fig. 1: The determination of the Group Electronegativity (χ) of 3'-OCF_3 in **1** through correlation plots of χ of the 3'- substituent as a function of the strength of the Gauche Effect ($\text{Æ}H°_{GE}$) of [X3'-C3'-C4'-O4'] fragment in **1** - **7**. The $\text{Æ}H°_{GE}$ values have been calculated by subtracting Æ$H°$ of the two-state N ⇄ S pseudorotational equilibrium of the reference compound 2',3'-dideoxythymidine (**9**) from Æ$H°$ calculated for a particular 3'-substituted thymidine nucleoside (**1** - **7**) (See also ref. 1i for the original pseudorotational analyses of temperature-dependent vicinal $^3J_{HH}$ for **2** - **7**). The experimentally calculated group electronegativities of 3'-OCF_3 in **1** according to Marriot's ($\chi_{Marriott,exp.}$, ---- line, ■ symbol for **2** - **7**, ● symbol for **1**), Inamoto's ($\chi_{Inamoto,exp.}$, —— line, ▫ symbol for **2** - **7**, ○ symbol for **1**) and Mullay's ($\chi_{Mullay,exp.}$, ····· line, ▲ symbol for **2** - **7**, ▼ symbol for **1**) scales have been calculated using the slopes and intercepts which define the best straight lines fitted using Profit program[26] through the pairs of χ versus $\text{Æ}H°_{GE}$ corresponding to **2** - **5** and **7**: For Marriott's scale: $\chi = 0.26 - 0.022\ \text{Æ}H°_{GE}$ (Eq. 1); For Mullay's scale: $\chi = 2.63 - 0.19\ \text{Æ}H°_{GE}$; For Inamoto's scale: $\chi = 2.25 - 0.08\ \text{Æ}H°_{GE}$.

Table 1: Vicinal Proton-Proton Coupling Constants for 3'-OCF_3-2',3'-dideoxythymidine (**1**) as a Function of Temperature[a]

T (K)	$^3J_{1'2'}$	$^3J_{1'2''}$	$^3J_{2'3'}$	$^3J_{2''3'}$	$^3J_{3'4'}$	$^3J_{4'5'}$	$^3J_{4'5''}$
278	7.4	6.5	6.9	3.3	3.5	3.6	4.5
288	7.5	6.5	6.8	3.4	3.7	3.6	4.5
298	7.5	6.4	7.0	3.3	3.6	3.7	4.5
308	7.4	6.5	7.0	3.3	3.6	3.7	4.6
318	7.5	6.5	7.0	3.3	3.6	3.7	4.7
328	7.5	6.5	6.9	3.3	3.7	3.8	4.6
338	7.5	6.5	7.0	3.3	3.6	3.8	4.7
348	7.4	6.6	7.0	3.3	3.6	3.9	4.7
358	7.4	6.5	7.0	3.3	3.6	3.8	4.8

[a] The tabulated $^3J_{HH}$ values have been extracted from one-dimensional ^1H spectra of a 5mM D_2O solution (pD Å 7.0) of **1** recorded at 500.13 MHz on a Bruker DRX spectrometer. They have been verified with help of the DAISY program for simulation of spin systems[22].

for 2',3'-dideoxythymidine (**9**) (ÆH° = 5.4 kJ/mol) yields the strength ($ÆH°_{GE}$ = -5.4 kJ/mol) of the gauche effect of [3'-OCF_3-C3'-C4'-O4'] in **1**, which is much reduced compared with that of 3'-OH in **3** (-7.2 kJ/mol), 3'-OMe in **4** (-7.5 kJ/mol), 3'-NO_2 in **5** (-7.8 kJ/mol), 3'-OPO_3H^- in **6** (-8.0 kJ/mol) and 3'-F in **7** (-11.3 kJ/mol) (Table 2) [1i].

We have estimated the group electronegativity of 3'-OCF_3 in **1**, expressed in Marriott's scale [$\chi_{Marriott,exp.}(OCF_3)$ = 0.38 ± 0.05], Inamoto's inductive substituent parameter scale [$\iota_{Inamoto,exp.}(OCF_3)$ = 2.64 ± 0.10] and Mullay's scale [$\chi_{Mullay,exp.}(OCF_3)$ = 3.66 ± 0.05] by using the equations defining our correlation plots[1i] of experimental $ÆH°_{GE}$ as a function of group electronegativity for **2** - **5** and **7** (Fig. 1). In each of the electronegativity scales, the group electronegativity of 3'-OCF_3 in **1** is found to be larger than that of 3'-NH_2[27] in **2**, but slightly smaller than that of 3'-OH in **3** or 3'-OMe in **4** and clearly reduced in comparison with that of 3'-NO_2 in **5**, 3'-OPO_3H^- in **6** and 3'-F in **7** (Table 2).

It is noteworthy that our experimentally determined (*i.e.* using calibration plots shown in Fig. 1) inductive substituent parameter value of 3'-OCF_3 ($\iota_{Inamoto,exp.}(OCF_3)$ = 2.64 ± 0.10) is quite comparable to the theoretical value calculated by Inamoto and Masuda themselves[2f] [$\iota_{Inamoto.theor.}(OCF_3)$ = 2.79].

Table 2. Estimation of the group electronegativity of 3'-OCF_3 in **1** from the strength of the [3'-OCF_3-C3'-C4'-O4'] gauche effect using 3'-substituted-2',3'-dideoxythymidine derivatives **2** - **7** as references.

		Thermodynamics of the two-state N ⇄ S equilibrium in **1** - **7**					Gauche effect of 3'-X	Theoretically determined or experimentally refined group electronegativity of 3'-X in **1** - **7**			
Cmpd	3'-X	ΔH° (kJ/mol)[a]	ΔS° (J/molK)[a]	-TΔS° (kJ/mol)[a]	ΔG^{298} (kJ/mol)[a]	$\Delta_{\%S}$ (358-278K)[a]	$\Delta H°_{GE}$ (kJ/mol)[b]	Marriott's scale[c] $\chi_{Marriott,theor/exp}$	Refined scale[d] $\chi_{thiswork,refined}$	Mullay's scale[e] $\chi_{Mullay,theor.}$	Inamoto's scale[f] $^{1}\chi_{Inamoto,theor/exp}$
1	OCF_3	0.0 (0.4)	7.5 (1.5)	-2.2 (0.4)	-2.3 (0.6)	0	-5.4	0.48 / 0.38 ± 0.05	0.38	3.66 ± 0.05	2.79 / 2.64 ± 0.10
2	NH_2	2.6 (0.1)	-1.9 (0.7)	+0.6 (0.2)	+3.2 (0.2)	+5	-2.8	0.33	0.32	3.15	2.47
3	OH	-1.8 (0.3)	-0.9 (0.5)	+0.3 (0.1)	-1.5 (0.3)	-4	-7.2	0.43	0.42	3.97	2.79
4	OMe	-2.1 (0.1)	+1.1 (0.8)	-0.3 (0.2)	-2.4 (0.2)	-4	-7.5	0.44	0.43	4.03	2.82
5	NO_2	-2.4 (0.1)	+3.7 (1.1)	-1.1 (0.3)	-3.5 (0.3)	-3	-7.8	0.4	0.43	4.08	2.75
6	OPO_3H^-	-2.6 (0.1)	-4.3 (0.4)	+1.3 (0.1)	-1.3 (0.1)	-3	-8.0	0.48 / 0.44 ± 0.1	0.44	4.12 ± 0.02	- / 2.8 ± 0.2
7	F	-5.9 (0.3)	-2.3 (0.8)	+0.7 (0.2)	-5.2 (0.4)	-6	-11.3	0.52	0.51	4.73	3.1

[a] The enthalpy (ΔH°, in kJ/mol) and entropy (ΔS°, in J/molK; -TΔS° in kJ/mol at 298K) contributions to the free-energy (ΔG^{298} at 298K, in kJ/mol) of the pseudorotational equilibrium in **2** - **7** have been taken from ref. 1i. For **1**, ΔH° and ΔS° have been calculated using our published procedure based upon pseudorotational analyses of temperature-dependent vicinal $^{3}J_{HH}$ (See the experimental section for the detail of the conformational analyses and Table 1 for the experimental coupling constants). ΔG^{298} at 298K for **1** has been calculated as follows: $\Delta G^{298} = \Delta H° - T\Delta S°$. The standard deviations of ΔH°, ΔS°, -TΔS° and ΔG^{298} are given in parentheses. %S (278K - 358K) is the change in the population of S-type pseudorotamers from 278K to 358K. For **2** - **7**, Δ %S (278K - 358K) has been taken from ref. 1i. For **1**, at a particular temperature T (K) (*i.e.* 278K or 358K), the population of S-type pseudorotamers has been calculated using the relation: $\%S\ (T) = 100 * [\exp(-\Delta G^{T}/RT)] / [\exp(-\Delta G^{T}/RT) + 1]$. [b] $\Delta H°_{GE}$ have been calculated by

subtracting $\Delta H°$ characterizing the N $\rightleftarrows$ S equilibrium of the sugar moiety in **1** - **7** from that obtained for **9** (ref. 1i). [c] The theoretical group electronegativities of NH_2, OH, OMe, NO_2 and F have been taken from ref. 8b. For OCF_3, and OPO_3H^-, we have used the original methodology from Marriott , *i.e.* H-OCF_3 and H-OPO_3H^- have been optimized ab initio with Gaussian 94 program[27]. $\chi_{Marriott,theor.}$ (OCF_3) (0.48) and $\chi_{Marriott,theor.}$ (OPO_3H^-) (0.48) have been taken as the atomic electron population on the constituent H in H-OCF_3 and H-OPO_3H^- respectively. The experimental group electronegativities of OCF_3, and OPO_3H^- in Marriott's scale have been calculated from the relation: $\chi_{Marriott,exp.} = 0.26 - 0.022\ \Delta H°_{GE}$, where $\Delta H°_{GE}$ represents the strength of the gauche effect of [3'-OCF_3-C3'-C4'-O4'] and [3'-OPO_3H^- -C3'-C4'-O4'] in **1** and **6** (see ref. 1i), respectively. [d] The group electronegativities in our refined scale have been estimated using the relation: $\chi_{thiswork,refined} = 0.26 - 0.022\ \Delta H°_{GE}$, which is simply the best fit line through the pairs [$\chi_{Marriott,theor.}$, $\Delta H°_{GE}$] for compounds **2** - **5** and **7** (see ref. 1i). [e] The theoretical group electronegativities of NH_2, OH, OMe, NO_2 and F have been taken from ref. 8a. For OCF_3, and OPO_3H^- , $\chi_{Mullay,exp}$ have been calculated from the relation: $\chi_{Mullay,exp.} = 2.63 - 0.19\Delta H°_{GE}$, where $\Delta H°_{GE}$ represents the strength of the gauche effect of [3'-OCF_3-C3'-C4'-O4'] and [3'-OPO_3H^- -C3'-C4'-O4'] in **1** and **6** (see ref. 1i) respectively. [f] The theoretical inductive substituent parameters values of NH_2, OH, OMe, NO_2 and F have been taken from ref. 8f For OCF_3 and OPO_3H^- , $\iota_{Inamoto,exp}$ have been calculated from the relation: $\iota_{Inamoto,exp} = 2.25 - 0.08\Delta H°_{GE}$, where $\Delta H°_{GE}$ represents the strength of the gauche effect of [3'-OCF_3-C3'-C4'-O4'] and [3'-OPO_3H^- -C3'-C4'-O4'] in **1** and **6** (see ref. 1i), respectively. The theoretical inductive substituent parameter value for OCF_3 [$\iota_{Inamoto,theor}$] has been taken from ref. 8f.

In order to validate our newly determined $\chi_{Marriott,exp.}(OCF_3)$ value (0.38 ± 0.05), we have applied Marrott's original procedure[2b] in the case of H-OCF_3: Using Gaussian 94 program[21], we have optimized *ab initio* the geometry of H-OCF_3 at HF/6-31G* level. The theoritical group electronegativity of OCF_3 [$\chi_{Marriott,theor.}(OCF_3)$ = 0.478] in H-OCF_3 has been taken as the charge density on the constituent hydrogen atom determined by Mulliken population analysis using the optimized H-OCF_3 structure. This theoretical estimate, rather close to that already published by Marriott in the case of F in H-F [$\chi_{Marriott,theor.}(F)$ = 0.517] is clearly *larger* than our experimentally determined value [$\chi_{Marriott,exp.}(OCF_3)$ = 0.38 ± 0.05, see above and Fig. 1].

The comparison of theoretically estimated χ [$\chi_{Marriott,theor.}(OCF_3)$ in Marriott's scale] and ι values [$\iota_{Inamoto,theor.}(OCF_3)$ in Inamoto's scale] shows some inconsistency: Marriott's theoretical scale (0.48) predicts that OCF_3 is more electronegative than OH (0.43) and OMe (0.44), whereas in the case of Inamoto's theoretical scale there are only negligible differences between the ι values for OH, OMe and OCF_3 (2.80 ± 0.02). In the case of $\chi_{Marriott,theor.}$ scale, OCF_3 is expected to have an electronegativity intermediate between that of OMe and that of F (0.517). On the other hand, in the case of $\iota_{Inamoto,theor.}$ scale, OCF_3 ressembles much more OH and OMe, and it is therefore much less electronegative than fluorine [$\iota_{Inamoto,theor.}(F)$ = 3.10].

Our experimental data [$\iota_{Inamoto,exp}$ = 2.64 ± 0.10, $\chi_{Marriott,exp}$ = 0.38 ± 0.05] have clearly shown that the 3'-OCF_3 in **1** drives the two-state N⇄S pseudorotational equilibrium in 3'-OCF_3-2',3'-dideoxythymidine **1** much less efficiently toward S-type conformations [ÆH°$_{GE}$ = -5.4 kJ/mol] than 3'-F in 3'-fluorothymidine (**7**) [ÆH°$_{GE}$ = -11.3 kJ/mol]. In fact, this desire to adopt S-type conformation is twice weaker in **1** in comparison with 3'-fluoro-2',3'-dideoxythymidine (**7**). Therefore, our results seem to be much more in agreement with Inamoto's theoretical ι scale than with Marriott's theoretical χ scale.

This has prompted us to readjust the theoretical electronegativity values in Marriott's scale [$\chi_{Marriott,theor.}$] specifically in the case of X3'-2',3'-dideoxythymidine nucleosides by recalculating them [refined electronegativities in Marriot's scale are

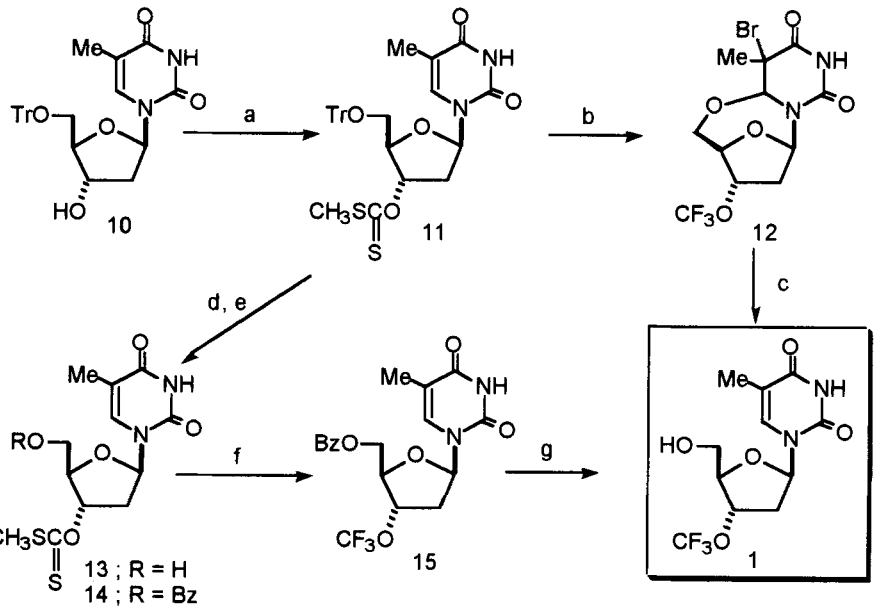
designed by: $\chi_{thiswork,refined}$] using the equation of the best line fitted through our original plot of $\chi_{Marriott,theor.}$ values as a function of experimental $\text{Æ}H°_{GE}$ for **1** - **7** (Table 1, Eq. 1):

$$\chi_{thiswork,refined} = 0.26 - 0.022\ \text{Æ}H°_{GE} \quad \ldots. \quad \text{Eq. 1.}$$

Using this procedure, we have now found: $\chi_{thiswork,refined}$ (NH_2[27]) = 0.32; $\chi_{thiswork,refined}$ (OH) = 0.42; $\chi_{thiswork,refined}$ (OCH_3) = 0.43; $\chi_{thiswork,refined}$ (NO_2) = 0.43; $\chi_{thiswork,refined}$ (OPO_3H^-) = $\chi_{Marriott,exp}$ (OPO_3H^-) = 0.44; $\chi_{thiswork,refined}$ (F) = 0.51; $\chi_{thiswork,refined}$ (OCF_3) = $\chi_{Marriott,exp.}$ (OCF_3) = 0.38.

(D) Chemistry

Introduction of the trifluoromethyl group at the 3'-OH was basically adopted from a recently published method.[23] Treatment of 5'-*O*-tritylthymidine (**10**) with CS_2 and MeI in a mixture of 50% NaOH and CH_2Cl_2 at room temperature gave **11** (82%), which is a



reagents and conditions (a) CS_2, MeI, 2 M NaOH/CH_2Cl_2 (1:1), rt, 82%; (b) DBH, HF/pyridine, CH_2Cl_2, -78 °C-0 °C, 15%; (c) Zn, AcOH, rt, 73%; (d) TFA, MeOH, rt, 94%; (e) BzCl, pyridine, rt, 96%; (f) HF/pyridine, NIS, 56%; (g) NH_3/MeOH, rt, 86%.

starting material of the trifluoromethylation reaction. Reaction of **11** with pyridinium poly(hydrogen fluoride) (HF/pyridine) in the presence of 1,3-dibromo-5,5-dimethylhydantoin (DBH) did not give the desired 3'-*O*-trifluoromethyl thymidine derivative, but 1-(2-deoxy-3-*O*-trifluoromethyl-β-D-ribofuranosyl)-O^6,5'-anhydro-5-bromo-5,6-dihydrothymine (**12**) in 15% yield. Compound **12** was however easily converted into the desired 3'-*O*-trifluoromethylthymidine (**1**) in 73% yield upon its treatment with Zn in AcOH. Many attempts to improve the yield of **12** failed.

To improve the yield of **1**, deprotection of **11** was first tried with trifluoroacetic acid in MeOH to give **13**, and the resulting 5'-OH of **13** was benzoylated to afford **14** in 90% yield in two steps. Reaction of **14** with HF/pyridine in the presence of DBH (3 equiv) at -78 °C to 0 °C in CH_2Cl_2 gave the desired trifluoromethyl derivative **15** only in 26% yield. Attempts to improve the yield of **15**, addition of increased amount of the oxidant and changing the reaction temperature, were not fruitful. However, when *N*-iodosuccinimide (NIS, 3 equiv), but not *N*-chlorosuccinimide or *N*-bromosuccinimide, was used as an oxidant, the yield of **15** was improved to 56%. Treatment of **15** in NH_3/MeOH (saturated at 0 °C) at room temperature gave the desired **1** in 86% yield.

Experimental Section

Melting points are uncorrected. ^1H and ^{13}C NMR spectra were recorded at 270, 400, and 500 MHz (^1H), at 67.8 and 125 MHz (^{13}C). Chemical shifts are reported in ppm downfield from TMS (^1H and ^{13}C), and *J* values are given in Hertz. Mass spectra were obtained by the electron ionization (EI) or fast atom bombardment (FAB) methods. Thin-layer chromatography was done on Merck coated plate $60F_{254}$. Silica gel chromatography was done with Merck silica gel 5715. Reactions were carried out under an argon atmosphere.

1-(2-Deoxy-3-*O*-methylxanthyl-5-*O*-trityl-β-D-ribofuranosyl)thymine (11). A solution of **10** (10.0 g, 20.7 mmol) in CH_2Cl_2 (35 mL) was added to a mixture of tetrabutylammonium bromide (667 mg, 2.07 mmol) in aqueous NaOH (50%, 15 mL) and CS_2 (15 mL), and the solution was stirred at room temperature for 15 min. Methyl iodide (1.42 mL) was then added to the above mixture, which was further stirred at the same temperature for 14 h. The mixture was diluted with $CHCl_3$ (200 mL), which was washed with H_2O (100 mL x 2) and brine (50 mL x 2), dried (Na_2SO_4), and concentrated to dryness in vacuo. The residue was purified on a silica gel column, which was eluted with hexane:EtOAc = 1:1) to give **11** (9.7 g, 82% as a yellowish solid, which was crystallized from hexane/EtOAc): mp 189-191 °C; ^1H-NMR ($CDCl_3$) δ 8.10 (s, 1 H), 7.65 (s, 1 H), 7.45-7.22 (m, 15 H), 6.50 (dd, 1 H, *J* = 5.5, 9.2 Hz), 6.20 (d, 1 H, *J* = 6.0 Hz), 4.3 (dd, 1 H, *J* = 2.5, 2.5 Hz), 3.65 (dd, 1 H, *J* = 2.5, 10.5 Hz), 3.46 (dd, 1H, *J* = 2.5, 10.5 Hz), 2.7 - 2.5 (m, 2 H), 2.59 (s, 3 H), 1.57 (s, 3 H); ^{13}C-NMR ($CDCl_3$) δ 215.25, 163.67, 150.44,

143.16, 135.11, 128.55, 128.07, 127.46, 111.69, 87.78, 83.92, 64.00, 37.98, 19.30, 11.68; FAB-LRMS *m/z* 575 (MH^+); FAB-HRMS calcd for $C_{31}H_{30}N_2O_5S_2$ 575.1598, found 575.1694.

Anal.calcd for $C_{31}H_{30}N_2O_5S_2$: C, 64.80; H, 5.22; N, 4.87; S, 11.10. Found: C, 64.50; H, 5.42; N, 4.92; S, 10.99.

1-(2-Deoxy-3-*O*-trifluoromethyl-β-D-ribofuranosyl)-O^6,5'-anhydro-5-bromo-5,6-dihydrothymine (12). A solution of **11** (234 mg, 0.41 mmol) in CH_2Cl_2 (1.5 mL) was added to a solution of DBH (350 mg, 1.23 mmol) in CH_2Cl_2 and then HF/pyridine (820 μL) was added to the mixture at 78 °C under Ar. The whole was stirred at 0 °C for 1 h and diluted with $CHCl_3$, which was washed with aqueous saturated $NaHCO_3$ and H_2O. The separated organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was purified on a silica gel column, which was eluted with hexane:EtOAc = 3:1 to give **12** (35 mg, 15% as a solid): ^{1}H-NMR ($CDCl_3$) δ 7.40 (s, 1 H), 6.19 (d, 1 H, *J* = 6.0 Hz), 5.23 (s, 1 H), 4.82 (ddd, 1 H, *J* = 1.4, 5.1, 7.7 Hz), 4.64 (br s, 1 H), 4.09 (d, 1 H, *J* = 12.8 Hz), 3.81 (dd, 1 H, *J* = 2.4, 12.8 Hz), 2.95 (dd, 1 H, *J* = 7.7, 15.1 Hz), 2.53 (ddd, 1 H, *J* = 5.1, 6.0, 15.1 Hz), 1.56 (s, 3 H); EI-LRMS *m/z* 389 (M^+), 391 (M^++2). Anal. calcd for $C_{11}H_{12}F_3BrN_2O_5 \cdot 0.25H_2O$: C, 33.57; H, 3.20; N, 7.12. Found: C, 33.27; H, 3.34; N, 7.05.

1-(2-Deoxy-3-*O*-methylxanthyl-β-D-ribofuranosyl)thymine (13). A solution of **11** (2.0 g, 3.5 mmol) in MeOH (10 mL) containing TFA (400 μL) was heated under reflux for 2 days. The mixture was neutralized with aqueous saturated $NaHCO_3$ and the whole was partitioned between EtOAc (300 mL) and H_2O (40 mL x 2). The separated organic phase was dried (Na_2SO_4) and concentrated to dryness in vacuo. The residue was purified on a silica gel column, which was eluted with 3% MeOH in $CHCl_3$ to give **13** (1.1 g, 94% as a solid, which was crystallized from MeOH/$CHCl_3$): mp 209-210 °C; ^{1}H-NMR (DMSO-d_6) δ 11.40 (s, 1 H), 7.80 (s, 1 H), 6.23 (dd, 1 H, *J* = 7.8, 7.8 Hz), 5.95 (br s, 1 H), 5.31 (s, 1 H), 3.79-3.63 (m, 2 H), 2.61 (s, 3 H), 2.52 - 2.42 (m, 2 H), 1.80 (s, 3 H); ^{13}C-NMR (DMSO-d_6) δ 214.61, 163.63, 150.44, 135.72, 109.77, 84.54, 84.11, 83.81, 61.22, 36.49, 18.64, 12.25; EI-LRMS *m/z* 332 (M^+). Anal. calcd for $C_{12}H_{16}N_2O_5S_2$: C, 43.36; H, 4.85; N, 8.42; S, 19.29. Found: C, 43.47; H, 4.84; N, 8.27; S19.26.

1-(5-*O*-Benzoyl-2-deoxy-3-*O*-methylxanthyl-β-D-ribofuranosyl)thymine (14). Benzoyl chloride (420 μL, 3.6 mmol) was added to a solution of **13** (1.1 g, 3.3 mmol) in pyridine (20 mL) at 0 °C, and the mixture was stirred at room temperature for 4 h. Ice-water was added to the mixture, which was diluted with EtOAc (200 mL). The whole was washed with H_2O (20 mL x 4), and the separated organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was purified on a silica gel column, which was eluted with hexane:EtOAc = 1:1 to give **14** (1.4 g, 96% as a solid, which was crystallized from hexane/EtOAc): mp 79-82 °C; ^{1}H-NMR ($CDCl_3$) δ 8.42 (s, 1 H), 8.01-7.40 (m, 5 H), 7.25 (d, 1 H, *J* = 1.3 Hz), 6.42 (dd, 1 H, *J* = 5.0, 8.9 Hz), 6.11 (d, 1 H, *J* = 6.2 Hz), 4.77 (dd, 1 H, *J* = 2.8, 12.0 Hz), 4.68 (dd, 1 H, *J* = 3.3, 12.0 Hz), 4.59 (dd, 1 H, *J* = 2.8, 3.3 Hz), 2.75 (dd, 1 H, *J* = 5.0, 15.0 Hz), 2.61 (s, 3 H), 2.32 (ddd, 1 H, *J* = 6.2, 8.9, 15.0 Hz), 1.60 (s, 3 H); ^{13}C-NMR ($CDCl_3$) δ 215.32, 165.89, 163.52, 150.29, 134.30, 129.41, 129.18, 128.76, 111.66, 85.03, 82.73, 82.22, 64.33, 37.84, 19.44, 12.07; EI-LRMS *m/z* 436 (M^+). Anal. calcd for $C_{12}H_{16}N_2O_5S_2$: C, 43.36; H, 4.85; N, 8.42; S, 19.29. Found: C, 43.47; H, 4.84; N, 8.27; S, 19.26.

1-(5-*O*-Benzoyl-2-deoxy-3-*O*-trifluoromethyl-β-D-ribofuranosyl)thymine (15). A solution of NIS (481 mg, 2.1 mmol) in CH_2Cl_2 (4 mL) was added to HF/pyridine (1.4 mL) and then a solution of **14** (300 mg, 0.7 mmol) in CH_2Cl_2 (4 mL) was added at -78 °C under Ar. The mixture was stirred at 0 °C for 30 min and diluted with EtOAc (200 mL), which was washed with aqueous saturated $NaHCO_3$ (25 mL x 2), H2O (25 mL x 2), and brine (25 mL x 2). The separated organic phase was dried (Na_2SO_4) and concentrated to dryness in vacuo. The residue was purified on a silica gel column, which was eluted with hexane:EtOAc = 1:1 to give **15** (151 mg, 53% as a foam): IR (Nujol) 1220 cm^{-1} (-CF_3); ^{1}H-NMR ($CDCl_3$) δ 8.58 (s, 1 H), 8.02 (m, 2 H), 7.63 (m, 1 H), 7.49 (m, 2 H), 7.15 (d, 1 H), 6.27 (dd, 1 H, *J* = 6.0, 7.8 Hz), 5.02 (m, 1 H), 4.71 (dd, 1H, *J* = 3.0, 11.9), 4.53 (dd, 1 H, *J* = 2.7, 11.9 Hz), 4.49 (m, 1 H), 2.68 (ddd, 1 H, *J* = 3.3, 6.0, 14.5 Hz), 2.17 (s, 3 H); ^{13}C-NMR ($CDCl_3$) δ 165.82, 163.29, 150.15, 134.68, 133.73, 129.79, 129.45, 129.14, 129.02, 128.71, 121.26, 111.59, 85.37, 85.00, 81.83, 63.30, 37.97, 12.11; EI-LRMS *m/z* 414 (M^+); EI-HRMS calcd for $C_{18}H_{16}F_3N_2O_6$ 414.1039, found 414.1057.

1-(2-Deoxy-3-*O*-trifluoromethyl-β-D-ribofuranosyl)thymine (1). (a) Zn dust (66 mg, 1.0 mmol) was added to a solution of **12** (130 mg, 0.33 mmol) in AcOH (5 mL). The mixture was stirred at room temperature for 25 min and the insoluble materials were removed by filtration through Celite. The filtrate was concentrated to dryness, and the residue was purified on a silica gel column, which was eluted with $CHCl_3$:EtOH = 15:1 to give **1** (76 mg, 73% as solid). (b) Compound **15** (35 mg, 0.1 mmol) was treated with saturated NH_3/MeOH (saturated at 0 °C, 2 mL) at room temperature overnight. The solvent was removed in vacuo, and the residue was purified on a silica gel column, which was eluted with 3% EtOH in $CHCl_3$ to give **1** (22 mg, 86% as a solid, which was crystallized from MeOH/$CHCl_3$): mp 198-199 °C; IR (Nujol) 1220 cm^{-1} (-CF_3); ^{1}H-NMR (D_2O, 85 °C, 0.6 mg/mL) δ 7.58 (d, 1 H, *J* = 1.4 Hz), 6.26 (dd, 1 H, *J* = 6.8, 7.3 Hz), 5.07 (ddd, 1 H, *J* = 2.9, 3.9, 4.9 Hz), 4.30 (ddd, 1 H, *J* = 2.9,), 3.87 (dd, 1 H, *J* = 3.9, 12.5 Hz), 3.82 (dd, 1 H, *J* = 4.9, 12.5 Hz), 2.69 (ddd, 1 H, *J* = 3.4, 6.8, 15.1 Hz), 2.56 (ddd, 1 H, *J* = 6.9, 7.3, 15.1 Hz), 1.91 (s, 3 H); ^{13}C-NMR (DMSO-d_6) δ 163.64, 150.41, 135.88, 121.11, 109.76, 83.75, 83.45, 78.81, 60.45, 36.28, 12.17; EI-LRMS *m/z* 310 (M^+); EI-HRMS calcd for $C_{11}H_{13}F_3N_2O_5$ 310.0776, found 310.0756. Anal. calcd for $C_{11}H_{13}F_3N_2O5 \cdot 0.25\ H_2O$: C, 41.98; H, 4.32; N, 8.90. Found: C, 42.01; H, 4.26; N, 8.82.

Conformational analysis of 1 based on ^{1}H-NMR spectra at 500 MHz. One dimensional ^{1}H spectra of **1** (5 mM D_2O solution) have been recorded at 500 MHz throughout the 278 K - 358 K temperature range at pD = 6.5 in order to examine the effect of 3'-OCF_3 substituent on the drive the pseudorotational equilibrium of the constituent pentofuranosyl moiety. $^3J_{HH}$s have been extracted from the temperature-dependent 1D spectra and simulated using DAISY program[22] (Table 1).

Pseudorotational Analyses of Temperature-dependent $^3J_{HH}$ to Determine the Thermodynamics of the Two-state N⇄S Equilibrium in 1. The temperature-dependent $^3J_{HH}$ (Table 1) have been interpreted in terms of a two-state N⇄S pseudorotational equilibrium using PSEUROT[24] program. The PSEUROT algorithm is based on the following steps: (i) The user's input consists of starting ("guess") values for

the Phase Angle (P) and the Puckering Amplitude (Ψ_m) of both N- and S-type conformers and their relative populations. A section is also devoted to the input of the electronegativities of the substituents on all H-C-C-H fragments[25] Depending upon the user's input, the starting values for P and Ψ_m will be either refined or constrained during the PSEUROT optimization (*vide infra*). (ii) From the starting P and Ψ_m values of each of the N- and S-type sugars, the endocyclic torsions (ν_0 [C4'-O4'-C1'-C2'], ν_1 [O4'-C1'-C2'-C3'], ν_2 [C1'-C2'-C3'-C4'], ν_3 [C2'-C3'-C4'-O4'], ν_4 [C3'-C4'-O4'-C1']) are calculated basing on the pseudorotation law[17]. (iii) These endocyclic torsion angles are in turn converted into the corresponding proton-proton torsions (Φ_{HH}) using simple linear relationships. (iv) With the help of Altona *et al.* generalized Karplus-type equation[24c,d], each Φ_{HH} is translated into an individual $^3J_{HH}$. (v) PSEUROT calculates time-averaged $^3J_{HH}$ values from individual $^3J_{HH}$s basing upon the two-state N⇄S equilibrium hypothesis. (vi) The experimental and calculated time-averaged $^3J_{HH}$s are compared pairwise. In the following iterative steps, P and $\Psi_m$and/or their populations are randomly changed. The discrepancy between experimental and calculated $^3J_{HH}$ is monitored and optimized. (vii) The best fit geometries and populations of the N and S conformers are printed out together with the error analysis which shows individual differences between experimental and calculated $^3J_{HH}$ as well as the overall r.m.s. error.

The PSEUROT calculations for **1** have been performed either by constraining simultaneously (i) Ψ_m of both N- and S-type pseudorotamers to an identical value, which has been varied by 1° steps in the range from 29° to 45° or (ii) P (in the range from -30° to 30° in 10° steps) and Ψ_m (Ψ_m = 29°, 32° and 35°) of the minor N-type pseudorotamer. (iii) For each constrained geometry, the propagation of the error (σ = 0.1 Hz) inherent to each experimental $^3J_{HH}$ has been taken into account by constructing 650 sets of random input couplings, in such a way that over all the sets of inputs, the values for this $^3J_{HH}$ were normally distributed around the experimentally measured value (see Table 2). Each of these sets of couplings has been used to perform a PSEUROT calculation. From the temperature-dependent populations of N-type and S-type pseudorotamers in **1** produced by the PSEUROT analyses, we have subsequently calculated the thermodynamics of the two-state N⇄S pseudorotational equilibrium in **1** from van't Hoff type analyses (Table 2).

Acknowledgments

We thank Swedish Natural Science Research Council (NFR), Swedish Board for Technical Development (NUTEK) and Swedish Engineering Research Council (TFR) for generous financial support. Thanks are due to the Wallenbergstiftelsen, Forskningsrådsnämnden, and University of Uppsala for funds for the purchase of 600 and 500 MHz Bruker DRX NMR spectrometers.

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