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Unprecedented Lability of the 5′-*O*-*tert*-Butyldimethylsilyl Group from 3′-Spiro-5″-(4″-acylamino-1″,2″-oxathiole-2″,2″-dioxide) Nucleoside Derivatives via Neighboring Group Participation of the 4″-Acylamino Residue

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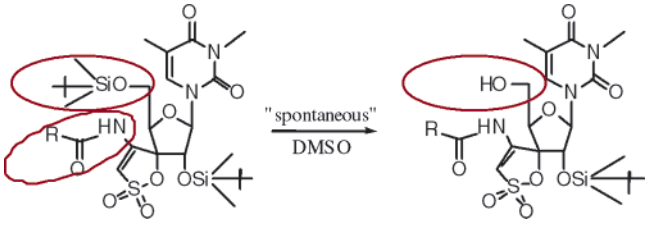

Scarce examples of exceptionally mild desilylation of *tert*-butyldimethylsilyl (TBDMS) ether groups by neighboring group participation have been previously described. Here, we investigate, in detail, the discovery of the unusual lability of the 5′-TBDMS group on 4″-acylamino TSAO derivatives in DMSO solution. The synthesis and comparative chemical stability studies in different solvents of a variety of 4″-substituted TSAO derivatives bearing different carbonyl functionalities are reported. Modifications have also been performed at the 5′-position of the TSAO molecule to gain insight into the structural requirements for the desilylation to occur. The role of the solvent has also been studied. Additionally, NMR and theoretical investigations have been carried out to get further insight into the conformational, geometric, and/or electronic parameters that may play a role in the "spontaneous" release of the 5′-TBDMS group. A silyl hydrolysis mechanism involving neighboring group participation of the 4″-acylamino group is proposed.

Introduction

HIV-1-specific nonnucleoside reverse transcriptase inhibitors (NNRTIs) have gained in the last years an increasing momentum in the therapy of HIV infections.[1−3] Among them, [2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-*β*-D-ribofuranosyl]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) nucleosides (TSAOs) are so far the only NNRTIs[4,5] that seem to interact at the interface between the p51 and p66 subunits of HIV-1 RT.[6] The prototype compound of this family is the thymine derivative TSAO-T (**1**), and one of the most selective compounds is the 3-*N*-methyl-substituted derivative TSAO-m^{3}T (**2**) (Figure 1). TSAO compounds consistently select for Glu138Lys mutant HIV-1 strains, in cell culture. This mutation is important at the level of the p51 subunit of HIV-1 RT.[7]

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(1) De Clercq, E. *Chem. Biodivers.* **2004**, *1*, 44−64.
(2) Pauwels, R. *Curr. Opin. Pharmacol.* **2004**, *4*, 1−10.
(3) Tarby, C. M. *Curr. Top. Med. Chem.* **2004**, *4*(10), 1045−1057.
(4) (a) Balzarini, J.; Pérez-Pérez, M. J.; San-Félix, A.; Schols, D.; Perno, C. F.; Vandamme, A. M.; Camarasa, M. J.; De Clercq, E. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 4392−4396. (b) Camarasa, M. J.; Pérez-Pérez, M. J.; San-Félix, A.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1992**, *35*, 2721−2727.
(5) Camarasa, M. J.; San-Félix, A.; Pérez-Pérez, M. J.; Velázquez, S.; Alvarez, R.; Chamorro, C.; Jimeno, M. L.; Pérez, C.; Gago, F.; De Clercq, E.; Balzarini *J. Carbohydr. Chem.* **2000**, *19*, 451−469.
(6) Rodríguez-Barrios, F.; Pérez, C.; Lobatón, E.; Velázquez, S.; Chamorro, C.; San-Félix, A.; Pérez-Pérez, M. J.; Camarasa, M. J.; Pelemans, H.; Balzarini, J.; Gago, F. *J. Med. Chem.* **2001**, *44*, 1853−1865.

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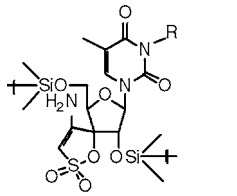
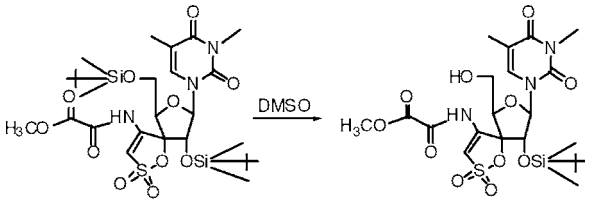


1 TSAO-T R = H
2 TSAO-m^{3}T R = CH_3

FIGURE 1. Structure of TSAO-T (**1**) and TSAO-m^{3}T (**2**).



3 **4**

FIGURE 2. Desilylation of compound **3** in DMSO.

As a part of a Medicinal Chemistry program on this unique family of NNRTIs, we prepared novel TSAO derivatives targeted at TSAO-resistant HIV-1 strains[8] that contain carbonyl groups at the 4″-position of the spiro moiety. Among them, compound **3** bearing an *N*-methoxalyl moiety at the 4″-position (Figure 2) was highly active against HIV-1 replication ($EC_{50(CEM)} = 0.06 \pm 0.02\ \mu M$).[8] To determine whether the antiviral activity of **3** was due to the intact compound or whether compound **3** could be partially acting by releasing TSAO-m^{3}T **2** (through the hydrolysis of the amide bond at the 4″-position), compound **3** was subjected to the conditions required for the antiviral assays. Thus, the compound was dissolved in DMSO and was further diluted by addition of 10% fetal calf serum. Under these conditions, **3** was converted to a more polar compound that was identified as the 5′-deprotected analogue **4** (Figure 2) as a result of the release of the 5′-silyl moiety whereas the amide bond at the 4″-position was completely stable. This was an unexpected behavior. It should be mentioned that the 5′-TBDMS moiety in TSAO molecules is essential for antiviral activity, and desilylation renders inactive compounds.[4] Desilylation of compound **3** was also observed in DMSO-d_6 solution on standing in an NMR tube. New signals corresponding to the 5′-OH derivative **4** appeared in only 2 h (ratio of **3** to **4** was 5:1), and after 24 h, the ratio of **3** to **4** was 1:1. This facile desilylation was never previously observed, with more than 800 TSAO derivatives prepared so far.[9]

These results prompted us to investigate the nature of this unexpectedly facile desilylation in detail. Scarce examples of exceptionally mild desilylation of TBDMS groups by neighboring group participation have been described.[10,11] In this paper, we investigate the behavior, in DMSO solution, of selected examples of 4″-N-substituted TSAO derivatives bearing different carbonyl functionalities. Analogues of **3** in which other groups have replaced the TBDMS moiety at the 5′-position have also been prepared and studied. Our study is aimed at assessing (i) the structural requirements at both the 4″-NH and 5′-O positions of the TSAO molecules for the desilylation process to occur; (ii) the importance of the configuration and/or conformation of the molecule in the desilylation process; and (iii) the influence of the solvent in the process. With all the results collected from the experiments, a neighboring group participation of the 4″-amide proton in the process is proposed together with a possible mechanism of the desilylation.

Results and Discussion

Synthesis. As mentioned before, the present study started after our finding that the *N*-methoxalyl derivative **3** gradually decomposed in DMSO solution to release the 5′-TBDMS group. To investigate this unprecedented extremely easy elimination reaction in detail, the previously described N-substituted TSAO derivatives **5**−**15**[8] (Figure 3), bearing carbonyl groups of a different nature, were included in the study.

Also, novel TSAO derivatives were prepared as follows. The synthesis of the *N*-trifluoroacetyl derivative **17** (bearing a highly electron-withdrawing carbonyl substituent) was initially attempted by reaction of TSAO-m^{3}T **2** (Scheme 1) with trifluoroacetyl anhydride at 80 °C in a sealed pressure tube in the presence of DMAP, according to our previously described acylation method.[8] Under these conditions, after the acid workup, to eliminate the DMAP, the 3″-trifluoroacetyl nucleoside **16** (Scheme 1) was obtained in low yield (12%) together with complex reaction mixtures of unidentified products. This result contrasts with the regioselectivity observed in the previously described acylation reactions of TSAO-m^{3}T[8] in which acylation occurred exclusively at the amino group of the 4″-position. A careful and detailed analysis of this reaction showed the initial formation (as detected from TLC) of both N-acylated **17** and C-acylated **16** derivatives followed by decomposition of the *N*-acyl derivative **17** in the acid workup. However, when the reaction of **2** with trifluoroacetyl anhydride was performed in the presence of a polymer-bound base (*p*-TBD 1,5,7-triazabicyclo [4.4.0]dec-5-ene)[12] that could be easily removed by filtration to avoid the acid workup, the N-acylated compound **17** was isolated in good yield (83%) (Scheme 1).

Interestingly, when the *N*-trifluoroacetyl derivative **17** was dissolved in DMSO, the 5′-O-deprotected derivative **18** was formed, as detected by NMR, but quickly transformed into the novel tricyclic nucleoside **19** (Scheme 1). Formation of this compound could be explained by attack of the 5′-OH to the 4″-position of the aminosultone ring. The structure of this tricyclic nucleoside **19** was unequivocally assigned from mono- and bidimensional ^1H and ^{13}C NMR (1D and 2D) techniques. Similar tricyclic structures have been recently described by our group.[13,14]

Next, modifications were performed at the 5′-position by replacing the 5′-TBDMS group of compounds **3** with other 5′-

(7) Jonckheere, H.; Taymans, J. M.; Balzarini, J.; Velázquez, S.; Camarasa, M. J.; Desmyter, J.; De Clercq, E.; Anné, J. *J. Biol. Chem.* **1994**, *269*, 25255−25258.

(8) De Castro, S.; Lobatón, E.; Pérez-Pérez, M. J.; San-Félix, A.; Cordeiro, A.; Andrei, G.; Snoeck, R.; De Clercq, E.; Balzarini, J.; Camarasa, M. J.; Velázquez, S. *J. Med. Chem.* **2005**, *48*, 1158−1168.

(9) Camarasa, M. J.; San-Félix, A.; Velázquez, S.; Pérez-Pérez, M. J.; Gago, F.; Balzarini, J. *Curr. Top. Med. Chem.* **2004**, *4*, 945−963.

(10) Kawahara, S.; Wada, T.; Sekine, M. *J. Am. Chem. Soc.* **1996**, *118*(40), 9461−9468.

(11) Greco, M. N.; Zhong, H. M.; Marynoff, B. E. *Tetrahedron Lett.* **1998**, *39*, 4959−4962.

(12) Ingate, S.; Pérez-Pérez, M. J.; De Clercq, E.; Balzarini, J.; Camarasa, M. J. *Antiviral Res.* **1995**, *27*, 281−299.

(13) Chamorro, C.; Luengo, S. M.; Bonache, M. C.; Velázquez, S.; Pérez-Pérez, M. J.; Camarasa, M. J.; Gago, F.; Jimeno, M. L.; San-Félix, A. *J. Org. Chem.* **2003**, *68*, 6695−6704.

(14) Bonache, M. C.; Chamorro, C.; Cordeiro, A.; Camarasa, M. J.; Jimeno, M. L.; San-Félix, A. *J. Org. Chem.* **2004**, *69*(25), 8758−8766.

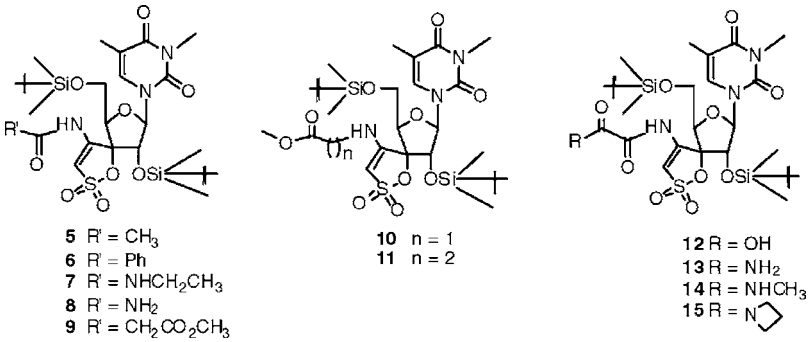
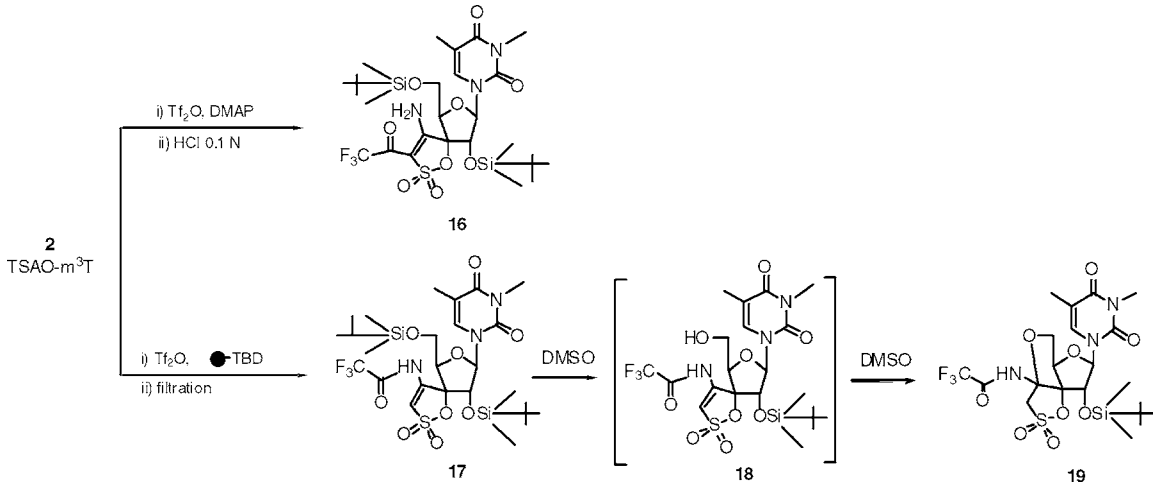


FIGURE 3. Structures of N-substituted TSAO derivatives **5**−**15**.

SCHEME 1



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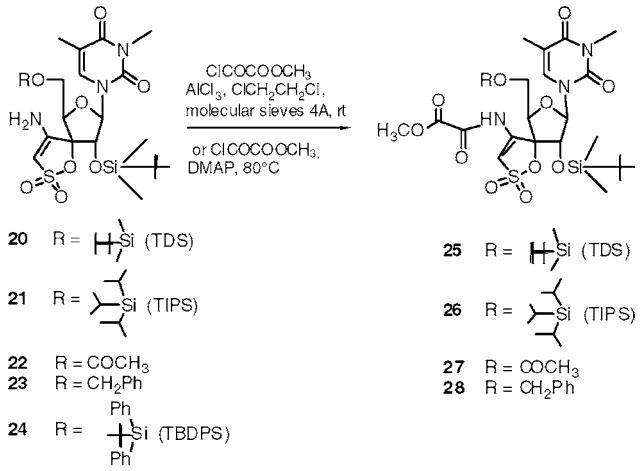


silyl, acetyl, and benzyl groups to study the stability of groups of a different nature. The 5′-*O*-*tert*-hexyldimethylsilyl (TDS), the 5′-*O*-triisopropilsilyl (TIPS), the 5′-*O*-acetyl, and the 5′-*O*-benzyl *N*-methoxalyl TSAO derivatives **25**−**28** (Scheme 2) were prepared by reaction of the corresponding 5′-O-protected TSAO derivatives **20**−**23**[15] with methyl oxalyl chloride, in the presence of $AlCl_3$ or DMAP, in 70%, 13%, 48%, and 50% yields, respectively. However, treatment of the more hindered 5′-*O*-*tert*-butyldiphenylsilyl (TBDPS) derivative **24** with methyl oxalyl chloride under various acylation conditions failed, and only unreacted starting material was recovered.

Stability Studies in Solution. Comparative chemical stability studies of the 5′-TBDMS group on the 4″-*N*-acyl or 4″-*N*-alkyl TSAO derivatives **5**−**15** (Figure 3)[8] and compounds **17** and **25**−**28** were carried out in DMSO, and their conversion into the corresponding 5′-O-deprotected derivatives was followed by HPLC. In all the experiments, the 4″-*N*-methoxalyl TSAO derivative **3** was included as a reference compound. Our results are shown in Figures 4 and 5. It should be noted that TSAO-m^{3}T **2** was completely stable in DMSO solution, and no release of the 5′-TBDMS moiety was detected.

Figure 4 shows the desilylation rate of 4″-*N*-acyl-substituted TSAO derivatives (**5**−**9** and **17**) in which the electron-withdrawing ability of the carbonyl substituent at the 4″-amino group has been varied [from the electron-donating NH_2 (**8**) to

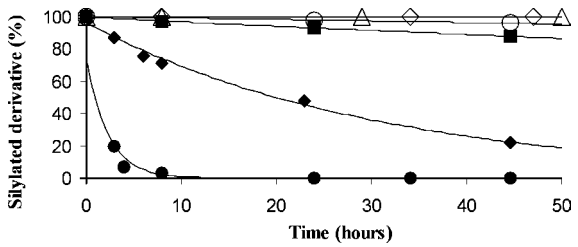


FIGURE 4. Stability studies of the 5′-TBDMS group on 4″-acyl TSAO derivatives **3** (◆ $COCO_2CH_3$), **5** (■ $COCH_3$), **6** (△ COPh), **8** (◇ $CONH_2$), **9** (○ $COCH_2CO_2CH_3$), and **17** (● $COCF_3$). Data for compounds **7**, **10**, and **11** (stability similar to that for compounds **6** and **8**) are omitted for clarity.

(15) Ley, S. V.; Baxendale, I. R.; Beam, R. N.; Jackson, P. S.; Leach, A. G.; Longbottom, D. A.; Nesi, M.; Scott, J. S.; Storer, I.; Taylon, S. J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3815−4195.

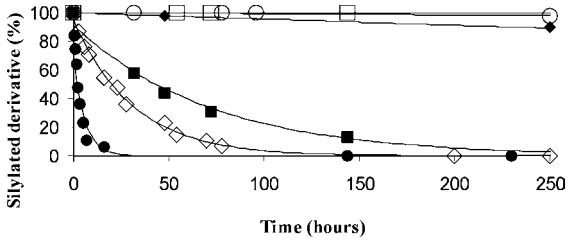


FIGURE 5. Stability studies of the 5′-TBDMS group on 4″-methoxalyl derivative **3** in different solvents: dimethyl sulfoxide (◇), dimethylacetamide (■), acetonitrile (○), chloroform (□), acetone (◆), and dimethyl sulfoxide/water 9:1 (●).

the strongly electron-accepting CF_3 group (**17**)]. Also, 4″-*N*-alkyl TSAO derivatives in which the carbonyl was attached to the 4″-amino group through one (**10**) or two methylenes (**11**) were included to study the influence of a β- or γ-carbonyl group on the hydrolysis rate of the 5′-TBDMS group. As shown in Figure 4, in general, the higher that the electrophilic character of the 4″-carbonyl substituent is, the faster the 5′-TBDMS deprotection. Thus, the 4″-*N*-trifluoroacetylamino derivative **17** proved to be much more unstable than the 4″-*N*-methoxalylamino derivative **3** and the other 4″-N-substituted derivatives **5**−**11** and **17** were much more stable. On the other hand, the higher stability of the 5′-TBDMS group in the 4″-*N*-methoxycarbonylmethylamino derivative **10** or the 4″-*N*-methoxycarbonylethylamino derivative **11** compared with that in the 4″-*N*-methoxalyl derivative **3** or the 4″-*N*-trifluoroacetyl **17** suggests that an amido proton at the 4″-position seems to be involved in the desilylation process.

Studies on the hydrolysis rate of the 5′-TBDMS group in the 4″-*N*-oxalyl-substituted TSAO derivatives **12**−**15** in DMSO showed that the reference 4″-*N*-methoxalyl derivative **3** was the most unstable 4″-*N*-oxalyl analogue, whereas compounds **12**−**15** were much more stable (Figure 1, Supporting Information).

We also studied the influence of the solvent on the stability of the 5′-TBDMS group of **3**. A variety of aprotic solvents differing in polarity and basicity, such as the strongly basic polar DMSO [Donicity number (DN) of 29.8, μ 3.96 (Debye)][16] or DMA (dimethylacetamide with a DN of 27.8, μ 3.80),[16] the moderately basic and polar acetonitrile (DN of 14, μ 3.47),[16] the relatively basic and less polar acetone (DN of 17.0, μ 2.87)[16] as well as the nonpolar chloroform (μ 1.15),[16] were examined. The results are shown in Figure 5. Protic solvents such as water could not be used because of insolubility of the highly lipophilic nucleoside **3** in water. However, mixtures of DMSO/H_2O could be included in the study. As shown in Figure 5, the desilylation rate was strongly solvent dependent. The relative rate of 5′ desilylation of compound **3** showed the order DMSO + H_2O > DMSO > DMA ≫ acetone > acetonitrile. Although mixtures of DMSO/H_2O resulted in the highest silyl hydrolysis rate, addition of water to acetonitrile showed no effect on the silyl hydrolysis rate (data not shown). Interestingly, in chloroform, the compound was very stable (see Figure 5). Thus, basicity and polarity of the solvent seem to play an important role in the desilylation of compound **3**.

(16) (a) Politzer, P.; Murray, J. S. Quantitative treatments of solute/solvent interactions. *Theoretical and computational chemistry*; Elsevier Science B. V.: 1994; Vol. I, pp 183−197. (b) Barthel, J.; Wachter, R.; Gores, H. H. *Modern aspects of electrochemistry*; Plenum: Englewood Cliffs, NJ, 1979; Vol. 13.

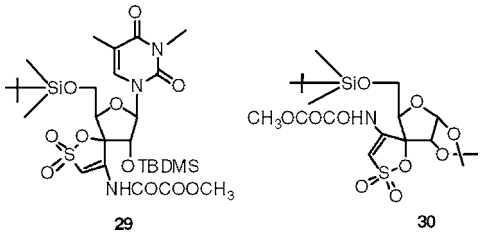


FIGURE 6. Structures of compounds **29** and **30**.

The rate of desilylation of 4″-*N*-methoxalyl compounds **25**−**28** bearing different groups at the 5′-position was also studied (Figure 2, Supporting Information). The hydrolysis rate depends on the sterical hindrance of the silyl group at the 5′-position (**3** > **25** > **26**), being that the 5′-*O*-triisopropil derivative **26** is the most stable silyl analogue. Under the same conditions, the 5′-acyl derivative **27** and the 5′-benzyl analogue **28** were also stable.

The above-described results indicate that the presence of certain substituents at both the 5′- and 4″-positions is required for the desilylation process to occur, which is a process that is favored in certain solvents. These results point to a neighboring group participation of the amide residue at the N-4″ position in the desilylation process. To address this issue, we included in the study the *N*-methoxalyl nucleoside derivative of xylo configuration **29**, in which the intramolecular neighboring group participation is not possible (Figure 6). Compound **29** was synthesized in 53% yield by reaction of *xylo*-TSAO-m^{3}T[4b] with methoxy alyl chloride in the presence of $AlCl_3$ according to our previously described method.[8]

This compound was stable in DMSO solution (Figure 3, Supporting Information) in contrast with the *N*-methoxalyl nucleoside derivative of ribo configuration **3**. The stability of the 5′-TBDMS group in the xylo nucleoside derivative **29**, compared with the prototype ribo nucleoside **3**, points to a direct interaction between the 4″-substituent and the 5′-silyloxy functionalities.

Finally, the behavior of the 1,2-isopropiliden-4′-*N*-methoxalyl sugar analogue **30** (Figure 3, Supporting Information) having a ribo configuration in DMSO solution was also studied. However, in contrast to the ribo nucleoside derivative **3**, the sugar derivative **30** in DMSO solution also proved to be stable. This suggests that the desilylation process may play a role in the neighboring group at the 4″-position and also that the distance between the 4″-amido group and the 5′-silyloxy functionalities may be important.

NMR Conformational Analysis in Solutions of 3 and 30. To assess this point, a comparative conformational analysis of the ribose ring and the exocyclic C4′−C5′ bond, in solution using NMR techniques, of the 4″-*N*-methoxalyl nucleoside **3** and the 4″-*N*-methoxalyl sugar analogue **30** both having a ribo configuration was performed.

The conformation in a solution [$(CD_3)_2CO$] of compounds **3** and **30** was studied by ^{1}H- and ^{13}C NMR spectroscopy. By using the concept of pseudorotation, we obtained information about the geometry of the furanose ring. The existence in these molecules of only one interprotonic coupling constant, $^3J_{H1',H2'}$, precluded the possibility of using the Altona's PSEUROT program,[17] so we used the added information provided by $^3J_{(C,H)}$ values. The procedure developed previously in our group,[18] similar to PSEUROT,[17] relates the vicinal carbon−proton coupling constant values ($J_{H1',H2'}$, $J_{C4',H2'}$, $J_{C4',H1'}$, $J_{C2',H4'}$, and

TABLE 1. Pseudorotational Parameters of 3 and 30

	3		30	
	exptl[a]	calcd	exptl[a]	calcd
$J_{(H1',H2')}$	8.1	7.4	3.9	3.9
$J_{(C4',H2')}$	2.0	0.5	5.8	6.3
$J_{(C4',H1')}$	1.1	2.5	1.5	0.6
$J_{(C2',H4')}$	<0.5	0.7	3.1	3.0
$J_{(C1',H4)}$	<0.5	0.5	<0.5	0.6
P[b]	126		18	
τ_μ[c]	40		50	
rms[d]	1.15		1.02	

[a] Measured in $(CD_3)_2CO$ at 300 MHz. Coupling values are in hertz. Errors for $^3J_{H,H} = \pm 0.1$ Hz and errors for $^3J_{C,H} = \pm 0.2$ Hz. [b] Pseudorotation angle (P). [c] Maximum out of plane pucker. [d] Root mean square.

$J_{C1',H4'}$) to the pseudorotational parameters P and τ_μ. Using this method, we calculated the pseudorotational parameters for **3** and **30** (Table 1). These calculations showed that the sugar ring of nucleoside **3** exhibits a marked preference for the $_1E$ conformation ($P = 126°$ and $\tau_\mu = 40$), and the furanose ring of the *N*-methoxalyl sugar analogue **30** exhibits a marked preference for the ^3E conformation ($P = 18°$ and $\tau_\mu = 50$).

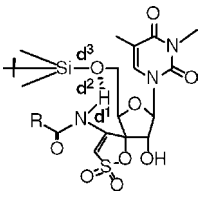
In addition, the populations of the conformers of the exocyclic hydroxymethyl groups were determined from $^3J_{(H4,H5proR)}$ and $^3J_{(H4,H5proS)}$, using sets of values of the couplings for the pure gauche+, gauche−, and trans conformations calculated from the Karplus equation parametrized by Altona et al.[19] The results (Table 1, Supporting Information) showed the prevalence of the gauche+ conformation in both compounds.

Using the major conformers for **3** and **30** as the starting geometry, we performed a geometry optimization by semiempirical methods. According to the AM1 calculations, the distance between the amide group at the 4″-position of the spiro moiety and the 5′-TBDMS group in the nucleoside **3** ($d = 2.25$ Å) is shorter than that in the *N*-methoxalyl sugar analogue **30** ($d = 2.72$ Å), depending on the furanose ring conformation of each compound (Figure 4, Supporting Information).

However, for other 4″-N-substituted nucleoside derivatives **5**−**15**, the $J_{H1',H2'}$ coupling constants were very similar and were found to be in the range 6.5−8.5 Hz.[8] In addition, a positive NOE between H-1′ and H-4′ was observed in all the compounds (data not shown). These experimental data were indicative of a marked preference for conformations in the eastern part of the pseudorotational circuit.[20] Thus, no significant conformational differences of the furanose ring in solution between the 4″-*N*-methoxalyl nucleoside **3** and the other 4″-N-substituted nucleoside analogues **5**−**15** were observed. Therefore, geometric and/or electronic effects that might account for the observed differences in the 5′ desilylation rate were next investigated using theoretical calculations.

Theoretical Calculations. A comparative theoretical study using DFT (density functional theory) calculations was carried out with the 4″-*N*-trifluoroacetyl derivative **17**, the 4″-*N*-methoxalyl prototype **3**, and the 4″-*N*-acetyl derivative **5** which represent selected examples of the most, intermediate, and least labile systems, respectively, bearing 4″-carbonyl substituents of a different nature. First, the B3LYP/3-21G(d) whole structures were modeled by using as starting models both the NMR results and the AM1 calculations (any attempt to use the B3LYP/6-31G(d) method was discharged because of huge CPU requirements). A subsequent reduction of complexity was accomplished in the above-mentioned derivatives by replacing the 2′-TBDMS with a hydroxyl (structures **17a**, **3a**, and **5a** (Table 2) bearing 4″-amide functions substituted with CF_3, $COOCH_3$, and CH_3 groups, respectively), whereas the DFT calculation was changed from B3LYP/3-21G(d) to B3LYP/6-31G(d). The results obtained were comparable at the two different calculation levels.

TABLE 2. Selected Optimized Geometric Parameters of Simplified 4″-*N*-Acylamino Compounds 17a, 3a, and 5a Using B3LYP/6-31G(d) Calculations



compound	R	$d^1_{4''-N-H}$ (Å)	$d^2_{4''-NH-5'-OSi}$ (Å)	$d^3_{5'-O-Si}$ (Å)
17a	CF_3	1.046	1.649	1.708
3a	$COOCH_3$	1.043	1.713	1.703
5a	CH_3	1.034	1.735	1.700

Table 2 shows the most relevant geometrical parameters of simplified compounds **17a**, **3a**, and **5a** that may account for the observed desilylation process. These are the distance of the 4″-N−H bond (d^1) that gives a measure of the acidity of this amido group, the distance between the 4″-NH and 5′-OSi (d^2) functionalities that could show the degree of interaction between the 4″-amido hydrogen and the nucleophilic 5′-oxygen, and the distance of the 5′-O−Si bond (d^3), which could be related to the lability of this bond.

The results showed that a reasonable correlation between desilylation and geometric parameters could be found in the three selected examples. Thus, as shown in Table 2, the higher electrophilic character of the 4″-carbonyl substituent (CF_3 > $COOCH_3$ > CH_3) in compounds **17a**, **3a**, and **5a** is observed as a larger distance of the 4″-N−H bond (d^1), a shorter distance between the hydrogen of the 4″-amido group and the oxygen of the 5′-silyloxy functionality (d^2), and a larger distance of the 5′-O−Si bond (d^3). These facts may reflect a higher acidity of the amide hydrogen (it means a more electronically "naked" proton), which could then interact in a more favorable way with the nucleophilic 5′-oxygen, and a higher lability of the 5′-O−Si bond. In this way, the trifluoroacetyl derivative **17a** (bearing the most electroattracting substituent) was the molecule that showed the largest N−H distance, the shorter intramolecular distance between the 4″-NH and the 5′-OSi, and the longest 5′-O−Si distance and, therefore, a more labile bond in concordance with the experimental results.

Under these assumptions and for simplicity, complex model studies between the further simplified *N*-amido-substituted sultones **17b**, **3b**, and **5b** (Figure 7) and a methoxytrimethylsilane moiety were carried out using ab initio B3LYP/6-31G+(d) calculations. The results are shown in Figure 7.

In the optimized structures, the $(CH_3)_3Si-O-CH_3$ plane of the silicon compound takes a perpendicular position with respect

(17) (a) Alvarez, R.; Jimeno, M. L.; Gil, T.; Pérez-Pérez, M. J.; Camarasa, M. J. *Nucleosides Nucleotides* **1997**, *16*, 1033−1036. (b) Alvarez, R.; Jimeno, M. L.; Gago, F.; Balzarini, J.; Pérez-Pérez, M. J.; Camarasa, M. J. *Antiviral Chem. Chemother.* **1998**, *9*, 333−340.

(18) de Leeuw, F. A. A. M.; van Beuzekom, A. A.; Altona, C. *J. Comput. Chem.* **1983**, *4*, 438−448.

(19) Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783−2792.

(20) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.

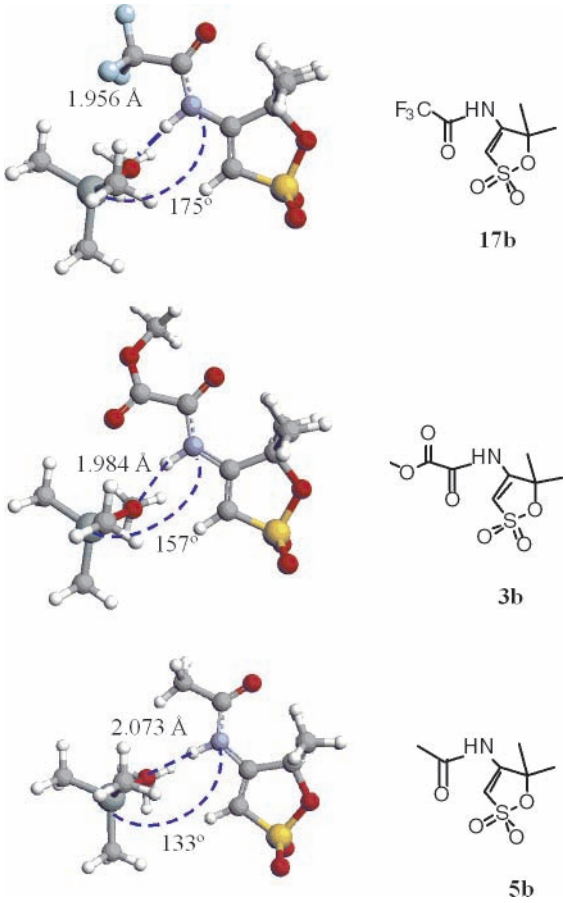


FIGURE 7. Complex model study between the further simplified *N*-amido-substituted sultones **17b**, **3b**, and **5b** and a methoxytrimethylsilane moiety.

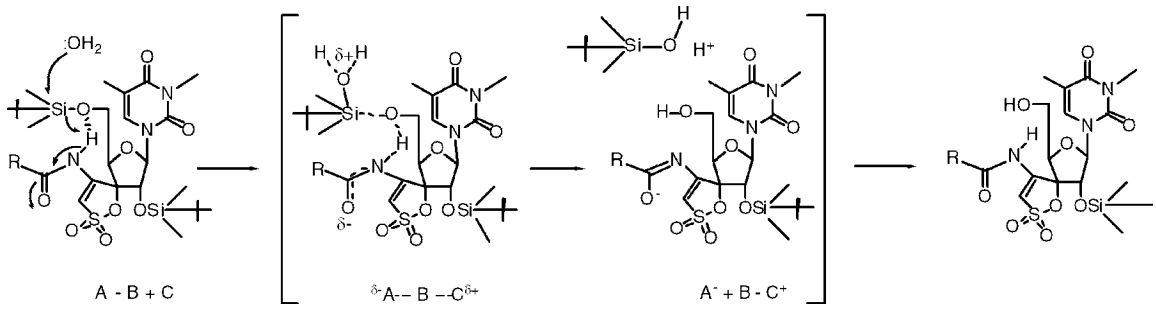
to the plane of the amide−sultone group to minimize steric interactions, whereas the amide group stays coplanar with the sultone ring. The hydrogen-bond strength in this position is maximized for the *N*-trifluoroacetyl derivative **17b** (the distance is 1.956 Å, and the angle of N−H−O is 175°). For the *N*-acetyl derivative **5b**, this interaction is much weaker (the distance is 2.073 Å, and the angle of N−H−O is 133°). In particular, this closer angle implies that transmission of the electronic density from the oxygen to the 4″-NH is less efficient and, therefore, that oxygen is more linked to the silicon atom resulting in a more stable 5′-O−Si bond.

Proposed Mechanism of Desilylation. Finally, all the above-mentioned results led us to propose the mechanism of desilylation of 4″-acylamino TSAO derivatives, depicted in Scheme 3. This mechanism involves a neighboring group participation of the 4″-NH amide proton in the unexpectedly fast hydrolysis of the 5′-TBDMS group. The interaction between an acidic 4″-amido proton and the nucleophilic oxygen of the 5′-silyloxy functionality, according to the theoretical studies mentioned above, may result in a longer distance of the 5′-O−Si bond and a higher lability of this bond toward nucleophilic attack of water assisted by molecules of solvent. Moreover, this 4″-NH−5′-OSi interaction would also enhance charge delocalization throughout the 4″-carbonyl group to facilitate desilylation. In the reaction, the charge distribution of the reaction species may change from "A−B + C" to "$A^- + B-C^+$" through a transition state "$[A^{d-}\cdots B\cdots C^{d+}]$" where A−B and C refer to RO-TBDMS and H_2O, respectively. Thus, it is likely that the desilylation reaction should proceed faster in polar basic solvents such as DMSO or DMA that may favor the proposed transition state. In fact, it could be hypothesized that the effect of these types of solvents, with high dipolar moments and high dielectric constant values,[16] could enhance the ability of the silicon group to interact with nucleophilic substances, i.e., H_2O, to break the O−Si bond. In this context, a quantum mechanical (QM) simulation was carried out to determine the influence of the solvent on this process. The self-consistent reaction field (SCFR), a solvent model employed to model molecular systems in solution, was highly CPU costly for the TSAO molecules, and therefore, an approximation was used. In this context, the interaction zero-point (zp) electronic energy was calculated among a trimethylsilyl cation and several solvents. The obtained values of −28.36, −10.02, and −4.92 kcal/mol for DMSO, acetone, and chloroform, respectively, confirm that the ability of DMSO to interact with the positive silicon atom is much higher than that for other solvents. Thus, a plausible explanation is that the 4″-NH−5′-O- intramolecular interaction mentioned above weakened the 5′-O−Si bond. If, in addition, the solvent molecules, with high dipolar moments and dielectric constant values, enclosed the *tert*-butyldimethylsilyl moiety further weakening the energy of this bond, these two factors together may facilitate the water attack and the final hydrolysis of the 5′-O−Si bond experimentally observed in DMSO or related solvents.

Conclusions

Here, we investigated, in detail, the unprecedented lability of the 5′-TBDMS group on 4″-acylamino TSAO derivatives in DMSO solution. Stability studies in DMSO solution of a variety of 4″-substituted TSAO derivatives showed that the presence of an acidic 4″-amido proton, a TBDMS group at the 5′-position, and a ribo configuration of the nucleoside are key structural requirements for the desilylation to occur. The polarity and basicity of the solvent also play a crucial role in the "spontane-

SCHEME 3



ous" release of the 5′-TBDMS group. Theoretical studies suggest a correlation among the acidity of the 4″-amido proton, which depends on the nature of the 4″-carbonyl substituent, the intramolecular 4″-NH−5′-OSi distance, and the distance of the 5′-O−Si bond and, therefore, the lability of the 5′-TBDMS group. Thus, the highest acidity of the 4″-amide proton in the 4″-trifluoroacetyl derivative **17** correlates with the shortest intramolecular 4″-NH−5′-OSi distance and the longest 5′-O−Si distance, thus resulting in the most unstable compound. All the results prompted us to propose a silyl hydrolysis mechanism involving neighboring group participation of the acidic 4″-amide proton further assisted by certain solvents. Our results complement scarce previous findings that suggested that neighboring group participation between TBDMS and phosphate[10] or NH and carboxyl groups[11] is involved in exceptionally mild desilylation of TBDMS groups. Taken together, all these studies suggest a generality for the enhanced hydrolytic cleavage of a silyloxy functionality by neighboring group participation of acidic hydrogen atoms of a different nature.

Experimental Section

Chemical and NMR Procedures. Microanalyses were obtained with a CHN-O-RAPID instrument. Electrospray mass spectra were measured on a quadropole mass spectrometer equipped with an electrospray source (LC/MS HP 1100). ^{1}H-1D-NMR spectra were recorded with a spectrometer operating at 300 MHz with Me_4Si as the internal standard. ^{13}C-1D-NMR spectra were recorded with a spectrometer operating at 75 MHz with Me_4Si as the internal standard. 2D spectra were recorded on a Unity 500 instrument working at 499.88 MHz (^{1}H) and 125.71 MHz (^{13}C). 2D inverse proton-detected heteronuclear shift correlation spectra, g-HSQC, and g-HMBC were obtained with the following conditions: Data were collected in a 4096 × 128 matrix with a spectral width of 8000 Hz in the proton domain and 25 000 Hz in the carbon domain and processed in a 4096 × 512 matrix. The g-HSQC experiment was optimized for a one-bond heteronuclear coupling constant of 145 Hz. The g-HMBC experiment was optimized for long-range coupling constants of 8 Hz. Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F_{254}. Separations on silica gel were performed by preparative centrifugal circular thin-layer chromatography (CCTLC) on a Chromatotron (Kiesegel 60 PF_{254} gipshaltig, layer thickness of 1 mm, flow rate of 5 mL/min). Flash column chromatography was performed with silica gel 60 (230−400 mesh). Analytical HPLC was carried out on a 484 system using a μBondapak C_{18} (3.9 × 300 mm: 10 mm). Isocratic conditions were used: mobile phase CH_3CN/H_2O (0.05% TFA); flow rate of 1 mL/min; detection, UV (254 nm). All retention times are quoted in minutes.

Acetonitrile, 1,2-dichloromethane, and 1,2-dichloroethane were dried by refluxing over calcium hydride.

[1-[2′-*O*-(*tert*-Butyldimethylsilyl)-*β*-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-methoxalylamino-1″,2″-oxathiole-2″,2″-dioxide) (4). A solution of compound **3** (20 mg, 0.029 mmol) in DMSO/H_2O, 9:1 (2 mL), was stirred for 2 h at room temperature. The DMSO was liophilized, and the residue was purified by CCTLC on the Chromatotron (dichloromethane/methanol, 10:1) to give **4** (13 mg, 80%). ^{1}H NMR [$(CD_3)_2CO$] δ: 0.70 (s, 9H, *t*-Bu), 1.90 (d, 3H, J = 1.1 Hz, CH_3-5), 3.18 (s, 3H, CH_3-3), 3.87 (s, 3H, CO_2-CH_3), 3.79−3.91 (m, 2H, 2H-5′), 4.41 (s, 1H, H-4′), 4.60 (d, 1H, $J_{1',2'}$ = 7.8 Hz, H-2′), 5.28 (bs, 1H, OH), 6.08 (d, 1H, H-1′), 7.57 (s, 1H, H-6), 7.93 (d, 1H, H-3″), 11.60 (bs, 1H, NH-4″).

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-*β*-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-amino-3″-trifluoroacetyl-1″,2″-oxathiole-2″,2″-dioxide) (16). To a solution of TSAO-m^{3}T (**2**) (120 mg, 0.2 mmol) in dry 1,2-dichloroethane (14 mL) was added trifluoroacetyl anhydride (56 mL, 0.4 mmol) and DMAP (248 mg, 2.04 mmol). The mixture was stirred in a pressure tube for 0.5−24 h at 80 °C. The reaction mixture was allowed to cool to room temperature. Then, dichloromethane was added and the solution was successively washed with 1 N HCl (1 × 20 mL), water (1 × 20 mL), and brine (1 × 20 mL). The organic phase was dried (Na_2SO_4), filtered, and evaporated to dryness. The final residue was purified by CCTLC on the Chromatotron (hexane/ethyl acetate, 3:1) to give 0.016 g (12%) of **16** as a white amorphous solid. ^{1}H NMR [$(CD_3)_2CO$] δ: 0.78, 0.87 (2s, 18H, 2*t*-Bu), 1.90 (s, 3H, CH_3-5), 3.25 (s, 3H, CH_3-3), 4.07 (dd, 1H, $J_{4',5'a}$ = 4.9, $J_{5'a,5'b}$ = 12.7 Hz, H-5′a), 4.13 (dd, 1H, $J_{4',5'a}$ = 3.4, $J_{5'a,5'b}$ = 12.7 Hz, H-5′b), 4.29 (dd, 1H, H-4′), 5.02 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-2′), 6.08 (d, 1H, H-1′), 7.49 (s, 1H, H-6), 9.16 (s, 1H, NHa), 9.83 (s, 1H, NHb). ^{13}C NMR [$(CD_3)_2CO$] δ: 13.1 (CH_3-5), 18.3, 18.7 [$(CH_3)_2$−*C*−Si], 25.5, 26.1 [$(CH_3)_2$−C−Si], 28.0 (CH_3-3), 61.5 (C-5′), 76.8 (C-2′), 85.2 (C-4′), 88.4 (C-1′), 90.4 (C-3′), 103.6 (C-3″), 111.6 (C-5), 116.6 (c, ^1J = 287.6 Hz, CF_3), 134.6 (C-6), 152.0 (C-2), 163.2 (C-4), 167.2 (C-4″), 173.0 (c, ^2J = 38.9 Hz, *C*O−CF_3). MS (ESI$^+$): *m*/*z* 701 [M + 1]$^+$. Anal. Calcd for $C_{27}H_{44}F_3N_3O_9SSi_2$: C, 46.33; H, 6.34; N, 6.00. Found: C, 46.19; H, 6.25; N, 5.90.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-*β*-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-trifluoroacetylamino-1″,2″-oxathiole-2″,2″-dioxide) (17). A solution of TSAO-m^{3}T (**2**) (120 mg, 0.2 mmol) in dry dichloromethane (14 mL) was reacted with trifluoroacetyl anhydride (56 mL, 0.4 mmol) in the presence of 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) polymer-bound (126 mg, 1.0 mmol) for 30 min at 0 °C. The resin was filtered, and the filtrate was evaporated to dryness. The final residue was purified by CCTLC on the Chromatotron (dichloromethane/methanol, 10:1) to give 0.18 g of **17** (83%) as a white amorphous solid. ^{1}H NMR [$(CD_3)_2CO$] δ: 0.81, 0.89 (2s, 18H, 2*t*-Bu), 2.03 (s, 3H, CH_3-5), 3.28 (s, 3H, CH_3-3), 4.08 (dd, 2H, $J_{4',5'a}$ = 4.1, $J_{5'a,5'b}$ = 8.5 Hz, H-5′), 4.29 (t, 1H, H-4′), 5.08 (d, 1H, $J_{1',2'}$ = 6.8 Hz, H-2′), 5.67 (d, 1H, H-1′), 7.60 (s, 1H, H-6), 10.96 (s, 1H, NH). ^{13}C NMR [$(CD_3)_2CO$] δ: 12.3 (CH_3-5),17.8, 18.6 [$(CH_3)_2$−*C*−Si], 25.0, 25.7 [$(CH_3)_2$−C−Si], 27.34 (CH_3-3), 61.22 (C-5′), 73.11 (C-2′), 83.44 (C-4′), 90.18 (C-3′), 94.12 (C-1′), 110.2 (C-3″), 111.2 (C-5), 129.6 (c, ^1J = 266.7 Hz, CF_3), 137.2 (C-6), 141.0 (C-4″), 151.6 (C-2), 156.2 (c, ^2J = 83.8 Hz, *C*O−CF_3), 162.5 (C-4). MS (ESI$^+$): *m*/*z* 701 [M + 1]$^+$. Anal. Calcd for $C_{27}H_{44}F_3N_3O_9SSi_2$: C, 46.33; H, 6.34; N, 6.00. Found: C, 46.21; H, 6.15; N, 5.86. HPLC: RT = 14.92 min (70:30).

[1-[2′-*O*-(*tert*-Butyldimethylsilyl)-*β*-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-trifluoroacetylamino-1″,2″-oxathiole-2″,2″-dioxide) and O$^{5'}$,4″-Cyclo[1-[2′-*O*-(*tert*-butyldimethylsilyl)-*β*-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-trifluoroacetylamino-1″,2″-oxathiolane-2″,2″-dioxide) (18 and 19). A solution of **17** (140 mg, 0.2 mmol) in DMSO (14 mL) was stirred at room temperature and monitored by HPLC until complete disappearance of the starting material. The DMSO was liophilized, and the residue was purified by CCTLC on the Chromatotron (dichloromethane/methanol, 20:1). From the fastest moving fractions, compound **18** (0.028 g, 29%) was identified as a white foam. ^{1}H NMR [$(CD_3)_2CO$] δ: 0.80 (s, 9H, *t*-Bu), 1.89 (s, 3H, CH_3-5), 3.27 (s, 3H, CH_3-3), 4.10 (m, 2H, H-5′), 4.49 (t, 1H, J = 1.2 Hz, H-4′), 5.02 (d, 1H, $J_{1',2'}$ = 8.1 Hz, H-2′), 6.01 (d, 1H, H-1′), 7.80 (s, 1H, H-3″), 7.89 (s, 1H, H-6), 11.45 (bs, 1H, NH). ^{13}C−RMN [$(CD_3)_2CO$] δ: 13.1 (CH_3-5), 18.3 [$(CH_3)_2$−*C*−Si], 25.6 [$(CH_3)_2$−C−Si], 28.0 (CH_3-3), 61.6 (C-5′), 75.0 (C-2′), 85.6 (C-4′), 90.1 (C-3′), 95.7 (C-1′), 111.6 (C-3″), 112.1 (C-5), 136.2 (C-6), 152.5 (C-2), 163.3 (C-4). MS (ESI$^+$): *m*/*z* 586 [M + 1]$^+$. Anal. Calcd for $C_{21}H_{30}F_3N_3O_9SSi$: C, 43.07; H, 5.16; N, 7.18. Found: C, 42.85; H, 4.95; N, 6.98. HPLC: RT = 2.39 min (65:35). From the slowest moving band, 60 mg (51%) of **19** was isolated as a white foam. ^{1}H NMR [$(CD_3)_2CO$] δ: 0.87 (s, 9H, *t*-Bu), 1.89 (s, 3H, CH_3-5), 3.27 (s, 3H, CH_3-3), 4.28 (dd, 1H, $J_{4',5'a}$ = 1.97, $J_{5'a,5'b}$ = 11.0 Hz, H-5′), 4.33 (AB system, 2H, J = 14.6 Hz, H-3″), 4.53 (dd, 1H, $J_{4',5'a}$ = 4.9, $J_{5'a,5'b}$ = 11.0 Hz, H-5′), 4.87 (dd, 1H, H-4′), 4.90 (d, 1H, $J_{1',2'}$ = 5.4 Hz, H-2′), 5.71 (d, 1H, H-1′), 7.59 (s, 1H, H-6), 9.79 (bs,

1H, NH). ^{13}C NMR [$(CD_3)_2CO$] δ: 12.3 (CH_3-5), 17.9 [$(CH_3)_2-C-$Si], 25.3 [$(CH_3)_2-C-$Si], 27.3 (CH_3-3), 53.6 (C-3″), 71.7 (C-5′), 72.4 (C-2′), 84.1 (C-4′), 96.8 (C-1′), 97.5 (C-4″), 98.5 (C-3′), 111.0 (C-5), 137.1 (C-6), 151.5 (C-2), 162.7 (C-4). MS (ESI$^+$): *m*/*z* 586 [M + 1]$^+$. Anal. Calcd for $C_{21}H_{30}F_3N_3O_9SSi$: C, 43.07; H, 5.16; N, 7.18. Found: C, 42.91; H, 5.00; N, 7.00. HPLC: RT = 3.19 min (65:35).

[1-[2′-*O*-(*tert*-Butyldimethylsilyl)-5′-*O*-(triisopropilsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (21). A solution of TSAO-m^{3}T 5′-OH[4b] (200 mg, 0.4 mmol), triisopropilsilyl chloride (0.29 mL, 1.36 mmol), and DMAP (166 mg, 1.36 mmol) in dry acetonitrile (40 mL) was stirred at 80 °C for 10 h, and the solvent was evaporated at dryness. The residue was redissolved in ethyl acetate and was succesively washed with 1 N HCl (1 × 20 mL), water (1 × 20 mL), and brine (1 × 20 mL). The organic phase was dried (Na_2SO_4), filtered, and evaporated. The final residue was purified in the Chromatotron (hexane/ethyl acetate, 3:1) to yield 126 mg (50%) of **21** as a white foam. ^{1}H NMR [$(CD_3)_2CO$] δ: 0.82, 1.11, 1.13 (s, 27H, *t*-Bu, *i*-Pr), 1.11, 1.13 (s, 18H, 6CH_3), 1.15 (m, 3H, 3CH), 1.97 (s, 3H, CH_3-5), 3.27 (s, 3H, CH_3-3), 4.17 (dd, 2H, $J_{4',5'a}$ = 8.5, $J_{5'a,5'b}$ = 12.1 Hz, H-5), 4.38 (t, 1H, H-4′), 4.66 (d, 1H, $J_{1',2'}$ = 8.1 Hz, H-2′), 5.79 (s, 1H, H-3″), 6.09 (d, 1H, H-1′), 6.53 (s, 1H, NH_2), 7.51 (s, 1H, H-6). ^{13}C NMR [$(CD_3)_2CO$] δ: 11.7 (CH_3-5), 12.3 [$(CH_3)_2-CH-$Si], 17.6 [$(CH_3)_2-C-$Si], 25.0 [$(CH_3)_3-C-$Si], 30.0 (CH_3-3), 62.7 (C-5′), 74.8 (C-2′), 84.7 (C-4′), 87.5 (C-1′), 91.7 (C-3″), 110.6 (C-5), 133.9 (C-6), 151.6 (C-2), 162.6 (C-4″). MS (ESI$^+$): *m*/*z* 646 [M + 1]$^+$. Anal. Calcd for $C_{28}H_{51}N_3O_8SSi_2$: C, 52.06; H, 7.96; N, 6.51. Found: C, 52.01; H, 7.88; N, 6.12.

[1-[2′-*O*-(*tert*-Butyldimethylsilyl)-5′-*O*-(*tert*-hexyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-methoxalylamino-1″,2″-oxathiole-2″,2″-dioxide) (25). According to the acylation method previously described by us,[8] a solution of [1-[2′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(*tert*-hexyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (**20**)[12] (120 mg, 0.2 mmol) in dry 1,2-dichloroethane (4 mL) was treated with methyl oxalyl chloride (22 μL, 0.24 mmol) and aluminum chloride (32 mg, 0.24 mmol) in the presence of 4-Å molecular sieves (1.8 g). The reaction mixture was stirred for 1 h at room temperature and then was quenched with ice water (10 mL). The mixture was stirred for an additional hour and filtered through a Celite pad. The organic layer was separated, washed several times with water (3 × 10 mL), dried (Na_2SO_4), filtered, and evaporated to dryness. The final residue obtained after the workup was purified in the Chromatotron (hexane/ethyl acetate, 2:1) to give 98 mg (70%) of **25** as a white foam. ^{1}H NMR [$(CD_3)_2CO$] δ: 0.78, 0.86 (s, 21H, 3*t*-Bu), 1.93 (s, 3H, CH_3-5), 3.28 (s, 3H, CH_3-3), 3.97 (s, 3H, CH_3O), 4.07 (dd, 1H, $J_{4',5'a}$ = 4.2, $J_{5'a,5'b}$ = 12.5 Hz, H-5′a), 4.17 (dd, 1H, $J_{4',5'a}$ = 4.6, $J_{5'a,5'b}$ = 12.7 Hz, H-5′b), 4.35 (t, 1H, H-4′), 4.97 (d, 1H, $J_{1',2'}$ = 7.6 Hz, H-2′), 5.94 (d, 1H, H-1′), 7.54 (s, 1H, H-6), 7.73 (s, 1H, H-3″), 10.38 (s, 1H, NH). ^{13}C NMR [$(CD_3)_2CO$] δ: 11.4 (CH_3-5), 16.8 [$(CH_3)_2-CH-$C], 17.2, 19.2 [$(CH_3)_2-C-$Si], 24.7 [$(CH_3)_2-C-$Si], 26.5 (CH_3-3), 60.4 (C-5′), 72.6 (C-2′), 83.0 (C-4′), 88.9, 90.7 (C-3′, C-1′), 108.4, 109.9 (C-5, C-3″), 134.1 (C-6), 139.8 (C-4″), 150.5 (C-2), 155.2, 158.6 (*CO*−*CO*), 161.7 (C-4). MS (ESI$^+$): *m*/*z* 705 [M + 1]$^+$. Anal. Calcd for $C_{28}H_{47}N_3O_{11}SSi_2$: C, 48.78; H, 6.87; N, 6.09. Found: C, 48.56; H, 6.72; N, 5.96. HPLC: RT = 19.72 min (65:35).

[1-[2′-*O*-(*tert*-Butyldimethylsilyl)-5′-*O*-(triisopropilsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-methoxalylamino-1″,2″-oxathiole-2″,2″-dioxide) (26). Compound **21** (120 mg, 0.2 mmol) was treated with methyl oxalyl chloride (52 μL, 0.56 mmol) and DMAP (146 mg, 1.20 mmol) in dry 1,2-dichloroethane (16 mL) in a pressure tube for 0.5 h at 80 °C. The reaction was stirred at 80 °C for 30 min. Then, 1,2-dichloroethane (20 mL) was added and the solution was successively washed with 1 N HCl (1 × 20 mL), water (1 × 20 mL), and brine (1 × 20 mL). The organic phase was dried (Na_2SO_4), filtered, and evaporated to dryness. The final residue was purified in the Chromatotron (hexane/ethyl acetate, 3:1). From the fastest moving fractions, 18 mg (13%) of **26** was isolated as a white foam. ^{1}H NMR [$(CD_3)_2CO$] δ: 0.84 (s, 9H, *t*-Bu), 1.09 (m, 18H, 3*i*-Pr), 1.93 (s, 3H, CH_3-5), 3.32 (s, 3H, CH_3-3), 3.99 (s, 3H, CH_3O), 4.12 (dd, 1H, $J_{4',5'a}$ = 6.2, $J_{5'a,5'b}$ = 12.4 Hz, H-5′a), 4.21 (dd, 1H, $J_{4',5'a}$ = 4.7, $J_{5'a,5'b}$ = 12.4 Hz, H-5′b), 4.37 (dd, 1H, H-4′), 5.85 (d, 1H, $J_{1',2'}$ = 6.8 Hz, H-2′), 5.16 (d, 1H, H-1′), 7.64 (s, 1H, H-6), 7.74 (s, 1H, H-3″), 10.19 (s, 1H, NH). ^{13}C NMR [$(CD_3)_2CO$] δ: 11.3 (CH_3-5), 15.7 [$(CH_3)_2-CH-$Si], 17.3 [$(CH_3)_2-C-$Si], 25.9 [$(CH_3)_3-C-$Si], 30.3 (CH_3-3), 63.5 (C-5′), 70.2 (C-2′), 85.7 (C-4′), 88.6, 91.2 (C-3′, C-1′), 109.5, 110.4 (C-5, C-3″), 120.6 (C-6), 140.9 (C-4″), 149.8 (C-2), 155.5, 160.6 (*CO*−*CO*), 161.3 (C-4). MS (ESI$^+$): *m*/*z* 733 [M + 1]$^+$. Anal. Calcd for $C_{31}H_{53}N_3O_{11}SSi_2$: C, 50.86; H, 7.30; N, 5.74. Found: C, 50.72; H, 7.12; N, 5.39. HPLC: RT = 14.02 min (60:40). From the slowest moving fractions, 36 mg (30%) of unreacted starting material was identified.

[1-[5′-*O*-Acetyl-2′-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-methoxalylamino-1″,2″-oxathiole-2″,2″-dioxide) (27). [1-[2′-*O*-(*tert*-Butyldimethylsilyl)-5′-*O*-acetyl−β−D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)[4b] (**22**) (0.05 g, 0.085 mmol) was treated with methyl oxalyl chloride (9 μL, 0.2 mmol) and aluminum chloride (13.3 mg, 0.1 mmol) following the acylation procedure described for compound **25**. The final residue was purified in the Chromatotron (hexane/ethyl acetate, 3:1) to give 27.2 mg (48%) of **27** as a white amorphous solid. ^{1}H NMR [$(CD_3)_2CO$] δ: 1.89 (s, 3H, CH_3-5), 3.16 (s, 3H, CH_3-3), 3.72 (s, 3H, CH_3O), 4.51 (s, 3H, H-5′, H-4′), 5.10 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-2′), 5.98 (d, 1H, H-1′), 7.60 (s, 1H, H-6), 7.94 (s, 1H, H-3″), 10.52 (s, 1H, NH). Anal. Calcd for $C_{24}H_{35}N_3O_{13}SSi$: C, 45.49; H, 5.57; N, 6.63; S, 5.06. Found: C, 45.32; H, 5.39; N, 6.50; S, 4.83.

[1-[5′-*O*-Benzyl-2′-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-methoxalylamino-1″,2″-oxathiole-2″,2″-dioxide) (28). A solution of nucleoside **23**[12] (120 mg, 0.2 mmol) in dry 1,2-dichloroethane (4 mL) was acylated with methoxalyl chloride (22 μL, 0.24 mmol) and aluminum trichloride (32 mg, 0.24 mmol) in the presence of 4-Å molecular sieves (1.8 g) according to the procedure described for compound **25**. The reaction mixture was stirred for 24 h at room temperature. The final residue was purified in the Chromatotron (hexane/ethyl acetate, 2:1) to give 66 mg (50%) of **28** as a white foam. ^{1}H NMR [$(CD_3)_2CO$] δ: 0.79 (s, 3H, *t*-Bu), 1.52 (s, 3H, CH_3-5), 3.22 (s, 3H, CH_3-3), 3.83 (s, 3H, CH_3O), 4.02 (c, 1H, $J_{5'a,5'b}$ = 9.2 Hz, H-5′), 4.55 (s, 1H, H-4′), 4.79 (d, 1H, $J_{1',2'}$ = 9.1 Hz, H-2′), 4.80 (s, 2H, CH_2), 6.33 (d, 1H, H-1′), 7.45 (m, 5H, Ph), 7.62 (s, 1H, H-3″), 7.82 (s, 1H, H-6), 10.84 (bs, 1H, NH). ^{13}C NMR [$(CD_3)_2CO$] δ: 13.1 (CH_3-5), 19.5, 19.6 [$(CH_3)_2-C-$Si], 24.6, 27.8 [$(CH_3)_2-C-$Si], 29.0 (CH_3-3), 49.5 (OCH_2), 55.8 (OCH_3), 61.2 (C-5′), 73.6 (C-2′), 84.2, 89.1 (C-1′, C-4′), 92.3 (C-3′), 111.5 (C-3″), 113.2 (C-5), 127.3, 128.4, 131.2 (Ar), 134.1 (C-6), 135.0 (Ar), 139.6 (C-4″), 152.4 (C-2), 155.8, 159.2 (*CO*−*CO*), 163.2 (C-4). MS (ESI$^+$): *m*/*z* 667 [M + 1]$^+$. Anal. Calcd for $C_{29}H_{39}N_3O_{11}SSi$: C, 52.32; H, 5.90; N, 6.31. Found: C, 52.00; H, 5.75; N, 6.25.

[1-[2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-xilofuranosyl]-3-*N*-(methyl)thymine]-3′-spiro-5″-(4″-methoxalylamino-1″,2″-oxathiole-2″,2″-dioxide) (29). According to the acylation method described for compound **25**, a solution of methoxalyl chloride (22 μL, 0.24 mmol) and $AlCl_3$ (16 mg, 0.12 mmol) in dry 1,2-dichloroethane (2 mL) was added dropwise to a solution of [1-[2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-xilofuranosyl]-3-*N*-(methyl)-thymine]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide)[4b] (120 mg, 0.2 mmol) in dry 1,2-dichloroethane (4 mL) in the presence of 4-Å molecular sieves (0.9 g). The final residue after workup was purified by CCTLC on the Chromatotron (hexane/ethyl acetate, 2:1) to give 70 mg (53%) of **29** as a white foam. ^{1}H NMR [$(CD_3)_2CO$] δ: 0.82, (s, 9H, TBDMS), 0.86 (s, 9H, TBDMS), 1.92 (d, 3H, CH_3-5), 3.26 (s, 3H, CH_3-3), 3.94 (s, 3H, CO_2-CH_3), 3.96 (d,

2H, $J_{4',5'a}$ = 6.0 Hz, 2H-5′), 4.69 (d, 1H, $J_{1',2'}$ = 3.9 Hz, H-2′), 4.93 (t, 1H, H-4′), 6.26 (d, 1H, H-1′), 7.41 (s, 1H, H-6), 7.65 (s, 1H, H-3″), 9.51 (bs, 1H, NH). MS (ESI$^+$): *m*/*z* 691 [M + 1]$^+$. Anal. Calcd for $C_{28}H_{47}N_3O_{11}SSi_2$: C, 48.74; H, 6.87; N, 6.09. Found: C, 48.59; H, 6.62; N, 5.79.

[5-*O*-(*tert*-Butyldimethylsilyl)-1,2-*O*-isopropiliden-α-D-ribofuranosyl]-3-spiro-5′-(4′-methoxalylamino-1″,2″-oxathiole-2″,2″-dioxide) (30). To a solution of [5-*O*-(*tert*-butyldimethylsilyl)-1,2-*O*-isopropiliden-α-D-ribofuranosyl]-3-spiro-5′-(4′-amino-1″,2″-oxathiole-2″,2″-dioxide)[21] (80 mg, 0.2 mmol) in dry acetonitrile (2 mL), (dimethylamino)pyridine (73 mg, 0.6 mmol) and methyl oxalyl chloride (26 μL, 0.28 mmol) were added. The reaction was stirred at 80 °C for 30 min. Then, 1,2-dichloroethane (20 mL) was added and the solution was successively washed with 1 N HCl (1 × 20 mL), water (1 × 20 mL), and brine (1 × 20 mL). The organic phase was dried (Na_2SO_4), filtered, and evaporated to dryness. The final residue was purified by CCTLC on the Chromatotron (hexane/ethyl acetate, 2:1) to give 0.050 g (51%) of **30** as a white foam. ^{1}H NMR [$(CD_3)_2CO$] δ: 0.86 (1s, 18H, 2*t*-Bu), 1.35, 1.58 (s, 6H, $2CH_3$−C), 3.95 (s, 3H, CH_3−OCO), 3.99 (d, 2H, $J_{4,5a}$ = 3.9, $J_{5a,5a}$ = 12.7 Hz, H-5a), 4.10 (d, 2H, $J_{4,5b}$ = 2.4, $J_{5a,5a}$ = 12.7 Hz, H-5′), 4.45 (t, 1H, H-4′), 4.82 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-2), 6.16 (d, 1H, H-1), 7.49 (s, 1H, H-3″), 9.37 (bs, 1H, NH). ^{13}C NMR [$(CD_3)_2$-CO] δ: 18.0 [$(CH_3)_2$−*C*−Si], 25.6 [(*C*H_3)$_2$−C−Si], 25.8 (2*C*H_3−C), 54.0 (CH_3−O), 59.2 (C-5), 78.6, 81.4 (C-4′) (C-3′), 103.9 (C-3), 106.7 (C-2), 114.4 (C-1), 143.0 (CO), 154.8 (C-4″), 159.4 (CO). MS (ESI$^+$): *m*/*z* 494 [M + 1]$^+$. Anal. Calcd for $C_{19}H_{31}NO_{10}SSi$: C, 46.23; H, 6.33; N, 2.84. Hallado: C, 45.95; H, 6.00; N, 2.56. HPLC: RT = 7.97 min (65:35).

Computational Methods. Computational chemistry was carried out by first drawing the molecules in the desktop of Cerius2 and optimizing the structures at the AM1 level of theory. Subsequently, electronic energies and structures were calculated by full optimization, without any geometrical constraint, by using the Becke's three-parameter hybrid functional[22] and using the Lee et al.[23] correlation functional with the 3-21G(d) basis set (B3LYP/3-21G(d)) and the 6-31G(d) basis set (B3LYP/6-31G(d)).[24] Frequency calculations were used for all minimized structures to determine that satisfactory minima were obtained. HOMO energies and charge values (CGHelp) were determined by doing a single-point calculation with the hybrid B3LYP/6-31G+(d,f).[25]

The complex model study was carried out by placing the three structures **17b**, **3b**, and **5b** close to the methoxytrimethylsilane moiety, and they were allowed to minimize without any geometrical restriction. Then, the B3LYP/6-31G(d)-optimized structures were additionally modeled by calculating a B3LYP/6-31G+(d,f) single point to determine their zero-point electronic energies and CGhelp charges.

To determine the influence of the solvent on this process, a quantum mechanical (QM) simulation was carried out. Solvation calculations in several solvents, DMSO, chloroform, and acetone, were carried out using the B3LYP density functional method, the 6-31G* basis set, and the PCM model of SCRF theory[26−29] by using the Gaussian 2003 (SCRF = PCM, solvent = item) keyword; also, the corresponding gas-phase calculations were computed. The solvation zero-point electronic energies were calculated by mere subtraction of the PCM energies and the gas-phase ones. The interaction zero-point (zp) electronic energy was calculated among a trimethylsilyl cation and several solvents. The obtained values for the studied solvents were calculated by the equation $DE^{zp}_{interaction} = E^{zp}_{cation+solvent} - (E^{zp}_{cation} + E^{zp}_{solvent})$.

Semiempirical model, AM1 and PM3, calculations were performed with MOPAC version 6.0.[30] The Gaussian 98 and Gaussian 98W program packages were used throughout this work.[31]

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Supporting Information Available: Four additional figures showing the hydrolysis rate of the 5′-TBDMS group in the 4″-N-substituted TSAO derivatives **12**−**15** and **25**−**30** and the AM1 major conformers of compounds **3** and **30** obtained from NMR experimental data. Additional table showing populations and *J* couplings (Hz) of the gauche+, gauche−, and trans conformers calculated from the Karplus equation parametrized by Altona et al. for compounds **3** and **30**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) Velázquez, S.; Chamorro, C.; Pérez-Pérez, M. J.; Alvarez, R.; Jimeno, M. L.; Martín-Domenech, A.; Pérez, C.; Gago, F.; De Clercq, E.; Balzarini, J.; San-Félix, A.; Camarasa, M. J. *J. Med. Chem.* **1998**, *41*, 4636−4647.

(22) Becke, A. D. *J. Chem. Phys*. **1993**, *98*, 5648−5654.

(23) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785−789.

(24) Hehre, W. J.; Radom, L.; Schleyer, P. V. R.; Pople, J. A. *Ab Initio Molecular Orbital Theory*; John Wiley & Sons: New York, 1986; Chapter 4.

(25) Foresman, J. B.; Frisch, Æ *Exploring Chemistry with Electronic Structure Methods*, 2nd ed.; Gaussian Inc., Pittsburgh, 1996.

(26) Miertus, S.; Scrocco, E.; Tomasi, J. *Chem. Phys.* **1981**, *55*, 117−129.

(27) Cancès, M. T.; Mennucci, B.; Tomasi, J. *J. Chem. Phys.* **1997**, *107*, 3032−3041.

(28) Cossi, M.; Barone, V.; Mennucci, B.; Tomasi, J. *Chem. Phys. Lett.* **1998**, *286*, 253−260.

(29) Cossi, M.; Scalmani, G.; Rega, N.; Barone, V. *J. Chem. Phys.* **2002**, *117*, 43−54.

(30) Stewart, J. P. P. *MOPAC*: *Quantum Chemistry Program Exchange*, revision 6.0; 1990.

(31) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, revision A.6; Gaussian, Inc.: Pittsburgh, PA, 1998.