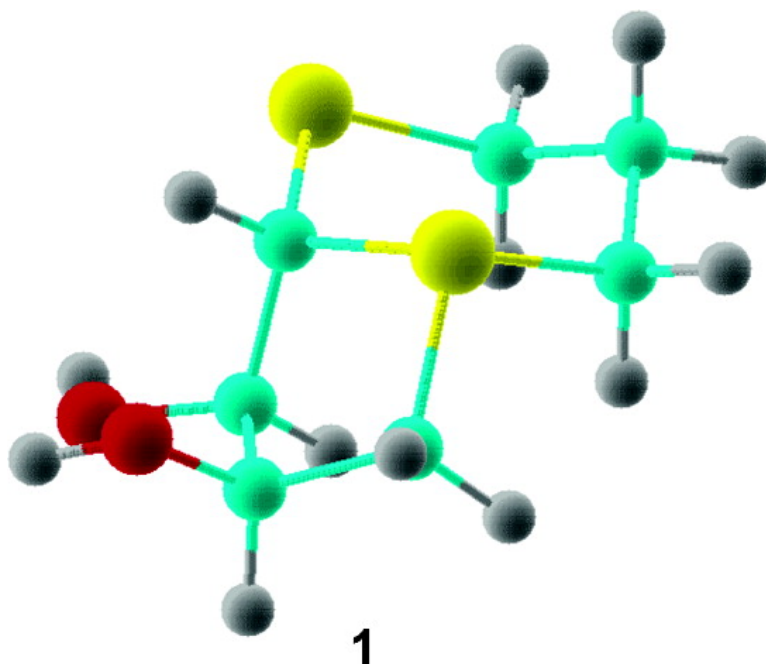Communication

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The Structure and Conformational Behavior of Sulfonium Salt Glycosidase Inhibitors in Solution: A Combined Quantum Mechanical NMR Approach

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Received January 16, 2004; E-mail: siriward@olemiss.edu; rwoods@ccrc.uga.edu
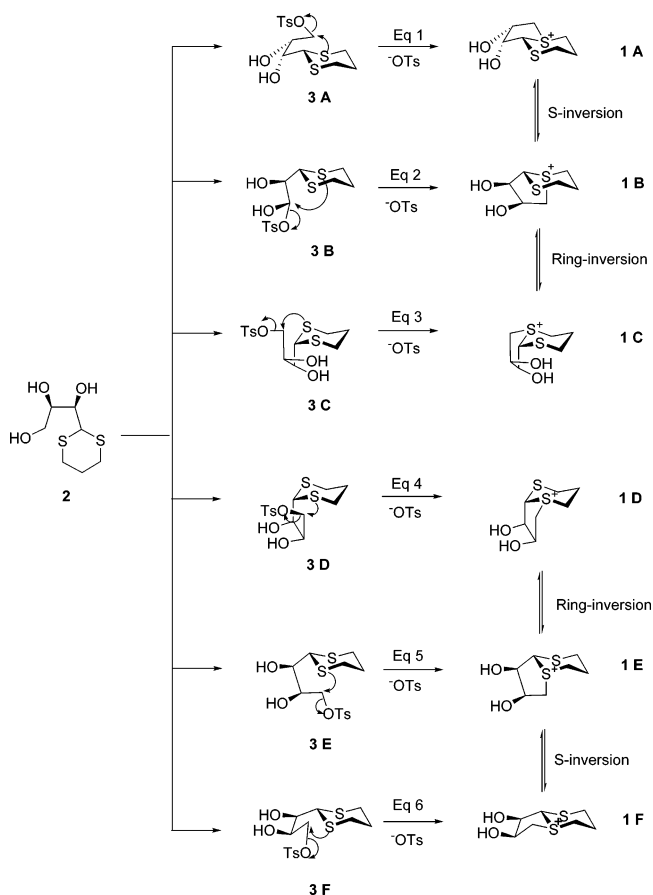
The drive to obtain compounds able to inhibit glycosidase activity potently and selectively has seen the synthesis of some very creatively designed molecules in the past decade. Clues as to the mode-of-action of the inhibited enzymes could be gleaned from consideration of the natural inhibitor structures, and these insights began increasingly to serve in the conception of novel inhibitors. A case in point is the natural mannosidase inhibitor swainsonine.[1–3] The activity of the azasugar swainsonine was proposed early on to arise from its resemblance, when protonated, to the mannosyl cation, which is presumed to be an intermediate in the enzymatic hydrolysis of mannosyl linkages. Consequently, the search for chemically stable mimics of the cationic intermediate has been a frequent component in the design of mannosidase inhibitors.[4–6] This led us to conjecture that properly designed sulfonium salts might also be effective inhibitors of glycosidases, which was confirmed for the very first time with a synthetic pyrolizidine analog.[7] More recently, the discovery of two naturally occurring polyhydroxy sulfonium salts that are potent inhibitors of glycosidases[8,9] has validated our original premise. The recent literature has seen a race not only to synthesize the natural sulfonium salts,[10,11] but also to design synthetic analogues.[12] However, establishing the structures of these synthetic salts and elucidating their solution conformations can be less than straightforward.

We have recently added to the sulfonium salt class of inhibitors with the synthesis of a bicyclic structure, namely, (1*R*,6*R*,7*R*,8*S*)-7,8-dihydroxy-5-thia-1-thioniabicyclo[4.3.0]nonane chloride (**1**).[13] In this analogue of swainsonine, the bridgehead nitrogen is replaced by a positively charged sulfur. This compound has been found to potently and selectively inhibit α-mannosidases. Sulfonium salt **1** was obtained as one compound by the reaction of the thioacetal **2** with tosyl chloride in pyridine (Scheme 1). Peaks associated with the expected tosylate precursor **3** were observed by ^{13}C NMR spectroscopy, but the compound could not be isolated.[13]

In the absence of X-ray diffraction or neutron scattering data, the conformational analysis of such complex charged structures is not trivial. For example, there are no empirical equations for describing the relationship between torsion angles containing protonated sulfurs and NMR *J*-coupling constants. Nor are there established relationships between NMR chemical shifts and molecular structures for these salts. Since several crystallization attempts failed to produce crystals suitable for diffraction analysis, the development of a reliable method to elucidate structures of inhibitors, such as **1**, was undertaken.

A confident assignment would underpin any attempt to rationalize the factors governing the observed formation of a sole product in a ring closure, which otherwise appears to have access to several modes of cyclization. To address this problem, we have performed finite perturbation ab initio quantum mechanical calculations to compute the *J*-couplings for all possible isomers of **1**.[14]

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Scheme 1

Calculations performed using density functional theory (DFT) have been shown to give reasonable NMR properties for relatively large molecules, while at the same time reducing CPU times relative to calculations performed at more sophisticated levels.[15,16] Only the Fermi contact term was considered here, as it makes the dominant contribution to vicinal H−H and C−H couplings.[17]

Geometry optimizations were performed for each of the proposed cyclization products **1A**−**1F** at the ab initio HF/6-311G** level; single-point conformational energies were also computed with the 6-311++G** basis set at the HF, MP2, and B3LYP levels (see Table 1). In all cases, the calculations predict the same trends. Notably, hydroxyl group orientation influenced the relative energies only modestly, and regardless of hydrogen bond orientation or estimated solvation effects, conformers **1A** and **1D** were consistently predicted to be the lowest in energy. Being separated by less than 1.5 kcal/mol, these conformations would be indistinguishable at room temperature, and each might be expected to contribute to the observed NMR properties.

Table 1. Single Point Relative Energies[a] Calculated with the 6-311++G** Basis Set for **1**, as a Function of Quantum Level

	quantum level		
conformation	HF	B3LYP	MP2
1A	0	0	0
1B	9.77 (8.26)	8.91 (7.65)	9.54 (8.69)
1C	9.46 (7.90)	8.10 (6.80)	8.01 (7.42)
1D	1.10 (0.34)	1.08 (0.38)	1.37 (1.44)
1E	6.85 (6.87)	5.91 (5.92)	6.86 (7.57)
1F	11.25 (9.86)	9.58 (8.38)	10.88 (10.42)

[a] In kilocalories per mole. Calculations performed with the Gaussian 98 program;[18] values in parentheses computed with the PCM[19] reaction field model for DMSO.

Table 2. Experimental and Computed J-Coupling Values[a] for **1**, Calculated at the B3LYP/6-311G** Level

conformation	$^1J_{CH}$	$^3J_{H6-C-S+-C}$	$^3J_{H6-C-S-C}$	$^3J_{H6-C-C-H}$
1A	144.0	−0.2	4.6	2.4
1B	162.8	4.1	0.8	4.5
1C	145.9	1.6	3.2	5.6
1D	154.1	3.9	5.2	8.9
1E	169.8	3.8	0.5	0.7
1F	152.2	1.1	2.9	6.8
experimental	151.9 ± 1	4.2 ± 0.7	5.2 ± 0.7	10.6 ± 0.3

[a] In Hz. Calculations performed with the Gaussian 98 program.[18]

Table 3. Computed[a] and Experimental[b] NOE Intensities for **1**

conformation	H7–H2ax	H7–H4ax	H8–H2ax	H8–H4ax
1A	0.7	0.6	<0.1	0.1
1B	0.4	0.3	<0.1	<0.1
1C	1.1	1.3	<0.1	0.1
1D	5.3	5.2	0.1	0.1
1E	0.3	<0.1	<0.1	<0.1
1F	0.3	<0.1	<0.1	0.1
experimental	4.8	3.7	0.9	n.a.

[a] Values are relative to the zero-mixing time diagonal peaks. Calculations performed with the CORMA program[22] with a mixing time of 500 ms. [b] Measured from 2D-NOESY experiments with a 500 ms mixing time.

Scalar $^3J_{HH}$-couplings were measured from 1D proton spectra and $^3J_{CH}$-couplings from the ^{13}C proton-decoupled and the 2D HSQMBC[20] spectra of salt **1** in DMSO-d_6 at 303 K. The NMR data showed a large $^3J_{H6-H7}$ value, consistent with an anti-relationship between those protons. In the absence of Karplus-type empirical equations for the remaining $^3J_{CH}$ values associated with the C–S–C–H and C–S$^+$–C–H sequences, a traditional empirical interpretation of the J-coupling data could not be performed. The quantum-computed J-couplings are reported in Table 2.

While the computed J-couplings are somewhat sensitive to the DFT functional,[21] the predicted and experimental data show remarkable overall agreement only with conformation **1D**. Structure **1D** corresponds to a cis-fused bicyclic system, in which H6 is equatorial (as suggested by the large $^1J_{C6-H6}$ value) and in which H-6 and H-7 adopt an anti-relationship, as expected from the $^3J_{H6-H7}$ value.

To complement the quantum J-data, the NMR nuclear Overhauser effect (NOE) intensities for all the conformations of **1** were computed using a full relaxation matrix approach and are presented in Table 3.

The experimental NOE data showed strong intensities between H7 and H2 and H4 of the six-membered ring, suggesting a conformation, such as **1B**, **1C**, **1D**, or **1E**, in which H7 is close to H2 and H4. Conformations with an anti-relationship between H6 and H7, such as **1E** or **1F**, could be discounted on the basis of the lack of NOEs between H-7–H-2ax and H-7–H-4ax. Only structure

1D gave rise to significant NOEs between these protons, consistent with the experimental data.

In summary, we have demonstrated the utility and accuracy of a computational approach based on a combination of well-established quantum mechanical methods. The most significant advantage of the quantum NMR calculations is that they do not rely on pre-existing empirical Karplus-type relationships, but rather may be applied a priori to novel molecular structures. Furthermore, we have shown that neither traditional experimental NMR methods nor gas-phase quantum mechanical calculations alone were able to unambiguously predict an experimentally consistent structure. It was only after the combination of the quantum-derived NMR J-coupling values and the NOE data that a complete picture of the conformational properties of this complex system could be formulated.

Supporting Information Available: Cartesian and key internal coordinates and absolute energies for each conformation of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA049700K