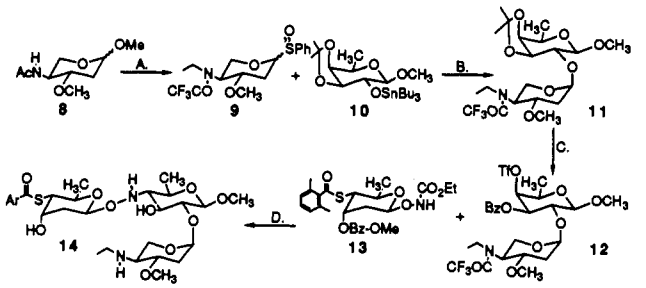
Scheme II[a]



[a] (A) 1. $LiAlH_4$–THF–Et_2O, 0 °C, 10 min, then room temperature, 20 h. 2. $(CF_3CO)_2O$–pyr, 5 h, room temperature (64% yield for two steps). 3. BF_3Et_2O–PhSH–CH_2Cl_2, −40 °C, 2 h, then 0 °C, 30 min (91%). 4. mCPBA–CH_2Cl_2, −78 °C, 1 h, then −20 °C, 30 min (95%). (B) 1.5 equiv of **10**, Tf_2O–Et_2O, −60 °C, 10 min, then 0 °C, 20 min ($\alpha:\beta$ > 12:1, 70%). (C) 1. CH_3OH(wet)–TsOH, room temperature, 30 min (90%). 2. BzCl–DMAP–Et_3N–CH_2Cl_2, −50 °C, 4 h (75%). 3. Tf_2O–pyr–CH_2Cl_2, room temperature, 30 min (95%). (D) 1. 1.1 equiv of **12**, NaH–HMPA–Et_2O, −20 °C, 10 min, then 0 °C, 1 h (87%). 2. NaOH(solid)–MeOH, 0 °C, 4 h, then room temperature, 30 min (50%).

extremely mild conditions (Scheme I) to give **7**.[7] The A3 benzoyl group is removed first, and the free hydroxyl then apparently facilitates deprotection of the urethane, obviating the use of strong base.

We have also found that the requisite glycosyl urethanes (e.g., **6**) can be synthesized directly simply by treating the corresponding activated glycosyl sulfoxides with readily available *N*-hydroxyurethane.[8] Thus, in the context of oligosaccharide synthesis the carboethoxy group on nitrogen plays two key roles: first, it deactivates the nitrogen so that glycosylation only takes place on oxygen; and second, it facilitates formation of an anion on nitrogen so that subsequent S_N2 displacement takes place cleanly. We have applied this general strategy for constructing N–O linked disaccharides to a synthesis of the core trisaccharide found in both calicheamicin and esperamicin (Scheme II). The acetylated 4-amino sugar **8**[9] was converted to its corresponding sulfoxide **9**, which was coupled using our sulfoxide glycosylation method[10] to fucose derivative **10**[11] to produce the α-linked disaccharide **11** stereospecifically (70%). Deprotection of the isopropylidene and selective benzoylation at C3 followed by triflation at C4 gave **12**, which was then coupled stereospecifically (87% yield) with glycosyl urethane derivative **13**.[7] The resulting trisaccharide was deprotected in one step to give **14**.[12]

N–O glycosidic linkages have been found in the oligosaccharides of two extremely potent antitumor agents. We have developed a general method to incorporate N–O linkages into oligosaccharides stereospecifically. We can now begin to study the importance of N–O linked oligosaccharides in DNA recognition.[13]

Acknowledgment. This work was supported by the National Institutes of Health and funds obtained from an ONR Young

(7) The structure was assigned by using a combination of one- and two-dimensional NMR; selected ^1H NMR data for **7** (δ, $CDCl_3$, 500 MHz): 4.65 (B ring H1, d, J = 8.6), 4.54 (A ring H1, d, J = 3.6), 2.34 (A ring H4, t, J = 9.9), 1.32 (A ring H6, d, J = 6.3).

(8) Kim, S.-H.; Yang, D.; Kahne, D. Manuscript in preparation.

(9) Kahne, D.; Yang, D.; Lee, M. D. *Tetrahedron Lett.* **1990**, *31*, 21.

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(11) Schuler, H. R.; Slessor, K. N. *Can. J. Chem.* **1977**, *55*, 3280. Formation of the tributyltin salt was carried out in toluene with 0.5 equiv of $(Bu_3Sn)_2O$ and crushed 4A molecular sieves at 70 °C for 1 h.

(12) The structure was assigned by comparison with one- and two-dimensional NMR data obtained for the calicheamicin oligosaccharide. See: Walker, S.; Valentine, K. G.; Kahne, D. *J. Am. Chem. Soc.* **1990**, *112*, 6428. Selected ^1H NMR data for **14** (δ, CD_3OD, 500 MHz): 5.39 (E ring H1, s), 5.06 (B ring H1, dd, J = 10.0, 1.2), 4.90 (A ring H1, d, J = 7.3), 4.90 (B ring H3, s), 3.49 (E ring OCH_3, s), 3.37 (A ring OCH_3, s), 2.29 (aromatic CH_3, s), 2.24 (A ring H4, t, J = 9.9), 1.37 (B ring H6, d, J = 6.0), 1.35 (A ring H6, d, J = 6.0), 1.16 (E ring CH_3CH_2, t, J = 7.0).

(13) See following paper in this issue.

Conformational Analysis of the N–O Bond in the Calicheamicin Oligosaccharide

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Received December 21, 1990

There is currently a great deal of interest in understanding the relationship between structure and function in oligosaccharides.[1] While cell-surface carbohydrates have been extensively studied, far less attention has been paid to carbohydrates that bind to DNA.[2] We are engaged in a study of the calicheamicin oligosaccharide in an effort to delineate the structural features important for DNA recognition.[3] The calicheamicin oligosaccharide contains an N–O linkage between rings A and B.[4] The effects of an N–O linkage on the shape of an oligosaccharide chain have never been studied and we have therefore undertaken a conformational analysis of the N–O bond in calicheamicin. Preliminary results indicate that it enforces an unusual curved conformation in the central portion of the molecule. We think this enforced curvature may be crucial for tight binding in the minor groove.

Hydroxylamine has a remarkable conformational profile.[5] It has a 2-fold rotational barrier and there is a large energy difference between conformers at the two energy minima.[6] In the low-energy conformer the O–H bond eclipses the nitrogen lone pair (Figure 1A, **1**). In the other conformer the O–H bond is anti to the nitrogen lone pair (**2**).[6] The conformers can interconvert (e.g., **1** → **2**) by inversion at nitrogen as well as rotation. In an N,O-disubstituted hydroxylamine where either of the substituents is chiral (e.g., calicheamicin), there are potentially four energetically distinct conformers around an N–O bond (Figure 1B, **3**–**6**), and the barriers to both rotation and inversion are appreciable.[5-7] To

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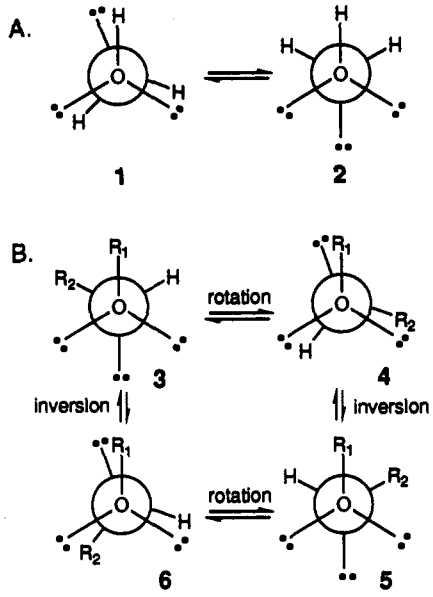


Figure 1. (A) Conformers of hydroxylamine; (B) conformers around an N,O-disubstituted hydroxylamine. If either R_1 or R_2 contains a chiral unit, **3** & **5** and **4** & **6** are pairs of diastereomers.

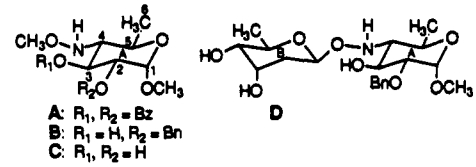
better understand how the N–O bond influences the conformation of the calicheamicin oligosaccharide, we synthesized model systems **A–D**[8] and studied their behavior at low temperature by NMR (Table I).

For all cases, only two sets of proton resonances could be frozen out. The free energy of activation for conformational interchange is approximately 10–11 kcal/mol for all four compounds. It is not clear whether the observed barrier is due to inversion or rotation.[10] Failure to observe a second barrier—and therefore the other two conformers—could, in principle, be due to chemical shift degeneracy between pairs of conformers or an activation barrier too low to be accessible by DNMR. However, ab initio calculations on *N,O*-dimethylhydroxylamine show a 6.7 kcal/mol energy difference between the eclipsed (lower energy) and staggered conformers.[11] It is therefore unlikely that the two staggered conformers in compounds **A–D** (**3** and **5**, Figure 1) are significantly populated even at room temperature.

The two eclipsed conformers frozen out at low temperature are unequally populated. The population distribution (**K**, Table I) depends on solvent and on variations in substituents. Compound **D**, the closest model system for the central disaccharide of calicheamicin, shows the largest conformational bias, with a ratio of approximately 6:1 in both methanol and acetone. We expect that the calicheamicin oligosaccharide also exists as a mixture of eclipsed conformers (**4** and **6**), with only a small energy difference (<1 kcal) between the two conformers at room temperature. Nevertheless, in both eclipsed conformers the N–O bond holds the A and B sugar rings (R_1 and R_2 in Figure 1) at the unusual dihedral angle of ~120°, imparting a gentle curvature to the central portion of the molecule. It seems likely that one of these eclipsed conformers is involved in binding in the minor groove of DNA: the high barriers to both rotation and inversion mean that a significant deviation from the idealized 120° angle upon binding would cost a great deal of energy.

Our analysis suggests that the N–O bond in calicheamicin, because of its unusual conformational profile and rigidity, plays a key role in controlling the shape of the oligosaccharide chain. Our conclusions are borne out by a crystal structure of a fragment of the calicheamicin oligosaccharide, which shows that the C–N–O–C bond does indeed adopt an eclipsed conformation.[4,12] We think it likely that the enforced curvature imparted in the calicheamicin oligosaccharide by the N–O linkage is important for tight binding in the minor groove.

Table I[a]



compd	solvent	$\Delta\nu$, Hz	T_c, °C	K (−88 °C)	$\Delta G^{\ddagger}$- (major → minor), kcal/mol
A	acetone-d_6	57[c]	−75	2.2	9.9
	CD_3OD	56[c]	−73	1.6	9.9
	CD_2Cl_2	55[c]	−83	5.4	9.8
B	acetone-d_6	57[c]	−69	4.0	10.4
	CD_3OD	57[c]	−64	1.9	10.3
	CD_2Cl_2	56[c]	−73	3.9	10.2
C[b]	acetone-d_6	58[c]	−65	3.3	10.5
	CD_3OD	56[c]	−64	1.6	10.4
D[b]	acetone-d_6	12[d]	−72	5	10.9
	CD_3OD	32[e]	−64	5.6	11.0

[a] Spectra were taken on a Jeol GSX 500 MHz spectrometer. $\Delta G^{\ddagger}$ values were estimated from the coalescence temperature with use of a modified formula for exchange between two uncoupled and unequally populated sites.[9] [b] Insoluble at low temperature in CD_2Cl_2. [c] Frequency difference between C_6 methyl doublets. [d] Frequency difference between A_1 methoxy singlets. [e] Frequency difference between A_4 triplets.

Acknowledgment. We thank Professor M. Francl (Bryn Mawr) for advice on the ab initio calculations and Professor K. Wiberg (Yale) for a copy of CHELPG. This work was supported by a Cyanamid/Princeton grantlet, The Parenteral Drug Association Foundation for Pharmaceutical Sciences (fellowship for S.W.), the Searle Scholars Program/The Chicago Community Trust, the National Institutes of Health, and funds from an ONR Young Investigator Award.

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(8) Overlap of proton resonances and the presence of other bonds with high rotational barriers make it difficult to use DNMR to study the N–O bond in calicheamicin itself. Compounds **A–D** were synthesized following the methods described in the preceding communication. Selected ^{1}H NMR data (δ, CD_3OD, 500 MHz): **A**, 3.03 (H4, t, J = 10.0), 1.64 (H6, d, J = 6.4); **B**, 2.23 (H4, t, J = 10.0), 1.27 (H6, d, J = 6.4); **C**, 2.23 (H4, t, J = 10.0), 1.28 (H6, d, 6.3); **D**, 4.96 (B ring H1, dd, J = 1.9, 10.1), 2.20 (A ring H4, t, J = 10.0), 1.26 (A ring H6, d, J = 6.4).

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(10) A combined process of torsion and inversion is possible in principle. However, the transition state for the combined process is expected to be significantly higher in energy than either process alone.[5b]

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(12) In the crystal structure the C–N–O–C angle is 135°.[4] On the basis of symmetry arguements, a minor deviation from the idealized 120° angle was expected: Hounshell, W. D.; Dougherty, D. A.; Mislow, K. *J. Am. Chem. Soc.* **1990**, *100*, 3149.