The present findings indicate an additional factor that contributes to the surface-seeking tendencies of proline.

Entry of the proline analogue into solution is accompanied by an entropy change that is less negative than the entropy change for solution of the norvaline analogue by 4.7 cal/(deg mol). In seeking to understand the origin of this favorable effect, it seems reasonable to suppose that the ring system of proline suffers relatively little loss of internal mobility when it enters the structured environment of solvent water, compared with the flexible side chains of conventional amino acids. Similarly, the entry of cyclohexane into water is accompanied by an entropy change that is more favorable than the entropy change of solvation of *n*-hexane by 3 cal/(deg mol).[9-11] In proline derivatives, this effect appears to be more than sufficient to compensate for the substantial loss of hydrophilic character that results from the absence of an NH proton, rendering proline more hydrophilic than residues with noncyclic hydrocarbon side chains of similar size.

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Construction of Glycosidic N-O Linkages in Oligosaccharides

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Calicheamicin γ^1 (Figure 1) is an extremely potent antitumor antibiotic that cleaves DNA sequence specifically.[1] The calicheamicin oligosaccharide, which has been implicated in DNA binding, contains an unusual N-O linkage between rings A and B. We report here a general method to introduce N-O linkages into oligosaccharides. We apply this method to the stereoselective construction of the core trisaccharide found in calicheamicin (and in the related antibiotic esperamicin A_1).[2]

When we began this work there were no general methods to construct N-O linked oligosaccharides. Recent reports in model systems suggested the possibility of introducing hydroxylamine linkages into oligosaccharides by reducing the corresponding oxime.[3] However, it appears that the stereochemical outcome of oxime reduction in oligosaccharides is unpredictable.[4] We felt that one way to ensure control of the C-N bond stereochemistry would be to do an S_N2 displacement on an appositely placed C-O bond.

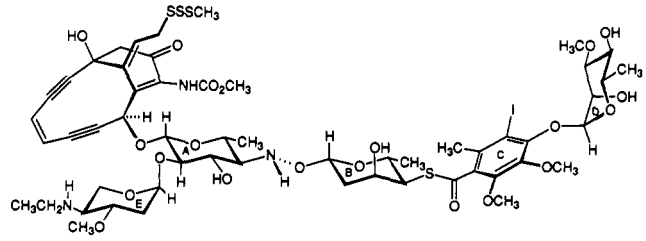
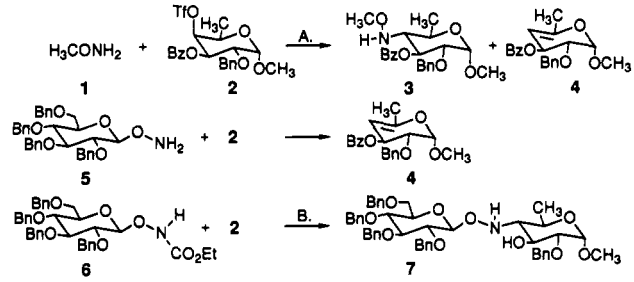


Figure 1. Calicheamicin γ^1.

Scheme I[a]



[a] (A) Excess 1-DMF, 1.5 h, room temperature (20%) (B) 1. NaH-Et_2O-HMPA, 30 min, room temperature (82%). (2) NaOH (solid)-MeOH, 1 h, room temperature (80%).

In our initial investigations we used *O*-methylhydroxylamine (**1**) to displace the axial C4 triflate **2** (Scheme I). The desired product **3** was obtained stereospecifically in 20% yield along with eliminated material (**4**, 10%). Attempts to increase the yield by changing the reaction conditions were not successful. Moreover, when sterically more demanding groups were put on oxygen the yield decreased significantly. We were unable to obtain any disaccharide when perbenzylated glucose hydroxylamine **5**[5] was used as a nucleophile.

In retrospect, these results were not surprising: S_N2 displacements with neutral nucleophiles are extremely difficult in sugar systems because the many oxygen substituents deactivate the ring. However, anions such as azide and thiolate effect rapid displacement,[6] so we reasoned that an anionic hydroxylamine derivative might work better. Accordingly, **5** was converted to **6** with ethyl chloroformate (CH_2Cl_2-saturated $NaHCO_3$, room temperature, 20 min, 100% yield). Urethane **6** was deprotonated and coupled stereospecifically to triflate **2** (82% yield; no elimination product formed under these reaction conditions). We were delighted to find that the coupled product can be deprotected under

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(4) For example, Nicolaou et al. report stereoselective formation of the hydroxylamine linkage in a model system of the central portion of the calicheamicin oligosaccharide.[3c] In spite of this extremely close analogy, they obtain a 2:1 mixture in favor of the wrong isomer when the same strategy is applied in a synthesis of the calicheamicin oligosaccharide. See: Nicolaou, K. C.; Groneberg, R. D.; Miyazaki, T.; Stylianides, N. A.; Schulze, T. J.; Stahl, W. *J. Am. Chem. Soc.* **1990**, *112*, 8193.

(5) Synthesized by Mitsunobu reaction with *N*-hydroxyphthalimide ((a) DEAD-THF-Ph_3P, room temperature 2 h. (b) N_2H_4-MeOH, 71% based on 45% conversion). See: Grochowski, E.; Jurczak, J. *Carbohydr. Res.* **1976**, *50*, C15.

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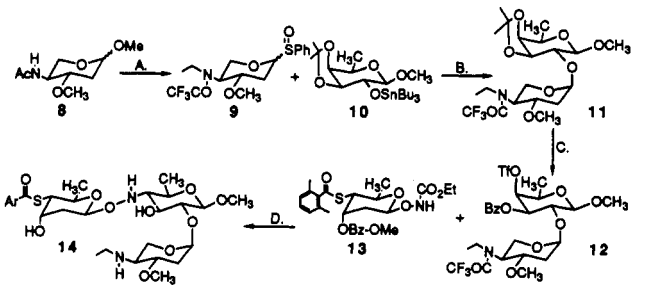
Scheme II[a]



[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).

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(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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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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