extended to the measurement of secondary KIE and those reactions involving heavy isotopes such as ^{13}C and ^{18}O (e.g., bis anionic oxy-Cope rearrangement).

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The Anomalous Hydrophilic Character of Proline

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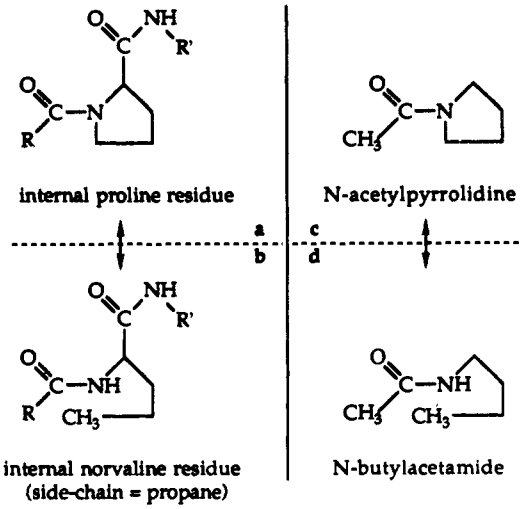
Amino acid residues vary greatly in their affinities for watery surroundings, as indicated by free energies of solvation of their side chains, determined by measuring their equilibria of transfer from neutral aqueous solution to the vapor phase[1] or to a nonpolar solvent such as cyclohexane.[2] These differences have proven useful in identifying portions of transmembrane proteins that are likely to be located within the lipid bilayer,[3] in analyzing the biological properties of peptide analogues and mutant proteins, and in attempting to understand the relationship between the three-dimensional structures of proteins and their amino acid sequences.[4]

Because amino acids and their derivatives do not enter truly nonpolar environments in quantities that can be detected, measurements of water-to-vapor and water-to-cyclohexane distribution coefficients have been confined to compounds of the type R-H, where R represents the side chain of an amino acid. Thus far, these scales of hydrophilic character have remained incomplete because the "side chain" of proline cannot be represented in this way (Scheme I).

The physical properties of proline might be considered to be comparable with those of valine or norvaline, nonpolar amino acids with which it shares a side chain containing three carbon atoms (Scheme I). However, amides of secondary amines are typically 10–20-fold less hydrophilic than amides of primary amides,[5] so that an internal proline residue would be expected to be correspondingly less hydrophilic (or more hydrophobic) than a valine or norvaline residue at a similar position. It seemed desirable to put this possibility to an experiment test.[6]

Scheme I shows a means of circumventing the difficulty of describing the side chain of proline by a side chain R. *N*-Acetylpyrrolidine (c) and *N*-butylacetamide (d) are structurally related to each other as an internal proline residue (a) is related to an internal norvaline residue with a normal propyl group as a side chain (b). If the water-to-vapor or water-to-cyclohexane distribution coefficients of *N*-acetylpyrrolidine and *N*-butylacetamide were known, then it should be possible to apply their ratio to the value for *n*-propane, to arrive at an approximate value for the effective distribution coefficient of the side chain of an internal proline residue,[7] placing it on a common side with the side chains of the other amino acids.

Scheme I[a]



[a] An internal proline residue (a) is related in structure to an internal norvaline residue (b) as *N*-acetylpyrrolidine (c) is related to *N*-butylacetamide (d).

Table I. Equilibria of Transfer from Vapor to Water ($K_{v\to w}$) and from Cyclohexane to Water ($K_{chx\to w}$)

	N-acetylpyrrolidine	*N*-butylacetamide
$K_{v\to w}$ (25 °C)	1.54 (±0.3) × 10^7	6.7 (±1.2) × 10^6
$\Delta G_{v\to w}$ (25 °C)	−9.77 ± 0.15 kcal/mol	−9.28 ± 0.12 kcal/mol
$K_{chx\to w}$ (8 °C)	459 ± 26	217 ± 15
$K_{chx\to w}$ (17 °C)	240 ± 4	130 ± 1.6
$K_{chx\to w}$ (25 °C)	172 ± 8	79 ± 0.3
$K_{chx\to w}$ (37 °C)	94.3 ± 2.4	40 ± 0.4
$\Delta G_{chx\to w}$ (25 °C)	−3.04 ± 0.03 kcal/mol	−2.58 ± 0.002
$\Delta H_{chx\to w}$ (25 °C)	−9.26 ± 0.3 kcal/mol	−10.2 ± 0.2 kcal/mol
$\Delta S_{chx\to w}$ (25 °C)	−20.9 ± 1.0 cal/(deg/mol)	−25.6 ± 0.6 cal/(deg/mol)
$T\Delta S_{chx\to w}$ (25 °C)	−6.22 ± 0.3 kcal/mol	−7.62 ± 0.3 kcal/mol

N-Acetylpyrrolidine and *N*-butylacetamide were prepared by treatment of the parent amines with acetic anhydride-*1*-^{14}C, followed by redistillation to remove the last traces of radioactive acetate. Water-to-vapor distribution coefficients were determined at 25 °C by dynamic vapor pressure measurements;[1] and water-to-cyclohexane distribution coefficients were determined by distribution measurements using radioactivity as a means of analysis[2] and also by proton NMR analysis.[2] These experiments were performed at 8, 17, 25, and 37 °C to determine enthalpies of transfer. The results are shown in Table I.

Solvation by water of *N*-butylacetamide was accompanied by a more negative enthalpy change than solvation *N*-acetylpyrrolidine, in accord with expectations based on their differing H-bonding capabilities. However, solvation of *N*-acetylpyrrolidine was accompanied by a very much less negative entropy change, more than fully compensating for this difference. As a result, *N*-acetylpyrrolidine was roughly twice as hydrophilic as *N*-butylacetamide. Proline often appears in solvent-exposed positions in proteins, in part because its structure excludes it from occupying internal positions in α-helices and β-structures but allows it to occur at reverse turns that tend to be found near protein surfaces.[8]

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(3) Kyte, J.; Doolittle, R. F. *J. Mol. Biol.* **1982**, *157*, 105.
(4) For a comprehensive review, see: Edsall, J. T.; McKenzie, H. A. *Adv. Biophys.* **1983**, *16*, 53.
(5) *N,N*-Dimethylacetamide exceeds *N*-methylacetamide by a factor of 13 in its water-to-vapor distribution coefficient (Wolfenden, R. *Biochemistry* **1978**, *17*, 201–204, and by a factor of 20 in its water-to-cyclohexane distribution coefficient (Gibbs, P.; Wolfenden, R., unpublished).
(6) Octanol–water distribution measurements on acylamino acid amides have suggested that proline residues may be more polar than valine (Yunger, L. M.; Cramer, R. D., III. *Mol. Pharmacol.* **1981**, *20*, 602). However, wet octanol has been found to interact specifically with certain heterocyclic solutes (ref 2) so that the meaning of this observation is uncertain.
(7) In most cases, free energies of solvation appear to be additive: Butler, J. A. V. *Trans. Faraday Soc.* **1937**, *33*, 229–237. Hine, J.; Mookerjee, P. K. *J. Org. Chem.* **1975**, *40*, 292–230. Cabani, S.; Gianni, P.; Mollica, V.; Lipori, L. *J. Solution Chem.* **1981**, *10*, 563–595.
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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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Registry No. Proline, 147-85-3; *N*-acetylpyrrolidine, 4030-18-6; *N*-butylacetamide, 1119-49-9.

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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.

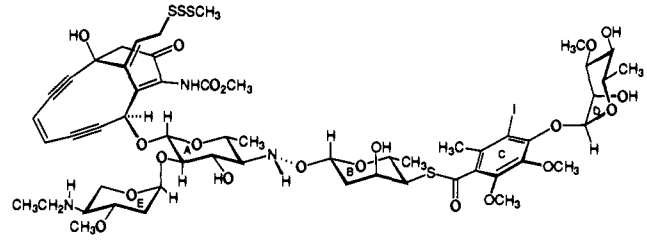
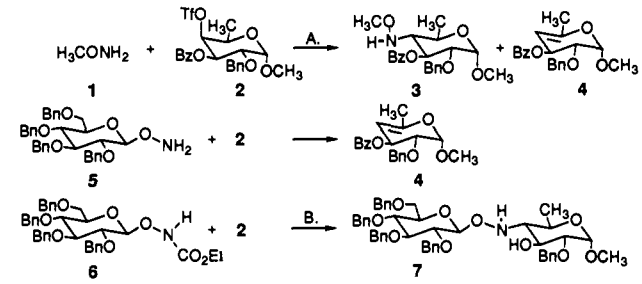


Figure 1. Calicheamicin γ^1.

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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