Conformation of the gummy polysaccharide from corm sacs of *Watsonia pyramidata*

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X-ray diffraction patterns have been obtained from drawn fibres of the gummy polysaccharide found in the corm sacs of *Watsonia* species. The polysaccharide consists of 1,4 linked β-D-xylose units, highly substituted with very short side chains. The X-ray reflections index on a trigonal unit cell and the evidence suggests that the molecule crystallizes as a left-handed, three-fold helix with an axially projected rise of 0.495 nm. The unit cell dimensions are dependent on relative humidity and contract from $a = b = 1.40$ nm at high relative humidity, to $a = b = 1.34$ nm when dried. Little variation is observed for the chain axis repeat, c. Contraction is accompanied by a loss of crystallinity.

INTRODUCTION

Polysaccharides are often found as excudates on plant stems and a variety of species have been investigated[1]. A group of these jelly-like substances are based upon a xylan backbone, to which are attached short chains of arabinose, galactose and glucuronic acid residues[2]. The chemical structure of the polysaccharide found in the vesicles of the corm sacs (technically stems) of *Watsonia pyramidata* has been investigated by Shaw and Stephen[3]. They reported that the polysaccharide was composed of a linear backbone of 1,4 di-equatorially linked β-D-xylose residues, highly substituted at positions 2 and 3 with L-arabinofuranose and some D-galacto-pyranose residues.

X-ray diffraction studies of fibres drawn from the gel revealed well oriented and highly crystalline X-ray patterns[4,5]. Furthermore the measured spacings and distribution of intensities favoured an extended three-fold helical conformation packing in a trigonal array. The axial advance per monomer (h) of 0.49 nm was similar to that reported for β(1 → 4)-D-xylan[6,7]

In this contribution we have scrutinized the X-ray diffraction results in more detail and using computed model building procedures have investigated a number of stereochemically feasible models conforming to three-fold helical symmetry.

EXPERIMENTAL

Material and sample preparation

Corms of *Watsonia pyramidata* (Andr.) Stapf. (order, Liliales; family, Iridaceae) were gathered in late summer when the polysaccharide content is greatest. The corms were cut open to reveal the gum-containing vesicles. Using tweezers, thin strands, approximately 200–500 μm in diameter, were drawn from the gummy substance and allowed to air dry. After drying for an hour the fibres were examined under a polarizing microscope and a selection made of those showing the best uniformity of colour and sharpness of extinction. These fibres were then held taut between clamps and kept at constant humidity ready for examination with X-rays.

X-ray diffraction

Specimens were irradiated with nickel-filtered CuKα radiation either from a Hilger and Watts Microfocus or an Elliott Rotating Anode X-ray generator. Various pinhole collimators were used in the range 150–500 μm diameter. The X-ray photographs for the 'dry' sample were obtained with the camera evacuated to a pressure of 10^{-2} torr. To obtain the X-ray photograph of the 'wet' sample, the relative humidity in the camera was maintained at 76% with saturated salt (sodium chloride) solution in a hydrogen filled camera.

The X-ray patterns were internally calibrated by dusting the specimens with calcite, which yields a diffraction ring of spacing 0.3035 nm.

Molecular model building

Molecular models were generated using a linked-atom description similar to that reported by Arnott and Wonocott[8]. The positions of the atoms were defined in terms of the internal bond lengths, bond angles and torsion angles of the molecule. Stereochemical parameters for the pyranose ring were taken from the average set given by Arnott and Scott[9] for the $^4C_1$ chair pyranose residue. Parameters for the furanose ring were obtained from those given by Brown and Levy[10] by reflection of the atomic coordinates of the ring atoms and modification of side groups.

Bond lengths and bond angles were held constant, including the glycosidic bond angles which were given the value 116.5°. This defines the pyranose rings and side groups as rigid bodies and leaves the glycosidic torsion angles of the

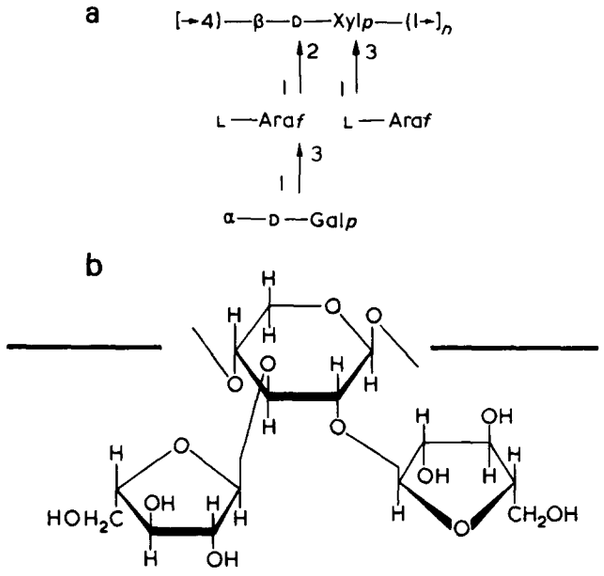

Figure 1 'Gummy polysaccharide from *Watsonia pyramidata*. (a) Chemical repeat; (b) possible schematic representation

backbone and side groups as explicit variables. These parameters were varied to generate plots showing the combinations of glycosidic torsion angles which would give a minimum number of contacts and also regions where certain interresidue hydrogen bonds could be formed. These parameters were varied so as to produce a model with the appropriate helical symmetry and pitch, within the limits previously defined by the steric contacts. This was accomplished following the method outlined by Guss *et al.*[11] and minimizing Φ, where:

$$\Phi = \Sigma\epsilon_j + \Sigma\lambda_h G_h$$

The second summation contains a series of Lagrangian multipliers, λ_h, and constraint expressions, G_h, that we wish to become zero. Through their use, helical continuity, pitch and symmetry were imposed on the model.

Between certain atoms there is the possibility of an attractive interaction such as a hydrogen bond. In these cases, if interatomic distance, d_j falls between specified limits ${}_A d_j$ and ${}_B d_j ({}_A d_j < d_j < {}_B d_j)$ then the term:

$$\epsilon_j = {}_I k_j ({}_I d_j - d_j)^2 \qquad {}_A d_j \leqslant d_j \leqslant {}_B d_j$$

is also considered, where ${}_I d_j$ is the 'ideal' value for the interaction and ${}_I k_j$ is the corresponding weighting constant. Values for the weighting constants and the distances that define the interactions were taken from Guss *et al.*[11].

CHEMICAL STRUCTURE

Analyses by Shaw and Stephen[3] established the structure of the gummy polysaccharide to have a 1,4 linked β-D-xylose backbone substituted on 9 out of 10 residues at C(2) and C(3). The side-chains may be summarized as follows (the approximate number per 10 xylose residues is given in parentheses):

$$\text{L} - \text{Ara}f - (1 \rightarrow \qquad (12)$$

$$\alpha - \text{D-Gal}p - (1 \rightarrow 3) - \text{L} - \text{Ara}f - (1 \rightarrow \qquad (2)$$

$$\alpha - \text{D-Gal}p - (1 \rightarrow 3) - \alpha - \text{L} - \text{Ara}f(1 \rightarrow 2)$$

$$- \text{L} - \text{Ara}f(1 \rightarrow \qquad (5)$$

In addition there are three remaining L-Araf units within the side chains, two of them 2-linked and one 3-linked (*Figure 1*). For trial models a xylose backbone with L-arabinofuranose residues substituted at the O(2) and O(3) was assumed. Conformational maps were computed for the backbone linkages (*Figure 2a*) and also for these two side

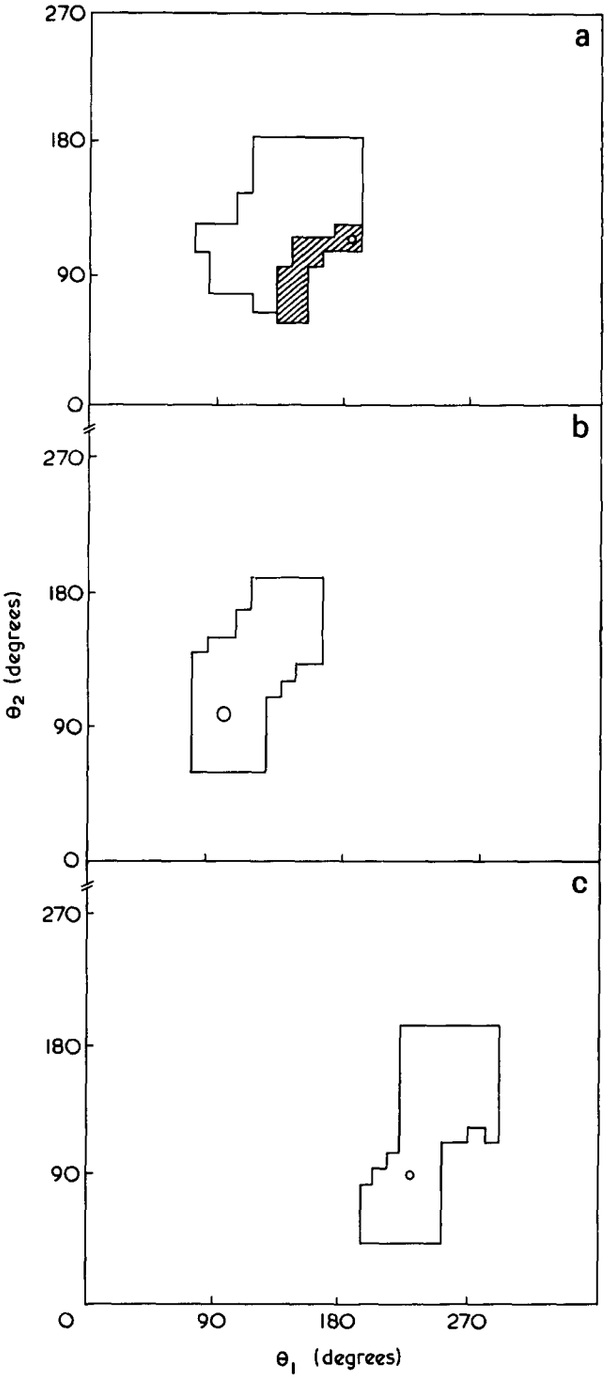

Figure 2 Steric maps and possible hydrogen bonds for the backbone linkages. (a) Final position of the trial model is marked (O), as are the final positions of the two side groups. (a) β-D-Xylan-(1—4)-β-D-xylan linkage [xylan O(3)—O(5)]: θ_1 = C(2)—C(1)—O—C(4); θ_2 = C(1)—O—C(4)—C(3); (b) α-L-arabinose-(1—2)-β-D-xylan: θ_1 = C(1)—C(2)—O—C(1); θ_2 = C(2)—O—C(1)—C(2); (c) α-L-arabinose-(1—3)-β-D-xylan: θ_1 = C(2)—C(3)—O—C(1); θ_2 = C(3)—O—C(1)—C(2)

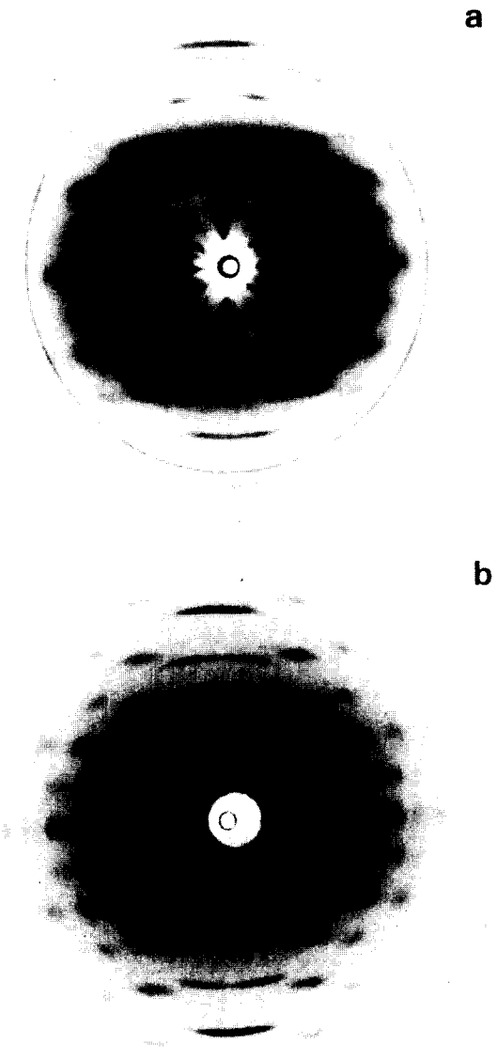

Figure 3 X-ray diffraction pattern of *Watsonia* polysaccharide. (a) Dry; (b) at 76% r.h.

group residues (*Figures 2b* and *2c*). The possibility of stabilizing hydrogen bonds, in a less highly substituted model was also considered. A hydrogen bond in the xylose backbone was considered feasible if the O(3)–O(5) distance was less than 0.3 nm. In further refinement of trial models, hydrogen bond distances of 0.28 nm were generally imposed.

RESULTS

Backbone

The X-ray diffraction patterns obtained from *Watsonia* polysaccharide showed variation with humidity (*Figure 3*). At both low and high relative humidities the layer line spacing was 1.485 nm, with meridional reflections occurring only on those layer lines with $l = 3n$. This repeat is about 10% less than the maximum theoretical extension in common with a large number of polysaccharides[6] and suggests a fairly extended structure.

The reflections index on a trigonal unit cell whose dimensions, perpendicular to the chain axis, increase with relative humidity. The dimensions of the unit cell vary from $a = b =$ 1.34 nm for dry fibres to $a = b = 1.40$ nm at 76% relative humidity. The simplest interpretation consistent with the basic feature of the X-ray pattern is a three-fold helix. Thus trial models of the helical backbone (without attached saccharide side appendages in this initial investigation) were computer generated to fit the projected repeating distance (h) and the three-fold symmetry. Both right- and left-handed models were generated and compared for short contacts[12]. All right-handed models were found to have unacceptable steric clashes across the glycosidic linkage. However, a left-handed model could be built which was stereochemically acceptable and also allowed the formation of a hydrogen bond of the type O(3)–H$\cdots$O(5) between adjacent residues, similar to that proposed by Nieduszynski and Marchessault[7]. The torsion angles at the glycosidic linkage are marked on the conformational map (*Figure 2a*) and computer drawn projections of the model are shown in *Figure 4a*. The fractional coordinates of the asymmetric unit are tabulated in *Table 1*.

Side chains

Since the composition and placing of the short side chains is not fully known, models were first generated with one L-arabinofuranose residue at the O(2), and retaining the intra-residue hydrogen bond in the backbone (*Figure 4b*). A second arabinose residue was then added to the model at the O(3) so that there were no short contacts between the side groups and the backbone (*Figure 4c*). The values of the torsional angles at each glycosidic linkage for the final model are marked on the conformational maps (*Figures 2b* and *c*) and computer drawn projections are shown (*Figure 4c*).

DISCUSSION AND CONCLUSIONS

The quality of the X-ray diffraction patterns illustrated in *Figure 3* indicates a much greater regularity of structure than is suggested by the chemical analyses (see section on Chemical structure). It therefore seems likely that the crystalline regions have a certain regularly repeating structure and that any variations of this repeat are accommodated in the amorphous phase. In this way both X-ray observations and chemical analysis can be made compatible. It is probable that those portions of the chains with regular structure are able to aggregate together forming crystalline micelles which may operate as junction zones to provide the gel-like characteristic behaviour of this substance; the non-regular regions existing in an amorphous environment and giving the necessary connectivity for any gel-like texture. A similar model for the association behaviour in solution has also been suggested[13].

Owing to the uncertainties regarding the precise chemical composition of the crystalline domain, the number of saccharide units attached as side appendages is in some doubt and therefore so is the number of chains passing through each unit cell. The density of the sample was recorded to be 1.52 g/ml in the 'dry' condition, although this is still likely to contain a certain amount of water of crystallization. The volume of the unit cell relating to this 'dry' state is computed to be 2.309 nm^3 which would give the following values for the three models examined: 5.3 chain segments for a single xylan backbone as in *Figure 4a;* 2.67 chain segments for xylan backbone plus one arabinose side group as in *Figure 4b,* and 1.78 chain segments for backbone plus two arabinose side groups as in *Figure 4c.* Since the space group is likely to be $P3_2$ (or possibly $P3_221$ or $P3_212$) the single backbone model (*Figure 4a*) would need 6 chains and no water, a rather tight squeeze and one would also have expected the basal plane of the unit cell to reduce accordingly,

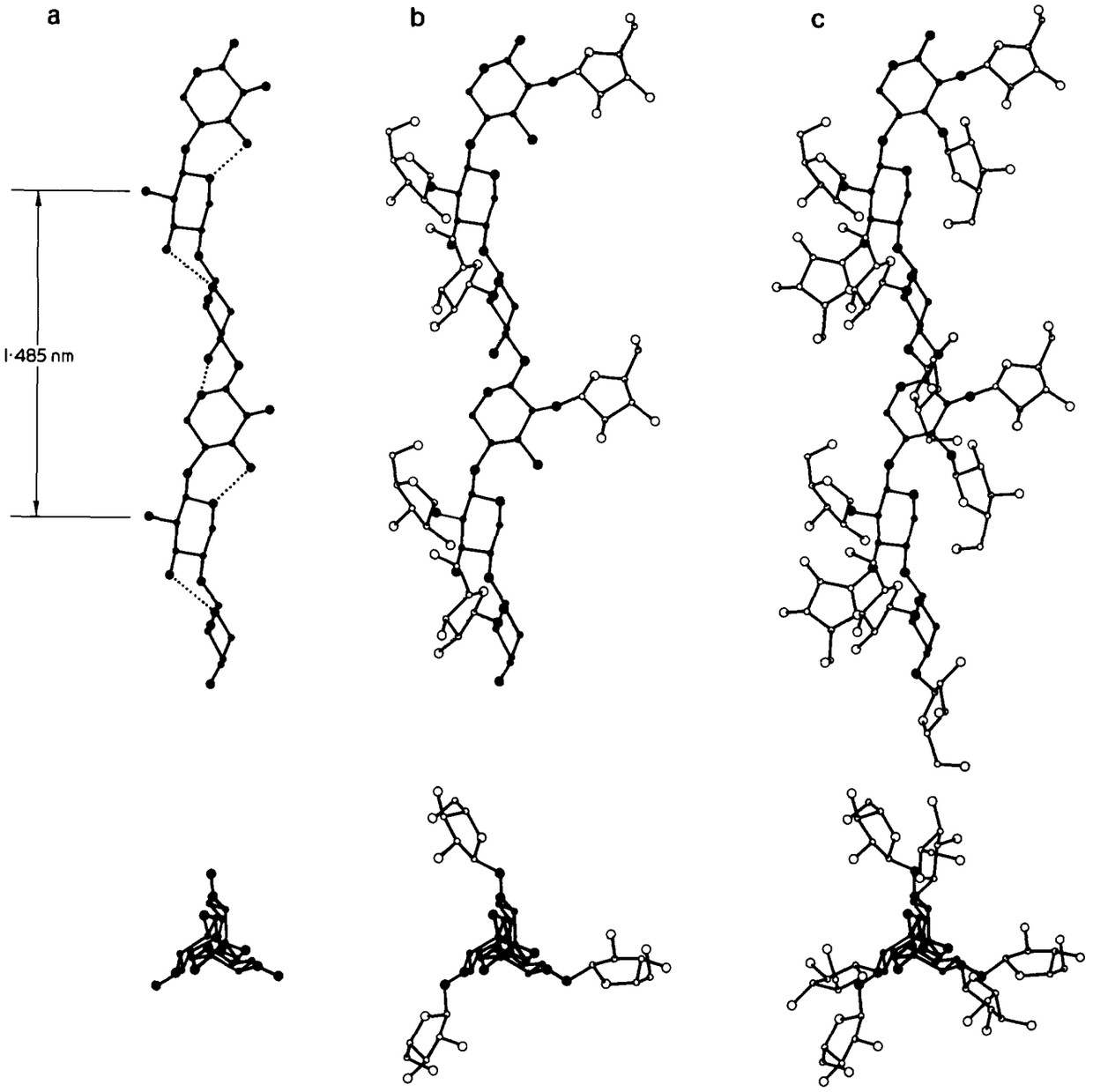

Figure 4 Projections of proposed molecular conformation. Hydrogen bonds are indicated by dotted lines. (a) Xylan backbone; (b) backbone and one L-arabinose side group; (c) backbone and two L-arabinose side groups

contrary to observation. (The next quantum jump down in chain number would be to 3 chains which would require more water than reasonably expected. Also this model is the least compatible with the chemical evidence.) The model with one arabinose side group looks reasonable; 2 chains can pass through the unit cell and still allow for a reasonable amount of water of crystallization to maintain local order even at backing pump pressure. The model with 2 arabinose side groups would again need some squeezing and no water to fit into the unit cell. (It should be remembered that measured densities are usually somewhat lower than ideal (or calculated) owing to imperfections in the sample and so some leeway is possible in these estimations.) One could, of course, only put 1 chain in the unit cell for this model and include water. Such a model would be fairly compatible with the chemical evidence. It is even possible to place a single chain with 3 saccharide units attached without violating density criteria. In this case one would need to attach an additional saccharide unit on to the end of one of the side arabinose units shown in *Figure 4c*.

At this level of the analysis we cannot easily distinguish between any of the last three models: i.e., containing 1, 2 or 3 saccharide units attached to the backbone. However the model shown in *Figure 1a* does not fit the evidence in a convincing manner and can probably be ruled out. We have attempted to take the analysis a stage further by calculating the cylindrically-averaged Fourier transforms of the various models and comparing the results with the observed diffraction intensities. However with such a strong interference function superimposed on the observed Fourier transform (and in one case with 2 chains in the unit cell) we were unable to make any clear delineation between the

Table 1 Fractional coordinates of the asymmetric repeating unit for the most acceptable 3_2 helical conformation

Atom	Fractional coordinates		
	x	*y*	*z*
D-Xylan residue:			
O(1)	0.0846	0.1182	0.0184
C(1)	0.0968	0.0652	0.0932
C(2)	0.1337	0.1454	0.1724
C(3)	0.1368	0.0875	0.2584
C(4)	0.0258	−0.0182	0.2731
C(5)	−0.0072	−0.0905	0.1888
O(5)	−0.0098	−0.0286	0.1122
O(2)	0.2401	0.2385	0.1536
O(3)	0.1606	0.1606	0.3333
1→2 linked Arabinofuranose residue:			
C(2)	0.2419	0.3369	0.1232
C(3)	0.2710	0.4268	0.1963
C(4)	0.3298	0.5340	0.1422
C(5)	0.3912	0.5058	0.0710
C(6)	0.4053	0.5621	−0.0188
O(4)	0.4041	0.6267	0.1957
O(2)	0.3271	0.3866	0.0589
O(3)	0.1821	0.4145	0.2494
O(6)	0.3049	0.5519	−0.0524
1→3 linked Arabinofuranose residue:			
C(2)	0.2477	0.1737	0.3921
C(3)	0.3651	0.2691	0.3667
C(4)	0.4214	0.3060	0.4581
C(5)	0.3254	0.2898	0.5185
C(6)	0.3323	0.2586	0.6147
O(4)	0.5096	0.4184	0.4577
O(2)	0.2254	0.2024	0.4771
O(3)	0.4234	0.2439	0.3029
O(6)	0.3536	0.1689	0.6216

models examined. If some further chemical analyses were performed and supplemented with n.m.r. observations giving more confidence to the chemical repeat within the crystalline domains then a structure determination would be possible.

The *Watsonia* polysaccharide clearly exhibits domains containing a regularly repeating sequence. The chain is able to form a stereochemically feasible 3_2 helical conformation similar to that proposed for xylan hydrate[7] and incorporating an intraresidue stabilizing hydrogen bond.

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