Crystal Structure of the Lectin from *Dioclea grandiflora* Complexed with Core Trimannoside of Asparagine-linked Carbohydrates*

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The seed lectin from *Dioclea grandiflora* (DGL) has recently been shown to possess high affinity for 3,6-di-D-mannopyranosyl-a-D-mannopyranose, the core trimannoside of asparagine-linked carbohydrates, but lower affinity for biantennary complex carbohydrate. In the previous paper, the thermodynamics of DGL binding to deoxy analogs of the core trimannoside and to a biantennary complex carbohydrate were determined by isothermal titration microcalorimetry. The data suggest that DGL recognizes specific hydroxyl groups of the trimannoside similar to that of the jack bean lectin concanavalin A (ConA) (Gupta, D. Dam, T. K., Oscarson, S., and Brewer, C. F. (1997) *J. Biol. Chem.* 272, 6388–6392). However, the thermodynamics of DGL binding to certain deoxy analogs and to the complex carbohydrate are different from that of ConA. In the present paper, the x-ray crystal structure of DGL complexed to the core trimannoside was determined to a resolution of 2.6 Å. The overall structure of the DGL complex is similar to the structure of the ConA-trimannoside complex (Naismith, J. H., and Field, R. A. (1996) *J. Biol. Chem.* 271, 972–976). The location and conformation of the bound trimannoside as well as its hydrogen-bonding interactions in both complexes are nearly identical. However, differences exist in the location of two loops outside of the respective binding sites containing residues 114–125 and 222–227. The latter residues affect the location of a network of hydrogen-bonded water molecules that interact with the trisaccharide. Differences in the arrangement of ordered water molecules in the binding site and/or protein conformational differences outside of the binding site may account for the differences in the thermodynamics of binding of the two lectins to deoxy analogs of the trimannoside. Molecular modeling studies suggest how DGL discriminates against binding the biantennary complex carbohydrate relative to ConA.

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Lectins derived from the seeds of leguminous plants are a well known group of carbohydrate-binding proteins (cf. Refs. 1 and 2). Concanavalin A (ConA),† the lectin from *Canavalia enisformis* (jack bean), is the best known member of the Leguminosae lectins because of its numerous biological applications related to its carbohydrate binding activity (cf. Ref. 3). The affinity of ConA for simple saccharides possessing Man or Glc residues has been known for over 60 years (cf. Ref. 4). However, the high affinity of ConA for certain N-linked carbohydrates is due to its specificity for 3,6-di-D-(a-D-mannopyranosyl)-a-D-mannopyranose, the core trimannoside present in N-linked carbohydrates (5, 6). The binding epitopes of the trimannoside have recently been investigated by isothermal titration microcalorimetry using deoxy analogs of methyl 3,6-di-D-(a-D-mannopyranosyl)-a-D-mannopyranoside (1 in Fig. 1) (7, 8). The results indicate the involvement of specific hydroxyl groups on all three residues of the trimannoside in binding to ConA. Importantly, the solution isothermal titration microcalorimetry data agree with the recently reported 2.3-Å x-ray crystal structure of ConA complexed to the trimannoside (9, 10).

A lectin with similar physical properties to ConA has been isolated from the seeds of *Dioclea grandiflora* lectin (DGL) (Brazilian mucuna tree) (11, 12). Like ConA, DGL is a Man/Glc-specific member of the Diocleinae subtribe of Leguminosae lectins (13). DGL has 81% amino acid sequence identity with ConA (Fig. 2) (14, 15), and the amino acid residues of the sugar binding site are conserved in both lectins (Tyr12, Pro13, Asn14, Thr15, Asp16, Leu99, Asp208, and Arg228). Recently, DGL was shown to bind with high affinity to the core trimannoside (16, 17). Furthermore, hemagglutination inhibition studies using deoxy analogs of 1 suggested that DGL and ConA recognize similar epitopes of the trisaccharide (17). However, DGL was also shown to possess relatively weak affinity for a biantennary complex carbohydrate (2 in Fig. 1), while ConA shows high affinity for the oligosaccharide (13, 17). In the previous paper (18), isothermal titration microcalorimetry measurements showed differences in the thermodynamics of DGL and ConA binding to certain deoxy analogs of 1 as well as their thermodynamics of binding to 2 (8, 18). Differences have also been reported in the biological properties of both lectins, including different histamine release activities in rat peritoneal mast cells (19). Indeed, the histamine release activities of nine Diocleinae subtribe lectins including DGL and ConA have been recently shown to correlate with their relative affinities for pentasaccharide 2 (13). Since DGL and ConA showed the lowest and highest affinities, respectively, for 2 (13) and the lowest and highest histamine release activities, respectively, a comparison of structures of these two Diocleinae lectins could provide insight into the molecular basis of their
different binding specificities and biological activities. In the present study, the crystal structure of DGL complexed with 1 has been determined to a resolution of 2.6 Å. The results allow comparison of the structure of the DGL-trimannoside complex with the structure of the corresponding ConA complex. The possible structural basis for the differences in the thermodynamic binding data of the two lectins for certain deoxy analogs of 1 (18) is also addressed. Last, molecular modeling has been used to investigate differences in the binding specificities of the DGL and ConA for 2 and hence their biological activities.

**EXPERIMENTAL PROCEDURES**

DGL was isolated from *D. grandiflora* seeds obtained from northeastern Brazil (Albano Ferreira Martin Ltd., Sao Paulo, Brazil) as described previously (11), and the amino acid sequence was verified. Crystals of DGL were produced at 4 °C using the hanging drop vapor diffusion technique (20). The well contained 0.6 M NaCl, 50 mM Hepes, pH 7.2, 5 mM CaCl\(_2\), and 5 mM MnCl\(_2\). The drop contained 25 mg/ml protein and 5 mM sugar.

An x-ray diffraction data set was collected to a resolution of 2.6 Å at room temperature using a single crystal of size 0.4 × 0.5 × 0.8 mm. Diffractometer data to 2.4 Å were included. The x-ray source used was a Rigaku RU-200 generator with a copper rotating anode (CuK\(_\alpha\) = 1.54 Å). The x-ray detector used was a Siemens X-1000A multiwire. The raw data were integrated and scaled with the SADIE/SAINT software package (Siemens Inc., Madison, WI). Data collection statistics are described previously (11), and the amino acid sequence was verified. 2

## RESULTS AND DISCUSSION

The x-ray crystal structure of DGL complexed with trimannoside 1 has been determined to a resolution of 2.6 Å. The thermodynamics of DGL binding to 1 and deoxy analogs of 1 obtained in the previous paper (18) can now be compared with the structure of the DGL complex with 1. In addition, the structural and thermodynamic data for DGL can be compared with corresponding structural (9, 10) and thermodynamic data for ConA (8). Determination of the structure of the DGL complex with 1 also permits molecular modeling of the interactions of the lectin with complex carbohydrate 2 (Fig. 1) and a comparison with the recently determined structure of ConA complexed to 2 (25).

**Structure of DGL**—The overall structure of DGL determined by x-ray crystallography consists of four identical 237-residue subunits, each of which fold into two \( \beta \)-sheets (Fig. 3A). The DGL tetramer is formed by the combination of two perpendicular two-fold symmetry axes, creating a molecule with 222 symmetry. The structural fold and subunit association of DGL consists of residues 1–237. The subunits of the asymmetric unit are related by a nearly perfect (180.1°) noncrystallographic two-fold axis (only a 0.23-Å deviation in \( \alpha \)-carbon position). In addition, the two subunits of the asymmetric unit are related to two other subunits through a perpendicular crystallographic two-fold axis, with the resulting tetramer possessing 222 symmetry.

Initial phases for the structure factor data were obtained using the molecular replacement technique. The atomic coordinates of a single subunit of ConA (Protein Data Bank, entry code 5CNA) were used as the search model (with all sugar atoms and water molecules removed). Solutions for the rotation function were obtained using AMoRE (21) incorporated into the CCP4 package (22). Subsequent Patterson correlation refinement of the rotation function solutions and determination of the translation function solutions were performed with XPLOR (23).

Additional rigid body refinement with XPLOR gave an \( R \) value of 37.7%, and \( R_{	ext{free}} \) of 36.3%. Electron density maps were then calculated, and the location of bound trimannoside 1 could be clearly identified, verifying the correctness of the molecular replacement solution.

The appropriate amino acid changes were made to convert the molecular model replacement of ConA to DGL, and simulated annealing refinement was performed (\( R \) value = 26.7%; \( R_{	ext{free}} \) = 34.3%) using XPLOR. Throughout all refinements, each subunit of the dimer was allowed to refine independently, i.e. noncrystallographic symmetry restraints were not applied. The correctness of the model was verified with a series of XPLOR simulated annealing omit maps. Two molecules of 1 (one for each subunit of the dimer) and 190 ordered water molecules were placed in the model over the course of cycling between manual model building using the program CHAIN (24) and XPLOR positional refinement followed by individual temperature factor refinement (final \( R \) value = 18.9%, final \( R_{	ext{free}} \) = 25.2%). Model refinement statistics are in Table I.

In order to compare the final structure of the DGL-trimannoside complex with the ConA-trimannoside complex (PDB entry code 5CVN (9) and the ConA-pentasaccharide (2) complex (PDB entry code 1TEI (25)), the atomic coordinates of the ConA complexes were superimposed onto that of DGL using the Dabsch method (26, 27) applied to the \( \alpha \)-carbon positions. In order to compute the positions of the ordered water molecules between the DGL-trimannoside complex and the ConA-trimannoside complex, a composite set of coordinates was created for a single subunit of the ConA-trimannoside complex by combining all of the water molecule positions from different superimposed subunits of the asymmetric unit and then eliminating redundant positions.

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2 C. F. Brewer and X.-J. Tang, unpublished results.
binding site of DGL, which is a negatively charged pocket, while the central Man and α(1,3) Man residues fit into a shallower negatively charged groove. As shown in Fig. 5A, two conserved residues, which contribute to the negatively charged surface, Asp16 and Asp208, directly interact with the trimannoside. The side chain of Arg228 forms the only positively charged area on this surface. A conserved ordered water molecule (W39) is involved in the indirect interaction between the side chain of Arg228 and 1, which is also observed in ConA (9). The presence of conserved ordered water molecules has also been observed in other members of the family of leguminous plant lectins (cf. 29).

Comparison of the Thermodynamic and Structural Data of DGL and ConA—Fig. 6 shows the changes in the enthalpy (ΔDH) of binding of DGL to deoxy analogs of 1, relative to 1 (18) and ConA (8). Changes of −1 kcal mol⁻¹ or greater in the ΔDH values for individual deoxy analogs suggest loss of binding at the position of substitution. The predicted interactions of specific hydroxyls of 1 with ConA derived from the ΔDH data for lectin binding to the deoxy analogs has recently been shown to agree well with the x-ray crystal structure of the ConA complex with 1 (Fig. 5) (9). The x-ray structure in Fig. 5A and the thermodynamic data in Fig. 6 show that the 3-, 4-, and 6-hydroxyls on the α(1,6) Man arm, the 2- and 4-hydroxyls of the central Man residue, and the 3- and 4-hydroxyls of the α(1,3) Man residue of 1 are involved in binding to ConA.

The overall pattern of ΔDH data for DGL binding to the deoxy analogs of 1 is similar to that for ConA (Fig. 6). Furthermore, with one exception, all of the interactions of DGL with 1 indicated by the solution thermodynamic data are present in the crystal structure of the DGL complex (Fig. 5A). The exception involves interactions at the 2-OH on the α(1–6) arm of 1. Unlike ConA, which shows a small 0.7 kcal mol⁻¹ change in ΔDH for the 2-deoxy α(1,6) arm analog of 1 (relative to 1), the thermodynamic data for DGL indicates a significant change in ΔDH (3.4 kcal mol⁻¹) for the same analog, suggesting the involvement of the 2-hydroxyl on the α(1,6) arm of 1 in binding. The crystal structure of the DGL complex with 1, however, shows no direct protein-carbohydrate binding interactions at this site. The reduction in binding indicated by the thermodynamic data for the α(1,6) Man 2-deoxy analog appears to reflect indirect binding to the protein of the 2-hydroxyl at this position in 1 via a water molecule in the binding site. Details of this interaction are discussed below.

Although the overall pattern of ΔDH data for DGL in Fig. 6 is similar to that for ConA, the magnitude of the ΔDH data for certain analogs of the α(1,6) arm of 1 is different. Thus, the 3-, 4-, and 6-deoxy α(1–6) Man analogs of 1 possess ΔDH values that are nearly twice as great for DGL (−6.1 kcal mol⁻¹) as for ConA (−3.2 kcal mol⁻¹). Table II lists the hydrogen bonding and polar contact residue assignments and distances for DGL and ConA with 1. It is clear that the two protein complexes are nearly identical, both in terms of the residues involved in binding to 1 as well as the number of hydrogen bonds and their distances to 1. Therefore, differences in the ΔDH values of the two lectins for the 3-, 4-, and 6-deoxy α(1–6) Man analogs of 1 are not due to differences in the direct lectin-carbohydrate hydrogen-bonding interactions (Fig. 5A). Furthermore, the −2.9 kcal mol⁻¹ difference in the average ΔDH values for the 3-, 4-, and 6-deoxy α(1–6) analogs binding to DGL (6.1 kcal mol⁻¹) and to ConA (3.1 kcal mol⁻¹) (Fig. 6) is nearly the same as the −2.7 kcal mol⁻¹ difference in ΔDH values for the 2-deoxy α(1,6) analog binding to DGL (3.4 kcal mol⁻¹) versus to ConA (0.7 kcal mol⁻¹) (Fig. 6). This suggests a common mechanism underlying the differences in the thermodynamics of binding of all four α(1–6) deoxy analogs to the two lectins. In this regard, it is interesting that the average ΔDH values for the 3-, 4-, and

### Table II

| Lectin | α(1,6) Man Residue | α(1,3) Man Residue | α(1,6) Trisaccharide | α(1,3) Trisaccharide |
|--------|--------------------|--------------------|----------------------|----------------------|
| DGL    |                    |                    |                      |                      |
| ConA   |                    |                    |                      |                      |

### Fig. 1

Structures of methyl 3,6-di-O-(α-(1,6)-mannopyranosyl)-α-(1,3)-mannopyranoside (1) and biantennary complex carbohydrate (2).

### Fig. 2

Amino acid sequence alignment between DGL and ConA displaying 81% identity.
6-deoxy α(1–6) analogs are nearly the same magnitude for each lectin. The relatively constant ΔΔH values of the 3-, 4-, and 6-deoxy α(1–6) analogs for each lectin occur despite the different number and type of hydrogen bonds at each respective position in the parent trisaccharide. This further suggests a common thermodynamic mechanism of binding of the 3-, 4-, and 6-deoxy α(1–6) analogs for each lectin.
and 6-deoxy α(1–6) analogs to each lectin.

Fig. 7 shows the superposition of the atomic coordinates of the binding site regions of DGL and ConA for 1. There are four amino acid differences found within a more extended area surrounding the ligand, which are residue 21 (Asn in DGL, Ser in ConA), residue 168 (Asn in DGL, Ser in ConA), residue 205 (Glu in DGL, His in ConA), and residue 226 (Gly in DGL, Thr in ConA). These residues are indirectly involved in ligand binding. They interact with a network of hydrogen-bonded ordered water molecules, which in turn directly interact with 1.

As shown in Fig. 7, the largest deviation in ordered water molecule organization appears near the shift in residues 222–227. In ConA, the side chain of Thr226 is oriented to make direct hydrogen bond interactions with the ordered water molecule network, and the smaller side chain of Ser168 accommodates a water between itself and Thr226. In DGL, residue 226 is a Gly, and ordered water molecules fill the space of the missing side chain. Importantly, this network of hydrogen-bonded ordered water molecules directly interacts with the hydroxyl oxygens at positions 2 and 3 of the α(1,6) arm of 1, and thermodynamic data indicate that the strength and specificity of DGL and ConA binding differ at these positions in 1 (18). Thus, the differences observed in the ∆ΔH values for DGL and ConA binding to the 2-, 3-, 4-, and 6-deoxy α(1–6) Man analogs of 1 may be due to altered structural water molecules in this region of the binding sites of the lectins.

Alternatively, DGL and ConA may undergo different conformational transitions upon binding 1 and the deoxy analogs that may contribute to the observed differences in the ∆ΔH values of the analogs. In any case, it is clear that differences in the ∆ΔH values of the 2-, 3-, 4-, and 6-deoxy α(1–6) Man analogs of 1 binding to DGL and ConA are not due to direct protein-ligand interactions.

**Nonlinear Contributions of ∆ΔH and ∆ΔG of the Deoxy Ana-

**TABLE II**

| Sugar residue | Protein/Water | DGL distances | ConA distances |
|---------------|--------------|---------------|---------------|
| α(1,6) Man    |              |               |               |
| O-3           | Arg228 N     | 2.9           | 2.9           |
| O-4           | Asn14 ND2    | 2.8           | 2.9           |
| O-4           | Asp208 OD2   | 2.9           | 2.7           |
| O-4           | Arg226 N     | 3.2 (3.5)     |               |
| O-5           | Leu89 N      | 2.9           | 2.9           |
| O-6           | Leu99 N      | 2.9           | 3.1           |
| O-6           | Tyr101 N     | 3.0           | 3.1           |
| O-6           | Asp208 OD1   | 3.3           | 2.9           |
| Central Man   |              |               |               |
| O-2           | OW39         | 2.8           | 2.6           |
| O-2           | Asp16 OD1    | (3.7)         | 3.1           |
| O-4           | Tyr12 OH     | 2.7           | 2.8           |
| α(1,3) Man    |              |               |               |
| O-3           | Pro13 O      | 3.0           | 2.9           |
| O-3           | Thr15 N      | 2.9           | 2.8           |
| O-3           | Thr15 OG     | 2.9           | 2.9           |
| O-4           | Thr15 OG1    | 2.6           | 3.1           |
| O-4           | Asp16 N      | 2.9           | 3.0           |
| O-4           | Asp16 OD1    | 2.9           | 3.3           |
logs of 1 to Binding—The x-ray structure of DGL with 1, together with the ΔΔH data shown in Fig. 6, demonstrates that the enthalpy contributions to binding of individual hydroxyl groups of 1 determined from the corresponding monodeoxy analogs are nonlinear. For example, the magnitude of loss in ΔΔH values for DGL binding to the 3-, 4-, and 6-deoxy α(1–6) Man analogs of 1, relative to 1, are nonlinear. The ΔΔH value for the 3-deoxy α(1–6) Man analog is 5.5 kcal mol⁻¹, and Table II shows that there is a single hydrogen bond from Arg228 Nt to the 3-OH of 1 at this position. On the other hand, the ΔΔH value for the 4-deoxy α(1–6) Man analog is 5.3 kcal mol⁻¹, and

Table II shows that there are three hydrogen bonds from the protein to the 4-OH at this position, involving Asn14¹ ND2, Asp208 OD2, and Arg228 N. Similar nonlinear relationships exist between the magnitude of the ΔΔH data for the other deoxy analogs and the distances of the hydrogen bonds and the nature of the donor-acceptor pairs in the hydrogen bonds in Table II. Thus, the observed ΔΔH value for each deoxy analog does not represent direct measurements of the enthalpy contribution to binding at each hydroxyl group of 1, but rather differential contributions of the solvent and protein to the thermodynamics of binding of the analogs. Similar conclusions can be made from the ΔΔG values for each analog (18). These findings support similar conclusions regarding the nonlinear nature of the observed ΔΔH values of the deoxy analogs binding to DGL based on the relative loss in ΔΔH of a tetra(deoxy) analog of 1 as compared with the sum of the losses in ΔΔH of the corresponding monodeoxy analogs binding (18). These results confirm similar conclusions regarding the binding of ConA to deoxy analogs of 1 (8).

Binding of DGL and ConA to Complex Carbohydrate 2—While DGL and ConA possess similar high affinities for 1, isothermal titration microcalorimetry measurements show that ConA possesses 30-fold higher affinity for 2 than DGL (18, 30). The recently determined x-ray crystal structure of ConA complexed with 2 reveals that the β(1,2) GlcNAc residue on the α(1,6) arm of the pentasaccharide fits into an extended groove of ConA and makes hydrogen-bond contacts on both sides of the sugar ring (25) (Fig. 8). The specific interactions of the β(1,2) GlcNAc residue on the α(1,6) arm are between the 3-hydroxyl and the side chain of Thr226, the 4-hydroxyl and the backbone carbonyl oxygen of Gly224, and the 7-hydroxyl and the side chain of Ser168 (Table III). Superposition of the x-ray crystal structure of DGL bound to 1 onto that of ConA complex with 2 reveals that proper contacts between DGL and the penta­saccharide 1 analogs. The solid bars show the DGL data, and the hatched bars represent the ConA data (18). The errors associated with ΔΔH are ±0.2–0.4 kcal mol⁻¹.

FIG. 6. ΔΔH values of DGL and ConA binding to deoxy trimannoside 1 analogs. The solid bars show the DGL data, and the hatched bars represent the ConA data (18). The errors associated with ΔΔH are ±0.2–0.4 kcal mol⁻¹.

FIG. 7. Stereo view of the superposition of the trimannoside binding region of the DGL (cyan) and ConA (violet). The trimannoside is shown as a green and red ball-and-stick model. Only the locations where ConA deviates from DGL are visible in the figure. The amino acids differ in only four places: in DGL, Asn2¹, Asp186, Glu205, and Gly226; in ConA, Ser2¹, Ser168, His205, and Thr226. In ConA, the side chain of Thr226 is turned outward toward a network of hydrogen-bonded ordered water molecules, and the backbone of residues 222–227 is shifted by almost 3.0 Å from the same region in DGL. This structural difference results in a slightly differing water network between the two lectins. This figure was generated with the program Insight II.
charide are prevented due to key amino acid differences at residue 226 (Thr in ConA, Gly in DGL) and at residue 168 (Ser in ConA, Asn in DGL) and the shift in the backbone of residues 222–227 (Fig. 8). Superposition of the structure of trimannoside 1 in DGL with the structure of complex carbohydrate 2 bound to ConA (25) is also shown in Fig. 8. Importantly, the core trimannoside moiety of 2 bound to ConA deviates by less than 0.5 Å from the position of 1 bound to DGL, indicating similar binding of this moiety in both complexes. However, the β1,2 GlcNAc residue on the α(1–6) arm of 2 modeled into DGL reveals contacts different from those observed in the ConA complex. In DGL, the side chain is missing from residue 226, and therefore no hydrogen-bond can be formed to the O-3 of the β1,2 GlcNAc residue. In addition, the backbone carbonyl oxygen of Gly224 is too far to make a proper hydrogen bond to the O-4 of the GlcNAc. These differences in the interac-
tions of the β1,2 GlcNAc residue on the α(1–6) arm of 2 in DGL appear to explain the 30-fold lower affinity of the lectin relative to ConA. The observed interactions of the β1,2 GlcNAc residue on the α(1–3) arm of 2 in DGL (Fig. 8) suggest little interference in binding of this region of the complex.

The contact differences for the β1,2 GlcNAc residue on the α(1,6) arm of 2 in DGL and ConA also explain why DGL fails to bind the disaccharide GlcNAcβ1–2Man, while ConA binds well to the disaccharide (17). The GlcNAcβ1–2Man moiety on the α(1,6) arm of 2 occupies the so-called monosaccharide binding site in DGL. Therefore, the same unfavorable interactions would occur for the disaccharide. The favorable interactions observed in the ConA complex with 2 at this site explains the ability of the lectin to bind the disaccharide. These differences also appear to explain why ConA can bind longer chain analogs of 2, while DGL cannot. 3

Summary—The x-ray crystal structure of D. grandiflora lectin complexed with trimannoside 1 has been determined to a resolution of 2.6 Å. The thermodynamics of DGL binding 1 and deoxy analogs of 1 (18) have been compared with the structure of the DGL complex with 1. With the exception of the lack of direct protein-carbohydrate contacts at the 2-OH position on the α(1,6) arm of 1 suggested by the thermodynamic data, the x-ray structure of the complex agrees with the thermodynamic data. The direct binding site contacts between DGL and 1 observed in the x-ray crystal structure are nearly identical to those for ConA and the core trimannoside (9). Differences in the thermodynamics of binding of DGL and ConA to 1 and deoxy α(1,6) Man analogs of 1 are not due to differences in direct lectin-carbohydrate interactions. Rather, these differences may be due to the altered...
location of ordered water molecules in the binding site and/or conformational changes outside of the binding site. Differences in the binding specificities of DGL and ConA for complex carbohydrate 2 appear to be due to relatively unfavorable contact interactions of the β(1,2) GlcNAc residue on the α(1–6) arm of 2 in DGL.

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