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Structural Studies on 2-Acetamido-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine and 2-Acetamido-6-*O*-(α-L-fucopyranosyl)-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine by 360-MHz Proton-Magnetic-Resonance Spectroscopy

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The 360-MHz proton magnetic resonance spectra of 2-acetamido-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine (GlcNAcβ1 → Asn) and 2-acetamido-6-*O*-(α-L-fucopyranosyl)-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine (Fucα1 → 6GlcNAcβ1 → Asn) in deuterium oxide were completely interpreted. The chemical shifts and coupling constants were refined by simulation of the spectra. By means of an adapted Karplus equation the pyranose ring conformation of the sugars was calculated.

The change of the geminal coupling constant $J_{6a,6b}$ in the *N*-acetylglucosamine residue of Fucα1 → 6GlcNAcβ1 → Asn with respect to GlcNAcβ1 → Asn is proposed to be characteristic for the (1 → 6) glycosidic linkage.

In recent years considerable progress has been made in the elucidation of the primary structure of the carbohydrate chains in glycopeptides [1]. The interaction of these glyco parts and proteins or other carbohydrate chains, seems to be an important aspect of the biological role of glycoproteins. For example, the life span of circulating cells and glycoproteins is to a large extent determined by the intactness of the carbohydrate chains. This implies that destroying systems are able to recognize the molecular details of these carbohydrate moieties [2,3]. Probably, molecule-membrane interactions are involved in these processes. Carbohydrate chains of glycoproteins and glycolipids may be involved in membrane-membrane interactions (*cf.* Sharon [4]).

To gain insight into the molecular events which occur in these interaction processes, knowledge about the spatial structure in solution of the carbohydrate parts of glycoconjugates is indispensable. It is the aim of our study to investigate to what extent high-resolution proton magnetic resonance (^{1}H-NMR) spectroscopy can furnish relevant data in this respect.

Since carbohydrate chains of glycoproteins are usually rather complex structures, we start with the investigation of relatively simple parts of these molecules, followed by the study of compounds of gradually increasing complexity.

In this paper some structural details are described of GlcNAcβ1 → Asn which represents a frequently occurring type of connexion between carbohydrate and polypeptide chains. Furthermore we report on the structure of Fucα1 → 6GlcNAcβ1 → Asn, which forms part of the carbohydrate chains in immunoglobulins like IgA [5,6], IgE [5,6] and IgG [6–9] and in porcine thyroglobulin (T. Osawa, personal communication).

MATERIALS AND METHODS

Chemicals

GlcNAcβ1 → Asn was obtained from Cyclochemical, Los Angeles, California 90001; Fucα1 → 6GlcNAcβ1 → Asn was isolated from urine of a patient with fucosidosis [10].

Deuterium oxide, for NMR spectroscopy, degree of deuteration 99.75%, was obtained from E. Merck, Darmstadt; sodium 2,2-dimethyl-2-silapentane-5-sulfonate from Merck, Sharp and Dohme, Canada Limited (Montreal, Canada).

Abbreviations. Fuc, fucose; GlcNAc, *N*-acetylglucosamine; Asn, asparagine; NMR, nuclear magnetic resonance.

Proton Exchange

GlcNAcβ1 → Asn and Fucα1 → 6GlcNAcβ1 → Asn were dissolved in ^2H_2O and after standing at room temperature for 6 h lyophilized. This procedure was repeated five times to get a high degree of proton exchange. Finally the samples were dissolved in ^2H_2O at concentrations of 0.5 and 0.2 M respectively.

Proton Magnetic Resonance Spectroscopy

^{1}H-NMR spectroscopy was performed on a Bruker HX-360 spectrometer, operating at 360 MHz in the continuous wave mode at a probe temperature of 25 °C.

Spectrum Simulation

Spectrum simulations were run on a 16 k Varian 620 i computer coupled with the Varian XL-100 spectrometer using the modified spin simulation program SIMEQ II-16/3 (M. J. A. de Bie, personal communication). Chemical shifts are given relative to sodium 2,2-dimethyl-2-silapentane-5-sulfonate (indirectly to acetone in ^2H_2O; $\delta = 2.225$ ppm) with an accuracy of 0.005 ppm. The accuracy of the coupling constants is about 0.05 Hz.

The first-order spectral parameters were checked and refined by calculation of the theoretical spectra until a good agreement was obtained between observed and calculated spectra. The vicinal and geminal

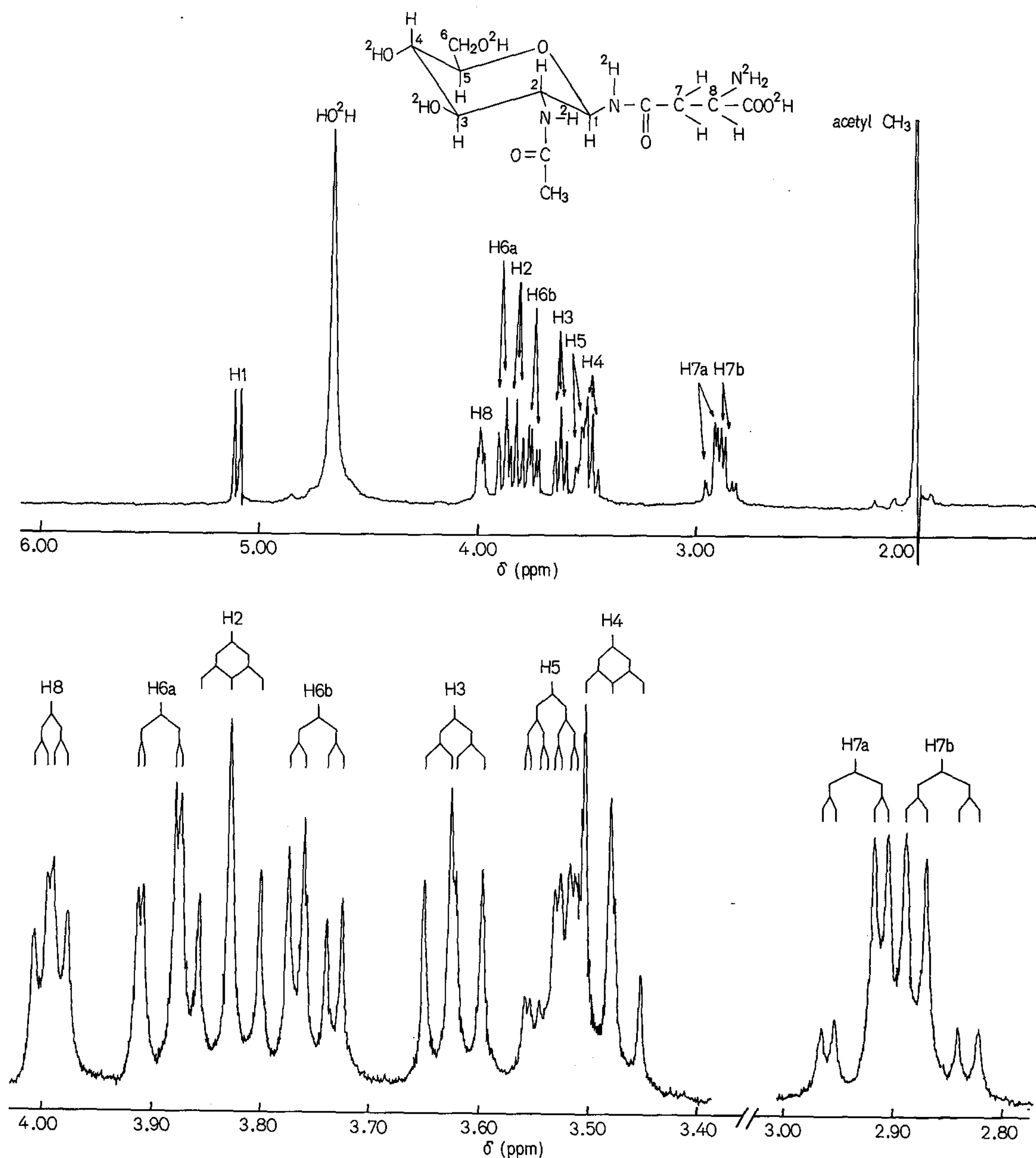


Fig. 1. ^{1}*H-NMR spectrum of GlcNAcβ1 → Asn in* ^2H_2O *at 360 MHz*

coupling constants were taken positive and negative respectively.

RESULTS AND DISCUSSION

The 360-MHz ^{1}H-NMR spectra of GlcNAcβ1 $\rightarrow$Asn and Fucα1$\rightarrow$6GlcNAcβ1$\rightarrow$Asn, recorded in ^2H_2O, are given in Fig. 1 and 2. The assignments of the proton resonances, together with the splitting patterns are indicated in the figures. The refined ^{1}H-NMR data are summarized in Tables 1 and 2.

The coupling constant $J_{1,2}$ (9.8 Hz) in N-acetylglucosamine of both compounds is indicative of a β-glycosidic linkage [11]. The anomeric proton H_1 resonates at a rather low field in view of its axial position; the chemical shift lies in the region usually

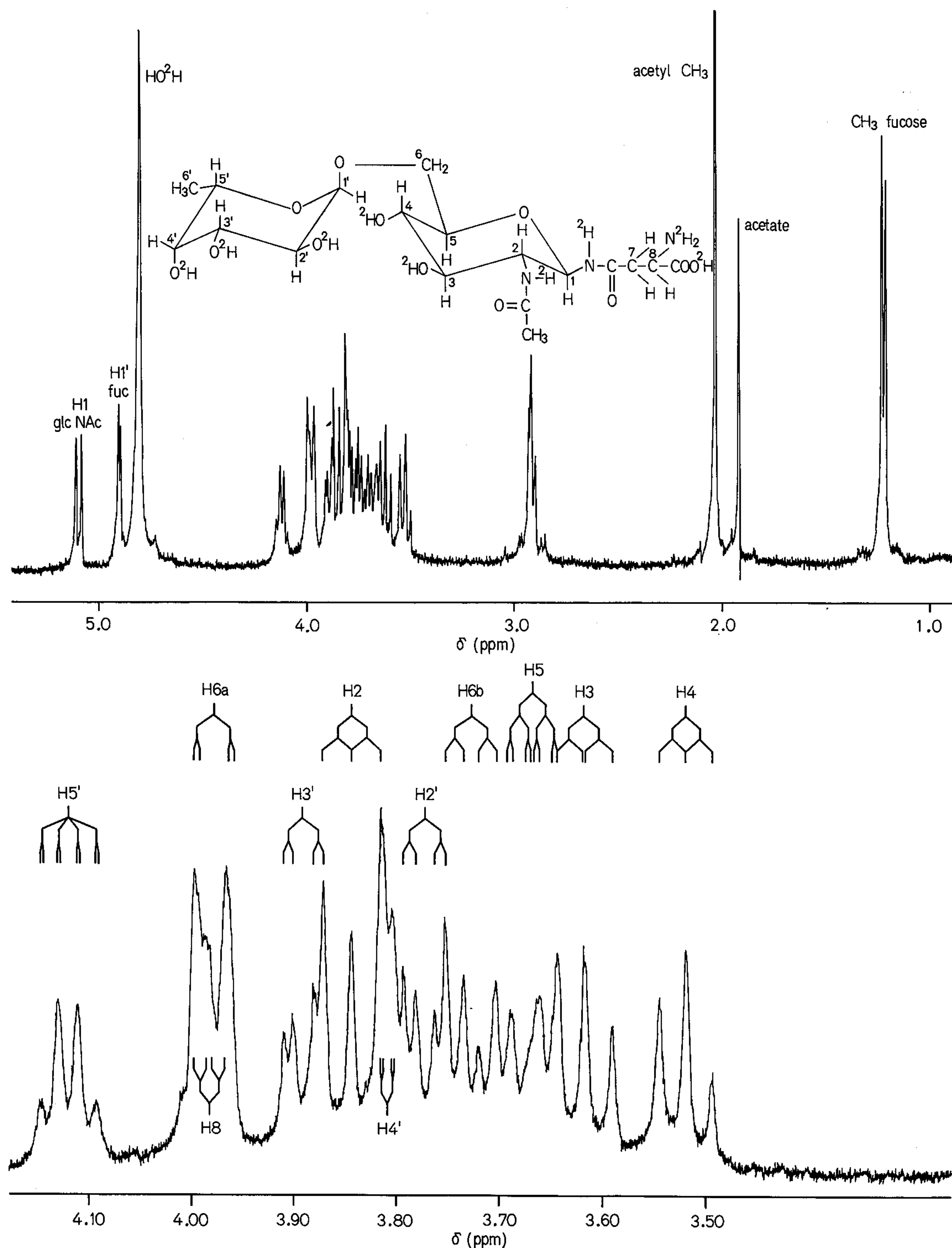
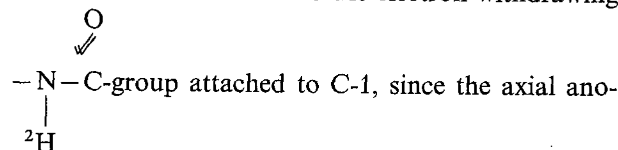


Fig. 2. *^{1}H-NMR spectrum of Fucα1$\rightarrow$6GlcNAcβ1$\rightarrow$Asn in ^2H_2O at 360 MHz*

found for equatorial anomeric protons. Probably this feature must be ascribed to the electron-withdrawing



$-\mathrm{N(^2H)}-\mathrm{C(=O)}$-group attached to C-1, since the axial anomeric proton of *N*-acetylglucosamine, free and *O*-glycosidically bound, is found in the region $\delta = 4.50-4.75$ ppm. The equatorial anomeric proton of free *N*-acetylglucosamine in ^2H_2O resonates at $\delta = 5.18$ ppm (L. Dorland, unpublished results). On the basis of the coupling constant $J_{1',2'}$ (3.75 Hz) in the fucose residue, it can be concluded that fucose is α-glycosidically bound.

Table 1. *Chemical shifts for GlcNAcβ1 → Asn and Fucα1 → 6GlcNAcβ1 → Asn in* ^2H_2O

Residue	Proton	Chemical shift of GlcNAcβ1 → Asn	Chemical shift of Fucα1 → 6GlcNAcβ1 → Asn
		ppm	
L-Fucose	1′		4.90
	2′		3.78
	3′		3.89
	4′		3.81
	5′		4.12
	6′		1.21
N-Acetyl-D-glucosamine	1	5.09	5.09
	2	3.83	3.84
	3	3.62	3.62
	4	3.48	3.53[a]
	5	3.53	3.67[a]
	6a	3.89	3.98[a]
	6b	3.75	3.73
L-Asparagine	7a	2.93	2.94
	7b	2.87	2.90
	8	3.99	3.98
N-Acetyl		2.02	2.02
HO^2H		4.65	4.81

[a] Significant shift increments.

The attachment of fucose to GlcNAcβ1 → Asn at C-6 of *N*-acetylglucosamine, gives rise to small changes in the chemical shifts of H-4, H-5 and H-6a (Table 1). The change is significantly larger for H-5 than for H-6a, although the latter proton is attached to the carbon atom involved in the glycosidic linkage. A steric effect might be held responsible for this. The type of bonding was unambiguously proven by ^{13}C-NMR spectroscopy and by methylation analysis, gas-liquid chromatography/mass spectrometry of the partially methylated alditol acetates [12,13] (and L. Dorland, unpublished results).

This observation illustrates that the position of the glycosidic linkage can hardly be assigned on the basis of ^{1}H-NMR only. The increment rules developed by De Bruyn *et al.* [14] for the assignment of the glycosidic linkage in various di-glucopyranoses cannot simply be extended to other sugars and even in their series several deviations occur. Interestingly, the geminal coupling constant $J_{6a,6b}$ of Fucα1 → 6GlcNAcβ1 → Asn is larger than in GlcNAcβ1 → Asn. From the data presented by De Bruyn *et al.* [14] it is clear that the (1 → 6) linked disaccharides which they investigated show a similar effect. Therefore it is reasonable to suggest that the value of the geminal coupling constant can be used for the assessment of a (1 → 6) glycosidic linkage. To a certain extent the geminal coupling constant $J_{6a,6b}$ is a reflexion of the preferred conformation of the C-5–C-6 fragment. It is conceivable that a large substituent at C-6 influences the ratio of the three staggered rotamers which are considered to be the most important of the various possible conformations. On the other hand the electronegativity of the substituent influences also the value of $J_{6a,6b}$ but estimation of the electronegativities shows that the replacement of hydrogen by a glycosyl moiety should have only a small effect. Further studies are necessary before a definite conclusion can be drawn on the preferred conformation of the C-5–C-6 fragment. De Bruyn *et al.* [14] noted that the values of the coupling constants $J_{5,6a}$ and $J_{5,6b}$ undergo inversion upon substitution at C-6. However, this is not the case in Fucα1 → 6GlcNAcβ1 → Asn.

The conformation of the sugar rings was deduced by calculation of the dihedral angles (ϕ) between the

Table 2. *Coupling constants of GlcNAcβ1 → Asn and Fucα1 → 6GlcNAcβ1 → Asn in* ^2H_2O

Compound	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$	$J_{7a,8}$	$J_{7b,8}$	$J_{7a,7b}$
	Hz									
GlcNAcβ1 → Asn	9.80	10.60	9.10	10.40	2.40	5.55	−12.70	4.00	6.90	−17.80
Fucα1 → 6	3.75	10.30	3.40	0.60	6.55					
GlcNAcβ1 → Asn	9.85	9.85	9.50	9.50	2.10	5.55	−11.40	3.80	6.30	−17.25

Table 3. *Calculated dihedral angles $\phi_{HH'}$ for* N*-acetyl-*D*-glucosamine and* L*-fucose in GlcNAcβ1 → Asn and Fucα1 → 6GlcNAcβ1 → Asn*

Compound	ϕ_{12}	ϕ_{23}	ϕ_{34}	ϕ_{45}
	degrees			
GlcNAcβ1 → Asn	162	167	154	166
Fucα1 → 6	45	167	48	83
GlcNAcβ1 → Asn	163	159	157	157

protons in the ring fragments H−C−C′−H′ from the vicinal coupling constants $J_{HH'}$ by means of an adapted Karplus equation [15]:

$$J_{HH'} = (6.6 - 1.0\cos\phi + 5.6\cos 2\phi)\,(1 - \sum_{i=1}^{i=4} f_i\,\Delta X_i).$$

where $\Delta X_i = X_R\text{-}X_H$ represents the difference in electronegativity X between a substituent R and hydrogen. The factor f_i amounts 0.15 when the dihedral angle θ between R and H in H−C−C′−R is larger than 90° and 0.05 when θ is smaller than 90°. The following values for X were used [16,17]: $X_H = 2.1$; X_R for R is ring O = 3.3, for R is −C−O = 2.5, for R is $-O^2H$ = 3.4, for R is $-N^2HCOCH_3$ = 3.2 and for R is −C−N⟨ = 2.5.

The values obtained for the dihedral angles are given in Table 3. The angles are for GlcNAc in both compounds in agreement with the C1(D) chair conformation, wherein the ring is somewhat flattened with respect to the ideal chair conformation [17]. The angles of L-fucose correspond well to the 1C(L) conformation and a similar ring flattening is observed [18].

The proton shifts of asparagine in GlcNAcβ1 → Asn are in accordance with the chemical shifts found for the β-methylene and α-methine protons in free asparagine as a 'Zwitterion' [19]. In comparison to Asn in GlcNAcβ1 → Asn the β-methylene protons of Asn in Fucα1 → 6GlcNAcβ1 → Asn show a very small downfield shift. This indicates a slightly more cationic character resulting from a lower pH value.

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REFERENCES

1. Montreuil, J. (1975) *Pure Appl. Chem. 42*, 431−477.
2. Bocci, V. (1976) *Experientia (Basel) 32*, 135−140.
3. Jancik, J. & Schauer, R. (1974) *Hoppe-Seyler's Z. Physiol. Chem. 355*, 395−400.
4. Sharon, N. (1975) *Complex Carbohydrates, Their Chemistry, Biosynthesis, and Functions*, pp. 182−190, Addison-Wesley Publishing Company, London.
5. Baenziger, J., Kornfeld, S. & Kochwa, S. (1974) *J. Biol. Chem. 249*, 1897−1903.
6. Baenziger, J. & Kornfeld, S. (1974) *J. Biol. Chem. 249*, 7270−7281.
7. Kornfeld, R., Keller, J., Baenziger, J. & Kornfeld, S. (1971) *J. Biol. Chem. 246*, 3259−3268.
8. Tai, T., Ito, S., Yamashita, K., Muramatsu, T. & Kobata, A. (1975) *Biochem. Biophys. Res. Commun. 65*, 968−974.
9. Chéron, A., Fournet, B., Spik, G. & Montreuil, J. (1976) *Biochimie (Paris) 58*, 927−942.
10. Strecker, G., Fournet, B., Spik, G., Montreuil, J., Durand, P. & Tondeur, M. (1977) *C.R. Hebd. Séances Acad. Sci. Sér. D, Sci. Nat. (Paris)* in press.
11. Lemieux, R. U. & Stevens, J. D. (1966) *Can. J. Chem. 44*, 249−262.
12. Björndal, H., Hellerqvist, C. G., Lindberg, B. & Svensson, S. (1970) *Angew. Chem. Int. Ed. Engl. 9*, 610−619.
13. Stellner, K., Saito, H. & Hakomori, S. (1973) *Arch. Biochem. Biophys. 155*, 464−472.
14. De Bruyn, A., Anteunis, M. & Verhegge, G. (1975) *Bull. Soc. Chim. Belg. 84*, 721−734.
15. Streefkerk, D. G., de Bie, M. J. A & Vliegenthart, J. F. G. (1973) *Tetrahedron, 29*, 833−844.
16. Cavanaugh, J. R. & Dailey, B. P. (1961) *J. Chem. Phys. 34*, 1099−1107.
17. Streefkerk, D. G., de Bie, M. J. A. & Vliegenthart, J. F. G. (1974) *Carbohyd. Res. 33*, 339−349.
18. Streefkerk, D. G., de Bie, M. J. A. & Vliegenthart, J. F. G. (1974) *Carbohyd. Res. 38*, 47−59.
19. Roberts, G. C. K. & Jardetzky, O. (1970) *Adv. Protein Chem. 24*, 447−545.

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