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THE SYNTHESES OF VARIOUS 1-*N*-(L-ASPART-4-OYL)-GLYCOSYLAMINES AND THEIR ANALOGS

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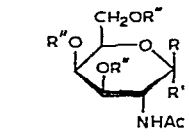
ABSTRACT

Aspartoylglycosylamines and their analogs having structures similar to 2-acetamido-1-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine, in which aspartic acid is linked to glycosylamines from 2-acetamido-2-deoxy-D-galactose, D-galactose, and D-mannose, and to cyclohexylamine and aniline, respectively, were synthesized. These syntheses were accomplished by condensing *O*-acetylglycosylamines, aniline, and cyclohexylamine with an aspartic acid derivative having blocked 1-carboxyl and 2-amino groups, followed by removal of the protecting groups. The stability of the synthetic compounds in acidic and alkaline media was investigated.

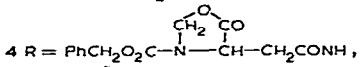
INTRODUCTION

In many glycoproteins, the carbohydrate residue is linked to the protein backbone through an aspartoylglycosylamine compound[1]. This compound is 2-acetamido-1-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine (**19**), which has been isolated from several glycoproteins[2], and is cleaved into aspartic acid, ammonia, and 2-acetamido-2-deoxy-D-glucose by an enzyme participating in glycoprotein catabolism, (L-aspart-4-oyl)glycosylamine amido hydrolase[3,4]. *N*-(Aspartoyl)glycosylamines containing carbohydrates other than 2-acetamido-2-deoxy-D-glucose have not been shown to occur in glycoproteins, nor have their properties been investigated, except for *N*-(L-aspart-4-oyl)-β-D-glucopyranosylamine. This compound was synthesized by Marks and Neuberger[5] who investigated its stability in acidic and alkaline media.

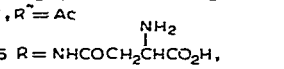
We synthesized the aspartoylglycosylamines **6**, **12**, and **18**, in which 2-acetamido-2-deoxy-D-galactose, D-galactose, and D-mannose, respectively, constitute the carbohydrate residues. These compounds may serve as reference substances for new aspartoylglycosylamines which might occur in glycoproteins, since these carbohydrate constituents are frequently found in glycoproteins. The properties of the synthetic compounds were investigated, particularly the stability in acidic and alkaline media. Analogous compounds, in which aniline or cyclohexylamine is linked to the 4-carboxyl group of aspartic acid, were also synthesized and subjected to similar conditions.



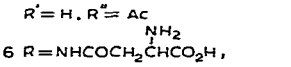
1 R = H, R' = Cl, R'' = Ac
2 R = N_3, R' = H, R'' = Ac
3 R = NH_2, R' = H, R'' = Ac



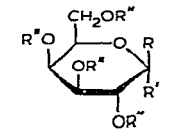
4 R = $PhCH_2O_2C$—N—CH—CH_2CONH, R' = H, R'' = Ac



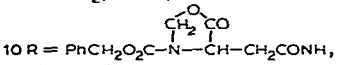
5 R = $NHCOCH_2CHCO_2H$, R' = H, R'' = Ac



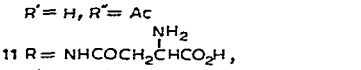
6 R = $NHCOCH_2CHCO_2H$, R' = H, R'' = H



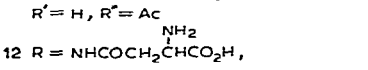
7 R = H, R' = Br, R'' = Ac
8 R = N_3, R' = H, R'' = Ac
9 R = NH_2, R' = H, R'' = Ac



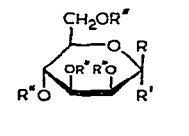
10 R = $PhCH_2O_2C$—N—CH—CH_2CONH, R' = H, R'' = Ac



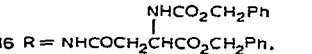
11 R = $NHCOCH_2CHCO_2H$, R' = H, R'' = Ac



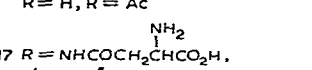
12 R = $NHCOCH_2CHCO_2H$, R' = H, R'' = H



13 R = H, R' = Cl, R'' = Ac
14 R = N_3, R' = H, R'' = Ac
15 R = NH_2, R' = H, R'' = Ac



16 R = $NHCOCH_2CHCO_2CH_2Ph$, R' = H, R'' = Ac



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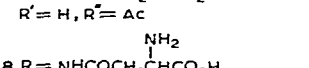
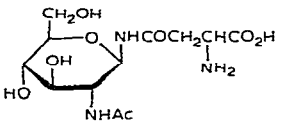
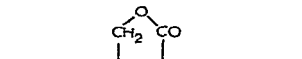


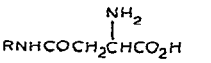
18 R = $NHCOCH_2CHCO_2H$, R' = H, R'' = H



19



20 R = OH
21 R = HNPh
22 R = HNC_6H_{11}



23 R = Ph
24 R = C_6H_{11}

RESULTS AND DISCUSSION

Two types of aspartic acid derivatives were used, 1-benzyl *N*-(benzyloxycarbonyl)-L-aspartate[5] for the synthesis of *N*-(L-aspart-4-oyl)-*β*-D-mannopyranosylamine (**18**), and 3-benzyloxycarbonyl-5-oxo-4-oxazolidinacetic acid (**20**)[6] for the synthesis of 2-acetamido-1-*N*-(L-aspart-4-oyl)-2-deoxy-*β*-D-galactopyranosylamine (**6**), *N*-(L-aspart-4-oyl)-*β*-D-galactopyranosylamine (**12**), *N*-(L-aspart-4-oyl)aniline (**23**) and *N*-(L-aspart-4-oyl)cyclohexylamine (**24**). The latter reagent had the advantage that a pure preparation can readily be obtained, whereas the former requires a separation from the 4-benzyl derivative. However, this advantage was compensated by cleavage of the oxazolidine ring during catalytic hydrogenation, resulting in a lower yield, as compared to that obtained by removal of the benzyl group. Thus, both aspartic acid derivatives are equally usable. Glycosylamines esterified with *O*-acetyl groups were prepared *via* their corresponding halides and azides. Synthesis of the fully protected 2-acetamido-2-deoxy-*α*-D-galactopyranosyl chloride (**1**) followed the route described by Tarasiejska and Jeanloz[7]. Treatment of **1** with silver azide gave the amorphous azide **2** which was reduced to the amine **3**.

2,3,4,6-Tetra-*O*-acetyl-*α*-D-galactopyranosyl bromide (**7**) was prepared from the corresponding pentaacetate according to the procedure described by Lemieux for 2,3,4,6-tetra-*O*-acetyl-*α*-D-glucopyranosyl bromide[8]. Its conversion into the amine (**9**) *via* the azide (**8**) followed the conditions described by Bertho[9].

2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl chloride (**13**) was prepared according to Bonner[10]. Treatment of **13** with silver azide gave a syrupy azide **14**, which was reduced to the crystalline amine **15**.

Glycosylamines protected with *O*-acetyl groups, as well as aniline and cyclohexylamine, were condensed with either of the aspartic acid derivatives in the presence of dicyclohexylcarbodiimide. The amorphous reaction products were treated without further purification to remove the protecting groups, the *O*-acetyl groups of the 2-acetamido-2-deoxy-D-galactose derivative **5** with acid and those of the D-galactose and D-mannose derivatives **11** and **17** with alkali. The compounds were purified by chromatography or by electrophoresis or by both, and then crystallized. *N*-(L-Aspart-4-oyl)aniline (**23**), was obtained in amorphous form, but it was pure on examination by electrophoresis, and *N*-(L-aspart-4-oyl)cyclohexylamine (**24**) was obtained in crystalline form. The 2-acetamido-2-deoxy-D-glucose derivative **19** was synthesized as described previously[11]. A β-D-anomeric configuration was assigned to **19**, based on the synthesis from 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide, whose anomeric configuration has been firmly established[12], through a pathway in which no change in the configuration at C-1 can occur. The isorotation rule was successfully applied to **19** (in the fully-protected form)[13], and its optical rotatory dispersion (o.r.d.) has been measured[14]. The anomeric configuration of the asparagine derivatives **6**, **12**, and **18** was determined by the isorotation rules (Table I). From the A values, we concluded that all the synthetic compounds have a β-D configuration.

TABLE I

MOLAR OPTICAL ROTATIONS AND *A* VALUES OF VARIOUS *N*-L-(ASPART-4-OYL)GLYCOSYLAMINES

N-L-(*Aspart-4-oyl*)*glycosylamines*	$[M]_D \times 10^{-2}$, (*degrees*)	$B \times 10^{-2}$	$A \times 10^{-2}$
2-Acetamido-2-deoxy-β-D-glucopyranosylamine (**19**)	+84	+202[a]	−118
2-Acetamido-2-deoxy-β-D-galactopyranosylamine (**6**)	+20	+221[a]	−201
β-D-Galactopyranosylamine (**12**)	+31	+184[b]	−153
β-D-Mannopyranosylamine (**18**)	−89	+26[a]	−115
β-D-Glucopyranosylamine (**19**)	−51[c]	+118[a]	−169

[a]Values calculated from the values for methyl α- and β-D-glycosides (ref. 19). [b]Value calculated from the values for α- and β-anomers (ref. 20). [c]Value from ref. 5.

The o.r.d. spectra (Fig. 1) of the asparagine derivatives **19** and **6** were similar, but no Cotton effect, at about 205 nm, was observed for the 2-acetamido-2-deoxy-D-glucose compound **6**. The spectra of the D-galactopyranosyl and the D-mannopyranosyl derivatives (**12** and **18**, respectively) differed somewhat from those of the hexosamine derivatives **19** or **6**, but the positive Cotton effect was seen at 231 mn, which may correspond to that occuring at 227 nm for **19** and **6** and which is attributed to the β-D anomeric configuration. Thus, o.r.d. spectra also indicate a β-D anomeric configuration for all the synthetic *N*-(L-aspart-4-oyl)glycosylamines.

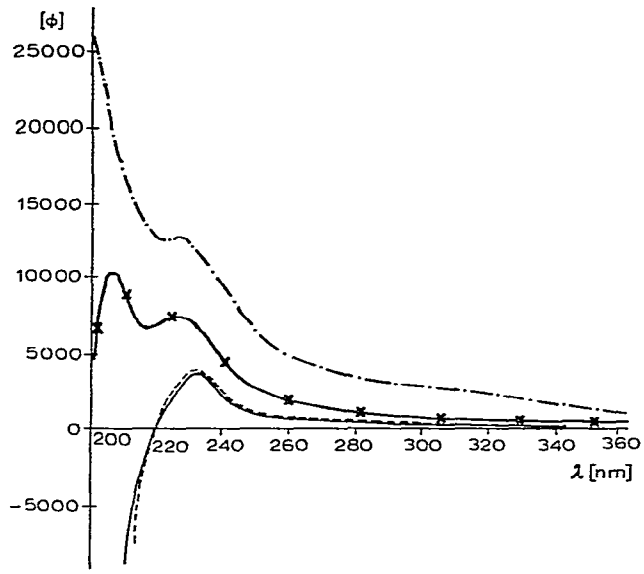


Fig. 1. O.r.d. spectra of 2-acetamido-1-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine (**19**) (×—×), 2-acetamido-1-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-galactopyranosylamine (**6**) (.—.), *N*-(L-aspart-4-oyl)-β-D-galactopyranosylamine (**12**) (— — —), and *N*-(L-aspart-4-oyl)-β-D-mannopyranosylamine (**18**) (———), in water at 28–29°.

N.m.r. spectra were complex, but some of the signals were again indicative of a β-D anomeric configuration for the aspartoylglycosylamines examined. Both hexosamine compounds **19** and **6** had similar spectra with respect to the anomeric proton; doublets were discernible at 5.09 p.p.m. for **19** and at 5.04 p.p.m. for **6** (at about 50°) both with spacings of 8-9 Hz. The spectrum of the D-galactose derivative **12** in the region from 4.8 to 5.1 p.p.m. was complicated, but a doublet at 4.92 p.p.m. (at about 50°) with a spacing of 8.7 Hz was considered to be a signal arising from the anomeric proton. The D-mannose derivative **18** showed a doublet-like signal at 5.25 p.p.m., but the observed small spacing, only about 1 Hz, which has also been recorded for α- or β-D-mannopyranosides[15], did not permit any conclusion as to the anomeric configuration.

Stabilities toward acid and alkali. — Stabilities of the L-(aspart-4-oyl)amine linkages in the synthetic compounds were compared in M hydrochloric acid and 0.2M sodium hydroxide solutions at 100°. The stabilities were expressed in terms of rate constants (k) for the release of aspartic acid obtained from curves following first order kinetics. The values for the hydrolyses of **19** and **6** should represent the release of L-aspartic acid from both the parent forms and their *N*-deacetylated forms [2-amino-1-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine and 2-amino-1-(L-aspart-4-oyl)-2-deoxy-β-D-galactopyranosylamine, respectively], since it has been shown from kinetic studies of the acid hydrolysis of **19** that L-aspartic acid is initially and mainly

released from **19** with the concomitant release of ammonia. Then it is released from *N*-deacetylated **19** at a slower rate[16,17].

As shown in Table II, the stabilities of the (L-aspart-4-oyl)amine linkages in various synthetic compounds in the presence of acid were nearly the same, except for *N*-(L-aspart-4-oyl)cyclohexylamine (**24**) which was exceedingly stable. The value for **19** is similar to that reported by Marshall[16], $20 \times 10^{-3} \cdot min^{-1}$ in 2M hydrochloric acid at 100°.

TABLE II

STABILITIES OF VARIOUS *N*-(L-ASPART-4-OYL) DERIVATIVES IN ACIDIC AND ALKALINE MEDIA AT 100°

Glycosylamines and analogs	*Hydrolysis rates in M hydrochloric acid,* k $(min^{-1}) \times 10^3$	*Hydrolysis rates in 0.2M sodium hydroxide,* k $(min^{-1}) \times 10^3$
2-Acetamido-2-deoxy-β-D-glucopyranosylamine (**19**)	3.2	1.4
2-Acetamido-2-deoxy-β-D-galactopyranosylamine (**6**)	4.0	1.8
β-D-Galactopyranosylamine (**12**)	6.2	2.8
β-D-Mannopyranosylamine (**18**)	6.2	51.3
Aniline (**23**)	7.1	2.2
Cyclohexylamine (**24**)	0.46	0.15

Under alkaline conditions, the rate constants for the hydrolyses of the 1-*N*-(L-aspart-4-oyl)glycosylamines **19**, **6**, **12**, and *N*-(L-aspart-4-oyl)aniline (**23**) were of the same order, $1\text{–}3 \times 10^{-3} \cdot min^{-1}$, whereas that of the D-mannose derivative **18** was extremely large, $51.3 \times 10^{-3} \cdot min^{-1}$, and that of *N*-(L-aspart-4-oyl)cyclohexylamine (**24**) was very small. The marked instability of **18** in the presence of alkali may well be due to the effect of the axial hydroxyl group at C-2. The value for **19** is similar to that reported by Austen and Marshall[18], $2.8 \times 10^{-3} \cdot min^{-1}$ under the same conditions.

(L-Aspart-4-oyl)glycosylamine amido hydrolase isolated from hog serum[3] or kidney[4] was capable of releasing L-aspartic acid from the synthetic (L-aspart-4-oyl)-glycosylamines. *N*-(L-Aspart-4-oyl)aniline (**23**) and *N*-(L-aspart-4-oyl)cyclohexylamine (**24**) were not degraded by the enzyme, but were competitive inhibitors. Details of the enzymatic studies will be published elsewhere.

EXPERIMENTAL

General procedures. — Melting points were determined with a Mitamura micro melting-point apparatus and are uncorrected. Optical rotations were determined in a semimicro tube, 0.5-dm long, using a Rex polarimeter. O.r.d. spectra were recorded with a JASCO Model ORD/UV-5. N.m.r. spectra were obtained with a Varian spectrophotometer A-60, for fresh deuterium oxide solutions of samples with sodium 2,2-dimethyl-2-silapentane-5-sulfonate as the internal reference. I.r. spectra were recorded for KBr discs on a Hitachi spectrophotometer Model EP-I. Elementary

analyses were performed at the Elementary Analysis Center of Kyoto University. High-voltage paper electrophoresis, which was used to check the purity of the synthetic compounds as well as to purify compound **18**, was performed in a pyridine–acetic acid buffer, pH 3.6, with a pyridine concentration of 1% at a potential gradient of 50 V/cm. Electrophoresis was carried out for 1.5 h, on paper strips cooled with hexane, after which the spots were stained with ninhydrin. Hydrolyses of the synthetic compounds by acid or alkali were followed by the determination of the release of aspartic acid with a Hitachi amino acid analyzer Model KLA 3B.

*2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-galactopyranosyl azide* (**2**). — 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl chloride (**1**) was synthesized from 2-amino-2-deoxy-D-galactose hydrochloride *via* 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α,β-D-galactopyranose according to the method of Tarasiejska and Jeanloz[7]. A suspension of silver azide in dry chloroform (100 ml) was prepared by mixing sodium azide (1.0 g) and silver nitrate (2.5 g) in water (100 ml), then successively washing the precipitate with water, ethanol, ether, and chloroform. A solution of **1** (0.8 g) in chloroform (50 ml) was added to the suspension, and the mixture was boiled for 90 min under reflux, then cooled, and filtered. The filtrate was evaporated to dryness. The residue, showing an i.r. absorption band at 2120 cm^{-1} characteristic of the azide group, was used for the next reaction without purification.

*2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-galactopyranosylamine* (**3**). — Hydrogen gas was introduced for 7 h into a solution of **2** in ethyl acetate (100 ml) at room temperature and atmospheric pressure in the presence of platinum oxide (120 mg) as catalyst. The suspension was filtered, and from the filtrate, after concentration *in vacuo*, **3** was obtained as an amorphous deposit by addition of petroleum ether. The sample (400 mg, 53% based on **1**) was used for the next reaction without purification.

*2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-(3-benzyloxycarbonyl-5-oxo-4-oxazolidinacetyl)-2-deoxy-β-*D*-galactopyranosylamine* (**4**). — The aspartic acid derivative, 3-benzyloxycarbonyl-5-oxo-4-oxazolidinacetic acid (**20**), was prepared by heating *N*-(benzyloxycarbonyl)-L-aspartic acid (5.0 g) for 30 min at reflux with paraformaldehyde (750 mg) and *p*-toluenesulfonic acid (190 mg) in benzene (150 ml) according to the method of Itoh[6]. The reaction mixture was washed with water, and then the aspartic acid derivative was extracted with 0.5M sodium hydrogen carbonate (3 × 50 ml). The combined extract was acidified to pH 4-5 with M hydrochloric acid, and the resulting free acid was extracted with ethyl acetate. Evaporation of the solution gave syrupy **20**.

Compound **20** (500 mg), dissolved in dichloromethane (50 ml), was mixed with **3** (400 mg) and dicyclohexylcarbodiimide (500 mg) in dichloromethane (50 ml) under vigorous stirring, and the mixture was kept at room temperature overnight. The *N,N'*-dicyclohexylurea which had formed was filtered off and the filtrate was evaporated. The residue was dissolved in hot methanol, and the solution was concentrated and cooled. After removal of the precipitate (*N,N'*-dicyclohexylurea), the filtrate was evaporated. The residue (**4**) was used for the next reaction without purification.

*2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-(L-aspart-4-oyl)-2-deoxy-β-*D*-galactopyrano-*

sylamine (**5**) and *2-acetamido*-1-N-(L-*aspart-4-oyl*)-*2-dexoy-β*-D-*galactopyranosylamine* (**6**). — Hydrogen gas was introduced for 5 h into a solution of **4** in methanol (100 ml) under stirring at atmospheric pressure and room temperature in the presence of palladium-black (300 mg) as a catalyst. The catalyst was filtered off and the filtrate was evaporated. The residue was dissolved in ethanol, and ether was added to the solution to give amorphous **5** (1.0 g). A suspension of **5** in M sulfuric acid (55 ml) was kept at room temperature for 40 h. After the insoluble materials had been filtered off, the filtrate was applied to a Dowex-1(X-8, AcO^-) column (2.0 × 100 cm) and eluted with water. Ninhydrin-positive fractions were collected and evaporated. The residue was crystallized from water–ethanol to give needles (**6**, 150 mg, 38% based on **3**). Recrystallization from water–ethanol gave 93 mg of crystals, which on paper electrophoresis showed the presence of acidic contaminants. Further purification was achieved by passing an aqueous solution of **6** through a Dowex-1(X-8, AcO^-) column (1.6 × 10 cm). From the effluent, 34 mg of pure crystals of **6** was obtained, m.p. 207–108°; $[\alpha]_D^{29}$ +6.1° (*c* 1.08, water); i.r. data: ν_{max}^{KBr} 3310 (NH and OH), 1625 (amide I), 1537 (amide II), 1312 (amide III), 1087 (C–O–C) cm^{-1}.

Anal. Calc. for $C_{12}H_{21}N_3O_8 \cdot 1.5H_2O$: C, 39.77; H, 6.81; N, 11.60. Found: C, 39.54; H, 6.69; N, 11.66.

2,3,4,6-Tetra-O-*acetyl-1*-N-(*3-benzyloxycarbonyl-5-oxo-4-oxazolidinacetyl*)-*β*-D-*galactopyranosylamine* (**10**). — 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**7**) was synthesized by the procedure used by Lemieux[8] for the corresponding D-glucopyranosyl bromide. Compound **7** (10 g) was converted into 2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl azide[9] (**8**, 4.5 g), and then into 2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosylamine[9] (**9**, 3.5 g) by procedures similar to those used for **3**. Compound **9** (3.5 g) was then condensed with **20** (2.9 g) using dicyclohexylcarbodiimide (1.9 g) in dichloromethane, as described for **4**, which gave amorphous **10** (2.3 g, 44% based on **9**).

N-(L-*Aspart-4-oyl*)-*β*-D-*galactopyranosylamine* (**12**). — After catalytic hydrogenation of **10** (1.2 g in 150 ml of ethyl acetate) in the presence of palladium-black under conditions similar to those used for **5**, the amorphous product, 2,3,4,6-tetra-*O*-acetyl-*N*-(L-aspart-4-oyl)-*β*-D-galactopyranosylamine (**11**), was treated with 0.6M lithium hydroxide (17.5 ml) for 1.5 h at room temperature. The mixture was neutralized with acetic acid and filtered, and the solution evaporated. The residue was dissolved in a small amount of water, from which crude **12** was precipitated with ethanol. Crystallization from water–ethanol gave 350 mg of needles (61%, based on **10**). Further purification with Dowex 1(X-8, AcO^-), as described for **6**, and recrystallization from water–ethanol gave 216 mg of crystals which were homogeneous on paper electrophoresis, m.p. 224–227°; $[\alpha]_D^{30}$ +10.4° (*c* 1.05, water); i.r. data: ν_{max}^{KBr} 3270 (NH and OH), 1698, 1580 (COO^-), 1660 (amide I), 1524 (amide II), 1320 (amide III), and 1075 (C–O–C) cm^{-1}.

Anal. Calc. for $C_{10}H_{18}N_2O_8$: C, 40.81; H, 6.16; N, 9.52. Found: C, 40.56; H, 6.24; N, 9.26.

2,3,4,6-Tetra-O-*acetyl-β*-D-*mannopyranosylamine* (**15**). — 2,3,4,6-Tetra-*O*-ace-

tyl-α-D-mannopyranosyl chloride (**13**) was synthesized from D-mannose *via* 1,2,3,4,6-penta-*O*-acetyl-β-D-mannopyranose according to the method of Bonner[10]. Compound **13** (5.0 g) was treated with silver azide, as described for **2**, and the resulting syrupy 2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl azide (**14**) was reduced in the presence of platinum oxide to give crystalline **15** (1.8 g, 38% based on **13**), m.p. 141–142°; $[\alpha]_D^{28} - 16.9°$ (c 2.00, chloroform).

Anal. Calc. for $C_{14}H_{21}NO_9$: C, 48.41; H, 6.10; N, 4.03. Found: C, 48.70; H, 6.12; N, 3.93.

N-(L-*Aspart-4-oyl*)-β-D-*mannopyranosylamine* (**18**). — Solutions of 1-benzyl *N*-(benzyloxycarbonyl)-L-aspartate[5] (2.0 g) and dicyclohexylcarbodiimide (1.4 g), each in 50 ml dichloromethane, were added to a solution of **15** (1.8 g) in dichloromethane (50 ml), and the mixture was kept for 43 h at 25°. Crystalline 2,3,4,6-tetra-*O*-acetyl-*N*-[1-benzyl N-(benzyloxycarbonyl)-L-aspart-4-oyl]-β-D-mannopyranosylamine (**16**, 2.9 g, 81% based on **15**) was isolated from the reaction mixture by procedures similar to those described[11] for the synthesis of **19**. The crystals, however, did not show a sharp melting point (156–182°), probably because of contamination with *N,N'*-dicyclohexylurea, which could not be removed by recrystallization. The sample was used in the next reaction without further purification. A solution of **16** (1.3 g) in methanol (100 ml) was hydrogenated in the presence of palladium-black (150 mg) for 6 h. The mixture was filtered and the solution evaporated. The residue (**17**) was suspended in water–methanol (3 ml), 0.6M lithium hydroxide (24 ml) was added, and the suspension was kept for 1.5 h at room temperature. After filtration, the mixture was applied to a Dowex-50 (X-8, 20–50 mesh, H^+) column (1.6 × 18 cm) and eluted with M ammonium hydroxide. The ninhydrin-positive fractions (revealed by spot tests after the evaporation of ammonia) were lyophilized. The residue, dissolved in water, was applied to a column of Dowex 1 (X-8, 100–200 mesh, 1.6 × 15 cm, AcO^-) and eluted with water. The effluent showing a positive ninhydrin reaction was lyophilized and the residue was purified by preparative paper electrophoresis. Ninhydrin-positive bands having mobilities corresponding to **12** were eluted with the same buffer as used for the electrophoresis, and the eluate was passed through a Sephadex G-10 column (1.2 × 100 cm) equilibrated with water. The effluent positive in the ninhydrin reaction was evaporated. Crystallization from water–ethanol gave, after several months, needles (**18**). Recrystallization from water–ethanol afforded 102 mg (8.0% based on **16**), m.p. 208–211°; $[\alpha]_D^{30} - 30.1°$ (c 1.05, water); i.r. data: ν_{max}^{KBr} 3320 (NH and OH), 1625 (amide I), 1530 (amide II), 1305 (amide III), and 1070 (C–O–C) cm^{-1}.

Anal. Calc. for $C_{10}H_{18}N_2O_8 \cdot 2H_2O$: C, 36.36; H, 6.71; N, 8.48. Found: C, 36.66; H, 6.68; N, 8.36.

N-(L-*Aspart-4-oyl*)*aniline* (**23**). — 3-Benzyloxycarbonyl-5-oxo-4-oxazolidinacetanilide (**21**) was prepared from aniline (500 mg) and **20** (1.2 g), as described for **4**. Hydrogen gas was introduced into a solution of the condensation product (**21**) in methanol (150 ml) in the presence of palladium-black (150 mg). After filtration, the solution was evaporated, and the residue was dissolved in hot water (40 ml). Ethanol–ethyl acetate was added to the solution to give a precipitate which was redissolved in

hot water. Concentration of the solution gave an amorphous deposit (113 mg, 10.0% based on aniline) which was homogeneous on paper electrophoresis; i.r. data: ν_{max}^{KBr} 3250, 2980 (NH), 1646 (amide I), 1594 (CO_2^-), 1534 (amide II), 1305 (amide III), 749, and 688 (aromatic) cm^{-1}.

Anal. Calc. for $C_{10}H_{12}N_2O_3$: C, 57.68; H, 5.81; N, 13.46. Found: C, 57.77; H, 5.96; N, 13.42.

N-(L-*Aspart-4-oyl*)*cyclohexylamine* (**24**). — 3-Benzyloxycarbonyl-5-oxo-4-oxazolidinacetamidocyclohexane (**22**) was prepared from cyclohexylamine (500 mg) and **20** (1.8 g), as described for **21**. The product was dissolved in methanol (100 ml) and hydrogenated, as described for **21**. After filtration, the solution was evaporated, and the residue was extracted with water. Ethanol was added to the aqueous solution to give a slight turbidity. After several days, the mixture gave needles (132 mg, 12.0% based on cyclohexylamine) that were homogeneous on paper electrophoresis, m.p. 204–205°; i.r. data: ν_{max}^{KBr} 3360 (NH and OH), 3000 (NH), 1658 (amide I), 1570 (amide II or CO_2^-), and 1315 (amide III) cm^{-1}.

Anal. Calc. for $C_{10}H_{18}N_2O_3 \cdot 0.5H_2O$: C, 53.86; H, 8.58; N, 12.58. Found: C, 54.19; H, 8.57; N, 12.32.

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