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STUDIES ON 2-AMINO-2-DEOXY-D-GLUCOSE DERIVATIVES.
PART XV. SYNTHESIS OF 1-*N*-ACYL-2-ACYLAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYLAMINES*

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The carbohydrate–peptide bond in glycopeptides isolated from hen-egg albumin[1–4] and from other sources[5–7] was shown, by comparison of the physical properties of synthetic and natural products[8–13], to be an amido linkage between the β-carboxyl group of L-aspartic acid and the amino group of 2-acetamido-2-deoxy-β-D-glucopyranosylamine. Synthesis of 2-acetamido-1-*N*-(β-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine was performed[8] by condensation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosylamine with α-benzyl *N*-(benzyloxycarbonyl)-L-aspartate, followed by removal of the protecting groups from the product.

In an earlier paper from our laboratory[14], a new synthesis of 2-acetamido-2-deoxy-1-*N*-glycyl-β-D-glucopyranosylamine, *via* the key intermediate 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosylamine[15] (**1**), was presented. In the present paper, this method is used for the synthesis of 1-*N*-acyl-2-acylamino-2-deoxy-β-D-glucopyranosylamines, wherein one of the two acylamino groups is an acetamido group and the other is an (aminoacyl)amino group. Compound **1** was prepared by heating 2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-glucose in methanolic ammonia in a sealed tube. Treatment of **1** with 60% acetic acid for 1 h at 100° gave only 2-acetamido-2-deoxy-D-glucose (papergram[16]) and none of its 2-epimer.

Condensation of **1** with suitably protected amino acid derivatives (*N*-benzyloxycarbonyl-L-alanine, *N*,*N*′-dibenzyloxycarbonyl-L-lysine, α-benzyl *N*-benzyloxycarbonyl-L-aspartate, and β-benzyl *N*-benzyloxycarbonyl-L-aspartate) in pyridine, at room temperature, by the carbodiimide method afforded 1-*N*-(protected aminoacyl)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosylamines (**2**, **3**, **4**, and **5**) in 30–60% yield. Their assigned structures were supported by their characteristic i.r. absorptions (urethan, amido, or ester carbonyl groups) and by their positive Fehling's reaction. The benzylidene group of these compounds was resistant to hydrogenolysis, but was removed by hydrolysis with 60% acetic acid for 1 or 2 h at 100°. 1-*N*-(Protected aminoacyl)-2-acetamido-2-deoxy-β-D-glucopyranosylamines (**6**, **7**, **8**, and **9**) thus obtained in about 80% yield were catalytically hydrogenolyzed to give the 2-acetamido-1-*N*-aminoacyl-2-deoxy-β-D-glucopyranosylamines (**10**, **11**, **12**, and **13**) in 60–80% yield.

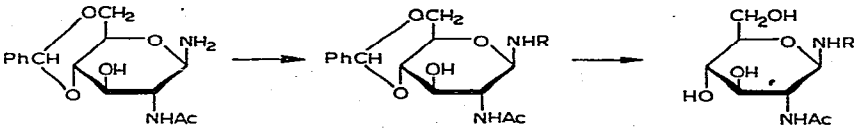
*Part of this work was presented at the International Symposium on the Chemistry of Natural Products held in April 1964 at Kyoto, Japan.

TABLE I

1-*N*-AMINOACYL-2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYLAMINES AND THEIR DERIVATIVES

Compound	*Formula*	*M.p. (dec.) degrees*	*Optical rotation*		c	*Carbon*		*Hydrogen*		*Nitrogen*		*Yield* %
			$[\alpha]_D^{23}$ *degrees*	*Solvent*		*Calc.* %	*Found* %	*Calc.* %	*Found* %	*Calc.* %	*Found* %	
2	$C_{26}H_{31}N_3O_8$	—	—	—	—	60.81	60.53	6.08	5.92	8.18	8.07	50
3	$C_{37}H_{44}N_4O_{10}$	228–229	0.0	C_5H_5N	1.0	63.05	62.69	6.29	6.27	7.95	7.73	30
4	$C_{34}H_{37}N_3O_{10}$	214–216	−0.6	$HCONMe_2$	1.0	63.05	63.07	5.76	5.91	6.49	6.67	55
5	$C_{34}H_{37}N_3O_{10}$	224	−26.9	$HCONMe_2$	1.0	63.05	63.25	5.76	5.97	6.49	6.73	45
6	$C_{19}H_{27}N_3O_8$	198–199	14.4	$HCONMe_2$	1.0	53.64	53.77	6.40	6.63	9.88	9.90	75
7	$C_{30}H_{40}N_4O_{10}$	219–220	0.0	$HCONMe_2$	1.0	58.43	58.02	6.54	6.28	9.09	9.36	79
8	$C_{27}H_{33}N_3O_{10}$	188–189	13.6	$HCONMe_2$	1.0	57.95	57.84	5.94	6.02	7.51	7.62	80
9	$C_{27}H_{33}N_3O_{10}$	218–220	−15.1	$HCONMe_2$	1.0	57.95	57.74	5.94	5.98	7.51	7.90	80
10	$C_{11}H_{21}N_3O_6 \cdot H_2O$	200	43.8	H_2O	1.5	42.71	42.74	7.49	7.45	13.59	13.33	80
11	$C_{14}H_{28}N_4O_6 \cdot 2H_2O$	syrup	17.8	H_2O	1.0	43.73	43.84	8.39	8.37	14.57	14.51	90
12	$C_{12}H_{21}N_3O_8 \cdot 0.5H_2O$	232–235	25.0	H_2O	1.5	41.26	41.90	6.44	6.38	12.21	11.93	70
13	$C_{12}H_{21}N_3O_8 \cdot 1.5H_2O$	230–240	20.6	H_2O	1.5	39.77	39.96	6.68	7.06	11.59	11.18	70

The above-mentioned reactions were also applied to the preparation of 2-[(aminoacyl)amino]-2-deoxy-β-D-glucopyranosylacetamides in which the (aminoacyl)amino group is a glycylamino and a L-lysylamino group (**30** and **31**).

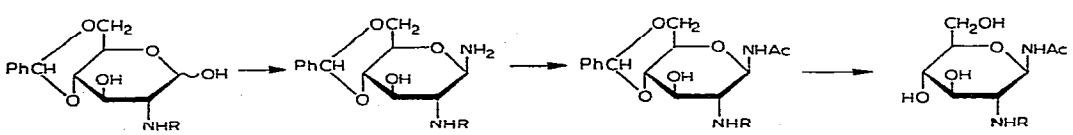


1 **2–5** **6–13**

2 and **6**, R = *N*-(benzyloxycarbonyl)-L-alanyl
3 and **7**, R = *N*,*N*′-di(benzyloxycarbonyl)-L-lysyl
4 and **8**, R = α-benzyl *N*-(benzyloxycarbonyl)-β-L-aspartyl
5 and **9**, R = β-benzyl *N*-(benzyloxycarbonyl)-α-L-aspartyl
10, R = L-alanyl
11, R = L-lysyl
12, R = β-L-aspartyl
13, R = α-L-aspartyl

Benzylidenation of 2-[(*N*-benzyloxycarbonylglycyl)amino]- and 2-{[*N*,*N*′-(dibenzyloxycarbonyl)-L-lysyl]amino}-2-deoxy-D-glucose, which were synthesized by the method of Kochetkov *et al.*[17], afforded, in 60% yield, the corresponding 4,6-*O*-benzylidene derivatives (**14** and **18**), which gave in 35–50% yield 4,6-*O*-benzylidene-2-[(*N*-benzyloxycarbonylglycyl)amino]- and 4,6-*O*-benzylidene-2-{[*N*,*N*-di(benzyloxycarbonyl)-L-lysyl]amino}-2-deoxy-β-D-glucopyranosylamine (**15** and **19**) by treatment with methanolic ammonia in a sealed tube. The anomeric configurations of **15** and **19** were assumed to be β-D from their specific rotations.

The procedure outlined above was unsuccessful, however, in the case of the aspartic acid derivative. The crude syrupy 2-{[α-benzyl *N*-(benzyloxycarbonyl)-L-aspartyl]amino}-2-deoxy-D-glucose, prepared by condensation of α-benzyl *N*-(benzyloxy-



14,18,22,26 **15,19,23,27** **16,20,24,28** **17,21,25,29–35**

14, **15**, **16**, and **17**, R = *N*-(benzyloxycarbonyl)glycyl
18, **19**, **20**, and **21**, R = *N*,*N*′-di(benzyloxycarbonyl)-L-lysyl
22 and **34**, R = α-benzyl *N*-(benzyloxycarbonyl)-β-L-aspartyl
23, **24**, and **25**, R = *N*-(benzyloxycarbonyl)-β-L-asparaginyl
26, **27**, **28**, and **29**, R = benzyloxycarbonyl
30, R = glycyl
31, R = L-lysyl
32, R = β-L-asparaginyl
33, R = H·HCl
35, R = β-L-aspartyl

TABLE II

2-[(AMINOACYL)AMINO]-2-DEOXY-β-D-GLUCOPYRANOSYLACETAMIDES AND THEIR DERIVATIVES

Compound	*Formula*	*M.p. (dec.) degrees*	*Optical rotation*		c	*Carbon*		*Hydrogen*		*Nitrogen*		*Yield* %
			$[\alpha]_D^{23}$ *degrees*	*Solvent*		*Calc.* %	*Found* %	*Calc.* %	*Found* %	*Calc.* %	*Found* %	
14	$C_{23}H_{26}N_2O_8$	217–219	15.0	$HCONMe_2$	1.0	60.25	60.25	5.72	5.62	6.11	6.18	52
15	$C_{23}H_{27}N_3O_7$	187–189	−26.5	$HCONMe_2$	1.0	60.38	59.53	5.95	5.88	9.19	9.07	42
16	$C_{25}H_{29}N_3O_8$	254–255	6.6	$HCONMe_2$	1.0	60.11	60.02	5.85	6.09	8.41	8.68	55
17	$C_{18}H_{25}N_3O_8$	214–215	18.2	AcOH	1.0	52.55	52.14	6.13	6.58	10.21	10.42	89
18	$C_{35}H_{41}N_3O_{10}$	185–187	15.8	$HCONMe_2$	1.0	63.33	63.08	6.23	6.31	6.33	6.33	60
19	$C_{35}H_{42}N_4O_9$	201–202	−27.4	$HCONMe_2$	1.0	63.43	63.27	6.39	6.50	8.45	8.32	50
20	$C_{37}H_{44}N_4O_{10}$	234–237[a]	10.5	$HCONMe_2$	1.0	63.05	63.10	6.29	6.19	7.95	7.92	80
21	$C_{30}H_{40}N_4O_{10}\cdot H_2O$	164–166[a]	11.0	AcOH	1.0	56.85	57.23	6.67	6.98	8.83	8.54	80
30	$C_{10}H_{19}N_3O\cdot 0.5(COOH)_2\cdot H_2O$	205	44.7	H_2O	1.5	38.82	39.09	6.47	6.79	12.34	12.16	70
31	$C_{14}H_{28}N_4O_6\cdot 2HCl$	184–185	29.4	H_2O	1.5	39.92	40.22	7.16	7.39	13.33	13.25	60

[a]No dec.

carbonyl)-L-aspartate and 2-amino-2-deoxy-D-glucose in presence of *N,N'*-dicyclohexylcarbodiimide in aqueous acetone, was directly benzylidenated to give 4,6-*O*-benzylidene-2-{[*N*-(benzyloxycarbonyl)-β-L-aspartyl]amino}-2-deoxy-D-glucopyranose (**22**) in crystalline form. When the condensation was performed in aqueous pyridine, the analytical results for the benzylidene derivative were consistent with those of a lactone derivative, in which the benzyl ester of the aspartyl moiety had been hydrolyzed and the resulting carboxyl group had formed a lactone with a hydroxyl group of the sugar moiety; the i.r. spectrum showed a characteristic absorption at 1725 cm^{-1} instead of the ester group absorption of **22** at 1745 cm^{-1}. After treatment with methanolic ammonia, both benzylidene derivatives gave the same glucosylamine, namely, 4,6-*O*-benzylidene-2-{[*N*-(benzyloxycarbonyl)-β-L-asparaginyl]amino}-2-deoxy-β-D-glucopyranosylamine (**23**), in which the α-carboxyl group converted spontaneously into a carbamoyl group. Although **23** was transformed into 2-[(β-L-asparaginyl)amino]-2-deoxy-β-D-glucopyranosylacetamide (**32**) through the series of reactions mentioned previously, the carbamoyl group could not be successfully hydrolyzed into a carboxyl group without cleavage of the glycosidic bond.

In order to link the β-L-aspartyl group to the 2-amino group of 2-amino-2-deoxy-D-glucosylamine after *N*-acetylation at C-1, 4,6-*O*-benzylidene-2-[(benzyloxycarbonyl)amino]-2-deoxy-D-glucose (**26**), obtained by benzylidenation of 2-[(benzyloxycarbonyl)amino]-2-deoxy-D-glucose[18] was transformed into 2-[(benzyloxycarbonyl)amino]-2-deoxy-β-D-glucopyranosylacetamide (**29**) *via* 4,6-*O*-benzylidene-2-[(benzyloxycarbonyl)amino]-2-deoxy-β-D-glucopyranosylamine (**27**) and its *N*-acetyl derivative **28**. The benzyloxycarbonyl group of **29** was hydrogenolyzed, and the resulting 2-amino-2-deoxy-β-D-glucopyranosyl hydrochloride (**33**) was then condensed with α-benzyl *N*-benzyloxycarbonyl-L-aspartate to give 2-{[*N*-(benzyloxycarbonyl)-β-L-aspartyl]amino}-2-deoxy-β-D-glucopyranosylacetamide (**34**) which was again hydrogenolyzed to give the desired product, 2-[(β-L-aspartyl)amino]-2-deoxy-β-D-glucopyranosylacetamide (**35**).

4,6-*O*-Benzylidene-2-[(benzyloxycarbonyl)amino]-2-deoxy-D-glucopyranose (**26**), **27**, and **29** were fully acetylated to give the corresponding 1,3-di-*O*-acetyl, *N*acetyl-*O*-acetyl, and 3,4,6-tri-*O*-acetyl derivatives, respectively, and the last-named compound was tranformed into 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranosylacetamide hydrochloride.

The behavior of **12** during chromatography and electrophoresis was compared with that of the 2-acetamido-2-deoxy-D-glucose–L-aspartic acid compound isolated from hen-egg albumin. In these tests, the two compounds were found to be identical. On the other hand, no difference between **12** and **35** could be found in these tests. Recently, Micheel *et al.*[19] reported that the hydrolysis of **12** in N hydrochloric acid for 1 h at 100° gives the α-D anomer of **12** and 2-[(β-L-aspartyl)amino]-2-deoxy-D-glucose. Such an anomerization and $N \rightarrow N$ acyl migration complicates the reaction. However, the hydrolysis rate of each *N*-acetyl and *N*-aspartyl group in compounds **12** and **35**, in which the two groups are interchanged, should be different. The results of this study will be reported later.

TABLE III

2-(β-L-ASPARTYL)AMINO-4,6-*O*-BENZYLIDENE-2-DEOXY-D-GLUCOSE, 2-(β-L-ASPARAGINYL)AMINO-2-DEOXY-β-D-GLUCOPYRANOSYLACETAMIDE AND THEIR DERIVATIVES

Compound	*Formula*	*M.p. (dec.) degrees*	*Optical rotation*			*Carbon*		*Hydrogen*		*Nitrogen*		*Yield*
			$[\alpha]_D^{23}$ *degrees*	*Solvent*	c	*Calc.* %	*Found* %	*Calc.* %	*Found* %	*Calc.* %	*Found* %	%
22	$C_{32}H_{34}N_2O_{10}$	166–168	−26.0	$HCONMe_2$	1.0	63.36	63.03	5.65	5.77	4.62	4.79	50
23	$C_{25}H_{30}N_4O_8$	208–209	−15.4	$HCONMe_2$	1.0	58.36	58.70	5.88	6.10	10.89	10.80	28
24	$C_{27}H_{32}N_4O_9$	252–254	0.0	$HCONMe_2$	1.0	58.26	57.92	5.80	6.09	10.07	10.28	50
25	$C_{20}H_{28}N_4O_9$	237–238	53.0	AcOH	1.5	51.27	50.82	6.02	6.30	11.96	11.81	80
32	$C_{12}H_{22}N_4O_7 \cdot H_2O$	190–192	—	—	—	40.91	40.65	6.87	6.98	15.92	15.72	70

TABLE IV

4,6-*O*-BENZYLIDENE-2-[(BENZYLOXYCARBONYL)AMINO]-2-DEOXY-D-GLUCOSE, 2-AMINO-2-DEOXY-β-D-GLUCOPYRANOSYLACETAMIDE, 2-(β-L-ASPARTYL)AMINO-2-DEOXY-β-D-GLUCOPYRANOSYLACETAMIDE AND THEIR DERIVATIVES

Compound	*Formula*	*M.p. (dec.) degrees*	*Optical rotation*			*Carbon*		*Hydrogen*		*Nitrogen*		*Yield*
			$[\alpha]_D^{23}$ *degrees*	*Solvent*	c	*Calc.* %	*Found* %	*Calc.* %	*Found* %	*Calc.* %	*Found* %	%
26	$C_{21}H_{23}NO_7$	240	−18.0	$HCONMe_2$	1.0	62.83	62.68	5.78	5.99	3.49	3.60	72
27	$C_{21}H_{24}N_2O_6$	195–196	−39.0	$HCONMe_2$	1.0	62.99	63.00	6.04	6.26	7.00	7.27	50
28	$C_{23}H_{26}N_2O_7$	278–279	−13.0	$HCONMe_2$	1.0	62.43	62.13	5.92	6.64	6.33	6.73	90
29	$C_{16}H_{22}N_2O_7$	206	28.0	MeOH	1.5	54.23	54.46	6.26	6.45	7.91	7.96	85
33	$C_8H_{16}N_2O_5 \cdot HCl \cdot H_2O$	176	0.0	H_2O	1.5	36.16	36.35	6.78	7.16	10.55	10.66	95
34	$C_{27}H_{33}N_3O_{10}$	222–223	26.0	$(Me_2N)_3PO$	1.0	57.95	58.18	5.95	6.28	7.51	7.77	40
35	$C_{12}H_{21}N_3O_8 \cdot 1.5H_2O$	270	23.6	H_2O	1.5	41.90	41.67	6.44	6.91	12.20	12.54	60

EXPERIMENTAL

Melting points are uncorrected. The solutions were evaporated under diminished pressure at a bath temperature not exceeding 45°. Optical rotations were measured in a 0.5-dm tube. The content of water of crystallization was calculated from the analytical values; some values were ascertained by titration with Fischer's reagent[20] of the water liberated in 0.5 h into a nitrogen atmosphere at 150°. For example, the water content of compounds **12** and **35** was estimated as 2.92% (theoretical value, 2.60%) and 6.99% (theoretical value, 7.64%), respectively.

1-N-(*Protected aminoacyl*)-*2-acetamido-4,6-O-benzylidene-2-deoxy-β-*D-*glucopyranosylamines* (**2**, **3**, **4**, *and* **5**). — To a stirred mixture of 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosylamine (1 mole)[14] and protected amino acid (1 mole) in pyridine was added a conc. solution of *N,N'*-dicyclohexylcarbodiimide (1.5 mole) in pyridine. The solution was stirred for several h at room temperature. *N,N'*-Dicyclohexylurea was filtered off, and the filtrate was evaporated. The residue was washed with hot ethanol and precipitated from *N,N*-dimethylformamide by addition of ether to give an amorphous solid.

1-N-(*Protected aminoacyl*)-*2-acetamido-2-deoxy-β-*D-*glucopyranosylamines* (**6**, **7**, **8**, and **9**), *2-deoxy-2-*[(*protected aminoacyl*)*amino*]-*β-*D-*glucopyranosylacetamides* (**17**, **21** *and* **25**), *and 2-*[(*benzyloxycarbonyl*)*amino*]-*2-deoxy-β-*D-*glucopyranosylacetamide* (**29**). — A suspension of **2**, **3**, **4**, **5**, **16**, **20**, **24**, or **28** (1 part) in 60% acetic acid (about 10 parts) was heated at 100° for 1 or 2 h until the compound was completely dissolved. In the case of glycine, L-alanine, and L-aspartic acid derivatives, the solutions were directly evaporated, and the residues were crystallized from 70% aq. ethanol. The L-lysine derivative was easily obtained as crystals by addition of water to the hydrolyzate, and was recrystallized from ethanol–ether.

Compound **29** was acetylated with acetic anhydride in pyridine to give 3,4,6-tri-*O*-acetyl-2-[(benzyloxycarbonyl)amino]-2-deoxy-β-D-glucopyranosylacetamide, m.p. 233° (dec.), $[\alpha]_D^{23}$ −18° (*c* 1.0, chloroform), yield 87%.

Anal. Calc. for $C_{22}H_{28}N_2O_{10}$: C, 54.99; H, 5.87; N, 5.83. Found: C, 54.64; H, 6.10; N, 6.10%.

1-N-(*Aminoacyl*)-*2-acetamido-2-deoxy-β-*D-*glucopyranosylamines* (**10**, **11**, **12** and **13**) and 2-[(*aminoacyl*)*amino*]-*2-deoxy-β-*D-*glucopyranosylacetamides* (**30**, **31**, **32**, *and* **35**). — A suspension of **6**, **7**, **8**, **9**, **17**, **21**, **25**, or **34** (1 part) in 50% aq. ethanol (about 10 parts), occasionally containing acetic acid (a few parts), was hydrogenolyzed in the presence of pailadium–charcoal. As the reaction proceeded, the compounds dissolved completely. The catalyst was filtered off, and the filtrate was evaporated. The residue was crystallized from water–ethanol, usually as a hydrate or a salt with hydrochloric acid or oxalic acid.

4,6-O-Benzylidene-2-{[N-(*benzyloxycarbonyl*)*aminoacyl*]*amino*}-*2-deoxy-*D-*glucoses* (**14** *and* **18**) *and 4,6-O-benzylidene-2-*[(*benzyloxycarbonyl*)*amino*]-*2-deoxy-*D-*glucose* (**26**).— The preparation of **26** is described to illustrate the method.

A mixture of 2-[(benzyloxycarbonyl)amino]-2-deoxy-D-glucose (50 g)[18], benzal-

dehyde (300 ml), and anhydrous zinc chloride (70 g) was shaken for 24 h at room temperature. The mixture was extracted twice with petroleum ether (300 ml). The residue was stirred with ice and water (1000 ml), filtered, and washed with petroleum ether and then with water. The ppt. was triturated with a small amount of methanol, filtered and then recrystallized from pyridine–methanol (yield 45 g).

The corresponding *N*-(benzyloxycarbonyl)glycyl and *N,N'*-di(benzyloxycarbonyl)-L-lysyl derivatives were recrystallized from ethanol and methanol, respectively, sometimes resulting in a gelatinous mass.

Compound **26** was acetylated with acetic anhydride in pyridine to give 1,3-di-*O*-acetyl-4,6-*O*-benzylidene-2-[(benzyloxycarbonyl)amino]-2-deoxy-D-glucopyranose, m.p. 246–247° (dec.), $[\alpha]_D^{23}$ 0° (*c* 0.8, *N,N*-dimethylformamide), yield 96%.

Anal. Calc. for $C_{25}H_{27}NO_9$: C, 61.85; H, 5.60; N, 2.89. Found: C, 61.66; H, 5.42; N, 2.84%.

2-{[α-Benzyl N-(benzyloxycarbonyl)-β-L-aspartyl]amino}-4,6-O-benzylidene-2-deoxy-D-glucose (**22**). — To an ice-cold mixture of 2-amino-2-deoxy-D-glucose hydrochloride (10 g, 46.5 mmoles) in N sodium hydroxide (46 ml) and α-benzyl *N*-benzyloxycarbonyl-L-aspartate (16.5 g, 45.3 mmoles) in acetone (140 ml) was added dropwise *N,N'*-dicyclohexylcarbodiimide (28.6 g, 139 mmoles) in acetone (40 ml). After the reaction mixture had been stirred for several h at room temperature, the ppt. (*N,N'*-dicyclohexylurea and sodium chloride, 28.7 g) was filtered off, and a few drops of acetic acid were added to the filtrate. The solution was stirred again for a few h, and the urea was filtered off (8.1 g). The filtrate was evaporated and the residue was dissolved in hot ethanol (50 ml). The solution was kept overnight in a refrigerator and the crystalline product was filtered off. One half of it (3.5 g) was washed with acetone and ethyl acetate, and recrystallized from ethanol–acetone. After filtration of a small amount of ppt., evaporation of the filtrate to one half gave *O*-(α-benzyl *N*-benzyloxycarbonyl-L-aspartyl)-*N,N'*-dicyclohexylpseudourea as needles, m.p. 97–98°, yield 1.5 g.

Anal. Calc. for $C_{32}H_{41}N_3O_6$: C, 68.18; H, 7.33; N, 7.46. Found: C, 68.13; H, 7.34; N, 7.53%.

The ethanolic mother liquor was evaporated, and the resulting syrup (10 g), which could not be crystallized, was condensed with benzaldehyde to give the crystalline product (**22**), which was recrystallized from ethanol (yield 9 g).

When the condensation of 2-amino-2-deoxy-D-glucose with the aspartate derivative was performed in pyridine–water, however, the resulting benzylidene compound showed m.p. 179–181°, $[\alpha]_D^{23}$ −26° (*c* 1.1, *N,N*-dimethylformamide). Analytical values were in agreement with those calculated for a lactone of a benzylidene-benzyloxycarbonyl-aspartylaminodeoxy-hexose.

Anal. Calc. for $C_{25}H_{26}N_2O_9$: C, 60.23; H, 5.65; N, 5.62. Found: C, 59.66; H, 5.28; N, 5.75%.

4,6-O-Benzylidene-2-[(N-benzyloxycarbonylaminoacyl)amino]-2-deoxy-β-D-glucopyranosylamines (**15** *and* **19**) *and 4,6-O-benzylidene-2-[(benzyloxycarbonyl)amino]-2-deoxy-β-D-glucopyranosylamine* (**27**). — Compounds **14**, **18**, or **22** (1 part) were heated with saturated methanolic ammonia (15–20 parts) for 2 h to 2 days at 70–90°

in a sealed tube, until the starting material was completely dissolved. The presence of a small amount of ammonium chloride accelerated the reaction. The solution was gradually cooled to room temperature, and the crystals precipitated were filtered. In all cases, the product was analyzed and used for the next reaction without recrystallization. The glycosidic configuration of these compounds was determined to be β-D from their rotational values.

Compound **27** was acetylated with acetic anhydride in pyridine to give 3-*O*-acetyl-4,6-*O*-benzylidene-2-[(benzyloxycarbonyl)amino]-2-deoxy-β-D-glucopyranosylacetamide, m.p. 298° (dec), $[\alpha]_D^{23}$ −8.2° (*c* 1.0, *N*,*N*-dimethylformamide), yield 96%.

Anal. Calc. for $C_{25}H_{28}N_2O_8$: C, 61.97; H, 5.83; N, 5.78. Found: C, 62.07; H, 5.88; N, 6.20%.

4,6-O-Benzylidene-2-{[N-(benzyloxycarbonyl)-β-L-asparaginyl]amino}-2-deoxy-β-D-glucopyranosylamine (**23**). — A suspension of **22** (4 g) in saturated methanolic ammonia (130 ml) was heated for 2 days in a sealed tube at 60–70°, and the gelatinous ppt. was completely dissolved. After cooling, the ppt. was filtered and dried. It darkened gradually from about 190° on, and decomposed at 209°. The same compound was obtained in a lower yield (20%) from the lactone of 4,6-*O*-benzylidene-2-{[*N*-(benzyloxycarbonyl)-β-L-aspartyl]amino}-2-deoxy-D-glucose by the same procedure.

4,6-O-Benzylidene-2-deoxy-2-[(protected aminoacyl)amino]-β-D-gluco-pyranosylacetamides (**16**, **20**, *and* **24**) *and 4,6-O-benzylidene-2-[(benzyloxycarbonyl)amino]-2-deoxy-β-D-glucopyranosylacetamide* (**28**). — To a solution or suspension of **15**, **19**, **23**, or **27** in *N*,*N*-dimethylformamide–methanol was added under refrigeration 2 equimolar amounts of acetic anhydride, and the solution was kept for several h at room temperature. The acetylation was usually exothermic, the suspended mass dissolved at once, and then the corresponding *N*-acetyl derivatives were precipitated. Dilution of the reaction mixture with water increased the yield of the product, which was usually recrystallized from *N*,*N*-dimethylformamide–ether.

2-Amino-2-deoxy-β-D-glucopyranosylacetamide hydrochloride (**33**). — A suspension of **29** (5 g) in 50% aq. methanol (80 ml) was hydrogenated in the presence of 10% palladium–charcoal (1 g) and an equimolar amount of hydrochloric acid until the starting material was completely dissolved. After removal of the catalyst, the filtrate was evaporated to give quantitatively the hydrochloride. The pure crystals, which were recrystallized from ethanol–water, decomposed at 176° after gradual browning from 135° on.

2-{[α-Benzyl N-(benzyloxycarbonyl)-β-L-aspartyl]amino}-2-deoxy-β-D-glucopyranosylacetamide (**34**). — A solution of **33** (3.6 g, 14 mmoles) in water (30 ml) was neutralized with triethylamine (1.52 g, 15 mmoles) in acetone (30 ml) and α-benzyl *N*-benzyloxycarbonyl-L-aspartate (7.3 g, 13 mmoles) was added. To the resulting solution was added dropwise, with stirring, *N*,*N'*-dicyclohexylcarbodiimide (5.35 g, 26 mmoles) in acetone (40 ml), and the mixture was kept one day at room temperature. The resulting *N*,*N'*-dicyclohexylurea was filtered off, and the filtrate was evaporated. The residual syrup was triturated with ether (150 ml), and then with water. The residue (4.8 g) was extracted with ethyl acetate (150 ml), and the extract was evaporated to

give an amorphous mass (3.5 g). Crystallization from ethanol (450 ml) afforded the pure product (2.3 g), m.p. 222–223° (dec). The mother liquor gave an additional crop (1.0 g), m.p. 209–211° (dec.). From the ether extract and water washings, α-benzyl *N*-benzyloxycarbonyl-L-aspartate (4.3 g) and the starting material (0.7 g) were recovered.

3,4,6-Tri-O-acetyl-2-amino-2-deoxy-β-D-glucopyranosylacetamide hydrochloride. — A solution of 3,4,6-tri-*O*-acetyl-2-[(benzyloxycarbonyl)amino]-2-deoxy-β-D-glucopyranosylacetamide (2.4 g) in chloroform (40 ml) and methanol (10 ml) was treated with an equimolar amount of conc. hydrochloric acid (0.8 ml); 5% palladium–charcoal (1 g) was added, and the mixture was hydrogenated. After 10 min, the catalyst was filtered off, and concentration of the filtrate gave fine needles (1.8 g) which gave a positive Beilstein test, m.p. 242–243° (dec.), $[\alpha]_D^{23}$ 40° (*c* 0.5, methanol).

Anal. Calc. for $C_{14}H_{23}N_2O_8Cl$: C, 43.92; H, 6.05; N, 7.51. Found: C, 44.07; H, 6.32; N, 7.29%.

Comparison of the synthetic products **12**, **13**, *and* **35** *with the product isolated from hen-egg albumin.* — Compounds **12**, **13**, and **35** were compared by chromatographic and electrophoretic procedures with the 2-acetamido-2-deoxy-D-glucose–L-aspartic acid compound isolated from hen-egg albumin (Table V). The values observed for **13** were slightly different from those of the other compounds. After treatment with a 1% solution of ninhydrin in acetone–pyridine (49:1)[21] only **13** showed a purple spot, whereas the others gave brown spots.

TABLE V

RATE OF MIGRATION OF COMPOUNDS **12**, **13**, AND **35**, AND OF THE NATURAL PRODUCT ISOLATED FROM HEN-EGG ALBUMIN ON PAPER CHROMATOGRAMS AND ELECTROPHORETOGRAM

Compound	*Paper chromatography*[a]		*Paper electrophoresis*[b]	
	Conditions[c]			
	A	*B*	*C*	*D*
12	0.47	0.40	3.9	5.2
13	0.52	0.41	4.1	5.6
35	0.48	0.39	3.9	5.0
Natural product	0.47	0.40	3.9	5.1

[a]R_F value. [b]Distance of migration in cm. [c]A, 2-methylpropionic acid–0.5N aq. ammonia (5:3). B, phenol–water (10:1). C, 5N acetic acid, 20 volts cm^{-1}, 3.5 h. D, borate buffer (pH 8.0), 20 volts cm^{-1}, 2 h.

SUMMARY

2-Acetamido-1-*N*-[(aminoacyl)amino]-2-deoxy-β-D-glucopyranosylamines, whose aminoacyl groups L-alanyl, L-lysyl, β-L-aspartyl, and α-L-aspartyl, were synthesized by condensation of 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosylamine with suitably protected amino acids in presence of *N*,*N*′-dicyclohexylcarbodiimide, followed by removal of the protecting groups.

This method was applied to the synthesis of 2-[(aminoacyl)amino]-2-deoxy-β-D-

glucopyranosylacetamides using 2-[(protected aminoacyl)amino]-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosylamines as intermediates these were obtained, in the case of neutral and basic amino acid derivatives, by amination of the corresponding starting materials with methanolic ammonia.

For the acidic amino acid derivative, however, the protected carboxyl group was converted into the amido group by amination. 2-Amino-2-deoxy-D-glucopyranosylacetamide hydrochloride was synthesized from 4,6-*O*-benzylidene-2-[(benzyloxycarbonyl)amino]-2-deoxy-D-glucose by similar reactions, and then it was condensed with a protected amino acid. 2-[(β-L-Aspartyl)amino]-2-deoxy-β-D-glucopyranosylacetamide, thus obtained, was compared with 2-acetamido-1-[*N*-(β-L-aspartyl)amino]-2-deoxy-β-D-glucopyranosylamine, obtained from ovalbumin, and no significant difference could be found.

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REFERENCES

1 L. W. CUNNINGHAM, B. J. NUENKE, AND R. B. NUENKE, *Biochim. Biophys. Acta*, 26 (1957) 660.
2 F. R. JEVONS, *Nature*, 181 (1958) 1346.
3 P. JOHANSEN, R. D. MARSHALL, AND A. NEUBERGER, *Nature*, 181 (1958) 1345.
4 E. D. KAVERZNEVA AND V. P. BOGDANOV, *Biokhimiya*, 27 (1962) 273.
5 J. L. SIMKIN, E. R. SKINNER, AND H. S. SESHADRI, *Biochem. J.*, 90 (1964) 316.
6 T. H. PLUMMER, Jr., AND C. H. W. HIRS, *J. Biol. Chem.*, 239 (1964) 2530.
7 S. KAMIYAMA AND K. SCHMID, *Biochim. Biophys. Acta*, 63 (1962) 266.
8 G. S. MARKS, R. D. MARSHALL, AND A. NEUBERGER, *Biochem. J.*, 87 (1963) 274.
9 R. D. MARSHALL AND A. NEUBERGER, *Biochemistry*, 3 (1964) 1596.
10 I. YAMASHINA, K. BAN-I AND M. MAKINO, *Biochim. Biophys. Acta*, 78 (1963) 382.
11 F. MICHEEL, E. A. OSTMANN, AND G. PIELMEIER, *Tetrahedron Letters*, (1963) 115.
12 V. P. BOGDANOV, E. D. KAVERZNEVA, AND A. P. ANDREYEVA, *Biochim. Biophys. Acta*, 83 (1964) 69.
13 H. TSUKAMOTO, A. YAMAMOTO, AND C. MIYASHITA, *Biochem. Biophys. Res. Commun.*, 15 (1964) 151.
14 J. YOSHIMURA AND H. HASHIMOTO, *Nippon Kagaku Zasshi*, 85 (1964) 239.
15 C. H. BOLTON AND R. W. JEANLOZ, *J. Org. Chem.*, 28 (1963) 3228.
16 R. KUHN AND R. BROSSMER, *Ann.*, 616 (1958) 221.
17 N. K. KOCHETKOV, V. A. DEREVITSKAYA, L. M. LIKHOSHERSTOV, N. V. MOLODTSOV, AND S. G. KARA-MURZA, *Tetrahedron*, 18 (1962) 273.
18 E. CHARGAFF AND E. BOVARNICK, *J. Biol. Chem.*, 118 (1937) 426.
19 F. MICHEEL, Y. TANAKA, AND K. R. RÖMER, *Tetrahedron Letters*, (1964) 3913.
20 K. FISCHER, *Angew. Chem.*, 48 (1935) 394.
21 A. P. FLETCHER, G. S. MARKS, R. D. MARSHALL, AND A. NEUBERGER, *Biochem. J.*, 87 (1963) 265.