GLYCOPEPTIDES

COMMUNICATION 13. SYNTHESIS OF O-(AMINO-ACYL) DERIVATIVES OF N-ACETYLGLUCOSAMINE*

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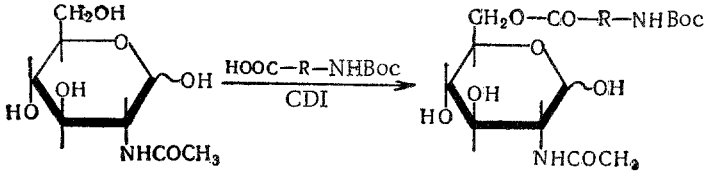
The elucidation of the nature of the linkage between the peptide and polysaccharide parts of natural glycopeptides is substantially facilitated by a study of the properties of model amino-acyl derivatives of monosaccharide derivatives, which are distinguished by the nature of the amino acid, of the monosaccharide, and of the type of bond between them. It is known that in natural glycopeptides, and also in other mixed biopolymers, amino sugars provide some of the most usual places for the linkage of the peptide chain to the polysaccharide [1-3]. We have previously prepared compounds with an amide type of bond—N-(amino-acyl) derivatives of glucosamine and galactosamine [4, 5]; in this paper we report on the synthesis of amino-acyl derivatives of N-acetylglucosamine, namely, 6-O-(amino-acyl) derivatives of N-acetylglucosamine (2-acetamido-2-deoxyglucose). The synthesis of these compounds was carried out by the method which we described earlier for the preparations of O-(amino-acyl) derivatives of neutral monosaccharides [5, 6], namely by the condensation of N-acetylglucosamine with (benzyloxycarbonylamino) acids in dry pyridine in presence of dicyclohexylcarbodiimide (CDI) in the cold.

The fairly universal carbodiimide method for the synthesis of amino-acyl derivatives of sugars was found to be very convenient in this case. The results show that the condensation of (benzyloxycarbonylamino) acids with unsubstituted N-acetylglucosamine goes predominantly at the primary hydroxyl with formation of 6-O-(amino-acyl) derivatives. When equimolecular amounts of the reactants are condensed, the formation of the 6-O-(amino acyl) derivative is accompanied by the formation of a small amount (10-15%) of the product of condensation at one of the secondary hydroxyls, which was found to be the 3-O-derivative (shown by periodate oxidation). When the condensation is carried out with 100% excess of N-acetylglucosamine the only reaction product, obtained in 50-60% yield, is the 6-O-(amino-acyl) derivative of glucosamine,



By the use of this variant of the synthesis we obtained N-(benzyloxycarbonyl) derivatives of 6-O-glycyl-, 6-O-alanyl-, 6-O-(6-aminohexanoyl)-, and also 6-O-diglycyl-N-acetylglucosamines. The structures of these compounds were confirmed by positive tests with the aniline phthalate reagent and silver nitrate (presence of free glycosidic hydroxyl), with fluorescein (presence of the benzyloxycarbonyl group), and the infrared spectra, which showed the presence of bands in the region 1740-1755 cm^{-1} (ester carbonyl), in the region 1675-1710 cm^{-1} (carbonyl group), and in the region 1675-1710 cm^{-1} (urethan carbonyl).

Final confirmation of the structures of the amino-acyl derivatives was obtained by periodate oxidation carried out for the case of N-acetyl-6-O-(N-benzyloxycarbonylglycyl)glucosamine. It is known [7] that the quantitative

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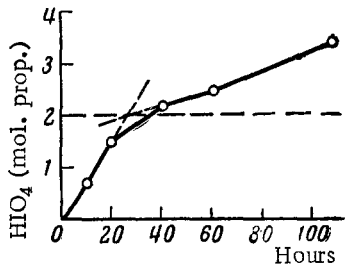


Fig. 1. Periodate oxidation of N-acetyl-6-O-(N-benzyloxycarbonylglycyl)glucosamine.

oxidation of N-acetylglucosamine at pH 7 and room temperature is very much complicated by processes of overoxidation due to the oxidation of acetamidomalonaldehyde formed as an intermediate product. Lowering of the oxidation temperature to 5° usually reduces the rate of overoxidation greatly. In view of this we carried out the periodate oxidation of the amino-acyl derivative of N-acetylglucosamine in the cold (5°) at pH 4. Under these conditions overoxidation still occurred, though at a relatively low rate, and to estimate the amount of periodic acid lost we determined the approximate kinetics of the oxidation (Fig. 1). It will be seen from Fig. 1 that the curve for the relation of periodic acid consumption to time has a sharp break corresponding to the consumption of 2 molecular proportions of periodic acid. Also, the N-acetyl-(N-benzyloxycarbonylglycyl)glucosamine obtained does not react with benzaldehyde, which excludes the structure of a 3-O-derivative. It has thus been proved unequivocally that the compound obtained is a 6-O-derivative.

To elucidate the question of the structure of the by-product formed in the amino-acylation of N-acetylglucosamine when equimolecular proportions of the reactants are used, the reaction mixture, without isolation of the products, was treated with benzaldehyde in presence of zinc chloride, as we have described earlier [8]. Analysis of the mixture by thin-layer chromatography showed the presence of not only the main product—N-acetyl-6-O-(N-benzyloxycarbonylglycyl)glucosamine—but also small amounts of the benzylidene derivative of another amino-acyl derivative, which could only be a 3-O-(amino-acyl) derivative. This was confirmed by a direct comparison on thin-layer chromatograms of this substance with a known sample of N-acetyl-4,6-O-benzylidene-3-O-(N-benzyloxycarbonylglycyl)glucosamine prepared by the condensation of N-acetyl-4,6-O-benzylideneglucosamine with N-(benzyloxycarbonyl)glycine under the conditions of carbodiimide condensation. Hence, the by-product in the amino-acylation of N-acetylglucosamine is the 3-O-(amino-acyl) derivative.

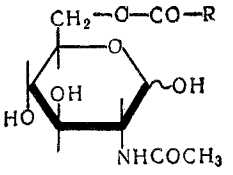
To pass from benzyloxycarbonyl derivatives to amino acyl derivatives with an unsubstituted amino group we eliminated the benzyloxycarbonyl group by hydrogenolysis in presence of oxalic acid, for 6-O-(amino-acyl) derivatives of N-acetylglucosamine are highly labile and cannot withstand the action of strong acids. The resulting N-acetyl-6-O-(amino-acyl)glucosamine oxalates were chromatographically and electrophoretically homogeneous, gave a positive test with ninhydrin, and gave positive tests for free hydroxyl with silver nitrate and aniline phthalate. Their structures were confirmed also by the infrared spectra, which contained an intense band at 1755 cm^{-1} (ester grouping) and a band at 1610 cm^{-1} (carbonyl of ionized oxalic acid). Judging from the behavior of the condensation products in the course of hydrogenation, they are more stable than the analogous products of condensation with D-glucose [9]. More detailed data on the stability of the compounds will be published shortly.

EXPERIMENTAL

Paper chromatography was carried out on Whatman No. 3 paper. The chromatograms were descending. Mobile phase: butyl alcohol—acetic acid—water, 4 : 1 : 1, upper layer (System I); ethyl acetate—pyridine—acetic acid—water, 5 : 5 : 3 : 1 (System II). Thin-layer chromatography on silica gel was carried out in the system chloroform—methanol, 8 : 2 (System III). Electrophoresis was carried out in the buffer: pyridine (2 ml)—acetic acid (4 ml)—water (to 1 liter), pH 4.2-4.5, voltage 900-1000. The spots were detected with ninhydrin, silver nitrate, fluorescein, aniline phthalate, and potassium periodate-cuprate.

N-Acetyl-6-O-[(benzyloxycarbonylamino)-acyl]-D-glucosamines. 0.015 mole of dicyclohexylcarbodiimide was added to a solution of 0.01 mole of N-acetyl-D-glucosamine and 0.005 mole of the N-(benzyloxycarbonyl) derivative of the amino acid or peptide (glycine, L-alanine, 6-aminohexanoic acid, diglycine) in 120 ml of dry pyridine, and the mixture was left for 48 h at 5°. Pyridine was vacuum-distilled off to dryness, the residue was treated with a mixture of 50 ml of water and 25 ml of ether, insoluble 1,3-dicyclohexylurea was filtered off, and the aqueous layer was extracted with five 25-ml portions of ether; the ether extracts were washed with two 25-ml portions of water, which were then combined with the main aqueous solution. The aqueous solution, slightly acidified with acetic acid, was extracted with butyl alcohol (eight 25-ml portions), and the butyl alcohol extracts were washed with water (three 15-ml portions) and evaporated to dryness in a vacuum. The yield of reaction product, which was somewhat contaminated with unchanged N-acetyl-D-glucosamine, was 50-60%. The product was purified by crystallization from a mixture of absolute ethanol and dry ethyl acetate. Data on the N-acetyl-6-O-[(benzyloxycarbonylamino)-acyl]-D-glucosamines obtained are given in Table 1.

TABLE 1. N-Acetyl-6-O-[(benzyloxycarbonylamino)-acyl]-D-glucosamines



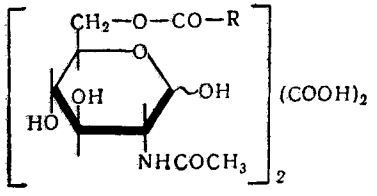
R	Molecular formula	M.p.,* °C	$[\alpha]_D^{18}$ †	Found, %		Calcd., %		R_f ‡		Yield of chromatographically pure substance, %
				C	H	C	H	a	b	
Boc-NH-CH_2—	$C_{18}H_{24}N_2O_9$	169,5—171	+45,90 (*C* 0,503, CH_3OH)	52,39 52,41	6,01 5,91	52,42	5,86	0,59	0,37	38
L-Boc-NH-CH-CH_3	$C_{19}H_{26}N_2O_9$	166,5—167,5		53,15 53,08	6,39 6,18	53,51	6,13	0,67	0,43	12
Boc-NH-$(CH_2)_5$-	$C_{22}H_{33}N_2O_9$	161—163	+46,02 (*C* 0,474, CH_3OH)	55,57	6,91	55,57	7,08	0,74	0,49	30
Boc-NH-CH_2-CO-NH-CH_2-	$C_{20}H_{27}N_3O_{10}$	127—128	+43,85 (*C* 0,497, CH_3OH)	51,20 51,29	6,00 6,08	51,17	5,79	0,47	0,23	27

* The melting point was determined in a Kofler block.

† Equilibrium mixture.

‡ a) Paper chromatography in System I; b) thin-layer chromatography in System III.

TABLE 2. N-Acetyl-6-O-(amino-acyl)-D-glucosamines



R	Molecular formula	M.p.* °C (decomp.)	$[\alpha]_D^{18}$ †	Found, %		Calculated, %		R_f ‡	Yield of chromatographically pure substance, %
				C	H	C	H		
NH_2-CH_2-	$C_{22}H_{38}N_4O_{18}$	108–110	+ 31,66 (*c* 0,476, water)	41,22 41,34	6,25 6,43	40,86	5,92	0,52	64,3
L-$NH_2-CH(CH_3)-$	$C_{24}H_{40}N_4O_{18}$	100–102	+ 16,18 (*c* 0,512, water)	43,22 43,33	6,59 6,78	42,85	5,96	0,59	74,1
$NH_2-(CH_2)_5-$	$C_{30}H_{54}N_4O_{18}$	86–87	+ 25,69 (*c* 0,487, water)	47,32	7,17	47,48	7,17	0,68	76
$NH_2-CH_2-CO-NH-CH_2-$	$C_{26}H_{44}N_6O_{20}$	119–121	+ 29,96 (*c* 0,499, water)	41,20 41,40	6,09 6,18	41,05	5,83	0,50	70

* The melting point was determined in a Kofler block.

† Equilibrium mixture.

‡ Paper chromatography in System II.

Identification of N-acetyl-4,6-O-benzylidene-3-O-[N-(benzyloxycarbonyl)glycyl]glucosamine formed in the condensation. 1 mmole of dicyclohexylcarbodiimide was added to a solution of 0.7 mmole of N-acetyl-4,6-O-benzylideneglucosamine and 0.7 mmole of N-(benzyloxycarbonyl)glycine in 30 ml of dry pyridine, and the mixture was stirred for one day at 20°. In thin-layer chromatography of the reaction mixture on silica gel in the system chloroform–methanol, 9 : 1, we obtained two spots, which were developed with aniline phthalate and concentrated sulfuric acid. One spot, R_f 0.35, corresponded to unchanged N-acetyl-4,6-O-benzylideneglucosamine (known sample), and the other, R_f 0.54, corresponded to the product of condensation at the only free secondary hydroxy (at C-3) {N-acetyl-4,6-O-benzylidene-3-O-[N-(benzyloxycarbonyl)glycyl]glucosamine}.

In the condensation of 0.1 mole of N-acetylglucosamine with 0.1 mole of N-(benzyloxycarbonyl)glycine in presence of 0.15 mole of dicyclohexylcarbodiimide in dry pyridine we obtained by the procedure described above 300 mg of pure N-acetyl-6-O-[N-(benzyloxycarbonyl)glycyl]glucosamine and 495 mg of a mixture of this 6-O-(amino-acyl) derivative (R_f 0.29 in thin-layer chromatography on silica gel in the system chloroform–methanol, 9 : 1) and what was thought to be the 3-O-(amino-acyl) derivative (R_f 0.41).

The 495 mg of mixture was dissolved in 5 ml of freshly distilled benzaldehyde in presence of freshly roasted $ZnCl_2$, and the mixture was stirred at 20° for 2.5 h. To remove benzaldehyde the reaction mixture was extracted with three 15-ml portions of petroleum ether; the precipitated oil was treated with a mixture of 15 ml of ether and 15 ml of water, and the ether layer was separated and evaporated; the residue was dissolved in methanol, and the methanolic solution was chromatographed on silica gel in the system chloroform–methanol, 9 : 1. Detection with aniline phthalate and concentrated sulfuric acid showed the presence of three spots: R_f 0.29, corresponding to the 6-O-(amino-acyl) derivative; R_f 0.41–the 3-O-(amino-acyl) derivative; and R_f 0.54, corresponding to N-acetyl-4,6-O-benzylidene-3-O-[N-(benzyloxycarbonyl)glycyl]glucosamine, which was prepared above.

N-Acetyl-6-O-(amino-acyl)glucosamines (oxalates). 0.15 mmole of the N-acetyl-6-O-[(benzyloxycarbonylamino)-acyl]-D-glucosamine was dissolved in 2 ml of 75% methanol, 0.09 mmole of oxalic acid and 35 mg of $Pd/BaSO_4$ were added, and hydrogenation was carried out for two hours. The catalyst was separated by centrifugation. The filtrate was diluted with five times its volume of dry acetone, and the flocculent precipitate that formed was centrifuged off, dissolved in the least possible amount of water (2-3 drops), and reprecipitated with dry acetone. The oil was rubbed out three times in absolute acetone until it was converted into a powder, and residual acetone was removed in a vacuum. Data on the substances obtained are given in Table 2.

Oxidation of N-acetyl-6-O-[N-(benzyloxycarbonyl)glycyl]-D-glucosamine with periodic acid. 20.9 g of N-acetyl-6-O-[N-(benzyloxycarbonyl)glycyl]-D-glucosamine was dissolved in 40 ml of acetate buffer of pH 4.0, 10 ml of periodic acid solution (5.5 g/liter) was added, and the mixture was left at 5° in the dark for 100 h. After definite intervals of time 5-ml aliquots were taken and neutralized with 0.5 N NaOH; 2 ml of 4% borax solution and 2 ml of 10% KI solution were added, and the solution was titrated with sodium arsenite. The results are shown in Fig. 1.

SUMMARY

By the condensation of N-(benzyloxycarbonylamino) acids with N-acetylglucosamine in presence of dicyclohexylcarbodiimide 6-O-(amino-acyl) derivatives of N-acetylglucosamine–models of one of the possible types of linkages in natural glycopeptides–were prepared.

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