SYNTHESIS OF MODIFIED LYSOZYME INHIBITORS BY THE OXAZOLINE METHOD

S. É. Zurabyan, V. V. Pimenova,
E. A. Shashkova, and A. Ya. Khorlin

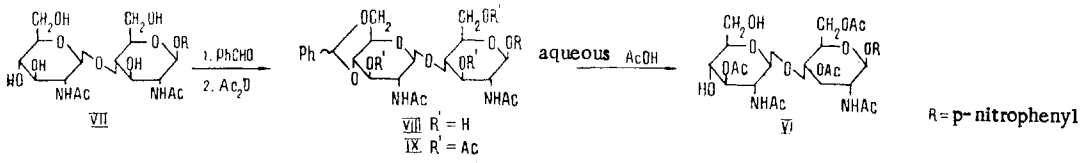
UDC 577.150.9 + 577.150.4

We have previously [1] investigated the interaction of lysozyme with low-molecular-weight inhibitors containing β-(1 → 4)- and β-(1 → 6)-glucosaminidic bonds. We showed that the trioside GNAc-(1 → 4)-GNAc-(1 → 6)-GNAc-$OC_6H_4NO_2$* (I) possesses a high affinity for the active center of lysozyme and is hydrolyzed by the latter. Later, glycosides of the trisaccharides GNAc-(1 → 3)-GNAc-(1 → 6)-GNAc $O_6H_4NO_2$ (II) [2] and GNAc-(1 → 4)-GNAc-(1 → 4)-GNAc-$OC_6H_4NO_2$ (III), isomeric with (I), were synthesized.

To widen the range of synthetic substrates and inhibitors of lysozyme we have performed the synthesis of glycosides of new oligosaccharides with monosaccharide residues connected by β-(1→4)- and β-(1-6)-glucosaminide bonds, namely: GNAc-(1 → 6)-GNAc-(1 → 4)-GNAc-$OC_6H_4NO_2$ (IV) and GNAc-(1 → 4)-GNAc-(1 → 6)-GNAc- (1 → 4)-GNAc-$OC_6H_4NO_2$ (V). In addition, we have studied the inhibiting action of these compounds and also the synthesized triosides (II) and (III) on the lysis of the bacterial walls of Micrococcus lysodeikticus by lysozyme.

For the synthesis of compounds (IV) and (V) we selected the oxazoline method, by means of which various 1,2-trans-2-acetamido-2-deoxyglycosides can be obtained, including oligosaccharides with various positions of the glycosidic linkages between the monosaccharide residues [2-4].

It has been reported [3] that in compounds containing unsubstituted hydroxy groups at the C-4 and C-6 atoms of the pyranose ring, the primary hydroxy group is glucosylated almost exclusively. Consequently, as the aglycone component in the synthesis of the trioside (IV) and the tetraoside (V) we selected the partial acetate of p-nitrophenyl-β-chitobioside (VI). This glycoside was obtained by converting di-N-acetyl-p-nitrophenyl-β-chitobioside (VII) into the benzylidene derivative (VIII) and then into its acetate (IX), with the subsequent removal of the benzylidene protective group by heating in glacial acetic acid.

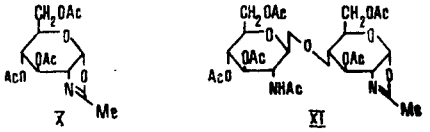


The stage of the glycosylation of (VI) with the oxazoline derivatives of (X) and (XI) was complicated by the low solubility of the component to be glycosylated in the solvents usually used [2-5] in this reaction. Because of this, the selection of a suitable solvent was the decisive factor in the synthesis of derivatives of higher oligosaccharides of amino sugars.

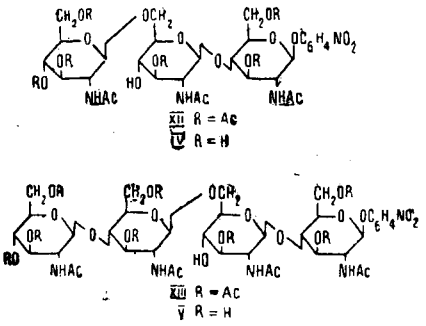
* GNAc represents 2-acetamido-2-deoxy-β-D-glucopyranosyl.

M. M. Shemyakin Institute of the Chemistry of Natural Compounds, Academy of Sciences of the USSR.
Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 689-693, November-December, 1971. Original article submitted July 8, 1971.

© 1971 Consultants Bureau, a division of Plenum Publishing Corporation, 227 West 17th Street, New York, N. Y. 10011. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording or otherwise, without written permission of the publisher. A copy of this article is available from the publisher for $15.00.



The performance of the reaction in highly polar solvents such as N,N-dimethylformamide (DMFA) or dimethyl sulfoxide did not lead to satisfactory results. The best glycosylation of (VI) was achieved in anhydrous nitromethane, the solubility in which the aglycone component was still fairly low. The reaction was performed with a 1.5- to 2-fold molar amount of the oxazolines (X) and (XI) in the presence of p-toluenesulfonic acid at 90-130°C. After the chromatographic separation of the reaction mixture and deacetylation, the desired products (IV) and (V) were obtained with yields of 14 and 13%, respectively. We were unable completely to avoid the formation of by-products, which were not studied in detail.



The glycosides (IV) and (V) were shown to be individual compounds on paper chromatograms [in the butanol–ethanol–water (10 : 2 : 5) system] (R_{GNAc} 1.22 and 0.84, respectively), and differed in chromatographic mobility from the corresponding glycosides with regular β-(1 → 4) bonds—tri-N-acetylchitotrioside (R_{GNAc} 1.17) and tetra-N-acetylchitotetraoside (R_{GNAc} 0.81).

The structure of the glycosides (IV) and (V) was shown by periodate oxidation and the Smith degradation of these sugars [7]. In oxidation, 2.0 equivalents of periodate were consumed, and in the products of the Smith degradation p-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside was identified. Both these facts indicate the presence of a (1 → 6)-glucosaminidic bond in the glycosides synthesized.

Information on the inhibiting action of the triosides (I-III) obtained previously and of the glycosides (IV) and (V) on the lysis of the bacterial cells of M. lysodeikticus by lysozyme is given in Table 1.

Attention is attracted by the similar values of $K_{i\,(appt)}$ for the triosides (I-III). The high inhibiting activity of trioside (I) has been reported previously [1]. It proved to be close to the activity of trioside (III), containing only β-(1 → 4)-glucosaminidic bonds, i.e., in this case, in contrast to the biosides GNAc-

TABLE 1. $[I]_{50}$ Values and Calculated* Values for $K_{i\,(appt)}$ for Various Inhibitors

Substance	Inhibitor	$[I]_{50}\times10^3$ M	$K_{i\,(appt)}{}^{*}\times 10^5$ M
I	GNAc-(1→4)-GNAc-(1→6)-GNAc-OR†	0,4	1,5[1]
II	GNAc-(1→3)-GNAc-(1→6)-GNAc-OR	0,4	1,5
III	GNAc-(1→4)-GNAc-(1→4)-GNAc-OR	0,25	0,9
IV	GNAc-(1→6)-GNAc-(1→4)-GNAc-OR	4,5	16,2
V	GNAc-(1→4)-GNAc-(1→6)-GNAc-(1→4)-GNAc-OR	0,65	2,3
	Tetra-N-acetylchitotetraose	0 1	0.36

* Calculated from the relation $K_{i\,(appt)} = K'_{i\,(appt)}\,[I]_{50}/[I]'_{50}$, which applies to competing inhibitors.

† R = p-nitrophenyl.

(1 → 4)-GNAc-$OC_6H_4NO_2$ and GNAc-(1 → 6)-GNAc-$OC_6H_4NO_2$ [1] the replacement of the β-(1 → 4)-glucosaminidic bond between the first and second (from the reducing end) monosaccharide units by a β-(1 → 6) bond has practically no effect on the affinity of the inhibitors for the active center of the lysozyme. The replacement of the β-(1 → 4)-glucosaminidic bond between the second and third monosaccharide residues by a β-(1 → 3) bond likewise has no effect on the inhibiting activity of the trioside. On the basis of these results it is possible to put forward the hypothesis of the absence in lysozyme of a strict specificity for the positions of the glucosaminidic bonds between the monosaccharide residues.

However, in those cases where the β-(1 → 4)-glucosidic bond between the second and third monosaccharide units was replaced by a β-(1 → 6) bond [as in glycosides (IV) and (V)], a considerable decrease in inhibiting effect is found. Triosides (I) and (IV) differ only by the sequence of β-(1 → 4)- and β-(1 → 6) glucosaminidic bonds, but this has a decisive influence on their affinity for the active center of the lysozyme. This fact permits the assumption that the sequence of positions of the glucosaminidic bonds in the oligosugars and their glycosides, determining the conformation of the carbohydrate chains of these modified substrates and inhibitors, plays a fundamental, if not decisive, role.

EXPERIMENTAL

The solvents were evaporated in vacuum at temperatures not exceeding 35°C. The melting points (corrected) were determined on a Kofler block and the specific rotations on a Perkin-Elmer 141 polarimeter. Thin-layer chromatography (TLC) was performed on silica containing 5% of gypsum, and paper chromatography (PC, descending) on "Leningrad medium" paper in the butanol–ethanol–water (10 : 2 : 5) system. The sugars were revealed with concentrated H_2SO_4 (TLC) and with diphenylamine or benzidine periodate (PC); the nitrophenyl glycosides were found specifically by means of a solution of sodium methoxide with subsequent heating. Periodate oxidation was performed with 0.01 N KIO_4, the consumption of periodate being determined iodometrically [6]. Degradation was carried out by Smith's method [7]. Analytical samples were dried at 25°C and a pressure of 10^{-6} mm for 6 h.

p-Nitrophenyl 2-Acetamido-4-O-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranoside (VIII). A mixture of 3.4 g of p-nitrophenyl di-N-acetyl-β-chitobioside (VII) [8], 25 ml of freshly distilled benzaldehyde, and 3.75 g of anhydrous zinc chloride was stirred at room temperature for two days. Then 20 ml of hexane and 20 ml of water were added to the reaction mixture and it was stirred for another 1 h. The hexane layer was decanted off, and the precipitate was filtered off and washed with water. Recrystallization from DMFA yielded 2.2 g (57%) of chromatographically pure [TLC; chloroform–methanol (4 : 1)] benzylidene derivative (VIII) with mp 261-264°C (decomp.), $[\alpha]_D^{20}$ −42° (c 0.15; DMFA).

Found %: C 55.1; H 5.7. $C_{29}H_{34}N_3O_{13}$. Calculated %: C 55.1; H 5.4.

p-Nitrophenyl 2-Acetamido-4-O-(2-acetamido-3-O-acetyl-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranoside (IX). Compound (VIII) (1.1 g) was acetylated with 10 ml of acetic anhydride in a mixture of 20 ml of pyridine and 15 ml of DMFA at 50°C for 24 h. This furnished 1.28 g (yield 97%) of the acetate (IX) with mp 305-306.5°C (decomp., from DMFA), $[\alpha]_D^{20}$ −40° (c 0.15; DMFA), $R_{cpd.(VIII)}$ 2.15 [TLC; chloroform–methanol (9 : 1)].

Found %: C 55.0; H 5.3. $C_{35}H_{41}N_3O_{16}$. Calculated %: C 55.3; H 5.4.

p-Nitrophenyl 2-Acetamido-4-O-(2-acetamido-3-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranoside (VI). A suspension of 4.68 g of (IX) in 300 ml of 60% acetic acid was stirred at 100°C for 30 min. The solution was evaporated to dryness and traces of benzaldehyde and acetic acid were eliminated by distillation with water. The residue was recrystallized from nitromethane. This gave 3.2 g (yield 77%) of the tri-O-acetate (VI), mp 236-237°C, $[\alpha]_D^{20}$ −35° (c 0.15; DMFA), $R_{cpd.(IX)}$ 0.43 [TLC, chloroform–methanol (9 : 1)].

Found %: C 49.7; H 5.5. $C_{28}H_{37}N_3O_{16}$. Calculated %: C 50.1; H 5.5.

p-Nitrophenyl O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 6)-O-(2-acetamido-3-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranoside (XII). A suspension of 0.40 g of (VI), 0.39 g of 2-methyl-(3',4',6'-tri-O-acetyl-1,2-dideoxy-α-D-glucopyrano) [2',1' : 4,5]-2-oxazoline (X) [9] and about 20 mg of p-toluenesulfonic acid in 13 ml of absolute nitromethane was heated in a sealed tube at 120-130°C for 30 min. Then the reaction mixture was

cooled, and 0.24 g of the starting material (VI) was filtered off. The filtrate was evaporated and the residue was chromatographed on silica (column 1.5 × 50 cm), the product being eluted by the gradient method with solvents ranging from chloroform to chloroform–methanol (12 : 1). From the fractions containing a substance with $R_{cpd.(VI)}$ 1.3 [TLC, chloroform–methanol (12 : 1)] 95 mg of the acetylated trioside (XII) was isolated. Yield 14% on the (VI) originally charged; mp 273.5-275°C (decomp.) from nitromethane, $[\alpha]_D^{20}$ −44° (c 0.2; DMFA).

Found %: C 50.6; H 5.6. $C_{42}H_{56}N_4O_{24}$. Calculated %: C 50.4; H 5.6.

p-Nitrophenyl O-(2-Acetamido-2-deoxy-β-D-glycopyranosyl)-(1 → 6)-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside (IV). A suspension of 180 mg of the acetate (XII) in 3 ml of absolute methanol was treated with 0.5 ml of 1 M sodium methoxide and was stirred at room temperature for 1 h. Then it was diluted with 3 ml of water, and the solution was deionized with KU-2 cation-exchange resin (H^+ form) and evaporated to dryness. Recrystallization of the residue from aqueous methanol gave 130 mg (yield 96%) of the trioside (IV) with mp 257.5-259°C, $[\alpha]_D^{20}$ −2° (c 0.21; water), R_{GNAc} 1.22 (PC).

Found %: C 47.0; H 6.3. $C_{30}H_{14}N_4O_{18} \cdot H_2O$. Calculated %: C 47.0; H 6.0.

The trioside (IV) absorbed 2.0 equivalents of KIO_4 in 16 h at 25°C.

p-Nitrophenyl O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-O-(2 acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 6)-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside (V). A suspension of 1.0 g of (VI), 1.4 g of 2-methyl-[4'-O-(2'-acetamido-3',4',6'-tri-O-acetyl-2'-deoxy-β-D-glucopyranosyl)-3',6'-di-O-acetyl-1,2-dideoxy-α-D-glucopyrano][2',1' : 4,5]-2-oxazoline (XI) [9], and about 30 mg of p-toluenesulfonic acid in 140 ml of absolute nitromethane was heated at 80-90°C with stirring for 1.5 h. The reaction mixture was evaporated to dryness and the residue was chromatographed on silica (column 1.5 × 50 cm) with elution by the gradient method using solvents ranging from chloroform to chloroform–methanol (9 : 1). The fractions containing substances with $R_{cpd.(VI)}$ 1.0 [TLC, chloroform–methanol (9 : 1)] were combined and evaporated. The mixture of the starting material (VI) and the product (XIII) obtained in this way (0.96 g) was treated with 3 ml sodium methoxide in 10 ml of absolute methanol at 25°C with stirring for 1 h. The mixture was diluted with water to dissolve the precipitate and was neutralized with KU-2 cation-exchange resin (H^+ form). The resin was filtered off and washed with water, and the solution was evaporated. Preparative chromatography on Whatman 3 MM paper gave 0.18 g (yield 13%) of the tetraoside (V), mp 248-249°C (from methanol), $[\alpha]_D^{20}$ −28° (c 0.2; water), R_{GNAc} 0.84 (PC).

Found %: C 47.1; H 6.4. $C_{38}H_{57}N_5O_{23} \cdot H_2O$. Calculated %: C 47.0; H 6.1.

The tetraoside (V) absorbed 2.0 equivalents of KIO_4 in 16 h at 25°C.

Inhibition of the Lysis of the Bacterial Walls of M. lysodeikticus. These experiments were performed by the procedure described previously [1].

CONCLUSIONS

The following glycosides of tri- and tetrasaccharides have been synthesized by the oxazoline method: GNAc-(1 → 6)-GNAc-(1 → 4)-GNAc-$OC_6H_4NO_2$ and GNAc-(1 → 4)-GNAc-(1 → 6)-GNAc-(1 → 4)-GNAc-$OC_6H_4NO_2$.

Information has been given on the inhibition by these compounds and other triosides of the lysis of the bacterial walls of M. lysodeikticus by lysozyme.

It has been shown that a change in the positions of the glucosaminidic bonds between the monosaccharide units in the modified inhibitors does not deprive them of their affinity for the active center of lysozyme, but their inhibiting activity depends substantially on the sequence of position of the glucosaminidic bonds.

LITERATURE CITED

1. E. A. Shashkova, I. V. Bikha, A. A. Vichutinskii, and A. Ya. Khorlin, Biokhimiya, 35, 124 (1970).
2. S. É. Zurabyan, T. S. Antonenko, and A. Ya. Khorlin, Carbohydrate Res., 15, 21 (1970).
3. S. É. Zurabyan, T. P. Volosyuk, and A. Ya. Khorlin, Carbohydrate Res., 9, 215 (1969).

4. T. S. Antonenko, S. É. Zurabyan, and A. Ya. Khorlin, Izv. Akad. Nauk SSSR, Ser. Khim., 1970, 2766.
5. W. L. Salo and H. G. Fletcher, J. Org. Chem., 34, 3189 (1969).
6. L. M. Likhosherstov and L. É. Brossar, Khim. Prirodn. Soedin., 3, 7 (1967).
7. I. J. Goldstein, G. W. Hay, B. A. Lewis, and F. Smith, in: Methods of Carbohydrate Chemistry, Academic Press, Vol. 5 (1965), p. 361.
8. S. É. Zurabyan, T. P. Volosyuk, and A. Ya. Khorlin, Izv. Akad. Nauk SSSR, Ser. Khim., 1968, 1612.
9. A. Ya. Khorlin, M. L. Shul'man, S. É. Zurabyan, I. M. Privalova, and Yu. L. Kopaevich, Izv. Akad. Nauk SSSR, Ser. Khim., 1968, 2094.