Bull. Chem. Soc. Jpn., 59, 1419—1423 (1986)

Synthesis of *β*(1—4)-Linked Disaccharides of *N*-Acetylglucosamine and *N*-Acetylmuramic Acid by Their Direct Condensation

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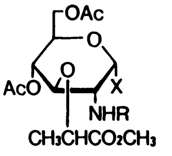
(Received January 14, 1986)

As a basic study in the synthetic approach to immunoactive cell wall peptidoglycan whose backbone is a *β*(1—4)-linked saccharide of alternating glucosamine and muramic acid, methods for the direct condensation of these sugar components were studied and *O*-(*N*-acetyl-*β*-muramyl)-(1→4)-*N*-acetyl-D-glucosamine and *O*-(*N*-acetyl-*β*-D-glucosaminyl)-(1→4)-*N*-acetylmuramic acid were synthesized. In the synthesis of the latter disaccharide, previous formation of an ester bond between the carboxyl group of muramic acid and the 6-hydroxyl group of glucosamine proved to facilitate the glycoside bond fromation between these sugar moieties.

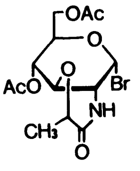
In the preceding paper, we reported syntheses of two disaccharide-dipeptides which correspond to partial structures of cell wall peptidoglycan, i.e., *O*-(*N*-acetyl-*β*-D-glucosaminyl)-(1→4)-*N*-acetylmuramyl-L-alanyl-D-isoglutamine and *O*-(*N*-acetyl-*β*-muramyl-L-alanyl-D-isoglutamine)-(1→4)-*N*-acetyl-D-glucosamine.[1] Both of these structures contain additional one D-glucosamine unit linked to either position 1 or 4 of the muramic acid residue of *N*-acetylmuramyl-L-alanyl-D-isoglutamine which is the minimum structure responsible for the immunostimulating activity of cell wall peptidoglycan. Since cell wall fragments which retain long glycan chains generally show higher activity than the muramyl dipeptide, we expected similar enhancement of the activity for these disaccharide dipeptides as well. However, the result of biological tests revealed that only one additional glucosamine unit is not sufficient and a longer glycan structure would be required for significant enhancement of the activity.[1]

In this respect, we were interested in the synthesis of a larger cell wall structure whose backbone consists of an alternating glycan of glucosamine and muramic acid. For construction of such a glycan structure, direct coupling of the component sugar seemed to be necessary.[2] However, no direct synthesis of saccharides of muramic acid has been reported yet. We thus examined this approach and describe in this paper a preparation of both sequences of *β*(1—4) disaccharide consisting of glucosamine and muramic acid by their direct condensation.

The synthesis of the disaccharide in which muramic acid is located on the non-reducing side, i.e., O-(*N*-acetyl-*β*-muramyl)-(1→4)-*N*-acetyl-D-glucosamine, was performed as follows. At first, several glycosyl halogenides **1**—**4** were prepared[3] and subjected to reaction with glucosamine derivatives (**5** or **6**) in the presence of mercury(II) cyanide. However, no disaccharide was obtained in any combination of reaction partners, halogenides being transformed quantitatively to the corresponding oxazolines.[4] Therefore, glycosidation with oxazoline derivatives was next attempted. The oxazoline **7** derived from peracetylated methyl ester of muramic acid was subjected to acid-catalyzed reaction[5] with the glycosyl acceptor **5** or **6** as above. Again in these cases, no disaccharide was obtained. After prolonged reaction period, **7** was either hydrolyzed or changed to the corresponding glycal. Such low ability of the muramic acid derivative **7** to act as glycosyl donor seemed to be attributed to the steric hindrance caused by the lactic acid moiety, which might cover the upper face of the molecules, hindering the access of



1 X=Cl , R=Ac
2 X=Br , R=Ac
3 X=Br , R=CHCl₂CO



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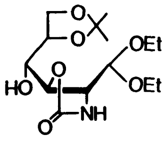

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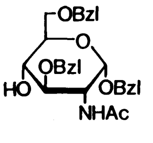

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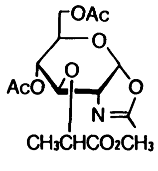

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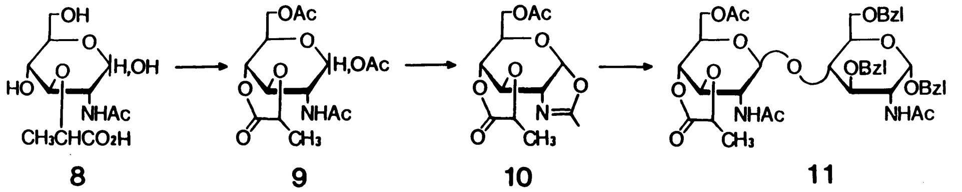


8 **9** **10** **11**

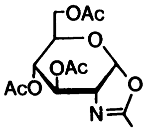
the glycosyl acceptor from the β-side.[6]

Relaxation of this steric hindrance could be expected in the oxazoline of an intramolecular ester form **10** where the lactic acid moiety is kept away from C-1. Formation of this ester also serves for the simultaneous protection of both carboxyl and 4-hydroxyl groups of the muramic acid moiety. In fact, by coupling of **10** with the glucosamine derivative **6** the desired β(1—4) disaccharide **11**[7] was obtained in 21% yield. The relatively low yield of **11** could be ascribed to the known very poor reactivity of the 4-hydroxyl group of glucosamine in the pyranoside form.

The next target of the synthesis was another disaccharide in which muramic acid is the reducing component. The first attempt to condense the simple peracetylated oxazoline **12** with the muramic acid derivative **13** failed to give a disaccharide. In addition to the known low reactivity of the 4-hydroxyl group described above, a steric hindrance due to the lactic acid moiety in **13** might be responsible in this case, too. Therefore, a novel device was required to facilitate the coupling. We thus tried to connect the two components prior to the glycosidation reaction with an ester linkage which can be formed more readily than a glycosidic bond. The muramic acid possesses a carboxyl function which can be used for this connection. The glycosidation reaction then becomes an intramolecular process and hence could be expected to proceed more easily. In the field of peptide chemistry, several examples of similar approaches had been described where two components to be coupled had been brought close together by auxiliary groups.[8,9]

Examination of molecular models indicated that an ester linkage between the carboxyl group of the muramic acid residue and the 6-hydroxyl groups of glucosamine is sterically most favorable for the formation of β(1—4) glycoside. According to this strategy, a synthetic precursor required for the glycosidation reaction, i.e., an oxazoline with an ester-linked muramic acid, was prepared as follows. The 6-hydroxyl group of the glucosamine derivative **14** was esterified with the protected muramic acid **17**.[10] Acetylation of the remaining free 4′-hydroxyl group in the product **18** followed by cleavage of the acetonide and 6-*O*-monoacetylation afforded **21**. All these transformations proceeded in satisfactory yields. When **21** was subjected to the standard conditions for allyl–propenyl isomerization described by Gent and Gigg[11] (in the presence of DABCO in ethanol–benzene–water), cleavage of the inter-sugar ester linkage occurred preferentially. This side reaction could be avoided by using dry benzene as solvent and eliminating DABCO. The propenyl glycoside **22** thus obtained was then treated with mercury (II) salt[12] to give the desired oxazoline **23** as syrup.

Glycosidation was effected in the presence of *p*-toluenesulfonic acid at 50 °C. The product was purified after cleavage of the ester linkage by methanolysis followed by re-*O*-acetylation.[13] The peracetylated methyl ester of *O*-(β-glucosaminyl)-(1→4)-muramic acid benzyl glycoside (**24**) was obtained in 20% yield from the oxazoline **23**. In contrast, no disaccharide was obtained at all under similar reaction conditions as described above in the reaction of the two corresponding monosaccharide units **12** and **13** which were not linked previously together with an ester bond. Consequently, the presence of the ester linkage which kept the two sugar moieties



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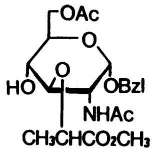

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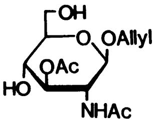

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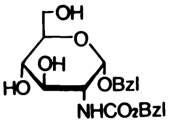

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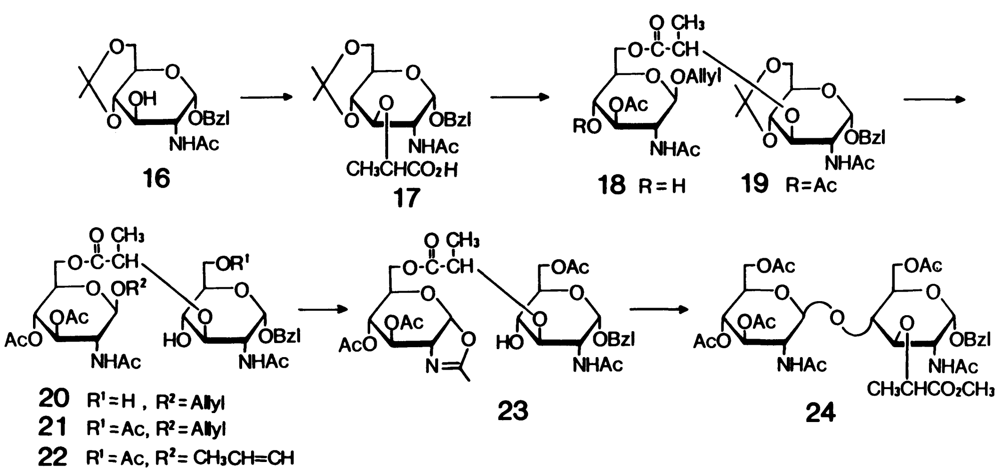

in close proximity to each other certainly favored the formation of the desired glycoside bond in the above experiment. Thus, this is the first example of the so-called "entropic activation"[9] in glycosidation reaction.

Since both sequences of β(1—4) disaccharide were prepared by direct condensation of glucosamine and muramic acid as described in this work, the basic problem was solved in the synthesis of cell wall glycan which consists of the alternating these two sugar moieties. Furthermore, the receipt of "entropic activation" demonstrated in this work seems to have wide applicability. Its some application even for glycosides without muramic acid were already attempted[14] by linking sugar moieties with dicarboxylic acids and the result will be reported in details elsewhere.

Experimental

All melting points are uncorrected. ^{1}H NMR spectra were measured for $CDCl_3$ solutions with tetramethylsilane as an internal standard (δ, ppm). Optical rotations were measured with a Perkin-Elmer 141 polarimeter. FD-MS spectra were obtained with Si-emitter on JEOL O1-SG mass spectrometer. Column chromatography and preparative thin-layer chromatography were carried out with Kieselgel 60 (0.063—0.2 mm) and with pre-coated Kieselgel F_{254} plates (0.5 mm thickness), E. Merck.

2-Acetamido-1,6-di-*O*-acetyl-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-D-glucopyranose (Intramolecular Ester Form) (9). Acetic anhydride (10.0 ml, 106 mmol) was added to a suspension of *N*-acetylmuramic acid (**8**) (4.05 g, 15.3 mmol) in THF (130 ml). After the mixture had been stirred at 30 °C for 13 h and cooled to room temperature, pyridine (20 ml) was added to it. The mixture was stirred at room temperature for additional 8 h. The product isolated by usual work-up was recrystallized from $CHCl_3$–ether; 4.33 g (79%); mp 200—205 °C(decomp); $[\alpha]_D^{23}$ +151° (*c* 1.00, $CHCl_3$), ^{1}H NMR (100 MHz): 6.40 (1H, d, J=8 Hz, NH); 6.20 (1H, d, J=3 Hz, H-1), 2.15, 2.10, and 1.95 (each 3H, s, $COCH_3$), 1.50 (3H, d, J=7 Hz, lactyl CH_3). Found: C, 49.93; H, 5.87; N, 3.83%. Calcd for $C_{15}H_{21}NO_9$: C, 50.14; H, 5.89; N, 3.90%.

6-*O*-Acetyl-2-amino-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-1-α-*O*:2-*N*,*N*-ethylidyne-D-glucopyranose (Intramolecular Ester Form) (10). Compound **9** (4.25 g, 11.8 mmol) was dissolved in a saturated solution of dry HBr in CH_2Cl_2 (80 ml), and the mixture was left stand at room temperature for 13 h. After the solvent had been evaporated in vacuo. The residue was dried in vacuo over KOH, overnight, and dissolved again in dry CH_2Cl_2 (80 ml). Pyridine (20 ml) was added to the solution. The mixture was stirred at room temperature for 1 h, washed with H_2O, dried ($MgSO_4$) and evaporated in vacuo to give syrup. It was used for the next reaction without further purification after co-evaporation in vacuo three times with toluene. ^{1}H NMR (60 MHz): 6.0 (1H, d, J=7 Hz, H-1), 2.10 and 2.15 (each 3H, s, oxazoline CH_3 and CH_3CO), 1.60 (3H, d, J=7 Hz, lactyl CH_3).

Benzyl 4-*O*-[2-Acetamido-6-*O*-acetyl-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-β-D-glucopyranosyl]-2-acetamido-3,6-di-*O*-benzyl-α-D-glucopyranoside (Intramolecular Ester Form) (11). *p*-Toluenesulfonic acid monohydrate (80 mg, 0.42 mmol) was dehydrated by heating at 110 °C in vacuo. To this was added a solution of **10** (obtained from 4.25 g of **9** as described above) and benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**6**) (1.82 g, 3.70 mmol) in dry CH_2ClCH_2Cl (50 ml). The mixture was heated at 70 °C for 13 h. After evaporation of the solvent in vacuo, the product was purified by silica-gel column chromatography and recrystallized from $CHCl_3$–acetone; 604 mg (21% based on **6**); mp 262—265 °C (decomp); $[\alpha]_D^{20}$ +114° (*c* 1.01, $CHCl_3$). ^{1}H NMR (100 MHz): 7.40, 7.30, and 7.25 (each 5H, s, aromatic), 1.90, 1.85, and 1.70 (each 3H, s, CH_3CO), 1.45 (3H, d, J=7 Hz, lactyl CH_3). FD-MS: m/z 791 $(M+H)^+$, 813 $(M+Na)^+$. Found: C, 63.66; H, 6.34; N, 3.55%. Calcd for $C_{42}H_{50}N_2O_{13}$; C, 63.79; H, 6.37; N, 3.54%.

Benzyl 2-Benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside (15). To a suspension of 2-benzyloxycarbonylamino-2-deoxy-D-glucose[15] (120 g, 0.384 mol) in benzyl alcohol (900 ml) heated at 100 °C, a solution of dry HCl (29.9 g) in benzyl alcohol (180 ml) was added. The mixture was stirred at 100 °C till a homogeneous solution was obtained. After cooling, the mixture was poured into ether (1 l). The precipitate obtained on addition of hexane (2 l) was filtered and recrystallized form EtOH; 144 g (77%); mp 168—170 °C; $[\alpha]_D^{13}$ +123° (*c* 0.98, MeOH).[16]

Benzyl 2-Acetamido-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside (16).[10] Compound **15** (20.0 g, 59.8 mmol) was hydrogenolyzed with Pd-black in a mixture of MeOH and THF (1:1, 400 ml). When the starting material disappeared as checked with TLC, the catalyst was filtered off, and acetic anhydride was (10.0 ml, 106 mmol) added to the filtrate. After 1 h at room temperature, the solvent was evaporated in vacuo. The residue was recrystallized from EtOH to give the acetamido derivative; 16.4 g (94%).

To a suspension of this product (10.0 g, 26.6 mmol) in dry acetone (150 ml), were added 2,2-dimethoxypropane (3.0 ml, 28 mmol) and *p*-toluenesulfonic acid monohydrate (0.25 g). The mixture was stirred at room temperature for 4 h and worked up as usual to give **16** as amorphous solid; 10.4 g (88% from **15**); $[\alpha]_D^{13}$ +98.3° (*c* 0.97, $CHCl_3$). ^{1}H NMR (60 MHz): 7.20 (5H, s, aromatic), 4.87 (1H, d, J=2.5 Hz, H-1), 1.67 and 1.47 (each 3H, s, isopropylidene CH_3).

Benzyl 2-Acetamido-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside (17).[10] Sodium hydride (60% oil dispersion; 1.65 g, 68.4 mmol) was added to a solution of **16** (12.0 g, 34.2 mmol) in dry dioxane (700 ml). The mixture was heated to 60 °C and (*S*)-2-chloropropionic acid (8.00 g, 68.4 mmol) was added to it. Stirring was then continued at 60 °C for 1 h. After the excess NaH had been decomposed with ice, the solvent was evaporated in vacuo and the residue extracted with $CHCl_3$ after acidification. Usual work-up afforded a syrup which was dissolved in AcOEt and treated with an ethereal solution of CH_2N_2. Complete evaporation of the volatile materials (bath 50 °C at 5 mmHg) afforded a crystalline residue, which was once washed with a mixture of ether–hexane (5:1) and then hydrolyzed with KOH (2.0 g, 36 mmol) in MeOH (120 ml) at room temperature. Removal of the solvent after neutralization with Amberlite IRC-CG50 (H^+) afforded amorphous solid; 11.9 g (82%); $[\alpha]_D^{16}$ +127.3° (*c* 0.92, $CHCl_3$). ^{1}H NMR (90 MHz): 9.05 (1H, broad s, COOH), 7.95 (1H, d, J=2 Hz, NHAc), 7.28 (5H, s, aromatic), 5.35 (1H, d, J=2.5 Hz, H-1), 4.35 (1H, q, $CH_3C\underline{H}$(O)–COOH), 2.05 (3H, s, CH_3CO), 1.51 and 1.40 (each 3H, s, isopropylidene CH_3), 1.42 (3H, d, J=6.8 Hz, $C\underline{H}_3CH$(O)–COOH).

Allyl 2-Acetamido-3-*O*-acetyl-2-deoxy-*β*-D-glucopyranoside 6-*O*-Esterified with Benzyl 2-Acetamido-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-4, 6-*O*-isopropylidene-*α*-D-glucopyranoside (18). Dicyclohexylcarbodiimide (3.80 g, 18.4 mmol) and 4-dimethylaminopyridine (0.02 g) were added to an ice-cooled solution of allyl 2-acetamido-3-*O*-acetyl-2-deoxy-*β*-D-glucopyranoside (**14**) (5.00 g 16.5 mmol) and **17** (7.70 g, 18.2 mmol) in a mixture of THF (20 ml) and dioxane (100 ml). After the mixture had been stirred at room temperature for 24 h, the product isolated by usual work-up and silica-gel column chromatography ($CHCl_3$–MeOH 30:1) was recrystallized from EtOAc–hexane; 10.0 g (86%); mp 107—111 °C; $[\alpha]_D^{16}$ +61.1° (c 1.11, $CHCl_3$). Found: C, 56.57; H, 6.99; N, 3.87%. Calcd for $C_{34}H_{48}N_2O_{14}\cdot 3/4H_2O$: C, 56.54; H, 6.91; N, 3.88%.

Allyl 2-Acetamido-3,4-di-*O*-acetyl-2-deoxy-*β*-D-glucopyranoside 6-*O*-Esterified with Benzyl 2-Acetamido-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-4, 6-*O*-isopropylidene-*α*-D-glucopyranoside (19). Compound **18** (2.36 g, 3.33 mmol) was acetylated with acetic anhydride in pyridine. After usual work-up, the product was recrystallized from EtOAc–hexane; 1.89 g (76%); mp 168—170 °C; $[\alpha]_D^{16}$ +75.6° (c 0.93, $CHCl_3$). FD-MS: m/z 750 (M^+). Found: C, 57.55; H, 6.73; N, 3.70%. Calcd for $C_{36}H_{50}N_2O_{15}$: C, 57.59; H, 6.71 N, 3.73%.

Allyl 2-Acetamido-3,4-di-*O*-acetyl-2-deoxy-*β*-D-glucopyranoside 6-*O*-Esterified with Benzyl 2-Acetamido-6-*O*-acetyl-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-*α*-D-glucopyranoside (21). Compound **19** (4.10 g, 5.47 mmol) was heated at 90 °C in 90% AcOH (100 ml) for 30 min. Evaporation of the solvent followed by co-evaporation with toluene in vacuo and trituration with ether afforded **20** as crystals, which were collected by filtration; 3.71 g (96%), mp 180—181 °C, $[\alpha]_D^{16}$ +58.4° (c 1.07, MeOH).

This compound (4.00 g, 5.6 mmol) was dissolved in pyridine (100 ml) and treated with acetic anhydride (2.3 ml, 23 mmol) under ice-cooling for 3 h. Usual work-up followed by purification with a silica-gel column ($CHCl_3$–MeOH, 30:1) gave an amorphous solid; 3.8 g (90%); $[\alpha]_D^{16}$ +98.4° (c 0.92, $CHCl_3$). Found: C, 55.52; H, 6.52; N, 3.67%. Calcd for $C_{35}H_{48}N_2O_{16}\cdot 1/4H_2O$: C, 55.51; H, 6.46; N, 3.70%.

1-Propenyl 2-Acetamido-3,4-di-*O*-acetyl-2-deoxy-*β*-D-glucopyranoside 6-*O*-Esterified with Benzyl 2-Acetamido-6-*O*-acetyl-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-*α*-D-glucopyranoside (22). Compound **21** (2.00 g, 2.66 mmol) was heated under reflux with chlorotris(triphenylphosphine)rhodium(I) (200 mg) in anhyd benzene under Ar atmosphere for 16 h. After evaporation of the solvent in vacuo, the product was isolated by silica-gel column chromatography ($CHCl_3$–MeOH, 30:1) as pale yellow solid; 1.34 g (67%); $[\alpha]_D^{16}$ +9.06° (c 1.02, $CHCl_3$). ^1H NMR (90 MHz): 6.30—5.88 (1H, m, $OC\underline{H}=CHCH_3$), 1.53 (3H, dd, $OCH=CHC\underline{H}_3$).

3,4-Di-*O*-acetyl-2-amino-2-deoxy-1-*α*-*O*: 2-*N*,*N*-ethylidyne-D-glucopyranose 6-*O*-Esterified with Benzyl 2-Acetamido-6-*O*-acetyl-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-*α*-D-glucopyranoside (23). After a solution of **22** (800 mg, 1.1 mmol) in anhyd CH_3CN (15 ml) had been stirred with powdered $CaSO_4$ (300 mg) at room temperature, $HgCl_2$ (450 mg, 1.7 mmol) and HgO (450 mg, 2.1 mmol) were added to it and the whole mixture was stirred at 60 °C under Ar atmosphere for 2 h. Usual work-up and column chromatographic purification on silica gel ($CHCl_3$–MeOH, 50:1) afforded colorless syrup; 313 mg (41%). ^1H NMR (100 MHz): 5.99 (1H, d, J=7.8 Hz, H-1 of oxazoline form). FD-MS: m/z 694 (M^+).

Benzyl 4-*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-*β*-D-glucopyranosyl)-2-acetamido-6-*O*-acetyl-2-deoxy-3-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-*α*-D-glucopyranoside (24). Compound **23** (225 mg, 0.32 mmol) was heated in dry CH_2ClCH_2Cl (4.5 ml) in the presence of *p*-toluenesulfonic acid (2 mg) at 50 °C for 16 h. The mixture was cooled and then treated with ethereal solution of CH_2N_2. The solvent was evaporated in vacuo and the residue was treated with 0.1 M† NaOMe (20 ml). After 3 h, the mixture was neutralized with Dowex 50X8 (H^+) and evaporated in vacuo. The residue was dried by co-evaporation with benzene and then acetylated with acetic anhydride (0.4 ml) and pyridine (5 ml). The product obtained by usual work-up followed by silica-gel column chromatography ($CHCl_3$–MeOH, 50:1) was recrystallized from EtOAc–ether; 47.9 mg (20%); mp 192—194 °C; $[\alpha]_D^{11}$ +102° (c 1.04, $CHCl_3$). FD-MS: m/z 768 (M^+). Found: C, 54.63; H, 6.32; N, 3.68%. Calcd for $C_{35}H_{48}N_2O_{17}$: C, 54.68; H, 6.29; N, 3.64%.

References

1) S. Kusumoto, K. Yamamoto, M. Imoto, M. Inage, M. Tsujimoto, S. Kotani, and T. Shiba, *Bull. Chem. Soc. Jpn.*, the preceding paper.

2) In our preceding synthesis,[1] the disaccharides of muramic acid and glucosamine were prepared via a glucosamine disaccharide by converting one of its sugar moieties into muramic acid. However, application of the same approach to a larger glycan seems to be not practical because complete and regio-controlled introduction of a desired number of 1-carboxyethyl ethers to a glucosamine oligosaccharide seems to be difficult.

3) The chloride (**1**) was prepared by treating *N*-acetylmuramic acid methyl ester with acetyl chloride, while the bromides were prepared by action of HBr in CH_2Cl_2 on the corresponding 1-*O*-acetates. Those of dichloroacetyl and intramolecular amide types were derived via removal of the *N*-acetyl group with $Et_3O\cdot BF_4$. *N*-Acetylmuramic acid itself was prepared according to Flowers and Jeanloz with slight modification as described for the preparation of **17**; *J. Org. Chem.*, **28**, 2983 (1963).

4) The glucosamine components were recovered unchanged. In case of **4**, the decomposition product could not be identified.

5) *p*-Toluenesulfonic acid, trifluoromethanesulfonic acid or $FeCl_3$ was used in CH_2ClCH_2Cl at 80 °C.

6) The same reason might also apply to the halogenides (**1**—**4**) described above.

7) For further confirmation of the structure, **11** was coupled with benzyl L-alanyl-D-isoglutaminate after alkaline hydrolysis. Hydrogenolytic deprotection of the coupling product afforded *O*-(*N*-acetyl-*β*-muramyl-L-alanyl-D-isoglutamine)-(1→4)-*N*-acetyl-D-glucosamine which was iden-

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† $1M=1\ mol\ dm^{-3}$.

tical with the compound obtained in our previous work.1)

8) a) I. Ugi, *Angew. Chem.*, **74**, 9 (1962); b) H. v. Zychlinsky, I. Ugi, and D. Marquarding, *Angew. Chem.*, **86**, 517 (1974).

9) D. S Kemp, *Biopolymers*, **20**, 1793 (1981) and references cited in it.

10) A. Hasegawa, Y. Kaneda, M. Amano, M. Kiso, and I. Azuma, *Agric. Biol. Chem.*, **42**, 2187 (1978).

11) P. A. Gent and R. Gigg, *J. Chem. Soc., Perkin Trans. 1*, **1973**, 1858.

12) M . A. Nashed, C. W. Slife, M. Kiso, and L. Anderson, *Carbohydr. Res.*, **82**, 237 (1980).

13) When the condensation product was subjected directly to preparative silica gel TLC, a considerable cleavage of the inter-sugar ester linkage occurred to give a mixture.

14) A part of the preliminary result was already presented at the National Meeting of the Chemical Society of Japan, Kyoto 1983, Proceeding Vol. 2, p. 1151.

15) K. Heins, K. Propp, R. Harrison, and H. Paulsen, *Chem. Ber.*, **100**, 2655 (1967).

16) Though a small amount of the β-anomer was detected on TLC, this product was used for the following reaction.