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Note

A simple approach to the synthesis of muramic acid and isomuramic acid: ^{1}H and ^{13}C NMR characterisation

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Abstract

A simple and efficient synthesis of 2-amino-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-D-glucose (muramic acid, **6**) and its stereoisomer 2-amino-3-*O*-[(*S*)-1-carboxyethyl]-2-deoxy-D-glucose (isomuramic acid, **7**) from methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (**1**) is described. Condensation of the O-3 oxyanion of **1** with an excess of methyl (*R*,*S*)-2-bromopropionate, followed by alkaline hydrolysis of the crude product and subsequent acidification, afforded crystalline methyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-[(*R*,*S*)-1-carboxyethyl]-2-deoxy-α-D-glucopyranoside (**2**), in 72% yield, as a mixture of diastereomers. Esterification of **2** with an excess of diazomethane afforded quantitatively the corresponding mixture of epimeric esters, which were very easily separated by column chromatography on silica gel, giving pure (*R*) and (*S*) epimeric esters. Removal of the benzylidene and acetyl groups by acid hydrolysis gave, respectively, muramic acid (**6**), in 95% yield and isomuramic acid (**7**), in 93% yield. ^{1}H and ^{13}C NMR data are given. © 1997 Elsevier Science Ltd.

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Muramic acid (**6**) has been shown to be the main component of the peptidoglycan chain, which constitutes a building unit of the bacterial cell walls and spores [1]. It was first isolated from the spore peptide of *Bacillus megaterium* [2] and subsequently characterised by synthesis [3,4] as 2-amino-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-D-glucose. Later it was shown that this amino sugar is present in the cell walls of most, if not all, bacteria [5,6]. The discovery in 1974 [7] that the minimal adjuvant active structure of bacterial peptidoglycans is the muramyl dipeptide *N*-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) has generated a great deal of interest in muramic acid. The potential utilisation of a simple, well-defined synthetic adjuvant molecule as MDP, showing maxi-

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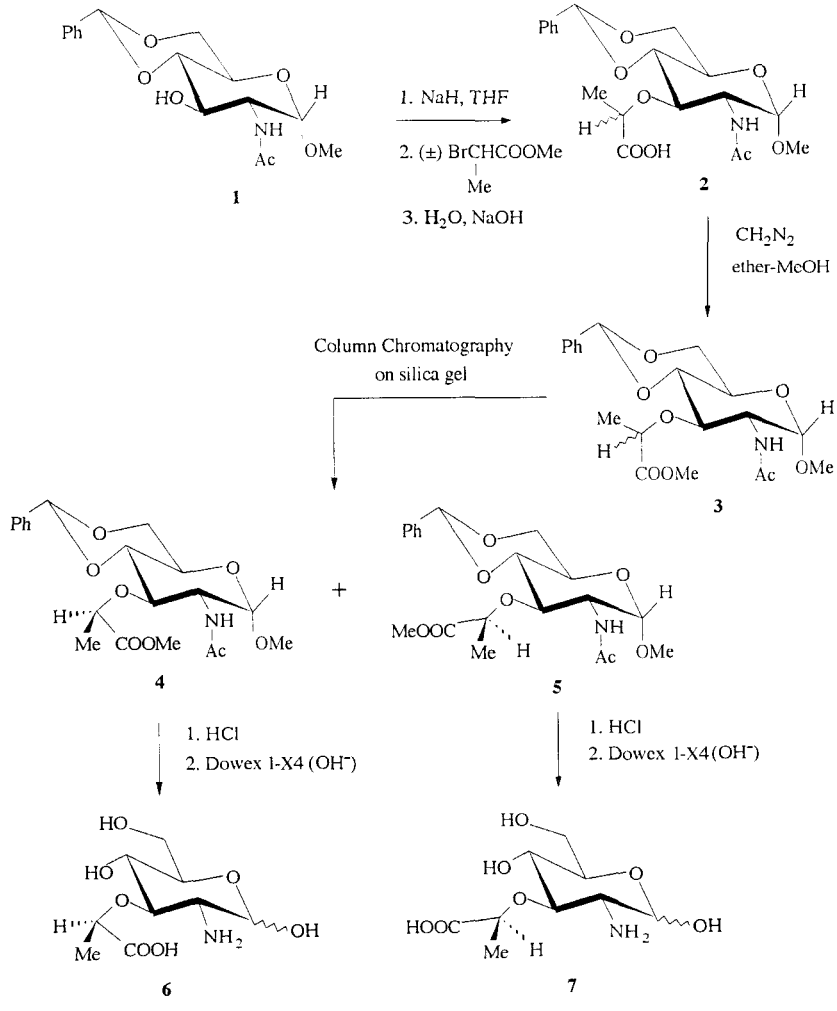
mal stimulation of the immune response but little toxicity and other side effects, characterising, for example, the well-known Freund's complete adjuvant (FCA) [8], has heralded a new era in immunology, especially in the field of vaccination.

Isomuramic acid (**7**) was first reported as a synthetic isomer of muramic acid [9,10] and its synthesis was accomplished in parallel with the latter. Recently *N*-acetylisomuramic acid and *N*-acetylmuramic acid have been identified as constituents of the O-specific polysaccharides of *Proteus penneri* 62 [11] and *Yersinia ruckerii* II [12], respectively.

The syntheses of muramic acid reported in the literature [9,13–16] are based on condensation of D-glucosamine derivatives with a 2-halogeno-propionic acid derivative, forming an ether link with the hydroxyl group at C-3. In most of these syntheses, methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (**1**) has been used as starting material. Alkylation of the unprotected O-3 group of **1** with racemic 2-halogeno-propionic acid derivatives led to the formation of a mixture of epimeric (*R*) and (*S*) ethers (muramic and isomuramic acid derivatives), which, after removal of the protective groups by acid hydrolysis, gave the corresponding acids. All the methods described require the separation of the epimeric mixture in order to obtain the above acids as pure diastereomers. This separation has been carried out either (*a*) at the final stage of the synthesis on the mixture of muramic and isomuramic acids [4,17] which, as amphoteric substances, are separable by the use of suitably buffered cation-exchange resins, or (*b*) after partial hydrolysis of the condensation products, for example, on the diastereomeric mixture of ethyl 2-acetamido-3-*O*-[(*R*,*S*)-1-carboxyethyl]-2-deoxy-β-D-glucopyranoside [13], also using ion-exchange chromatography. Both methods require tedious chromatography and give low yields.

Improvement in the synthesis of muramic acid was achieved by the use of enantiopure (*S*)-2-chloropro-



Scheme 1.

pionic acid as the alkylating agent in the crucial condensation step [9]. It was found that when (*S*)-2-chloropropionic acid was condensed with an alkoxide, it underwent Walden inversion. Therefore, the condensation of **1** with pure (*S*)-2-chloropropionic acid led stereospecifically to muramic acid, with the (*R*) configuration of the lactic acid moiety. This method avoids chromatographic separation, but the preparation of the required reagent in its optically active form is cumbersome [18,19].

An alternative procedure for the synthesis of muramic acid [10,14,20] involved alkylation on O-3 using ethyl (*R*,*S*)-2-bromopropionate as alkylating agent on the appropriate oxazoline derived from 2-benzamido-2-deoxy-5,6-*O*-isopropylidene-α-D-glucofuranose, followed by separation of the (*R*) and (*S*) epimeric acids by fractional crystallisation of their benzylthiouronium salts and subsequent hydrolysis. This is not a method of choice, since many steps are carried out before the purification.

Beginning a new research programme on the immunological properties of immunogens containing derivatives of muramic acid, the availability of this acid appeared of great interest. Furthermore, for future biochemical studies in this area, we shall need multigram quantities of pure synthetic muramic acid. All the previously reported syntheses have at least one associated difficulty, either a tedious and time-consuming chromatographic separation on ion-exchange resins or the use of an optically pure alkylating agent. The latter method is still not appealing, since the preparation of (*S*)-2-halogeno-propionic acid starting from L-alanine [18], or by a resolution method [19], is complicated and expensive. We have therefore undertaken a study of the synthesis of muramic acid and at the same time of its diastereoisomer, isomuramic acid, seeking a more convenient procedure. We focused our effort on investigating an efficient chromatographic separation of the condensation products of **1** with racemic 2-halogeno-propionic acid, before the removal of the protective groups. Indeed, the diastereoisomeric acids **2** and particularly the corresponding esters **3** are molecules of low polarity, soluble in organic solvents (e.g., dichloromethane, ethyl ether), and therefore a chromatographic separation on common chromatographic supports (e.g., silica gel or alumina) was expected to be easy and efficient.

In this paper we report the preparation of pure muramic and isomuramic acids, starting from common raw materials, based on the separation of the epimeric mixture of esters **3** by a simple column chromatography step. The subsequent removal of the protective groups leads to pure muramic acid (**6**) and isomuramic acid (**7**). As very little is known in the literature about the NMR spectra of **6** and **7**, we have also carried out the complete assignment of the signals in the ^{1}H and ^{13}C NMR spectra of **6** and **7** and their precursors by two-dimensional COSY and HETCOR spectroscopy. The reaction sequence is shown in Scheme 1.

The starting compound **1** was prepared from D-glucosamine hydrochloride through methyl 2-acetamido-2-deoxy-α-D-glucopyranoside, which was treated with benzaldehyde according to the Neuberger procedure [21] and the improvement proposed by Kent and Strange [17]. Treatment of **1** with an excess of sodium hydride in refluxing tetrahydrofuran and then condensation with methyl (*R*,*S*)-2-bromopropionate gave, after hydrolysis of the excess of hydride, an epimeric mixture of methyl esters **3** along with a considerable quantity of the corresponding acids **2** produced from the partial saponification of **3**. The crude product was consequently fully saponified with aqueous alkali and the acid **2** was isolated after acidification. Recrystallisation of the crude product from methanol gave pure methyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-[(*R*,*S*)-1-carboxyethyl]-2-deoxy-α-D-glucopyranoside (**2**) with an epimeric configuration of the lactic acid moiety in an (*R*):(*S*) ratio of ca. 2:1, as judged by the ^{1}H NMR spectra. Attempts to separate the above acid epimers by column chromatography proved to be very difficult; they were successful only in microscale quantities, because of the low solubility of this acid.

Esterification with an excess of an ethereal solution of diazomethane in a methanolic solution of **2** led quantitatively to the corresponding methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-[(*R*,*S*)-1-methoxycarbonylethyl]-α-D-glucopyranoside (**3**), which was purified by recrystallisation from dichloromethane–light petroleum. The ^{1}H NMR spectrum of **3** showed that a 2:1 mixture of epimers had been obtained. Chromatographic fractionation was successfully applied to the epimeric esters **3**, on silica gel, using appropriate mixtures of dichloromethane and ether as eluents, and furnished the (*R*) (**4**) and (*S*) (**5**) esters in the ratio 2:1. After recrystallisation pure samples of the epimeric esters were obtained. Comparison of the physical data of **4** with the literature [9,15] confirms the assignment of this compound as the (*R*) isomer and therefore compound **5** corresponds to the (*S*) isomer.

Structure identification of compounds **4** and **5** by

^1H and ^{13}C NMR spectroscopy was accomplished by a combination of 1D spectra and two-dimensional ^1H–^1H COSY and ^{13}C–^1H HETCOR experiments. The ^1H and ^{13}C NMR chemical shifts and the $^3J_{1,2}$ coupling constants are listed in Table 1. The $^3J_{1,2}$ value of 3.2 Hz is indicative of ax–eq coupling and thus confirms the α configuration. The most significant differences between the chemical shifts for the **4** and **5** diastereoisomers were observed for protons H-2 (δ 3.81 and 4.27, respectively), H-1 (δ 5.13 and 4.70), $COOCH_3$ (δ 3.69 and 3.33), NH (δ 7.51 and 5.67), and $CHCH_3$ (δ 4.47 and 4.10). Once the ^1H NMR spectra of compounds **4** and **5** had been fully assigned, the ^{13}C signals could be assigned by 2D ^{13}C–^1H Heteronuclear Shift Correlation (HETCOR) experiments. The most significant chemical shift differences (Table 1) for the (*R*) and (*S*) isomers were observed for carbons C-3 (83.2 and 81.7 ppm, respectively), C-5 (74.6 and 77.8 ppm), C-2 (53.8 and 55.2 ppm), and C-1 (97.5 and 99.0 ppm). The assignment of the acetamido (175.7 and 173.2 ppm) and ester (171.5 and 169.7 ppm) carbonyl signals was based on the known value for the $NHCOCH_3$ group of compound **1**.

Removal of all protective groups from compounds **4** and **5** was effected by means of 6 M HCl and led to the hydrochloride salts of muramic acid and isomuramic acid, respectively. An aqueous solution of muramic acid hydrochloride was treated with Dowex 1-X4(OH^- form) to pH 6, to form the free amino sugar. Freeze-drying of the filtrate gave crude crystalline muramic acid (**6**), which was found to be homogeneous by TLC and to behave identically with an authentic sample. When isomuramic acid hydrochloride was treated in a similar manner, isomuramic acid (**7**) was obtained, shown to be homogeneous by TLC.

The acids **6** and **7** were easily differentiated by TLC on silica gel using 1-BuOH–EtOH–H_2O (4:5:1) as eluent. The corresponding spots were visualised with ninhydrin or methanolic 5% H_2SO_4 at 100 °C. Under these conditions **6** showed a greater mobility (R_f 0.4) than **7** (R_f 0.3).

Because of the very poor crystallisation properties of **6** and **7**, further purification was accomplished efficiently by preparative TLC, in order to obtain analytical samples of **6** and **7**. The optical rotation and melting point of **6** are in accordance with those

Table 1
NMR data (δ in ppm, ^3J in Hz) in $CDCl_3$, 25 °C [a]

Position	250-MHz ^1H NMR		62.9-MHz ^{13}C NMR	
	4	**5**	**4**	**5**
CH-1	5.13, d $J_{1,2}$ 3.2	4.70, d $J_{1,2}$ 3.7	97.5	99.0
CH-2	3.81, m	4.27, m	53.8	55.2
CH-3	3.66, m	3.66, m	83.2	81.7
CH-4	3.62, m	3.63, m	62.6	62.6
CH-5	3.76, m	3.73, m	74.6	77.8
CH-6a	3.76, m	3.69, m	69.03	68.9
CH-6b	4.22, m	4.23, m	–	–
CH–CH_3	4.47, q J_{CH,CH_3} 7	4.10, q J_{CH,CH_3} 7	75.2	76.7
CH–*CH_3*	1.36, d J_{CH,CH_3} 7	1.26, d J_{CH,CH_3} 7	18.7	19.2
*CH_3*CON	2.01, s	1.97, s	22.8	23.3
O*CH_3*	3.31, s	3.20, s	55.3	55.2
COO*CH_3*	3.69, s	3.33, s	52.3	51.4
Ph–*CH*	5.53, s	5.42, s	101.3	101.5
Ph *o,m*	7.33, m	7.32, m	*o* 129.7 *m* 128.3	*o* 129.1 *m* 128.1
Ph *p*	7.39, m	7.39, m	*p* 125.8 C-1 137.3	*p* 126.2 C-1 137.1
NH	7.51, d $J_{NH,H-2}$ 3.5	5.67, d $J_{NH,H-2}$ 9	–	–
*CO*N	–	–	175.7	173.2
COO	–	–	171.5	169.7

[a] s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

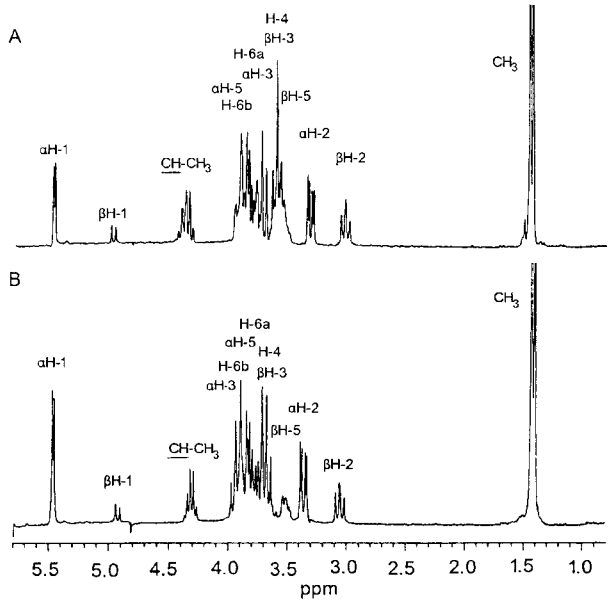


Fig. 1. 250-MHz ^{1}H NMR spectrum of a solution of A, muramic acid (**6**) and B, isomuramic acid (**7**), in D_2O, 25 °C.

already reported in the literature for the corresponding (*R*) isomer [9,20]. The molecular weight of **6** and **7**, derived from mass spectral analysis, is 251, in agreement with the proposed structure. The identification of **6** and **7** by ^{1}H NMR spectroscopy (Fig. 1) was complicated by the overlapping of the signals of the ring protons H-3, H-4, and H-5 with the H-6 protons and also by the presence of the mixture of α and β anomers, resulting after the deblocking of the anomeric methyl glucoside. Using 2D ^{1}H–^{1}H COSY and then ^{13}C NMR and ^{13}C–^{1}H HETCOR spectroscopy, both α and β anomers were fully assigned. The ^{1}H and ^{13}C NMR chemical shifts and the ^3J coupling constants are listed in Table 2. The equatorially oriented H-1 proton (α anomer) appears at higher frequency than the axially oriented H-1 proton (β anomer). The $^3J_{1,2}$ coupling constant (3 or 8 Hz) also indicates the α or β configuration (eq–ax or ax–ax, respectively). Thus α H-1 was assigned to δ 5.41 and 5.48, and β H-1 to δ 4.91 and 4.93, for compounds **6** and **7**, respectively. Using H-1 protons as the starting points in the ^{1}H–^{1}H COSY spectrum, we could identify protons α H-2 and β H-2; consequently, all protons were unequivocally assigned. The values of 9 Hz for $^3J_{2,3}$, $^3J_{3,4}$, and $^3J_{4,5}$ are consistent with the ax–ax configuration of D-glucopyranose. The ^{13}C NMR assignment could be deduced by the ^{13}C–^{1}H HETCOR spectrum by means of the correlation to the corresponding ^{1}H NMR signals. From Table 2, we note that the most significant chemical shift differences for (*R*) and (*S*) isomers were for H-3, C-3, C-4, and *CH*–CH_3.

It should be noted that all analytical and spectroscopic data of synthetic **6** are indistinguishable from those obtained from an authentic sample of muramic acid.

In this work we have been able to achieve the preparation of pure muramic acid and isomuramic acid, starting from common raw materials, by the application of a simple and efficient chromatographic

Table 2
NMR data (δ in ppm, ^3J in Hz) in D_2O, 25 °C [a]

Position	250-MHz ^{1}H NMR				62.9-MHz ^{13}C NMR			
	6		**7**		**6**		**7**	
	α	β	α	β	α	β	α	β
CH-1	5.41, d $J_{1,2}$ 3	4.91, d $J_{1,2}$ 8	5.48, d $J_{1,2}$ 3	4.93, d $J_{1,2}$ 8	91.7	95.7	92.0	95.6
CH-2	3.27, dd $J_{2,3}$ 9	2.97, dd $J_{2,3}$ 9	3.37, dd $J_{2,3}$ 9	3.08, dd $J_{2,3}$ 9	56.2	58.4	55.9	58.3
CH-3	3.68, t $J_{3,4}$ 9	3.52, t $J_{3,4}$ 9	3.95, t $J_{3,4}$ 9	3.72, t $J_{3,4}$ 9	80.9	82.6	79.5	81.5
CH-4	3.56, t $J_{4,5}$ 9	3.56, t $J_{4,5}$ 9	3.67, t $J_{4,5}$ 9	3.67, t $J_{4,5}$ 9	73.0	73.2	71.7	71.7
CH-5	3.85, m	3.45, m	3.88, m	3.49, m	74.1	78.6	74.5	78.8
CH-6a	3.75, m	3.75, m	3.76, m	3.76, m	62.8	62.8	62.9	62.9
CH-6b	3.83, m	3.83, m	3.93, m	3.93, m	–	–	–	–
CH–CH_3	4.28, q J_{CH,CH_3} 7	4.28, q J_{CH,CH_3} 7	4.39, q J_{CH,CH_3} 7	4.39, q J_{CH,CH_3} 7	81.9	81.9	79.9	79.9
CH–*CH*$_3$	1.39, d J_{CH,CH_3} 7	1.39, d J_{CH,CH_3} 7	1.42, d J_{CH,CH_3} 7	1.42, d J_{CH,CH_3} 7	21.6	21.6	21.3	21.3
COO	–	–	–	–	182.3	182.3	181.5	181.5

[a] s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiplet.

process. This approach proved to be far superior to those reported previously and could be performed for the production of pure acids on a multigram scale.

1. Experimental

General methods.—Melting points were measured in capillaries with a Büchi 510 apparatus and are uncorrected. IR spectra were recorded on a Nicolet Magna IR-550 Fourier-tranform spectrophotometer for KBr disks (Sigma). Mass spectra of compounds **4–7** were recorded by direct inlet on a Fisons VG Biotech instrument. Optical rotations were determined at 20–25 °C, on a Perkin–Elmer 141 polarimeter.

^{1}H NMR spectra were recorded on a Bruker AC-250 MHz instrument, at 25 °C, in $CDCl_3$ using the residual $CHCl_3$ peak as reference, or in D_2O using external 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) as reference. 2D ^{1}H–^{1}H COSY spectra were acquired with a 60° excitation pulse, and 32 scans for each of 256 experiments with 2K data points and 1.22 Hz/PT digital resolution in each direction. For each FID, non-shifted sine-bell apodisation was applied before the Fourier transformation and the spectra were finally symmetrised over the diagonal. 2D ^{13}C–^{1}H HETCOR spectra were acquired using standard pulse sequences with 500 scans for each of 64 experiments with 1K data points. Before the Fourier transformation, $\pi/2$-shifted sine-bell apodisation was applied in each direction.

Analytical TLC was carried out on Merck Silica Gel 60-F_{254} precoated aluminum sheets in the following solvent systems: *A*, 4:5:1 1-butanol–EtOH–water; *B*, 7:3 CH_2Cl_2–ethyl ether; *C*, 10:0.5 $CHCl_3$–MeOH; *D*, EtOAc. Detection of the spots was performed with methanolic 5% H_2SO_4 at 100 °C or ninhydrin. Preparative TLC (PLC) was performed using Merck precoated Silica Gel 60-F_{254} plates (2 mm; 20 × 20 cm), using solvent *A*. Column chromatography was carried out on Merck Silica Gel (0.063–0.200 mm), using solvents *B* and *D*. Light petroleum refers to that fraction boiling in the range 40–60 °C. All solvents used were either spectroscopic grade or distilled prior to use. Extracts were dried over Na_2SO_4.

Dowex 1-X4 (Cl^-) resin, purchased from Merck, was treated with aqueous NaOH before use. NaH, fine powder dispersed in mineral oil at 80% concentration, was purchased from Fluka. Methyl (*R,S*)-2-bromopropionate was purchased from Merck, and muramic acid from Sigma.

Methyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-[(R)-1-methoxycarbonylethyl]-α-D-glucopyranoside (**4**) *and methyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-[(S)-1-methoxycarbonylethyl]-α-D-glucopyranoside* (**5**).—To a solution of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (**1**) [17] (3.4 g, 10.5 mmol) in dry THF (250 mL) was added NaH (2.5 g, 84 mmol) under dry N_2 and the mixture was stirred under gentle reflux for 2 h. After the reaction mixture had been cooled to 40 °C, a solution of methyl (*R,S*)-2-bromopropionate (4.5 g, 27 mmol) in dry THF (10 mL) was added dropwise. Stirring was continued for 2 h under gentle reflux and then at room temperature overnight to complete the reaction. Ice–water (20 mL) was then added and the mixture was concentrated in vacuo to a thick orange syrup, which was dissolved in water (100 mL) and washed with light petroleum (3 × 30 mL) to remove the excess of bromoester. The crude solid (5.2 g) contained a mixture of acid **2** and ester **3**, as revealed when chromatographed on TLC (solvent *D*). Full saponification was performed by warming (50 °C) the crude product for 30 min in a mixture of MeOH (25 mL) and aqueous 2 M NaOH (5 mL). The yellowish alkaline solution thus obtained was cooled (0–5 °C) and acidified with 6 M hydrochloric acid, affording a white precipitate which was separated by filtration, washed thoroughly with water, and dried to give the crude acid **2** (3.8 g). TLC (solvent *C*, elution four times) revealed that **2** was a mixture of two major components of R_f 0.5 and 0.7. Recrystallisation from MeOH gave needles (2.75 g, 72%); mp 254–256 °C; $[\alpha]_D^{20}$ +109° (*c* 1, MeOH).

A cold solution of **2** (2.4 g, 60 mmol) in MeOH (15 mL) was esterified by addition of a slight excess of CH_2N_2 in ether. The solvent was evaporated under reduced pressure to afford a white solid which, after crystallisation from 1:2 CH_2Cl_2–light petroleum, gave the crystalline product **3** (2.3 g, 93%); mp 194–195 °C. TLC (solvent *B*, twofold elution or solvent *D*, one elution) revealed that **3** was a mixture of two major components of R_f 0.5 and 0.7. These components were completely separated by column chromatography as described below.

Crude **3** (4 g) was subjected to column chromatography (60 g of silica gel; column, 70 cm × 1.5 cm). Solvent *B* was applied first and fractions with R_f 0.7 were combined and evaporated to give a white solid (2.4 g, 60%), homogeneous on TLC (solvent *B*, twofold elution). This compound was subsequently recrystallised from MeOH to give the ester **4**; mp 208–209 °C (dec), lit. 210–211 °C [15]; $[\alpha]_D^{20}$ +114°

(*c* 1, $CHCl_3$), lit. $[\alpha]_D^{24}$ +106° ($CHCl_3$) [15]; IR: ν_{max} 3293 (NH), 3121 (aromatic), 2932 (methyl), 1734 (C=O), 1652 (Amide I), 1564 (Amide II), 1369, 1129 cm^{-1}; MS (*m/z*): 409 (M^+), 350, 263, 187. Compound **4** was fully characterised from NMR spectral data (Table 1).

Further elution of the column with solvent *D* and evaporation of the fractions with R_f 0.5 afforded a white solid (1.4 g, 35%), homogeneous on TLC (solvent *B*, twofold elution), which on recrystallisation from MeOH gave the ester **5**; mp 261–262 °C (dec); $[\alpha]_D^{20}$ +13° (*c* 0.75, EtOH) and $[\alpha]_D^{20}$ +22.5° (*c* 1, $CHCl_3$); IR: ν_{max} 3293 (NH), 3121 (aromatic), 2932 (methyl), 1734 (C=O), 1652 (Amide I), 1564 (Amide II), 1369, 1129 cm^{-1}; MS (*m/z*): 409 (M^+), 350, 263, 187. NMR spectral data are listed in Table 1.

2-Amino-3-O-[(R)-1-carboxyethyl]-2-deoxy-D-glucose (muramic acid, **6**).—A solution of pure **4** (2 g, 4.9 mmol) in 6 M HCl (40 mL) was heated under reflux for 4 h. The reaction mixture was then extracted with ether (3 × 15 mL) in order to remove benzaldehyde and then decolourised with charcoal. The colourless solution thus obtained was concentrated to dryness in vacuo. The residue (1.8 g) was redissolved in a small amount of distilled water and neutralised by stirring with Dowex 1-X4 (OH^-) resin to pH 6. The resin was then filtered off, and the solution was decolourised again with a small amount of charcoal and lyophilised to yield **6** (1.1 g, 93%) as a pale-yellow solid. This product was homogeneous on TLC (solvent *A*, R_f 0.4) with a mobility identical to that of an authentic sample.

In order to obtain an analytical sample, compound **6** (100 mg) was repurified on PLC (solvent *A*) to give, after extraction with EtOH of the silica zone of R_f 0.35, dilution with distilled water, and lyophilisation, pure muramic acid (70 mg); mp 146–148 °C (dec), lit. 152–154 °C [9], 150 °C [20]; $[\alpha]_D^{25}$ +106° (*c* 1, water), lit. +103° (water) [9], +114° (water) [20]; IR: ν_{max} 3406 (NH and OH), 2932 (methyl) cm^{-1}; MS (*m/z*): 251 (M^+), 234, 184, 144. NMR spectral data of this compound (Table 2) are identical with those obtained for an authentic sample of muramic acid.

2-Amino-3-O-[(S)-1-carboxyethyl]-2-deoxy-D-glucose (isomuramic acid, **7**).—The previous procedure applied to the pure ester **5** (1 g, 2.5 mmol) afforded **7** (0.5 g, 85%). PLC purification of **7** (100 mg, solvent *A*) gave, after extraction with EtOH of the silica zone of R_f 0.25, dilution with distilled water, and lyophilisation, pure isomuramic acid (60 mg); mp 130–131 °C; $[\alpha]_D^{25}$ +40° (*c* 0.8, water); IR: ν_{max} 3406 (NH and OH), 2932 (methyl) cm^{-1}; MS (*m/z*): 251 (M^+), 234, 184, 144; NMR spectral data are presented in Table 2.

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