Liebigs Ann. Chem. **1986**, 37–45

Muramyl Peptides, 1

Stereochemically Pure Derivatives of Muramic and Isomuramic Acids

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Received May 28, 1985

An improved, large-scale preparation is described, leading from 2-acetamido-2-deoxy-D-glucose to benzyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-[(R)-1-carboxyethyl]-2-deoxy-α-D-glucopyranoside (α-Benzyl *N*-acetyl-4,6-*O*-benzylidenemuramic acid, **5r**) and its (*S*)-isomer (α-Benzyl *N*-acetyl-4,6-*O*-benzylideneisomuramic acid, **5s**) *via* chromatographic separation of their methyl esters (**4r** and **4s**). The high yield and complete separation of **4r** and **4s** allow a reliable assessment of the low stereoselectivity of the lactyl ether synthesis step from racemic 2-chloropropionic acid. Similarly, syntheses employing pure enantiomers of 2-chloropropionic acid were disclosed as not completely stereospecific. Coupling products with L- and D-alanine esters were prepared for both **5r** and **5s**.

Muramylpeptide, 1. – Stereochemisch einheitliche Derivate der Muramin- und Isomuraminsäuren

Eine verbesserte Synthese im großen Maßstab führt von 2-Acetamido-2-desoxy-D-glucose zu Benzyl-2-acetamido-4,6-*O*-benzylidene-3-*O*-[(R)-1-carboxyethyl]-2-desoxy-α-D-glucopyranosid (α-Benzyl-*N*-acetyl-4,6-*O*-benzylidenmuraminsäure **5r**) und ihrem (*S*)-Isomeren (α-Benzyl-*N*-acetyl-4,6-*O*-benzylidenisomuraminsäure, **5s**) über eine chromatographische Trennung ihrer Methylester (**4r** und **4s**). Die hohe Ausbeute und die vollständige Trennung von **4r** und **4s** erlaubt eine verläßliche Aussage über die niedrige Stereoselektivität der Lactyletherbildung durch racemische 2-Chlorpropionsäure während der Synthese. Synthesen mit Hilfe von (*R*)- oder (*S*)-Chlorpropionsäure sind ebenfalls nicht stereospezifisch. Von beiden Säuren **5r** und **5s** wurden Kupplungsprodukte mit L- und D-Alaninestern dargestellt.

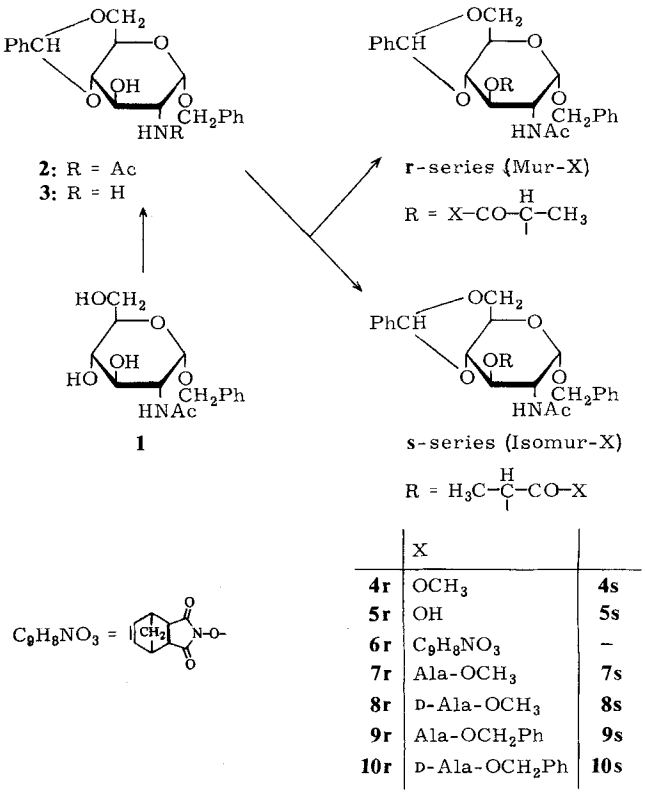
The presence of muramic acid in bacterial cell wall peptidoglycans is well established, and structures have been elucidated for many of them[1]. Several groups[2–7] have synthesized muramyl peptide subunits of these structures and/or artificial modifications of these subunits, which exhibit biological activities, such as immunomodulation or arthritogenesis[8,9]. The first problem in the synthesis of muramyl peptides is the reliable supply of a stereochemically pure muramic acid

0170–2041/86/0101–0037 $ 02.50/0

derivative, suitable for coupling to amino acid or peptide amino ends. As we started to work in this field, it occurred to us that it was rather difficult to unequivocally ascertain the degree or absence of contamination of the most frequently used muramic acid derivative, benzyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-[(R)-1-carboxyethyl]-2-deoxy-α-D-glucopyranoside (**5r**) by its (*S*)-1-carboxyethyl ("*iso*muramic") diastereomer (**5s**).

In the earliest synthesis[10] of this muramic acid derivative, a specific optical rotation of +115 (MeOH) was listed, along with +94 ($CHCl_3$) for its methyl ester **4r**. A later publication from the same laboratory[11] lists the corresponding rotations at +98 and +100, respectively.

Scheme 1



	X	
4r	OCH_3	**4s**
5r	OH	**5s**
6r	$C_9H_8NO_3$	–
7r	Ala-OCH_3	**7s**
8r	D-Ala-OCH_3	**8s**
9r	Ala-OCH_2Ph	**9s**
10r	D-Ala-OCH_2Ph	**10s**

We found, after complete chromatographic separation, rotations of +117 and +126, respectively. Thus, for the later synthesis[11] from the *Jeanloz* laboratory, "stereoselectivity of a degree, which ... has not yet been encountered", apparently was claimed on the basis of 76% yield of a stereochemically impure compound, synthesized from racemic 2-chloropropionic acid. Similarly, tacit or stated assumptions[10,12–14] that stereochemically pure muramic acid derivatives or analoga must result from syntheses employing optically pure either (*R*)- or (*S*)-chloropropionic acids, prepared by one of us[12], appear overly simplistic in view of a well established duality of the nucleophilic substitution mechanism for 2-bromopro-

pionic acid[15–17], which is S_N2 (inversion) competing with a neighboring group reaction *via* an α-lactone (retention). For instance, the earliest synthesis of compound **4r** *via* (*S*)-chloropropionic acid[10], gave a product that according to our optical data was compound **4s** up to 40%. The sterical purity of protected muramic acid derivatives is, however, important for the synthesis of muramyl peptides with potential biological activities, which depend crucially upon the stereochemistry of the substrates. Isomuramic acid initially present in small proportions in a synthetic product may be unintentionally enriched in subsequent purification and fractionation procedures, since isomuramic acid derivatives are generally less soluble and higher melting than their corresponding muramic acid derivatives. On the other hand, deprotected isomuramyl peptides are more easily hydrolized[18] than muramyl peptides. During the final cleavage of certain protective groups, and during the handling of large muramyl peptides in aqueous solutions, preferential hydrolysis of isomuramyl peptides may contaminate an already stereochemically heterogeneous substrate further with a potentially biologically active high molecular mass peptide which may be difficilt to remove.

In view of the noticed and projected difficulties described above, a synthesis *via* racemic 2-chloropropionic acid of a diastereomeric mixture of compounds **5r, s** and the development of a reliable preparative and analytical method for their separation appeared to be the best foundation for work in the area of muramyl peptides.

Discussion

The synthesis of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside[19] (**2a**) was simplified and adapted for large-scale use. In the work-up from the acidic glucosidation, aqueous potassium carbonate was substituted for lead carbonate, and benzyl alcohol was removed subsequently *in vacuo*. This operation made possible easier recovery of benzyl alcohol for reuse, smaller amounts of 2-isopropoxypropane for precipitation of the glycoside, and an increased proportion of α-anomer in the product (**1a, b**). In the subsequent preparation of the 4,6-*O*-benzylidene derivative **2a**, with only four equivalents of benzaldehyde and triethyl orthoformate as the condensing agent, the product crystallized directly from the reaction mixture in high anomeric purity. This new method eliminates the need for the customary fifty-fold excess of benzaldehyde in the zinc chloride method and the attendant large amounts of ether or petroleum ether in its work-up. Whenever the solubilities of the benzylidene derivatives are low, the new method should be applicable to other sugars and more expensive substituted benzaldehydes. The preparation of the mixture of blocked muramic acid methyl esters (**4r, s**) makes use of the low solubility of the sodium salts of the blocked muramic acids in saturated sodium chloride solution; unpolar impurities are at this stage conveniently removed with ether, while polar impurities remain in the aqueous solution. The purified sodium salts are conveniently methylated with methyl iodide, eliminating preparation of a mixture of acids and a costly methylation with diazomethane at this stage. The methyl esters were obtained in high yield and purity as shown by thin-layer chromatography on silica gel with dioxane/CCl_4 (1:2). This analytical system was also adapted for column chromatography separation and gave complete separation with empty eluant fractions between the two diastereomers **4r** and **4s** with a high compound/silica gel ratio (1:40). The

isomuramic acid derivative easily crystallized in the column. This made necessary a particular mode of loading the column with prefractionated adsorbates (see Experimental) in order to avoid clogging of the column. Chromatographic materials were easily recovered for reuse. It was also possible to base a separation by fractional crystallization on the dioxane/CCl_4 system, but we found the chromatographic separation more reproducible. Another solvent system, dichloromethane/diethyl ether[20] did not give clean separations of isomers (**4r, s**). The pure, protected methyl esters of muramic (**4r**) and isomuramic (**4s**) acids were easily hydrolized by aqueous sodium hydroxide in dioxane. Whenever the resulting sodium salts were desired for a particular experiment, they were easily salted out by NaCl from their aqueous solutions, as described for their mixture. The preparation of the free acids proceeded especially well in the described manner in a sonic bath with rapid stirring. The fine dispersion of the precipitate during acidification makes an excess of acid unnecessary, so that the benzylidene group is not endangered. Also, inclusions of salts or other impurities in the emerging precipitate were reliably prevented. The coupling of the protected muramic acids **5r** and **5s** with D- or L-alanine esters followed established methods for peptide synthesis. Scheme 1 shows the chemical conversions and Table 1 presents the physical constants of the various α-benzyl *N*-acetyl-4,6-*O*-benzylidenemuramic and -isomuramic acid derivatives which are, if possible, compared to previously published values.

In several cases (**5s, 7r, 8r**) we found melting points that were substantially higher than published values. For two derivatives of importance to the synthesis of naturally occurring muramyl peptides (**4r** and **7r**) we found substantially higher optical rotations, indicating contamination by isomuramic acid derivatives in prior preparations. In two cases (**5s** and **9r**) we were unable to prepare solutions of the concentrations indicated in the literature, since our products proved to be much less soluble. The R_F values quoted in Table 1 show that the muramyl-L-alanine derivatives **7r** and **9r** can easily be distinguished from their likely contaminates (**7s, 8r** resp. **9s, 10r**) by TLC with CCl_4/dioxane (2:1). The isomuramic acid derivatives (**7s, 8s** resp. **9s, 10s**), however, show very similar mobilities.

P. G. thanks the *University of the Pacific* for a sabbatical year grant for research at Medizinische Hochschule, Hannover, where this work was carried out. The authors thank Mr. *G. Jonissek* and Mr. *B. Weitzel* of the *Medizinische Hochschule* for excellent experimental support. The authors are grateful for support by the *Deutsche Forschungsgemeinschaft* and the *Fonds der Chemischen Industrie.*

Experimental

Thin-layer chromatography (TLC) was carried out on silica gel GF, "Uniplates" by Analtech, New Jersey, USA, on layers of 250 μ with CCl_4/dioxane (2:1) as the developing solvent. Compounds were applied to the plates as solutions in anhydrous, purified dioxane. Spots were visualized under UV light, and subsequently also by spraying of the plates with 10% H_2SO_4 and charring at 120 °C. Preparative chromatography was done on columns (diameter/length 1:20) with "Kieselgel 60", 0.063–0.200 mm particle size, from E. Merck, Darmstadt, W. Germany. Elemantary analysis were determined by *B. Diercks* of Medizinische Hoch-

schule, Hannover, with a Mod. 1106 Carlo Erba Elemental Analyzer. Optical rotations were measured in concentrations of 0.5–1.0 at 20–25°C in 1-dm cuvettes with a Perkin-Elmer 141 polarimeter. All optical rotations were positive. Melting points were obtained with a Büchi melting-point apparatus (model "Dr. Tottoli") and are uncorrected.

Benzyl 2-acetamido-2-deoxy-α,β-D-glucopyranosides (**1a, b**): Into a suspension of finely powdered 2-acetamido-2-deoxy-D-glucose (72 g, 0.325 mol) in benzyl alcohol (1.6 l) was rapidly introduced gaseous HCl until the temperature had risen by 13°C. This corresponds to an uptake of about 30 g of HCl. The mixture was heated and was stirred for 2 h at 85°C. An aspirator vacuum was applied, with continued stirring, to remove most of the water that had formed. After the solution had cooled by this evaporation, it was stirred at 70°C for another hour. The solution was then cooled to 0°C and was treated for about 12 h with a solution of K_2CO_3 (200 g) in water (300 ml) with gentle stirring. The upper organic phase was separated, was dried with Na_2SO_4, and was concentrated in a rotary evaporator at 0.3 Torr until a thick oil remained. Benzyl alcohol was reclaimed by distillation of the condensate. The residual oil was stirred with a mixture of 2-isopropoxypropane and pentane (1:1, 2 l). The resulting precipitate was filtered off, was air-dried, was dissolved in hot ethanol, and was crystallized at −10°C. Crystals were filtered off and were washed with diethyl ether to give crude **1a, b** (74 g). The ethanolic mother liquors were evaporated, and the residue was treated with the ether washings from the first fraction. At −10°C a second fraction (3 g) was obtained. The total yield (76%; $[\alpha]^{20} = 159$; $c = 0.8$ in H_2O) obtained by this procedure contains only about 5% of β-glycoside, while about 13% is normal[21]. Apparently much β-glycoside remains in the aqueous K_2CO_3 solution.

Benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (**2a**). – A) Compound **1a, b** (12.4 g, 0.04 mol) was dried by suspension in anhydrous ethanol (20 ml) and toluene (80 ml) followed by rotary evaporation *in vacuo*. To the dry residue were added anhydrous dimethylformamide (40 ml), anhydrous dioxane (40 ml), triethyl orthoformate (20 ml), benzaldehyde (16 ml), and toluenesulfonic acid (1 g). The mixture was mechanically stirred at room temperature and became clear after about 10 min. Soon a new precipitation became visible, which was completed by stirring the suspension at room temperature for 20 h. Absolute diethyl ether (100 ml) was added. The mixture was stirred for 1 h at 0°C and was suction filtered. After being washed with diethyl ether, the filter cake was dried *in vacuo* to give **2a** (12.1 g, 76%; $R_F = 0.38$; $[\alpha]^{20} = 108$; $c = 1$ in pyridine) with only traces of the β-anomer **2b** ($R_F = 0.23$) by TLC ($CHCl_3$/EtOH; 20:1). The filtrate was concentrated *in vacuo* and treated with diethyl ether to give additional α,β-mixture (2.4 g, 15%) which may be separated *via* the 3-*O*-acetyl derivative[19].

B) To a cold solution of compound **3a** (26 g, 73 mmol) in a mixture of $CHCl_3$ (200 ml), methanol (400 ml), and pyridine (16 ml) was added dropwise with stirring acetic anhydride (20 ml). After being stirred for about 12 h at 0°C, the mixture was suction filtered. The filter cake was washed with methanol and diethyl ether (25.4 g). The concentrated mother liquors gave another fraction (2.2 g). Both fractions were recrystallized from dioxane/2-propanol (1:1) to give pure **2a** (25.3 g, 87%).

Benzyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (**3a**): The compound was prepared as described[19]. A trace amount of the β-anomer was removed at this stage because it is more soluble and lower melting. This is in contrast to all other known related[19] anomeric pairs.

Benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-[(R,S)-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside (**4r, s**): Compound **2a** (20 g, 0.05 mol), in a three-necked round-bottom

2-l flask, was suspended in benzene (150 ml), which was distilled off. The dry residue was dissolved in anhydrous dioxane. The solution was treated with NaH (16.8 g of a 50% suspension in mineral oil, which was removed and replaced by anhydrous toluene) in small portions. The mixture was stirred for 1 h at 80°C. At 65°C, a solution of racemic 2-chloropropionic acid (16 g) in anhydrous dioxane (60 ml) was slowly added with stirring. After being heated and stirred for 2 h at 60°C, the mixture was treated by dropwise addition of water (160 ml) over 30 min at 80°C. The two-phase mixture was evaporated *in vacuo* to dryness. To the residue was added ethanol (100 ml), which was distilled off. The residue was extracted with ether and was vigorously stirred with a saturated, aqueous solution of NaCl (200 ml), and was kept for about 12 h at 4°C. The precipitate was suction filtered, was washed with a little saturated NaCl solution, and was dissolved in warm water (600 ml). The murky solution was extracted with diethyl ether (3 × 100 ml), was treated at 80°C with activated charcoal, was filtered, was concentrated to about 100 ml, was saturated with solid NaCl (35 g), and was kept for about 12 h at 4°C. The purified sodium salts of compounds **5r, s** were filtered off, were dried, and were dissolved in anhydrous dimethylformamide (200 ml), which was evaporated *in vacuo*. The solution of the residue in anhydrous dimethylformamide (200 ml) was methylated by being stirred with methyl iodide (14 g) at room temperature for 3 d. Dimethylformamide was evaporated *in vacuo*. the residue was shaken with distilled water, was filtered off, and was dried to give **4r, s** (22.6 g, 93%).

Column chromatographic separation of compounds **4r, s**: At 60°C, a solution of compound **4r, s** (17.0 g, 0.035 mol) in dioxane (100 ml) was added to a suspension of silica gel (100 g) in CCl_4 (200 ml) with stirring. The mixture was suction filtered (residue 1) into a suspension of silica gel (100 g) in CCl_4 (200 ml). The suspension in the filter flask was filtered again (residue II). The filtrate was evaporated *in vacuo*. the residue was dissolved in CH_2Cl_2. The solution was added to a suspension of silica gel (100 g) in CCl_4 (200 ml). This mixture was freed in a rotary evaporator of CH_2Cl_2 (suspension III). A column was prepared from an *in vacuo* deaerated suspension of silica gel (400 g) in CCl_4. Onto the top of this column was given suspension III, followed by residues II, and I in CCl_4 suspensions. The column was eluted with CCl_4, containing dioxane (%): 1 l (5%), 2 l (10%), 2 l (20%), 1 l (25%), 2 l (40%), 2 l (60%), followed by pure dioxane, 1 l, and dioxane with 10% methanol, 1 l. Fractions 1–53 (130 ml each) were collected, followed by fractions of 340 ml. Fractions 28–43, containing **4r** (muramic acid derivative), were pooled and evaporated. The residue was recrystallized from methanol to give **4r** (9.5 g, 56% recovery; m.p. 210°; $[\alpha]^{20} = 126°$; $c = 1$ in $CHCl_3$). Fractions 44–47 were empty. Fractions 48–58, containing **4s** (isomuramic acid derivative), on evaporation gave a residue which was recrystallized from dioxane/CCl_4 to give **4s** (6.3 g, 37% recovery; m.p. 250°; $[\alpha]^{20} = 52$; $c = 0.5$ in $CHCl_3$). – When the purified sodium salt mixture of **5r, s** was treated with HCl in the manner described below, and the resulting mixture of free acids (**5r, s**) was esterified with diazomethane, as described[13], methyl esters (**4r** and **4s**) with identical physical constants were obtained after chromatographic separation.

Recovery of chromatographic materials: Silica gel from the column was reclaimed for future use by heating it (100°C) in 3 N HCl, with stirring, for 5 h followed by suction filtration, washing with distilled water and drying at 140°C. Dioxane/CCl_4 mixtures from the rotary evaporation of the fractions were suitably pooled, were dried with $MgSO_4$, and finally were purified and were made anhydrous by being given over a column of molecular sieve (3 Å). The composition of the mixtures can be reliably estimated with the assumption that the refractive index of the mixtures is a linear function of the percentage composition, including the refractive indices of pure CCl_4 and pure dioxane. Addition of dioxane or CCl_4 produced

mixtures suitable for future column separations. Attempted fractional distillation of dioxane/CCl_4 mixtures at normal pressure produced considerable decomposition with evolution of HCl.

Benzyl 2-acetamido-4,6-O-benzylidene-3-O-[(R)-1-carboxyethyl]-2-deoxy-α-D-glucopyranoside (**5r**): Compound **4r** (9.7 g, 0.02 mol) was dissolved in dioxane (200 ml) at 40°C. Aqueous 0.5 N NaOH (100 ml) was added dropwise with stirring. After 1 h, the mixture was evaporated to dryness *in vacuo*. the residue was dissolved in water (400 ml). The reaction vessel was placed in an ice-cooled ultrasonic bath and the solution was stirred with ice (200 g). Hydrochloric acid (1 N) was added slowly with rapid stirring and sonication until pH = 3 was reached and maintained for 10 min. The precipitate was suction filtered, was washed free of acid with cold distilled water, was washed with a small amount of cold (−10°C) ethanol, was dried over $CaCl_2$ and soda lime *in vacuo*, and was recrystallized from ethanol to give **5r** [9.2 g, 98%, m.p. 229−230°C, $[\alpha]^{20}$ = 117 (*c* = 0.5 in MeOH), $[\alpha]^{20}$ = 120 (*c* = 1 in pyridine)]. − Na salt: m.p. 260°C, $[\alpha]^{20}$ = 103 (*c* = 0.5 in DMF).

Benzyl 2-acetamido-4,6-O-benzylidene-3-O-[(S)-1-carboxyethyl]-2-deoxy-α-D-glucopyranoside (**5s**): Compound **4s** was subjected to the preceding preparative procedure to give **5s** [98%, m.p. 292°C, $[\alpha]^{20}$ = 62 (*c* = 1 in pyridine)], insoluble in MeOH. − Na salt: m.p. 340°C, $[\alpha]^{20}$ = 53 (*c* = 0.5 in DMF).

Benzyl 2-acetamido-4,6-O-benzylidene-3-O-[(R)-1-carboxyethyl)]-2-deoxy-α-D-glucopyranoside (HONB ester, **6r**): Dicyclohexylcarbodiimide (4 g, 20 mmol) in anhydrous tetrahydrofuran (50 ml) was slowly added to a solution of compound **5r** (7.45 g, 15.8 mmol) and *N*-hydroxy-5-norbornene-2,3-dicarboximide (HONB, 3.7 g, 18 mmol) in anhydrous tetrahydrofuran (100 ml) with stirring at 0°C. After being stirred at room temperature for about 12 h the suspension was evaporated *in vacuo*. The residue was stirred in ethyl acetate (400 ml) and the mixture was filtered (3.9 g dicyclohexylurea). The filtrate was stirred with saturated $NaHCO_3$ for 1 h. The organic phase was separated, was dried with $MgSO_4$, and was evaporated to dryness. The residue was stirred with diethyl ether and was filtered off to give compound **6r** (9.5 g, 95%), which was dissolved in CH_2Cl_2 (40 ml) and chromatographed on a column of silica gel (500 g) with CCl_4/dioxane (2:1) as the eluant to give purified **6r** [9 g, 90%, m.p. 125°C, $[\alpha]^{20}$ = 87 (*c* = 0.5 in DMF)]. Elemental analysis (Table 1) indicated a composition with one additional mole of water.

Coupling of α-benzyl N-acetyl-4,6-O-benzylidenemuramic (or isomuramic) acid with L- (or D-) alanine methyl ester hydrochloride (or benzyl ester toluenesulfonate). − General procedures: All eight compounds (Table 1) were synthesized by both methods, A and B, in yields of 80−95%.

A) Compound **5r** (or **5s**; 0.472 g, 1 mmol) was dissolved in the minimal amount of warm CH_2Cl_2, was cooled to 0°C, and was treated with anhydrous 1-hydroxybenzotriazole (0.142 g, 1.05 mmol) and dicyclohexylcarbodiimide (DCCl; 0.217 g, 1.05 mmol). After 1 h at 0°C and 1 h at 20°C, dicyclohexylurea (DCU) was filtered off. the filtrate was added at 0°C slowly to a stirred cold solution of the alanine ester salt (1 mmol) and *N*-ethylmorpholine (0.13 ml, 1 mmol) in CH_2Cl_2. After 3 d at 20°C, additional DCU was filtered off, and the filtrate was washed successively with 10% aqueous potassium hydrogen carbonate (3 × 20 ml), 10% aqueous citric acid (3 × 20 ml), and H_2O (2 × 20 ml), was dried with Na_2SO_4, and was evaporated *in vacuo*. The residue was recrystallized from ethanol or dimethylformamide/water.

B) A solution of the alanine ester salt (1.05 mmol) in CH_2Cl_2 was treated successively with *N*-ethyldiisopropylamine (0.18 ml, 1.05 mmol), with *N*-hydroxysuccinimide (121 mg, 1.05

Table 1. Derivatives of α-benzyl *N*-acetyl-4,6-*O*-benzylidenemuramic acid (**r**-series, Mur-X) and -isomuramic acid (**s**-series, Isomur-X)

	X[a] (R_F)[b]	M.p. [°C] (Lit. value)	$[\alpha]_D$, solvent (Lit. value)	Mol. formula (Mol. mass)		Analyses C	H	N
4r	-OMe (0.70)	210 (212)[10]	126, $CHCl_3$ (94, $CHCl_3$)[10]	$C_{26}H_{31}NO_8$ (485.5)	Calc.	64.31	6.44	2.89
					Found	64.26	6.53	2.94
4s	-OMe (0.40)	254 (253)[11]	53, $CHCl_3$ (62, $CHCl_3$)[13] (54, $CHCl_3$)[11]	$C_{26}H_{31}NO_8$ (485.5)	Calc.	64.31	6.44	2.89
					Found	64.15	6.48	2.85
5r	-OH	229 (237)[10]	117, MeOH; 120, pyridine (115, MeOH)[10] (98, MeOH)[11]	$C_{25}H_{29}NO_8$ (471.5)	Calc.	63.68	6.20	2.97
					Found	63.52	6.20	3.10
5s	-OH	292 (275)[13]	insol. MeOH; 62, pyridine (79, MeOH)[13] (64, pyridine)[13]	$C_{25}H_{29}NO_8$ (471.5)	Calc.	63.68	6.20	2.97
					Found	63.85	6.05	2.98
6r	$-C_9H_8NO_3$	125 (123)[18]	86, DMF (86, DMF)[18]	$C_{34}H_{36}N_2O_{10} \cdot H_2O$ (650.7)	Calc.	62.75	5.88	4.31
					Found	62.68	5.74	4.20
7r	-Ala-OMe (0.39)	234 (226)[21]	81, CH_2Cl_2; 110, DMF (83, DMF)[21]	$C_{29}H_{36}H_2O_9$ (556.6)	Calc.	62.58	6.52	5.03
					Found	62.20	6.55	5.00
7s	-Ala-OMe (0.28)	270	35, CH_2Cl_2; 50, DMF	$C_{29}H_{36}N_2O_9$ (556.6)	Calc.	62.58	6.52	5.03
					Found	62.40	6.08	5.01
8r	-D-Ala-OMe (0.76)	209 (194)[21]	98, CH_2Cl_2; 114, DMF (112, DMF)[21]	$C_{29}H_{36}N_2O_9$ (556.6)	Calc.	62.58	6.52	5.03
					Found	62.09	6.60	4.91
8s	-D-Ala-OMe (0.24)	236	59, CH_2Cl_2; 67, DMF	$C_{29}H_{36}N_2O_9$ (556.6)	Calc.	62.58	6.52	5.03
					Found	62.40	6.10	5.01
9r	-Ala-PCH_2Ph (0.52)	220 (216)[22]	70, CH_2Cl_2; 80, DMF (86, DMF)[22]	$C_{35}H_{40}N_2O_9$ (632.7)	Calc.	66.44	6.37	4.43
					Found	66.20	6.50	4.37
9s	-Ala-OCH_2Ph (0.36)	233	40, CH_2Cl_2; 49, DMF	$C_{35}H_{40}N_2O_9$ (632.7)	Calc.	66.44	6.37	4.43
					Found	66.02	6.30	4.41
10r	-D-Ala-OCH_2Ph (0.68)	216	87, CH_2Cl_2; 143, DMF	$C_{35}H_{40}N_2O_9$ (632.7)	Calc.	66.44	6.37	4.43
					Found	66.66	6.24	4.12
10s	-D-Ala-OCH_2Ph (0.34)	200	56, CH_2Cl_2; 66; DMF	$C_{35}H_{40}N_2O_9$ (632.7)	Calc.	66.44	6.37	4.43
					Found	66.37	6.68	4.29

[a] See Scheme 1. — [b] In CCl_4/dioxane (2:1).

mmol), with a saturated solution of compound **5r** (or **5s**; 0.472 g, 1 mmol) in CH_2Cl_2, and with 2-chloropyridine methoiodide (0.3 g, 1.18 mmol). After being stirred for about 12 h at 20°C, the resulting clear, yellow solution was extracted and worked-up as described under A).

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