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METHYL β-GLYCOSIDES OF *N*-ACETYL-6-*O*-(ω-AMINOACYL)MURAMYL-L-ALANYL-D-ISOGLUTAMINES, AND THEIR CONJUGATES WITH MENINGOCOCCAL GROUP C POLYSACCHARIDE

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ABSTRACT

Spacer arms 2.1–3.7 nm (21–37 Å) long were prepared, and coupled with the methyl β-glycoside of *N*-acetylmuramyl-L-alanyl-D-isoglutamine benzyl ester, to give blocked 6-acylates. Deprotection was effected with palladium chloride and triethylsilane. Chemical conjugates of MDP-meningococcal group C polysaccharide were then synthesized, in attempts to enhance the immunogenicity of the polysaccharide antigen.

INTRODUCTION

N-[2-*O*-(2-Acetamido-2,3-dideoxy-D-glucopyranos-3-yl)-D-lactoyl]-L-alanine-D-isoglutamine ["*N*-acetylmuramyl-L-alanyl-D-isoglutamine", MDP) is the minimal, adjuvant-active structure capable of replacing whole mycobacterial cells in complete Freund's adjuvant for increasing levels of humoral antibodies against a given antigen and for inducing delayed hypersensitivity[1]. In contrast to other mycobacterial fractions, MDP is also adjuvant-active in aqueous medium by the parenteral or the oral route[2]. MDP has been rendered immunogenic when coupled to protein carriers *via* carbodiimide or phenyl isothiocyanate intermediates[3]. When rabbits were immunized with the conjugates, they gave high anti-MDP titers, and the antibodies produced could recognize free, synthetic MDP. In addition to high anti-MDP titers, very high anti-carrier titers were also obtained, as compared to controls immunized with carrier alone, showing that chemical conjugation had not abolished the adjuvant potential of MDP.

Sela and co-workers[4] studied the combination of synthetic antigens with adjuvants, either by simple mixing or by covalent attachment, *via* synthetic carriers. From their model study, they found that the immunogenicity of a synthetic MS-2 coliphage-coat protein fragment linked to multi[poly(DL-alanyl)-poly(L-lysine)] was greatly increased by its chemical attachment to a disaccharide tetrapeptide isolated from the peptidoglycan of *Bacillus megaterium*. In another study[5], the immunogenicity of the synthetic polypeptide poly(L-Tyr, L-Glu)-poly(DL-Ala)–poly(L-Lys) chemically linked to MDP was also greatly enhanced when injected in aqueous solution into

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mice. The antigen alone, administered under the same conditions, did not lead to antibody production. A mixture of the antigen with MDP was much less effective in eliciting the immune responses. It is thus possible, by covalent combination with MDP, to convert poor immunogens, including desired vaccines, into more-efficient ones.

We now report syntheses of the methyl β-glycosides of *N*-acetyl-6-*O*-(ω-aminoacyl)muramyl-L-alanyl-D-isoglutamines having spacer-arm lengths of 2.1–3.7 nm (21–37Å), and their chemical coupling to meningococcal group C polysaccharide[6] in attempts to enhance the immunogenicity of the polysaccharide antigen.

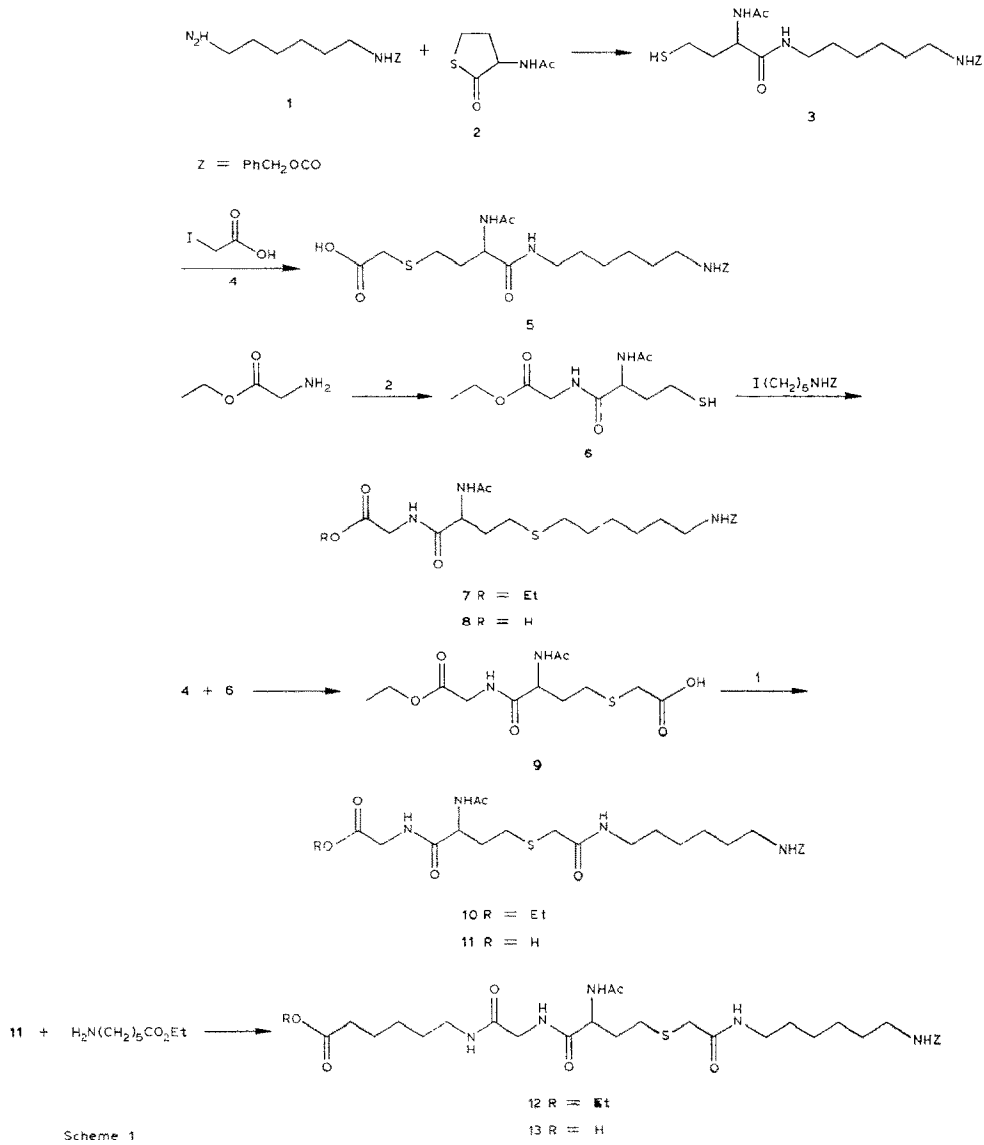
RESULTS AND DISCUSSION

MDP can be chemically attached to bacterial polysaccharides, *via* spacer arms, by three different routes. First, the glutamyl 4-carboxyl group can be coupled to the carboxyl group of meningococcal polysaccharides *via* α,ω-diaminoalkanes; this approach has already been described[7]. The second route is by the use of ω-aminoalkyl β-glycosides, described separately[8]. The third route is *via* the 6-hydroxyl group of MDP; attachment here by an ester linkage is particularly attractive, in view of the known, potent adjuvanticity of *N*-acetyl-6-*O*-acylmuramyl-L-alanyl-D-isoglutamines[9]. Moreover, it has also been reported that owl monkeys were effectively protected against a human malaria parasite after immunization with an appropriate antigen in liposomes containing the 6-*O*-stearoyl derivative[10].

In this study, the weak immunogen, meningococcal group C polysaccharide was chosen as a model for condensations with such 6-*O*-(ω-aminoacyl) derivatives as **21**, **23**, **25**, and **27**. A number of other *N*-acetyl-6-*O*-(ω-aminoacyl)muramyl-L-alanyl-D-isoglutamines have already been reported as potential immunoadjuvants[11]. In view of the importance of spacer arms in affinity chromatography[12], and in biological systems[13,14], we set out to prepare the appropriately protected spacer arms **5** (2.2), **8** (2.1), **11** (2.6), and **13** (3.7 nm) for chemical couplings with the glycoside of benzyl α-*N*-acetylmuramyl-L-alanyl-D-isoglutamine benzyl ester[9] or the methyl β-glycoside **14**. The synthesis of these spacer arms is outlined in Scheme 1.

These spacer arms are considered to be superior to the more readily accessible poly(methylene) types, which have a strong tendency to coil in aqueous milieu, thus lessening their effective lengths. Moreover, other, similarly prepared, spacer arms were reported to be useful in another study[13].

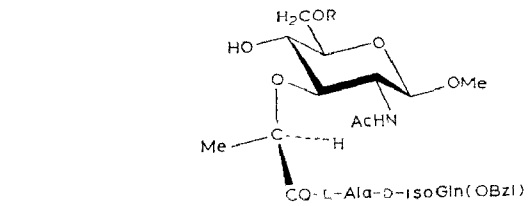
Initially, the spacer **5** was coupled with the benzyl α-glycoside of *N*-acetylmuramyl-L-alanyl-D-isoglutamine benzyl ester[9] in the presence of DCC and 4-(dimethylamino)pyridine[15], to give **20**. Considerable difficulty was encountered in the deprotection of **20**. For example, hydrogenolysis of this blocked compound in the presence of palladium oxide or palladium hydroxide, or both, in glacial acetic acid was found to be very slow, and efforts to force the reaction to completion, *e.g.*, by multiple catalyst changes, and variation of the pressure and the temperature, resulted in loss of the 6-*O*-acyl spacer arm. In one instance, 6-*O*-acetyl-MDP, formed by



Scheme 1

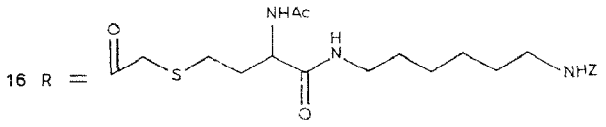
trans-esterification, was isolated as a major product by column chromatography. In order to circumvent the difficulty in removing the benzyl glycoside group, the methyl β-glycoside **14** was used as the starting material. This was reasonable, as the methyl β-glycoside of *N*-acetylmuramyl-L-alanyl-D-isoglutamine (**14**) has been shown[16] to have adjuvant activity comparable to that of MDP. Thus, condensations of **14** with the spacers **5**, **8**, **11**, and **13** gave **16**–**19**, respectively, in 60–70% yield.

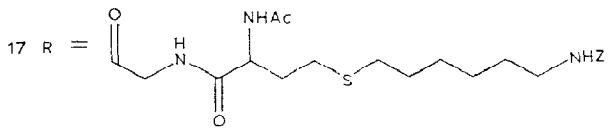
To establish whether the isolated products **16**–**19** were 6-acylates, they were treated with acetic anhydride in pyridine, to yield the respective 4-acetates, as indicated by their 300-MHz, n.m.r. spectra; *e.g.*, the signal for H-4 of the 4-*O*-acetyl

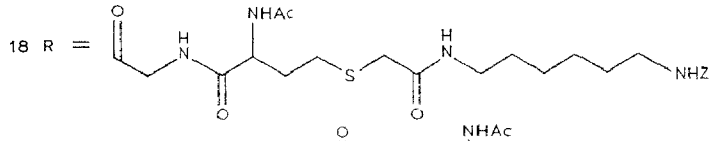


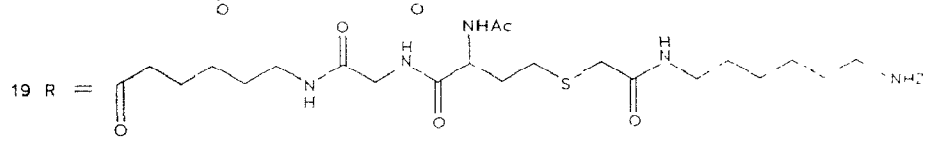
14 R = H

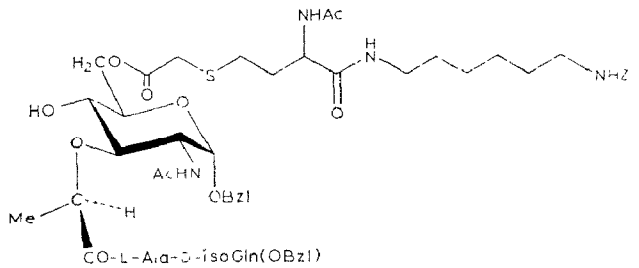
15 R = Ac





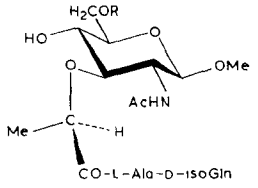




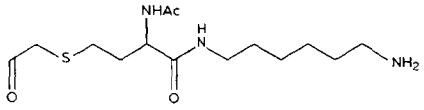


20

derivative of **17** appeared at δ 4.96 as a triplet (J 9.0 Hz). Compounds **16–19** were successfully deprotected with triethylsilane and palladium chloride[17], albeit in low yields, to give **21**, **23**, **25**, and **27**, respectively. The amino-containing ligands (**21**, **23**, **25**, and **27**) were then coupled with meningococcal group C polysaccharide *via* the *N*-hydroxysuccinimide active ester, to give the conjugates **22**, **24**, **26**, and **28**, respectively, as fluffy solids.



21 R =



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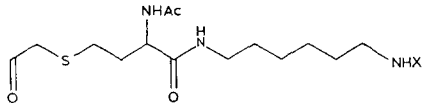

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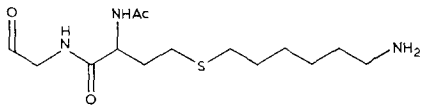
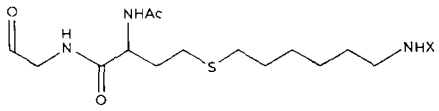
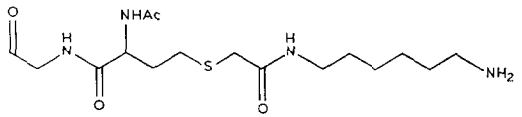


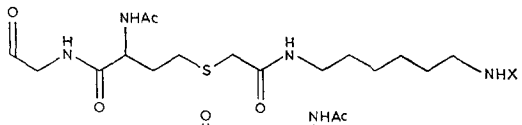
24 R =



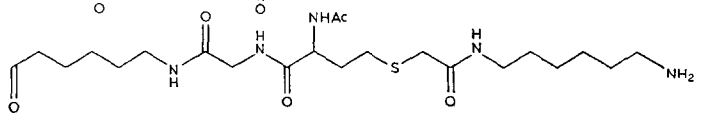
25 R =



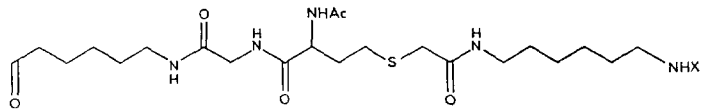
26 R =



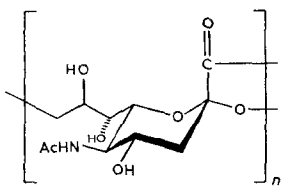
27 R =



28 R =



X =



Spinco analysis of the conjugates indicated the presence of 9–11 % of MDP. The molecular sizes of the MDP–polysaccharide conjugates were found to decrease somewhat (K_d 0.3–0.35) compared to meningococcal group C polysaccharide (K_d 0.24), but these values are still well below K_d 0.5, where the antigen may begin to lose its immunogenicity. In the antigen–antibody, quantitative precipitin assay, these conjugates were all recognized by anti-meningococcal group C polysaccharide antibody. The conjugates **22** (spacer-arm length, 2.2 nm) and **24** (2.1 nm) were as antigenic as the native polysaccharide whereas the conjugates **26** (2.6 nm) and **28** (3.7 nm) were definitely more antigenic than the others. These conjugates were tested *in vivo* for their ability to enhance the serum bactericidal antibody response of mice, but no enhancement of immunogenicity was observed, despite the positive, *in vitro* results.

EXPERIMENTAL

General methods. — Melting points were determined with a Thomas Hoover Unimelt apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer Model 241 polarimeter. Thin-layer chromatography (t.l.c.) was performed on silica gel GF_{254} (Analtech) plates, and the spots were detected with a ceric sulfate (1 %)–sulfuric acid (10 %) spray. Column chromatography was conducted on silica gel 60 (70–230 mesh, ASTM). N.m.r. spectra were recorded for solutions in chloroform-*d* (unless stated otherwise) at 300 MHz, with tetramethylsilane as the internal standard. Conventional processing consisted of drying organic solutions with anhydrous sodium sulfate, filtration, and evaporation of the filtrate under diminished pressure. Antigen–antibody quantitative precipitin assay was performed on immunodiffusion plates. Solvent *A* was 4:4:1 (v/v) $CHCl_3$–CH_3OH–H_2O.

1-Amino-6-(benzyloxycarbonylamino)hexane (**1**) *hydrochloride*. — Benzyl chloroformate (12.0 g, 0.07 mol) was added dropwise to a stirred solution of 1,6-diaminohexane (15.0 g, 0.13 mol) in methanol (50 mL) kept at pH 3 with 12M HCl. During the addition, the pH was maintained at 3–4 by adding 2.5M NaOH. After being stirred for 18 h, the mixture was filtered, and the filtrate was evaporated *in vacuo* to a residue that was suspended in water, and the suspension basified to pH 13 with 2.5M NaOH. Chloroform was added to the solution, and the organic layer was separated, dried, and evaporated to an oil that was dissolved in dichloromethane. Dry hydrogen chloride was bubbled into the solution at 0 ° to pH 1.0. The product was allowed to crystallize, and the suspension filtered, to give **1** · HCl as needles (7.0 g, 35 %), m.p. 220–221 °. An analytical sample was obtained by recrystallization from ethyl acetate–methanol; m.p. 223–224 °.

Anal. Calc. for $C_{14}H_{23}ClN_2O_2$: C, 58.68; H, 8.08; Cl, 12.63; N, 9.77. Found: C, 58.46; H, 8.17; Cl, 12.23; N, 9.61.

1-(N-Acetyl-DL-homocysteinylamino)-6-(benzyloxycarbonylamino)hexane (**3**). — *N*-Acetyl-DL-homocysteine thiolactone (**2**; 1.53 g, 9.6 mmol) was added to a solution of **1** hydrochloride (2.75 g, 9.6 mmol) and triethylamine (1.35 mL) in ethanol (50 mL). The mixture was stirred under nitrogen for 4 h, and evaporated *in vacuo* to a

residue that was partitioned between chloroform (150 mL) and aq. sodium hydrogencarbonate solution (100 mL). The organic layer was dried, and evaporated to a crystalline mass which was purified by column chromatography on silica gel with 24:1 (v/v) chloroform–methanol as the eluant. Crystallization, and recrystallization, from ethyl acetate–methanol afforded pure compound **3** (2.0 g, 51%); m.p. 135–137°.

Anal. Calc. for $C_{20}H_{31}N_3O_4S$: C, 58.65; H, 7.63; N, 10.26; S, 7.83. Found: C, 58.72; H, 7.69; N, 10.60; S, 8.00.

*1-[N-Acetyl-S-(carboxymethyl)-*DL*-homocysteinylamino]-6-(benzyloxycarbonylamino)hexane* (**5**). — Lead acetate trihydrate (0.48 g, 1.3 mmol) was added to a stirred solution of **3** (1.03 g, 2.5 mmol) and iodoacetic acid (**4**) (0.72 g, 2.5 mmol) in 75% ethanol (28 mL), and a yellow precipitate formed almost immediately. The mixture was stirred for 4 h at room temperature, filtered through Celite, and the solid washed with 75% ethanol. The filtrates were combined, and evaporated *in vacuo* to a syrup which was placed on a column of silica gel and eluted with 475:25:1 (v/v) chloroform–methanol–glacial acetic acid. Compound **5** was isolated as a syrup that crystallized on standing (1.0 g, 85%). Recrystallization from ethyl acetate–methanol afforded pure **5**, m.p. 128–130°.

Anal. Calc. for $C_{22}H_{33}N_3O_6S$: C, 56.51; H, 7.11; N, 8.99; S, 6.86. Found: C, 56.67; H, 7.30; N, 9.01; S, 6.98.

Ethyl N-(N-*acetyl-*DL*-homocysteinyl)glycinate* (**6**). — A mixture of glycine ethyl ester hydrochloride (25.1 g, 0.18 mol), **2** (28.6 g, 0.18 mol), and triethylamine (25 mL, 0.18 mol) in chloroform (300 mL) was stirred under nitrogen for 4 h at room temperature. Aqueous sodium hydrogencarbonate solution was added, and the organic layer was separated, washed with water, dried, and evaporated to a crystalline mass. Recrystallization from ethanol–ethyl ether afforded needles of **6** (39.5 g, 84%); m.p. 112°; *m/z* 262 (M^+).

Anal. Calc. for $C_{10}H_{18}N_2O_4S$: C, 45.79; H, 6.92; N, 10.68; S, 12.22. Found: C, 46.24; H, 7.27; N, 10.49; S, 12.56.

Ethyl N-{N-*acetyl*-S-[*6-(benzyloxycarbonylamino)hexyl*]-DL *homocysteinyl}glycinate* (**7**). — A mixture of **6** (2.17 g, 8.3 mmol), 6-(benzyloxycarbonylamino)hexyl iodide[18] (2.92 g, 8.1 mmol), and sodium hydrogensulfite (1.0 g, 9.7 mmol) in ethanol (15 mL) was stirred under nitrogen for 2 d at room temperature, and evaporated *in vacuo* to a residue that was partitioned between chloroform and water. The organic layer was successively washed with aq. sodium thiosulfate and water, dried, and evaporated to an oil that was purified by column chromatography on silica gel with 4:1 (v/v) chloroform–ethyl acetate as the eluant. Compound **7** crystallized from ethyl acetate, to give pure material (2.8 g, 7%); m.p. 99–100°; *m/z* 495 $(M)^+$ and 450 $(M^+ - OCH_2CH_3)$.

Anal. Calc. for $C_{24}H_{37}N_3O_6S$: C, 58.16; H, 7.52; N, 8.48; S, 6.47. Found: C, 58.23; H, 7.74; N, 8.33; S, 6.52.

N-{N-*Acetyl*-S-[*6-(benzyloxycarbonylamino)hexyl*]-DL-*homocysteinyl}glycine* (**8**). — Sodium hydroxide (2.5M; 0.9 mL) was added to a solution of **7** (1.1 g, 2.2 mmol) in 2:1 (v/v) ethanol–water (5 mL). After 4 h at room temperature, the solution

was diluted with water (20 mL), and acidified with conc. HCl to pH 1.0. The product was extracted into chloroform, and the extracts were combined, dried, and evaporated *in vacuo* to a residue that crystallized from ethanol–ethyl acetate, to afford pure **8** (1.0 g, 97%); m.p. 117°.

Anal. Calc. for $C_{22}H_{33}N_3O_6S$: C, 56.51; H, 7.11; N, 8.99; S, 6.86. Found: C, 56.96; H, 7.50; N, 8.80; S, 6.51.

Ethyl N-[N-*acetyl*-S-(*carboxymethyl*)-DL-*homocysteinyl*]*glycinate* (**9**). – A mixture of **6** (10.0 g, 38.1 mmol), **4** (7.8 g, 49.1 mmol), and lead(II) acetate dihydrate (2.59 g, 7.96 mmol) in ethanol (35 mL) was stirred under nitrogen overnight at room temperature. Potassium iodide (5.0 g, 30.1 mmol) was then added, and stirring was continued for 30 min. The mixture was filtered, and the solid washed with aqueous ethanol. The filtrates were combined, and evaporated *in vacuo* to a residue that was partitioned between chloroform and aq. sodium carbonate (pH 10). The basic, aqueous layer was acidified with 6M HCl to pH 1.0, and the solution was extracted with 1:9 (v/v) ethanol–chloroform. The extracts were dried, and evaporated to an oil that crystallized from ethyl acetate–ethyl ether to afford pure **9** (8.1 g, 66%); m.p. 115–116°; *m/z* 320 (M^+) and 302 ($M^+ - H_2O$).

Anal. Calc. for $C_{12}H_{20}N_2O_6S$: C, 44.99; H, 6.29; N, 8.74; S, 10.00. Found: C, 45.02; H, 6.45; N, 8.86; S, 10.27.

Ethyl N-[N-*acetyl*-S-{2-[6-(*benzyloxycarbonylamino*)*hexylamino*]-2-*oxoethyl*}-DL-*homocysteinyl*]*glycinate* (**10**). – *p*-Nitrophenol (0.96 g, 6.9 mmol) was added to a solution of **9** (2.04 g, 6.6 mmol) and dicyclohexylcarbodiimide (DCC; 1.54 g, 7.5 mmol) in dry *N,N*-dimethylformamide (DMF; 10 mL). The mixture was stirred for 3 h at room temperature, filtered, and the filtrate treated with **1** (1.68 g, 6.7 mmol) for 2 h. It was then evaporated *in vacuo* to a residue that was placed on a column of silica gel, and eluted with 9:1 (v/v) chloroform–methanol. The product isolated crystallized from ethyl acetate, to give **10** (2.49 g, 69%); m.p. 116–117°; *m/z* 552 (M^+) and 507 ($M^+ - OCH_2CH_3$).

Anal. Calc. for $C_{26}H_{40}N_4O_7S$: C, 56.74; H, 7.29; N, 10.14; S, 5.80. Found: C, 56.97; H, 7.46; N, 10.54; S, 5.59.

N-[N-*Acetyl*-S-{2-[6-(*benzyloxycarbonylamino*)*hexylamino*]-2-*oxoethyl*}-DL-*homocysteinyl*]*glycine* (**11**). – Sodium hydroxide (2.5M; 0.7 mL) was added to a solution of **10** (0.93 g, 1.7 mmol) in 2:1 (v/v) ethanol–water (5 mL). After 4 h at room temperature, the solution was diluted with water (20 mL), and acidified with conc. HCl to pH 1.0. The product was extracted into chloroform, and the extracts were combined, dried, and evaporated *in vacuo* to a residue that crystallized from ethanol–ethyl acetate, to afford pure **11** (0.83 g, 93%); m.p. 137–139°.

Anal. Calc. for $C_{24}H_{36}N_4O_7S$: C, 54.97; H, 6.91; N, 10.68; S, 6.11. Found: C, 55.35; H, 7.23; N, 10.53; S, 6.38.

N-[N-*Acetyl*-S-{2-[6-(*benzyloxycarbonylamino*)*hexylamino*]-2-*oxoethyl*}-DL-*homocysteinyl*]-N-(*5-ethoxycarbonylpentyl*)*glycinamide* (**12**). – *p*-Nitrophenol (0.51 g, 3.7 mmol) was added to a solution of **11** (1.55 g, 3.0 mmol) and DCC (0.7 g, 3.4 mmol) in DMF (5 mL). After being stirred for 3 h at room temperature, the mixture was

filtered, and the filtrate was added to a stirred solution of ethyl 6-aminohexanoate hydrochloride (1.28 g, 6.5 mmol) in DMF (5 mL) containing triethylamine (0.5 mL, 6.8 mmol). The mixture was stirred overnight at room temperature, and evaporated *in vacuo* to a residue that was placed on a column of silica gel, and eluted with 9:1 (v/v) chloroform–methanol. The product was isolated as an oil that crystallized from ethyl acetate, to afford pure **12** (1.59 g, 81%); m.p. 125–127°.

Anal. Calc. for $C_{32}H_{51}N_5O_8S$: C, 57.72; H, 7.71; N, 10.52; S, 4.82. Found: C, 57.89; H, 7.99; N, 10.28; S, 4.93.

N-[N-*Acetyl*-S-{2-[6-(*benzyloxycarbonylamino*)*hexylamino*]-2-*oxoethyl*}-DL-*homocysteinyl*]-N-(5-*carboxypentyl*)*glycinamide* (**13**). — Compound **12** (0.55 g, 0.83 mmol) was treated with sodium hydroxide (2.5M; 0.3 mL) in 2:1 (v/v) ethanol–water (3 mL), and processed in the usual way, to give **13** (0.39 g, 74%); m.p. 110–112° (EtOH–EtOAc).

N-[2-O-(*Methyl 2-acetamido-2,3-dideoxy-β*-D-*glucopyranosid-3-yl*)-D-*lactoyl*]-L-*alanine*-D-*isoglutamine benzyl ester* (*methyl β-glycoside of* N-*acetylmuramyl*-L-*alanyl*-D-*isoglutamine benzyl ester*) (**14**). — A suspension of the methyl β-glycoside of *N*-acetyl-4,6-*O*-benzylidenemuramyl-L-alanyl-D-isoglutamine benzyl ester[9] (1.67 g, 2.4 mmol) in 60% acetic acid (25 mL) was boiled under reflux for 2 h. The solution was cooled, and evaporated *in vacuo* to an oil that crystallized from methanol, to afford pure **14** (0.97 g, 63%); m.p. 207–210° (dec.), $[\alpha]_D^{27}$ +8.1° (*c* 1.35, methanol); n.m.r. ($CDCl_3$–CD_3OD): δ 1.38, 1.41 (2 d, *J* 6.5 Hz, 2 $CHCH_3$), 1.96 (s, NHAc), 2.50 (t, CH_2CO), 3.49 (s, OCH_3), 4.32 (d, $J_{1,2}$ 8.5 Hz, H-1), and 5.16 (s, $CH_2C_6H_5$).

Anal. Calc. for $C_{27}H_{40}N_4O_{11} \cdot CH_3OH$: C, 53.50; H, 7.05; N, 8.91. Found: C, 53.42; H, 6.77; N, 9.31.

General procedure for 6-O-acylation of **14**. — A mixture of **14** (1.0 mmol), a carboxylic acid (1.2 mmol), DCC (1.2 mmol), and 4-(dimethylamino)pyridine (0.04 mmol) in dry DMF (5 mL) was stirred under nitrogen overnight at room temperature, filtered, and the filtrate evaporated *in vacuo* to a residue. The product was usually purified by column chromatography on silica gel, with 9:1 (v/v) chloroform–methanol as the eluant.

Compound **16** was prepared from **14** and **5** in 58% yield; $[\alpha]_D^{27}$ +10.8° (*c* 2.6, DMF).

Anal. Calc. for $C_{49}H_{71}N_7O_{16}S \cdot CH_3OH$: C, 55.70; H, 7.01; N, 9.09; S, 2.97. Found: C, 55.67; H, 6.96; N, 8.81; S, 2.93.

Compound **17** was prepared from **14** and **8** in 72% yield; $[\alpha]_D^{27}$ +10.1° (*c* 1.05, methanol); n.m.r. ($CDCl_3$–CD_3OD): δ 1.38, 1.41 (2 d, 2 $CHCH_3$), 2.46–2.64 (m, CH_2SCH_2 and CH_2CO), 3.16 (t, CH_2NHZ), 4.34 (d, $J_{1,2}$ 8.0 Hz, H-1), 5.11, 5.16 (2 s, 2 $CH_2C_6H_5$), and 7.37 and 7.38 (aromatic).

Anal. Calc. for $C_{49}H_{71}N_7O_{16}S \cdot H_2O$: C, 55.31; H, 6.91; N, 9.21. Found: C, 55.70; H, 7.05; N, 9.17.

Compound **18** was prepared from **14** and **11** in 72% yield; $[\alpha]_D^{27}$ +8.4° (*c* 1.05, methanol); n.m.r. ($CDCl_3$–CD_3OD): δ 1.37, 1.40 (2 d, 2 $CHCH_3$), 2.50 (t,

$CH_2OCOBzl$), 2.65 (t, CCH_2S), 5.11, 5.15 (2 s, 2 $CH_2C_6H_5$), and 7.37 and 7.38 (aromatic).

Anal. Calc. for $C_{51}H_{74}N_8O_{17}S \cdot CH_3OH$: C, 55.02; H, 6.92; N, 9.87; S, 2.82. Found: C, 54.94; H, 7.24; N, 9.85; S, 3.05.

Compound **19** was prepared from **14** and **13** in 66% yield; $[\alpha]_D^{27}$ +11.3° (*c* 1.2, methanol); n.m.r. ($CDCl_3$–CD_3OD): δ 1.38, 1.40 (2 d, 2 $CHCH_3$), 1.99, 2.04 (2 s, 2 NHAc), 2.38 (t, $OCOCH_2$), 2.51 (m, $CH_2OCOBzl$), 2.66 (m, CCH_2S), 5.10, 5.16 ($OCH_2C_6H_5$), and 7.37 and 7.38 (aromatic).

Compound **20** was prepared from the benzyl α-glycoside of *N*-acetylmuramyl-L-alanyl-D-isoglutamine benzyl ester[9] and **5** in 65% yield; $[\alpha]_D^{27}$ +60.5° (*c* 1.04, DMF).

Anal. Calc. for $C_{55}H_{75}N_7O_{16}S$: C, 58.86; H, 6.74; N, 8.74; S, 2.86. Found: C, 58.80; H, 6.83; N, 8.71; S, 2.59.

General procedure for deprotection of 6-O-acyl-MDP analogs (**16–19**). — A mixture of the 6-*O*-acyl-MDP (100 mg), palladium chloride (100 mg), triethylsilane (1 mL), and triethylamine (200 μL) in dry oxolane (10 mL) was heated, with stirring, overnight at 40°. Methanol (1 mL) was added, and the mixture was stirred for 0.5 h at room temperature, and evaporated to a residue that was taken up in methanol. The suspension was filtered, and the filtrate was evaporated to a syrup which was chromatographed on a column of silica gel, using chloroform–methanol–water (6:4:1, and then 4:4:1, v/v) as eluant. The product isolated was usually a glass.

Compound **21** was prepared from **16** in 35% yield; $[\alpha]_D^{27}$ 0° (*c* 1.1, methanol); R_F 0.23 (solvent *A*); n.m.r. (D_2O): δ 1.39, 1.45 (2 d, 2 $CHCH_3$), 1.99, 2.06 (2 s, 2 NHAc), 2.29 (t, CH_2COOH), 2.73 (m, SCH_2C), 3.50 (s, OCH_3), and 4.46 (d, $J_{1,2}$ 8.5 Hz, H-1).

Compound **23** was prepared from **17** in 24% yield; $[\alpha]_D^{27}$ −0.9° (*c* 1.3, methanol); R_F 0.15 (solvent *A*); n.m.r. (D_2O): δ 1.39, 1.46 (2 d, 2 $CHCH_3$), 2.0, 2.10 (2 s, 2 NHAc), 2.30 (t, CH_2COOH), 2.58–2.80 (m, CCH_2SCH_2C), 3.03 (t, CH_2NH_2), and 3.52 (s, OCH_3).

Compound **25** was prepared from **18** in 39% yield, $[\alpha]_D^{27}$ −1.9° (*c* 1.6, methanol); R_F 0.15 (solvent *A*). An analytical sample was converted into the carbonate (salt).

Anal. Calc. for $C_{36}H_{62}N_8O_{15}S \cdot H_2CO_3$: C, 47.22; H, 6.85; N, 11.90. Found: C, 47.24; H, 7.19; N, 11.45.

Compound **27** was prepared from **19** in 9% yield, $[\alpha]_D^{27}$ −3.6° (*c* 2.3, methanol); R_F 0.15 (solvent *A*).

Hydrogenolysis of **20**. — A solution of **20** (100 mg) in glacial acetic acid (5 mL) containing palladium hydroxide (100 mg) was hydrogenolyzed at 40 lb.in.$^{-2}$ overnight at room temperature, the progress of the reaction being monitored by t.l.c. The catalyst was filtered off, another batch of palladium hydroxide (100 mg) was added, and the mixture was again hydrogenolyzed under the same conditions. This process was repeated several times, until all of the **20** had disappeared; the catalyst was then filtered off, and washed with glacial acetic acid. The filtrates were combined, and evaporated *in vacuo* to a residue which was placed on a column of silica gel, and eluted with 6:4:1 (v/v) $CHCl_3$–CH_3OH–H_2O. Fractions containing the major

product were combined, and evaporated to a syrup which was dissolved in D_2O, and the solution freeze-dried (18 mg). This compound was identified as 6-*O*-acetyl-MDP by 300-MHz, n.m.r. spectroscopy; n.m.r. (D_2O): δ 1.38, 1.43 (2 d, J 6.5 Hz, 2 $CHCH_3$), 1.98 (s, NHAc), 2.14 (s, OAc), 2.29 (t, CH_2COOH), and 5.20 (d, $J_{1,2}$ 3.5 Hz, H-1).

General procedure for coupling of ligands **21**, **23**, **25**, *and* **27** *to meningococcal group C polysaccharide.* — A suspension of meningococcal group C polysaccharide (50 mg, containing 0.16 mmol of *N*-acetylneuraminic acid residues), *N*-hydroxysuccinimide (2.0 mg, 19 μmol) and 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride (4.0 mg, 19 μmol) in dry DMF (1 mL) was stirred for 3 h at 0°. A solution of the ligand (**21**, **23**, **25**, or **27**; 16 μmol) in DMF (1 mL) was added to the foregoing mixture, and the suspension was stirred for another 3 h at 0°. The mixture was diluted with water (4 mL), and dialyzed against distilled water, with three changes of solvent. The solution was then lyophilized, to give the conjugate as a fluffy solid. Spinco analyses of the conjugates indicated the presence of 9–11% of MDP.

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