Synthesis of Lipophilic Muramyl Dipeptide Derivatives *via* *O*-Aminoacyl Intermediates[1)]

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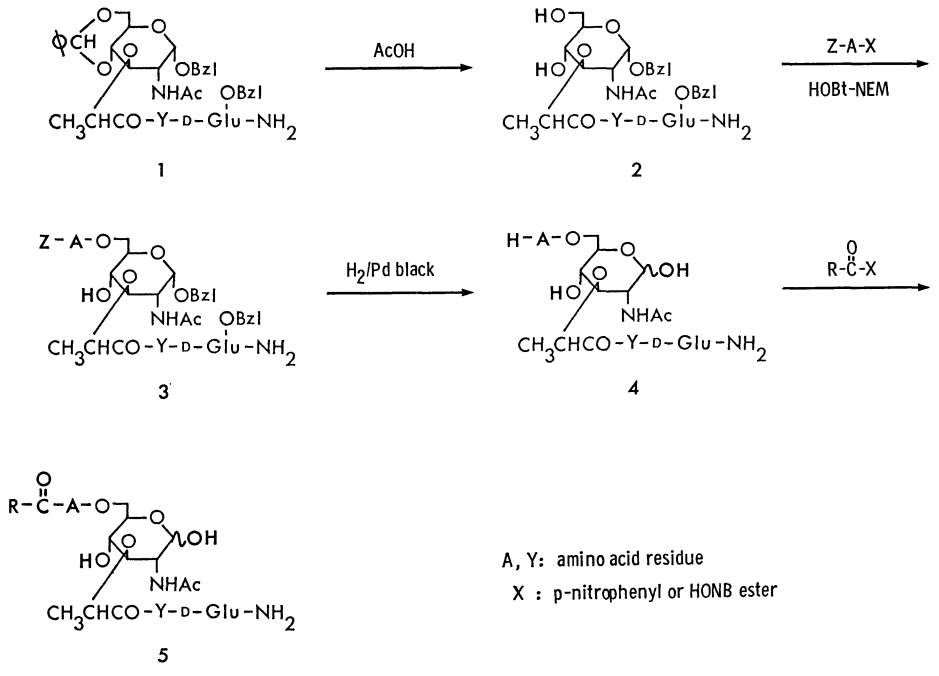
Incorporation of an amino acid into the 6 position of the muramic acid moiety of muramyl dipeptide has been achieved, providing an effective means for the synthesis of various new derivatives. Six new muramyl dipeptide derivatives with an *N*-acylated amino acid were synthesized using these intermediates and their immunological activity was examined. All the derivatives exhibited adjuvant activity on the induction of delayed-type hypersensitivity to *N*-acetyl-3-(4-arsonophenylazo)-L-tyrosine.

N-Acetylmuramyl-L-alanyl-D-isoglutamine (muramyl dipeptide or MDP) was found to be of the adjuvant-active minimal structure of bacterial cell wall peptidoglycans.[2)] Efforts to develop a new antitumor agent by modifying the MDP molecule led to the discovery of mycoloyl derivatives[3)] and synthetic mycoloyl-mimic long-chain fatty acid derivatives.[4)] Recently it was found that quinonyl derivatives of MDP display potent antitumor activity on the suppression of tumor growth (Meth-A fibrosarcoma) in syngeneic BALB/c female mice.[5)]

In these compounds, the 6 position of the muramyl moiety of MDP was acylated to render a lipophilic nature to the molecule. Acylation with mycolic acids, however, involves several steps and the use of 18-crown-6,[3)] the type of acid that can be introduced being restricted by the final deprotection by catalytic hydrogenolysis. The synthesis of quinonyl MDP derivatives also requires catalytic hydrogenolysis in the final stage.[5)] Thus, we tried to incorporate benzyloxycarbonylamino acids into the 6 position of the muramic acid moiety as a linking unit to introduce a lipophilic acyl group (Scheme 1). After the hydrogenolytic deprotection of all protecting groups, *O*-aminoacyl derivatives of MDP (**4**) can be coupled with various carboxylic acids as well as sulfonic and phosphoric acids, providing a valuable means for the synthesis of new derivatives of MDP. The insertion of various amino acids would affect the immunological activity.

In this paper, we describe the synthesis of six new MDP derivatives *via* *O*-aminoacyl intermediates (**4**) and their immunological activities.

The starting protected MDP derivatives (**1**) were synthesized according to the method described in a previous paper.[6)] Heating of **1** in aqueous acetic acid removed the benzylidene moiety to give partially protected MDP derivatives[7)] (**2**), which were then coupled with benzyloxycarbonylamino acid *p*-nitrophenyl ester in the presence of 1-hydroxybenzotriazole (HOBt) and *N*-ethylmorpholine (NEM) in *N*,*N*-



Scheme 1.

TABLE 1. YIELDS AND PHYSICOCHEMICAL PROPERTIES OF THE INTERMEDIATES **2**, **3**, AND **4**[a]

Compd	A	Y	Yield (%)	Mp/°C	$[\alpha]_D$[b] (temp)	Formula	Found (Calcd) (%) C	H	N
2a	—	Aib[c]	98	113(d)	+92.2°(20)	$C_{34}H_{46}N_4O_{11}$	59.09 (59.46	6.93 6.75	7.92 8.16)
2b	—	Ser	67	188—191	+89.8°(23)	$C_{40}H_{50}N_4O_{12}\cdot 1/2H_2O$	61.07 (60.98	6.38 6.53	7.00 7.11)
2c	—	Ala	93	222—223(d)[d]	+103.6°(23)[d]	$C_{33}H_{44}N_4O_{11}\cdot 1/2H_2O$	58.17 (58.14	6.48 6.65	8.11 8.22)
3a	Gly	Aib[c]	68	67(d)	+75.8°(23)	$C_{44}H_{55}N_5O_{14}$	60.25 (60.19	6.25 6.31	7.95 7.98)
3b	Leu	Aib[c]	57	98—100	+61.3°(20)	$C_{48}H_{63}N_5O_{14}$	61.63 (61.72	6.80 6.80	7.63 7.50)
3c	Leu	Ser	62	177—178	+58.7°(23)	$C_{54}H_{67}N_5O_{15}$	63.01 (63.20	6.49 6.58	6.71 6.83)
3d	Aud[e]	Ala	60	144—145	+67.3°(21)	$C_{52}H_{71}N_5O_{14}$	63.18 (63.07	7.31 7.23	6.97 7.07)
3e	β-Ala	Ala	71	152—154	+75.5°(23)	$C_{44}H_{55}N_5O_{14}$	59.79 (60.19	6.31 6.31	7.82 7.98)
4a	Gly	Aib[c]	69	128(d)	+61.0°(23)	$C_{22}H_{37}N_5O_{12}\cdot 1/2H_2O$	46.26 (46.15	6.93 6.69	12.29 12.23)
4b	Leu	Aib[c]	99	150—153(d)	+56.9°(20)	$C_{26}H_{45}N_5O_{12}\cdot 3H_2O$	46.14 (46.35	7.45 7.63	10.20 10.40)
4c	Leu	Ser	99	154(d)	+28.8°(23)[f]	$C_{25}H_{43}N_5O_{13}\cdot H_2O$	47.00 (46.93	7.19 7.09	10.52 10.94)
4d	Aud[e]	Ala	88	120(d)	+20.7°(21)	$C_{30}H_{53}N_5O_{12}\cdot H_2O$	51.85 (51.93	8.00 7.99	9.82 10.10)
4e	β-Ala	Ala	99	87—91(d)	+33.2°(23)	$C_{22}H_{37}N_5O_{12}\cdot 1/2H_2O$	46.46 (46.15	6.99 6.69	11.95 12.23)

a) Amino acid symbols denote the L-configuration unless otherwise stated. b) Solvent; DMF, *c* 0.5. c) Aib: α-aminoisobutyric acid. d) Lit,[7] mp 221.5—223 °C (d); $[\alpha]_D^{22}$ +93.6° (*c* 0.5, DMF). e) Aud: 11-aminoundecanoic acid. f) Solvent: H_2O, *c* 0.5 (after 25 h).

dimethylformamide (DMF) in 50—70% yields. α, β, or ω-Amino acid was coupled as a linking unit (Table 1). This procedure was originally adopted by Klausner and Chorev for the preparation of depsipeptides.[8] Efficient acylation of MDP components was accomplished with a twofold excess of protected amino acid active esters in a minimum amount of DMF. HOBt and NEM were added in a fourfold excess over the amount of MDP components. The resulting protected *O*-aminoacyl derivatives (**3**) were subjected to catalytic hydrogenolysis in acetic acid or methanol with palladium black as a catalyst in order to remove all the protecting groups. The physicochemical properties of the *O*-aminoacyl derivatives (**4**) thus obtained are given in Table 1 together with those of partially protected MDP derivatives (**2**) and protected *O*-aminoacyl derivatives (**3**).

The *O*-aminoacyl derivatives have been coupled with various carboxylic acids such as 10-(2,3-dimethoxy-5-methyl-1,4-benzoquinon-6-yl)decanoic acid[9] (**5a** and **5c**), 9-(2-methyl-1,4-naphthoquinon-3-yl)nonanoic acid[9] (**5b**), 3-(2,3-dimethoxy-5-methyl-1,4-benzoquinon-6-yl)propionic acid[9] (**5d**), all-*trans*-5,9,13,17-tetramethyl-4,8,12,16-octadecatetraenoic acid[10] (geranylgeranylacetic acid, **5e**), and retionic acid[11] (**5f**), by means of the active ester method in an attempt to obtain a variety of immunologically active compounds, especially with autitumor activity. Structures of carboxylic residues of **5a**—**f** are given in Table 2. Purification of the final products was carried out by column chromatography over silica gel and Sephadex LH-20. The structures of the compounds are given in Table 2 together with their physicochemical properties.

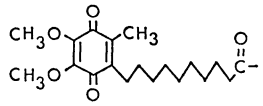
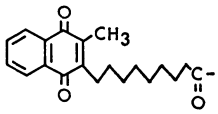
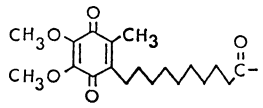
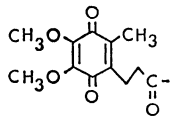
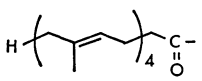
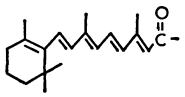
Their adjuvant activity on the induction of delayed-type hypersensitivity to *N*-acetyl-3-(4-arsonophenylazo)-L-tyrosine (ABA–Tyr) in guinea pigs was assayed according to a method described earlier.[6] All the compounds tested were found to be immunoadjuvant active, compounds **5a**, **5b**, and **5d** being more active than MDP (Table 3). Attachment of the lipophilic quinonyl moiety to the MDP molecule with 11-aminoundecanoic acid as a linking unit (**5d**) was found to be most favorable. Replacement of L-alanine of MDP by α-aminoisobutyric acid combined with the incorporation of quinonyl acids (**5a** and **5b**) also had a favorable effect on the biological potency.

The results show that the approach should provide a practical means for obtaining various MDP derivatives with immunological activities.

Experimental

Melting points were taken in open capillaries and are uncorrected. Optical rotations were determined with a Perkin-Elmer Model 141 polarimeter. All chemicals and solvents were of reagent grade and used without further purification. The reactions were monitored on TLC with Merck F_{254} silica gel plates, which were developed with

Table 2. Yields and physicochemical properties of MDP derivatives 5[a]

Compd	RCO-	A	Y	Yield (%)	$[\alpha]_D$[b] (temp, solvent)	Formula	Found(Calcd) (%) C	H	N
5a		Gly	Aib[c]	55	+30.2° (23, EtOH)	$C_{41}H_{63}N_5O_{13}\cdot 3H_2O$	52.14 (52.21	7.20 7.37	7.25 7.34)
5b		Leu	Aib[c]	47	+23.4° (20, EtOH)	$C_{45}H_{71}N_5O_{17}\cdot H_2O$	55.60 (55.59	7.49 7.57	7.14 7.21)
5c		Leu	Ser	56	+16.7° (23, H_2O)[d]	$C_{44}H_{69}N_5O_{13}\cdot 2H_2O$	53.40 (53.27	7.09 7.42	7.05 7.06)
5d		Aud[e]	Ala	51	+25.4° (23, 70%EtOH)	$C_{42}H_{65}N_5O_{17}\cdot H_2O$	54.42 (54.24	7.38 7.26	7.50 7.53)
5e		β-Ala	Ala	34	+25.8° (27, 70%EtOH)	$C_{44}H_{71}N_5O_{13}\cdot 2H_2O$	57.59 (57.81	7.95 8.27	7.62 7.66)
5f[f]		β-Ala	Ala	27	+23.9° (27, H_2O)[d]	$C_{42}H_{63}N_5O_{13}\cdot 5H_2O$	53.47 (53.85	7.19 7.86	7.43 7.48)

a) Amino acid symbols denote the L-configuration unless otherwise stated. b) *c* 0.5. c) Aib: α-aminoisobutyric acid. d) Optical rotation determined when mutarotation was completed (25 h). e) Aud: 11-aminoundecanoic acid. f) Preparation carried out in a dark place.

Table 3. Adjuvant activity of MDP derivatives for the induction of delayed-type hypersensitivity to ABA–Tyr in guinea pigs

Compound[a]	Skin reaction (mm±SE) 24 h	48 h
5a	20.5±0.8	19.5±1.4
5b	19.6±0.4	18.8±0.6
5c	18.0±1.0	18.2±1.7
5d	20.5±0.5	22.3±1.2
5e	18.8±0.7	18.6±1.1
5f	18.9±0.5	16.3±0.4
MDP	20.0±0.4	17.3±0.4
Control 1 (ABA–Tyr+FIA)[b]	9.0±1.6	1.8±1.2
Control 2 (ABA–Tyr only)	0	0

a) Dose: MDP, 100 μg; **5**, equimolar to MDP. b) FIA: Freund's incomplete adjuvant.

$CHCl_3$–acetone–MeOH (10 : 3 : 2, v/v) for compounds **2**, $CHCl_3$–MeOH–AcOH (18: 2: 1, v/v) for **3**, *n*-BuOH–AcOH–EtOAc–H_2O (1:1:1:1, v/v) for **4** and **5**, and EtOAc–pyridine–H_2O–AcOH (30 : 10 : 5 : 3, v/v) for **5**. Evaporation was carried out in a rotary vacuum evaporator under reduced pressure below 45 °C.

N-Acetyl-1-O-benzyl-α-muramyl-α-aminoisobutyryl-D-isoglutamine Benzyl Ester (2a). *N*-Acetyl-1-*O*-benzyl-4,6-*O*-benzylidene-α-muramyl-α-aminoisobutyryl-D-isoglutamine benzyl ester[5] (8.60 g, 11.1 mmol) was suspended in 75% AcOH (200 ml). The suspension was heated on a boiling water bath for 30 min, during which it gradually became clear. After evaporation of the solvent, the residue was flushed with H_2O and dissolved in a mixture of EtOAc (150 ml) and *n*-BuOH (50 ml). The solution was then washed successively with satd NaCl, 5% $NaHCO_3$–satd NaCl (1 : 1, v/v) and satd NaCl, and then dried over anhyd Na_2SO_4. Concentration followed by addition of Et_2O gave a white precipitate of **2a**: 6.90 g, 90.5% (Table 1).

Other partially protected MDP derivatives (2b and 2c) were prepared from appropriate protected MDP derivatives in a similar manner.

N-Acetyl-1-O-benzyl-6-O-benzyloxycarbonylglycyl-α-muramyl-α-aminoisobutyryl-D-isoglutamine Benzyl Ester (3a). A mixture of **2a** (412 mg, 0.6 mmol), benzyloxycarbonylglycine *p*-nitrophenyl ester (396 mg, 1.2 mmol), HOBt (324 mg, 2.4 mmol) and NEM (0.31 ml, 2.4 mmol) in DMF (3 ml) was stirred at room temperature for 40 h. After evaporation of the solvent, the residue was dissolved in EtOAc (15 ml) and the solution was washed with 5% $NaHCO_3$ (1×10 ml) then satd NaCl (1×10 ml), and dried over anhyd Na_2SO_4. The residue was purified by column chromatography of silica gel (30 g) with $CHCl_3$–acetone–MeOH (10 : 3 : 2, v/v) as an eluent, giving **3a**, 356 mg, 67.6% (Table 1).

Other protected MDP derivatives (3b—3e) were prepared from appropriate *N*-protected amino acid *p*-nitrophenyl ester and the partially protected MDP derivatives in a similar manner.

N-Acetyl-6-O-glycylmuramyl-α-aminoisobutyryl-D-isoglutamine (4a). Compound **3a** (307 mg, 0.55 mmol) was hydrogenated in acetic acid (10 ml) with palladium black as a

catalyst at room temperature for 3 h. After filtration and evaporation, the residue was purified by column chromatography of Sephadex LH-20 (1.8 cm × 45 cm) with EtOH–0.1 M AcOH (3 : 2, v/v) as an eluent, giving **4a**, 184 mg, 69.3% (Table 1).

*Other MDP derivatives with an amino acid (**4b—4e**)* were prepared from appropriate protected MDP derivatives with an *N*-protected amino acid in a similar manner.

N-*Acetyl-6*-O-[*10-(2,3-dimethoxy-5-methyl-1,4-benzoquinon-6-yl) decanoylglycyl*]*muramyl-α-aminoisobutyryl*-D-*isoglutamine (**5a**)*. 10-(2,3-Dimethoxy-5-methyl-1,4-benzoquinon-6-yl)decanoic acid *p*-nitrophenyl ester (47.4 mg, 0.1 mmol) was added to a solution of **4a** (56.4 mg, 0.1 mmol) and NEM (12.8 μl, 0.1 mmol) in DMF (1 ml). The mixture was stirred at room temperature for 16 h and evaporated. The residue was applied onto silica-gel column, the column being developed with EtOAc–pyridine–H_2O–AcOH (30 : 20 : 5 : 3, v/v) as an eluent. The fractions containing the product were collected and evaporated. The residue was rechromatographed over Sephadex LH-20 with EtOH–0.1 M AcOH (3: 2, v/v) as an eluent, giving **5a**, 50.5 mg, 55.4% (Table 2).

Other MDP derivatives with an N-*acylated amino acid (**5b—5f**)* were prepared from appropriate carboxylic acid active esters and MDP derivatives with an amino acid in a similar manner.

Biological Assays. Determination of the adjuvant activity on the induction of delayed-type hepersentivity was carried out according to the reported method.[6]

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10) Geranylgeranylacetic acid was synthesized from geranylgeranyl bromide and diethyl malonate in the presence of NaH by the conventional method.

11) Purchased from SIGMA Chemical Company, St. Louis, Mo. 63178, U. S. A.