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Synthesis of immunologically Active Muramyl Dipeptide Derivatives containing a Quinonyl Moiety *via* Aminoacyl Intermediates[1]

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3-(2,3-Dimethoxy-5-methyl-1,4-benzoquinon-6-yl)propanoic acid, 10-(2,3-dimethoxy-5-methyl-1,4-benzoquinon-6-yl)decanoic acid, 6-(2,3,5-trimethyl-1,4-benzoquinon-6-yl)-hexanoic acid, 9-(2-methyl-1,4-naphthoquinon-3-yl)nonanoic acid were coupled to N-acetyl-6-O-aminoacylmuramyl-α-aminoisobutyryl-D-isoglutamines by the active ester method. The aminoacyl residues used were Gly, β-Ala, L-Pro, L-Leu, D-Leu, Ahx and Aud. Most of the resulting derivatives showed more potent adjuvant activity than N-acetylmuramyl-L-alanyl-D-isoglutamine, an active constituent of mycobacterial cell wall peptidoglycan, for the induction of delayed-type hypersensitivity. The strength of the activity was affected by the combination of quinonyl acids and linking amino acids, and the present study indicated that the incorporation of an ω-(2,3-dimethoxy-5-methyl-1,4-benzoquinon-6-yl)alkanoyl moiety through Leu was most favorable.

Keywords——adjuvant; muramyl dipeptide; delayed-type hypersensitivity; structure/activity; quinonyl acids

The recent finding that N-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) is the effective minimal structure of bacterial cell walls in Freund's complete adjuvant has led to the synthesis of MDP and its analogs by several laboratories.[2] Extensive studies on various biological activities exerted by MDP and a variety of its analogs have also been undertaken and correlations between activity and chemical structure have been established.[3] On the basis of these findings, further attempts are being made to find a practical approach to the immunotherapy of infections[4] and tumors[5] with these chemically well-defined compounds.

Previously, we reported the synthesis of MDP derivatives which suppressed syngenic tumor growth (Meth-A fibrosarcoma) in BALB/c female mice.[6] All these active compounds possessed the ω-(2,3-dimethoxy-5-methyl-1,4-benzoquinon-6-yl)alkanoyl[7] substituent in the 6-position of the muramic acid moiety. In yet another attempt to synthesize compounds with potent immunological activity, we tried to introduce various labile carboxylic acids by inserting an amino acid between the carboxylic acids and MDP or modified MDP moieties.[8] Namely, an N-protected amino acid was incorporated into the 6-position of the muramic acid moiety of suitably protected MDP derivatives by the active ester method in the presence of 1-hydroxybenzotriazole and N-ethylmorpholine. After hydrogenolytic deprotection of all the protecting groups, the resulting aminoacyl intermediate could be condensed with carboxylic acids under mild conditions to give various types of MDP derivatives (Chart 1). Potent adjuvant activities of the derivatives possessing an alkanoyl group substituted with 2,3-dimethoxy-5-methyl-1,4-benzoquinone at its ω-position were found . This observation has stimulated our interest in studying the structure-activity relationships of the quinonyl acid moiety as well as the linking amino acid moiety, both incorporated at the 6-position of the muramic acid moiety of MDP.

We also reported that the replacement of L-Ala in MDP with Aib resulted in an increase of the immunological potency of MDP,[9] and 10-(2,3-dimethoxy-5-methyl-1,4-benzoquinon-

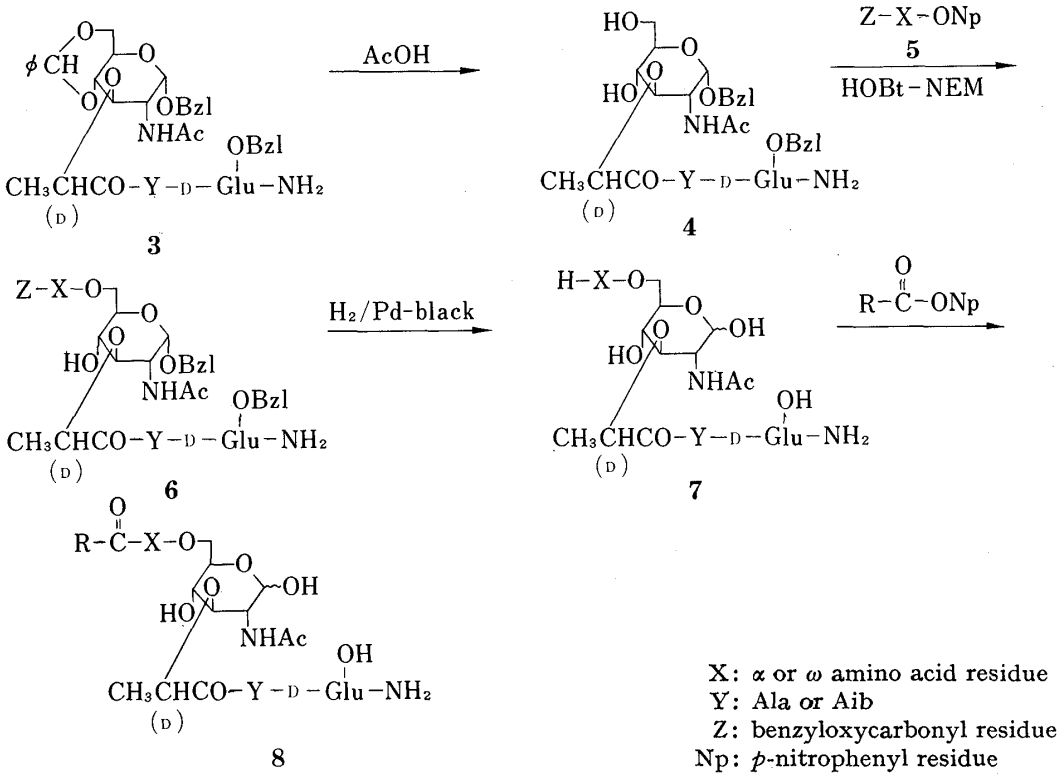


Chart 1

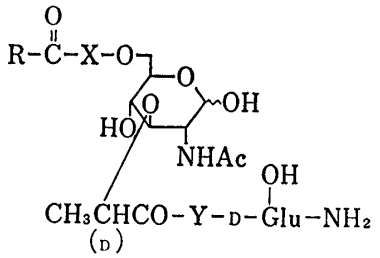
6-yl)decanoyl derivatives of this compound with amino acids as the linking unit exhibited even higher biological potency.[8] These results directed our attention to the synthesis of various quinonyl derivatives of MDP, in which L-Ala was replaced with Aib, in addition to those of natural MDP

In this paper, we describe the synthesis and immunological evaluation of twelve new MDP derivatives with quinonyl-aminoacyl groups. The compounds synthesized are listed in Table I.

Chemistry

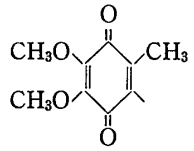
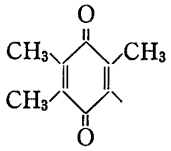
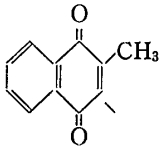
The starting *tert*-butoxycarbonyl dipeptide benzyl ester (**1**) was prepared by the N-hydroxy-5-norbornene-2,3-dicarboximide (HONB) active ester method.[10] Treatment of **1** with trifluoroacetic acid followed by coupling with N-acetyl-1-O-benzyl-4,6-O-benzylidene-α-muramic acid HONB ester (**2**)[9] gave protected glycopeptides (**3**). Compound **3** (**a**—**g**)[11] was precipitated from the reaction mixture. Filtration and washing gave the desired product in pure form. It is noteworthy that acylation of the sterically hindered Aib residue resulted in a good yield in the synthesis of **3** (**h**—**p**). Compound **3** (**h**—**p**) was highly soluble in organic solvents compared with the corresponding protected muramyl dipeptides in which Aib was replaced with a usual amino acid such as L-Ala or L-Val. Therefore, purification of **3** (**h**—**p**) was carried out by repeated extraction with ethyl acetate. After selective removal of the benzylidene-protecting group by treatment with 75% acetic acid at 100 °C, the resulting N-acetyl-1-O-benzyl-α-muramyl dipeptide benzyl ester (**4**) was acylated at the 6-position of the muramyl moiety with benzyloxycarbonylamino acid *p*-nitrophenyl ester (**5**) in the presence of 1-hydroxybenzotriazole and N-ethylmorpholine[12] giving compound **6**. All the protecting groups were removed by hydrogenolysis over palladium black in acetic acid giving N-acetyl-6-O-aminoacylmuramyl dipeptide derivatives (**7**). The physicochemical properties of the intermediates are listed in Table II. Condensation of **7** with a quinonyl acid by the active ester method afforded the final product (**8**). Purification of **8** was carried out by silica gel column

TABLE I. Structure and Adjuvant Activity of MDP Derivatives for the Induction of Delayed-type Hypersensitivity to ABA–Tyr in Guinea Pigs



Compound	R[b]	X	Y	Skin reaction at 48 h[c] (mm)
8a	Q–$(CH_2)_2$–	β-Ala	L-Ala	14.7
8b	Q–$(CH_2)_9$–	Ahx	L-Ala	19.9
8c	Q–$(CH_2)_2$–	Gly	L-Ala	16.5
8d	Q–$(CH_2)_9$–	β-Ala	L-Ala	19.6
8e	Q–$(CH_2)_2$–	Pro	L-Ala	18.4
8f[a]	Q–$(CH_2)_2$–	Aud	L-Ala	22.3
8g	Q–$(CH_2)_9$–	L-Leu	L-Ala	16.1
8h[a]	Q–$(CH_2)_9$–	Gly	Aib	19.5
8i[a]	Q–$(CH_2)_9$–	L-Leu	Aib	21.7
8j	Q–$(CH_2)_2$–	L-Leu	Aib	22.6
8k	E–$(CH_2)_5$–	L-Leu	Aib	18.2
8l	K–$(CH_2)_8$–	L-Leu	Aib	18.8
8m	Q–$(CH_2)_9$–	D-Leu	Aib	21.1
8n	Q–$(CH_2)_2$–	D-Leu	Aib	21.2
8o	Q–$(CH_2)_9$–	Aud	Aib	11.3
8p	Q–$(CH_2)_2$–	Aud	Aib	20.4
MDP				17.3
Control 1 (ABA–Tyr+FIA)				1.8
Control 2 (ABA–Tyr only)				0

a) These compounds were reported previously.[8]

b) Q:  E:  K: 

c) Dose: MDP, 100 μg; **8**, equimolar to MDP.

chromatography followed by gel filtration on Sephadex LH-20. The physicochemical properties of the analogs synthesized are listed in Table III.

Biological Results

The adjuvant activities of these synthetic analogs **8** for the induction of delayed-type hypersensitivity to N-acetyl-3-(4-arsonophenylazo)-L-tyrosine (ABA-Tyr) in guinea pigs were assayed by a method described earlier.[13] The results are listed in Table I. When the link unit was other than Leu, the derivatives with a small link unit and a short quinonyl acid (**8a**, **8c** and **8e**) showed reduced potency. The compound in which both link unit and quinonyl acid had long chains (**8o**) also showed weak activity, whereas those with a long link unit and a short quinonyl acid (**8f** and **8p**) or with a short link unit and a long acid moiety (**8b** and **8d**) exhibited high potency. This indicated that the chain length of the substituent at the 6-position as a whole was critical to the activity. When the linking amino acid was L- or D-Leu, however, the chain length of the substituents did not affect the activity (**8i** *vs.* **8j** and **8m** *vs.* **8n**). Of the compounds with L-Leu, those with the 2,3-dimethoxy-5-methyl-1,4-benzoquinonyl

moiety (**8i** and **8j**) had superior activity to those with the 2,3,5-trimethyl-1,4-benzoquinonyl (**8k**) or the 2-methyl-1,4-naphthoquinonyl moiety (**8l**). The optical properties of the linking amino acid also had no effect on the adjuvanticity (compare **8i** and **8j** with **8m** and **8n**, respectively).

TABLE II. Yields and Physicochemical Properties of newly Synthesized Intermediates **6** and **7**[a]

Compound	X	Y	Yield (%)	mp (°C)	$[\alpha]_D^{23}$[b]	Formula	Analysis (%) Calcd (Found) C	H	N
6b	Ahx	L-Ala	57.2	161—162	+74.8°	$C_{47}H_{61}N_5O_{14}$	61.36 (61.49	6.68 6.69	7.61 7.37)
6c	Gly	L-Ala	65.4	192—193	+80.2°	$C_{43}H_{53}N_5O_{14}$	59.78 (59.65	6.18 6.01	8.11 7.96)
6e	L-Pro	L-Ala	45.3	110—112	+61.1°	$C_{46}H_{57}N_5O_{14}$	61.11 (61.15	6.36 6.45	7.75 7.53)
6g	L-Leu	L-Ala	41.1	184	+67.0°	$C_{47}H_{61}N_5O_{14}$	61.36 (61.05	6.68 6.66	7.61 7.89)
6(m, n)	D-Leu	Aib	63.1	89—91	+78.9°	$C_{48}H_{63}N_5O_{14}$	61.72 (61.77	6.80 6.98	7.50 7.37)
6(o, p)	Aud	Aib	50.0	62—64	+63.2°	$C_{53}H_{73}N_5O_{14}$	63.39 (63.30	7.33 7.49	6.98 7.17)
7b	Ahx	L-Ala	81.3	96—97	+24.0°	$C_{25}H_{43}N_5O_{12}\cdot 1.5H_2O$	47.46 (47.50	7.33 7.34	11.07 10.57)
7c	Gly	L-Ala	84.4	104—107	+48.8°	$C_{21}H_{35}N_5O_{12}\cdot 0.5H_2O$	45.16 (45.55	6.50 6.35	12.54 12.49)
7e	L-Pro	L-Ala	100	159	+37.6°	$C_{24}H_{39}N_5O_{12}\cdot 1.5H_2O$	46.75 (47.06	6.87 7.00	11.35 11.57)
7g	L-Leu	L-Ala	91.8	134—138	+40.0°	$C_{25}H_{43}N_5O_{12}\cdot H_2O$	48.14 (47.89	7.27 7.52	11.23 10.74)
7(m, n)	D-Leu	Aib	100	145—147 (d)	+50.2°	$C_{26}H_{45}N_5O_{12}\cdot 3H_2O$	46.35 (46.08	7.63 7.32	10.40 10.06)
7(o, p)	Aud	Aib	96.0	119—120 (d)	+57.2°	$C_{31}H_{55}N_5O_{12}\cdot 2H_2O$	51.29 (51.31	8.19 8.18	9.65 9.55)

a) Compounds reported previously[8] were: **6(a, d)**, **6f**, **6h**, **6(i—l)**, **7(a, d)**, **7f**, **7h**, and **7(i—l)**.
b) $c=0.5$, DMF.

TABLE III. Yields and Physicochemical Properties of MDP Derivatives **8**

Compound	Yield (%)	$[\alpha]_D^{23}$[a]	Formula	Analysis (%) Calcd C	H	N	Found C	H	N
8a	56.6	+31.2°	$C_{34}H_{49}N_5O_{17}\cdot 3H_2O$	47.82	6.49	8.20	47.66	6.11	8.23
8b	80.0	+28.6°	$C_{44}H_{69}N_5O_{17}$	55.15	7.47	7.31	54.97	7.24	7.25
8c	67.1	+33.3°	$C_{33}H_{47}N_5O_{17}\cdot H_2O$	49.31	6.15	8.71	49.20	6.27	8.63
8d	53.5	+29.0°	$C_{41}H_{63}N_5O_{17}\cdot H_2O$	54.29	7.22	7.72	54.13	7.21	7.50
8e	50.9	+10.4°	$C_{36}H_{51}N_5O_{17}\cdot 3H_2O$	49.13	6.53	7.96	49.22	5.98	8.05
8g	61.2	+16.2°	$C_{44}H_{69}N_5O_{17}\cdot H_2O$	55.02	7.49	7.42	55.15	7.47	7.31
8j	57.3	+26.2°	$C_{38}H_{57}N_5O_{17}\cdot H_2O$	51.17	6.89	7.85	51.22	6.52	7.76
8k	49.5	+26.6°	$C_{41}H_{63}N_5O_{15}\cdot H_2O$	55.70	7.41	7.92	55.65	7.58	8.06
8l	38.0	+20.2°	$C_{46}H_{67}N_5O_{15}\cdot 2H_2O$	57.19	7.41	7.25	57.11	7.21	7.26
8m	34.1	+37.6°	$C_{45}H_{71}N_5O_{17}\cdot 1.5H_2O$	55.09	7.60	7.14	54.91	7.28	7.29
8n	55.0	+39.4°	$C_{38}H_{57}N_5O_{17}\cdot 2H_2O$	51.17	6.89	7.85	51.18	6.62	7.78
8o	56.0	+28.5°	$C_{50}H_{81}N_5O_{17}\cdot 1.5H_2O$	57.12	8.05	6.66	57.08	8.10	6.67
8p	59.4	+32.0°	$C_{43}H_{67}N_5O_{17}\cdot H_2O$	54.70	7.37	7.42	54.89	7.42	7.46

a) $c=0.5$, EtOH.

The activities of **8i** and **8p** with Aib in the peptide moiety were quite different from those of the corresponding L-Ala compounds (**8g** and **8f**) when each compound had the same substituent, *viz.*, the 10-(2,3-dimethoxy-5-methyl-1,4-benzoquinon-6-yl)decanoyl-L-leucyl or 3-(2,3-dimethoxy-5-methyl-1,4-benzoquinon-6-yl)propionyl-11-aminoundecanoyl moiety, although the adjuvant activity of the unsubstituted Aib analog was found to be higher than that of MDP.[8] These results show that the combination of quinonyl compound and linking amino acid as well as a substituted amino acid with L-Ala in the MDP molecule affects the adjuvant activity. In our present study, incorporation of ω-(2,3-dimethoxy-5-methyl-1,4-benzoquinon-6-yl)alkanoyl groups through Leu into the Aib analog of MDP (**8i**, **8j**, **8m** and **8n**) gave the best result.

In conclusion, most of these synthetic compounds displayed more potent activity than MDP, demonstrating the apparent effectiveness of quinonyl compounds linked through an amino acid. Further studies are in progress in our laboratories to examine the antitumor activity of these compounds.

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 were used without further purification. The reactions were monitored by TLC on Merck F_{254} Silica gel plates, which were generally developed with $CHCl_3$–acetone–MeOH (10: 3: 2, v/v) and $CHCl_3$–MeOH–AcOH (18: 2: 1, v/v) for compounds **1**, **3**, **4** and **6**, 1-BuOH–AcOH–AcOEt–H_2O (1: 1: 1: 1, v/v) for **7** and **8**, and with AcOEt–pyridine–H_2O–AcOH (30: 10: 5: 3, v/v) for **8**. Solutions were concentrated in a rotary vacuum evaporator under reduced pressure at temperatures below 45°C.

***tert*-Butoxycarbonyl-α-aminoisobutyryl-D-isoglutamine Benzyl Ester [1(h—p)]**——*tert*-Butoxycarbonyl-D-isoglutamine benzyl ester (7.4 g, 22 mmol) was dissolved in trifluoroacetic acid (30 ml) at room temperature. After 20 min, trifluoroacetic acid was removed by evaporation and the residue was triturated with petroleum ether (p.ether)–Et_2O (1: 1). The resulting powder was filtered off and dried over NaOH pellets. This trifluoroacetate was dissolved in acetonitrile (100 ml) together with *tert*-butoxycarbonyl-α-aminoisobutyric acid HONB ester (7.30 g, 20 ml) then Et_3N (3.80 ml) was added. The mixture was stirred for 15 h at room temperature. The solution was concentrated and the residue was extracted with AcOEt (50 ml). The organic layer was washed successively with 5% $NaHCO_3$, 10% citric acid solution, and water, then dried over Na_2SO_4. The solution was evaporated to dryness, and the residue was triturated with p.ether giving crystals which were recrystallized from AcOEt–p.ether: 7.75 g (91.9%). mp 112—113°C. $[\alpha]_D^{27}$ +13.2° (c=0.5, EtOH). *Anal.* Calcd for $C_{21}H_{31}N_3O_6$: C, 59.87; H, 7.41; N, 9.97. Found: C, 59.81; H, 7.75; N, 9.84.

***tert*-Butoxycarbonyl-L-alanyl-D-isoglutamine Benzyl Ester [1(a—g)]**——This compound was prepared from *tert*-butoxycarbonyl-L-alanine N-hydroxysuccimide ester and *tert*-butoxycarbonyl-D-isoglutamine benzyl ester in the manner described for **1(h—p)**: 83.2%. mp 140—141°C. (lit.[14] mp 137.5—138.5°C). $[\alpha]_D^{20}$ +8.3° (c=0.5, MeOH). Found: C, 59.41; H, 7.23; N, 10.25.

N-Acetyl-O-benzyl-4,6-O-benzylidene-α-muramyl-L-alanyl-D-isoglutamine Benzyl Ester [3(a—g)]——Compound **1(a—g)** (12.2 g, 30 mmol) was treated with trifluoroacetic acid (60 ml) for 30 min at room temperature. The mixture was evaporated to dryness, and the residue was washed with a mixture of Et_2O and p.ether (1: 1) by decantation. The resulting semisolid material was neutralized with Et_3N (4.2 ml) under cooling, and a solution of **2** (19.0 g, 30 mmol) in acetonitrile (350 ml) was added immediately. After a while, crystals began to precipitate. The mixture was left for 20 h at room temperature, then Et_2O (500 ml) was added and the resulting crystals were collected: 21.9 g (96.1%). mp 223—225°C (dec.) (lit.[14] mp 240°C (dec.)). $[\alpha]_D^{20}$ +89.2° (c=1.0, DMF). *Anal.* Calcd for $C_{40}H_{48}N_4O_{11}$: C, 63.15; H, 6.36; N, 7.40. Found: C, 62.91; H, 6.21; N, 7.25.

N-Acetyl-1-O-benzyl-4,6-O-benzylidene-α-muramyl-α-aminoisobutyryl-D-isoglutamine Benzyl Ester [3(h—p)]——Compound **1(h—p)** (3.20 g, 7.59 mmol) was treated with trifluoroacetic acid (20 ml) for 30 min at room temperature. The mixture was evaporated to dryness, and the residue was triturated with a mixture of Et_2O and p.ether (1: 1). The resulting powder was collected. The trifluoroacetate thus obtained was dissolved in acetonitrile (25 ml) and the solution was neutralized with Et_3N (1.17 ml) under cooling. To this was added **2** (4.80 g, 7.59 mmol) and the mixture was stirred for 20 h at room temperature. The mixture was evaporated to dryness, and the residue was dissolved in AcOEt (100 ml). The solution was washed with 5% citric acid solution and water, then dried over Na_2SO_4. The solvent was evaporated off and the residue was triturated with p.ether. The resulting powder was collected by filtration: 5.10 g (86.7%). mp 93°C. $[\alpha]_D^{21}$ +87.7° (c=1.0, DMF). *Anal.* Calcd for $C_{41}H_{50}N_4O_{11}$: C, 63.55; H, 6.51; N, 7.23. Found: C, 63.82; H, 6.88; N, 6.87.

N-Acetyl-1-O-benzyl-α-muramyl-α-aminoisobutyryl-D-isoglutamine Benzyl Ester [4(h—p)]——A suspension of 3(h—p) (4.50 g, 5.8 mmol) in 75% acetic acid (100 ml) was heated on a boiling water bath for 30 min. In the course of the reaction, the mixture gradually became clear. The mixture was evaporated to dryness, and the residue was flushed with water then toluene (twice each). The residual material was recrystallized from EtOH–Et_2O–p.ether: 3.90 g (97.9%). mp 113°C. (dec.). $[\alpha]_D^{20}$ +92.2° (c=0.5, DMF). *Anal.* Calcd for $C_{34}H_{46}N_4O_{11}$: C, 59.49; H, 6.75; N, 8.16. Found: C, 59.09; H, 6.93; N, 7.92.

N-Acetyl-1-O-benzyl-α-muramyl-L-alanyl-D-isoglutamine Benzyl Ester [4(a—g)]——This compound was prepared in a manner similar to that used for 4(h—p): 93%. mp 222—223°C. $[\alpha]_D^{23}$ +103.6° (c=0.5, DMF) (lit.[15] mp 221.5—223°C. $[\alpha]_D^{22}$ +93.6° (c=0.5, DMF)). Found: C, 58.17; H, 6.48; N, 8.11.

N-Acetyl-1-O-benzyl-6-O-(N-benzyloxycarbonyl-6-aminohexanoyl)-α-muramyl-L-alanyl-D-isoglutamine Benzyl Ester (6b)——A mixture of 4(a—g) (3.36 g, 5 mmol), benzyloxycarbonyl-6-aminohexanoic acid *p*-nitrophenyl ester (3.68 g, 10 mmol), 1-hydroxybenzotriazole (2.70 g, 20 mmol) and N-ethylmorpholine (2.56 ml, 20 mmol) in DMF (30 ml) was stirred for 16 h at room temperature. The solution was filtered and the solvent was evaporated off. The residue was dissolved in AcOEt and the solution was washed with 5% $NaHCO_3$, 0.5 N HCl then water, and dried over Na_2SO_4. After removal of the solvent by evaporation, the residue was purified by column chromatography on silica gel with $CHCl_3$–acetone–MeOH (10: 3: 2, v/v) as the eluent. The fractions containing the desired product were collected and the solvent was evaporated off. The residue was reprecipitated from AcOEt–Et_2O.

The other compounds were prepared in a similar manner. The yields and physicochemical data of **6b**, **6c**, **6e** and **6m—6p** are given in Table II.

N-Acetyl-6-O-(6-aminohexanoyl)-muramyl-L-alanyl-D-isoglutamine (7b)——Compound **6b** (374 mg, 0.4 mmol) was hydrogenated in AcOH (10 ml) with palladium black as a catalyst for 3 h at room temperature. The reaction mixture was filtered and evaporated to dryness. The residue was flushed with toluene and reprecipitated from EtOH–Et_2O.

The other compounds were prepared in a similar manner. The yields and physicochemical data of **7b**, **7c**, **7e** and **7m—7p** are given in Table II.

N-Acetyl-6-O-[3-(2,3-dimethoxy-5-methyl-1,4-benzoquinon-6-yl)propionyl-β-alanyl]muramyl-L-alanyl-D-isoglutamine (8a)——3-(2,3-Dimethoxy-5-methyl-1,4-benzoquinon-6-yl)propionic acid *p*-nitrophenyl ester (117 mg, 0.3 mmol) was added to a solution of **7a** (170 mg, 0.3 mmol) and N-ethylmorpholine (77 μl, 0.6 mmol) in DMF (3 ml). The mixture was stirred for 16 h at room temperature and evaporated to dryness. The residue was applied to a column (1.2×12 cm) of Silica gel and the column was developed with AcOEt–pyridine–H_2O–AcOH (30: 20: 5: 3, v/v). The fractions containing the product were collected and the solvent was evaporated off. The residue was rechromatographed over Sephadex LH-20 (column size: 1.5×45 cm) to carry out desalting, with EtOH–0.1 M AcOH (3: 2, v/v) as the eluent. The fractions containing the desired product were collected and EtOH was evaporated off. The residual aqueous solution was lyophilized giving a yellow powder.

The other MDP derivatives (**8b—8e** and **8j—8p**) were prepared in a similar manner. Their yields and the physicochemical data are given in Table III.

Biological Assays——Determination of the adjuvant activity on the induction of delayed-type hypersensitivity was carried out according to the method described earlier.[13]

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References and Notes

1) Abbreviations used are those recommended by the IUPAC-IUB Commission on Biochemistry Nomenclature in July 1965 and 1966; *Biochemistry* **5**, 2435 (1966); *ibid.*, **6**, 362 (1967). Other abbreviations: Aib=α-aminoisobutyric acid, Ahx=6-aminohexanoic acid, Aud=11-aminoundecanoic acid.
2) F. Ellouz, A. Adam, R. Ciorbaru, and E. Lederer, *Biochem. Biophys. Res. Commun.*, **59**, 1317 (1974); S. Kotani, Y. Watanabe, F. Kinoshita, T. Shimono, I. Morisaki, T. Shiba, S. Kusumoto, Y. Tarumi, and K. Ikenaka, *Biken J.*, **18**, 105 (1975).
3) Review: P. Dukor, L. Tarcsay, and G. Bascbang, Annual Reports in Medicinal Chemistry, Vol. 14, ed. by H.-J. Hess, Academic Press, New York, 1979, p. 146.
4) L. Chedid, M. Parant, F. Parant, P. Lefrancier, J. Choay, and E. Lederer, *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 2089 (1977).
5) T. Shiba, S. Okada, S. Kusumoto, I. Azuma, and Y. Yamamura, *Tetrahedron Lett.*, **1978**, 4899.
6) S. Kobayashi, T. Fukuda, I. Imada, M. Fujino, I. Azuma, and Y. Yamamura, *Chem. Pharm. Bull.*, **27**, 3193 (1979).
7) I. Azuma, K. Sugimura, Y. Yamamura, R. Bando, M. Watanabe, I. Imada, and H. Morimoto, *Internat. J. Vit. Nutr. Res.*, **48**, 255 (1978).
8) S. Kobayashi, T. Fukuda, H. Yukimasa, I. Imada, M. Fujino, I. Azuma, and Y. Yamamura, *Bull. Chem. Soc. Jpn.*, **53**, 2917 (1980).

9) S. Kobayashi, T. Fukuda, H. Yukimasa, M. Fujino, I. Azuma, and Y. Yamamura, *Bull. Chem. Soc. Jpn.*, **53**, 2570 (1980).
10) M. Fujino, S. Kobayashi, M. Obayashi, T. Fukuda, S. Shinagawa, and O. Nishimura, *Chem. Pharm. Bull.*, **22**, 1857 (1974).
11) Arabic numbers (Chart 1) representing intermediates and the final products were lettered **a**, **b**, **c**, *etc.* on the following principle in this text. The desired final products were each lettered according to their substituents as shown in Table I. The intermediates which gave **8b** were lettered **b**, such as **7b**, **6b**, *etc.* When a couple of compounds, **8a** and **8d**, for example, were prepared from a common intermediate, this intermediate was designated as **7(a,d)**. Thus, compound **3(a—g)** represents N-acetyl-1-O-benzyl-4,6-O-benzylidene-α-muramyl-L-alanyl-D-isoglutamine benzyl ester, the common intermediate for the synthesis of **8a**, **8b**, ..**8g**.
12) *a*) Y.S. Klausner and M. Chorev, *Chem. Commun.*, **1975**, 973; *b*) When the coupling reaction was performed in pyridine by using DCC in the presence of a catalytic amount of *p*-toluenesulfonic acid [K. Holmberg and B. Hansen, *Acta. Chem. Scand.*, **B 33**, 410 (1979)], a 4,6-di-O-acylated minor by-product was also obtained. In this case, however, the formation of the by-product was not observed. Although direct evidence could not be obtained, the compounds obtained should be 6-O-acylated. *cf.* ref. 15.
13) M. Uemiya, K. Sugimura, T. Kusama, I. Saiki, M. Yamawaki, I. Azuma, and Y. Yamamura, *Infect. Immun.*, **24**, 83 (1979).
14) S. Kusumoto, Y. Tarumi, K. Ikenaka, and T. Shiba, *Bull. Chem. Soc. Jpn.*, **49**, 533 (1976).
15) S. Kusumoto, S. Okada, K. Yamamoto, and T. Shiba, *Bull. Chem. Soc. Jpn.*, **51**, 2122 (1978).