Muramyl Dipeptide Derivatives with Multiprenylacetyl Group. Synthesis and Immunological Activities[1)]

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Several linear and branched all-*trans*-multiprenylacetic acids were synthesised, and introduced to the 6 position of the sugar moiety of muramyl dipeptide and its analog, *via* an amino acid as a linking unit. Compared with the saturated stearoyl derivative, all the compounds having a multiprenylacetyl group exhibited more potent adjuvant activity on the induction of delayed-type hypersensitivity to *N*-acetyl-3-(4-arsonophenylazo)-L-tyrosine. The derivatives with larger branched side chains tended to have increased activity.

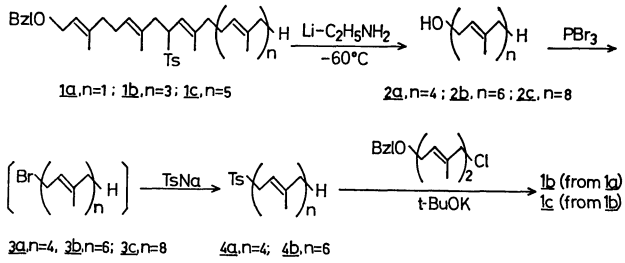
Following the finding that *N*-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) is the effective minimal component of bacterial cell walls eliciting the function of Freund's complete adjuvant,[2)] many studies on the synthesis and evalutation of the activity of MDP and its analogs have been carried out.[3)] During these investigations, several attempts to apply these chemically well-defined compounds to immunotherapy of cancer and infectious disease have been undertaken. Particularly some derivatives with a lipophilic substituent introduced to the 6 position of the carbohydrate moiety of MDP showed interesting results. For example, 6-*O*-mycoloyl-*N*-acetylmuramyl-L-alanyl-D-isoglutamine, in which the mycoloyl moiety was of either natural[4)] or synthetic origin,[5)] and 6-*O*-quinonyl-*N*-acetylmuramyl-L-valyl-D-isoglutamine methyl esters[6)] suppressed syngeneic tumor growth (Meth-A fibrosarcoma in BALB/c female mice). Furthermore, 6-*O*-stearoyl-*N*-acetylmuramyl-L-alanyl-D-isoglutamine protected the mice from infections.[7)] The potent adjuvant activity of MDP for the induction of delayed-type hypersensitivity to *N*-acetyl-3-(4-arsonophenylazo)-L-tyrosine (ABA–Tyr) in guinea pigs was also retained in these compounds. The results indicate that the lipophilicity of the residues introduced to the MDP molecule plays an important role in potentiating the immunological activity of MDP. The incorporated fatty acid may furnish an affinity for the membranes of macrophages and/or lymphocytes to the molecule: the precise role of the quinone nucleus moiety in the quinonyl MDP derivatives is left to be solved in future.

In order to investigate further the role of the lipophilicity, we tried to introduce a lipophilic unsaturated carboxylic acid to the 6 position of the muramyl moiety of MDP. Thus, nine multiprenylacetic acids (**7**) with different chain lengths were first synthesized. In view of lability of these carboxylic acids to the final hydrogenolytic deprotecting procedure, a coupling of them with amino group of 6-*O*-aminoacyl-*N*-acetylmuramyl dipeptides (**8**)[8)] was attempted under mild conditions to give the desired compounds. The versatility of these compounds (**8**) as intermediates for the synthesis of MDP derivatives has been demonstrated.[8,9)]

In this paper, we describe the synthesis and immunological evaluation of eight new polyprenylacetyl-amino-acylated MDP derivatives (**9**). The compounds synthesized are presented in Table 3.

Chemistry

Synthesis of Multiprenyl Alcohols (2). All-*trans*-1-benzyloxy-3,7,11,15-tetramethyl-9-(*p*-tolylsulfonyl)-2,6,10,14-hexadecatetraene (**1a**)[10)] served as material for the synthesis of multiprenyl alcohols containing more than four isoprenyl units (**2a**—**c**) as shown in Scheme 1. All-*trans*-3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraen-1-ol (**2a**) was prepared by reductive elimination of the sulfonyl and benzyl group of **1a** with lithium in ethylamine at −60 °C in 95.6% yield.



Scheme 1.

Compound **2a** was then converted to all-*trans*-1-bromo-3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraene (**3a**) by treatment with PBr_3. Without further purification, the bromo compound (**3a**) was allowed to react with sodium *p*-toluenesulfinate dihydrate to give all-*trans*-3,7,11,15-tetramethyl-1-(*p*-tolylsulfonyl)-2,6,10,14-hexadecatetraene (**4a**) in an overall yield of 85%. Compound **4a** was coupled with *trans,trans*-1-benzyloxy-8-chloro-3,7-dimethyl-2,6-octadiene (**5**)[10)] in the presence of *t*-BuOK to give all-*trans*-1-benzyloxy-3,7,11,15,19,23-hexamethyl-9-(*p*-tolylsulfonyl)-2,6,10,14,18,22-tetracosahexaene (**1b**) (89%) which was subjected to reduction with lithium in ethylamine, giving all-*trans*-3,7,11,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaen-1-ol (**2b**) in 82% yield. The multiprenyl chain elongation of **2b** to all-*trans*-3,7,11,15,19,23,27,31-octamethyl-2,6,10,14,18,22,26,30-triacontaoctaen-1-ol (**2c**) was performed by repeating the above mentioned procedure (**2b**→**3b**→**4b**→**1c**→**2c**). Physicochemical properties of the multiprenyl alcohols (**2**) and their intermediates are listed in Table 1.

Table 1. Yields and data of elemental analysis of multiprenylalcohols (**2**) and their intermediates (**1** and **4**)

Compound	n	Yield %	Formula	Found (Calcd) (%) C	H	S
1a	1	91.0	$C_{34}H_{46}O_3S$	76.24 (76.36	8.89 8.67	6.19 6.00)
1b	3	88.9	$C_{44}H_{62}O_3S$	78.66 (78.75	9.46 9.31	4.69 4.78)
1c	5	86.4	$C_{54}H_{78}O_3S$	80.46 (80.34	9.79 9.74	3.98 3.97)
2a	4	95.6	$C_{20}H_{34}O$	82.95 (82.69	12.17) 11.80)	
2b	6	82.0	$C_{30}H_{50}O$	84.75 (84.44	12.19 11.81)	
2c[a]	8	71.5	$C_{40}H_{66}O$	85.56 (85.34	12.13 11.82)	
4a	4	84.7	$C_{27}H_{40}O_2S$	75.78 (75.78	9.73 9.47	7.30 7.48)
4b	6	77.2	$C_{37}H_{56}O_2S$	78.75 (78.66	10.02 9.99	5.72 5.68)

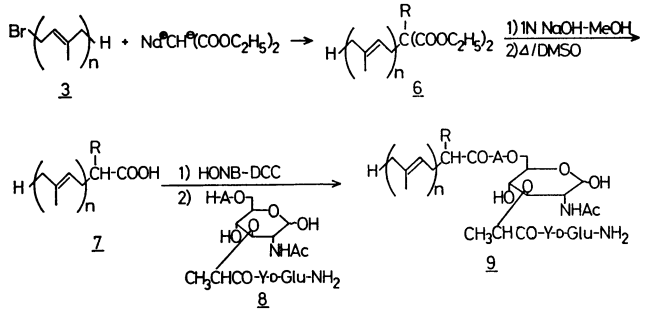
a) Wax with low mp.

Table 2. Yields and data of elemental analysis of multiprenylacetic acids (**7**) and their intermediates (**6**)

Compound	n	R	Yield %	Formula	Found (Calcd) (%) C	H
6a	4	H	52.0	$C_{27}H_{44}O_4$	74.97 (75.02	10.35 10.26)
6a′	4		13.4	$C_{47}H_{76}O_4$	79.86 (80.12	10.89 10.79)
6b	6	H	38.0	$C_{37}H_{60}O_4$	78.48 (78.12	10.75 10.75)
6b′	6		44.0	$C_{67}H_{108}O_4$	82.27 (82.32	11.20 11.14)
6c[a]	8	H	40.7	$C_{47}H_{76}O_4$	80.29 (80.06	11.05 10.87)
6c′[a]	8		16.6	$C_{87}H_{140}O_4$	83.80 (83.59	11.37 11.29)
6d	2	H	54.2	$C_{17}H_{28}O_4$	69.17 (68.89	9.67 9.52)
6d′	2		27.1	$C_{27}H_{44}O_4$	75.23 (74.95	10.47 10.25)
7a	4	H	73.2	$C_{22}H_{36}O_2$	79.46 (79.46	11.20 10.91)
7a′	4		92.9	$C_{42}H_{68}O_2$	83.13 (83.38	11.52 11.32)
7b[a]	6	H	100	$C_{32}H_{52}O_2$	81.74 (81.99	11.50 11.18)
7b′[a]	6		74.6	$C_{62}H_{100}O_2$	85.12 (84.86	11.77 11.49)
7c[a]	8	H	82.0	$C_{42}H_{68}O_2$	83.44 (83.38	11.47 11.32)
7c′[a]	8		96.0	$C_{82}H_{132}O_2$	85.62 (85.65	11.79 11.57
7d	2	H	89.9	$C_{12}H_{20}O_2$	73.29 (73.42	10.57 10.27)
7d′	2		85.6	$C_{22}H_{36}O_2$	79.46 (79.46	11.02 10.91)
7e	3	H	—[11]	$C_{17}H_{28}O_2$	77.55 (77.22	10.64 10.68)

a) Wax with low mp.

Synthesis of Mutiprenylacetic Acids (7). All-*trans*-5,9,13-trimethyl-4,8,12-tetracosatrienoic acid (**7d**) was synthesized according to the method reported by Fujita *et al.*[11] The eight other multiprenylacetic acids with different chain lengths were synthesized from multiprenyl alcohols (**2**, n=2—8) by the malonic ester method as shown in Scheme 2.

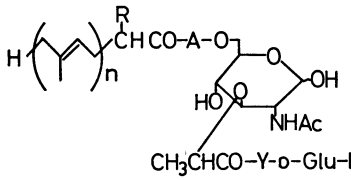


Scheme 2.

Multiprenyl alcohols (**2**) were converted to the corresponding bromides (**3**, n=2—8) by treatment with PBr_3. Using the resulting suitable multiprenyl bromides, diethyl malonate was alkylated in the presence of NaH to give the diethyl, mono- and disubstituted malonate (**6**). The molar ratio of the products of mono- (**6b**) and disubstituted (**6b'**) malonate was 2:1 when one equivalent of **3b**, for example, was allowed to react with diethyl malonate in the presence of one equivalent of NaH in DMF at room temperature for 2 h. The combined yield of **6b** and **6b'** was 82.1% based on **3b**. The reaction conditions that might affect the overall yield as well as the ratio of mono- and disubstituted compounds were not further exaimned since the result obtained was satisfactory for the present study. After the separation of the products by means of silica gel column chromatography with hexane–diisopropyl ether (15:1) as an eluent, each product was hydrolyzed in 1 M KOH (5 equivalent)–MeOH–THF (2:5:1) under reflux. The resulting dicarboxylic acids were subjected to decarboxylation in DMSO at 140—150 °C to give the straight and branched chain multiprenylacetic acids, *i.e.*, all-*trans*-5,9,13,17,21,-25-hexamethyl-4,8,12,16,20,24-hexacosahexaenoic acid (**7b**) and 2-(all-*trans*-3,7,11,15,19,23-hexamethyl-2,6,-10,14,18,22-tetracosahexaenyl)-all-*trans*-5,9,13,17,21,25-hexanethyl-4,8,12,16,22,24-hexacosahexaenoic acid (**7b'**). Physicochemical properties of the multiprenylacetic acids synthesized by this procedure are listed in Table 2 together with those of the intermediates (**6**).

Synthesis of 6-O-Multiprenylacylaminoacyl MDP Derivatives (9). The carboxylic acids prepared above were then converted to *N*-hydroxy-5-norbornene-2,3-

Table 3. Yields and physicochemical properties of MDP derivatives **9**, **10** and **11**



Compound	n	R	A	Y	Yield %	$[\alpha]_D$ (temp, c, solvent)	Formula	Found (Colcd) (%) C	H	N
9d	2	H	β-Ala	L-Ala	56.0	+28.2[b] (24, 0.5, H_2O)	$C_{34}H_{55}N_5O_{13}$ H_2O	53.71 (53.74	7.43 7.56	8.99 9.21)
9e	3	H	β-Ala	L-Ala	49.5	+38.4 (23, 0.5, DMF)	$C_{39}H_{63}N_5O_{13}$ H_2O	56.24 (56.57	7.96 7.91	8.43 8.45)
9a[a]	4	H	β-Ala	L-Ala	34.0	+25.8 (27, 0.4, EtOH)	$C_{44}H_{71}N_5O_{13}$ $2H_2O$	57.59 (57.81	7.95 8.27	7.62 7.66)
9b	6	H	L-Leu	Aib	17.9	+20.0 (25, 0.4, EtOH)	$C_{58}H_{95}N_5O_{13}$ H_2O	64.06 (64.00	8.96 8.98	6.32 6.44)
9c	8	H	L-Leu	Aib	14.1	+17.5 (23, 0.3, EtOH)	$C_{68}H_{111}N_5O_{13}$ H_2O	66.21 (66.68	9.38 9.30	5.73 5.72)
9d'	2		β-Ala	L-Ala	13.2	+24.7[b] (24, 0.3, H_2O)	$C_{45}H_{71}N_5O_{15}$ H_2O	57.07 (57.48	7.81 7.82	7.64 7.49)
9a'	4		β-Ala	L-Ala	28.0	+20.0 (27, 0.3, EtOH)	$C_{64}H_{103}N_5O_{13}$ $2H_2O$	64.58 (64.28	9.08 9.09	6.00 5.90)
9b'	6		L-Leu	Aib	13.5	+15.4 (25, 0.4, EtOH)	$C_{88}H_{143}N_5O_{13}$ H_2O	70.46 (70.60	9.79 9.76	4.59 4.68)
10	Stearoyl		β-Ala	L-Ala	66.3	+27.4[b] (27, 0.5, 70% EtOH)	$C_{40}H_{71}N_5O_{13}$ $2H_2O$	55.71 (55.47	8.88 8.73	8.12 8.09)
11[a]	Retinoyl		β-Ala	L-Ala	27.0	+23.9[b] (27, 0.5, H_2O)	$C_{42}H_{63}N_5O_{13}$ $5H_2O$	53.47 (53.85	7.19 7.86	7.43 7.48)

a) These compounds were reported previously.[8] b) Optical rotation was determined when mutarotation was completed (25 h).

dicaboximide (HONB)[12] active ester by dicyclohexylcarbodiimide (DCC) and coupled with 6-*O*-aminoacyl-*N*-acetylmuramyl dipeptide (**8**)[8] to give multiprenylacetyl derivatives of MDP with an amino acid as a linking unit (**9**) (Scheme 2). In this study, 6-*O*-β-alanyl-*N*-acetylmuramyl-L-alanyl-D-isoglutamine (**8a**) as an analog of a natural MDP type and 6-*O*-L-leucyl-*N*-acetylmuramyl-α-aminoisobutyryl-D-isoglutamine (**8b**) as an analog of an artificial MDP type with higher activity were used. In this acylation, the carboxylic acids with higher molecular weight gave the lower yields. Particularly the acylation with **7c'** could not be accomplished in spite of many trials. In addition to the steric effect, the large difference in the polarities of the two reactants (**7c'** and **8b**) may prevent access of each component to the reacting point. Stearoyl (**10**) and retinoyl (**11**) residues were introduced to **8a** in a similar manner for comparison of the biological activity. The resulting products were purified by column chromatography on silica gel with AcOEt–pyridine–AcOH–H_2O (80:10:3:5) as a solvent system. Rechromatography using Sephedex LH-20 with EtOH as an eluent gave the pure products listed in Table 3.

Biology

The adjuvant activity of these synthetic MDP analogs for the induction of delayed-typed hypersensitivity to *N*-acetyl-3-(4-arsonophenylazo)-L-tyrosine (ABA–Tyr) in guinea pigs was assayed by a method described earlier.[13] The reslults are shown in Table 4.

All the compounds reported in this paper showed more potent activity than the saturated stearoyl derivative (**10**). Among them, five compounds (**9b**, **9c**, **9b'**, **9d'**, **11**) revealed higher activity than MDP. The high activity of **9e**, in which the carbon number in the side chain is comparable to that of the stearoyl derivative (**10**), showed that the presence of the multiprenyl structure in the carbon chain is favorable for the activity. The activity of **9d'** is higher than **9a**, indicating that the branched structure of the chain is also favorable. The remarkable tendency for the compounds with larger side chains (**9b**, **9c**, and **9b'**) to possess increased activity is interesting although there was no clear-cut relation between the chain length and the activity. The high activity of retinoyl derivative (**11**), in spite of its small carbon number in the chain, suggested that there is some additional immunological function caused by retinoyl moiety as in the case of quinonyl derivatives[9] of MDP.

These results show that the introduction of lipophilic unsaturated carboxylic acids leads to the potentiation of the immunological activity of MDP, thus providing another approach to the development of a new family of immunologically active compounds.

Table 4. Adjuvant activity of MDP derivatives **9**, **10**, and **11** in delayed-type hypersensitivity to ABA-Tyr (100 μg) in guinea pigs

Compound[a]	Skin reaction (mm±s.e.)	
	24 h	48 h
9d	22.8±0.6	23.5±1.7
9e	25.0±1.3	26.3±1.6
9a	22.1±1.2	24.1±1.4
9b	24.3±0.7	28.9±1.3
9c	26.4±1.2	26.6±1.3
9d'	25.8±0.9	29.8±2.0
9a'	24.0±1.6	26.1±1.3
9b'	26.1±1.4	30.9±2.6
10	21.1±1.4	23.1±2.0
11	24.1±0.9	28.1±1.4
MDP	23.9±1.1	26.9±1.2
Control (ABA-Tyr+FIA)[b]	0	0

a) Dose: 100 μg. b) FIA: Freund's incomplete adjuvant.

Experimental

Optical rotations were determined with a Perkin-Elmer Model 141 polarimeter. NMR spectra were obtained on Varian EM-360 and EM-390 spectrometers. All chemicals and solvent were reagent grade and used without further purification. The reactions were monitored on TLC with Merck F_{254} silica-gel plates. Evaporation was carried out in a rotary evaporator under reduced pressure at temperatures below 45 °C.

All-trans-*3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraen-1-ol (2a).* Li (10.9 g, 2 g atom) was dissolved in $EtNH_2$ (300 ml) at −60 °C under N_2. After the solution became blue, a solution of all-*trans*-1-benzyloxy-3,7,11,15-tetramethyl-9-*p*-tolylsulfonyl-2,6,10,14-hexadecatetraene (**1a**) (21 g, 393 mmol) in anhydrous THF (40 ml) was added dropwise for 30 min. The mixture was stirred for 15 min at −60 °C, while blue color was kept. Isoprene (5 ml) and MeOH (100 ml) were carefully added to quench the excess Li. After the careful addition of water (500 ml), the organic solvents were evaporated. The residual aqueous solution was extracted with diisopropyl ether (150 ml×3). The organic layer combined was washed with water, and dried over Na_2SO_4. The solvent was evaporated and the resulting residue was purified by column chromatography (7×15 cm) on SiO_2 using hexane–diisopropyl ether (5:3, and then 1:2) as solvent: 10.9 g (95.6%) (Table 1). NMR ($CDCl_3$): 1.62 (9H, s), 1.70 (6H, s), 1.91—2.18 (12H, m), 4.15 (2H, d), 5.0—5.2 (3H, m), 5.43 (1H, t).

Other Multiprenyl Alcohols (2b and *2c)* were prepared from **1b** and **1c** in a similar manner.

All-trans-*3,7,11,15-tetramethyl-1-(*p-*tolylsulfonyl)-2,6,10,14-hexadecatetraene (4a).* To a solution of **2a** (14.4 g, 49.6 mmol) in absolute THF (70 ml) was added a solution of PBr_3 (5.6 g, 20.7 mmol) in THF (70 ml) dropwise at −7—−10 °C. Then the mixture was stirred at the same temperature for 15 min. The solvent was evaporated and the residue was dissolved in hexane–diisopropyl ether (1:1, 200 ml). The solution was successively washed with 5% $NaHCO_3$ and water, and then dried over Na_2SO_4. The solvent was evaporated to give all-*trans*-1-bromo-3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraene (**3a**). Without further purification, **3a** was dissolved in DMF (150 ml), and sodium *p*-toluenesulfinate tetrahydrate (24.8 g, 99.2 mmol) was added. After stirring for 1 h at room temperature, the mixture was diluted with hexane–diisopropyl ether (1:1, 400 ml), washed with water, and then dried over Na_2SO_4. After evaporation of the solvents, the resulting residue was purified by silica gel chromatography (6×25 cm) using hexane–diisopropyl ether (2:1) as solvent: 18.0 g (84.7%) (Table 1). NMR ($CDCl_3$): 1.36 (3H, d), 1.61 (9H), 1.68

(3H, s), 1.98—2.25 (12H, m), 2.44 (3H, s), 3.78 (2H, d), 5.10 (3H, m), 5.18 (1H, t), 7.31 and 7.74 (4H).

Compound ***4b*** was prepared from 2b in a similar manner.

*All-*trans*-1-benzyloxy-3,7,11,15,19,23-hexamethyl-9-(*p*-tolylsulfonyl)-2,6,10,14,18,22-tetracosahexaene (**1b**).* To a solution of **4a** (17.7 g, 41.3 mmol) and *trans,trans*-1-benzyloxy-8-chloro-3,7-dimethyl-2,6-octadiene (**5**) (13.1 g, 47 mmol) in absolute THF (100 ml)–DMF (12 ml) was added *t*-BuOK (6.96 g, 62 mmol) at −20 °C. After stirring for 20 min at the same temperature, the mixture was diluted with hexane–diisopropyl ether (1:1, 400 ml), and washed with 5% phosphoric acid (400 ml) and water, and then dried over Na_2SO_4. The solvent was evaporated, and the residue was chromatographed on a silica-gel column (5.5×20 cm) with hexane–diisopropyl ether (1:1) as an eluent: 24.6 g (88.9%) (Table 1). NMR ($CDCl_3$): 1.25 (3H, d), 1.55 (3H, s), 1.62 (12H, s), 1.70 (3H, s), 1.85—2.16 (16H, m), 2.44 (3H, s), 2.88 (2H, dd), 3.80 (1H, dd), 4.01 (2H, d), 4.50 (2H, s), 4.90 (1H, d), 4.95—5.24 (4H, m), 5.37 (1H, t), 7.30 and 7.71 (4H, m), 7.33 (5H, s).

Compound ***Ic*** was prepared from **4b** and **5** in a similar manner.

*Ethyl 2-Ethoxycarbonyl-all-*trans*-5,9,13,17,21,25-hexamethyl-4,8,12,16,20,24-hexacosahexaenate (**6b**)* and *Ethyl 2-(All-*trans*-3,7,11,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaenyl)-2-ethoxycarbonyl-all-*trans*-5,9,13,17,21,25-hexamethyl-4,8,12,16,20,24-hexacosahexaenate (**6b′**).* All-*trans*-3,7,11,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaen-1-ol (**2b**) (2.13 g, 5 mmol) was converted to the corresponding bromo-compound (**3b**) in a manner similar to that described in the synthesis of **4a**. To a solution of diethyl malonate (800 mg, 5 mmol) in absolute DMF (10 ml) NaH (240 mg, 5 mmol; 50% suspension in mineral oil) was added under N_2 with stirring. After stirring for 20 min at room temperature, the solution was cooled to −10 °C. A solution of **3b** in absolute THF (10 ml) was added dropwise at −10—−6 °C. After 30 min, the mixture was allowed to react at room temperature and stirring was continued for an additional 2 h. Water (100 ml) was carefully added and the solution was extracted with diisopropyl ether (100 ml). The organic layer was washed with water and dried over Na_2SO_4. The solvent was evaporated and the resulting residue was chromatographed on a silica-gel column (4.2×15 cm) with hexane–diisopropyl ether (15:1) to give pure **6b** and **6b′**: 1.09 g (38.0%) and 1.08 g (44.0%) (Table 2). NMR ($CDCl_3$) for 6b:1.27 (6H, t), 1.64 (21H, s), 1.88—2.19 (20H, m), 2.57 (4H, dd), 3.33 (1H, t), 4.17 (4H, q), 4.92—5.62 (6H, m).

*Other Esters (**6a**, **6a′**, **6c**, **6c′**, **6d**, and **6d′**)* were prepared from the appropriate alcohols and diethyl malonate in a similar manner. Geraniol for the preparation of **6d** and **6d′** was purchased from Wako Pure Chemical Industries, LTD, Osaka.

*2-(All-*trans*-3,7,11,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexenyl)-all-*trans*-5,9,13,17,21,25-hexamethyl-4,8,12,16,20,24-hexacosahexaenoic Acid (**7b′**).* A solution of **6b′** (1.08 g, 1.1 mmol) in 1 M KOH–MeOH–THF (2:5:1, 22 ml) was heated under reflux for 100 h. After cooling to room temperature, 0.5 M HCl (50 ml) was added and the solution was extracted with AcOEt (40 ml). The organic layer was washed with water, dried over Na_2SO_4, and then evaporated. The residue (dicarboxylic acid) was dissolved in DMSO (5 ml) and the solution was heated at 150 °C for 20 min, during which time the evolution of CO_2 was completed. After cooling to room temperature, the mixture was diluted with H_2O (30 ml) and extracted with AcOEt–diisopropyl ether (1:1, 50 ml). The organic layer was washed with water, dried over Na_2SO_4, and evaporated. The resulting residue was chromatographed on a silica-gel column (3.8×6 cm) with hexane–diisopropyl ether (4:1): 720 mg (74.6%). (Table 2). NMR ($CDCl_3$): 1.61 (42H, s), 1.95—2.45 (45H, m), 4.95—5.21 (12H, m).

*Other Multiprenylacetic Acids (**7a**, **7a′**, **7b**, **7c**, **7c′**, **7d**, and **7d′**)* were prepared from the corresponding esters in a similar manner.

*N-Acetyl-6-O-[[2-(all-*trans*-3,7,11,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaenyl)-all-*trans*-5,9,13,17,21,25-hexamethyl-4,8,12,16,20,24-hexacosahexaenoyl]-L-leucyl]muramyl-α-aminoisobutyryl-D-isoglutamine (**9b′**).* To a solution of **7b′** (87.7 mg, 0.1 mmol) and HONB (21.6 mg, 0.12 mmol) in AcOEt–acetonitrile (1:1, 2 ml) was added DCC (24.7 mg, 0.12 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h and then at room temperature for 3 h. After filtration of precipitates, the solvent was evaporated. The resulting active ester, *N*-acetyl-6-*O*-(L-leucyl)muramyl-α-aminoisobutyryl-D-isoglutamine (**8**)[8] (62 mg, 0.1 mmol) and NEM (26 μl) were dissolved in DMF (1 ml), and the mixture was stirred at 60 °C for 70 h. After evaporation of the solvent, the residue was purified by chromatography on a column of silica gel (2×12 cm) with AcOEt–pyridine–AcOH–water (80:10:3:5), followed by rechromatography on a column of Sephadex LH-20 (1.5×90 cm) with EtOH as an eluent: 20 mg (13.5%) (Table 3).

*Other MDP Derivatives with Acylated Amino Acid (**9**)* listed in Table 3 were prepared from the appropriate carboxylic acids and MDP derivatives with an amino acid in a similar manner.

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

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References

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