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Synthesis, and the Adjuvant and Tumor-Suppressive Activities of Quinonyl Muramyl Dipeptides[1)]

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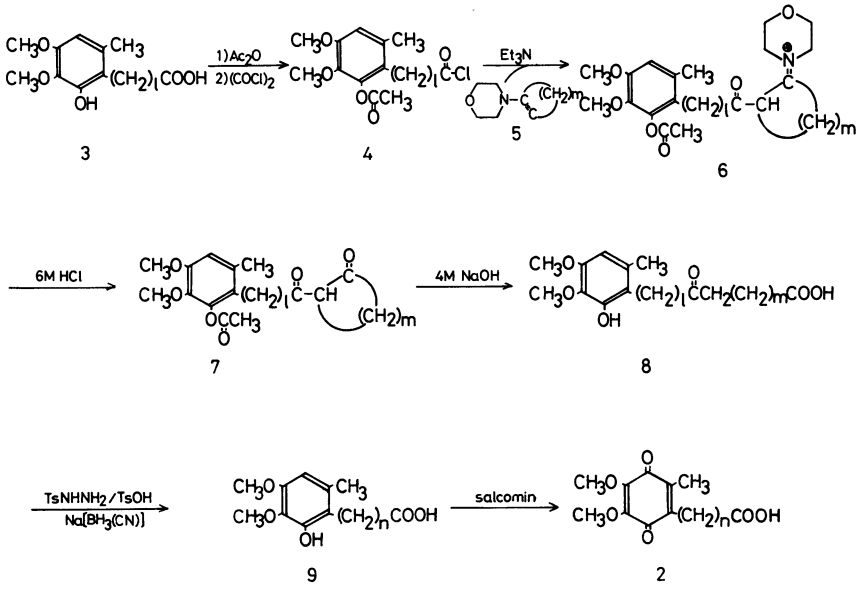
ω-(1,4-Benzoquinon-2-yl)alkanoic acids, 2-[10-(5,6-dimethoxy-3-methyl-1,4-benzoquinon-2-yl)decyl]-3-hydroxytetracosanoic acid, all-*trans*-5,9,13,17-tetramethyl-4,8,12,16-octadecatetraenoic acid, and stearic acid were coupled to the 6-*O*-position of the carbohydrate moiety of muramyl dipeptide alkyl esters, and 6-*O*-aminoacyl-muramyl dipeptide methyl esters. The aminoacyl residues used were Gly, Leu, Ahx, and Aud. New synthetic methods were developed for ω-(1,4-benzoquinon-2-yl)alkanoic acids such as 22-(5,6-dimethoxy-3-methyl-1,4-benzoquinon-2-yl)docosanoic acid, and the α-branched ω-(1,4-benzoquinone-2-yl) β-hydroxy acid, 2-[10-(5,6-dimethoxy-3-methyl-1,4-benzoquinon-2-yl)decyl]-3-hydroxytetracosanoic acid. The effects of the resulting quinonyl, multiprenylacetyl, and stearoylmuramyl dipetides on the induction of delayed-type hypersensitivity to ABA-Tyr in guinea pigs and the tumor(meth-A)-suppressive activity in syngeneic BALB/c female mice were measured. The results revealed that all these muramyl-dipeptide derivatives retained the adjuvant activity whereas the potent tumor-suppressive activity was observed only in quinonylmuramyl dipeptides, indicating that the 5,6-dimethoxy-3-methyl-1,4-benzoquinone ring is a requisite for the manifestation of the tumor-suppressive activity. The lipophilicity-hydrophilicity balance of the molecule was also important. Among the compounds tested, *N*-acetyl-6-*O*-[10-(5,6-dimethoxy-3-methyl-1,4-benzoquinon-2-yl)decanoyl]muramyl-L-valyl-D-isoglutamine methyl ester showed the most potent tumor-suppressive activity. This compound also showed tumor-regressive activity in guinea pigs, and hence is a good candidate for further studies.

The immunotherapy of cancer, especially with *Mycobacterium tubeculosus Bacillus Calmette-Guèrin* (BCG), originally incorporated into clinical studies by Mathé and his colleagues, was investigated in many centers.[2)] The observation that BCG has serious side effects has stimulated clinical trials of less toxic material such as the cell wall skelton of BCG and that of *Nocardia*;[3)] the potent immunological activity of these materials is well established.[4)] Consequently, the discovery that *N*-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) is able to replace mycobacterial whole cells in Freund's complete adjuvant stimulated efforts to develop a new antitumor agent by modifying this immunoactive minimal component of the cell wall peptidoglycan.[5)] In 1980 McLaughlin *et al.* reported that some synthetic MDP analogues, when administered in combination with trehalose dimycolate (TDM) as oil-in-water emulsion, caused line-10 hepatocellular-carcinoma regression in strain 2 guinea pigs.[6)] It was postulated that the presence of TDM enabled the water-soluble MDP analogues to disperse efficiently in the organic phase, and hence facilitated their antitumor activity. In an earlier attempt, Yamamura *et al.* reported that the incorporation into the MDP molecule of mycolic acid, a lipophilic constituent of the mycobacterial cell wall, resulted in a weak, compared with BCG cell wall skeleton, but distinct manifestation of the antitumor activity as determined by Meth-A fibrosarcoma suppression in syngeneic BALB/c female mice.[7)] Later, Kusumoto *et al.* found that the Meth-A fibrosarcoma suppressive effect of the 6-*O*-mycoloyl derivatives of MDP was unaffected when mycolic acid was replaced by a synthetic mycolic acid-mimic α-branched β-hydroxy fatty acid with 48 carbon atoms.[8)] These findings suggest that the lipophilicity-hydrophilicity balance of the molecule may be required for antitumor activity and the application of an MDP derivative with more favorable characteristics for cancer immunotherapy is possible.

In our efforts to obtain an agent for cancer immunotherapy, we tried to incorporate immunologically active lipophilic carboxylic acids, such as quinonyl[9)] and multiprenylacetic acids,[10)] into the MDP molecule using 6-*O*-aminoacyl MDP intermediates, which provided an effective means for introducing these labile carboxylic acids.[11)] Most of the resulting compounds, especially those having a 10-(5,6-dimethoxy-3-methyl-1,4-benzoquinon-2-yl)decanoyl group, exhibited a favorable biological effect on the induction of delayed-type hypersensitivity to *N*-acetyl-3-(4-arsonophenylazo)-L-tyrosine (ABA-Tyr) in guinea pigs.[11)] The apparent tumor-suppressive effect of the methyl esters of these derivatives has also been established.[12)] In this paper, we describe further the synthesis and immunological activities of quinonyl muramyl dipeptide alkyl ester derivatives **1** and a new general procedure for the synthesis of quinonyl acids, including mycoloyl-type quinonyl acid, having a long hydrocarbon chain. [A mycoloyl-type quinonyl acid (quinonylmycolic acid in this paper) is an α-branched β-hydroxyalkanoic acid having a quinone ring at the ω-position (**25**).]

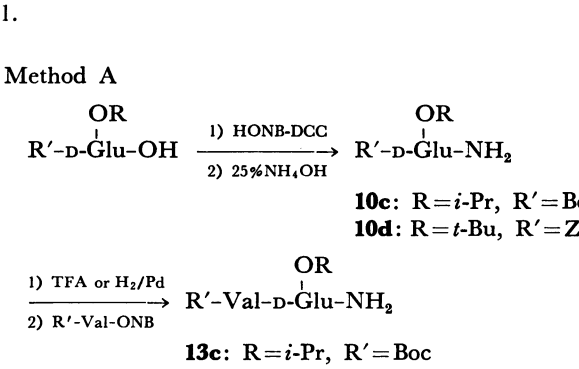
Chemistry. Six ω-(5,6-dimethoxy-3-methyl-1,4-benzoquinon-2-yl)alkanoic acids, QS-3, 6, 10, 16, 22, and 34 (**2**)[13)] and quinonylmycolic acid (**25**) were prepared. QS-3 (**2a**), 6 (**2b**), and 10 (**2c**) were prepared by the reported procedure.[14)] Since a convenient synthetic sequence of QS-16 (**2d**), 22 (**2e**), and 34 (**2f**) with long hydrocarbon chains has not been described, we developed a general synthetic method for those quinonyl acids (Scheme 1): ω-(3,4-dimethoxy-2-hydroxy-6-methylphenyl)alkanoic acid **3**[14)] was acetylated and then converted to the carbonyl chloride **4** by treatment with oxalyl dichloride. The chloride **4** was condensed



Scheme 1.

with an enamine **5**,[15] having an appropriate ring size, to give a condensation product **6**. After successive hydrolysis with 6 M HCl and 4 M NaOH, the carbonyl group of **8** was reduced with sodium cyanotrihydroborate in the presence of *p*-toluenesulfonohydrazide[16] to give **9**. When a 6-membered enamine (1-morpholinocyclohexene **5a**, m=4) and a 12-membered enamine (1-morpholinocyclododecene **5b**, m=10) are used, 6 and 12 carbon atoms can be extended, respectively [**3a**(l=9)→**9a**(n=15) and **3a**→**9b**(n=21)], in this conversion. By oxidation of **9a** with (*N*,*N*′-disalicylideneethylenediaminato)cobalt(II) (salcomin)[17] the target compound **2** was obtained. In this oxidation, potassium nitrosodisulfonate (Fremy's salt) used for the synthesis of quinonyl acids, such as QS-10 with a short hydrocarbon chain, could not be used for the synthesis of those with a long hydrocarbon chain because the yield was low or the reaction failed to proceed.[14] Compound **9b** (n=21), newly obtained by this procedure, was used as the starting material to synthesize **2f** (n=33). These findings demonstrate the accomplishment of a facile chain elongation of the alkyl side chain in quinonyl acids.

The synthesis of partially protected muramyl dipeptide alkyl esters **16** and their condensation with these carboxylic acids were carried out as outlined in Schemes 2 and 3: Secondary and tertiary alkyl ester analogues **13c** and **d** were prepared in a manner similar to the synthesis of the methyl ester analogue **13a**,[18] *i.e.* D-Glu was esterified at the γ-carboxyl group initially. After α-amino protection and amidation, either Boc-Val-ONB or *Z*-Val-ONB was coupled with α-amino-deprotected D-isoGln isopropyl and *t*-butyl ester to give **13c** and **d**, respectively (Scheme 2, Method A). Other Boc-Val-D-isoGln alkyl ester analogues (**13b**, **e**, and **f**) were prepared by esterification of Boc-Val-D-isoGln (**12**) with an appropriate alcohol; 6-chloro-1-(*p*-chlorophenylsulfonyloxy)benzotriazole (CCBT)[19]



Scheme 2.

was used as a coupling agent. Dipeptide **12** was obtained by catalytic hydrogenolysis from Boc-Val-D-isoGln benzyl ester (**11**)[18] (Scheme 2, Method B).

Boc-Val-D-isoGln alkyl esters **13** thus obtained were deblocked by TFA and coupled with the 1-*O*-benzyl-4,6-*O*-benzylidene-*N*-acetyl-α-muramic acid HONB ester (**14**).[18] In the case of compound **13d**, the *Z* group was removed by hydrogenolysis. The 4,6-*O*-benzylidene group of the muramyl moiety of the resulting protected glycopeptide **15** was removed by treatment with hot 75% acetic acid to give the partially protected muramyl dipeptide alkyl esters **16**, to which were condensed the above-mentioned quinonyl acids **2** by DCC in a mixture of pyridine and DMF. The yield from this procedure of quinonyl acids having short

† 1 M=1 mol dm^{-3}.

TABLE 1. YIELDS AND PHYSICOCHEMICAL PROPERTIES OF INTERMEDIATE DIPEPTIDES **13**
Boc-Y-D-Glu(OR)-NH_2

Compd	Y	R	Method[a]	Yield %	Mp θ_m/°C	$[\alpha]_D$ (c 0.5) (Temp., Solvent)	Formula	Analysis Found(Calcd) C	H	N
13a[b]	Val	Me	A	84.3	117—119	+8.6° (23, EtOH)	$C_{16}H_{29}N_3O_6$	53.72 (53.46	8.10 8.13	11.44 11.69)
13b	Val	Et	B	43.9	128—129	+8.4° (23, DMF)	$C_{17}H_{31}N_3O_6$	54.71 (54.76	8.21 8.37	11.31 11.25)
13c	Val	*i*-Pr	A	79.6	144—145	+10.2° (23, DMF)	$C_{18}H_{33}N_3O_6$	55.73 (55.79	8.59 8.58	10.60 10.84)
13d[c]	Val	*t*-Bu	A	68.1	210—211	+19.1° (23, DMF)	$C_{22}H_{33}N_3O_6$	60.51 (60.67	7.56 7.64	9.60 9.65)
13e	Val	*n*-Hex	B	22.0	94— 95	+9.4° (23, DMF)	$C_{21}H_{39}N_3O_6 \cdot 1/2H_2O$	57.66 (57.51	8.92 9.19	9.96 9.58)
13f	Val	St	B	37.0	88— 89	+4.0° (23, DMF)	$C_{33}H_{63}N_3O_6$	66.26 (66.29	11.10 10.62	7.10 7.03)
13g	Ser(Bzl)	Me	A	71.2	103—104	+7.0° (22, EtOH)	$C_{21}H_{31}N_3O_7$	57.05 (57.65	7.15 7.14	9.55 9.61)
13h	Thr(Bzl)	Me	A	93.1	131—132	+7.0° (22, EtOH)	$C_{22}H_{33}N_3O_7$	58.71 (58.52	7.43 7.37	9.25 9.31)

a) See text. b) Compound **13a** was reported previously: Ref. 18. c) Boc is replaced by Z in this compound.

TABLE 2. YIELDS AND PHYSICOCHEMICAL PROPERTIES OF PROTECTED AND PARTIALLY PROTECTED MURAMYL DIPEPTIDES **15** AND **16**

Compd	Y	R	Yield %	Mp θ_m/°C	$[\alpha]_D$ (Temp)[a]	Formula	Analysis Found(Calcd) C	H	N
15a[b]	Val	Me	92.0	242(d)	+86.1° (22)	$C_{36}H_{48}N_4O_{11} \cdot H_2O$	59.35 (59.16	6.80 6.90	7.70 7.68)
15b	Val	Et	74.3	>255	+98.4° (23)	$C_{37}H_{50}N_4O_{11}$	61.00 (61.14	6.97 6.93	7.65 7.71)
15c	Val	*i*-Pr	83.2	>255	+93.9° (23)	$C_{38}H_{52}N_4O_{11}$	61.89 (61.60	7.27 7.08	7.63 7.56)
15d	Val	*t*-Bu	75.3	186—188(d)	+97.4° (23)	$C_{39}H_{54}N_4O_{11}$	62.20 (61.92	7.25 7.21	7.56 7.42)
15e	Val	*n*-Hex	76.2	>255	+93.4° (23)	$C_{41}H_{58}N_4O_{11}$	62.74 (62.79	7.33 7.47	7.23 7.16)
15f	Val	St	84.8	249(d)	+67.5° (23)	$C_{53}H_{82}N_4O_{11} \cdot H_2O$	65.81 (65.67	8.66 8.76	5.90 5.78)
15g	Ser(Bzl)	Me	80.0	277—288	+92.0° (21)	$C_{41}H_{50}N_4O_{12}$	62.25 (62.26	6.35 6.37	7.15 7.09)
15h	Thr(Bzl)	Me	85.5	248	+92.0° (21)	$C_{42}H_{52}N_4O_{12}$	62.27 (62.67	6.44 6.51	7.20 6.96)
16a	Val	Me	73.0	242	+111.3° (23)	$C_{29}H_{44}N_4O_{11}$	55.59 (55.76	7.16 7.10	8.97 8.70)
16b	Val	Et	95.7	235	+110.5° (23)	$C_{30}H_{46}N_4O_{11}$	56.04 (56.41	7.22 7.26	8.61 8.77)
16c	Val	*i*-Pr	85.6	207—208	+111.2° (23)	$C_{31}H_{48}N_4O_{11} \cdot 1/2H_2O$	56.26 (56.26	7.46 7.31	8.43 8.46)
16d	Val	*t*-Bu	69.2	197—199	+110.3° (23)	$C_{32}H_{50}N_4O_{11} \cdot 1/2H_2O$	56.84 (56.84	7.55 7.60	8.19 8.27)
16e	Val	*n*-Hex	78.3	210—212	+101.6° (23)	$C_{34}H_{54}N_4O_{11} \cdot 1/2H_2O$	57.99 (58.02	7.91 7.88	7.95 7.96)
16f	Val	St	83.8	231(d)	+85.1° (23)	$C_{46}H_{78}N_4O_{11} \cdot 1/2H_2O$	63.94 (64.01	9.19 9.11	6.47 6.49)
16g	Ser(Bzl)	Me	80.0	224—225	+99.2° (21)	$C_{34}H_{46}N_4O_{12}$	57.96 (58.10	6.51 6.60	8.22 7.97)
16h	Thr(Bzl)	Me	85.7	181—182	+102.4° (21)	$C_{35}H_{48}N_4O_{12} \cdot 1/2H_2O$	57.95 (57.91	6.66 6.81	7.65 7.72)

a) Solvent, DMF (c 0.5). b) Compound **15a** was reported previously: Ref. 18.

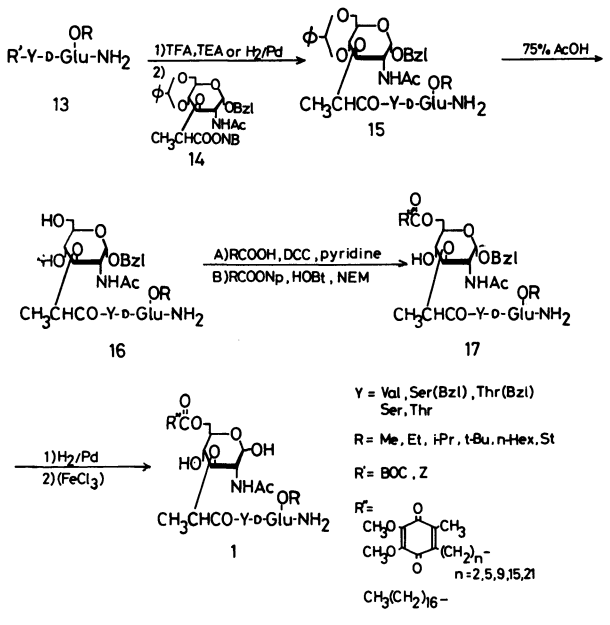
TABLE 3. YIELDS AND PHYSICOCHEMICAL PROPERTIES OF PROTECTED QUINONYL MURAMYL DIPEPTIDES **17**

Compd	R''	Y	R	Yield %	Mp θ_m/°C	$[\alpha]_D^{23}$ a) (Solvent)	Formula	Analysis Found(Calcd) C	H	N
17a	QS-10	Val	Me	75.8	172—175	+70.2° (MeOH) b)	$C_{48}H_{70}N_4O_{16}\cdot 1/2H_2O$	59.87 (59.55)	6.99 7.39	6.14 5.79)
17b	QS-10	Val	Et	41.1	177	+66.7° ($CHCl_3$)	$C_{49}H_{72}N_4O_{16}\cdot 1/2H_2O$	59.73 (59.92)	7.32 7.49	5.85 5.71)
17c	QS-10	Val	*i*-Pr	56.0	177—179	+69.0° (EtOH)	$C_{50}H_{74}N_4O_{16}$	60.89 (60.83)	7.67 7.56	5.76 5.68)
17d	QS-10	Val	*t*-Bu	53.3	157	+65.6° (EtOH)	$C_{51}H_{76}N_4O_{16}$	61.16 (61.18)	7.65 7.65	5.61 5.61)
17e	QS-10	Val	*n*-Hex	36.9	174—176	+46.9° ($CHCl_3$)	$C_{53}H_{80}N_4O_{16}$	61.75 (61.85)	7.74 7.84	5.37 5.44)
17f	QS-10	Val	St	30.6	180	+38.4° ($CHCl_3$)	$C_{65}H_{104}N_4O_{16}\cdot H_2O$	64.22 (64.22)	8.61 8.79	5.21 4.61)
17g	QS-3	Val	Me	37.6	189—190	+60.0° ($CHCl_3$)	$C_{41}H_{56}N_4O_{16}\cdot 1/2H_2O$	56.72 (56.60)	6.66 6.61	6.47 6.44)
17i	QS-16	Val	Me	60.4	188—190	+59.2° (DMF)	$C_{54}H_{82}N_4O_{16}\cdot 1/2H_2O$	61.45 (61.63)	7.93 7.95	5.51 5.33)
17j	QS-22	Val	Me	27.8	182—184	+52.8° (DMF)	$C_{60}H_{94}N_4O_{16}\cdot 1/2H_2O$	63.78 (63.41)	8.93 8.42	5.42 4.93)
17k	QS-10	Ser(Bzl)	Me	72.3	162—165	+65.4° (EtOH) b)	$C_{53}H_{72}N_4O_{17}$	61.67 (61.37)	7.15 7.00	5.13 5.40)
17l	QS-10	Thr(Bzl)	Me	74.9	143	+59.6° (EtOH) b)	$C_{54}H_{74}N_4O_{17}\cdot 1/2H_2O$	61.02 (61.17)	7.05 7.13	5.40 5.29)
17m	St	Val	Me	31.2	214	+56.0° ($CHCl_3$) c)	$C_{47}H_{78}N_4O_{12}$	62.99 (63.35)	8.74 8.82	6.35 6·29)

a) *c* 0.5. b) Temperature=21 °C. c) *c* 0.4; Temperature=24 °C.

alkyl chains (**2a**: n=2; **2c**: n=9) reached up to 70%. The yield of quinonyl acids with long alkyl chains (**2d**: n=15; **2e**: n=21) was 35 and 25%, respectively, and that of **2f** (n=33) was much lower. Stearic acid was also condensed to *N*-acetyl-1-*O*-benzyl-α-muramyl-L-valyl-D-isoglutamine methyl ester (**16a**) in a similar manner to obtain *N*-acetyl-6-*O*-stearoylmuramyl-L-valyl-D-isoglutamine methyl ester (**1o**) to evaluate the effect of the quinonyl moiety on biological activities.

Alternatively, the partially protected muramyl dipeptide alkyl esters **16** were condensed with the *p*-nitrophenyl ester of the quinonyl acids in the presence of HOBt and NEM in DMF at room temperature for 2 d. This procedure was also used for the condensation of benzyloxycarbonyl amino acids with the partially protected muramyl dipeptide **16a** to obtain compounds with a linking amino acid between a lipophilic carboxylic acid and muramyl dipeptide moiety[10] (Scheme 6, **16a**→**33**). The protected quinonyl muramyl dipeptide alkyl esters **17** were then hydrogenated in acetic acid or methanol with palladium black as a catalyst. The hydroquinonyl group was oxidized by $FeCl_3$ in methanol to the desired 6-*O*-quinonyl-*N*-acetylmuramyl-L-valyl-D-isoglutamine alkyl esters **1** (Scheme 3). Compound **1h** was obtained in a moderate yield directly by the coupling of quinonyl acid **2b** (n=5) with *N*-acetylmuramyl-L-valyl-D-isoglutamine methyl ester[18] using DCC as a condensing agent. Compounds **1m** and **n** having a Ser of Thr residue instead of the Val residue of **1a** were synthesized similarly with Boc-Ser(Bzl)-D-isoGln methyl ester (**11g**) or Boc-Thr(Bzl)-D-isoGln methyl ester (**11h**) as a starting material, respectively.



Scheme 3.

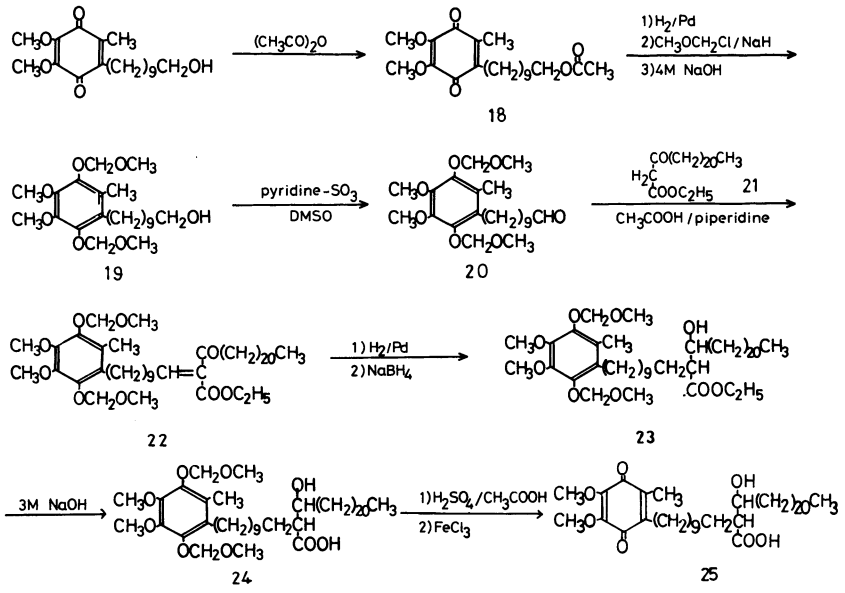
We also developed a novel general synthetic route for quinonylmycolic acid **25** with 10-(5,6-dimethoxy-3-methyl-1,4-benzoquinon-2-yl)decyl acetate (**18**)[14] as a starting material (Scheme 4). Compound **18** was hydrogenated and the hydroxyl groups of the resulting hydroquinone were methoxymethylated by methoxymethyl chloride in the presence of NaH and subsequent hydrolysis with 4M NaOH gave the protected quinonyl alcohol **19**. Compound **19** was oxidized with

dimethyl sulfoxide using pyridine-sulfur trioxide complex to the aldehyde **20**, which was then condensed with the ethyl 3-oxoalkanoate **21**[20] in the presence of acetic acid-piperidine to produce the condensation product **22**. Catalytic hydrogenation of the double bond of **22** and subsequent reduction with sodium borohydride afforded the protected quinonylmycolic acid ethyl ester **23**, which was then hydrolyzed with 3M NaOH to yield

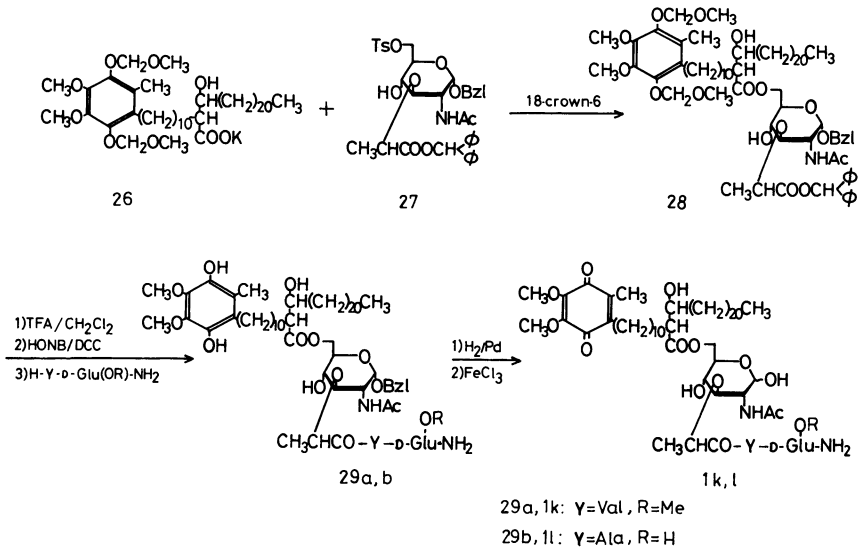
TABLE 4. YIDLDS AND PHYSICOCHEMICAL PROPERTIES OF QUINONYL MURAMYL DIPEPTIDES **1**

Compd	R''	Y	R	Yield %	Mp θ_m/°C	$[\alpha]_D^{23}$ a) (Solvent)	Formula	Analysis Found (Calcd) C	H	N
1a	QS-10	Val	Me	73.0	188—189	+35.2° (EtOH) b)	$C_{41}H_{64}N_4O_{16}$	56.47 (56.67	7.42 7.42	6.43 6.45)
1b	QS-10	Val	Et	77.9	—	+33.0° (EtOH)	$C_{42}H_{66}N_4O_{16}\cdot H_2O$	55.67 (55.98	7.53 7.61	6.07 6.22)
1c	QS-10	Val	*i*-Pr	69.1	194—195	+35.4° (EtOH)	$C_{43}H_{68}N_4O_{16}$	57.39 (57.57	7.75 7.64	6.14 6.25)
1d	QS-10	Val	*t*-Bu	53.3	192—193	+35.6° (EtOH)	$C_{44}H_{70}N_4O_{15}$	57.73 (58.00	7.83 7.75	6.16 6.16)
1e	QS-10	Val	*n*-Hex	57.3	200—202	+39.2° (EtOH)	$C_{46}H_{74}N_4O_{16}\cdot H_2O$	57.59 (57.72	7.91 8.00	6.06 5.85)
1f	QS-10	Val	St	67.7	—	+20.3° ($CHCl_3$)	$C_{58}H_{100}N_4O_{16}\cdot 1/2H_2O$	62.23 (62.28	8.93 9.10	5.18 5.01)
1g	QS-3	Val	Me	67.1	167	+42.9° (EtOH)	$C_{34}H_{50}N_4O_{16}\cdot H_2O$	51.57 (51.76	6.63 6.65	7.12 7.10)
1h	QS-6	Val	Me	38.8	174 (d)	+43.8° (EtOH)	$C_{37}H_{56}N_4O_{16}\cdot 1/2H_2O$	53.95 (54.06	6.98 6.99	6.76 6.82)
1i	QS-16	Val	Me	67.6	193—195	+33.1° (EtOH)	$C_{47}H_{76}N_4O_{16}\cdot H_2O$	58.33 (58.13	8.14 8.10	5.81 5.78)
1j	Q-22	Val	Me	48.2	204	+28.6° (EtOH)	$C_{53}H_{88}N_4O_{16}\cdot H_2O$	60.42 (60.32	8.65 8.60	5.35 5.31)
1k	Qmy c)	Val	Me	33.0	—	+26.9° (EtOH)	$C_{65}H_{112}N_4O_{17}$	63.64 (63.90	9.32 9.42	4.59 4.34)
1l	Qmy c)	Ala	H	33.4	—	+21.2° (EtOH)	$C_{62}H_{106}N_4O_{17}\cdot 3H_2O$	60.25 (60.36	9.11 9.15	4.82 4.54)
1m	QS-10	Ser	Me	72.6	149—150	+39.2° (MeOH) d)	$C_{39}H_{60}N_4O_{17}$	54.81 (54.66	7.01 7.06	6.80 6.54)
1n	QS-10	Thr	Me	78.0	147—148	+37.2° (MeOH) b)	$C_{40}H_{62}N_4O_{17}$	55.38 (55.16	7.40 7.18	6.79 6.43)
1o	St	Val	Me	96.3	178—181 (d)	+34.9° (EtOH)	$C_{40}H_{72}N_4O_{12}\cdot H_2O$	57.31 (57.39	8.81 9.15	6.89 6.69)

a) *c* 0.5. b) Temperature = 21 °C. c) Quinonylmycoloyl. d) Temperature = 22 °C.



Scheme 4.



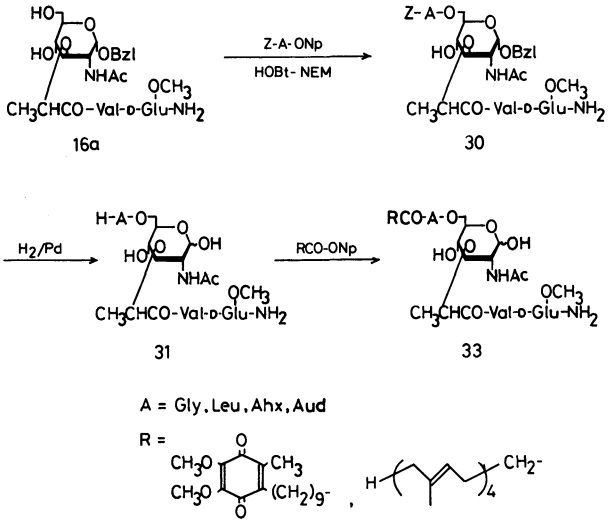
Scheme 5.

24. The protecting methoxymethyl group of **24** was then removed by heating at 100 °C for 10 min in a mixture of 2M H_2SO_4 and acetic acid. The resulting hydroquinone was reoxidized with $FeCl_3$ in dioxane to the desired quinonylmycolic acid **25**.

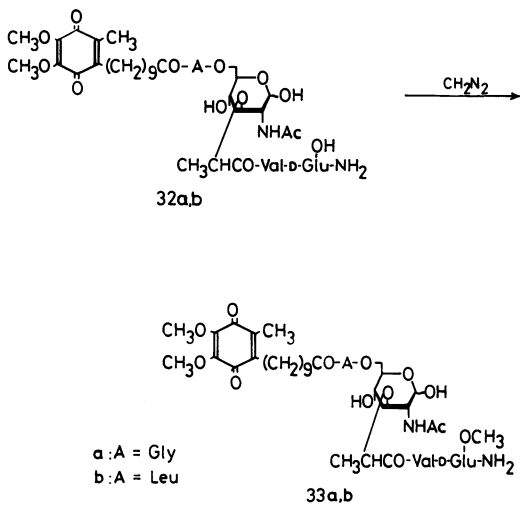
The synthesis of 6-*O*-quinonylmycoloylmuramyl dipeptides **1k** and **l** was carried out as shown in Scheme 5 as in the case for the synthesis of 6-*O*-mycoloylmuramyl dipeptides,[7] since the condensation of **25** with *N*-acetyl-1-*O*-benzyl-α-muramyl dipeptides by DCC in a mixture of pyridine and DMF did not proceed. Protected quinonylmycolic acid **24** was converted to the potassium salt **26**, which was then condensed with *N*-acetyl-1-*O*-benzyl-6-*O*-tosyl-α-muramic acid diphenylmethyl ester (**27**)[7] in the presence of 18-crown-6. The diphenylmethyl and methoxymethyl protecting groups of **28** were then removed by TFA. The resulting carboxylic acid was activated by the HONB-DCC method[21] and condensed *in situ* with the dipeptides to give the protected quinonylmycoloylmuramyl dipeptide **29**. The removal of the benzyl protecting group by catalytic hydrogenolysis and reoxidation of the hydroquinone ring by $FeCl_3$ produced target compound **1**.

The methyl ester with a linking amino acid between a lipophilic acid and the muramyl dipeptide moiety **33a—d** were prepared by acylating the corresponding 6-*O*-aminoacylmuramyl dipeptide methyl esters **31** with *p*-nitrophenyl ester of 10-(5,6-dimethoxy-3-methyl-1,4-benzoquinon-2-yl)decanoic acid (Scheme 6). *N*-Acetyl-6-*O*-(all-*trans*-5,9,13,17-tetramethyl-4,8,12,16-octadecatetraenoyl-L-leucyl)muramyl-L-valyl-D-isoglutamine methyl ester (**33e**) was also obtained in this procedure. The esters **33a** and **b** could also be obtained by treating the corresponding γ-carboxyl-free derivatives **32** with diazomethane in methanol as exemplified in Scheme 7. The γ-carboxyl-free derivatives **32** were obtained according to the procedure reported earlier.[11]

The purification of all these desired products **1** was carried out by column chromatography on silica

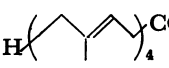


Scheme 6.



Scheme 7.

TABLE 5. YIELDS AND PHYSICOCHEMICAL PROPERTIES OF QUINONYL AND MULTIPRENYL MURAMYL DIPEPTIDES **33** AND THEIR INTERMEDIATES **30** AND **31**

Compd	R	A	Yield %	Mp θ_m/°C	$[\alpha]_D$ (c 0.5) (Temp., Solvent)	Formula	Analysis Found(Calcd) C	H	N
30a	—	Gly	59.1	189—190	+84.8° (21, DMF)	$C_{39}H_{53}N_5O_{14}$	57.35 (57.41	6.58 6.55	8.13 8.59)
30b	—	Leu	71.1	232—234	+72.2° (22, DMF)	$C_{43}H_{61}N_5O_{14}$	58.98 (59.23	7.19 7.05	8.10 8.03)
30c	—	Ahx	71.8	189—191	+78.6° (21, DMF)	$C_{43}H_{61}N_5O_{14}$	59.26 (59.23	7.10 7.05	8.25 8.03)
30d	—	Aud	68.2	177—179	+74.4° (22, DMF)	$C_{48}H_{71}N_5O_{14}$	61.22 (61.19	7.72 7.60	7.60 7.44)
31a	—	Gly	80.5	—	+36.8° (21, 95%EtOH)	$C_{24}H_{41}N_5O_{12}\cdot H_2O$	47.60 (47.28	7.13 7.11	11.55 11.49)
31b	—	Leu	82.6	105—108	+45.3° (23, DMF)	$C_{28}H_{49}N_5O_{12}\cdot CH_3COOH$	50.97 (50.91	7.73 7.55	10.00 9.90)
31c	—	Ahx	74.9	122—124	+39.6° (21, EtOH)	$C_{28}H_{49}N_5O_{12}\cdot 1/2H_2O$	51.46 (51.21	7.84 7.68	10.61 10.67)
31d	—	Aud	82.7	121—122	+41.5° (21, EtOH)	$C_{33}H_{59}N_5O_{12}\cdot H_2O$	53.78 (53.86	8.13 8.36	9.76 9.52)
32a	QS-10	Gly	77.1	175—177 (d)	+26.0° (22, 85%EtOH)	$C_{42}H_{65}N_5O_{17}\cdot 2H_2O$	53.36 (53.20	7.21 7.34	7.54 7.39)
32b	QS-10	Leu	46.7	103—106	+19.4° (22, EtOH)	$C_{46}H_{73}N_5O_{17}\cdot 3/2H_2O$	55.56 (55.52	7.43 7.70	6.93 7.04)
33a	QS-10	Gly	24.0	190—191	+23.0° (22, EtOH)	$C_{43}H_{67}N_5O_{17}\cdot H_2O$	54.69 (54.71	7.51 7.37	7.69 7.42)
33b	QS-10	Leu	40.7	193—195 (d)	+21.0° (23, EtOH)	$C_{47}H_{75}N_5O_{17}$	57.07 (57.48	7.78 7.70	7.15 7.13)
33c	QS-10	Ahx	48.7	176—177 (d)	+31.2° (21, EtOH)	$C_{47}H_{75}N_5O_{17}\cdot 1/2H_2O$	56.98 (56.95	7.72 7.73	6.91 7.07)
33d	QS-10	Aud	57.1	185—186 (d)	+32.6° (21, EtOH)	$C_{52}H_{85}N_5O_{17}$	59.06 (59.35	8.25 8.14	6.44 6.66)
33e		Leu	76.9	93— 97	+23.3° (23, EtOH)	$C_{50}H_{83}N_5O_{13}\cdot H_2O$	61.49 (61.26	8.70 8.74	7.16 7.15)

gel and Sephadex LH-20. The physicochemical properties of the intermediates and the desired MDP derivatives are shown in Tables 1—5.

Measurement of Adjuvant and Tumor Suppressive Activities. The adjuvant activity of the synthetic MDP derivatives **1** and **33** on the induction of delayed-type hypersensitivity to ABA-Tyr in guinea pigs was assayed by the following method.[12] Hartley guinea pigs each weighing 300—500 g were immunized in the footpads (all four) with a total of 50 μg of ABA-Tyr in Freund's incomplete adjuvant containing the synthetic MDP derivatives. Five animals were used per group. Control groups were immunized with ABA-Tyr alone in Freund's incomplete adjuvant (Control 1) or with Freund's incomplete adjuvant alone (Control 2). After 2 weeks, skin tests were performed with 50 or 100 μg of ABA-Tyr-bovine serum alubmin (ABA-BSA) prepared by the method of Tabachnick and Sobotka.[22] Skin reaction were measured 24 and 48 h after an intradermal injection of the test antigen. The results are shown as the average diameter (mm) ± the standard error (SE) of the skin reaction (induration) of five guinea pigs.

The evaluation of the suppressive activity on a transplantable tumor in syngeneic mice was carried out according to the procedure reported previously.[12] A mixture of tumor cells (Meth-A, 1×10^5 cells) and the synthetic MDP derivatives suspended in phosphate-buffered saline was inoculated intradermally into the flanks of BALB/c female mice. Tumor size at the site of the inoculation and survival rates were examined every week. The results were obtained 4 weeks after the fibrosarcoma inoculation. Animals with tumors less than 2 mm were considered to be tumor free; such mice were then rechallenged by Meth-A (1×10^5 cells) and observed for an additional 2 weeks to confirm the immunotherapeutic activity of the test materials.

Results and Discussion

Some lipid-soluble vitamins, such as retinol, α-tocopherol, and ubiquinones, are known to exert diverse immunological effects.[23] The metabolites of ubiquinones and their synthetic related compounds are also lipophilic and possess various biological characteristics of ubiquinones including the enhancement of humoral immune response.[24] It has been argued that, among a series of MDP derivatives, the lipophilicity or lipophilicity-hydrophilicity balance is a fundamental requirement for the expression of the antitumor activity.[8,25] It was reported that 6-*O*-mycoloyl-MDP exhibited a tumor-suppressive effect, but was unable to

enhance humoral antibody formation.[26] Therefore, the incorporation of lipophilic and immunologically active quinonyl acids, which enhance humoral immune response,[24] may produce some additional favorable effects in MDP. These were the considerations that led us to synthesize several quinonyl derivatives of MDP in the hope of creating an effective antitumor agent. We examined the adjuvant activity of the compounds on the induction of delayed-type hypersensitivity to ABA-Tyr in guinea pigs. The suppression test of a transplantable tumor in syngeneic mice was carried out on those compounds that exhibited potent adjuvant activity. In this study, the Ala residue in MDP was replaced mostly with the Val residue since *N*-acetylmuramyl-L-valyl-D-isoglutamine showed the most potent activity on inducing delayed-type hypersensitivity.[18] Some other lipophilic MDP analogues, such as **1o** and **33e**, were also synthesized and tested to evaluate the effect of the quinonyl group on the adjuvant and tumor-suppressive activities.

Compounds **1a—f** were synthesized and examined to assess the effect produced by varying the ester substituent at the γ-carboxyl group of the isoglutamine moiety. Ester functionality was varied in the sequence of methyl, ethyl, isopropyl, *t*-butyl, hexyl, and stearyl (octadecyl). As shown in Table 6 (Expt. 1), the adjuvant activity of all the ester derivatives were more potent than MDP; the isopropyl homologue **1c** showed maximum activity. Although an ester substituent bigger than the *t*-butyl group (**1d**) caused a slight decrease in activity, there was little variation in activity between each ester set. This indicates a reduced dependence of the adjuvant activity on steric factors and/or the lipophilicity of the ester substituents at the γ-carboxyl group of the isoglutamine moiety.

The extent of increase in the adjuvant activity was appreciably affected by the chain length in the quinonyl acid moiety (Table 6; Expt. 2). Among the normal alkyl groups investigated (**1a**, and **1g—j**), activity was optimal for the decanoyl derivative **1a** and decreased for the longer chain derivatives **1i** and **1j**. Decreasing activity was a function of chain length. Compounds with shorter chain length, **1g** and **1h**, also exhibited less potent adjuvant activity than **1a**. The lower activity of these compounds could be interpreted as a consequence of either an inadequate lipophilicity of the molecule or an inadequate spacial arrangement of the quinone ring and MDP moiety. In the quinonylmycoloyl series, the adjuvant activity of the methylated Val derivative **1k** is less potent than even MDP whereas the γ-carboxyl-free MDP derivative **1l** showed the most potent activity (Table 6; Expt. 2). This finding also indicates that excess lipophilicity of the molecule causes decreased adjuvant activity.

In the tumor-suppressive activity, as in the adjuvant activity, most of the ester homologues showed comparable potent activity whereas the chain length of the quinonyl acid moiety greatly affected the activity; thus some parallelism exists between the adjuvant and the tumor-suppressive activity (data not shown). Among the quinonyl muramyl dipeptide ester derivatives,

Table 6. Adjuvant activity of quinonyl MDP derivatives **1** on the induction of delayed-type hypersensitivity to ABA-Tyr in guinea pigs

Compd[a]	R	n	Skin reaction with ABA-Tyr	
			24 h	48 h
Expt. 1			(mm)	(mm)
1a	Me	9	23.8±1.0	19.9±0.9
1b	Et	9	24.1±0.5	19.0±0.6
1c	*i*-Pr	9	23.5±0.8	20.6±0.9
1d	*t*-Bu	9	21.9±0.8	19.1±1.4
1e	*n*-Hex	9	21.6±0.6	19.3±0.4
1f	St	9	20.1±0.8	19.8±1.1
MDP			20.7±0.5	18.1±0.4
Control 1 (ABA-Tyr+FIA[c])			3.0±1.0	0
Control 2 (FIA[c] alone)			0	0
Expt. 2				
1g	Me	2	18.6±0.5	16.4±1.4
1h	Me	5	18.9±0.7	15.1±0.7
1a	Me	9	18.3±1.0	17.8±0.6
1i	Me	15	17.4±0.6	17.1±0.5
1j	Me	21	17.9±0.8	13.8±1.0
1k	Me	Quinonylmycoloyl	16.6±1.0	15.1±0.7
1l[b]	H	Quinonylmycoloyl	17.1±0.5	19.1±0.8
MDP			16.4±0.3	16.6±0.9
Control 1 (ABA-Tyr+FIA[c])			5.8±0.7	0
Control 2 (FIA[c] alone)			0	0

a) Dose: 100 μg. b) In this compound the dipeptide moiety is Ala-D-isoGln. c) FIA: Freund's incomplete adjuvant.

methyl ester homologue **1a** showed the most potent activity when 5×10^5 Meth-A cells were used (Table 7). Therefore, compound **1a** was further modified and tested for adjuvant and tumor-suppressive activity. The compounds synthesized for this purpose were those in which the Val residue of **1a** was replaced with Ser (**1m**) and Thr (**1n**), and those having a linking amino acid between the quinonyl acid and muramyl-dipeptide moiety (**33**).

The effects of these synthetic MDP derivatives **1m**, **1n**, and **33** together with those of the most immunoadjuvant active analogues of MDP, *N*-acetylmuramyl-L-valyl-D-isoglutamine (**34**) and its methyl ester (**35**),[18] on the induction of delayed-type hypersensitivity to ABA-Tyr is shown in Table 8. The results for 6-*O*-stearoyl (**1o**) and 6-*O*-multiprenyl (**33e**) derivatives, and nonmethylated quinonyl MDP derivatives having Gly (**32a**) and Leu (**32b**) as the linking amino acids are also included for comparison. The effect on Meth-A suppression is shown in Table 9.

The 10-(5,6-dimethoxy-3-methyl-1,4-benzoquinon-2-yl)decanoyl group incorporated directly into the 6-*O*-position of the muramyl moiety (**1a**) maintained the adjuvant activity of the parent molecules **34** and **35**. Differences in activity among **1a**, **1m**, and **1n**, in which the Ala of MDP was replaced with Val, Ser, and Thr, reflect differences in activity observed among the corresponding parent MDP analogues, *i.e.*, *N*-acetylmuramyl-L-valyl, L-seryl, and L-threonyl-D-isoglutamine.[18] These derivatives also showed potent tumor-suppressive activity even though they were administered as a suspension in phosphate-buffered saline whereas mycoloyl-MDP was reported to be active when administered as an oil-in-water emulsion.[7] The activity developed by **1m** and **1n** was somewhat lower than that of **1a**. The potency of all three derivatives was in good accordance with that of their adjuvant activity when 5×10^5 Meth-A cells were used (Table 9, Expt. 1). A dose response was obtained for **1a** (Table 9, Expt. 2). The induction of systemic tumor immunity that suppressed tumor growth in mice was demonstrated by the reinoculation of 1×10^5 Meth-A cells. The adjuvant activity of stearoyl-MDP derivative **1o** diminished slightly and its apparent low tumor-suppressive activity indicated that not only lipophilicity but some other biological

TABLE 7. TUMOR-SUPPRESSIVE ACTIVITY OF QUINONYL MDP DERIVATIVES

Compd[b]	Primary suppression[a]	
	Meth-A (1×10^5)	Meth-A (5×10^5)
1a	10/10	6/10
1b	10/10	1/10
1c	10/10	2/10

a) Figures represent the ratio of tumor-free mice to surviving mice. b) Dose: 100 μg.

TABLE 8. ADJUVANT ACTIVITY OF MDP DERIVATIVES ON THE INDUCTION OF DELAYED-TYPE HYPERSENSITIVITY TO ABA-Tyr IN GUINEA PIGS

Compd	Skin reaction with ABA-Tyr (mm±SE)	
Expt. 1	24 h	48 h
1a	24.4±1.2	19.2±0.9
1m	23.8±0.6	19.1±0.4
1n	21.1±0.5	17.3±0.9
1o	22.5±0.6	18.4±0.4
34	23.6±0.8	19.4±0.7
35	24.1±0.8	19.7±0.4
Control 1	3.3±1.4	0
Control 2	0	0
Expt. 2		
33a	21.7±0.5	20.6±0.6
33b	21.3±0.7	19.7±0.9
33c	23.9±0.7	18.3±1.2
33d	22.2±0.5	15.5±1.2
33e	22.9±0.8	20.2±1.0
34	22.0±0.4	19.9±0.8
Control 1	3.0±1.2	0
Control 2	0	0

TABLE 9. TUMOR-SUPPRESSIVE ACTIVITY OF MDP DERIVATIVES

Compd.	Dose (μg)	Primary suppression[a]	Rechallenge[a,b]
Expt. 1			
1a	100	10/10 (6/10)[c]	5/10
1m	100	3/10 (4/10)[c]	2/3
1n	100	6/10 (0/10)[c]	6/6
34	100	0/10	—
35	100	0/9	—
Control	—	0/10 (0/10)[c]	—
Expt. 2			
1a	300	10/10	8/9
1a	100	4/10	3/3
Control	—	0/10	—
Expt. 3			
1a	100	6/10	ND
	20	2/10	ND
33a	100	8/10	ND
	20	0/10	—
33b	100	5/10	ND
	20	2/10	ND
Control	—	0/9	—
Expt. 4			
1a	100	8/10	ND
1o	100	2/9	ND
33a	100	1/10	ND
33b	100	4/10	ND
33c	100	2/10	ND
33d	100	2/10	ND
33e	100	2/8	ND
Control	—	0/10	—

a) Figures represent the ratio of tumor-free mice to surviving mice. b) ND=not determined. c) Meth-A: 5×10^5 cells.

activities rendered by quinonyl moiety, such as interaction with the cell surface membranes, are necessary for the development of tumor-suppressive activity.

Most of the quinonyl muramyl dipeptides with a linking amino acid between the quinonyl acid and the muramyl moiety (**33**) also exhibited adjuvant activity comparable to that of **34**. The lower activity of **33d**, with 11-aminoundecanoic acid as a linking unit, is compatible with the result obtained for nonmethylated quinonyl muramyl dipeptides with a long chain linking unit.[9] The tumor-suppressive activity of these quinonyl muramyl dipeptides **33** was also demonstrated (Table 9, Expt. 3). Compounds **33c** and **33d** are equipotent in tumor-suppressive activity in spite of the latter's diminished adjuvant activity. Among these quinonyl muramyl dipeptides with a linking amino acid, **33b** is almost as active as **1a**. The result for **33a** was variable. The retention of the adjuvant activity by the multiprenyl derivative **33e** showed that the quinonyl moiety is not a requisite for maintaining the induction activity for delayed-type hypersensitivity. The low activity in tumor suppression of **33e**, however, indicated the importance of the quinonyl moiety for the development of tumor suppression as observed in the case of stearoyl-MDP **1o**. Parent muramyl dipeptide analogues **34** and **35** did not cause any tumor-suppressive activity either.

From these results, we conclude that quinonyl muramyl dipeptide **1a** has the most potent adjuvant and tumor-suppressive activities. It is of particular interest that **1a**, when injected into the established tumor as an oil-in-water emulsion on day 2, 5, 8, and 15 after intradermal inoculation, caused complete regression of the line-10 hepatocellular carcinoma in strain 2 guinea pigs.[27] Metastasis was not observed. Although more extensive search to find an effective immunotherapeutic agent for cancer by modifying natural MDP is required, the potent adjuvant, tumor-suppressive, and tumor-regressive effects of **1a** make it a good candidate for further studies.

Experimental

Melting points were taken in open capillaries and are uncorrected. Optical rotations were determined with a Perkin Elmer model 141 polarimeter. ^{1}H NMR spectra were obtained on a Varian EM-390 spectrometer with tetramethylsilane as an internal standard. 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 pre-coated plates, which were generally developed with $CHCl_3$–acetone–EtOH (70:30:3, v/v) for compound **11**, $CHCl_3$–acetone–MeOH (10:3:2, v/v) for **1**, **15**, **16**, **17**, **30**, and **33**, AcOEt–pyridine–H_2O–AcOH (30:10:5:3, v/v) for **1**, **31**, and **33**, and *n*-BuOH–AcOH–AcOEt–H_2O (1:1:1:1, v/v) for **1** and **33**. Solution were concentrated in a rotary vacuum evaporator under reduced pressure at temperatures below 45 °C.

*10-(2-Acetoxy-3,4-dimethoxy-6-methylphenyl)decanoyl Chloride (**4a**).* A solution of 10-(3,4-dimethoxy-2-hydroxy-6-methylphenyl)decanoic acid (**3a**)[14] (12.6 g, 37.1 mmol) in Ac_2O (30 ml) was combined with concd H_2SO_4 (1 ml), and the mixture was stirred for 1.5 h at room temperature. Water (10 ml) was then added carefully and the stirring was continued for 2 h. After the removal of the solvent, the residue was dissolved in AcOEt (100 ml), and the solution was washed with 5% $NaHCO_3$. The aqueous layer was back-washed with AcOEt (100 ml). The extracts were combined, washed succesively with 1M HCl and water, and dried ($NaSO_4$). The solvent was evaporated to give an oily acetyl compound (13.5 g, 95.6%) which was dissolved in benzene (20 ml). To this was added oxalyl dichloride (9.25 ml, 0.1 mol) dropwise at room temperature, then the mixture was stirred at 50 °C for 1 h. After the removal of the solvent, the residue was flushed with benzene, and dried (NaOH pellets): 13.0 g (91.8%) ^{1}H NMR ($CDCl_3$): δ=1.28—1.90 (14H, m), 2.25—2.73 (8H, m), 2.91 (2H, t), 3.85 (3H, s), 3.90 (3H, s), and 6.72 (1H, s).

*22-(3,4-Dimethoxy-2-hydroxy-6-methylphenyl)-13-oxodocosanoic Acid (**8b**).* A solution of **4a** (7.98 g, 20 mmol) in $CHCl_3$ (4 ml) was added dropwise to a mixture of 1-morpholinocyclododecene (**5b**)[28] (6.29 g, 24 mmol) and Et_3N (3.08 ml, 22 mmol) at 0 °C over a period of 30 min, and the reaction mixture was stirred at room temperature for 15 h. To this were added $CHCl_3$ (5 ml) and 6M HCl (10 ml); the mixture was heated under reflux for 5 h, cooled to room temperature, diluted with water (20 ml), and extracted with $CHCl_3$ (2×30 ml). The combined extracts were washed with water, and dried (Na_2SO_4). After the removal of the solvent, the residue was purified by column chromatography (6×11 cm) on silica gel using $CHCl_3$ as an eluant to give 2-[10-(2-acetoxy-3,4-dimethoxy-6-methylphenyl)decanoyl]cyclododecanone (**7b**): 8.63 g (79.2%). Then, a solution of compound **7b** in EtOH (8 ml) was added to hot 4M NaOH (7.8 ml) and the mixture was heated for 10 min under reflux, cooled to room temperature, acidified to pH 2 with 6M HCl, and extracted with AcOEt (30 ml). The extract was washed with water, dried (Na_2SO_4), and concentrated to give the residue, which was recrystallized from ether–petroleum ether: 6.12 g (74.4%). mp 63—64 °C. ^{1}H NMR ($CDCl_3$): δ=1.17—1.90 (32H, m), 2.22—2.78 (11H, m), 3.88 (3H, s), 3.92 (3H, s), and 6.40 (1H, s).

Found: C, 71.57; H, 10.17%. Calcd for $C_{31}H_{52}O_6$: C, 71.50; H, 10.07%.

*16-(3,4-Dimethoxy-2-hydroxy-6-methylphenyl)-7-oxohexadecanoic Acid (**8a**).* This compound was synthesized from **4a** and **5a** in a manner similar to that of **8b**: 35.9%. mp 55—57 °C.

Found: C, 68.64; H, 9.40%. Calcd for $C_{25}H_{40}O_6$: C, 68.77; H, 9.23%.

*34-(3,4-Dimethoxy-2-hydroxy-6-methylphenyl)-13-oxotetracontanoic Acid (**8c**).* This compound was synthesized from **4b**, which was derived from **3b** and **5b** in a manner similar to that of **8b**: 32.7%. mp 92—93 °C.

Found: C, 74.88; H, 11.29%. Calcd for $C_{43}H_{76}O_6$: C, 74.95; H, 11.12%.

*22-(3,4-Dimethoxy-2-hydroxy-6-methylphenyl)docosanoic Acid(**9b**).* Compound **8b** (5.21 g, 10 mmol), *p*-toluenesulfonohydrazide (2.33 g, 12.5 mmol) and cyclohexane (25 ml) were added to a solution of DMF–sulfolane (25 ml—25 ml) containing *p*-toluenesulfonic acid (250 mg) at 100 °C. After 5 min, Na[BH_3(CN)] (2.51 g, 41 mmol) was added. The mixture was stirred for 20 min at the same temperature and the solution was diluted with brine, and extracted with ether (2×100 ml). The extracts were combined, washed with water, 0.5M HCl and water successively, and dried (Na_2SO_4). After the removal of the solvent, the residue was recrystallized from a small amount of ether: 3.13 g (58.6%). mp 67—68 °C (lit,[14] mp 67—68 °C).

Found: C, 73.76; H, 10.78%. Calcd for $C_{31}H_{54}O_5$: C, 73.47; H, 10.74%.

*16-(3,4-Dimethoxy-2-hydroxy-6-methylphenyl)hexadecanoic Acid (**9a**).* This compound was synthesized from **8a** in a manner similar to that of **9b**: 57.4%. mp 45 °C.

Found: C, 71.13; H, 10.13%. Calcd for $C_{25}H_{42}O_5$: C, 71.05;

H, 10.02%.

*34-(3,4-Dimethoxy-2-hydroxy-6-methylphenyl)tetracontanoic Acid (**9c**).* This compound was synthesized from **8c** in a manner similar to that of **9b**: 70.6%. mp 93 °C.

Found: C, 76.73; H, 11.91%. Calcd for $C_{42}H_{78}O_5$: C, 76.50; H, 11.65%.

*22-(5,6-Dimethoxy-3-methyl-1,4-benzoquinon-2-yl)docosanoic Acid (**2e**).* A mixture of **9b** (2.0 g, 3.95 mmol) and (N,N'-disalicylideneethylenediaminato)cobalt(II) (salcomin 325 mg, 1 mmol) in DMF (10 ml) was stirred under oxygen at room temperature for 24 h. The solvent was evaporated and the residue was dissolved in AcOEt (50 ml). The insolubles were filtered and the filtrate was washed succesively with 0.5 M HCl and water, and dried (Na_2SO_4). After the removal of the solvent, the residue was purified on a column (4.5×11 cm) of silica gel using $CHCl_3$ as an eluent and then recrystallized from EtOH: 1.04 g (50.6%). mp 83—84 °C (lit,[14] mp 86—87 °C).

Found: C, 71.44; H, 10.18%. Calcd for $C_{31}H_{52}O_6$: C, 71.50; H, 10.07%.

*16-(5,6-Dimethoxy-3-methyl-1,4-benzoquinon-2-yl)hexadecanoic Acid (**2d**).* This compound was synthesized from **9a** in a manner similar to that of **2e**: 58.9%. mp 70—71 °C.

Found: C, 68.89; H, 9.30%. Calcd for $C_{25}H_{40}O_6$: C, 68.77; H, 9.23%.

*34-(5,6-Dimethoxy-3-methyl-1,4-benzoquinon-2-yl)tetracontanoic Acid (**2f**).* This compound was synthesized from **9c** in a manner similar to that of **2e**: 21.3%. mp 89—90 °C.

Found: C, 74.73; H, 11.09%. Calcd for $C_{43}H_{76}O_6$: C, 74.95; H, 11.12%.

N-(t-Butoxycarbonyl)-D-glutamic Acid γ-Isopropyl Ester DCHA Salt. Thionyl chloride (2 ml, 27 mmol) was added dropwise to isopropyl alcohol (40 ml) at −10 °C. To this was added D-glutamic acid (3.0 g, 20 mmol), and the mixture was stirred at room temperature for 3 days. After the insolubles were removed by filtration, ether (200 ml) was added to the filtrate to give D-glutamic acid γ-isopropyl ester HCl salt (1.6 g, 35.4%). Without further purification, the salt (1.5 g, 6.64 mmol) was dissolved in water (5 ml) and the resulting solution was combined with DMF solution (5 ml) of *O-t*-butyl *S*-(4,6-dimethyl-2-pyrimidinyl) thiocarbonate (1.91g, 7.97 mmol). Et_3N (1.84 ml) was then added at 4 °C, and the mixture was stirred at room temperature for 20 h, diluted with water (20 ml), and extracted with ether (2×30 ml). The pH of the aqueous layer was adjusted to 2 with 2M HCl under cooling and the mixture was extracted with AcOEt (50 ml). The AcOEt layer was washed with 1M HCl and water succesively, and dried (Na_2SO_4). After the removal of the solvent, the residue (1.90 g) was converted to DCHA salt in ether by adding DCHA (1.3 ml): 2.50 g (80.0%). mp 131 °C. $[\alpha]_D^{23}$ −7.1° (*c* 0.5, DMF).

Found: C, 63.57; H, 10.01; N, 6.08%. Calcd for $C_{25}H_{46}N_2O_6$: C, 63.80; H, 9.85; N, 5.95%.

*N-(t-Butoxycarbonyl)-D-isoglutamine Isopropyl Ester (**10c**).* *N*-(*t*-Butoxycarbonyl)-D-glutamic acid γ-isopropyl ester DCHA salt (704 mg, 1.5 mmol) was converted to free carboxylic acid by the usual method. The resulting oil and *N*-hydroxysuccinimide (207 mg, 1.8 mmol) were dissolved in CH_3CN (10 ml). To this was added DCC (371 mg, 1.8 mmol) at 4 °C, and the mixture was stirred at room temperature for 2 h. After the removal of the precipitates by filtration, 25% aqueous ammonia (0.6 ml) was added under cooling and the mixture was stirred at 4 °C for 30 min. The solvent was evaporated and the resulting crystals were washed with hot water: 375 mg (86.8%). mp 148—150 °C. $[\alpha]_D^{23}$ −3.2° (*c* 0.5, DMF).

Found: C, 54.60; H, 8.75; N, 9.63%. Calcd for $C_{13}H_{24}N_2O_5$: C, 54.15; H, 8.39; N, 9.72%.

*N-(Benzyloxycarbonyl)-D-isoglutamine t-Butyl Ester (**10d**).* This compound was synthesized in a similar manner to that described for 10c: 67.8%. mp 113 °C. $[\alpha]_D^{22}$ −2.5° (*c* 0.5, DMF).

Found: C, 61.13; H, 7.23; N, 8.36%. Calcd for $C_{17}H_{24}N_2O_5$: C, 60.70; H, 7.19; N, 8.33%.

*N-(t-Butoxycarbonyl)-L-valyl-D-isoglutamine (**12**).* *t*-Butoxycarbonyl-L-valyl-D-isoglutamine benzyl ester (**11**)[18] (7.50 g, 17.2 mmol) was hydrogenated in MeOH (150 ml) using palladium black as a catalyst at room temperature for 6 h. After the removal of the catalyst by filtration, the filtrate was concentrated to give the residue which was reprecipitated from AcOEt–petroleum ether: 6.00 g (quantitative). mp 89—90 °C. $[\alpha]_D^{23}$ +5.8° (*c* 0.5, DMF).

Found: C, 51.96; H, 8.10; N, 12.11%. Calcd for $C_{15}H_{27}N_3O_6$: C, 52.16; H, 7.88; N, 12.17%.

*N-(t-Butoxycarbonyl)-L-valyl-D-isoglutamine Isopropyl Ester (**13c**).* Compound **10c** (173 mg, 0.6 mmol) was treated with TFA (2 ml) at room temperature for 20 min. After the removal of TFA, the residual oil was washed with a mixture of ether and petroleum ether (1 : 1, v/v) by decantation. The resulting oil was then dissolved in CH_3CN (5 ml) together with *N*-(*t*-butoxycarbonyl)-L-valine HONB ester (277 mg, 0.6 mmol), Et_3N (0.1 ml) was added, and the mixture was stirred at room temperature for 15 h. After the usual work-up, the product was crystallized from petroleum ether: 185 mg (79.6%). The physicochemical data are given in Table 1.

*N-(Benzyloxycarbonyl)-L-valyl-D-isoglutamine t-Butyl Ester (**13d**).* Compound **10d** (202 mg, 0.6 mmol) was hydrogenated in MeOH (10 ml) with palladium black as a catalyst at room temperature for 3 h. After the removal of the catalyst by filtration, the fltrate was concentrated to dryness. The residue was dissolved in CH_3CN (10 ml) together with *N*-(benzyloxycarbonyl)-L-valine HONB ester (248 mg, 0.6 mmol), and the mixture was stirred at room temperature for 15 h. To this was added 5% citric acid solution, and the resulting crystals were collected by filtration and recrystallized from MeOH–AcOEt–petroleum ether: 178 mg (68.1%). The physicochemical data are given in Table 1.

*N-(t-Butoxycarbonyl)-L-valyl-D-isoglutamine Octadecyl Ester (**13f**).* Compound **12** (1.73 g, 5 mmol) was combined with Et_3N (0.84 ml, 6 mmol) in DMF (5 ml) at 4 °C, and to this was added 6-chloro-1-(*p*-chlorophenylsulfonyloxy) benzotriazole[19] (2.06 g, 6 mmol) with stirring. Soon after, the mixture became clear and stirring was continued at room temperature for 4 h. A solution of 1-octadecanol (4.05 g, 15 mmol) and Et_3N (0.7 ml) in CH_2Cl_2 (30 ml) was then added at 4 °C and the mixture was stirred at room temperature for additional 24 h. After the usual work-up, the residue was recrystallized from AcOEt–petroleum ether: 1.10 g (37.0%).

Compounds **13b** and **e** were prepared similarly from appropriate alcohols and **12**. The yields and physicochemical data are given in Table 1.

*N-(t-Butoxycarbonyl)-O-benzyl-L-seryl-D-isoglutamine Methyl Ester (**13g**).* *N*-(*t*-Butoxycarbonyl)-D-isoglutamine methyl ester[18] (0.65 g, 2.5 mmol) was treated with TFA (5 ml) at room temperature for 20 min. After the removal of TFA, the residue was triturated with ether. The resulting oily D-isoglutamine methyl ester trifluoroacetate was dried over NaOH pellets, dissolved in AcOEt (5 ml), and neutralized with Et_3N (0.35 ml) under cooling. A solution of *N*-(*t*-butoxycarbonyl)-*O*-benzyl-L-serine HONB ester (1.14 g, 2.5 mmol) in AcOEt (5 ml) was then added, and the mixture was stirred at room temperature for 16 h. After the usual work-up, the residue was recrystallized from AcOEt–ether: 776 mg (71.2%).

Compound **13h** was prepared in a similar manner. The yield and physicochemical data are given in Table 1.

*N-Acetyl-1-O-benzyl-4,6-O-benzylidene-α-muramyl-L-valy-D-isoglutamine t-Butyl Ester (**15d**).* Compound **13d** (160

mg, 0.38 mmol) was hydrogenated in MeOH (20 ml) with palladium black as a catalyst at room temperature for 2 h. After the removal of the catalyst by filtration, the filtrate was concentrated to give the residue which was dissolved in CH_3CN (10 ml) together with **14** (240 mg, 0.38 mmol). The mixture was left at room temperature for 15 h, then the resulting crystals were collected and recrystallized from CH_3CN–ether: 216 mg (75.3%). The physicochemical data are given in Table 2.

N-Acetyl-1-O-benzyl-4,6-O-benzylidene-α-muramyl-O-benzyl-L-seryl-D-isoglutamine Methyl Ester (15g). Dipeptide **11h** (0.44 g, 1 mmol) was treated with TFA (6 ml) at room temperature for 20 min. After the removal of TFA, the residue was triturated with a mixture of ether and petroleum ether (1:1, v/v). The resulting powder was filtered, dried over NaOH pellets, and dissolved in THF (4 ml). After neutralization with Et_3N (0.14 ml) under cooling, this was combined with a solution of *N*-acetyl-1-*O*-benzyl-4,6-*O*-benzylidene-α-muramic acid HONB ester (**14**) (0.63 g, 1 mmol) in THF (2 ml). The mixture was then stirred at room temperature for 16 h, and the gel was filtered and washed thoroughly with THF. The solid obtained was reprecipitated from EtOH–DMF: 0.55 g 80.0%.

Compounds **15a—c**, **e**, **f**, and **h** were prepared in a similar manner. The yields and physicochemical data of **15** are given in Table 2.

N-Acetyl-1-O-benzyl-α-muramyl-L-valyl-D-isoglutamine Methyl Ester (16a). *N*-Acetyl-1-*O*-benzyl-4,6-*O*-benzylidene-α-muramyl-L-valyl-D-isoglutamine methyl ester[18] (10.1 g, 14.4 mmol) in 75% acetic acid (200 ml) was heated in a boiling water bath for 20 min. After the removal of the solvent, the residue was flushed with water and then toluene, and reprecipitated from EtOH–ether: 0.84 g (73.0%).

Compound **16b—h** were prepared in a similar manner. The yields and physicochemical data of **16** are given in Table 2.

N-Acetyl-1-O-benzyl-6-O-[10-(5,6-dimethoxy-3-methyl-1,4-benzoquinon-2-yl)decanoyl]-α-muramyl-L-valyl-D-isoglutamine Methyl Ester (17a). *Method A.* Compound **16a** (1.25 g, 2 mmol) in DMF (10 ml) and 10-(5,6-dimethoxy-3-methyl-1,4-benzoquinon-2-yl)decanoic acid (0.85 g, 2.4 mmol) in dry pyridine (4 ml) was combined. To this was added DCC (0.83 g, 4 mmol) under cooling and the mixture was stirred at 4 °C for 6 d. After the usual work-up, the crude material was recrystallized from a mixture of MeOH and ether: 1.47 g (75.8%). *Method B.* Compound **16a** (0.25 g, 0.4 mmol) and 10-(5,6-dimethoxy-3-methyl-1,4-benzoquinon-2-yl)decanoic acid *p*-nitrophenyl ester (0.38 g, 0.8 mmol), HOBt (0.22 g, 1.6 mmol), and NEM (0.21 g, 1.6 mmol) were dissolved in DMF (2 ml) and the mixture was stirred at room temperature for 2 d. After the removal of the solvent, the residue was applied onto a silica-gel column which was eluted with $CHCl_3$–MeOH–AcOH (18:2:1, v/v) as an eluting solvent. The resulting **17a** was further purified by column chromatography of Sephadex LH-20 (1.5×90 cm) with EtOH as an eluent: 0.19 g (49.1%).

Compounds **17b—g**, **i**, and **j** were synthesized in a manner similar to that described in method B. Compounds **17k—m** were synthesized in a manner similar to that described in method A and purified as described in method B. The yields and physicochemical data of **17** are given in Table 3.

N-Acetyl-6-[10-(5,6-dimethoxy-3-methyl-1,4-benzoquinon-2-yl)decanoyl]muramyl-L-valyl-D-isoglutamine Methyl Ester (1a). Compound **17a** (192 mg, 0.2 mmol) was hydrogenated in MeOH (4 ml) with palladium black as a catalyst at room temperature for 4 h and the catalyst was removed. The methanol solution of the resulting hydroquinone was combined with an aqueous solution of $FeCl_3 \cdot 6H_2O$ (1 g), and the mixture was stirred at room temperature for 30 min. To this was added AcOEt (50 ml), and the AcOEt layer was washed with water (15 ml) and dried (Na_2SO_4). After the removal of the solvent, the crystalline residue was purified by column chromatography of Sephadex LH-20 (1.8×44 cm) with a mixture of EtOH and 0.1 M AcOH (3:2, v/v) as an eluent. Recrystallization from EtOH–ether–petroleum ether gave **1a**: 127 mg (73.0%).

Compounds **1b—g** and **i—m** were synthesized in a similar manner. The yields and physicochemical data of **1** are given in Table 4.

N-Acetyl-6-O-[6-(5,6-dimethoxy-3-methyl-1,4-benzoquinon-2-yl)hexanoyl]muramyl-L-valyl-D-isoglutamine Methyl Ester (1h). 6-(5,6-Dimethoxy-3-methyl-1,4-benzoquinon-2-yl)hexanoic acid (**2b**) (35.6 mg, 0.12 mmol) and *N*-acetylmuramyl-L-valyl-D-isoglutamine methyl ester[18] (53.3 mg, 0.1 mmol) were dissolved in a mixture of DMF and pyridine (0.4 ml each). To this was added DCC (41.2 mg, 0.2 mmol), and the solution was stirred at room temperature for 30 h. The precipitate formed was filtered and the filtrate was concentrated to give the residue which was purified by column chromatography on silica gel (15 g) using $CHCl_3$–MeOH–acetone (50:9:6, v/v) as an eluant. Recrystallization from EtOH–ether gave **1h**: 31.5 mg (38.8%). The physicochemical data are given in Tabel 4.

Ethyl 3-Oxotetracosanoate (21).[20] A solution of diethyl malonate (21.7 g, 0.12 mol) in EtOH (13 ml) was slowly added dropwise to a suspension of magnesium (4.1 g, 0.17 mol) in a mixture of EtOH (4 ml) and CCl_4 (0.2 ml), maintaining a vigorous reaction. Ether (50 ml) was then added, and the mixture was refluxed for 3 h. After the removal of the solvent, the residue was flushed with benzene to remove EtOH and the residue was dissolved in ether (60 ml). To this was added over a period of 30 min an ethereal solution (50 ml) of docosanoyl chloride (0.15 mol), and the mixture was stirred at 50 °C for 2 h and at room temperature for 15 h. After 1M H_2SO_4 (300 ml) was added carefully, the mixture was extracted with ether (300 ml). The organic layer was washed with water, dried (Na_2SO_4), and concentrated to give 2-ethoxycarbonyl-3-oxotetracosanoic acid ethyl ester (75.4 g, 93.5%). This was dissolved in DMSO (50 ml) containing *p*-toluenesulfonic acid (2.1 g) and the mixture was heated at 200—210 °C in an oil bath for 20 min. After the evolution of CO_2 gas had ceased, the mixture was allowed to cool to room temperature, diluted with water (500 ml), and extracted with ether (500 ml). The extract was washed with water, dried (Na_2SO_4), and concentrated to give the residue, which was purified by column chromatography on silica gel (700 g) using hexane–diisopropyl ether (9:1, v/v) as an eluting solvent: 27.9 g (45.2%).

Found: C, 76.31; H, 12.23%. Calcd for $C_{26}H_{50}O_3$: C, 76.04; H, 12.27%.

10-(5,6-Dimethoxy-3-methyl-1,4-benzoquinon-2-yl)decyl Acetate (18). A solution of 10-(5,6-dimethoxy-3-methyl-1,4-benzoquinon-2-yl)-1-decanol[14] (50.4 g, 0.15 mol) and pyridine (48.6 ml, 0.6 mol) in CH_2Cl_2 (250 ml) was combined with acetic anhydride (42.6 ml, 0.45 mol) at 4 °C, and the mixture was stirred at room temperature for 15 h. Water (50 ml) was then added and the mixture was stirred at room temperature for 30 min. After the removal of the solvent, AcOEt (300 ml), $NaHCO_3$ (60 g) and water (200 ml) were added to the residue, and the mixture was stirred at room temperature for additional 1 h. The organic layer was washed succesively with 1M HCl and water, and dried (Na_2SO_4). After the removal of the solvent, the residue was triturated with petroleum ether to give **18** as a crystal: 51.5 g (90.7%). mp 39—40 °C.

Found: C, 66.35; H, 8.53%. Calcd for $C_{21}H_{32}O_6$: C, 66.30; H, 8.48%.

10-(3,4-Dimethoxy-2,5-dimethoxymethyloxy-6-methylphenyl)-1-decanol (19). Compound **18** (30.3 g, 80 mmol) was

hydrogenated in DMF (300 ml) with 5% palladium on carbon as a catalyst at room temperature for 2 h and the catalyst was removed by filtration. To the filtrate was added NaH (7.04 g, 0.18 mol) under nitrogen. After being stirred for 30 min, the mixture was combined with methoxymethyl chloride (13.4 ml, 0.18 mol) at 4 °C, and the stirring was continued at room temperature for additional 15 h. The reaction mixture was then diluted with water (2 l) and extracted with ether (500 ml). The extract was concentrated to give an oil (40 g) which was dissolved in EtOH (200 ml) and treated with 4M NaOH (60 ml). The mixture was stirred at room temperature for 15 h, diluted with water (1 l), and extracted with ether (300 ml). The extract was washed with water, dried (Na_2SO_4), and concentrated to give a residue which was purified by column chromatography on silica gel (300 g) with $CHCl_3$–MeOH (50:1, v/v) as an eluting solvent: 27.4 g (80.3%).

Found: C, 63.24; H, 9.35%. Calcd for $C_{23}H_{40}O_7 \cdot 1/2H_2O$: C, 63.13; H, 9.45%.

*10-(3,4-Dimethoxy-2,5-dimethoxymethyloxy-6-methylphenyl)-decanal (**20**).* A solution of pyridine-sulfur trioxide complex (30.7 g, 0.19 mol) in DMSO (120 ml) was added dropwise to a solution of **19** (27.4 g, 64.2 mmol) and Et_3N (44.9 ml, 0.32 mol) in DMSO (60 ml) over a period of 1 h, and the mixture was stirred at room temperature for 1 h. The mixture was poured into ice-cold water (500 ml) and extracted with ether (500 ml). The extract was washed with water, dried (Na_2SO_4), and concentrated to give a residue which was purified by column chromatography on silica gel (300 g) with $CHCl_3$: 22.1 g (81.1%).

Found: C, 64.29; H, 9.07%. Calcd for $C_{23}H_{38}O_7$: C, 64.76; H, 8.98%.

*2-[10-[3,4-Dimethoxy-2,5-bis(dimethoxymethoxy)-6-methylphenyl]decylidene]-3-oxotetracosanoic Acid Ethyl Ester (**22**).* A solution of **21** (14.4 g, 35 mmol) and **20** (14.9 g, 35 mmol) in anhydrous benzene (40 ml) was combined with AcOH (0.35 ml) and piperidine (0.18 ml), and the mixture was heated under reflux to remove the water formed azeotropically. After the removal of the solvent, the residue was purified by column chromatography on silica gel (450 g) with $CHCl_3$–MeOH (9:1, v/v): 18.8 g (65.6%). ^1H NMR ($CDCl_3$): δ=0.88 (3H, t), 1.10—1.72 (57H, b), 2.20 (3H, s), 2.34—2.81 (4H, m), 3.59 (6H, s), 4.20 (2H, q), 5.03 (4H, s), 5.33—5.71 and 6.60—7.00 (1H, m).

Found: C, 71.18; H, 10.53%. Calcd for $C_{49}H_{89}O_9 \cdot 1/2H_2O$: C, 71.06; H, 10.46%.

*2-[10-[3,4-Dimethoxy-2,5-bis(dimethoxymethoxy)-6-methylphenyl]decyl]-3-hydroxytetracosanoic Acid Ethyl Ester (**23**).* Compound **22** (18.4 g, 22.5 mmol) was hydrogenated in a mixture of dioxane (200 ml) and EtOH (150 ml) with palladium black as a catalyst at room temperature for 8 h. The catalyst was removed by filtration and the filtrate was concentrated. The resulting residue was dissolved in a mixture of dioxane (120 ml) and MeOH (40 ml), and to this was added $NaBH_4$ (851 mg, 22.5 mmol) with stirring. After 30 min, the pH of the mixture was adjusted to 3 with 5% citric acid. The mixture was then diluted with water (200 ml) and extracted with AcOEt (200 ml). The extract was washed with water, dried (Na_2SO_4), and concentrated to give a crystalline residue which was recrystallized from MeOH: 16.9 g (91.2%). mp 42—44 °C.

Found: C, 71.75; H, 11.30%. Calcd for $C_{49}H_{90}O_9$: C, 71.48; H, 11.02%.

*2-[10-[3,4-Dimethoxy-2,5-bis(dimethoxymethoxy)-6-methylphenyl]decyl]-3-hydroxytetracosanoic Acid (**24**).* A solution of **23** (16.5 g, 20 mmol) in a mixture of EtOH (70 ml) and dioxane (50 ml) was combined with 3M NaOH (30 ml), and the mixture was heated under reflux for 2 h. After the solution was cooled to room temperature, the pH was adjusted to 3 with 10% citric acid. The mixture was diluted with water (300 ml) and extracted with AcOEt (150 ml). The extract was washed with water, dried (Na_2SO_4), and concentrated to give a residue which was purified by column chromatography on silica gel (200 g) with $CHCl_3$–MeOH (50:1, v/v): 14.3 g (89.9%). mp 60 °C. ^1H NMR ($CDCl_3$): δ=0.87 (3H, t), 1.07—1.80 (58H, broad s), 2.20 (3H, s), 2.34—2.82 (3H, m), 3.28—3.50 (1H, m), 3.59 (6H, s), and 5.00 (4H, s).

Found: C, 70.86; H, 11.01%. Calcd for $C_{47}H_{86}O_9$: C, 70.99; H, 10.90%.

*2-[10-(5,6-Dimethoxy-3-methyl-1,4-benzoquinon-2-yl)decyl]-3-hydroxytetracosanoic Acid (**25**).* A solution of **24** (1.59 g, 2 mmol) in hot AcOH (20 ml) was combined with 2M H_2SO_4 (2 ml), and the mixture was stirred at 100 °C for 10 min. After the removal of the solvent, the residue was dissolved in AcOEt (50 ml) and the solution was washed with water. The solvent was evaporated and the residue was dissolved in dioxane (30 ml). To this was added a solution of $FeCl_3 \cdot 6H_2O$ (2.70 g, 10 mmol) in water, and the mixture was stirred at room temperature for 1 h, diluted with water (100 ml), and extracted with AcOEt (50 ml). The extract was washed with water, dried (Na_2SO_4), and concentrated to give a residue which was crystallized from MeOH: 1.03 g (73.1%). mp 72—73 °C ^1H NMR ($CDCl_3$): δ=0.89 (3H, t), 1.10—1.67 (58H, broad s), 2.00 (3H, s), 2.30—2.61 (3H, m), 3.73—3.93 (1H, m), and 3.98 (6H, s).

Found: C, 73.01; H, 10.87%. Calcd for $C_{43}H_{76}O_7$: C, 73.25; H, 10.87%.

*N-Acetyl-1-O-benzyl-6-O-[2-[10-[3,4-dimethoxy-2,5-bis(dimethoxymethoxy)-6-methylphenyl]decyl]-3-hydroxytetracosanoyl]-α-muramic Acid Diphenylmethyl Ester (**28**).* A solution of **24** (3.18 g, 4 mmol) in $CHCl_3$ (30 ml) was made basic to phenolphthalein with a solution of 0.5 M KOH in MeOH (8.1 ml) and then the solvent was removed to give a waxy potassium salt **26**. This salt (833 mg, 1 mmol) was dissolved, together with *N*-acetyl-1-*O*-benzyl-6-*O*-(*p*-tolyluenesulfonyl)-α-muramic acid diphenylmethyl ester (**27**)[7] (703 mg, 1 mmol) and 18-crown-6 (60 mg, 0.23 mmol), in anhydrous toluene (40 ml). After being stirred at 80 °C for 4 h, the mixture was concentrated to give a residue which was purified by column chromatography on silica gel (30 g) with $CHCl_3$–MeOH (99:1, v/v): 1.05 g (79.1%).

Found: C, 69.47; H, 9.03; N, 1.10%. Calcd for $C_{78}H_{119}NO_{16} \cdot H_2O$: C, 69.66; H, 9.07; N, 1.04%.

*N-Acetyl-6-O-[2-[10-(5,6-dimethoxy-3-methyl-1,4-benzoquinon-2-yl)decyl]-3-hydroxytetracosanoyl]muramyl-L-valyl-D-isoglutamine Methyl Ester (**1k**).* A solution of **28** (1.0 g, 0.75 mmo) and anisole (1.1 ml) in CH_2Cl_2 (10 ml) was combined with TFA (10 ml) at 4 °C with stirring and the stirring was continued for additional 1 h. After the removal of the solvent, the residue was dissolved in ether (30 ml) and the solution was washed with water, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (20 g) with $CHCl_3$–acetone–MeOH (10:3:2, v/v) as an eluting solvent to give *N*-acetyl-1-*O*-benzyl-6-*O*-[2-[10-(2,5-dihydroxy-3,4-dimethoxy-6-methylphenyl)decyl-3-hydroxytetracosanoyl]-α-muramic acid (500 mg, 62.2%). This acid (214 mg, 0.2 mmol) was dissolved in THF (3 ml) together with L-valyl-D-isoglutamine methyl ester hydrochloride (65 mg, 0.27 mmol), Et_3N (38 μl, 0.27 mmol), and HONB (54 mg, 0.3 mmol). To this was added DCC under cooling, and the mixture was stirred at room temperature for 15 h. After the removal of the precipitate by filtration, the filtrate was concentrated and the residue was dissolved in AcOEt (30 ml). The solution was washed successively with 5% citric acid and water, dried (Na_2SO_4), and concentrated to give a residue which was purified by column chromatography on silica gel (5 g) with $CHCl_3$–MeOH (97:3, v/v) as an eluant. The resulting crystalline

protected glycopeptide **29a** (106 mg) was hydrogenated in a mixture of THF (2 ml) and AcOH (1 ml) with palladium black as a catalyst at room temperature for 3 h and the free glycopeptide obtained was dissolved in dioxane (10 ml). To this was added a solution of $FeCl_3 \cdot 6H_2O$ (270 mg, 1 mmol) in a small amount of water, and the mixture was stirred at room temperature for 3 min to oxidize the hydroquinone ring. After the removal of the solvent, the residue was dissolved in AcOEt (20 ml). The solution was washed with water, dried (Na_2SO_4), and concentrated to give a residue which was lyophilized from *t*-BuOH: 88 mg (33.0%). The physicochemical data are given in Table 4.

N-Acetyl-6-O-[2-[10(5,6-dimethoxy-3-methyl-1,4-benzoquinon-2-yl)decyl]-3-hydroxytetracosanoyl]muramyl-L-alanyl-D-isoglutamine (11) was synthesized in a manner similar to that of **1k**. The yield and physicochemical data are given in Table 4.

N-Acetyl-1-O-benzyl-6-O-(benzyloxycarbonyl-L-leucyl)-α-muramyl-L-valyl-D-isoglutamine Methyl Ester (30b). A mixture of **16a** (31.2 g, 5 mmol), benzyloxycarbonyl-L-leucine *p*-nitrophenyl ester (3.86 g, 10 mmol), HOBt (2.70 g, 20 mmol) and NEM (2.56 ml, 20 mmol) in DMF (50 ml) was stirred at room temperature for 60 h. After the removal of the solvent, the residue was purified by column chromatography of silica gel with $CHCl_3$–MeOH (19:1, v/v) as an eluent. Reprecipitation from MeOH–ether gave **30b**: 3.1 g (71.7%).

Compounds **30a**, **c**, and **d** were synthesized in a similar manner. The yields and physicochemical data are given in Table 5.

N-Acetyl-6-O-(L-leucyl)muramyl-L-valy-D-isoglutamine Methyl Ester (31b). Compound **30b** (0.3 g, 0.35 mmol) was hydrogenated in AcOH (10 ml) with palladium black as a catalyst at room temperature for 4 h, and the catalyst was removed by filtration. After the removal of the solvent, the residue was reprecipitated from a mixture of MeOH and ether: 0.19 g (82.6%).

Compounds **31a**, **c**, and **d** were synthesized in a similar manner. The yields and physicochemical data are given in Table 5.

N-Acetyl-6-O-[10-(5,6-dimethoxy-3-methyl-1,4-benzoquinon-2-yl)decanoyl-L-leucyl]muramyl-L-valyl-D-isoglutamine Methyl Ester (33b). *Method A.* A mixture of **31b** (51.8 mg, 0.08 mmol), 10-(5,6-dimethoxy-3-methyl-1,4-benzoquinon-2-yl)decanoic acid *p*-nitrophenyl ester (41.7 mg, 0.088 mmol), and NEM (0.012 ml, 0.09 mmol) in DMF (0.5 ml) was stirred at room temperature for 60 h. After the removal of the solvent, the residue was applied onto a silica-gel column (2× 12 cm) which was eluted with AcOEt–pyridine–H_2O–AcOH (130:10:5:3, v/v). The resulting crude **33b** was further purified by column chromatography of Sephdex LH-20 (1.5×90 cm) with EtOH–0.1 M AcOH (3:2, v/v) as an eluent and lyophilized from *t*-BuOH: 32 mg (40.7%). *Method B.* *N*-Acetyl-6-*O*-[10-(5,6-dimethoxy-3-methyl-1,4-benzoquinon-2-yl)decanoyl-L-leucyl]muramyl-L-valyl-D-isoglutamine[29] (32b, 20 mg) was dissolved in MeOH (0.3 ml). To this was added excess ethereal diazomethane solution dropwise under cooling and the mixture was stirred at 4 °C for 30 min. After the removal of the solvent, the residue was purified as described in method A: 13.8 mg (67.0%).

Compounds **33a**, **c**, and **d** were synthesized in a similar manner as described in method A, and compounds **33a** and **e** in method B. The yields and physicochemical data of **33** are given in Table 5.

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