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Chemical Synthesis and Adjuvant Activity of N-Acetylmuramyl-L-alanyl-D-isoglutamine (MDP) Analogs[1,2]

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Twenty-two kinds of N-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) analogs were synthesized and their adjuvant activity on the induction of delayed-type hypersensitivity to ABA-N-acetyl-L-tyrosine was examined. The L-alanine residue of MDP could be replaced with certain other amino acid residues without loss of activity. The structure–activity relationship of these compounds is discussed.

With respect to replacement of the L-alanine residue of MDP in connection with adjuvant activity, it was shown that (1) amino acids having a suitable side chain were effective, (2) basic amino acids were unfavorable, (3) aromatic amino acids were unfavorable, and (4) acidic amino acids were effective.

The D-isoglutamine residue of MDP was considered to be essential for the adjuvant activity. The adjuvant activity was decreased by esterification with methanol of the D-glutamic acid residue of MDP and related N-acetylmuramyldipeptides, but the adjuvant activity of D-glutamic acid diamide analogs was similar to that of MDP and its analogs.

Keywords——muramyl dipeptide; immunoadjuvant activity; chemical synthesis of MDP; MDP methyl ester; MDP acid amide

N-Acetylmuramyl-L-alanyl-D-isoglutamine (MDP) has been shown to be the minimal structure of bacterial cell walls required for immunoadjuvant activity.[3] Synthetic MDP was shown to have adjuvant activity for the development of delayed-type hypersensitivity and enhancement of circulating antibody formation when administered in a water-in-oil emulsion with an antigen.[4] MDP has also been reported to enhance nonspecific immunity to *Klebsiella pneumoniae* infection,[5] mitogenic activity in mouse spleen cells[6] and to induce polyclonal activation *in vitro*.[7]

Accordingly, analogs and derivatives of MDP have been synthesized and the relationship between chemical structure and biological activity of these synthetic N-acetylmuramyldipeptides was examined in comparison with that of MDP.[8] The results suggested that the L-alanine residue of MDP could be replaced with certain other amino acid residues, but that the D-glutamic acid residue was essential for the adjuvant activity of these N-acetylmuramyldipeptides. N-Acetylmuramyl-L-seryl-D-isoglutamine (MurNAc-L-Ser-D-isoGln) as well as MDP was shown to be a potent adjuvant for immune responses, but N-acetylmuramyl-glycyl-D-isoglutamine (MurNAc-Gly-D-isoGln) was less active as an adjuvant for the induction of delayed-type hypersensitivity in guinea pigs.[4b,9] Furthermore, it has also been demonstrated that N-acetylmuramyl-L-valyl-D-isoglutamine (MurNAc-L-Val-D-isoGln) was as adjuvant-active as MDP and MurNAc-L-Ser-D-isoGln on immune response in guinea pigs and mice.[9,10,13] However, MurNAc-D-Ala-D-isoGln was inactive as an adjuvant.[4b,9a,11]

This paper describes the chemical synthesis and adjuvant activity on the induction of delayed-type hypersensitivity in guinea pigs of several kinds of muramyldipeptides in which the L-alanine residue of MDP had been replaced with other amino acid residues and in which

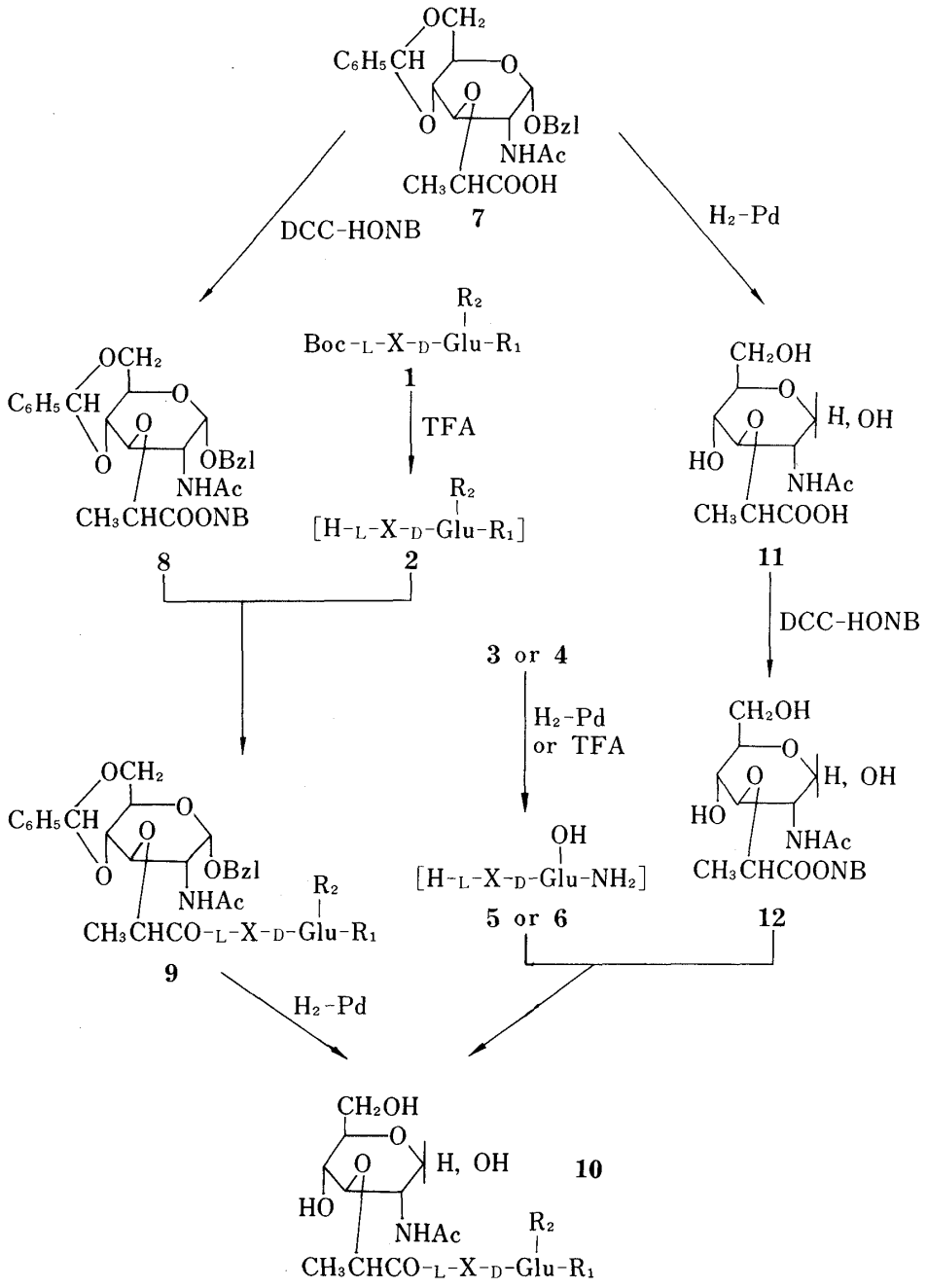

Fig. 1. Synthetic Procedures

the D-glutamic acid residue of MDP analogs had been modified chemically. The compounds synthesized for this study are listed in Table III.

Chemistry

MDP analogs were synthesized by the previously reported procedure using DCC–HONB as a coupling agent at each step.[12–14] The Boc and Z groups were generally used for protection of the α- and ε-amino groups, respectively. The Bzl group was used for the protection of side chain hydroxyl and carboxyl groups, the nitro group for the δ-guanidino group of arginine, and the tosyl group for the imidazole ring of histidine. The Boc group was selectively removed by treatment with TFA prior to each coupling. The tosyl group was removed with HOBt.[15]

The protected dipeptides (**1**) were prepared by coupling of protected Boc amino acid HONB ester with D-isoglutamine benzyl ester, methyl ester, D-glutamic acid diamide, or dimethyl ester. Compound **1i** was prepared by coupling N^{α}-Boc-N^{G}-nitro-L-arginine and D-isoglutamine benzyl ester in the presence of DCC–HOBt to prevent lactam formation.[16] The yields and physicochemical characteristics of these protected dipeptides are summarized in Table I.

TABLE I. Yields and Properties of Protected Dipeptides **1**, **3** and **4**

Compd.	X	R_1	R_2	Yield (%) (Recryst. solv.)	mp/°C	$[\alpha]_D^{22}$ ($c=0.5$, DMF)	Formula	Analysis (%) Calcd (Found) C	H	N
1a	Phe	NH_2	OBzl	78.4 ($CHCl_3$–Et_2O)	136—138	+34.8°[a]	$C_{26}H_{33}N_3O_6$	64.58 (64.29)	6.88 (6.81)	8.69 (8.66)
1b	C-Gly	NH_2	OBzl	77.6 (EtOH–Et_2O)	168—170	+10.9°	$C_{25}H_{37}N_3O_6$	63.13 (63.37)	7.84 (7.98)	8.84 (9.11)
1c	Leu	NH_2	OBzl	87.5 (ether–pet. ether)	108—110	−3.3°	$C_{23}H_{35}N_3O_6$	61.45 (61.75)	7.85 (7.80)	9.35 (9.03)
1d	Ile	NH_2	OBzl	71.2 (AcOEt–pet. ether)	152—153	+5.9°	$C_{23}H_{35}N_3O_6$	61.45 (61.69)	7.85 (7.82)	9.35 (9.10)
1e	Gln	NH_2	OBzl	85.0 (EtOH–Et_2O)	154—155	−0.7°	$C_{22}H_{32}N_4O_7$	56.88 (56.91)	6.94 (6.91)	12.06 (11.95)
1j	His	NH_2	OBzl	33.8 (Et_2O–pet. ether)	Hygroscopic	+2.5°	$C_{23}H_{31}N_5O_6$	58.34 (58.19)	6.60 (6.95)	14.79 (14.54)
1k	Trp	NH_2	OBzl	90.9 (AcOEt)	98—99	+4.3°	$C_{28}H_{34}N_4O_6$	64.35 (64.12)	6.56 (6.75)	10.72 (10.36)
1f	Glu(OBzl)	NH_2	OBzl	70.4 (AcOEt–pet. ether)	118—120	−0.8°	$C_{29}H_{37}N_3O_8$	62.69 (62.44)	6.71 (6.63)	7.56 (7.85)
1g	Lys(Z)	NH_2	OBzl	95.0 (AcOEt–pet. ether)	100—101	−2.5°	$C_{31}H_{42}N_4O_8$	62.19 (62.13)	7.07 (7.05)	9.36 (9.30)
1h	Tyr(OBzl)	NH_2	OBzl	75.5 ($CHCl_3$–Et_2O)	165—167	+5.4°	$C_{33}H_{39}N_3O_7$	67.21 (67.12)	6.67 (6.69)	7.13 (6.96)
1i	Arg($^{G}NO_2$)	NH_2	OBzl	74.4 (80% EtOH)	161—164	+3.3°	$C_{23}H_{35}N_7O_8$	51.39 (51.35)	6.56 (6.54)	18.24 (17.98)
1m	Lys(Ac)	NH_2	OBzl	71.0 (AcOEt–pet. ether)	136—138	−9.3°	$C_{25}H_{38}N_4O_7$	59.27 (59.36)	7.56 (7.65)	11.06 (10.96)
1n	Lys(Z)	NH_2	OCH_3	88.5 (AcOEt–pet. ether)	123—125	−4.9°	$C_{25}H_{38}N_4O_8$	57.45 (57.34)	7.33 (7.35)	10.72 (10.73)
1o	Lys(Ac)	NH_2	OCH_3	74.5 (EtOH–Et_2O)	151—153	−9.8°	$C_{19}H_{34}N_4O_7$	53.01 (52.88)	7.96 (7.92)	13.02 (13.03)
1v	Phe	NH_2	OCH_3	73.6 (AcOEt–pet. ether)	137—139	+5.3°	$C_{20}H_{29}N_3O_6$	58.95 (58.94)	7.17 (7.11)	10.31 (10.33)
1s	Ala	OCH_3	OCH_3	69.3 (Et_2O–pet. ether)	65—66	+3.7°	$C_{15}H_{26}N_2O_7$	52.01 (52.32)	7.57 (7.84)	8.09 (8.25)
1t	Val	NH_2	NH_2	82.4 (EtOH–Et_2O)	167—168	+4.5°	$C_{15}H_{28}N_4O_5 \cdot 1/4H_2O$	51.63 (51.40)	8.23 (8.04)	16.06 (15.94)
1u	Val	OCH_3	OCH_3	84.1 (Et_2O–pet. ether)	72—73	+11.8°	$C_{17}H_{30}N_2O_7$	54.53 (54.87)	8.08 (8.24)	7.48 (7.66)
1w	Phe	NH_2	NH_2	79.5 (EtOH–Et_2O)	193—195	−1.2°	$C_{19}H_{28}N_4O_5 \cdot 1/2H_2O$	56.84 (56.74)	7.28 (7.01)	13.96 (13.68)
1x	Phe	OCH_3	OCH_3	81.0 (EtOH–Et_2O)	97—99	+10.2°	$C_{21}H_{30}N_2O_7$	59.70 (59.72)	7.16 (7.12)	6.63 (6.70)
1p	Lys(Z)	NH_2	NH_2	78.4 (EtOH–Et_2O)	165—167	−6.2°	$C_{24}H_{37}N_5O_7 \cdot 1/4H_2O$	56.29 (56.38)	7.38 (7.30)	13.68 (13.72)
1q	Lys(Z)	OCH_3	OCH_3	83.3 (Et_2O–pet. ether)	78—80	+5.7°	$C_{26}H_{39}N_3O_9$	58.08 (58.00)	7.31 (7.21)	7.82 (7.67)
3	Z-Trp-D-Glu(OBzl)-NH_2			77.5 (EtOH–Et_2O)	187—188	−1.3°	$C_{31}H_{32}N_4O_6$	66.89 (66.88)	5.80 (5.62)	10.07 (9.95)
4	Boc-Met-D-Glu-NH_2			57.4 (AcOEt–pet. ether)	127—129	−8.9°	$C_{15}H_{27}N_3O_6S$	47.73 (47.68)	7.21 (7.05)	11.13[b] (10.99)

a) In EtOH.
b) S: Calcd, 8.50%, Found, 8.48%.

The protected dipeptides (**2**) were coupled with benzyl 2-acetamido-4,6-O-benzyliden-3-O-[1-(*R*)-carboxyethyl]-2-deoxy-α-D-glucopyranoside HONB ester (**8**)[11] to give protected muramyl dipeptide analogs (**9**). The yields and physicochemical charateristics of **9** are summarized in Table II. The final deprotection of all protecting groups of **9** was carried out by catalytic hydrogenation. When the solubility of the starting material in AcOH was low, as in the cases of **9a—d**, **9g—i** and **9m—x**, hydrolytic removal of the benzylidene moiety from

Table II. Yields and Properties of Protected MDP Analogs **9**

Compd.	X	R_1	R_2	Yield (%) (Recryst. solv.)	mp/°C	$[\alpha]_D^{22}$ ($c=0.5$, DMF)	Formula	Analysis (%) Calcd (Found) C	H	N
9a	Phe	NH_2	OBzl	71.7 (DMF–EtOH–Et_2O)	267—269	+102.4°	$C_{46}H_{52}N_4O_{11}$	66.01 (66.14	6.26 6.30	6.69 6.71)
9b	C-Gly	NH_2	OBzl	73.0 (DMF–EtOH–Et_2O)	289—291	+64.6°[a]	$C_{45}H_{56}N_4O_{11}$	65.20 (64.98	6.81 6.82	6.76 6.59)
9c	Leu	NH_2	OBzl	71.2 (DMF–EtOH–Et_2O)	241—244	+91.9°	$C_{43}H_{54}N_4O_{11}$	64.32 (64.09	6.78 6.78	6.98 6.75)
9d	Ile	NH_2	OBzl	79.9 (DMF–EtOH–Et_2O)	274—275.5	+93.1°	$C_{43}H_{54}N_4O_{11}$	64.32 (64.32	6.78 6.80	6.98 6.81)
9e	Gln	NH_2	OBzl	73.4 (DMF–EtOH)	258—259	+88.3°	$C_{42}H_{51}N_5O_{12}$	61.67 (61.30	6.29 6.29	8.56 8.52)
9j	His	NH_2	OBzl	50.2 (DMF–EtOH–Et_2O)	223—225	+87.4°	$C_{43}H_{50}N_6O_{11}\cdot 1/2H_2O$	61.79 (61.53	6.15 6.22	10.06 10.17)
9k	Trp	NH_2	OBzl	69.6 (DMF–EtOH–Et_2O)	218—220	+106.5°	$C_{48}H_{53}N_5O_{11}$	65.81 (65.62	6.10 6.08	8.00 8.21)
9f	Glu(OBzl)	NH_2	OBzl	74.8 (DMF–EtOH–Et_2O)	220—222	+79.8°	$C_{49}H_{56}N_4O_{13}\cdot 1/2H_2O$	64.10 (64.10	6.26 6.10	6.10 6.18)
9g	Lys(Z)	NH_2	OBzl	77.7 (DMF–EtOH)	251—253	+75.2°	$C_{51}H_{61}N_5O_{13}$	64.34 (64.30	6.46 6.45	7.36 7.16)
9h	Tyr(OBzl)	NH_2	OBzl	74.2 (DMF–EtOH–Et_2O)	260—262	+86.1°	$C_{53}H_{58}N_4O_{12}$	67.50 (67.43	6.20 6.13	5.94 6.08)
9i	Arg($^GNO_2$)	NH_2	OBzl	73.0 (80% EtOH)	197—200	+73.7°	$C_{43}H_{54}N_8O_{13}$	57.97 (57.67	6.11 6.18	12.58 12.66)
9m	Lys(Ac)	NH_2	OBzl	83.7 (DMF–EtOH–Et_2O)	243—245	+85.3°	$C_{45}H_{57}N_5O_{12}$	62.85 (62.75	6.68 6.67	8.14 8.08)
9n	Lys(Z)	NH_2	OCH_3	68.5 (DMF–EtOH–Et_2O)	238—240	+82.5°	$C_{45}H_{57}N_5O_{13}\cdot H_2O$	60.46 (60.72	6.65 6.44	7.83 7.71)
9o	Lys(Ac)	NH_2	OCH_3	79.5 (DMF–EtOH–Et_2O)	252—254	+86.8°	$C_{39}H_{53}N_5O_{12}$	59.75 (59.66	6.82 6.85	8.94 8.94)
9v	Phe	NH_2	OCH_3	71.1 (DMF–EtOH–Et_2O)	294—296 (decomp.)	+100.9°	$C_{40}H_{48}N_4O_{11}$	63.14 (63.08	6.36 6.36	7.36 7.24)
9r[c]	Ala	NH_2	NH_2	82.1 (DMF–EtOH–Et_2O)	269—271 (decomp.)	+104.5°	$C_{33}H_{43}N_5O_{10}\cdot 1/4H_2O$	58.78 (58.62	6.50 6.53	10.39 10.29)
9s	Ala	OCH_3	OCH_3	76.2 (DMF–Et_2O)	231—232	+100.0°	$C_{35}H_{45}N_3O_{12}$	60.07 (59.89	6.48 6.46	6.01 5.94)
9t	Val	NH_2	NH_2	86.0 (DMF–EtOH)	280—283 (decomp.)	+86.1°[b]	$C_{35}H_{47}N_5O_{10}\cdot 1/2H_2O$	59.47 (59.55	6.85 6.77	9.91 9.99)
9u	Val	OCH_3	OCH_3	68.7 (DMF–EtOH–Et_2O)	246—247	+98.9°	$C_{37}H_{49}N_3O_{12}$	61.06 (60.84	6.77 6.74	5.77 5.73)
9w	Phe	NH_2	NH_2	83.1 (HMPA–DMF–EtOH)	278—280 (decomp.)	+68.1°[a]	$C_{39}H_{47}N_5O_{10}\cdot 1/4H_2O$	62.43 (62.44	6.38 6.28	9.33 9.40)
9x	Phe	OCH_3	OCH_3	67.0 (DMF–EtOH–Et_2O)	213—215	+92.3°	$C_{41}H_{49}N_3O_{12}$	63.47 (63.46	6.37 6.33	5.42 5.43)
9p	Lys(Z)	NH_2	NH_2	88.4 (DMF–EtOH)	252—254 (decomp.)	+81.0°	$C_{44}H_{56}N_6O_{12}\cdot 1/2H_2O$	60.74 (60.90	6.60 6.39	9.66 9.51)
9q	Lys(Z)	OCH_3	OCH_3	62.9 (DMF–EtOH–Et_2O)	195—197	+81.3°	$C_{46}H_{58}N_4O_{14}$	62.01 (61.79	6.56 6.46	6.29 6.32)

a) In HMPA.
b) In HMPA–DMF (1:1, v/v).
c) Ref. (12c).

TABLE III. Yields and Properties of MDP Analogs **10**

Compd[a]	X	R_1	R_2	Yield (%)	$[\alpha]_D^{22}$ ($c=0.5$, EtOH)	Formula	Analysis (%) Calcd (Found) C	H	N
10a	Phe	NH_2	OH	87.9	+77.9°	$C_{25}H_{36}N_4O_{11}\cdot 1/2H_2O$	51.98 (51.86	6.46 6.72	9.70 9.30)
10b	C-Gly	NH_2	OH	94.5	+40.2°	$C_{24}H_{40}N_4O_{11}\cdot 3/2H_2O$	49.05 (49.27	7.38 7.44	9.54 9.56)
10c	Leu	NH_2	OH	88.6	+38.3°	$C_{22}H_{38}N_4O_{11}\cdot 3/2H_2O$	47.05 (47.19	7.36 7.18	9.98 9.87)
10d	Ile	NH_2	OH	84.7	+40.4°	$C_{22}H_{38}N_4O_{11}\cdot 2H_2O$	46.31 (46.27	7.42 7.41	9.82 9.68)
10e	Gln	NH_2	OH	53.1	+46.2°[b]	$C_{21}H_{35}N_5O_{12}\cdot H_2O$	44.44 (44.40	6.57 6.87	12.34 11.98)
10f	Glu	NH_2	OH	89.3	+48.0°	$C_{21}H_{34}N_4O_{13}\cdot 1/2H_2O$	44.36 (44.29	6.38 6.62	9.86 10.09)
10g	Lys	NH_2	OH	95.0	+36.7°[b]	$C_{22}H_{39}N_5O_{11}\cdot 3/2CH_3COOH\cdot 1/2H_2O$	46.29 (46.33	7.15 7.38	10.80 10.99)
10h	Tyr	NH_2	OH	56.1	+67.7°	$C_{25}H_{36}N_4O_{12}\cdot 1/2H_2O$	50.58 (50.29	6.28 6.47	9.44 9.34)
10i	Arg	NH_2	OH	80.4	+35.7°[b]	$C_{22}H_{39}N_7O_{11}\cdot 1/2CH_3COOH\cdot 2H_2O$	42.92 (42.78	7.05 7.16	15.23 15.37)
10j	His	NH_2	OH	87.6	+57.1°	$C_{22}H_{34}N_6O_{11}\cdot 1/2CH_3COOH\cdot H_2O$	45.54 (45.79	6.31 6.21	13.86 13.99)
10k	Trp	NH_2	OH	22.1	+59.4°	$C_{27}H_{37}N_5O_{11}\cdot 3H_2O$	49.01 (49.05	6.55 6.26	10.59 10.34)
10l	Met	NH_2	OH	30.5	+42.5°	$C_{21}H_{36}N_4O_{11}\cdot 1/2H_2O$	44.91 (44.85	6.64 6.91	9.98[d] 9.77)
10m	Lys(Ac)	NH_2	OH	Quantitative	+55.4°	$C_{24}H_{41}N_5O_{12}\cdot 1/2H_2O$	47.99 (47.89	7.05 6.93	11.66 11.43)
10n	Lys	NH_2	OCH_3	69.0	+54.4°	$C_{23}H_{41}N_5O_{11}\cdot CH_3COOH\cdot 1/2H_2O$	47.46 (47.67	7.33 7.34	11.07 11.02)
10o	Lys(Ac)	NH_2	OCH_3	67.3	+64.2°	$C_{25}H_{43}N_5O_{12}\cdot 1/2H_2O$	48.85 (49.08	7.24 7.11	11.40 11.47)
10v	Phe	NH_2	OCH_3	60.2	+75.5°	$C_{26}H_{38}N_4O_{11}\cdot 1/2H_2O$	52.78 (52.60	6.64 6.57	9.47 9.38)
10r[e]	Ala	NH_2	NH_2	90.9	+35.4°	$C_{19}H_{33}N_5O_{10}\cdot 2H_2O$	43.26 (43.61	7.07 6.81	13.28 13.27)
10z[e]	Ala	OCH_3	OCH_3	76.2	+39.3°	$C_{21}H_{35}N_3O_{12}\cdot 2.5H_2O$	44.52 (44.62	7.12 7.03	7.42 7.30)
10t	Val	NH_2	NH_2	94.9	+45.4°[c]	$C_{21}H_{37}N_5O_{10}\cdot 1/2CH_3COOH\cdot 3/2H_2O$	45.82 (45.50	7.34 7.20	12.15 12.03)
10u	Val	OCH_3	OCH_3	70.2	+54.7°[c]	$C_{23}H_{39}N_3O_{12}\cdot 3/2H_2O$	47.91 (48.26	7.34 7.00	7.29 7.06)
10w	Phe	NH_2	NH_2	84.3	+31.6°[c]	$C_{25}H_{37}N_5O_{10}\cdot 2H_2O$	49.74 (49.42	6.85 6.75	11.60 11.30)
10x	Phe	OCH_3	OCH_3	56.2	+54.1°	$C_{27}H_{39}N_3O_{12}\cdot 1/2CH_3COOH\cdot H_2O$	52.08 (52.13	6.71 6.46	6.51 6.58)
10p	Lys	NH_2	NH_2	82.0	+31.4°	$C_{22}H_{40}N_6O_{10}\cdot CH_3COOH\cdot 2.5H_2O$	44.09 (43.88	7.56 7.29	12.86 12.82)
10q	Lys	OCH_3	OCH_3	70.4	+30.9°	$C_{24}H_{42}N_4O_{12}\cdot CH_3COOH\cdot 2H_2O$	46.28 (45.97	7.47 7.37	8.30 8.33)

a) All these compounds are colorless hygroscopic powders and do not show definite melting points.
b) In 75% aqueous EtOH.
c) In DMF.
d) S: Calcd, 5.71%; Found, 5.55%.
e) Ref. (12c).

9 was effected in 60% AcOH at 100° prior to hydrogenolysis. The final products were purified by column chromatography on Sephadex LH-20 then lyophilized to give hygroscopic powder. The yields and physicochemical characteristics of **10** are summarized in Table III.

During hydrogenolytic deprotection of the tryptophan analog (**9k**) in acetic acid or N,N'-dimethylformamide (DMF), TLC showed the generation of unknown by-products as well as the desired product **10k**, because degradation of the indole ring of tryptophan had presumably occurred. All efforts to isolate **10k** were unsuccessful. Therefore, direct coupling of N-acetylmuramic acid (**11**) with L-tryptophyl-D-isoglutamine (**5**) was attempted to obtain **10k** without the final deprotection procedure.

After the hydrogenolytic deprotection of **3** over palladium black in methanol, the resulting deprotected free dipeptide **5** was coupled with **11** which had been converted into the HONB active ester (**12**) prior to the coupling. Although the HONSu ester of benzyl N-acetylmuramide was reported to be easily converted into an intramolecular ester,[17] this was not observed on thin layer chromatography (TLC) during the condensation of **5** with **11** when the HONB active ester was used.

Compound **101** was also obtained by direct coupling of **11** with the deprotected dipeptide **6** as described above, since sulfur-containing peptides usually poisoned the catalyst. The final product thus obtained was purified by successive column chromatography on Amberlite CG-120, Amberlite XAD-2, and Sephadex LH-20.

Measurement of Adjuvant Activity

The adjuvant activity of these synthetic MDP analogs for the induction of delayed-type hypersensitivity to ABA–Tyr was examined in guinea pigs by a method described earlier.[9b] Briefly, four Hartley guinea pigs (weighing 300—500 g) in each group were immunized by injection into the four footpads of 50 μg of ABA–Tyr in Freund's incomplete adjuvant (FIA) with synthetic N-acetylmuramyldipeptides. Control groups were immunized with ABA-N-acetyl-L-tyrosine alone in FIA (control 1) or FIA alone (control 2). After 2 weeks, skin tests were performed with 100 or 50 μg of ABA-bovine serum albumin (ABA–BSA), prepared by the method of Tabachinick and Sobotka,[18] and skin reactions were measured 24 hr and 48 hr after intradermal injection of test antigen.

Results and Discussion

As shown in Table IV, the MDP analogs, **10l**, **10f**, **10e**, **10c** and **10d** had adjuvant activities similar to or more potent than that of MDP in this immune system. In the cases of **10l**, **10f**, **10e**, **10a** and **10c** necrotic skin reaction in addition to very strong induration and erythema reactions was observed at the injected site in guinea pigs, as in the case of MDP and the L-valine analog. On the other hand, lower activities were seen with the **10g**, **10i** and **10j** analogs. The analogs of **10a**, **10h**, **10k** and **10b** had clear adjuvanticity, but were less active than MDP, especially with a 10 μg dose.

These results suggest that increased lipophilicity and steric bulk of the side chain of the amino acid adjacent to the muramic acid moiety may play an important role in the development of adjuvant activity, as suggested in our previous paper.[13] The weak adjuvant activities of **10a**, **10b**, **10h**, **10j** and **10k** in comparison with that of MDP indicate that too large a side chain or aromaticity of these amino acid residues was also unfavorable for the adjuvant activity. **10f** and **10e** had similar adjuvant activity to MDP, but **10g**, **10i** and **10j** were less active than MDP, though the bulkiness of the side chain of L-lysine is comparable to that of L-methionine. These results suggest that an acidic moiety has no effect on the activity, but the introduction of a basic of amino acid is unfavorable for the adjuvant activity of N-acetylmuramyldipeptides. It may be considered that the amino or imino group of the side chains of L-lysine, L-histidine and L-arginine interacts with the carboxyl group of the D-isoglutamine residue and interferes

TABLE IV. Adjuvant Activities of N-Acetylmuramyldipeptides on the Induction of Delayed-type Hypersensitivity to ABA–N-acetyl-L-tyrosine in Guinea Pigs

Compd.	MDP analogs of (Amino acid residue of X[a])	Dose (μg)	Skin reaction with ABA–BSA (50 μg)[b] at 24 hr (mm in diameter ± S.E.)	at 48 hr	Necrotic[c] reaction
Exp. I					
MDP	Ala	100	21.3±0.5	18.6±1.2	1/4
		10	20.0±1.0	18.9±0.7	
	Val	100	23.1±1.0	21.7±1.2	3/4
		10	22.0±1.0	20.4±0.6	
10l	Met	100	24.5±0.7	20.9±0.4	1/4
		10	22.3±0.6	19.0±0.5	
10f	Glu	100	22.8±0.7	19.0±0.8	2/4
		10	20.1±0.9	16.3±0.8	
10e	Gln	100	21.5±0.9	20.4±1.1	1/4
		10	21.3±0.4	19.0±0.9	
10g	Lys	100	(14.7±1.3)	(9.3±1.9)	
		10	(5.5±0.2)	0	
10i	Arg	100	20.6±0.6	15.9±1.4	
		10	(16.5±0.9)	(8.8±1.1)	
10j	His	100	(10.8±2.3)	0	
		10	(9.8±2.1)	(2.7±2.7)	
10a	Phe	100	21.6±1.1	20.3±1.1	2/4
		10	(13.8±1.4)	(8.8±2.3)	
10h	Tyr	100	24.4±0.8	20.2±0.6	
		10	(18.1±1.7)	(7.3±2.8)	
10k	Trp	100	22.4±0.9	19.1±0.9	
		10	(18.3±1.3)	(13.1±1.0)	
Exp. II					
	Val	100	21.2±0.6	19.8±0.5	2/4
		10	20.0±0.5	18.8±0.5	1/4
10c	Leu	100	22.4±0.8	19.6±0.8	1/4
		10	21.9±0.5	16.8±0.8	
10d	Ile	100	21.3±0.6	19.1±0.5	
		10	20.8±0.6	16.4±1.5	
10b	C–Gly	100	19.9±1.0	15.9±1.1	
		10	20.5±0.6	13.3±0.8	
Control 1 (ABA–Tyr+FIA)			0	0	
Control 2 (FIA alone)			0	0	

a) MurNAc-L-X-D-Gln(OH)-NH_2.
b) The data indicate the average skin reaction (erythema) and standard error (S.E.) of four guinea pigs; parentheses indicate the size of faint erythema.
c) No. of guinea pigs with necrotic skin reaction/No. of guinea pigs tested.

with the adjuvant activity of these N-acetylmuramyldipeptides.. This possibility is supported by the results in Table V.

As shown in Table V, the adjuvant activities of MurNAc-L-Lys-D-isoGln analogs (**10m**, **10o**) in which the amino group of the L-Lys residue was blocked by acetylation were more potent than that of **10g**. Furthermore, the activities of **10p** and **10q** in which the free carboxyl group of the D-isoGln residue was blocked were more potent than that of **10g**.

It has already been shown that the D-glutamic acid residue is essential for the adjuvant activity of MDP and its analogs.[3b,5,6,8,9a] In this experiment, we examined the effect of chemical modification of the D-glutamic acid residue of MDP and its analogs. As shown in Table V, D-isoglutamine methyl ester or D-glutamic acid dimethyl ester analogs (**10v**, **10n**, **10o**, **10z**, **10u**, **10x**) were less active than the corresponding MDP analogs (**10a**, **10g**, **10m**, MDP, L-Val analog). The adjuvant activities of D-glutamic acid diamide analogs (**10r**, **10t**, **10w**) were similar to or slightly lower than those of the corresponding D-isoglutamine analogs (MDP, L-Val analog, **10a**). These results are similar to those described previously.[4,5]

Table V. Adjuvant Activity of N-Acetylmuramyldipeptides on the Induction of Dealyed-type Hypersensitivity to ABA-N-acetyl-L-tyrosine in Guinea Pigs

Compd.	MDP analogs[a] X	R_1	R_2	Dose (μg)	Skin reaction with ABA-BSA (50 μg) at 24 hr (mm in diameter±S.E.)	at 48 hr (mm in diameter±S.E.)
Exp. I						
MDP	Ala	NH_2	OH	100	19.2±1.2	20.1±1.4
10a	Phe	NH_2	OH	100	18.5±0.9	17.1±1.4
10v	Phe	NH_2	OCH_3	100	18.9±2.0	13.1±3.2
10g	Lys	NH_2	OH	100	(7.9±2.0)	0
10m	Lys(Ac)	NH_2	OH	100	18.9±1.0	15.1±0.8
10n	Lys	NH_2	OCH_3	100	0	0
10o	Lys(Ac)	NH_2	OCH_3	100	15.5±2.0	10.4±2.4
	Control (ABA-Tyr+FIA)				0	0
Exp. II						
MDP	Ala	NH_2	OH	100	20.5±1.3	16.5±1.6
10r	Ala	NH_2	NH_2	100	19.6±1.0	20.4±1.8
10z	Ala	OCH_3	OCH_3	100	15.9±2.0	8.1±2.2
	Val	NH_2	OH	100	22.3±1.1	21.6±1.5
10t	Val	NH_2	NH_2	100	21.1±1.0	19.9±1.0
10u	Val	OCH_3	OCH_3	100	19.4±1.2	18.8±2.0
10a	Phe	NH_2	OH	100	17.0±1.0	12.3±2.8
10w	Phe	NH_2	NH_2	100	13.3±1.1	(4.8±1.1)
10x	Phe	OCH_3	OCH_3	100	10.2±6.4	(3.0±1.9)
10g	Lys	NH_2	OH	100	6.1±1.5	(4.5±1.0)
10p	Lys	NH_2	NH_2	100	19.3±1.1	9.6±0.8
10q	Lys	OCH_3	OCH_3	100	17.8±1.2	14.5±2.9
	Control (ABA-Tyr+FIA)				0	0

See Table IV for the methods.

R_2
|
a) MurNAc-L-X-D-Glu-R_1.

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. Silica gel 60 (Merck), Sephadex LH-20 (Pharmacia) and Amberlite XAD-2 (Rohm and Haas) were used for column chromatography.

The reactions were monitored by TLC with Merck F_{254} silica gel pre-coated plates, which were generally developed with $CHCl_3$–MeOH–AcOH (180: 20: 1, v/v) for protected dipeptides (**1**, **3** and **4**) and protected muramyldipeptides (**9**), and with AcOEt–pyridine–H_2O–AcOH (30: 10: 5: 3, v/v) and *n*-BuOH–AcOH–AcOEt–H_2O (1: 1: 1: 1, v/v) for muramyl dipeptide analogs (**10**).

Solutions were concentrated by evaporation under reduced pressure with an outside bath temperature of below 40°.

Boc-L-cyclohexylGly——L-CyclohexylGly[19] (200 mg, 1.3 mmol), Boc-N_3 (223 mg, 1.56 mmol), and Et_3N (0.44 ml, 3.12 mmol) were combined in a mixture of DMF (5 ml) and H_2O (5 ml) at room temperature and the solution was stirred for 24 hr at 40°. H_2O (30 ml) was then added to the solution and the mixture was washed with AcOEt (20 ml×2). The AcOEt layer was extracted with 5% $NaHCO_3$ solution (10 ml). All the aqueous layers were combined, acidified to pH 4 with 1 N HCl at 0°, and extracted with AcOEt (20 ml×3). The extract was washed with H_2O–NaCl solution (20 ml×2), dried over Na_2SO_4, and concentrated. The residue was triturated with pet. ether to give 265 mg of white crystals (79.1%): mp 81—83°; $[\alpha]_D^{22}$ −2.3° (c=0.5, EtOH). *Anal.* Calcd for $C_{13}H_{23}NO_4$: C, 60.67; H, 9.01; N, 5.44. Found: C, 60.59; H, 8.89; N, 5.52.

Boc-Lys(Ac)OH——Boc-Lys(Ac)OH was prepared from Lys(Ac)OH[20] (1.51 g, 8 mmol) by using Boc-N_3. Recrystallization from Et_2O–pet. ether: 1.32 g (57.2%); mp 142—143°; $[\alpha]_D^{22}$ −22.1° (c=0.5, DMF). *Anal.* Calcd for $C_{13}H_{24}N_2O_5$: C, 54.15; H, 8.53; N, 9.72. Found: C, 54.12; H, 8.53; N, 9.74.

Boc-D-Glu(NH_2)NH_2——Boc-D-Glu (1.24 g, 5 mmol) and *p*-nitrophenol (1.39 g, 10 mmol) were dissolved in THF (20 ml), DCC (2.06 g, 10 mmol) was added at 0°, and the solution was stirred in a refrigerator overnight. The resulting dicyclohexylurea was filtered off and 28% NH_4OH (5 ml) was added to the filtrate. The solution was stirred for 3 hr at room temperature. The solvent was evaporated off, and the residue

was dissolved in H_2O (100 ml). The solution was neutralized with 10% aqueous H-COOH, washed with AcOEt (20 ml×2), and subjected to lyophilization. The residue was recrystallized from EtOH–Et_2O to give 0.95 g of white crystals (77.5%): mp 134—136°; $[\alpha]_D^{22}$ −4.6° (c=0.5, EtOH). *Anal.* Calcd for $C_{16}H_{19}N_3O_4$: C, 48.97; H, 7.81; N, 17.13. Found: C, 48.98; H, 7.83; N, 17.06.

D-Glu(OMe)OMe·HCl——D-Glu(OMe)OMe·HCl was prepared from D-Glu by using HCl–MeOH and recrystallized from EtOH–Et_2O: 6.93 g (96.3%): mp 86—89°; $[\alpha]_D^{22}$ −26.0° (c=1, EtOH). *Anal.* Calcd for $C_{17}H_{14}ClNO_4$: C, 39.72; H, 6.67; Cl, 16.75; N, 6.62. Found: C, 39.62; H, 6.75; Cl, 16.65; N, 6.75.

Boc-L-Phe-D-Glu(OBzl)NH_2 (1a)——Boc-L-Phe-HONB[14] (1.28 g, 3.1 mmol), D-Glu(OBzl)NH_2·TFA (1.19 g, 3.4 mmol) and Et_3N (0.48 ml) were combined in CH_3CN (25 ml) at 0° and the solution was stirred for 6 hr at room temperature. After removal of the solvent by evaporation, the residue was dissolved in AcOEt and the solution was washed with 5% $NaHCO_3$, 1 N HCl, and H_2O–NaCl successively, then dried over Na_2SO_4 and concentrated. The residue was recrystallized from $CHCl_3$–Et_2O to give 1.14 g of white crystals (69.8%): mp 136—138°; $[\alpha]_D^{22}$ +34.8°(c=0.5, EtOH). *Anal.* Calcd for $C_{26}H_{33}N_3O_6$: C, 64.44; H, 7.07; N, 8.67. Found: C, 64.29; H, 6.81; N, 8.66.

Boc-Arg(NO_2)-D-Glu(OBzl)NH_2 (1i)——Boc-Arg(NO_2) (0.64 g, 2 mmol), D-Glu(OBzl)NH_2·TFA (0.70 g, 2 mmol), HOBt (0.41 g, 3 mmol), and Et_3N (0.28 ml) were combined in CH_3CN–DMF (15 ml–15 ml). DCC (0.52 g, 25 mmol) was then added to the solution at 0° and the solution was stirred overnight at room temperature. After removal of the solvent by evaporation, the residue was dissolved in $CHCl_3$–*n*-BuOH (50 ml–10 ml). The solution was washed with 1 N HCl, 5% $NaHCO_3$, and H_2O–NaCl solution successively, then dried over Na_2SO_4 and concentrated. The residue was recrystallized from 80% EtOH to give 0.80 g of white crystals (74.4%): mp 161—164°; $[\alpha]_D^{22}$ +3.3° (c=0.5, DMF). *Anal.* Calcd for $C_{23}H_{35}N_7O_8$: C, 51.39; H, 6.56; N, 18.24. Found: C, 51.35; H, 6.54; N, 17.98.

Boc-His(Tos)-D-Glu(OBzl)NH_2 (1j)——Boc-His(Tos)-OH [prepared from 1.18 g (2 mmol) of Boc-His(Tos)-OH·DCHA], HONB (0.39 g, 2.2 mmol), DCC (0.45 g, 2.2 mmol) were combined in CH_3CN (30 ml) at 0°, and the solution was stirred overnight at room temperature. N,N′-Dicyclohexylurea was removed by filtration and the filtrate was evaporated to dryness. The residual Boc-His(Tos)ONB, D-Glu(OBzl)NH_2·TFA (0.7 g, 2 mmol), and Et_3N (0.28 ml) were combined in CH_3CN (30 ml) at 0° and the solution was stirred overnight at room temperature. After removal of the solvent by evaporation, the residue was dissolved in AcOEt (50 ml), and the solution was washed with 1 N HCl solution (20 ml×2). Boc-His(Tos)-D-Glu(OBzl)NH_2 was found in the organic layer, while the desired detosyl product (**1j**) was in the aqueous layer. The work-up of this aqueous layer is described later. The AcOEt layer was washed with 5% $NaHCO_3$ (20 ml×2) and H_2O–NaCl solution (20 ml×2), dried over Na_2SO_4 and concentrated. Boc-His(Tos)-D-Glu(OBzl)NH_2 thus obtained and HOBt (0.41 g, 3 mmol) were combined in THF (15 ml) and the solution was stirred overnight at room temperature. After removal of the solvent by evaporation, the residue was dissolved in 0.5 N HCl (30 ml) and the aqueous solution was washed with AcOEt (15 ml×2), neutralized with $NaHCO_3$, and extracted with AcOEt (20 ml×2). The extract was washed with 5% $NaHCO_3$ (20 ml×2) and H_2O–NaCl (20 ml×2), dried over Na_2SO_4, and concentrated to give an oily material.

In the meantime, the aqueous layer mentioned above was neutralized with $NaHCO_3$, and extracted with AcOEt (20 ml×2). The AcOEt layer was washed with H_2O–NaCl solution (20 ml×2), dried over Na_2SO_4 and concentrated to give an oily material.

These oily materials were combined and triturated with Et_2O–pet. ether to give 0.32 g of hygroscopic white powder: $[\alpha]_D^{22}$ +2.5° (c=0.5, DMF). *Anal.* Calcd for $C_{23}H_{31}N_5O_6$: C, 58.34; H, 6.60; N, 14.79. Found: C, 58.19; H, 6.95; N, 14.54.

Boc-Val-D-Glu(NH_2)NH_2 (1t)——Boc-D-Glu(NH_2)NH_2 (0.25 g, 1 mmol) was dissolved in TFA (2 ml) and the solution was allowed to stand for 20 min at room temperature. TFA was then evaporated off and the residue was triturated with Et_2O. After decantation, the residue was dried under reduced pressure in the presence of NaOH pellets. D-Glu(NH_2)NH_2·TFA thus obtained was dissolved in DMF (10 ml) and the solution was neutralized with Et_3N (0.14 ml) at 0°. Next, Boc-Val-HONB[13] (0.38 g, 1 mmol) was added, and the solution was stirred for 24 hr at room temperature. After removal of the solvent by evaporation, the residue was dissolved in a small portion of $CHCl_3$ and subjected to silica gel column chromatography (solvent: $CHCl_3$: MeOH: AcOH=180: 20: 1, v/v). Fractions containing the product were collected and concentrated. The residue was recrystallized from EtOH–Et_2O to give 0.28 g of white crystals (82.4%) mp 167—168°; $[\alpha]_D^{22}$ +4.5° (c=0.5, DMF). *Anal.* Calcd for $C_{15}H_{28}N_4O_5 \cdot 1/4H_2O$: C, 51.63; H, 8.63; N, 16.06. Found: C, 51.40; H, 8.04; N, 15.94.

Boc-Ala-D-Glu(NH_2)NH_2 (1r)——This compound was prepared in a manner similar to that described for **1t**. Its physicochemical properties, however, were not determined since all efforts to crystallize **1r** were unsuccessful.

The analogous protected dipeptides (**1b—k**, **1m—o**, **1q—v**, and **1x**) were all prepared in a manner similar to that described for **1a**. The other analogous protected dipeptides (**1p** and **1w**) were prepared in a manner similar to that described for **1t**. The yields and physicochemical properties are summarized in Table I.

Z-Trp-D-Glu(OBzl)NH_2 (3)——Z-Try-HONB (1.00 g, 2 mmol), D-Glu(OBzl)NH_2·TFA (0.70 g, 2 mmol), and Et_3N (0.28 ml) were combined in CH_3CN (20 ml) at 0° and the solution was stirred overnight at 0°. After removal of the solvent by evaporation, the residual solid was collected by filtration, and recrystallized

from EtOH–Et_2O to give 0.86 g of white crystals (57.4%): mp 187—188°; $[\alpha]_D^{22}$ −1.3° (c=0.5, DMF). *Anal.* Calcd for $C_{31}H_{32}N_4O_6$: C, 66.89; H, 5.80; N, 10.07. Found: C, 66.88; H, 5.62; N, 9.95.

Try-D-Glu(OH)NH_2 (5)——Compound **3** (278 mg, 0.5 mmol) was hydrogenolyzed with palladium black as a catalyst in methanol at room temperature. After the catalyst had been removed by filtration, the filtrate was concentrated to give **5**, which was used without further purification. The physicochemical properties of this compound were not determined.

Boc-Met-D-Glu(OH)NH_2 (4)——Boc-Met-Opcp (1.49 g, 3 mmol), D-Glu(OH)NH_2·TFA (0.78 g, 3 mmol), and Et_3N (0.42 ml) were combined in CH_3CN (30 ml) at 0° and the solution was stirred overnight at room temperature. The solvent was evaporated off, the residue was dissolved in AcOEt and the solution was washed with H_2O. The H_2O layer was acidified with 1 N HCl at 0° and extracted with AcOEt. The AcOEt extracts were combined, washed with H_2O–NaCl, dried over Na_2SO_4 and concentrated. The residue was triturated with Et_2O and recrystallized from AcOEt–pet. ether to give 0.65 g of white crystals (57.4%); mp 127—129°; $[\alpha]_D^{22}$ −8.9° (c=0.5, DMF). *Anal.* Calcd for $C_{15}H_{27}N_3O_6S$: C, 47.73; H, 7.21; N, 11.13; S, 8.50. Found: C, 47.68; H, 7.05; N, 10.99; S, 8.48.

Met-D-Glu(OH)NH_2·TFA (6)——Compound **4** (0.38 g, 1 mmol) was dissolved in TFA (10 ml) containing anisole (1 ml) and the solution was allowed to stand at room temperature for 10 min. After removal of the solvent, the residue was triturated with Et_2O and filtered to give **6** as the trifluoroacetate (hygroscopic white powder), which was used without further purification. The physicochemical properties of this compound were not determined.

N-Acetyl-1-O-benzyl-4,6-O-benzylidene-α-muramyl-L-Phe-D-Glu(OBzl)NH_2 (9a)——Compound **1a** (0.97 g, 2 mmol) was dissolved in TFA (10 ml) and the solution was allowed to stand at room temperature for 20 min. After removal of the solvent, the residue was triturated with Et_2O–pet. ether to give white crystals. Phe-D-Glu(OBzl)NH_2·TFA (**2a**) thus obtained was dissolved in CH_3CN (30 ml) and the solution was neutralized with Et_3N (0.28 ml) at 0°. Next, a CH_3CN solution (20 ml) of N-acetyl-1-O-benzyl-4,6-O-benzylidene-α-muramide HONB ester[13] was added, and the mixture was stirred overnight at room temperature. After removal of the solvent by evaporation, the residue was collected by filtration, and recrystallized from DMF–EtOH–Et_2O to give 1.14 g of white crystals (71.7%): mp 267—269°; $[\alpha]_D^{22}$ +102.4° (c=0.5, DMF). *Anal.* Calcd for $C_{46}H_{52}N_4O_{11}$: C, 66.01; H, 6.26; N, 6.69. Found: C, 66.14; H, 6.30; N, 6.71.

The analogous protected N-acetylmuramyl dipeptides (**9b—k, 9m—x**) were all prepared in a manner similar to that described for **9a**. The yields and physicochemical properties of these compounds are summarized in Table II.

MurNAc-Phe-D-Glu(OH)NH_2 (10a)——Compound **9a** (0.84 g, 1 mmol) was heated in 60% AcOH (50 ml) on a boiling water bath for 30 min. The solvent was evaporated off, and the residue was freed from benzaldehyde by codistillation with water. The residue was then hydrogenolyzed with palladium black as a catalyst in acetic acid (50 ml) for 72 hr at room temperature. After the catalyst had been removed by filtration, the filtrate was concentrated and the residue was dissolved in EtOH–0.1 N AcOH (1.2 ml—0.8 ml). The solution was applied to a column of Sephadex LH-20 (2×90 cm), which was eluted with the same solvent system. Fractions (2 g each) corresponding to the main peak were collected and the solvent was evaporated off. The residue was dissolved in water (20 ml) and lyophilized to give 0.50 g of hygroscopic white powder (87.9%): $[\alpha]_D^{22}$ +77.9° (c=0.5, EtOH). *Anal.* Calcd for $C_{25}H_{36}N_4O_{11}\cdot 1/2H_2O$: C, 51.98; H, 6.46; N, 9.70. Found: C, 51.86; H, 6.72; N, 9.30.

MurNAc-Trp-D-Glu(OH)NH_2 (10k)——N-Acetylmuramic acid (146 mg, 0.5 mmol), HONB (108 mg, 0.6 mmol) and DCC (124 mg, 0.6 mmol) were combined in DMF (2 ml) at 0° and the solution was stirred for 2 hr at 0°. After N,N′-dicyclohexylurea had been removed by filtration, the filtrate was again cooled, **5** (223 mg, 0.5 mmol) and Et_3N (0.14 ml, 1.0 mmol) were added, and the solution was stirred for 48 hr at 0°. After removal of the solvent, the residue was subjected to silica gel column chromatography (solvent: AcOEt–pyridine–H_2O–AcOH=30: 10: 5: 3, v/v). The fractions containing the desired product were collected and the solvent was evaporated off. The residue was dissolved in water and the solution was applied to a column of Amberlite CG-120 (H^+, 1×1 cm). The passed solution and water washings were combined and concentrated. The residue was applied to a column of Amberlite XAD-2 (2×20 cm) which was eluted with H_2O–EtOH (gradient: H_2O/EtOH=100 ml/100 ml). The fractions containing the product were combined and concentrated. The residue was dissolved in a mixture of EtOH (1.2 ml) and 0.1 N AcOH (0.8 ml), and the solution was applied to a column of Sephadex LH-20 (2×90 cm), which was eluted with the same solvent system. Individual fractions (2 g each) were collected and the fractions containing pure product were combined and concentrated. The residue was dissolved in water and lyophilized to give 67 mg of white powder (22.1%): $[\alpha]_D^{22}$ +59.4° (c=0.5, EtOH). *Anal.* Calcd for $C_{27}H_{37}N_5O_{11}\cdot 3H_2O$: C, 49.01; H, 6.55; N, 10.59. Found: C, 49.05; H, 6.26; N, 10.34.

MurNAc-Met-D-Glu(OH)NH_2 (10l)——This compound was prepared in a manner similar to that described for **10k** without the Amberlite XAD-2 column chromatography purification step: 118 mg (30.5%): $[\alpha]_D^{22}$ +42.5° (c=0.5, EtOH). *Anal.* Calcd for $C_{21}H_{36}N_4O_{11}S\cdot 1/2H_2O$: C, 44.91; H, 6.64; N, 9.98; S, 5.71. Found: C, 44.85; H, 6.91; N, 9.77; S, 5.55.

The analogous N-acetylmuramyl dipeptides (**10b—j, 10m—x**) were prepared in a manner similar to that described for **10a** either with (**9c** and **9g—x**) or without (**9e, 9f** and **9j**) the pretreatment in 60% AcOH at 100°.

Compounds **9b** and **9d** were treated with DMF–60% AcOH (1: 1) prior to catalytic hydrogenolysis. The yields and physicochemical properties of **10** are summarized in Table III.

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References and Notes

1) Presented in part at the 15th Joint Meeting of the Tuberculosis Panel of the US-Japan Co-operative Medical Science Program, Tokyo, Oct., 1980.
2) The abbreviations used are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: *Biochemistry*, **5**, 2458 (1966); *ibid.*, **6**, 362 (1967); *ibid.*, **11**, 1726 (1972). Z=benzyloxycarbonyl; DCC=dicyclohexylcarbodiimide; HOBt=1-hydroxybenzotriazole; TFA=trifluoroacetic acid; Boc=*tert*-butoxycarbonyl; HONB=N-hydroxy-5-norbornene-2,3-dicarboximide; Bzl=benzyl; DCHA=dicyclohexylamine; Et_3N=triethylamine; HONSu=N-hydroxysuccinimide; ABA-Tyr=N-acetyl-3-(4-arsonophenylazo)-L-tyrosine; MurNAc=N-acetylmuramyl; HOpcp=pentachlorophenol; pet. ether=petroleum ether.
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