Tetrahedron Letters No. 49, pp 4899 - 4902.

0040-4039/78/1201-4899$02.00/0

SYNTHESIS OF LONG CHAIN FATTY ACID ESTERS OF *N*-ACETYLMURAMYL-L-ALANYL-D-ISOGLUTAMINE IN RELATION TO ANTITUMOR ACTIVITY[1)]

Shoichi Kusumoto, Masaru Inage, and Tetsuo Shiba*
Department of Chemistry, Faculty of Science, Osaka University
Toyonaka, Osaka 560, Japan

Ichiro Azuma and Yuichi Yamamura
The Third Department of Internal Medicine, Medical School, Osaka University
Fukushima-ku, Osaka 553, Japan

Our synthetic study revealed that *N*-acetylmuramyl-L-alanyl-D-isoglutamine (1) is the minimum effective structure required for the immuno potentiating ability of various bacterial cell walls. However, the muramyl dipeptide (1) showed no antitumor activity based on immuno reaction, whereas cell wall of *Mycobacterium bovis* BCG itself has such activity, being used for immunotherapy of cancer. In view of this fact as well as the high lipid content of BCG cell wall, we had an expectation that addition of lipophilic character to the molecule of muramyl dipeptide (1) might produce the antitumor activity. This was shown to be true in our recent work in the case of 6-*O*-mycoloyl muramyl dipeptide (2a) which was prepared by coupling of muramyl dipeptide (1) with natural mycolic acid isolated from bacterial cells.[2,3)] However, there still remained some ambiguities concerning the question whether a certain structural variation or the heterogeneity in the natural mycolic acid[2)] is required for the activity, and also the possibility that the activity is derived even from an accompanying impurity can not be excluded. Therefore, we now prepared a new series of 6-*O*-acyl-*N*-acetylmuramyl-L-alanyl-D-isoglutamines (2b-f) by use of pure synthetic fatty acids of high molecular weights in order to clarify the structural requirement for the activity in the fatty acid moiety.

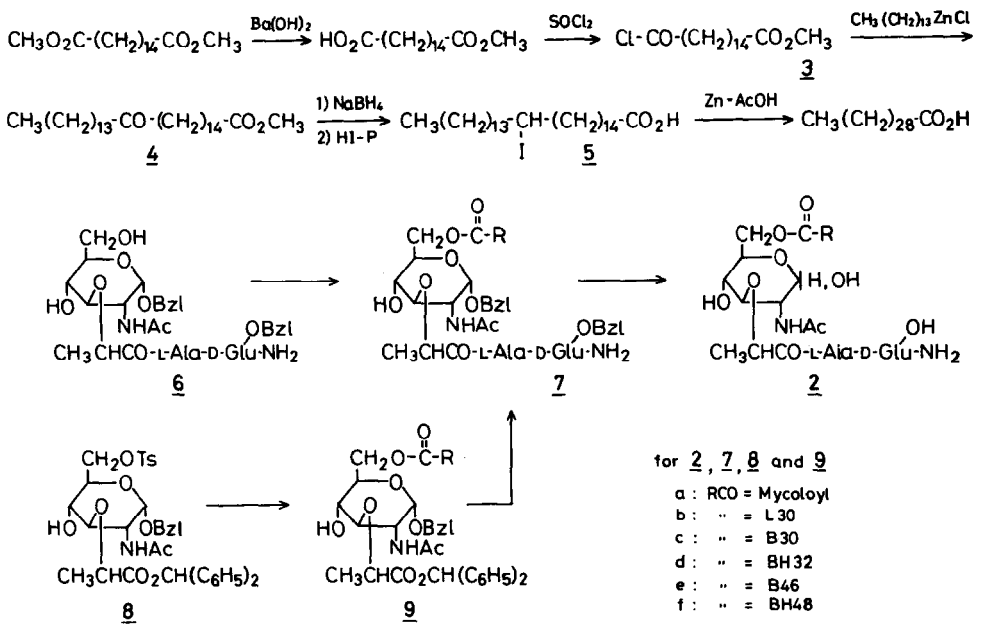
In this investigation, we used five fatty acids of three different types; i) linear (L), ii) α-branched (B), and iii) α-branched β-hydroxylated (BH) ones.[4)] The BH-type acids represent the essential structure of natural mycolic acid. We chose the carbon numbers of 30 to 48 for these acids, which correspond approximately to the range of molecular size of natural corynomycolic and nocardomycolic acid, respectively.[2)]

Triacontanoic acid[5)] of L-type was prepared from commercially available hexadecandioic acid as shown in the scheme. The monoester monochloride (3) of the dicarboxylic acid was coupled with myristyl zinc chloride to give a straight

chain keto ester (4) (60%, mp 77-78°C)[5] according to the known method for the compound of a similar type.[6] Since direct Huang-Minlon reduction of the carbonyl function in 4 did not proceed well, 4 was converted into the saturated acid in the following three steps, *i.e.*, reduction with $NaBH_4$ (refluxing in chloroform-ethanol (1:1)) followed by conversion into the iodo acid (5) (64% from 4, mp 65-67°C)[5] and then reductive removal of the iodine atom.

The B-type acids were obtained by alkylation of diethyl malonate followed by hydrolysis and decarboxylation. Thus, 2-tetradecylhexadecanoic acid[5] and 2-docosyltetracosanoic acid[5] were prepared by using myristyl bromide and docosyl bromide, respectively. On the other hand, BH-type acids were synthesized according to Polonsky and Lederer.[7] Thus, 3-hydroxy-2-tetradecyloctadecanoic acid[5] and 3-hydroxy-2-docosylhexacosanoic acid[5] were prepared *via* Claisen condensation of methyl palmitate and methyl tetracosanoate, respectively. These two acids were used as racemic mixtures of each two diastereomers[7] for further coupling reaction with muramic acid.

The L- and B-type acids without second functional groups in the molecules could be converted into their acid chlorides, which could be used for the direct acylation of the 6-hydroxyl group in 1-α-*O*-benzyl-*N*-acetylmuramyl-L-alanyl-D-isoglutamine benzyl ester (6) as described previously in the synthesis of 6-*O*-stearoyl derivative.[8] In fact, the reaction of 6 with 2-tetradecylhexadecanoyl chloride in a mixture of pyridine and tetrahydrofuran afforded the protected 6-*O*-acyl muramyl dipeptide (7c) (45%, mp 173-174°C).[5] However, this reaction was troublesome to proceed because of the poor reactivity and the low solubility of the acid chloride as compared to acylation with usual fatty acids



of less carbon atoms. Therefore, such a synthetic route seemed not to be preferable to be applied for B46 and L30 acids with poorer solubilities than B30 acid.

As the result, the novel procedure employed for the preparation of mycoloyl derivatives[2] must be adopted also for introduction of B46 and L30 acids as well as the two BH-type acids. When 1-α-*O*-benzyl-6-*O*-tosyl-*N*-acetylmuramic acid diphenylmethyl ester (8) was heated with the potassium salt of each synthetic acid in the presence of 18-crown-6 in boiling benzene, the corresponding 6-*O*-acyl-1-α-*O*-benzyl-*N*-acetylmuramic acid diphenylmethyl ester (9)[5] was formed in a good yield. After removal of the diphenylmethyl group in 9 with trifluoroacetic acid, the product was condensed with L-alanyl-D-isoglutamine benzyl ester by means of dicyclohexylcarbodiimide–*N*-hydroxysuccinimide to give 6-*O*-acyl-1-α-*O*-benzyl-*N*-acetylmuramyl-L-alanyl-D-isoglutamine benzyl ester (7).[5] The final deprotection was carried out by hydrogenolysis of 7b-f with palladium black catalyst in tetrahydrofuran to give the desired 6-*O*-acyl-*N*-acetylmuramyl-L-alanyl-D-isoglutamines (2b-f).[5] The physical properties of these products and their synthetic intermediates (7b-f) are summarized in Table 2.

Concerning the biological activity, most of the 6-*O*-acyl muramyl dipeptides prepared in this study showed antitumor activity as shown in Table 3. It should be noticed that two of them (2e and f) are the first pure synthetic compounds

Table 1. Synthetic fatty acids used in this study

Abbreviation[4]	Name	Structure	Mp (°C)
L30 acid	Triacontanoic acid	$CH_3-(CH_2)_{28}-CO_2H$	97 - 99
B30 acid	2-Tetradecylhexadecanoic acid	$CH_3-(CH_2)_{13}-\underset{\displaystyle CH_3-(CH_2)_{13}}{\overset{}{\underset{}{CH}}}-CO_2H$	73.5 - 75
BH32 acid	3-Hydroxy-2-tetradecyl-octadecanoic acid	$CH_3-(CH_2)_{14}-\underset{\displaystyle CH_3-(CH_2)_{13}}{\overset{\displaystyle OH}{\underset{}{CH}}}-CO_2H$	72 - 75
B46 acid	2-Docosyltetracosanoic acid	$CH_3-(CH_2)_{21}-\underset{\displaystyle CH_3-(CH_2)_{21}}{\overset{}{\underset{}{CH}}}-CO_2H$	87 - 89
BH48 acid	3-Hydroxy-2-docosyl-hexacosanoic acid	$CH_3-(CH_2)_{22}-\underset{\displaystyle CH_3-(CH_2)_{21}}{\overset{\displaystyle OH}{\underset{}{CH}}}-CO_2H$	89 - 90

Table 2. Yields and physical constants of 7 and 2

	Yield (%)	$[\alpha]_D$*1	Mp (°C)		Yield (%)	$[\alpha]_D$*2	Mp (dec,°C)
7b	63	+52.2°	181 - 182	2b	96	+30.7°	185 - 186
7c*3	45	+52.9°	173 - 174	2c	86	+32.8°	152 - 155
7d	59	+47.4°	176 - 178	2d	89	+30.8°	170 - 172
7e	84	+34.8°	161 - 163	2e	93	+22.7°	150
7f	78	+43.3°	171 - 173	2f	82	+22.0°	169 - 170

*1 *c* 1 in $CHCl_3$. *2 *c* 0.5 in $CHCl_3$-methanol (1:1).
*3 Prepared by the acid chloride method; see text.

possessing significant activity based on immuno reaction. Moreover, the following important elucidations were obtained on the relationship between the structures and the activity of acyl muramyl dipeptides from this synthetic study. i) The heterogeneity and the presence of double bonds, cyclopropane rings or methoxy groups involved in natural mycolic acids are not essential for the activity. ii) Acyl groups with carbon numbers more than 46 are more favorable than those around 30. iii) The presence of the α-side chain and the β-hydroxyl group enhances the antitumor activity more or less.

Table 3. Antitumor activity of synthetic 6-*O*-acyl *N*-acetylmuramyl-L-alanyl-D-isoglutamine*1

Compound	Dose (μg)	Suppression of tumor*2
2b (RCO = L30)	100	0/10
2c (" = B30)	100	1/10
2d (" = BH32)	100	2/10
2e (" = B46)	100	4/10
2f (" = BH48)	100	7/10
2a (" = Nocardomycoloyl)	100	5/10
control	0	0/10

*1 A mixture of 20-methylcholanthrene-induced ascites tumor cells (2×10^5) and oil attached each compound was innoculated intradermally into BALB/c mice.
*2 No. of tumor-free mice/No. of mice treated.

References and Footnotes

1) This work was presented at the 37th Annual Meeting of the Chemical Society of Japan, Kanagawa, April, 1978; cf. Abstract p.1121.
2) a) S. Kusumoto, S. Okada, T. Shiba, I. Azuma, and Y. Yamamura, *Tetrahedron Lett.*, 1976, 4287. b) T. Shiba, S. Okada, S. Kusumoto, I. Azuma, and Y. Yamamura, *Bull. Chem. Soc. Jpn.*, 51, in press (1978).
3) Y. Yamamura, I. Azuma, K. Sugimura, M. Yamawaki, M. Uemiya, S. Kusumoto, S. Okada, and T. Shiba, *Proc. Jpn. Acad.*, 53, 63 (1977).
4) Following abbreviations are used for the synthetic acid in this study. The capital letters L, B, and H indicate "linear" "α-branched", and "β-hydroxylated", respectively. The structures of the individual acids are represented by combination of these symbols and the total numbers of their carbon atoms. For instance, "L30 acid" stands for triacontanoic acid and "BH32 acid" for 3-hydroxy-2-tetradecyloctadecanoic acid.
5) Satisfactory elemental analysis was obtained.
6) R. R. Reinhard and J. A. Dixon, *J. Org. Chem.*, 30, 1450 (1965).
7) J. Polonsky and E. Lederer, *Bull. Soc. Chim. Fr.*, 1954, 504.
8) S. Kusumoto, S. Okada, K. Yamamoto, and T. Shiba, *Bull. Chem. Soc. Jpn.*, 51, 2122 (1978).

(Received in Japan 7 September 1978)