SYNTHESIS OF BIOLOGICALLY ACTIVE PENTAPEPTIDE ANALOGS OF THE N-TERMINAL PART OF LIPOPROTEIN FROM THE OUTER MEMBRANE OF ESCHERICHIA COLI

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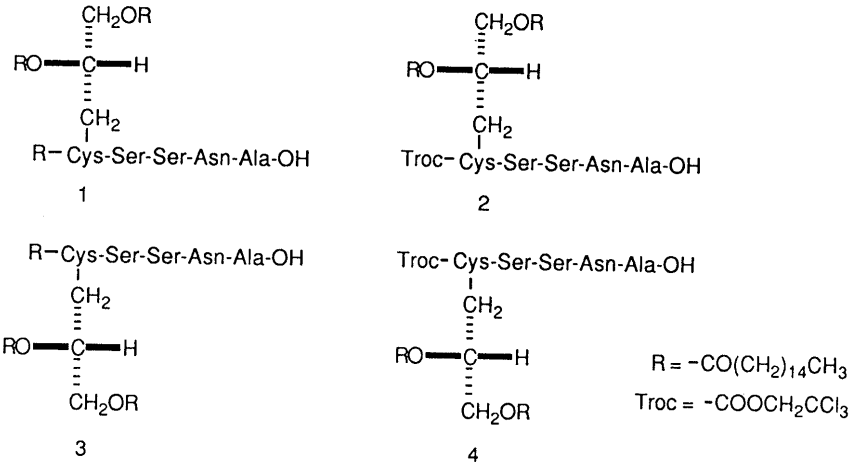
Newly synthesized lipopentapeptide derivatives with (R)-glycerol moieties showed higher mitogenic activities than those with the (S)-configuration.

KEYWORDS peptide synthesis; lipoprotein segment; mitogenic activity; chiral glycerol derivative; S-[2,3-bis(palmitoyloxy)propyl]-N-trichloroethoxycarbonyl pentapeptide

The lipoprotein[1] from the outer membrane of Escherichia coli and other Enterobacteriaceae is a potent polyclonal activator for B lymphocytes. To determine the molecular structure responsible for the biological activities of lipoprotein, a series of oligopeptide analogs of its N-terminal part were synthesized.[2,3] S-[2,3-bis(palmitoyloxy)-(2-RS)-propyl]-N-palmitoyl-(R)-cysteinyl-(S)-seryl-(S)-seryl-(S)-asparaginyl-(S)-alanine was an active mitogen and polyclonal B lymphocyte activator in vitro and in vivo.[4~6] It also supplements Salmonella vaccins.[7] In this paper we describe a new synthesis of S-[2,3-bis(palmitoyloxy)-(2R and 2S)-propyl]-N-palmitoyl-(R)-cysteinyl-(S)-seryl-(S)-asparaginyl-(S)-alanine (1 and 3) and their N-(2,2,2-trichloroethoxycarbonyl)(2 and 4) by using the N-(2,2,2-trichloroethoxycarbonyl)cysteinyl intermediates, which prevents the racemization of their cysteinyl parts in the condensation steps.



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The compounds 1, 2, 3 and 4 were synthesized according to the reaction sequence shown in Chart 1. The starting material 5 was prepared according to the method reported by K. H. Wiesmuller et al.[2] N-protection of 5 with 2,2,2-trichloroethoxychloroformate (3 eq) in pyridine followed by reduction with dithioerythritol (4 eq) in $CHCl_3$ in the presence of triethylamine (3 eq) afforded 7, which was used without further purification. Reaction of 7 with (R)-8[8] in dimethylformamide in the presence of N,N-diisopropylethylamine (4 eq) gave 9 (55% from 6). Esterification of 9 with palmitoyl chloride(2 eq) and N,N-diisopropylethylamine (4 eq) in CH_2Cl_2 in the presence of a catalytic amount of 4-dimethylaminopyridine followed by deprotection of the tert-butyl group of 10 with trifluoroacetic acid afforded 11 in 69% yield from (R)-8. Compound 13 was obtained in 61% yield by coupling 11 with the pentapeptide 12[9] in DMF using dicyclohexylcarbodiimide (1.1 eq) and 1-hydroxybenzotriazole (2 eq) as a coupling agent according to the method[2] reported by K. H. Wiesmuller et al. Deprotection of all tert-butyl groups of 13 was carried out by treatment with trifluoroacetic acid to give 2[10] in 45% yield. The trichloroethoxycarbonyl group of 13 was removed by treatment with zinc in acetic acid to give 14, which was then acylated with palmitoyl chloride and N,N-diisopropylethylamine in CH_2Cl_2 to afford 15. The final deprotection of all tert-butyl groups of 15 was carried out by treatment with trifluoroacetic acid to give 1[11] (53% yield from 13). In the same way the compounds 3[12] and 4[13] were synthesized by using (S)-8[14] in place of (R)-8. The structures of 1, 2, 3 and 4 were supported by elemental analysis and confirmed by analysis of the IR, ^{1}H-NMR and FABMASS spectra. The chemical purity of 1,2,3 and 4 were determined to be 99.4% (t_R=4.31 min), 99.4%(t_R=4.36 min), 99.9%(t_R=4.28 min) and 99.4%(t_R=4.35 min) respectively by high performance liquid chromatography (HPLC) using an Asahipak column ODP-50 [0.6 x 15 cm, λ=210 nm, 0.1% TFA/CH_3CN $^{16}/_{84}$ (7.5 min) → $^{0}/_{100}$(12.5 min),

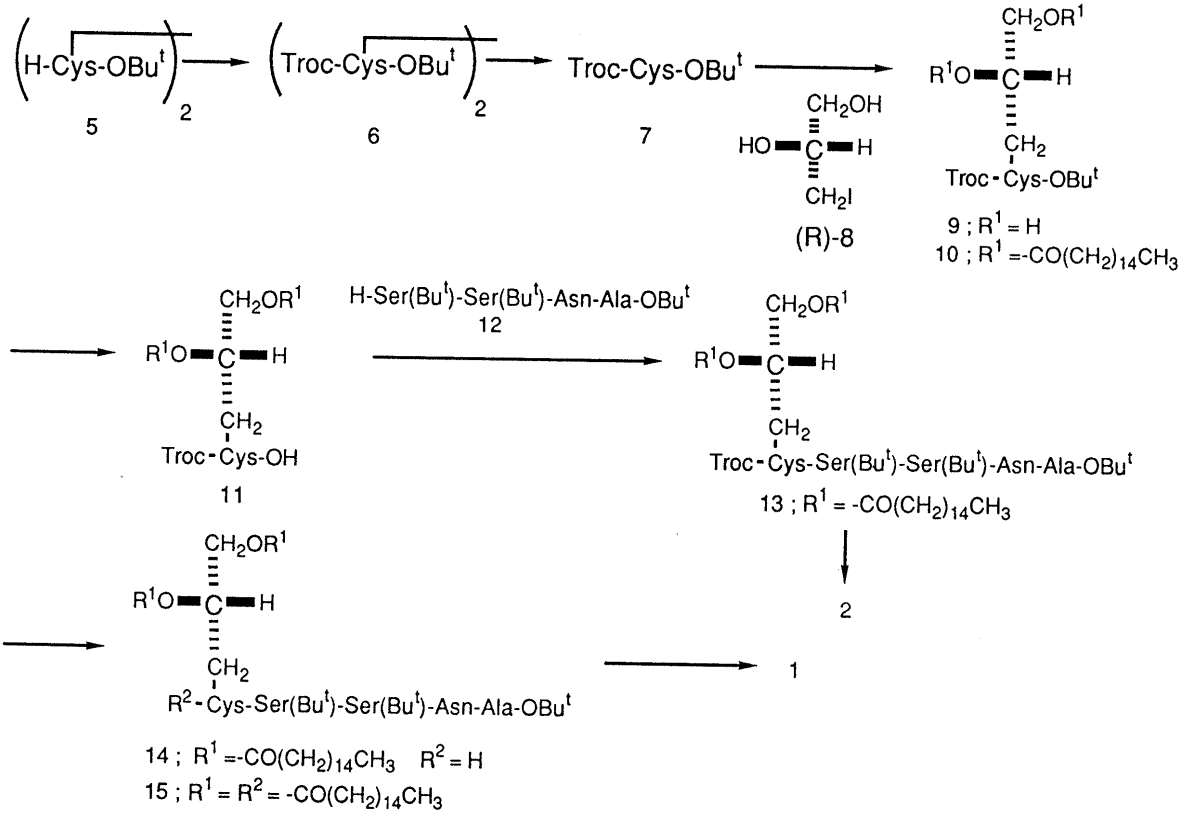

Chart 1

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flow rate 1.0 ml/min]. The mitogenic activities of all the lipopentapeptides 1, 2, 3 and 4 were measured. Compounds 1 and 4 had the same degree of activity and the activity of 2 was greatly enhanced. While the compound 3 activity was weak. These results indicate that the natural [(2R)-propyl] type 1 has a higher activity than the unnatural [(2S)-propyl] type 3 and that the Troc derivative increases mitogenic activity.

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8) (R)-8 was synthesized from (S)-1-O-tosyl-2-benzyl-glycerol[15] in 65% yield, by deprotection of benzyl group (H_2,Pd/C) and subsequent iodidation with NaI (3 eq) in a pressure bottle. mp 35~37 ℃ $[\alpha]_D$= -6.0°(C=1.15 ,$CHCl_3$), IR (KBr): 3334 (OH).
9) 12 was synthesized from Z-Asn-Ala-OBu^t [16] in the following steps [i. removal of Z-group (H_2,Pd/C), ii. condensation of Z-Ser(Bu^t)-OH and H-Asn-Ala-OBu^t, iii. condensation of Z- Ser(Bu^t)-OH and H-Ser(Bu^t)-Asn-Ala-OBu^t, iv. removal of Z-group (H_2, Pd/C)], which was identical with the corresponding authentic sample by Bessler[2] in every respect (^{1}H-NMR, mp, Rf, FABMASS).
10) mp 205~207 ℃ (white powder from $CHCl_3$:MeOH=1:1), $[\alpha]_D$=+9.20°(C=1.00 ,$CHCl_3$), FABMASS: m/z (M+H)$^+$ 1205, IR (KBr): 3300 (OH, NH), 1736 (O=C-O), 1662, 1537 (CONH).
11) mp 211~213 ℃ (white powder from $CHCl_3$:MeOH=1:1), $[\alpha]_D$=+56.5°(C=1.02 ,$CHCl_3$), FABMASS: m/z (M+H)$^+$ 1270, IR (KBr): 3284 (OH, NH), 1732 (O=C-O), 1627, 1550 (CONH).
12) mp 210~212 ℃ (white powder from $CHCl_3$:MeOH=1:1), $[\alpha]_D$=-28.3°(C= 0.86 ,$CHCl_3$), FABMASS: m/z (M+H)$^+$ 1270, IR (KBr): 3296 (OH, NH), 1736 (O=C-O), 1639, 1538 (CONH).
13) mp 204~207 ℃ (white powder from $CHCl_3$:MeOH=1:1), $[\alpha]_D$=+16.6°(C=1.00 ,$CHCl_3$), FABMASS: m/z (M+H)$^+$ 1205, IR (KBr): 3302 (OH, NH), 1737 (O=C-O), 1629, 1538 (CONH).
14) (S)-8 was synthesized starting from (S)-1-O-tosyl-2-benzyl glycerol in 51% yield in the following steps [i. protection with methoxymethyl chloride (1.2 eq), ii. deacetylation with NaOH, iii. tosylation with tosyl chloride, iv. demethoxymethylation with HCl, v. removal of benzyl group (H_2, Pd/C), vi. iodidation with NaI in a pressure bottle.]. mp 35~37 ℃, $[\alpha]_D$= +6.0° (C=1.35, $CHCl_3$), IR:(KBr) 3334 (OH)
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