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Synthesis and Absolute Configuration of a Novel Aminoglycoglycerolipid, Species-Specific Major Immunodeterminant of *Mycoplasma fermentans*

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Abstract: In order to determine the absolute configuration of a novel aminoglycoglycerolipid isolated from *Mycoplasma fermentans*, the possible two diastereomers were stereoselectively synthesized by using *(S)*- and *(R)*-glycidols as the key building block. Their ^{1}H-^{1}H-cosy spectra compared with those of the natural product allowed us to determine the absolute configuration of the glycolipid.

Key Words: amino-alcohols, glycolipids, immunodeficiency, phospholipids, phosphoramidites

Recently, two novel phosphocholine-containing glycoglycerolipids were isolated as the main cell membrane lipid components of *Mycoplasma fermentans* [1-3].[1] They have a unique common structure bearing a phosphocholine at C-6 of α-D-glucopyranosyl glyceride called GGPL-I and GGPL-III [2] (or MfGL-I and MfGL-II [3]), respectively. The latter has a higher structure carrying a 2-amino-1,3-propanediol (serinol) between the phosphocholine and the C-6-phosphate (Figure 1). They are determined to be species-specific major immunodeterminants of *M. fermentans* [7], and their specific antibodies are detected widely in the sera of HIV-infected individuals [8]. It is also observed that GGPL-III enhances fusogenecity of *M. fermentans* with

[1] Isolation of GGPL-I was reported by Matsuda et al (lit. 1), whereas that of GGPL-III by the same group (lit. 2) and Zaehringer *et al* (lit. 3) independently at nearly the same time. *M. fermentans* is suspected to have certain pathogenic roles in rheumatoid arthritis disorders (lit. 4) and the progression of human immunodeficiency virus (HIV) diseases (lit. 5,6), though the underlying mechanism is not clarified at the molecular level.

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Molt-3 lymphocytes [9]. These observations have suggested the pathological importance of GGPL-I and GGPL-III and their major roles in *M. fermentans*-host cell interactions. As part of our research project to develop artificial glycoconjugate polymers which adsorb certain pathogenic bacteria and viruses [10,11], our interest is directed to the potential utility of these new aminoglycolipids. In this communication, we describe the diastereoselective synthesis of the two GGPL-III stereoisomers to determine the absolute configuration of the natural product.

Although the absolute structure of GGPL-I was already established by its stereoselective synthesis [12], that of GGPL-III remains ambiguous in the segment of 2-amino-1,3-propanediol (serinol). To clarify the absolute configuration, we performed the diastereoselective synthesis of both diastereo isomers, *(S)*-GGPL-III and *(R)*-GGPL-III (Scheme 3) by using 2-azido-3 (or 1-)-*O*-*p*-methoxyphenyl-1,3-propanediols *(S)*-**6** and *(R)*-**6** as key synthetic precursors. They were designed taking into account their ease of preparation from commercially available *(R)*- and *(S)*-glycidol as summarized in Scheme 1. They are obtained as crystalline solids[1] in optically pure forms (^{1}H-NMR analysis using a chiral derivatizing agent *(S)*-TBMB carboxylic acid [13]). In order to verify the utility of *(S)*- and *(R)*-**6** for the synthesis of GGPL-III,

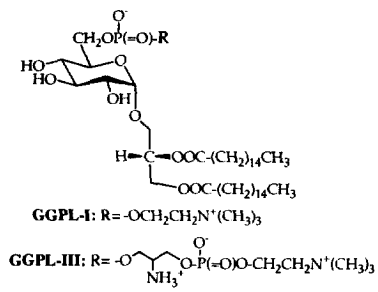
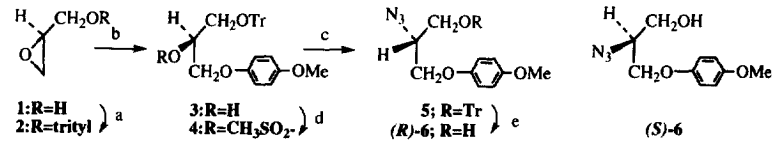


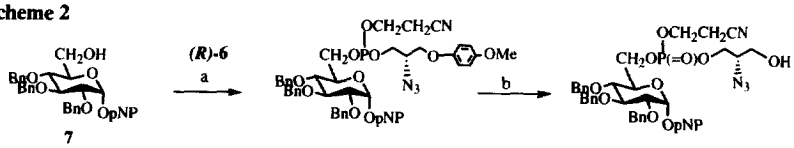
Figure 1 Structures of GGPL-I and GGPL-III

Scheme 1



a: TrCl/pyridine-CH_2Cl_2, 0℃. b: Sodium *p*-methoxyphenoxide/DMF, 100℃, 99%.c:MsCl/Pyridine-CH_2Cl_2,99%. d:NaN_3/DMF,100℃, 85%. e:Amberlyst-R15 (H^+)/MeOH, 55 ℃ then Et_3N, 93%.

Scheme 2



a: 2-cyanoethyl-N,N,N',N'-tetraisopropylphosphorodiamidite, 1*H*-tetrazole, MS4A then *(R)*-**6** / CH_2Cl_2, 0℃.
b: silver (II) bis(pyridine-2,6-dicarboxylate)monohydrate/MeCN/H_2O/MeOH, rt (75 % yield from **7**).

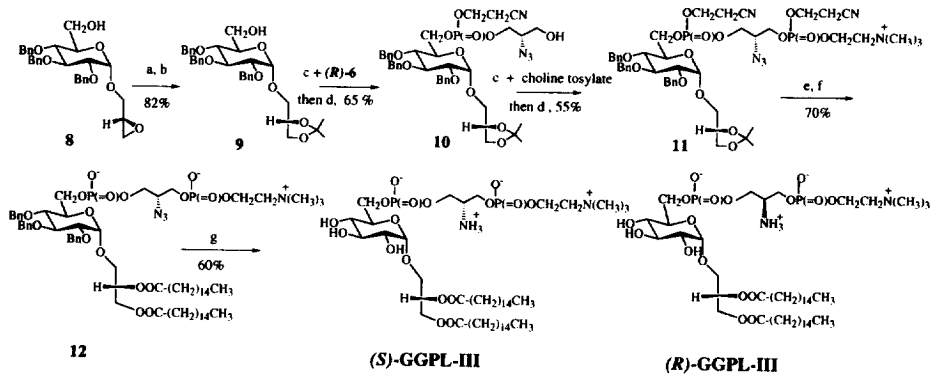
they each were preliminarily coupled with *p*NP 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **7** by a phosphoramidite method [14,15] (Scheme 2). This experiment enabled us to find a silver (II) dipicolinate-H_2O complex [16] as an effective agent which is able to oxidize both the phosphite and the *p*-methoxyphenylether in

[1] Physical data of (R)-**6**:mp=58~60℃, $[\alpha]_D$=+38.8° (c 0.52, MeOH), Anal Calcd. C 53.80; H 5.88; N 18.81.Found C 53.69; H 5.80; N 18.75. (S)-**6**:mp=59~59.5℃, $[\alpha]_D$=-37.8° (c 0.50, MeOH), Anal Found C 53.53; H 5.72; N 18.70. ^{1}H-NMR (CDCl3, 400 MHz); 3.78 (3H, s, $-OCH_3$), 4.13 and 4.08 (2H, dd, 6.3 and 10.0 Hz, $-CH_2$-O-pMP), 3.85 and 3.78 (2H, m, multiple spin couplings of ABX type), 3.90 (1H, m, -HCN$_3$).

an CH_3CN-H_2O-MeOH mixture; when the oxidant was used in CH_3CN-CH_2Cl_2-MeOH, selective oxidation at the phosphite moiety resulted.

For the synthesis of both *(R)*- and *(S)*-GGPL-III, 3-*O*-[2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl]-*(S)*-glycidol **8** [12] was used as the other key synthetic precursor to construct the segment of 3-*O*-(α-D-glucopyranosyl)-*sn*-glyceride. The *(S)*-glycidol was selected as the aglycon assuming that the natural GGPL-III has the same *(2S)*-configuration at the glyceride moiety as GGPL-I [12]. Epoxide opening for **8** with cessium acetate in DMF followed by usual deacetylation and isopropylidenation procedures gave 1,2-*O*-isopropylidene-3-*O*-(2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-*sn*-glycerol **9**[1]. Successive introduction of a 2-cyanoethyl *N*-diisopropylphosphoramidite and then *(R)*-**6** was carried out in a previously established manner, which was followed by oxidation by the silver (II) complex *in situ* to afford **11** in 55 % yield. Introduction of dipalmitates to the glycerol moiety was performed after removal of the 2-cyanoethyl group by aqueous ammonia in methanol [17] and then isopropylidene by 5% trifluoroacetic acid in methanol to afford **12** in 70% yield. [**12**: FAB-MS; 1367 $(M+Na)^+$, 1343 $(M-1)^-$ in *m*-nitrobenzylalcohol]. Catalytic hydrogenation in an 1% acetic acid in methanol afforded the desired *(S)*-GGPL-III as amorphous powder after being purified by silica gel (eluted by CH_3COOH-H_2O-CH_3OH-$CHCl_3$) and then ODS (CH_3CN-CH_3OH-*n*-BuOH) columns. In the same way, *(R)*-GGPL-III was prepared by using *(S)*-**6**.

Scheme 3



a: AcOCs/DMF, 80℃ then NaOMe/MeOH. b: *p*-TsOH/acetone, rt. c: 2-cyanoethyl-*N,N,N',N'*-tetraisopropyl phosphorodiamidite, 1*H*-tetrazole, MS4A/CH_2Cl_2, 0℃. d: silver (II) bis(pyridine-2,6-dicarboxylate)monohydrate / MeCN/H_2O/MeOH, rt. e: aq.NH_4OH/MeOH then CF_3COOH/MeOH/H_2O. f: palmitoyl chloride, dimethylamino pyridine/pyridine. g: H_2, $Pd(OH)_2$/CH_3COOH/MeOH, rt.

FAB-MS [1049 $(M+1)^+$ in triethanolamine] and the ^{1}H-NMR spectra of synthetic *(R)*- and *(S)*-GGPL-III accorded with the proposed primary structure of GGPL-III [2,3]. ^{1}H-NMR signals of all glyceride protons of *(R)*- and *(S)*-GGPL-III completely matched in both chemical shifts and coupling constants with those of the natural product. This means that the natural GGPL-III has the *(2S)*-configuration at the glyceride moiety as GGPL-I does [12]. On the other hand, the ^{1}H-NMR signals of the serinol methylene protons (indicated as **A** and **B** in Figure 2) showed an apparent difference in the chemical shifts; the methylene signals of the *(R)*-isomer shift at higher fields than those of the *(S)*-isomer. The chemical shifts of the natural product well accorded with

[1] Gigg et al has reported an alternative synthetic way to the compound **9** (lit. 16).

those of the *(S)*-isomer, though slight deviations are observable[1] in the shape of overlapping signals at 3.45~3.70 ppm. These observations allow us to conclude that natural GGPL-III has the *(S)*-configuration at the serinol moiety.

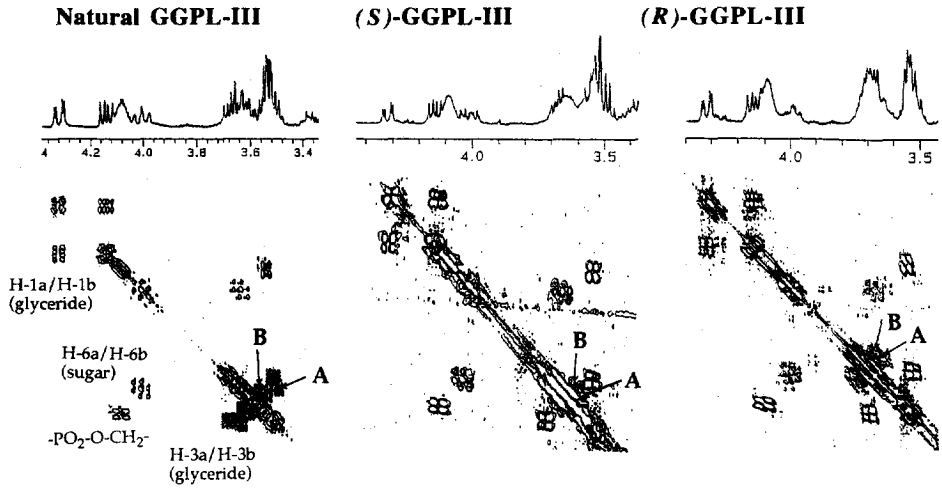


Figure 2. Partial ^{1}H-^{1}H COSY NMR spectra of natural and synthetic GGPL-IIIs (DMSO-d_6, 50 ℃). Serinol methylene protons are signed with A (-$\underline{CH_2}$-CH(NH_2)-CH_2-) and B (-CH_2-CH(NH_2)-$\underline{CH_2}$-).

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[1] The ^{1}H-NMR spectrum of GGPL-III shows strong solvent or concentration dependence (lit. 2 and 3) arising possibly from its high ionic and surfacactive structure. The measurement was, therefore, performed under the same conditions (temperature, solvent and concentration) for synthetic *(R)*- and *(S)*-isomers as far as possible. The slight NMR deviation between the natural GGPL-III and the synthetic *(S)*-isomer may be due to the measurement conditions or to contamination by ions or solvents.