ORGANIC LETTERS

2006
Vol. 8, No. 5
943−946

Total Synthesis of Geranylgeranylglyceryl Phosphate Enantiomers: Substrates for Characterization of 2,3-*O*-Digeranylgeranylglyceryl Phosphate Synthase

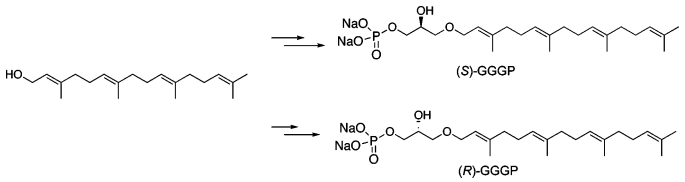
Honglu Zhang,[†] Kyohei Shibuya,[‡] Hisashi Hemmi,[‡] Tokuzo Nishino,[‡] and Glenn D. Prestwich*,[†]

Department of Medicinal Chemistry, The University of Utah, 419 Wakara Way, Suite 205, Salt Lake City, Utah 84108-1257, and Department of Biomolecular Engineering, Tohoku University, Aoba-yama 07, Sendai, Miyagi 980-8579, Japan

gprestwich@pharm.utah.edu

Received December 20, 2005

ABSTRACT



To determine the enantioselectivity of (*S*)-2,3-di-*O*-geranylgeranylglyceryl phosphate synthase (DGGGPS) from the thermoacidophilic archaeon *Sulfolobus solfataricus*, we developed an efficient enantioselective route to the enantiomeric geranylgeranylglyceryl phosphates (*R*)-GGGP and (*S*)-GGGP. Previous routes to these substrates involved enzymatic conversions due to the lability of the polyprenyl chains toward common phosphorylation reaction conditions. The synthesis described herein employs a mild trimethyl phosphite/carbon tetrabromide oxidative phosphorylation to circumvent this problem. In contrast to previous results suggesting that only (*S*)-GGGP can act as the prenyl acceptor substrate, both (*R*)-GGGP and (*S*)-GGGP were found to be substrates for DGGGPS.

All living organisms have been classified into three primary kingdoms: archaebacteria, eubacteria, and eukaryotes.[1] Although the membrane lipids in eubacteria and eukaryotes are composed of glyceryl esters of fatty acids, archaebacterial membrane lipids contain isopranyl glyceryl ethers.[2,3] The core structures of archaeal membrane lipids, including diether and bipolar tetraether lipids, contain fully reduced C_{20} or C_{25} prenyl groups.[4,5] Biosynthesis of the isoprene moieties in the core lipids follows the mevalonate pathway used also by eubacteria and eukaryotes.[6−8] The prenyl transfer reactions responsible for building the isoprenoid diphosphates, e.g., geranylgeranyl diphosphate (GGPP), are catalyzed by a

* To whom correspondence should be addressed. Phone: +1-801-585-9051. Fax: +1-801-585-9053.
[†] The University of Utah.
[‡] Tohoku University.

(1) Woese, C. R. *Sci. Am.* **1981**, *244*, 98−122.
(2) Langworthy, T. A.; Tornabene, T. G.; Holzer, G. *Zentralbl. Bakteriol., Mikrobiol. Hyg., Abt. I, Orig. C.* **1982**, *3*, 228−244.
(3) Langworthy, T. A.; Pond, J. L. *Syst. Appl. Microbiol.* **1986**, *7*, 253−257.
(4) Kates, M. *Prog. Chem. Fats Other Lipids* **1978**, *15*, 301−342.
(5) Heathcock, C. H.; Finkelstein, B. L.; Aoki, T.; Poulter, C. D. *Science* **1985**, *229*, 862−864.
(6) De Rosa, M.; De Rosa, S.; Gambacorta, A. *Phytochemistry* **1977**, *16*, 1909−1912.
(7) De Rosa, M.; Gambacorta, A.; Nicolaus, B. *Phytochemistry* **1980**, *19*, 791−793.
(8) Moldoveanu, N.; Kates, M. *Biochim. Biophys. Acta* **1988**, *960*, 164−182.

10.1021/ol0530878 CCC: $33.50
Published on Web 01/28/2006

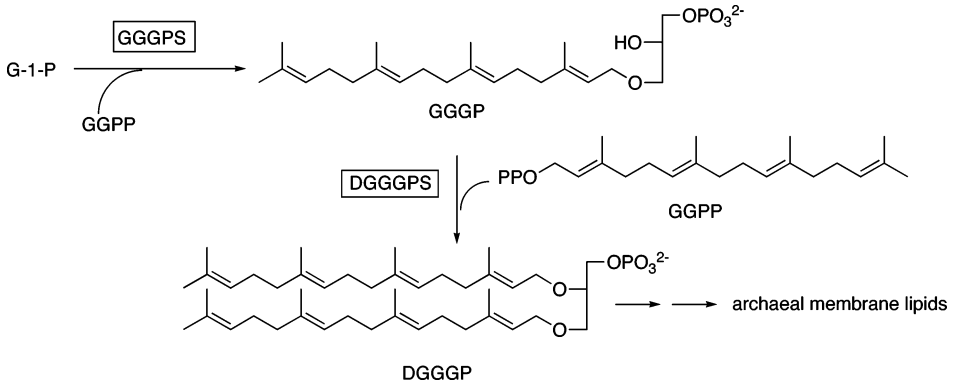


Figure 1. Biosynthesis of DGGGP, a key intermediate for archaeal membrane lipids. Key: G-1-P, (*S*)-glyceryl phosphate; GGGPS, (*S*)-GGGP synthase; DGGGPS, DGGGP synthase.

family of prenyltransferases.[9−12] As shown in Figure 1, the biogenesis of the core structure of the archaeal membrane lipids starts with the prenyl transfer reaction catalyzed by (*S*)-3-*O*-geranylgeranylglyceryl phosphate [(*S*)-GGGP] synthase, which selectively uses (*S*)-glyceryl phosphate as the prenyl acceptor (Figure 1). Then, the product is utilized as the presumed acceptor substrate for the biosynthesis of (*S*)-2,3-di-*O*-geranylgeranylglyceryl phosphate (DGGGP), an advanced intermediate of archaeal membrane lipids.[13]

To date, only an enzyme-assisted synthesis of (*S*)-GGGP has been reported.[11] An enantiospecific chemical synthesis of both individual enantiomers of GGGP was required to validate this biosynthetic hypothesis, in part because of the acid-sensitive geranylgeranyl group of GGGP. To fully characterize the substrate selectivity of DGGGP synthase (DGGGPS), we developed a mild and effective route to the two GGGP enantiomers. The instability of GGGP indeed posed significant challenges, and the biological results with the enantiomers were unexpected.

The synthesis of (*S*)-GGGP (**8**) is summarized in Scheme 1. Treatment of the (2*E*,6*E*,10*E*)-geranylgeraniol **1** with Ph_3P/CBr_4 at 25 °C afforded geranylgeranyl bromide **2**.[14] Next, (*S*)-solketal was alkylated with geranylgeranyl bromide by using KH as base, to give ether **3** in 73% yield.[11] The reported HCl/THF method to remove the acetonide[13] resulted in a complex mixture containing the desired product in low yield. The desired diol **4** was thus prepared in 75% yield using *p*-TsOH in methanol.[15,16] To obtain selective phosphorylation of the primary hydroxyl, we first tried a strategy involving protection of the secondary hydroxyl as a silyl ether. Thus, both hydroxyl groups of diol **4** were protected as TBS ethers (TBSCl, imidazole, anhydrous DMF).[15,16] Subsequently, the more labile primary TBS ether was selectively removed at room temperature by using HF·Py (HF·Py/Py/THF = 1:2:5). Unfortunately, phosphorylation of the primary alcohol under standard conditions (dimethylphosphoryl chloride, *t*-BuOK, CH_2Cl_2)[15,16] failed to give the desired product because of the lability of the polyene system.

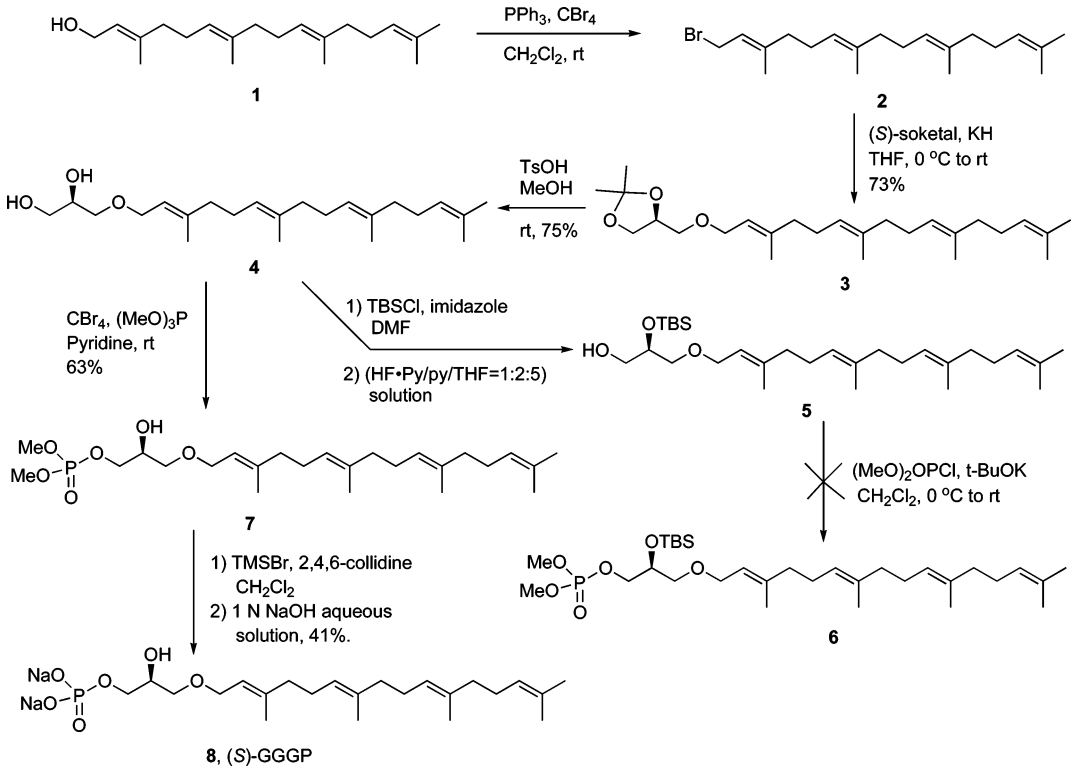
A second strategy proved more successful. The use of the trimethyl phosphite/carbon tetrabromide oxidative phosphorylation method[17] was deemed sufficiently mild to permit phosphorylation without damage to the geranylgeranyl moiety. Treatment of diol **4** with 1.1 equiv of CBr_4 and 1.2 equiv of $P(OMe)_3$ gave selective phosphorylation of the primary alcohol to give the protected phosphate **7**. Essentially no bisphosphate product was detected.

The next challenge in this synthesis was liberation of the free phosphate monoester from the protected triester. We first tried TMSBr, a standard deprotecting reagent for removal of methyl and ethyl groups in the synthesis of acyl-migration-prone lysophosphatidic acid derivatives.[15,16] However, GGGP did not survive this strong Lewis acid. By using a solution of TMSBr in 2,4,6-trimethylpyridine (*sym*-collidine),[18−20] we obtained the desired monophosphate in the acidic form. Titration with 1 N aq NaOH afforded (*S*)-GGGP (**8**) as the stabilized sodium salt.

To determine the enantioselectivity of DGGGPS, both the enantiomers (*S*)-GGGP (**8**) and (*R*)-GGGP (**12**) were re-

(9) Poulter, C. D. In *Biochemistry of Cell Walls and Membranes in Fungi*; Kuhn, P. J., Trinci, A. P. J., Jung, M. J., Goosey, M. W., Copping, L. G., Eds.; Springer-Verlag: Berlin, Heidelberg, 1990; pp 169−188.
(10) Poulter, C. D. In *Biosynthesis of Isopreniod compounds*; Rilling, H. C., Porter, J. W., Spurgeon, S. L., Eds.; Wiley: New York, 1981; Vol. I, pp 161−224.
(11) Zhang, D.; Poulter, C. D. *J. Am. Chem. Soc.* **1993**, *115*, 1270−1277.
(12) Ohnuma, S.-i.; Suzuki, M.; Nishino, T. *J. Biol. Chem.* **1994**, *269*, 14792−14797.
(13) Hemmi, H.; Shibuya, K.; Takahashi, Y.; Nakayama, T.; Nishino, T. *J. Biol. Chem.* **2004**, *279*, 50197−50203.
(14) Tokumasu, M. A. H.; Hiraga, Y.; Kojima, S.; Ohkta, K. *J. Chem. Soc., Perkin Trans. 1* **1999**, 489−496.
(15) Xu, Y.; Prestwich, G. D. *J. Org. Chem.* **2002**, *67*, 7158−7161.
(16) Xu, Y.; Qian, L.; Prestwich, G. D. *J. Org. Chem.* **2003**, *68*, 5320−5330.
(17) Oza, V. B.; Corcoran, R. C. *J. Org. Chem.* **1995**, *60*, 3680−3684.
(18) Cermak, D. M.; Wiemer, D. F.; Lewis, K.; Hohl, R. J. *Bioorg. Med. Chem.* **2000**, *8*, 2729−2737.
(19) Macchia, M.; Jannitti, N.; Gervasi, G.; Danesi, R. *J. Med. Chem.* **1996**, *39*, 1352−1356.
(20) Magnin, D. R.; Biller, S. A.; Dickson, J. K., Jr.; Logan, J. V.; Lawrence, R. M.; Chen, Y.; Sulsky, R. B.; Ciosek, C. P., Jr.; Harrity, T. W.; Jolibois, K. G.; et al. *J. Med. Chem.* **1995**, *38*, 2596−2605.

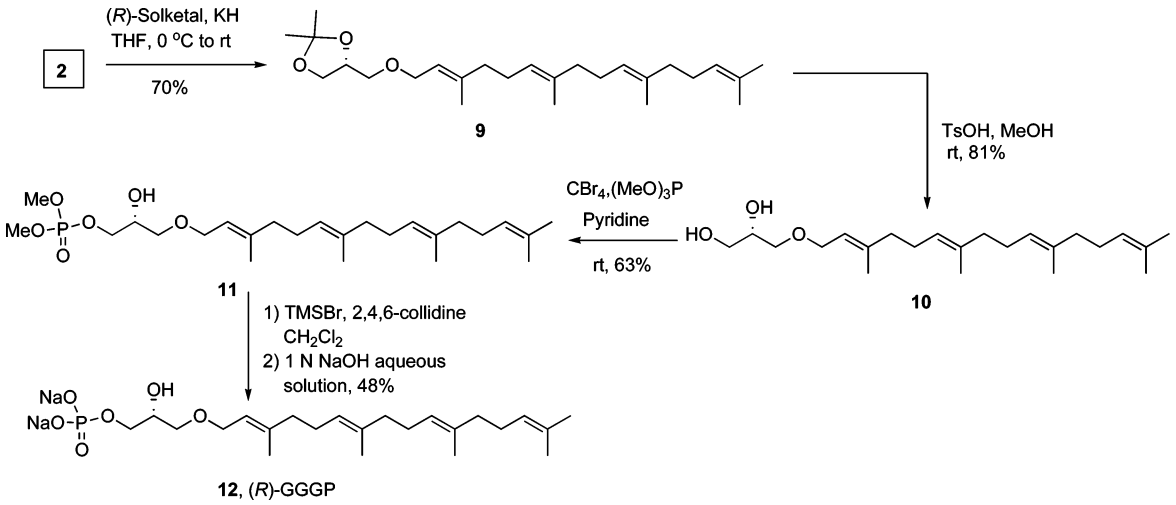
Scheme 1. Synthesis of (*S*)-GGGP (**8**)



quired. Starting with (*R*)-solketal, (*R*)-GGGP (**12**) was synthesized using the successful route as summarized in Scheme 2.

DGGGPS is a member of the UbiA prenyltransferase family that can catalyze the transfer of a prenyl group to its biological acceptor substrate (*S*)-GGGP (**8**). With the enantiomeric substrates (*R*)-GGGP (**12**) and (*S*)-GGGP (**8**) in hand, we determined the activity of DGGGPS toward each of these substrates. From the results of radio HPLC analysis (Figure 2) and reversed-phase TLC analysis (Figure 3), we found that the DGGGP and presumably its enantiomer were formed in the reactions using (*S*)-GGGP (**8**) and (*R*)-GGGP (**12**), respectively. In these reactions, the starting reagent [^{14}C]-GGPP was formed first from [^{14}C]isopentenyl diphosphate and (*E*,*E*)-farnesyl diphosphate by the activity of GGPS. Then, [^{14}C]-GGPP was used as the prenyl donor substrate for DGGGPS. Thus, the results demonstrated that the C_{20}-prenyl group of GGPP could be transferred to either of the two GGGP enantiomers by the action of DGGGPS. (*S*)-GGGP seems to be marginally preferred in Figure 2,

Scheme 2. Synthesis of (*R*)-GGGP (**12**)



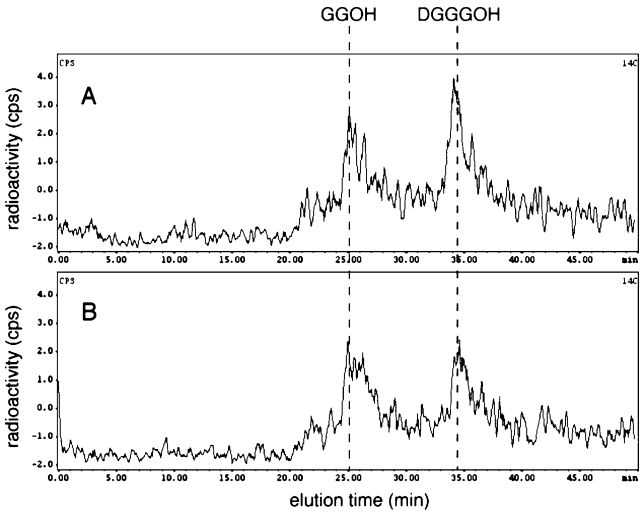


Figure 2. Radio HPLC analysis of 1-butanol extracts from the enzyme reaction with [^{14}C]-GGPP synthase and DGGGPS, using 0.5 nmol [^{14}C]-isopentenyl diphosphate, 0.5 nmol (*E*,*E*)-farnesyl diphosphate, 0.4 μmol (*S*)-GGGP (A), or (*R*)-GGGP (B) as substrates. Key: GGOH, geranylgeraniol; DGGGOH, digeranylgeranylglycerol.

whereas the (*R*)-enantiomer appeared to be preferred in Figure 3. These results are reproducible but qualitative; the GGGPs produced are quite labile. Nevertheless, to our surprise, both (*R*)-GGGP (**12**) and (*S*)-GGGP (**8**) were accepted at a comparable extent as substrates for DGGGPS. During the biosynthesis of archaeal membrane lipids, GGGPS catalyzes the transfer of prenyl groups from GGPP to (*S*)-glyceryl phosphate in the formation of (*S*)-GGGP (**8**), and the ether linkage between both (*S*)-GGGP (**8**) and another geranylgeranyl group is formed under the control of DGGGPS. GGGPSs are known to have strict substrate preferences: (*R*)-glyceryl phosphate is a very poor substrate.[13,21] Thus, our results strongly suggest that the chirality of the archaeal membrane lipid is determined by GGGPS, not by DGGGPS. However, (*R*)-GGGP (**12**) and (*S*)-GGGP (**8**) will be important tools for more detailed analysis of the specific activity and enantioselectivity of DGGGPS in future studies.

(21) Zhang, D.; Poulter, C. D. *J. Org. Chem.* **1993**, *58*, 3919−3922.

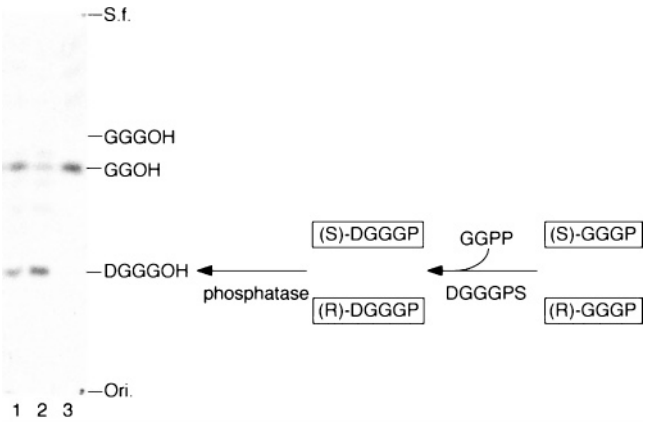


Figure 3. Autoradiogram of TLC from left to right: (1) (*S*)-GGGP; (2) (*R*)-GGGP; (3) without an acceptor substrate. Key: GGGOH, geranylgeranylglycerol; GGOH, geranylgeraniol; DGGGOH, digeranylgeranylglycerol; Ori, origin; S.f., solvent front.

In conclusion, (*S*)-GGGP and (*R*)-GGGP were each synthesized by a five-step procedure starting from the (2*E*,6*E*,10*E*)-geranylgeraniol and the appropriate enantiomer of solketal. A regioselective phosphorylation of diol **4** was achieved using $CBr_4/P(OMe)_3$, and the instability problem of the geranylgeranyl group was circumvented by judicious selection of mild reaction conditions. The LKC18 reversed-phase TLC analysis and radio HPLC analysis have shown that the DGGGPS can catalyze the transfer of a prenyl group to the secondary hydroxy groups of both (*R*)-GGGP and (*S*)-GGGP.

Acknowledgment. We thank Dr. Y. Xu (University of Utah) and G. Jiang (University of Utah) for many helpful discussions. We thank the NIH (NS29632 to G.D.P.) and grants-in-aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan for financial support of this work.

Supporting Information Available: Experimental procedures and characterization for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0530878