Tetrahedron Letters, Vol. 34, No. 19, pp. 3037-3040, 1993
Printed in Great Britain

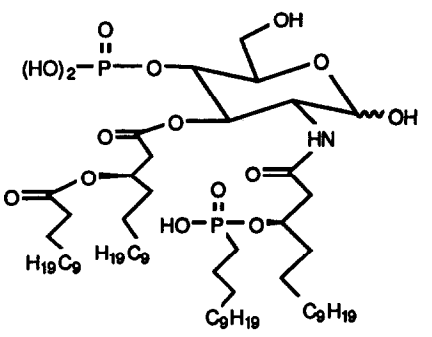
0040-4039/93 $6.00 + .00
Pergamon Press Ltd

Synthesis Of Phosphonate Analogs Of Lipid X

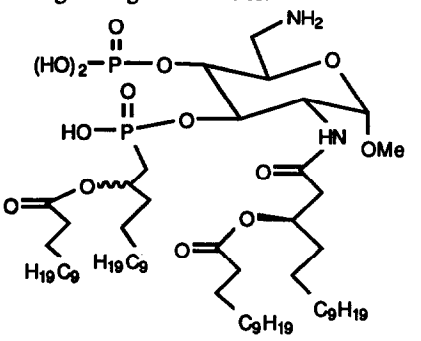
K. Balreddy*#, L. Dong, D. M. Simpson and R. Titmas.
IGEN Inc, 1530 East Jefferson Street, Rockville, MD 20852.

Abstract: Simple synthetic approaches for novel phosphonate analogs 1 and 2 (TS analogs) of lipid X are described.

Lipid-A, a constituent of lipopolysaccharides of gram negative bacterial cell wall, has been shown to be a major causative agent in the induction of septic shock.[1] Apart from its endotoxic property, lipid A has also been shown to exhibit beneficial properties such as immunostimulation, antitumor and antiviral activities.[2] Lipid X, a biosynthetic intermediate of lipid A has some of the beneficial properties of lipid A, but is non-toxic. For this reason many studies have been performed on the biological activities of lipid X and lipid A analogs.[2,3] These studies have shown that the number and position of the fatty acyl groups of lipid A play an important role in the induction of septic shock. Catalytic antibodies capable of hydrolyzing esters have been generated using haptens in which the ester group has been replaced with a phosphonate analog.[4] We therefore synthesized phosphonate analogs 1 and 2 of lipid X so as to generate catalytic antibodies that will cleave the fatty acyl groups from lipid A and so reduce its endotoxic activity.[5] In addition, these novel compounds may exhibit other interesting biological activities.



1



2

Figure 1

For the synthesis of transition state analogs 1 and 2 (Fig. 1) building units 3,[6] 4,[7] 5, 6[8] and 7 (Fig. 2) were prepared first.

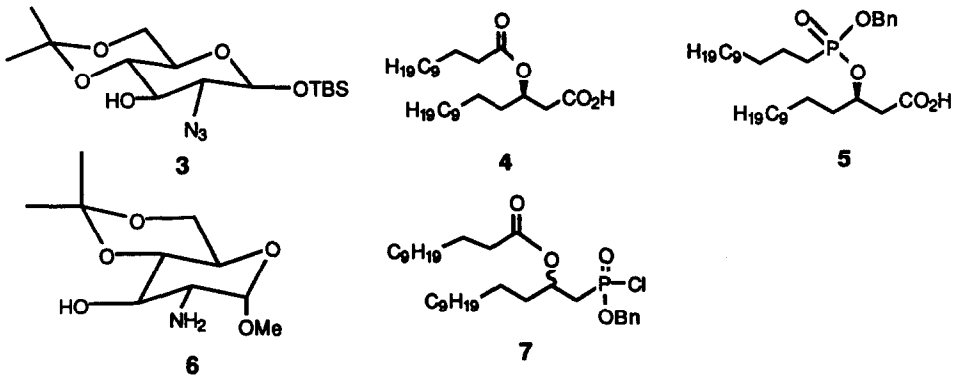
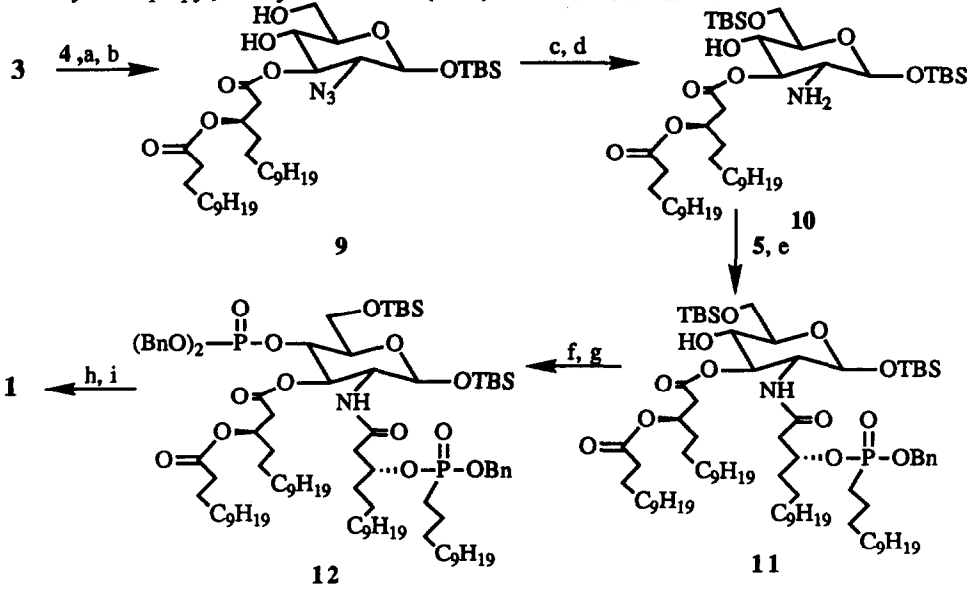


Figure 2

Preparation of TS analog **1** was achieved by the following sequence of reactions (Scheme 1). The hydroxy compound **3** was condensed with **4** (DCC, DMAP) to give the corresponding ester, which, after deprotection of acetonide, yielded the diol **9**. The primary hydroxyl of diol **9** was selectively protected as the ter-butyldimethylsilyl ether (TBS)which, on hydrogenation (Pd-C, methanol), afforded the amino compound **10**. Condensation of compound **10** with acid **5** in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) furnished the amide **11**.

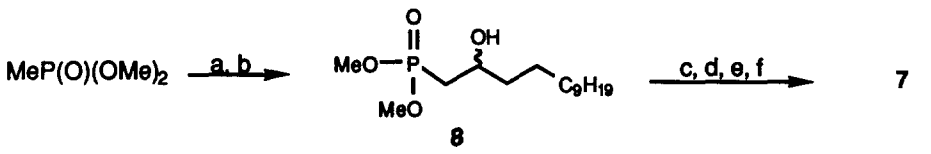


Scheme 1

(a) DCC, DMAP, CH_2Cl_2, 82%, (b) TFA, CH_2Cl_2, 84%, (c) TBSCl (1 eq), imidazole, DMF, 87%, (d) H_2, Pd-C (10%), Methanol, 96%, (e) EDC, HOBT 49%, (f) N,N-diisopropylamino dibenzyl phosphite, tetrazole, CH_2Cl_2 (g) *m*-CPBA, 91%, (h) H_2, Pd-C (10%), Methanol, 90%, (i) HF-pyridine, 51%.

The hydroxyl of compound **11** was phosphorylated using N,N-diisopropylamino dibenzyl phosphite[9] followed by oxidation (*m*-CPBA) to give the dibenzyl phosphonate **12**. The phosphonate **12** was converted to compound **1** by hydrogenation followed by desilylation (HF-pyridine).

The requisite phosphonochloridate **7** for the preparation of TS analog **2** was prepared starting from dimethyl methylphosphonate (Scheme 2). Dimethyl methylphosphonate was treated with n-butyllithium at -78 ^{0}C followed by reacting with methyl laurate to give the corresponding ketophosphonate which, after reduction ($NaBH_4$, methanol) afforded the hydroxy phosphonate **8**. Hydroxy phosphonate **8** was condensed with lauric acid (DCC, DMAP) to give the dimethyl phosphonate ester. The phosphonate ester was demethylated (TMSI) to yield the phosphonic diacid which was selectively monobenzylated[10] (BnOH, CCl_3CN, pyridine) to give the monoacid. The resulting monoacid compound was reacted with PCl_5 to furnish the phosphonochloridate **7**.



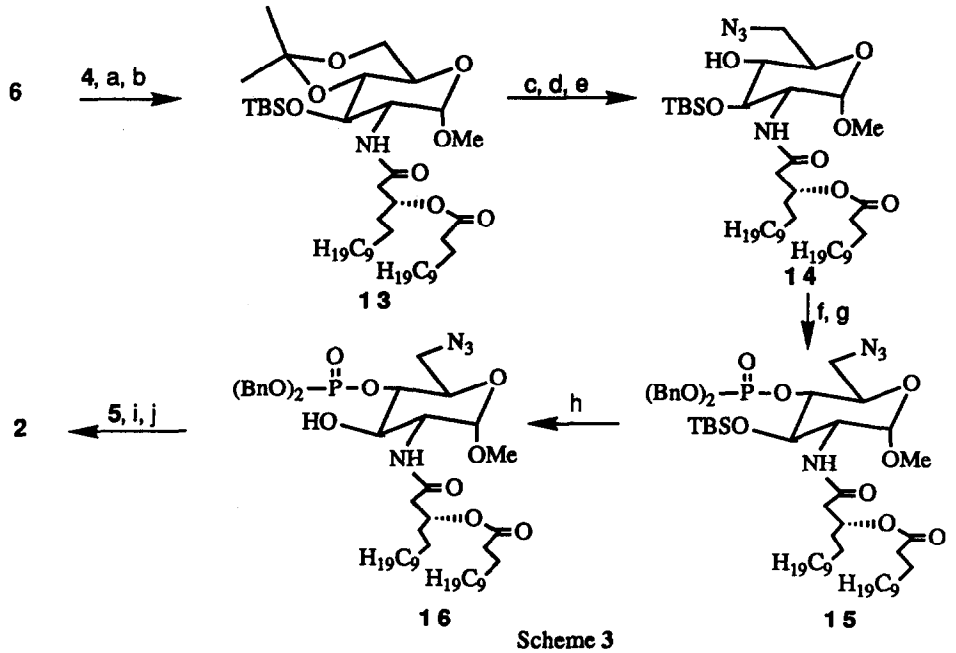
8

Scheme 2

(a) n-BuLi, THF, -78 ^{0}C, Methyl laurate, 64%, (b) $NaBH_4$, MeOH, 94%, (c) Lauric Acid, DCC, CH_2Cl_2, 82%, (d) TMSI, CH_2Cl_2, 82%, (e) BnOH, CCl_3CN, $CHCl_3$, Pyridine, 64%, (f) PCl_5, $CHCl_3$.

Compound **2** was prepared starting from the amino compound **6** (Scheme 3). The amino compound **6**, on condensation with **4** (EDC, HOBT, 68%), gave the corresponding amide which was silylated to obtain compound **13**. Deprotection of the acetonide group of compound **13** with TFA furnished the corresponding diol. The primary hydroxyl of diol was first converted to tosylate (TsCl) and subsequently to azide (NaN_3, DMF) to afford azido compound **14**. The hydroxyl group of **14** was phosphorylated[9] to give dibenzylphosphate **15**. Compound **15**, after desilylation (PTSA, MeOH,0 ^{0}C), yielded the hydroxy compound **16**. Compound **16** was coupled with phosphonochloridate **7** (DMAP) to give the phosphonate which, after hydrogenation afforded compound **2**.

In conclusion we have prepared the new phosphonate analogs of lipid X **1** and **2**,[11] which will serve as transition state analogs for eliciting catalytic antibodies having esterase activity with the potential to detoxify lipid A.[12] The biological activities of these compounds will be published elsewhere.



Scheme 3

Reagents: (a) EDC, HOBT, 68%. (b) TBSCl, Imidazole, DMF, 84%. (c) TFA, CH_2Cl_2, 93%, (d) TsCl, DMAP, CH_2Cl_2, 83%, (e) NaN_3, DMF, 78%, (f) N,N-diisopropylamino dibenzyl phosphite, tetrazole, CH_2Cl_2 (g) *m*-CPBA, 82%, (h) PTSA, Methanol, 59%, (i) DMAP, Et_3N, CH_2Cl_2, 56%, (j) H_2, Pd-C (10%), ethyl acetate, 78%.

References and Notes

Present address: Du Pont de nemours & Co. Stine-Haskell Research Center, Newark, DE 19714-0030.

1. (a) Westphal, O.; Luderitz, O.; Eichenberger, E.; Keiderling, W. *Z. Naturforsch.* **1952**, *76*, 536. (b) Galanos, C.; Rietschel, E. T.; Luderitz, O.; Westphal, O.; Kim, Y. B.; Watson, D. W. *Eur. J. Biochem.* **1972**, *31*, 230.
2. (a) Kumazawa, Y.; Matsuura, M.; Nakatsura-Watanabe, Y.; Fukumoto, M.; Nishimura, C.; Homma, J. Y.; Inage, M.; Kusumoto, M.; Shiba, T *Eur. J. Immunol.* **1984**, *14*, 109. (b) Proctor, R. A.; Will, J. A.; Burhop, K. E.; Raetz, C. R. H. *Infec. Immunity* **1986**, *52*, 905. (c) Homma, J. Y.; Matsuura, M.; Kumazawa, Y. *Drugs of the Future* **1989**, *14*, 645, and references cited therein.
3. (a) Miyamoto, M.; Baker, M. L.; Lewis, M. D. *Tetrahedron Lett.* **1992**, *33*, 3725. and references cited therein.(b) Vyplel, H.; Scholz, D.; Loibner, H.; Bednarik, K.; Schaller, H.; *Tetrahedron lett.* *1992*, *33*, 1261.
4. Phosphonate based TS analogs: Lerner, R. A.; Benkovic, S. J.; Schultz, P. G. *Science*, **1991**, *252*, 659.
5. Detoxification of lipid A by the enzyme acyloxyacyl hydrolase: Munford, R. S.; Hall, C. L. *Science* **1986**, *234*, 203.
6. Preparation of azido sugar 3: (a) Lemieux, R. U.; Ratcliffe, R. M. *Can. J. Chem.* **1979**, *57*, 1244. (b) Schmidt, R. R.; Grunler, G. *Carbohydr. Res.* **1985**, *135*, 203. (c) Kinzy, W.; Schmidt, R. R. *Carbohydr. Res.* **1975**, *166*, 265.
7. Preparation of acids **4** and **5** will be described elsewhere.
8. Preparation of amino compound **6**: Imoto, M.; Yoshimura, H.; Shimamoto, T.; Sakaguchi, N.; Kusumoto, S.; Shiba, T. *Bull. Chem. Soc Jpn.* **1987**, *60*, 2205.
9. Yu, K-L.; Frazer-Reid, B. *Tetrahedron Lett.* **1988**, *29*, 979.
10. Neumann, J.-M.; Herve, M.; Debouzy, J.-C.; Guerra, F. I.; Gouyette, C.; Dupraz, B.; Dihn, T. H. *J. Am. Chem. Soc.* **1989**, *111*, 4270.
11. All the new compounds gave satisfactory spectral data.
12. Generation of catalytic antibodies for the detoxification of LPS: Darsley, M.; Kamireddy, B.; Bauxbaum, S.; Cai, Z.; Dong, L.; Simpson, D.; Sugasawara, R.; Titmas, R.; Zerby, D.; Poster presented at the *2nd Annual Meeting of Endotoxins*, **1992**, Vienna, Austria.

(Received in USA 4 November 1992; accepted 12 February 1993)