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Synthesis of Injectable Antifungal Sch 59884

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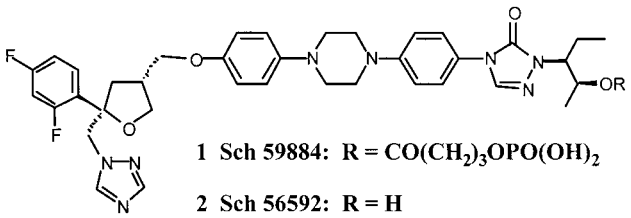
Abstract:

A synthesis for the preparation of multikilogram quantities of the injectable antifungal Sch 59884 is described.

Opportunistic fungal infections in immune compromised individuals have resulted in a renewed interest in potent antifungals. These infections can cause swallowing difficulties in these individuals, and therefore injectable antifungals are desirable. Sch 59884, **1**, is one such potent azole injectable antifungal,[1] and large amounts of this phosphate were needed to further evaluate its potential as an injectable antifungal. We describe here our work towards the development of a practical synthetic route which allowed for the preparation of multikilogram batches **1**.



1 Sch 59884: R = $CO(CH_2)_3OPO(OH)_2$

2 Sch 56592: R = H

Sch 59884 is derived from the oral antifungal Sch 56592, **2**.[2] This compound is comprised of four stereogenic centers, two of which emanate from the synthetically challenging 2,4-substituted tetrahydrofuran moiety. As such, its preparation is lengthy and expensive. To minimize the economic burden of the preparation of **1**, it was necessary that incorporation of **2** should come at a late stage, and that the subsequent synthetic transformations should be very high-yielding. This reasoning led to the convergent synthesis of **1** which is shown in Scheme 1.

The availability of 4-hydroxy sodium butyrate, **3**, made it a suitable starting material for the preparation of **1**. For the phosphate moiety, many commercially viable trivalent as well as pentavalent phosphorylating reagents were considered. The trivalent reagents phosphorylate well, and they would require subsequent oxidation. Since Sch 56592 is unstable to the oxidation conditions, the use of these reagents was deemed incompatible for the preparation of **1**. Either the cost of the commercially available pentavalent phosphorylating reagents or the conditions required for the removal of the protecting groups inherent in them (incompatible with Sch 59884 stability) deterred us from using them. Since hydrogenolysis was known to be compatible with Sch 56592,[2] we chose dibenzyl phosphate as the preferred phosphate moiety for the preparation of **1**. Initially direct phosphorylation of **3** with dibenzylchlorophosphate (DBCP, its preparation described later in this contribution) was attempted with the expectation that the mixed anhydride formed from the reaction of **3** and DBCP could eventually be hydrolyzed to the desired phosphoester **6,** or ideally, the mixed anhydride could be used to for the direct synthesis of **7**. In reality these attempts led to a mixture of inseparable products, and this approach was abandoned. Next, a literature survey revealed no example for the selective protection of alcohol function of **3**. At this stage conditions reported[3] for the carboxylic acid moiety protection of amino acids were applied to **3**. Thus, reaction of *p*-nitrobenzylbromide with **3** resulted in the formation of **4**. Although the major product of this reaction was the desired product **4**, several intensely colored byproducts also formed, and purification of **4** from this reaction mixture via a plant-friendly procedure proved difficult. Thus, it was decided to progress with crude **4** for its subsequent coupling with DBCP.

DBCP is a commercially unavailable, reactive, unstable reagent.[4,5] Several methods for its preparation have been reported.[4a−e] On the basis of our laboratory work it was concluded that the method described in ref 4a could be scaled up, it would not require purification of unstable DBCP, and it did not generate hazardous waste. Hence, this procedure was optimized for a scale-up in the plant. Calorimetric evaluation of the literature procedure for its preparation via the addition of *N*-chlorosuccinamide (NCS) to a cold (0 °C)

(1) (a) Bennett, F.; Ganguly, A.; Girijavallabhan, V. M.; Patel, N. M.; Saksena, A. K. New Piperazinyl-phenyl Triazolyl-4-hydroxybutanoate Compound Useful for Treating Systemic Fungal Infections. U.S. Patent 6,043,245, March 28, 2000. (b) Girijavalabhan, V. M.; Bennett, F.; Saksena, A. K.; Lovey, R. G.; Wang, H.; Pike, R. E.; Patel, N.; Loebennerg, D.; Nomier, A. A.; Lin, C. C.; Ganguly, A. K. Sch 59884, A Water-Soluble Prodrug of Sch 56592 for Intravenous Formulations. *Interscience Conference on Antimicrobial Agents and Chemotherapy*; American Society for Microbiology: Washington, DC, 1999, Paper 1932.

(2) Andrews, D. R.; Gala, D.; Gosteli, J.; Gunter, F.; Mergelsberg, I.; Sudhakar, A. Process for the Preparation of Triazolones. U.S. Patent 5,625,064, April 29, 1997.

(3) Perich, J. W.; Alewood, P. F.; Johns, R. B. *Aust. J. Chem*. **1991**, *44*, 253.

(4) (a) Atherton, F. R. *Biochem. Prep*. **1957**, *5*, 1. (b) Asai, S.; Nakamura, H.; Tanabe, M.; Sakamoto, K. *Ind. Eng. Chem. Res*. **1994**, *33*, 1687. (c) Silverberg, L. J.; Dillon, J. L.; Vemishetti, P.; Sleezer, P. D.; Discordia, R. P.; Hartung, K. B.; Gao, Q. *Org. Process Res. Dev*. **2000**, *4*, 34. (d) Silverberg, L. J.; Dillon, J. L.; Vemishetti, P. *Tetrahedron Lett*. **1996**, *37*, 771. (e) Oza V. B.; Corcoran, R. C. *J. Org. Chem*. **1995**, *60*, 3680.

(5) (a) *Encyclopedia of Reagents for Organic Synthesis*; Paquette, L. A., Ed.; John Wiley and Sons: West Sussex, England, 1995; Vol. 3, p 1536. (b) Kenner, G. W.; Todd, A. R.; Weymouth, F. J. *J. Chem. Soc*. **1952**, 3675. (c) Atherton, F. R.; Howard, H. T.; Todd, A. R. *J. Chem. Soc*. **1948**, 1106. (d) Personal communication with manufacturer of phosphorous reagents suggested that traces of benzyl alcohol can trigger an autodecomposition of DBCP. Thus, it was deemed safe to consume this reagent immediately after its preparation.

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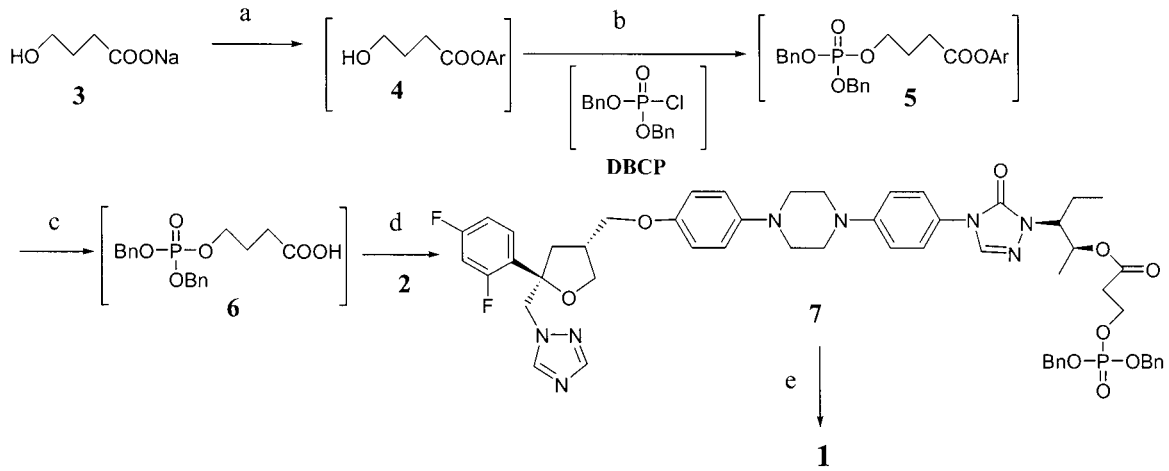
Scheme 1. **Synthesis of Sch 59884**[a]



[a] (a) DMF, *p*-nitrobenzylbromide.(b) Toluene, pyridine, −20 °C. (c) Na_2S/THF/H_2O/0−5 °C or THF/Zn/HCl/rt. (d) THF/DMAP/TSCl. (e) THF/Pd/C/HCOOH or THF/Pd/C/H_2 60−100 psi, 50−60 °C.

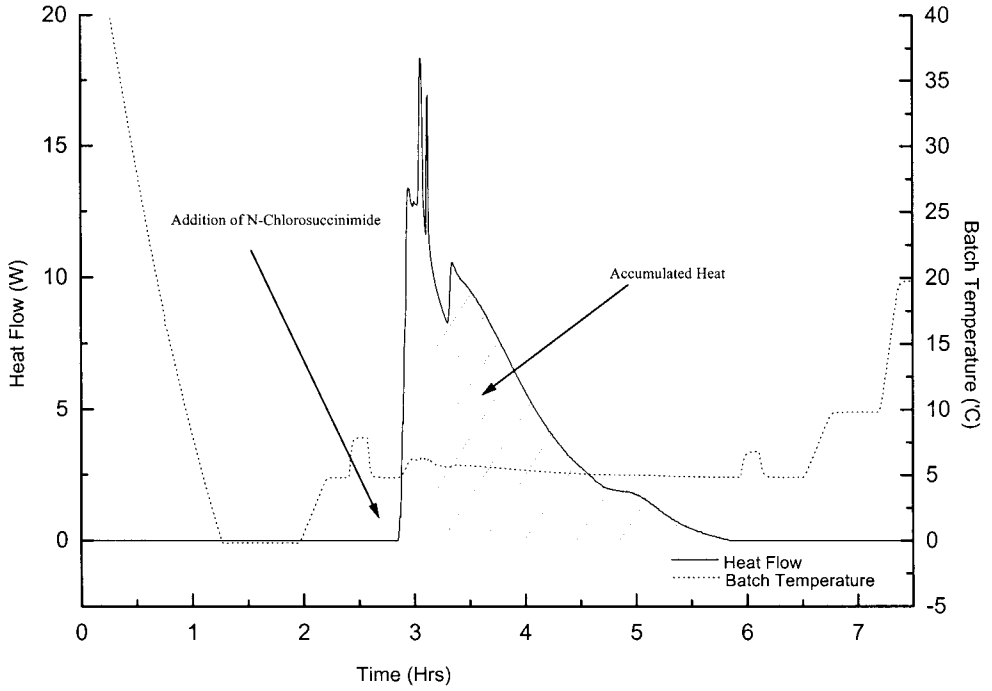


Figure 1. **Literature preparation of DBCP.**

solution of dibenzyl phosphite in toluene (in place of benzene which is an undesirable solvent due to its toxicity) showed that this procedure was unsuitable for large-scale work. This reaction was quite exothermic with an adiabatic temperature rise of 56.5 °C, and at this temperature a large amount of heat was released from this reaction mixture over several hours past the addition of NCS (Figure 1). In view of this the reagent was prepared by the controlled addition of commercially available dibenzyl phosphite to a suspension of NCS in toluene at 20−25 °C. Under the warmer conditions the reaction was rapid, and no heat accumulation was seen in laboratory experiment with single reagent addition (Figure 2). Since the instability of this reagent prevents its purification via distillation, it was desirable that the preparation of this reagent be as clean and complete under the modified conditions as it was under the literature conditions.

The laboratory reaction was conveniently monitored with ReactIR (Figure 3) for the consumption of the phosphite as well as the formation of the phosphate. As shown in the figure, ReactIR confirmed that the product was stable under the reaction conditions. Alternatively, ^{31}P NMR can also be utilized to ascertain the progress of this reaction. This technique confirmed that the conversion of the phosphite to the phosphate was quantitative in both cases,[8] and that the latter technique was more convenient for monitoring of the reaction; hence, it was practiced during the scale-up. In

(6) (a) Na_2S: Lamment, S. R.; Ellis, A. I.; Chauvette, R. R.; Kukolja, S. *J. Org. Chem.* **1978**, *43*, 1243. (b) $Na_2S_2O_5$: Perich, J. W.; Reynolds, E. C. *Synlett* **1991**, 577. (c) NaS_2O_4: Guibe-Jample, E.; Wakselman, M. *Synth. Commun.* **1982**, *12*, 219.

(7) (a) Chauvette, R. R.; Pennington, P. A. *J. Med. Chem.* **1975**, *18*, 403. (b) Pless, J.; Guttmann, S. In *Peptides 1966*; H.-C. Beyermann, Ed.; North-Holland: Amsterdam, 1967; p 50.

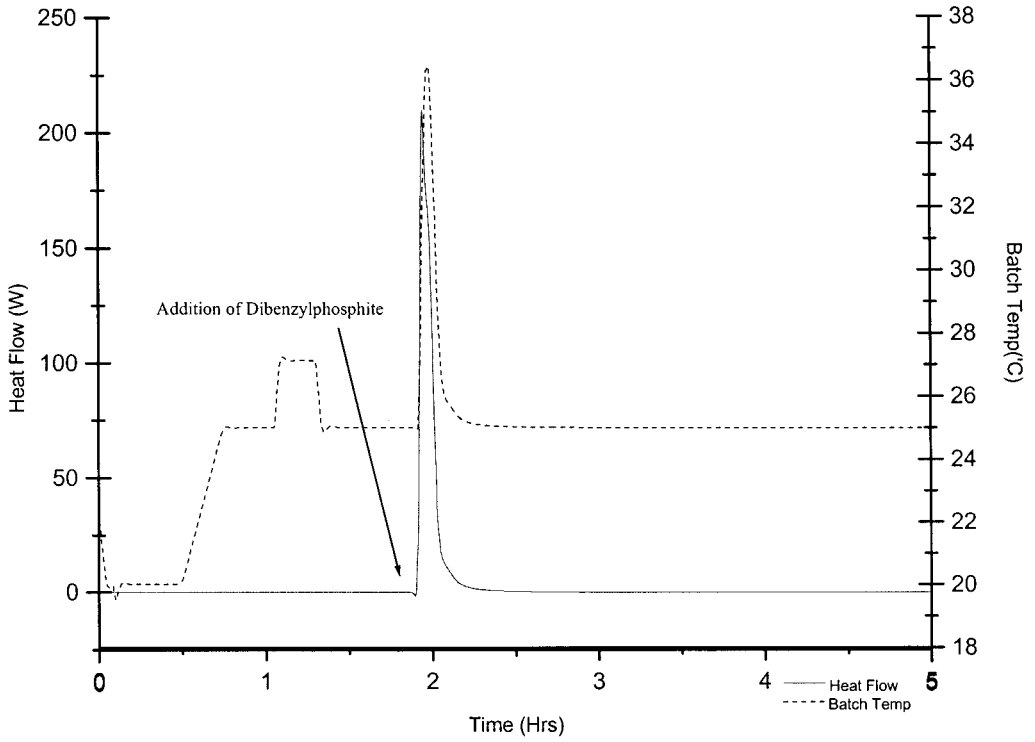


Figure 2. **Modified preparation of DBCP.**

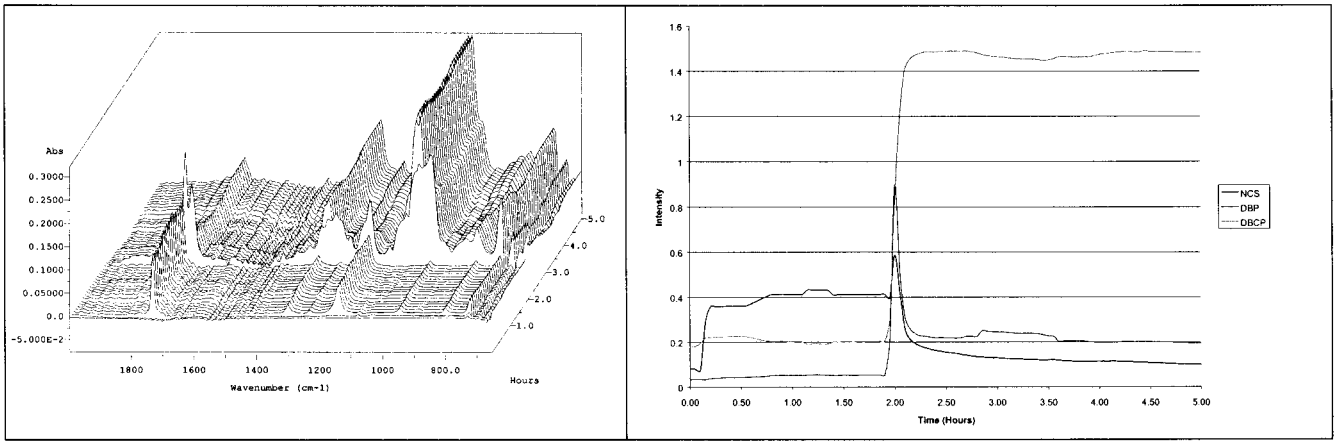


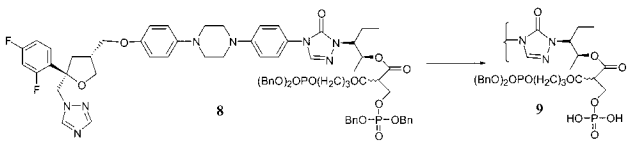
Figure 3. **Laboratory analysis of DBCP formation via ReactIR.**

practice this reagent was prepared in situ and consumed promptly after its preparation. Coupling of DBCP with **4** in the presence of pyridine progressed well. It was noticed that the residual NCS, if present, led to chlorinated **7** (absolute structure unknown). On the other hand, process stressing showed that residual dibenzyl phosphite, did not generate new impurities in the subsequent steps. Hence, as noted in the Experimental Section, for practical purposes NCS was the limiting reagent in the preparation of DBCP. Regardless of the method of preparation of **5**, a convenient, nonchromatographic purification of compound **5** proved difficult, as it carried the impurities formed during the conversion of **3** to **4**, as well as the ones emanating from their reaction with DBCP. Realizing that compound **6**, a carboxylic acid, could allow for removal of neutral organic impurities by acid/base treatments, it was decided to use crude **5** for further reaction even though the purification burden was likely to be significantly large at this stage. In the early stages of this work, the sodium sulfide, NaS_2O_4, and $Na_2S_2O_8$ procedures[6] as well as the Zn/HCl procedure reported in the literature[7] were applied for the removal of the *p*-nitrobenzyl group. On a small scale the Na_2S procedure quickly led to the desired product, whereas the Zn/HCl procedure appeared irreproducible, primarily due to the heterogeneity of the reaction mixture. To quickly meet the initial urgent kilogram need of the drug substance the former procedure was piloted. Scale-up of this procedure in the pilot plant required a larger excess of sodium sulfide compared to that needed for laboratory results. This resulted in a significant amount of

(8) Under moisture-free conditions, a small amount of the heterogeneous reaction mixture in toluene was cooled to room temperature and added to d_6-DMSO and subjected to ^{31}P NMR analysis. For this mixture a 10 ppm shift (from ca. 34−24 ppm) was seen upon conversion of the phosphite to the phosphate. Typically, the reaction was considered complete when no signal for the phosphite could be detected (implying >98% conversion).

decomposition of the product due to longer than expected exposure to basic conditions and necessitated a partial chromatographic purification of **5** prior to its use. It was also noticed that the removal of the benzyl groups of **7** required prolonged hydrogenation time when Na_2S was used for the removal of the *p*-nitrobenzyl group of **5**. This was attributed to residual sulfur-containing impurities that poisoned the catalyst. Thus, an alternate to Na_2S was much needed for a viable larger-scale process.

Ongoing laboratory worked suggested that both compounds **5** and **6** were stable to acidic conditions. This prompted us to reevaluate the removal of the *p*-nitrobenzyl group of **5** via the use of a heterogeneous Zn/HCl procedure. In fact, under these conditions, crude **5** was converted to **6** in reasonably good yields. Initial addition of HCl to the reaction mixture generated a large amount of heat and hydrogen gas. This reaction also produced some polymeric beads. The initial rapid heat and gas generation issues were overcome in the plant by efficient heat removal and slow charging of HCl. The size of the polymeric beads was controlled by rapid agitation of the reaction mixture. Although highly pure **6** could not be obtained from this reaction, suitably pure (92%) **6** was obtained after acid/base workup, and this was then reacted with **2** to form **7**. Compound **7** is a solid, and it was readily purified by *i*-PrAc/heptane or *i*-PrOH/water precipitation. For the coupling of compounds similar to acid **6** with 2° alcohol **2** it was known in our laboratory that the carbodiimide reagents worked well, but the removal of the byproducts was difficult. Conversion of the acids to their acid chlorides (with thionyl chloride, or oxalyl chloride) led to highly colored products, which too were difficult to purify. In some cases the contaminant interfered with the removal of the benzyl groups. On the other hand, the mixed anhydride procedure via the use of DMAP and TsCl typically led to good quality product in high yields, and hence this procedure was chosen for the coupling of **6** with **2**.[9] This procedure worked in high (90%) yield both in the laboratory and in the plant. Interestingly enough, during the scale-up in the plant, up to 3% of compound **8** also formed at this stage. As the subsequent publications will show, the formation of this compound occurs during the esterification stage, and it is not due to a byproduct that contaminated **6** in pilot plant. Precipitation of **7** removed most of the impurities that were carried over in crude **6**, but it did not remove **8**. This was removed as the diphosphate **9** after deprotection of the benzyl groups as discussed below.



The deprotection of **7** in the laboratory was initially attempted via hydrogenation (50% water wet 5% Pd/C, THF). This conversion was slow even at elevated temperatures (~60 °C and up to 100 psi H_2 pressure). This conversion proceeded smoothly and reproducibly in the laboratory via transfer hydrogenation (toluene, 50% water wet 5% Pd/C, HCOOH). When scaled up in the plant, the reaction proceeded smoothly; however, the isolated product consistently showed a high amount of residual sodium. Control experiments in the laboratory followed by testing of the catalyst established that sodium salts in the catalyst were converted to sodium salt of **1** during debenzylation, resulting in the high sodium content of crude **1**.

Thus, the removal of bis-butyrophosphate **9**, resulting from debenzylation of **8**, and the removal of sodium in crude **1** was necessary in the plant batches to obtain high-quality **1**. It was clear that the removal of sodium would be possible either via the use of a mineral acid or via the use of a large excess of organic acids (e.g., sulfonic or phosphoryl acids, by driving the equilibration of sodium towards the added acid). During this removal the hydrolysis of **1** back to **2** had to be rigorously contained, as **2** could interfere with the dissolution of **1** to a clear injectable solution. After much work, an aqueous acid slurry procedure was developed which allowed for the removal of sodium (as sodium chloride) with minimal hydrolysis of **2** and an excellent recovery of **2**.

Similarly, several dozen conditions were evaluated prior to identifying the aqueous EtOH slurry procedure which allowed for the removal the bis-phosphate resulting from the debenzylation of **8**. Removal of the above impurities resulted in >98% crude **1**, which at this stage was highly insoluble, and had **2** as a major impurity. This made the final crystallization of **1** extremely challenging. Again, a program had to be undertaken to identify a plant-friendly procedure for crystallization of **1** which would result in a reproducible polymorph of **1**. A THF/DME procedure was identified, successfully implemented in the plant to prepare several multikilogram batches of **1**, and is described in the Experimental Section. Compound **1** is a stable solid which can be stored at room temperature for months.

Last, it was decided to develop laboratory procedures that can allow for preparation of water soluble forms of this compound preferably without the use of organic solvents in which **1** was soluble (refluxing THF/^{i}PrOH, hot MeOH, CH_2Cl_2).[10] Two salts, the disodium and the di-NMG (*N*-methyl-D-glucamine) salts were of interest. It was discovered that free acid **1** could be suspended in water at room temperature with no detectable degradation for several hours. This fact was used to make the above salts, whereby **1** and the base were suspended in water and vigorously stirred. Under these conditions **1** gradually was converted to its water soluble salt. The end point was a clear aqueous solution of salts of **1** allowing for the formation of homogeneous salts. Lyo-

(9) This procedure, developed for other Schering compounds, was forwarded by Drs. W. Leong and D. Andrews. Initial work can be found in: (a) Brewster, J. H.; Ciotti, C. J. *J. Am. Chem. Soc*. **1955**, *77*, 6214. (b) Adams, W.; Baeza, J.; Liu, J.-C. *J. Am. Chem. Soc*. **1972**, *99*, 2000.

(10) Compound **1** was fairly soluble in methylene chloride (an environmentally restricted solvent), whereas it required large quantities of refluxing THF/*i*-PrOH. It was reasonably soluble in warm MeOH; however, solvolysis to **2** was a problem. Furthermore, a strict limit on residual solvents in the drug substance was anticipated for an injectable product. Thus, water would be an ideal solvent.

(11) HPLC conditions: Phenomenex Ultracarb ODS (5 μ), 0.02 M aqueous ammonium acetate:CH_3CN::35:65 to 65:35 over 33 min at 1 mL/min; detection at 254 nm.

philization of this solution led to the solid salts. Since the sodium salt was hygroscopic whereas the di-NMG salt as a hydrate was stable, the latter was selected as the salt of choice. For practical purposes **1** was delivered as a crystalline, stable, solid drug substance. The preparation of the di-NMG salt, along with the necessary sterile filtration and formulation required for an injectable pharmaceutical, was incorporated in the formulation of **1** by the formulation group.

In summary, a plant-suitable synthesis of injectable antifungal Sch 59884 was devised. Unanticipated issues that surfaced during its scale-up were resolved, and several kilograms of this compound were prepared.

Experimental Section

Preparation of 4-*O*-(Dibenzyl)phosphoryl]-butyric Acid (6). Preparation of this compound was carried out in four stages.

Stage A: Preparation of (4-Hydroxybutyric acid)*p*-nitrobenzoate (4). A solution of 1.0 kg (4.6 mol) of 4-nitrobenzyl bromide in 3.0 L of dimethylformamide (DMF) was added slowly to a suspension of 0.7 kg (5.6 mol) of 4-hydroxybutyric acid sodium salt in 2.0 L of DMF while maintaining a temperature <30 °C. The reaction was heated to 35−40 °C and the temperature maintained for about 2 h. The reaction was monitored by TLC (silica gel, 1:1 hexane/ethyl acetate), and when complete the reaction was cooled to room temperature and partitioned between 4 L of toluene and 10 L of water. The addition of water was exothermic, and the temperature of the reaction mixture was held below 30 °C during addition. After separation of the layers, the aqueous layer was extracted with an additional 4 L of toluene. The toluene layers were combined and washed with 2 L of 10% sodium chloride and concentrated in vacuo to about 2 L (the batch temperature was not allowed to exceed 40 °C). The solution of **4** in toluene was stored at room temperature under nitrogen for use in Stage C. Typical yield was 0.8 kg (3.3 mol) of active per 2 L of solvent as determined by HPLC.

Stage B: Preparation of Dibenzylchlorophosphate (DBCP). Two liters (9 mol) of dibenzyl phosphite in 9.4 L of toluene was added to a suspension of 1.1 kg (8.2 mol) of *N*-chlorosuccinimide in 9.4 L of toluene at 25 °C over 6 h while the temperature was maintained between 20 and 25 °C. The reaction mixture was held between 20 and 25 °C for 3 h, sampled, and analyzed by ^{31}P NMR. Upon reaction completion, the solution was filtered under closed, inert conditions to remove the solid byproduct succinimide and washed with 3 L of toluene. The resultant filtrate was cooled to −10 °C and used soon after; its preparation is described below in Stage C. For its use it was assumed that the yield for this step was quantitative (2.4 kg, 8.1 mol) in 21 L of toluene.

Stage C: Preparation of {4-*O*-[(Dibenzyl)phosphoryl]-butyric acid}*p*-nitrobenzyl. (5). A solution of 2.4 kg (8.1 mol) of dibenzyl chlorophosphate in 21 L of toluene (prepared in Stage B) was cooled to −15 to −20 °C, and the solution of **4** (prepared in Stage A) followed by 0.67 L (8.7 mol) of pyridine was added to it while the reaction temperature was maintained below 0 °C. The reaction mixture was stirred at 0 °C for about 4 h, and then it was warmed to room temperature over about 8 h and stirred for an additional 8 h. The reaction was monitored by HPLC, and when the reaction was complete, the mixture was washed with 2 × 11 L of 10% sodium chloride solution, 2 × 7.5 L of 10% sodium bicarbonate solution, and 1.1 L of 10% sodium chloride solution. The toluene layer was concentrated in vacuo to about 2 L (batch temperature was not allowed to exceed 40 °C). The solution of **5** in toluene under nitrogen atmosphere was stored at room temperature prior to its use for the subsequent step. Typical yield for the two steps was 70% (1.6 kg, 3.2 mol active **5** in 4.6 kg solution as determined by HPLC vs a working reference standard) and was used without purification for subsequent reactions.

^{1}H NMR ($CDCl_3$) δ = 8.15 (2H, d, J = 8.8 Hz), 7.45 (2H, d, J = 8.8 Hz), 5.15 (2H, S), 3.64, t, J = 9.6 Hz), 2.48 (2H, t, J = 7.8 Hz), 1.9−1.8 (2H, m), 1.64(1H, s).

Stage D: Preparation of the Title Compound 6. The removal of *p*-nitrobenzyl group from **5** was accomplished via either procedure (a), which was less desirable for plant scale, or procedure (b).

(a) Na_2S Procedure. A solution of **5** (1.0 kg active, 2.0 mol) in toluene as prepared above was concentrated to remove most of the solvent via vacuum distillation. The resultant solution was dissolved in 21 L of tetrahydrofuran and cooled to 5 °C. A solution of 1.4 kg (6.0 mol) of sodium sulfide nonahydrate in 12 L of water was added to the batch while a batch temperature of <10 °C was maintained. The batch was allowed to react for 1 h and was checked for completion by TLC. Upon reaction completion, 11 L of TBME was added, and after thorough agitation the layers were settled and split. The aqueous layer was washed with an additional 9 L of TBME. The pH of the aqueous layer was adjusted to 4.7−4.8 with 5% aqueous HCl while a temperature of <5 °C was maintained. The aqueous layer was then extracted twice with 11 L of TBME, and the two organic washes were combined and distilled to 5 L. The solution was used "as-is" in the next step.

(b) Zn/HCl Procedure. Five liters of 4 M HCl was added slowly to an ice-cooled, stirred solution of 8 L of DMF, 1.0 kg (2.0 mol) of "active" **5**, and 0.56 kg of zinc (8.6 mol, ACS grade 99.8%+, −10+50 mesh). The HCl was added at a rate such that the temperature of the reaction was ≤20 °C (note: initial addition of the HCl solution produces the greatest temperature increase.). Upon reaction completion, a cloudy yellow solution was produced. Cooling was maintained for an additional 10 min, and the reaction was allowed to warm to ambient temperature over 1−4 h. The reaction was generally allowed to continue overnight, at which time all of the zinc had dissolved, and the reaction mixture was a clear yellow solution. Upon reaction completion as determined by HPLC, 10 L of TBME and 0.5 kg of Celite was added to the reaction. Sixteen liters of water was then slowly added to the reaction mixture and stirred for 10−15 min. The resulting suspension was filtered through a sparkler containing a pad of Celite (0.5 kg) and washed with an additional 5 L of TBME. The resultant solution was

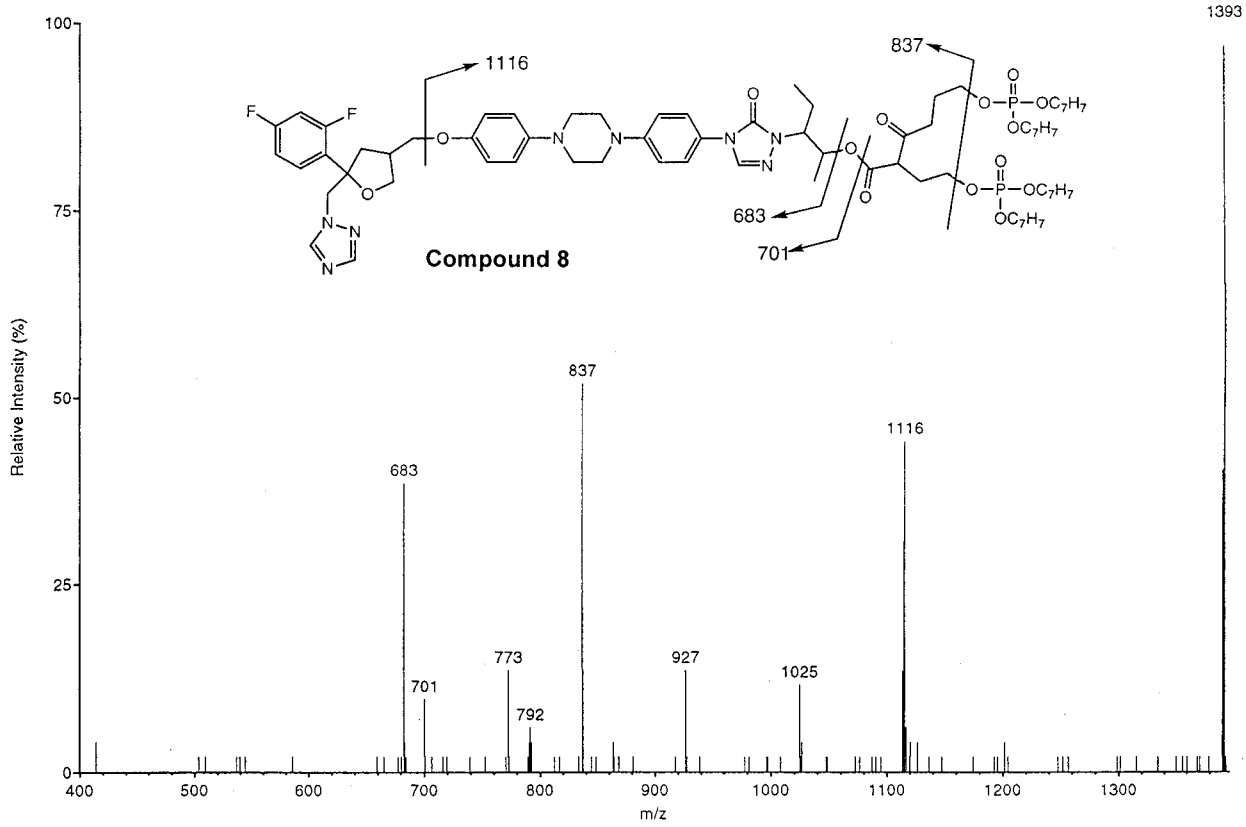


Figure 4.

split, and the aqueous layer was washed with an additional 10 L of TBME. The pH of the aqueous was approximately 1.5−2.5. The organic layers were combined and washed with 10 and 5 L of 10% $NaHCO_3$ respectively. The pH of the aqueous extracts was between 8.5 and 8.0. The basic aqueous layers were combined with 5 L of TBME and slowly brought to pH 4.8−5.0 with 4 N HCl (approximately 4 L). The solution was stirred 10−15 min while the pH was maintained at 4.8−5.0. The layers were split, and the aqueous layer was washed with an additional 5 L of TBME. The final two organic layers were combined and distilled to 4 L of volume. The yield of **6** was 66% corrected for purity, the purity was 92% versus working reference standard **9** (which was prepared by preparative flash chromatography: SiO_2 and gradient 1−2% MeOH in CH_2Cl_2). This was used without purification for the next step.

^{1}H NMR (d_6-DMSO) δ = 7.4−7.3 (10H, m), 5.03 (4H, d, J = 8.2 Hz), 3.99 (2H, q, J = 6.5, 13.6 Hz), 2.27 (2H, t, J = 7.3 Hz), 1.8 (2H, m); HRMS FAB MS *m*/*z* 365.1154 ($[M + H]^+$, calcd 365.11).

Preparation of (−)-2(*S*)-4[-4[-4[-4[[(*R*-*cis*)-5,2,4-Difluorophenyl)tetrahydro-5-(1H-1,2,4-triazol-1-yl-methyl)-3-furanyl]methoxy]phenyl]1-piperazinyl]-phenyl-4,5-dihydro-5-oxo-1H-1,2,4-triazol-1yl]-1(*S*)-methylbutyl[[(phenylmethoxy)carbonyl]amino]-acetate (7). *p*-Toluene sulfonyl chloride (PTS-Cl) [0.43 kg (1.85 mequiv, molar equivalent compared to **2**)] dissolved in 3.5 L of tetrahydrofuran was added to a solution of 1.0 kg (1.0 mequiv) of **2**, 0.73 kg (1.40 mequiv) of **6** (its solution weight was 7.4 kg), 0.56 kg (4.0 mequiv) of of (dimethyl-amino)pyridine in 11 L of tetrahydrofuran cooled to a temperature between 0 and 3 °C. The PTS-Cl solution was added at a rate to maintain the reaction temperature between 0 and 3 °C. The reaction was allowed to warm slowly over 5 h to room temperature, upon which it showed consumption of **2** as determined by HPLC. Upon reaction completion 7 L of water and 24 L of isopropyl acetate were added to the reaction mixture. The aqueous layer was brought to a pH of 0.9−1.0 with aqueous 5%HCl, and the layers were separated. The acidic aqueous layer was extracted with an additional 7 L of isopropyl acetate. The combined organic layers were washed sequentially with 8 L of a 10% sodium bicarbonate and 8 L of sodium chloride solution. The resulting organic solution was then concentrated to approximately 10 L and treated with 0.1 kg of Darco. The resultant mixture was refluxed for 10 min, cooled to room temperature, and filtered. The Darco cake was washed with additional 6 L of isopropyl acetate to minimize product loss. The organic solution was reheated to reflux, and 8.6 L of hexanes was added slowly while a temperature between 85 and 89 °C was maintained. The reaction was cooled to a temperature between 50 and 55 °C over about 4 h and finally cooled to ambient temperature over an additional 4 h. The resultant solids were filtered and washed with 1:1 isopropyl acetate/hexanes and dried in vacuo at room temperature to give product as an off-white solid. The isolated yield of **7** was 90% corrected for purity which was 97.5% as measured against reference standard.

^{1}H NMR (DMSO) δ = 8.41 (1H, s), 83.8 (1H, s), 7.84 (1H, s), 7.53 (2H, d, J = 9.0 Hz), 7.5−7.3 (12H, m), 7.11 (2H, d, J = 9.2 Hz), 7.05 (1H, dt, J = 2.3, 8.5 Hz), 6.99 (2H, d, J = 9.2 Hz), 6.86 (2H, d, J = 9.1 Hz), 4.86 (2H, dd,

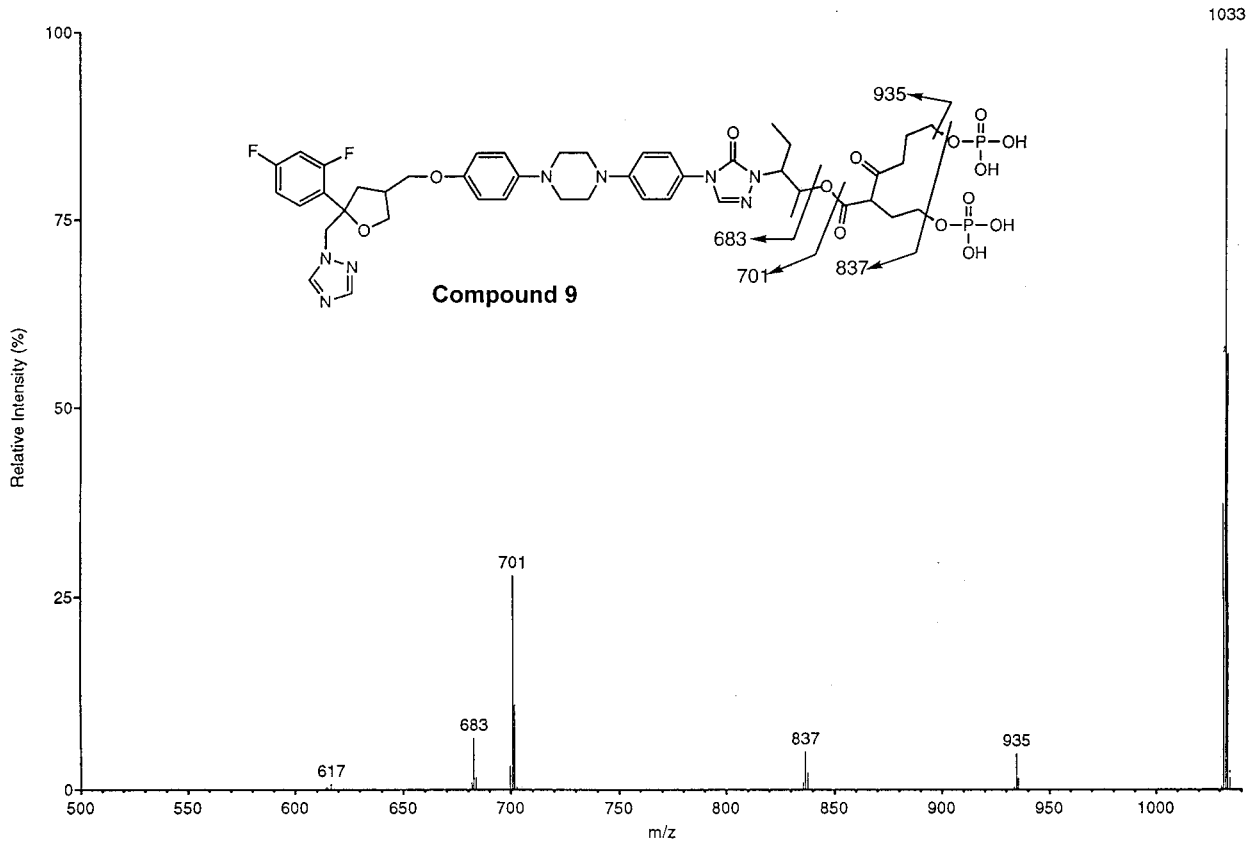


Figure 5.

J = 14.6, 21.6 Hz), 4.2−4.1 (2H, m), 3.96 (2H, q, J = 6.5, 13.3 Hz), 3.8 (2H, m), 3.7 (1H, m), 3.39 (3H, s), 3.3 (3H, m), 3.2 (3H, m), 2.6 (1H, m), 2.5−2.4 (1H, m), 2.3 (2H, m), 2.19 (1H, dd, J = 8.0, 13.1 Hz), 1.8−1.7 (3H, m), 1.27 (3H, d, J = 6.4 Hz), 0.83 (3H, t, J = 7.2 Hz). Anal. Calcd for ($C_{55}H_{61}O_9N_8F_2P$): C, 63.09; H, 5.87; N, 10.70. Found: C, 63.24; H, 5.94; N, 10.73. MS (FAB) 1047 $(M + H)^+$.

Preparation of 2,5,-Anhydro-1,3,4-trideoxy-2-*C*-(2,4-difluorophenyl)-4-[[4-[4-[4-[1-[(1*S*,2*S*)-1-ethyl-2-[1-oxo-4-(phosphonooxy)butoxy]propyl]-1,5-dihydro-5-oxo-4H-1,2,4-triazol-4-yl]phenyl]-1-piperazinyl]phenoxy]methyl]-1-(1H-1,2,4-triazol-1yl)-D-threopentitol (1). Under nitrogen, 1.0 kg of **7**, and 0.5 kg of 50% water wet 5% Pd/C were suspended in 10 L of toluene and cooled to 15 °C. One liter of formic acid was added to the reaction mixture over 15−20 min while the reaction temperature was maintained between 15 and 20 °C. The biphasic mixture was vigorously agitated for an additional 3 h (NOTE: The reaction time was heavily dependent upon which method was used to deprotect the PNB ester. If the Na_2S procedure was used, the reaction must be stirred for 18 h to ensure completion). After confirming reaction completion via HPLC analysis, 10 L of *i*-PrOH and 0.2 kg of siliceous earth were added to the reaction mixture, which was then filtered through 0.45 μm filter to obtain a clear solution. The solution was next filtered through a 0.2 μm pore-sized filter. Both filters were washed with a solution of 0.05 L of formic acid in 6 L of *i*-PrOH to minimize product loss. This solution was concentrated via distillation under atmospheric pressure until the internal temperature reached 100 °C (the volume was approximately 3 L), and then it was cooled to 75 °C. A solution of 0.1 L of THF in 10L of *i*-PrOH was separately warmed to 65 °C and transferred to the hot reaction solution. The resulting solution was held at 65 °C for 30 min and then cooled to 55 °C and held for 1 h. The solution was cooled to 0 °C slowly over 3 h, during which the product crystallized. The batch was collected by filtration. This was washed with 5 L of cold *i*-PrOH. The wet cake was sucked dry and checked for sodium content. If high sodium levels were found, the product was reslurried in 5 L of dilute aqueous HCl (to pH 2.0) at 5−10 °C for 30 min, refiltered, and washed sequentially with water and IPA. The wet cake was sucked dry, transferred to a vacuum oven, and dried until the *i*-PrOH content was <0.2% (LOD). Isolated yields ranged from 0.65 to 0.67 kg (79−81%). See below for analytical data.

Removal of Na and the Diphosphate impurity 9. Fifteen liters of water heated to 65 ± 5 °C was added to a suspension of 1.0 kg of **1** in 10.4 L of 2B ethanol heated to reflux. The temperature of the mixture was maintained at 70 °C or above throughout this addition. The addition of water resulted in a clear solution. The solution was cooled to 50−55 °C over approximately 1.5 h, held at this temperature for 1.5 h, and seeded if necessary for crystallization. Upon crystallization the mixture was cooled to room temperature and stirred for 12 h. The solids were filtered and washed with water and dried under reduced pressure at 40−50 °C until the ethanol content was less than 0.2% w/w. The solids were stirred as a slurry in 20 L of water, and the pH was adjusted to 2.9 ± 0.1 with 5% HCl. The slurry was agitated slowly overnight,

filtered, and washed with water until a pH of >4.0 was reached for the wash. The wet cake was transferred to a vacuum oven and dried at 40−45 °C.

Recrystallization of 1. Under nitrogen, for 1.0 kg of **1**, 20 L of THF was used. The mixture of **1** and THF was stirred and heated to reflux for 30 min to ensure complete dissolution. The mixture was recooled to ambient temperature, and 0.05 kg of activated carbon and 0.75 kg of siliceous earth were added. The mixture was reheated to reflux for 30 min. The mixture was cooled to 40 °C and filtered through 0.45 μm followed by 0.2 μm pore-sized filters. The reaction vessel and the filters were washed with 5 L of THF to maximize recovery. The combined solution was concentrated at atmospheric pressure to a volume of approximately 5 L. Separately, 30 L of 1,2-dimethoxyethane (DME) was heated to 55 °C, passed through a 0.2 μm pore size filter, and added to the above solution. The resultant solution was reheated to reflux for 30 to 60 min, during which the product crystallized. The mixture was then slowly cooled to room temperature over 6−8 h, followed by cooling to 0−5 °C for 1 h. The product was collected by filtration. The reaction mixture and the filter cake were rinsed with an additional 5 L of DME. The filter cake was transferred to a draft oven where it was dried under reduced pressure at 30 °C until the residual solvent analysis was <0.2% (GC). Isolated yields ranged from 0.76 to 0.82 kg (76−82%); mp 175−176 °C. $[\alpha_D] = -54.2°$ (c=10 mg/mL, CH_2Cl_2); ^{1}H NMR (d_6-DMSO): 8.35 (s, 1H); 8.32 (s, 1H); 7.79 (s, 1H); 7.45 (m, 2H); 7.30 (m, 2H); 7.10 (m, 2H); 6.95 (m, 3H); 6.80 (m, 2H); 5.08 (m, 1H); 4.60 (m, 2H); 4.05 (m, 2H); 3.70 (m, 5H); 3.30 (m, 4H); 3.15 (m, 4H); 2.55 (m, 1H); 2.40 (m, 1H); 2.30 (m, 2H); 2.15 (m, 1H); 1.73 (m, 4H); 1.25 (m 3H); 0.80 (m, 3H). HRMS (calcd for M + H): 867.3406; (obsd): 867.3428; Elem. Anal. (calcd): 56.81 C, 5.70 H, 12.93 N; (obsd): 56.55 C, 5.73 H, 12.70 N.

Preparation of Water-Soluble Salt of 1: Sch 59884·2 NMG. In a clean, dry flask, 100 g of **1** (115 mmol) and 45 g of *N*-methyl-D-glucamine (NMG, 231 mmol) were combined and diluted with 1.45 L of H_2O. The contents were agitated until all solids dissolved (30 min). The solution was filtered through a 0.45 μm pore-sized filter. The clear solution was then flash frozen (dry ice/2-propanol) and lyophylized overnight to afford 143 g (113 mmol, 99%) of the di-NMG salt of 59884. MS (calcd for M + H): 1258.3; (obsd): 1258.4 (main fragment at 867 for parent acid). Elem. Anal. (calcd for monohydrate): 51.80 C, 6.72 H, 10.98 N; (obsd): 51.51 C, 6.70 H, 10.83 N.

Identification of Compounds 8 and 9. Liquid chromatography/mass spectrometry (LC/MS) analyses to identify process-related impurities were performed on a Sciex API3 mass spectrometer operated in the positive ion electrospray mode. For all LC/MS analyses, the orifice potential was set to 80 v. Tandem MS spectra were acquired using argon as the collision gas at CGT = 170 and the orifice potential set to 60 v. Liquid chromatography was performed using Shimadzu 10-AD HPLC pumps and a YMC ODS-A 4.6 mm × 150 mm column. Mobile phase A was 20 mM ammonium acetate, and mobile phase B was acetonitrile. A linear gradient from 30 to 65% B in 15 min at a flow rate of 1 mL/min was used to elute components off the column.

LC/MS analysis of **7** (t_R 29.0 min, MW 1046) detected **8** (Figure 4) as a significant impurity eluting at 33.1 min. The molecular weight of 1392 Da recorded for **8** is 346 Da greater than that for **7**, suggesting an additional butyrodibenzyl phosphate group. The MS/MS fragmentation spectrum shows loss of two dibenzyl phosphate groups to give an ion at *m*/*z* 837. Further fragmentation of *m*/*z* 837 at the ester bond generates ion *m*/*z* 701 representing the alcohol **2**. The difference of 136 mass units between ion *m*/*z* 837 and 701 corresponds to the mass expected for the bis-butyro group. Debenzylation of **8** generated the bis-butyrophosphate **9** (see Figure 5) with a molecular weight of 1032 Da. The MS/MS fragmentation spectrum showing sequential loss of two phosphate groups to give ions *m*/*z* 935 and 837 followed by loss of the bis-butyro group to give ion *m*/*z* 701 confirmed **9** as the bis-butyrophosphate.

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