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Development of an Alternative Process for the Manufacture of a Key Starting Material for Cefovecin Sodium

Timothy Norris,*,† Isao Nagakura,‡ Hiromasa Morita,‡ Grant McLachlan,§ and Joe Desneves§

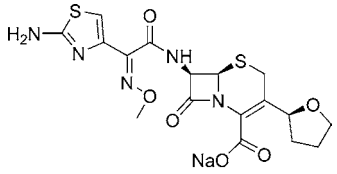
Chemical Research and Development, Pfizer Inc Global Research and Development, Eastern Point Road, Groton, Connecticut 06340, U.S.A., Pfizer Nagoya, 5-2, Taketoyo-cho, Chita-gun, Aichi, 470-2393 Japan, and Institute of Drug Technology, 45 Wadhurst Drive, Boronia, VIC 3155, Australia

Abstract:

A process has been developed for the use of trimethylphosphite for the formation of the six-membered 3,6-dihydro-2*H*-[1,3]-thiazine ring in the cephem architecture by an intramolecular Horner−Emmons−Wadsworth condensation. The process is a suitable alternative to the traditional Wittig process, which uses trimethylphosphine. The process developed is a highly telescoped reaction pathway consisting of at least six known reaction intermediates that was scaled for production use to produce 2.

Introduction

Cefovecin **1** is a potent stable antibiotic targeted for companion animals. Cefovecin features a chiral tetrahydrofuran ring substituent at C-3, which is responsible for the unique activity and stability profile. The synthesis of Cefovecin is described in the patent literature.[1] Starting from penicillin G this synthesis consists of 13 transformations, many of which are telescoped steps. The intermediate products are often variable mixtures of diastereoisomers. It is not until the cephem intermediate **2** is reached that a single crystalline diastereoisomer is obtained. As a result, this material was targeted as a key control gate in the synthesis, and therefore the synthesis is critical to the establishment of a commercial process.



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***cefovecin* sodium**

Parallel conversions from penicillin G *via* penicillin G sulfoxide and rearrangement with trialkyl phosphites lead to the starting material for the synthesis of **3** and related derivatives; this chemistry is reported in the literature.[2] The original synthetic procedure using Wittig chemistry derives from the patent literature[3] and is currently used on a large-scale basis for manufacture of **2** from **3** involving five telescoped steps, in which none of the intermediates are isolated, as shown in Scheme 1.

The β-lactam **3** is converted to a chloro compound using thionyl chloride. After aqueous workup the product **4** is reacted with trimethylphosphine (Me_3P) to form the phosphonium salt **5**. This is unusual because the standard phosphine reagents are tributylphosphine or triphenylphosphines.[4] Triphenylphosphine is unreactive with **4**, and tributlyphosphine and triethylphosphite gave poor yields. Me_3P solves this problem and was used successfully on a large scale. A sodium bicarbonate wash gives the ylide **6**, which reacts intramolecularly with the ketone moiety to form the six-membered ring, over 24 h. The cyclised product **7** is treated with phosphorous pentachloride and isobutanol to achieve removal of the phenacyl protecting group. Compound **2** precipitates from the reaction solution in high purity.

Results and Discusion

Horner−Emmons−Wadsworth Approach. There are a number of disadvantages to the use of Me_3P in this process. First, Me_3P is expensive, contributing approximately 25% to the total cost of goods for the process. Me_3P is also extremely hazardous, as it is highly flammable and toxic with environmental issues. The yields from the reaction were highly variable, and the intermediates are relatively unstable.

An alternative to Me_3P involves the use of trimethylphosphite [$P(OMe)_3$] in the synthesis. The synthetic approach with $P(OMe)_3$ is presented in Scheme 2. Steps one and five are the same as those in the Me_3P route. Replacement of Me_3P by $P(OMe)_3$ leads to the formation of a phosphonate ester **8**, which then undergoes cyclisation to **7**.

Chlorination. Chlorination of **3** using thionyl chloride in dichloromethane with 2-picoline gives **4** in near quantitative yield. The optimum conditions for the $SOCl_2$ charge was found to require 1.1 equiv, based on the initial charge of **3**. Lower charges gave incomplete conversion to **4**.

In order to avoid side-product formation the reaction must be cooled. Cooling a solution of **3** and 2-picoline in dichloromethane from ambient temperature to −20 °C

* Author for correspondence. E-mail: timothy.norris@pfizer.com.
† Pfizer Inc. Global Research and Development.
‡ Pfizer Nagoya.
§ Institute of Drug Technology.

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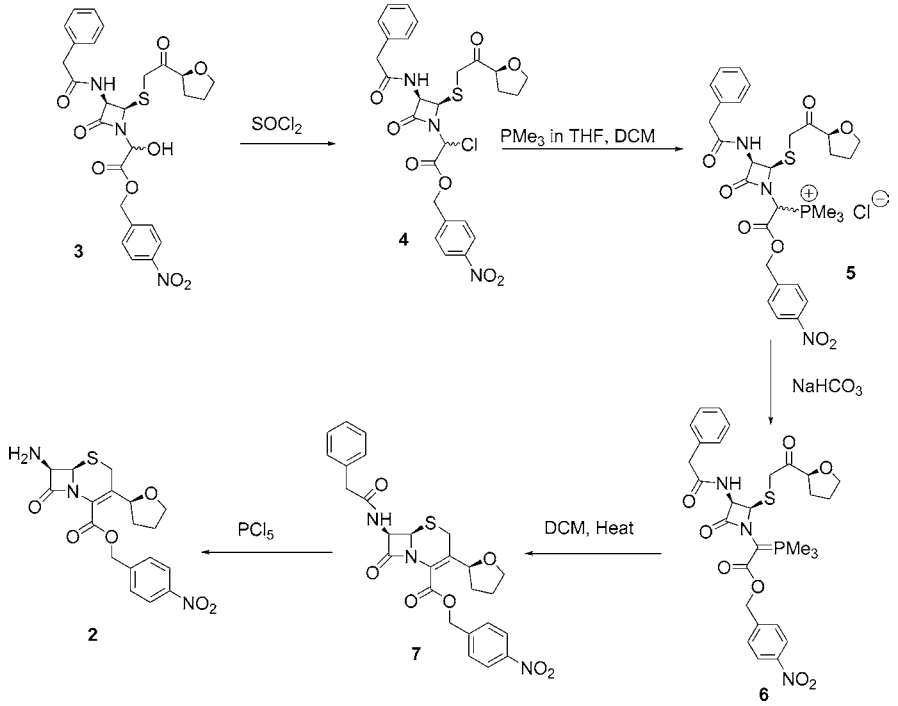
Scheme 1. **Synthesis of 2 via the trimethyl phosphine (Me_3P) route**



resulted in some precipitation. Addition of thionyl chloride to this suspension at −20 °C gave a higher amount of unreacted starting material, which could not be chlorinated by addition of excess thionyl chloride. Therefore, a portion of the total thionyl chloride charge (10%) was added before precipitation commenced, at −15 °C. The solution was then cooled to −20 °C, and the remaining thionyl chloride was added slowly at or below this temperature. The product is significantly more soluble in dichloromethane, and therefore no precipitation was observed using this procedure.

3 and **4** are diastereomeric mixtures of the hydroxy and chloro epimers, respectively. TLC of the chlorination reaction mixture showed clean conversion of **3** to **4**, with a small amount of unreacted **3** and baseline material. None of the diastereomers were resolved. The four possible diastereomers were resolved using reversed-phase HPLC. However, the RP-HPLC result was not consistent with the TLC. It indicated that the reaction mixture contained approximately 50% of product **4** (mainly one epimer) and 50% of starting material **3**, also mainly one epimer. Normal-phase HPLC was consistent with the TLC and showed the reaction conditions used gave greater than 90% conversion to **4**, with 3−10% of **3** remaining. These observations suggested that one epimer of the product hydrolyses rapidly in the RP-HPLC, whereas the other is relatively stable.

Fortunately, although the reaction was quenched into saturated brine and dried over magnesium sulfate before proceeding with the phosphonate formation, the workup procedure did not cause any significant hydrolysis of **4**.

Halide Exchange and Formation of Phosphonate 8. Phosphonates are commonly prepared by reaction of an alkyl halide with the trialkylphosphite (Arbuzov reaction) or by reaction of an alkali metal derivative of the dialkyl phosphate (Michaelis reaction). The Arbuzov reaction offers simpler reaction conditions[5] and was developed for the preparation of phosphonate **8**.

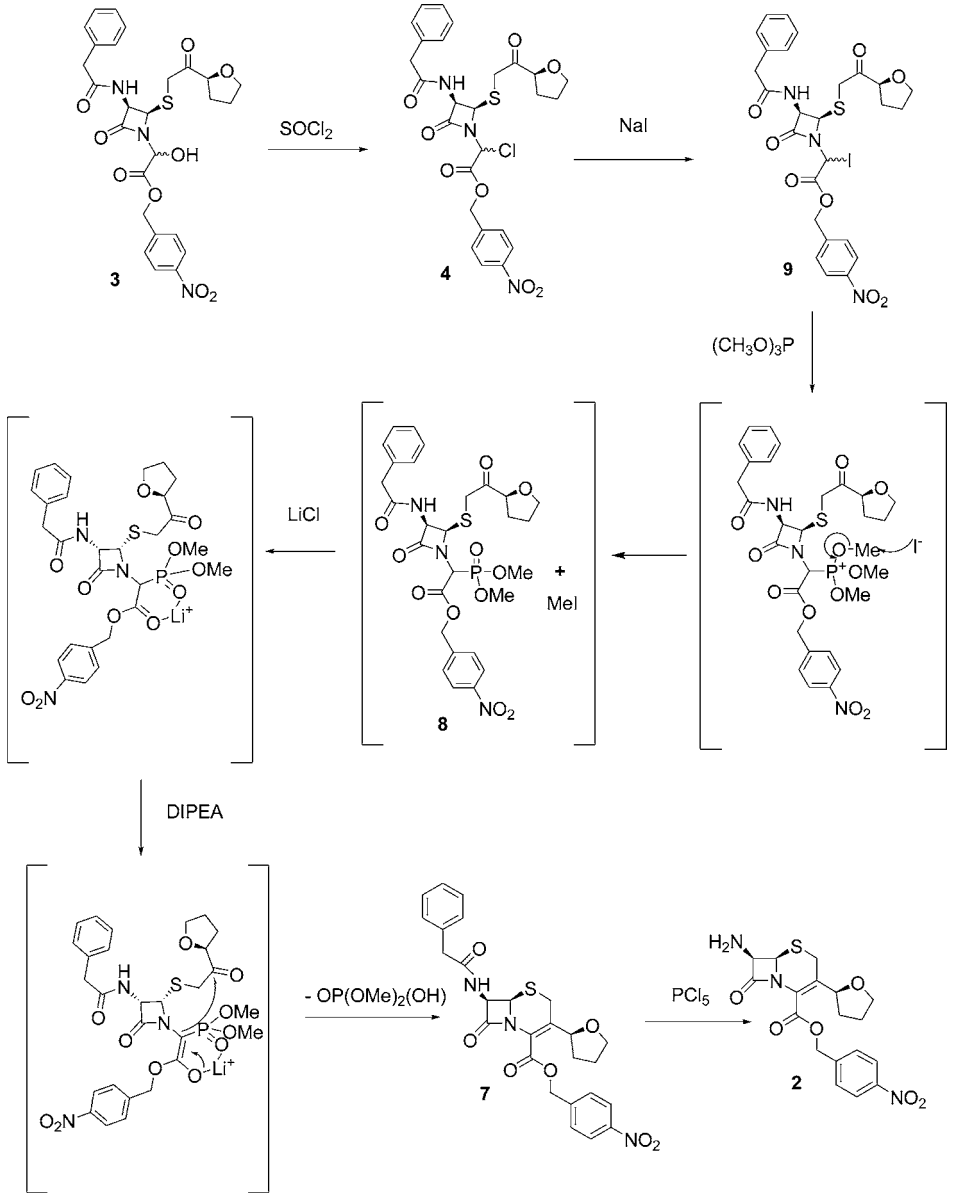
Trimethylphosphite, triethylphosphite, and tributylphosphite do not react with the chloro compound **4**, and therefore the chloride was converted into iodide by reaction with sodium iodide (Finkelstein reaction). This was initially performed by adding sodium iodide to the **4** reaction solution, after the aqueous workup and drying. This procedure gave inconsistent yields and purities of the iodo compound **9**, due to the low solubility of sodium iodide in dichloromethane. Dosing known amounts of water into the reaction mixture and analysis by KF titration showed that when the dichloromethane contained sufficient water to dissolve enough sodium iodide to allow the reaction to proceed, significant hydrolysis occurred.

Alternative solvents for the Finkelstein reaction were evaluated. The use of acetone (and other ketone containing solvents, e.g., methyl ethyl ketone) was avoided due to its potential to compete with the internal ketone during the cyclization of **8**. Acetonitrile was found to be a good solvent for the halide exchange in terms of both product yield and quality. Some degradation occurred if the **4** reaction solution was evaporated to dryness and the residue dissolved in acetonitrile. However, the halide exchange reaction could be performed by drying and then concentration of the **4** reaction solution after workup and drying and then diluting with acetonitrile, followed by addition of the sodium iodide.

The charge of sodium iodide is critical to the yield of **8** (and **9**). Insufficient sodium iodide (e.g., due to lack of availability due to solubility issues) results in a yield reduction through incomplete reaction of **4**. Excess sodium iodide (greater than the optimum charge noted below) causes decomposition of **8**, after its formation by reaction of the

(5) Boutagy J.; Thomas, R. *Chem. Rev.* **1974**, *1*, 87−99.

Scheme 2. **Synthesis of 2 via the trimethyl phosphite [P(OMe)$_3$] route**



excess iodide anions with the phosphonate methyl groups, as shown in Figure 1. The optimum charge was determined as 1.05 mol equiv, based on the initial charge of **3**.[6] Halide exchange is complete within minutes of the addition of sodium iodide.

Our experience with the Wittig synthesis suggested that the use of the least sterically hindered trialkylphosphite for the Arbuzov reaction with **9** would be advantageous. In our hands, only trimethylphosphite [P(OMe)$_3$] gave good conversion of **9** to the corresponding phosphonate derivative.

The phosphonate **8** was prepared by addition of P(OMe)$_3$ to the solution of **9**. The reaction of P(OMe)$_3$ with **9** is exothermic, and this required careful control since the rate of production of the phosphate impurity was increased with temperature (as shown in Figure 1). The exotherm was controlled by cooling the solution of **9** to below 5 °C before the addition and by slow addition of the P(OMe)$_3$ as a solution in dichloromethane.

The optimum P(OMe)$_3$ charge was found to be 1.45 mol equiv, based on the initial charge of **3**. Lower charges of P(OMe)$_3$ gave incomplete conversion of **9** to **8**, and higher charges gave rise to problems later in the synthesis (in the PCl_5 deprotection) due to the telescopic design of the process.

The phosphonate is fully formed after reaction for 1.5 h at room temperature. An HPLC solution assay for **8** was developed, which showed a yield of 75% from **3**. It was important to determine the content of **8** in the reaction mixture so that subsequent reagent charges could be based on the result.

Cyclization. The cephem six-membered ring cyclization is performed by addition of lithium chloride[7] and an organic

(6) The amount of the phosphate impurity in the reactions with minimal or no excess sodium iodide was initially very low. It was found to increase in the reaction solutions on storage for 24 h at ambient temperature if excess sodium iodide was present. However, **8** solutions may be held at lower temperatures (4 °C and −20 °C) for up to 4 days.

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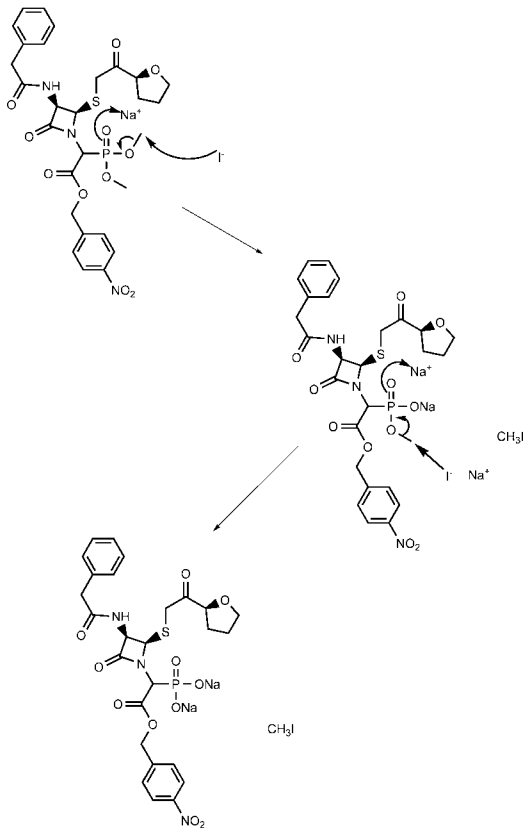

Figure 1. **Proposed mechanism for the formation of the phosphate impurity.**

soluble base to the **8** reaction solution. The reaction proceeds via formation of a (stabilized) phosphonate anion, which cyclizes internally to give the product, **7**, which contains the fully formed bicyclic cephalosporin nucleus.

At least 2 mol equiv of lithium chloride were required for successful cyclization. Excess lithium chloride had no deleterious effect.

A number of bases were investigated, and diisopropylethylamine, DIPEA, was found to be the most successful.[8] Weaker bases than DIPEA were unsuccessful, probably because they are not able to deprotonate the phosphonate.

A major difference between the phosphite and phosphine routes is the potential for $\Delta 2-3$ double bond isomerisation during the cyclisation step in the phosphite method. Isomerization of the double bond in the cephalosporin ring is promoted by a base.[9] In the Wittig synthesis the ylide is formed by treatment of the phosphonium salt in dichloromethane with aqueous sodium bicarbonate. The organic phase is separated and allowed to cyclize at ambient temperature, which takes up to 16 h. Since DIPEA is a stronger base than bicarbonate and it is difficult to remove from the reaction until after the cyclization, the DIPEA charge is critical. The amount of isomerization is directly related to the DIPEA charge. The optimal amount of DIPEA is in the range 1.20 to 1.50 equiv, based on the mole amount of **8**. This ensures complete reaction and minimizes the formation of the double-bond isomer. The amount of **8** in the reaction solution was determined by HPLC assay, and the DIPEA and lithium chloride charges were based on the result.

After addition of DIPEA and lithium chloride the solution was stirred at ambient temperature to effect the cyclization, which required more than 16 h to go to completion. The use of a higher reaction temperature and/or significantly longer reaction times led to an increase in side products and lower yield.

It was found that residual water in the cyclization reaction mixture resulted in the formation of impurities and lower yields. Therefore, the solution of **8** was dried over magnesium sulfate before addition of the lithium chloride and DIPEA.[10] The yield of **7** from **3** in the above reaction is comparable to that in the original Me_3P route.[11]

Deprotection. The deprotection uses the standard conditions in cephalosporin chemistry, phosphorous pentachloride, picoline, and then isobutanol.[12] Compounds **9** and **8** require the presence of acetonitrile in the reaction solution. However, it was necessary to remove the acetonitrile prior to proceeding with the final deprotection of compound **7** reaction because acetonitrile reacts with the phosphorous pentachloride, and it increases the solubility of the product (**2**) in the reaction mixture and results in a lower yield.[13]

There were two opportunities to remove the acetontrile: after formation of **8** or after formation of **7**. There are two methods for removal of the acetontrile: distillation and phase extraction. Studies found that removal of the acetonitrile after formation of **8** by distillation affected the product impurity profile and yield of **7**. Likewise, removal of acetonitrile by extraction of the reaction mixture containing **8**, prior to cyclisation, leads to emulsions, low yield, and recovery and issues with the reaction water content when proceeding to the subsequent cyclisation step. Thus the only step available to remove the acetonitrile was immediately prior to the deprotection of **7**. The reaction mixture was extracted with acid solution to remove the DIPEA salts, followed by brine, and this removed some of the acetonitrile. The reaction mixture was then distilled twice, to ensure complete removal of acetonitrile.

Two major issues with the conversion of **7** to **2** are the residual water content and control of reaction temperature/exotherms. These are common to both the phosphite and phosphine routes. The water content needs to be low, and this is achieved through the distillation procedure to remove the acetonitrile.

In addition, it has been found that the deprotection reaction works consistently well on **7** that has been isolated and purified[14] but is variable when using **7** produced via this telescoped series of reactions from **3**. This suggests that some

(8) DBU and other soluble bases can also be used successfully. The use of hetergeneous bases was not successful.
(9) Base causes double bond isomerization.
(10) Compound **8** interferes with the KF reagents, and the water content of the reaction solution was unknown. The magnesium sulfate was sufficient to provide a clean reaction.
(11) See ref 6.
(12) Standard deprotection conditions in cephalosporin chemistry.
(13) If the product does not precipitate from the reaction mixture, it decomposes reasonably rapidly.
(14) A reference standard of **7** was prepared by coupling of **2** with phenylacetic acid.

other component(s) in the reaction solution have a detrimental effect on the deprotection reaction.

Dimethylphosphate (DMP) is a byproduct of the cyclization reaction. DMP and the excess $P(OMe)_3$ were shown to negatively effect the deprotection and are not removed by the aqueous workup of **7**. Based on this observation the excess $P(OMe)_3$ charge used in the preparation of **8** from **9** was kept at a minimum, which was found to be 1.45. There is some data to suggest that 10 Å molecular sieves remove the phosphorous compounds from the reaction mixture.

Conclusion

The phosphite route has been shown to be robust. On a 50 g scale three lots of **2** were prepared in very similar yields (50−55%). The overall yield is comparable with the best achievable with the phosphine method. Batches of **2** prepared by the Horner−Emmons−Wadsworth method were found to have a similar impurity profile to that produced by the original phosphine (Wittig) method. They have been used to prepare Cefovecin **1** that met all of the current test specifications for drug substance release, and the process was subsequently scaled for production operations.

Experimental Section

(6*R*,7*R*)-4-Nitrobenzyl 7-Amino-8-oxo-3-((*S*)-tetrahydrofuran-2-yl)-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylate Hydrochloride Salt, 2. Compound **3** (51.19 g) (80% potency, 73.4 mmol) was dissolved in dichloromethane (750 mL). 2-Picoline (11.8 mL) (119.5 mmol, 1.63 equiv) was added, and the solution was cooled to −15 °C. Thionyl chloride (7.6 mL) (104.19 mmol, 1.42 equiv) was added in one portion (over approximately 3 min). The reaction was stirred for 1 h below −20 °C. It was washed with 20% brine solution (2 × 250 mL) and dried over 40 g of magnesium sulfate, for 10 min at ambient temperature. The desiccant was filtered off and washed with 100 mL of dichloromethane. The filtrate was concentrated to 150 mL on a rotary evaporator at less than 35 °C. Acetonitrile (150 mL) was added, and the solution was further concentrated to 200 mL at less than 35 °C. The solution was cooled to less than 5 °C. Sodium iodide (11.59 g) (119.5 mmol, 1.05 equiv to 3) was charged followed by trimethylphosphite (12.6 mL) (106.8 mmol, 1.45 equiv to 3) dissolved in dichloromethane (10 mL), added dropwise over 10 min. The temperature was maintained at or below 5 °C during the addition. No exotherm was observed on this scale. The solution was allowed to warm to room temperature over 1.5 h. The phosphonate content was determined by HPLC assay (36.49 g, 56.2 mmol). This corresponds to a yield of 76.5% for the two steps. Dichloromethane (500 mL) was added (total volume approximately 700 mL). Activated carbon (17 g) and magnesium sulfate (20.1 g) were added, and the mixture was stirred for 10 min. The mixture was clarified by filtration through a bed of celite, and the celite was washed with dichloromethane (150 mL). The phosphonate content was determined by HPLC assay (36.5 g, 56.1mmol). Lithium chloride (5.11 g) (120.5 mmol, 2.15 equiv relative to the phosphonate) and DIPEA (12.6 mL) (72.3 mmol, 1.29 equiv relative to the phosphonate) were added. The solution was stirred at ambient temperature for 16 h. The reaction solution was successively washed with 1% aqueous hydrochloric acid (400 mL) and 20% brine solution (2 × 400 mL). The organic phase was dried with powered 4 Å molecular sieves (22.3 g) and celite (20.3 g). The desiccant was decanted off through a plug of silica G (43 g) and washed with dichloromethane (200 mL). The solution was concentrated to a thick oil on a rotary evaporator at less than 35 °C, and dichloromethane (350 mL) was added. This solution was then reconcentrated to a thick oil on a rotary evaporator at less than 35 °C, and dichloromethane (350 mL) was added. The water content was determined to be 140 ppm. The **7** content was determined by HPLC assay as 25.76 g (49.2 mmol, 67.0% yield from **3**, 87.6% for the cyclization). The solution was cooled to −55 °C, and phosphorous pentachloride (30.4 g) (147.4mmol, 3.0 equiv of **7**) was charged. After 5 min, 2-picoline (29 mL) (293.6mmol, 6.0 equiv of **7**) was added, maintaining the temperature below −40 °C. An exotherm was observed. The solution was stirred for 1 h below −20 °C. At this stage the reaction was a thick slurry. It was cooled to below −50 °C, and isobutanol (205 mL, 2.02mol) was charged. This caused the reaction to warm to −30 °C. The solution was allowed to warm to ambient temperature, and after stirring for 1 h, a seed crystal of **2** was added. The solution was stirred for 16 h in a closed system to avoid evaporation of the dichloromethane. The solid was collected by filtration. The solid was washed with dichloromethane (2 × 100 mL). The solid was dried to constant weight at 40 °C under high vacuum to give **2** (18.4 g) (41.64mmol, 56.7% yield from **3**, 84.6% yield from **7**).

Mp 202 °C decomp. ^{1}H NMR (400 MHz, D_2O/CD_3CN): δ 1.62 (m, 1H), 1.85 (m, 2H), 2.15 (m, 1H), 3.37 (d, 1H, *J* 17.6 Hz), 3.57 (d, 1H, *J* 17.6 Hz), 3.7(m, 1H), 3.85 (m, 1H), 4.91 (dd, 1H), 4.95 (d, 1H, *J* 4.9 Hz), 5.13 (d, 1H, *J* 4.9 Hz), 5.31 (dd, 2H), 7.58 (d, 2H, *J* 8.9 Hz), 8.18 (d, 2H, *J* 8.9 Hz). ^{13}C NMR (100 MHz, D_2O/CD_3CN): δ 24.90, 26.92, 32.71, 67.59, 55.96, 59.34, 70.58, 78.21, 123.02, 143.80, 124.81, 130.08, 142.13, 148.84, 161.86, 162.59. MS (*m*/*z*) 406.3 amu (M + H), $C_{18}H_{20}ClN_3O_6S$ requires: C, 48.92; H, 4.70; N, 9.36; S, 7.26. Found: C, 49.00; H, 4.74; N, 9.44; S, 6.84%

Characterization and isolation of normally nonisolated intermediates found between **3** and **2** in the synthetic pathway are available from the patent literature.[15]

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