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Practical Synthesis of the New Carbapenem Antibiotic Ertapenem Sodium

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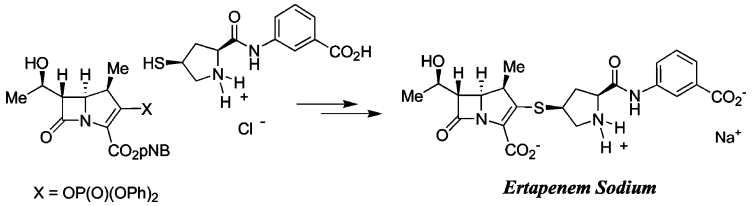

A practical synthesis for the large-scale production of the new carbapenem antibiotic, [4*R*,5*S*,6*S*]-3-[[(3*S*,5*S*)-5-[[(3-Carboxyphenyl)amino]carbonyl]-3-pyrrolidinyl]thio]-6-[(1*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid monosodium salt (ertapenem sodium, **1**), has been developed. The synthesis features the novel use of 1,1,3,3-tetramethylguanidine as base for the low-temperature reaction of a thiol, derived from *trans*-4-hydroxy-L-proline, with the carbapenem nucleus activated as the enol phosphate. Hydrogenolysis of a *p*-nitrobenzyl ester is effected using a palladium on carbon catalyst to give an overall yield for the two steps of 90%. The use of bicarbonate in the hydrogenolysis was key in providing protection of the pyrrolidine amine as the sodium carbamate improving both the performance of the reaction and the stability of the product. This discovery made processing at manufacturing scale possible. Experimental evidence for the formation of the sodium carbamate is provided. A remarkably expedient process for the simultaneous purification and concentration of the aqueous product stream relies on ion-pairing extraction for the removal of the water-soluble 1,1,3,3-tetramethylguanidine. Crystallization then affords 59−64% overall yield of the monosodium salt form of the product.

Introduction

The rising emergence of bacteria resistant to existing antibacterial therapy is responsible for the prevailing interest in new agents for the fight against these threats to human health.[1] The carbapenem class of antibiotics has become widely used in treating infections caused by a broad range of pathogens.[2] The development of new carbapenems is an important part of our effort to address the problem of multi-drug-resistant bacteria and has produced new antibiotics with improved properties for the treatment of human infections.[3]

Imipenem, the first carbapenem antibiotic approved for clinical use, has a remarkable spectrum of activity and remains on the last line of defense in treatment of many of the most serious infections.[4] As a consequence of poor solution stability toward hydrolysis and susceptibility to enzymatic inactivation, however, imipenem must be administered four times daily along with cilastatin, an inhibitor of the enzyme, human renal dehydropeptidase-1 (DHP-1), which is responsible for inactivation of the antibiotic.[5]

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(1) (a) Goldhaber, M. *Science* **1994**, *266*, 1462. (b) Verhoef, J. *Adv. Exp. Med. Biol.* **2003**, *531*, 301−13.

(2) (a) Coulton, S.; Hunt, E. In *Progress in Medicinal Chemistry*; Ellis, G. P., Luscombe, D. K., Eds.; Elsevier Science: 1996; Vol. 33, p 99−145. (b) Hashizume, T.; Morishima, H. *Drugs Future* **2000**, *25*, 833−841.

(3) Sunagawa, M.; Sasaki, A. *Heterocycles* **2001**, *54*, 497−528.

(4) (a) Kahan, J. S.; Kahan, F. M.; Goegelman, R.; Currie, S. A.; Jackson, M.; Stapley, E. O.; Miller, T. W.; Miller, A. K.; Hendlin, D.; Mochales, S.; Hernandez, S.; Woodruff, H. B.; Birnbaum, J. *J. Antibiot. (Tokyo)* **1979**, *32*, 1−12. (b) Kropp, H.; Sundelof, J. G.; Kahan, J. S.; Kahan, F. M.; Birnbaum, J. *Antimicrob. Agents Chemother.* **1980**, *17*, 993−1000.

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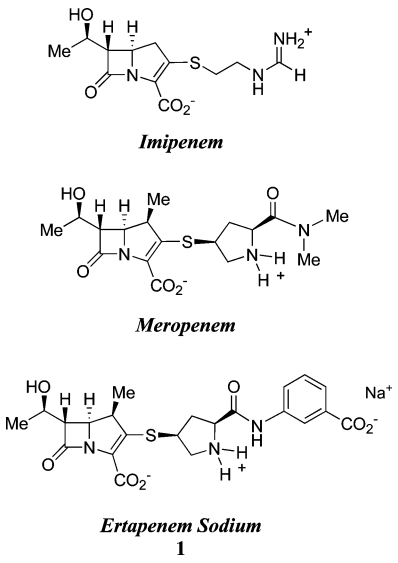


FIGURE 1.

Since imipenem was introduced in the 1980s, there has been a substantial effort to identify carbapenem antibiotics with improved pharmacokinetics while retaining a broad spectrum of activity. The introduction of a β-methyl substituent to the carbapenem nucleus proved to be a key advance in achieving this goal.[6] Meropenem, discovered at Sumitomo Pharmaceuticals, was the first carbapenem antibiotic to be developed for clinical use as single agent therapy.[7] Multiple daily dosing, however, was still required.

Ertapenem sodium[8] is a new broad-spectrum carbapenem antibiotic that has demonstrated efficacy against the growing number of cephalosporin-resistant bacteria.[9] In clinical trials, the antibiotic showed an improved pharmacokinetic profile in comparison with other carbapenems allowing single agent therapy and once-daily dosing.[10] Ertapenem sodium is the active ingredient in INVANZ which was recently approved in the United States as an IV or IM treatment for moderate to severe upper and lower respiratory tract, urinary tract, skin, obstetric, and gynecologic infections. While the β-methyl carbapenems clearly represent a significant advance in antibiotic therapy, these new agents challenge existing methods of synthesis. In the most convergent approach to these compounds, construction of a carbon−sulfur bond is made more difficult by the increased complexity of the reaction partners and the increased steric demands on bringing them together. The introduction of charged and nucleophilic functionality, while important in giving the antibiotic the desired properties, has made purification and isolation of the product more difficult. Herein, we describe our strategy and discoveries leading to a practical synthesis for the new antibiotic ertapenem sodium **1** allowing production on multi-kilogram scale.[11]

Results and Discussion

Ertapenem is an amino dicarboxylic acid and as such can exist in four forms in solution. The zwitterionic monocarboxylate and the dicarboxylate salts are the predominant forms in the pH range where the best solution stability is observed.[12] The monosodium salt is the predominant form in the range from pH 4 to 7; the disodium salt becomes the predominant form above pH 7. Ertapenem was found to crystallize as the monosodium salt from a concentrated aqueous solution at pH 5.5 on addition of a specific combination of alcohol solvents. Attempts to crystallize other salts were not successful. The monosodium salt, therefore, became the target for isolation of the product.

Since isolation of the product would require achieving a concentration in excess of 100 mg/mL, we examined the solution stability at that concentration and found that the rate of degradation in aqueous solution at 2 °C is about 1%/h across the pH range which affords the best solution stability (pH 5−8). Although hydrolysis contributes to the degradation of ertapenem, the formation of dimeric degradates accounts for most of the loss in this pH range at high concentration.[13]

As with Meropenem, attack of the pyrrolidine amine on the β-lactam is responsible for formation of dimeric degradates.[14] In the case of ertapenem, other degradates resulting from bimolecular reactions appear to arise from attack by the two carboxylates on the β-lactam, leading initially to anhydrides, which can then undergo intramolecular acyl transfer to give the more stable products that are observed.[15] The degradates have been characterized by NMR analysis of samples isolated by preparative HPLC.[12]

Recognizing the limitations that product stability would impose on purification and isolation of the product,

(5) Norrby, S. R.; Alestig, K.; Bjornegard, B.; Burman, L. A.; Ferber, F.; Huber, J. L.; Jones, K. H.; Kahan, F. M.; Kahan, J. S.; Kropp, H.; Meisinger, M. A.; Sundelof, J. G. *Antimicrob. Agents Chemother.* **1983**, *23*, 300−7.

(6) Shih, D. H.; Baker, F.; Cama, L.; Christensen, B. G. *Heterocycles* **1984**, *21*, 29−40.

(7) (a) Sunagawa, M.; Matsumura, H.; Inoue, T.; Fukasawa, M.; Kato, M. *J. Antibiot. (Tokyo)* **1990**, *43*, 519−32. (b) Sunagawa, M.; Matsumura, H.; Inoue, T.; Fukasawa, M.; Kato, M. *J. Antibiot. (Tokyo)* **1991**, *44*, 459−62. (c) Fukasawa, M.; Sumita, Y.; Harabe, E. T.; Tanio, T.; Nouda, H.; Kohzuki, T.; Okuda, T.; Matsumura, H.; Sunagawa, M. *Antimicrob. Agents Chemother.* **1992**, *36*, 1577−9.

(8) Ertapenem was discovered at Zeneca Pharmaceuticals (now AstraZeneca) and was licenced by Merck. Betts, M. J.; Davies, G. M.; Swain, M. L. Antibiotic Compounds. U.S. Patent 5,478,820, 1995.

(9) (a) Jones, R. N. *J. Chemother.* **2001**, *13*, 363−76. (b) Hilliard, N. J.; Johnson, C. N.; Armstrong, S. H.; Quarles, S.; Waites, K. B. *Int. J. Antimicrob. Agents* **2002**, *20*, 136−40.

(10) Gill, C. J.; Jackson, J. J.; Gerckens, L. S.; Pelak, B. A.; Thompson, R. K.; Sundelof, J. G.; Kropp, H.; Rosen, H. *Antimicrob. Agents Chemother.* **1998**, *42*, 1996−2001. (b) Majumdar, A. K.; Musson, D. G.; Birk, K. L.; Kitchen, C. J.; Holland, S.; McCrea, J.; Mistry, G.; Hesney, M.; Xi, L.; Li, S. X.; Haesen, R.; Blum, R. A.; Lins, R. L.; Greenberg, H.; Waldman, S.; Deutsch, P.; Rogers, J. D. *Antimicrob. Agents Chemother.* **2002**, *46*, 3506−11.

(11) For a recent review covering synthesis of carbapenems, see: Singh, G. S. *Mini-Rev. Med. Chem.* **2004**, *4*, 69−92. A scaleable procedure for preparation of the carbapenem doripenem, avoiding chromatographic purification, was recently reported: Nishino, Y.; Kobayashi, M.; Shinno, T.; Izumi, K.; Yonezawa, H.; Masui, Y.; Takahira, M. *Org. Process Res. Dev.* **2003**, *7*, 846−850.

(12) Crocker, L. S.; Wang, Y.; McCauley, J. A. *Org. Process Res. Dev.* **2001**, *5*, 77−79.

(13) Sajonz, P.; Natishan, T. K.; Wu, Y.; Williams, J. M.; Pipik, B.; DiMichele, L.; Novak, T.; Pitzenberger, S.; Dubost, D.; Almarsson, Ö. *J. Liq. Chrom. Relat. Technol.* **2001**, *24*, 2999−3015.

(14) Takeuchi, Y.; Sunagawa, M.; Isobe, Y.; Hamazume, Y.; Noguchi, T. *Chem. Pharm. Bull.* **1995**, *43*, 689.

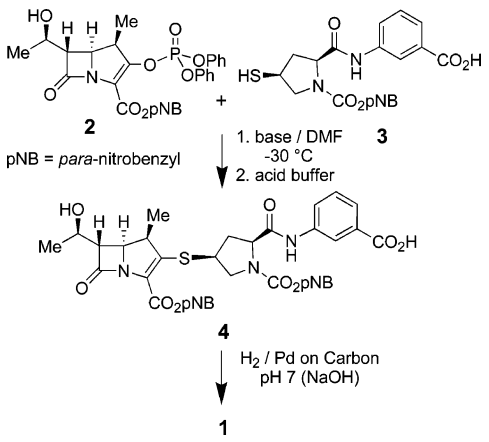
(15) An anhydride has been proposed as an intermediate in the bimolecular degradation of imipenem: (a) Smith, G. B.; Schoenewaldt, E. F. *J. Pharm. Sci.* **1981**, *70*, 272−6. (b) Ratcliffe, R. W.; Wildonger, K. J.; DiMichele, L.; Douglas, A. W.; Hajdu, R.; Goegelman, R. T.; Springer, J. P.; Hirshfield, J. *J. Org. Chem.* **1989**, *54*, 653−660. (c) Smith, G. B.; Dezeny, G. C.; Douglas, A. W. *J. Pharm. Sci.* **1990**, *79*, 732−40.

we set out to address this problem in design of the synthesis. Since the removal of protecting groups would likely detract from the yield overall and create byproducts that would have to be removed in purification of the product, we intended to minimize the use of protecting groups. At the outset, we realized the importance of each reaction consistently performing near perfection, not only to achieve the highest possible yield, but also to limit the need for operations to remove undesired side-products. The processing time associated with these operations was expected to be very costly in terms of degradation and, thus, yield. Herein we describe how this strategy led to an exceedingly practical synthesis which forms the basis for the manufacture of this important compound.

1st Generation Synthesis. Carbapenems related to imipenem have generally been constructed through reaction of a thiol with an activated form of the bicyclic carbapenem nucleus.[16] Since appearing in the first reported synthesis of a β-methyl carbapenem,[7] the enol phosphate **2** has seen extensive use in the preparation of a large number of carbapenems for testing as antibacterial agents[3,4] and is now commercially available in bulk quantities.[17] For the coupling reaction, functionality in the thiol has generally been protected to avoid competing reactions and to improve the organic solubility and stability of the product after coupling. The thiols required for the synthesis of ertapenem were derived through a highly efficient process starting with *trans*-4-hydroxyl-L-proline as previously reported.[18] This process allowed preparation of thiol substrates for the coupling reaction arrayed both with and without protecting groups at the carboxylic acid and amine functions. Initially, we chose to protect the amine as a *p*-nitrobenzyl carbamate allowing deprotection concurrent with hydrogenolysis of the *p*-nitrobenzyl ester. Considering the difference in reactivity between carboxylate and thiolate toward soft electrophiles, we expected that we would be able to define conditions for the coupling reaction favoring the desired reaction so that protection of the carboxylic acid would not be required.[19]

It was clearly important to consistently achieve high conversion in the coupling reaction to avoid carrying unreacted thiol into the hydrogenation where it would undoubtedly poison the catalyst.[20] Following standard conditions reported in the carbapenem literature, reactions of **3** with **2** using a moderate excess (2.1 equiv) of a tertiary amine base such as diisopropylethylamine or triethylamine in DMF at −30 °C proved to be slow, requiring 24−48 h to approach complete conversion (Scheme 1). Increasing the temperature significantly

SCHEME 1



compromised selectivity for the desired reaction. Although some improvement in rate could be achieved by using more base, the reduction in reaction time was modest and consistently achieving complete conversion remained a problem.

The first solution to this problem came from an experiment with a very different goal. After completion of the reaction, we found that three extractions were required to remove the amine salts and solvent (DMF) prior to hydrogenolysis of the protecting groups. It was necessary to perform extractions at this stage since the product of the hydrogenolysis would be water soluble and removal of DMF from the aqueous solution would not be practical. We were interested in avoiding these extractions, and it occurred to us that it might be possible to crystallize the product from the reaction mixture as a carboxylate salt if a suitable amine were used as base in the coupling. Filtration then would afford the product free of the coupling reaction solvent and all but one equiv of amine. Dicyclohexylamine often gives crystalline carboxylate salts. In the reaction using dicyclohexylamine as base, crystalline solids were observed, but these solids proved to be the diphenyl phosphate salt of dicyclohexylamine and not the salt of the carbapenem product. Although the experiment did not produce the desired result, we observed that the coupling reaction was much faster, achieving complete conversion in only 4 h. Diisopropylamine is considerably less expensive and lower in molecular weight compared with dicyclohexylamine and proved to be an excellent base for this reaction. We did not uncover any evidence for reaction with the β-lactam.

The rate increase observed in using dicyclohexylamine or diisopropylamine suggests that the secondary amines afford a substantially higher proportion of thiolate in the rate-limiting equilibrium between thiol and thiolate compared with the tertiary amine bases. The dissociation constants reported for diethylamine and triethylamine in DMF are 10.4 and 9.25, respectively.[21] The dissociation constant for thiol **3** in DMF was estimated to be 11.2.[22]

(16) An alternate, less convergent approach has been reported: Prashad, A. S.; Vlahos, N.; Fabio, P.; Feigelson, G. B. *Tetrahedron Lett.* **1998**, *39*, 7035−7038.

(17) Interest in the synthesis of 1-β-methyl carbapenems generated a variety of solutions to this problem: Berks, A. H. *Tetrahedron* **1996**, *52*. The carbapenem nucleus has become commercially available with the carboxylic acid protected as the *p*-nitrobenzyl ester from Nisso, Kaneka, and Takasago.

(18) (a) Brands, K. M. J.; Marchesini, G.; Williams, J. M.; Dolling, U.-H.; Reider, P. J. *Tetrahedron Lett.* **1996**, *37*, 2919−2922. (b) Brands, K. M.; Jobson, R. B.; Conrad, K. M.; Williams, J. M.; Pipik, B.; Cameron, M.; Davies, A. J.; Houghton, P. G.; Ashwood, M. S.; Cottrell, I. F.; Reamer, R. A.; Kennedy, D. J.; Dolling, U. H.; Reider, P. J. *J. Org. Chem.* **2002**, *67*, 4771−6.

(19) Pearson, R. G. *J. Am. Chem. Soc.* **1963**, *85*, 3533−3539.

(20) Rylander, P. N. *Catalytic Hydrogenation in Organic Synthesis*; Academic Press: New York, 1979.

(21) Demange-Guerin, G. *Talanta* **1970**, *17*, 1075−1084. The dissociation constant for diisopropylamine in DMF has not been reported.

(22) The dissociation constants for the carboxylic acid and thiol functions were determined as a function of DMF concentration in water by titration. Extrapolation to 100% DMF gave dissociation constants of 8.2 and 11.2, respectively. McCauley, J. A. Unpublished results (May 5, 1994). For validation of this approach, see: Sistovaris, N.; Hamachi, Y.; Kuriki, T. *Fresenius J. Anal. Chem.* **1991**, *340* 345−349.

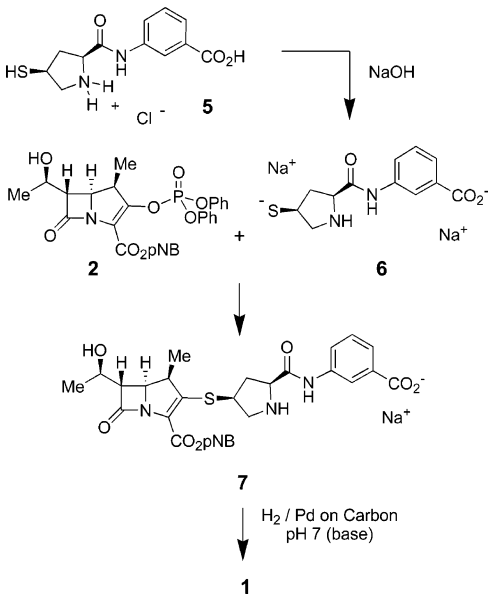
The use of secondary amines for formation of the C−S bond in the synthesis of carbapenems is unprecedented.

After complete conversion had been achieved, the reaction was quenched into a mixture of dilute phosphoric acid and ethyl acetate. DMF and amine salts were removed at this stage through multiple extractions. The ethyl acetate solution was then combined with Pd on carbon catalyst in water for hydrogenolysis of both *p*-nitrobenzyl ester and *p*-nitrobenzyl carbamate. The pH of this biphasic reaction was controlled between 7.1 and 7.2 during the reaction by the addition of NaOH.

Following hydrogenolysis, two unanticipated problems were encountered. The filtration to remove the catalyst was very slow and the level of Pd in the filtrate was unacceptably high. Both problems were overcome by adjusting the pH to <6 prior to filtration. The Pd level in the resulting filtrate was dramatically reduced (from >1000 to <2 ppm); the filtrate was no longer black but light yellow. At the lower pH, however, the product was less stable. Adjusting the pH to 7−8 improved solution stability, but the salts introduced in the two pH adjustments added to the load of impurities that had to be removed from the aqueous solution to allow isolation of the product. The time required for these operations contributed to the increasing level of degradates. In lab-scale experiments, the assay yield of ertapenem was 75 to 80%.[23] Yields were somewhat lower with more degradates formed on larger scale where each operation requires more time.

Following the catalyst filtration, the product was purified using a hydrophobic resin column. The best purification was achieved by first extracting residual ethyl acetate using dichloromethane. Ethyl acetate affected retention on the polystyrene-based column; whereas, residual dichloromethane did not. Since the product has charged functionality at the pH of the solution (pH 7−8) loaded onto the column, it is not readily adsorbed on the solid resin stationary phase and the presence of ethyl acetate adversely affects retention on the column. The column was eluted using dilute acetone (2%) in aqueous sodium bicarbonate (0.05 M) to give a solution of ertapenem with reduced levels of degradates, reaction byproducts, and salts in 80−85% recovery. The solution, however, was quite dilute (5−10 g/L), and significant concentration was required before the product could be crystallized. Nanofiltration, with diafiltration used to reduce the level of inorganic salts, allowed concentration of the aqueous solution at below 10 °C.[24] Initially, this concentration step was conducted at about pH 5.5 to allow more complete removal of salts. Later, we found that the pH of the rich cut could be adjusted to <6 using HCl and then back to pH 7 using NaOH to improve the stability of ertapenem during nanofiltration. These operations converted sodium bicarbonate, used in elution of the hydrophobic resin column, to sodium chloride which was more efficiently removed by nanofiltration. Nonetheless, the time required for these operations, particularly as the concentration increased, resulted in degradation of the product. When the desired concentration range was reached, the pH of the solution was adjusted to 5.5 using acetic acid and the monosodium salt was then crystallized from solution by adding methanol and 1-propanol. A costly recrystallization was typically required to produce pure product.

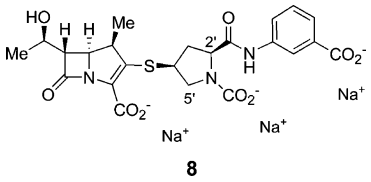
SCHEME 2



2nd Generation Synthesis. The productivity of the 1st generation synthesis was clearly limited by the hydrophobic resin column purification. The subsequent concentration (about 20×) that was needed in order to crystallize the product was inefficient and resulted in product degradation. The resin column was primarily introduced to remove byproducts of the hydrogenolysis reaction. We expected therefore that the potential for eliminating the resin column from the isolation process would be improved if the coupling reaction could be performed without a protecting group on the pyrrolidine amine. Furthermore, the pH adjustment, which had been introduced to control the level of solubilized palladium, increased the degradate and inorganic salt load thus limiting options for improving efficiency in purification and isolation of the product. Recovery in the recrystallization of **1**, which was often required to remove degradates from the isolated product, was only about 80% providing a clear incentive for devising a synthesis where recrystallization would not be required.

In our initial effort to make use of the hydrochloride salt **5**, we identified serious challenges (Scheme 2). The product of the coupling reaction was now an amino acid, and it was no longer possible to use simple extractions to remove DMF and amine salts after the coupling without substantial losses of product. Therefore, the solvent and salts from the coupling reaction would have to be removed after the hydrogenolysis. *N*-Ethylpyrrolidinone (NEP) was chosen as solvent to allow extraction from an aqueous solution containing ertapenem following hydrogenolysis. The list of bases suitable for use in the coupling and affording salts that could be removed from an aqueous solution was quite limited. We expected that sodium hydroxide would be a sufficiently strong base to give complete deprotonation of the thiol for the coupling

(23) For consistency, yields reported throughout are based on **2**.
(24) Antonucci, V.; Yen, D.; Kelly, J.; Crocker, L.; Dienemann, E.; Miller, R.; Almarrson, Ö. *J. Pharm. Sci.* **2002**, *91* (4), 923−932.



8

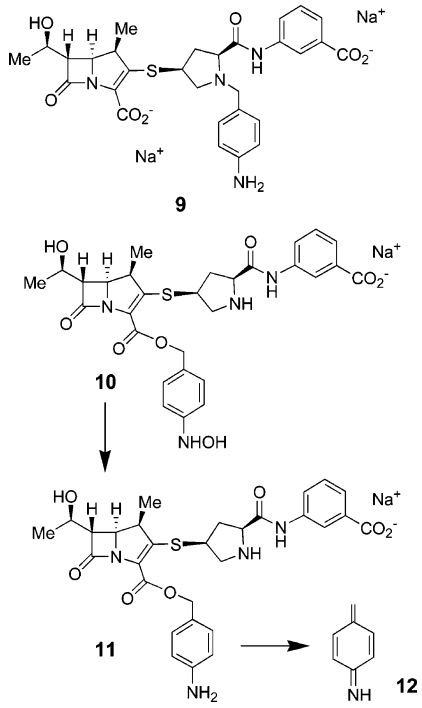
FIGURE 2.

and that sodium salts could be removed from the aqueous product stream by nanofiltration after the hydrogenolysis.

In proposing to use sodium hydroxide in this reaction, however, it was obvious that we would need to change the way the coupling reaction was carried out. The base could clearly not be added to a cold mixture of enol phosphate and thiol. Thus, it became necessary to form the thiolate separately to avoid hydrolysis of the carbapenem; addition of more than 3 equiv of a solution of sodium hydroxide in water to a solution of thiol **5** in NEP after rigorous degassing was found to ensure complete conversion in the subsequent coupling reaction. Any excess NaOH, however, led to hydrolysis of the carbapenem. We discovered that isopropyl acetate could be added to the thiolate solution to consume excess NaOH; the resulting sodium acetate and 2-propanol formed were innocuous in the coupling. When an NEP solution of the carbapenem enol phosphate **2** was added to the cold NEP solution of the thiolate, complete conversion could be achieved at low temperature. Good selectivity was observed as long as the temperature was maintained below −40 °C. The water introduced with the sodium hydroxide to ensure solubility of the sodium salts, however, caused the reaction mixture to become very viscous below −45 °C. After the reaction was complete, the mixture was quenched into aqueous buffer, and the resulting solution was used directly in the hydrogenation without extractive purification.

In preliminary experiments, the overall yield after hydrogenolysis was significantly lower than had been achieved with the 1st generation synthesis (45%, cf. 75−80%) even though the coupling reaction was performing well. Dimer degradates and one side-product were observed at significantly higher levels. It was at this stage that an important and timely discovery was made. It was found that the formation of dimer degradates arising from reaction of the pyrrolidine amine with the carbapenem was suppressed in solutions containing sodium bicarbonate. We proposed that this observation could be explained by the formation of the sodium carbamate **8** (Figure 2), which was subsequently confirmed by NMR.[25] The formation of the carbamate affords protection of the pyrrolidine amine inhibiting attack on the carbapenem.[26] The addition of 3 equiv of sodium bicarbonate prior to

SCHEME 3



9 **10** **11** **12**

hydrogenolysis suppressed the formation of dimer degradates as well as the main side-product of the reaction so that yields became comparable to those achieved in the 1st generation synthesis where the pyrrolidine amine had been protected as the *p*-nitrobenzyl carbamate.[27] The bicarbonate also serves to maintain the pH between 8 and 7 making it unnecessary to add sodium hydroxide during the hydrogenolysis.

Conclusive evidence for formation of the carbamate **8** was obtained in a $^{1}H-^{13}C$ heteronuclear multiple-bond correlation (HMBC) 2-D NMR experiment.[28] Three-bond correlations were observed between the carbamate carbonyl carbon and protons on the proximal pyrrolidine carbons (2′ and 5′).

The main side-product of the hydrogenolysis was later identified. Mass by LCMS (M + 1 = 581) suggested that the product was a *p*-aminobenzyl derivative of ertapenem. The fragmentation pattern, however, indicated that this compound was not simply the intermediate resulting from reduction of **7** (i.e. **11**, Scheme 3). Structure **9** is consistent with the fragmentation pattern in the mass spectrum and was subsequently confirmed by

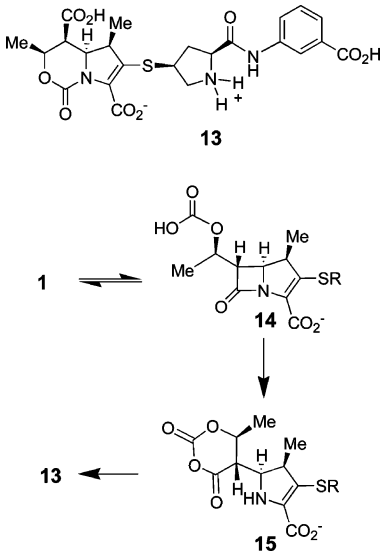
(25) The carbamate form of meropenem was also characterized. Almarsson, Ö.; Kaufman, M. J.; Stong, J. D.; Wu, Y.; Mayr, S. M.; Petrich, M. A.; Williams, J. M. *J. Pharm. Sci.* **1998**, *87*, 663−666.

(26) The use of carbon dioxide in protection of an amine is very limited. Formation of a carbamate in the hydrogenation of a nitro group to a primary amine was used to suppress hydrolysis of the nitro olefin starting material catalyzed by the amine product. Semikolenov, V. A.; Simokova, I. L.; Golovin, A. V.; Burova, O. A.; Simirnova, N. M. In *Heterogeneous Catalysis and Fine Chemicals*; Blaser, H. U., et al., Eds.; *Stud. Surf. Sci., Catal*. **1997**, *108*, 225−262. The influence of bicarbonate and carbon dioxide on reactions containing amines is often not recognized or considered in interpreting results.

(27) For background on the kinetics of carbamate formation see: (a) Caplow, M. *J. Am. Chem. Soc.* **1968**, *90*, 6795−6803. (b) Ewing, S. P.; Lockshon, D.; Jencks, W. P. *J. Am. Chem. Soc.* **1980**, *102*, 3072−3084. For characterization of carbamates by NMR, see: (c) Archer, R. A.; Kitchell, B. S. *J. Org. Chem.* **1966**, *31*, 3409−3410. (d) Lemieux, R. U.; Barton, M. A. *Can. J. Chem.* **1971**, *49*, 767−776. (e) Morrow, J. S.; Keim, P.; Gurd, F. R. N. *J. Biol. Chem.* **1974**, *249*, 7484−7494. (f) Pratt, R. F.; Dryjanski, M.; Wun, E. S.; Marathias, V. M. *J. Am. Chem. Soc.* **1996**, *118*, 8207. Methods for the preparation of alkyl carbamates from amines using carbon dioxide have been reported: (g) McGhee, W.; Riley, D.; Christ, K.; Pan, Y.; Parnas, B. *J. Org. Chem.* **1995**, *60*, 2820−2830. (h) Dean, D. C.; Wallace, M. A.; Marks, T. M.; Melillo, D. G. *Tetrahedron Lett.* **1997**, *38*, 919−922. (i) Salvatore, R. N.; Shin, S. I.; Nagel, A. S.; Jung, K. W. *J. Org. Chem.* **2001**, *66*, 1035−1037.

(28) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093−2094.

SCHEME 4



NMR analysis of an isolated sample.[29] This side-product most likely results from alkylation of the pyrrolidine amine during hydrogenolysis. Reduction of the nitro group gives the hydroxylamine **10** which is observed as an intermediate in the reaction mixture.[30] Further reduction of **10** would produce **11** which has not been observed. Fragmentation of **11** as depicted in Scheme 4 would produce **12** which would react readily with available nucleophiles.[31] Reaction at the pyrrolidine amine would give **9**. Formation of the carbamate **8** makes the pyrrolidine unavailable and thereby inhibits the formation of **9**.

Amines are known to affect the performance of palladium-catalyzed hydrogenation reactions.[32] Often hydrogenation reactions of compounds containing the amine function or producing an amine-containing product are performed under acidic conditions so that the amine is protonated limiting interaction with the catalyst. Although we do not have direct evidence of a beneficial effect on catalyst performance in this case due to the complexity of the reaction, it seems reasonable to believe that formation of a carbamate would also suppress interaction of an amine with the catalyst and thereby improve catalyst performance.

The use of bicarbonate in the process was beneficial overall, but there was one adverse consequence that we came to realize. One degradate, identified as the oxazinone **13** (Scheme 4), was the direct result of reaction of the carbapenem with carbon dioxide. A sample was prepared using high-pressure carbon dioxide and the structure confirmed by NMR and LCMS analysis. We propose that the β-lactam ring is opened as a result of the reaction of carbon dioxide at the secondary hydroxyl to give **14**. The resulting anhydride **15** would then undergo rearrangement to afford **13**.[33] This degradate is typically observed at about 0.5−1% by area relative to **1** in the hydrogenolysis reaction mixture. The rate of formation appears to be primarily dependent on the concentration of carbon dioxide as would be expected from the proposed mechanism.

A Practical Synthesis. We had made significant advances in the 2nd generation synthesis, and discoveries from this effort would develop into key elements for a practical synthesis. The coupling reaction, however, was clearly quite complex and not suitable for consistent, reliable production. There was still a potential strategic advantage in avoiding protection of the pyrrolidine amine. We had shown that protection was not required for the coupling reaction, but selectivity was very much dependent on temperature. Reducing the temperature further was not an option as long as sodium hydroxide was used as base. It was clear that a nonnucleophilic, organic-soluble base would offer significant advantages. 1,1,3,3-Tetramethylguanidine (TMG) proved to be an excellent alternative allowing short reaction times (less than 2 h) at temperatures as low as −60 °C. When TMG was used as base in the coupling reaction, the yield through hydrogenolysis improved from 75−80% to about 90%.

With the coupling and hydrogenolysis reactions performing well and the impurity load consequently reduced, the goal of eliminating the resin purification was beginning to appear attainable, but the challenges in avoiding the pH adjustment to reduce solubilized palladium levels and in removing TMG from the aqueous solution after the hydrogenolysis remained.

Control of the level of palladium in the filtrate after hydrogenolysis continued to require pH adjustment prior to filtration. This procedure added processing time and introduced inorganic salts that had to be removed in order to crystallize the product. More importantly, the rate of degradation in the reaction mixture increased substantially below pH 7 compounding the impurity load on purification. In trying to understand the source of solubilized palladium, a practical solution was realized. We suspected that colloidal Pd(0) was responsible for the black color of the filtrate, and this Pd(0) might arise from the reduction of Pd(II) that had been leached from the catalyst support by some ligand in the reaction mixture. If the Pd were reduced on the carbon support to Pd(0) prior to exposure to constituents of the reaction mixture capable of ligating Pd(II), the level of solubilized Pd might be reduced. Some improvement was observed in using commercially available prereduced catalysts, but the low levels required were realized only when the catalyst was reduced in the reactor and not exposed to air before introducing the substrate. When the catalyst was prereduced in this way, the reaction mixture could be filtered at pH 7−8 to give a yellow solution nearly free of palladium. Indeed, we subsequently showed that ertap-

(29) Fragments of mass 370 and 338 amu, corresponding to scission of the C−S bonds, were consistent with **9**. Consecutive losses of CO_2 (535 and 491 amu) were also observed suggesting the presence of two carboxylic acids.

(30) By LCMS M + 1 = 597 with fragments of mass 265 and 233 amu, corresponding to cission of the C−S bonds.

(31) (a) Filar, L. J.; Winstein, S. *Tetrahedron Lett.* **1960**, *1* (46), 9−16. (b) Domé, M.; Wakselman, M. *Bull. Soc. Chim. Fr.* **1975**, 576−582. (c) Le Corre, G.; Guibe-Jampel, E.; Wakselman, M. *Tetrahedron* **1978**, *34*, 3105−3112.

(32) Czech, B. P.; Bartsch, R. A. *J. Org. Chem.* **1984**, *49*, 4076−4078 and references therein.

(33) (a) Johnson, D. A.; Hardcastle, G. A., Jr. *J. Am. Chem. Soc.* **1961**, *83*, 3534−3535. The carbamate intermediate was subsequently characterized by NMR: See ref 27c,e.

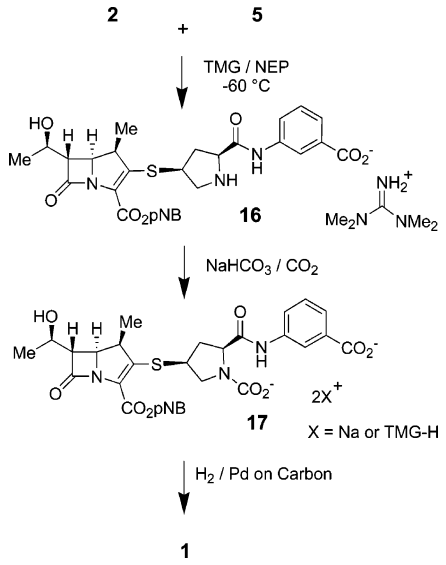
enem solubilizes palladium from an unreduced Pd-on-carbon catalyst.[34]

Ion exchange chromatography held some promise as a means for removing TMG. We found, however, that the loading would limit productivity and the high salt concentration required to elute the column would make extended diafiltration necessary.

A lead in solving the TMG removal problem came from an observation made in experiments intended to define an efficient means for extracting NEP from the aqueous solution. In screening a series of solvents, we found that 1 equiv of TMG (out of 3.3 charged to the coupling reaction) was readily extracted from the aqueous solution. The 1 equiv of diphenyl phosphate (DPP) present as a normal side product of the coupling reaction was extracted simultaneously. Additional extractions did not reduce the level of TMG in the aqueous layer. This observation suggested that the TMG was forming an organic soluble ion pair with DPP.[35] We found that other ion-pairing agents (lipophilic sulfonates and carboxylates) were not as efficient as DPP in partitioning the TMG into the organic layer and some created serious emulsion problems. Although commercial DPP could be added to improve extraction efficiency, we recognized that in production sufficient DPP would be produced in 3−4 batches to efficiently remove TMG for a single batch if the DPP were recycled into the process from the waste streams. An extraction protocol was developed that uses a solution of DPP in isoamyl alcohol to reduce the TMG level from about 3.5 to 0.2 equiv (relative to **1**) while concentrating the aqueous solution about 4x as needed for a direct crystallization of the product with minimal losses to the organic extract. This extraction also removes byproducts of the hydrogenolysis. Thus, this single operation eliminates both the inefficient resin column purification and nanofiltration concentration operations required in the earlier syntheses. The extraction is best performed as a continuous operation using a centrifugal extractor.[36]

With the basis for a practical synthesis established, we examined the details of each reaction to understand the controlling features and ensure optimal performance. The success of the coupling reaction derives from achieving near perfect chemoselectivity for the desired reaction. The enol phosphate contains two electrophilic sites, and the thiol contains three nucelophilic sites. The product of each of the possible combinations would retain the potential to react further. Under less than optimal conditions, many of the possible products of these undesired reactions are indeed observed. Although none of the side products have been unambiguously identified,

SCHEME 5



experiments were performed under conditions expected to favor specific reactions and the resulting side products were characterized by LCMS.

Addition of the carbapenem enol phosphate **2** or the preformed thiolate of **5** (Scheme 5) to a reaction mixture after conversion was complete resulted in the formation of products corresponding to adducts of each with the product of the coupling reaction (M + 1 = 1205 and 877, respectively). The latter product was correlated with a product after hydrogenolysis that was also observed when ertapenem **1** was reacted with **5** in water at pH 7.5. Warming a typical coupling reaction mixture after complete conversion had been achieved resulted in the increase of a product (M + 1 = 1221) believed to come from reaction of two molecules of the desired product **16**. When base was added at higher temperature (−25 rather than −60 °C), a second product believed to be the result of a reaction between the carbapenem enol phosphate **2** and thiol **5** (M + 1 = 861) is observed at higher than typical levels. Clearly, the ability to maintain the low temperature during the reaction is important in achieving high selectivity for the desired product and in minimizing further reaction of the product. It is also important to use a sufficiently strong base to give a fast reaction and to add the base as quickly as possible to minimize reactions between starting materials and product.

The amount of the disulfide **18**[18b] (Figure 3) corresponding to thiol **5** which was formed in the coupling reaction was variable even when the solvent was rigorously degassed prior to use. We found a report that *N*-methylpyrrolidinone (NMP) forms hydroperoxides upon exposure to air and NEP would be expected to do the

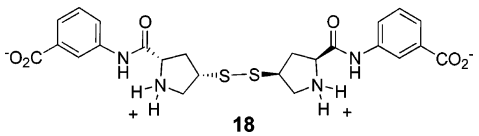


FIGURE 3.

(34) The level of palladium in solution for a slurry of 10% Pd on carbon in water containing ertapenem and 2.5 equiv of sodium bicarbonate (pH 7.1) was more than 100× higher than the control (Pd undetected < 0.3 mg/L).

(35) For a review covering the factors influencing partitioning for charged species see: Leo, A.; Hansch, C.; Elkins, D. *Chem. Rev.* **1971**, *71*, 525−616.

(36) (a) Jacobsen, F. M.; Beyer, G. H. *AIChE J.* **1956**, *2*, 283−289. (b) Todd, D. B.; De Cicco, R. W.; Daiser, H. R. *Chem. Eng. Prog.* **1965**, *61* (5), 74−77. (c) Podbielniak, W. J.; Kaiser, H. R.; Ziegenhorn, G. J. In *Chemical Engineering Progress Symposium Series: The History of Penicillin Production*; AIChE: New York, 1970; Vol. 66 (100), p 45−50. (d) Todd, D. B. *Chem. Eng.* **1972**, *July 24*, 152−158. (e) King, M. L.; Foreman, A. L.; Orella, C.; Pines, S. H. *Chem. Eng. Prog.* **1985**, *81* (May), 36−39.

TABLE 1. Bicarbonate and Hydrogenolysis Performance

entry[a]	$NaHCO_3$ (equiv)[b]	NaOH pH > 7[c] (equiv)	**1** yield[d] (%)	**10** area %[e]	**9** area %[e]	dimers[f] area %[e]
1	0	1.0[g]	77	0.6	5.9	4.4
2	1.0	0.8	85	0.0	3.7	1.6
3	2.0	0.3	90	0.0	1.4	1.0
4	3.0[g]	0	91	0.2	0.9	0.8
5	4.0	0	90	0.1	1.2	0.1

[a] The reactions were performed in a 1 L autoclave with the exception of entry 4 which was run in a 2 gal reactor. [b] $NaHCO_3$ was generated from NaOH and carbon dioxide. Equiv are based on NaOH charged. [c] NaOH added during the hydrogenolysis to maintain the pH above 7. [d] Yield based on **2**. [e] HPLC area % relative to **1**. [f] Degradates resulting from reaction of the pyrrolidine amine and β-lactam. [g] The pH was adjusted with NaOH to 6.5 prior to charging the coupling reaction mixture into the autoclave.

same.[37] The amount of disufide observed in the reaction could be reduced by storing the NEP under nitrogen, but some increase in comparison with what was present in the starting material was still observed. The addition of a small amount (1 mol %) of tri-*n*-butylphosphine prior to charging the thiol suppressed formation of the disulfide without adversely impacting performance of the hydrogenolysis.[38]

The use of bicarbonate in the hydrogenolysis reaction was key in achieving high yields with the unprotected thiol. The effect of bicarbonate on the hydrogenolysis was clearly demonstrated in a series of reactions using 0, 1, 2, 3, and 4 equiv of bicarbonate (Table 1). Sodium hydroxide was added during the reactions as needed to maintain the pH above 7. The yield improved with the increasing charge of bicarbonate from 77% without bicarbonate to 91% with 3 equiv. The increase in yield was a result of suppressed dimer formation (4.4 to 0.8 area %) and a reduction in **9** from about 5.9 to 1.2 area %. Although the level of dimers was further reduced by using 4 equiv of bicarbonate, the yield was not improved.

Conclusions

Ertapenem presented a significant challenge in developing a practical synthesis for manufacturing. Our strategy in minimizing the use of protecting groups created opportunities that led to an efficient and highly productive synthesis. By using TMG as base, the coupling reaction is completed at a rate and temperature that allows high selectivity for the desired product. The use of bicarbonate in the synthesis affords protection of a pyrrolidine amine as the carbamate allowing improvements in productivity and performance in the hydrogenolysis of a *p*-nitrobenzyl ester. The solution stability of the final product was also improved reducing the amount of degradation observed so that the extended time required in manufacturing could be tolerated. Overall, minimizing the burden on purification made it possible to use a novel ion-pairing extraction for purification and concentration prior to isolation of the product by crystallization. The innovation in developing this synthesis resulted in a practical and highly productive synthesis for manufacturing an unstable carbapenem.

Experimental Section

All commercially available materials were used as received. Compound **5** was prepared as described previously.[18b]

(4*R*,5*S*,6*S*,8*R*,2′*S*,4′*S*)-3-[[2-[[(3-Carboxyphenyl)amino]-carbonyl]pyrrolidin-4-yl]thio]-4-methyl-6-(1-hydroxyethyl)-7-oxo-1-azabicylclo[3.2.0]hept-2-en-2-carboxylic Acid Sodium Salt (Ertapenem Sodium 1). *N*-Ethylpyrrolidinone (NEP, 1.33 L) containing 0.5% (w/v) water was charged into a 3 L flask equipped with an air-driven overhead stirrer, thermocouple, and a nitrogen/vacuum inlet. The temperature was adjusted to about 0 °C and [4*R*,5*S*,6*S*,8*R*)-3-[(diphenoxyphosphinyl)oxy]-6-(1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-en-2-carboxylic acid (4-nitro-phenyl) methyl ester (**2**, 170.0 g, 0.286 mol) was charged. After inertion (vacuum/nitrogen), 0.7 mL of tri-*n*-butylphosphine followed by (2*S*-*cis*)-3-[[(4-mercapto-2-pyrrolidinyl)carbonyl]amino]benzoic acid monohydrochloride (**5**, 85.8 g, 0.280 mol) was charged. The flask was inerted (vacuum/nitrogen), and the solution was cooled to −55 to −60 °C. 1,1,3,3-Tetramethylguanidine (TMG, 110 g, 0.936 mol) was charged with vigorous agitation maintaining the internal temperature below −50 °C. The reaction mixture was maintained at −50 to −55 °C for 1 h and warmed to −40 °C. The reaction was complete as determined by HPLC analysis (YMCbasic; 250 × 4.6 mm; 5 μm particle size; UV detection at 254 nm, 0.05% phosphoric acid/acetonitrile 85:15 for 5 min then gradient to 20:80 over 15 min; 1.2 mL/min; relative retention times: **5**, 0.37; **18**, 0.38; diphenyl phosphate, 0.96; **16**, 1.00; **2**, 1.50).

A 2 gal autoclave equipped with a pH probe was charged with a slurry of 5% Pd on C catalyst (127 g, 48.3% dry wt, 29 mmol Pd) in 1.8 L of water. The vessel was purged with nitrogen and placed under hydrogen (70 psi) for 1 h, then vented and placed under nitrogen. Sodium hydroxide (5.0 N, 171 mL, 0.86 mol) was charged by pump maintaining the pH below 9 with carbon dioxide. The temperature was adjusted to 5 °C.

The coupling reaction mixture was charged into the autoclave maintaining the temperature below 15 °C and introducing carbon dioxide as needed to maintain the pH below 9. The reaction flask was rinsed with 113 mL of NEP, and the pH was adjusted to about 8 using carbon dioxide. The temperature was adjusted to 5 °C and the vessel was placed under hydrogen pressure (70 psi). The temperature was increased to 20 °C over 30 min. After 2 h, the pH was about 7, and the hydrogen was vented and the vessel was purged with nitrogen. The reaction mixture was cooled to 5 °C and activated carbon (19 g dry weight) was charged. The mixture was filtered using a stainless steel pressure filter, and the catalyst cake was washed with 0.5 L of water.

The combined filtrate and wash (3.8 L containing 128 g of product, 90% yield) was extracted with 9.5 L of isoamyl alcohol containing diphenylphosphoric acid (0.950 mol, 238 g), 50% NaOH (563 mmol, 45 g), and water (120 g) using a countercurrent centrifugal extractor.[39] The resulting aqueous solution was extracted with 2.5 L of isoamyl alcohol to give an aqueous solution of ertapenem (140 g/L).

The pH was adjusted to 5.5 with acetic acid. The product was crystallized by adding volumes of methanol and 1-propanol equal to the batch volume at −10 °C. The solid was isolated by filtration and washed with 0.8 L of a mixture of 2-propanol and water (85:15 v/v), then with 1.3 L of methyl acetate containing 2% (w/v) water at 5 °C. The solid was dried with a nitrogen stream under vacuum to a final water content of about 17%. The overall yield on multikilogram scale was

(37) (a) Drago, R. S.; Riley, R. *J. Am. Chem. Soc.* **1990**, *112*, 215. (b) Patton, D. E.; Drago, R. S. *J. Chem. Soc., Perkins Trans. 1* **1993**, 1611.

(38) Larger amounts of tri-*n*-butylphosphine poison the palladium catalyst affecting performance of the hydrogenolysis.

(39) On lab scale, two CINC V-2 single stage centrifugal separators (Costner Industries Texas, LP, formerly CINC.; http://www.cit-ind-.com/) were used in series.

59−64% based on **2**. On lab scale, yields were somewhat lower as a result of physical losses in the extraction step: HPLC analysis (YMCbasic; 250 × 4.6 mm; 5 μm particle size; UV detection at 225 nm, 0.05% phosphoric acid/acetonitrile 90:10 for 1 min then gradient to 60:40 over 19 min; 1.0 mL/min) relative retention times: TMG, 0.30; NEP, 0.68; hydrolysis product, 0.76; **13**, 0.84; ertapenem **1**, 1.00; **9**, 1.06; **10**, 1.11; dimer degradates, broad at 1.36; diphenyl phosphate, 1.53; **16**, 1.94. UV (nm, H_2O) 294; FT-IR (Nujol mull, cm^{-1}) 3650−3600, 1751, 1695, 1559, 1459, 1377, 771; ^1H NMR (500.13 MHz, dioxane as reference at $\delta = 3.75$) δ 7.86 (m, 1H), 7.71 (m, 1H), 7.65 (m, 1H), 7.47 (t, 1H, $J = 8.0$ Hz), 4.62 (t, 1H, $J = 8.3$ Hz), 4.21 (om, 1H), 4.18 (dd, 1H, $J = 9.5$, 2.4 Hz), 4.07 (m, 1H), 3.82 (dd, 1H, $J = 12.3$, 6.8 Hz), 3.47 (dd, 1H, $J = 12.3$, 5.6 Hz), 3.42 (dd, 1H, $J = 6.0$, 2.4 Hz), 3.31 (m, 1H), 3.02 (m, 1H), 2.20 (m, 1H), 1.27 (d, 3H, $J = 6.4$ Hz), 1.17 (d, 3H, $J = 6.8$ Hz); ^{13}C NMR (100.61 MHz) δ 177.3, 175.3, 168.4, 167.7, 138.4, 138.1, 137.0, 134.5, 130.0, 127.0, 124.9, 122.5, 65.9, 60.9, 59.5, 56.7, 53.2, 43.5, 41.4, 35.5, 20.9, 16.7; FTICR/MS calculated for $[C_{22}H_{25}N_3O_7S + H]^+$: 476.1486; found: 476.1498.

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Supporting Information Available: Experimental details for the preparation or isolation of a reference sample and characterization data supporting the proposed structures for **8**, **9**, and **13** are provided. This material is available free of charge via the Internet at http:// pubs.acs.org.

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