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A Practical Synthesis of an Anti-Methicillin Resistant *Staphylococcus aureus* Cephalosporin BMS-247243

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Abstract:

A practical synthesis of the anti-methicillin resistant *Staphylococcus aureus* cephem (6*R-trans*)-*E*-7-[[[[2,5-dichloro-4-[3-[(carboxymethyl)amino]-3-oxo-1-propenyl]phenyl]-thio]-acetyl]amino]-4-[[(2-carboxy-8-oxo-5-thia-1-azabicyclo-[4.2.0]oct-2-en-3-yl)methyl]thio]-2,6-dimethyl-1-[3-(4-methylmorpholino-4-yl)propyl]-1-pyridinium, hydroxide, inner salt (BMS-247243) was developed. A process was developed for the interchange of the iodide counterion in 3a to chloride 3b that was essential for an efficient synthesis of the C-3 side chain 4-mercaptopyridone 6b. Use of catalytic Bu_4NCl in the reaction of chlorocinnamide 14 with the Li-salt of methylthioglycolate formed the methyl ester of the C-7 side chain 12b in high yield. Reaction with the dianion of thioglycolic acid gave an increased level of the corresponding Michael addition byproduct that led to lower quality thermodynamic product 12b by the reverse reaction. Cephem nucleus 16 was acylated with the acid chloride of acid 12b in a biphasic system to circumvent the cumbersome workup involved in reactions mediated by carbodiimdes DCC or EDAC for the synthesis of diester 17. An unusual degradation product diacid 20 was obtained during the deprotection of diester 17 with TFA to amorphous diacid 19. Reaction of diacid 19 with 4-mercaptopyridone 6b formed BMS-247243 in moderate yield. Alternately, an efficient coupling of diester 17 with 4-mercaptopyridone 6b gave crystalline diester 21 with minimal (<1%) contamination of the double bond isomer 22. Double deprotection of diester 21 followed by crystallization furnished the double zwitterion BMS-247243 in high yield.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) and other antibiotic resistant strains of microbial pathogens continue to pose a tremendous menace to human life.[1−6] Major efforts continue for the discovery and development of new antibiotics to combat serious infections caused by these resistant bacteria.[1−3] Treatment of infections caused by MRSA with the glycopeptide vancomycin is jeopardized by the emergence of vancomycin-resistant enterococci (VRE).[5] A recently introduced combination of two streptogramins (Synercid) is the only drug for combating life-threatening infections caused by MRSA and VRE.[6] Over the years cephalosporins (cephems) have been extensively modified to construct anti-MRSA antibiotics due to their better safety profile.[1,2] The search for a potent anti-MRSA cephem at Bristol-Myers Squibb led to the design of the double zwitterion BMS-247243 (Figure 1).[7]

The retrosynthetic analysis for BMS-247243 is illustrated in Scheme 1. The core cephem intermediate **D** (R_1═$CHPh_2$), made by a combination of fermentation and chemical synthesis, is commercially available. As a general strategy the construction of the new cephems is implemented by sequential attachment of the C-3 and C-7 side chains to the cephem nucleus. Diester intermediate **B**, made by appendage of C-7 side chain **E** to the cephem nucleus **D**, can be deprotected to the corresponding diacid and coupled with C-3 side chain 4-mercaptopyridone **C** to furnish cephem BMS-247243. Alternately, diester **A** can be constructed by the coupling of diester **B** with 4-mercaptopyridone **C** and then doubly deprotected in a single step to BMS-247243. The C-7 side chain **E** can be made from trichloroester **F** that is assembled by Heck reaction of iodide **H** (Y = I) with

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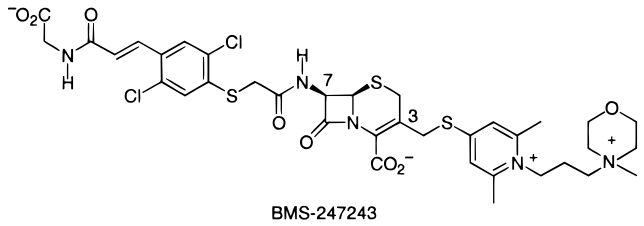

Figure 1. **Structure of cephem BMS 247243.**

cinnamic acid followed by amidation with a glycine ester. The C-3 side chain **C** can be prepared by condensation of 4-mercaptopyrone **I** [Z = S, made from γ-pyrone **I**, (Z = O)] with the amine **J**, that in turn can be prepared by quaternization of the primary N-protected morpholine derivative **K** with methyl iodide.

The original synthesis via coupling of diacid **B** ($R_1 = R_2 = H$) with 4-mercaptopyridone **C** provided BMS-247243 for biological testing and preliminary toxicology studies.[7] However, considerable improvements were necessary for the large-scale production of the drug substance needed for formulation and safety evaluation.

Results and Discussion

The C-3 (**6**, Scheme 2) and C-7 (**12**, Schemes 3 and 4) side chains were prepared separately and appended to the cephem derivatives. Reaction of acid **12b** with cephem core ACLH·HCl (**16**, Scheme 5) produced chlorodiester **17**. Diester **17** was converted to cephem BMS-247243 by two routes, (i) deprotect−couple (Scheme 6) and (ii) couple−deprotect (Scheme 7). The couple−deprotect route proved to be better and was optimized for the production of BMS-247243 on kilogram scale.

Synthesis of C-3 Side Chain 6. The quaternary ammonium intermediate **6** required for appendage of the C-3 side chain was prepared from commercially available aminopropylmorpholine **1** (Scheme 2). Boc protection of amine **1** followed by quaternization with CH_3I formed the quaternary ammonium salt **2** in nearly quantitative yield. Deprotection of **2** with TFA gave the TFA salt **3a**. This brown salt caused severe purification problems in the subsequent steps. The presence of the iodide counterion posed a difficult challenge in terms of improvement in yields and quality of the intermediates. Replacement of TFA by concentrated HCl in the deprotection step made the process more economical, but coloration due to the iodide counterion persisted. Treatment of the crude iodide obtained after deprotection with concentrated HCl with Bu_3BnNCl in a biphasic system (H_2O/CH_2Cl_2) resulted in an efficient exchange of the iodide counterion with chloride; Bu_3BnNI stayed in the CH_2Cl_2 layer.[8] After workup the HCl salt was obtained as a white solid in 99% crystallized yield. The process was improved further to eliminate the use of CH_2Cl_2. After quaternization of **1b**, the intermediate salt **2** was deprotected in situ with ethanolic HCl, and the resulting iodide/chloride mixed crystals were treated directly with Bu_3BnNCl in EtOH to complete the exchange. This significant improvement made subsequent coupling with 4-mercaptopyrone **5** more efficient. Thionation of 2,6-dimethyl-4-pyrone **4** (commercially available) with P_2S_5 (toluene, reflux) formed 4-mercaptopyrone **5** in 69% yield.[9] This reaction could not be scaled up even to 10 g input of **4** due to formation of highly colored product in low yield. With Lawesson's reagent[10] in toluene 4-mercaptopyrone **5** could be obtained in 87% crystallized yield. The procedure was modified to avoid an aqueous extractive workup, which caused exposure to a strong stench. The product was directly crystallized from MeOH or EtOH. This reaction was scaled up to produce several kilograms of **5**. The coupling of 4-mercaptopyrone **5** originally was performed with iodide **3a** using aqueous $NaHCO_3$ in EtOH. This process was not satisfactory due to difficulties in purification of the product. The use of chloride **3b** and an organic base, for example, Et_3N, in the coupling process formed 4-mercaptopyrone **6b** in 80−85% crystallized yields. This process was used to prepare several kilograms of the C-3 side chain **6b**.

Synthesis of C-7 Side Chain 12. The original six-step synthesis of the C-7 side chain produced acid **12b** in 11% overall yield (Scheme 3).[7] The iodination of trichloroaniline **7** (yield 44%) via diazotization needed further improvement due to the moderate yield.[11] The unsaturated acrylic acid side chain was introduced via Heck reaction with *tert*-butyl acrylate (**8** to **9**), and therefore, an extra deprotection step (**10** to **11a**) was required after thionation (**9** to **10**). An expensive activating agent, *N*-hydroxysuccinimide, was used for the amidation with glycine (**11a** to **12a**). The coupling reagent dicyclohexylcarbodiimide used in this step formed dicyclohexylurea that caused purification problems. Ester **12a** was hydrolyzed in a separate step to the desired acid **12b**. Thus, considerable opportunities for improvements in the synthesis of C-7 side chain **12b** were present.

Acid **12b** was produced in 43% overall yield by an alternate, more efficient four-step synthesis (Scheme 4). In the conversion of trichloroaniline **7** to iodide **8,** the amount of acetic acid was reduced by 40%, and the reaction was performed at 15−20 °C instead of 0 °C. A much better quality product was produced in 75−80% yields by this process. Direct iodination of 2,4,5-trichloroaniline with *N*-iodosuccinimide was not successful.[12] The same reaction with iodine and periodic acid formed a mixture of several products.[13] Heck coupling of iodide **8** with acrylic acid [Pd(OAc)$_2$, Et_3N or K_2CO_3, EtOAc] formed cinnamic acid **13** in 75−80% yields.[14−16]

The amidation of acid **13** with *tert*-butylglycine hydrochloride with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC, Et_3N, CH_2Cl_2) to amidoester **14** was an efficient, high-yielding process. However, workup was cumbersome due to the formation of a thick slurry of salts. Use of alternate solvents CH_3CN or EtOAc worsened

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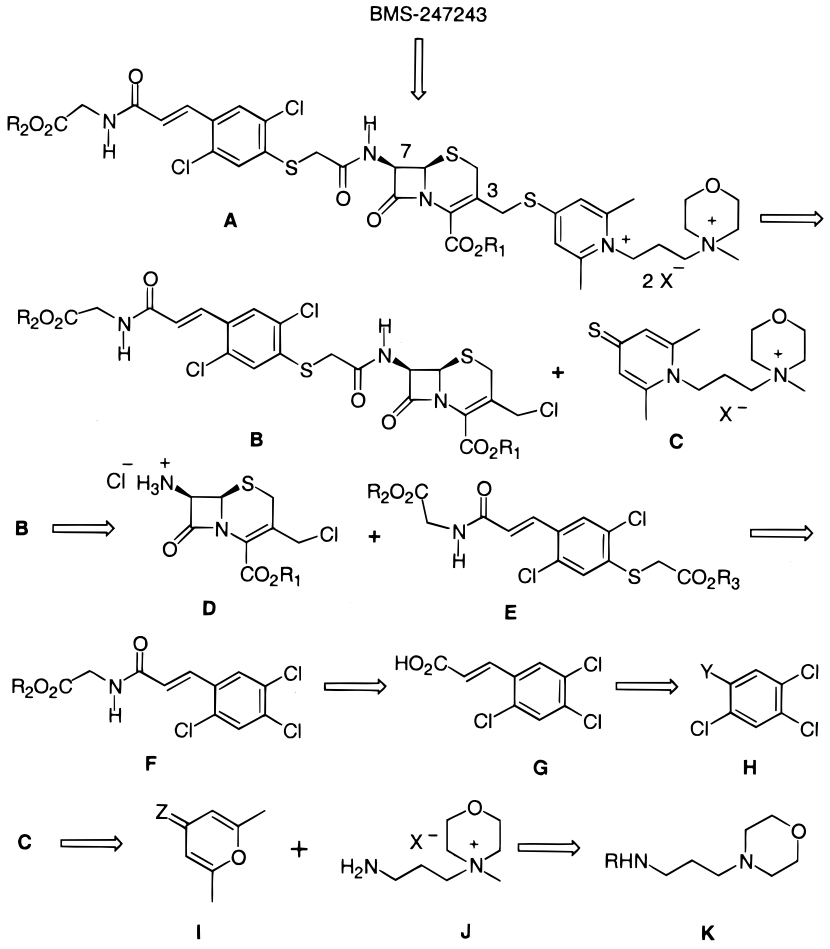
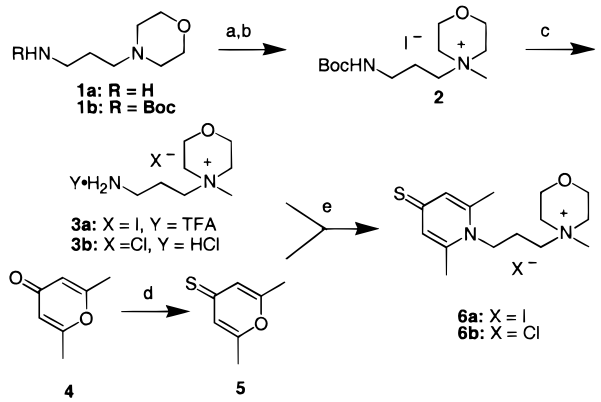
Scheme 1. **Retrosynthetic analysis of BMS-247243**

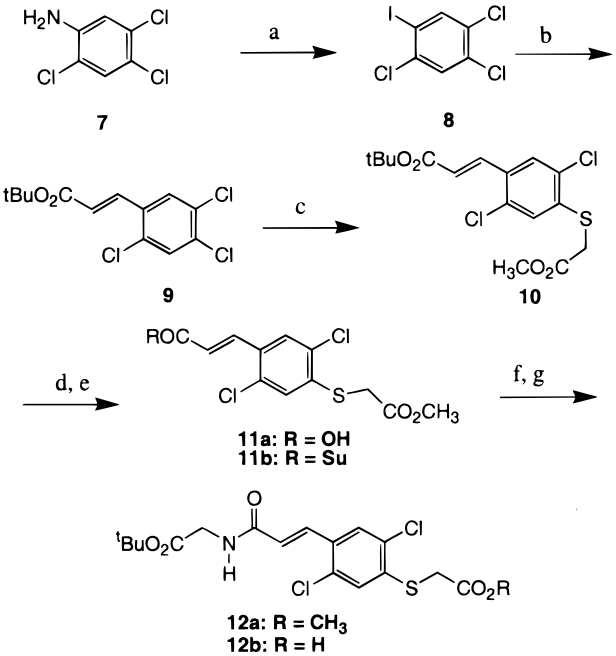


Scheme 2. **Synthesis of C-3 side chain 6**[a]



[a] Reaction conditions and yields: a. Boc_2O, EtOAc, 30−35 °C, 20 h; ∼100%. b. CH_3I, EtOH (or acetone), 40 °C, 20 h; 99%. c. TFA or conc. HCl, Bu_3BnNCl, 95 − 99%. d. Lawesson's reagent (p-$OCH_3C_6H_4PS_2)_2$, toluene, 30 °C, 2h; 87%. e. TEA, EtOH, reflux, 10 h; 80 − 85%.

Scheme 3. **Original synthesis of C-7 side chain 12b**[a]

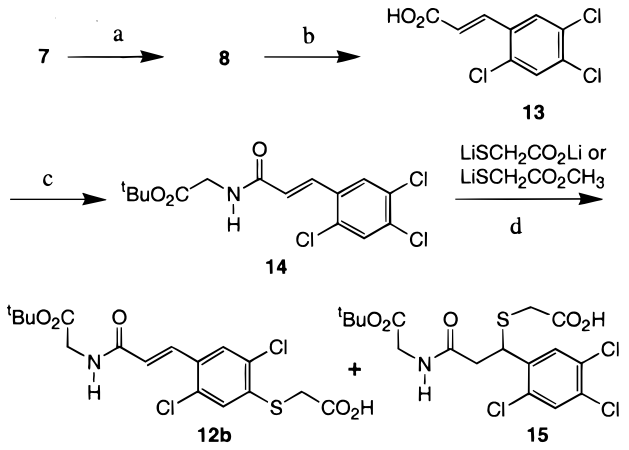


[a] Reaction conditions and yields: a. AcOH, HCl, $NaNO_2$, Kl; 44%. b. *tert*-Butyl acrylate, Bu_3N, $Pd(OAc)_2$, 80 °C; 75%. c. $NaSCH_2CO_2CH_3$, DMF; 75%. d. TFA, CH_2Cl_2; 79%. e. *N*-Hydoxysuccinimide (HOSu), DCC, THF; f. *tert*-Butyl glycine, THF. g. NaOH, CH_3OH, THF; 56% from **11a**.

the process. Coupling via the acid chloride of **13** gave superior results. Thus, the expensive EDAC was replaced by commercially available Vilsmeier reagent, forming amidoester **14** in 95% yield. Acid chloride formation was done in THF, thereby replacing an environmentally unfriendly solvent CH_2Cl_2. Furthermore, the organic base Et_3N needed for the coupling with EDAC was replaced by aqueous $KHCO_3$ in the acid chloride reaction.

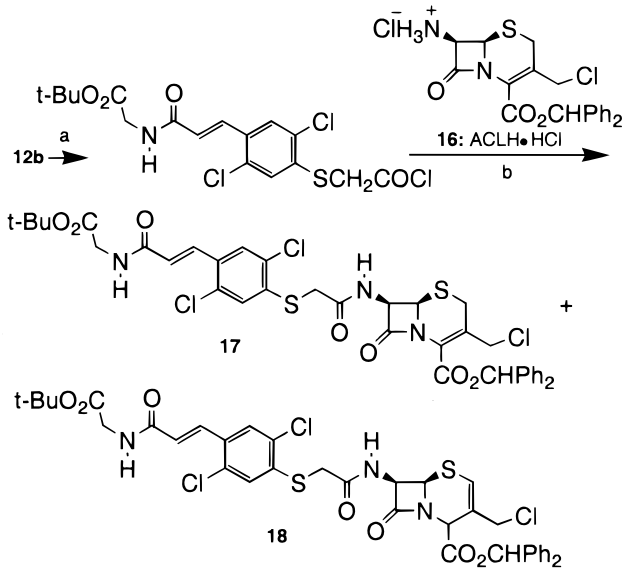
Displacement of the most reactive *p*-Cl atom in cinnamide **14** with the dianion of thioglycolic acid would provide acid **12b** in a single step. This process would also avoid the use of the strongly smelling reagent thioglycolic acid methyl

Scheme 4. **Alternate synthesis of C-7 side chain 12b**[a]



[a] Reaction conditions and yields: a. AcOH, HCl, $NaNO_2$, Kl; 75%. b. Acrylic acid, CH_3CN, $Pd(OAc)_2$, K_2CO_3, 80 °C; 80%. c. Vilsmeier reagent $[ClCH{=}NMe_2]^+Cl^-$, THF−H_2O, *tert*-butyl glycine; 96%. d. Bu_4NCl, DMF, −5 °C; aqueous LiOH, HCl; 75%.

Scheme 5. **Coupling of acid 12b with cephem nucleus 16**[a]



[a] Reaction conditions and yield: a. Vilsmeier reagent, THF or EtOAc. b. Aqueous $KHCO_3$ or $NaHCO_3$; 80%.

ester, which was used in the original synthesis (Scheme 3). Michael addition of dilithiothioglycolate to the cinnamate function formed byproduct **15**. Reaction of **14** with the dianion of thioglycolic acid (LiHMDS, DMF, 2 h) formed a mixture of **12b** and **15** in a ratio of 3.5:1.[17] Due to reversibility of the Michael reaction, this ratio changed to 18:1 over 20 h. However, several additional impurities were formed during this time. Use of Bu_4NCl (1.7 mol equiv) in this reaction minimized the formation of side products and gave acid **12b** in 61% yield. For comparison, we investigated the displacement reaction with the lithium salt of the thioglycolic acid methyl ester in the presence of a stoichiometric amount of Bu_4NCl.[7,18] This turned out to be a more efficient method for the preparation of **12b**. Furthermore, LiHMDS was replaced by LiO^tBu for salt formation, and

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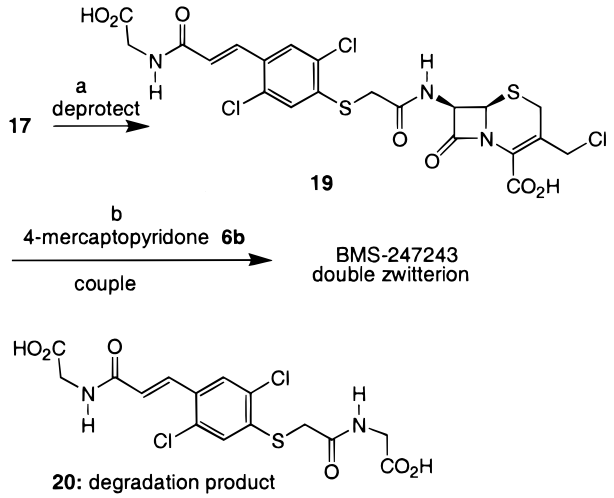
Scheme 6. **Synthesis of BMS-247243 via deprotect−couple route**[a]



[a] Reaction conditions and yields: a. TFA, anisole, CH_2Cl_2, 0 °C; 78%. b. Aqueous THF, 25 °C, 0.5 h; 70−80%.

the phase transfer agent Bu_4NCl (0.15 mol equiv) was used in only catalytic amount. The intermediate methyl ester formed in this reaction was saponified in situ with aqueous LiOH to produce **12b** in 81% yield. In-process HPLC indicated only a minor amount [area percent (AP) 2−5] of the Michael addition product. This alternate synthesis was used to prepare several kilograms of C-7 side chain **12b**.

The Coupling of C-7 Side Chain 12b with Cephem Nucleus 16. Acylation of cephem nucleus ACLH·HCl (**16**)[19] with the C-7 side chain **12b** initially was done with DCC in THF. Product **17** was formed in 80% yield, but there were purification problems due to the low solubility of product **17** in organic solvents and contamination by dicyclohexylurea. Carbodiimide is generally a reagent of choice for such reactions to avoid formation of the isomerization byproduct **18**, generally referred to as the Δ^2-isomer. Use of the water soluble carbodiimide reagent EDAC [CH_3CN/THF, *N*-methylmorpholine (NMM)] gave better quality product in ∼90% yield. The workup solvent volumes of reactions done without THF as cosolvent were enormous (>100 mL/g of product). Acylation of **16** via a mixed anhydride of acid **12b**, prepared with pivaloyl chloride/NMM, gave **17** in 80% crystallized yield. The Δ^2-isomer **18** was also produced (1−3%) in this reaction.[20a] Use of a stronger base, for example, Bu_3N, in this reaction increased the level of Δ^2-isomer **18** to 35%. Pure diester **17** is stable to double bond migration during crystallization by heating in solvents. However, in the presence of base diester **17** (CH_3CN, Bu_3N, 25 °C) underwent considerable isomerization to **18** (∼50%). This isomerization phenomenon is well-known,[21−23] and reversion of the wrong isomer back to the desired Δ^3-isomer requires

(19) ACLH·HCl was purchased from Otsuka Chemical Company, Japan.

(20) HPLC method: Wavelength 260 nm; solvent A: 0.2% aqueous phosphoric acid; solvent B: 90% acetonitrile/10% water; flow rate 1.5 ml/min. (a) Column: YMC S3 ODS-A 6.0 × 150 mm; start B = 0%, final B = 100%, gradient time = 20 min, hold time 10 min. (b) Column: YMC S3 Ph 6.0 × 150 mm; start B = 30%, final B = 55%, gradient time = 60 min. (c) same as method (a) but with gradient time = 30 min.

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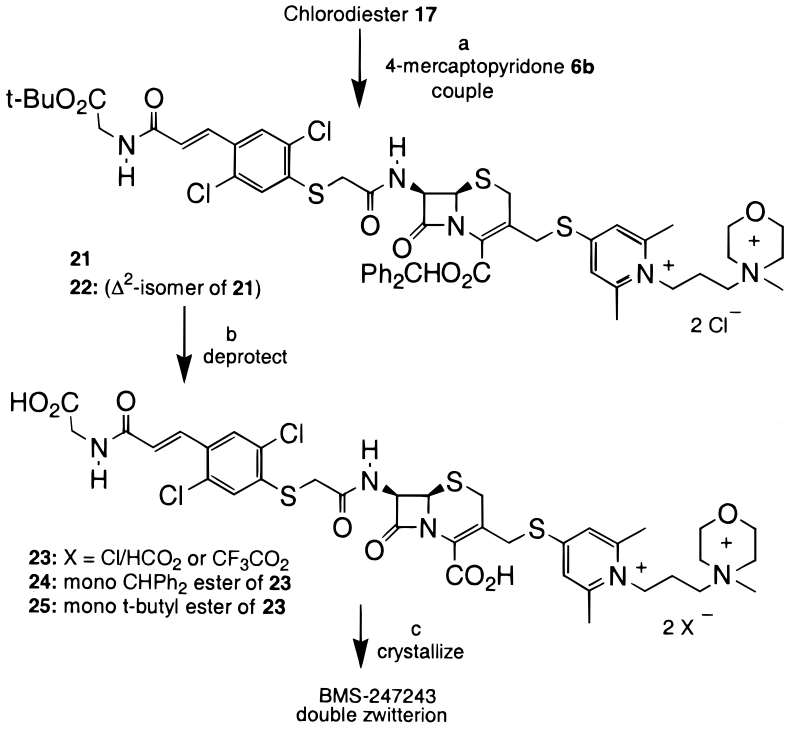
Scheme 7. **Alternate synthesis of cephem-BMS 247243 via couple−deprotect route**[a]



[a] Reaction conditions and yields: a. Aqueous acetone, 25 °C, 18 h; 80%. b. Formic acid, 98%, or TFA/Et_3SiH; 90−98%. c. Aqueous $NaHCO_3$ or NaOH; 65−70%.

a two-step process involving oxidation to the corresponding cephem sulfoxide followed by reduction.[24,25] Therefore, it was necessary to develop a coupling process which gave minimal isomerization and reproduced flawlessly on scale-up.

Coupling via the acid chloride of **12b** was investigated next. Reaction of the acid chloride made from acid **12b** with in situ formed Vilsmeier reagent [$(COCl)_2$, cat DMF, CH_2Cl_2] with cephem **16** in the presence of NMM produced diester **17** in 80% yield. Crystallized product contained 0.5−3% isomer **18**, although >5% isomerization occurred during the reaction.[20a]

An alternate biphasic coupling process was finally developed to minimize the rearrangement of the double bond during this reaction. Acid chloride made by the reaction of acid **12b** with commercially available Vilsmeier reagent was treated with cephem **16** to form diester **17** with HPLC AP ~95 in ~75−80% yields.[20a] Less than 3% isomerization to **18** occurred during this reaction. The workup was significantly improved, and the level of contamination of the Δ^2-isomer **18** in compound **17** was 0−1.5%. This process was used to prepare several kilograms of diester **17**.

Synthesis of Cephem BMS-247243 via Deprotect−Couple Route. In our original synthesis (Scheme 6) the double deprotection of diester **17** with TFA formed amorphous diacid **19** with HPLC AP 85−90 in 78% crude yield.[20c,26] Initially, this approach was preferred to avoid the use of intermediate **17**, which possessed a bulky ester substituent at the C-4 position that could cause increased isomerization of the double bond in the cephem nucleus. All efforts to crystallize diacid **19** for purification purposes were unsuccessful. The instability of this intermediate during attempted crystallization made the purification task all the more difficult. Alternate deprotection methods (TMSOTf, TMSI, HCl, MsOH) resulted in more complex reaction mixtures. An unusual degradation product diacid **20** was produced (3−10%) during deprotections with neat TFA.[27,28] The degradation of diester **17** to diacid **20** was caused by the presence of water in the TFA. Diester **17** actually was converted with 20% aqueous TFA to diacid **20** in over 70% yield. The coupling of diacid **19** with 4-mercaptopyridone **6b** in aqueous THF followed by treatment with base gave the double zwitterion cephem BMS-247243 with AP 90 in

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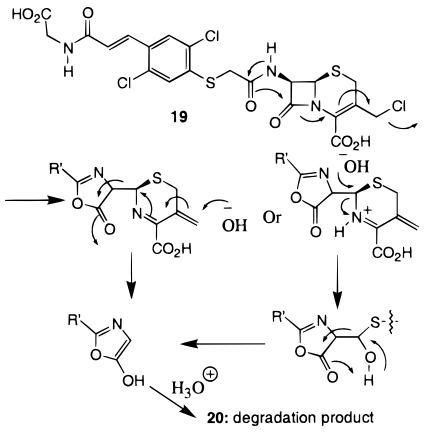
(26) Diacid **19** could not be analyzed by HPLC due to its instability. It was unstable also in DMSO, and therefore NMR spectra were taken in deuterated THF or DMF.
(27) Possible paths for the degradation of cephem diacid **19** to the diacid **20** are outlined below.



70−80% yield.[20c] The product was purified further by column chromatography over CHP-20.

There was no evidence of the formation of the corresponding Δ^2-isomer in the coupling process with diacid **19**. However, this advantage was severely offset by the inability of **19** to crystallize into a stable form. Thus, the last two steps in the synthesis were inverted to address this problem.

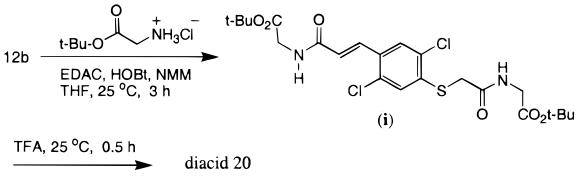
Alternate Synthesis of BMS-247243 via Couple−Deprotect Route. Coupling of diester **17** with 4-mercaptopyridone **6b** in aqueous THF formed dichloride salt **21** (Scheme 7) with AP >95 in 80% crystallized yield.[20a] Under these optimized reaction conditions, isomerization of the cephem nucleus to the Δ^2-isomer **22** was minimal (3%).[20b] This amount of isomeric impurity was reduced to below 0.5% after precipitation of the salt **21**. In CH_3CN the heterogeneous reaction mixture produced greater than 25% of the Δ^2-isomer **22**. The process in aqueous acetone was scaled up to produce large quantities of this final intermediate. The final deprotection of **21** with 98% formic acid[29] or TFA yielded disalt **23** (chloride and formate or trifluoroacetate) in 90−95% yields. Further treatment of the salt **23** with aqueous base (NaOH or $NaHCO_3$) at pH 6.0 produced the double zwitterion BMS-247243 with HPLC AP >98 as off-white crystals in 65−73% yield.[20c]

Deprotection reactions with formic acid did not completely remove the benzhydryl ester group (0.5−1.5 AP **24**), and extended exposure resulted in degradation of the product **21**. Addition of 2−4 mol equiv of TFA or HCl towards the end of the reaction produced increased amounts of the degradation product diacid **20** described above. On the other hand, reaction with TFA completely removed the benzhydryl ester, but mono-*tert*-butyl ester **25** (1−2%) contaminated the product.[30,31] Since the final crystallization of the double zwitterion BMS-247243 was performed in water, it was difficult to completely remove these more lipophilic monoesters. Addition of conventional carbenium ion scavengers, for example, anisole or thioanisole, did not improve the quality of the product in these reactions. It was surmised that the soluble isobutylene [or the corresponding trifluoroacetate derivative $(CH_3)_3CO_2CF_3$] was causing the reverse reaction at a low level. Heptane, insoluble in the reaction mixture, was used during deprotection reactions with TFA to extract out these moieties. Indeed, under these conditions the level of *tert*-butyl monoester **25** was reduced to below 0.2% during reaction with TFA. It was further found that the use of Et_3SiH in deprotections with TFA also produced product **23** with minimal amounts of the monoesters.[32]

We prepared over 2 kg of BMS-247243 by deprotection of diester **21** with 98% formic acid followed by crystallization of the double zwitterion from water.

Initially, double zwitterion BMS-247243 crystallized from a saline solution as a birefringent liquid crystal phase which contained only trace amounts of a three-dimensionally ordered crystalline component as determined by powder X-ray diffraction (PXRD). The lyophiles obtained originally after purification of the double zwitterion by column chromatography or the subsequent batches of these liquid crystal/amorphous solids were unstable to storage at ambient temperature; the AP decreased by 2−4% over a month.[20c] Salts of BMS-247243 made with different acids (2 equiv each of HCl, HNO_3, H_2SO_4, H_3PO_4, MSA, PTSA, or CSA), in the hope of obtaining a crystalline stable form, did not crystallize. Crystallization of the double zwitterion from the crude disalt **23** from water at pH 6.0 with seed crystals from the above partially crystalline material gave predominantly crystalline material [polyhydrates (4−11 H_2O) from different batches] with a better stability profile for the higher hydrates. Finally, a stable form of BMS-247243 was prepared serendipitously. Suspensions of the double zwitterion in water were stirred with different concentrations of NaCl (3, 6, and 9 mol equiv) for 63 h at 25 °C to prepare a stable form with a uniform amount of NaCl and H_2O. These samples contained variable amounts of NaCl (0.2−0.6 mol) and water (6−7 mol). It turned out that a similar experiment without NaCl produced material which after drying contained 10−11 mol of H_2O. This material proved stable for months at ambient temperature. These polyhydrates displayed similar PXRD patterns. Small and very thin spear shaped crystals were obtained after several recrystallizations of a batch of the double zwitterion from water. Single-crystal X-ray analysis of one of the larger crystals (<10 μ) based on intensity data from a synchrotron source revealed most of the molecular structure.[33] At least eight water molecules (A−H) have been identified in the crystal structure of BMS-247243 (Figure 2), and "empty space" considerations suggest an additional two or three other possible water sites. Each

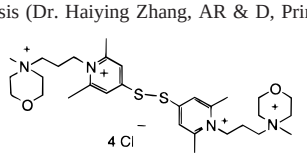
(28) The structure of the diacid **20** was confirmed by the following synthesis. Acylation of *tert*-butylglycine with monoacid ester **12b** formed diester (**i**) which on double deprotection gave diacid **20**.



(29) Halpern, B.; Nitecki, D. E. *Tetrahedron Lett.* **1967**, *8*, 3031.

(30) Diphenylmethyl ester hydrolyzed selectively on heating diester **21** in water at 80 °C. After 10 min the ratio of the mono-*tert*-butyl ester **21** to the diester **25** was 2.6:1.

(31) A minor amount (<0.1%) of the byproduct disulfide (**i**) was isolated by column chromatography of one of the partially deprotected batches to prepare a reference sample of monoester **25**. The structure is based on NMR spectra and MS/MS analysis (Dr. Haiying Zhang, AR & D, Princeton).



(32) Pearson, D. A.; Blanchette, M.; Baker, M. L.; Guindon, C. A. *Tetrahedron Lett.* **1989**, *30*, 2739.

(33) (a) Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Basic Energy Sciences, Office of Science, under Contract No. W-31-109-Eng-38. (b) Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk). (c) Data were collected at beamline 17-ID in the facilities of the Industrial Macromolecular Crystallography Association Collaborative Access Team (IMCA-CAT) at the Advanced Photon Source. These facilities are supported by the companies of the Industrial Macromolecular Crystallography

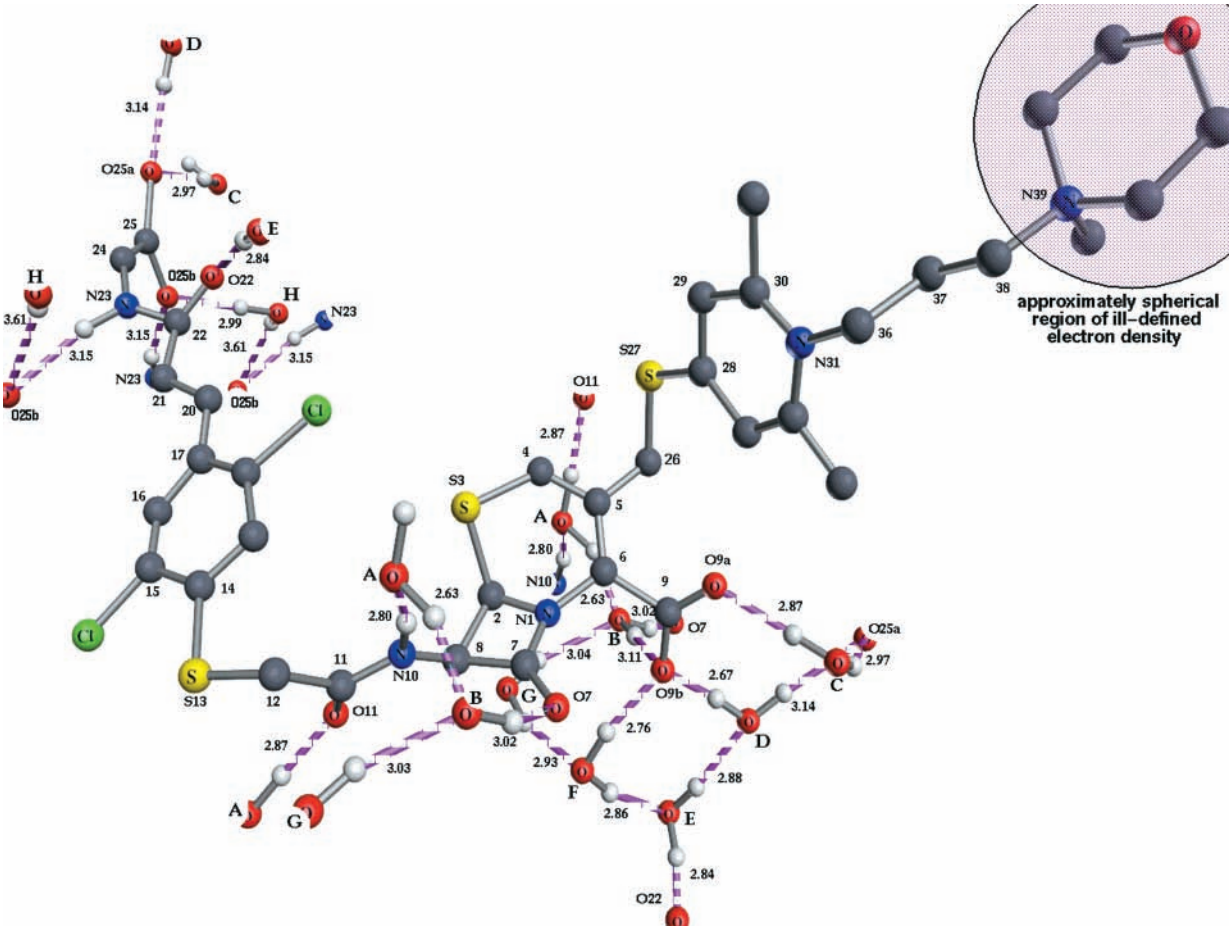


Figure 2. **Single-crystal X-ray analysis of BMS-247243.**

molecule of the double zwitterion is involved in at least 13 *inter*molecular H-bonds, of which only two involve molecule−molecule interactions [O25b ← (HN23, O25b) < −HN23]. Most of the water molecules are a receptor of one, and a donor for two, H-bonds. Water molecules C and G donate two but accept no H-bonds. Water H is not part of the seven-water-cluster (A−G) and appears to be involved in only one H-bond (to the poorly resolved carboxyl of the glycine). The powder XRD pattern simulated from the refined atomic coordinates in the single-crystal structure is in good agreement with the observed XRD pattern of the crystallized batches.

In summary we have developed a practical synthesis of the anti-MRSA cephem BMS-247243. Efficient methods were devised for the preparation of the C3 and C7 side chains **6b** and **12b,** respectively. These side chains were sequentially appended to the cephem nucleus ACLH with very high stereochemical efficiency to produce a crystalline final intermediate **21**. An efficient process was developed for the conversion of the diester **21** to the crystalline double zwitterion BMS-247243.

Association through a contract with Illinois Institute of Technology (IIT), executed through the IIT's Center for Synchrotron Radiation Research and Instrumentation.

Selected torsional angles (deg)

2-8-N10-11	8-N10-11-12	N10-11-12-S13
120	-177	152
11-12-S13-14	12-S13-14-15	16-17-20-21
-79	174	12
17-20-21-22	20-21-22-N23	21-22-N23-24
-172	-167	-176
22-N23-24-25	N23-24-25-O25b	N1-6-9-O9b
58	9	43
4-5-26-S27	5-26-S27-28	26-S27-28-29
52	95	-172
30-N31-36-37	N31-36-37-38	36-37-38-N39
-73	-178	-174

Experimental Section

All new compounds described in the Experimental Section were fully characterized. Analytical and spectral data for these compounds are for lab batches and are provided as Supporting Information. HPLC analysis results are described as area percent (AP).

4-[3-[[(Dimethylethoxy)carbonyl]amino]propyl]-4-morpholine (1b). To a solution of N-(3-aminopropyl)-morpholine **1a** (1.0 kg, 6.953 mol) in 4.0 L of EtOAc was added slowly a solution of Boc_2O (1.59 kg, 7.282 mol) in 4.0 L of EtOAc, keeping the reaction temperature at 30−35 °C by external cooling. **Caution:** The reaction was exothermic, and strong evolution of CO_2 was observed, which gradually slowed after completion of addition. The mixture was allowed to warm to room temperature. The reaction was monitored by HPLC (<1% **1a** after 3 h).[34a] The mixture was washed once with 5.0 L of half-saturated $NaHCO_3$ and once with 2.5 L of brine. The solvent was distilled off in a vacuum. Solid NaCl that precipitated during the concentration was filtered and then the solvent was removed to give 1.73 kg (109% yield) of compound **1b** as a light yellow oil. The product was used in the next reaction as soon as possible since it darkened on

storage at room temperature.

4-(3-Aminopropyl)-4-methylmorpholinium, Chloride Hydrochloride (3b). (Caution: Due to the use of CH_3I in this reaction, general safety rules should be followed). CH_3I (1.18 kg, 8.303 mol) was added in one portion to a solution of amine **1a** (1.58 kg, 6.92 mol) in 7.9 L of EtOH at room temperature. The reaction mixture was warmed to 35−40 °C and stirred at this temperature until the reaction was complete (22 h).[35] EtOH was distilled to half the volume (4.7 L); excess CH_3I was removed during this process. To this solution of salt **2** was added a solution of 12% ethanolic HCl (12.63 kg, 41.517 mol). The reaction was slightly exothermic and crystallization of the mixed chloride/iodide salt started in 0.5 h.[36a] After the solution stirred overnight at ambient temperature, the crystal suspension was cooled to 0 °C and stirred for 2 h. The solid was filtered and washed with 2.49 L of EtOH. The wet solid was suspended in 6.92 L of EtOH containing $BnBu_3NCl$ (2.27 kg, 7.265 mol), and the slurry was stirred overnight at ambient temperature. The crystal slurry was cooled to 0 °C, stirred for 2 h, and filtered. The product was washed twice with 2.5 L of cold EtOH and dried under vacuum at 25 °C to a constant weight to give 1.49 kg of salt **3b** (93% yield) as white to slightly yellow crystals. The crystals become slightly darker when stored in air.

2,6-Dimethyl-4-mercaptopyrone (5). To a solution of 2,6-dimethyl-γ-pyrone (100 g, 0.806 mol) in 2.0 L of toluene was added Lawesson's Reagent (162.91 g, 0.403 mol) at 25 °C. A light red suspension was obtained, and the temperature rose slightly to 30 °C. The mixture was agitated at room temperature for 1 h and monitored by TLC.[36b] Agitation was continued for another hour to ensure complete consumption of the starting material. Toluene was removed in a vacuum until a residual volume of 266 mL of was obtained. Water (300 mL) was added, and the remaining toluene was distilled off azeotropically at atmospheric pressure until the boiling point reached 100 °C. The aqueous phase of the distillate was poured back into the reactor. At the end of the distillation the organic phase was slightly red and the aqueous phase was slightly green. The reaction mixture was cooled to 50 °C and CH_3OH (1.2 L) was added. The mixture was heated to reflux to obtain a clear red solution and then cooled to 0−5 °C. During cooling, the product started to crystallize at 50 °C. After the solution stirred for 2 h in the cold, the product was filtered and washed with cold 50% aqueous MeOH (500 mL, 0 °C). The solid was dried under vacuum to give 96.9 g of 4-mercaptopyrone **5** (85% yield). This procedure was used to prepare several kilograms of **5**.

4-[3-(1,4-Dihydro-2,6-dimethyl-4-thioxopyridin-1-yl)-propyl]-4-methyl-morpholinium, Hydroxide, Inner Salt, Chloride (6b). Et_3N (1.051 kg, 10.382 mol) and 4-mercaptopyrone **5** (0.67 kg, 4.758 mol) were added to a suspension of amine hydrochloride **3b** (1.0 kg, 4.326 mol) in 9.5 L of EtOH at 20−25 °C. The mixture was refluxed (~78 °C)[34c] for 10 h and then cooled to ambient temperature and stirred overnight. The product was filtered, washed three times with 2.0 L of EtOH, and dried under vacuum at 30 °C to constant weight to give 1.10 kg of 4-mercaptopyridone **6b** (80% yield) as yellow crystals.

2,4,5-Trichloro-1-iodobenzene (8). A suspension of 2,4,5-trichloroaniline (140.0 g, 0.7126 mol) in 2.8 L of acetic acid was agitated and heated at 35−40 °C to obtain a clear solution. The solution was cooled to 15−20 °C and 140 mL of concentrated HCl was added, and the mixture was agitated for 0.5 h. A solution of $NaNO_2$ (49.17 g, 0.7126 mol) in 168 mL of H_2O was added rapidly with cooling at 15−20 °C. The reaction was complete after 0.5 h.[36c] The mixture was polish filtered with a Diacel filter, and the cake was washed with three 50 mL portions of acetic acid. A solution of KI (130.13 g, 0.7839 mol) in 672 mL of H_2O was added to the filtrate at 15−20 °C with cooling. (**Caution:** copious gas evolution ensued). After 0.5 h, $Na_2S_2O_3 \cdot 5H_2O$ (70.74 g, 0.285 mol) was added to the mixture and agitated for 0.5 h. The precipitate was filtered and then washed twice with 230 mL of AcOH/H_2O (3:1) and finally with 750 mL of H_2O until the filtrate remained colorless and registered pH 3−4. The wet product was dried under vacuum at 30 °C to constant weight to give 162.4 g (75% yield) iodide **8**.

2,4,5-Trichlorocinnamic Acid (13). 2,3,5-Trichloro-1-iodobenzene (150 g, 0.49 mol), K_2CO_3 (83 g, 0.60 mol), $Pd(OAc)_2$ (2.0 g, 0.009 mol), H_2O (850 mL), and acetonitrile (230 mL) were charged into a 3-L, three-necked round-bottomed flask. The mixture was heated to 70 °C, and acrylic acid (40.0 g, 0.56 mol) was added over a period of 15 min. The resulting black solution was stirred at 80 °C. After 3 h H_2O (2 L) and EtOAc (1 L) were added, and the pH was adjusted to 9 with NaOH. The temperature was held at 40 °C to effect a clean phase separation, and the aqueous phase was washed with EtOAc (600 mL) and acidified to pH 4 with HCl (12 N) to give a white slurry. After the addition of H_2O (2 L), the slurry was stirred for 1 h. The solid was filtered, washed with H_2O (300 mL) and heptane (300 mL), and then dried at 60 °C under vacuum for 16 h to give 93 g (79.5% yield) of the cinnamic acid **13** as a white solid.

***N*-[1-Oxo-3-(2,4,5-trichlorophenyl)-2-propenyl]glycine,1,1-dimethylethyl Ester (14).** Vilsmeier reagent (0.71 kg, 5.567 mol) was added to a suspension of acid **13** (1.0 kg, 3.976 mol) in 5.92 L of THF in a vessel at 20−24 °C under a nitrogen atmosphere. (**Caution**: Vilsmeier reagent is very sensitive to hydrolysis; handle under inert gas). Aliquots of the reaction mixture were quenched with CH_3OH to monitor acid chloride formation by HPLC.[34d] DMF·HCl oiled out (~2% of total volume) as a black oil during this process. The reaction was complete in 3 h. In a second vessel a solution of $KHCO_3$ (1.55 kg) was prepared in 4.34

(34) (a) HPLC. YMC ODS-AM, 5 μm, 120 A, 250 × 4.6 mm. Mobile phase: A: 0.01 M phosphate buffer, pH 7.0; B: CH_3CN. A:B 70:30, wavelength 205 nm, flow rate 1.2 mL/min; runtime 15 min. Retention time (t_R) **1a** 2.3 min, **1b** 7.0 min. (b) YMC ODS-AM, 5 μm, 120 A, 250 × 4.6 mm. t_R **2** 2.4 min, CH_3I 14.1 min. (c) YMC ODS-AM, 5 μm, 120 A, 250 × 4.6 mm. A: 0.2% H_3PO_4, B: CH_3CN, A:B 70:30, wavelength 210 nm, flow rate 1 mL/min, run time 20 min, t_R **5** 11.2 min, **6b**, 2.5 min. (d) YMC ODS-AQ, 5 μm, 250 × 4.6 mm, A: 0.1% TFA in H_2O, B: CH_3CN. A:B 35:65, wavelength 230 nm, flow rate 1.5 mL/min; runtime 20 min, t_R **13** 5.7 min, methyl ester of **13b,** 14.4 min, **14** 9.2. (e) Method same (d) but with 35% A and 65% B. t_R **14** 9.2 min, **12a** 6.2 min, **12b** 3.7 min, DMF 2.0 min.

(35) This reaction required 2 days at room temperature.

(36) Monitored by TLC: (a) Silica gel Si-60, EtOAc:AcOH:H_2O = 3:1:1, visualized with ninhydrin, R_f **2** 0.39, **3** 0.11. (b) EtOAc, R_f **4** 0.74, **5** 0.14. (c) EtOAc:heptane = 1:4, visualized by UV.

L of H_2O and 0.37 L of THF. A solution of glycine *tert*-butyl ester hydrochloride (0.8 kg, 4.771 mol) in 1.58 L of H_2O was added to the bicarbonate solution at such a rate that the temperature remained at 20−25 °C. CO_2 gas evolution and slight foaming occurred during this time. The mixture was stirred further for 0.5 h after completion of the addition. The acid chloride solution was then added slowly over 40 min to this solution of the amine at 20−25 °C. Faster addition caused some hydrolysis of the acid chloride. The mixture was stirred for ∼1.5 h, and reaction progress was monitored by HPLC.[34d] Water (11.84 L) was added slowly to the reaction mixture, keeping the temperature at 20−25 °C. The biphasic solution became a fine suspension over 0.5 h. After the solution stirred further for 0.5 h, the crystal slurry was filtered and washed twice with 4.93 L of H_2O. The product was dried to constant weight under vacuum at 50 °C to furnish 1.39 kg (96% yield) of **14** as off-white crystals (H_2O < 0.1% by KF).

[[2,5-Dichloro-4-[(*E*)-3-[[[(1,1-dimethylethoxy)carbonyl]methyl]amino]-3-oxo-propenyl]phenyl]thio]acetic acid, 12b. LiO-*t*-Bu (0.26 kg, 3.291 mol) and anhydrous Bu_4NCl (0.13 kg) were stirred in 8.6 L of DMF in a vessel at ambient temperature under a N_2 atmosphere. The thin suspension was cooled to −10 to −5 °C, and mercaptomethyl acetate (0.35 kg, 3.291 mol) was added slowly over 20 min. The mixture was stirred further for 45 min. In a separate vessel a suspension of chloroester **14** (1.0 kg, 2.742 mol) in 4.3 L of dry DMF (KF < 0.05% H_2O) was cooled to −10 to −5 °C with stirring under N_2 atmosphere. The solution of the thiolate anion was added over 0.5 h to this suspension keeping the temperature between −6 to −2 °C. The yellowish brown solution became an orange solution over 15 h. The formation of diester **12a** was monitored by HPLC.[34e] A solution of LiOH·H_2O (0.11 kg, 2.726 mol) in 5.47 L of H_2O was added slowly over 3 h to the solution of ester **12a** at −4 to 0 °C, maintaining the pH below 13.6. The addition was exothermic, and the temperature rose to 4 °C in the beginning. If the base is added in one portion, ∼5% of the corresponding diacid was produced. After 0.5 h at 0 °C, the pH of the mixture was adjusted to 8.5 by the addition of 0.17 L of 1 N HCl, and then 4.8 L of H_2O and 7.2 L of TBME were added at 5−22 °C. The organic phase was discarded, and 8.16 L of EtOAc was added to the aqueous phase. With stirring the pH was adjusted to 3.6−4.1 with 1.33 L of 1 N HCl at 28−36 °C. The product precipitated below 22 °C during the pH adjustment. The organic phase was separated, and the aqueous phase was extracted twice with 8.16 L of EtOAc at 30−35 °C. The temperature was increased to 50 °C if precipitation occurred during the extraction. The organic extracts were combined and washed twice with 4.8 L of H_2O at 45−50 °C. The solution was concentrated to 5 L volume under vacuum at 60 °C (KF <2.5% H_2O). Toluene (14.4 L) was added to the thick, yellowish white suspension at 60−70 °C. The crystal slurry was cooled to 20 °C over 1 h and stirred further for 1 h. The product was filtered, washed twice with 2 L of toluene/EtOAc (3:1), and dried under vacuum at <50 °C to constant weight to give 0.91 kg of acid **12b** (79% yield) as white crystals.

(6*R-trans*)-3-(Chloromethyl)-4-7-[[2-[3-[[[(1,1-dimethylethoxy)-carbonyl]methyl]amino]-3-oxopropenyl]-2,5-dichlorophenyl]-thio]acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid, Diphenylmethyl Ester (17). To a 3-L, round-bottomed flask equipped with an overhead stirrer, thermocouple, and nitrogen inlet was added acid **12b** (250.0 g, 0.595 mol) and THF (2 L). The slurry was cooled to 0−5 °C, and Vilsmeier reagent (105.0 g, 0.82 mol) was added in small portions. The reaction was stirred at 0−5 °C for 1 h to give a clear yellow solution of the acid chloride of **12b**. In a separate 12-L, round-bottomed flask $KHCO_3$ (250 g, 2.5 mol), THF (3 L), and water (1.25 L) were added followed by solid ACLH·HCl (267.5 g, 0.595 mol) to give a biphasic solution which was stirred for 30 min at 20−25 °C. The cold (0−5 °C) acid chloride solution of **12b** was added via a Teflon tube to the biphasic solution of ACLH over 20−30 min while the temperature was maintained at 19−25 °C. The pH of the biphasic solution varied from 8.2 to 7.5 over the addition. When the addition was complete, the biphasic solution was held for 1 h at 20−25 °C. The pH was adjusted to 3−4 with the addition of 6 N aqueous HCl, and the mixture was heated to 45 °C to effect a clean split. The clear, red organic phase was distilled (50 °C, in a vacuum) to a volume of approximately 3 L. The solution was cooled to 40 °C, at which point crystals formed. MTBE (2.5 L) was added, and the solution was cooled to room temperature and held overnight (∼14 h). The product was filtered, washed with MTBE (2 × 1 L), and dried under vacuum to give 436.7 g of diester **17** (90% yield) as an off-white solid, HPLC AP 94.9, Δ^2-isomer **18** AP 0.2.[20b]

(6*R-trans*)-4-[[[7-(*E*)[[[4-[3-[[(1,1-Dimethylethoxy)acetyl]-amino]-3-oxo-1-propenyl]-2,5-dichlorophenyl]thio]acetyl]-amino]-8-oxo-2-[(diphenylmethoxy)carbonyl]-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]thio]-2,6-dimethyl-1-[3-(4-methylmorpholino-4-yl)propyl]-1-pyridinium, Dichloride Diester (21). A solution of chlorodiester **17** (250 g, 0.3029 mol) and 4-mercaptopyridone **6b** (100.8 g, 0.318 mol) in 2.75 L of THF and 908 mL of deionized H_2O was stirred at room temperature for 24 h under a N_2 atmosphere. HPLC analysis showed 0.5 AP **17**, and ∼2.8 AP Δ^2-isomer **22**.[20a,b] The mixture was filtered to remove fine particles, and the filtrate was concentrated via distillation under vacuum at 50 °C until the mixture partially solidified. Acetone (7.0 L) was added over 15 min, and the resulting slurry was warmed to 50 °C to obtain a solution. The solution was cooled slowly; at 40 °C crystallization commenced. MTBE (1.7 L) was added via an addition funnel over 15 min at 40 °C, and the slurry was cooled slowly to 20 °C and stirred for an additional 2 h. The crystals were filtered and washed with 1.5 L of 5% H_2O/acetone, then 1.5 L of acetone, and finally 1.5 L of MTBE. The solid was dried in a vacuum for 90 h to yield 256.1 g (74.6%) of diester **21** as an off-white solid, HPLC AP 97.4, Δ^2-isomer **22** AP 0.8.[20b]

(6*R-trans*)-*E*-7-[[[[2,5-Dichloro-4-[3-[(carboxymethyl)-amino]-3-oxo-1-propenyl]phenyl]thio]acetyl]amino]-4-

[[(2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methyl]thio]-2,6-dimethyl-1-[3-(4-methylmorpholino-4-yl)propyl]-1-pyridinium, Dichloride (23). Formic acid (98%, 1.82 L) was added to diester **21** (588.4 g, 0.5343 mol), and the resulting solution was warmed to 35 °C and stirred at this temperature for 3 h. HPLC analysis showed less than 3 AP of a mixture of monoesters **24** and **25**.[20c] The solution was cooled to 10 °C and filtered through glass wool to remove some white solids. It was then added slowly with rapid stirring via an addition funnel to 14.5 L of ethyl acetate over a 45 min period. After the addition was complete, the resulting slurry was stirred for 1 h. The solid was filtered, washed with 3 L of ethyl acetate, and dried under vacuum at room temperature for 48 h to yield 503.5 g (∼91.5%) of diacid salt **23** (X = Cl/formate) as a light yellow solid with AP 92.1.[20c] An additional 0.93 kg of salt **23** was produced by this method. This product was converted to double zwitterion BMS-247243 without further purification.

(6*R*-*trans*)-*E*-7-[[[[2,5-Dichloro-4-[3-[(carboxymethyl)-amino]-3-oxo-1-propenyl]phenyl]thio]acetyl]amino]-4-[[(2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methyl]thio]-2,6-dimethyl-1-[3-(4-methylmorpholino-4-yl)propyl]-1-pyridinium, Hydroxide, Inner Salt (BMS-247243-01). Diacid salt **23** (1.4 kg, 1.535 mol) was dissolved in 11.2 L of deionized water while the pH was maintained between 1.8 and 2.0 with 1 N sodium hydroxide. The mixture was adjusted to pH 3.0 with 2 L of 1 N sodium hydroxide added over a 45 min period. The entire solution was polish filtered and the resulting solution adjusted to pH 3.8 with 1 L of 2 N sodium hydroxide added over a 30 min period. Seed crystals of double zwitterion BMS-247243 (0.1 g) were added, and the pH was adjusted to 6.5 with 500 mL of 2 N sodium hydroxide. After the solution stirred for about 1 h at room temperature, a thin slurry had formed, and the pH was 6.1. The pH was readjusted to 6.5 with 20 mL of 2 N sodium hydroxide. The resulting slurry was stirred for 16 h at room temperature after which time the slurry had thickened considerably. After the solution stirred overnight in an ice bath at 10−15 °C, crystals were filtered, washed with 3 L of cold (∼5 °C) deionized water and then with 1 L of absolute ethanol. The product was dried under vacuum at 30−35 °C with a nitrogen bleed for 48 h to give 0.861 kg (72.8%) of double zwitterion BMS 247243-01, mp 180−83 °C dec; HPLC, AP 98.7.[20c] Anal. Calcd for $C_{36}H_{41}N_5O_8S_3Cl_2 \cdot 6.0\ H_2O \cdot 0.06$ EtOH, C, 45.68; H, 5.66; N, 7.38; S, 10.13; Cl, 7.47; H_2O, 11.38. Found: C, 45.75; H, 5.72; N, 7.32; S, 9.99; Cl, 7.47; H_2O, 11.18 (KF).

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Supporting Information Available

Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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