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Development and Scale-Up of an Optimized Route to the ALK Inhibitor CEP-28122

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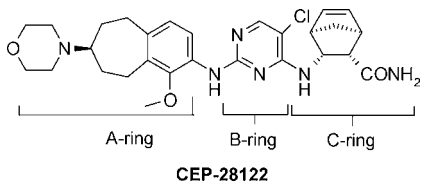
ABSTRACT: Evolution of the process strategies to prepare CEP-28122, an anaplastic lymphoma kinase (ALK) inhibitor, is presented. The initial medicinal chemistry route, used for the preparation of key supplies for biological screening, is reviewed. In addition, the process research and development of the final optimized process for manufacture of preclinical and clinical supplies is discussed. Details regarding a blocking group strategy for selective nitration; discovery of a one-pot transfer hydrogenation to effect a reductive amination, nitro group reduction, and dehalogenation; an enzymatic resolution of a critical intermediate; and the discovery of a novel, stable, in situ generated mixed mesylate hydrochloride salt of the API are disclosed.

INTRODUCTION

Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase (RTK) member of the insulin receptor superfamily identified as part of the NPM−ALK fusion gene in anaplastic large cell lymphoma (ALCL) with a t(2;5) chromosomal translocation.[1] ALK, when fused with NPM, is constitutively activated and shown to be involved in proliferation and survival of a variety of human cancers.[2] The aberrant signaling of ALK resulting from rearrangements or mutations/gene amplification leads to an "oncogenic addiction" which can be targeted with kinase inhibitors.[3] Crizotinib is the first ALK inhibitor to be approved and has shown a clinical impact with patients that are highly refractory.[4] The need for novel ALK inhibitors to impact emergence of resistance mechanisms as well as to provide improved kinase selectivity profiles is of great importance. CEP-28122 is a selective, potent ALK inhibitor, demonstrating robust antitumor efficacy in tumor xenograft mouse models, which advanced into preclinical development.[5] It is a complex small molecule comprised of three core subunits, two of which contain one or more chiral centers (Figure 1).



CEP-28122

Figure 1. ALK inhibitor CEP-28122.

The initial route to CEP-28122 employed in the medicinal chemistry group will be reviewed. The process development program undertaken to improve the synthesis for the preparation of 6.7 kg of drug substance for preclinical and initial clinical supplies will be detailed. Significant improvements include development of a blocking strategy for selective nitration; discovery of a one-pot transfer hydrogenation to effect a simultaneous reductive amination, nitro group reduction and dehalogenation; an enzymatic resolution of a key C-ring intermediate; and discovery of a novel, stable, in situ generated mixed mesylate hydrochloride salt of the API.

MEDICINAL CHEMISTRY ROUTE

The initial strategy for synthesis of CEP-28122 involved coupling three key structural elements, including a morpholine-substituted benzocycloheptane unit (A-ring), a diaminopyrimidine central core (B-ring), and a bicyclic amino amide fragment (C-ring). The synthesis of the CEP-28122 A-ring utilized the bromination of 1-methoxy-2,3-dimethylbenzene (**1**). Several methods were explored with the best results achieved using NBS and AIBN to provide **2** in good yield.[6] The formation of the fused arylcycloheptane ring, **3**, was accomplished using diethyl 1,3-acetone-dicarboxylate with sodium bicarbonate and tetra-n-butylammonium iodide in a biphasic solvent system.[7] The crude adduct was treated with ether to remove tetra-butylammonium iodide salts and this product stream was subjected to ester hydrolysis and decarboxylation conditions to provide **3**, in 33% yield over 2 steps, after purification. Nitration of **3** resulted in several regioisomers and dinitrated products in poor overall yield; however, they were separable using chromatography on silica gel. Reductive amination of **4** with morpholine led to the formation of **5** in good overall yield. Subsequent reduction of the nitro group was accomplished under standard hydrogenation conditions to give racemic A-ring **6** (Scheme 1).

The synthesis of the C-ring fragment, **13**, involved a [2 + 2] cycloaddition to give racemic **8**. Following *N*-lactam protection and amide hydrolysis, **10** was subjected to fractional recrystallization using (*S*)-1-phenylethylamine to provide the desired (1*S*,2*S*,3*R*,4*R*) enantiomer **11**.[8] Conversion of the acid functionality to the primary amide gave **12** and Boc-deprotection led to **13**.[9] CEP-28122 was assembled in a tandem S_NAr reaction sequence in which **13** was condensed with the trichloropyrimidine, **14**,

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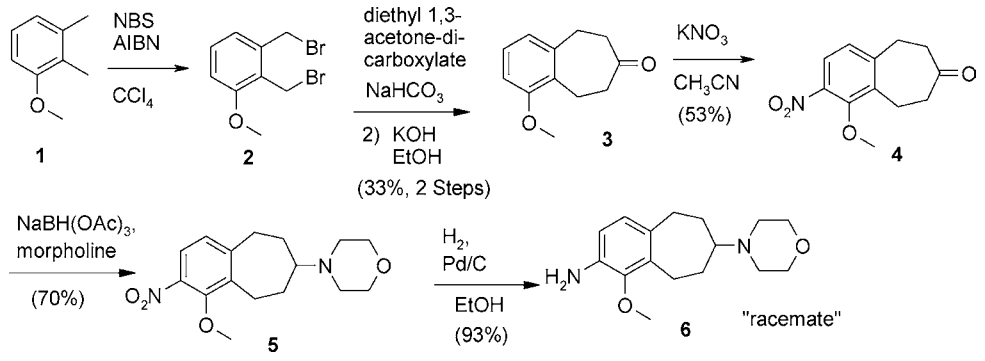
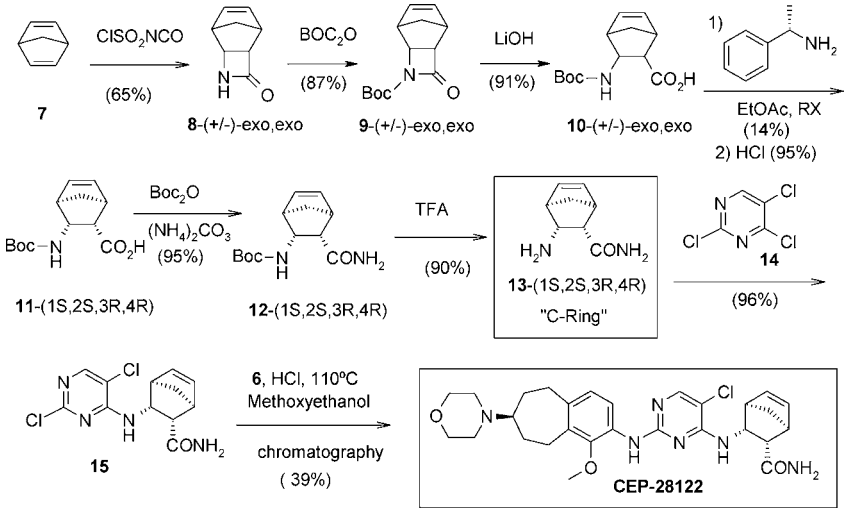
Scheme 1. Medicinal chemistry route to A-ring fragment of CEP-28122



Scheme 2. Medicinal chemistry route to C-ring and CEP-28122



followed by reaction with the A-ring fragment **6** (Scheme 2). A mixture of diastereomers was obtained and separated by reverse phase HPLC. An X-ray crystal structure of one of the diastereomers was solved to elucidate the absolute configuration about the stereogenic center of the A-ring fragment of CEP-28122.[5]

■ PROCESS DEVELOPMENT

A-Ring Synthesis. While the medicinal chemistry approach was acceptable to produce the racemic A-ring on gram scale, numerous challenges needed to be overcome for scale-up. Central to the development work was identifying a route that would accomplish this and obviate the HPLC separation throughout the API synthesis. A blocking group strategy that placed a substituent in the 4-position would allow for a selective nitration (Scheme 3). Furthermore, if this blocking group could be removed under subsequent reaction conditions, post nitration, the overall number of downstream steps would be unchanged. Selective halogenations of 2,3-dimethylanisole are known to occur para to the methoxy position with excellent selectivity.[10] Furthermore, hydrogenation conditions already in place for the nitro reduction could be further developed to remove this blocking group.

Initially, use of a bromide blocking group strategy was pursued because the tribromination of the anisole occurs in a single step[10a] and the bromo dehalogenation reaction is considerably more facile than the corresponding chloro variant. This strategy worked well through the decarboxylation step. However, nitration of the bromo variant resulted in a 2:1:1 mixture of ortho, para, and ortho/para overnitration products that resulted from *ipso* substitution.

A chloride at this position, with less leaving group capability, could eliminate this problem. As shown in Scheme 3, the chloride was efficiently introduced by reacting a solution of **1** in a small volume of chlorobenzene (PhCl) with sulfuryl chloride (SO_2Cl_2) and catalytic aluminum chloride ($AlCl_3$).[11] After aqueous workup, the crude reaction mixture in PhCl was treated with AIBN as a radical initiator prior to the portion-wise addition of *N*-bromosuccinimide (NBS), generating the monochloro-dibromo compound **17**. Eventually, this route was scaled up to produce a total of 31 kg of **17** representing a 74% yield over the two halogenation steps.

Early optimization of the dialkylation and decarboxylation steps focused on developing an efficient telescoped process that negated the need to isolate intermediate **18** and also employed a crystallization to avoid silica gel purification of **19**. During this development it was discovered that incomplete conversion using $NaHCO_3$ led to the presence of residual **17** in the decarboxylation step. Under the strongly basic, caustic conditions of the decarboxylation, residual **17** generated two dimerization impurities, **22** and **23** (Figure 2), that significantly impaired the performance of the downstream chemistry.[12] More reactive dialkylation reaction conditions using K_2CO_3 and tetrabutylammonium sulfate as the phase transfer catalyst were developed. These improved conditions allowed for the complete consumption of **17** and eliminated the formation of **22** and **23**.

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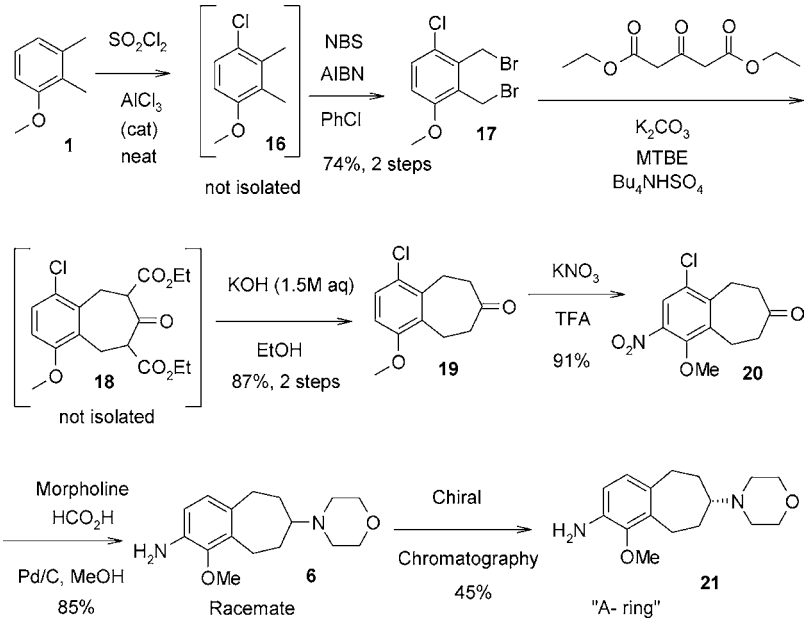
Scheme 3. Optimized A-ring synthesis



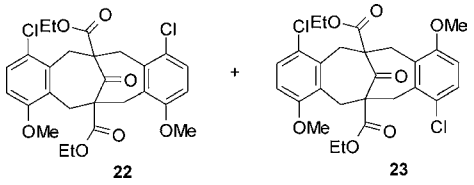


Figure 2. Dimerization impurities.

On scale-up, dibromide **17** was treated with K_2CO_3 and diethyl 1,3-acetonedicarboxylate under biphasic conditions with tetrabutylammonium sulfate. Upon complete consumption of **17** and after aqueous workup, the product mixture underwent decarboxylation in EtOH which resulted in direct crystallization of the product. Key to this process was the ratio of ethanol to water present in the reaction mixture. A simple filtration followed by vacuum drying provided 9.4 kg (87% yield) of **19** in 99.3 wt % purity.

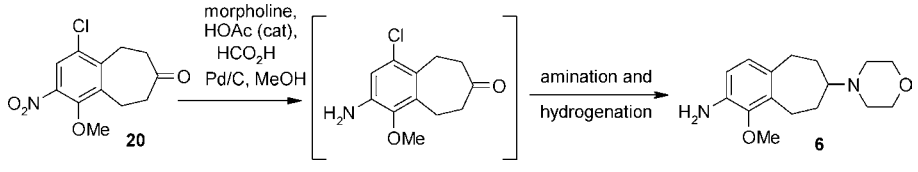
With the para-chloro blocking group in place, the subsequent nitration was completely selective with no *ipso* substitution. Initial nitration conditions used potassium nitrate (KNO_3) in concentrated sulfuric acid. This process proved difficult on scale-up as the reaction had to be quenched with water followed by neutralization of the acid, both highly exothermic and volume inefficient. A rapid screening of alternative nitrating reagents and reaction media was conducted. Trifluoroacetic acid (TFA) with KNO_3 provided a cleaner reaction profile and higher yield. The product could be crystallized directly upon addition of water thus avoiding the difficult aqueous workup and subsequent IPAc recrystallization.

In the pilot plant, it was necessary to carefully control temperature and to avoid the presence of excess nitrating agent. To accomplish this, a reactor containing solid KNO_3 and **19** was charged with five volumes of TFA at −12 °C. The exothermic reaction was readily controlled via a slow increase in the temperature, to 22 °C over 5 h, followed by a 6 h hold. The excess KNO_3 was quenched with water which induced precipitation of the product. This process was used to prepare a total of 9.9 kg of **20** with a typical purity of 99.1 wt %. The optimized yield for this improved process is 91% versus 46% when using the para bromo analogue in the nitration.

The most dramatic change to the A-ring synthesis was the elimination of the stepwise sequence to introduce the morpholine substituent, reduce the nitro group, and remove the halogen blocking group on the anisole ring. Early routes relied on a reductive amination with sodium triacetoxyborohydride to introduce the morpholine. On larger scale, despite the use of either molecular sieves or azeotropic drying with toluene, a significant amount of the secondary alcohol, the product from ketone reduction, was observed. This challenge coupled with the fact that the subsequent steps also required the addition of hydrogen or hydride warranted the investigation of a more direct route. At the onset, a reduction using hydrogen at atmospheric pressure was identified that effected four chemical transformations in a single step: reduction of the nitro group, enamine formation, reduction of the enamine, and dehalogenation. To avoid the use of hydrogen gas, a transfer hydrogenation variant was developed with similar results.[13]

By using LC/MS and syntheses of authentic samples of the likely reaction intermediates, the reaction pathway could be monitored (Scheme 4). Under the transfer hydrogenation

Scheme 4. One-pot enamine formation/reduction/dehalogenation/nitroreduction



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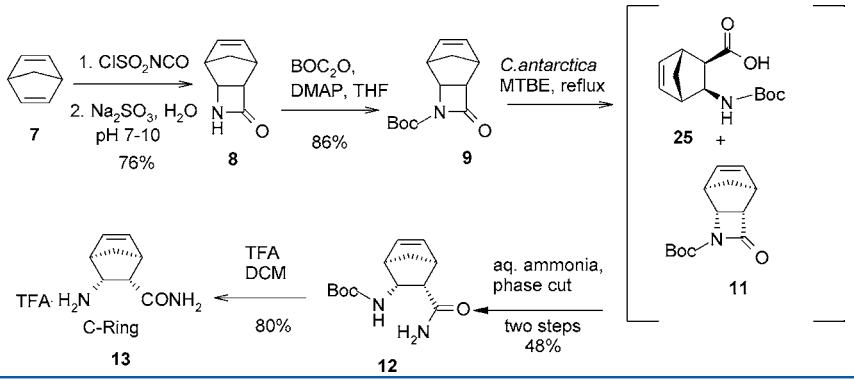
Scheme 5. Optimized synthesis of the "C-ring"



conditions, nitro reduction occurred almost immediately and tracked closely with the addition rate of the formate solution. While small amounts of the subsequent dechlorination were observed, the bulk of the material continued through the enamine formation and reduction pathway with the chloride attached. Both reaction pathways then slowly proceeded to the desired product.

Upon scale-up, formate solution generated from the reaction of formic acid and morpholine was added to substrate **20** in methanol (MeOH) with 10% Pd/C at 60 °C. Generating the formate in a separate vessel, prior to the hydrogenation, removed the latent exotherm that was discovered during development. The initiation of the transfer hydrogenation reaction was confirmed through observation of carbon dioxide evolution and the exotherm was controlled by the rate of formate addition. Gratifyingly, the one pot amination/hydrogenation efficiently generated racemate **6** in 85.4% overall yield averaging 96% per transformation. A total of 9.7 kg of material with a purity of 93.4−96 A% was obtained.

Chiral chromatography was developed to separate the resulting A-ring racemate prior to the final coupling reaction. Separation was carried out on a portion of the available racemate resulting in 3.8 kg of chiral amine **21** representing a 45% yield or 90% recovery of the desired isomer.

An overall yield of 50% for the synthesis of the racemate **6** was achieved in this scale-up. In so doing all chromatographic purifications except for the chiral purification of the final product were eliminated. This compares favorably to the 26−30% yield achieved in an earlier scale-up using a modified medicinal chemistry procedure which necessitated chromatography of **4**.

The chiral chromatographic purification on scale was time-consuming and expensive. An alternative strategy that relied on a classic diastereomeric salt resolution appeared to be a viable alternative. Using a wide variety of chiral acids and solvent systems, over 200 diastereomeric salt resolutions were screened on small scale. The lead results using tartaric acid derivatives in alcoholic solvents were further investigated. Subsequent to the campaign described herein, a two-step diastereomeric salt resolution was identified that gave enantiopure A-ring in better than 98% de. The first step forms a hemisalt with dibenzoyl-tartaric acid, **24** (Figure 3), in methanol resulting in the enriched A-ring with moderate diastereomeric excess. Recrystallization of this salt further upgrades the diastereomeric purity to >98% in 35−40% overall yield. Salt break to the free base is possible with aqueous NaOH followed by extraction with EtOAc.

C-Ring Synthesis. The original process used to generate the C-ring (Scheme 2) required seven steps and a tedious dia-

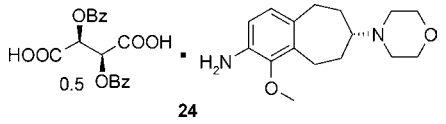
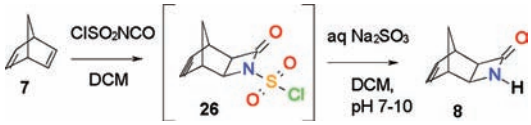


Figure 3. Dibenzoyl tartaric acid salt of the A-ring.

stereomeric salt resolution with seven recrystallization stages. The overall yield was low providing only 6.5% of **13** from **7**. Key issues included a variable yield in the [2 + 2] cycloaddition between norbornadiene and chlorosulfonyl isocyanate, a low yield in the diastereomeric salt resolution resulting from multiple recrystallizations, which were required to achieve >98% ee, and the stepwise introduction of the amino amide functionality. All of these issues were successfully addressed, using the process outlined in Scheme 5, reducing the overall number of steps to four and improving the yield of **13** from **7** to 25%.

The first step in the C-ring synthesis consists of two parts. A [2 + 2] cycloaddition between chlorosulfonyl isocyanate and 2,5-norbornadiene yields an intermediate chlorosulfonamide (Scheme 6). Intermediate **26** is then reduced with sodium

Scheme 6. [2 + 2] Cycloaddition/oxidation



sulfite to the β-lactam **8**.[14] Variability in the yield of this sequence was determined to be caused by the sodium sulfite reduction, in which the pH must be strictly controlled between 7 and 10 to avoid degradation of the β-lactam product. The use of a carbonate/bicarbonate buffer combined with a short reaction time led to more consistent results. On larger scale, addition of a methylene chloride (DCM) solution of the chlorosulfonamide to an aqueous buffered solution of sodium sulfite minimized contact time between the base and product. A rapid phase cut followed by a solvent switch to *tert*-butyl methyl ether (MTBE) afforded 8.9 kg (76%) of **8** as a granular white solid.

Preparation of **9** using standard BOC-protection conditions utilized di-t-butyldicarbonate in THF with catalytic DMAP. Following addition of water, the product was precipitated by distillation of THF. Filtration and drying yielded 12.7 kg (86%) of **9**.

The original process for the C-ring hydrolyzed the β-lactam to the amino acid **10** which was then resolved using R-(+)-phenethyl amine.[9] This process was highly inefficient and yielded only 28% of the available 50% of the desired isomer .

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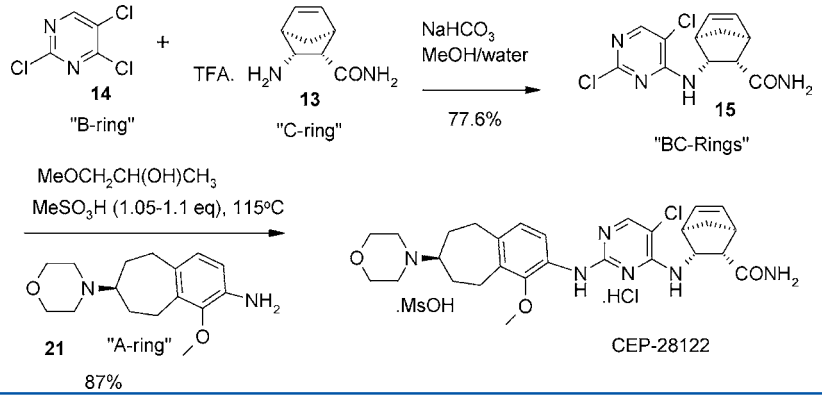
Scheme 7. Final synthesis of CEP-28122



A screen of alternative amines did not provide any suitable alternatives. Selective enzymatic hydrolysis of the β-lactam was investigated next. A screen of lipase, and β-lactamase enzymes yielded a promising hit, *Candida antarctica B.*[15] The pure enzyme, as well as several immobilized forms, was active in hydrolyzing only the undesired enantiomer to the amino acid. Optical purities of >98% ee were reproducibly obtained.

Operationally, racemic **9** was dissolved in MTBE and 75 wt % of Novozyme 435 (*C. antarctica*) resin beads was added along with 1.0 equivalent of water. The reaction was then aged at 50 °C, and could be run for up to nine days or until the hydrolysis was complete. The resin beads were removed by filtration and the filtrate, containing a mixture of the unwanted enantiomer as an amino acid and the desired enantiomer as a β-lactam, was treated with 5.0 equiv of aqueous ammonia at 35 °C for 2 h. The aqueous layer containing the salt of the undesired isomer was cleanly separated from the organic phase containing the desired product.[15d] A solvent switch to heptanes was used to precipitate **12**. Following filtration and drying, a total of 5.6 kg (48% or 98% of desired isomer) of **12** of >98% ee was obtained. A large amount of the enzyme resin was used in this process; however, we have shown in laboratory studies that the resin can be recycled without loss of activity at least six times thus reducing the overall enzyme cost significantly.

The final step in the C-ring synthesis is a straightforward removal of the BOC protecting group with 5 equiv of TFA in DCM. A solvent exchange into MTBE readily precipitates the product. A total of 4.6 kg of **13** was produced with a chemical purity of 99.1 A% and >98% ee. This simplified, efficient four-step higher-yielding process provides the C-ring compound **13** in 25% yield and is readily scalable to multikilogram quantities.

Final Coupling Reactions to yield CEP-28122. The final product is obtained by first coupling the C-ring, **13**, with the B-ring, 2,4,5-trichloropyrimidine **14**, then coupling this product with the A-ring **21** to form CEP-28122 as the mixed mono-mesylate monohydrochloride salt (Scheme 7).

No change was made to the chemistry of the coupling reaction to form the BC-ring; however, the workup was significantly simplified. In the original procedure, 70 volumes of water were used to quench the reaction which was extracted with ethyl acetate followed by concentration to dryness. We discovered that the volume efficiency could be dramatically improved. The reaction is now run in 15 volumes of 33% aqueous methanol with 3 equivalents of sodium bicarbonate at 45 °C. Once complete the temperature is raised to 65 °C and ~6 volumes of water is added to induce nucleation. After cooling to <5 °C the crystallized product is isolated through simple filtration. A total of 3.82 kg (77.6%) of **15** was obtained in 99.2 A% purity.

The final step to generate the API was initially challenging because a stable salt form was not yet known. Through a salt study it was discovered that a mixed monomesylate monohydrochloride bis salt form was extremely stable as well as compatible with the process to make the drug product. This salt was generated by combining the A-ring and BC-ring in 1-methoxy-2-propanol and heating the mixture to 115 °C. Methanesulfonic acid (1.05−1.1 equiv) was then added and the reaction aged until complete by HPLC assay. One equivalent of hydrogen chloride is generated in the reaction, the source of HCl for the salt. If methanesulfonic acid (MsOH) is present while the reaction is being brought to temperature it leads to degradation of the starting materials and if the temperature of the reaction is <90 °C coupling progress is sluggish. Typically after one-third of the MsOH has been added, the mixed salt begins to precipitate. Once the reaction is cooled, the product is isolated by filtration. However, the product must be protected humidity levels above 70% or an unstable monohydrate will form. A total of 6.8 kg (87%) of CEP-28122 mixed salt was prepared in this manner with a chemical purity of 97.9 A% and a chiral purity of 98.6 A%.

■ CONCLUSION

An efficient eight-step (longest linear), convergent, and scalable process was developed for the preparation of CEP-28122. Significant improvements in the synthesis of the two chiral fragments, the A and C rings were demonstrated. A selective nitration coupled with a one-pot enamine/transfer hydrogenation, followed by chiral chromatography was used to prepare the A-ring fragment. The C-ring fragment used an efficient enzymatic hydrolytic resolution of enantiomers that improved the yield of this fragment from 6.5% to 25%. Finally, the two fragments were brought together in a two-step coupling sequence that featured formation of a novel mixed mesylate/hydrochloride bis salt in the final API. The overall yield was improved from 4.26% to 19%. A total of 6.8 kg of material was produced for preclinical studies and phase I clinical supplies.

■ EXPERIMENTAL SECTION

HPLC spectra were collected on an Agilent 1100 series instrument using one of the following methods: Methods A and B used an Agilent Zorbax XDB C-18, 4.5 mm × 150 mm column, 1 mL/min. Method A: 0−100%−0% 0.1% TFA ACN/0.1% TFA water over 10 min, hold 3 mi, then 2 min to 0% used for IPC and assay of **1**, **16**, **17**, **18**, **19**, and **20**. Method B: 0−90% 0.1%

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TFA ACN/0.1% TFA water over 15 min, 40 °C, for IPC and assay of **7**, **8**, **9**, **11**, **12**, **13**, and **26**. Method C: Phenomenex Gemini-NX C18, 4.6 mm × 100 mm column, 1 mL/min, 40 °C, eluting with 15 mM, pH 9.0 ammonium chloride buffer/acetonitrile 95−50% 6 min, 50−35% next 6 min, 35−5% next 3 min hold 3 min then 5−95% 0.1 min hold 5 min used for IPC and assay of **13**, **14**, **15**, and **21**. Chiral Method D: Chiralpak AD-H 2.6 mm × 250 mm, 5 μm column, 1 mL/min, isocratic 7:3 heptane/EtOH, 25 min for chiral assay of **11**, **12**, **13**, and **25**. Chiral Method E: Chiralcel OJ-H, 4.6 × 250 mm, 5 μm column, 1 mL/min, isocratic 0.2% diethylamine, 15 min used for assay of **21**.

4-Chloro-2,3-dimethylanisole (16). Chlorobenzene (13.2 L) was added to aluminum chloride (127 g, 0.95 mol) followed by 2,3-dimethylanisole (13.0 kg, 95.4 mol) and was cooled to 13 °C. Sulfuryl chloride (8.11 L, 100 mol) was added, and the batch was warmed to 20 °C for 22 h. Chlorobenzene (79 L) and sodium bicarbonate (7.5% in water, 100 L) were charged sequentially at <35 °C. After stirring for 45 min, the layers were separated. The organic layer was dried over sodium sulfate (5.3 kg) for 1 h and filtered to provide a solution of **16** to be used directly in the subsequent step. LC analysis gave a 95.0% assay yield (90.6 mol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 (d, J = 8.76 Hz, 1H), 6.64 (d, J = 8.76 Hz, 1H), 3.79 (s, 3H), 2.32 (s, 3H), 2.18 (s, 3H).

4-Chloro-2,3-di(bromomethyl)anisole (17). To a solution of **16** in chlorobenzene (70.6 L of a 1 M solution, 70.6 mol) at 90 °C was added 2,2′-azobisisobutyronitrile (0.79 kg, 4.8 mol). *N*-Bromosuccinimide (32.3 kg, 181 mol) was added portion-wise over 1 h at 90−100 °C. After 1.5 h at 90 °C the batch was cooled to 25 °C, and $NaHCO_3$ (7.5% in water, 100 L) was added at <25 °C, and the batch was stirred for 45 min before separating the layers. The lower organic layer was concentrated by vacuum distillation (50−60 mmHg, 40−57 °C) to approximately 15 L, and methanol (52.7 L) was added at 40 °C resulting in the precipitation of product. A premixed solution of methanol (95.3 L) and water (36.8 L) was added over 1 h at <25 °C then stirred for 10 h at 25 °C and 3 h at −10 °C. The slurry was filtered, washed with a 1:1 methanol/water solution (30 L), and dried at 45 °C under vacuum with a low nitrogen purge to give 17.2 kg (52.4 mol) of **16** with 99.0 A% and 99.4 wt % purity (78.1%, 74.2% isolated yield from 2,3-dimethylanisole). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (d, J = 8.88 Hz, 1H), 6.82 (d, J = 8.92 Hz, 1H), 4.76 (s, 2H), 4.72 (s, 2H), 3.89 (s, 3H).

1-Chloro-4-methoxy-5,6,8,9-tetrahydro-benzocyclohepten-7-one (19). To a solution of **17** (15.9 kg, 48.4 mol), diethyl-1,3-acetonedicarboxylate (15.7 kg, 77.6 mol), and Bu_4NHSO_4 (1.65 kg, 4.86 mol) in MTBE (148 kg) was added over 40 min, at 20−27 °C, a solution of K_2CO_3 (20.2 kg) in DI H_2O (48.8 kg). After 23.5 h DI H_2O (47.6 kg) was added, and the layers were separated. The organic was washed with 20% (w/w) aqueous NaCl (32.1 kg) then concentrated to ∼30 L (120 to 154 mmHg), and the solvent was exchanged to (50.2 kg final solvent charge). A 1.5 M solution of KOH (146 kg) was added over 1 h at 20−24 °C. After stirring at 78 °C for 2 h the batch was cooled to 25 °C, stirred overnight then cooled to 0 °C ± 5 °C and held 3 h 20 min. The product was filtered, rinsed with 20% (v/v) H_2O in EtOH (70 kg), and dried (50−55 °C, 50 mm) to provide **19** as a white solid (9.43 kg, 42.1 mol, 87% yield) in 99.6 A% and 99.3 wt.% purity. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (d, J = 8.7 Hz, 1H), 6.75 (d, J = 8.7 Hz, 1H), 3.82 (s, 3H), 3.16−3.13 (m, 2H), 3.06−3.03 (m, 2H), 2.60−2.54 (m, 4H).

4-Chloro-1-methoxy-2-nitro-5,6,8,9-tetrahydro-benzocyclohepten-7-one (20). CAUTION: this reaction is exothermic with an adiabatic heat rise of 46 °C. No additional thermal events were noted up to 250 °C. A complete hazard evaluation determined that, to control the exotherm, the reaction had to start at a low temperature and be slowly warmed to initiate reaction. An inerted vessel was charged with **19** (9.0 kg, 40.1 mol) and potassium nitrate (4.250 kg, 42.0 mol). Trifluoroacetic acid (45.4 L) was added at −10 °C over 1 min and then warmed slowly to 22 °C over 3.5 h. A rapid exotherm to 36 °C was observed as the reaction went to completion. After cooling to 22 °C, DI water (135.1 L) was added at 17 °C. The tan slurry was stirred for 2 h, filtered, washed with DI water (45.0 L), and dried (RT, 50 mm) to obtain 9.96 kg of **20** with a purity of 99.1 wt % (9.87 kg corrected, 36.6 mol, 91.4% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (s, 1H), 3.90 (s, 3H), 3.22 (m, 2H), 3.13 (m, 2H), 2.65 (m, 4H).

1-Methoxy-7-morpholin-4-yl-6,7,8,9-tetrahydro-5*H*-benzocyclohepten-2-ylamine (6). To a solution of morpholine (12.10 kg, 139 mol, 10.5 equiv) in MeOH (24.8 kg, 5.5 vols) at 15 °C was added formic acid (5.40 kg, 117 mol, 8.8 equiv) over 10 min at <40 °C. This solution was added to a slurry of **20** (3.90 kg physical, 91 wt %, 3.55 kg corrected, 13.2 mol) and 10% Pd/C (4.45 kg, 125 wt %, 50% wet) in methanol (91.2 kg, 11 vols) at 65 °C over 1.5 h. After stirring at 60 °C for 1.5 h and cooling to 25 °C, the batch was filtered through Celite 545 (0.8 kg) which was washed extensively with EtOAc (30 kg). The MeOH in the filtrate was removed by vacuum (73−144 mmHg, 23−29 °C) before adding EtOAc (60.3 kg, 7.5 vols) and sodium bicarbonate solution (8% aqueous, 7.6 gal) and then separating the layers. The aqueous was extracted with 30 kg of EtOAc. The EtOAc phases were combined, dried over Na_2SO_4 (4 kg), filtered, and then concentrated to dryness in vacuo. The solids were dried under vacuum (50 mmHg, 50 °C) to an LOD of 0.73%, yielding 3.04 kg (11.27 mol, 85.4%) of **6** with 97.6 A% and 98.6 wt % purity before purification by chromatography. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.71 (d, J = 7.88 Hz, 1H), 6.51 (d, J = 7.96 Hz, 1H), 3.70 (s, 3H), 3.70 (broad s, 2H, [NH_2]), 3.69 (dd, J = 4.72, 4.72 Hz, 4H), 3.29 (dd, J = 7.27, 14.08 Hz, 1H), 2.74 (ddd, J = 1.24, 7.55, 14.49 Hz, 1H), 2.63−2.57 (m, 2H), 2.55 (dd, J = 4.64, 4.64 Hz, 4H), 2.30 (dd, J = 11.60, 14.04 Hz, 1H), 2.13−2.04 (m, 2H), 1.42−1.34 (m, 2H).

Chromatographic Purification of 1-Methoxy-(7*S*)-morpholin-4-yl-6,7,8,9-tetrahydro-5*H*-benzocyclohept-en-2-ylamine (11). A Novasep Hipersep HPLC system equipped with a 25 mm × 11 cm column packed with 20 μ CHIRALPAK AZ and eluting with 80/20/0/1 (by volume) hexanes/EtOAc/TEA at 570 mL/min was used to carry out the preparative chromatographic separation of several batches of the two enantiomers of **6**. Isolated 2.74 kg (45% of mass, 90% enantiomeric yield) of desired isomer **21**. The preparative chromatography was carried out under contract at Chiral Technologies Inc., 800 North Five Points Rd., West Chester, PA 19380.

Diastereomeric Salt Purification of 1-Methoxy-(7*S*)-morpholin-4-yl-6,7,8,9-tetrahydro-5*H*-benzocyclohept-en-2-ylamine (11). A mixture of **6** (5.00 g, 18.1 mmol. 1.0 equiv) in MeOH (50.0 mL) was heated to dissolution at 50 °C. A solution of dibenzoyl-*L*-tartaric acid (3.14 g, 8.76 mmol, 0.48 equiv, in 10 mL MeOH) was added via additional funnel and immediately seeded with enantiopure **11** (+)-DBTA salt (21 mg). A slurry formed rapidly and was heated at 55 °C 2 h, cooled to RT overnight then to <5 °C and stirred for 3 h. After filtration the white solid was washed with MeOH (12.5 mL) to yield 3.46 g (42%, 84% based on available **11**) at 94.9% de by chiral HPLC. Crude **11** DBTA salt (3.42 g) was slurried in

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MeOH (68.0 mL) at RT then rapidly heated to 80 °C. After stirring for 2 h it was cooled RT overnight, the white solid was filtered, and rinsed with MeOH (10.0 mL) to give 2.99 g (87% yield) with 99.3% de by chiral HPLC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.00−7.98 (m, 2H), 7.69−7.65 (m, 1H), 7.56−7.53 (m, 2H), 6.55 (d, J = 8.0 Hz, 1H), 6.40 (d, J = 7.9 Hz, 1H), 3.64−3.58 (m, 4H), 3.53 (s, 3H), 3.13−3.08 (m, 1H), 2.92−2.87 (m, 1H), 2.74 (m, 4 H), 2.57−2.43 (m, 3H), 2.21−2.05 (m, 3 H), 1.30−1.19 (m, 2H).

(±)-(1*S*,2*S*,5*R*,6*R*,)-3-Aza-tricyclo[4.2.1.0(2,5)]-non-7-en-one (8). To a stirred solution of 2,5-norbornadiene (8.0 kg, 86.9 mol) and DCM (41 L) at −15 °C was added over 1.5 h a solution of chlorosulfonyl isocyanate (12.5 kg, 86.9 mol) in DCM (16 L) at −15 ± 5 °C. After 0.5 h the batch was warmed to 15 °C, held for 16 h, and then cooled to <5 °C where DI water (23 L) was added over 5 min. After the mixture stirred for 45 min at <5.0 °C and warmed to 20 °C, the layers were separated. The aqueous was extracted with DCM (24 L), and the organic phases were combined, washed with brine (20 L), dried over $MgSO_4$, and filtered. Solids were rinsed with DCM (10 L), and the filtrate was concentrated to 53.05 kg (760 mm, 37 °C). This was then added over 45 min at 5 ± 5 °C to a solution of sodium sulfite (8.75 kg, 70.0 mol, 0.8 equiv), sodium bicarbonate (12.75 kg, 152 mol, 1.8 equiv), and sodium carbonate (7.55 kg, 70.0 mol, 0.8 equiv) in DI water (120 L). After stirring for 30 min at 15−20 °C the layers were separated. The aqueous was extracted with DCM (30 L), and the combined organics washed with brine (45 L), dried over $MgSO_4$, and filtered, and the solids were washed with DCM (10 L). A solvent exchange (760 mm, 37 °C) to heptane (105 L) precipitated the product which was isolated by filtration after cooling to 20 °C. The wetcake was washed [heptanes (3 × 10 L)], dried to constant weight at 50 mmHg and 45.0 °C to yield 8.89 kg (65.7 mol, 75.6%) of **8** as an off-white solid with 96.8 A%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02 (s, b, 1H), 6.23 (dd, J = 3.04, 5.64 Hz, 1H), 6.14 (dd, J = 3.24, 5.64 Hz, 1H), 5.75 (s, 1H), 3.33 (d, J = 3.88 Hz, 1H), 2.88 (dt, J = 1.44, 3.04 Hz, 1H), 2.80 (t, J = 1.16 Hz, 1H), 2.75 (s, 1H), 1.60 (d, J = 9.44 Hz, 1H), 1.48 (d, J = 9.44 Hz, 1H).

(±)-(1S, 2S, 5R, 6R)-4-Oxo-3-aza-tricyclo[4.2.1.0(2, 5)-non-7-ene-3-carboxylic acid tert-butyl ester (9). A solution of **8** (8.5 kg, 62.9 mol, 1.0 equiv) and DMAP (920 g, 7.52 mol, 0.12 equiv) in THF (68 L) was cooled to 2.0 °C, and a solution of di-*tert*-butyl dicarbonate (13.73 kg, 62.9 mol, 1.0 equiv) in THF (25 L) was added over 40 min. After warming to 20 °C and stirring for 19 h, to the mixture was then added DI water (68 L), and THF was removed by distillation (21 °C, 50−80 mmHg). The precipitated solids were filtered, washed with DI water (17 L), and dried (50 °C, 55 mmHg) to yield 12.68 kg (53.9 mol, 85.8%) of **9** with 97.2A% purity. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.3 (dd, J = 3.04, 5.60 Hz, 1H), 6.20 (dd, J = 3.24, 5.60 Hz, 1H), 3.78 (d, J = 4.52 Hz, 1H), 3.14 (t, J = 1.24 Hz, 1H), 3.07 (dt, J = 1.32, 4.48 Hz, 1H), 2.94 (s, 1H), 1.55 9d, J = 9.92 Hz, 1H), 1.45 (s, 9H), 1.43 (d, J = 9.90 Hz, 1H).

((1*R*,2*R*,3*S*,4*S*)-3-Carbamoyl-bicyclo[2.2.1]hept-5-en-2-yl)-carbamic Acid *tert*-Butyl Ester (12). To a mixture of **9** (12.5 kg, 53 mol, 1.0 equiv) and Novozyme 435 resin-immobilized *C. antarctica* lipase (8.8 kg, 75 wt %) in MTBE (112 L) was added DI water (956 g, 53 mol, 1.0 equiv); the mixture was heated to 50 ± 5 °C and held for 6 d, keeping the solids just suspended. After the batch was cooled to 25 °C, MTBE (13 L) was added, and the beads were removed by filtration and washed with MTBE (30 L). The filtrate was charged to a clean reactor through a 0.1 μ polishing filter and cooled to 16 °C. Then 28% NH_4OH (35 kg, 576 mol, 10.8 equiv) was added, and the mixture was heated to 30−34 °C. After 18 h at 30−34 °C, the mixture was cooled to 23 °C. The layers were separated, and the organic layer was washed with 10% NH_4OH (2 × 50 L). The product was crystallized through a solvent exchange of MTBE to heptane (88 L, 18 ± 3 °C, 100 mmHg) and isolated by filtration after cooling to 20 °C. The wet cake was washed with heptanes (22 L) and dried under vacuum (50 °C, 50 mmHg) to yield **12** (6.54 kg, 25.9 mol, 48%) in 98.3% ee and 94 A% chemical purity. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.47 (s, 1H), 7.03 (s, 1H), 6.39 (d, J = 8.64 Hz, 1H), 6.20 (m, 2H), 3.57 (t, J = 8.20 Hz, 1H), 2.7 (s, 1H), 2.53 (s, 1H), 2.36 (d, J = 8.28 Hz, 1H), 2.03 (d, J = 8.72 Hz, 1H), 1.37 (s, b, 10 H), 1.31 (d, J = 8.92 Hz, 1H).

((1*S*,2*S*,3*R*,4*R*)-3-Amino-bicyclo[2.2.1]hept-5-ene-2-carboxylic Acid Amide Trifluoroacetate Salt (13). To a solution of **12** (5.50 kg, 21.8 mol, 1.0 equiv) in DCM (44 L) at 13 °C was added over 1 h trifluoroacetic acid (12.5 kg, 8.1 L, 109 mol, 5.0 equiv). The batch was then warmed to 20 °C and stirred for 18.5 h. A solvent exchange to MTBE (55 L, 10 vols, 1 atm, max 56 °C) caused the product to precipitate as a white solid which was isolated by vacuum filtration after cooling to 25 °C. The wetcake was washed with MTBE (11 L) then dried to constant weight (50 mmHg, 50 °C) to yield **12** (4.63 kg, 17.4 mol, 80%) of 99 A% purity. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.94 (s, b, 3H), 7.86 (s, 1H), 7.31 (s, 1H), 6.33 (dd, J = 2.8, 5.56 Hz, 1H), 6.20 (dd, J = 3.04, 5.48 Hz, 1H), 3.08 (d, J = 7.72 Hz, 1H), 2.89 (s, b, 2H), 2.40 (d, J = 7.72 Hz, 1H), 2.13 (d, J = 9.32 Hz, 1H), 1.39 (d, J = 9.40 Hz, 1H).

(1S,2S,3R,4R)-3-(2,5-Dichloro-pyrimidin-4-ylamino)-bicyclo[2.2.1]hept-5-ene-2-carboxylic Acid Amide, BC-ring (15). To a mixture of sodium bicarbonate (4.2 kg, 50 mol, 3 equiv) in DI water (22.5) was charged **13** (4.55 kg, 17.1 mol, 1.0 equiv), methanol (45 L), and 2,4,5-trichloropyrimidine (3.1 kg, 16.9 mol, 0.99 equiv). The batch was stirred at 40 °C for 22 h and then heated to 60 °C where DI water (29.6 L) was charged over 0.5 h. The batch was cooled to 55 °C, seeded (3.7 g), and then cooled to 30 °C. After the mixture stirred 18 h at RT and cooled to 8 °C, the solids were filtered, washed with 6:7 MeOH/DI water (10 L), and dried under vacuum (50−100 mmHg, 50−55 °C) to constant weight, yielding 3.82 kg (12.8 mol, 77.6%) of **15** as a white solid with 99.2 A% purity. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.63 (d, J = 7.40 Hz, 1H), 8.21 (s, 1H), 7.89 (s, b, 1H), 7.34 (s, b, 1H), 6.34 (dd, J = 2.88, 5.60 Hz, 1H), 6.30 (dd, J = 2.96, 5.60 Hz, 1H), 3.99 (t, J = 7.20 Hz, 1H), 2.89 (s, 1H), 2.75 (s, 1H), 2.54 (s, b, 1H), 2.04 (d, J = 8.92 Hz, 1H), 1.40 (d, J = 8.96 Hz, 1H).

(1*S*,2*S*,3*R*,4*R*)-3-[5-Chloro-2-(*S*)-1-methoxy-7-morpholin-4-yl-6,7,8,9-tetrahydro-5*H*-benzocyclohepten-2-ylamino)-pyrimidin-4-ylamino]bicycle[2.2.1]hept-5-ene-2-carboxylic Acid Amide Methanesulfonic Acid Hydrochloride Salt (CEP-28122). To a solution of **15** (1.55 kg, 5.03 mol) in 1-methoxy-2-propanol (6 L) was charged **21** (1.46 kg, 5.02 mol, 1.0 equiv) in 1-methoxy-2-propanol (4 L), and the mixture was then heated to 100 °C, after which methanesulfonic acid (350 mL, 518 g, 5.4 mol) was added dropwise over 50 min at 104−109 °C. Solids were precipitated after approximately 100 mL of acid had been added. After heating the batch at 115 °C for 19 h an additional 15 mL (22.2 g, 23 mmol, 4.6%) of methanesulfonic acid was added, and then the mixture was heated 5 h. The slurry was cooled to <10 °C, and the

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product solids were isolated by filtration and washed with cold 1-methoxy-2-propanol until the filtrate was clear and colorless (12.0 L). The solids were then dried to constant weight (50 mmHg, 50 °C), yielding 2.94 kg (4.37 mol, 87.1%) of CEP-28122 monomesylate/monohydrochloride with 97.4 A% chemical purity and 97% ee. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.6 (s, b, 1H), 9.93 (s, b, 1H), 9.58 (s, b, 1H), 8.36 (s, 1H), 8.06 (s, 1H), 7.86 (d, J = 7.28 Hz, 1H), 7.48 (s, 1H), 7.09 (d, J = 8.36 Hz, 1H), 6.39 (dd, J = 2.88, 5.56 Hz, 1H), 6.23 (dd, J = 2.92, 5.52 Hz, 1H), 3.93 (m, 6H), 3.69 (s, 3H), 3.69 (s, b, 1H), 3.58 (m, 2H), 3.29 (m, b, 4H), 3.17 (m, 2H), 2.94 (m, 3H), 2.77 (t, J = 12.04 Hz, 1H), 2.53 (d, J = 8.00 Hz, 2H), 2.49 (d, b, J = 13.68 Hz, 2H), 2.34 (s, 3H), 1.96 (d, J = 8.80 Hz, 1H), 1.46 (m, b, 3H), 1.01 (d, J = 6.24 Hz, 1H). Anal. Calcd for $C_{29}H_{40}N_6O_6SCl_2$ (671.64): C, 51.86; H, 6.00; N, 12.51; Cl, 10.56. Found: C, 51.75; H, 6.07; N, 12.37; Cl, 10.57. Heavy metals <20 ppm.

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