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Process Development for a Key Synthetic Intermediate of LY2140023, a Clinical Candidate for the Treatment of Schizophrenia

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ABSTRACT: To fuel clinical development of the experimental CNS medicine LY2140023, we developed a scalable route for the multistep synthesis of a pivotal synthetic intermediate. The core of the conformationally restricted glutamic acid-based amino acid analogue was built via a Rh-catalyzed cyclopropanation of thiophene. Regioselective functionalization of the remaining double bond was achieved by a hydroboration/oxidation sequence followed by a Bucherer−Bergs reaction to give a hydantoin with the targeted L-glutamic acid configuration. Subsequent resolution, oxidation state, and protecting group manipulations gave the key intermediate in an overall nine-step scalable streamlined route starting from thiophene.

INTRODUCTION

L-Glutamic acid (**1**) is the primary excitatory amino acid neurotransmitter in the mammalian central nervous system (CNS), taking part in several physiological and pathophysiological processes (e.g., anxiety and schizophrenia). It influences neuronal transmission in part by activation of G-protein-coupled metabotropic glutamate (mGlu) receptors.[1−3] mGlu receptors are highly heterogeneous with respect to their structure, function, and localization within the central nervous system and represent promising targets for therapeutic intervention in a multiplicity of CNS disorders.[4−6]

Although altered glutamate neurotransmission has been linked to schizophrenia for decades, all commonly prescribed antipsychotics act on dopamine receptors.[7] The dense location of mGlu2/3 (group II) receptors in limbic and forebrain areas (associated with drug abuse, anxiety, and schizophrenia) suggests that these receptors may be alternative pharmacological targets for the development of new medications for these disorders.[8]

As part of a program aimed at identifying highly potent and selective agonists for mGlu2/3 receptors, a series of novel, conformationally restricted bicyclic glutamic acid-based amino acids have been investigated over the past two decades (Figure 1).[9−12]

Among these novel non-natural amino acids, bicyclic sulfone **8** (LY404039) was found to be a promising selective agonist for mGlu2/3 receptors showing antipsychotic potential both in animal studies and in humans.[12] However, **8** suffers from low human oral bioavailability. After a significant effort, methionine amide **9** (LY2140023) emerged as a suitable replacement. The amide linkage is hydrolyzed in vivo, affording the active mGlu2/3 receptor agonist **8** (Figure 2) with suitable bioavailability. Amide **9**, which is obtained from **8** by way of key intermediate amino diester **10**, has shown efficacy for the oral treatment for schizophrenia in a phase II clinical study.[12,13]

Interest in this novel antipsychotic agent prompted the development of a reliable, safe, and scalable process for the synthesis of intermediate dimethyl ester **10**. This work describes the development of processes that have now been successfully demonstrated at pilot plant scale, allowing practical multikilogram production of the API.

RESULTS AND DISCUSSION

Overview of the First-Generation Route. The initially described first-generation multistep laboratory scale synthesis of the free base of diester **10** is shown in Scheme 1.[11] The synthetic strategy was straightforward: thiophene and ethyl diazoacetate (**11**) were brought together in a rhodium(II)-catalyzed cyclopropanation[14] to fashion the bicyclo[3.1.0]hexane structural framework in one step while leaving a suitable handle for further functionalization. Hydroboration of the remaining olefin in **12** was followed by Swern oxidation,[15] affording ketone **14** as a substrate for an amino acid synthesis targeting the L-glutamate configuration. In the event, **14** was subjected to Bucherer−Bergs conditions[16] giving rise to a masked amino acid in the form of racemic hydantoin **15**, which was crystallized from a 4:1 mixture of diastereomers. Separation of enantiomers by diastereomeric salt formation and crystallization followed by salt break gave single isomer (−)-**15**. The sequence of hydantoin hydrolysis, exhaustive protection, and chromatographic purification provided sulfide **18** as a suitably soluble substrate for conversion to sulfone **19** upon treatment with *m*-CPBA in methylene chloride. Removal of the *tert*-butyl carbamate with trifluoroacetic acid followed by treatment with aqueous bicarbonate, extractive workup, and solvent

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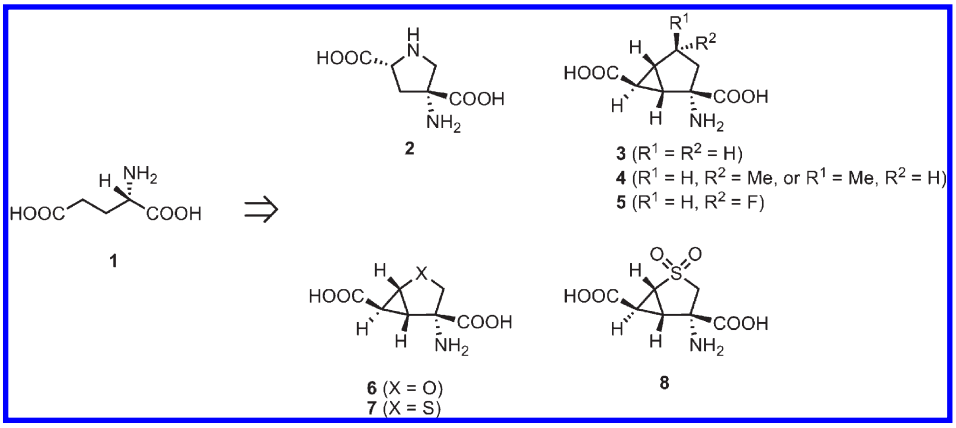


Figure 1. Structures of representative L-glutamic acid (**1**)-derived mGlu2/3 receptor agonists **2**−**8**.

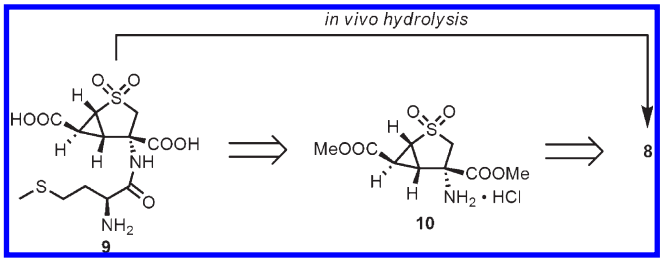


Figure 2. Relationship among LY2140023 (**9**), diester intermediate **10**, and agonist **8**.

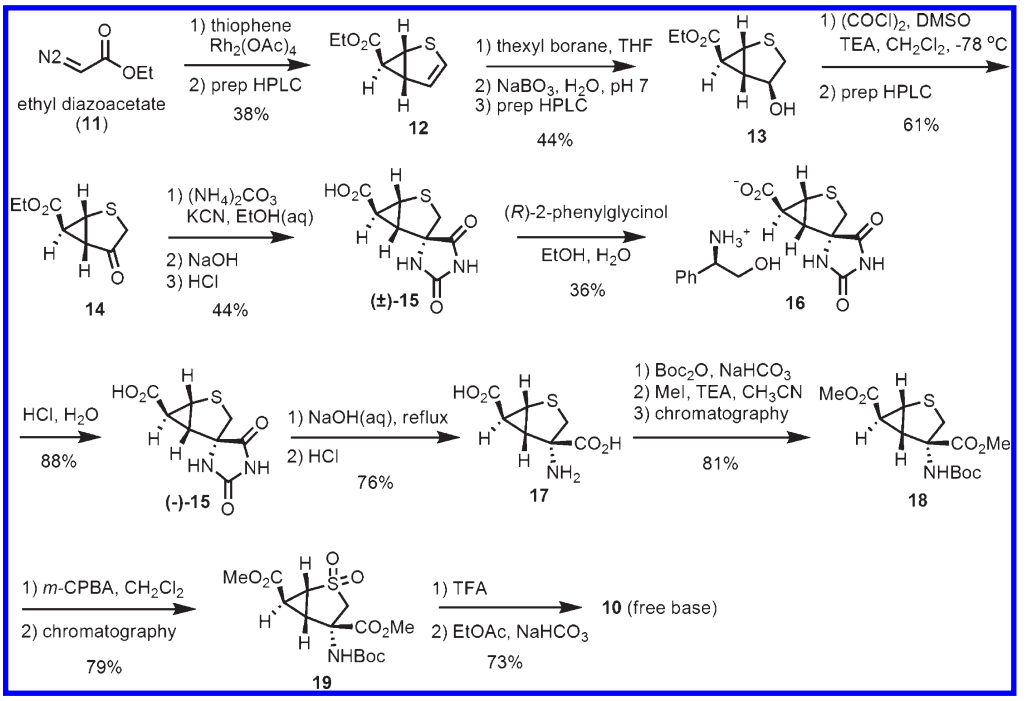
removal in vacuo gave the free base of amine **10** in approximately 12 chemical steps and 0.5% overall yield from **11**.

Transformation of the First-Generation Route into a Scalable Second-Generation Route. The first-generation synthesis, while strategically straightforward and logical, involved wasteful protection−deprotection steps together with several chromatographic purifications and low yields, making it unsuitable for multikilogram scale. Therefore, this route was developed and streamlined, resulting in a nine-step, chromatography free, higher-yielding second-generation route (Scheme 2).

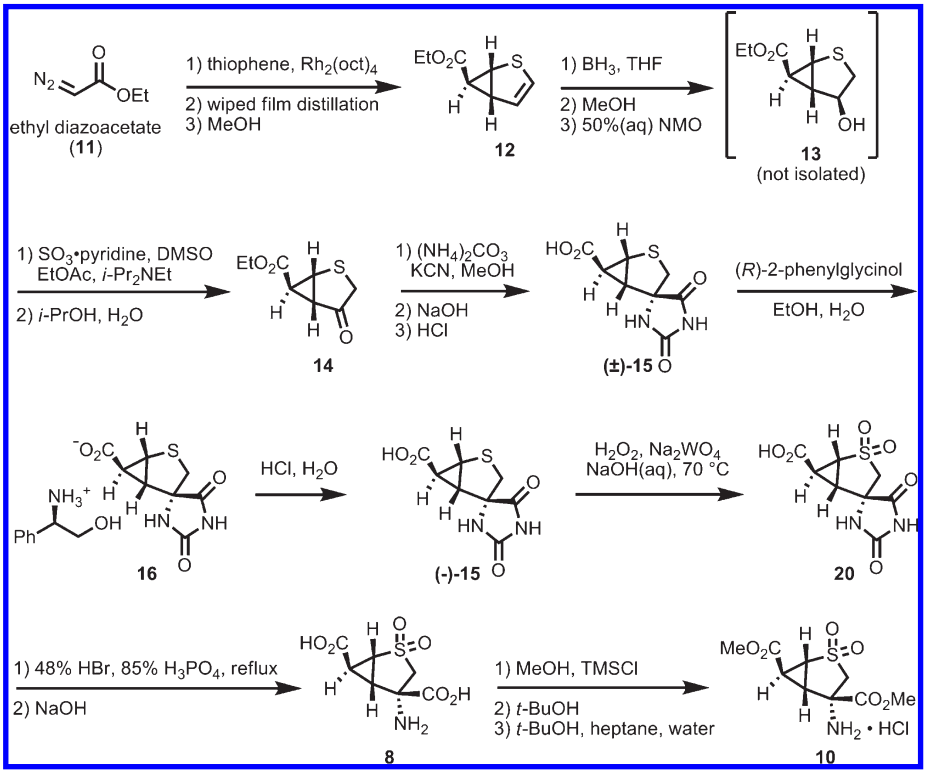
Cyclopropanation. The original rhodium(II) acetate (0.3 mol %)-catalyzed cyclopropanation of thiophene by ethyl diazoacetate suffered from a low yield after chromatographic purification. A catalyst screen was conducted in search of a more efficient transformation. We investigated less expensive copper catalysts such as $CuPF_6(CH_3CN)_4$ only to find that dimerization of ethyl diazoacetate was the predominant reaction pathway.[17] Dilution of the reaction mixture with large volumes of thiophene partially suppressed the dimerization but was not practical especially because the yield remained depressed compared to that for the rhodium(II) process. Additionally, asymmetric copper and rhodium catalyst systems were tested but provided lowered yields of racemic **12**.[17] We eventually turned to rhodium(II) octanoate as a catalyst because it was more readily available and less expensive. While its use in the cyclopropanation resulted in a yield similar to that with rhodium(II) acetate, a lower loading of 0.05 mol % could be realized. Following complete consumption of **11**, the thiophene solvent was removed by wiped film distillation; a second-pass distillation then provided cyclopropane **12** as a crude oil. Dissolution of the oil in methanol followed by cooling to low temperatures produced a slurry from which **12** was isolated in 30−35% yield and greater than 95% (w/w) purity as an off-white solid. It is noteworthy that the cyclopropanation reaction is stereoselective for the isomer having an *exo*-oriented carboethoxy substituent as shown (**12**).[14] The epimeric *endo* isomer has not been detected in either the reaction mixture or the isolated material. The process described herein has been transferred to multiple contract manufacturers whereby several metric tons of **12** have been produced to date.[18]

Hydroboration. Regioselective hydroxylation of the double bond of **12** could be achieved by a hydroboration[19] using $BH_3 \cdot THF$ in place of thexyl borane, followed by a methanol quench and subsequent oxidation to the corresponding secondary alcohol **13**. Moving away from the original procedure of using thexyl borane resulted in a simpler reaction sequence, and the workup process did not have to contend with the 2,3-dimethyl-2-butanol (thexyl alcohol) byproduct. As in the bulky thexyl borane process, the predominant byproduct is the β-epimeric alcohol ($\sim$98:2 β:α). The hydroboration required 1.3 equiv of $BH_3 \cdot$ THF to achieve full conversion in $\leq$6 h at 0 °C. Experiments with less borane resulted in longer reaction times and inferior yields, perhaps indicating that a portion of the borane is sequestered by process impurities or rendered inactive through complexation with the sulfur atom of sulfide **12**. Proton NMR analysis of the methanol-quenched hydroboration reaction mixture revealed a mixure of intermediates, making it difficult to discern if the major product is a mono- or dialkyl borane. After the methanol quench, oxidation of the methyl borinate/boronate mixture to alcohol **13** could be achieved by using a refluxing aqueous solution (50%) of *N*-methylmorpholine *N*-oxide (NMO). Aqueous NMO bears advantages over alkaline hydrogen peroxide and sodium perborate in terms of safety, byproduct profile (e.g., products of overoxidation to sulfoxide, or hydroxide-induced events such as ester hydrolysis and ring fragmentation to give **21**), and convenience (liquid charge vs solid perborate charge). The use of NMO is a direct corollary to the use of trimethylamine *N*-oxide as demonstrated by Kabalka.[20] We found trimethylamine *N*-oxide to work as well as NMO; however, the limited availability at multikilogram scale led us to select NMO for the oxidation. The oxidation did not occur with pyridine *N*-oxide at room temperature in contrast to amine *N*-oxides. Under these new conditions, alcohol **13** was obtained after extractive workup in 75−80% yield as a solution in EtOAc of sufficient purity to be used directly in the next step. Further purification of **12** was not necessary, and isolation would have been a challenge because of the low melting (mp 52 °C). It is

Scheme 1. First-Generation Synthesis of 10



Scheme 2. Second-Generation Synthesis of 10



worth noting that in experiments in which 4−5 equiv of methanol was added to quench the hydroboration mixture, NMO oxidation was usually complete within 4 h, whereas in the case of addition of a larger excess of methanol (>15 equiv), the subsequent oxidation required at least 10 h. This fact might be explained by competing coordination of methanol to the boron resulting in an increased reaction time. Lending some additional support to this hypothesis is the observation that a

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Table 1. Comparison of the Hydroboration of 12 Using 1.3 equiv of $BH_3 \cdot THF$ or $BH_3 \cdot DMS$

	T (°C)	t (h)	corrected yield (%)
$BH_3 \cdot THF$	0	2	70
$BH_3 \cdot DMS$	0	19	50
$BH_3 \cdot DMS$	5−10	6	68
$BH_3 \cdot DMS$	25	2	35

much faster rate of oxidation can be achieved (1.5 h at room temperature) by using crystalline, anhydrous NMO (Aldrich, 97%) where water is not present to compete with NMO for coordination to boron. Perhaps another explanation for the enhanced rate under anhydrous conditions results from a difference in rate of oxidation between boronate esters and boronic acids, the latter likely being the oxidation substrate in the aqueous environment at 60 °C.

Although the use of $BH_3 \cdot THF$ as the hydroboration agent for the conversion of **12** to **13** was found to be reliable on the lab and on the pilot plant scale in many settings, we sought to render the process suitable for execution in a manufacturing environment where the reagent's low autoignition temperature (90 °C) would be prohibitive because of safety policies. We therefore investigated the borane dimethyl sulfide complex ($BH_3 \cdot DMS$) as a possible substitution. Although known to be less hazardous (e.g., autoignition temperature of 206 °C) than the THF complex, it is therefore less reactive. Not surprisingly, significant differences were encountered between the two reagents as evidenced by the experimental data listed in Table 1.

As expected, when the reaction was conducted at the same temperature (0 °C), a significantly longer reaction time (19 h) was required to achieve full conversion of the starting material, resulting in a significant decrease in yield. In addition, at 25 °C full conversion was achieved for the DMS complex within 2 h, accompanied by a poor yield. The lowered yield is attributed to competitive reduction of the ethyl ester in the event of a prolonged reaction time or a higher reaction temperature. Fortunately, when the reaction was conducted at the midrange temperature of 5−10 °C, full conversion was usually achieved after 6−8 h, resulting in a comparable yield and purity as in the $BH_3 \cdot THF$ process. The quality of **13** had an important impact on the downstream steps. Using **13** with a low purity [<75% (w/w)] in the next step, oxidation (**13** → **14**), resulted in a low yield of a dark and tacky product that resulted in further reduced yields in the next steps due to poor crystallization efficiencies. Using **13** with a purity of >85% (w/w) resulted in **14**, a powdery yellow solid of high quality [>90% (w/w) in the assay] that performed well in the subsequent steps. Accordingly, the $BH_3 \cdot DMS$ process was performed successfully on the 60 kg scale, delivering **13** with a yield and quality similar to those of $BH_3 \cdot THF$ but in a safer manner.

Alcohol Oxidation. The oxidation to ketone **14** was initially achieved by a Swern oxidation (Scheme 1). However, the cryogenic temperatures and use of methylene chloride made this procedure undesireable for large scale production. Therefore, the process was changed to a Parikh−Doering oxidation[21] using $SO_3 \cdot$pyridine that could be conducted out at more practical temperatures potentially using less toxic solvents. Furthermore, unlike a Swern-type process using oxalyl chloride or trifluoroacetic anhydride as an activator, the Parikh−Doering method is amenable to conversion control through introduction of

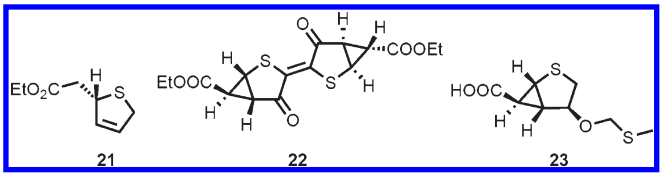

Figure 3. Some hydroboration and Parikh−Doering byproducts.

additional reagent in the event of a stall. After some brief experimentation, we settled on conducting the reaction in an EtOAc/DMSO mixture with Hünig's base and 2.5 equiv of $SO_3 \cdot$pyridine at 0 °C. The reaction was usually finished within 1−2 h, and the resulting **14** could be obtained by crystallization from aqueous or neat *i*-PrOH in ∼75% yield with a purity of >90% (w/w), which was sufficient for the subsequent steps. However, in the case of prolonged reaction times at elevated temperatures (room temperature or greater) with larger excesses of oxidant, the product began to overoxidize to dimers such as **22** (Figure 3). Additionally, the reaction was consistently plagued by the methyl thiomethyl ether byproduct **23** (∼5%) typical of alcohol oxidations using activated DMSO.[15b] With this knowledge, the hydroboration/Parikh−Doering telescope sequence was reliably conducted on a 30−120 kg scale.

Hydantoin Formation. The Bucherer−Bergs reaction to install the masked amino acid functionality was examined with the primary goal of increasing the yield without compromising safety for the inherently hazardous cyanide using step. The hydantoin formation reaction usually finished within 16 h at 30−35 °C, with care taken to maintain the headspace containing the gaseous reagents necessary for the transformation (e.g., hydrogen cyanide, ammonia, and carbon dioxide). Upon completion of the formation of hydantoin, the product mixture was comprised of ∼75% ethyl and ∼25% methyl esters because of exchange with the methanol solvent. Instead of isolation of the mixture, the esters were saponified in the same pot and subsequently crystallized when the pH was lowered to give desired isomer (±)-**15** together with 15−20% racemic diastereomer **24**. We have found that desired diastereomer (±)-**15** kinetically crystallizes out in ∼40−45% yield upon acidification of the saponification mixture, after which time (±)-**15** crystallizes along with (±)-**24** (i.e., **24** + **25**) as a 1:1 mixture. When the system is left to completely relax over 12−24 h, a yield of 65−70% for (±)-**15** was realized at a 300 gal scale. Unwanted isomers **24** and **25** are effectively removed in the next step, resolution (see below). An important safety measure was that the hydantoin forming reaction could be efficiently and reproducibly conducted with only 1 equiv of potassium cyanide, which led to reduced exposure through raw material and waste stream handling. With respect to the quality of ammonium carbonate,[22] we observed a significant influence on the yield. Using a batch of reagent with a low ammonia content (<30%, indicative of a high NH_4HCO_3 fraction), at least 3 equiv of "$(NH_4)_2CO_3$" was necessary to ensure >90% conversion of **14** to hydantoins. Using less would result in the full consumption of **14**, but only 75−80% of hydantoins were formed as a result of competing decomposition of starting material, leading to a decreased isolated yield of ∼55%. If $(NH_4)_2CO_3$ with a high content of ammonia (30−34%, indicative of a high $NH_4CO_2NH_2$ fraction) was used, only 2 equiv was necessary to ensure >90% conversion to hydantoins. As we found the ammonia content of $(NH_4)_2CO_3$ to vary from batch to batch,

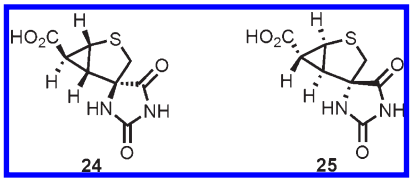

Figure 4. Diastereomeric hydantoins purged in the resolution of (±)-**15**.

approximately 3.5 equiv was often used to ensure consistent conversion to (±)-**15**.

Resolution and Salt Break. With a mixture of (±)-**15** and (±)-**24** in hand, we were gratified to find that resolution with 1.2 equiv of (*R*)-phenylglycinol in a 4:1 EtOH/H_2O mixture gave ammonium salt **16** with a high enantiomeric purity (>97:3 er for **15** vs its enantiomer) in up to 38% yield after isolation by crystallization. Furthermore, unwanted diastereomers **24** and **25** (Figure 4) were effectively removed. Subsequent acidification of an aqueous solution of **16** effected salt break with concomitant crystallization of free acid **15** in 90−95% yield on a 300 gal scale with a further upgrade in stereochemical purity to greater than 99.95:0.05 er and dr. With a high stereoisomeric purity established for hydantoin **15**, possessing four nonepimerizable stereocenters,[23] high stereoisomeric purity is ensured for downstream products.

Over the course of conducting several pilot plant campaigns utilizing the procedures described above for the sequence spanning the conversion of ketone **14** to hydantoin **15**, we generally found that the isolation of (±)-**15** via crystallization was a bottleneck on a plant scale with respect to cycle time and throughput. Slow crystallization rates were occasionally experienced as a function of decreased starting material purity. Under such circumstances, there could be substantial filtrate losses resulting in 5−10% lower than expected yields. Furthermore, the filtration and drying of (±)-**15** were often excessively time-consuming as the material frequently crystallized as a thick pastelike slurry of fine particles. To avoid the filtrate losses and eliminate long filtration and drying times, an extractive workup process was designed. After ester saponification with sodium hydroxide, the pH was lowered to 7, whereby an extraction with ethyl acetate was employed to remove organic-soluble impurities. Further lowering the aqueous layer pH to 1 and washing with tetrahydrofuran effectively extracted (±)-**15** with a <1% loss to the aqueous phase (72−74% corrected solution yield, total dr = 4:1). On the basis of solubility data, tetrahydrofuran was selected as the solvent for extraction of the racemic hydantoin acids **15** and **24**. Because of the high salt content of the reaction mixture, phase separation was not a problem. The tetrahydrofuran solution of hydantoins was then solvent exchanged into ethanol followed by dilution with H_2O, and treatment with (*R*)-phenylglycinol and triethylamine. As part of this telescope process development effort, we found that the amount of phenylglycinol could be reduced by substitution in part with an inexpensive achiral base such as triethylamine while the yield and enantiomeric purity were maintained. It was important that the total amount of base equaled the total amount of carboxylic acids present in the form of (±)-**15** and isomers **24** and **25**. Using 0.85 equiv of phenylglycinol combined with 0.35 equiv of Et_3N resulted in an isolated yield of 36−38% of **16** in the lab and on a pilot plant scale with an enantiomeric purity of >95:5 er for **15** versus its enantiomer. Execution of the overall process on a plant scale (40 kg) proceeded smoothly, achieving an average yield of 24% from **14** to (−)-**15** with improved cycle time and throughput.

Sulfide Oxidation. At the point of sulfone formation, the second-generation synthesis diverges significantly from the first-generation synthesis. The poor organic solubility of hydantoin **15** necessitated the deprotection−reprotection sequence upon going from **15** to sulfone **19** by way of amino diacid **17** and carbamate diester **18**. For instance, **15** is completely insoluble in typical organic solvents at reasonable temperatures and concentrations, whereas **18** is readily soluble in methylene chloride, for example, in which it can be efficiently oxidized with *m*-CPBA. Attempting to avoid the seemingly extra steps as well as methylene chloride and *m*-CPBA, we explored options for the direct conversion of sulfide **15** to sulfone **20**. Fortunately, the sodium salt of **15** is sufficiently soluble in an aqueous environment to allow the use of water-soluble oxidizing agents such as Oxone[24] or hydrogen peroxide in conjunction with metal catalysts (e.g., $MeReO_3$ or Na_2WO_4).[25] Ultimately, the conversion, yield, economy, and throughput for the hydrogen peroxide/tungstate system were found to be superior to those of other methods. Conducting the oxidation at 70 °C in the presence of 0.5 mol % sodium tungstate dihydrate ensured a satisfactory rate for complete conversion to sulfone with less than 0.5% intermediate sulfoxide remaining at the end of the reaction, typically 4 h after controlled addition of 35% hydrogen peroxide. An important aspect of the oxidation is pH control, which ensures that sulfur oxidation is competitive with base-induced peroxide decomposition. Using 3 equiv of 35% hydrogen peroxide with an initial reaction pH of 5−6 allowed for optimal performance without stalling. Isolated yields of 91−94% with purities of >99% (w/w) were obtained after the pH had been lowered to induce crystallization of acid **20** followed by filtration and drying. The process could be reproduced across multiple plant batches on a 45−75 kg scale. The typical level of tungsten content in the product was <300 ppm on a pilot plant scale. At such levels, further processing of **20** through the hydrolysis and crystallization of the next step ensured that the tungsten specification of 60 ppm for API **9** would be met (see below).

Hydantoin Hydrolysis. Previous experience with analogous molecules bearing the 5,5′-disubstituted hydantoin moiety has shown the hydrolysis to be a difficult transformation requiring harsh conditions whether in an alkaline or acidic environment (see below).[9] As a result, we were not optimistic that a direct conversion of hydantoin **20** to dimethyl ester **10** would be feasible; therefore, a two-step approach of hydantoin hydrolysis followed by esterification was pursued. The initial hydantoin cleavage process that we utilized for early material deliveries employed 50% sodium hydroxide at reflux. The conversion was slow, taking more than 40−50 h for acceptable starting material consumption, and the yield was only moderate at 80−85% because of the competitive production of multiple byproducts. The product could be crystallized upon lowering the pH to the range of its isoelectric point (pH 1−2). The desire for a faster reaction rate and better yield led to the exploration of alternative conditions for the hydrolysis such as strong mineral acids at reflux. The use of 48% HBr (8.9 M) as the reaction solvent gave a significantly improved yield versus that of the sodium hydroxide method. Of the acids screened, hydrobromic acid was superior in terms of rate and ease of workup. For example, there was a 2-fold rate increase over that of 9 M hydrochloric acid (18 h vs 36 h to reach completion), and the use of 9 M sulfuric acid, while

effecting a hydrolysis rate comparable to that of HBr, gave a product that was significantly contaminated with sodium bisulfate. The marked rate difference between HBr and HCl is presumably due to the higher acid concentration and temperature at reflux (8.9 and 5.5 M and 126 and 108 °C, respectively). Increasing the pH to the isoelectric point range upon completion of the hydrolysis, followed by filtration and drying, gave **8** (as its zwitterionic form) in 94−96% yields and >99% (w/w) purity at a 9−70 kg scale. The tungsten content at this stage was consistently less than 10 ppm. Interestingly, we found that the addition of 85% phosphoric acid to the hydrolysis reaction medium afforded, after pH adjustment, crystals of **8** with a rectangular shape that were more readily filtered. This is in contrast to the small, needlelike crystals of **8** that were obtained previously and which filtered poorly.

Esterification. Bis-esterification of diacid **8** was best conducted utilizing Fischer-type conditions to minimize waste and expense. The key to many such esterifications is the ability to adequately remove the water byproduct to drive the equilibrating mixture toward product formation, thereby maximizing conversion and yield. We found that typical mineral acids such as concentrated hydrochloric or sulfuric acids did not provide acceptable conversion. The use of gaseous hydrogen chloride would presumably give a higher level of conversion, but our preference was to avoid using gaseous reagents if possible because of the relative handling inconvenience. We found acceptable conversion could be achieved by refluxing **8** in methanol in the presence of trimethylsilyl chloride (3.5 equiv).[26] Typical conversions under these conditions were greater than 97% at scales from 22 to 80 kg. Over the course of the reaction, product **10** would crystallize out of solution. To achieve a higher crystallization yield, *tert*-butanol was added as an antisolvent. Apart from solubility considerations, *tert*-butanol was selected as the antisolvent because of its miscibility in the reaction system and inability to undergo ester exchange with the product. Once the resultant slurry had been cooled, filtered, and dried, dimethyl ester amine hydrochloride salt **10** was produced in 89−91% yield. The product at this stage was an unstable methanol solvate that would slowly convert to a stable hydrate form. The hydrate form performed much better downstream;[27] thus, we added a form conversion step to deliberately generate the hydrated species in a controlled manner. The form conversion consisted of reslurrying the methanol solvate in heptane followed by the addition of wet *tert*-butanol at room temperature to give the hydrated form of **10** in quantitative yield with purities of >97% (w/w). Heptane was chosen as the reslurry solvent to eliminate filtrate losses. However, the water was poorly dispersed when added directly to the heptane slurry, producing a clumpy heterogeneous product. When co-administered with *tert*-butanol, the water was well mixed throughout the slurry, giving the product as a uniform powder. Under these conditions, the conversion was facile, being limited by mass transfer according to the rate of water introduction. Because of the inability of X-ray powder diffraction to distinguish between the forms, we found that either Raman spectroscopy or ^{1}H NMR (monitoring the disappearance of methanol) could be employed to monitor the conversion. However, as the rate of conversion coincides with the rate of addition of water, monitoring the conversion is typically unnecessary.

CONCLUSION

A scalable and robust process for production of synthetic intermediate **10** was developed and successfully implemented at a pilot plant scale. The first generation process, which gave **10** in 12 steps and 0.4% yield, including multiple chromatographic purifications, was streamlined significantly by removing unnecessary steps and chromatographies. The process hazards were reduced and the space time yield was increased. The second-generation process, by which more than 1 metric ton of **10** has been afforded to date, consists of nine steps starting from thiophene and ethyl diazoacetate with a 4% overall yield.

EXPERIMENTAL SECTION

General. Monitoring of reaction progress and assay of the final compounds were conducted by HPLC and comparison to reference substances. The individual HPLC conditions can be found in the experimental procedures where applicable. Full characterization data, including HRMS data, are provided for new compounds **10** and **20**.

(±)-(1*R*,5*R*,6*S*)-2-Thiabicyclo[3.1.0]hex-3-ene-6-carboxylic Acid, Ethyl Ester (12). Caution! Ethyl diazoacetate is a toxic and explosive substance. Consult the MSDS and other safety-related information prior to handling. Follow appropriate safety precautions. A solution of ethyl diazoacetate (**11**) (300 g, 2.63 mol) in thiophene (1.6 L) was added over 8 h to a 45 °C solution of $Rh_2(oct)_4$ (0.968 g, 1.24 mmol) in thiophene (2 L). After being stirred for an additional 2−3 h, the reaction mixture was cooled to room temperature. Of the total mass (3985 g), 3240 g (81%) was concentrated by rotary evaporation to an orange-colored oil that was further distilled using a wiped film distillation apparatus at 1.5 Torr and 23 °C to remove residual thiophene prior to product distillation. The product was distilled at 120 °C and 1.3 Torr to produce 187 g (44% purity-corrected yield) of crude **12** as a yellow oil. After dissolution of a 23.5 g portion of the oil in MeOH (2 mL per 1 g of distillate) and after it had cooled to −10 °C, seed crystals were added and the resulting slurry was further cooled to −45 °C for 2−3 h. Filtration and washing with −45 °C MeOH (1 × 1 mL per gram of distillate) gave 12.6 g (31% overall yield) of **12** as an off-white solid:[28] mp 36 °C (DSC); FDMS M^+ = 170; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.09 (dd, 1H, J = 3 and 3 Hz), 1.26 (t, 3H, J = 7 Hz), 3.04 (ddd, 1H, J = 2.6, 3.3, and 7.5 Hz), 3.49 (ddd, 1H, J = 1.8, 3.0, and 7.5 Hz), 4.15 (q, 2H, J = 7 Hz), 5.88 (dd, 1H, J = 5.6 and 2.6 Hz), 6.15 (dd, 1H, J = 1.8 and 5.6 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.3, 23.9, 34.1, 39.0, 60.9, 122.9, 127.7, 174.1; FTIR (ATR) 3074 (w), 2985 (w), 1701 (s), 1553 (w), 1372 (s), 1349 (s), 1274 (s), 1219 (s), 1157 (s), 1009 (s), 796 (s) cm^{-1}. Reaction monitoring and product (**12**, retention time of ∼16 min) assay determination were conducted by HPLC analysis with UV detection at 215 nm. The instrument was equipped with an Atlantis d-C18, 3 μm, 150 mm × 4.6 mm column. The solvent system consisted of acetonitrile and water (5:95 ramped to 80:20 over 25 min) running at a flow rate of 1.5 mL/min.

(±)-(1*R*,5*S*,6*S*)-2-Thia-4-oxobicyclo[3.1.0]hexane-6-carboxylic Acid, Ethyl Ester (14) (borane−dimethylsulfide method). Neat $BH_3 \cdot DMS$[29] (71 mL, 0.77 mol) was added dropwise over 20 min to a cold (0 °C) solution of **12** (97 g, 0.57 mol) in THF (640 mL), keeping the temperature below 5 °C. The mixture was stirred for 5−6 h at 5−10 °C. After the mixture had cooled to −10 °C, MeOH (90 mL) was added dropwise over 30 min, keeping the temperature below 0 °C. After the mixture had been stirred at 0 °C for 30 min, an aqueous solution of *N*-methylmorpholine *N*-oxide [50% (w/w), 138 mL] was added over 30 min. The resulting mixture was refluxed for 4 h, cooled to room temperature, and diluted with EtOAc (1 L) and extracted with aqueous

sulfuric acid [5% (w/w), 580 mL]. The organic layer was washed with a 1:1 mixture of brine and NaOH (1 N) (2 × 1 L) and once with brine (500 mL). The organic layer was azeotropically distilled under vacuum to ensure a solution water content of <0.3% (w/w) by KF to ultimately provide ∼180 mL of a solution of **13** (∼70 g, ∼0.37 mol) in EtOAc that was directly used for the subsequent oxidation.

To the solution described above were added additional EtOAc (400 mL) and *N*,*N*-diisopropylethylamine (508 mL, 2.95 mol), and the mixture was cooled to 0 °C, followed by the addition of a solution of SO_3·pyridine [95% (w/w), 159 g, 0.95 mol] in DMSO (635 mL) over 10 min, keeping the temperature below 25 °C. After the mixture had been stirred at 0 °C for 1−2 h, aqueous HCl (3 N) was added over 10−15 min. After phase separation, the aqueous layer was re-extracted with EtOAc (430 mL), and the combined organic layers were washed sequentially with a saturated solution of $NaHCO_3$ (870 mL) and brine (575 mL). The EtOAc was replaced with *i*-PrOH by vacuum distillation to provide a solution of **14** in *i*-PrOH (180 mL). After cooling to 0 °C, the resulting yellow suspension was stirred for 10 h. After the mixture had been filtered, washed with cold *i*-PrOH (80 mL), and dried in vacuo, **14** was obtained as a yellow solid in 50% yield (59 g, 90% potency, 0.285 mol): mp 56−57 °C; FDMS M^+ = 186; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.27 (t, 3H, J = 7 Hz), 2.34 (dd, 1H, J = 3 and 3 Hz), 2.58−2.60 (m, 1H), 3.19−3.23 (m, 1H), 3.30 (d, 2H, J = 2 Hz), 4.16 (q, 2H, J = 7 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.2, 29.3, 30.8, 34.1, 34.9, 61.7, 168.8, 206.4; FTIR (ATR) 2991 (w), 1713 (s), 1402 (m), 1379 (m), 1268 (m), 1182 (s), 1005 (m), 830 (m) cm^{-1}. Reaction monitoring and product (**14**, retention time of ∼2.9 min) assay determination were conducted by HPLC analysis with UV detection at 215 nm. The instrument was equipped with an Atlantis d-C18, 3 μm, 50 mm × 4.6 mm column. The solvent system consisted of acetonitrile and water (20:80, isocratic) running at a flow rate of 2 mL/min.

(±)-(1*R*,5*S*,6*S*)-2-Thia-4-hydroxybicyclo[3.1.0]hexane-6-carboxylic Acid, Ethyl Ester (13) (borane−tetrahydrofuran method). To a −10 °C solution of **12** (35 kg, 206 mol) in 125 L of THF was added BH_3·THF (1 M in THF, 267 L, 267 mol) over 75 min at <0 °C.[29] After being stirred at −5 °C for 5−6 h, the mixture was cooled to −10 °C and treated with methanol (23 kg, 720 mol) over 1 h at <0 °C. After the mixture had been stirred at −5 °C for 30 min, 50% aqueous *N*-methylmorpholine *N*-oxide (58 kg, 247 mol) was added over 10−15 min, heated to 65 °C, and stirred for 5−6 h. The mixture was cooled to 10−15 °C, diluted with EtOAc (320 L), and extracted with 0.5 N HCl (1 × 350 L). The organic layer was washed with a 1:1 brine/1 N NaOH mixture (2 × 350 L) followed by brine (1 × 175 L). The product-containing organic layer was azeotropically distilled under vacuum to a target KF of 0.3%. The resulting ethyl acetate solution was forward processed as outlined for the borane−dimethylsulfide procedure described above. Reaction monitoring (product **13** retention time of ∼1.3 min) were performed by HPLC with UV detection at 215 nm. The instrument was equipped with an Atlantis d-C18, 3 μm, 50 mm × 4.6 mm column. The solvent system consisted of acetonitrile, trifluoroacetic acid, and water (20:80:0.05, isocratic) running at a flow rate of 2 mL/min. Product (**13**, retention time of ∼8.2 min) solution assay determination was conducted by HPLC analysis with UV detection at 215 nm. The instrument was equipped with an Atlantis d-C18, 3 μm, 150 mm × 4.6 mm column. The solvent system consisted of acetonitrile with 0.05% trifluoroacetic acid and water with 0.05% trifluoroacetic acid (5:95 ramped to 80:20 over 25 min) running at a flow rate of 1.5 mL/min.

(−)-(1′*R*,4*S*,5′*S*,6′*S*)-2,5-Dioxospiro[imidazolidine-4,4′-[2]thiabicyclo[3.1.0]hexane]-6′-carboxylic Acid [(−)-15] [telescope from 14 to (−)-15 with extractive isolation of (±)-15]. Caution! Potassium cyanide and hydrogen cyanide are extremely toxic substances. Consult the MSDS and other safety-related information prior to handling. Follow appropriate safety precautions. A mixture of $(NH_4)_2CO_3$ (30.7 g, 0.32 mol) and KCN (6.1 g, 0.093 mol) in 119 mL of methanol was stirred at room temperature for 75 min, at which time a solution of **14** (17.4 g, 0.093 mol) in 119 mL of methanol was introduced. The reaction vessel was closed, and the mixture was stirred at 30−35 °C for 16 h. After addition of H_2O (50 mL), the mixture was concentrated by vacuum distillation to 50 mL followed by the addition of aqueous NaOH [20% (w/w), 53 mL] and stirred for 2 h (25 °C). Concentrated HCl (16 mL) was added to adjust the pH to 7−8, followed by an extraction with EtOAc (55 mL). The aqueous phase was further acidified to pH 1 upon addition of concentrated HCl (16 mL). The resulting slurry was twice extracted with THF (100 mL + 50 mL) to give a solution of (±)-**15** (∼15 g, ∼0.066 mol, 70−74% yield) in THF that was directly used for the resolution. This solution was concentrated by vacuum distillation to ∼30 mL; EtOH (150 mL) was added and the mixture again vacuum distilled to ∼30 mL. After addition of EtOH (120 mL) and enough H_2O (10 mL in this case) to bring the total water content to 15 wt %, (*R*)-phenylglycinol (7.7 g, 0.056 mol) and triethylamine (3.2 mL, 0.023 mol) were added. The mixture was refluxed for 1 h and cooled to 0 °C over 9 h and stirred at 0 °C for an additional 5 h. After the sample had been filtered, washed with a cold EtOH/H_2O mixture (4:1), and dried in vacuo, **16** was isolated as a gray to brown solid (∼9 g, ∼0.025 mol) in ∼38% yield (for the resolution step).

This solid was then suspended in H_2O (54 mL), heated to 70−75 °C, and acidified with concentrated HCl (9 mL, target pH of <1). The resulting suspension was cooled to 0 °C and stirred for 3 h. After the mixture had been filtered, washed with cold H_2O, and dried in vacuo, (−)-**15** was obtained as an off-white solid (5.1 g, 0.022 mol) in 24% yield (based on **14**) and >99% ee and >99% de: $[\alpha]^{25}_D$ −202 (c = 1.0, MeOH); mp 330 °C (DSC); FDMS M^+ = 228; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.01 (t, 1H, J = 2 Hz), 2.29 (dd, 1H, J = 3 and 8 Hz), 2.69 (d, 1H, J = 12 Hz), 2.89 (dd, 1H, J = 3 and 8 Hz), 3.15 (d, 1H, J = 12 Hz), 8.10 (br s, 1H), 10.75 (br s, 1H), 12.52 (br s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 24.6, 29.7, 34.2, 34.9, 70.7, 156.0, 171.6, 175.0; FTIR (ATR) 3211 (m), 3079 (w), 1785 (w), 1727 (s), 1713 (s), 1702 (s), 1430 (m), 1397 (m), 1244 (m), 1179 (m), 1124 (m), 1022 (m), 882 (m), 731 (s), 715 (s), 631 (s) cm^{-1}. See the HPLC method information in the nontelescoped, individual procedures below.

(±)-(1′*R*,4*S*,5′*S*,6′*S*)-2,5-Dioxospiro[imidazolidine-4,4′-[2]-thiabicyclo[3.1.0]hexane]-6′-carboxylic Acid [(±)-15] [isolation of (±)-15 by crystallization]. Caution! Potassium cyanide and hydrogen cyanide are extremely toxic substances. Consult the MSDS and other safety-related information prior to handling. Follow appropriate safety precautions. A mixture of $(NH_4)_2CO_3$ (10.34 g, 107.6 mmol) and KCN (3.51 g, 53.9 mmol) in methanol (150 mL) was stirred at room temperature for 60−90 min in a closed vessel. Ketone **14** (10.0 g, 53.7 mmol) was charged in one portion as a solid and the flask resealed. The reaction mixture was allowed to stir at 30−32 °C for 20−24 h.

The methanol volume was reduced to approximately 20 mL by vacuum distillation, at which time 2.75 N NaOH (55 mL) was charged followed by stirring at 20−30 °C for 1−2 h. Treatment with water (55 mL) followed by adjustment of the pH from ∼13 to 2.0 using dropwise addition of concentrated (12 N) HCl (∼20 mL) and seeding with (±)-**15** resulted in product crystallization. The slurry was allowed to stir at room temperature for 1−2 h and then at 0−3 °C for 12−36 h. The product was isolated by suction filtration, rinsed with cold (0−3 °C) water (2 × 10 mL), and dried in a vacuum oven at 40−45 °C to afford 10.69 g of a solid consisting of a 4.6:1 ratio of (±)-**15** to (±)-**24**. The purity-corrected yield was 69% for (±)-**15**. Reaction monitoring and product (**12**, retention time of ∼8.6 min; **24**, retention time of ∼11 min) assay determination were conducted by HPLC analysis with UV detection at 210 nm. The instrument was equipped with an Atlantis d-C18, 3 μm, 150 mm × 4.6 mm column. The solvent system consisted of acetonitrile and water with 0.1% phosphoric acid (5:95 ramped to 45:55 over 30 min) running at a flow rate of 1.0 mL/min.

(−)-(1′*R*,4*S*,5′*S*,6′*S*)-2,5-Dioxospiro[imidazolidine-4,4′-[2]-thiabicyclo[3.1.0]hexane]-6′-carboxylic Acid [(−)-15] [resolution of (±)-15 that had been isolated by crystallization]. To a 4.3:1 diastereomeric mixture of hydantoins (±)-**15** and (±)-**24** (39.3 kg, 172 mol) along with 24.4 kg (178 mol) of (*R*)-phenylglycinol were added 540 L of ethanol and 135 L of water. The slurry was heated to 78−82 °C with stirring until complete dissolution was achieved. The mixture was allowed to cool to 0 °C over 7 h and held for 3 h. The slurry was filtered, washed with a 4:1 ethanol/water mixture (1 × 137 L), and blown with nitrogen for 4 h. Analysis of the wet cake revealed the presence of 13 wt % ethanol, 6 wt % water, 1.2 area % (+)-**15**, and 1.9 area % diastereomers **24** and **25** combined. The filter cake was dissolved with 137 L of water with stirring at 65−70 °C and then transferred and rinsed (20 L) into a glass-lined tank and cooled to 50−55 °C. Treatment with 32% HCl to pH ∼0 produced a slurry that was cooled to 0 °C over 2 h, and then stirred for 2 h, filtered, washed with 0 °C water (2 × 25 L), and dried in vacuo at 60 °C to give 13.2 kg of (−)-**15** (38% corrected yield from **14**) as a brown solid with high isomeric purity (>99.9:0.1 er and dr by HPLC area %). Product [(−)-**15**, retention time of ∼8.2 min; (+)-**15**, retention time of ∼5.0 min] enantiomeric purities were determined by HPLC analysis with UV detection at 215 nm. The instrument was equipped with a Chiralpak IA, 5 μm, 250 mm × 4.6 mm column. The solvent system was a hexane/ethanol/trifluoroacetic acid mixture [60:40:0.1 (v/v), isocratic] running at a flow rate of 1.0 mL/min.

(−)-(1′*R*,4*S*,5′*S*,6′*S*)-2,5-Dioxospiro[imidazolidine-4,4′-[2]-thiabicyclo[3.1.0]hexane]-6′-carboxylic Acid, 2′,2′-Dioxide (20). To a mixture of water (64.2 L) and 50% NaOH (13.4 kg) at 20−30 °C was added 0.32 kg of $Na_2WO_4 \cdot 2H_2O$ (0.97 mol) followed by 43 kg (189 mol) of (−)-**15**. The mixture (pH 5.1) was heated to 65−75 °C, at which time 35% H_2O_2 (55.7 kg, 573 mol) was added over 4 h to maintain the temperature range target. After being stirred at 70 °C for 4 h, the mixture was slowly acidified via addition of 32% HCl to a target pH of 0−1. The resultant slurry was cooled to 0 °C over 1 h and then stirred for 1 h, filtered, and washed with 20 °C *i*-PrOH (2 × 110 L). The wet cake was dried in vacuo at 50 °C to give 44.9 kg (91%) of **20** as an off-white solid: $[\alpha]^{25}_{D}$ −49 (*c* = 1.19, 1 N NaOH); mp 353 °C (DSC); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 2.39 (t, 1H, *J* = 4 Hz), 2.80 (dd, 1H, *J* = 7 and 4 Hz), 3.03 (d, 1H, *J* = 16 Hz), 3.74 (dd, 1H, *J* = 7 and 4 Hz), 3.85 (d, 1H, *J* = 15 Hz), 8.13 (s, 1H), 10.99 (s, 1H), 13.15 (s, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 21.9, 31.7, 44.2, 52.6, 62.7, 156.4, 169.9, 174.4; FTIR (KBr) 3317 (s), 3250 (s), 3211 (s), 3086 (w), 1791 (s), 1742 (s), 1713 (s), 1327 (s), 1192 (s), 1140 (s) cm^{-1}; HRMS *m*/*z* found 261.0172 $(M + H)^+$, $C_8H_9N_2O_6S$ requires 261.0176. Reaction monitoring and product (**20**, retention time of ∼6.1 min) assay determination were conducted by HPLC analysis with UV detection at 215 nm. The instrument was equipped with a Zorbax SB-C8, 3.5 μm, 150 mm × 4.6 mm column. The solvent system consisted of 5% (v) acetonitrile and 95% (v) 20 mM aqueous phosphate buffer containing 16.5 mM tetrabutylammonium chloride (pH 3) running at a flow rate of 1.5 mL/min (isocratic).

(−)-(1*R*,4*S*,5*S*,6*S*)-4-Amino-2-thiabicyclo[3.1.0]hexane-4,6-dicarboxylic Acid, 2,2-Dioxide (8). To aqueous 48% HBr (192.4 kg) and aqueous 85% H_3PO_4 (34.0 kg) was added 43 kg (165 mol) of **20**. The mixture was heated to 130 °C and allowed to stir for 25 h. After the mixture had cooled to 80 °C, H_2O (137 L) was added followed slowly by 90−95 L of 10 N NaOH. After the mixture had cooled further to 25 °C, the pH (1.7) was within the target range of 1.4−1.8 and did not require further adjustment. The slurry was cooled to 2 °C and allowed to stir for 4 h. Filtration, washing with 0 °C water (2 × 135 L), washing with 0 °C methanol (1 × 270 L), and vacuum drying at 65 °C gave 37 kg (94%) of **8** as an off-white solid: $[\alpha]^{20}_{D}$ −18 (*c* = 1.02, 1 N HCl); $[\alpha]^{20}_{D}$ −76 (*c* = 1.52, 1 N NaOH); mp 308 °C (DSC); FDMS $M^+ + 1 = 236$; ^{1}H NMR (D_2O, KOD) δ 2.22 (m, 1H), 2.63−2.66 (m, 1H) 3.08 (d, 1H, *J* = 14.9 Hz), 3.32−3.40 (m, 1H), 3.66 (d, 1H, *J* = 14.9 Hz); ^{13}C NMR (D_2O, KOD) δ 24.7, 32.0, 43.0, 54.6, 60.49, 174.2, 174.3; FTIR (KBr) 3439 (m), 3068 (m), 2995 (m), 2944 (m), 1709 (s), 1653 (m), 1542 (m), 1324 (s), 1248 (s), 1192 (s), 843 (m) cm^{-1}. Reaction monitoring and product (**8**, retention time of ∼2.7 min) assay determination were conducted by HPLC analysis with UV detection at 215 nm. The instrument was equipped with a Zorbax SB-C8, 3.5 μm, 150 mm × 4.6 mm column. The solvent system consisted of 5% (v) acetonitrile and 95% (v) 20 mM aqueous phosphate buffer containing 16.5 mM tetrabutylammonium chloride (pH 3) running at a flow rate of 1.5 mL/min (isocratic).

(−)-(1*R*,4*S*,5*S*,6*S*)-4-Amino-2-thiabicyclo[3.1.0]hexane-4,6-dicarboxylic Acid, Dimethyl Ester, 2,2-Dioxide, Hydrochloride (10). A slurry of **8** (18.2 kg, 98.8 wt % via HPLC assay, 76.4 mol) in MeOH (63 L) was heated to 48−53 °C followed by the addition of TMSCl (30.0 kg, 276 mol). The biphasic mixture was warmed to 55−60 °C and allowed to stir for 1−2 h, at which time the mixture was seeded with hydrate **10** (∼0.4 wt %) to induce product crystallization if spontaneous nucleation had not already occurred. After the mixture had been stirred for a total of 21 h, *t*-BuOH (63 L, warmed to 45−55 °C) was added over 30 min. The resultant slurry was cooled to 28 °C over 1 h and allowed to stir for an additional 3.5 h. The product was isolated by pressure filtration and washed with *t*-BuOH (1 × 90−95 L) and then dried at 50 °C to give the methanol solvate of **10**, which was directly converted to the hydrate form of **10** within the agitated filter dryer. Thusly, the dried filter cake was suspended in heptane (126 L) by being stirred at 25 °C and then was treated over 110 min with a solution of aqueous *t*-BuOH prepared from 23.8 kg of *t*-BuOH and 1.6 kg of water. After the mixture had been stirred at 25 °C for 75 min, pressure filtered, washed with heptane (1 × 30−35 L), and dried at 50 °C, **10** was isolated as a white solid in 91% yield (22.2 kg, 93.9 wt % HPLC assay, 69.6 mol): $[\alpha]^{25}_{D}$ −22 (*c* = 1.00, MeOH); $[\alpha]^{25}_{D}$ −21 (*c* = 1.01, H_2O); mp 194 °C (DSC); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.96 (dd, 1H, *J* = 7.2

and 4.2 Hz), 3.35 (t, 1H, J = 4.0 Hz), 3.70 (s, 3H), 3.79 (d, 1H, J = 15.2 Hz), 3.83 (s, 3H), 3.95−4.02 (m, 2H), 9.52 (br s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 167.8, 167.1, 58.2, 54.0, 52.7, 51.1, 42.7, 29.2, 21.0; FTIR (ATR) 3559 (w), 3182 (w), 3012 (w), 1753 (m), 1730 (s), 1649 (w), 1581 (w), 1324 (s), 1301 (s), 1210 (s), 1174 (s), 1119 (s), 767 (s) cm^{-1}; FIA-APCI-MS (positive ion mode) m/z (M + H) 264 (free base); water content 3.2% by KF; chloride content 11.4% by potentiometric titration ($AgNO_3$); HRMS m/z found 264.0533 $(M + H)^+$, $C_9H_{14}NO_6S$ (free base) requires 264.0536. Reaction monitoring and product (**10**, retention time of ∼6.6 min) assay determination were conducted by HPLC analysis with UV detection at 210 nm. The instrument was equipped with a Gemini C-18, 3 μm, 150 mm × 4.6 mm column. The solvent system was a 5:95 (v/v) acetonitrile/aqueous buffer [22 mM NaH_2PO_4 containing 10 mM tetrabutylammonium chloride (pH to 7.6 with NaOH)] mixture running at a flow rate of 1.0 mL/min (isocratic).

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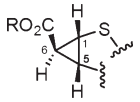
(22) Commercially available $(NH_4)_2CO_3$ is a mixture of NH_4HCO_3 and $NH_4CO_2NH_2$ in varying ratios. The quality in terms of ammonia content was determined according to the USP NF monograph for ammonium carbonate.

(23) In theory, C-6 would appear to be an epimerizable stereocenter because of the attachment of an "acidic" proton. However, several attempts to incorporate deuterium at C-6 of **12**, **13**, and **15** via base-induced enolization followed by quenching with D_2O were uniformly met with failure; the proteo starting material was recovered unchanged or suffered decomposition. From these results, we consider C-6 to be nonepimerizable.



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(27) For instance, the methanol solvate immediately became a semisolid, sticky mass upon exposure to the THF solvent used in the next step. The hydrate gave a free-flowing slurry by contrast.

(28) Refrigerated storage of **12** in protective packaging to exclude moisture and oxygen is recommended because of the low melting point and degradation potential.

(29) For an illustrative overview of the handling of boranes at scale, see: Atkins, W. J.; Burkhardt, E. R.; Matos, K. *Org. Process Res. Dev.* **2006**, *10*, 1292.