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Development of a Multigram Asymmetric Synthesis of 2-(*R*)-2-(4,7,10-Tris *tert*-Butylcarboxymethyl-1,4,7,10-tetraazacyclododec-1-yl)-pentanedioic Acid, 1-*tert*-Butyl Ester, (*R*)-*tert*-Bu_4-DOTAGA[1]

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Abstract:

A process for the multigram asymmetric synthesis of the chiral tetraazamacrocycle 2-(*R*)-2-(4,7,10-tris *tert*-butylcarboxymethyl-1,4,7,10-tetraazacyclododec-1-yl)-pentanedioic acid, 1-*tert*-butyl ester ((*R*)-*tert*-Bu_4-DOTAGA, 4) has been devised and demonstrated. The nine-step synthesis features an improved synthesis of 2-(*S*)-5-oxotetrahydrofuran-2-carboxylic acid, *tert*-butyl ester 8, the precursor to the novel alkylating agent (*S*)-5-benzyl 1-*tert*-butyl 2-(methylsulfonyloxy)pentanedioate 12, which was used to introduce an orthogonally protected chiral glutarate arm to the 1,4,7,10-tetraazacyclododecane (cyclen) nucleus in high optical purity. Cyclen derivative (*R*)-*t*-Bu_4-DOTAGA, 4, a key intermediate for the manufacture of a magnetic resonance imaging (MRI) candidate, was produced with high chemical (≥95%) and optical (ee ≥ 97%) purity. The process developed was successfully applied to the kilogram-scale cGMP synthesis of (*R*)-*t*-Bu_4-DOTAGA.

Introduction

Macrocyclic chelates of Gd(III) provide an advantage over open-chain chelates in that the macrocycle enhances the kinetic inertness and thermodynamic stability of the coordination complex.[2] There are several approved, commercially available MRI contrast agents based on tetraazamacrocyclic ligands (Figure 1): Gd-DOTA (Dotarem), **1**, Gd-HPDO3A (ProHance), **2**, and Gd-DO3AB (Gadovist), **3**. These commercial contrast agents exemplify the utility of tetraazamacrocycles, specifically cyclen (1,4,7,10-tetraazacyclododecane), in their application to MRI. In our continuing efforts to improve MRI technology, we have used ligand **4**, bearing a free carboxylate side chain as a handle for conjugation, as a precursor to more highly functionalized gadolinium chelate diagnostic agents.

Results and Discussion

Discovery Synthesis of 4. The initial synthesis of optically enriched **4** was based on a preparation described in the literature (Scheme 1).[3] A sample of **4** prepared using this synthetic route was converted to its (*R*)-(+)-α-methylbenzylamide **5** for stereochemical analysis by proton NMR. The proton NMR spectrum of this material showed that **5** prepared via this route was a 2:1 (*R*,*R*):(*R*,*S*) mixture of diastereomers, indicating that partial racemization of the alkylating agent or racemization during the alkylation reaction had occurred. It was clear that a key intermediate, such as **4**, would need to be prepared in a more optically homogeneous form for use in the manufacture of a drug candidate. Additionally, the single literature reference describing the synthesis of **4** reported its preparation on a scale of only 500 mg, without an evaluation of the optical purity of key intermediates or of the product. In order to support our MRI diagnostic development program, it was necessary to devise a synthetic route, which would produce **4** in multigram and kilogram quantities, having greatly improved enantiomeric excess (ee) and operational efficiency.

The basis for a more stereoselective asymmetric synthesis of **4** was found in the literature.[4] At Bristol-Myers-Squibb, Kang and co-workers reported the use of the triflate ester of benzyl-(L)-lactate to prepare DO3MA (2,2′,2″-(1,4,7,10-tetraazacyclododecane-1,4,7-triyl)tris-propanoic acid) by alkylation of a cyclen derivative. It was found that, whereas alkylation of *N*-formyl cyclen with 2-chloropropionic acid required temperatures of 85−90 °C and a 12 h reaction time in aqueous base, use of benzyl (L)-2-trifyloxy propionate alkylated this cyclen derivative at ambient temperature, in the presence of diisopropylethylamine, within 2.5 h (Scheme 2). The enhanced reactivity of the triflate relative to the chloride derivative allowed the reaction to proceed under far milder conditions, with the expectation that the possibility of racemization would be minimal.

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(1) During the preparation of this manuscript, a patent was submitted and issued covering the chemistry described in this publication: (a) Amedio, J. C.; Caravan, P. D.; Jacques, V.; Zhou, K. L.; Levy, S.; Kalogeropoulos, S.; Greenfield, M. WO2005/001415 (AN 2005 14659), 2005.

(2) Jacques, V.; Desreux, J. F. Synthesis of MRI Contrast Agents II. Macrocyclic Ligands. In *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*; Merbach, A. E., Toth, E., Eds.; John Wiley and Sons: New York, NY, 2001; pp 157−191.

(3) (a) Eisenweiner, K.; Powell, P.; Mäcke, H. R. *Bioorg. Med. Chem. Lett.* **2000**, 2133. (b) Mäcke, H. R.; Eisenweiner, K.; Powell, P. Patent WO 02/024235 A3 (AN 2002 240598), 2002.

(4) Kang, S. I.; Ranganathan, R. S.; Emswiler, J. E.; Kumar, K.; Gougoutas, J. Z.; Malley, M. F.; Tweedle, M. F. *Inorg. Chem.* **1993**, *32*, 2912.

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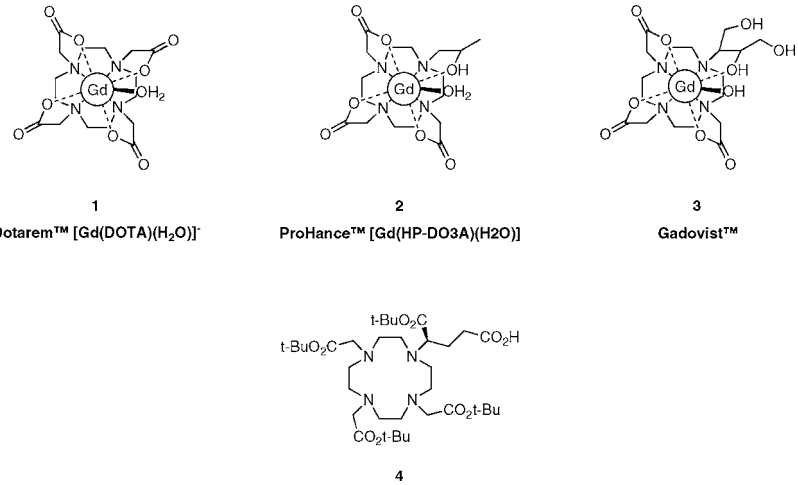
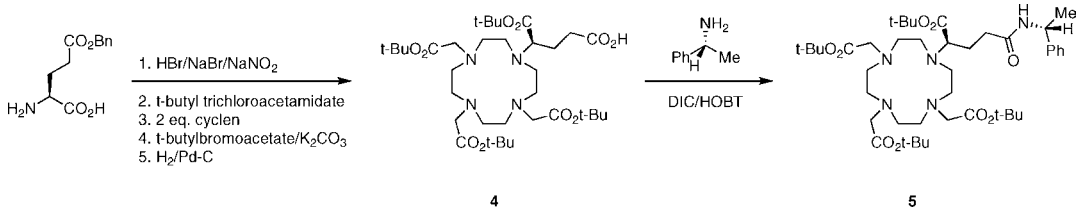
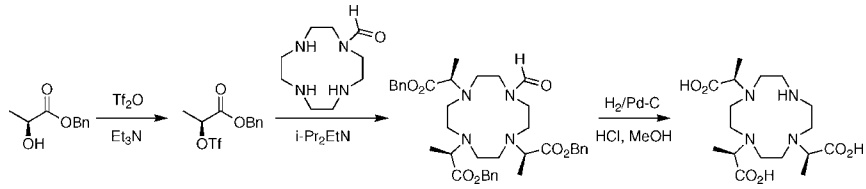


Figure 1. **Approved, commercially available MRI contrast agents based on 1,4,7,10-tetraazacyclododecane ligands, 1−3, and structure of protected tetraazamacrocyclic ligand 4.**

Scheme 1. **Initial discovery synthesis of ((*R*)-DOTAGA), 4, using a literature procedure and conversion to α-methylbenzylamide 5**



Scheme 2. **Synthesis of DO3MA as described by Kang and co-workers**[4]



Based on the above reasoning, it was expected that alkylation of cyclen with the triflate ester derived from (2)-(*S*)-1-*tert*-butyl-2-hydroxy-5-benzyl glutarate **9**, **(**Scheme 3) would result in **4** with significantly increased optical purity, as compared with **4** prepared using the literature route. The improved asymmetric synthesis of **4** is depicted in Scheme 3. The commercially available (*S*)-5-oxotetrahydrofuran-2-carboxylic acid **6** was converted to its acid chloride **7** using Vilsmeier reagent generated *in situ* from oxalyl chloride and catalytic DMF. Treatment of **7** with *tert*-butanol and 2,6-lutidine[5] gave the crude ester, **8**, which was purified by gradient elution from a silica plug (86% yield). The lactone was then hydrolyzed using one equivalent of 1 N potassium hydroxide, and the resulting carboxylate salt was alkylated with benzyl bromide in DMF,[6] giving the crude orthogonally protected diester, which was purified using column chromatography to give **9** in 82% yield. The triflate ester[7] of **9** was then generated *in situ*, at −40 °C, and added to a cooled solution of cyclen in dichloromethane, giving crude **10** after aqueous workup. Previous results indicated that the triflate was not sufficiently stable to be isolated. Fully alkylated cyclen **11** was then prepared (48% yield for three steps) by reacting crude **10** with *tert*-butyl bromoacetate. Hydrogenation to remove the benzyl protecting group gave **4** in 95% yield. A sample of **4** prepared via this route was converted to **5**, which was analyzed using proton NMR.

(5) Schmidt, U.; Braun, C.; Sutoris, H. *Synthesis* **1996**, 223.

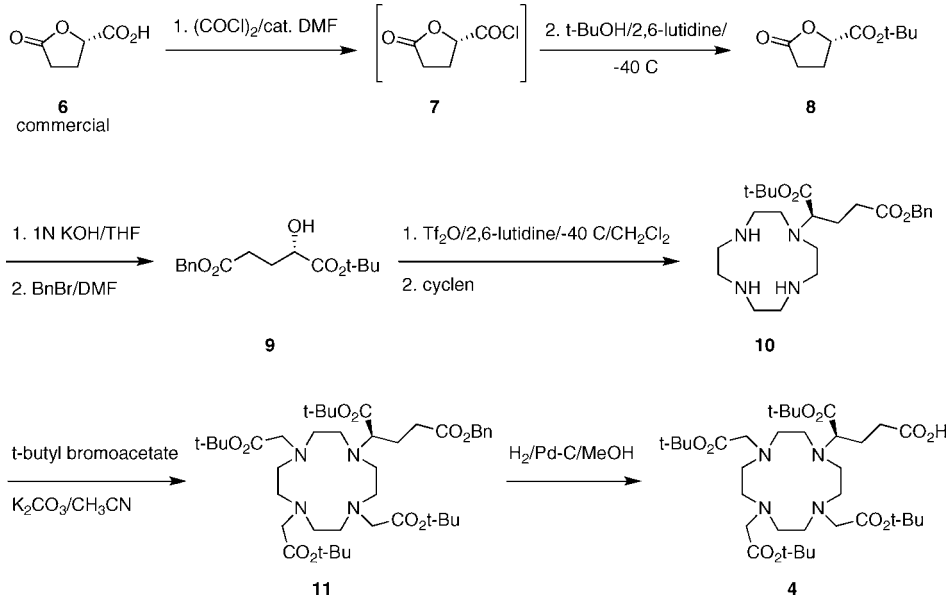
(6) (a) Lactone hydrolysis: Shin, I.; Lee, M.; Lee, M.; Jung, M.; Lee, W.; Yoon, J. *J. Org. Chem*, **2000**, *65*, 7667. (b) Benzyl bromide alkylation: Hoffmann, M.; Wasielewski, C. *Roczniki Chem.* **1975**, *49*, 151.

(7) The motivation for the generation and use of the triflate ester of **11** was based on results reported in ref 2.

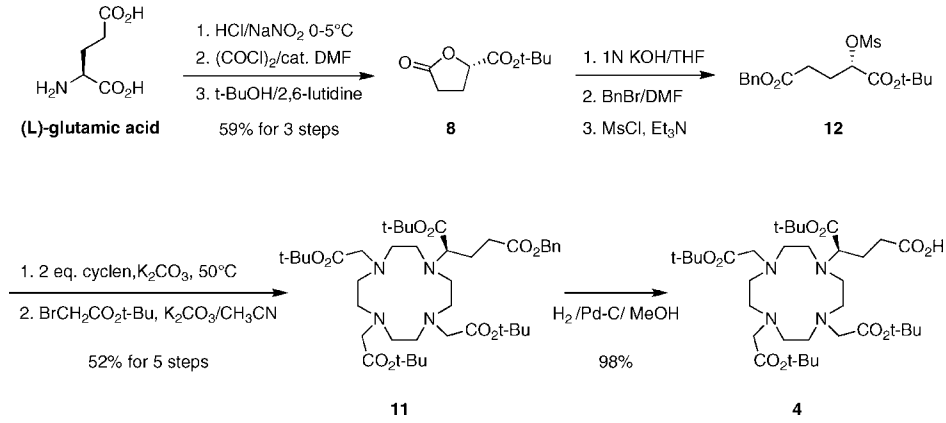
(8) (a) Doolittle, R. E.; Heath, R. R. *J. Org. Chem.* **1984**, *49*, 5041. (b) Vigneron, J. P.; Meric, R.; Larcheveque, M.; Debal, A.; Lallemend, J. Y.; Kunesch, G.; Zagatti, P.; Gallois, M. *Tetrahedron* **1984**, *40*, 3521. (c) Gringore, O. H.; Rouessac, F. P. *Organic Syntheses* **1985**, *63*, 121. (d) Okabe, M.; Sun, R. C.; Tam, S. Y. K.; Todaro, L. J.; Coffen, D. L. *J. Org. Chem.* **1988**, *53*, 4780. (e) Zamzow, M.; Hocker, H. *Macromol. Chem. Phys.* **1994**, *195*, 2381. (f) Cai, X.; Chorghade, M. S.; Fura, A.; Grewal, G. S.; Jauregi, K. A.; Lounsbury, H. A.; Scannel, R. T.; Yeh, C. G.; Young, M. A.; Yu, S.; Guo, L.; Moriarty, R. M.; Penmasta, R.; Rao, M. S.; Singhal, R. K.; Song, Z.; Staszewski, J. P.; Tuladhar, S. M.; Yang, S. *Org. Process Res. Dev.* **1999**, *3*, 73.

(9) Lactone carboxylic acid **6** was chosen as the optically enriched starting material for **9**. It was necessary to determine whether stereochemical integrity was maintained throughout the synthesis of **4**, starting from **6**. Analysis of **4** was done using HPLC, with a chiral stationary phase. The chromatographic resolution of **4** was not sufficient to obtain baseline resolution of the peaks corresponding to the enantiomers of **4** and allow a precise determination of optical purity. An analysis of a diastereomeric derivative of **4** prepared via the triflate ester of **9** using proton NMR was then undertaken. The diastereomer **(*R*,*R*)-5** was prepared by reacting **4** with (*R*)-(+)-α-methyl benzylamine in the presence of DIC and HOBt, as shown in Scheme 1 (see Experimental Section).

Scheme 3. **Second-generation asymmetric synthesis of 4**



Scheme 4. **Optimized multigram asymmetric synthesis of 4**

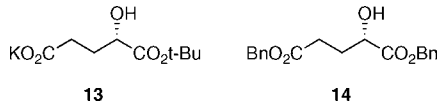


Preparation of **4** via the triflate route gave product having ee = 97 ± 2% (HPLC).[9] Thus, use of the triflate ester derived from **9** to alkylate cyclen resulted in the first highly stereoselective asymmetric synthesis of **4**.

Process Development and Optimization of the Synthesis of 4. The optimized laboratory multigram asymmetric synthesis of **4** is shown in Scheme 4. Numerous issues were identified for optimization of the synthesis of **4** for scale-up and eventual cGMP manufacture, including sourcing or production of **6**, generation of highly optically pure **8**, and improvement of the alkylation of cyclen with an orthogonally protected electrophilic (*S*)-2-hydroxyglutarate equivalent to produce **10**.

Conversion of (L)-Glutamic Acid to 6 and 8. Lactone acid **6** was identified as a very expensive starting material. With the knowledge that there was ample literature precedent for the preparation of this compound,[8] it was decided that **6** would be prepared from (L)-glutamic acid, via diazotization chemistry. Using a reported procedure[8e] (Scheme 4), (L)-glutamic acid was converted to **6** by treatment with sodium nitrite in the presence of excess HCl in 48% yield, based on 100 g of starting material in the initial experiment, thus demonstrating the potential for large-scale preparation. Practical difficulties with this chemistry were also identified. The aqueous reaction medium and the water solubility of the product necessitated the distillation of a large volume of water in order to isolate the product. Additionally, the water solubility of **6** made crystallization (initially from ether) unpredictable and very slow (24−48 h at −20 °C).

Significant improvements to the synthesis of **6** occurred after an analytical method was developed to assess the enantiomeric excess (ee) of samples of crude and purified **6** (chiral GC). It was discovered that crude **6** could be reproducibly prepared with ee ≥ 93%. The ability to prepare and carry forward **6** in crude form simplified the workup and isolation and improved the contained yield. The stereoselective conversion of (L)-glutamic acid to **6** depended critically on the rate of addition of the aqueous sodium nitrite solution to the acidic (L)-glutamic acid solution, and on maintaining an internal temperature range (0−5 °C) of the reaction mixture during this addition. An acceptable rate of addition was 1.5−3.0 mL/min (a total addition time of 3−5 h on a scale of hundreds of grams). If the sodium nitrite solution was added too rapidly (e.g., > 100 mL/h on a scale of 200 g starting (L)-glutamate), the quality and ee of the product decreased significantly, regardless of the temperature at which the reaction mixture was maintained. A significant amount of nitric oxide was produced, even at temperatures within the recommended range of 0−5 °C, and very low yields



13 14

Figure 2. **2-(*S*)-2-Hydroxyglutaric acid, dibenzyl ester, the major impurity present in crude 9.**

(20−30%) were obtained. Crude **6** was carried forward to the acid chloride intermediate **7**, at which point it was purified by vacuum distillation.[8a] A temperature range of 0−5 °C was acceptable for the reaction of **7** with *tert*-butanol to form the *tert*-butyl ester. After conversion to **8**, the crude material was purified by filtration through a plug containing a layer each of activated carbon, flash silica, and Celite, eluting with ethyl acetate. The filtrate was then concentrated, and the crude solid was recrystallized from a mixture of ethyl acetate and hexanes. This purification procedure resulted in reproducible production of **8** in isolated yields of 55−60%, having chemical purity ≥99% and ee ≥ 99%. This result was key to the development of a practical kilogram-scale synthesis of **4**, since stereochemistry is fixed at the formation of **6** and optical purity is a key specification for **4**. It was subsequently found that the recrystallization step was responsible for most of the enhancement in the optical purity of **8**, as compared with that of **6**, since use of the crude acid chloride **7** to prepare **8** gave purified material having ee identical to that obtained from distilled acid chloride.

Conversion of 8 to 9. Operationally, the chemistry used to convert **8** to **9** was quite straightforward. The major question concerned identification of reaction end points (i.e., reaction rate and the introduction of an in-process control), side reactions, and optimization of the chromatographic purification of **9**. An HPLC study indicated that the hydrolysis of the lactone was not complete after 1 h at ambient temperature, the time described for the reaction in the discovery synthesis and in literature reports.[6a] Since the workup consisted of removal of water *in vacuo* at elevated temperature, it is likely that the reaction was completed during concentration of the reaction mixture. *In situ* monitoring of the hydrolysis by infrared spectroscopy showed that it was complete after 2 h at 50 °C. During this interval, no hydrolysis of the *tert*-butyl ester was apparent. However, the major impurity from the subsequent alkylation reaction of the potassium carboxylate intermediate **13** was a dibenzylated hydroxy diester **14** (Figure 2). The above result indicates that formation of the dicarboxylate was occurring during the concentration of the reaction mixture *in vacuo* at elevated temperature, or that the *tert*-butyl ester was transesterified to the benzyl ester under the alkylation conditions.

One operational difficulty was the solubility of **13**, product of alkaline hydrolysis of **8**. Compound **13** was obtained as a crystalline solid after the removal of water from the lactone hydrolysis reaction mixture. The potassium carboxylate salt was not soluble in DMF, the solvent used for the subsequent reaction. After benzyl bromide was added to DMF, **13** adhered to the bottom and walls of the reaction vessel. In the presence of benzyl bromide, the carboxylate salt dissolved as it reacted. This created problems on the kilogram scale, since the reaction vessel was not identical to the flask used to concentrate the reaction mixture, and there was not a suitable organic solvent to transfer the **13** to a reaction vessel. It was unlikely that the presence of a small amount of water would affect the outcome of the alkylation reaction, so **13** was dissolved using a small amount of water and transferred to the reaction vessel, then diluted with DMF prior to the addition of benzyl bromide. The results for the reaction containing water were comparable to the results obtained for the original reaction run in dry DMF. On further scale-up, the hydrolysis reaction mixture was concentrated to leave an aqueous solution of **13**, which was diluted with DMF for the subsequent reaction.

Table 1. **Effect of base, temperature and solvent on alkylation of cyclen with 12**

entry	solvent	base	temperature, °C	HPLC area % conversion to **10** in 24 h (48 h)
1	CH_2Cl_2	K_2CO_3	25	12
2	DMF	K_2CO_3	25	29
3	CH_3CN	K_2CO_3	25	45 (67)
4	CH_3CN	*i*-Pr_2NEt	25	37 (57)
5	CH_3CN	Et_3N	25	30 (49)
6	CH_3CN	2,6-lutidine	25	30 (47)
7	DMF	K_2CO_3	50	80
8	CH_3CN	K_2CO_3	50	95
9	CH_3CN	K_2CO_3	100	72

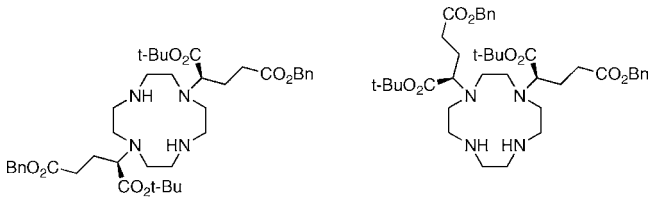
Conversion of 9 to 10. The *in situ* generation of the triflate ester of **9** and its conversion to monoalkylated cyclen **10** were identified as key steps at the initial scale-up stage. There were a number of drawbacks to using the triflate: (a) the stability of the triflate was unknown, but probably marginal, (b) the triflate was generated *in situ* and added to cyclen, thus affording little control over the quality and purity of the triflate used, and (c) the reaction was run at −40 °C, and this is not generally a desirable temperature for scale-up or pilot-plant production. In order to avoid these issues, an alternative alkylating agent, mesylate **12**, was studied.

Mesylate esters are less reactive than the corresponding triflates. By one estimate, a triflate ester is 40,000 times more reactive than its corresponding mesylate ester.[10] As expected, the reaction of **12** with cyclen was slow at ambient temperature using three different solvents and four different bases (Table 1). As indicated in Table 1, the alkylation reaction proceeded most rapidly in acetonitrile.

As shown in Table 1, the conversion of **12** to **10** at ambient temperature was low under all conditions examined. The set of conditions giving the highest conversion of **12** to **10** at ambient temperature was entry 3, using acetonitrile as the solvent and potassium carbonate as the base. The alkylation reaction run at 50 °C in acetonitrile in the presence of potassium carbonate (entry 8, Table 1), resulted in 95% conversion of **12** to **10** after 24 h. Running the reaction at 100 °C in acetonitrile using potassium carbonate as the base resulted in lower conversion of **12** to **10**, due to decomposition.

It is clear that alkylation of cyclen with the mesylate **12** gives superior results, in comparison with the alkylation of cyclen with the triflate. The use of the mesylate is advantageous in that (1) it is stable, up to approximately 50 °C, (2) it can be isolated in crude (yet highly pure) form via aqueous workup, and (3) its reaction with cyclen is controllable, giving **11** after

(10) (a) Crossland, R. K.; Wells, W. E.; Shiner, V. J., Jr. *J. Am. Chem. Soc.* **1971**, *93*, 4217. (b) Streitweiser, A., Jr.; Wilkins, C. L.; Kiehlman, E. *J. Am. Chem. Soc.* **1968**, *90*, 1598.



15 16

Figure 3. **Structures of the 1,4- and 1,7- bis-alkylated cyclen impurities.**

alkylation with *tert*-butyl bromoacetate in 84% yield (10-g scale), considerably improved over the yield of **11** (48%) obtained using the triflate and in identical quality. The significant improvement in the yield of **11** using **12** over that obtained using the triflate of **9** is most likely attributable to the increase in stability of the alkylating agent, and thus the actual quantity of alkylating agent available to alkylate cyclen.

The monoalkylation of cyclen was carried out by adding the mesylate **12** to a suspension of 2 equiv of cyclen in acetonitrile preheated to 50 °C in the presence of 1 equiv of potassium carbonate. Excess cyclen was necessary in order for the reaction to proceed optimally. Less than 2 equiv of cyclen resulted in the formation of significantly more bis-alkylated cyclen impurities **15** and **16** (Figure 3). The amounts of **15** and **16** were less than 5% when ≥2 equiv of cyclen were used.

In the aqueous workup to remove the residual cyclen, it was discovered that consecutive washes with a small amount of water and brine in a ratio of 1:5 and 1:15 (v/v), respectively, relative to the volume of the ethyl acetate solution of the crude **10**, removed all detectable residual cyclen and its mesylate salt to the limits of LC/MS detection (<0.1% of peak area of **10**). The removal of excess cyclen at this point was crucial to the quality of **11** obtained, since the side product, DOTA tetra-*tert*-butyl ester, was impossible to separate from a mixture with **11**. This reaction sequence was carried out on a scale of 10−100 g. No differences in extent of reaction or impurity profile were observed.

Conversion of 10 to 11. The use of mesylate **12** as the alkylating agent for cyclen allowed more control over the quality of **10** produced, which in turn resulted in higher yields of **11**, in comparison to the triflate chemistry. Three equivalents of *tert*-butyl bromoacetate were used, based on the potency of **10**. If the reaction was run with excess *tert*-butyl bromoacetate (>3 equivalents), overalkylation gave a quaternary ammonium salt. The overalkylated impurity could be converted back to the desired product **11** by heating an ethyl acetate solution at reflux.

The limited solubility of **11** in ethyl acetate allowed us to isolate and purify the crude material by trituration. Using a ratio of ethyl acetate to crude starting **10** of 5:1 (v/w), **11** with purity >95% (HPLC area) was consistently obtained. On ≥100 g scale, the yield of **11** (based on **9**) was 52−63% for three chemical transformations.

Conversion of 11 to 4. The hydrogenation of **11** was initially done in methanol, at 50 psi in the presence of 10% Pd−C by mass to give the free carboxylic acid **4** in 98% yield. Experiments were run at either 20 or 40 psi hydrogen pressure, and both were determined to be complete after less than 1 h.

Scale-up of the palladium-catalyzed hydrogenation to cleave the benzyl protecting group was straightforward. This hydrogenation was performed on up to a 50-g scale in a Parr shaker apparatus. Prewetted Pd−C was used in order to minimize fire hazard during the transfer of catalyst. It was crucial that residual methanol in **4** was minimal, since the next step was the conversion to various activated esters. Coevaporation of **4** with acetonitrile was necessary to ensure that any remaining methanol was removed (confirmed by proton NMR). The yield of **4** was 85−90%, in 95% purity by HPLC area. The stereochemical integrity of the chiral center in the side chain was maintained with minimal or no racemization, as determined by HPLC of the derivative **5** (96.8 ± 2.0% ee, based on HPLC analysis).

Preparation for cGMP Kilogram-Scale Manufacture of 4. The reduction to practice of the above process at an external vendor confirmed that the most problematic aspects were related to the conversion of (L)-glutamic acid to **6**. The yield of **8** was diminished compared to that originally observed, most likely due to prolonged heating of the reaction mixture from the formation of **6** during the removal of water and HCl, and also to decomposition during vacuum distillation of crude **7**. Since analysis of the process did establish that virtually all of the enhancement in ee observed in the conversion of **6** to isolated **8** was attributable to the purification using a carbon/silica/Celite plug, followed by recrystallization, the vacuum distillation of **7** was not necessary in order to obtain **8** with acceptable purity and ee. Subsequent to the reduction to practice, crude **6** was converted to crude **7**, which was used directly in the preparation of **8**. Intermediate **8** was then purified and isolated as described above. Aside from lower yields, the remainder of the reduction to practice synthesis of **4** proceeded as described for the multigram asymmetric synthesis of **4**. Intermediate **9** was converted to **10** using the mesylate chemistry previously described. The loss of significant amounts of **10** subsequent to aqueous washes during workup became apparent during the early scale-up of this chemistry. The volumes of water and brine used, relative to the large volume of ethyl acetate of the workup solution, were determined by screening of workup conditions and monitoring for the presence of residual cyclen using LC/MS and HPLC. Monoalkylated cyclen **10** prepared in the reduction to practice had a purity of 77% with 0.4% residual cyclen. Alkylation of cyclen with **12**, followed by alkylation of **10** with *tert*-butylbromoacetate provided **11** in 56% yield, based on **9**. Hydrogenation of **11** to give **4** was performed with Pd−C in a mixture of water and ethanol. The yield was 92%, based on mass. The product was 97.6% pure and had ee = 97.4%.

Scale-Up and Production of 6, 8, and 9. (L)-Glutamic acid was converted to **9** on a 1−2-kg scale, in preparation for production of starting material for the kg-scale cGMP manufacture of **4**. This intermediate scale-up indicated that the time-consuming removal of water to isolate crude **6** remained an issue. Scale-up of the conversion of (L)-glutamic acid to **6** to 5 kg revealed a hazard that was not apparent at the lower kg scale. Concentration of the reaction mixture *in vacuo* at 30 °C produced a large exotherm (a temperature increase of 40−50 °C) once most of the water had been removed, accompanied by vigorous gas evolution. Additionally, the use of ethyl acetate

as the extraction solvent for the crude concentrated residue of **6** resulted in transesterification of the open-chain 2-hydroxyglutaric acid present in the crude mixture, due to prolonged contact with residual HCl. Based on mass spectral data, the compound most likely to be responsible for the exotherm was the nitrite ester of 2-hydroxyglutaric acid. Decomposition of this compound would form **6** in the presence of acid, and gas evolution (presumably nitric oxide) would occur. An attempt was made to dissipate the exotherm by feeding portions of the partially concentrated reaction to boiling toluene and then removing the remaining water as an azeotrope. This treatment failed to decompose the energetic material present in the mixture, as evidenced by the continued presence of a vigorous exotherm in a sample analyzed by differential scanning calorimetry (DSC). Unfortunately, this heat treatment also resulted in the decomposition of a significant amount of crude **6** and in an unacceptable loss of optical purity (ee = 72% after treatment with boiling toluene). For the remaining kilogram-scale batches of **6**, the reaction mixture was concentrated in portions of not more than 8−10 L apiece, and each residue was set aside for later pooling in order to avoid thermal decomposition of material due to prolonged exposure of the accumulated product to heat. Crude **6** (still containing significant amounts of water, even after coevaporation with toluene) was then extracted with dichloromethane, dried with sodium sulfate, decanted and carried forward to the preparation of the acid chloride **7**. The purification of crude **8** was modified to accommodate the increased scale of the operations by first filtering through a silica plug and then passing the filtrate through an in-line cartridge of activated carbon, followed by partial concentration of the ethyl acetate solution and dilution with heptane to induce crystallization. The above modifications resulted in the ability to prepare multikilogram quantities of **8** in ≥99% ee and ≥99% purity. The yield for the conversion of L-glutamic acid to **8** on a multikilogram scale was 42%, compared with 56% for the same series of conversions performed on a 200-g scale. The scale-up of the conversion of **8** to **9** proceeded with essentially no issues. The reactions were run at 2.5−3.0-kg scale, giving a 54% yield of **9** based on assay. Purification of ∼1 kg of crude **9** was done by application to a 6-in. column containing 15 kg flash silica, and eluting with a gradient starting with pure heptane, stepping up to a maximum of 25% ethyl acetate/heptane. Therefore, six column chromatography runs were required to purify all of the intermediate **9** produced in the cGMP campaign.

Kilogram-Scale Conversion of 9 to 4. Monoalkylated cyclen **10** was prepared in two batches from two lots of **9**. No difficulties were encountered, but both lots of **10** were found to be out of specification (the quantity of the dialkylated cyclen impurity for one lot was 5.5%, while the release specification for this intermediate was <5.0%; purity of the second lot was 73.5%, while the release specification for purity of **10** was ≥75%). Use tests for the conversion to **11** were conducted with small samples of each nonconforming lot of **10**. Both samples gave **11** having purity >96.0%, exceeding the specification for **11**, set at ≥95.0%. The major impurities were the dialkylated cyclen products carried over from **10** (2.5−3.0%). Based on the results of these use tests, both nonconforming lots of **10** were released for further processing, since it was clear that the dialkylated cyclen impurity and assay specifications for **10** had been set too tightly. The yields of **10** for these lots were 72−73%, based on assay. The kilogram-scale preparation of **11** from **10** proceeded smoothly. The yield of **11** for both lots was 64%, giving a total of 5.6 kg of material.

Kilogram-scale conversion of **11** to **4** using catalytic hydrogenation proceeded without incident. The hydrogenation was carried out in a 5-gal stainless steel reactor, requiring four batches of ∼1.4 kg each of **11**. Two lots of **4** were produced, in 87.6% yield (2.11 kg) and 91.4% yield (2.35 kg), based on assay. Both lots had ee = 99.0%, based on HPLC.

Experimental Section

2-(*S*)-5-Oxotetrahydrofuran-2-carboxylic acid, *tert*-Butyl Ester (8). A solution of sodium nitrite (140 g, 2.03 mol) in water (320 mL) was added over 4 h to a mechanically stirred mixture of L-glutamic acid (200 g, 1.36 mol), dioxane (150 mL) and HCl (280 mL) in water (530 mL), maintained at an internal temperature of 0−5 °C. On completion of the addition, the mixture was warmed to room temperature (RT) and stirred for 2 h. Solvents were then removed *in vacuo* (50−55 °C and 30 Torr). ***Caution: While concentrating the above aqueous reaction mixture on a scale of 5 kg, an exotherm (40−50 °C temperature increase), accompanied by vigorous gas evolution, was observed. In subsequent runs, the reaction mixture was concentrated in smaller portions (≤ 10 L of reaction mixture concentrated at a time), which were combined in CH_2Cl_2 before being carried forward to the acid chloride.*** The residue was coevaporated with toluene (2 × 500 mL) to remove additional water, then ethyl acetate (1 L) was added, followed by sodium sulfate (100 g), and the mixture was stirred for 0.5 h. The ethyl acetate solution was decanted and filtered, and the solids were washed and stirred with additional ethyl acetate (1 L). The combined filtrates were concentrated *in vacuo*, and the resulting residue was further dried under high vacuum (1 Torr). The mass of crude residue containing **6** was 193 g, exceeding the mass of the theoretical yield by 16 g, attributable to solvent trapped by the viscous, syrupy residue. A sample of this residue was subjected to chiral GC analysis, which indicated the optical purity of this material to be 94% ee. LC/MS: $m/z = 131$ (MH^+).

Determination of the ee of crude **6** via chiral GC: ∼25 mg of crude **6** and ∼50 mg of dicyclohexylcarbodiimide (DCC) were dissolved in CH_2Cl_2 (∼1 mL), and methanol (2 drops) was added; the mixture was stirred 1 h, then filtered through a 0.45 μm Nylon syringe filter; injection volume: 1 μL; assayed on a 2,6-di-*O*-pentyl-3-propionyl capillary column, 40 mm × 0.25 mm; temperature 250 °C; split ratio 100:1; run time 20 min; t_R = 12.1 min ((*R*)-enantiomer), t_R = 13.7 min ((*S*)-enantiomer).

Oxalyl chloride (297 mL, 432 g, 3.40 mol) was added to a solution of crude **6** (assumed ∼177 g, 1.36 mol) and DMF (2.00 mL) in dry dichloromethane (800 mL) cooled to 0−5 °C in an ice bath. The mixture was warmed to RT after the addition was complete and stirred 2 h, then concentrated *in vacuo*. The residue was coevaporated with dichloromethane (1 L), was then transferred under inert atmosphere to a 500 mL distillation flask, and was subjected to vacuum distillation (short path still head/

condenser). The acid chloride **7** distilled at 86−96 °C at 1 Torr. The mass of distillate obtained was 154 g.

Acid chloride **7** (154 g, ∼1.04 mol) in dichloromethane (350 mL) was added over a period of 1.5 h to a stirred solution of *tert*-butanol (200 mL, 154 g, 2.08 mol) and 2,6-lutidine (145 mL, 133 g, 1.25 mol) in dichloromethane (800 mL) cooled to 0 °C. On completion of the addition, the mixture was warmed to RT and stirred overnight. The mixture was then poured into a separatory funnel and washed with water (2 × 800 mL), 10% aq citric acid (2 × 800 mL), saturated sodium bicarbonate (2 × 800 mL) and brine (2 × 800 mL), then dried (sodium sulfate), decanted and concentrated to a dark brown residue, which crystallized on standing. The crude residue was dissolved in ethyl acetate (150 mL) and applied to a plug composed of a wetted bed of Celite (bottom layer, 150 g), flash silica (middle layer, 150 g) and activated carbon (top layer, Darco, 100 mesh, 150 g). The loaded plug was eluted with ethyl acetate (3 L), and the filtrate was concentrated to a residue, which was crystallized by adding ethyl acetate (200 mL) followed by hexanes (600 mL), to give 134 g of **8** (53%) after drying at ambient temperature at 1 Torr for 3 h. A second crop of crystals (7.00 g, 3%) was obtained by concentrating to a residue and precipitating with hexanes (300 mL), giving a yield of 56% **8**, based on L-glutamic acid. The purity of the material was determined to be 99.7% by HPLC area integration. This material was determined to have ee ≥ 99% by chiral GC. ^{1}H NMR ($CDCl_3$): δ (ppm) = 4.8 (dd, 1 H), 2.8−2.2 (m, 4 H), 1.5 (s, 9 H). ^{13}C NMR: δ (ppm) = 176.3, 169.0, 83.0, 76.2, 27.9, 26.8, 25.9. Anal. Calcd for $C_9H_{14}O_4$: C, 58.05; H, 7.58. Found: C, 58.31; H, 7.72.

Determination of ee via chiral GC: injection volume: 1 μL of a 15 mg/mL solution of **8** in CH_2Cl_2; assayed on a 2,6-di-*O*-pentyl-3-propionyl capillary column, 40 mm × 0.25 mm; temperature 250 °C; split ratio 100:1; run time 60 min. t_R = 50.40 min ((*R*)-enantiomer), t_R = 52.45 min ((*S*)-enantiomer).

2-(*S*)-2-Hydroxypentanedioic Acid, 1-*tert*-Butyl-5-benzyl Ester (9). A solution of 1 N KOH (357 mL, 0.357 mol) was added in a single portion to a stirred solution of **8** (66.4 g, 0.357 mol) in THF (250 mL). The internal temperature rose to 37 °C. The mixture was heated and stirred at 40 °C for 2 h, then concentrated to a solid *in vacuo* (55 °C, <30 Torr), and dried under high vacuum (ambient temperature, < 5 Torr) overnight. The solid residue was suspended and stirred in DMF (650 mL), and benzyl bromide (44.6 mL, 64.2 g, 0.375 mol) was added. After stirring 8 h, the mixture was poured into ice water (1.5 L), then extracted with ethyl acetate (2 × 500 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The weight of the crude residue after removing ethyl acetate was 150 g, indicating the presence of approximately 45 g of DMF and excess benzyl bromide left to be removed. The remainder of the DMF was removed *in vacuo* using rotary evaporation with a high vacuum (<1 Torr) pump, leaving 101 g of crude **9**. This material was applied to a plug of flash silica (8 × 25 cm) and eluted with hexanes (1 L), 1:9 ethyl acetate/hexanes (v/v) (1 L) and 15:85 ethyl acetate/hexanes (v/v) (2 L). Fractions containing the product were pooled and concentrated to give 79.7 g (76%) of **9** as a light-yellow tinted oil. ^{1}H NMR ($CDCl_3$): δ (ppm) = 7.35 (m, 5 H), 5.13 (s, 2 H), 4.1 (m, 1 H), 2.88 (d, 1 H), 2.59−2.46 (m, 2 H), 2.2−2.1 (two m, 2 H), 1.48 (s, 9 H). ^{13}C NMR: δ (ppm) = 173.9, 173.0, 135.9, 128.6, 128.2, 128.19, 82.7, 69.6, 66.3, 29.8, 29.5, 28.0. Anal. Calcd for $C_{16}H_{22}O_5$: C, 65.29; H, 7.53. Found: C, 65.19; H, 7.74.

(2)-(*R*)-2-(1,4,7,10-Tetraazacyclododec-1-yl-pentanedioic Acid, 1-*tert*-Butyl-5-benzyl Ester (10). Methanesulfonyl chloride (38.9 mL, 62.2 g, 0.503 mol) was added to a stirred mixture of **9** (132 g, 0.449 mol) and Et_3N (70.1 mL, 60.0 g, 0.594 mol) in CH_2Cl_2 (1.5 L) cooled to 0−5 °C in an ice bath. After the addition was complete, the mixture was warmed to RT, stirred 0.5 h and checked by HPLC for completeness. After 1 h, water (500 mL) was added, the organic phase was separated and washed with brine (500 mL) then dried (Na_2SO_4), decanted and concentrated *in vacuo* to give **12** (163 g), sufficiently pure to be used in the next step. ^{1}H NMR ($CDCl_3$): δ (ppm) = 7.35 (m, 5 H), 5.13 (s, 2 H), 4.97 (dd, 1 H), 3.11 (s, 3 H), 2.57−2.14 (m, 4 H), 1.48 (s, 9 H).

The mesylate **12** (163 g, 0.431 mol), as a solution in CH_3CN (800 mL), was added over a period of 45 min to a stirred mixture of cyclen (153 g, 0.888 mol) and potassium carbonate (61.3 g, 0.432 mol) in CH_3CN (2.4 L) preheated to 50 °C, monitoring by HPLC for completeness. After 19 h, the reaction mixture was cooled to RT and filtered to remove potassium salts and cyclen mesylate. The filter cake was then washed with additional CH_3CN (2 × 150 mL), and the filtrate was concentrated to a red oil *in vacuo* and redissolved in ethyl acetate (4.8 L). The organic solution was washed with water (300 mL) and brine (100 mL). (If cyclen is detected in the organic phase after this initial set of washes (LC/MS, *m*/*z* = 173, MH^+ for cyclen), washing needs to be repeated.) The organic solution was then separated and dried (Na_2SO_4) and then decanted and concentrated *in vacuo* (water bath temperature <35 °C) to give 174 g of crude product (92% pure by HPLC area integration). ^{1}H NMR ($CDCl_3$): δ (ppm) = 7.36 (m, 5 H), 5.8 (s, 2 H), 3.24 (dd, 1 H), 2.65−2.35 (m, 20 H), 1.39 (s, 9 H). LC/MS (electrospray): *m*/*z* = 449 (MH^+).

2-(*R*)-2-(4,7,10-Tris *tert*-Butyloxycarbonylmethyl-1,4,7,10-tetraazacyclododec-1-yl)-pentanedioic Acid-1-*tert*-Butyl-5-benzyl Ester (11). Crude **10** (prepared from mesylate **12**, 174 g, ∼0.388 mol) and potassium carbonate (438 g, 3.18 mol) were dissolved/suspended in dry CH_3CN (2 L), then a solution of *tert*-butyl bromoacetate (173.0 mL, 1.17 mol) in CH_3CN (432 mL) was added over a period of 1 h. HPLC monitoring indicated that the reaction was incomplete. An additional portion of *tert*-butylbromoacetate (21.6 mL, 28.5 g, 0.146 mol) was then added, and stirring was continued for 17 h. The mixture was filtered, and the filtrate was concentrated *in vacuo*, dissolved in ethyl acetate (3 L), and washed with water (2 L), saturated sodium bicarbonate (1 L), and brine (1 L). After drying (Na_2SO_4), the solution was decanted and concentrated to a volume of 0.75−1 L *in vacuo*, at which point a fine, white precipitate formed. The precipitate was isolated by vacuum filtration, and the filter cake was washed with ethyl acetate (3 × 300 mL), then dried under high vacuum (<5 Torr) to remove traces of ethyl acetate to give 223 g (63.0% for three steps, formation of **12**, **10** and **11**) of fully alkylated cyclen **11**. A small sample of this material was repurified using preparative HPLC prior to submission for elemental analysis. The aqueous

eluent for preparative HPLC was 0.1% trifluoroacetic acid (TFA) in water, resulting in formation of the mono-TFA salt *in situ*. ^{1}H NMR ($CDCl_3$): δ (ppm) = 7.34 (m, 5 H), 5.08 (d, 2 H), 3.39−2.00 (m, 27 H), 1.43 (four s, 36 H). ^{13}C NMR: δ (ppm) = 175.0, 174.7, 173.0, 172.84, 172.81, 135.6, 128.6, 128.4, 128.3, 82.6, 81.94, 81.90, 81.88, 66.4, 59.9, 55.9, 55.8, 55.5, 52.7, 52.4, 48.6, 48.5, 48.1, 47.2, 44.3, 32.5, 27.9, 27.8, 27.7, 19.3. Anal. Calcd for $C_{42}H_{70}N_4O_{10}\cdot$TFA: C, 56.70; H, 8.00; N, 6.01. Found: C, 56.68; H, 7.96; N, 5.99.

2-(*R*)-2-(4,7,10-Tris-*tert*-Butyloxycarbonylmethyl-1-4-7-10-tetraazacyclododec-1-yl)-pentanedioic Acid, 1-*tert*-Butyl Ester (4). Palladium on carbon (dry, 3.00 g, ≈10% by mass) was added as a slurry in water (20 mL) to a methanol solution of **11** (30.0 g, 38.0 mmol, in 200 mL methanol) in a Parr pressure shaker bottle. The mixture was subjected to three cycles of vacuum and hydrogen purge, then pressurized to 25−30 psi hydrogen and shaken for 2 h. HPLC indicated that the only major species present was the desired product. After evacuating the system to remove hydrogen, the bottle was opened, and Celite (15 g) was added. The slurry was filtered through a methanol-wet bed of Celite (15 g), and the filtrate was concentrated (bath temperature <35 °C to a light-yellow syrup *in vacuo*. The syrup was coevaporated with acetonitrile (2 × 150 mL) in order to azeotrope out residual water and to remove any remaining methanol (confirmed by proton NMR), giving 21.8 g (82%) of an amorphous off-white solid after drying under high vacuum. HPLC: 95% pure by area integration. ^{1}H NMR ($CDCl_3$): δ (ppm) = 3.61−2.0 (6 m, 27 H), 1.45 (3 s, 36 H). ^{13}C NMR: δ (ppm) = 175.2, 175.0, 172.8, 172.7, 172.6, 82.3, 82.0, 81.94, 81.89, 60.0, 55.8, 55.7, 55.5, 52.6, 52.5, 48.5, 48.4, 48.0, 47.1, 44.1, 27.85, 27.83, 27.79, 20.1. Anal. Calcd for $C_{35}H_{64}N_4O_{10}\cdot$2.7 TFA: C, 47.22; H, 6.93; N, 5.51. Found: C, 47.31; H, 7.04; N, 5.53.

2-(*R*)-2-(4,7,10-Tris *tert*-Butylcarboxymethyl-1,4,7,10-tetraazacyclododec-1-yl)-pentanedioic Acid, 1-*tert*-Butyl Ester, 5-(*R*)-(+)α-Methylbenzylamide (5). (*R*)-(+)-α-Methylbenzylamine (129 μL, 121 mg, 1.00 mmol) was added to a stirred mixture of **4** (701 mg, 1.00 mmol), DIC (155 μL, 126 mg, 1.00 mmol) and HOBT (135 mg, 1.00 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred 3 h, then diluted with CH_2Cl_2 (20 mL), and washed with water (10 mL) and brine (10 mL), dried (Na_2SO_4), decanted, and concentrated to a crystalline residue (757 mg). A portion (250 mg) of this material was purified by preparative HPLC (25 mm C_4 silica, A: 0.1% TFA in water, B: 0.1% TFA in CH_3CN, gradient of 5 to 65% solvent B, monitoring at 220 nm, t_R = 23 min). ^{1}H NMR ($CDCl_3$): δ (ppm) = 8.52 (d, 1 H), 7.47−7.10 (m, 5 H), 5.03 (m, 1 H), 3.50−1.70 (m, 26 H), 1.41 (m, 39 H). ^{13}C NMR: δ (ppm) = 172.9, 172.8, 172.7, 172.5, 172.1, 145.0, 128.3, 126.6, 126.4, 82.1, 82.04, 82.00, 81.9, 60.6, 55.8, 55.5, 52.7, 52.67, 52.5, 49.2, 49.1, 48.5, 48.4, 48.0, 47.0, 46.97, 46.9, 35.1,27.9, 27.87, 27.8, 22.8, 21.9. LC/MS (electrospray): m/z = 805 (MH^+). Anal. Calcd for $C_{43}H_{73}N_5O_9\cdot H_2O$: C, 62.9; H, 9.14; N, 8.52. Found: C, 63.1; H, 8.90; N, 8.64.

HPLC analysis of the stereochemical composition of **5**: 150 mm × 4.6 mm CN column; temperature 30 °C; eluent 75:25 1.0 M K_3PO_4, pH = 2.5/acetonitrile; flow rate 1.2 mL/min; UV 220 nm; t_R = 18.7 min (**(*R*,*R*)-5**), t_R= 20.9 min (**(*S*,*R*)-5**, prepared as a standard).

Proton NMR Determination of Optical Purity of 5: In order to prepare **(*S*,*R*)-5**, **(*S*)-4** was synthesized from the *R*-enantiomer of **6** (97% ee, purchased from Aldrich) using the synthetic route used to prepare **4** (Scheme 4). Proton NMR spectra of crude **(*R*,*R*)-5** and **(*S*,*R*)-5** were recorded in $CDCl_3$. The amide NH signal of **5** (a doublet at approximately 8 ppm, J = 7 Hz) showed a difference in chemical shift for the (*R*,*R*)- and (*S*,*R*)-diastereomers. Two sets of amide NH peaks were present in the proton NMR spectrum of a sample containing both diastereomers of **5**. Subsequent to the proton NMR analysis of optical purity, an HPLC method was developed which provided accurate analysis of the stereochemical composition of **5** prepared using the triflate chemistry. The de purity of **5** was determined to be 97 ± 2%, comparable to the ee purity of the commercial **6** used as the starting material.

Conclusions

A multigram asymmetric synthesis of the tetraazamacrocyclic ligand precursor **4** was developed. Stereochemistry of **4** originated from (L)-glutamic acid, and stereochemical integrity was maintained throughout the synthesis. The chemistry developed was successfully extended to the kilogram-scale manufacture of **4**, giving the product in high chemical (95−99%) and optical (ee = 97 ± 2%) purity. The process developed for the preparation of **4** represents a significant improvement over the existing literature method, as regards scalability, economy, operational simplicity and stereoselectivity. This process has been applied to the multikilogram-scale cGMP manufacture of **4**.

Supporting Information Available

General experimental details and characterization data for **6**, **8**, **9**, **11**, and **4**, as well as chiral GC data supporting the ee reported for **6** and **8** and HPLC data supporting the de reported for **5** and the ee reported for **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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