Organic Process Research & Development **2004**, *8,* 587−592

Alternate Synthesis of a β-3 Adrenergic Receptor Agonist

Robert W. Scott,*,†,§ Darrell E. Fox,† John W. Wong,‡ and Michael P. Burns‡

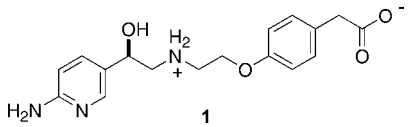
Pfizer Chemical Research and Development and Pfizer Bioprocess Research and Development, Eastern Point Road, Groton, Connecticut 06340, U.S.A.

Abstract:

Previously our group reported synthetic efforts used to synthesize kilogram quantities of the β-3 receptor agonist (*R*)-(4-(2-(2-(6-aminopyridin-3-yl)-2-hydroxyethylamino)ethoxy)phenyl)-acetic acid, 1. Additional research was conducted to explore an alternate chiral route with a streamlined protecting group scheme. The alternate asymmetric process and synthetic rationale are described.

Introduction

The preceding report outlined process research and scale-up for the β-3 receptor agonist (*R*)-(4-(2-(2-(6-aminopyridin-3-yl)-2-hydroxyethylamino)ethoxy)phenyl)acetic acid, **1**.[1]



1

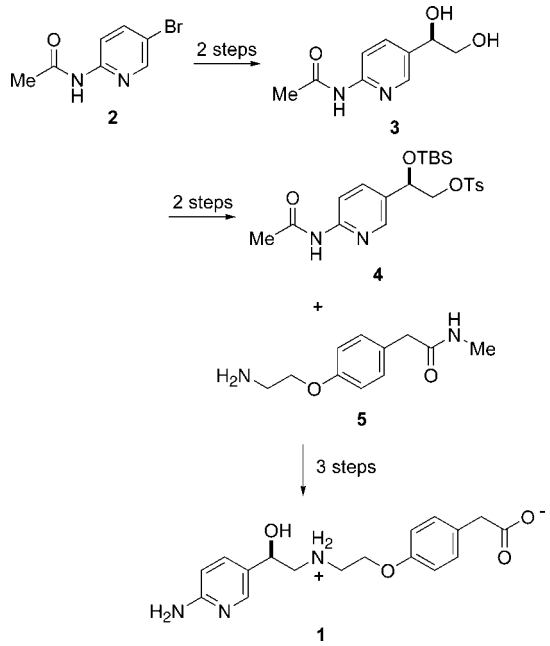
A key intermediate in the synthesis is tosylate **4**, which was used to alkylate amine **5** followed by appropriate deprotections to complete the synthesis (Scheme 1).

Although multiple bulk lots were produced by this route, the initial synthetic difficulties outlined in the previous contribution led us to consider alternate chemistry. In particular, the focus was on functionalization of the pyridine with an appropriate two-carbon extension containing the chiral center. The previous work had shown that reaction via epoxide chemistry (such as **6**) is unsuitable for two reasons: a significant amount of undesired α-opening of the epoxide accompanied the desired product during the amine alkylation, and extensive racemization was observed, which is believed to occur via an aza-quinone methide (**7**) (Scheme 2.).

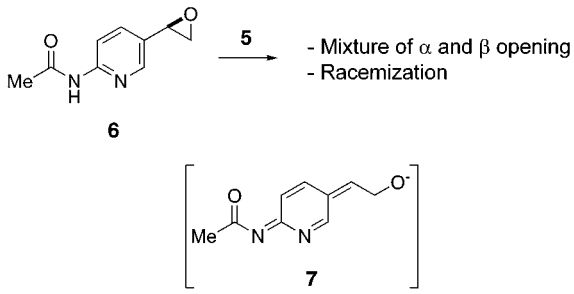
The sequence presented in the previous contribution successfully avoided the problems associated with the epoxide chemistry by utilizing tosylate **4**.

In search of increased synthetic efficiency, we explored a process that utilized an alternate source of chirality installation, would minimize use of protecting groups, and would avoid the issues of highly water-soluble intermediates that have complicated the chemistry in this series. By utilizing the 2,5-dimethylpyrrole to mask the aminopyridine,[2]

Scheme 1



Scheme 2



we hoped to address the solubility, protecting group issues, and epoxide problems simultaneously. It was reasoned that incorporation of the amine lone pair in the pyrrole aromatic system would improve the epoxide stability by destabilizing aza-quinone methide intermediates. The group is also expected to be tolerant to a broader range of reaction conditions than the amide-protected structures described previously.

Results and Discussion

2-Amino-5-bromopyridine (**8**) was converted to pyrrole **9** under standard Dean−Stark conditions with 2,5-hexanedi-

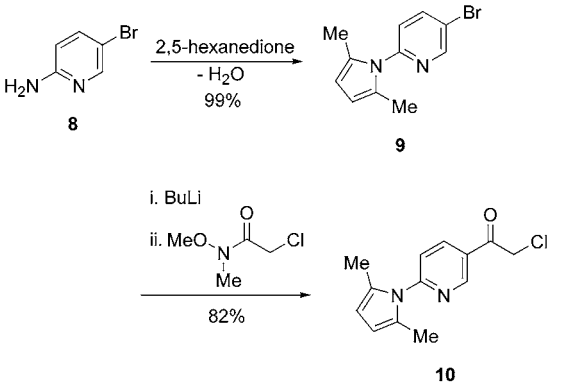
* Author for correspondence. E-mail: robert_scott@pfizer.com.
† Chemical Research and Development.
‡ Bioprocess Research and Development.
§ Current address: Pfizer Chemical Research and Development, La Jolla, CA 92121.

(1) Vanderplas, B. C.; DeVries, K. M.; Fox, D. E.; Raggon, J. W.; Snyder, M. W.; Urban, F. J. *Org. Process Res. Dev.* **2004**, *8*, 583−586.

(2) After the completion of this work, a similar approach to compound **10** was published in the patent literature: Ladouceur, G. H.; Connell, R. D.; Baryza, J.; Campbell, A.; Lease, T. G.; Cook, J. H. PCT Int. Appl. WO 99/32475, July 1, 1999.

10.1021/op0499775 CCC: $27.50
Published on Web 05/28/2004

Scheme 3

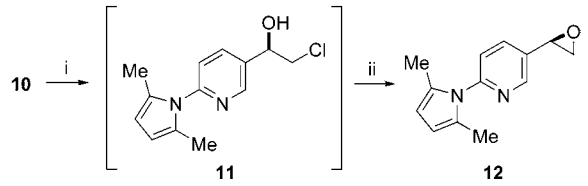


one.[3] Conversion of the bromide to the acylated pyridine via the Grignard reagent was only moderately successful. Lithiation was much more facile; however, the anion thus formed was unstable in THF.[4] The use of less-polar ethereal solvents minimized this problem. The anion was generated at low temperature (<−40 °C) by lithium−halogen exchange in MTBE. The anion was reacted with *N*-methoxy-*N*-methylchloroacetamide,[5] to provide the desired chloroketone **10** upon workup. A small amount of quenched anion (5−10%) was formed, and this impurity was removed by reslurry of the product in hexanes (Scheme 3).

With ready access to chloroketone **10**, various classes of chiral reducing agents and conditions were explored. B-Chlorodiisopinocampheylborane (DIP-Cl)[6] and oxazaborolidine-catalyzed borane reduction[7a] showed promising results and were amenable to scale-up. The subsequent chemistry for completion of the synthesis was slightly different for these two reagents, and both options were explored further. Additionally, bioreduction of **10** was considered as a future option, and initial investigations into this approach are described.

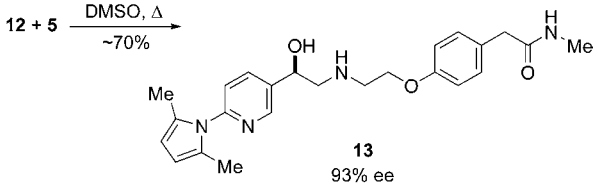
(−)-DIP-Cl Reduction. The reduction of **10** with commercially available DIP-Cl[8] produced chiral chorohydrin **11** in 93−95% ee at −25 °C. Reaction at 0 °C provided a slight erosion of selectivity to 90% ee. From a long-term process standpoint, a cost-effective reducing agent was desirable since the boron species is used quantitatively. DIP-Cl made from lower-grade (and much less expensive) pinene shows enhanced stereoselectivity as the (*d*/*l*) catalyst is less active.[9] The use of a commercially available heptane solution of DIP-Cl[10] which was produced from 80% ee pinene, provided the desired (*R*) reduction product in 91% ee. As described in the previous manuscript, even near-racemic **1** could be enriched to >99% ee by the final crystallization in the process; therefore, the chirality level produced by this route with the lower-grade pinene was sufficient to provide acceptable final product (Scheme 4).

Scheme 4



i. 1.3 (-)-DIPCl; ii. $NaBO_3 \cdot 4H_2O$, NaOH

Scheme 5



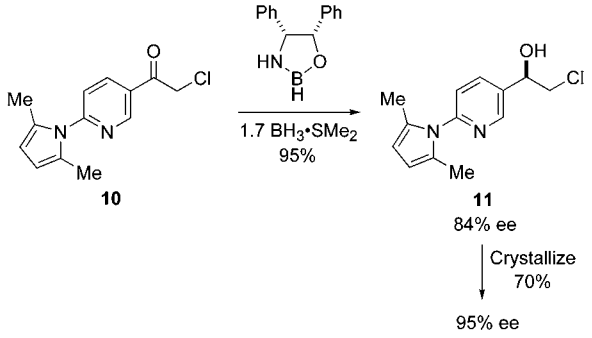
One liability of the DIP-Cl reaction is the need to safely oxidize the boron−carbon bonds of the reagent after reaction. As the desired substrate contains a pyridine ring, strongly oxidizing solutions of H_2O_2 and NaOH provided a mixture of products. Alternate oxidizing agents were screened (trimethylamine *N*-oxide, sodium percarbonate, and sodium perborate), and superior performance was observed with $NaBO_3 \cdot 4H_2O$.[11] The basic nature of these oxidations led to the slow formation of the epoxide from the product chlorohydrin. As a result, excess NaOH was added to complete the conversion to epoxide **12** during the oxidation reaction, allowing the isolation of a single compound. Unfortunately, epoxide **12** is an oil heavily contaminated with the pinene-related byproducts. This necessitates coupling the crude epoxide with amine **5** to provide **13**. Amine **5** was used in excess (2 equiv) to minimize dialkylated byproduct (Scheme 5).

The coupled product could be purified by dissolution into acidic aqueous media and washing the pinene materials away with an ethereal extraction. Basification of the aqueous layer followed by ethyl acetate extraction provided **13** sufficiently pure to continue with the synthesis.

Two key suppositions of our synthetic strategy were verified at this juncture. First, only the desired β-opened product could be detected during the alkylation, and there was no erosion in the chirality. This demonstrated that the dimethylpyrrole protecting group successfully altered the electronics of the system to allow the epoxide to be a practical coupling partner. Second, the product was freely soluble in organic solvent and easily extracted from the aqueous layer. This is in contrast to the previously disclosed acetate-protected aminopyridine which does not impart sufficient organic solubility, requiring the use of TBS or BOC protecting groups for clean isolation.[1]

(3) Bruekelman, S. P.; Leach, S. E.; Meakins, G. D.; Tirel, M. D. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2801−2807.
(4) Acylations in THF showed addition of the acyl group on multiple positions of the pyridine ring as well as significant proton quenching.
(5) Tillyer, R.; Frey, L. F.; Tschaen, D. M.; Dolling, U.-H. *Synlett* **1996**, 225−226.
(6) Chandrasekharan, J.; Ramachandran, P. V.; Brown, H. C. *J. Org. Chem.* **1985**, *50*, 5448−5450.
(7) (a) Corey, E. J.; Helal, C. J. *Angew. Chem., Int. Ed.* **1998**, *37*, 1986−2012 and references therein. (b) Methyl or methoxy-CBS-oxazaborolidine ligands provided 2−42% ee.
(8) Aldrich Chemical Co.
(9) (a) Simpson, P.; Tschaen, D.; Verhoeven, T. R. *Synth. Commun.* **1991**, *21*, 1705−1714. (b) King, A. O.; Corley, E. G.; Anderson, R. K.; Larsen, R. D.; Verhoeven, T. R.; Reider, P. J. *J. Org. Chem.* **1993**, *58*, 3731−3735.
(10) This material is available in bulk quantities from Callery Chemical Co. It is produced from the more readily avilable 80% ee pinene. An economic method of preparation has been described from the Merck laboratories: Zhao, M; King, A. O.; Larsen, R. D.; Verhoeven, T. R.; Reider, P. J. *Tetrahedron Lett.*, **1997**, *38*, 2641−2644.
(11) Kabalka, G. W.; Shoup, T. M.; Goudgaon, N. M. *J. Org. Chem.* **1989**, *54*, 5930−5933.

Scheme 6



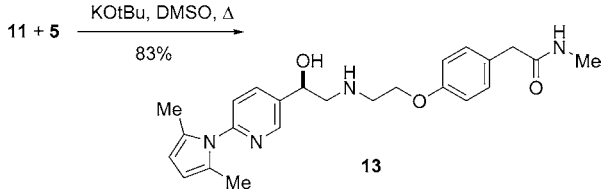
Oxazaborolidine-Catalyzed Reduction. Simultaneous to demonstration of the DIP-Cl route, ligand-catalyzed reduction was explored. These reactions provide an advantage from a scale-up perspective due to the clean chemical conversions and low catalyst load. However, a drawback was the uniformly lower ee's that were observed by ligand-catalyzed reductions for our system.

Quallich has reported that use of the 2-amino-1,2-diphenylethanol ligand is useful for reductions in acetylpyridine systems.[12] This result translated well to our synthesis, where this ligand was clearly superior to the diphenylprolinol-derived ligands.[7] Using this system, a 95% yield of product can be obtained in 84% ee. As compared to the DIP-Cl reductions, oxazaborolidine-catalyzed reduction is much cleaner, and the product chlorohydrin can be isolated directly and crystallized to provide enantiomeric enrichment. Two crops of product were obtained[13] for an overall yield of 67%, that is 95% ee (Scheme 6).

Bioreduction. Another option explored as a potential long-term solution to the chiral reduction was microbial reduction of chloroketone **10**. Microbial whole-cell reductions of aromatic ketones have been extensively studied,[14] and many examples, including α-haloketone reductions, have been reported in the literature.[15] High selectivity is a potential advantage that makes microbial reduction an attractive option. Other advantages of microbial reduction are the low cost of the biocatalyst and mild reaction conditions. However, a drawback of whole-cell conversions from a scale-up perspective is the difficulty that is often encountered in recovering product from dilute reaction mixtures. Solubility and toxicity of the substrate and/or product to the microorganism are two factors that limit substrate loading. Thus, identifying a whole-cell conversion that operates at high substrate concentration (∼10 g/L) would improve the potential for an economically feasible microbial process.

A microbial screen for reduction of ketone **10** revealed several organisms that selectively produced the (*R*)-alcohol. The most selective of these hits was the yeast *Zygosaccharomyces bailii* ATCC 38924. This organism has been reported for the stereoselective reduction of a ketone

Scheme 7



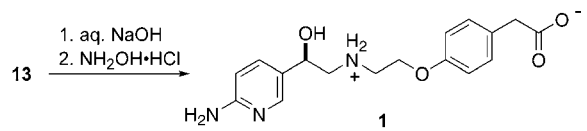
intermediate for a cholesterol-lowering agent.[16] Incubation of **10** with *Z. bailii* in 8-L fermentors resulted in a 57% yield of chlorohydrin **11** with 98% ee, starting from an initial substrate concentration of 2 g/L. After a reaction time of 20 h, product was recovered from fermentation broth by adsorption on to Amberlite XAD-4 resin, elution with ethyl acetate, and silica flash chromatography. The potential for higher substrate loading was demonstrated in a small-scale experiment using a concentrated slurry of *Z. bailii* cells in phosphate buffer. Under these conditions, a 76% yield was obtained with 98% ee starting with a substrate loading of 16 g/L. These results looked promising with respect to scale-up potential, and further improvements may be possible with the application of techniques such as extractive biocatalysis.[17,18]

Fragment Coupling. Coupling of amine **5** with chiral chlorohydrin **11** was carried out under our standard conditions, but the reaction was sluggish as compared to the previously described epoxide couplings. It was discovered that this reaction also proceeded by formation of the epoxide in situ, but the amine was a poor base for epoxide formation, and therefore reaction times were extended. By addition of a stoichiometric base that did not interfere with the coupling, reaction times were significantly shortened. Thus, addition of potassium *tert*-butoxide to a mixture of the chlorohydrin and amine followed by heating in DMSO provided clean coupling product **13** in 83% yield. Opening of the epoxide at the α center was not detected, and the coupling product was easily extracted from the aqueous phase without the need for further derivitization (Scheme 7).

Deprotection. Completion of the synthesis of the desired candidate required hydrolysis of the methylamide on the right-hand portion of the molecule and removal of the 2,5-dimethylpyrrole protecting group to unmask the aminopyridine. It was known from previous work that the amide deprotection can best be effected with basic rather than acidic hydrolysis.[1] We planned to remove the pyrrole group by exchange with hydroxylamine.[3] Both permutations for the order of these deprotections were successful; however, pyrrole removal as the last step provided the cleanest product. The final two-step procedure started with amide hydrolysis by NaOH in aqueous ethanol. The crude carboxylate compound was then treated with hydroxylamine to effect pyrrole removal, followed by filtration to provide **1**. Both reactions were high yielding; however, the physical properties

(12) Quallich, G. J.; Woodall, T. M. *Tetrahedron Lett.* **1993**, *34*, 4145−4148.
(13) First crop: 41% recovery, 97.2% ee; second crop: 29% recovery, 92% ee.
(14) D'Arrigo, P.; Pedrocchi-Fantoni, G.; Servi, S. In *Stereoselective Biocatalysis*; Patel, R. N., Ed.; Marcel Dekker: New York, 2000; pp 365−396.
(15) Patel, R. N.; Bannerjee, A.; Chu, L.; Brozozowski, D.; Nanduri, V.; Szarka, L. *J. Am. Oil Chem. Soc*. **1998**, *75*, 1473−1482.
(16) Homann, M. J.; Previte, E. Stereoselective Microbial Reduction for the Preparation of 1-(4-Fluorophenyl)-3(*R*)-[3(*S*)-hydroxy-3-(4-fluorophenyl)-propyl)]-4(*S*)-(4-hydroxyphenyl)-2-azetidinone. U.S. Patent 6,133,001, 2000.
(17) D'Arrigo, P.; Fuganti, C.; Fantoni, G. P.; Servi, S. *Tetrahedron* **1998**, 15017−15026.
(18) Vicenzi, J. T.; Zmijewski, M. J.; Reinhard, M. R.; Landen, B. E.; Muth, W. L.; Marler, P. G. *Enzyme Microb. Technol.* **1997**, *20*, 494−499.

Scheme 8



of **1** make it difficult to isolate in high recovery free from inorganics, especially when ethanol is used as the main solvent. The crude product was purified by redissolution in basic water, hot filtration to remove any insoluble material, and pH adjustment to neutral to precipitate the clean zwitterionic product **1** (Scheme 8).[19]

The material isolated by this sequence was of high chemical purity and chiral purity in a 58% yield for the two-step deprotection and crystallization process.

Conclusions

In summary, the sequence described provides the targeted drug candidate **1** in a six-linear step process in an overall yield of 26% from commercially available 2-amino-5-bromopyridine. The amine **5** requires four steps to synthesize. The key synthetic advance was the use of the 2,5-dimethylpyrrole protecting group for the aminopyridine. This functionality opened up new options in fragment coupling via epoxide chemistry adding electronic stability to the system to avoid racemization and undesired α-opening. In addition, the dimethylpyrrole added significant organic solubility to the compounds in this notoriously water-soluble series. Two options were developed for the key chiral reduction: an oxazaborolidine-catalyzed borane reduction and a microbial reduction. Both technologies were researched in the lab to the point that reasonable confidence has been gained towards future scale-up applicability. From a process standpoint, the synthesis is a promising and viable alternative to the large-scale synthesis described previously.

Experimental Section

All materials were purchased from commercial suppliers and used without further purification. All reactions were conducted under an atmosphere of nitrogen unless noted otherwise. NMR spectra were obtained on a Varian Unity 400 spectrometer. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, NY.

5-Bromo-2-(2,5-dimethylpyrrol-1-yl)-pyridine (9). To a solution of 2-amino-5-bromopyridine (89.87 g, 0.5194 mol) in toluene (500 mL) was added 2,5-hexanedione (85.0 mL, 0.724 mol). The reaction flask was equipped with a Dean–Stark apparatus and the solution heated to reflux for 14 h. The cooled reaction solution was extracted with H_2O (2 × 200 mL). The organic layer was added directly to a plug of SiO_2 (3 in. × 2 in.) and eluted with toluene (250 mL). This treatment removes a significant amount of color from the crude product. The resulting eluent was concentrated to a light amber oil, which solidified upon cooling to provide 128.76 g (99%) of **9**. ^{1}H NMR (400 MHz, $CDCl_3$): 2.13 (s, 6), 5.90 (s, 2), 7.12 (d, 1, J = 8.3), 7.93 (dd, 1, J = 2.5, 8.3), 8.65 (d, J = 2.5). ^{13}C NMR (100 MHz, $CDCl_3$): 13.21, 107.41, 118.92, 123.01, 128.60, 140.51, 150.43, 150.71. Anal. Calcd for $C_{11}H_{11}N_2Br$: C, 52.61; H, 4.42; N, 11.16. Found: C, 52.68; H, 4.28; N, 10.99.

5-(2-Chloroacetyl)-2-(2,5-dimethylpyrrol-1-yl)pyridine (10). A solution of **9** (100.00 g, 0.3982 mol) in MTBE (1.5 L) was cooled to −78 °C. To the solution was added BuLi (175 mL, 2.5 M in hexanes, 0.4375 mol) over 15 min, causing a rise in internal temperature from −78 to −70 °C. After 10 min, a solution of 2-chloro-*N*-methoxy-*N*-methylacetamide (63.10 g, 0.4587 mol) in MTBE (200 mL) was added over 10 min, causing a rise in internal temperature from −78 to −65 °C. After an additional 20 min, 1 M HCl (1 L) was added, and the cooling bath removed. The mixture was stirred vigorously for 2 h while warming to room temperature, and then the layers were separated. The aqueous layer was extracted with MTBE (400 mL), and the combined organic layers were extracted with 400 mL of brine (containing 30 mL of 12.5 M NaOH—this wash serves to free-base any protonated pyridine). The organic layer was dried with $MgSO_4$, filtered, and concentrated to provide crude **10** as a red oil. To the crude oil was added hexanes (500 mL) and the mixture stirred vigorously overnight. The mixture thus obtained was filtered, and the solids were rinsed with hexanes (2 × 100 mL) to provide 81.32 g (82%) of **10** as a tan, slightly sticky powder. This material should be stored cold, as prolonged storage at room temperature will cause darkening and impurity buildup. ^{1}H NMR (300 MHz, $CDCl_3$): 2.18 (s, 6), 4.69 (s, 2), 5.93 (s, 2), 7.33 (ap. d, 1, J = 8.3), 8.36 (ddd, 1, J = 1.3, 2.6, 8.5), 9.11−9.13 (m, 1). ^{13}C NMR (100 MHz, $CDCl_3$): 13.65, 45.48, 108.59, 121.16, 127.70, 128.88, 138.07, 149.78, 155.67, 189.41. Anal. Calcd for $C_{13}H_{13}N_2OCl$: C, 62.78; H, 5.27; N, 11.26. Found: C, 62.75; H, 5.11; N, 11.27. Mass Spec: AP^+ = 249.1, AP^- = 247.2

(*R*)-2-(2,5-Dimethylpyrrol-1-yl)-5-oxiranylpyridine (12). To a N_2-purged flask was added (−)-DIP-Cl [(−)-B-chlorodiisopinocampheylborane, 75.90 g, 0.2366 mol] which was rinsed into the flask with MTBE (50 mL). To the solution was added THF (112.5 mL), and the mixture cooled to approximately −30 °C. A solution of **10** (45.0 g, 0.181 mol) in THF (67.5 mL) was added dropwise over 10 min, and the internal temperature reached a maximum of −19 °C. The reaction was held between −30 and −23 °C for 6 h, after which HPLC analysis showed complete conversion to **11** (chiral HPLC of aliquot shows 93% ee). To the solution was added $NaBO_3 \cdot 4H_2O$ (27.8 g, 0.181 mol) followed by MTBE (175 mL). The mixture was stirred at room temperature overnight. To the reaction mixture was added 2 N NaOH (675 mL), and the mixture stirred at room temperature for 6 h. HPLC analysis shows complete conversion of **11** to the epoxide **12**. The layers were separated, and the aqueous layer was extracted with MTBE (560 mL). The combined organic layers were extracted with 1 N NaOH (225 mL) and brine (225 mL). The organic solution was dried ($MgSO_4$), filtered, and concentrated to a thick oil to provide 82.9 g of crude **12** which was contaminated with large amounts of pinene-derived material. The crude material was used in the subsequent step without further purification. ^{1}H NMR (400

(19) **1** has high solubility in both basic and acidic aqueous media but can be recovered with reasonable efficiency near neutral pH as the zwitterion.

MHz, $CDCl_3$): 2.11 (s, 6), 2.90 (dd, 1, J = 2.6, 5.3), 3.24 (dd, 1, J = 4.0, 5.3), 3.96 (dd, 1, J = 2.5, 4.0), 5.89 (s, 2), 7.20 (d, 1, J = 8.1), 7.66 (dd, 1, J = 2.5, 8.3), 8.56 (d, 1, J = 2.5).

13 (from 12). To a crude solution of **12** (74.6 g mixture, synthesized from 0.163 mol of **10**) in DMSO (140 mL) was added 2-[4-(2-amino-ethoxy)-phenyl]-*N*-methyl-acetamide **(5)**[20] (68.35 g, 0.3282 mol). The mixture was heated in an 85 °C bath for 8.5 h. Heating was discontinued and the solution allowed to cool and stir at room temperature overnight. The mixture was added to 0.5 M HCl (2.8 L), which caused a gum to separate out. The solution was extracted with diethyl ether (3 × 1 L), and the ether washes were discarded. The aqueous layer was decanted from the gum that settled out, and 1 M NaOH (2.1 L) was added. The aqueous layer was extracted with EtOAc (2 × 1.4 L), and the combined organic layers were extracted with brine (500 mL) and dried over K_2CO_3. The solution was filtered and concentrated to an oil, which solidified to a tacky solid upon storage to provide 43.38 g (63% from 9) of **13** which was sufficiently pure to carry forward in the synthesis. A small portion was purified by SiO_2 chromatography (MeOH/EtOAc/NEt_3) to provide an analytically pure sample. ^{1}H NMR (300 MHz, $CDCl_3$): 2.14 (s, 6), 2.77 (d, 3, J = 4.8), 2.84 (dd, 1, J = 9.5, 12.2), 3.07−3.21 (m, 3), 3.54 (s, 2), 4.13 (t, 2, J = 5.0), 4.84 (dd, 1, J = 3.3, 9.3), 5.47 (br s, 1), 5.92 (s, 2), 6.93 (d, 2, J = 8.7), 7.20 (d, 2, J = 8.7), 7.24 (d, 1, J = 8.1), 7.91 (dd, 1, J = 2.3, 8.1), 8.60 (d, 1, J = 2.2). ^{13}C NMR (100 MHz, $CDCl_3$): 13.15, 26.46, 42.70, 48.34, 56.69, 67.36, 69.37, 106.87, 114.96, 121.67, 127.34, 128.59, 130.67, 135.76, 136.68, 147.22, 151.36, 157.96, 172.06. Anal. Calcd for $C_{24}H_{30}N_4O_3$: C, 68.22; H, 7.16; N, 13.26. Found: C, 67.82; H, 7.39; N, 13.03.

(*R*)-5-(2-Chloro-1-hydroxyethyl)-2-(2,5-dimethylpyrrol-1-yl)-5-(2-chloro-1-hydroxyethyl)pyridine (11). To a solution of (1*S*,2*R*)-2-amino-1,2-diphenylethanol (0.2429 g, 1.139 mmol) in THF (75 mL) was added BH_3SMe_2 (∼10.0 M, 3.80 mL). This solution was allowed to stir at room temperature for 15 h, during which time hyrdogen was evolved. To the catalyst solution was added **10** (5.5134 g, 22.168 mmol) in THF (10 mL) via syringe pump over 3 h. After an additional 3 h, the reaction was quenched by the slow addition of water (15 mL), causing slow hydrogen evolution. One hour after the addition of water, the reaction solution was added to diisopropyl ether (50 mL), EtOAc (50 mL), and 2 N HCl (200 mL) and stirred well for 15 min. The phases were separated, and the aqueous phase was extracted with EtOAc (150 mL). The combined organic phases were further extracted with brine (125 mL) containing 12.5 M NaOH solution (1.5 mL). The solution was dried ($MgSO_4$), filtered, and concentrated to provide 5.27 g (95%) of crude product. Chiral HPLC showed this material to be a 91.8:8.2 mixture of desired (*R*) to undesired (*S*) enantiomers. The crude was crystallized from EtOAc/hexanes providing two crops of material, totalling 3.7098 g (67%) of material that was a 97.5:2.5 mixture of enantiomers. ^{1}H NMR (400 MHz, $CDCl_3$): 2.12 (s, 6), 2.85 (d, 1, J = 3.4), 3.7 (dd, 1, J = 8.6, 11.3), 3.83 (dd, 1, J = 3.6, 11.3), 5.02 (ddd, 1, J = 3.4, 3.4, 8.3), 5.89 (s, 2), 7.23 (d, 1, J = 9.2), 7.88 (dd, 1, J = 2.6, 8.3), 8.60 (d, 1, J = 2.4). ^{13}C NMR (100 MHz, $CDCl_3$): 13.11, 50.00, 71.06, 107.16, 121.92, 128.68, 135.18, 136.37, 147.34, 151.65. AP+ = 251.1. Anal. Calcd for $C_{13}H_{15}N_2OCl$: C, 62.28; H, 6.03; N, 11.17. Found: C, 62.09; H, 6.15; N, 11.28.

11 (Bioreduction of 10 in Fermentor Cultures of *Zygosaccharomyces bailii* ATCC 38924). Cultures of *Z. bailii* ATCC 38924 were obtained from the American Type Culture Collection (ATCC, Manassas, Virginia) and stored as glycerol suspensions of vegetative cells at −80 °C. A three-stage protocol was used to prepare cultures for the bioreduction of **10**. The medium used for all three stages contained 2% glucose, 0.5% soy flour, 0.5% NaCl, and 0.5% K_2HPO_4 (pH adjusted to 7.0 before autoclaving). Additionally, the medium for stages one and three contained 0.5% TWEEN 80. The initial-stage cultures were grown in 250-mL Erlenmeyer flasks containing 30 mL of growth medium. These flasks were inoculated with 0.2 mL of the glycerol suspensions of *Z. bailii* and agitated on a rotary shaker (210 rpm) for 24 h at 29 °C. Second-stage cultures were grown in two 2.8-L Fernbach flasks (500 mL of medium) and were each inoculated with the contents of a single first-stage culture and agitated (210 rpm) for 48 h at 29 °C. Final-stage cultures were grown in fermentor jars containing 8 L of medium. Two fermentors were each inoculated with one of the second-stage cultures and incubated at 29 °C with agitation at 300 rpm and aeration at 3 L min^{-1}. After 48 h, 16 g (potency of ∼80%) of **10** (200 g/L solution in DMSO) was added to each fermentor, and incubation continued for 20 h. At the end of this time, the broths from the two fermentors were combined and stirred overnight with 750 g of Amberlite XAD-4 resin. The resin was then collected on an 80 mesh screen, washed with 8 L of water, and extracted with 8 L of of EtOAc. The EtOAc extract was washed with water, dried over anhydrous magnesium sulfate, filtered, and concentrated under vacuum to yield the crude product. Flash chromatography on silica gel using EtOAc/hexanes mixtures followed by crystallization from EtOAc/hexanes afforded 14.7 g (57%) of **11** with an enantiomeric excess of 98%.

11 (Bioreduction of 10 with Washed Cells of *Z. bailii* ATCC 38924). A two-stage protocol was used to prepare *Z. bailii* cells for the bioreduction of **10**. In the first stage, a 300-mL Erlenmeyer flask containing 100 mL of growth medium (2% glucose, 2% peptone, 1.2% yeast extract, and 1.2% malt extract; pH adjusted to 6.0 before autoclaving) was inoculated with 1 mL of a glycerol suspension of *Z. bailii* cells and incubated at 29 °C on a rotary shaker (210 rpm) for 48 h. Second-stage cultures were set up in a manner identical to that of the first stage except that the glucose content of the medium was increased to 12%. Two second-stage cultures were each inoculated with 4 mL of the first stage culture and incubated under the conditions described for stage one. After 72 h, the contents of the two second-stage cultures were combined and centrifuged. The pellet containing the cells was washed with 50 mL of 0.1 M

(20) DeVries, K. M.; Dow, R. L.; Wright, S. W. PCT Int. Appl. WO98/21184, May 22, 1998.

phosphate buffer (pH 5.6) and resuspended with 12.5 mL of the same buffer. The bioreduction was carried out in two test tubes (16 × 125 mm), each containing 50 mg of **10**, 0.125 mL of DMF, 1.25 mL of 1% aqueous TWEEN 80, and 0.4 mL of an aqueous glucose solution (250 mg/mL). This mixture was sonicated for 10 min followed by the addition of 1.25 mL of the suspension of *Z. bailii* cells. The reaction mixture was agitated on a rotary shaker (210 rpm) at 37 °C for 21 h. The contents of the tubes were each diluted with methanol to a volume of 50 mL. These methanol extracts were analyzed by HPLC to reveal a 76% in situ yield of **11** with an ee of 98%.

13 (from 11). To a solution of **11** (9.99 g, 39.8 mmol) in DMSO (80 mL) was added **5**[20] (16.23 g, 77.93 mmol) followed by KO*t*-Bu (4.49 g, 40.0 mmol). The mixture was heated in an 80 °C bath for 20 h. After cooling, the mixture was added to water (300 mL) and EtOAc (300 mL). The phases were separated, and the organic layer was further extracted with water (2 × 200 mL). The organic phase was extracted with brine, dried (K_2CO_3), filtered, and concentrated to provide 13.93 g (83%) of **13** as a hard yellow solid. Characterization data are the same as listed previously.

(*R*)-(4-(2-(2-(6-Aminopyridin-3-yl)-2-hydroxyethylamino)ethoxy)phenyl)acetic Acid (1). In a 200-mL flask, 50 mL of absolute EtOH was added to **13** (13.26 g, 31.38 mmol). The solution was heated to 70 °C until all the solids had dissolved (30 min). To the solution was added 1.6 M NaOH (60 mL), and the bath temperature was raised to 100 °C. After 48 h, HPLC analysis showed only 2% starting material remaining. The solution was concentrated to half of the volume under vacuum while heating in a 50 °C bath. To the crude residue was added absolute EtOH (120 mL) and the solution heated to 80 °C. To the hot solution were added hydroxlyamine hydrochloride (10.80 g, 0.1554 mol) and water (10 mL), and heating continuted overnight. After a total heating time of 20 h, analysis of the crude solution showed some starting material remainng. An additional portion of hydroxlyamine hydrochloride (2.00 g, 0.0288 mol) was added, and heating continuted an additional 17 h. After cooling to room temperature, the heterogeneous solution was filtered, and the solids were rinsed with EtOH (40 mL) to provide 12.2624 g of crude **1**. To the crude material were added H_2O (60 mL) and NaOH (2.05 g), and the solution was heated to 70 °C causing most of the solids to dissolve. The hot solution was filtered through a scintered glass frit while hot to remove a very small quantity of insoluble material. The solution was cooled to room temperature, and 6 M HCl was added dropwise to reduce the pH to 6.7, after which stirring was continued for 30 min (note: solids began to come out of solution at pH = 9.5). The solids were filtered and rinsed with water (2 × 10−15 mL). The purified **1** was air-dried followed by drying under high vacuum to provide 5.9815 g (58%) of product which was clean by ^{1}H NMR analysis and HPLC (96% at 210 nm).

Acknowledgment

We thank James J. Cawley for work on the 8-L fermentation runs.

Received for review January 8, 2004.

OP0499775