Organic Process Research & Development **2008,** *12,* 823–830

Full Papers

AMD070, a CXCR4 Chemokine Receptor Antagonist: Practical Large-Scale Laboratory Synthesis

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Abstract:

An efficient and convergent four-step synthetic route to the CXCR4 chemokine receptor antagonist AMD070 (1) has been developed which employs only a single chromatographic step in the entire sequence. Novel reductive amination methods have been developed for the coupling of 2 and 3 in which a dehydrative imine formation is followed by reduction with an attenuated borohydride reagent (zinc chloride and sodium borohydride). Selective extraction methods were employed to purify synthetic intermediates and remove reagents and impurities. A procedure has also been developed to isolate 1 in a pure crystalline form.

Introduction

The CXCR4 chemokine receptor is a 7-transmembrane G-protein coupled receptor (GPCR) expressed on the surface of a variety of cell types including epithelial, endothelial, neuronal, and hematopoietic cells including CD4+ T cells, the target for HIV infection. The natural ligand for the receptor is the chemokine CXCL12 (SDF-1). The CXCR4/CXCL12 axis plays major roles in hematopoiesis, hematopoietic stem cell and lymphocyte trafficking and homing, and neonatal development.[1]

T-tropic strains of HIV utilize CXCR4 as a coreceptor during infection, enabling the entry of viral RNA into the human cell.[2] Through the use of a specific CXCR4 chemokine receptor antagonist, the interaction between HIV and the CXCR4 coreceptor can be interrupted, and infection can therefore be blocked.[3] Thus, a specific CXCR4 chemokine receptor could be of therapeutic benefit as an HIV entry inhibitor.

AMD070 (**1**) is a small-molecule CXCR4 antagonist.[4] Phase 1a data[5] showed that AMD070 is generally safe, well tolerated, and orally bioavailable. Preliminary clinical data also showed that AMD070 is active in HIV patients: 4/8 patients at a dose of 200 mg BID had significant reductions in CXCR4 viral load with an average reduction of 1.3 log.[6] These results are the first demonstration of clinical efficacy against HIV for an orally bioavailable CXCR4 antagonist.

A retrosynthetic analysis of **1** provides a convergent strategic route based on two disconnections and three starting materials (Scheme 1). The synthesis of 8-amino-5,6,7,8-tetrahydroquinoline,[7,8] and the isolation of the enantiopure (*S*)-isomer **2** from the corresponding racemate *via* enzymatic resolution[9] have been described previously. Installation of the two remaining frag-

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(1) (a) Zou, Y. R.; Kottmann, A. H.; Kuroda, M.; Taniuchi, I.; Littman, D. R. *Nature* **1998**, *393*, 595–599. (b) Nagasawa; Tachibana; K; Kishimoto, T. *Semin. Immunol.* **1998**, *10* (3), 179–85. (c) Murdoch, C. *Immunol. Rev.* **2000**, *177*, 175–184. (d) Lavi, E.; Strizki, J. M.; Ulrich, A. M.; Zhang, W.; Fu, L.; Wang, Q.; O'Conner, M.; Hoxie, J. A.; Gonzalez-Scarano, F. *Am. J. Pathol.* **1997**, *151* (4), 1035–1042. (e) Watt, S. M.; Forde, S. P. *Vox Sang.* **2008**, *94*, 18–32.

(2) (a) Caroll, R. G.; Riley, J. L.; Levine, B. L.; Feng, Y.; Kaushal, S.; Ritchey, D. W.; Bernstein, W.; Weislow, O. S.; Brown, C. R.; Berger, E. A.; June, C. J. *Science* **1997**, *276*, 273–276. (b) Schramm, B.; Penn, M. L.; Speck, R. F.; Chan, S. Y.; De Clerq, E.; Schols, D.; Connor, R. I.; Goldsmith, M. A. *J. Virol.* **2000**, *74*, 184–192.

(3) (a) Schols, D.; Claes, S.; De Clerq, E.; Hendrix, C.; Bridger, G.; Calandra, G.; Henson, G. W.; Fransen, S.; Huang, W.; Whitcomb, J. M.; Petropoulos, C. J. *AMD3100, a CXCR4 Antagonist, Reduced HIV Viral Load and X4 Levels in Humans*, presented at the 9th Conference on Retroviruses and Opportunistic Infections (CROI),February 24−28, 2002,Seattle, WA. (b) Hendrix, C. W.; Collier, A. C.; Lederman, M. M.; Schols, D.; Pollard, M.; Brown, S.; Jackson, J. B.; Coombs, R. W.; Glesby, M. J.; Flexner, C. W.; Bridger, G. J.; Badel, K.; MacFarland, R. T.; Henson, G. W.; Calandra, G. *J. Acquired Immune Defic. Syndr. Hum. Retrovirol.* **2004**, *37* (2), 1253–1262.

(4) Bridger, G.; Skerlj, R.; Kaller, A.; Harwig, C.; Bogucki, D.; Wilson, T. R.; Crawford, J.; McEachern, E. J.; Atsma, B.; Nan, S.; Zhou, Y.; Schols, D.; Smith, C. D.; Di Fluri, M. Preparation of Tertiary *N*-(5,6,7,8-Tetrahydro-8-quinolinyl)-*N*-(1H-benzimidazol-2-ylmethyl)amines and Analogs as Chemokine Receptor Modulators for Treatment of HIV or FIV. Int. Appl. WO2002034745, PCT, 2002.

(5) (a) Stone, N.; Dunaway, S.; Flexner, C.; Calandra, G.; Wiggins, I.; Conley, J.; Snyder, S.; Tierney, C.; Hendrix, C. W.; and the ACTG A5191 Study Team., Biologic activity of an orally bioavailable CXCR4 antagonist in human subjects, abstr 7225. XV International AIDS Conference, Bangkok, Thailand, 2004. (b) Stone, N.; Dunaway, S. B.; Flexner, C.; Tierney, C.; Calandra, G. B.; Becker, S.; Cao, Y.-J.; Wiggins, I. P.; Conley, J.; MacFarland, R. T.; Park, J.-G.; Lalama, C.; Snyder, S.; Kallungal, B.; Klingman, K. L.; Hendrix, C. W. *Antimicrob. Agents Chemother.* **2007**, *51* (7), 2351–2358.

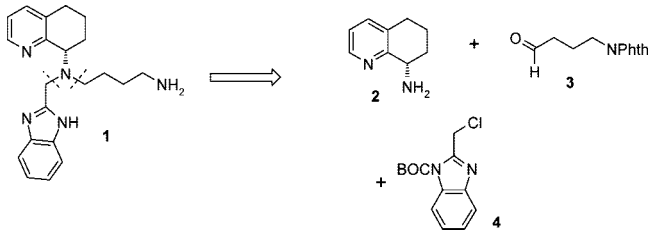
(6) In open-label dose-finding phase 2a clinical study conducted by Adult Aids Clinical Trial Group (ACTG Study A5210), patients were dosed twice daily for 10 consecutive days: Saag, M.; Rosenkranz, S.; Becker, S.; Klingman, K.; Kallungal, B.; Zadzikla, A.; Coakley, E.; Acosta, E.; Calandra, G.; Johnson, V.; and NIAID AIDS Clinical Trial Group. *Proof of concept of ARV activity and AMD11070 (an orally administered CXCR4 entry inhibitor): results of the first dosing cohort A studied in ACTG Protocol A5210. Abstr. 512*, presented at the 14th Conference on Retroviruses and Opportunistic Infections (CROI),February 25−28, 2007,Los Angeles, CA.

(7) Skupinska, K. A.; McEachern, E. J.; Skerlj, R. T.; Bridger, G. J. *J. Org. Chem.* **2002**, *67*, 7890–7893.

(8) McEachern, E. J.; Yang, W.; Chen, G.; Skerlj, R. T.; Bridger, G. J. *Synth. Commun.* **2003**, *33*, 3497.

10.1021/op8000993 CCC: $40.75
Published on Web 07/18/2008

Scheme 1



ments was planned to proceed *via* reductive amination to install **3**, and *N*-alkylation to install **4**.

The initial medicinal chemistry route to **1** is described in Scheme 2, and was used to provide material for preclinical evaluation.[10] The aminobutyraldehyde **3** was generated from 4-aminobutanol, after protection of the primary amine as a phthalimide[11] by oxidation with tetrapropylammonium perruthenate (TPAP)[12] to provide the aldehyde **3**. Installation of the tetrahydroquinoline fragment **2** was accomplished through established reductive amination methods.[13] The secondary amine **5** was purified by column chromatography before being subjected to an *N*-alkylation with *N*-BOC-2-chloromethylbenzimidazole[14] **4**, which, in turn, was synthesized from commercially available 2-chloromethylbenzimidazole. Removal of the phthaloyl protecting group was achieved with hydrazine, and the product **1** was initially isolated as the amorphous hydrochloride salt. Overall, the yield from commercial reagents was approximately 8% (six linear steps), and four of the six steps required column chromatography. Synthetic refinement would clearly be necessary to furnish the kilogram amounts of **1** necessary for preclinical and clinical evaluation.

Development of Practical Routes to Intermediates 2, 3, and 4. In the initial route, it was possible to generate racemic 8-amino-5,6,7,8-tetrahydroquinoline **2** from the corresponding unsubstituted 5,6,7,8-tetrahydroquinoline **7**, *via* formation of an *N*-oxide followed by a Boekelheide rearrangement[15] to install the alcohol functionality at the benzylic position in **8**, then functional group conversion to generate **2** (Scheme 3). However, the route was six steps long, and the yields were low (33%). Accordingly, we developed a preferred chromatography-free route using a regioselective oxime formation as a key transformation.[8] With the regioselective nitrosation of **7** to generate the oxime **9**, followed by a zinc-mediated reduction, it was possible to efficiently access the racemic amine **2** in two steps. Further refinement of reagent stoichiometries and reaction conditions enabled the safe and reliable large-scale preparation of the racemic amine **2** on scales up to 45 kg. Specifically, *n*-hexyllithium was selected as a preferred base over *n*-butyllithium for safety reasons (less pressurization of reaction vessel) and isoamyl nitrate was selected over *tert*-butyl nitrite due to its lower volatility and commercial availability in high-purity.

Subsequent resolution of the racemic amine **rac-2** with *Candida antarctica* lipase B (CaLB) to give the (*S*)-isomer **2**, and the corresponding (*R*)-isomer as the acetamide **10**, were based on our established procedures (Scheme 4).[9] Larger-scale resolutions were carried out in toluene instead of neat ethyl acetate or isopropyl ether, with ethyl acetate as the acylating agent, and afforded 41% yields (82% of theoretical maximum) of (*S*)-amine **2** in high enantiopurity (97% ee). Typically, the amine was isolated as the solid bis-HCl salt, due to its enhanced stability and handling convenience over the liquid freebase. It should be noted that it was possible, in small-scale proof of concept experiments, to isolate the acetamide **10**, racemize the stereocenter at elevated temperature (150 °C, neat), and hydrolyze the acetamide to provide **rac-2**, thus providing a potential pathway for recycling the reagent.[9]

Development of the aminoaldehyde (**3**) synthesis was initiated from 4-aminobutyric acid and made use of the phthaloyl protecting group[11] (Scheme 5) to generate **11**. Reduction of the acid was accomplished *via* hydrogenolysis of the acid chloride **12**.[16] A point of note is that significant variability in the efficiency of the hydrogenolysis during early development was traced to carryover of *p*-TsOH which was initially used in the formation of **11**. Reduction of the residual *p*-toluenesulfonic acid, or sulfonyl chloride after treatment with oxalyl chloride, provided 4-methylbenzenethiol as a potential catalyst poison. Its presence was confirmed by the isolation of the thioester side product[17] **13** as a contaminant in **3**. Once the *p*-TsOH was removed from the process, however, the hydrogenolysis became predictable and scaleable to provide up to 30-kg amounts of **3**. Interestingly, the reaction rate for the protection of the amine was only decreased by approximately 2-fold in the absence of *p*-TsOH. By using 10% Pd/C as a catalyst with 15 psi H_2(g) at ambient temperature, the hydrogenolysis reaction was complete in approximately 6 h. Both **11** and **3** were isolated as solids in high purity (>95% by HPLC) through precipitative workup procedures.

The benzimidazole fragment **4** was generated by protecting commercially available 2-chloromethylbenzimidazole with BOC.[14] Changing the reaction solvent from THF to DMF enabled the reaction to be performed at concentrations up to 2 M, and at an increased reaction rate. In THF, the reaction typically required 4−6 days to consume the reagent, whereas in DMF the reaction was typically complete in 12−16 h. Again, a precipitative workup was applied to isolate **4** in 96+% area purity by HPLC.

Assembly of 1. For the coupling steps, efforts were focused on optimizing reaction conditions to minimize the formation of side products so that chromatographic purifications could be avoided. Initial evaluation of the reductive amination-based

(9) (a) Skupinska, K. A.; McEachern, E. J.; Baird, I. R.; Skerlj, R. T.; Bridger, G. J. *J. Org. Chem.* **2003**, *68*, 3546–3551. (b) Bridger, G. J.; Skerlj, R. T.; Kaller, A.; Harwig, C.; Bogucki, D.; Wilson, T. R.; Crawford, J.; McEachern, E. J.; Atsma, B.; Nan, S.; Zhou, Y.; Schols, D.; Smith, C. D.; DiFluri, R. Preparation of azolulmethylaminotetrahydroquinolines and related compounds as chemokine receptor binding agents. PCT Int. Appl. WO 0222600, 2002.

(10) Bridger, G. J.; Pelus, L. M. Chemokine combinations to mobilize progenitor/stem cells. U.S. Pat. 20060035829, 2006.

(11) Hamilton, R.; Walker, B. J.; Walker, B. *Tetrahedron Lett.* **1993**, *34*, 2847–2850.

(12) Ley, S. V.; Marsden, S. P.; Norman, J.; Griffith, W. P. *Synthesis* **1994**, 639–666.

(13) Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Marynaoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849–3862.

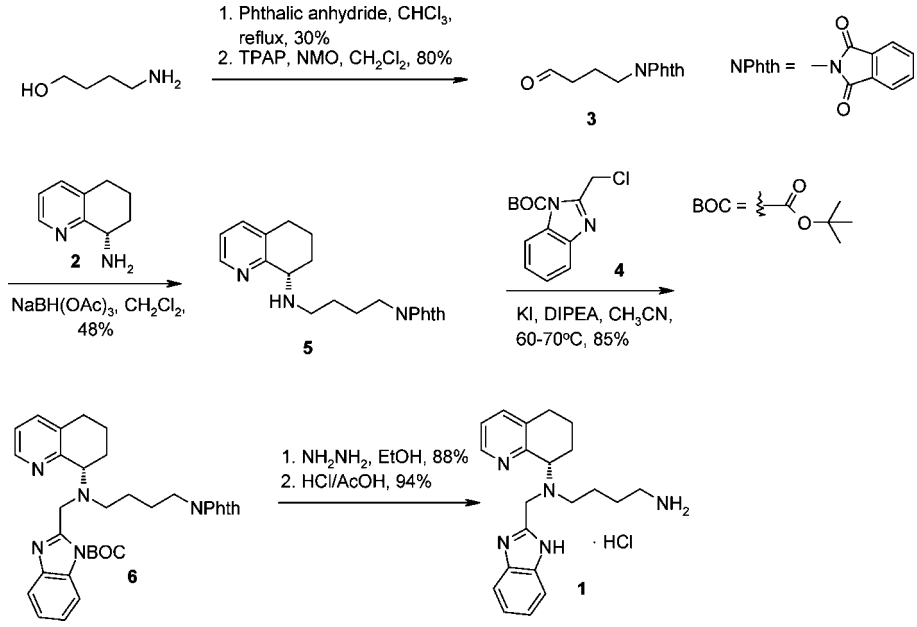
(14) An, H.; Wang, T.; Mohan, V.; Griffey, R. H.; Cook, P. D. *Tetrahedron* **1998**, *54*, 3999–4012.

(15) Beschke, H. *Aldrichimica Acta* **1978**, *11*, 13–16.

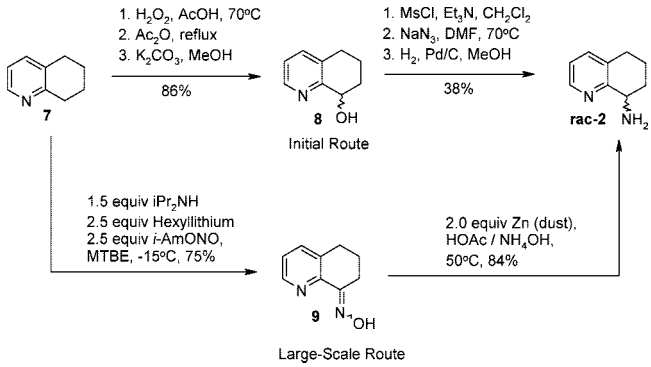
(16) Balenović, K.; Jambrešić, I.; Furić, I. *J. Org. Chem.* **1952**, *17*, 1459–1462.

(17) McElhinney, R. S.; McCormick, J. E.; Bibby, M. C.; Double, J. A.; Radacic, M.; Dumont, P. *J. Med. Chem.* **1996**, *39*, 1403–1412.

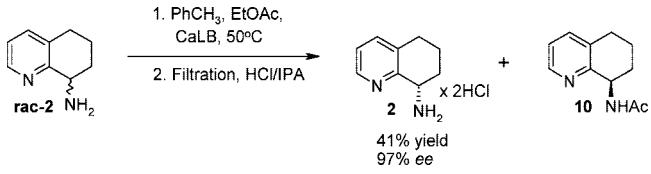
Scheme 2



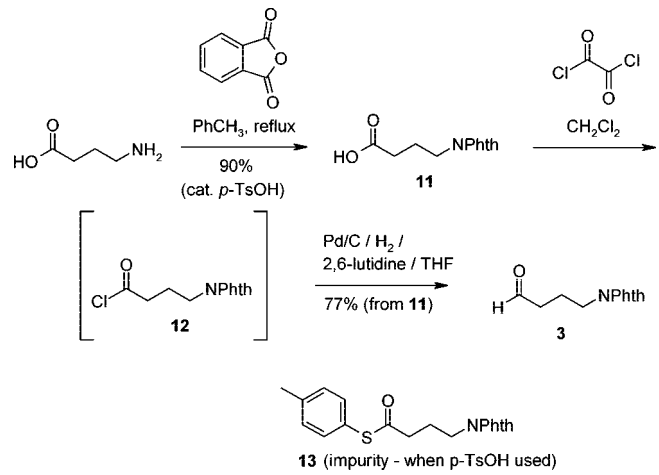
Scheme 3



Scheme 4



Scheme 5



coupling of **2** and **3** using sodium borohydride or sodium triacetoxyborohydride revealed that a complicated mixture of side products was formed (Scheme 6). In general, acidic reaction conditions favored the aldol-type self-condensation of **3** to form **15** (identified by ^{1}H NMR and LC/MS as a presumed mixture of E/Z isomers). The di-addition product **16** was formed if the aldehyde **3** was present in excess, or if the relative rate of imine **14** reduction was fast relative to the condensation of chiral amine **2** and *N*-phthaloyl aldehyde **3** (*i.e.* **5** was available as a reagent during the course of reaction). Overreduction (to generate **17**) occurred if sodium borohydride or other strong reducing agents were used.

In the initial small-scale syntheses using sodium triacetoxyborohydride, the practical solution to balance side-reactivity issues was to use 1.5 equiv of chiral amine reagent **2,** relative to the aldehyde **3,** to suppress di-addition. Following reaction workup, the desired secondary amine **5** was purified by silica gel column chromatography. With these methods, it was possible to isolate **5** in 45−70% yields from **3**. For large-scale synthesis, however, it was not feasible to use such an excess of the comparatively costly substrate **2** nor was it practical to employ silica gel column chromatography.

The first goal of the synthetic development was to attempt to carry out the reductive amination with an equimolar stoichiometry of **2** and **3**. Early attempts were based on a preformation of the imine **14** *via* a dehydrative coupling (monitored by ^{1}H NMR). An examination of various dehydrating agents and solvents led to the selection of potassium carbonate in THF as a preferred combination to form **14**. The basic conditions during dehydration suppressed aldol-type condensations, and the carbonate was easily removed by filtration. In comparison, when magnesium sulfate was used, it led to the formation of higher levels of impurities (>5 mol % by ^{1}H NMR).

Having established that the imine **14** could be prepared as a discrete reagent (formed in >95% conversions by ^{1}H NMR), various reducing agents were examined (Table 1). Unfortunately, the commercial hydride-based reagents proved to be

Scheme 6

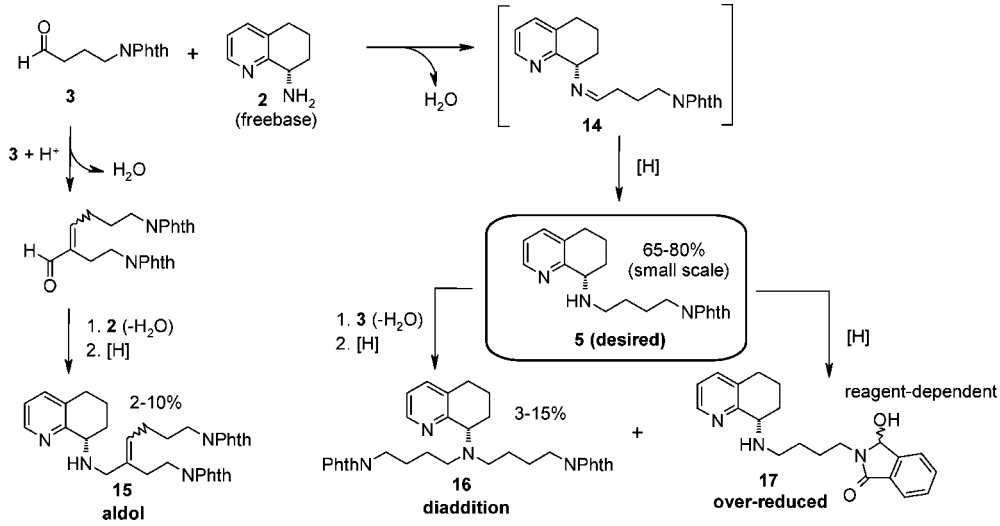
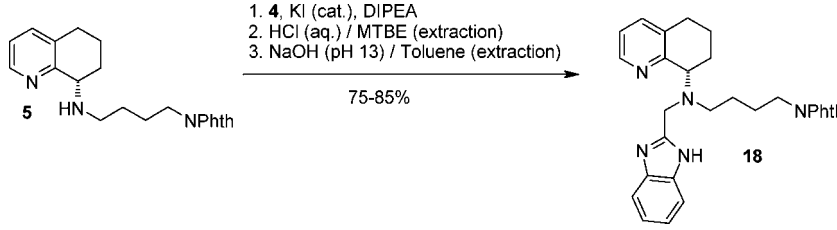


Table 1. **Reagent selection for reduction of imine 14**

entry	reagent/solvent/temperature	conversion to **5**[a] (%)	side products[b]
1	$NaBH(OAc)_3$ (1.5 equiv)/CH_2Cl_2 (or THF)/ambient	70−75	∼10% di-addition (**16**)
2	$NaBH_4$ (1.0 equiv)/THF/−20 °C to ambient	60	∼20% over-reduced (**17**)
3	$NaBH_4$ (1.0 equiv) + AcOH (1.5 equiv)/THF/−20 °C	80	<5% over-reduced (**17**)
4	$NaBH_4$ (1.0 equiv) + $ZnCl_2$ (1.1 equiv)/THF/−10 °C	80−90	∼2% aldol (**15**)

[a] Yields determined by ^{1}H NMR of reaction mixtures prior: product integral/imine + product integral. [b] Measured by ^{1}H NMR integration as fraction of product integral.

Scheme 7



unsuitable: sodium triacetoxyborohydride gave the di-addition side product (**16**) as an impurity, presumably *via* hydrolysis of the imine *in situ*. In comparison, use of sodium borohydride led to a degree of overreduction (**17**). Sodium cyanoborohydride was not considered for toxicological reasons. Conversely, attempts at catalytic hydrogenation (using Pd/C) were low-yielding.

We determined that the ideal chemical reducing agent should have a reducing activity between that of sodium triacetoxyborohydride and sodium borohydride. Indeed, evaluation of such attenuated borohydride agents described in literature reports, such as sodium acetoxyborohydride[18] and zinc borohydride[19] (reagents generated *in situ*) gave a cleaner, higher-yielding reaction profile. Continued experimentation led to the selection of a 1.1:1 ratio of zinc chloride:sodium borohydride as the optimal reducing agent (Table 1, entry 4), since it not only gave a clean, high-yielding reaction profile, but also enabled the reaction to be run at concentrations up to 1 M in substrate (vs the 0.1 M maximum using sodium acetoxyborohydride reagent).

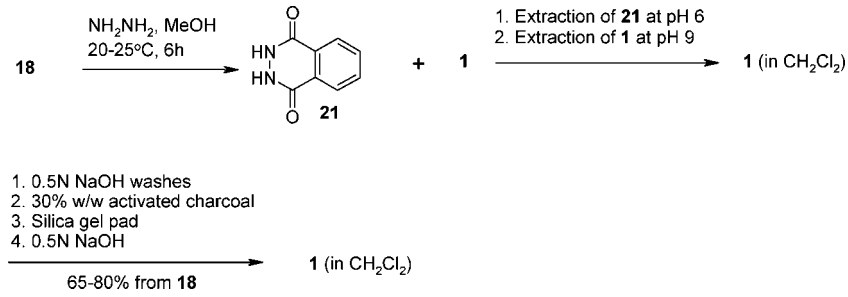
To avoid chromatography, a procedure was developed to isolate the secondary amine **5** as a solid in 90+% purity using diisopropyl ether as an antisolvent to induce precipitation. By controlling the solvent ratios and temperatures in the precipitative conditions, the product **5** could be isolated in sufficient purity (90+% area by HPLC) to eliminate the need for column chromatography. The process was successfully carried out on up 20-kg scales, with yields of approximately 65% from the amine **2** (bis-HCl salt).

Installation of the benzimidazole **4** was accomplished with only minor modifications of the early developed work (Scheme 7). The reaction was conducted at ∼0.5 M in acetonitrile at 50 °C, using *N*,*N*-diisopropylethylamine (DIPEA) as a base in the presence of catalytic potassium iodide. Careful control of stoichiometry, and an HPLC test for reaction completion was implemented to ensure complete reaction (typically 6−12 h). At the completion of the reaction, the carbamate protecting group was hydrolyzed with aqueous HCl, which also served to hydrolyze any residual benzyl chloride **4**. The tertiary amine then became soluble in the aqueous layer, and nonpolar impurities could be removed *via* a wash with *tert*-butyl methyl ether (MTBE). After adjusting the pH to approximately 13, the

(18) Reetz, T. *J. Am. Chem. Soc.* **1960**, *82*, 5039–5042.

(19) Bhattacharyya, S.; Chatterjee, A.; Duttachowdhury, S. K. *J. Chem. Soc., Perkin. Trans. 1* **1994**, 1–2.

Scheme 8



product **18** was selectively extracted into toluene. It should be noted that excessively basic conditions (pH ≥ 14) could lead to the partial hydrolysis of the phthalimide protecting group. The tertiary amine **18** was typically stored as a toluene stock solution at −10 °C.

The final goal of the synthetic development was to isolate a pure, stable, crystalline form of **1**. Although hydrazine is often used for cleaving the phthaloyl protecting group,[20] the high toxicity of the reagent provided a compelling incentive to search for alternatives. A number of amines were evaluated for the deprotection, including methylamine,[21] butylamine,[22] hydroxylamine hydrochloride[23] with sodium methoxide), ammonia, and ethylene diamine.[24] Generally, the reactions were conducted in alcohol solvents (ethanol or butanol), at both room and elevated (80 °C) temperatures. The introduction of the first equivalent of nucleophile—to form a mixed diamide—usually proceeded efficiently and could be tracked by NMR or LC/MS. The problem step was the displacement of **1** from the phthalic diamide with a second equivalent of nucleophile. In the case of methyl- and butylamine, the reaction proceeded to only 30−40% completion following overnight exposure to an excess (>15 equiv) of the reagent at 80 °C. The conversions with hydroxylamine and ammonia were even lower. Ethylenediamine was more reactive but was ultimately abandoned as a reagent since the pendant primary amine functionalities on the phthalic diamide side product would make extractive removal of the "impurity" from an aqueous solution of the polyamine **1** difficult. Attempts at using a reductive removal of the phthalimide with reagents such as sodium borohydride/acetic acid[25] were also unsuccessful due to racemization of the chiral center (presumably occurring upon exposure to hot acetic acid). Consequently, hydrazine remained the only practical reagent, in spite of the hazards, for the transformation at large scale.

Eventually, suitable conditions were developed for the reaction: eight equivalents of hydrazine in methanol at room temperature. Typically, the hydrolysis was complete in 16−24 h. The isolation of **1** was based on the selective extraction-based removal of the cyclic diamide side product **21**, as shown in Scheme 8: it was possible to selectively extract **21** into dichloromethane at pH 6, leaving **1** in the aqueous layer.

The workup consisted of repeated washes of the dichloromethane solution of **1** with dilute aqueous base (0.5 N NaOH) in order to remove residual hydrazine. Treatment with activated charcoal served to effectively decolorize the solution of **1**, and a silica gel pad (elution with 1% NH_4OH, 79% dichloromethane, and 20% methanol) was applied to remove residual low-polarity and high-polarity (baseline) impurities. At 50−70 g laboratory scale, approximately seven fractions of 500 mL each were collected, the product would be present in fractions 3−7, and the high-polarity impurity would remain on the column. It should be noted that the silica pad was successfully applied in the pilot plant (at 7-kg scale) by filling a Nutsche filter with silica and eluting 50-L fractions. A final wash with 0.5 M NaOH was applied to the pooled, concentrated fractions to ensure that **1** was not partially protonated, due to exposure to silica gel, and was later used in conjunction with an in-process, specifically developed HPLC test to ensure that the residual hydrazine level was <8 ppm. At this stage in the sequence, **1** was typically 98+% area pure by HPLC analysis of the solution.

Isolation of 1 as a Crystalline Solid. After extensive laboratory experimentation, ethyl acetate was selected as the recrystallization solvent, as it enabled the removal of residual dichloromethane from the solution of **1** *via* a vacuum distillation solvent exchange. The recrystallization was then conducted by heating to 65−67 °C to solubilize the substrate and then cooling to 40 °C and seeding to induce crystallization.

The crystallization of **1** was capable of rejecting some chemical impurities, though more importantly, it enhanced the enantiomeric excess (ee) of **1**. In small-scale laboratory trials at 50-g scale, it was possible to upgrade the ee from 85% to 98%. This property was effectively exploited in the large-scale (10 kg) conversion of a 90% ee batch of **1**, which yielded crystalline material with an enantiopurity of >99% ee. An X-ray crystal structure determination confirmed the crystalline nature of **1**, whose asymmetric centre had previously been determined to be (*S*) *via* analysis of **2** (as a *Re* complex).[9]

Conclusions

An efficient four-step synthetic route to crystalline **1** has been developed which employs only a single chromatographic step: a silica gel pad in the purification of **1** prior to crystallization. The key step in the sequence is a novel reductive amination, employing an attenuated borohydride reducing agent, between

(20) Sasaki, T.; Minamoto, K.; Itoh, H. *J. Org. Chem.* **1978**, *43*, 2320–2325.
(21) (a) Sen, S. E.; Roach, S. L. *Synthesis* **1995**, 756–758. (b) Ojima, I.; Lin, S.; Chakravarty, S.; Fenoglio, I.; Park, Y. H.; Sun, C.-M.; Appendino, G.; Pera, P.; Veith, J. M.; Bernacki, R. J. *J. Org. Chem.* **1998**, *63*, 1637–1645.
(22) Kanie, O.; Crawley, S. C.; Palcic, M. M.; Hindsgaul, O. *Carbohydr. Res.* **1993**, *243*, 139–164.
(23) Carroll, F. I.; Berrang, B.; Linn, C. P. *J. Med. Chem.* **1985**, *28*, 1564–1567.
(24) Wentworth, P.; Janda, K. D. *Synlett* **1997**, 537–543.
(25) Osby, J. O.; Martin, M. G.; Ganem, B. *Tetrahedron Lett.* **1984**, *25*, 2093–2096.

the chiral (*S*)-amine **2** and the aminobutyraldehyde **3** to afford the secondary amine **5**. *N*-alkylation with **4** provided the requisite protected tertiary amine **18**, which was purified *via* a pH-selective extraction. Hydrolysis of the phthalimide protecting group with hydrazine was followed by further pH-selective extraction methods to afford **1** in solution. In the final step, a crystallization procedure for the isolation of **1** was developed, which allows for the enhancement of both chemical and chiral purity. The synthetic methods described herein have been successfully applied on large scale (with minor modification) to supply 7-kg amounts of **1**.

It should be noted that an alternate procedure was subsequently developed and has been carried out on approximately 20-kg scale to provide a crystalline salt form of AMD070 without intermediate isolation of crystalline **1**; however, the synthetic development was suspended before the process for a high-purity conversion to the crystalline freebase could be developed. The procedure uses an *N*,*N*-di-BOC to protect the aminoaldehyde fragment, which enables the removal of hydrazine and the silica gel column from the synthetic process. We intend to report on these procedures in due course.

Experimental Section

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded using $CDCl_3$ solvent with TMS as an internal standard (unless otherwise noted). HPLC was performed using reversed-phase conditions.

Isolation of (*S*)-8-Amino-5,6,7,8-tetrahydroquinoline Free Base (2). (*S*)-8-amino-5,6,7,8-tetrahydroquinoline hydrochloride (80 g, 0.361 mol) was dissolved in deionized water (200 mL) and neutralized to pH 7 with a 10 N sodium hydroxide solution (~32 mL), then diluted with additional deionized water (100 mL). The mixture was extracted with dichloromethane (3 × 300 mL) until 8-acetamido-5,6,7,8-tetrahydroquinoline was not present by thin layer chromatography (TLC conditions: dichloromethane/methanol/ammonium hydroxide (88:10:2). 8-Acetamido-5,6,7,8-tetrahydroquinoline $R_f = 0.6$. (*S*)-8-amino-5,6,7,8-tetrahydroquinoline $R_f = 0.5$). The aqueous layer was adjusted to pH 13−14 with 10 N sodium hydroxide (30 mL) and was extracted with dichloromethane (3 × 300 mL). The combined organic layers were dried (sodium sulfate) and concentrated *in vacuo* to afford (*S*)-8-amino-5,6,7,8-tetrahydroquinoline (**2**, 51.3 g, 96%) as a dark-brown oil. Note: the amine freebase should be stored under an inert atmosphere at low temperatures (−10 °C or below) if not used immediately. ^{1}H NMR ($CDCl_3$) δ 1.64−1.84 (m, 2H), 1.94−2.01 (m, 1H), 2.14−2.23 (m, 1H), 2.69−2.87 (m, 2H), 3.99 (dd, 1H, J = 7.7, 5.3 Hz), 7.06 (dd, 1H, J = 7.7, 4.4 Hz), 7.36 (d, 1H, J = 7.5 Hz), 8.41 (d, 1H, J = 4.4 Hz). Chiral purity was determined by gas chromatography to be 97.5% ee (separated by chiral GC, J&W CycloSil B column, isothermally run at 130 °C for 40 min, (*S*)-(+)-enantiomer$_{rt}$ = 26.3 min, (*R*)-(−)-enantiomer$_{rt}$ = 28.7 min).

Preparation of 4-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)butyraldehyde (3). 4-Aminobutyric acid (35 g) and phthalic anhydride (52.5 g) were suspended in toluene (300 mL) and the reaction mixture was adjusted to reflux. Water was removed by a Dean−Stark trap. In-process NMR was performed to confirm the completion of the reaction (typically requires 6−12 h and is evaluated by comparison of α-proton resonances, expecting >95% conversion). The reaction was cooled to a maximum temperature of −8 °C and agitated at this temperature for ~1 h. The reaction mixture was filtered, and the filter cake was rinsed with toluene (−8 °C, 75 mL). The filter cake was dried under vacuum at a maximum temperature of 50 °C. The protected acid **11** was isolated in a yield of 84% (64 g, 99.5% HPLC purity). The acid **11** (66.4 g) was then dissolved in dichloromethane (280 mL), and oxalyl chloride (40 g) was added over ~8 h (to control the gas evolution). The completion of the reaction was confirmed by in-process NMR (α-proton resonances). The solution was then concentrated. Tetrahydrofuran (300 mL) and 2,6-lutidine (31.7 g) were added. The solution was transferred to a hydrogenator, rinsing forward with THF (2 × 30 mL). To this solution was added a slurry of 10% Pd/C (3.3 g) in dichloromethane (10 mL). The mixture was hydrogenated under 15 psi H_2 gas at 22 °C. After 6 h, in-process NMR analysis was performed to monitor conversion to the aldehyde (>95% conversion). The reaction mixture was filtered through a Celite pad, and the filter cake was rinsed with THF (30 mL). The filtrate was combined and concentrated to about 120 mL; 1 N HCl solution (100 mL) was added, and the resulting solution was agitated at 22 °C for 16 h. THF was removed under vacuum, and then dichloromethane (150 mL) was added. The organic phase was separated and the aqueous phase discarded. The organic phase was returned to the flask. An aqueous solution of 8% sodium bicarbonate (200 mL) was added, and the mixture was agitated for 60 min. The layers were separated, and the organic layer was concentrated to about 250 mL; then heptane (200 mL) was added. After agitating the mixture for 15 min the solution was concentrated to ~250 mL (to remove dichloromethane), and additional heptane (200 mL) was added. The mixture was adjusted to <5 °C and agitated at that temperature for 1 h. The solid was filtered, and the filter cake was rinsed with heptane (150 mL). The cake was dried under vacuum at ambient temperature. The yield of **3** was 77% (96.6% area HPLC purity). Spectral data matched literature[10] values: ^{1}H NMR ($CDCl_3$) δ 2.00 (m, 2H), 2.50 (t, 2H, J = 6.3 Hz), 3.73 (t, 2H, J = 6.3 Hz), 7.70 (m, 2H), 7.82 (m, 2H), 9.75 (s, 1H).

Preparation of *tert*-BOC-2-chloromethylbenzimidazole (4). 2-Chloromethylbenzimidazole (285 g, 1.71 mol) and di-*tert*-butyl dicarbonate (410 g, 1.88 mol) were suspended in DMF (850 mL). *N*,*N*-diisopropylethylamine (30 mL, 0.17 mol) was added to the agitated reaction mixture. The reaction mixture was agitated at 25 °C for 5 h (an in-process ^{1}H NMR confirmed >5:1 ratio of **4**:2-chloromethylbenzimidazole by comparison of benzylic resonances). Then additional di-*tert*-butyl dicarbonate (82 g, 0.36 mol) was added, and the reaction mixture was warmed to 40 °C and agitated for a further 6 h (until >97% conversion by ^{1}H NMR). Water (250 mL) was added, and the reaction mixture was agitated for 2 h at 40 °C. The reaction mixture was then concentrated under vacuum at 40 °C to approximately half-volume. The temperature was reduced to 22 °C, water (100 mL) and DMF (200 mL) were added, and the mixture agitated for 2 h, during which time a precipitate formed. The contents were filtered through a glass frit. The filter cake was washed with water (250 mL). The filter cake was

then dried under vacuum. The yield of (**4**) was 365 g (80%). By HPLC: 96% area pure. Spectral data matches literature[14] values: ^{1}H NMR ($CDCl_3$) δ 1.73 (s, 9H), 5.06 (s, 2H), 7.37 (m, 2H), 7.73 (dd, 1H, J = 4.1, 0.9 Hz), 7.98 (dd, 1H, J = 4.1, 0.9 Hz).

Preparation of Secondary *S*)-2-[4-(5,6,7,8-Tetrahydroquinolin-8-ylamino)butyl]isoindole-1,3-dione (5).

*Part 1. Imine Formation [(S)-2-[4-(5,6,7,8-Tetrahydroquinolin-8-ylamino)but-4-enyl]isoindole-1,3-dione (**14**)].* To a solution of 8-amino-5,6,7,8-tetrahydroquinoline (**2**, 50.0 g, 338 mmol, 1.0 equiv) in THF (1700 mL, [0.2 M]) in a 3-L flask was added 4-(*N*-phthalimidylamino)butanal (**3**, 73.3 g, 338 mmol, 1.0 equiv) and 325 mesh potassium carbonate (325 mesh, 46.6 g, 338 mmol, 1.0 equiv). The mixture was then stirred for 1 h and filtered. An NMR aliquot is used to monitor complete imine (**14**) formation, using the triplet at 4.31 ppm as a diagnostic resonance for product formation (reaction is deemed complete when <3 mol % **2** and **3** exist relative to **14**). ^{1}H NMR ($CDCl_3$) δ 1.76−2.19 (series of m, 6H), 2.35 (m, 2H), 2.78 (m, 2H), 3.73 (m, 2H), 4.31 (t, 1H, J = 5.1 Hz), 7.05 (dd, 1H, J = 7.8, 4.8 Hz), 7.38 (d, 1H, J = 7.8 Hz), 7.69 (m, 2H), 7.80 (m, 2H), 7.82 (t, 1H, J = 4.1 Hz), 8.38 (d, 1H, J = 4.8 Hz).

Part 2. Reducing Agent Formation. To a flask containing THF (160 mL) was added zinc(II) chloride (35.1 g, 257 mmol). A mild exotherm occurred upon dissolution. Sodium borohydride (8.85 g, 234 mmol) was then added slowly. The mixture was stirred for 1 h, during which time a homogeneous solution formed. The solution was cooled to −20 °C.

Part 3. Reduction. A solution of imine **14** (234 mmol) in THF (80 mL) was cooled to −20 °C, and was then added slowly to the cooled solution of zinc chloride and sodium borohydride *via* cannula, maintaining the internal temperature of the reaction flask between −10 and −20 °C. The reaction was then stirred at −15 °C for 2 h. The reaction completion was confirmed by ^{1}H NMR ($CDCl_3$) by the disappearance of the imine-specific triplet resonance in the proton NMR at 4.3 ppm, or the triplet at 7.82 ppm (<2 mol % residual reagent). A solution of 6 N aqueous HCl was added dropwise, maintaining the temperature below −5 °C, until the pH of the aqueous layer measured 2−3 (by pH paper). The reaction was allowed to warm to room temperature, and a solution of 13% aqueous sodium carbonate was added until the pH reached 4, as measured with pH paper. The reaction flask was placed under vacuum, and the THF solvent was removed by distillation. Water (345 mL) and dichloromethane (260 mL) were then added. The mixture was agitated, and then the aqueous and organic layers were separated. The organic layer was washed with concentrated aqueous ammonium hydroxide (110 mL) and then water (150 mL). The dichloromethane solution was concentrated to ∼75 mL under vacuum, then diisopropyl ether (520 mL) was added. **Caution:** It should be noted that diisopropyl ether is very flammable and can form explosive peroxides upon storage. As a matter of safety, the solvent should be tested for peroxide content with a commercial peroxide test strip prior to use. The solution was concentrated under vacuum to ∼160 mL and was then cooled slowly to −10 °C, with agitation, during which time a precipitate formed. The precipitate (**5**) was filtered, and washed with diisopropyl ether. After drying under vacuum, the desired product was obtained in a 68.6 g yield (84%) as a light-brown crystalline solid. ^{1}H NMR ($CDCl_3$) δ 1.59−2.17 (series of m, 8H), 2.74 (m, 4H), 3.72 (t, 2H, J = 7.2 Hz), 3.72 (m, 1H), 7.04 (dd, 1H, J = 7.8, 4.8 Hz), 7.35 (dd, 1H, J = 7.8, 0.6 Hz), 7.70 (m, 2H), 7.82 (m, 2H), 8.36 (dd, 1H, J = 4.8, 0.6 Hz). ^{13}C NMR ($CDCl_3$) δ 20.0, 27.0, 28.1, 29.1, 29.3, 47.7, 58.5, 122.2, 123.5 (2C), 132.5, 134.2 (2C), 137.3, 147.2, 158.3, 168.8 (2C). ES-MS *m*/*z* 350 (M + H); Purity by HPLC 94% area. Chiral purity 97% ee (by chiral HPLC).

Preparation of Tertiary Amine (*S*)-2-{4-[1-Methyl-1*H*-benzimidazol-2-ylmethyl)-(5,6,7,8-tetrahydroquinolin-8-yl)amino]butyl}isoindole-1,3-dione (18). A 5-L three-necked flask was charged with secondary amine **5** (65 g, 0.186 mol, 1.0 equiv), acetonitrile (0.37 L), *N*,*N*-diisopropylethylamine (48 mL, 0.28 mol, 1.5 equiv), *N*-BOC-2-chloromethylbenzimidazole (**4**, 52.0 g, 196 mmol, 1.05 equiv), and potassium iodide (3.08 g, 18.6 mmol, 0.1 equiv) in that order. The mixture was stirred mechanically at a 50 °C internal temperature under a nitrogen atmosphere for 4 h. A ^{1}H NMR of an aliquot indicated the reaction was complete (the appearance of a multiplet at 4.18 ppm ($CDCl_3$) is indicative of product formation, >95% conversion required), and the mixture was then allowed to cool to RT. Water (300 mL) and *tert*-butyl methyl ether (500 mL) were added. The pH was adjusted to pH 3 by addition of 6 N HCl (∼35 mL). The aqueous and organic layers were separated, the ether layer discarded, and the aqueous layer was stirred at RT for 8−16 h. Toluene (600 mL) was added, and the pH was adjusted to 13 with 10 M sodium hydroxide. The mixture was then filtered through a pad of diatomaceous earth (∼8 cm diameter × 1 cm depth), washing forward with approximately 50 mL of toluene. After separation of the aqueous and organic layers, the aqueous layer was extracted with toluene (500 mL). The combined organic layers were washed with 5% w/w aqueous sodium hydroxide (20 mL), and the tertiary amine **18** was held at −10 °C (or colder) as a stock solution. Yield 74% (by ^{1}H NMR estimate). A sample of the solution was concentrated: ^{1}H NMR ($CDCl_3$) δ 1.42−2.17 (series of m, 8H), 2.74 (m, 4H), 3.52 (t, 2H, J = 7.2 Hz), 3.98 (m, 1H), 4.02 (d, 1H, J = 16.8 Hz), 4.11 (d, 1H, J = 16.8 Hz), 7.04 (dd, 1H, J = 7.8, 4.8 Hz), 7.15 (m, 4H), 7.35 (d, 1H, J = 7.8 Hz), 7.40 (br s, 1H (N*H*)), 7.64 (m, 2H), 7.76 (m, 2H), 8.81 (d 1H, J = 4.8 Hz). ES-MS *m*/*z* 480.3 (M + H (LC/MS)); Purity by HPLC 93% area. Chiral Purity 97.5% ee by chiral HPLC.

Preparation of (*S*)-*N*′-(1*H*-Benzimidazol-2-ylmethyl)-*N*′-(5,6,7,8-tetrahydroquinolin-8-yl)butane-1,4-diamine (1, solution). Tertiary amine **18** (0.137 mol, in toluene) was concentrated under vacuum to a foam (0.25 equiv $PhCH_3$ by ^{1}H NMR). The residue was taken up in methanol (550 mL), transferred to a 2 L round bottomed flask, which was purged with nitrogen, and treated with hydrazine hydrate (63 mL, 1.1 mol, 8.0 equiv). The mixture was stirred mechanically in a capped vessel for 20 h at 25 °C (monitored by HPLC, >98% conversion required). The thick beige slurry was filtered through a glass frit (washing forward with 500 mL of dichloromethane). The filtrate was washed with 200 mL of 0.5 M NaOH. The aqueous layer was discarded. To the organic layer was added water (300 mL), and 6 M HCl was added until the pH was adjusted to 5−6 (with stirring). The layers were separated, and the organic

layer was discarded. To the aqueous layer was added activated charcoal (Norit G-60, 5 g), and the mixture was stirred for 1 h. The suspension was filtered, and to the filtrate (pale yellow) was added dichloromethane (200 mL). The pH of the aqueous layer was adjusted to 13 with the addition of 4 M NaOH. The layers were then separated, and the organics were concentrated. The residue was taken up in 50 mL of dichloromethane and was passed through a pad of silica gel (60 g, preconditioned with eluent: 15% methanol, 2% conc. NH_4OH in dichloromethane). The product was eluted with the preconditioning solvent mixture in fractions of ~500 mL volume. Typically 7−8 fractions were collected. The combined fractions containing product were concentrated, then taken up in 500 mL of dichloromethane, washed with 100 mL of 0.5 N NaOH and then water (100 mL). The organic layer was dried over sodium sulfate. The solution of **1** in dichloromethane was stored at low temperature (−10 °C or lower) in preparation for crystallization.

Crystallization of 1. The solution of **1** in dichloromethane (47.8 g, 0.137 mol theoretical yield) was concentrated to a volume of approximately 120 mL under vacuum (at 25 °C). Ethyl acetate (600 mL) was then added, and the mixture was concentrated under vacuum to a final volume of 350 mL. The solvent ratio was checked by ^{1}H NMR (if the dichloromethane content is >1 mol% with respect to ethyl acetate, the concentration cycle was repeated). The suspension was then heated, with stirring, to an internal temperature of 65−67 °C (over a period of 30−60 min). The mixture was then stirred at 65 °C for five minutes, until all solid material dissolved, and the solution was transparent and homogeneous. The solution was allowed to cool to 45 °C, at which point 30 mg of a sample of crystalline **1** was added to seed crystallization. The solution was allowed to slowly cool to room temperature (over 3 h), with stirring. The flask was cooled to 5 °C for 1 h. The off-white crystals were isolated by suction filtration (through filter paper), and the crystals were washed with 50 mL of cold (0 °C) ethyl acetate. The filter cake was placed under a cone of nitrogen and was allowed to dry for 5 min while still under vacuum pressure. The crystals were transferred to a flask and were placed under vacuum for 1 h. The crystals were placed in a 40 °C vacuum oven (<1.0 mmHg) and were dried for 24 h. The yield of (off-white) crystalline **1** was 32.45 g (67%). ^{1}H NMR ($CDCl_3$) δ 1.23−1.49 (m, 4H), 1.62−1.77 (m, 1H), 1.85−1.97 (m, 1H), 2.00−2.10 (m, 1H), 2.16−2.26 (m, 1H), 2.51 (t, 2H, J = 6.8 Hz), 2.54−2.62 (m, 1H), 2.67−2.78 (m, 1H), 2.81−2.92 (m, 1H), 7.15 (d, 1H, J = 7.6 Hz), 7.18−7.23 (m, 2H), 7.59 (br s, 1H), 8.60 (d, 1H, J = 4.4 Hz). ^{13}C NMR ($CDCl_3$) δ 21.8, 23.9, 26.4, 29.6, 31.4, 42.1, 49.8, 50.9, 62.2, 115.2, 121.8 (2C), 122.5, 134.9, 137.7 (2C), 147.0 (2C), 157.0, 157.9. ES-MS *m/z* 350 (M + H). Chemical purity: 99.4% (area HPLC). Chiral purity: >99% ee (chiral HPLC). Melting point: 108−110 °C. Anal. Calcd for $C_{11}H_{27}N_5$: C, 72.17; H, 7.79; N, 20.04. Found: C, 72.07; H, 7.71; N, 19.95.

Acknowledgment

We thank the following for their contributions: Raylo Chemicals Inc. (Edmonton, AB, Canada) and Aptuit (formerly Evotec, Abingdon, Oxon, UK) for collaborative development and for large-scale manufactures of AMD070 under GMP conditions; the analytical staff at AnorMED for their method development and analyses; Brian Patrick (University of British Columbia) for the crystal structure determination.

Supporting Information Available

Characterization and purity data for new compounds (**1**, **5**, **6**, and **18**), representative spectra for known compounds; CIF file and crystal structure report for **1**. This material is available free of charge *via* the Internet at http://pubs.acs.org.

Received for review April 22, 2008.

OP8000993