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Commercialization and Late-Stage Development of a Semisynthetic Antifungal API: Anidulafungin/D-Fructose (Eraxis)

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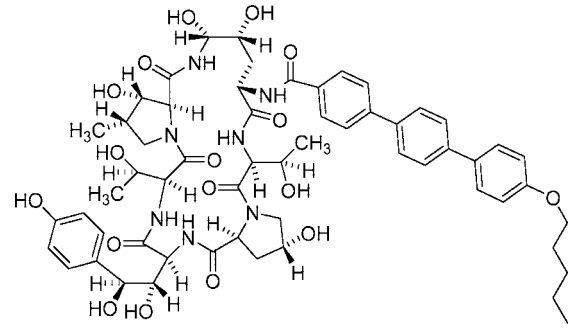
Abstract:

Many years ago anidulafungin 1 was identified as a potentially useful medicine for the treatment of fungal infections. Its chemical and physical properties as a relatively high molecular weight semisynthetic derived from echinocandin B proved to be a significant hurdle to its final presentation as a useful medicine. It has recently been approved as an intravenous treatment for invasive candidaisis, an increasingly common health hazard that is potentially life-threatening. The development and commercialization of this API, which is presented as a molecular mixture of anidulafungin and D-fructose is described. This includes, single crystal X-ray structures of the starting materials, the echinocandin B cyclic-peptide nucleus (ECBN·HCl) and the active ester 1-({[4″-(pentyloxy)-1,1′:4′,1″-terphenyl-4-yl]carbonyl}oxy)-1*H*-1,2,3-benzotriazole (TOBt). Details of the structure and properties of starting materials, scale-up chemistry and unusual crystallization phenomena associated with the API formation are discussed.

Introduction

Anidulafungin, **1** is a recently approved semisynthetic antifungal[1] that is used to treat invasive infections, such as candidemia, other forms of candidia infections, and esophogeal candidiasis. The drug substance has been developed as a molecular mixture of anidulafungin with D-fructose that does not have a fixed stoichiometry because of the weak nature of the bonding forces between anidulafungin and D-fructose. The commercial synthesis, intermediate structures, and late-stage development of anidulafungin drug substance are described as well as the unusual characteristics of the cocrystallization[3] with D-fructose to form the bulk drug substance final form used to prepare the lyophilized dosage in the individual sterile pre-injection vials.



1
anidulafungin

Discussion

ECBN hydrochloride. Echinocandin B, **2**, is a metabolic product[4] produced by *Aspergillus nidulans var. echinulatus*, A 32204 with reported antibiotic and antifungal properties. Echinocandin B, **2**, has been deacylated enzymatically using an *Actinoplanes utahensis* culture, which removes the linoleoyl function from the α-amino group of the *N*-terminal dihydroxyornithine unit to yield cyclic peptide, echinocandin B nucleus, which was isolated as the hydrochloride salt, ECBN·HCl, **3** (Scheme 1) after treatment with dilute hydrochloric acid.[5]

A single crystal X-ray structure of **3** has not previously been reported. A trial structure was obtained by direct methods. ECBN contains 6 amino acid fragments and the cyclic polypeptide contains 5 standard amide links. The rare structural feature is an *N*-carboxyhemiaminaldiol functional group linking the ornithine derived moiety [(2*S*,4*R*,5*R*)-2,5-diamino-4,5-dihydroxypentanoic acid] and the methylproline derived moiety [(2*S*, 3*S*, 4*S*)-3-hydroxy-4-methylproline-2-carboxylic acid] in the cyclic peptide. This trial structure refined routinely up until a point.[6] The molecular architecture of ECBN·HCl, **3** is shown in Figure 1 and a stereoscopic presentation with position of water molecules in Figure 2. The 21 membered ring adopts a strain free conformation that is stable despite containing an unusual linkage. This linkage is stable to neutral and mildly acidic environments, but opens hydrolytically at pH > 8. As the pH of the environment increases the rate of ring opening is exponentially increased. Survival at pH 12 or higher is a matter of minutes.

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(1) Trade name Eraxis (United States of America), Ecalta (European Union).

(2) (a) Bergogne-Berezin, E. *Antibiotiques* **2007**, *9* (3), 212–215. (b) Debono, M.; Turner, W. W.; LaGrandeurL; Burkhardt, F. J.; Nissen, J. S.; Nichols, K. K.; Rodriguez, M. J.; Zweifel, M. J.; Zeckner, D. J. *J. Med. Chem.* **1995**, *38* (17), 3271–3281. (c) DeBono, M; Gordee, R. S. *Annul. Rev. Microbiol.* **1994**, *48*, 471–497. See pp 477−486 on glucan synthase inhibitors.

(3) Larew, L. A.; Milton, N; Sabatowski, J. L.; Moder, K. P. U.S. Patent 7,041,637 B2, May 9, 2006.

(4) Benz, F.; Knüsel, F.; Nüesch, J.; Treichler, H.; Voser, W.; Nyfeler, R.; Keller-Schierlexin, W. *Helv. Chim. Acta* **1974**, *57*, 2459–2477.

(5) (a) Boeck, L. D.; Fukuda, D. S.; Abbott, B. J.; DeBono, M. *J. Antibiot.* **1989**, *42* (3), 382–388. (b) DeBono, M.; Abbott, B. J.; Fukuda, D. S.; Barnhart, M.; Willard, K. E.; Molloy, R. M.; Michel, K. H.; Turner, J. R.; Butler, T. F.; Hunt, A. H. *Antibiotics* **1989**, *42* (3), 389–397.

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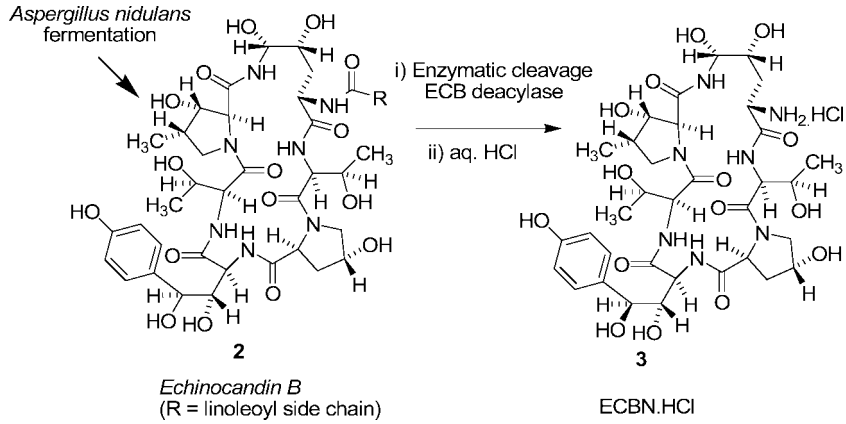
Scheme 1. **Formation of ECBN·HCl, 3**



The single crystal X-ray data presented above was obtained directly from crystals selected from bulk material produced by recovery from the fermentation process biomass. This substrate was the starting material for the synthetic part of the process.

ECBN·HCl, **3**, has a molecular weight 834.27 and is bristling with functionality, which provides a challenge and imposes limitations on the type of solvents and reagents that can be used to synthetically modify the natural product. As previously mentioned, **3** contains a rare structural feature, the *N*-carboxyhemiaminaldiol functional group. This group is the most reactive part of the cyclic peptide ring system and is subject to hydrolytic cleavage, which results in ring-opening and loss of biological activity. The safe operating pH range for **3** at room temperature (~20−25 °C) is between pH 3−5; between pH 7−8 after 30 h ~30−50% of **3** is lost to hydrolysis. Above pH 8 the rate of hydrolysis becomes very rapid, and at pH 10 ECBN loss to hydrolysis is ~95% after <20 h. The situation is clearly illustrated in Figure 3.

The initial product of hydrolysis, **4**, is thought to be the open-chain species formed by hydrolysis of the *N*-carboxyhemiaminaldiol functional group, while the final isolable product, **5**, is the result of formation of a pyrrolidino ring on the end of the peptide chain. Compounds **4** and **5** have different retention times on HPLC but the same molecular formula $C_{34}H_{51}N_7O_{15}$ (Scheme 2). Mass spectral observation confirms the molecular weight of ECBN **3** and **5** are the same, 797.81, but full detailed structural confirmation of **4** and **5** have not been obtained because of the complexity of these molecules. The above observations would be consistent with observations reported for the hydrolysis of other echinocandin B-related compounds similar to anidulafungin where an analogous cascade is reported.[7] Determination of the kinetics of hydrolysis of ECBN·HCl helped define the process robustness for plant operation during the coupling step. It was also used in development of alternative routes that may have been needed to avoid use of 1-*N*-hydroxybenzotriazole, HOBt.

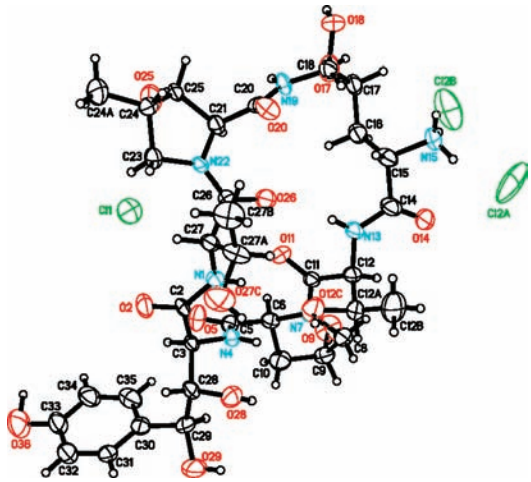


Figure 1. **Molecular architecture of ECBN·HCl, 3 in crystalline state.**

The HPLC generated kinetics for the formation of hydrolysis product **5** are shown in Figure 4 at pH 10 (20−25 °C), and this chemistry can be used to isolate **5**. The low concentration steady-state impurity is presumed to be **4**. Compound **5** and its analogue **9** (discussed later) were the focal points when considering reaction conditions for manufacture, and the process was designed to minimize and control the formation of these open-chain entities.

Crystalline **3** produced from the fermentation recovery process with physical properties as described above is used directly in the coupling process with the active ester, TOBt to provide anidulafungin **1**.

Active Ester, TOBt, 7. Terphenyl acid **6**[8] obtained by synthesis[9] as outlined by some examples in Scheme 3 is the starting material for the production of the active ester **7** abbreviated as TOBt.[10] The terphenyl acid unit when coupled to the free primary amine in **3** via an amide linkage greatly enhances the antifungal activity of anidulafungin **1** compared to the native echinochandin B which has an analogous linoleyl amide side chain. The large-scale process for the production of

(6) A difference map revealed that the chloride was disordered. On further examination a new difference map revealed a number of water molecules of crystallization. It had been noted that crystals of this material lost their ability to diffract X-rays when allowed to stand at room temperature. To avoid this degradation the crystals were stored at 23 to > 50°C and were quickly sealed in epoxy before data were collected. Crystals treated in this manner gave a good diffraction pattern to a resolution of 1 Å. The degradation of the crystals was most likely due to the loss of water. This made the estimation of the amounts of water prone to error. On the basis of thermal parameters it was estimated that 5.5 water molecules were present in the crystal structure. The water was located in nine sites. Two of these sites were estimated at full occupancy and seven at half occupancy (hence, the estimate of 5.5 water molecules).

(7) Balkovec, J. M.; Black, R., M.; Hammond, J. V.; Heck, J. V.; Zambias, G. A.; Bartizal, K.; Kropp, H.; Trainor, C.; Schwartz, R., E., C.; McFadden, D. C.; Nollstadt, K. N.; Pittarelli, L., A.; Powles, M., A.; Schmatz, D., M. *J. Med. Chem.* **1992**, *35* (1), 194–198, See page 196.

Figure 2. **Stereoscopic picture of ECBN·HCl, 3, with position of water molecules present in crystal.**

TOBt, **7**, is described in the Experimental Section. There are of course a number of ways to form the amide bond link. Several groups have studied this problem including ourselves. The intrinsic solubility properties of ECBN, **3**, which prefers aqueous solvent mixtures are not compatible with most derivatives of **6**; the latter are practically insoluble in any media except a few powerful aprotic solvents such as DMF and DMSO in which slight solubility is observed. This solvent incompatibility provided a problem for most derivatives of **6** other than **7** when the workup phase was explored. This was not obvious at first sight, but it became apparent when experience was gained in laboratory manipulations. It is the main reason why the number of methods to couple ECBN·HCl, **3**, with a derivative of **6** is quite restricted. Active ester **7** the HOBt derivative of **6** provides a suitable intermediate for the key coupling reaction in the restricted field of practical operation.

It was determined that **7** could be conveniently prepared from **6** by reacting with HOBt in the presence of triethylamine (TEA) and methanesulfonyl chloride (MsCl) using chlorobenzene as solvent. With **6** as the limiting reagent, the operating ranges established for this reaction were as follows. Relative to **6** the molar equivalents for each reagent was; TEA (5.0−7.0), HOBt (1.4−1.8), MsCl (2.35−2.65), and chlorobenzene reaction solvent ~18 ± 4 L kg^{-1}. Reaction temperatures and time were 30−45 °C and 1.5−3.0 h, respectively, lower temperatures naturally requiring longer reaction times. Crystalline product could be filtered directly from the reaction media, which were then washed and reslurried. Residual chlorobenzene was removed by reslurrying in ethanol to produce a product with an HPLC purity, typically >98%. A large-scale operational procedure is noted in the Experimental Section.

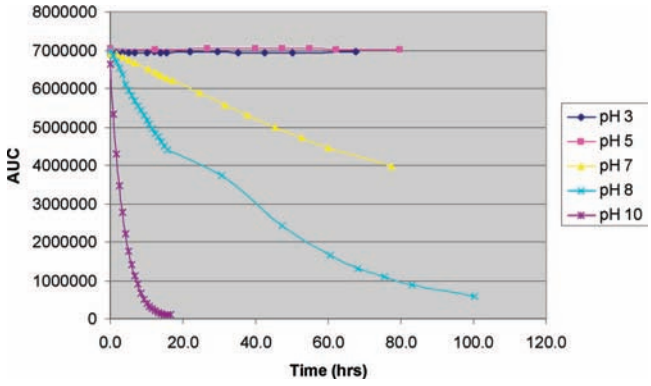


Figure 3. **HPLC ECBN Degradation curves from hydrolysis at various pH (20−25 °C).**

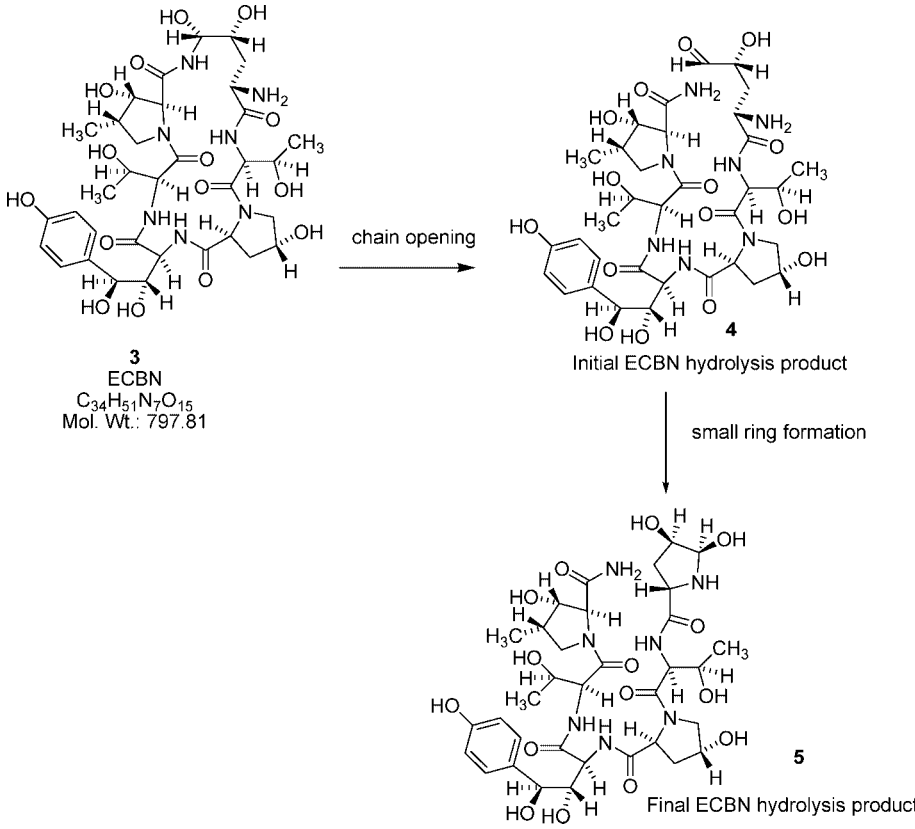
HOBt derivatives of carboxylic acids are not often unambiguously characterized. It is reported[11] that these compounds can exist in two interconvertible forms, both of which are capable of reacting with amines to yield amides. It is possible for the product to be linked to the HOBt unit via a ring nitrogen atom, the compound in this case existing as an *N*-oxide zwitterion **8**, or via the hydroxy function as **7**. Since TOBt is an intermediate used in pharmaceutical synthesis, it was important to fully prove its structure. IR spectroscopy showed that the isolated TOBt material had a strong C═O stretching frequency at 1774 cm^{-1}, which is consistent with an *O*-acyl structure as in **7** rather than the *N*-oxide structure **8**[12] (Scheme 4). Further confirmation of the structure of ester **7** was obtained from a crystal obtained directly from a typical laboratory run. It was not especially recrystallized before being subjected to single-crystal X-ray analysis (Figure 5).

Dry HOBt gives a positive drop hammer test result and thus has a potential for an explosive event in certain special circumstances such as accidental excessive heating in the solid state; as a result, the commercially available monohydrate form of HOBt was used in larger-scale operations where this risk is significantly lowered. It was additionally determined that HOBt containing 20% water could also be used in the synthesis of **7**, although this was determined to be unnecessary for the mode of operation described in the Experimental Section. The drop hammer test was also used to determine the explosive potential of TOBt, **7**, and this compound was found to give a negative response to this test. Under the conditions of formation, isolation, and drying described there is no explosive hazard with this heavily ballasted *N*-hydroxybenotriazole-containing molecule (Scheme 5).

Anidulafungin. Since its first synthesis **1** has been produced in a number of ways,[13] for example formation of an active 2,4,5-trichlorophenol ester of **6** using DCC (dicyclohexylcarbodiimide), removal of dicyclohexylurea, and subsequent reaction with **3** in DMF. For preparation of API for clinical use the reaction

(8) 4″-(Pentyloxy)-1,1′:4′,1″-terphenyl-4-carboxylic acid.
(9) (a) Scherer, S.; Haber, S. Patent WO 00/50375, August 31, 2000. (b) Milton, N.; Moder, K., P.; Sabatowski, J., L.; Sweetana, S. A. U.S. Patent 6,960,564 B2, November 1, 2005.
(10) 1-({[4″-(Pentyloxy)-1,1′:4′,1″-terphenyl-4-yl]carbonyl}oxy)-1H-1,2,3-benzotriazole.
(11) (a) Katritzky, A. R.; Malhorta, N.; Fan, W-Q; Anders, E *J. Chem. Soc., Perkin Trans. 2* **1991**, 1545–1547. (b) Singh, J; Fox, R.; Wong, M; Kissick, T. P.; Moniot, J. L. *J. Org. Chem.* **1988**, *53* (1), 205–208.
(12) The IR C═O stretching frequency for a *N*-oxide structure such as **8** would be expected to fall in the range 1681−1710 cm^{-1} based on literature precedents.

Scheme 2. **Proposed hydrolysis pathway of ECBN·HCl, 3**



of activated ester **7** and HCl salt **3** have been used exclusively, but the methodology has been refined to improve yield and minimize side-product formation. Figure 3 shows that the ECBN peptide, **3**, starts to show an increasing tendency to undergo hydrolysis at pH > 5 to form **5**. Similarly, **1** when formed in reaction can analogously form **9**, via an open-chain compound analogous to compound **4**. See Schemes 2 and 5.

Bearing in mind the pH degradation profile for **3** (Figure 4) a suitable buffer for facilitating coupling between **3** and **7** should be only slightly alkaline, KH_2PO_4 in aqueous acetone provides a suitable medium for reaction at pH ~7.2. Under these conditions at 50−55 °C, **3** and **7** react smoothly to form **1** with minimal formation of reaction side products **9** and **10**. Compound **9** is the open-chain hydrolysis product analogous to **5**, and compound **10** is the bis-terphenyl acid derivative formed by over-reaction of the activated ester **7** at the phenolic functional group in **3**. These two substances are familiar side products that a researcher in this field will encounter when optimizing or route scouting for methodologies to form **1** from

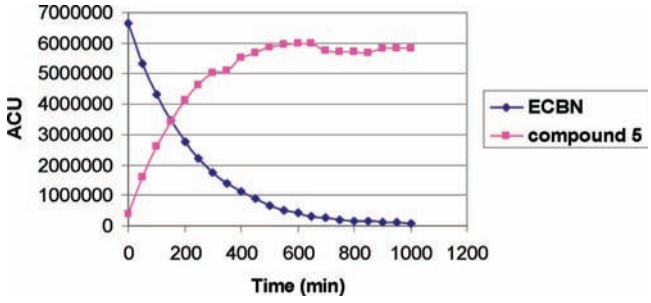
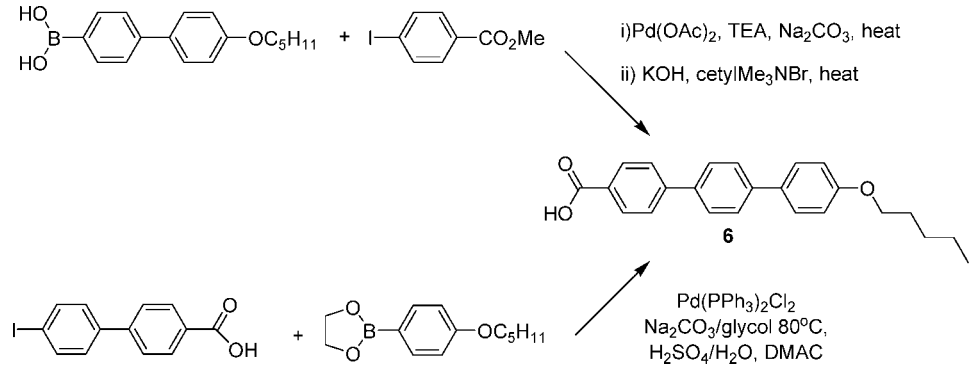


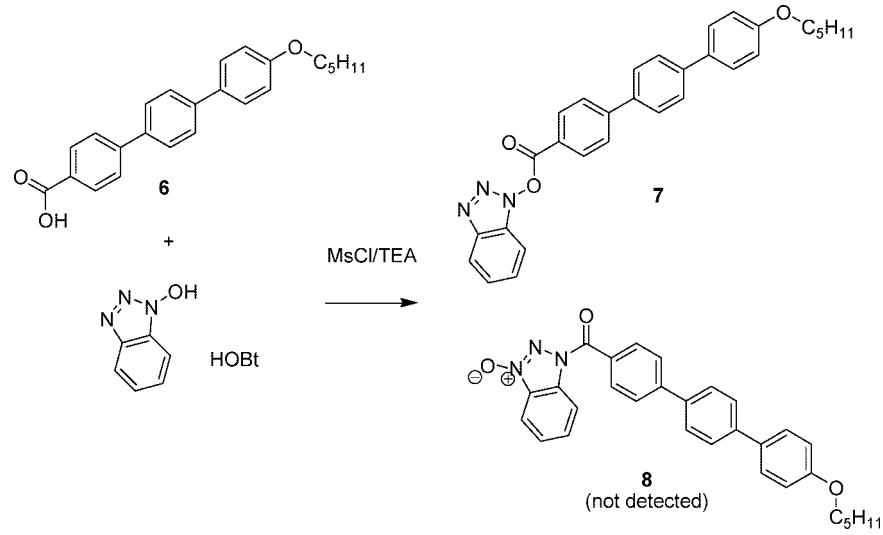
Figure 4. **HPLC kinetics for degradation of ECBN and formation of hydrolysis product 5.**

activated esters, mixed anhydrides, or acyl derivatives of terphenyl acid **6** and cyclic peptide **3**. Thus, relative to **3** the limiting reagent, **7** (1.0−0.95 mol), was reacted in the presence of KH_2PO_4 (1.1−1.3 mol) in aqueous acetone (H_2O/acetone, ~1:4 (v/v) ~20−22 L kg^{-1} **3**) at 45−55 °C for ~2.5−4 h to form a hazy solution of **1** with some **9** and **10** side products. The haze is a result of the presence of compounds **10** and **6** which are even more insoluble than **1** in a given solvent media. In first-generation processes, the reaction medium was initially quenched into acetonitrile to precipitate crude **1**. Crude **1** was redissolved in a ternary mixture of water, acetone, and acetonitrile. This resulted in a non-robust process stream that often prematurely precipitated side products **10** and **6**. As a result, the carbon filter cartridge used to remove these and other trace impurities from the product stream often blinded and required constant intervention. In the laborious first-generation protocol three isolations were required to provide **1** in technical API form; as a result, excessive production of **9** was an inconvenience that ensued in workup manipulations. Although **9** could be removed easily at the anidulafungin/D-fructose processing step, its excessive formation can lead to expensive yield loss of costly **1**. The second-generation process was much more efficient and high yielding.[14] In this case workup was simplified by cooling the reaction solution to room temperature and carbon treating the acetone−water medium. This produces a stable solution of **1** largely free of **6**, **10**, and other process-related trace impurities. The carbon-treated solution was directly quenched into acetonitrile to precipitate technical anidulafungin, **1**. On isolation, washing, and drying, further treatment consists of water wash/reslurry, reisolation, and redrying. The overall

Scheme 3. **Synthesis of starting material 6**



Scheme 4. **Synthesis of 7 and possible alternate structure 8**



outcome results in a more solvent efficient and operationally efficient robust process that reduces environmental burden. An example of a large-scale operation is noted in the Experimental Section which includes in the particular example cited a final wash with methanol.

Anidulafungin/D-Fructose API. The final phase of the process is the purification of anidulafungin by crystallization and isolation of a nonstoichiometric fructose complex of the API.[15] The processing time for this step is an important consideration due to the decomposition of the API in solution. Degradation studies established the decomposition rate of ~0.9% h^{-1} at 55 °C, the temperature required for complete dissolution and clarification (compulsory for an intravenously delivered drug). More importantly, purging studies showed that the total time at this temperature should not exceed ~3.5 h for reliable rejection of impurity **9** (analogous to formation of **5** observed in ECBN degradation chemistry). A robust methodology was developed whereby quality could be maintained by a well-controlled crystallization process.

Characterization of first-generation API indicated a material of low crystallinity, with discernible wide angle X-ray scattering (WAXS) peaks only at low angles and a pronounced glass transition at 109 °C, indicating a solid-state structure disordered at dimensions longer than a few molecules. Polarized light microscopy showed a collection of small (~2 μm) fragments, indicative either of massive attrition or very little growth during crystallization (Figure 6). Features such as these were of concern in that they can negatively impact filtration, washing of the product cake, drying, and the long-term stability of the material. In fact, the first-generation process utilized an unusually large wash of 30 L kg^{-1} API, to mitigate potential emergence of some of these types of problems.

The solubility curve of API in a fructose/methanol solvent was established by infrared measurements in the mode of attenuated total reflectance. Quantification of the solubility behavior enabled experiments to be conducted from known concentrations of supersaturated solutions. One approach for a temperature-sensitive material was to perform a "crash cool" crystallization by rapid heat removal through either a small stirred tank or a heat exchanger. Crystallization rates were observed to be sluggish even from supersaturated solutions.[16] At the more concentrated end of the supersaturated solution zone (metastable solution zone), however, secondary nucleation was entirely suppressed as evidenced by particles composed of crystal lamellae radiating outward from a core nucleus, with the crystallite density highest near the center and decreasing with the distance from the nucleus (Figure 7). This behavior is characteristic of crystallites growing at very high rates and is well-known in melt crystallizations where growth occurs with spherulitic morphology at a rapidly advancing crystallization front.[17] As the supersaturated solution concentration decreases, the growth rate slows, and the morphology changes to individual plates, which are more fluid and less susceptible to heterogeneous growth on reactor surfaces. Surprisingly, these quite

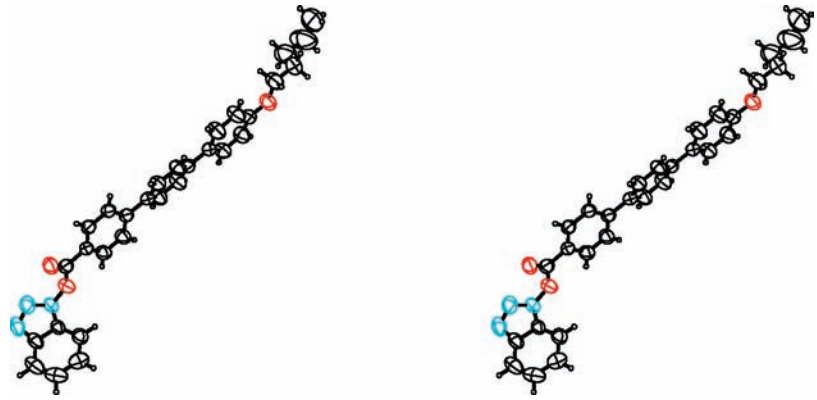


Figure 5. **Structure of TOBt, 7 , in crystalline state from single-crystal X-ray data.**

different crystallization histories and morphologies have no discernible impact on product quality.

Production of large high-quality crystals can be achieved when the temperature is lowered to a few degrees below the saturation temperature (~38 °C) and held for 2−4 h. Crystals grown under these conditions do not exhibit a discernible glass transition (Figure 8), and the X-ray data show peaks representative of a much more highly organized structure[18] (Figure 9). It is interesting to note, however, that despite the microscopic appearance of highly crystalline material all attempts to measure the enthalpy of crystallization calorimetrically (both in the solid and solution state) have been unsuccessful.

Refinement of Second-Generation Crystallization Process. While 3.5 h is a reasonable period in which to fulfill the desired crystallization operations outlined above, it is not of sufficient duration to provide a long cushion in the event of a process upset or other delay. It would be advantageous if the processing could be performed at significantly lower temperature, as a 10 °C decrease to 45 °C reduces the decomposition rate in solution by a factor of about 8. If the material is processed in a slurry to slurry transition, the decomposition rate is reduced further by an amount proportional to the mass fraction of product in the slurry. Thus, at 45 °C it is estimated that the decomposition rate of a slurry to slurry transition would be a factor of 12 less than at 55 °C. Lower temperatures would continue this trend even more dramatically.

Experiments were carried out performing complex formation in a slurry to slurry transition. These were explored in small-scale experiments from 30 to 45 °C. Technical anidulafungin transforms completely into anidulafungin/D-fructose material in <1 h under all the conditions studied. With increasing slurry time and temperature, Ostwald ripening leads to easily filtering crystallites within 2 h (Figure 10).

Clarification, however, is necessary for this API to render it speck-free from extraneous particulate matter, but armed with the above information the initial dissolution process operation was carried out at the lower end of the allowable process range to facilitate minimal formation of **9** during processing as a practical compromise.[21] The example of a large-scale operation is noted in the Experimental Section.

The anidulafugin/D-fructose solid is somewhat enigmatic and not typical when compared to lower-molecular weight API molecules. As a result, its classification as crystalline or amorphous solid is not definitive as it can exhibit properties of both states. It can be clearly seen that during the final purification and crystallization steps that the solid phase has initially many crystalline features, and it exhibits normal crystal growth characteristics during initiation of nucleation (Figure 7). It has been shown that the purified anidulafungin obtained as the loosely bound nonstoichiometric solid has a slower rate of degradation than the technical material, which has a very similar HPLC profile. However, examination of dry bulk material by powder X-ray diffraction shows that the solid is largely amorphous in nature with few clearly defined peaks. Thus far we have been unable to obtain and preserve (even cryogenically) one of the initially formed crystals from methanol or other solvents for sufficient time to obtain a crystal structure. The process described provides a reproducible solid with D-fructose content in the range 7−20%. This substrate is then used to produce a sterile, lyophilized solid for patient presentation.

Experimental Section

Large-Scale Preparation of 1-({[4″-(Pentyloxy)-1,1′:4′,1″-terphenyl-4-yl]carbonyl}oxy)-1H-1,2,3-benzotriazole, 7. 4″-(Pentyloxy)-1,1′:4′,1″-terphenyl-4-carboxylic acid **6** (19.6 kg, 54.9 mol, 1.0 equiv), HOBt monohydrate (13.8 kg [assay 88.5%], 89.8 mol, 1.6 equiv), chlorobenzene (385 kg), and TEA (30.4 kg, 302 mol, 5.5 equiv) were charged to a suitable clean, dry, glass-lined reactor equipped with agitator, condenser, heating/cooling jacket, and appropriate controls. Agitation was

(13) For early examples: (a) Debono, M. Turner, W. W. LaGrandeur, LBurkhardt, F. J. Nissen, J. S. Nichols, K. K. Rodriguez, M. J. Zweifel, M. J. Zeckner, D. J. *J. Med. Chem.* **1995**, *38* (17), 3271–3281. See specifically the Experimental Section starting on page 3278.

(14) First-generation processes produce **1** in about 65% yield; on the same calculation basis, second-generation processes yield ~85%.

(15) This provides a stable form that best suits the requirements of formulation to produce a sterile lyophilized solid in a dispensing vial.

(16) This may be due to the tendency of the macromolecule to ultimately form a largely disordered solid when characterized after drying when solvent molecules are removed from the crystal matrix. Any apparent crystalline ordered form initially formed rapidly reverts to a disordered form on standing.

(17) Bassett, D. C. *CRC Crit. Rev. Solid State Mater. Sci.* **1984**, *12*, 97–163.

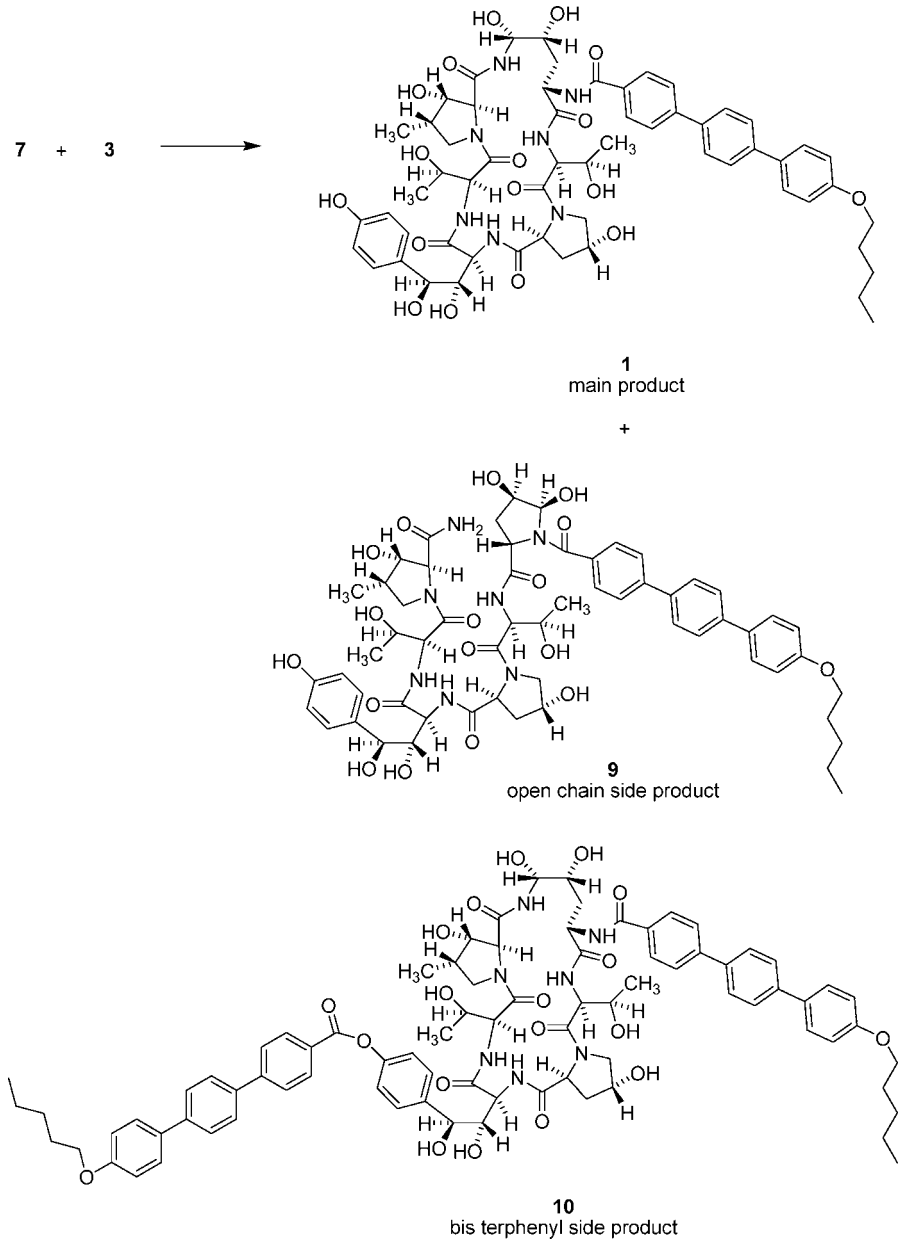
(18) Crash-cooled solid anidulafungin/D-fructose products from supersaturated solutions do not possess such well-defined powder X-ray diffraction peaks.

(19) Photomicrographs: upper left: 42 °C, upper right: 37 °C, lower left: 30 °C, and lower right: 20 °C.

(20) The large glass transition observed in first-generation material has been eliminated. First- and second-generation anidulafungin/D-fructose materials do not exhibit a definitive melting transition.

(21) Even if more **9** is produced at the top end of the temperature range, it is readily purged by the protocol described.

Scheme 5. **Coupling reaction to form anidulafungin, 1**



commenced with the vessel contents held at 30−35 °C for 1 h. The initial slurry formed almost a complete solution. MsCl (15.9 kg, 138 mol, 2.5 equiv) was slowly added to the reaction vessel over ∼3 h such that the vessel internal contents did not exceed 40 °C. The reaction mixture was stirred in the temperature range 35−40 °C for ∼4 h. On completion of the reaction the resultant crystal slurry was cooled to 20−30 °C, granulated for a period and filtered on a horizontal plate pressure filter/dryer, and washed with chlorobenzene (55 kg) and 3A ethanol (33 kg). The solids were partially dried and ∼37.4 kg obtained. These were added to 3A ethanol (236 kg) at 20−30 °C to form a crystal slurry, refiltered, washed with 3A ethanol (131 kg), and dried at 25−35 °C until GC assay indicated the residual ethanol was ≤1%. Yield 22.9 kg, 88%. Assay (HPLC) >97%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.96 (t, 3H), 1.42 (m, 2H), 1.48 (q, 2H), 1.84 (q, 2H), 4.03 (t, 2H), 7.02 (d, 2H), 7.47 (t, 1H), 7.51 (d, 1H), 7.57 (d, 1H), 7.60 (d, 2H), 7.72 (d, 2H), 7.76 (d, 2H), 7.88 (d, 2H), 8.13 (d, 1H), 8.37 (d, 2H). IR (cm^{-1}) ν_{max}; 1774 (C═O).

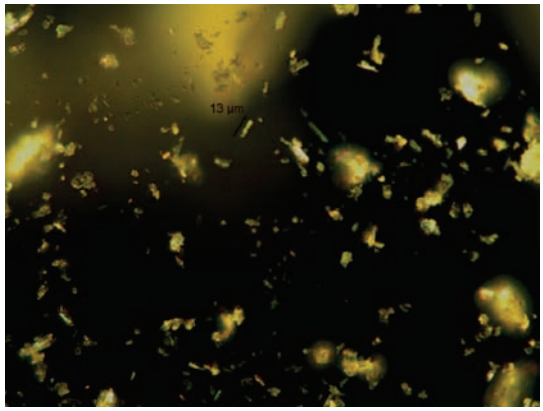


Figure 6. **Anidulafungin API produced in first-generation process.**

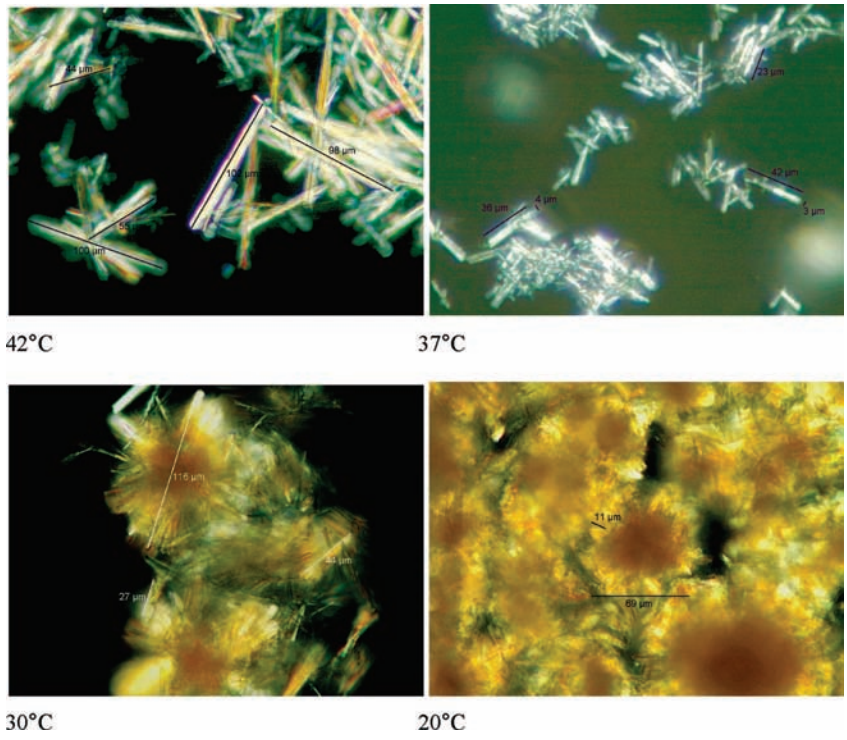


Figure 7. **Formation of solid anidulafungin/D-fructose from highly supersaturated solutions at various temperatures.**[19]

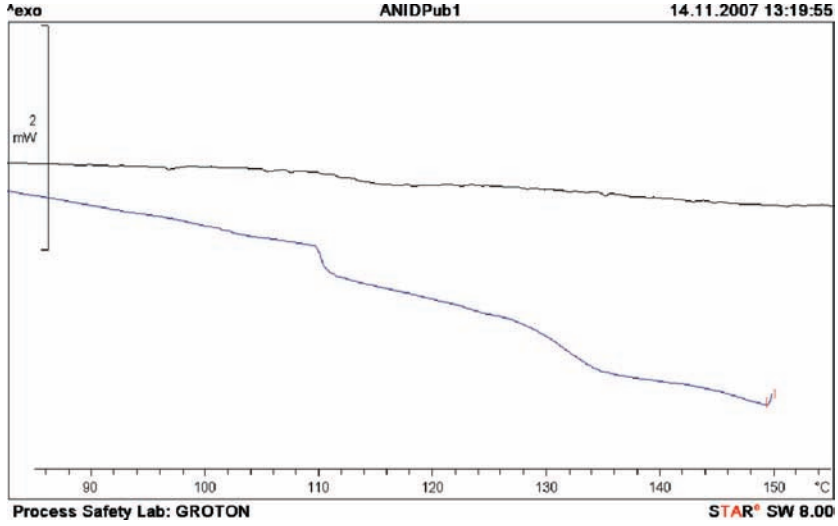


Figure 8. **DSC traces for first-generation (bottom) and second-generation (top) anidulafungin/D-fructose nonstoichiometric complex.**[20]

Large-Scale Preparation of Anidulafugin, 1. ECBN·HCl, **3** (10.7 kg, 8.7 kgA based on assay, 1.0 equiv), TOBt, **7** (4.7 kg, 0.95 equiv), KH_2PO_4 (2.2 kg, 1.22 equiv), acetone (146 L), and water (33L) were charged to a suitable reaction vessel and stirred together at 55 ± 5 °C for 3 h [HPLC control check]. At the end of the reaction period the reaction mixture was cooled to the temperature range 20−25 °C and recirculated around an activated carbon cartridge system until the level of side product **10** was <0.1%. The carbon-treated reaction solution was quenched into acetonitrile (74 kg). The precipitated product was filtered, washed with acetonitrile, and dried. Yield 9.2 kg, 86%. The dried product was re-slurried in water (~210 L) for a period at room temperature, filtered, washed with a small quantity of methanol (20 kg), and redried. Yield 7.6 kg, (71%). ^{1}H NMR (700 MHz, d_6-DMSO) δ 0.91 (t, 3H), 1.12 (d, 3H), 1.36 (m, 2H), 1.41 (m, 2H), 1.74 (p, 2H), 1.88 and 1.97 (overlapped, 2H), 3.85 (overlapped, 1H), 4.01 (t, 2H), 4.35 (overlapped, 1H), 4.44 (m, 1H), 4.76 (m, 1H), 4.80 (m, 1H), 5.02 (m, 1H), 5.07 (d, 1H), 5.52 (d, 1H), 7.04 (d, 1H), 7.66 (d, 1H), 7.74 (d, 1H), 7.80 (d, 1H), 7.82 (d, 1H), 7.97 (d, 1H), 8.01 (d, 1H), 8.14 (broad s, 1H), 8.60 (d, 1H). IR (cm^{-1}) KBr ν_{max}; 3450 (O−H), 2932 (C−H), 2871 (C−H), 1632 (C═O), 1517 (Ar), 1488 (Ar), 1248 (C−O), 821 (C−H out-of-plane bending Ar 2 adj H's).

Large-Scale Preparation of 1/D-Fructose Nonstoichiometric Complex. Technical anidulafungin, **1** (7.6 kg, 1.0 equiv), D-fructose (7.8 kg, 6.5 equiv), and disodium citrate sesquihydrate (15.6g, 0.009 equiv) were dissolved in 64 L of methanol at 50−55 °C. The solution was clarified and the line rinsed with

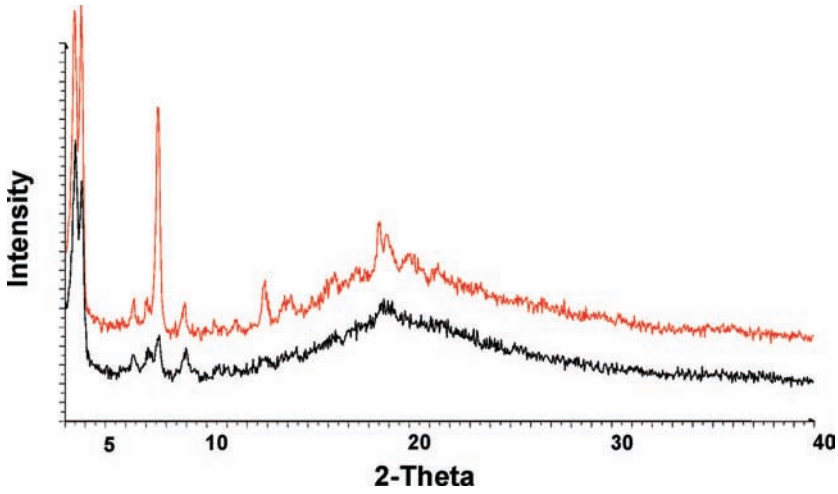


Figure 9. **WAXS data for second-generation process (top, red plot) and first-generation process anidulafungin/D-fructose nonstoichiometric complex (bottom, black plot).**

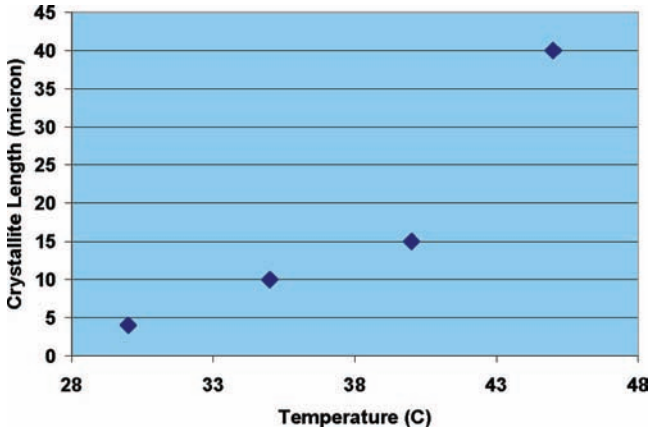


Figure 10. **Anidulafungin/D-fructose crystallite dimension after 2-h slurry in methanol.**

11 L of methanol chase. The solution was adjusted to 42 °C, seeded, and stirred between 38−44 °C for 1 h. The slurry was cooled at 6 °C h^{-1} to a final range of 0−5 °C, and then stirred for two additional hours. The slurry was filtered and washed with a solution of D-fructose (1.8 kg) in methanol (230 L). The cake was subsequently dried with nitrogen to NMT 0.5% methanol and NMT 6% water. Yield: 6.3 kg (74%).

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Supporting Information Available

Crystallographic information files; description of HPLC conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

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