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An Efficient Large-Scale Synthesis of EDP-420, a First-in-Class Bridged Bicyclic Macrolide (BBM) Antibiotic Drug Candidate

Guoyou Xu, Datong Tang,* Yonghua Gai, Guoqiang Wang, Heejin Kim, Zhigang Chen, Ly T. Phan, Yat Sun Or, and Zhe Wang

Enanta Pharmaceuticals, Inc., 500 Arsenal Street, Watertown, Massachusetts 02472, U.S.A.

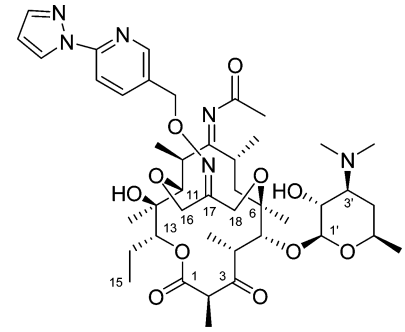
Abstract:

A multistep, practical, and cost-effective synthesis of novel bridged bicyclic macrolide drug candidate EDP-420 (1) is described. Starting from inexpensive and commercially available erythromycin A 9-oxime, the current chemical process involves a series of transformations: triacetylation, Pd-catalyzed *O,O*-bis-allylation (bridge formation), acid-catalyzed sugar cleavage, oxime reduction, acetylation, Os-catalyzed bridge olefin oxidative cleavage, Corey−Kim oxidation, bridge oxime formation, deprotection, and final purification. Multikilogram quantities have been synthesized.

Introduction

EDP-420 (**1,** Figure 1),[1] entered phase II clinical trials in early 2006 for the treatment of community-acquired pneumonia. First in its class, **1** is a bridged bicyclic macrolide (BBM) that was designed and discovered by Enanta to have optimal pharmacokinetics (PK) and to provide broad treatment against respiratory pathogens, including several multidrug resistant strains for which traditional macrolides, penicillins, and fluoroquinolones are no longer effective.

EDP-420 has broad activity against respiratory pathogens including macrolide resistant *Streptococcus pneumoniae*, PRSP, *S. pyogenes*, *Haemophilus influenzae* and atypicals. In addition, this compound broadens the spectrum of existing macrolide antibiotics by demonstrating excellent *in vitro* activity against many strains of multidrug resistant *S. pneumoniae*, including strains resistant to the traditional macrolides, penicillins and fluoroquinolones. Extensive comparative *in vivo* efficacy studies were performed between EDP-420 and telithromycin against *H. influenzae*, *S. pneumoniae*, *Staphylococcus aureus*, and *S. pyogenes.* In these studies, EDP-420 consistently performed equal to or better than telithromycin.[2] EDP-420 also possesses an excellent PK profile across multiple species, with a half-life 2−3 times longer as compared to those of clarithromycin and telithromycin.[3] On the basis of these observations, we expect EDP-420 will be a once daily dosed drug.



EDP-420, **1**

Figure 1. **Chemical structure of EDP-420 (1).**

Like the classic macrolide drug erythromycin A,[4] **1** also features a polyfunctionalized 14-membered lactone ring. However, **1** is a 3-keto macrolide molecule (known as a ketolide) bearing a unique 6,11-*O,O*-three-carbon bridged (*E*)-oxime side-chain subunit. The structural complexity constitutes a formidable challenge for supplying the active pharmaceutical ingredient (API) in high purity for preclinical toxicity studies and clinical trials. Erythromycin A 9-oxime **2**, derived from erythromycin A via an oxime formation process and commercially available in bulk quantity, was chosen as the starting material. The construction of **1** requires the 6,11-bridge formation as the key step, (Figure 2). Our process optimization focused on this key step with the ultimate goal of pilot-plant production of **1** in multikilogram quantities.

Results and Discussion

6,11-*O,O*-Bridge Formation. Using bis(Boc)-protected allylic diol **3** as the dielectrophile, we developed a novel bridging reaction for erythromycin A-derived macrolide *via* a palladium(0)-catalyzed tandem 6-*O* and 11-*O*-allylic dialkylation (Scheme 1).[5] This sequence first required protection of the otherwise reactive hydroxyls. Thus, commercially available **2** was first protected as its 9,2′,4″-triacetate **5** by reacting with Ac_2O in THF in the presence of triethylamine and catalytic amount of DMAP. Isolation and characterization of some of the impurities established that there were one monoacetate (9-oxime acetate), two diacetate (9,2′-diacetate and 9,4″-diacetate), and possibly one tetraacetate (9,2′,4″,11-tetra-acetate). However,

* Author for correspondence. E-mail: dtang@enanta.com.

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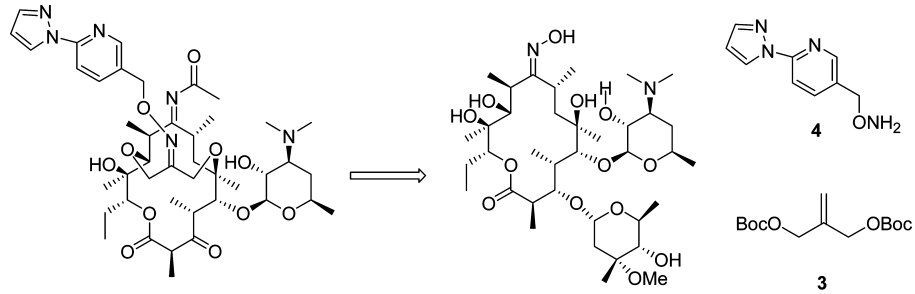
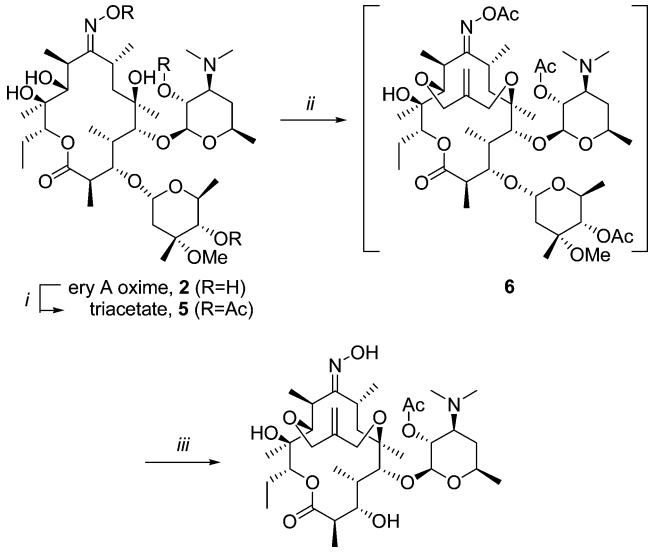


Figure 2. **Retrosynthetic analysis of EDP-420.**

Scheme 1. **Strategic 6,11-*O,O*-bridge construction via Pd-catalyzed diallylation[a]**



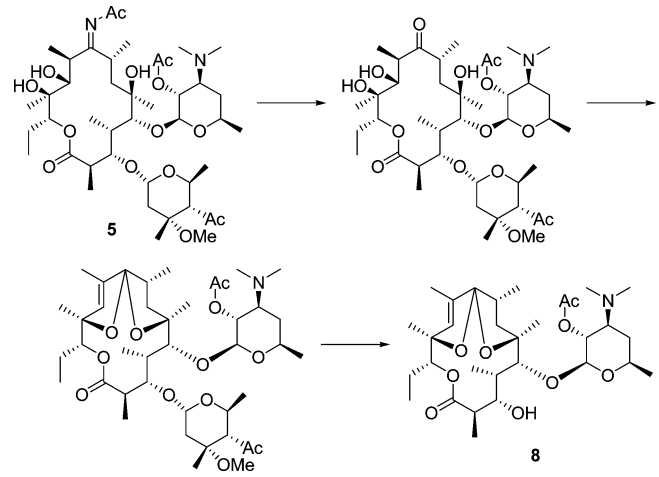
[a] Conditions: (i) Ac_2O, DMAP, TEA, rt, then crystallization from MeCN (80%); (ii) 3, $Pd_2(dba)_3$, dppb, THF, reflux; (iii) 1 N aq HCl, 60 °C, then crystallization from MeCN (60% from 5).

since the partial hydrate of the triacetate **5** was highly crystalline when recrystallized from MeCN, the impurities were efficiently removed in solution. The recrystallized chemical yield for triacetylation on multikilogram scale in the plant was 70−90%.

Treatment of the resulting triacetate **5** with bis(Boc)-protected diol **3** in the presence of tris(dibenzylideneacetone)dipalladium(0) [$Pd_2(dba)_3$, 2 mol %] as catalyst and catalytic 1,4-bis(diphenylphosphino)butane [dppb, 4 mol %] as ligand smoothly effected a regioselective tandem diallylation at the 6,11-hydroxyl groups through the "Pd−π-allyl complex" transition state to afford the desired 6,11-*O,O*-bridged macrolide key intermediate **6**. Without isolation, the 3-cladinose sugar fragment and the 9-oxime acetate were selectively hydrolyzed with 1 M HCl to give the bridged olefin 9-oxime **7**, which was easily isolated by crystallization from acetonitrile on multikilogram-scale preparation (Scheme 1).

The bridging reagent bis(Boc)-protected diol **3** was quantitatively prepared on multikilogram scale by reacting 2-methylene-1,3-propanediol with Boc_2O with phase-transfer catalysis (PTC) in aqueous NaOH and CH_2Cl_2. The crude product was typically contaminated with 5 mol % of unreacted Boc_2O, which did not affect the subsequent reaction. Therefore, the crude product **3** was telescoped directly into the next step—the macrolide bridge formation.

Scheme 2. **Formation of the major byproduct isolated in the bridge formation step**

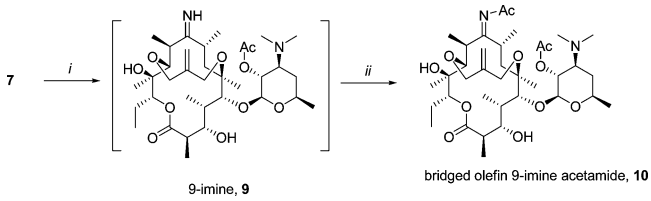


Like most of Pd (0)-catalyzed chemistry, the macrolide bridge formation reaction was highly oxygen- and moisture-sensitive. In addition, both the purity and dryness of the triacetate **5** were critical for a high yield in this reaction. Otherwise, up to 15% of a "dehydrated ketal" byproduct, erythralosamine 2′-acetate **8,** would be isolated after the subsequent acidic sugar cleavage reaction. The byproduct **8** was a well-known degradation product from erythromycins[6] and was formed, in this study, from the unreacted triacetate and its impurities through 9-hydrolysis, 6,9- and 12,9-hemiketal formation, dehydration, spiroketal formation, and cladinose sugar cleavage (Scheme 2). To minimize this unproductive pathway, the crystallized triacetate **5** was first dried azeotropically from toluene before the Pd-catalyzed bridge formation reaction. This minimized the formation of **8**. At 4 kg scale, the overall isolated yield of bridged olefin 9-oxime **7** was 55−65% based on the triacetate **2**. The chemical purity of **7** was above 95 area % by HPLC analysis.

Oxime Reduction to Imine Acetamide Intermediate 10. Aqueous $TiCl_3$ is a useful reagent for the reduction of oximes to imines.[7] Bridged 9-oxime intermediate **7** was reduced with 20% $TiCl_3$ solution in 3% aqueous HCl to 9-imine **9** (Scheme 3). Surprisingly, the imine functionality of intermediate **9** was very stable under these aqueous acidic condition. Conventional

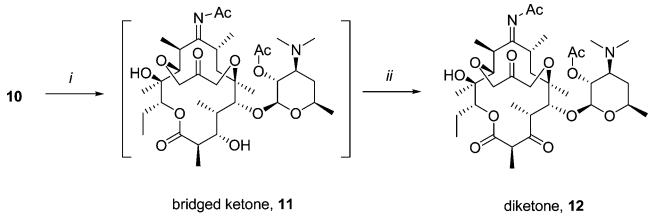
(6) Flynn, E. H.; Sigal, M. V.; Wiley, P. F.; Gerzon, K. J. *Am Chem. Soc.* **1954**, *76*, 3121.
(7) (a) Timms, G. H.; Wildsmith, E. *Tetrahedron Lett.* **1971**, *12*, 195. (b) Gasparski, C. M.; Ghosh, A.; Miller, M. J. *J. Org. Chem.* **1992**, *57*, 3546.

Scheme 3. **Bridged olefin 9-imine acetamide, 10**[a]



[a] Conditions: (i) $TiCl_3$ in aq HCl, EtOH, rt, then basification; (ii) Ac_2O, CH_2Cl_2, rt, then crystallization from EtOAc/heptanes (85% from **7**).

Scheme 4. **Late-stage intermediate diketone, 12**[a]



[a] Conditions: (i) OsO_4, $NaIO_4$, acetone/H_2O; (ii) NCS, DMS, CH_2Cl_2, −20 °C, then TEA, crystallization from EtOH (55% from 10).

hydrolysis leading to the corresponding 9-ketone under various conditions investigated in our laboratories proved to be difficult. We believe that the stability of this imine group is due to the constrained conformation of the macrocycle that results in 8,10-methyl groups blocking one side of the imine while the bridge blocks the other side. Due to the presence of the neighboring "dimethyl amine" group on the desosamine sugar moiety, aqueous acid conditions prevented the otherwise expected hydrolysis of the 2′-acetate.

Subsequent basification and extraction produced the 9-imine **9** as a solution in EtOAc. This imine intermediate solution was solvent exchanged to CH_2Cl_2 and treated with Ac_2O to produce the bridged olefin 9-imine acetamide **10**. For a typical 4-kg-scale operation, the overall yield for this one-step process was 85−90% after crystallization from EtOAc/heptanes.

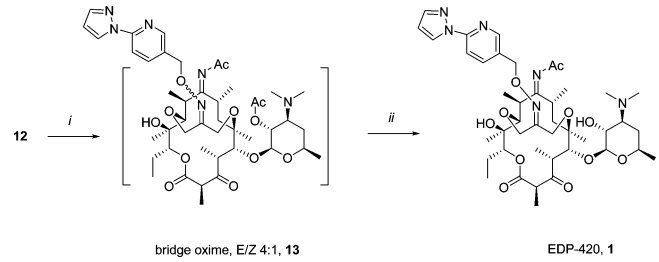
Late-Stage Intermediate Diketone 12. Diketone **12** was prepared from bridged olefin 9-imine acetamide **10** *via* a 2-step one-pot process, *i.e.*, oxidative cleavage of bridge olefin to intermediate **11** followed by oxidation at the 3 position, (Scheme 4). If the reactions were reversed, overall yield was lower. The exocyclic olefin's oxidative cleavage reaction[8] was conducted by using OsO_4 (0.28 mol %)-catalyzed $NaIO_4$ in aqueous acetone to give ∼85% yield (solution NMR analysis). Alternatively, ozonolysis in EtOAc/MeOH at low temperature with a Me_2S reductive workup also worked very well in comparable product yield and purity. Although ozone is more typically used for olefin cleavages,[9] we chose OsO_4-catalyzed $NaIO_4$ cleavage for the multikilogram-scale reaction primarily due to equipment limitations in our kilolab.

3-Oxidation was first carried out by a Dess−Martin periodinane oxidation by our discovery medicinal chemistry team.

(8) Pappo, R.; Allen, D. S., Jr.; Lemieux, R. U.; Johnson, W. S. *J. Org. Chem.* **1956**, *21*, 478.

(9) (a) Bailey, P. S. *Ozonation in Organic Chemistry*; Academic: New York, 1978; Vol. 1. Bailey, P. S. *Ozonation in Organic Chemistry*; Academic: San Diego, 1982; Vol. 2. (b) Razumovskii, S. D.; Zaikov, G. E. *Ozone and Its Reactions with Organic Compounds*; Elsevier: Amsterdam, 1984.

Scheme 5. **Bridge oxime formation to EDP-420**[a]



[a] Conditions: (i) **4**, HCl, EtOH/H_2O, 0 to 5 °C; (ii) MeOH, rt, then crystallization from EtOH/H_2O (40% from **12**).

It is well-known that the Dess−Martin oxidation is a mild, clean, and usually high-yielding reaction. However, in addition to its cost issue, we were uncomfortable about using it for a large-scale process due to its potentially explosive nature. Instead, we screened some commonly used oxidation reagents[10] that would be most suitable for a large-scale preparation, including the TPAP [Pr_4NRuO_4]−NMO reagent and the "activated" DMSO oxidations, i.e., the Swern and Moffatt reactions. These reagents either led to incomplete conversions or significant side reactions. However, the Corey−Kim reaction[11] provided the answer. Corey−Kim oxidation is a mild and selective reaction particularly suitable for complex alcohols, such as macrolide molecules, in which *N*-chlorosuccinimide (NCS) is first reacted with dimethyl sulfide (DMS) to form the NCS−DMS complex reagent. The stoichiometry of NCS [optimized as 1.30 equiv to intermediate **11**] was critical for the product purity. Excess NCS resulted in 10−15% yields of 12-methythiomethyl (MTM) ether byproduct formation. Because the preparation of diketone **12** was a two-step, one-pot process (Scheme 4) without isolation of the intermediate **11**, the solution yield (typically ∼78% for olefin cleavage step) of **11** was determined by ^1H NMR analysis. On the basis of its solution yield, the NCS stoichiometry was adjusted accordingly.

For multikilogram preparation, we also screened the solvent and temperature. The optimal Corey−Kim conditions were using methylene chloride at −15 to −20 °C. In the plant, 4 kg batches (16 batches) achieved a 55% overall average yield (two steps) after crystallization from EtOH in 95 area % purity (NMR and HPLC analysis).

Bridge Oxime Formation to EDP-420 (1). The final operational step was a two-step, one-pot process (Scheme 5) without isolation of the bridge oxime *E/Z* mixture **13**. The oxime was formed by treating the diketone **12** with hydroxylamine **4** in EtOH. After aqueous workup (basification and extraction), the *E/Z* mixture (∼4:1) of the oximes was telescoped into the next step: methanolysis for deprotection at the 2′ position. The oxime mixture was then purified by crystallization from aq EtOH to provide crude EDP-420. On typical kilogram scale, overall isolated yields for crude **1** were 40−42% in 95 area % HPLC purity. Most of the undesired *Z*-isomer remained in the mother liquor and was thus easily removed by filtration during recovery of the crystallized **1**.

EDP-420 has the *E*-oxime configuration on the 6,11-bridge. It was obvious that the ability to generate a high *E/Z* ratio for

(10) For a recent review about alcohol oxidation by activated DMSO and related reactions, see: Tidwell, T. T. *Synthesis* **1990**, 857–870.

(11) Corey, E. J.; Kim, C. U.; Misco, P. F. *Org. Synth.* **1978**, *58*, 122.

Table 1. **Acid-catalyzed formation of bridge oxime 13: *E*/*Z* ratio[a]**

entry	acid	pK_a	oxime *E*/*Z* ratio by HPLC
1	none[b]		0.7
2	L-malic acid	3.4	1.2
3	$MeSO_3H$	−2	1.6
4	*p*-TsOH	−2.8	1.6
5	H_3PO_4	2.1	1.9
6	CSA	1.2	2.5
7	BF_3 or $ZnCl_2$[c]	−	3.0
8	HCl	−7	4.0
9	HBr/HI	−9/−10	4.0
10	H_2SO_4	−9	4.3
11	$HClO_4$[d]	−10	8.0

[a] Experiments were carried out on 2 g scale in EtOH/H_2O at 0 to 5 °C for 2 h except where noted. [b] In toluene at rt for 2 days, <65% conversion. [c] In EtOAc or EtOH/THF. [d] In aq EtOH/MeCN at −35 °C for 7 h.

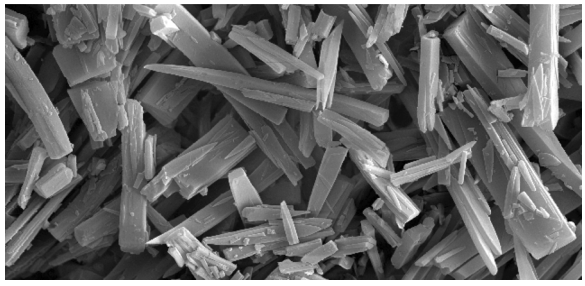


Figure 3. **SEM crystal image of polymorphic form I for EDP-420 API, 1.**

the oxime formation step would translate into overall higher yields and in general be a superior process. We found that the oxime *E*/*Z* ratio for diketone **12** was dependent on the pK_a of the acid or Lewis acid catalysts (Table 1). The highest *E*/*Z* ratio (8:1, 89% *E*) was achieved by using perchloric acid as catalyst. Unfortunately, in addition to being a hazardous reagent, it also required low temperature (−35 °C) and longer reaction time. Aqueous HCl as the acid catalyst for the bridge oxime formation (*E*/*Z* ratio 4:1, 80% *E*) was still a better fit in our facilities for our large-scale preparations in spite of the inherent yield loss.

The osmium level was measured by inductively coupled plasma (ICP) spectroscopy in the crude EDP-420 as usually <1 ppm, which attained our acceptance critieria for GMP clinical material. In cases when osmium level was high (up to 10 ppm), we found that washing the crude API solution in EtOAc with 3% sodium metabisulfite in aqueous saturated $NaHCO_3$ followed by recrystallization in EtOH/water effectively reduced the Os level to <1 ppm.

Final Purification of EDP-420. Conventional recrystallization from various solvents proved insufficient to achieve the limit of >98% HPLC purity, required for clinical use. Instead, we developed a purification process using the L-malic acid salt after screening numerous acids. Our optimized process involved L-malic acid salt formation in EtOAc/IPA, recrystallization, salt release, and then final recrystallization from aq EtOH to afford **1** as a monohydrate in >98% HPLC area % purity and in 75−80% overall yield based on crude API. Our largest plant scale for the production ofAPI **1** was 3 kg per batch. While EDP-420 possesses multiple polymorphs, this technique yielded the desired form (Figure 3) reproducibly.

In conclusion, we have developed a practical 10-step chemical process for the multikilogram scale preparation of EDP-420 API. All isolated intermediates were obtained by crystallization and filtration. This efficient process has been successfully demonstrated at the pilot plant scale.

Experimental Section

General Methods. All solvents were purchased from Aldrich. All reagents were used as purchased. MS experiments were carried out on an Agilent 6210 Time-of-Flight in ESI + mode using direct flow injection. Mobile phases are A: water + 0.1% formic acid and B: ACN + 0.1% formic acid. ^1H and ^{13}C NMR spectra were recorded on a Varian INOVA 500 spectrometer operating at 500 and 125 MHz, respectively. Two-dimensional (2D) NMR spectra were acquired using standard pulse sequences. HPLC method used for determination of the purity of **1**: L-column ODS, 5 μm, 4.6 mm × 250 mm, a constant temperature of about 40 °C; detector 210 nm; mobile phase A is a mixture of 0.005 mol/L KH_2PO_4 buffer (pH 8.0) and acetonitrile (3:2). Mobile phase B is a mixture of 0.012 mol/L KH_2PO_4 buffer (pH 8.0) and acetonitrile (1:3). Gradient program: HPLC program: 0−5 min (70% A); 5−40 min (70 to 0% A); 40−65 min (0% A); 65−65.01 min (0 to 70% A); 65.01 to 80 min (70% A). Flow rate 1.0 mL/min. t_R for **1** ≈ 30 min.

2-Methylene-1,3-propanediol bis(*tert*-butyl Carbonate) (3). A solution of Boc_2O (6.6 kg, 30.24 mol) in CH_2Cl_2 (15 L) was treated with 2-methylene-1,3-propanediol (1.0 kg, 11.35 mol) and followed by $(n\text{-}Bu)_4NHSO_4$ (0.641 kg, 1.888 mol). This was agitated vigorously and cooled to 12−15 °C. A solution of 6 N aq NaOH (13.2 L) was added over 2 h at 25−30 °C. The resulting two-phase mixture was agitated at 25 °C for 2−3 h. The aqueous layer was separated, and additional $(n\text{-}Bu)_4NHSO_4$ (64 g, 0.1888 mol, 10% of the initial amount), Boc_2O (0.66 kg, 3.024 mol, 10% of the initial amount), and CH_2Cl_2 (2 L) were added. A solution of 6 N aq NaOH (1.32 L, 10% of the initial amount) was added over 0.5−1 h at 25−30 °C. This was agitated at 20−28 °C for 3−4 h. After separation of phases, the organic phase was washed with H_2O (3 × 8 L) and concentrated *in vacuo* until water content (Karl Fischer) of the residue was NMT 100 ppm. (If necessary, anhydrous EtOAc (5 L) was added and distilled *in vacuo* to dry the residue azeotropically) The crude **3** was obtained as an oil in quantitative yield. ^1H NMR ($CDCl_3$, 500 MHz): δ 5.21 (s, 2 H), 4.50 (s, 4 H), 1.39 (s, 18 H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 153.4, 138.5, 117.5, 82.2, 66.3, 26.8.

Erythromycin A 9-Oxime 9,2′,4″-triacetate (5). A solution of erythromycin A 9-oxime (**2**) (1.0 kg, 1.335 mol) in EtOAc (4 L) was treated with TEA (0.744 L, 5.338 mol) followed by DMAP (48.9 g, 0.40 mol). This was agitated vigorously and Ac_2O (0.441 L, 4.674 mol) was added over a period of 1−2 h at below 40 °C. This was agitated for 2 h at 20−28 °C. Additional TEA (74.4 mL, 0.5338 mol, 10% of initial amount) and additional Ac_2O (44.1 mL, 0.4674 mol, 10% of initial amount) was added over 30 min at <35 °C. This was further agitated at 20−28 °C for 1.5−2 h. After the reaction was complete as indicated by HPLC analysis (conversion >97%), it was diluted with EtOAc (6 L), quenched with saturated aq $NaHCO_3$ (3 L), and agitated at 20−28 °C for 10 min. The

aqueous phase was separated and discarded. The organic phase was washed with saturated aq $NaHCO_3$ (3 L), followed by 15% aq NaCl (3 L), and concentrated *in vacuo* to 3−4 L of remaining volume. The resulting solution was taken up in acetonitrile (4 L) and concentrated *in vacuo* to 3−4 L of remaining volume. Acetonitrile (2 L) was added and the solution was concentrated *in vacuo* until crystallization occurred (final volume 3−4 L). Upon formation of crystals, the slurry was agitated at 10−15 °C for at least 2 h. The crystals were collected by filtration and dried under vacuum until constant weight to afford crystalline triacetate**5** with a typical yield of 80%. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.15 (1 H, 13-OCH), 4.98 (1 H, 1″-OCHO), 4.79 (1 H, 2′-OCH), 4.68 (2 H, 1′-OCHO and 4″-OCH), 4.33 (1 H, 11-OH), 4.31 (1 H, 5″-OCH), 3.89 (1 H, 3-OCH), 3.77 (1 H, 11-OCH), 3.74 (1 H, 5′-OCH), 3.67 (1 H, 8-CH), 3.45 (1 H, 5-OCH), 3.35 (3 H, 3″-OCH_3), 3.19 (1 H, 12-OH), 2.88 (1 H, 2-CH), 2.78 (1 H, 10-CH), 2.74 (1 H, 3′-NCH), 2.42 and 1.66 (2 H, 2″-CH_2), 2.29 (6 H, 3′-NCH_3 × 2), 2.17 (3 H, 9═$NOCOCH_3$), 2.11 (3 H, 4″-$OCOCH_3$), 2.06 (3 H, 2′-$OCOCH_3$), 1.95 and 1.48 (2 H, 14-CH_2), 1.93 (1H, 4-CH), 1.72 and 1.34 (2 H, 4′-CH_2), 1.51 (2 H, 7-CH_2), 1.43 (3 H, 6-CH_3), 1.27 (3 H, 10-CH_3), 1.19 (6 H, 2-CH_3 and 5′-CH_3), 1.18 (3 H, 12-CH_3), 1.14 (3 H, 3″-CH_3), 1.13 (3 H, 5″-CH_3), 1.11 (3 H, 8-CH_3), 0.95 (3 H, 4-CH_3), 0.85 (3 H, 15-CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 178.7, 175.4, 170.4, 170.0, 168.3, 100.6, 96.3, 83.4, 79.4, 79.0, 77.4, 74.9, 74.0, 72.6, 72.2, 70.1, 67.7, 63.6, 63.4, 49.5, 45.0, 40.7, 39.2, 37.4, 35.7, 34.5, 31.5, 28.6, 26.9, 21.7, 21.7, 21.5, 21.4, 21.1, 20.2, 18.7, 18.6, 16.7, 16.1, 15.0, 10.9, 9.3. HRMS Calcd for $C_{43}H_{74}N_2O_{16}$ (MH^+) 875.5111. Found 875.5141.

3-Descladinose-6,11-*O*,*O*-bridged Erythromycin A 9-Oxime 2′-acetate (7). A solution of **5** (1.0 kg, 1.143 mol) in anhydrous THF (5 L) was treated with a solution of **3** (0.62 kg, 2.150 mol) in anhydrous THF (2 L). This was degassed by bubbling a steady stream of dry N_2 gas for 5−10 min. 1,4-Bis(diphenylphosphino) butane (dppb) (19.5 g, 45.7 mmol, 4 mol %) and tris(dibenzylideneacetone)dipalladium(0) [$Pd_2(dba)_3$] (20.8 g, 22.7 mmol, 2 mol %) were added sequentially. The resulting mixture was immediately degassed as previously described. This was then heated, while being agitated, to reflux over the period of about 2 h and then held at the reflux temperature for 6 h at which point the reaction was complete as indicated by HPLC analysis (conversion >97%). After cooling to room temperature, the reaction mixture was filtered through a 2 in pad of silica gel (0.1−0.25 kg) to remove most of the Pd catalyst, phosphine ligand, and other polar impurities. The silica gel pad was washed with fresh THF (1 L). The combined filtrate was concentrated *in vacuo* to 1.5−2 L of remaining volume to afford a solution of **6** in THF. This was treated with 1 N HCl (8 L), heated to 60 °C over a period of 1−2 h and agitated at 58−62 °C for additional 2 h at which point the reaction was complete as indicated by HPLC analysis (conversion >97%). After cooling to room temperature, this was washed with MTBE (2 × 4 L). The aqueous solution was diluted with EtOAc (2 L) and 50 wt % aq K_2CO_3 (about 0.8 kg of solid K_2CO_3 in H_2O) was added at 22−28 °C to pH 9−10. The resulting mixture was extracted with EtOAc (2 × 4 L). The combined organic phase was washed with H_2O (4 L) and concentrated *in vacuo* to 1.5−2 L of remaining volume. MeCN (4−6 L) was added and the solution was concentrated *in vacuo* to 1.5−2 L of remaining volume. The concentrated residue was agitated at 40−45 °C under atmospheric pressure until crystallization occurred. The resulting slurry was cooled to 0−4 °C over 3−4 h and held for at least 2 h. The crystals were collected by filtration, washed with chilled MeCN (0.25 L) and dried under vacuum until constant weight to afford crystalline **7**. The typical yield for this 2-step one-pot process was 50−65%. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.09 and 4.98 (2 H, 19═CH_2), 4.97 (1 H, 13-OCH), 4.78 (1 H, 2′-OCH), 4.74 (1 H, 1′-OCHO), 4.43 (1 H, 11-OCH), 4.39 and 4.07 (2 H, 16-CH_2), 3.90 and 3.67 (2 H, 18-CH_2), 3.84 (1 H, 8-CH), 3.74 (1 H, 5-OCH), 3.50 (1 H, 5′-OCH), 3.43 (1 H, 3-OCH), 2.82 (1 H, 12-OH), 2.79 (1 H, 3′-NCH), 2.73 (1 H, 2-CH), 2.62 (1 H, 10-CH), 2.43 (1 H, 4-CH), 2.31 (6 H, 3′-NCH_3 × 2), 2.08 (3 H, 2′-$OCOCH_3$), 2.03 and 1.46 (2 H, 14-CH_2), 1.77 and 1.35 (2 H, 4′-CH_2), 1.59 and 1.32 (2 H, 7-CH_2), 1.38 (3 H, 6-CH_3), 1.24 (3 H, 5′-CH_3), 1.23 (3 H, 2-CH_3), 1.22 (6 H, 10-CH_3 and 12-CH_3), 0.97 (6 H, 4-CH_3 and 8-CH_3), 0.89 (3 H, 15-CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 175.0, 170.1, 166.1, 143.5, 119.2, 99.6, 81.5, 79.5, 78.3 (3-OCH and 13-OCH), 77.2, 76.0, 73.9, 71.7, 68.9, 65.7, 63.4, 44.1, 40.9, 37.5, 36.1, 34.2, 31.3, 25.8, 23.3, 21.8, 21.4, 20.1, 19.5, 17.3, 15.6, 14.6, 11.9, 7.9. HRMS Calcd for $C_{35}H_{60}N_2O_{11}$ (MH^+) 685.4270. Found 685.4409.

3-Descladinose-6,11-*O*,*O*-bridged Erythromycin A 9-Imine Acetamide 2′-Acetate (10). A solution of **7** (1.0 kg, 1.460 mol) in EtOH (2 L) was treated with 20% $TiCl_3$ solution in 3% aqueous HCl (2.847 kg or 2.33 L, 3.691 mol) over 1 h at 25−35 °C. This was agitated at 25−30 °C for 3 h or until the reaction was complete by HPLC analysis (conversion >97%). The reacton mixture was diluted with 8−12 °C H_2O (15 L) and treated with 50 wt % aq NaOH (0.466 L) over 0.5−1 h at <35 °C to pH 6−7, and then was further basified with saturated aq K_2CO_3 (about 0.666 L) at <35 °C to pH 9−10. This was extracted with CH_2Cl_2 (4 × 4 L). The combined extracts were concentrated *in vacuo* to 2−2.5 L of remaining volume. The resulting concentrated solution of 9-imine **9** in CH_2Cl_2 was treated with Ac_2O (0.277 L, 2.936 mol), and the 9-acetylation reaction was complete at ambient temperature within 2 h as indicated by HPLC analysis (conversion >97%). The solvent was exchanged by repeated EtOAc addition (2 or 3 × 3 L) and distillation *in vacuo* until crystallization occurred at 1.5−2 L of remaining volume. Heptanes (1.5 to 2 L) was added at 40−45 °C. The slurry was cooled to 0−4 °C over 2 h and held for 2 h. The crystals were collected by filtration, washed with chilled EtOAc/heptanes (1:2, 0.3 L), and dried under vacuum at 45 °C until constant weight to afford crystalline **10**. The average yield (2 kg/batch, 10 batches) for this two-step, one-pot process was 84%. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.18 (2 H, 19═CH_2), 4.93 (1 H, 13-OCH), 4.75 (1 H, 2′-OCH), 4.74 (1 H, 1′-OCHO), 4.59 (1 H, 11-OCH), 4.13 and 3.60 (2 H, 18-CH_2), 4.08 and 4.52 (2 H, 16-CH_2), 3.74 (1 H, 5-OCH), 3.48 (1 H, 5′-OCH), 3.43 (1 H, 3-OCH), 2.84 (1 H, 12-OH), 2.73 (1 H, 2-CH), 2.72 (1 H, 3′-NCH), 2.66 (1 H, 10-CH), 2.55 (1 H, 8-CH), 2.43 (1 H, 4-CH), 2.26 (6 H, 3′-NCH_3 × 2), 2.12 (3 H, 9═$NCOCH_3$), 2.07 (3 H, 2′-$OCOCH_3$), 2.02 and 1.46 (2 H, 14-CH_2), 1.73 and 1.33 (2 H, 4′-CH_2), 1.69 and 1.39 (2 H,

7-CH_2), 1.31 (3 H, 6-CH_3), 1.26 (3 H, 10-CH_3), 1.23 (9 H, 2-CH_3, 12-CH_3 and 5′-CH_3), 1.10 (3 H, 8-CH_3), 0.97 (3 H, 4-CH_3), 0.91 (3H, 15-CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 184.3, 176.4, 174.5, 169.7, 141.8, 122.3, 99.6, 81.5, 78.6, 78.0 (3-OCH and 13-OCH), 76.3, 75.9, 73.8, 71.7, 68.9, 65.8, 63.2, 43.7, 40.9, 39.9, 39.2, 38.5, 35.5, 31.1, 25.6, 23.3, 21.7, 21.2, 19.8, 19.5, 17.2, 15.8, 14.6, 11.8, 7.8. HRMS Calcd for $C_{37}H6_2N_2O_{11}$ (MH^+) 711.4426. Found 711.4431.

3-Descladinose-6,11-*O*,*O*-bridged Ketone Erythromycin A 9-Imine Acetamide 2′-Acetate (12). A solution of **10** (4.0 kg, 5.628 mol) in acetone (7 L) was treated with $NaIO_4$ (2.648 kg, 12.38 mol) and followed by H_2O (14 L). A 4 wt % aq OsO_4 (96 mL, 15.72 mmol, 0.28 mol %) solution was added during a period of 5 to 10 min. (**Caution: OsO_4 is highly poisonous, even at low exposure levels, and must be handled with appropriate precautions!**) This was an exothermic reaction and the temperature gradually rose to 40−45 °C over the period of 1 h., The reaction was complete after additional 2−3 h at 35−42 °C as indicated by HPLC (conversion >97%). If the diol intermediate persisted, additional 10% $NaIO_4$ (264 g, 1.236 mol) was added and the reaction mixture was agitated for 1 h. After cooling to 20−25 °C, the reaction mixtue was diluted with EtOAc (40 L), treated with saturated aq $NaHCO_3$ (16 L), and the resulting two-phase mixture was agitated vigorously for 15 min. The organic phase was separated and the remaining aq suspension was further extracted with EtOAc (2 × 16 L). The combined organic extracts were treated with a mixed solution of $Na_2S_2O_5$ (0.40 kg, 2.104 mol) in H_2O (1.5 L) and saturated aq $NaHCO_3$ (16 L) to remove remaining oxidizing reagents. After agitation and separation, the organic phase was washed with 15% aq NaCl (16 L) and concentrated *in vacuo* to 7−8 L of remaining volume. Toluene (8 L) was added and distilled *in vacuo* to dry the material azeotropically. CH_2Cl_2 (16 L) was added and concentrated again *in vacuo* to 7−8 L of remaining volume to give a solution of **11** in CH_2Cl_2., The typical yield for this olefin oxidative cleavage step was 78% as determined by ^{1}H NMR analysis. A suspension of NCS (752 g, 5.632 mol) in CH_2Cl_2 (12 L) was agitated and cooled to −15 °C. Me_2S (580 mL, 7.88 mol) was added dropwise over 25 min at −13 to −15 °C. After 15 min, the mixture was cooled to −20 °C. This was treated with the above concentrated CH_2Cl_2 solution of **11** over 1 h at −18 to −20 °C. After agitating for 3 h, Et_3N (784 mL, 5.624 mol) was added over 20 min at −15 to −18 °C by controlling the addition rate. After 1 h, the reaction mixture was warmed to 5−10 °C and then diluted with EtOAc (64 L) and washed with saturated aq $NaHCO_3$ (2 × 20 L) and 15% aq NaCl (20 L). The organic phase was concentrated *in vacuo* to 5−6 L of remaining volume. The solvent was swapped to EtOH by charging EtOH (about 10 L) while continuously concentrating the organic solution *in vacuo* until crystallization occurred. The resulting slurry was gradually cooled to 0−4 °C and held for 2 h. The crystals were collected by filtration, washed with chilled EtOH (0.6 L at 5 °C), and dried under vacuum at 30 °C until at constant weight to afford 2.08 kg (52% overall yield for two steps) of **12**. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.93 (1 H, 13-OCH), 4.78 (1 H, 2′-OCH), 4.63 (1 H, 11-OCH), 4.53 (1 H, 1′-OCHO), 4.41 and 3.56 (2 H, 18-CH_2), 4.34 and 3.95 (2 H, 16-CH_2), 4.24 (1 H, 5-OCH), 4.00 (1 H, 2-CH), 3.65 (1 H, 5′-OCH), 3.44 (1 H, 4-CH), 2.87 (1 H, 3′-NCH), 2.83 (1 H, 10-CH), 2.67 (1 H, 12-OH), 2.64 (1 H, 8-CH), 2.36 (6 H, 3′-NCH_3 × 2), 2.10 (3 H, 9═$NCOCH_3$), 2.08 and 1.57 (2 H, 14-CH_2), 2.07 (3 H, 2′-$OCOCH_3$), 1.84 and 1.38 (2 H, 4′-CH_2), 1.79 and 1.49 (2 H, 7-CH_2), 1.35 (3 H, 6-CH_3), 1.33 (3 H, 2-CH_3), 1.32 (3 H, 12-CH^3), 1.29 (3 H, 10-CH_3), 1.28 (3 H, 5′-CH_3), 1.27 (3 H, 4-CH_3), 1.17 (3 H, 8-CH_3), 0.92 (3 H, 15-CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 205.5, 204.9, 184.2, 175.8, 169.8, 169.4, 99.8, 79.9, 79.2, 79.0, 75.9 (12-OC and 16-CH_2), 74.9, 71.2, 69.2, 68.9, 63.7, 51.1, 45.3, 40.7, 39.7, 38.8, 36.8, 30.9, 25.3, 23.1, 21.6, 21.4, 20.1, 19.7, 17.1, 15.6, 14.3, 12.9, 11.6. HRMS Calcd for $C_{36}H_{58}N_2O_{12}$ (MH^+) 711.4063. Found 711.4066.

(9*E*)-9-(Acetylimino)-3-de[(2,6-dideoxy-3-C-methyl-3-*O*-methyl-α-L-ribo-hexopyranosyl)oxy]-9-deoxo-3-oxo-6,11-*O*-[(2*E*)-2-({[6-(1*H*-pyrazol-1-yl)pyridin-3-yl]methoxy}imino)-propane-1,3-diyl]erythromycin, EDP-420 (1). A solution of hydroxylamine **4**[12] (308 g, 1.619 mol) in EtOH (2.76 L) was treated with 1 N aq HCl (2.76 L) at <25 °C by adjusting the addition rate. The mixture was cooled to 0−5 °C and **12** (1.0 kg, 1.407 mol) was added at 0−5 °C. The mixture became a clear solution and was agitated for 0.5−1 h, at which point the reaction was complete as indicated by HPLC analysis (conversion >97%). This was warmed to 15−20 °C, diluted with EtOAc (7 L) and washed with saturated aq $NaHCO_3$ (6 L). The aqueous phase was back-extracted with EtOAc (3 L). The combined organic extracts were concentrated *in vacuo* to 1−1.5 L to give a solution of **13** (oxime *E*/*Z* ratio ∼4:1 by HPLC) in EtOAc. The solvent was swapped to MeOH by charging MeOH (4 L) and continuously concentrating *in vacuo* to 1.5−2 L. Additional MeOH (2 L) was added, and the resulting solution was filtered. The filtrate (**13** in MeOH) was agitated at ambient temperature for 15−18 h, after which the 2′-deacetylation reaction was complete as indicated by HPLC analysis (conversion >97%). The reaction mixture was concentrated *in vacuo* to 1−2 L. The solvent was swapped to EtOH by charging EtOH (5 L) and concentration to 2.5−3 L. H_2O (3 L) was slowly added until crystallization occurred while maintaining an internal temperature of 70−80 °C. The hot slurry was gradually cooled to 20−22 °C over a period of 3−4 h. The crystals were collected by filtration, washed with EtOH/H_2O (1:2, 0.3 L), and dried under vacuum at 50−70 °C to afford crystalline crude **1** [typical yield: 65%, typical purity of the desired *E*-oxime product: >90 area % by HPLC, oxime *E*/*Z* ratio >30:1].

Purification of (9*E*)-9-(Acetylimino)-3-de[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-ribo-hexopyranosyl)oxy]-9-deoxo-3-oxo-6,11-*O*-[(2*E*)-2-({[6-(1*H*-pyrazol-1-yl)pyridin-3-yl]methoxy}imino)propane-1,3-diyl]erythromycin, EDP-420 (1). Crude **1** (1.26 kg, 1.5 mol) was dissolved in ethyl acetate (3.2 L) at 60−70 °C and was treated with a solution of L-malic acid (0.24 kg, 1.79 mol) in ethyl acetate (3.2 L) at 55−65 °C. After 10 min 2-propanol (6.4 L) was added. This was cooled to 18−23 °C over the period of 2−3 h and held for at least 1 h. The crystals were collected by filtration, washed with ethyl acetate/2-propanol (1:1, 0.6 L), and dried under vacuum at 40−45 °C until at constant weight. This was dissolved in ethyl

(12) Tang, D.; Qiu, Y.; Kim, H.; Or, Y. S.; Wang, Z. U.S. Patent 7,538,225, 2009.

acetate (6.4 L) at 70−80 °C. 2-Propanol (6.4 L) was added at 60−65 °C. The hot slurry was cooled to 18−23 °C over 2−3 h and held for 1 h. The crystals were collected by filtration, washed with ethyl acetate/2-propanol (1:1, 0.6 L), and dried under vacuum at 40−45 °C until at constant weight. A suspension of this crystalline solid in ethyl acetate (10 L) was slowly treated with saturated aqueous sodium bicarbonate solution (6.0 L) at 18−25 °C. After 20 min, the layers were separated. The aqueous solution was extracted with ethyl acetate (7.0 L). The combined organic extracts were washed with brine (6.0 L), concentrated *in vacuo* to 4 L of remaining volume, and filtered. The filtrate was concentrated *in vacuo* to 1−2 L. The solvent was swapped to EtOH by charging ethanol (5.0 L) and concentrating to 2.5−3 L of remaining volumes. H_2O (3 L) was slowly added over 0.5 h until crystallization occurred while maintaining an internal temperature of 70−80 °C. The hot slurry was gradually cooled to 20−22 °C over a period of 3−4 h and held for at least 2 h. The crystals were collected by filtration, washed with EtOH/H_2O (1:1, 0.5 L), and dried under vacuum at 40−45 °C to afford 0.8 kg (63% overall yield) of **1** as a white crystalline solid in >98 area % purity by HPLC analysis. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.58 (1 H, −CH═N in pyrazole), 8.42 (1 H, −CH═N in pyridine), 7.96 (1 H, −CH═ in pyridine), 7.85 (1 H, −CH═ in pyridine), 7.75 (1 H, −CH═N in pyrazole), 6.47 (1 H, −CH═ in pyrazole), 5.31 (2 H, ArCH_2O), 4.72 (1 H, 11-OCH), 4.64 and 4.51 (2 H, 16-CH_2), 4.63 (1 H, 13-OCH), 4.47 (1 H, 5-OCH), 4.35 (1 H, 1′-OCHO), 3.99 (1 H, 2-CH), 3.98 and 3.55 (2 H, 18-CH_2), 3.61 (1 H, 5′-OCH), 3.46 (1 H, 4-CH), 3.18 (1 H, 2′-OCH), 2.80 (1 H, 10-CH), 2.75 (1 H, 12-OH), 2.62 (1 H, 8-CH), 2.49 (1 H, 3′-NCH), 2.28 (6 H, 3′-NCH_3 × 2), 2.11 (3 H, 9═NAc), 2.01 and 1.46 (2 H, 14-CH_2), 1.81 and 1.68 (2 H, 7-CH_2), 1.69 and 1.24 (2 H, 4′-CH_2), 1.41 (3 H, 4-CH_3), 1.38 (3 H, 6-CH_3), 1.33 (6 H, 2-CH_3 and 10-CH_3), 1.29 (3 H, 12-CH_3), 1.26 (3 H, 5′-CH_3), 1.16 (3 H, 8-CH_3), 1.00 (3 H, 15-CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 205.8, 184.2, 177.8, 167.7, 153.9, 151.1, 148.3, 142.2, 139.3, 131.2, 127.3, 112.3, 108.0, 103.0, 79.4, 79.1, 76.5, 75.6, 74.7, 73.2, 70.6, 69.7, 66.2, 63.0, 62.9, 50.7, 46.2, 40.6, 40.5, 38.7, 37.2, 28.6, 25.4, 23.9, 21.6, 20.3, 19.6, 18.0, 15.1, 14.1, 13.8, 13.0. HRMS Calcd for $C_{43}H_{64}N_6O_{11}$ (MH^+) 841.4706. Found 841.4704.

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Supporting Information Available

^{1}H and ^{13}C NMR spectra and assignments for all new isolated compounds **1**, **7**, **10**−**13**. Two-dimensional NMR spectra (COSY, HMBC, HMQC, NOESY, TOCSY) for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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