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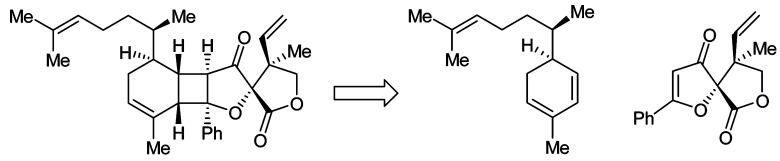

biyouyanagin A **ent-zingiberene** **hyperolactone C**

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Total Synthesis, Revised Structure, and Biological Evaluation of Biyouyanagin A and Analogues Thereof

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Abstract: Isolated from *Hypericum* species *H. chinese L. var. salicifolium*, biyouyanagin A was assigned structure **1a** or **1b** on the basis of NMR spectroscopic analysis. This novel natural product exhibited significant anti-HIV properties and inhibition of lipopolysaccharide-induced cytokine production. Described herein are the total syntheses of biyouyanagin A and several analogues (**3**−**11**), structural revision of biyouyanagin A to **2b**, and the biological properties of all synthesized compounds. The total synthesis proceeded through cascade sequences that efficiently produced enantiomerically pure key building blocks **15b** (*ent*-zingiberene) and **18** (hyperolactone C) and featured a novel [2 + 2] photoinduced cycloaddition reaction which occurred with complete regio- and stereoselectivity. Biological investigations with the synthesized biyouyangagins A (**2**−**11**) and hyperolactones C (**12**−**16**) revealed that the activity of biyouyanagin A most likely resides in its hyperolactone C structural domain.

Introduction

The *Hypericum* species *H. chinese L. var. salicifolium* has been used in Japan as a folk medicine for the treatment of female disorders for centuries.[1] Investigations with this plant led to the discovery of biyouyanagin A, a substance which was originally assigned structure **1a** or **1b** (Figure 1) on the basis of NMR spectroscopic analysis.[2] Biyouyanagin A exhibited significant and selective inhibitory activity against HIV replication in H9 lymphocytes (EC_{50} = 0.798 μg mL^{-1}) as compared to noninfected H9 lymphocytes (EC_{50} >25 μg mL^{-1}), thus demonstrating a therapeutic index (TI) of greater than 31.3.[2] In addition, this substance inhibited strongly lipopolysaccharide (LPs)-induced cytokine production at 10 μg mL^{-1} [IL-10 = 0.03; IL-12 = 0.02; tumor necrosis factor-α (TNF-α) = 0.48].[2] In a recent communication we reported the total synthesis and structural revision of biyouyanagin A (**2b**) and its 24-epimer, 24-*epi*-biyouyanagin A (**2a**, Figure 2).[3] In this article we report our detailed investigations in the biyouyanagin field, including

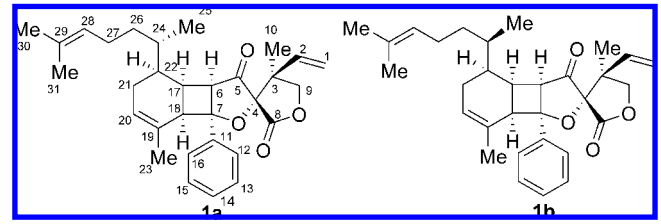

Figure 1. Originally proposed structures for biyouyanagin A.

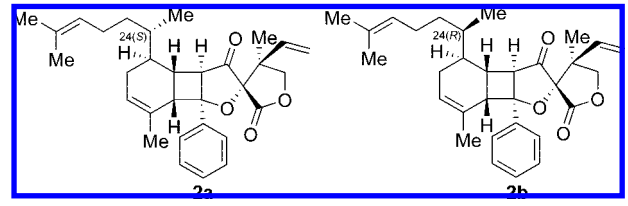

Figure 2. Revised structures of biyouyanagin A (**2b**) and 24-*epi*-biyouyanagin A (**2a**).

an X-ray crystallographic analysis of biyouyanagin A (**2b**) and the synthesis and biological evaluation of several analogues (**3**−**16**, Figure 3) of this novel natural product.

Results and Discussion

By virtue of its novel molecular architecture, the remaining ambiguity shadowing its structure, and its significant biological properties, biyouyanagin A presented itself as an attractive synthetic target. Of particular interest was the verification of the all-*cis* stereochemistry of the substituents around the cyclobutane ring, an arrangement that looked rather odd at the

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⊥ University of California, San Diego.

(1) Murakami, K. *Tokushima-ken Yakusouzukan* **1984**, 102–103.
(2) Tanaka, N.; Okasaka, M.; Ishimaru, Y.; Yakaishi, Y.; Sato, M.; Okamoto, M.; Oshikawa, T.; Ahmed, S. U.; Consentino, L. M.; Lee, K.-H. *Org. Lett.* **2005**, *7*, 2997–2999.
(3) Nicolaou, K. C.; Sarlah, D.; Shaw, D. M. *Angew. Chem., Int. Ed.* **2007**, *46*, 4708–4711.

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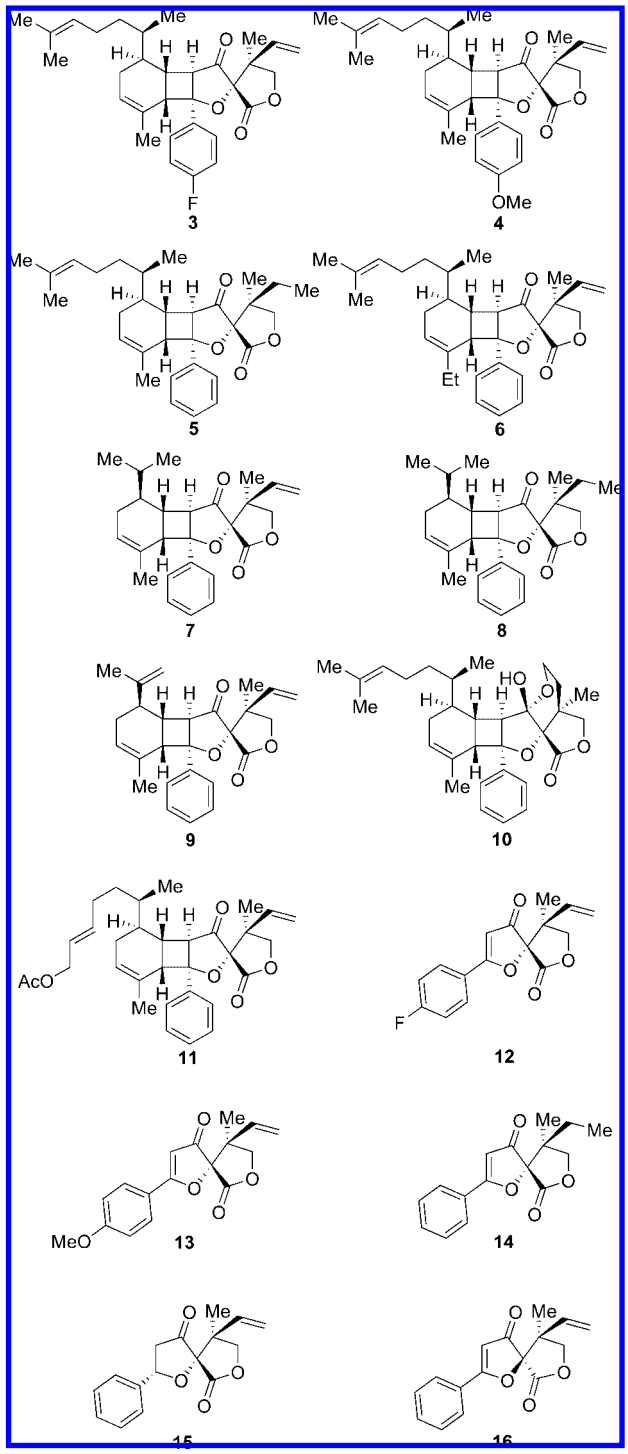


Figure 3. Synthesized biyouyanagin A (**3**−**11**) and hyperolactone C (**12**−**16**) analogues.

outset,[2] given the steric congestion associated with it. Another impetus for undertaking the total synthesis of biyouyanagin A was to advance further the advent of cascade reactions[4] and exploit recent developments in organocatalysis[5] for total synthesis purposes.

(4) For a recent review article on cascade reactions in total synthesis, see: (a) Nicolaou, K. C.; Edmonds, D. J.; Bulger, P. G. *Angew. Chem., Int. Ed.* **2006**, *45*, 7134–7186.

(5) Berkessel, A.; Gröger, H. *Asymmetric Organocatalysis: From Biomimetic Concepts to Applications in Asymmetric Synthesis*; Wiley-VCH, Weinheim, 2006; p 454.

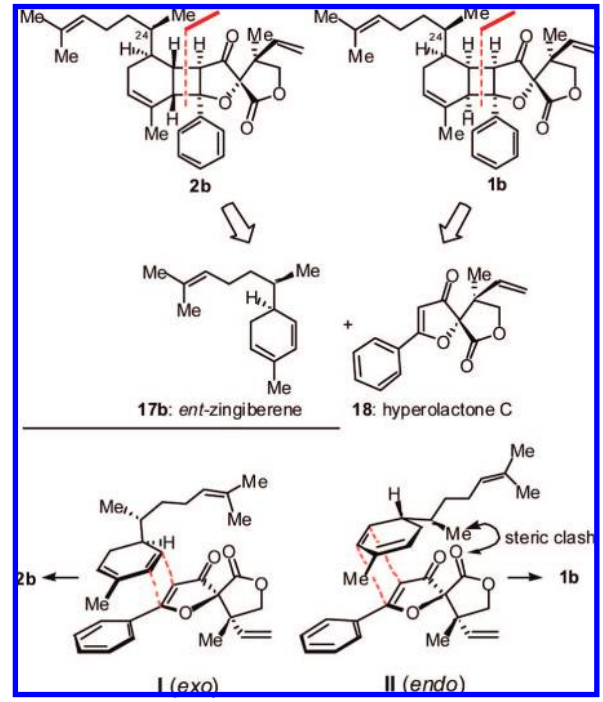
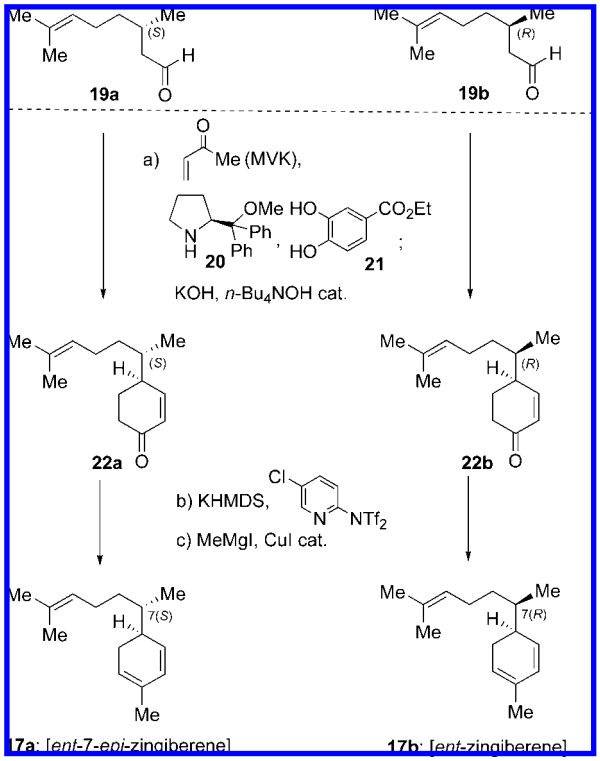


Figure 4. Retrosynthetic analysis of biyouyanagin A and transition states of the proposed photoinduced [2 + 2] cycloaddition reaction. For simplicity, only the 24(*R*) isomers (**1b** and **2b**) are shown.

Retrosynthetic Analysis. While there are myriad ways to disassemble the biyouyanagin A molecule retrosynthetically, the one made possible by a retro [2 + 2] cycloaddition reaction (Figure 4) is both aesthetically and practically most appealing. In the synthetic direction such a reaction can, in principle, be realized by irradiation with UV light, although no precedent existed at the outset of this work for the photoinduced [2 + 2] cycloaddition of substrates such as the two components defined by the proposed cyclobutane disconnection (i.e., triene **17b** or its 7-epimer **17a** and enone **18**, Figure 4). If successful, however, this approach would consititute a highly convergent strategy for the total synthesis of the natural product and might also have implications in its biosynthesis. To be sure, however, this rather obvious hypothesis had been proposed as a plausible biosynthetic pathway toward biyouyanagin A by its discoverers.[2] In considering such a scenario, inspection of the two transition states that could lead to the biyouyanagin A molecule (**I**: *exo* and **II**: *endo*, Figure 4) was instructive. Thus, formation of the *cis*, *cis*, *cis*, *cis* structure (**1a** or **1b**) originally proposed for biyouyanagin A[2] requires the *endo* transition state **II**, an arrangement that suffers from severe steric congestion between the γ-lactone moiety of the enone and the side chain of the triene component as demonstrated by manual molecular models and shown in Figure 4. On the other hand, the alternative arrangement of the reacting components as shown in the *exo* transition state **I** is free of such unfavorable interactions. This realization created a suspicion in our minds with regards to the structure of biyouyanagin A as proposed in the isolation paper.[2] Specifically, we began to favor the *cis*, *cis*, *trans*, *trans* stereochemistry as shown in structure **2b**, although the cloud of ambiguity over the configuration of the C-24 stereocenter (see structure **2a**) remained. In addition, the NOE interactions reported for biyouyanagin A,[2] in conjunction with manual molecular models, did not exclude the *cis*, *cis*, *trans*, *trans* structure **2b** (or **2a**), a fact that fueled further our skepticism about the true structure of the natural product. It was with this

Scheme 1. Synthesis of Terpenoids **17a** and **17b**[a]

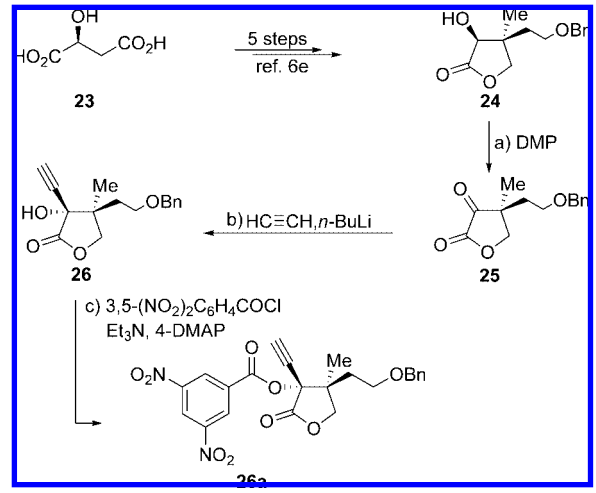


[a] Reagents and conditions: (a) **19a** or **19b** (1.0 equiv), MVK (1.5 equiv), **20** (5 mol%), ethyl 3,4-dihydroxybenzoate (20 mol%), 0 °C, 24 h; then KOH (0.1 N aq., 1.0 equiv), *n*-Bu_4NOH (40% aq., cat.), Et_2O/THF/H_2O (3:1:3), reflux, 6 h, 72% yield, 93% *de* for **22a**; 68% yield, 86% *de* for **22b**; (b) KHMDS (1.5 equiv), THF, −78 °C, 3 h; then Comins reagent (1.5 equiv), THF, −78 °C, 1 h; (c) MeMgI (3.0 M in Et_2O, 1.5 equiv), CuI (2 mol%), THF, 0 °C, 15 min, 80% over the two steps. MVK = methyl vinyl ketone; THF = tetrahydrofuran; KHMDS = potassium hexamethyldisilazanide; Tf = trifluoromethanesulfonyl.

background that we embarked on the synthetic journey to biyouyanagin A, whose true structure became our immediate puzzle to solve.

Construction of Building Blocks for Biyouyanagin A. Having defined the required building blocks for the devised [2 + 2] photocycloaddition strategy toward biyouyanagin A, their construction evolved as shown in Scheme 1 (**17a**: *ent*-7-*epi*-zingiberene and **17b**: *ent*-zingiberene) and Schemes 2 and 3 (**18**: hyperolactone C). Interestingly, hyperolactone C (**18**) is already known in the literature as a naturally occurring substance,[6] while **17a** and **17b** are enantiomers of natural terpenes (7-*epi*-zingiberene and zingiberene, respectively).[7] The initial

Scheme 2. Synthesis of Propargyl Alcohol **26**[a]



[a] Reagents and conditions: (a) DMP (2.0 equiv), CH_2Cl_2, 25 °C, 5 h, 92%; (b) acetylene, *n*-BuLi, THF, −78 °C, 1 h, 92%, 3:1 d.r.; (c) 3,5-dinitrobenzoyl chloride (1.2 equiv), Et_3N (1.2 equiv), 4-DMAP (0.1 equiv), CH_2Cl_2, 25 °C, 3 h, 98%. DMP = Dess−Martin periodinane; 4-DMAP = 4-dimethylaminopyridine.

choice of these particular enantiomeric forms of **17a** and **17b** was based on the assumption that the hyperolactone C structural motif of biyouyanagin A would be of the same absolute configuration as the naturally occurring hyperolactone C,[6] a postulate that, in turn, suggested the absolute configuration of the terpenoid fragments as shown.

The synthesis of the terpenoid fragments **17a** (*ent*-7-*epi*-zingiberene) and **17b** (*ent*-zingiberene) begun from (*S*)-citronellal (**19a**) and (*R*)-citronellal (**19b**), respectively, and proceeded according to the sequence depicted in Scheme 1. Thus, enamine-mediated 1,4-addition of (*S*)-citronellal (**19a**) to methyl vinyl ketone (MVK) facilitated by the proline-derived catalyst **20** (5 mol%)[8] and ethyl 3,4-dihydroxybenzoate (**21**) as a cocatalyst (20 mol%)[9] furnished, after an intramolecular aldol condensation (KOH, *n*-Bu_4NOH cat.) of the resulting ketoaldehyde product, 24(*S*) enone **22a** in 72% overall yield and 93% de. The conversion of the latter compound to the desired building block **17a** (*ent*-7-*epi*-zingiberene) proceeded smoothly and in 80% overall yield through a Kumada coupling of the corresponding vinyl triflate (KHMDS, Comins reagent)[10] with MeMgI (CuI cat.).[11] A similar route starting from (*R*)-citronellal (**19b**) and MVK, and proceeding through intermediate enone **22b** (68% yield, and 86% de), furnished *ent*-zingiberene (**17b**), the other targeted terpenoid fragment (80% overall yield for the last two steps).

The synthesis of hyperolactone C (**18**, Scheme 2)[6e] began with (*S*)-malic acid (**23**) and proceeded, sequentially, through lactone **26** (Scheme 2) and spirolactone intermediate **27** (Scheme 4). As shown in Scheme 2, (*S*)-malic acid was converted in five steps, and by literature procedures,[6e] to hydroxy lactone **24**, which was oxidized with DMP to ketolactone **25** in 92% yield. The latter compound reacted with lithium acetylide (generated from acetylene and *n*-BuLi in THF at −78 °C) to afford, stereoselectively, propargylic alcohol **26** (92% yield, *ca.*

(6) For selected papers on hyperolactone C, see: (a) Isolation and structure: Aramaki, Y.; Chiba, K.; Tada, M. *Phytochemistry* **1995**, *38*, 1419–1421. (b) X-ray structure: Crockett, S. L.; Schuhly, W.; Belaj, F.; Khan, I. A. *Acta Crystallogr., Sect. E* **2004**, *60*, o2174−o2176. (c) Synthesis: Ichinari, D.; Ueki, T.; Yoshihara, K.; Kinoshita, T. *Chem. Commun.* **1997**, 1743–1743. (d) Ueki, T.; Ichinari, D.; Yoshihara, K.; Morimoto, Y.; Kinoshita, T. *Tetrahedron Lett.* **1998**, *39*, 667–668. (e) Ueki, T.; Doe, M.; Tanaka, R.; Morimoto, Y.; Yoshihara, K.; Kinoshita, T. *J. Heterocycl. Chem.* **2001**, *38*, 165–172. (f) Kraus, G. A.; Wei, J. *J. Nat. Prod.* **2004**, *67*, 1039–1040.

(7) For selected papers on zingiberenes, see: (a) Eschenmoser, A.; Schinz, H. *Helv. Chim. Acta* **1950**, *33*, 171–177. (b) Arigoni, D.; Jeger, O. *Helv. Chim. Acta* **1954**, *37*, 881–883. (c) Joshi, G. D.; Kulkarni, S. N. *Indian J. Chem.* **1965**, *3*, 91–92. (d) Uhde, G.; Ohloff, G. *Helv. Chim. Acta* **1972**, *55*, 2621–2625. (e) Breeden, D. C.; Coates, R. M. *Tetrahedron* **1994**, *50*, 11123–11132. (f) Bhonsle, J. B.; Deshpande, V. H.; Ravindranathan, T. *Indian J. Chem., Sect. B* **1994**, *33*, 313–316.

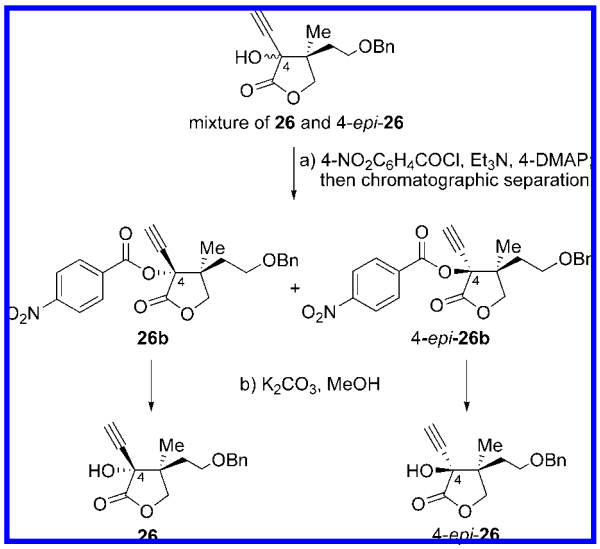
(8) Enders, D.; Kipphardt, H.; Gerdes, P.; Brena-Valle, L. J.; Bhushan, V. *Bull. Soc. Chim. Belg.* **1988**, *97*, 691–704.

(9) Chi, Y.; Gellman, S. H. *Org. Lett.* **2005**, *7*, 4253–4256.

(10) Comins, D. L.; Dehghani, A. *Tetrahedron Lett.* **1992**, *33*, 6299–6302.

(11) Karlström, A. S. E.; Rönn, M.; Thorarensen, A.; Bäckvall, J.-E. *J. Org. Chem.* **1998**, *63*, 2517–2522.

Scheme 3. Separation of Propargyl Alcohols **26** and 4-*epi*-**26** (Biyouyanagin Numbering)[a]



[a] Reagents and conditions: (a) 3,5-dinitrobenzoyl chloride (1.2 equiv), Et_3N (1.2 equiv), 4-DMAP (0.1 equiv), CH_2Cl_2, 25 °C, 3 h; (b) K_2CO_3 (5.0 equiv), MeOH, 0 → 25 °C, 30 min, 98%.

Figure 5. ORTEP drawing of compound **26a** with the thermal ellipsoids at 30% probability level.

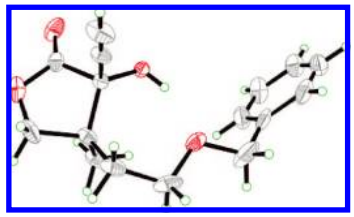
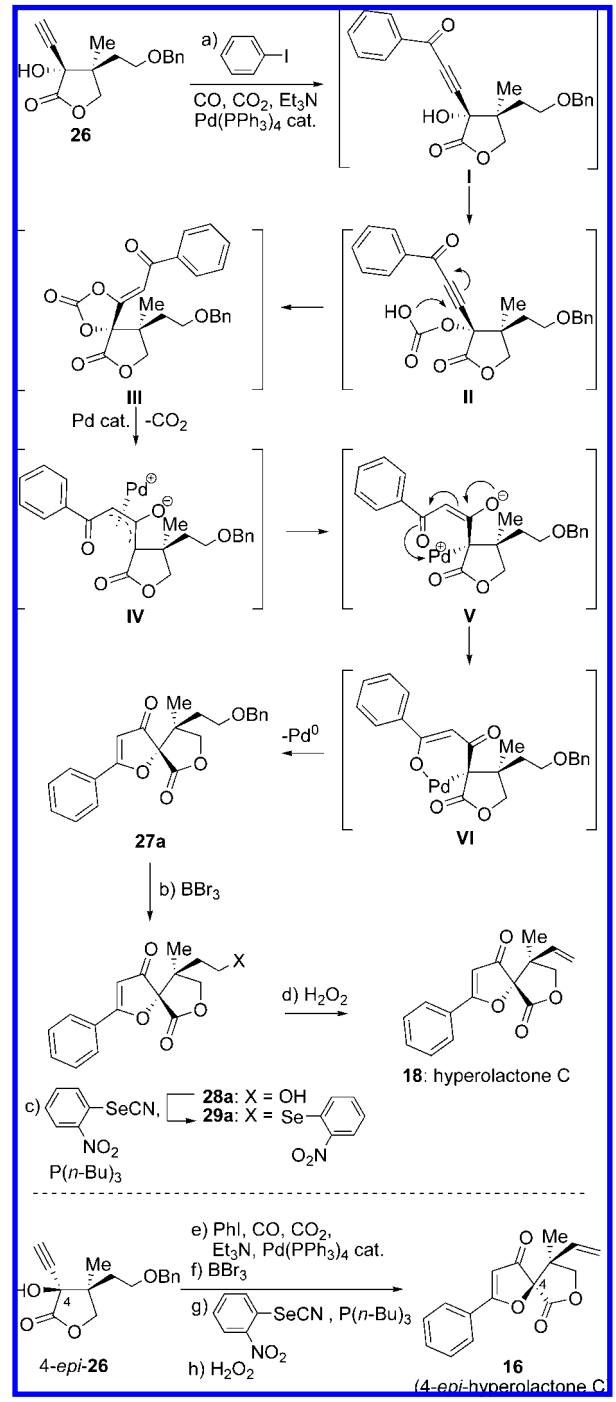


Figure 6. ORTEP drawing of compound **26** with the thermal ellipsoids at 30% probability level.

3:1 isomeric ratio). Although this mixture could not be conveniently resolved chromatographically, the desired stereoisomer could be isolated easily by fractional crystallization from CH_2Cl_2/hexanes (62% yield). Alternatively, the two isomers could be separated by flash column chromatography of their 4-nitrobenzoates (4-nitrobenzoyl chloride, Et_3N, 4-DMAP, 95% combined yield), and then the two free alcohols (**26** and 4-*epi*-**26**) were released after hydrolysis (K_2CO_3, MeOH, 98% yield, Scheme 3). The stereoselectivity observed in this reaction is attributed to the presumed chelation control exerted by the benzyloxy group. The stereochemistry of **26** was initially confirmed by an X-ray crystallographic analysis (see ORTEP drawing, Figure 5) of its 2,4-dinitrobenzoate (**26a**, mp 159−160 °C, toluene; obtained by reaction of **26** with 2,4-dinitrobenzoyl chloride in the presence of Et_3N and 4-DMAP, 98% yield) and subsequently by an X-ray crystallographic analysis performed on the compound itself (**26**), which crystallized upon prolonged standing in a mixture of CH_2Cl_2 and hexanes (mp 84−86 °C, CH_2Cl_2/hexanes; see ORTEP drawing, Figure 6).

Scheme 4. Synthesis of Hyperolactone C (**18**) and 4-*epi*-Hyperolactone C (**16**) through Palladium-Catalyzed Cascade Sequences (Biyouyanagin Numbering)[a]



[a] Reagents and conditions: (a) $Pd(PPh_3)_4$ (5 mol%), PhI (1.2 equiv), CO (200 psi), CO_2 (200 psi), Et_3N, 100 °C, 5 h, 79%; (b) BBr_3 (1.5 equiv), CH_2Cl_2, −78 °C, 30 min, 95%; (c) *o*-$NO_2C_6H_4SeCN$ (1.2 equiv), P(*n*-Bu)$_3$ (1.2 equiv), THF, 25 °C, 4 h, 90%; (d) H_2O_2 (30% aq., 10 equiv), THF, 0 → 25 °C, 1 h, 85%, (e) same as (a), 71%; (f) same conditions as for (b); (g) same conditions as for (c); (h) same conditions as for (d), 71% over the three steps.

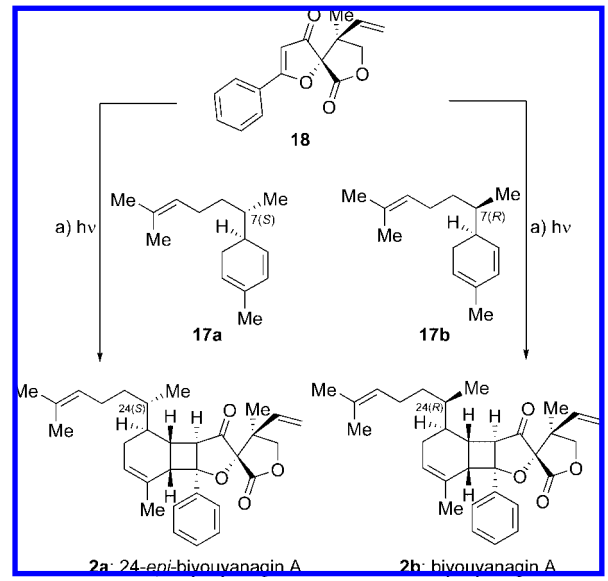
What was developed next was a highly efficient and pleasing cascade sequence that produced spirolactone **27a** from propargylic alcohol **26** and phenyl iodide in one pot. This remarkable palladium-catalyzed cascade was brought about by $Pd(PPh_3)_4$ (5 mol%) and Et_3N under an atmosphere of a mixture of CO and CO_2 (200 psi) as depicted in Scheme 4 (79% yield, single

Figure 7. ORTEP drawing of compound **27a** with the thermal ellipsoids at 30% probability level.

diastereomer).[12] An X-ray crystallographic analysis of **27a** (mp 176−177 °C, toluene, see ORTEP drawing, Figure 7) allowed its unambiguous stereochemical assignment. The retention of stereochemistry at the spirocenter of product **27a** as compared to the starting substrate (**26**) of the cascade sequence is consistent with the proposed mechanism involving intermediates **I**−**VI** shown in Scheme 4. Specifically, it is postulated[12] that a Sonogashira-type process under the reaction conditions allows the initial formation of acetylenic ketone **I**, which absorbs a molecule of CO_2 to furnish species **II**, an intermediate that serves as the precursor to cyclic carbonate **III**, whose palladium-catalyzed extrusion of CO_2 leads to the π-palladium complex **IV** ↔ **V**, stereospecifically. Finally, rearrangement of **V**, as shown, leads to palladacycle **VI**, which readily loses Pd^0 to afford the observed product (**27a**) with retention of stereochemistry. The completion of the synthesis of hyperolactone C (**18**) required debenzylation (BBr_3), selenenylation [*o*-$(NO_2)C_6H_4$-SeCN, *n*-Bu_3P], and oxidation/*syn*-elimination (H_2O_2), a sequence that proceeded in 73% overall yield {$[\alpha]_D^{25} = -272.8$ ($c = 0.69$, $CHCl_3$); lit.,[6b] $[\alpha]_D^{25} = -270.7$ ($c = 0.11$, $CHCl_3$)} as shown in Scheme 4. Similarly, 4-*epi*-hyperolactone C (**16**) was synthesized from 4-*epi*-**26** without loss of integrity of stereochemistry (Scheme 4).

Total Synthesis of Biyouyanagin A and 24-*epi*-Biyouyanagin. Having established enantioselective and practical routes to fragments **17a** and **17b** as well as **18**, the next task became their photoinduced fusion into the biyouyanagin A framework (Scheme 5). To this end, each of the two terpenoid fragments **17a** and **17b** (4.0 equiv) were separately mixed with hyperolactone C (**18**, 1.0 equiv) and irradiated with UV light in the presence of 2′-acetonaphthone[13] (1.0 equiv) as a triplet sensitizer in a concentrated CH_2Cl_2 solution in a quartz cell (320 nm filter) at 5 °C for 8 h. Under these conditions, we were pleased to observe regio- and stereoselective formation of the desired cyclobutane product in each case, leading to **2a** (48% yield, based on **18**) and **2b** (54% yield, based on **18**). These compounds exhibited NMR spectroscopic data consistent with their structures, with **2b** revealing identical signals to those reported for the natural product, biyouyanagin A.[2] Crucial NOE data (see Figure 8) for this compound, however, supported **2b**, rather than the originally proposed structure **1b**. Particularly diagnostic for the *cis*, *cis*, *trans*, *trans* stereochemistry were the NOEs between H-6 and H-17, H-6 and H-22, and H-17 and H-22. Note that adjacent protons of the cyclobutane ring may exhibit an NOE, even if they are *trans* to each other, as it is the case here. In addition, the indicated NOEs between the aromatic and C-23 methyl protons (see Figure 8) revealed the *syn* orientation of these substituents. The absolute structures of **2a**

Scheme 5. Completion of the Total Synthesis of 24-*epi*-Biyouyanagin A (**2a**) and Biyouyanagin A (**2b**)[a]



[a] Reagents and conditions: (a) **17a** or **17b** (4.0 equiv), 2′-acetonaphthone (1.0 equiv), CH_2Cl_2 (0.5 M for **18**), 320 nm filter, 5 °C, 8 h, 48% for **2a**, 54% for **2b**.

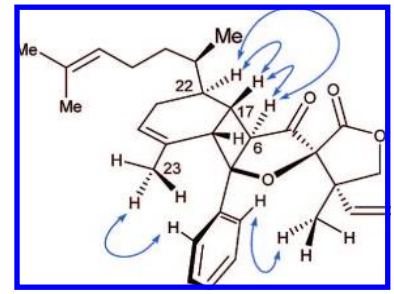


Figure 8. Selected NOEs exhibited by biyouyanagin A (**2b**).

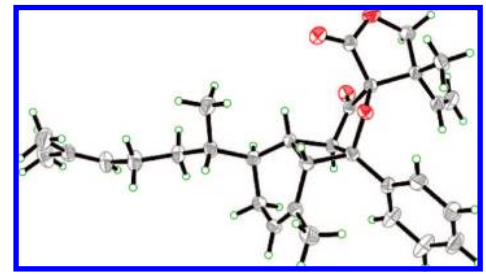


Figure 9. ORTEP drawing of compound **2a** with the thermal ellipsoids at 30% probability level.

(mp 94−95 °C, hexanes) and **2b** (mp 75−76 °C, hexanes) were ultimately solved by X-ray crystallographic analysis (see ORTEP drawings, Figures 9 and 10, respectively), which unambiguously established the complete structure of biyouyanagin A as **2b**. Its absolute stereochemistry was deduced from those of the starting materials [(*R*)-citronellal and (*S*)-malic acid] employed for the synthesis of the natural enantiomer {$[\alpha]_D^{25} = -256.6$ ($c = 0.80$, $CHCl_3$); lit.,[2] $[\alpha]_D^{25} = -240.0$ ($c = 0.50$, $CHCl_3$)}.

Design and Synthesis of Biyouyanagin A Analogues. With an efficient route to the biyouyanagin A molecule and the establishment of its relative and absolute stereochemistry, we then turned our attention to the application of the developed synthetic technology to the construction of designed analogues

(12) Inoue, Y.; Ohuchi, K.; Yen, I.-F.; Imaizumi, S. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 3518–3522.
(13) (a) Cantrell, T. S. *J. Org. Chem.* **1974**, *39*, 3063–3070. (b) Schenck, G. O.; Kuhls, J.; Krauch, C. H. *Justus Liebigs Ann. Chem.* **1966**, *693*, 20–43.

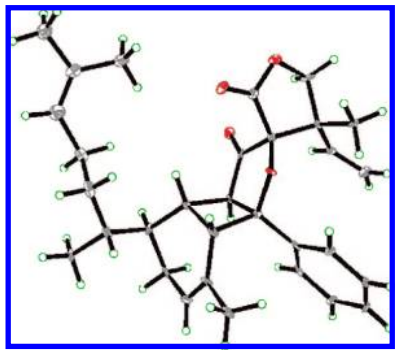


Figure 10. ORTEP drawing of compound **2b** with the thermal ellipsoids at 30% probability level.

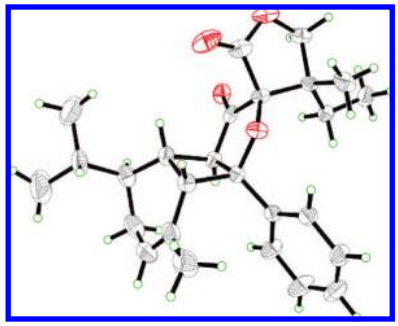
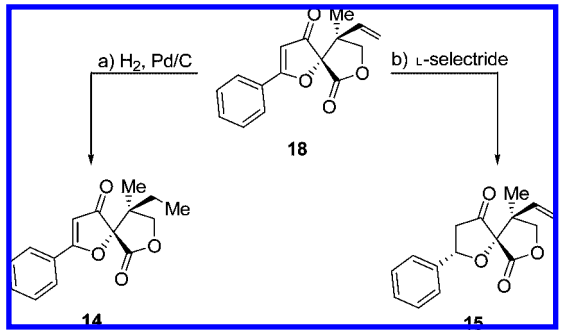


Figure 11. ORTEP drawing of compound **8** with the thermal ellipsoids at 30% probability level.

Scheme 6. Synthesis of Enone **14** and Ketone **15**[a]



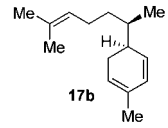
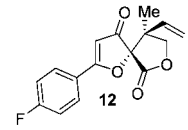
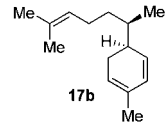
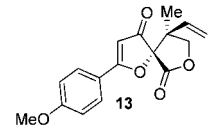
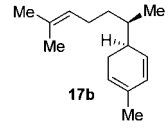
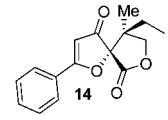
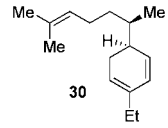
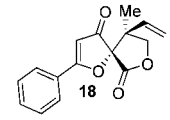
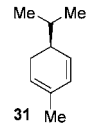
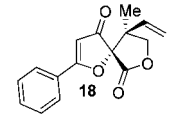
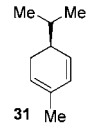
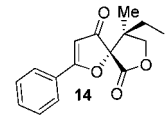
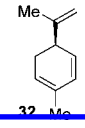
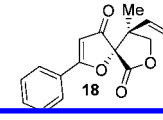
[a] Reagents and conditions: (a) H_2 (1 atm), 10 wt % Pd/C (5 mol%), MeOH, 25 °C, 3 h, 99%; (b) L-Selectride (3.0 equiv), THF, −78 → −10 °C, 1 h, 70%.

for biological investigations. The biyouyanagin A analogues **3**−**11** (Figure 3) were designed in order to take advantage of the flexibility and scope of the developed synthetic strategy and to test the effect of substituents at C-3, C-7, C-19, and C-22, while the truncated structures **12**−**16** (hyperolactone C analogues) were designed in order to explore structure activity relationships within the hyperolactone C (**18**) structural motif, which was suspected to be the active pharmacophore of the biyouyanagin A molecule.

The required building blocks were constructed by procedures either as described above (**30**, **12**, **13**) for biyouyanagin A or as reported previously in the literature (**31**,[14] **32**[15]), except for enone **14** and ketone **15**. Enone **14** was obtained from hydrogenation of hyperolactone C (**18**) with H_2 (1 atm) and 10 mol% Pd/C cat. in quantitative yield, while a stereoselective 1,4-reduction of **18** with L-selectride gave ketone **15** in 70% yield (Scheme 6). Analogues **3**−**9** (see Table 1 and Figure 2) were synthesized from the corresponding terpenoid dienes and enone components through [2 + 2] photocycloaddition reactions as indicated in Table 1. The structures of these products were consistent with their spectroscopic data; that of **8** was further confirmed by X-ray crystallographic analysis (mp 170−171 °C, hexanes; see Figure 11 for ORTEP drawing).

The hexacyclic compound **10** was unexpectedly obtained (40% yield) upon irradiation of a mixture of terpenoid component **17b** and enone **28a** in the presence of 2′-acetonaphthone through the presumed intermediacy of the initially formed adduct **33** as shown in Scheme 7. The spontaneous ring closure of the

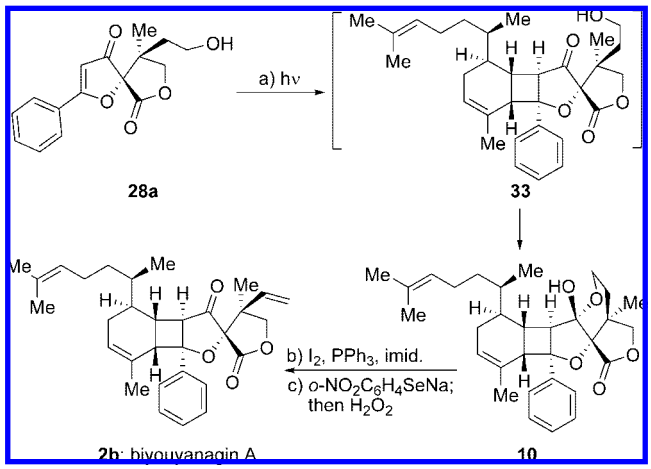
Table 1. Syntheses of Biyouyanagin A Analogues by [2 + 2] Photocycloaddition[a]

entry	terpenoid component	enone component	product (%yield)
1			**3** (49)
2			**4** (15)
3			**5** (43)
4			**6** (53)
5			**7** (49)
6			**8** (46)
7			**9** (50)

[a] Reactions were carried out by irradiating (quartz cell, 320 nm filter) a concentrated solution (0.5 M) of the terpenoid (4.0 equiv) and the enone (1.0 equiv) components in CH_2Cl_2 at 5 °C for 8 h.

(14) Sen, A.; Grosch, W. *Flavour Frag. J.* **1990**, *5*, 233–234.
(15) Buttrus, N. H.; Cornforth, J.; Hitchcock, P. B.; Kumar, A.; Stuart, A. S. *J. Chem. Soc., Perkin Trans. 1* **1987**, 851–857.

Scheme 7. Synthesis of Biyouyanagin A Analogue **10**[a]



[a] Reagents and conditions: (a) **17b** (4.0 equiv), 2′-acetonaphthone (1.0 equiv), CH_2Cl_2 (0.5 M for **28a**), 320 nm filter, 5 °C, 8 h, 40%; (b) I_2 (3.0 equiv), PPh_3 (3.0 equiv), imidazole (4.0 equiv), CH_2Cl_2; (c) *o*-$NO_2C_6H_4SeNa$ (2.0 equiv), THF; then H_2O_2 (30% aqueous, 10 equiv), THF, 25 °C, 5 h, 51% over the three steps.

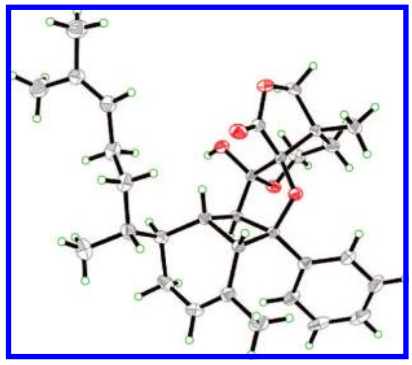


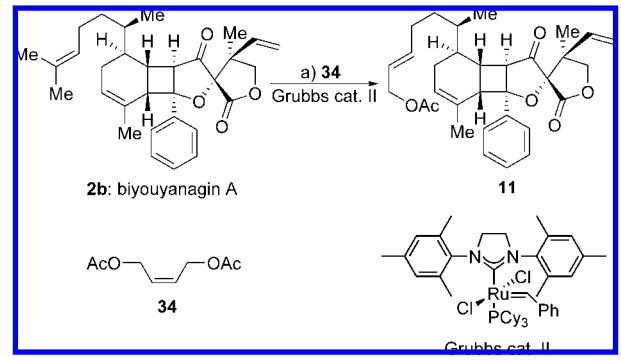
Figure 12. ORTEP drawing of compound **10** with the thermal ellipsoids at 30% probability level.

hydroxyl moiety upon the carbonyl function and the stability of its lactol form (**10**) are clearly favored within the latter molecule, as opposed to the architecture of the starting enone, which apparently (^1H and ^{13}C NMR spectroscopic analysis) remains open as shown. The structure of compound **10** was initially based on its spectroscopic data and its facile conversion to biyouyanagin A (**2b**) through iodination (I_2, PPh_3, imid.), followed by sequential exposure of the resulting iodide to *o*-$NO_2C_6H_4SeNa$ and H_2O_2 (51% overall yield for the three steps). Ultimately, an X-ray crystallographic analysis of **10** (mp 127−128 °C, CH_2Cl_2/hexanes) unambiguously established its assigned structure (see ORTEP drawing, Figure 12).

Finally, compound **11** was constructed from biyouyanagin A (**2b**) by a cross metathesis with (*Z*)-2-butene-1,4-diol diacetate (**34**, Scheme 8). This remarkably chemoselective reaction was brought about, in 73% yield, by exposure of a mixture of **2b** and **34** (5.0 equiv) to Grubbs catalyst II (10 mol%) in a CH_2Cl_2 solution at 40 °C.[16] Interestingly, no products arising from cross metathesis with any of the other olefinic bonds of biyouyanagin A (**2b**) were observed.

Biological Evaluation of Biyouyanagin A and Analogues. The synthesized compounds were tested for their inhibitory activity against HIV-1 replication in MT-2 lymphocytes and their cytotoxicity against noninfected MT-2 lymphocytes using the TZM-bl/luciferase assay and AZT as a control. As seen in Table 2, synthetic biyouyanagin A (**2b**) exhibited significant activity against HIV-1 replication in this assay (IC_{50} = 26 μM) and lower cytotoxicity against the MT-2 lymphocytes, scoring a therapeutic index (TI) of 7.5. The lower inhibitory potency observed in these experiments compared to those reported by Tanaka et al. (IC_{50} = 1.7 μM)[2] may be due to sensitivity differences in the two assays employed. 24-*epi*-Biyouyanagin A (**2a**) and the other biyouyanagin A analogues listed in Table 2 (and shown in Figures 2–4) displayed similar activities against HIV-1 replication and cytotoxicities against the MT-2 lymphocytes (IC_{50} = 19−31 μM; CC_{50} = 143−512 μM) and TIs (7.5−18.9), with analogues **6** and **10** being the most potent against HIV-1 (IC_{50} = 19 μM) and analogue **11** possessing the most favorable TI (18.9). Interestingly, however, we discovered that hyperolactone C (**18**, Figure 4) and its analogues (**12**−**16**, Figure 3) showed comparable, and in some cases more potent, anti-HIV activities compared to biyouyanagin A and its analogues. Thus, hyperolactone C (**18**, entry 17), itself, exhibited an IC_{50} value of 29 μM against HIV-1 infected cells and a CC_{50} value of 925 μM (TI = 32.0). The most potent member of the series against HIV-1 replication was found to be hyperolactone

Scheme 8. Synthesis of Analogue **11** via Olefin Cross Metathesis[a]



[a] Reagents and conditions: Grubbs II cat. (10 mol%), **34** (5.0 equiv), CH_2Cl_2, 40 °C, 24 h, 74%.

Table 2. Anti-HIV-1 Activities and Cytotoxicities of Biyouyanagin A and Analogues[a]

entry	compd	HIV-1 IC_{50}[b] (μM)	MT-2 CC_{50}[c] (μM)	TI[d]
1	AZT	0.078	>50	>641
2	**2a**	20	285	14.4
3	**2b**	26	198	7.5
4	**3**	25	444	17.5
5	**4**	25	268	10.8
6	**5**	20	197	10.0
7	**6**	19	213	11.1
8	**7**	23	333	14.4
9	**9**	31	232	7.5
10	**10**	19	143	7.5
11	**11**	27	512	18.9
12	**12**	16	1301	80.0
13	**13**	12	417	35.6
14	**14**	34	1377	40.0
15	**15**	23	459	20.0
16	**16**	35	462	13.3
17	**18**	29	925	32.0

[a] Average value from quintuplicate experiments. [b] IC_{50} is the concentration at which 50% inhibition of HIV-1 replication is observed as measured by a TZM-bl/luciferase assay. [c] CC_{50} is the concentration at which 50% inhibition of cell metabolism is observed as measured by a colorimetric XTT assay. [d] Therapeutic index (CC_{50}/IC_{50}).

(16) Chatterjee, A. K.; Choi, T. L.; Sanders, D. P.; Grubbs, R. H. *J. Am. Chem. Soc.* **2003**, *125*, 11360–11370.

C analogue **13** (IC_{50} = 12 μM), possessing a *para*-methoxy group on its phenyl moiety. The most impressive compound of the class, however, turned out to be hyperolactone C analogue **12** which exhibited a TI of 80.0 (IC_{50} = 16 μM; CC_{50} = 1301 μM). With a fluoride residue in its structure, this relatively small molecule may serve as a new lead for a drug discovery program in the anti-HIV area of pharmaceutical research.

Conclusion

Relying on a highly convergent strategy, the described total synthesis of biyouyanagin A and its 24-epimer highlights the importance of cascade reactions in total synthesis and the continuing role the latter discipline plays in natural products chemistry and biology. Thus, based on both organocatalysis and metallocatalysis, the developed synthetic technology culminated in the revision of the originally assigned structure of biyouyanagin A and determination of its absolute stereochemistry. It also delivered a series of analogues of the natural product, whose biological evaluation established the first structure−activity relationships in the field and led to the discovery that hyperolactone C, a plausible biogenetic precursor of biyouyanagin A, possesses a higher and significant potency against HIV-1 replication. Based on these biological results and considering the efficiency of the established synthetic strategy, further investigations into chemical biology and medicinal chemistry may be warranted.

Acknowledgment. Dedicated to Professor Duilio Arigoni on the occasion of his 80th birthday. We thank Dr. D. H. Huang and Dr. L. Pasterneck for NMR spectroscopic assistance and Dr. Siuzak and Dr. R. Chadha for mass spectrometric and X-ray crystallographic assistance, respectively. Financial support for this work was provided by the National Institutes of Health (USA) and the Skaggs Institute for Chemical Biology and A*STAR (postdoctoral fellowship to T.R.W.).

Supporting Information Available: Experimental procedures and full compound characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

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