Biyouyanagin A

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Total Synthesis and Revised Structure of Biyouyanagin A**

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Dedicated to Professors Albert Eschenmoser and Duilio Arigoni

Biyouyanagin A (**1**, Figure 1) is a naturally occurring substance that was recently isolated from the leaves of *H. chinense L.* var. *salicifolium*, a *Hypericum* species used in Japan as a folk medicine for the treatment of female disorders.[1] This compound exhibited significant and selective inhibitory activity against HIV replication in H9 lymphocytes ($EC_{50} = 0.798\ \mu g\,mL^{-1}$) compared with noninfected H9 lymphocytes ($EC_{50} > 25\ \mu g\,mL^{-1}$), which amounts to a therapeutic index (TI) of greater than 31.3. Furthermore, biyouyanagin A strongly inhibited lipopolysaccharide (LPS)-induced cytokine production (at $10\ \mu g\,mL^{-1}$, IL-10 = 0.03; IL-12 = 0.02; tumor necrosis factor−α (TNF−α) = 0.48). In view of the potential of biyouyanagin A as a biological tool and a drug-discovery lead, and to clarify the remaining structural ambiguity (the stereochemistry at the C24 position), we set out to synthesize the two epimers **1a** and **1b** (Figure 1). Herein we report the total synthesis of both the (24*S*) and (24*R*) epimers of biyouyanagin A in their enantiomerically pure form, and the full structural elucidation of the natural product, which required not only assignment of the *R* configuration at the C24 position, but also revision of the stereochemistry at the C17 and C18 positions (**2a** and **2b**, Figure 1).

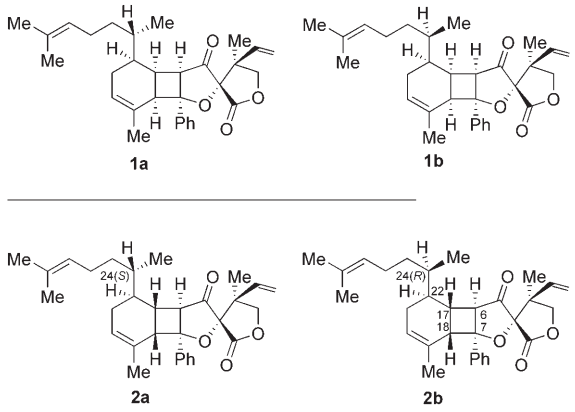
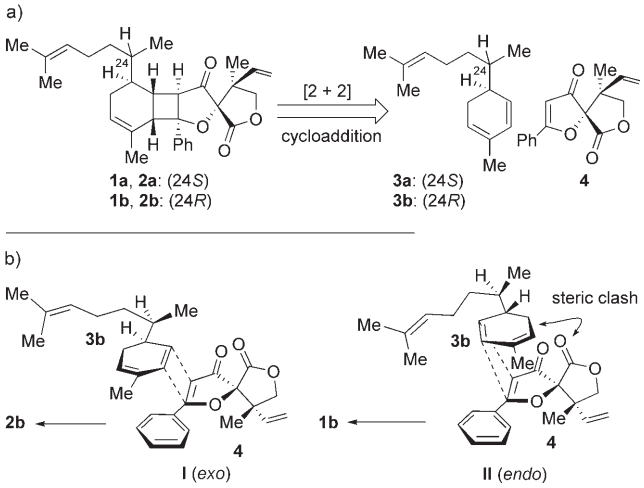


Figure 1. Originally proposed (**1a** and **1b**) structures of biyouyanagin A and revised (**2a** and **2b**) structures.

In our quest to synthesize biyouyanagin A, we decided to employ the rather apparent, but unprecedented [2+2] photocycloaddition reaction that involved the two required components, diene **3a** (*ent*-7-epizingiberene)[2] or **3b** (*ent*-zingiberene)[2] and enone **4**,[3] as shown retrosynthetically in Scheme 1a. Proposed as a biosynthetic pathway,[1] this route had the advantage of optimum convergency, but left the stereochemical outcome of the [2+2] cycloaddition open.



Scheme 1. a) Biogenetically-inspired retrosynthetic analysis of biyouyanagin A. b) *Exo* (**I**) and *endo* (**II**) arrangements of the reactants **3b** and **4** potentially form **2b** or **1b**, respectively.

Indeed, based on steric constraints, the *exo* arrangement, as in **I** (Scheme 1b), should be favored over the *endo* arrangement, as in **II**, in any [2+2] cycloaddition reaction between **3a** or **3b** and **4**, unless the two components were forced to combine by enzymes or artificial tethering.

Careful consideration of the reported NOE interactions for biyouyanagin A (**1a** or **1b**)[1] left its stereochemical assignment ambiguous at best, for the reported interactions of H6 with H17 and H22 could, based on molecular models, have also been explained by structure **2a** or **2b**. It was with this reasoning that we developed the hypothesis that the

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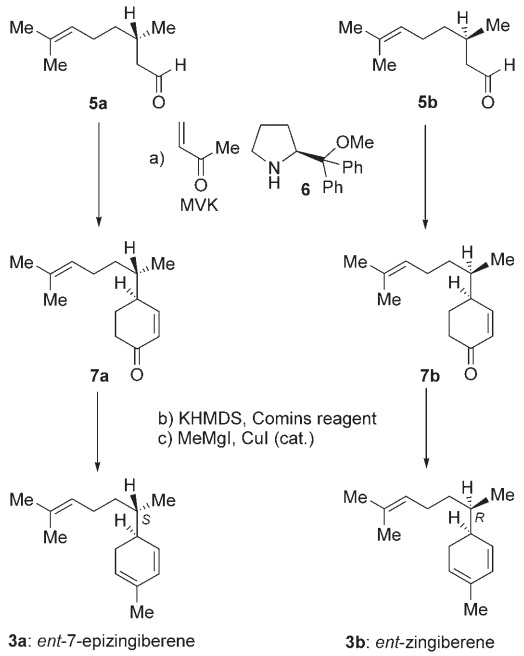
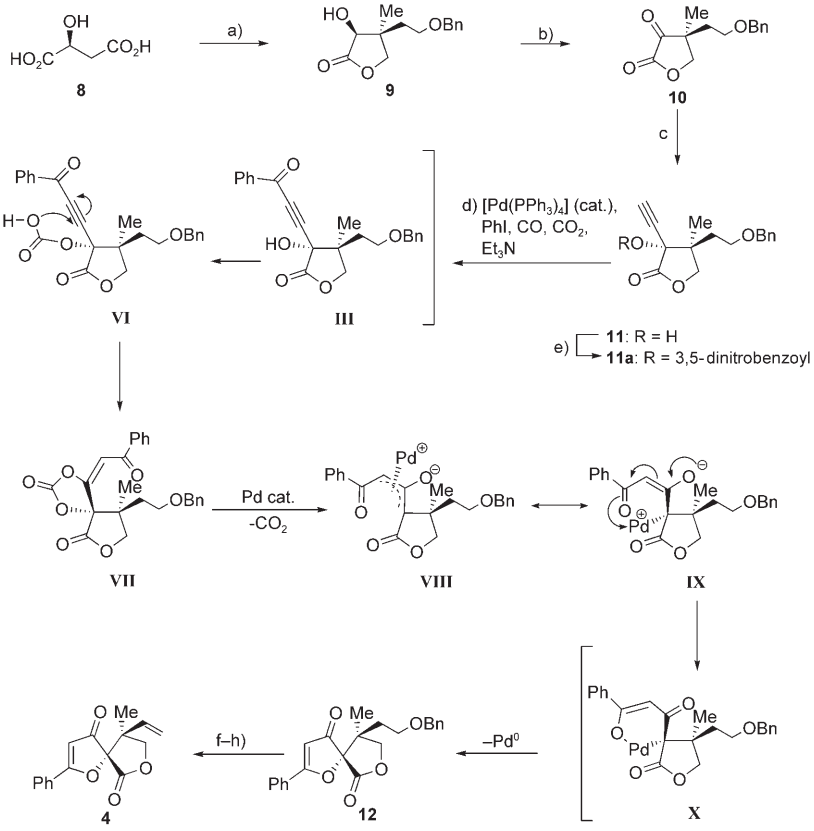


Scheme 2. Synthesis of **3a** and **3b**. Reagents and conditions: a) **5a** or **5b** (1.0 equiv), MVK (1.5 equiv), **6** (5 mol%), ethyl 3,4-dihydroxybenzoate (20 mol%), 0°C, 24 h; then KOH (0.1 N aq, 1.0 equiv), nBu$_4$NOH (40% aq, cat.), Et$_2$O/THF/H$_2$O (3:1:3), reflux, 6 h, 72% yield, 93% *de* for **7a**; 68% yield, 86% *de* for **7b**; 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$_2$O, 1.5 equiv), CuI (2 mol%), THF, 0°C, 15 min, 80% (2 steps). KHMDS = potassium hexamethyldisilazanide.

structure of biyouyanagin A could very well be one of the two diastereoisomers **2a** or **2b**, and that these structures could be directly reached by photoinduced [2+2] cycloaddition between the two partners **3a** or **3b** and **4**.

The required terpenoid structures **3a** and **3b** were swiftly synthesized from (*S*)- and (*R*)-citronellals (**5a** and **5b**, respectively) through asymmetric α functionalization as shown in Scheme 2. Thus, enamine-mediated Michael addition of **5a** to methyl vinyl ketone (MVK), which employed the proline-derived catalyst **6** and ethyl 3,4-dihydroxybenzoate as co-catalyst,[4] followed by an intramolecular aldol condensation within the initially formed ketoaldehyde, resulted in the formation of the 24*S* enone **7a**[5] (72% yield, 93% *de*). Regioselective transformation of the latter compound to the corresponding enol triflate,[6] followed by copper-catalyzed methylenation,[7] furnished one of the desired terpenes **3a** ((22*R*, 24*S*), *ent*-7-epizingiberene; 80% overall yield) while a similar route starting with **5b** gave the other terpene **3b** ((22*R*, 24*R*), *ent*-zingiberene, through the enone **7b**, which was obtained from **5b** in 68% yield and 86% *de*). Purification at the enone stage served to obtain pure samples of **3a** and **3b**.

Hyperolactone C[3] (**4**) was synthesized from L-malic acid (**8**) as shown in Scheme 3. Thus, the hydroxylactone **9**, which was obtained from **8** according to literature procedures,[3e] was oxidized to dione **10** (DMP, 92%), which reacted chemo- and stereoselectively (chelation control) with lithium acetylide to afford propargylic alcohol **11** as the major product, together with its minor diastereomer (79% total yield, ca. 3:1 ratio). A palladium-catalyzed carbonylative insertion cascade with this mixture, which involving PhI, CO, and CO$_2$, led to the spirolactone **12** as a 3:1 mixture with its diastereomer (77% overall yield), from which it was separated either by chromatography or crystallization from toluene.[8] Proof of the retention of stereochemistry in this cascade sequence came from X-ray analysis of the 3,5-dinitrobenzyl derivative **11a**, prepared from **11**, 3,5-dinitrobenzoyl chloride, NEt$_3$, and DMAP (m.p. 159°C, toluene) and product **12** (m.p. 176°C, toluene) (Figure 2).[9] This observation can be explained by the mechanism shown in Scheme 3, in which the presumed intermediate **III** is converted into the palladium allyl species **VIII** with the approach of the palladium species from the least sterically demanding face (that is, opposite to the benzyloxy-



Scheme 3. Synthesis of hyperolactone C (**4**). Reagents and conditions: a) Ref. [3e]; b) DMP (2.0 equiv), CH$_2$Cl$_2$, 25°C, 5 h, 92%; c) acetylene, nBuLi, THF, −78°C, 1 h, 79%, 3:1 d.r.; d) [Pd(PPh$_3$)$_4$] (5 mol%), PhI, CO (200 psi), CO$_2$ (200 psi), Et$_3$N, 100°C, 5 h, 77%; e) 3,5-dinitrobenzoyl chloride (1.2 equiv), NEt$_3$ (1.2 equiv), DMAP (0.1 equiv), CH$_2$Cl$_2$, 25°C, 3 h, 87%; f) BBr$_3$ (1.5 equiv), CH$_2$Cl$_2$, −78°C, 30 min; g) *o*-NO$_2$PhSeCN (1.2 equiv), P(nBu)$_3$ (1.2 equiv), THF, 25°C, 4 h; h) H$_2$O$_2$ (30% aq, excess), THF, 25°C, 1 h, 73% (3 steps). DMAP = 4-dimethylaminopyridine, DMP = Dess–Martin periodinane.

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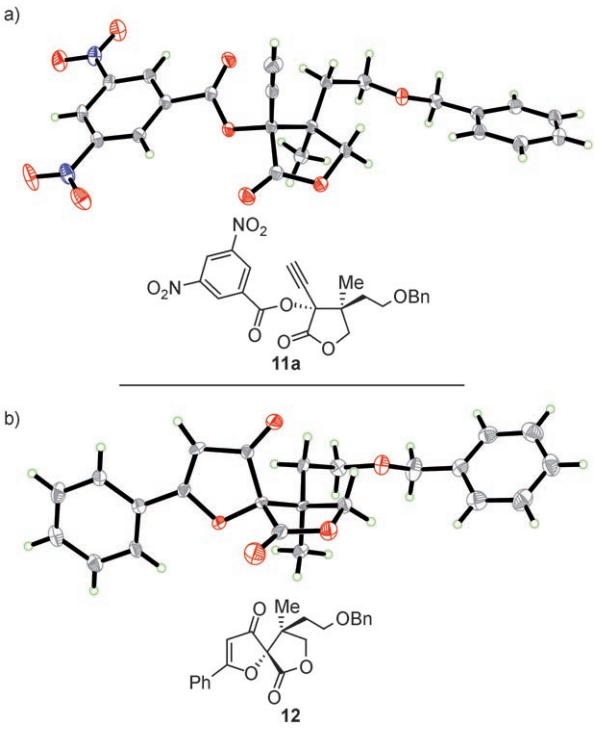
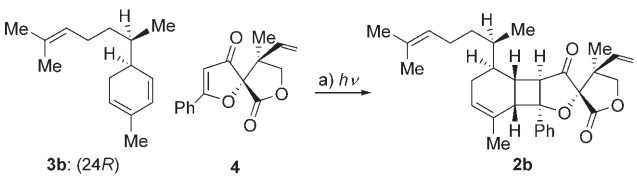


Figure 2. ORTEP views of a) **11a** and b) **12** with thermal ellipsoids set at the 30% level. Bn = benzyl.

ethylene group of the tertiary carbon) as in **IX** and **X**, from which palladium is extruded to form spirolactone **12**.

The pure stereoisomer of **12**, was then converted into hyperolactone C (**4**) by a three-step sequence that involved debenzylation (BBr_3), selenation, and oxidation/*syn* elimination (79% overall yield).

With the required fragments in hand, the stage was now set to investigate the key [2+2] photoinduced cycloaddition reaction.[10] Irradiation of a mixture of **4** and **3a** (24*S*) or **3b** (24*R*) in the presence of 2′-acetonapthone as a triplet sensitizer and in a quartz cell (> 320 nm filters) as a reaction vessel, led to rapid chemo-, regio-, and stereoselective cyclobutane formation through hetero-coupling to give **2a** (24*S*) or **2b** (24*R*), of which **2b** proved to be the natural product.[11] The reaction was carried out in concentrated CH_2Cl_2 solution with a fourfold excess of the less valuable terpene added in portions (Scheme 4).



Scheme 4. Completion of the total synthesis of biyouyanagin A (**2b**). Reagents and conditions: a) **4** (1.0 equiv), **3b** (4.0 equiv), 2′-acetonaphthone (1.0 equiv), CH_2Cl_2, 25 °C, 5 h, 46%.

The spectroscopic data (^1H and ^{13}C NMR, MS, IR data) and optical rotation of this product were consistent with those reported for the natural product (Table 1). However, the full NOE data for this compound supports the structure **2b**, rather than the originally proposed structure **1b**. Crucially, the absence of a strong NOE interaction between H18 and the closest protons on the aromatic ring eliminates the proposed structures **1a** and **1b**.[12] The confusion apparently arose from the assumption that the NOE interaction observed between H6 and H17 is indicative of a *syn* arrangement between these protons, whereas this is not necessarily the case. Furthermore, the observed NOE interaction between H6 and H22 is indicative of *anti*, rather than *syn* arrangement, as these hydrogen atoms are in closer proximity within the *anti* structures **2a** or **2b**, than they are within the *syn* structures **1a** or **1b**.

Table 1: Selected physical properties for **2a** and **2b**.

2a: R_f = 0.62 (silica gel, hexane/EtOAc 2:1); $[\alpha]_D^{25} = -126.6$ (c = 0.08, $CHCl_3$); IR (thin film): $\tilde{\nu}$ = 2923, 1792, 1742, 1104. ^1H NMR (600 MHz, $CDCl_3$): δ = 7.34–7.27 (m, 5H), 5.46 (m, 1H), 5.22 (dd, J = 17.4, 10.8 Hz, 1H), 5.08 (br.t, J = 6.6 Hz, 1H), 4.78 (d, J = 10.8 Hz, 1H), 4.72 (d, J = 9.0 Hz, 1H), 4.60 (d, J = 17.4 Hz, 1H), 3.98 (d, J = 8.4 Hz, 1H), 3.44 (d, J = 8.4 Hz, 1H), 3.19 (dd, J = 6.0, 1.2 Hz, 1H), 3.12 (ddd, J = 8.4, 5.4, 5.4 Hz, 1H), 2.19 (m, 1H), 2.03 (m, 1H), 2.01 (m, 1H), 1.90 (m, 1H), 1.69 (d, J = 1.2 Hz, 3H), 1.67 (m, 1H), 1.60 (s, 3H), 1.46–1.37 (m, 2H), 1.31 (s, 3H), 1.16 (m, 1H), 1.00 (s, 3H), 0.91 ppm (d, J = 7.2 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 209.5, 171.7, 139.6, 134.5, 131.5, 131.4, 127.7, 127.7, 125.9, 124.6, 123.6, 118.4, 93.1, 89.6, 73.7, 51.7, 50.1, 49.0, 38.8, 35.5, 34.9, 34.6, 25.7, 25.5, 22.7, 21.7, 20.1, 17.8, 17.3 ppm. HRMS (ESI-TOF): m/z calcd for $C_{31}H_{38}O_4Na^+$, 497.2662; found 497.2657.
2b: R_f = 0.62 (silica gel, hexane/EtOAc 2:1); $[\alpha]_D^{25} = -256.6$ (c = 0.80, $CHCl_3$); [lit.,[1] $[\alpha]_D^{25} = -240.0$ (c = 0.50, $CHCl_3$)]; IR (thin film): $\tilde{\nu}$ = 2926, 1792, 1743, 1173 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ = 7.37–7.26 (m, 5H), 5.46 (m, 1H), 5.22 (dd, J = 17.6, 11.2 Hz, 1H), 5.11 (br.t, J = 6.8 Hz, 1H), 4.80 (d, J = 11.2 Hz, 1H), 4.71 (d, J = 8.8 Hz, 1H), 4.61 (d, J = 17.6 Hz, 1H), 3.99 (d, J = 8.4 Hz, 1H), 3.48 (d, J = 8.4 Hz, 1H), 3.16 (d, J = 6.0, 1.2 Hz, 1H), 3.02 (ddd, J = 8.4, 6.0, 6.0 Hz, 1H), 2.09 (m, 1H), 2.02 (m, 1H), 1.99 (m, 1H), 1.94 (m, 1H), 1.73 (m , 1H), 1.70 (s, 3H), 1.61 (s, 3H), 1.46 (m, 1H), 1.44 (m, 1H), 1.31 (s, 3H), 1.20 (m, 1H), 1.02 (s, 3H), 0.82 ppm (d, J = 6.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 209.6, 171.6, 139.6, 134.5, 131.4, 131.4, 127.7, 127.7, 125.9, 124.5, 123.8, 118.4, 93.0, 89.7, 73.6, 51.8, 50.3, 49.0, 38.7, 35.9, 35.0, 34.9, 25.8, 25.7, 23.4, 21.7, 20.1, 17.7, 16.8 ppm. HRMS (ESI-TOF): m/z calcd for $C_{31}H_{39}O_4^+$, 475.2843; found 475.2845.

In conclusion, a 12-step total synthesis of biyouyanagin A has been accomplished and led to the stereochemical reassignment of the natural product from **1a** or **1b**, to **2b**. In addition to the structural revision, the described chemistry renders the natural substance readily available for further biological investigations and opens the way to design analogues for studies for structure-activity relationships. It should also be noted that terpenoid **3a** similarly reacted with **4** under the same photolytic conditions to afford (24*S*)-*epi*-biyouyanagin A (**2a**); a substance that may occur naturally as well.

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Keywords: domino reactions · inhibitors · photocycloaddition · structure elucidation · total synthesis

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[11] Cyclobutane formation could also be observed at a slower rate in the absence of the sensitizer.

[12] The absence of a strong NOE interaction between H18 and the closest aromatic protons (*ortho*) is in contrast to the interaction observed between H6 and these protons (ca. 1:128 ratio, 400 MHz, irradiation), which supports a *trans* relationship between H18 and the phenyl group, while the *cis* relationship between H6 and the phenyl group is firmly established.

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