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Biomimetic Total Synthesis of Meiogynin A, an Inhibitor of Bcl-xL and Bak Interaction

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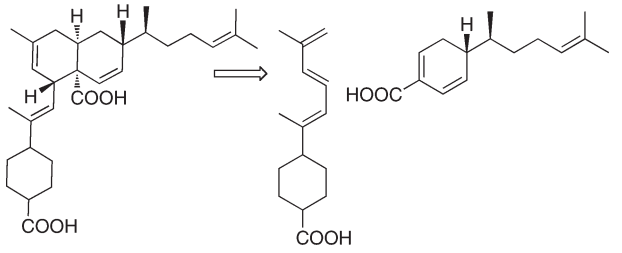

A short, convergent, and selective synthesis of meiogynin A, an inhibitor of the antiapoptotic protein Bcl-xL, has been performed. This synthesis, based on a biomimetic approach, allowed the determination of its absolute configuration. Three isomers of meiogynin A have also been elaborated. One of these was found to be three times more potent than the natural compound.

Apoptosis is the programmed cell death (PCD) used by multicellular organisms to regulate tissue homeostasis through the elimination of useless or potentially harmful cells. Disruption of this process is implicated in many kinds of diseases, such as cancer. The Bcl-2 family of proteins governs the commitment to PCD at the mitochondrion (the intrinsic apoptotic pathway). It is divided into antiapoptotic such as Bcl-2 or Bcl-xL and proapoptotic proteins such as Bax, Bad, or Bim.[1] In an effort to find potent inhibitors of the antiapoptotic protein Bcl-xL, we have recently isolated a new dimeric sesquiterpenoid by a bioassay-guided screening of Malaysian plants extracts.[2] This compound, meiogynin A (**1**) (Figure 1), isolated from the bark of *Meiogyne cylindrocarpa*, acts as an antagonist of the Bcl-xL/Bak association. Only its relative configuration could be determined, and the relative configuration of the methyl group at C-1″ was proposed using conformational analysis. Meiogynin A possesses an original substituted *cis*-decalin carbon skeleton with a carboxylic acid at the ring junction and five asymmetric centers. Compound **2**, the epimer at C-1 of meiogynin A, was also isolated in the active fraction as a minor compound but exhibited a less potent affinity for Bcl-xL. The biological properties and unique structure of meiogynin A (**1**) led us to consider its total synthesis, not only to determine its absolute configuration but also to have access to various analogues for SAR studies. We hypothesized (Figure 1) that the biosynthesis of compounds **1** and **2** may involve a Diels−Alder reaction between a racemic bisabolatriene acid unit **3** and a chiral zingiberene unit **4**[3,4] either in an endo (compound **1**) or in an exo mode (compound **2**). That was supported by the identification of α-bisabolol, a putative precursor of **3** and **4**, by GC−MS analysis of the essential oil of the bark of *M. cylindrocarpa*.[2]

In order to validate this biomimetic pathway, and to assess unambiguously the absolute configuration of the five asymmetric centers, we have performed a short, convergent, and selective total synthesis of meiogynin A **1** based on a final Diels−Alder reaction between triene **3** and four dienophiles (4*R*,1′*R*), (4*R*,1′*S*), (4*S*,1′*S*), and (4*S*,1′*R*) **4**, potential precursors of meiogynin A and diastereomers. The selective synthesis of triene **3**, with a good control of the *E*,*E* configuration of the double bonds, was performed in six steps from commercial *trans*-1,4-cyclohexanedimethanol **5** (Scheme 1). After mono TBS-alcohol protection,[5] the remaining free alcohol was easily oxidized to the aldehyde **6** using Dess−Martin periodinane. A Seyferth−Gilbert homologation was then carried out using the Bestmann−Ohira reagent[6] to give the alkyne **7** in a very good yield (94%).

In order to settle the trisubstituted double bond, a Neigishi carboalumination reaction[7] was performed on compound **7**, using Lipshutz's conditions.[8] With iodine as the electrophile, the desired **8-*E*** compound was obtained along with 8% of its inseparable **8-*Z*** isomer (77% yield). When using NBS, no *Z* isomer could be detected in the crude mixture, but the reaction was not reproducible and yields were much lower (from 20 to 58%). Direct Suzuki coupling of compound **8** with pinacol boronate ester **12** elaborated from the commercial alkyne **11** using Schwartz's catalyst[9] led to the corresponding triene **9**. Unfortunately, that compound was unstable to purification conditions, which meant that a tandem carboalumination−coupling reaction[10] could not be envisaged, in our case, because of the fragileness of this derivative.

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(9) Pereira, S.; Srebnik, M. *Organometallics* **1995**, *14*, 3127–3128.

(10) See, for example: Lipshutz, B. H.; Butler, T.; Lower, A.; Servesko, J. *Org. Lett.* **2007**, 3737–3740.

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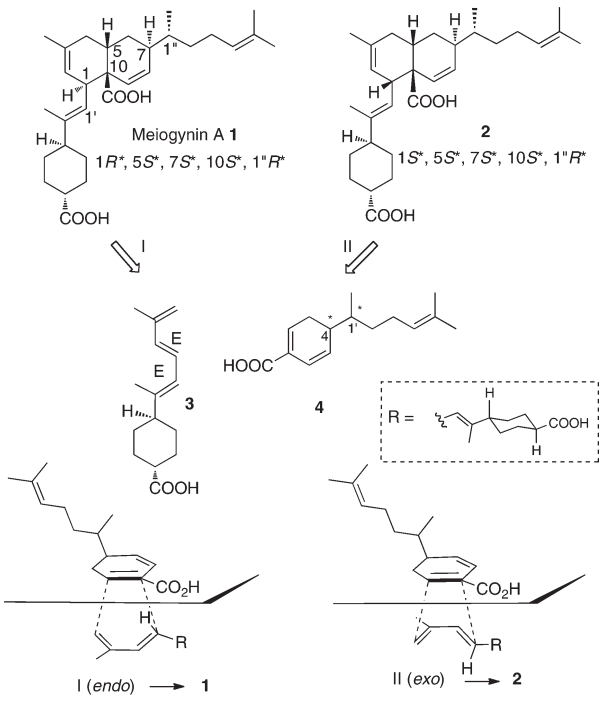


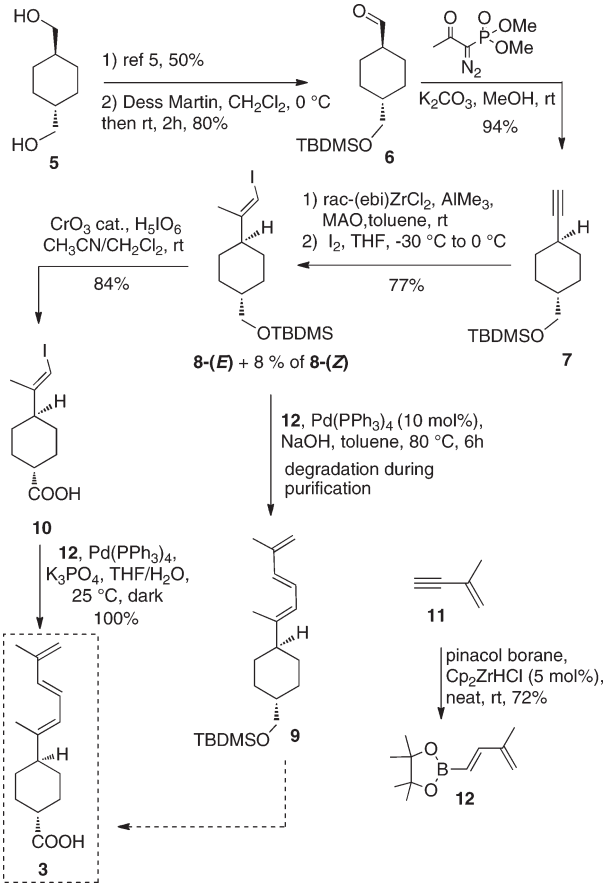
FIGURE 1. Biomimetic hypothesis.

The TBDMS ether **8** was thus selectively oxidized to the acid **10** using a modified one-pot procedure with CrO_3−H_5IO_6.[11] The minor *Z* isomer **10** could be partially removed at this step (ratio *E*/*Z* 95/5).

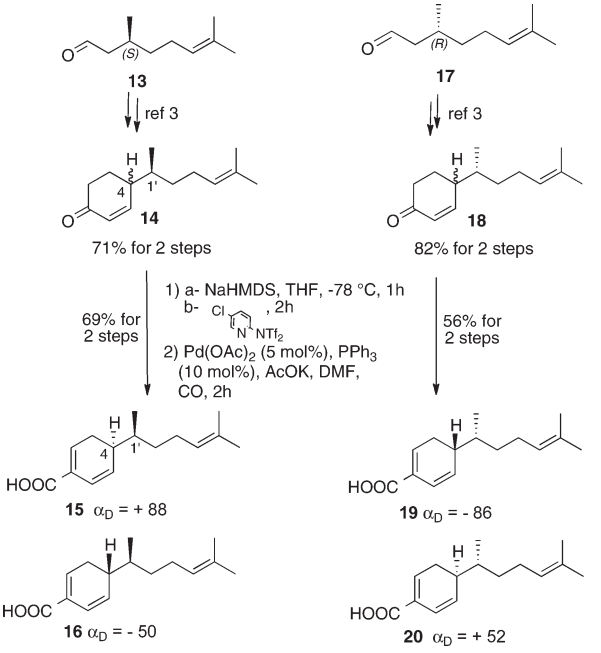
The vinyl iodide thus obtained was engaged in a Suzuki coupling with pinacol boronate ester **12**. The best conditions were found to be as follows: tetrakis(triphenylphosphine) palladium, K_3PO_4, THF/H_2O at 25 °C for 16 h. Triene **3** was unstable when purified by conventional methods (normal- or reversed-phase column chromatography), but thanks to its acid function, it could be obtained pure by an acid−base extraction. Compound **3** was also light sensitive, and performing the Suzuki reaction in the dark increased yield from 62% to 100% after acid−base extraction. Expected triene **3** was obtained with an overall yield of 24% for 6 steps.

By analogy with the previous results of Hagiwara[3] and Nicolaou,[12] a synthesis of the four potential chiral dienophiles was envisaged from (*R*)- and (*S*)-citronellal via the formation of the cyclohexenone intermediates **14** and **18** by a Robinson annulation (Scheme 2). The Robinson annulation was first carried out in a nonselective manner to give **14** and **18** as a mixture of diastereomers.[3] A carbonylation[13] was performed on their enol triflates and led to the formation of the expected dienophiles **15**, **16** and **19**, **20**. The two mixtures of diastereomers were separated at that stage[14] to yield four pure compounds. These compounds were prone to aromatization at room temperature but could be stored for weeks under argon at −20 °C. In parallel, the synthesis of cyclohexenones **21** and **22** was performed, according to Nicolaou's procedure (Scheme 3).[12] A proline-derived

SCHEME 1. Synthesis of Triene 3



SCHEME 2. Synthesis of Chiral Dienophiles 15, 16, 19, and 20



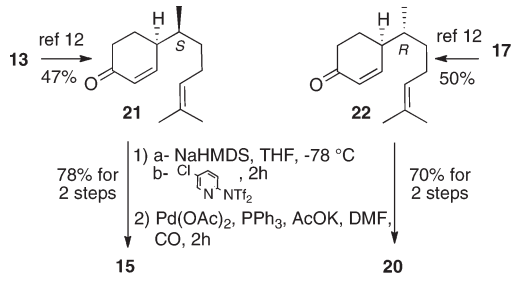
(11) Zhang, S.; Xu, L.; Trudell, M. L. *Synthesis* **2005**, *16*, 1757–1760.

(12) (a) Nicolaou, K. C.; Sarlah, D.; Shaw, D. M. *Angew. Chem., Int. Ed.* **2007**, *46*, 4708–4711. (b) Nicolaou, K. C.; Wu, T. R.; Sarlah, D.; Shaw, D. M.; Rowcliffe, E.; Burton, D. R. *J. Am. Chem. Soc.* **2008**, *130*, 11114–11121.

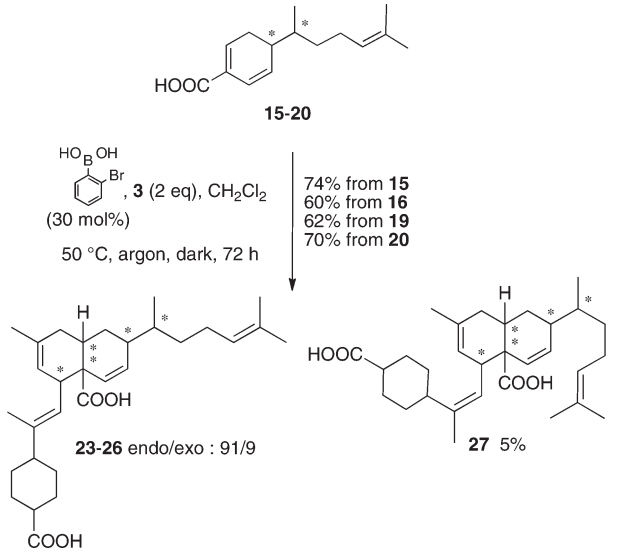
(13) Freskos, J. N.; Laneman, S. A.; Reilly, M. L.; Ripin, D. H. *Tetrahedron Lett.* **1994**, *35*, 835–838.

(14) Conditions of separation of dienophiles: Hypercarb column; eluant: water + 0.1% formic acid/MeOH + 0.1% formic acid 10/90.

SCHEME 3. Synthesis of Chiral Dienophiles 15 and 20



SCHEME 4. Final Cycloaddition and Synthesis of Compounds 23−26



catalyst[15] and 3,4-dihydroxybenzoate as a cocatalyst were used for the enamine-mediated Michael addition of (*S*)-citronellal **13** and (*R*)-citronellal **17** to methyl vinyl ketone and was followed by an intramolecular aldol condensation of the resulting ketoaldehyde product.[16] Their subsequent transformation into acids **15** and **20** allowed the unambiguous identification of each of the four previous synthesized dienophiles **15**, **16**, **19**, and **20**.[17]

The final Diels−Alder (DA) reactions between triene **3** and pure dienophiles **15**, **16**, **19**, and **20** were carried out using 2-bromobenzeneboronic acid as an organocatalyst as described by Hall et al. (Scheme 4).[18] In our case, the reaction had to be performed at 50 °C, since no cycloadducts were observed at room temperature. The reaction was completed within 72 h with 30 mol % of catalyst (because of the two acid functions, the reaction was much slower with a smaller load of catalyst). Cycloadducts were obtained in reasonable yields ranging from 60 to 74% (Scheme 4). As, to our knowledge, methyl 1,3 cyclohexadiene-2-carboxylate has only been used

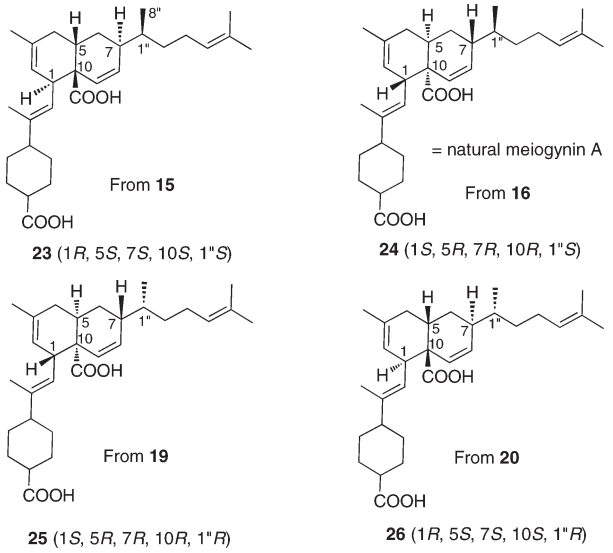


FIGURE 2. Meiogynin A **24** and its isomers.

once, in an hetero-Diels−Alder reaction, and as a diene,[19] we were pleased to see that compounds **15**, **16**, **19**, and **20** react solely as dienophiles. In addition, the chemo, regio, and facial selectivities were total as only the less hindered diene of **3** reacts with these dienophiles, anti to their lateral chain. The observed diastereoselectivities of the DA were also very satisfying as the expected products were obtained as major compounds along with only 9% of their exo isomers (in the same proportion as in the active fraction of the plant extract).

In addition, 5% of adducts **27** arising from a cycloaddition between dienophiles and remaining *E*,*Z*-triene **3** were formed.[20] The endo isomers **23**−**26** could be isolated by preparative HPLC (Figure 2).[21]

The spectroscopic data (^{1}H and ^{13}C NMR) of these products showed noticeable differences in chemical shifts for H1″ (0.77 ppm for natural meiogynin A and compounds **24** and **26** and 0.79 ppm for compounds **23** and **25**), C8″ (15.5 ppm for natural meiogynin A and **24** and **26** and 16.1 ppm for **23** and **25**), C6 (25.4 ppm and 27.6 ppm), and C7 (36.1 ppm and 36.5 ppm). These data and the comparison of optical rotations for compounds **24** and **26**, allowed us to determine compound **24** (1*S*,5*R*,7*R*,10*R*,1″*S*) to be meiogynin A. Nevertheless, having unambiguously established the absolute configuration of natural meiogynin A by its total synthesis, we were surprised to observe that its optical rotation ($[\alpha]_D$ −200; *c* = 0.1 in CH_2Cl_2) was considerably different from that which we reported previously for the same compound obtained from its natural source ($[\alpha]_D$ −281; *c* = 0.12 in CH_2Cl_2).[2] However, after further, careful purification of the natural compound, its optical rotation was now practically identical ($[\alpha]_D$ −195; *c* = 0.1 in CH_2Cl_2) to the synthetic product, thereby confirming our configurational assignment.

The binding affinity of compounds **23**−**26** was evaluated on Bcl-xL by competition against the fluorescent-tagged BH3 domain of the protein Bak, as described[22] and compared

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(16) In our case, the observed de (determined by HPLC) for the enamine-mediated Michael addition of (*S*)-citronellal **13** and (*R*)-citronellal **17** to methyl vinyl ketone were disappointing (60% de from **13** and 56% de from **17**).

(17) The catalyst derived from (*R*)-proline should give (1′*S*,4*R*)-**14** and (1′*R*,4*R*)-**18** as major isomers, precursors of dienophiles **16** and **19**.

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(20) Ratios of these compounds were determined by HPLC.

(21) Conditions of separation of endo/exo adducts: Sunfire column; eluant: MeOH + 0.1% TFA/ ACN + 0.1% TFA/ H_2O + 0.1% TFA 10/70/20.

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TABLE 1. Binding Affinities of Natural Meiogynin A and Compounds 23−26 for the Antiapoptotic Protein Bcl-xL

entry	compd	IC_{50}[a,c] (μM)	K_i[b,c] (μM)
1	**1**	10.7 ± 1.6	8.3 ± 1.2
2	**23**	129 ± 17	100 ± 13
3	**24**	11.6 ± 0.5	9 ± 0.4
4	**25**	2.9 ± 0.7	2.3 ± 0.5
5	**26**	52 ± 12	40 ± 9

[a] IC_{50} is the concentration of compound inducing 50% of inhibition of the binding of the labeled reference compound Bak to Bcl-xL. [b] K_i is the concentration corresponding to 50% of inhibition of the binding of the labeled reference compound Bak to Bcl-xL and corrected for experimental conditions according to Cheng and Prussof.[23] [c] Values are reported as the means of two independent determinations.

with that of a pure sample of natural meiogynin A. The activity of compound **24** was similar to that of natural meiogynin **1**, and interestingly, one of its diastereomers (compound **25**, with an opposite configuration at C1″) was three times more potent (Table 1).

In conclusion, we have performed a short (11 steps), convergent, and selective synthesis of meiogynin A (**1**) based on a biomimetic hypothesis with only one protection step. We also have elaborated three isomers **23**, **25**, and **26**. An in vitro affinity displacement assay revealed that compound **25** exhibited a more potent affinity for Bcl-xL than the natural product, showing how important it is to control the configuration of all the asymmetric centers. Pharmacomodulations on these interesting compounds are currently underway in our laboratory.

Experimental Section

General Procedure for the Cycloaddition Reactions. Triene **3** (2.00 equiv), dienophiles **15**, **16**, **19**, or **20** (1.00 equiv), and 2-bromobenzeneboronic acid (0.3 equiv) were dissolved in wet dichloromethane and stirred, in the dark, under argon, at 50 °C for 3 days. The solvent was removed in vacuo, and the crude mixture was purified by flash chromatography on a reversed-phase column (Versapack C18 cartridge) to afford the expected endo adduct along with 9% of its exo isomer and 5% of **27**. The endo/exo adducts were separated by preparative HPLC.

(**1*S*,5*R*,7*R*,10*R*,1″*S*)-24.** From triene **3** (38 mg, 0.16 mmol) and dienophile **16** (19 mg, 0.08 mmol) in dichloromethane (0.3 mL) was obtained desired compound **24** (along with 9% of the *exo* adduct and 5% of **27**) after purification (22.5 mg, 60%). Compound **24** was obtained pure after preparative HPLC (Sunfire column, eluant: MeOH + 0.1% TFA/CH_3CN + 0.1% TFA/H_2O + 0.1% TFA 10/70/20), t_R 20.3 min: $[\alpha]^{25}_D$ −200 (*c* 0.1, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.65 (d, J = 10.1 Hz, 1H), 5.59 (d, J = 10.1 Hz, 1H), 5.17 (br. s, 1H), 5.12 (t, J = 7.0 Hz, 1H), 4.96 (d, J = 10.6 Hz, 1H), 3.18 (m, 1H), 2.56 (m, 1H), 2.35 (m, 1H), 2.26 (t, J = 12.1 Hz, 1H), 2.02 (m, 1H), 1.99 (m, 1H), 1.95 (m, 1H), 1.93 (m, 1H), 1.91 (m, 1H), 1.87 (m, 1H), 1.81 (m, 1H), 1.79 (m, 1H), 1.78 (m, 1H), 1.71 (s, 3H), 1.67 (m,1H), 1.66 (s, 3H), 1.65 (s, 3H), 1.63 (s, 3H), 1.57 (m, 1H), 1.51 (m, 1H), 1.50 (m, 1H), 1.42 (m, 1H), 1.33 (m, 1H), 1.26 (m, 1H), 1.19 (m, 1H), 1.13 (m, 1H), 0.77 (d, J = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 182.2, 180.8, 140.1, 133.7, 133.4, 131.2, 129.8, 124.7, 123.2, 120.1, 48.6, 46.3, 43.2, 42.8, 36.3, 36.1, 34.1, 31.6, 30.7, 30.1, 29.3, 28.6, 27.9, 25.9, 25.7, 25.4, 23.3, 17.6, 15.5, 14.6; IR (film): 2921, 2853, 1702 cm^{-1}; MS (ES^+, CH_2Cl_2/MeOH) m/z = 491.0 $[M + H]^+$; HRMS (ES^+) calcd for $C_{30}H_{44}O_4Na$ 491.3137, found 491.3153.

(**1*S*,5*R*,7*R*,10*R*,1″*R*)-25.** From triene **3** (40 mg, 0.17 mmol) and dienophile **19** (20 mg, 0.08 mmol) in dichloromethane (0.3 mL) was obtained desired compound **25** (along with 9% of the *exo* adduct and 5% of **27**) after purification (24.5 mg, 62%). Compound **25** was obtained pure after preparative HPLC (Sunfire column, eluant: MeOH + 0.1% TFA/CH_3CN + 0.1% TFA/H_2O + 0.1% TFA 10/70/20), t_R 20.2 min: $[\alpha]^{25}_D$ −220 (*c* 0.1, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.64 (d, J = 10.2 Hz, 1H), 5.55 (d, J = 10.2 Hz, 1H), 5.12 (br. s, 1H), 5.05 (t, J = 7.0 Hz, 1H), 4.92 (d, J = 10.0 Hz, 1H), 3.13 (m, 1H), 2.51 (m, 1H), 2.26 (m, 1H), 2.21 (t, J = 12.0 Hz, 1H), 1.97 (m, 1H), 1.94 (m, 1H), 1.90 (m, 1H), 1.88 (m, 1H), 1.86 (m, 1H), 1.84 (m, 1H), 1.80 (m, 1H), 1.77 (m, 1H), 1.74 (m,1H), 1.65 (s, 3H), 1.61 (s, 3H), 1.60 (s, 3H), 1.59 (m,1H), 1.57 (s, 3H), 1.55 (m, 1H), 1.43 (m, 1H), 1.40 (m, 1H), 1.38 (m, 1H), 1.31 (m, 1H), 1.26 (m, 1H), 1.19 (m, 1H), 1.13 (m, 1H), 0.79 (d, J = 6.6 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 182.5, 181.2, 139.9, 133.2, 132.1, 131.0, 130.1, 124.6, 123.0, 119.9, 48.5, 46.1, 43.1, 42.8, 36.5, 36.4, 33.5, 31.4, 30.5, 29.8, 29.2, 28.4, 27.8, 27.6, 25.8, 25.5, 23.2, 17.5, 16.1, 14.5; IR (film): 2921, 2849, 1700 cm^{-1}; MS (ES^+, CH_2Cl_2 / MeOH) m/z = 491.0 $[M + H]^+$; HRMS (ES^+) calcd for $C_{30}H_{44}O_4Na$ 491.3137, found 491.3155.

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Supporting Information Available: Complete experimental procedures, ^{1}H and ^{13}C spectra for all new compounds, and HPLC chromatograms for final compounds **23−26**. This material is available free of charge via the Internet at http://pubs.acs.org.

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