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Tuning the Acyclic Ether Moiety of Anticancer Agent AA005 with Conformationally Constrained Fragments

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Abstract: A new series of anticancer annonaceous acetogenin mimetics were designed, synthesized, and evaluated based on our previously developed compound AA005, in which a variety of conformationally constrained fragments were introduced. Parallel syntheses of all new compounds were accomplished by replacement of the acyclic bis-ether functionality of AA005 with certain conformationally constrained fragments. Slight effects to the anticancer activity were exerted by altering stereochemistries in the middle modification region. Similar to AA005, most newly synthesized mimetics were found to exhibit potent activities against breast cancer cells, and showed satisfactory selectivities between cancerous and non-cancerous cells. An *N*,*N*′-dimethyl bis-amide compound **67** exhibits 30 times more potency against MDA-MB-468 cells than its parent molecule AA005. This study indicates that the introduction of appropriate conformational constraints is a useful optimizing tool for this class of anticancer agents. Successes in the bis-amide analogues of AA005 make this unique class of anticancer agents much simpler and more flexible for future further developments.

Keywords: anticancer agents · conformational constraints · mimicry · organic chemistry · parallel synthesis

Introduction

Annonaceous acetogenins are a large family of fatty acid derived natural products that feature an α,β-unsaturated γ-methylbutenolide at one terminal, one to three THF rings in the middle region, and a hydrocarbon chain between these two functional parts, as well as another hydrocarbon chain at the other terminal.[1,2] Many members of this family exhibit a broad spectrum of biological activities, among which the most impressive is their anticancer activity (cytotoxicity). They were considered to be the most powerful inhibitors of complex I (NADH: ubiquinone oxidoreductase) in mitochondria.[3] Due to their potent bioactivities and unique structures, we have been engaged in modifying natural acetogenins (e.g. bullatacin) into the corresponding mimetics with simpler structures for several years. In our previous successful studies,[4,5] both the THF rings of natural bullatacin (a representative of this family) were replaced by an ethylene glycol ether unit. These studies finally led to our invention of AA005 (Scheme 1). This bis-ether compound not only shows potent antitumor activities against a variety of human cancer cells in the 50–100 nM range, but also exhibits significant selectivities between a number of human normal and cancerous cells. To generate further improved analogues of AA005, a parallel fragment-assembly strategy was thus developed in a systematic fashion.[6] However, all our previous works used the same protocol, in which the conformationally constrained bis-THF rings of natural acetogenin were simply replaced by linear and rotation-free ethylene-glycol-ether functionalities. Little is known about the conformational contributions to the bioactivity when changing the THF rings of natural products to the ethylene glycol ethers of AA005.

To explore the above, a number of new AA005-like molecules with various conformational constraints were designed (Scheme 1). The structure of AA005 indicated that incorporation of conformationally constrained fragments into the middle ether-bond region of AA005 is feasible by our previously developed strategy.[6] Among these, one series contain a 1,2-disubstituted ethylene glycol unit and exist in either the linear (e.g. compounds **1**, **2**,[7] Scheme 1) or cyclic state (e.g. compounds **3**–**6**). The other series are equipped with a bis-amide moiety to alter the corresponding ether function-

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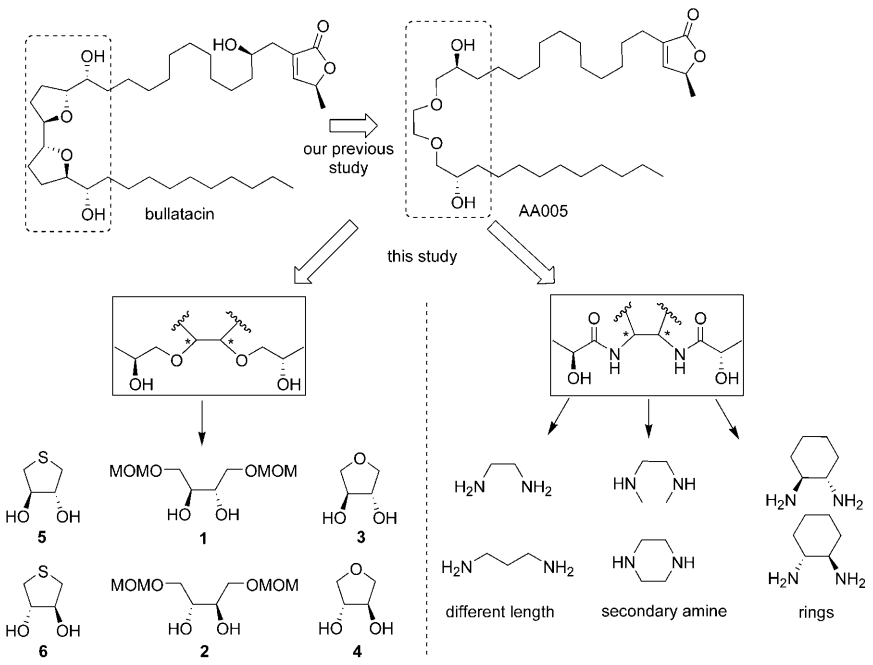


Scheme 1. Design of new AA005-like molecules with conformational constraints. MOM = methoxymethyl.

ality in AA005. Amides are generally more rigid in conformation than the corresponding ether compounds. Their conformations are, therefore, more constrained at various extents. Relative to the corresponding ether bonds, formation of the amide bonds is more efficient and convenient in the synthesis. In addition, the further expansion of structural diversity can be easily achieved by simply altering the diamine fragments. With the above advantageous considerations, several 1,2-diamines, 1,3-diamines, and 1,2-disubstituted ethylene glycol fragments were chosen for constructing new AA005-like molecules in this work.

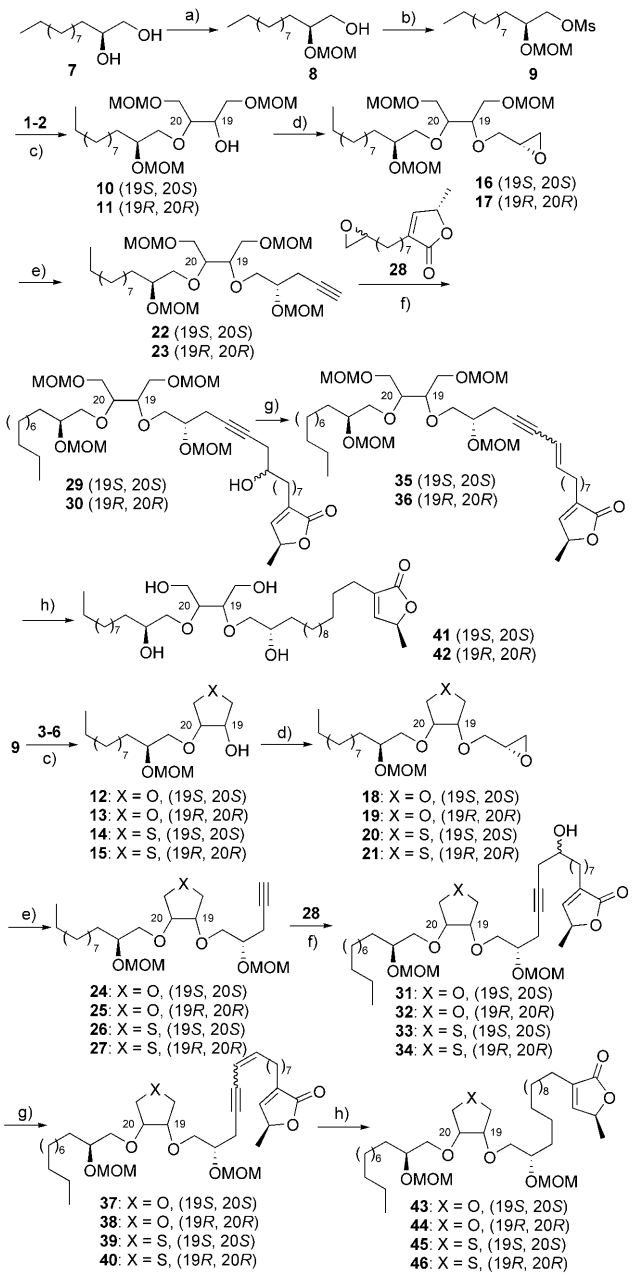
Results and Discussion

Chemical synthesis: Synthesis of the first series of compounds with an ether linkage is depicted in Scheme 2. Selective protection of the secondary alcohol of known diol **7**[4] afforded the corresponding MOM ether **8**, the remaining primary alcohol of which was then converted to the mesylate **9**. Successive *O*-alkylation of diols **1–6** with mesylate **9** followed by (−)-(*R*)-epichlorohydrin[8] afforded the building blocks **16–21** (in parallel). Regioselective opening of **16–21** by the lithium salt of trimethylsilyl acetylene in the presence of $BF_3{\cdot}Et_2O$[9] followed by treatment with MOMCl and removal of TMS with TBAF afforded compounds **22–27**. Deprotonation of the terminal alkynes **22–27** with *n*BuLi followed by treatment with epoxide **28** in the presence of $BF_3{\cdot}Et_2O$ at −78 °C gave the whole skeleton precursors **29–34**. Elimination of the newly born hydroxyl group with MsCl, Et_3N, and DBU afforded compounds **35–40**. Selective reduction of the middle triple and double bonds was achieved with tosyl hydrazide based conditions. Global deprotection of the MOM ethers with $BF_3{\cdot}Et_2O$ in Me_2S[10] provided the final products **41–46**.

Accordingly, bis-amide compounds were constructed with sequential couplings of two carboxylic acids (**47** and **52**) with certain diamine fragments. Synthesis of the left carboxylic acid **47** and the right carboxylic acid **52** is shown in Scheme 3. Initial direct oxidation of the primary alcohol of diol **7** by using a TEMPO-based method[11] (TEMPO = 2,2,6,6-tetramethylpiperidene *N*-oxyl) failed, giving very complicated results. A two-step alternative, Dess–Martin oxidation of **8** followed by treatment with sodium chlorite[12] afforded the desired carboxylic acid **47**. Synthesis of the other carboxylic acid **52** started from an inexpensive commercially available material, *cis*-eruic acid (Scheme 3). By using a known procedure,[5] diol **48** could be prepared in a large scale. Iterative protections of the primary alcohol and secondary alcohol of **48** afforded the methyl ester **49**. The α,β-unsaturated lactone moiety was constructed by a three-step sequence. Aldol condensation of compound **49** with *O*-THP-(*S*)-lactadehyde, hydrolysis of *O*-THP protecting groups, and in situ lactonization, followed by β-elimination gave compound **50**. Removal of the TBDPS group with HF/pyridine 1:1 followed by a two-step oxidation afforded the second acid **52**.

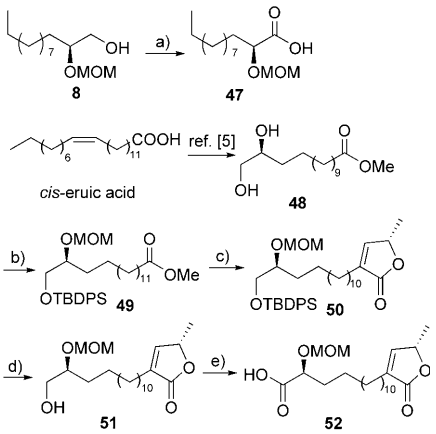
Sequential assembly of the corresponding fragments into the final products was carried out in parallel under standard amide-formation conditions (EDCI and HOBt) to give diamides **59–64** (Scheme 4). The final global deprotection of the MOM ethers of **59–64** was performed in a dichloromethane solution containing 30% trifluoroacetic acid and afforded the targets **65–70** in satisfactory yields.

Biological evaluation: The preliminary screening of all newly synthesized AA005 analogues revealed that most compounds exhibited comparable or more active potencies than their parent AA005 against a number of cancer cell lines. Similar to AA005, they are inactive to human normal hepatic cell line HL7702. Such cell-type selectivity has been observed in our previous studies on AA005 and (4*R*)-hydroxy-AA005,[5,13] though the detailed mechanisms are still unclear.[14] Further evaluation of their inhibitory activities against human breast cancer cell lines MDA-MB-435 and MDA-MB-468, and non-cancerous human mammary epithelial cells (HMEC) was accomplished by WST methods, by using AA005 as a positive reference. The inhibitory poten-

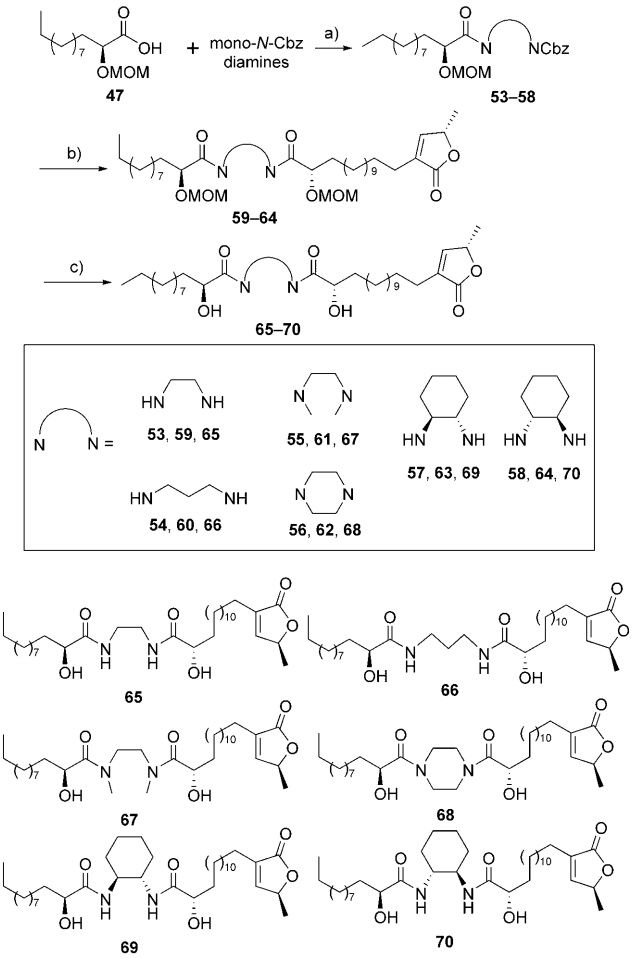


Scheme 2. Syntheses of new mimetics **41**–**46**: a) i) $HC(OCH_3)_3$, (*D*)-CSA, CH_2Cl_2; ii) DIBALH in toluene, CH_2Cl_2, 0 °C, 88 % over 2 steps; b) MsCl, Et_3N, CH_2Cl_2, 100 %; c) NaH, DMF, 80 °C, 79–82 %; d) 50 % NaOH, Bu_4NHSO_4, (*R*)-epichlorohydrin, hexane, 60–70 %; e) i) *n*BuLi, BF_3•Et_2O, trimethylsilyacetylene, THF, −78 °C; ii) MOMCl, *i*Pr_2NEt, CH_2Cl_2; iii) TBAF, THF, 0 °C, 85–88 % over 3 steps; f) *n*-BuLi, BF_3•Et_2O, **30**, THF, −78 °C, 58–68 %; g) MsCl, Et_3N then DBU, CH_2Cl_2, 60–65 %; h) i) $TsNHNH_2$, NaOAc, DME/H_2O, reflux; ii) conc. HCl, MeOH, 43–57 % over 2 steps. CSA = 10-camphorsulfonic acid; DIBALH = diisobutylaluminum hydride; MsCl = methanesulfonyl chloride; TBAF = tetrabutylammonium fluoride; DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene; DME = 1,2-dimethoxyethane.

cies of compounds **41**–**46** and **65**–**70**, in terms of IC_{50} values, are listed in Table 1. All compounds except **69** and **70** show low micromolar and submicromolar potencies against the human breast cancer cells MDA-MB-468, whereas they are



Scheme 3. Syntheses of acids **47** and **52**: a) i) DMP, CH_2Cl_2; ii) $NaClO_2$, KH_2PO_4, 2-methyl-2-bulene, *t*BuOH/H_2O 4:1, 90 % over 2 steps; b) i) TBDPSCl, imidazole, CH_2Cl_2; ii) MOMCl, *i*Pr_2NEt, CH_2Cl_2, 90 % over 2 steps; c) i) LDA, *O*-THP-(*S*)-lactaldehyde, THF; ii) 10 % H_2SO_4, THF; ii) $(CF_3CO)_2O$, Et_3N, 60 % over 3 steps; d) HF/pyridine 1:1, THF, RT, 75 %; e) i) DMP, CH_2Cl_2; ii) KH_2PO_4, $NaClO_2$, 2-methyl-2-butene, *t*BuOH/H_2O, 86 % in 2 steps. TBDPSCl = *tert*-butyldiphenylchlorosilane; LDA = lithium diisopropylamide; DMP = Dess–Martin periodinane; THP = tetrahydropyran.



Scheme 4. Syntheses of amide compounds **65**–**70**: a) EDCI, HOBt, CH_2Cl_2, 85–90 %; b) i) Pd/C, H_2 (1 atm), MeOH; ii) **52**, EDCI, HOBt, 73–90 % in 2 steps; c) 30 % TFA, CH_2Cl_2, 57–70 %. EDCI = *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; HOBt = 1-hydroxybenzotriazole hydrate; TFA = trifluoroacetic acid.

Table 1. Bioactivity screening of newly synthesized AA005 analogues.[a–c]

Entry	Compounds	IC_{50} [μM]		
		MDA-MB-435	MDA-MB-468	HMEC
1	AA005	>100	5.932	82.11
2	**41**	6.467	0.830	14.25
3	**42**	4.170	1.005	10.86
4	**43**	5.500	0.994	13.60
5	**44**	11.24	1.630	17.68
6	**45**	25.69	2.559	20.63
7	**46**	18.40	3.007	17.95
8	**65**	>100	2.953	>100
9	**66**	>100	2.019	>100
10	**67**	3.784	0.218	15.11
11	**68**	12.61	0.858	70.00
12	**69**	>100	11.81	>100
13	**70**	>100	61.59	>100

[a] AA005 was used as a positive control. [b] MDA-MB-435: human breast cancer cell; MDA-MB-468: human breast cancer cell; HMEC: non-cancerous human mammary epithelial cells. [c] Inhibition of cell growth by the listed compounds in MDA-MB-435, MDA-MB-468, and HMEC cells was determined by using a WST assay.

less active against the other type of human breast cancer cells MDA-MB-435. Eight samples presented at least 10 times less potency, and four of them are inactive (IC_{50} > 100 μM) against noncancerous HMEC, when compared to MDA-MB-468. Compound **67**, the most potent compound in this study, exhibits 69 times more potency against MDA-MB-468 than HMEC cells.

Based on their cytotoxicities against MDA-MB-468, some conclusions can be drawn with relevance to their structural features. In general, all the newly synthesized compounds are more active than their parent AA005, with the exception of two more rigid compounds **69** and **70** (Table 1, entries 12 and 13). Among them, compound **67** (entry 10) exhibited the highest potency with an IC_{50} of 0.218 μM, 30-times greater than that of AA005. Obviously, the introduction of appropriate conformational constraints into the middle ether region of AA005 is a useful strategy for the improvement of anticancer activity. Furthermore, compound **67** (entry 10, SI = 69) shows a better selectivity for inhibitory effects between cancerous MDA-MB-468 cells and noncancerous HMEC cells than its parent AA005 (entry 1, SI = 14).

For the first series of compounds with ether functionalities (Table 1, entries 2–7), either in the open or cyclic state, the bioactivities are similar. Only limited improvements were achieved. The best of this series are compounds **41** and **43** ($IC_{50} \approx 1$ μM, entries 2 and 4), as both of these compounds acquired a potency 5–6 times that of AA005. However, much more different results were observed in the second series, the amide-containing compounds. It was the first opportunity to introduce nitrogen atom(s) into the middle region of the AA005 skeleton. Among the six nitrogen-containing compounds, we can find the best (**67**; $IC_{50} = 0.218$ μM, entry 10) and worst compound (**70**; $IC_{50} = 61.59$ μM, entry 13) in this study. Linear amides **65** (with 1,2-ethanediamine as the linkage) and **66** (with 1,3-propanediamine as the linkage) slightly improved their cytotoxicities (entries 8 and 9). More interestingly, a great extent of enhancement (approximately 14 times) was achieved when both amides of **65** were N-methyalted (compound **67**, entry 10). A similar but more rigid compound **68** with a 1,4-piperazine moiety (entry 11) could not afford a better record, though it is 2–3 times more potent than amides **65** and **66**. To our surprise, introduction of 1,2-*trans*-cyclohexanediamine into the skeleton of AA005 yielded negative results (entries 12 and 13). Generally, the amide is more rigid in conformation than the corresponding ether, even in the acyclic states. Based on the above results, it can be concluded that conformational constraint is a double-edged sword for the bioactivity of such AA005-like molecules: An appropriate conformational constraint with slightly rigid properties, such as that introduced into **67**, is mostly favorable. Second, stereochemistry affects the bioactivity of these AA005-like compounds little or to a very limited extent. A similar phenomenon was also observed in the naturally occurring annonaceous acetogenins with the hydroxylated bis-THF ring(s), the stereochemistries of which hardly affect the inhibitory potency.[15] In this study, for instance, pairs of diastereomerically different (enantiomers, each in the modification region) compounds, **41** and **42** (entries 2 and 3), **43** and **44** (entries 4 and 5), and **45** and **46** (entries 6 and 7), exhibit almost the same potencies. The two worst compounds in this study, **69** and **70** (entries 12 and 13), gave only a five times potency difference against MDA-MB468. In addition, the nature of heteroatoms (O, S, or N) introduced in these compounds is not crucial to the bioactivity, though nitrogen-containing compounds give relatively better results.

Conclusion

A number of new analogues based on anticancer annonaceous acetogenin mimetic AA005 were designed and synthesized by the introduction of conformational constraints to the linear skeleton of AA005. Biological results demonstrate that stereochemical variations in the modification region exerted little effects on the bioactivity. Most AA005 analogues in this study were found to be more potent against breast cancer cell line MDA-MB-468 than the other cancer cell line MDA-MB-435 and showed satisfactory selectivity to noncancerous cell line HMEC. The generally improved bioactivities clearly indicate that the introduction of appropriate conformational constraints into the linear AA005 skeleton is a useful optimizing tool for this unique class of anticancer agents. In addition, success with the amide analogues of AA005 makes the AA005-based anticancer agents much simpler to synthesize and more flexible for future further development.

Experimental Section

General methods: Optical rotations were measured by a Perkin–Elmer 341 MC polarimeter at room temperature. IR spectra were obtained by using a Fourier-transform IR spectrometer (FTIR). ^{1}H NMR spectra

were recorded with Varian EM and Bruker AMX machines at 300, 400, or 500 MHz and are reported in ppm (δ) downfield relative to TMS as the internal standard, and ^{13}C NMR spectra were recorded at 100 and 150 MHz and assigned in ppm (δ). HRMS spectra were recorded by using either a Kratos Concept instrument, a Q-Tof micro instrument, or an APEXIII 7.0 TESLA FTMS. Elemental analysis was preformed by using an Elemental VARIO EL apparatus. All melting points were uncorrected. Flash column chromatography was performed on silica gel (10–40 μm).

General procedure for 29–34: *n*BuLi (1.6 M in hexanes, 2 mmol) at −78 °C was added to a solution of **22–27** (2 mmol) in THF (5 mL). After the mixture had been stirred for 30 min, $BF_3{\cdot}Et_2O$ (2 mmol) was added. After an additional 30 min at −78 °C, a solution of **28** (2 mmol) in THF (5 mL) was added dropwise. The reaction mixture was quenched after 2 h by the addition of aq. NH_4Cl solution and subsequently extracted with ethyl acetate (3×25 mL). The combined organic layers were washed with water and brine, concentrated, and then purified by column chromatography with EtOAc/petroleum ether 1:5 to 1:2 as the eluent to give compounds **29–34**, respectively.

*Data for **29***: Yellowish oil; yield: 58%; IR (film): $\tilde{\nu}$=3487, 2926, 2856, 2249, 1757, 1465, 1320, 1314, 1152, 1111, 1038, 919, 733 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ=0.88 (3H, t, J=7.0 Hz), 1.26 (24H, m), 1.40 (3H, d, J=6.8 Hz), 1.54 (6H, m), 2.26 (2H, t, J=7.3 Hz), 2.47 (4H, m), 3.36 (6H, s), 3.37 (3H, s), 3.38 (3H, s), 3.37 (1H, m), 3.56–3.85 (12H, m), 4.62 (4H, s), 4.63 (1H, d, J=6.7 Hz), 4.72 (2H, s), 4.76 (1H, d, J=6.8 Hz), 4.99 (1H, dq, J=1.5, 6.7 Hz), 6.99 ppm (1H, d, J=1.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ=173.7, 148.8, 134.1, 96.6, 95.9, 95.8, 79.4, 79.1, 78.2, 76.4, 74.8, 73.8, 69.9, 66.9, 55.37, 55.33, 55.1, 36.2, 32.0, 31.7, 29.6, 29.5, 29.3, 29.2, 29.1, 29.0, 27.7, 27.2, 25.5, 25.3, 25.0, 22.5, 22.1, 19.0, 13.9 ppm; MS (ESI): m/z: 825 [M^++Na]; HRMS (ESI): m/z: calcd for $C_{43}H_{78}O_{13}Na$: 825.5334; found: 825.5362.

*Data for **30***: Yellowish oil; yield: 58%; IR (film): $\tilde{\nu}$=3494, 2927, 2856, 2249, 1757, 1465, 1319, 1214, 1152, 1037, 919, 756, 734 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ=0.88 (3H, t, J=7.0 Hz), 1.26 (24H, m), 1.40 (3H, d, J=6.8 Hz), 1.53 (6H, m), 2.26 (2H, t, J=7.0 Hz), 2.47 (4H, m), 3.36 (6H, s), 3.37 (3H, s), 3.38 (3H, s), 3.37 (1H, m), 3.62 (8H, m), 3.76 (4H, m), 4.62 (4H, s), 4.64 (1H, d, J=6.8 Hz), 4.72 (2H, m), 4.77 (1H, d, J=6.8 Hz), 4.99 (1H, dq, J=1.5, 6.8 Hz), 6.99 ppm (1H, d, J=1.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ=173.7, 148.8, 134.1, 96.6, 96.0, 95.8, 79.3, 79.1, 76.5, 74.9, 74.0, 72.3, 69.9, 66.9, 55.36, 55.33, 55.1, 36.2, 32.0, 31.7, 29.6, 29.5, 29.3, 29.2, 29.1, 28.9, 27.7, 27.2, 25.5, 25.2, 25.0, 22.5, 22.1, 19.0, 13.9 ppm; MS (ESI): m/z: 825 [M^++Na]; HRMS (ESI): m/z: calcd for $C_{43}H_{78}O_{13}Na$: 825.5334; found: 825.5301.

*Data for **31***: Yellowish oil; yield: 60%; IR (film): $\tilde{\nu}$=3489, 2926, 2856, 1756, 1467, 1319, 1214, 1151, 1105, 1033, 919 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ=0.88 (3H, t, J=7.0 Hz), 1.26 (24H, m), 1.40 (3H, d, J=6.8 Hz), 1.53 (4H, m), 2.27 (2H+1OH, m), 2.45 (4H, m), 3.37 (3H, s), 3.38 (3H, s), 3.51 (2H, m), 3.67 (4H, m), 3.80 (3H, m), 3.92 (2H, m), 3.98 (2H, m), 4.64 (1H, d, J=6.8 Hz), 4.73 (3H, m), 4.99 (1H, dq, J=1.5, 6.7 Hz), 6.99 ppm (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ=173.0, 148.1, 133.4, 95.2, 95.1, 82.8, 77.7, 75.4, 73.8, 71.4, 70.6, 70.4, 69.7, 69.3, 54.7, 54.6, 35.5, 31.2, 31.1, 28.9, 28.8, 28.6, 28.5, 28.4, 28.3, 27.0, 26.6, 24.8, 24.6, 24.3, 21.8, 21.3, 18.4, 13.3 ppm; MS (ESI): m/z: 719 [M^++Na]; HRMS (ESI): m/z: calcd for $C_{39}H_{68}O_{10}Na$: 719.4704; found: 719.4726.

*Data for **32***: Yellowish oil; yield: 60%; IR (film): $\tilde{\nu}$=3489, 2927, 2856, 1757, 1465, 1319, 1213, 1152, 1106, 1035, 919 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ=0.88 (3H, t, J=7.0 Hz), 1.26 (24H, m), 1.40 (3H, d, J=6.8 Hz), 1.53 (4H, m), 2.27 (2H+1OH, m), 2.46 (4H, m), 3.38 (3H, s), 3.39 (3H, s), 3.50 (2H, m), 3.62 (2H, m), 3.67 (2H, m), 3.81 (3H, m), 3.94 (4H, m), 4.65 (1H, d, J=6.8 Hz), 4.72 (3H, m), 4.99 (1H, dq, J=1.6, 6.8 Hz), 7.00 ppm (1H, d, J=1.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ=173.0, 148.1, 133.4, 95.3, 95.1, 82.8, 82.7, 77.8, 75.5, 73.9, 71.5, 70.6, 70.5, 69.8, 69.2, 54.7, 54.6, 35.5, 31.2, 31.0, 28.8, 28.7, 28.6, 28.5, 28.4, 28.2, 27.0, 26.5, 24.8, 24.6, 24.3, 21.8, 21.2, 18.3, 13.2 ppm; MS (ESI): m/z: 719 [M^++Na]; HRMS (ESI): m/z: calcd for $C_{39}H_{68}O_{10}Na$: 719.4704; found: 719.4733.

*Data for **33***: Yellowish oil; yield: 67%; IR (film): $\tilde{\nu}$=3489, 2927, 2855, 1756, 1465, 1318, 1204, 1152, 1105, 1036,, 918 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ=0.87 (3H, t, J=7.2 Hz), 1.25–1.53 (30H, m), 1.40 (3H, d, J=6.9 Hz), 2.25 (2H+1OH, m), 2.46 (4H, m), 2.83 (2H, m), 2.96 (1H, d, J=3.9 Hz), 3.01 (1H, d, J=3.9 Hz), 3.37 (3H, s), 3.38 (3H, s), 3.45–3.69 (7H, m), 3.81 (1H, m), 4.06 (2H, m), 4.64 (1H, d, J=6.6 Hz), 4.71 (2H, s), 4.74 (1H, d, J=6.6 Hz), 4.99 (1H, dq, J=1.5, 6.6 Hz), 6.98 ppm (1H, d, J=1.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ=173.9, 148.9, 134.2, 96.0, 95.9, 84.6, 84.4, 78.8, 78.4, 77.4, 76.2, 74.7, 72.2, 70.6, 70.1, 55.56, 55.51, 36.3, 32.87, 32.82, 31.97, 31.92, 29.7, 29.5, 29.4, 29.3, 29.2, 29.1, 27.8, 27.3, 25.6, 25.4, 25.1, 22.7, 22.1, 19.2, 14.1 ppm; MS (ESI): m/z: 735 (M^++Na); HRMS (ESI): m/z: calcd for $C_{39}H_{68}O_9SNa$: 735.4476; found: 735.4484; elemental analysis calcd (%) for $C_{39}H_{68}O_9S$: C 65.70, H 9.61; found: C 66.00, H 9.61.

*Data for **34***: Yellowish oil; yield: 68%; IR (film): $\tilde{\nu}$=3496, 2927, 2855, 1757, 1465, 1357, 1318, 1204, 1152, 1106, 1036, 918 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ=0.86 (3H, t, J=7.2 Hz), 1.25–1.53 (30H, m), 1.39 (3H, d, J=6.9 Hz), 2.25 (2H+1OH, m), 2.46 (4H, m), 2.82 (2H, m), 2.97 (1H, d, J=4.2 Hz), 3.01 (1H, d, J=4.2 Hz), 3.37 (3H, s), 3.38 (3H, s), 3.43–3.69 (7H, m), 3.80 (1H, m), 4.05 (2H, m), 4.63 (1H, d, J=6.6 Hz), 4.71 (2H, s), 4.74 (1H, d, J=6.6 Hz), 4.99 (1H, dq, J=1.8, 6.6 Hz), 6.98 ppm (1H, d, J=1.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ=173.9, 148.9, 134.2, 96.1, 95.9, 84.6, 84.4, 78.8, 77.4, 76.4, 74.7, 72.3, 70.7, 70.1, 55.5, 36.3, 32.8, 32.0, 31.9, 29.7, 29.5, 29.4, 29.3, 29.2, 29.1, 27.8, 27.3, 25.6, 25.3, 25.1, 22.7, 22.1, 19.2, 14.1 ppm; MS (ESI): m/z: 735 [M^++Na]; HRMS (ESI): m/z: calcd for $C_{39}H_{68}O_9SNa$: 735.4476; found: 735.4481; elemental analysis calcd (%) for $C_{39}H_{68}O_9S$: C 65.70, H 9.61; found: C 65.69, H 9.85.

General procedure for compounds 35–40: MsCl (1 mmol) in CH_2Cl_2 (2 mL) was added to a stirred solution of **29–34** (0.5 mmol) and Et_3N (1.5 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The reaction mixture was stirred at RT for 30 min, and then DBU (1 mmol) was added. The mixture was stirred for a further 2 h, and was then quenched with water (5 mL) and extracted with CH_2Cl_2 (3×20 mL). The combined organic layers were washed with HCl (1 N), water, and brine, and then dried over Na_2SO_4. Removal of the solvent followed by column chromatography with EtOAc/petroleum ether 1:3 to 1:1 as the eluent gave compounds **35–40**, respectively.

*Data for **35***: Yellowish oil; yield: 62%; IR (film): $\tilde{\nu}$=2928, 2856, 1759, 1465, 1319, 1214, 1152, 1110, 1039, 919 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ=0.88 (3H, t, J=7.0 Hz), 1.26 (24H, m), 1.40 (3H, d, J=6.8 Hz), 1.52 (6H, m), 2.06 (1H, m), 2.26 (3H,m), 2.62 (2H, m), 3.35 (6H, s), 3.37 (3H, s), 3.39 (3H, s), 3.53–3.87 (13H, m), 4.62 (5H, m), 4.74 (3H, m), 4.99 (1H, dq, J=1.6, 6.8 Hz), 5.43 (1H, m), 5.81 (0.55H, dt, J=10.7, 7.3 Hz), 6.04 (0.46H, dt, J=15.8, 7.0 Hz), 6.99 ppm (1H, d, J=1.5 Hz); MS (ESI): m/z: 807 [M^++Na]; HRMS (ESI): m/z: calcd for $C_{43}H_{76}O_{12}Na$: 807.5229; found: 807.5254.

*Data for **36***: Yellowish oil; yield: 62%; IR (film): $\tilde{\nu}$=2928, 2856, 1759, 1465, 1401, 1319, 1214, 1153, 1111, 954, 919 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ=0.88 (3H, t, J=7.0 Hz), 1.26 (24H, m), 1.40 (3H, d, J=6.8 Hz), 1.52 (6H, m), 2.06 (1H, m), 2.26 (3H,m), 2.62 (2H, m), 3.36 (6H, s), 3.37 (3H, s), 3.39 (3H, s), 3.37 (2H, m), 3.53–3.87 (11H, m), 4.63 (5H, m), 4.74 (3H, m), 4.99 (1H, dq, J=1.6, 6.8 Hz), 5.43 (1H, m), 5.81 (0.55H, dt, J=10.7, 7.3 Hz), 6.04 (0.46H, dt, J=15.8, 7.0 Hz), 6.99 ppm (1H, d, J=1.5 Hz); MS (ESI): m/z: 807 [M^++Na]; HRMS (ESI): m/z: calcd for $C_{43}H_{76}O_{12}Na$: 807.5229; found 807.5233.

*Data for **37***: Yellowish oil; yield: 60%; IR (film): 3489, 2926, 2856, 1756, 1467, 1319, 1214, 1151, 1105, 1033, 919 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ=0.88 (3H, t, J=7.0 Hz), 1.26 (24H, m), 1.40 (3H, d, J=6.8 Hz), 1.53 (4H, m), 2.27 (2H+1OH, m), 2.45 (4H, m), 3.37 (3H, s), 3.38 (3H, s), 3.51 (2H, m), 3.67 (4H, m), 3.80 (3H, m), 3.92 (2H, m), 3.98 (2H, m), 4.64 (1H, d, J=6.8 Hz), 4.73 (3H, m), 4.99 (1H, dq, J=1.5, 6.7 Hz), 6.99 ppm (1H, s); MS (ESI): m/z: 719 [M^++Na]; HRMS (ESI): m/z: calcd for $C_{39}H_{68}O_{10}Na$: 719.4704; found: 719.4726.

*Data for **38***: Yellowish oil; yield: 60%; IR (film): $\tilde{\nu}$=2927, 2855, 1758, 1464, 1356, 1318, 1216, 1152, 1105, 1037, 918 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ=0.88 (3H, t, J=7.1 Hz), 1.26 (24H, m), 1.40 (3H, d, J=6.8 Hz), 1.53 (4H, m), 2.06 (1H, m), 2.26 (3H, m), 2.61 (2H, m), 3.37 (3H, s), 3.39 (3H, s), 3.49 (2H, m), 3.78 (2H, m), 3.85 (1H, m), 3.94 (4H, m), 4.64 (1H, d, J=6.8 Hz), 4.73 (3H, m), 5.00 (1H, dq, J=1.5,

6.8 Hz), 5.43 (1H, m), 5.83 (0.55H, dt, J=10.6, 7.4 Hz), 6.05 (0.45H, dt, J=15.2, 7.4 Hz), 6.99 ppm (1H, s); MS (ESI): m/z: 701 [M^++Na]; HRMS (ESI): m/z: calcd for $C_{39}H_{66}O_9Na$: 701.4599; found: 701.4588.

Data for ***39***: Yellowish oil; yield: 65%; IR (film): $\tilde{\nu}$=2926, 2854, 1758, 1465, 1318, 1152, 1105, 1037, 918 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ= 0.88 (3H, t, J=7.2 Hz), 1.26–1.53 (30H, m), 1.41 (3H, d, J=6.9 Hz), 2.07 (1H, m), 2.26 (2H, t, J=7.5 Hz), 2.26 (1H, m), 2.58 (1H, m), 2.64 (1H, m), 2.86 (2H, m), 2.99 (1H, d, J=3.6 Hz), 3.02 (1H, d, J=6.6 Hz), 3.38 (3H, s), 3.39 (3H, s), 3.46–3.71 (5H, m), 3.85 (1H, m), 4.08 (2H, m), 4.65 (1H, d, J=6.6 Hz), 4.73 (2H, s), 4.74 (1H, d, J=6.6 Hz), 4.99 (1H, dq, J=1.8, 6.6 Hz), 5.44 (1H, m), 5.83 (0.55H, dt, J=11.1, 7.5 Hz), 6.05 (0.45H, dt, J=15.9, 6.9 Hz), 6.99 ppm (1H, d, J=1.5 Hz); MS (ESI): m/z: 717 [M^++Na]; HRMS (ESI): m/z: calcd for $C_{39}H_{66}O_8SNa$: 717.4370; found: 717.4399.

Data for ***40***: Yellowish oil; yield: 65%; IR (film): $\tilde{\nu}$=2926, 2854, 1758, 1465, 1318, 1152, 1105, 1037, 918 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ= 0.88 (3H, t, J=7.2 Hz), 1.25–1.53 (30H, m), 1.41 (3H, d, J=6.9 Hz), 2.07 (1H, m), 2.26 (2H, t, J=7.5 Hz), 2.26 (1H, m), 2.58 (1H, m), 2.64 (1H, m), 2.82 (1H, dd, J=3.3, 6.3 Hz), 2.87 (1H, dd, J=3.3, 5.4 Hz), 2.99 (1H, d, J=4.2 Hz), 3.02 (1H, J=4.2 Hz), 3.38 (3H, s), 3.39 (3H, s), 3.44–3.71 (5H, m), 3.84 (1H, m), 4.08 (2H, m), 4.65 (1H, d, J=6.6 Hz), 4.73 (2H, s), 4.74 (1H, d, J=6.6 Hz), 4.99 (1H, dq, J=1.5, 6.6 Hz), 5.45 (1H, m), 5.83 (0.55H, dt, J=11.1, 7.5 Hz), 6.06 (0.45H, dt, J=14.4, 7.8 Hz), 6.99 ppm (1H, d, J=1.5 Hz); MS (ESI): m/z: 717 [M^++Na]; HRMS (ESI): m/z: calcd for $C_{39}H_{66}O_8SNa$: 717.4370; found: 717.4382.

General procedure for compounds 41–46: NaOAc (22.0 mmol) in water (30 mL) was added over 5 h to a stirred solution of **35–40** (0.15 mmol) and *p*-toluenesulfonyl hydrazide (18.6 mmol) in dimethoxyethane (25 mL) under reflux. The mixture was then cooled down to RT, poured into water, and extracted with ether (3×20 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was directly used in the next step without further purification. Concentrated HCl (0.5 mL) was added to a stirred solution of the above crude product in methanol (2 mL) at 0°C. Then, the reaction mixture was warmed to RT and stirred for 24 h. Finally, the mixture was quenched with sat. $NaHCO_3$ at 0°C. The mixture was extracted with ethyl acetate (3×15 mL) and the extracts were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography with EtOAc/petroleum ether 1:5 to 1:1 as the eluent to afford compounds **41–46**, respectively.

Data for ***41***: White wax; yield: 46%; $[\alpha]_D^{25}$=−16.1 (c=0.6 in $CHCl_3$); IR (film): $\tilde{\nu}$=3353, 2925, 2851, 1749, 1471, 1326, 1201, 1144, 1113, 1033, 880, 720 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.88 (3H, t, J=7.0 Hz), 1.26–1.50 (38H, m), 1.40 (3H, d, J=6.8 Hz), 1.54 (2H, m), 2.26 (2H, t, J= 8.0 Hz), 3.18 (4OH, brs), 3.44 (2H, m), 3.65 (6H, m), 3.79 (4H, m), 4.99 (1H, dq, J=1.6, 6.8 Hz), 6.99 ppm (1H, d, J=1.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ=173.9, 148.9, 134.3, 80.6, 77.4, 75.2, 70.7, 60.5, 33.1, 31.9, 29.7, 29.68, 29.63, 29.5, 29.4, 29.3, 29.2, 29.1, 27.4, 25.5, 25.1, 22.6, 19.1, 14.0 ppm; MS (ESI): m/z: 637 [M^++Na]; HRMS (ESI): m/z: calcd for $C_{35}H_{66}O_8Na$: 637.4649; found: 637.4678.

Data for ***42***: White wax; yield: 54%; $[\alpha]_D^{25}$=−0.11 (c=1.45 in $CHCl_3$); IR (film): $\tilde{\nu}$=3356, 2920, 2851, 1753, 1469, 1321, 1084, 1029, 889, 721 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.88 (3H, t, J=7.0 Hz), 1.26–1.50 (38H, m), 1.40 (3H, d, J=6.8 Hz), 1.54 (2H, m), 2.26 (2H, t, J= 8.0 Hz), 3.39 (2H, m), 3.44 (4OH, brs), 3.75 (10H, m), 4.99 (1H, dq, J= 1.6, 6.8 Hz), 6.99 ppm (1H, d, J=1.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ=173.9, 148.9, 134.2, 80.8, 77.4, 75.5, 71.0, 60.7, 32.9, 31.8, 29.68, 29.66, 29.60, 29.5, 29.4, 29.3, 29.2, 29.1, 27.3, 25.6, 25.1, 22.6, 19.1, 14.0 ppm; MS (ESI): m/z: 637 [M^++Na]; HRMS (ESI): m/z: calcd for $C_{35}H_{66}O_8Na$: 637.4649; found: 637.4676.

Data for ***43***: White wax; yield: 57%; $[\alpha]_D^{25}$=+12.5 (c=0.95 in $CHCl_3$); IR (film): $\tilde{\nu}$=3500, 2919, 2850, 1747, 1467, 1323, 1138, 1122, 1079, 1027, 880, 722 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ=0.88 (3H, t, J=7.1 Hz), 1.26 (32H, m), 1.40 (3H, d, J=6.8 Hz), 1.44 (6H, m), 1.54 (2H, m), 2.26 (2H+2OH, m), 3.31 (2H, m), 3.52 (2H, m), 3.73 (2H, m), 3.79 (2H, m), 3.94 (2H, m), 3.99 (2H, m), 4.99 (1H, dq, J=1.6, 6.8 Hz), 6.99 ppm (1H, J=1.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ=173.8, 148.8, 134.2, 83.4, 77.3, 73.9, 71.4, 70.3, 70.2, 33.0, 31.8, 29.6, 29.5, 29.27, 29.23, 29.1, 27.3, 25.4, 25.1, 22.6, 19.1, 14.0 ppm; MS (ESI): m/z: 619 [M^++Na]; HRMS (ESI): m/z: calcd for $C_{35}H_{64}O_7Na$: 619.4568; found: 619.4524.

Data for ***44***: White wax; yield: 57%; IR (film): $\tilde{\nu}$=3500, 2920, 2851, 1743, 1467, 1325, 1147, 1074, 1031, 953, 727 cm^{-1}; $[\alpha]_D^{25}$=+0.14 (c=0.8 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ=0.88 (3H, t, J=7.1 Hz), 1.26 (32H, m), 1.40 (3H, d, J=6.8 Hz), 1.44 (6H, m), 1.54 (2H, m), 2.26 (2H, t, J=7.7 Hz), 2.30 (2OH, brs), 3.33 (2H, m), 3.51 (2H, m), 3.77 (4H, m), 3.94 (2H, m), 3.99 (2H, m), 4.99 (1H, dq, J=1.5, 6.8 Hz), 6.99 ppm (1H, J=1.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ=173.8, 148.8, 134.3, 83.4, 77.3, 73.9, 71.4, 70.3, 33.0, 31.8, 29.6, 29.5, 29.2, 29.1, 27.3, 25.4, 25.1, 22.6, 19.1, 14.0 ppm; MS (ESI): m/z: 619 [M^++Na]; HRMS (ESI): calcd for $C_{35}H_{64}O_7Na$: 619.4568; found: 619.4562.

Data for ***45***: White wax; yield: 46%; $[\alpha]_D^{25}$=+38.1 (c=2.0 in $CHCl_3$); IR (film): $\tilde{\nu}$=3498, 2926, 2850, 1745, 1468, 1324, 1166, 1121, 1029, 953, 863, 721 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ=0.88 (3H, t, J=7.1 Hz), 1.26–1.56 (40H, m), 1.41 (3H, d, J=6.8 Hz), 2.26 (2H, t, J=7.9 Hz), 2.47 (2OH, brs), 2.84 (2H, m), 3.00 (2H, m), 3.36 (2H, dd, J=7.9, 9.4 Hz), 3.53 (2H, dd, J=2.8, 9.6 Hz), 3.74 (2H, m), 4.08 (2H, m), 4.99 (1H, dq, J=1.4, 6.6 Hz), 6.99 ppm (1H, d, J=1.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ=173.8, 148.8, 134.2, 83.9, 77.3, 73.8, 70.1, 33.0, 32.9, 32.2, 31.8, 29.6, 29.4, 29.2, 29.1, 27.3, 25.4, 25.1, 22.6, 19.1, 14.0 ppm; MS (ESI): m/z: 635 [M^++Na]; HRMS (ESI): m/z: calcd for $C_{35}H_{64}O_6SNa$: 635.4315; found: 635.4331.

Data for ***46***: White wax; yield: 43%; IR (film): $\tilde{\nu}$=3463, 2924, 2851, 1750, 1467, 1320, 1175, 1087, 1027, 948, 869, 722 cm^{-1}; $[\alpha]_D^{25}$=−29.0 (c= 1.55 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ=0.88 (3H, t, J=7.0 Hz), 1.26–1.56 (40H, m), 1.41 (3H, d, J=6.8 Hz), 2.26 (2H, t, J=7.7 Hz), 2.83 (2H, m), 2.99 (1H, d, J=3.4 Hz), 3.02 (1H, d, J=3.6 Hz), 3.33 (2H, dd, J=8.5, 8.5 Hz), 3.59 (2H, dd, J=2.9, 9.5 Hz), 3.74 (2H, m), 4.09 (2H, m), 4.99 (1H, q, J=6.6 Hz), 6.99 ppm (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ=173.7, 148.8, 134.2, 84.5, 77.3, 74.3, 70.5, 33.0, 32.9, 32.0, 31.8, 29.5, 29.4, 29.2, 29.1, 27.3, 25.4, 25.1, 22.6, 19.1, 14.0 ppm; MS (ESI): m/z: 635 [M^++Na]; HRMS (ESI): m/z: calcd for $C_{35}H_{64}O_6SNa$: 635.4315; found: 635.4332.

General procedure for 59–64: Compounds **53–58** (1 mmol) in methanol (10 mL) were hydrogenated under H_2 (1 atm) in the presence of 10% Pd/C at room temperature for 4 h. After removal of solid through Celite and evaporation of solvents, the crude product was directly used in the next step. The mixture of this crude product, compound **52** (0.19 g, 0.5 mmol), and HOBt (0.13 g, 1.0 mmol) in dry CH_2Cl_2 (15 mL) was treated with EDCI (0.19 g, 1.0 mmol) at 0°C. The reaction mixture was stirred for 4 h at RT, quenched with saturated NH_4Cl, and extracted with ethyl acetate. The extracts were washed with saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography with EtOAc/petroleum ether 1:5 to 1:1 as the eluent to give compounds **59–64**, respectively.

Data for ***59***: White solid; yield: 77%; $[\alpha]_D^{25}$=−37.8 (c=1.90 in $CHCl_3$); IR (film): $\tilde{\nu}$=3291, 3077, 2920, 2851, 1745, 1660, 1547, 1471, 1382, 1323, 1233, 1161, 1104, 1051, 1036, 958, 920, 719 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ=0.88 (3H, t, J=7.1 Hz), 1.25 (34H, m), 1.40 (3H, d, J= 6.8 Hz), 1.54 (2H, m), 1.75 (4H, m), 2.26 (2H, t, J=8.0 Hz), 3.39 (6H, s), 3.47 (4H, m), 4.03 (2H, m), 4.62 (2H, d, J=6.6 Hz), 4.65 (2H, d, J= 6.6 Hz), 4.99 (1H, q, J=6.8 Hz), 6.99 (1H, s), 7.02 ppm (2H, brs); ^{13}C NMR (125 MHz, $CDCl_3$): δ=173.8, 173.5, 148.8, 134.3, 96.2, 77.9, 77.3, 56.0, 39.2, 32.9, 31.8, 29.5, 29.4, 29.3, 29.2, 29.1, 27.3, 25.1, 24.8, 22.6, 19.1, 14.0 ppm; MS (ESI): m/z: 691 [M^++Na]; HRMS (MALDI): m/z: calcd for $C_{37}H_{68}N_2O_8Na$: 691.4867; found: 691.4854.

Data for ***60***: White solid; yield: 90%; $[\alpha]_D^{25}$=−40.6 (c=2.40 in $CHCl_3$); IR (film): $\tilde{\nu}$=3295, 2923, 2852, 2822, 1755, 1652, 1535, 1468, 1434, 1371, 1319, 1215, 1152, 1128, 1095, 1038, 919, 723, 670 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ=0.88 (3H, t, J=7.1 Hz), 1.25 (32H, m), 1.40 (3H, d, J=6.8 Hz), 1.52 (2H, m), 1.73 (6H, m), 2.26 (2H, t, J=8.0 Hz), 3.30 (4H, m), 3.40 (6H, s), 4.05 (2H, m), 4.67 (2H, d, J=6.6 Hz), 4.69 (2H, d, J=6.6 Hz), 5.00 (1H, dq, J=1.6, 6.8 Hz), 6.99 (1H, d, J=1.6 Hz), 7.06 ppm (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ=173.7, 173.0, 148.8, 134.2, 96.2, 77.9, 77.2, 56.0, 35.3, 33.0, 31.8, 29.9, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 27.3, 25.1, 24.8, 22.6, 19.1, 14.0 ppm; MS (ESI): m/z: 705 [M^+

+Na]; HRMS (MALDI): *m/z*: calcd for $C_{38}H_{70}N_2O_8Na$: 705.5024; found: 705.5035.

Data for ***61***: Yellowish oil; yield: 75%; $[\alpha]_D^{25}=-53.8$ ($c=1.60$ in $CHCl_3$); IR (film): $\tilde{\nu}=2925$, 2854, 1756, 1655, 1466, 1406, 1318, 1151, 1105, 1039, 919, 722 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta=0.84$ (3H, t, $J=7.1$ Hz), 1.22 (34H, m), 1.37 (3H, d, $J=6.8$ Hz), 1.52 (6H, m), 2.23 (2H, t, $J=7.0$ Hz), 2.96 (1H, s), 3.07 (5H, s), 3.33 (6H, s), 3.31 (1H, m), 3.48 (2H, m), 3.69 (1H, m), 4.32 (2H, m), 4.59 (4H, m), 4.95 (1H, dq, $J=1.6$, 6.8 Hz), 6.96 ppm (1H, d, $J=1.5$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=$ 173.7, 172.2, 148.7, 134.2, 95.5, 73.8, 55.87, 55.80, 55.7, 45.3, 35.2, 32.3, 31.8, 29.5, 29.4, 29.2, 29.1, 27.3, 25.6, 25.5, 25.1, 22.5, 19.1, 14.0 ppm; MS (ESI): *m/z*: 719 [M^++Na]; HRMS (MALDI): *m/z*: calcd for $C_{39}H_{72}N_2O_8Na$: 719.5180; found: 719.5162.

Data for ***62***: Yellowish oil; yield: 73%; $[\alpha]_D^{25}=-28.4$ ($c=3.32$ in $CHCl_3$); IR (film): $\tilde{\nu}=2925$, 2854, 1756, 1651, 1463, 1371, 1318, 1216, 1153, 1105, 1041, 920, 722 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta=0.81$ (3H, t, $J=$ 7.0 Hz), 1.19 (32H, m), 1.33 (3H, d, $J=6.8$ Hz), 1.46 (4H, m), 1.64 (4H, m), 2.19 (2H, t, $J=7.9$ Hz), 3.30 (6H, s), 3.58 (8H, m), 4.30 (2H, s), 4.57 (4H, s), 4.92 (1H, dq, $J=1.4$, 6.8 Hz), 6.92 ppm (1H, d, $J=1.4$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=173.7$, 170.7, 148.7, 134.2, 95.7, 75.3, 55.9, 45.3, 45.0, 42.2, 32.7, 31.7, 29.5, 29.4, 29.3, 29.2, 29.1, 27.3, 25.5, 25.0, 22.5, 19.1, 14.0 ppm; MS (ESI): *m/z*: 717 [M^++Na]; HRMS (MALDI): *m/z*: calcd for $C_{39}H_{70}N_2O_8Na$: 717.5024; found: 717.5034.

Data for ***63***: White solid; yield: 73%; $[\alpha]_D^{25}=-62.0$ ($c=3.35$ in $CHCl_3$); IR (film): $\tilde{\nu}=3269$, 3086, 2923, 2853, 1757, 1648, 1552, 1467, 1319, 1219, 1107, 1041, 919, 721 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta=0.80$ (3H, t, $J=7.0$ Hz), 1.18 (38H, m), 1.33 (3H, d, $J=6.8$ Hz), 1.47 (2H, m), 1.64 (6H, m), 1.95 (2H, d, $J=12.1$ Hz), 2.19 (2H, t, $J=7.9$ Hz), 3.28 (6H, s), 3.63 (2H, m), 3.88 (2H, m), 4.44 (2H, d, $J=6.8$ Hz), 4.55 (2H, d, $J=$ 6.8 Hz), 4.92 (1H, dq, $J=1.5$, 6.8 Hz), 6.68 (2H, brs), 6.91 ppm (1H, d, $J=1.4$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=173.7$, 172.6, 148.7, 134.2, 96.0, 77.4, 55.9, 52.69, 52.64, 32.9, 32.4, 31.8, 29.4, 29.3, 29.2, 29.1, 27.3, 25.1, 24.6, 24.5, 22.5, 19.1, 14.0 ppm; MS (ESI): *m/z*: 745 [M^++Na]; HRMS (MALDI): *m/z*: calcd for $C_{41}H_{74}N_2O_8Na$: 745.5337; found: 745.5342.

Data for ***64***: White solid; yield: 75%; $[\alpha]_D^{25}=-22.7$ ($c=3.45$ in $CHCl_3$); IR (film): $\tilde{\nu}=3278$, 3075, 2924, 2854, 1758, 1643, 1526, 1467, 1319, 1288, 1154, 1103, 1032, 920, 721 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta=0.80$ (3H, t, $J=7.0$ Hz), 1.18 (38H, m), 1.33 (3H, d, $J=6.8$ Hz), 1.49 (4H, m), 1.70 (4H, m), 2.03 (2H, d, $J=11.8$ Hz), 2.19 (2H, t, $J=7.9$ Hz), 3.32 (6H, s), 3.59 (2H, m), 3.88 (2H, m), 4.56 (4H, s), 4.92 (1H, dq, $J=1.6$, 6.8 Hz), 6.77 (2H, brs), 6.91 ppm (1H, d, $J=1.5$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=173.7$, 173.2, 148.7, 134.2, 96.3, 78.2, 56.1, 52.9, 52.8, 33.4, 32.3, 31.8, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 27.3, 25.5, 25.1, 24.5, 22.6, 19.1, 14.0 ppm; MS (ESI): *m/z*: 745 [M^++Na]; HRMS (MALDI): *m/z*: calcd for $C_{41}H_{74}N_2O_8Na$: 745.5337; found: 745.5333.

General procedure for 65–70: A solution of compounds **59–64** (0.10 mmol) in CH_2Cl_2 (2 mL) was treated with TFA (1 mL) at 0°C. After stirring at RT for 1 h, the solvent was removed under reduced pressure. The residue was purified by column chromatography with CH_2Cl_2/MeOH 20:1 to 10:1 to give compounds **65–70**, respectively.

Data for ***65***: White wax; yield: 70%; $[\alpha]_D^{25}=-21.2$ ($c=0.42$ in $CHCl_3$); IR (film): $\tilde{\nu}=3404$, 3298, 2918, 2849, 2530, 2457, 1747, 1618, 1482, 1468, 1429, 1378, 1319, 1260, 1104, 1081, 1027, 951, 721 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$/MeOD): $\delta=0.88$ (3H, t, $J=7.1$ Hz), 1.26 (34H, m), 1.41 (3H, d, $J=6.8$ Hz), 1.55 (4H, m), 1.78 (2H, m), 2.26 (2H, t, $J=$ 8.0 Hz), 3.30 (2H, m), 3.46 (2H, m), 3.98 (2H, m), 5.00 (1H, dq, $J=1.2$, 6.8 Hz), 7.05 ppm (1H, $J=1.5$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$/MeOD): $\delta=174.2$, 172.3, 147.3, 131.8, 75.6, 69.5, 51.1, 36.6, 32.1, 29.6, 27.3, 27.2, 27.1, 27.0, 26.8, 25.1, 22.9, 20.3, 16.7 ppm; MS (ESI): *m/z*: 603 [M^++Na]; HRMS (MALDI): *m/z*: calcd for $C_{33}H_{60}N_2O_6Na$: 603.4343; found: 603.4373.

Data for ***66***: White wax; yield: 65%; $[\alpha]_D^{25}=-11.0$ ($c=0.60$ in $CHCl_3$); IR (film): $\tilde{\nu}=3401$, 3299, 2919, 2848, 2523, 2456, 1748, 1614, 1538, 1484, 1468, 1437, 1376, 1317, 1080, 1027, 934, 721 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$/MeOD): $\delta=0.88$ (3H, t, $J=7.0$ Hz), 1.26 (34H, m), 1.40 (3H, d, $J=6.8$ Hz), 1.56 (4H, m), 1.72 (2H, m), 1.79 (2H, m), 2.26 (2H, t, $J=$ 7.8 Hz), 3.21 (2H, m), 3.37 (2H, m), 4.02 (2H, m), 5.00 (1H, q, $J=$ 6.8 Hz), 7.01 (1H, s), 7.24 ppm (2H, brs); ^{13}C NMR (125 MHz, $CDCl_3$/MeOD): $\delta=175.6$, 174.1, 149.1, 134.2, 77.5, 71.9, 34.5, 31.8, 29.5, 29.4, 29.3, 29.2, 29.1, 29.0, 27.3, 25.1, 25.0, 22.5, 19.0, 13.9 ppm; MS (ESI): *m/z*: 617 [M^++Na]; HRMS (MALDI): *m/z*: calcd for $C_{34}H_{63}N_2O_6Na$: 595.4680; found: 595.4678.

Data for ***67***: White wax; yield: 57%; $[\alpha]_D^{25}=+10.3$ ($c=0.90$, $CHCl_3$); IR (film): $\tilde{\nu}=3412$, 2924, 2852, 2457, 1755, 1642, 1466, 1387, 1318, 1286, 1199, 1080, 1027, 722 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta=0.88$ (3H, t, $J=7.0$ Hz), 1.26 (36H, m), 1.40 (3H, d, $J=6.8$ Hz), 1.54 (4H, m), 2.26 (2H, t, $J=8.0$ Hz), 3.01 (6H, m),3.56 (4H+2OH, m), 4.30 (2H, m), 4.99 (1H, dq, $J=1.5$, 6.8 Hz), 7.01 ppm (1H, d, $J=1.2$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=175.2$, 173.8, 148.8, 134.3, 77.3, 68.1, 45.3, 34.9, 34.7, 31.8, 29.6, 29.5, 29.4, 29.3, 29.1, 27.4, 25.3, 25.1, 25.0, 22.6, 19.2, 14.0 ppm; MS (ESI): *m/z*: 631 [M^++Na]; HRMS (MALDI): *m/z*: calcd for $C_{35}H_{64}N_2O_6Na$: 631.4656; found: 631.4685.

Data for ***68***: White wax; yield: 70%; $[\alpha]_D^{25}=-2.43$ ($c=0.90$ in $CHCl_3$); IR (film): $\tilde{\nu}=3419$, 2924, 2853, 1754, 1641, 1467, 1395, 1319, 1283, 1255, 1082, 1022, 721 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta=0.88$ (3H, t, $J=$ 7.0 Hz), 1.29 (32H, m), 1.34 (3H, d, $J=6.8$ Hz), 1.49 (5H, m), 1.59 (3H, m), 2.26 (2H, t, $J=8.0$ Hz), 3.43 (5H+2OH, m), 3.70 (2H, m), 3.83 (1H, m), 4.36 (2H, m), 4.99 (1H, dq, $J=1.4$, 6.8 Hz), 6.98 ppm (1H, d, $J=$ 1.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=173.8$, 148.8, 134.3, 77.3, 68.0, 44.6, 42.2, 35.1, 31.8, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 27.4, 25.1, 24.9, 22.6, 19.2, 14.0 ppm; MS (ESI): *m/z*: 629 [M^++Na]; HRMS (MALDI): *m/z*: calcd for $C_{35}H_{62}N_2O_6Na$: 629.4500; found: 629.4513.

Data for ***69***: White wax; yield: 70%; $[\alpha]_D^{25}=-61.2$ ($c=1.25$ in $CHCl_3$); IR (film): $\tilde{\nu}=3281$, 3090, 2920, 2851, 1753, 1649, 1618, 1552, 1467, 1320, 1202, 1147, 1115, 1082, 1027, 950, 856, 721 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta=0.88$ (3H, t, $J=7.1$ Hz), 1.29 (38H, m), 1.40 (3H, d, $J=$ 6.8 Hz), 1.55 (4H, m), 1.72 (4H, m), 1.94 (2H, m), 2.26 (2H, t, $J=$ 8.0 Hz), 3.72 (2H, m), 3.98 (2H, m), 4.99 (1H, dq, $J=1.6$, 6.8 Hz), 6.98 (1H, d, $J=1.4$ Hz), 7.02 ppm (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=$ 175.8, 173.8, 148.8, 134.3, 77.3, 72.2, 53.2, 34.4, 32.0, 31.8, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 27.4, 25.2, 25.1, 24.7, 22.6, 19.1, 14.0 ppm; MS (ESI): *m/z*: 657 [M^++Na]; HRMS (MALDI): *m/z*: calcd for $C_{37}H_{66}N_2O_6Na$: 657.4813; found: 657.4814.

Data for ***70***: White wax; yield: 70%; $[\alpha]_D^{25}=+20.5$ ($c=0.95$ in $CHCl_3$); IR (film): $\tilde{\nu}=3340$, 3260, 2919, 2451, 1752, 1651, 1617, 1539, 1467, 1320, 1202, 1114, 1082, 1027, 721 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta=0.87$ (3H, t, $J=7.1$ Hz), 1.25 (38H, m), 1.40 (3H, d, $J=6.8$ Hz), 1.54 (4H, m), 1.77 (4H, m), 2.01 (2H, m), 2.26 (2H, t, $J=8.0$ Hz), 3.69 (2H, m), 4.00 (2H, m), 4.99 (1H, dq, $J=1.5$, 6.8 Hz), 6.88 (2H, brs), 6.98 ppm (1H, d, $J=1.4$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=175.0$, 173.9, 148.98, 134.3, 77.4, 72.0, 53.3, 35.0, 31.90, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 27.4, 25.1, 24.6, 22.6, 19.1, 14.0 ppm; MS (ESI): *m/z*: 657 [M^++Na]; HRMS (MALDI): *m/z*: calcd for $C_{37}H_{66}N_2O_6Na$: 657.4813; found: 657.4822.

WST cell growth assay: The anticancer activities of newly synthesized AA005 analogues were determined using the MDA-MB-468 and and MDA-MB-435 human breast cancer cell lines. To test their selectivity, normal mammary epithelial cells (HMEC) were used. Cells were seeded in 96-well flat bottom cell culture plates at a density of 3000–4000 cells per well with different concentrations of the tested compounds and incubated for four days. Cell growth inhibition was determined using the WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium monosodium salt; Dojindo Molecular Technologies Inc., Gaithersburg, Maryland). WST-8 was added at a final concentration of 10% to each well, and then the plates were incubated at 37°C for 2–3 h. The absorbance of the samples was measured at 450 nm using a Molecular Device Reader. Concentration of the compounds that inhibited cell growth by 50% (IC_{50}) was calculated by comparing absorbance in the untreated cells (DMSO control) and the cells treated with the tested compounds.

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[1] Reviews see: a) A. Cave, B. Figadere, A. Laurens, D. Cortes, *Fortschr. Chem. Org. Naturst.* **1997**, *70*, 81–288; b) F. Q. Alali, X.-X. Liu, J. L. McLaughlin, *J. Nat. Prod.* **1999**, *62*, 504–540; c) A. Bermejo, B. Figadère, M. C. Zafra-Polo, I. Barrachina, E. Estornell, D. Cortes, *Nat. Prod. Rep.* **2005**, *22*, 269–303; d) T.-S. Hu, Y.-L. Wu, Z.-J. Yao in *Medicinal Chemistry of Bioactive Natural Products* (Eds.: X.-T Liang, W.-S. Fang), Wiley, Hoboken, **2006**, Chapter 10, pp. 399–441.

[2] Recent examples on the total synthesis of annonaceous acetogenins: a) G. Keum, C. H. Hwang, S. B. Kang, Y. Kim, E. Lee, *J. Am. Chem. Soc.* **2005**, *127*, 10396–10399; b) J. M. Tinsley, W. R. Roush, *J. Am. Chem. Soc.* **2005**, *127*, 10818–10819; c) S. Takahashi, N. Ogawa, H. Koshino, T. Nakata, *Org. Lett.* **2005**, *7*, 2783–2786; d) J. M. Tinsley, E. Mertz, P. Y. Chong, R. F. Rarig, W. R. Roush, *Org. Lett.* **2005**, *7*, 4245–4248; e) K. A. Keaton, A. J. Phillips, *J. Am. Chem. Soc.* **2006**, *128*, 408–409; f) D. P. Curran, Q. Zhang, C. Richard, H. Lu, V. Gudipati, C. S. Wilcox, *J. Am. Chem. Soc.* **2006**, *128*, 9561–9573; g) M. T. Crimmins, Y. Zhang, F. A. Diaz, *Org. Lett.* **2006**, *8*, 2369–2372; h) T. R. Hoye, B. M. Eklov, J. Jeon, M. Khoroosi, *Org. Lett.* **2006**, *8*, 3383–3386.

[3] a) J. L. Landolt, K. I. Ahammadsahib, R. M. Hollingworth, R. Barr, F. L. Crane, N. L. Buerck, G. P. McCabe, J. L. McLaughlin, *Chem.-Biol. Interact.* **1995**, *98*, 1–13; b) R. José, T. Tormo, R. Gallardo, D. Aragón, E. E. Cortes, *Chem.-Biol. Interact.* **1999**, *122*, 171–183.

[4] B.-B. Zeng, Y. Wu, Q. Yu, Y.-L. Wu, Y. Li, X.-G. Chen, *Angew. Chem.* **2000**, *112*, 2010–2013; *Angew. Chem. Int. Ed.* **2000**, *39*, 1934–1937.

[5] B.-B. Zeng, Y. Wu, S. Jiang, Q. Yu, Z.-J. Yao, Z.-H. Liu, H.-Y. Li, Y. Li, X.-G. Chen, Y.-L. Wu, *Chem. Eur. J.* **2003**, *9*, 282–290.

[6] S. Jiang, Y. Li, X.-G. Chen, T.-S. Hu, Y.-L. Wu, Z.-J. Yao, *Angew. Chem.* **2004**, *116*, 333–338; *Angew. Chem. Int. Ed.* **2004**, *43*, 329–334.

[7] Z.-J. Yao, Y.-L. Wu, *J. Org. Chem.* **1995**, *60*, 1170–1175; further conversions of the known materials, [(4*S*,5*S*)-2,2-dimethyl-1,3-dioxolane-4,5-diyl]dimethanol and [(4*R*,5*R*)-2,2-dimethyl-1,3-dioxolane-4,5-diyl]dimethanol into the desired building blocks **1** and **2** were accomplished by routine operations of protecting groups.

[8] Y. L. Huerou, J. Doyon, R. L. Grée, *J. Org. Chem.* **1999**, *64*, 6782–6790.

[9] M. Yamaguchi, I. Hirao, *Tetrahedron Lett.* **1983**, *24*, 391–394.

[10] a) T.-S. Hu, Q. Yu, Y.-L. Wu, Y. K. Wu, *J. Org. Chem.* **2001**, *66*, 853–861; b) J. A. Marshall, M. Z. Chen, *J. Org. Chem.* **1997**, *62*, 5996–6000.

[11] a) M. Ito, M. Yamanaka, N. Kutsumura, S. Nishiyama, *Tetrahedron* **2004**, *60*, 5623–5634; b) K.-G. Liu, S. Yan, Y.-L. Wu, Z.-J. Yao, *Org. Lett.* **2004**, *6*, 2269–2272.

[12] I. Paterson, A. D. Findlay, G. J. Florence, *Org. Lett.* **2006**, *8*, 2131–2134.

[13] H.-X. Liu, G.-R. Huang, H.-M. Zhang, S. Jiang, J.-R. Wu, Z.-J. Yao, *ChemBioChem* **2007**, *8*, 172–177.

[14] S. Jiang, Z.-H. Liu, G. Sheng, B.-B. Zeng, X.-G. Cheng, Y.-L. Wu, Z.-J. Yao, *J. Org. Chem.* **2002**, *67*, 3404–3408.

[15] a) H. Miyoshi, M. Ohshima, H. Shimada, T. Akagi, H. Iwamura, J. L. McLaughlin, *Biochim. Biophys. Acta* **1998**, *1365*, 443–452; b) H. Makabe, A. Miyawaki, R. Takahashi, Y. Hattori, H. Konno, M. Abe, H. Miyoshi, *Tetrahedron Lett.* **2004**, *45*, 973–977; c) H. Makabe, Y. Hattori, Y. Kimura, H. Konno, M. Abe, H. Miyoshi, A. Tanaka, T. Oritani, *Tetrahedron* **2004**, *60*, 10651–10657; d) D. Fujita, N. Ichimaru, M. Abe, M. Murai, T. Hamada, T. Nishioka, H. Miyoshi, *Tetrahedron Lett.* **2005**, *46*, 5775–5779.

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