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Synthesis of OSW-1 analogs with modified side chains and their antitumor activities

Lehua Deng,[a] Hao Wu,[b] Biao Yu,[a,*] Manrong Jiang[b] and Jiarui Wu[b]

[a]*State Key Laboratory of Bio-organic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China*
[b]*Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China*

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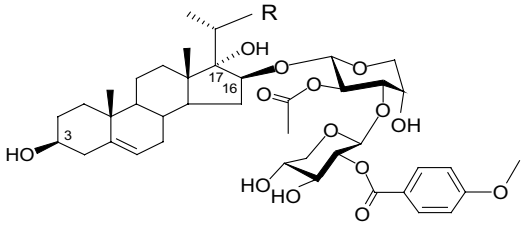
Abstract—Four analogs of OSW-1 (**1**–**4**) with modified side chains on the steroidal skeleton were synthesized following modification of our previous route for the total synthesis of OSW-1. Testing of the analogs against growth of tumor cells demonstrated that the 22-one function and the full length of the side chain of OSW-1 were not required for the antitumor action of OSW-1.

Since 1992, a small family of cholestane saponins featuring a novel 3β,16β,17α-trihydroxycholest-5-en-22-one aglycone with a sugar residue at the 16-OH has been disclosed, by the group of Sashida and co-workers, from the bulbs of *Ornithogalum saudersiae* and taxonomically related plants of the lily family.[1,2] Those saponins have attracted a great attention due to their potent antitumor activities.[2] As the major and representative member, OSW-1 was tested against the NCI (the US National Cancer Institute) 60 cell lines, showing 10–100 times more potency compared to those of the clinically applied anticancer agents, for example, cisplatin, as positive controls.[2a] Comparing the structure–activity relationship (SAR) of OSW-1 and its natural congeners, requirement of the 16-*O*-disaccharide moiety of OSW-1 for its significant cytotoxic activity was clearly revealed; those with modified sugar residue showed much less activities.[2] Especially, removal of the acetyl (Ac) and the 4-methoxybenzoyl (MBz) groups on the disaccharide moiety diminished the cytotoxicity by three order of magnitude.[2] Substitution with a glucose on the 3-OH, a site remote to the 16-*O*-disaccharide, did not affect apparently the cytotoxic activity.[2] However, synthetic glycosides bearing the acyl disaccharide but disparate steroid aglycones of OSW-1 did not show any cytotoxicity.[3] Inversion of the C-16 configuration, where the disaccharide is attached, was also not allowed to retain the significant cytotoxic activity of OSW-1.[4] Considering the similarity of the cytotoxicity profile and molecular structure of the OSW-1 aglycone with that of the cephalostatins, a related mechanism of action involving formation of C22-oxocarbenium ions was suggested.[5] To examine the importance of the side chain, especially of the 22-one function, we synthesized OSW-1 analogs **1**–**4** (Fig. 1) with modified side chains on the steroidal skeleton and tested their antitumor activities.

Three routes toward the total synthesis of OSW-1 have been developed, with the major differences being in the preparation of the aglycone.[6–9] Adopting modification of our own procedure,[6] we synthesized the desired



R = -C(=O)$CH_2CH_2CH(CH_3)_2$ (**OSW-1**)
-$CH_2CH_2CH_2CH(CH_3)_2$ (**1**)
-CH(OH)$CH_2CH_2CH(CH_3)_2$ (**2**)
-CH(=O)$CH_2CH_2CH_3$ (**3**)
-CH(=O)CH_3 (**4**)

Figure 1. OSW-1 and its analogs (**1**–**4**) with modified side chains.

Keywords: OSW-1; Analogs; Side chain; Synthesis; Antitumor.
* Corresponding author. E-mail: byu@mail.sioc.ac.cn

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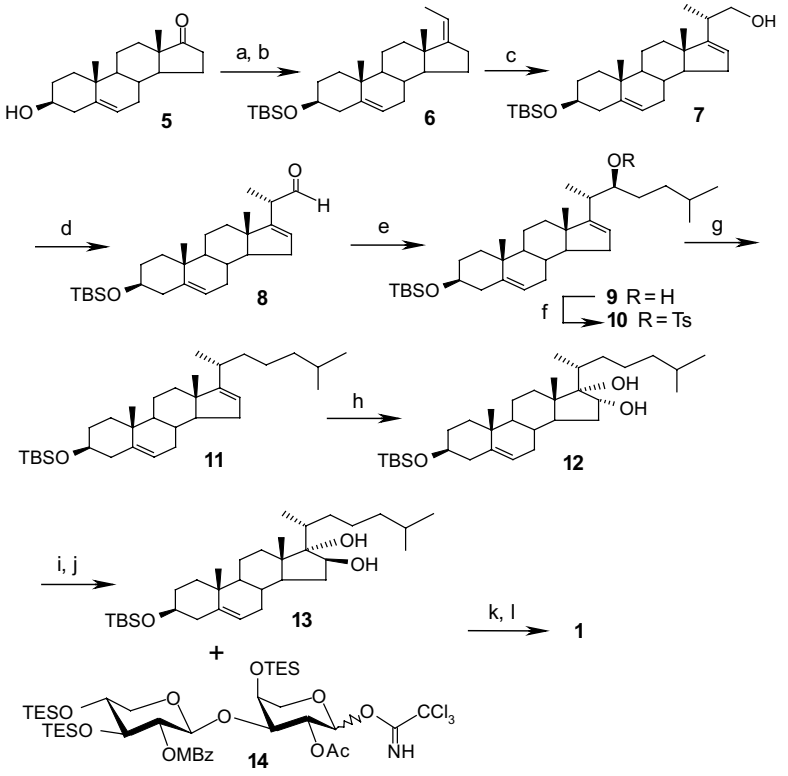
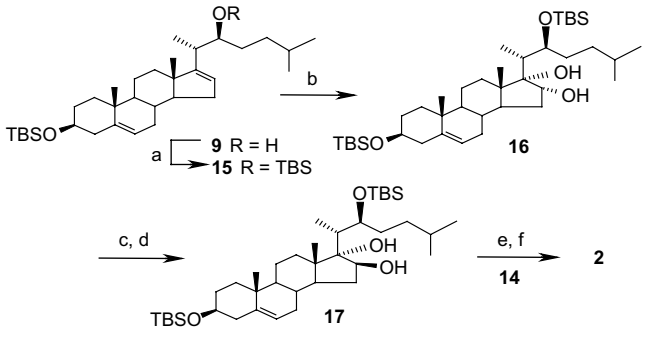


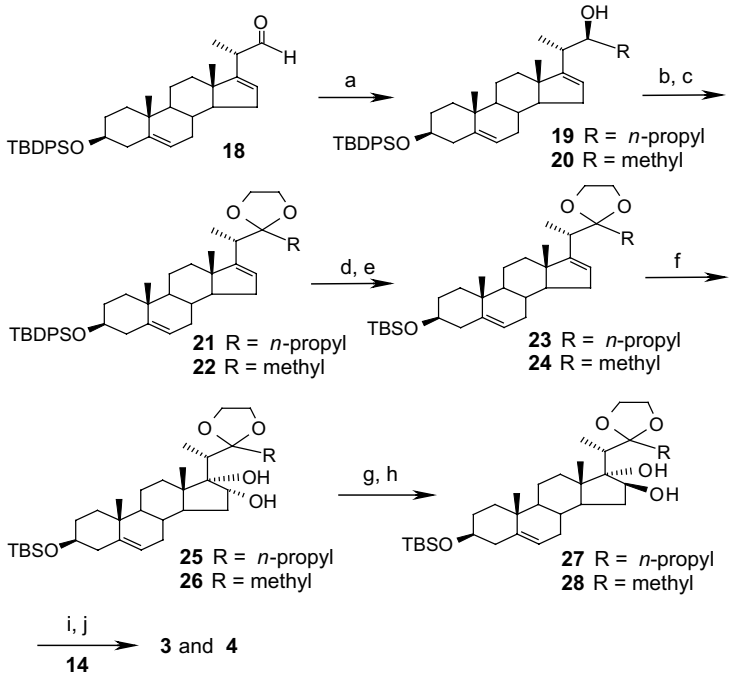
Scheme 1. Synthesis of the 22-deoxy-OSW-1 (**1**). Reagents and conditions: (a) $Ph_3P^+EtBr^-$, *t*-BuOK, THF, reflux, 88%; (b) TBSCl, imidazole, DMF, rt, 97%; (c) $(CH_2O)_n$, $BF_3{\cdot}Et_2O$, CH_2Cl_2, rt, 73%; (d) Dess–Martin periodinane, CH_2Cl_2, rt, 94%; (e) 3-methylbutyl magnesium bromide, Et_2O, rt, 77%; (f) TsCl, pyridine, 0 °C to rt; (g) $LiAlH_4$, THF, reflux, 45% (two steps); (h) OsO_4, pyridine–THF, −35 °C to rt; then H_2S (gas), 51%; (i) Swern oxidation, 79%; (j) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, THF, 0 °C, 52%; (k) **14**, TMSOTf (0.05 equiv), 4 Å MS, CH_2Cl_2, −40 °C, 58%; (l) $Pd(CN)_2Cl_2$, acetone–water (v/v, 20:1), rt, 51%.

OSW-1 analogs **1**–**4** (Schemes 1–3). Depicted in Scheme 1 is the preparation of compound **1**, an OSW-1 analog with the 22-one being reduced into CH_2. Starting from 5-androsten-3β-ol-17-one **5**, a similar sequence as that employed in the OSW-1 synthesis was followed to introduce the C-17 side to provide 22-ol **9**, that is, Wittig reaction, protection of the 3-OH with TBS ether, Prins reaction, oxidation of the resulting 22-OH into 22-aldehyde, followed by a Grignard addition. A difference from the previous synthesis is the use of TBS protection (instead of TBDPS protection) on the 3-OH, which tolerated the Prins reaction (**6** → **7**) under controlled conditions. Removal of the 22-OH (on **9**) was achieved via reduction of its tosylate **10** with $LiAlH_4$, albeit in a moderate yield (45% for two steps). Again, a similar sequence as that employed in the OSW-1 synthesis was used to convert diene **11** into 16β,17α-diol **13**, that is, selective dihydroxylation of the 16,17-ene with OsO_4 (1.0 equiv), Swern oxidation of the 16α-OH, and reduction of the resulting 16-one. Guo and Fuchs have found that the yields for the dihydroxylation of the 16,17-ene and the stereoselectivity for the reduction of the 16-one were highly dependent on the substitution on the C-22.[9] Fortunately, treatment of **11** with OsO_4 (1.0 equiv) provided the 16α,17α-diol **12** in a satisfactory 51% yield; and reduction of the 16-one with $NaBH_4$/$CeCl_3$ gave the desired 16β-ol **13** as a major product (52%), with the 16α isomer (**12**) being obtained in 18% yield. The final coupling and deprotection procedures were also borrowed from those in the OSW-1 synthesis. Thus, coupling of diol **13** with disaccharide trichloroacetimidate **14** provided the corresponding glycoside in 58% yield, which was subjected to deprotection of the silyl groups with $Pd(CN)_2Cl_2$,[10] affording the desired compound **1** in 51% yield.



Scheme 2. Synthesis of the 22-hydroxy-OSW-1 (**2**). Reagents and conditions: (a) TBSCl, imidazole, DMF, rt, 97%; (b) OsO_4, pyridine–THF, −35 °C to rt; then H_2S (gas), 78%; (c) TPAP, NMO, 4 Å MS, CH_2Cl_2, rt, 95%; (d) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, THF, 0 °C, 64%; (e) **14**, TMSOTf (0.05 equiv), 4 Å MS, CH_2Cl_2, −40 °C, 70%; (f) $Pd(CN)_2Cl_2$, acetone–water (v/v, 20:1), rt, 71%.

The desired 22-OH analog of OSW-1 (**2**) was synthesized from 22α-ol **9**, which was the only stereoisomer



Scheme 3. Synthesis of the OSW-1 analogs with shorter side chains (**3** and **4**). Reagents and conditions: (a) *n*-propyl (or methyl) magnesium bromide, Et_2O, 0 °C, 84% (for **19**); 83% (for **20**); (b) PDC, 4 Å MS, CH_2Cl_2–DMF, rt, 79% (for oxidation of **19**); 75% (for oxidation of **20**); (c) $HOCH_2CH_2OH$, $CH(OEt)_3$, *p*-TsOH·H_2O, rt, 86% (for **21**); 98% (for **22**); (d) TBAF, THF, rt; (e) TBSCl, imidazole, DMF, rt, 62% (for **23**, two steps); 90% (for **24**, two steps); (f) OsO_4, pyridine–THF, −40 °C to rt; then H_2S (gas), 34% (for **25**); 38% (for **26**); (g) TPAP, NMO, 4 Å MS, CH_2Cl_2, rt, 72% (for substrate **25**); 97% (for substrate **26**); (h) $NaBH_4$, $CeCl_3·7H_2O$, THF, 0 °C, 54% (for **27**); 53% (for **28**); (i) **14**, TMSOTf (0.05 equiv), 4 Å MS, CH_2Cl_2, −40 °C, 75% (for glycosylation of **27**); 74% (for glycosylation of **28**); (j) $Pd(CN)_2Cl_2$, acetone–water (v/v, 20:1), rt, 53% (for **3**); 51% (for **4**).

being isolated in the Grignard addition (**8** → **9**)[6] (Scheme 2). Protection of the 22-OH on **9** with TBS ether gave diene **15**. Then, a similar route for the preparation of **1** from **11** (Scheme 1) was adopted for conversion of **15** to **2**. Dihydroxylation of **15** with OsO_4 (1.0 equiv) gave 16α,17α-diol **16** in a better yield (78%) compared to that for **11** → **12**. Reduction of the corresponding 16-one with $NaBH_4/CeCl_3$ afforded the desired 16β-ol product **17** in 64% yield, with the 16α isomer (**16**) being isolated in 29% yield. A modification in the synthesis is the use of TPAP/NMO[11] for oxidation of the 16α-OH (of diol **16**), which was more convenient to perform than the previous Swern oxidation and gave excellent yield of the 16-one (95%).

OSW-1 analogs with shorter side chains on the steroidal skeleton (**3** and **4**) were prepared starting from aldehyde **18**, an intermediate in the OSW-1 synthesis[6] (Scheme 3). Grignard addition of **18** with *n*-propyl or methyl magnesium bromide provided 22-ol **19** or **20** in good yield. Then, similar transformations as those used in the OSW-1 synthesis were followed to furnish analogs **3** and **4**. The use of TPAP/NMO for oxidation of the 16α-OH (of diols **25** and **26**) avoided the cleavage of the 22-ethylene glycol ketal, which occurred in the previous Swern oxidation conditions in the OSW-1 synthesis. In the reduction of the resulting 16-ones with $NaBH_4/CeCl_3$, the desired 16β-ols (**27** and **28**) were obtained in 54% and 53% yields, respectively, with considerable amount of the 16α isomers (**25** and **26**) being recovered (29% and 18%, respectively).

The in vitro antitumor activities of the synthetic OSW-1 and its analogs **1**–**4** against AGS (stomach cancer cells), 7404 (liver carcinoma cells), and MCF-7 (breast cancer cells) were evaluated by the standard MTT assay[12] using cisplatin as a positive control. The results are listed in Table 1. The IC_{50} values of cisplatin against these three cell lines used in our assays are consistent with those determined by others.[13–15] OSW-1 showed 70–170 times

Table 1. Cytotoxic activities of OSW-1, its analogs **1**–**4**,[16] and cisplatin against tumor cells[a]

Tumor cells[b]	IC_{50} (μM)					
	1	**2**	**3**	**4**	OSW-1	Cisplatin
AGS	1.38	7.26	1.92	6.98	1.42	24.1
7404	0.063	1.86	0.032	2.90	0.10	8.37
MCF-7	0.060	1.79	0.020	6.61	0.27	18.7

[a] The standard MTT assay was followed.[12]

[b] AGS: human stomach cancer cell line; 7404: human liver carcinoma cell line; MCF-7: human breast cancer cell line.

higher potency than cisplatin. Surprisingly, analog **1**, with the 22-one (of OSW-1) being saturated into a CH_2, thus formation of the putative C22-oxocarbenium is impossible, was slightly more potent than OSW-1 against the tested three cancer cell lines. While the 22-OH analog **2** was less potent by 30-fold (for 7407 and MCF-7) than **1**. The full length of the cholestane side chain was also not essential to the antitumor activity; congener **3**, with the two terminal methyl groups (of OSW-1) being removed, was slightly more potent than OSW-1. However, the shorter congener **4**, with the terminal *iso*-butyl group (of OSW-1) being removed, was significantly less potent than OSW-1.

In summary, OSW-1 analogs (**1**–**4**) with modified side chains on the steroidal skeleton were synthesized following modification of our previous procedure for the total synthesis of OSW-1. Antitumor activity test of these compounds demonstrated that the side chain of OSW-1 tolerated certain modifications without affecting apparently the significant antitumor potency of OSW-1. Especially, the antitumor activity of OSW-1 was independent of the 22-one function, which was previously proposed to be crucial to the antitumor action of OSW-1 saponins (and the cephalostatins).[5]

Acknowledgements

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References and notes

1. Kubo, S.; Mimaki, Y.; Terao, M.; Sashida, Y.; Nikaido, T.; Ohmoto, T. *Phytochemistry* **1992**, *31*, 3969.
2. (a) Mimaki, Y.; Kuroda, M.; Kameyama, A.; Sashida, Y.; Hirano, T.; Oka, K.; Maekawa, R.; Wada, T.; Sugita, K.; Beutler, J. A. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 633; (b) Kuroda, M.; Mimaki, Y.; Yokosuka, A.; Sashida, Y.; Beutler, J. A. *J. Nat. Prod.* **2001**, *64*, 88; (c) Kuroda, M.; Mimaki, Y.; Yokosuka, A.; Sashida, Y. *Chem. Pharm. Bull.* **2001**, *49*, 1042; (d) Kuroda, M.; Mimaki, Y.; Yokosuka, A.; Hasegawa, F.; Sashida, Y. *J. Nat. Prod.* **2002**, *65*, 1417.
3. (a) Ma, X.; Yu, B.; Hui, Y.; Xiao, D.; Ding, J. *Carbohydr. Res.* **2000**, *329*, 495; (b) Ma, X.; Yu, B.; Hui, Y.; Miao, Z.; Ding, J. *Carbohydr. Res.* **2001**, *334*, 159.
4. Ma, X.; Yu, B.; Hui, Y.; Miao, Z.; Ding, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2153.
5. Guo, C.; LaCour, T. G.; Fuchs, P. L. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 419.
6. Deng, S.; Yu, B.; Lou, Y.; Hui, Y. *J. Org. Chem.* **1999**, *64*, 202.
7. (a) Yu, W.; Jin, Z. *J. Am. Chem. Soc.* **2001**, *123*, 3369; (b) Yu, W.; Jin, Z. *J. Am. Chem. Soc.* **2002**, *124*, 6576.
8. (a) Morzycki, J. W.; Gryszkiewicz, A.; Jastrzebska, I. *Tetrahedron Lett.* **2000**, *41*, 3751; (b) Morzycki, J. W.; Wojtkielewicz, A. *Carbohydr. Res.* **2002**, *337*, 1269.
9. For the first synthesis of the aglycone of OSW-1, see: Guo, C.; Fuchs, P. L. *Tetrahedron Lett.* **1998**, *39*, 1099.
10. Lipshutz, B. H.; Pollart, D.; Monforte, J.; Kotsuki, H. *Tetrahedron Lett.* **1985**, *26*, 705.
11. Paquette, L. A.; Wang, H.; Su, Z.; Zhao, M. *J. Am. Chem. Soc.* **1998**, *121*, 5213.
12. Kuroda, M.; Mimaki, Y.; Sashida, Y.; Hirano, T.; Oka, K.; Dobashi, A.; Li, H.; Harada, N. *Tetrahedron* **1997**, *53*, 11549.
13. Shen, D.; Akiyama, S.; Schoenlein, P.; Pastan, P.; Gottesman, M. *Br. J. Cancer* **1995**, *71*, 676.
14. Kim, Y.; Song, R.; Chung, H.; Jun, M.; Sohn, Y. *J. Inorg. Biochem.* **2004**, *78*, 78.
15. Kwon, Y.; Whang, K.; Park, Y.; Kim, K. *Bioorg. Med. Chem.* **2003**, *11*, 1669.
16. Analytical data for compounds **1**–**4**. Compound **1**: $[\alpha]_D^{25}$ −17.2 (*c* 0.40, CH_3OH); ^1H NMR (300 MHz, C_5D_5N): δ 8.28 (d, $J = 8.8$ Hz, 2H), 7.04 (d, $J = 8.8$ Hz, 2H), 6.54 (br d, $J = 5.3$ Hz, 1H), 6.21 (s, 1H), 6.15 (br s, 1H), 5.84 (t, $J = 6.8$ Hz, 1H), 5.67 (t-like, $J = 8.4$, 7.8 Hz, 1H), 5.34 (br d, $J = 3.9$ Hz, 1H), 5.08 (d, $J = 8.0$ Hz, 1H), 4.77 (s, 1H), 4.74 (d, $J = 7.1$ Hz, 1H), 4.44 (br s, 1H), 3.70 (s, 3H), 2.57 (br d, $J = 6.6$ Hz, 2H), 2.44 (m, 1H), 2.35 (q, $J = 6.9$ Hz, 1H), 1.93 (s, 3H), 1.20 (d, $J = 6.8$ Hz, 3H), 1.05 (s, 3H), 1.02 (s, 3H), 0.90 (d, $J = 6.5$ Hz, 2H), 0.89 (d, $J = 6.3$ Hz, 2H); ^{13}C NMR (75 MHz, C_5D_5N): δ 169.16, 165.51, 163.84, 141.97, 132.39, 121.22, 114.06, 103.51, 102.43, 87.95, 86.24, 80.70, 76.19, 75.11, 71.79, 71.32, 70.80, 68.49, 66.96, 66.19, 55.46, 50.48, 49.42, 47.11, 43.54, 40.49, 37.82, 36.91, 36.03, 35.67, 34.13, 33.25, 32.27, 28.26, 25.67, 23.07, 22.83, 21.02, 19.59, 14.33, 13.19; HRMS (MALDI) calcd for $C_{47}H_{70}O_{14}Na$ ($M+Na^+$): 881.46654; found: 881.46578. Compound **2**: $[\alpha]_D^{25}$ −13.6 (*c* 0.60, CH_3OH); ^1H NMR (300 MHz, C_5D_5N): δ 8.28 (dd, $J = 8.8$, 1.4 Hz, 2H), 7.03 (dd, $J = 9.0$, 1.4 Hz, 2H), 6.54 (br d, $J = 5.3$ Hz, 1H), 6.21 (s, 1H), 6.25–6.05 (br s, 1H), 5.79 (t, $J = 7.9$ Hz, 1H), 5.66 (t-like, $J = 7.6$, 8.8 Hz, 1H), 5.34 (br d, $J = 3.9$ Hz, 1H), 5.12 (d, $J = 7.7$ Hz, 1H), 4.75 (d, $J = 6.5$ Hz, 1H), 4.42 (br s, 1H), 3.70 (s, 3H), 2.58 (br d, $J = 7.6$ Hz, 2H), 2.45 (m, 1H), 2.35 (q, $J = 6.9$ Hz, 1H), 1.92 (s, 3H), 1.33 (d, $J = 7.0$ Hz, 3H), 1.05 (br s, 6H), 0.95 (d, $J = 6.6$ Hz, 3H), 0.89 (d, $J = 6.6$ Hz, 3H); ^{13}C NMR (75 MHz, C_5D_5N): δ 169.13, 165.46, 163.84, 141.97, 132.36, 121.19, 114.06, 103.24, 102.02, 88.77, 88.24, 80.50, 76.02, 74.99, 74.49, 71.80, 71.29, 70.70, 68.28, 66.81, 66.07, 55.44, 50.45, 48.92, 47.00, 43.51, 38.08, 37.78, 36.90, 36.18, 35.55, 34.07, 33.33, 32.60, 32.25, 28.64, 23.05, 22.86, 20.90, 19.59, 13.21, 8.50; HRMS (MALDI) calcd for $C_{47}H_{70}O_{15}Na$ ($M+Na^+$): 897.46125; found: 897.46070. Compound **3**: $[\alpha]_D^{25}$ −38.6 (*c* 0.25, CH_3OH); ^1H NMR (300 MHz, C_5D_5N): δ 8.31 (d, $J = 8.8$ Hz, 2H), 7.06 (d, $J = 8.8$ Hz, 2H), 5.66 (t, $J = 8.1$ Hz, 1H), 5.50 (t-like, $J = 7.1$, 6.6 Hz, 1H), 5.36 (d, $J = 4.1$ Hz, 1H), 5.09 (d, $J = 7.4$ Hz, 1H), 4.76 (s, 1H), 4.55 (d, $J = 5.8$ Hz, 1H), 4.35 (br s, 1H), 4.32–4.06 (m, 6H), 3.72 (s, 3H), 3.12 (q, $J = 7.2$ Hz, 1H), 2.59 (d, $J = 7.4$ Hz, 2H), 1.93 (s, 3H), 1.23 (d, $J = 7.1$ Hz, 3H), 1.05 (s, 3H), 0.97 (s, 3H), 0.81 (d, $J = 7.3$ Hz, 3H); ^{13}C NMR (75 MHz, C_5D_5N): δ 218.68, 169.28, 165.43, 163.91, 141.94, 132.43, 121.08, 114.16, 103.75, 100.78, 88.54, 85.74, 81.18, 76.40, 75.16, 72.05, 71.30, 70.73, 67.10, 65.22, 55.52, 50.19, 48.54, 46.52, 42.95, 37.79, 36.88, 34.65, 32.74, 32.25, 29.99, 20.82, 19.61, 17.04, 13.66, 11.73; HRMS (MALDI) calcd for $C_{45}H_{64}O_{15}Na$ ($M+Na^+$): 867.41448; found: 867.41375. Compound **4**: $[\alpha]_D^{25}$ −30.0 (*c* 0.30, CH_3OH); ^1H NMR (300 MHz, C_5D_5N): δ 8.29 (d, $J = 8.5$ Hz, 2H), 7.03 (d, $J = 8.5$ Hz, 2H), 5.67 (t, $J = 8.4$ Hz, 1H), 5.59 (t-like, $J = 8.2$, 6.9 Hz, 1H), 5.36 (d, $J = 4.4$ Hz, 1H), 5.11 (d, $J = 7.7$ Hz, 1H), 4.64 (s, 1H), 4.59 (d, $J = 6.6$ Hz, 1H), 4.42 (br s, 1H), 4.35–4.10

(m, 6H), 3.69 (s, 3H), 3.14 (q, $J = 7.4$ Hz, 1H), 2.58 (d, $J = 7.4$ Hz, 2H), 2.07 (s, 3H), 1.92 (s, 3H), 1.18 (d, $J = 7.1$ Hz, 3H), 1.04 (s, 3H), 0.92 (s, 3H); ^{13}C NMR (75 MHz, C_5D_5N): δ 216.82, 169.34, 165.51, 163.87, 141.95, 132.42, 121.04, 114.10, 103.61, 101.02, 89.12, 85.55, 81.27, 76.36, 75.19, 71.88, 71.30, 70.80, 68.19, 67.04, 65.93, 55.49, 50.13, 48.51, 46.47, 43.50, 37.76, 36.87, 33.94, 32.63, 32.25, 29.96, 28.88, 20.92, 19.58, 13.66, 11.67; HRMS (MALDI) calcd for $C_{43}H_{60}O_{15}Na$ ($M+Na^+$): 839.38300; found: 839.38245.