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Design, synthesis and biological evaluation of novel, simplified analogues of laulimalide: modification of the side chain

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Abstract—Novel, simplified analogues of the microtubule-stabilizing anticancer agent laulimalide, including the first derivatives with unnatural side chains, were designed by molecular modelling, synthesized by a late-stage diversification strategy, and evaluated in vitro for growth inhibition of human ovarian carcinoma cell lines (A2780, A2780/AD10).
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Pacific marine sponges (*Fasciospongia rimosa*, *Hyatella* sp. and *Spongia mycofijiensis*) are the natural source of laulimalide (**1**, Fig. 1), a 20-membered macrolide of polyketide origin.[1,2] It is a highly potent antimitotic agent, which inhibits proliferation of a range of human cancer cell lines, including multidrug resistant cells, at nanomolar concentrations.[3] Like Taxol® (paclitaxel), it acts by microtubule stabilization and disruption of mitotic spindle formation.[3a] However, it appears to have a different, as yet undefined, binding site on tubulin,[4,5] is superior in its ability to circumvent P-glycoprotein-mediated drug resistance, and retains activity against paclitaxel resistant cells.[3a] This promising biological profile renders laulimalide a highly attractive lead for the development of new anticancer agents, however, this is severely hampered by its low natural abundance. Despite considerable synthetic efforts, which have culminated in a multitude of total syntheses,[2,6,7] this supply problem still remains, such that the development of simplified and more readily available analogues retaining biological activity is an important goal.

So far, only a limited range of analogues, relying on modifications at C2–C3, C8, C15–C17 and C20 have been reported, all of which are however significantly less active.[7] Recently, we have developed a novel synthetic approach towards laulimalide using a versatile

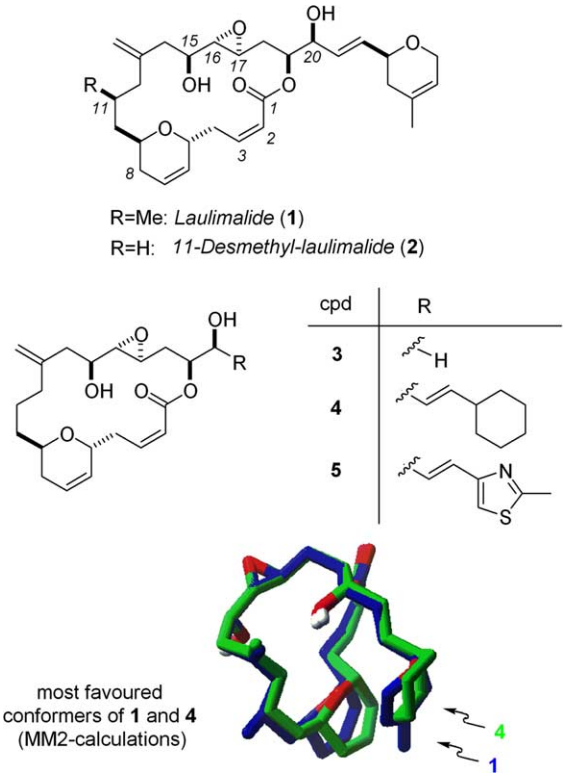

Figure 1. Laulimalide and simplified analogues thereof.

Keywords: Laulimalide; Analogues; Anticancer agent; Nozaki–Kishi coupling; Modelling.
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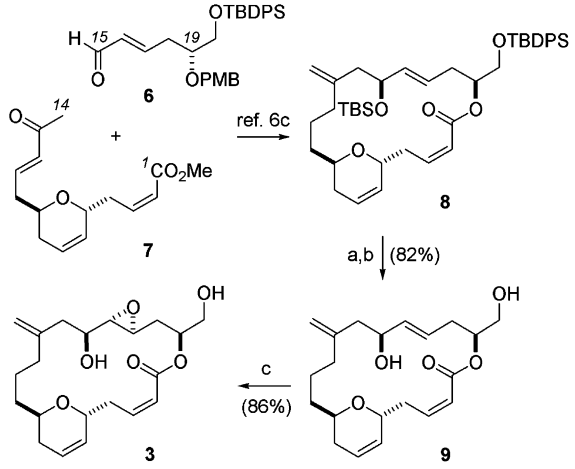
Nozaki–Kishi coupling[8] for late-stage introduction of the dihydropyran side chain and successfully applied this procedure to access 11-desmethyl-laulimalide (**2**) and related structures.[7d] Herein, we describe an improved protocol for the preparation of 11-desmethyl-laulimalide, report on the design and synthesis of the first laulimalide analogues with unnatural (or truncated) side chains (**3**–**5**), and disclose the cancer cell growth inhibitory activity of all of these compounds relative to other microtubule-stabilizing agents (laulimalide, paclitaxel and discodermolide).

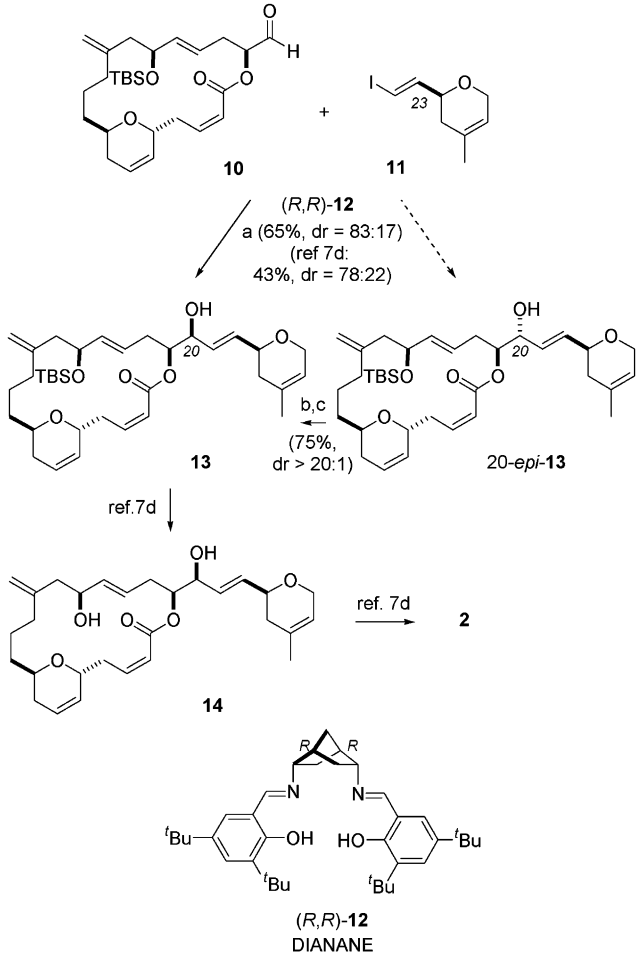
Our starting point for rational analogue design was conformational analysis (MM2, MacroModel 8.0) of laulimalide and related simplified structures, which was carried out both in vacuo and in solution (chloroform, water).[9] These calculations revealed that deletion of the 11-methyl group only has a minor impact on the respective 3D structures. Likewise, changes in the side chain, for example, by replacing the dihydropyran with a cyclohexyl group (Fig. 1), or its complete removal, only had a minor influence on the macrocyclic conformation, as expected. These findings prompted us to evaluate the general importance of the side chain and the 11-methyl group on biological activity, together with preparing and evaluating side chain modified analogues **3**–**5**.[10]

As a first target, we decided to delete the side chain altogether and access macrocyclic epoxide **3**. Its preparation started from known lactone **8** (Scheme 1), which was synthesized in a convergent and scalable manner using our previously established route from D-malic acid derived C15–C19 building block **6** and the C1–C14 subunit **7**.[6c] Stepwise deprotection of the primary and the secondary hydroxyls of **8** using TBAF and H_2SiF_6[11] revealed the secondary allylic alcohol **9** in a straightforward sequence. A highly selective Sharpless epoxidation[6c,e] completed the synthesis of the desired epoxide **3**.[12]

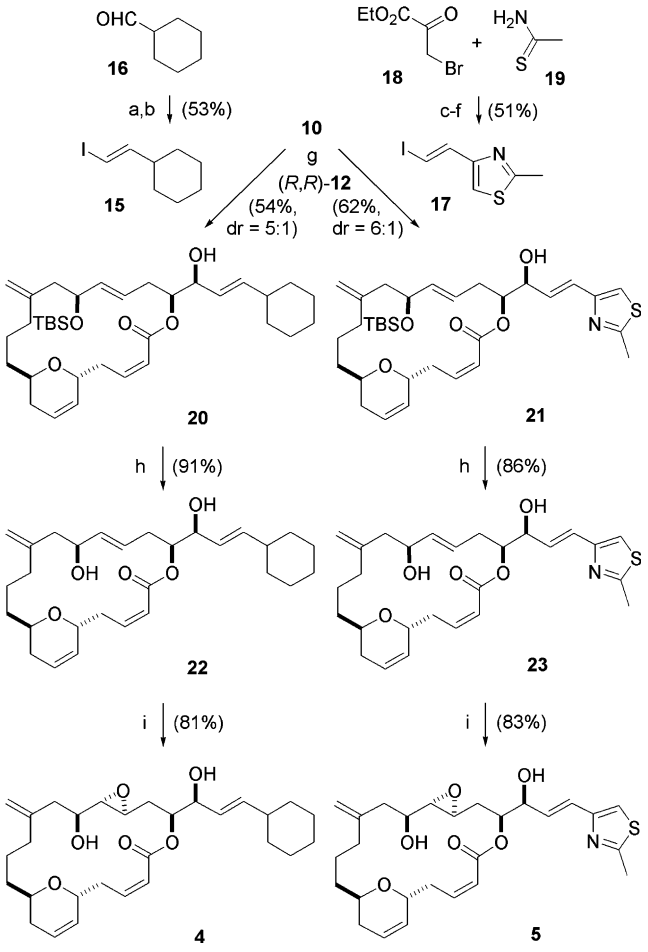


Scheme 1. Synthesis of macrocyclic epoxide **3**. Reagents and conditions: (a) TBAF/AcOH (pH 7), THF, 0 °C to rt; (b) H_2SiF_6, CH_3CN, rt; (c) (+)-DIPT, $Ti(OiPr)_4$, *t*-BuOOH, CH_2Cl_2, −20 °C.

The key step in our recent synthesis of 11-desmethyl-laulimalide (**2**, Scheme 2) was a catalytic asymmetric Nozaki–Kishi coupling of macrocyclic aldehyde **10** with vinyl iodide **11** in the presence of DIANANE-type ligand (*R*,*R*)-**12**.[7d,8,13] This overturned the unfavourable substrate-based selectivity in this transformation and gave the desired allylic alcohol **13** as the major diastereomer. As an improvement to this protocol, we found that increasing the nickel content (from 2 to 10 mol % $NiCl_2$) significantly speeds up this reaction.[14] Together with a modified procedure for cleaving the intermediate TMS ether by use of HF/pyridine, instead of the previously used two-step procedure,[7d,8] led to a 50% enhancement of the chemical yield (65% vs 43%) together with slightly improving the diastereomeric ratio (83:17 vs 78:22). The minor C20 epimer is not lost as any unfavourable diastereomeric mixture can be used by employing an oxidation–reduction sequence, as shown for 20-*epi*-**13**. Here, it was found that K-Selectride not only leads to higher chemoselectivity but also better diastereoselectivity, as compared to previously used L-Selectride and allows access to diastereomerically pure **13** (dr > 20:1 vs 9:1).[15] Completion of the synthesis of 11-desmethyl-laulimalide



Scheme 2. Synthesis of 11-desmethyl-laulimalide (**2**) by asymmetric Nozaki–Kishi coupling. Reagents and conditions: (a) (i) (*R*,*R*)-**12** (10 mol%), $CrCl_2$ (10 mol%), $NiCl_2$ (10 mol%), Et_3N (20 mol%), Mn, TMSCl, THF, rt; (ii) HF/pyridine, THF, 0 °C; (b) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 to −10 °C; (c) K-Selectride, THF, −78 °C.



Scheme 3. Synthesis of the side chain analogues **4** and **5** via common late-stage intermediate **10**. Reagents and conditions: (a) $(MeO)_2POC(N_2)C(O)Me$, K_2CO_3, MeOH, rt; (b) (i) DIBALH, hexane, −40 °C to 50 °C; (ii) I_2, hexane/THF, −40 °C to rt; (c) (i) $KHCO_3$, DME, rt; (ii) $(CF_3CO)_2O$, pyridine, DME, 0 °C to rt; (d) DIBALH, CH_2Cl_2, −78 °C; (e) $(MeO)_2POC(N_2)C(O)Me$, K_2CO_3, MeOH, rt; (f) (i) Cp_2ZrHCl, CH_2Cl_2, rt; (ii) I_2, CH_2Cl_2, rt; (g) (i) (*R*,*R*)-**12** (10 mol%), $CrCl_2$ (10 mol%), $NiCl_2$ (10 mol%), Et_3N (20 mol%), Mn, TMSCl, THF, rt; (ii) HF/pyridine, THF, 0 °C; (h) HF/pyridine, THF; 0 °C to rt; (i) (+)-DIPT, $Ti(OiPr)_4$, *t*-BuOOH, CH_2Cl_2, −20 °C.

via **14** by regio- and diastereoselective Sharpless epoxidation proceeded under our previously reported conditions.[7d,16]

With these improved protocols in hand, we then proceeded to synthesize the target laulimalide derivatives with simplified, unnatural side chains using aldehyde **10** as a common intermediate (Scheme 3). The cyclohexyl analogue **4** was chosen as a sterically similar, but electronically different mimic of the authentic dihydropyran ring, while the thiazole analogue **5** was selected for generation of novel structural hybrids with the epothilones.[17] Vinyl iodide **15** was readily available from aldehyde **16** by homologation with the Ohira–Bestmann reagent,[18] hydroalumination of the derived alkyne and trapping of the intermediate organometallic species with iodine.[19] Generation of **17** likewise involved use of the Ohira–Bestmann reagent on a thiazole aldehyde obtained by cyclocondensation[20] and subsequent reduction[21] from bromoketone **18** and thioacetamide **19**. This time, hydroiodination was effected by hydrozirconation and subsequent treatment with iodine.[22] Catalytic, asymmetric Nozaki–Kishi coupling of macrocyclic aldehyde **10** with both **15** and **17** proceeded smoothly and with preparatively useful yields, in the presence of ligand (*R*,*R*)-**12**, to give **20** and **21**, respectively.[23] Notably, the diastereoselectivities were improved as compared to the coupling with vinyl iodide **11**, which might be attributed to an influence from the additional C23 stereocentre or, in the case of **17**, the possibility for further coordination of the thiazole ring to the catalytically active intermediate chromium(II)-species. Subsequent TBS deprotection gave **22** and **23**, which was followed again by Sharpless epoxidation to produce **4** and **5**, respectively, in a highly selective manner.[12]

For biological evaluation of the foregoing analogues, we first checked if they could promote the polymerization of tubulin for microtubule assembly, that is, modify the critical concentration (Table 1).[24] For this purpose, pure GTP-tubulin was incubated, in a glycerol

Table 1. Effect on in vitro tubulin assembly and inhibitory effects of ligands on growth of human ovarian carcinoma cells

Compds	Critical concentration GAB[a,b,c] (μM)	Cytotoxicity A2780 IC_{50}[d] (nM)	Cytotoxicity A2780/AD10 IC_{50}[d] (nM)
Laulimalide (**1**)	0.72 ± 0.1	3.4 ± 1.0	7.5 ± 0.7
2	1.1 ± 0.1	50 ± 13	74 ± 14
3	3.0 ± 1.2	40,000 ± 1000	44,000 ± 1500
4	1.4 ± 0.2	9000 ± 310	8300 ± 2300
5	3.6 ± 0.4	na	na
9	3.4 ± 0.3	na	na
14	1.8 ± 0.3	430 ± 70	1100 ± 230
22	3.8 ± 0.6	na	na
23	3.4 ± 0.4	na	na
DMSO	3.3 ± 0.1	na	na
Paclitaxel	0.46 ± 0.24	1.1 ± 0.3	1500 ± 60
Discodermolide	0.51 ± 0.09	10 ± 0.1	100 ± 19

[a] GAB: 3.4 M glycerol, 10 mM phosphate, 1 mM ethylene glycol-bis(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 1 mM GTP, 6 mM $MgCl_2$ and pH 6.7 buffer.

[b] Average of four measurements (with standard errors).

[c] All the data measured at 37 °C.

[d] IC_{50} were determined after three days exposure to drugs using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide cell proliferation assay[24] and values (± standard error) are means of at least four independent experiments. Na = not active below 50 μM.

containing buffer (GAB-1mM GTP) at 37 °C, in the presence of 10% excess of each of the ligands. Under these conditions, tubulin can assemble in the absence of exogenous ligands with a critical concentration of ~3.3 μM. Out of the analogues examined, only 11-desmethyl-laulimalide (**2**), its desepoxy-precursor **14** and the cyclohexyl analogue **4**, with laulimalide (**1**) as a control, significantly increased tubulin assembly, having critical concentrations of 1.1 ± 0.1 μM, 1.8 ± 0.3 μM, 1.4 ± 0.2 μM and 0.72 ± 0.1 μM, respectively, (analogue **3** only modified the critical concentration weakly, 3.0 ± 1.2 μM). As in the previous studies, paclitaxel (critical concentration = 0.46 ± 0.2 μM) modified assembly more powerfully than laulimalide and its analogues.[3a] All the polymers observed were microtubules.

Table 1 also summarizes the inhibitory effect of laulimalide and its analogues, as compared with paclitaxel and discodermolide, on the growth of the ovarian carcinoma cell line A2780 and the multidrug-resistant (MDR, P-glycoprotein overexpressing) cell line A2780/AD10.[25] The parental A2780 cells were 3-fold more sensitive to paclitaxel than to laulimalide. However, the MDR cell line exhibited a strong resistance to paclitaxel (1360-fold), while laulimalide had only a small reduction in potency of 2.2-fold. Among the laulimalide analogues tested, the most potent is its desmethyl-derivative **2**, followed by its desepoxy precursor **14**, the cyclohexyl analogue **4** and the side chain truncated analogue **3**. Significantly, analogue **2** was only 1.5-fold less potent in the MDR cell as compared to the parental line. Moreover, 11-desmethyl-laulimalide (**2**) is of similar potency to discodermolide[26] and represents one of the most active laulimalide analogues tested so far.[7] The observation that removal of the epoxide leads to loss in activity is in agreement with previous data.[3a,7b] The other compounds were essentially inactive having an IC_{50} above 50 μM.

In conclusion, we have developed a series of analogues of laulimalide with simplifications in the macrocycle (deletion of the 11-methyl group) and also the side chain (truncation or substitution). The synthesis of these compounds was enabled by a late-stage diversification strategy relying on asymmetric Nozaki–Kishi methodology. The most potent analogue with low nanomolar IC_{50} values was 11-desmethyl-laulimalide. Truncation or substitution of the side chain, however, leads to significant loss of activity, which suggests that this must be part of the pharmacophore region. These results, combined with other analogue studies,[7] suggest that it may be possible to simplify the laulimalide structure, yet still retain potency.

Acknowledgements

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3: $[\alpha]_D^{20}$ −96 (*c* 0.047, CH_2Cl_2); ^{1}H NMR δ (700 MHz, CD_3OD): 6.42 (ddd, *J* = 11.5, 10.4, 3.4 Hz, 1H), 5.94 (ddd, *J* = 11.5, 2.6, 1.1 Hz, 1H), 5.86 (ddt, *J* = 10.3, 5.6, 2.2 Hz, 1H), 5.76 (ddt, 10.4, 2.9, 1.2 Hz, 1H), 5.15–5.12 (m, 1H), 4.83 (br s, 1H), 4.80 (d, *J* = 1.5 Hz, 1H), 4.34 (dt, *J* = 11.3, 2.4 Hz, 1H), 4.04 (ddd, *J* = 8.5, 3.9, 2.1 Hz, 1H), 3.82 (dddd, 17.0, 10.3, 10.2, 1.0 Hz, 1H), 3.69–3.65 (m, 1H), 3.59 (dd, *J* = 11.8, 4.5 Hz, 1H), 3.56 (dd, *J* = 11.8, 5.3 Hz, 1H), 3.00 (ddd, *J* = 7.3, 4.6, 2.2 Hz, 1H), 2.89 (t, *J* = 2.2 Hz, 1H), 2.22 (dq, *J* = 17.0, 3.1 Hz, 1H), 2.15 (dd, *J* = 15.6, 3.8 Hz, 1H), 2.12 (ddd, *J* = 14.4, 4.7, 2.2 Hz, 1H), 2.07 (dt, *J* = 14.3, 7.5 Hz, 1H), 2.01 (dt, *J* = 14.9, 7.5 Hz, 1H), 1.98–1.86 (m, 3H), 1.57 (ddd, *J* = 14.4, 11.1, 7.9 Hz, 1H), 1.49–1.42 (m, 3H), 1.42–1.36 (m, 1H); ^{13}C NMR δ (126 MHz, CD_3OD): 167.3, 150.8, 147.7, 130.1, 126.1, 121.7, 111.5, 74.5, 72.4, 68.5, 67.8, 64.7, 62.5, 53.1, 39.1, 38.6, 36.6, 34.7, 34.2, 32.4, 25.8; HRMS-EI *m*/*z*: measured 401.1954 ($[M+Na]^+$, calcd 401.1940 for $C_{21}H_{30}O_6Na$); MS-EI *m*/*z* (relative intensity): 401 ($[M+Na]^+$, 100), 219 (47); FTIR ν_{max}: 3351, 2925, 1716, 1647, 1420, 1081, 814 cm^{-1}. **4**: $[\alpha]_D^{24}$ −182 (*c* 0.050, CH_2Cl_2); ^{1}H NMR δ (500 MHz, CD_3OD): 6.39 (ddd, *J* = 11.4, 10.4, 3.5 Hz, 1H), 5.94 (dd, *J* = 11.5, 1.4 Hz, 1H), 5.90–5.80 (m, 1H), 5.72 (*J* = ddd, 10.1, 2.4, 1.1 Hz, 1H), 5.62 (dd, *J* = 15.6, 6.9 Hz, 1H), 5.34 (ddd, *J* = 15.6, 6.6, 1.1 Hz, 1H), 5.10 (ddd, *J* = 11.9, 11.4, 4.7 Hz, 1H), 4.79 (s, 1H), 4.72 (s, 1H), 4.58 (br s, 1H), 4.31 (d, *J* = 11.4 Hz, 1H), 4.07 (dd, *J* = 5.8, 5.4 Hz, 1H), 3.91 (ddd, *J* = 8.7, 3.9, 2.1 Hz, 1H), 3.81 (ddd, *J* = 16.4, 10.1, 10.1 Hz, 1H), 3.70–3.60 (m, 1H), 2.95 (ddd, *J* = 7.3, 4.4, 2.2 Hz, 1H), 2.81 (dd, *J* = 2.2, 2.0 Hz, 1H), 2.20–1.85 (m, 9H), 1.75–1.60 (m, 5H), 1.52 (ddd, *J* = 14.3, 11.4, 7.9 Hz, 1H), 1.45–1.0 (m, 10H); ^{13}C NMR δ (100 MHz, CD_3OD): 167.2, 150.7, 147.8, 140.3, 130.1, 127.2, 126.1, 121.7, 111.5, 74.6, 74.5, 73.9, 68.5, 67.9, 62.7, 53.4, 41.8, 39.0, 38.4, 36.6,34.3, 34.3, 34.0, 33.9, 32.4, 27,3, 27.0, 25.7. HRMS-ES *m*/*z*: measured 487.3036 (MH^+, calcd 487.3060 for $C_{29}H_{43}O_6$). MS-ES *m*/*z* (relative intensity): 509 (100), 487 (46). FTIR ν_{max}: 3426, 2922, 1719, 1168, 810 cm^{-1}. **5**: $[\alpha]_D^{24}$ −198 (*c* 0.033, CH_2Cl_2); ^{1}H NMR δ (500 MHz, CD_3OD): 7.18 (s, 1H), 6.67 (dd, *J* = 15.7, 1.3 Hz, 1H), 6.50 (dd, *J* = 15.7, 5.8 Hz), 6.44 (ddd, *J* = 11.5, 10.4, 3.5 Hz, 1H), 5.99 (ddd, *J* = 11.5, 2.5, 1.3 Hz, 1H), 5.89 (dddd, *J* = 10.3, 4.3, 1.9, 1.9 Hz, 1H), 5.78 (dddd, *J* = 10.3, 2.8, 2.8, 1.1 Hz, 1H), 5.26 (ddd, *J* = 11.4, 4.7, 1.9 Hz, 1H), 4.61 (br s, 1H), 4.87 (s, 1H), 4.79 (s, 1H), 4.36 (m, 2H), 4.07 (ddd, *J* = 8.5, 3.9, 2.2 Hz, 1H), 3.82 (dddd, *J* = 16.9, 11.2, 11.2, 1.1 Hz, 1H), 3.73 (dddd, *J* = 9.0, 9.0, 3.2, 3.1 Hz, 1H), 3.04 (ddd, *J* = 7.9, 4.4, 2.3 Hz, 1H), 2.92 (dd, *J* = 2.2, 2.2 Hz, 1H), 2.65 (s, 3H), 2.26 (ddd, *J* = 14.2, 4.4, 1.73 Hz, 1H), 2.18–1.82 (m, 7H), 1.64 (ddd, *J* = 14.2, 11.4, 1.9 Hz, 1H), 1.55–1.20 (m, 5H); ^{13}C NMR δ (100 MHz, CD_3OD): 168.5, 167.2, 154.2, 150.5, 147.7, 131.6, 130.1, 126.1, 125.4, 121.6, 116.6, 111.4, 74.5, 73.7, 73.6, 68.5, 67.8, 62.5, 53.3, 39.1, 38.5, 36.5, 34.2, 33.8, 32.4, 25.7, 18.7; HRMS-ES *m*/*z*: measured 502.2268 (MH^+, calcd 502.2263 for $C_{27}H_{36}NO_6S$); MS-ES *m*/*z* (relative intensity): 502 (MH^+, 100), 343 (52); FTIR ν_{max}: 3413, 2922, 1717, 1178, 1079, 810 cm^{-1}.
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