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Synthesis of novel discodermolide analogues with modified hydrogen-bonding donor/acceptor sites

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Abstract—A series of novel structural analogues of the potent microtubule-stabilizing anticancer agent discodermolide were synthesised, with modifications in the C16–C20 region to create new oxygenated H-bonding donor/acceptor sites for tubulin binding. By starting from an advanced C9–C24 intermediate, fully synthetic discodermolide analogues, incorporating either an additional hydroxyl group **3**, an oxetane **4** or a cyclic carbonate **5**, were obtained in 10 or 11 steps by using a versatile aldol construction of the C6–C7 bond.

As a structurally unique antimitotic agent, the marine-derived polyketide discodermolide (**1**, Fig. 1) is a promising candidate for clinical development in cancer chemotherapy.[1–4] Sharing a similar microtubule-stabilizing mechanism of action to Taxol/paclitaxel (**2**),[3a–d] discodermolide inhibits the proliferation of numerous cancer cell lines, including those that are Taxol-resistant, and shows synergy with Taxol.[3e] Moreover, in hollow fibre and xenograft mouse models, discodermolide induces significant growth inhibition of human tumours in vivo.[4] Its potential as a new chemotherapeutic agent for the treatment of solid tumours, including drug-resistant breast and ovarian cancer, has recently led to discodermolide entering Phase I clinical trials. Due to the low isolation yield from the deep-sea sponge source,[2] total synthesis presently offers the only viable means of drug supply. This situation has stimulated considerable interest in developing a practical synthetic route for producing discodermolide,[1] thus also enabling access to novel analogues for biological evaluation. Recently, studies focussed on exploring structure–activity relationships for discodermolide, with the aim of defining a pharmacophore model, have been reported by several groups.[4,5]

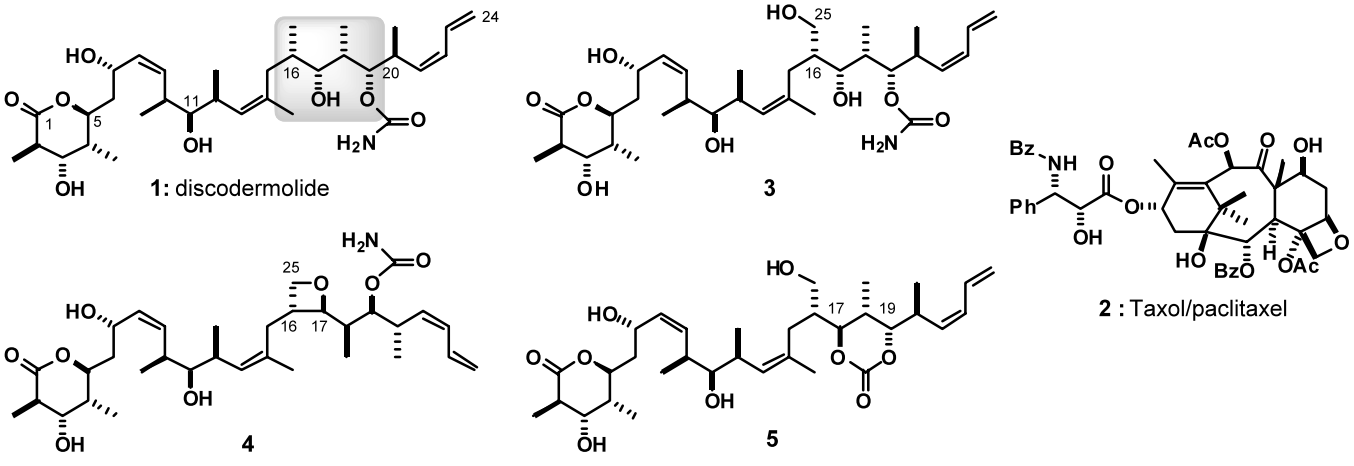

Figure 1.

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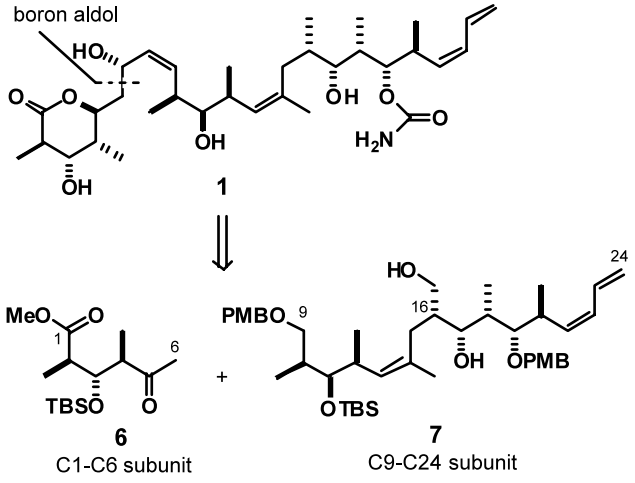
In parallel with our efforts to develop a highly practical synthesis of discodermolide,[1a,6] we have previously prepared a range of epimeric and truncated analogues.[5a] As an extension to this work, we now report the total synthesis of the more elaborate discodermolide analogues **3**, **4** and **5**, where these feature novel oxygenated hydrogen-bonding donor/acceptor sites within the C16–C20 region (as indicated by the shading in **1**), potentially enhancing interactions with the binding site in β-tubulin. Notably, the cyclic carbonate **5** arises from an unexpected acid-mediated cyclisation of the oxetane analogue **4**.

In the SAR studies on discodermolide reported originally by Schreiber and co-workers,[5b] the natural (*S*)-configuration at the C16 methyl-bearing stereocentre proved to be essential for retaining biological activity. To extend these studies further into the C16–C20 region, we envisaged adapting our total synthesis of (+)-discodermolide (Scheme 1) that employs the methyl ketone **6** and 1,3-diol **7** as C1–C6 and C9–C24 subunits, respectively.[6a,b] In this work, the required methyl substitution at C16 was secured by performing a controlled deoxygenation on **7**. This approach presents a convenient opportunity for analogue chemistry if the oxygenation associated with the C16 position is retained, enabling the modification of the hydrogen bonding donor/acceptor sites in this region of the linear polyketide backbone.



Scheme 1.

To this end, the preparation of the hydroxymethyl analogue **3** (with one additional H-bond donor/acceptor site) and the oxetane-containing analogue **4** (with one H-bond donor site removed) was initially undertaken. In the latter case, the introduction of the Lewis and Brønsted basic oxetane also introduces a conformational lock, preventing rotation about the C16–C17 bond, as well as conferring some superficial resemblance to the D-ring oxetane in paclitaxel (**2**). Otherwise, these localised structural changes were not expected to lead to any severe perturbation of the conformational preferences over the rest of the molecule. In this regard, modelling studies (Macromodel 7.2)[7] indicated that both the pentaol **3** and oxetane **4** largely mimicked the conformational preferences of discodermolide (**1**),[8] favouring a similar low energy U-shaped arrangement (Fig. 2).

The strategy adopted for the preparation of discodermolide analogues **3** and **4** relied on a late-stage aldol coupling between the methyl ketone **6** and the appropriate (*Z*)-enal containing the modified C7–C24 region. As shown in Scheme 2, the synthesis of the first analogue **3**, having a hydroxymethyl substituent at C16, started out from the 1,3-diol **7**.[6] *Bis*-silylation of **7** with TBSOTf/2,6-lutidine, followed by oxidative cleavage of both PMB ethers with DDQ, gave the diol **8** (90%). Selective oxidation of the primary alcohol using TEMPO/PhI(OAc)$_2$[9] and a Still–Gennari HWE olefination[10] of the resulting aldehyde generated the desired (*Z*)-enoate **9** exclusively in 84% yield. Following installation of the carbamate moiety at the C19 hydroxyl in **9** under standard conditions ($Cl_3CC(O)NCO$; K_2CO_3, MeOH),[11] DIBAL-H reduction of the methyl ester to provide the corresponding alcohol and subsequent Dess–Martin oxidation afforded (*Z*)-enal **10** (96%).

Enolisation of methyl ketone **6** with (+)-Ipc$_2$BCl/Et$_3$N in Et$_2$O,[6a,b,12] followed by addition to aldehyde **10**, proceeded in 65% yield with 8:1 *dr* in favour of the desired (7*S*)-configured adduct **11**. In this complex aldol coupling, the chiral boron reagent is needed to overturn the strong inherent facial bias of the aldehyde

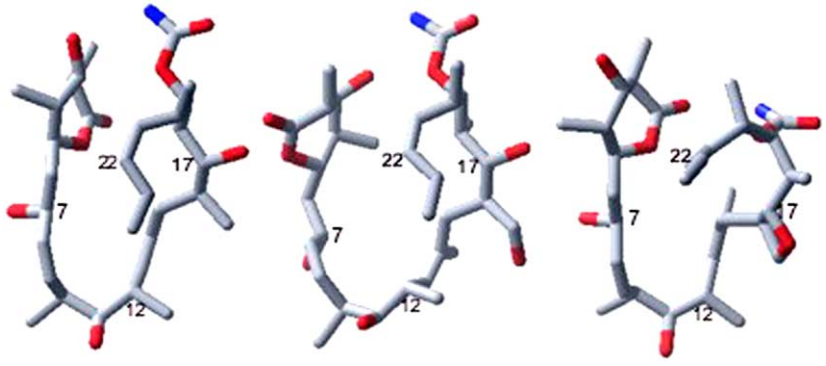
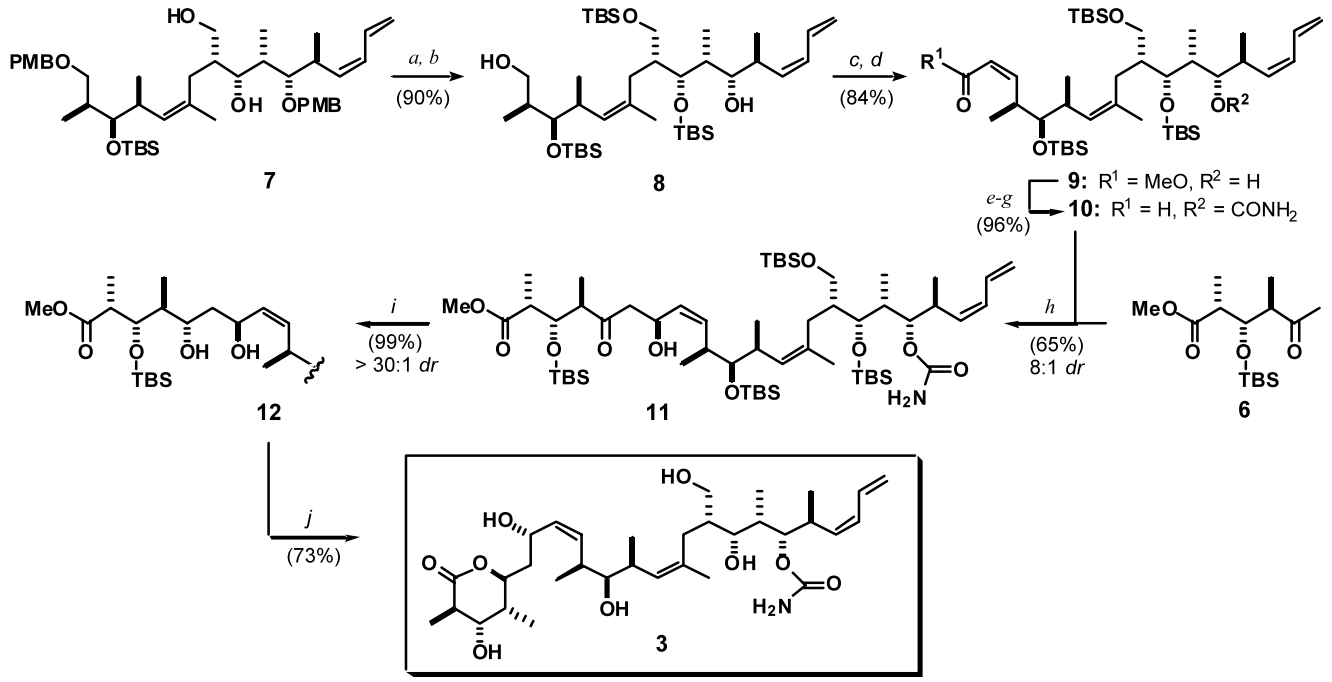


Figure 2. Energy minimised conformations of **1**, **3** and **4**.[7,8]



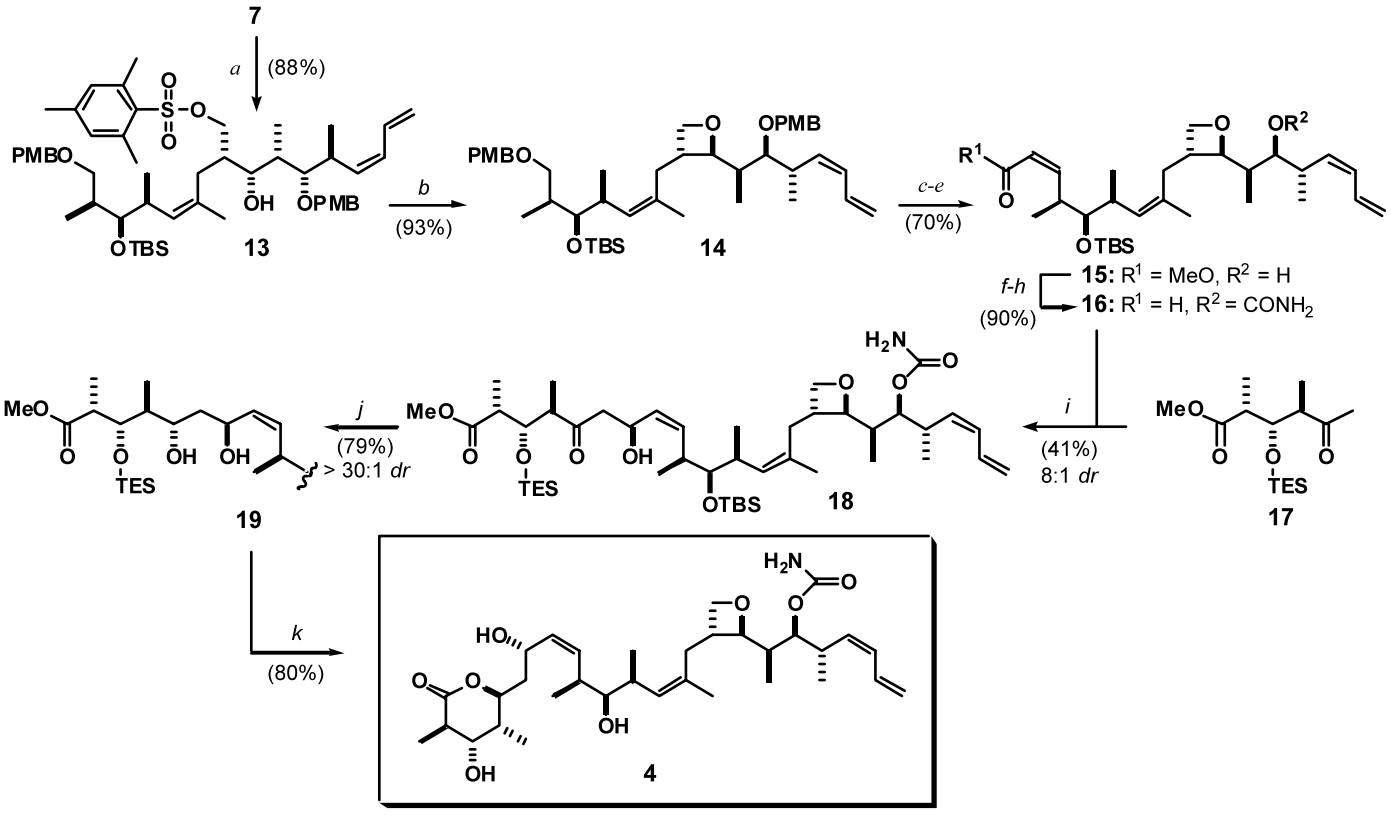
Scheme 2. *Reagents and conditions*: (a) TBSOTf, 2,6-lutidine, CH_2Cl_2, −78 to 20°C, 3 h; (b) DDQ, CH_2Cl_2/pH 7 buffer, 0°C, 2 h; (c) cat. TEMPO, $PhI(OAc)_2$, CH_2Cl_2, 20°C, 4 h; (d) $(CF_3CH_2O)_2P(O)CH_2CO_2Me$, K_2CO_3, 18-C-6, PhMe, −20 to 0°C, 3 h; (e) $Cl_3CC(O)NCO$, CH_2Cl_2, 20°C, 30 min; K_2CO_3, MeOH, 20°C, 2 h; (f) DIBAL-H, CH_2Cl_2, −78°C, 4 h; (g) Dess–Martin periodinane, CH_2Cl_2, 20°C, 30 min; (h) **6**, (+)-Ipc_2BCl, Et_3N, Et_2O, 0°C, 1.5 h; **10**, −78 to −20°C, 16 h; (i) $Me_4NBH(OAc)_3$, MeCN/AcOH, −20°C, 1 h; (j) 3 M HCl, MeOH, 20°C, 72 h.

component. Subsequent hydroxyl-directed 1,3-*anti* reduction[13] of the β-hydroxy ketone **11** with $Me_4NBH(OAc)_3$ then provided diol **12** smoothly (99%). Final treatment of this diol **12** with 3 M HCl in MeOH (72 h) caused deprotection of the four TBS ethers with concomitant lactonisation to afford the desired C16-hydroxymethyl analogue **3** (73%).[14] Overall, this synthesis of pentaol **3** proceeded in 10 steps and 30% yield from diol **7** (Scheme 2).

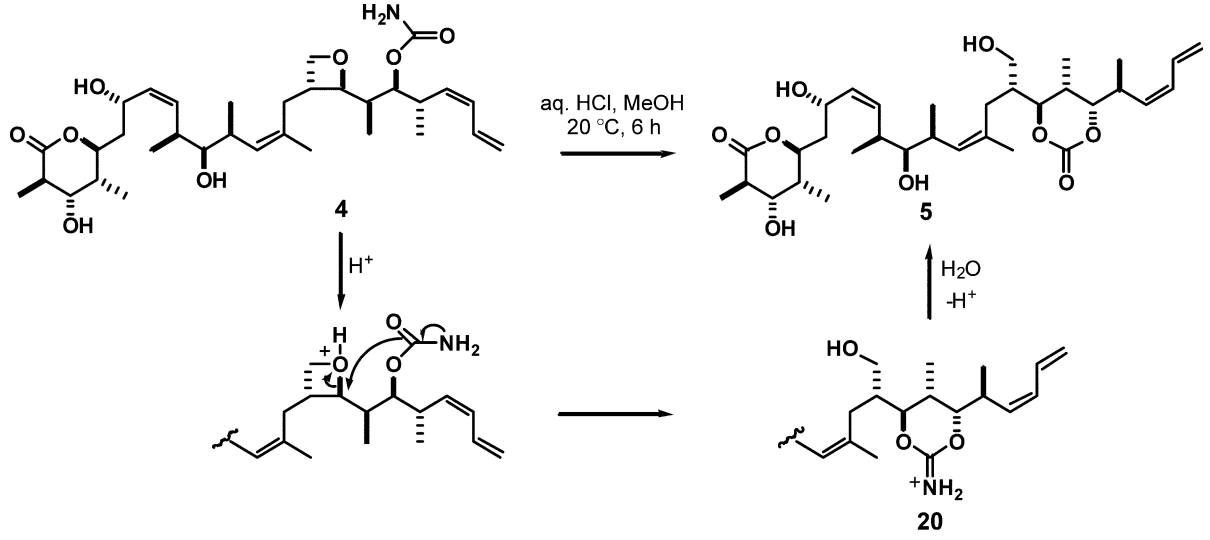
Our synthetic plan for the more challenging discodermolide analogue **4** relied upon the early introduction of the oxetane moiety (Scheme 3). Thus, selective sulfonation of 1,3-diol **7** with 2,6-mesitylenesulfonyl chloride and Et_3N gave **13** in 88% yield. In the course of our discodermolide synthesis,[6b] treatment of **13** with Super-Hydride®, with a view to reductively displacing the sulfonate group, unexpectedly led to the formation of oxetane **14**. This fortuitous reaction proved less useful on a preparative scale, where a complex mixture of products was obtained. After some optimisation, we found that generation of the potassium alkoxide of **13** with KO*t*-Bu in THF led to clean cyclisation to afford **14** in 93% yield.

Removal of the PMB ethers in **14**, selective oxidation and a (*Z*)-selective HWE reaction then provided ester **15**. Following introduction of the carbamate and oxidation state adjustment, the aldehyde **16** was obtained in readiness for aldol coupling with the C1–C6 subunit. In this case, however, the presence of the acid-sensitive oxetane functionality necessitated a reassessment of the protecting group strategy. Thus, the TBS ether at the C3 hydroxyl in methyl ketone **6** was first replaced with the more labile TES ether, as in **17**, in order to avoid undesired oxetane opening in the final desilylation step.[15] Under our standard conditions, the aldol coupling between **16** and **17** proceeded in 41% yield with 8:1 *dr* in favour of the (7*S*)-adduct **18**. Following controlled 1,3-*anti* reduction to give **19**, brief treatment with 3M HCl in MeOH (1 h) then provided the target oxetane analogue **4** (62%).[14] Altogether, this sequence proceeded in 11 steps and 13% yield from 1,3-diol **7**.

Fortuitously, a further structurally novel discodermolide analogue was isolated from the preceding acid-mediated deprotection step to form the oxetane analogue **4**. Initially, the presence of a minor byproduct in the reaction mixture was detected and, following its isolation by normal-phase HPLC purification, NMR analysis suggested opening of the oxetane ring and loss of the carbamate moiety. On a preparative scale, treatment of oxetane **4** with aq. HCl/MeOH for 6 h led to clean conversion into the same byproduct (79%), where the structure was assigned as the cyclic carbonate **5** (Scheme 4). Mechanistically, **5** presumably arises by acid-catalysed regioselective opening of the oxetane at the C17 position by the carbonyl oxygen of the adjacent carbamate group, followed by hydrolysis of the resulting imino carbonate intermediate **20**. Detailed NMR analysis of **5** indicated that this intramolecular oxetane opening reaction proceeded with inversion of configuration at C17.[16]



Scheme 3. *Reagents and conditions*: (a) 2,4,6-$Me_3(C_6H_2)SO_2Cl$, Et_3N, CH_2Cl_2, 20°C, 20 h; (b) *t*-BuOK, THF, 0°C, 30 min; (c) DDQ, CH_2Cl_2/pH7 buffer, 0°C, 2 h; (d) cat. TEMPO, $PhI(OAc)_2$, CH_2Cl_2, 20°C, 4 h; (e) $(CF_3CH_2O)_2P(O)CH_2CO_2Me$, K_2CO_3, 18-C-6, PhMe, −20 to 0°C, 3 h; (f) $Cl_3CC(O)NCO$, CH_2Cl_2, 20°C, 30 min; K_2CO_3, MeOH, 20°C, 2 h; (g) DIBAL-H, CH_2Cl_2, −78°C, 4 h; (h) Dess–Martin periodinane, CH_2Cl_2, 20°C, 30 min; (i) **17**, (+)-Ipc_2BCl, Et_3N, Et_2O, 0°C, 1.5 h; **16**, −78 to −20°C, 16 h; (j) $Me_4NBH(OAc)_3$, MeCN/AcOH, −20°C, 1 h; (k) 3 M HCl, MeOH, 20°C, 1 h.



Scheme 4.

In summary, we have synthesised three novel analogues **3**, **4** and **5** by adapting our versatile aldol-based route to discodermolide.[6] Biological evaluation of these compounds regarding their cell growth inhibitory activities and tubulin polymerization properties may provide useful information in helping to define the preferred binding mode of discodermolide to β-tubulin, and these studies will be reported in due course.

Acknowledgements

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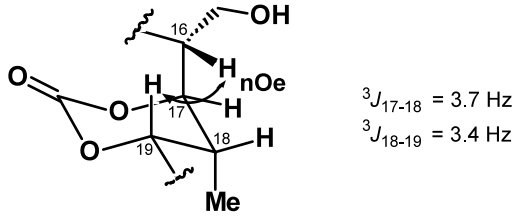
14. All new compounds gave spectroscopic data in agreement with the structures indicated. Analogue **3** had ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 6.61 (1H, ddd, J=16.8, 10.8, 10.4 Hz, H_{23}), 6.04 (1H, app t, J=11.0 Hz, H_{22}), 5.53 (1H, dd, J=11.0, 8.0 Hz, H_8), 5.41 (1H, dd, J=10.7, 10.5 Hz, H_9), 5.34 (1H, dd, J=10.4, 10.3 Hz, H_{21}), 5.24 (2H, m, H_{13}, H_{24A}), 5.13 (1H, d, J=10.3 Hz, H_{24B}), 4.74 (1H, ddd, J=10.3, 8.0, 3.4 Hz, H_7), 4.70–4.58 (4H, m, H_5, H_{19}, $CONH_2$), 3.82 (1H, br d, J=11.0 Hz, H_{25A}), 3.73 (1H, dd, J=3.8, 3.8 Hz, H_3), 3.58–3.54 (2H, m, H_{17}, H_{25B}), 3.20 (1H, dd, J=6.8, 4.3 Hz, H_{11}), 3.00 (1H, ddq, J=10.1, 6.8, 6.8 Hz, H_{20}), 2.78 (1H, ddq, J=9.7, 6.7, 6.7 Hz, H_{10}), 2.66 (1H, qd, J=7.1, 4.4 Hz, H_2), 2.63–2.56 (1H, m, H_{12}), 2.14–1.87 (8H, m, H_4, H_{6A}, H_{15A}, H_{15B}, H_{18}, 3×OH), 1.78–1.61 (5H, m obs, H_{6B}, 14-Me, OH), 1.33 (3H, d, J=7.3 Hz, 2-Me), 1.08 (3H, d, J=7.1 Hz, 4-Me), 1.05–0.99 (9H, d, 10-Me, 18-Me, 20-Me), 0.97 (3H, d, J=7.0 Hz, 12-Me); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 173.8, 157.3, 134.5, 133.3, 132.9, 132.5, 132.0, 130.6, 130.1, 118.2, 79.5, 79.0, 73.2, 64.3, 43.1, 40.9, 39.1, 37.7, 36.2, 35.7, 35.3, 35.1, 31.2, 31.1, 30.9, 29.7, 23.3, 18.3, 17.5, 15.6, 15.3, 12.6, 8.4; HRMS (ES+) calcd for $C_{33}H_{55}NO_9Na$ $[M+Na]^+$ 615.3775, found 615.3765. Analogue **4** had ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 6.62 (1H, ddd, J=16.9, 10.8, 10.5 Hz, H_{23}), 6.03 (1H, app t, J=11.0 Hz, H_{22}), 5.53 (1H, dd, J=11.1, 7.7 Hz, H_8), 5.44 (1H, dd, J=10.7, 10.3 Hz, H_9), 5.29 (1H, dd, J=10.6, 10.4 Hz, H_{21}), 5.21 (1H, dd, J=16.8, 1.2 Hz, H_{24A}), 5.15–5.09 (2H, m, H_{13}, H_{24B}), 4.73 (1H, ddd, J=8.3, 8.3, 3.2 Hz, H_7), 4.65–4.59 (2H, m, H_5, H_{19}), 4.58 (2H, br s, $CONH_2$), 4.53 (1H, dd, J=7.9, 6.1 Hz, H_{25A}), 4.25–4.21 (2H, m, H_{17}, H_{25B}), 3.74 (1H, dd, J=4.1, 3.9 Hz, H_3), 3.19 (1H, dd, J=6.6, 5.2 Hz, H_{11}), 2.98 (1H, ddq, J=9.0, 6.9, 6.9 Hz, H_{20}), 2.81–2.65 (3H, m, H_2, H_{10}, H_{16}), 2.63 (1H, ddq, J=10.1, 6.9, 6.9 Hz, H_{12}), 2.46 (1H, dd, J=13.7, 10.6 Hz, H_{15A}), 2.36 (1H, dd, J=13.8, 5.0 Hz, H_{15B}), 2.22–2.00 (4H, m, H_4, 3×OH), 1.86 (1H, ddq, J=13.5, 6.8, 3.4 Hz, H_{18}), 1.73 (1H, ddd, J=14.4, 9.4, 2.4, H_{6A}), 1.72 (1H, ddd, J=14.2, 10.7, 3.3 Hz, H_{6B}), 1.62 (3H, s, 14-Me), 1.33 (3H, d, J=7.2 Hz, 2-Me), 1.09 (3H, d, J=6.9 Hz, 18-Me), 1.05 (3H, d, J=6.9 Hz, 10-Me), 1.04–1.00 (6H, m, 4-Me, 20-Me), 0.97 (3H, d, J=6.8 Hz, 12-Me); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 173.7, 156.6,

134.0, 133.8, 132.9, 132.2 (2C), 130.2, 130.1, 117.9, 89.5, 79.0, 77.2, 75.6, 73.7, 73.2, 64.7, 43.1, 41.0, 40.5, 37.7, 36.1, 35.7, 35.6, 35.0, 34.7, 23.4, 18.4, 17.4, 15.6, 15.3, 12.5, 8.3; HRMS (ES+) calcd for $C_{33}H_{53}NO_8Na$ $[M+Na]^+$ 614.3669, found 614.3671.

15. On acid treatment of **19**, rapid deprotection of the two TBS groups and the TES group was accomplished in 80% yield in 1 h. In contrast, the TBS ether at C3 on the δ-lactone of discodermolide requires prolonged treatment with acid (2–3 days) for complete removal and this was found to be incompatible with the oxetane group.
16. Analysis of the ^1H NMR data of cyclic carbonate **5** enabled the C17 configuration to be determined unambiguously. Strong nOe contacts were observed between H_{19} and H_{16}, which is consistent with the preferred chair-like conformation depicted below. The small vicinal coupling constants, $^3J_{17-18}=3.7$ Hz and $^3J_{18-19}=3.4$ Hz, are also in good agreement with the proposed stereostructure.



Analogue **5** had ^1H NMR (500 MHz, $CDCl_3$) δ_H 6.61 (1H, ddd, $J=16.8$, 11.0, 10.4 Hz, H_{23}), 6.13 (1H, app t, $J=10.9$ Hz, H_{22}), 5.60–5.53 (2H, m, H_8, H_9), 5.38 (1H, dd, $J=10.4$, 10.3 Hz, H_{21}), 5.28 (1H, d, $J=16.8$ Hz, H_{24A}), 5.23–5.19 (2H, m, H_{13}, H_{24B}), 4.70 (1H, dddd, $J=6.8$, 6.5, 3.4, 3.3 Hz, H_7), 4.66 (1H, ddd, $J=11.4$, 10.5, 2.1 Hz, H_5), 4.33 (1H, dd, $J=7.8$, 3.7 Hz, H_{17}), 4.30 (1H, dd, $J=7.8$, 3.4 Hz, H_{19}), 3.77 (1H, dd, $J=4.6$, 4.0 Hz, H_3), 3.66 (1H, dd, $J=11.0$, 3.4 Hz, H_{25A}), 3.55 (1H, dd, $J=10.7$, 6.0 Hz, H_{25B}), 3.24 (1H, app t, $J=5.6$ Hz, H_{11}), 3.00 (1H, ddq, $J=9.2$, 7.3, 7.3 Hz, H_{20}), 2.82–2.76 (1H, m, H_{10}), 2.69 (1H, qd, $J=7.5$, 5.0 Hz, H_2), 2.58 (1H, ddq, $J=7.2$, 3.7, 3.5 Hz, H_{18}), 2.51 (1H, ddq, $J=9.7$, 6.4, 6.4 Hz, H_{12}), 2.33 (1H, dd, $J=13.7$, 11.4 Hz, H_{15A}), 2.20–2.12 (1H, m, H_{15B}), 2.06–1.99 (2H, m, H_4, H_{16}), 1.87 (1H, ddd, $J=14.5$, 9.2, 2.3 Hz, Me_{6A}), 1.80–1.56 (6H, m, 4×OH, H_{6B}, 14-Me), 1.34 (3H, d, $J=7.2$ Hz, 2-Me), 1.16 (3H, d, $J=7.1$ Hz, 18-Me), 1.10 (3H, d, $J=7.0$ Hz, 4-Me), 1.08–1.05 (6H, m, 10-Me, 20-Me), 1.01 (3H, d, $J=6.7$ Hz, 12-Me); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 173.9, 149.2, 134.1, 132.9, 132.1, 131.8, 131.7 (2C), 130.7, 118.9, 85.4, 82.3, 79.2, 77.0 (obs), 72.9, 64.2, 60.9, 42.8, 41.2, 40.8, 36.1, 35.9, 35.8, 35.3, 29.5, 29.1, 23.2, 18.9, 17.2, 15.9, 15.4, 12.5, 11.8; HRMS (ES+) calcd for $C_{33}H_{52}O_9Na$ $[M+Na]^+$ 615.3509, found 615.3516.