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Spirobicyclo[2.2.2]octane derivatives: mimetics of baccatin III and paclitaxel (Taxol)†‡

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The formylated spirobyclic alcohol **8a** was computer modeled to be a mimetic of paclitaxel. In this model, the formyl group was used as a truncated paclitaxel side chain in order to reduce the computational work. Compound **8c**, carrying the paclitaxel side chain, was synthesized in six steps from optically active 1,3-diketone **12**. Microtubule stabilization was not observed for **8c**, indicating that the model needs to be adjusted.

Introduction

Paclitaxel (Taxol) **1a** and docetaxel (Taxotere) **1b** (Fig. 1), are well known microtubule stabilizing anticancer agents[1,2] and paclitaxel has been prepared by several independent total synthetic routes.[3–10] However, ingenious as they may be, these syntheses require many and complex synthetic steps. Indeed, the diterpenoid core of these bio-active molecules, *i.e.* baccatin III and its derivatives, is still a major synthetic challenge.

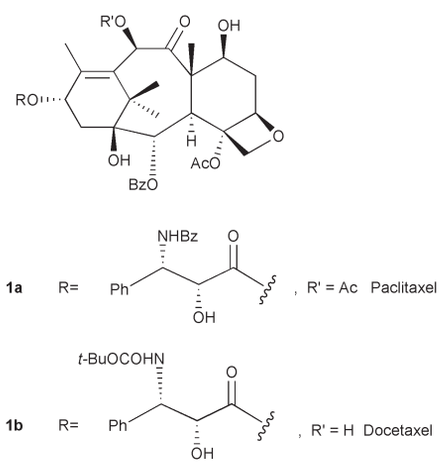

Fig. 1

In addition to paclitaxel, docetaxel and modified taxanes, *e.g.* **2**,[11] **3**,[12] **4**[13] and **5**,[14] a number of non-taxoids have been shown to be microtubule stabilizers (Fig. 2). Thus, the bicyclic epoxide **6** was reported to be a microtubule stabilizer.[15] Howarth *et al.* reported microtubule stabilization of guanosine derivative **7** carrying the paclitaxel side chain (R in formula **1a**).[16] Very recently, Snyder *et al.* showed that a taxane derivative **5**, lacking the oxetane ring, was potent as a microtubule stabilizer *in vitro*, but had a much lower cytotoxicity than paclitaxel.[14] It should also be noted that a number of other non-taxanes have been found to be microtubule stabilizers (epothilones, sarcodictyin, synstab A, jatrophanes and others).[17,18]

Structure activity data on paclitaxel analogs indicate that several functional group variations are allowed at the northern half without seriously affecting the microtubule stabilization. Thus, one would expect that a "Taxol mimetic" would not strictly have to mimic these parts of the molecule. It seems more critical, however, that the side-chain and in particular the 2′-hydroxyl is properly positioned. Also the 2-benzoyloxy, the 4-acetoxy and the oxetane moieties seem to be necessary for the activity of Taxol and related compounds. This aspect has recently been discussed.[19,20]

Several compounds of widely varying structures show microtubule activity and there seems to be considerable opportunity to develop alternative structures having taxane-like properties. Since the taxane diterpenoid skeleton (baccatin III) is quite rigid and rather difficult to synthesize, we looked for simple rigid replacements of this structure that might be easier to synthesize and, decorated with the necessary pharmacophores, would possibly have the same or similar biological activity as paclitaxel itself. Moreover, in the long run it is necessary to develop new alternatives to taxoid anticancer drugs due to the occurrence of multidrug resistance.[21]

Results and discussion

We first made a computer investigation to establish which conformation of paclitaxel/docetaxel would be most suitable as a template for comparison of new structures. Preferably, this would be the bioactive conformation. Nogales and co-workers have determined the structure of the α,β-tubulin heterodimer (the basic structural unit of microtubule) bound to paclitaxel. A resolution of 3.7 Å was obtained by electron crystallography of zinc-induced tubuline sheets.[22,23] This apparently shows the location of the binding site of paclitaxel. However, the structure lacks the degree of resolution to allow the determination of a more detailed bioactive conformation. Therefore, we decided to use crystal structures of paclitaxel and docetaxel, since these must be low energy conformations. A crystal structure of docetaxel was reported by Gueritte in 1990[24] and later Mastropaolo published the crystal structures of two different conformers of paclitaxel, A and B.[25] In order to determine which one to apply as our template we made an overlay of these structures in which we also included our own energy minimised version of paclitaxel (Fig. 3). The Macromodel computer program was used for this purpose.[26]

As can be seen, there are no major differences between the rigid cores and it is almost exclusively the side chain that differs in its orientations. Snyder *et al.* have addressed this problem and suggested, by using both theoretical and experimental

† Electronic supplementary information (ESI) available: Table of results of the alkylation of compound **12**, copies of the ^{1}H NMR and ^{13}C NMR spectra for all compounds, NOESY spectra for compounds **13a**, **13b** and the *O*-alkylated major products, the overlay between **8b** and paclitaxel A and the overlay of the different conformers of **8a**/**8b**. See http://www.rsc.org/suppdata/ob/b4/b409678a/

‡ Taken in part from the PhD thesis of Fredrik Almqvist, Lund University, 1996.

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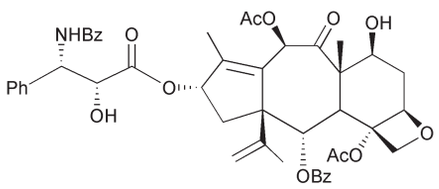

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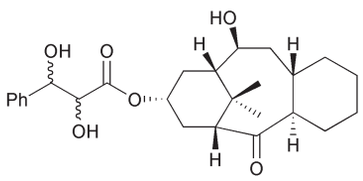

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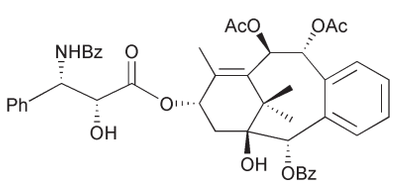

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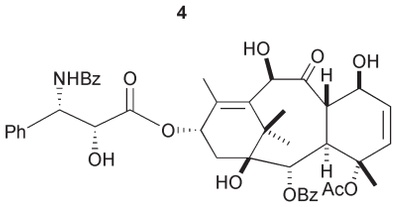

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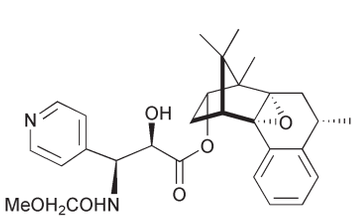

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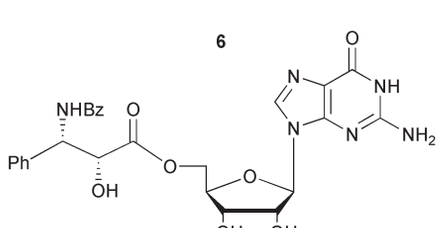

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Fig. 2 Examples of structures showing biological activity comparable to paclitaxel.

evidence that it is the T-paclitaxel conformation that fits into the β-tubulin binding site.[27–29] It is of particular interest that it is not the close contact between the phenyl groups emanating from the 3′-position and the 2-O-benzoate (hydrophobically collapsed conformer) that is important but the rather close contact between the former and the 4-OAc methyl group.[29] However, along with the suggestion of Swindell *et al.*[30] we reasoned that the side chain should have the same or similar degrees of freedom to orient itself in all of these and also in new structures. Thus, we concluded that any of the above structures were useful as our template for the taxane core and we decided to use paclitaxel A.

A number of MM3-energy minimised rigid structural motifs were then checked for structural similarity with paclitaxel A by using the MacMimic computer program.[31] Based on this preliminary test it appeared that spirobicyclo frameworks

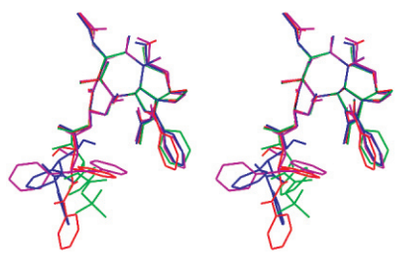
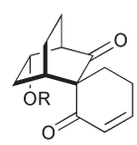


Fig. 3 Stereoview of overlay containing crystal structure of docetaxel, crystal structures of two different conformers of paclitaxel, A and B, and the calculated minimum energy conformation of paclitaxel. Color code: green = docetaxel; blue = paclitaxel A, red = paclitaxel B; magenta = energy minimized paclitaxel.

would be interesting candidates and the best fit to the taxane diterpenoid core was found for **8** (Fig. 4).[32] In fact, Kuwajima *et al.* indicated that spirobicyclics would constitute a new lead to taxane-like biologically active compounds and also that one of their compounds obtained by an unexpected Cope-rearrangement of a tricyclic framework, **9**, exhibited the same potency as verapamil as a MDR (multi drug resistant) reversing agent.[33]



8a: Spiro cyclohexenone ring in back position (see Fig. 6), R = formyl
8b: Spiro cyclohexenone ring in front position (See Fig. 6) R = formyl
8c: R = see 1a

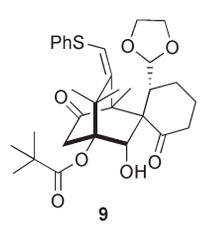


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Fig. 4

Further computational work was made by use of the MacroModel computer program.[26] Thus, the individual candidate structures were energy minimised (for details see Experimental). In order to simplify the calculations the paclitaxel side chain was replaced by a formic ester. Comparison of paclitaxel A and several spirobicyclic structures (overlay) resulted in the identification of **8a** as a fair mimetic of the southern part of paclitaxel A. In these overlays the highlighted six atoms shown in Fig. 5 were used as contact points to comply with the pharmacophore model (13-O, 2-O, 4-O, and the oxetane ring, as mentioned).

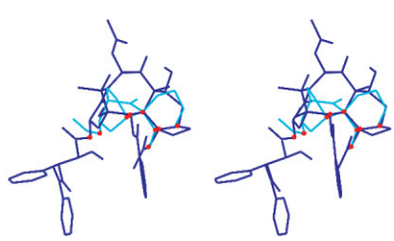


Fig. 5 Overlay between paclitaxel A and **8a**, rms: 0.415.

When performing the energy minimisations of the mimetics another conformer of **8a** was found, namely **8b**, which had approximately the same steric energy as **8a**. However, a much worse overlay rms value was obtained for **8b**/paclitaxel A (rms 0.729) than for **8a**/paclitaxel A (rms 0.415). The difference in geometry of **8a** and **8b** is a flip of the six-membered spiro ring

(Fig. 6). With a calculated energy difference of 0.01 kcal mol^{-1} between **8a** and **8b**, they would approximately equally populate the equilibrium conformations. We assume that the ring flip should have a low energy barrier, thus, allowing the best fitting structure, **8a**, to be attached to the microtubule binding-site.

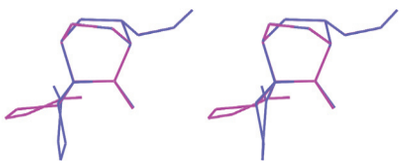


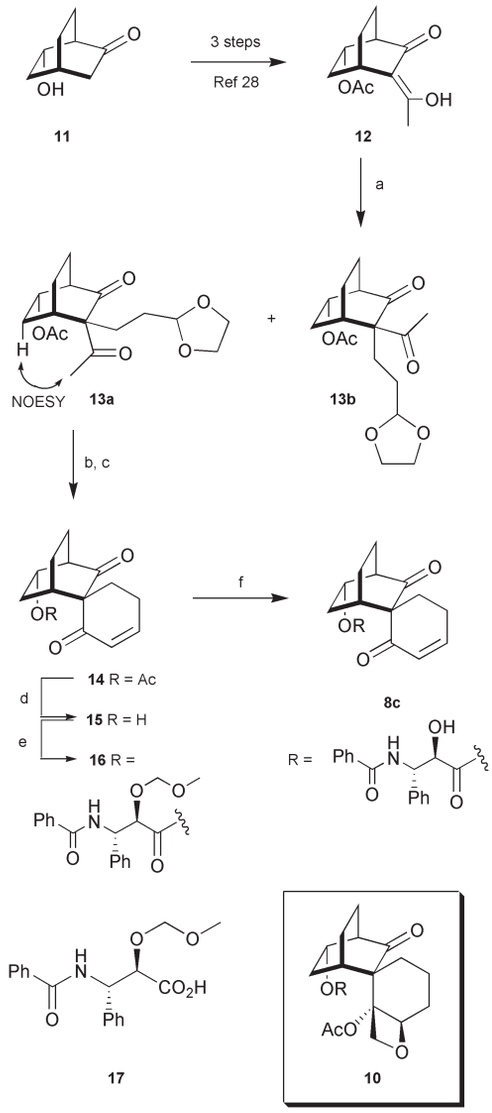
Fig. 6 Stereoview of the overlays of **8a** and **8b**. **8a**: spiro ring in back position (blue), **8b**: spiro ring in front position (magenta).

In the synthetic planning of **8c** (carrying the paclitaxel side chain) we imagined that bicyclo[2.2.2]octane-2,6-dione could be used as a starting material, since it was available to us in substantial amounts for other projects.[34–36] Thus, the class of spirobicyclo derivatives such as **8** appeared to be easy to synthesize and even transformable into **10** (Scheme 1) or closely related compounds. A few other spirobicyclic derivatives of similar structures have been synthesized earlier,[33,37–40] but these compounds can not easily be used for our purpose.

We here report a six step synthesis of optically active **8c**, starting from **12**, which was prepared from easily available optically active (>96% ee) keto-alcohol **11**[34] (Scheme 1).

Alkylation of **12** with 2-(2-iodo)-1,3-dioxolane using Cs_2CO_3 as a base provided **13a**, which carried the necessary functionalities and number of carbon atoms for the subsequent aldol cyclization. Preference for alkylation of the *exo*-face was achieved due to the steric influence of the *endo*-acetoxy group, although to a lesser extent than we hoped. The *exo*:*endo* ratio in the best case was 80:20, which was not improved by the use of the corresponding TBDMS-protected derivative.[41] In addition to the C-alkylation products substantial amounts of two O-alkylation products were formed in all cases, despite the use of several methods that were recommended to avoid reactions at oxygen.[42–46] Hydrolysis of the two O-alkylation products regenerated **12** but only product **13a** was used for further synthesis. Fortunately, column chromatography allowed purification of **13a**, which was isolated in 45% yield. A NOESY cross peak due to the proximity of the methyl group of the acetyl group and the *endo*-5H (indicated in Scheme 1 with an arrow) clearly supported the structure of **13a**. Several other combinations of bases and solvents were used in order to improve the selectivity without success. The C/O alkylation ratios varied between 44:56 and 67:33 and the *exo*:*endo* ratios between 64:36 and 80:20 (see Electronic Supplementary Information†). To avoid the O-alkylation problem we also applied a Mukaiyama-type reaction[47,48] between the TMS-enol ethers of **12** (mixture of isomers)[49] and the dimethyl acetal of acrolein, but without success. It only resulted in the recovery of **12**.

Aqueous acid treatment of **13a** could conceivably lead to hydrolysis of the two protective groups (the acetal and the acetate) followed directly by an acid catalyzed aldol cyclization to give the spiro-anellated cyclohexenone ring of **15**. However, neither the reaction conditions (1 M HCl, THF), that we had previously used for a similar purpose,[50] nor several other acidic reagents (PPTS in acetone water, wet silica, oxalic acid in dichloromethane and $PdCl_2(CH_3CN)_2$ (cat.) in wet acetone[51]) were effective in hydrolyzing the acetal unit. Curiously, treatment of **13a** with 1 M HCl in acetone did result in a slow conversion into the corresponding aldehyde (60%) but the desired cyclization was not observed. Stronger acid and higher temperature destroyed the material. An efficient method for acetal hydrolysis was finally found to be treatment of **13a** with 80% acetic acid at 65 °C. Even though these conditions did not lead to a combined aldol cyclization and acetate hydrolysis, an almost quantitative yield of the corresponding aldehyde was obtained. The aldol cyclization to give **14** was then performed simply by changing the medium to toluene–TsOH (cat.)



Scheme 1 (a) Cs_2CO_3, 2-(2-iodoethyl)-1,3-dioxolane, toluene, 100 °C, 3 h, **13b** (9%):**13a** (45%) 20:80 and two O-alkylation products; (b) acetic acid (80%), 65 °C, 3 h; (c) TsOH, toluene, Soxhlet apparatus (4 Å molecular sieves), reflux, 3 h, 85% from **13a**; (d) K_2CO_3, MeOH:H_2O 9:1, room temperature, 2 h, 86%; (e) DMAP, DCC, **17**, CH_2Cl_2, 0 °C to room temperature, 3 h, 55%; (f) Me_2BBr, CH_2Cl_2, −70 °C to −50 °C, 2 h, 70%.

followed by azeotropic removal of water. Subsequent basic ester hydrolysis of **14** gave **15** in 73% yield over three steps.

Next, the side chain of paclitaxel, in the form of (2*R*,3*S*)-*N*-benzoyl-2-*O*-(methoxymethyl)-phenylisoserine (**17**), available in our laboratory from earlier work in the taxane field,[52] was attached to **15** by DCC/DMAP treatment according to an earlier report by Greene *et al.* for similar cases.[53] Finally, **8c** was obtained after removal of the MOM protecting group by Me_2BBr[54] in 39% yield over two steps. Some of the 2′-epimer (*ca.* 10% of the yield) was also formed, but was removed by chromatographic separation (HPLC).

Compound **8c** dissolved in DMSO:water 25:75 was submitted to a microtubule polymerization assay but did not show any microtubule stabilization. Microtubule proteins (3 mg ml^{-1}) were assembled at 30 °C in the presence or absence of 0.305 or 0.61 mM compound **8c** dissolved in DMSO:water 25:75. Compound **8c** inhibited the assembly slightly compared to the control. However, no Taxol-like effects were seen. Microtubules disassembled rapidly upon lowering of the temperature to 4 °C, and no decreased lag-phase or increase in assembly rate was seen either if **8c** was added initially or reassembled again at 30 °C after disassembly at 4 °C.

The failure of **8c** to stabilize microtubule is most likely due to the lack of a benzoate group corresponding to the 2-benzoate

in paclitaxel and also to the absence of the oxetane/4-OAc grouping. Efforts to include these features will be reported in due course.

Experimental

Computations

Energy minimisations were initiated by running a full structure energy minimisation using the Polak–Riber Conjugate gradient (PRCG), (MM3*), followed by a Monte Carlo search (1000 cycles). Finally, a Full Matrix Newton Raphson (FMNR) minimisation was performed with all the conformers found within 1 kcal mol^{-1} from the global minimum in the Monte Carlo search. To investigate the impact of the force field, two minimisation series were made after the PRCG minimisation, using two different force fields (MM3* and the Merck Molecular Force Field (MMFF)). Since the core structure is rather rigid, flexibility was only expected for the side chain and the spiro cyclohexenone ring. As predicted, the obtained conformers from the two series showed a low degree of flexibility. From the sequence using MM3*, six conformers were found (ΔE = 0.64 kcal mol^{-1}) with a total of five different orientations of the formyl group and two for the spiro ring. For the MMFF sequence, however, only four conformers were found (ΔE = 0.54 kcal mol^{-1}), with two orientations of the formyl group and two for the spiro ring. When comparing the lowest energy conformer from each minimisation sequence, they showed different orientations for both the formyl group and the spiro ring (see Electronic Supplementary Information†). However, since the energy difference between all the conformers in each sequence was found to be very small, one can assume an equilibrium between the different conformers. Therefore, when searching for the structure with best resemblance with paclitaxel A, all the obtained low energy conformers were analysed and **8a** from the MMFF sequence was found to be a good mimetic. No solvent effects were taken into account in these methods, which were implemented in the MacroModel™ version 7.1 molecular modelling software (Schrödinger, Inc., Portland).[26] The software was executed on a Silicon Graphics CMNB024B model O2™ workstation (Silicon Graphics, Inc., Mountain View, CA).

General

GC analyses were performed on a Varian 3400 gas chromatograph equipped with a DBwax (J&W Scientific) capillary column (30 m, 0.25 mm i.d., 0.25 μm stationary phase). NMR spectra were recorded on a Bruker DRX 400 spectrometer at 400 MHz (^{1}H) and at 100 MHz (^{13}C) using $CDCl_3$ as solvent. Signals from the solvent were used as reference lines (δ 7.26 (^{1}H) and 77.0 (^{13}C)) and J values are given in Hz. Infrared spectra were recorded on a Shimadzu 8300 FTIR instrument. Optical rotations were measured with a Perkin Elmer 241 polarimeter at 21 °C at the sodium D-line. Chromatographic separations were performed on Matrex Amicon normal phase silica gel 60 (0.035–0.070 mm) and for thin layer chromatography Merck pre-coated TLC plates silica gel 60 F-254, 0.25 mm were used. After elution the TLC plates were sprayed with a solution of *p*-methoxybenzaldehyde (26 mL), glacial acetic acid (11 mL), concentrated sulfuric acid (35 mL) and 95% ethanol (960 mL) and the compounds were visualized upon heating. All solvents were dried and distilled according to standard procedures,[55] and the reactions were performed in septum-capped, oven-dried flasks under an atmospheric pressure of argon. Organic extracts were dried using Na_2SO_4 throughout. Mass spectra were recorded on a JEOL JMDX 303 spectrometer.

(1*R*,3*R*,4*S*,6*S*)-3-Acetyl-3-(2-(2-oxolanyl)ethyl)-6-acetoxybicyclo[2.2.2]octan-2-one (13a) and (1*R*,3*S*,4*S*,6*S*)-3-acetyl-3-(2-(2-oxolanyl)ethyl)-6-acetoxybicyclo[2.2.2]octan-2-one (13b)

A solution of **12**[41] (554 mg, 2.47 mmol) in toluene (6 mL) was added to Cs_2CO_3 (1.14 g, 3.50 mmol) in toluene (6 mL) at room temperature. 2-(2-Iodoethyl)-1,3-dioxolane (1.20 g, 6.14 mmol, 2.5 equiv.) was then added (the flask was covered with aluminium foil) and the mixture was heated at 100 °C for 3 h whereafter ice was added together with a small amount of water. The mixture was extracted with EtOAc (3 × 50 mL) and then the combined organic phase was washed with brine (2 × 15 mL), dried and the solvent was removed at reduced pressure. The residue was purified by column chromatography (SiO_2, heptane : EtOAc 1 : 1) to give a mixture of the C- and O-alkylated isomers (761 mg, 95%). Repeated chromatography gave the following compounds in order of elution:

13b. (75 mg, 9%); R_f 0.30; $[\alpha]_D$ −103 (c 0.9 in $CHCl_3$); ν_{max}(neat)/cm^{-1} 2950, 2880, 1730, 1690, 1355, 1225; δ_H ($CDCl_3$) 5.16 (1 H, ddd, J 9.3 Hz, 4.1 Hz and 2.0 Hz), 4.82 (1 H, t, J 4.1 Hz), 3.80–3.97 (4 H, m), 2.58 (1 H, m), 2.50 (1 H, m), 2.24 (3 H, s), 2.00–2.21 (3 H, m), 1.93 (3 H, s), 1.66–1.91 (5 H, m), 1.40–1.57 (2 H, m); δ_C ($CDCl_3$) 213.23, 204.16, 169.61, 115.43, 103.43, 70.96, 67.08, 65.01, 65.00, 47.15, 31.73, 30.44, 27.71, 27.46, 27.03, 21.13, 20.89, 19.59; HRCIMS (CH_4) Calcd. for $C_{17}H_{25}O_6$ 325.1651. Found 325.1652.

13a. (360 mg, 45%); R_f 0.24; $[\alpha]_D$ +52 (c 1.6 in $CHCl_3$); ν_{max}(neat)/cm^{-1} 2950, 2880, 1735, 1695, 1235; δ_H ($CDCl_3$) 5.10 (1 H, dt, J 9.8 Hz, 3.2 Hz), 4.81 (1 H, t, J 4.3 Hz), 3.80–3.95 (4 H, m), 2.53 (1 H, m), 2.42 (1 H, m), 2.25 (3 H, s), 2.00–2.16 (2 H, m), 2.02 (3 H, s), 1.93 (1 H, m), 1.61–1.84 (5 H, m), 1.44–1.55 (2 H, m); δ_C ($CDCl_3$) 212.09, 205.59, 170.07, 103.61, 71.80, 66.82, 64.96, 64.93, 47.54, 32.45, 30.44, 28.23, 27.75, 27.47, 21.56, 21.11, 18.95; HRCIMS (CH_4) Calcd. for $C_{17}H_{25}O_6$ 325.1651. Found 325.1653.

The 2-O-alkylated major product was obtained from a forerun and a NOESY experiment (see below) indicated the position of the alkylation; (95 mg, 12%); R_f 0.43; $[\alpha]_D$ −21 (c 1.1 in $CHCl_3$); ν_{max}(neat)/cm^{-1} 2940, 2860, 1720, 1670, 1595, 1230; δ_H ($CDCl_3$) 5.04 (1 H, t, J 4.8 Hz), 4.98 (1 H, dt, J 8.7 Hz, 3.1 Hz), 4.11 (2 H, m), 3.85–3.99 (4 H, m), 3.46 (1 H, m), 3.26 (1 H, m), 2.40 (3 H, s), 2.11 (2 H, m), 2.01 (3 H, s), 1.99 (1 H, m), 1.63 (1 H, m), 1.50 (1 H, m), 1.38 (1 H, m), 1.19 (2 H, m); δ_C ($CDCl_3$) 193.74, 170.58, 166.08, 119.88, 101.57, 73.22, 64.97, 64.91, 64.49, 35.26, 35.25, 34.14, 31.65, 27.75, 23.74, 22.09, 21.16; HRCIMS (CH_4) Calcd. for $C_{17}H_{25}O_6$ 325.1651. Found 325.1652. The two O-alkylated products were taken together and converted back to the starting material.

The NOESY spectra of **13a**,**13b** and the major O-alkylated products were recorded using a mixing time of 800 ms. The time domain data sets were accumulated over a sweep width of 1611 Hz using 2048 complex data points in the t2 dimension. The repetition delay was 1.0 s and 64 scans were collected for each t1 increment (256 in total). Total experimental time was 11 h 18 min.

(1*R*,3*S*,4*S*,6*S*)-Spiro[6-acetoxybicyclo[2.2.2]octan-2-one-3,1′-cyclohex-3′-en]-2′-one (14)

Compound **13a** (200 mg, 0.62 mmol) was dissolved in 80% acetic acid (25 mL) and heated at 65 °C for 3 h. The solution was cooled to room temperature and then concentrated at reduced pressure. In order to remove remaining water the residue was co-evaporated three times with toluene under reduced pressure. Neat TsOH (*ca.* 5 mg) was added to a solution of the crude aldehyde in toluene (30 mL) and the resulting mixture was heated at reflux temperature for 3 h in a Soxhlet apparatus containing activated 4 Å molecular sieves in the thimble. Half-saturated aqueous $NaHCO_3$ was added at 0 °C, and the aqueous phase was extracted with EtOAc. The combined organic solution was dried and then concentrated at reduced pressure. The residue was chromatographed (SiO_2, heptane : EtOAc 1 : 1) to give **14** (138 mg, 85%) as a white solid; mp 139–141 °C; $[\alpha]_D$ −146 (c 0.3 in $CHCl_3$); ν_{max}(KBr)/cm^{-1} 2960, 2935, 1730, 1660, 1235, 1030; δ_H ($CDCl_3$) 6.89 (1 H, m),

5.95 (1 H, ddd, *J* 10.2, 2.6 and 1.6 Hz), 5.13 (1 H, dt, *J* 9.8 Hz, 3.4 Hz), 2.54 (1 H, m), 2.34–2.51 (3 H, m), 2.15–2.32 (3 H, m), 2.09 (1 H, m), 2.01 (3 H, s), 1.86 (1 H, dq, *J* 14.8, 3.1 Hz), 1.78 (1 H, m), 1.62 (1 H, m), 1.50 (1 H, m); δ_C ($CDCl_3$) 211.79, 198.82, 170.06, 148.72, 128.58, 71.27, 61.77, 46.83, 31.30, 30.41, 26.93, 22.59, 21.13, 21.06, 18.47; HRCIMS (CH_4) Calcd. for $C_{15}H_{19}O_4$ 263.1283. Found 263.1281.

(1*R*,3*S*,4*S*,6*S*)-Spiro[6-hydroxybicyclo[2.2.2]octan-2-one-3,1′-cyclohex-3′-en]-2′-one (15)

Solid K_2CO_3 (10 mg, 0.07 mmol) was added all at once to a solution of **14** (100 mg, 0.38 mmol) in MeOH : H_2O 9 : 1 (20 mL). The mixture was kept at room temperature for 2 h and then the solution was concentrated to approximately 4 mL at reduced pressure. The resulting solution was extracted several times with EtOAc and the combined organic phase was dried and concentrated at reduced pressure. The residue was chromatographed (SiO_2, heptane : EtOAc 1 : 4) to give **15** (72 mg, 86%) as a white solid; mp 135–136 °C; $[\alpha]_D$ −43 (*c* 0.5 in $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3020–3500, 2950, 2910, 1720, 1650, 1215; δ_H ($CDCl_3$) 6.88 (1 H, m), 5.93 (1 H, dt, *J* 10.2, 1.8 Hz), 4.23 (1 H, dt, *J* 9.6 Hz, 3.0 Hz), 2.45 (1 H, m), 2.27–2.41 (4 H, m), 2.14 (1 H, ddd, *J* 14.8, 9.5 and 3.0 Hz), 2.05 (1 H, m), 1.88 (1 H, dq, *J* 14.7 Hz, 3.1 Hz), 1.79 (1 H, s), 1.56–1.73 (3 H, m), 1.45 (1 H, m); δ_C ($CDCl_3$) 213.51, 199.38, 148.87, 128.55, 69.60, 61.97, 51.13, 33.13, 30.58, 26.92, 22.69, 21.09, 18.43; HRCIMS (CH_4) Calcd. for $C_{13}H_{17}O_3$ 221.1178. Found 221.1174.

(2*R*,3*S*)-*N*-Benzoyl-3-phenylisoserine ester with (1*R*,3*S*,4*S*,6*S*)-spiro[6-hydroxybicyclo[2.2.2]octan-2-one-3,1′-cyclohex-3′-en]-2′-one (8c)

DMAP (*ca.* 3 mg) and DCC (51 mg, 0.25 mmol) were added to a solution of **15** (46 mg, 0.21 mmol) and **17**[52,53] (83 mg, 0.25 mmol) in CH_2Cl_2 (1.5 mL) at 0 °C. The cooling bath was removed and the mixture was stirred at room temperature for 3 h. After filtration, the filtrate was concentrated at reduced pressure and then the residue was chromatographed (SiO_2, heptane : EtOAc 1 : 2) to give **16** (61 mg, 55%) as a white solid; mp 198–200 °C; $[\alpha]_D$ −43 (*c* 2.1 in $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3380, 3000, 2950, 1740, 1720, 1660, 1645, 1520, 1035; δ_H ($CDCl_3$) 7.83 (2 H, m), 7.44–7.56 (3 H, m), 7.35 (4 H, m), 7.26 (1 H, m), 7.06 (1 H, d, *J* 9.2 Hz), 6.89 (1 H, m), 5.92 (1 H, dt, *J* 10.1 Hz, 1.2 Hz), 5.71 (1 H, dd, *J* 9.2, 2.2 Hz), 5.23 (1 H, dt, *J* 9.8, 3.3 Hz), 4.63 (1 H, d, *J* 6.9 Hz), 4.57 (1 H, d, *J* 2.3 Hz), 4.46 (1 H, d, *J* 6.9 Hz), 2.87 (3 H, s), 2.53 (1 H, m), 2.07–2.51 (7 H, m), 1.85 (1 H, dq, *J* 15.2 Hz, 2.0 Hz), 1.75 (1 H, m), 1.60 (1 H, m), 1.46 (1 H, m); δ_C ($CDCl_3$) 211.61, 199.00, 169.06, 166.85, 149.44, 138.19, 134.03, 131.79, 128.68, 128.51, 128.27, 127.66, 127.06, 126.42, 95.67, 76.51, 72.77, 61.94, 55.76, 54.49, 46.79, 31.07, 30.42, 26.95, 22.73, 20.94, 18.52; HRCIMS (CH_4) Calcd. for $C_{31}H_{34}O_7N$ 532.2335. Found 532.2333.

Me_2BBr (158 μL, 1.85 M in CH_2Cl_2, 292 μmol) was added to a solution of **16** (39 mg, 73 μmol) in CH_2Cl_2 (3 mL) at −70 °C. The solution was stirred for 2 h at −50 °C and then the reaction mixture was quenched by addition of aqueous $NaHCO_3$ (sat., 3 mL). The aqueous phase was extracted with CH_2Cl_2 and then the combined organic phase was washed with brine, dried and the solvent was removed under reduced pressure. The residue was chromatographed (HPLC, Nucleosil Silica, 500 × 10 mm, heptane : EtOAc 1 : 3) to give **8c** (24 mg, 70%) as a white foam; $[\alpha]_D$ −55 (*c* 1.2 in $CHCl_3$); ν_{max}(ZnSe)/cm^{-1} 3374, 3031, 2951, 2876, 1728, 1660, 1520, 1114; δ_H ($CDCl_3$) 7.77 (2 H, m), 7.27–7.54 (8 H, m), 6.97 (1 H, bd, *J* 8.8 Hz), 6.91 (1 H, ddt, *J* 10.1 Hz, 3.0 Hz, 1.7 Hz), 5.94 (1 H, dq, *J* 10.2 Hz, 1.1 Hz), 5.67 (1 H, dd, *J* 9.4 and 1.9 Hz), 5.27 (1 H, dt, *J* 9.9 and 3.2 Hz), 4.62 (1 H, d, *J* 2.0 Hz), 3.42 (1 H, s), 2.54 (1 H, m), 2.06–2.50 (7 H, m), 1.98 (1 H, dq, *J* 15.3 Hz, 2.9 Hz), 1.76 (1 H, m), 1.62 (1 H, m), 1.47 (1 H, m); δ_C ($CDCl_3$) 211.85, 199.00, 172.04, 166.86, 149.50, 138.35, 133.94, 131.84, 128.75, 128.68, 128.29, 127.92, 127.02, 126.83, 73.68, 73.06, 62.02, 54.46, 46.83, 30.66, 30.42, 26.96, 22.75, 20.97, 18.47; HRCIMS (CH_4) Calcd. for $C_{29}H_{30}O_6N$ 488.2073. Found 488.2078.

Isolation of microtubule proteins and assembly studies

Cow brain microtubule proteins were isolated by two cycles of assembly–disassembly as described in Wallin *et al.*[56] Assembly was measured at +30 °C. Microtubule proteins were drop-frozen and kept in liquid nitrogen until used in experiments. Protein concentrations were determined according to Lowry *et al.*,[57] with bovine serum albumin as a standard.

Assembly–disassembly of microtubules were measured in a temperature-controlled spectrophotometer at 350 nm. The assembly was induced by addition of 1 mM GTP and increasing the temperature from 4 °C to 30 °C.

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