Pergamon

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Tetrahedron 59 (2003) 9979–9984

TETRAHEDRON

Synthesis and biological evaluation of fluorescently labeled epothilone analogs for tubulin binding studies

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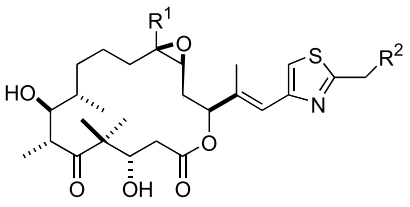
Received 12 August 2003; revised 9 October 2003; accepted 9 October 2003

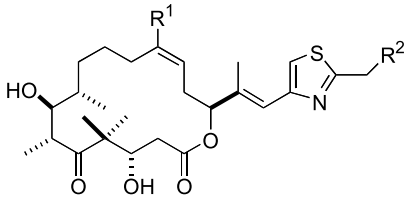
Abstract—The two fluorescently labeled epothilones **14** and **15** have been synthesized using a modification of Nicolaou's macrolactonization and Stille coupling strategy. The cytotoxicities of the compounds were 6.1 and 2.7 μg/mL, respectively, against the A2870 ovarian cancer cell line, and 0.5 and 1.0 μg/mL, respectively, against the PC-3 prostate cancer cell line. The critical concentration of tubulin was 0.5 and 1.0 μM in the presence of **14** and **15**, respectively, compared with 0.3 μM for paclitaxel. The fluorescent properties of the two molecules in solution and bound to microtubules are described.

1. Introduction

An exciting new discovery in the cancer chemotherapy area has been the emergence of the epothilones as potential new chemotherapeutic agents. Though the gross structure was revealed in a German patent by Hofle et al. 1993,[1] scientific interest in them was greatly stimulated by the discovery of their tubulin polymerization activity in 1995.[2] Studies of the bioactivity of epothilones A and B have shown that they stabilize microtubules in the same way as paclitaxel, but that they were superior to paclitaxel in treating vinblastin-resistant CCRF-CEM tumors in mice.[3] Also epothilone D was curative against paclitaxel-resistant CCRF-CEM tumors.[4] The chemistry, biology and structure activity relationships of the epothilones have been extensively reviewed.[5,6]



$R^1 = R^2 = H$	Epothilone A
$R^1 = CH_3$, $R^2 = H$	Epothilone B
$R^1 = H$, $R^2 = OH$	Epothilone E
$R^1 = CH_3$, $R^2 = OH$	Epothilone F



$R^1 = R^2 = H$	Epothilone C
$R^1 = CH_3$, $R^2 = H$	Epothilone D
$R^1 = H$, $R^2 = OH$	Desoxyepothilone E
$R^1 = CH_3$, $R^2 = OH$	Desoxyepothilone F

Not only do epothilones act as tubulin-polymerization agents in the same way that paclitaxel does, but they also compete for the same binding site on the polymer, since they act as competitive inhibitors for the binding of [^{3}H] paclitaxel to tubulin polymer.[7] Recently, a bridged paclitaxel was prepared as a proposed paclitaxel–epothilone hybrid construct. It was shown to promote tubulin assembly nearly as well as paclitaxel, although it was significantly less cytotoxic that either compound alone.[8] This finding was taken as lending support to the hypothesis that paclitaxel and epothilone share a common pharmacophore, but the activity differences noted suggest that critical aspects of the pharmacophore have yet to account for ligand–protein binding. For this reason a study of the interaction of the epothilones with tubulin polymer will not only provide information on the conformation of the epothilones on the tubulin polymer, but may also well provide further

Keywords: epothilone; tubulin; fluorescence; bioactivity.
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doi:10.1016/j.tet.2003.10.024

information on the nature of the paclitaxel tubulin interaction.

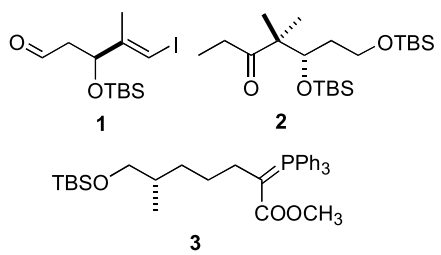
Knowledge of the molecular conformation helps in the development of drugs with the necessary three-dimensional features to bind to the protein target, but with minimal complexity in their structures. Although an X-ray structure of epothilone B has been published,[9] it does not define the structure of the molecule in the bound state on tubulin. Recently, the Taylor group investigated the conformational properties of epothilones in solution, based on computational and 2D NMR methods. They concluded that epothilone A and B prefer two distinct conformations,[10] but this work did not address the conformation of epothilone on the tubulin polymer. We thus elected to approach the question of the interaction of epothilone and tubulin through fluorescence spectroscopic studies using the FRET technique and a REDOR NMR study, similar to the previous studies we have done for paclitaxel.[11]

Fluorescence spectroscopy is widely used to investigate ligand–receptor interactions in biological systems.[12–14] The studies are performed using fluorescently labeled molecules. In this paper we describe the synthesis and cytotoxicity of epothilone analogs and two of their fluorescent congeners. Based on previous structure–activity studies of epothilones,[5,6] structural changes at C_{21} and C_{26} are less detrimental to their activity than changes at other locations, and so we envisioned attaching the fluorescent labels at these sites. Our synthesis was based primarily on Nicolaou's macrolactonization and Stille coupling strategy.[15]

2. Results and discussion

The synthesis of advanced precursor **4** was accomplished from the fragments **1**, **2**, and **3**, which were in turn prepared from commercially available starting materials using literature procedures.[15–17]

The vinyl iodide (**4**) on coupling with stannanes, **5**[15] and **6** (4.5 equiv.) in the presence of $Pd(AcCN)_2Cl_2$ provided the epothilone analogs **7** and **8**,[18] respectively.



Having synthesized the epothilone analogs with a C_{26} hydroxyl group, incorporation of the fluorescent tags remained. We decided to use the 3-aminobenzoyl or 3-(*N*,*N*-dimethylamino)benzoyl group as fluorescent tags, which are the smallest organic fluorophores routinely used in biological systems. Such environmentally sensitive groups on paclitaxel have been successfully used to monitor ligand binding to microtubules, to probe the polarity of the binding site environment, and as donor fluorophores in measurements of FRET.[11] The manipulation was envisioned in two steps, namely coupling of **7** or **8** with *m*-nitrobenzoic acid, followed by reduction of the nitro group. The EDCI (ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride) coupling of epothilone analog **7** with 3-nitrobenzoic acid at room temperature provided the desired derivative **10** in nearly 60% yield, but reduction of the nitro group to the amino group under various hydrogenation conditions only gave complex mixtures of products. It was clear from the proton NMR spectra of the crude product mixture that the double bonds and/or the lactone ring were being affected, and we were unable to find hydrogenation conditions that were selective for the nitro group. Since the supply of compound **10** was exhausted by these preliminary experiments, we elected to pursue a protecting group strategy, starting with *N*-protected benzoic acids. Most of the *N*-protecting groups require strongly acidic or strongly basic reagents, for their deprotection.[19] Of the available amino protecting groups, the trimethylsilylethoxycarbonyl (Teoc) and fluorenemethyloxycarbonyl (Fmoc) groups require comparably mild conditions for their deprotection.[19] The coupling of 3-(*N*-trimethylsilylethoxycarbonyl) amino benzoic acid, prepared from 3-aminobenzoic acid with succinimidyl Teoc in aqueous dioxane,[20] with **7** furnished **11** in nearly 70% yield. Regrettably, however, deprotection posed unprecedented problems. Deprotection of **11** with HF·Py, in pyridine, at 0–60°C gave only TBS deprotected product (**12**), with the N-Teoc group intact. With TBAF at 0°C, the result was the same, and higher temperatures gave a complex mixture. Evidently the lactone ring of epothilone **11** was not compatible with TBAF at high temperatures (Scheme 1).

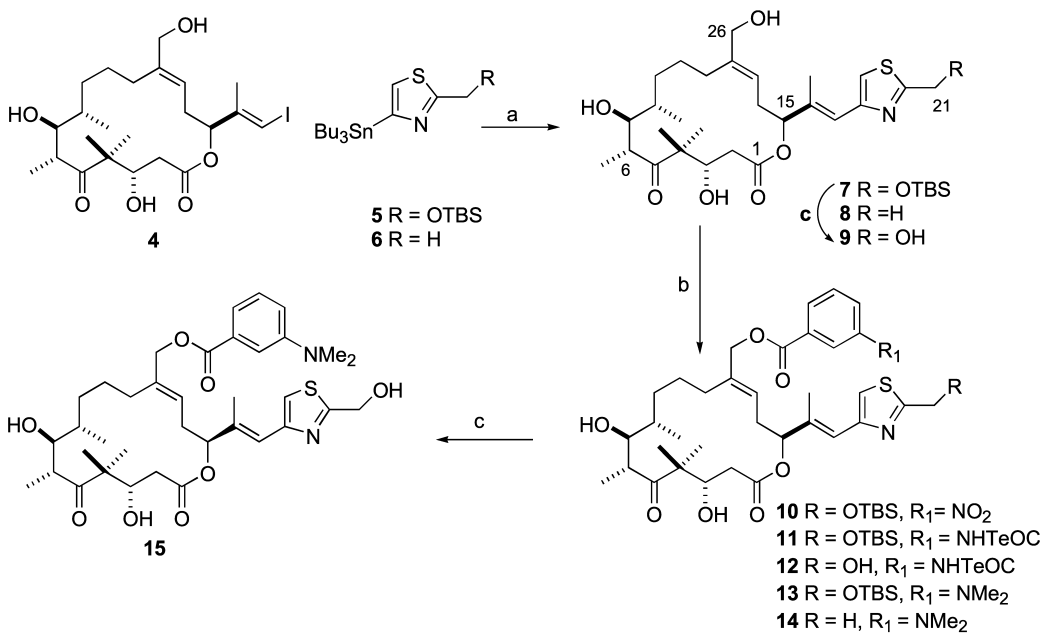
In view of the difficulties encountered in the synthesis of the free amino derivative, we elected to prepare the *N*,*N*-dimethylamino benzoate derivatives as our first target compounds for fluorescence studies.

The analogs **7** and **8** were subjected to EDCI coupling with 3-(*N*,*N*-dimethylamino)benzoic acid to furnish TBS ether **13** and compound **14**. Deprotection of **13** with HF·Py in pyridine at 0°C provided **15** in 92% yield.

The cytotoxicities of the epothilone analogs **8**, **9**, **14**, and **15** were determined towards the A2780 ovarian cancer and PC-3 prostate cancer cell lines (Table 1). Compound **8** was the most cytotoxic, with an activity comparable to that of paclitaxel in the A2780 cell line. The 21-hydroxyl derivative **9** was less active, as were the fluorescent derivatives **14** and **15**. Although these analogs are less cytotoxic than most of the naturally occurring epothilones, their diminished cytotoxicity should not prove a barrier to carrying out the proposed binding studies.

Table 1. Cytotoxicities of epothilones **8**, **9**, **14**, and **15**

Compound	IC_{50}, A2780 (μM)	IC_{50}, PC-3 (μM)
Paclitaxel	0.23	0.05
8	0.59	0.25
9	4.0	1.1
14	6.1	0.50
15	2.7	1.0



Scheme 1. (a) $Pd(AcCN)_2Cl_2$, DMF, rt (69% for **7**, 80% for **8**). (b) **7**, 3-(*N*,*N*-dimethylamino)benzoic acid, EDCl, DMAP, CH_2Cl_2, (**13**, 79%) **8**, 3 (*N*,*N*-dimethylamino)benzoic acid, EDCl, DMAP, CH_2Cl_2, (**14**, 54%). (c) **13**, HF–Py in Py, THF, 0°C, 92–95%.

All compounds induced tubulin to assemble into normal microtubules, as confirmed by electron microscopy. Paclitaxel binding to polymerized tubulin affects the conformation of the protein in a way that favors tubulin assembly, i.e. by increasing the equilibrium constant for polymer growth (K_p). The reciprocal of the critical concentration is a very close approximation of K_p.[21] The critical concentration of tubulin in the presence of **14** and **15** was 0.5 and 1.0 μM, respectively, compared to 0.3 μM for paclitaxel. These data indicate that microtubule binding by these ligands and paclitaxel affect the conformation of tubulin in the same manner.

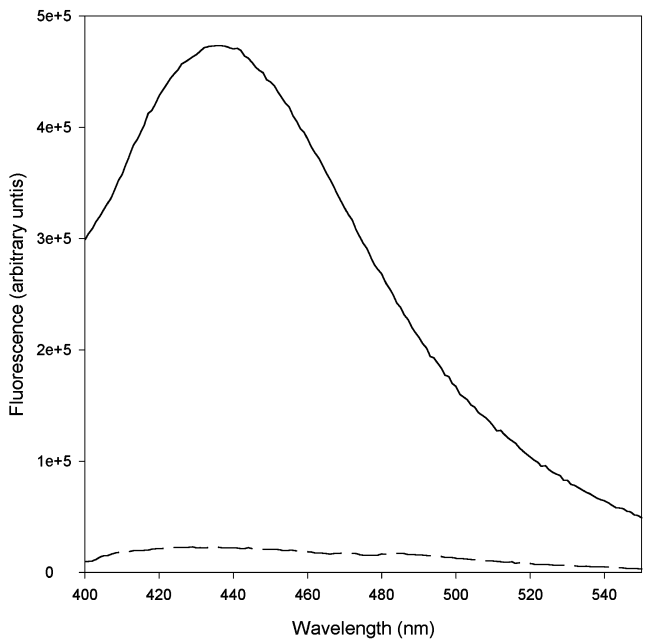


Figure 1. Emission spectrum of **15** bound to microtubules. Tubulin (15 μM) in buffer (0.1 M PIPES, 1 mM $MgSO_4$, 2 mM EGTA, 0.1 mM GTP, pH 6.90) was assembled by incubation at 37°C for 30 min. Compound **15** in DMSO was added to a final concentration of 0.3 μM. The emission spectrum is shown (solid curve). The emission spectrum of 30 μM **15** in ethanol is shown for comparison (dashed curve). The excitation wavelength was 320 nm, and the background has been subtracted in each spectrum. Comparable results were obtained with **14**.

The fluorescent properties of **14** and **15** were examined as a function of solvent and of microtubule binding. The Stokes shift was a linear function of solvent polarity (E_T30). The optical properties of the fluorophore can therefore, be described by general solvent effects.[22] The emission spectrum of **15** in the presence of excess polymerized tubulin is shown in Figure 1. The emission maximum of the microtubule-bound species was similar to that of the fluorophore in DMSO, indicating that the fluorophore's environment on the microtubule is of intermediate polarity.

Microtubule binding also greatly increased the quantum yield of the fluorophore; this property will be useful for quantitative analysis of the microtubule binding parameters of the fluorescent epothilone and for FRET studies.

3. Conclusions

We have synthesized two pairs of epothilone analogs with their fluorescent conjugates (**8**, **14** and **9**, **15**) for comparative fluorescent study; the environment of the fluorophore on the microtubule is in a region of intermediate polarity.

4. Experimental

4.1. General experimental procedures

^1H and ^{13}C NMR spectra were obtained on Varian Unity 400 or JEOL Eclipse 500 spectrometers in $CDCl_3$ at 400 and

100 MHz or at 500 and 125 MHz, respectively. HRFAB and LRFAB mass spectra were obtained on a JEOL HX-110 instrument. NMR and other characterization data for known compounds were identical with reported values.

4.1.1. Compound 4. $[\alpha]_D=-32°$ (*c* 0.2, $CHCl_3$), literature value $[\alpha]_D=-32.1$ (*c* 0.2, $CHCl_3$).[15]

4.1.2. Thiazole 5. Compound **5** was prepared by the literature procedure.[15]

4.1.3. Synthesis of thiazole 6. (a) To a solution of 2,4-dibromo-thiazole (1 g, 4.13 mmol) in ether (20 mL, 0.2 M) was added dropwise *n*-BuLi (1.6 M, 2.84 mL, 4.54 mmol, 1.1 equiv.) at −78°C for 5 min. After complete addition, the reaction mixture was stirred for 45 min. $MeSO_3CF_3$ (0.934 mL, 8.26 mmol, 2 equiv.) was added and stirred for 15 min. Sat. $NaHCO_3$ was added to quench the reaction, followed by water, and the mixture was extracted with ether (3×20 mL). The combined organic extracts were dried and evaporated. The resultant crude mixture was purified by chromatography over silica gel, eluting with 5% ether in hexane, to furnish 2-methyl-4-bromothiazole (300 mg, 40%). ^{1}H NMR (400 MHz): δ 7.00 (s, 1H), 2.68 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.4, 124.2, 116.3, 19.5. (b) To a solution of the above compound (280 mg, 1.55 mmol) in ether (10 mL) was added dropwise *n*-BuLi (1.6 M, 1.73 mL, 1.1 equiv.) at −78°C and the resulting solution was stirred for 15 min at −78°C. Tributyltin chloride (0.53 mL, 1.89 mmol, 1.2 equiv.) was added and the solution stirred for 2 h. Hexane was added and the mixture filtered through a short plug of silica gel, eluting with 30% EtOAc in hexane. Evaporation of the solvent and purification of the resulting crude product on preparative TLC over silica gel with 4% EtOAc in hexane gave **6** (250 mg, 40% yield). ^{1}H NMR (400 MHz): δ 7.18 (s, 1H), 2.78 (s, 3H), 1.58 (m, 6H), 1.36 (m, 6H), 1.12 (m, 6H), 0.88 (t, *J*=7 Hz, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.0, 159.5, 125.2, 29.2, 27.4, 18.9, 13.8, 10.3. HRFABMS *m/z* 390.1299; calcd for $C_{16}H_{32}NSSn$, 390.1279.

4.1.4. Synthesis of 7.[15] To a solution of compound **4** (24 mg, 0.044 mmol) and $Pd(AcCN)_2Cl_2$ (3 mg) was added a degassed solution of stannane **5** (105 mg, 0.20 mmol, 4.5 equiv.) in DMF (1 mL) at room temperature, and the resulting solution was stirred for 24 h. The reaction mixture was then filtered through a short plug of silica gel, eluting with EtOAc. The EtOAc was concentrated and the resulting residue was purified by preparative TLC on silica gel, eluting with 58% EtOAc in hexane, to provide **7** (19.3 mg, 69%). $[\alpha]_D=-44.4°$ (*c* 0.5, $CHCl_3$). ^{1}H NMR (500 MHz) δ 7.06 (s, 1H), 6.58 (s, 1H), 5.44 (dd, *J*=9, 5 Hz, 1H), 5.26 (dd, *J*=9.5, 2 Hz, 1H), 4.90 (s, 2H), 4.25 (m, 1H), 4.06 (d, *J*=13 Hz, 1H), 4.02 (d, *J*=13 Hz, 1H), 3.69 (bs, 1H), 3.42 (d, *J*=5.7 Hz, 1H), 3.15 (qd, *J*=6.8, 3 Hz, 1H), 2.97 (bs, 1H), 2.68 (ddd, *J*=15, 9.7, 9.7 Hz, 1H), 2.46 (dd, *J*=15, 10 Hz, 1H), 2.29 (m, 2H), 2.06 (m, 1H) 2.05 (s, 3H), 1.63 (s, 3H), 1.67 (m, 1H), 1.65 (s, 3H), 1.32 (s, 3H), 1.28 (m, 3H), 1.18 (d, *J*=6.8 Hz, 3H), 1.06 (s, 3H), 0.99 (d, *J*=7.2 Hz, 3H), 0.95 (s, 9H), 0.12 (s, 6H). ^{13}C NMR (125 MHz) δ 220, 172.7, 170.5, 152.3, 142.1, 138.7, 122.0, 119.7, 116.2, 78.8, 74.0, 72.4, 66.5, 63.2, 53.7, 41.9, 39.8, 38.1, 32.2, 31.9, 28.1, 28.0, 27.0, 25.9, 25.6, 22.9, 18.3, 17.7, 16.1, 16.0, 13.8, −5.2. HRFABMS *m/z* 638.35620; calcd for $C_{33}H_{56}NO_7SSi$, 638.3547.

4.1.5. Synthesis of 8. Treatment of **4** with thiazole **6** by the same procedure as described above for reaction of **4** and **5**, furnished **8**[18] with some minor tin impurities (7.5 mg, 80%). $[\alpha]_D=-25.0°$ (*c* 0.185, $CHCl_3$). ^{1}H NMR (400 MHz) δ 6.96 (s, 1H), 6.60 (s, 1H), 5.44 (dd, *J*=7, 5 Hz, 1H), 5.24 (dd, *J*=7, 2 Hz, 1H), 4.30 (m, 1H), 4.15 (dd, *J*=13, 2 Hz, 1H), 4.06 (dd *J*=13, 2 Hz, 1H), 3.69 (bs, 2H), 3.19 (qd, *J*=6.8, 3 Hz, 1H), 3.01 (bs, 1H), 2.68 (s, 3H), 2.52 (ddd, *J*=16, 7, 7 Hz, 1H), 2.45 (dd, *J*=15, 10 Hz, 1H), 2.36–2.30 (m, 3H), 2.10 (m, 1H), 2.06 (s, 3H), 1.80 (bs, 1H), 1.75 (s, 3H), 1.35 (s, 3H), 1.18 (d, *J*=6.8 Hz, 3H), 1.06 (s, 3H), 1.01 (d, *J*=7.2 Hz, 3H). ^{13}C NMR (100 MHz) δ 220.9, 170.5, 165.3, 152.0, 142.1, 139.0, 122.0, 119.4, 115.8, 78.5, 74.0, 72.2, 66.4, 53.9, 41.7, 39.8, 38.1, 32.2, 31.8, 28.0, 25.4, 23.0, 19.2, 17.7, 16.2, 16.0, 13.3. HRFABMS *m/z* 508.2730; calcd for $C_{27}H_{42}NO_6S$, 508.2733.

4.1.6. Synthesis of 9. To a solution of **7** (7.1 mg, 0.011 mmol), in THF (1.4 mL) was added a stock solution of HF·Py in pyridine, prepared by addition of HF·Py (0.1 mL) to pyridine (0.28 mL) in THF (0.5 mL, at 0°C, and the resulting solution was stirred for 2.5 h at 0°C. Sat. $NaHCO_3$ solution was added to quench the reaction followed by extraction with EtOAc (15 mL×3). The combined organics were dried and evaporated. The resulting residue was purified by preparative TLC on silica gel, eluting with EtOAc, to give **9** (5.5 mg, 95%). $[\alpha]_D=-27.8°$ (*c* 0.165, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (s, 1H), 6.61 (s, 1H), 5.46 (dd, *J*=9, 5 Hz, 1H), 5.28 (d, *J*=7.2 Hz, 1H), 4.9 (s, 2H), 4.27 (d, *J*=8.4 Hz, 1H), 4.08 (d, *J*=13.2 Hz, 1H), 4.00 (d, *J*=13.2 Hz, 1H), 3.68 (dd, *J*=4, 2.6 Hz, 1H), 3.55 (bs, 1H), 3.15 (qd, *J*=7, 2.5 Hz, 1H), 3.00 (bs, 1H), 2.65 (m, 1H), 2.48 (d, *J*=11 Hz, 1H), 2.45 (d, *J*=11 Hz, 1H), 2.35 (dd, *J*=14, 3 Hz, 1H), 2.29 (d, *J*=2.4 Hz, 1H), 2.25 (m, 1H), 2.12 (m, 1H), 2.05 (s, 3H), 1.75 (m, 2H), 1.34 (m, 1H), 1.32 (s, 3H), 1.15 (d, *J*=6.8 Hz, 3H), 1.05 (s, 3H), 1.00 (d, *J*=7.2 Hz, 3H). ^{13}C NMR (100 MHz) δ 220.7, 170.4, 142.1, 139.4, 133.1, 127.3, 121.6, 118.9, 116.6, 78.5, 74.1, 72.2, 66.4, 53.8, 41.9, 39.8, 38.1, 31.9, 31.8, 28.3, 25.6, 23.1, 17.9, 16.2, 16.0, 13.5. HRFABMS *m/z* 544.4951; calcd for $C_{27}H_{41}NO_7SNa$, 546.2501.

4.1.7. Synthesis of 13. To a solution of 3-dimethyl-aminobenzoic acid (6.7 mg, 0.042 mmol) and DMAP (2–3 mg) in CH_2Cl_2 (1 mL) was added **7** (10 mg, 0.01564 mmol) in CH_2Cl_2 (1 mL), followed by EDCI (10 mg, 0.052 mmol, 1.2 equiv. to acid) and the resulting solution was stirred for 6 h. EtOAc was then added to quench the reaction and the solution concentrated. The resulting residue was purified by preparative TLC on silica gel, eluting with 30% EtOAc in hexane gave **13** (9.8 mg, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, *J*=2.6 Hz, 1H), 7.37 (d, *J*=7.6 Hz, 1H), 7.25 (t, *J*=8.8 Hz, 1H), 6.98 (s, 1H), 6.88 (dd, *J*=8, 2 Hz, 1H), 6.54 (s, 1H), 5.56 (dd, *J*=9, 6 Hz, 1H), 5.27 (dd, *J*=9, 5 Hz, 1H), 4.92 (s, 3H), 4.82 (d, *J*=12.7 Hz, 1H), 4.64 (d, *J*=12.7 Hz, 1H), 4.26 (d, *J*=10 Hz, 1H), 3.69 (bs, 1H), 3.16 (qd, *J*=6.8, 2.4 Hz, 2H), 2.98 (s, 6H), 2.89 (bs, 1H), 2.49 (m, 1h), 2.45 (m, 2H), 2.27 (m, 2H), 2.19 (m, 1H) 2.08 (s, 3H), 1.74 (bs, 3H), 1.38

(m, 1H), 1.37 (s, 3H), 1.25 (s, 1H), 1.17 (d, J=6.8 Hz, 3H), 1.06 (s, 3H), 1.00 (d, J=7 Hz, 3H), 0.96 (s, 9H), 0.13 (s, 6H). ^{13}C NMR (100 MHz) δ 220.7, 172.6, 170.4, 167.2, 152.4, 150.69, 138.4, 137.3, 131.0, 129.2, 124.5, 119.8, 117.6, 117.0, 116.3, 113.4, 78.4, 77.4, 74.2, 72.4, 68.0, 63.3, 53.6, 42.0, 40.7, 39.7, 38.2, 32.1, 31.8, 29.9, 28.6, 25.9, 25.6, 22.9, 18.4, 18.3, 16.07, 16.03, 13.6, −5.1. HRFABMS m/z 785.4243; calcd for $C_{42}H_{65}N_2O_8SSi$ 785.4231.

4.1.8. Synthesis of 14. Treatment of **8** by the same as described above yielded **14** (5.2 mg, 54%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, J=2.6 Hz, 1H), 7.36 (d, J=7.8 Hz, 1H), 7.26 (t, J=7.8 Hz, 1H), 6.90 (s, 1H), 6.89 (d, J=3 Hz, 1H), 6.58 (s, 1H), 5.58 (dd, J=9, 5.7 Hz, 1H), 5.26 (dd, J=8.7, 1.9 Hz, 1H), 4.80 (d, J=12.7 Hz, 1H), 4.66 (d, J=12.7 Hz, 1H), 4.31 (bd, J=11.3 Hz, 1H), 3.68 (m, 2H), 3.16 (qd, J=6.8, 2.2 Hz, 2H), 2.98 (s, 6H), 2.88 (s, 1H), 2.69 (s, 3H), 2.66 (m, 1H), 2.42 (m, 2H), 2.28 (m, 3H), 2.06 (s, 3H), 1.78 (bs, 1H), 1.38 (m, 2H), 1.35 (s, 3H), 1.26 (s, 1H), 1.16 (d, J=6.8 Hz, 3H), 1.05 (s, 3H), 1.00 (d, J=7 Hz, 3H). ^{13}C NMR (100 MHz) δ 220.9, 170.4, 167.1, 151.1, 137.3, 131.0, 129.3, 124.9, 117.1, 115.7, 113.5, 78.1, 77.4, 74.2, 71.9, 68.1, 54.0, 41.7, 40.8, 39.8, 38.3, 32.1, 31.8, 30.0, 28.6, 25.5, 23.1, 19.0, 17.6, 16.5, 15.9, 13.5. HRFABMS m/z 655.3412; calcd for $C_{36}H_{51}N_2O_7S$, 655.3417.

4.1.9. Synthesis of 15. To a solution of **13** (6.4 mg, 0.0081 mmol), in THF (1.5 mL) was added a stock solution of HF·Py in pyridine, prepared by addition of HF·Py (0.1 mL) to pyridine (0.28 mL) in THF (0.5 mL, at 0°C, and the resulting solution was stirred for 6 h at 0°C. Aqueous $NaHCO_3$ was added to quench the reaction, and the resulting solution was extracted with EtOAc (3×15 mL). The combined organic extracts were dried and evaporated. The resulting residue was purified by preparative TLC on silica gel, eluting with 65% EtOAc in hexane, to give **15** (5 mg, 92%). $[\alpha]_D$=−21.8° (c 0.11, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, J=2 Hz, 1H), 7.36 (d, J=7.6 Hz, 1H), 7.25 (t, J=6.8 Hz, 1H), 7.0 (s, 1H), 6.90 (dd, J=7.5, 2.6 Hz, 1H), 6.55 (s, 1H), 5.60 (dd, J=9, 6.4 Hz, 1H), 5.30 (dd, J=8.3, 2.4 Hz, 1H), 4.89 (d, J=3.5 Hz), 4.83 (d, J=13 Hz, 1H), 4.65 (d, J=13 Hz, 1H), 4.28 (m, 1H), 3.68 (bd, J=3.7 Hz, 1H), 3.45 (d, J=6 Hz, 1H), 3.26 (bs, 1H), 3.16 (qd, J=2.68, 1H), 2.97 (s, 6H), 2.88 (d, J=2 Hz, 1H), 2.66 (m, 1H), 2.46 (m, 2H), 2.26 (m, 3H), 2.0 (m, 3H), 1.70 (m, 2H), 1.68 (s, 3H), 1.37 (m, 1H), 1.36 (s, 3H), 1.25 (s, 1H), 1.17 (d, J=6.8 Hz, 3H), 1.06 (s, 3H), 1.00 (d, J=7 Hz, 3H). ^{13}C NMR (100 MHz) δ 220.5, 170.3, 170.0, 167.2, 152.4, 150.7, 138.8, 137.2, 129.2, 127.7, 124.2, 119.2, 117.7, 117.2, 116.7, 113.4, 78.2, 77.4, 74.4, 72.2, 68.0, 62.1, 53.7, 42.0, 40.7, 39.7, 38.2, 31.9, 31.7, 28.6, 25.6, 23.8, 18.0, 16.2, 15.9, 13.7. HRFABMS m/z 671.3350; calcd for $C_{36}H_{51}N_2O_8S$, 671.3336.

4.2. Biological data and fluorescence measurements

MAP-free bovine brain tubulin was used for all in vitro studies. The ability of the paclitaxel derivatives to induce purified tubulin to assemble into microtubules was assessed by light scattering (apparent absorption at 350 nm), and all experiments were carried out at 37°C in PMEG buffer containing 4% DMSO. The critical concentration of tubulin was determined in the presence of 20 μM paclitaxel or 28 μM **14** or **15**. The concentrations of ligand were chosen to ensure that the receptor site was saturated with ligand.[23] The extent of assembly was measured at different tubulin concentrations (0.5–6 μM). Critical concentrations were calculated from the x-intercepts of plots of apparent $A_{350\ nm}$ vs tubulin concentration.

Absorption and emission spectra of the fluorophores in solvent and bound to microtubules were measured and the Stokes shifts were calculated in standard ways, described in detail in Han et al.[24] for a fluorescent derivative of paclitaxel.

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