Paclitaxel Analogues from *Taxus* × *media* cv. Hicksii

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The roots of T. × *media* Rehd. cv. Hicksii gave three novel analogues of paclitaxel modified at the *N*-acyl residue (*N*-debenzoyl-*N*-α-methylbutyryl paclitaxel and *N*-debenzoyl-*N*-cinnamoyl paclitaxel, **1b** and **1c**, respectively) or at the ester group at C-2 (2-debenzoyl-2-tigloyl paclitaxel, **1d**). Compounds **1b** and **1d** showed reduced cytotoxicity and tubulin binding compared to paclitaxel, while **1c** retained substantial activity in these assays.

Over the past few years, there has been extensive structure−activity work in the paclitaxel (Taxol, **1a**) area, spurred by present difficulties in the clinical administration of this natural product and by its limited activity against multiple-drug resistant tumors.[1] Apart from docetaxel (Taxotere),[2] none of the semisynthetic analogues has so far reached the status of human clinical trials. Nevertheless, these studies have established a database of structure−activity relationships broad enough to assist the mapping of the taxoid recognition site on tubulin[3] and to direct the design of combinatorial libraries for targeting purposes.[4] Comparatively little systematic work has been done instead on the occurrence of natural analogues of paclitaxel in yew extracts, although early studies have indicated the presence of compounds with a different *N*-acylation pattern (e.g., cephalomannine[5] and paclitaxel C derivatives[6]). Naturally occurring analogues of plant prototypes can still prove useful in medicine, with relevant examples being the compound pairs morphine−codeine, quinine−quinidine, and vinblastine−vincristine. The study of "natural diversity" within the paclitaxel pharmacophore might thus afford interesting clues not only to track the biogenetic pathway to the natural product, but also for drug design and for the many emerging nononcological applications of paclitaxel-like compounds.[7] As part of a systematic search for paclitaxel analogues in yew extracts,[8] we report here the characterization of three analogues having structural features not yet addressed in studies aimed at the semisynthetic modification of the natural product.

Results and Discussion

Compound **1b** was obtained by repeated separation (RP-18 column chromatography) of the mother liquors of paclitaxel obtained from the roots of *T.* × *media* Rehd. cv. Hicksii. HRMS established a molecular weight of 833 amu for **1b**, corresponding to the formula $C_{45}H_{55}NO_{14}$. The ^{1}H NMR spectrum displayed signals indicative of a baccatin III derivative esterified with a *N*-substituted phenylisoserine side chain. The *N*-acyl residue was identified as α-methylbutyric acid on the basis of diagnostic ^{1}H NMR signals [deshielded multiplet at δ 2.20, coupled to a methyl signal (δ 1.10) and to the two diastereotopic protons of a methylene (δ 1.64 and 1.39), in turn coupled to another methyl (δ 0.87)]. These assignments were confirmed by ^{13}C NMR spectroscopy (Table 2). Thus, **1b** is *N*-debenzoyl-*N*-(2-methylbutanoyl)paclitaxel. The α-methylbutyryl residue is typically found bound to the 14-hydroxyl of the terpenoid core of taxoids, and has not yet been detected on the side chain.[8] The 2-methylbutanoyl moiety of taiwanxan was shown by X-ray crystallography to have an *S* configuration at C-2.[9] No attempt has been made to establish the configuration of this moiety in **1b**. Compound **1b** showed reduced cytotoxicity and tubulin binding compared to *N*-debenzoyl-*N*-butanoylpaclitaxel[10] and paclitaxel itself (Table 3), showing that α-branching on the *N*-acyl group is detrimental for activity.

Compounds having a MS fragmentation pattern indicative of the gross structures **1c** and **1d** were also detected by HPLC−MS of the fractions containing **1b**. Thus, the detection of diagnostic $[M + H]^+$, $[MH - ScOH]^+$, and $[ScOH + H]^+$ peaks at *m*/*z* 880, 586, and 312 for **1c** and *m*/*z* 832, 547, and 286 for **1d** suggested that **1d** had the side chain of paclitaxel, and **1c** a side chain where, assuming the connectivity of phenylisoserine, the *N*-benzoyl group was replaced by a cinnamoyl moiety. The $[MH - ScOH]^+$ peak and its further fragmentations suggested that **1c** was a derivative of baccatin III, while in **1d** the benzoyl group was replaced by a C-5 unsaturated acid having the molecular weight (100) of one of the so-called false hemiterpenoid acids.[11] Among the substituents fulfilling these requirements, senecic and angelic acids seemed unlikely, being unknown in yew constituents and typical of higher plants instead. Tiglic acid seemed instead a plausible candidate, because it occurs in cephalomannine-type yew alkaloids.[8] Furthermore, 2-debenzoyl-2-tiglioyl-10-deacetylbaccatin III has been reported as a yew constituent.[12] The very low concentration of compounds **1c** and **1d** (ca. 0.02 and 0.05% of the mother liquors, respectively) precluded their isolation. To confirm the tentative identifications, compounds corresponding to **1c** and **1d** were synthesized, and their HPLC profiles and MS fragmentations were compared to those of the natural products. The synthesis of **1c** was executed according to an established procedure for the synthesis of *N*-debenzoyl-*N*-acyl derivatives of paclitaxel already employed for the synthesis of other natural analogues.[13] Thus, 7-triethylsilyl (TES) baccatin III was esterified with the protected oxazolidine **2**, obtained from (2*R*,3*S*)-phenylisoserine by Schotten−Baumann amidation with cinnamoyl chloride and reaction with 2,4-dimethoxybenzaldehyde.[14] After removal of the

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Table 1. ^{1}H NMR Data for Compounds **1b**−**1d** ($CDCl_3$, *J* in Hz)[a,b]

position	1b	1c	1d
2	5.69 d (7.2)	5.67 d (7.1)	5.49 d (7.1)
3	3.79 d (7.2)	3.8′ d (7.1)	3.70 d (7.1)
5	4.95 dd (9.5, 1.8)	4.93 dd (9.5, 2.0)	4.94 dd (9.5, 2.0)
6α	2.55 m	2.48 m	2.54 ddd
6*β*	1.88 m	1.85 m	1.90 m
7	4.39 m	4.39 m	4.35 m
10	6.31 s	6.28 s	6.24 s
13	6.17 tq (9.1, 9.1, 1.0)	6.23 tq (8.7, 8.7, 1.5)	6.20 tq (8.7, 8.7, 1.5)
14α,*β*	2.29 m	2.28 m	2.24 m
16	1.14 s	1.12 s	1.09 s
17	1.23 s	1.22 s	1.22 s
18	1.88 d	1.82 d	1.76 d
19	1.68 s	1.68 s	1.64 s
20α	4.29 d (8.0)	4.29 d (7.9)	4.38 d (7.9)
20*β*	4.21 d (8.0)	4.19 d (7.9)	4.16 d (7.9)
2′	4.65 br d (2.9)	4.74 br d (2.8)	4.76 br d (2.8)
3′	5.59 dd (8.9, 2.9)	5.70 dd (7.0, 2.8)	5.75 dd (7.0, 2.8)
OAc-4	2.37 s	2.38 s	2.27 s
OAc-10	2.23 s	2.20 s	2.22 s
NH-3′	7.06 d	6.46 d	6.96 d
Ph-3′	7.32−7.28 m	7.30−7.18 m	7.30−7.08 m

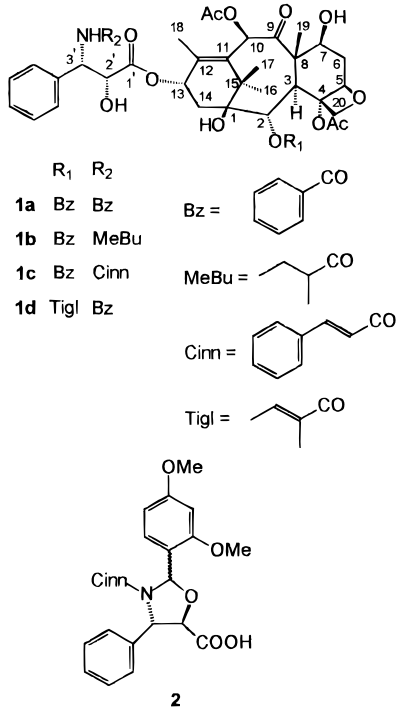
[a] Other signals (*δ*) for **1b**: Bz-2, 8.11 (AA′), 7.61 (BB′), 7.51 (C); *N*-α-MeBu, 2.20 (qd, *J* = 6.7, 1.4 Hz, H-2), 1.64 (m, H-3a), 1.39 (ddq, *J* = 13.3, 7.5, 1.4 Hz, H-3b), 0.87 (t, *J* = 7.4 Hz, H-4), 1.10 (d, *J* = 6.7 Hz, 2-Me). For **1c**: Bz-2, 8.10 (AA′), 7.65 (BB′), 7.25 (C); *N*-Cinn, 6.45 (d, *J* = 15.6 Hz, H-2), 5.70 (d, *J* = 15.6 Hz, H-3), 7.24−8.10 (3-Ph). For **1d**: Tigl-2, 7.03 (qq, *J* = 7.0, 1.2 Hz, H-3), 1.88 (d, *J* = 1.2 Hz, 2-Me), 1.87 (d, *J* = 7.0 Hz, 3-Me), *N*-Bz, 7.30−7.81 (m). [b] Significant NOEs (s = strong, m = medium, w = weak) in DMSO−H_2O for **1d** as representative: 4-acetyl, 3′-phenyl (s), 4-acetyl, H-3 tigl (w); 4-acetyl, H-3′ (m); 4-acetyl, H-2′ (m); 4-acetyl, tigl α-methyl (m); 3′-Ph, tigl-α-methyl (m); 3′-Phe, H-4 tigl (w).

Table 2. ^{13}C NMR Data for Compounds **1b**−**1d** ($CDCl_3$)[a]

carbon	1b	1c	1d
1	78.6 s	78.3 s	78.6 s
2	74.9 d	74.5 d	71.7 d
3	45.5 d	45.3 d	45.1 d
4	81.0 s	80.7 s	80.6 s
5	84.3 d	84.0 d	84.0 d
6	35.6 t	35.2 t	35.1 t
7	71.9 d	73.1 d	72.0 d
8	58.4 s	57.9 s	58.1 s
9	203.7 s	203.4 s	203.1 s
10	75.6 d	75.2 d	75.1 d
11	138.5 s	134.1 s	132.8 s
12	142.1 s	141.6 s	141.4 s
13	72.0 d	71.5 d	72.7 d
14	35.5 t	35.2 t	35.0 t
15	43.2 s	42.7 s	42.6 s
16	22.5 q	21.4 q	21.2 q
17	26.6 q	26.3 q	26.4 q
18	14.0 q	14.4 q	14.3 q
19	9.5 q	9.1 q	9.0 q
20	76.4 t	76.0 t	76.0 t
1′	172.8 s	172.4 s	172.3 s
2′	73.4 d	71.5 d	73.9 d
3′	54.4 d	54.8 d	54.5 d
Ph (*ipso*)-3′	133.0 s	128.9 s	133.3 s
Ph (*ortho*)-3′	126.8 d	129.8 d	126.6 d
Ph (*meta*)-3′	128.6 d	128.3 d	128.3 d
Ph (*para*)-3′	127.7 d	133.3 d	131.6 d

[a] Assignments confirmed by HMBC and HMQC experiments. Other signals. For **1b**: Bz-2, 166.6 (s), 129.3 (s, *ipso*), 130.1 (d, *ortho*), 128.7 (d, *meta*), 133.4 (d, *para*); OAc-4, 170.1 (s), 22.6 (q); OAc-10, 171.0 (s), 26.8 (q); *N*-MeBu, 176.1 (s, C-1), 42.9 (d, C-2), 27.0 (t, C-3), 14.0 (q, C-4), 17.4 (q, 2-Me). For **1c**: Bz-2, 166.4 (s), 128.9 (s, *ipso*), 129.8 (d, *ortho*), 128.3 (d, *meta*), 133.3 (d, *para*); OAc-4, 170.1 (s), 22.1 (q); OAc-10, 170.8 (s), 20.3 (q); *N*-Cinn, 165.7 (s), 119.5 (d, C-2), 141.7 (d, C-3), 137.8 (s, *ipso*), 127.4 (d, *ortho*), 128.4 (d, *meta*), 127.7 (d, *para*). For **1d**: Tigl-2, 168.0 (s, C-1), 127.5 (s, C-2), 139.8 (d, C-3), 13.6 (q, C-4), 11.5 (q, C-2 Me); OAc-4, 169.8 (s), 22.0 (q); OAc-10: 170.8 (s), 20.3 (q); *N*-Bz, 166.6 (s), 137.6 (s, *ipso*), 126.6 (d, *ortho*), 128.6 (d, *meta*), 127.9 (d, *para*).

protecting groups with HCl in methanol, **1c** was obtained as a colorless powder. The synthesis of **1d** required the replacement of the 2-benzoyl group of paclitaxel or baccatin III with a tigloyl group. Several semisynthetic 2-debenzoyl-2-acyl derivatives of paclitaxel are known,[15] but attempts to esterify 13-dehydro-7-TES-2-debenzoylbaccatin III with tiglic acid were reportedly hampered by the increased steric demand caused by the α-substituent on the acyl moiety.[16] We were thus surprised to observe that 2-debenzoyl-2′-TBS-7-TES-paclitaxel[17] (TBS = *tert*-butyldimethylsilyl) could instead be smoothly esterified with tiglic acid. After removal of the protecting groups, **1d** was eventually obtained as a colorless powder. The reasons for the higher reactivity of 2-debenzoylpaclitaxel derivatives compared to the corresponding 2-debenzoyl-13-dehydrobaccatin III derivatives are not immediately obvious.



Synthetic **1c** and **1d** showed HPLC profiles and MS fragmentation patterns matching those of the natural

Table 3. In vitro Biological Evaluation of Paclitaxel Analogues **1b**−**1d**[22]

compound	microtubule assembly assay [ED_{50}/ED_{50}(paclitaxel)]	MCF7 [ED_{50}/ED_{50}(paclitaxel)]	MCF7−R [ED_{50}/ED_{50}(paclitaxel)]
1b	6.7[a]	>10	*c*
1c	1.2	1.3	1.2
1d	1.1	12[b]	*c*

[a] Corresponding value for paclitaxel D: 2.04.[10a] [b] Corresponding value for 2-debenzoyl-2-senecyoylpaclitaxel: 0.65.[16] [c] ED_{50} > 5000 nM (for paclitaxel ED_{50} = 1940 nM).

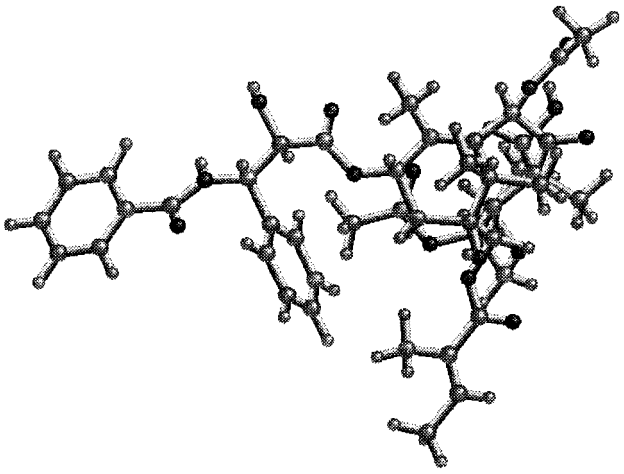


Figure 1. Ball-and-stick representation (from Sybyl 6.4) of **1d** in a conformation consistent with the NOESY data in DMSO−H_2O.

products, backing up the assignment of **1c** as *N*-debenzoyl-*N*-cinnamoylpaclitaxel and **1d** as 2-debenzoyl-2-tigloylpaclitaxel (= isocephalomannine). Compound **1d** is the first natural analogue of paclitaxel with a modified ester group at C-2.

The cinnamoyl and the tigloyl residues are widespread among taxoids,[8] but their location in **1c** and **1d** is unusual, since the cinnamoyl group is typically bound via an ester bond to the terpenoid core[8] and the tigloyl residue via an amide bond to the side chain.[8,12] It was thus interesting to evaluate the biological activity of these compounds, which feature structural elements not yet addressed among semisynthetic taxoid analogues. Compound **1d** displayed decreased cytotoxicity and tubulin binding compared to both paclitaxel and its 2-debenzoyol-2-senecioyl analogue (Table 3),[16] showing that α-branching is detrimental for activity also on the acyl moiety at C-2. On the other hand, the *N*-debenzoyl-*N*-cinnamoyl analogue (**1c**) displayed cytotoxicity and tubulin binding comparable to those of paclitaxel, suggesting that vinylation of the *N*-benzoyl group is well tolerated.

The side chain of paclitaxel adopts a different conformation in apolar and polar solvents, where hydrophobic clustering of the acyl residues at C-2, C-4, and the side chain takes place (hydrophobic collapse).[18] Though the relevance of this conformational change for tubulin binding is still unclear, the three new analogues **1b**−**1d** differ from paclitaxel in one of the structural elements involved in the "hydrophobic collapse," and their conformational behavior was thus investigated. All three analogues showed evidence of hydrophobic clustering in DMSO−H_2O, as shown by diagnostic NOEs between the 3′-phenyl and the acyl moiety at C-2 (Table 1). Interestingly, in **1d**, the strongest NOEs of the tigloyl moiety at C-2 with the 3′-phenyl and the 4-acetyl were, in regard to the α-methyl, resulting in a cluster more closely packed than that obtainable by interaction(s) of the terminal methyl (Figure 1). This type of clustering is presumably the one taking place in the more active 2′-senecioyl analogue.

Experimental Section

General Experimental Procedures. Melting points were determined on a Büchi SMP-20 apparatus and are uncorrected. IR spectra were recorded on a Perkin−Elmer model 237 spectrophotometer. ^1H and ^{13}C NMR spectra were obtained on a Bruker AM 400 spectrometer (400 and 100 MHz, respectively). ^1H and ^{13}C NMR chemical shifts refer to C*H*Cl_3 at 7.26 ppm, and *C*DCl_3 at 77.0 ppm, respectively. NOESY spectra were acquired on a Bruker AM-500 instrument, operating at 500.13 MHz for ^1H, at a temperature of −10 °C. Samples (2 mg) were dissolved in 75% DMSO-d_6−25% D_2O. The mixing time was 400 ms. HRMS were taken on a MAT 95ST Finnigan MAT apparatus (70 eV, EI mode). Positive DCI/NH_3 spectra were obtained on a Finnigan-MAT 4610 quadrupole instrument equipped with superincos data system and a ppinici device, using the direct exposure probe. Si gel 60 (70−230 mesh, Merck) and LiChroprep RP-18 (25−40 μm, Merck) were used for open column chromatography on normal and reversed-phase, respectively.

Plant Material. Commercial roots of *T.* × *media* Rehd. cv. Hicksii were employed. A voucher specimen is kept at the Botanical Department, Indena S.p.A., Settala (sample BE 14200, BA 47498).

Liquid Chromatography−Thermospray Mass Spectrometry. The HPLC−MS system used included a Waters 600-MS pump/system controller and Waters 486 tunable UV/vis detector. Injections were performed with a Waters 717 plus autosampler. A Zorbax SB-CN (250 × 4.6 mm i.d., 5 μm) column was used. The mobile phase composition was eluent A (H_2O−MeOH 8:2), eluent B (CH_3CN−MeOH 8:2), in gradient conditions (eluant A from 99% to 35% in 65 min). The flow rate was 1 mL min^{-1}, and the UV detector was set at 227 nm. The liquid chromatography system was connected to a Finnigan-MAT TSQ triple quadrupole mass spectrometer equipped with a TSP-2 thermospray interface and 5100 DEC station with an ICIS data system. Mass spectrometer conditions were optimized in order to achieve maximal sensitivity. Typical values were as follows: source block temperature 230 °C, vaporizer temperature 70 °C, repeller voltage 30 V, discharge 1800 V, filament off-mode. The electron multiplier and dynode voltages were set to 2000 V and 15 kV, respectively. Preamplifier sensitivity was −8−10 A/V. Positive thermospray spectra from *m*/*z* 200 to 1000 (scan time 1.5 s) were obtained scanning the Q3 analyzer and acquiring in centroid mode.

Analysis of the Mother Liquors of Paclitaxel from *T.* × *media* cv. Hicksii−Isolation of 1b. Paclitaxel was isolated from the roots of *T.* × *media* cv. Hicksii according to the method previously described,[19] and was recrystallized from aqueous MeOH. HPLC−MS analysis of the mother liquors showed, along with known analogues,[20] three unidentified phenylisoserine esters, accounting for ca. 0.05, 0.02, and 0.5% of the material. To isolate the most abundant of these compounds, a sample of mother liquors (8.5 g, obtained from the recrystallization of 28 g of paclitaxel, in turn, obtained from 260 kg of plant material) was repeatedly separated by column chromatography on reversed-phase si gel, using a CH_3CN−H_2O−MeOH (4:4:2) eluent mixture. Eventually, 350 mg of **1b** were obtained (yield 0.014 × 10^{-3} %). The two other unidentified peaks were very close to the peaks of cephalomannine (**1d**, 0.05%) and 7-epipaclitaxel (**1c**, 0.02%). Attempts to achieve a concentration of these minor compounds by repeated crystallizations failed.

***N*-Debenzoyl-*N*-(2-methylbutyryl)paclitaxel (1b):** white powder, mp 226 °C; $[\alpha]^{25}_D$ −48° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3438, 2961, 1730, 1714, 1638 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; CIMS (NH_3) *m/z* 851 $[M + NH_4]^+$ (3), 729 $[M - PhCOOH]^+$ (2), 586 $[M - ScH]^+$ (10), 568 $[M - ScOH]^+$ (7), 464 [*m/z* 586 − PhCOOH]$^+$ (100), 446 [*m/z* 464 − $H_2O]^+$ (41), 283 $[ScOH + NH_4]^+$ (93), 266 $[ScOH + H]^+$ (86), 248 [*m/z* 266 − $H_2O]^+$ (44); HREIMS *m/z* 833.3619 $[M]^+$ (0.5) (calcd for $C_{45}H_{55}NO_{14}$, 833.3622).

Synthesis of *N*-Debenzoyl-*N*-cinnamoylpaclitaxel (1c). (a) Synthesis of (2*S*,3*R*)-*N*-cinnamoylphenylisoserine 2,4-dimethoxybenzal derivative **2**: A solution of of *N*-BOC-2*R*,3*S*-phenylisoserine methyl ester (2.0 g, 6.8 mmol)[21] in MeOH (30 mL) was treated with concentrated HCl (2 mL). The mixture was heated while being stirred in an oil bath for 8 h. The solvent was then removed under vacuum and the residue diluted with CH_2Cl_2 (25 mL) and saturated with $NaHCO_3$ (25 mL). After stirring for 10 min at room temperature, cinnamoyl chloride (1.35 g, 8.1 mmol, 1.19 mol equivalents) was added. Stirring was continued for 3 h at room temperature, and the reaction was then worked up by dilution with H_2O and extraction with CH_2Cl_2. The residue obtained after drying ($MgSO_4$) and removal of the solvent was dissolved in dry THF (50 mL) and treated with pyridinium-*p*-toluenesulfonate (90 mg) and with an excess 2,4-dimethoxybenzaldehyde dimethylacetal (10 g). After distillation at ambient pressure to remove ca. half of the solvent, the solution was evaporated, and the residue was dissolved in MeOH (60 mL) and treated with a solution of K_2CO_3 (1.4 g) in H_2O (3.0 mL). After being stirred overnight at room temperature, the solvent was evaporated, and the residue partitioned between H_2O and EtOAc. The H_2O phase was then acidified (5% $KHSO_4$) and extracted with EtOAc to afford crude **2**. The latter was used for the next step without further purification.

(b) Synthesis of **1c** from **2** and 7-TES-baccatin III: To a solution of **2** in toluene (60 mL), DCC (1 g), and DMAP (200 mg) were added. After being stirred for 10 min at room temperature, 7-TES-baccatin III (750 mg) was added. The solution was stirred at 70 °C for 2 h, filtered, and evaporated. The residue was dissolved in 0.2 N MeOH hydrochloric acid (30 mL) and stirred for 1 h. After evaporation of the solvent, the residue was taken up in CH_2Cl_2 and washed with saturated $NaHCO_3$ and brine. After drying (Na_2SO_4) and evaporation of the solvent, the residue was purified by column chromatography (Si gel, CH_2Cl_2−EtOH, 98:2 as eluent) to afford 750 mg **1c** as a white powder: mp 180 °C (dec); $[\alpha]^{25}_D$ −16.6° (*c* 0.90, MeOH); IR (KBr) ν_{max} 3412, 2936, 1724, 1661, 1626 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; CIMS (NH_3) *m/z* 897 $[M + NH_4]^+$ (3), 880 $[M + H]^+$ (4), 862 [*m/z* 880 − $H_2O]^+$ (1), 586 $[M - ScH]^+$ (100), 568 $[M - ScOH]^+$ (17), 551 [*m/z* 862 − ScOH]$^+$(15), 526 [*m/z* 586 − AcOH]$^+$ (36), 509 [*m/z* 551 − $CH_3CO]^+$ (37), 387 [*m/z* 509 − BzOH]$^+$ (26), 329 $[ScOH + NH_4]^+$ (19), 312 $[ScOH + H]^+$ (20); HREIMS *m/z* 879.3470 $[M]^+$ (1) (calcd for $C_{49}H_{53}NO_{14}$, 879.3466).

Synthesis of 2-Debenzoyl-2-tigloylpaclitaxel (Isocephalomannine, 1d). To a solution of 2′-TBS-7-TES-2-debenzoylpaclitaxel[16] (1.5 g, 1.5 mMol) in CH_2Cl_2−toluene (1:2), tiglic acid (3.0 g, 30 mMol, 20 mol equivalents), DMAP (1.8 g, 15 mMol, 10 mol equivalents), and DCC (8.2 g, 40 mmol, 26 mol equivalents) were added, and the solution was refluxed (100 °C) for 16 h. After cooling to room temperature, the reaction was worked up by dilution with toluene and filtration. The filtrate was washed sequentially with 3% HCl, saturated $NaHCO_3$, and brine. After drying (Na_2SO_4) and evaporation, the residue was filtered through a short Si gel column to remove the excess tiglic acid (hexane−EtOAc, 7:3 as eluent). The residue was then dissolved in pyridine (7 mL), and the solution was cooled to 0 °C and pyridinium hydrogen fluoride (1.4 mL) was added. After stirring at 0 °C for 3 h and then at room temperature for 12 h, the reaction was worked up by the addition of saturated $NaHCO_3$ and extraction with Et_2O. The organic phase was washed with 3% HCl and brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (hexane−EtOAc, 1:1 as eluent) to give 180 mg **1d** as a colorless powder: mp 232 °C (dec); $[\alpha]^{25}_D$ −44° (*c* 0.70, MeOH); IR (KBr) ν_{max} 3439, 1731, 1717, 1647 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; CIMS (NH_3) *m/z* 849 $[M + NH_4]^+$ (3), 832 $[M + H]^+$ (1), 564 [*m/z* 849 − ScOH]$^+$ (16), 547 [*m/z* 832 − ScOH]$^+$ (9), 504 [*m/z* 564 − AcOH]$^+$(15), 487 [*m/z* 547 − AcOH]$^+$ (9), 464 [*m/z* 564 − TiglOH]$^+$ (29), 447 [*m/z* 547 − TiglOH]$^+$ (65), 387 [*m/z* 447 − AcOH]$^+$ (13), 303 $[ScOH + NH_4]^+$ (100), 286 $[ScOH + H]^+$ (51), 268 [*m/z* 286 − $H_2O]^+$ (10), 240 [268 − CO]$^+$ (59); HREIMS *m/z* 831.3471 $[M]^+$ (1) (calcd for $C_{45}H_{53}NO_{14}$, 831.3466).

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