Stereospecific Synthesis and Biological Evaluation of Monodesmethyl Metabolites of (+)-13a-(*S*)-Deoxytylophorinine as Potential Antitumor Agents

Pengfei Yu,[1] Haining Lv,[1] Chao Li, Jinhong Ren, Shuanggang Ma, Song Xu, Xiaoguang Chen, Shishan Yu*

State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, P. R. of China
Fax +86(10)63017757; E-mail: yushishan@imm.ac.cn

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Abstract: Three major monodesmethyl metabolites of (+)-13a-(*S*)-deoxytylophorinine were synthesized stereospecifically and their configurations at C-13a were determined. Biological assays revealed that one of the metabolites, 3-*O*-desmethyl-13a-(*S*)-deoxytylophorinine, had a higher cytotoxic potency than the parent compound or the positive controls doxorubicin (Adriamycin) and paclitaxel (Taxol).

Key words: alkaloids, medicinal chemistry, antitumor agents, stereoselective synthesis

Historically, natural products and their derivatives have served as major sources of therapeutic agents and lead molecules in drug discovery. In the early nineteenth century, about 80% of all drugs were obtained from roots, bark, or leaves. More recently, over 70% of antitumor compounds are either natural products or derivatives thereof.[2–4] For example, the phenanthroindolizidine alkaloids are pentacyclic natural products found primarily in the plant families *Cynanchum*, *Pergularia*, and *Tylophora* and in some genera of the *Asclepiadaceae*.[5–7] These alkaloids are known for their extensive bioactivities, especially their profound antitumor activities.[8–12] However, side effects have limited their application as antitumor drugs. For example, in the early 1960s, the clinical candidate tylocrebrine was found to be toxic to the central nervous system (CNS), causing disorientation and ataxia.[13] As a result, no phenanthroindolizidine alkaloid is currently used in clinical treatment. Novel phenanthroindolizidine alkaloids and their derivatives with potent antitumor activities and reduced CNS toxicities are therefore very attractive targets for pharmaceutical research.

The phenanthroindolizidine alkaloid (+)-13a-(*S*)-deoxytylophorinine [(*S*)-**1**; Figure 1] was originally isolated from the roots of *Tylophora atrofolliculata* and *T. ovata*, and it was found to possess potent anticancer activities both *in vitro* and *in vivo*.[14] Moreover, (*S*)-**1** penetrates the blood–brain barrier and distributes in brain tissues without obvious CNS toxicity.[15] This compound therefore aroused our particular attention and interest. We recently reported a synthesis, biological evaluation, and mechanistic study on (*S*)-**1** and its derivatives.[16] The biological results showed that this alkaloid and some of its derivatives possessed potent cytotoxic activities through blockage of the PI3K and MAPK signaling transduction pathways and by interference with progression of the cell cycle. These findings identified (*S*)-**1** and its derivatives as promising antitumor chemotherapeutic agents and prompted us to investigate them further.

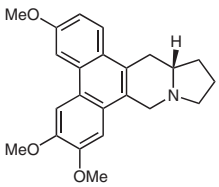

Figure 1 Chemical structure of (*S*)-**1**

To demonstrate the potential of (*S*)-**1** as a candidate for cancer treatment clinical trials, the absorption, distribution, metabolism, and excretion properties of this compound were investigated. The compound is susceptible to metabolism by rat liver microsomes, and the metabolite mixture is as potent as the parent compound (data not shown). Compared with the parent compound, some metabolites of (*S*)-**1** might have similar or greater levels of cytotoxic activity.

Recently, Abliz and co-workers investigated the main metabolites of (*S*)-**1** in rat urine, and they identified 6-*O*-desmethyldeoxytylophorinine (**2**), 7-*O*-desmethyldeoxytylophorinine (**3**), and 3-*O*-desmethyldeoxytylophorinine (**4**) by integrated rapid-resolution liquid chromatography–tandem mass spectrometry.[17] However, the configurations of these compounds at C-13a and their activities could not be determined because of the limited quantities that were available, thereby necessitating the synthesis of these metabolites and their enantiomers (Scheme 1). Furthermore, with these compounds in hand, structure–activity relationships for (*S*)-**1** and its metabolites could be systematically studied.

We synthesized 6-*O*-desmethyldeoxytylophorinine [(*S*)-**2** or (*R*)-**2**] and 7-*O*-desmethyldeoxytylophorinine [(*S*)-**3** or (*R*)-**3**], two of the main metabolites of (*S*)-**1**, for the first time. This synthesis was accomplished by random desmethylation of (*S*)-**1** or its enantiomer (–)-13a-(*R*)-deoxytylophorinine [(*R*)-**1**] with magnesium iodide under solvent-free conditions (Scheme 2).[18] The demethylated positions in (*S*)-**2**, (*R*)-**2**, (*S*)-**3**, and (*R*)-**3** were determined

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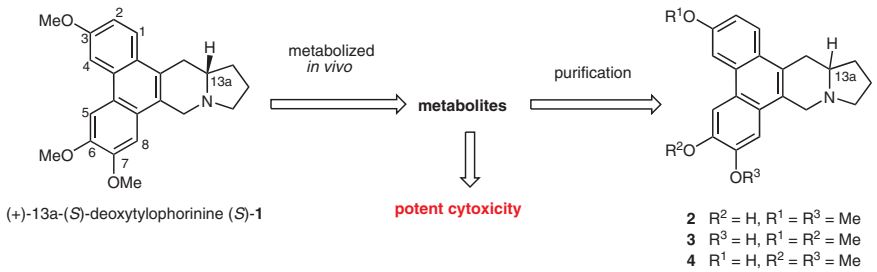
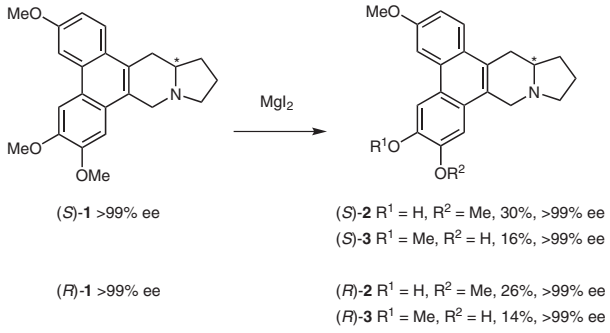


Scheme 1 Metabolism of (*S*)-**1**. The mixture of metabolites of (*S*)-**1** from rat-liver microsomes showed a comparable cytotoxic potency to that of the parent compound. After purification, the main metabolites in rat urine were identified by an integrated rapid-resolution liquid chromatography–tandem mass spectrometric approach to be 6-*O*-desmethyldeoxytylophorinine (**2**), 7-*O*-desmethyldeoxytylophorinine (**3**), and 3-*O*-desmethyldeoxytylophorinine (**4**).



Scheme 2 Syntheses of (*S*)-**2**, (*R*)-**2**, (*S*)-**3**, and (*R*)-**3**

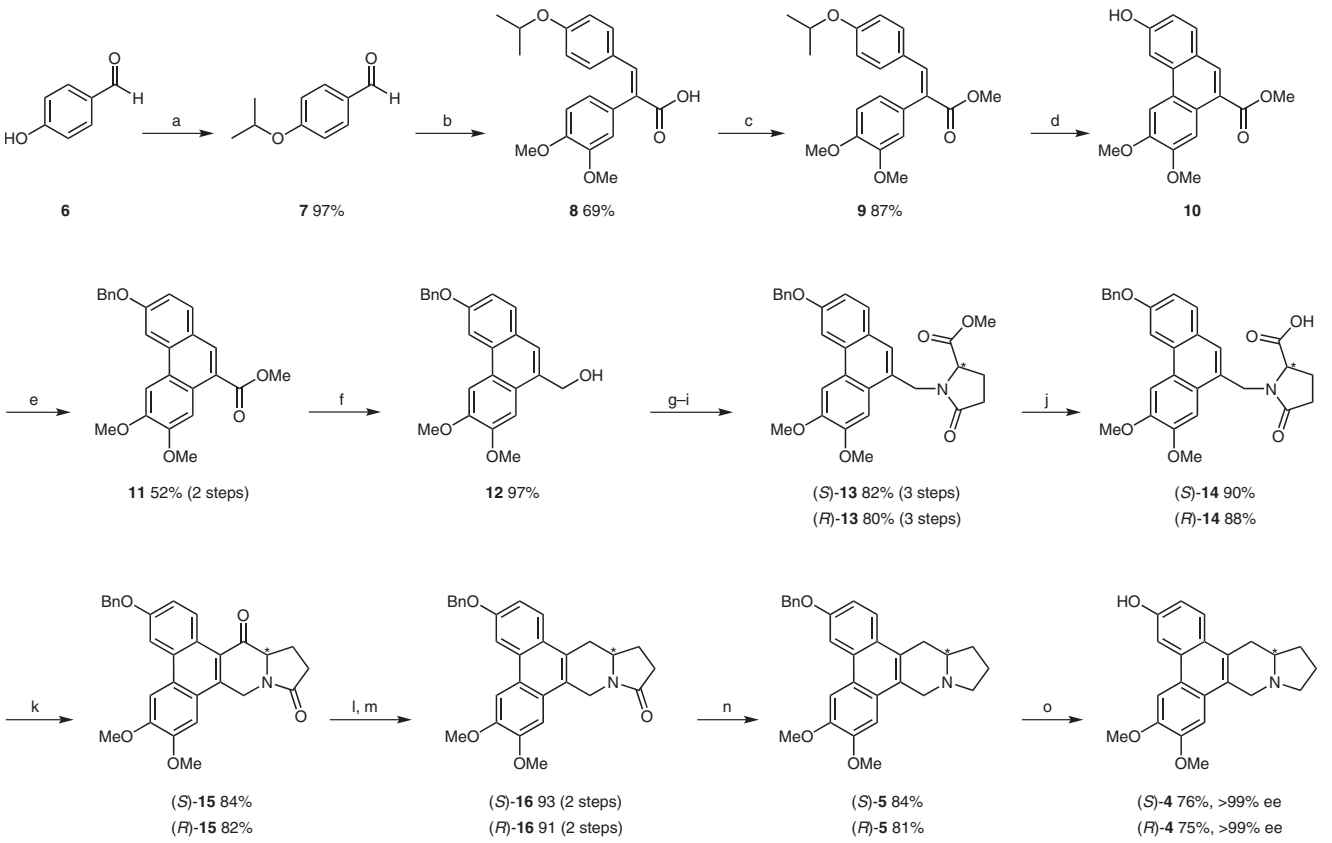
by means of nuclear Overhauser effect (NOE) experiments (see Supporting Information).

Circular dichroism (CD) spectra of **1**, **2**, and **3** were recorded (see Supporting Information) to determine whether the configuration at C-13a was changed during random demethylation. The CD spectra of (*S*)-**1**, (*S*)-**2**, and (*S*)-**3** displayed positive Cotton effects in the region 270–280 nm, showing that the absolute configuration at C-13a was *S*.[19] Moreover, the CD spectra of (*R*)-**2** and (*R*)-**3** displayed negative Cotton effects in this region, as did the parent compound (*R*)-**1**. Therefore, the configuration at C-13a was retained during random demethylation.

Having accomplished stereospecific syntheses of the C-6 and C-7 desmethyl metabolites of (*S*)-**1**, we also prepared 3-*O*-desmethyldeoxytylophorinine [(*S*)-**4** or (*R*)-**4**]. Our synthetic route to this compound was similar to that adopted by Ikeda,[19] but with a different protecting group and different intramolecular oxidative coupling conditions. The enantiomeric excess (ee) of the final product was more than 99%. As shown in Scheme 3, the hydroxy group of 4-hydroxybenzaldehyde (**6**) was protected with an isopropyl group to give 4-isopropoxybenzaldehyde (**7**). The reason for selecting isopropyl as the protecting group was that aluminum(III) chloride has been reported to cleave isopropyl aryl ethers while leaving methyl aryl ethers intact.[20] We surmised that the Lewis acid iron(III) chloride, an oxidative coupling reagent commonly used in the synthesis of phenanthrene rings, would function in the same manner as aluminum(III) chloride in this respect. After Perkin condensation and esterification, the intermediate **9** was subjected to iron(III) chloride catalyzed intramolecular oxidative coupling to construct the phenanthrene ring. Gratifyingly, removal of the isopropyl group and oxidative coupling were accomplished simultaneously. After benzylation of the hydroxy group in the 3-position and application of analogous steps to those used in the synthesis of (*S*)-**1**,[16,21–24] we obtained compound (*S*)-**5**, a novel derivative of (*S*)-**1** with a benzyloxy group at C-3. The target compound (*S*)-**4** was obtained by catalytic hydrogenation of (*S*)-**5** in 11% overall yield and >99% ee. Compounds (*R*)-**4** and (*R*)-**5** were prepared by using the same procedures and materials as (*S*)-**4** and (*S*)-**5**, except for the use of dimethyl D-glutamate hydrochloride as the building block (ee >99%).

Because the C-13a configurations of the metabolites had not been determined previously[17] as a result of the limited quantities available in rat urine, the purified metabolites of (*S*)-**1** and the synthesized monodesmethyl compounds were subjected to chiral-HPLC analysis (CHIRALPAK AD-H column, 4.6 × 250 mm, 5 μm). By comparing the retention time of each metabolite with that of the corresponding synthetic enantiomer, the C-3 desmethyl metabolite of (*S*)-**1** was found to be identical to (*S*)-**4**, and the C-6 desmethyl metabolite and C-7 desmethyl metabolite were found to be identical to (*S*)-**2** and (*S*)-**3**, respectively. These results showed that the configuration at C-13a was unchanged during metabolism.

Having successfully synthesized the optically pure metabolites and derivatives of 13a-(*S*)-deoxytylophorinine and their enantiomers, we were interested in investigating the cytotoxic activities of these compounds [(*S*)-**1**–(*S*)-**5** and (*R*)-**1**–(*R*)-**5**] with five human cancer cell lines: A375 (human malignant melanoma cell line), SH-SY5Y (human neuroblastoma cell line), HepG2 (human hepatocellular cancer cell line), SKOV3 (human ovarian cancer cell line), and U251 (human glioblastoma cancer cell line). This assay was conducted by an *in vitro* method using 3-(4,5-dimethyl-1,3-thiazol-2-yl)-2,5-diphenyl-2*H*-tetrazol-3-ium bromide> (MTT). Doxorubicin (Adriamycin) and paclitaxel (Taxol) were used as positive controls. The concentrations (in nM) that inhibited cell growth by 50% (IC_{50}) are listed in Table 1.



Scheme 3 Syntheses of (*S*)-**4**, (*R*)-**4**, (*S*)-**5**, and (*R*)-**5**. *Reagents and conditions*: a) *i*-PrBr, KI, K_2CO_3, DMF; b) Ac_2O, Et_3N; c) $(ClCO)_2$, py, CH_2Cl_2, then MeOH, py; d) $FeCl_3$, 4-Å MS, CH_2Cl_2; e) BnBr, K_2CO_3, acetone; f) $LiAlH_4$, THF; g) NaI, TMSCl, MeCN–1,4-dioxane; h) dimethyl L-glutamate hydrochloride [to give (*S*)-**13**] dimethyl D-glutamate hydrochloride [to give (*R*)-**13**], K_2CO_3, MeCN–1,4-dioxane; i) AcOH, MeOH; j) TFA, aq acetone; k) $(COCl)_2$, DMF, CH_2Cl_2, then $SnCl_4$, CH_2Cl_2; l) $NaBH_4$, EtOH; m) TESH, $BF_3{\cdot}Et_2O$, CH_2Cl_2; n) $LiAlH_4$, THF; o) H_2, 10% Pd/C, HCO_2H, MeOH.

Table 1 *In Vitro* Cytotoxicities of 13a-(*S*)-Deoxytylophorinine, Its Metabolites, and Its Derivatives Against Cancer Cell Lines

Compound	IC_{50}[a] (nM)				
	A375	SH-SY5Y	HepG2	SKOV3	U251
(*S*)-**1**	160 ± 30	510 ± 380	160 ± 90	100 ± 30	850 ± 300
(*R*)-**1**	>5000	>5000	>5000	510 ± 360	>5000
(*S*)-**2**	650 ± 450	1010 ± 280	1140 ± 840	150 ± 80	1080 ± 670
(*R*)-**2**	>5000	>5000	>5000	>5000	>5000
(*S*)-**3**	2270 ± 1480	2100 ± 1110	2270 ± 480	190 ± 110	740 ± 580
(*R*)-**3**	2800 ± 670	2400 ± 150	2800 ± 900	140 ± 50	1560 ± 1010
(*S*)-**4**	0.011 ± 0.012	0.0054 ± 0.0073	0.011 ± 0.021	0.00020 ± 0.00015	0.011 ± 0.0094
(*R*)-**4**	2510 ± 1080	3530 ± 1980	2510 ± 450	430 ± 290	1040 ± 900
(*S*)-**5**	2030 ± 990	3190 ± 2520	2030 ± 1150	360 ± 60	2680 ± 990
(*R*)-**5**	>5000	>5000	>5000	>5000	>5000
doxorubicin	53 ± 45	250 ± 190	280 ± 120	>5000	320 ± 170
paclitaxel	28 ± 33	480 ± 250	21 ± 13	13 ± 28	4.5 ± 3.6

[a] The IC_{50} values were all measured after 72 h treatment. Each value represents the mean value ± the standard deviation of three independent experiments performed in triplicate.

Most of the synthesized compounds presented marked cytotoxic activities *in vitro*, except for (*R*)-**2** and (*R*)-**5**. Interestingly, compounds with an *S*-configuration at C-13a showed greater potencies than their enantiomers. Therefore, the stereochemistry at C-13a plays a fundamental role in the biological activity. However, the difference in activity between each pair of enantiomers also depended on the substituents present on the phenanthrene ring. For example, (*S*)-**3**, the C-7 desmethyl metabolite of (*S*)-**1**, displayed only a slightly more potent cytotoxicity than its enantiomer (*R*)-**3** against human cancer cell lines other than SKOV3. However, the C-3 desmethyl metabolite (*S*)-**4** showed a cytotoxic activity that was five orders of magnitude greater than that of its enantiomer. Moreover, (*S*)-**4** showed a much higher cytotoxicity than the parent compound, or even the positive controls doxorubicin and paclitaxel. This was probably because the hydroxy group at C-3 is capable of forming hydrogen bonds with the active site of the biological target. The two new C-3-benzyloxy derivatives (*S*)-**5** and (*R*)-**5** had low activities, presumably due to their lack of planarity and to steric hindrance.

In conclusion, three optically pure compounds [3-*O*-desmethyl-13a-(*S*)-deoxytylophorinine, 6-*O*-desmethyl-13a-(*S*)-deoxytylophorinine, and 7-*O*-desmethyl-13a-(*S*)-deoxytylophorinine] and their enantiomers were synthesized stereospecifically. By comparing the retention times of the three purified metabolites with those of the synthetic enantiomers in chiral HPLC, we confirmed that the configuration at C-13a remains unchanged during metabolism. A subsequent biological evaluation demonstrated that the C-3 desmethyl metabolite of (*S*)-**1** possessed excellent cytotoxic activities against five human cancer cell lines. This showed that the presence of a hydrogen-bond donor at C-3 increased the potency of the lead compound. This research further underlines the importance of studying the metabolism of natural products for clues to new drug candidates.

All materials and reagents were obtained from commercial sources and used without further purification unless otherwise stated. THF was distilled from Na and benzophenone, MeCN was distilled from 4 Å MS, and CH_2Cl_2 was distilled from P_2O_5. All these distillations were performed under dry N_2, immediately before the solvent was used. Melting points were determined on an XT5B micromelting point apparatus (Beijing Keyi Electric Light Instrument Factory, Beijing) and are uncorrected. NMR spectra were recorded in $CDCl_3$, DMSO-d_6 or pyridine-d_5 on a Varian INOVA 500 MHz spectrometer equipped with a cold probe. Chemical shifts (δ) are reported in ppm. The coupling constants (*J*) are reported in Hz. High-resolution mass spectra were recorded with an Agilent Technologies 6250 Accurate-Mass Q-TOF LC/MS spectrometer. Optical rotations were measured at r.t. with a PerkinElmer 341 MC polarimeter at the Na D line. Purities and optical purities of the synthesized compounds were assessed by HPLC on Xtimate C_{18} (4.6 × 250 mm, 5 μm) or Chiralpak AD-H columns (4.6 × 250 mm, 5 μm), respectively.

6-*O*-Desmethyl-13a-(*S*)-deoxytylophorinine [(*S*)-2] and 7-*O*-desmethyl-13a-(*S*)-deoxytylophorinine [(*S*)-3]; Typical Procedure

A mixture of Mg powder (114 mg, 1.14 mmol), I_2 (560 mg, 1.14 mmol), and anhyd Et_2O (20 mL) was refluxed under argon with stirring for 0.5 h in darkness. The mixture was then cooled to r.t. The clear soln was added to a soln of (+)-13a-(*S*)-deoxytylophorinine [(*S*)-**1**; 200 mg, 0.55 mmol] in anhyd CH_2Cl_2 (20 mL), and the mixture was stirred for 0.5 h. The solvent was removed under reduced pressure, and the residue was heated at 80 °C for 0.5 h in darkness. AcOH (5 mL), sat. aq $Na_2S_2O_3$ (20 mL), and 10% MeOH–CH_2Cl_2 (50 mL) were added and the mixture was stirred for 1 h then filtered. The filter cake was washed with 10% MeOH–CH_2Cl_2 (3 × 30 mL). The organic layer was separated, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by flash column chromatography [silica gel, CH_2Cl_2–MeOH (80:1 to 30:1)] to give (*S*)-**2** and (*S*)-**3**.

(*S*)-2

Pale-yellow solid; yield: 58 mg (30%); mp 211.5–212.7 °C (dec.); $[\alpha]_D^{24}$ +98.0 (*c* 0.1, $CHCl_3$); >99% ee [flow rate 1.0 mL/min, 18% *i*-PrOH–hexane (0.2% Et_3N), t_R = 16.59 min]; purity: >99% (HPLC) [flow rate 1.0 mL/min, 32% MeCN–H_2O (0.2% Et_3N)].

^{1}H NMR (500 MHz, pyridine-d_5): δ = 11.59 (s, 1 H), 8.68 (s, 1 H), 8.26 (d, *J* = 1.1 Hz, 1 H), 8.04 (d, *J* = 9.0 Hz, 1 H), 7.50 (s, 1 H), 7.43 (dd, *J* = 9.0, 1.1 Hz, 1 H), 4.85 (d, *J* = 14.7 Hz, 1 H), 3.96 (s, 3 H), 3.90 (s, 3 H), 3.73 (d, *J* = 14.7 Hz, 1 H), 3.43–3.34 (m, 2 H), 3.04–2.91 (m, 1 H), 2.47–2.39 (m, 1 H), 2.38–2.30 (m, 1 H), 2.14–2.06 (m, 1 H), 1.96–1.82 (m, 1 H), 1.80–1.63 (m, 2 H).

^{1}H NMR (600 MHz, DMSO-d_6): δ = 9.36 (s, 1 H), 8.03 (s, 1 H), 7.89 (d, *J* = 9.0 Hz, 1 H), 7.85 (d, *J* = 1.8 Hz, 1 H), 7.18 (s, 1 H), 7.17 (dd, *J* = 9.0, 1.8 Hz, 1 H), 4.53 (d, *J* = 15.0 Hz, 1 H), 3.95 (s, 3 H), 3.94 (s, 3 H), 3.51–3.49 (m, 1 H), 3.33–3.32 (m, 2 H), 2.77–2.72 (m, 1 H), 2.35–2.31 (m, 2 H), 2.14–2.13 (m, 1 H), 1.87–1.81 (m, 2 H), 1.64–1.60 (m, 1 H).

^{13}C NMR (125 MHz, pyridine-d_5): δ = 158.7, 150.7, 148.3, 131.5, 127.1, 127.0, 126.6, 126.1, 125.9, 125.4, 116.6, 109.7, 105.1, 104.5, 61.0, 56.2, 55.7 (2 × C), 54.8, 34.5, 32.1, 22.5.

HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{22}H_{24}NO_3$: 350.1751; found: 350.1760.

(*S*)-3

Pale-green solid; yield: 30 mg (16%); mp 172.7–173.9 °C (dec.); $[\alpha]_D^{24}$ +93.0 (*c* 0.1, $CHCl_3$); >99% ee [flow rate 1.0 mL/min, 18% *i*-PrOH–hexane (0.2% Et_3N), t_R = 16.61 min]; purity: >99% (HPLC) [flow rate 1.0 mL/min, 32% MeCN–H_2O (0.2% Et_3N)].

^{1}H NMR (500 MHz, pyridine-d_5): δ = 11.62 (br s, 1 H), 8.41 (s, 1 H), 8.39 (s, 1 H), 8.09–8.03 (m, 1 H), 7.81 (s, 1 H), 7.44 (d, *J* = 8.9 Hz, 1 H), 4.69 (d, *J* = 14.8 Hz, 1 H), 4.00 (s, 3 H), 3.93 (s, 3 H), 3.66–3.58 (m, 1 H), 3.42–3.30 (m, 2 H), 3.03–2.91 (m, 1 H), 2.42–2.33 (m, 1 H), 2.32–2.24 (m, 1 H), 2.14–2.03 (m, 1 H), 1.94–1.82 (m, 1 H), 1.78–1.62 (m, 2 H).

^{13}C NMR (125 MHz, pyridine-d_5): δ = 158.8, 149.5, 149.2, 132.0, 127.8, 127.6, 126.7, 126.3, 126.2, 124.4, 115.8, 109.1, 105.6, 105.5, 60.9, 56.4, 55.9, 55.7, 54.9, 34.5, 32.1, 22.4.

HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{22}H_{24}NO_3$: 350.1751; found: 350.1746.

6-*O*-Desmethyl-13a-(*R*)-deoxytylophorinine [(*R*)-2] and 7-*O*-Desmethyl-13a-(*R*)-deoxytylophorinine [(*R*)-3]

These compounds were prepared in a similar manner, starting from (*R*)-**1**.

(*R*)-2

Pale-yellow solid; yield: 52 mg (26%); mp 221.2–222.8 °C (dec.); $[\alpha]_D^{24}$ –113.0 (*c* 0.1, $CHCl_3$); >99% ee [flow rate 1.0 mL/min, 18% *i*-PrOH–hexane (0.2% Et_3N), t_R (minor) = 16.58 min, t_R (major)

= 19.41 min]; purity: >99% (HPLC) [flow rate 1.0 mL/min, 32% MeCN–H_2O (0.2% Et_3N)].

^{1}H NMR (500 MHz, pyridine-d_5): δ = 11.57 (s, 1 H), 8.69 (s, 1 H), 8.26 (d, *J* = 1.7 Hz, 1 H), 8.05 (d, *J* = 9.0 Hz, 1 H), 7.51 (s, 1 H), 7.43 (dd, *J* = 9.0, 1.7 Hz, 1 H), 4.86 (d, *J* = 14.7 Hz, 1 H), 3.96 (s, 3 H), 3.90 (s, 3 H), 3.74 (d, *J* = 14.6 Hz, 1 H), 3.43–3.36 (m, 2 H), 3.04–2.94 (m, 1 H), 2.48–2.39 (m, 1 H), 2.38–2.31 (m, 1 H), 2.16–2.06 (m, 1 H), 1.96–1.84 (m, 1 H), 1.80–1.64 (m, 2 H).

^{13}C NMR (125 MHz, pyridine-d_5): δ = 158.7, 150.7, 148.3, 131.5, 127.1, 127.0, 126.6, 126.1, 125.9, 125.4, 116.6, 109.7, 105.1, 104.5, 61.0, 56.2, 55.7 (2 × C), 54.8, 34.5, 32.1, 22.5.

HRMS (ESI): *m/z* [M + H]$^+$ calcd for $C_{22}H_{24}NO_3$: 350.1751; found: 350.1757.

(*R*)-3

Pale-green solid; yield: 26 mg (14%); mp 177.6–178.9 °C (dec.); $[\alpha]_D^{24}$ –91.0 (*c* 0.1, $CHCl_3$); >99% ee [flow rate 1.0 mL/min, 18% *i*-PrOH–hexane (0.2% Et_3N), t_R (minor) = 16.22 min, t_R (major) = 32.98 min]; purity: >99% (HPLC) [flow rate 1.0 mL/min, 32% MeCN–H_2O (0.2% Et_3N)].

^{1}H NMR (500 MHz, pyridine-d_5): δ = 11.63 (br s, 1 H), 8.42 (s, 1 H), 8.40 (d, *J* = 2.4 Hz, 1 H), 8.06 (d, *J* = 9.0 Hz, 1 H), 7.80 (s, 1 H), 7.44 (dd, *J* = 9.0, 2.4 Hz, 1 H), 4.71 (d, *J* = 14.8 Hz, 1 H), 4.00 (s, 3 H), 3.93 (s, 3 H), 3.76–3.71 (m, 1 H), 3.43–3.32 (m, 2 H), 3.03–2.92 (m, 1 H), 2.45–2.35 (m, 1 H), 2.34–2.26 (m, 1 H), 2.15–2.04 (m, 1 H), 1.95–1.82 (m, 1 H), 1.79–1.63 (m, 2 H).

^{13}C NMR (125 MHz, pyridine-d_5): δ = 158.8, 149.5, 149.2, 132.0, 127.7, 127.6, 126.3, 126.2 (2 × C), 124.4, 115.8, 109.1, 105.6, 105.5, 61.0, 56.4, 55.9, 55.7, 54.8, 32.1, 30.5, 22.4.

HRMS (ESI): *m/z* [M + H]$^+$ calcd for $C_{22}H_{24}NO_3$: 350.1751; found: 350.1757.

4-Isopropoxybenzaldehyde (7)

A mixture of 4-hydroxybenzaldehyde (**6**; 100.00 g, 0.80 mol), *i*-PrBr (115.34 mL, 1.20 mol), anhyd K_2CO_3 (169.80 g, 1.20 mol), anhyd KI (13.52 g, 0.08 mol), and DMF (500 mL) was stirred at 55 °C for 6 h. A second portion of *i*-PrBr (38.45 mL, 0.40 mol) was then added and the mixture was stirred for a further 12 h at 55 °C, cooled to r.t., diluted with H_2O (200 mL), and filtered. The aqueous phase was extracted with EtOAc (3 × 150 mL). The organic phases were combined and washed sequentially with 10% aq NaOH, H_2O, and brine then dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give a pale-yellow oil; yield: 128.30 g (97%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.85 (s, 1 H), 7.80 (d, *J* = 8.7 Hz, 2 H), 6.96 (d, *J* = 8.7 Hz, 2 H), 4.66 (hept, *J* = 6.0 Hz, 1 H), 1.36 (d, *J* = 6.1 Hz, 6 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 190.9, 163.3, 132.2 (2 × C), 129.7, 115.7, 70.4, 22.0.

HRMS (ESI): *m/z* [M + Na]$^+$ calcd for $C_{10}H_{12}NaO_2$: 187.0730; found: 187.0729.

(2*E*)-2-(3,4-Dimethoxyphenyl)-3-(4-isopropoxyphenyl)acrylic Acid (8)

A mixture of aldehyde **7** (50.00 g, 0.80 mol), (3,4-dimethoxyphenyl)acetic acid (57.47 g, 0.29 mol), Et_3N (49.00 mL, 0.35 mol), and Ac_2O (70.00 mL, 0.73 mol) was refluxed with stirring under argon for 8 h, then cooled to r.t. The mixture was diluted with EtOAc (500 mL) and washed with H_2O (3 × 300 mL). The organic phase was dried, filtered, and concentrated under reduced pressure to give a pale-yellow solid; yield: 70.35 g (69%); mp 164.0–165.2 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.40 (br s, 1 H), 7.65 (s, 1 H), 7.03 (d, *J* = 8.6 Hz, 2 H), 6.96 (d, *J* = 8.2 Hz, 1 H), 6.79–6.71 (m, 3 H), 6.67 (d, *J* = 8.2 Hz, 1 H), 4.63–4.54 (m, 1 H), 3.78 (s, 3 H), 3.66 (s, 3 H), 1.21 (d, *J* = 6.0 Hz, 6 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 168.8, 158.1, 148.7, 148.1, 138.5, 132.0 (2 × C), 130.3, 128.9, 126.6, 121.7, 115.1 (2 × C), 113.1, 111.9, 69.1, 55.5, 55.4, 21.7.

HRMS (ESI): *m/z* [M – H]$^-$ calcd for $C_{20}H_{21}O_5$: 341.1394; found: 341.1397.

Methyl (2*E*)-2-(3,4-Dimethoxyphenyl)-3-(4-isopropoxyphenyl)acrylate (9)

Oxalyl chloride (45.90 mL, 0.53 mol) was added in portions to a soln of acid **8** (120.00 g, 0.35 mol) in CH_2Cl_2 (500 mL) at r.t. Pyridine (8.70 mL, 0.11 mol) was then added and the mixture was stirred for 1 h. The solvent was removed under reduced pressure and the residue was treated with pyridine (43.30 mL, 0.53 mol) and MeOH (150 mL) at 0 °C, then kept at r.t. for 0.5 h. The mixture was filtered, washed with MeOH (3 × 30 mL), and concentrated in vacuo to give a white solid; yield: 108.93 g (87%); mp 113.9–115.1 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.77 (s, 1 H), 7.01 (d, *J* = 8.8 Hz, 2 H), 6.91 (d, *J* = 8.2 Hz, 1 H), 6.80 (dd, *J* = 8.2, 1.8 Hz, 1 H), 6.75 (d, *J* = 1.7 Hz, 1 H), 6.67 (d, *J* = 8.8 Hz, 2 H), 4.51 (hept, *J* = 5.9 Hz, 1 H), 3.94 (s, 3 H), 3.82 (s, 3 H), 3.79 (s, 3 H), 1.30 (d, *J* = 6.1 Hz, 6 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 169.0, 159.0, 149.3, 148.7, 140.5, 132.7 (2 × C), 129.5, 128.9, 127.1, 122.3, 115.5 (2 × C), 113.0, 111.6, 70.0, 56.1, 56.0, 52.5, 22.2 (2 × C).

HRMS (ESI): *m/z* [M + Na]$^+$ calcd for $C_{21}H_{24}NaO_5$: 379.1516; found: 379.1528.

Methyl 3-(Benzyloxy)-6,7-dimethoxyphenanthrene-9-carboxylate (11)

Ester **9** (10.00 g, 0.03 mol) was added to a mixture of anhyd $FeCl_3$ (18.58 g, 0.11 mol), 4 Å MS (30 g), and CH_2Cl_2 (300 mL) at 0 °C, and the mixture was stirred for 8 h. The reaction was then quenched with sat. aq $NaHCO_3$ (150 mL) and filtered. The organic layer was collected, dried (Na_2SO_4), filtered, and concentrated. The crude hydroxy ester **10** was used in the next step without further purification.

A mixture of crude hydroxy ester **10**, BnBr (5.20 mL, 0.04 mol), K_2CO_3 (7.91 g, 0.06 mol), and acetone (100 mL) was refluxed for 3 h, cooled, diluted with H_2O (100 mL), and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash column chromatography [silica gel, PE–EtOAc–CH_2Cl_2 (20:4:1)] to give **11** as a pale-yellow solid; yield: 5.91 g (52%); mp 133.8–134.9 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.66 (s, 1 H), 8.46 (s, 1 H), 7.92 (d, *J* = 2.0 Hz, 1 H), 7.88 (d, *J* = 8.8 Hz, 1 H), 7.82 (s, 1 H), 7.57–7.54 (m, 2 H), 7.47–7.42 (m, 2 H), 7.40–7.36 (m, 1 H), 7.31 (dd, *J* = 8.8, 2.3 Hz, 1 H), 5.32 (s, 2 H), 4.11 (s, 3 H), 4.09 (s, 3 H), 4.03 (s, 3 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 168.4, 159.6, 150.1, 149.1, 136.9, 133.6, 132.1, 131.4, 129.0 (2 × C), 128.5, 127.8 (2 × C), 125.3, 125.2, 124.6, 122.0, 116.6, 107.1, 105.6, 103.4, 70.7, 56.1 (2 × C), 52.3.

HRMS (ESI): *m/z* [M + Na]$^+$ calcd for $C_{25}H_{22}NaO_5$: 425.1359; found: 425.1362.

[3-(Benzyloxy)-6,7-dimethoxy-9-phenanthryl]methanol (12)

A soln of ester **11** (19.76 g, 49.10 mmol) in THF (300 mL) at 0 °C was added dropwise to a suspension of $LiAlH_4$ (3.80 g, 98.20 mmol) in THF (20 mL), and the mixture was stirred for 0.5 h at 0 °C. The reaction was then quenched with H_2O (20 mL) and 10% aq HCl (200 mL). The mixture was filtered and the filtrate was extracted with CH_2Cl_2 (3 × 200 mL). The combined organic phase was dried, filtered, and concentrated to give a white powder; yield: 17.85 g (97%); mp 190.7–191.5 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.95 (d, *J* = 1.9 Hz, 1 H), 7.87 (s, 1 H), 7.80 (d, *J* = 8.7 Hz, 1 H), 7.63 (s, 1 H), 7.58–7.53 (m, 3 H), 7.46–7.41 (m, 2 H), 7.39–7.35 (m, 1 H), 7.30–7.28 (m, 1 H), 5.30 (s, 2 H), 5.13 (s, 2 H), 4.11 (s, 3 H), 4.08 (s, 3 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 157.8, 149.7, 149.1, 137.2, 131.7, 131.5, 130.4, 128.9 (2 × C), 128.4, 127.9 (2 × C), 126.0, 125.9, 125.1, 124.8, 116.1, 105.9, 105.1, 104.0, 70.7, 65.0, 56.2 (2 × C).

HRMS (ESI): *m/z* [M + Na]$^+$ calcd for $C_{24}H_{22}NaO_4$: 397.1410; found: 397.1413.

Methyl (2*S*)-1-{[3-(Benzyloxy)-6,7-dimethoxy-9-phenanthryl]methyl}-5-oxopyrrolidine-2-carboxylate [(*S*)-13]

A mixture of alcohol **12** (9.97 g, 26.63 mmol), NaI (8.14 g, 53.25 mmol), 1,4-dioxane (500 mL), and MeCN (250 mL) was stirred at 60 °C for 0.5 h. The mixture was then cooled to r.t., treated with TMSCl (5.17 mL, 39.94 mmol), and stirred at r.t. for 1 h. Dimethyl L-glutamate hydrochloride (8.45 g, 39.94 mmol) and anhyd K_2CO_3 (11.26 g, 79.88 mmol) were added and the mixture was stirred for another 8 h. The mixture was then partitioned between H_2O (200 mL) and CH_2Cl_2 (200 mL). The organic layer was separated, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was directly purified by flash column chromatography [silica gel, PE–EtOAc (3:1)] to give a pale-yellow oil to which AcOH (50 mL) and MeOH (100 mL) were quickly added. The resulting mixture was stirred at 60 °C for 4 h then concentrated in vacuo. The residue was washed with Et_2O (3 × 5 mL), filtered, and dried to give a white powder; yield: 10.84 g (82%); mp 172.4–173.7 °C; $[\alpha]_D^{24}$ +54.8 (*c* 1.0, $CHCl_3$).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.93 (d, *J* = 2.1 Hz, 1 H), 7.84 (s, 1 H), 7.76 (d, *J* = 8.7 Hz, 1 H), 7.63 (s, 1 H), 7.57–7.53 (m, 2 H), 7.47–7.41 (m, 3 H), 7.39–7.35 (m, 1 H), 7.30–7.27 (m, 1 H), 5.51 (d, *J* = 14.6 Hz, 1 H), 5.30 (s, 2 H), 4.41 (d, *J* = 14.5 Hz, 1 H), 4.10 (s, 3 H), 4.05 (s, 3 H), 3.84 (dd, *J* = 9.3, 3.3 Hz, 1 H), 3.59 (s, 3 H), 2.66–2.56 (m, 1 H), 2.44–2.36 (m, 1 H), 2.20–2.07 (m, 1 H), 2.03–1.95 (m, 1 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 174.8, 172.4, 158.0, 150.0, 149.2, 137.2, 131.7, 130.3, 129.0 (2 × C), 128.4, 127.8 (2 × C), 127.7, 126.8, 126.1, 125.6, 124.9, 116.2, 105.9, 105.5, 103.8, 70.8, 58.7, 56.6, 56.2, 52.5, 45.0, 30.1, 23.0.

HRMS (ESI): *m/z* [M + Na]$^+$ calcd for $C_{30}H_{29}NNaO_6$: 522.1887; found: 522.1898.

(2*S*)-1-{[3-(Benzyloxy)-6,7-dimethoxy-9-phenanthryl]methyl}-5-oxopyrrolidine-2-carboxylic Acid [(*S*)-14]

A mixture of ester (*S*)-**13** (1.18 g, 2.36 mol), TFA (5.40 mL, 120.10 mmol), H_2O (5 mL), and acetone (13 mL) was stirred at 60 °C for 12 h. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography [silica gel, CH_2Cl_2–MeOH (100:1)] to give a white solid; yield: 1.15 g (90%); mp 253.4–254.3 °C; $[\alpha]_D^{24}$ +46.3 (*c* 1.0, DMF).

^{1}H NMR (500 MHz, DMSO-d_6): δ = 13.01 (s, 1 H), 8.17 (s, 1 H), 8.07 (s, 1 H), 7.84 (d, *J* = 8.7 Hz, 1 H), 7.60–7.56 (m, 2 H), 7.52 (s, 1 H), 7.49 (s, 1 H), 7.45–7.40 (m, 2 H), 7.35 (t, *J* = 7.2 Hz, 1 H), 7.29 (dd, *J* = 8.7, 1.6 Hz, 1 H), 5.39 (d, *J* = 14.6 Hz, 1 H), 5.37 (s, 2 H), 4.23 (d, *J* = 14.6 Hz, 1 H), 4.03 (s, 3 H), 3.88 (s, 3 H), 3.67 (dd, *J* = 9.2, 3.2 Hz, 1 H), 2.46–2.37 (m, 1 H), 2.37–2.28 (m, 1 H), 2.18–2.07 (m, 1 H), 1.93–1.83 (m, 1 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 174.2, 173.2, 157.3, 149.4, 148.9, 137.2, 130.9, 130.0, 128.5 (2 × C), 128.0 (2 × C), 127.9, 126.6, 126.3, 125.4, 124.9, 124.4, 116.3, 105.5, 105.0, 104.6, 69.7, 57.9, 55.9, 55.5, 43.6, 29.3, 22.3.

HRMS (ESI): *m/z* [M – H]$^-$ calcd for $C_{29}H_{26}NO_6$: 484.1766; found: 484.1770.

(13a*S*)-3-(Benzyloxy)-6,7-dimethoxy-13,13a-dihydrodibenzo[*f*,*h*]pyrrolo[1,2-*b*]isoquinoline-11,14(9*H*,12*H*)-dione [(*S*)-15]

Oxalyl chloride (0.86 mL, 9.94 mmol) and DMF (0.30 mL) were added to a soln of acid (*S*)-**14** (3.84 g, 7.91 mmol) in anhyd CH_2Cl_2 (200 mL). The mixture was stirred at r.t. for 1 h then heated to reflux. A soln of $SnCl_4$ (2.35 mL, 19.80 mmol)) in anhyd CH_2Cl_2 (50 mL) was added in five portions, one every hour, and the mixture was then maintained at reflux for an additional 2 h. The mixture was then cooled to r.t. and 1 M HCl (150 mL) was added. The organic layer was separated, washed with H_2O (3 × 50 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by flash column chromatography [silica gel, CH_2Cl_2–MeOH (100:1)] to give a pale-yellow solid; yield: 3.10 g (84%); mp 209.7–210.9 °C; $[\alpha]_D^{24}$ +117.3 (*c* 0.3, $CHCl_3$).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.36 (d, *J* = 9.4 Hz, 1 H), 7.94 (d, *J* = 2.5 Hz, 1 H), 7.81 (s, 1 H), 7.57–7.54 (m, 2 H), 7.46–7.41 (m, 2 H), 7.40–7.35 (m, 2 H), 7.31 (s, 1 H), 5.73 (d, *J* = 17.9 Hz, 1 H), 5.30 (s, 2 H), 4.68 (d, *J* = 17.6 Hz, 1 H), 4.48–4.40 (m, 1 H), 4.13 (s, 3 H), 4.09 (s, 3 H), 2.68–2.48 (m, 4 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 195.5, 174.3, 157.7, 152.0, 150.3, 137.4, 137.1, 131.4, 129.4, 129.0 (2 × C), 128.5, 128.1, 127.8 (2 × C), 123.5, 123.0, 122.7, 116.8, 106.4, 104.6, 104.0, 70.7, 61.5, 56.5, 56.3, 41.0, 30.3, 21.0.

HRMS (ESI): *m/z* [M + Na]$^+$ calcd for $C_{29}H_{25}NNaO_5$: 490.1625; found: 490.1617.

(13a*S*)-3-(Benzyloxy)-6,7-dimethoxy-12,13,13a,14-tetrahydrodibenzo[*f*,*h*]pyrrolo[1,2-*b*]isoquinolin-11(9*H*)-one [(*S*)-16]

$NaBH_4$ (1.38 g, 35.77 mmol) was added to a stirred soln of (*S*)-**15** (8.36 g, 17.88 mmol) in a mixture of CH_2Cl_2 (150 mL) and MeOH (150 mL), and the resulting mixture was stirred for 15 min. The reaction was then quenched with sat. aq NH_4Cl (150 mL). The organic layer was separated, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (100 mL), and TESH (5.80 mL, 35.77 mmol) and $BF_3{\cdot}Et_2O$ (6.83 mL, 53.68 mmol) were added. The mixture was stirred for another 4 h and then sat. aq $NaHCO_3$ (60 mL) was added. The organic layer was separated, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by flash column chromatography [silica gel, CH_2Cl_2–MeOH (100:1)] to give a pale-yellow solid; yield: 7.54 g (93%); mp 237.8–238.7 °C; $[\alpha]_D^{24}$ +187.0 (*c* 1.0, $CHCl_3$).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.99 (d, *J* = 2.3 Hz, 1 H), 7.94 (d, *J* = 9.1 Hz, 1 H), 7.85 (s, 1 H), 7.57–7.53 (m, 2 H), 7.46–7.41 (m, 2 H), 7.39–7.35 (m, 1 H), 7.32 (dd, *J* = 9.0, 2.4 Hz, 1 H), 7.19 (s, 1 H), 5.33 (d, *J* = 17.0 Hz, 1 H), 5.31 (s, 2 H), 4.57 (d, *J* = 17.0 Hz, 1 H), 4.10 (s, 3 H), 4.07 (s, 3 H), 4.00–3.91 (m, 1 H), 3.59–3.53 (m, 1 H), 2.92–2.84 (m, 1 H), 2.66–2.60 (m, 2 H), 2.59–2.51 (m, 1 H), 2.08–1.97 (m, 1 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 174.5, 157.3, 150.0, 149.0, 137.2, 130.9, 129.0 (2 × C), 128.4, 127.8 (2 × C), 125.4, 125.2, 125.1, 125.0, 123.7, 122.5, 115.8, 106.6, 104.1, 103.1, 70.8, 56.4, 56.2, 53.6, 41.4, 33.5, 30.4, 25.5.

HRMS (ESI): *m/z* [M + Na]$^+$ calcd for $C_{29}H_{27}NNaO_4$: 476.1832; found: 476.1836.

(13a*S*)-3-(Benzyloxy)-6,7-dimethoxy-9,11,12,13,13a,14-hexahydrodibenzo[*f*,*h*]pyrrolo[1,2-*b*]isoquinoline [(*S*)-5]

$LiAlH_4$ (88 mg, 2.28 mmol) was added to a suspension of ketone (*S*)-**16** (520 mg, 1.14 mmol) in THF (50 mL), and the mixture was refluxed with stirring in darkness for 2 h. The mixture was then allowed to cool to r.t. and the reaction was quenched with acetone (1 mL) and H_2O (20 mL). The mixture was filtered, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by flash column chromatography [silica gel, CH_2Cl_2–MeOH (60:1)] to give a white solid; yield: 421 mg (84%); mp 205.5–206.6 °C (dec.); $[\alpha]_D^{24}$ +101.0 (*c* 1.0, $CHCl_3$); 99% ee [flow rate 1.0 mL/min, 22% *i*-PrOH–hexane (0.2% Et_3N), t_R = 30.25 min]; purity: >99% (HPLC) [flow rate 1.0 mL/min, 40% MeCN–H_2O (0.2% Et_3N)].

^{1}H NMR (500 MHz, pyridine-d_5): δ = 8.53 (d, *J* = 1.6 Hz, 1 H), 8.36 (s, 1 H), 8.09 (d, *J* = 9.0 Hz, 1 H), 7.65 (d, *J* = 7.6 Hz, 2 H), 7.57–7.54 (m, 1 H), 7.50–7.42 (m, 3 H), 7.37 (t, *J* = 7.3 Hz, 1 H), 5.40 (s, 2 H), 4.83 (d, *J* = 14.7 Hz, 1 H), 4.02 (s, 3 H), 3.97 (s, 3 H), 3.72 (d, *J* = 14.8 Hz, 1 H), 3.45–3.36 (m, 2 H), 3.05–2.92 (m, 1 H), 2.47–

2.38 (m, 1 H), 2.38–2.31 (m, 1 H), 2.18–2.06 (m, 1 H), 1.96–1.84 (m, 1 H), 1.81–1.65 (m, 2 H).

^{13}C NMR (125 MHz, pyridine-d_5): δ = 158.0, 151.0, 149.9, 138.6, 131.6, 129.4 (2 × C), 128.8, 128.7 (2 × C), 128.1, 127.0, 126.8, 126.7, 126.3, 124.6, 116.7, 107.1, 105.8, 104.9, 71.0, 60.9, 56.5, 56.3, 55.7, 54.8, 34.6, 32.1, 22.5.

HRMS (ESI): *m/z* [M + H]$^+$ calcd for $C_{29}H_{30}NO_3$: 440.2220; found: 440.2244.

3-*O*-Desmethyl-13a-(*S*)-deoxytylophorinine [(*S*)-4]
A mixture of benzyloxy derivative (*S*)-**5** (300 mg, 0.68 mmol), 10% Pd/C (50 mg), HCO_2H (40 mL), and MeOH (20 mL) was stirred at 70 °C in darkness under H_2 (1.0 atm) for 12 h. The mixture was then filtered and concentrated in vacuo. The residue was purified by flash column chromatography [silica gel, CH_2Cl_2–MeOH (60:1 then 40:1)] to give a white solid; yield: 188 mg (76%); mp 205.5–206.9 °C (dec.); $[\alpha]_D^{24}$ +126.6 (*c* 1.0, DMF); >99% ee [flow rate 1.0 mL/min, 18% *i*-PrOH–hexane (0.2% Et_3N), t_R (major) = 14.55 min, t_R (minor) = 31.77 min]; purity: >99% (HPLC) [flow rate 1.0 mL/min, 25% MeCN–H_2O (0.2% Et_3N)].

^{1}H NMR (500 MHz, pyridine-d_5): δ = 11.84 (s, 1 H), 8.52 (s, 1 H), 8.17 (s, 1 H), 8.09 (d, *J* = 8.8 Hz, 1 H), 7.63 (d, *J* = 8.8 Hz, 1 H), 7.45 (s, 1 H), 4.82 (d, *J* = 14.7 Hz, 1 H), 3.95 (s, 3 H), 3.93 (s, 3 H), 3.72 (d, *J* = 14.5 Hz, 1 H), 3.45 (d, *J* = 14.1 Hz, 1 H), 3.39 (t, *J* = 8.3 Hz, 1 H), 3.07–2.97 (m, 1 H), 2.48–2.39 (m, 1 H), 2.35 (q, *J* = 8.5 Hz, 1 H), 2.16–2.06 (m, 1 H), 1.96–1.83 (m, 1 H), 1.80–1.63 (m, 2 H).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.17 (s, 1 H), 7.95 (d, *J* = 2.0 Hz, 1 H), 7.93 (s, 1 H), 7.84 (d, *J* = 8.8 Hz, 1 H), 7.18 (s, 1 H), 7.10 (dd, *J* = 8.8, 2.0 Hz, 1 H), 4.57 (d, *J* = 15.2 Hz, 1 H), 3.99 (s, 3 H), 3.93 (s, 3 H), 3.56 (d, *J* = 15.2 Hz, 1 H), 3.38–3.34 (m, 2 H), 2.81–2.75 (m, 1 H), 2.50–2.39 (m, 2 H), 2.18–2.13 (m, 1 H), 1.91–1.85 (m, 2 H), 1.69–1.62 (m, 1 H).

^{13}C NMR (125 MHz, pyridine-d_5): δ = 157.7, 150.8, 149.6, 132.0, 128.2, 126.7, 126.3, 126.0, 125.7, 124.4, 117.7, 108.0, 105.4, 104.9, 61.0, 56.3, 56.1, 55.8, 54.8, 34.6, 32.1, 22.5.

HRMS (ESI): *m/z* [M + H]$^+$ calcd for $C_{22}H_{24}NO_3$: 350.1751; found: 350.1758.

Methyl (2*R*)-1-{[3-(Benzyloxy)-6,7-dimethoxy-9-phenanthryl]methyl}-5-oxopyrrolidine-2-carboxylate [(*R*)-13]
This was prepared in a similar manner to (*S*)-**13**, but starting from dimethyl D-glutamate hydrochloride. Subsequent steps leading to the synthesis of (*R*)-**4** were identical to those applied to the corresponding enantiomeric compounds.

White solid; yield: 9.68 g (80%); mp 172.0–174.5 °C (dec.); $[\alpha]_D^{24}$ –54.1 (*c* 1.0, $CHCl_3$).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.93 (d, *J* = 1.8 Hz, 1 H), 7.84 (s, 1 H), 7.76 (d, *J* = 8.7 Hz, 1 H), 7.63 (s, 1 H), 7.57–7.53 (m, 2 H), 7.47–7.41 (m, 3 H), 7.39–7.35 (m, 1 H), 7.30–7.27 (m, 1 H), 5.51 (d, *J* = 14.6 Hz, 1 H), 5.30 (s, 2 H), 4.41 (d, *J* = 14.5 Hz, 1 H), 4.10 (s, 3 H), 4.05 (s, 3 H), 3.84 (dd, *J* = 9.1, 3.1 Hz, 1 H), 3.59 (s, 3 H), 2.66–2.56 (m, 1 H), 2.47–2.34 (m, 1 H), 2.17–2.06 (m, 1 H), 2.05–1.93 (m, 1 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 174.9, 172.5, 158.0, 150.0, 149.2, 137.2, 131.7, 130.3, 129.0 (2 × C), 128.4, 127.8 (2 × C), 127.7, 126.8, 126.1, 125.6, 124.9, 116.2, 105.9, 105.5, 103.8, 70.8, 58.7, 56.6, 56.2, 52.5, 45.0, 30.1, 23.0.

HRMS (ESI): *m/z* [M + Na]$^+$ calcd for $C_{30}H_{29}NNaO_6$: 522.1887; found: 522.1899.

(2*R*)-1-{[3-(Benzyloxy)-6,7-dimethoxy-9-phenanthryl]methyl}-5-oxopyrrolidine-2-carboxylic Acid [(*R*)-14]
White solid; yield: 2.15 g (88%); mp 257.7–258.9 °C; $[[\alpha]_D^{24}$ –43.1 (*c* 1.0, DMF).

^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.99 (br s, 1 H), 8.17 (s, 1 H), 8.07 (s, 1 H), 7.84 (d, *J* = 8.7 Hz, 1 H), 7.60–7.56 (m, 2 H), 7.52 (s, 1 H), 7.49 (s, 1 H), 7.46–7.40 (t, *J* = 7.5 Hz, 2 H), 7.35 (t, *J* = 7.3 Hz, 1 H), 7.29 (dd, *J* = 8.7, 1.7 Hz, 1 H), 5.30 (d, *J* = 14.7 Hz, 1 H), 5.37 (s, 2 H), 4.23 (d, *J* = 14.7 Hz, 1 H), 4.03 (s, 3 H), 3.88 (s, 3 H), 3.67 (dd, *J* = 9.3, 3.2 Hz, 1 H), 2.46–2.37 (m, 1 H), 2.37–2.28 (m, 1 H), 2.18–2.10 (m, 1 H), 1.92–1.84 (m, 1 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 174.2, 173.2, 157.3, 149.4, 148.9, 137.2, 130.9, 130.0, 128.5 (2 × C), 128.0 (2 × C), 127.9, 126.6, 126.4, 125.4, 124.9, 124.4, 116.3, 105.5, 105.0, 104.6, 69.7, 57.9, 55.9, 55.5, 43.7, 29.3, 22.3.

HRMS (ESI): *m/z* [M – H]$^-$ calcd for $C_{29}H_{26}NO_6$: 484.1766; found: 484.1770.

(13a*R*)-3-(Benzyloxy)-6,7-dimethoxy-13,13a-dihydrodibenzo[*f*,*h*]pyrrolo[1,2-*b*]isoquinoline-11,14(9*H*,12*H*)-dione [(*R*)-15]
Pale-yellow solid; yield: 2.95 g (82%); mp 214.0–215.2 °C; $[\alpha]_D^{24}$ –126.3 (*c* 1.0, $CHCl_3$).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.35 (d, *J* = 9.4 Hz, 1 H), 7.93 (d, *J* = 2.4 Hz, 1 H), 7.80 (s, 1 H), 7.56–7.51 (m, 2 H), 7.45–7.40 (m, 2 H), 7.39–7.34 (m, 2 H), 7.30 (s, 1 H), 5.71 (d, *J* = 17.9 Hz, 1 H), 5.29 (s, 2 H), 4.67 (d, *J* = 17.9 Hz, 1 H), 4.47–4.39 (m, 1 H), 4.12 (s, 3 H), 4.08 (s, 3 H), 2.66–2.49 (m, 4 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 195.5, 174.3, 157.7, 152.0, 150.2, 137.4, 137.1, 131.4, 129.4, 129.0 (2 × C), 128.5, 128.1, 127.8 (2 × C), 123.5, 123.0 (2 × C), 122.7, 116.8, 106.4, 104.6, 104.0, 70.7, 61.5, 56.5, 56.3, 41.0, 30.3, 21.0.

HRMS (ESI): *m/z* [M + Na]$^+$ calcd for $C_{29}H_{25}NNaO_5$: 490.1625; found: 490.1628.

(13a*R*)-3-(Benzyloxy)-6,7-dimethoxy-12,13,13a,14-tetrahydrodibenzo[*f*,*h*]pyrrolo[1,2-*b*]isoquinolin-11(9*H*)-one [(*R*)-16]
Pale-yellow solid; yield: 4.59 g (91%); mp 236.9–238.0 °C; $[\alpha]_D^{24}$ –195.0 (*c* 1.0, $CHCl_3$).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.00 (s, 1 H), 7.94 (d, *J* = 9.0 Hz, 1 H), 7.85 (s, 1 H), 7.55 (d, *J* = 7.4 Hz, 2 H), 7.46–7.42 (m, 2 H), 7.39–7.35 (m, 1 H), 7.32 (dd, *J* = 9.1, 2.1 Hz, 1 H), 7.19 (s, 1 H), 5.34 (d, *J* = 17.0 Hz, 1 H), 5.31 (s, 2 H), 4.57 (d, *J* = 16.7 Hz, 1 H), 4.10 (s, 3 H), 4.07 (s, 3 H), 4.00–3.92 (m, 1 H), 3.59–3.53 (m, 1 H), 2.94–2.83 (m, 1 H), 2.68–2.50 (m, 3 H), 2.07–1.99 (m, 1 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 174.5, 157.3, 150.0, 149.0, 137.2, 130.9, 129.0 (2 × C), 128.4, 127.8 (2 × C), 125.4, 125.2, 125.1, 125.0, 123.7, 122.5, 115.8, 106.6, 104.1, 103.1, 70.7, 56.4, 56.2, 53.6, 41.4, 33.5, 31.2, 25.5.

HRMS (ESI): *m/z* [M + Na]$^+$ calcd for $C_{29}H_{27}NNaO_4$: 476.1832; found: 476.1834.

(13a*R*)-3-(Benzyloxy)-6,7-dimethoxy-9,11,12,13,13a,14-hexahydrodibenzo[*f*,*h*]pyrrolo[1,2-*b*]isoquinoline [(*R*)-5]
Pale-yellow solid; yield: 215 mg (81%); mp 204.3–205.0 °C (dec.); $[\alpha]_D^{24}$ –109.9 (*c* 1.0, $CHCl_3$); >99% ee [flow rate 1.0 mL/min, 22% *i*-PrOH–hexane (0.2% Et_3N), t_R (minor) = 30.26 min, t_R (major) = 48.30 min]; purity: >99% (HPLC) [flow rate 1.0 mL/min, 40% MeCN–H_2O (0.2% Et_3N)].

^{1}H NMR (500 MHz, pyridine-d_5): δ = 8.54 (d, *J* = 2.4 Hz, 1 H), 8.37 (s, 1 H), 8.09 (d, *J* = 9.0 Hz, 1 H), 7.65 (d, *J* = 7.4 Hz, 2 H), 7.56 (d, *J* = 2.4 Hz, 1 H), 7.49–7.43 (m, 3 H), 7.40–7.35 (m, 1 H), 5.40 (s, 2 H), 4.84 (d, *J* = 14.7 Hz, 1 H), 4.02 (s, 3 H), 3.97 (s, 3 H), 3.73 (d, *J* = 14.7 Hz, 1 H), 3.45–3.36 (m, 2 H), 3.04–2.95 (m, 1 H), 2.48–2.40 (m, 1 H), 2.39–2.31 (m, 1 H), 2.17–2.08 (m, 1 H), 1.97–1.85 (m, 1 H), 1.81–1.65 (m, 2 H).

^{13}C NMR (125 MHz, pyridine-d_5): δ = 158.0, 151.0, 149.9, 138.6, 131.6, 129.4 (2 × C), 128.8, 128.7 (2 × C), 128.1, 127.0, 126.8, 126.7, 126.3, 124.6, 116.7, 107.1, 105.8, 104.9, 71.0, 61.0, 56.5, 56.3, 55.7, 54.8, 34.6, 32.1, 22.5.

HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{29}H_{30}NO_3$: 440.2220; found: 440.2237.

3-*O*-Desmethyl-13a-(*R*)-deoxytylophorinine [(*R*)-4]

White solid; yield: 152 mg (75%); mp 205.0–206.2 °C (dec.); $[\alpha]_D^{24}$ –130.4 (*c* 1.0, DMF); >99% ee [flow rate 1.0 mL/min, 18% *i*-PrOH–hexane (0.2% Et_3N), t_R (minor) = 14.59 min, t_R (major) = 30.63 min]; purity: >99% (HPLC) [flow rate 1.0 mL/min, 25% MeCN–H_2O (0.2% Et_3N)].

^{1}H NMR (500 MHz, pyridine-d_5): δ = 11.86 (s, 1 H), 8.52 (d, *J* = 2.3 Hz, 1 H), 8.18 (s, 1 H), 8.09 (d, *J* = 8.8 Hz, 1 H), 7.63 (dd, *J* = 8.8, 2.1 Hz, 1 H), 7.46 (s, 1 H), 4.83 (d, *J* = 14.7 Hz, 1 H), 3.95 (s, 3 H), 3.93 (s, 3 H), 3.72 (d, *J* = 14.7 Hz, 1 H), 3.48–3.42 (m, 1 H), 3.41–3.36 (m, 1 H), 3.07–2.97 (m, 1 H), 2.48–2.40 (m, 1 H), 2.38–2.31 (m, 1 H), 2.15–2.06 (m, 1 H), 1.96–1.84 (m, 1 H), 1.80–1.64 (m, 2 H).

^{13}C NMR (125 MHz, pyridine-d_5): δ = 157.7, 150.8, 149.6, 132.0, 128.2, 126.7, 126.3, 126.0, 125.7, 124.4, 117.7, 108.0, 105.4, 104.9, 61.0, 56.3, 56.1, 55.8, 54.8, 34.6, 32.1, 22.5.

HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{22}H_{24}NO_3$: 350.1751; found: 350.1754.

Cytotoxicity Assays

A375, SH-SY5Y, HepG2, and SKOV3 cancer cell lines were obtained from ATCC (Manassas, VA). U251 was obtained from the Cell Culture Center, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and Peking Union Medical College (Beijing). The cytotoxicities of the synthesized compounds against the cancer cell lines were evaluated by the MTT method *in vitro* with doxorubicin and paclitaxel as positive controls. A375, SH-SY5Y, HepG2, SKOV3, and U251 cells were seeded on 96-well polystyrene cell-culture plates at a density of 2×10^4 cells/mL (100 μL). After incubation for 24 h at 37 °C in a 5% CO_2 atmosphere, the cells were treated with six different concentrations of the test compounds for 72 h. The drug-containing medium was then removed and replaced with culture medium containing 0.5 mg/mL MTT (100 μL) and incubated for 4 h. Formazan blue, formed from the MTT, was then extracted with DMSO (180 mL) and its absorbance was determined by using a Spectra Max190 instrument at 570 nm.

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Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synthesis.

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(1) These authors contributed equally to this study.
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