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Synthesis of the marine alkaloid caulersin

Niklas Wahlström,[a,b] Birgitta Stensland[c] and Jan Bergman[a,b,*]

[a]*Unit for Organic Chemistry, CNT, Department of Biosciences at Novum, Karolinska Institute, Novum Research Park, SE-141 57 Huddinge, Sweden*
[b]*Södertörn University College, SE-141 04 Huddinge, Sweden*
[c]*Preformulation and Biopharmaceutics, Solid State Analysis/SBBG B314:3, Astrazeneca PAR & D, SE-151 85 Södertälje, Sweden*

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Abstract—A three-step synthesis of caulersin (**3**) from indole-2-acetic acid methyl ester and indole-2-carbonyl chloride is described. As the spectral data of the synthetic sample differed from those reported for the natural product, the structure was determined by X-ray crystallography.

1. Introduction

In connection with previous synthetic studies of indolocarbazoles,[1] the highly potent aryl hydrocarbon receptor ligand indolo[3,2-*b*]carbazole-6,12-dione (**1**) and the corresponding 2,3-*b* isomer (**2**) have been synthesized via new ring closing strategies (Fig. 1).[2] Utilizing a similar ring closing strategy, the related bisindole marine natural product caulersin (**3**), was now considered as a synthetic target. Caulersin was isolated in 1997 from the algae *Caulerpa serrulata*,[3] and represents the only natural product isolated so far containing a bisindole structure bridged by a central troponoid framework. A lengthy synthesis of caulersin (seven steps, 14% reported overall yield) was published in 1999.[4] A structure related to caulersin, the bisindole caulerpin (**4**), isolated from several different green and red algae,[5–8] has showed moderate antitumor activity.[9] Furthermore, **4** acts as a plant growth regulator[10] and has also been shown to inhibit the multixenobiotic resistance (MXR) pump in algae, thus enhancing toxicity of xenobiotics.[11] The synthesis of **4** was reported by Maiti and Thomson who treated indole-2-acetic acid methyl ester with a Vilsmeier salt to produce a low yield of **4**.[12,13] In an oxidative transformation of **4**, several oxygenated adducts were isolated.[14] During the analysis of these structures, caulersin (**3**) was suggested as one of the stabile intermediates formed in the mass spectrometer. As no natural ring-transformed adduct from **4** has been isolated, the possibility of a product–precursor relationship between **3** and **4** does exist. With the objective of subjecting **3** to further biological studies, large quantities of **3** were needed. This encouraged the development of a shorter and higher yielding synthesis of **3**, something that now has been accomplished.

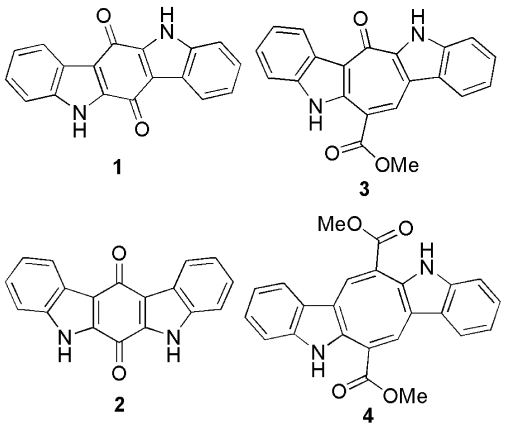

Figure 1. Indolo[3,2-*b*]carbazole-6,12-dione (**1**), indolo[2,3-*b*]carbazole-6,13-dione (**2**), caulersin (**3**) and caulerpin (**4**).

2. Results and discussion

Retrosynthetic analysis of **3** leads to the 2,3-diindolyl ketone **5** by excision of a carbon atom, probably as formaldehyde, from the seven-membered ring. Further scission of **5** gives indole-2-acetic acid methyl ester (**6a**)[15] and indole-2-carboxylic acid (**7**) (Scheme 1).

In practice, treatment of indole-2-acetic acid methyl ester (**6a**) with dimethylaluminium chloride,[16,17] followed by addition of indole-2-carbonyl chloride produced the 3-acylated bisindole **5** in 35% yield together with two other

Keywords: Caulersin; Marine alkaloid; Vilsmeier salt; Indole.
* Corresponding author. Tel.: +46-8-608-9204; fax: +46-8-608-1501; e-mail address: jabe@biosci.ki.se

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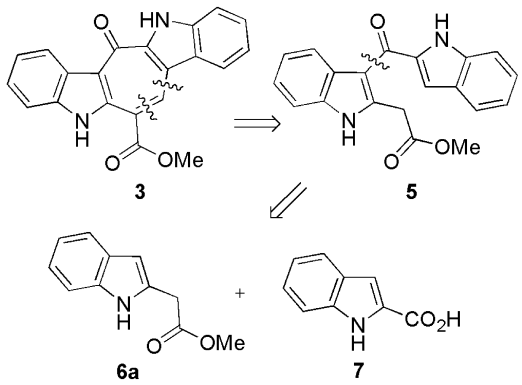
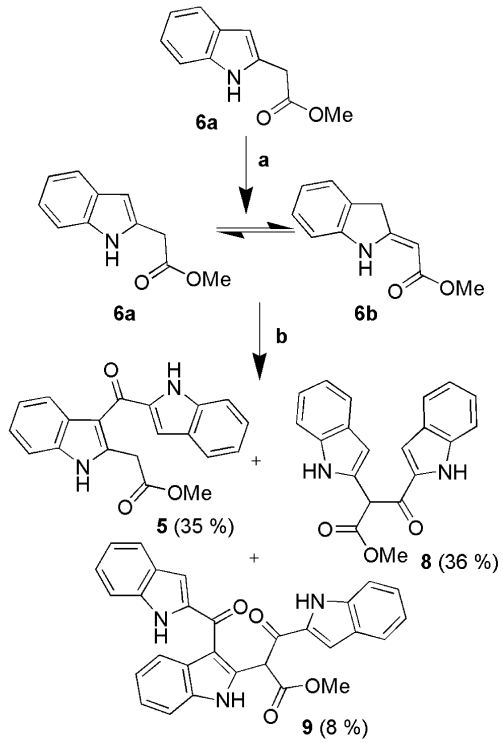
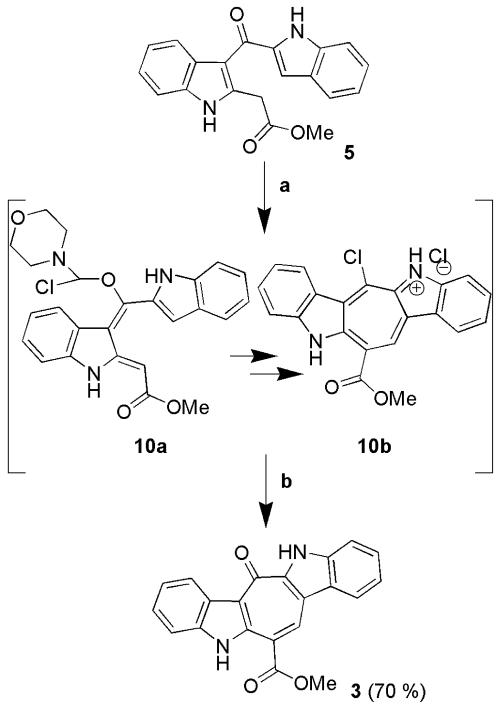


Scheme 1. Retrosynthetic analysis of caulersin (**3**).



Scheme 2. (a) Me_2AlCl, CH_2Cl_2; (b) indole-2-carbonyl chloride.

products arising from the tautomer **6b** (Scheme 2). The acid sensitive indole **6a** undergoes partial isomerization to the enamine **6b** which in turn reacts with indole-2-carbonyl chloride, producing **8** (36%) together with the doubly acylated product **9** (8%), obviously a secondary product of **8**. Initial reaction between **5** and 1 equiv. of chloromethylenemorpholinium chloride,[18] in dichloroethane at 60 °C failed to give **3**. Increasing the number of the Vilsmeier salt equivalents to 3 produced, upon cooling, a red solid. The chemical shift of one of the aromatic doublets and the aromatic singlet were shifted downfield in the ^{1}H NMR spectrum of this intermediate, and the aliphatic triplet had disappeared. This sparingly soluble intermediate was rapidly transformed into **3** in the NMR-tube, no ^{13}C NMR data could therefore be obtained. However, when the ethyl ester analogue of **5** was treated with chloromethylenemorpholinium chloride a red solid formed that proved to be slightly more stable, something that enabled the isolation of the ethyl ester derivative of **10b** as the corresponding free base. Mass spectrometry of the free base showed the



Scheme 3. (a) Chloromethylenemorpholinium chloride 3 equiv., 21–60 °C; (b) DMSO, H_2O, 50 °C.

presence of a chlorine atom incorporated in the structure. This information, suggests that **10a** is the initial product formed (Scheme 3). Secondly, another Vilsmeier salt equivalent reacts with **10a** and is thus further transformed into **10b**. Hydrolysis of **10b** in DMSO/water at 50 °C for 14 h then produced **3** as an orange solid in 70% yield. The total yield of caulersin in this facile three-step procedure was 25%.

When the spectral data of **3** were compared with those recorded for the natural product the proton spectra proved to be essentially identical. However, the reported melting points and the IR spectra differed. During the melting point measurement we noted that the consistency of the powder of **3** changed only marginally at the reported melting point (269–274 °C),[3,4] to a more golden color. The sample finally melted between 352–355 °C, 70 °C higher than previously reported. As other rigid and planar bisindoles exemplified by **1** and **2** have high melting points (>400 °C),[2] the higher melting point recorded in our hands seems reasonable.

When the crystals were analyzed with IR-spectroscopy a similar pattern of peaks, but not identical, could be seen as compared to the material before sublimation. This was attributed to the different packing of the crystals before and after sublimation. In comparison with the previously synthesized material and the isolated natural product, there were small differences for the two NH functionalities of the unsublimed sample. ^{1}H and ^{13}C NMR spectroscopy showed that in solution, the sublimed crystals were identical with the unsublimed material. In the ^{13}C spectrum several differences with the reported data were observed. This is best exemplified by the absence of the chemical shift reported for the position of carbon 20 (C-20) at 146.8 ppm. Instead, a signal at 114.6 ppm was assigned for C-20 (Fig. 2). The ^{13}C spectrum of the isolated **3** obtained from

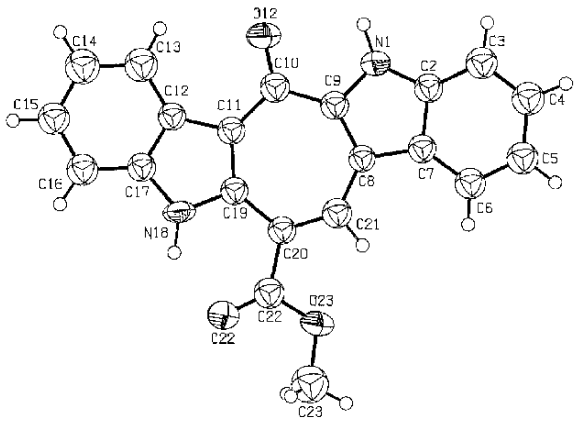
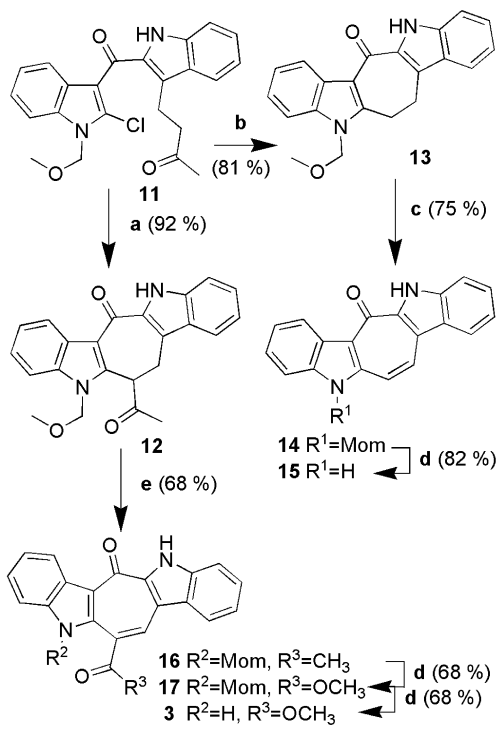


Figure 2. Crystal structure of caulersin (**3**) with atom numbering. CCDC reference number 210210.

Su,[19] had a considerably lower signal to noise ratio compared with the spectrum from our synthetic product. Furthermore, the signal with the lowest intensity, a singlet at 119.3 ppm was not accounted for. We now undertook a detailed 2D NMR study of compound **3**, which enabled us to assign all carbon and hydrogen atoms in the molecule. To further establish the structure, small fine yellow crystals of **3** were obtained by sublimation at 285 °C and 0.35 mbar. The X-ray diffraction study clearly showed that the structure was correct (Fig. 2). Worth to mention here is the extremely high packing coefficient[20] (72.6%), a figure which indicates that the molecules are exceptionally well packed in the crystal.

To verify the structure of the previously reported **3** and the isolated natural product,[3] the synthesis of Fresneda and co-workers was repeated.[4] *o*-Azidobenzaldehyde[21,22] and *N*-methoxymethyl-3-acetyl-2-chloroindole[23] was condensed to produce the expected chalcone which when heated in xylenes, formed the corresponding bisindole ketone. This ketone was treated with methyl vinyl ketone and boron trifluoride diethyl etherate in nitromethane to give the Michael adduct **11**[4] and a small amount of 5-nitro-2-pentanone.[24] During this last reaction several portions of the boron reagent and the methyl vinyl ketone were added to complete the reaction, in contrast to the single addition reported. In the subsequent ring closing reaction with potassium *tert*-butoxide/*tert*-butanol (*t*-BuOK/*t*-BuOH, 2.5 equiv.) the cyclized seven-ring bridged bisindole **12** could be obtained via a modification of the workup procedure (Scheme 4). When several equivalents of *t*-BuOK were used, the unexpected deacylation of **12** readily took place, to produce the deacylated heptacycle **13**. The retention values of **11** and **13** in several different eluent systems are similar, thus precluding the use of TLC to monitor the progress of the reaction. In the selective formation of **13** in 81% yield, multiple equivalents of *t*-BuOK were used. Bisindole **13** was dehydrogenated with DDQ in benzene to give the aromatized molecule **14** in 76% yield. Subsequent deprotection of **14** was carried out with 6 M HCl in refluxing ethanol to produce the parent bisindolotropolone **15** in 82% yield. Dehydrogenation of **12** with DDQ produced the aromatized molecule **16** that was subsequently subjected to treatment with fresh potassium hypochlorite (KOCl) solution.[4,25] The bisindole ester **17** was isolated in 68% using the procedure of Fresneda.[25] The ester **17** was then heated in MeOH with 6 M HCl, to give **3** in 68%, with similar spectroscopic data with material obtained via Scheme 3.



Scheme 4. (a) 2.5 equiv. *t*-BuOK/*t*-BuOH/PhH, reflux; (b) 8.5 equiv. *t*-BuOK/*t*-BuOH/PhH, reflux; (c) DDQ, PhH, reflux; (d) EtOH, 6 M HCl, reflux; (e) DDQ, PhH, reflux; (f) 3 equiv. KOCl, THF/MeOH, 0–21 °C, 5 h;[25] (g) MeOH, 6 M HCl, reflux, 6 h.[25]

3. Conclusions

A simple three-step synthesis in 25% total yield of the marine natural product caulersin (**3**) has been developed by cyclisation of the simple ketoester **5** with the Vilsmeier reagent chloromethylenemorpholinium chloride to yield the central seven-membered ring ketone after in situ treatment with water in DMSO. Unambiguous determination of its structure via an X-ray diffraction study confirmed the structure of **3** and thus verified the new data obtained in this work. The samples of **3** prepared via Schemes 3 and 4 are identical. The main difference between the reported natural product,[3] the previous synthesis of **3**,[4] and this work, is the melting point and ^{13}C NMR data presented here.

4. Experimental

4.1. General aspects

NMR spectra were obtained on a Bruker Avance 300 DPX spectrometer (Bruker, Newark, DE) operating at 300 MHz and a Jeol Eclipse+500 FT NMR spectrometer (Jeol Ltd, Tokyo, Japan) operating at 500 MHz. Spectra were recorded in acetone-d_6, DMSO-d_6 or $CDCl_3$, using the solvent as internal standard at 300 or 500 MHz for ^1H and 75 or 125 MHz for ^{13}C at 298 K if not stated otherwise; δ values are given in ppm and coupling constants are reported in Hz.

IR spectra were recorded on a Perkin–Elmer FT-IR 1600 or on a ThermoNicolet Avatar 330 FT-IR spectrophotometer. Melting points were determined using the capillary method on a Büchi B-545, an Electrothermal IA9200 and on a Heizbank koffler hotbench and are uncorrected. Mass spectra were recorded using an LC/MS system operating in the electron spray ionization (ESI) mode at 70 eV. The elemental analyses were performed by H. Kolbe Microanalytisches Laboratorium, Mühlheim an der Ruhr, Germany. Sublimation was carried out on a Thermal Gradient Sublimer, Esoteric Chemicals AB, Sweden. All reagents were of standard quality and used as received from Lancaster, Aldrich, or Merck. Solvents were purified by distillation or were of HPLC grade. Dichloromethane, dichloroethane and *tert*-butanol were distilled from CaH_2 and stored over activated molecular sieves prior to use. Benzene and *n*-heptane were stored over sodium. Chromatographic separations were performed on silica gel 60 (230–400 mesh). Reactions were monitored by thin-layer chromatography, on silica gel coated plates with a fluorescent indicator.

4.2. Synthesis of compounds 5, 7 and 8

1.0 M Me_2AlCl in hexanes (15.80 mmol, 15.8 mL) was added dropwise to a cold (−40 °C) solution of indole-2-acetic acid methyl ester[15] (**6a**) (2.30 g, 12.16 mmol) in dry CH_2Cl_2 (40 mL) under nitrogen. The temperature was then allowed to rise to −10 °C over 45 min before being lowered to −20 °C whereupon a solution of indole-2-carbonyl chloride (2.84 g, 15.80 mmol) in CH_2Cl_2 (20 mL) was added dropwise at such a rate as to keep the temperature below −10 °C. The temperature was then gradually increased to 21 °C over 16 h. The reaction mixture was poured out on iced water (100 mL) in a 500 mL beaker. After the initial frothing, the yellow suspension was diluted with water (200 mL) and stirred for 1 h. The suspension was extracted with EtOAc (3×100 mL) and filtered through celite and washed with a little EtOAc. The combined organic phases were washed with water (100 mL) and brine (100 mL) before drying over Na_2SO_4. All water phases (approximately 500 mL) were extracted with $CHCl_3$ (2×300 mL), the combined $CHCl_3$ phases were washed with water (200 mL), brine (200 mL) and then dried over Na_2SO_4. The dried organic phases were combined and evaporated to dryness to form an orange solid. This solid was subjected to column chromatography on silica with a gradient eluent system of EtOAc/hexane (20–50%) to give **8** in 0.56 g, **9** in 0.08 g and **5** in 1.07 g. Fractions with more than one substance were combined and evaporated and subjected to a second column chromatography with the gradient eluent Et_2O/hexane (70–90%) gave **8** in 0.91 g, **9** in 0.40 g, and finally **5** in 0.35 g. Compound **8** was isolated in a total yield of 1.47 g (36%), and the trimeric product **9** in 0.48 g (8%) and finally the bisindole **5** in 1.42 g (35%).

4.2.1. 2,3-Bis-(1*H*-indol-2-yl)-3-oxo-propionic acid methyl ester (8). White solid. Mp 179.5–182.5 °C (Et_2O/hexane); IR (KBr) 3346, 3052, 2954, 1721, 1660, 1636, 1518, 1428, 1343, 1276, 1138, 751 cm^{-1}; ^1H NMR (300 MHz; DMSO-d_6) δ 11.96 (1H, s), 11.17 (1H, s), 7.71 (1H, d, J=8.0 Hz), 7.61 (1H, d, J=1.0 Hz), 7.48–7.44 (2H, m), 7.40 (1H, d, J=8.1 Hz), 7.31 (1H, dd, J=7.7, 7.2 Hz), 7.11–7.03 (2H, m), 6.95 (1H, dd, J=7.3, 7.2 Hz), 6.45 (1H, d, J=0.7 Hz), 6.21 (1H, s), 3.73 (3H, s); ^{13}C NMR (75 MHz; DMSO-d_6) δ 184.5 (s), 168.3 (s), 138.4 (s), 136.5 (s), 133.8 (s), 130.9 (s), 127.5 (s), 126.7 (s), 126.3 (d), 122.9 (d), 121.2 (d), 120.6 (d), 119.8 (d), 119.0 (d), 112.8 (d), 111.5 (d), 111.4 (d), 102.0 (d), 53.5 (d), 52.7 (q). MS ESI m/z $[M+1]^+$ 333, $[M-1]^-$ 331. Anal. Calcd for $C_{20}H_{16}N_2O_3$: C, 72.3; H, 4.85; N, 8.4. Found C, 72.2; H, 4.9; N, 8.4.

4.2.2. 2-[3-(1*H*-Indole-2-carbonyl)-1*H*-indol-2-yl]-3-(1*H*-indol-2-yl)-3-oxo-propionic acid methyl ester (9). Yellow solid. Recrystallized from CH_2Cl_2/*c*-hexane. Mp 130 °C dec.; IR (KBr) 3382, 3061, 2951, 1738, 1646, 1619, 1591, 1521, 1438, 1342, 1174, 1138, 745 cm^{-1}; ^1H NMR (300 MHz; DMSO-d_6) δ 12.23 (1H, s), 12.00 (1H, s), 11.98 (1H, s), 7.70–7.58 (4H, m), 7.53–7.44 (3H, m), 7.33–7.27 (2H, m), 7.21 (1H, dd, J=7.7, 7.2 Hz), 7.12–7.04 (4H, m), 6.69 (1H, s), 3.75 (3H, s); ^{13}C NMR (75 MHz; DMSO-d_6) δ 183.9 (s), 182.3 (s), 167.7 (s), 138.5 (s), 137.6 (s), 136.8 (s), 136.0 (s), 136.0 (s), 133.8 (s), 126,8 (s), 126.7 (s), 126.4 (d), 125.4 (s), 125.1 (d), 122.9 (d), 122.6 (d), 123.0 (d), 120.6 (2×d), 120.3 (d), 120.1 (d), 114.4 (s), 112.8 (d), 112.6 (d), 112.5 (d), 111.3 (d), 109.9 (d), 52.9 (q), 52.3 (d); MS ESI m/z $[M+1]^+$ 476; FABHRMS Calcd for $C_{29}H_{22}N_3O_4$ 476.1610 $[M+H]^+$ found 476.1608.

4.2.3. [3-(1*H*-Indole-2-carbonyl)-1*H*-indol-2-yl]acetic acid methyl ester (5). Yellow solid. Mp 171 °C dec. (EtOAc/heptane); IR (KBr) 3313, 3175, 1720, 1597, 1435, 1342, 749 cm^{-1}; ^1H NMR (300 MHz; DMSO-d_6) δ 12.05 (1H, s), 11.84 (1H, s), 7.72 (1H, d, J=7.9 Hz), 7.50–7.47 (2H, m), 7.27 (1H, dd, J=8.0, 7.2 Hz), 7.20 (1H, dd, J=8.0, 7.1 Hz), 7.13–7.04 (2H, m), 7.02 (1H, d, J=1.2 Hz), 4.08 (2H, s), 3.62 (3H, s); ^{13}C NMR (75 MHz; DMSO-d_6) δ 182.1 (s), 169.7 (s), 138.1 (s), 137.4 (s), 136.9 (s), 135.1 (s), 126.8 (s), 126.1 (s), 124.7 (d), 122.4 (d), 122.2 (d), 120.8 (d), 120.1 (d), 120.0 (d), 114.0 (s), 112.5 (d), 111.7 (d), 109.4 (d), 51.9 (q), 33.1 (t); MS ESI m/z $[M-1]^-$ 331. Anal. Calcd for $C_{20}H_{16}N_2O_3$: C, 72.3; H, 4.85; N, 8.4. Found C, 72.1; H, 5.0; N, 8.2.

4.2.4. 6,11-Dihydro-6-oxo-5*H*-cyclohepta[1,2-*b*:4,5-*b*′]diindole-12-carboxylic acid methyl ester. (Caulersin) (3). Solid chloromethylenemorpholinium chloride (427 mg, 2.51 mmol) was added to a suspension of [3-(1*H*-indole-2-carbonyl)-1*H*-indol-2-yl]acetic acid methyl ester (**5**) (278 mg, 0.84 mmol) in dry dichloroethane (20 mL) at 21 °C under nitrogen. The resulting suspension was stirred for 30 min at 30–35 °C, then heated at 50 °C for 4 h and finally at 60 °C for 90 min. The solvent was removed under reduced pressure at 50 °C and the resulting dark red solid suspended in a mixture of DMSO (12 mL) and water (2 mL). The suspension was stirred at 50 °C for 14 h and then poured out on water/brine (1:1, 150 mL). The resulting brown precipitate was collected and washed with water, and then EtOAc until an almost clear filtrate was obtained. The solid was dried at 55 °C/0.35 mbar to give caulersin (**3**) (202 mg, 70%) as an orange powder. This sample changes color to golden crystals at 275–280 °C and melts at 352–355 °C. (lit.[3] mp 269–270 °C, lit.[4] mp 273–274 °C); IR (KBr) 3320, 3169, 1689, 1534, 1496, 1422, 1267, 1241 cm^{-1}; after sublimation; IR (KBr) 3348, 3241, 1667, 1540, 1492, 1418, 1270, 1240 cm^{-1}; for atom numbering

see Fig. 2. ^{1}H NMR (500 MHz; DMSO-d_6) δ 13.10 (1H, s, N*H*, N-1), 12.36 (1H, s, N*H*, N-18), 9.14 (1H, s, C*H*, C-21), 9.06 (1H, d, C*H*, C-13, J=8.2 Hz), 8.36 (1H, d, C*H*, C-6, J=7.8 Hz), 7.95 (1H, d, C*H*, C-16, J=7.8 Hz), 7.77 (1H, d, C*H*, C-3, J=7.8 Hz), 7.57 (1H, dd, C*H*, C-4, J=7.8, 7.4 Hz), 7.51 (1H, dd, C*H*, C-15, J=7.8, 6.9 Hz), 7.43 (1H, dd, C*H*, C-5, J=7.8, 7.3 Hz), 7.40 (1H, dd, C*H*, C-14, J=7.8, 7.4 Hz), 4.09 (3H, –OCH_3, C-23, s); ^{13}C NMR (125 MHz; DMSO-d_6) δ 172.4 (s, C-10), 168.0 (s, C-22), 140.9 (s, C-9), 138.6 (s, C-19), 136.9 (s, C-2), 136.4 (s, C-17), 129.7 (d, C-21), 126.9 (s, C-7), 126.7 (d, C-4), 126.1 (s, C-12), 125.8 (d, C-15), 123.5 (d, C-13), 122.0 (d, C-5), 121.7 (d, C-14), 120.3 (d, C-6), 119.3 (s, C-11), 114.6 (s, C-20), 114.4 (s, C-8), 113.2 (d, C-3), 112.4 (d, C-16), 53.0 (q, C-23); MS ESI m/z [M+1]$^+$ 343. Calcd for $C_{21}H_{14}N_2O_3$, C, 73.7; H, 4.1; N, 8.2. Found C, 73.8; H, 4.0; N, 8.1.

The NMR data of **3** after being heated up to its melting point was identical with the data given above.

4.2.5. Data of Caulersin (3) obtained via Scheme 4. Yellow solid prepared with the method of Fresneda.[25] Mp 349.0–352.5 °C; IR (neat) 3347, 3231, 1667, 1540, 1490, 1417, 1269, 1240 cm^{-1}; ^{1}H NMR (500 MHz; DMSO-d_6) δ 13.10 (1H, s), 12.36 (1H, s), 9.15 (1H, s), 9.06 (1H, d, J=8.2 Hz), 8.36 (1H, d, J=8.2 Hz), 7.95 (1H, d, J=7.8 Hz), 7.77 (1H, d, J=7.8 Hz), 7.58 (1H, dd, J=7.8, 7.4 Hz), 7.51 (1H, dd, J=7.8, 7.4 Hz), 7.44 (1H, dd, J=7.8, 7.4 Hz), 7.40 (1H, dd, J=7.8, 7.4 Hz), 4.09 (3H, s); ^{13}C NMR (125 MHz; DMSO-d_6) δ 172.4 (s), 168.0 (s), 140.9 (s), 138.6 (s), 136.8 (s), 136.4 (s), 129.7 (d), 126.9 (s), 126.7 (d), 126.1 (s), 125.8 (d), 123.5 (d), 122.0 (d), 121.7 (d), 120.3 (d), 119.3 (s), 114.6 (s), 114.3 (s), 113.2 (d), 112.3 (d), 53.0 (q);

4.2.6. Compound 10b. An aliquot from the reaction between **5** and chloromethylenemorpholinium chloride was withdrawn from reaction mixture (before removal of solvent) and quickly filtered under nitrogen, washed with small amounts of dry dichloroethane. The NMR-sample was prepared immediately before the spectrum was recorded. ^{1}H NMR (300 MHz; DMSO-d_6) δ 14.41 (1H, bs), 14.03 (1H, bs), 10.12 (1H, s), 9.40 (1H, d, J=8.3 Hz), 8.88 (1H, d, J=8.0 Hz), 8.35 (1H, d, J=8.1 Hz), 8.14–7.96 (3H, m), 7.82–7.73 (2H, m), 4.45 (3H, s).

4.2.7. 5,11,12,13-Tetrahydro-11-(methoxymethyl)-6*H*-cyclohepta[1,2-*b*:4,5-*b*′]diindol-6-one (13). *t*-BuOK (1.95 g, 16.53 mmol) in *t*-BuOH (50 mL) was added to a solution of **11** (1.69 g, 4.13 mmol) in anhydrous benzene (80 mL) under a flow of nitrogen. The solution was heated at reflux for 1.5 h, when additional *t*-BuOK (2.2 g, 18.65 mmol) was added and the reflux was continued for another hour. After cooling the reaction mixture was washed with saturated NH_4Cl (50 mL) and then extracted with EtOAc (3×50 mL). The combined organic phases were washed with water (50 mL) then brine (75 mL) before drying over $MgSO_4$. The solvents were evaporated to give a yellow solid, which was recrystallized from dichloroethane/heptane to give **13** as golden crystals (1.69 g, 81%). Mp 214–216 °C; IR (KBr) 3283, 3059, 2928, 2823, 1564, 1460, 1419, 1228, 1102, 1047, 747 cm^{-1}; ^{1}H NMR (300 MHz; $CDCl_3$) δ 9.31 (1H, s), 8.67–8.64 (1H, m), 7.71 (1H, d, J=8.1 Hz), 7.51–7.45 (2H, m), 7.38–7.32 (3H, m), 7.18 (1H, t, J=7.5 Hz), 5.63 (2H, s), 3.47–3.43 (2H, m), 3.37–3.33 (5H, m); ^{13}C NMR (75 MHz; $CDCl_3$) δ 179.7 (s), 148.0 (s), 137.2 (s), 136.3 (s), 134.2 (s), 127.6 (s), 127.5 (s), 125.9 (d), 123.8 (d), 123.3 (d), 123.0 (d), 120.8 (d), 120.2 (d), 119.9 (s), 116.1 (s), 112.3 (d), 109.5 (d), 74.2 (t), 56.4 (q), 25.3 (t), 22.0 (t); MS ESI m/z [M+1]$^+$ 331. Anal. Calcd for $C_{21}H_{18}N_2O_2$: C, 76.3; H, 5.5; N, 8.5. Found C, 76.2; H, 5.4; N, 8.6.

4.2.8. 6,11-Dihydro-11-(methoxymethyl)-6-oxo-5*H*-Cyclohepta[1,2-*b*:4,5-*b*′]diindole (14). DDQ (0.666 g, 2.94 mmol) was added portionwise to a suspension of **13** (0.485 g, 1.47 mmol) in benzene (30 mL) at 21 °C. The resulting suspension was heated at reflux for 80 min, then cooled to 21 °C and treated with 0.4 M KOH (250 mL), and extracted with EtOAc (3×100 mL). The combined organic phases were washed with water (2×100 mL), then brine (2×100 mL), and dried over $MgSO_4$. Concentration of the solvents to dryness followed by crystallization from dichloroethane afforded **14** (362 mg, 75%) in two crops as a light yellow-greenish solid. Mp 251.5–254.0 °C dec.; IR (KBr) 3287, 3053, 2996, 2900, 1616, 1599, 1577, 1527, 1505, 1415, 1323, 1234, 1113, 1055, 910, 887, 760, 738, 711 cm^{-1}; ^{1}H NMR (300 MHz; DMSO-d_6) δ 12.65 (1H, s), 9.11 (1H, d, J=7.8 Hz), 8.41–8.35 (2H, m), 7.95 (1H, d, J=8.2 Hz), 7.82 (1H, d, J=11.4 Hz), 7.73 (1H, d, J=8.2 Hz), 7.60–7.50 (2H, m), 7.44 (1H, dd, J=7.5, 7.4 Hz), 7.36 (1H, dd, J=7.7, 7.3 Hz), 6.05 (2H, s), 3.30 (3H, s); ^{13}C NMR (75 MHz; DMSO-d_6) δ 172.8 (s), 142.2 (s), 139.6 (s), 138.2 (s), 136.8 (s), 126.5 (d), 126.2 (s), 126.0 (s), 126.0 (d), 125.0 (d), 123.8 (d), 122.1 (d), 120.9 (d), 120.8 (d), 119.2 (s), 117.3 (s), 112.8 (d), 112.6 (d), 110.0 (d), 73.6 (t), 55.6 (q); MS ESI m/z [M+1]$^+$ 329 [M−1]$^-$ 327. Anal. Calcd for $C_{21}H_{16}N_2O_2$: C, 76.8; H, 4.9; N, 8.5. Found C, 76.7; H, 4.85; N, 8.4.

4.2.9. 6,11-Dihydro-6-oxo-5*H*-cyclohepta[1,2-*b*:4,5-*b*′]-diindole (15). The bisindole **14** (164 mg, 0.50 mmol) was suspended in EtOH (15 mL) and 6 M HCl (10 mL) and heated at reflux for 8.5 h. Upon cooling to 21 °C a yellow solid precipitated. The reaction mixture was diluted with water (175 mL) and then filtered. The precipitate filtered off and washed with an excess of water. The solid was recrystallized from EtOAc to give the bisindolotropolone **15** (117 mg, 82%) in two crops as a yellow powder. Mp 398.5 °C (dec.) IR (KBr) 3252, 1756, 1598, 1558, 1514, 1408, 1216, 740, 707 cm^{-1}; ^{1}H NMR (300 MHz; DMSO-d_6) δ 12.54 (1H, s), 12.45 (1H, s), 8.99 (1H, s, J=7.9 Hz), 8.32–8.26 (2H, m), 7.72 (1H, d, J=8.2 Hz), 7.62 (1H, d, J=8.6 Hz), 7.59 (1H, d, J=11.1 Hz), 7.52–7.47 (2H, m), 7.40–7.31 (2H, m); ^{13}C NMR (75 MHz; DMSO-d_6) δ 172.9 (s), 142.6 (s), 139.2 (s), 136.6 (s), 136.5 (s), 126.8 (s), 126.3 (s), 126.1 (d), 125.4 (d), 124.4 (d), 123.5 (d), 121.2 (d), 120.7 (d), 120.6 (d), 117.9 (s), 116.8 (s), 115.0 (d), 112.7 (d), 111.0 (d); MS ESI m/z [M+1]$^+$ 285, [M−1]$^-$ 283. Anal. Calcd for $C_{19}H_{12}N_2O$: C, 80.3; H, 4.25; N, 9.85. Found C, 80.35; H, 4.3; N, 9.7.

4.2.10. 4-[2-[[2-Chloro-1-(methoxymethyl)-1*H*-indol-3-yl]carbonyl]-1*H*-indol-3-yl]-2-butanone (11). Methyl vinylketone (1.32 g, 18.8 mmol) in $MeNO_2$ (15 mL) was added at 21 °C to a suspension of [2-chloro-1-(methoxymethyl)-1*H*-indole-3-yl]-1*H*-indol-2-yl methanone[4] (2.12 g,

6.26 mmol) in $MeNO_2$ (40 mL) under nitrogen. The suspension was cooled to −20 °C and $BF_3{\cdot}OEt_2$ (87 mg, 0.61 mmol) in EtOH (1.0 mL) was added dropwise. The reaction temperature was then allowed to rise to 0 °C and kept at this temperature for 3 h, before further 92 mg of $BF_3{\cdot}OEt_2$ in EtOH (0.5 mL) was added. After 15 h at 0 °C more methyl vinylketone (960 mg) in $MeNO_2$ (1 mL) was added followed by $BF_3{\cdot}OEt_2$ (140 mg) in EtOH (0.3 mL). After 2 h, a saturated solution of $NaHCO_3$ (100 mL) was added, followed by extraction with CH_2Cl_2 (4×50 mL). The combined organic phases were washed with water (100 mL), then brine (100 mL), and dried over $MgSO_4$. Evaporation produced a reddish residue that was subjected to column chromatography (eluent Et_2O/hexane, 8:2) to give 5-nitro-2-pentanone (200 mg) and the bisindole **11** (1.69 g, 66%) identical with the product previously reported.[4]

4.2.11. 5-Nitro-2-pentanone.[24] ^{1}H NMR (300 MHz; $CDCl_3$) δ 4.41 (2H, t, J=6.6 Hz), 2.58 (2H, d, J=6.8 Hz), 2.21 (2H, m), 2.14 (3H, s); ^{13}C NMR (75 MHz; $CDCl_3$) δ 206.6 (s), 74.7 (t), 39.4 (t), 30.1 (q), 21.2 (t)

4.2.12. 12-Acetyl-5,11,12,13-tetrahydro-11-(methoxymethyl)-6*H*-cyclohepta[1,2-*b*:4,5-*b*′]diindol-6-one (12). *t*-BuOK (412 mg, 3.67 mmol) in *t*-BuOH (35 mL) was added to a solution of **11** (600 mg, 1.47 mmol) in benzene (50 mL) at 21 °C. The mixture was then heated at reflux for 70 min and then cooled (10 °C) and poured into saturated aqueous NH_4Cl (25 mL) and water (50 mL). The phases were extracted with EtOAc (3×50 mL) and the combined organic phases were dried over $MgSO_4$, and then evaporated to give a yellow solid. Titruation with Et_2O produced **12** (511 mg) as yellow crystals, with benzene inclined in the crystals in a ratio 1.00:0.39 as determined by ^{1} H NMR spectroscopy. Conventional drying of the solid failed to remove the benzene. The etheral phases were concentrated to 10 mL and stirred for 1 h at 21 °C to give another crop of **12** (30 mg), free of benzene. In total 502 mg (92%) (without benzene) was obtained of the diindolyl ketone **12**. Crystallization from CH_2Cl_2/hexane produced crystals with identical spectroscopic characteristics as with those reported previously.[4]

4.2.13. 12-Acetyl-5,11-dihydro-11-(methoxymethyl)-6*H*-cyclohepta[1,2-*b*:4,5-*b*′]diindol-6-one (16). DDQ (366 mg, 1.61 mmol) was added in one portion to a suspension of **12** (300 mg, 0.81 mmol) in benzene (125 mL) at 21 °C. The mixture was refluxed for 90 min and then additional DDQ (180 mg) was added. The reflux was continued for 4.5 h and then allowed to cool to 21 °C. 1 M NaOH (200 mL) and EtOAc (100 mL) was added to the solution. The organic solvents were separated and the aqueous phase extracted with EtOAc (2×50 mL). Drying of the organic solvents over $MgSO_4$ and removal of the solvents with evaporation produced a yellow solid. This solid was boiled in Et_2O (60 mL), filtered and then dried to give the yellow bisindole **16** (186 mg). The filtrate was then evaporated and the remaining solid crystallized from EtOAc/heptane to produce more of **16** (16 mg). The total yield of **16** was 202 mg (68%). Mp 225–230 °C; IR (KBr) 3426, 3249, 2926, 1663, 1577, 1550, 1416, 1245, 1089, 746 cm^{-1}; ^{1}H NMR (300 MHz; acetone-d_6) δ 11.91 (1H, s), 9.19 (1H, d, J=8.0 Hz), 8.69 (1H, s), 8.42 (1H, d, J=8.0 Hz), 7.86 (1H, d, J=8.2 Hz), 7.80 (1H, d, J=8.3 Hz), 7.60–7.54 (2H, m), 7.46–7.40 (2H, m), 5.75 (2H, s), 2.96 (3H, s), 2.90 (3H, s); ^{13}C NMR (75 MHz; acetone-d_6) δ 200.4 (s), 174.5 (s), 141.6 (s), 139.6 (s), 139.3 (s), 138.1 (s), 130.6 (s), 128.3 (s), 127.8 (d), 127.6 (s), 127.1 (d), 126.7 (d), 125.1 (d), 123.8 (s), 123.4 (d), 122.7 (d), 121.7 (d), 116.1 (s), 113.9 (d), 111.5 (d), 78.5 (t), 55.9 (q), 29.0 (q); MS ESI m/z $[M+1]^+$ 371, $[M-1]^-$ 369.

4.2.14. 11-(Methoxymethyl)-6-oxo-5*H*-cyclohepta[1,2-*b*:4,5-*b*′]diindole-12-carboxylic acid methyl ester (17). Yellow solid prepared with the method of Fresneda.[25] Mp 246.5–250.5 °C, (lit.[4,25] mp 255–256 °C); IR (neat) 1705, 1579, 1245 cm^{-1}; ^{1}H NMR (300 MHz; $CDCl_3$) δ 12.14 (1H, s), 9.40 (1H, d, J=7.8 Hz), 8.08 (1H, s), 8.23 (1H, d, J=7.9 Hz), 7.81 (1H, d, J=8.1 Hz), 7.96–7.59 (2H, m), 7.55–7.49 (2H, m), 7.42 (1H, dd, J=7.6, 7.3 Hz), 5.78 (2H, s), 4.07 (3H, s), 2.96 (3H, s); ^{13}C NMR (75 MHz; $CDCl_3$) δ 174.2 (s), 169.4 (s), 141.4 (s), 139.9 (s), 138.6 (s), 137.3 (s), 129.6 (d), 127.4 (d), 127.4 (s), 127.0 (s), 126.3 (d), 125.0 (d), 123. 4 (d), 122.3 (d), 120.7 (d), 119.2 (s), 116.4 (s), 113.3 (d), 110.9 (d), 78.5 (t), 56.2 (q), 53.3 (q); MS ESI m/z $[M+1]^+$ 387, $[M-1]^-$ 385.

4.3. X-ray crystallography study

CCDC reference number 210210. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax:+44-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

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