Synthesis of (+)-Thiersindole C

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This paper is dedicated to Prof. V. Gotor on the occasion of his 60th birthday.

Abstract: The indole diterpene alkaloid (+)-thiersindole C has been synthesised from *ent*-halimic acid. Firstly was elaborated the bicyclic system, secondly a Fischer indolization was used to obtain the north side chain and finally elongation of the south side chain was achieved with an isoprene unit. The synthesis of (+)-thiersindole C has corroborated the absolute configuration for the natural product (–)-thiersindole C. The synthesized (+)-thiersindole C showed antitumor activity against a number of human tumor cell lines with an IC_{50} in the range of 10^{-5} M.

Key words: indole diterpene alkaloids, thiersindole C, *ent*-halimic acid

The indole diterpene alkaloids isolated from fungi show structural diversity[1] and bioactivities such as insecticidal, tremorgenic or pollen growth inhibitory activity among others.[2]

Penicillium thiersii is a new *Penicillium* species that colonize sclerotia or stromata of wood decay fungi.[3] Extracts of *P. thiersii* cultures show potent insecticidal activity, and chemical studies of this species led to the discovery of indole diterpenoids such as thiersinines A and B[4] with a new ring system, and more recently three new indole diterpenoids thiersindoles A–C[3] with quite different structures from those isolated previously in this species (Figure 1).

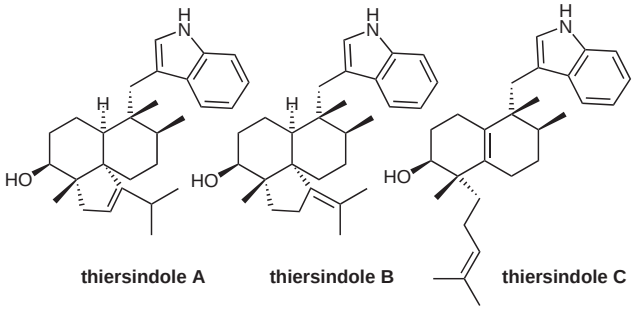

Figure 1 Thiersindoles A–C structures.

Thiersindoles A–C structures and their relative stereochemistries were determined by analysis of NMR data (^{1}H and ^{13}C, HMQC, HMBC and NOESY). The absolute stereochemistry of thiersindole B was assigned by application of the modified Mosher NMR method. The other two thiersindoles were assumed to posses the same absolute stereochemistry.[3]

In this paper we show the synthesis of thiersindole C, confirming the proposed structure and absolute configuration,[3] from the natural product *ent*-halimic acid (**1**)[5] (Figure 2). This compound, whose structure and absolute configuration are known, and which has been previously used by us for the synthesis of bioactives products such as *ent*-halimanolides,[6] *ent*-agelasine C,[7] chettaphanins I[8] and II[9] and sesterterpenolides,[10] was chosen as starting material for the synthesis of thiersindole C.

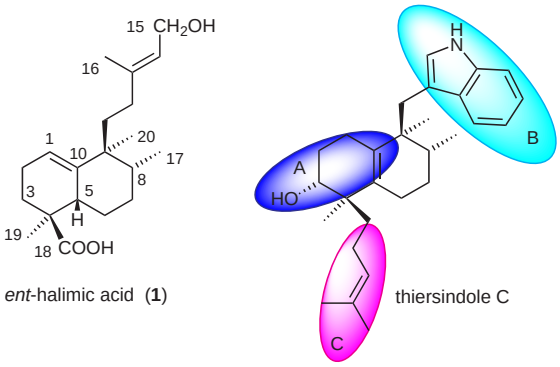

Figure 2 Structures for *ent*-halimic acid and thiersindole C.

The main transformations for the synthesis of thiersindole C from *ent*-halimic acid are (Figure 2):

(a) Functionalization at C-3 and isomerization of the anular double bond to the tetrasubstituted position.

(b) Elaboration of the indole unit on the north side chain.

(c) Addition of an isoprenic unit at C-18 in order to form the south side chain.

The retrosynthetic patway is shown in Scheme 1. Elaboration of the south side chain of thiersindole C can be achieved from the intermediate **4** (Scheme 1), via elongation by a one-carbon unit and then by a four-carbons

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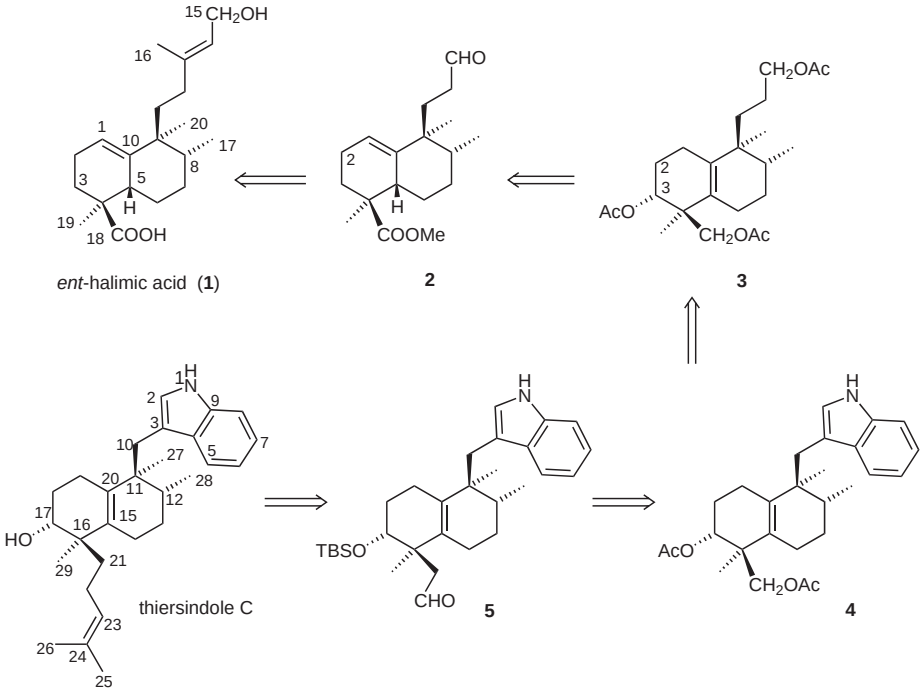


Scheme 1

fragment, by displacement of a good leaving group such as iodide or tosylate group to give **5**. The north side chain can be elaborated by Fischer's methodology with an aldehyde derivative from the intermediate **3**. The functionalization of the bicyclic system can be achieved with the trinorderivative **2**, previously obtained from *ent*-halimic acid (**1**)[5] by allylic oxidation at C-2 position, subsequent oxygenation at C-3, and removal of the carbonyl group at C-2. Ulterior migration of the double bond to C5–C10 will give the intermediate **3**, after changing to the adequate functional groups.

The synthesis of thiersindole C was carried out in three parts:

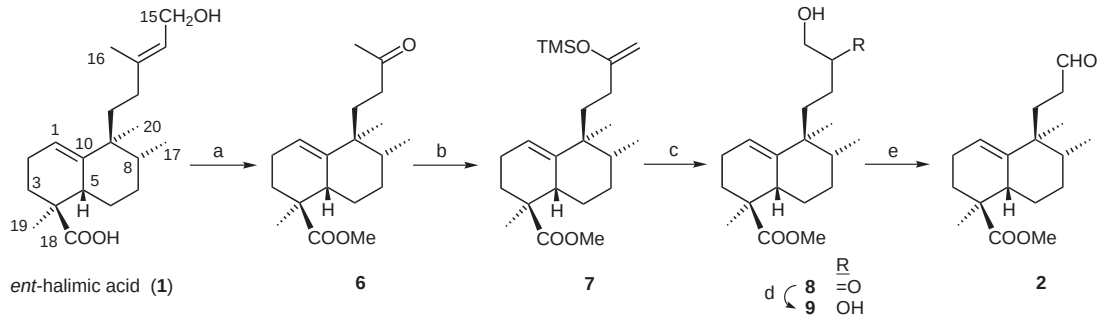
1) Synthesis of the intermediate **3** functionalized at C-3 and tetrasubstituted double bond.
2) Synthesis of the indole intermediate **4**.
3) Synthesis of thiersindole C, and final transformations.

Synthesis of Intermediate 3 Functionalized at C-3 and Tetrasubstituted Double Bond

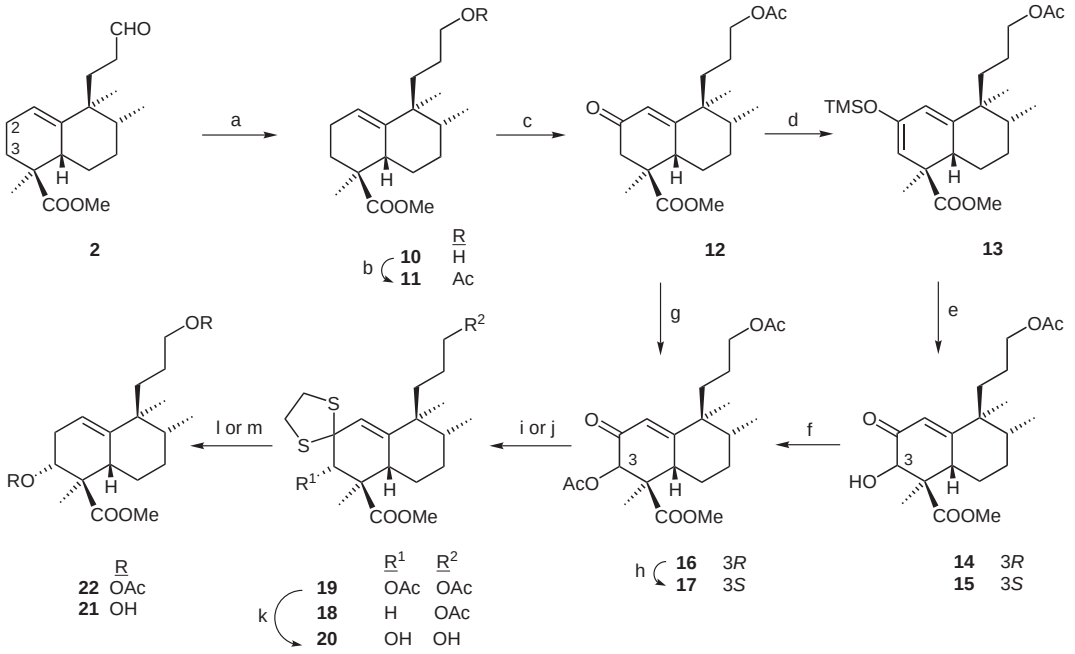
This stage included the degradation of the side chain of *ent*-halimic acid (**1**) by three carbon atoms to elaborate the derivative **2**, and then functionalization on C-3, followed by isomerization of the double bond.

Methylketone **6** was obtained from *ent*-halimic acid (**1**; Scheme 2), following our protocol.[10] Trapping the kinetic enolate of **6** by treatment with LDA and TMSCl gave the silylenolether **7** which by oxidation with OsO_4–NMO[9,11] produced the α-hydroxyketone **8**. Reduction of the last compound with $NaBH_4$ followed by cleavage of the diol unit of **9** with $Pb(OAc)_4$ gave the required aldehyde **2**.

Functionalization at C-3 (Scheme 3) was achieved by oxidation of C-2. Subsequent removal of the carbonyl group obtained gave the intermediate **21** (Scheme 3).



Scheme 2 *Reagents and conditions*: (a) Ref. 10; (b) LDA, TMSCl, THF, –78 ºC, 30 min (96%); (c) OsO_4, NMO, *t*-BuOH, THF, H_2O, r.t., overnight (97%); (d) $NaBH_4$, EtOH, r.t., 30 min (93%); (e) $Pb(OAc)_4$, C_6H_6, r.t., 20 min (98%).



Scheme 3 *Reagents and conditions*: (a) $NaBH_4$, EtOH, r.t., 30 min (95%); (b) Ac_2O, pyridine, r.t., overnight (98%); (c) Na_2CrO_4, Ac_2O, AcOH, NaOAc, C_6H_6, 55 °C, overnight (71%); (d) LDA, TMSCl, THF, –78 °C, 30 min (95%); (e) OsO_4, NMO, *t*-BuOH, THF, H_2O, r.t., overnight; (f) Ac_2O, pyridine, r.t., overnight (**12**: 14%; **16**: 38%; **17**: 38%, from **13**, two steps); (g) $Mn(OAc)_3{\cdot}2H_2O$, C_6H_6, 130 °C, overnight (**16**: 48%; **17**: 49%); (h) NaH, THF, 70 °C, 1 h (88%, based on recovered starting material); (i) ethane-1,3-dithiol, CH_2Cl_2, $BF_3{\cdot}Et_2O$, r.t., overnight (**18**: 11%; **19**: 83%); (j) 1,2-ethanedithiobis(trimethylsilane), ZnI_2, Et_2O, –20 °C → r.t. (**19**, 92%); (k) K_2CO_3–MeOH (3%), r.t., overnight (96%); (l) **19** to **22**: Ni (Raney), EtOH, 50 °C, 2 h (**22**: 65%; **11**: 31%); (m) **20** to **21**: Ni (Raney), EtOH, 50 °C, 2 h (96%).

Reduction of **2** with $NaBH_4$ followed by acetylation of the hydroxyderivative **10** led to the acetoxyderivative **11** that was treated with Na_2CrO_4 to afford **12** in good yield.

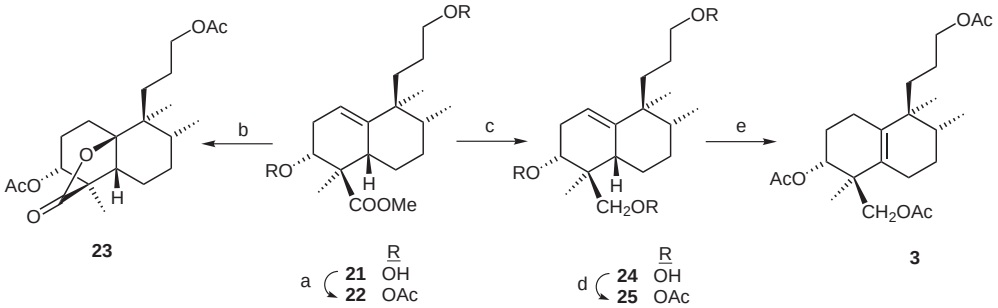
Oxidation at C-3 in **12** was carried out in two different ways:

a) Trapping the enolate derived from the kinetic deprotonation of enone with chlorotrimethylsilane provided the *O*-trimethylsilyldienol ether **13**. Oxidation of the later compound with OsO_4 (or MCPBA) led to the mixture of dihydroxy derivatives **14** and **15**, which without further isolation were characterized as the acetyl derivatives **16** and **17**.

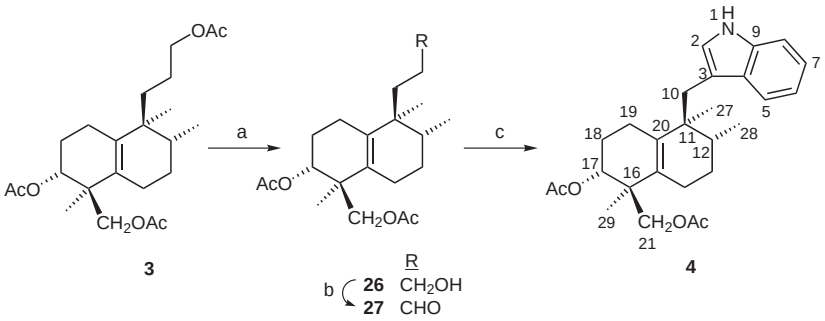
b) Better results were obtained by reaction of **12** with $Mn(OAc)_3$[12] that gave **16** and **17** in an excellent yield. The undesirable isomer **16** could be recycled into **17**, by treatment with NaH that gave a mixture of **16** and **17**, with **17** being the major component.

Once we had introduced the hydroxy group at C-3, we proceeded to reduce the carbonyl group at C-2. Treatment of **17** with ethanedithiol in the presence of $BF_3{\cdot}Et_2O$ led to compounds **18** (11%) and **19** (83%). However, best results were obtained when the reaction of **17** took place with 1,2-ethanedithiobis(trimethylsylane) and ZnI_2,[13] affording **19** in a 92% yield. If dithiane **19** was made to react with Ni Raney[14] a mixture of **22** and **11** was obtained, however if diol **20**, (obtained by alkaline hydrolysis of **19** with K_2CO_3), reacted with Ni Raney,[14] it afforded only the diol **21** in an excellent yield (77% from **17**).

Once the C-3 goup had been oxygenated, the only task that remained was to isomerize the annular double bond (Scheme 4). This isomerization in acidic media has been achieved before for other derivatives of *ent*-halimic acid,[15] but it must not be done in the absence of the methoxycarbonyl at C-18, because if the reaction takes place



Scheme 4 *Reagents and conditions*: (a) Ac_2O, pyridine, r.t., overnight (98%); (b) HI, C_6H_6, 85 °C, 8 h (75%); (c) $LiAlH_4$, Et_2O, r.t., 1 h (96%); (d) Ac_2O, pyridine, r.t., overnight (98%); (e) HI, C_6H_6, 85 °C, 8 h (92%).



Scheme 5 (a) K_2CO_3–MeOH (0.7%), 0 °C, 5 h (94%); (b) CrO_3, pyridine, CH_2Cl_2, r.t., 1 h (99%); (c) $PhNHNH_2$, AcOH, r.t., 2 h, and 130 °C, 2 h (93%).

with this functionality, γ-lactones 10,18-olides are generated, as showed by reaction of the acetyl derivative **22** with HI that gave lactone **23** (Scheme 4). That is the reason we decided to obtain the acetyl derivative **25**, originated by $LiAlH_4$ reduction of **21** and subsequent acetylation of triol **24** that led to the desired triacetate **3** by acidic treatment of **25**.

Synthesis of Indole Intermediate 4

To introduce the indole system in the side chain of the triacetate **3**, it was necessary to obtain the aldehyde **27** and make use of the Fischer reaction.[16] (Scheme 5).

Chemoselective hydrolysis of **3** with K_2CO_3 afforded the diacetyl derivative **26**, which by oxidation with CrO_3 in pyridine gave the aldehyde **27** in nearly quantitative yield.

Reaction of **27** with phenylhydrazine in AcOH gave the indole derivative **4**[17a] in a very good yield. The X-ray experiments confirmed the absolute configuration of **4**[17b] (Figure 3).

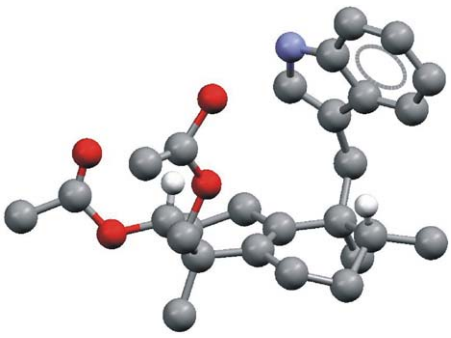


Figure 3 ORTEP view of compound **4**.

Synthesis of Thiersindole C and Final Transformations

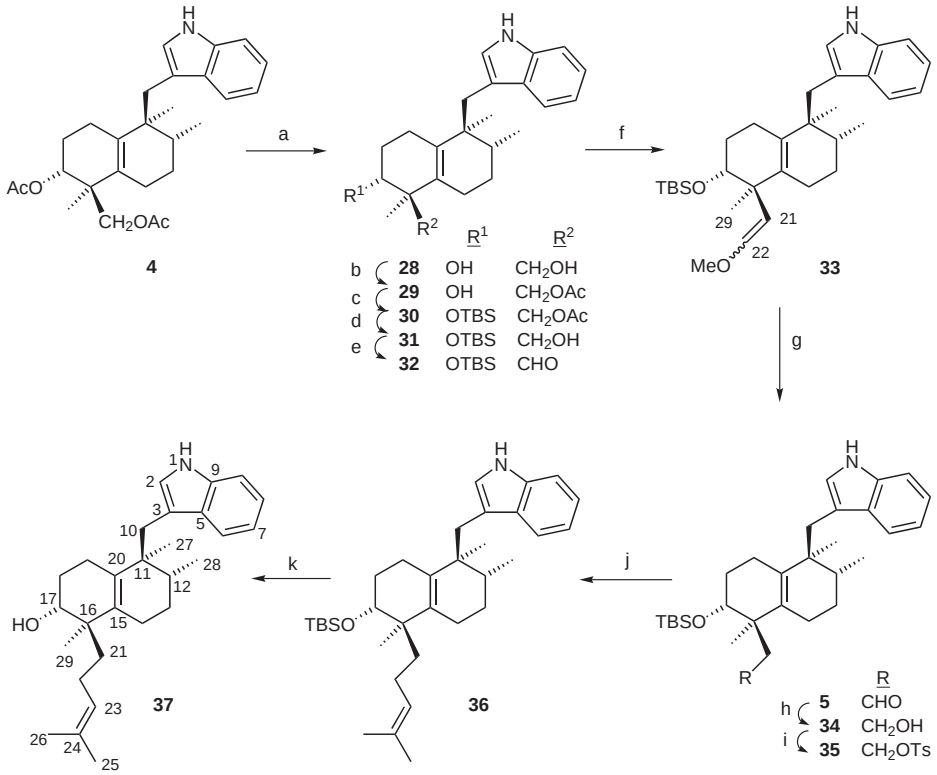
Finally, the transformation of **4** into thiersindole C required elongation of the south chain with an isoprenic unit. It was necessary to distinguish both acetoxy groups and protect C-3. Due to neopentyl character of the primary hydroxy group it was necessary to introduce the remaining five carbons in two steps, first elongation by one carbon and then by four carbons (Scheme 6).

Hydrolysis of **4** led to diol **28** that by acetylation with one equivalent of Ac_2O in pyridine afforded the monoacetyl derivative **29**. Protection of the hydroxy group at C-3, with TBDMSOTf gave **30**, that by hydrolysis generated the hydroxy derivative **31**, which was oxidized with tetrapropylammonium perruthenate (TPAP)–NMO to give the aldehyde **32**. Reaction of **32** with methoxymethylenetriphenylphosphonium chloride in the presence of NaHMDS[18] gave **33** that by careful hydrolysis with *p*-TsOH led to the aldehyde **5**.

Reduction of **5** with $NaBH_4$ produced the hydroxy derivative **34** that by treatment with TsCl in pyridine gave the tosylate **35**. Reaction of the latter with the appropriate cuprate[19] afforded **36**. Final deprotection of **36** with TBAF gave **37**[20] ($[\alpha]_D^{22}$ +54) whose physical data are exactly the same as those describe by Gloer et al. for (–)-thiersindole C ($[\alpha]_D^{22}$ –42)[3] (Figure 1) except for the sign of the optical rotation which it is exactly the opposite.

In this manner the structure for thiersindole C was confirmed and the absolute configuration for the natural product was corroborated. Further studies on the synthesis of thiersindoles A and B are currently under progress.

The in vitro antitumor activity of (+)-thiersindole C was determined by the XTT assay in different human tumor cell lines,[21] including HeLa (human epitheloid cervix carcinoma), A549 (human lung carcinoma), HT-29 (human colon adenocarcinoma), and HL-60 (human acute myeloid leukemia). The synthesized (+)-thiersindole C inhibited proliferation of HeLa (IC_{50} = 25.8 ± 3.1 μM), A549 (IC_{50} = 28.0 ± 3.2 μM), HT-29 (IC_{50} = 30.5 ± 2.2 μM) and HL-60 (IC_{50} = 26.2 ± 2.8 μM) cells. These data show that compound **37** inhibits proliferation of various human tumor cells, including leukemic and solid tumor cells, with an IC_{50} in the range of 25 μM. These results indicate that this compound has approximately the same antitumor potency as several diterpenes such as tubingensins A and B.[22]



Scheme 6 *Reagents and conditions*: (a) K_2CO_3–MeOH (1%), 0 ºC, 8 h (97%); (b) Ac_2O (1 equiv), pyridine, r.t., overnight (**4**: 7%; **29**: 89%); (c) TBDMSOTf, 2,6-lutidine, CH_2Cl_2, 0 ºC to r.t., 45 min (98%); (d) $LiAlH_4$, Et_2O, r.t., 1 h (97%); (e) TPAP–NMO, CH_2Cl_2, r.t., 2 h (96%); (f) $MeOCH_2PPh_3Cl$, NaHMDS, THF, –78 ºC, 20 min (94%); (g) *p*-TsOH, Me_2CO, H_2O (0.03 M), r.t., overnight (95%); (h) $NaBH_4$, EtOH, r.t., 1 h (96%); (i) TsCl, pyridine, r.t., 2 h (97%); (j) 2-methylpropenylmagnesium bromide, $ClCuSMe_2$, THF, –45 ºC to r.t., 4 h (86%); (k) TBAF, THF, 70 ºC, 3 h (77%).

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(17) (a) Numbering for compound **4** agrees with that for the thiersindole C skeleton. (b) Crystal data for **4**: $C_{27}H_{35}NO_4$, monoclinic, space group $P2_1$ (no 4), a = 7.4100 (15) Å, b = 10.169 (2) Å, c = 16.045 (3) Å, α = γ = 90°, β = 97.11 (3)°, V = 1199.7 (4) Å^3, Z = 2, D_c = 1.211 Mg/m^3, μ(Cu – $K_α$) = 0.640 mm^{-1}, F(000) = 472. The number of reflections collected was 1963, of which 937 were considered to be observed with I > 2σI. The structure was determined by direct methods using the SHELXTL^{TM} suite of programs. Hydrogen atoms were placed in calculated positions. Full-matrix least squares refinement based on F^2 with anisotropic thermal parameters for the non-hydrogen atoms led to agreement factors, R_1 = 0.0721 and $ωR_2$ = 0.1823. Crystallographic data (excluding structure factors) for the structure reported in this paper has been deposited at the Cambridge Crystallographic Data Centre as supplementary material no. CCDC-628164.

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(20) **(+)-Thiersindole C(37)**: $[α]_D^{22}$ +54.0 (*c* = 0.20, CH_2Cl_2). IR (film): 3411, 3056, 2927, 1457, 1375, 1261, 1094, 1046, 1012, 995, 740 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.89 (br s, 1 H, H-1), 7.60 (d, *J* = 7.9 Hz, 1 H, H-5), 7.32 (d, *J* = 8.1 Hz, 1 H, H-8), 7.16 (ddd, *J* = 1.1, 7.2, 8.1 Hz, 1 H, H-7), 7.10 (ddd, *J* = 1.1, 7.2, 7.9 Hz, 1 H, H-6), 7.05 (s, 1 H, H-2), 5.10 (br t, *J* = 6.0 Hz, 1 H, H-23), 3.82 (dd, *J* = 3.7, 11.1 Hz, 1 H, H-17), 2.92 (d, *J* = 15.8 Hz, 1 H, H_A-10), 2.84 (d, *J* = 15.8 Hz, 1 H, H_B-10), 2.37 (m, 1 H, H_A-14), 2.25 (m, 1 H, H_B-14), 2.02 (m, 1 H, H_A-19), 2.00 (m, 1 H, H_A-22), 1.73 (m, 2 H, H_B-19, H_B-22), 1.71 (m, 1 H, H-12), 1.70 (m, 1 H, H_A-18), 1.68 (s, 3 H, Me-26), 1.60 (m, 1 H, H_A-21), 1.55 (s, 3 H, Me-25), 1.49 (m, 1 H, H_A-13), 1.39 (m, 1 H, H_B-13), 1.36 (m, 1 H, H_B-21), 1.03 (s, 3 H, Me-27), 0.98 (s, 3 H, Me-29), 0.86 (d, *J* = 6.8 Hz, 3 H, Me-28). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 121.4 (C-2), 113.1 (C-3), 128.9 (C-4), 118.7 (C-5), 119.1 (C-6), 121.6 (C-7), 110.8 (C-8), 135.4 (C-9), 31.2 (C-10), 42.1 (C-11), 33.3 (C-12), 27.2 (C-13), 24.8 (C-14), 134.7 (C-15), 43.1 (C-16), 80.0 (C-17), 27.5 (C-18), 24.9 (C-19), 135.0 (C-20), 35.6 (C-21), 23.1 (C-22), 124.8 (C-23), 131.1 (C-24), 25.6 (C-25), 17.7 (C-26), 21.7 (C-27), 16.7 (C-28), 20.4 (C-29). HRMS (ESI): *m*/*z* [M$^+$ + Na] calcd for $C_{28}H_{39}NONa$: 428.2924; found: 428.2928.

(21) The in vitro antitumor activity for compound **37**, (+)-thiersindole C, was determined by measurement of its cytostatic and cytotoxic properties in human tumor cell lines by the XTT assay, in which the metabolic activity of viable cells was assessed. Cells were incubated in RPMI-1640 (HL-60) or DMEM (HeLa, A549, HT-29) culture medium containing 10% fetal calf serum, in the absence and in the presence of the indicated compound at a concentration range of 10^{-4} to 10^{-8} M in 96-well plates, and following a 72-h incubation at 37 °C in a humidified atmosphere of air–CO_2 (19:1) the XTT assay was performed. Measurements were done in triplicate, and the IC_{50} value, defined as the drug concentration required to cause 50% inhibition in the cellular proliferation with respect to the untreated controls, were determined. Values shown are means ±SE of four independent determinations.[22]

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