Rational Drug Design and Synthesis of a Highly Selective Nonpeptide δ-Opioid Agonist, (4a*S**,12a*R**)-4a-(3-Hydroxyphenyl)-2-methyl-1,2,3,4,4a,5,12,12a-octahydropyrido[3,4-*b*]acridine (TAN-67)

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We designed highly selective non-peptide agonists for the δ-opioid receptor. On the basis of the "message–address" concept in this field and the accessory site hypothesis, a novel class of heterocycle-fused octahydroisoquinoline derivatives were synthesized. One of these compounds [(4a*S,12a*R**)-4a-(3-hydroxyphenyl)-2-methyl-1,2,3,4,4a,5,12,12a-octahydropyrido[3,4-*b*]acridine, TAN-67 (2)] showed high selectivity for the δ-opioid receptor (K_i=1.12 nM) in guinea-pig cerebrum with a 2070-fold lower affinity for the μ-opioid receptor and a 1600-fold lower affinity for the κ-opioid receptor. TAN-67 was a potent δ-opioid receptor agonist with an IC_{50} value of 6.61 nM in the mouse vas deferens assay that was reversed by naltrindole (NTI) (K_e=0.21). Moreover, TAN-67 was shown to have antinociceptive activity following subcutaneous administration in the mouse acetic acid abdominal constriction assay that was antagonized by NTI (δ_1- and δ_2-antagonist) and 7-benzylidinenaltrexone (δ_1-antagonist), but not by naltriben (δ_2-antagonist). This systemically applicable non-peptide agonist will be useful for elucidating the pharmacological properties of the δ-opioid receptor.**

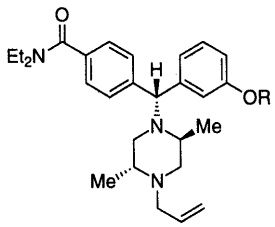
Key words δ-opioid agonist; message–address concept; accessory site; TAN-67; δ_1-selective agonist

Three types of opioid receptors (μ, δ, κ) are now well established not only by pharmacological studies but also by molecular biological studies.[1] Although many highly selective and potent δ opioid receptor agonists are presently available for studies on this receptor,[2] almost all of them are peptides related to enkephalin (the most popular one is DPDPE ([D-Pen^2, D-Pen^5]enkephalin))[3] and their administration in animal studies is limited to intra cerebral ventricle (i.c.v.) or intrathecal (i.t.) injection. Because of this limitation, the pharmacological study of the δ opioid receptor has not progressed rapidly. Moreover, each type of opioid receptor contains some subtypes,[4] *e.g.*, δ_1 and δ_2, which complicates the study of their pharmacological functions.

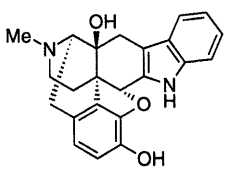
Recently, nonpeptide δ opioid agonists BW373U86,[5] SNC80,[6] OMI[7] and SIOM[8] have been described (Fig.1), but more selective (including subtype selectivity) and potent agonists are still needed to investigate the properties of the δ opioid receptor. Here, we report the design and syntheses of nonpeptide, highly selective, and potent δ opioid receptor agonists which are novel, heterocycle-fused, octahydroisoquinoline derivatives (this class of compounds has also been independently investigated by the SmithKline Beecham group[9]).

Design Rationale Naltrindole (NTI, **7**) was originally reported as a potent and selective nonpeptide δ opioid antagonist.[10] The rationale for the design of this antagonist was based on the "message–address" concept[11,12] for the recognition of ligands by receptors and on the idea that the phenyl group of Phe^4 of leucine–enkephalin functions as part of the address for interaction with the δ opioid receptor. In the model for the binding of NTI with the δ-opioid receptor, we considered the three pharmacophore binding sites at the morphinan moiety (message part) and the one δ-opioid receptor specific interactions at the address part (Fig. 2). The possible pharmacophore binding sites of the morphinan part would have the ability for ionic interaction with the cationic part of NTI (protonated 17-nitrogen), a π–π interaction with the aromatic moiety, and a hydrogen bond with the 3-hydroxy group, respectively. The selective binding of NTI to the δ-opioid receptor is due to an additional π–π interaction of the indole aromatic ring of the address part with the δ-receptor, because the cyclohexeno derivative (where the benzene moiety of the indole ring is converted to a cyclohexene ring) is deficient in selectivity and potency for the δ-opioid receptor.[13] This binding model for NTI in combination with the accessory site[14] model led us to the design of novel selective δ-opioid receptor agonists.

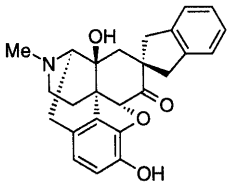
In general, receptors can change their structure as they fit into the structure of ligands ("induced fit") when the agonist binds them. This change leads to the next signal transduction so that the agonist shows a pharmacological effect. When the antagonist has an extra structural part that interferes with the



(±) - BW373U86 : R = H
(+) - SNC80 : R=Me



OMI



SIOM

Fig. 1

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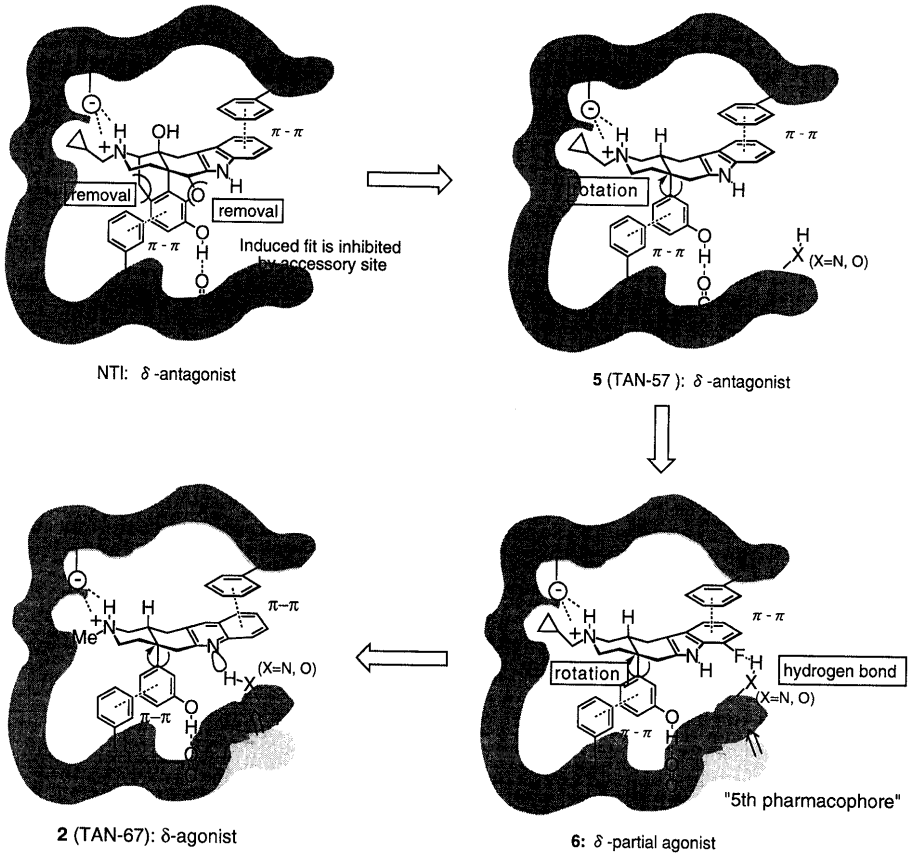


Fig. 2. Possible Binding Model of Each Ligand with the δ-Receptor

structural change of the receptor, it does not show such an effect even if it binds a receptor. The structural site for participating in the interference of "induced fit" is called an "accessory site," which is usually a hydrophobic and sterically hindered site. The structural difference between an antagonist and agonist lies mainly in whether it has an accessory site or not. From this point of view, we paid attention to the phenol part of NTI which is fixed by the 4,5-epoxy and 10-methylene moieties. The conformationally fixed phenol moiety could disturb the approach (induced fit) of the receptor for NTI. In order to evaluate this hypothesis, we synthesized the compound **5** (TAN-57; Fig. 2).[15] Compound **5** has a freely rotatable phenol ring (4,5-epoxy and 10-methylene moieties were removed from NTI) which can rotate to a suitable position by induced fit for the agonistic interaction with the receptor.

Contrary to our expectation, compound **5** showed no agonist activity in the mouse vas deferens (MVD) test up to 10 mM. However, after investigating the structure–activity relationships in this series of compounds, we at last discovered the remarkably potent but partial δ opioid agonist **6** with a 7-F-substituent (max 61.7±5.1% inhibition [n=4], up to 60 nM; 50% inhibition 57.2±6.8 nM [n=4] in the MVD test). Furthermore, none of the compounds, including the 7-Cl, Br, Me, NO_2, and 8-, 9- or 10-F derivatives, showed agonist activity.[16] From these observations, we assumed that the significant character of the fluoro-substituted compound is derived from its hydrogen bonding ability, because only the fluorine atom can form a hydrogen bond among these substituents. The specific position (only 7-, but not 8-, 9- or 10-) of the substituent for the agonist activity implies the importance of the hydrogen bond at this position for changing the receptor shape to lead to agonist activity (Fig. 2). In other words, this final binding site, which we call a "fifth pharmacophore", should be dominant for its agonist character. We then designed a more favorable structure for the hydrogen bond with the fifth pharmacophore site to give a tighter fit with the receptor. On the basis of the above hypothesis, we synthesized compounds which are fused quinoline or quinoxaline to decahydroisoquinoline. These compounds have a lone pair electron on a nitrogen atom in the quinoline or quinoxaline rings in order to form a hydrogen bond with the receptor more effectively than with the 7-fluoro-indolo compound **6**. Furthermore, the phenol ring in these compounds can rotate to remove the steric hindrance with the receptor in the case of binding to the fifth pharmacophore.

Compound **2** (TAN-67), designed as mentioned above, was shown to be highly selective and to have potent δ-opioid agonist activity. The presumed model for the binding of TAN-67 with the δ-opioid receptor is shown in Fig. 2. The five pharmacophore interactions should be important for its selectivity and potency.

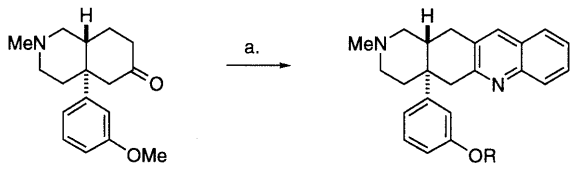
Chemistry Compound **9** was synthesized by treatment of compound **8**[17] with *o*-aminobenzaldehyde in ethanol under acidic condition (Chart 1).

Compounds **11** and **13** were synthesized from compound **9** through the steps shown in Chart 2. Thus, compound **9** was transformed to the vinyl carbamate derivative **10a** or the 2,2,2-trichloroethyl carbamate derivative **10b** using chloroformate in 1,2-dichloroethane with Proton Sponge™ (1,8-bis(dimethylamino)naphthalene).[18] Then **10a** or **10b** was subjected to acidic or reductive conditions to give compound

11, which was condensed with cyclopropanecarbonyl chloride to afford the amide derivative **12**. The *N*-cyclopropylmethyl compound **13** was synthesized by successive metal hydride reduction of **12**.

The quinoxalino compound **20** was synthesized from the new diketo intermediate **18**[19] by condensation with 1,2-diaminobenzene. The diketo compound **18** was made from compound **14** in a manner similar to that reported by Zimmerman and colleagues[17] through the steps shown in Chart 3.

All of these 4a-methoxyphenyl compounds **9**, **11**, **13**, and **20** were demethylated to give the 4a-hydroxyphenyl compounds **2**, **3**, **1**, and **4**, respectively using *n*-PrSH/*tert*-BuOK or BBr_3 in order to evaluate their pharmacological properties.



8

(a) o-$C_6H_4(NH_2)(CHO)$, $MeSO_3H$, EtOH (b) *n*-PrSH, *t*-BuOK, DMF

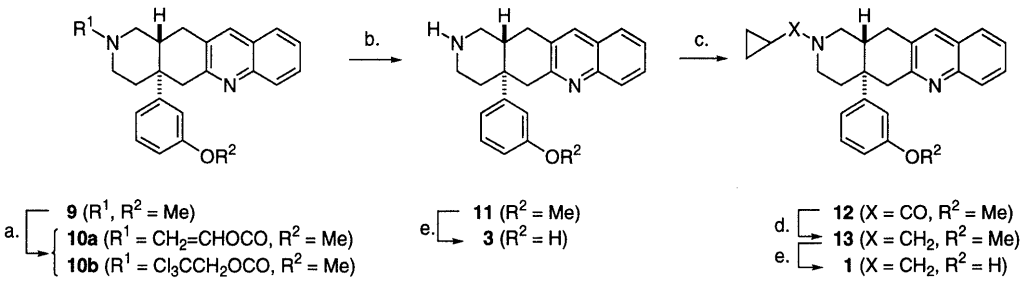
Chart 1

Pharmacological Results

Binding Studies The opioid receptor binding activity of selected compounds was determined with a radio-ligand competition assay in guinea pig brain membranes using a modification of the method of Werling *et al.*[20] ^{3}[H]-[D-Ala^2, MePhe^4, Gly-ol^5]enkephalin (DAMGO, 1 nM), ^{3}[H]-DPDPE (1 nM) and ^{3}[H]-ethylketocyclazocine (EKC, 2 nM) were used to label μ, δ, and κ binding sites, respectively. Nonspecific binding was defined as binding in the presence of 5 μM naloxone (μ-receptor), 2 μM DADLE (δ-receptor) or 5 μM EKC (κ-receptor). DAMGO (100 nM) and DPDPE (200 nM) were included in the incubation mixture with ^{3}[H]-EKC to prevent binding to the μ and δ sites, respectively. The binding data are expressed as K_i values (Table 1).

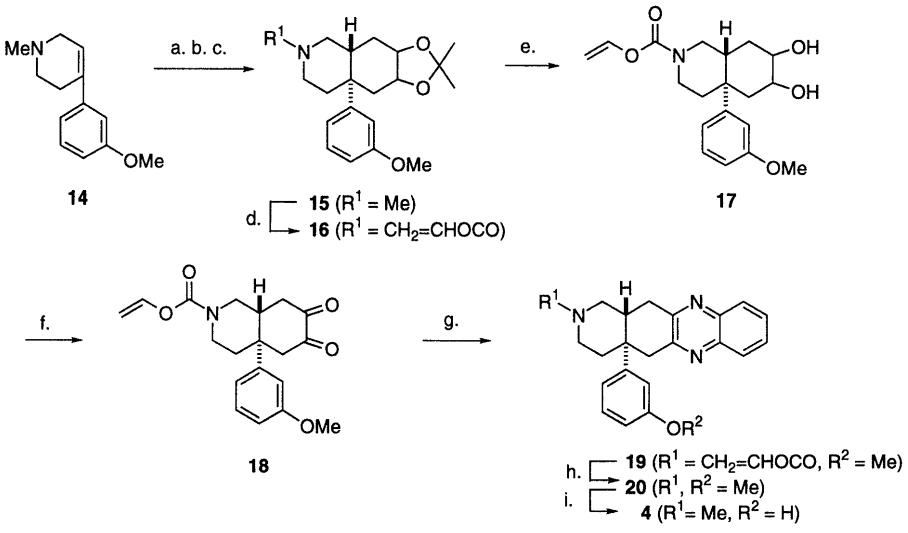
All of the compounds in Table 1 had a lower K_i value for the δ-opioid receptor than for the μ- and κ-opioid receptors. Compound **2**, named TAN-67, had the highest selectivity with a K_i ratio of 2070 for μ/δ and 1600 for κ/δ.

Smooth Muscle Experiments The opioid agonist potencies of the target compounds were determined on electrically stimulated guinea-pig ileum (GPI) and MVD preparations (Table 2). The agonist activities are expressed as IC_{50} values and dose ratio (IC_{50} ratio) which is the IC_{50} of the agonist in the presence of the antagonist, naloxone (μ), nor-binaltorphimine (nor-BNI) (κ), and NTI (δ), respectively, divided by



(a) CH_2=CHOCOCl, Proton Sponge™, CH_2Cl_2 or Cl_3CCH_2OCOCl, Proton Sponge™, CH_2Cl_2 (b) HCl / MeOH or Zn, AcOH (c) cyclo-C_3H_5COCl, Et_3N, THF (d) DIBAL, toluene (e) *n*-PrSH, *tert*-BuOK, DMF

Chart 2



14 **17** **18**

(a) (1) *n*-BuLi, THF (2) 2,2-dimethyl-4,5-bis(bromomethyl)-1,3-dioxolane, THF (b) NaI, K_2CO_3, DMF (c) $NaBH_4$, HCl/MeOH (d) CH_2=CHOCOCl, Proton Sponge™, CH_2Cl_2 (e) HCl / MeOH (f) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2 (g) o-$C_6H_4(NH_2)_2$, EtOH (h) DIBAL, toluene (i). BBr_3, CH_2Cl_2

Chart 3

Table 1. Opioid Receptor Binding[a]

Compd	K_i (nM)			K_i selectivity ratio	
	δ	μ	κ	μ/δ	κ/δ
1	0.90±0.39	175± 65	42± 15	194	47
2 (TAN-67)	1.12±0.42	2320±720	1790±602	2071	1600
3	2.41±0.86	452±112	2130±765	188	884
4	4.32±1.50	664±222	—[b]	154	—
5 (TAN-57)	3.50±0.98	71.6± 19	951±302	21	271
DPDPE[c]	4.74±1.35	911±251	—[b]	192	—

a) Each value is mean±SEM of at least three determinations. *b*) Undetectable. *c*) δ-Selective opioid peptide.

Table 2. Opioid Agonist Activity

Compd	GPI	MVD		
	IC_{50} (nM)	IC_{50} (nM)	NTI DR[a]	K_e (nM)[b]
1	10733± 1357	0.25±0.02	30.8	0.67
2 (TAN-67)	26470± 3211	6.61±1.25	96.2	0.21
3	135026±11025	88.7±9.3	165	0.12
4	114425± 9806	65.7±8.6	89.7	0.23
DPDPE[c]	11300± 1085	3.93±0.49	76.5	0.26

a) DR (Dose Ratio): (the IC_{50} in the presence of NTI)/(the IC_{50} without NTI). The concentration of NTI was 20 nM. *b*) K_e value=(the concentration of NTI)/(DR-1). *c*) δ-Selective opioid peptide.

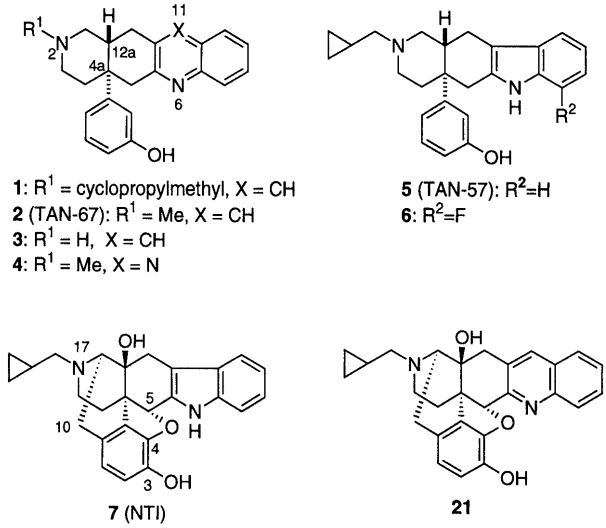


Fig. 3

the control IC_{50} of the agonist. The K_e value was calculated from the equation K_e=[NTI]/(IC_{50} ratio−1) when the response was antagonized by NTI (20 nM).

The compounds in Table 2 showed potent agonist activity, *i.e.*, the IC_{50} values were 0.25—88.7 nM in the MVD test. When they were tested after treatment of the preparation with NTI, a δ-selective antagonist, the IC_{50} value was significantly increased. On the other hand, using the GPI preparation, the tested compounds showed very weak agonist activity which was not reversed by naloxone (20 nM) or nor-BNI (20 nM). These results show that these compounds are effective δ-selective agonists.

Acetic Acid Writhing Study The antinociceptive effect was evaluated using the acetic acid abdominal constriction test. Each mouse was injected i.p. with 0.7% acetic acid in a volume of 10 ml/kg, 30 min after s.c. administration of the test compound. The number of abdominal constrictions was counted during the 10—20 min period after the injection of the test compound.

Subcutaneous administration of TAN-67 caused a decrease in the number of constrictions in a dose-dependent manner with an ED_{50} (95% confidence limits) of 31.4 (14.2—69.4) mg/kg. Thus TAN-67 has an antinociceptive effect even when administered peripherally.

Discussion

We designed and synthesized the δ-opioid receptor agonist TAN-67 based on the "message–address" concept and the accessory site hypothesis. In addition, we demonstrated the importance of the "fifth pharmacophore", which is involved in hydrogen bonding at the 6-nitrogen of octahydropyrido[3,4-*b*]acridine framework of TAN-67. TAN-67 showed high selectivity in a receptor binding test and remarkable potency in a MVD assay as a δ-selective agonist. Moreover, TAN-67 administered subcutaneously at doses from 3 to 100 mg/kg produced a dose-dependent inhibition of the acetic acid-induced abdominal constriction response, and this antinociceptive response was mediated by the stimulation of δ_1-, but not by δ_2-, μ-, or κ-opioid receptors, because the effect was blocked by the s.c. pretreatment with δ_1-antagonist 7-benzylidinenaltrexone (BNTX), but not by naltriben[21] (NTB, δ_2), β-funaltrexamine (β-FNA, μ), or nor-BNI (κ).[21] This selectivity was also confirmed by the intrathecal administration during the tail-flick test using the optically pure form of TAN-67 ((−)-TAN-67).[22] In addition, we had already shown that TAN-67 has a high binding affinity for the cloned human δ-opioid receptor expressed on Chinese hamster ovary (CHO) cells and that it has full and potent agonist activity in the forskolin-stimulated adenylate cyclase activity test.[23]

All compounds in Table 1 have lower K_i values for the δ-opioid receptor rather than for the μ- and κ-opioid receptors. Compound **1** having a cyclopropylmethyl substituent on nitrogen (2-position) was a more potent agonist in the MVD assay with a high degree of dose-shift when antagonized by NTI. The N-H compound **3** was also an agonist in the MVD assay; therefore, the full agonist character and δ-selectivity of TAN-67 was not very dependent on the N-substituent.

The 10-times lower potency of the octahydropyrido[3,4-*b*]phenazine derivative, compound **4**, compared to that of TAN-67 can be explained by the lower electron-donating property of the nitrogen in the quinoxaline ring.

As mentioned in the design rationale section, both the fea-

tures of a freely rotatable 4a-hydroxyphenyl group and of a lone pair electron capable of forming a hydrogen bond with the receptor as the "fifth pharmacophore" are important for the agonist activity of TAN-67. In fact, the conversion of the indole moiety of NTI to the quinoline group which is compound **21** reported by Portoghese and colleagues,[13] resulted in merely a weak antagonist. Therefore, even if the lone pair electron as the fifth pharmacophore is positioned at this point, such a compound will not be a δ-opioid agonist without a freely rotatable 4a-hydroxyphenyl group. Only when the 4a-hydroxyphenyl moiety rotates to remove the steric hindrance, the δ-opioid receptor can access the fifth pharmacophore site in the ligand and change its shape.

On the other hand, a compound with a freely rotatable 4a-hydroxyphenyl group and without the lone pair electron as the "fifth pharmacophore" was not an agonist. This is the case, mentioned in the design section, with compound **5** (TAN-57) which was shown to be an antagonist. Only a 7-fluoro derivatives (**6**) of TAN-57, which has a lone pair electron on the fluorine atom that is capable of forming a hydrogen bond with the receptor, has weak and partial agonist activity. These observations lead us to assume the importance of the "fifth pharmacophore".

Conclusions

We have described non-peptide highly selective and potent δ (δ_1)-opioid agonists. The rational design for these useful ligands was based on the "message–address" concept and the accessory site hypothesis. In addition, the importance of a lone pair of electrons as the "fifth pharmacophore" was also demonstrated. Moreover, these systemically administrable non-peptide agonists will be of practical use for elucidating the pharmacological properties of the δ-opioid receptor.

Experimental

General Melting points were determined on a Yanaco MP-500D melting point apparatus and were uncorrected. Nuclear magnetic resonance (NMR) data were taken on JEOL JNM-EX-90 (90 MHz), JEOL GX-400 (400 MHz) or JEOL GSX-500 (500 MHz) spectrometers and reported in δ (ppm) downfield from tetramethylsilane (TMS). Infrared (IR) spectra were determined on a JASCO FT/IR-5000 as KBr pellets or neat. Mass spectra (MS) were obtained on a JEOL JMS-D-300, JEOL JMS-D-303 or VG ZAB-HF instruments applying an electric ionization (EI) method or a fast atom bombardment (FAB) ionization method. Elemental analyses were determined with a Heraeus CHN-ORAPID for carbon, hydrogen and nitrogen, Kyoto Electronics AT-118 for chlorine, and Yokogawa IC-7000 for sulfur. Elemental analyses were within 0.4% of the theoretical values. The progress of the reactions and purity of final products were determined on Merck Silica Gel Art.5715. Column chromatography was carried out using Merck Silica Gel (70—230 mesh) or Merck Lobar columns (Art. 10401) or Pharmacia Sephadex LH-20 with the indicated eluents.

Dimethylformamide (DMF), CH_2Cl_2, and dimethyl sulfoxide (DMSO) were distilled from CaH_2 and EtOH was distilled from magnesium ethoxide. Tetrahydrofuran (THF) was distilled from benzophenone ketyl just prior to use.

(4a*S,12a*R**)-4a-(3-Methoxyphenyl)-2-methyl-1,2,3,4,4a,5,12,12a-octahydropyrido[3,4-*b*]acridine (9)** A mixture of **8** (295.2 mg, 1.08 mmol) and 2-aminobenzaldehyde (399.8 mg, 3.30 mmol) was added to dry EtOH (5 ml), and the resulting mixture was treated with $MeSO_3H$ (0.18 ml, 2.75 mmol), and then refluxed for 17 h. The mixture was cooled to room temperature, and quenched with a saturated solution of $NaHCO_3$ (10 ml) and H_2O (10 ml). The mixture was extracted with EtOAc (50, 30 ml). The organic layer was washed with a saturated solution of NaCl (20 ml), dried ($MgSO_4$), and evaporated *in vacuo*. The residue was chromatographed on silica gel, eluting with NH_4OH-saturated $CHCl_3$–$CHCl_3$ (1 : 2—1 : 1) and the resulting material was washed with ether to give 306.0 mg (79.0%) of **9**: mp 178.5—179 °C (EtOAc). IR (KBr) cm^{-1}: 3392, 2940, 2806, 1605, 1578, 1493, 1286, 1245, 1044, 777, 770. NMR ($CDCl_3$, 400 MHz) δ: 1.96—2.06 (1H, m), 2.12—2.20 (1H, m), 2.25—2.32 (1H, m), 2.38 (3H, s), 2.59—2.80 (3H, m), 2.92—2.97 (1H, m), 3.09—3.18 (2H, m), 3.22—3.32 (1H, m), 3.68 (3H, s), 3.76 (1H, d, J=16.5 Hz), 6.55—6.61 (1H, m), 7.03—7.12 (3H, m), 7.37—7.42 (1H, m), 7.53—7.58 (1H, m), 7.61—7.66 (1H, m), 7.77 (1H, s), 7.91 (1H, d, J=8.5 Hz). MS (FAB) 359 ($(M+H)^+$). *Anal.* Calcd for $C_{24}H_{26}N_2O$: C, 80.41; H, 7.31; N, 7.81. Found: C, 80.48; H, 7.32; N, 7.92.

(4a*S,12a*R**)-4a-(3-Hydroxyphenyl)-2-methyl-1,2,3,4,4a,5,12,12a-octahydropyrido[3,4-*b*]acridine·Dimethane Sulfonate (2·2MeSO$_3$H, TAN-67)** To a solution of *tert*-BuOK (309.1 mg, 2.75 mmol) in dry DMF (6 ml) was added *n*-propanethiol (6 ml), and the solution was refluxed for five minutes. After cooling to room temperature, a solution of **9** (197.5 mg, 0.551 mmol) in dry DMF (4 ml) was added and the resulting solution was refluxed for 4 h. After the solution was cooled to room temperature, the solvent was removed *in vacuo*. The residue was treated with H_2O (10 ml) and extracted with $CHCl_3$–MeOH (3 : 1, 3×30 ml). The combined organic layers were washed with a saturated NaCl solution (10 ml), dried ($MgSO_4$), and evaporated *in vacuo*. The residue was treated with MeOH (10 ml) and an insoluble material (**2**, 24.3 mg) was filtered off. The filtrate was concentrated and adjusted to pH=2 with a solution of $MeSO_3H$ in MeOH. After removing the solvent *in vacuo*, the residue was chromatographed on Sephadex LH-20 and eluted with MeOH to give 180.0 mg (73.7%) of **2**·2MeSO$_3$H. **2**: mp 260—265 °C (dec.). IR (KBr) cm^{-1}: 3032, 2930, 2804, 1618, 1582, 1493, 1454, 1421, 1352, 1267, 1249, 1147, 779, 756. NMR (DMSO-d_6, 500 MHz) δ: 1.91—2.00 (1H, m), 2.09 (1H, t, J=12.2 Hz), 2.30 (1H, d, J=13.4 Hz), 2.35 (3H, s), 2.60—2.67 (1H, m), 2.68—2.75 (1H, d, J=11.6 Hz), 2.75—2.84 (1H, m), 2.94—3.02 (1H, m), 3.08 (1H, d, J=16.5 Hz), 3.20—3.42 (3H, m), 3.63 (1H, d, J=16.5 Hz), 6.42—6.48 (1H, m), 6.92—7.01 (3H, m), 7.47 (1H, t, J=7.3 Hz), 7.58—7.64 (1H, m), 7.77 (1H, d, J=8.5 Hz), 7.82 (1H, d, J=8.6 Hz), 8.00 (1H, s). MS (FAB) 345 ($(M+H)^+$). HR-MS Calcd for $C_{23}H_{25}N_2O$ 345.1967, Found 345.1967. **2**·2MeSO$_3$H: mp 154—157 °C. *Anal.* Calcd for $C_{23}H_{24}N_2O \cdot 2CH_3SO_3H \cdot H_2O$: C, 54.14; H, 6.18; N, 5.05; S, 11.56. Found: C, 53.94; H, 6.22; N, 5.05; S, 11.62.

(4a*S,12a*R**)-4a-(3-Methoxyphenyl)-2-vinyloxycarbonyl-1,2,3,4,4a,5,12,12a-octahydropyrido[3,4-*b*]acridine (10a)** To a solution of **9** (300 mg, 0.837 mmol) and Proton Sponge™ (179.4 mg, 0.837 mmol) in dry CH_2Cl_2 (4 ml) at 0 °C under an argon atmosphere was added dropwise vinyl chloroformate (0.21 ml, 0.837 mmol). After stirring at room temperature overnight, the reaction mixture was quenched with 1 N HCl (50 ml) and extracted with CH_2Cl_2 (15, 10 ml). The organic layer was washed with 1 N HCl (2×50 ml), H_2O (30 ml) and a saturated NaCl solution (30 ml), dried ($MgSO_4$), and evaporated *in vacuo* to give a pale yellow amorphous compound (336.5 mg). The amorphous substance was washed with ether to give 261.5 mg (75.4%) of **10a**: mp 160—163 °C. IR (KBr) cm^{-1}: 2922, 1755, 1437, 1245, 1151, 756. NMR ($CDCl_3$, 400 MHz) δ: 1.85—1.98 (1H, m), 2.30—2.40 (1H, m), 2.50—2.62 (1H, m), 2.88—3.05 (1H, m), 3.12—3.40 (3H, m), 3.47—3.60 (2H, m), 3.74 (3H, s), 4.06—4.19 (1H, m), 4.24—4.40 (1H, m), 4.50 (1H, d, J=5.9 Hz), 4.82 (1H, d, J=14.2 Hz), 6.65 (1H, dd, J=8.3, 2.0 Hz), 7.00—7.10 (2H, m), 7.10—7.19 (1H, m), 7.19—7.30 (2H, m), 7.58—7.70 (1H, m), 7.70—7.90 (2H, m), 8.10—8.30 (1H, m). MS (EI) *m/z* 414 (M^+). HR-MS Calcd for $C_{26}H_{26}N_2O_3$ 414.1943, Found 414.1939.

(4a*S,12a*R**)-4a-(3-Methoxypheny)-2-(2,2,2-trichloroethoxycarbonyl)-1,2,3,4,4a,5,12,12a-octahydropyrido[3,4-*b*]acridine (10b)** To a solution of **9** (64.0 mg, 0.179 mmol) and Proton Sponge™ (57.5 mg, 0.269 mmol) in dry CH_2Cl_2 (1 ml) at 0 °C under an argon atmosphere was added dropwise 2,2,2-trichloroethyl chloroformate (52.8 mg, 0.249 mmol). After stirring at room temperature for 2.5 h, the reaction mixture was quenched with 1 N HCl (10 ml) and extracted with EtOAc (2×30 ml). The organic layer was washed with 1 N HCl (10 ml), H_2O (30 ml) and a saturated NaCl solution (20 ml), dried ($MgSO_4$), and evaporated *in vacuo* to give a pale yellow oil (111.5 mg). The oil was chromatographed on silica gel, eluting with cyclohexane–EtOAc (3 : 1—2 : 1) to give 76.2 mg of **10b** as an oil. The oil was crystallized from EtOAc (1.0 ml)–*n*-hexane (1.4 ml) and filtered to give 50.6 mg (54.4%) of **10b**: mp 183—184 °C (EtOAc–*n*-hexane). IR (KBr) cm^{-1}: 2944, 1719, 1601, 1493, 1437, 1423, 1241, 1131, 1040, 803, 756, 710. NMR ($CDCl_3$, 400 MHz) δ: 1.91 (1H, td, J=13.2, 4.4 Hz), 2.30—2.35 (1H, m), 2.48—2.60 (1H, m), 3.02—3.22 (4H, m), 3.48—3.67 (1H, m), 3.67 (3H, s), 3.73—3.81 (1H, m), 4.05—4.16 (1H, m), 4.23—4.31 (1H, m), 4.73—4.84 (2H, m), 6.62 (1H, d, J=8.3 Hz), 6.98—7.07 (1H, m), 7.08—7.20 (2H, m), 7.39—7.44 (1H, m), 7.55—7.62 (1H, m), 7.66 (1H, d, J=7.8 Hz), 7.79 (1H, d, J=5.9 Hz), 7.92 (1H, d, J=8.3 Hz). MS (EI) *m/z* 518 (M^+). *Anal.* Calcd for $C_{26}H_{25}Cl_3N_2O_3$: C, 60.07; H, 4.85; N, 5.39; Cl, 20.46. Found: C, 60.21; H, 5.04; N, 5.25; Cl, 20.25.

(4a*S,12a*R**)-2-Cyclopropylmethylcarbonyl-4a-(3-methoxyphenyl)-**

1,2,3,4,4a,5,12,12a-octahydropyrido[3,4-*b*]acridine (12) Method A: A solution of **10a** (214.6 mg, 0.518 mmol) in MeOH (2 ml) was treated with *ca.* 8 N HCl–MeOH (2 ml) and refluxed for 0.5 h under an argon atmosphere. After the solution was cooled to 0 °C, the solution was quenched with a saturated $NaHCO_3$ solution (6 ml) and H_2O (10 ml), and then the mixture was extracted with CH_2Cl_2 (2×20 ml). The organic layer was washed with a solution of saturated NaCl (10 ml), dried ($MgSO_4$), and evaporated *in vacuo* to give 187.5 mg (quantitative yield) of **11**.

To a solution of the residue in dry THF (5 ml) was added Et_3N (122.9 mg, 1.21 mmol) and cyclopropanecarbonyl chloride (133.3 mg, 1.27 mmol) at 0 °C. The mixture was stirred continuously at room temperature for 3 h, and then the reaction mixture was quenched with a saturated $NaHCO_3$ solution (6 ml) and extracted with CH_2Cl_2 (2×10 ml). The organic layer was washed with a saturated NaCl solution (10 ml), dried ($MgSO_4$), and evaporated *in vacuo*. The residue was chromatographed on silica gel and eluted with cyclohexane–EtOAc (1 : 3—1 : 4) to give 169.6 mg (79.4%) of **12**: mp 227—228 °C (CH_2Cl_2–ether). IR (KBr) cm^{-1}: 2944, 1636, 1439, 1257, 1232, 1050, 781, 754. NMR ($CDCl_3$, 400 MHz) δ: 0.72—0.85 (2H, m), 0.95—1.10 (2H, m), 1.74—2.00 (2H, m), 2.30—2.63 (2H, m), 2.97—3.22 (3H, m), 3.22—3.40 (1.5H, m), 3.67 (3H, s), 3.73—3.89 (1.5H, m), 4.05—4.14 (0.6H, m), 4.16—4.26 (0.4H, m), 4.32—4.42 (0.4H, m), 4.62—4.72 (0.6H, m), 6.63 (1H, d, J=7.3 Hz), 6.96—7.15 (3H, m), 7.36—7.46 (1H, m), 7.53—7.70 (2H, m), 7.79 (1H, s), 7.88—7.97 (1H, m). MS (EI) *m/z* 412 (M^+). HR-MS Calcd for $C_{27}H_{28}N_2O_2$ 412.2151, Found 412.2164.

Method B: A solution of **10b** (900 mg, 1.73 mmol) in acetic acid (10 ml) was treated with Zn (2.08 g, 31.8 mmol) and stirred at room temperature overnight. The insoluble material was filtered off through a Celite pad by washing with MeOH and the filtrate was evaporated *in vacuo*. To the residue was added a saturated solution of $NaHCO_3$ and the mixture was extracted with $CHCl_3$ (3×50 ml). The organic layer was washed with a saturated $NaHCO_3$ solution (30 ml), H_2O (30 ml) and a saturated NaCl solution (30 ml), dried ($MgSO_4$), and evaporated *in vacuo* to give a yellow brown amorphous compound (615.8 mg). The amorphous substance was collected by filtration and washed with ether to give 265.9 mg of **11**.

A solution of the amorphous substance in dry THF (1 ml) was treated with Et_3N (0.32 ml, 2.32 mmol) and cyclopropanecarbonyl chloride (0.18 ml, 1.93 mmol) at 0 °C and the reaction mixture was stirred at room temperature for 30 min. After cooling to 0 °C, the reaction mixture was quenched with a saturated $NaHCO_3$ solution (10 ml) and extracted with CH_2Cl_2 (2×10 ml). The organic layer was washed with a saturated solution of NaCl (10 ml), dried ($MgSO_4$), and evaporated *in vacuo*. The residue (299.9 mg) was chromatographed on silica gel and eluted with cyclohexane–EtOAc (1 : 4—1 : 6) to give 189.5 mg (26.6%) of **12.**

(4a*S,12a*R**)-2-Cyclopropylmethyl-4a-(3-methoxyphenyl)-1,2,3,4,4a,5,12,12a-octahydropyrido[3,4-*b*]acridine (13)** To a solution of **12** (136.0 mg, 0.330 mmol) in dry toluene (2 ml) at −75 °C was added a solution of 1.5 M DIBAL–toluene (2 ml, 3 mmol) pre-cooled at −78 °C under an argon atmosphere. The mixture was stirred below −55 °C for 1.5 h and treated with a saturated $NaHCO_3$ solution (4 ml). After the addition of CH_2Cl_2 (20 ml) and H_2O (10 ml) followed by stirring at room temperature, the insoluble material was filtered off through a Celite pad. The organic layer was separated from the filtrate and the aqueous layer was extracted with CH_2Cl_2 (2×20 ml). The organic layer was washed with a saturated NaCl solution (10 ml), dried ($MgSO_4$), and evaporated *in vacuo*. The residue (149.1 mg) was chromatographed on silica gel and eluted with 5—10% MeOH–$CHCl_3$ and recrystallized from EtOAc–*n*-hexane to give 46.4 mg of **13** as crystals and 74.2 mg of **13** as filtrate (yield 91.7%): mp 208—212 °C (amorphous before recrystallization), 77—80 °C (crystal as **13**·0.7H_2O recrystallized from EtOAc–*n*-hexane). IR (KBr) cm^{-1}: 2912, 1607, 1582, 1493, 1238, 1046, 783, 772, 706. NMR ($CDCl_3$, 400 MHz) δ: 0.10—0.20 (2H, m), 0.55 (2H, d, J=7.8 Hz), 0.88—1.00 (1H, m), 2.02—2.21 (2H, m), 2.25—2.43 (3H, m), 2.64—2.82 (2H, m), 2.93—3.00 (1H, m), 3.11—3.32 (4H, m), 3.68 (3H, s), 3.75 (1H, d, J=16.6 Hz), 6.54—6.61 (1H, m), 7.02—7.10 (3H, m), 7.37—7.43 (1H, m), 7.53—7.59 (1H, m), 7.64 (1H, d, J=7.8 Hz), 7.78 (1H, s), 7.91 (1H, d, J=8.8 Hz). MS (EI) *m/z* 398 (M^+).

(4a*S,12a*R**)-2-Cyclopropylmethyl-4a-(3-hydroxyphenyl)-1,2,3,4,4a,5,12,12a-octahydropyrido[3,4-*b*]acridine·Dimethane Sulfonate (1·2-$MeSO_3H$)** To a solution of *tert*-BuOK (216.6 mg, 1.93 mmol) in dry DMF (2.5 ml) under an argon atmosphere there was added *n*-propanethiol (0.24 ml, 2.70 mmol) and the solution was refluxed for five minutes. After cooling to room temperature, a solution of **13** (154.0 mg, 0.386 mmol) in dry DMF (3.5 ml) was added and the resulting solution was refluxed for 2 h. After the reaction was completed, the mixture was cooled to 0 °C, and quenched with acetic acid (0.3 ml), then acetic acid and the solvent were removed *in vacuo*. The residue was quenched with a saturated $NaHCO_3$ solution (20 ml) and extracted with $CHCl_3$–EtOH (3 : 1, 2×30 ml). The organic layer was washed with H_2O (10 ml) and a saturated NaCl solution (10 ml), dried ($MgSO_4$), and evaporated *in vacuo*. The residue obtained was treated with MeOH (6 ml) and a solution of $MeSO_3H$ (200 mg) in MeOH. The insoluble material was filtered off and the filtrate was concentrated *in vacuo*. The residue was chromatographed on Sephadex LH-20, eluted with MeOH, and the resulting material was washed with ether (2×4 ml) and *n*-hexane (2×4 ml), and then filtered to give 178.5 mg of **1**·2$MeSO_3H$ as a pale yellow amorphous substance and 11.0 mg of the filtrate (85.1% yield). **1**: mp >250 °C (dec.). IR (KBr) cm^{-1}: 3400, 2914, 1580, 1493, 1423, 1238, 777, 764. NMR (CD_3OD, 400 MHz) δ: 0.26—0.38 (2H, m), 0.68 (2H, d, J=7.8 Hz), 1.00—1.10 (1H, m), 2.02—2.15 (2H, m), 2.42—2.58 (2H, m), 2.68—2.81 (3H, m), 3.10—3.42 (5H, m), 3.42—3.55 (1H, m), 3.66 (1H, d, J=16.1 Hz), 6.45—6.52 (1H, m), 6.91—7.05 (3H, m), 7.43—7.50 (1H, m), 7.58—7.64 (1H, m), 7.70 (1H, d, J=8.3 Hz), 7.83 (1H, d, J=8.3 Hz), 8.03 (1H, s). MS (FAB) 385 ($(M+H)^+$). 1·2$MeSO_3H$: mp 140—143 °C. *Anal.* Calcd for $C_{26}H_{28}N_2O\cdot 2CH_3SO_3H\cdot 1.6H_2O$: C, 55.53; H, 6.53; N, 4.63; S, 10.59. Found : C, 55.50; H, 6.32; N, 4.60; S, 10.73.

(4a*S,12a*R**)-4a-(3-Hydroxyphenyl)-1,2,3,4,4a,5,12,12a-octahydropyrido[3,4-*b*]acridine·Dimethane Sulfonate (3·2$MeSO_3H$)** To a solution of *tert*-BuOK (102.7 mg, 0.915 mmol) in dry DMF (2 ml) under an argon atmosphere there was added *n*-propanethiol (0.15 ml, 1.28 mmol) and the solution was refluxed for five minutes. After cooling to room temperature, a solution of **11** (62.9 mg, 0.183 mmol) in dry DMF (4 ml) was added and the resulting solution was refluxed for 3 h. After the reaction was completed, the mixture was cooled to room temperature, and quenched with acetic acid (1 ml), then acetic acid and the solvent were removed *in vacuo*. The residue was quenched with a saturated solution of $NaHCO_3$ (4 ml), H_2O (4 ml), and a saturated solution of NaCl (6 ml). The mixture was extracted with hot $CHCl_3$–MeOH (5 : 2, 2×20 ml). The combined organic layers were dried ($MgSO_4$) and evaporated *in vacuo*. The residue was treated with $CHCl_3$–MeOH (5 : 2, 4 ml) and a solution of $MeSO_3H$ (88.8 mg, 0.924 mmol) in MeOH at 0 °C. The solvent was removed *in vacuo* and the residue was chromatographed on Sephadex LH-20 and eluted with MeOH to give 54.5 mg (57.0%) of **3**·2$MeSO_3H$; mp 163—169 °C. IR (KBr) cm^{-1}: 3400, 2934, 2798, 1599, 1466, 1197, 1052, 785, 561, 536. NMR (DMSO-d_6+CD_3OD, 500 MHz) δ: 2.08 (1H, td, J=13.9, 3.7 Hz), 2.46 (6H, s), 2.45—2.53 (1H, m), 2.59—2.68 (1H, m), 2.75—2.85 (1H, m), 3.30 (1H, d, J=13.2 Hz), 3.36—3.48 (3H, m), 3.48—3.56 (2H, m), 3.75 (1H, d, J=17.2 Hz), 6.56 (1H, dd, J=7.9, 1.6 Hz), 6.91—6.94 (1H, m), 6.98 (1H, d, J=7.9 Hz), 7.07 (1H, t, J=7.9 Hz), 7.78 (1H, t, J=7.3 Hz), 7.95 (1H, t, J=7.3 Hz), 8.03 (1H, d, J=8.2 Hz), 8.12 (1H, d, J=8.2 Hz), 8.71 (1H, s). MS (FAB) 331 ($(M+H)^+$). *Anal.* Calcd for $C_{22}H_{22}N_2O\cdot 2CH_3SO_3H\cdot H_2O$: C, 53.32; H, 5.97; N, 5.18; S, 11.86. Found: C, 53.04; H, 5.65; N, 5.10; S, 11.48.

(4a*R,8a*S**)-8a-(3-Methoxyphenyl)-2,2,6-trimethyl-3a,4,4a,5,6,7,8,8a,9,9a-decahydro-1,3-dioxolo[4,5-*g*]isoquinoline (15)** To a stirred solution of **14** (10.09 g, 49.65 mmol) in THF (100 ml) under an argon atmosphere was added 1.637 N *n*-BuLi (30.3 ml, 49.65 mmol) at −10 °C. After stirring for 0.5 h, the mixture was cooled to −78 °C. The solution was added to a stirred solution of 2,2-dimethyl-4,5-bis(bromomethyl)-1,3-dioxolane (28.60 g, 99.3 mmol) in THF (70 ml) at −78 °C. The resulting mixture was stirred for 1 h, quenched with H_2O (50 ml) and a saturated $NaHCO_3$ solution (50 ml), and extracted with EtOAc (2×100 ml). The organic layer was washed with a saturated NaCl solution (50 ml), dried (Na_2SO_4), filtered, and evaporated to give an oil (40.48 g).

To a solution of the residue in DMF (80 ml) under an argon atmosphere were added NaI (37.21 g, 248.3 mmol) and anhydrous K_2CO_3 (34.31 g, 248.3 mmol). After heating at 100—150 °C for 2.5 h, the mixture was cooled to room temperature, filtered, and concentrated *in vacuo* at below 40 °C. The residue was treated with H_2O (50 ml) and a saturated $NaHCO_3$ solution (50 ml), and extracted with EtOAc (3×100 ml). The organic layer was washed with a saturated NaCl solution (50 ml), dried (Na_2SO_4), filtered, and evaporated to give an oil (30.25 g).

To a solution of the oil in MeOH (90 ml) was added sodium cyanoborohydride (13.47 g, 198.6 mmol). The mixture was cooled below −15 °C, then added dropwise with *ca.* 4 N HCl–MeOH solution (18 ml, 72.0 mmol), and stirred for 0.5 h. The mixture was then quenched with a saturated $NaHCO_3$ solution (50 ml), concentrated, treated with H_2O (50 ml), and extracted with EtOAc (2×100 ml). The organic layer was washed with H_2O (3×50 ml) and a saturated NaCl solution (50 ml), dried (Na_2SO_4), filtered, and evaporated to give an oil. The oil was chromatographed on silica gel and eluted with 2—30% MeOH–$CHCl_3$ to give 4.42 g (26.9%) of **15**: IR (neat) cm^{-1}: 3372, 2940, 1607, 1582, 1460, 1288, 1236, 1122, 1050, 847, 785. NMR ($CDCl_3$,

500 MHz) δ: 1.27, 1.29 (3H, s), 1.39, 1.40 (3H, s), 1.57—1.64 (0.5H, m), 1.79—1.88 (0.5H, m), 1.90—2.17 (3H, m), 2.17—2.30 (1.5H, m), 2.33, 2.35 (3H, s), 2.45, 2.51 (1H, m), 2.60—2.75 (2H, m), 2.78—2.86 (1H, m), 2.88—3.03 (1.5H, m), 3.12—3.17 (0.5H, m), 3.52—3.57 (0.5H, m), 3.74—3.85 (0.5H, m), 3.81 (3H, s), 3.92—3.98 (0.5H, m), 6.71—6.77 (1H, m), 6.89—7.03 (2H, m), 7.21—7.25 (1H, t, J=7.9 Hz). MS m/z 331 (M^+). HR-MS Calcd for $C_{20}H_{29}NO_3$ 331.21474, Found 331.21286.

(4a*S,8a*R**)-4a-(3-Methoxyphenyl)-6,7-dioxo-2-(vinyloxycarbonyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline (18)** A solution of **15** (500 mg, 1.508 mmol) in dry CH_2Cl_2 (3 ml), which was dried by azeotropic distillation with toluene (2×2 ml), was added to a solution of vinyl chloroformate (0.38 ml, 4.53 mmol) and Proton Sponge™ (1.62 g, 7.54 mmol) in dry CH_2Cl_2 (8 ml) in an ice-bath under an argon atmosphere. The resulting mixture was stirred at room temperature for 1 h. The solvent was evaporated *in vacuo* and ether (10 ml) was added. The insoluble material was filtered off and the filtrate was washed with 1 N HCl (3×30 ml) and a saturated NaCl solution (30 ml), dried, and evaporated to give a yellow oil (502.5 mg). The oil was dissolved in MeOH (6 ml), cooled in an ice-bath, and treated with *ca.* 4 N HCl–MeOH (0.9 ml, 3.6 mmol). After stirring for 2.5 h, the mixture was quenched with a saturated $NaHCO_3$ solution (10 ml) and extracted with EtOAc (50, 30 ml). The organic layer was washed with a saturated NaCl solution (20 ml), dried ($MgSO_4$), filtered, and evaporated to give 348.4 mg (77.1%) of **17** as an amorphous substance.

To a solution of oxalyl chloride (80.7 mg, 0.636 mmol) in dry CH_2Cl_2 (1 ml) at −78 °C was added dropwise dry DMSO (58.7 mg, 0.751 mmol) under an argon atmosphere. After stirring for 10 min, the solution was treated with a solution of **17** (100.3 mg, 0.289 mmol) in dry CH_2Cl_2 (1 ml). The solution was stirred at −78 °C for 20 min, followed by addition of Et_3N (0.24 ml, 1.734 mmol). After additional stirring for 1 h, the resulting mixture was treated with saturated $NaHCO_3$ solution (6 ml) and extracted with EtOAc (2×15 ml). The organic layer was washed with a saturated NaCl solution (10 ml), dried ($MgSO_4$), filtered, and evaporated. The residue was chromatographed on silica gel and eluted with cyclohexane–EtOAc (4 : 1) to give 46.4 mg (46.8%) of **18**: IR (KBr) cm^{-1}: 3364, 2944, 1702, 1676, 1601, 1435, 1274, 1236, 1203, 1156, 1042, 884, 787. NMR ($CDCl_3$, 500 MHz) δ: 1.73—1.94 (1H, m), 2.25—2.35 (1H, m), 2.46—2.68 (2H, m), 3.03 (1H, d, J =16.2 Hz), 3.12—3.19 (1H, m), 3.45—3.66 (1H, m), 3.77, 3.79 (3H, s), 3.96—4.10 (1H, m), 4.22—4.36 (1H, m), 4.43—4.51 (1H, m), 4.72—4.86 (1H, m), 5.87—6.00 (1H, m), 6.06—6.22 (1H, m), 6.69—6.75 (1H, m), 6.78—6.89 (2H, m), 7.06—7.26 (2H, m). MS (El) m/z 343 (M^+). *Anal.* Calcd for $C_{19}H_{21}NO_5$: C, 66.46; H, 6.16; N, 4.08. Found: C, 66.57; H, 6.32; N, 4.22.

(4a*S,12a*R**)-4a-(3-Methoxyphenyl)-2-(vinyloxycarbonyl)-1,2,3,4,4a,5,12,12a-octahydropyrido[3,4-*b*]phenazine (19)** A mixture of **18** (450 mg, 1.31 mmol) and *o*-phenylenediamine (397.5 mg, 3.93 mmol) in EtOH (8 ml) was refluxed for 2 h under an argon atmosphere. After the mixture was cooled to room temperature, it was quenched with 1 N HCl (20 ml), and extracted with $CHCl_3$ (2×30 ml). The organic layer was washed with 1 N HCl (20 ml) and a saturated NaCl solution (20 ml), dried ($MgSO_4$), filtered, and then evaporated *in vacuo* to give 586.9 mg (quantitative yield) of **19** as an amorphous substance: mp 191—193 °C. IR (KBr) cm^{-1}: 2918, 1717, 1649, 1609, 1578, 1491, 1460, 1439, 1408, 1270, 1253, 1234, 1152, 1052, 876, 766, 708. NMR ($CDCl_3$, 500 MHz) δ: 1.87—1.96 (1H, m), 2.30—2.38 (1H, m), 2.62—2.70 (1H, m), 2.88—3.03 (1H, m), 3.16 (1H, d, J=16.5 Hz), 3.35—3.45 (2H, m), 3.55—3.64 (1H, m), 3.68 (3H, s), 3.78 (1H, d, J=16.5 Hz), 4.06—4.16 (1H, m), 4.27—4.41 (1H, m), 4.46—4.54 (1H, m), 4.77—4.90 (1H, m), 6.62 (1H, dd, J=7.9, 2.5 Hz), 6.95—7.07 (2H, m), 7.11 (1H, t, J=7.9 Hz), 7.22—7.30 (1H, m), 7.61—7.67 (2H, m), 7.88—7.96 (2H, m). MS (El) m/z 415 (M^+). HR-MS Calcd for $C_{25}H_{25}N_3O_3$ 415.19173, Found 415.19197.

(4a*S,12a*R**)-4a-(3-Methoxyphenyl)-2-methyl-1,2,3,4,4a,5,12,12a-octahydropyrido[3,4-*b*]phenazine (20)** To a solution of **19** (174.9 mg, 0.421 mmol) in dry toluene (11 ml) at −78 °C under an argon atmosphere was added a toluene solution of diisobutylaluminum hydride (DIBAL, 1.5 M, 1.02 ml, 1.53 mmol). After stirring at −78 °C for 10 min, MeOH (2 ml) was added to the solution. The mixture was treated with a saturated $NaHCO_3$ solution (2 ml) and $CHCl_3$ (20 ml). The insoluble material was filtered off and a mixture of saturated $NaHCO_3$ solution (20 ml) and $CHCl_3$ (20 ml) was added to the filtrate. The organic layer was separated and the aqueous layer was extracted with $CHCl_3$ (50 ml). The organic layer was washed with a saturated NaCl solution (20 ml), dried ($MgSO_4$), and evaporated *in vacuo*. The residue was chromatographed on silica gel and eluted with 2—50% MeOH–$CHCl_3$ to give 103.9 mg (68.7%) of **20**: mp 177—179 °C (dec., $CHCl_3$–ether). IR (KBr) cm^{-1}: 3384, 2940, 2798, 1607, 1578, 1489, 1462, 1437, 1288, 1245, 1048, 876, 779, 764, 708. NMR ($CDCl_3$, 400 MHz) δ: 2.03—2.18 (2H, m), 2.28—2.30 (1H, m), 2.41 (3H, s), 2.72—2.77 (1H, m), 2.77—2.89 (2H, m), 3.02—3.07 (1H, m), 3.17 (1H, d, J=16.8 Hz), 3.32—3.41 (1H, m), 3.41—3.52 (1H, m), 3.69 (3H, s), 3.75 (1H, d, J=16.8 Hz), 6.56—6.61 (1H, m), 7.03—7.12 (3H, m), 7.58—7.66 (2H, m), 7.87—7.93 (2H, m). MS m/z 359 (M^+). HR-MS Calcd for $C_{23}H_{25}N_3O$ 359.20092, Found 359.20105.

(4a*S,12a*R**)-4a-(3-Hydroxyphenyl)-2-methyl-1,2,3,4,4a,5,12,12a-octahydropyrido[3,4-*b*]phenazine·Methane Sulfonate (4·$MeSO_3H$)** A solution of **20** (117.1 mg, 0.326 mmol) in dry CH_2Cl_2 (7 ml) was added to a solution of 1 M BBr_3 (0.96 ml, 0.96 mmol) in dry CH_2Cl_2 (1 ml) in an ice-bath. After stirring at room temperature for 20 min, a mixture of a concentrated solution of NH_4OH (20 ml) and ice was added to the reaction mixture. The resulting mixture was extracted with $CHCl_3$–MeOH (3 : 1, 3×50 ml). The organic layer was washed with a saturated NaCl solution (10 ml), dried ($MgSO_4$), and evaporated *in vacuo*. The residue was chromatographed on silica gel and eluted with 5—10% MeOH–$CHCl_3$ to give a solid (138.7 mg). The solid was treated with a solution of $MeSO_3H$ in MeOH, and further chromatographed on Sephadex LH-20 and eluted with MeOH to give 32.9 mg (22.8%) of **4**·$MeSO_3H$. **4**: mp >220 °C (dec.). IR (KBr) cm^{-1}: 3220, 2966, 1584, 1491, 1466, 1263, 1098, 1025, 872, 801, 768, 708. NMR (CD_3OD, 500 MHz) δ: 2.12—2.23 (1H, m), 2.54—2.71 (3H, m), 2.80 (3H, s), 2.90—3.00 (1H, m), 3.23—3.30 (1H, m), 3.40—3.60 (4H, m), 3.68 (1H, d, J=16.5 Hz), 4.59 (1H, br s), 6.52 (1H, dd, J=7.9, 1.2 Hz), 6.93—7.01 (2H, m), 7.04 (1H, t, J=7.9 Hz), 7.68—7.75 (2H, m), 7.85—7.94 (2H, m). MS (FAB) 346 ($(M+H)^+$). HR-MS Calcd for $C_{22}H_{24}N_3O$ 346.1919, Found 346.1905. **4**·$MeSO_3H$: mp 160–163 °C. *Anal.* Calcd for $C_{22}H_{23}N_3O \cdot CH_3SO_3H \cdot 0.3H_2O$: C, 61.81; H, 6.22; N, 9.40; S, 7.17. Found: C, 62.15; H, 6.29, N, 9.38, S, 7.24.

References and Notes

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