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ISOLATION, STRUCTURE CHARACTERIZATION, AND SYNTHESIS OF STABILIZED 1,2,3,4-TETRAHYDROISOQUINOLINE MARINE NATURAL PRODUCT FROM POTASSIUM CYANIDE PRETREATED THAI TUNICATE, *ECTEINASCIDIA THURSTONI*

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This paper is dedicated to Professor Victor Snieckus of Queen's University on the occasion of his 77th birthday.

Abstract – A simple 1,2,3,4-tetrahydroisoquinoline marine natural product (**2a**) together with two known compounds (**3** and **4**) was isolated from a low polar fraction of the crude extract of KCN pretreated Thai tunicate *Ecteinascidia thurstoni*. The structure of **2a** was determined by X-ray crystallographic analysis. We also reported an eight-step large-scale preparation of **2a** from isovanillin (**9**).

INTRODUCTION

In our ongoing search for new anticancer metabolites from marine animals, we found that the Thai tunicate, *Ecteinascidia thurstoni*,¹ produces ecteinascidin 743 (**1a**), a novel metabolite that has received considerable attention for its strong in vivo activity (Figure 1).^{2,3} However, there are few structure-activity relationship (SAR) studies of this fascinating target because it can be isolated in only trace amounts due to its instability, i.e., **1a** easily decomposes during the extraction and isolation process.^{4,5} We solved this problem by converting **1a** having a very unstable α -amino alcohol functionality

at C-21 into a stable α -aminonitrile compound by pretreatment with KCN to generate **1b** and its *S*-oxide **1c**.¹ The availability of **1b** enabled us to prepare several ecteinascidin analogues having enhanced antitumor activity. We have already reported the preparation of 6'-*O*-ester derivatives⁶ and 2'-*N*-amide derivatives⁷ along with their biological activities.

In our continuing SAR studies of ecteinascidin marine natural products, we found simple 1,2,3,4-tetrahydroisoquinoline (**2a**) along with two known compounds (**3** and **4**). We present here the structure of **2a**, which was elucidated by spectroscopic analyses and X-ray crystallographic analysis. We also report an eight-step synthesis of **2a** from commercially available isovanillin (**9**) in 56% overall yield.

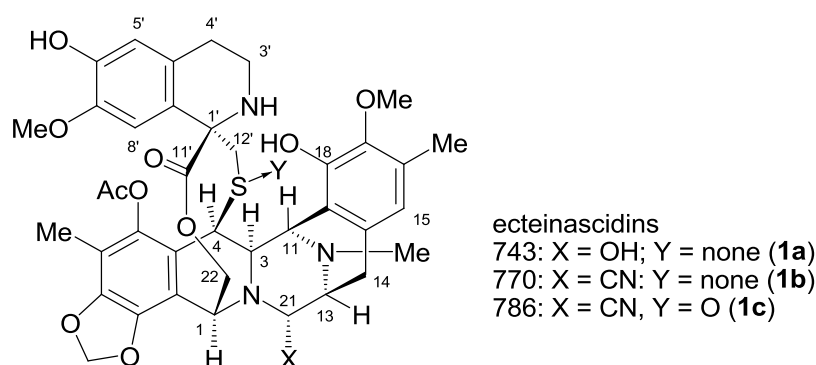


Figure 1. Structures of ecteinascidin marine natural products

RESULTS AND DISCUSSION

The Thai tunicate *E. thurstoni* was collected by SCUBA diving around the southeastern coast of Phuket Island in October 2011. The collected animals (51.3 kg, wet weight) were homogenized with phosphate buffer solution to adjust pH to 7, and then aqueous KCN was added. The resulting mixture was macerated with methanol and a residue (32.28 g) was obtained by using our standard extraction and separation process. The residue was subjected to Sephadex LH-20 column chromatography to give ecteinascidins 770 (**1b**: 359.0 mg) and 786 (**1c**: 319.8 mg). The most lipophilic fraction (4.55 g) in the Sephadex LH-20 column chromatography was subjected to flash column chromatography on silica gel several times to give **2a** (10.0 mg), **3** (8.7 mg), and **4** (3.6 mg) (Figure 2).

New compound **2a** was confirmed to have the molecular formula $C_{12}H_{12}N_2O_2$ by HREIMS. All proton and carbon signals were assigned after extensive NMR measurements using COSY, HMQC, and HMBC techniques. The molecular formula of **2a** indicated seven degrees of unsaturation and the detected resonance attributable to six olefinic carbons and one nitrile carbon in **2a** accounted for five degrees of unsaturation. Thus, **2a** was presumed to have two rings. It displayed simple 1H -NMR signals at δ 3.85 (3H, s, OCH_3), δ 5.54 (1H, br s, D_2O exchangeable OH proton), δ 3.70 and δ 3.71 (each 2H, s, methylene

protons neighboring nitrogen), δ 6.51 and δ 6.81 (each 1H, s, aromatic protons), and δ 2.83 (4H, s like protons). Acetylation of **2a** afforded monoacetate **2b**, which confirmed the presence of one hydroxyl group. Both ^1H - and ^{13}C -NMR spectral data of **2a** and **2b** in Table 1 showed correspondence with the NMR data reported for the synthetic compound 2-(6,7-dimethoxy-3,4-dihydroisoquinoline-2(1H)-yl)acetonitrile (**5**).⁸ The NMR data for **2a** revealed the presence of only one methoxy group, suggesting that **2a** is an *O*-demethyl analogue of **5**. It was not possible to define the relative positions of the unassigned hydroxyl and methoxy groups at C-6 and C-7. Finally, the structure elucidation of **2a** was completed by single crystal X-ray diffraction analysis (Figure 3).

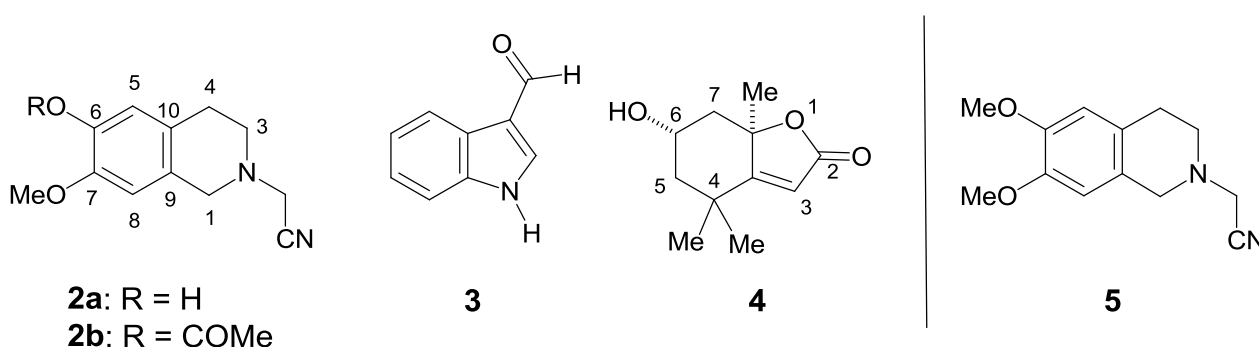


Figure 2. Structures of several minor marine natural products from Thai *Ecteinascidia thurstoni*

Table 1. NMR data for compounds **2a,b** and **5** in CDCl_3

No	2a			2b			5 ^{*1}	
	δ_{H}	δ_{C}	HMBC	δ_{H}	δ_{C}	δ_{H}	δ_{C} ^{*2}	
1	3.71 (2H, s)	53.9 CH ₂	C3, C8, C9, C10, C2a	3.70 (2H, s)	54.0 CH ₂	3.71 (2H, s)	53.8	
3	2.83 (2H, s)	49.8 CH ₂	C1, C4, C2a, C10	2.84 (2H, s)	49.6 CH ₂	2.86 (2H, s)	49.8	
4	2.83 (2H, s)	28.3 CH ₂	C3, C5, C9, C10	2.84 (2H, s)	28.1 CH ₂	2.86 (2H, s)	28.5	
5	6.67 (1H, s)	114.2 CH	C4, C7, C9	6.80 (1H, s)	122.7 CH	6.60 (1H, s)	111.3	
6		144.3 C			138.3 C		147.8	
7		145.1 C			149.3 C		147.4	
8	6.51 (1H, s)	108.5 CH	C1, C6, C10	6.62 (1H, s)	110.2 CH	6.53 (1H, s)	109.3	
9 ^{*3}		124.4 C			131.6 C		124.9	
10 ^{*3}		125.7 C			125.4 C		125.0	
NCH ₂	3.70 (2H, s)	46.0 CH ₂	C1, C3, CN	3.70 (2H, s)	45.9 CH ₂	3.71 (2H, s)	45.9	
CN		114.7 C			114.6 C		114.7	
OCH ₃	3.85 (3H, s)	55.9 CH ₃	C7	3.79 (3H, s)	56.0 CH ₃	3.85 (6H, s)	55.8 55.9	
COCH ₃				2.30 (3H, s)	20.6 CH ₃			
COCH ₃					169.2 C=O			
OH	5.54 (1H, br s)							

*1 ^1H -NMR measurement at 200 MHz and ^{13}C -NMR measurement at 50.3 MHz. See reference 8.

*2 Individual carbon signals chemical shifts were presented in reference 8.

*3 Assignments were interchangeable.

Compound **3** (mp 198-199.5 °C) was identified as the commercially available indole-3-carboxaldehyde based on its characteristic spectroscopic data.⁹ Loliolide **4** (C₁₁H₁₆O₃) was also identified from its spectroscopic data and by comparison with published data,^{10,11} including its optical rotation.¹² (-)-Loliolide (**4**) was previously isolated from a variety of higher plants and a few marine animals, including the sea hare *Dolabella ecaudata*,¹³ the marine sponge *Sphaciospongia* sp.,¹⁴ and the soft coral *Simularia capillosa*.¹⁵ However, as *D. ecaudata* is known to concentrate algal metabolites, **4** isolated from it may be of algal origin.¹⁶

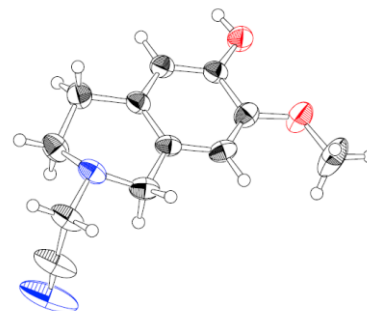


Figure 3. ORTEP drawing of Compound **2a**

We were very interested in the structure of **2a** because its 1,2,3,4-tetrahydroisoquinoline ring has the same substituents as the northwestern part of the ecteinascidin framework. It also possesses a smallest cyanonitrile compound that enables the formation of potent electrophilic iminium ion species **A**, which has been implicated in the formation of covalent bonds with DNA and bio macromolecules (Chart 1).¹⁷ In this context, we directed our attention towards establishing a practical method for the synthesis of **2a**.

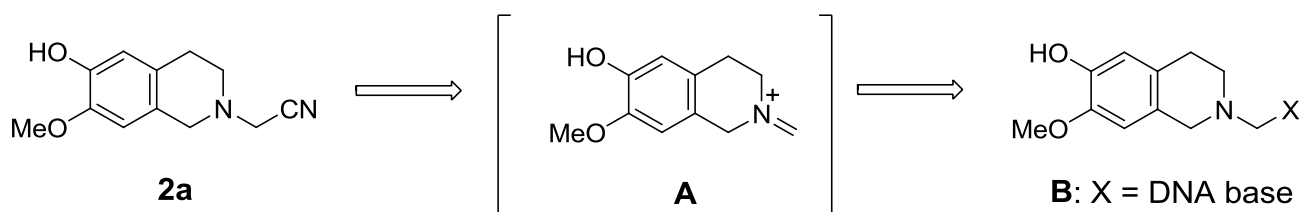


Chart 1. Plausible reaction pathway for generation of a DNA binding compound **B** via iminium ion **A** from **2a**

6-Hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline (**6a**) was obtained by selective demethylation of corresponding dimethoxy compound **7a**¹⁸ or **7b** via **6b**,¹⁹ which was prepared by the Pomeranz-Fritsch cyclization. As we were able to prepare 6-hydroxy-7-methoxyisoquinolinemethanol (**8**)²⁰ in ten steps from vanillin in 26% overall yield using an isopropyl group for phenol protection, we set our sights on developing another synthetic process for large-scale preparation (Figure 4).²¹

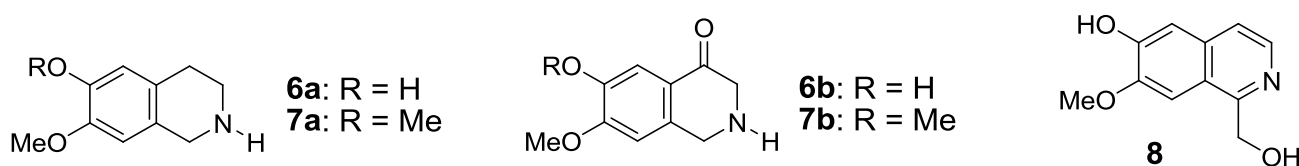
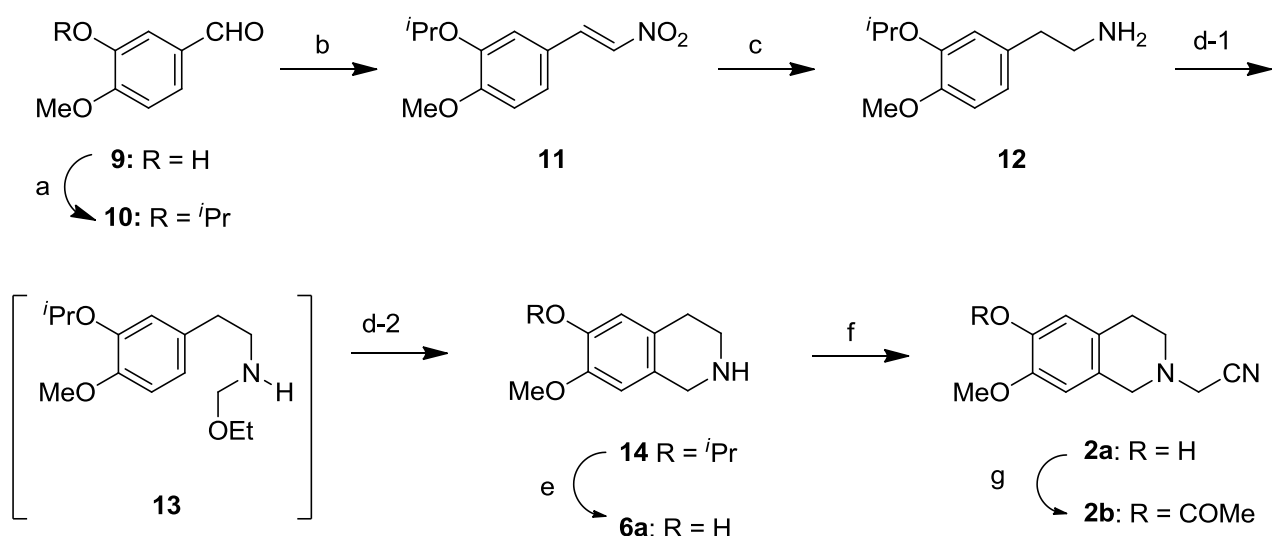


Figure 4

Alkylation of isovanillin (**9**) with isopropyl bromide in the presence of K_2CO_3 in DMF followed by the Henry condensation gave β -nitrostyrene (**11**) in 94% yield (Scheme 1). This material was then employed in a two-step reduction (1. Hydride reduction of alkene; 2. Nitro group reduction with $NaBH_4$ - $NiCl_4$ ²²) to afford phenethylamine (**12**) in 95% overall yield. The construction of 1,2,3,4-tetrahydroisoquinoline (**14**) from **12** was accomplished by means of our modified Pictet-Spengler reaction.²³ The reaction of **12** with a large excess of paraformaldehyde in the presence of K_2CO_3 in ethanol at 25 °C for 5 h gave *O,N*-acetal (**13**),²⁴ which was subsequently treated with trifluoroacetic acid (TFA) at 25 °C for 1.5 h to provide **14** in 92% overall yield. The structure of **14** was determined from the 1H -NMR spectrum that displayed signals of H-5 as a singlet at δ 6.62 ppm and H-8 as a singlet at δ 6.51 ppm. Treatment of **14** with $TiCl_4$ in CH_2Cl_2 at 25 °C for 5 h gave **6a** (84%), which was identical with authentic spectral data.¹⁸ Finally, the reaction of **6a** with 37% aqueous formaldehyde and KCN in 1N HCl solution at 25 °C for 5 h gave **2a** in 81% yield. **2a** was confirmed to be identical with the natural product by direct comparison of IR, EIMS, 1H -NMR, and ^{13}C -NMR data, together with their melting points. Furthermore, its acetate (**2b**) prepared from synthetic **2a** was also identical with that of authentic **2b** prepared from natural **2a**.



Scheme 1. a) K_2CO_3 , *i*PrBr, DMF, 80 °C, 1 h, 100%; b) NH_4OAc , $MeNO_2$, reflux, 3 h, 94%; c) (i) $NaBH_4$, SiO_2 , *i*PrOH- $CHCl_3$, 25 °C, 4 h; (ii) $NiCl_2 \cdot 6H_2O$, $NaBH_4$, MeOH-THF, -17 °C, 30 min, 95% (2 steps); d-1) $(HCHO)_n$, K_2CO_3 , EtOH, 25 °C, 5 h; d-2) TFA, 25 °C, 1.5 h, 92% (2 steps); e) $TiCl_4$, CH_2Cl_2 , 25 °C, 5 h, 84%; f) 37% $HCHO$ - H_2O , KCN, 1N HCl aq., rt, 5 h, 81%; g) acetic anhydride, pyridine, 25 °C, 7 h, 94%.

CONCLUSION

We found a stable analog of 1,2,3,4-tetrahydroisoquinoline marine natural product (**2a**) along with two known compounds, indole-3-carboxaldehyde (**3**) and loliolide (**4**), from KCN pretreated *E. thurstoni*. In addition, we developed a practical method for the synthesis of simple natural product **2a** from isovanillin

(**9**) in 56% overall yield. Unfortunately, **2a** did not show any cytotoxicity when tested in vitro using three representative human solid tumor cell lines (HCT116 human colon carcinoma, QG56 human lung carcinoma, and DU145 prostate carcinoma). Nevertheless, we remain very interested in the biosynthetic precursors of ecteinascidin marine natural products and will continue to conduct studies related to them.

EXPERIMENTAL

All melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotation was measured with a Horiba-SEPA polarimeter. IR spectra were obtained with a Shimadzu Prestige-21/IR Affinity-1 Fourier Transform Infrared (FT-IR) spectrometer. ^1H - and ^{13}C -NMR spectra were recorded on a JEOL JNM-LAMBDA 500 NMR spectrometer at 500 MHz for ^1H and 125 MHz for ^{13}C , and on a JEOL AL-400 spectrometer at 400 MHz for ^1H and 100 MHz for ^{13}C . NMR spectra were measured in CDCl_3 or $\text{DMSO}-d_6$ and the chemical shifts were recorded in δ_{H} values relative to $(\text{CH}_3)_4\text{Si}$ (TMS) as the internal standard. Mass spectra were recorded on a JMS-700 instrument with a direct inlet system operating at 70 eV. Elemental analyses were conducted on a YANACO MT-6 CHN CORDER elemental analyzer.

Isolation of Three Minor Marine Natural Products from KCN Pretreated *E. thurstoni*. The tunicate *Ecteinascidia thurstoni* was collected by SCUBA around the southeastern coast of Phuket Island at depths range from 1 to 5 m in October 2011 and frozen until used. The collected animals (51.3 kg, wet weight) were homogenized with aqueous buffer solution and adjusted to pH between 6.8 and 7.2. 10% aqueous KCN was added and the resulting mixture was stirred at 30 °C for 5 h. Use of our standard extraction and separation process provided ecteinascidin 770 (**1b**: 359.0 mg, 7.0×10^{-5} % of wet weight) and ecteinascidin 786 (**1c**: 319.8 mg, 6.2×10^{-5} % of wet weight) from the crude extract (32.4 g). The most lipophilic fraction (4.55 g) was purified by silica gel flash column chromatography several times to give compounds **2** (10.0 mg), **3** (8.7 mg), and **4** (3.6 mg).

2-(6-Hydroxy-7-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)acetonitrile (**2a**). Recrystallization of colorless powder from benzene afforded **2a** as pale yellow prisms, mp 165-166 °C. IR ν_{max} (KBr) 3400~2600, 2247, 1611, 1535, 1427, 1402, 1382, 1348, 1290, 1252, 1215, 1204, 1130, 1118, 1094, 1024, 941, 862 cm^{-1} . δ_{H} (400 MHz) 2.83 (4H, br s, 3- H_2 and 4- H_2), 3.70 (2H, s, NCH_2), 3.71 (2H, s, 1- H_2), 3.85 (3H, s, OCH_3), 5.54 (1H, br s, 6-OH), 6.51 (1H, s, 8-H), 6.67 (1H, s, 5-H). δ_{C} (100 MHz) 28.3 (C-4), 46.0 (NCH_2), 49.8 (C-3), 53.9 (C-1), 55.9 (OCH_3), 108.5 (C-8), 114.2 (C-5), 114.7 (CN), 124.4 (C-10), 125.7 (C-9), 144.3 (C-6), 145.1 (C-7). EIMS m/z (%): 218 (M^+ , 74), 217 (58), 191 (8), 151 (11), 150 (100), 135 (11), 107 (10). HREIMS m/z 218.1058 (M^+ , calcd for $\text{C}_{12}\text{H}_{12}\text{N}_2\text{O}_2$, 218.1055).

Indole-3-carboxaldehyde (3). Recrystallization from EtOAc/hexane gave **3** as colorless needles, mp 198-199.5 °C (Lit.⁹ mp 193-198 °C). IR ν_{\max} (KBr) 3169, 2931, 1636, 1614, 1446, 1244, 1128, 1083 cm^{-1} . δ_{H} (400 MHz, DMSO- d_6) 7.20 (1H, dt, $J = 6.8$ and 1.5 Hz, C-5), 7.25 (1H, dt, $J = 6.8$ and 1.5 Hz, C-6), 7.50 (1H, dd, $J = 6.8$, 1.5 Hz, C-7), 8.07 (1H, dd, $J = 6.8$, 1.5 Hz, C-4), 8.29 (1H, s, C-2), 9.92 (1H, s, CHO), 12.13 (1H, br s, NH). EIMS m/z (%): 145 (M^+ , 100), 144 (87), 116 (18). Anal. Calcd for $\text{C}_9\text{H}_7\text{NO}$: C 74.47, H 4.86, N 9.65. Found: C 74.35, H 5.87, N 9.59.

Loliolide (4). Colorless amorphous powder. IR $[\alpha]_{\text{D}}^{22} -64.4$ (c , 0.06, MeOH); Lit.¹⁰ $[\alpha]_{\text{D}}^{23} -67.9$ (c , 0.88, MeOH). ν_{\max} (KBr) 3435, 2924, 1734, 1720, 1622, 1273, 1265, 1234, 1193, 1161, 1099, 1028, 962, 868 cm^{-1} . δ_{H} (500 MHz) 1.27 and 1.47 (each 3H, s, 4- CH_3 x 2), 1.54 (1H, dd, $J = 14.7$, 3.7 Hz, 5-H), 1.78 (3H, s, 7a- CH_3), 1.78 (1H, dd, $J = 13.3$, 4.0 Hz, 7-H), 1.98 (1H, dt, $J = 14.7$, 2.9 Hz, 5-H), 2.46 (1H, dt, $J = 13.3$, 2.5 Hz, 7-H), 4.33 (1H, quint, $J = 3.4$ Hz, 6-H), 5.70 (1H, s, 3-H). δ_{C} (125 MHz) 26.5 (4- CH_3), 27.0 (7a- CH_3), 30.7 (4- CH_3), 35.9 (C-4), 45.6 (C-7), 47.3 (C-5), 66.9 (C-6), 86.6 (C-7a), 113.0 (C-3), 171.8 (C-2), 182.3 (C-3a). EIMS m/z (%): 196 (M^+ , 40), 178 (95), 153 (24), 140 (56), 135 (35), 112 (23), 111 (100), 109 (27), 95 (24), 67 (21), 57 (20), 43 (48). HREIMS m/z 196.1105 (M^+ , calcd for $\text{C}_{11}\text{H}_{16}\text{O}_3$, 196.1099).

Acetylation of 2a. A solution of **2a** (3.9 mg, 0.018 mmol) and acetic anhydride (0.2 mL) in dry pyridine (1.0 mL) was stirred at 25 °C for 1 h. The reaction mixture was diluted with water (10 mL) and extracted with EtOAc (10 mL x 3). The combined extracts were washed with brine (10 mL), dried, and concentrated in vacuo to give a residue (6.3 mg), which was subjected to chromatography on a silica gel (2 g) column with hexane-EtOAc (2:1) as the eluent to give **2b** (3.5 mg, 75.0%) as a pale yellow solid.

ν_{\max} (KBr) 2941, 2841, 2234, 1755, 1622, 1516, 1464, 1429, 1369, 1333, 1294, 1267, 1227, 1200, 1132, 1096, 1022, 914, 868 cm^{-1} . δ_{H} (400 MHz) 2.30 (3H, s, COCH_3), 2.84 (4H, br s, 3- H_2 and 4- H_2), 3.70 (2H, s, NCH_2), 3.76 (2H, s, 1- H_2), 3.79 (3H, s, OCH_3), 6.62 (1H, s, 8-H), 6.80 (1H, s, 5-H). δ_{C} (100 MHz) 20.6 (COCH_3), 28.1 (C-4), 45.9 (NCH_2), 49.6 (C-3), 54.0 (C-1), 56.0 (OCH_3), 110.2 (C-8), 114.6 (CN), 122.7 (C-5), 125.4 (C-10), 131.6 (C-9), 138.3 (C-6), 149.3 (C-7), 169.2 (CO). EIMS m/z (%): 260 (M^+ , 22), 218 (49), 217 (53), 191 (11), 151 (11), 150 (100). HREIMS m/z 260.1163 (M^+ , calcd for $\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_3$, 260.1161).

X-Ray Structure Determination of Compound 2a. Crystals of **2a** ($\text{C}_{12}\text{H}_{14}\text{N}_2\text{O}_2$) belong to orthorhombic space group $\text{P}2_12_12_1$ (#19) with $a = 6.4469(2)$ Å, $b = 8.8558(2)$ Å, $c = 19.8617(5)$ Å, $V = 1133.95(5)$ Å³, $Z = 4$, and $D_{\text{calcd}} = 1.278$ g/cm³. X-Ray intensities were measured with a Rigaku R-Axis RAPID diffractometer in the graphite-monochromatic $\text{CuK}\alpha$ radiation mode ($\lambda = 1.54187$ Å). The final cycle of

the full-matrix least-squares refinement was based on 2080 unique reflections ($2\theta < 136.4^\circ$) and 146 variable parameters and converged with unweighted and weighted agreement factors of $R = 0.0507$, $R_w = 0.1164$, and $R_1 = 0.0445$ for $I > 2.0\sigma(I)$ data. The drawing of the molecule was made by ORTEP as shown in Figure 3. CCDC-No. 898,447 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif.

3-Isopropoxy-4-methoxybenzaldehyde (10). A mixture of isovanillin (**9**) (10.0 g, 65.7 mmol), isopropyl bromide (18.7 mL, 0.197 mmol), and anhydrous K_2CO_3 (27.4 g, 0.197 mmol) in DMF (100 mL) was heated at $80^\circ C$ for 1 h. After the reaction mixture was cooled to $25^\circ C$, it was diluted with water (200 mL) and extracted with Et_2O (300 mL x 3). The combined extracts were washed with brine (70 mL), dried, and concentrated in vacuo to give a residue, the chromatography of which on a silica gel column with hexane-EtOAc (9:1-4:1) gave **10** (13.0 g, quantitative yield) as a colorless oil.

ν_{max} ($CHCl_3$) 3017, 2936, 2841, 1982, 1686, 1585, 1508, 1435, 1267, 1240, 1132, 1111, 1024, 1001, 808 cm^{-1} . δ_H (400 MHz) 1.40 (6H, d, $J = 6.0$ Hz, $CH(CH_3)_2$), 3.94 (3H, s, OCH_3), 4.65 (1H, sept, $J = 6.0$ Hz, $CH(CH_3)_2$), 6.98 (1H, d, $J = 8.3$ Hz, 5-H), 7.42 (1H, d, $J = 2.0$ Hz, 2-H), 7.45 (1H, dd, $J = 8.3, 2.0$ Hz, 6-H), 9.84 (1H, s, CHO). δ_C (100 MHz) 21.9 ($CH(CH_3)_2$), 56.1 (OCH_3), 71.3 (OCH), 110.9 (CH), 112.7 (CH), 126.4 (CH), 130.0 (C), 147.8 (C), 155.6 (C), 190.9 (CHO). EIMS m/z (%): 194 (M^+ , 26), 153 (10), 152 (100), 151 (99). HREIMS m/z 194.0938 (M^+ , calcd for $C_{11}H_{14}O_3$, 194.0943).

3-Isopropoxy-4-methoxy- β -nitrostyrene (11). A stirred solution of **10** (12.80 g, 65.9 mmol) and NH_4OAc (5.24 g, 65.9 mmol) in nitromethane (192 mL) was heated under reflux for 3 h. After the reaction mixture was cooled to $0^\circ C$, it was diluted with water (200 mL) and extracted with Et_2O (500 mL x 3). The combined extracts were washed with brine (200 mL), dried, and concentrated in vacuo to give a residue, recrystallization of which from MeOH gave **11** (13.87 g, 88.7%) as pale yellow prisms. The mother liquor was purified by column chromatography with hexane-EtOAc (9:1) to give an additional amount of **11** (0.87 g, 5.6%) (14.74 g, 94.3%), mp $117-118^\circ C$.

ν_{max} (KBr) 3111, 2981, 2968, 1626, 1593, 1514, 1493, 1433, 1333, 1261, 1231, 1168, 1138, 1113, 1026, 1005, 959, 806 cm^{-1} . δ_H (400 MHz) 1.40 (6H, d, $J = 6.2$ Hz, $CH(CH_3)_2$), 3.92 (3H, s, OCH_3), 4.57 (1H, sept, $J = 6.2$ Hz, $CH(CH_3)_2$), 6.92 (1H, d, $J = 8.3$ Hz, 5-H), 7.05 (1H, d, $J = 2.0$ Hz, 2-H), 7.17 (1H, dd, $J = 8.3, 2.0$ Hz, 6-H), 7.51 (1H, d, $J = 13.7$ Hz, $ArCH=CHNO_2$), 7.95 (1H, d, $J = 13.7$ Hz, $ArCH=CHNO_2$). δ_C (100 MHz) 21.9 ($CH(CH_3)_2$), 56.0 (OCH_3), 71.8 (OCH), 111.9 (CH), 115.0 (CH), 122.7 (C), 124.6 (CH), 135.0 (CH), 139.4 (CH), 147.8 (C), 154.2 (C). EIMS m/z (%): 237 (M^+ , 75), 195 (100), 149 (15), 148 (64), 133 (27), 89 (14). HREIMS m/z 237.0997 (M^+ , calcd for $C_{12}H_{15}NO_4$, 237.1001). Anal. Calcd

for C₁₂H₁₅NO₄: C 60.75, H 6.37, N 5.90. Found: C 60.83, H 6.45, N 5.84.

2-(3-Isopropoxy-4-methoxyphenyl)ethylamine (12). NaBH₄ (8.03 g, 212 mmol) was added to a mixture of **11** (9.50 g, 65.9 mmol) and silica gel (96.20 g, 0.16 mol) in 2-propanol (180 mL) and CHCl₃ (600 mL) over 15 min at 0 °C, and this mixture was vigorously stirred at 25 °C for 4 h. The reaction mixture was diluted with acetic acid (50 mL) at 0 °C. Then, a large amount of inorganic precipitate was removed by filtration and washing with CHCl₃ and MeOH. The combined filtrates were concentrated in vacuo and the residue was diluted with 5% aqueous NaHCO₃ solution (500 mL) and then extracted with CH₂Cl₂ (500 mL x 3). The combined extracts were washed with brine (500 mL), dried, and concentrated in vacuo to give a nitroalkane as a colorless oil, which was used without further purification. An analytical sample was obtained by column chromatography (elution with 9:1 hexane-EtOAc).

ν_{\max} (KBr) 2974, 1591, 1551, 1518, 1443, 1377, 1258, 1234, 1142, 1117, 1028, 964, 874 cm⁻¹. δ_{H} (400 MHz) 1.36 (6H, d, $J = 6.2$ Hz, CH(CH₃)₂), 3.24 (2H, t, $J = 7.3$ Hz, ArCH₂), 3.83 (3H, s, OCH₃), 4.50 (1H, sept, $J = 6.2$ Hz, CH(CH₃)₂), 4.57 (2H, t, $J = 7.3$ Hz, CH₂NO₂), 6.74 (1H, d, $J = 2.0$ Hz, 2-H), 6.75 (1H, dd, $J = 8.8, 2.0$ Hz, 6-H), 7.82 (1H, d, $J = 8.8$ Hz, 5-H). δ_{C} (100 MHz) 22.0 (CH(CH₃)₂), 33.0 (ArCH₂), 56.0 (OCH₃), 71.6 (OCH), 76.6 (CH₂NO₂), 112.3 (CH), 116.5 (CH), 121.1 (CH), 128.0 (C), 147.5 (C), 149.8 (C). EIMS m/z (%): 239 (M⁺, 26), 197 (9), 151 (25), 150 (100), 135 (14), 91 (13). HREIMS m/z 239.1155 (M⁺, calcd for C₁₂H₁₇NO₄, 239.1158).

A mixture of the above product and nickel chloride hexahydrate (38.80 g, 0.16 mol) in MeOH/THF (2:1, 690 mL) was stirred at -17 °C. NaBH₄ (12.90 g, 368 mmol) was added to the above mixture over 30 min and stirring was continued at the same temperature for 30 min. The reaction mixture was diluted with water (450 mL) and filtered through a Celite pad. The filter cake was carefully washed with CHCl₃ (200 mL) and the combined filtrates were concentrated in vacuo. The residue was diluted with benzene (300 mL) and extracted with 1N aqueous HCl (300 mL x 3). The combined aqueous layer was made alkaline with concentrated NH₄OH and then extracted with MeOH/CHCl₃ (1:19, 700 mL x 3). The combined extracts were washed with brine (500 mL), dried, and concentrated in vacuo. The residue was subjected to SiO₂ chromatography with concentrated NH₄OH-MeOH-CHCl₃ (1 : 9 : 90) as eluent to give amine (**12**: 8.00 g, 95.0%) as a pale yellow oil.

ν_{\max} (CHCl₃) 3011, 2980, 2936, 1587, 1512, 1443, 1423, 1373, 1260, 1138, 1109, 1030, 806 cm⁻¹. δ_{H} (400 MHz) 1.36 (6H, d, $J = 6.2$ Hz, CH(CH₃)₂), 1.54 (2H, br s, NH₂), 2.67 (2H, t, $J = 6.8$ Hz, ArCH₂), 2.93 (2H, t, $J = 6.8$ Hz, CH₂NO₂), 3.83 (3H, s, OCH₃), 4.52 (1H, sept, $J = 6.2$ Hz, CH(CH₃)₂), 6.74 (1H, dd, $J = 7.8, 2.0$ Hz, 6-H), 6.75 (1H, d, $J = 2.0$ Hz, 2-H), 6.82 (1H, d, $J = 7.8$ Hz, 5-H). δ_{C} (100 MHz) 22.1 (CH(CH₃)₂), 39.4 (ArCH₂), 43.6 (CH₂NH₂), 56.0 (OCH₃), 71.4 (OCH), 112.2 (CH), 116.9 (CH), 121.3

(CH), 132.3 (C), 147.2 (C), 148.9 (C). EIMS m/z (%): 209 (M^+ , 26), 180 (35), 179 (13), 138 (100), 137 (45), 123 (15). HREIMS m/z 209.1415 (M^+ , calcd for $C_{12}H_{19}NO_2$, 209.1416).

6-Isopropoxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline (14). A mixture of amine **12** (7.49 g, 35.8 mmol), paraformaldehyde (3.39 g, 107 mmol), and K_2CO_3 (19.9 g, 143 mmol) in EtOH (300 mL) was stirred at 25 °C for 5 h. After the inorganic precipitate was removed by filtration, the filter cake was washed with EtOH (100 mL) and the combined filtrates were concentrated in vacuo to afford *O,N*-acetal (**13**),²³ which was used in the next step without further purification.

A solution of the above product in trifluoroacetic acid (300 mL) was stirred at 25 °C for 1.5 h. The reaction mixture was poured into water (500 mL), pH was brought to 9-10 with concentrated NH_4OH , and extraction was carried out with $CHCl_3$ (700 mL x 4). The combined extracts were washed with brine (500 mL), dried, and concentrated in vacuo. The residue was subjected to silica gel chromatography with concentrated NH_4OH -MeOH- $CHCl_3$ (1 : 9 : 90) as eluent to give amine (**14**: 7.25 g, 92.0%) as a pale yellow oil.

ν_{max} ($CHCl_3$) 3017, 2978, 2933, 2837, 1609, 1514, 1466, 1383, 1327, 1300, 1254, 1206, 1103, 1026, 800 cm^{-1} . δ_H (400 MHz) 1.35 (6H, d, $J = 6.2$ Hz, $CH(CH_3)_2$), 1.78 (1H, br s, NH), 2.69 (2H, t, $J = 5.9$ Hz, 4- H_2), 3.11 (2H, t, $J = 5.9$ Hz, 3- H_2), 3.81 (3H, s, OCH_3), 3.93 (2H, s, 1- H_2), 4.46 (1H, sept, $J = 6.2$ Hz, $CH(CH_3)_2$), 6.51 (1H, s, 8-H), 6.62 (1H, s, 5-H). δ_C (100 MHz) 22.1 ($CH(CH_3)_2$), 28.6 (C-4), 44.0 (C-3), 48.0 (C-1), 56.0 (OCH_3), 71.6 (OCH), 109.8 (C-5), 117.0 (C-8), 126.5 (C), 128.5 (C), 145.6 (C), 148.8 (C). EIMS m/z (%): 221 (M^+ , 50), 179 (13), 178 (66), 151 (14), 150 (100), 135 (9). HREIMS m/z 221.1422 (M^+ , calcd for $C_{13}H_{19}NO_2$, 221.1416).

6-Hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline (6a). A stirred solution of **14** (1.10 g, 4.96 mmol) in dry CH_2Cl_2 (100 mL) was cooled in ice water and $TiCl_4$ (1.6 mL, 14.87 mmol) was added dropwise for 10 min. This mixture was then stirred at 25 °C for 5 h. After the reaction mixture was poured into water (400 mL), pH was brought to 7 with saturated aqueous $NaHCO_3$ solution. The mixture was extracted with MeOH/ $CHCl_3$ (1 : 9; 600 mL x 4). The combined extracts were washed with brine (600 mL), dried, and concentrated in vacuo to give a solid, recrystallization of which from EtOH afforded **6a** (774.0 mg, 83.8%) as pale yellow needles, mp 211-212 °C (Lit.,²⁵ mp 214-216 °C).

ν_{max} (KBr) 3289, 2986, 2914, 2833, 1604, 1530, 1469, 1456, 1412, 1352, 1325, 1306, 1273, 1248, 1221, 1115, 1070, 1031, 962, 856, 839, 814 cm^{-1} . δ_H (400 MHz, $DMSO-d_6$) 2.69 (2H, m, 4- H_2 , the signals overlapped with solvent signal), 2.85 (2H, t, $J = 5.9$ Hz, 3- H_2), 3.68 (3H, s, OCH_3), 3.70 (2H, s, 1- H_2), 6.43 (1H, s, 8-H), 6.51 (1H, s, 5-H). δ_C (100 MHz, $DMSO-d_6$) 28.0 (C-4), 43.5 (C-3), 47.4 (C-1), 55.6

(OCH₃), 109.9 (C-5), 115.6 (C-8), 126.6 (C), 126.8 (C), 144.4 (C), 145.7 (C). EIMS *m/z* (%): 179 (M⁺, 72), 178 (71), 151 (15), 150 (100), 135 (21), 107 (15). HREIMS *m/z* 179.0941 (M⁺, calcd for C₁₀H₁₃NO₂, 179.0941). Anal. Calcd for C₁₀H₁₂NO₂: C 67.02, H 7.31, N 7.82. Found: C 67.19, H 7.30, N 7.73.

2-Cyanomethyl-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline (2a). A stirred solution of **6a** (400.0 mg, 2.232 mmol) and 37% aqueous formaldehyde (0.5 mL, 6.696 mmol) in 1N aqueous HCl (3.2 mL) solution was cooled on ice-water. KCN (741.5 mg, 11.16 mmol) was added in one portion and the resulting mixture was stirred at 25 °C for 5 h. The reaction mixture was diluted with water (7.0 mL) and extracted with CHCl₃ (20 mL x 3). The combined extracts were washed with brine (20 mL), dried, and concentrated in vacuo to give a residue (409.8 mg). The aqueous layer was neutralized with 1N aqueous HCl solution and extracted with MeOH/CHCl₃ (1:9; 20 mL x 4). The combined extracts were washed with brine, dried, and concentrated in vacuo to give an additional residue (94.1 mg). The combined crude products were recrystallized from benzene to give **2a** (396.0 mg, 81.0%) as dark yellow prisms, mp 165.5-167 °C.

ν_{\max} (KBr) 3400~2600, 2247, 1611, 1535, 1427, 1402, 1383, 1348, 1325, 1290, 1252, 1215, 1203, 1130, 1119, 1094, 1024, 941, 862 cm⁻¹. δ_{H} (400 MHz) 2.84 (4H, br s, 3-H₂ and 4-H₂), 3.70 (2H, s, NCH₂), 3.70 (2H, s, 1-H₂), 3.85 (3H, s, OCH₃), 5.52 (1H, br s, OH), 6.51 (1H, s, 8-H), 6.67 (1H, s, 5-H). δ_{C} (100 MHz) 28.3 (C-4), 45.9 (NCH₂), 49.9 (C-3), 54.1 (C-1), 56.0 (OCH₃), 108.6 (C-8), 114.2 (C-5), 114.7 (CN), 124.4 and 125.7 (C-9 and C-10), 144.3 (C-6), 145.1 (C-7). EIMS *m/z* (%): 218 (M⁺, 57), 217 (54), 151 (12), 150 (100), 135 (13), 107 (9). HREIMS *m/z* 218.1058 (M⁺, calcd for C₁₂H₁₄N₂O₂, 218.1055). Anal. Calcd for C₁₂H₁₄N₂O₂: C 66.03, H 6.47, N 12.84. Found: C 66.40, H 6.45, N 12.76.

6-Acetoxy-2-cyanomethyl-7-methoxy-1,2,3,4-tetrahydroisoquinoline (2b). A solution of **2a** (50.0 mg, 0.23 mmol) and acetic anhydride (0.3 mL) in dry pyridine (1.2 mL) was stirred at 0 °C for 7 h. The reaction mixture was diluted with water (20 mL) and extracted with EtOAc (25 mL x 3). The combined extracts were washed with brine (20 mL), dried, and concentrated in vacuo to give a solid, recrystallization of which from Et₂O afforded **2b** (55.8 mg, 94.0%) as colorless prisms, mp 124-125 °C.

ν_{\max} (KBr) 2943, 2843, 2814, 2229, 1761, 1622, 1520, 1468, 1433, 1368, 1333, 1292, 1267, 1226, 1206, 1196, 1128, 1096, 1022, 914, 868 cm⁻¹. δ_{H} (500 MHz) 2.30 (3H, s, COCH₃), 2.84 (4H, br s, 3-H₂ and 4-H₂), 3.70 (2H, s, NCH₂), 3.76 (2H, s, 1-H₂), 3.79 (3H, s, OCH₃), 6.62 (1H, s, 8-H), 6.80 (1H, s, 5-H). δ_{C} (125 MHz) 20.6 (COCH₃), 28.1 (C-4), 45.9 (NCH₂), 49.6 (C-3), 54.0 (C-1), 56.0 (OCH₃), 112.7 (C-8), 114.6 (CN), 122.7 (C-5), 125.4 (C-10), 131.6 (C-9), 138.4 (C-6), 149.3 (C-7), 169.2 (CO). EIMS *m/z* (%): 260 (M⁺, 22), 218 (49), 217 (53), 191 (11), 151 (11), 150 (100). HREIMS *m/z* 260.1160 (M⁺, calcd for

C₁₄H₁₆N₂O₃, 260.1161). Anal. Calcd for C₁₄H₁₆N₂O₃: C 64.60, H 6.20, N 10.76. Found: C 64.76, H 6.28, N 10.71.

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