Supplementary Information

UNEXPECTED REDUCTION OF INDOLE DOUBLE BOND IN MITRAGYNINE USING *N*-BUTYLSILANE AND CATALYTIC TRIS(PENTAFLOROPHENYL)BORANE

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Equipments

Various analytical methods such as UV-Visible, IR, NMR, mass spectroscopy, CHN analysis, HPTLC and DSC melting point were carried out to characterize the isolated pure (E)-methyl 2-((2S,3S,12bS)-3-ethyl-8-methoxy-1,2,3,4,6,7,7a,12,12a,12b-decahydroindolo[2,3-a]quinolizin-2-yl)-3-methoxyacrylate free base. UV-Visible spectroscopy was carried out using Shimadzu UV 160-A double beam spectrophotometer (Kyoto, Japan) using the HPLC grade methanol as a blank. FTIR spectra were recorded using KBr pellets by a Thermal Scientific Nicolet 6700 spectrophotometer with Omnic software (Franklin, USA). ^1H -NMR and ^{13}C -NMR were performed using 400 MHz and 100 MHz Bruker spectrometer (Karlsruhe, Germany), respectively. One-dimensional and two-dimensional NMR experiments were carried out on a Bruker 600 FT-NMR (Darmstadt, Germany) operating at 25°C. The samples were dissolved in CDCl_3 and the chemical shifts were recorded with reference to that of tetramethylsilane (0, ppm). GC-MS spectra were taken using HP 6890A GC system (Santa Clara, USA) with HP-5MS Polyimide coated capillary column (30 m x 0.25 mm i.d. x 0.1 μm), heated from 100°C to 280°C at 10°C / min, 10 Kpa helium at 1.00 mL/min of flow rate and an injection volume of 1 μL with split ratio of 5 : 1. CHN elemental analysis was conducted using Perkin Elmer CHN Analyzer Model 2400-2 (Massachusetts, USA). HPTLC study was performed on a 0.50 mm thick Silica gel 60 G_{254} (10 cm x 20 cm) using a mobile phase hexane:ethyl acetate (92:8 v/v). TLC plates were then visualized under UV light (254 and 366 nm). CD spectra was measured at 250C on a Jasco-715 CD spectropolarimeter. All CD spectra were recorded with the standard sensitivity , a band width of 2.0 nm, a data pitch of 0.5 nm and a scan velocity of 20 nm/min. The CD spectra was calibrated using (1R)-(-)-10-camphorsulphonic acid and (R)-(-)-Pantolactones according to Miles et al., 2008. The solvent was always recorded on the same day as the analytical sample. Evaluation of the data was performed with the program “Spectra Analysis” of the Jasco-715

instruments. The wavelength Range was from 190 nm to 350 nm. The scan Speed is Medium and response time was Medium. The number of scans was 16-32. DSC Pyris 6 (Mettler Toledo, Switzerland) differential scanning calorimetry with STARe software (Mettler Toledo) was used for melting point measurement. Indium (melting point 156.6°C and melting enthalpy 28.45J/g) was chosen for standard calibration of instrumental cell constant and temperature, in order to obtain correct and precise determination result. The DSC thermogram was plotted from 30 °C to 300 °C and the analysis was performed at a heating rate of 10 °C per minute. The glass transition temperature, T_g was measured using the mid point method. Approximately 4.500 ± 0.500 mg of samples was weighed precisely and transferred accurately into the DSC crucible pans with seal with pre-pin lid. The crucibles was Capped and packed by moulding machine before fixing into the DSC furnace. After cooling by liquid nitrogen (99.99 %), the sample was heated from 30°C to 300°C at the velocity of 10°C/min. The heating and cooling stages were running at 20mL/min purge gas and 50mL/min shield gas (nitrogen gas) ambience. The method of 30 °C to 300 °C@10 °C/min N₂ with Blank was used. A background calibration with blank empty pan at both reference and sample sensors was performed prior to the analysis.

A) Silane Reduced Mitragynine

1. ^1H NMR-Aliphatic Expanded Region

2. ^1H NMR – Expanded Aromatic Region

3. ^1H NMR –Methoxy group

1H-SRIA1

4. ^1H NMR full

1H-SRA2 in CDCl3
Proton CDCl3

5 ^{13}C NMR Full Spectrum

6. Dept-90

DEPT90 SR1A in CDCl₃
C13DEPT90

Current Data Parameters
NAME SR1ADEPT90
EXPNO 16
PROCNO 1

F2 - Acquisition Parameters
Date_ 20100812
Time 8.40
INSTRUM spect
PROBHD 5 mm PABBO BI
PULPROG DEPT90
TD 65536
SOLVENT CDCl₃
NS 256
DS 4
SWH 29761.904 Hz
FIDRES 0.454131 Hz
AQ 1.1010548 sec
RG 188.56
DW 16.800 usec
DE 6.50 usec
TE 296.8 K
CNST2 145.0000000
CNST11 1.0000000
D1 2.00000000 sec
D20 0.00689655 sec

===== CHANNEL f1 ==
NUC1 13C
P1 10.25 usec
P2 20.50 usec
PLW1 74.00000000 W
SFO1 125.7703643 MHz

===== CHANNEL f2 ==
CPDPRG2 waltz16
NUC2 1H

7. Dept-45

DEPT45 SR1A in CDCl₃
C13DEPT45 CDCl₃

Current Data Parameters
NAME SR1ADEPT45
EXPNO 19
PROCNO 1

F2 - Acquisition Parameters
Date_ 20100812
Time 9.52
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG DEPT45
TD 65536
SOLVENT CDCl₃
NS 256
DS 4
SWH 29761.904 Hz
FIDRES 0.454131 Hz
AQ 1.1010548 sec
RG 188.56
DW 16.800 usec
DE 6.50 usec
TE 296.8 K
CNST2 145.0000000
CNST11 1.0000000
D1 2.00000000 sec
D20 0.00689655 sec

===== CHANNEL f1 =====
NUC1 13C
P1 10.25 usec
P2 20.50 usec
PLW1 74.00000000 W
SFO1 125.7703643 MHz

===== CHANNEL f2 =====
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec

8. DEPT-135

DEPT135 in CDCl₃
C13DEPT135 CDCI3

Current Data Parameters
NAME SR1ADEPT135
EXPNO 18
PROCNO 1

F2 - Acquisition Parameters
Date_ 20100812
Time 9.36
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG DEPT135
TD 65536
SOLVENT CDCl₃
NS 256
DS 4
SWH 29761.904 Hz
FIDRES 0.454131 Hz
AQ 1.1010548 sec
RG 188.56
DW 16.800 usec
DE 6.50 usec
TE 296.8 K
CNST2 145.000000
CNST11 1.000000
D1 2.00000000 sec
D20 0.00689655 sec

===== CHANNEL f1 =====
NUC1 13C
P1 10.25 usec
P2 20.50 usec
PLW1 74.00000000 W
SFO1 125.7703643 MHz

9. APT ¹³C

APT 13C SR1A in CDCl₃
C13APT CDCI3

Current Data Parameters
NAME SR1A13APT13C
EXPNO 20
PROCNO 1

F2 - Acquisition Parameters
Date_ 20100812
Time 10.25
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG APT13C
TD 65536
SOLVENT CDCl₃
NS 256
DS 4
SWH 29761.904 Hz
FIDRES 0.454131 Hz
AQ 1.1010548 sec
RG 188.56
DW 16.800 usec
DE 6.50 usec
TE 296.8 K
CNST2 145.000000
CNST11 1.000000
D1 2.00000000 sec
D20 0.00689655 sec

===== CHANNEL f1 =====
NUC1 13C
P1 10.25 usec
P2 20.50 usec
PLW1 74.00000000 W
SFO1 125.7703643 MHz

===== CHANNEL f2 =====
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PLW2 15.00000000 W
PLW12 0.51337999 W
SFO2 500.1320005 MHz

F2 - Processing parameters
SI 32768
SF 125.7577876 MHz
WDW EM
SSB 0

10. HSQC-Aromatic Expanded Region

11. HSQC-Aromatic Expanded Region

12. HSQC-Methoxy group

13. HSQC -Expanded aliphatic Region

14. HSQC-Expanded Aliphatic Region

15 HSQC –Aliphatic Expanded Region

16. HSQC-Aliphatic Expanded Region

17. HSQC-Aliphatic Expanded Region

18. HSQC-Aliphatic Expanded Region

19. HSQC-Aliphatic Expanded Region

20. HSQC-Full Spectrum

21 HSQC-Aromatic Expanded Region

22. DQF-Cosy-Aromatic Expanded Region

23. DQF-Cosy-Aliphatic Expanded Region

24. DQF-Cosy-Aliphatic Expanded Region

25. DQF-Cosy-Aliphatic Expanded Region

26. DQF-Cosy-Aliphatic Expanded Region

27. DQF-Cosy-Aliphatic Expanded Region

28 DQF-Cosy-Expanded Aliphatic Region

29 DQF-Cosy-Full spectrum

30. Tocsy-Expanded Aromatic Region

[illegible]

1D ^1H NMR spectrum (top) and 1D ^{13}C NMR spectrum (bottom) of Silane-Reduced-Mitragynine. The 2D COSY spectrum (middle) shows correlations between the two. The chemical structures illustrate the proton and carbon numbering systems used for the assignments.

Silane-Reduced-Mitragynine Proton Numbering System

Silane-Reduced-Mitragynine Carbon Numbering System

33 NOESY-Full spectrum

Figure 34 NOESY-Aliphatic expansion region

37- UV spectrum

Spectrum Peak Pick Report

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Data Set: RawData - C:\Users\Pengguna\Desktop\smit.spc 0.1855/L

Measurement Properties
Wavelength Range (nm.): 190.00 to 400.00
Scan Speed: Medium
Sampling Interval: 0.2
Auto Sampling Interval: Enabled
Scan Mode: Single

Instrument Properties
Instrument Type: UV-1800 Series
Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 340.8 nm
S/R Exchange: Normal

Attachment Properties
Attachment: None

Sample Preparation Properties
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

No.	P/V	Wavelength	Abs.	Description
1	⊕	390.80	0.027	
2	⊕	339.80	0.160	
3	⊕	335.40	0.157	
4	⊕	278.80	3.192	
5	⊕	237.00	3.448	
6	⊕	389.60	0.026	
7	⊕	337.40	0.156	
8	⊕	253.60	1.824	
9	⊕	196.40	0.698	

Sup 1 HPTLC fingerprint of the silane reduced mitragynine and the impurity / side product

Parameters	TLC conditions / results	
	Silane Reduced Mitragynine	Impurity / Side Product
Quantity sample applied	2 ul	2 ul
Detector	12 W VL. 6.1C UV detector	12 W VL. 6.1C UV detector
	6 W : 254 nm	6 W : 254 nm
	6 W : 365 nm	6 W : 365 nm
Stationary Phase	Silica Gel 60G ₂₅₄	Silica Gel 60G ₂₅₄
Mobile Phase	Hexane : Ethyl acetate (92: 8)	Hexane : Ethyl acetate (92 : 8)
Applied Concentration, mg/ml	0.05	0.05
Elution Length, cm	8.0-10.0	8.0-10.0
hR _f , cm	52.0	90.0
Visual appearance of Band :		
White light	Yellowish	Yellowish
UV 365	Yellowish	Yellowish
UV 254	Brownish	Brownish
Iodine	Yellowish brown	Yellowish brown
Dragendorff reagent	Orange	Orange

Sup. 2 Summary of 1-D NMR data for silane reduced Mitragynine

Hydrogen No.	Carbon No.	Chemical Shift, δ , ppm					
		^1H	^{13}C	CH_3	CH_2	CH	C
H-18	C-23	0.87	12.81	12.81			
H-17	C-22	1.26, 1.29	19.25		19.25		
H-4	C-4	1.86, 1.87	23.55		23.55		
H-11	C-13	1.67, 1.77	29.74		29.74		
H-12	C-14	2.00	31.98			31.98	
H-5	C-5	3.08	34.32			34.32	
H-19	C-20	1.97, 2.04	40.36		40.36		
H-13	C-17	3.70	51.36	51.36			
H-3	C-3	2.52, 2.54	53.55		53.55		
H-10	C-12	3.87	55.30	55.30			
H-16	C-21	1.19	57.33			57.33	
H-1	C-1	3.02	66.82			66.82	
H-15	C-19	3.73	61.59	61.59			
H-2	C-2	2.60	64.22			64.22	
H-8	C-10	6.46	99.78			99.78	
H-6	C-8	6.91	104.25			104.25	
-	C-15	-	111.25				111.25
-	C-6	-	117.55				117.55
H-7	C-9	7.00	122.03			122.03	
-	C-11	-	137.34				137.34
-	C-7	-	154.53				154.53
H-14	C-18	7.43	160.60			160.60	

-	C-16	-	169.14				169.14
H-9 (N-H)		5.23	-				
Total	Total	32	23	4	5	9	5

Sup 3 Summary of 2D-NMR data for silane reduced mitragynine

Hydrogen No.	Carbon No.	Chemical Shift, δ			
		HSQC C-H	HMBC C-H'	DQF COSY H-H'	TOCSY H-H'
H-18	C-23	H18 (0.87)- C23 (12.81)	H18 (0.87)- C23 (12.81) H17 (1.26, 1.29)- C22 (19.25) H16(1.19)- C21(57.33) H19(1.97,2.04)- C20(40.36) H12(2.00)- C14(31.98)	H18(0.87)- H17(1.26,1.29)	H18(0.87)- H17(1.26,1.29)
H-17	C-22	H17 (1.26, 1.29)- C22 (19.25)	H17 (1.26, 1.29)- C22 (19.25) H18 (0.87)- C23 (12.81) H16(1.19)- C21(57.33) H19(1.97,2.04)- C20(40.36) H12(2.00)- C14(31.98)	H17(1.26,1.29)- H16(1.19) H17(1.26,1.29)- H18(0.87)	H17(1.26,1.29)- H16(1.19) H17(1.26,1.29)- H18(0.87)
H-4	C-4	H4(1.86, 1.87)- C4(23.55)	H4(1.86, 1.87)- C4(23.55) H3(2.52, 2.54)- C3(53.55) H5(3.08)- C5(34.32) H1(3.01)- C1(66.82)	H4(2.76, 2.87)- H3(3.09, 3.32)	H4(2.76, 2.87)- H3(3.09, 3.32)
H-11	C-13	H11(1.67, 1.77)- C13(29.74)	H11(1.67, 1.77)- C13(29.74) H12(2.00)-	H11(1.67,1.77)- H2(2.60) H11(1.67, 1.77)-	H11(1.67,1.77)- H2(2.60) H11(1.67, 1.77)-

			C14(31.98) H2(2.60)– C2(64.22) H1(3.01)– C1(66.82) H16(1.19)– C21(57.33)	H12(2.00)	H12(2.00)
H-12	C-14	H12(2.00)– C14(31.98)	H12(2.00)– C14(31.98)	H12(2.00)– H11(1.67, 1.77) H12(2.00)– H16(1.19)	H12(2.00)–H11(1.67, 1.77) H12(2.00)–H16(1.19)
H-5	C-5	H5(3.08)–C5(34.32)	H5(3.08)– C5(34.32)	H7(7.00)–H6(6.91)	H7(7.00)–H6(6.91)
H-19	C-20	H19(1.97,2.04)– C20(40.36)	H19(1.97,2.04)– C20(40.36)	H19(1.97,2.04)– H16(1.19)	H19(1.97,2.04)– H16(1.19)
H-13	C-17	H13(3.70)– C17(51.36)	H13(3.70)– C17(51.36)	-	-
H-3	C-3	H3(2.52, 2.54)– C3(53.55)	H3(2.52, 2.54)– C3(53.55)	H3(3.09, 3.32)– H4(2.76, 2.87)	H3(3.09, 3.32)– H4(2.76, 2.87)
H-10	C-12	H10(3.87)– C12(55.30)	H10(3.87)– C12(55.30)	-	-
H-16	C-21	H16(1.19)– C21(57.33)	H16(1.19)– C21(57.33)	H16(1.19)– H12(2.00) H16(1.19)– H17(1.26,1.29) H16(1.19)– H19(1.97,2.04)	H16(1.19)– H12(2.00) H16(1.19)– H17(1.26,1.29) H16(1.19)– H19(1.97,2.04)
H-1	C-1	H1(3.02)– C1(66.82)		H1(3.02)–H5(3.08) H1(3.02)–H2(2.60)	H1(3.02)–H5(3.08) H1(3.02)–H2(2.60)
H-15	C-19	H15(3.73)– C19(61.59)	H1(3.02)–C1(66.82)	-	-
			H15(3.73)– C19(61.59)		
H-2	C-2	H2(2.60)– C2(64.22)	H2(2.60)–C2(64.22)	H2(2.60)–H1(3.01)	H2(2.60)–H1(3.01)
H-8	C-10	H8(6.46)– C10(99.78)	H8(6.46)– C10(99.78)	H8(6.46)–H7(7.00)	H8(6.46)–H7(7.00)
H-6	C-8	H6(6.91)– C8(104.25)	H6(6.91)– C8(104.25)	H6(6.91)–H7(7.00)	H6(6.91)–H7(7.00)
-	C-15	-		-	-
-	C-6	-		-	-
H-7	C-9	H7(7.00)– C9(122.03)	H7(7.00)– C9(122.03)	H7(7.00)–H6(6.91) H7(7.00)–H8(6.46)	H7(7.00)–H6(6.91)

				H7(7.00)-H8(6.46)	
-	C-11	-	-	-	-
-	C-7	-	-	-	-
H-14	C-18	H14(7.43)– C18(160.60)	H14(7.43)– C18(160.60)	-	-
-	C-16	-	-	-	-
H-9 (N-H)	-	-	-	-	-
32	23				

Sup 4 Comparison between the chemical shift values of ¹H NMR for mitragynine and silane-reduced-mitragynine

Parameters	Chemical Shift, δ (ppm)	
	Pure Mitragynine ¹⁵	Silane-reduced-mitragynine
H-18	0.87 (3H, dd, J=7.5 Hz)	0.87 (3 H, dd, J= 7.5 Hz, -CH ₃)
H-16	1.64(1H,br-d, J=11.3)	1.19 (1H, m, -CH)
H-17 α	1.30(1H,m, CH ₂)	1.26 (1H, m, -CH ₂)
H-17 β	1.75-1.80 (2H, m)	1.29 (1H, m, -CH ₂)
H-11 α	1.75-1.80 (2H, m)	1.67 (1H, m, -CH ₂)
H-11 β	2.40-2.55 (3H,m)	1.77 (1H, m, -CH ₂)
H-4 α	2.90-3.00(2H, m)	1.86 (1H, m, -CH ₂)
H-4 β	3.04-3.07(3H,m)	1.87 (1H, m, -CH ₂)
H-19 α	2.40-2.55 (3H,m)	1.97 (1H, m, -CH ₂)
H-12	3.04-3.07(3H,m)	2.00 (1H, m, -CH)
H-19 β	3.04-3.07(3H,m)	2.04 (1H, m, -CH ₂)
H-3 α	2.40-2.55 (3H,m)	2.52 (1H, m, -CH ₂)
H-3 β	2.90-3.00(2H, m)	2.54 (1H, m, -CH ₂)
H-1	-	3.02 (1H,m, -CH)
H-5	-	3.08 (1H, m, -CH)
17-OCH ₃	3.68 (3H,s,22-OCH ₃)	3.70 (3H, s,17-OCH ₃)
19-OCH ₃	3.78(3H,s,17-OCH ₃)	3.73 (3H, s, 19-OCH ₃)
10-OCH ₃	3.84(3H,s,9-OCH ₃)	3.86 (3H, s, 10-OCH ₃)
H-8	6.41(1H,d,J=7.9)	6.46 (1H, d, J=7.7 Hz, Ar-H)
H-6	6.89 (1H,d,J=7.9 Hz)	6.91 (1H, d, J=8.1 Hz, Ar-H)
H-7	6.91(1H,dd, J=7.9, 7.9 Hz)	7.00 (1H, t, Ar-H)
H-14	7.53 (1H,s,)	7.43 (1H, s, Ar-H)
N-H (H9)	7.68(1H,br-s,N-H)	5.23 (1H, br-s, N-H)
Solvent CDCl ₃	7.26	7.26

Sup 5 Comparison between the chemical shift values of ^{13}C NMR for Mitragynine and Silane-reduced-mitragynine

Parameters	Chemical Shift, δ (ppm)	
	Pure Mitragynine ¹⁵	Silane-reduced-mitragynine
C-1	133.69	66.82
C-2	61.33	64.22
C-3	53.85	53.55
C-4	22.78	23.55
C-5	107.91	34.32
C-6	117.72	117.55
C-7	154.49	154.53
C-8	104.26	104.25
C-9	121.91	122.03
C-10	99.80	99.78
C-11	137.33	137.34
C-13	29.78	29.74
C-14	31.99	39.80
C-15	111.55	111.25
C-18	160.65	160.60
C-23	12.95	12.81
C-22	14.22	19.25
C-21	39.95	40.36
C-20	57.77	57.33
C-16	169.36	169.14
C-17	51.45	51.36
C-12	55.40	55.30
C-19	61.65	57.33

Sup. 6 Medium pressure chromatography setting

Method 10008.10: **Synthetic analogue of Mitragynine**

Separation

Flowrate [ml/min] **20**
 Rack **FC30 (30x50ml)**
 Column **Glass Column 26/230- 044036**
☒ **Precolumn used**
 Pressure min: **0** bar
 max: **50** bar
 Tube Volume [ml] **50**
 Solvent Setup:
 Solvent B Ethyl Acetate
 Solvent A Hexane

Start %B	End %B	min	s
0	0	15	0
2	2	15	0
4	4	15	0
6	6	25	0
8	8	25	0
10	10	10	0
15	15	10	0
25	25	5	0
50	50	5	0

Conditioning

Flowrate [ml/min] **20**
 Rack **FC30 (30x50ml)**
 Column **Glass Column 26/230- 044036**
☒ **Precolumn used**
 Pressure min: **0** bar
 max: **50** bar
 Tube Volume [ml] **50**
 Solvent Setup:
 Solvent B
 Solvent A Hexane

Start %B	End %B	min	s
0	0	0	59

Total Time: 00:00:59

Sup. 7 Purification fractions and fractions containing pure silane reduced mitragynine

Sup. 8 Postulated mechanism of indole reduction

Sup. 9 DSC melting point of silane reduced mitragynine

Sup. 10 Pure silane reduced mitragynine GC-MS peak

Sup. 11a 2D-HPTLC densitogram profile of the crude reaction product with silane reduced mitragynine ($R_f=0.52$)

Sup. 11b 3D-HPTLC densitogram profile of the crude reaction product with silane reduced mitragynine ($R_f=0.52$)

Sup. 12a&12b Silane Reduced Mitragynine (Proton Numbering System and Carbon Numbering System)

Fig. 8a : Silane Reduced Mitragynine
(Proton Numbering System)

Fig. 8b : Silane Reduced Mitragynine
(Carbon Numbering System)