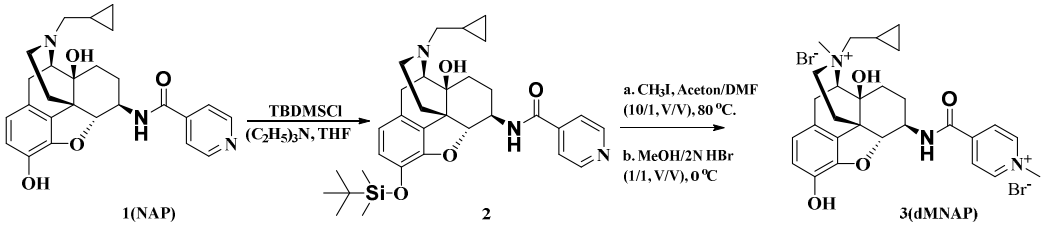
Supporting Information

Methylation Products of 6β-N-heterocyclic substituted naltrexamine derivatives as potential peripheral opioid receptor modulators

Yi Zheng[a], Samuel Obeng[a], Huiqun Wang[a], David L. Stevens[b], Essie Komla[b], Dana E. Selley[b], William L. Dewey[b], Hamid I. Akbarali[b], Yan Zhang[a]*

[a] Department of Medicinal Chemistry, Virginia Commonwealth University, 800 E. Leigh Street, Richmond, Virginia 23298, United States
[b] Department of Pharmacology and Toxicology, Virginia Commonwealth University, 1112 East Clay Street, Richmond, Virginia 23298, United States

A. The synthesis of dMNAP



Scheme 1. The synthesis procedure of dMNAP(compound 3).

1. 17-Cyclopropylmethyl-14*β*-hydroxy-4,5*α*-epoxy-6*β*-[(4′-pyridyl)acetamido]-3*β*-tert-butyldimethylsilyl)oxy morphinan (compound 2)

tert-Butyldimethylsilyl chloride (227 mg, 1.5 mmol) was added slowly to the mixture of NAP (100 mg, 0.25 mmol) and trimethylamine (152 mg, 1.5 mmol) in THF at 0 ^{o}C. Then, the reaction temperature was warmed to room temperature and kept it stirring for three days at that temperature. Three days later, the reaction was quenched with water and extracted with dichloromethane. The organic phase was collected, dried using Na_2SO_4 and concentrated under reduce pressure to get the reaction crude. The crude was purified with silica gel to get compound 2 (105 mg) as brown solid in 54 % yield.

Mass Spectrum: $[M+ H]^+$, m/z calc. 561.79 obs. 562.3062.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.90 (d, J = 7.8 Hz, 1H, exchangeable), 8,73 and 8.72 (dd, 1 H each, J = 1.6 Hz, 2H), 7.73 and 7.32 (dd, 1 H each, J = 1.6 Hz, 2H), 6.61 (s, 2H), 5.25 (s, 1H), 4.91 (s, 1H), 4.74 (d, J = 7.8 Hz, 1H), 3.71-3.63 (m, 1H), 3.05 (d, , J = 6.08 Hz, 2H), 2.99 (s, 1H), 2.65-2.59 (m, 2H), 2.40-2.30 (m, 2H), 2.23-2.15 (m, 1H), 2.00-1.94 (m, 1H), 1.85-1.76 (m, 1H), 1.64-1.60 (m, 1H), 1.50 (d, , J = 13.36 Hz, 1H), 1.37-1.31 (m, 1H), 1.26 (d, J = 13.04 Hz, 2H), 0.85 (s, 9H), 0.48 (d, J = 7.32 Hz, 2H), 0.48-0.47 (m, 2H), 0.05 (s, 3H), 0.04 (s, 3H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 164.14, 150.11, 144.72, 141.37, 137.93, 131.64, 126.19, 121.29, 121.00, 118.65, 90.47, 69.47, 61.59, 58.28, 51.73, 47.03, 43.51, 30.05, 25.71, 25.35, 24.29, 22.25, 217.70, 9.14, 3.58, 3.46, -0.01, -3.30, -4.79, -4.92.

IR (Diamond, cm^{-1}): V_{max} 2927.62, 2855.22, 1645.75, 1607.83, 1539.71, 1495.21, 1471.46, 1436.78, 1407.52, 1333.15, 1271.20, 1253.78, 1187.13, 1165.60, 1130.22, 1096.87, 1037.43, 1003.75, 985.85, 997.40, 953.66, 882.43, 866.20, 836.31, 781.14, 681.31.

2. 17-Cyclopropylmethyl-3,14*β*-dhydroxy-4,5*α*-epoxy-17-methyl-6*β*-[(4′-methylpyridyl)acetamido] morphinan (compound 3, dMNAP)

Iodomethane (482 mg, 3.4mmol) was added to a solution of compound 2 (130 mg, 0.23 mmol) in actone/dimethylformamide. Then, the reaction temperature was rose to

80-85 ^{o}C and stirred for one week at that temperature. After that, the mixture was concentrated under reduce pressure and the reaction crude was used directly for next step.

The reaction crude was dissolved again in methanol and 2N HBr(V/V, 1:1) at 0 ^{o}C and stirred for one hour at that temperature. One hour later, the solvent was removed under reduce pressure. Then, the residue was recrystallized with methanol and diethyl ether to get 90 mg compound 3 as dark yellow solid in 81% yield.

Mass Spectrum: $[M\text{-}CH_3]^+$, m/z calc. 477.26 obs. 462.2483. mp 154.1-155.5 ^{o}C

^{1}H NMR (400 MHz, DMSO-d_6): δ 9.54 (d, J = 7.84 Hz, 1H, exchangeable), 9,41 (s, 1H, exchangeable), 9.18 (d, J = 6,68 Hz, 2H), 8.45 (d, J = 6.68 Hz, 2H), 6.74 (s, 2H), 5.97 (s, 1H, exchangeable), 4.82 (d, J = 7.76 Hz, 1H), 4.41 (s, 3H), 3.88-3.83 (m, 2H), 3.73-3.69 (m, 1H), 3.60 (s, 2H), 3.55 (s, 1H), 3.50 (s, 1H), 3.17 (d, J = 1.92 Hz, 1H), 3.10 (s, 3H), 2.94-2.88 (m, 1H), 2.77-2.71 (m, 1H), 2.16 (t, 1H), 2.00-1.90 (m, 1H), 1.78 (d, J = 13.64 Hz, 1H), 1.57 (d, J = 10.4 Hz, 2H), 1.50-1.43 (m, 1H), 1.24-1.19 (m, 2H), 0.79-0.77 (m, 1H), 0.74-0.68 (m, 1H), 0.64-0.60 (m, 1H), 0.410.36 (m, 1H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 161.19, 147.24, 146.55, 142.00, 141.87, 129.57, 125.24, 119.95, 119.64, 118.09, 89.64, 71.79, 71.56, 70.92, 57.28, 54.43, 54.39, 54.35, 53.10, 51.77, 34.25, 31.14, 27.42, 24.48, 23.24, 5.79, 3.81, 2.76.

IR (Diamond, cm^{-1}): V_{max} 3225.15, 3043.45, 2961.28, 2160.95, 1644.16, 1610.69, 1547.79, 1504.06, 1483.22, 1456.05, 1410.17, 1320.82, 1269.15, 1229.92, 1153.83, 1126.07, 1054.32, 1031.27, 988.08, 944.85, 915.31, 878.13, 854.01, 810.74, 790.64, 749.60.

HPLC
Method: C:\star\data\YY\PHPLC\METHODS\C8 column\60H2O40MeCN1ml20MIN230nm_dMNMP.mth
Sample: Manual Sample
Injection Date: Fri Nov 03 15:48:07 2017
Manual injection
Operator: Yan Zhang Group
Instrument: Varian Star #1
Notes:

Misc Info

Workstation: D9288P71
Bus Address: 44
Run Time: 0.000 to 20.000 min.

Recalc Info

Recalc Method: C:\star\data\YY\PHPLC\METHODS\C8 column\60H2O40MeCN1ml20MIN230nm_dMNMP.mth
Recalc Date: Fri Nov 03 16:08:12 2017
Operator: Yan Zhang Group
Instrument: Varian Star #1
Notes:
Sample Rate: 20.000 Hz.
Measurement Type: 1 = Area
Calculation Type: 1 = %
Normalize Results: No

Peak Info for Channel 1
---

44 Peaks (tR	Timeoffset	RRT	Sepcode	Width	Counts	Result	Name)
2.399	0.000	0.0000	TF	0.00	342364320	96.519551	
10.258	0.000	0.0000	TF	0.00	13682469	3.480449	

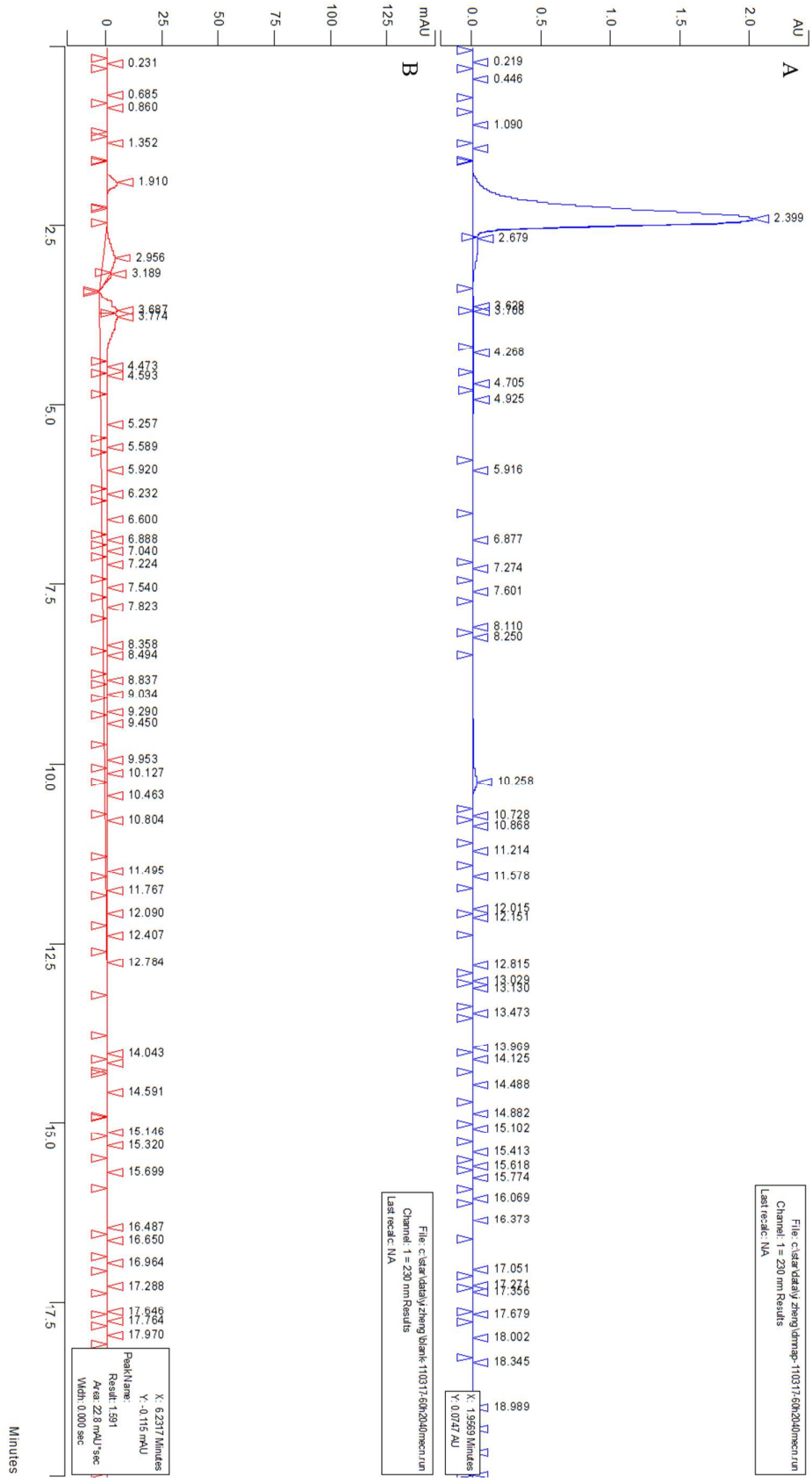
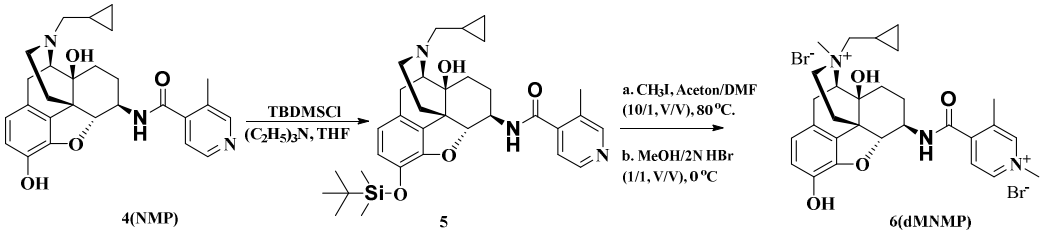


Figure S1. The HPLC of dMNAP (A) and background (B).

B. The synthesis of dMNMP



Scheme 2. The synthesis procedure of dMNMP(compound 6).

1. 17-Cyclopropylmethyl-14*β*-hydroxy-4,5*α*-epoxy-6*β*-{[4′-(3′-methylpyridyl)carboxamido]}-3*β*-tert-butyldimethylsilyl)oxy morphinan (compound 5)

tert-Butyldimethylsilyl chloride (398 mg, 2.64 mmol) was added slowly to the mixture of NMP (200 mg, 0.44 mmol) and trimethylamine (312 mg, 3.08 mmol) in THF at 0 ^{o}C. Then, the mixture was warmed to room temperature and stirred for three days at that temperature. Three days later, the reaction was quenched with water and extracted with dichloromethane. The organic phase was collected, dried with Na_2SO_4 and concentrated under reduce pressure to get the reaction crude. The crude was purified with silica gel to get compound 5 (105 mg, 83.3%) as brown solid.

Mass Spectrum: $[M+ H]^+$, m/z calc. 575.32 obs. 576.3323

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.68 (d, J = 7.96 Hz, 1H), 8.49 (s, 1H), 8.47 (d, J = 4.92 Hz, 1H), 7.21 (d, J = 4.88 Hz, 1H), 6.62 (s, 2H), 4.88 (s, 1H), 4.57 (d, J = 7.76 Hz, 1H), 3.64-3.58 (m, 1H), 3.16 (d, J = 5.24 Hz, 1H), 3.03 (d, J = 5.0 Hz, 2H), 2.98 (s, 1H), 2.62-2.58 (m, 2H), 2.34 (d, J = 3.2 Hz, 1H), 2.31 (s, 3H), 2.19-2.11 (m, 1H), 1.98-1.93 (m, 1H), 1.82-1.73 (m, 2H), 1.69-1.64 (m, 1H), 1.50 (d, J = 13.48 Hz, 1H), 1.36-1.30 (m, 1H), 1.23 (d, J = 9.96 Hz, 1H), 0.92 (s, 9H), 0.87-0.83 (m, 2H), 0.47 (d, J = 7.2 Hz, 2H), 0.12 (d, J = 2.44 Hz, 6H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 166.23, 151.03, 147.01, 144.60, 143.43, 137.70, 131.40, 129.37, 126.01, 121.12, 120.46, 118.46, 90.37, 69.27, 61.40, 58.09, 51.27, 46.86, 43.31, 29.98, 29.79, 25.23, 24.17, 22.06, 17.56, 15.64, 8.95, 3.38, 3.27, -0.19, -4.91.

IR (Diamond, cm^{-1}): V_{max} 3270.15, 2928.00, 2856.28, 1645.17, 1607.78, 1538.35, 1495.49, 1471.62, 1436.92, 1391.63, 1333.00, 1270.70, 1256.83, 1187.68, 1163.62, 1130.08, 1097.12, 1037.23, 1003.57, 985.94, 952.98, 938.48, 883.98, 861.66, 838.26, 782.43, 748.88, 676.36.

2. 17-Cyclopropylmethyl-3,14*β*-dihydroxy-4,5*α*-epoxy-6*β*-{[4′-methyl-(3′-methylpyridyl)]carboxamido} morphinan (compound 6, dMNMP)

Iodomethane (322 mg, 2.27mmol) was added to a solution of compound 5 (85 mg, 0.15 mmol) in actone/dimethylformamide. Then, the reaction temperature was rose to

80-85 ^{o}C and stirred for one week at that temperature. After that, the mixture was concentrated under reduce pressure to get the crude for next step.

The crude was dissolved again in methanol and 2N HBr(V/V, 1:1) at 0 ^{o}C and stirred for one hour at that temperature. One hour later, the mixture was concentrated under reduce pressure. The residue was then recrystallized with methanol and diethyl ether to get compound 6 (42 mg) as dark yellow solid in 58.2% yield.

Mass Spectrum: $[M\text{-}CH_3]^+$, m/z calc. 491.28, obs. 476.2595 mp 151.4-153.4 ^{o}C

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.36 (s, 1H), 9.07 (s, 1H), 8.97 (d, J = 6.16 Hz, 1H), 8.05 (d, J = 6.16 Hz, 1H), 6.78-6.72 (m, 1H), 6.68-6.66 (m, 1H), 6.28 (s, 1H), 4.70 (d, J = 7.72 Hz, 1H), 4.35 (s, 3H), 3.94-3.83 (m, 2H), 3.67-3.61 (m, 2H), 3.37 (s, 1H), 3.11 (s, 3H), 3.05 (d, J = 6.4 Hz, 1H), 2.91-2.86 (m, 1H), 1.99-1.90 (m, 1H), 1.83-1.81 (m, 1H), 1.69-1.62 (m, 1H), 1.47-1.38 (m, 2H), 1.24-1.22 (m, 1H), 1.11-1.07 (m, 1H), 0.79-0.76 (m, 1H), 0.73-0.67 (s, 1H), 0.65-0.60 (m, 1H), 0.55-0.50 (m, 1H), 0.43-0.36 (m, 1H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 163.86, 150.26, 146.66, 143.58, 141.98, 141.42, 135.11, 129.47, 124.79, 120.59, 119.65, 118.11, 117.97, 89.38, 71.59, 69.60, 54.33, 51.46, 47.78, 45.63, 34.12, 27.20, 22.98, 16.18, 5.67, 5.05, 2.59, 0.03.

IR (Diamond, cm^{-1}): V_{max} 3187.75, 3033.80, 2161.15, 1657.66, 1548.98, 1502.76, 1463.13, 1427.81, 1384.16, 1325.29, 1270.29, 1236.87, 1155.32, 1125.73, 1092.61, 1053.21, 1032.03, 1013.46, 987.35, 944.40, 917.64, 883.60, 836.92, 829.35, 797.34, 779.96, 748.02, 680.76.

HPLC
Method: C:\star\data\YY\PHPLC\METHODS\C8 column\50H2O50MeCN1ml25MIN230nmdMNAP.mth
Sample: Manual Sample
Injection Date: Fri Nov 03 10:22:09 2017
Manual injection
Operator: Yan Zhang Group
Instrument: Varian Star #1
Notes:

Misc Info

Workstation: D9288P71
Bus Address: 44
Run Time: 0.000 to 20.000 min.

Recalc Info

Recalc Method: C:\star\data\YY\PHPLC\METHODS\C8 column\50H2O50MeCN1ml25MIN230nmdMNAP.mth
Recalc Date: Fri Nov 03 10:42:40 2017
Operator: Yan Zhang Group
Instrument: Varian Star #1
Notes:
Sample Rate: 20.000 Hz.
Measurement Type: 1 = Area
Calculation Type: 1 = %
Normalize Results: No

Peak Info for Channel 1

---

1 Peaks (tR	Timeoffset	RRT	Sepcode	Width	Counts	Result	Name)
2.214	0.000	0.0000	BB	9.60	70580024	100.000000	

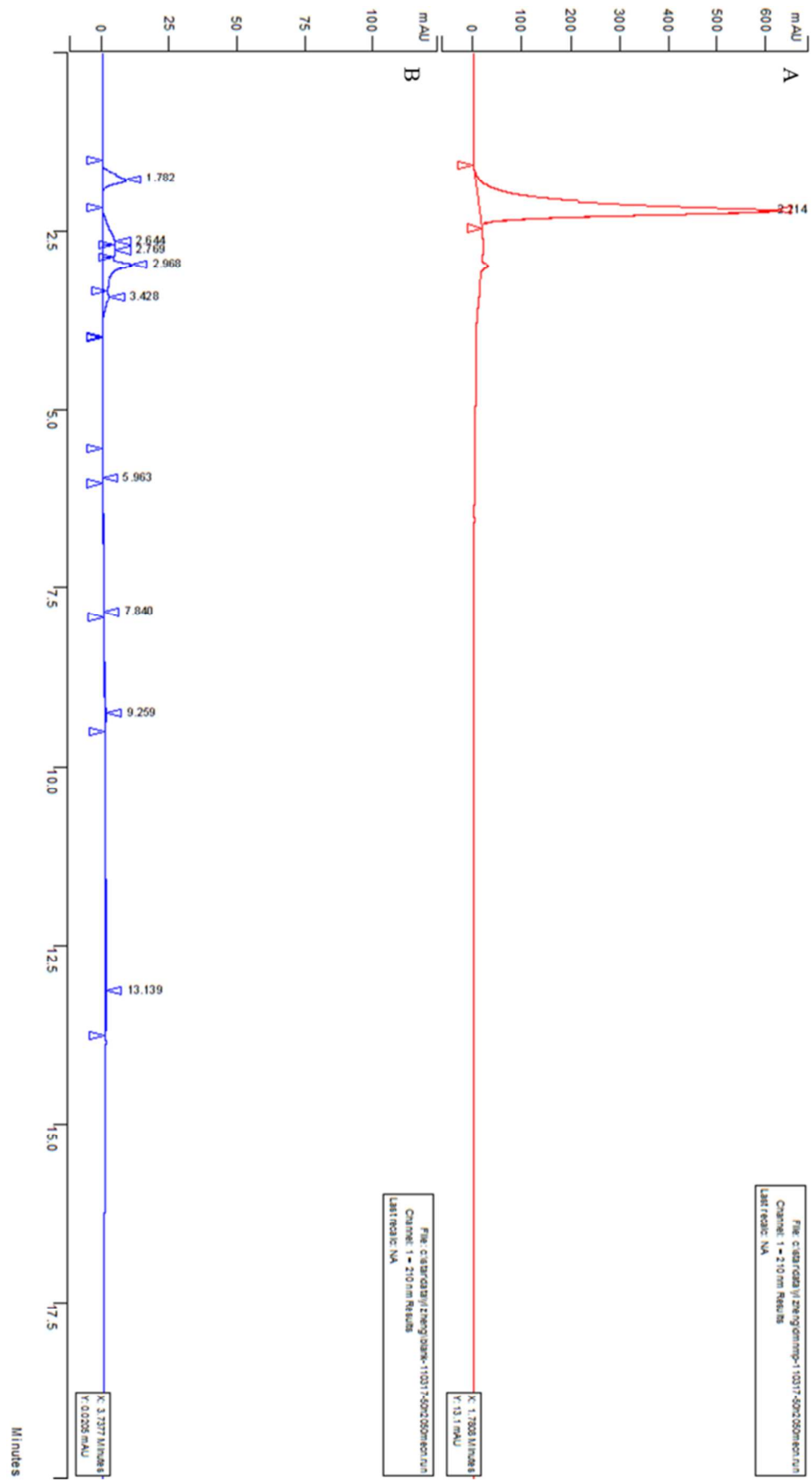


Figure S2. The HPLC of dMNMP (A) and background (B).

Table S1. The binding results of dMNAP and dMNMP with somatostatin receptor (NET), peripheral benzodiazepine receptor (PBR), serotonin transporter (SERT), and sigma receptors (Sigma).

Compounds	NET	PBR	SERT	Sigma 1	Sigma2 PC12
dMNAP	14.1	-10.3	-10.9	5.1	9.2
dMNMP	23.4	16.5	6.4	-1.8	-16.7

Data represent mean % inhibition (N = 4 determinations) for compound tested at receptor subtypes. Significant inhibition is considered > 50%. In cases where negative inhibition (-) is seen, this represents a stimulation of binding. Occasionally, compounds at high concentrations will non-specifically increase binding. The default concentration for primary binding experiments is 10 uM.

Table S2. The binding results of dMNAP and dMNMP with histamine receptors (H1-H4) and muscarinic acetylcholine receptors (M1-M4).

Compounds	H1	H2	H3	H4	M1	M2	M3	M4
dMNAP	26.5	17.5	18.1	-3.8	30.6	31.5	30.4	3.7
dMNMP	21.6	23.7	15.2	-13.6	-11.4	-1.4	17.2	6.7

Data represent mean % inhibition (N = 4 determinations) for compound tested at receptor subtypes. Significant inhibition is considered > 50%. In cases where negative inhibition (-) is seen, this represents a stimulation of binding. Occasionally, compounds at high concentrations will non-specifically increase binding. The default concentration for primary binding experiments is 10 uM.

Table S3. The binding results of dMNAP and dMNMP with dopamine receptor and GABAA receptor

Compounds	D1	D2	D3	D4	D5	DAT	GABAA
dMNAP	-4.2	-11.8	-0.7	-3.2	-2.1	0.7	16.3
dMNMP	7.5	-14.9	10.2	2.7	-1.4	-4.6	27.0

Data represent mean % inhibition (N = 4 determinations) for compound tested at receptor subtypes. Significant inhibition is considered > 50%. In cases where negative inhibition (-) is seen, this represents a stimulation of binding. Occasionally, compounds at high concentrations will non-specifically increase binding. The default concentration for primary binding experiments is 10 uM.

Table S4. The binding results of dMNAP and dMNMP with serotonin receptor.

Compounds	5-HT 1A	5-HT 1B	5-HT 1D	5-HT 1E	5-HT 2A	5-HT 2B	5-HT 2C	5-HT 3	5-HT 7
dMNAP	4.6	3.1	-9.1	3.9	-4.5	-4.8	13.8	-27.4	18.7
dMNMP	-2.3	4.6	-8.2	13.8	-13.8	-16.9	15.6	-27.8	16.6

Data represent mean % inhibition (N = 4 determinations) for compound tested at receptor subtypes. Significant inhibition is considered > 50%. In cases where negative inhibition (-) is seen, this represents a stimulation of binding. Occasionally, compounds at high concentrations will

non-specifically increase binding. The default concentration for primary binding experiments is 10 uM.

Table S5. The binding results of dMNAP and dMNMP with adrenergic receptor, Sympathetic receptor, and BZP rat brain site.

Compounds	Alpha1A	Alpha1B	Alpha1D	Alpha2A	Alpha2B	Alpha2C	Beta1	Beta3	BZP Rat Brain Site
dMNAP	1.0	0.9	-2.5	13.7	5.7	31.9	-18.8	-2.1	-4.2
dMNMP	-7.1	-1.5	-5.1	17.8	-0.2	19.0	-7.6	-10.8	11.7

Data represent mean % inhibition (N = 4 determinations) for compound tested at receptor subtypes. Significant inhibition is considered > 50%. In cases where negative inhibition (-) is seen, this represents a stimulation of binding. Occasionally, compounds at high concentrations will non-specifically increase binding. The default concentration for primary binding experiments is 10 uM.

Table S6. The measured shortest distances between atoms on critical amino acid residues and atoms on the ligands of dMNAP and dMNMP in the inactive and active MOR and KOR from docking studies.

Residue	Receptor	Atom of residue	Atom of ligand	Distance (Å)
$D^{3.32}$	Inactive MOR	N21@dMNAP	OD2@$D^{3.32}$	5.8
		N21@dMNMP	OD2@$D^{3.32}$	4.0
	Inactive KOR	N21@dMNAP	OD2@$D^{3.32}$	6.4
		N21@dMNMP	OD2@$D^{3.32}$	6.4
	Active MOR	N21@dMNAP	OD2@$D^{3.32}$	3.8
		N21@dMNMP	OD2@$D^{3.32}$	3.9
	Active KOR	N21@dMNAP	OD2@$D^{3.32}$	3.9
		N21@dMNMP	OD2@$D^{3.32}$	4.5
$W^{6.48}$	Inactive MOR	C36@dMNAP	CZ3@$W^{6.48}$	3.5
		C36@dMNMP	CZ2@$W^{6.48}$	3.4
	Inactive KOR	C36@dMNAP	CZ2@$W^{6.48}$	4.4
		C36@dMNMP	CZ2@$W^{6.48}$	4.3
	Active MOR	C36@dMNAP	CZ3@$W^{6.48}$	3.9
		C36@dMNMP	CZ3@$W^{6.48}$	3.6
	Active KOR	C36@dMNAP	CZ3@$W^{6.48}$	3.2
		C24@dMNMP	CH2@$W^{6.48}$	3.7
$Y^{7.43}$	Inactive MOR	C63@dMNAP	CZ@$Y^{7.43}$	4.1
		C63@dMNMP	CZ@$Y^{7.43}$	5.3
	Inactive KOR	C63@dMNAP	CZ@$Y^{7.43}$	5.0
		C63@dMNMP	CZ@$Y^{7.43}$	4.8

	Active MOR	C63@dMNAP	CZ@$Y^{7.43}$	4.3
		C63@dMNMP	CZ@$Y^{7.43}$	4.1
	Active KOR	C63@dMNAP	CZ@$Y^{7.43}$	3.7
		C63@dMNMP	CZ@$Y^{7.43}$	3.7

Table S7. The measured shortest distances between atoms on critical amino acid residues and atoms on the ligands of dMNAP and dMNMP in the active MOR and KOR from docking studies.

Residue	Receptor	Atom of residue	Atom of ligand	Distance (Å)
$D^{3.32}$	Active MOR	N21@dMNAP	OD2@$D^{3.32}$	3.8
		N21@dMNMP	OD2@$D^{3.32}$	3.9
	Active KOR	N21@dMNAP	OD2@$D^{3.32}$	3.9
		N21@dMNMP	OD2@$D^{3.32}$	4.5
$W^{6.48}$	Active MOR	C36@dMNAP	CZ3@$W^{6.48}$	3.9
		C36@dMNMP	CZ3@$W^{6.48}$	3.6
	Active KOR	C36@dMNAP	CZ3@$W^{6.48}$	3.2
		C24@dMNMP	CH2@$W^{6.48}$	3.7
$Y^{7.43}$	Active MOR	C63@dMNAP	CZ@$Y^{7.43}$	4.3
		C63@dMNMP	CZ@$Y^{7.43}$	4.1
	Active KOR	C63@dMNAP	CZ@$Y^{7.43}$	3.7
		C63@dMNMP	CZ@$Y^{7.43}$	3.7

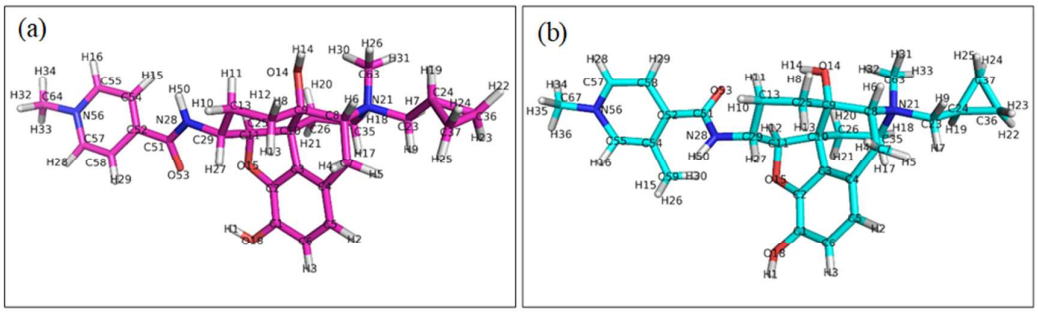


Figure S3. The chemical structures of dMNAP (a) and dMNMP (b) with notations from the docking studies.