Synthesis and Evaluation of New ^{18}F-Labelled Thienylcyclohexylpiperidine (TCP) Analogues as Radioligands for the NMDA Receptor-Channel Complex

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SUMMARY

We have synthesized new fluorine-18 labelled derivatives of thienylcyclohexylpiperidine (TCP), a non-competitive antagonist of NMDA receptor, which binds to the phencyclidine (PCP) binding site located within the receptor-associated ion channel. The mesylate precursors for (1*S**,2*R**)-2-(hydroxymethyl)- and (1*S**,2*R**)-2-(methoxymethyoxymethyl)-1-(*N*-piperidyl)-1-[2-(2'-[^{18}F]fluoroethyl)thiophenyl]cyclohexane, [^{18}F]**4** and [^{18}F]**19**, respectively, were prepared from 2-hydroxycyclo-hexanone. Radiochemical syntheses were done by displacement of the mesylates by [^{18}F]fluoride ion with no-carrier-added [K/2.2.2]$^{+}$^{18}F^{-} in 4-4.5 % radiochemical yields with specific activity of >31 GBq/mol. In the biodistribution studies with [^{18}F]**4** and [^{18}F]**19**, no selective accumulation of radioactivity was observed. Low affinities of these ligands to the NMDA receptor were also shown in *in vitro* binding experiments.

Keywords: NMDA receptor, TCP, fluorine-18, ^{18}F-labelled ligand

INTRODUCTION

The NMDA (N-methyl-D-aspartate) receptor is one type of neuronal ionotropic receptors for L-glutamate, the fast excitatory neurotransmitter in the mammalian central nervous system (CNS).[1] The NMDA receptors are widely distributed in the brain and spinal cord, with the highest densities in the cerebral cortex and hippocampus. The NMDA receptors have received increasing attention because of an unusual feature, as excessive activation of the receptor can lead to over-excitation of the target neurons to the point of cell death, probably caused by an excess accumulation of intracellular Ca^{2+}.[2] It also seems likely that the NMDA receptors contribute importantly to the etiology and progression of many neurological disease states such as those following traumatic head or spinal cord injury, stroke, perinatal ischaemia, in hypoglycemic conditions, or Alzheimer's and Huntington's diseases.[3] Thus, there has been great interest in the development of useful radioligands for imaging the NMDA receptor in living human brain by non-invasive tomographic techniques. Several radio-labelled ligands have been developed for *in vivo* studies with PET or SPECT,[4] but none of these ligands has proved to be useful for imaging the NMDA receptor.

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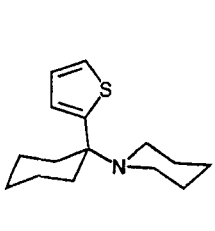
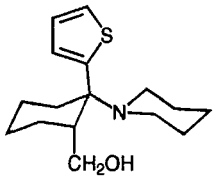
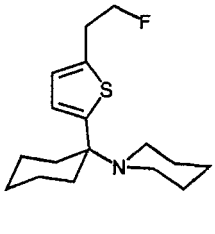
In our previous work aimed at the development of new [^{18}F]labelled radioligands for PET studies, we synthesized analogues of 1-[1-(2-thienyl)cyclohexyl]piperidine (TCP, **1**), a non-competitive antagonist of NMDA receptor, which binds to the phencyclidine (PCP) binding site located within the receptor-associated ion channel. Among them, 1-[1-(2-thienyl)-2-hydorxymethylcyclohexyl]piperidine (*cis*-HTCP, **2**) showed relatively high *in vitro* affinity to NMDA receptor (IC_{50}=16 nM) in displacement of the binding of [^{3}H]TCP to rat nervous membranes.[5] But the synthesis of the corresponding [^{18}F]-labelled compound was unsuccessful. On the other hand, the [^{18}F]labelled TCP analogue with (2'-fluoroethyl)-2-thienyl group (^{18}FE-TCP, **3**) showed 35% and 46% specific binding in the hippocampus and striatum, respectively, in the biodistribution study with rats,[6] although its non-labelled correspondence showed somewhat lower *in vitro* affinity with IC_{50}=61 nM compared to **2**. As part of further research for positron-labelled radioligands based on the TCP molecule, new TCP analogues (**4**) having both a hydroxymethyl group at C2 of the cycohexane and a fluoroethyl substituent at C5 of the thiophene ring were designed. This paper describes the synthesis and *in vitro* evaluation of **4** as a TCP antagonist as well as the *in vivo* biodistribution of [^{18}F]labelled-**4** in rats.



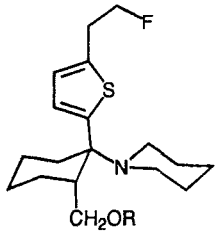
TCP (**1**)



cis-HTCP (**2**)



FE-TCP (**3**)

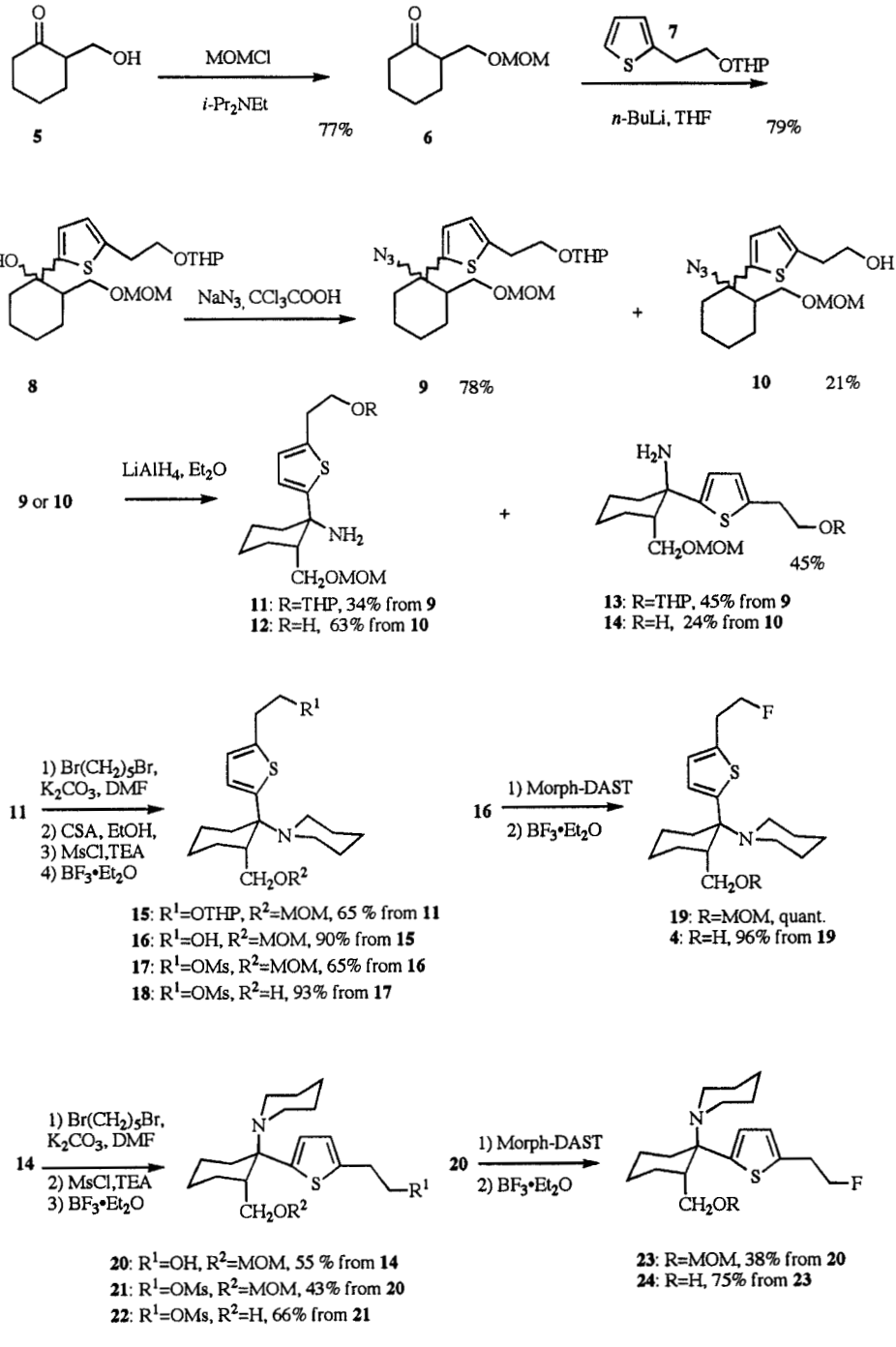


4a: R=CH_2OCH_3
4b: R=H

CHEMISTRY

The non-radioactive fluoroethyl-substituted analogue **4** was synthesized starting from 2-(2'-hydroxy-ethyl)thiophene and 2-ethoxycarbonylcyclohexanone as previously reported[5,6] (Scheme 1). During the azide introduction to **8**, deprotected **10** (21%) was obtained together with the desired **9** (78%). Successive transformation of the functional groups of **9**, including reduction with $LiAlH_4$, piperidine formation, deprotection of THP, mesylation, then deprotection of MOM produced **18**, which is the precursor for [^{18}F]fluorination. Non-radiolabelled **4** was prepared directly from alcohol derivative **16** *via* direct fluorination with morpholinosulphur trifluoride, because **4** could not be derived from **18** by the usual displacement reaction with fluoride anion, such as KF-Kriptofix2.2.2 or *n*-Bu_4NF. The corresponding non-radiolabelled stereoisomer **24** was derived from **10** by a similar sequence of reactions through **14** with non-protected hydroxy group. These non-radiolabelled fluorinated derivatives (**4** and **24**) were used for *in vitro* evaluation as NMDA ligands as well as the standard in HPLC comparison in radiosynthesis.

The radiosynthesis was carried out with the mesylate precursor (**17** and **18**) in dry acetonitrile using no-carrier-added [K/2.2.2]$^+$^{18}F$^-$ as the radiofluorinating agent. The closed TPX vessel was heated in an oil bath at 90°C for 15 min. The mixture was briefly cooled and passed through a silica gel Sep-Pak cartridge, then purified by HPLC with a reverse-phase ODS column. The overall radiochemical yields were 4-4.5% at the end of the synthesis (not corrected for decay) following an overall preparation time of 74-85 min.



5

6

8

9 78%

10 21%

9 or **10**

11: R=THP, 34% from **9**
12: R=H, 63% from **10**

13: R=THP, 45% from **9**
14: R=H, 24% from **10**

11

15: R^1=OTHP, R^2=MOM, 65 % from **11**
16: R^1=OH, R^2=MOM, 90% from **15**
17: R^1=OMs, R^2=MOM, 65% from **16**
18: R^1=OMs, R^2=H, 93% from **17**

16

19: R=MOM, quant.
4: R=H, 96% from **19**

14

20: R^1=OH, R^2=MOM, 55 % from **14**
21: R^1=OMs, R^2=MOM, 43% from **20**
22: R^1=OMs, R^2=H, 66% from **21**

20

23: R=MOM, 38% from **20**
24: R=H, 75% from **23**

Scheme 1. Synthesis of Flurorinated TCP Analogues.

In vitro and *in vivo* evaluation

In vitro affinities for the PCP recognition sites on the NMDA receptor complex were determined by displacement of [^{3}H]TCP binding to rat brain homogenates (Table 1).[6,7] Introduction of the substituents both to the C2 cyclohexane and the thiophene ring decreased affinity significantly, showing a remarkable contrast to the previous results in that hydroxymethyl on the cyclohexane ring (**2**)[5] and fluoroethyl on the thiophene ring (**3**)[6] caused a slight decrease in affinity to NMDA receptors (Table 2). Although this binding affinity did not seem to be suitable for developing the corresponding [^{18}F]fluorinated ligands for *in vivo* study, we moved on to investigate further the *in vivo* biodistribution study of [^{18}F]**4** and [^{18}F]**19**, because there are some examples where low-affinity radioligands show relatively high selective accumulation into the target receptors.[8]

Table 1. Evaluation of *in vitro* binding of TCP analogues to NMDA receptor

Compound	IC_{50}(nM)
16	>10000
17	694±52
18	222±51
19	>10000
4	1508±51
20	>10000
21	>10000
22	4624±243
24	>10000
TCP (**1**)	
cis-HTCP (**2**)	7.94±0.003
FE-TCP (**3**)	16±2
	61±9

Values are means with S.E.M. of 2-4 experiments. Non-specific binding is defined as [^{3}H]TCP binding in the presence of 100 μM PCP.

The biodistribution of ^{18}F activity in the various tissues in the Wistar male rats following intravenous administration of [^{18}F]**4** is shown in Table 2. Initial uptake in the whole brain was 0.20 %dose/g at 5 min., falling to 0.03 %dose/g at 60 min. with highest uptake of 0.25 %dose/g at 10 min. Highest uptake was observed in the kidney with relatively slow clearance. Clearance from the blood was very rapid. Accumulation of radioactivity in the bone was initially low, but increased gradually to 0.17 %dose/g at 60 min., indicating that *in vivo* defluorination was not significant. Brain regional distribution did not show selective accumulation in any tissues throughout the time investigated. The result of the same biodistribution study with [^{18}F]**19**, which is more hydrophobic than [^{18}F]fluorinated **4**, is shown in Table 3. The highest uptake in the brain was observed at 10 min. and gradually cleared at 60 min. As with the biodistribution of [^{18}F]**4**, selective accumulation was not observed. Next, for blocking studies, animals were injected through the tail vein with *cis*-HTCP (**2**) 5 min. prior to the injection of [^{18}F]**4** or [^{18}F]**19**, and the biodistributions of radioactivity were investigated at 10 min. post-injection of the radioligands (Table 4). No blocking effect of *cis*-HTCP (**2**) was observed for all the tissues investigated, clearly indicating that the radioactivity was distributed due to non-selective binding.

In conclusion, it turned out that the new ligands [^{18}F]**4** or [^{18}F]**19** were not suitable as *in vivo* radioligands for PET studies of NMDA receptor. Recent studies have suggested that hydrophobic compounds tend to penetrate membranes to bind membrane-bound receptors and that this mechanism

may cause nonselective *in vivo* distribution.[9] Non-specific biodistribution obtained with hydrophobic radioligands including our results may be due to this penetration route. It will be necessary to use more hydrophilic molecules as lead compounds for developing useful *in vivo* radioligands for NMDA receptor.

Table 2. Biodistribution of radioactivity in tissue of [^{18}F]**4**.[a]

Tissue	5 min.	10 min.	15 min.	60 min.
Brain	0.20±0.10	0.25±0.02	0.15±0.06	0.03±0.02
Hippocampus	0.18±0.09	0.24±0.01	0.16±0.06	0.03±0.02
Striatum	0.15±0.11	0.25±0.02	0.15±0.05	0.04±0.03
Cerebral cortex	0.20±0.11	0.25±0.03	0.16±0.08	0.05±0.03
Cerebellum	0.18±0.09	0.25±0.02	0.14±0.05	0.05±0.03
Lung	0.78±0.45	0.53±0.29	0.64±0.26	0.15±0.06
Liver	0.25±0.13	0.41±0.16	0.48±0.29	0.11±0.07
Kidney	0.99±0.46	1.53±0.23	1.00±0.53	0.23±0.10
Heart	0.30±0.16	0.43±0.06	0.23±0.06	0.07±0.04
Bone	0.06±0.04	0.13±0.01	0.16±0.06	0.17±0.10
Blood	0.04±0.01	0.20±0.08	0.08±0.04	0.02±0.02

a) Tissue radioactivity was expressed %dose/g with means±S. D. form three rats.

Table 3. Biodistribution of radioactivity in tissue of [^{18}F]**19**.[a]

Tissue	5 min.	10 min.	15 min.	60 min.
Brain	0.13±0.13	0.31±0.04	0.15±0.05	0.05±0.04
Hippocampus	0.12±0.11	0.33±0.04	0.15±0.07	0.05±0.05
Striatum	0.14±0.15	0.28±0.02	0.15±0.04	0.04±0.04
Cerebral cortex	0.14±0.14	0.35±0.04	0.16±0.05	0.05±0.04
Cerebellum	0.12±0.12	0.28±0.04	0.14±0.05	0.04±0.04
Lung	0.50±0.45	1.18±0.24	0.61±0.23	0.24±0.21
Liver	0.10±0.11	0.52±0.12	0.24±0.07	0.20±0.18
Kidney	0.66±0.64	2.37±0.16	1.27±0.44	0.45±0.39
Heart	0.20±0.18	0.37±0.06	0.17±0.06	0.07±0.06
Bone	0.02±0.02	0.08±0.01	0.17±0.13	0.20±0.34
Blood	0.09±0.013	0.12±0.01	0.08±0.02	0.04±0.04

a) Tissue radioactivity was expressed %dose/g with means±S. D. form three rats.

Table 4. Effect of Pre-injection of *cis*-HPTC (**2**) on the biodistribution of radioactivity in tissue of [^{18}F]**4** and [^{18}F]**19**.[a]

	[^{18}F]**4**		[^{18}F]**19**	
Tissue	Pre-injected	control	Pre-injected	control
Brain	0.26±0.08	0.26±0.06	0.29±0.09	0.36±0.05
Hippocampus	0.25±0.08	0.24±0.06	0.32±0.08	0.33±0.05
Striatum	0.24±0.08	0.22±0.04	0.31±0.11	0.38±0.16
Cerebral cortex	0.28±0.08	0.28±0.06	0.37±0.08	0.45±0.10
Cerebellum	0.22±0.07	0.23±0.06	0.25±0.11	0.34±0.10
Lung	0.91±0.14	0.88±0.18	1.19±0.18	1.78±0.62
Liver	0.52±0.16	0.49±0.02	0.48±0.25	0.54±0.18
Kidney	1.63±0.61	1.63±0.22	1.72±0.85	2.53±0.72
Heart	0.35±0.10	0.37±0.06	0.35±0.05	0.52±0.18
Bone	0.11±0.04	0.11±0.05	0.31±0.32	0.22±0.12
Blood	0.16±0.06	0.14±0.03	0.17±0.07	0.14±0.05

a) Rats were pretreated with *cis*-HPTC (**2**) (1.7 μmol/kg) or saline vehicle alone, 5 min prior to the injection of [^{18}F]**4** or [^{18}F]**19**. Each value represents the mean±S. D (%dose/g) from three rats.

Experimental

^{1}H-NMR spectra were obtained on a Varian Unity 500, JNM GX-270, or Hitachi R-1200 spectrometers with $SiMe_4$ as an internal standard in $CDCl_3$, and IR spectra were recorded with a JASCO IR Report 100 spectrometer. Low resolution FD mass spectra were obtained on a JEOL JMS-300 spectrometer, and high resolution FAB mass spectra (HRFABMS) were obtained on a JEOL NMS-SX 102-SX spectrometer. Ultraviolet spectra were obtained on a Hitachi 220A spectrometer. Fluorine-18 was produced from 16% enriched [^{18}O]H_2O by the $^{18}O(p,n)^{18}F$ reaction as described previously.[6] Aminopolyether (Kryptofix 2.2.2) supported potassium[^{18}F]fluoride ([K/2.2.2]$^+$$^{18}F^-$) was prepared by the addition of $K_2CO_3 \cdot 1.5H_2O$ (1 mg) and Kryptofix 2.2.2 (4 mg) to the irradiated water in a TPX (polymethylpentene) vessel and subsequent removal of the water by co-evaporation with dry acetonitrile as reported previously.[6] Radiochemical yields were expressed as the percentage of the initial activity of the [^{18}F]fluorinating agent.

2-(Methoxymethyloxymethyl)cyclohexanone (6). A mixture of 2-hydroxymethylcyclo-hexanone (200 mg, 1.56 mmol), N,N-diisopropylethylamine (0.54 mL, 3.12 mmol), and chloromethylmethyl ether (0.18 mL, 2.34 mmol) in CH_2Cl_2 (2 mL) was stirred at room temperature for 18 h. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and the whole was successively washed with sat'd aq. $NaHCO_3$, water, and brine, then dried over Na_2SO_4. Filtration and evaporation gave a crude oil which was chromatographed on a silica gel column (hexane:AcOEt=4:1) to provide **6** as a colorless oil (208 mg, 77%). ^{1}H NMR δ: 1.40~2.50 (9H, m), 3.36 (3H, s), 3.52~3.94 (2H, m), 4.62 (2H, s). IR (neat) cm^{-1}: 1705.

1-[2-(2'-Tetrahydropyranyloxyethyl)thienyl]-2-(methoxymethyloxymethyl)-cyclohexanol (8) A solution of n-BuLi (5.85ml, 9.2 mmol) was added into a solution of 2-(2'-tetrahydropyranyloxyethyl)thiophene (**7**, 1.41g, 8.3 mmol) in THF (23 mL) at -78°C, and the reaction mixture was stirred at 0°C for 30 min, then cooled at -78°C again. A solution of **6** (1.41 g/6 ml, 8.3 mmol) in THF was added into the above reaction mixture and the whole was allowed to warm up to 15 °C, then the mixture was stirred at the same temperature for 2 h. Ice was added to the reaction mixture, and the whole was extracted with ether, then the organic layer was dried over Na_2SO_4. Filtration and evaporation gave a crude oil which was chromatographed on a silica gel column (hexane:AcOEt=4:1) to provide **8** as a pale yellow oil (2.53 g, 79%). ^{1}H NMR δ: 1.35~2.04 (15H, m), 3.03 (2H, t ,J=7.2), 3.28 (3H, s), 3.40~4.10 (7H, m), 4.50 (2H, s), 4.60 (1H, bs), 6.69 (2H, s). IR (neat) cm^{-1}: 3470, 3050. FDMass (m/e):384 (M$^+$).

1-[2-(2'-Tetrahydropyranyloxyethyl)thienyl]-2-(methoxymethyloxymethyl)-cyclohexaneazido (9) and 1-[2-(2'-Hydroxyethyl)thienyl]-2-(methoxymethyloxymethyl)cyclohexaneazido (10) A solution of **8** (672mg, 1.74 mmol) in $CHCl_3$ (34 mL) was added to a solution of NaN_3 (2.53g, 35 mmol) and Cl_3COOH (6.1mL, 35 mmol) in $CHCl_3$ (34 mL) at 0°C, and the reaction mixtrue was stirred for 2h at 0°C and 17 h at room temperature. Ice was added to the reaction mixture and the whole was extracted with CH_2Cl_2. The organic layer was washed with sat'd aq. $NaHCO_3$ and dried over Na_2SO_4. Filtration and evaporation gave a crude oil which was chromatographed on a silica gel column (hexane:AcOEt=5:1) to provide **9** as a pale yellow oil (310 mg, 78%) and the isomer **10** (120 mg, 21%) . **9**: ^{1}H NMR δ: 1.30~2.21 (15H, m), 3.07 (2H, t, J=6.6), 3.28 (3H, s), 3.35~4.13 (6H, m), 4.49 (2H, s), 4.57 (1H, bs), 6.72 (1H, d J=3.8), 6.81 (1H, d J=3.8). IR (neat) cm^{-1}: 2100. FDMass (m/e): 409 (M$^+$), 366 (M$^+$-HN_3). **10**: ^{1}H NMR δ: 1.20~2.2 (9H, m), 2.97 (2H, t, J=5.9), 3.24~3.34 (3H, m), 3.50~3.72 (2H, m), 3.84 (2H, t, J=6.0), 4.46~4.60 (2H, m), 6.70 (1H, d J=3.6), 6.81 (1H, d J=3.6). IR (neat) cm^{-1}: 2100, 3060, 3400. FDMass (m/e): 325 (M$^+$).

(1S*,2R*)-2-(Methoxymethyloxymethyl)-1-amino-1-[2-(2'-tetrahydropyranyloxyethyl)thiophenyl]cyclohexane (11) and (1R*,2R*)-2-(Methoxymethyloxymethyl)-1-amino-1-[2- (2'-tetrahydropyranyloxyethyl)-thiophenyl]cyclohexane (13) A solution of **9** (5.59 g, 13.6 mmol) in ether (130 mL) was added to a suspension of $LiAlH_4$ (778 mg) at 0°C and the whole was heated under reflux for 8 h. The reaction mixture was quenched by successive addition of water (0.78 mL), 15% aq NaOH (0.78 mL), and water (2.34 mL), then the whole

was filtered through a celite pad. The filtrate was evaporated to give a crude oil which was chromatographed on a silica gel column (hexane:AcOEt=1:1) to afford **11** (1.77g, 33.6%) as a pale yellow oil and **12** (2.35 g, 44.7%) together with the starting material **9** (669 mg, 12%). **11**: ^{1}H NMR δ: 1.39~1.97 (15H, m), 3.04 (3H, t, J=7.02), 3.27 (3H, s), 3.35~4.13 (8H, m), 4.49 (2H, s), 4.62 (1H, bs), 6.71 (2H, s). IR (neat) cm^{-1}: 3050, 3380. FDMass (m/e): 325 (M^+). **13**: ^{1}H NMR δ, 1.30~2.09 (15H, m), 3.04 (3H, t, J=6.2), 3.28 (3H, s), 3.42~4.15 (8H, m), 4.49 (2H, s), 4.61 (1H, bs), 6.63~6.70 (2H, s). IR (neat) cm^{-1}: 3050, 3400. FDMass (m/e): 325 (M^+).

(1S*,2R*)-2-(Methoxymethyloxymethyl)-1-amino-1-[2-(2'-hydroxyethyl)thiophenyl]cyclohexane (12) and (1R*,2R*)-2-(methoxymethyloxy-methyl)-1-amino-1-[2-(2'-hydroxyethyl)thiophenyl]cyclohexane (14) The azido **10** (1.47 g, 4.52 mmol) was subjected to the same reduction with $LiAlH_4$ by the same procedure as described above. The crude mixture was chomatographed on a silica gel colunm (hexane:AcOEt=1:1) to afford **12** (855 mg, 63%) as a colorless oil and **14** (320 mg, 24%) as a colorless oil together with the starting material **10** (248 mg, 17%). **12**: ^{1}H NMR δ: 1.52~2.07 (9H, m), 3.00 (2H, t, J=6.2), 3.27 (3H, s), 3.30~3.43 (5H, m), 3.83 (2H, t, J=6.3), 4.50 (2H, s), 6.68 (1H, d J=3.8), 6.80 (1H, d J=3.8). IR (neat) cm^{-1}: 3050, 3400. FDMass (m/e): 299 (M^+). **14**: ^{1}H NMR δ: 1.50~2.03 (9H, m), 2.99 (2H, t, J=6.4), 3.26 (3H, s), 3.36~3.42 (5H, m), 3.82 (2H, t, J=6.4), 4.48 (2H, s), 6.68 (1H, d J=4.1), 6.75 (1H, d J=4.1). IR (neat) cm^{-1}: 3050, 3350. FDMass (m/e): 299 (M^+).

(1S*,2R*)-2-(Methoxymethyloxymethyl)-1-(*N*-piperidyl)-1-[2-(2'-tetrahydropyranyloxyethyl)thiophenyl]cyclohexane (15) A mixture of **11** (1.13g, 2.9 mmol) and 1,5-dibromopentane (0.21 mL) in DMF (11 mL) was heated at 65°C for 1h, then K_2CO_3 was added. The mixture was heated at 65°C for 7h, and 1,5-dibromopentane (0.63 mL) was added, then the mixture was heated at the same temperature for an additional 9 h. The reaction mixture was extrated with ether (23 mL), and washed successively with 10% aq K_2CO_3 and water, then dried over Na_2SO_4. Filtration and evaporation gave a crude oil which was chromatographed on a silica gel column (hexane:AcOEt=2:1) to provide 15 (870 mg, 65%) as a colorless oil. **15**: ^{1}H NMR δ: 1.54~1.64 (20H, m), 2.24~2.28 (4H, m), 2.68~2.72 (1H, m), 3.07 (2H, dd, J=6.93,J=0.66), 3.39 (3H, s), 3.61~3.75 (4H, m), 3.93~3.97 (2H, m), 4.63 (1H, d, J=3.3), 4.67 (1H, d, J=10.6), 4.69 (1H, d, J=10.6), 6.60 (1H, d, J=3.63), 6.75 (1H, d, J=3.63). IR (neat) cm^{-1}: 3060. FAB MASS (m/e): 451(M^+)

(1S*,2R*)-2-(Methoxymethyloxymethyl)-1-(*N*-piperidyl)-1-[2-(2'-hydroxyethyl)thiophenyl]cyclohexane (16) A mixture of **15** (782 mg, 1.73 mmol) and 10-camphor sulfonic acid (436 mg) in methanol (20 mL) was stirred at room temperature for 21 h. The reaction mixture was neutralized with NaOH (136 mg) and the solvent was evaporated to give a crude mixture which was chromatographed on an alumina column (AcOEt) to give **16** (577 mg, 90%) as a colorless oil. ^{1}H NMR δ: 1.25~1.90 (15H, m), 2.08~2.27 (4H, m), 2.69~2.71 (1H, m), 3.04 (2H, t, J=6.71), 3.39 (3H, s), 3.71 (1H, t, J=10.38), 3.87 (2H, t, J=6.72), 3.96 (1H, dd, J=3.67, J=10.4), 4.67 (1H, d, J=17.1), 4.69 (1H, d, J=17.1), 6.63 (1H, d, J=3.66), 6.75 (1H, d, J=3.66) 15H. IR (neat) cm^{-1}: 3400.

(1S*,2R*)-2-(Methoxymethyloxymethyl)-1-(*N*-piperidyl)-1-[2-(2'-methansulfonyloxy-ethyl)thiophenyl]cyclohexane (17) Methanesulfonyl chloride (82 μL) was added to a solution of **16** (388 mg, 1.06 mmol) and triethylamine (0.29 mL) in dry ether (15 mL) at 0°C, and the mixture was stirred at the same temperature for 1h. The reaction mixture was extracted with AcOEt (46 mL), and washed successively with brine and water, then dried over Na_2SO_4. Filtration and evaporation gave a crude oil which was chromatographed on a silica gel column (hexane:AcOEt=5:1) to afford **17** (205 mg, 43%) as a colorless oil together with recovered **16** (130 mg, 34 %. ^{1}H NMR δ: 1.23~1.54 (14H, m), 2.23~2.27 (4H, m), 2.67~2.70 (1H, m), 2.94 (3H, s), 3.23 (2H, td, J=6.93,J=0.66), 3.40 (3H, s), 3.71 (1H, t, J=10.2), 3.96 (1H, dd, J=3.63, J=9.9), 4.43 (2H, t, J=6.93), 4.67 (1H, d, J=10.6), 4.69 (1H, d, J=10.6), 6.63 (1H, d, J=3.3), 6.80 (1H, d, J=3.63). IR (neat) cm^{-1}: 3070. FAB MASS (m/e): 445(M^+)

(1S*,2R*)-2-(Hydroxymethyl)-1-(*N*-piperidyl)-1-[2-(2'-methansulfonyloxy-ethyl)thiophenyl]cyclohexane (18). BF_3Et_2O (69 μL) was added to a solution of **17** (25 mg) in Me_2S (0.3 mL) at 0°C, and the mixture was stirred at the same temperature for 10 min. The reaction mixture was quenched with sat'd aq. $NaHCO_3$, and extracted with AcOEt. The organic layer was washed with brine and dried over Na_2SO_4. Filtration and evaporation gave a crude oil which was chromatographed on a silica gel column (CH_2Cl_2:ether=1:1) to provide **18** (20.9 mg, 93%) as a colorless oil. ^{1}H NMR δ: 1.18~1.76 (15H, m), 1.97~2.20 (4H, m), 2.82~2.85 (1H, m), 2.97 (3H,

s), 3.20 (2H, dt ,J=0.66, 6.93), 3.61 (1H, dd, J=3.96, 11.55), 4.34 (1H, t ,J=11.05), 4.43 (2H, t, J=6.93), 6.73 (1H, d, J=3.63), 6.82 (1H, d, J=3.63). IR (neat) cm^{-1}: 3400, 3060. FAB Mass (m/e) 401(M+). HR FABMass (m/e) calcd for $C_{19}H_{31}O_4NS_2$: 401.1695. Found 401.1693.

(1S*,2R*)-2-(Methoxymethyloxymethyl)-1-(*N*-piperidyl)-1-[2-(2'-fluoroethyl)thiophenyl]cyclohexane (19). Morpholinosulphur trifluoride (85 μL) was added to a solution of **16** (127 mg, 0.285 mmol) in CH_2Cl_2 (4 mL) at -78°C, and the reaction mixture was allowed to warm up to room temperature, then stirred for 24 h. The reaction mixture was quenched with sat'd aq. $NaHCO_3$, and extracted with CH_2Cl_2. The organic layer was washed with brine and dried over Na_2SO_4. Filtration and evaporation gave a crude oil which was chromatographed on a alumina column (hexane: CH_2Cl_2=2:1) to produce 19 (64.5 mg, 50%) as a colorless oil together with the recovered 16 (62.2 mg, 49%). ^1H NMR δ:, 1.33~1.49 (12H, m), 1.62 (2H, bs), 1.87~1.91 (4H, m), 2.69~2.71 (1H, m), 3.18 (2H ,dt, J=23.8, J=6.1), 3.39 (3H, s), 3.71 (1H, t, J=10.4), 3.96 (1H, dd, J=3.67, J=9.77), 4.63 (2H, dt, J=36.0, J=6.1), 4.68 (1H, d ,J=11.3), 4.69 (1H ,d, J=11.3), 6.63 (1H, d, J=3.36), 6.78 (1H, d, J=3.36). IR (neat) cm^{-1}: 3050. FAB mass (M+) 369

(1S*,2R*)-2-(Hydroxymethyl)-1-(*N*-piperidyl)-1-[2-(2'-fluoroethyl)thiophenyl]-cyclohexane (4). BF_3Et_2O (50 μL) was added to a solution of **19** (14.3 mg) in Me_2S (0.3 mL) at 0°C, and the mixture was stirred at the same temperature for 40 min. The reaction mixture was quenched with sat'd aq. $NaHCO_3$, and extracted with AcOEt. The organic layer was washed with brine and dried over Na_2SO_4. Filtration and evaporation gave a crude oil which was chromatographed on an alumina column (ether) to provide **4** (12.1 mg, 96%) as a colorless oil. ^1H NMR δ: 1.15~1.86 (15H, m), 2.01~2.28 (4H, m), 2.83~2.93 (1H, m), 3.20 (2H, ddt ,J=23.04, 0.66, 6.27), 3.62 (1H, dd, J=3.80, 11.72), 4.35 (1H, t ,J=11.05), 4.66 (2H, dt, J=46.85, 6.27), 6.72 (1H, d, J=3.63), 6.80 (1H, d, J=3.63). IR (neat) cm^{-1}: 3400, 3060. FAB Mass (m/e): 326 (M+). HR FABMass (m/e): calcd for $C_{18}H_{28}ONSF$: 325.1875. Found:325.1873.

(1R*,2R*)-2-(Methoxymethyloxymethyl)-1-(*N*-piperidyl)-1-[2-(2'-hydroxyethyl)-thiophenyl]cyclohexane (20). Amine **14** (320 mg) was subjected to the same procedure as described for **15** to produce **20** (216 mg, 55%). ^1H NMR δ: 1.37~2.06 (15H, m), 2.36~2.40 (4H, m), 3.01 (2H ,t, , J=6.11), 3.30 (3H, s), 3.42 (2H, t, J=6.71), 3.45~3.47 (1H, m), 3.66 (1H, t, J=6.1, 3.84 (1H, t, J=6.1), 4.53 (1H ,d, J=6.4), 4.55 (1H, d, J=6.4), 6.69 (1H, d, J=4.3), 6.71 (1H, d, J=4.3). IR (neat) cm^{-1}: 3400, 3050. FD mass (m/e): 367(M+).

(1R*,2R*)-2-(Methoxymethyloxymethyl)-1-(*N*-piperidyl)-1-[2-(2'-methansulfonyl-oxyethyl)thiophenyl]cyclohexane (21) Alcohol **20** (45 mg, 0.12 mmol) was subjected to the same procedure as described for **17** to produce **21** (23.4 mg, 43%). ^1H NMR δ: 1.36~1.94 (15H, m), 2.26~2.37 (4H, m), 2.93 (3H, s), 3.20 (2H, t, J=6.27), 4.25 (1H, t ,J=6.27), (1H, t ,J=6.77), (2H, t, J=6.77), 4.54 (2H, s), 6.71 (1H, d, J=3.63), 6.74 (1H, d, J=3.63). IR (neat) cm^{-1}: 3050. FD Mass (m/e): 445(M+).

(1R*,2R*)-2-(Hydroxymethyl)-1-(*N*-piperidyl)-1-[2-(2'-methansulfonyloxyethyl)-thiophenyl]cyclohexane (22) MOM ether **21** (20.5 mg, 0.046 mmol) was subjected to the same procedure as described for **18** to produce **22** (12.3 mg, 66%). ^1H NMR δ: 1.25~2.08 (15H, m), 2.27~2.49 (4H, m), 2.71~2.82 (1H, m), 2.97 (3H, s), 3.24 (2H, t ,J=6.60), 3.58 (1H, dd, J=5.78, 11.38), 3.80 (1H, t ,J=11.73), 4.43 (2H, t, J=6.93), 6.81 (1H, d, J=3.47), 7.01 (1H, d, J=3.47). IR (neat) cm^{-1}: 3400. FAB Mass (M+) 402. HR FABMass (m/e) calcd for $C_{19}H_{31}O_4NS_2$: 401.1695. Found 401.1693.

(1R*,2R*)-2-(Methoxymethyloxymethyl)-1-(*N*-piperidyl)-1-[2-(2'-fluoroethyl)-thiophenyl]cyclohexane (23). Alcohol **20** (41 mg, 0.011 mmol) was subjected to the same procedure as described for **19** to produce **24** (11.4 mg, 28%) together with the recovered **20** (10.7 mg, 26%). ^1H NMR δ: 1.25~2.19 (15H, m), 2.27~2.38 (4H, m), 3.16 (2H, dt, J=22.4, 6.6), 3.30 (3H, s)), 3.47 (2H, t ,J=6.93), 4.55 (2H, s), 4.62 (2H, dt, J=46.9, 6.6), 6.69 (1H, d, J=3.63), 6.72 (1H, d, J=3.63). IR (neat) cm^{-1}: 3050. FD Mass (m/e): 369 (M+).

(1R*,2R*)-2-(Hydroxymethyl)-1-(*N*-piperidyl)-1-[2-(2'-fluoroethyl)thiophenyl]-cyclohexane (24). MOM ether **23** (19 mg, 0.051 mmol) was subjected to the same procedure as described for **4** to produce **24** (12.5 mg, 75%). ^1H NMR δ: ^1H NMR δ: 1.18~1.91 (15H, m), 2.23~2.49 (4H, m), 2.69~2.80 (1H, m), 3.19 (2H, ddt ,J=22.77, 0.66, 6.6), 3.56 (1H, dd, J=11.4, 5.77), 3.83 (1H, t, J=10.73), 4.65 (2H, dt, J=46.85, 6.6), 6.79 (1H, d, J=3.46), 7.00 (1H, d,

J=3.46). IR (neat) cm^{-1}: 3380, 3070. FD Mass (m/e) 326(M+). HR FABMass (m/e): calcd for $C_{18}H_{28}ONSF$: 325.1876. Found: 325.1854.

(1S*,2R*)-2-(Hydroxymethyl)-1-(*N*-piperidyl)-1-[2-(2'-[^{18}F]fluoroethyl)thiophenyl]cyclohexane ([^{18}F]4). A solution of the mesylate **18** (0.2 mg) in 300 μL of acetonitrile was added to a TPX vessel containing the [K/2.2.2]$^{+}$^{18}F^{-}(1-2 mCi). The closed vessel was heated in an oil bath at 90°C for 15 min. The mixture was briefly cooled and passed through a silica gel Sep-Pak cartridge. The product, eluted with AcOEt (3 mL), was evaported, and the residue was purified by HPLC (Column: MegaPak SIL C18-10 7.5X250mm+ YMC GEL ODS AQ-324 S-5 10X300mm; Gard column: Waters Resolve C18 Guard-Pak, Solvent: CH_3CN:H_2O:cHCl=400:600:1; Flow rate: 3.4ml/min). A radioactive peak (t_R=40 min) corresponding to the retention time of the authentic **4** was collected. The overall radiochemical yield was 4 % at the end of the synthesis (not corrected for decay) following an overall preparation time of 85 min. The specific activity of the product was estimated by UV spectroscopy to be >31GBq/mol. The solvent was evaporated under vacuum azeotropically with ethanol three times, and the residual radioactive material was dissolved in saline which was used for animal experiments.

(1S*,2R*)-2-(Methoxymethyloxymethyl)-1-(*N*-piperidyl)-1-[2-(2'-[^{18}F]fluoroethyl)thiophenyl]cyclohexane ([^{18}F]19). The radioligand [^{18}F]**19** was prepared from **17** by the same procedure as described for [^{18}F]**4**. The crude product was purified by HPLC (Column: YMC GEL ODS AQ-324 S-5 10X300mm; Gard column: Waters Resolve C18 Guard-Pak, Solvent: CH_3CN:H_2O:cHCl =400:800:1; Flow rate: 3.4ml/min). A radioactive peak (t_R=18 min) corresponding to the retention time of the authentic **19** was collected. The overall radiochemical yield was 4.5 % at the end of the synthesis (not corrected for decay) following an overall preparation time of 74 min. The specific activity of the product was estimated by UV spectroscopy to be >31GBq/mol. The solvent was evaporated under vacuum azeotropically with ethanol three times, and the residual radioactive material was dissolved in saline which was used for animal experiments.

In vitro binding assay. *In vitro* affinities for the PCP recognition sites on the NMDA receptor complex were determined by displacement of [^{3}H]TCP binding to rat brain homogenates as described previously.[6,7] The forebrains were obtained from Wistar male rats (200-250 g). The incubation medium of the homogenate consisted of 0.5 mL of 5 mM Tris-HCl buffer containing 5 nM [^{3}H]TCP (47.8 Ci/mmol), 100 μg membrane protein, various amounts of test compounds, 30 mM L-glutamate and 100 μM glycine. Non-specific binding was determined in the presence of 100 μM PCP. The IC_{50} values to displace specific [^{3}H]TCP binding were determined from concentration-inhibition curves.

***In vivo* studies.** Wistar male rats (190-280 g) given standard laboratory food and water *ad libitum* were used in this investigation. Aliquots of [^{18}F]**4** or [^{18}F]**19** in a volume of 100 μL with activities ranging from 166-400 kBq (corresponding to 5.35-13 μmol of the ligands), were injected through the tail vein of unaneaesthetized rats, the experiments being in accordance with the code of practice of our faculty. After given time intervals the animals were killed by cervical dislocation under light ether anaesthesia. Samples of blood and the organs of interest were taken, weighed and assayed for radioactivity in a Packard auto-gamma 500 scintillation counter. The brain was dissected into cerebellum, cortex, striatum, hippocampus and the remaining brain tissue. Percent injected dose per gram (%dose/g) was calculated for each tissue. For blocking studies, animals were injected through the tail vein with either 1.7 μmol/kg of *cis*-2-hydroxy-1-(N-piperidyl)-1-(2-thienyl)-cyclohexane or saline vehicle alone, 5 min prior to the injection of [^{18}F]**4** or [^{18}F]**19**. Ten minutes later, the rats were killed as described above, and the regional radioactivity was determined. Student's t-test was used for statistical analysis.

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