

Copper-Catalyzed Amination of (Bromophenyl)ethanolamine for a Concise Synthesis of Aniline-Containing Analogues of NMDA NR2B Antagonist Ifenprodil

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CHEMISTRY

2-Bromo-1-(4-bromophenyl)propan-1-one¹

To 4-bromopropiophenone (14.01 g, 65.74 mmol) in carbon tetrachloride (200 mL) in a two-necked flask of 500 mL equipped with magnetically stirrer and addition funnel was added dropwise over 1.5 h bromine (3.4 mL, 66.36 mmol) in carbon tetrachloride (50 mL). The bromine colour dissipated essentially on contact with the solution. The mixture was stirred for 1.5 h, then HBr was removed from the solution with a stream of nitrogen and HBr gas was trapped by bubbling the effluent nitrogen gas through a stirred NaOH solution (1N, 200 mL). The reaction mixture was washed twice with water, then with saturated NaHCO₃, water and brine, dried over MgSO₄ and concentrated under vacuum to yield the titled compound as a white solid (18.5 g, 100%) used without further purification. Mp 85 °C (lit. 5-88 °C). R_f 0.60 (heptanes/ethyl acetate 50/50). ¹H NMR (CDCl₃, 400 MHz) δ 7.89 (d, J = 8.6 Hz, 2H), 7.63 (d, J = 8.6 Hz, 2H), 5.22 (q, J = 6.2 Hz, 1H), 1.90 (d, J = 6.2 Hz, 3H). ¹³C NMR (CDCl₃, 100.6 MHz) δ 192.5, 132.9, 131.2, 130.6, 129.1, 41.3, 20.1. IR (cm⁻¹) 1678, 1583, 1396, 1249, 1163, 1068, 1010, 842, 743,

534. ESI⁺/MS/MS *m/z* (%) 293.2 ([M(C₉H₈⁷⁹Br⁸¹BrO)+H]⁺, 100). Anal. calcd for C₉H₈Br₂O C, 37.02, H, 2.76; found C, 37.10, H, 2.86.

2-(4-Benzylpiperidin-1-yl)-1-(4-bromophenyl)propan-1-one 14

A solution of 2-bromo-1-(4-bromophenyl)propan-1-one (17.5 g, 59.7 mmol), 4-benzylpiperidine (10.5 mL, 59.7 mmol) and triethylamine (16.6 mL, 119.4 mmol) in ethanol (400 mL) was refluxed overnight (22 h). The reaction mixture was cooled to r.t. and a precipitate was formed. Filtration of the precipitate yielded the titled compound as a white powder which was used without further purification (18.9 g, 82%). Mp 84 °C. Rf 0.55 (ethyl acetate/heptanes 50/50). ¹H NMR (CDCl₃, 400 MHz) δ 7.9 (d, *J* = 8.6 Hz, 2H), 7.46 (d, *J* = 8.6 Hz, 2H), 7.18-6.98 (m, 5H), 3.88 (q, *J* = 6.8 Hz, 1H), 2.78-2.74 (m, 1H), 2.57-2.52 (m, 1H), 2.38 (d, *J* = 6.8 Hz, 2H), 2.41-2.21 (m, 1H), 2.01-1.91 (m, 1H), 1.55-1.25 (m, 3H), 1.12 (d, *J* = 6.8 Hz, 3H), 1.20-1.00 (m, 2H). ¹³C NMR (CDCl₃, 100.6 MHz) δ 199.7, 140.6, 137.5, 132.2, 131.1, 130.6, 129.0, 128.1, 125.7, 65.2, 51.9, 47.9, 43.1, 37.8, 32.7, 32.3, 10.1. IR (cm⁻¹) 2925, 2348, 1677, 1581, 1214, 774, 696. ESI⁺/MS/MS *m/z* (%) 386.2 ([M(C₂₁H₂₄⁷⁹Br NO)+H]⁺, 30), 202.2 (100), 183.0 (25), 132.1 (90). ESI⁺/HRMS calcd for C₂₁H₂₅⁷⁹BrNO 386.1120; found 386.1118. Anal. calcd for C₂₁H₂₄BrNO C, 65.29, H, 6.26, N, 3.63; found C, 64.52, H, 6.57, N, 3.88.

(1*R,2*R**)-2-(4-Benzylpiperidin-1-yl)-1-(4-bromophenyl)propan-1-ol 13a**

To a slurry of sodium borohydride (1.3 g, 34 mmol) in ethanol (150 mL) was added dropwise under nitrogen 2-(4-benzylpiperidin-1-yl)-1-(4-bromophenyl)propan-1-one (5 g, 12.9 mmol) in ethanol (200 mL) while monitoring that the pH of the solution was maintained at 7.5 or greater at all times. After an overnight stirring, the mixture was concentrated in vacuo. The residue was partitioned between ethyl acetate and water, and the phases were separated. After extraction twice with ethyl acetate, the combined organic layers were washed with brine, dried over magnesium sulfate and concentrated under vacuum to yield a crude residue corresponding to a mixture of the title compound and its (1*R**,2*S**) diastereoisomer (80/20 ratio). Separation was carried out by purification by flash column chromatography on silica gel to afford the desired product **13a** as a white solid (3.9 g, 78%). Mp 108.3 °C. Rf 0.68 (ethyl acetate/heptanes 50/50). ¹H NMR (CDCl₃, 400 MHz) δ 7.47 (d, *J* = 8.0 Hz, 2H), 7.46-7.16 (m, 7H), 4.20 (d, *J* = 9.6 Hz, 1H), 2.80-2.72 (m, 1H), 2.68-2.61 (m, 1H), 2.58 (d, *J* = 7.2 Hz, 2H), 2.59-2.50 (m, 2H), 2.12-2.07 (m, 1H), 1.75-1.72 (m, 2H), 1.66-1.48 (m, 1H), 1.46-1.32 (m, 1H), 1.30-1.26 (m, 1H), 0.75 (d, *J* = 6.8 Hz, 3H). ¹³C NMR (CDCl₃, 100.6 MHz) δ 141.4, 140.5, 131.3, 129.0,

128.2, 127.8, 125.9, 121.4, 73.7, 66.6, 52.8, 44.4, 43.2, 38.2, 33.0, 32.5, 7.9. IR (cm⁻¹) 3306, 2921, 2820, 1667, 1444, 1137, 1082, 1008, 809, 741, 698, 537. ESI⁺/MS/MS m/z (%) 388.2 ([M(C₂₁H₂₆⁷⁹BrNO)+H]⁺, 49), 370.2 (100). ESI⁺/HRMS calcd for C₂₁H₂₇⁷⁹BrNO 388.1276; found 388.1272. Anal. calcd for C₂₁H₂₆BrNO C, 64.95, H, 6.75, N, 3.61; found C, 65.10, H, 7.11, N, 3.51.

(1*R,2*S**)-2-(4-Benzylpiperidin-1-yl)-1-(4-bromophenyl)propan-1-ol 13b**

Obtained as minor diastereoisomer (0.95 g, 19%) during the synthesis of (1*R**,2*R**) aryl bromide **13a**. Mp 135.5 °C. Rf 0.27 (ethyl acetate/heptanes, 50/50). ¹H NMR (CDCl₃, 400 MHz) δ 7.42 (d, J = 8.0 Hz, 2H), 7.26-7.22 (m, 2H), 7.16-7.05 (m, 5H), 4.74 (d, J = 4.3 Hz, 1H), 2.99-2.96 (m, 1H), 2.66-2.61 (m, 2H), 2.48 (d, J = 7.2 Hz, 2H), 2.22-2.15 (m, 1H), 2.01-1.95 (m, 1H), 1.64-1.54 (m, 2H), 1.48-1.44 (m, 1H), 1.28-1.15 (m, 3H), 0.74 (d, J = 6.8 Hz, 3H). ¹³C NMR (CDCl₃, 100.6 MHz) δ 141.3, 140.6, 131.0, 129.1, 128.2, 127.8, 125.8, 120.4, 71.8, 64.1, 52.8, 49.3, 43.2, 38.0, 32.9, 32.6, 10.3.

(1*R,2*R**)-1-(4-Aminophenyl)-2-(4-benzylpiperidin-1-yl)propan-1-ol 1a**

To imine **23a** (390 mg, 0.80 mmol) in methanol (18 mL) was added sodium acetate (155 mg, 1.92 mmol) and hydroxylamine hydrochloride (100 mg, 1.44 mmol). The mixture was stirred at r.t. for 2 h and concentrated in vacuo. The crude product was partitioned between dichloromethane and an aqueous solution of sodium hydroxide (2M). The phases were separated and the aqueous layer was extracted twice with dichloromethane. The combined organic layers were dried over MgSO₄ and concentrated in vacuo. The crude residue was purified onto silica gel column chromatography to yield the titled compound as a clear yellow solid (155 mg, 60%).

BIOLOGY

HEK293 cell culture and transfection

Human embryonic kidney 293 (HEK 293) cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum (FCS), 100 IU/ml penicillin and 100 µg/ml streptomycin (Invitrogen, Cergy, France). HEK-293 cell were transfected with the cDNA encoding for NR1-1A and NR2B. The rat NR1 (NR1-1A) and NR2B-GFP cDNA clones were generous gifts of Dr. S. Vicini. For transfections, HEK 293 cells grown to ~70% confluency were incubated with 2 µg of cDNA encoding for desired subunit for 1 h in serum-free DMEM in the presence of the Transfast reagent (Promega,

Paris, France). Cells were then incubated with serum-containing DMEM for 24-48 h prior to imaging. DMEM was supplemented with the NMDA antagonists 2-amino-5-phosphonopentanoic acid (at 100 μ M, racemic mixture; Tocris, UK) to avoid the toxicity associated with NMDA receptor expression.²

Calcium imaging

Transfected HEK cells were loaded for 30-45 min at 37 °C with 10 μ M Fura-2/AM and 0.2% pluronic acid (F-127, Invitrogen, Cergy Pontoise, France) and incubated for an additional 15 min at r.t. in HEPES and Bicarbonate-Buffered Saline Solution (HBBSS) containing (in mM) 116 NaCl, 5.4 KCl, 1.8mM CaCl₂, 0.8 MgSO₄, 1.3 NaH₂PO₄, 12 HEPES, 5.5 glucose, 25 bicarbonates and 10 μ M Glycine at pH = 7.45. Experiments were performed at r.t. with continuous perfusion at 2 ml/min with peristaltic pump, on the stage of a Nikon Eclipse inverted microscope equipped with a 100 W mercury lamp and oil immersion Nikon X40 objective with 1.4 numerical aperture. Fura-2 (excitation: 340/380, emission: 510) ratio images were acquired every 2 seconds with a digital camera (Princeton Instruments, Trenton, New Jersey) and digitized (512/512 pixels) using Metafluor 6.3 (Universal Imaging Corporation, Chester, USA). Fluorescence ratios (340/380 nm) were converted to intracellular Ca²⁺ concentrations using the following formula :

$$[Ca^{2+}]_i = K_d[(R-R_{min})/(R_{max}-R)]F_o/F$$

where R is the ratio for observed 340/380 fluorescence ratio, R_{min} is the ratio for a Ca²⁺-free solution, R_{max} is the ratio for a saturated Ca²⁺ solution, K_d = 135 nM (the dissociation constant for the fura-2), F_o is the intensity of a Ca²⁺-free solution at 380 nM, and F_s is the intensity for a saturated Ca²⁺ solution at 380 nM.³ All stimulation protocols (NMDA at 100 μ M during 30 sec) were separated by a minimum of 5 min to allow the full recovery to the basal level of [Ca²⁺]_i. Because the slow rate of inhibition,⁴ ifenprodil (*erythro* or *threo* prepared as described)⁵ and analogues **1a-12a** were applied 60 sec prior and during NMDA application. The integrated area under the curve (AUC) for changes in [Ca²⁺]_i was calculated on a 2-min period after the beginning of drug application. The two control responses were averaged and used to calculate the percentage of inhibition induced by compound application.

Data analysis

Dose-response curves were analyzed by using the Origin (USA). Data generated from the various experiments were analysed for statistical significance by using ANOVA followed

by a Bonferroni/Dunn test ($p < 0.05$).*, indicates significantly different from NMDA alone; #, indicates significantly different from NMDA + erythro-ifenprodil.

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