

## *Electronic Supplementary Information*

# Dendrimeric Tetravalent Ligands for the Serotonin- Gated Ion Channel

*Marco Paolino,<sup>a</sup> Laura Mennuni,<sup>b</sup> Germano Giuliani,<sup>a</sup> Maurizio Anzini,<sup>a</sup> Marco Lanza,<sup>b</sup>  
Gianfranco Caselli,<sup>b</sup> Chiara Galimberti,<sup>b</sup> Maria Cristina Menziani,<sup>c</sup> Alessandro Donati,<sup>a</sup> Andrea  
Cappelli.<sup>a,\*</sup>*

<sup>a</sup>Dipartimento di Biotecnologie, Chimica e Farmacia and European Research Centre for Drug  
Discovery and Development, Università degli Studi di Siena, Via A. Moro 2, 53100 Siena, Italy

<sup>b</sup>Rottapharm Biotech S.r.l., Via Valosa di Sopra 3, 20900 Monza, Italy

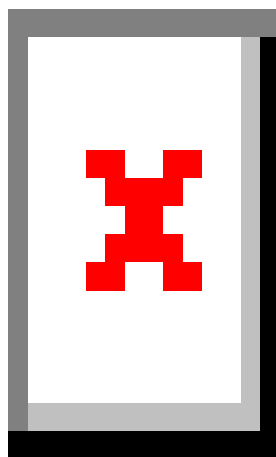
<sup>c</sup>Dipartimento di Scienze Chimiche e Geologiche, Università degli Studi di Modena e Reggio  
Emilia, Via Campi 183, 41100 Modena, Italy

## 1. Chemistry.

### 1.1 Synthesis of the shortly tethered 5-HT<sub>3</sub>R ligands.

The preparation of tetravalent ligand **TETRA-S** was performed through the reaction sequence shown in Scheme 1. The primary amino groups of first generation poly(propylene imine) (PPI) dendrimer **PPI-G1** were allowed to react exhaustively with acyl chloride **1** (ref 1) to obtain dendrimer derivative **2** bearing four 2-chloroquinoline units. This compound was transformed into target tetravalent ligand **TETRA-S** by reaction with *N*-methylpiperazine.

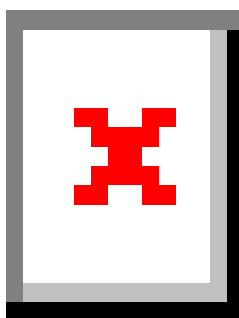
Scheme 1



**Reagents:** (i) TEA, CHCl<sub>3</sub>; (ii) *N*-methylpiperazine.

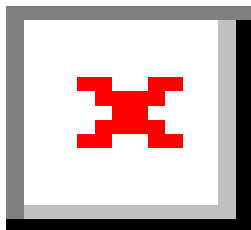
The synthesis of the two reference compounds **DIME-S** and **MONO-S** (Schemes 2 and 3) was performed by applying the same methodology used to obtain the tetravalent compound **TETRA-S**. In particular, the preparation of bivalent ligand **DIME-S** started from *N*<sup>1</sup>-(3-aminopropyl)-*N*<sup>1</sup>-methylpropane-1,3-diamine **3** (Scheme 2) as a model of the pincer system formed by two arms of the PPI dendrimer directly connected with tertiary nitrogen atom. On the other hand, monovalent ligand **MONO-S** was prepared from 3-(dimethylamino)-1-propylamine **5** (Scheme 3) that was considered to represent the model of the dendrimeric arm.

### Scheme 2



**Reagents:** (i) **1**, TEA, CHCl<sub>3</sub>, (ii) *N*-methylpiperazine.

### Scheme 3

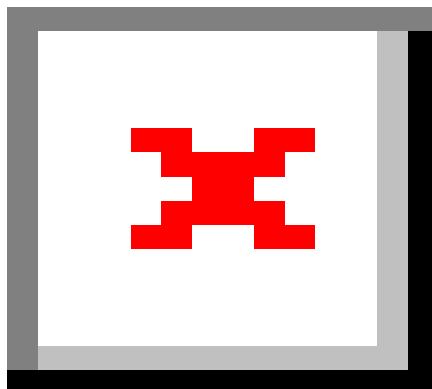


**Reagents:** (i) **1**, TEA, CHCl<sub>3</sub>; (ii) *N*-methylpiperazine.

#### **1.2 Synthesis of 5-HT<sub>3</sub>R ligands bearing long spacers.**

The preparation of tetravalent ligand **TETRA-L** was performed by means of the convergent approach reported in the Scheme 4. The key step in the synthesis was the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction between the propargyl groups of the dendrimer derivative **PPI-G1-Prop** and the azido group of arylpiperazine compound **7** (compound **P1** in the paper).

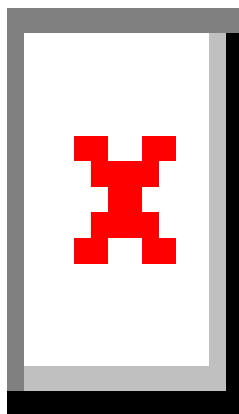
#### Scheme 4



**Reagents:** (i) DIPEA, CuBr, DMF, MW (150 W, 60 °C, 5 x 10 min).

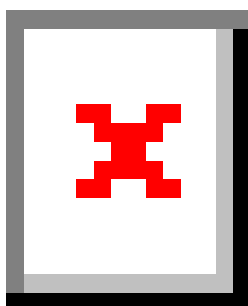
The preparation of the two reference compounds **DIME-L** and **MONO-L** (Schemes 5 and 6) was performed by applying the same CuAAC methodology used to obtain the tetravalent compound **TETRA-L** to synthons **8** and **9**, respectively.

### Scheme 5



**Reagents:** (i) DIPEA, CuBr, THF, MW (150 W, 60 °C, 5 x 10 min).

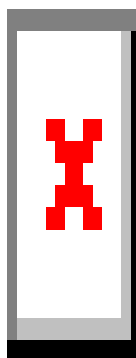
### Scheme 6



**Reagents:** (i) DIPEA, CuBr, THF, MW (150 W, 60 °C, 5 x 10 min).

The required arylpiperazine synthon **7** was prepared starting from acid chloride **1** following the multistep sequence reported in the Scheme 7. The reaction of compound **1** with O-(2-aminoethyl)-O'-(2-azidoethyl)heptaethylene glycol (**10**, commercially available from Aldrich and showing an oligomeric purity  $\geq 90\%$ ) gave the expected amide derivative **11**, which was readily converted into piperazinyl derivate **7**.

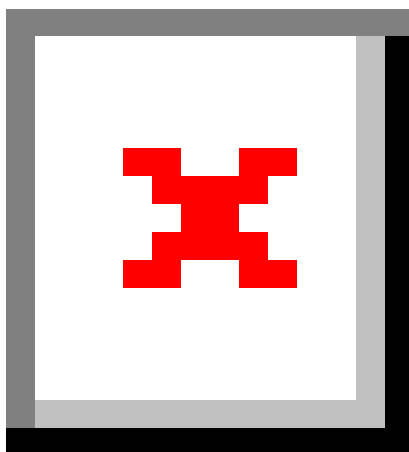
### Scheme 7



**Reagents:** (i) **1**, CH<sub>2</sub>Cl<sub>2</sub>, TEA; (ii) *N*-methylpiperazine.

The preparation of the remaining required synthons (**PPI-G1-Prop**, **8**, **9**) is depicted in Scheme 8. The introduction of the propargyl moiety on the dendrimeric primary amine groups of **PPI-G1** was performed by reaction with carbamate **13**, which was obtained by reaction of propargylamine **12** with bis-*p*-nitrophenyl carbonate. Similarly, the reaction of **13** with primary amine groups of **3** and **5** was used to synthesize simplified scaffolds **8** and **9**, respectively.

## Scheme 8



**Reagents:** (i) bis-*p*-nitrophenyl carbonate, CH<sub>2</sub>Cl<sub>2</sub>; (ii) **13**, THF; (iii) **13**, CH<sub>2</sub>Cl<sub>2</sub>.



## 2. Experimental Procedures

### 2.1 Synthesis.

All chemicals used were of reagent grade. Yields refer to purified products and are not optimized. Melting points were determined in open capillaries on a Gallenkamp apparatus and are uncorrected. Merck silica gel 60 (230-400 mesh) or aluminium oxide 90 active neutral (70-230 mesh) were used for column chromatography. Merck TLC plates, silica gel 60 F<sub>254</sub> were used for TLC. NMR spectra were recorded with a Bruker AC 200, a Bruker DRX-400 AVANCE, or a Bruker DRX-600 AVANCE spectrometer in the indicated solvents (TMS as internal standard): the values of the chemical shifts are expressed in ppm and the coupling constants (*J*) in Hz. The purity of compounds **TETRA-S**, **DIME-S**, **MONO-S**, **TETRA-L**, **DIME-L**, **MONO-L** and **7** was assessed by RP-HPLC and was found to be higher than 95%. An Agilent 1100 Series system equipped with a Zorbax Eclipse XDB-C8 (4.6 x 150 mm) column was used in the HPLC analysis. UV detection was achieved at 254 nm.

Mass spectra were recorded on an Agilent 1100 LC/MSD operating with an electrospray source. High-resolution mass spectrometry (HRMS) measurements were carried out on a Thermo LTQ Orbitrap instrument. Operating conditions for the ESI source were as follows: spray voltage + 4.2 kV; capillary temperature 275 °C; sheath gas (nitrogen) flow rate, ca. 0.75 L/min. Methanolic solutions of the different compounds (ca. 1 x 10<sup>-4</sup> M) have been introduced via direct infusion at a flow rate of 5 µL/min.

*N,N',N'',N'''*-[3,3',3'',3''']-[Butane-1,4-diylbis(azanetriyl)]tetrakis(propane-3,1-diyl)]tetrakis(2-chloro-3-methylquinoline-4-carboxamide) (**2**).

A mixture of 2-chloro-3-methyl-4-quinolinecarboxylic acid chloride **1** (ref 1) (0.47 g, 1.96 mmol) in chloroform (20 mL) with first generation poly(propyleneimine) dendrimer **PPI-G1** (0.10 g, 0.32 mmol) and triethylamine (TEA, 0.36 mL, 2.6 mmol) was stirred at room temperature for 48 h. The reaction mixture was then partitioned between chloroform and a saturated solution of NaHCO<sub>3</sub> in

water and the organic layer was dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by flash chromatography with ethyl acetate-TEA (9:1) as the eluent to give compound **2** as a white solid (0.22 g, yield 61%, mp 211-212 °C). <sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>): 1.09 (br s, 4H), 1.67 (br s, 8H), 2.21 (br s, 4H), 2.28 (s, 12H), 2.40 (br s, 8H), 3.45 (br q, *J* = 5.9, 8H), 7.23 (br t, 4H), 7.37-7.66 (m, 12H), 7.80 (d, *J* = 8.2, 4H). MS(ESI): *m/z* 1131 (M + H<sup>+</sup>).

***N,N',N'',N'''*-[3,3',3'',3''']-[Butane-1,4-diylbis(azanetriyl)]tetrakis(propane-3,1-diyl)]tetrakis[3-methyl-2-(4-methylpiperazin-1-yl)quinoline-4-carboxamide] (TETRA-S).**

A mixture of dendrimer derivative **2** (0.15 g, 0.13 mmol) in *N*-methylpiperazine (5.0 mL) was heated at 130-140 °C under argon for 4 h. After cooling to room temperature, the reaction mixture was partitioned between chloroform and brine made basic with 1M NaOH (basic brine). The aqueous layer was extracted with chloroform and the combined extracts were dried over sodium sulfate and concentrated under reduced pressure. Purification of the residue by chromatography with aluminium oxide as the stationary phase and ethyl acetate-TEA-methanol (7:2:1) as the eluent gave compound **TETRA-S** as a white solid (0.12 g, yield 67%, mp 161-162 °C). HPLC: acetonitrile-methanol-(1% acetic acid-water) (4:3:3), flux 0.5 mL/min, retention time = 1.14 min, purity = 98%. <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>): 1.37 (br s, 4H), 1.67 (m, 8H), 2.19 (s, 12H), 2.21 (s, 12H), 2.37 (br s, 4H), 2.47 (br m, 24H), 3.12 (br s, 16H), 3.34 (br m, 8H), 7.32 (t, *J* = 7.5, 4H), 7.52 (m, 8H), 7.71 (d, *J* = 8.3, 4H), 8.58 (t, *J* = 5.4, 4H). <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD): 1.47 (br s, 4H), 1.82 (m, 8H), 2.27 (s, 12H), 2.32 (s, 12H), 2.47 (br s, 4H), 2.59 (m, 24H), 3.23 (br s, 16H), 3.50 (t, *J* = 6.7, 8H), 7.33 (t, *J* = 7.5, 4H), 7.56 (m, 8H), 7.79 (d, *J* = 8.2, 4H). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>): 15.8, 25.1, 27.3, 37.8, 46.3, 49.8, 51.9, 54.0, 55.1, 120.9, 122.3, 124.9, 127.8, 129.1, 145.4, 161.1, 167.2. HRMS (ESI): *m/z* calculated for [C<sub>80</sub>H<sub>108</sub>N<sub>18</sub>O<sub>4</sub> + H<sup>+</sup>] requires 1385.8874, found 1385.8882.

***N,N'*-[3,3'-(Methylazanediyl)bis(propane-3,1-diyl)]bis(2-chloro-3-methylquinoline-4-carboxamide) (4).**

A mixture of 2-chloro-3-methyl-4-quinolinecarboxylic acid chloride **1** (ref 1) (0.40 g, 1.67 mmol) in chloroform (20 mL) containing *N*<sup>1</sup>-(3-aminopropyl)-*N*<sup>1</sup>-methylpropane-1,3-diamine (**3**, 0.12 g, 0.83 mmol) and TEA (0.46 mL, 3.3 mmol) was stirred at room temperature for 48 h. The reaction mixture was then partitioned between chloroform and a saturated solution of NaHCO<sub>3</sub> in water and the organic layer was dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by flash chromatography with ethyl acetate-TEA (9:1) as the eluent to give compound **4** as a colorless oil (0.45 g, yield 98%). <sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>): 1.92 (m, 4H), 2.34 (s, 6H), 2.39 (s, 3H), 2.77 (br s, 4H), 3.54 (q, *J* = 6.2, 4H), 7.34 (br t, 2H), 7.40-7.61 (m, 6H), 7.82 (d, *J* = 8.0, 2H). MS(ESI): *m/z* 552 (M + H<sup>+</sup>).

***N,N'*-(3,3'-(Methylazanediyl)bis(propane-3,1-diyl))bis(3-methyl-2-(4-methylpiperazin-1-yl)quinoline-4-carboxamide) (DIME-S).**

A mixture of compound **4** (0.38 g, 0.69 mmol) in *N*-methylpiperazine (5.0 mL) was heated at 130-140 °C under argon for 4 h. After cooling to room temperature, the reaction mixture was partitioned between chloroform and basic brine. The aqueous layer was extracted with chloroform and the combined extracts were dried over sodium sulfate and concentrated under reduced pressure. Purification of the residue by chromatography with aluminium oxide as the stationary phase and ethyl acetate-methanol (9:1) as the eluent gave compound **DIME-S** as a pale yellow solid (0.30 g, yield 64%, mp 106-107 °C). HPLC: acetonitrile-methanol-(1% acetic acid-water) (4:3:3), flux 0.5 mL/min, retention time = 1.25 min, purity = 99.6%. <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>): 1.70 (m, 4H), 2.14 (s, 3H), 2.20 (s, 6H), 2.22 (s, 6H), 2.37 (t, *J* = 6.9, 4H), 2.46 (m, 8H), 3.13 (br s, 8H), 3.31 (overlap H<sub>2</sub>O, 4H), 7.34 (t, *J* = 7.5, 2H), 7.53 (m, 4H), 7.71 (d, *J* = 8.2, 2H), 8.59 (t, *J* = 5.6, 2H). <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD): 1.86 (m, 4H), 2.29 (s, 3H), 2.31 (s, 12H), 2.57 (t, *J* = 7.3, 4H), 2.63 (br s, 8H), 3.27 (br s, 8H), 3.51 (t, *J* = 6.7, 4H), 7.36 (t, *J* = 7.6, 2H), 7.56 (t, *J* = 7.8, 2H), 7.60 (d, *J*

= 8.2, 2H), 7.79 (d,  $J = 8.3$ , 2H).  $^{13}\text{C}$ -NMR (100 MHz, DMSO- $d_6$ ): 15.8, 27.3, 37.5, 42.3, 46.3, 49.8, 55.1, 55.4, 120.9, 122.3, 124.9, 127.8, 129.1, 145.4, 161.1, 167.2. HRMS (ESI):  $m/z$  calculated for  $[\text{C}_{39}\text{H}_{53}\text{N}_9\text{O}_2 + \text{H}^+]$  requires 680.4395, found 680.4400.

### **2-Chloro-*N*-[3-(dimethylamino)propyl]-3-methylquinoline-4-carboxamide (6).**

A mixture of 2-chloro-3-methyl-4-quinolinecarboxylic acid chloride **1** (ref 1) (0.20 g, 0.83 mmol) in chloroform (20 mL) with  $N^1,N^1$ -dimethyl-1,3-propanediamine (**5**, 0.085 g, 0.83 mmol) and TEA (0.23 mL, 1.65 mmol) was stirred at room temperature for 24 h. The reaction mixture was then partitioned between chloroform and a saturated solution of  $\text{NaHCO}_3$ . The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by flash chromatography with ethyl acetate-TEA (9:1) as the eluent to give compound **6** as a white solid (0.25 g, yield 98%, mp 73-74 °C).  $^1\text{H}$ -NMR (200 MHz,  $\text{CDCl}_3$ ): 1.86 (m, 2H), 2.20 (s, 6H), 2.49 (s, 3H), 2.55 (t,  $J = 6.2$ , 2H), 3.68 (q,  $J = 5.9$ , 2H), 7.53 (t,  $J = 7.5$ , 1H), 7.67 (t,  $J = 7.7$ , 1H), 7.76 (d,  $J = 8.7$ , 1H), 7.78 (br t, 1H), 7.96 (d,  $J = 8.4$ , 1H). MS(ESI):  $m/z$  306 ( $\text{M} + \text{H}^+$ ).

### ***N*-[3-(Dimethylamino)propyl]-3-methyl-2-(4-methylpiperazin-1-yl)quinoline-4-carboxamide (MONO-S).**

A mixture of 2-chloroquinoline derivative **6** (0.20 g, 0.654 mmol) in *N*-methylpiperazine (5.0 mL) was heated at 130-140 °C under argon for 4 h. After cooling to room temperature, the reaction mixture was partitioned between chloroform and basic brine. The aqueous layer was extracted with chloroform and the combined extracts were dried over sodium sulfate and concentrated under reduced pressure. Purification of the residue by chromatography with aluminium oxide as the stationary phase and ethyl acetate-methanol (9:1) as the eluent gave compound **MONO-S** as a pale yellow oil, which crystallized on standing (0.15 g, yield 63%). HPLC: acetonitrile-methanol-(1% acetic acid-water) (4:3:3), flux 0.5 mL/min, retention time = 1.33 min, purity = 99.7%.  $^1\text{H}$ -NMR (400 MHz, DMSO- $d_6$ ): 1.65 (m, 2H), 2.10 (s, 6H), 2.22 (s, 3H), 2.25 (m, 5H), 2.47 (m, 4H), 3.17

(br s, 4H), 3.32 (overlap H<sub>2</sub>O, 2H), 7.36 (t,  $J = 7.5$ , 1H), 7.54 (m, 2H), 7.72 (d,  $J = 8.3$ , 1H), 8.58 (t,  $J = 5.6$ , 1H). <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD): 1.85 (m, 2H), 2.25 (s, 6H), 2.36 (s, 3H), 2.38 (s, 3H), 2.45 (m, 2H), 2.66 (br s, 4H), 3.32 (br s, 4H), 3.50 (t,  $J = 6.9$ , 2H), 7.40 (m, 1H), 7.56-7.63 (m, 2H), 7.81 (d,  $J = 8.3$ , 1H). <sup>13</sup>C-NMR (100 MHz, DMSO-d<sub>6</sub>): 15.8, 27.2, 37.4, 45.4, 46.3, 49.8, 55.1, 57.0, 121.0, 122.3, 125.0, 127.8, 129.2, 145.4, 161.1, 167.2. HRMS (ESI):  $m/z$  calculated for [C<sub>21</sub>H<sub>31</sub>N<sub>5</sub>O + H<sup>+</sup>] requires 370.2601, found 370.2605.

***N*-(26-Azido-3,6,9,12,15,18,21,24-octaoxahehexacosyl)-2-chloro-3-methylquinoline-4-carboxamide (11).**

A mixture of acid chloride **1** (ref 1) (0.28 g, 1.17 mmol) in dichloromethane (14 mL) and TEA (5.0 mL) with *O*-(2-aminoethyl)-*O'*-(2-azidoethyl)heptaethylene glycol (**10**, commercially available from Aldrich and showing oligomeric purity  $\geq 90\%$ , 0.50 g, 1.14 mmol) was stirred at room temperature for 30 min and then concentrated under reduced pressure. The residue was purified by flash chromatography with ethyl acetate-TEA (9:1) as the eluent to give pure compound **11** as a colorless oil (0.73 g, yield 97%). <sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>): 2.50 (s, 3H), 3.33-3.79 (m, 36H), 7.03 (br s, 1H), 7.53 (t,  $J = 8.1$ , 1H), 7.65 (t,  $J = 7.9$ , 1H), 7.79 (d,  $J = 8.0$ , 1H), 7.95 (d,  $J = 8.4$ , 1H). MS(ESI):  $m/z$  664 (M + Na<sup>+</sup>).

***N*-(26-Azido-3,6,9,12,15,18,21,24-octaoxahehexacosyl)-3-methyl-2-(4-methylpiperazin-1-yl)quinoline-4-carboxamide (7).**

A mixture of the 2-chloroquinoline derivative **11** (0.11 g, 0.17 mmol) in *N*-methylpiperazine (3.0 mL) was heated at 130-140 °C under argon for 7 h. After cooling to room temperature, the reaction mixture was partitioned between chloroform and basic brine. The aqueous layer was extracted with chloroform and the combined extracts were dried over sodium sulfate and concentrated under reduced pressure. Purification of the residue by flash chromatography with ethyl acetate-TEA (8:2) as the eluent gave compound **7** as a pale yellow oil (0.079 g, resa 66%). HPLC: acetonitrile-

methanol-(0.1% formic acid-water) (2:3:5), flux 1.0 mL/min, retention time = 3.36 min, purity = 97.7%. <sup>1</sup>H-NMR (600 MHz, CDCl<sub>3</sub>): 2.38 (s, 3H), 2.39 (s, 3H), 2.63 (br s, 4H), 3.34 (br s, 4H), 3.36 (m, 2H), 3.39-3.66 (m, 30H), 3.74 (m, 4H), 6.74 (br s, 1H), 7.34 (t, *J* = 7.6, 1H), 7.54 (t, *J* = 7.6, 1H), 7.67 (d, *J* = 8.2, 1H), 7.82 (d, *J* = 8.4, 1H). <sup>13</sup>C-NMR (150 MHz, CDCl<sub>3</sub>): 15.8, 39.5, 46.2, 49.5, 50.7, 55.1, 69.7, 70.0, 70.5, 121.2, 121.9, 124.1, 124.8, 127.9, 128.8, 144.1, 145.8, 160.8, 168.1. HRMS (ESI): *m/z* calculated for [C<sub>34</sub>H<sub>55</sub>N<sub>7</sub>O<sub>9</sub> + H<sup>+</sup>] requires 706.4134, found 706.4140.

#### **4-Nitrophenyl prop-2-ynylcarbamate (13).**

A mixture of propargylamine **12** (0.66 mL, 10.3 mmol) in dichloromethane (20 mL) containing bis(4-nitrophenyl)carbonate (3.13 g, 10.3 mmol) was stirred at room temperature for 24 h. The reaction mixture was then concentrated under reduced pressure to give a bright yellow solid, which was purified by washing with diethyl ether to provide carbamate **13** as a yellow needle-like solid (1.2 g, yield 53%, mp 121-122 °C, literature<sup>2</sup>: 123-125 °C). <sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>): 2.31 (t, *J* = 2.5, 1H), 4.08 (dd, *J* = 2.5, 8.0, 2H), 5.31 (br t, 1H), 7.32 (d, *J* = 9.2, 2H), 8.24 (d, *J* = 9.0, 2H).

#### **1,1',1'',1'''-[3,3',3'',3''']-[Butane-1,4-diylbis(azanetriyl)]tetrakis(propane-3,1-diyl)tetrakis[3-(prop-2-ynyl)urea] (PPI-G1-Prop).**

A mixture of DAB-Am-4, poly(propylenimine) dendrimer, generation 1, **PPI-G1** (0.072 g, 0.227 mmol) in THF (20 mL) containing carbamate **13** (0.20 g, 0.908 mmol) was stirred at room temperature for 24 h. The precipitate was collected by filtration and purified by washing with THF to give pure compound **PPI-G1-Prop** as a pale yellow solid (0.12 g, yield 82%, mp 156-157 °C). <sup>1</sup>H-NMR (600 MHz, DMSO-*d*<sub>6</sub>): 1.32 (br s, 4H), 1.46 (br m, 8H), 2.30 (br m, 12H), 2.99 (br m, 12H), 3.76 (br s, 8H), 6.01 (br s, 4H), 6.17 (br s, 4H). <sup>13</sup>C-NMR (150 MHz, DMSO-*d*<sub>6</sub>): 25.2, 28.4, 29.8, 38.7, 51.9, 54.2, 73.3, 83.5, 158.6. HRMS (ESI): *m/z* calculated for [C<sub>32</sub>H<sub>52</sub>N<sub>10</sub>O<sub>4</sub> + H<sup>+</sup>] requires 641.4246, found 641.4249.

### **1,1'-[3,3'-(Methylazanediyl)bis(propane-3,1-diyl)]bis[3-(prop-2-ynyl)urea] (8).**

A mixture of *N*<sup>1</sup>-(3-aminopropyl)-*N*<sup>1</sup>-methyl-1,3-propanediamine (0.15 g, 1.03 mmol) in chloroform (20 mL) with carbamate **13** (0.46 g, 2.09 mmol) was stirred at room temperature for 24 h. The reaction mixture was then concentrated under reduced pressure and the residue was purified by washing with diethyl ether to give compound **8** as a pale yellow solid (0.25 g, yield 79%, 125-126 °C). <sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>): 1.65 (m, 4H), 2.16-2.20 (m, 5H), 2.42 (t, *J* = 5.9, 4H), 3.27 (q, *J* = 5.9, 4H), 3.96 (dd, *J* = 2.3, 7.8, 4H), 5.71 (br t, 2H), 6.01 (br t, 2H). MS( EI): *m/z* 308 (M + H<sup>+</sup>).

### **1-[3-(Dimethylamino)propyl]-3-(prop-2-ynyl)urea (9).**

A mixture of *N*<sup>1</sup>,*N*<sup>1</sup>-dimethyl-1,3-propanediamine (0.15 g, 1.47 mmol) in chloroform (20 mL) with carbamate **13** (0.32 g, 1.45 mmol) was stirred at room temperature for 24 h. The reaction mixture was then concentrated under reduced pressure and the residue was purified by flash chromatography with ethyl acetate-methanol (9:1) as the eluent to give compound **9** as a colourless oil (0.25 g, yield 94%). <sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>): 1.61 (m, 2H), 2.18 (m, 7H), 2.33 (t, *J* = 6.3, 2H), 3.20 (q, *J* = 7.6, 2H), 3.93 (dd, *J* = 2.4, 7.6, 2H), 5.30 (br s, 1H), 6.17 (br s, 1H). MS(EI): *m/z* 184 (M + H<sup>+</sup>).

### **Tetravalent ligand TETRA-L.**

In a microwave tube, a mixture of the dendrimer **PPI-G1-Prop** (24 mg, 0.037 mmol) in DMF (5.0 mL) containing azide **7** (110 mg, 0.156 mmol), CuBr (3.0 mg, 0.021 mmol) and DIPEA (4.0 μL, 0.023 mmol) was exposed to microwave irradiation into a CEM Discover instrument for 50 min (5 x 10 min, T = 60 °C, W = 150). The reaction mixture was then concentrated under reduced pressure and the resulting oily residue was dissolved into a mixture of water-33% NH<sub>4</sub>OH (1:1). The aqueous solution was extracted with chloroform and the organic layer was dried over sodium sulfate and concentrated under reduced pressure. The yellow oily residue was purified by washing with

ethyl acetate to obtain pure tetravalent compound **TETRA-L** as a yellow oil (74 mg, yield 58%). HPLC: methanol-(0.1% formic acid-water) (4:6), flux 1.0 mL/min, retention time = 1.51 min, purity = 96.6%. <sup>1</sup>H-NMR (600 MHz, DMSO-d<sub>6</sub>): 1.31 (bs s, 4H), 1.45 (br s, 8H), 2.25 (s, 12H), 2.29 (s, 24H), 2.53 (br s, 16H), 2.99 (br m, 8H), 3.20 (br s, 16H), 3.43-3.60 (m, 128H), 3.77 (br t, 8H), 4.20 (d, *J* = 5.3, 8H), 4.46 (br t, 8H), 5.98 (br s, 4H), 6.27 (br s, 4H), 7.38 (t, *J* = 7.7, 4H), 7.58 (m, 4H), 7.62 (d, *J* = 8.1, 4H), 7.75 (d, *J* = 8.4, 4H), 7.83 (s, 4H), 8.66 (t, *J* = 5.6, 4H). <sup>13</sup>C-NMR (150 MHz, DMSO-d<sub>6</sub>): 16.2, 25.1, 28.5, 35.8, 38.6, 39.6, 46.7, 50.1, 50.3, 51.9, 54.1, 55.6, 69.7, 70.5, 70.7, 121.5, 122.7, 123.7, 125.5, 125.6, 128.2, 129.6, 145.9, 146.7, 158.9, 161.6, 167.9. HRMS (ESI): *m/z* calculated for [C<sub>168</sub>H<sub>272</sub>N<sub>38</sub>O<sub>40</sub> + 2H<sup>+</sup>] requires 1732.0282, found 1732.0283.

### **Bivalent ligand DIME-L.**

In a microwave tube, a mixture of **8** (32 mg, 0.104 mmol) in THF (5.0 mL) containing azide **7** (150 mg, 0.213 mmol), CuBr (7.0 g, 0.049 mmol) and DIPEA (9.0 μL, 0.052 mmol) was exposed to microwave irradiation into a CEM Discover instrument for 40 min (4 x 10 min, T = 60 °C, W = 150). The reaction mixture was then concentrated under reduced pressure and the resulting oily residue was dissolved into a mixture of water-33% NH<sub>4</sub>OH (1:1). The aqueous solution was extracted with chloroform and the organic layer was dried over sodium sulfate and concentrated under reduced pressure. The yellow oily residue was purified by chromatography with aluminium oxide as stationary phase and ethyl acetate-methanol (9:1) as the eluent to obtain bivalent ligand **DIME-L** as a yellow oil (75 mg, yield 42%). HPLC: acetonitrile-methanol-(0.1% formic acid-water) (2:3:5), flux 1.0 mL/min, retention time = 1.95 min, purity = 97.5%. <sup>1</sup>H-NMR (600 MHz, DMSO-d<sub>6</sub>): 1.51 (m, 4H), 2.16 (br s, 3H), 2.26 (s, 6H), 2.29 (s, 6H), 2.35 (m, 4H), 2.53 (br s, 8H), 3.02 (q, *J* = 6.4, 4H), 3.20 (br s, 8H), 3.44-3.61 (m, 64H), 3.78 (t, *J* = 5.3, 4H), 4.20 (d, *J* = 5.7, 4H), 4.47 (t, *J* = 5.3, 4H), 6.02 (br s, 2H), 6.32 (br s, 2H), 7.38 (m, 2H), 7.58 (m, 2H), 7.62 (d, *J* = 8.2, 2H), 7.75 (d, *J* = 8.3, 2H), 7.83 (s, 2H), 8.66 (t, *J* = 5.5, 2H). <sup>13</sup>C-NMR (150 MHz, DMSO-d<sub>6</sub>):



16.2, 28.1, 35.9, 38.3, 39.6, 42.2, 46.7, 50.2, 55.3, 55.5, 69.7, 70.7, 121.5, 122.8, 123.7, 125.4, 125.6, 128.2, 129.6, 145.9, 146.8, 158.9, 161.6, 167.9. HRMS (ESI):  $m/z$  calculated for  $[C_{83}H_{135}N_{19}O_{20} + H^+]$  requires 1719.0204, found 1719.0211.

***N*-[26-[4-[[3-[3-(dimethylamino)propyl]ureido]methyl]-1*H*-1,2,3-triazol-1-yl]-3,6,9,12,15,18,21,24-octaoxahexacosyl]-3-methyl-2-(4-methylpiperazin-1-yl)quinoline-4-carboxamide (MONO-L).**

In a microwave tube, a mixture of **9** (26 mg, 0.14 mmol) in THF (5.0 mL) containing azide **7** (100 mg, 0.14 mmol), CuBr (10 mg, 0.070 mmol), and DIPEA (12  $\mu$ L, 0.069 mmol) was exposed to microwave irradiation into a CEM Discover instrument for 40 min (4 x 10 min, T = 60 °C, W = 150). The reaction mixture was then concentrated under reduced pressure and the resulting oily residue was dissolved in a mixture of water-33% NH<sub>4</sub>OH (1:1). The aqueous solution was extracted with chloroform and the organic layer was dried over sodium sulfate and concentrated under reduced pressure. The yellow oily residue was purified by chromatography with aluminium oxide as the stationary phase and ethyl acetate-TEA-methanol (7:2:1) as the eluent to obtain monovalent ligand **MONO-L** as a yellow oil (88 mg, yield 71%). HPLC: acetonitrile-methanol-(0.1% formic acid-water) (2:3:5), flux 1.0 mL/min, retention time = 1.57 min, purity = 97.8%. <sup>1</sup>H-NMR (600 MHz, DMSO-*d*<sub>6</sub>): 1.51 (m, 2H), 2.17 (s, 6H), 2.27 (m, 5H), 2.31 (s, 3H), 2.54 (br s, 4H), 3.02 (q,  $J$  = 6.6, 2H), 3.22 (br s, 4H), 3.44-3.68 (m, 32H), 3.80 (t,  $J$  = 5.3, 2H), 4.22 (d,  $J$  = 5.7, 2H), 4.49 (t,  $J$  = 5.3, 2H), 5.96 (t,  $J$  = 5.6, 1H), 6.30 (br s, 1H), 7.39 (m, 1H), 7.59 (m, 1H), 7.63 (d,  $J$  = 8.2, 1H), 7.76 (d,  $J$  = 8.2, 1H), 7.85 (s, 1H), 8.67 (t,  $J$  = 5.6, 1H). <sup>13</sup>C-NMR (150 MHz, DMSO-*d*<sub>6</sub>): 16.2, 28.6, 35.9, 38.4, 39.6, 45.8, 46.7, 50.1, 50.2, 55.6, 57.4, 69.7, 70.7, 121.5, 122.8, 123.7, 125.4, 125.6, 128.2, 129.6, 145.9, 146.8, 158.9, 161.6, 167.9. HRMS (ESI):  $m/z$  calculated for  $[C_{43}H_{72}N_{10}O_{10} + H^+]$  requires 889.5506, found 889.5517.

## 2.2 *In Vitro* Binding Assays.

Male Wistar rats (Harlan, Italy) weighing 275-300 g were used. Animal care and handling throughout the experimental procedures were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). Animals were sacrificed by decapitation, brains were rapidly removed, cerebral cortical tissues and hippocampi dissected and used for binding assay preparation according to Nelson *al.* ref 3, with slight modification. The cerebral membrane preparation was finally suspended in Hepes buffer 50 mM, pH 7.4, just before the binding assay was performed, in order to obtain 0.5 mg protein/sample. [<sup>3</sup>H]-BRL43694 (Granisetron; s.a. 81 Ci/mmol; PerkinElmer Life Science Products) binding, at the concentration corresponding to  $K_d$  value, was assayed in polystyrene multiwell plate (24 well) for 30 min at 25 °C in final incubation volumes of 1.0 mL. The specific binding of the tritiated ligand was defined as the difference between the binding in the absence (total binding) and in the presence of 30  $\mu$ M unlabelled 5-HT (non-specific binding). It represented in an average 70% of the total binding.

Incubation was stopped by rapid filtration under vacuum through Unifilter GF/B glass fiber filters plate, presoaked in Hepes buffer 50 mM, pH 7.4, containing 0.1% polyethyleneimine by means of a cell harvester. Filters were immediately rinsed three times with cold buffer, dried for two hours at room temperature, then 0.2 mL of Micriscint50 (PerkinElmer) were added and after at least two hours stabilization period, radioactivity was determined. Competition experiments were analyzed by non linear regression fitting using GraphPhad software (version 6 for Windows), in order to obtain the  $IC_{50}$  value (the concentration of unlabelled drug that caused 50% inhibition of specific binding). Apparent affinity constants ( $K_i$ ) were derived from the  $IC_{50}$  values according to the Cheng and Prusoff equation.<sup>4</sup>

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