Supporting Information

Synthesis of Specific Bivalent Probes that functionally interact with 5-HT$_4$ Receptor Dimers

Olivier Russo,[†, ♠,⌘] Magali Berthouze, [‡, ♠,⌘], Mireille Giner,[†, ♠] Jean-Louis Soulier,[†, ♠] Lucie Rivail,[†, ♠] Sames Sicsic,[†, ♠], Frank Lezoualc'h,[‡, ♠] Ralf Jockers,[1-2] Isabelle Berque-Bestel[†, ♠, *]

[†] CNRS UMR C 8076 (BioCIS), Molécules Fluorées et Chimie Médicinale;

[‡] INSERM UMR S769, Signalisation et Physiopathologie Cardiaque;

[♠] IFR-141, Faculté de Pharmacie, Université Paris XI, 5, rue Jean-Baptiste Clément, 92296 Châtenay-Malabry Cedex, France.

[1] Institut Cochin, Université Paris Descartes, CNRS (UMR 8104), Department of Cell Biology, Paris, France.

[2] Inserm, U567, Paris, France.

[⌘] Participate equally to this work.

[*] to whom correspondence should be addressed: Isabelle Berque-Bestel, INSERM U869, Univ Victor Segalen Bordeaux 2, Bordeaux, F-33076, France.
Phone: +33 1 57 57 09 45, Fax: +33 1 57 57 10 15
isabelle.bestel@bordeaux.inserm.fr

Contents Supporting Information

Chemistry experimental (including spectroscopic analyses)
Table of elemental analyses

Chemistry experimental

4-(*tert*-butoxycarbonyl(prop-2-ynyl)amino)butanoic acid (2b).

Same procedure as described for **2a**, using **1b**. 77% yield, pale yellow solid. Rf (cHex/AcOEt 7/3 + 0.5% AcOH) = 0.33. ^{1}H NMR (200 MHz) δ 9.92 (bs, 1H), 4.01 (bs, 2H), 3.38 (t, *J* = 7.0 Hz, 2H), 2.37 (t, *J* = 7.0 Hz, 2H), 2.19 (t, *J* = 2.4 Hz, 1H), 1.89 (m, *J* = 7.0 Hz, 2H), 1.45 (s, 9H). ^{13}C NMR (50 MHz) δ 178.4, 155.0, 80.5, 79.4, 71.4, 45.6, 36.1, 31.0, 28.1, 22.9. F = 86°C

4,4'-(3,3'-(1,4-phenylene)bis(prop-2-yne-3,1-diyl))bis(*tert*-butoxycarbonylazanediyl) dibutanoic acid (3b).

Same procedure as described for **3a**, using **2b**. 62% yield, amber oil. Rf (cHex/AcOEt 6/4 + 0.5% AcOH) = 0.20. ^{1}H NMR (200 MHz) δ 9,68 (bs, 2H), 7.31 (s, 4H), 4.24 (bs, 4H), 3.43 (t, *J* = 7.0 Hz, 4H), 2.39 (t, *J* = 7.0 Hz, 4H), 1.94 (m, 4H), 1.46 (s, 18H). ^{13}C NMR (50 MHz) δ 178.8, 155.1, 131.4, 122.6, 86.7, 82.8, 80.5, 45.8, 36.9, 31.0, 28.2, 23.0.

4,4'-(3,3'-(1,3-phenylene)bis(prop-2-yne-3,1-diyl))bis(*tert*-butoxycarbonylazanediyl) dibutanoic acid (4).

Same procedure as described for **3a**, using **2b** and 1,3-diiodobenzene. 46% yield, amber oil. Rf (cHex/AcOEt 6/4 + 0.5% AcOH) = 0.18. ^{1}H NMR (200 MHz) δ 9.56 (bs, 2H), 7.37 (s, 1H), 7.26-7.08 (m, 3H), 4.29 (bs, 4H), 3.47 (t, *J* = 7.0 Hz, 4H), 2.45 (t, *J* = 7.0 Hz, 4H), 2.00 (m, 4H), 1.51 (s, 18H). ^{13}C NMR (50 MHz) δ 178.8, 155.1, 134.7, 131.3, 128.2, 123.0, 85.6, 82.4, 80.4, 45.8, 36.9, 31.1, 28.2, 20.6.

4,4'-(3,3'-(1,4-phenylene)bis(propane-3,1-diyl))bis(*tert*-butoxycarbonylazanediyl) dibutanoic acid (5b).

Same procedure as described for **5a**, using **3b**. 97% yield, yellow oil. ^{1}H NMR (200 MHz) δ 7.48 (bs, 2H), 7.08 (s, 4H), 3.21 (m, 8H), 2.56 (m, 4H), 2.32 (m, 4H), 1.82 (m, 8H), 1.43 (s, 18H). ^{13}C NMR (50 MHz) δ 178.3, 155.9, 139.2, 128.4, 79.8, 47.2, 46.5, 32.9, 31.4, 30.1, 28.6, 23.8.

4,4'-(3,3'-(1,3-phenylene)bis(propane-3,1-diyl))bis(*tert*-butoxycarbonylazanediyl) dibutanoic acid (6)

Same procedure as described for **5a**, using **4**. 99% yield, yellow oil. ^{1}H NMR (200 MHz) δ 7.82 (s, 1H), 7.55, 7.33 (m, 3H), 3.75 (m, 8H), 3.10 (m, 4H), 2.79 (m, 4H), 2.37 (m, 8H), 1.98 (s, 18H). ^{13}C NMR (50 MHz) δ 177.7, 155.9, 142.0, 128.5, 128.3, 126.0, 79.5, 47.4, 47.1, 33.3, 32.7, 28.6, 24.6, 24.5.

Dimer 8b

Same procedure as described for **8a**, using **4** and **7a**. 40% yield, white hygroscopic foam. ^{1}H NMR (200 MHz) δ 7.83 (s, 2H), 7.46-7.27 (m, 4H), 6.63 (m, 2H), 6.33 (s, 2H), 4.63 (bs, 4H), 4.41(t, *J* = 5.8 Hz, 4H), 4.25 (bs, 4H), 3.85 (m, 8H), 3.46 (t, *J* = 6.2 Hz, 4H), 2.98 (bs, m, 4H), 2.79 (t, *J* = 5.8 Hz, 4H), 2.39-2.19 (m, 8H), 1.97 (m, 8H), 1.52 (m, 22H). ^{13}C NMR (50 MHz) δ 171.7, 169.5, 169.4, 160.1, 147.9, 155.4, 155.4, 133.2, 134.5, 131.3, 128.3, 123.0, 109.8, 98.1, 85.8, 82.2, 80.4, 61.9, 56.6, 55.9, 52.4, 46.0, 45.7, 37.1, 33.6, 31.8, 28.3, 24.2. MS (ESI) m/z = 1175 $[M+H]^+$. Anal. ($C_{60}H_{80}N_8O_{12}Cl_2$, 2 H_2O), C, H, N.

Dimer 8c

Same procedure as described for **8a**, using **3b** and **7a**. 42% yield, white hygroscopic foam. ^{1}H NMR (400 MHz) δ 7.71 (s, 2H), 7.26 (s, 4H), 6.61 (bs, 2H), 6.24 (s, 2H), 4.70 (bs, 4H), 4.30 (t, *J* = 5.8 Hz, 4H), 4.15 (bs, 4H), 3.72 (s, 6H), 3.63 (m, 2H), 3.34 (t, *J* = 6.6 Hz, 4H), 2.91-2.85 (m, 4H), 2.69 (t, *J* = 5.8 Hz, 4H), 2.27-2.10 (m, 8H), 1.89-1.82 (m, 8H), 1.42 (s, 18H), 1.18 (m, 4H). ^{13}C NMR (100 MHz) δ 171.8, 169.5, 164.4, 160.1, 148.2, 155.4, 133.1, 131.4, 122.6, 109.7, 98.1, 87.0, 82.6, 80.4, 61.8, 56.5, 55.8, 52.4, 46.0, 45.7, 37.1, 33.5, 31.7, 28.3, 24.2. MS (ESI) m/z = 1175 $[M+H]^+$. Anal. ($C_{60}H_{80}N_8O_{12}Cl_2$, 5 H_2O), C, H, N.

Dimer 8d

Same procedure as described for **8a**, using **4** and **7b**. 25% yield, white hygroscopic foam. ^{1}H NMR (200 MHz) δ 7.72 (s, 2H), 7.35-7.12 (m, 4H), 6.22 (s, 2H), 4.52 (bs, 4H), 4.30 (t, *J* = 6.0 Hz, 4H), 4.14 (bs, 4H), 3.74 (s, 6H), 3.34 (t, *J* = 6.6 Hz, 4H), 3.05-2.91 (m, 8H), 2.68 (t, *J* = 6.0 Hz, 4H), 2.10 (m, 4H), 1.90 (m, 4H), 1.60 (m, 4H), 1.41-1.21 (m, 28H). ^{13}C NMR (50 MHz) δ 171.7, 169.5, 164.4, 160.2, 147.9, 155.4, 133.2, 134.5, 131.3, 128.4, 123.0, 109.8, 98.2, 85.9, 82.2, 80.4, 61.7, 56.7, 55.9, 53.5, 45.7, 44.9, 37.2, 35.5, 33.4, 29.6, 28.3, 24.2. MS (ESI) m/z = 1204 $[M+H]^+$. Anal. ($C_{62}H_{84}N_8O_{12}Cl_2$, 5 H_2O)C, H, N.

Dimer 8e

Same procedure as described for **8a**, using **3b** and **7b**. 17% yield, white hygroscopic foam. ^{1}H NMR (200 MHz) δ 7.76 (s, 2H), 7.30 (s, 4H), 6.27 (s, 2H), 4.59 (bs, 4H), 4.34 (t, *J* = 5.8 Hz, 4H), 4.19 (bs, 4H), 3.78 (s, 6H), 3.39 (t, *J* = 6.4 Hz, 4H), 3.10 (m, 4H), 2.95 (m, 4H), 2.73 (t, *J* = 5.8 Hz, 4H), 2.10 (m, 4H), 1.90 (m, 4H), 1.65 (m, 4H), 1.45-1.14 (m, 28H). ^{13}C NMR (50 MHz) δ 172.4, 169.5, 164.4, 160.2, 148.0, 155.4, 133.1, 131.4, 122.6, 109.8, 98.1, 87.0, 82.6, 80.4, 61.7, 56.7, 55.9, 53.5, 45.8, 44.9, 37.2, 35.5, 33.4, 29.6, 28.3, 24.2. MS (ESI) m/z = 1204 $[M+H]^+$. Anal. ($C_{62}H_{84}N_8O_{12}Cl_2$, 2.5 H_2O), C, H, N.

Dimer 9a

Same procedure as described for **8a**, using **5a** and **7b**. 37% yield, beige hygroscopic foam. ^{1}H NMR (200 MHz) δ 7.78 (s, 2H), 7.05 (s, 4H), 6.28 (s, 2H), 4.54 (bs, 4H), 4.35 (t, *J* = 5.9 Hz, 4H), 3.79 (m, 10H), 3.27 (m, 4H), 3.12 (m, 4H), 2.95 (m, 4H), 2.73 (t, *J* = 5.9 Hz, 4H), 2.53 (m, 4H), 2.07 (m, 4H), 1.81 (m, 4H), 1.65 (m, 4H), 1.42 (m, 22H), 1.36-1.16 (m, 2H). ^{13}C

NMR (50 MHz) δ170.0, 164.6, 160.4, 155.2, 148.1, 139.0, 133.4, 131.0, 128.9, 128.4, 110.0, 109.7, 98.4, 80.9, 62.03, 57.0, 56.2, 53.6, 52.0, 48.7, 44.9, 35.8, 32.7, 29.8, 28.4. MS (ESI) m/z = 1156 $[M+H]^+$. Anal. ($C_{58}H_{84}Cl_2N_8O_{12}$, 0.5 H_2O), C, H, N.

Dimer 9b

Same procedure as described for **8a**, using **6** and **7a**. 62% yield, yellow hygroscopic foam. ^1H NMR (200 MHz) δ 7.96 (s, 2H), 7.32 (m, 4H), 6.47 (s, 2H), 4.79 (bs, 4H), 4.52 (t, *J* = 5.7 Hz, 4H), 3.98 (m, 8H), 3.39 (m, 8H), 3.10 (m, 4H), 2.91 (t, *J* = 5.7 Hz, 4H), 2.73 (m, 4H), 2.55-2.22 (m, 8H), 2.19-1.87 (m, 8H), 1.84-1.50 (m, 22H), 1.49-1.27 (m, 4H). ^{13}C NMR (50 MHz) δ171.8, 164.4, 160.1, 156.1, 148.0 141.6, 133.1, 128.3, 128.2, 125.7, 109.8, 109.4, 98.1, 79.5, 62.1, 56.6, 55.9, 52.5, 46.8, 46.1, 33.5, 33.1, 30.0, 28.3, 26.5, 24.5. MS (ESI) m/z = 1206 $[M+Na]^+$. Anal. ($C_{60}H_{88}Cl_2N_8O_{12}$, 2 H_2O), C, H, N.

Dimer 9c

Same procedure as described for **8a**, using **5b** and **7a**. 49% yield, white hygroscopic foam. ^1H NMR (200 MHz) δ7.74 (s, 2H), 7.03 (s, 4H), 6.70 (m, 2H), 6.26 (s, 2H), 4.64 (bs, 4H), 4.30 (t, *J* = 5.5 Hz, 4H), 3.76 (m, 8H), 3.17 (m, 8H), 2.87 (m, 4H), 2.68 (t, *J* = 5.5 Hz, 4H), 2.50 (m, 4H), 2.38-2.00 (m, 8H), 1.99-1.62 (m, 12H), 1.39 (m, 22H). ^{13}C NMR (50 MHz) δ172.0, 164.6, 160.3, 156.2, 148.2, 139.1, 133.3, 128.3, 109.9, 109.5, 98.3, 79.6, 62.3, 56.8, 56.1, 52.6, 46.9, 46.3, 45.7, 33.6, 32.8, 32.1, 30.2, 28.5, 24.7. MS (ESI) m/z = 1206 $[M+Na]^+$. Anal. ($C_{60}H_{88}Cl_2N_8O_{12}$, H_2O), C, H, N.

Dimer 9d

Same procedure as described for **8a**, using **6** and **7b**. 54% yield, white hygroscopic foam. ^1H NMR (200 MHz) δ7.84 (s, 2H), 7.16 (m, 4H), 6.35 (s, 2H), 5.89 (m, 2H), 4.68 (bs, 4H), 4.42 (t, *J* = 5.5 Hz, 4H), 3.86 (s, 6H), 3.43-2.90 (m, 16H), 2.80 (t, *J* = 5.5 Hz, 4H), 2.61 (m, 2H), 2.28-2.07 (m, 8H), 1.97-1.65 (m, 8H), 1.63-1.23 (m, 30H). ^{13}C NMR (50 MHz) δ 172.8, 164.6, 160. 4, 156.5, 148.2, 141.8, 133.3, 128.6, 128.4, 125.9, 110.0, 109.5, 98.3, 79.7, 62.0, 57.0, 56.1, 53.7, 47.0, 45.1, 35.7, 33.6, 33.3, 29.9, 28.5, 26.7, 24.7. MS (ESI) m/z = 1212 $[M+H]^+$. Anal. ($C_{62}H_{92}Cl_2N_8O_{12}$, 3 H_2O), C, H, N.

Dimer 9e

Same procedure as described for **8a**, using **5b** and **7b**. 48% yield, white hygroscopic foam. ^1H NMR (200 MHz) δ7.72 (s, 2H), 6.96 (s, 4H), 6.25 (s, 2H), 4.67 (bs, 4H), 4.30 (t, *J* = 5.8 Hz, 4H), 3.75 (s, 6H), 3.11 (m, 12H), 2.94 (m, 4H), 2.68 (t, *J* = 5.8 Hz, 4H), 2.50 (m, 2H), 2.25-1.95 (m, 8H), 1.87-1.55 (m, 8H), 1.54-1.12 (m, 30H). ^{13}C NMR (50 MHz) δ 172.7, 164.6, 160.3, 156.4, 1483, 141.8, 133.2, 125.8, 109.8, 109.3, 98.3, 79. 6, 62.0, 56.9, 56.0, 53.7, 47.0, 45.0, 35.7, 33.5, 33.2, 30.2, 29.9, 28.5, 26.7, 24.6. MS (ESI) m/z = 1212 $[M+H]^+$. Anal. ($C_{62}H_{92}Cl_2N_8O_{12}$, 4 H_2O), C, H, N.

Table of elemental analyses

Compound	%	C	H	N
8a	Calcd	58.37	6.86	9.39
	found	58.36	6.96	9.55
8b	Calcd	56.90	7.18	8.85
	found	56.83	7.09	8.73
8c	Calcd	56.90	7.18	8.85
	found	57.26	6.89	9.00
8d	Calcd	57.53	7.33	8.66
	found	57.48	6.98	8.70
8e	Calcd	59,60	7,19	8,97
	found	59.45	7.41	8.93
9a	Calcd	59.78	7.37	9.62
	found	59.76	7.48	9.53
9b	Calcd	59.05	7.61	9.18
	found	59.17	7.49	9.01
9c	Calcd	59.93	7.56	9.32
	found	59.88	7.87	9.21
9d	Calcd	58.80	7.82	8.85
	found	58.81	8.23	8.71
9e	Calcd	57.97	7.86	8.73
	found	58.26	8.05	8.69
13	Calcd	62.33	8.43	8.55
	found	62.07	8.32	8.58