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Supporting Information

Fluorescent Amyloid β peptide ligand derivatives as potential diagnostic tools for Alzheimer disease

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Contents:

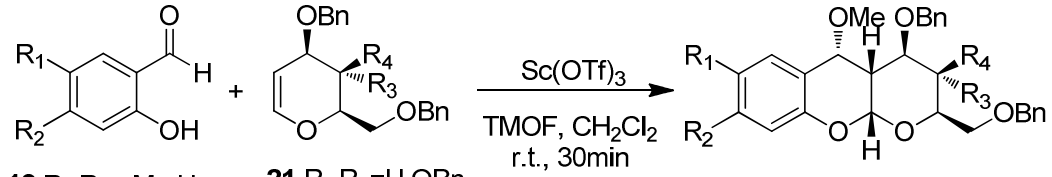
Synthesis

General Remarks

All solvents were dried with molecular sieves for at least 24h prior to use. Thin layer chromatography (TLC) was performed on silica gel 60 F254 plates (Merck) with detection using UV light when possible, or by charring with a solution of concd. H_2SO_4/EtOH/H_2O (5:45:45) or a solution of $(NH_4)_6Mo_7O_{24}$ (21 g), $Ce(SO_4)_2$ (1 g), concd. H_2SO_4 (31 mL) in water (500 mL). Flash column chromatography was performed on silica gel 230-400 mesh (Merck). ^1H and ^{13}C NMR spectra were recorded at 25°C unless otherwise stated, with a Varian Mercury 400 MHz instrument. Chemical shift assignments, reported in ppm, are referenced to the corresponding solvent peaks. HRMS were recorded on a QSTAR elite LC/MS/MS system with a nanospray ion source. Optical rotations were measured at room temperature using an Atago Polax-2L polarimeter and are reported in units of 10^{-1} deg·cm2·g^{-1}.

General synthetic strategy for the synthesis of protected compounds **22** and **23**: Airoldi, C.; Cardona, F.; Sironi, E.; Colombo, L.; Salmona, M.; Silva, A.; Nicotra, F.; La Ferla, B. *Chemical Communications* **2011**, *47*, 10266.

A mixture containing the appropriate O-hydroxybenzaldehyde (**19-20**) (2.5 equiv.), trimethylorthoformiate (2.5 equiv.) and scandium triflate (3% mol) in CH_2Cl_2 is stirred at r.t. for 20 min. The mixture is then cooled at 0°C and tri-*O*-benzyl-D-galactal (**21**) is added. The reaction is then left stirring at r.t. for 30 min. The reaction is then diluted with CH_2Cl_2, washed with water, dried over Na_2SO_4, filtrated and the solvent is removed under reduced pressure. The crude is purified by flash chromatography, Toluene/AcOEt (9.75:0.25) to afford pure compounds **(22-23)**.



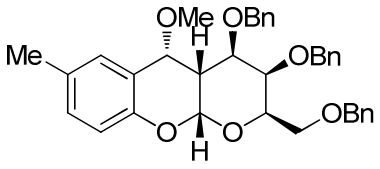
19 R_1,R_2= Me,H
20 R_1,R_2= H, Me

21 R_3,R_4=H,OBn

22 R_1,R_2,R_3,R_4= Me,H,H,OBn
23 R_1,R_2,R_3,R_4= H,Me,H,OBn

Compounds (22): yield 91%, C5 R/S 95/5

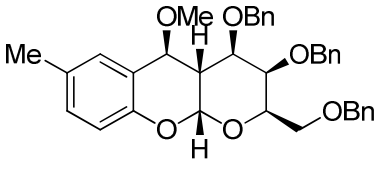
(22R)



22R

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (d, *J* = 7.1 Hz, 1H, H6), 7.31 – 7.21 (m, 15H, Ar), 6.99 (d, *J* = 7.3 Hz, 1H, H8), 6.72 (d, *J* = 8.2 Hz, 1H, H9), 5.61 (d, *J* = 2.9 Hz, 1H, H10a), 4.96 (d, *J* = 11.4 Hz, 1H, OCH_2Ph), 4.74 (d, *J* = 4.5 Hz, 1H, H5), 4.62 – 4.33 (m, 5H, OCH_2Ph), 4.18 (t, *J* = 6.4 Hz, 1H, H2), 3.80 (s, 1H, H3), 3.66 (dd, *J* = 11.2, 2.6 Hz, 1H, H4), 3.61 (dd, *J* = 6.3, 4.1 Hz, 2H, CH_2O), 3.57 (s, 3H,OMe), 3.33 – 3.23 (m, 1H, H4a), 2.31 (s, 3H, Me). ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.98, 139.05, 138.93, 138.16, 130.59, 129.64, 128.61, 128.43, 128.40, 128.36, 128.13, 127.97, 127.75, 127.52, 126.52, 121.97, 115.37, 97.63, 76.92, 76.33, 75.66, 75.16, 73.93, 73.71, 72.69, 71.65, 69.15, 57.1, 34.78, 20.99. $[\alpha]_D^{20}$= -5,2 (c=1, $CHCl_3$); MS: m/z calcd for $[M + H]^+$ = 567.3, $[M + Na]^+$ = 589.3, $[M + K]^+$ = 605.2; found $[M + H]^+$ = 567.6, $[M + Na]^+$ = 589.5, $[M + K]^+$ = 605.6.

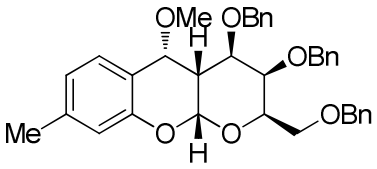
(22S)



22S

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.13 (m, 15H, Ar), 7.01 (dd, *J* = 8.3, 1.7 Hz, 1H, H8), 6.82 (s, 1H, H6), 6.75 (d, *J* = 8.3 Hz, 1H, H9), 5.66 (d, *J* = 3.2 Hz, 1H, H10a), 4.92 (d, *J* = 11.5 Hz, 1H, OCH_2Ph), 4.65 – 4.42 (m, 5H, OCH_2Ph), 4.37 – 4.30 (d, *J* = 2.0 Hz 1H, H5), 4.26 – 4.17 (m, 1H, H2), 3.98 (s, 1H, H3), 3.72 – 3.63 (m, 2H, CH_2O), 3.38 (s, 3H, ArOMe), 3.36 – 3.29 (dd, *J* = 2.38, 11.78 Hz, 1H, H4), 3.06 – 2.93 (m, 1H, H4a), 2.26 (s, 3H, Me). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.53, 138.81, 138.10, 137.77, 131.35, 131.03, 129.91, 128.64, 128.60, 128.54, 128.49, 128.26, 128.21, 128.01, 127.93, 127.82, 118.18, 116.64, 94.89, 75.00, 74.84, 74.80, 73.79, 71.55, 71.47, 71.35, 68.96, 56.42, 37.60, 29.93, 20.77. $[\alpha]_D^{20}$= -2,1 (c=1, $CHCl_3$); MS: m/z calcd for $[M + H]^+$ = 567.3, $[M + Na]^+$ = 589.3, $[M + K]^+$ = 605.2; found $[M + H]^+$ = 567.6, $[M + Na]^+$ = 589.5, $[M + K]^+$ = 605.6.

Compound (23): yield 45%, C5 R/S 100/0

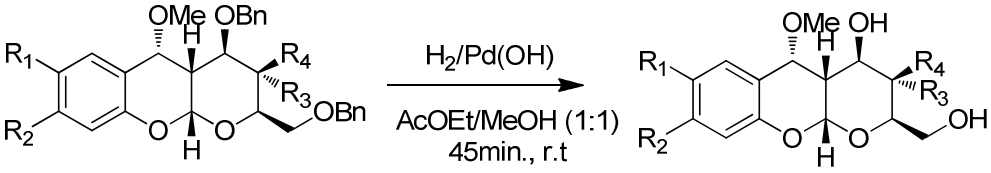


23

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, *J* = 7.8 Hz, 1H, H6), 7.37 – 7.20 (m, 15H, Ar), 6.81 (d, *J* = 7.8 Hz, 1H, H7), 6.66 (s, 1H, H9), 5.63 (d, *J* = 2.8 Hz, 1H, H10a), 4.97 (d, *J* = 11.4 Hz, 1H, OCH_2Ph), 4.74 (d, *J* = 4.2 Hz, 1H, H5), 4.62 – 4.34 (m, 5H, OCH_2Ph), 4.19 (t, *J* = 6.3 Hz, 1H, H2), 3.81 (s, 1H, H3), 3.66 (dd, *J* = 11.1, 2.6 Hz, 1H, H4), 3.62 (dd, *J* = 6.2, 4.6 Hz, 2H, CH_2O), 3.56 (s, 3H, OMe), 3.34 – 3.21 (m, 1H, H4a), 2.31 (s, 3H, Me). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.11, 139.20, 139.07, 139.00, 138.19, 128.61, 128.44, 128.41, 128.37, 128.12, 127.97, 127.94, 127.75, 127.51, 126.10, 122.28, 119.48, 116.04, 97.74, 76.22, 75.74, 75.16, 74.03, 73.70, 72.84, 71.69, 69.16, 57.06, 34.89, 21.41. $[\alpha]_D^{20}$= -6,2 (c=1, $CHCl_3$). MS: m/z calcd for $[M + K]^+$ = 605.2; found [$[M + K]^+$ = 605.2.

General synthetic strategy for the synthesis of compounds **5** and 1**2** (C5 R only): Airoldi, C.; Cardona, F.; Sironi, E.; Colombo, L.; Salmona, M.; Silva, A.; Nicotra, F.; La Ferla, B. *Chemical Communications* **2011**, *47*, 10266.

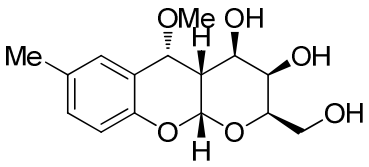
To a 6mM solution of the protected compound in AcOEt/MeOH 1:1, previously degassed, $Pd(OH)_2$ 5% mol is added and the reaction mixture is stirred under H_2 atmosphere for 45min.-1.5 h. Then the catalist is removed by filtration and the solvent evaporated under reduced pressure to afford pure compounds (**5** and **12**)**.**



22 R_1,R_2,R_3,R_4= Me,H,H,OBn
23 R_1,R_2,R_3,R_4= H,Me,H,OBn

5 R_1,R_2,R_3,R_4= Me,H,H,OH
12 R_1,R_2,R_3,R_4= H,Me,H,OH

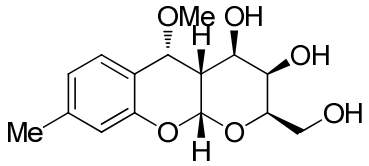
Compound (5): yield 95%



5

^{1}H NMR (400 MHz, CD_3OD) δ 7.24 (s, 1H, H6), 6.98 (d, *J* = 8.2 Hz, 1H, H8), 6.66 (d, *J* = 8.3 Hz, 1H, H9), 5.57 (d, *J* = 3.0 Hz, 1H, H10a), 3.98 (t, *J* = 6.0 Hz, 1H, H2), 3.83 – 3.78 (m, 2H, H3 and H4), 3.77 (dd, *J* = 6.0, 2.9 Hz, 2H, CH_2O), 3.68 (s, 3H, OMe), 2.96 – 2.89 (m, 1H, H4a), 2.26 (s, 3H, Me). ^{13}C NMR (101 MHz, CD_3OD) δ 150.14, 130.22, 129.54, 126.29, 120.97, 115.09, 96.65, 77.67, 72.66, 67.62, 67.37, 61.64, 57.73, 34.59, 19.57. $[\alpha]_D^{20}$= +13,3 (c=1, $CHCl_3$); MS: m/z calcd for $[M + Na]^+$ = 319.1, $[M + K]^+$ = 335.1; found $[M + Na]^+$ = 319.4, $[M + K]^+$ = 335.4.

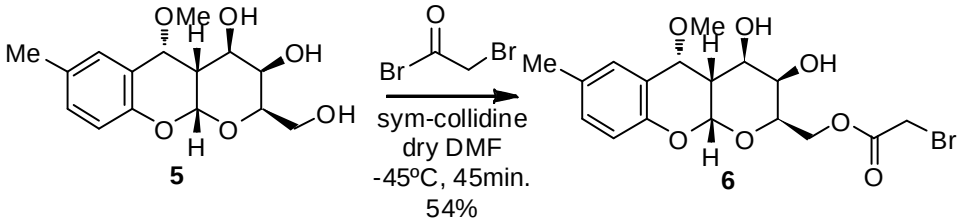
Compound (12): yield 97%



12

^{1}H NMR (400 MHz, CD_3OD) δ 7.29 (d,*J* = 7.8 Hz, 1H, H6), 6.76 (d, *J* = 7.8 Hz, 1H, H7), 6.60 (s, 1H, H9), 5.57 (d, *J* = 3.0 Hz, 1H, H10a), 4.85 (d, *J* = 4.7 Hz, 1H, H5), 3.98 (t, *J* = 5.9 Hz, 1H, H3), 3.83 – 3.73 (m, 4H, CH_2O, H2, H4), 3.67 (s, 3H, OMe), 2.96 – 2.85 (m, 1H, H4a), 2.25 (s, 3H, Me). ^{13}C NMR (101 MHz, CD_3OD) δ 156.14, 143.22, 133.19, 129.62, 125.66, 122.29, 119.72, 100.65, 81.54, 76.64, 71.24, 65.63, 61.61, 38.51, 23.91. $[\alpha]_D^{20}$= +8,3 (c=1, $CHCl_3$); MS: m/z calcd for $[M + Na]^+$ = 319.1; found $[M + Na]^+$ = 319.3.

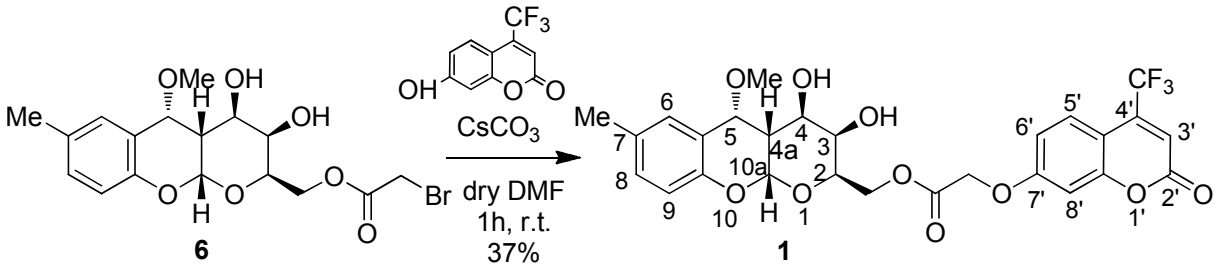
Compound (6)



To a solution of compound **5** (90mg/0,303mmol) and 2,4,6-trimethylpyridine (0,424mmol/ 56uL) in dry DMF (1,68mL) with stirring at -45ºC a solution of Bromoacetyl Bromide (0,394mmol/34uL) in

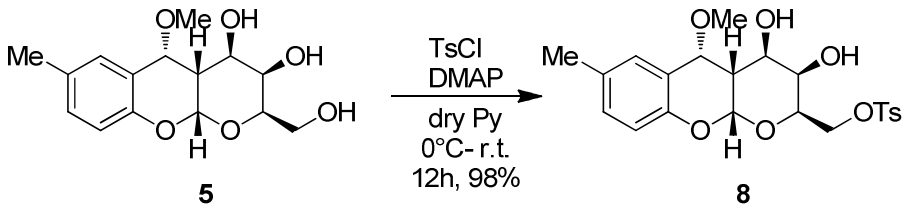
dry Toluene (200uL) was added. Stirring was continued for 40min. at -45ºC, and the the mixture was allowed to warm to room temperature. Toluene (12mL) was added, and the solids filtered off, and the filtrate concentrated. The residue was purified by flash chromatography Toluene/EtOAc (7:3) and EtOAc, affording 54% (68mg) of a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (s, 1H, H-6), 7.02 (d, *J* = 8.2 Hz, 1H, H-8), 6.74 (d, *J* = 8.2 Hz, 1H, H-9), 5.55 (d, *J* = 3.0 Hz, 1H, H-10a), 4.81 (d, *J* = 4.7 Hz, 1H, H-5), 4.50 (d, *J* = 6.0 Hz, 1H, C*H*$_2$), 4.28 (t, *J* = 6.0 Hz, 1H, H-2), 4.04 – 3.95 (m, 1H, H-4), 3.95 – 3.84 (m, *J* = 7.2 Hz, 3H, C*H*$_2$Br and H-3), 3.73 (s, 3H, OC*H*$_3$), 2.98 – 2.82 (m, 1H, H-5a), 2.30 (s, 3H, C*H*$_3$).^{13}C NMR (101 MHz, CD_3OD) δ 171.71, 153.78, 134.36, 133.53, 130.41, 125.02, 119.10, 100.24, 81.30, 75.25, 74.11, 73.98, 68.85, 61.41, 52.39, 52.18, 51.96, 51.75, 51.54, 51.32, 51.11, 43.85, 29.27, 23.52.

Compound (1)



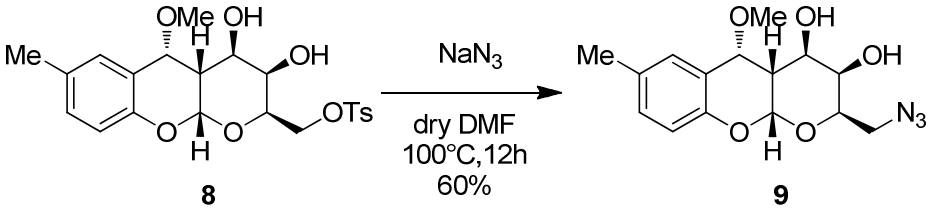
A solution of 7-hydroxycoumarin-4-trifluoromthyl (41mg/0,18mmol) in dry DMF (2mL) was added to a stirred solution of compound **6** (50mg/0,12mmol) in dry DMF (1mL). To the reaction mixture was added $CsCO_3$ (43mg/0,13mmol) and the resulting solution was stirred at r.t. for 1h, after which was poured into a solution of CH_2Cl_2-H_2O. The organic phase was washed with 1M NaOH solution and aqueous layer was washed with CH_2Cl_2. The combined organic layers were dried and concentrated under reduce pressure. The crude product was purified by flash chromatography Toluene/EtOAc (6:4), affording 37% (25mg) of a white solid. ^{1}H NMR (400 MHz, DMSO) δ 7.61 (d, *J* = 9.0 Hz, 1H, H-5´), 7.25 – 7.12 (m, 2H, H-8´,H-6), 7.09 (dd, *J* = 9.0, 2.3 Hz, 1H, H-6´), 6.95 (d, *J* = 8.4 Hz, 1H, H-8), 6.86 (s, 1H, H-3´), 6.63 (d, *J* = 8.4 Hz, 1H, H-9), 5.57 (d, *J* = 2.5 Hz, 1H, H-10a), 5.03 (s, 2H, -OCOC*H*$_2$-), 4.82 (d, *J* = 4.7 Hz, 1H, H-5), 4.39 – 4.26 (m, 1H, C*H*$_2$), 4.30 – 4.2 (m, 2H, C*H*$_2$, H-3), 4.10 – 3.98 (m, 1H, H-2), 3.62 – 3.54 (m, 1H, H-4), 3.53 (s, 3H, OC*H*$_3$), 2.90 – 2.76 (m, 1H, H-4a), 2.21 (s, 3H, C*H*$_3$).

Compound (8)



A solution of compound **5** (482mg/1,63mmol) in dry Pyridine (3,3mL) was cooled to 0ºC in a ice bath with stirring. A solution of p-toluensulfonyl chloride (574mg/3mmol) in dry Pyridine (3,8mL) was then added dropwise and stirring was continued at r.t. until the reaction seemed completed by TLC. After 12h, the solvent was then removed in vacuum to afford a crude product which was purified by flash chromatography, Petroleum Ether/ EtOAc (5:5), yielding 98% (719mg) of a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, *J* = 8.1 Hz, 2H, OTs), 7.34 (d, *J* = 8.1 Hz, 2H, OTs), 7.19 (s, 1H, H-6), 7.01 (d, *J* = 8.3 Hz, 1H, H-8), 6.71 (d, *J* = 8.3 Hz, 1H, H-9), 5.46 (d, *J* = 2.8 Hz, 1H, H-10a), 4.76 (d, *J* = 5.0 Hz, 1H, H-5), 4.39 - 4.21 (m, 2H, C*H*$_2$O), 3.94 (m, 2H, H-4 and H-2), 3.83 (s, 1H, H-3), 3.69 (s, 3H, OMe), 2.82 (m, 1H, H-4a), 2.44 (s, 3H, Me), 2.29 (s, 3H, C*H*$_3$).^{13}C NMR (101 MHz, $CDCl_3$) δ 149.60, 145.05, 133.05, 131.06, 130.51, 130.05, 128.31, 126.68, 120.04, 116.11, 95.95, 78.50, 77.54, 77.22, 76.91, 69.55, 68.98, 66.88, 66.63, 59.64, 34.70, 21.85, 20.87. $[\alpha]_D^{20}$= +4,1 (c=1, CH_3CH_2OH); MS: m/z calcd for $[M + Na]^+$ = 473.5; found $[M + Na]^+$ = 473.1

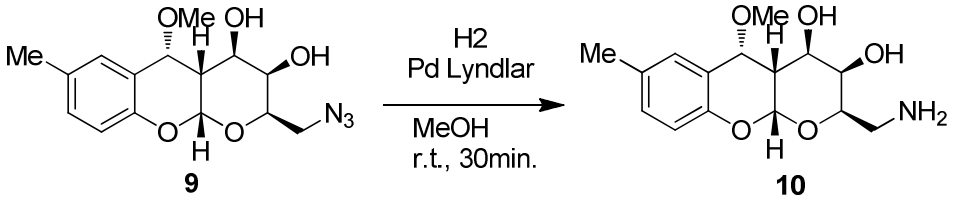
Compound (9)



To a solution of compound **8** (100mg/0,22mmol) in dry DMF (1mL), a solution of NaN_3 (101mg/1,55mmol) in dry DMF (0,5mL) was added and stirring was continued at 100ºC for 12h. Upon cooling to r.t., the colorless precipitate was filtrate ad the solvent was evaporated to dryness. The crude product was purified by flash chromatography, Petroleum Ether/EtOAc (6:4) affording 60% (42mg) of a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (s, 1H, H-6), 7.02 (d, *J* = 8.1 Hz, 1H, H-8), 6.75 (d, *J* = 8.1 Hz, 1H, H-9), 5.56 (d, *J* = 2.7 Hz, 1H, H-10a), 4.80 (d, *J* = 5.0 Hz, 1H, H-5), 4.16 (t, *J* = 5.9 Hz, 1H, H-2), 3.98 (d, *J* = 2.9 Hz, 1H, H-4), 3.83 (s, 1H, H-3), 3.72 (s, 3H,

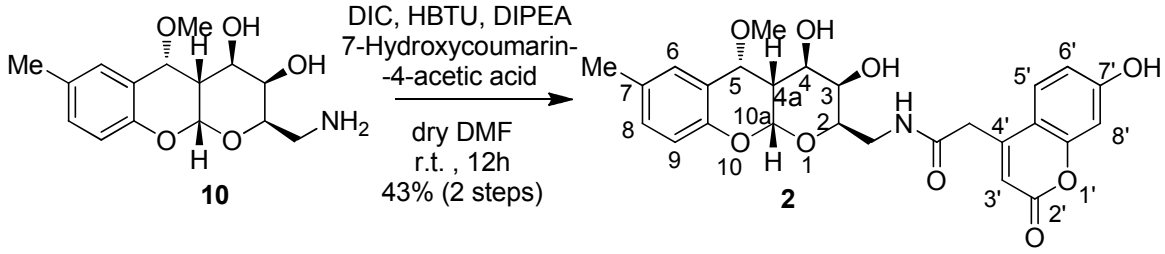
OCH_3), 3.71 – 3.66 (m, 1H, CH_2O), 3.51 (dd, *J* = 12.7, 5.9 Hz, 1H, CH_2O), 2.93-2.84 (m, 1H, H-4a), 2.30 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.67, 131.05, 130.53, 126.71, 120.06, 116.14, 96.06, 78.53, 77.57, 77.25, 76.93, 70.67, 67.25, 67.15, 59.72, 51.50, 34.69, 20.92. $[\alpha]_D^{20}$= -10,3 (c=1, CH_3CH_2OH); MS: m/z calcd for $[M + Na]^+$ = 344.3; found $[M + Na]^+$ = 344.1.

Compound (10)



To a solution of compound **9** (30mg/0,093mmol) in MeOH (6mL) was added Lindlar catalyst (5%), and the mixture was hydrogenated at 1atm and r.t. for 30min. Filtration and concentration gave amine 10 in a quantitative yield ^{1}H NMR (400 MHz, CD_3OD) δ 7.23 (d, *J* = 2.4 Hz, 1H, H-6), 6.98 (d, *J* = 8.1 Hz, 1H, H-9), 6.64 (dd, *J* = 8.2, 2.4 Hz, 1H, H-8), 5.59 (d, *J* = 3.0 Hz, 1H, H-10a), 4.86 (d, *J* = 5.0 Hz, 1H, H-5), 3.97-3.90 (m, 1H, H-2), 3.88 – 3.77 (m, 1H, H-4), 3.74 (dd, *J* = 9.7, 5.3 Hz, 1H, H-3), 3.68 (s, 3H, OMe), 3.06 – 3.00 (m, 1H, CH_2O), 2.97 – 2.90 (m, 1H, H-4a), 2.87 (dd, *J* = 13.3, 4.7 Hz, 1H, CH_2O), 2.29 – 2.23 (m, 3H, CH_3).

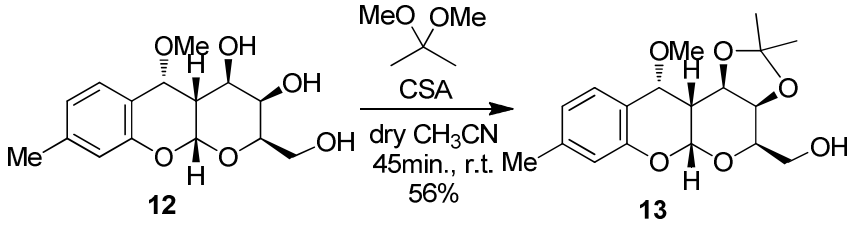
Compound (2)



Working in anhydrous conditions, compound **10** (30mg/0,093mmol), 7-hydroxycoumarin-4-acetic acid (24,6mg/0,112mmol) and HBTU (53,1mg/0,14mmol) were dissolved in dry DMF (1,3ml). To this reaction mixture DIPEA (48µL/0,28mmol) was added at r.t. and after 10 min. DIC (0,14mmol/22µL) was added at 0ºC. The final reaction mixture was stirred at room temperature until the reaction seemed completed by TLC. After 12h, the solvent was then removed in vacuum to afford a crude product, which was purified by flash chromatography, $CHCl_3$/MeOH (9,5:0,5), yielding 43% (20 mg) of a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.59 (d, *J* = 8.8 Hz, 1H, H-

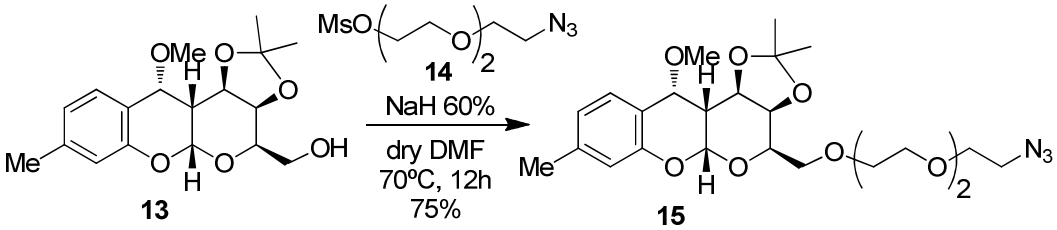
5‘), 7.23 (s, 1H, H-6), 6.97 (d, *J* = 7.5 Hz, 1H, H-8), 6.76 (d, *J* = 8.8 Hz, 1H, H-6‘), 6.63 (m, 2H, H-9 and H-8‘), 6.16 (s, 1H, H-3‘), 5.54 (d, *J* = 2.8 Hz, 1H, H-10a), 4.85 (d, *J* = 4.9 Hz, 1H, H-5), 4.02 (m, 1H, H-2), 3.81-3.71 (m, 3H, H-4 and C*H*$_2$C=O), 3.67 (bs, 4H, OC*H*$_3$ and H-3), 3.57 (dd, *J* = 13.8, 4.5 Hz, 1H, C*H*$_2$O), 3.44 (dd, *J* = 13.5, 8.5 Hz, 1H,C*H*$_2$O), 2.90-2.84 (m, 1H, H-4a), 2.26 (s, 3H, C*H*$_3$).^{13}C NMR (101 MHz, CD3OD) δ 170.35, 162.86, 156.03, 150.04, 130.21, 129.55, 126.27, 126.16, 120.88, 115.22, 114.65, 110.71, 110.37, 102.95, 96.59, 77.58, 70.49, 68.05, 67.12, 67.00, 57.64, 48.44, 48.23, 48.02, 47.81, 47.59, 47.38, 47.17, 40.72, 34.36, 29.59, 19.57. $[\alpha]_D^{20}$= +16,6 (c=1, CH_3CH_2OH); MS: m/z calcd for [M + Na]$^+$ = 520.5; found [M + Na]$^+$ = 520.2

Compound (13)



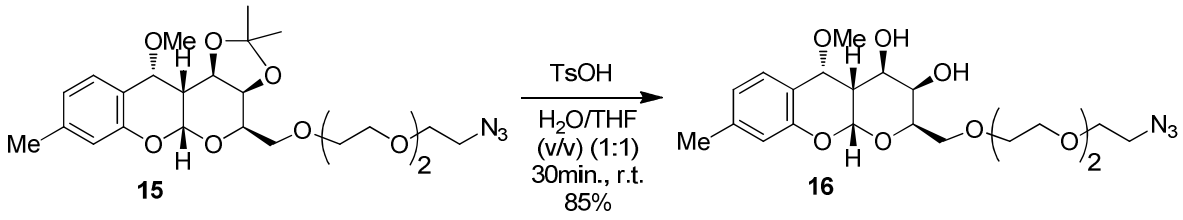
To a solution of compound **12** (500mg/1,69mmol) in dry CH_3CN (8,0mL), acetone dimetheylacetal (6,76mmol/0,4mL) and CSA (1%mmol) were added under argon. The reaction mixture remained under magnetic stirring for 45min. at room temperature, after which was added Et_3N to neutralize the CSA. The reaction was then concentrated under reduce pressure and the crude product purified by flash chromatography, Petroleum Ether/EtOAc (6:4) affording 56%(336mg) of a yellow solid.
^{1}H NMR (400 MHz, CD_3OD) 7.30 (d, *J* = 7.9 Hz, 1H, H-6), 6.78 (d, *J* = 7.9 Hz, 1H, H-7), 6.63 (s, 1H, H-9), 5.52 (d, *J* = 3.8 Hz, 1H, H-10), 4.68 (d, *J* = 5.2 Hz, 1H, H-5), 4.31 (m, 1H, H-2), 4.15 (dd, *J* = 5.6, 2.4 Hz, 1H, H-3), 4.12 – 4.06 (m, 1H, H-4), 3.80 (dd, *J* = 6.1, 3.5 Hz, 2H, C*H*$_2$O), 3.54 (s, 3H,OC*H*$_3$), 2.79 – 2.66 (m, 1H, H-4a), 2.26 (s, 3H, Ar C*H*$_3$), 1.53 (s, 3H, C*H*$_3$), 1.29 (s, 3H, C*H*$_3$). ^{13}C NMR (101 MHz, CD_3OD) δ 152.12, 139.13, 127.03, 121.80, 118.72, 115.86, 108.61, 95.52, 75.27, 71.43, 70.87, 69.47, 61.45, 55.97, 48.21, 48.00, 47.78, 47.57, 47.36, 47.14, 46.93, 37.67, 26.92, 24.85, 19.75.MS: m/z calcd for [M + Na]$^+$ = 359.3; found [M + Na]$^+$ = 359.3.

Compound (15)



To a 60 % suspension of sodium hydride in oil (75 mg, 2,67mmol) an argon atmosphere dry DMF (10 mL) was added. The suspension was cooled to 0ºC with stirring and a solution of compound **13** (300mg/0,89mmol) in dry DMF (3 ml) and compound **14** (570mg/1,79mmo) in dry DMF (3ml) were added. The mixture was stirred at 70ºC for 12 h. After cooling to 0ºC, the reaction was quenched by slowly addition of MeOH (4 ml) and stirred for more 20 min. The reaction mixture was diluted with EtOAc, and washed with water, dried (NaSO4) and concentrated. The residue was purified by flash chromatography, Petroleum Ether/EtOAc (3:7), yielding 75% of a brownish oil. MS: m/z calcd for $[M + Na]^+ = 516.4$; found $[M + Na]^+ = 516.4$.

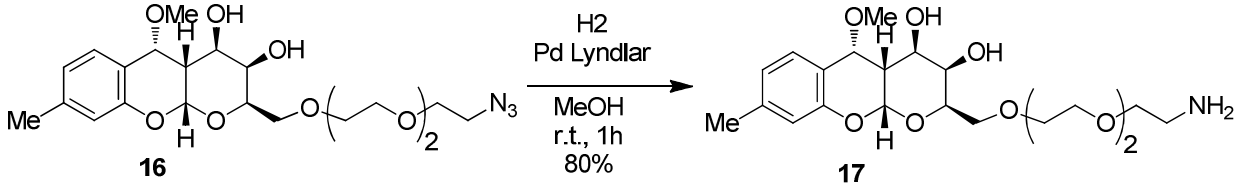
Compound (16)



To a solution of compoud **13** (100mg/0,22mmol) in CH_3CN:H_2O (10:2) (10mL),p-toluenesulfonic acid (0,42mg/0,002mmol) was added. The final reaction mixture was stirred at room temperature until the reaction seemed completed by TLC. After 30min., Et3N is added and the solvent was then removed in vacuum to afford a crude product which was purified by flash chromatography, Petroleum Ether/EtOAc (2:8), yielding 85% of a brownish oil ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (d, *J* = 7.7 Hz, 1H, H-6), 6.79 (d, *J* = 7.7 Hz, 1H, H-7), 6.65 (s, 1H, H-9), 5.57 (d, *J* = 2.8 Hz, 1H, H-10a), 4.79 (d, *J* = 4.8 Hz, 1H, O*CH*$_3$), 4.19 (t, *J* = 5.4 Hz, 1H, H-2), 3.96 (dd, *J* = 14.6, 3.8 Hz, 3H, H-3, H-4), 3.83 (dd, *J* = 9.0, 5.7 Hz, 2H, H-1''), 3.77 – 3.57 (m, 13H, triethyleneglycol, C*H*$_3$O), 3.40 (t, *J* = 5.0 Hz, 2H, H-7''), 2.94 (dd, *J* = 11.5, 6.7 Hz, 2H, H-4a), 2.29 (s, 3H, C*H*$_3$). ^{13}C NMR

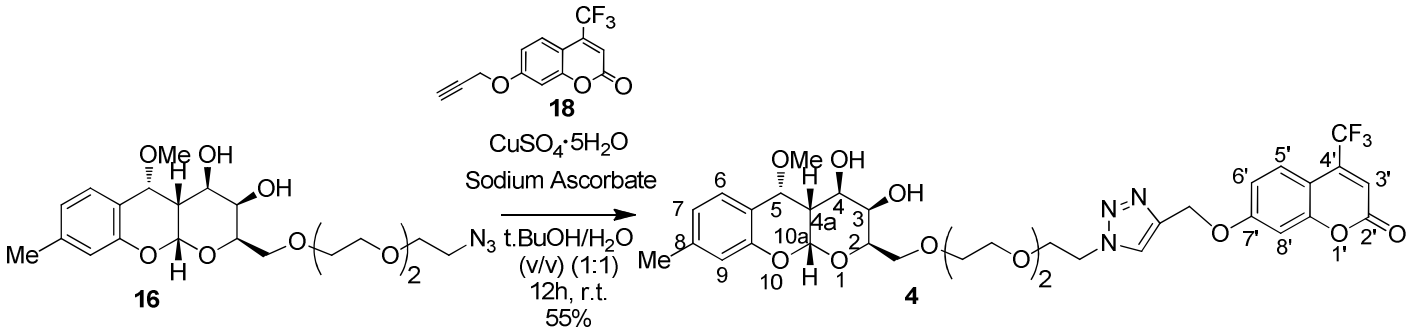
(101 MHz, $CDCl_3$) δ 151.71, 139.79, 126.07, 122.23, 117.45, 116.30, 96.15, 78.29, 78.29, 77.33, 77.33, 77.01, 77.01, 76.69, 76.69, 70.63, 70.42, 70.02, 67.37, 67.12, 59.27, 50.66, 34.58, 29.69.

Compound (17)



To a solution of compound **16** (80mg/0,20mmol) in $CHCl_3$:MeOH (1:1) (5mL) was added Lindlar catalyst (5%), and the mixture was hydrogenated at 1atm and r.t. for 1h. Filtration and concentration gave amine 17 in 80% yield as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (d, *J* = 7.8 Hz, 1H, H-6), 6.78 (d, *J* = 7.7 Hz, 1H, H-7), 6.64 (bs, 1H, H-9), 5.57 (d, *J* = 2.2 Hz, 1H, H-10a), 4.78 (d, *J* = 4.4 Hz, 1H, H-5), 4.17 (t, *J* = 5.6 Hz, 1H, C*H*$_{2PEG}$), 4.00 – 3.58 (m, 15H, H-2, H-3, H-4, C*H*$_2$O, 5 C*H*$_{2PEG}$, C*H*$_3$O), 3.54 (t, *J* = 4.7 Hz, 2H, C*H*$_{2PEG}$), 3.05 – 2.94 (m, 1H, H-4a), 2.88 (bs, 2H, C*H*$_{2PEG}$), 2.28 (s, 3H, C*H*$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.18, 137.10, 123.49, 119.60, 115.04, 113.67, 93.76, 75.59, 74.76, 74.45, 74.13, 69.93, 68.25, 67.99, 67.93, 67.92, 67.75, 67.57, 64.57, 64.28, 56.63, 38.73, 32.05, 18.59. MS: m/z calcd for $[M + H]^+$ = 429; found $[M + H]^+$ = 428.9

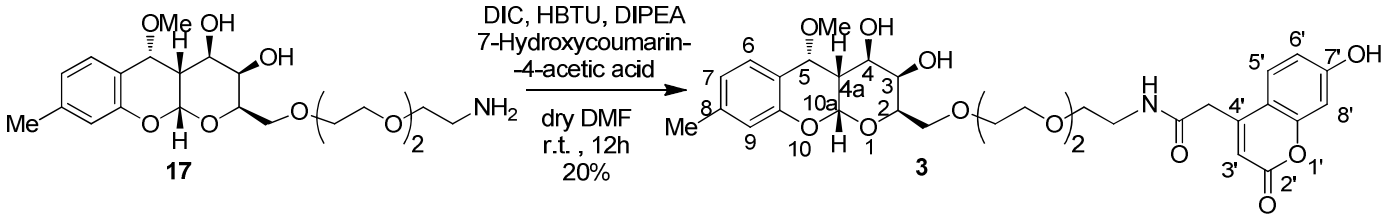
Compound (4)



To a vigorously stirring suspension of compound **18** (72mg/0,27mmol) in 1:1 t-BuOH/H2O, Copper(II) sulfate pentahydrate (9mg/0,035mmol) and sodium ascorbate (14mg/0,069mmol) were added. The reaction mixture was stirred for 10min at room temperture and then compound 16 (110mg/0,23mmol) was added. The suspension was stirred vigorously at r.t. for 12h. At this time TLC indicated completion of the reaction. Distilled water was added, and the aqueous layer was extracted with CH_2Cl_2. The combined extracts were dried, filtered and evaporated to afford a brownish oily solid. The product was then purified using column chromatography using $CHCl_3$ as

eluant, which afforded the expected compound **4** as a brownish solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8,26 (s, 1H, H8'), 7.65 (dd, *J* = 9.0, 1.8 Hz, 1H, H-5'), 7.25 (d, *J* = 7.8 Hz, 1H, H-6), 7.14 (d, *J* = 2.5 Hz, 1H, H-8'), 7.07 (dd, *J* = 9.0, 2.5 Hz, 1H, H-6'), 6.72 (d, *J* = 7.8 Hz, 1H, H-7), 6.68 (s, 1H, H-3'), 6.47 (s, 1H, H-9), 5.52 (d, *J* = 3.0 Hz, 1H, H-10a), 5.32 (s, 2H, H-9'), 4.80 (d, *J* = 4.8 Hz, 1H, H-5), 4.67 – 4.57 (m, 2H, H-6''), 4.08 (t, *J* = 5.9 Hz, 1H, H-2''), 3.95 – 3.87 (m, 2H, H-7''), 3.79 – 3.52 (m, 15H, H-3'',H-4'', H-5'', H-1'', H-2, H-3, H-4, OCH_3), 2.95 – 2.85 (m, 1H, H-4a), 2.22 (s, 3H, ArCH_3).^{13}C NMR (400 MHz, CD_3OD) δ 162.28, 156.17, 151.80, 142.19, 139.03, 125.96, 125.70, 125.46, 121.79, 112.24, 115.37, 113.56, 107.00, 102.22, 96.43, 76.35, 70.90, 70.51, 70.33, 70.40, 70.09, 70.04, 68.87, 67.41, 66.94, 66.90, 61.60, 61.62, 57.49, 50.17, 34.30, 19.76. ^{19}F NMR (400 MHz, CD_3OD) δ -62.0. MS: m/z calcd for $[M + H]_+$ = 721.7; found $[M + H]^+$ = 721.7.

Compound (3)



Working in anhydrous conditions, compound **17** (50mg/0,13mmol), 7-hydroxycoumarin-4-acetic acid (31mg/0,16mmol) and HBTU (68,26mg/0,20mmol) were dissolved in dry DMF (2ml). To this reaction mixture DIPEA (0,39mmol/67µL) was added at r.t. and after 10 min. DIC (0,20mmol/31µL) was added at 0ºC. The final reaction mixture was stirred at room temperature until the reaction seemed completed by TLC. After 12h, the solvent was then removed in vacuum to afford a crude product which was purified by flash chromatography, CH_2Cl_2/Acetone (5:5), yielding 20% (15mg) of a brown color solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.61 (d, *J* = 9.0 Hz, 1H, H-5'), 7.28 (d, *J* = 8.0 Hz, 1H, H-6), 6.80 (dd, *J* = 9.0, 2.1 Hz, 1H, H-6'), 6.75 (d, *J* = 8.0 Hz, 1H, H-7), 6.70 (d, *J* = 2.1 Hz, 1H, H-8'), 6.56 (s, 1H, H-3'), 6.19 (bs, 1H, H-9), 5.54 (d, *J* = 3.0 Hz, 1H, H-10a), 4.10 (t, *J* = 5.7 Hz, 1H, CH_{2PEG}), 4.86 (1H, H-5, under H_2O signal), 3.85 – 3.48 (m, 18H, H-9', H-2. H-3, H-4, 4 CH_{2PEG}, OCH_3, CH_2O), 3.43 – 3.38 (m, 2H, CH_{2PEG}), 2.97 – 2.83 (m, 1H, H- 4a), 2.24 (s, 3H, CH_3). ^{13}C NMR (101 MHz, CD_3OD) δ 169.56, 162.02, 161.61, 155.36, 151.85, 151.24, 139.06, 126.13, 125.72, 121.57, 118.06, 115.39, 112.97, 111.71, 111.59, 102.21, 96.44, 77.34, 70.81, 70.39, 70.30, 70.17, 70.09, 69.85, 68.94, 67.39, 66.91, 57.44, 48.21, 47.99, 47.78, 47.57, 47.35, 47.14, 46.93, 39.37, 38.78, 34.30, 19.77. MS: m/z calcd for $[M + H]^+$ = 629; found $[M + Na]^+$ = 629.

NMR binding studies

NMR experiments were recorded on a Varian 400-MHz Mercury. A batch of Aβ1-42 was selected that contained pre-amyloidogenic seeds highly toxic to N2a cells. Immediately before use, lyophilized Aβ1-42 was dissolved in 10 mM NaOD in D_2O at a concentration of 160μM, then diluted 1:1 with 20 mM phosphate buffer, pH 7.4 containing one of the tested compounds. Compounds **2**, **3** and **4** were dissolved in PB, pH 7.4, and added to the peptide solution; for compound **4** 5% of d-DMSO was added. The pH of each sample was verified with a Microelectrode (Mettler Toledo) for 5 mm NMR tubes and adjusted with NaOD or DCl. All pH values were corrected for isotope effect. Basic sequences were employed for ^{1}H, ^{19}F, 2D-TOCSY, 2D-NOESY and STD experiments. For STD, a train of Gaussian-shaped pulses each of 50 ms was employed to saturate selectively the protein envelope; the total saturation time of the protein envelope was adjusted by the number of shaped pulses and was varied between 3 s and 0.3 s.

Sections staining

Brain tissue from Tg CRND8 mice encoding a double mutant form of amyloid precursor protein 695 (KM670/671NL + V717F) under the control of the PrP gene promoter (Chishti et al., 2001) were dissected and were fixed in Carnoy's and embedded in paraffin.

Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L. No. 116, G.U. Suppl. 40, Feb. 18, 1992, Circolare No. 8, G.U., 14 Luglio 1994) and international laws and policies (EEC Council Directive 86/609, OJ L 358, 1 Dec.12, 1987; NIH Guide for the Care and use of Laboratory Animals, U.S. National Research Council, 1996). All efforts were made to minimize the number of animals used and their suffering.

Seven-micrometer-thick serial sections of paraffin-embedded blocks from the temporal cortex were mounted on gelatin coated microscope slides and used for staining. Paraffin sections were subjected to two incubations of 5 min in xylene, an incubation of 10 min each in 100% - 96% -70% EtOH for the complete dewaxing and two successive incubations of 5 min in water. Tissue sections were covered with a solution 3 μM of thioflavine T or 6 μM of compound **3** dissolved in water. After 30 min of incubation, the sections were washed 3 times for 5 min in water and 5 min each in 70% - 96% - 100% EtOH. The sections were then incubated 5 min in xylene before adding the coverslip. Sections were visualized by fluorescent fluoromicroscope M-3204CCCD (OlympusBX61) equipped with the filters FITC (Ex 488 nm) for detecting thioflavine T and DAPI (Ex 405 nm) for detecting test compounds.

Transport Experiments

Trans-endothelial-electrical resistance (TEER) were measured by EVOMX meter, STX2 electrode, World Precision Instruments, Sarasota, Florida.

Fluorescence measurements were done using a Cary Eclipse spectrofluorimeter (Varian Inc., Palo Alto, California).

The radioactivity assay were achieved by means of a Tri-Carb 2200 CA Liquid Scintillation Analyzer (Packard Instrument Co. Inc., Rockville, MD)

The differences were evaluated for statistical significance using Student's t-test.

For transport experiments across a cell monolayer, hCMEC/D3 were seeded in a 12-well Transwell® inserts coated with type I collagen. 0.5 mL of cell suspensions containing 2.0 • 105cells were added to the upper (donor) chamber which was inserted into the lower (acceptor) chamber containing 1.0 mL of the culture medium. A cell monolayer was usually formed 14 days after seeding judged by three criteria: (1) the cells formed a confluent monolayer without visible spaces between cells under a light microscope; (2) the height of the culture medium in the upper chamber had to be at least 2 mm higher than that in the lower chamber for at least 24 h; and (3) a constant TEER (trans endothelial electrical resistance) value, measured using an EVOM Endohm chamber. Wells were used when TEER value was higher than 50 $\Omega \cdot cm^2$. Trans-endothelial permeability coefficient (PE) was calculated as reported by Bickel U. (*NeuroRx: The Journal of the American Society for Experimental NeuroTherapeutics* ,Vol. 2, 15–26, January 2005):

After adding the test substance to the donor compartment, repeated samples are taken from the donor compartment over the desired time course. The concentration measured in these samples and the known volumes of the compartments

(V_{donor} and $V_{acceptor}$) are used to calculate the incremental clearance volumes ΔV_{Cl} for each time point:

$$\Delta V_{Cl} = C_{acceptor} \cdot V_{acceptor} / C_{donor}$$

As long as the concentration in the acceptor compartment is small and ΔV_{Cl} increases in linear manner, the

slope of the line can be interpreted as the PS product for unidirectional transfer. With the known exchange surface,

S, (filter area) the permeability may be obtained as P = PS/S. Finally, a correction needs to be made for
the permeability of the cell-free filter:

$1/P_{endothel} = 1/P_{totall} - 1(P_{filter})$

With $P_{endothel}$ being equal to PE.

After adding the test substance to the upper compartment, samples were taken from the lower compartment at different times (0-60-180min) for liquid scintillation counting (C14 sucrose) and for fluorescence assays (compound **3**). At the end of the experiments, TEER and [14C]sucrose PE were re-determined in order to prove no occurrence of adverse effects on tight junction function due to sample application.