Confocal Microscopy Imaging of NR2B-Containing NMDA Receptors Based on Fluorescent Ifenprodil-Like Conjugates

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SUPPORTING INFORMATION

1. CHEMISTRY

General. Dry THF and Et_2O were displayed by using a MBraun SPS 800 solvent purification system. All other chemicals obtained from commercial suppliers were used as received. Reactions were purified by chromatography column with Merck silica gel Geduran Si 60 (0.040–0.063 nm). Thin layer chromatography (TLC) were carried out on silica gel 60 F254 (1.1 mm, Merck) with spot detection under UV light and approriate staining reagent (I_2, $KMnO_4$ or ninhydrin). Melting points (Mp) were obtained on an Electrothermal IA9000 capillary apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded at room temperature on Bruker DPX 250 or DRX 400 spectrometers. The chemical shifts are calibrated to TMS (δ H 0.00) or residual proton and carbon resonance of the solvent $CDCl_3$ (δ H 7.26 and δ C 77.16). Infrared (IR) spectra were recorded on a Thermo Nicolet 380 FT-IR instrument. Mass spectra were recorded on QTOF Micro Waters instruments. Elemental analyses were performed with a C, H, N, S, O Thermoquest apparatus. Analyses and semi-preparative purifications by high-performance liquid chromatography (HPLC) were performed on a Waters system, equipped with a 600 gradient pump and a photodiode array detector (λ 210 to 750 nm) using a reverse-phase MS C18 Waters Bondapak column (7.8 x 300 mm, 10 µm) eluting with acetonitrile (ACN) and aqueous trifluoroacetic acid (TFA, 0.1%, pH 2) [System A : ACN 20% (15 min), then linear gradient from 20 to 80% (20 min), then 80% (5 min), then linear gradient from 80 to 20% (5 min) and 20% (5 min) at a flow rate of 3

mL/min ; System B : ACN 25% (20 min), then linear gradient from 25 to 85% (30 min), then 85% (10 min), then linear gradient from 85 to 25% (10 min) and 25% (10 min) at a flow rate of 3 mL/min ; System C : ACN 0% (5 min), then linear gradient from 0 to 90% (45 min), then 90% (10 min), then linear gradient from 90 to 0% (10 min) and 0% (10 min) at a flow rate of 3 mL/min]. Ultraviolet (UV) absorption and fluorescence emission spectra were recorded on a Perkin-Elmer spectrophotometer LS 55 using ligands at 0.003 mM in solution in H_2O containing 0.3% DMSO.

tert-Butyl-6-[(1R*,2R*)-1-(4-benzyloxyphenyl)-2-(4-benzylpiperidin-1-yl)propylamino]hexylcarbamate (9)

To a stirred solution of *O*-benzylated (*threo*)-ifenprodil[1] (2.1 g, 5 mmol) in dry THF (60 mL) was added triethylamine (2.1 mL, 15 mmol) at rt. After cooling to 0 °C, methanesulfonyl chloride (1 mL, 12.9 mmol) was added dropwise. The resulting reaction mixture became more visquous and stirring was maintained for 30 min at 0 °C. Triethylamine (1.4 mL, 10 mmol) then mono-Boc protected 1,6-hexyldiamine[2] (2.7 mg, 12.5 mmol) were added successively and the resulting mixture was stirred at 0 °C to rt for 20 h. The reaction mixture was partitioned between CH_2Cl_2 and water. After separation and extraction with CH_2Cl_2, the combined organic layers were washed with saturated $NaHCO_3$ then brine, dried over $MgSO_4$ and concentrated under vacuum. The residue was purified by column chromatography over silica gel (ethyl acetate: heptane 50: 50) to yield the titled compound **9** as a white solid (2.70 g, 88%). Mp 98 °C. Rf 0.13 (heptane/ethyl acetate 50/50). ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.39-7.07 (m, 12H), 6.84 (d, J = 8.8 Hz, 2H), 4.97 (s, 2H), 4.42 (bs, 2H), 3.21 (d, J = 9.8 Hz, 1H), 3.02-2.95 (m, 2H), 2.60-2.10 (m, 8H), 2.02-1.91 (m, 2H), 1.78-1.52 (m, 2H), 1.48-1.02 (m, 19H), 0.52 (d, J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$, 62.9 MHz) δ 158.4, 156.4, 141.3, 137.6, 135.8, 129.7, 129.5, 128.9, 128.5, 128.3, 128.0, 126.1, 114.8, 79.4, 70.4, 66.0, 64.9, 53.2, 48.0, 44.6, 43.8, 41.0, 39.0, 33.6, 33.4, 30.5, 30.2, 28.9, 27.8, 27.4, 9.1. IR ν (cm^{-1}) 3378, 3279, 2924, 1691, 1510, 1452, 1235, 1225, 1166, 730, 696. ESI$^+$/MS/MS m/z (%) 614 ($[M+H]^+$, 58), 398 (100). ESI$^+$/HRMS calcd for $C_{39}H_{56}N_3O_3$ 614.4322; found 614.4321. Anal. calcd for $C_{39}H_{55}N_3O_3$ C, 76.31, H, 9.03, N, 6.85; found C, 76.57, H, 9.45, N, 7.12.

4-{(1R*,2R*)-[1-(6-Aminohexylamino)-2-(4-benzylpiperidin-1-yl)]propyl}phenol (10)

To a solution of compound **9** (298 mg, 0.486 mmol) in acetic acid (9 mL) was added HBr (47% in water, 0.4 mL, 7.2 mmol). The mixture was refluxed overnight. After cooling to rt, aqueous NaOH (3M) was added until pH 12. After extraction with CH_2Cl_2, the organic layer was dried over $MgSO_4$ then

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[2] a) Morrell, A.; Placzek, M. S.; Steffen, J. D.; Antony, S.; Agama, K.; Pommier, Y.; Cushman, M. *J. Med. Chem*. **2007**, *50*, 2040. b) Callahan, J. F.; Shton-Shue, D.; Bryan, H. G.; Bryan, W. M.; Heckman, G. D.; Kinter, L. B.; McDonald, J. E.; Moore, M. L.; Schmidt, D. B.; Silvestri, J. S. *J. Med. Chem*. **1989**, *32*, 391.

concentrated under vacuum. The residue was purified by column chromatography over silica gel (CH_2Cl_2/MeOH/NH_4OH 90/10/0.5) to yield the expected compound **10** as a white solid (118 mg, 57%). Mp 58 °C. Rf 0.29 (CH_2Cl_2/MeOH/NH_4OH 80/20/0.5). ^1H NMR ($CDCl_3$, 400 MHz) δ 7.32-7.28 (m, 2H), 7.22-7.15 (m, 5H), 6.74 (d, J = 8.4 Hz, 2H), 4.55 (bs, 4H), 3.32 (d, J = 10.0 Hz, 1H), 2.74-2.69 (m, 5H), 2.55 (d, J = 7.2 Hz, 2H), 2.53-2.33 (m, 3H), 2.10-2.00 (m, 1H), 1.75-1.62 (m, 2H), 1.53-1.33 (m, 5H), 1.31-1.12 (m, 6H), 0.63 (d, J = 6.4 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 157.3, 141.2, 130.0, 129.5, 128.6, 126.2, 116.2, 65.8, 64.0, 49.7, 47.3, 44.8, 43.7, 41.8, 33.3, 32.6, 29.3, 27.3, 26.7, 9.3. IR ν (cm-1) 3400, 2925, 1514, 1452, 1260, 1145, 834, 744, 698. ESI^+/MS/MS m/z (%) 424 ($[M+H]^+$, 24), 308 (100). ESI^+/HRMS calcd for $C_{27}H_{42}N_3O$ 424.3328; found 424.3316. Hydrochloride salt of **10** was obtained as a white solid after stirring in diethyl ether (5 mL) with HCl in diethyl ether (1M, 2 mL) for 30 min. Anal. calcd for $C_{27}H_{41}N_3O$,3HCl C, 60.16, H, 9.35, N, 7.80, found C, 59.91, H, 8.79, N, 7.52.

***N*-{6-[(1R*,2R*)-2-(4-Benzylpiperidin-1-yl)-1-(4-hydroxyphenyl)propylamino]hexyl}-5-dimethylaminonaphthalene-1-sulfonamide (4)**

To a mixture of hydrochloride salt of phenylethylenediamine **10** (25 mg, 47 µmol) and triethylamine (35 µL, 250 µmol) in CH_2Cl_2 (5 mL) cooled to -5 °C was added slowly dansyl chloride (13 mg, 46 µmol) in CH_2Cl_2 (5 mL). The final solution was stirred at -5 °C for 7 h and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel (CH_2Cl_2/MeOH/NH_4OH 90/10/0.1) to yield **4** as a beige solid (14 mg, 47%). Rf 0.45 (CH_2Cl_2/MeOH/NH_4OH 90/10/0.1). t_R 29.0 min (HPLC system A). ESI^+/MS/MS m/z (%) 657.4 ($[M+H]^+$, 100), 308.2 (72). ESI^+/HRMS calcd for $C_{39}H_{53}N_4O_3S$ 657.3838; found 657.3809.

***N*-{6-[(1R*,2R*)-2-(4-Benzylpiperidin-1-yl)-1-(4-hydroxyphenyl)propylamino]hexyl}-5(6)-fluoresceincarboxamide (5)**

To a solution of 5-carboxyfluorescein *N*-succinimidyl ester (4.5 mg, 9.7 µmol) in *N*-methyl-2-pyrrolidinone NMP (200 µL) was added a solution of phenylethylenediamine **10** (5 mg, 11.8 µmol) and *N*,*N*-diisopropylethylamine DIEA (10 µL, 59 µmol) in 200 µL of NMP. The reaction was protected from the light with aluminium foil and stirred at rt for 18 h. The reaction mixture was diluted with water (2.5 mL) and purified by semi-preparative HPLC (injection volumes 600-700 µL) to yield **5** as a yellow powder after being lyophilized (4.5 mg, 46%). HPLC purity > 95%; t_R 24.1 min (system C). ESI^+/MS/MS m/z (%) 780.4 ($[M(C_{48}H_{51}N_3O_7)+H]^+$, 100), 473.2 (20). ESI^+/HMRS calcd for $C_{48}H_{52}N_3O_7$ (MH^+) 782.3805, found 782.3789.

_N_-(_N_-{6-[(1R*,2R*)-2-(4-Benzylpiperidin-1-yl)-1-(4-hydroxyphenyl)propylamino]hexyl}-5-aminocarbonylpentyl)-5(6)-fluoresceincarboxamide (6)

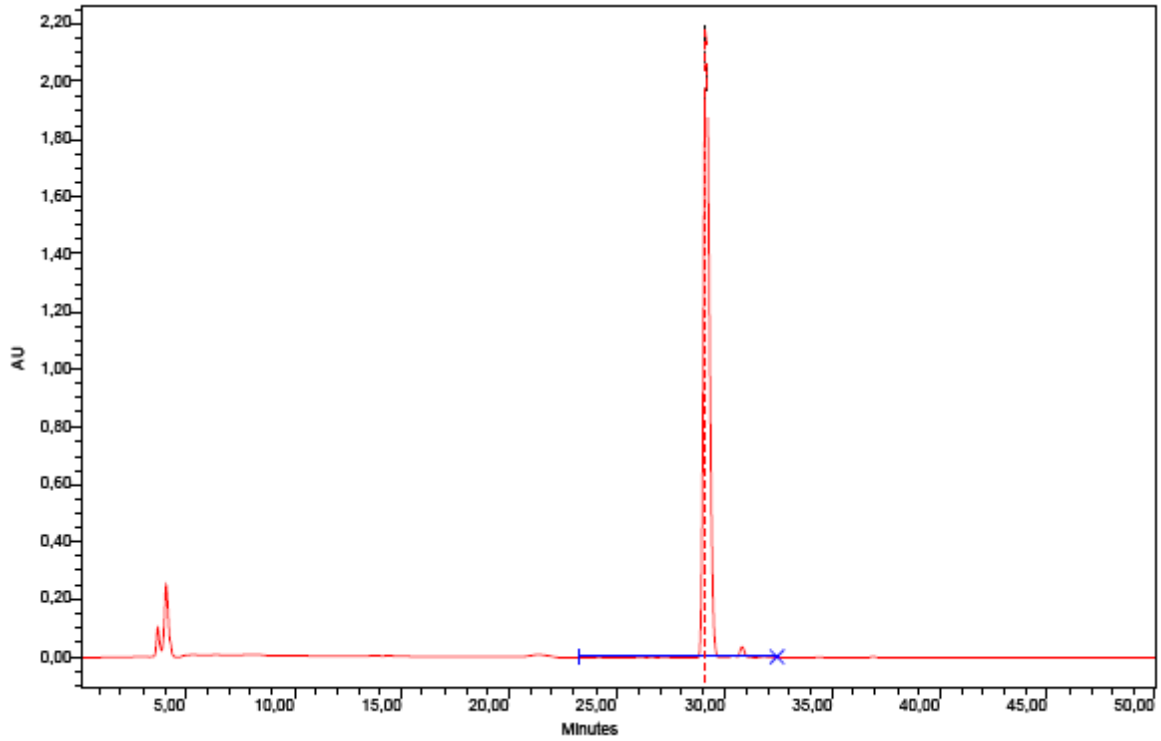
To a solution of 6-[fluorescein-5(6)-carboxamido]hexanoic acid *N*-hydroxysuccinimidyl ester (6.1 mg, 10.4 μmol) in *N*-methyl-2-pyrrolidinone NMP (150 μL) was added a solution of phenylethylenediamine **10** (5.3 mg, 9.8 μmol) and *N*,*N*-diisopropylethylamine DIEA (3.5 μL, 20.2 μmol) in 200 μL of NMP. The reaction was protected from the light with aluminium foil and stirred at rt for 18 h. The reaction mixture was diluted with water (2.3 mL) and purified by semi-preparative HPLC (injection volumes 600-800 μL) to yield **6** as a yellow powder after being lyophilized (5 mg, 45%). HPLC purity > 95%; t_R 24.3 min (system C). ESI$^+$/MS/MS m/z (%) 895.8 ([M($C_{54}H_{62}N_4O_8$)+H]$^+$, 65), 588.5 (20), 308.3 (100). ESI$^+$/HMRS calcd for $C_{54}H_{63}N_4O_8$ (MH^+) 895.4646, found 895.4666.

2-{5-[-1-(6-{6-[(1R*,2R*)-2-(4-Benzylpiperidin-1-yl)-1-(4-hydroxyphenyl)propylamino]hexyl}-5-aminocarbonylpentyl-3,3-dimethyl-5-sulfo-1,3-dihydro-2H-indol-2-ylidene]penta-1,3,5-trienyl}-1-ethyl-3,3-dimethyl-5-sulfo-3H-indolinium (7)

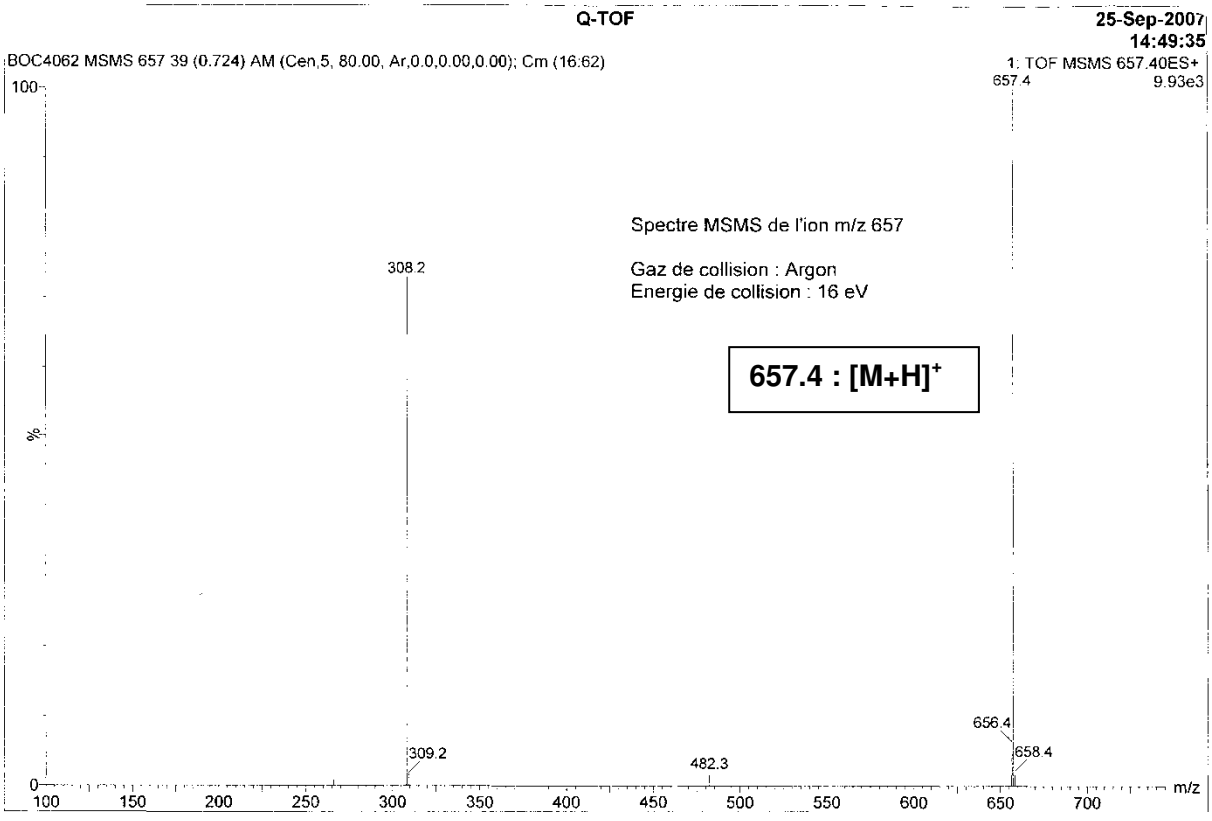
To a mixture of cyanine (Cy5) dye succinimidyl ester[3] Cy5.0-CO_2Su (4.6 mg, 6.09 μmol) in *N*-methyl-2-pyrrolidinone NMP (100 μL) was added a solution of NMP (100 μL) containing the hydrochloride salt of phenylethylenediamine **10** (3.9 mg, 7.3 μmol) and *N*,*N*-diisopropylethylamine DIEA (5.0 μL, 30.5 μmol). The reaction was protected from the light and stirred at rt for 3h. The reaction mixture was purified by semi-preparative HPLC to yield **7** as a blue powder after being lyophilized (4.1 mg, 53%). HPLC purity > 95%; t_R 32.6 min (system B). ESI$^+$/MS/MS m/z (%) 553.9 ([M($C_{60}H_{78}N_5O_8S_2$)+2Na]$^{2+}$, 23), 542.9 ([M+Na+H]$^{2+}$, 53), 531.9 ([M+2H]$^{2+}$, 100).

[3] Mujumdar, R. B.; Ernst, L. A.; Mujumdar, S. R. ; Lewis, C. J.; Waggoner, A. S. *Bioconjugate Chem.* **1993**, *4*, 105.

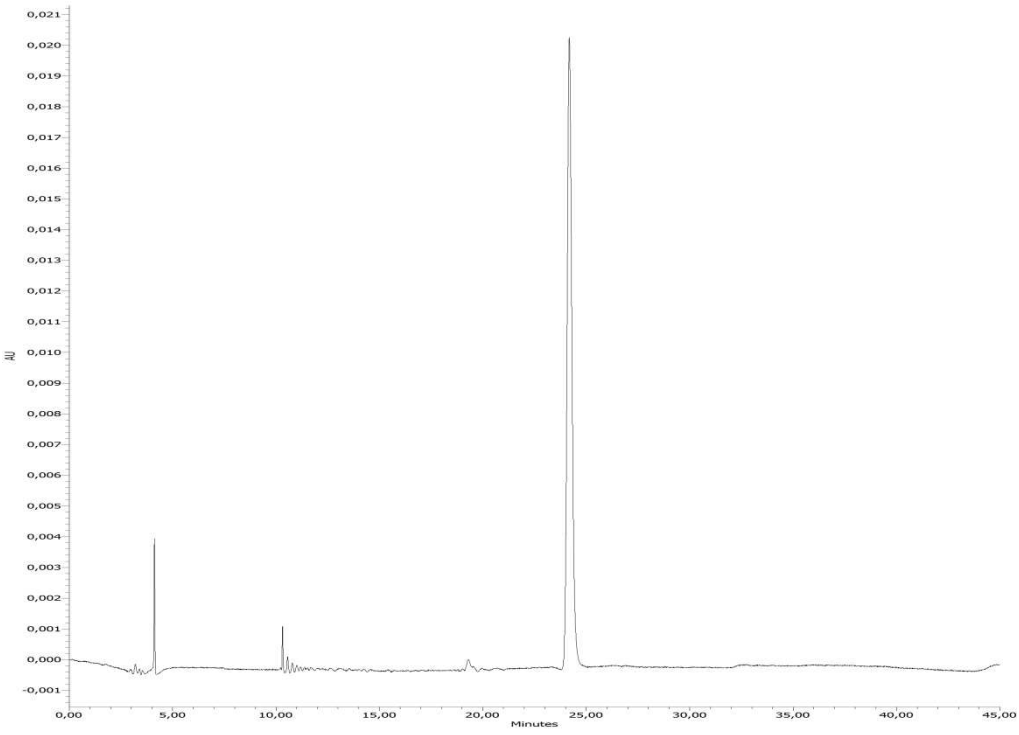
HPLC Chromatogram for dansyl-ligand 4



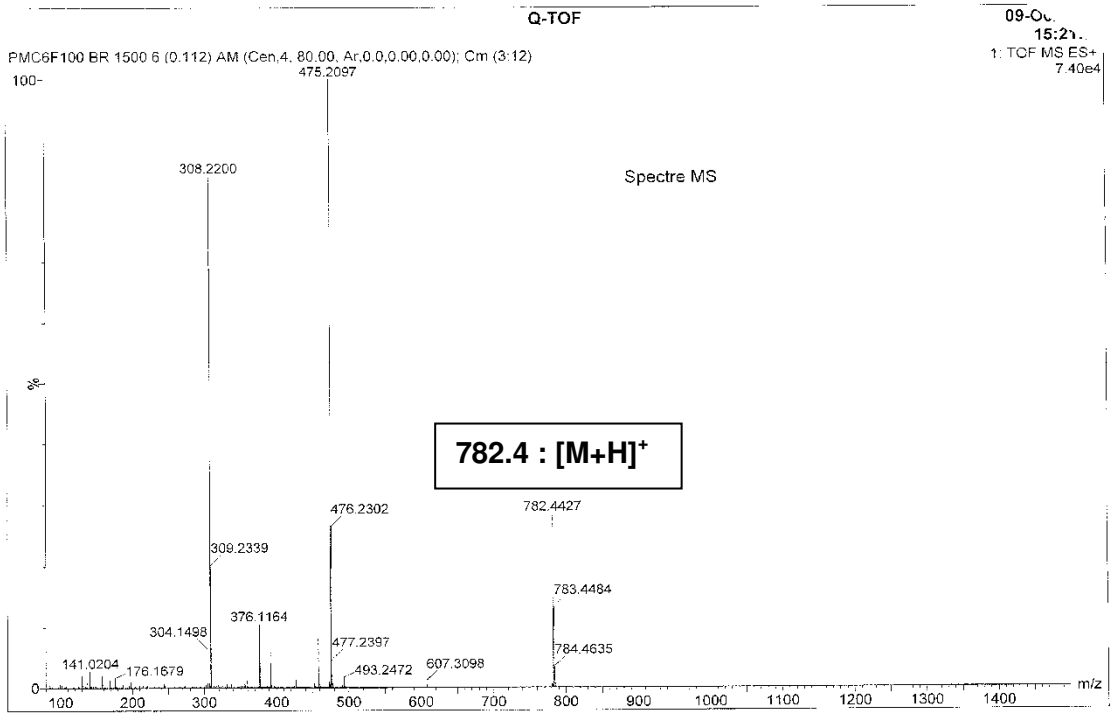
Mass spectrum for dansyl-ligand 4



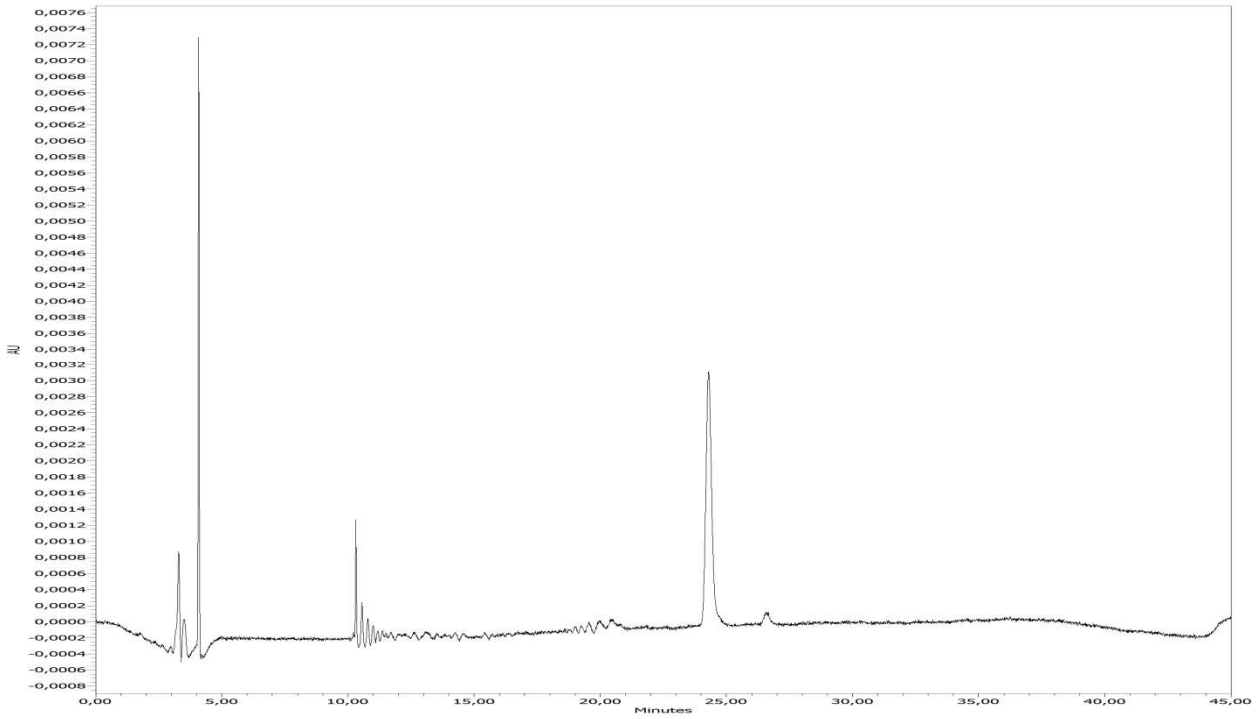
HPLC Chromatogram for fluorescein-ligand 5



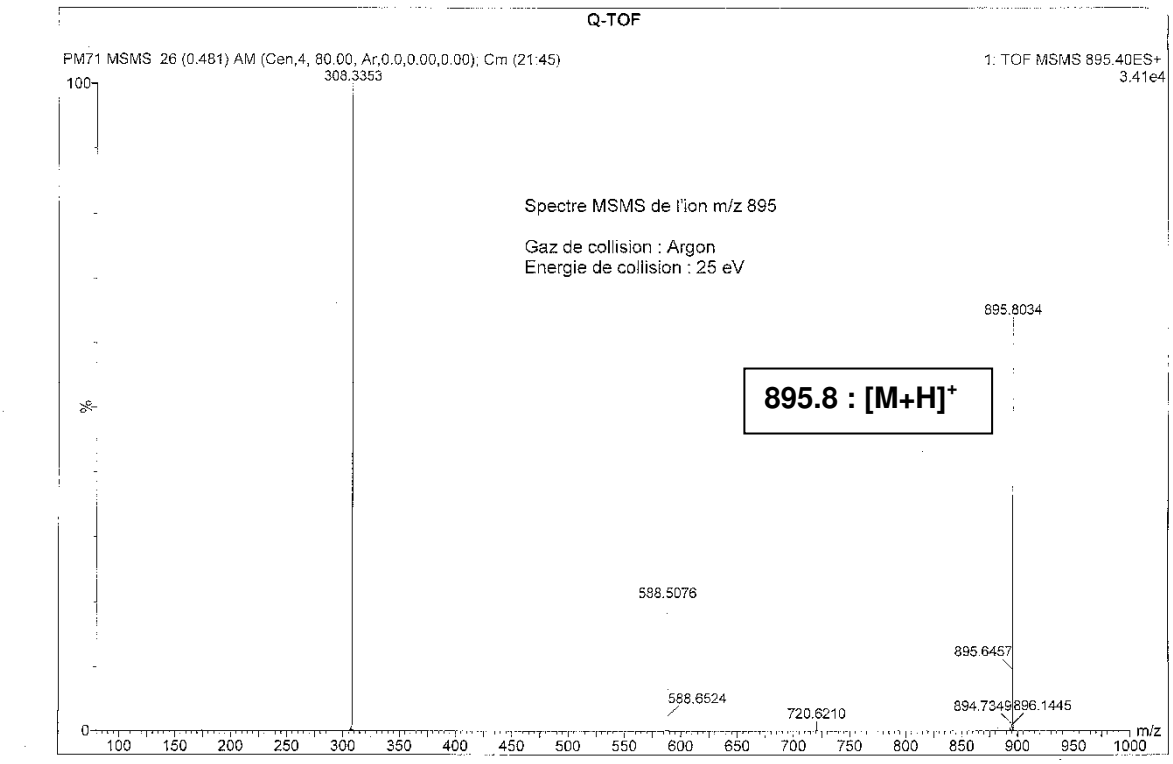
Mass spectrum for fluorescein-ligand 5



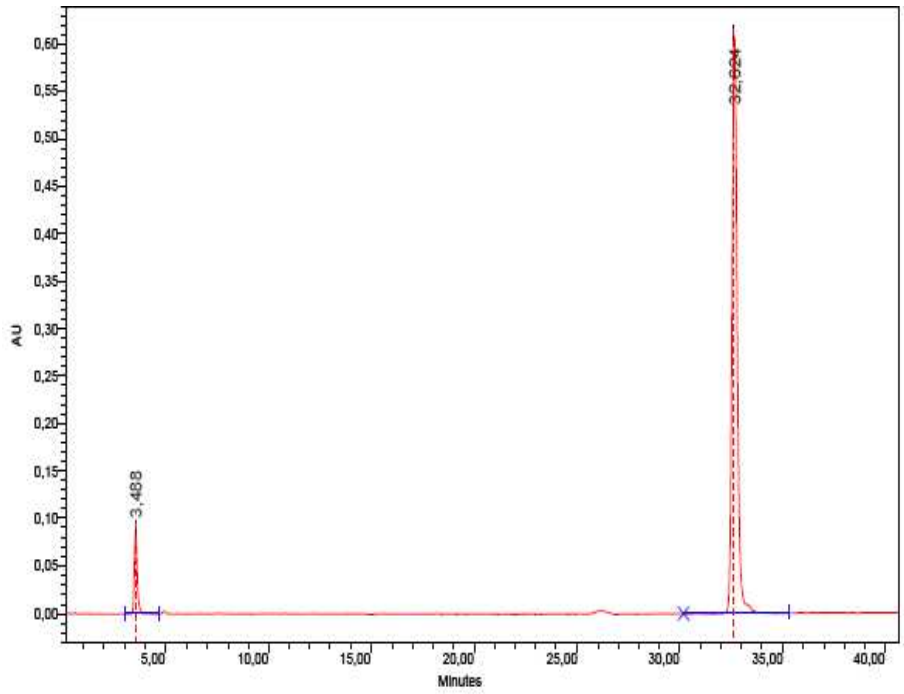
HPLC Chromatogram for fluorescein-ligand 6



Mass spectrum for fluorescein-ligand 6



HPLC Chromatogram for Cy5-ligand 7



Mass spectrum for Cy5-ligand 7