Glycosylated porphyrin dimers as robust ratiometric temperature sensors

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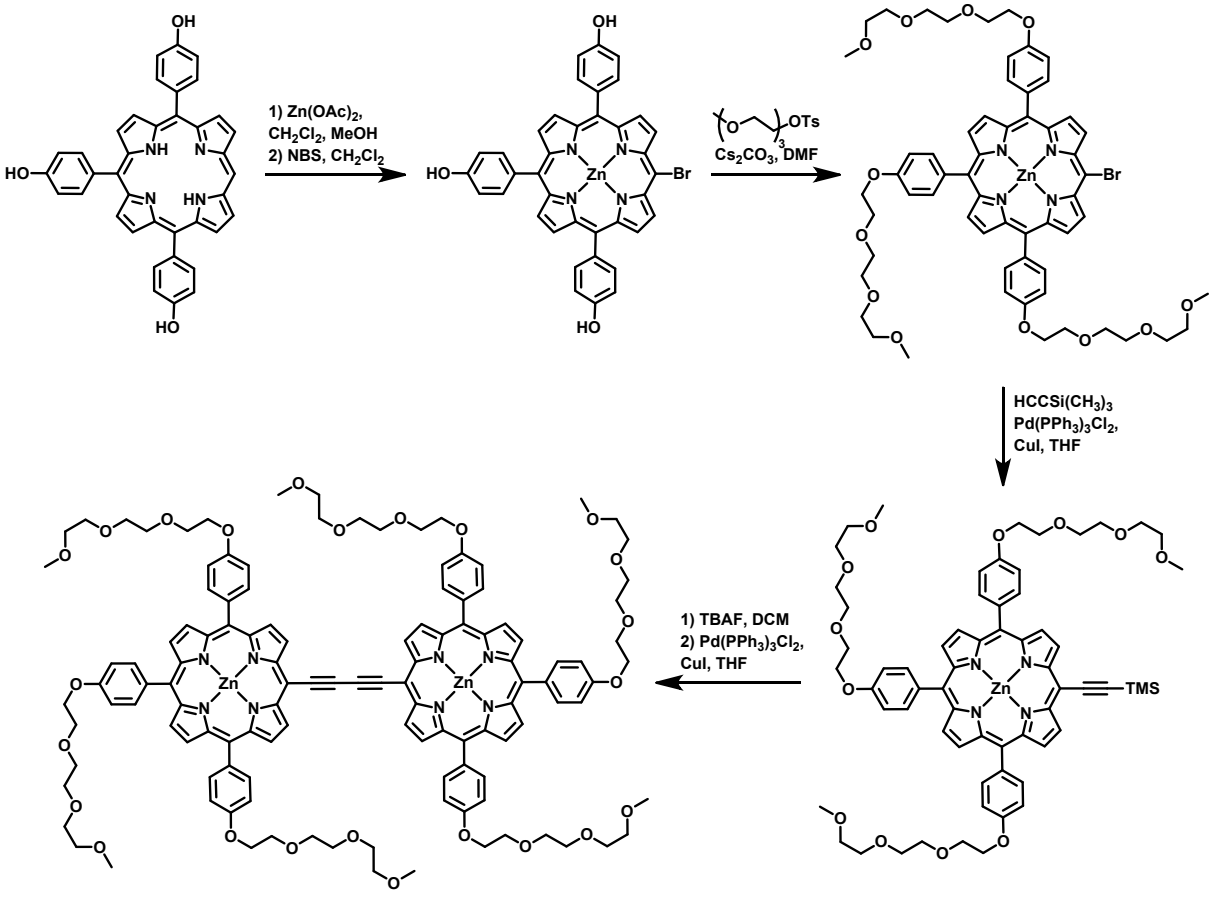
Reagents and Materials

All solvents used were reagent grade. The following reagents have been abbreviated: dimethyl formamide (DMF), dimethylsulfoxide (DMSO), 2-(2-(2-methoxyethoy)ethoxy)ethanol tosylate **14** (CAS [77544-60-6]) was purchased from Aldrich and used without purification. DMF was distilled under slow argon flow and kept over 4 Å sieves. Column chromatography was performed with the indicated solvents using E. Merck silica gel 60 (particle size 0.035-0.070 mm). Macherey-Nagel precoated plates (SIL G-200, 2 mm) were used for preparative thin-layer chromatography. Yields refer to chromatographically and spectroscopically pure compounds. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker AC300 spectrometer (300 MHz) at ambient temperature using an internal deuterium lock. Chemical shift values are given in ppm relative to tetramethyl silane (TMS). Acidic impurities in $CDCl_3$ were removed by treatment with anhydrous K_2CO_3. Assignments of the resonance to individual protons are based on integration and selective homonuclear correlation (COSY). Heteronuclear multiple coherence (HMQC, HMBC) spectra were also obtained for all compounds and allow assignments of the resonance to carbon atoms. Quantitative UV-visible spectra were recorded with a a Agilent Cary 300 spectrophotometer. Fluorescence spectra were recorded using Cary Eclipse spectrophotometer apparatus. Measurements were performed at room temperature with solutions of OD < 0.1 to avoid re-absorption of the emitted light, and data were corrected with a blank and from the variations of the detector with the emitted wavelength. Melting points were determined on Electrothermal 1100 element. Microanalyses and MALDI-TOF analyses were performed respectively by the ICSN-CNRS Elemental Analysis Center and Mass Spectrometry Center (Imagif) at Gif-sur-Yvette, France.

Synthesis

1. Synthesis of dimer 1

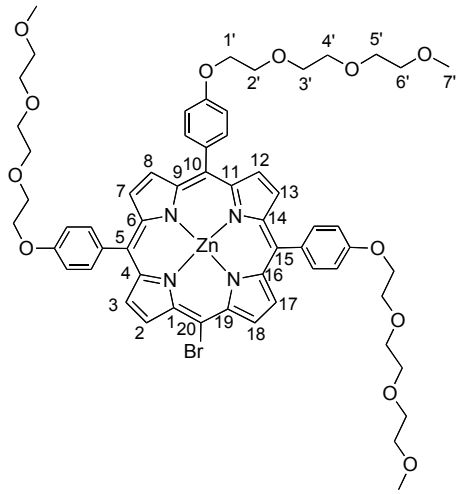
The synthesis of compounds **2-5** was previously published in reference[i]. Dimer **1** was produced following a similar synthesis to the glycosylated dimers, described in Scheme 1. Previously described 5,10,15-tri-(4-{[(methoxy)-ethoxy]-ethoxy}-ethoxy)phenyl-20-trimethylsilylacetylenylporphyrinate Zinc (II) was deprotected with ter-butylammonium fluoride (TBAF) to afford the unstable free alkyne analog which was engaged, without further purification, in a Glaser homocoupling reaction in the presence of copper (I) iodide, bis-triphenylphosphine-bis-chloride palladium (II) in tetrahydrofuran (THF) and triethylamine (NEt_3). The expected compound was isolated with a 60% yield.



Scheme 1: Synthesis of compound **1**.

2. Procedures

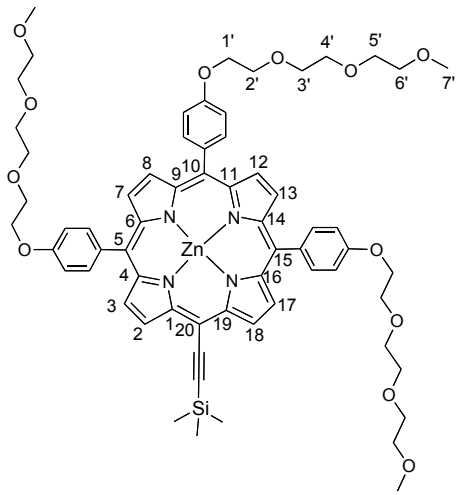
5-bromo-10,15,20-tri(para-2-(2-(2-methoxyethoy)ethoxy)ethanoxyphenyl porphyrin zinc complex.



5-bromo-10,15,20-tri-para-hydroxyphenyl porphyrin zinc complex (660 mg, 0.87 mmol, 1 eq.) in 100 mL anhydrous DMF, was combined with 2-(2-(2-methoxyethoy)ethoxy)ethanol tosylate (4.15 g, 13.05 mmol, 15 eq.) and Cs_2CO_3 (12.76 g, 40 mmol, 45 eq.). The mixture was stirred under argon at room temperature for 24 h. It was concentrated under vacuum then taken up with a mixture of water and ethyl acetate (2/1, v/v) and the organic layer separated. The aqueous layer was extracted with ethyl acetate. The combined organic extracts were washed with water (2x), dried over magnesium sulfate, filtered and the solvent evaporated under vacuum. The product was purified by three crystallizations from CH_2Cl_2/n-heptane then purified by preparative chromatography to afford the desired compound as a purple solid (913 mg, 88%). UV-vis in CH_2Cl_2: λ_{max} nm (ε $mM^{-1}.cm^{-1}$): 429 (207.0), 564 (7.5), 605 (5.0). Melting point > 300°C. ^1H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 9.74 (d, J = 4.6 Hz, 2H, H-2, 18), 8.95 (d, J = 4.6 Hz, 4H, H-3, 17), 8.86 (m, 4H, H-7, 8, 12, 13), 8.16-7.97 (m, 6H, H-o-phenyl), 7.20-7.05 (m, 6H, H-m-phenyl), 4.00-3.90 (m, 6H, H-1'), 3.62-3.50 (m, 6H, H-2'), 3.46-3.30 (m, 6H, H-3'), 3.29-3.19 (m, 6H, H-4'), 3.19-3.12 (m, 6H, H-5'), 3.01-2.88 (m, 6H,

H-6'), 2.75 (s, 9H, H-7'). $^{13}C-NMR$ ($CDCl_3$, 75.3 MHz) δ (ppm): 158.9 (C-*p*-phenoxy), 152.0 (C-4, 16), 150.1 (C-6, 9, 11, 14), 159.9 (C-1, 19), 135.0 (C-*o*-phenoxy), 135.0 (C-1-phenoxy), 132.7 (C-3, 17), 132.0 (C-7, 8, 12, 13), 130.7 (C-2, 18), 120.5 (C-5, 10, 15), 112.2 (C-*m*-phenoxy), 98.5 (C-20), 68,1-64.3 (C-1', 2', 3', 4', 5', 6'), 52.9 (C-7'). MS (MALDI-TOF) m/z: $[M]^+$ Calcd for $C_{59}H_{65}BrN_4O_{12}Zn$ 1164.31; Found 1164.31.

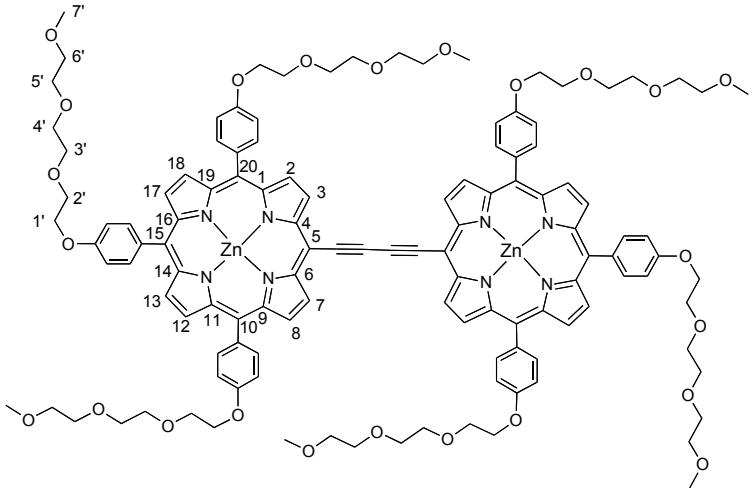
5-allyl-10,15,20-tri(para-2-(2-(2-methoxyethoy)ethoxy)ethanoxyphenyl porphyrin zinc complex.



5-bromo-10,15,20-tri(para-2-(2-(2-methoxyethoy)ethoxy)ethanoxyphenyl porphyrin zinc complex (0.80 g, 0.69 mmol, 1 eq.) was combined with $Pd(PPh_3)_2Cl_2$ (47 mg, 69 mmol, 0.1 eq.) and CuI (12 mg, 69 mmol, 0.1 eq.). The flask was purged with argon for 10 min before the solids were dissolved in dry THF (15 mL) and dry Et_3N (2.5 mL). The solution was deoxygenated with argon for 5 min and frozen at 77 K. Trimethysilylacetylene (390 mg, 4.1 mmol, 6 eq.) was added and the frozen mixture was left under argon flow for 1h. The gas was then allowed to escape and the crude solution warmed to room temperature was stirred for 16 h under argon after which the reaction was quenched with water. It was then extracted with CH_2Cl_2, washed three times with water and once with brine. The organic phase was dried over anhydrous magnesium sulfate, filtered and the solvents removed under reduced pressure. The title compound was obtained after

purification over silica gel chromatography (CH_2Cl_2/MeOH, 99/1, v/v) as a blue powder (560 mg, 69% yield). UV-vis in CH_2Cl_2: λ_{max} nm (ε $mM^{-1}.cm^{-1}$): 435 (259.8), 572 (9.2), 619 (11.6). Melting point > 300°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm) 9.76 (d, J = 4.6 Hz, 2H, H-2, 18), 8.99 (d, J = 4.6 Hz, 4H, H-3, 17), 8.86 (m, 4H, H-7, 8, 12, 13), 8.16-7.97 (m, 6H, H-o-phenyl), 7.20-7.05 (m, 6H, H-m-phenyl), 4.05-3.96 (m, 6H, H-1'), 3.66-3.53 (m, 6H, H-2'), 3.41-3.32 (m, 6H, H-3'), 3.28-3.20 (m, 6H, H-4'), 3.19-3.10 (m, 6H, H-5'), 3.05-2.98 (m, 6H, H-6'), 2.92 (s, 9H, H-7'), 0.64 (s, 9H, $Si(CH_3)_3$). ^{13}C-NMR ($CDCl_3$, 75.3 MHz) δ (ppm) 158.7 (C-*p*-phenoxy), 151.6 (C-4,16), 150.3 (C-6, 9, 11, 14), 150.1 (C-1,19), 135.3 (C-*o*-phenoxy), 135.2 (C-1-phenoxy), 132.2 (C-3,17), 131.8 (C-7,8,12,13), 130.2 (C-2,18), 120.2 (C-5,10,15), 112.3 (C-*m*-phenoxy), 98.5 (C-20), 95.6 ($\underline{C}CSiMe_3$), 93.7 ($C\underline{C}SiMe_3$), 69.8-65.5 (C-1',2',3',4',5',6'), 54.2 (C-7'), 0.0 ($SiCH_3$). MS (MALDI-TOF) m/z: $[M]^+$ Calcd for $C_{64}H_{74}N_4O_{12}SiZn$ 1182.44; Found 1182.44.

Dimer 1

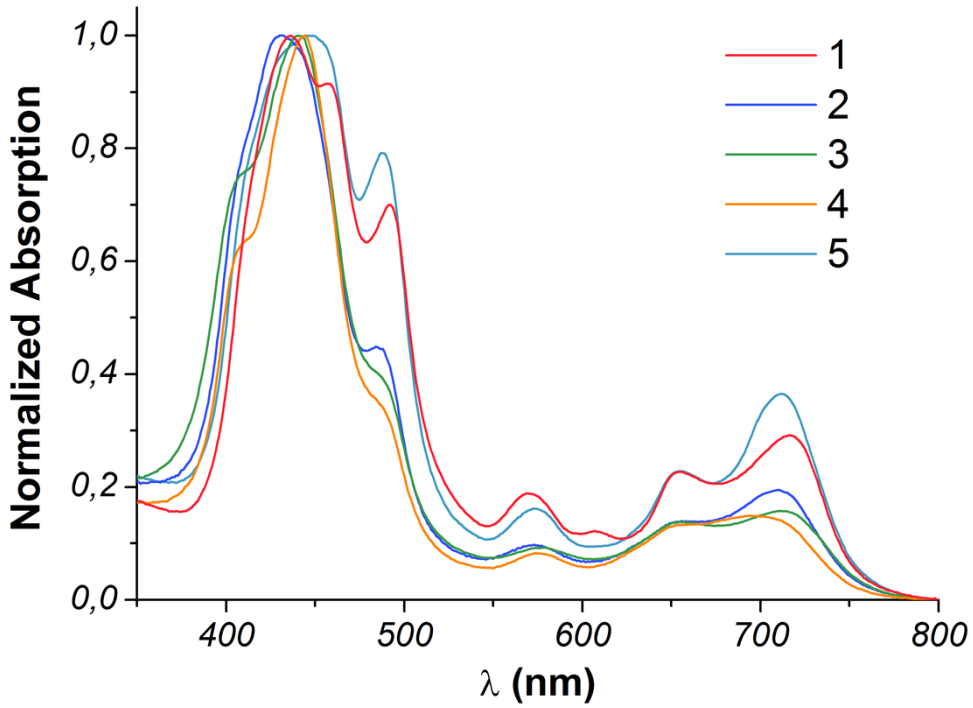


5-allyl-10,15,20-tri(para-2-(2-(2-methoxyethoy)ethoxy)ethanoxyphenyl porphyrin zinc complex (175 mg, 147 µmol, 1 eq.) was dissolved in dry THF (80 mL) and a solution of tetrabutylammonium fluoride at 1 M in THF (0.112 mL, 112 µmol, 2.6 eq.) was slowly added. The reaction was stirred at room temperature for 30 min. Calcium chloride was then added to quench the reaction. The organic layer was concentrated under vacuum, the residue

was dissolved in CH_2Cl_2 and the solution was washed with repeatedly with water and dried over anhydrous $MgSO_4$ then filtered. The generated product was combined with copper (I) iodide (6.5 mg, 0.03 mmoL, 0.23 eq.) and dichlorobis-(triphenylphosphine)-palladium (II) (23.8 mg, 34 µmol, 0.23 eq.) in anhydrous THF (20 mL) and dry Et_3N (20 mL). The reaction mixture was stirred at room temperature overnight and poured into an erlenmeyer containing H_2O and CH_2Cl_2. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were washed with water, dried over anhydrous $MgSO_4$, filtered and concentrated under vacuum. The product was purified by silica gel chromatography using DCM/EtOH (95:5). The desired porphyrin was finally isolated after three crystallizations in AcOEt/n-heptane as purple powder (156 mg, 60%). Melting point > 300°C. ^{1}H−NMR (THF-d_5, 300 MHz) δ (ppm): 9.82 (d, 4.2 Hz, 4H, H-3, H-7), 8.91 (d, 4.2 Hz, 4H, H-2, H-8), 8.68 (s, 8H, H-12, H-13, H-17, H-18), 7.99-7.91 (m, 12H, o-phenoxy), 7.19-7.13 (m, 12H, m-phenoxy), 4.20 (broad, 12H, H-1'), 3.82 (broad, 12H, H-2''), 3.60-3.37 (m, 48H, H-3', H-4', H-5', H-6'), 3.18 (s, 18H, H-7'). ^{13}C−NMR (THF-d_8, 75.3 MHz) δ (ppm): 158.5 (C-*p*-phenoxy), 152.8 (C-4,6), 150.7 (C-1, C-9), 149.7-149.5 (C-11, C-14, C-16, C-19), 134.85 (C-*o*-phenoxy), 134.76 (C-1 phenoxy), 132.3 (C-2, C-8), 131.3-130.8 (C-12, C-13, C-17, C-18), 129.56 (C-3, C-7), 123.0 (C15), 121.6 (C-10, C-20), 112.0 (C-*m*-phenoxy), 96.6 (C-5), 87.4 (triple bond), 81.0 (triple bond), 71.6-67.3 (C-3', C-4', C-5', C-6'), 69.36 (C-2'), 67.28 (C-1'), 57.6 (C-7'). MS (MALDI-TOF) m/z: $[M]^+$ Calcd for $C_{112}H_{130}N_8O_{24}Zn_2$ 2218.78; Found $[M]^+$ 2218.79. HRMS (MALDI-TOF) (m/z) $[M]^+$ Calcd for $C_{112}H_{130}N_8O_{24}Zn_2$ 2218.7781; Found 2218.7794.

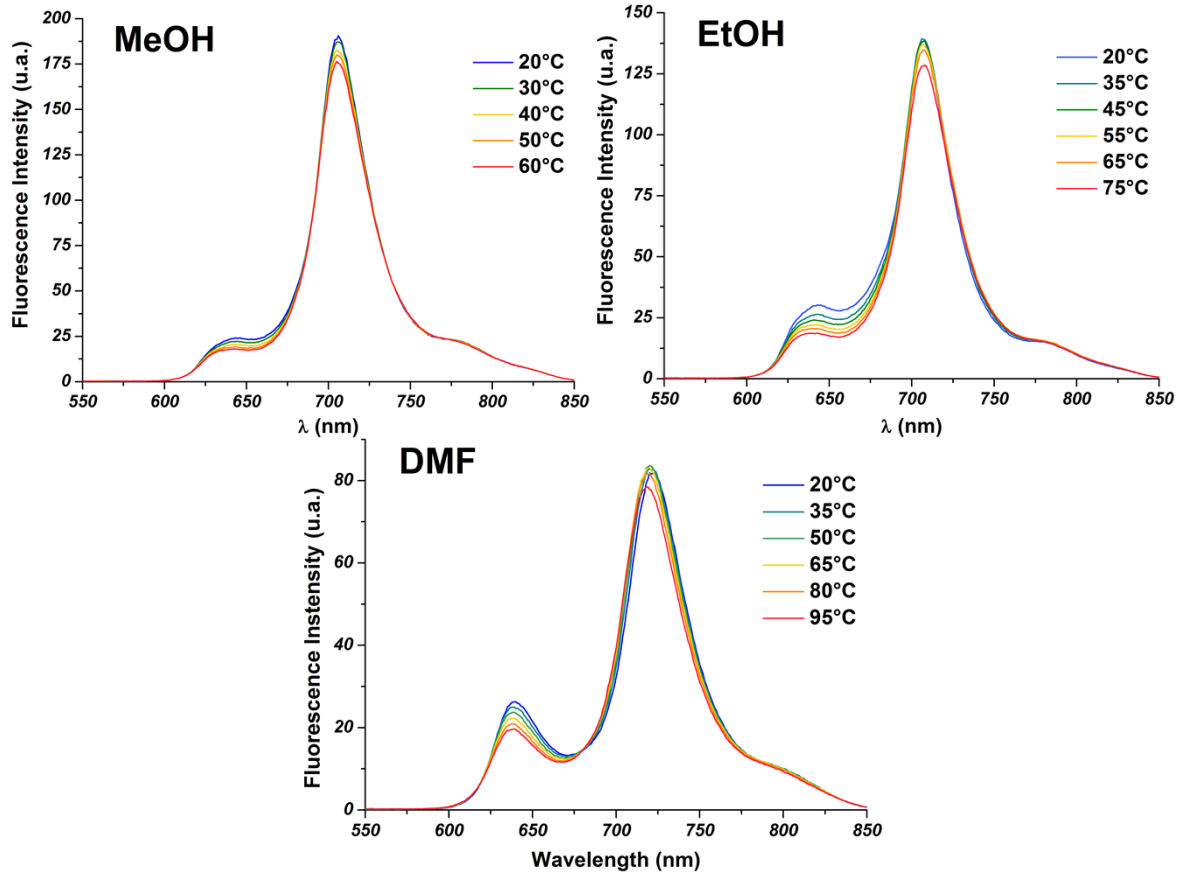
Absorption of dimers 1-5 in water

UV/Vis spectra were recorded at 10^{-5} M in the desired solvent. Spectra of compounds **2-5** have already been presented and discussed in reference [1] and are reproduced here for practical reasons.



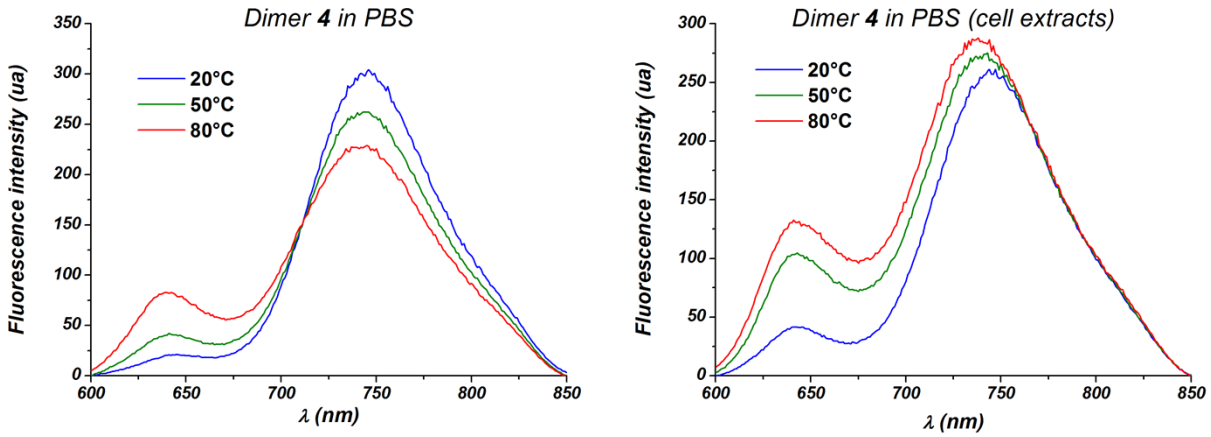
Emission of dimer 2 in polar organic solvents and influence of temperature.

Similar results were observed for dimers **3-5**. All compounds were excited at 450 nm.



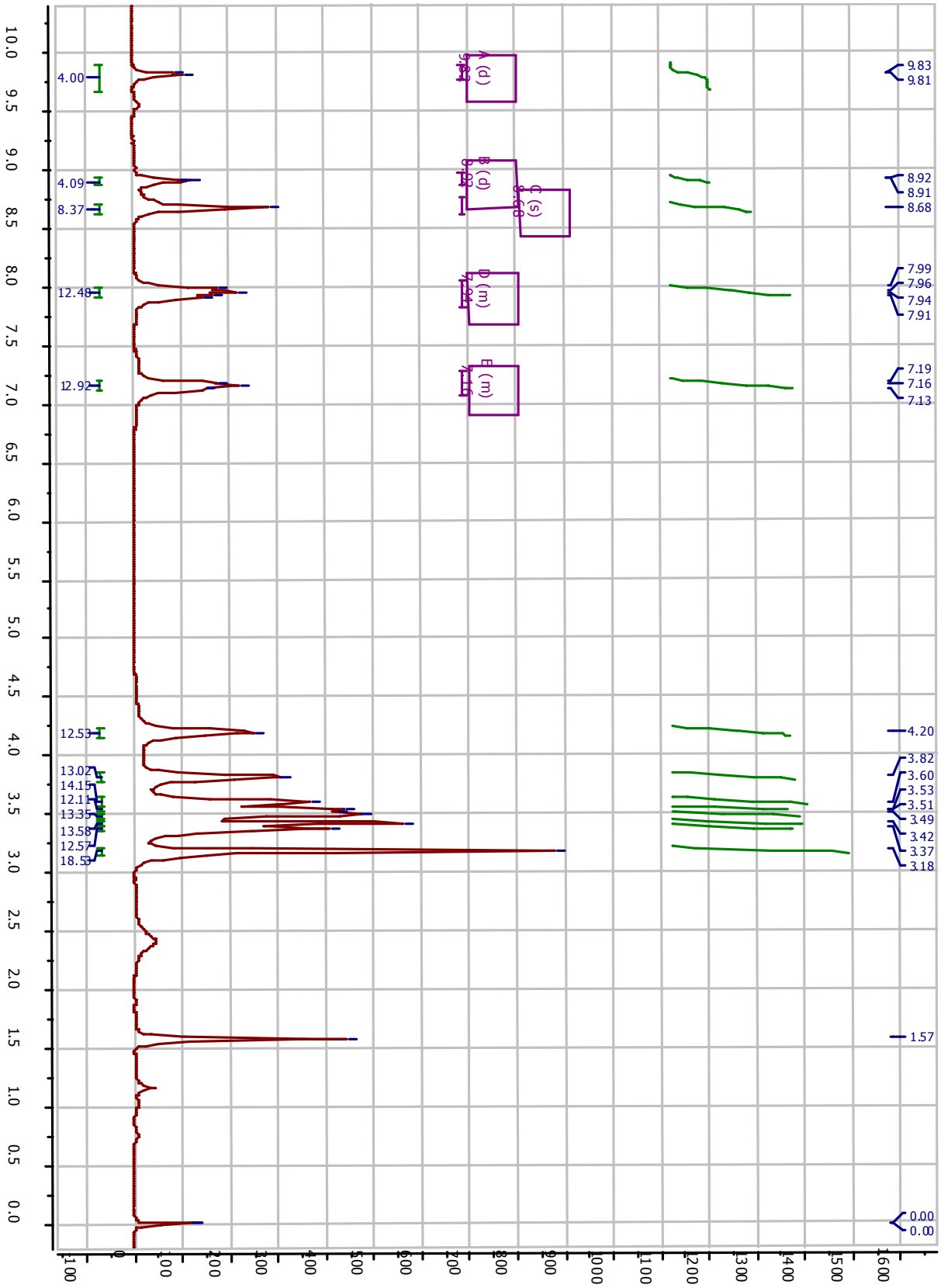
Competition with biological elements in PBS.

Two solutions of dimer **4** at 1 µM in PBS were prepared. Two solutions of dimer 4 at 1 µM in PBS were prepared. To one of them was added 5 µL of a buffer (HEPES pH 7.6 20mM, glycerol 20%, NaCl 500mM, MgCl 2 1.5mM, EDTA 0.2 mM, DTT 1mM and an antiprotease cocktail) containing about 50 µg of nuclear proteins from U-87 MG (ATCC® HTB-14) cells, a glioblastoma cell line. The other solution was diluted accordingly to obtain final solutions of identical concentrations. The fluorescence emission spectra were taken in the usual conditions.

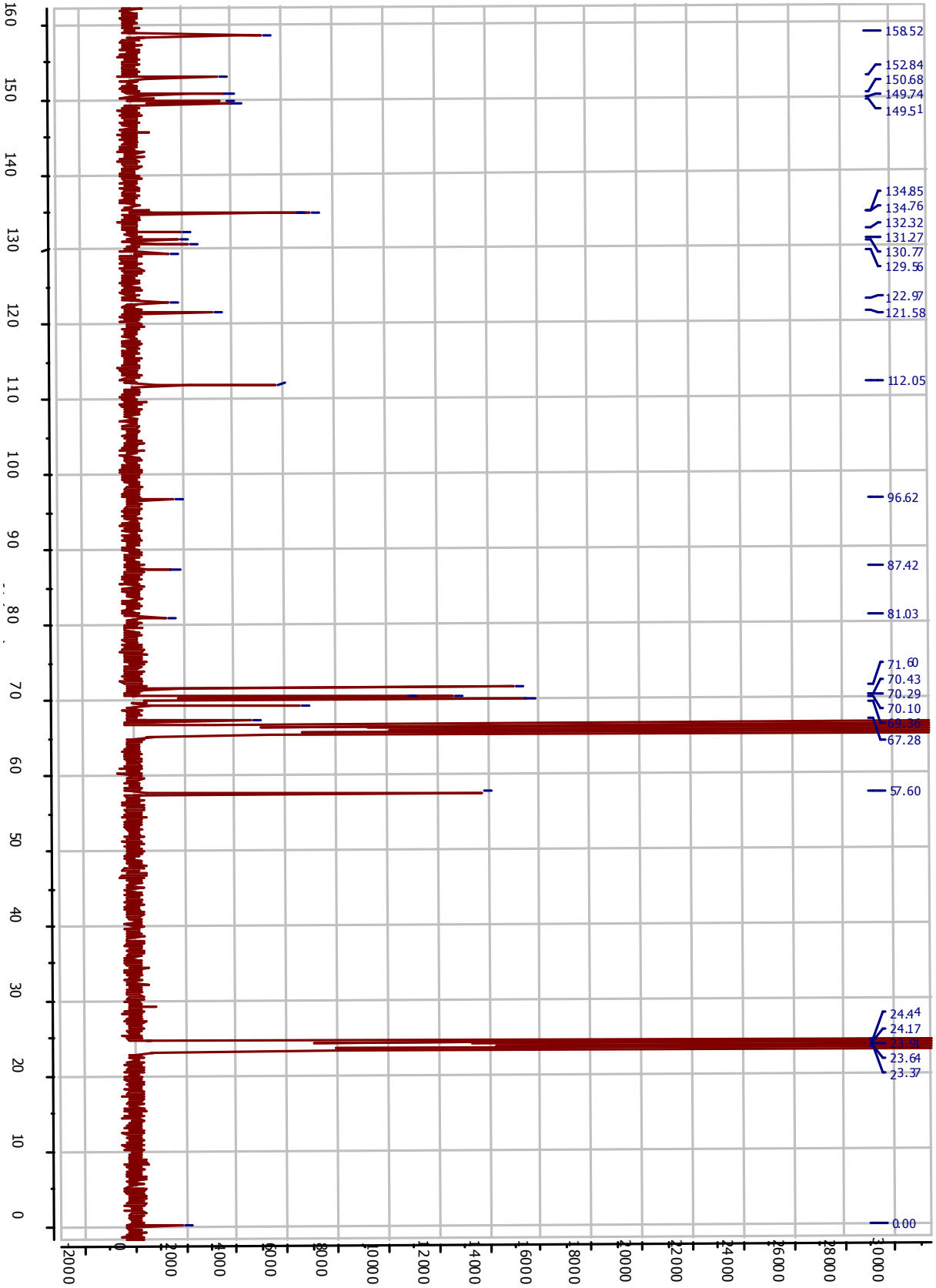


As anticipated, the addition of biological compounds completely alters the fluorescence response of the compounds as illustrated by the loss of the isoemissive point at 715 nm. This result shows the necessity of the encapsulation of our compounds for applications in biological media.

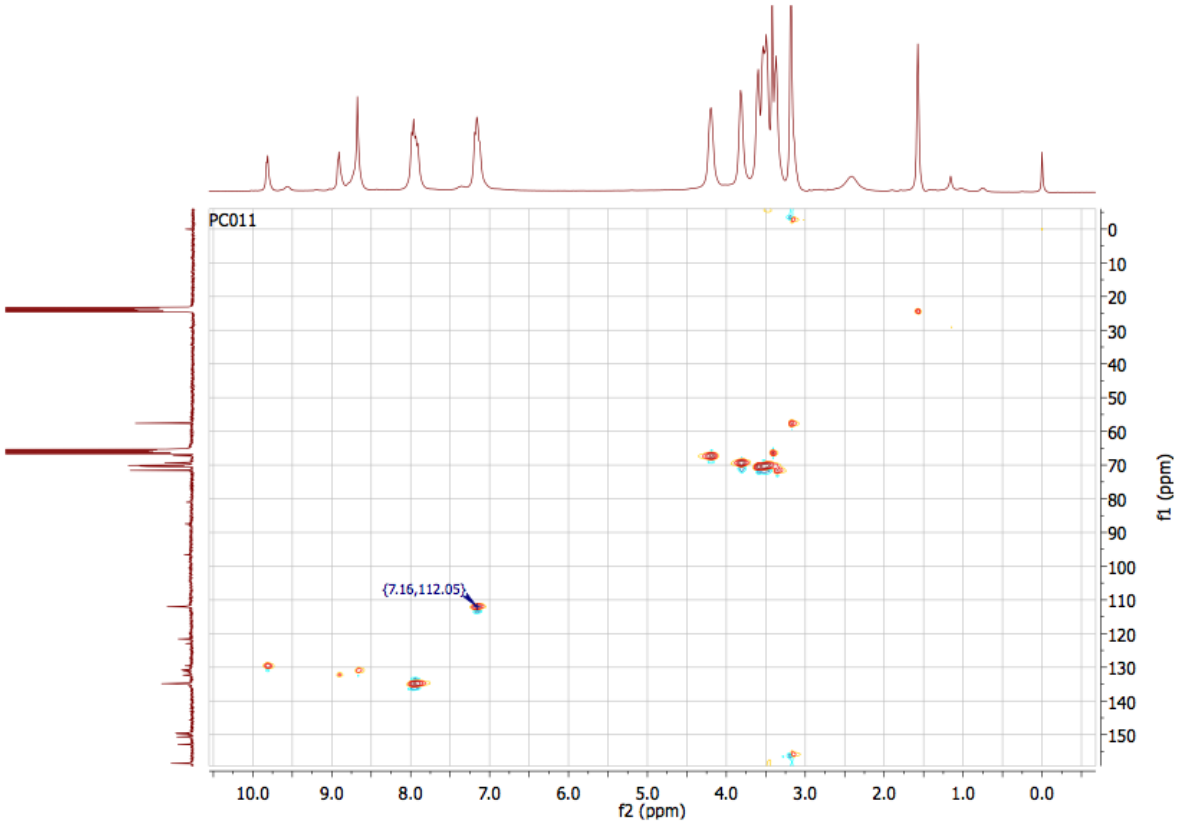
Dimer 1 ^{1}H NMR



Dimer 1 ^{13}C NMR



Dimer 1 HMQC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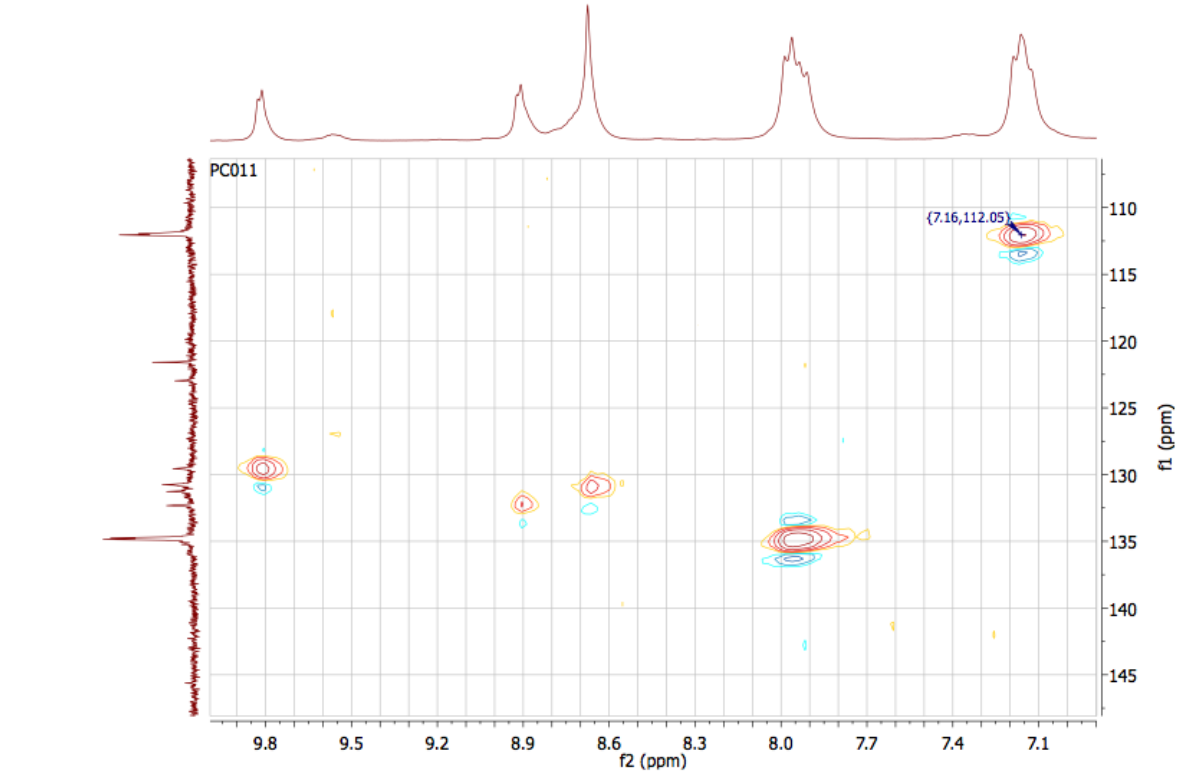
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f2 (ppm)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
f1 (ppm)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f1 (ppm)
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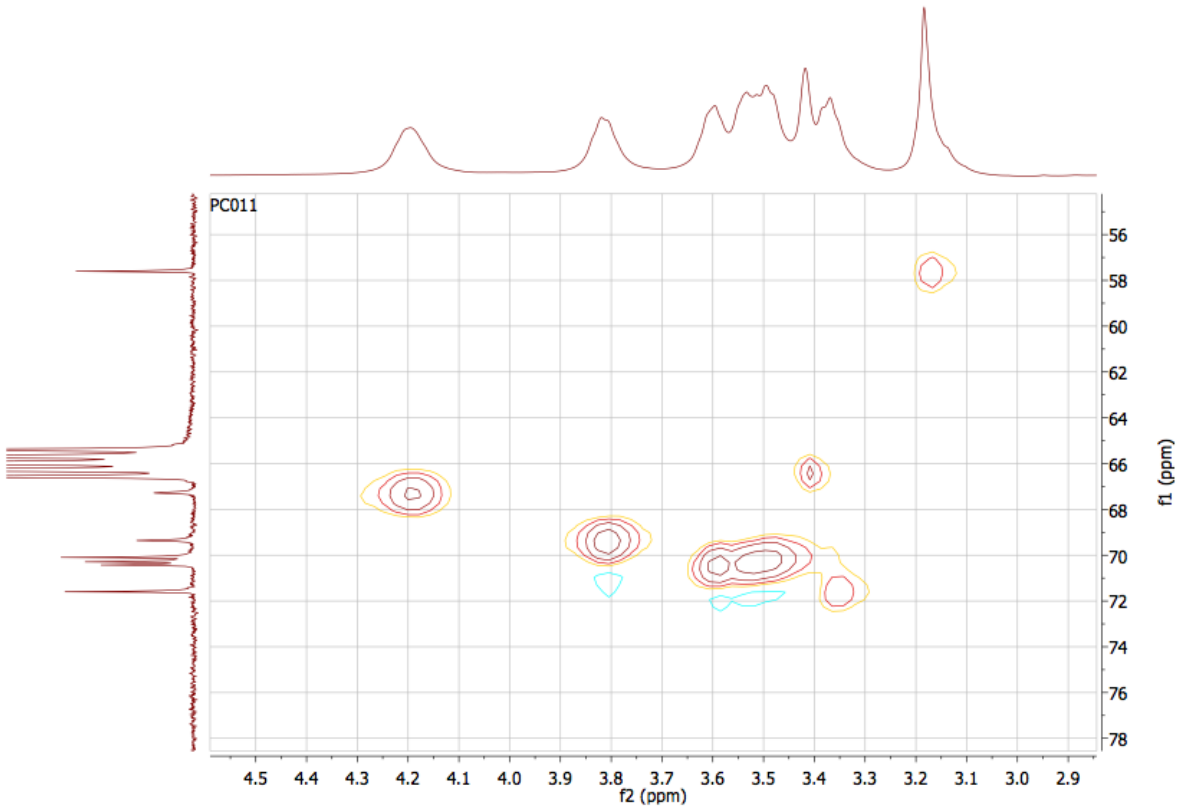
7.7
7.4
7.1
f2 (ppm)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66
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72
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76
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4.1
4.0
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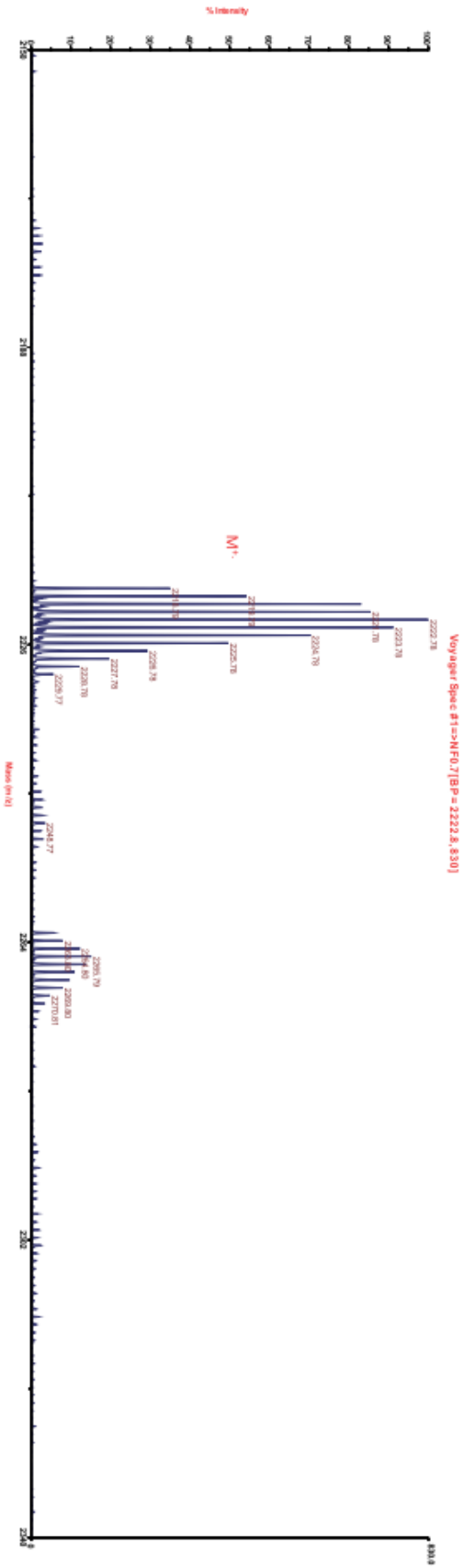
3.1
3.0
2.9
f2 (ppm)

Dimer 1 MS (MALDI-TOF)



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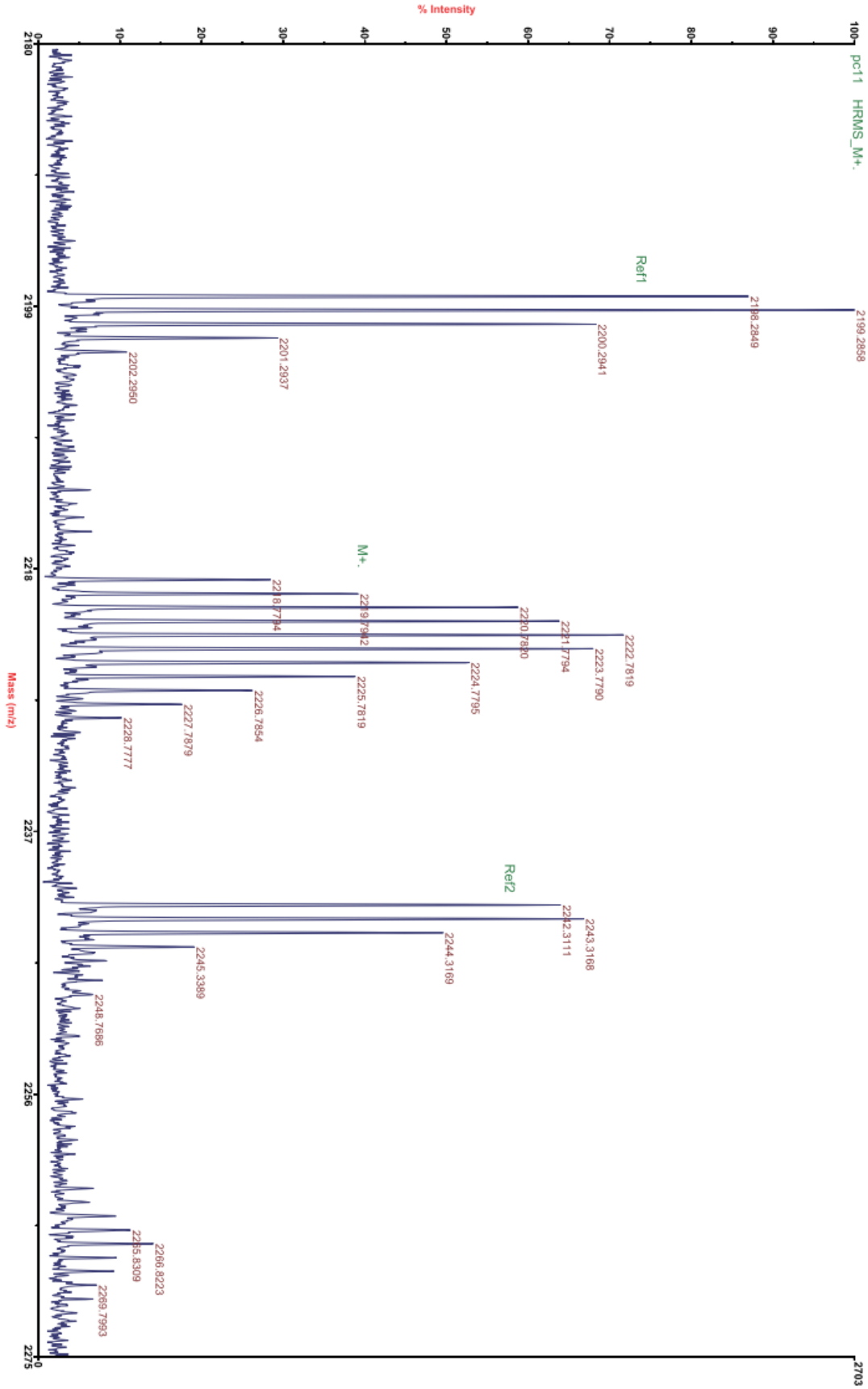

[i] G. Garcia, F. Hammerer, F. Poyer, S. Achelle, M.-P. Teulade-Fichou and P. Maillard, *Bioorg. Med. Chem.*, 2012, **21**, 153-165.