Supporting Information for

Targeting Cancer Cells with Folic Acid-Iminoboronate Fluorescent Conjugates

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1. General Information

All reactions were performed in oven-dried glassware or in sterilized 1.5-2 mL eppendorf tubes. Reaction mixtures were analyzed by thin layer chromatography TLC using coated silica gel plates (Merck, aluminium sheets, silica gel 60 F254), and visualization of TLC spots was effected using UV and phosphomolybdic acid solution. NMR spectra were recorded in a Bruker AMX 300 or 400 using $CDCl_3$, D_2O or DMSO as solvent and $(CH_3)_4Si$ (^1H) as internal standard. All coupling constants (*J* values) are expressed in Hz. All reagents were purchased from either Aldrich or Alfa-Aesar and used without further purification. UV-visible spectra were recorded on a Shimadzu UV-1603 UV-visible spectrometer with a temperature controlled (25 ºC) cell holder. Mass spectra and HRMS were performed in a FTICR-MS Bruker Daltonics Apex Qe, Apollo II combi source, 7 Tesla Magnet, operated in positive ionization mode.

2. Chemistry Results

2.1 – Boronated core compound **4** synthesis

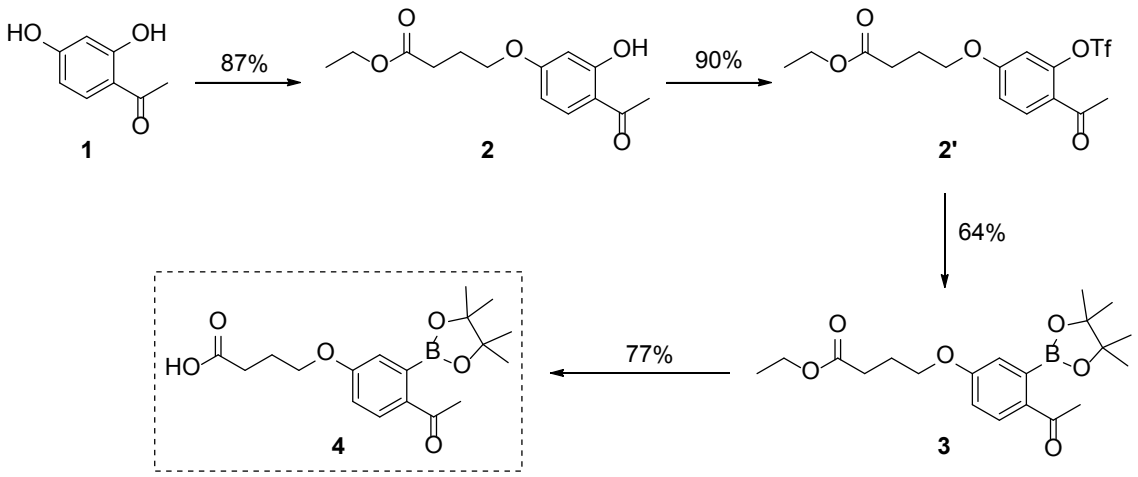

Figure S1 – Synthesis of compound **4**

2.1.1 – Synthesis of compound **2**

Literature basis - Al-Smadi, M.; Hanold, N.; Kalbitz, H.; Meier, H.; *Synthesis*, **2009**, 15, 2539

In a round bottom flask (A), 2',4'-dihydroxyacetophenone (5.0 g, 32.9 mmol) and potassium carbonate (4.5 g, 32.9 mmol) were heated in acetone to reflux for 2 hours. In a separate round bottom flask (B), ethyl 4-bromobutyrate (4.7 mL, 32.9 mmol) and sodium iodide (5.4 g, 36.1 mmol) were stirred in acetone at room temperature for 2 hours. Then, flask B was transferred to flask A and refluxed for 20 hours. After that, acetone was evaporated to dryness and 200 mL of water were added and extracted with diethyl ether (3*200 mL). The solution was dried (Na_2SO_4), concentrated and purified by column chromatography (silica gel, hexane:diethyl ether (9:1)) to give a viscous oil that solidified below 20ºC; compound **2** (7.6 g, 87%)

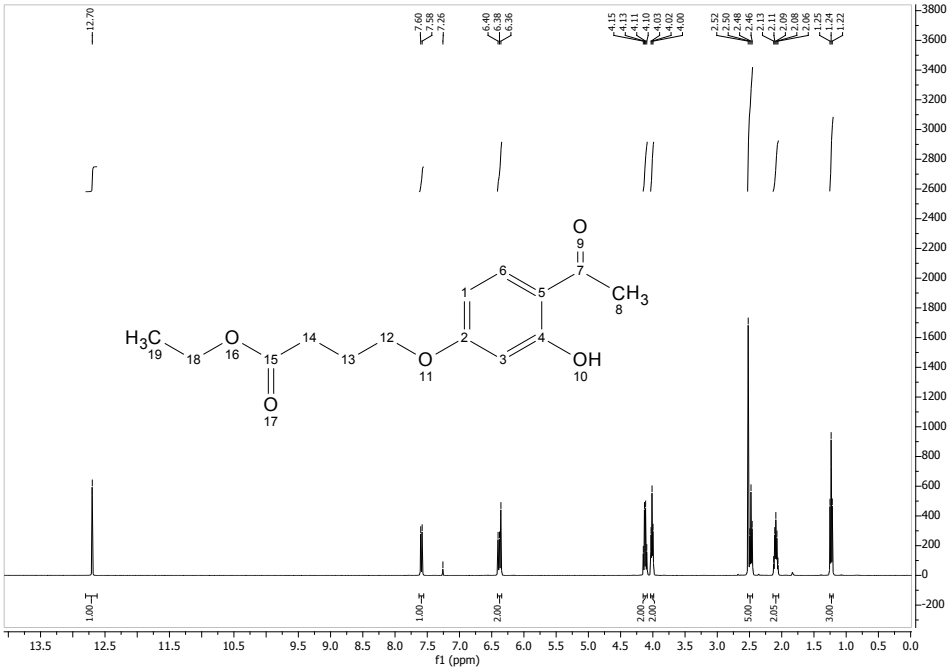

Figure S2 – ^{1}H-NMR for compound **2**

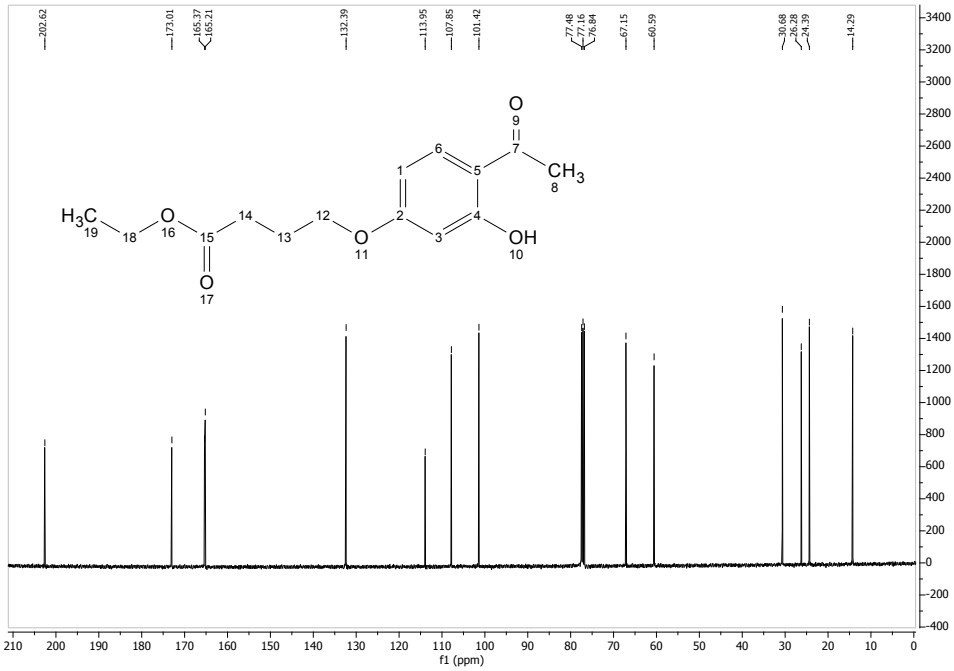

Figure S3 – ^{13}C-NMR of compound **2**

^{1}H NMR (400 MHz, $CDCl_3$): δ 12.70 (s, 1H, $C_{arom}O\underline{H}$ (10)), 7.59 (d, J = 8.9 Hz, 1H, $C\underline{H}_{arom}$ (6)), 6.39 (d, J = 8.9 Hz, 1H, $C\underline{H}_{arom}$ (1)), 6.36 (s, 1H, $C\underline{H}_{arom}$ (3)), 4.12 (q, J = 7.1 Hz, 2H, $OC\underline{H}_2CH_3$ (18)), 4.02 (t, J = 6.1 Hz, 2H, $OC\underline{H}_2CH_2$ (12)), 2.52 (s, 3H, $COC\underline{H}_3$ (8)), 2.48 (t, J = 7.2 Hz, 2H, $CH_2C\underline{H}_2CO$ (14)), 2.09 (p, J = 6.6 Hz, 2H, $CH_2C\underline{H}_2CH_2$ (13)), 1.24 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$ (19)).

^{13}C NMR (101 MHz, $CDCl_3$): δ 202.62 (s, $C\underline{C}OCH_3$ (7)), 173.01 (s, $CH_2\underline{C}OO$ (15)), 165.37 (s, $CH\underline{C}_{arom}OHC$ (4)), 165.21 (s, $(CH)_2\underline{C}_{arom}OCH_2$ (2)), 132.39 (s, $\underline{C}H_{arom}$ (6)), 113.95 (s, $CH\underline{C}_{arom}COCOH$ (5)), 107.85 (s, $\underline{C}H_{arom}$ (1)), 101.42 (s, $\underline{C}H_{arom}$ (3)), 67.15 (s, $O\underline{C}H_2CH_2$ (12)), 60.59 (s, $O\underline{C}H_2CH_3$ (18)), 30.68 (s, $CH_2\underline{C}H_2CO$ (14)), 26.28 (s, $CO\underline{C}H_3$ (8)), 24.39 (s, $CH_2\underline{C}H_2CH_2$ (13)), 14.29 (s, $CH_2\underline{C}H_3$ (19)).

HMRS (ESI): *m/z* calculated $[M+Na]^+$ = 289.10464, found $[M+Na]^+$ = 289.10334.

2.1.2 – Synthesis of compound **2'**

Literature basis - Luker, T.; Bonnert, R.; Paine, S.W.; Schmidt, J.; Sargent, C.; Cook, A.R.; Cook, A.; Gardiner, P.; Hill, S.; Weyman-Jones, C.; Patel, A.; Thom, S.; Thorne, P.; *J. Med. Chem.* **2011** , 54 (6), 1779

In a round bottom flask, N-Phenyltrifluoromethanesulfonimide (3.05 g, 8.5 mmol) was added portion wise to a solution of **1** (3.0 g, 11.3 mmol) and triethylamine (4.7 mL, 33.8 mmol) in DMF (27 mL) and the reaction stirred for 2 h. Water was added and the mixture extracted with diethyl ether. The organic layer was washed with water, LiCl (aq), brine, dried (Na_2SO_4), concentrated and purified by column chromatography (silica gel, hexane:diethyl ether (4:1 – 1:1)) to give **2'** as a white solid (3.9 g, 90%).

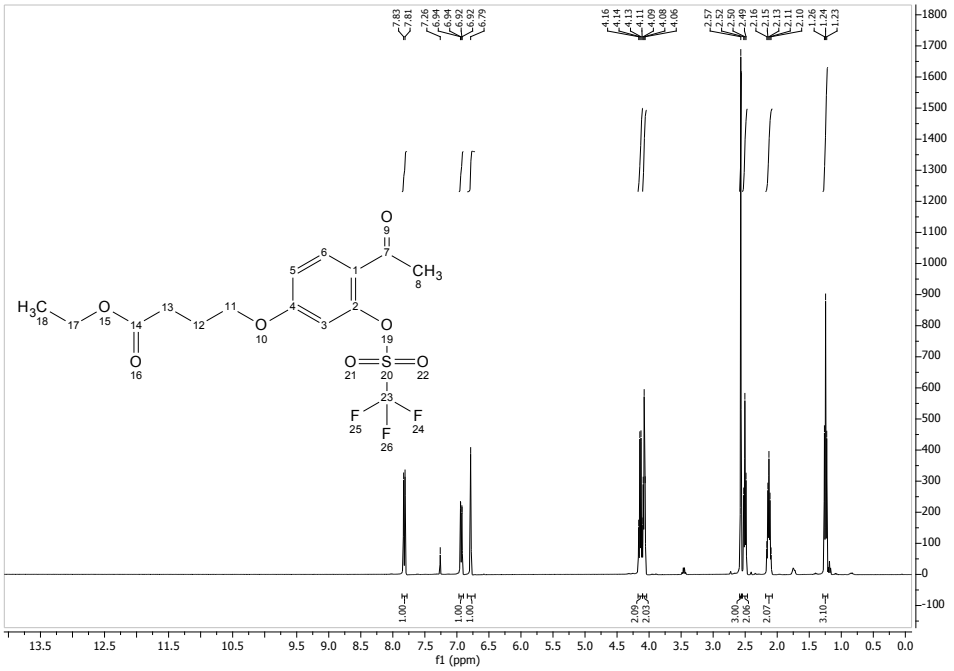

Figure S4 – ^{1}H-NMR for compound **2'**

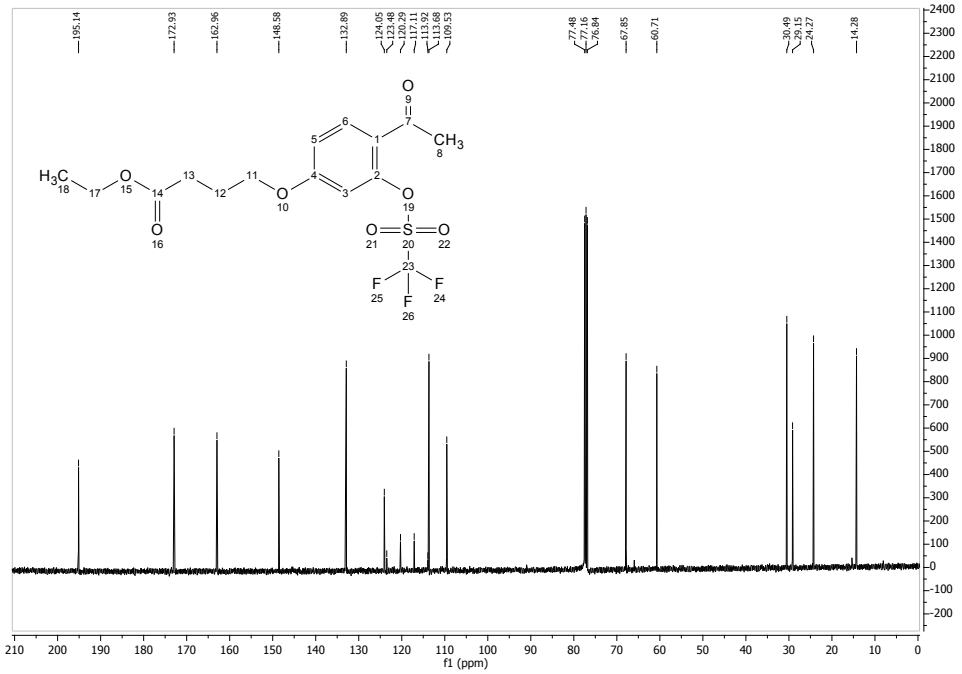

Figure S5 – ^{13}C-NMR of compound **2'**

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (d, *J* = 8.8 Hz, 1H, C<u>H</u>$_{arom}$ (6)), 6.93 (d, *J* = 8.8 Hz, 1H, C<u>H</u>$_{arom}$ (5)), 6.79 (s, 1H, C<u>H</u>$_{arom}$ (3)), 4.14 (q, *J* = 7.1 Hz, 2H, OC<u>H</u>$_2$CH$_3$ (17)), 4.08 (t, *J* = 6.1 Hz, 2H, OC<u>H</u>$_2$CH$_2$ (11)), 2.57 (s, 3H, COC<u>H</u>$_3$ (8)), 2.50 (t, *J* = 7.1 Hz, 2H, CH$_2$C<u>H</u>$_2$CO (13)), 2.13 (p, *J* = 6.6 Hz, 2H, CH$_2$C<u>H</u>$_2$CH$_2$ (12)), 1.24 (t, *J* = 7.1 Hz, 3H, CH$_2$C<u>H</u>$_3$ (18)).
^{13}C NMR (101 MHz, $CDCl_3$): δ 195.14 (s, C<u>C</u>OCH$_3$ (7)), 172.93 (s, CH$_2$<u>C</u>OO (14)), 162.96 (s, (CH)$_2$<u>C</u>$_{arom}$OCH$_2$ (4)), 148.58 (s, CH<u>C</u>$_{arom}$OTfC (2)), 132.89 (s, <u>C</u>H$_{arom}$ (6)), 124.05 (s, CH<u>C</u>$_{arom}$COCOTf (1)), 118.70 (q, SO$_2$<u>C</u>F$_3$ (23)), 113.68 (s, <u>C</u>H$_{arom}$ (5)), 109.53 (s, <u>C</u>H$_{arom}$ (3)), 67.85 (s, O<u>C</u>H$_2$CH$_2$ (11)), 60.71 (s, O<u>C</u>H$_2$CH$_3$ (17)), 30.49 (s, CH$_2$<u>C</u>H$_2$CO (13)), 29.15 (s, CO<u>C</u>H$_3$ (8)), 24.27 (s, CH$_2$<u>C</u>H$_2$CH$_2$ (12)), 14.28 (s, CH$_2$<u>C</u>H$_3$ (18)).
HMRS (ESI): *m/z* calculated $[M+K]^+$ = 437.027867, found $[M+K]^+$ = 437.027219.

2.1.3 – Synthesis of compound **3**

Literature basis - Patent: Anacor Pharmaceuticals, Inc.; GlaxoSmithKline; US2010/256092; (**2010**); (A1) English

In a flame dried round bottom flask, under inert atmosphere, **2'** (0.5 g, 1.25 mmol), bis(pinacolato)diboron (0,6 g, 2.51 mmol), [1,1'-bis(diphenylphosphino)ferrocene]palladium (II) chloride (0,1 g; 0,125 mmol) and sodium acetate (0,3 g, 3,76 mmol) were flushed 3 times with Argon. Then, 8 mL of previously distilled dioxane (8 mL) were added and degassed for 10 minutes with bubbling Argon. The reaction mixture was heated at 95 ºC for 4 hours, after which the solvent was concentrated in vacuo and the crude purified by flash column chromatography (silica gel, dichloromethane:diethyl ether (19:1 – 9:1)) to give **3** as a white solid (0,3 g, 64%).

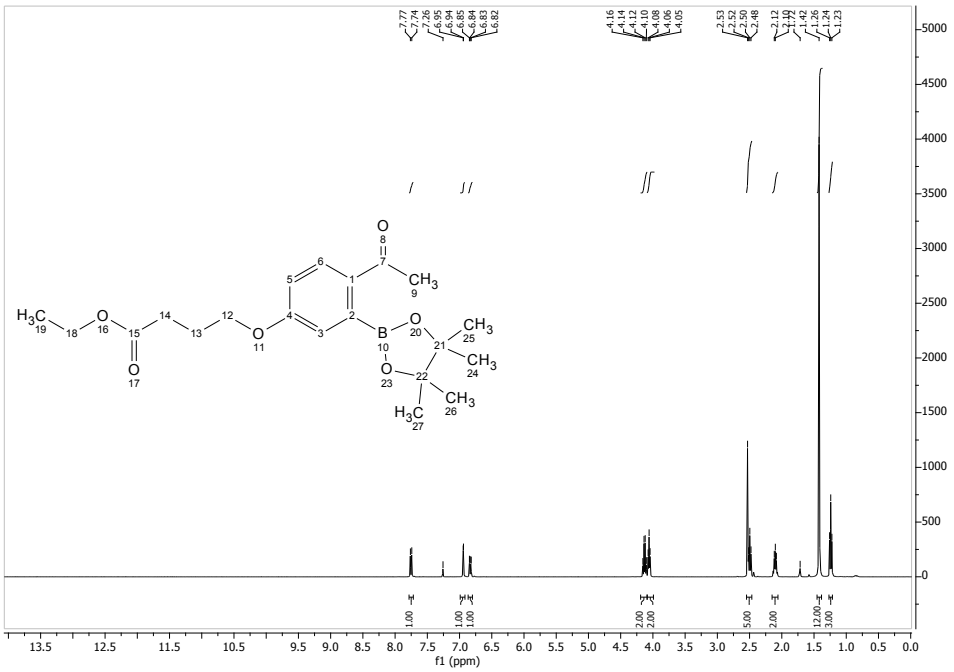

Figure S6 – ^{1}H-NMR for compound **3**

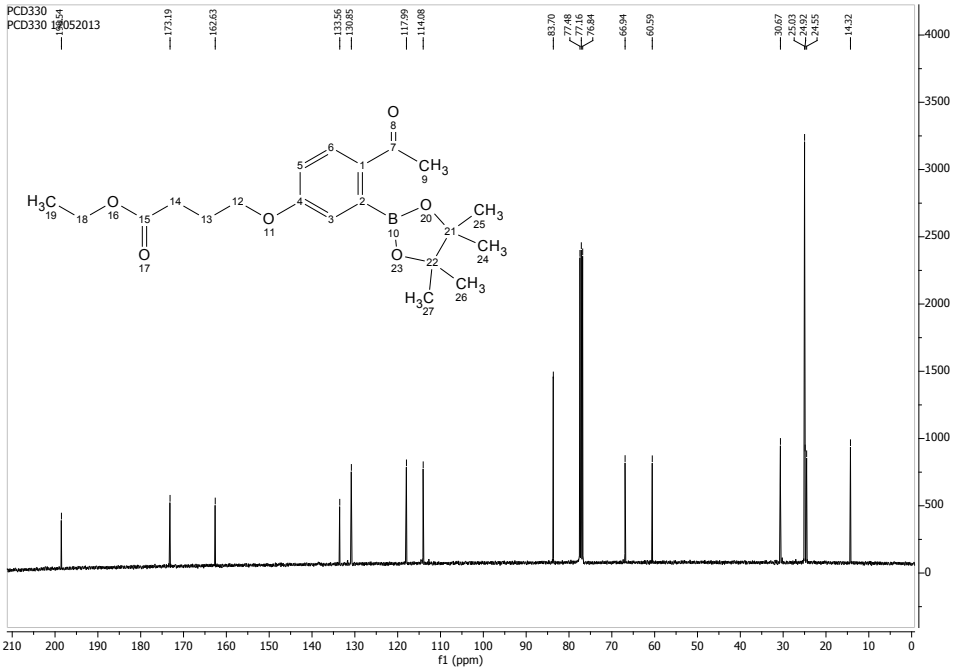

Figure S7 – ^{13}C-NMR of compound **3**

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 (d, *J* = 8.6 Hz, 1H, C$\underline{H}_{arom}$ (6)), 6.93 (s, 1H, C$\underline{H}_{arom}$ (3)), 6.82 (d, *J* = 8.6 Hz, 1H, C$\underline{H}_{arom}$ (5)), 4.11 (q, *J* = 7.1 Hz, 2H, OC$\underline{H}_2$CH$_3$ (18)), 4.04 (t, *J* = 6.0 Hz, 2H, OC$\underline{H}_2$CH$_2$ (12)), 2.54 – 2.45 (m, 5H, COC$\underline{H}_3$ (9), CH$_2$C$\underline{H}_2$CO (14)), 2.12 – 2.05 (m, 2H, CH$_2$C$\underline{H}_2$CH$_2$ (13)), 1.41 (s, 12H, CC$\underline{H}_3$ (24-27)), 1.23 (t, *J* = 7.1 Hz, 3H, OCH$_2$C$\underline{H}_3$ (19)).
^{13}C NMR (101 MHz, $CDCl_3$): δ 198.54 (s, C$_{arom}\underline{C}$OCH$_3$ (7)), 173.16 (s, CH$_2\underline{C}$OO (15)), 162.57 (s, $\underline{C}_{arom}$OCH$_2$ (4)), 133.47 (s, CH$_3$CO$\underline{C}_{arom}$ (1)), 130.79 (s, $\underline{C}H_{arom}$ (6)), 117.94 (s, $\underline{C}H_{arom}$ (3)), 113.98 (s, $\underline{C}H_{arom}$ (5)), 83.63 (s, $\underline{C}$(CH$_3$)$_2$ (21,22)), 66.87 (s, O$\underline{C}$H$_2$CH$_2$ (12)), 60.54 (s O$\underline{C}$H$_2$CH$_3$ (18)), 30.60 (s, CH$_2\underline{C}$H$_2$CO (14)), 24,95 (s, C($\underline{C}$H$_3$)$_2$ (24-27)), 24.54 (s, $\underline{C}$H$_3$COC$_{arom}$ (9)), 24.47 (s, CH$_2\underline{C}$H$_2$CH$_2$ (13)), 14.32 (s, OCH$_2\underline{C}$H$_3$(19)).
^{13}C-HMBC 138.5 (s, $\underline{C}_{arom}$B (2))
HMRS (ESI): *m/z* calculated $[M+H]^+$ = 377.213364, found $[M+H]^+$ = 377.211988.

2.1.4 – Synthesis of compound **4**

Literature basis - Griffin, D.R.; Andrea M. Kasko, A.M.; *J. Am. Chem. Soc.* **2012**, 134, 13103

In a round bottom flask, under inert atmosphere, **3** (1.3 g, 3.4 mmol) was stirred in a solution of trifluoroacetic acid (1 mL) and water (9.5 mL) at 90 ºC for 3 hours, after which the solvent was concentrated in vacuo. The product was purified by flash column chromatography (silica gel, dichloromethane:diethyl ether 9:1 – 1:1) and recrystallized in diethyl ether to afford **4** (0.9 g, 77%).

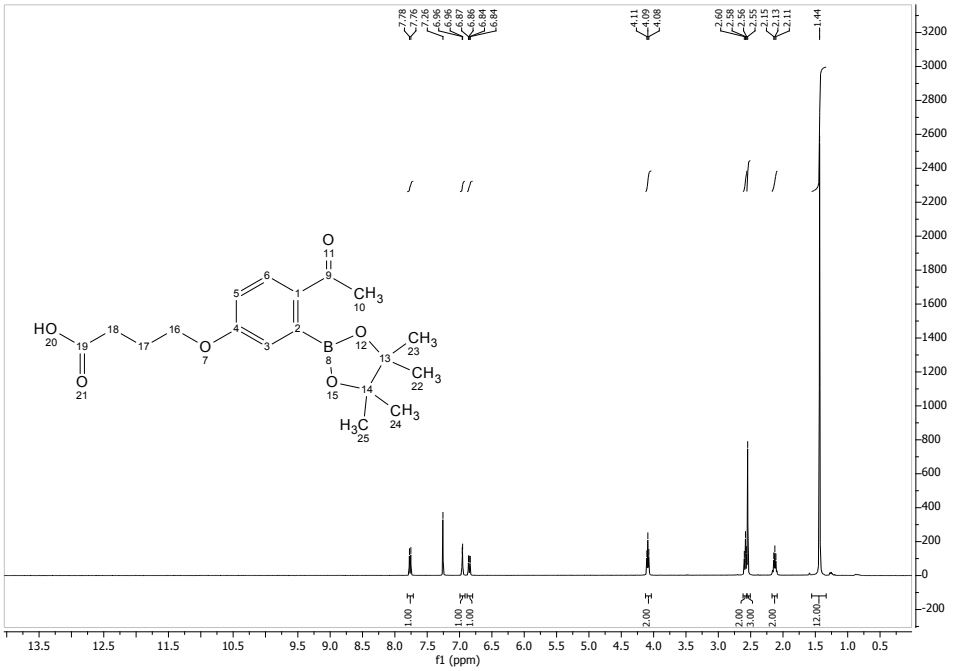

Figure S8 – ^{1}H-NMR for compound **4**

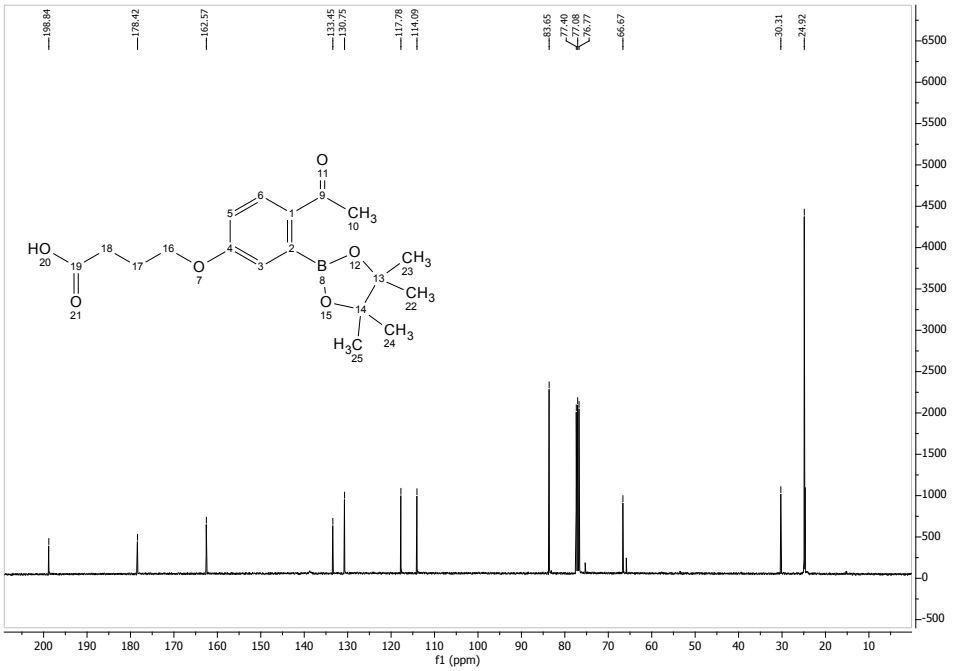

Figure S9 – ^{13}C-NMR for compound **4**

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (d, *J* = 8.6 Hz, 1H, C<u>H</u>$_{arom}$ (6)), 6.96 (d, *J* = 2.3 Hz, 1H, C<u>H</u>$_{arom}$ (3)), 6.85 (dd, *J* = 8.6, 2.3 Hz, 1H, C<u>H</u>$_{arom}$ (5)), 4.09 (t, *J* = 6.0 Hz, 2H, OC<u>H</u>$_2$CH$_2$ (16)), 2.58 (t, *J* = 7.1 Hz,2H, CH$_2$C<u>H</u>$_2$COOH (18)), 2.55 (s, 3H, COC<u>H</u>$_3$ (10)), 2.12 – 2.05 (m, 2H, CH$_2$C<u>H</u>$_2$CH$_2$ (17)), 1.44 (s, 12H, CC<u>H</u>$_3$ (22-25)).

^{13}C NMR (101 MHz, $CDCl_3$): δ 198.92 (s, C$_{arom}$<u>C</u>OCH$_3$ (9)), 178.49 (s, CH$_2$<u>C</u>OO (19)), 162.64 (s, <u>C</u>$_{arom}$OCH$_2$ (4)), 133.53 (s, CH$_3$CO<u>C</u>$_{arom}$ (1)), 130.83 (s, <u>C</u>H$_{arom}$ (6)), 117.86 (s, <u>C</u>H$_{arom}$ (3)), 114.16 (s, <u>C</u>H$_{arom}$ (5)), 83.73 (s, <u>C</u>(CH$_3$)$_2$ (13,14)), 66.75 (s, O<u>C</u>H$_2$CH$_2$ (16)), 30.39 (s, CH$_2$<u>C</u>H$_2$CO (18)), 24.99 (s, C(<u>C</u>H$_3$)$_2$ (22-25)), 24.77 (s, CO<u>C</u>H$_3$ (10)), 24.28 (s, CH$_2$<u>C</u>H$_2$CH$_2$ (17)).

^{13}C-HMBC 138.7 (s, <u>C</u>$_{arom}$B (2))

HMRS (ESI): *m/z* calculated $[M+H]^+$ = 349.182029, found $[M+H]^+$ = 349.181109.

2.2 – Fluorescent boronated species

2.2.1 – Synthesis of boronate ester 5'

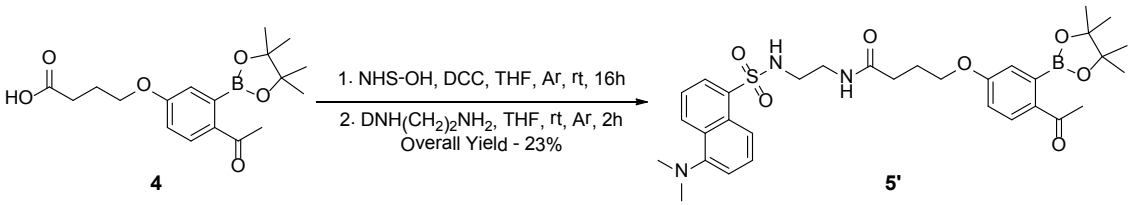

Figure S10 – Synthesis of compound **5'**

2.2.1.1 – Synthesis of 2-Dansylaminoethylamine (**DNH(CH$_2$)$_2$NH$_2$**)

Literature basis - Sun, W.; Bandmann, H., Schrader, T.; *Chem. Eur. J.* **2007**, 13, 7701

In a round bottom flask, 1,2-ethylenediamine (7.4 mL, 111 mmol) was diluted in dichloromethane previously distilled over CaH_2 (25 mL) and cooled to 0 ºC, while stirring. A solution of dansyl chloride (1.0 g, 3.7 mmol) in dichloromethane 16 mL was added dropwise. The mixture was stirred while warming to room temperature. It was subsequently acidified with HCl (1 M) and then extracted with dichloromethane (3x20 mL). The organic layer was dried ($MgSO_4$) and concentrated to afford **DNH(CH$_2$)$_2$NH$_2$** as a light yellow solid (1.02 g, 94%).

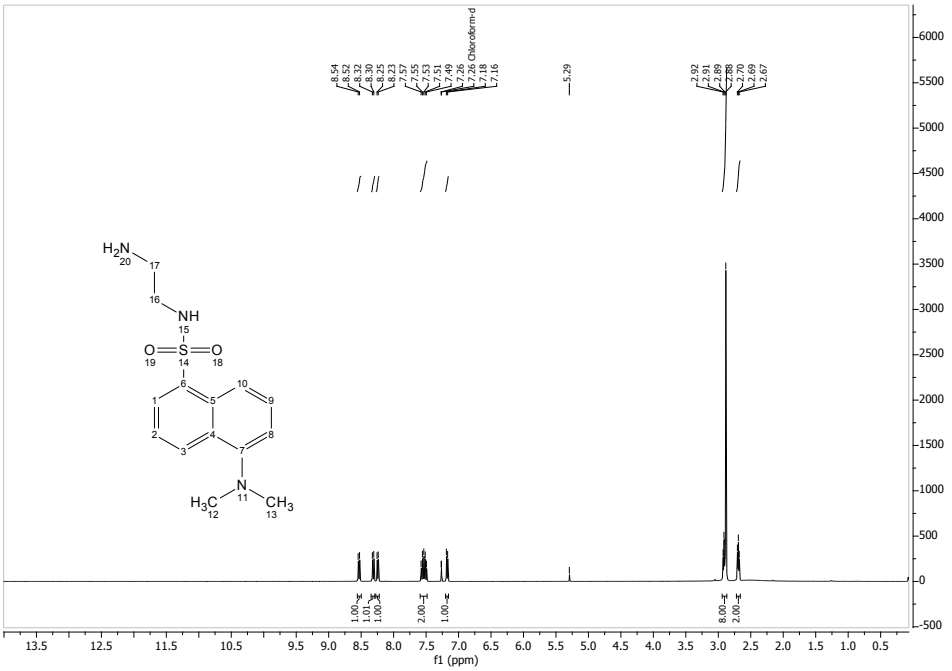

Figure S11 – ^{1}H-NMR for compound **DNH(CH$_2$)$_2$NH$_2$**

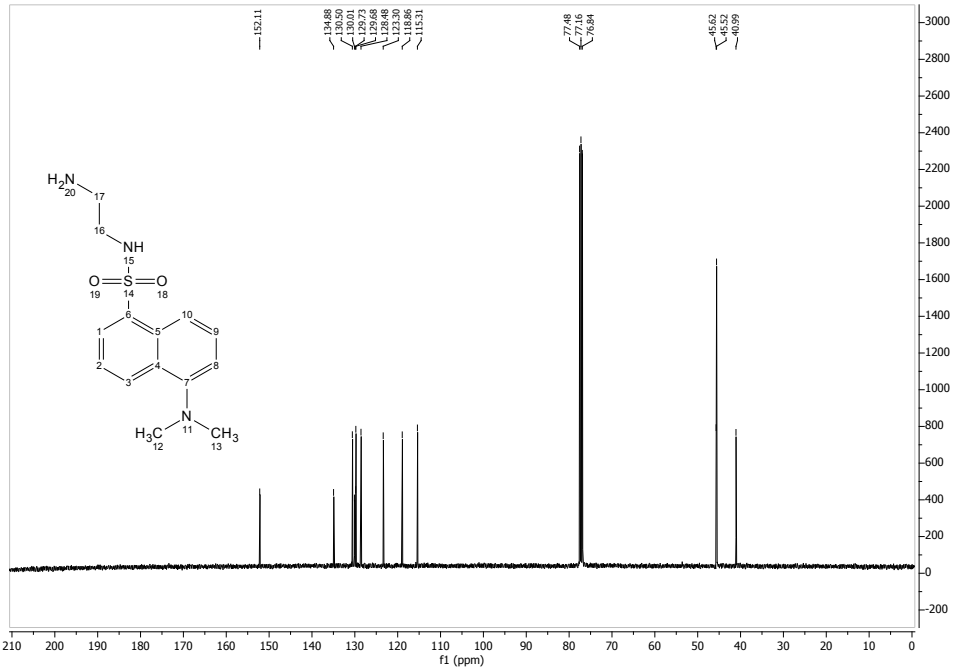

Figure S12 – ^{13}C-NMR for compound **DNH(CH$_2$)$_2$NH$_2$**

^{1}H NMR (400 MHz, CDCl$_3$): δ 8.53 (d, *J* = 8.5 Hz, 1H), 8.31 (d, *J* = 8.5 Hz, 1H), 8.24 (d, *J* = 7.5 Hz, 1H), 7.57 – 7.49 (m, 2H), 7.17 (d, *J* = 7.5 Hz, 1H), 2.93 – 2.86 (m, 8H), 2.69 (t, *J* = 5.7 Hz, 2H).
^{13}C NMR (101 MHz, CDCl$_3$): δ 152.11 (s), 134.88 (s), 130.50 (s), 130.01 (s), 129.73 (s), 129.68 (s), 128.48 (s), 123.30 (s), 118.86 (s), 115.31 (s), 45.62 (s) 45.52 (s), 40.99 (s).
NMR characterization in accordance with the one described in literature

2.2.1.2 – Amidation to afford boronate ester **5'**

Literature basis - Park, S. Y.; Oh, K. T.; Oh, Y. T.; Oh, N. M.; Youn, Y. S.; Lee, E. S. *Chem. Commun*, **2012**, 48, 2522

In a round bottom flask, under inert atmosphere, **4** (100 mg, 0.29 mmol), *N,N'*-dicyclohexylcarbodiimide (119 mg, 0.57 mmol) and *N*-hydroxysuccinimide (66 mg, 0.29 mmol) were mixed at room temperature in 5 mL of previously distilled tetrahydrofuran and stirred overnight. DNH(CH$_2$)$_2$NH$_2$ (101 mg, 0.34 mmol) was added and the solution was stirred for 2 hours at room temperature. After which the solvent was concentrated in vacuo and redissolved in ethyl acetate, which was then washed with water and brine. The product was further purified by flash column chromatography (silica gel, ethyl acetate:hexane 1:1 – 1:0) to afford **5'** as a light yellow solid (41 mg, 23%).

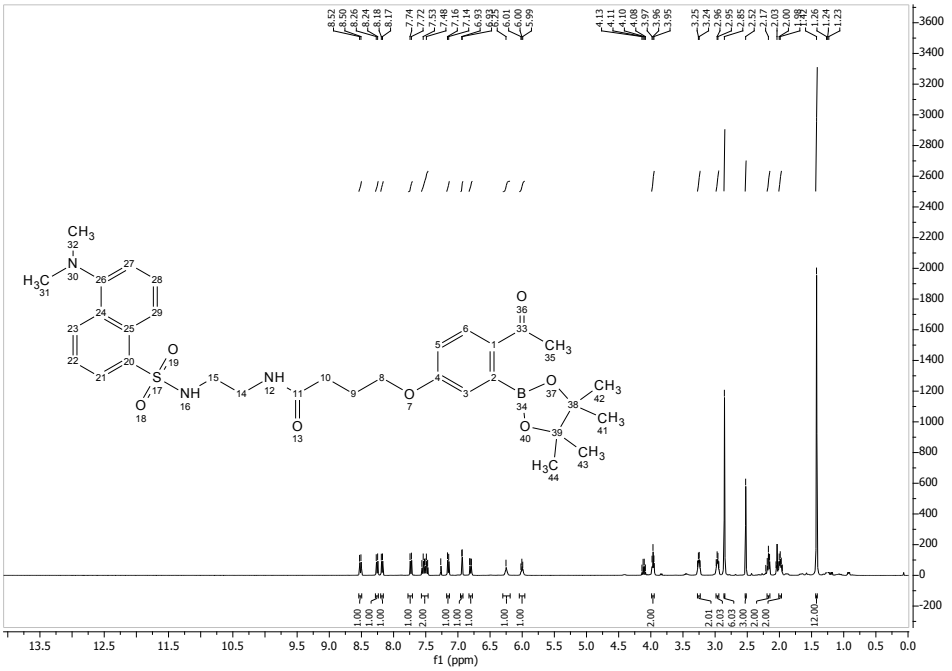

Figure S13 – ^{1}H-NMR for compound **5'**

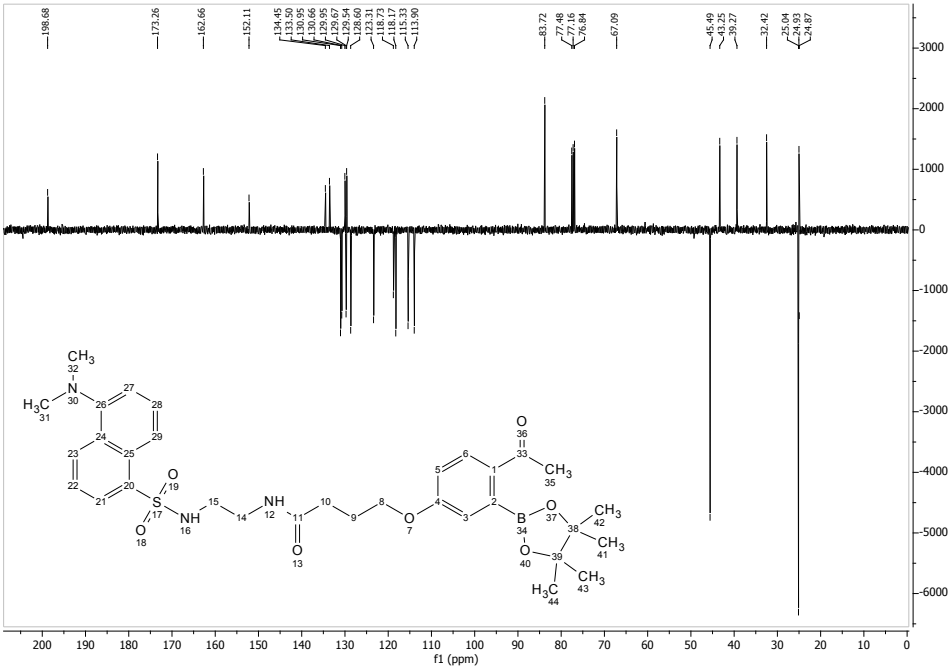

Figure S14 – ^{13}C-NMR for compound **5'**

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.51 (d, *J* = 8.5 Hz, 1H, C<u>H</u>$_{arom}$ (23)), 8.25 (d, *J* = 8.6 Hz, 1H, C<u>H</u>$_{arom}$ (29)), 8.18 (d, *J* = 7.2 Hz, 1H, C<u>H</u>$_{arom}$ (21)), 7.73 (d, *J* = 8.6 Hz, 1H, C<u>H</u>$_{arom}$ (6)), 7.51 (dt, *J* = 19.6, 8.0 Hz, 2H, C<u>H</u>$_{arom}$ (28,22)), 7.15 (d, *J* = 7.6 Hz, 1H, C<u>H</u>$_{arom}$ (27)), 6.93 (d, *J* = 1.9 Hz, 1H, C<u>H</u>$_{arom}$ (3)), 6.80 (dd, *J* = 8.6, 1.7 Hz, 1H, C<u>H</u>$_{arom}$ (5)), 6.25 (s, 1H, CON<u>H</u>CH$_2$ (12)), 6.00 (t, *J* = 5.6 Hz, 1H, CH$_2$N<u>H</u>SO$_2$ (16)), 3.96 (t, *J* = 5.8 Hz, 2H, OC<u>H$_2$</u>CH$_2$ (8)), 3.25 (dd, *J* = 10.3, 5.3 Hz, 2H, CONHC<u>H$_2$</u> (14)), 2.96 (dd, *J* = 10.3, 5.3 Hz, 2H, C<u>H$_2$</u>NHSO$_2$ (15)), 2.85 (s, 6H, C$_{arom}$N(C<u>H$_3$</u>)$_2$ (31,32)), 2.52 (s, 3H, COC<u>H$_3$</u> (35)), 2.17 (t, *J* = 7.2 Hz, 2H, CH$_2$C<u>H$_2$</u>CONH (10)), 2.00 – 1.96 (m, 2H, CH$_2$C<u>H$_2$</u>CH$_2$ (9)), 1.42 (s, 12H, C(C<u>H$_3$</u>)$_2$ (41-44)).

^{13}C NMR (101 MHz, $CDCl_3$): δ 198.68 (s, C$_{arom}$<u>C</u>OCH$_3$ (33)), 173.26 (s, CH$_2$<u>C</u>ONH (11)), 162.66 (s, <u>C</u>$_{arom}$OCH$_2$ (4)), 152.11 (s, C$_{arom}$N(CH$_3$)$_2$ (26)), 134.45 (s, CH<u>C</u>$_{arom}$SO$_2$ (20)), 133.50 (s, CH$_3$CO<u>C</u>$_{arom}$ (1)), 130.95 (s, <u>C</u>H$_{arom}$ (6)), 130.66 (s, <u>C</u>H$_{arom}$ (23)), 129.95 (s, CN<u>C</u>$_{arom}$CH (24)), 129.67 (s, <u>C</u>H$_{arom}$CSO$_2$ (21)), 129.54 (s, CHC$_{arom}$CSO$_2$ (25)), 128.60 (s, <u>C</u>H$_{arom}$ (28)), 123.31 (s, <u>C</u>H$_{arom}$ (22)),

118.73 (s, $\underline{C}H_{arom}$ (29)), 118.17 (s, $\underline{C}H_{arom}$ (3)), 115.33 (s, $\underline{C}H_{arom}CNCH_3$ (27)), 113.90 (s, $\underline{C}H_{arom}$ (5)), 83.72 (s, $\underline{C}(CH_3)_2$ (38,39)), 67.09 (s, $O\underline{C}H_2CH_2$ (8)), 45.49 (s, $C_{arom}N(\underline{C}H_3)_2$ (31,32)), 43.25 (s, $\underline{C}H_2NHSO_2$ (15)), 39.27 (s, $CONH\underline{C}H_2$ (14)), 32.42 (s, $CH_2\underline{C}H_2CONH$ (10)), 24,96 (s, $C(\underline{C}H_3)_2$ (41-44)), 24,93 (s, $CH_2\underline{C}H_2CH_2$ (9)), 24,87 (s, $CO\underline{C}H_3$ (35)).
^{13}C-HMBC 138.6 (s, $\underline{C}_{arom}B$ (2))
HMRS (ESI): *m/z* calculated $[M+K]^+ = 662.247383$, found $[M+K]^+ = 662.247221$.

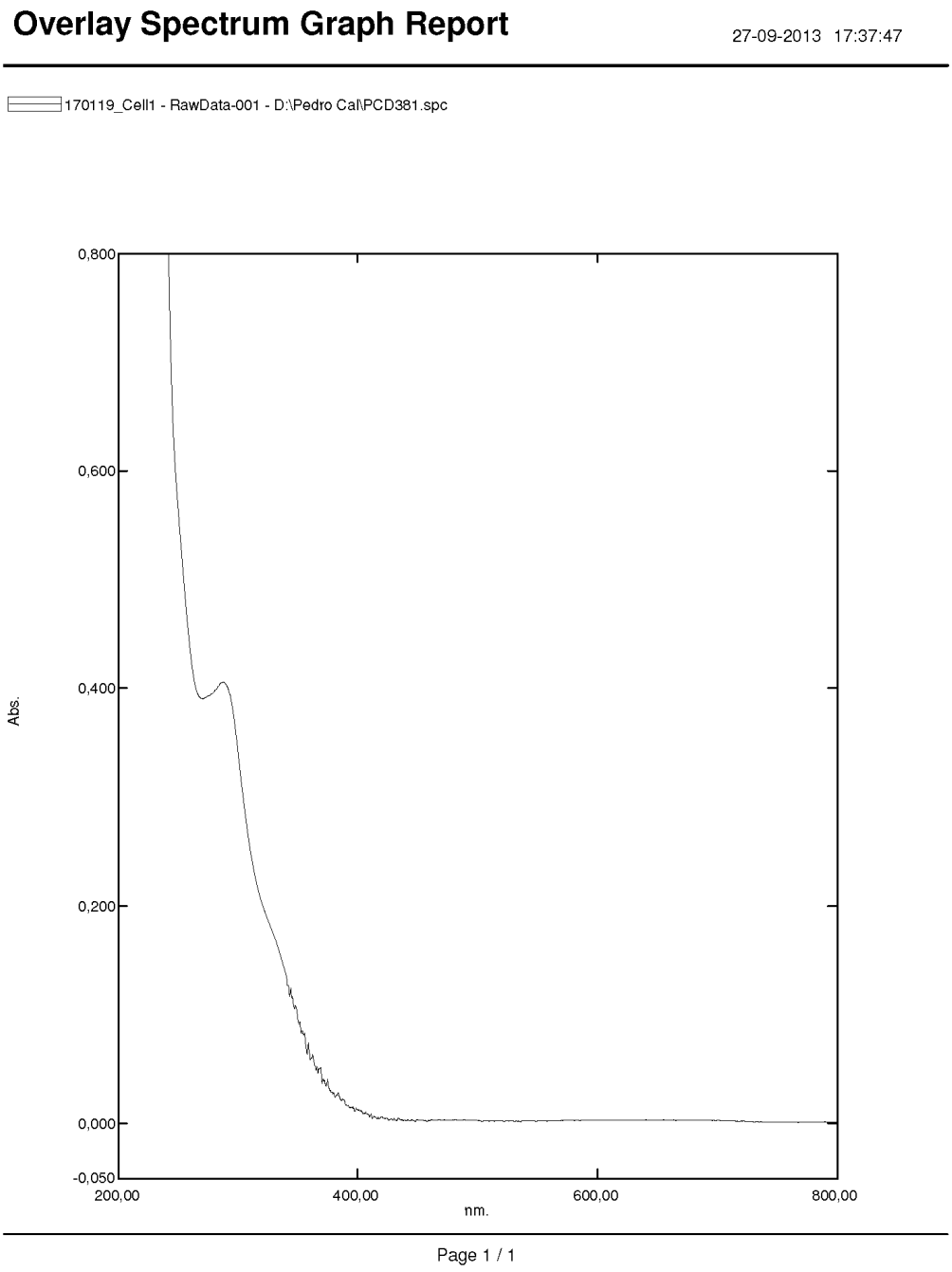

Figure S15 – UV spectrum for compound **5'**

λ_{max} = 290nm; Absorbance (Abs_{max})= 0,404; Molar absorption coefficient (ε)= 8080 $M^{-1}cm^{-1}$

2.2.2 – Synthesis of boronate ester 6'

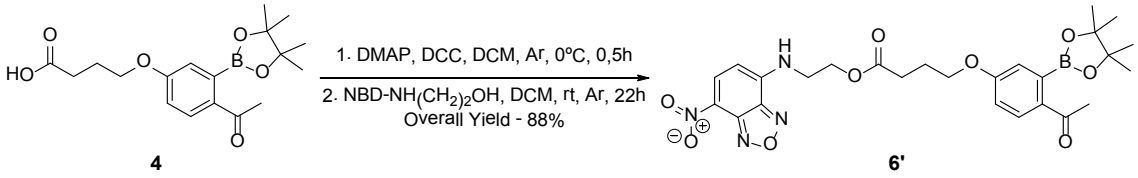

Figure S16 – Synthesis of compound **6'**

2.2.2.1 – Synthesis of 4-(2-Hydroxyethylamino)-7-nitro-2,1,3-benzoxadiazole (**NBD-NH(CH$_2$)$_2$OH**)

Literature basis - Onoda, M.; Uchiyama, S., Santa, T., Imai, K.; *Analytical Chemistry* **2002**, 74(16), 4089

In a round bottom flask, under inert atmosphere, 4-Chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl) (100 mg, 0.50 mmol) was dissolved in acetonitrile (10 mL). After the addition of 2-ethanolamine (200 µL) in acetonitrile (5 mL), the solution was stirred at room temperature for 30 min. The reaction mixture was concentrated and the residue was purified by column chromatography (silica gel, dichloromethane-methanol (1:0 – 19:1)) to afford **NBD-NH(CH$_2$)$_2$OH** as an orange solid in 49% yield. This compound was immediately used after being synthesized.

2.2.2.2 – Esterification to afford boronate ester **6'**

Literature basis - Liu, Z.; Hu, J.; Sun, J.; He, G.; Li, Y.; Zhang, G.; *Journal of Polymer Science Part A: Polymer Chemistry* **2010,** 48 (16), 3573

In a round bottom flask, under inert atmosphere, **4** (40 mg, 0.11 mmol), *N,N'*-dicyclohexylcarbodiimide (71 mg, 0.34 mmol) and 4-dimethylaminopyridine (2 mg, 0.01 mmol) were mixed at 0ºC in 1mL of previously distilled acetonitrile and stirred for 30 minutes. A solution of NBD-NH(CH$_2$)$_2$OH (50 mg, 0.23 mmol) in 1 mL of previously distilled acetonitrile was added via a syringe and the solution was stirred for 22 hours at room temperature. The solvent was, then, concentrated in vacuo and the residue was purified by flash column chromatography (silica gel, dichloromethane:acetone 1:0 – 9:1) and recrystallized with acetone/hexane to afford **6'** as an orange solid (56 mg, 88%).

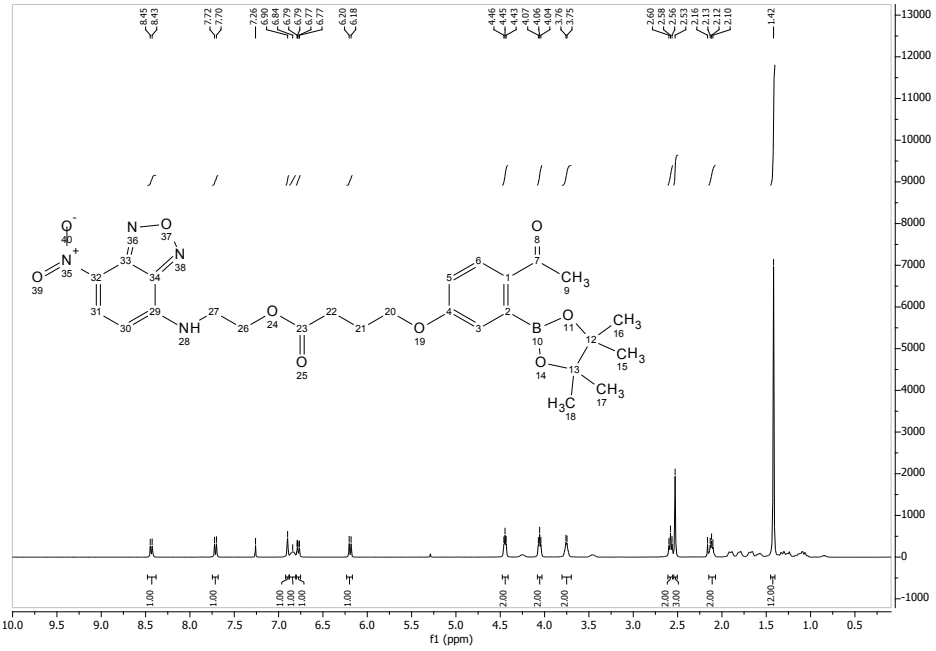

Figure S17 – ^{1}H-NMR for compound **6'**

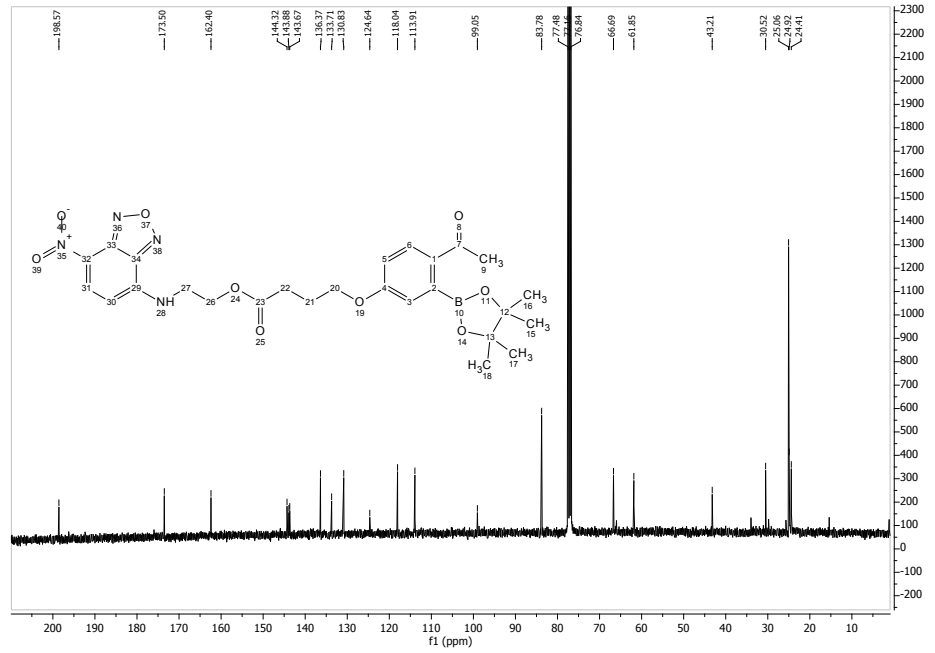

Figure S18 – ^{13}C-NMR for compound **6'**

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.44 (d, *J* = 8.6 Hz, 1H, CHC<u>H</u>CNO$_2$ (31)), 7.71 (d, *J* = 8.6 Hz, 1H, C<u>H</u>$_{arom}$ (6)), 6.90 (s, 1H, C<u>H</u>$_{arom}$ (3)), 6.84 (s, 1H, CH$_2$N<u>H</u>C (28)), 6.78 (d, *J* = 8.6 Hz, 1H, C<u>H</u>$_{arom}$ (5)), 6.19 (d, *J* = 8.6 Hz, 1H, NHCC<u>H</u>CH (30)), 4.45 (t, *J* = 5.0 Hz, 2H, OC<u>H</u>$_2$CH$_2$NH (26)), 4.06 (t, *J* = 5.7 Hz, 2H, OC<u>H</u>$_2$CH$_2$ (20)), 3.75 (d, *J* = 5.0 Hz, 2H, OCH$_2$C<u>H</u>$_2$NH (27)), 2.58 (t, *J* = 7.1 Hz, 2H, CH$_2$C<u>H</u>$_2$COO (22)), 2.53 (s, 3H, COC<u>H</u>$_3$ (9)), 2.15 – 2.07 (m, 2H, CH$_2$C<u>H</u>$_2$CH$_2$ (21)), 1.42 (s, 12H, CC<u>H</u>$_3$ (15-18)).

^{13}C NMR (101 MHz, $CDCl_3$): δ 198.57 (s, C$_{arom}$<u>C</u>OCH$_3$ (7)), 173.50 (s, CH$_2$<u>C</u>OO (23)), 162.40 (s, <u>C</u>$_{arom}$OCH$_2$ (4)), 144.32 (s, 2x<u>C</u>NO (33,34)), 143.88 (s, C<u>C</u>NO$_2$CH (32)), 143.67 (s, C<u>C</u>NHCH (29)), 136.37 (s, CH<u>C</u>HCNO$_2$ (31)), 133.71 (s, CH$_3$CO<u>C</u>$_{arom}$ (1)), 130.83 (s, <u>C</u>H$_{arom}$ (6)), 118.04 (s, <u>C</u>H$_{arom}$ (3)), 113.91 (s, <u>C</u>H$_{arom}$ (5)), 99.05 (s, NHC<u>C</u>HCH (30)), 83.78 (s, <u>C</u>(CH$_3$)$_2$ (12,13)), 66.69 (s, O<u>C</u>H$_2$CH$_2$ (20)), 61.85 (s, O<u>C</u>H$_2$CH$_2$NH (26)), 43.21 (s, OCH$_2$<u>C</u>H$_2$NH (27)), 30.52 (s, CH$_2$<u>C</u>H$_2$CO (22)), 25.06 (s, C(<u>C</u>H$_3$)$_2$ (15-18)), 24.92 (s, CO<u>C</u>H$_3$ (9)), 24.41 (s, CH$_2$<u>C</u>H$_2$CH$_2$ (21)).

^{13}C-HMBC 138.5 (s, <u>C</u>$_{arom}$B (2))

HMRS (ESI): *m/z* calculated $[M+Na]^+$ = 577.208100, found $[M+Na]^+$ = 577.207605.

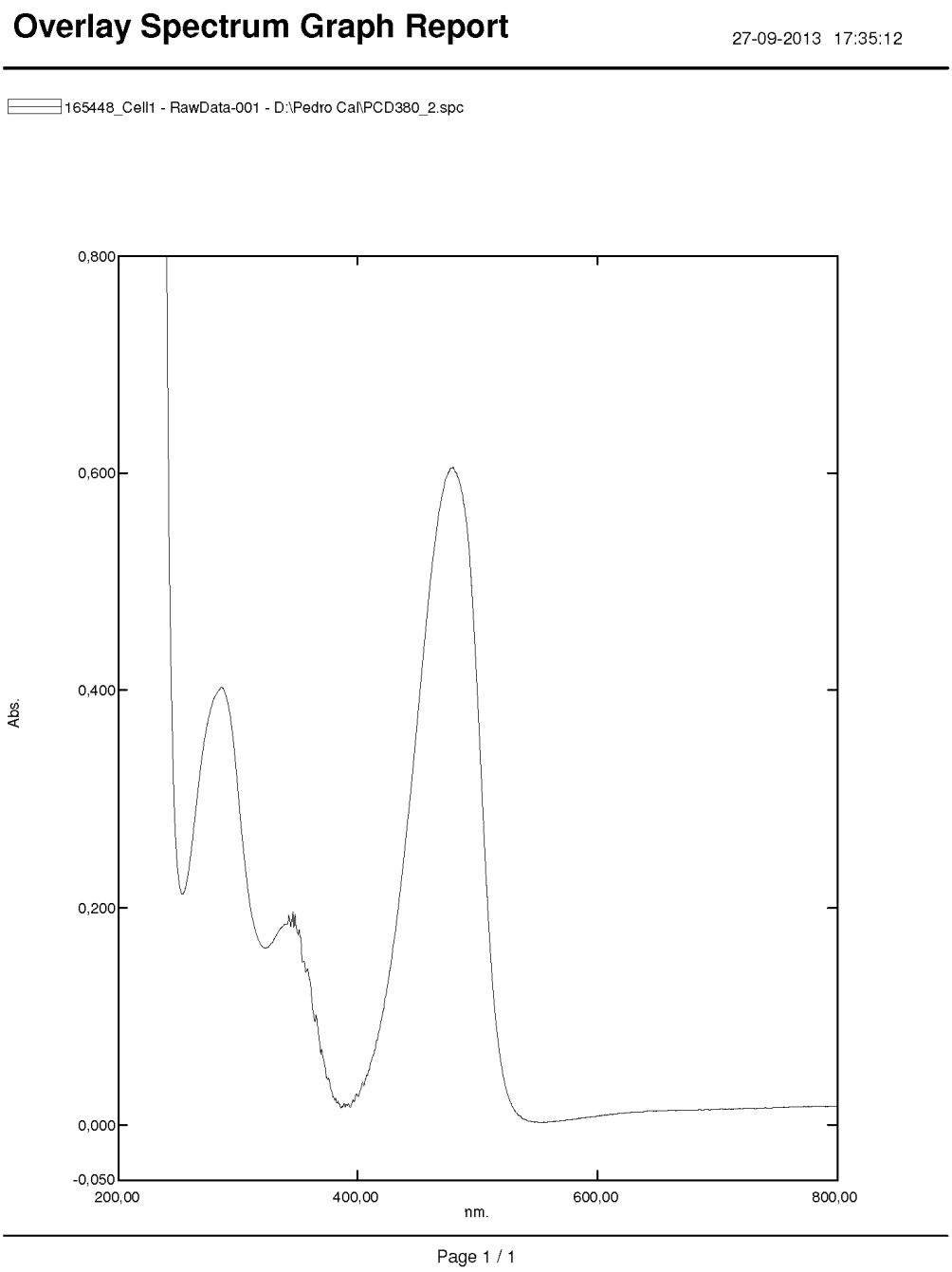

Figure S19 – UV spectrum for compound **6'**

λ_{max} = 480 nm; Absorbance (Abs_{max})= 0,604; Molar absorption coefficient (ε)= 12080 $M^{-1}cm^{-1}$

2.3 – Synthesis of EDA-FA and reaction with 5 and 6

2.3.1 – Synthesis of N-(2-aminoethyl) folic acid (EDA-FA)

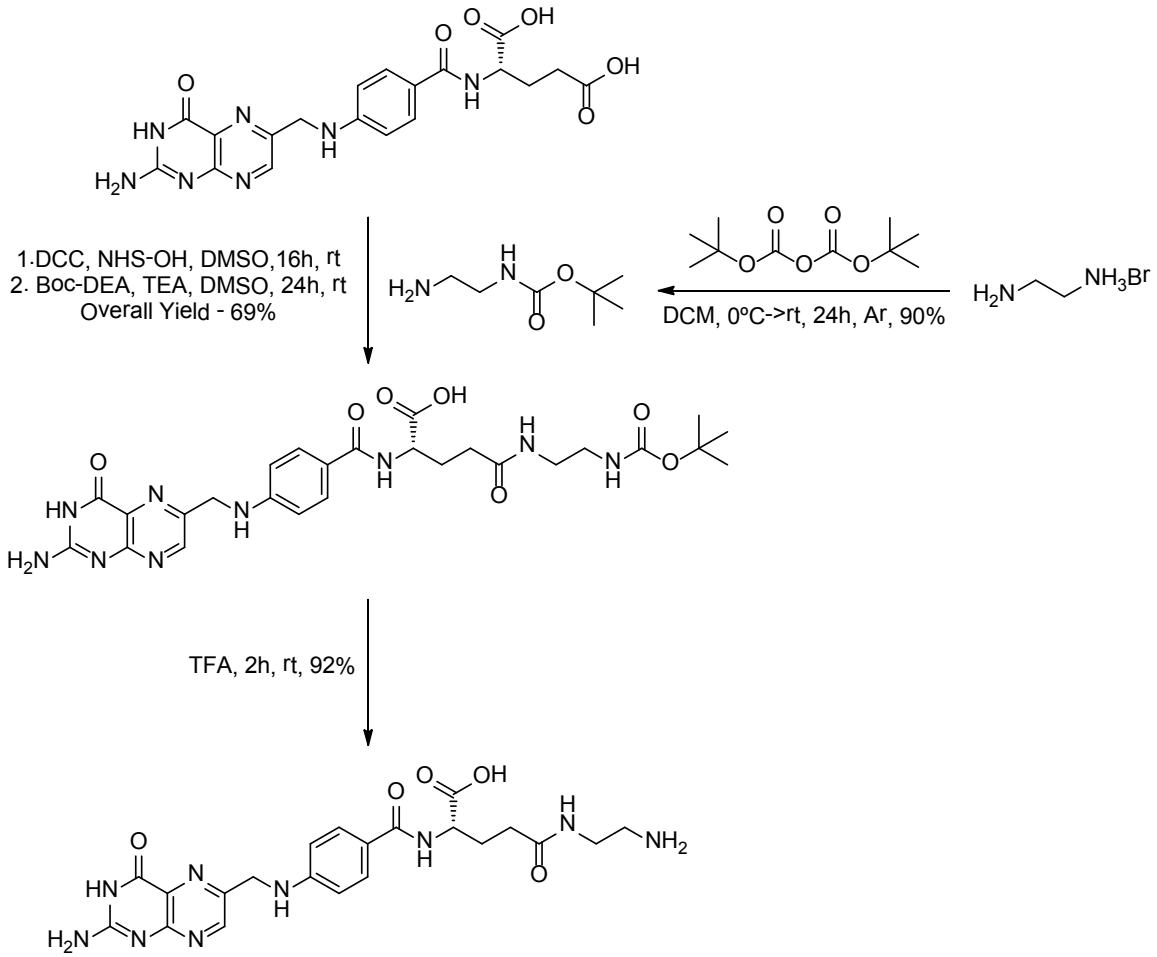

Figure S20 – Synthesis of compound **EDA-FA**

The derivative of folic acid (N-(2-aminoethyl) folic acid (EDA-FA)) was synthesized according to an existing procedure obtaining 69% in the first step and 92% in the second one. (Rutnakornpituk, M.; Puangsin, N.; Theamdee, P.; Rutnakornpituk, B.; Wichai, U.; *Polymer*, **2011**, 52, 987).
HMRS (ESI): *m/z* calculated $[M+H]^+ = 484.205141$, found $[M+H]^+ = 484.205640$.

2.3.2 – Pinacol deprotection and reaction with EDA-FA

2.3.2.1 – Compound **5** and **6** synthesis
Literature basis - Pennington, T.E.; Kardiman, C.; Hutton, C.A.; *Tetrahedron Letters* **2004**, 45, 6657

Treatment of the boronate ester with 5 equiv of polystyrene–boronic acid in acetonitrile/1 M HCl (9:1) for 18 h yielded 27% of compound **5** and 53% of compound **6**, after evaporating

solvents and performing a flash column chromatography (silica gel, dichloromethane:acetone 4:1 – 0:1). These compounds were immediately used after deprotection.

2.3.2.2 – Reaction with **EDA-FA**

Compound 5 and 6 were dissolved in DMSO, as well as EDA-FA. From these solutions compounds 5 and 6 (100 μM) reacted with 100 μM in $NH_4CH_3CO_2$ buffer (50,0 mM, pH 7.0) at room temperature. After 10 minutes, the reactions mixtures were evaluated by performing an ESI-FTICR-MS and the conjugated species were detected:

- **HRMS for compound 5 with EDA-FA (ESI):** *m/z* calculated $[M+H-H_2O]^+ = 989.39022$, found $[M+H-H_2O]^+ = 989.39107$.
- **HRMS for compound 6 with EDA-FA (ESI):** *m/z* calculated $[M+H]^+ = 938.33545$, found $[M+H]^+ = 938.33917$.

2.4 – Aboronated core VS boronate core – Equilibrium

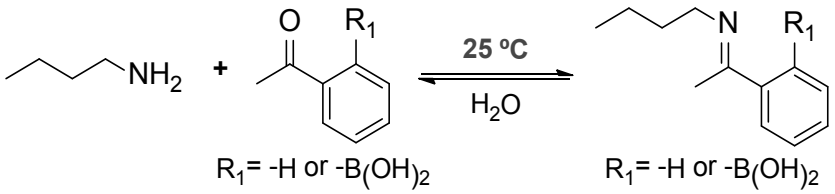

Figure S21 – Equilibrium of 2-acetylbenzene derivatives with 1-butylamine in aqueous medium

As a preliminary result, the aboronated core – acetophenone – and the boronated core – 2-acetylbenzeneboronic acid – were evaluated in terms of imine stability once these were reacted with 1-butylamine – an alkylic model for lysine (Figure S21).

General procedure:
2-Acetylbenzeneboronic acid or acetophenone (0,33mmol) was added to a 10mL round bottom flask and then dissolved in 2mL of H_2O. Afterwards, 1-butylamine (33μL, 0,33 mmol) was added to the same flask and these compounds reacted for 16 hours at 25ºC. At that time, one drop of the reaction mixture was taken from the media and diluted in D_2O in order to perform a ^{1}H-NMR and evaluate the reaction's yield based on the comparison of the signal from α-protons of 1-butylamine and the same protons from imine, which differ from ~2.5 ppm to ~3.6 ppm, respectively. Figure S22 shows ^{1}H-NMR for 2-acetylbenzeneboronic acid and Figure S23 shows ^{1}H-NMR for acetophenone.

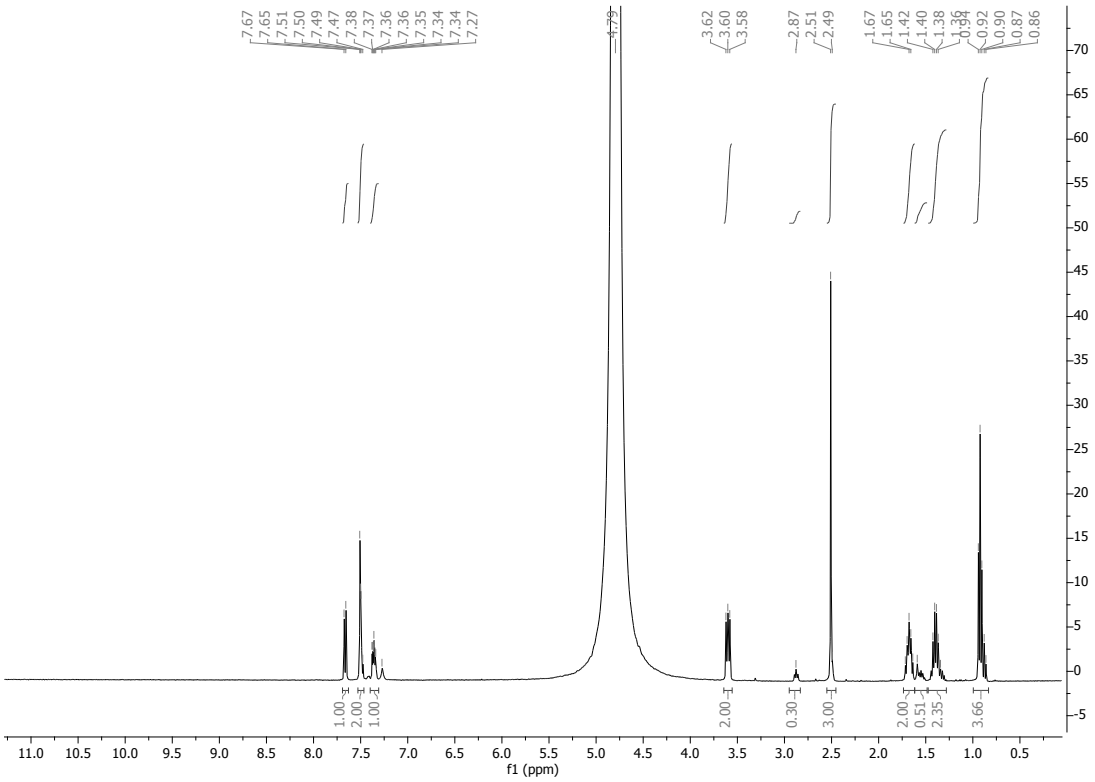

Figure S22 – ^{1}H-NMR for 2-acetylbenzeneboronic acid and 1-butylamine in water after 16h of reaction (in D_2O)

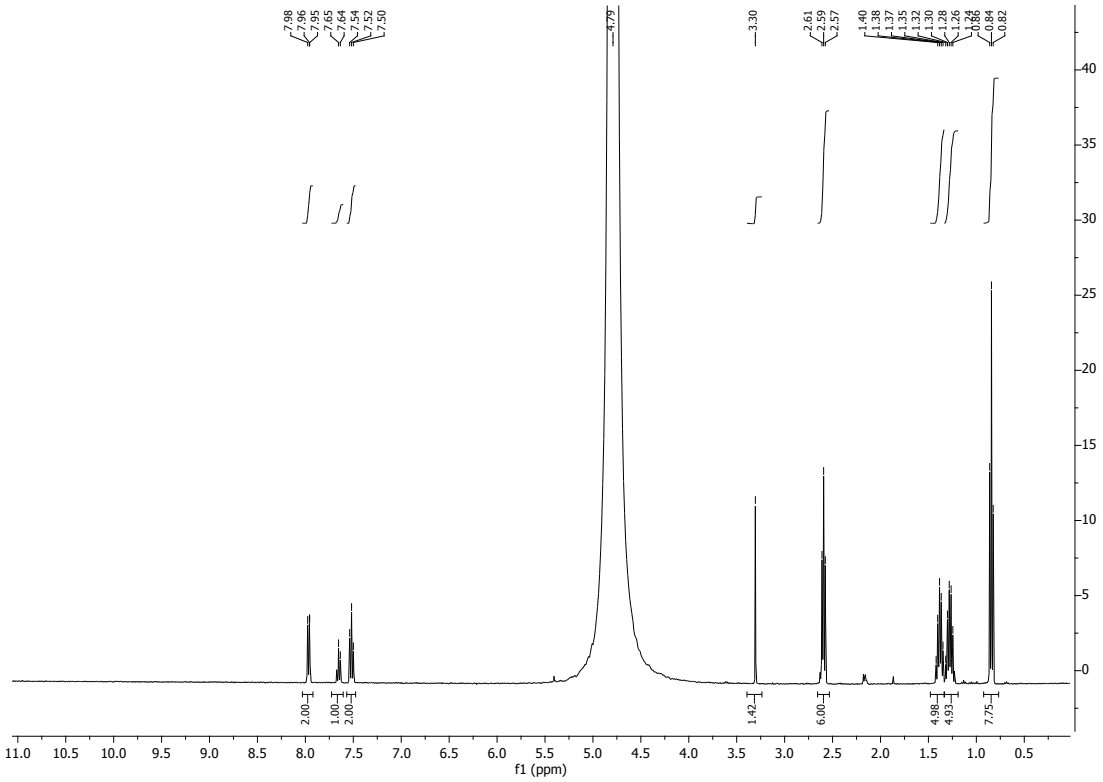

Figure S23 – ^{1}H-NMR for acetophenone and 1-butylamine in water after 16h of reaction (in D_2O)

2.5 – Aboronated compound 14 synthesis

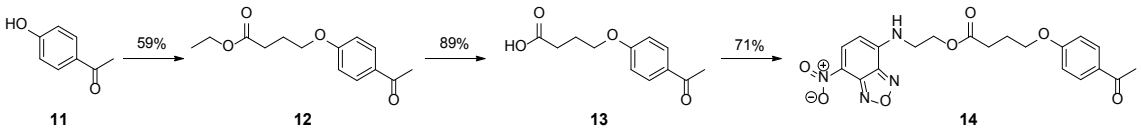

Figure S24 – Synthesis of compound **14**

Once the imine's stability of the aboronated species was studied, a fluorescent probe was synthesized to serve as a blank for the biological results.

2.5.1 – Synthesis of compound **12**

Literature basis - Al-Smadi, M.; Hanold, N.; Kalbitz, H.; Meier, H.; *Synthesis*, **2009**, 15, 2539

In a round bottom flask (A), 4'-hydroxyacetophenone (0.5 g, 3.6 mmol) and potassium carbonate (0.5 g, 3.6 mmol) were heated in acetone to reflux for 3 hours. In a separate round bottom flask (B), ethyl 4-bromobutyrate (0.8 mL, 5.5 mmol) and sodium iodide (0.8 g, 5.5 mmol) were stirred in acetone at room temperature for 2 hours. Then, flask B was transferred to flask A and refluxed for 14 hours. After that, acetone was evaporated and 200 mL of water were added and extracted with diethyl ether (3*200 mL). The solution was dried (Na_2SO_4), concentrated and purified by column chromatography (silica gel, hexane:diethyl ether (9:1)) to give a viscous oil that solidified below 20ºC; compound **12** (0.54 g, 59%).

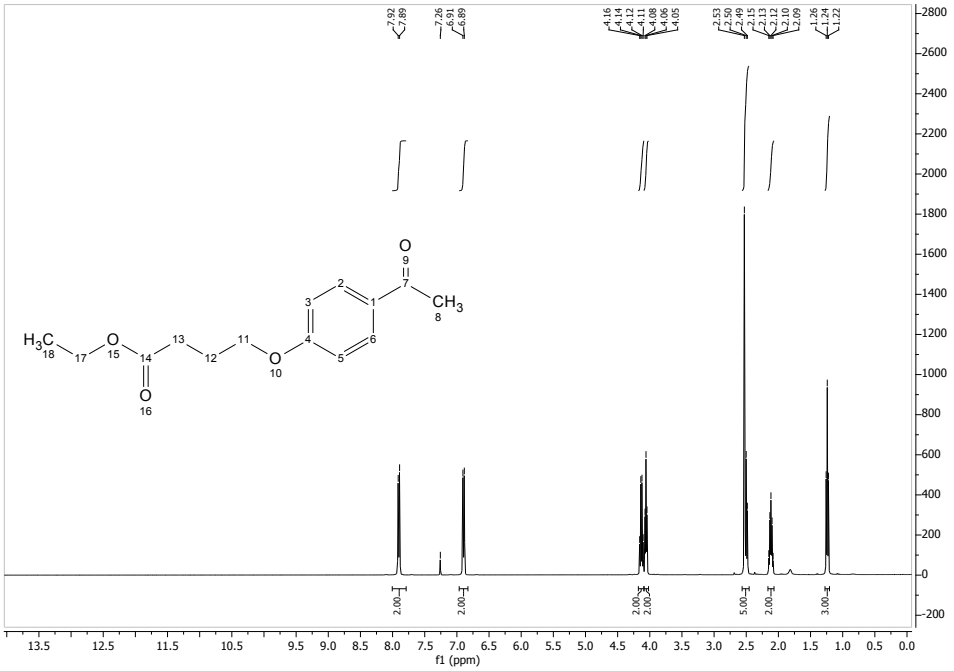

Figure S25 – ^{1}H-NMR for compound **12**

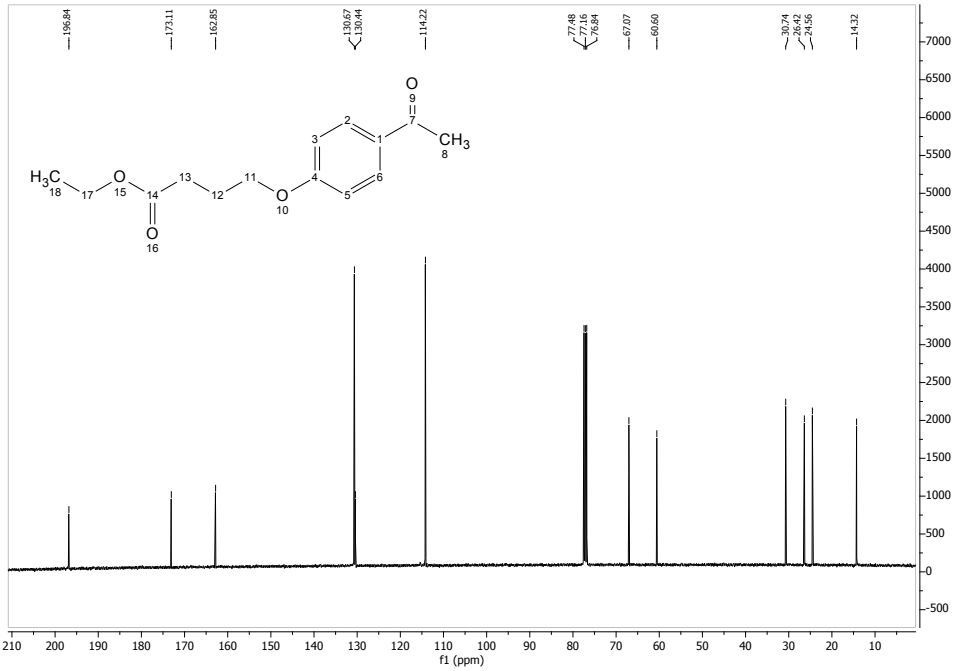

Figure S26 – ^{13}C-NMR for compound **12**

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (d, *J* = 8.7 Hz, 2H, C<u>H</u>$_{arom}$ (2,6)), 6.90 (d, *J* = 8.7 Hz, 2H, C<u>H</u>$_{arom}$ (3,5)), 4.13 (q, *J* = 7.1 Hz, 2H, OC<u>H</u>$_2$CH$_3$ (17)), 4.06 (t, *J* = 6.1 Hz, 2H, OC<u>H</u>$_2$CH$_2$ (11)), 2.57 – 2.46 (m, 5H, COC<u>H</u>$_3$ (8)), COC<u>H</u>$_2$CH$_2$ (13)), 2.12 (p, *J* = 6.6 Hz, 2H, CH$_2$C<u>H</u>$_2$CH$_2$ (12)), 1.24 (t, *J* = 7.1 Hz, 3H, OCH$_2$C<u>H</u>$_3$ (18)).
^{13}C NMR (101 MHz, $CDCl_3$): δ 196.84 (s, C$_{arom}$<u>C</u>OCH$_3$ (7)), 173.11 (s, O<u>C</u>OCH$_2$ (14)), 162.85 (s, CH<u>C</u>$_{arom}$OCH$_2$ (4)), 130.67 (s, <u>C</u>H$_{arom}$ (2,6)), 130.44 (s, CH<u>C</u>$_{arom}$COCH (1)), 114.22 (s, <u>C</u>H$_{arom}$ (3,5)), 67.07 (s, O<u>C</u>H$_2$CH$_2$ (11)), 60.60 (s, O<u>C</u>H$_2$CH$_3$ (17)), 30.74 (s, CO<u>C</u>H$_2$CH$_2$ (13)), 26.42 (s, CO<u>C</u>H$_3$ (8)), 24.56 (s, CH$_2$<u>C</u>H$_2$CH$_2$ (12)), 14.32 (s, OCH$_2$<u>C</u>H$_3$ (18)).
HMRS (ESI): *m/z* calculated $[M+Na]^+$ = 273.109730, found $[M+Na]^+$ = 273.108601.

2.5.2 – Synthesis of compound **13**

Literature basis - Griffin, D.R.; Andrea M. Kasko, A.M.; *J. Am. Chem. Soc.* **2012**, 134, 13103

In a round bottom flask, **12** (0.3 g, 1.2 mmol) was stirred in a solution of trifluoroacetic acid (0.35 mL) and water (3.35 mL) at 90 ºC for 3 hours. The product precipitates during the reaction and it was filtrated and washed with water and acetone to afford **13** as a white solid (0.24 g, 89%).

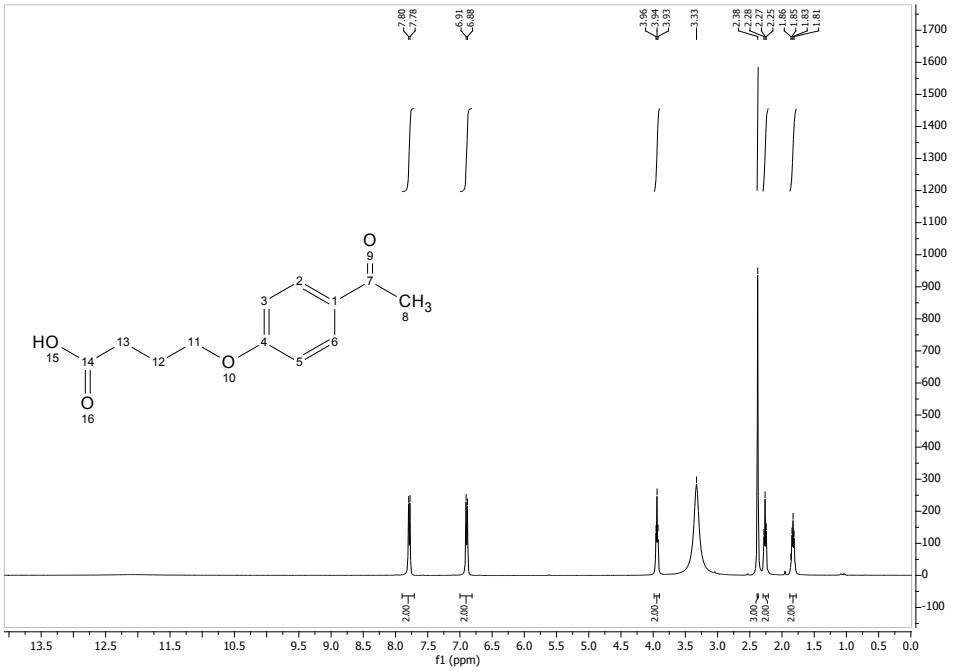

Figure S27 – ^{1}H-NMR for compound **13**

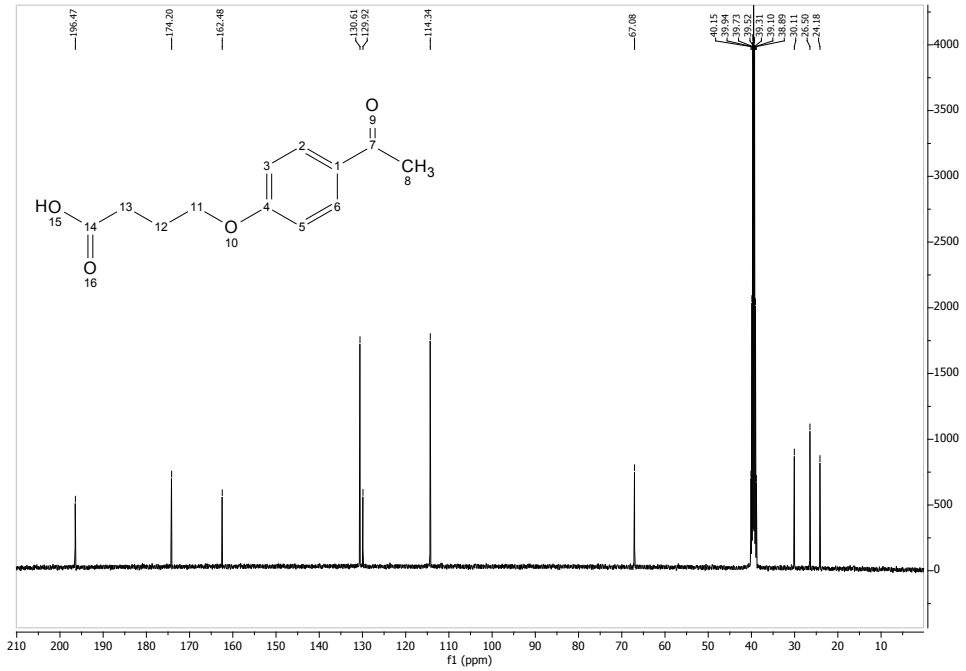

Figure S28 – ^{13}C-NMR for compound **13**

^{1}H NMR (400 MHz, DMSO): δ 7.79 (d, *J* = 8.5 Hz, 2H, C$\underline{H}_{arom}$ (2,6)), 6.90 (d, *J* = 8.5 Hz, 2H, C$\underline{H}_{arom}$ (3,5)), 3.94 (t, *J* = 6.3 Hz, 2H, OC$\underline{H}_2$CH$_2$ (11)), 2.38 (s, 3H COC$\underline{H}_3$ (8)), 2.27 (t, *J* = 7.2 Hz, 2H, COC$\underline{H}_2$CH$_2$ (13)), 1.86 – 1.81 (m, 2H, CH$_2$C$\underline{H}_2$CH$_2$ (12)).
^{13}C NMR (101 MHz, DMSO): δ 196.47 (s, C$_{arom}\underline{C}$OCH$_3$ (7)), 174.20 (s, O$\underline{C}$OCH$_2$ (14)), 162.48 (s, CH$\underline{C}_{arom}$OCH$_2$ (4)), 130.61 (s, $\underline{C}$H$_{arom}$ (2,6)), 129.92 (s, CH$\underline{C}_{arom}$COCH (1)), 114.34 (s, $\underline{C}$H$_{arom}$ (3,5)), 67.08 (s, O$\underline{C}$H$_2$CH$_2$ (11)), 30.11 (s, CO$\underline{C}$H$_2$CH$_2$ (13)), 26.50 (s, CO$\underline{C}$H$_3$ (8)), 24.18 (s, CH$_2\underline{C}$H$_2$CH$_2$ (12)).
HMRS (ESI): *m/z* calculated [M+Na]$^+$ = 245.078430, found [M+Na]$^+$ = 245.077406.

2.5.3 – Synthesis of compound **14**

Literature basis - Liu, Z.; Hu, J.; Sun, J.; He, G.; Li, Y.; Zhang, G.; *Journal of Polymer Science Part A: Polymer Chemistry* **2010,** 48 (16), 3573

In a round bottom flask, under inert atmosphere, **13** (25 mg, 0.11 mmol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (65 mg, 0.34 mmol) and 4-dimethylaminopyridine (2 mg, 0.01 mmol) were mixed at 0 ºC in 1 mL of previously distilled dichloromethane and stirred for 1 hour. A solution of NBDNH$(CH_2)_2$OH (50 mg, 0.23 mmol) in 0.5 mL of previously distilled acetone was added via a syringe and the solution was stirred for 5 hours at room temperature. Then, the solvent was concentrated in vacuo and the residue was dissolved in dichloromethane and washed with a solution of 5% $NaHCO_3$. The organic phases were dried ($MgSO_4$) and concentrated to a residue that was further purified by column chromatography (silica gel, dichloromethane:ethyl acetate 1:0 – 9:1) to afford **14** as an orange solid (34 mg, 71%).

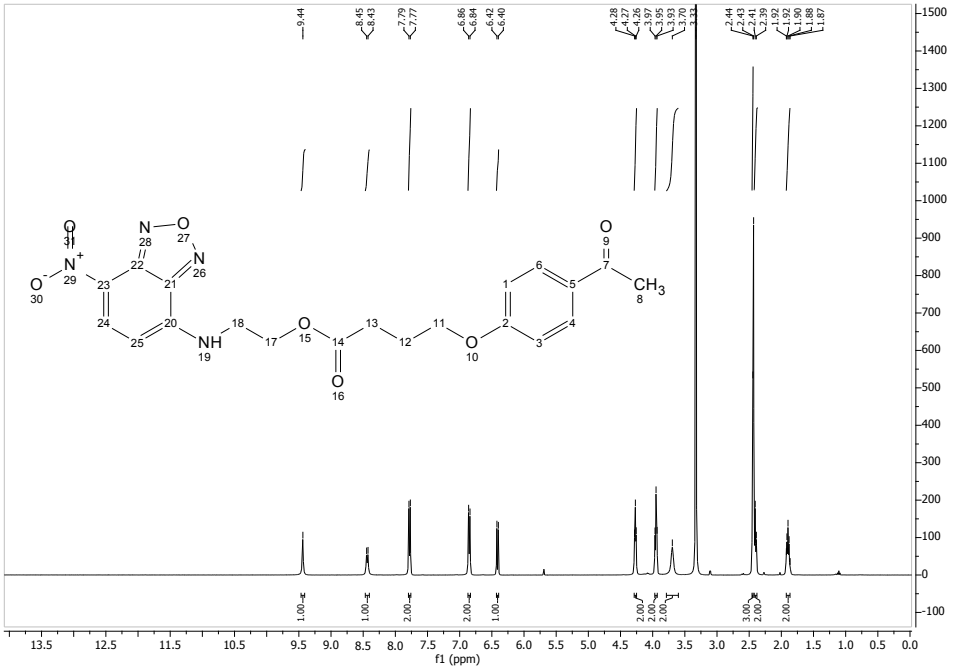

Figure S29 – ^{1}H-NMR for compound **14**

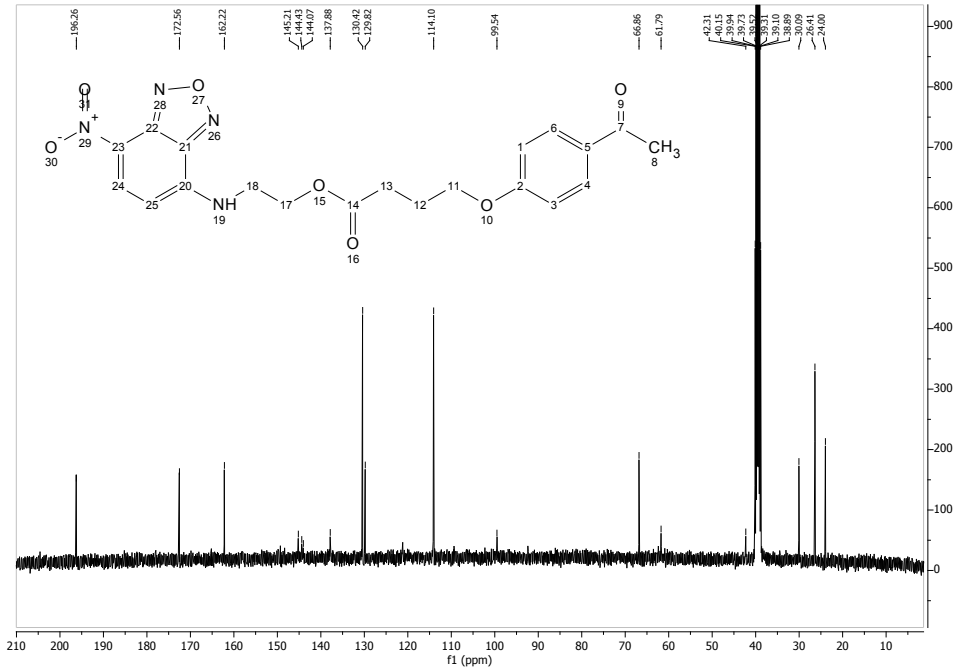

Figure S30 – ^{13}C-NMR for compound **14**

^{1}H NMR (400 MHz, DMSO): δ 9.44 (s, 1H, CNHCH$_2$ (19)), 8.44 (d, *J* = 9.0 Hz, 1H, CHCH$_{arom}$CNO$_2$ (24)), 7.78 (d, *J* = 8.6 Hz, 2H, CH$_{arom}$ (2,6)), 6.85 (d, *J* = 8.6 Hz, 2H, CH$_{arom}$ (3,5)), 6.41 (d, *J* = 9.0 Hz, 1H, NHCCH$_{arom}$CH (25)), 4.27 (t, *J* = 5.0 Hz, 2H, NHCH$_2$CH$_2$O (17)), 3.95 (t, *J* = 6.3 Hz, 2H, OCH$_2$CH$_2$ (11)), 3.70 (s, 2H, NHCH$_2$CH$_2$O (18)), 2.44 (s, 3H, COCH$_3$ (8)), 2.40 (t, *J* = 7.1 Hz, 2H, COCH$_2$CH$_2$ (13)), 1.92 – 1.87 (m, 2H, CH$_2$CH$_2$CH$_2$ (12)).
^{13}C NMR (101 MHz, DMSO): δ 196.26 (s, CCOCH$_3$ (7)), 172.56 (s, OCOCH$_2$ (14)), 162.22 (s, CHC$_{arom}$OCH$_2$ (4)), 145.21 (s, 2xC$_{arom}$NO (21,22)), 144,43 (s, CC$_{arom}$NO$_2$CH (23)), 144,07 (s, CC$_{arom}$NHCH$_2$ (20)), 137.88 (s, CHCH$_{arom}$CNO$_2$ (24)), 130.42 (s, CH$_{arom}$ (2,6)), 129.82 (s, CHC$_{arom}$COCH (1)), 114.10 (s, CH$_{arom}$ (3,5)), 99.54 (s, NHCCH$_{arom}$CH (25)), 66.86 (s, OCH$_2$CH$_2$ (11)), 61.79 (s, NHCH$_2$CH$_2$O (17)), 42.31 (s, NHCH$_2$CH$_2$O (18)), 30.09 (s, COCH$_2$CH$_2$ (13)), 26.41 (s, COCH$_3$ (8)), 24.00 (s, CH$_2$CH$_2$CH$_2$ (12)).
HMRS (ESI): *m/z* calculated [M+Na]$^+$ = 451.122420, found [M+Na]$^+$ = 451.121982.

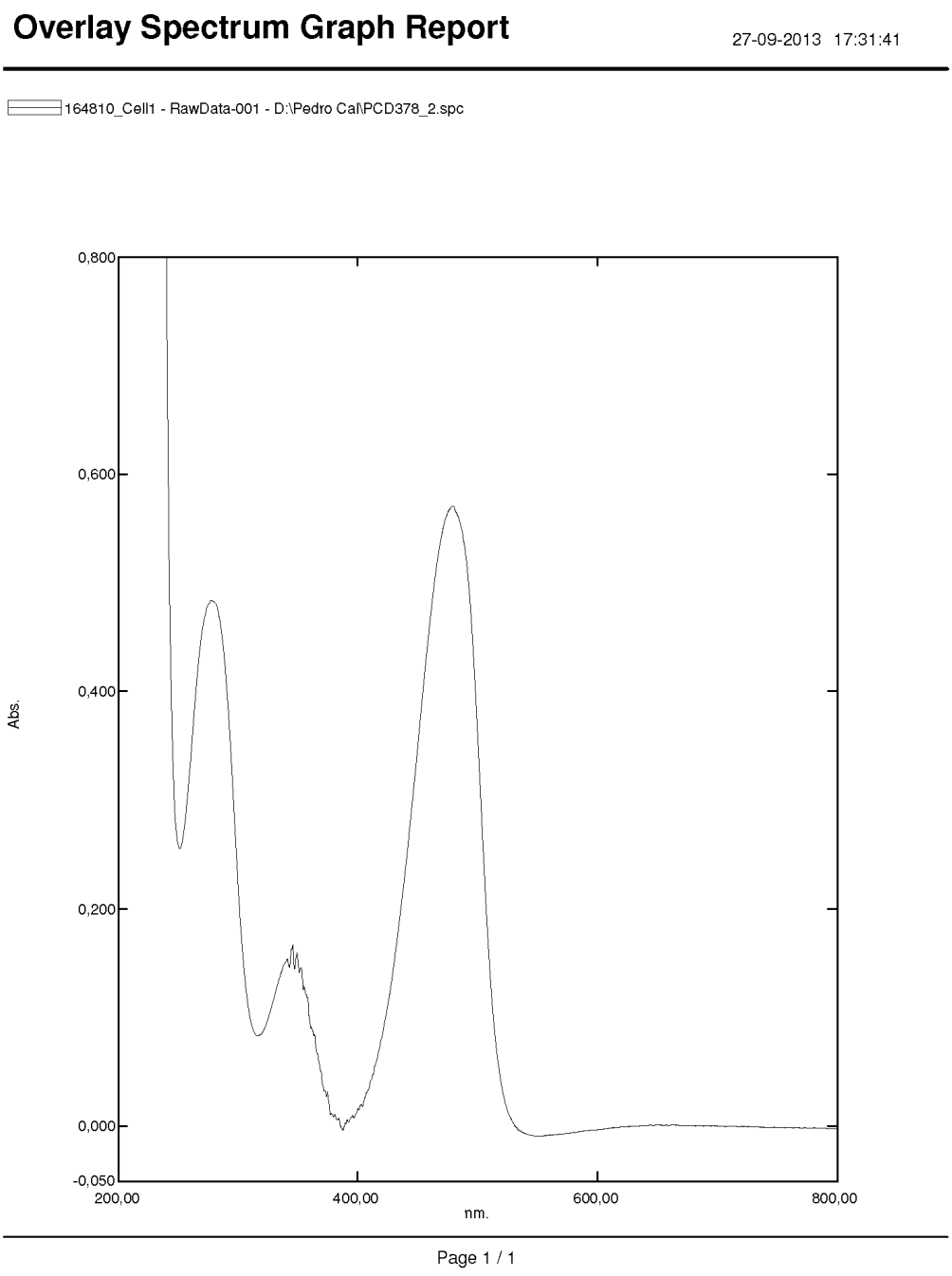

Figure S31 – UV spectrum for compound **14**

λ_{max} = 480 nm; Absorbance (Abs_{max})= 0,570; Molar absorption coefficient (ε)= 11400 $M^{-1}cm^{-1}$

3. Biochemistry Results

Lysozyme, from chicken egg white, was purchased from Sigma-Aldrich and used without further purification.

General Procedure for reactions with lyzosyme:

Compounds 5, 6 and 14 dissolved in DMSO (200 µM) reacted with 10 µM of lysozyme in $NH_4CH_3CO_2$ buffer (50,0 mM, pH 7.0) at room temperature. After 10, 30 minutes or 2 hours of reaction the solutions were evaluated by performing an ESI-FTICR-MS and the conjugated species were detected, up to 4 modifications. Results are shown in Figures S29-S46 and tables S1-S4.

3.1 – Unmodified Lysozyme

Reaction	Deconvoluted Mass	Molecule	Abundance	Abundace [%]
Lys	14296.77169	$[M+H]^+$	1815955	100.00

Table S1 – Data for the most relevant deconvoluted species (unmodified lyzosyme)

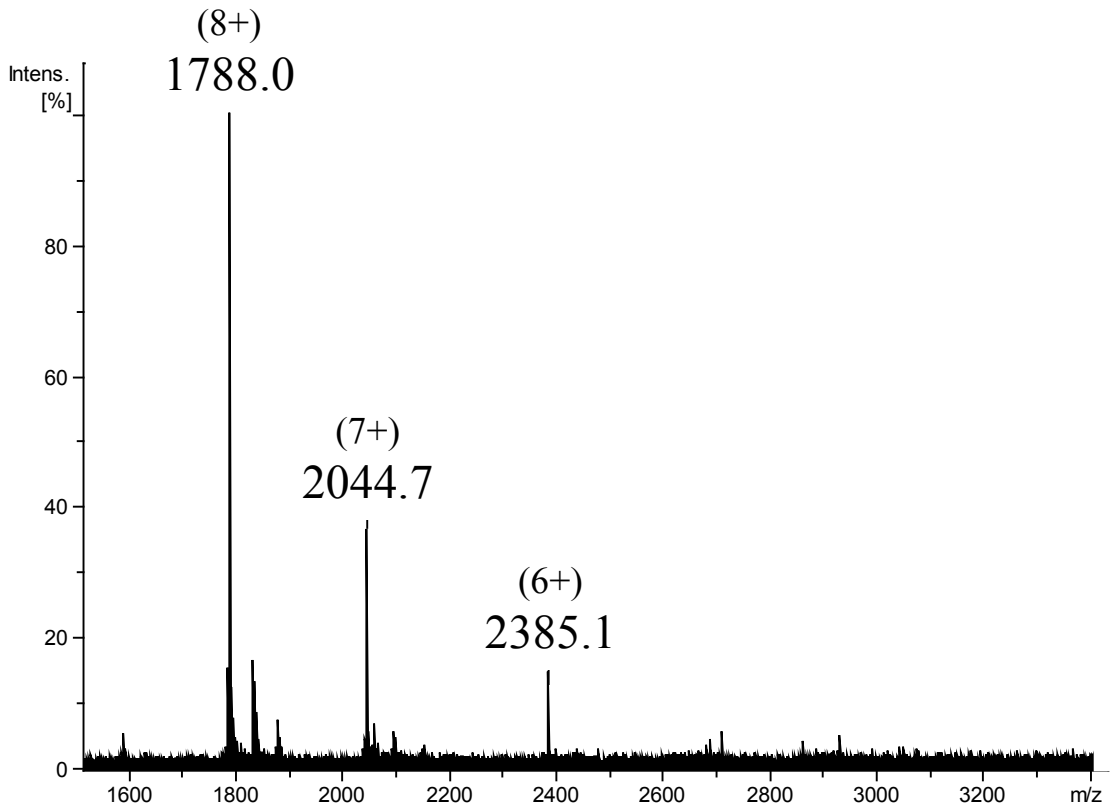

Figure S32 – Lyzosyme (10,0 µM) with 1% (v/v) DMSO in acetate buffer (50,0 mM, pH 7.0) – Positive Mode of ESI-FTICR-MS full spectrum

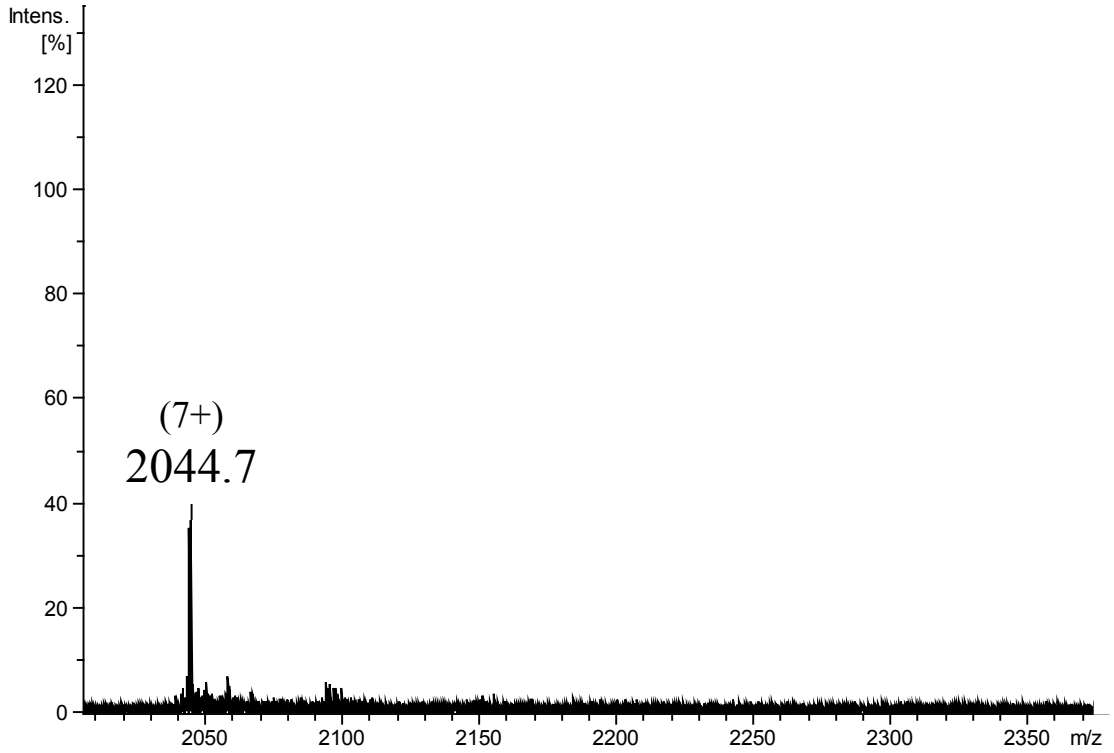

Figure S33 – Lyzosyme (10,0 µM) with 1% (v/v) DMSO in acetate buffer (50,0 mM, pH 7.0) – Positive Mode of ESI-FTICR-MS zoomed spectrum (7+) charge state (*m/z* 2000 – 2400)

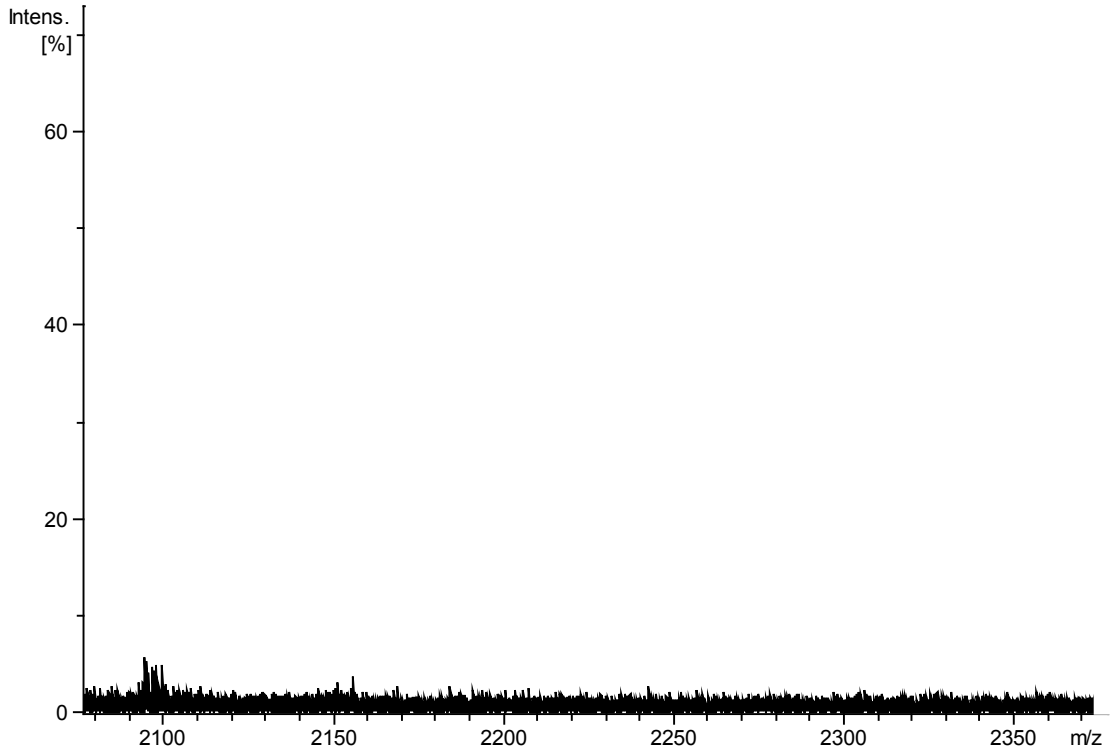

Figure S34 – Lyzosyme (10,0 µM) with 1% (v/v) DMSO in acetate buffer (50,0 mM, pH 7.0) – Positive Mode of ESI-FTICR-MS zoomed spectrum (7+) charge state (*m/z* 2060 – 2400)

3.2 – Lysozyme reaction with compound 5

Reaction	Deconvoluted Mass	Molecule	Abundance	Abundace [%]
Lys	14296.86852	$[M+H]^+$	17286140	100.00
Lys + **5**	14803.04136	$[M+H-H_2O]^+$	4950278	28.64

Table S2 – Data for the most relevant deconvoluted species (reaction with compound **5**)

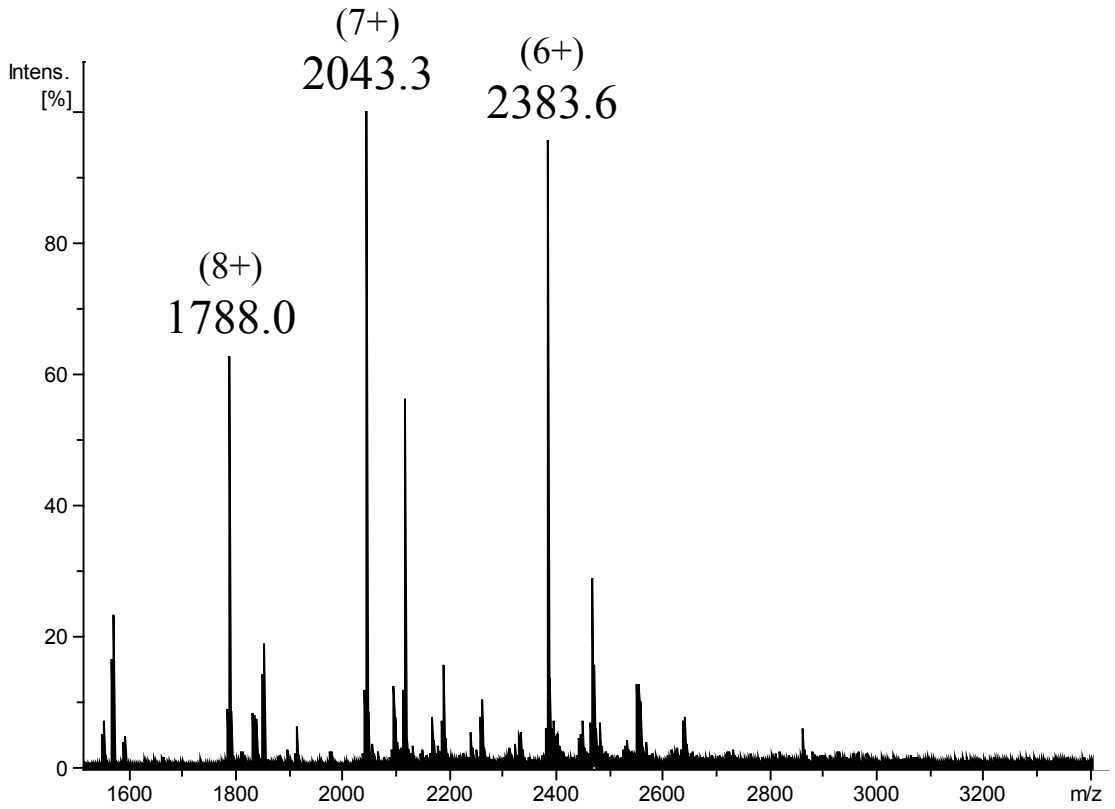

Figure S35 – Lyzosyme (10,0 µM) with compound **5** dissolved in DMSO (200,0 µM) in acetate buffer (50,0 mM, pH 7.0) – Positive Mode of ESI-FTICR-MS full spectrum

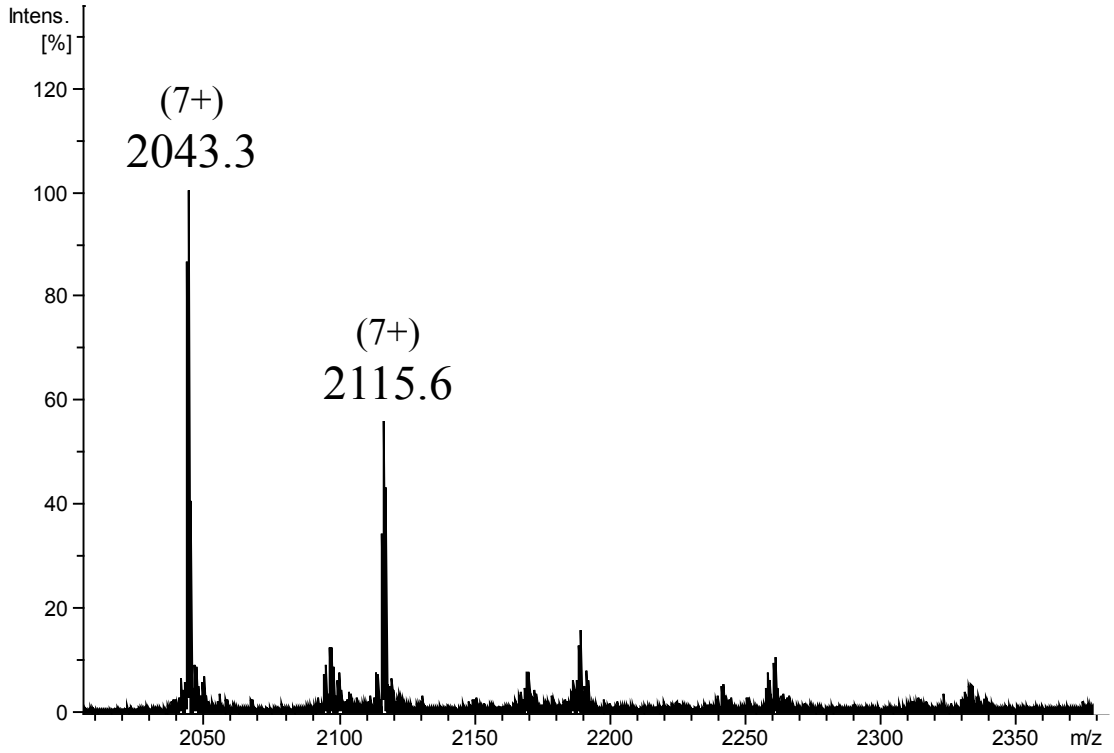

Figure S36 – Lyzosyme (10,0 µM) with compound **5** dissolved in DMSO (200,0 µM) in acetate buffer (50,0 mM, pH 7.0) – Positive Mode of ESI-FTICR-MS zoomed spectrum (7+) charge state (*m/z* 2000 – 2400)

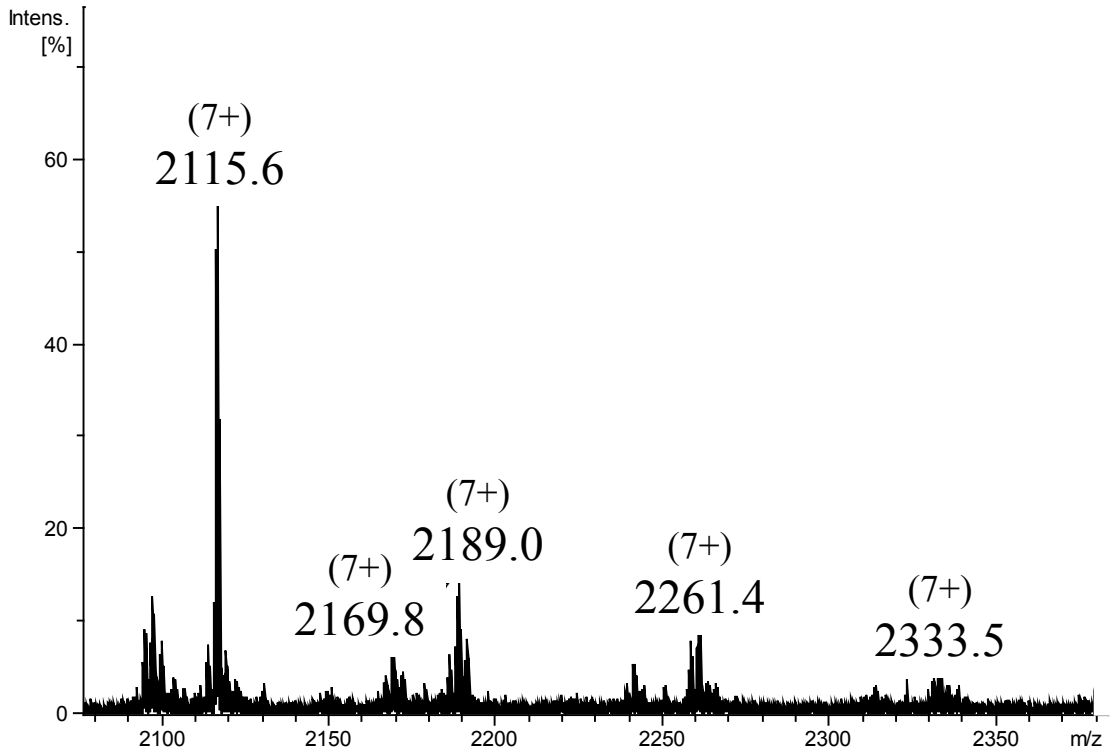

Figure S37 – Lyzosyme (10,0 µM) with compound **5** dissolved in DMSO (200,0 µM) in acetate buffer (50,0 mM, pH 7.0) – Positive Mode of ESI-FTICR-MS zoomed spectrum (7+) charge state (*m/z* 2060 – 2400)

3.3 – Lysozyme reaction with compound 6

Reaction	Deconvoluted Mass	Molecule	Abundance	Abundace [%]
Lys	14296.84145	$[M+H]^+$	7490357	100.00
Lys + **6**	14732.91497	$[M+H-H_2O]^+$	1133658	15.13

Table S3 – Data for the most relevant deconvoluted species (reaction with compound **6**)

3.3.1 – After 30 minutes of reaction

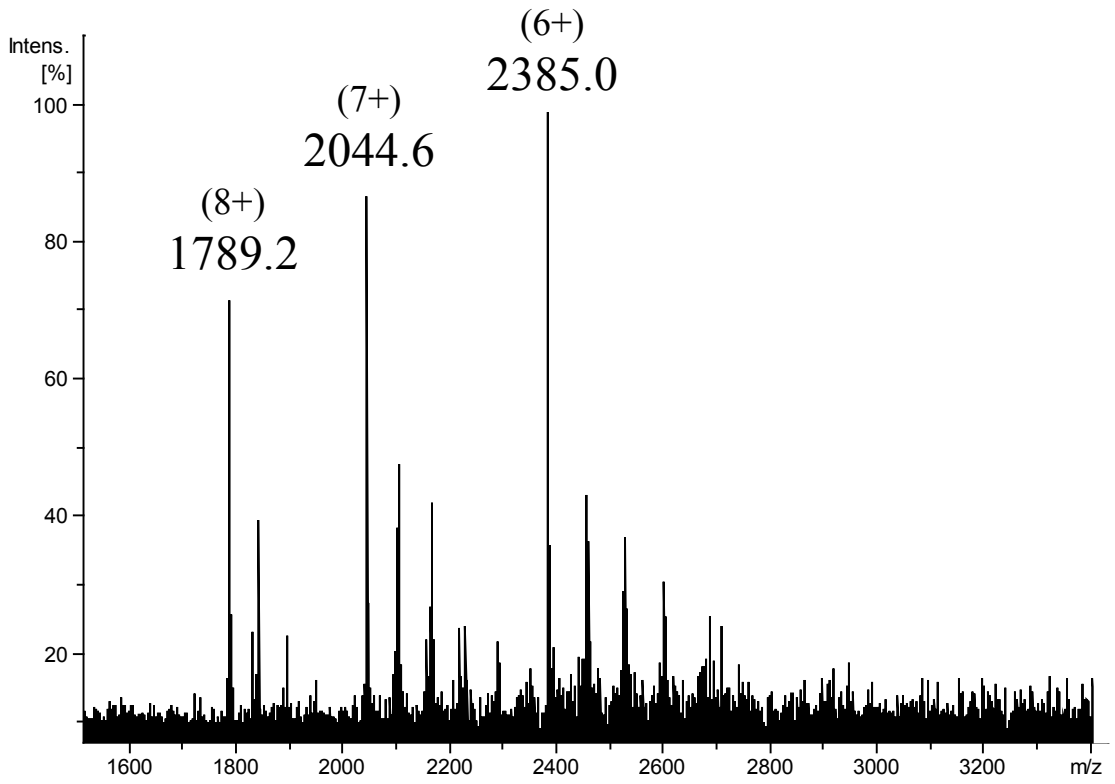

Figure S38 – Lyzosyme (10,0 µM) with compound **6** dissolved in DMSO (200,0 µM) in acetate buffer (50,0 mM, pH 7.0) after 30 minutes – Positive Mode of ESI-FTICR-MS full spectrum

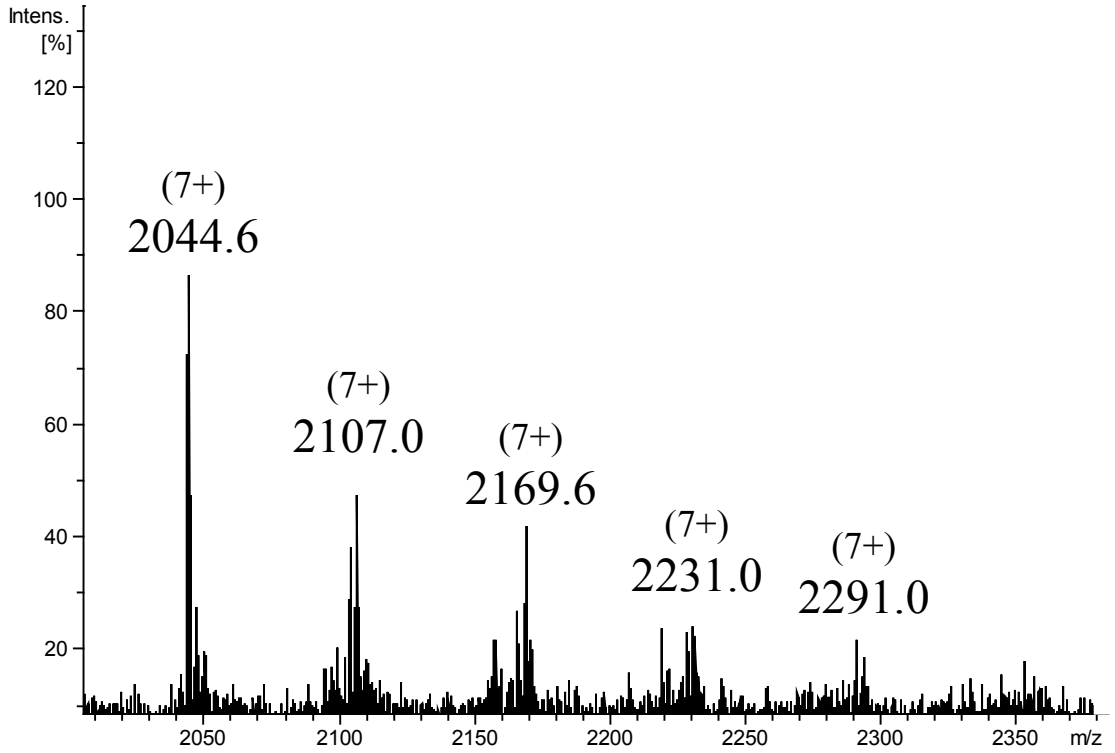

Figure S39 – Lyzosyme (10,0 µM) with compound **6** dissolved in DMSO (200,0 µM) in acetate buffer (50,0 mM, pH 7.0) after 30 minutes – Positive Mode of ESI-FTICR-MS
Zoomed spectrum (7+) charge state (*m/z* 2000 – 2400)

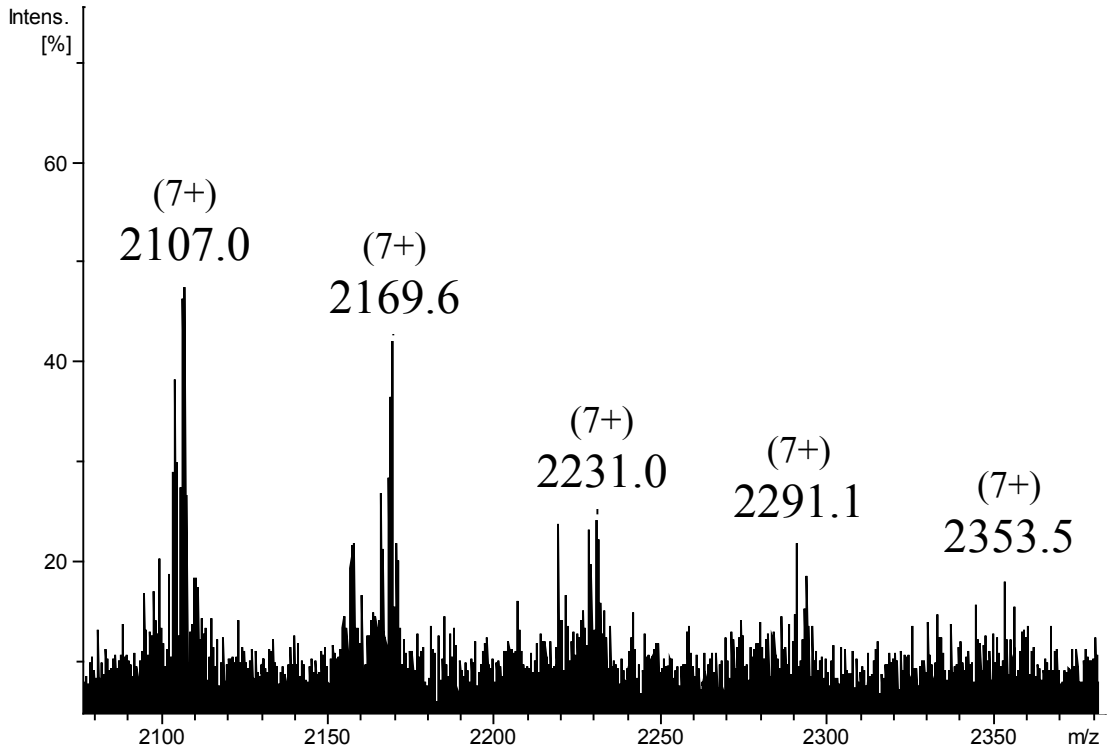

Figure S40 – Lyzosyme (10,0 µM) with compound **6** dissolved in DMSO (200,0 µM) in acetate buffer (50,0 mM, pH 7.0) after 30 minutes – Positive Mode of ESI-FTICR-MS
Zoomed spectrum (7+) charge state (*m/z* 2060 – 2400)

3.3.2 – After 2 hours of reaction

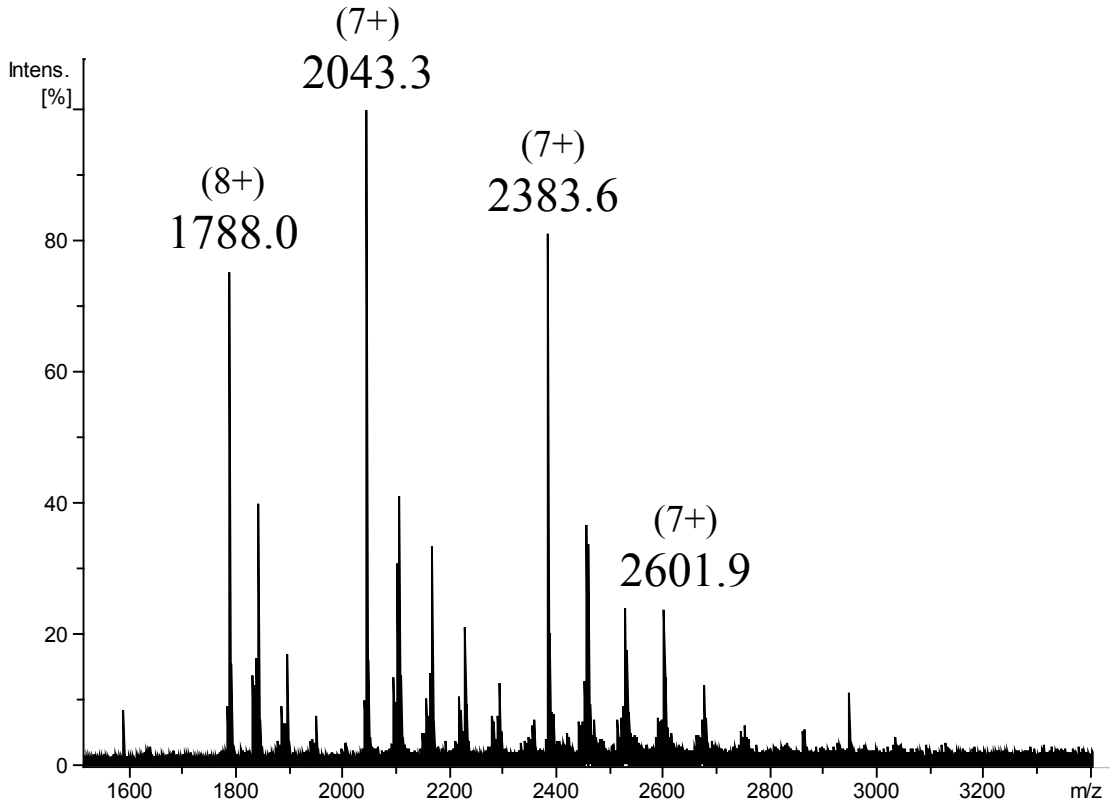

Figure S41 – Lyzosyme (10,0 µM) with compound **6** dissolved in DMSO (200,0 µM) in acetate buffer (50,0 mM, pH 7.0) after 2 hours – Positive Mode of ESI-FTICR-MS full spectrum

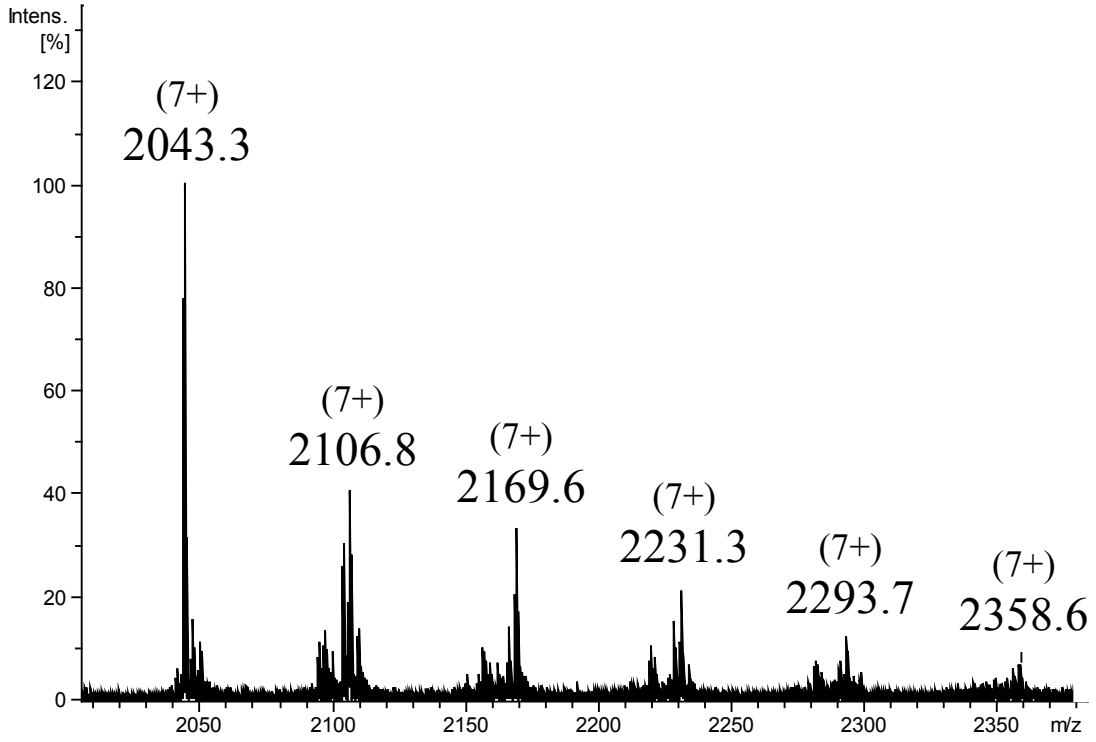

Figure S42 – Lyzosyme (10,0 µM) with compound **6** dissolved in DMSO (200,0 µM) in acetate buffer (50,0 mM, pH 7.0) after 2 hours – Positive Mode of ESI-FTICR-MS
Zoomed spectrum (7+) charge state (*m/z* 2000 – 2400)

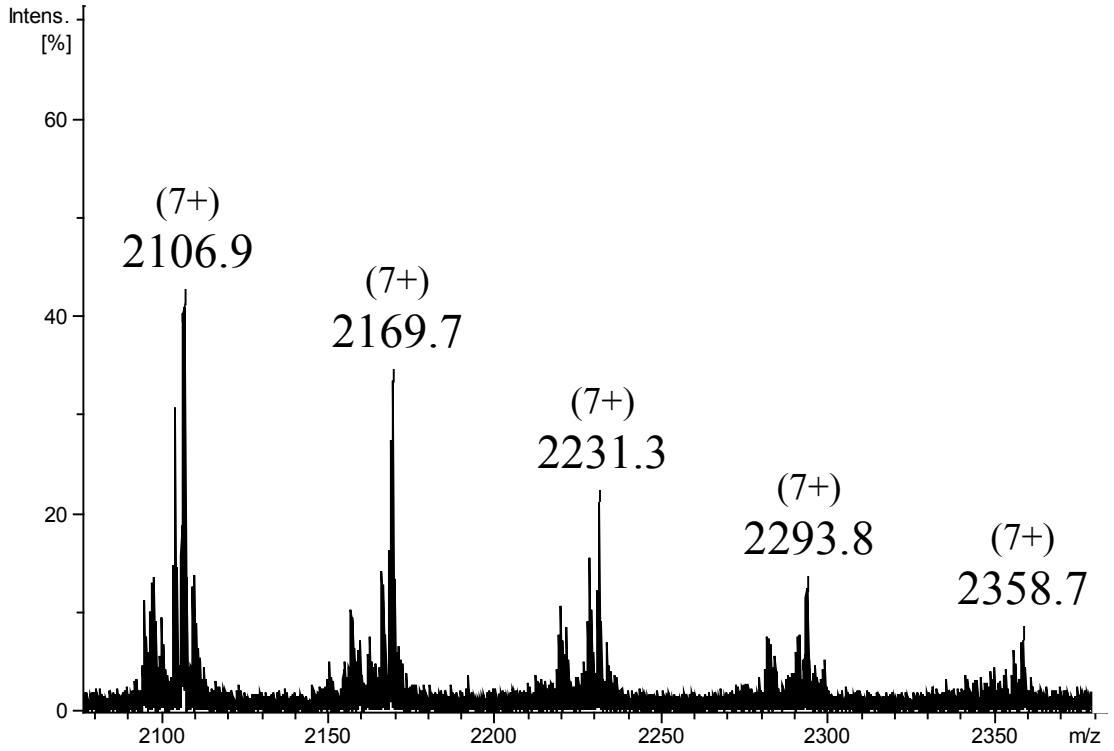

Figure S43– Lyzosyme (10,0 µM) with compound **6** dissolved in DMSO (200,0 µM) in acetate buffer (50,0 mM, pH 7.0) after 2 hours – Positive Mode of ESI-FTICR-MS
Zoomed spectrum (7+) charge state (*m/z* 2060 – 2400)

3.4 – Lysozyme reaction with compound 14

Reaction	Deconvoluted Mass	Molecule	Abundance	Abundace [%]
Lys	14296.77072	[M+H]+	38352376	100.00
Lys + **14**	14687.05288	$[M+H-H_2O]^+$	2494221	6.50

Table S4 – Data for the most relevant deconvoluted species (reaction with compound **14**)

3.4.1 – After 30 minutes of reaction

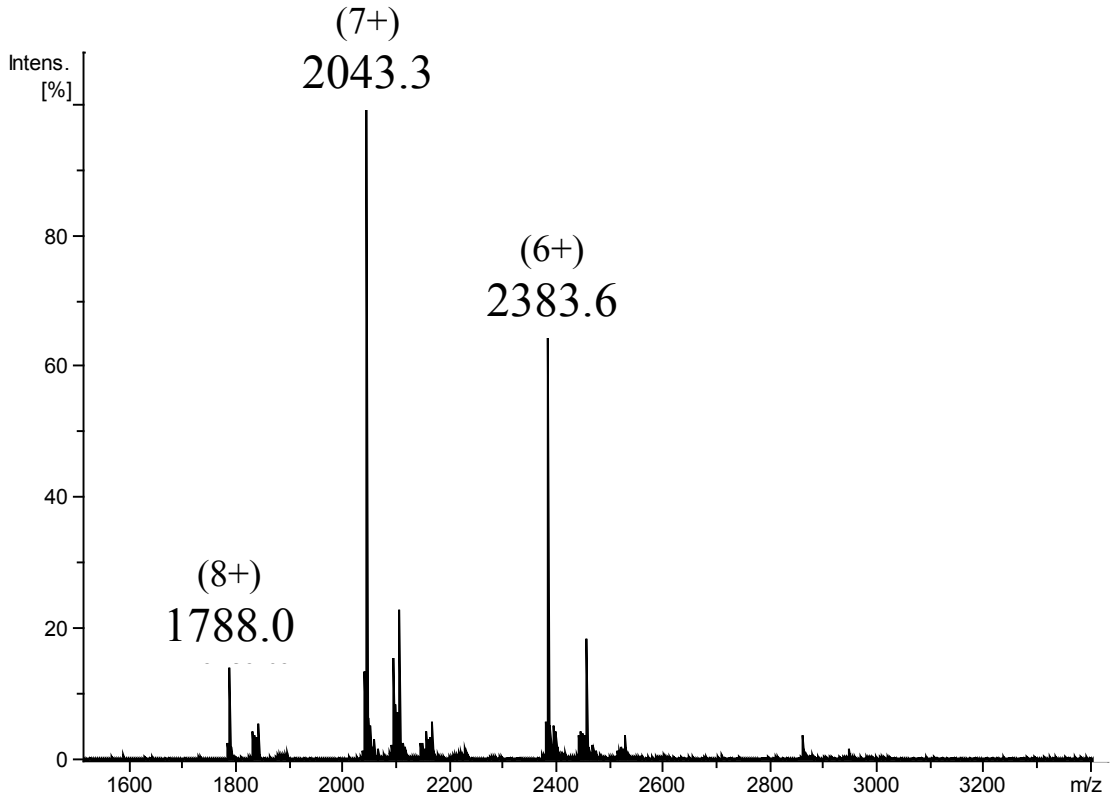

Figure S44 – Lyzosyme (10,0 µM) with compound **14** dissolved in DMSO (200,0 µM) in acetate buffer (50,0 mM, pH 7.0) after 30 minutes –
Positive Mode of ESI-FTICR-MS full spectrum

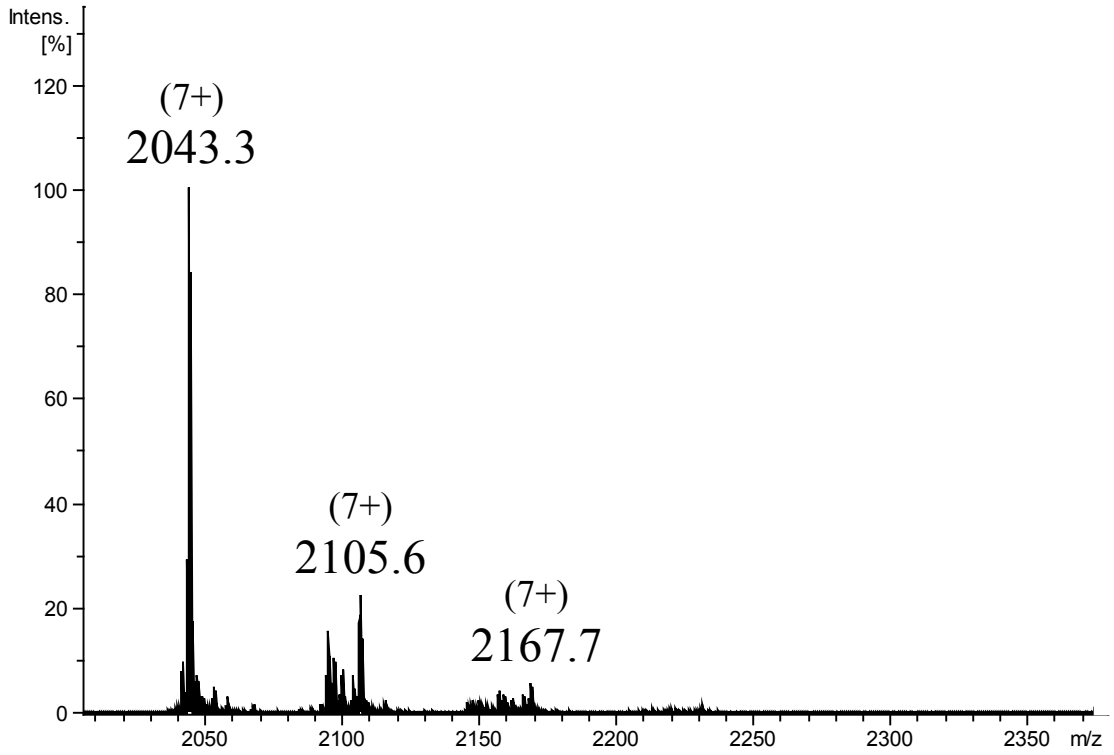

Figure S45 – Lyzosyme (10,0 µM) with compound **14** dissolved in DMSO (200,0 µM) in acetate buffer (50,0 mM, pH 7.0) after 30 minutes – Positive Mode of ESI-FTICR-MS
Zoomed spectrum (7+) charge state (*m/z* 2000 – 2400)

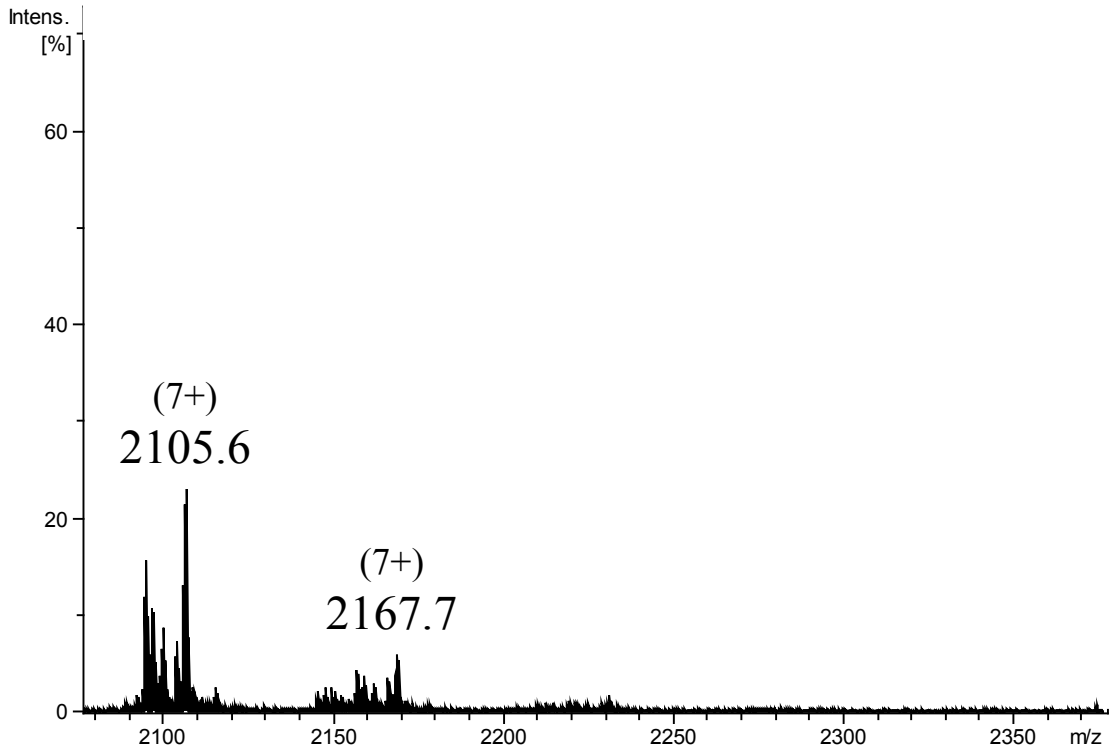

Figure S46 – Lyzosyme (10,0 µM) with compound **14** dissolved in DMSO (200,0 µM) in acetate buffer (50,0 mM, pH 7.0) after 30 minutes – Positive Mode of ESI-FTICR-MS Zoomed spectrum (7+) charge state (*m/z* 2060 – 2400)

3.4.2 – After 2 hours of reaction

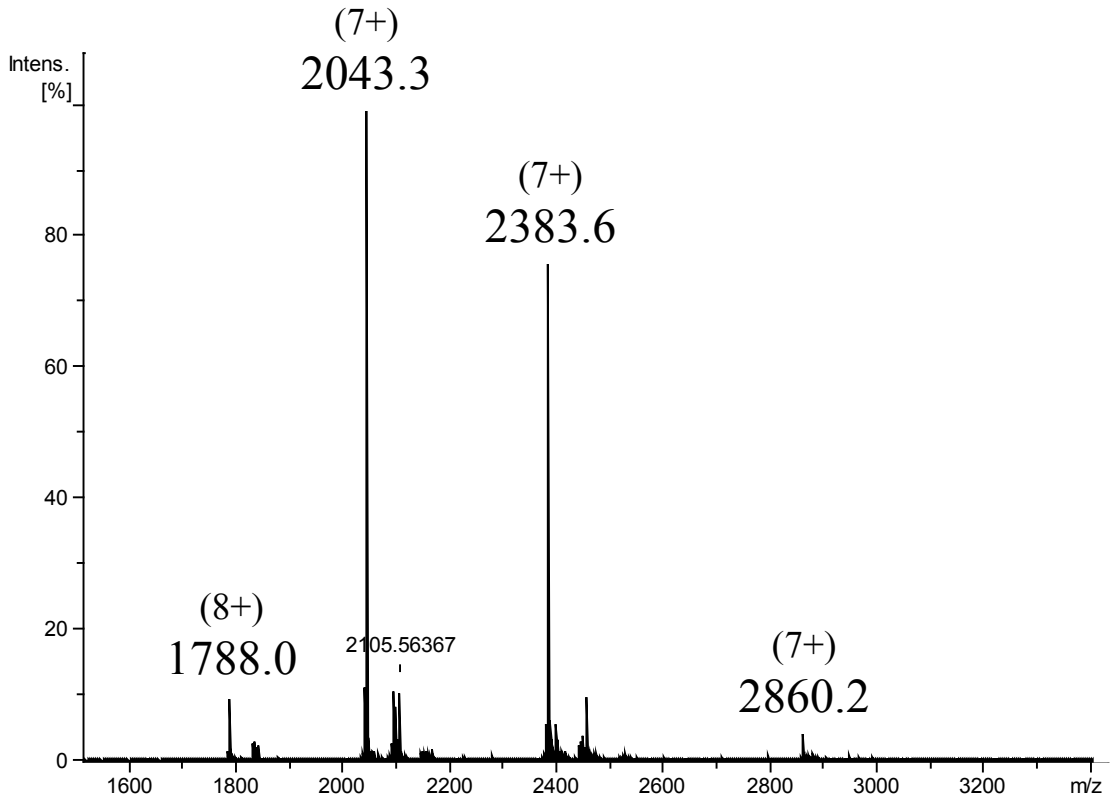

Figure S47 – Lyzosyme (10,0 µM) with compound **14** dissolved in DMSO (200,0 µM) in acetate buffer (50,0 mM, pH 7.0) after 2 hours – Positive Mode of ESI-FTICR-MS full spectrum

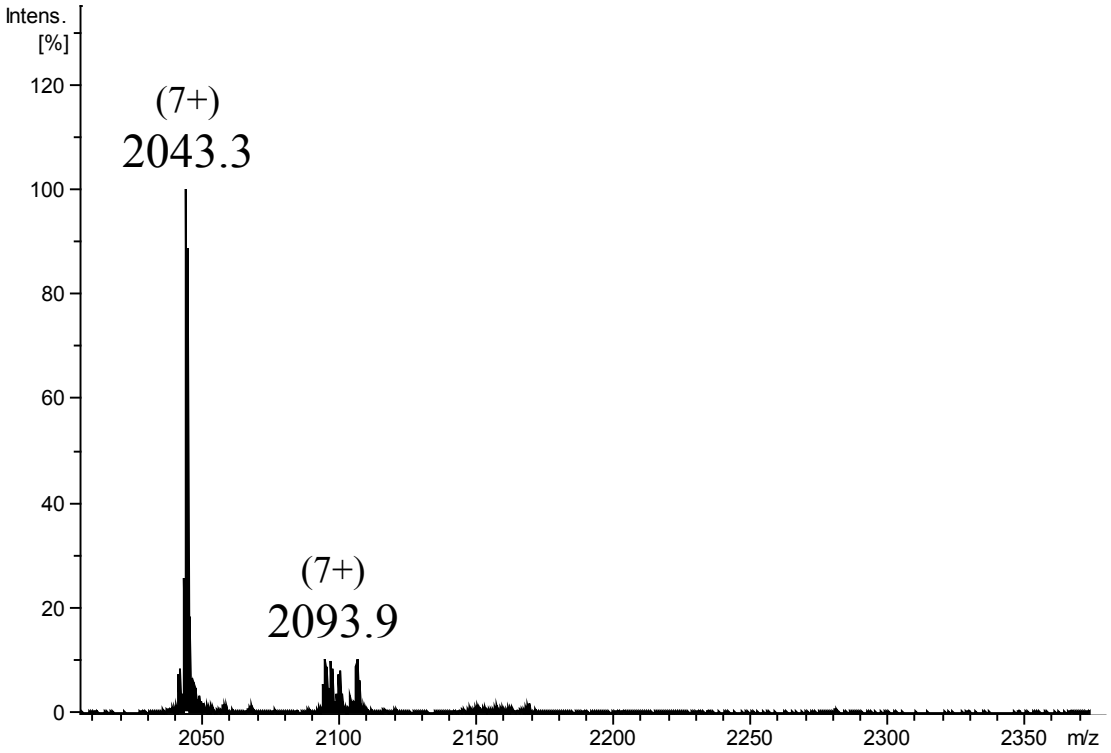

Figure S48 – Lyzosyme (10,0 µM) with compound **14** dissolved in DMSO (200,0 µM) in acetate buffer (50,0 mM, pH 7.0) after 2 hours – Positive Mode of ESI-FTICR-MS Zoomed spectrum (7+) charge state (*m/z* 2000 – 2400)

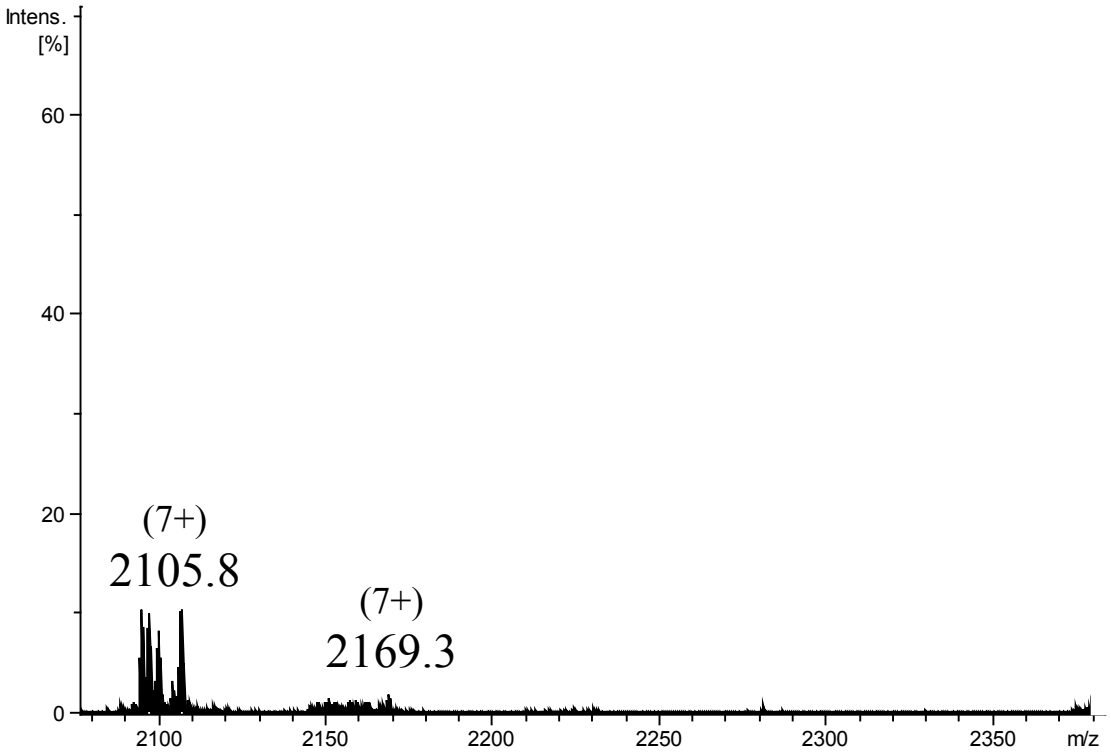

Figure S49 – Lyzosyme (10,0 µM) with compound **14** dissolved in DMSO (200,0 µM) in acetate buffer (50,0 mM, pH 7.0) after 2 hours – Positive Mode of ESI-FTICR-MS Zoomed spectrum (7+) charge state (*m/z* 2060 – 2400)

4. Biological Results

Cell staining procedure: Cell lines were culture in RPMI-1640 medium with phenol red supplemented with 10% fetal bovine serum and antibiotic antimycotic solution (100 units/ml penicillin, 0,1 mg/ml streptomycin and 0,25 mg/ml amphotericin B) at 37 ºC and under a 5% CO_2 atmosphere.

Cells were plated on a Slide 8 well plate and incubated for 2 days in the incubator. Prior to the addition of the cells the chambers were coated with poly-L-Lysine for one hour and then each well washed with fresh cell culture medium. Samples to be tested were added at approximately 20 μM concentration in phenol-red free RPMI-1640 medium for 4 hours. When pre-treatment with FA-ethylenediamine was employed, the concentration used was 71,4 μM and the incubation time was 1 hour. Cells were washed and their membrane stained with WGA-Alexa 594 dye for 15-20 minutes. After this staining treatment, the cells were washed and fresh phenol-red free RPMI-1640 medium was added. Cell imaging was carried on a Leica TCS-SP5 Multiphoton/Confocal Fluorescence Microscope equipped with a continuous Ar ion laser (458, 476, 488, 496 and 514 nm) and a Helium-Neon laser (633 nm).