Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Salen/salan metallic complexes as redox labels for biomolecules

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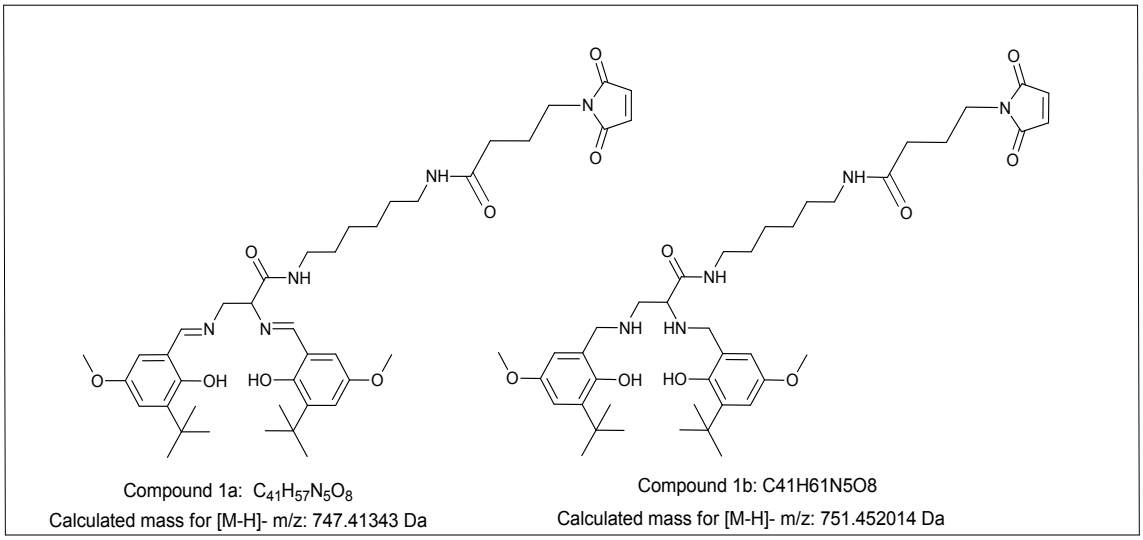
MATERIALS AND METHODS

- ### Reagents and Materials

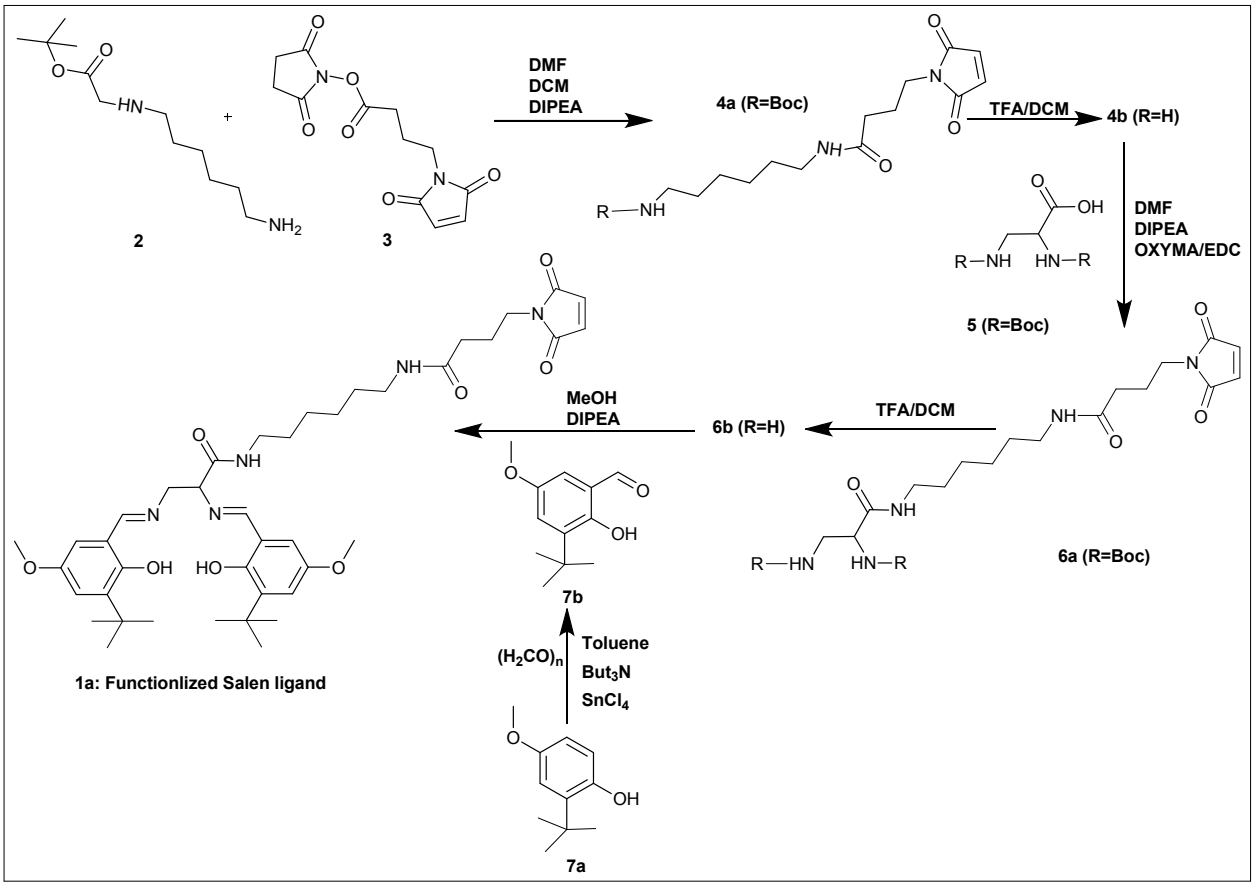
N-Boc-1,6-hexanediamine hydrochloride, *N*,*N*-Diisopropylethylamine(DIPEA), 2-(tert-butyl)-4-methoxyphenol, Tin(IV) chloride ($SnCl_4$), Paraformaldehyde, 2,5-Dimethylfuran, Copper (II) acetate, Vanadyl acetylacetonate were purchased from Sigma-Aldrich (France). N-succinimidyl 4-Maleimidobutyrate was acquired from Bachem (Switzerland). Boc-D-Dap(Boc)-OH*DCHA, N-Ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC*HCl), and Oxyma Pure were procured by Iris (Deutschland). The organic solvents required for syntheses were purchased from VWR (France) except DMF form Carlo Erba (France). The aptamer sequence was synthesized by the Swiss DNA Company Microsynth. All the ^1H NMR spectra were recorded using a JEOL 400 MHz spectrometer and the NMR solvents were purchased from Eurisotop (France). Liquid chromatography analyses were achieved using a Thermo Fisher Scientific LC/MS device, Accela HPLC coupled to a LCQ Fleet equipped with an electrospray ionization source and a 3D ion-trap analyzer. Mass spectral analysis was carried out using Electrospray ionization mass spectrometry (ESI-MS) (Thermo Scientific, France). The results of Aptamer – Markers coupling were checked by uHPLC Vanquish Thermo coupled to mass detector Orbitrap, QExactive Plus Thermo equipped with an electrospray ionization heated source ESI.

- **Chemical synthesis**



Schema S1 Functionalized Salen 1a / Salan 1b ligands

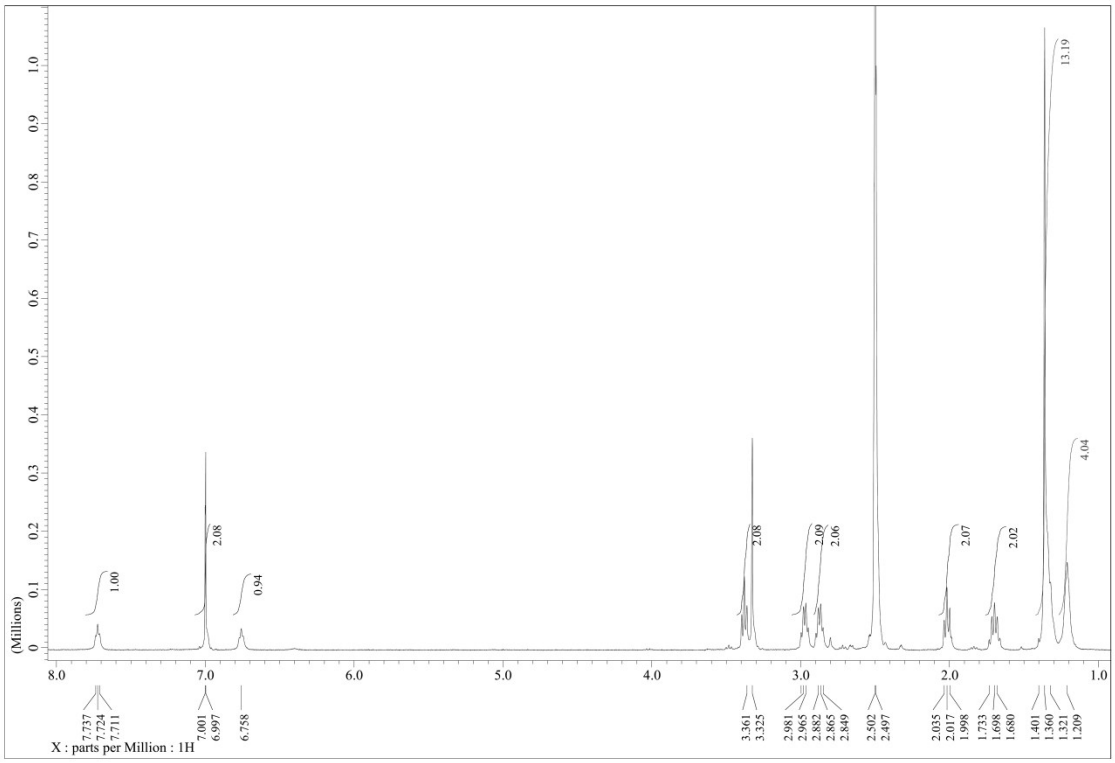
Salen ligand synthesis



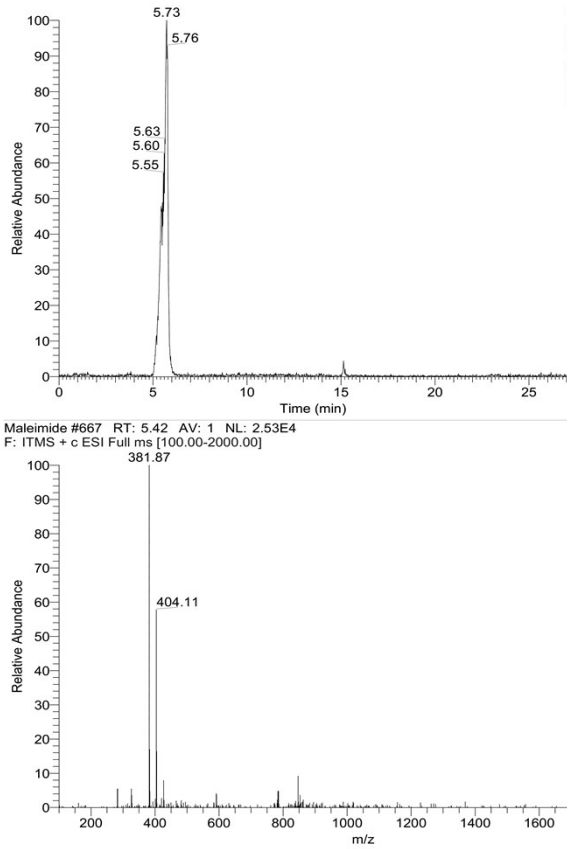
Scheme S2 Synthesis route for Salen ligand

Compound 4a

N-succinimidyl 4-Maleimidobutyrate (756 mg, 2.7 mmol) and N-Boc 1,6-hexadiamino hydrochlorate (455 mg, 1.8mmol) were suspended in a mixture of dichloromethane (DCM) and DimEthylformamide (DMF) (3 mL/3 mL), with DIPEA (5.4mmol). After 1h of stirring at room temperature, ethyl acetate was added and the resulting solution was washed twice with HCl, twice with Na_2CO_3 and twice with saturated NaCl solution. The organic phase is dried over anhydrous magnesium sulfate ($MgSO_4$), filtered and finally evaporated under low pressure. The crude compound was purified by column chromatography with dichloromethane/methanol (9.8/0.2) as eluent, yielding 650mg of compound **4a** (92%). 1H RMN 400 MHz (DMSO-d6) δ/ppm: 7.72 (t, 1H, NH), 7.00 (d, 2H, H-maleimide group), 6.75 (t, 1H, NH), 3.36 (m, 2H, CH_2-CH_2-N), 2.98 (q, 2H, NH-CH_2-CH_2), 2.86 (q, 2H, NH-CH_2-CH_2), 2.01 (t, 2H,CO-CH_2-CH_2-), 1.69 (m, 2H,-CH_2-CH_2-CH_2), 1.40 -1.32 (13H, 1.38 (s, 9H, CH_3 butyl group) 1.36 (m, 4H, CH_2-CH_2-CH_2), 1.20 (m, 4H, CH_2-CH_2-CH_2). Calculated for $C_{27}H_{45}N_5O_8$ $[M+H]^+$ m/z: 382.23; $[M+Na]^+$ m/z: 404.21; found m/z: $[M+H]^+$ m/z: 381.87; $[M+Na]^+$ m/z: 404,11.



NMR spectrum of Compound **4a**



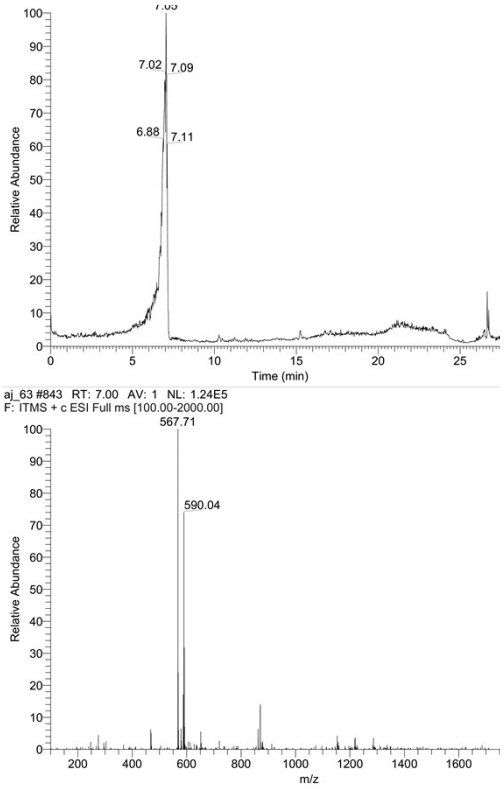
LC/MS chromatogram of compound **4a**

Compound 4b

For the Boc deprotection, Compound **4a** was suspended in 3 mL DCM and 3 mL trifluoroacetic acid (TFA) and stirred for 1h. The resulting product (compound **4b**) was washed with cyclohexane three times to eliminate the TFA.

Compound 6a

Boc-D-Dap(Boc)-OH*DCHA (130 mg, 0.38 mmol) was added to a stirred solution of compound 4b (85 mg, 0.25 mmol), EDC (118 mg, 0.62 mmol), Oxyma (88 mg, 0.62mmol) and DIPEA (175µl, 1 mmol) in 3 mL of DMF at room temperature. After stirring overnight, the mixture was supplemented with ethyl acetate (20 mL) and washed successively with HCl (0.5M, 1 x 5 mL), H_2O (1 x 5 mL), $NaHCO_3$ (10%, 1 x 5 mL), H_2O (2 x 5 mL) and Brine (1 x 5ml). The organic phase is dried over $MgSO_4$, filtered and evaporated under low pressure yielding 125mg of compound 6a (89%). Calculated for $C_{27}H_{45}N_5O_8$ $[M+H]^+$ m/z: 568.33; $[M+Na]^+$ m/z/590.31; $[M+K]^+$ m/z:606.42; found m/z: $[M+H]^+$ m/z: 567.94; $[M+Na]^+$ m/z: 590.24.



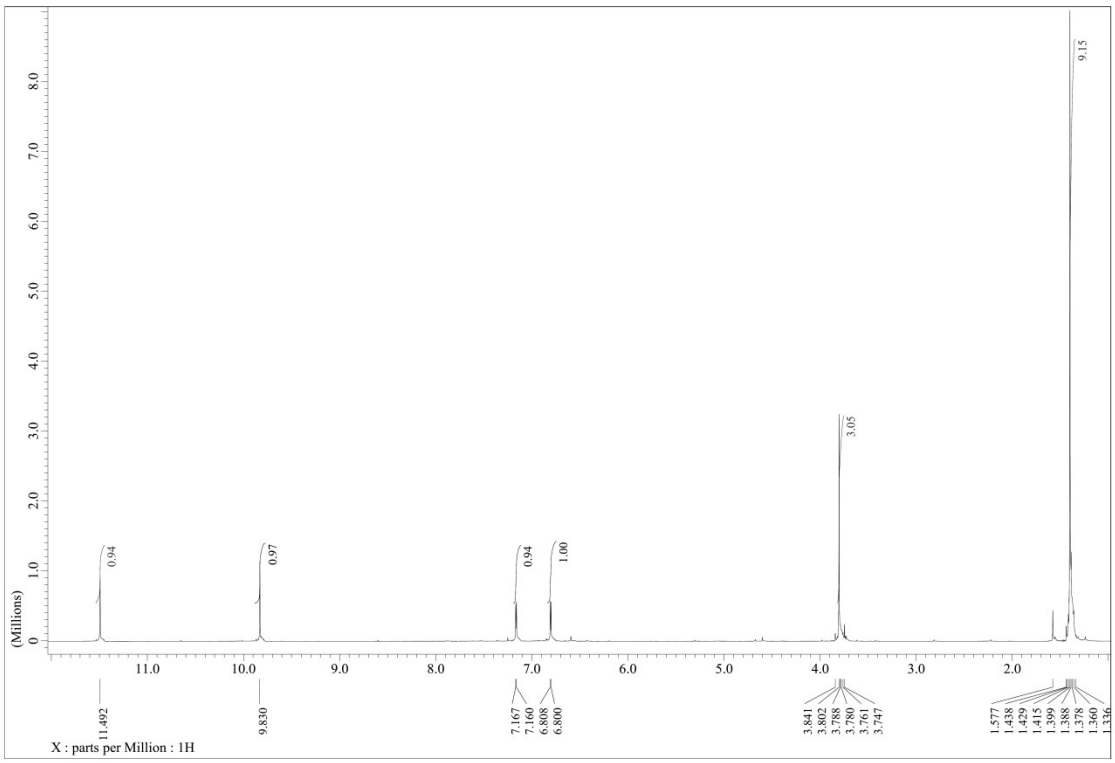
LC/MS chromatogram of compound **6a**

Compound 6b

For the Boc deprotection, compound **6a** was suspended in 5 mL DCM and 5 mL TFA and stirred for 3h. The resulting product (compound **6b**) was washed with cyclohexane three times.

Compound 7b

2-(tert-butyl)-4-methoxyphenol (compound **7a**) (13.7 mmol, 2.5mg) was dissolved in a solution of Toluen, tributylamine (11 mmol, 2.5 mL) and $SnCl_4$ (1.38 mmol, 0.16 mL). After 20min of stirring, Paraformaldehyde (1 g, 33.3 mmol) was added and the solution was stirred overnight at 100°C. The reaction mixture was mixed with 140 mL of HCl (1M) and stirred for 45min at room temperature. The resulting solution was washed first with diethyl ether (4*100mL) and brine (1 x20ml). The organic phase is dried over anhydrous $MgSO_4$, filtered and evaporated under low pressure. The crude compound was purified by column chromatography with cyclohexane/ethyl acetate (4/6) as eluent, leading to 1.9 g (67%) of compound **7b**. ^{1}H RMN 400 MHz (Chloroform-D) δ/ppm: 11.49 (s, 1H, C-CHO), 9.83 (s, 1H, OH), 7.16 - 7.80 (s, 1H, ArH), 3.78 (s, 3H, O-CH_3), 1.39 -1.35 (m, 9H, CH_3-butyl group).

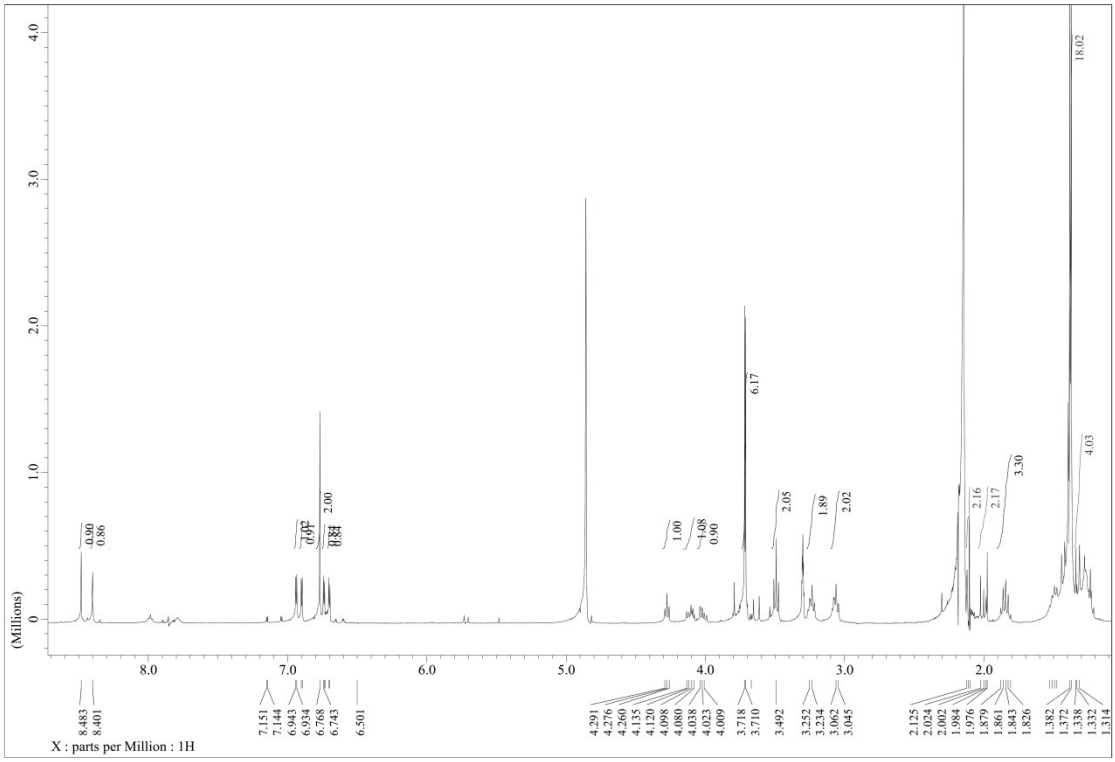


NMR spectrum of Compound **7a**

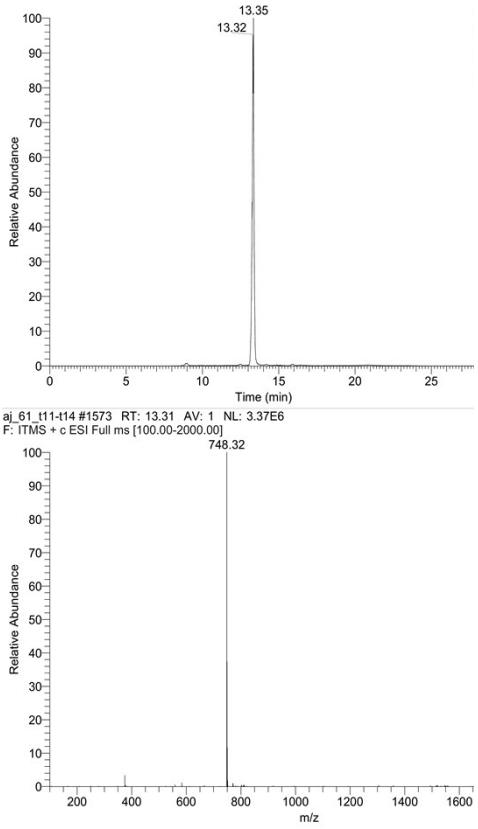
Compound 1a

3-(tert-Butyl)-2-hydroxy-5-methoxybenzaldehyde (compound **7b**) (125 mg, 0.6 mmol), dissolved in 3ml methanol, was added dropwise to the mixture of Compound **6b** (150mg, 0.264 mmol) in 2 mL methanol with DIPEA (0.56 mL, 0.6 mmol). After stirring overnight at room temperature, the reaction mixture was diluted with 10 mL of water, acidified with acetic acid (0.8 mmol). The resulting solution was supplemented with 20 mL ethyl acetate, washed with water (2 x 5ml) and finally the organic phase was dried with $MgSO_4$ filtered and the solvent was eliminated under low pressure. The crude compound was purified by column chromatography with dichloromethane/methanol (9.5/0.5) as eluent, leading to 150 mg (76%) of compound **1a.** ^{1}H RMN 400 MHz (MeOH-D3) δ/ppm: 8.48 (s, 1H, ArH), 8.40 (s, 1H, ArH), 6.94 – 6.93 (d, 1H, ArH), 6.902 (d, 1H, ArH), 6.76 (d, 2H, H-maleimide group), 6.74 – 6.74 (d, 1H, ArH), 6.704 – 6.769 (d,1H, ArH), 4.291 – 4.260 (t, 1H, CH_2-CH-N), 4.13 – 4.08 (m, 1H, -CH-CH_2-N),4.038 – 3.99 (m, 1H, CH-CH_2-N), 3.71 – 3.70 (6H, 3.718 (s, 3H, O-CH_3), 3.710 (s, 3H, O-CH_3)) , 3.49 (t, 2H, CH_2-CH_2-N), 3.25 – 3.04 (4H, 3.23 (m, 2H, CH_2-CH_2-NH), 3.06 (q,2H, CH_2-CH_2-NH), 2.12 – 2.10 (t, 2H, CH_2-CH_2-CO), 2.02 – 1.97 (m, 2H, CH_2-CH_2-CH_2), 1.87 (m, 4H, CH_2-CH_2-CH_2), 1.39-1.31 (22H, 1.38 (s, CH_3 butyl group), 1.37 (s, CH_3 butyl group),

1.31 (q, 4H, CH_2-CH_2-CH_2)). Calculated for $C_{41}H_{61}N_5O_8$ $[M+H]^+$ m/z: 748.42, found $[M+H]^+$ m/z: 748.32.

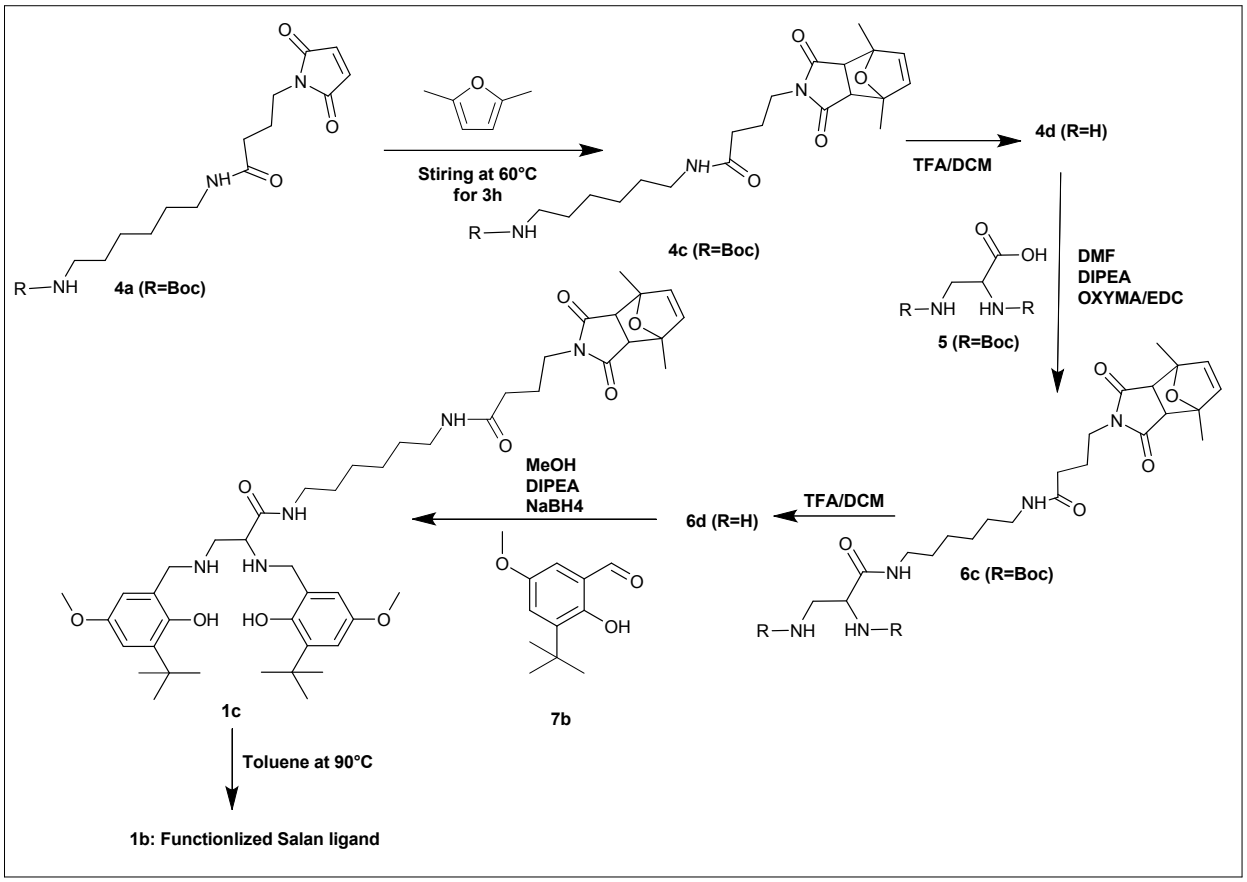


NMR spectrum of compound **1a**



LC/MS chromatogram of compound **1a**

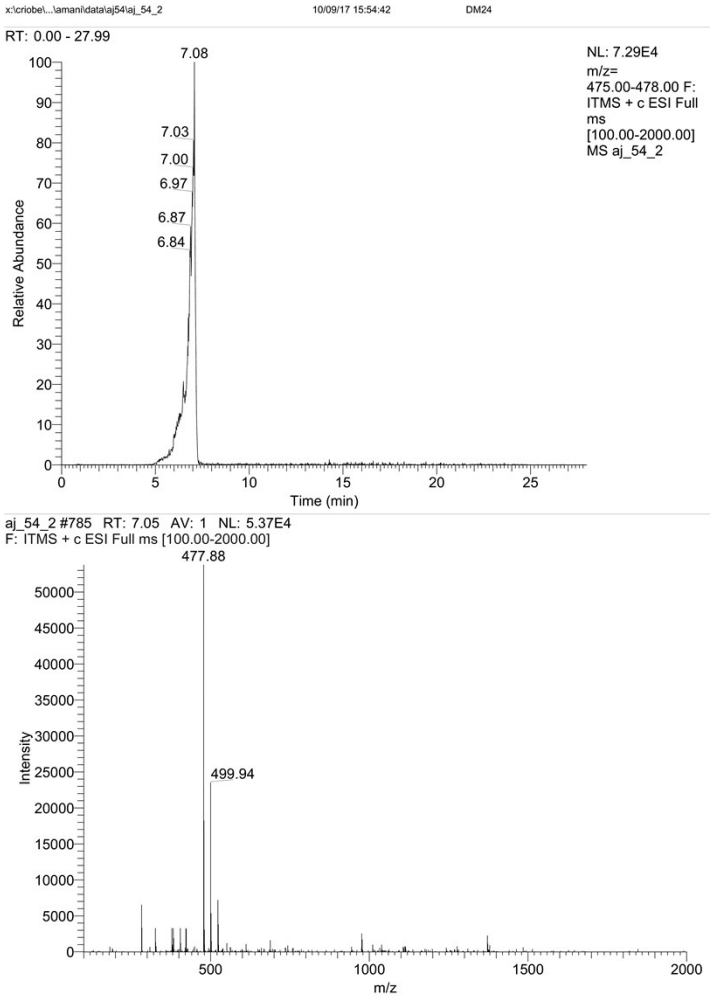
Salan ligand synthesis



Schema S3 Synthesis route for Salan ligand

Compound 4c

Diels alder reaction was carried out to protect the maleimide group of compound **4a** (100 mg, 0.25 mmol) dissolved in 3 mL 2,5-Dimethylfuran. The reaction mixture was stirred for 3h at 60°C. The crude compound was purified by column chromatography with dichloromethane/methanol (9.5/0.5) as eluent, leading to 112 mg (95%) of compound **4c.** Calculated for $C_{25}H_{37}N_3O_6$ $[M+H]^+$ m/z: 478.29, $[M+Na]^+$ m/z: 500.27; found m/z: $[M+H]^+$ m/z: 477.88, $[M+Na]^+$ m/z :499.94.



LC/MS chromatogram of compound **4c**

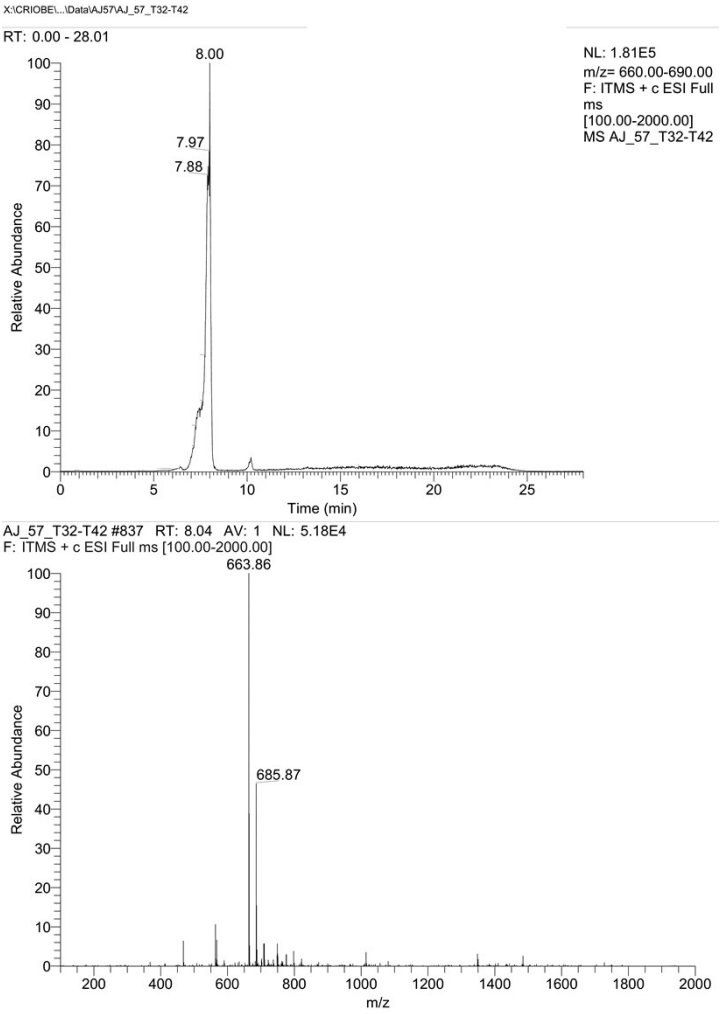
Compound 4d

For the Boc deprotection, Compound **4c** was suspended in 2 mL DCM and 2 mL TFA and stirred for 1h. The resulting product (compound **4d**) was washed with cyclohexane three times.

Compound 6c

Boc-D-Dap(Boc)-OH*DCHA (compound 5) (110 mg, 0.36 mmol) was added to a stirred solution of compound **4d** (70 mg, 0.18 mmol), EDC (83 mg, 0.54 mmol), Oxyma (51 mg, 0.36mmol) and DIPEA (125µl, 0.72mmol) in 3 mL of DMF at room temperature. After stirring overnight, the mixture was supplemented with ethyl acetate (20 mL) and washed successively with HCl (0.5M, 1 x 5 mL), H_2O (1 x 5 mL), $NaHCO_3$ (10%, 1 x 5 mL), H_2O (2 x 5 mL) and Brine (1 x 5ml). The organic phase is dried over $MgSO_4$, filtered and evaporated under low pressure. The crude compound 6c was purified by column chromatography with

dichloromethane/methanol (9.75/0.25) as eluent, yielding 80mg of compound **6c** (68%). Calculated for $C_{33}H_{51}N_5O_9$ $[M+H]^+$ m/z: 664.39; $[M+Na]^+$ m/z: 686.37 found m/z: $[M+H]^+$ m/z: 663.86; $[M+Na]^+$ m/z 685.87.



LC/MS chromatogram of compound **6c**

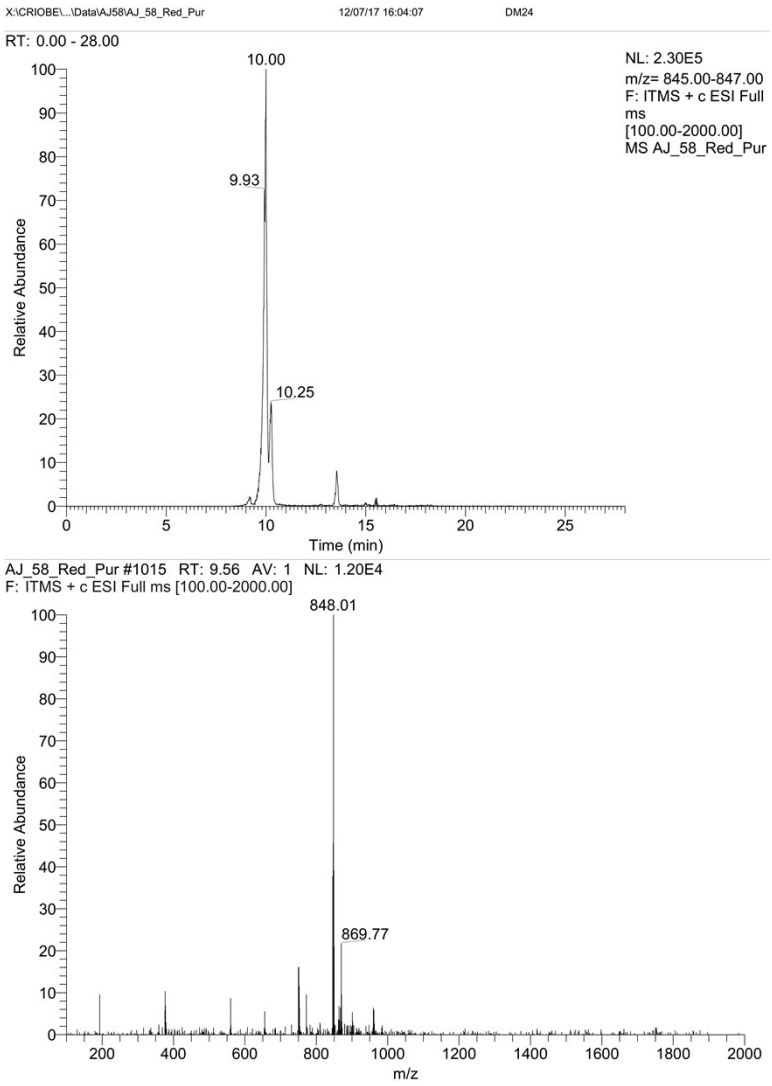
Compound 6d

For the Boc deprotection, compound **6c** was suspended in 5 mL DCM and 5 mL TFA and stirred for 3h. The resulting product (compound **6d**) was washed with cyclohexane three times.

Compound 1c

3-(tert-Butyl)-2-hydroxy-5-methoxybenzaldehyde (compound **7b**) (50 mg, 0.25 mmol), dissolved in 2 mL MeOH, was added dropwise to the mixture of Compound **6d** (80mg, 0.12 mmol) in 2 mL MeOH with DIPEA (0.83 mL, 0.48 mmol). After stirring overnight at room

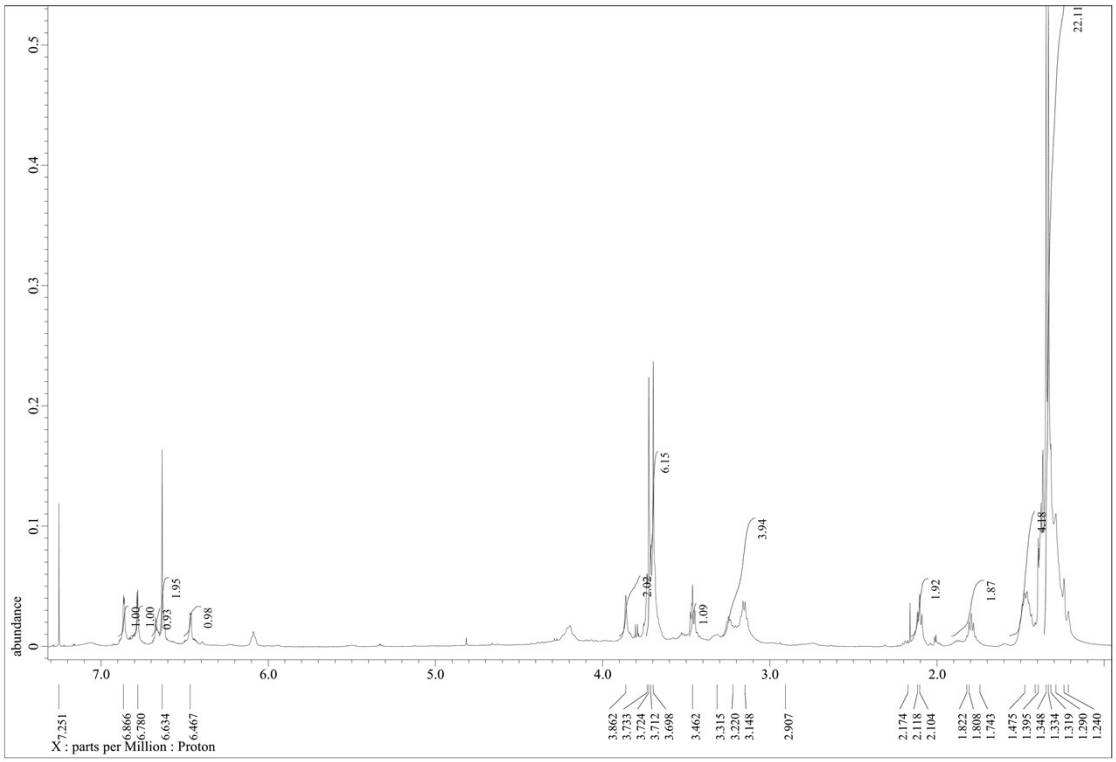
temperature, the reaction mixture was cooled and Sodium borohydride (18.2 mg, 0.422 mmol) was added. After stirring for 1h, the solvent was evaporated; the resulting product was suspended in 10 mL water and acidified with acetic acid (0.48 mmol). The mixture was supplemented with 20 mL ethyl acetate, washed with water (2 x 5ml) and finally the organic phase was dried with $MgSO_4$ filtered and the solvent was eliminated under low pressure. The crude compound was purified by column chromatography with dichloromethane/methanol (9.8/0.2) as eluent, leading to 70mg (69%) of compound **1c.** Calculated for $C_{47}H_{67}N_5O_9$ $[M+H]^+$ m/z: 848.51, $[M+Na]^+$ m/z: 870.49 found m/z: $[M+H]^+$ m/z: 848.01, $[M+Na]^+$ m/z: 869.77.



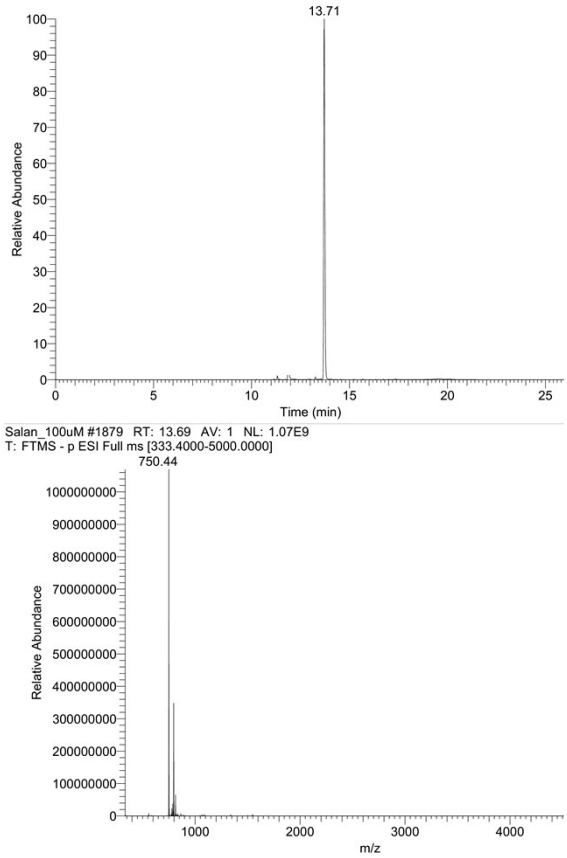
LC/MS chromatogram of compound **1c**

Compound 1b

Compound **1a** was finally obtained by a reverse Diels-Alder reaction for maleimide group deprotection. Compound **1c** (50mg, 0.05 mmol) was suspended in 3ml anhydrous toluene at 90°C and stirred overnight. The solvent was evaporated and purification by column chromatography was carried out with dichloromethane/methanol (9.5/0.5) as eluent, leading to 15 mg (40%) of compound **1b.** ^{1}H RMN 400 MHz (Chloroform-D) δ/ppm: 6.86 (d,1H, ArH), 6.78 (d,1H,ArH), 6.69 (d, 1H, ArH), 6.63 (d, 2H, H-maleimide group), 6.46 (d, 1H, ArH), 6.088 (t, 1H, NH – CH_2), 4.23 (t, 1H, CH_2-CH_2-N), 3.86 (t,1H, , CH_2-CH_2-N), 3.75 – 3.66 (6H, 3.71 (s, 3H, O-CH_3), 3.69 (s, 3H, O-CH_3)), 3.46 (t, 1H, CH_2-CH-NH), 3.25 – 3.12 (4H, 3.23 (t, 2H, CH_2-CH_2-NH), 3.14 (t, 2H, CH_2-CH_2-NH)), 2.11 (t, 2H, CH_2-CH_2-CO), 1.80 (m, 2H, CH_2-CH_2-CH_2), 1.47 (m, 4H, CH_2-CH_2-CH_2), 1.39 – 1.21 (22H, 1.37 (m, 4H, CH_2-CH_2-CH_2), 1.36 (s, 9H, CH_3 butyl group), 1.34 (s, 9H, CH_3 butyl group), 1.27 (q, 4H, CH_2-CH_2-CH_2)). Calculated for $C_{41}H_{61}N_5O_8$ $[M-H]^-$ m/z: 750.45 found m/z: $[M-H]^-$ m/z: 750.44.



NMR spectrum of compound **1b**



LC/MS chromatogram of compound **1b**

Copper salen complex synthesis

Compound 8

Copper (II) acetate (5.19 mg, 0.026mmol), dissolved in 2 mL methanol was added to solution of Salen ligand (compound **1a**) (20 mg, 0.026 mmol) in 1 mL methanol. The reaction mixture was heated at reflux for 1h. The resulting solution was dried under low pressure. Copper salen complex was obtained as dark blue solid.

Oxo-vanadium salen complex synthesis

Compound 9

Vanadyl acetylacetonate (6.9mg, 0.026mmol), dissolved in 2 mL methanol, was added to Salen ligand (compound **1a**) (20 mg, 0.026 mmol) solution was dissolved in 1 mL methanol. The reaction mixture was heated at reflux for 1 hour. The resulting solution was dried under low pressure. Oxo-vanadium salen complex was obtained as dark green solid.

Oxo-vanadium salan complex synthesis

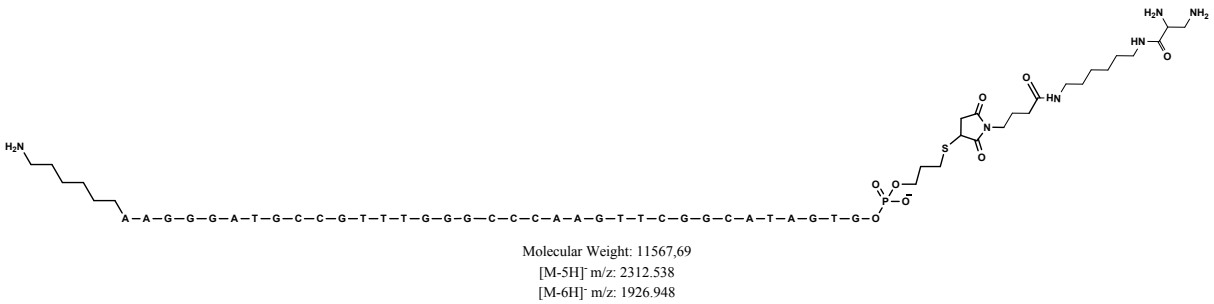
Compound 10

Vanadyl acetylacetonate (0.69 mg, 0.0026 mmol), dissolved in 1 mL methanol, was added to Salen ligand (compound **1b**) (2 mg, 0.0026 mmol) solution was dissolved in 1 mL methanol. The reaction mixture was heated at reflux for 1 hour. The resulting solution was dried under low pressure. Oxo-vanadium salan complex was obtained as dark green solid.

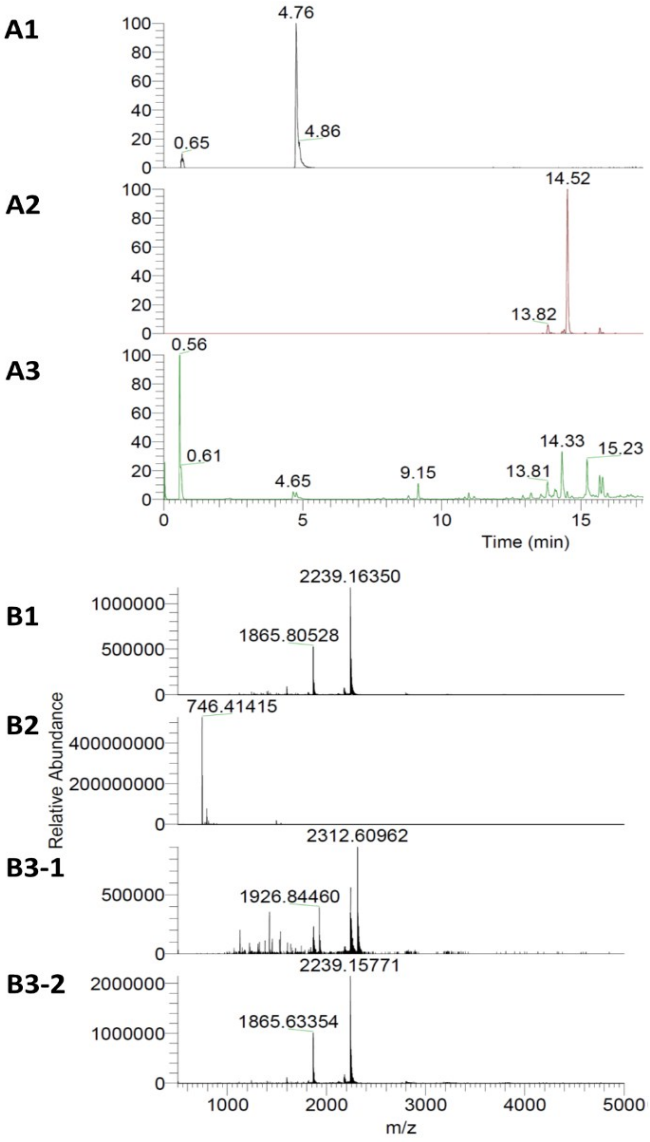
Markers-Aptamer coupling

Linearized aptamer (10µL, 11 nmol) was mixed with desired compound (**1a**, **1b**, **10**) (90µL, 22 nmol). The reaction solution was stirred overnight at 45°C. Obtained compound were respectively compound **11**, **12**, **13.**

Compound 11: Calculated for $C_{393}H_{506}N_{143}O_{223}P_{36}S^-$ $[M-5H]^-$ m/z: 2388.634, $[M-6H]^-$ m/z: 1990.362 found m/z: $C_{369}H_{478}N_{143}O_{219}P_{36}S^-$ (compound 14) $[M-5H]^-$ m/z: 2312.60962, $[M-6H]^-$ m/z: 1926.84460, $C_{352}H_{449}N_{138}O_{215}P_{36}S^-$ (Free Aptamer) $[M-5H]^-$ m/z: 2239.15771, $[M-6H]^-$ m/z: 1865.63354.



Compound **14**: Coupling of the Aptamer with a degradation product of Salen ligand



LC/HRMS of E2 aptamer (A1, B1) ; Salen 1a (A2, B2) ; compound 11 (A3, B3).

Compound 12:

Calculated for C393H508N143O223P36S- [M-5H]- m/z: 2389.44, [M-6H]- m/z: 1991.03 found m/z: [M-5H]- m/z: 2389.45207, [M-6H]- m/z: 1991.04880.

Compound 13:

Calculated for $C_{393}H_{506}N_{143}O_{224}P_{36}SV^-$ $[M-5H]^-$ m/z: 2402.022, $[M-6H]^-$ m/z: 2001.5183 found m/z: $[M-5H]^-$ m/z: 2402.24902, $[M-6H]^-$ m/z: 2001.87732.

- **UV-Vis spectroscopy Characterization**

Compounds were diluted in MeOH to a concentration of 0.125mM. The absorption spectra were recorded over the wavelength range 200-900 nm.

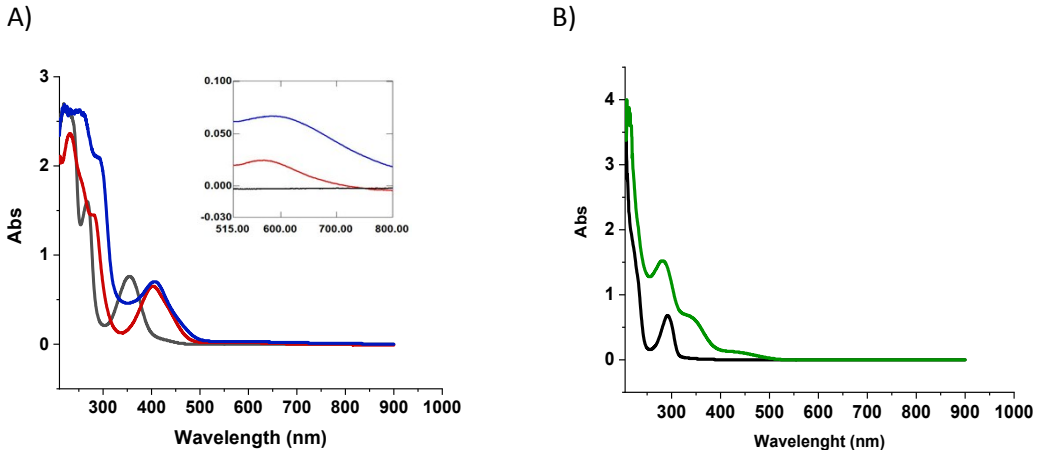


Figure 1(A) UV-Vis spectra of salen 1a (black), Cu-salen 8 (red) and VO-salan 9 (Blue). (B) UV-Vis spectra of salan 1b (black) and VO-salan 10 (green)

- **Cyclic Voltammetry measurements**

The complexes were dissolved in methanol at a concentration of 2mM. Then, a drop of 3µL of the complexe solution (6 nmol) was adsorbed on the surface of the working electrode of the SPCE. The CV measurements were performed with PBS buffer (0.1M) (NaCl, KCl, Na_2HPO_4 and KH_2PO_4). Potential values were given vs Ag/AgCl with a scan rate equal to 0.1V/s.

- **Aptasensor's design**

First, the functionalization of the working electrode of the SPCE was carried out by electrochemical reduction of the diazonium salts (2mM 4-Aminobenzoic acid and 0.1M NaNO2). Carboxyl groups formed on the surface was activated by EDC / NHS. The VO-Salan labeled, Salan labeled and label free aptamers were diluted in PBS to a concentration of 20 µM and a heat treatment (8 min at 90 ° C, 4 min at 0 ° C and 15 min at room temperature) was applied to ensure the linearization of aptamer sequence. A drop of 20µl of the different aptamers solutions was placed on the working electrode. After 4 hours at room temperature and under humid atmosphere, a peptide bond between the NH2 of the aptamer and the activated carboxyl group on the surface was formed. The electrodes were then washed with PBS buffer and square wave voltammetry (SWV) measurements were performed. The last step consist in dropping a solution of E2 (8, 4 and 0.05 µM) prepared in Tris buffer (selection buffer of the used aptamer) on the electrode and leave it for 1 hour at room temperature and under a humid atmosphere. Finally, SWV measurements were achieved.