Electronic Supporting Information

Bisphosphonate Ligand Mediated Ultrasensitive Capacitive Protein Sensor: Complementary Match of Supramolecular and Dynamic Chemistry

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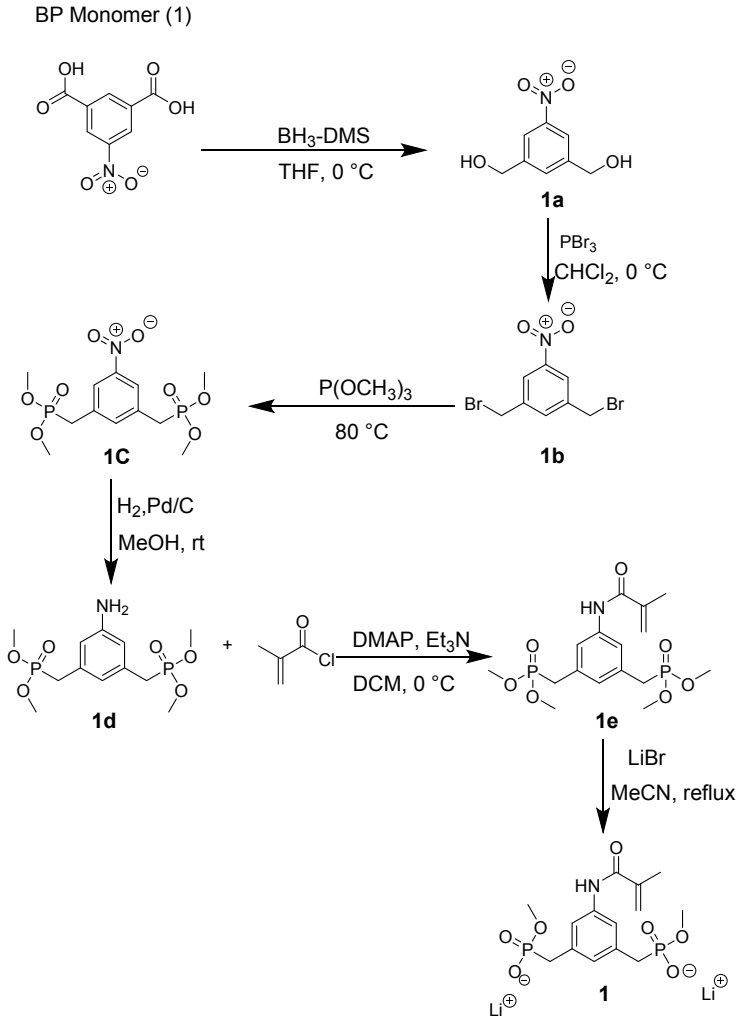
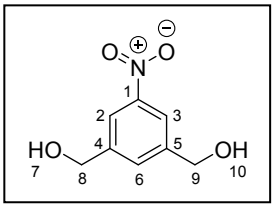
Table of Contents



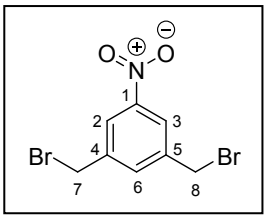
Scheme 1. Synthesis of 5-(Methacryloylamino)-m-xylylene bisphosphonic acid dimethylesterdilithium salt (**1**, **BP**) monomers

Synthesis of Functional Monomer

3, 5-bis - (hydroxymethyl) nitroxylylene (1a) 10 g of 5-nitroisophthalic acid (47.36 mmol, 1.0 equivalent) was dissolved in 100 mL dry THF and the mixture was cooled to 0 °C. 94.72 mL borane dimethyl sulfide complex solution (2.0 M BH_3-DMS in THF) (14.39 g, 189.44 mmol, 4 equivalent) was then added dropwise using dropping funnel to the reaction mixture under argon atmosphere. The resulting reaction was stirred at 0 °C for 1 hr and left to stir for additional 48 h at room temperature. The unreacted borane complex was neutralized by dropwise addition of 100 mL methanol. After the removal of excess solvent, the yellow oily residue was dissolved in 500 mL ethyl acetate and washed with 100 mL saturated $NaHCO_3$, water and brine respectively. The organic layer was dried with $MgSO_4$ and then solvent was removed by using rotavap. The crude compound **1a** was purified using silica gel column chromatography with a mixture of ethyl acetate: cyclohexane/ 5:1 gives (5.65 g, 81% yield) 1a as pale-yellow crystals was obtained. **^{1}H-NMR** (600 MHz, MeOD-d_4) δ [ppm] = **8.14** (s, 2H, H-2; H-3), **7.72** (s, 1H, H-6), **4.72** (s, 4H, H-8, H-9). **^{13}C-NMR** (150.92 MHz, MeOD-d_4) δ [ppm] = **149.92** (C-1), **145.48** (C-4, C-5), **131.67** (C-6), **120.88** (C-2, C-3), **63.94** (C-8, C-9).

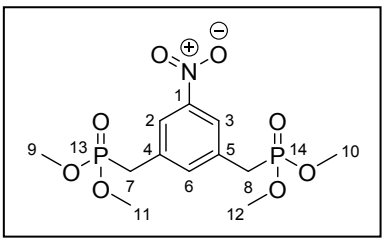


3, 5-bis-(bromomethyl) nitroxylylene (1b) 5.0 g of 3, 5-bis-(hydroxymethyl) nitroxylene **1a** (27.3 mmol, 1 equivalent) was dissolved in 30 mL dry dichloromethane and cooled to 0 °C. To this solution, 6.5 ml (69.2 mmol, 2.5 equivalent) of phosphorus tribromide in 10 mL dry dichloromethane was added dropwise. The reaction

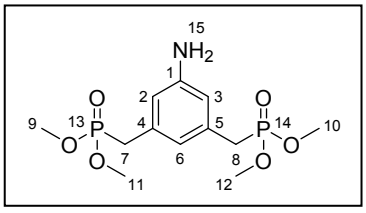


mixture was stirred for 1 h at 0 °C and then stirred for 3 days at room temperature. The resulting solution was poured into 200 g of crushed ice to neutralize the remaining phosphorus tribromide. The resulting suspension was then extracted with (4 x 100 mL) diethyl ether. The collected organic layers were washed twice with 50 mL saturated $NaHCO_3$, water and brine respectively. The organic layer was then dried with Na_2SO_4 and the excess ether was removed *in vacuo* to give (7.46 g, 91 % yield) **1b** as a pale-yellow powder. **^{1}H-NMR** (600 MHz, $CDCl_3$) δ [ppm] = **8.19** (s, 2H, H-2; H-3), **7.75** (s, 1H, H-6), **4.52** (s, 4H, H-8, H-9). **^{13}C-NMR** (150.92 MHz, $CDCl_3$) δ [ppm] = **148.45** (C-1), **140.30** (C-4, C-5), **135.19** (C-6), **123.61** (C-2, C-3), **30.57** (C-7, C-8).

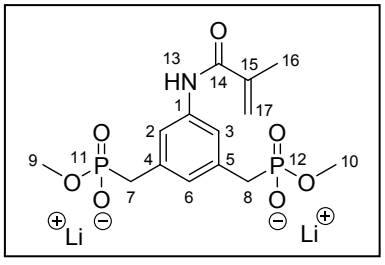
5-Nitro-m-xylylene bisphosphonic acid tetramethylester (1c) 5.1 g (16.5 mmol, 1 equivalent) of 3, 5-bis-(bromomethyl) nitro-xylylene (**1b)** was dissolved in 15 mL (127 mmol, 7.7 equivalent) trimethylphosphite. This solution was stirred for 5 h at 80 °C and was further stirred overnight at room temperature. The excessive trimethylphosphite was removed via condensation. The residue was dissolved in cyclohexane and stirred for 1 h. The obtained solid was filtered and recrystallized in hexane/ethyl acetate mixture to give (5.4 g; 89% yield) **1c** as solid compound. **^{1}H-NMR** (600 MHz, $CDCl_3$) δ [ppm] = **8.03** (s, 2H, H-2; H-3), **7.59** (s, 1H, H-6), **3.71** (d, ^3J = 10.9 Hz, 12H, H-9, H-10, H-11, H-12), **3.23** (d, ^2J = 22.0 Hz, 4H, H-7, H-8).**^{13}C-NMR** (150.92 MHz, $CDCl_3$) δ [ppm] = **148.42** (C-1), **136.98** (C-4, C-5), **133.84** (C-2, C-3), **123.19** (C-6), **53.00** (C-9, C-10, C-11, C-12), **32.43** (d, $J_{C,P}$ = 138.9 Hz, C-7, C-8). **^{31}P-NMR** (242.92 MHz, $CDCl_3$) δ [ppm] = **26.80** (P-13, P-14).



5-Amino-m-xylylene bisphosphonic acid tetramethyl ester (1d) 0.45 g (1.27 mmol, 1 equivalent) of 5-Nitro-*m*-xylylene bisphosphonic acid tetramethylester (**1c)** and 12 mg of palladium on Carbon was dispersed in 20 mL absolute ethanol. The hydrogen gas was placed into reaction vessel by flushing and the reaction mixture was stirred for two days at room temperature. The Pd/C was removed via filtration over celite before the solvent was removed *in vacuo* to give the product **1d** (0.4 g, 97% yield) was a white solid. **^{1}H-NMR** (600 MHz, $CDCl_3$) δ [ppm] = **6.61** (s, 1H, H-6), **6.58** (s, 2H, H-2; H-3), **4.14** (bs, 2H, H-15), **3.66** (d, ^3J = 10.8 Hz, 12H, H-9, H-10, H-11, H-12), **3.04** (d, ^2J = 21.8 Hz, 4H, H-7, H-8). **^{13}C-NMR** (150.92 MHz, $CDCl_3$) δ [ppm] = **146.09** (C-1), **132.17** (C-4, C-5), **122.06** (C-2, C-3), **115.87** (C-6), **53.06** (C-9, C-10, C-11, C-12), **32.76** (d, $J_{C,P}$ = 138.1 Hz, C-7, C-8). **^{31}P-NMR** (242.92 MHz, $CDCl_3$) δ [ppm] = **26.86** (P-13, P-14).



5-(Methacryloylamino)-m-xylylenebisphosphonic acid tetramethylester (1e) 2.05 g (6.1 mmol, 1 equivalent) of 5-Amino-*m*-xylylene bisphosphonic acid tetramethylester (**1d**), 0.95 mL (0.69 g, 6.8 mmol, 1.1 equivalent) triethylamine and a catalytic amount of 4-(*N,N*-dimethylamino)-pyridine was dissolved in 50 mL dry dichloromethane. A solution of 0.88 mL (0.95 g, 9.1 mmol, 1.5 equivalent) methacryloyl chloride in 20 mL dry dichloromethane was added dropwise at 0 °C within 1 h. The reaction mixture was stirred for an additional hour at room temperature. The reaction mixture was washed several times with 0.6 M NaOH and then dried with anhydrous Na_2SO_4. The solvent was evaporated in vacuum oven. The crude product was purified by silica gel column chromatography with dichloromethane/methanol 19:1 solvent mixture and the obtained product **1e** (1.4 g, 57 % yield) was yellow oil. **^{1}H-NMR** (600 MHz, $CDCl_3$) δ [ppm] = **7.89** (s, 1H, H-15), **7.44** (s, 2H, H-2, H-3), **6.94** (s, 1H, H-6), **5.77** (s, 1H, H-19a), **5.42** (s, 1H, H-19b), **3.65** (d, ^3J = 10.8 Hz, 12H, H-9, H-10, H-11, H-12), **3.09** (d, ^2J = 21.9 Hz, 4H, H-7, H-8), **2.00** (s, 3H, H-18). **^{13}C-NMR** (150.92 MHz, $CDCl_3$) δ [ppm] = **166.72** (C-16), **140.48** (C-17), **138.49** (C-1), **132.16** (C-4, C-5), **126.66** (C-6), **120.04** (C-19), **119.98** (C-2, C-3), **52.86** (C-9, C-10, C-11, C-12), **32.38** (d, $J_{C,P}$ = 137.9 Hz, C-7, C-8), **18.60** (C-18). **^{31}P-NMR** (242.92 MHz, $CDCl_3$) δ [ppm] = **26.28** (P-13, P-14).



5-(Methacryloylamino)-m-xylylenebisphosphonic acid dimethylester dilithium salt (BP; 1) 1.05 g (2.59 mmol, 1 equivalent) of 5-(Methacryloylamino)-*m*-xylylenebisphosphonic acid tetramethylester (**1e**) was dissolved in 50 mL absolute acetonitrile. To the solution 655 mg (7.54 mmol, 2.9 equivalent) of Lithium Bromide was added and the reaction mixture was refluxed overnight. The resulting

reaction mixture was centrifuged to recover the solid pellet. The pellet was washed with (4 x 15 mL) diethyl ether and dried *in vacuo* to gave (0.97 g, 96 % yield) off-white solid product (**1**). **^{1}H-NMR** (600 MHz, D_2O) δ [ppm] = **7.22** (s, 2H, H-2, H-3), **7.05** (s, 1H, H-6), **5.81** (s, 1H, H-17a), **5.56** (s, 1H, H-17b), **3.54** (d, ^{3}J = 10.3 Hz, 6H, H-9, H-10), **3.04** (d, ^{2}J = 20.4 Hz, 4H, H-7, H-8), **2.01** (s, 3H, H-16). **^{13}C-NMR** (150.92 MHz, D_2O) δ [ppm] = **173.46** (C-14), **142.26** (C-15), **139.21** (C-1), **138.19** (C-4, C-5), **130.61** (C-6), **124.04** (C-19), **124.00** (C-2, C-3), **54.36** (d, $^{2}J_{C,P}$ = 6.0 Hz, C-9, C-10), **35.87** (d, $J_{C,P}$ = 129.4 Hz, C-7, C-8), **20.30** (C-16). **^{31}P-NMR** (242.92 MHz, D_2O) δ [ppm] = **26.31** (P-11, P-12).
HRMS (pos. APCI, MeOH): Calculated for $C_{14}H_{21}NO_7P_2$, M+nH: 378.0866; Found: 378.0848.

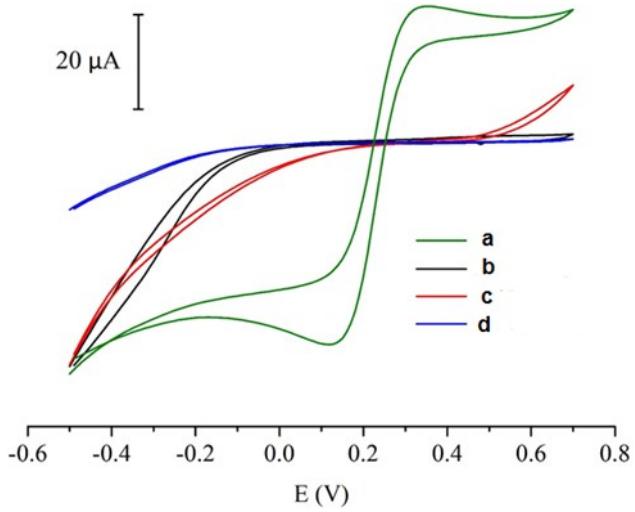


Fig. S1 Cyclic voltammetry for monitoring the insulating effect of the different modification steps on the gold electrode using ferro/ferricyanide solution. (a) Bare gold electrode; (b) gold electrode after electropolymerization of tyramine; (c) gold electrode after surface acryloyl chloride; (d) trypsin Imprinted electrode after treatment with 1-dodecanethiol. The scan range was from -0.5 V to +0.7 V (vs. Ag/AgCl) at scan rate of 0.1 Vs^{-1}.

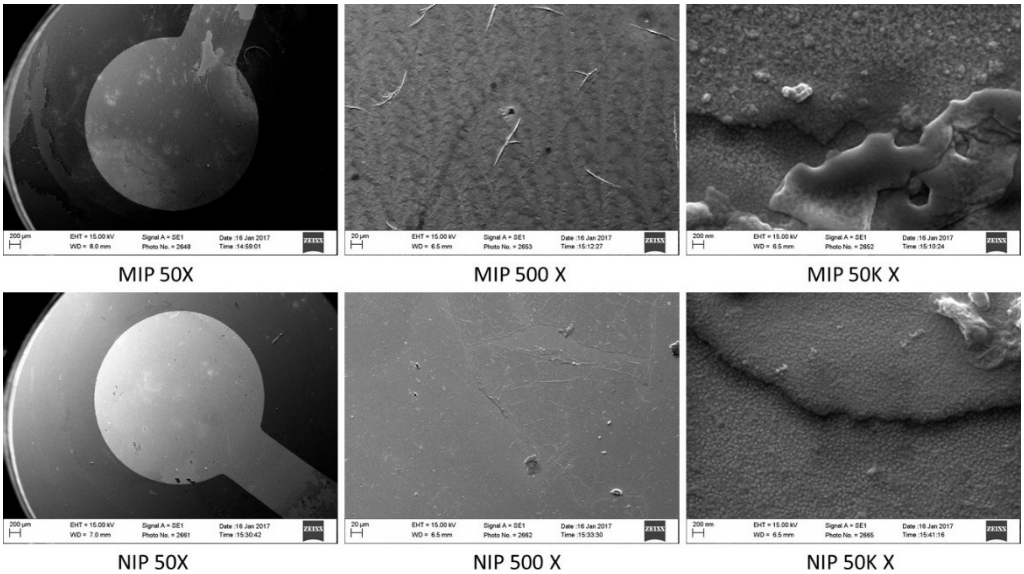


Fig. S2 Scanning electronic microscopic (SEM) images of MIP and NIP capacitive sensor surfaces at different magnifications.

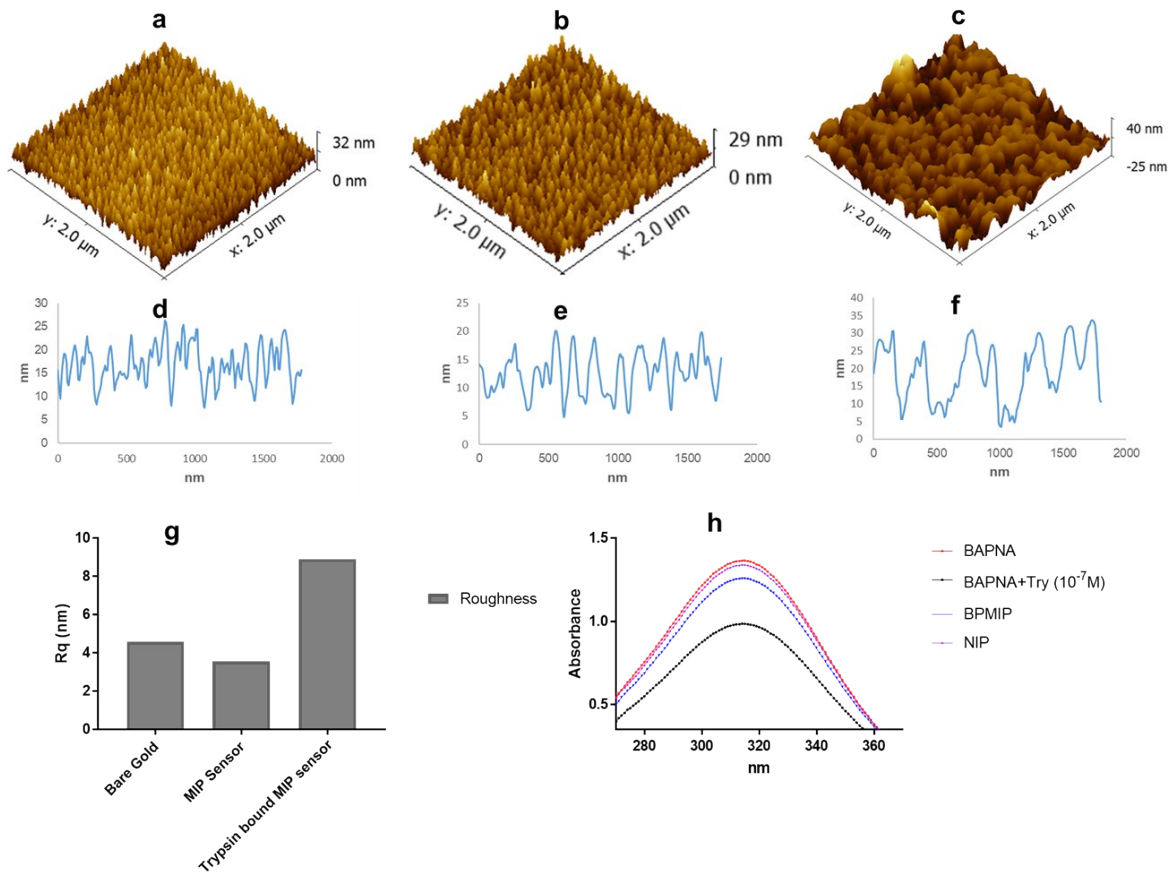


Fig. S3 Atomic Force Microscopic (AFM) images of capacitive sensor surfaces a) bare gold, b) MIP sensor, c) trypsin bound MIP sensor; d, e, f, g) roughness profile of bare gold electrode, MIP sensor, Trypsin bound MIP sensor; h) Enzyme activity of Trypsin bound MIP and NIP sensors.

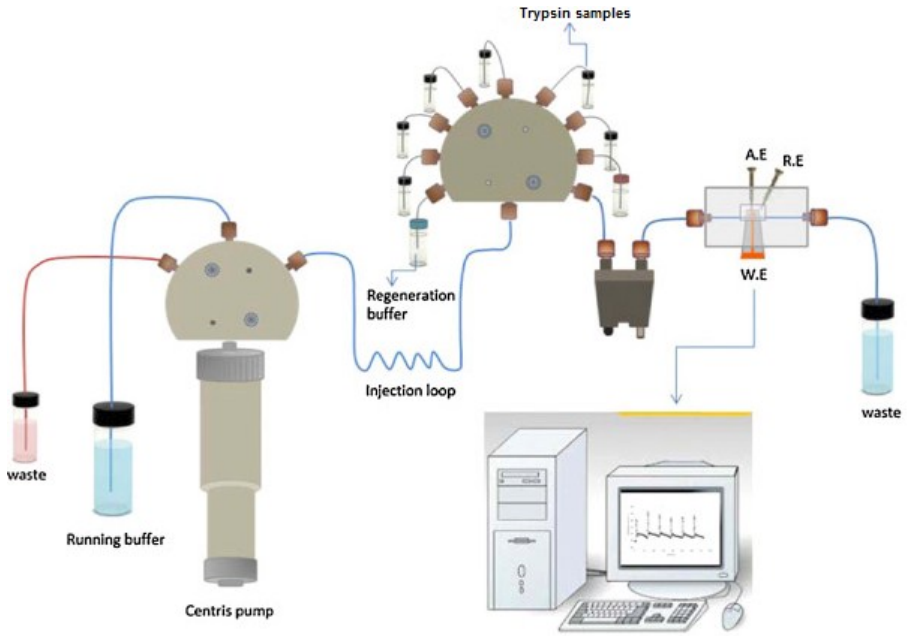


Fig. S4 Schematic diagram showing the capacitive sensor with an automated flow injection system[1].

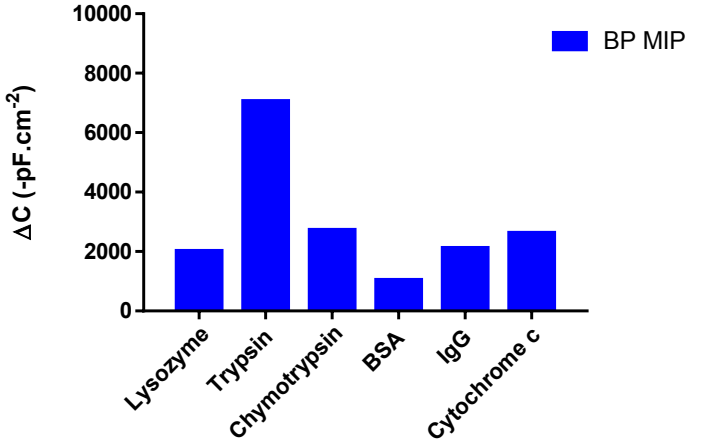


Fig. S5 Selectivity of Trypsin-imprinted capacitive biosensor based on BP monomer in 100 mM phosphate buffer pH 7.4 against competing proteins. Chymotrypsin (pI – 8.3, 25.6 KDa), Bovine Serum Albumin (pI – 5.8; 66.5 KDa), Cytochrome C (pI – 10.0±0.5; 12.3 KDa), Lysozyme (pI – 11.35; 14.3 KDa), IgG (pI – 6.85 ± 1; 155 – 160 KDa) and trypsin (pI – 10.1±0.5; 23.3 KDa) in singular manner Conditions: protein concentration 1.0 mg/mL (3.9×10^{-5} M for chy, 1.5×10^{-5} M for BSA, 7.0×10^{-5} M for lyz and 8.1×10^{-5} for cyt c, 4.3×10^{-5} M for try).

Table S1. Selectivity coefficients of trypsin-MIP and NIP electrode

Functional Monomer (FM)	Proteins	ΔC (pF) MIP	ΔC (pF) NIP	Selectivity coefficient k (MIP)	Selectivity coefficient k (NIP)	Relative selectivity coefficient (k')
BP	Lysozyme	3947	1544	4.0	2.48	1.63
	Trypsin	15920	3822	-	-	-
	Chymotrypsin	1539	3056	10.4	1.25	8.28
	BSA	2640	1522	6.0	2.51	2.41
	IgG	3851	2516	4.1	1.52	2.72
	Cytochrome C	3034	3631	5.3	1.05	4.99
Without FM*	Lysozyme	1128	2011	6.5	1.02	6.39
	Trypsin	7331	2044	-	-	-
	Chymotrypsin	10	1966	733.1	1.04	705.1
	BSA	694	1266	10.6	1.61	6.54
	Cytochrome C	2118	3014	3.5	0.68	5.10

Δ: capacitance change for the trypsin-MIP and NIP electrodes (BP as FM in 50 mM Phosphate Buffer pH: 7.4. K: selectivity coefficient for trypsin verses competing proteins, k': relative selectivity coefficient for trypsin-MIP electrode versus NIP electrode]. *: data was obtained in 10 mM phosphate buffer pH- 7.4 from our previous publication[2]. The MIP electrode without functional monomer failed to perform in > 10 mM phosphate buffer (pH-7.4).

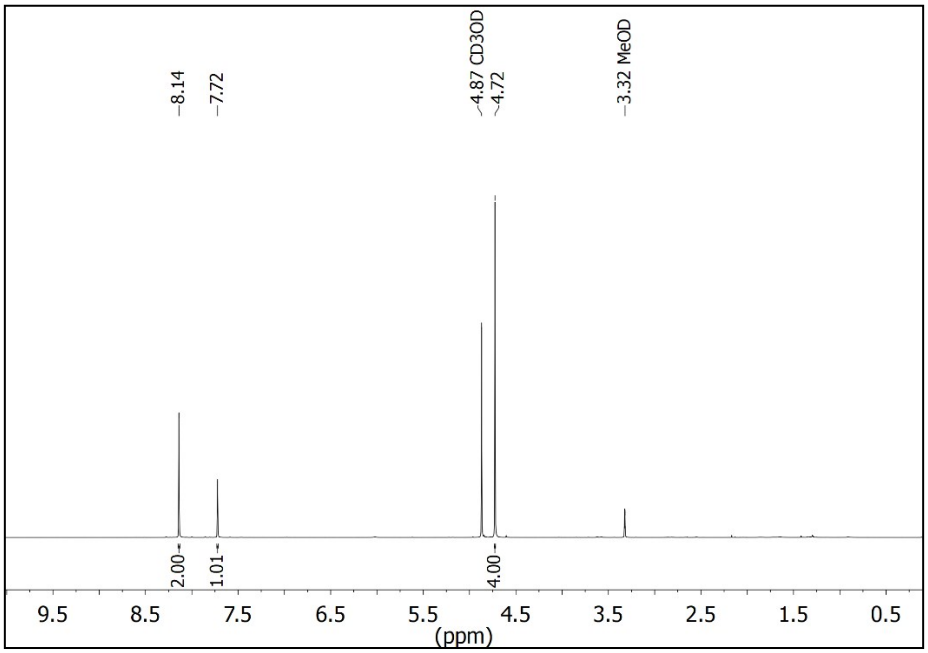
Table S2 Contact angle changes of stamp and gold sensor during modification step (measured in triplicates)

Glass	WCA, °	Gold electrode	WCA, °
Bare glass	56.68±2.35	Tyramine polymer	66.27±1.85
Acid/base treatment	25.17±2.17	Acryloyl modification	53.77±2.54
APTES Modified	81.26±0.82	After polymerization	79.27±2.65
GA-Modified	74.98±1.11		
Trypsin Immobilized	64.05±1.44		
After peeling off	76.33±1.88		

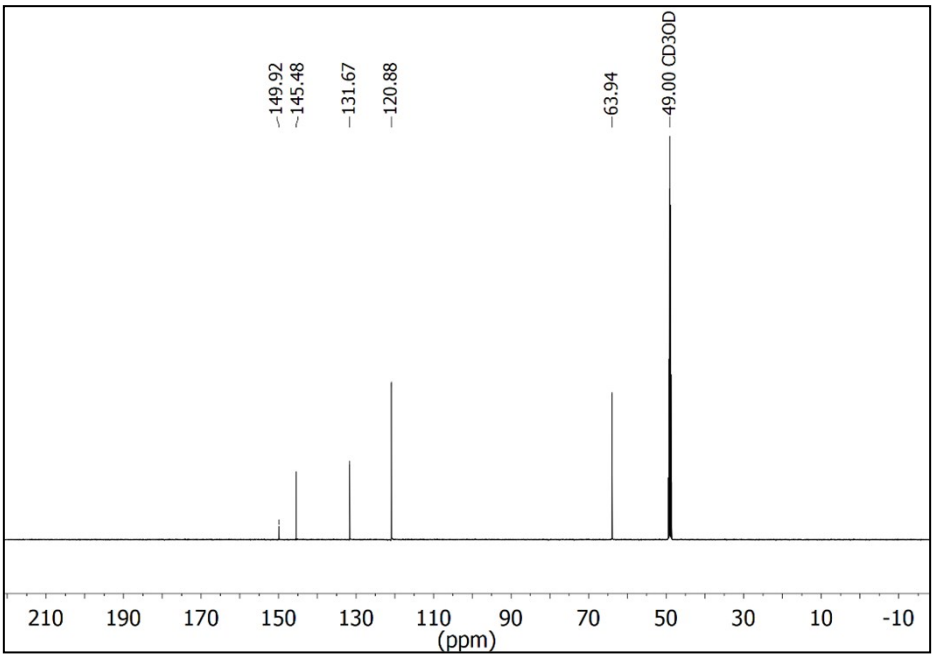
Table S3 Comparison of analytical performances of different techniques used for trypsin detection

Sensing principle	**Sensor preparation method**	**Limit ofdetection (LOD)**	**Ref**
Colorimetric sensor	Employing cytochrome c as an enzyme substrate, 3,3',5,5'-tetramethylbenzidine as a chromogenic reagent	1.9 x 10^{-10} M	Zhang and Du 2016[3]
Liquid crystal (LC) sensor system	BSA immobilized gold grids as the enzymatic substrate	4.3 x 10^{-10} M	Schyrr et al. 2014[4]
Interferometric sensor	Combination of chemically functionalized nanoporous anodic alumina photonic films and reflectometric interference spectroscopy	1.07 x 10^{-6} M	Arredondo et al. 2012[5]
Localized SPR	Integration of light weight and simple laboratory optical components with the camera module of the smartphone	1.10 x 10^{-6} M	Dutta et al. 2016[6]
Fluorescence detection system	Cell phone based portable bioassay platform	3.9 x 10^{-11} M	Reddy et al. 2012[7]
Flow injection analysis-QCM biosensors	Immobilizing trypsin inhibitor as a specific ligand	0.16 x 10^{-6} M	Huang et al. 2015[8]
Turn-on chemiluminescent biosensing platform	Using streptavidin-modified magnetic beads	1.0 x 10^{-11} M	Zhang et al. 2014[9]
Fluorescence based sensing	Functional high surface area scaffolds based on cellulose nanocrystals and polyvinyl alcohol	10.7 x 10^{-6} M	Osman et al. 2013[10]
Electrochemical immunosensor	Multiwall carbon nanotubes (MWCNTs)-composite modified electrode	0.86 x 10^{-13} M	Yi et al. 2014[11]
Fluorescent sensors	Quantum-dot based	0.42 x 10^{-9} M	Zhang et al. 2014[12]
QCM	Surface imprinting (molecular imprinting)	43 x 10^{-10} M	Hayden et al. 2006[13]
Square wave voltammetric sensor	1,2-benzoquinone electrochemical reduction on gelatin coated screen printed electrodes	4.3 x 10^{-10} M	Stoytcheva et al. 2012[14]
Self-powered sensor	Signals the presence of trypsin via a light-emitting diode (LED) that is visible to the unaided eye	2.1 x 10^{-8} M	Zaccheaoand Crooks 2011[15]
Amperometric sensor	Two layer configuration; a polymer-glucose oxidase inner layer and a gelatin outer layer	42 x 10^{-12} M	Ionescu et al. 2006[16]

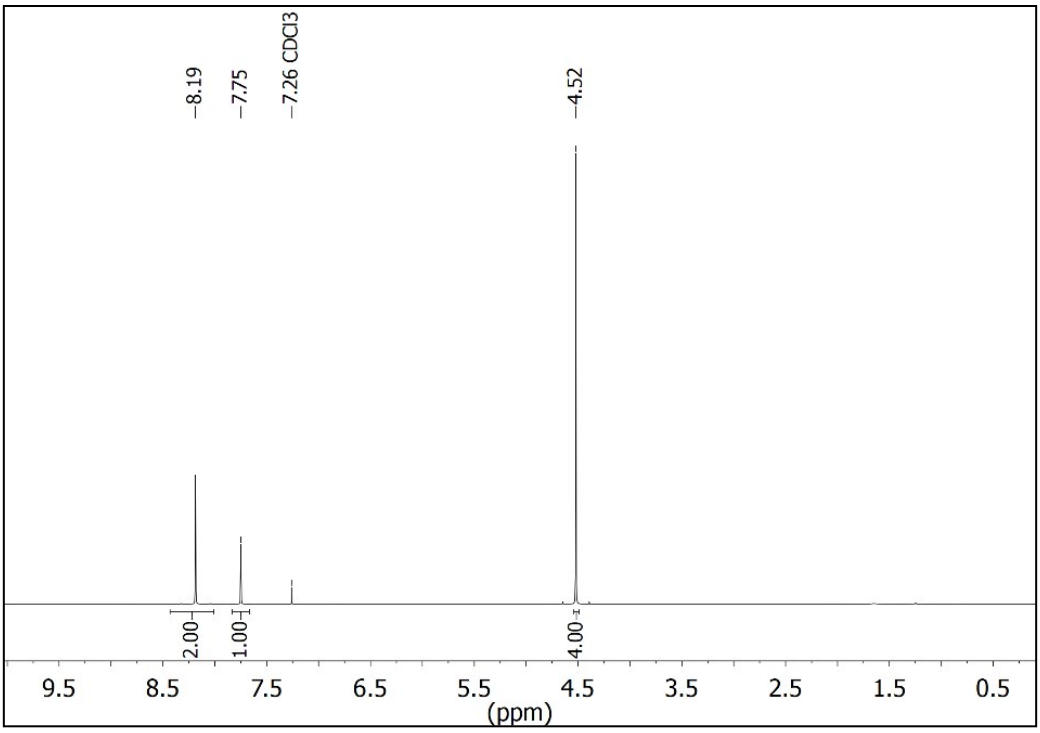
Electrochemical method	Gelatin coated glassy carbon electrode	4.3 x 10^{-10} M	Tan and Wei 2015[17]
Capacitive biosensor	Microcontact imprinting of trypsin to the electrode	3.0 x 10^{-13} M	Ertürk and Mattiasson et al. 2016[2]
Capacitive biosensor	Microcontact imprinting of trypsin using bisphosphonate based functional monomer to the electrode	1 x 10^{-13} M	In this study



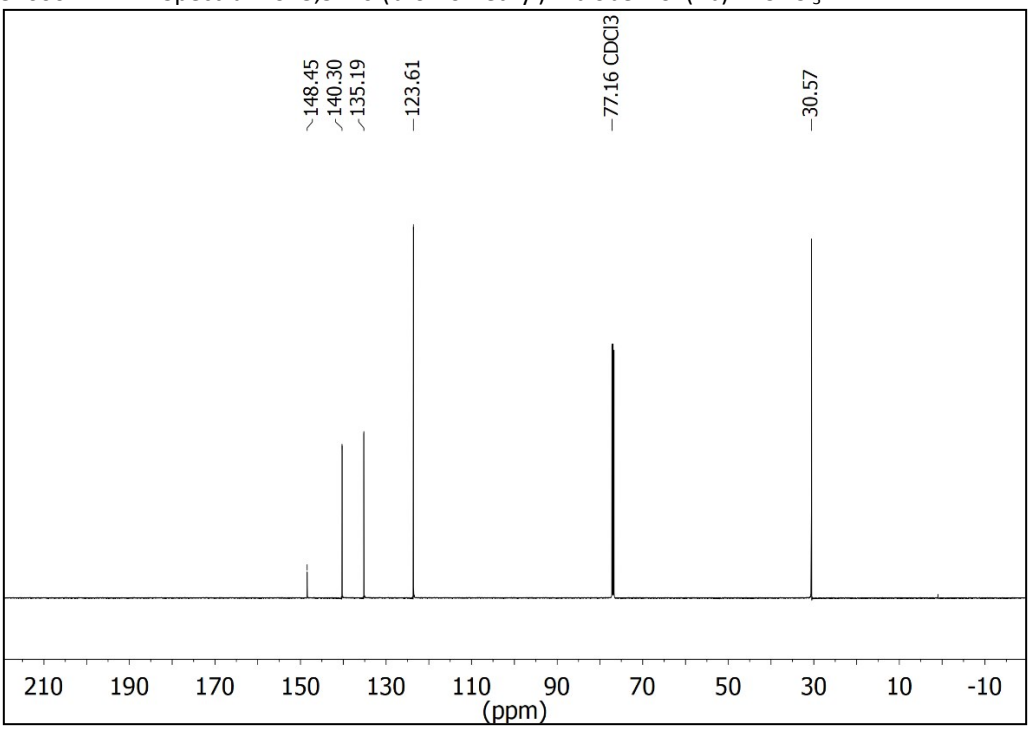
Spectra 1. 600 MHz ^{1}H-Spectrum of 3,5-Bis-(hydroxymethyl)-nitrobenzol (**1a**) in MeOD.



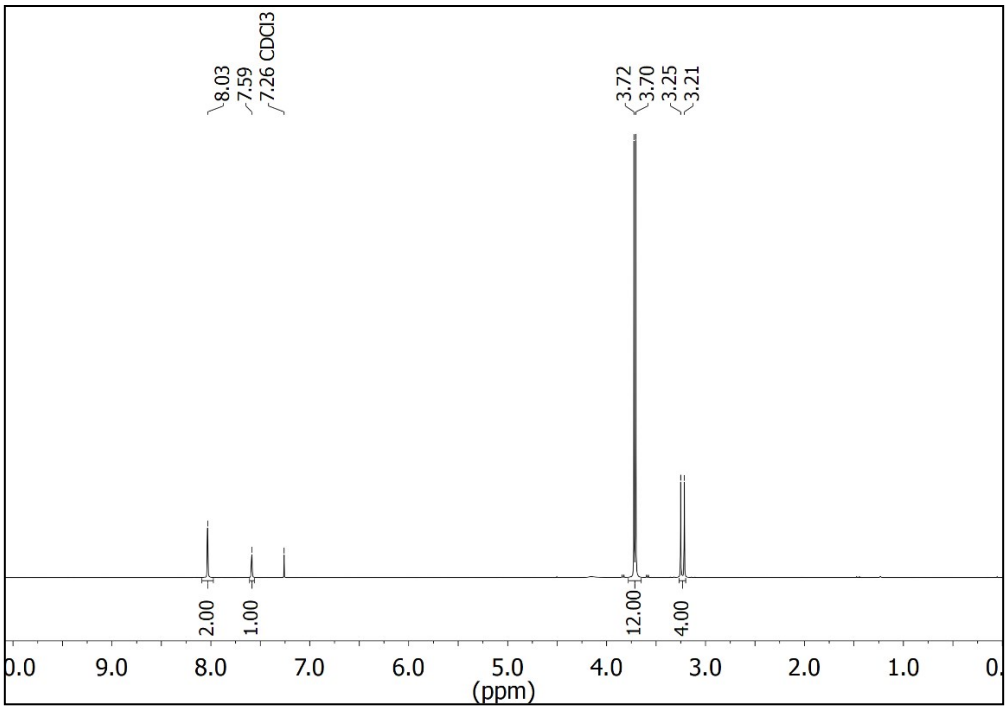
Spectra 2. 150.92 MHz ^{13}C-Spectrum of 3,5-Bis-(hydroxymethyl)-nitrobenzol (**1a**) in MeOD.



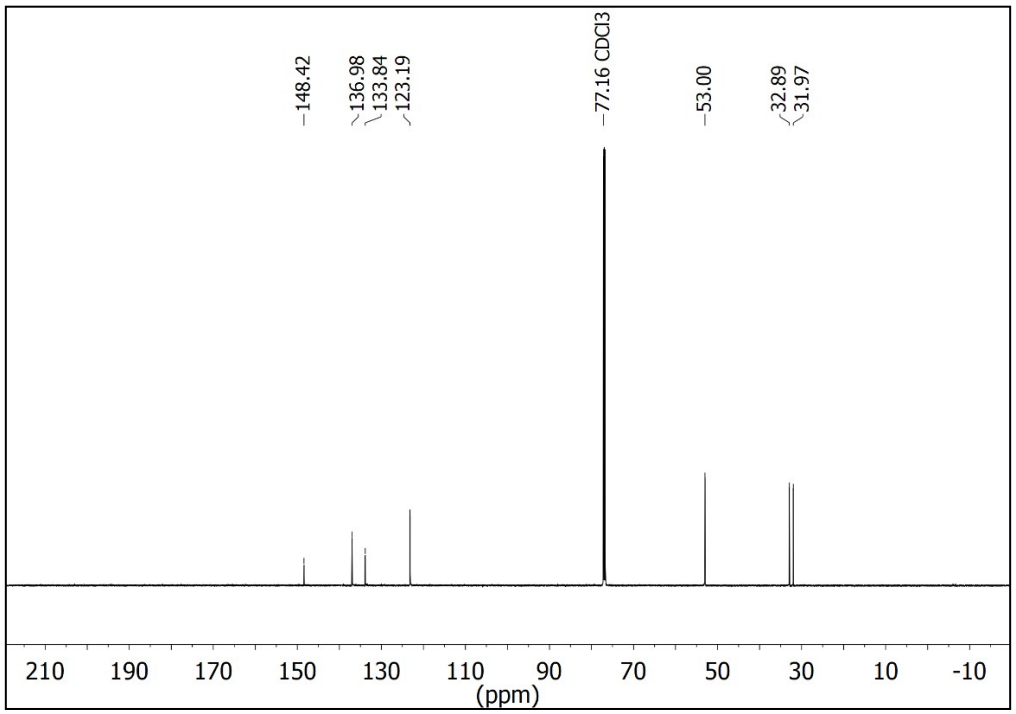
Spectra 3. 600 MHz ^{1}H-Spectrum of 3,5-Bis-(bromomethyl)-nitrobenzol (**1b**) in $CDCl_3$.



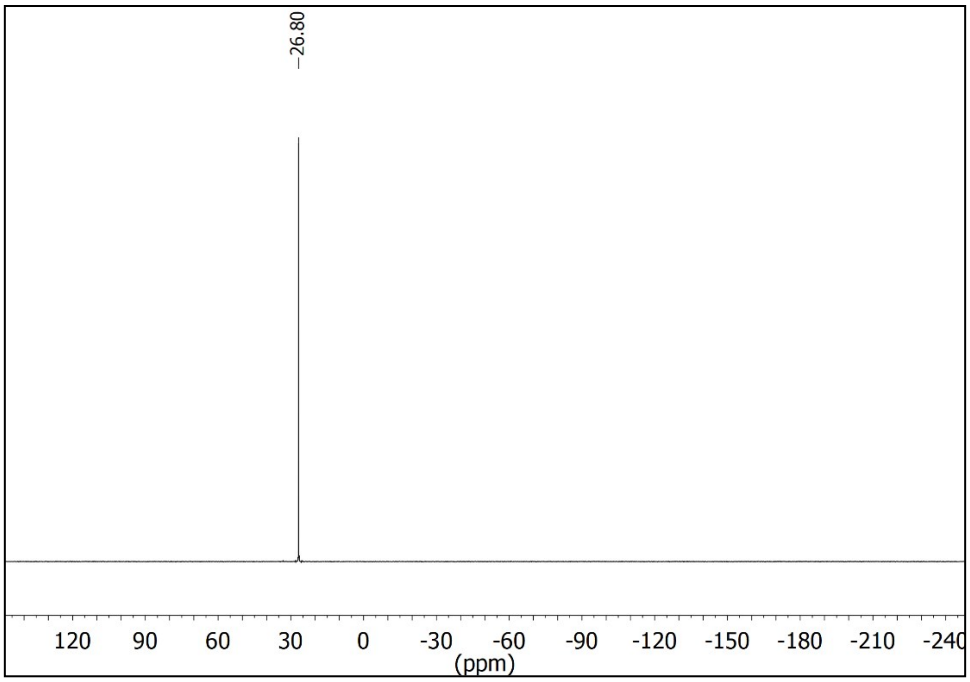
Spectra 4. 150.92 MHz ^{13}C-Spectrum of 3,5-Bis-(bromomethyl)-nitrobenzol (**1b**) in $CDCl_3$.



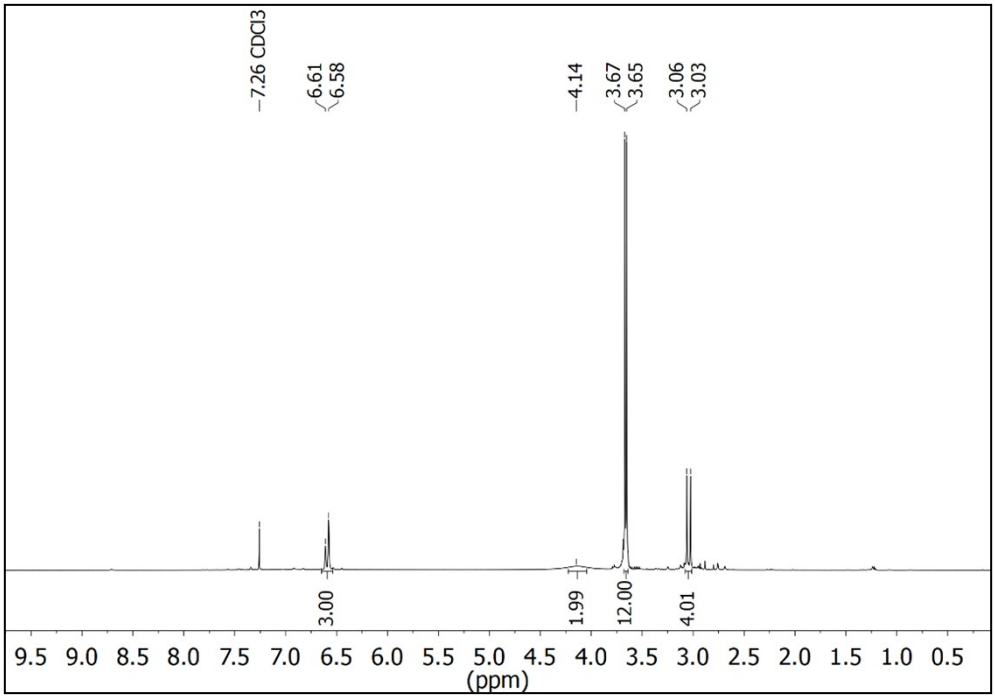
Spectra 5. 600 MHz ^{1}H-Spectrum of 3,5-Bis-(dimethoxy-phosphorylmethyl)-nitrobenzol (**1c**) in $CDCl_3$.



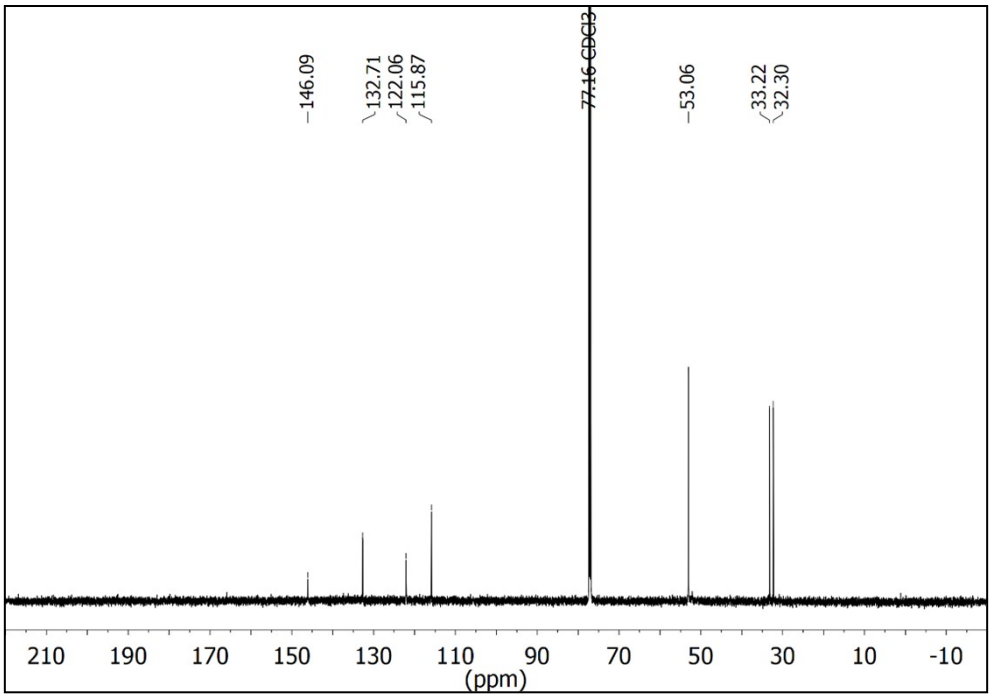
Spectra 6. 150.92 MHz ^{13}C-Spectrum of 3,5-Bis-(dimethoxy-phosphorylmethyl)-nitrobenzol (**1c**) in $CDCl_3$.



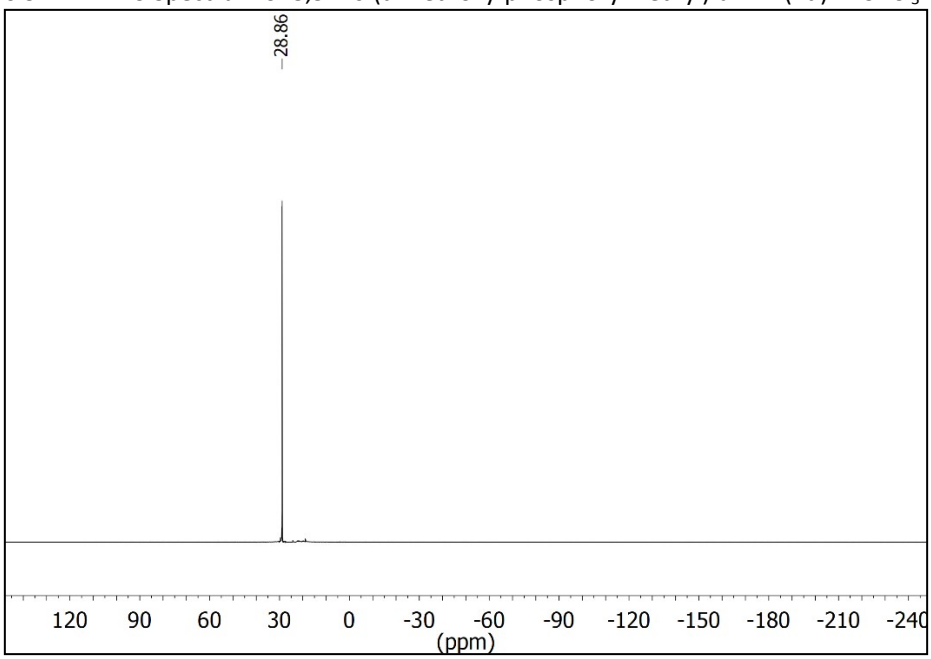
Spectra 7. 242.92 MHz ^{31}P-Spectrum of 3,5-Bis-(dimethoxy-phosphorylmethyl)-nitrobenzol (**1c**) in $CDCl_3$.



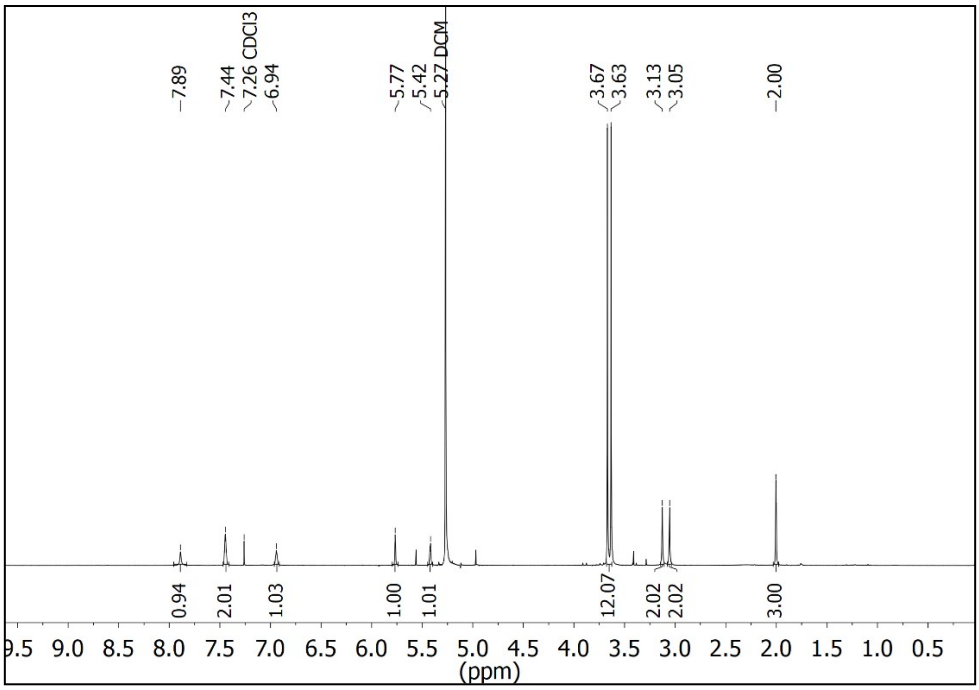
Spectra 8. 600 MHz ^{1}H-Spectrum of 3,5-Bis-(dimethoxy-phosphorylmethyl)-anilin (**1d**) in $CDCl_3$.



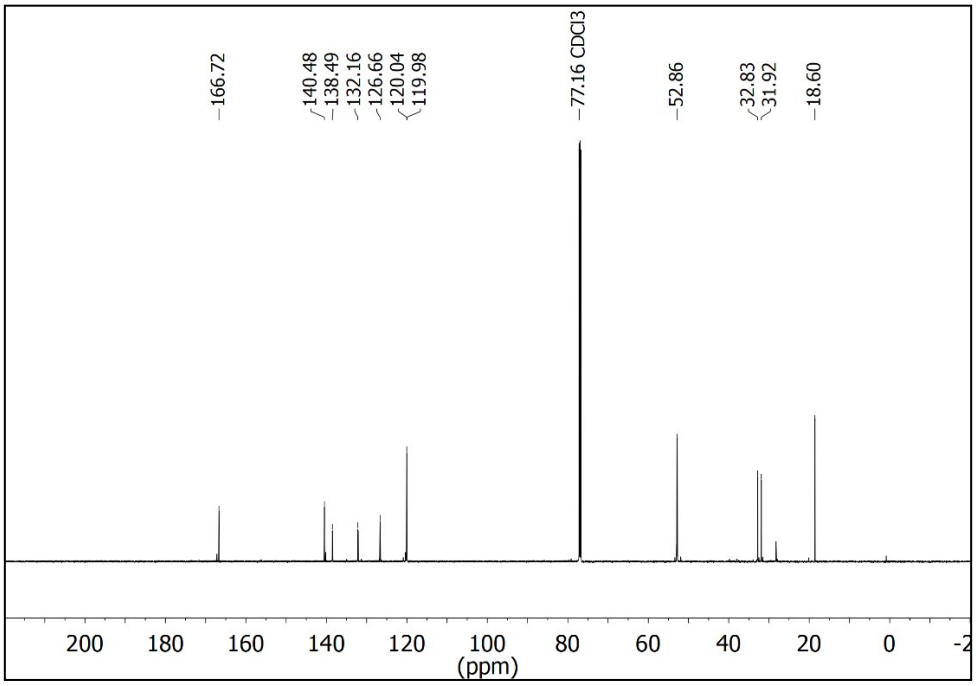
Spectra 9. 150.92 MHz ^{13}C-Spectrum of 3,5-Bis-(dimethoxy-phosphorylmethyl)-anilin (**1d**) in $CDCl_3$.



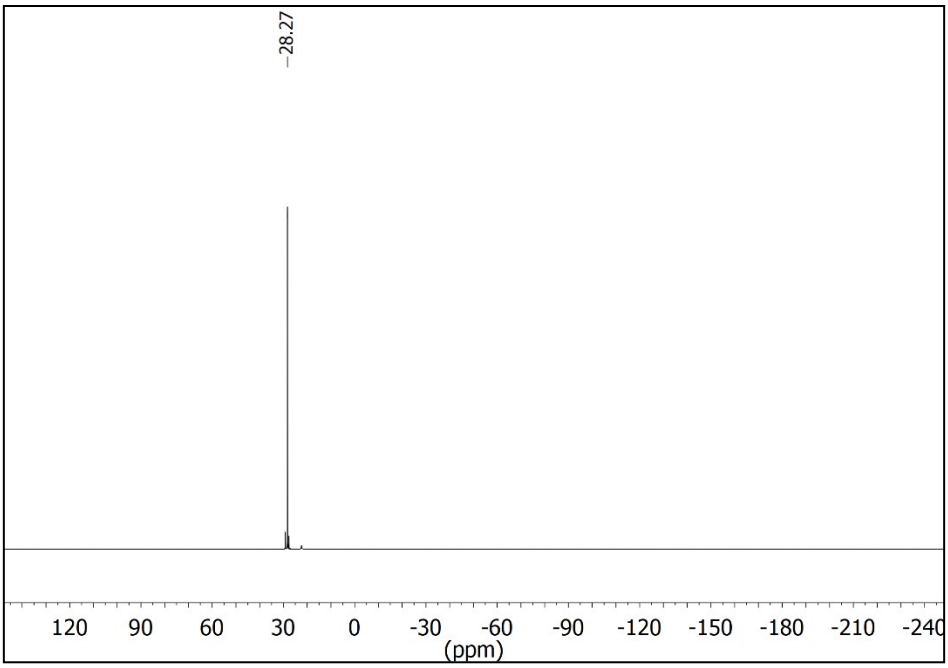
Spectra 10. 242.92 MHz ^{31}P-Spectrum of 3,5-Bis-(dimethoxy-phosphorylmethyl)-anilin (**1d**) in $CDCl_3$.



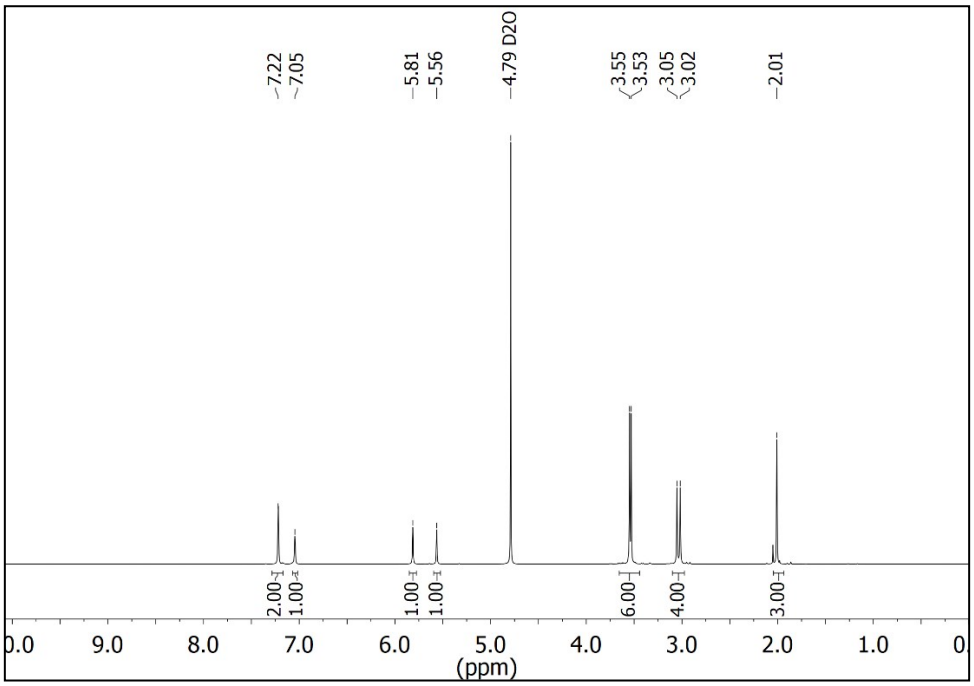
Spectra 11. 600 MHz ^{1}H-Spectrum of *N*-Methacryl-[3,5-Bis(dimethoxy-phosphorylmethyl)]-phenyl (**1e**) in $CDCl_3$.



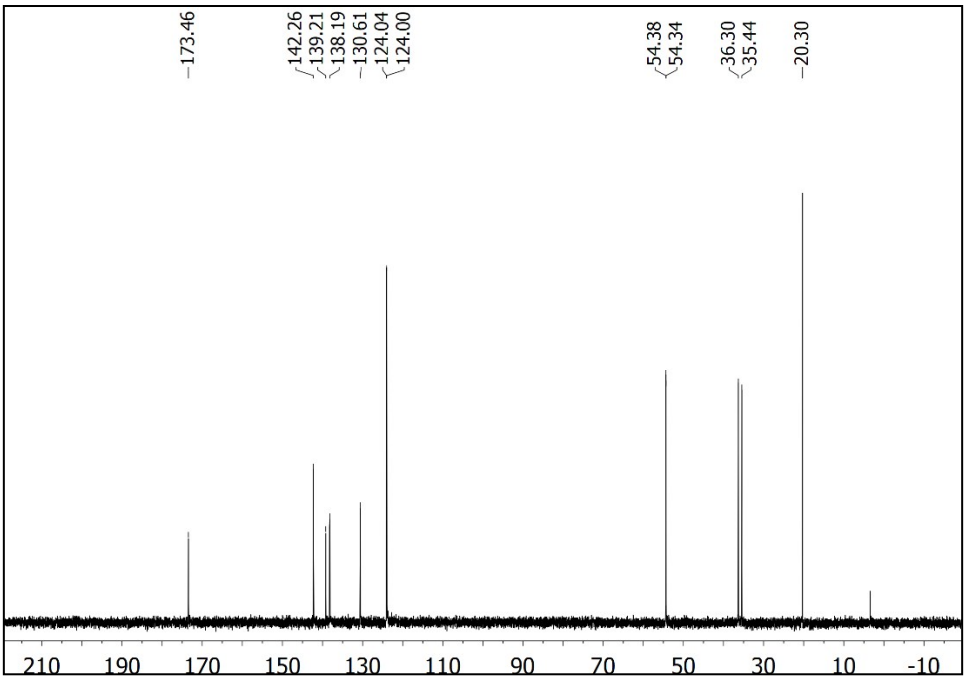
Spectra 12. 150.92 MHz ^{13}C-Spectrum of *N*-Methacryl-[3,5-Bis(dimethoxy-phosphorylmethyl)]-phenyl (**1e**) in $CDCl_3$.



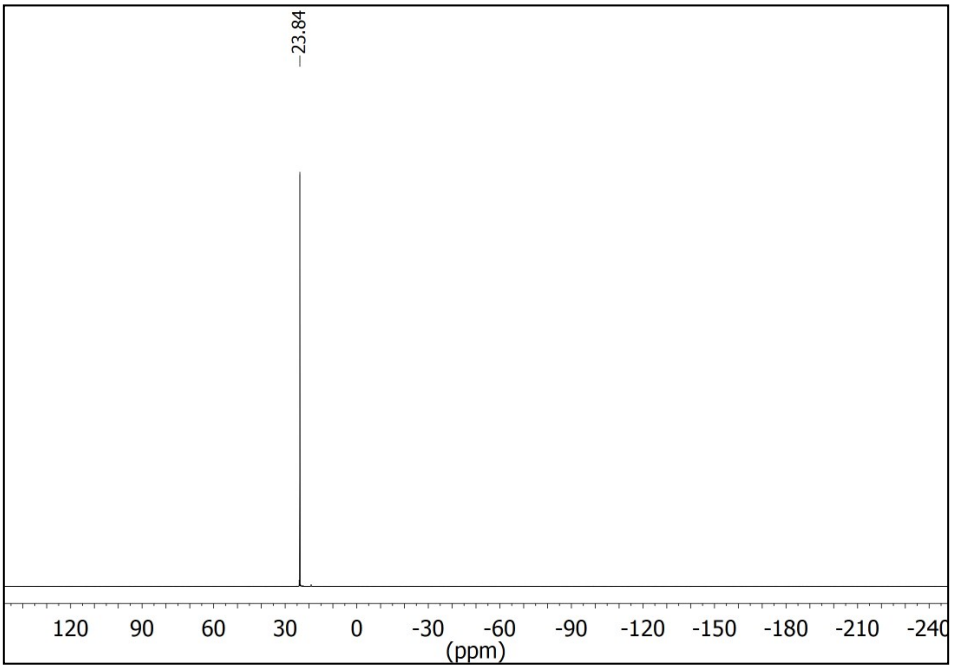
Spectra 13. 242.92 MHz ^{31}P-Spectrum of *N*-Methacryl-[3,5-Bis(dimethoxy-phosphorylmethyl)]-phenyl (**1e**) in $CDCl_3$.



Spectra 14. 600 MHz ^{1}H-Spectrum of Dilithium-*N*-Methacryl-[3,5-Bis(methoxy-phosphorylmethyl)]-phenyl (**1**) in D_2O.



Spectra 15. 150.92 MHz ^{13}C-Spectrum of Dilithium-*N*-Methacryl-[3,5-Bis(methoxy-phosphorylmethyl)]-phenyl (**BP, 1**) in D_2O.



Spectra 16. 242.92 MHz ^{31}P-Spectrum of Dilithium-*N*-Methacryl-[3,5-Bis(methoxy-phosphorylmethyl)]-phenyl (**BP, 1**) in D_2O.

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