Electronic Supplementary Material (ESI) for Materials Advances.

Supporting Information

for

Toll Like Receptor-based Electrochemical Sensors via N-Heterocyclic Carbene-modified Surfaces: Towards Improved Sensing of DNA Molecules

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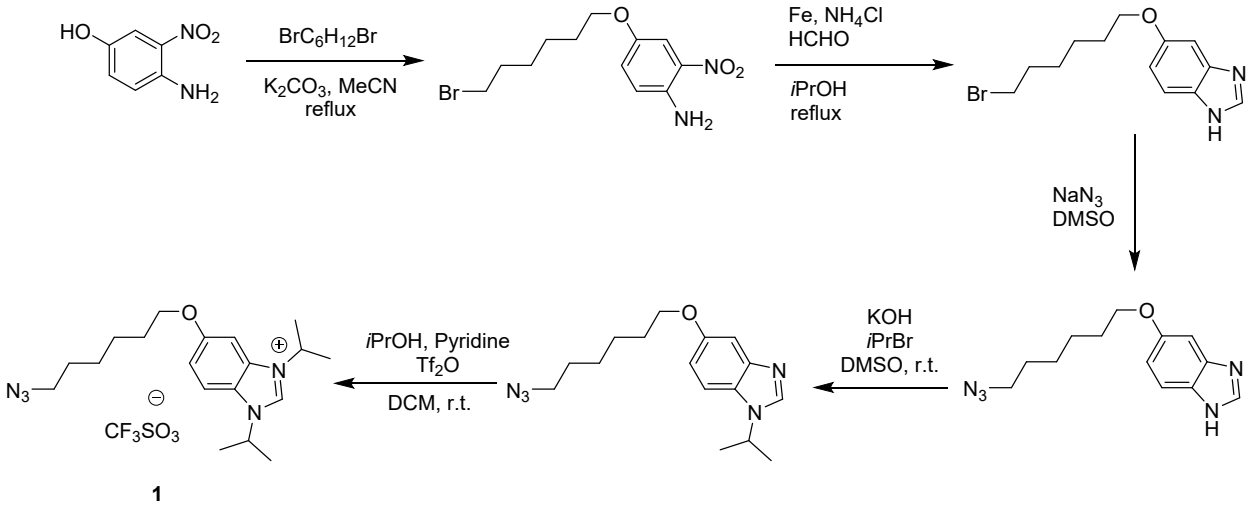
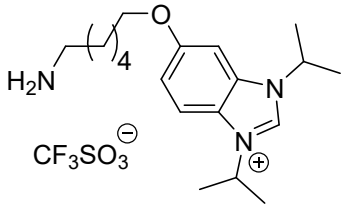
Detailed Synthesis



Scheme S1. Synthesis of Key Compound **1**.

The key compound **1** was synthesized following published procedure (Scheme 1).[1]

Synthesis of NHC 2 via Staudinger Reduction.



PPh_3 (0.276 g, 1.05 mmol) and compound **1** (0.260 g, 0.526 mmol) were dissolved in a mixture of THF/H_2O (10/1, 11 mL). The resulting clear orange solution was refluxed in argon atmosphere for 20 h. After that, the reaction mixture was cooled down to r.t. and volatiles were removed under reduced pressure. A cloudy orange oil was obtained which was then dissolved in minimum volume of CH_2Cl_2 and triturated in hexanes (3×10 mL) and Et_2O (3×10 mL), consecutively. The resulting clear red oil was dissolved again in a minimum volume of CH_2Cl_2, passed through a short celite plug, and concentrated to obtain **2** as a dark red sticky oil (0.187 g, 72%).

^{1}H NMR (500 MHz, CD_3CN) δ 8.83 (s, 1H, $CH_{(aromatic)}$), 7.77 (d, *J* = 9.2 Hz, 1H, $CH_{(aromatic)}$), 7.29 (d, *J* = 2.3 Hz, 1H, $CH_{(aromatic)}$), 7.25 (dd, *J* = 9.2, 2.3 Hz, 1H, $CH_{(aromatic)}$), 5.45 (s, 2H, NH_2), 4.89 (hept, *J* = 6.7 Hz, 2H, $(CH_3)_2$-CH-N), 4.11 (t, *J* = 6.6 Hz, 2H, O-CH_2), 2.61 (t, *J* = 6.5 Hz, 2H, H_2N-CH_2), 1.87 – 1.78 (m, 4H), 1.65 (d, *J* = 6.7 Hz, 12H, N-CH-$(CH_3)_2$), 1.53 – 1.45 (m, 2H), 1.42 (qd, *J* = 5.0, 2.2 Hz, 2H) ppm. ^{13}C NMR (126 MHz, CD_3CN) δ 159.53, 137.23, 133.37, 126.15, 123.28, 120.73, 115.40, 97.37, 69.92, 52.26, 51.82, 41.82, 31.81, 29.48, 26.95, 26.22, 21.98, 21.93 ppm. ^{19}F NMR (471 MHz, CD_3CN) δ –79.33 ppm. m/z calcd for $C_{19}H_{30}ON_3^+$ $[M]^+$ 318.25399, found 318.25229.

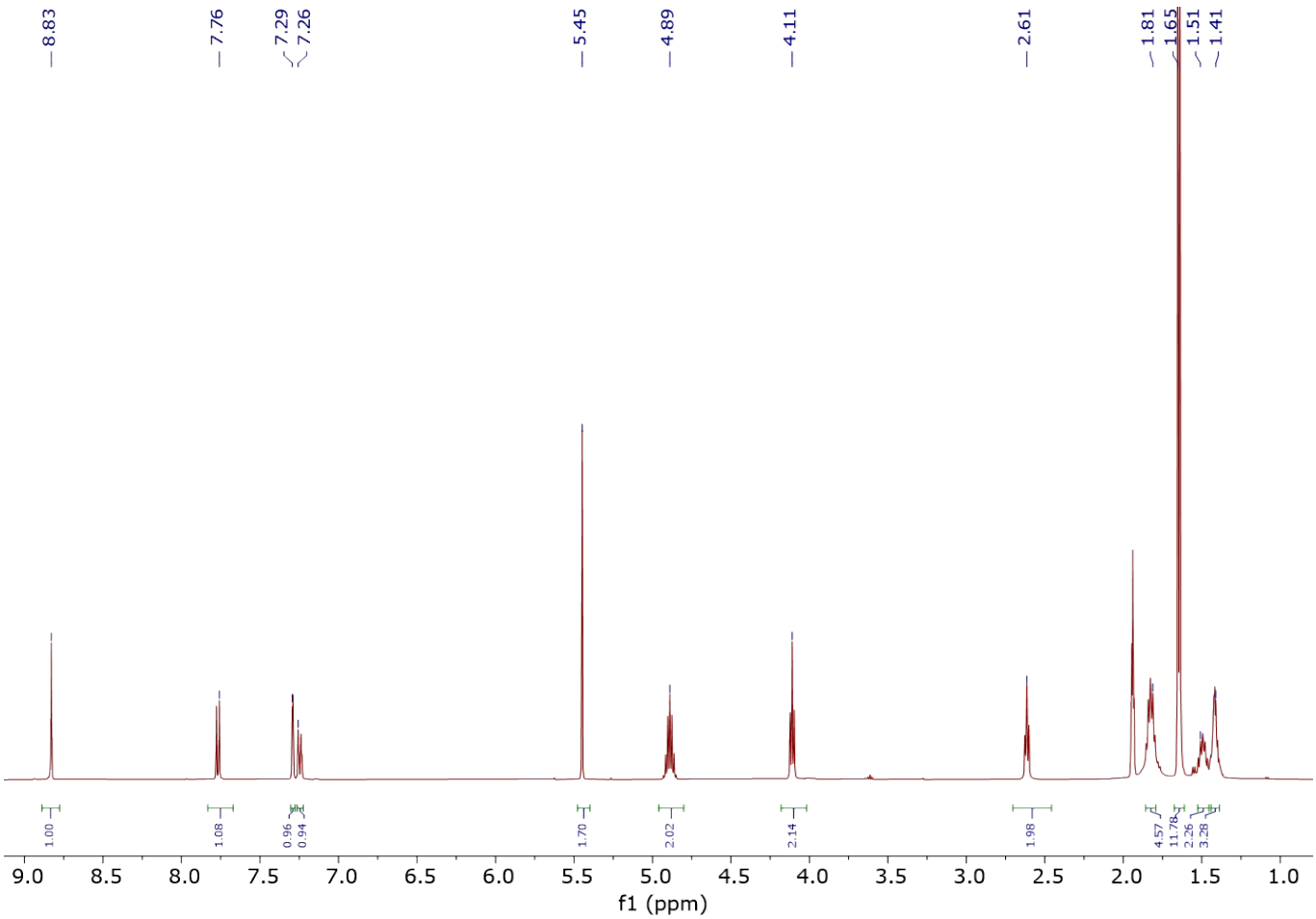


Figure S1. ^{1}H NMR of compound **2** in CD_3CN.

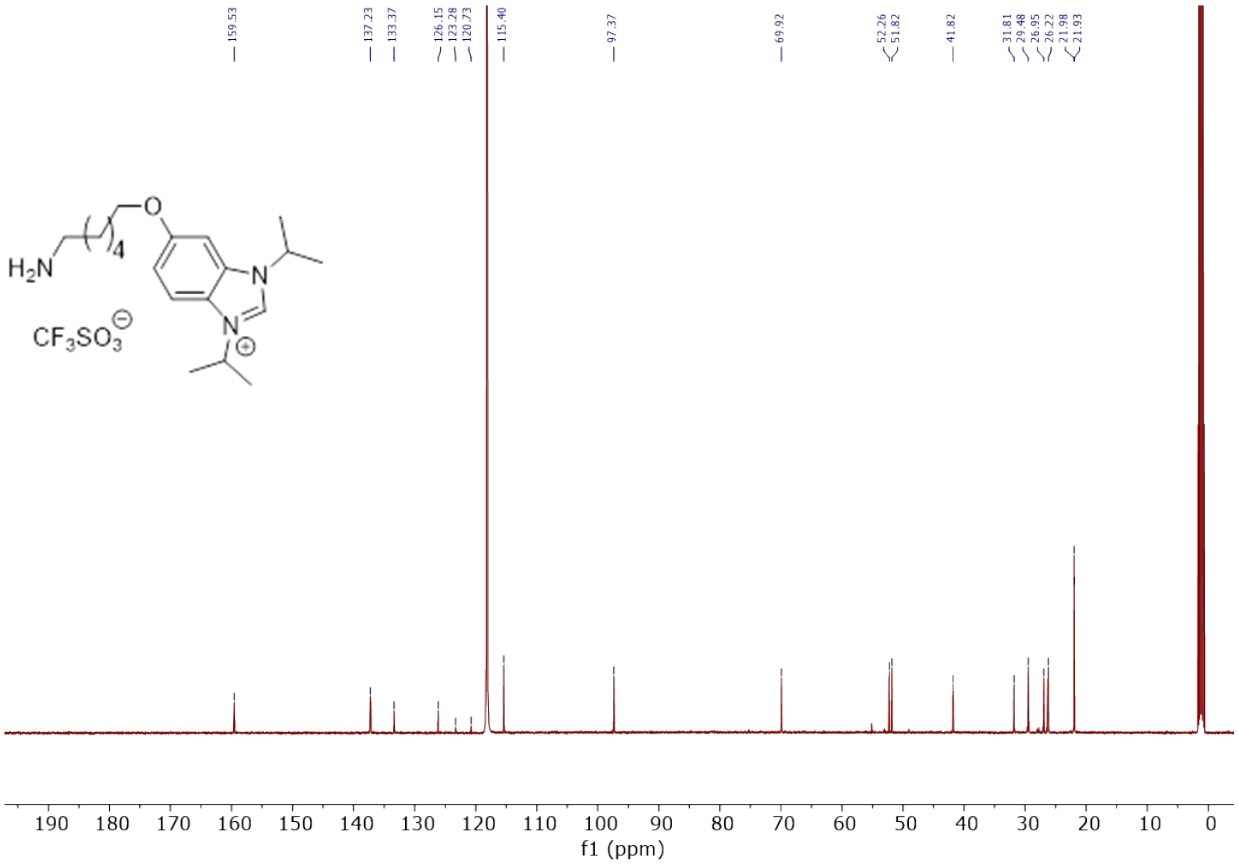


Figure S2. $^{13}C\{^{1}H\}$ NMR of compound **2** in CD_3CN.

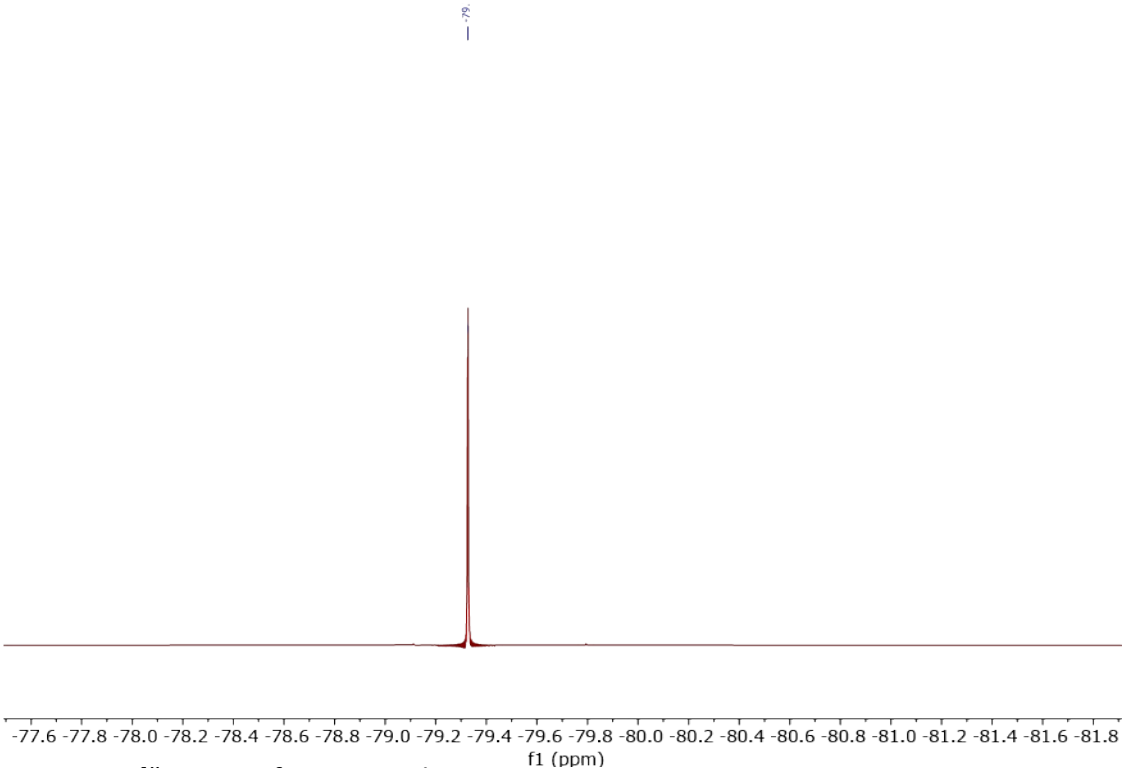


Figure S3. ^{19}F NMR of compound **2** in CD_3CN.

Simulation Model.

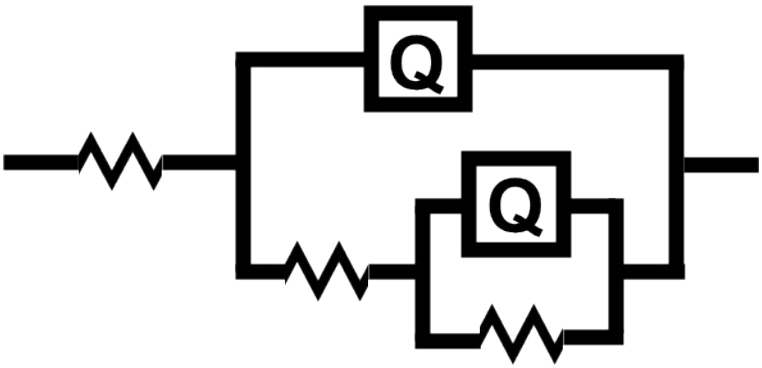


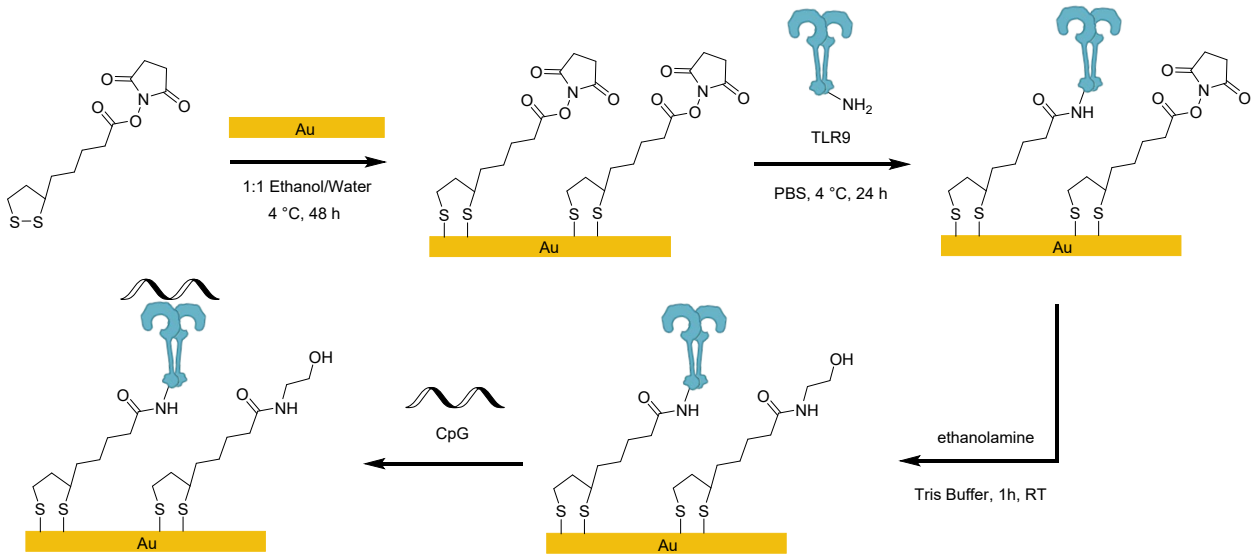
Figure S4. Simulator Model r(q(r(qr)) used for the fitting of impedance data to obtain Q and R values

Table S1: *Figure 6 p-values between the sample and storage/contaminants.*

	No TLR	Pam3CSK4	Cl307	4 week storage
p-values at 5 µM	0.01	0.004	0.02	0.05
p-values at 10 µM	0.007	0.008	0.02	0.08
p-values at 20 µM	0.005	0.004	0.01	0.05

lipoic acid n-hydroxysuccinimide ester based TLR9 sensor performance

lipoic acid n-hydroxysuccinimide ester monolayers were prepared by dissolving the said compound in 1:1 ethanol/water solution, and a clean gold substrate was immersed in this solution for 48 hours at 4 °C. The modified surface was washed with 1:1 ethanol/water solution. Then, the surface was immersed in 250 µg/mL of TLR9 in PBS solution overnight at 4 °C, then rinsed with PBS solution. The modified substrates were then immersed in 1 M ethanolamine in 50 mM Tris(pH 8.4) for 1 hour at room temperature, then rinsed with PBS solution before the exposure to CpG.[2, 3]



Scheme S2: Gold surface functionalization with 1-lipoic acid n-hydroxysuccinimide ester, followed by the immobilization of TLR9 with a primary amine side chain, the blocking stage with blocking achieved by exposure to ethanolamine, and the detection stage to various concentrations of CpG.

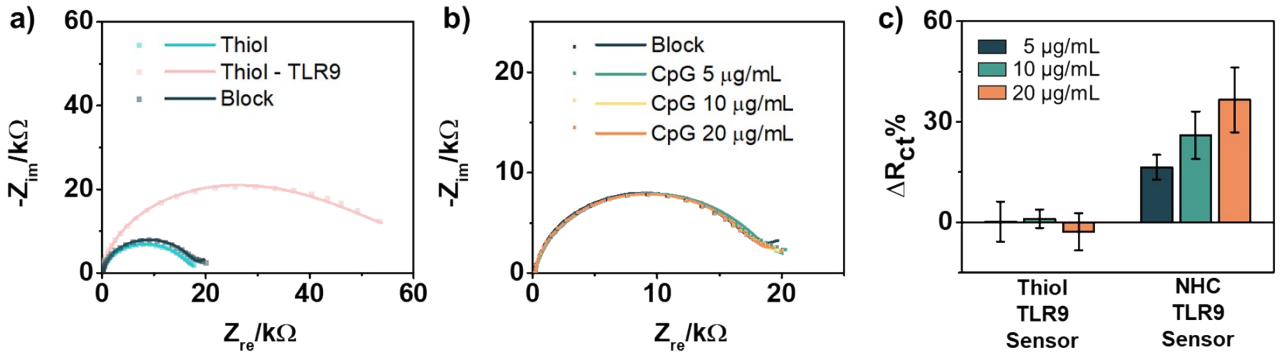


Figure S5: a) EIS measurements of **Thiol-TLR9** sensor construction stages. b) EIS measurements of CpG detection. c) Average ΔR_{ct}% values for three sensors (thiols and NHC based) to different concentrations of CpG. The error bars represent the standard deviation based on the three electrode replicates for the average.

Reference

1. Singh, I.; Lee, D. S.; Huang, S.; Bhattacharjee, H.; Xu, W.; McLeod, J. F.; Crudden, C. M.; She, Z., N-Heterocyclic Carbenes Meet Toll-like Receptors. *Chemical Communications* **2021,** *57*, 8421.

2. McLeod, J.; Park, C.; Cunningham, A.; O'Donnell, L.; Brown, R. S.; Kelly, F.; She, Z., Developing a toll-like receptor biosensor for Gram-positive bacterial detection and its storage strategies. *Analyst* **2020,** *145* (18), 6024-6031.

3. She, Z.; Topping, K.; Ma, T.; Zhao, T.; Zhou, W.; Kamal, A.; Ahmadi, S.; Kraatz, H.-B., Detection of the Lipopeptide Pam3CSK4 Using a Hybridized Toll-like Receptor Electrochemical Sensor. *Analytical Chemistry* **2017,** *89* (9), 4882-4888.