Host-Guest Systems on the Surface of Functionalized Superparamagnetic Iron Oxide Nanoparticles (SPIONs) Utilizing Hamilton Receptors and Cyanurate Derivative Molecules

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Synthesis

Cyanurate Phosphonate (CyaPEst)

Procedure

The solution of bromophosphonate 1 (3 g, 10 mmol) in 50 mL DMF was mixed with cyanuric acid 2 (2.7 g, 21 mmol). DBU (1.5 mL, 10 mmol) was added to the mixture. Then the mixture was heated up at 70 °C under inert atmosphere for overnight. The mixture was cooled to room temperature. To the mixture was poured 300 mL of DCM to precipitate the excess of cyanuric acid and let the sediment settled. The precipitate was filtered. The solution was evaporated using rotary evaporator. Into the residue was added EtOAc 100 mL. The organic mixture was washed with water (50 mL) 5 times and one time with brine (50 mL). The organic phase was dried over MgSO₄. The solvent then was evaporated. The crude product was purified with silica column chromatography using the first eluent DCM/MeOH (9:1) to get rid out yellowish side product and followed by second eluent EtOAc/Cyclohexane (4:1) + 5% MeOH to yield the product as a white solid (2 g, 0.6 mmol, 60%).

Physical data

Rₓ=0.44 (EtOAc/Cyclohexane 4:1 + 5% MeOH); ¹H NMR (400 MHz, DMSO-d₆): δ=11.37 (s, 2H; N-H), 4.02-3.89 (m, 4H; P-O-CH₂), 3.61 (t, ³J_H,H=8 Hz, 2H; N-CH₂), 1.72-1.63 (m, 2H; P-CH₂), 1.52-1.24 (m, 8H; CH₂), 1.21 (t, ³J_H,H=8 Hz, 6H; CH₃); ¹³C NMR (101 MHz, DMSO-d₆): δ=149.8 (CONH), 148.6 (CONH), 60.7 (d, ³J_C,P=6 Hz; COP), 29.4 (d, ³J_C,P=15 Hz; CP), 27.1 (s, CH₂), 25.6 (s, CH₂), 25.1 (s, CH₂), 23.7 (s, CH₂), 21.9 (d, ³J_C,P=6 Hz; CCP), 16.3 (d, ³J_C,P=5 Hz; CCOP); ³¹P NMR (162 MHz, DMSO-d₆): δ=31.90 (s); FTIR (ATR): ν˜= 623, 663, 684, 700, 734, 744, 761, 798, 908, 962, 1024, 1051, 1095, 1132, 1224, 1238, 1296, 1327, 1363, 1390, 1440, 1506, 1583, 1604, 1674, 2866, 2951, 3267 cm⁻¹; MS (MALDI-dctb): m/z calcd for C₁₃H₂₄N₃O₆P⁺H⁺: 350.3312 [M+H]⁺; found: 350.4095 [M+H]⁺;

NMR spectra

Figure S1. ¹H-NMR of Cyanurate Phosphonate CyaPEst (RT, 400 MHz, DMSO-d₆)
Figure S2. $^{13}$C-NMR of Cyanurate Phosphonate CyaPEst (RT, 101 MHz, DMSO-d$_6$)

Figure S3. $^{31}$P-NMR of Cyanurate Phosphonate CyaPEst (RT, 161 MHz, DMSO-d$_6$)
Cyanurate Phosphonic Acid (CyaPAc)

Procedure

Under protection of inert atmosphere, phosphonate cyanurate CyaPEst (570 mg, 1.63 mmol) was dissolved in 25 mL dried DCM. The mixture was stirred and heated to 35 °C. To the solution was added TMS-Br (0.645 mL, 4.89 mmol). The solution was stirred for 3 hours at 35 °C. After that, the solvent was evaporated and to the residue was added anhydrous MeOH (1.9 mL, 48.9 mmol). The mixture was stirred for one hour at 40 °C, in which white solid was formed. To the solid stuff was added 5 drops of water and then the solid was dried under vacuum to yield the final product as white solid (480 mg, 1.6 mmol, 98%).

Physical data

$^1$H NMR (400 MHz, DMSO- $d_6$): δ=11.37 (s, 2H; N-H), 9.00 (broad-s, 2H; POH), 3.61 (t, $^3J_{H,H}$=8 Hz, 2H; N-CH$_2$), 1.52-1.19 (m, 10H; CH$_2$); $^{13}$C NMR (101 MHz, DMSO-d$_6$): δ=149.8 (CONH), 148.6 (CONH), 29.6 (d, $^3J_{C,P}$=16 Hz; CP), 28.1 (s, CH$_2$), 27.1 (s, CH$_2$), 26.8 (s, CH$_2$), 25.8 (s, CH$_2$), 22.6 (d, $^3J_{C,P}$=4 Hz; CCP); $^{31}$P NMR (162 MHz, DMSO-d$_6$): δ=26.46 (s); FTIR (ATR): $\tilde{\nu}$= 667, 684, 717, 740, 758, 792, 812, 848, 931, 954, 997, 1070, 1093, 1168, 1205, 1219, 1246, 1319, 1373, 1406, 1417, 1460, 1606, 1649, 1681, 1728, 1745, 1764, 2794, 2864, 2927, 3088, 3201, cm$^{-1}$; MS (ESI-negative polarity): m/z calcd for C$_9$H$_{15}$N$_3$O$_6$P-H$: 292.0698 [M-H]$^-$; found: 292.0730 [M-H]$^-$; elemental analysis calcd (%) for C$_9$H$_{16}$N$_3$O$_6$P: C 36.87, H 5.50, N 14.32; found: C 36.82, H 5.54, N 13.69.

NMR Spectra

Figure S4. $^1$H-NMR of Cyanurate phosphonic acid CyaPAc (RT, 400 MHz, DMSO-d$_6$)
Figure S5. $^{13}$C-NMR of Cyanurate phosphonic acid CyaPAc (RT, 101 MHz, DMSO-d$_6$)

Figure S6. $^{31}$P-NMR of Cyanurate Phosphonic Acid CyaPAc (RT, 162 MHz, DMSO-d$_6$)
Hamilton Receptor Phosphonate (HamPEst)

Procedure

The ethynyl Hamilton Ham-01 (170 mg, 0.3 mmol) was dissolved in 50 mL dried ethanol. To the solution was added azido phosphonate 3 (132 mg, 0.5 mmol) which priorly dissolved in 10 mL dried ethanol. To the mixture was added sodium ascorbate (10 mg, 0.05 mmol) in 1 mL deionized water. The mixture was stirred and heated up to 40 ºC for 15 minutes. Then to the mixture was added CuSO₄·5H₂O (1.25 mg, 0.005 mg) in 1 mL deionized water. The reaction was stirred at 40 ºC for overnight. After completion, the solvent was evaporated, and the crude was purified with silica column chromatography using EtOAc/DCM (4:1) as eluent. The final product was achieved as white solid (190 mg, 0.24 mmol, 76%).

Physical data

\( R_I = 0.1 \) (EtOAc/DCM 4:1); \(^1^H\) NMR (400 MHz, DMSO-\(d_6\)): \( \delta = 10.58 \) (s, 2H; N-H), 10.05 (s, 2H; N-H), 8.80 (s, 1H; Triazole-H), 8.62 (d, \( J_{CH} = 4 \) Hz, 2H; Ar-H), 8.45 (s, 1H; Ar-H), 7.89-7.80 (m, 6H; Py-H), 4.45 (t, \( J_{CH} = 8 \) Hz, 2H; N-CH₂), 4.02-3.88 (m, 4H; P-O-CH₂), 2.31 (s, 4H; Neopentyl-H), 1.92-1.26 (m, 10H; CH₂), 1.20 (t, \( J_{CH} = 8 \) Hz, 6H; Phosphonate-CH₃), 1.02 (s, 18H; Neopentyl-CH₂); \(^{13}^C\) NMR (101 MHz, DMSO-\(d_6\)): \( \delta = 170.9 \) (C=O), 165.0 (C=O), 150.5 (C-pyridine), 150.0 (C-pyridine), 145.1 (C-phenyl), 140.0 (C-pyridine), 135.0 (C-pyridine), 131.5 (C-phenyl), 127.6 (C-phenyl), 126.6 (C-triazole), 110.5 (C-pyridine), 110.0 (C-phenyl), 60.7 (d, \( J_{CH} = 6 \) Hz; COP), 49.6 (C-triazole), 49.0 (CH₂-neopentyl), 30.9 (C-neopentyl), 29.5 (CH₂-neopentyl), 29.3 (s, CH₂), 29.1 (d, \( J_{CH} = 16 \) Hz; CP), 25.3 (s, CH₂), 25.0 (s, CH₂), 23.6 (s, CH₂), 21.8 (d, \( J_{CH} = 5 \) Hz; CCP), 16.3 (d, \( J_{CH} = 5 \) Hz; CCP); \(^3^P\) NMR (162 MHz, DMSO-\(d_6\)): \( \delta = 31.84 \) (s); FTIR (ATR): \( \nu = 623, 663, 684, 700, 734, 744, 761, 798, 908, 962, 1024, 1051, 1095, 1132, 1153, 1224, 1238, 1296, 1327, 1363, 1390, 1440, 1506, 1583, 1604, 1674, 2866, 2951, 3267 cm \(^{-1}\)); MS (MALDI-dctb): \( m/z \) calcd for C\(_{92}\)H\(_{68}\)N\(_8\)O\(_4\)P+H\(^+\): 832.4270 [M+H]\(^+\); found: 832.4716 [M+H]\(^+\)

NMR Spectra

![NMR Spectra](image)

Figure S7. \(^1^H\)-NMR of Click-Hamilton Receptor HamPEst (RT, 400 MHz, DMSO-\(d_6\))
Figure S8. $^{13}$C-NMR of Click-Hamilton Receptor HamPEst (RT, 101 MHz, DMSO-$d_6$)

Figure S9. $^{31}$P-NMR of Click Hamilton Receptor HamPEst (RT, 162 MHz, DMSO-$d_6$)
Hamilton Receptor Phosphonic Acid (HamPAc)

Procedure

Under inert atmosphere, phosphonate Hamilton receptor HamPEst (150 mg, 0.18 mmol) was dissolved in 15 mL dry DCM. The mixture was stirred and heated up to 35 °C. To the mixture was added TMS-Br (65.8 mg, 0.43 mmol) and the reaction was continued to stir for 3 hours at 35 °C. After completion, the solvent was evaporated, and the residue was added dry MeOH (0.4 mL, 10 mmol) in which white precipitate formed. The mixture was stirred and heated at 40 °C for 1 hour and then added two drops of water to precipitate the product. The final product was collected and dried under vacuum as white solid (135 mg, 0.174 mmol, 96%).

Physical data

$^1$H NMR (600 MHz, RT, DMSO-d$_6$): $\delta$=11.08 (s, 2H; N-H), 10.59 (s, 2H; N-H), 8.85 (s, 1H; Triazole-H), 8.68 (d, $^3$J$_{HH}$=1.5 Hz, 2H; Ar-H), 8.52 (s, 1H; Ar-H), 7.98 (t, $^3$J$_{HH}$=8.1 Hz, 2H; Py-H), 7.73 (dd, 4H; $^3$J$_{HH}$=8.1 Hz, Py-H), 5.83 (s-broad, 2H; P-OH), 4.45 (t, $^3$J$_{HH}$=7 Hz, 2H; N-CH$_2$), 2.35 (s, 4H; Neopentyl-H), 1.91-1.86 (m, 2H; CH$_2$), 1.53-1.25 (m, 8H; CH$_2$), 1.03 (s, 18H; Neopentyl-CH$_3$); $^13$C NMR (151 MHz, DMSO-d$_6$): $\delta$=171.8 (C=O), 165.6 (C=O), 149.2 (C-phenyl), 148.7 (C-phenyl), 144.9 (C-pyridine), 141.9 (C-phenyl), 134.6 (C-pyridine), 131.6 (C-pyridine), 128.0 (C-pyridine), 126.9 (C-triazole), 122.4 (C-phenyl), 110.2 (C-phenyl), 109.6 (C-phenyl), 49.7 (C-triazole), 49.1 (CH$_2$-neopentyl), 31.0 (C-neopentyl), 29.5 (CH$_3$-neopentyl), 29.4 (s, CH$_3$), 29.3 (d, $^3$J$_{CCP}$=15 Hz; CP), 27.7 (s, CH$_3$), 26.8 (s, CH$_3$), 25.4 (s, CH$_3$), 22.5 (d, $^3$J$_{CCP}$=5 Hz; CCP); $^{31}$P NMR (162 MHz, DMSO-d$_6$): $\delta$=26.41 (s); FTIR (ATR): $\tilde{\nu}$= 653, 677, 719, 744, 767, 802, 852, 887, 908, 929, 947, 972, 1064, 1091, 1126, 1149, 1178, 1220, 1247, 1323, 1367, 1398, 1435, 1477, 1554, 1570, 1610, 1639, 1680, 2590, 2870, 2906, 2953, 3037, 3269 cm$^{-1}$; MS (MALDI-dctb): $m/z$ calcd for C$_{38}$H$_{50}$N$_9$O$_7$P$^+$: 776.3644 [M+H]$^+$; found: 776.4190 [M+H]$^+$

NMR Spectra

Figure S10. $^1$H-NMR of Click-Hamilton Receptor Phosphonic Acid HamPAc (RT, 600 MHz, DMSO-d$_6$)
Figure S11. $^{13}$C-NMR of Click-Hamilton Receptor Phosphonic Acid HamPAC (RT, 151 MHz, DMSO-$d_6$)

Figure S12. $^{31}$P-NMR of Click-Hamilton Receptor Phosphonic Acid HamPAC (RT, 162 MHz, DMSO-$d_6$)
HBC Cyanurate (CyaHBC)

**Procedure**

Under inert atmosphere, iodo-hexabenzocoronene 4 (50 mg, 54 μmol) was dissolved in anhydrous THF (20 mL). To the solution was added NET₃ (10 mL), Pd(PPh₃)₂Cl₂ (2 mg, 2.7 μmol), and Cul (5.2 mg, 27 μmol). Then the ethynyl-Cyanurate Cya-04 (11.2 mg, 48.6 μmol) in 10 mL anhydrous THF was added to the mixture. The mixture was stirred for 15 minutes at room temperature to homogenize the system and subsequently heated up under reflux at 70 °C for overnight. After the completion, the solvent was evaporated, and the residue was purified using short silica column. The first eluent was DCM to wash the orange-ring impurity and followed by EtOAc as second eluent to collect the yellow fraction. The collected fraction was dried out from solvent and rinsed with MeOH followed by pentane. The collected stuff was dried under vacuum to yield yellow solid as final product (40 mg, 39 μmol, 88%).

**Physical data**

Rₓ=0.5 (EtOAc): ¹H NMR (400 MHz, RT, THF-d₈): δ=10.72 (s, 2H; N-H), 9.37-9.29 (m, 12H; Ar-H), 7.88 (d, ¹Jᵥ=8 Hz, 2H; Ar-H), 7.47 (d, ¹Jᵥ=8 Hz, 2H; Ar-H), 1.89 (s, 9H, CH₃), 1.87 (s, 18H, CH₃), 1.85 (s, 18H, CH₃); ¹³C NMR (151 MHz, RT, DMSO-d₆:THF-d₈=2/1): δ= 149.75 (C=O), 148.92 (C=O), 148.11 (C=O), 134.74 (C-Ar), 131.85 (C-Ar), 129.94 (C-Ar), 129.55 (C-Ar), 129.23 (C-Ar), 129.15 (C-Ar), 128.76 (C-Ar), 124.19 (C-Ar), 123.16 (C-Ar), 122.21 (C-Ar), 122.12 (C-Ar), 121.92 (C-Ar), 119.97 (C-Ar), 119.23 (C-Ar), 119.19 (C-Ar), 119.03 (C-Ar), 118.68 (C-Ar), 118.42 (C-Ar), 117.86 (C-Ar), 92.14 (C-sp), 94.72 (C-sp), 35.53 (-CCH₃), 35.43 (-CCH₃), 35.36 (-CH₃), 31.73 (-CH₃), 31.68 (-CH₃), 31.63 (-CH₃); FTIR (ATR): ν= 613, 628, 686, 721, 746, 758, 812, 837, 864, 923, 941, 1022, 1039, 1103, 1130, 1166, 1201, 1224, 1263, 1311, 1361, 1371, 1394, 1409, 1419, 1506, 1577, 1606, 1701, 1751, 2208, 2382, 2744, 2866, 2902, 2953, 3072, 3095, 3172 cm⁻¹; UV/Vis (DCB): λ_max (ε)= 368 nm (134340 mol⁻¹·dm³·cm⁻¹); fluorescence (DCB): λex = 368 nm; λ_em=475, 496, 505 nm; MS (MALDI/dctb): m/z calcd for C₁₀₇H₆₃N₄O₉⁺: 1031.3325 [M+H]⁺; found: 1031.4921 [M+H]⁺

**NMR Spectra**

Figure S13. ¹H-NMR of HBC-Cyanurate CyaHBC (400 MHz, RT, THF-d₈)
Figure S14. $^{13}$C-NMR of HBC-Cyanurate (151 MHz, RT, DMSO-$d_6$:THF-$d_8$/2:1)
HBC-Hamilton (HamHBC)

Procedure

Under inert atmosphere, iodo-hexabenzocoronene 4 (50 mg, 54 µmol) was dissolved in anhydrous THF (50 mL). To the solution was added NEt$_3$ (20 mL), Pd(PPh$_3$)$_2$Cl$_2$ (5 mg, 2.7 µmol), PPh$_3$ (1 mg, 27 µmol), and Cul (3 mg, 16.2 µmol). Then the ethynyl-Hamilton Ham-01 (35 mg, 60 µmol) was dissolved in anhydrous THF (20 mL) and NEt$_3$ (5 mL), and subsequently added to the mixture. The reaction was stirred at room temperature for overnight. After that, the solvent was evaporated, and the residue was purified using silica column chromatography with eluent Cyclohexane/EtOAc (3:2). The clean fraction was collected and dried under vacuum to obtain the final product as yellow solid (71 mg, 52 µmol, 96%).

Physical data

$R_t$=0.6 (Cyclohexane/EtOAc 3:2); $^1$H NMR (400 MHz, RT, CDCl$_3$): δ=9.37-9.29 (m, 12H; Ar-H), 8.37 (s, 2H; Ar-H), 8.32 (s, 2H; N-H), 8.26 (s, 1H; Ar-H), 8.00 (d, $^J_{H,H}$=4 Hz, 4H; Py-H), 7.75 (d, $^J_{H,H}$=4 Hz, 2H; Py-H), 7.68 (s, 2H; N-H), 2.29 (s, 4H; CH$_2$), 1.85 (s, 18H; CH$_3$), 1.54 (s, 9H; CH$_3$), 1.56 (s, 18H; CH$_3$), 1.14 (s, 18H; CH$_3$); $^1$C NMR (151 MHz, RT, CDCl$_3$): δ=170.41 (C=O), 163.35 (C=O), 149.95, 149.37, 149.29, 149.22, 149.13, 141.09, 135.49, 133.64, 131.07, 130.76, 130.56, 130.45, 130.40, 129.48, 126.08, 125.85, 125.22, 124.74, 123.85, 123.81, 123.79, 121.16, 120.80, 120.51, 119.95, 119.63, 119.44, 119.39, 119.06, 118.98, 110.38, 109.88, 94.27 (C-sp$^3$), 87.97 (C-sp$^3$), 52.04 (CH$_3$), 36.00 (CH$_3$), 35.95 (CH$_3$), 35.91 (CH$_3$), 32.37 (CH$_3$), 32.27 (CH$_3$), 32.24 (CH$_3$), 31.54 (CH$_3$), 30.04 (CH$_3$); FTIR (ATR): $\nu$'= 630, 711, 736, 798, 866, 923, 941, 987, 1016, 1039, 1082, 1126, 1153, 1201, 1222, 1240, 1261, 1294, 1363, 1367, 1394, 1444, 1496, 1583, 1604, 1683, 2866, 2904, 2953, 3429 cm$^{-1}$; UV/Vis (DCB): $\lambda_{max}$ (e) = 370 nm (134880 mol$^{-1}$dm$^3$cm$^{-1}$); fluorescence (DCB): $\lambda_{em}$=370 nm; $\lambda_{ex}$=476, 497, 507 nm; MS (MALDI/dctb): m/z calcd for C$_{94}$H$_{52}$N$_6$O$_4$H$^+$: 1369.7253 [M+H]$^+$, found: 1369.6600 [M+H]$^+$

NMR Spectra

Figure S15. $^1$H-NMR of HBC-Hamilton Receptor (RT, 400 MHz, CDCl$_3$)
Figure S16. $^{13}$C-NMR of HBC-Hamilton Receptor (RT, 151 MHz, CDCl$_3$)
Functionalization of Nanoparticles

Surface Area

Average particle size (d) = 8 nm as referred to the specification of product from the vendor,

Density \( (\rho) \) of \( \text{Fe}_3\text{O}_4 \) = 5.17 g/cm\(^3\)

Thus,

Volume \( (V) \) of a SPION \( = \frac{4}{3} \times \pi \times r^3 = \frac{4}{3} \times 3.14 \times (4 \text{ nm})^3 = 267.95 \text{ nm}^3 \)

Mass of a SPION \( = \rho \times V = 5.17 \frac{\text{g}}{\text{cm}^3} \times 267.95 \text{ nm}^3 = 5.17 \frac{\text{g}}{\text{cm}^3} \times 267.95 \times 10^{-21} \text{ cm}^3 = 1.385 \times 10^{-18} \text{ g/particle} \)

Number of particles in 1 g of SPIONs \( = \frac{1}{1.385 \times 10^{-18} \text{ g}} = 7.22 \times 10^{17} \text{ Nanoparticles/g} \)

Surface area \( (A) \) of a SPION \( = 4 \times \pi \times r^2 = 4 \times 3.14 \times (4 \text{ nm})^2 = 200.96 \text{ nm}^2/\text{nanoparticle} \)

Specific Surface area \( (\text{SSA}) \) per gram of SPIONs \( = 7.22 \times 10^{17} \frac{\text{Nanoparticles}}{\text{g}} \times \frac{200.96 \text{ nm}^2}{\text{nanoparticle}} = 1.45 \times 10^{20} \text{ nm}^2/\text{g} \)

**Grafting density was calculated using formula:**

\[
\theta = \frac{\text{wt}}{100 - \text{wt}} \times \frac{\text{NA}}{\text{MW} \times \text{SSA}}
\]

Where is:

\( \text{Wt} \) = % mass loss from TGA measurement

\( \text{NA} \) = Avogadro number

\( \text{MW} \) = molecular weight of organic ligands

\( \text{SSA} \) = calculated specific surface area

Figure S17. TEM of pristine \( \text{Fe}_3\text{O}_4 \)
Fe₃O₄@CyaPAC

Procedure

Fe₃O₄-NPs from stock solution with concentration of 30 mg/mL was taken out 1 mL and dispersed in 23 mL solution mixture of THF/MeOH (4:1) to gain the final concentration of 1.25 mg/mL in total 24 mL volume. The dispersion was sonicated for 15 minutes at 20 °C to homogenize it. Then the dispersion was divided equally into 6 centrifugation glass tubes, each tube contains 5 mg of NPs from 4 mL dispersion (1.25 mg/mL). Then the NPs were centrifugated at 12500 g for 1 hour at 20 °C. The supernatant was decanted and the residues were mixed with the phosphonic acid cyanurate CyaPAC solutions accordingly to perform functionalization process. Functionalization process was done by mixing the Fe₃O₄-NPs with phosphonic acid cyanurate solutions. Prior to this step, the phosphonic acid cyanurate was prepared in 6 different concentration in THF/MeOH (4:1) solution mixture to yield certain concentration. After that, the cyanurate combined with Fe₃O₄-NPs dispersions were sonicated for 15 minutes at 20 °C, followed by centrifugation at 12500 g for 1 hour at 20 °C. The supernatant was decanted and the residue was subsequently washed two more times to get rid out the excess unbound cyanurate molecules. The washing cycle was done by dispersing the NPs residue in 4 mL THF/MeOH (4:1) mixture, then sonicated for 15 minutes at 20 °C, followed by centrifugation at 12500 g for 1 hour at 20 °C. After decantation of the solution, the final functionalized Fe₃O₄-NPs are dried in the drying oven for overnight at 75 °C to get solid cyanurate functionalized Fe₃O₄-NPs that can be dispersed accordingly and used further.

Table S1. Used cyanurate phosphonic acid CyaPAC ligands for functionalization of SPIONs

| Fe₃O₄-NPs | Phosphonic acid cyanurate CyaPAC ligand |
|----------|---------------------------------------|
| Mass (mg) | Mass (mg) | Molecular weight (g/mol) | Mol (mmol) | Volume (mL) | Concentration (mM) |
| 5         | 1.17      | 293.21                  | 0.004      | 4           | 1               |
| 5         | 5.86      | 293.21                  | 0.020      | 4           | 5               |
| 5         | 11.7      | 293.21                  | 0.040      | 4           | 10              |
| 5         | 17.59     | 293.21                  | 0.060      | 4           | 15              |
| 5         | 23.45     | 293.21                  | 0.080      | 4           | 20              |
| 5         | 35.18     | 293.21                  | 0.120      | 4           | 30              |

Figure S18. TEM picture of cyanurate functionalized SPIONs Fe₃O₄@CyaPAC.
DLS and Zeta Potential

The size distribution of functionalized Fe$_3$O$_4$@CyaPAc nanoparticles was determined using DLS technique. The dispersion of functionalized Fe$_3$O$_4$@CyaPAc was prepared in concentration of 0.125 mg/mL (0.0125 wt%) in THF/MeOH (4:1) solution mixture. The sample was put in glass cuvette and measured using Malvern ZetaSizer. The measurement was carried out 3 times per sample.

The Zeta potential of functionalized Fe$_3$O$_4$@CyaPAc nanoparticles was determined using ZetaSizer from Malvern equipped with a dip cell in quartz cuvette. The dispersion of functionalized Fe$_3$O$_4$@CyaPAc nanoparticles was prepared in concentration of 0.125 mg/mL (0.0125 wt%) in 1 mL of THF/MeOH (4:1) solution mixture. The measurement was carried out utilizing Hückel equation, with applied voltage of 5 V, using 100 times scan number and slow mode analysis. The measurement was repeated 3 times in which fresh sample was used in each repetition. The average number from 3 measurements were used as zeta potential value of Fe$_3$O$_4$@CyaPAc dispersions in various ligand concentrations.

TGA

The functionalization degree was determined using thermogravimetry analysis (TGA). The dried functionalized Fe$_3$O$_4$-NPs was placed in aluminum oxide crucible and heated up to 1000 °C under atmospheric gas mixture with heating rate 30 °C/min. The percentage of mass loss was used to determine the functionalization degree and coverage surface density.

| Concentration (mM) | Mass loss (%) | Surface Area* (nm$^2$/g) | Grafting density** (nm$^{-2}$) | 1/concentration | 1/grafting density |
|-------------------|--------------|---------------------------|-------------------------------|----------------|-------------------|
| 1                 | 8.81         | 1.45*10e20                | 1.36                          | 1000           | 0.735             |
| 5                 | 8.39         | 1.45*10e20                | 1.29                          | 200            | 0.775             |
| 10                | 9.24         | 1.45*10e20                | 1.44                          | 100            | 0.694             |
| 15                | 9.14         | 1.45*10e20                | 1.42                          | 66.67          | 0.704             |
| 20                | 9.88         | 1.45*10e20                | 1.55                          | 50             | 0.645             |
| 30                | 9.34         | 1.45*10e20                | 1.46                          | 33.3           | 0.684             |

Plotting the mass loss versus the concentration of ligands used to functionalize the iron oxide nanoparticles, a plateau was achieved at 9.8% of mass loss at concentration 20 mM. At this condition, the surface of Fe$_3$O$_4$ NPs was saturated by CyaPAc molecules with grafting density about 2 molecule/nm$^2$.

\[ \theta = \frac{wt}{100 - wt} \times \frac{NA}{MW \times SSA} = \frac{9.8}{100 - 9.8} \times \frac{6.02 \times 10^{23} \text{mol}^{-1}}{293.21 \text{g/mol} \times 1.45 \times 10^{20} \frac{\text{nm}^2}{\text{g}}} \]

\[ = 1.55 \text{ molecules/nm}^2 \]

Assuming a single nanoparticle as a sphere with average size of 8 nm, thus at saturation condition there are approximately 310 CyaPAc molecules attached to the surface of a single SPION.

Surface area (A) of a SPION = \(4 \times \pi \times r^2 = 4 \times 3.14 \times (4 \text{ nm})^2 = 200.96 \text{ nm}^2/\text{nanoparticle} \)

\[ \text{in average} = A \times \theta = 200.96 \frac{\text{nm}^2}{\text{NP}} \times 1.55 \frac{\text{molecule}}{\text{nm}^2} = \sim 300 \frac{\text{molecule}}{\text{NP}} \]
**Fe₃O₄@HamPAc**

**Procedure**

Fe₃O₄-NPs from stock solution with concentration of 30 mg/mL was taken out 1 mL and dispersed in 23 mL solution mixture of DMSO/EtOH (3:1) to gain the final concentration of 1.25 mg/mL in total 24 mL volume. The dispersion was sonicated for 15 minutes at 20 °C to homogenize it. Then the dispersion was divided into 6 centrifugation glass tube, each tube contains 5 mg of NPs from 4 mL dispersion (1.25 mg/mL). Then the NPs were centrifugated at 12500 g for 1 hour at 10 °C. The supernatant was decanted and the residues were mixed with the phosphonic acid Hamilton receptor solutions accordingly to perform functionalization process. Functionalization process was done by mixing the Fe₃O₄-NPs with phosphonic acid Hamilton receptor solutions. Prior to this step, the phosphonic acid Hamilton receptor HamPAc was prepared in 6 different concentration in DMSO/EtOH (3:1) solution mixture to yield certain concentration. After that, the Hamilton receptor combined with Fe₃O₄-NPs dispersions were sonicated for 15 minutes at 20 °C, followed by centrifugation at 12500 g for 1 hour at 10 °C. The supernatant was decanted and the residue was subsequently washed twice to get rid out the excess unbound cyanurate molecules. The washing cycle was done by dispersing the NPs residue in 4 mL dried EtOH, then sonicated for 15 minutes at 20 °C, followed by centrifugation at 12500 g for 1 hour at 10 °C. After decantation of the solution, the final functionalized Fe₃O₄-NPs are dried in the drying oven for overnight at 75 °C to get solid Hamilton receptor functionalized Fe₃O₄-NPs that can be dispersed accordingly and used further.

| Table S2. Used Hamilton receptor phosphonic acid HamPAc ligands for functionalization of SPIONs |
|---------------------------------------------------------------|
| **Fe₃O₄-NPs** | **Phosphonic acid Hamilton receptor ligand** |
| Mass (mg) | Mass (mg) | Molecular weight (g/mol) | Mol (mmol) | Volume (mL) | Concentration (mM) |
| 5 | 3.2 | 775.85 | 0.004 | 4 | 1 |
| 5 | 9.3 | 775.85 | 0.012 | 4 | 3 |
| 5 | 15.7 | 775.85 | 0.020 | 4 | 5 |
| 5 | 25.3 | 775.85 | 0.032 | 4 | 8 |
| 5 | 31.0 | 775.85 | 0.040 | 4 | 10 |
| 5 | 46.5 | 775.85 | 0.060 | 4 | 15 |

**Figure S19.** TEM picture of functionalized Fe₃O₄@HamPAc NPs.
**DLS and Zeta Potential**

The size distribution of functionalized Fe$_3$O$_4$@HamPAc nanoparticles was determined using DLS technique. The dispersion of functionalized Fe$_3$O$_4$@HamPAc nanoparticles was prepared in concentration of 0.2 mg/mL (0.02 wt%) in DMSO/EtOH (3:1) solution mixture. The sample was put in glass cuvette and measured using Malvern ZetaSizer. The measurement was carried out 3 times per sample.

The Zeta potential of functionalized Fe$_3$O$_4$@HamPAc nanoparticles was determined using ZetaSizer from Malvern, equipped with a dip cell, and using quartz cuvette. The dispersion of functionalized Fe$_3$O$_4$@HamPAc nanoparticles was prepared in concentration of 0.2 mg/mL (0.02 wt%) in 1 mL of DMSO/EtOH (3:1) solution mixture. The measurement was carried out utilizing Hückel equation, with applied voltage 5-10 V, using 100 times scan number and slow mode analysis. The measurement was repeated 3 times in which fresh sample was used in each repetition. The average number from 3 measurements were used as zeta potential value of Fe$_3$O$_4$@HamPAc dispersions in various ligand concentrations.

**TGA**

The functionalization degree was determined using thermogravimetry analysis (TGA). The dried functionalized Fe$_3$O$_4$-NPs was placed in aluminum oxide crucible and heated up to 1000 °C under atmospheric gas mixture with heating rate 30 °C/min. The percentage of mass loss was used to determine the functionalization degree and coverage surface density.

| Concentration (mM) | Mass loss (%) | Surface Area* (nm$^2$/g) | Grafting density** (nm$^2$/M$^{-1}$) | 1/concentration | 1/grafting density |
|-------------------|---------------|---------------------------|-------------------------------------|-----------------|-------------------|
| 1                 | 5.81          | 1.45*10e20                | 0.33                                | 1000            | 3.03              |
| 3                 | 7.26          | 1.45*10e20                | 0.42                                | 333.5           | 2.38              |
| 5                 | 9.01          | 1.45*10e20                | 0.53                                | 200             | 1.88              |
| 8                 | 11.33         | 1.45*10e20                | 0.68                                | 125             | 1.47              |
| 10                | 11.53         | 1.45*10e20                | 0.69                                | 100             | 1.44              |
| 15                | 12.08         | 1.45*10e20                | 0.73                                | 66.7            | 1.34              |

Plotting the mass loss versus the concentration of ligands used to functionalize the iron oxide nanoparticles, a plateau was achieved at 12% of mass loss at concentration 15 mM. At this condition, the surface of Fe$_3$O$_4$ NPs was saturated by HamPAc molecules with grafting density about 0.73 molecule/nm$^2$.

\[
\theta = \frac{wt}{100 - wt} \times \frac{NA}{MW \times SSA} = \frac{9.8}{100 - 9.8} \times \frac{6.02 \times 10^{23} \text{mol}^{-1}}{775.85 \text{g/mol} \times 1.45 \times 10^{20} \text{nm}^2} \text{g}^{-1}
\]

\[= 0.73 \text{ molecules/nm}^2\]

Assuming a single nanoparticle as a sphere with average size of 8 nm, thus at saturation condition there are approximately 150 HamPAc molecules attached to the surface of a single SPION.

Surface area (A) of a SPION = \(4 \times \pi \times r^2 = 4 \times 3.14 \times (4 \text{ nm})^2 = 200.96 \text{ nm}^2/\text{nanoparticle}\)

in average = \(A \times \theta = 200.96 \text{ nm}^2/\text{NP} \times 0.73 \frac{\text{molecule}}{\text{nm}^2} = \sim 150 \frac{\text{molecule}}{\text{NP}}\)
NMR Study of Host-Guest Interactions

Job’s Plot

Figure S20. (a). general scheme of hydrogen bond interactions among Hamilton receptors and cyanurate molecules, (b). NMR titration study to determine the complexation of HamPEst with CyaPEst, (c). Job’s plot of interaction among HamPEst with CyaPEst confirming 1 to 1 complexation.

Chemical shift data

Figure S21. Relative position of active protons of HamPEst in various organic solvents (a). in ACN-$d_3$ where NH1 and NH2 at 9.11 and 8.57 ppm, (b). in CDCl$_3$ where NH1 and NH2 at 8.42 and 8.22 ppm, and (c). in DMSO-$d_6$ where NH1 ad NH2 at 10.56 and 10.03 ppm.
Figure S22. NMR titration among HamPEst with CyaPEst in various polar aprotic organic solvents containing oxygen atoms (a). Acetone-$d_6$, (b) THF-$d_8$, and (c). DMSO-$d_6$ did not show any hydrogen bond interactions.
UV-Vis of HamHBC and CyaHBC

**Figure S23.** Dilution series of UV-Vis spectra of HamHBC in DCB with the regression linear.

**Figure S24.** Dilution series of UV-Vis spectra of CyaHBC in DCB with the regression linear.
Fluorescence of HamHBC and CyaHBC

Figure S25. Emission spectra of HamHBC in DCB excited at 370 nm in concentration range between 10 till 700 nM.

Figure S26. Emission spectra of CyaHBC in DCB excited at 368 nm in concentration range between 40 till 700 nM.
Fluorescence Titration of functionalized SPIONs in DCB

**Figure S27.** (a). Fluorescence titration data of Fe$_3$O$_4$@HamPAc with CyaHBC in DCB showing quenching effect, (b). fitting of fluorescence titration results at 425 nm.

**Figure S28.** (a). Fluorescence titration data of Fe$_3$O$_4$@CyAPAc with HamHBC in DCB showing increasement of emission intensity from HBC moiety, (b). fitting of fluorescence titration results at 425 nm.