Electronic Supplementary Information (ESI)

[12]aneN₃-based multifunctional compounds as fluorescent probes and nucleic acids delivering agents

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6. Spectra
1. Spectroscopic properties of 1a-1e

**Fig. S1** The absorption spectra (50 μM) and fluorescence (10 μM) spectra of 1a-1e in water-Tris-HCl buffer (1 mM, pH = 7.2).

**Table S1** The fluorescent intensity changes of 1a-1e after addition of Cu$^{2+}$ ions

| Probes | Fluorescent Intensity (F₀, before addition of Cu$^{2+}$) | Fluorescent Intensity (F, after addition of Cu$^{2+}$) | Fluorescent Changes (F₀ / F) |
|--------|----------------------------------------------------------|-----------------------------------------------------|----------------------------|
| 1a     | 605.86                                                   | 60.91                                               | 9.95                      |
| 1b     | 810.36                                                   | 17.15                                               | 47.25                     |
| 1c     | 339.10                                                   | 57.22                                               | 5.93                      |
| 1d     | 866.29                                                   | 13.60                                               | 63.69                     |
| 1e     | 582.98                                                   | 39.97                                               | 14.59                     |

**Fig. S2** Selectivity studies of 1b with Cu$^{2+}$ in the presence of other metal ions. Blue bars represent the addition of the competing metal ion (100 μM) to the solution of the 1 (10 μM). Red bars represent the addition of Cu$^{2+}$ (30 μM) to the solution containing other metal ions.
Fig. S3 Plots of fluorescence intensity of 1a-1e as a function of [Cu$$^{2+}$$]/[I]. The standard deviations obtained by fluorescence responses of 1a-1e were determined to be σ = 1.17, 0.63, 1.08, 0.13 and 0.34 for Cu$$^{2+}$$, therefore, the detection limits were calculated by the formula (3σ/k) and given the results of 1.21×10^-8 M, 7.48×10^-9 M, 4.40×10^-8 M, 1.23×10^-9 M and 2.36×10^-9 M, respectively.
Fig. S4 Job's plot showing the 1:1 (1a-1d/Cu$^{2+}$) and 1:2 (1e/Cu$^{2+}$) complex stoichiometry, mole fraction of Cu$^{2+}$ $X = [\text{Cu}^{2+}]/([\text{Cu}^{2+}]+[1])$, $[\text{Cu}^{2+}]+[1] = 30 \mu M$ in Tris-HCl buffer.
Fig. S5 $^1$H NMR of compound 1b upon titration of Cu(ClO$_4$)$_2$ (CD$_3$SOCD$_3$)

Fig. S6 The proposed binding modes of 1 and Cu$^{2+}$

2. Characterization of 1a-1e/RNA (DNA) complexes
Fig. S7 (A1-E1) Mean diameter and (A2-E2) zeta potential of 1a-1e/DNA complexes and 1a-1e/RNA complexes obtained at various concentrations by DLS.

3 Cell uptake of 1a-1e/RNA (DNA) complexes

| Concentration | Image |
|---------------|-------|
| 10 μM         | A1    |
| 15 μM         | A2    |
| 20 μM         | A3    |
| 25 μM         | A4    |

Fig. S7 Fluorescence microscope images of HeLa cells transfected with Cy5-labeled siRNA (9 μg/mL) by 1b at different concentrations. A1-A4: BF, B1-B4: red channels, C1-C4: green channels.

| Weight Ratio | Image |
|--------------|-------|
| 2/1          | A1    |
| 5/1          | A2    |
| 10/1         | A3    |
| 15/1         | A4    |

Fig. S8 Fluorescence microscope images of HeLa cells transfected with Cy5-labeled siRNA (9 μg/mL) by 25 KDa PEI at different weight ratios. A1-A4: BF, B1-B4: red channels.
Fig. S9 Fluorescence microscope images of HeLa cells transfected with Cy5-labeled siRNA (9 μg/mL) by lipofectamine 2000 at different weight ratios. A1-A4: BF, B1-B4: red channels.

Fig. S10 Fluorescence microscope images of HepG2 cells transfected with Cy5-labeled siRNA (9 μg/mL) by MFCs 1a-1e at the concentration of 20 μM, 25 kD PEI and lipofectamine 2000 as positive control. A1-E1: red channels, A2-E2: green channels.
Fig. S11 Fluorescence microscope images of U2Os cells transfected with Cy5-labeled siRNA (9 μg/mL) by MFCs 1a-1e at the concentration of 20 μM, 25 kD PEI and lipofectamine 2000 as positive control. A1-E1: red channels, A2-E2: green channels.

Fig. S12 Fluorescence microscope images of MC3T3-E1 cells transfected with Cy5-labeled siRNA (9 μg/mL) by MFCs 1a-1e at the concentration of 20 μM, 25 kD PEI and lipofectamine 2000 as positive control. A1-E1: red channels, A2-E2: green channels.
4. Cytotoxicity

![Graphs showing cell viability data](image)

**Fig. S13** Cytotoxicities of the complexes of MFCs 1a-1e/DNA at different concentrations on HeLa, HepG2, U2Os and MC3T3-E1 cells.

5. Spectroscopic data of compounds

**4a**: 59%; $^1$H NMR (400 MHz, CDCl$_3$) δ 8.59 (d, $J$ = 7.2 Hz, 1H), 8.48 (dd, $J$ = 17.1, 8.1 Hz, 2H), 7.70 (t, $J$ = 7.9 Hz, 1H), 7.38 (s, 2H), 7.28 - 7.09 (m, 4H), 5.45 (s, 4H), 4.15 (t, $J$ = 7.2 Hz, 2H), 3.83 - 3.76 (m, 8H), 3.33 (s, 16H), 3.19 (s, 3H), 2.65 (s, 3H), 2.42 (s, 8H), 1.88 - 1.82 (m, 12H), 1.73 - 1.65 (m, 2H), 1.45 (s, 38H), 0.96 (t, $J$ = 7.2 Hz, 3H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 169.68, 164.15, 163.76, 155.96, 143.89, 137.52, 136.45, 132.15, 130.98, 130.44, 129.94, 127.91, 126.38, 125.16, 122.93, 115.55, 114.88, 79.07, 53.01, 49.56, 46.52, 45.30, 43.70, 41.90, 39.85, 37.62, 30.11, 28.36, 25.96, 20.22, 13.73; IR (KBr, cm$^{-1}$): 3338.86, 3127.11, 2967.17, 2931.93, 1690.36, 1649.70, 1581.93, 1416.57, 1359.64, 1245.78, 1169.88, 776.81; EI-MS calcd. For C$_{73}$H$_{109}$N$_{15}$O$_{11}$ (M+H)$^+$: 1372.8, found 1373.0.

**4b**: 78%; $^1$H NMR (400 MHz, CDCl$_3$) δ 8.57 (d, $J$ = 7.3 Hz, 1H), 8.41 (d, $J$ = 8.4 Hz, 1H), 8.28 (s, 1H), 7.73 (s, 2H), 7.64 (t, $J$ = 7.9 Hz, 1H), 7.34 (s, 7H), 7.15 (s, 1H), 6.58 (d, $J$ = 8.5 Hz, 1H), 5.52 (s, 4H), 4.19 - 4.12 (m, 2H), 3.89 (s, 2H), 3.70 (s, 4H), 3.55 (s, 2H), 3.30 - 3.24 (m, 16H), 2.39 (s, 8H), 1.90 - 1.66 (m, 14H), 1.42 (d, $J$ = 13.1 H, 38H), 0.96 (t, $J$ = 7.3 Hz, 3H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 168.55, 164.39, 163.90, 156.20, 149.97, 144.08, 136.55, 135.81, 134.13, 130.72, 129.45, 127.07, 124.42, 122.41, 120.11, 109.37, 103.27, 79.24, 53.12, 49.65, 46.87, 45.31, 43.90, 39.73, 39.22, 30.21, 28.38, 25.84, 25.40, 20.29, 13.78; IR (KBr, cm$^{-1}$): 3379.86, 3127.11, 2967.17, 2931.93, 1690.36, 1649.70, 1581.93, 1416.57, 1359.64, 1245.78, 1169.88, 776.81; EI-MS calcd. For C$_{71}$H$_{105}$N$_{15}$O$_{11}$ (M+H)$^+$: 1344.8, found 1344.9.

**4c**: 57%; $^1$H NMR (400 MHz, CDCl$_3$) δ 8.61 (d, $J$ = 7.1 Hz, 1H), 8.54 (d, $J$ = 8.0 Hz, 1H), 8.40 (d, $J$ = 8.4 Hz, 1H), 7.76 - 7.72 (m, 1H), 7.41 (s, 2H), 7.31 (s, 2H), 7.24 (d, $J$ = 4.0 Hz, 2H), 5.56 (s, 4H), 4.31
(t, J = 6.7 Hz, 1H), 4.20 - 4.15 (m, 2H), 4.07 (s, 1H), 3.79 (s, 4H), 3.31 (s, 18H), 2.44 - 2.42 (m, 8H), 1.88 - 1.83 (m, 12H), 1.73 - 1.67 (m, 4H), 1.44 (s, 38H), 1.28 - 1.21 (m, 2H), 0.98 (d, J = 7.3 Hz, 3H);

\(^{13}\)C NMR (101 MHz, D\(_2\)O) \(\delta\) 166.26, 165.02, 161.60, 161.15, 153.66, 152.17, 141.72, 134.54, 134.26, 129.71, 129.66, 128.53, 128.27, 127.13, 126.99, 126.19, 125.60, 124.01, 123.59, 120.82, 120.26, 115.24, 112.96, 76.67, 62.88, 50.54, 50.39, 47.10, 44.28, 42.82, 41.33, 37.45, 27.93, 27.61, 25.87, 24.75, 23.46, 17.73, 16.54, 11.21, 11.08; IR (KBr, cm\(^{-1}\)) : 3343.72, 3127.11, 2969.88, 2926.51, 1690.36, 1657.83, 1590.06, 1416.57, 1365.06, 1229.52, 1161.75, 784.94;

EI-MS calcd. For C\(_{73}\)H\(_{107}\)N\(_{15}\)O\(_{11}\) (M+H): 1370.8, found 1370.8.

7d: 42%; \(^{1}\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.58 (d, J = 7.0 Hz, 1H), 8.45 (d, J = 8.0 Hz, 1H), 8.25 (d, J = 8.3 Hz, 1H), 7.63 - 7.59 (m, 3H), 7.38 (s, 2H), 7.32 (s, 1H), 6.71 (d, J = 8.1 Hz, 1H), 6.61 (s, 1H), 5.76 (s, 1H), 5.52 (s, 4H), 4.16 (s, 2H), 3.74 (s, 4H), 3.58 - 3.10 (m, 20H), 2.41 (s, 8H), 1.87 - 1.39 (m, 60H), 0.97 (t, J = 6.8 Hz, 3H); \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 166.36, 164.64, 164.06, 156.26, 149.89, 136.84, 136.46, 134.41, 130.93, 129.82, 126.77, 124.38, 122.88, 120.33, 109.64, 104.04, 79.31, 53.25, 49.85, 46.97, 45.39, 43.95, 43.28, 39.85, 39.71 30.28, 29.60, 29.38, 28.53, 27.88, 26.39, 26.29, 26.04, 20.36, 13.83; IR (KBr, cm\(^{-1}\)) : 3433.43, 2929.22, 1638.86, 1579.22, 1384.04, 1362.35, 1251.20, 1167.17, 1104.82, 641.2; EI-MS calcd. For C\(_{75}\)H\(_{113}\)N\(_{15}\)O\(_{11}\) (M+H): 1400.8, found 1400.6.

7e: 36%; \(^{1}\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.49 (d, J = 6.3 Hz, 1H), 8.37 (d, J = 7.6 Hz, 1H), 8.17 (d, J = 6.9 Hz, 1H), 7.60 (s, 2H), 7.52 (s, 1H), 7.27 (s, 1H), 7.20 (s, 1H), 6.62 (d, J = 8.3 Hz, 2H), 5.65 (s, 1H), 5.48 (s, 2H), 4.31 (s, 2H), 4.08 (s, 2H), 3.67 (s, 2H), 3.39 - 3.32 (m, 4H), 3.21 - 3.16 (m, 8H), 2.33 (s, 4H), 1.73 (s, 8H), 1.61 (s, 4H), 1.47 (s, 2H), 1.37 (s, 20H), 0.89 (s, 3H); \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 166.61, 164.67, 164.11, 156.30, 149.72, 137.18, 136.56, 136.13, 134.39, 130.97, 129.98, 129.83, 126.41, 126.32, 126.90, 126.41, 126.48, 128.45, 123.02, 120.32, 109.92, 104.12, 79.40, 53.94, 53.48, 49.88, 47.22, 45.42, 44.05, 43.28, 39.90, 39.69, 30.30, 29.63, 29.47, 28.55, 28.45, 26.36, 26.25, 20.39, 13.83; IR (KBr, cm\(^{-1}\)) : 3438.86, 2926.51, 2099.70, 1638.86, 1579.22, 1549.40, 1384.04, 1359.64, 1248.49, 1164.46, 1115.66, 779.52; EI-MS calcd. For C\(_{53}\)H\(_{74}\)N\(_{12}\)O\(_{7}\) (M+H): 991.5, found 991.3.
6. Spectra

$^1$H NMR spectrum of compound 4a (solvent: CDCl$_3$)

$^{13}$C NMR spectrum of compound 4a (solvent: CDCl$_3$)
IR spectrum of compound 4a

Ms spectrum of compound 4a
$^1$H NMR spectrum of compound 4b (solvent: CDCl$_3$)

$^{13}$C NMR spectrum of compound 4b (solvent: CDCl$_3$)
IR spectrum of compound 4b

Ms spectrum of compound 4b (solvent: CDCl₃)
$^{1}H$ NMR spectrum of compound 4c (solvent: CDCl$_3$)

$^{13}$C NMR spectrum of compound 4c (solvent: CDCl$_3$)
IR spectrum of compound 4c

Ms spectrum of compound 4c
$^1$H NMR spectrum of compound 7d (solvent: CDCl$_3$)

$^{13}$C NMR spectrum of compound 7d (solvent: CDCl$_3$)
IR spectrum of compound 7d (solvent: CDCl₃)

Ms spectrum of compound 7d (solvent: CDCl₃)
$^1$H NMR spectrum of compound 7e (solvent: CDCl$_3$)

$^{13}$C NMR spectrum of compound 7e (solvent: CDCl$_3$)
IR spectrum of compound 7e (solvent: CDCl$_3$)

Ms spectrum of compound 7e (solvent: CDCl$_3$)
$^1$H NMR spectrum of compound 1a (solvent: D$_2$O)

$^{13}$C NMR spectrum of compound 1a (solvent: D$_2$O)
**IR spectrum of compound 1a**

**Elemental Composition Report**

**Single Mass Analysis**

Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for I-FIT = 2

Monoisotopic Mass, Even Electron Ions

535 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 0-60  H: 0-80  N: 0-15  O: 0-10

GYG097.38 (0.518)

TOF MS ES+

1.16e+002

| Minimum | Maximum | Mass  | Calc. Mass | mDa | PPM | DBE | I-FIT | Formula |
|---------|---------|-------|------------|-----|-----|-----|-------|----------|
| 972.6427| 972.6412| 1.5   | 1.5        | 1.5 | 22.5| 0.2 |       | C53 H70 N15 O3 |
| 972.6452| -2.6    | -2.6  | -2.6       | -2.6| -2.6| -2.6|       | C58 H78 N13 O0 |

**HRMs spectrum of compound 1a**
$^1$H NMR spectrum of compound 1b (solvent: D$_2$O)

$^{13}$C NMR spectrum of compound 1b (solvent: D$_2$O)
Elemental Composition Report

**Single Mass Analysis**

Tolerance = 5.0 PPM  DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
671 formula(e) evaluated with 4 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-80  H: 0-80  N: 0-15  O: 0-10

OYO98B 46 (0.851)
TOP MS ES+

**HRMs spectrum of compound 1b**
$^1$H NMR spectrum of compound 1c (solvent: D$_2$O)

$^{13}$C NMR spectrum of compound 1c (solvent: D$_2$O)
IR spectrum of compound 1c

Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 ppm / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
540 formula(e) evaluated with 4 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-80 H: 0-80 N: 0-16 O: 0-10

Elemental composition calculated as C55H72N2O12

HRMs spectrum of compound 1c
$^1$H NMR spectrum of compound 1d (solvent: D$_2$O)

$^{13}$C NMR spectrum of compound 1d (solvent: D$_2$O)
IR spectrum of compound 1d

Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Macromolecular Mass, Even Electron Ions
6011 formulas evaluated with 27 results within limits (up to 50 closest results for each mass)

CyC-144 (6.204)
TOF MS ES+

HRMs spectrum of compound 1d

30
$^1$H NMR spectrum of compound 1e (solvent: D$_2$O)

$^{13}$C NMR spectrum of compound 1e (solvent: D$_2$O)
IR spectrum of compound \(1e\)

Elemental Composition Report

**Single Mass Analysis**

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions

4915 formula(s) evaluated with 11 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 0-100  H: 0-120  N: 0-15  O: 0-10  I: 0-4

GVG-144S 25 (0.463)

TOF MS ES+

| Mass   | Calc. Mass | mDa | PPM | DBE | i-FIT | Formula |
|--------|------------|-----|-----|-----|-------|---------|
| 791.4839 | 791.4839 | 0.2 | 0.3 | 7.5 | 34.4 | C45 H76 O3 I |
| 791.4844 | 791.4844 | -0.3 | -0.4 | 5.8 | 87.1 | C30 H72 N12 O4 I |
| 791.4847 | 791.4847 | -0.6 | -0.8 | 14.5 | 28.6 | C46 H67 N2 O9 |
| 791.4833 | 791.4833 | 0.8 | 1.0 | 20.5 | 28.9 | C43 H59 N12 O3 |
| 791.4832 | 791.4832 | -1.4 | -1.4 | 7.5 | 76.8 | C31 H63 N14 O10 |
| 791.4828 | 791.4828 | 1.3 | 1.6 | 27.5 | 6.1 | C58 H63 O2 |
| 791.4860 | 791.4860 | -1.9 | -2.4 | 19.5 | 22.3 | C47 H63 N6 O5 |
| 791.4820 | 791.4820 | 2.1 | 2.7 | 15.5 | 36.2 | C42 H63 N8 O7 |
| 791.4842 | 791.4842 | 2.9 | 3.7 | 8.5 | 42.9 | C41 H72 N6 O1 |
| 791.4871 | 791.4871 | -3.0 | -3.8 | -0.5 | 74.3 | C38 H76 N6 O6 I |
| 791.4873 | 791.4873 | -3.2 | -4.0 | 24.5 | 16.9 | C48 H59 N10 O |

HRMs spectrum of compound \(1e\)

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