Supporting Information

Synthesis of bifunctional molecules containing [12]aneN₃ and coumarin moieties as effective DNA condensation agents and new non-viral gene vectors

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1. Synthesis and characterization

1.1 Synthesis route

Scheme S1  Syntheses of 1-6: i) a) CHCl₃, overnight; b) 3M HCl, reflux 12 h; ii) THF/H₂O, CuSO₄·5H₂O, sodium ascorbate, overnight; iii) CH₃COCl, CH₃OH.
1.2 Syntheses of the Compounds 1, 2-7-12

1.2.1 Syntheses of compound 1, 2, 7

Compound 1, 2, 7 were syntheses according the literature[1, 2].

1): Yield: 61%. M.p.: 206 °C-208 °C. ¹H NMR (400 MHz, D₂O) δ 7.56 (s, 5H), 4.38 (s, 2H), 3.47 – 3.26 (m, 12H), 2.32 – 2.11 (m, 6H). ¹³C NMR (101 MHz, D₂O) δ 131.12, 130.59, 129.61, 128.54, 58.97, 46.77, 42.29, 41.19, 20.59, 17.62. ESI-MS Calcd. for C₁₆H₂₈N₃(M+H)+: 262.2, found: 262.4.

2): Yield: 82%. M.p.: 199 °C-200 °C. ¹H NMR (400 MHz, D₂O) δ 7.76 – 7.61 (m, 4H), 4.49 (s, 4H), 3.53 – 3.43 (m, 8H), 3.43 – 3.32 (m, 16H), 2.35 – 2.18 (m, 12H). ¹³C NMR (101 MHz, D₂O) δ 133.72, 133.14, 130.69, 129.75, 58.28, 46.85, 42.25, 41.11, 20.54, 17.57. ESI-MS Calcd. for C₂₆H₄₉N₆(M+H)+: 445.4, found: 445.8.

7): Yield: 44%. ¹H NMR (400 MHz, CDCl₃) δ 3.38 (d, J = 2.2 Hz, 2H), 3.26 – 3.33 (m, 8H), 2.51 – 2.54 (m, 4H), 2.15 (t, J = 2.2 Hz, 1H), 1.88 – 1.75 (m, 6H), 1.46 (s, 18H).

1.2.2 Synthesis of compound 8

Compound 8 was synthesis according to literature[3].

8): Yield: 97%. ¹H NMR (400 MHz, CDCl₃): 7.25 (s, 3H), 4.40 (s, 6H).

1.2.3 Synthesis of compound 12

Compound 12 was synthesis according to literature[4].

12a): Yield: 86%. ¹H NMR (400 MHz, CDCl₃) δ 7.65 (d, J = 9.5 Hz, 1H), 7.40 (d, J = 8.5 Hz, 1H), 6.96 – 6.85 (m, 2H), 6.28 (d, J = 9.5 Hz, 1H), 4.76 (d, J = 2.4 Hz, 2H), 2.58 (t, J = 2.4 Hz, 1H).

12b): Yield: 83%. ¹H NMR (400 MHz, CDCl₃) δ 7.52 (dd, J = 7.6, 1.8 Hz, 1H), 6.96 – 6.89 (m, 2H), 6.16 (d, J = 1.0 Hz, 1H), 4.76 (d, J = 2.4 Hz, 2H), 2.57 (t, J = 2.4 Hz, 1H), 2.40 (d, J = 1.0 Hz, 3H).

1.2.4 Syntheses of compound 9-11

Azide compound 8 (0.44 g, 1.8 mmol) and compound 7 (1.12 g, 2.7 mmol) were added into THF/H₂O (v/v = 2:1), CuSO₄·5H₂O (0.045 g, 0.18 mmol) and sodium ascorbate (0.044 g, 0.37 mmol) were also added into the solution as catalysis. The mixture was stirred over night at room temperature, saturated with NaCl, and extracted with ethyl acetate. The organic layers were washed once with brine, dried over Na₂SO₄ and evaporated under reduced pressure. The crude products were purified by flash chromatography on silica gel with PE/Acetone (2:1) to yield the Boc-protected compound 9-11 as pale yellow solid.

9): 0.31 g, Yield: 12%. M.p.: 35 °C-36 °C. ¹H NMR (400 MHz, CDCl₃) δ 7.36 (s, 3H), 7.06 (s, 3H), 5.45 (s, 6H), 3.75 (s, 6H), 3.52 – 3.17 (m, 24H), 2.55 – 2.33 (m, 12H), 2.07 – 1.61 (m, 18H), 1.42 (s, 54H). ¹³C NMR (101 MHz, CDCl₃) δ 156.36, 144.42, 137.19, 127.12, 122.77, 79.36, 53.26, 49.81, 46.97, 45.48, 43.96, 28.56, 27.35, 26.17. IR (KBr, cm⁻¹): 2967, 2930, 1727, 1690, 1476, 1454, 1414, 1365, 1283, 1165, 1073. ESI-MS Calcd. for C₇₂H₁₂₇N₁₈O₁₃(M+2H)+: 1473.0,
found: 1473.2.

**10): 0.75 g, Yield: 40 %. M.p.: 79 °C-81 °C. **$^1$H NMR (400 MHz, CDCl$_3$) δ 7.35 (s, 2H), 7.12 (s, 3H), 5.51 (s, 4H), 4.31 (s, 2H), 3.77 (s, 4H), 3.38 – 3.30 (m, 16H), 2.42 – 2.34 (m, 8H), 1.84 – 1.81 (m, 12H), 1.43 (s, 36H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 156.43, 144.61, 137.86, 136.92, 127.50, 126.90, 122.58, 79.42, 54.08, 53.49, 49.93, 46.96, 45.55, 43.99, 28.62, 27.28, 26.29. IR (KBr, cm$^{-1}$): 3433, 2976, 2929, 2097, 1682, 1484, 1412, 1368, 1164, 1044. HR-MS Calcd. for C$_{53}$H$_{88}$N$_{15}$O$_8$ (M+H)$^+$: 1062.6940, found: 1062.6906.

**11): 0.30 g, Yield: 26 %. M.p.: 52 °C-64 °C. **$^1$H NMR (400 MHz, CDCl$_3$) δ 7.34 (s, 1H), 7.26 (s, 1H), 7.21 (s, 2H), 5.56 (s, 2H), 4.36 (s, 4H), 3.77 (s, 2H), 3.61 – 3.03 (m, 8H), 2.72 – 2.19 (m, 4H), 2.19 – 1.53 (m, 6H), 1.43 (s, 18H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 156.38, 144.53, 137.48, 136.50, 127.94, 127.19, 122.42, 79.36, 54.21, 53.62, 49.93, 46.97, 45.55, 43.99, 28.58, 27.29, 26.27. IR (KBr, cm$^{-1}$): 2974, 2926, 2098, 1686, 1478, 1454, 1414, 1365, 1248, 1165, 1048. HR-MS Calcd. for C$_{31}$H$_{49}$N$_{12}$O$_4$ (M+H)$^+$: 653.4000, found: 653.4011.

2. Agarose gel electrophoresis (Time effects)

**Figure S1.** Agarose gel electrophoresis assay to investigate the pUC18 DNA condensation induced by different time of 3 in 50mM Tris-HCl buffer (pH = 7.4, 37 °C). [DNA] = 9 μg/mL, Lane 1, 8, 9: DNA control, 0, 4, 12 h; lane 2-7: [3]= 60 μM, 0.5, 1, 2, 3, 4, 12 h.
3. Dynamic light scattering

**Figure S2.** Hydrodynamic diameter distributions of pUC18 DNA particles condensed by 3-6 at different concentrations. The DNA concentration is 1 μg/mL.
4. EB displacement assay

**Figure S3.** Fluorescence quenching curves of EB bound CT-DNA by 1-6 in 5 mM Tris-HCl/50 mM NaCl (pH 7.4, $\lambda_{ex}=537$ nm, [EB] = 20 μM, [DNA] = 100 μM, 25.0 °C). The arrows show the intensity changes on increasing the concentration of the condensing agents.
5. Cellular uptake study

![Fluorescence microscope image](image)

**Figure S4.** Fluorescence microscope image (40×) of Hela cells transfected with FITC-DNA by NLS-free 4b at different DOPE ratios and concentrations. The concentration of FITC-DNA was 5 μg/dish. (a-d) [4b] = 60 μM, the 4b/DOPE ratios are 1:0, 2:1, 1:1, 1:2, respectively; (e-f) 4b/DOPE = 1:1, [4b] = 40, 60 μM, respectively; (g) NLS-plasmid assemblies control; (h) Lipofectamine 2000™.
6. References

7. Spectra of the compounds synthesized

7.1 Spectra data for compound 7

7.2 Spectra data for compound 8
7.3 Spectra data for compound 9
7.4 Spectra data for compound 10
### 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 = 3

#### Monoisotopic Mass, Even Electron Ions
140 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
- C: 0-55
- H: 0-100
- N: 0-15
- O: 0-8

#### TOF MS ES+

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7.5 Spectra data for compound 11
Elemental Composition Report

Single Mass Analysis
Tolerance = 2.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
246 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0.41  H: 0-50  N: 0-15  O: 0-5
VP-NiSSBOC 6 (0.102)
TOF MS ES+

7.27e+003

Minimum: 653.4011
Maximum: 654.4155

Mass Calc. Mass mDa PPM DBE i-FIT Formula
653.4011 653.4000 1.1 1.7 13.5 19.9 C31 H49 N12 O4
7.6 Spectra data for compound 12a

7.7 Spectra data for compound 12b
7.8 Spectra data for compound 1
7.9 Spectra data for compound 2Br-2
7.10 Spectra data for compound 2

![Spectra diagram]
7.11 Spectra data for compound 3Br-3

- 6.111
- 7.184
- 4.790
- 3.9577
- 3.788
- 3.321
- 2.922
- 2.2705
- 2.795
- 7.922
7.12 Spectra data for compound 3
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 = 3

Monoisotopic Mass, Even Electron Ions
328 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-40  H: 0-80  N: 0-10  O: 0-6

YP-PHT129N3HCL-130525 10 (0.170)
TOF MS ES+

Minimum:
Maximum:
Mass  Calc. Mass  mDa  PPM  DBE  i-FIT  Formula
628.5770  628.5781  -1.1  -1.7  5.5  108.0  C40 H74 N3 O2
628.5754  1.6  2.5  6.5  198.6  C36 H70 N9
7.13 Spectra data for compound 4Boc-4a
7.14 Spectra data for compound 4a
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 = 3

Monoisotopic Mass, Even Electron Ions
3445 formula(e) evaluated with 5 results within limits (up to 50 closest results for each mass)

Elements Used:
C: 0-70  H: 0-80  N: 0-20  O: 0-5  Br: 0-3

**6J-XDSC-13CL 5 (0.83)**
TOF MS ES+

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| 862.5317| 862.5317   | 0.5  | 0.6 | 21.5 | 8.1  | 2.34e+003
| 862.5327| 862.5327   | -0.5 | -0.6| 6.5  | n/a  | 2.34e+003
| 862.5312| 862.5312   | 1.0  | 1.2 | 28.5 | 25.12 | C60 H68 N3 O2
| 862.5343| 862.5343   | -2.1 | -2.4| 20.5 | 4.5  | C49 H68 N9 O5 |
7.15 Spectra data for compound 4Boc-4b

[Image of spectroscopic data with peaks and chemical structure]
7.16 Spectra data for compound 4b
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 = 3

Monoisotopic Mass, Even Electron Ions
3452 formula(e) evaluated with 5 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-70  H: 0-80  N: 0-20  O: 0-5  Br: 0-3

4J-I-DOS-HCL 4 (0.074)
TOF MS ES+

1.18e+00

Minimum: 10.0  3.0  -1.5
Maximum: 90.0

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7.17 Spectra data for compound 2Boc-5a
7.18 Spectra data for compound 5a

![Spectra data for compound 5a](image-url)
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 = 3

Monoisotopic Mass, Even Electron Ions
533 formula(e) evaluated with 4 results within limits (up to 50 closest results for each mass)

Elements Used:
- C: 0-50
- H: 0-80
- N: 0-15
- O: 0-10

G2F-1-49S 2 (0.037)
TOF MS/MS

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7.19 Spectra data for compound 2Boc-5b
7.20 Spectra data for compound 5b
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 = 3

Monoisotopic Mass, Even Electron ions
417 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-50  H: 0-80  N: 0-15  O: 0-10
QZF-2-XDS 6 (0.111)
TOF MS ES+

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Minimum: 10.0  Maximum: 5.0  Tolerance: 5.0 PPM  DBE: min = -1.5, max = 50.0

Page 1
7.21 Spectra data for compound 6
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 = 3

Monoisotopic Mass, Even Electron Ions
3442 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-70  H: 0-80  N: 0-20  O: 0-5  Br: 0-3

3CiOCK-MCl 4 (0.111)
TOF MS ES+

Minimum: 871.00  Maximum: 874.50

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