Supporting information

Effect of backbone flexibility on covalent template-directed synthesis of linear oligomers

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General experimental details.

All the reagents and materials used in the synthesis of the compounds described below were bought from commercial sources, without prior purification. Dry THF and CH$_2$Cl$_2$ were obtained from a solvent purification system (Pure Solv™, Innovative Technology, Inc.). Anhydrous DMF was purchased from Sigma-Aldrich. Thin layer chromatography was carried out using silica gel 60F (Merck) on glass plates. Flash chromatography was carried out on an automated system (Combiflash Rf+ or Combiflash Rf Lumen) using prepacked cartridges of silica (25μ PuriFlash® columns). All NMR spectroscopy was carried out on a Bruker 400 MHz DPX400, 400 MHz AVIII400, 500 MHz DCH cryoprobe or 500 MHz TCI Cryoprobe spectrometer using the residual solvent as the internal standard. All chemical shifts (δ) are quoted in ppm and coupling constants given in Hz. Splitting patterns are given as follows: s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet). FT-IR spectra were measured on a PerkinElmer Spectrum One spectrometer equipped with an ATR cell. Melting points were measured in a Mettler Toledo MP50 Melting Point System. UPLC analysis of samples was performed using Waters Acquity H-class UPLC coupled with a single quadrupole Waters SQD2. ACQUITY UPLC CSH C18 Column, 130Å, 1.7 μm, 2.1 mm X 50 mm was used as the UPLC column. The conditions of the UPLC method are as follows: gradients of water +0.1% formic acid (solvent A) and acetonitrile +0.1% formic acid (solvent B=) as specified in each case. Flow rate: 0.6 ml/min; Column temperature of 40°C; Injection volume of 2 µL. The signal was monitored at 254 nm. HRMS analysis was performed in a Waters LCT Premier equipped with a TOF mass analyser and W optics for enhanced resolution, using 50% aqueous acetonitrile with 0.25% formic acid as mobile phase. Compounds 2$^{51}$, 3$^{51}$, 7$^{53}$, 12$^{51}$, 16$^{52}$, 17$^{51}$ and 19$^{52}$ have been previously described.
Synthesis and characterization of described compounds

Compound 4.

To a solution of 3 (0.346 g, 0.84 mmol) in MeOH (5 mL) was added 2 M NaOH solution (1.09 mL, 2.19 mmol). The reaction was stirred overnight at room temperature and then the solution was carefully quenched with 5% dilute HCl and extracted with EtOAc (3x) followed by washing with H₂O and brine. The organic layer was dried over anhydrous MgSO₄ and concentrated under vacuum, affording the corresponding carboxylic acids (0.325 g, 97 %) as a light yellow solid. To this material, 4-((tert-butyldimethylsilyl)oxy)phenol (0.183 g, 0.82 mmol), EDC (0.172 g, 0.90 mmol) and DMAP (0.010 g, 0.08 mmol) were added and dissolved under N₂ atmosphere in dry CH₂Cl₂ (10 ml). The solution was stirred under N₂ atmosphere at room temperature for 1 h. The crude was diluted with EtOAc (20 mL) and washed with 5% aq. soln. HCl (3x), H₂O (1x) and brine. The organic phase was dried with MgSO₄ and concentrated in vacuo. Compound 4 was obtained as a yellow oil (0.493 g, quantitative; overall yield: 97%) as a clear oil.

¹H NMR (400 MHz, CDCl₃): δH = 8.24 (d, 2H, J = 8.0 Hz), 7.70 (bs, 2H), 7.07 (d, 2H, J = 9.0 Hz), 6.87 (d, 2H, J = 9.0 Hz), 4.51 and 4.17 (bs, 4H, splitting is due to the existence of rotamers around the amide bond; only a singlet would be expected in the absence of rotamers), 2.31 (s, 1H), 1.09 (m, 21H), 1.00 (s, 9H), 0.22 (s, 6H).

¹³C NMR (100.6 MHz, CDCl₃): δC = 172.3, 170.9, 153.7, 152.7, 144.8, 137.3, 130.5, 130.4, 127.4, 122.4, 120.8, 96.9, 85.1, 77.7, 72.7, 38.5, 33.5, 25.8, 19.5, 18.7, 11.3, -4.3.

HRMS (ES+): calcd for C₃₅H₅₀N₄O₄Si₂ 604.3278 [M+H]+, found 604.3280 [M+H]+.

FT-IR (ATR): νmax 2930, 2864, 1740, 1653, 1502, 1462, 1258, 1191, 1075, 915 and 775 cm⁻¹.
$^{1}$H-NMR (400 MHz, CDCl$_3$) Compound 4.
$^{13}\text{C-NMR (100.6 MHz, CDCl}_3\text{)}$ Compound 4.
Compound 5.

1,4-bis(azidomethyl)benzene\textsuperscript{4d} (0.1578 g, 8.39 mmol), Cu(CH\textsubscript{3}CN\textsubscript{4})PF\textsubscript{6} (0.044 g, 0.08 mmol) and TBTA (0.044 g, 0.08 mmol) were mixed in a round-bottom flask and, under N\textsubscript{2}, THF (120 mL) was added. A solution of 4 (0.506 g, 0.84 mmol) in THF (2 mL) was added and the reaction was stirred overnight at room temperature. The solvent was evaporated to dryness and the crude was purified by flash column chromatography on silica gel (gradient from 10\% to 100\% of EtOAc in Pet. Ether) to afford compound 5 (0.452 g, 68\%) as a foam, and the corresponding disubstituted compound (0.050 g, 8\%).

\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): \(\delta_H = 8.20 \text{ (d, 2H, } J = 8.5 \text{ Hz)}, 7.69 \text{ (d, 2H, } J = 8.5 \text{ Hz)}, 7.66 \text{ (s partially overlapped, 1H)}, 7.33 \text{ (m, 4H)}, 7.07 \text{ (d, 2H, } J = 9.0 \text{ Hz)}, 6.87 \text{ (d, 2H, } J = 9.0 \text{ Hz)}, 5.53 \text{ (s, 2H)}, 4.85 \text{ and 4.11 (bs, 4H), 4.36 (s, 2H), 1.10 (m, 21H), 0.99 (s, 9H), 0.21 (s, 6H).}

\textsuperscript{13}C NMR (125.7 MHz, CDCl\textsubscript{3}): \(\delta_C = 170.2, 164.8, 153.6, 144.8, 139.8, 136.4, 134.7, 131.4, 130.7, 130.6, 130.4, 129.1, 128.8, 127.5, 123.6, 122.4, 120.8, 101.9, 77.6, 54.4, 54.0, 40.6 \text{ and 40.3, 25.8, 18.8, 18.4, 11.3, -4.3.}

HRMS (ES\textsuperscript{+}): calcd for C\textsubscript{43}H\textsubscript{58}N\textsubscript{7}O\textsubscript{4}Si\textsubscript{2} 792.4089 [M+H]\textsuperscript{+}, found 792.4084 [M+H]\textsuperscript{+}.

FT-IR (ATR): \(\nu_{\text{max}} 2923, 2852, 2099, 1738, 1642, 1502, 1259, 1192 \text{ and 773 cm}^{-1}.\)
$^1$H-NMR (400 MHz, CDCl$_3$) Compound 5
$^{13}$C-NMR (125.7 MHz, CDCl$_3$) Compound 5.
Compound 6.

A solution of 5 (0.452 g, 0.57 mmol) in dry THF (4 mL) was treated at 0 °C with TBAF solution (1M in THF, 1.14 mL, 1.14 mmol). The reaction was stirred for 5 minutes and quenched with 5% soln. HCl and extracted with EtOAc (3x) followed by washing with H₂O and brine. The organic layer was dried over anhydrous MgSO₄ and concentrate under vacuum. The crude material was purified by flash column chromatography on silica gel (gradient from 10% to 100% of EtOAc in Pet. Ether) to afford compound 6 (0.284 g, 95%) as a light yellow solid.

**Melting point**: 154-156 °C.

**¹H NMR (400 MHz, CDCl₃)**: δ_H = 8.22 (d, 2H, J = 8.0 Hz), 7.66 (s partially overlapped, 1H), 7.64 (m, 2H), 7.34 (m, 4H), 7.07 (d, 2H, J = 9.0 Hz), 6.86 (d, 2H, J = 9.0 Hz), 5.54 (s, 2H), 5.20, 4.86, 4.67 and 4.10 (bs, 4H, splitting is due to the existence of rotamers around the amide bond; only two singlets would be expected in the absence of rotamers), 4.36 (s, 2H), 2.36 and 2.29 (bs, 1H).

**¹³C NMR (100.6 MHz, CDCl₃)**: δ_C = 170.4, 164.9, 154.0, 144.2, 143.9, 139.6, 132.5, 130.5, 129.1, 128.8, 127.6, 127.5, 126.0, 123.7, 122.6, 116.3, 78.3, 73.7, 54.4, 54.0. N-CH₂ peaks are not listed due to broadness caused by the existence of rotamers.

**HRMS (ES+)**: calcd for C₂₈H₂₄N₇O₄ 522.1890 [M+H]**, found 522.1891 [M+H]**.

**FT-IR (ATR)**: ν_max 2922, 2851, 2098, 1732, 1635, 1506, 1264, 1193, 1077 and 774 cm⁻¹.
$^1$H-NMR (400 MHz, CDCl$_3$) Compound 6.
$^{13}$C-NMR (100.6 MHz, CDCl$_3$) Compound 6.
Compound 7 (0.072 g, 0.06 mmol), compound 6 (0.065 g, 0.12 mmol), EDC (0.026 g, 0.14 mmol) and DMAP (0.001 g, 0.006 mmol) were dissolved under N₂ atmosphere in dry CH₂Cl₂ (3 ml). The solution was stirred overnight under N₂ atmosphere at room temperature. The crude was diluted with EtOAc (5 mL) and washed with 5% aq. soln. HCl (3x), H₂O (1x) and brine. The organic phase was dried with MgSO₄ and concentrated in vacuo. The crude material was purified by flash column chromatography on silica gel (gradient from 0% to 4% of MeOH in CH₂Cl₂) to afford pre-ZIP 8 (0.073 g, 54%) as a foam.

H NMR (400 MHz, CDCl₃): δH = 8.26 (m, 8H), 7.84 (d, 4H, J = 7.5 Hz), 7.77-7.67 (m, 8H), 7.65 and 7.58 (s, 2H, splitting is due to the existence of rotamers around the amide bond, only a singlet would be expected in the absence of rotamers), 7.44 (t, 2H, J = 1.5 Hz), 7.39-7.33 (m, 12H), 7.30 (s, 8H), 7.13 (d, 4H, J = 1.5 Hz), 5.54 (m, 12H), 4.89, 4.69 and 4.58 (bs, 12H, splitting is due to the existence of rotamers around the amide bond; only two singlets would be expected in the absence of rotamers), 4.39 (s, 4H), 4.39 and 4.12 (bs, 4H, splitting is due to the existence of rotamers around the amide bond; only a singlet would be expected in the absence of rotamers), 2.39 and 2.29 (bs, 2H, splitting is due to the existence of rotamers around the amide bond; only a singlet would be expected in the absence of rotamers), 1.33 (s, 36H).

C NMR (125.7 MHz, CDCl₃): δC = 170.7, 164.5, 152.1, 152.0, 148.6, 148.5, 144.2, 140.6, 135.5, 135.4, 133.7, 130.7, 130.6, 130.5, 129.1, 128.8, 128.1, 127.5, 124.0, 123.9, 123.6, 123.4, 123.1, 122.9, 122.8, 122.5, 78.4, 73.7, 55.1, 54.4, 54.0, 53.9, 44.2, 43.9, 40.2, 39.6, 35.1, 31.5.

HRMS (ES+): calcd for C₁₂₂H₁₁₉N₂₈O₁₂ 2167.9562 [M+H]+, found 2167.9568 [M+H]+.

FT-IR (ATR): νmax 2960, 2931, 2102, 1736, 1633, 1260, 1172, 1072 and 749 cm⁻¹.
$^{1}$H-NMR (400 MHz, CDCl$_3$) pre-ZIP 8.
$^{13}$C-NMR (125.7 MHz, CDCl$_3$) pre-ZIP 8.
COSY (CDCl\textsubscript{3}) pre-ZIP 8
Compound 10.

9^{62} (0.470 g, 1.86 mmol), EDC (0.464 g, 2.42 mmol) and DMAP (0.023 g, 0.19 mmol) were dissolved in dry CH₂Cl₂ (10 ml). Amine derivative 2 (0.532 g, 1.86 mmol) and TEA (0.260 mL, 1.86 mmol) were added under N₂ atmosphere and the solution was left stirring at room temperature for 2 h. The crude was diluted with EtOAc (50 mL) and washed with 5% aq. soln. HCl (3x), H₂O (1x) and brine. The organic phase was dried with MgSO₄ and concentrated in vacuo. The crude material was purified by flash column chromatography on silica gel (gradient from 0% to 10% of EtOAc in Pet. Ether) to afford 10 (0.855 g, 95 %) as a clear oil.

\(^1\)H NMR (400 MHz, CDCl₃): \(\delta_H = 7.51\) (d, 2H, \(J = 8.5\) Hz), 6.85 (d, 2H, \(J = 8.5\) Hz), 4.38 and 4.35 (bs, 4H, splitting is due to the existence of rotamers around the amide bond; only a singlet would be expected in the absence of rotamers), 2.27 (bs, 1H) 1.09 (m, 2H), 0.99 (s, 9H), 0.21 (s, 6H).

\(^{13}\)C NMR (100.6 MHz, CDCl₃): \(\delta_C = 170.7, 157.7, 129.2, 127.5, 119.9, 101.8, 78.5, 76.2, 72.4, 25.6, 18.6, 18.2, 11.1, -4.4.\) N-CH₂ peaks are not listed due to broadness caused by the existence of rotamers.

HRMS (ES+): calcd for C₂₈H₄₆NO₂Si₂: 484.3067 [M+H]^+, found 484.3062 [M+H]^+.

FT-IR (ATR): \(\nu_{max} 2941, 2864, 1650, 1605, 1510, 1253, 911\) and 841 cm\(^{-1}\).
$^1$H-NMR (400 MHz, CDCl$_3$) Compound 10.
$^{13}$C-NMR (100.6 MHz, CDCl$_3$) Compound 10.

- 170.72
- 157.73
- 129.23
- 127.51
- 119.94
- 101.81

- 78.48
- 77.32
- 77.01
- 76.69
- 76.30
- 72.43

- 39.44
- 34.88
- 25.62
- 18.58
- 16.22
- 11.14
- -4.40
Compound S1.

1-Azido-4-iodobenzene (0.109 g, 0.45 mmol), Pd(PPh₃)₄ (0.005 g, 0.006 mmol) and Cul (0.003 g, 0.013 mmol) were mixed under N₂ and THF/TEA 1:1 (5 mL) was added. Then, 10 (0.154 g, 0.32 mmol) dissolved in THF (1 mL) was added. After stirring at 30 °C for 2 h, the reaction was quenched with 0.1 N HCl soln. and extracted with EtOAc (2x). The combined organic phase was washed with 0.02 M EDTA soln. and brine, dried over MgSO₄ and the solvent evaporated. The crude material was purified by flash column chromatography on silica gel (gradient from 0% to 5% of EtOAc in Pet. Ether) to afford S1 (0.118 g, 62%) as a clear oil, recovering starting material 10 (0.015 g, 10%).

¹H NMR (400 MHz, CDCl₃): δ_H = 7.53 (d, 2H, J = 8.5 Hz), 7.43 (d, 2H, J = 8.5 Hz), 6.97 (d, 2H, J = 8.5 Hz), 6.86 (d, 2H, J = 8.5 Hz), 4.60 (bs, 1H), 4.37 (bs, 1H), 1.09 (m, 21H), 0.99 (s, 9H), 0.22 (s, 6H).

¹³C NMR (100.6 MHz, CDCl₃): δ_C = 170.9, 157.9, 161.0, 133.5, 129.4, 127.8, 125.1, 120.1, 119.1, 102.1, 88.7, 84.3, 80.1, 25.8, 18.8, 18.4, 11.3, -4.2. N-CH₂ peaks are not listed due to broadness caused by the existence of rotamers.

HRMS (ES−): calcd for C₃₄H₄₅N₄O₂Si₂ 601.3394 [M+H]⁺, found 601.3432 [M+H]⁺.

FT-IR (ATR): ν_max 2928, 2864, 2128, 2096, 1646, 1605, 1506, 1255, 912 and 840 cm⁻¹.
\[ ^1\text{H-NMR (400 MHz, CDCl}_3\text{) Compound S1.} \]
$^{13}$C-NMR (100.6 MHz, CDCl$_3$) Compound S1.
Compound 11.

Compound S1 (0.049 g, 0.08 mmol) was dissolved in dry THF (1 mL) under N$_2$ atmosphere. TBAF (1M in THF, 0.16 mL, 0.16 mmol) was added and the reactions stirred at room temperature for 15 min. THF was evaporated and the crude dissolved in EtOAc and washed with 0.1 N HCl soln. (3x), H$_2$O (1x) and brine. The obtained residue was dried under high vacuum for 3 h, yielding 11 as a light-yellow foam (0.027 g, quantitative).

$^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_H$ = 10.0 (bs, 1H), 7.51 (d, 2H, $J = 8.5$ Hz), 7.40 (d, 2H, $J = 8.5$ Hz), 7.13 (d, 2H, $J = 8.5$ Hz), 6.84 (d, 2H, $J = 8.5$ Hz), 4.46 (bs, 1H), 4.28 (bs, 1H), 3.37 (s, 1H).

$^1$H NMR (100.6 MHz, DMSO-$d_6$): $\delta_C$ = 169.9, 159.4, 139.9, 133.2, 129.2, 124.9, 119.5, 118.4, 115.1, 85.0, 83.1, 79.3, 75.3, 48.6.

HRMS (ES+): calcd for C$_{19}$H$_{15}$N$_{4}$O$_3$ 331.1195 [M+H]$^+$, found 331.1216 [M+H]$^+$.

FT-IR (ATR): $\nu_{\text{max}}$ 3255, 2922, 2852, 2130, 2098, 1631, 1601, 1514 and 1276 cm$^{-1}$. 
$^1$H-NMR (400 MHz, DMSO-d$_6$) Compound 11.
$^1$H-NMR (100.6 MHz, DMSO-$d_6$) Compound 11.
Compound 13.

![Chemical Structure of Compound 13](image)

1-Azido-4-iodobenzene (0.051 g, 0.21 mmol), Pd(PPh₃)₄ (2 mg, 0.002 mmol) and Cul (0.3 mg, 0.002 mmol) were mixed under N₂ and THF/TEA 1:1 (4 mL) was added. Then, 12 (0.086 g, 0.17 mmol) dissolved in THF (1 mL) was added. After stirring at 30 °C for 45 min, the reaction was quenched with 0.1 N HCl soln. and extracted with EtOAc (2x). The combined organic phase was washed with 0.02 M EDTA soln. and brine, dried over MgSO₄ and the solvent evaporated. The crude material was purified by flash column chromatography on silica gel (gradient from 0% to 40% of EtOAc in Pet. Ether) to afford compound 13 (0.089 g, 84%) as a brown oil.

$^1$H NMR (400 MHz, CDCl₃): $\delta_H = 8.09$ (d, 2H, $J = 7.5$ Hz), 7.62 (m, 3H), 7.42 (m, 3H), 7.10 (s, 2H), 6.99 (d, 2H, $J = 7.5$ Hz), 5.50 (s, 2H), 4.90, 4.67, 4.58 and 4.28 (bs, 4H, splitting is due to the existence of rotamers around the amide bond; only two singlets would be expected in the absence of rotamers), 3.93 (s, 3H), 1.30 (s, 18H).

$^{13}$C NMR (125.7 MHz, CDCl₃): $\delta_C = 170.2, 166.4, 151.9, 143.8, 140.6, 139.4, 133.7, 133.5, 132.9, 129.9, 127.3, 123.6, 123.0, 122.5, 119.2, 84.5, 84.0, 55.0, 52.5, 40.3, 34.5, 35.0, 31.5.

HRMS (ES+): calcd for C₃₆H₄₀N₇O₃ 618.3193 [M+H]+, found 618.3201 [M+H]+.

FT-IR (ATR): $\nu_{max}$ 2954, 2127, 2095, 1723, 1637, 1275, 1248, 1108 and 750 cm⁻¹.
$^1$H-NMR (400 MHz, CDCl$_3$) Compound 13.
$^{13}$C-NMR (125.7 MHz, CDCl$_3$) Compound 13.
Compound S2.

Compound 13 (0.064 g, 0.10 mmol) and compound 9 (0.052 g, 0.10 mmol) were dissolved in anhydrous THF (6 mL) under N\textsubscript{2} atmosphere. Then, TBTA (5 mg, 0.01 mmol) and Cu(CH\textsubscript{3}CN)\textsubscript{4}PF\textsubscript{6} (3.7 mg, 0.01 mmol) were dissolved in THF (2 mL) and this solution added to the reaction mixture. After 16 h of stirring at room temperature, the solvent was evaporated and the crude purified by flash column chromatography on silica gel (gradient from 10% to 100% of EtOAc in Pet. Ether) to afford S2 (0.113 g, 97 %) as a foam. A minor impurity is observed in the NMR spectra.

\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): \(\delta_{\text{H}} = 8.18 \text{ (m, 1H)}, 8.09 \text{ (m, 4H)}, 7.76 \text{ (d, 4H, } J = 8.0 \text{ Hz)}, 7.62 \text{ (m, 6H)}, 7.43 \text{ (s, 2H)}, 7.11 \text{ (s, 4H)}, 5.51 \text{ (s, 4H)}, 4.92, 4.77, 4.74, 4.65, 4.59, 4.33 \text{ (bs, 8H)}, 3.94 \text{ (s, 3H)}, 3.92 \text{ (s, 3H)}, 1.31 \text{ (s, 36H).}

\textsuperscript{13}C NMR (100.6 MHz, CDCl\textsubscript{3}): \(\delta_{\text{C}} = 176.1, 166.5, 166.5, 152.0, 143.8, 143.1, 133.7, 133.5, 1230.0, 127.8, 127.3, 125.1, 123.0, 122.5, 120.4, 96.1, 95.1, 55.1, 52.5, 44.5, 40.7, 39.2, 35.0, 34.5, 31.5.

HRMS (ES+): calcd for C\textsubscript{66}H\textsubscript{76}N\textsubscript{11}O\textsubscript{6} 1118.5980 [M+H]\textsuperscript{+}, found 1118.6074 [M+H]\textsuperscript{+}.

FT-IR (ATR): \(\nu_{\text{max}} = 2955, 1725, 1636, 1434, 1277, 1248 \text{ and 751 cm}^{-1}.\)
$^1$H-NMR (400 MHz, CDCl$_3$) Compound S2.
$^{13}$C-NMR (100.6 MHz, CDCl$_3$) Compound S2.
COSY (CDCl$_3$) Compound S2.
HSQC (CDCl₃) Compound S2
HMBC (CDCl₃) Compound S2
Compound 14.

Compound S2 (0.103 g, 0.09 mmol) was dissolved in MeOH/THF 1:1 (2 mL) and 2 N NaOH soln. (0.275 mL, 0.55 mmol) was added. After 16 h of stirring at room temperature, the solvent was evaporated and crude dissolved in H2O and acidified with 1 N HCl soln. to pH 2-3. The aqueous phase was extracted with EtOAc (3x) and the combined organic phase was washed with brine, dried over MgSO4 and evaporated to dryness. The obtained yellow solid, 14 (0.100 g, quantitative), was used without further purification.

Melting point: 146-148 °C.

1H NMR (400 MHz, CDCl3): δH = 8.34 (m, 1H), 8.09 (m, 4H), 7.74 (m, 6H), 7.65 (d, 2H, J = 7.5 Hz), 7.53 (d, 2H, J = 7.5 Hz), 7.42 (s, 2H), 7.12 (s, 4H), 5.52 (s, 4H), 4.95, 4.81, 4.70, 4.65 and 4.33 (bs, 8H, splitting is due to the existence of rotamers around the amide bond; only three singlets would be expected in the absence of rotamers), 1.29 (s, 36H).

13C NMR (100.6 MHz, CDCl3): δC = 176.4, 171.4, 169.8, 166.6, 133.5, 133.4, 133.4, 130.5, 130.5, 127.7, 127.4, 123.1, 123.1, 122.6, 120.3, 85.6, 82.9, 55.1, 35.0, 33.8, 31.5. N-CH2 peaks are not listed due to broadness caused by the existence of rotamers.

HRMS (ES+): calcd for C64H72N11O8 1090.5667 [M+H]+, found 1090.5776 [M+H]+.

FT-IR (ATR): νmax 2962, 1713, 1635, 1603, 1247 and 735 cm⁻¹.
$^1$H-NMR (400 MHz, CDCl$_3$) Compound 14.
$^{13}$C-NMR (100.6 MHz, CDCl$_3$) Compound 14.
Pre-ZIP 15.

A solution of 14 (0.078 g, 0.072 mmol), 11 (0.052 g, 0.158 mmol), EDC (0.041 g, 0.215 mmol) and DMAP (3 mg, 0.022 mmol) in dry CH\textsubscript{2}Cl\textsubscript{2} (5 mL) under N\textsubscript{2} atmosphere was stirred at room temperature for 16 h. The reaction was diluted with EtOAc (5 mL) and washed with 0.1 N HCl soln. (3x), H\textsubscript{2}O (1x) and brine. The organic phase was dried with MgSO\textsubscript{4} and concentrated in vacuo. The crude material was purified by flash column chromatography on silica gel (gradient from 0% to 4% of MeOH in CH\textsubscript{2}Cl\textsubscript{2}) to afford 15 (0.086 g, 70 %) as a light-yellow foam.

\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): \(\delta_H = 8.24\) (m, 6H), 7.98 (m, 2H), 7.86 (d, 2H, \(J = 8.0\) Hz), 7.73 (m, 9H), 7.60 (m, 3H), 7.43 (m, 5H), 7.31 (m, 4H), 7.12 (s, 4H), 6.97 (d, 2H, \(J = 8.5\) Hz), 5.52 (s, 4H), 4.94-4.37 (m, 16H), 2.36 and 2.04 (bs, 2H), 1.30 (s, 36H).

\textsuperscript{13}C NMR (100.6 MHz, CDCl\textsubscript{3}): \(\delta_C = 170.8, 170.0, 164.1, 164.0, 152.4, 151.9, 144.5, 143.6, 142.8, 140.6, 140.5, 136.7, 133.6, 133.4, 132.4, 130.6, 130.5, 129.0, 128.1, 123.0, 122.42, 122.0, 120.3, 119.1, 85.5, 84.1, 84.0, 83.6, 78.3, 72.9, 55.0, 44.3, 40.3, 39.6, 38.4, 35.0, 31.5.

HRMS (ES\textsuperscript{+}): calcd for C\textsubscript{102}H\textsubscript{86}N\textsubscript{19}O\textsubscript{8} 1714.7684 [M+H]\textsuperscript{+}, found 1714.7640 [M+H]\textsuperscript{+}.

FT-IR (ATR): \(\nu_{\text{max}} 2964, 2127, 2095, 1739, 1635, 1249, 1200\) and 747 cm\textsuperscript{-1}. 
$^1$H-NMR (400 MHz, CDCl$_3$) Compound 15.
$^{13}$C-NMR (100.6 MHz, CDCl$_3$) Compound 15.
Compound S3.

17 (0.035 g, 0.042 mmol), Cu(CH\textsubscript{3}CN)\textsubscript{4}PF\textsubscript{6} (0.002 g, 0.006 mmol) and TBTA (0.003 g, 0.006 mmol) were mixed in a round-bottom flask and, under N\textsubscript{2}, THF (2 mL) was added. A solution of 1-(azidomethyl)-3,5-di-tert-butylbenzene\textsuperscript{S6} (0.016 g, 0.063 mmol) in THF (1 mL) was added and the reaction was stirred overnight at room temperature. The solvent was evaporated to dryness and the crude was purified by flash column chromatography on silica gel (gradient from 5% to 85% of EtOAc in Pet. Ether) to afford S3 (0.043 g, 94%) as a foam.

\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): \(\delta\textsubscript{H} = 8.08\) (m, 3H), 7.85 (d, 2H, \(J = 8.0\) Hz), 7.74 (d, 2H, \(J = 8.0\) Hz), 7.70 (s, 1H), 7.60 (t, 2H, \(J = 7.5\) Hz), 7.68 and 7.53 (s, 1H, splitting is due to the existence of rotamers around the amide bond; only a singlet would be expected in the absence of rotamers), 7.42 (m, 1H), 7.40 (m, 1H), 7.32 (m, 4H), 7.10 (s, 2H), 7.08 and 7.07 (s, 2H, splitting is due to the existence of rotamers around the amide bond; only a singlet would be expected in the absence of rotamers), 4.61 and 4.55 (s, 2H, splitting is due to the existence of rotamers around the amide bond; only a singlet would be expected in the absence of rotamers), 3.91 (s, 3H), 3.85 (s, 3H), 1.30 (s, 18H), 1.28 (s, 18H).

\textsuperscript{13}C NMR (100.6 MHz, CDCl\textsubscript{3}): \(\delta\textsubscript{C} = 169.3, 166.4, 166.2, 152.0, 151.9, 143.8, 139.6, 139.5, 133.8, 133.7, 131.61, 129.9, 129.4, 128.8, 127.8, 124.0, 122. 9, 122.5, 122.3, 121.2, 55.0, 54.9, 52.4, 46.3, 44.2, 35.0, 31.5. N-CH\textsubscript{2} peaks are not listed due to broadness caused by the existence of rotamers.

HRMS (ES\textsuperscript{+}): calcd for C\textsubscript{63}H\textsubscript{74}N\textsubscript{11}O\textsubscript{6} 1080.5824 [M+H]\textsuperscript{+}, found 1080.5872 [M+H]\textsuperscript{+}.

FT-IR (ATR): \(\nu\textsubscript{max} 2954, 2926, 2864, 1725, 1642, 1519, 1278, 1249, 1110\) and 755 cm\textsuperscript{-1}.
\[ ^1\text{H-NMR (400 MHz, CDCl}_3\text{)} \text{ Compound S3.} \]
$^{13}$C-NMR (100.6 MHz, CDCl$_3$) Compound S3.
Compound 18.

Compound S1 (0.059 g, 0.054 mmol) was dissolved in THF/MeOH/H$_2$O 2.5:1:1 (2 mL) and LiOH (0.011 g, 0.273 mmol) was added. After 6 h of stirring at room temperature, the crude diluted with H$_2$O and acidified with 1 M HCl soln. to pH 2-3. The aqueous phase was extracted with EtOAc (3x) and the combined organic phase was washed with brine, dried over MgSO$_4$ and evaporated to dryness. The obtained yellow solid 18 (0.052 g, 91%) was used without further purification.

$^1$H NMR (500 MHz, DMSO-$d_6$): $\delta_H = 8.68$ and 850 (bs, 1H, splitting is due to the existence of rotamers around the amide bond; only a singlet would be expected in the absence of rotamers), 8.23 (s, 1H), 8.19 (s, 1H), 7.91 (bs, 2H), 7.76 (d, 2H, $J = 7.5$ Hz), 7.70 (bs, 2H), 7.53 and 7.38 (bs, 2H, splitting is due to the existence of rotamers around the amide bond; only a singlet would be expected in the absence of rotamers), 7.32 (m, 6H), 7.13 (s, 2H), 7.06 (s, 2H), 5.56 (s, 2H), 5.3 (s, 2H), 5.16 (s, 2H), 4.74, 4.66 and 4.49 (s, 4H, splitting is due to the existence of rotamers around the amide bond; only two singlets would be expected in the absence of rotamers), 1.24 (s, 18H), 1.19 (s, 18H).

$^{13}$C NMR (126 MHz, DMSO-$d_6$): $\delta_C = 170.4, 169.2, 150.8, 150.7, 144.2, 143.1, 142.8, 137.9, 135.4, 135.3, 134.4, 129.3, 129.2, 129.0, 128.7, 127.8, 125.9, 124.0, 123.9, 121.9, 121.7, 121.5, 120.3, 53.3, 53.2, 44.9, 43.8, 39.5, 34.5, 34.4, 31.2, 31.1.

HRMS (ES+): calcld for C$_{61}$H$_{70}$N$_{11}$O$_6$ 1052.5511 [M+H]$^+$, found 1052.5493 [M+H]$^+$.

FT-IR (ATR): $\nu_{\text{max}}$ 2956, 2924, 2863, 1713, 1636, 1600, 1519, 1407, 1249, 1049 and 1025 cm$^{-1}$.
$^1$H NMR (500 MHz, DMSO-$d_6$) Compound 18.
$^{13}$C NMR (126 MHz, DMSO-$d_6$) Compound 18.
A solution of 18 (0.052 g, 0.050 mmol), 19 (0.044 g, 0.149 mmol), EDC (0.028 g, 0.149 mmol) and DMAP (5 mg, 0.045 mmol) in dry CH\(_2\)Cl\(_2\) (5 mL) under N\(_2\) atmosphere was stirred at room temperature for 16 h. The reaction was diluted with EtOAc (5 mL) and washed with 0.1 N HCl soln. (3x), H\(_2\)O (1x) and brine. The organic phase was dried with MgSO\(_4\) and concentrated in vacuo. The crude material was purified by flash column chromatography on silica gel (gradient from 0% to 4% of MeOH in CH\(_2\)Cl\(_2\)) to afford 20 (0.025 g, 31%) as a light-yellow foam. This molecule is not stable upon long-term storage, due to azide-alkyne cycloaddition reaction occurring even in the absence of copper (I) catalysts, yielding a mixture of regioisomers. NMR spectra show the existence of minor impurities corresponding to these adducts.

\(^1\text{H NMR (400 MHz, CDCl}_3\text{):}\) \(\delta_H = 8.17\ (m, 3H), 7.95\ (m, 2H), 7.82\ (m, 2H), 7.71\ (s, 1H), 7.69\) and 7.56 (s, 1H, splitting is due to the existence of rotamers around the amide bond; only a singlet would be expected in the absence of rotamers), 7.63 (m, 2H), 7.40 (m, 10H), 7.15 (d, 2H, \(J = 8.5\) Hz), 7.10 (m, 8H), 7.03 (d, 2H, \(J = 8.5\) Hz), 6.95 (d, 2H, \(J = 8.5\) Hz), 6.91 (d, 2H, \(J = 8.5\) Hz), 5.52 (m, 4H), 5.15 (s, 2H), 4.72-4.57 (m, 8H, some splitting is due to the existence of rotamers around the amide bond), 2.26 (m, 2H), 1.30 (s, 18H), 1.28 (s, 18H).

\(^{13}\text{C NMR (100.6 MHz, CDCl}_3\text{):}\) \(\delta_C = 170.8, 170.7, 169.2, 169.1, 164.0, 163.7, 152.1, 152.0, 151.9, 144.5, 143.7, 142.9, 140.5, 140.4, 139.5, 139.4, 139.4, 135.4, 135.3, 133.8, 133.7, 132.7, 130.6, 130.6, 130.5, 130.0, 129.3, 129.0, 128.9, 128.1, 124.0, 123.1, 123.1, 123.0, 122.5, 122.4, 121.3, 121.2, 120.1, 120.0, 78.8, 72.8, 55.1, 55.0, 46.4, 44.2, 43.8, 40.1, 39.6, 39.4, 35.0, 31.5.

**HRMS (ES\(^+\)):** calcd for C\(_{93}\)H\(_{90}\)N\(_{19}\)O\(_8\) 1601.7253 [M+H]\(^+\), found 1601.7174 [M+H]\(^+\).

**FT-IR (ATR):** \(\nu_{\text{max}}\) 2960, 2930, 2865, 2123, 2095, 1642, 1505, 1260, 1201, 1067 and 750 cm\(^{-1}\).
$^1$H-NMR (400 MHz, CDCl$_3$) Compound 20.
$^\text{13C-NMR (100.6 MHz, CDCl$_3$)}$ Compound 20.
Cyclic duplex 22.

Compound 8 (25 mg, 0.012 mmol) was dissolved, under N₂, in dry THF (50 mL). Cu(CH₃CN)₄PF₆ (4 mg, 0.012 mmol) and TBTA (6 mg, 0.012 mmol) were added and the reaction was stirred overnight at room temperature. The reaction was diluted with EtOAc and washed with 0.02 M NaEDTA neutral aq. soln.(2x) and brine (1x). The organic layer was dried over MgSO₄ and the solvent evaporated to dryness. The crude was purified by flash column chromatography on silica gel (gradient from 0% to 15% of MeOH in CH₂Cl₂) to afford compound 22 (14 mg, 57%) as a white solid.

**Melting point:** 201-202 °C.

**¹H NMR (500 MHz, CD₃CN):** δH = 8.13 (bs, 8H), 7.82-7.54 (m, 18H), 7.43 (s, 2H), 7.32-7.23 (m, 18H), 7.11 (s, 4H), 5.48 (m, 16 H), 4.63-4.43 (bs, 16H), 1.27 (s, 36H).

**¹³C NMR (125.7 MHz, CD₃CN):** δC = 171.2, 171.1, 165.5, 165.5, 152.6, 149.6, 145.0, 144.7, 144.1, 142.3, 137.1, 136.2, 136.1, 131.3, 131.1, 129.8, 129.5, 129.4, 128.3, 124.4, 124.2, 123.9, 123.4, 123.4, 55.0, 54.0, 53.9, 45.0, 44.9, 40.8, 40.7, 35.6, 31.6.

**HRMS (ES+):** calcd for C₁₂₂H₁₁₉N₂₈O₁₂ 2167.9562 [M+H]^+, found 2167.9565 [M+H]^+.

**FT-IR (ATR):** νmax 3017, 2961, 1736, 1633, 1262, 1217, 1174 and 751 cm⁻¹.
$^1$H-NMR (500 MHz, CD$_3$CN) Compound 22.
$^{13}$C-NMR (125.7 MHz, CD$_3$CN) Compound 22.
COSY (CD$_3$CN) Compound 22.
HSQC (CD$_3$CN) Compound 22.
HMBC (CD₃CN) Compound 22.
Pre-ZIP 15 (10 mg, 5.8·10⁻³ mmol) was dissolved, under N₂, in dry THF (120 mL). Cu(CH₃CN)₄PF₆ (4 mg, 0.012 mmol) and TBTA (6 mg, 0.012 mmol) were added and the reaction was stirred overnight at room temperature. The solvent was evaporated and the reaction mixture was purified by flash column chromatography on silica gel (gradient from 0% to 5% of MeOH in CH₂Cl₂). The fractions corresponding to the product were combined, dried and filtered through a pad of silica using EtOAc as solvent, to remove traces of copper. Cyclic duplex 25 (7 mg, 70%) was afforded as a white solid, containing TBTA as a minor impurity.

^1H NMR (500 MHz, DMSO-d₆): δH = 8.84 (m, 3H), 8.29 (s, 1H), 8.26 (s, 1H), 8.24 (m, 4H), 7.91 (m, 2H), 7.85 (m, 5H), 7.74 (m, 3H), 7.60 (d, 2H, J = 8.0 Hz), 7.50 (m, 8H), 7.35 (m, 2H), 7.16 (s, 2H), 7.12 (m, 6H), 5.59 (s, 2H), 5.58 (s, 2H), 5.25-4.19 (m, 16H), 1.23 (s, 18H).

^13C NMR (125.7 MHz, DMSO-d₆): δC = 169.7, 169.6, 169.4, 169.2, 163.7, 163.7, 151.5, 151.4, 150.7, 143.7, 143.0, 143.0, 141.0, 140.5, 136.2, 135.9, 135.8, 133.0, 132.2, 132.0, 130.2, 130.0, 129.6, 128.7, 128.0, 127.7, 127.1, 124.2, 124.1, 124.0, 122.0, 121.9, 121.7, 120.0, 119.1, 87.9, 87.9, 83.5, 53.3, 47.1, 43.7, 40.8, 40.1, 39.6, 39.5, 34.5, 34.5, 31.2, 31.1.

HRMS (ES+): calcld for C₁₀₂H₉₅N₁₉O₈Na 1736.7509 [M+Na]^+, found 1736.7529 [M+Na]^+.

FT-IR (ATR): νmax 2956, 2925, 2856, 1740, 1639, 15118, 1455, 1260, 1204 and 841 cm⁻¹.
$^1$H-NMR (500 MHz, DMSO-$d_6$) Compound 25.
$^{13}$C NMR (125.7 MHz, DMSO-$d_6$) Compound 25.
COSY (DMSO-\textit{d}_6) Compound 25.
HSQC (DMSO-\textit{d}_6) Compound 25.
HMBC (DMSO-\textit{d}_6) Compound 25.
Synthesis of trimer pre-ZIP S7 (backbone B)

Compound S4 was synthesized in moderate yield by palladium-catalysed coupling of 3 and 4-azido-1-iodobenzene (Scheme S1). The CuAAC reaction of two equivalents of S4 with methyl 4-(di(prop-2-yn-1-yI)carbamoyl)benzoate\(^{[53]}\) yielded 3-mer S5 in very good yield. Hydrolysis of the methyl ester, followed by cleavage of the alkyne protecting group and capping with 1-(azidomethyl)-3,5-di-tert-butylbenzene afforded 3-mer template S6. The coupling of S6 with three equivalents of 11 gave pre-ZIP S7 in good yield.

**Scheme S1**
Compound S4.

1-Azido-4-iodobenzene (0.278 g, 1.14 mmol), Pd(PPh₃)₄ (0.012 g, 0.001 mmol) and CuI (0.002 g, 0.001 mmol) were mixed under N₂ and THF/TEA 1:1 (5 mL) was added. Then, 3 (0.425 g, 1.03 mmol) dissolved in THF (1 mL) was added. After stirring at room temperature for 2 h, the reaction was quenched with 0.1 N HCl soln. and extracted with EtOAc (2x). The combined organic phase was washed with 0.02 M EDTA soln. and brine, dried over MgSO₄ and the solvent evaporated. The crude material was purified by flash column chromatography on silica gel (gradient from 0% to 15% of EtOAc in Pet. Ether) to afford S4 (0.300 g, 55%) as a clear oil, recovering starting material 1 (0.085 g, 20%).

¹H NMR (400 MHz, CDCl₃): δH = 8.10 (d, 2H, J = 8.0 Hz), 7.66 (d, 2H, J = 8.0 Hz), 7.43 (d, 2H, J = 8.0 Hz), 6.97 (d, 2H, J = 8.0 Hz), 4.72 and 4.60 (bs, 2H, splitting is due to the existence of rotamers around the amide bond; only a singlet would be expected in the absence of rotamers), 4.38 and 4.19 (bs, 2H, splitting is due to the existence of rotamers around the amide bond; only a singlet would be expected in the absence of rotamers), 3.94 (s, 3H), 1.09 (s, 21H).

¹³C NMR (100.6 MHz, CDCl₃): δC = 169.9, 166.4, 161.0, 133.5, 131.9, 130.0, 127.3, 125.1, 119.2, 101.4, 87.3, 83.7, 79.3, 52.5, 39.5, 34.7, 18.7, 11.3.

HRMS (ES+): calcd for C₃₀H₃₇N₄O₃Si 529.2635 [M+H]⁺, found 529.2614 [M+H]⁺.

FT-IR (ATR): νmax 2923, 2865, 2130, 2094, 1729, 1649 and 1278 cm⁻¹.
$^1\text{H-NMR (400 MHz, CDCl}_3\text{)}$ Compound S4.
$^{13}$C-NMR (100.6 MHz, CDCl$_3$) Compound S4.
Compound S5.

S4 (0.408 g, 0.77 mmol) and methyl 4-((di(prop-2-yn-1-yl)carbamoyl)benzoate[53] (0.099 g, 0.39 mmol) were dissolved in anhydrous THF (5 mL) under N₂ atmosphere. Then, TBTA (20 mg, 0.04 mmol) and Cu(CH₃CN)₂PF₆ (15 mg, 0.04 mmol) were dissolved in THF (2 mL) and this solution added to the reaction mixture. After 16 h of stirring at room temperature, the solvent was evaporated and the crude purified by flash column chromatography on silica gel (gradient from 0% to 70% of EtOAc in Pet. Ether) to afford S5 (0.431 g, 85 %) as a foam.

¹H NMR (400 MHz, CDCl₃): δ_H = 8.23 and 8.14 (bs, 2H, splitting is due to the existence of rotamers around the amide bond; only a singlet would be expected in the absence of rotamers), 8.11 (d, 6H, J = 8.5 Hz, 3-H), 7.81 (d, 2H, J = 8.0 Hz, 2-H), 7.75 (d, 4H, J = 8.5 Hz, 2-H), 7.68 (m, 4H, 2'-H), 7.63 (m, 4H, 3'-H), 4.84, 4.76, 4.71, 4.62, 4.41 and 4.33 (bs, 12H, N-CH₂, splitting is due to the existence of rotamers around the amide bond; only three signa would be expected in the absence of rotamers), 3.95 (s, 6H, OCH₃), 3.93 (s, 3H, OCH₃), 1.10 (s, 42H, TIPS).

¹³C NMR (100.6 MHz, CDCl₃): δ_C = 171.2, 170.0, 166.4, 139.5, 139.2, 133.5, 132.0, 131.8, 130.0, 127.9, 127.3, 122.2, 121.3, 120.3, 101.2, 86.8, 85.6, 83.1, 52.6, 44.8, 44.4, 39.9, 39.6, 34.9, 18.8, 11.3.

HRMS (ES+): calcd for C₇₅H₈₆N₉O₉Si₂ 1312.6087 [M+H]⁺, found 1312.5963 [M+H]⁺.

FT-IR (ATR): ν_max 2944, 2864, 1723, 1638, 1434, 1276, 1248 and 751 cm⁻¹.
H-NMR (400 MHz, CDCl₃) Compound S5.

\[ \text{S5} \]
$^{13}$C-NMR (100.6 MHz, CDCl$_3$) Compound S5.
Compound S6.

To a solution of S5 (0.134 g, 0.10 mmol) in MeOH (2.5 mL) was added NaOH 2 N solution (0.500 mL, 1.00 mmol). The reaction was stirred overnight at room temperature and then the solution was carefully quenched with 5% dilute HCl and extracted with EtOAc (3x) followed by washing with H2O and brine. The organic layer was dried over anhydrous MgSO4 and concentrate under vacuum, affording carboxylic acid 3-mer template (0.130 g, quantitative), used without further purification. This derivative was dissolved in THF (2 mL) and treated with TBAF solution (1 M in THF, 0.210 mL, 0.210 mmol). The reaction was stirred for 48h and quenched with 5% soln. HCl and extracted with EtOAc (3x) followed by washing with H2O and brine. The organic layer was dried over anhydrous MgSO4 and concentrate under vacuum. The crude was dissolved in dry THF (5 mL) and Cu(CH3CN)4PF6 (0.008 g, 0.02 mmol) and TBTA (0.011 g, 0.02 mmol) were added. A solution of 1-(azidomethyl)-3,5-di-tert-butylbenzene (0.075 g, 0.31 mmol) in THF (2 mL) was added and the reaction was stirred overnight at room temperature. The solvent was evaporated to dryness and the crude was purified by flash column chromatography on silica gel (gradient from 0% to 10% of MeOH with 1% AcOH in CH2Cl2) to afford S6 (0.131 g, 89%) as a light-yellow solid.

Melting point: 159-160 °C.

1H NMR (400 MHz, CD3OD): δH = 8.44 (m, 2H), 8.07 (m, 6H), 7.72 (m, 4H), 7.60 (d, 4H, J = 8.0 Hz), 7.46 (d, 4H, J = 8.5 Hz), 7.38 (s, 2H), 7.31 (m, 4H), 7.15 (s, 4H), 7.15 (m, 4H), 5.55 (m, 4H), 4.72, 4.57, 4.30 and 3.75 (bs, 12H, partially overlapped by solvent signal), 1.22 (s, 36H).

13C NMR (100.6 MHz, CD3OD): δC = 173.3, 168.9, 152.8, 140.8, 136.7, 135.9, 134.2, 131.1, 130.7, 130.0, 129.6, 129.0, 128.4, 128.1, 125.7, 123.5, 123.3, 121.3, 121.3, 86.4, 84.9, 55.5, 54.9, 45.8, 44.5, 41.8, 41.4, 36.2, 35.7, 31.8.

HRMS (ES+): calcd for C84H86N15O9 1449.6766 [M+H]+, found 1449.6733 [M+H]+.

FT-IR (ATR): νmax 2959, 2925, 2854, 1711, 1634, 1258, 1047 and 753 cm⁻¹.
$^1$H-NMR (400 MHz, CD$_3$OD) Compound S6.
$^{13}$C-NMR (100.6 MHz, CD$_3$OD) Compound S6.
A solution of S6 (0.026 g, 0.018 mmol), 11 (0.016 g, 0.048 mmol), EDC (0.013 g, 0.069 mmol) and DMAP (0.8 mg, 0.007 mmol) in dry CH2Cl2 (2 mL) under N2 atmosphere was stirred at room temperature for 16 h. The reaction was diluted with EtOAc (5 mL) and washed with 0.1N HCl soln. (3x), H2O (1x) and brine. The organic phase was dried with MgSO4 and concentrated in vacuo. The crude material was purified by flash column chromatography on silica gel (gradient from 0% to 4% of MeOH in CH2Cl2) to afford S7 (0.033 g, 77 %) as a light-yellow foam.

$^1$H NMR (400 MHz, CDCl3): $\delta_H = 8.28$ (m, 8H), $7.93$ (d, 2H, $J = 8.5$ Hz), $7.78$ (m, 6H), $7.70$ (m, 10H), $7.63$ (m, 6H), $7.44$ (m, 7H), $7.32$ (m, 7H), $7.12$ (s, 4H), $6.98$ (d, 4H, $J = 8.0$ Hz), $5.53$ (s, 4H), $4.97$-$4.21$ (m, 24H), $2.35$ (m, 3H), $1.31$ (s, 36H).

$^{13}$C NMR (100.6 MHz, CDCl3): $\delta_C = 171.0$, 170.1, 170.0, 164.2, 164.1, 152.5, 152.0, 144.5, 143.7, 140.6, 140.5, 133.7, 133.5, 132.6, 132.6, 130.7, 129.1, 128.2, 127.6, 123.7, 123.1, 122.5, 122.1, 120.4, 119.2, 85.7, 84.0, 83.9, 83.7, 55.1, 40.3, 37.5, 35.1, 33.9, 33.5, 31.5.

HRMS (ES+): calcd for C141H123N27O12 1193.4939 [M+2H]$^2+$, found 1193.4987 [M+2H]$^2+$.

FT-IR (ATR): $\nu_{max}$ 2957, 2923, 2853, 2127, 2096, 1739, 1639, 1526, 1454, 1428, 1380, 1307, 1237, 1223, 1192, 1154, 1101, 1040, 755 cm$^{-1}$. 

S71
$^1$H-NMR (400 MHz, CDCl$_3$) Pre-ZIP S7.
$^{13}$C-NMR (100.6 MHz, CDCl$_3$) Pre-ZIP S7.
CuAAC reaction of pre-ZIP 8.

Scheme S2 shows the possible products for the CuAAC reaction of pre-ZIP 8 performed at high dilution. Firstly, the linear duplex 21 must be formed and then cyclised to form cyclic duplex 22.

The reaction was performed as follow: Compound 8 (25 mg, 0.012 mmol) was dissolved, under N₂ atmosphere, in dry THF (50 mL). Cu(CH₃CN)₂PF₆ (4 mg, 0.012 mmol) and TBTA (6 mg, 0.012 mmol) were added and the reaction was stirred at room temperature under N₂ atmosphere. Aliquots of the reaction were taken at different times, diluted with EtOAc and washed with 0.2 M EDTA soln. and then analysed by UPLC to assess the progress of the reaction (Figure S1). After 21 h of reaction, the solution was diluted with EtOAc and washed with 0.02 M NaEDTA neutral aq. soln.(2x) and brine (1x). The organic layer was dried over MgSO₄ and the solvent evaporated to dryness. The crude was purified by flash column chromatography on silica gel (gradient from 0% to 15% of MeOH in CH₂Cl₂) to afford compound 22 (14 mg, 57%) as a white solid.
Figure S1. A. UPLC traces for the CuAAC reaction of 1,4-bis(triazolyl)methylbenzene derivative 8 (250 µM of 8 and Cu-TBTA in THF) after 3h and 21h. Conditions: C18 column at 40 °C (254 nm) using water + 0.1% formic acid (A) and CH₃CN + 0.1% formic acid (B) with flow rate of 0.6 ml/min; Gradient of 0-3 minutes 5%-95%B + 2 minutes 95%B. B. Mass spectra of 22.

Compound 22 was fully characterised by ¹H NMR, ¹³C NMR, IR and HRMS (see page S91). Key evidence of the formation of a cyclic product was the absence of acetylene protons in ¹H NMR and azide stretching bands in IR, when compared to the starting material 8 (Figure S2).

Figure S2. A. Partial ¹H NMR (400 MHz, CDCl₃) of pre-ZIP 8 (top) and cyclic duplex 22 (bottom), showing the absence of acetylene protons in the latter. B. FT-IR (ATR) of pre-ZIP 8 (top) and cyclic duplex 22 (bottom), showing the absence of azide stretching band in the latter.
As shown in Scheme S3, basic hydrolysis of 22 provided template 7 and cyclic 2-mer 23. The reaction was performed as follow: A solution of 22 (5 mg, 2.3·10⁻³ mmol) in MeOH (2 mL) was treated with 2 M NaOH solution (0.020 mL, 4.6·10⁻² mmol). After 16 h of stirring at room temperature, the solution was acidified with 1 M HCl soln. to pH 2-3 and extracted with EtOAc (3x). The combined organic phase was washed with brine, dried over MgSO₄ and evaporated to dryness. The obtained residue was analysed without further purification, corresponding to a mixture of cyclic-2-mer 23 and template 7 (Figure S3).
Figure S3. A. UPLC traces of cyclic duplex 22 (top) and crude of the hydrolysis of 22 (bottom). Conditions: C18 column at 40 °C (254 nm) using water + 0.1% formic acid (A) and CH$_3$CN + 0.1% formic acid (B) with flow rate of 0.6 ml/min; Gradient of 0-3 minutes 5%-95%B + 2 minutes 95% B. B. Mass spectra of cyclic 2-mer 23. C. Mass spectra of template 8.
**CuAAC reaction of pre-ZIP 15.**

Scheme S4 shows the possible products for the CuAAC reaction of pre-ZIP 15 performed at high dilution. Firstly, the linear duplex 24 must be formed both in the parallel and antiparallel arrangement as the backbone is directional. Then, further CuAAC reaction of 24 would afford cyclic duplex 25.

The reaction was performed as follow: Compound 15 (10 mg, 5.8·10⁻³ mmol) was dissolved, under N₂ atmosphere, in dry THF (120 mL). Cu(CH₃CN)₄PF₆ (2 mg, 5.8·10⁻³ mmol) and TBTA (3 mg, 5.8·10⁻³ mmol) were added and the reaction was stirred at room temperature under N₂ atmosphere. Aliquots of the reaction were taken at different times, diluted with EtOAc and washed with 0.2 M EDTA soln. and then analysed by UPLC to assess the progress of the reaction (Figure S4). After 96 h of reaction, the solvent was evaporated and the reaction mixture was purified by flash column chromatography on silica gel (gradient from 0% to 5% of MeOH in CH₂Cl₂). The fractions corresponding to the product were combined, dried and filtered through a pad of silica using EtOAc as solvent, affording cyclic duplex 25 (7 mg, 70%).
Figure S4. A. UPLC traces for the CuAAC reaction of p-triazolylethynylbenzene derivative 15 (50 µM of 15 and Cu-TBTA in THF) after 22 h and 96 h. Conditions: C18 column at 40 °C (254 nm) using water + 0.1% formic acid (A) and CH₃CN + 0.1% formic acid (B) with flow rate of 0.6 ml/min; Gradient of 0-3 minutes 5%-95%B + 2 minutes 95% B. B. Mass spectra of 25.

Compound 25 was fully characterised by ¹H NMR, ¹³C NMR, IR and HRMS (see page S94). Key evidence of the formation of a cyclic product was the absence of acetylene protons in ¹H NMR and azide stretching bands in IR, when compared to the starting material 15 (Figure S5).
Figure S5. A. Partial \(^1\)H NMR (400 MHz, CDCl\(_3\)) of pre-ZIP 15 (top) and cyclic duplex 24 (bottom), showing the absence of acetylene protons in the latter. B. FT-IR (ATR) of pre-ZIP 15 (top) and cyclic duplex 25 (bottom), showing the absence of azide stretching band in the latter.

As shown in Scheme S5, basic hydrolysis of 25 provided template 14 and cyclic 2-mer 26. The reaction was performed as follow: A solution of 25 (5 mg, 2.9·10\(^{-3}\) mmol) in MeOH (1 mL) was treated with 2 M NaOH solution (0.030 mL, 5.8·10\(^{-2}\) mmol). After 20 min of stirring at room temperature, the solution was acidified with 1 M HCl soln. to pH 2-3 and extracted with EtOAc (3x). The combined organic phase was washed with brine, dried over MgSO\(_4\) and evaporated to dryness. The obtained residue was analysed without further purification, corresponding to a mixture of cyclic-2-mer 26 and template 14 (Figure S6).

Scheme S5.
Figure S6. A. UPLC traces of cyclic duplex 25 (top) and crude of the hydrolysis of 25 (bottom). Conditions: C18 column at 40 °C (254 nm) using water + 0.1% formic acid (A) and CH$_3$CN + 0.1% formic acid (B) with flow rate of 0.6 ml/min; Gradient of 0-3 minutes 5% -95%B + 2 minutes 95% B. B. Mass spectra of cyclic 2-mer 26. C. Mass spectra of template 14.
CuAAC reaction of pre-ZIP 20.

Scheme S6 shows the possible products for the CuAAC reaction of pre-ZIP 20 performed at high dilution. Firstly, the linear duplex 27 must be formed both in the parallel and antiparallel arrangement as the backbone is directional. Then, further CuAAC reaction of 27 would lead to intermolecular adducts such as 28. Cyclisation of the linear duplex 27 to form cyclic 2-mer duplex is not possible due to the high ring strain associated to such process.

The reaction was performed as follow: Compound 20 (4 mg, 2.5·10⁻³ mmol) was dissolved, under N₂ atmosphere, in dry THF (50 mL). Cu(CH₃CN)₃PF₆ (0.9 mg, 2.5·10⁻³ mmol) and TBTA (1.2 mg, 2.5·10⁻³ mmol) were added and the reaction was stirred at room temperature under N₂ atmosphere. Aliquots of the reaction were taken at different times, diluted with EtOAc and washed with 0.2 M EDTA soln. and then analysed by UPLC to assess the progress of the reaction (Figure S7). After 22 h of reaction, duplex 27 is the major product formed and correspond to the linear duplex structure bearing alkyne and azide functionalities because it accumulates and is then consumed. Mass spectra of 27 is shown in Figure S8A. After 9 days of reaction, 27 reacted completely to give intermolecular adducts, such as 28, although multiple adducts are possible. Confirmation of the formation of intermolecular adducts is given by the mass spectra shown in Figure S8B. The triply charged ion with a mass of 1068.2 can only correspond to an intermolecular adduct with molecular mass of 3199.4.
**Figure S7.** UPLC traces for the CuAAC reaction of p-triazolylaniline derivative 20 (50 µM of 20 and Cu-TBTA in THF) after 3h, 22 h, 3 days and 9 days. **Conditions:** C18 column at 40 °C (254 nm) using water + 0.1% formic acid (A) and CH₃CN + 0.1% formic acid (B) with flow rate of 0.6 ml/min; Gradient of 0-2 minutes 65% -100%B + 1 minute 100% B.
Basic hydrolysis of the aliquots taken from the reaction at different times proved to be an alternative and efficient way to analyse the progress of the reaction. As shown in Scheme S7, basic hydrolysis of the aliquot taken after 22 h of reaction, containing 27 as the major product, would provide template 18 and linear 2-mer 26. In contrast, basic hydrolysis of the aliquot taken after 9 days of reaction, containing intermolecular adducts such as 28, would provide template 18 and cyclic 4-mer 29 among larger cyclic species.

The reaction was performed as follow: Aliquots taken from the reaction at different times were diluted with EtOAc, washed with 0.2 M EDTA soln. and the solvent evaporated. The crude was dissolved in THF (1 mL) and 1 M LiOH soln. (3-4 drops) was added. After 15 min of reaction at room temperature, the reaction was quenched with diluted HCl soln., extracted with EtOAc and analyse by UPLC. Figure S9A shows the chromatogram of the crude after hydrolysis of the aliquot taken after 22 h of reaction (top), and the mass spectra of the peak with a retention time of 1.94 min, corresponding to the linear 2-mer. Figure S9B shows the chromatogram of the crude after hydrolysis of the aliquot taken after 9 days of reaction (top), and the mass spectra of the peak with a retention time of 1.61 min, corresponding to the cyclic 4-mer 29.
Scheme S7.

duplex (27, only antiparallel shown)
Exact mass: 1599.71

Cu(CH$_2$CN)$_2$PF$_6$
TBTA, THF

intermolecular adduct (28)
Exact mass: 3199.43

LIOH

linear 2-mer
Exact mass: 584.19

template (18)
Exact mass: 1051.54

LIOH

cyclic 4-mer (29)
Exact mass: 1168.38
Figure S9. A. UPLC trace for the hydrolysis of CuAAC reaction crude of 20 after 22 h (top) and mass spectra of linear 2-mer (bottom). B. UPLC trace for the hydrolysis of CuAAC reaction crude of 20 after 9 days (top) and mass spectra of cyclic 4-mer 29 (bottom). Conditions: C18 column at 40 °C (254 nm) using water + 0.1% formic acid (A) and CH3CN + 0.1% formic acid (B) with flow rate of 0.6 ml/min; Gradient of 0-4 minutes 5% -100%B + 1 minute 100% B.
CuAAC reaction of pre-ZIP S7.

Scheme S8 shows a schematic representation of the ZIP reaction for the trimer Pre-ZIP S7.

![Scheme S8](image)

The reaction was performed as follow: Compound S7 (5 mg, 5.8·10^{-3} mmol) was dissolved, under N₂ atmosphere, in dry THF (120 mL). Cu(CH₃CN)₄PF₆ (2 mg, 5.8·10^{-3} mmol) and TBTA (3 mg, 5.8·10^{-3} mmol) were added and the reaction was stirred at room temperature under N₂ atmosphere. Aliquots of the reaction were taken at different times, diluted with EtOAc and washed with 0.2 M EDTA soln. and then analysed by UPLC to assess the progress of the reaction (Figure S10).

After 64 h of reaction, basic hydrolysis of the ZIP product provided template S6 and a mixture of cyclic 2-mer 26 and cyclic 3-mer S8. The reaction was performed as follow: A solution of ZIP product (3 mg, 2.9·10^{-3} mmol) in MeOH (1 mL) was treated with 2 M NaOH solution (0.030 mL, 5.8·10^{-2} mmol). After 20 min of stirring at room temperature, the solution was acidified with 1 M HCl soln. to pH 2-3 and extracted with EtOAc (3x). The combined organic phase was washed with brine, dried over MgSO₄ and evaporated to dryness. The obtained residue was analysed without further purification, corresponding to a mixture of cyclic-2-mer 26, cyclic 3-mer S7 and template 14 (Figure S10).
Figure S10. UPLC traces for the CuAAC reaction of pre-ZIP S7 (50 µM in THF) after 16 h and 64 h, and after subsequent hydrolysis. Conditions: C18 column at 40 °C (254 nm) using water + 0.1% formic acid (A) and CH3CN + 0.1% formic acid (B); Gradient of 0-3 minutes 5% -95%B + 2 minutes 95% B.
Molecular modelling calculations

General details

Molecular mechanics calculations were performed using MacroModel implemented in Maestro 11 (Schrodinger release 2016-4). Structures were simplified by replacing the capping groups by methyl and phenyl groups. The structures were minimized first, and the minimized structures were then used as the starting molecular structure for the MacroModel conformational search. The force field used was MMFFs with CHCl₃ solvation. The charges were defined by the force field library and no cut off was used for non-covalent interactions. A Polak-Ribiere Conjugate Gradient (PRCG) was used and each minimisation was subjected to 10,000 iterations with a convergence threshold of 0.01. Conformational searches were performed using 100 steps per rotatable bond (maximum number of steps of 10,000). Images were created using PyMol.

Estimation of the ring strain.

The ring strain for the ZIP and 2-mer macrocyclization reactions was estimated by using the strain energy of the product ($E_{strain}$), as shown in Scheme S9. The disconnection of the triazole rings into the corresponding azide and alkyne was not used due to problems in the parameterisation of azide moieties in the MMFFs force-field. The disconnection of the macrocyclic structures was done through the benzyl-triazole or phenyl-triazole bonds. This hypothetical transformation provides a method for calculating $E_{Bond}$ as the energy difference between two fragments and the connected oligomer backbone (Equation 1). The energy contribution associated with benzyl-triazole or phenyl-triazole bond connection ($E_{Bond}$) was subtracted from the difference between the energy of the macrocyclic product ($E_{macrocycle}$) and the energy of the linear intermediate ($E_{linear}$) (Equation 2).

Scheme S9.

\[ E_{Bond} = E_{product} - (2 \times E_{fragment}) \]  
(Eq.1)

\[ E_{strain} = E_{macrocycle} - E_{linear} - E_{Bond} \]  
(Eq.2)
1,4-bis(triazolyl)methylbenzene backbone (A)

**Bond**

\[ E_{\text{bond}} = E_{\text{product}} - (E_{\text{fragmentA}} + E_{\text{fragmentB}}): \ 334.3 - (197.5 + 49.6) = 87.1 \text{ kJ} \cdot \text{mol}^{-1} \]

**ZIP**

\[ E_{\text{strain}} = E_{\text{ZIP}} - E_{\text{preZIP}} - E_{\text{bond}} = 939.2 - 831.2 - 87.1 = 20.9 \text{ kJ} \cdot \text{mol}^{-1} \]

**2-mer macrocyclization**

\[ E_{\text{strain}} = E_{\text{macrocycle}} - E_{\text{linear}} - E_{\text{bond}} = 539.3 - 424.7 - 87.1 = 27.5 \text{ kJ} \cdot \text{mol}^{-1} \]
**3-mer macrocyclization**

\[
E_{\text{strain}} = E_{\text{macrocycle}} - E_{\text{linear}} = 790.7 - 664.3 - 87.1 = 39.3 \text{ kJ\cdot mol}^{-1}
\]

**p-triazolylethynylbenzene backbone (B)**

**Bond**

\[
E_{\text{bond}} = E_{\text{product}} - (E_{\text{fragment A}} + E_{\text{fragment B}}) = 203.9 - (60.9 + 49.6) = 93.4 \text{ kJ\cdot mol}^{-1}
\]
Parallel

\[ \text{ZIP parallel } E = 699.4 \text{ kJ·mol}^{-1} \]

\[ \text{Pre-ZIP parallel } E = 580.7 \text{ kJ·mol}^{-1} \]

\[ E_{\text{strain parallel}} = E_{\text{ZIP parallel}} - E_{\text{pre-ZIP parallel}} - E_{\text{bond}} = 699.4 - 580.7 - 93.4 = 25.2 \text{ kJ·mol}^{-1} \]

\[ E_{\text{strain antiparallel}} = E_{\text{ZIP antiparallel}} - E_{\text{pre-ZIP antiparallel}} - E_{\text{bond}} = 689.1 - 575.0 - 93.4 = 20.7 \text{ kJ·mol}^{-1} \]

Average \( E_{\text{strain}} = (25.2 + 20.7)/2 = 23.0 \text{ kJ·mol}^{-1} \)

Antiparallel

\[ \text{ZIP antiparallel } E = 689.1 \text{ kJ·mol}^{-1} \]

\[ \text{Pre-ZIP antiparallel } E = 575.0 \text{ kJ·mol}^{-1} \]

2-mer macrocyclization

\[ \text{linear } E = 163.4 \text{ kJ·mol}^{-1} \]

\[ \text{2-mer macrocycle } E = 271.1 \text{ kJ·mol}^{-1} \]

\[ E_{\text{strain}} = E_{\text{macrocycle}} - E_{\text{linear}} - E_{\text{bond}} = 271.1 - 163.4 - 93.4 = 14.3 \text{ kJ·mol}^{-1} \]
3-mer macrocyclization

\[
\begin{align*}
E_{\text{bond}} + E_{\text{strain}} &= 467.1 \text{ kJ} \cdot \text{mol}^{-1} \\
E_{\text{bond}} + E_{\text{strain}} &= 407.8 \text{ kJ} \cdot \text{mol}^{-1} - 280.2 \text{ kJ} \cdot \text{mol}^{-1} - 93.4 \text{ kJ} \cdot \text{mol}^{-1} = 34.2 \text{ kJ} \cdot \text{mol}^{-1}
\end{align*}
\]

\[E_{\text{strain}} = E_{\text{macrocycle}} - E_{\text{linear}} - E_{\text{bond}} = 407.8 - 280.2 - 93.4 = 34.2 \text{ kJ} \cdot \text{mol}^{-1}\]

\[p\text{-triazolylaniline backbone (C)}\]

Bond

\[
\begin{align*}
E_{\text{bond}} + E_{\text{strain}} &= 467.1 \text{ kJ} \cdot \text{mol}^{-1} \\
E_{\text{bond}} + E_{\text{strain}} &= 187.0 \text{ kJ} \cdot \text{mol}^{-1}
\end{align*}
\]

\[E_{\text{bond}} = E_{\text{product}} - (2 \times E_{\text{fragment}}) = 467.1 - (2 \times 187.0) = 93.0 \text{ kJ} \cdot \text{mol}^{-1}\]
$E_{strain\ parallel} = E_{ZIP\ parallel} - E_{preZIP\ parallel} - E_{bond} = 1219.6 - 1100.1 - 93.0 = \text{26.5 kJ·mol}^{-1}$

$E_{strain\ antiparallel} = E_{ZIP\ antiparallel} - E_{preZIP\ antiparallel} - E_{bond} = 1215.7 - 1099.8 - 93.0 = \text{22.9 kJ·mol}^{-1}$

Average $E_{strain} = (26.5 + 22.9)/2 = \text{24.7 kJ·mol}^{-1}$

2-mer macrocyclization

$E_{strain} = E_{macrocycle} - E_{linear} - E_{bond} = 671.8 - 467.1 - 93.0 = \text{111.7 kJ·mol}^{-1}$
3-mer macrocyclization

\[ E_{\text{bond}} + E_{\text{strain}} = 867.6 \text{ kJ·mol}^{-1} \]

\[ E_{\text{macrocycle}} = 867.6 \text{ kJ·mol}^{-1} \]

\[ E_{\text{linear}} = 747.3 \text{ kJ·mol}^{-1} \]

\[ E_{\text{strain}} = E_{\text{macrocycle}} - E_{\text{linear}} - E_{\text{bond}} = 867.6 - 747.3 - 93.0 = 27.3 \text{ kJ·mol}^{-1} \]

Ring strain calculations for 2-mer macrocycle attached to the template strand.

1,4-bis(triazolyl)methylbenzene backbone (A)
In the same way as in the previous section, these structures were used to perform molecular mechanics conformational searches with the MMFFs force-field and CHCl₃ solvation, the energies of the lowest conformations were used to calculate the ring strain for the
macrocyclization reaction. Figure S11 compares the ring strain energies for macrocyclization reaction for the simplified structures used in the previous section and those shown above.

![Bar chart comparing ring strain energies for macrocyclization reaction](image)

**Figure S11.** Comparison of the calculated ring strain energies for the macrocyclization reaction for the structures shown in the previous section (simplified structures) and those shown in the previous page (full structures)
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