Synthesis and Aggregation Behavior of Biohybrid Amphiphiles Composed of a Tripeptidic Head Group and a Polystyrene Tail


Supporting information

Experimental procedure for peptides 5 and 6.

Scheme S1 Synthesis of H-Gly-Gly-Arg-AMC (5) and butynoyl-functionalized Gly-Gly-Arg-AMC (6) (i) AMC, POCl₃, pyridine (dry), -15 °C (2 h) to r.t. (1 h) (68%); (ii) 2.6 M HCl in EtOAc, r.t., 2 h for B (97%) and 4 h for 5 (98%); (iii) Boc-Gly-Gly-OH (1.02 equiv.), H-Arg-AMC (B), DMF, EDC.HCl (1.1 equiv.) DMAP (2 equiv.), 0 °C to r.t., 16 h (86%); (iv) 2 M NaOH, dioxane/H₂O (1:1 v/v), r.t., 18 h (63%).

Materials

Unless otherwise stated, all chemicals were obtained from commercial sources and used without further purification. Analytical thin layer chromatography (TLC) was performed on Merck precoated silica gel 60 F-254 plates (layer thickness 0.25 mm) with
visualization by ultraviolet (UV) irradiation at $\lambda = 254$ nm and/or $\lambda = 366$ nm and/or staining with KMnO$_4$ or Ninhydrin.

**Methods**

NMR spectra were recorded on *Bruker DMX300* (300 MHz and 75 MHz for $^1$H and $^{13}$C, respectively) and *Varian inova 400* (400 MHz) spectrometers. $^1$H NMR chemical shifts ($\delta$) are reported in parts per million (ppm) relative to a residual proton peak of the solvent; $\delta = 3.31$ for CD$_3$OD. Multiplicities are reported as: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), dt (doublet of triplets), dq (double quartet), ddd (double, double doublet), ddt (double, double triplet) or m (multiplet). Broad peaks are indicated by the addition of br. Coupling constants are reported as a $J$ value in Hertz (Hz). The number of protons (n) for a given resonance is indicated as nH, and is based on spectral integration values. $^{13}$C NMR chemical shifts ($\delta$) are reported in ppm relative to CD$_3$OD ($\delta = 49.0$). IR spectra were recorded on an *ATI Matson Genesis Series FTIR* spectrometer fitted with an ATR cell. The vibrations ($\nu$) are given in cm$^{-1}$. Electrospray ionisation time-of-flight (ESI-ToF) spectra were measured with a *JEOL AccuToF*. Elemental analyses were carried out using a *Carlo Erba Instruments CHNS-O EA 1108* element analyzer. FCPC (fast centrifugal partition chromatography) purification was performed on a *Kromaton Prep-FCPC A200* complete system equipped with 200 mL rotor, an Arman gradient high pressure pump 100 mL/min, an *Alltech ELSD2000* detector with flow split and an *Advantec MFS CHF122SB* fraction collector. The flow rate of the mobile phase used during purifications was 5.0 mL/min.

**Boc-Arg-AMC·HCl (A)**

To a cold solution (-15 °C) of Boc-Arg-OH·HCl (4.64 g, 5.0 mmol) and 7-amino-4-methylcoumarin (875 mg, 5.0 mmol) in pyridine (15 mL), phosphoryl chloride (0.51 ml, 5.5 mmol) was added drop wise. A color change going from yellow to orange was observed. The mixture was stirred for 2 hours at -15 °C, then allowed to warm to room
temperature and stirred for an additional hour. The reaction mixture was quenched with water (15 mL). The solvents were evaporated under reduced pressure. The crude reaction mixture was purified by FCPC chromatography. The product was obtained as a light yellow powder (1.59 g, 68%). $R_f = 0.65$ (n-BuOH/H$_2$O/AcOH 4:1:1 v/v). $^1$H-NMR (300 MHz, CD$_3$OD) : 7.70 (d, $J = 2.13$, 1H), 7.60 (d, $J = 11.6$ Hz, 1H), 7.38 (d, $J = 12$ Hz, 1H), 6.13 (d, $J = 1.6$ Hz, 1H), 4.16-4.10 (m, 1H), 3.12 (t, $J = 8.8$ Hz, 2H), 2.36 (s, 3H), 1.59 - 1.85 (m, 4H), 1.45 (s, 9H). $^{13}$C-NMR (50 MHz, CD$_3$OD) : 173.6, 163.2, 158.6, 158.0, 155.2, 143.3, 126.7, 117.2, 113.6, 108.0, 80.9, 56.4, 42.1, 30.6, 28.8, 26.5, 18.6. HRMS (ESI+) m/z calcd for C$_{21}$H$_{29}$N$_5$O$_5$ [M+H]$^+$ 432.2247, found: 432.2247.

**H-Arg-AMC·2HCl (B)**

To a suspension of Boc-Arg-AMC·HCl (A, 1.18 g, 2.53 mmol) in Et$_2$O (50 ml), a solution of hydrochloric acid in EtOAc (2.6 M, 10 mL) was added. The suspension was stirred overnight at room temperature after which the solvents were removed in vacuo. The product was re-suspended in Et$_2$O (20 mL), stirred for 2 hours and collected by filtration to yield a white powder (993 mg, 97%). $R_f = 0.17$ (n-BuOH/H$_2$O/AcOH 4:1:1 v/v). $^1$H-NMR (400 MHz, CD$_3$OD) $\delta$: 7.91 (s, 1H), 7.76 (d, $J = 8.8$ Hz, 1H), 7.55 (d, $J = 8.4$ Hz, 1H), 6.27 (s, 1H), 4.18-4.11 (m, 1H), 3.27 (t, $J = 6.8$ Hz, 2H), 2.47 (s, 3H), 1.95-2.15 (m, 2H), 1.68-1.82 (m, 2H). $^{13}$C-NMR (50 MHz, CD$_3$OD) : 169.0, 155.4, 155.2, 142.6, 127.0, 117.7, 117.3, 113.9, 108.3, 54.8, 41.8, 29.8, 25.5, 18.7. Anal. C$_{16}$H$_{21}$N$_5$O$_2$ · 3.0 HCl calculated C 45.15% H 5.69% N 16.45%, measured C 45.12% H 5.43% N 16.57%.

**Boc-Gly-Gly-Arg-AMC·HCl (C)**

H-Arg-AMC·2HCl (B, 993 mg, 2.46 mmol), Boc-Gly-Gly-OH (696 mg, 3.0 mmol) and 4-(dimethylamino)pyridine (DMAP, 611 mg, 5.0 mmol) were dissolved in DMF (20 mL). The mixture was cooled to 0 °C and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl, 531 mg, 2.8 mmol) was added in small portions. The mixture
was stirred for 15 min at 0 °C and 16 hours at room temperature. The solvent was removed under reduced pressure and the crude reaction mixture was purified by FCPC. The product was obtained as an off-white solid (1.13 g, 86%). $R_f = 0.48$ ($n$-BuOH/H$_2$O/AcOH 4:1:1 v/v). $^1$H-NMR (300 MHz, CD$_3$OD) $\delta$: 7.82 (d, $J = 1.9$ Hz, 1H), 7.71 (d, $J = 8.7$ Hz, 1H), 7.48 (dd, $J = 2.1$, 8.6 Hz, 1H), 6.24 (s, 1H), 4.57 (t, $J = 8.9$ Hz, 1H), 3.92 (s, 2H), 3.75 (s, 2H), 3.22 (t, $J = 7.0$ Hz, 2H), 2.45 (s, 3H), 2.05-1.91 (m, 1H), 1.85-1.60 (m, 3H), 1.42 (s, 9H). $^{13}$C-NMR (75 MHz, CD$_3$OD) $\delta$: 173.8, 172.5, 172.1, 163.2, 158.7, 155.33, 155.27, 143.3, 126.7, 117.4 (2C), 113.7, 108.2, 81.0, 55.1, 45.0, 43.9, 42.0, 30.0, 28.8, 26.4, 18.6. HRMS (ESI+) $m/z$ calcd for C$_{25}$H$_{36}$N$_7$O$_7$ [M+H]$^+$ 546.2676, found: 546.2641

H-Gly-Gly-Arg-AMC·2HCl (5)

To a suspension of Boc-Gly-Gly-Arg-AMC·HCl (C, 1.00 g, 1.72 mmol) in Et$_2$O (30 mL) was added HCl in EtOAc (2.5 mL, 2.6 M). The reaction mixture was stirred 4 hours at room temperature. The resulting gum was dried in vacuo and re-suspended in Et$_2$O (20 mL). The resulting solid was filtered off, washed with Et$_2$O (2 × 10 mL) and dried in vacuo. Product 5 was obtained as an off-white solid (870 mg, 98%). $R_f = 0.20$ ($n$-BuOH/H$_2$O/AcOH 4:1:1 v/v). $^1$H-NMR (400 MHz, CD$_3$OD) $\delta$: 7.86 (d, $J = 2.0$ Hz, 1H), 7.72 (d, $J = 8.7$ Hz, 1H), 7.55 (dd, $J = 8.7$, 2.1 Hz, 1H), 6.25 (d, $J = 1.2$ Hz, 1H), 4.56 (m, 1H), 4.03 (s, 2H), 3.84 (s, 2H), 3.25 (t, $J = 7.0$ Hz, 2H), 2.46 (d, $J = 1.2$ Hz, 3H) 2.08-1.95 (m, 1H), 1.86-1.58 (m, 3H). $^{13}$C-NMR (50 MHz, CD$_3$OD) $\delta$: 172.5, 171.4, 168.1, 163.0, 158.4, 155.1, 154.9, 142.9, 126.4, 117.1, 117.0, 113.4, 107.8, 55.2, 43.3, 41.8, 41.5, 29.9, 26.2, 18.4. Anal. calcd. for C$_{20}$H$_{27}$N$_7$O$_5$ · 3.5 HCl: C 41.79% H 5.36% N 17.06%, measured C 41.72% H 5.71% N 17.17%. HRMS (ESI+) $m/z$ calcd for C$_{20}$H$_{28}$N$_7$O$_5$ [M+H]$^+$ 446.2152, found: 446.2176.
N-3-Butyn-1-yl-Gly-Gly-Arg-AMC (6)

To a solution of H-Gly-Gly-Arg-AMC·2HCl (5, 258.5 mg, 0.5 mmol) in a H₂O/dioxane mixture (5:1, v/v) was added 2M NaOH until the pH reached 9-10. Under a nitrogen atmosphere 3-butyne-chloroformate (62.3 µL 0.55 mmol) was added and the mixture was allowed to stir for 5 hrs at room temperature. Completion of the reaction was monitored by TLC: BuOH/H₂O/AcOH (4:1:1). The product was lyophilised and purified by FCPC using an n-BuOH/H₂O system. After purification the product was obtained as a white solid (185 mg, 63%). $R_f = 0.48$ (n-BuOH/H₂O/AcOH, 4:1:1). $^1$H-NMR (300 MHz, CD₃OD) $\delta$ (ppm): 7.88 (d, $J = 1.8$ Hz, 1H), 7.73 (d, $J = 8.7$ Hz, 1H), 7.57 (dd, $J = 8.8$, 2.1 Hz, 1H), 6.25 (d, $J = 1.2$ Hz, 1H), 4.58 (dd, $J = 9.0$, 4.8 Hz, 1H), 4.12 (dt, $J = 6.8$, 1.5 Hz, 2H), 3.92 (d, $J = 7.8$ Hz, 2H), 3.80 (s, 2H), 3.24 (t, $J = 7.0$ Hz, 2H), 2.50 (td, $J = 6.9$, 3.5 Hz, 2H), 2.48 (d, $J = 1.2$ Hz, 3H), 2.30 (t, $J = 2.3$, 1H), 2.09-1.95 (m, 1H), 1.92-1.65 (m, 3H). $^{13}$C-NMR (75 MHz, CD₃OD) $\delta$ (ppm): 173.5, 172.5, 172.1, 163.2, 158.7, 155.4, 155.3, 143.4, 126.8, 117.5, 117.4, 113.8, 108.3, 107.6, 71.1, 64.5, 55.0, 45.3, 43.9, 42.0, 30.1, 26.4, 19.9, 18.6. HRMS (ESI+) m/z calcd for C$_{25}$H$_{31}$N$_7$NaO$_7$ [M+Na]$^+$ 564.2483, found: 564.2467.
Self-assembly of biohybrid 9b in water using the injection method at 50 °C

Figures S1 and S2 show TEM and cryoSEM images of the aggregates obtained from compound 9b using the injection method at 50 °C. Following this method, the formation of holes was found to be more pronounced than for the methods at room temperature.

Figure S1. TEM image of aggregates formed by compound 9b after injecting a THF solution of the compound into water at 50 °C. The arrows indicate the holes.
Figure S2. cryoSEM image of aggregates formed by compound 9b after injecting a THF solution of the compound into water at 50 °C. The arrows indicate the holes.