Supplementary Information for

Photoreversible formation of nanotubes in water from an azobenzene-appended L-ValylGlycine amphiphilic derivative

Diego Navarro-Barreda, César A. Angulo-Pachón, Francisco Galindo and Juan Felipe Miravet*

Departamento de Química Inorgánica y Orgánica, Universitat Jaume I, Av. Vicent Sos Baynat s/n. Castellón, 12071, Spain.

E-mail: miravet@uji.es

Table of Contents

General methods

NMR spectra were recorded on an Agilent VNMR System spectrometer (500 MHz for ^{1}H-NMR, 125 MHz ^{13}C-NMR) or Bruker Avance III HD spectrometers (400 MHz and 300 MHz for ^{1}H-NMR, 101 MHz and 75 MHz for ^{13}C-NMR) in the indicated solvent at 30 °C. Mass spectra were recorded with a mass spectrometry triple quadrupole Q-TOF Premier spectrometer (Waters) with simultaneous Electrospray and APCI Probe. The ultraviolet-visible (UV-vis) absorption spectra and the fluorescence intensity were measured with a JASCO V- 630 UV–vis spectrophotometer and a JASCO FP-8300 fluorometer, respectively. Dynamic Light Scattering (DLS) measurements were recorded using a Zetasizer Nano ZS (Malvern Instruments, UK), using 3 mL disposable poly(methyl methacrylate) cuvettes (10 mm optical path length). Analyses were carried out using a He-Ne laser (633 nm) at a fixed scattering angle of 173°. Automatic optimization of beam focusing and attenuation was applied for each sample. The results were reported as the average of three measurements. Transmission electron microscopic (TEM) images were obtained using a JEOL 2100 electron microscope with thermionic gun LaB6 100 kV equipped with a GatanOrius high-resolution CCD camera. TEM samples were prepared over carbon formvar copper grids. Cryogenic transmission electron microscopic (Cryo-TEM) images were examined using a a Gatan 626 cryo-holder (Gatan Company, California, USA) on a FEI Tecnai G2 Spirit BioTwin (ThermoFisher Scientific company, Oregon, USA). The images were recorded digitally with a Xarosa camera (EMSIS GmbH, Münster, Germany) under low-dose conditions. Samples were irradiated with UV and visible light using three 365 nm UV LEDs (1200 mW flux output at 2.7 W power dissipation, LZ1- 00UV00) and three 457 nm Blue LEDs (50 lumen nominal flux at 3.3W power dissipation, LZ1-00B202), respectively. LEDs were placed at 8 cm from the cuvette. Ultrasonication was carried out in a Elmasonic S 60 H device.

Preparation of the nanotubes

In a representative example, 2.0 mg (0.0044 mmol) of **SucValGlyAzo** in 1.0 mL of Tris buffer (0.1 M, pH 7.4) were sonicated (37 kHz, Elmasonic S 60 H, 220-240 V) for 25 minutes at room temperature. Subsequently, 1.66 mL of additional buffer was added (final concentration of **SucValGlyAzo** = 1.7 mM) and the mixture was sonicated again for 25 minutes at room temperature. Finally, the resulting mixture was filtered through a 0.45 μm nylon filter membrane to afford a monomodal size distribution when measured by DLS.

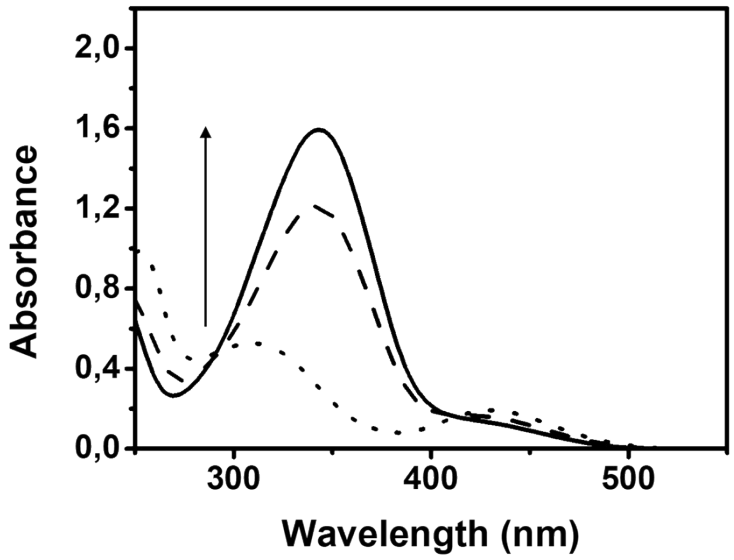


Fig. S1. Absorption spectra of **SucValGlyAzo** (1.3 mM, 0.1 M Tris buffer, pH 7.4, 25 ºC) obtained in the study of the thermal *cis-trans* conversion at 25 ºC. Dotted line: irradiation for 30 seconds with 365 nm light. Dashed line: 60 minutes of thermal equilibration at 25 ºC. Solid line: 120 minutes of thermal equilibration.

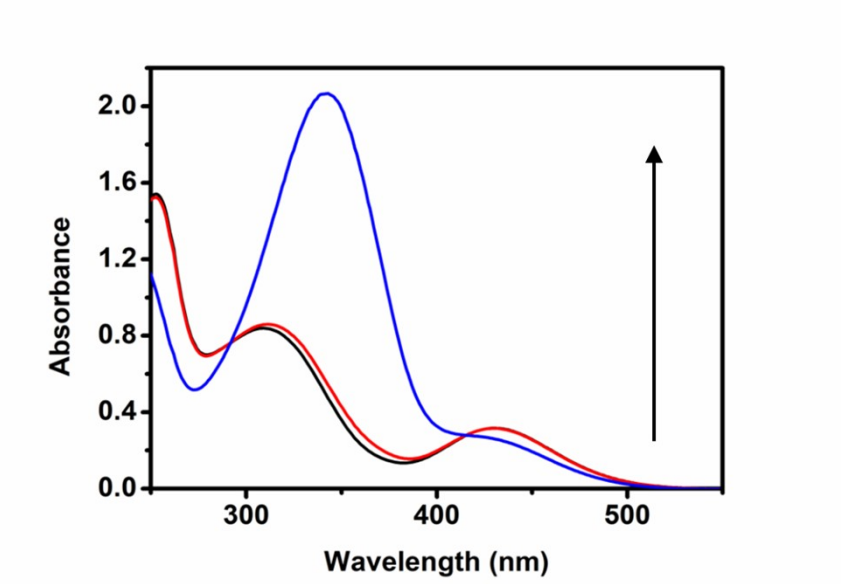


Fig. S2. Absorption spectra of **SucValGlyAzo** (1.7 mM, 0.1 M Tris buffer, pH 7.4, 25 ºC) obtained in the study of the Vis light promoted cis-trans conversion at 25 ºC. Black line: irradiation of the initial sample for 30 seconds with 365 nm light. Red line: After resting in the dark for 10 minutes Blue line: After irradiation for 30 seconds with 457 nm light

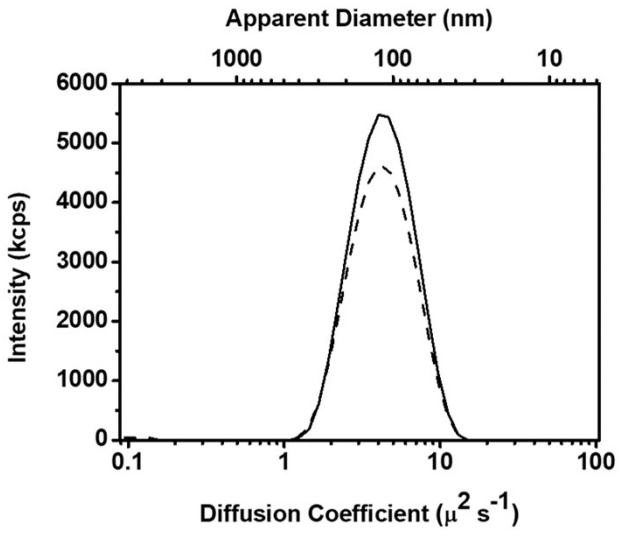


Fig. S3. Diffusion coefficient and apparent particle diameter distribution obtained by DLS for **SucValGlyAzo** (1.7 mM, 0.1 M Tris buffer, pH 7.4, 25 ºC). Solid line: fresh sample. Dashed line: Sample stored for 16 h at 4 ºC.

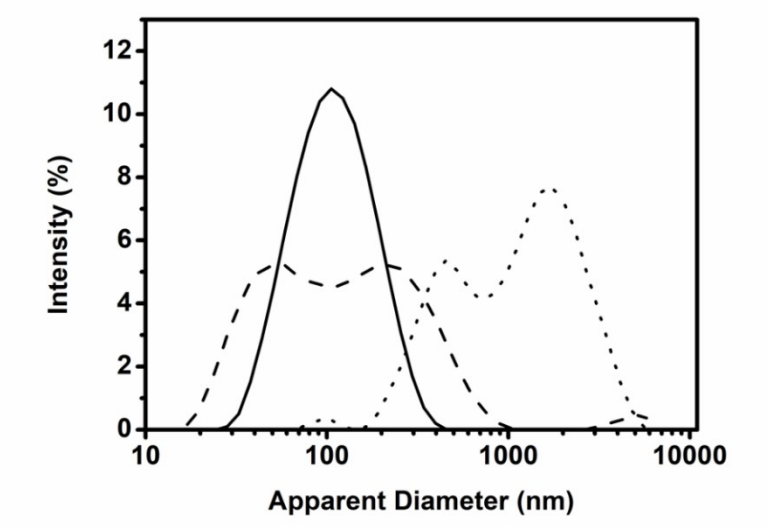


Fig. S4. Apparent diameter distribution obtained by DLS for **SucValGlyAzo** in different aqueous buffers (1.7 mM, 0.1 M buffer, pH 7.4, 25 ºC). Dotted line: phosphate buffer; dashed line: HEPES; solid line: Tris. The apparent hydrodynamic diameters given are collected after filtration (0.45 μm mesh filter).

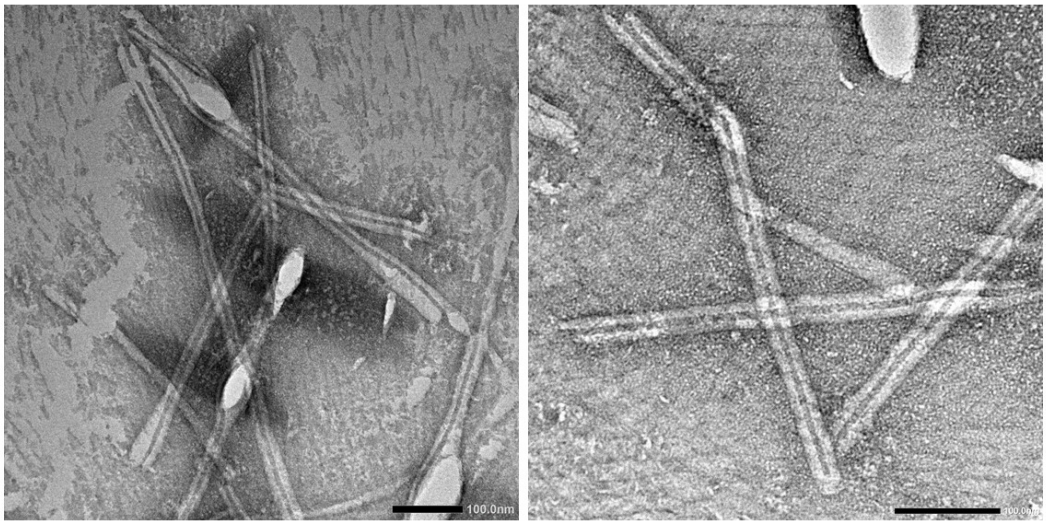


Fig. S5. TEM images of the nanotubes formed by **SucValGlyAzo** collected before filtration (1.7 mM, 0.1 M Tris buffer, pH 7.4; uranyl acetate was used as a staining agent). Scale bar: 100 nm.

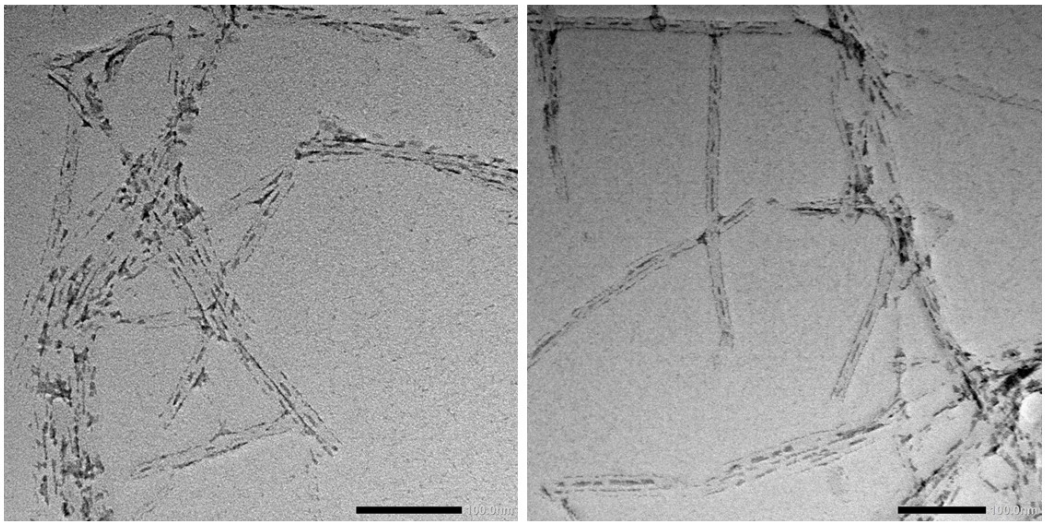


Fig. S6. TEM images of the nanotubes formed by **SucValGlyAzo** collected after UV light (1.7 mM, 0.1 M Tris buffer, pH 7.4; uranyl acetate was used as a staining agent). Scale bar: 100 nm.

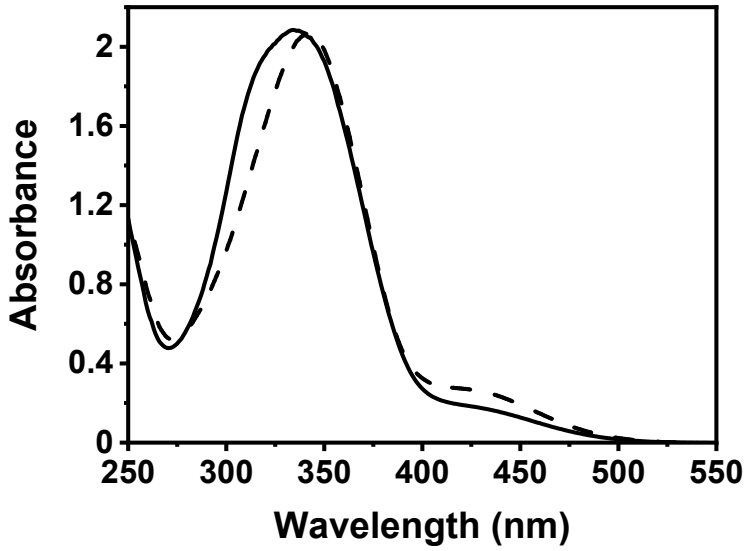


Fig. S7. Absorption spectra of **SucValGlyAzo** (1.7 mM, 0.1 M Tris buffer, pH 7.4, 25 ºC) obtained before irradiation with UV light (solid line) and after *trans-cis-trans* conversion process triggered by successive irradiation for 30 seconds with 3565 UV-light and 457 nm Vis-light (dashed line).

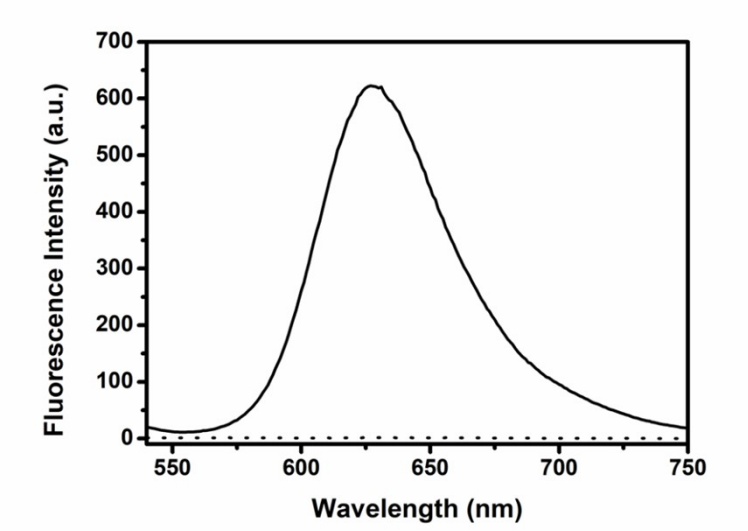
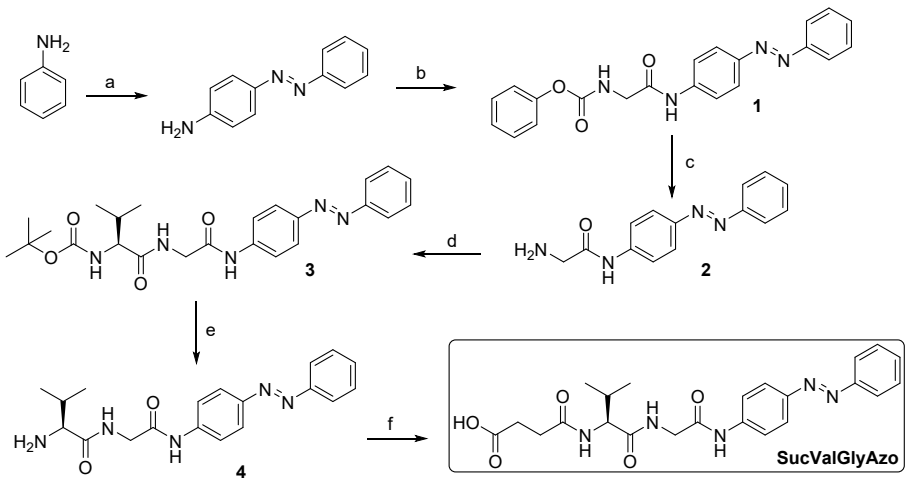


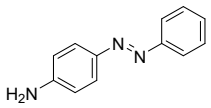
Fig. S8. Fluorescence emission spectra of Nile Red (10 μM) in the presence (solid line) and absence (dotted line) of **SucValGlyAzo** nanotubes (1.7 mM, 0.1 M Tris buffer, pH 7.4, 25 ºC).

Synthesis of SucValGlyAzo



Scheme S1. Synthesis of **SucValGlyAzo**. Reagents: a) $NaNO_3$, HCl, NH_3, H_2O; b) *N*-carbobenzyloxyglycine, $(CO)_2Cl_2$, Et_3N; c) HBr 33 wt.% in acetic acid; d) BocValOH, DIPEA, EDCI, ethyl (hydroxyimino)cyanoacetate; e) TFA; f) Succinic anhydride, Na_2CO_3.

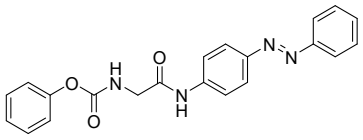
4-aminoazobenzene



Aniline (0.92 g, 10 mmol) was added dropwise to a solution of concentrated HCl (37%, 3 mL) in deionized water (30 mL) at 0 °C. Then, a sodium nitrite solution (0.70 g, 10.1 mmol) in water (5 mL) was added slowly for 10 min. The mixture was stirred at 0 °C for 60 min to further a yellow transparent diazonium salt solution. Aniline (0.93 g, 10.1 mmol) and 1 N HCl (10 mL) was dissolved in water (30 mL) under vigorous stirring at 0 °C. Then, to this resulting mixture, the diazonium salt solution previously obtained was added dropwise at 0 °C. The system was kept being stirred at 5°C for 3 h. Once, 1 M NH_3 (30 mL) was added slowly, precipitating an orange solid. The precipitate was

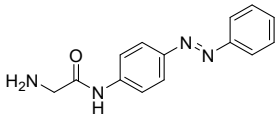
filtered off and washed with sodium hydrogen carbonate solution (pH≈8). The precipitate was collected by filtration, washed with deionized water three times, and dried under vacuum, yielding the product as an orange crystalline solid. Yield 72%; ^{1}H NMR and ^{13}C NMR spectra were consistent with those described in the literature;[1] HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{12}H_{11}N_3$ 198.1031; found, 198.1034.

Synthesis of benzyl (E)-(2-oxo-2-((4-(phenyldiazenyl) phenyl)amino)ethyl) carbamate - (1)



Commercially available *N*-carbobenzyloxyglycine (2.34 g, 11 mmol, 1.08 eq) was dissolved in anhydrous dichloromethane (37 mL) in a two-neck flask. Then, oxalyl chloride (5.6 mL; 11.2 mmol, 1.098 eq) and DMF (90 µL, 8.0 µL/mmol oxalyl chloride) were added dropwise. The mixture was stirred at 0 °C for 2 hours under N_2 atmosphere. After that, 4-aminoazobenzene (2.0 g, 10.2 mmol, 1 eq) in anhydrous dichloromethane (100 mL) was added dropwise at room temperature. Then, triethylamine (1.64 mL, 11 mmol, 1.08 eq) was added and the mixture reaction was stirred overnight under N_2 atmosphere. Then, the solvent was removed under reduced pressure, yielding a dark red slurry. The crude was washed with acid 1 M HCl, 1 M (200 mL), 0.1 M NaOH (200 mL) and finally, with destilled water until neutral pH. The product was dried in vacuum at 30 °C for 24 h. The compound **1** was obtained as an orange solid (yield 83%); ^{1}H NMR (500 MHz, DMSO-d_6, δ): 10.29 (s, 1H), 7.93-7.80 (m, 4H), 7.63 – 7.47 (m, 6H), 7.46 – 7.21 (m, 5H), 5.07 (s, 2H), 3.88 (d, J = 5.9 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6, δ): 168.5, 156.6, 152.0, 147.5, 142.0, 137.0, 131.0, 129.4, 128.3, 127.8, 127.7, 123.7, 122.3, 119.3, 65.5, 44.2; HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{22}H_{20}N_4O_3$ 389.1613; found, 389.1614.

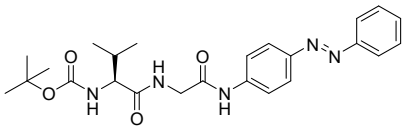
(E)-2-amino-N-(4-(phenyldiazenyl)phenyl)acetamide - (2)



Hydrogen bromide solution, 33 wt.% in acetic acid (27.4 mL, 3.3 mL/ mmol Cbz), was added to 1 (3.22 g, 8.29 mmol). The reaction was stirred under N_2 atmosphere at 0 °C for 30 minutes. After that,

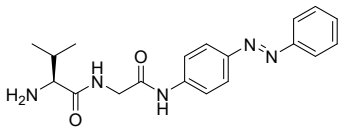
the mixture reaction was further stirred at room temperature overnight. Diethyl ether was added dropwise until the formation of a red precipitate. The redish solid obtained was filtered off under vacuum and the residue was dissolved in water (100 mL). The resulting solution was basified with solid sodium hydroxide at 0 °C and the precipitate filtered off and washed with water (300 mL) twice. The compound **2** was dried under reduced pressure at 50 °C overnight. The crude, as orange solid, was used for the next step without further purification. ^{1}H NMR (300 MHz, DMSO-d_6, δ): 9.98 (s, 1H), 7.95 – 7.81 (m, 4H), 7.64 – 7.53 (m, 3H), 7.46 – 7.18 (m, 4H), 3.78 (d, J = 9.2 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6, δ): 172.5, 152.0, 147.5, 141.9, 131.0, 129.4, 123.7, 122.3, 119.2, 45.6.

Tert-butyl (S,E)-(3-methyl-1-oxo-1-((2-oxo-2-((4-(phenyldiazenyl) phenyl)amino)ethyl)amino) butan-2-yl) carbamate - (3)



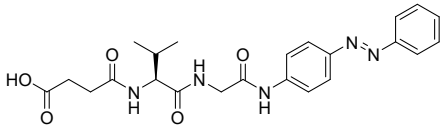
Amine **2** (1.45 g, 5.70 mmol, 1 eq.), DIPEA (1.19 mL, 6.84 mmol, 1.2 eq.) and comercially available *N*-(*tert*-butoxycarbonyl)-L-valine (1.24 g, 5.70 mmol, 1 eq.) were dissolved in anhydrous DMF (150 mL) at room temperature. Then, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (1.19 g, 6.21 mmol, 1.09 eq.) and ethyl (hydroxyimino)cyanoacetate (0.91 g, 6.21 mmol, 1.09 eq.) were added and the reaction was stirred at room temperature overnight. Once, water was added until precipitation occurred. The orange solid obtained was filtered under vacuum and the residue was washed with 1 M HCl (200 mL) and 0.1 M NaOH (200 mL). Finally, the solid was washed with distilled water (400 mL) until neutral pH and dried under reduced pressure at 50 °C overnight. The compound **3** was obtained as an orange-brown solid (yield 82%). ^{1}H NMR (400 MHz, DMSO-d_6, δ, signals for the *E* isomer): 10.20 (s, 1H), 8.29 (t, J= 4.8 Hz, 1H), 7.95 – 7.80 (m, 6H), 7.64 – 7.49 (m, 3H), 3.96 (m, 1H), 3.85 (m, 2H), 1.97 (m, 1H), 1.40 (s,1H), 0.92– 0.88(dd, J= 7.6 Hz, 6.4 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6, δ, signals for the *E* isomer): 172.2, 168.3, 155.9, 152.2, 147.8, 142.0, 131.2, 129.6, 123.9, 122.5, 119.5, 78.4, 60.1, 43.0, 30.4, 28.4, 19.4, 18.39; HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{24}H_{31}N_5O_4$ 454.2454; found, 454.2447.

(S,E)-2-amino-3-methyl-N-(2-oxo-2-((4-(phenyldiazenyl)phenyl) amino)ethyl)butanamide - (4)



To a solution of 3 (1.83 g, 4.04 mmol, 1 eq.) in dichloromethane (40 mL), trifluoroacetic acid (8.1 mL, 2 mL/ mmol Boc) were added dropwise and stirred at room temperature under N_2 atmosphere overnight. Then, solvent was removed under reduced pressure and the residue (dark oil liquid) was dissolved with 0.2 M NaOH (200 mL) and extracted three times with chloroform (200 mL). The organic phase was concentrated until dryness. The compound **4** was obtained as an orange solid (yield 90%); ^{1}H NMR (500 MHz, DMSO-d_6, δ, signals for the *E* isomer): 10.33 (s, 1H), 8.24 (s, 1H), 7.92 – 7.78 (m, 6H), 7.60 – 7.48 (m, 3H) 3.97 (m, 2H), 3.06 (d, *J*= 5.0 Hz, 1H), 1.97 (m, 1H), 0.93 (d, *J*= 7.0 Hz, 6H); ^{13}C NMR (126 MHz DMSO-d_6, δ, signals for the *E* isomer): 175.1, 168.3, 152.5, 147.6, 142.0, 131.0, 129.4, 123.7, 122.3, 119.2, 59.9, 42.7, 31.4, 19.6; HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{19}H_{23}N_5O_2$ 354.1930; found, 354.1926.

Synthesis of (S,E)-4-((3-methyl-1-oxo-1-((2-oxo-2-((4-(phenyldiazenyl)phenyl) amino)ethyl) amino)butan-2-yl)amino)-4-oxobutanoic acid – (**SucValGlyAzo**)



A solution of 4 (1.42 g, 4.04 mmol, 1 eq.) in THF (120 mL) was treated with solid Na_2CO_3 (1.50 g,14.14 mmol, 3.5 eq.) at room temperature under N_2 atmosphere. Succinic anhydride (808 mg, 8.08 mmol, 2 eq.) dissolved in THF (60 mL) was added dropwise at room temperature. The resulting mixture was stirred overnight. Once, the solution was concentrated under reduced pressure and the crude residue was dissolved in water (100 mL). Then, hydrochloric acid (concentrated grade) was added dropwise at 0 °C until complete precipitation occurred. The solid obtained was filtered off under vacuum, and the residue was washed with water (300 mL) until neutral pH. The compound was dried under reduced pressure at 50 °C overnight. The compound **SucValGlyAzo** was obtained as an orange brown solid (yield 85%); ^{1}H NMR (400 MHz, DMSO-d_6, δ, signals for the *E* isomer): 12.07 (s, 1H), 10.11 (s, 1H), 8.36 (s, 1H), 8.02 (d, J= 7.2 Hz, 1H), 7.95 – 7.82 (m, 6H) , 7.62 – 7.53 (m, 3H), 4.14 (t, J= 6.4 Hz, 1H), 3.94 (d, J= 4.4 Hz, 2H), 2.49 (s, 4H), 2.03 (m, 1H), 0.92 (d, J= 6.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6, δ, signals for the *E* isomer): 173.9, 171.8, 171.7, 168.2, 152.0,

147.6, 141.7, 131.0, 129.4, 123.7, 122.3, 119.3, 58.5, 42.8, 30.0, 29.9 , 29.2, 19.2; HRMS (ESI-TOF) m/z: $[M-H]^-$ calcd for $C_{23}H_{27}N_5O_5$ 452.1934; found, 452.1937.

NMR spectra

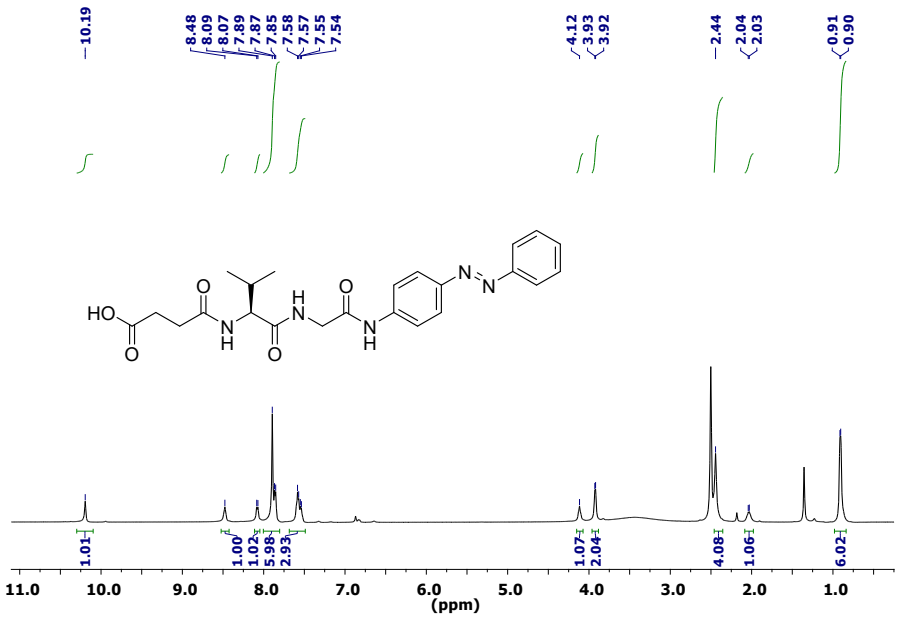


Fig. S7. ^{1}H NMR of **SucValGlyAzo**.

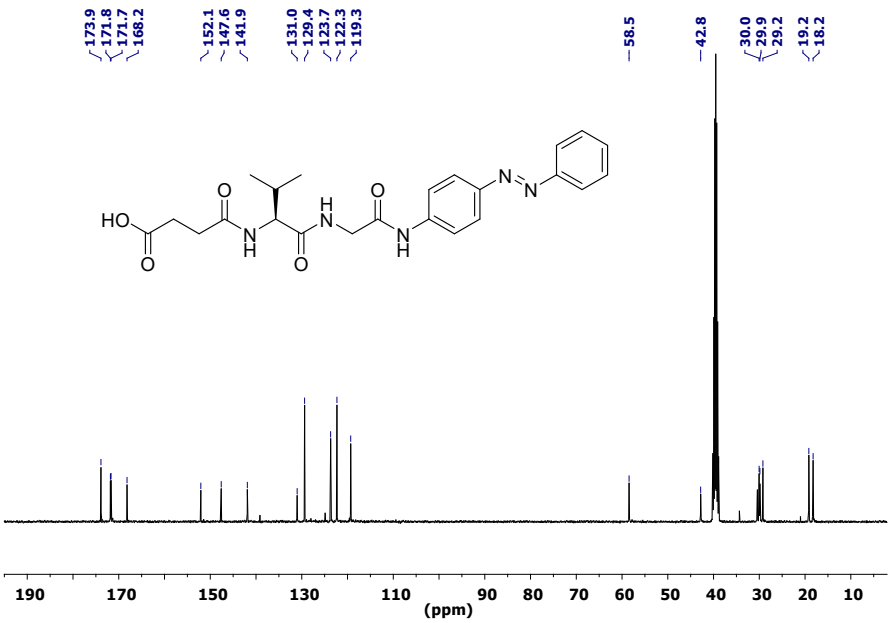


Fig. S8. ^{13}C NMR of **SucValGlyAzo**.

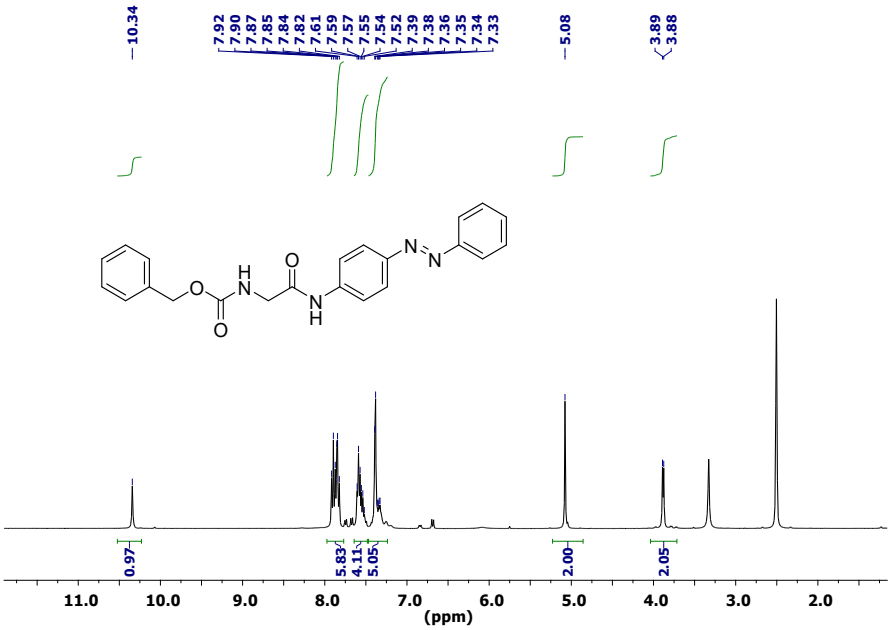


Fig. S9. ^{1}H NMR of 1.

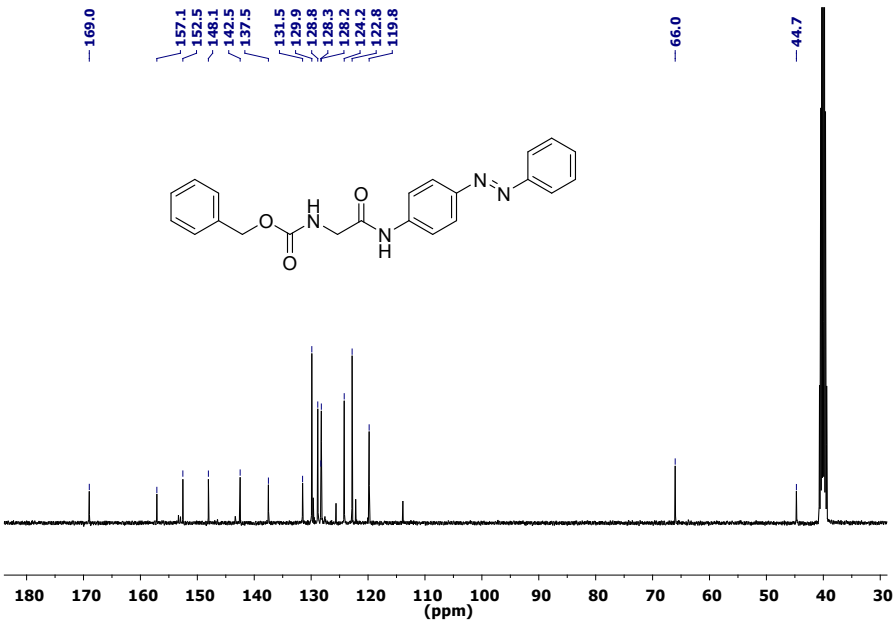


Fig. S10. ^{13}C NMR of 1.

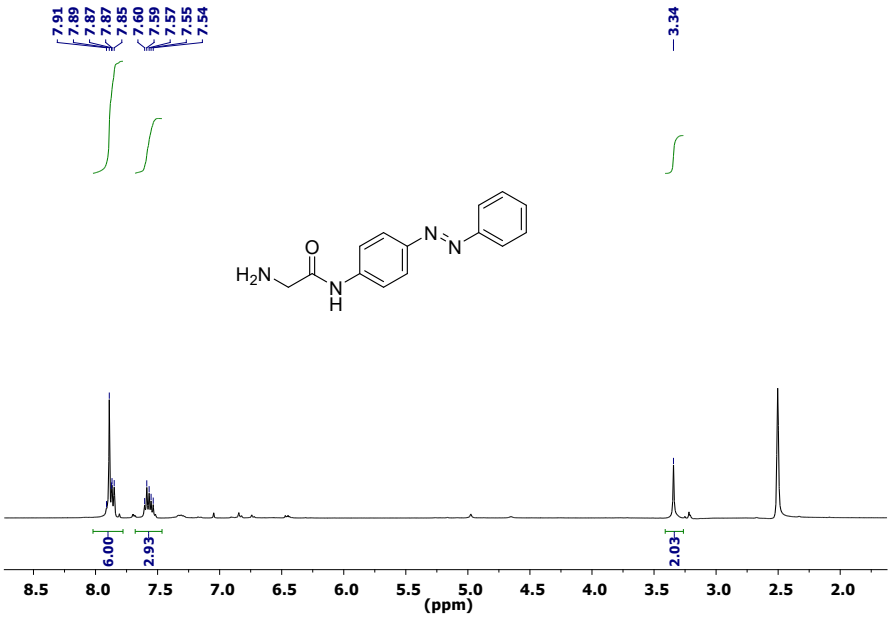


Fig. S11. ^{1}H NMR of 2.

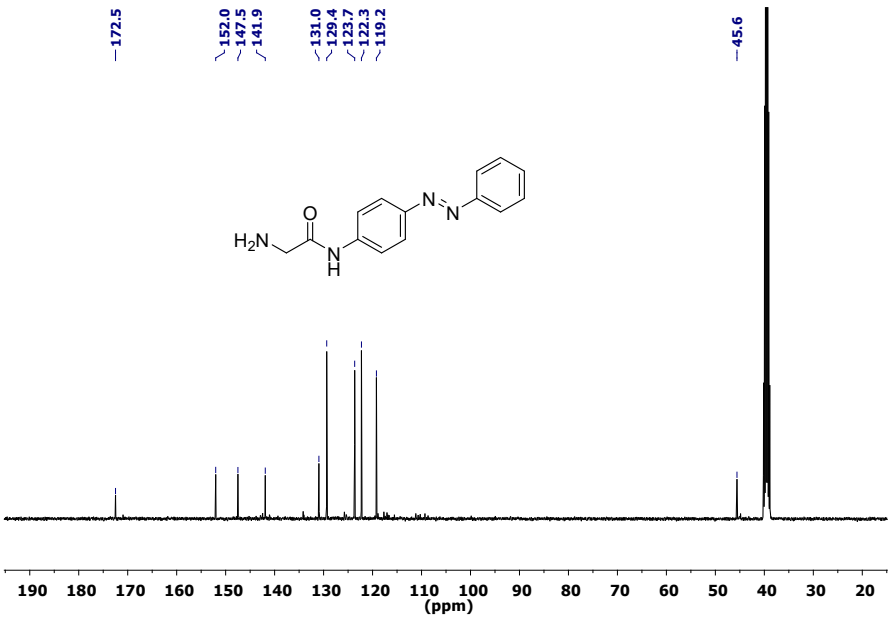


Fig. S12. ^{1}H NMR of 2.

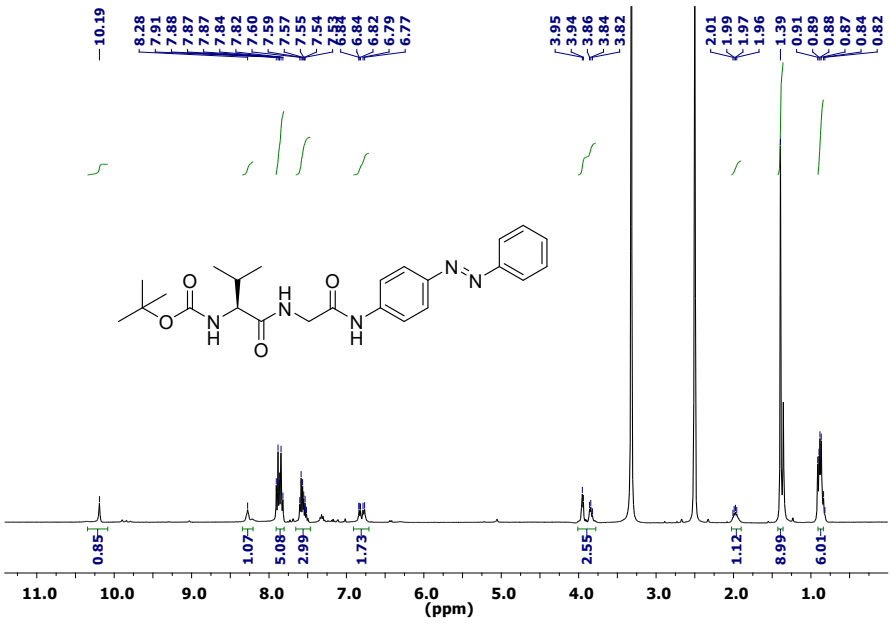


Fig. S13. ^{1}H NMR of 3.

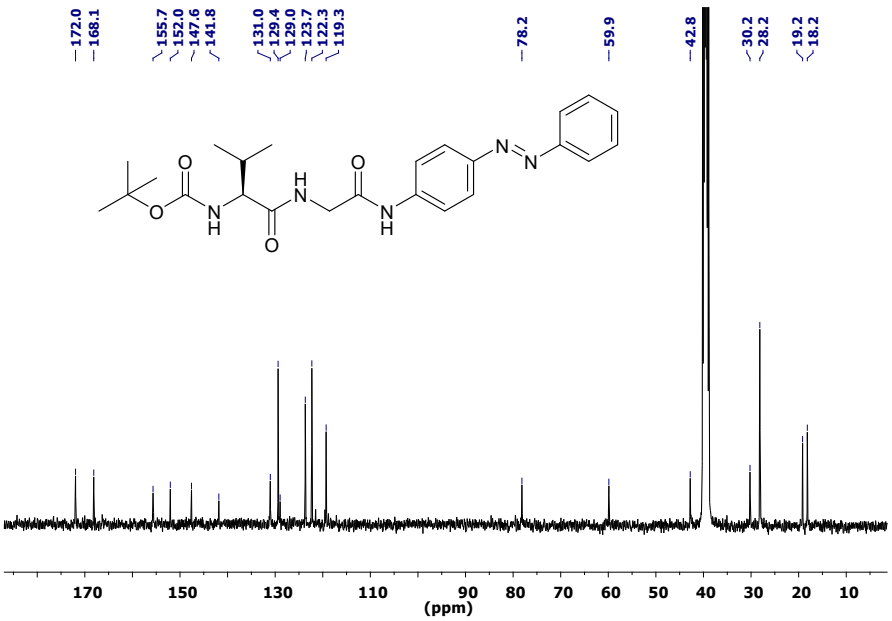


Fig. S14. ^{13}C NMR of 3.

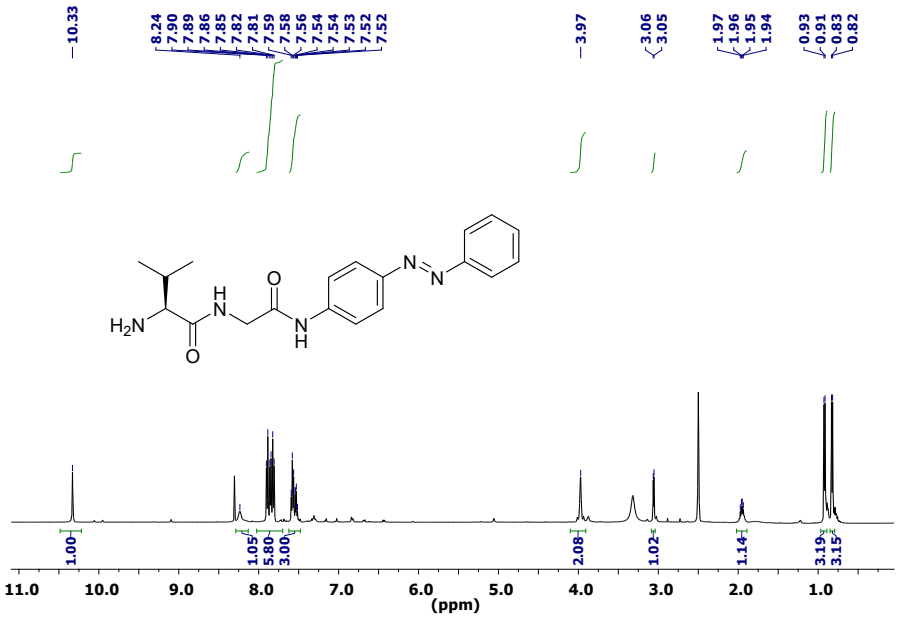


Fig. S15. ^{1}H NMR of 4.

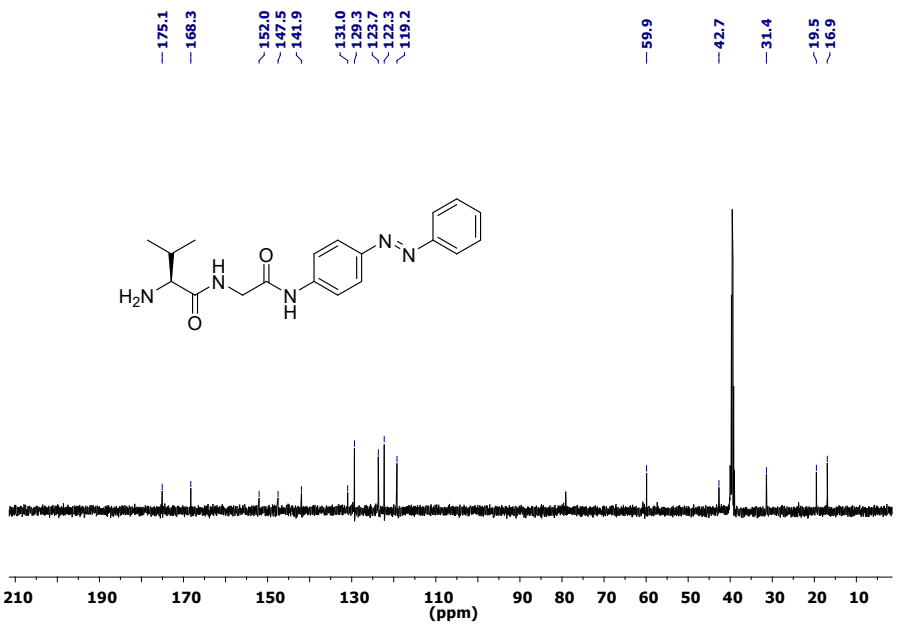


Fig. S16. ^{13}C NMR of 4.

References

1 M. Schönberger and D. Trauner, *Angew. Chemie - Int. Ed.*, 2014, **53**, 3264–3267.