Electronic Supplementary Information (ESI) for

Triazole-linked polyamides and polyesters derived from cholic acid

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Experimental Section

Materials

Cholic acid (CA, 98%), propargyl amine (98%), propargyl alcohol (99%), 1-ethyl-3-(3dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl, crystalline), 1hydroxybenzotriazole monohydrate (HOBt, 98%), sodium azide (NaN₃, 99.5%), copper (I) bromide (CuBr, 99.999%), copper (II) sulfate pentahydrate (CuSO₄· 5 H₂O, 98%), 2-methyl-2-propanol (*t*-BuOH, 99,7%); N,N,N',N",N" pentamethyldiethylene triamine (PMDETA) (99%), lithium bromide (LiBr, 99%), and sodium ascorbate (SA, 98%) were purchased from Aldrich and used as received. Tosyl chloride (TsCl, 98%, Aldrich) was purified by recrystallization from chloroform and petroleum ether twice prior to use. Pyridine (Py, 99.8%, Aldrich) and N,N-dimethylformamide (DMF, 99.8%, Aldrich) were dried over CaH₂ under dry nitrogen atmosphere for 24 h and distilled under reduced pressure prior to use.

Methods

¹H NMR spectra were measured on a Bruker AV-400 spectrometer operating at 400 MHz, and ¹³C NMR spectra on a Bruker AV-500 spectrometer operating at 125 MHz. DMSO-d₆ was used as the solvent, which also served as an internal reference (2.50 ppm for ¹H and 39.43 ppm for ¹³C). FT-IR spectra were recorded on TA Instruments Nicolet 6700 spectrometer equipped with attenuated total reflectance (ATR) accessory. High-resolution mass spectrometry (HRMS) was performed by LC/MSD TOF system (Agilent Technologies) in positive electrospray ionization (ESI) mode. Size exclusion chromatography (SEC) was performed on a system consisting of a Waters 600E pump, a Waters 717Plus autosampler, a set of two columns (Waters HR6, pore size 1×10^6 Å, and Phenomenex Phenogel, pore size 1×10^4 Å), a Dawn EOS multi-angle laser light scattering detector, and an Optilab rEX interferometric refractometer (both from Wyatt, $\lambda = 690$ nm). The eluent (DMF, 0.01 M LiBr) flow rate was 1.0 mL·min⁻¹ and the temperature of the columns 25 °C. The dn/dc value of cholic acid polymers was determined to be 0.0885 mL·g⁻¹ at 690 nm in DMF (25 °C).

Thermal and mechanical analyses

Thermogravimetric analyses (TGA) were performed on a Hi-Res TGA 2950 thermogravimetric analyzer (TA Instruments) under N₂ atmosphere. The decomposition temperature T_d was defined as the onset of decomposition. Differential scanning calorimetry (DSC) measurements were carried out on a TA Q1000 differential scanning calorimeter (TA Instruments) with a heating rate of 10.0 °C·min⁻¹. The glass transition temperature T_g was determined from the second cycle and defined as the inflection point in the change of the heat capacity.

Polymer films were prepared by evaporating a concentrated polymer solution in DMF (100 mg·mL⁻¹) in a PTFE mold (1.5 × 2 cm) at 145 °C under atmospheric pressure for 24 h and curing them for 1 h at 160 °C and for 1 h at 180 °C. Smaller rectangular samples (4 mm × 10 mm) were cut from these films and used for dynamic mechanical tests (dimensions of the films were measured with a digital caliper with a precision of 0.01 mm). Mechanical tests were performed on a DMA 2980 dynamic mechanical analyzer from TA Instruments. For controlled force (stress-strain) experiments, a preload force of 0.01 N and a force ramp of 0.2 N·min⁻¹ to 18 N were used. Dynamic experiments to measure T_g were performed at 1 Hz, a preload force of 0.01 N was applied to the sample subjected to the heating rate of 2 °C·min⁻¹, and an oscillation amplitude of 2 µm. The T_g values determined by DMA were defined as the onset in the change of the storage modulus.

Syntheses of cholic acid derivatives 4a and 4b (structures 1-5, Scheme S1)

Propargylation of cholic acid (1)

Compounds **2a** (amide) and **2b** (ester) were synthesized by a procedure modified from the one by Gonnade and coworkers.^[a] Cholic acid (1, 2.45 g, 6.00 mmol) was placed in a flamedried round-bottom flask equipped with a stir bar. Propargyl amine (0.78 ml, 12.0 mmol) or propargyl alcohol (0.70 ml, 12.0 mmol) in dry DMF (10 mL) was added to the flask under nitrogen atmosphere. The solution was cooled down to 0 °C and degassed with nitrogen for 10-15 min, after which EDC·HCl (1.73 g, 9.0 mmol) and HOBt (0.41 g, 3.00 mmol) were

^a N.S Vatmurge, B.G. Hazra, V.S. Pore, F. Shirazi, M.V. Deshpande, S. Kadreppa, S. Chattopadhyay, R.G. Gonnade, *Org. Biomol. Chem.*, 2008, **6**, 3823-3830.

added. After 30 minutes, the mixture was allowed to warm up to room temperature and stirred for 24 h. The reaction was quenched by pouring the mixture into a beaker filled with crushed ice, and extracted with ethyl acetate (3×100 mL). The combined extracts were washed with water (100 mL), 5% LiBr (aq. 50 mL), and brine (50 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. Column chromatography with ethyl acetate : hexane (9:1 or 4:1), followed by vacuum-drying afforded pure alkyne compounds **2a** and **2b** as white solids in yields of 84 and 81 %, respectively.

N-Propargyl-3α,7α,12α-trihydroxy-5β-cholan-24-amide (2a) ATR-FTIR v_{max} (cm⁻¹): 3392, 3301, 2926, 2864, 2113, 1649; HRMS (ESI) *m*/*z*: calculated for C₂₇H₄₄NO₄ 446.3265, found 446.3275 (M + H⁺).

Propargyl-3*α*,7*α*,12*α*-trihydroxy-5β-cholan-24-oate (2b) ATR-FTIR v_{max} (cm⁻¹): 3396, 3290, 2929, 2872, 2097, 1737; HRMS (ESI) *m/z*: calculated for C₂₇H₄₆NO₅ 464.3371, found 464.3384 (M + NH₄⁺).

Tosylation of compounds 2a and 2b

A modified procedure from Gouin and Zhu was applied.^[b] Compound **2a** (2.00 g, 4.50 mmol) or **2b** (1.90 g, 4.26 mmol) was dissolved in anhydrous pyridine (10 mL) in a flame-dried round-bottom flask with a stir bar, the solution was cooled down to 0 °C using an ice-water bath and degassed for 10-15 min under nitrogen atmosphere. A cold pyridine solution (10 mL) of p-tosyl chloride (0.88 g, 4.50 mmol) or (0.83 g, 4.26 mmol) was added dropwise to the mixture within 15 min. The reaction mixture was kept stirring at 0 °C for 1 h after the addition, then warmed up to room temperature and stirred overnight. The solvent was removed under reduced pressure and the residue dissolved in ethyl acetate (150 mL). The resulting solution was washed with water (3×50 mL) and brine (2×50 mL), and dried over anhydrous sodium sulfate. The solvent was removed to give crude **3a** and **3b** as white solids (yields 86 and 88 %, respectively), which were used for the next step of monomer synthesis without further purification.

^b S. Gouin, X.X. Zhu, Steroids, 1996, 61, 664-669.



Scheme S1. Structures of cholic acid (1), its derivatives (2-4), and polymers (5).

Azidation of compounds 3a and 3b

A literature procedure was adapted for the synthesis.^[c] A suspension of **3a** (2.00 g, 3.43 mmol) or **3b** (2.00 g, 3.42 mmol) and sodium azide (0.67 g, 10.3 mmol) in DMF (10 mL) was heated at 45 °C for 4 days. After filtration, the reaction was quenched by adding crushed ice

^c V. del Amo, L. Siracusa, T. Markidis, B. Baragaña, K.M. Bhattarai, M. Galobardes, G. Naredo, M.N. Pérez-Payán, A.P. Davis, *Org. Biomol. Chem.*, 2004, **2**, 3320-3328.

(50 mL), and extracted with ethyl acetate (3×50 mL). The combined extracts were washed with water (3×50 mL) and brine (2×50 mL), and dried over anhydrous sodium sulfate. The filtered solution was concentrated under reduced pressure. Column chromatography with ethyl acetate : hexane (4:1), followed by three recrystallizations from a mixture of ethyl acetate and hexane at 0 °C. Vacuum-drying afforded pure compounds **4a** and **4b** as white solids in yields of 74 and 71 %, respectively.

N-Propargyl-3β-azido-7α,12α-dihydroxy-5β-cholan-24-amide (4a) ATR-FTIR v_{max} (cm⁻¹): 3417, 3306, 2924, 2867, 2095, 1651; HRMS (ESI) *m/z*: calculated for C₂₇H₄₃N₄O₃ 471.3330, found 471.3345, (M + H⁺).

Propargyl-3β-azido-7α,12α-dihydroxy-5β-cholan-24-oate (4b) ATR-FTIR v_{max} (cm⁻¹): 3435, 3290, 2926, 2870, 2094, 1731; HRMS (ESI) *m/z*: calculated for C₂₇H₄₅N₄O₄ 489.3435, found 489.3437, (M + NH₄⁺).

Syntheses of polyamide 5a and polyester 5b

Anhydrous DMF, 5.0 mL, was added via a N₂-purged syringe to the flame-dried roundbottom flask containing compound **4a** (320 mg, 0.68 mmol) or **4b** (335 mg, 0.71 mmol) and degassed with nitrogen for 15-20 min. Next, N,N,N',N",N"-pentamethyldiethylene triamine (PMDETA, 0.72 mL, 3.40 mmol) was introduced via a N₂-purged syringe to the monomer solution. The resulting mixture was transferred into the another flame-dried flask containing copper(I) bromide (CuBr, 2.0 mg, 0.014 mmol) under nitrogen atmosphere equipped with a stir bar and a condenser. The mixture was stirred under nitrogen at 80 °C for 120 h in dark. After cooling to room temperature, the polymer was purified by precipitation from a DMSO : DMF mixture (1:1) into acetone. Vacuum-drying afforded a slightly yellow solid (ca. 280 mg) in yields of 88 and 84 % for **5a** and **5b**, respectively.

Synthesis of polyamide 5a'

The solvent tert-butanol (t-BuOH, 35 mL) was placed in a round-bottom flask equipped with a stir bar and a condenser, and degassed by bubbling nitrogen gas for 30 min. Compound **4a** (250 mg, 0.53 mmol) was added while stirring the solution vigorously under nitrogen. Sodium ascorbate (SA, 63.1 mg, 0.11 mmol) in 1.0 mL of deionized water was introduced to the mixture, followed by dropwise addition of copper sulfate pentahydrate (CuSO₄·5H₂O, 39.8 mg, 0.053 mmol) in 2.0 mL of deionized water. The reaction was carried out at 60 °C under nitrogen for 120 h in dark. After cooling to room temperature, the solvent

was removed under vacuum and a minimum amount of DMSO was added to dissolve the product, followed by precipitation (twice) in water. The resulting product was isolated by freeze-drying. The polymer was re-dissolved in dimethyl sulfoxide (DMSO, 1 mL), precipitated in ethyl acetate, and dried under vacuum, affording a slightly yellow solid, yield 48 %.

Spectroscopic data of the polymers

Polyamide 5a. ATR-FTIR υ_{max} (cm⁻¹): 3377, 2933, 2867, 1650. **Polyester 5b.** ATR-FTIR υ_{max} (cm⁻¹): 3424, 2942, 2867, 173, 1660. **Polyamide 5a'.** ATR-FTIR υ_{max} (cm⁻¹): 3435, 3307, 2935, 2869, 2097, 1654.

Table S1. ¹H and ¹³C NMR chemical shifts (in ppm) of the cholic acid-containing compounds and of the polymers in DMSO-d₆.

Proton	2a	3 a	4 a	5a	5a'	2b	3 b	4b	5b
3	3.18	4.25	3.99	4.59	4.59; 3.99*	3.18	4.25	3.99	4.63
7	3.61	3.58	3.62	3.64	3.64	3.62	3.58	3.62	3.64
12	3.78	3.75	3.78	3.79	3.80	3.78	3.76	3.78	3.78
18	0.58	0.56	0.58	0.58	0.58	0.59	0.57	0.59	0.56
19	0.81	0.78	0.85	0.79	0.85	0.81	0.78	0.85	0.78
21	0.92	0.92	0.92	0.92	0.92	0.93	0.91	0.92	0.90
25	8.19	8.19	8.19	8.22	8.20	-	-	-	-
26	3.82	3.81	3.81	4.27	4.27	4.67	4.67	4.67	5.10
28	3.06	3.06	3.06	-	3.06*	3.52	3.51	3.51	-
28'	-	-	-	7.91	7.90	-	-	-	8.17

Carbon	2a	4 a	5a	5a'	2b	4 b	5b
3	70.31	58.26	55.69	55.69; 58.26*	70.32	58.36	55.90
7	66.13	66.05	66.08	66.08	66.13	66.15	66.07
12	70.89	70.88	70.91	70.91	70.87	70.96	70.87
18	12.25	12.23	12.24	12.24	12.22	12.30	12.19
19	22.53	22.63	22.66	22.65	22.51	22.73	22.64
21	17.00	17.00	17.04	17.04	16.77	16.88	16.80
24	172.26	172.24	172.41	172.40	172.49	172.58	172.95
26	31.46	31.45	31.54	31.54	51.33	51.44	56.94
27	81.31	81.30	-	81.31*	78.47	78.57	-
27'	-	-	121.72	121.73	-	-	123.77
28	72.60	72.59	-	72.60*	77.33	77.43	-
28'	-	-	144.35	144.38	-	-	144.36

* values for the end group



Figure S1. (A) FTIR spectra of compound **4a** (black), and the polyamides **5a'** (red) and **5a** (blue), (B) FTIR spectra of compound **4b** (black) and the polyester **5b** (green).



Figure S2. Size exclusion chromatograms (SEC) of the polyamides **5a'** and **5a**, and the polyester **5b**. Eluent: DMF; flow rate: $1 \text{ mL} \cdot \text{min}^{-1}$; temperature: 25 °C.



Figure S3. ¹H NMR spectra of the heterofunctional α -propargyl ester- ω -azide cholic acid derivative **4b** (bottom) and the corresponding polyester **5b** (top) in DMSO-d₆. The insets correspond to the expanded regions of the spectra marked with dashed lines.



Figure S4. ¹H NMR spectra of the heterofunctional α -propargyl amide- ω -azide cholic acid derivative **4a** (bottom) and the corresponding polyamide **5a'** (top) in DMSO-d₆. The insets correspond to the expanded regions of the spectra marked with dashed lines.



Figure S5. (A) Traces of thermogravimetric analysis (TGA) of the polymers; (B) Heating and cooling curves of the second thermal cycles of the polymers by differential scanning calorimetry (DSC). Heating and cooling rate: 10 °C·min⁻¹. The arrow points to the glass transition temperature (T_g) of **5b**. Polyamides **5a'** (solid), **5a** (dash-dots) and polyester **5b** (dashes) in both figures.



Figure S6. Stress-strain curves of the polyamide **5a** (dashes) and the polyester **5b** (solid) obtained at 30 °C.