Supplementary Material (ESI) for Chemical Communications

Archaeosomes based on synthetic tetraether-like lipids as novel versatile gene delivery systems

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Supporting information

Synthetic part

I. Materials and reagents

General Methods. All commercially available chemicals were used without further purification, and solvents were carefully dried and distilled prior to use. All nonhydrolytic reactions were carried out under a nitrogen atmosphere. Analytical TLC was performed on Merck 60 F_{254} silica gel nonactivated plates and visualization was done with UV light both short and long range (254nm and 365nm), or by spraying with a 5% H_2SO_4 solution in EtOH or a ninhydrine solution followed by charring with a heat gun. ^1H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively. Tetramethylsilane was used as reference for ^1H NMR. High resolution mass spectra were determined on a MS/MS ZabSpec TOF Micromass spectrometer (Electrospray positive mode, SM-ESI+).

II. Experimentals and spectral data

Neutral tetraether GR:

To a suspension of potassium hydride (1.072 g, 8 mmol, 4 equiv) in dry THF (10 mL) was added a solution of alcohol **4** (2.78 g, 6 mmol, 3 equiv) in dry THF (11 mL) at 0 °C under a vigourous agitation and the reaction mixture was stirred at room temperature for 1 h. A solution of ditriflate **5** (0.85 g, 1.1 mmol, 1 equiv) in dry THF was added and the reaction mixture was stirred at room temperature overnight under nitrogen atmosphere. Water was added and the aqueous phase was extracted with Et_2O. The combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (PE/EtOAc: 99:1) to yield the corresponding dibenzylated

tetraether (1.32 g, 45%) as a colorless oil. R_f = 0.66 (PE/EtOAc: 90:10). $[\alpha]^{20}_D$: +1.7° (*c* 1.10, $CHCl_3$). A mixture of this protected diol (0.239 g, 1.72 mmol, 1 equiv) in THF/EtOH (6 mL, 1:1) and palladium dihydroxide (25 mg, 10% w/w) was stirred at room temperature for 12 h under hydrogen atmosphere. The reaction mixture was filtered on celite and the solvents were evaporated under reduced pressure to yield GR (0.177 g, 85%) as a colorless oil. R*f* = 0.2 (petroleum ether/ ethyl acetate, 8/2 v/v). $[\alpha]^{20}_D$ +9.1 (*c* 1, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ 0.83-0.87 (m, 30H), 0.90-0.98 (dt, 1H), 1.06-1.66 (m, 90H), 1.68-1.78 (m, 2H), 1.91-1.99 (m, 1H), 2.13-2.24 (m, 2H), 3.28 (d, 4H), 3.39 (t, 4H), 3.43 (t, 8H), 3.50-3.70 (m, 10H). ^{13}C-NMR ($CDCl_3$): δ 19.7-19.8, 22.7, 22.8, 24.4-24.6, 26.2, 26.3, 28.1, 28.9, 29.6-29.9, 32.9, 34.0, 37.1-37.6, 39.4, 39.8, 63.2, 68.7, 70.9-71.9, 75.7, 78.3. Elemental analysis calcd (%) for $C_{77}H_{154}O_8$: C, 76.56; H, 12.85. Found: 76.08; H, 12.60.

Dicationic tetraether GRcat:

A mixture of diamine **6** (90 mg, 0.06 mmol, 1 equiv) and activated glycine betaine **7** (60 mg, 0.27 mmol, 3 equiv) in DMF/Et_3N (11 mL, 10:1) was stirred at room temperature overnight. The reaction mixture was concentrated under reduced pressure and the residue was purified by flash chromatography on silica gel ($CHCl_3$/acetone/MeOH/NH_4OH: 8:6:5:1) to yield GRcat (75 mg, 80%) as a red gum. R*f* = 0.4 ($CHCl_3$/CH_3COCH_3/CH_3OH/NH_4OH, 8/6/5/1 v/v/v/v). $[\alpha]^{20}_D$ +13.1 (*c* 0.81, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ 0.83-0.87 (m, 30H), 0.90-2.24 (m, 96H), 3.18-3.60 (m, 26H), 3.20 (s, 18H), 5.04 (s, 4H); ^{13}C-NMR ($CDCl_3$): δ 19.7-19.8, 22.7, 22.8, 24.3-39.4, 24.9-43.2, 54.7, 65.3, 68.6-77.3. SM-ESI+: m/z calcd for $C_{87}H_{176}N_4O_8$ 702.67441, found 702.6745 [M^{++}].

HPTLC measurements

I. Methods

HPTLC plates were pre-washed with methanol to eliminate interference from material intrinsic to the plate, dried at 120°C for 30 min and cooled in a dessiccator. Standards were prepared by dissolving appropriate amount of considered lipid pairs (MM18 and DOPE, MM18 and GR) in chloroform. Aqueous solutions of liposomes and archaeosomes were lyophilized and the resulting dried lipid mixtures were dissolved in an appropriate volume of chloroform.

Standards and samples corresponding to one lipid pair were spotted using the Automated TLC Sampler 4 from Camag.

Plates were developed in a HPTLC tank with the following solvent mixtures :

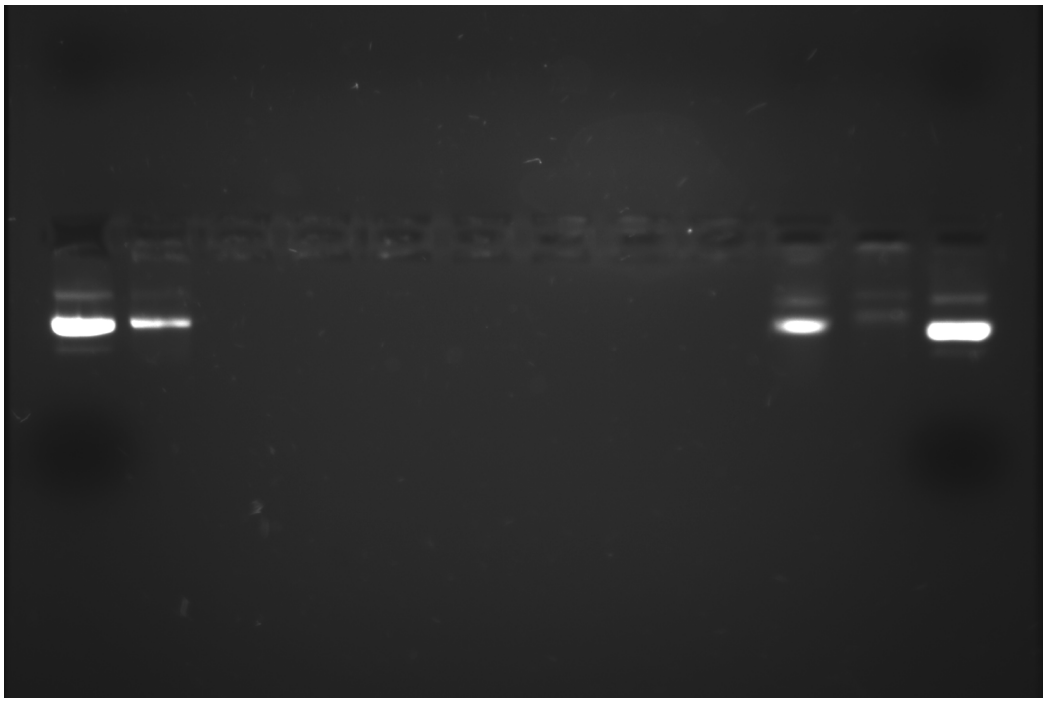
- MM18/DOPE lipid pair : CH_2Cl_2 / MeOH 60/40 + 7% NH_4OH
- MM18/GR lipid pair : CH_2Cl_2 / MeOH 80/20 + 7% NH_4OH for MM18 and CH_2Cl_2 / MeOH 95/15 for GR.

After drying, the plates were derivatized with a primuline solution and scanned from the origin to the solvent front by using a TLC scanner 3 densitometer from Camag. Plates were scanned at 366 nm (deuterium source) in fluorescence mode.

II. Experimental data: Lipid compositions of liposomes and archaeosomes

	% MM18	% Co-lipid
MM18/DOPE 5%	92.8	7.2
MM18/DOPE 15%	90.2	7.8
MM18/GR 5%	95.8	4.2
MM18/GR 15%	89.5	10.5

Gel retardation: binding between GRcat and pDNA



Samples from the left to the right:

- Uncomplexed plasmid DNA
- Lipoplexes (GR Cat/pDNA) with increasing charge ratios (+/-) : 1, 2, 3, 4, 5, 6, 7 and 8
- polyplexes (PEI 25 K/pDNA) with charge ratio 1 and 2
- Uncomplexed plasmid DNA