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Introduction of cyclic guanidines into cationic lipids for non-viral gene delivery

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Abstract

In order to study the impact of chemical modifications of lipopolyamines on their gene delivery properties, we have introduced cyclic guanidines into the polyamine moiety. These lipopolyamino-cycloguanidines can be easily obtained by reacting polyamines with 2-methylmercapto-2-imidazolinium iodide or 2-methylmercapto tetrahydropyrimidinium iodide. These lipopolyamino-cycloguanidines constitute a novel family of cationic lipids.

Keywords: polyamine; cyclic guanidine; cationic lipid; gene delivery.

Lipopolyamines form well defined self-assembling complexes with DNA, and are of great interest as carriers for gene delivery. However, their efficiency remains to be improved.[1,2] We and others, have recently explored the introduction of guanidium groups as cationic heads into our lipopolyamines.[3–9] Transformation of amines into guanidines can be performed using *O*-methylisouronium sulfate or *S*-methylisothiouronium sulfate.[10] Under these conditions, we were surprised to always obtain a mixture of two compounds. The first was the expected aliphatic lipopolyamino-guanidine (see **1** and **3** in Fig. 1) and the second product was a lipopolyamino-cycloguanidine, which was obtained as a side reaction product,[11] (see **2** and **4** in Fig. 1**)**.

The first family of aliphatic guanidines has been further developed using *N*,*N*′-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea, which reacts selectively with primary amine to give exclusively the aliphatic product in good yields.[4] The increasing interest in amidinium containing cationic lipids as gene delivery vectors by different groups,[3–9] prompted us to develop an extended family of cationic lipids bearing these cyclic guanidines. Difficulties for separating the mixture of cyclic (**2**, **4**) and aliphatic (**1**, **3**) products, and the low yield obtained for each of the products, prompted us to use an alternative synthetic procedure for acceding to cyclic guanidines. The cyclic guanidines (aminoimidazolines)

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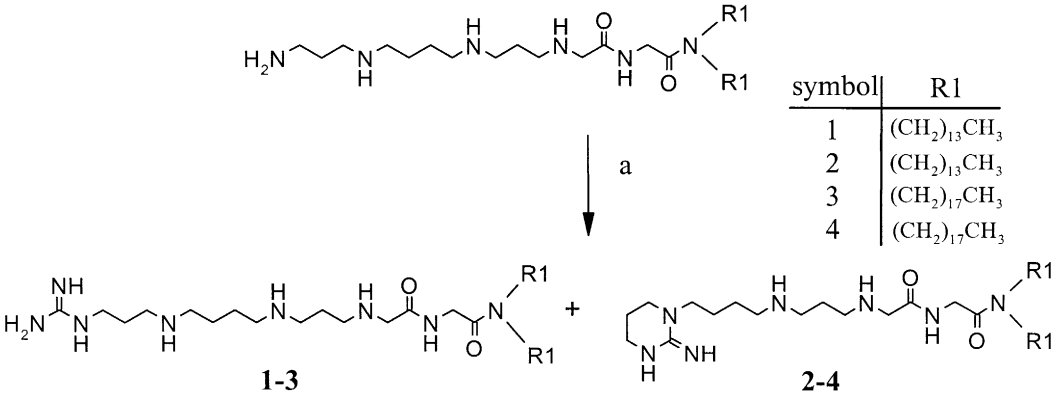

Fig. 1. Synthesis of libraries of lipopolyamino-cycloguanidines/lipopolyamino-guanidines. a: *O*-methyl-isourea or *S*-methyl isothiourea in MeOH/H_2O, TEA, 40°C, HPLC separation (see Ref. 14 for structural data)

were previously used as a ribonuclease active site model system and their ability to cleave RNA was tested in those model compounds.[12] From these previous synthetic methods, we have found a specific reaction to obtain directly a cyclic guanidine starting from primary amino groups using either 2-methylmercapto-2-imidazolinium iodide (to give a five member ring guanidine in products **5**–**6**) or 2-methylmercapto tetrahydropyrimidinium iodide (to give a six member ring guanidine in products **7**–**8**).[13,14] The feasibility of this method for polyamines was assessed here by the synthesis of various geometrically differing lipopolyamino-cycloguanidines, starting from their lipopolyamine counterparts using either commercially available 2-methylmercapto-2-imidazolinium iodide (*n*=1) or 2-methylmercapto tetrahydropyrimidinium iodide (*n*=2) synthesised in our laboratory as previously described[13] (see Fig. 2).

Briefly, the lipopolyamine was dissolved in dichloromethane at room temperature under an argon atmosphere. Triethylamine and 2-methylmercapto-2-imidazolinium iodide or 2-methylmercapto tetrahydropyrimidinium iodide were added in one portion. The evolution of methyl–sulfide was immediately remarked upon. The progress of the reaction was monitored by HPLC. All the reactions were completed after 12 h at 25°C. The solvent was evaporated, and Boc protecting groups were cleaved using TFA for products **6**–**8**. After evaporation the products were directly purified by HPLC using C4 reverse phase column with a gradient of water/acetonitrile. Yields were between 34–68% after HPLC purification. Products were characterised by analytical HPLC,[15] and by NMR and mass spectra.[16,17]

In conclusion, we have synthesised a novel cationic lipid family bearing cyclic guanidines as cationic head. The feasibility of the synthetic method was demonstrated by the synthesis of various geometrically differing cationic lipids using three different reagents. The biological evaluation and physico-chemical characterisation of various lipopolyamino-cycloguanidines are currently ongoing and will be described elsewhere.

Acknowledgements

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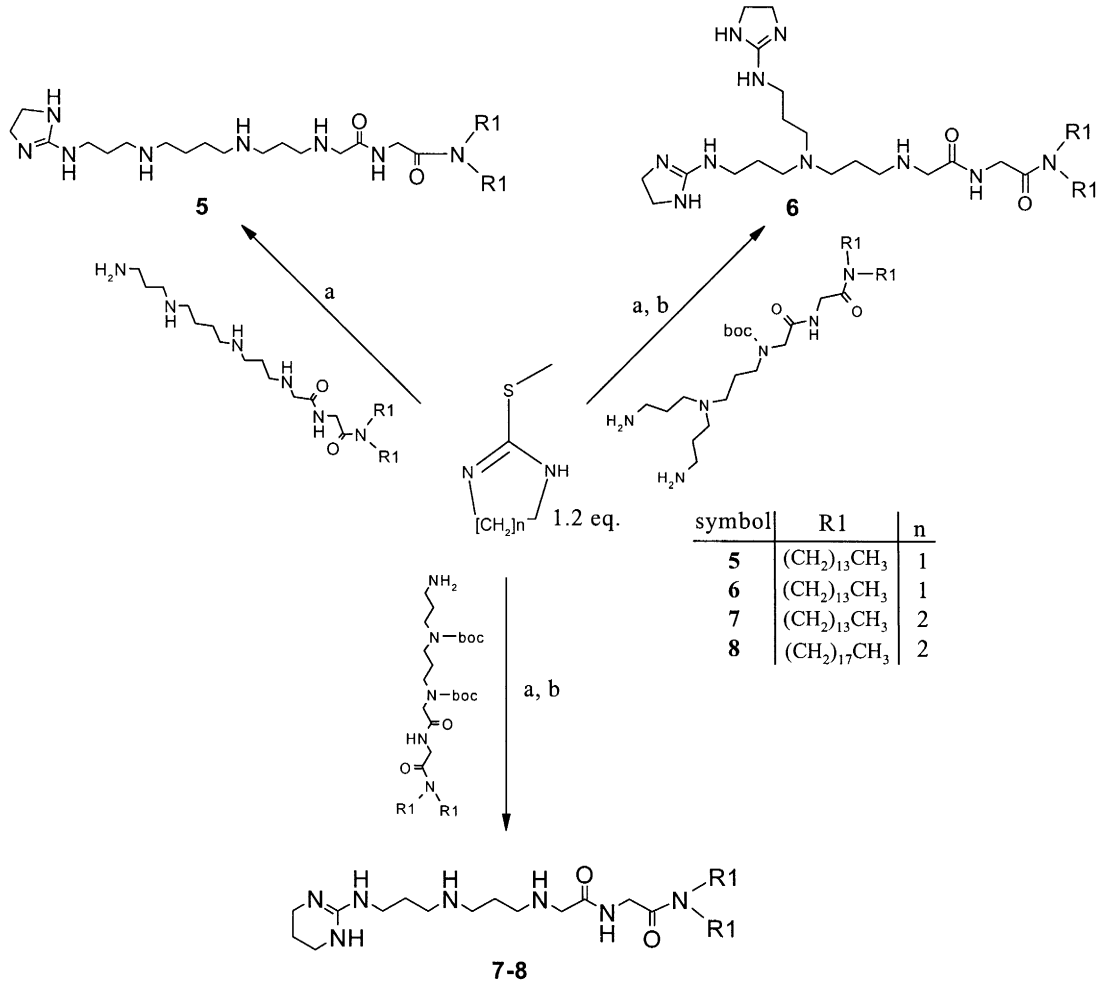

Fig. 2. Synthesis of lipopolyamino-cycloguanidines. a: dichloromethane, TEA (1.3 equiv.), 20°C, overnight, HPLC purification. b: TFA/DCM (1:1) 1 h 20°C (see Ref. 15 for structural data)

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14. Kane, J. M.; Carr, A. A.; Cheng, H. C.; Dubley, M. W.; Rampe, D.; Staeger, M. A. *Bioorg. Ed. Chem. Lett*. **1994**, *4*, 351–354.
15. Analytical HPLC were performed on a Merck–Hitachi gradient pump equipped with a AS-2000A autosampler, an L-6200A Intelligent pump and a UV–vis detector L-4000 with tunable wavelength set at 220 nm. Mobil phases were: H_2O (0.1% TFA) and MeCN (0.08% TFA). Column BU-300 aquapore butyl 7 μ, 300A 300×4.6 mm from Perkin–Elmer, gradient H_2O/MeCN: 3 min [40/60], 3–20 min [0/100], 35 min [0/100], flow: 1 mL/min.
16. *Product **1***: Yield 22%, HPLC, R_t=9.84 min, ^{1}H NMR (400 MHz, $(CD_3)_2SO$-d_6, δ in ppm): 0.87 (t, J=7 Hz, 6H: CH_3 from lipid chains); 1.15–1.40 (m, 44H: $(CH_2)_{11}$ central methylenes from lipid chains); 1.46 and 1.55 (2 m, 2H each: 1 CH_2 from lipid chains, respectively); 1.63 (m, 4H: 2 CH_2 central from butilene); 1.81 and 1.95 (2 m, 2H each: CH_2 centrals from propylene); 2.85–3.10 (m, 10H: 2 NCH_2 butylene-2 NCH_2 from one propylene and 1 of the 2 NCH_2 of the other propylene); 3.15–3.25 (m, 6H: last NCH_2 from remaining propylene and NCH_2 from lipid chains); 3.82 (bb, 2H: NCH_2CON); 4.04 (d, J=5 Hz, 2H: $CONCH_2CON$ from Gly); 7.00–7.60; 8.60–8.75 and 9.00 (vbb and 2 bb, 3H–5H and 2H, respectively: NH_3^+ $CFCOO^-$ -$NH_2^+CF3COO^-$ and C=NH); 7.78 (bt, J=5.5 Hz, 1H: N=CNH); 8.65 (bb: 1H CONH). MH^+: 751. *Product **2***: Yield 19.5%, HPLC, R_t=10.76 min, ^{1}H NMR (400 MHz, $(CD_3)_2SO$-d_6, δ in ppm): 0.88 (t, J=7 Hz, 6H: CH_3 from lipid chains); 1.15–1.40 (m, 44H: $(CH_2)_{11}$ central methylenes from lipid chains); 1.45 and 1.55–165 (2 m, 2H each: 1 CH_2 from lipid chains, respectively); 1.59 (m, 4H: 2 CH_2 central from butylene); 1.91 and 1.97 (2 m, 2H each: CH_2 central from propylene); 2.85–3.10 (m, 10H: 2 NCH_2 butylene-2 NCH_2 from one propylene and 1 of the 2 NCH_2 of the other propylene); 3.23 and 3.30–3.50 (2 m, respectively, 5H and 1H: last NCH_2 from remaining propylene and NCH_2 from lipid chains); 3.79 (bb, 2H: NCH_2CON); 4.03 (d, J=5 Hz, 2H: $CONCH_2CON$ from Gly); 7.27 and 8.40–9.00 (respectively bs and bb, 2H and 4H: $NH_2^+CF3COO^-$, NH^+ $CF3COO^-$and 2=NH); 7.8 and 8.61 (respectively s and bs, 1H each: NHC=N and CONH). MH^+: 734. *Product **3***: Yield 20.1%, HPLC, R_t=14.94 min, ^{1}H NMR (400 MHz, $(CD_3)_2SO$-d_6, δ in ppm): 0.86 (t, J=7 Hz, 6H: CH_3 from lipid chains); 1.24 (m, 44H: $(CH_2)_{15}$ central methylenes from lipid chains); 1.43 and 1.53 (2 m, 2H each: 1 CH_2 from lipid chains, respectively); 1.63 (m, 4H: 2 CH_2 central from butylene); 1.81 and 1.96 (2 m, 2H each: CH_2 centrals from propylene); 2.85–3.10 and 3.22 (2 m, 16H: NCH_2 butylene-NCH_2 propylene and NCH_2 from lipid chains); 3.81 (bb, 2H: NCH_2CON); 4.03 (d, J=4.5 Hz, 2H: $CONCH_2CON$ from Gly); 7.32–7.97; 8.62–8.75 and 9.02 (bb, t, t, bb and bb, respectively: acidic protons). MH^+: 863. *Product **4***: Yield 20.8%, HPLC, R_t=15.99 min, ^{1}H NMR (400 MHz, $(CD_3)_2SO$-d_6, δ in ppm): 0.88 (t, J=6.5 Hz, 6H: CH_3 from lipid chains); 1.24 (m, 60H: CH_2 central methylenes from lipid chains); 1.35–1.70 (m, 4H: 1 CH_2 from lipid chains); 1.57 (m, 4H: 2 CH_2 central from butylene); 1.88 and 1.96 (2 m, 2H each: CH_2 central from propylene and CH_2 central from ring); 2.85–3.35 (2 m, 16H: 8 NCH_2); 3.81 (bs, 2H: NCH_2CON); 4.03 (d, J=5 Hz, 2H: $CONCH_2CON$ from Gly); 7.25 and 7.84 (respectively, s and bs, 1H each: 2 NH from ring); 8.61 (t, J=5.5 Hz, 1H: NHCO); 8.70 and 9.02 (2 bs, 1H each: 2 NH). MH^+: 846.
17. *Product **5***: Yield 48%, HPLC, R_t=8.90 min, ^{1}H NMR (400 MHz, $(CD_3)_2SO$-d_6 with some drops of CD_3COOD-d_4, δ in ppm): 0.87 (t, J=7 Hz, 6H: CH_3 from lipid chains); 1.15–1.40 (m, 44H: $(CH_2)_{11}$ central methylenes from lipid chains); 1.45 and 1.55 (2 m, 2H each: 1 CH_2 from lipid chains, respectively); 1.65 (m, 4H: 2 CH_2 central from butylene); 1.80–1.95 (m, 4H: CH_2 central from propylene); 2.85–3.05 (m, 10H: 2 NCH_2 butylene–2 NCH_2 from one propylene and 1 of the 2 NCH_2 of the other propylene); 3.24 (m, 6H: last NCH_2 from remaining propylene and NCH_2 from lipid chains); 3.56 (s, 2H: NCH_2CON); 3.62 (s, 4H: NCH_2CH_2N); 4.02 (d, J=5 Hz, 2H: $CONCH_2CON$ from Gly). MH^+: 777. *Product **6***: Yield 34%, HPLC, R_t=10.07 min, ^{1}H NMR (400 MHz, $(CD_3)_2SO$-d_6, at a temperature of 383 K, δ in ppm): 0.92 (t, J=7 Hz, 6H: CH_3 from lipid chains); 1.25–1.45 (m, 44H: $(CH_2)_{11}$ central methylenes from lipid chains); 1.57 (m, 4H: 1 CH_2 from lipid chains, respectively); 1.70–1.90 (m, 6H: 2 CH_2 central from propylene); 2.50–3.40 (m, 16H: 2 NCH_2 from propylene and the NCH_2 from lipid chains); 3.68 (s, 8H: 2 NCH_2CH_2N); 3.72 (bs, 2H: NCH_2CON); 4.06 (d, J=5 Hz, 2H: $CONCH_2CON$ from Gly). MH^+: 831. *Product **7***: Yield 38%, HPLC, R_t=8.42 min, ^{1}H NMR (400 MHz, $(CD_3)_2SO$-d_6, δ in ppm): 0.86 (t, J=7 Hz, 6H: CH_3 from lipid chains); 1.10–1.35 (m, 44H: $(CH_2)_{11}$ central methylenes from lipid chains); 1.44 and 1.53 (2 m, 2H each: 1 CH_2 from lipid chains, respectively); 1.80–2.00 (m, 6H: CH_2 central from propylene and CH_2 from 1,4,5,6–tetrahydro-pyrimidine); 2.80–3.10 (m, 10H: NCH_2 from propylene and the NCH_2 from 1,4,5,6-tetrahydro-pyrimidine); 3.15–3.45 (m,

6H: $=NCH_2$ from 1,4,5,6-tetrahydro-pyrimidine and NCH_2 from lipid chains); 3.81 (bb, 2H: NCH_2CON); 4.04 (d, J=5 Hz, 2H: $CONCH_2CON$ from Gly); 7.89–8.62–8.75 and 9.01 (4 bs, 8H: acidic protons). MH^+: 720. *Product **8***: Yield 68%, HPLC, R_t=15.83 min, ^1H NMR (400 MHz, $(CD_3)_2SO$-d_6, δ in ppm): 0.88 (t, J=7 Hz, 6H: CH_3 from lipid chains); 1.15–1.35 (m, 60H: $(CH_2)_{15}$ central methylenes from lipid chains); 1.46 and 1.54 (2 m, 2H each: 1 CH_2 from lipid chains, respectively); 1.80–2.00 (m, 6H: CH_2 central from propylene and CH_2 from 1,4,5,6-tetrahydro-pyrimidine); 2.85–3.05 (m, 10H: NCH_2 from propylene and the NCH_2 from 1,4,5,6-tetrahydro-pyrimidine); 3.15–3.45 (m, 6H: $=NCH_2$ from 1,4,5,6-tetrahydro-pyrimidine and NCH_2 from lipid chains); 3.81 (bb, 2H: NCH_2CON); 4.04 (d, J=5 Hz, 2H: $CONCH_2CON$ from Gly); 7.88–8.61–8.74 and 8.99 (4 bs, 8H: acidic protons). MH^+: 832.