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Synthesis and stability study of the new pentammonio lipid pcTG90, a gene transfer agent

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Abstract

The cationic lipid pcTG90 has been prepared from (*S*)-1-aminopropane-2,3-diol by *N*-acylation with *N*-protected 18-amino-3,7,11,15-tetraazaoctadecanoic acid and *O*-acylation with oleic acid. The former acid could be obtained from 1,3-propanediamine via tetraprotected caldopentamine. The stability of the cationic lipid in HEPES buffer has been studied.

Keywords: polyamines; amphiphilic compounds.

In the course of our program on the design, synthesis, and evaluation of new cationic lipids for gene transfer,[1] we have shown on a family of amphiphilic triacylglycerols with tri-, tetra-, and pentammonio polar heads that the highest transfection efficacy in in vitro assays could be obtained with the lipid **pcTG56**, in which glycerol bears two oleoyl groups and a pentammonio polar head.[2] However, this compound showed slow degradation in the 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) buffer solution used for formulation studies. The hydrolysis of the *sn*-3 ester function seemed to be involved since 1,2-dioleoyl glycerol was identified among the degradation products. Therefore, we have decided to prepare the amide analogue **pcTG90** in which the same polar head is linked to the hydrophobic moiety of the molecule by an amide function which we expected to be more resistant to hydrolysis. Since in vivo tests required multigram amounts of cationic lipids, we have also worked out a shorter and more efficient synthesis for **pcTG90** than the one used for **pcTG56**.[2]

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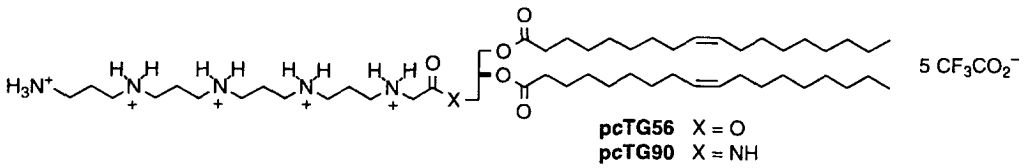
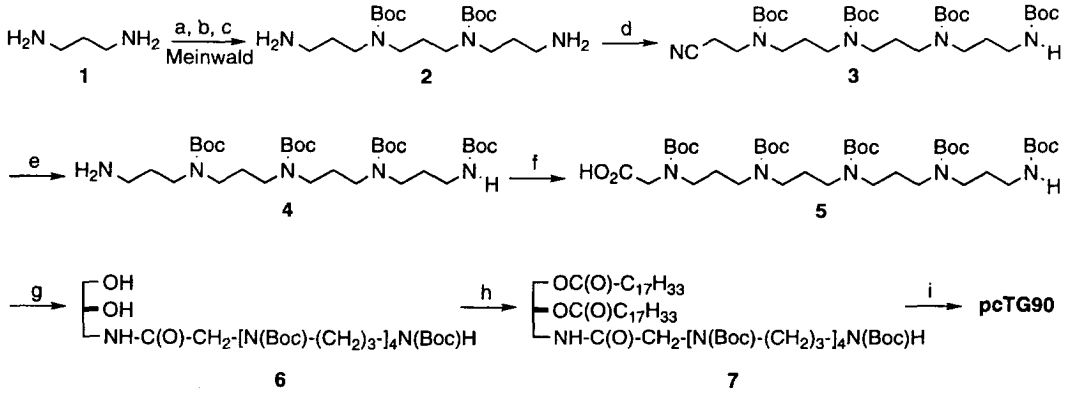


The preparation of **pcTG90** required first the obtention of the *N*-protected pentamino acid **5** and hence that of the tetraprotected caldopentamine **4**.[3] By subjecting commercially available 1,3-propanediamine **1** to the three-step sequence — cyanoethylation, *t*-butoxycarbonyl (Boc) protection of the amines, and reduction of the cyano groups — described earlier by Meinwald,[4,5] we obtained the diprotected thermine **2** (Scheme 1). This compound could then be monocyanoethylated by reaction with one equivalent of acrylonitrile and, after treatment with ditertiobutyl dicarbonate, gave the nitrile **3** which was reduced to the amine **4**[6] by catalytic hydrogenation on Raney nickel. This sequence furnished **4** in 43% overall yield from **1** on a multigram scale and was found to be more convenient than other syntheses of such tetraprotected pentamines.[7] Three additional steps in one pot — imine formation by reaction of amine **4** with glyoxylic acid, catalytic hydrogenation on palladium, and protection of the resulting secondary amine with Boc — led to the *N*-protected pentamino acid **5**[6] in 73% yield.



Scheme 1. (a) CH_2=CHCN (2.0 equiv.), EtOH, rt, 16 h (yield: 88%); (b) $(Boc)_2O$ (2.2 equiv.), $(i\text{-Pr})_2NEt$ (2.0 equiv.), rt, 4 h (98%); (c) H_2 (gas bag), Raney Ni, EtOH, NaOH (2.5 equiv.), rt, 16 h (88%); (d) CH_2=CHCN (1.0 equiv.; dropwise addition), EtOH, 0°C, then rt, 16 h; then concentrate in vacuo and add $(Boc)_2O$ (2.2 equiv.), $(i\text{-Pr})_2NEt$ (2.0 equiv.), THF, rt, 3 h (63%); (e) H_2 (gas bag), Raney Ni, EtOH, NaOH (1.5 equiv.), rt, 16 h (91%); (f) OHC-CO_2H (1.1 equiv.), MeOH, rt, 0.5 h; then H_2 (gas bag), 10% Pd/C, rt, 4 h; then flush with Ar and add $(Boc)_2O$ (1.5 equiv.), $(i\text{-Pr})_2NEt$ (1.5 equiv.), rt, 3 h (73%); (g) *N*-hydroxysuccinimide (1.1 equiv.), DCC (1.1 equiv.), dioxane, rt, 16 h; then (*S*)-1-aminopropane-2,3-diol (2.0 equiv.) in DMF, rt, 2 h (79%); (h) oleic acid (2.2 equiv.), DCC (2.2 equiv.), DMAP (0.1 equiv.), CH_2Cl_2, rt, 16 h (62%); (i) CF_3CO_2H:CH_2Cl_2 (1:1, v/v), 0°C, 3 h (91%)

The synthesis of **pcTG90** was then undertaken from (*S*)-1-aminopropane-2,3-diol, prepared by treatment of commercially available (*R*)-glycidol with cold aqueous ammonia.[8] *N*-Acylation with acid **5**, activated as its *N*-succinimidyl ester, and *O*-acylation with oleic acid in the presence of 1,3-dicyclohexylcarbodiimide (DCC) led to compound **7**[6] which, by removal of the Boc protecting groups, gave **pcTG90**.[9]

Stability studies consisting of the storage of **pcTG56** and of **pcTG90** in HEPES buffer at 4°C and at 21°C for variable periods of time and in the determination of the percentage of recovered cationic lipid were carried out.[10] They showed a higher stability at room temperature for **pcTG90** than for **pcTG56** (Table 1). **pcTG90** was also tested as a gene transfer agent and exhibited high levels of transfection activity in vitro and in vivo.[11]

Table 1
Percentage of recovered **pcTG56** and **pcTG90** after storage in HEPES buffer

Storage time (days)	Recovered **pcTG56** after storage at 4°C	Recovered **pcTG56** after storage at 21°C	Recovered **pcTG90** after storage at 4°C	Recovered **pcTG90** after storage at 21°C
5	94%	66%	95%	98%
15	93%	37%	91%	95%
33	80%	11%		94%

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2. Unpublished results from these laboratories.
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5. In our hands, reduction of the cyano groups by catalytic hydrogenation on Raney nickel (Bergeron, R. J.; Garlich, J. R. *Synthesis* **1984**, 782–784) gave better yields than those reported by Meinwald for the reduction with $LiAlH_4$.
6. ^1H NMR (200 MHz, $CDCl_3$). Amine **4**: δ 1.43 and 1.45 (2 s, 36H, *t*-Bu-), 1.60–1.82 (m, 8H, -N(Boc)-CH_2-CH_2-), 2.71 (m, 2H, -CH_2-NH_2), 3.02–3.35 (m, 14H, -N(Boc)-CH_2-). Acid **5**: δ 1.44 (br s, 45H, *t*-Bu-), 1.58–1.84 (m, 8H, -N(Boc)-CH_2-CH_2-), 3.02–3.38 (m, 16H, -N(Boc)-CH_2-), 3.86 and 3.94 (2 m, 2H, -CH_2-CO_2H). Lipid **7**: δ 0.88 (t, *J*=6.4 Hz, 6H, Me-), 1.27 and 1.29 (2 br s, 40H, -CH_2-), 1.43, 1.44 and 1.46 (3 s, 45H, *t*-Bu-), 1.53–1.82 (m, 12H, -O-C(O)-CH_2-CH_2- and -N(Boc)-CH_2-CH_2-), 2.00 (m, 8H, -CH_2-CH=), 2.30 (t, *J*=7.5 Hz, 4H, -O-C(O)-CH_2-), 3.02–3.30 (m, 16H, -N(Boc)-CH_2-), 3.50 (m, 2H, -CH_2-NH-C(O)-), 3.81 (br s, 2H, -NH-C(O)-CH_2-N(Boc)-), 4.10 and 4.25 (2 dd, *J*=12.1, 5.8, 4.0 Hz, 2H, -CH_2-O-C(O)-), 5.08 (m, 1H, >CH-O-C(O)-), 5.34 (m, 4H, -CH=).
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9. Lipid **pcTG90**: ^1H NMR (200 MHz, $CDCl_3$-CF_3CO_2D) δ 0.87 (t, *J*=6.4 Hz, 6H, Me-), 1.27 and 1.28 (2 br s, 40H, -CH_2-), 1.58 (m, 4H, -O-C(O)-CH_2-CH_2-), 2.00 (m, 8H, -CH_2-CH=), 2.12–2.46 (m, 12H, -NH_2^+-CH_2-CH_2- and -O-C(O)-CH_2-), 3.20 (m, 16H, -NH_2^+-CH_2-), 3.50 (m, 2H, -C(O)-NH-CH_2-), 3.98 (m, 2H, -NH_2^+-CH_2-C(O)-NH-), 4.23 (m, 2H, -CH_2-O-C(O)-), 5.20 (m, 1H, >CH-O-C(O)-), 5.34 (m, 4H, -CH=). FAB-MS (*m*-nitrobenzyl alcohol) *m/z* 905.9 (100%, $[M+H]^+$); 677.6 (27%, $[M-\{H(NHCH_2CH_2CH_2)_4\}+2H]^+$); 620.6 (82%, $[M-\{NH_2(CH_2CH_2CH_2NH)_4CH_2C(O)\}+2H]^+$); 356.4 (78%, $[M-\{NH_2(CH_2CH_2CH_2NH)_4CH_2C(O)\}$-oleoyl group+3H]$^+$); 283.3 (75%, [oleic acid+H]$^+$). M stands for $C_{53}H_{104}N_6O_5$=904.8.
10. The cationic lipid (2 mg) was dissolved in 1:1 dimethylsulfoxide:ethanol (40 µl) and 20 mM HEPES buffer (pH 7.5, 160 µl) was added. Aliquots (20 µl) of this solution were stored in sealed tubes: one series was kept at 4°C and another at 21°C. After dilution with isopropanol (40 µl), vortexing, and addition of 99.9:0.1 water:CF_3CO_2H (40 µl), they were analysed by HPLC (Supelcosil ABZ+Plus column; elution with mixtures of 0.15% CF_3CO_2H in water (A) and 0.05% CF_3CO_2H in isopropanol (B): 50% A/50% B for 8 min, then 50% to 100% B gradient for 12 min, then 100% B for 10 min; detection at 205 nm).
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