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The facile solid-phase synthesis of cholesterol-based polyamine lipids

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Abstract—A facile solid-phase methodology for the production of cholesterol-based polyamines useful in mediating nucleic acid delivery for gene therapy is described. The methodology is compatible with a range of polyamines producing a library of lipids in excellent yields (>87%) and purity.

Polyamines are a class of naturally occurring compounds that display excellent nucleic acid binding and condensing properties. This fact has been exploited in the design of cationic lipids (cytofectins) for nonviral liposomal gene therapy.[1] Here, these lipids as components of cationic liposomes, aid complexation of negatively charged nucleic acids thereby generating nucleic acid delivery vectors known as lipoplexes or LD's (liposome–DNA). The overall positive charge of the lipoplexes is important for initiating cell entry and release of the complexed nucleic acid into the cell cytoplasm. There have been a vast number of cationic lipids detailed over the last 15 years, many of these based on the structures of naturally occurring linear polyamines.[2] Although the exact mechanism by which these compounds help mediate transfection is still poorly understood, evidence in the literature suggests that the success of these reagents arises from the abnormally low pK_a's (p$K_a \leqslant 7$) of the polyamines, a direct result of the number of amino groups present and the methylene spacings between them.[3–5] Of particular interest recently has been the cationic lipid CDAN 1 (Scheme 1). CDAN is a cholesterol-based polyamine lipid with unnaturally occurring 2–3–2 methylene spacings, which when combined with the natural helper lipid DOPE forms an exceptionally effective transfection agent (Trojene™), capable of transfecting a wide range of cells.[4,6]

Despite the increasing interest in cationic lipids, the synthetic routes to these compounds are frequently tedious and time consuming. The highly polar yet lipophilic nature of these compounds makes for difficult purifications and low overall yields (<25%).[4,5,7] Here we propose a new solid-phase strategy to these target molecules, which allows facile production of a library of cholesterol-based polyamine lipids in excellent yields (>87%) and purity.

The strategy employs 2-chlorotrityl chloride resin **2** (0.4 mmol g^{-1}) as a solid support and protecting group for one primary amine on the starting material, and utilises the high selectivity of 2-acetyldimedone (Dde-OH) as a protecting group for the second primary amine.[8] The overall route for synthesis of the lipids is depicted in Scheme 1, using CDAN as a representative example.

The general synthesis procedure developed is as follows. Firstly, resin (2-chlorotrityl chloride [purchased from Argonaut, UK] **2**, 0.4 mmol g^{-1}) was loaded by treatment with excess polyamine (in the case of CDAN, *N*,*N*′-bis(2-aminoethyl)-1,3-propane diamine is used). Any unreacted 2-chlorotrityl groups were quenched with MeOH to prevent subsequent side reactions. Excess polyamine was isolated by filtration and easily recycled. The remaining free primary amine sites were subsequently reacted with excess 2-acetyldimedone, forming

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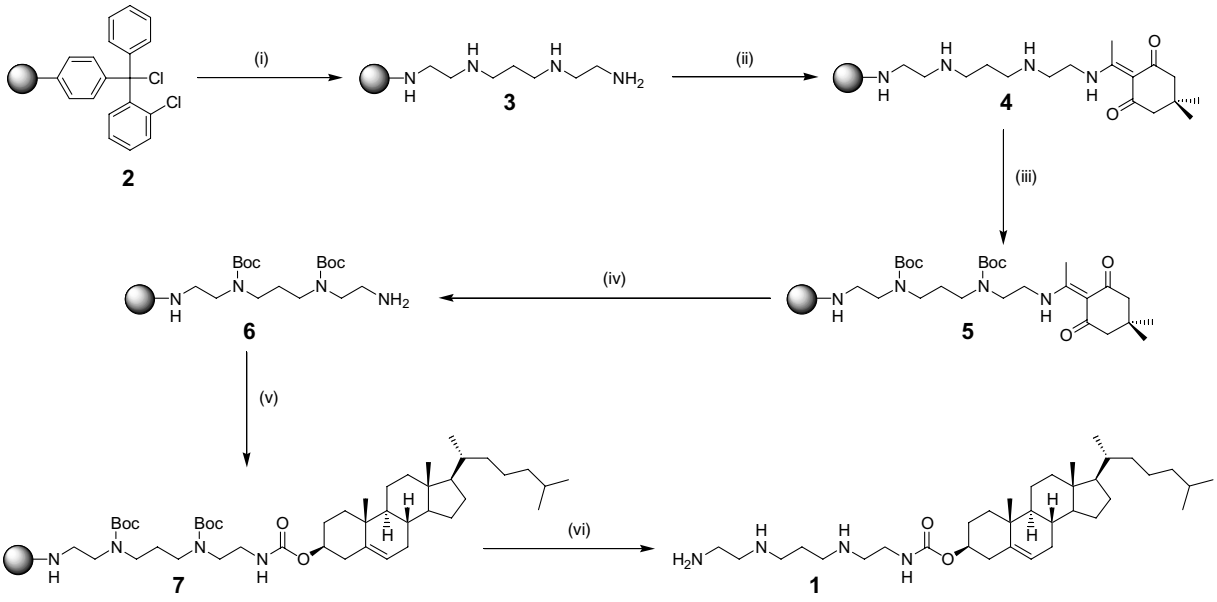
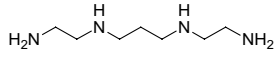
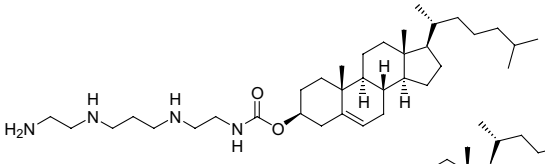
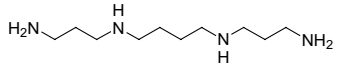
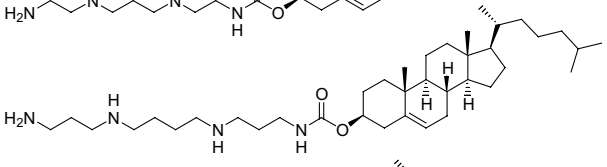
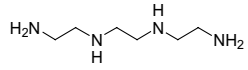
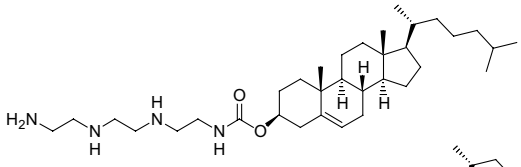
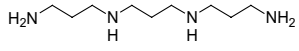
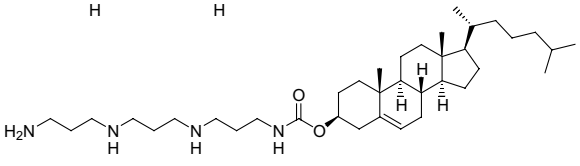


Scheme 1. Reagents and conditions: (i) Polyamine (10 equiv), CH_2Cl_2, rt, 2 h, then MeOH (1000 equiv) rt, 10 min; (ii) Dde-OH (10 equiv), DMF, rt 12 h; (iii) Boc_2O (5 equiv per free amine), NEt_3 (2 equiv per free amine), CH_2Cl_2, rt, 4 h; (iv) 2% hydrazine hydrate in DMF, rt, 10 min (repeat step×2); (v) cholesterol chloroformate (10 equiv), NEt_3 (3 equiv), rt, 4 h; (vi) 50% TFA in CH_2Cl_2, rt, 1 h, 93%.

Table 1

Polyamine		Product		% Yield (% purity[11])	Literature % overall yield
	**8**		**1**	93% (96%)	16%[5], 10%[4,7]
	**9**		**10**	87% (95%)	14%[5]
	**11**		**12**	93% (96%)	NA
	**13**		**14**	92% (95%)	24%[5]

a hydrogen bond stabilised enamine **4**. The presence of the protected primary amine was confirmed by the Kaiser test.[†] Free secondary amines were then protected with *tert*-butyloxycarbonyl (Boc) groups and the Dde protected primary amine was selectively deprotected upon treatment with 2% hydrazine hydrate in DMF. The assembly of the lipids was completed by treating the liberated primary amine **6** with cholesterol chloroformate. The final products were cleaved from the resin by treatment with 50% TFA in CH_2Cl_2. The filtrate was collected, concentrated in vacuo and the resulting oils were lyophilised to give CDAN as a pale solid in excellent yield and purity. This methodology is compatible with a series of polyamines (see Table 1), giving a

[†] Kaiser test[9]: To a few resin beads, add two drops of each of the following solutions: (a) 80 g phenol in 20 mL of EtOH, (b) 2 mL of 0.00 M aqueous KCN in 98 mL pyridine, (c) 5 g ninhydrin in 100 mL EtOH. Heat the resulting solution containing the resin beads to 80 °C for 2 min (CAUTION hazardous materials). Blue beads indicate the presence of primary amines.

range of cholesterol-based polyamine lipids. The purity of the lipids was assessed by HPLC and ^{1}H and ^{13}C NMR.[10]

In conclusion, we have demonstrated a novel solid-phase methodology for the production of cholesterol-based polyamine lipids, which allows production of a library of lipids in excellent yield and purity.

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10. Sample data for production of CDAN **1**. FTIR (CH_2Cl_2) ν_{max} 3584–3245, 2937, 2868, 1695, 1538, 1469, 1379, 1251, 1133 and 1014 cm^{-1}. ^{1}H NMR (400 MHz, d_6-DMSO) δ 0.65 (3H, s, 18-CH_3), 0.83–0.85 (2×3H, d, $J = 6.4$ Hz, overlapping 1.6 Hz, 27-CH_3, 26-CH_3), 0.89–0.90 (3H, d, $J = 6.4$ Hz, 21-CH_3), 0.97 (3H, s, 19-CH_3), 0.97–2.03 (28H, m, 1-CH_2, 2-CH_2, 4-CH_2, 7-CH_2, 8-CH, 9-CH, 11-CH_2, 12-CH_2, 14-CH, 15-CH_2, 16-CH_2, 17-CH, 20-CH, 22-CH_2, 23-CH_2, 25-CH, 5′-CH_2), 2.17–2.34 (2 H, m, 24-CH_2), 2.92–3.32 (12H, m, 1′-CH_2, 2′-CH_2, 4′-CH_2, 6′-CH_2, 8′-CH_2, 9′-CH_2), 4.27–4.39 (1H, m, 3-CH), 5.30–5.37 (1H, m, 6-CH). ^{13}C NMR (400 MHz, d_6-DMSO) δ 11.6 (18-CH_3), 18.5 (21-CH_3), 18.9 (19-CH_3), 20.50 (11-CH_2), 22.3 (26-CH_3), 22.3 (5′-CH_2), 22.6 (27-CH_3), 23.1 (23-CH_2), 23.8 (15-CH_2), 27.3 (25-CH), 27.7 (2-CH_2, 16-CH_2 overlapping), 31.2 (7-CH_2), 31.3 (8-CH), 35.1 (20-CH, 1′-CH_2 overlapping), 35.6 (22-CH_2), 35.9 (10-C), 36.5 (1-CH_2), 36.6 (9′-CH_2), 38.2 (24-CH_2), 38.8 (4-CH_2), 39.1 (12-CH_2), 41.8 (13-C), 43.9 (4′-CH_2), 44.1 (6′-CH_2), 46.4 (2′-CH_2, 8′-CH_2 overlapping), 49.4 (9-CH), 55.5 (17-CH), 56.1 (14-CH), 73.4 (3-CH), 121.9 (6-CH), 139.6 (5-C), 155.8 (NCOO); m/z (FAB +ve) 573 (M+H); FAB-MS m/z for $C_{35}H_{65}N_4O_2$ (M+H) calculated 573.5108, found 573.5103. HPLC analysis $t_R = 22.4$ min, column: Vydac C-4 peptide (214TP54), mobile phases H_2O (0.1% TFA), MeCN (0.1% TFA), MeOH, gradient H_2O/MeCN/MeOH, 0 min [100/0/0], 1–15 min [0/100/0], 25 min [0/100/0], 25.1 min [0/0/100], 45 min [0/0/100], 45.1 min [100/0/0], 60 min [100/0/0]; flow 1 mL/min. HPLC analysis of lipids 10, 12, 14, using identical HPLC conditions, gave $t_R = 22.7$, 22.3 and 22.5 min, respectively.
11. Purity determined by HPLC and ^{1}H NMR.