Aminoglycoside-Derived Cationic Lipids for Gene Transfection: Synthesis of Kanamycin A Derivatives

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Cationic lipids are currently actively investigated as an alternative approach to recombinant viruses for gene transfer studies and gene therapy applications. Basically, they rely on the formation of lipid/DNA aggregates via electrostatic interactions between their cationic headgroup and the negatively charged DNA. The development of new amphiphilic structures should allow to shed light on their still poorly understood structure/activity relationship and thereby help to design improved vectors. It appears that aminoglycosides, which are natural polyamines known to bind to nucleic acids, provide a favourable scaffold for the synthesis of a variety of cationic lipids because of their structural features and multifunctional nature. The synthesis and full characterization of a series of lipophilic derivatives of the aminoglycoside antibiotic kanamycin A, mainly kanamycin–cholesterol conjugates, are reported herein.

Introduction

Gene therapy consists in delivering nucleic acids into somatic cells of patients to treat both genetic and acquired diseases.[1] Some positive results have already been reported, but the clinical trials performed to date have clearly indicated that a prerequisite for successful gene therapy is the use of an efficient and safe gene delivery system.[2] Although viral vectors have been shown particularly efficient for gene transfer,[3] they suffer from a number of inconveniences including immunogenicity, toxicity, safety issues and problems in industrial quality control and upscaling.[2] To overcome these difficulties, alternative approaches have been developed, using cationic polymers or cationic lipids.[4] Spontaneous formation of self-assembled vector/DNA complexes is in both cases due to electrostatic interactions between the positively charged vector and the negatively charged phosphate groups of DNA. Cationic lipids, which are composed of a cationic headgroup linked via a spacer to a hydrophobic moiety and are mostly formulated as liposomes with neutral helper lipids such as dioleoylphosphatidylethanolamine (DOPE), are particularly attractive because it is relatively easy to synthesize a great variety of reagents with favourable features. Thus, numerous cationic lipids have been developed over the last decade.[5a,6] However, although cationic liposomes have already been used in clinical gene therapy trials for cancer[7] and cystic fibrosis,[8] there is a clear requirement for improved, newer generation lipids, especially because of the relatively low gene transfer efficiency of the current lipids when compared with viral vectors. Thus, a major research effort is still devoted to the discovery of new reagents and delivery systems able to carry nucleic acids into the cell nucleus. As a part of our work aimed at discovering such compounds,[9] we report here a new approach based on the use of an aminoglycoside scaffold.

Aminoglycoside antibiotics constitute a large family of clinically important drugs used in the treatment of gram-negative infections.[10] They effect their antibacterial activity by interfering with ribosomal function (via binding to rRNA), which ultimately results in the disruption of protein biosynthesis.[11] These antibiotics have also been shown to interact with a variety of other RNA molecules such as group I introns,[12] hammerhead ribozymes,[13] and the HIV-1's TAR[14] and RRE[15] regulatory domains. Aminoglycosides have in fact been shown to be rather non specific RNA binders that recognize numerous similar three-dimensional RNA structures and have therefore become an important starting point for the study of RNA−small molecules recognition.[16]

Most of the naturally occurring aminoglycosides are structurally characterized by amino sugars glycosidically linked to an aminocyclitol which, in most cases, is 2-deoxystreptamine. There are actually several types of 2-deoxystreptamine derivatives: monosubstituted derivatives (such as neamine), 4,5-disubstituted (neomycin type derivatives),

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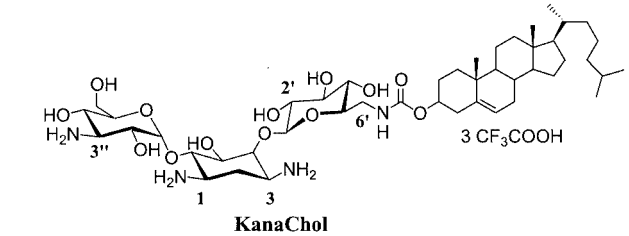
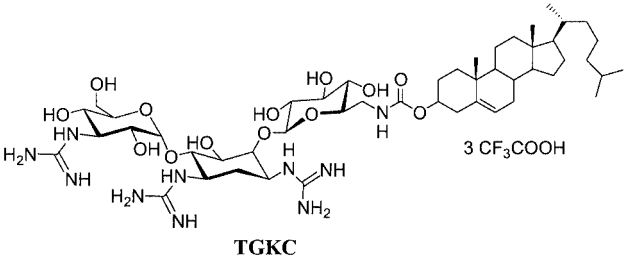
and 4,6-disubstituted (kanamycin, tobramycin, and gentamycin derivatives). Aminoglycosides carry up to six amino groups, which are predominantly charged at physiological pH,[17] and bind with high affinity to anions and nucleic acids via electrostatic and hydrogen bonding interactions.[18] It has recently been shown that the 1,3-hydroxyamine motifs often found in their structure interact strongly with both the phosphodiester backbone and the Hoogsteen face of guanine.[19] Taken together, these features make aminoglycosides very promising as versatile frameworks for the synthesis of a variety of novel cationic lipids for gene transfection into eukaryotic cells. Indeed, selective acylation of one or several of their functional groups should provide aminoglycosides with the lipophilic properties required for their use as vectors for gene transfer. In addition, the structural diversity of aminoglycosides also provides a means to investigate in detail the structure/activity relationships of these new transfection reagents by allowing to evaluate the influence of specific features on transfection efficiency (nature and number of the hydrophobic subunits, geometry of the molecules, number of positive charges, etc.).

Along these lines, we have first prepared a cationic cholesterol derivative, KanaChol-6′, simply termed KanaChol herein, characterized by a kanamycin A polar headgroup. It was expected that, because of its three free amine groups, this molecule would bind to and condense DNA at physiological pH and that the cholesterol subunit would in addition facilitate the cellular uptake of the lipoplexes generated. Moreover, we have also synthesized reagent TGKC (**T**ri**G**uanidinium−**K**anamycin−**C**holesterol), the fully guanidinylated derivative of KanaChol.



KanaChol



TGKC

Indeed, we,[9a−9d] as well as others,[20] have previously exploited the favourable features of the guanidinium group for DNA binding and recent reports have indicated that conversion of aminoglycosides to their fully guanidinylated derivatives[21] or to polyarginine conjugates[22] greatly enhances their RNA affinity. This beneficial effect may be all the more useful considering that aminoglycosides bind double-stranded DNA with low affinity.[18d,23] In addition, guanidinium groups are key to natural and synthetic transporters; arginine oligomers and artificial peptoids have indeed recently been shown to easily enter cells and tissues, thus enhancing uptake of drugs.[24] Finally, it should be stressed that, in KanaChol as well as in TGKC, the aminoglycoside headgroup is linked to the cholesterol moiety via a carbamoyl bond. Indeed, carbamoyl bonds, which also link together the headgroup and the lipid moiety in numerous cationic lipids including the widely used DC-Chol and our own BGTC,[9a] have been reported to be both chemically stable and biodegradable.[9a−9c,25]

Transfection studies showed that reagent KanaChol could mediate efficient gene transfection into a variety of mammalian cell lines when used either alone or as a liposomal formulation with the neutral phospholipid dioleoylphosphatidylethanolamine (DOPE).[26] Cationic TGKC/DOPE liposomes were also efficient for in vitro transfection. Finally, kanamycin-cholesterol/DOPE lipoplexes were found to be also efficient for gene transfection into the mouse airways in vivo via intranasal instillation.[26] Therefore, in a second step of our work, in order to explore the relationships between structure and transfection activity of kanamycin-derived lipids and so to develop more efficient vectors, we prepared a series of new kanamycin-lipid conjugates with a spacer and/or a hydrophobic anchor different from those in KanaChol. We report herein the synthesis and the full characterization of these different compounds.

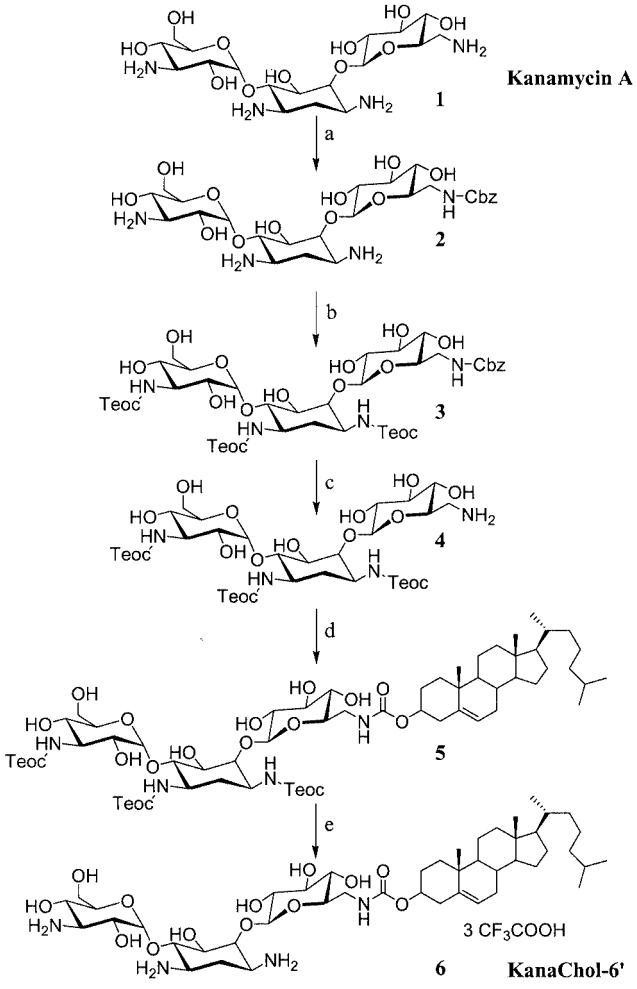
Results and Discussion

KanaChol and TGKC

To test our approach for designing new vectors for gene transfer, we first chose to prepare a kanamycin A derivative because this molecule contains only one aminomethyl group and thus a selective monoacylation of this amino group seemed easily feasible. Indeed, within the four amine functions present in kanamycin A, the 6′ position is known to be the most reactive (6′-NH_2 > 1-NH_2 > 3-NH_2 > 3′′-NH_2).[27] Therefore we tried a direct reaction between kanamycin A and cholesteryl chloroformate that gave an inseparable complex mixture. We were thus led to undertake a longer synthesis involving the selective protection of the 6′-NH_2 group by an efficient selective acylating agent, the protection of the amine functions in position 1, 3, and 3′′, then the deprotection of the 6′-NH_2 group followed by its reaction with the cholesteryl chloroformate and, finally, the deprotection of the amine functions in position 1, 3, and 3′′.

The different approaches reported for the selective protection of amino groups of aminoglycosides have been reviewed.[27] Among them, procedures based on the difference in reactivity of the amino groups towards weak acylating agents seem the most attractive. Particularly, the reagent, *N*-(Boc-*O*)-5-norbornene-*endo*-2,3-dicarboximide, which greatly favours reaction at unhindered amines, was claimed ideally suited for application to aminoglycoside chemistry.[28] Indeed, using the commercially available Cbz derivative, instead of the Boc one, we obtained the monoprotected

compound **2** with a 73% yield after chromatographic purification over silica (Scheme 1). The second step of the synthesis involved the full protection of the three remaining amines.



Scheme 1. Synthesis of KanaChol: (a) *N*-Benzyloxycarbonyloxy-5-norbornene-2,3-dicarboximide, Et_3N, DMSO/H_20 (1:1), 4 days, 73%; (b) 2-(Trimethylsilyl)ethyl *p*-nitrophenyl carbonate, Et_3N, dioxane/H_2O (2:1), 55 °C, 48 h, 73%; (c) H_2, Pd/C(10%), CH_3OH, 93%; (d) Cholesteryl chloroformate, Et_3N, THF/DMF (5:2), room temp., 48 h, 77%; (e) Trifluoroacetic acid, 0 °C, 40 mn, 97%

Although the classical Boc protection was reported to work,[29] it resulted in our hands in a complex mixture of components bearing two to six Boc groups as shown by MALDI-TOF mass spectroscopy experiments. On the contrary, the use of the protective reagent 2-(trimethylsilyl)ethyl *p*-nitrophenyl carbonate, (Teoc-O-Np),[30] in slight excess at 55 °C, allowed a good retrieval of the tri-Teoc protected compound **3** (73% yield). Then, the benzyloxycarbonyl group of **3** was removed by a classical hydrogen reduction on supported Pd/C (10%), giving the free aminomethyl derivative **4** with yields up to 93%. The coupling of cholesteryl chloroformate with compound **4** led to the conjugate **5** which was easily worked up (77% yield). Finally, the kanamycin−cholesteryl conjugate **6** (= KanaChol) was ob-

Table 1. ^{1}H Chemical shifts and peak assignments for compounds **1** and **2**

H atom	**2**[a]	**1**[a]	Δ[b]
1	2.86	2.83	0.03
2_a	1.10	1.16	−0.06
2_e	1.83	1.90	−0.07
3	2.65	2.83	−0.18
4	3.18	3.25	−0.07
5	3.56	3.60	−0.04
6	3.23	3.19	0.04
1′	5.13	5.27	−0.14
2′	3.55	3.52	0.03
3′	3.64	3.64	0.00
4′	3.25	3.24	0.01
5′	3.75	3.71	0.04
$6'_a$	3.59	2.93	**0.66**
$6'_b$	3.26	2.71	**0.55**
1′′	5.01	4.98	0.03
2′′	3.52	3.44	0.08
3′′	3.03	2.94	0.09
4′′	3.35	3.26	0.09
5′′	3.90	3.85	0.05
6′′	3.76	3.71	0.05
2′′′	5.09		
4′′′; 8′′′	7.43		
5′′′; 7′′′	7.43		
6′′′	7.43		

[a] Chemical shift values (δ) are given in ppm. Assignments for close lying signals are tentative and may be interchanged. [b] $\Delta = \delta_2 - \delta_1$.

Table 2. ^{13}C chemical shifts and peak assignments for compounds **1** and **2**

C atom	**2**[a]	**1**[a]	Δ[b]
1	50.7	52.9	−2.2
2	35.1	37.8	−2.7
3	49.4	51.4	−2.0
4	88.7	89.7	−1.0
5	74.4	76.6	−2.2
6	87.4	90.3	−2.9
1′	100.8	101.9	−1.1
2′	72.2	74.3	−2.1
3′	73.2	75.4	−2.2
4′	71.4	73.5	−2.1
5′	72.0	75.3	−3.3
6′	41.9	44.0	−2.1
1′′	100.3	102.5	−2.2
2′′	71.8	74.3	−2.5
3′′	54.6	56.7	−2.1
4′′	69.2	71.7	−2.5
5′′	72.4	74.5	−2.1
6′′	60.5	62.7	−2.2
1′′′	158.7		
2′′′	67.3		
3′′′	136.8		
4′′′; 8′′′	129.2; 129.0		
5′′′; 7′′′	129.0; 128.8		
6′′′	128.6		

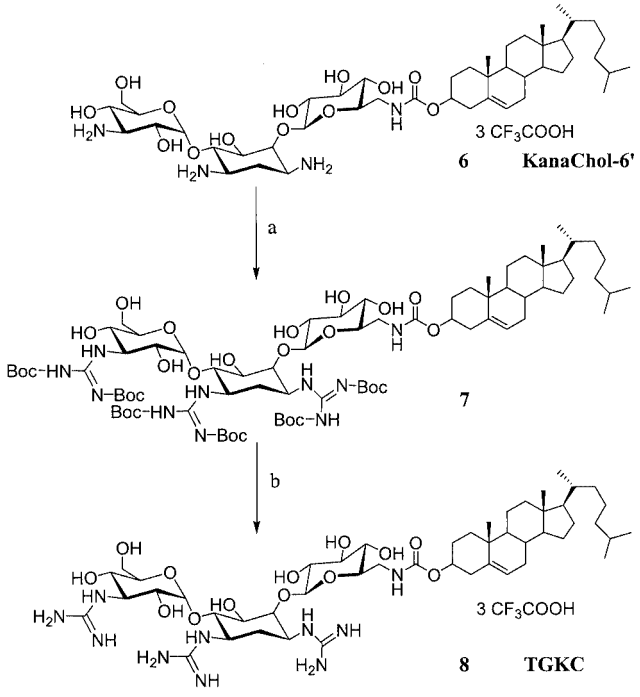
[a] Chemical shift values (δ) are given in ppm. Assignments for close lying signals are tentative and may be interchanged. [b] $\Delta = \delta_2 - \delta_1$.

tained in high yield (97%) by treatment of the Teoc protected compound **5** with trifluoroacetic acid.

Although the order of reactivity of the amino groups of kanamycin A seems well established,[27] we thought it necessary to confirm the structure of **2**, by spectroscopic methods, to make sure that the protecting group was on position 6′.

The complete study of compound **2** [and of kanamycin A free base (**1**) as a reference] through NMR spectroscopy in D_2O (^1H, ^{13}C, COSY, and HMQC on a BrukerAMX-400, 25 °C) enabled us to assign unambiguously almost all the signals for both protons and carbons (Tables 1 and 2, values found for kanamycin A free base are consistent with those found in literature, see reference[31,32]). The ^1H NMR spectrum of compound **2** revealed the presence of the characteristic groups of both the carbobenzyloxy moiety at chemical shifts (δ) of 5.09 (benzylic CH_2) and 7.43 ppm (aromatic protons) and the kanamycin A moiety. The latter was mainly characterized by the anomeric hydrogens at chemical shifts of 5.01 (H-1′′) and 5.13 ppm (H-1′) and by the two protons on the unsubstituted position of the deoxystreptamine ring (at 1.10 and 1.83 ppm). The integration ratio of the characteristic signals of the two moieties together with the mass spectrum results confirmed the presence of a mono-protected aminoglycoside.

The presence of a unique signal in the carbonyl region (at 158.71 ppm) in the ^{13}C NMR spectrum, as well as the fact that each carbon on the kanamycin A moiety has been assigned unambiguously, strongly support the hypothesis of a unique isomer. Furthermore, the two dimensional spectra and the coupling constants (data not shown) are fully consistent with the presence of a unique isomer. The comparison between the chemical shifts of **2** and those of kanamycin A free base **1** shows a downfield shift for the protons on the 6′ position of a least 0.5 ppm which is not observed on any of the other methylene protons adjacent to an amino group (3′′, 1, and 3). This difference in the chemical shifts signal indicates that compound **2** has only been acylated on the 6′ position as expected.



Scheme 2. Synthesis of TGKC: (a) *N*,*N*′-bis(*tert*-butoxycarbonyl-*N*′′-triflyl)guanidine, Et_3N, dioxane/H_2O (10:1), 4 days, 77%; (b) Trifluoroacetic acid, 0 °C, 4 h, 60%

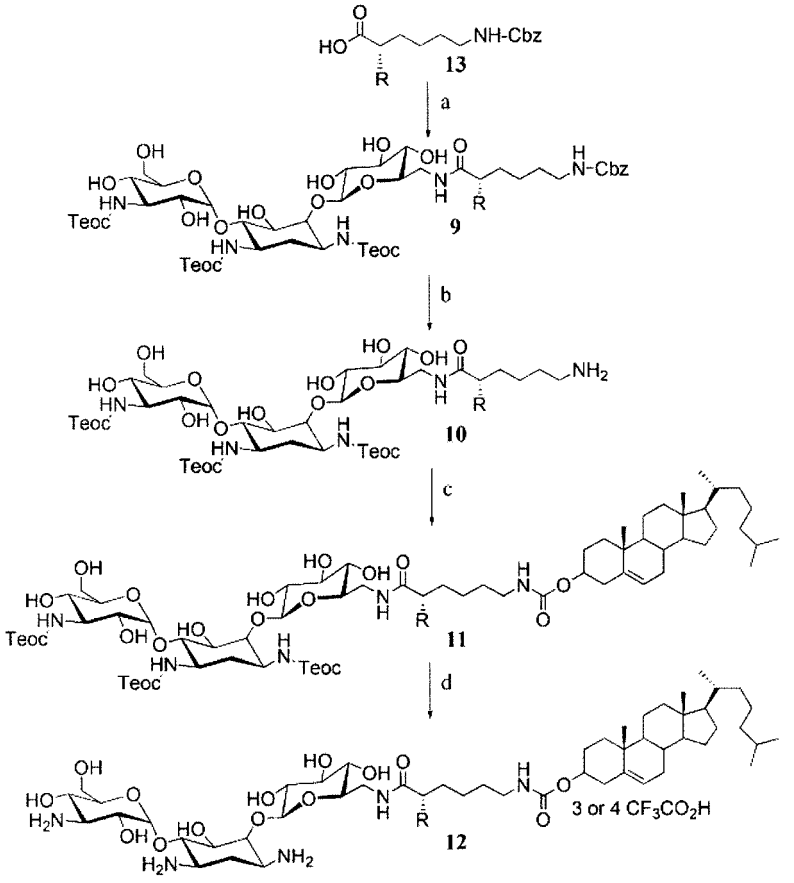
To obtain the guanidinium derivative **8** of KanaChol, named TGKC (**T**ri**G**uanidinium−**K**anamycin−**C**holesterol) by analogy with BGTC (**B**is**G**uanidinium−**T**ren−**C**holesterol)[9a], we used *N*,*N*′-bisBoc-*N*′′-triflylguanidine[33] as described by Goodman et al. and obtained the triguanidino Boc-protected compound **7** as an off-white solid (77%) which was subsequently treated with trifluoroacetic acid (60%yield) (Scheme 2). This last step was tricky because it is not easy to remove the six Boc groups without observing a certain amount of cleavage of the carbamate function.

KanaCapChol, KanaLysChol, KanaLysDiChol, and KanaSucDODA

KanaCapChol (**12a**), KanaLysChol (**12b**), and KanaLysDiChol (**12c**) were obtained from the same protected aminomethyl precursor **4**, by reaction with the commercially available *N*-protected amino acylating agents **13a**−**c** followed by straightforward deprotection and treatment with cholesteryl chloroformate (Scheme 3).

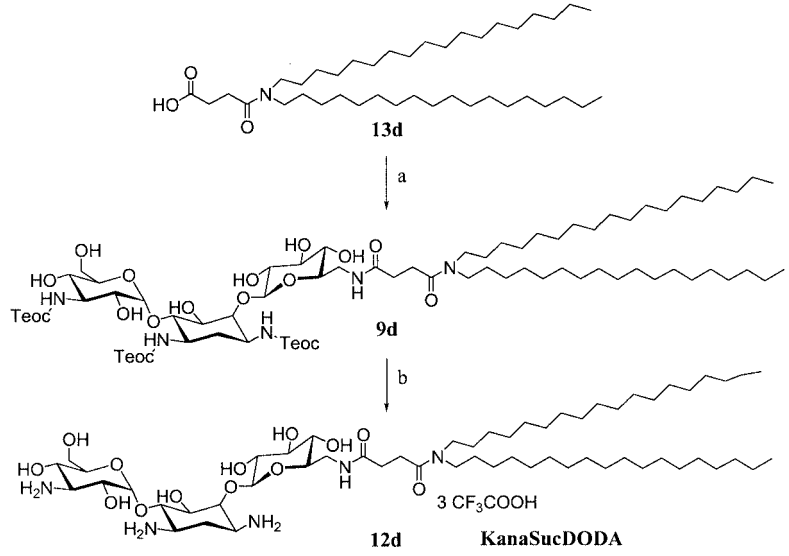
KanaSucDODA (**12d**) resulted from the reaction of **4** with *N*,*N*-dioctadecylsuccinamide **13d**, prepared from succinic anhydride and dioctadecylamine[34] (Scheme 4). In all cases, the final compound was isolated as its trifluoroacetate salt by deprotection of the amine groups of the aminoglycoside fragment by trifluoroacetic acid.

During these synthetic studies, a secondary reaction worth noting was observed. In some cases, mass spectroscopy studies of the resulting product of the catalytic hydrogenation of *N*-benzyloxycarbonyl-protected aminoglycoside revealed the presence, besides the desired compound of mass M, of a derivative corresponding to M + 12. Most of the time this unexpected compound was present in small quantities but, sometimes, it happened to be predominant. Indeed, it has previously been reported that a side-reaction of *N*-alkylation of amino groups occurs during palladium-catalyzed hydrogenolytic cleavage of *N*-benzyloxycarbonyl protecting groups;[35] in the presence of palladium catalyst and oxygen, alcohols, such as methanol, ethanol and benzylic alcohol, are readily converted into the corresponding aldehydes which combine with amino groups to form Schiff bases; the latter giving alkylamines upon catalytic hydrogenation. In our case, the formol generated by the oxidation of methanol, necessary for the solubilization of the aminoglycoside derivative, combines with the liberated amino group to form an intermediate imine and the favourable geometry of the aminoglycoside ring leads it subsequently to react intramolecularly with the closest hydroxyl function on position 4′ to form an oxazolidine ring. Despite the difficulty of purifying a mixture of so similar

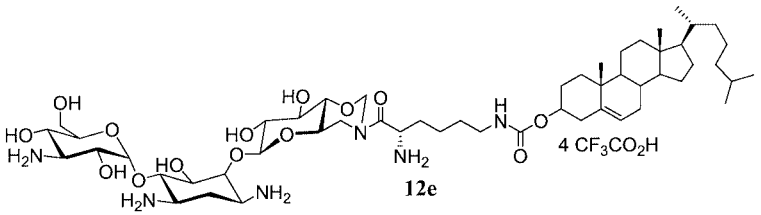


R =	13	9	10	11	12	
a	H	H	H	H	H	(KanaCapChol)
b	NH-Boc	NH-Boc	NH-Boc	NH-Boc	NH_2	(KanaLysChol)
c	NH-Cbz	NH-Cbz	NH_2	NH-COO-Chol	NH-COO-Chol	(KanaLysDiChol)

Scheme 3. Synthesis of KanaCapChol, KanalysChol, and KanaLysDiChol. a) HOAt/EDC, **4**, DMF; b) H_2/Pd/C, room temp.; c) cholesteryl chloroformate, Et_3N, DMF/THF; d) CF_3CO_2H



Scheme 4. Synthesis of KanaSucDODA. a) HOAt/EDC, **4**, DMF/CH_2Cl_2; b) CF_3CO_2H



12e

products, compound **12e** has been isolated, besides **12b**, in a pure state and its structure was confirmed by NMR studies and mass results. Of note, this side reaction can be prevented by adding water to methanol, removing oxygen from the solvents and using as little as possible palladium during the hydrogenolytic deprotection.

Conclusion

Using the natural antibiotic kanamycin A as starting material, we have prepared and fully characterized a set of lipidic aminoglycoside derivatives. These various syntheses were chiefly undertaken with a view to develop novel cationic lipids characterized by an aminoglycoside-based headgroup for gene transfection. Indeed, it is generally agreed that the nature of the positive headgroup plays a key role in the transfection activity of a given cationic lipid, the characteristics (such as the hydrophobicity/hydrophilicity balance, length of the spacer arm, ...) of the entire lipid molecule determining however its in toto efficiency. As indicated above, transfection experiments have shown that reagent KanaChol was indeed efficient for gene transfection in vitro and into the mouse airways in vivo.[26] The transfection activity of the other kanamycin-derived lipids will be reported elsewhere, the data obtained thus far suggesting that adequate modification of the spacer arm may favourably impact on the transfection efficiency.[36]

Experimental Section

General Remarks: All commercially available chemicals were reagent grade and were used without further purification. Kanamycin A free base was prepared from the corresponding monosulfate salt (purchased from Aldrich Chemical Co) by use of Amberlite IRA 400 (OH^-) strongly basic ion-exchange resin. Unless otherwise indicated, all the reactions were performed at room temperature. Flash chromatography employed Merck silica gel [Kieselgel 60 (0.040−0.063 mm)]. Analytical TLC was performed with 0.2 mm silica-coated aluminium sheets, visualization by UV light or by spraying either a solution of ninhydrin (0.3% in weight in *n*-butanol containing 3% acetic acid in volume) or a iodine solution (0.1 M in 10% sulfuric acid aqueous solution). Melting points were determined on an Electrothermal 9100 apparatus. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance300 spectrometer.

MALDI/TOF stands for Matrix Assisted Laser Desorption Ionisation coupled with Time Of Flight mass spectroscopy. MALDI mass spectra were recorded with a PerSeptive Biosystems Voyager Elite (Framingham MA, USA) time-of-flight mass spectrometer. This instrument is equipped with a nitrogen laser (337 nm), a delayed extraction and a reflector. It was operated at an accelerating potential of 20 kV in reflector mode. The mass spectra shown represent an average over 256 consecutive laser shots (3 Hz repetition rate). Peptides were used to calibrate the mass scale using the two points calibration software 3.07.1 from PerSeptive Biosystems. The analyte solutions were prepared in methanol or THF at a concentration of 2 $g \cdot L^{-1}$. The matrix, 2,5-dihydroxybenzoic acid (2,5-DHB) was from Sigma (France) and used without further purification. It was dissolved in methanol or THF (15 $g \cdot L^{-1}$). Five microliters of analyte solution were mixed with 50 μL of matrix solution. 2 microliters of sodium iodide solution (5 $g \cdot L^{-1}$ in methanol or THF) were added to induce cationisation in some experiments. One microliter of the final solution was deposited onto the sample stage and allowed to dry in air.

IR spectra were obtained on a Bruker Vector 22 FT-IR spectrometer. The microanalyses were performed at the Service De Microanalyse de l'Université Pierre et Marie Curie (Paris).

6′-*N*-(Benzyloxycarbonyl)kanamycin A (2): *N*-Benzyloxycarbonyloxy-5-norbornene-2,3-dicarboximide (Cbz-Nor, 1.29 g, 4.12 mmol, 1 equiv.) and triethylamine (575 μL, 4.12 mmol, 1 equiv.) were added to a solution of kanamycin A free base (2 g, 4.12 mmol) in water (20 mL) and dimethyl sulfoxide (200 mL). The mixture was stirred at room temperature overnight, upon which it was concentrated in vacuo to dryness. Flash column chromatography of the residue (silica gel, CH_2Cl_2/MeOH/NH_4OH, 5:4:1) afforded the desired product as a white solid (1.86 g, 72.9%). R_f = 0.22 (CH_2Cl_2/MeOH/NH_4OH, 5:4:1). m.p. 179 °C dec. $[\alpha]_D^{25}$ = +9.73 (*c* = 1.17, H_2O). ^1H & ^{13}C NMR: see Tables 1 and 2. IR (KBr, thin film): ν_{max} (cm^{-1}) = 3357 (br), 2925, 1699 (s), 1541, 1456, 1362, 1267, 1049 (s). MS: *m*/*z* MALDI-TOF [MH^+] 619.3, [MNa^+] 641.3. FAB$^+$ *m*/*z* [MH^+] 619.2. $C_{26}H_{42}N_4O_{13}\cdot 2H_2O$ (654.6): calcd. C 47.70, H 7.08, N 8.56; found C, 47.75, H 7.09, N 8.24.

1,3,3′′-Tris-*N*-(trimethylsilylethoxycarbonyl)-6′-*N*-(benzyloxycarbonyl)kanamycin A (3): To a solution of monoprotected kanamycin A (**2**) (0.373 g, 0.6 mmol) in water (5 mL) and dioxane (12 mL) was added triethylamine (350μL, 2.5 mmol, 4 equiv.) and 2-(trimethylsilyl)ethyl *p*-nitrophenyl carbonate (Teoc-O-Np, 0.717 g, 2.5 mmol, 4 equiv.). The mixture was stirred at 55 °C for 48 h, then the volatiles were removed in vacuo. The solid residue was dissolved in ethyl acetate (40 mL) and washed subsequently several times by a 1 N sodium hydroxide solution (40 mL) and a saturated sodium chloride aqueous solution. The organic layer was concentrated to dryness and flash column chromatography of the solid residue (CH_2Cl_2/MeOH, 91:9) afforded the desired product as a white solid (0.465 g, 73.3%). R_f = 0.31 (CH_2Cl_2/MeOH, 90:10). m.p. 222−223 °C dec. $[\alpha]_D^{25}$ = +5.60 (*c* = 0.38, CH_3OH). ^1H NMR (300 MHz, CD_3OD, 25 °C): δ = 7.34 (s, 5 H, arene-H), 5.09 (m, 4 H, CH_2-arene overlapping with two anomeric-H), 4.15 (m, 7 H, O-CH_2-CH_2 and sugar ring proton), 3.80−3.31 (overlapping multiplets, 15 H, various ring protons), 3.19 (t app, *J* = 9.4 Hz, 1 H), 2.06 (partially resolved multiplet, *J* = 12.6 Hz, 1 H, 2-H equatorial), 1.53 (partially resolved multiplet, *J* = 12.6 Hz, 1 H, 2-H axial), 1.00 (m, 6 H, CH_2-Si), 0.06 (s, 27 H, CH_3-Si) ppm.

^{13}C NMR (75 MHz, CD_3OD, 25 °C): δ = 158.69, 158.01, 157.53, 157.20, 136.92, 128.10, 127.62, 127.47, 101.34, 98.52, 84.34, 81.13, 75.30, 73.28, 72.92, 72.65, 71.09, 70.68, 70.60, 68.21, 66.21, 62.71, 62.51, 60.94, 56.46, 50.71, 49.91, 41.17, 34.34, 29.28, 17.30, 17.27, 17.16, −2.78, −2.82, −2.84 ppm. IR (KBr, thin film) ν_{max} (cm^{-1}) = 3335 (br), 2954, 1697 (vs), 1542 (s), 1457, 1419, 1251 (s), 1140, 1043 (s), 939. MS: *m*/*z* MALDI-TOF [MNa^+] 1073.7, [MK^+] 1089.7.

1,3,3′′-Tris-*N*-(trimethylsilylethoxycarbonyl)kanamycin A (4): To a solution of compound **3** (0.3 g, 0.29 mmol) in methanol (25 mL, 10% H_2O, degazed under nitrogen) was added Pd/C (10%, 30 mg). After 1 h stirring in hydrogen atmosphere, the suspension was filtered through a pad of celite and concentrated in vacuo to dryness to yield a white solid (0.23 g, 88%). m.p. 229−230 °C dec. $[\alpha]_D^{25}$ = +6.62 (*c* = 0.62, CH_3OH). ^{1}H NMR (300 MHz, CD_3OD, 25 °C): δ = 5.21 (m, 1 H, anomeric proton), 5.10 (partially resolved multiplet, *J* = 2.7 Hz, 1 H, anomeric proton), 4.16 (m, 7 H, O-C*H*$_2$-CH_2 and sugar ring proton), 3.81−3.31 (overlapping multiplets, 13 H, various ring protons), 3.19 (t app, *J* = 9.3 Hz, 1 H), 3.01 (partially resolved multiplet, *J* = 12.3 Hz, 1 H), 2.69 (partially resolved multiplet, *J* = 13.3, 6.1 Hz, 1 H), 2.07 (m, 1 H, 2-H equatorial), 1.57 (partially resolved multiplet, *J* = 12.6 Hz, 1 H, 2-H axial), 1.01 (m, 6 H, CH_2-Si), 0.06 (s, 27 H, CH_3-Si) ppm. ^{13}C NMR (75 MHz, CD_3OD, 25 °C): δ = 158.70, 157.56, 157.05, 100.96, 98.58, 83.91, 81.39, 75.42, 73.50, 72.96, 72.71, 72.56, 71.57, 70.59, 68.19, 62.76, 62.55, 60.96, 56.45, 50.68, 49.84, 42.34, 34.31, 17.37, 17.31, 17.20, −2.60, −2.65, −2.69 ppm. IR (KBr, thin film) ν_{max} (cm^{-1}) = 3357 (br), 2954, 1695 (vs), 1543 (s), 1419, 1251 (s), 1143, 1042 (vs), 939. MS: *m*/*z* MALDI-TOF [MH^+] 917.7, [MNa^+] 939.7, [MK^+] 955.7.

Compound 5: To a solution of compound **4** (1.655 g, 1.8 mmol) in tetrahydrofuran (75 mL) and dimethylformamide (30 mL) was added triethylamine (1.5 equiv., 377 μL, 2.7 mmol) and cholesteryl chloroformate (1.5 equiv., 1.215 g, 2.7 mmol). The reaction mixture became cloudy with time and was allowed to stir for 48 h. Upon this time, evaporation under reduced pressure was performed until only dimethylformamide remained. Water was then added to this solution and a huge white precipitate was formed. After filtration, flash column chromatography (silica gel, CH_2Cl_2/MeOH, 91:9) afforded the desired product as a white solid (1.857 g, 77.3%). R_f = 0.39 (CH_2Cl_2/MeOH, 90:10). m.p. 250−251 °C. $[\alpha]_D^{25}$ = +4.95 (*c* = 0.36, $CHCl_3$). ^{1}H NMR (300 MHz, CD_3OD/$CDCl_3$, 25 °C): δ = 5.36 (m, 1 H, 6-H from cholesteryl moiety), 5.03 (m, 2 H, anomeric protons), 4.43 (m, 1 H, 3-H from cholesteryl moiety), 4.21−4.05 (m, 7 H, CH_2-O-C=O and sugar ring proton), 3.82−3.29 (m, 15 H), 3.16 (t app, *J* = 9.3 Hz, 1 H), 2.33 (m, 2 H), 2.17 (partially resolved multiplet, *J* = 11.7 Hz, 1 H), 2.04−1.86 (m, 6 H), 1.59−0.84 (m, 39 H, CH_2-Si and various sugar ring and cholesteryl protons), 0.68 (s, 3 H, 18-H from cholesteryl moiety), 0.03 (s, 27 H, CH_3-Si) ppm. ^{13}C NMR (75 MHz, CD_3OD, 25 °C): δ = 158.89, 157.80, 157.46, 157.04, 139.68, 122.43, 101.48, 98.69, 84.51, 81.93, 77.65, 76.08, 75.28, 74.77, 7339, 73.14, 72.62, 71.18, 70.55, 68.82, 63.05, 61.38, 56.67, 56.50, 56.11, 50.67, 50.07, 49.85, 42.21, 40.99, 39.69, 39.40, 38.44, 38.26, 36.92, 36.47, 36.07, 35.70, 31.82, 31.77, 29.47, 28.06, 27.84, 27.10, 23.66, 22.35, 22.10, 20.93, 18.97, 18.36, 17.63, 17.49, 17.44, 11.51, −1.98, −2.06 ppm. IR (KBr, thin film) ν_{max} (cm^{-1}) = 3384 (br), 2953 (s), 1697 (vs), 1542 (s), 1458, 1420, 1251 (s), 1142, 1045 (vs), 948. MS: *m*/*z* MALDI-TOF [MNa^+] 1352.1, [MK^+] 1368.1. $C_{64}H_{116}N_4O_{19}Si_3{\cdot}2H_2O$ (1365.9): calcd. C 56.28, H 8.86, N 4.10; found C, 56.14, H 8.62, N 3.92.

Trifluoroacetate Salt 6; Kanachol (KANAmycin A−CHOLesterol Conjugate): Compound **5** (0.102 g, 0.076 mmol) was suspended in trifluoroacetic acid (2 mL) in an ice-saline bath. After 40 min under 0 °C, the mixture was concentrated to dryness. A foamy solid was obtained after several co-evaporation with methanol. The solid residue was dissolved in water and subsequent lyophilyzation of the solution afforded KanaChol as a fluffy white powder in 97% yield (0.063 g). R_f = 0.32 (CH_2Cl_2/MeOH/NH_4OH, 5:4:1). m.p. 198 °C dec. $[\alpha]_D^{25}$ = +0.68 (*c* = 0.27, H_2O). ^{1}H NMR (300 MHz, CD_3OD/$CDCl_3$, 25 °C): δ = 5.40 (m, 1 H, 6-H from cholesteryl moiety), 5.28 (partially resolved multiplet, *J* = 3.9 Hz, 1 H, anomeric proton), 5.11 (partially resolved multiplet, *J* = 3.6 Hz, 1 H, anomeric proton), 4.40 (m, 2 H), 3.93−3.29 (m, 15 H), 3.18 (t app, *J* = 9.3 Hz, 1 H), 2.55 (partially resolved multiplet, *J* = 12.3 Hz, 1 H), 2.34 (m, 2 H), 2.09−1.88 (m, 6 H), 1.65−0.88 (m, 33 H, various sugar ring and cholesteryl protons), 0.73 (s, 3 H, 18-H from cholesteryl moiety) ppm. ^{13}C NMR (75 MHz, CD_3OD, 25 °C): δ = 157.65, 139.86, 122.04, 100.05, 84.05, 80.75, 74.50, 73.94, 73.11, 72.04, 71.93, 71.24, 68.77, 66.17, 60.39, 56.72, 56.17, 55.52, 50.22, 49.67, 42.09, 41.35, 39.71, 39.28, 38.23, 37.98, 36.87, 36.35, 35.96, 35.70, 31.82, 27.90, 27.73, 23.89, 23.53, 21.76, 21.52, 20.75, 18.39, 17.83, 10.89 ppm. IR (KBr, thin film) ν_{max} (cm^{-1}) = 3421 (br), 2951, 1679 (vs), 1530, 1437, 1383, 1206 (s), 1139 (s), 1054 (s). MS: *m*/*z* MALDI-TOF [MH^+] 897.9, [MNa^+] 919.9. $C_{52}H_{83}N_4O_{19}F_9$ 4·5H_2O (1320.3): calcd. C 47.31, H 7.02, N 4.24; found C, 47.00, H 6.69, N 4.09.

Compound 7: To a solution of kanachol (**6**) (100 mg, 0.08 mmol) in water (0.5 mL) was added dioxane (5 mL) and *N*,*N*′-bisBoc-*N*′′-triflylguanidine (284 mg, 0.72 mmol, 9 equiv.) in alternating portions so that the solution remained clear. After 5 minutes triethylamine (9 equiv., 101 μL, 0.72 mmol) was added. The solution was stirred at room temperature for 4 days after which the volatiles were removed in vacuo. The residue was partitioned between water (10 mL) and chloroform (20 mL). The aqueous layer was separated and extracted with chloroform (2 × 10 mL). The combined organic layer was washed with brine, dried with Na_2SO_4, and concentrated in vacuo. Flash column chromatography (1% methanol in ethyl acetate) afforded the desired product as a white solid (100 mg, 77%). R_f = 0.45 (CH_2Cl_2/MeOH, 90:10). m.p. > 300 °C. $[\alpha]_D^{25}$ = +1.54 (*c* = 0.46, $CHCl_3$). ^{1}H NMR (300 MHz, CD_3OD, 25 °C): δ = 5.43 (m, 1 H, anomeric proton), 5.21 (m, 1 H, 6-H from cholesteryl moiety), 5.14 (m, 1 H, anomeric proton), 4.45−4.24 (m, 3 H), 4.14−4.05 (m, 1 H), 3.86−3.28 (m, 12 H, various sugar ring and cholesteryl protons), 3.13 (m, 1 H), 2.35−0.83 (m, 97 H, CH_3 from Boc, cholesteryl, and sugar ring protons), 0.68 (s, 3 H, 18-H from cholesteryl moiety) ppm. IR (KBr, thin film) ν_{max} (cm^{-1}) = 3327 (br), 2936, 1784, 1724 (s), 1647 (s), 1421, 1369, 1312, 1237, 1152 (vs), 1055 (s). MS: *m*/*z* MALDI-TOF [MNa^+] 1647.2, [MNa^+ − 1 Boc] 1546.9, [MNa^+ − 2 Boc] 1447.1, [MNa^+ − 3 Boc] 1347.5, [MNa^+ − 4 Boc] 1247.3.

Trifluoroacetate Salt 8 of the Tri-Guanidinium Kanamycin A Cholesterol Conjugate (TGKC): Compound **7** (20 mg, 0.012 mmol) was suspended in trifluoroacetic acid (1 mL) in an ice-saline bath. After 4 h the mixture was concentrated to dryness After several co-evaporation with methanol, the solid residue was dissolved in water and subsequent lyophilyzation of the solution afforded 10.1 mg (0.007 mmol, 60%) of TGKC. m.p. 128−129 °C dec. $[\alpha]_D^{25}$ = +2.17 (*c* = 0.23, DMSO). ^{1}H NMR (300 MHz, CD_3OD, 25 °C): δ = 5.39 (m, 1 H, anomeric proton), 5.26 (m, 1 H, 6-H from cholesteryl moiety), 5.09 (m, 1 H, anomeric proton), 4.36 (m, 2 H), 4.09 (m, 1 H), 3.80−3.20 (m, 14 H, various sugar ring and cholesteryl protons), 3.28 (m, 1 H), 2.60−0.80 (m, 33 H, various sugar ring and cholesteryl protons), 0.73 (s, 3 H, 18-H from cholesteryl moiety) ppm. IR (KBr, thin film) ν_{max} (cm^{-1}) = 3425 (br), 2936, 1677 (vs),

1438, 1384, 1206 (s), 1138 (s), 1040. MS: *m/z* MALDI-TOF [MH^+] 1023.8, [MNa^+] 1045.9.

Compound 9a: To a solution of amine **4** (340 mg, 0.38 mmol) in DMF (15 mL) in an ice bath was added subsequently acid **13a** (111 mg, 0.41 mmol, 1.1 equiv.), 1-hydroxy-7-azabenzotriazole (HOAt, 62 mg, 0.45 mmol, 1.2 equiv.), and *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC, 109 mg, 0.57 mmol, 1.5 equiv.). The mixture was stirred 0 °C for 2 h, then 12 h at room temperature until the solution became incolore. The solution was then partitioned between ethyl acetate and water and the organic layer was washed subsequently by an aqueous solution of hydrogen chloride (1%), a solution of sodium carbonate (0.2 M), and a saturated sodium choride aqueous solution. The organic layer was dried with Na_2SO_4, concentrated to dryness, and flash column chromatography (7.5% MeOH in CH_2Cl_2) of the solid residue afforded the desired product as a white powder (307 mg, 71%). R_f = 0.23 (CH_2Cl_2/MeOH, 90:10). ^{1}H NMR (300 MHz, CD_3OD, 25 °C): δ = 7.34 (m, 5 H, arene-H), 5.15−5.06 (m, 4 H, CH_2-arene overlapping with anomeric protons), 4.25−4.10 (m, 7 H, CH_2-O-C=O and sugar ring proton), 3.82−3.32 (m, 15 H), 3.13 (m, 3 H), 2.28 (t app, *J* = 7.3 Hz, 2 H), 2.06 (m, 1 H), 1.68−1.30 (m, 7 H), 1.00 (m, 6 H, CH_2-Si), 0.06 (m, 27 H, CH_3-Si) ppm. ^{13}C NMR (75 MHz, CD_3OD, 25 °C): δ = 175.67, 158.74, 158.68, 157.52, 157.48, 157.20, 137.08, 128.11, 127.58, 127.40, 101.27, 98.57, 84.29, 81.41, 75.25, 73.10, 73.01, 72.78, 71.11, 70.88, 70.59, 68.25, 65.96, 62.74, 62.57 61.01, 56.50, 50.68, 49.90, 40.31, 39.58, 35.46, 29.24, 26.10, 25.34, 17.40, 17.31, 17.22, −2.64, −2.67 ppm. MS: *m/z* MALDI-TOF [MNa^+] 1186.5.

1,3,3′′-Tris-*N*-(trimethylsilylethoxycarbonyl)-6′-*N*-(6-aminohexanoyl)kanamycin A (10a): To a solution of **9a** (118 mg, 0.10 mmol) in methanol (10% H_2O) (5 mL, degazed under nitrogen) was added Pd/C (10%, 12 mg). The solution was degazed and after 1 h stirring in hydrogen atmosphere, the suspension was filtered through a pad of celite and concentrated in vacuo to dryness to yield a white solid (85 mg, 81.4%). ^{1}H NMR (300 MHz, CD_3OD, 25 °C): δ = 5.13−5.08 (m, 2 H, anomeric protons), 4.25−4.10 (m, 7 H, CH_2-O-C=O and sugar ring proton), 3.82−3.32 (m, 15 H), 3.13 (t app, *J* = 9.4 Hz, 1 H), 2.72 (m, 2 H), 2.31 (t app, *J* = 7.3 Hz, 2 H), 2.06 (m, 1 H), 1.71−1.39 (m, 7 H), 1.00 (m, 6 H, CH_2-Si), 0.06 (m, 27 H, CH_3-Si) ppm. ^{13}C NMR (75 MHz, CD_3OD, 25 °C): δ = 174.16, 157.23, 156.06, 155.75, 99.80, 97.08, 82.83, 79.75, 73.78, 71.62, 71.48, 71.31, 69.60, 69.11, 66.71, 61.22, 61.03, 59.47, 54.95, 49.21, 48.39, 43.48, 39.15, 38.01, 29.63, 27.78, 25.15, 24.63, 24.05, 23.89, 15.87, 15.79, 15.68, −4.20, −4.25 ppm. MS: *m/z* MALDI-TOF [MNa^+] 1052.7.

Compound 11a: To a solution of compound **10a** (80 mg, 0.07 mmol) in THF/DMF (9:1, 10 mL) was added triethylamine (1.5 equiv., 16 μL, 0.11 mmol) and cholesteryl chloroformate (1.5 equiv., 52 mg, 0.11 mmol). The reaction mixture was stirred for 12 h. Upon this time, the volatiles were removed in vacuo and flash column chromatography of the solid residue (7.5% methanol in dichloromethane) afforded the desired product as a white solid (100 mg, 89.2%). R_f = 0.30 (CH_2Cl_2/MeOH, 90:10). ^{1}H NMR (300 MHz, CD_3OD/$CDCl_3$, 25 °C): δ = 5.38 (m, 1 H, 6-H from cholesteryl moiety), 5.03 (m, 2 H, anomeric protons), 4.40 (m, 1 H, 3-H from cholesteryl moiety), 4.21−4.06 (m, 7 H, CH_2-O-C=O and sugar ring proton), 3.82−3.32 (m, 15 H), 3.22−3.06 (m, 3 H), 2.39−2.24 (m, 4 H), 2.15 (m, 1 H), 2.05−1.80 (m, 6 H), 1.66−0.80 (m, 44 H), 0.62 (s, 3 H, 18-H from cholesteryl moiety), 0.04 (m, 27 H, CH_3-Si) ppm. ^{13}C NMR (75 MHz, CD_3OD/$CDCl_3$, 25 °C): δ = 175.73, 158.84, 157.47, 157.19, 157.15, 139.79, 122.25, 101.36, 98.65, 84.41, 81.73, 77.75, 75.20, 74.25, 73.02, 72.80, 71.22, 70.57, 68.67, 62.99, 61.30, 56.68, 56.50, 56.11, 50.65, 50.10, 46.68, 42.20, 40.27, 39.70, 39.38, 38.44, 36.93, 36.44, 36.06, 35.71, 31.82, 31.75, 29.47, 29.32, 28.05, 27.82, 26.22, 25.36, 24.08, 23.65, 22.28, 22.03, 20.90, 18.88, 18.30, 17.59, 17.46, 17.41, 11.44, −2.16, −2.19 ppm. MS: *m/z* MALDI-TOF: [MNa^+] 1464.8.

Trifluoroacetate Salt 12a; KanaCapChol: Compound **11a** (42 mg, 0.029 mmol) was suspended in trifluoroacetic acid (2 mL) in an ice-saline bath. After 45 min under 0 °C, the mixture was concentrated to dryness. After several co-evaporation with methanol, the solid residue was dissolved in water and subsequent lyophilyzation of the solution afforded **12a** as a fluffy white powder in 92% yield (36 mg). R_f = 0.32 (CH_2Cl_2/MeOH/NH_4OH, 5:4:1). ^{1}H NMR (300 MHz, CD_3OD, 25 °C): δ = 5.40 (m, 1 H, 6-H from cholesteryl moiety), 5.31 (partially resolved multiplet, *J* = 3 Hz, 1 H, anomeric protons), 5.11 (partially resolved multiplet, *J* = 2.7 Hz, 1 H, anomeric protons), 4.39 (m, 1 H, 3-H from cholesteryl moiety), 4.10−3.32 (m, 16 H), 3.26−3.06 (m, 3 H), 2.55 (partially resolved multiplet, *J* = 12 Hz, 1 H), 2.34−2.22 (m, 4 H), 2.08−1.80 (m, 6 H), 1.66−0.88 (m, 38 H), 0.73 (s, 3 H, 18-H from cholesteryl moiety) ppm. ^{13}C NMR (75 MHz, CD_3OD, 25 °C): δ = 175.99, 157.31, 139.87, 122.02, 100.19, 97.29, 84.02, 80.57, 74.10, 73.80, 72.97, 72.15, 71.98, 71.74, 71.19, 98.77, 66.10, 60.43, 56.73, 56.18, 55.51, 50.23, 49.76, 42.11, 40.13, 39.97, 39.73, 39.29, 38.31, 36.89, 36.34, 35.99, 35.72, 35.51, 31.82, 31.63, 29.36, 29.28, 27.89, 27.74, 26.06, 25.23, 23.91, 23.56, 21.80, 21.55, 20.76, 18.39, 17.87, 10.94 ppm. MS: *m/z* MALDI-TOF [MNa^+] 1032.7, [MH^+] 1010.7.

Compounds **9b−12b and 9c−12c** were prepared according to the procedures described for **9a−12a**.

Compound 9b: Yield 85.3%. R_f = 0.28 (CH_2Cl_2/MeOH, 90:10). ^{1}H NMR (300 MHz, CD_3OD, 25 °C): δ = 7.34 (m, 5 H, arene-H), 5.17 (m, 1 H, anomeric proton), 5.08 (m, 3 H, CH_2-arene overlapping with anomeric proton), 4.22−4.09 (m, 8 H, CH_2-O-C=O, $H_α$ from linker and sugar ring proton), 3.81−3.32 (m, 15 H), 3.13 (m, 3 H), 2.07 (m, *J* = 12.6 Hz, 1 H), 1.85−1.25 (m, 16 H), 1.01 (m, 6 H, CH_2-Si), 0.06 (m, 27 H, CH_3-Si) ppm. ^{13}C NMR (75 MHz, CD_3OD, 25 °C): δ = 174.49, 158.69, 157.56, 157.16 156.51, 137.04, 128.09, 127.57, 127.40, 101.02, 98.51, 83.77, 81.28, 79.29, 75.38, 73.11, 72.97, 72.71, 71.05, 70.60, 68.19, 65.68, 62.75, 62.53, 60.94, 56.45, 54.82, 50.73, 49.81, 40.06, 39.91, 34.20, 31.68, 29.17, 27.44, 22.79, 17.37, 17.27, 17.17, −2.74, −2.77 ppm. MS: *m/z* MALDI-TOF [MNa^+] 1301.6.

Compound 10b: Yield 89%. ^{1}H NMR (300 MHz, CD_3OD, 25 °C): δ = 5.17 (m, 1 H, anomeric proton), 5.09 (partially resolved multiplet, *J* = 2.7 Hz, 1 H, anomeric proton), 4.25−4.10 (m, 8 H, CH_2-O-C=O, $H_α$ from linker and sugar ring proton), 3.80−3.32 (m, 15 H), 3.13 (partially resolved multiplet, *J* = 9.4 Hz, 1 H), 2.69 (t app, *J* = 6.9 Hz, 2 H), 2.06 (partially resolved multiplet, *J* = 12.3 Hz, 1 H), 1.85−1.30 (m, 16 H), 1.01 (m, 6 H, CH_2-Si), 0.06 (m, 27 H, CH_3-Si) ppm. ^{13}C NMR (75 MHz, CD_3OD, 25 °C): δ = 174.53, 158.72, 157.55, 157.16, 156.47, 101.05, 98.58, 83.87, 81.32, 79.26, 75.39, 73.09, 72.98, 72.72, 71.05, 70.59, 70.50, 68.17, 62.73, 62.53, 60.95, 56.39, 54.83, 50.72, 49.81, 40.64, 39.25, 34.27, 31.83, 31.35, 27.45, 22.88, 17.38, 17.29, 17.17, −2.71, −2.75 ppm. MS: *m/z* MALDI-TOF [MNa^+] 1167.8.

Compound 11b: Yield 86%. R_f = 0.32 (CH_2Cl_2/MeOH, 90:10). ^{1}H NMR (300 MHz, CD_3OD, 25 °C): δ = 5.41 (m, 1 H, 6-H from cholesteryl moiety), 5.17 (m, 1 H, anomeric proton), 5.09 (partially resolved multiplet, *J* = 3 Hz, 1 H, anomeric proton), 4.40 (m, 1 H, 3-H from cholesteryl moiety), 4.22−4.06 (m, 8 H, CH_2-O-C=O, $H_α$ from linker and sugar ring proton), 3.80−3.32 (m, 15 H), 3.15−3.09 (m, 3 H), 2.34 (m, 2 H), 2.09−1.82 (m, 7 H), 1.75−0.88

(m, 54 H), 0.74 (s, 3 H, 18-H from cholesteryl moiety), 0.07 (m, 27 H, CH_3-Si) ppm. ^{13}C NMR (75 MHz, CD_3OD, 25 °C): δ = 172.99, 157.19, 156.01, 155.79, 155.64, 155.01, 138.37, 120.56, 99.49, 97.02, 82.27, 79.80, 77.76, 73.88, 72.56, 72.56, 71.62, 71.48, 71.22, 69.57, 69.10, 66.72, 61.24, 61.01, 59.46, 55.24, 54.94, 54.69, 53.34, 49.22, 48.72, 48.34, 40.63, 38.38, 38.25, 37.82, 36.85, 35.41, 34.86, 34.52, 34.25, 32.72, 30.34, 30.16, 27.75, 36.45, 26.26, 25.68, 22.45, 22.09, 21.32, 20.36, 20.11, 19.30, 16.98, 16.43, 15.90, 15.78, 15.69, 9.51, −4.21, −4.26 ppm. MS: *m*/*z* MALDI-TOF [MNa^+] 1580.1.

Trifluoroacetate Salt 12b; KanaLysChol: Yield 96%. R_f = 0.18 (CH_2Cl_2/MeOH/NH_4OH, 5:4:1). ^{1}H NMR (300 MHz, CD_3OD, 25 °C): δ = 5.40 (m, 1 H, 6-H from cholesteryl moiety), 5.35 (partially resolved multiplet, *J* = 3.9 Hz, 1 H, anomeric proton), 5.11 (partially resolved multiplet, *J* = 3.6 Hz, 1 H, anomeric proton), 4.38 (m, 1 H, 3-H from cholesteryl moiety), 3.99−3.32 (m, 16 H), 3.19−3.09 (m, 3 H), 2.55 (partially resolved multiplet, *J* = 12.3 Hz, 1 H), 2.32 (m, 2 H), 2.08−1.85 (m, 8 H), 1.72−0.88 (m, 38 H), 0.74 (s, 3 H, 18-H from cholesteryl moiety) ppm. ^{13}C NMR (75 MHz, CD_3OD, 25 °C): δ = 167.94, 156.26, 138.25, 120.49, 98.74, 95.57, 84.46, 78.77, 72.66, 72.25, 71.37, 70.68, 70.34, 69.81, 69.66, 67.16, 64.53, 58.93, 55.14, 54.58, 53.89, 51.52, 48.64, 48.19, 40.51, 38.60, 38.14, 37.70, 36.71, 35.29, 34.76, 34.39, 34.13, 30.23, 30.04, 29.41, 27.64, 26.33, 26.29, 26.15, 22.32, 21.96, 20.21, 19.96, 19.17, 16.79, 16.27, 9.34 ppm. MS: *m*/*z* MALDI-TOF [MNa^+] 1047.6.

Compound 9c: Yield 87.3%. R_f = 0.28 (CH_2Cl_2/MeOH, 90:10). ^{1}H NMR (300 MHz, CD_3OD, 25 °C): δ = 7.34 (m, 10 H, arene-H), 5.20 (m, 1 H, anomeric proton), 5.10−5.06 (m, 5 H, CH_2-arene overlapping with anomeric proton), 4.19−4.10 (m, 8 H, CH_2-O-C=O, $H_α$ from linker and sugar ring proton), 3.80−3.32 (m, 15 H), 3.12 (m, 3 H), 2.04 (partially resolved multipet, *J* = 12.6 Hz, 1 H), 1.80−1.30 (m, 7 H), 1.00 (m, 6 H, CH_2-Si), 0.06 (m, 27 H, CH_3-Si) ppm. ^{13}C NMR (75 MHz, CD_3OD, 25 °C): δ = 172.55, 157.22, 156.09, 155.70, 135.56, 135.21, 126.70, 126.62, 126.39, 126.10, 126.02, 125.93, 92.28, 97.04, 81.97, 79.96, 73.93, 71.65, 71.55, 71.18, 69.60, 69.39, 69.13, 66.76, 64.98, 64.53, 61.29, 61.08, 59.51, 55.01, 53.75, 49.24, 48.31, 38.58, 38.08, 32.76, 30.02, 27.88, 27.66, 21.26, 15.89, 15.82, 15.73, −4.51, −4.55 ppm. MS: *m*/*z* MALDI-TOF [MNa^+] 1335.7.

1,3,3′′-Tris-*N*-(trimethylsilylethoxycarbonyl)-6′-*N*-(2,6-diaminohexanoyl)kanamycin A (10c): Yield 91%. ^{1}H NMR (300 MHz, CD_3OD, 25 °C): δ = 5.16 (m, 1 H, anomeric proton), 5.09 (m, 1 H, anomeric proton), 4.21−4.10 (m, 8 H, CH_2-O-C=O, $H_α$ from linker and sugar ring proton), 3.81−3.32 (m, 15 H), 3.14 (partially resolved multiplet, *J* = 9.4 Hz, 1 H), 2.75 (t app, *J* = 7.0 Hz, 2 H), 2.06 (m, 1 H), 1.75−1.30 (m, 7 H), 1.02 (m, 6 H, CH_2-Si), 0.06 (m, 27 H, CH_3-Si) ppm. ^{13}C NMR (75 MHz, CD_3OD, 25 °C): δ = 177.16, 158.73, 157.57, 157.20, 101.19, 98.62, 84.14, 81.31, 75.36, 73.09, 73.00, 72.71, 70.92, 70.61, 70.45, 68.19, 62.73, 62.55, 60.97, 56.40, 54.62, 50.71, 49.78, 40.42, 39.13, 34.74, 34.34, 30.67, 22.56, 17.38, 17.29, 17.18, −2.71, −2.74 ppm. MS: *m*/*z* MALDI-TOF [MNa^+] 1067.7, [MH^+] 1045.6.

Compound 11c: Yield 60.9%. R_f = 0.32 (CH_2Cl_2/MeOH, 90:10). ^{1}H NMR (300 MHz, CD_3OD/$CDCl_3$, 25 °C): δ = 5.37 (m, 2 H, 6-H from cholesteryl moieties), 5.17 (m, 1 H, anomeric proton), 5.03 (partially resolved multiplet, *J* = 3.3 Hz, 1 H, anomeric proton), 4.43 (m, 2 H, 3-H from cholesteryl moieties), 4.17−4.00 (m, 8 H, CH_2-O-C=O, $H_α$ from linker and sugar ring proton), 3.82−3.32 (m, 15 H), 3.13−3.06 (m, 3 H), 2.32 (m, 4 H), 2.13 (m, 1 H), 2.05−1.72 (m, 12 H), 1.66−0.85 (m, 77 H), 0.70 (s, 6 H, 18-H from cholesteryl moieties), 0.04 (m, 27 H, CH_3-Si) ppm. ^{13}C NMR (75 MHz, CD_3OD/$CDCl_3$, 25 °C): δ = 174.19, 158.85, 157.46, 157.10, 156.86, 139, 73, 139.56, 122.65, 122.34, 100.66, 98.64, 83.35, 82.02, 77.75, 75.49, 74.83, 74.30, 73.05, 72.68, 71.15, 70.57, 68.68, 62.99, 61.31, 56.77, 56.65, 56.49, 56.26, 56.18, 55.03, 50.68, 50.05, 46.66, 42.23, 39.79, 39.71, 39.39, 38.46, 36.89, 36.47, 36.41, 36.10, 35.78, 31.91, 31.79, 29.47, 29.29, 28.09, 27.98, 27.82, 27.78, 24.15, 24.08, 23.74, 22.32, 22.30, 22.04, 20.94, 19.01, 18.92, 18.33, 17.62, 17.46, 11.56, 11.46, 8.28, −2.12, −2.19 ppm. MS: *m*/*z* MALDI-TOF [MNa^+] 1892.4.

Trifluoroacetate Salt 12c ; KanaLysDiChol: Yield 93.3%. R_f = 0.44 (CH_2Cl_2/MeOH/NH_4OH, 5:4:1). ^{1}H NMR (300 MHz, CD_3OD, 25 °C): δ = 5.40 (m, 2 H, 6-H from cholesteryl moieties), 5.33 (m, 1 H, anomeric proton), 5.12 (m, 1 H, anomeric proton), 4.43 (m, 2 H, 3-H from cholesteryl moieties), 4.25−4.00 (m, 2 H, sugar ring proton and $H_α$ from linker), 3.94−3.32 (m, 15 H), 3.22−3.06 (m, 3 H), 2.54 (m, 1 H), 2.34 (m, 4 H), 2.19−1.75 (m, 12 H), 1.70−0.90 (m, 71 H), 0.75 (s, 6 H, 18-H from cholesteryl moieties) ppm. ^{13}C NMR (75 MHz, CD_3OD, 25 °C): δ = 174.64, 160.75, 157.76, 139.72, 122.28, 100.29, 98.45, 83.84, 80.92, 74.68, 74.24, 73.65, 72.75, 71.95, 70.98, 68.72, 66.10, 60.49, 60.49, 56.82, 56.67, 56.35, 56.25, 55.54, 55.17, 50.0349.86, 42.21, 39.84, 39.69, 39.36, 38.97, 38.54, 38.42, 36.95, 36.78, 36.44, 36.35, 36.09, 35.80, 31.95, 31.74, 31.53, 29.44, 28.06, 27.80, 27.71, 24.13, 24.02, 23.76, 22.15, 21.88, 20.89, 18.90, 18.75, 18.21, 11.51, 11.29, 8.01 ppm. MS: *m*/*z* MALDI-TOF [MNa^+] 1459.9.

***N*-Succinyl(dioctadecyl)amine (13):** To a solution of dioctadecylamine (522 mg, 1 mmol) in pyridine (10 mL) was added succinic anhydride (200 mg, 2 mmol, 2 equiv.). The solution was refluxed for 12 h. The volatiles were removed in vacuo and the solid residue was resuspended in dichloromethane and washed with chlorhydric acid (1 N) followed by water. Recrystallisation in acetone afforded the desired produt as a white solid (566 mg, 90.9%). Mp found 66−68 °C, ref.[34] 56 °C. R_f = 0.86 (CH_2Cl_2/MeOH, 90:10). ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C): δ = 3.24 (quint., *J* = 6.1 Hz, 4 H, CH_2N), 2.65 (m, 4 H, CH_2CO), 1.6−1.1 (m, 64 H), 0.88 (m, 6 H, CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$, 25 °C): δ = 171.47, 48.09, 46.45, 31.80, 29.56, 29.23, 28.77, 27.95, 27.58, 26.93, 26.82, 22.55, 13.92.

Compound 9d: The compound was synthesized according to the procedure used for compounds **9a**−**c** with slight modifications. To a solution of amine **3** (154 mg, 0.168 mmol) in DMF/CH_2Cl_2 (1:1, 20 mL) in an ice bath was added subsequently the acid **13** (115 mg, 0.184 mmol, 1.1 equiv.), HOAt (27 mg, 0.201 mmol, 1.2 equiv.) and EDC (48 mg, 0.251 mmol, 1.5 equiv.). The mixture was stirred 0 °C for 2 h, then 12 h at room temperature. The solution was washed as described for **9a** with dichloromethane instead of ethyl acetate and flash column chromatography (CH_2Cl_2/MeOH, 92.5:7.5) of the solid residue afforded the desired product as a white powder (193 mg, 75.4%). R_f = 0.37 (CH_2Cl_2/MeOH, 90:10). ^{1}H NMR (300 MHz, CD_3OD/$CDCl_3$, 25 °C): δ = 5.29 (m, 1 H, anomeric proton), 5.22 (m, 1 H, anomeric proton), 4.50−4.04 (m, 8 H, CH_2-O-C=O and sugar ring protons), 4.00−3.38 (m, 17 H), 3.34 (t, *J* = 9.3 Hz, 2 H), 2.89 (m, 2 H), 2.76 (m, 2 H), 2.44 (partially resolved multiplet, *J* = 11.1 Hz, 1 H), 1.9−1.7 (m, 5 H, -CH_2- from DODA overlapping with sugar ring proton), 1.49 (m, 60 H, -CH_2- from DODA), 1.21 (partially resolved multiplet, *J* = 7.9 Hz, 6 H, CH_2-Si), 1.10 (partially resolved multiplet, *J* = 6.6 Hz, 6 H, -CH_3 from DODA), 0.26 (m, 27 H, CH_3-Si) ppm. ^{13}C NMR (75 MHz, CD_3OD/$CDCl_3$, 25 °C): δ = 174.13, 171.88, 158.82, 157.46, 157.06, 101.07, 98.63, 83.88, 81.82, 77.80, 75.43, 73.14, 73.04, 72.70, 71.18, 70.93, 70.59, 68.58, 62.94, 61.24, 56.46, 53.33, 50.75, 46.28, 39.92, 31.74, 30.56, 29.68, 29.47, 29.44, 29.35, 29.22, 29.15, 28.99, 28.63,

28.18, 26.84, 26.68, 22.43, 17.59, 17.43, 17.39, 13.44, −2.26, −2.31 ppm. MS: *m*/*z* MALDI-TOF [MNa^+] 1543.1.

Trifluoroacetate Salt 12d ; KanaSucDODA: Compound **11d** was deprotected according to the procedure used for **12a**. Yield 95.5%. R_f = 0.39 (CH_2Cl_2/MeOH/NH_4OH, 5:4:1). ^1H NMR (300 MHz, $CD_3OD/CDCl_3$, 25 °C): δ = 5.29 (m, 1 H, anomeric proton), 5.22 (m, 1 H, anomeric proton), 4.00−3.20 (m, 19 H), 3.17 (m, 2 H), 2.68 (m, 2 H), 2.54 (m, 3 H), 1.96 (partially resolved multiplet, *J* = 12.4 Hz, 1 H), 1.7−1.5 (m, 4 H, -CH_2- from DODA), 1.30 (m, 60 H, -CH_2-from DODA), 0.91 (partially resolved multiplet, *J* = 6.6 Hz, 6 H, CH_3 from DODA) ppm. ^{13}C NMR (75 MHz, $CD_3OD/CDCl_3$, 25 °C): δ = 174.15, 172.35, 100.25, 98.03, 83.84, 80.95, 73.70, 72.96, 72.57, 71.96, 71.25, 68.76, 65.97, 60.33, 55.52, 53.71, 49.83, 48.03, 46.23, 40.20, 31.71, 30.36, 29.45, 29.42, 29.34, 29.29, 29.27, 29.13, 29.02, 28.43, 27.81, 27.36, 26.70, 26.51, 22.37, 13.11 ppm. MS: *m*/*z* MALDI-TOF [MNa^+] 1110.8, [MH^+] 1088.8.

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