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# Homologation of Polyamines in the Synthesis of Lipo-Spermine Conjugates and related Lipoplexes

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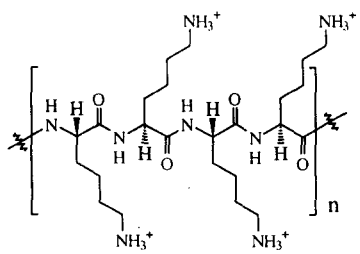
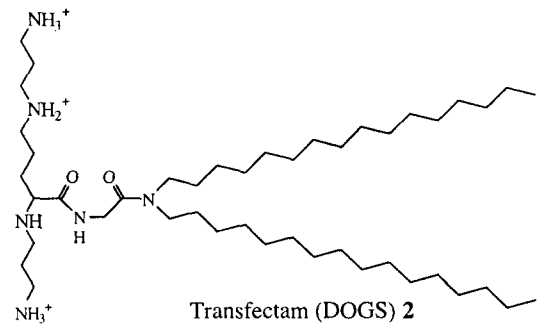
***Abstract:*** Polyamine amides are useful in gene delivery as synthetic (non-viral) vectors or mimics of polycationic histones. The application of a homologation strategy, based upon reductive alkylation, allows unsymmetrical polyamine amides to be prepared in good yield. The interaction of this polyamine amide with calf thymus DNA was demonstrated in an ethidium bromide fluorescence quenching assay.

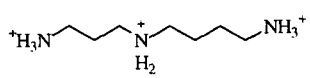

Gene delivery is an established protocol for the introduction into cells of polynucleic acids *in vitro*.[1] However, although gene therapy has the potential to correct a variety of disorders, including inflammation, cancer, neurodegeneration, and cystic fibrosis. Even with more than 200 clinical trials underway worldwide, there is still no single outcome that points to a success story.[1a,1b] One current major problem is the vector which carries the DNA into the cell. Non-viral vectors represent safe and efficient gene transfer strategies[1c,1d] which, unlike viral vectors, do not elicit immune responses.[1e] Poly-L-lysine[2] (n = 55-450) **1** and transfectam[3,4] (DOGS, **2**) are examples of non-viral vectors capable of delivering DNA to cells *in vitro* and *in vivo* through DNA condensation,[5,6] non-specific cell binding and internalisation. Spermidine **3** and spermine **4** contain a 3-4 methylene spacing between the amino functional groups which means that these molecules are essentially fully protonated at physiological pH (i.e. ammonium ions). Therefore, they should interact readily with the DNA phosphate backbone, causing condensation by charge neutralisation.[7]



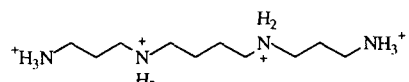
poly-L-lysine **1**



Transfectam (DOGS) **2**



spermidine **3**



spermine **4**

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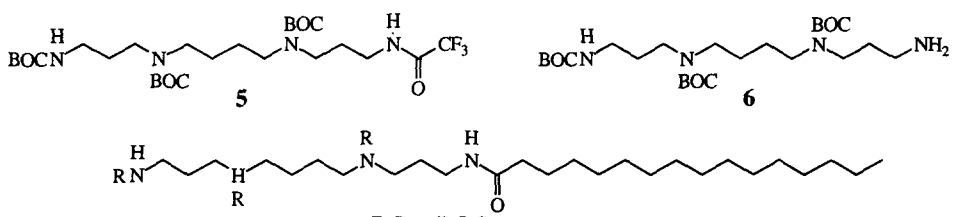
However, these polyamine-DNA interactions are readily reversible under physiological conditions[8] and form one of the plethora of roles played by spermidine **3** and spermine **4** *in vivo*, together with polycationic histones.[5,9] Structure-activity relationship studies (for a review, see: Blagbrough *et al.*[7]) with polyamines have shown that these molecules are ideally suited to bind to and then condense DNA.[10] In order to reinforce these effects, it is apparently beneficial if a lipid is covalently bound to the polyamine moiety, such a lipid can be cholesterol,[10,11] a bile acid,[12] or an aliphatic chain.[3,4] In the preceding *Letter*,[13] we reported the rapid and efficient synthesis of unsymmetrically protected tri-BOC-spermine using trifluoroacetyl as a protecting group. In this *Letter*, we report the preparation of lipo-spermidine **8** and lipo-spermine **13** using reductive alkylation as the key step for the homologation of polyamines and the preparation of unsymmetrical polyamine amides which are charged at physiological pH and therefore interact with DNA[2-12] to form lipoplexes.[1b] We are utilising the charge distribution found in the natural polyamine spermine **4** as a biomimetic warhead for the efficient condensation of plasmid DNA, an essential first step in non-viral gene delivery.

Spermine **4** was selectively protected on one of the primary amino functional groups by reaction with ethyl trifluoroacetate (1.0 eq., MeOH, -78 °C for 1 h then to 0 °C over 1 h), to afford a mixture containing predominantly mono-trifluoroacetamide, but also di-trifluoroacetamide. Immediately, in this solution, the remaining three amino functional groups were BOC protected with di-*tert*-butyl dicarbonate (4 eq., 0 °C to 25 °C over 1 h) to afford compound **5**. The TFA protecting group was then cleaved by increasing the pH to 11 with conc. aq. ammonia, stirring (25 °C, 15 h) to afford, after flash chromatography over silica gel (DCM-MeOH-conc. $NH_4OH$ 70:10:1 to 50:10:1 v/v/v), $N^1$, $N^2$, $N^3$-tri-*tert*-BOC-spermine **6** (50 %). *N*-Acylation of protected spermine **6** with hexadecanoic acid (1.2 eq., palmitic acid), mediated by DCC (1.5 eq.) and catalytic 1-hydroxybenzotriazole (HOBt) (0.2 eq., DMF, 40 °C, 12 h) afforded, after purification over silica gel (EtOAc-hexane 50:50 to 60:40 v/v), tri-BOC protected acylated spermine **7** (95 %). Deprotection by treatment with TFA (90:10 TFA-DCM v/v, 25 °C, 1 h) gave the polytrifluoroacetate salt of polyamine amide **8** (60 %), a spermidine **3** equivalent carrying three charges at physiological pH.



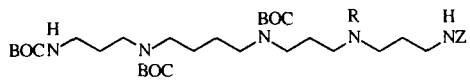
**5** **6**



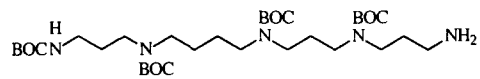
**7** R = BOC
**8** R = $H_2^+$

3-Aminopropan-1-ol was Z-protected under Schotten-Baumann conditions (1.1 eq. Z-Cl, 1 M aq. NaOH, DCM, 0 to 25 °C, 4 h, 60 %). Swern oxidation of the primary alcohol was then achieved by reaction with DMSO activated with oxalyl chloride (DCM, -78 °C), to afford, after purification over silica gel (EtOAc), 3-carbobenzoxyaminopropanal (82 %).

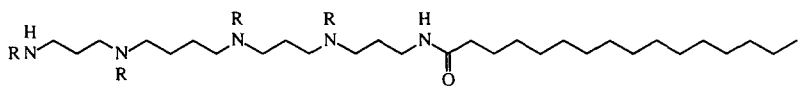
Reductive alkylation of the primary amine in **6** (1.2 eq.) with this aldehyde (1.0 eq., 1.5 eq. $NaCNBH_3$, cat. $CH_3COOH$, anhydrous MeOH, 25 °C, 24 h) gave protected amine **9** which was purified over silica gel (DCM-MeOH-conc. $NH_4OH$ 100:10:1 v/v/v). Secondary amine ($N^4$) was BOC-protected (1.2 eq. $(BOC)_2O$, DMF, 25 °C, 1 h, then quenched with $NH_4OH$) to form fully protected polyamine **10**. Selective removal of the Z carbamate was then achieved by hydrogenolysis (Pearlman's catalyst $Pd(OH)_2$, MeOH, 25 °C, 12 h) to yield, after purification over silica gel (DCM-MeOH-conc. $NH_4OH$ 150:10:1 to 100:10:1 v/v/v), protected polyamine **11** (47 %). *N*-Acylation of primary amine **11** with palmitic acid (1.2 eq., 1.5 eq. DCC, 0.2 eq. HOBt, DMF, $N_2$, 40 °C, 12 h) afforded, after purification over silica gel (EtOAc-hexane 50:50 to 60:40 v/v), poly-BOC protected polyamine amide **12** (95 %). Deprotection by treatment with TFA in DCM (10:90 TFA-DCM v/v, 25 °C, 2 h) gave the polytrifluoroacetate salt of lipo-spermine polyamine amide **13** (55 %).



**9** R = H
**10** R = BOC

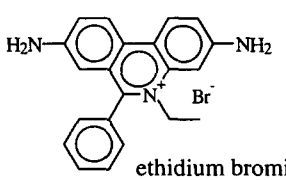


**11**

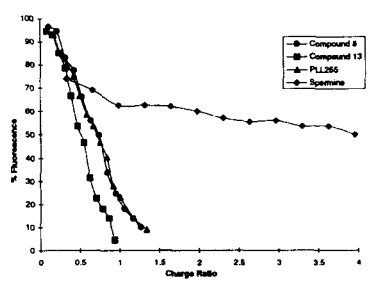


**12** R = BOC
**13** R = $H_2^+$

Lipo-polyamine conjugates **8** and **13** interact with DNA (forming lipoplexes) as demonstrated by an ethidium bromide (EthBr) **14** fluorescence quenching assay.[14a] Prevention of EthBr binding to DNA is a method of studying the binding behaviour of polyamines with nucleic acids.[14-16] While the modes of binding to DNA of aliphatic polyamines and EthBr (a polyaromatic intercalator) are certainly different, this assay does offer a qualitative comparison of the DNA-binding ability of similar classes of compounds.[15,16] We have used poly-L-lysine **1** (n = 255) and spermine **4** as our standards.[14e] Lipopolyamines **8** and **13** interact with calf thymus DNA in a manner consistent with DNA condensation and lipoplex formation. The $IC_{50}$s were determined (see graph) and are respectively 0.75 and 0.52 (charge ratio) compared to 0.75 and >4 (charge ratio) for poly-lysine **1** and spermine **4** respectively. Thus, lipo-spermidine **8** is essentially equipotent with the standard poly-L-lysine **1**. Lipo-spermine **13** has significantly higher binding-affinity for DNA than either **8** or **1**, and all three of these molecules have higher binding-affinity for DNA than free (un-conjugated) spermine **4**.



ethidium bromide **14**



Behr and co-workers have highlighted the key role played by spermine **4**, and established that many properties besides DNA binding strength and compaction are important for efficient gene transfer.[4] Optimisation of the lipid moiety will require the preparation of generations of analogues.[10] Furthermore, there is no obvious correlation between *in vitro* activity and *in vivo* potency with respect to gene delivery.[1b] Our approach allows unsymmetrical polyamine amides to be readily prepared on a gram scale without resorting to multiple chromatographic purification procedures which will be useful in this optimisation procedure.[13]

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