Organic & Biomolecular Chemistry

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: A. K. Yadav, N. Dey, S. Chatterjee, M. Ganguli and M. Fernandes, *Org. Biomol. Chem.*, 2017, DOI: 10.1039/C7OB02552A.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/obc

View Article Online
DOI: 10.1039/C7OB02552A

Organic Biomolecular Chemistry

COMMUNICATION

Dendrimeric Amide- and Carbamate-linked Lysine-based Efficient Molecular Transporters

Received 00th *January* 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Amit Kumar Yadav,[a,b] Namit Dey,[c] Sabyasachi Chattopadhyay,[c] Munia Ganguli[c,*] and Moneesha Fernandes[a,b,*]

Amide- and carbamate-linked dendrimeric oligomers are reported as molecular transporters. They effectively complex with pDNA and transport it into cells at an efficiency superior to Lipofectamine, when complexation is carried out by incubation overnight. The carbamate-linked K2C, is superior to amide-linked K2A; their pDNA complexes have very low associated cytotoxicity.

Cationic molecular transporters, including mainly peptides, are growing in importance to solve the problem of efficiently delivering cell-impermeable cargo molecules such as drugs, nucleic acids or diagnostic agents into cells. Besides peptides, oligoureas,[1] oligocarbamates,[2] peptoids[3] and even cationic polymers[4] have been explored for cell penetration. Among the cell-penetrating peptides (CPPs),[5] the presence of cationic arginine residues and their spatial presentation along the peptide backbone have been shown to be instrumental in deciding the ability to penetrate cells.[6]

Dendrimers have a polyvalent structure that present a concentration of several functionalities of the same type, enabling amplification of interactions with complementary groups. They can be used for drug encapsulation and controlled delivery; the degree of encapsulation generally increases with the dendrimer generation. Cationic dendrimers are also highly water-soluble and dendrimeric peptides are more resistant to proteolytic degradation than linear peptides, thus increasing their half-life in biological systems. They can, therefore, function as transporters for the delivery of cargo molecules into cells.[7] Some commonly known dendrimeric polymers that are capable of cell penetration include the poly(amido amine) (PAMAM),[8] poly(propylene imine) (PPI),[9] poly(lysine)-based dendrimers[10] and poly(ethoxyethylglycinamide) (PEE-G).[11] However, in the clinical context, there are still issues related to toxicity, bioavailability and biocompatibility that limit their application.[12] Therefore, there exists a need for development of new efficient molecular transporters that are non-toxic, freely water-soluble, are resistant to degradative enzymes, and that can carry cargo molecules inside cells.

We describe herein, the synthesis, cell penetration and cargo delivery properties of new lysine-based dendrimers that contain either amide- or carbamate- linkages at their branch points. The synthesis of a generation-2 dendrimeric structure is described (Figure 1), that results in the presentation of eight terminal guanidinium functionalities, for cell penetration. Carbamate linkages were chosen on the basis of our earlier report,[2b] where we showed that (R-X-R)-motif oligocarbamates displayed highly desirable cell penetration properties.

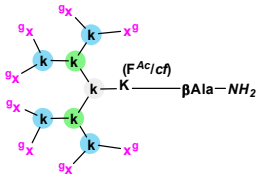


Figure 1. Schematic representation of the designed amide- and carbamate-linked dendrimers. The coloured circles represent the branch points, where the linkages were either amide or carbamate in K2A or K2C respectively; green and blue circles represent the two different dendrimer generations. βAla = β-alanine, K = lysine, F^{Ac} = *N*-acetyl phenylalanine, *cf* = 5(6)-carboxyfluorescein, lower case letters k and x represent residues with either amide- or carbamate- linkages at the attachment points; k = lysine or lysine-derived carbonate monomer and x = 6-aminohexanoic acid or 6-aminohexanoic acid-derived carbonate monomer, g = guanidinium group.

The dendrimers were designed to have terminal guanidinium groups, known to promote cell penetration, appended through 6-aminohexanoic acid-based spacers. A generation-2 dendrimeric structure was selected as it would result in the presentation of eight guanidinium groups, comparable to the same number of arginine residues in our control oligomer, $(R\text{-}X\text{-}R)_4$ (X = 6-aminohexanoic acid), one of the best-known synthetic cell-penetrating peptides for cargo delivery.[13] The dendrimers are shown to be able to penetrate cells, complex

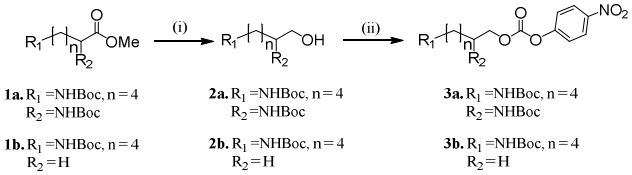
[a] *Organic Chemistry Division, CSIR-National Chemical Laboratory (CSIR-NCL), Dr. Homi Bhabha Road, Pune 411008, India. E-mail: m.dcosta@ncl.res.in*
[b] *Academy of Scientific and Innovative Research (AcSIR), CSIR-NCL Campus, Pune, India*
[c] *CSIR-Institute of Genomics and Integrative Biology, Mathura Road, New Delhi 110020, India. E-mail: mganguli@igib.in*

Electronic Supplementary Information (ESI) available: experimental procedures, chemical structures, NMR & MALDI-TOF spectra, cell studies. See DOI: 10.1039/x0xx00000x

View Article Online
DOI: 10.1039/C7OB02552A

with plasmid DNA (pDNA) cargo and transport it efficiently into cells, with negligible associated cytotoxicity.

The carbonate monomers **3a** and **3b**[2b] for the synthesis of the carbamate-linked dendrimers were synthesized according to Scheme 1. The ester functions in N^{α}-Boc-L-Lys(N-Boc)-methyl ester **1a** and *N*-Boc-6-aminohexanoic acid methyl ester **1b** were reduced by *in situ*-generated $LiBH_4$ to yield the corresponding alcohols **2a** and **2b** respectively. The hydroxyl groups in **2a** and **2b** were each activated as their *p*-nitrophenyl carbonates by treating with *p*-nitrophenylchloroformate in dry pyridine and CH_2Cl_2 to get the *N*-Boc-protected monomers **3a** and **3b**[2b] respectively, amenable for use in oligocarbamate synthesis.



Scheme 1. Synthesis of carbonate monomers for carbamate-linked dendrimer synthesis. Reagents and conditions: (i) $NaBH_4$:LiCl (1:1) (2.5 equiv), THF:EtOH (3:4) (**2a**, 90%; **2b**, 92%); (ii) *p*-nitrophenyl chloroformate (1.2 equiv), dry pyridine (2.5 equiv), CH_2Cl_2 (**3a**, 80%; **3b**, 85%).

The generation-2 dendrimers of this study were synthesized in a divergent strategy by solid phase synthesis, described in Schemes 2 and S1 (ESI). For the carbamate-linked dendrimers (Scheme 2), the *N*-Boc-protected *p*-nitrophenyl activated carbonate monomers **3a** & **3b** were incorporated at appropriate positions during synthesis on the solid support, while for the amide-linked dendrimers (ESI, Scheme S1), N^{α}-Boc-Lys(N^{ε}-Boc)-COOH and *N*-Boc-6-aminohexanoic acid were employed. MBHA resin was used as the solid support and Boc-chemistry protocols were used to assemble the dendrimers by following repetitive cycles of deprotection, neutralization and coupling. β-Alanine was first coupled to the solid support as a spacer, to give **4**, followed by an orthogonally-protected lysine residue, yielding **5**, to enable differential attachment of fluorescein or phenylalanine prior to cleavage from the support. The deprotection and coupling steps were monitored by the Kaiser test. For the coupling step, pre-activated monomers in the form of *p*-nitrophenyl carbonates were used for generation of carbamate linkages (Scheme 2), while appropriate coupling agents were used with the designated *N*-Boc-protected amino acids for the generation of amide linkages (ESI, Scheme S1). Accordingly, resin-linked compounds **6C** through **9C** were obtained bearing carbamate linkages, while compounds **6A** through **9A** contained amide linkages. For the synthesis of fluorescently-labeled dendrimers (Scheme 3), **9A** or **9C** was first treated with TFA to cleave the Boc-protecting groups, followed by guanidinylation of the resulting amine using 1*H*-pyrazole-1-carboxamidine hydrochloride in the presence of Hünig's base, to obtain **10A** and **10C** respectively. Removal of the Fmoc-protecting group of the lysine-N^{ε}-amino group was achieved by treatment with piperidine, and subsequent coupling with 5(6)-carboxyfluorescein using HOBt and diisopropylcarbodiimide (DIPCDI) as the coupling agents yielded the resin-linked fluoresceinylated dendrimers **11A** and **11C** respectively. In the absence of carboxyfluorescein, L-phenylalanine (Phe) was coupled to the N^{ε}-amino group of lysine in **9A** or **9C** (Scheme 3) to facilitate the calculation of concentration by UV-absorbance. In this case, the N^{α}-amino group of phenylalanine was subsequently acetylated (**12A** and **12C** respectively) to rule out its contribution towards the cationic charges of the dendrimer. TFA-mediated cleavage of the Boc-protecting groups in **12A** and **12C**, followed by guanidinylation, yielded the resin-linked dendrimers **13A** and **13C** respectively. The synthesized dendrimers **11A**, **11C**, **13A** and **13C** were cleaved from the solid support using TFA-TFMSA cleavage protocol to obtain **K2A-cf**, **K2C-cf**, **K2A** and **K2C** respectively. The chemical structures of the dendrimers are illustrated in the ESI (Figure S1). They were purified by RP-HPLC and characterised by MALDI-TOF mass spectroscopic analysis (Table 1), after re-checking their purity by analytical RP-HPLC on a C18 column.

Table 1. Lysine-based dendrimers of the study.

Sr. No.	Name	Dendrimer	MALDI-TOF Mass	
			Calcd.	Obsd.
1.	K2A	{[(*C(=NH$_2^+$)NH$_2$*-X)$_2$-K]$_2$-K}$_2$-K-K(Phe-*Ac*)-βAla-*NH$_2$*(amide)	2542.74	2547.29
2.	K2A-*cf*	{[(*C(=NH$_2^+$)NH$_2$*-X)$_2$-K]$_2$-K}$_2$-K-K(*cf*)-βAla-*NH$_2$*(amide)	2711.71	2717.16
3.	K2C	{[(*C(=NH$_2^+$)NH$_2$*-x)$_2$-k]$_2$-k}$_2$-k-K(Phe-*Ac*)-βAla-*NH$_2$*(carbamate)	2992.90	2998.39
4.	K2C-*cf*	{[(*C(=NH$_2^+$)NH$_2$*-x)$_2$-k]$_2$-k}$_2$-k-K(*cf*)-βAla-*NH$_2$*(carbamate)	3163.81	3170.11

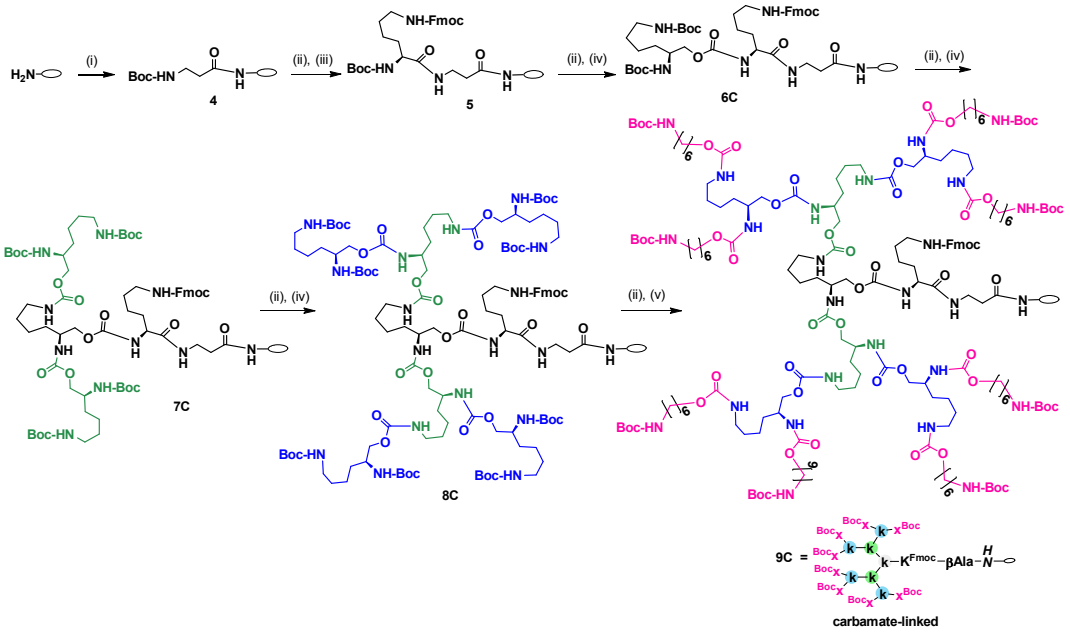
K = lysine, X = 6-aminohexanoic acid, k = carbamate-linked lysine derivative from **3a**, x = carbamate-linked 6-aminohexanoic acid derivative from **3b**, *C(=NH$_2^+$)NH$_2$*- = guanidinylation at N-terminus of dendrimer, Phe-*Ac* = N^{α}-acetyl phenylalanine, *cf* = 5(6)-carboxyfluorescein, βAla = β-alanine at the C-terminus of dendrimer.

CD spectroscopic studies were carried out to study the secondary structural features of the dendrimers. Spectra were recorded in water and in the presence of TFE (Figure 2). The CD spectrum of the carbamate-linked dendrimer, K2C, was almost featureless, probably a result of the flexibility associated with the introduction of the carbamate linkage, as observed earlier for (r-x-r)-motif carbamates.[2b] On the other hand, the CD spectrum of the amide-linked dendrimer, K2A, that showed a maximum at 216 nm and a minimum at 201 nm in water, changed significantly in the presence of TFE, when it displayed minima at 206 nm and 228 nm. Thus, the less structured and flexible K2C, with its cationic guanidinium groups, might be more amenable to adopt conformations favouring interactions with cellular membranes resulting in better cellular entry.

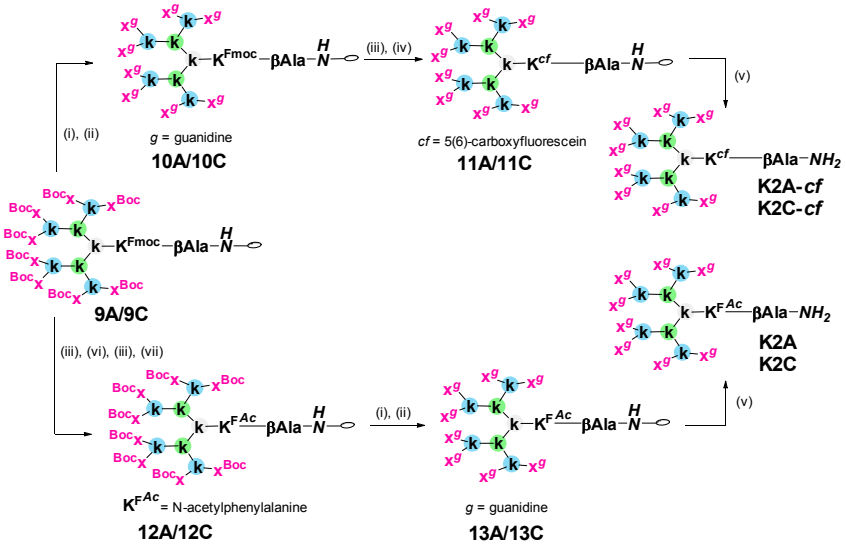
View Article Online
DOI: 10.1039/C7OB02552A

Organic Biomolecular Chemistry

COMMUNICATION



Scheme 2. Solid phase synthesis of carbamate-linked lysine-based dendrimer precursor **9C**. Reagents and conditions: (i) Boc-βAla-OH (3.0 equiv), HOBt (3.0 equiv), TBTU (3.0 equiv), DIPEA (3.0 equiv), DMF; (ii) 50 % TFA/CH_2Cl_2; (iii) Boc-Lys(Fmoc)-OH (3.0 equiv), HOBt (3.0 equiv), TBTU (3.0 equiv), DIPEA (3.0 equiv), DMF; (iv) **3a** (3.0 equiv), DIPEA (3.0 equiv), DMF; (v) **3b** (3.0 equiv), DIPEA, DMF.



Scheme 3. Synthesis of guanidinylated and fluorescein-labeled dendrimers. Reagents and conditions: (i) 50 % TFA/CH_2Cl_2; (ii) 1H-pyrazole carboxamidine hydrochloride (10.0 equiv per amine), DIPEA (10.0 equiv per amine), DMF; (iii) 20 % piperidine/DMF; (iv) 5(6)-carboxyfluorescein (10.0 equiv), HOBt (10.0 equiv), DIPCDI (10.0 equiv), DMF; (v) TFA, TFMSA, 1, 2-ethane dithiol, thioanisole; (vi) Fmoc-Phe-OH (3.0 equiv), HOBt (3.0 equiv), TBTU (3.0 equiv), DIPEA (3.0 equiv), DMF; (vii) Ac_2O (10.0 equiv), pyridine.

View Article Online
DOI: 10.1039/C7OB02552A

Organic Biomolecular Chemistry

COMMUNICATION

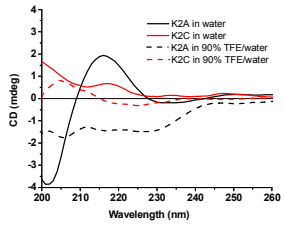


Figure 2. CD spectra of the K2A and K2C oligomers (500 µM) in water and 90% TFE/water.

The ability of the dendrimers to penetrate cells was evaluated in CHO-K1 and HaCaT cells. As seen in Figure 3, the dendrimers were able to penetrate cells efficiently, with almost 100 % cells showing the presence of internalized dendrimers with high mean fluorescence intensity. The percentage of fluorescent cells was similar to the corresponding non-dendrimeric oligomer $(R\text{-}X\text{-}R)_4$ in CHO-K1 and HaCaT cells; and the mean intensities were either slightly lower or slightly higher than the non-dendrimeric counterpart indicating the efficiency of the designed dendrimers.

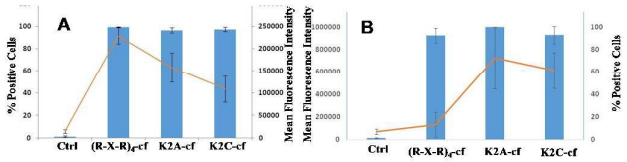


Figure 3. Uptake of the dendrimers in (A) CHO-K1 and (B) HaCaT cells. The cells were treated with the dendrimers (5 µM) for 4 h at 37 °C.

The ability of the dendrimers to carry pDNA cargo into cells was next evaluated. Each dendrimer in the present study bears eight guanidinium groups that are positively-charged at physiological pH. The cationic nature of the dendrimers conveniently allows their complexation with negatively-charged cargo such as DNA through charge interactions. Further, the efficacy of the cargo is significantly dependent on its release from the molecular transporters, once inside the cells. We therefore evaluated the ability of the dendrimers to form complexes with pDNA; subsequent release of the pDNA was studied through exposure to anionic challenge by the addition of heparin. Thus, K2A and K2C were separately pre-mixed with pDNA at different charge ratios (Z+/-, N:P; positively-charged N in dendrimer to negatively-charged phosphate in DNA). The resulting complexes were analysed by their mobility in agarose gels in an electrophoretic mobility shift assay (Figure 4). The carbamate-linked K2C was found to be more efficient at condensing DNA, than the corresponding amide-linked K2A. Almost complete condensation was observed for K2C at charge ratio charge ratio 5, while at a charge ratio of 10, no uncondensed pDNA was observed. In comparison, with K2A, a small amount of uncondensed pDNA was visible even at the charge ratio of 10. On the whole, the charge ratio 10 was considered suitable for pDNA complexation and used in all further cargo delivery studies. The release of complexed pDNA from its complexes with dendrimers was studied by exposure to anionic challenge, achieved by addition of increasing heparin concentrations (Figure 5). Complete release of pDNA was observed from the complexes formed by K2A at a ratio of 1:5 (dendrimer:heparin, w/w), while no release was observed from the K2C complexes even at a ratio of 1:10 (dendrimer:heparin, w/w). This could account for the lower transfection efficiency observed for this dendrimer with complexes prepared by 1 h incubation as described in subsequent sections. However, when the complexes were prepared by incubation for 24 h, very high release of pDNA was observed from the K2C complexes even at lower heparin concentrations. This indicates a better stability-release balance in case of the K2C complexes in comparison to K2A complexes.

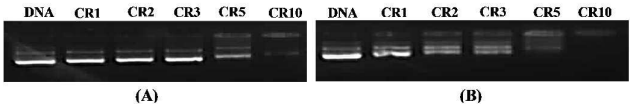


Figure 4. DNA condensation profile for complexes of (A) K2A and (B) K2C dendrimers with pDNA at increasing charge ratios.

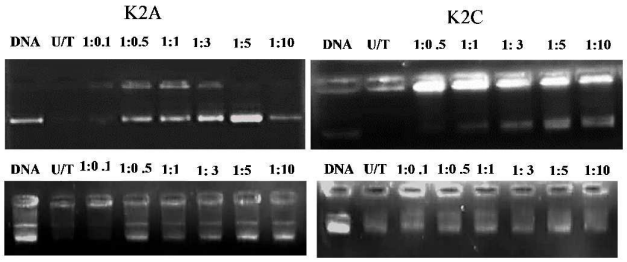


Figure 5. Heparin-induced pDNA release study from the complexes with K2A (left panels) and K2C (right panels). The complexes were prepared by incubating the dendrimers with pDNA for 1 h (upper panels) or overnight (lower panels) respectively. U/T = untreated.

Transfection was studied in CHO-K1 and HaCaT cells that were treated with complexes pre-formed by mixing K2A or K2C dendrimers with pMIR-Report luciferase plasmid DNA (Figure 6 and 7 respectively). For complex formation, a charge ratio of 10 was used. Lipofectamine™ 2000 was used as a standard transfection agent for comparison. K2C-pDNA complexes were found to be more efficient at transfection in comparison to

View Article Online
DOI: 10.1039/C7OB02552A

K2A-pDNA complexes in both cell lines studied. When the complexes were prepared by overnight incubation, the transfection efficiency was even higher than that of Lipofectamine™ 2000. Given the fact that the transfection efficiency of Lipofectamine™ 2000 *in vivo* is limited by its high associated cytotoxicity, this study illustrates the potential of the K2C dendrimer as a molecular transporter that can be used at a lower concentration, with minimal cytotoxic effects.

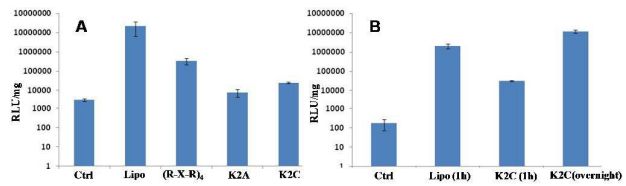


Figure 6. Transfection of CHO-K1 cells by pMIR-Report luciferase complexes with K2A and K2C dendrimers. (A) Dendrimer-pDNA complexes were prepared by incubation for 1 h. (B) Comparison of transfection when K2C-pDNA complexes were prepared by incubation for 1 h or overnight.

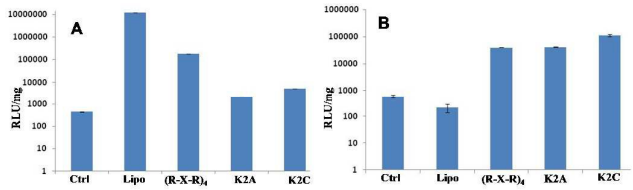


Figure 7. Transfection of HaCaT cells by pMIR-Report luciferase complexes with K2A and K2C dendrimers. $(R\text{-}X\text{-}R)_4$/Dendrimer-pDNA complexes were prepared by incubation (A) for 1 h or (B) overnight.

The cytotoxicity of the K2A and K2C dendrimers alone and in the form of complexes with pDNA was assessed by the standard MTT cell viability assay (Figures 8 and S2 (ESI)). While K2A by itself showed a slight toxic effect in CHO-K1 cells (Figure 8C), the cytotoxicity of the dendrimer-pDNA complexes was minimal even after 24h. This data is consistent with that obtained for the $(R\text{-}X\text{-}R)_4$-amide and -carbamate oligomers,[2b] making K2A and K2C advantageous over commercial transfection agents such as Lipofectamine, which displayed considerable cytotoxicity in comparison.[2b]

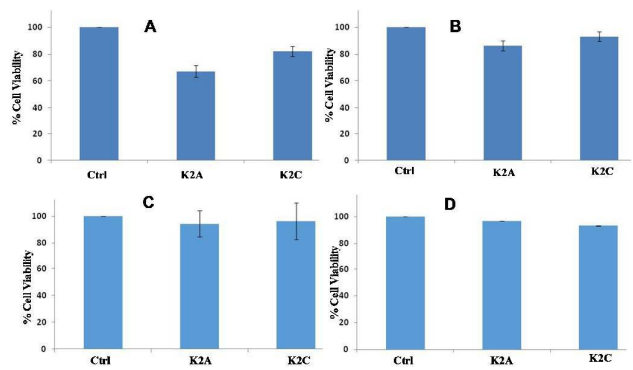


Figure 8. Effect of dendrimers (left panels) and dendrimer-pDNA complexes (right panels) on cell viability after 4 h in CHO-K1 (A and B respectively) and HaCaT cells (C and D respectively).

HaCaT cells were treated with the fluorescein-labeled dendrimers, K2A-*cf* and K2C-*cf*, following which, the cells were imaged to show the intracellular localization of the dendrimers. As seen in Figure S3 (ESI), K2C-*cf* was taken up to a larger extent than K2A-*cf*. Further, the fluorescence appears to be distributed in the cytoplasm as well as in the nucleus and nucleoli, illustrating their cell penetration efficiency.

Conclusions

In summary, we have designed and synthesized efficient lysine-based generation-2 dendrimers bearing amide- or carbamate-linkages at the branch points. The dendrimers possess good cell-penetration properties, and are capable of delivering cargo molecules such as pDNA into cells at an efficiency that is comparable or even better than the standard transfection agent, Lipofectamine, when their complexes with pDNA were prepared by incubation overnight. Taken together with their lower cytotoxicity, this makes them worthy candidates for further development as molecular transporters.

Conflicts of interest

There are no conflicts to declare.

Notes and references

‡ MG and MF gratefully acknowledge research funding (BSC0302) from CSIR, New Delhi. AKY thanks CSIR, New Delhi, for a research fellowship.

1 N. Tamilarasu, H. Ikramul, M. R. Tariq, *J. Am. Chem. Soc.*,1999, **121**, 1597-1598.
2 (a) P. Wender, J. Rothbard, T. Jessop, E. Kreider, B. Wylie, *J. Am. Chem. Soc.*, 2002, **124**, 13382-13383. (b) K. M. Patil, R. J. Naik, Rajpal, M. Fernandes, M. Ganguli, V. A. Kumar, *J. Am. Chem. Soc.*, 2012, **134**, 7196−7199.
3 (a) P. Wender, D. Mitchell, K. Pattabiraman, E. Pelkey, L. Steinman, J. Rothbard, *Proc. Natl. Acad. Sci., USA*, 2000, **97**, 13003-13008. (b) W. Huang, J. Seo, J. Lin, A. Barron, *Mol. BioSyst*., 2012, **8**, 2626-2628. (c) T. Schröder, K. Schmitz, N. Niemeier, T. Balaban, H. Krug, U. Schepers, S. Bräse, *Bioconjugate Chem*., 2007, **18**, 342-354.
4 (a) Y. Kim, S. Binauld, and M. H. Stenzel, *Biomacromolecules*, 2012, **13**, 3418−3426 (b) G. Gasparini, E. K. Bang, G. Molinard, D. V. Tulumello, S. Ward, S. O. Kelley, A. Roux, N. Sakai, S. Matile, *J. Am. Chem. Soc*., 2014, **136**, 6069−6074. (c) N. J. Treat, D. Smith, C. Teng, J. D. Flores, B. A. Abel, A. W. York, F. Huang, C. L. McCormick, *ACS Macro Lett*., 2012, **1**, 100−104.
5 (a) S. Abes, H. Moulton, P. Clair, P. Prevot, D. Youngblood, R. Wu, P. Iversen, B. Lebleu, *J. Controlled Release*, 2006, **116**, 304-313. (b) P. A. Wender, M. A. Huttner, D. Staveness, J. R. Vargas, A. F. Xu, *Mol. Pharmaceutics*, 2015, **12**, 742−750.
6 (a) J. B. Rothbard, E .Kreider, C. L. VanDeusen, L. Wright , B. L. Wylie, P. A. Wender, *J. Med. Chem*., 2002, **45**, 3612-3618. (b) Y. Demizu, M. Oba, K. Okitsu, H. Yamashita, T. Misawa, M. Tanaka, M. Kurihara, S. H. Gellman, *Org. Biomol. Chem*., 2015, **13**, 5617–5620. (c) N. E. Zhou, B. -Y. Zhu, B. D. Sykes, R. S. Hodges, *J. Am. Chem. Soc*., 1992, **114**, 4320-4326.
7 (a) A. F. Saleh, A. Arzumanov, R. Abes, D. Owen, B. Lebleu, M.J. Gait, *Bioconjugate Chem*. 2010, **21**, 1902–1911. (b) A. Eggimann, E. Blattes, S. Buschor, R. Biswas, S. M. Kammer, T.

View Article Online
DOI: 10.1039/C7OB02552A

Darbre, J. -L. Reymond, *Chem. Commun*., 2014, **50**, 7254-7257.
8 D. A. Tomalia, H. Baker, J. Dewald, M. Hall, G. Kallos, S. Martin, J. Roeck, J. Ryder, P. Smith*, Polym. J*., 1985, **17**, 117−132.
9 E. Buhleier, W. Wehner, F. Vogtle, *Synthesis*, 1978, **2**, 155−158.
10 (a) C. Rao, J. P. Tam, *J. Am. Chem. Soc*., 1994, **16**, 6975−6976. (b) J. P. Tam, *Proc. Natl. Acad. Sci. U. S. A*., 1988, **85**, 5409−5413. (c) J. Zhao, R. Zhou, X. Fu, W. Ren, L. Ma, R. Li, Y. Zhao, L.Guo, *Arch. Pharm. Chem. Life Sci*., 2014, **347**, 469–477.
11 S. Toms, S. M. Carnachan, I. F. Hermans, K. D. Johnson, A. A. Khan, S. E. O'Hagan, C. -W. Tang, P. M. Rendle, *ChemMedChem*., 2016, **11**, 1583–1586.
12 M. L. Watala, C. Watala. *J. Pharm. Sci*., 2015, **104**, 2–14.
13 (a) R. Abes, H. M. Moulton, P. Clair, S. -T. Yang, S. Abes, K. Melikov, P. Prevot, D. S. Youngblood, P. L. Iversen, L. V. Chernomordik, B. Lebleu, *Nucleic Acids Res*., 2008, **36**, 6343-6454. (b) N. Jearawiriyapaisarn, H. M. Moulton, B. Buckley, J. Roberts, P. Sazani, S. Fucharoen, P. L. Iversen, R. Kole, *Mol. Ther*., 2008, **16**, 1624-1629.

View Article Online
DOI: 10.1039/C7OB02552A

Table of Contents entry

Graphical abstract

Carbamate- and amide-linked generation-2 dendrimeric oligomers transport pDNA into cells very efficiently when complexed by incubation overnight.