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Benzylic Viologen Dendrimers: Review of Synthesis, Properties and Applications

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Abstract

Synthesis of benzylic viologen dendrimers, their guest complexation, photo physical and biological applications has been reviewed. Divergent and convergent approaches reported by Walder et al and Balzani et al for the synthesis of benzylic viologen dendrimers are discussed. Electron sponge behaviour and their use as electron storage devices is elaborated. CT (charge transfer) complexation with guest anionic dye molecules and their use as charge storing devices are discussed. It has been shown that these dendrimers sequentially complex organic anions guided through molecular recognition. Anions with matching symmetry showed sharp chemical shifts in ^{1}H NMR titrations. Existence of potential gradient is explained for such preferential complexation mechanism. Thus the sequential complexation of organic anions inside these dendrimers as evidenced by ^{1}H NMR titrations are briefly discussed. Further the biological applications focusing on their antiviral, antibacterial and antifungal activities reported so far has been addressed.

KEYWORDS

Dendrimers • polycations • Viologen • host-guest chemistry • Redox Gradient

1. Introduction:

Dendrimers are highly branched, symmetrical 3D macromolecules that emanate from a central core, branch along the branching units and end with a peripheral group giving rise to a defined architecture. Due to their high symmetry and monodispersity, they serve as a special class of polymers whose intrinsic properties are knowable. Though much of the early researches have focused on the synthetic methodology,[1] researchers have soon realised their potential to host guest molecules via different interactions such as [1]H bonding,[2] ionic interactions,[3] van der waals,[4] hydrophobic,[5] etc. Both dendrimers and its guest complexed supramolecular form shows wide range of physical and biological applications transforming it to a highly inter- or multidisciplinary area. Wide variety of dendrimers has been synthesized till date to address specific issues in the area of physics, chemistry and biology. Apart from highly symmetrical dendrimers, hybrid dendrimers[6] are known comprising of different chemical entities that are covalently bound giving rise to a dendrimer like architecture. Hybrid dendrimers are also classified under dendritic macromolecules due to their defined architecture, monodispersity, shape and molecular weight resemblance. Among all, dendrimers bearing redox active functionalities at the core or along the branches or at the periphery attracts more attention towards photo physical applications. Presence of such redox active functionalities generate a built-in redox gradient.[7] Oxidation or reduction of such molecules will show electron funnelling or antenna behavior which mimic natural photosystems.[8] Previous studies have shown that dendrimers containing viologen subunit at the core and electron-donating branches along the periphery mimic natural light harvesting systems.[9]

Dendrimers built of viologen subunits will be more attractive as i) viologens (4,4'-bipyridinium salts) are well-known electrochromic materials[10] and in addition they show

photochromism in the presence of a photosensitizer,[11] ii) due to their strong ability to accept electrons[12] they form strong and stable CT (charge-transfer) complexes with donor molecules.[11-13] Different research groups have reported on viologen functionalized dendrimers (at the core,[14] along the branches[12,15] or at the periphery[16]) and utilised them for variety of physical studies. Besides these, dendrons containing viologen subunits at the apical position have been successfully synthesized and self-assembled.[17] .

To date, massive number of reviews addressing the synthetic strategies including job-specific functionalization, guest-complexation, wide range of physical and biological applications, various analytical methods used for characterization, and monitoring guest-complexation, has been published.[18] The focus of the present review is to address on the benzylic viologen dendrimers, where we will summarize the so far reported synthetic methods (both convergent and divergent approach), their interesting electron sponge behavior, internal CT complexation upon reduction, CT complexation with guest molecules, step-wise complexation of organic anions and biological applications such as antiviral, antibacterial, antifungal activities and their application as gene delivering agents.

2. Synthesis

Benzylic viologen dendrimers were first reported by Walder et al via divergent approach using preformed branching units as shown in scheme 1 and 2.[15b] A year later, they reported on the synthesis of benzylic viologen dendrimers with a diphenylviologen core and compared their size, reduction induced pimerization and charge trapping behavior.[15a] In 2010, Walder et al discussed on the convergent and divergent strategies for the synthesis of benzylic viologen dendrimers along with their key intermediate synthesis (scheme 1).[19] Their corresponding convergent approach was reported by Balzani et al.[11-12] Convergent synthesis of hybrid phosphorus-viologen dendrimers with benzylicviologen branching units was reported by

Majoral et al.[20] The divergent approach reported by Walder et al and the convergent approach reported by Balzani et al will be discussed in detail in the following section.

Scheme 1

a) Divergent approach:

The key starting materials for the synthesis of benzylic viologen dendrimers were 1,3,5-tris(bromomethyl)benzene, its dihydroxy precursor (**V_1**) and 4,4'-dipyridyl (scheme 1).[19] Selective alkylation was carried out to yield the central core (**P_0**.3PF_6) where one end of the dipyridyl was quarternized and the other end remain free for further alkylation.[21] This could be achieved by using large excess of 4,4'-dipyridyl and slow addition. On the other hand 1-(bromomethyl)-3,5-bis(hydroxymethyl)benzene (**V_1**) and alkyl- or benzylbromide were selectively quarternized to yield the divergent dendron (**V_2**.PF_6) and end group (**E**.PF_6) respectively following the same approach. For zeroth generation, central core with pyridyl end was reacted with the corresponding alkyl or benzyl bromide. For higher generations, **P_0**.3PF_6 was reacted with **V_1** to yield **P_1**.6PF_6. Bromination of the corresponding product using HBr-HOAc gives the benzyl bromide terminated dendrimer which was then functionalized with end group **E**. Higher generations could be achieved by repeating the reaction sequence in the same way as mentioned above, i.e., quarternization of the brominated **P_1**.6PF_6 with **V_2**.PF_6, activation of the hydroxy group to benzylic bromide and further quarternization with the end group E. In this method, repetitive reactions were performed on a central core in sequence. Upto 3 generations have been reported so far. It is notable that generations beyond 3 are not possible due to steric crowding and solubility issues.

Scheme 2

Walder et al reported on the ethyl and 4-tert-butylbenzyl terminated benzylic viologen dendrimers, where the latter showed better solubility.[15b,15c] Asaftei et al reported on the bis(hydroxymethyl)benzyl- and thymine terminated benzylic viologen dendrimers following the same approach.[22] Bongard et al reported on the alkylammonium and alkylcarboxyl terminated benzylic viologen dendrimers as well as the synthesis of phenylbutyl viologen dendrimers using the same approach.[23]

b) Convergent approach:

Balzani et al reported on the convergent synthesis of benzylic viologen dendrimers terminated with a bulky tetraarylmethane groups[12] and aryloxy groups.[11] The key convergent dendron was synthesized by reacting 1,3-bis(bromomethyl)-5-(hydroxymethyl)benzene and 4,4'-dipyridyl in a selective manner to yield $\mathbf{R'_1}.2PF_6$.[19] The so obtained salt was then quarternized with the end group to give $\mathbf{R'_2}.4PF_6$. Activation at the benzylic alcohol position using $SOCl_2$ or $CBr_4/PPh_3/THF$ gave the corresponding benzylic chloride or bromide precursor which was then quarternized with $\mathbf{P_0}.3PF_6$ to yield generation 1 dendrimer $\mathbf{R'_9}.18PF_6$. Reaction of $\mathbf{R'_2}.4PF_6$ with the key convergent dendron $\mathbf{R'_1}.2PF_6$ yielded $\mathbf{R'_6}.12PF_6$. The benzylic alcohol thus obtained was activated using $SOCl_2$ or via Appel reaction and reacted further with the central core $\mathbf{P_0}.3PF_6$ to yield the generation 2 dendrimer $\mathbf{R'_{21}}.42PF_6$. The yields reported by this method[11-12,24] were relatively poor and this could be attributed to:

1) the use of Cl^- as the leaving group for quarternization of dpy (dipyridyl), where Cl^- is down in the order TsO^- or $I^- > Br^- > Cl^-$

2) limited solubility of viologen salts in THF which subsequently reduces the yield of Appel reaction.

Nevertheless the above mentioned problems could be easily surmounted by using HBr-HOAc as a brominating agent, where the transformation to benzylic bromide occurs in a homogeneous medium.

Tetraarylmethane,[12,24] aryloxy[11] and 4-*tert*-butyl[15c] terminated dendrimers as PF_6 salts are soluble in DCM, CH_3CN, CH_3COCH_3, DMF, DMSO whereas ethyl terminated Walder dendrimers[15b] are insoluble in DCM and soluble in rest of the solvents. Although all these dendrimers carry identical positive charge, their peripheral groups play an important role in determining their solubility and electron transfer behavior (section 4b).

Scheme 3

Synthesis of hybrid phosphorus-viologen dendrimers has been addressed in a review article by Majoral et al.[25]

3. Guest Interactions:

a) Counting the number of seats available for the guest molecules, guest binding and guest release

Balzani et al successfully guest complexed eosin (dianion) molecules onto the benzylic viologen dendrimers.[12] Authors observed 1:1 complexation (K_{ass} > 10^6 M^{-1}) between the viologen subunits and the eosin anions. The guest complexed supramolecular form did not show any fluorescence due to the static quenching by viologen subunits and there was a strong perturbation in the visible absorption band. CT (Charge Transfer) band is indicated by a weak absorption tail arising at 580 nm (Figure 1).

Figure 1

Further it is evident from the fluorescent titrations that fluorescent anions can be titrated precisely, i.e., generation 1 dendrimer containing 9 viologen subunits (dication) hosts up 9 eosin (dianion) molecules (fluorescence off) and generation 2 dendrimer containing 21

viologen subunits hosts up 21 eosin molecules. Anions were added in substoichiometric amounts to large excess and in each case the fluorescence spectra was recorded, no fluorescence (due to static quenching by viologen subunits) until the end point was observed and beyond the end point there is a gradual increase in fluorescent intensity (Figure 2). Thus one could exactly count the number of seats available for eosin molecule from fluorescent titrations.

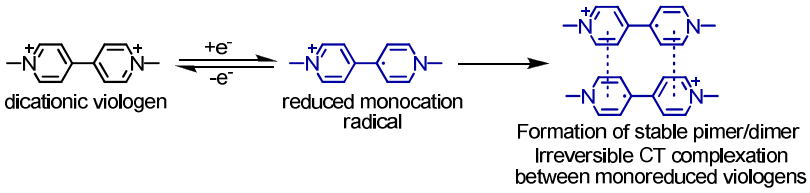
Figure 2

Authors further exemplified that these supramolecular complexes (1:1 viologen subunits in dendrimer:eosin, fluorescence off) can be destroyed by the addition of excess Cl^- ions (2×10^{-2} M TBA.Cl). Excess Cl^- ion displaces the complexed eosin anion from the dendritic pockets (fluorescence on) and eosin could be successfully complexed again by adding silver trifluoroacetate (fluorescence off) (Figure 3). Results indicate that benzylic viologen dendrimers have defined number of seats available for the guest anions indicating that they act as polyvalent scaffolds. Further, number of guest anions bound to the dendrimers was obtained precisely from the fluorescent titrations and authors showed that one could reversibly bind the guest molecules and release them by chemical triggering.

Figure 3

The above results suggests that one could precisely titrate and bind the anionic drug molecules for site-specific drug or gene delivery applications provided benzylic viologen dendrimers exhibit biocompatibility and biodegradability. In photo physical point of view, one could precisely complex donor dye molecules, photochemical excitation will inject the electrons onto the viologen subunits, whereby the viologen moieties will act as an electron trap. Upon oxidation, the reduced viologen dendrimer will release back the electrons , thereby the host dendrimer act as an electron sponge as illustrated by Walder et al.[15b] However these applications are limited due to the fast dimerization of viologen monocation radical to a

stable dimer (internal charge transfer complexation between two monocation radicals which is irreversible) as shown below



....eq (1)

b) Stepwise sequential complexation

Walder et al first reported on the sequential complexation of guest anions into cationic dendrimers based on trimethylenedipyridinium subunit using ^{1}H NMR titrations.[26] Authors have added the guest anions in sub-stoichiometric amounts to large excess and recorded the ^{1}H NMR spectrum. Plot of chemical shift vs. added anion equivalents showed that predominant shift change occurs at the sub-shell equivalence point illustrating that these dendrimers preferentially complexes anions in a shell-by-shell fashion.[15c,26] Previous ^{1}H NMR titration studies gave useful information on the formed supramolecular complex, i.e., upon complexation, either the host or the guest molecule will show upfield or downfield shift due to the change in their chemical environment, comparison of the spectral changes with that of the free host/guest protons will give us the necessary information about the formed supramolecular complex.[27] Walder et al noticed that the guest anions filled up the host dendrimers in a sequential fashion starting from the innermost core towards the periphery and their observations were evident from ^{1}H NMR titrations. Upon guest complexation authors observed that their dendrimers shrink to 30% of its original volume and opens up again by DOSY (diffusion ordered spectroscopy) and MM^{+} modelling. To cross check their observation and to elucidate the mechanism further, they had synthesized benzylic viologen dendrimers (**DPy**) with 4-*tert*-butylbenzyl end groups (**DPy$_0$**[generation 0], **DPy$_1$** [generation 1] and **DPy$_2$** [generation 2]) and extended their studies with benzene sulfonate (**BS**)

(monoanion), anthraquinone-disulfonate (**AQDS**) (dianion), Naphthalene-disulfonate (**NDS**) (dianion) and pyranine (**Pyr**) (trianion).[15c] Monitored host protons of **DPy_1** are shown in Figure 4. All these anions were complexed onto these cationic sites (trimethylenedipyridinium and benzylic viologen dendrimers) successfully (1:1 complexation between the cationic subunits and dianions was observed) and it has been shown that anions with matching symmetry showed predominant shifts in ^{1}H NMR titrations suggesting sequential filling (dianions), whereas mono- and tri anions could only be successfully titrated. The end points of sub-shell filling for mono and trianions are not that sharp predicting that apart from electrostatic interactions, molecular recognition also plays an important role (Figure 5, Table 1). An illustrative example of sequential filling is evident from ^{1}H NMR titrations as shown in Figure 5 and the results of titrations with different anions are summarized in Table 1.

Figure 4

The host dendrimers **DPy_0**, **DPy_1** and **DPy_2** has 1, 2 and 3 sub-shells respectively. Each dendrimer carries 6, 18 (6+12) and 42 (6+12+24) positive charges respectively. Each of these dendrimer can host 6, 18 (6+12) and 42 (6+12+24) monovalent anions or 3, 9 (3+6) and 21 (3+6+12) divalent anions or 2, 6 (2+4) and 14 (2+4+8) trivalent anions respectively (Figure 5, Table 1). Anions were added in sub-stoichiometric amounts to large excess and in each case ^{1}H NMR spectrum was recorded. Plot of chemical shift vs. added anion equivalents gave the NMR titration plots as shown in Figure 5. ^{1}H NMR titration curve shows that predominant peak shift (either peak splitting or peak merging (upfield or downfield shifts)) occurs either at the end point of sub-shell filling or closer to it (Figure 5). The results obtained from ^{1}H NMR titrations (Experimental) and MM+ simulations (Model) were shown in Table 1. Authors even observed overcharging in some cases, i.e., the host dendrimers responded to its chemical environment even beyond the theoretical end point, depicting that

these dendrimers interact with large excess of anions in some cases.[15c,26] The results obtained from ^{1}H NMR titrations and MM+ simulations are very different than the one obtained from fluorescent titrations. Fluorescent titrations gave the exact number of seats available for anion complexation (section 3a), whereas ^{1}H NMR titrations are governed by molecular recognition phenomena and it gives information on the number of seats available on the sub-shells and preferential sub-shell filling of guest molecules.

Figure 5

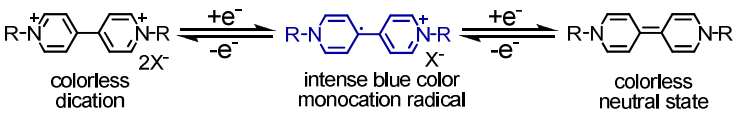
Table 1

Authors further performed gas phase MM^+ MD calculations and found the same "inside-out" filling mechanism supporting their experimental results. Thus, authors showed that these dendrimers act as an electrostatic attractor. Since the charges in the periphery are in fast motion and tainted as compared to the charges in the center, these dendrimers prefers to complex starting from the centre towards the periphery reflecting the fact that there is a built-in redox gradient. Besides these, one could generate radial chemical gradient within these dendrimers and could further tune it by sequentially completing different guest molecules.

4. Physical Applications:

a) Electron Sponge behaviour

Viologens undergo two-electron reduction in two steps, i.e., each viologen unit can take up to 2 electrons in two steps starting from the dicationic state through a monocation radical to a neutral species (eq.2). The intermediary monocation radical undergoes internal dimerization, also called "pimerization" which is irreversible (formation of a stable internal charge transfer complex as shown in eq. 1).[10a,15a,15b]



.....eq (2)

Walder et al demonstrated that benzylic viologen dendrimers can act as an electron sponge. Benzylic viologen dendrimers of generation 0 (6+), generation 1 (18+), generation 2 (42+) and generation 3 (90+) can undergo reduction in two steps and can take up to 6 (3+3) electrons, 18 (9+9) electrons, 42 (21+21) electrons and 90 (45+45) electrons respectively. Oxidation of the neutral moiety will give back the electrons, thus demonstrating the use of these dendrimers as an electron storage device or electron sponges. However their behaviour is very limited due to the fact that the formed cation monoradical will immediately dimerize (irreversible CT complexation between the viologen monocation radical) forming a stable dimer species. Dimerization is distance dependent, and it increases with increasing dendrimer generation.[15b] This factor also affects the electrochromic behavior of these molecules. Thus the electron storage property or electron storage behavior as well as electrochromism of viologen dendrimers were limited to few cycles.

Possible way to stop such an unwanted dimerization can be achieved by introducing *tert*-butyl groups on the bipyridinium moiety which will impart steric hindrance, thus it will prevent radical dimerization, a similar approach used in stabilizing TEMPO radicals. This approach will subsequently increase their electron sponge or electron storage behavior as well as their electrochromism.

b) Charge trapping behavior

Walder et al were able to reduce all the viologen subunits of their benzylic viologen dendrimers electrochemically.[15a,15b] On the other hand Balzani et al reported on the electrochemical, chemical and photochemical reduction of viologen dendrimers (Table 2).[24] In these experiments authors were able to reduce only the fraction of the viologen units

(Table 2) and they attributed such observation to the bulky tetraarylmethane peripheral groups. Authors further explained that upon reduction,

i) the dendrimers shrink due to the reduced electric charge and decreased number of counter ions

ii) dimerization of the monocation radical

iii) formation of donor-acceptor complexes between reduced viologen and the bulky peripheral groups. These factors limit further viologen reduction to a greater extent.[24]

Table 2

In another report, Balzani et al used relatively small aryloxy peripheral groups, and were able to complex eosin successfully. Number of sites available for eosin complexation were obtained from fluorescent titrations demonstrating their use as charge-storing devices.[11] Nevertheless authors were able to reduce only fraction of the viologen units in these dendrimers indicating that the charge pooling is incomplete, in other words authors observed charge trapping. Authors further explained that they observe charge trapping due to the presence of bulky terminal groups (both tetraarylmethane and aryloxy) whereas in ethyl terminated dendrimers[15b] all the viologen units are reducible.[24]

Scheme 4

Such charge trapping is also reported by Walder et al,[15a] electron trapping behavior was observed for benzylic viologen dendrimers (scheme 1 & 2) as well as for viologen dendrimers with radial redox gradient (scheme 4) based on spectroelectrochemistry and electrochemical redox reactions.[15a] Dendrimers shown in scheme 4 has two different viologen subunits. The central core is a diphenyl viologen (dpv) whose reduction potentials are ~-0.1 V for the first reduction and ~-0.35 V for the second reduction and branching units are well-known dibenzyl viologen (dbv), ~-0.3 V and ~-0.7 V. This difference in electrochemical potentials generate a radial redox gradient in these dendrimers, i.e., the peripheral dibenzyl

viologens are easily accessible to the electrode surface, if an electron is injected onto the peripheral units, it should efficiently reach the central core thereby the diphenylviologen gets reduced. On the other hand the oxidation process is shown to be really slow due to the difference in electrochemical potentials of dpv and dbv, which is 120 mV. This difference leads to an electron trapping behavior, i.e., the central core gets easily reduced, however the oxidation is difficult due to the activation barrier of 120 mV, thereby these dendrimers act as a charge trapping device. Similar charge trapping behavior was noticed in benzylic viologen dendrimers shown in scheme 1 and 2. Thus, authors stated that, the peripheral groups are easily accessible to the electrode surface thus they get reduced and oxidised easily, and due to the existence of gradient, electrons flow to the buried inner core easily, on contrary oxidation becomes difficult due to their inaccessibility and thus they trap the electrons.

5. Biological Applications

a) Antibacterial and Antifungal activities

Majoral et al evaluated the antimicrobial properties of hybrid viologen-phosphorous dendrimers (scheme 5).[25,28] Although authors have focused on different biological applications of these macromolecules,[29] we restrict our focus to the toxicity assays and the antimicrobial properties of these dendrimers.

Scheme 5

Invitro cytotoxicity and hemolytic effect were evaluated and authors have shown that dendrimers **1** and **8** were least cytotoxic and hemolytic whereas the dendrimers **6** and **7** with higher charge interacted strongly with the membranes and are more hemolytic and cytotoxic. Further they correlated the effect of surface groups, surface charge and size of the molecules for the observed hemolytic effect and cytotoxicity, and following conclusions have been drawn

1. Dendrimers with aldehyde end groups showed less cytotoxicity and hemolytic activity than the dendrimers with phosphonate ends.

2. PEGylated dendrimer **8** showed least cytotoxicity and least hemolysis.

3. Dendrimers with higher charge were more cytotoxic and showed more hemolysis.

Dendrimer **1**, **4** and **8** are not toxic to B14 cells (control cell line) but are very cytotoxic towards cancer cell line N2a. Authors revealed that all these dendrimers inhibited the bacterial growth of gram positive strain Staphylococcus aureus and gram negative strains Escherichia Coli and Proteus vulgaris, and in addition they also inhibited the growth of fungus Candida albicans in the range of 50-80%, predicting their use as antimicrobial agent.[28,30] However, their use as antimicrobial agents in real life applications is highly debatable due to their pronounced toxicity. They are discussed in detail in the following section.

b) Antiviral activities

Asaftei et al showed that benzylic viologen dendrimers with ethyl end groups (Scheme 1,2 and 4) are effective in inhibiting viral replication (HIV-1 strain in MT-4 cells) and benzylic viologen dendrimers with diphenylviologen core shows better adhesion on the viral envelope.[22a] Of course both these dendrimers showed moderate antiviral activity. The mechanism of action is shown to be purely electrostatic. It is well-known that the host cells expresses heparan sulphate on its surface and thus heparan sulphate plays an important role in the cellular entry of the virus.[22a] Polycationic dendrimers are supposed to bind to these heparan sulphate thereby inhibiting the growth of HIV (Human Immunodeficiency Virus) and HSV (Herpes Simplex Virus) strains. The invitro results showed that the action of inhibition is only moderate which was attributed to the low expression of heparan sulphate on these cells. Authors further showed that number of charge and the distance between the cationic subunits are important factors for antiviral activities.[22a]

Authors further extended their study with polycationic benzylic viologen dendrimers with ethyl and thymine end groups and systematically studied the HIV-1(strain IIIB) replication in MT-4 cells by interactions with the CXCR4 HIV co-receptor.[22b] It is shown that both thymine and ethyl end groups showed similar activities however thymine ended dendrimers showed increased hydrogen bonding interactions with CXCR4 co-receptor. Increasing positive charge in dendrimers lead to non-specific membrane binding, and this in fact increases the cytotoxicity. Authors showed that these dendrimers does not show significant inhibition of HIV-1 strains and they concluded that one should avoid polycationic benzylic viologen dendrimers to suppress cytotoxicity and non specific membrane binding.[22b] Further aspects that restrict their use in invitro or invivo biological studies are discussed in the following section.

c) As Vectors for nucleic acid delivery

Bongard et al used zeroth, first and second generation viologen dendrimers bearing ethyl (neutral), alkyl carboxyl (anion terminated) and alkyl ammonium (cation terminated) groups for invitro transportation of oligo-nucleotides, ribonucleic acids and single-or double-stranded DNA into eukaryotic cells.[23a,23c,23d] Authors were able to efficiently bind the nucleic acids to these dendrimers, formed dendriplex (nucleic acid : dendrimer complex) that were detected electrochemically. Authors showed that the dendriplex formation is pH independent and they could be transfected into eukaryotic cells; however the transfection efficiency reported were very poor due to the cytotoxicity and rigidity of viologen moieties. Further, authors showed that alkyl carboxyl terminated dendrimers increased transfection efficiency and less cytotoxicity than the neutral ethyl terminated dendrimers. Ammonium terminated dendrimers showed higher cytotoxicity (non-specific interactions) and poor transfection efficiency. However the transfection efficiencies were much much lower (~10-<20%) in

comparison with the transfection results obtained with cationic PAMAM dendrimers (~80%). Poor results obtained can be corroborated to the following factors:

1) Previous studies have shown that viologen subunit can intercalate into the double-stranded DNA minor groove[31]

2) Non-specific interactions shown by polycations

3) relatively low electrochemical potential of viologen subunits, which could effectively participate in redox reactions or trap single electrons in biological systems leading to higher cytotoxicity and non-biocompatibility[32]

4) relative rigidity of benzylic viologen dendrimers, Bongard et al further showed that increasing the alkyl chain length and use of pyridinium dendrimers instead of viologen can improve transfection efficiency.[23b]

Very recently Asaftei et al has reported on the transfection of oligonucleotides using thymine terminated benzylic viologen dendrimers.[33] Thymine as an end group provides extra hydrogen bonding, thus thymine terminated dendrimers showed improved DNA condensation and transfection. However, higher generation dendrimers were highly cytotoxic, and only lower generation dendrimers showed improved transfection efficiency. Further authors showed that they could target cancer cells using TNFα plasmid.

All the above mentioned applications demonstrate that viologen dendrimers show antimicrobial activities from very less extent to moderate range. They are well known herbicides, and hence they are toxic to living organisms including human. Their real life biological applications would still be questionable due to the following factors,

i) they are well known herbicides and beyond that they pose risks for human health[34,32]

ii) they are known to intercalate onto the minor grooves of double stranded DNA[31] iii) their relative low redox potentials allow them to participate in redox processes occurring in living systems where paraquat arrests the biological reactions by trapping a single electron

iv) their rigid structural backbone imposes further biocompatibility issues

v) the persistent positive charge will involve in non-specific interactions leading to cytotoxicity

vi) their biodegradability in living systems is highly questionable

It is easier said than done to rectify these problems, hence viologen dendrimer based biological applications will still remain appalling. Conversely researchers have reported on their applications towards gene therapy, antiviral and antimicrobial properties, from methodological point of view, they are great contributions, yet a real life application in this scenario will be far far away.

6. Conclusions and Outlook

Benzylic viologen dendrimers are a class of cationic dendrimers with redox active repeating units. Since their first report on synthesis, lot of exciting studies have been carried out. Balzani et al showed that these dendrimers form strong CT complexes with donor molecules and such complexation could be monitored by fluorescent titrations and ^{1}H NMR titrations. Precisely one could calculate the number of sites available for complexation from fluorescent and ^{1}H NMR titrations. Even step-wise complexation can be performed as shown by Walder et al, which allows a precise control over complexation of different anions (^{1}H NMR titrations). Energy or Redox Gradient could be generated from these architectures which is interesting from the photo physical point of view. Electron storage and electrochromic applications needs further focus as they are limited by dimerization of the formed monocation radical. Several groups have explored their antiviral behaviour, antibacterial and antifungal activities even used them as gene delivering vectors yet invitro applications or application in living systems is highly questionable owing to their toxicity. In general, they are a class of interesting compounds which could potentially mimic biological redox systems, light

harvesting systems and sensing applications. Very less has been explored so far and thus the system needs further investigations.

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Figures and Figure Captions

Figure 1

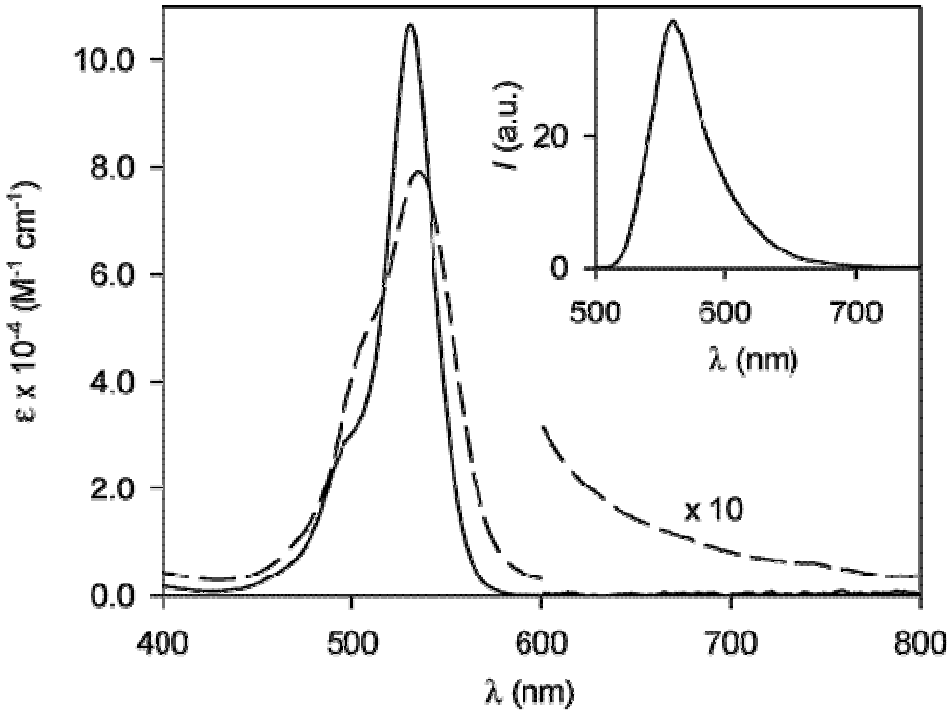


Figure 1. Full line: absorption and (inset) fluorescence spectra of the tetrabutylammonium salt of eosin dianion $\mathbf{D_1}^{2-}$ recorded in dichloromethane. Dashed line: absorption spectrum observed after addition of a stoichiometric amount of dioctyl viologen $\mathbf{A_1}^{2+}$ (as its PF_6^- salt); the fluorescence band is completely quenched. Reprinted with permission from Ref.[12] Copyright (2004) American Chemical Society.

Figure 2

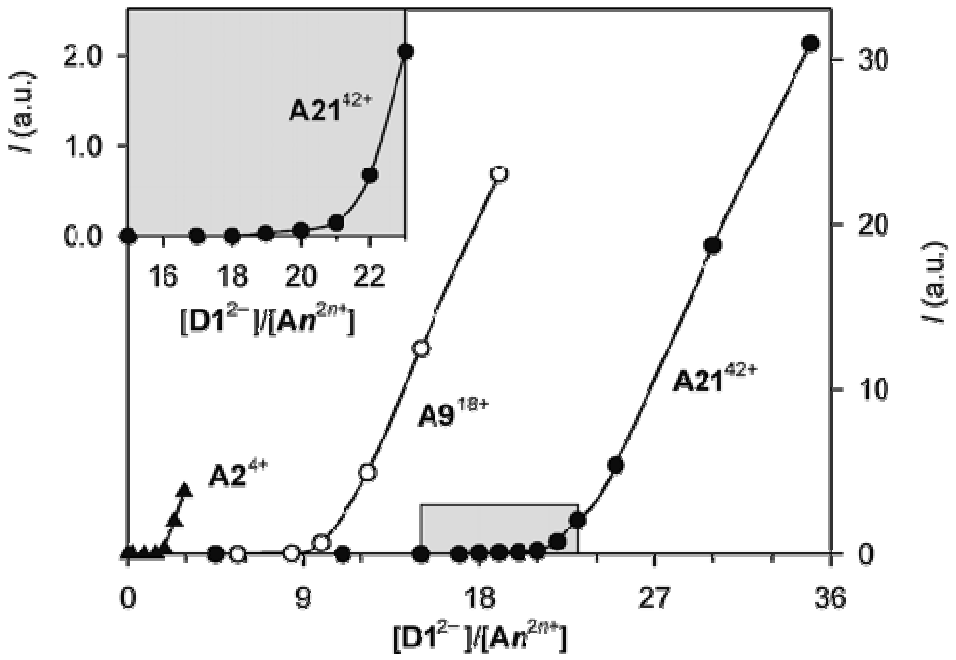


Figure 2. Fluorescent titration experiments performed in dichloromethane solutions. Diagram of the intensity of the eosin $\mathbf{D_1}^{2-}$ fluorescence band (λ_{ex} = 500 nm; λ_{em} = 560 nm) as a function of the $[\mathbf{D_1}^{2-}]/[\mathbf{A}_n{}^{2n+}]$ ratio (*n* is the number of viologen units in each compound). Reprinted with permission from Ref.[12] Copyright (2004) American Chemical Society.

Figure 3

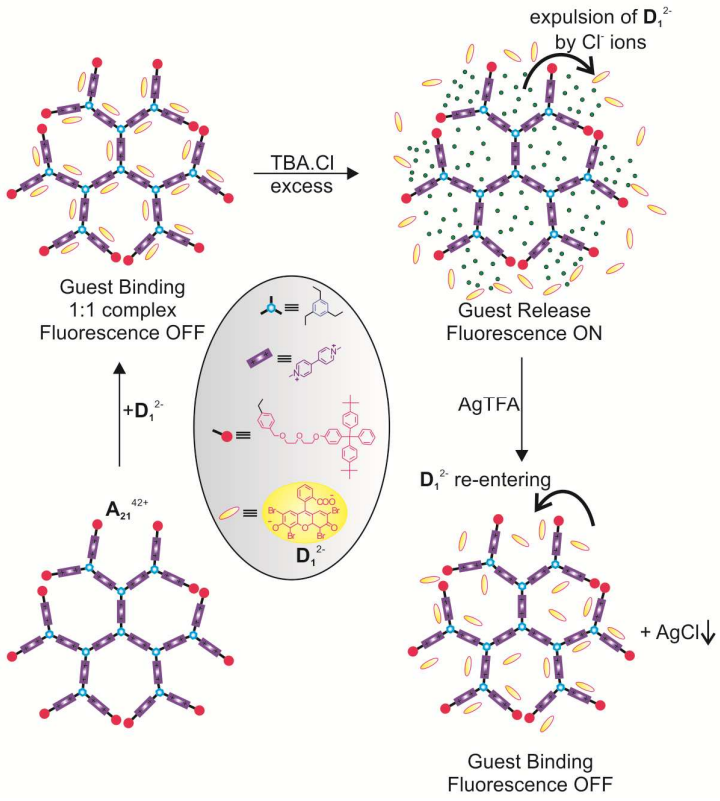


Figure 3. Illustration of Guest Binding and Guest release

Figure 4

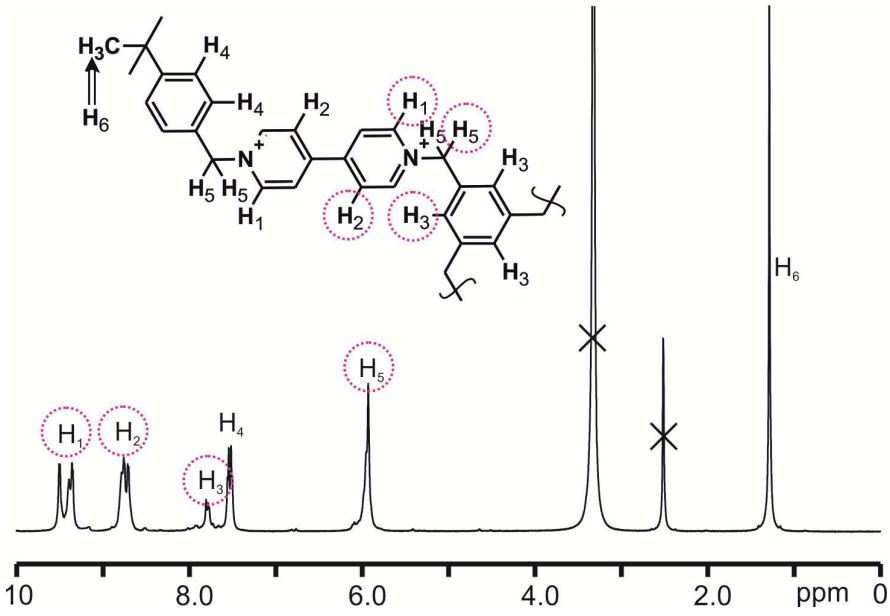


Figure 4. Monitored host protons of **DPy$_1$** (in the absence of anions) in ^{1}H NMR titrations. Reproduced partly with permission from Ref.[15c] Copyright (2011) American Chemical Society.

Figure 5

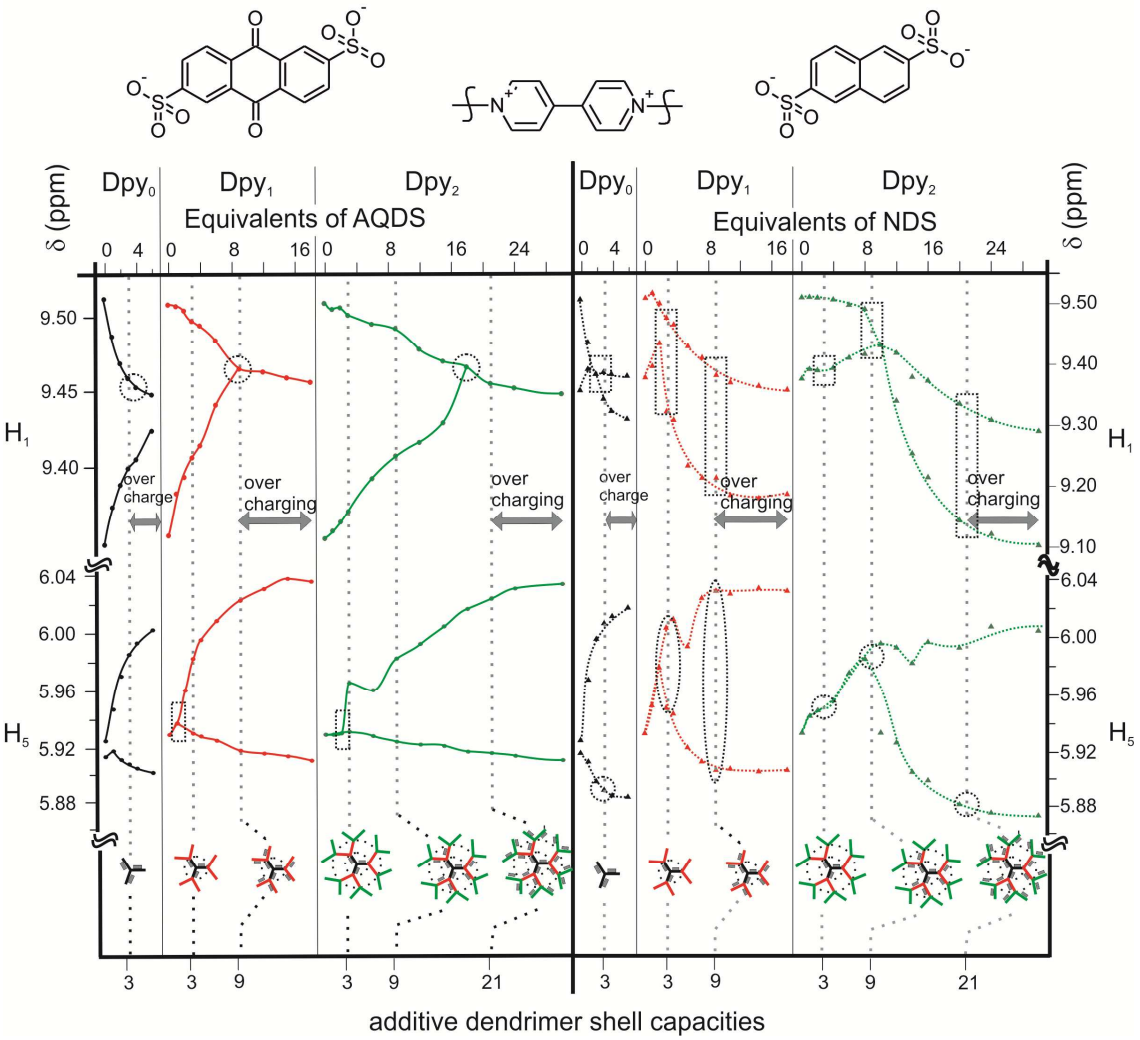
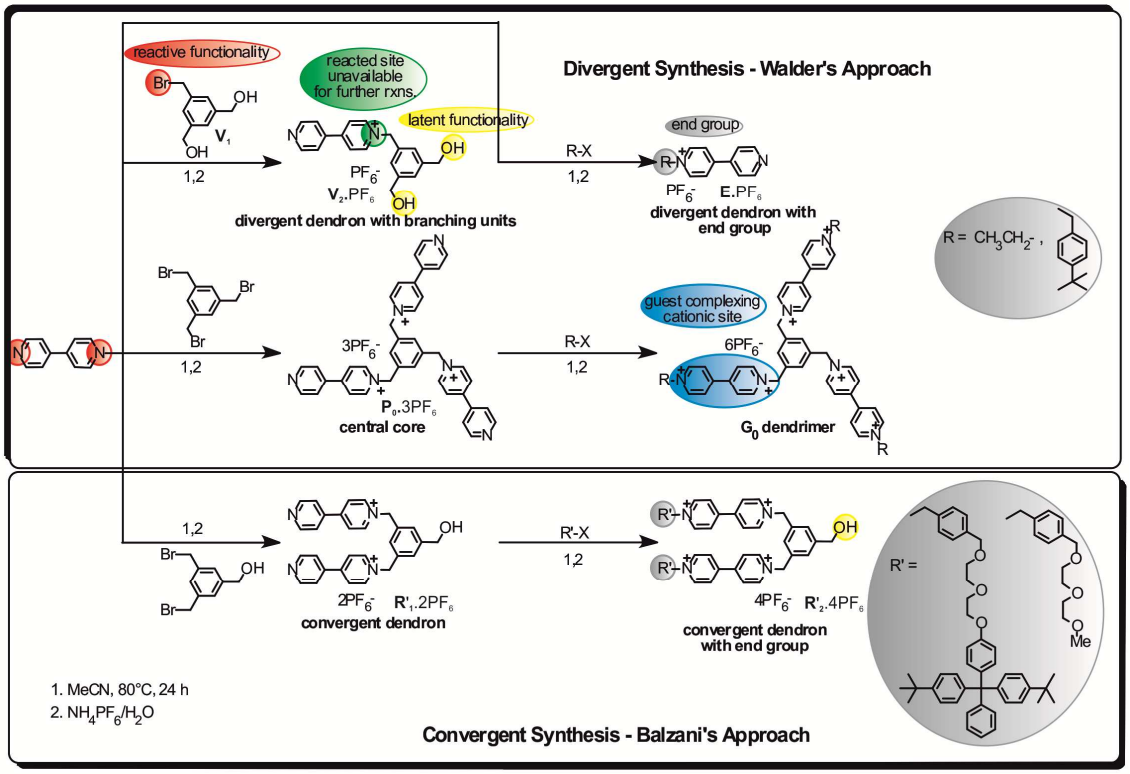


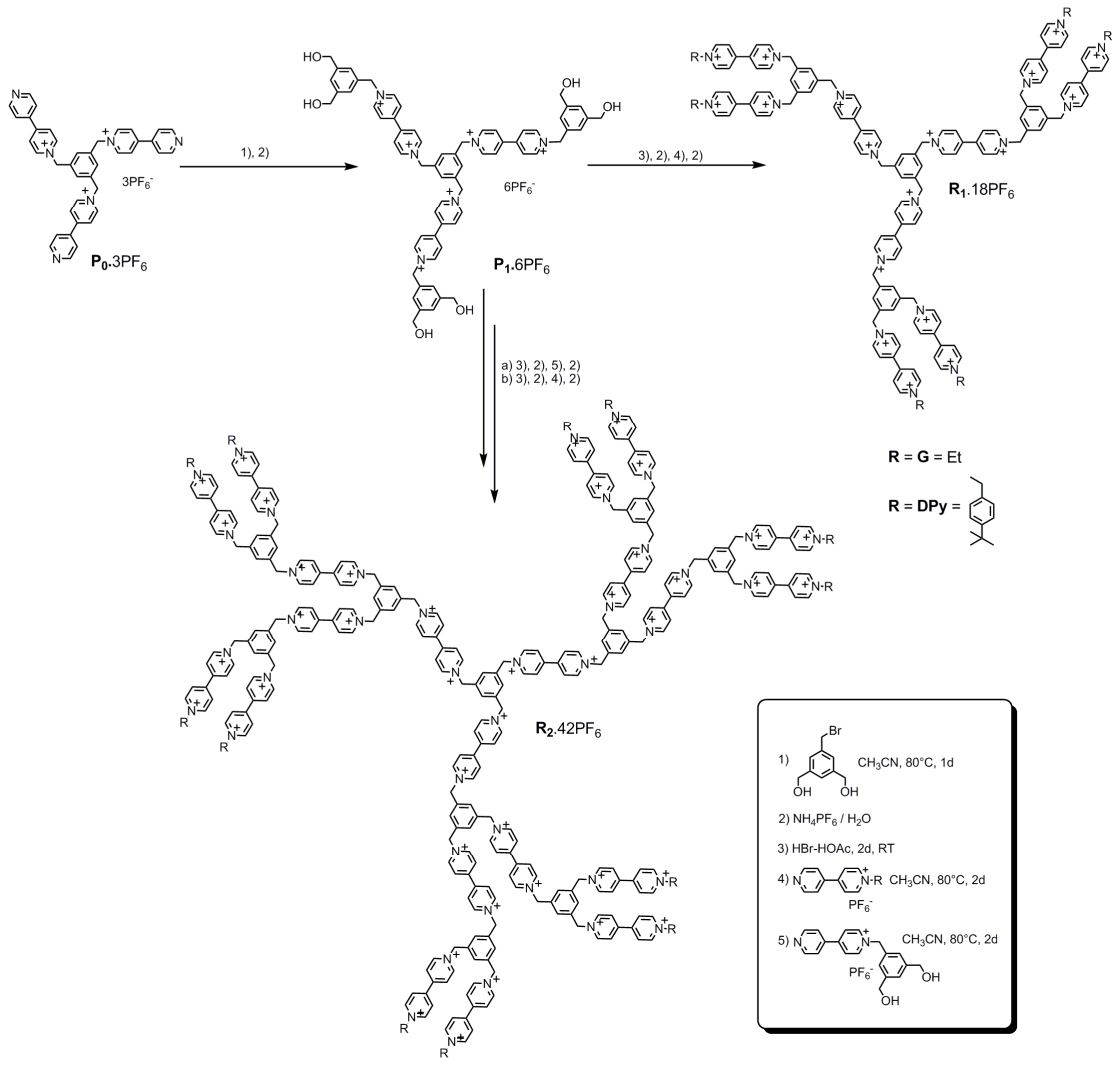
Figure 5. Plot of ^{1}H NMR peaks H_1,and H_5 of **DPy$_{0\text{-}2}$** vs. **AQDS** (left column) and **NDS** (right column) equivalent additions; [**DPy$_0$**] = 3.3 mM; [**DPy$_1$**] = 1.2 mM; [**DPy$_2$**] = 0.5mM in DMSO-d_6. Key: dotted lines, dendrimer sub-shell capacities; circles, sub-shell appearance; triangles, end point appearance; black arrows, innermost shell localized response. Adapted with permission from Ref.[15c] Copyright (2011) American Chemical Society.

Schemes and Scheme Captions

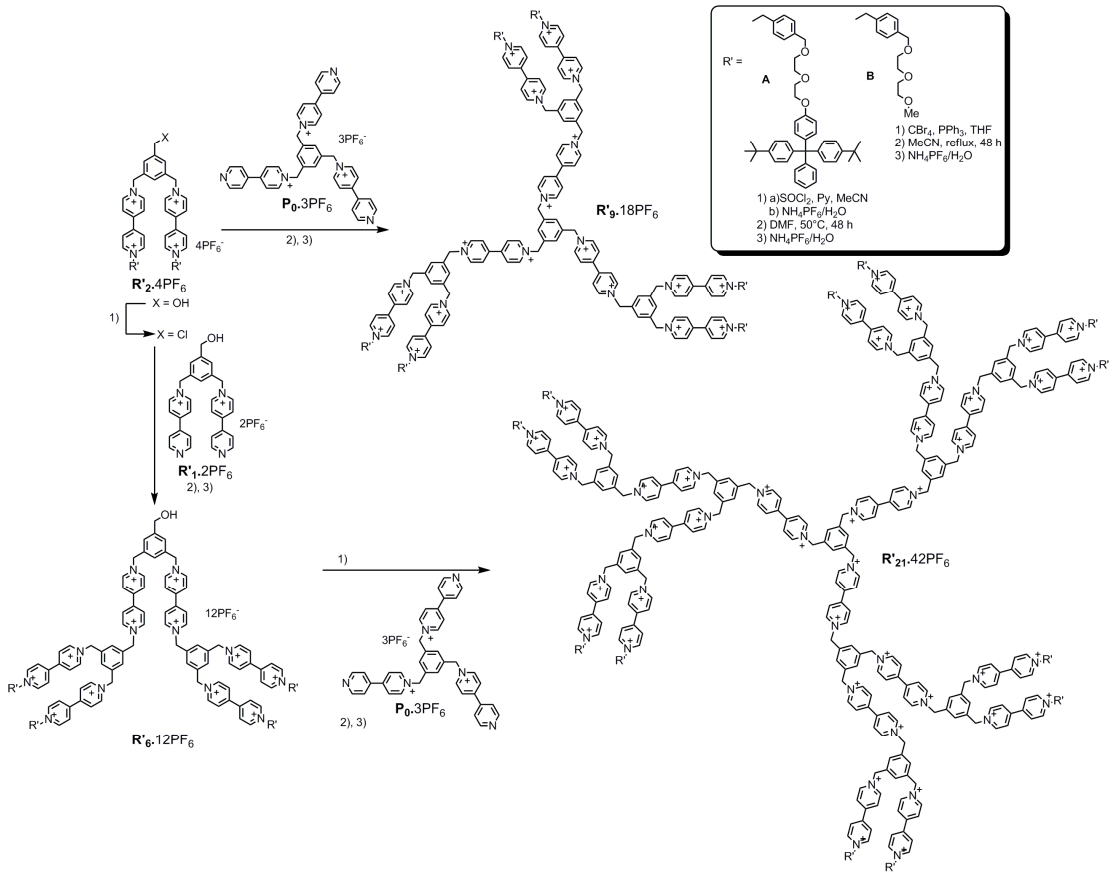
Scheme 1: Benzylic Viologen Dendrimers' key intermediate synthesis[19]



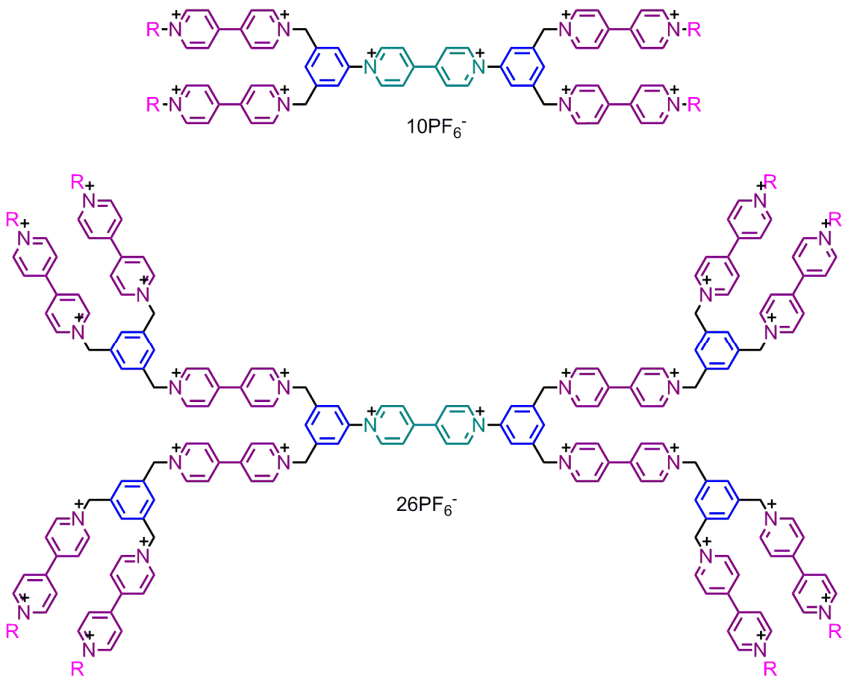
Scheme 2: Divergent approach for the synthesis of benzylic viologen dendrimers reported by Walder et al.[15b,15c]



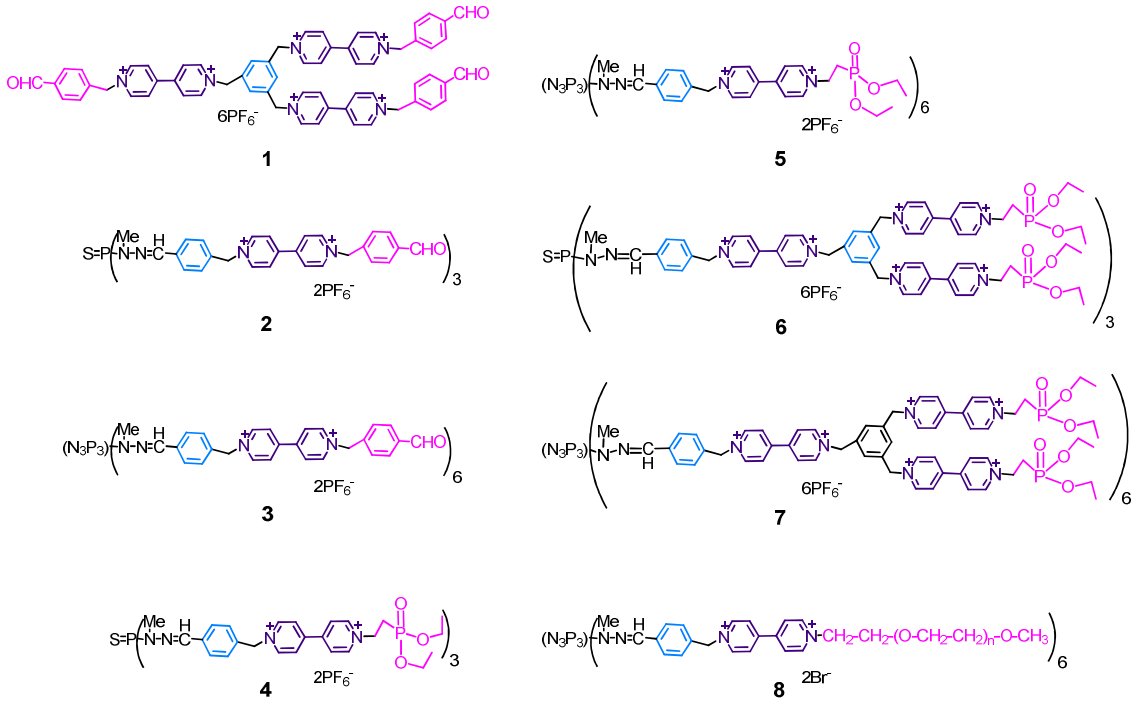
Scheme 3: Convergent approach for the synthesis of benzylic viologen dendrimers reported by Balzani et al.[11,12]



Scheme 4: Viologen dendrimer with radial redox gradient[15a]



Scheme 5: Chemical structure of Hybrid Viologen-Phosphorus Dendrimers. Adapted with permission from Ref.[28] Copyright (2002) American Chemical Society.



Tables

Table 1. Observed and theoretical sub-shell capacities. Reproduced with permission from Ref.[15c] Copyright (2011) American Chemical Society.

Hosts	No. of guest anions in the 1st | 2nd | 3rd dendrimer sub-shell [a]							
	Monoanion		Dianions				Trianion	
	BS		**NDS**		**AQDS**		**Pyr**	
	Exp. observed [d]	Model	Exp. observed [d]	Model	Exp. observed [d]	Model	Exp. observed [d]	Model
DPy0	~6-9[c]	6	2-3[c]	3	3[c]	3	2	2
DPy1	~4-6 | ~12	6 | 12	3 | 6	3 | 6	3 | 6[c]	3 | 6	2 | 3-5	2 | 4
DPy2	~5 | ~12 | -	6 | 12 | 24	3 | 6 | ~12 [c]	3 | 6 | 12	~2 | ~6 | ~12[c]	3 | 6 | 12	2 | -[b]	2 | 4 | 8

[a] individual sub-shell capacities, in contrast to eq. 1-3, where additive sub-shell capacities are used;
[b] precipitation
[c] overcharging
[d] interpretation of results from ^{1}H NMR titrations
[e] **BS** – Benzene Sulfonate; **NDS** – Naphthalene-2,6-disulfonate; **AQDS** – Anthraquinone-2,6-disulfonate; **Pyr** - Pyranine

Table 2. Number of exchanged electrons in electrochemical, chemical and photochemical reductions[11,15b,24]

Dendrimers	Number of exchanged electrons			
	$n_{expected}$	$n_{electrochemical\ redn.}$	$n_{chemical\ redn.}$	$n_{photochemical\ redn.}$
$\mathbf{A_9}^{18+}$	9	5[a,b]	4[b,c]	4[b,d]
$\mathbf{A_{21}}^{42+}$	21	14[a,b]	9[b,c]	13[b,d]
$\mathbf{B_9}^{18+}$	9	-	-	4.7[b,d]
$\mathbf{B_{21}}^{42+}$	21	-	-	8.5[b,d]
$\mathbf{G_0}$	3	2.9[e]	-	-
$\mathbf{G_1}$	9	9.1[e]	-	-
$\mathbf{G_2}$	21	20.4[e]	-	-
$\mathbf{G_3}$	45	43.4[e]	-	-

[a] MeCN; TBA.PF_6 – supporting electrolyte; glassy carbon – working electrode; Ag wire – quasi reference electrode; Pt spiral – counter electrode.

[b] The estimated error on the number of exchanged electrons is ±20%.

[c] Degassed MeCN, stoichiometric amounts of bis(benzene)chromium, [$Cr(\eta^6\text{-}C_6H_6)_2$] to obtain monoreduced viologen.

[d] Degassed CH_2Cl_2, irradiation with 365 nm light, 9-methylanthracene as a photosensitizer and triethanolamine as a sacrificial reductant.

[e] DMF; TBA.PF_6 – supporting electrolyte; glassy carbon – working electrode; Ag/AgCl– reference electrode; Pt wire – counter electrode.

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(21) Anion exchange is carried out whenever and whereever necessary. Please follow the schemes for details.

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TOC Graphical Abstract:

Dendrimers containing benzylic viologen branching units, their guest complexation, photophysical and biological applications has been reviewed.