Polycations: Syntheses of Polyammonium Strings as Antibacterial Agents

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Abstract: A series of polyammonium species, each bearing two to ten cationic sites within the covalent structure, have been synthesized. For these species quaternary ammonium sites are located in a linear array at regular intervals along a “string” backbone with free-floating anions. The polycationic species exhibit antibacterial activity using strains of E. coli.

Antibacterial activity has been associated with polycationic species which can increase the permeability of the outer membrane of bacteria. For example, poly(quaternary)phosphonium and poly(quaternary)-ammonium materials in which the cationic sites are immobilized by being covalently bound to an insoluble resin have been noted to act as significant antibacterial agents. The antibacterial activity of polypeptides bearing variable numbers of lysine residues, and of polyamines capable of being protonated to polycationic states has been reviewed. In the present effort polycationic (quaternary ammonium) species have been synthesized. These species are soluble in aqueous media and bear cationic sites arrayed in a linear manner along an unbranched chain (“strings”). In preliminary bacteriological studies, these species have been demonstrated to exhibit significant antibacterial activity against E. coli.

Polycationic strings have been constructed using the diamin "dabco" (1,4-diazabicyclo[2.2.2]octane) as the precursor reagent for the generation of the quaternary ammonium sites. The precursor reagents for linking the quaternary ammonium ion sites are the unbranched ω-halo-1-alkanols of two, three and eight carbons in length.

The dicaticonic focus sites 1a-c for the elaboration of the polycationic strings were prepared by the dialkylation of dabco using an excess of the appropriate ω-halo-1-alkanol in acetonitrile solution. Use of ethyl acetate as the solvent resulted in the precipitation of the monocationic products 2a-c in which only one of the two nucleophilic sites of dabco had been alkylated, precluding continued alkylation (Scheme 1).

\[
\begin{align*}
1a-c & \xrightarrow{SOCl_2} \text{Cl(CH}_2_2)_n - N^+ - N^- - \text{Cl}^- \\
& \xrightarrow{CH_3CN} 2a-c
\end{align*}
\]

Scheme 2

Continued elaboration of 4a-c proceeds by repetition of the two steps shown in Scheme 2, generation of a new hexactionic α,ω-dichloride 5a-c followed by reaction with the appropriate monocationic 2a-c to form the decacationic α,ω-diol 6a-c (Scheme 3).

\[
\begin{align*}
4a-c & \xrightarrow{SOCl_2} \text{Cl(CH}_2_2)_n - N^+ - N^- - \text{Cl}^- \\
& \xrightarrow{CH_3CN} 5a-c
\end{align*}
\]

Scheme 3

All new materials synthesized exhibit NMR spectra in accord with their proposed structures. All materials synthesized are significantly hygroscopic, adsorbing sizable quantities of water immediately upon exposure to room air. Quantitative combustion analyses are in accord with the proposed formulations upon adsorption of water. Data are noted for the series of compounds \( n = 2,3 \), \( n = 3,7 \) and \( n = 8,5 \). All spectra were measured using a Bruker 400 MHz DPX400 instrument and combustion analyses were performed by Schwarzkopf Microanalytical Laboratories, Woodside, NY.

Typical Experimental Procedures

α,ω-diol-diacaticonic salts 1a-c

Dabco (1 equivalent amount) and an ω-chloro-1-alkanol (2.36 equivalent amounts) are placed in a flask fitted with heating oil bath, magnetic stirrer and reflux condenser along with solvent acetonitrile (4 x the combined masses of the reagents). The reaction mixture is heated at reflux for 27 hr, after which time the voluminous white precipitate is collected by filtration and washed with ethanol and dried in vacuo.

Scheme 1

Elaborations of the species 1a-c to longer polycationic strings proceed by initial reaction with thionyl chloride in chloroform to generate the dichloride 3a-c, from which the hexactionic α,ω-diols 4a-c are readily generated by reaction with the appropriate 2 in acetonitrile. The solubilities of 3a-c in acetonitrile are low, but of sufficient magnitude to allow for dissolution and precipitation of 4a-c.

\[
\begin{align*}
\text{HO(CH}_2_2)_n - N^+ - N^- - \text{Cl}^- & \xrightarrow{\text{ethyl acetate}} 2a-c
\end{align*}
\]
collected by suction filtration through sintered glass, washed with ethyl acetate, and dried under high vacuum. (Material transfer, as with all of these preparations, must be made rapidly as material rapidly liquefies upon adsorption of water from atmosphere.) Typical yields of pure \( \alpha, \omega \)-diol-dicarboxylic acids 1a-c: 70-80%.

\( \omega \)-hydroxy-monocationic salts 2a-c

Dabaco (1 equivalent amount) and an \( \omega \)-chloro-1-alkanol (0.2 equivalent amount) and solvent ethyl acetate (4 x the combined masses of the reagents) are placed in a flask fitted with a magnetic stirrer. The reaction mixture is stirred at ambient temperature for 24 hr, after which time the white precipitate is collected by suction filtration through sintered glass, washed with ethyl acetate and anhydrous ether, and dried under high vacuum. Typical yields of the pure \( \omega \)-hydroxy-monocationic salts 2a-c: 55-65%.

\( \alpha, \omega \)-dichloride-dicationic salts 3a-c

In a flask fitted with heating oil bath, magnetic stirrer and reflux condenser are placed 1 (1 equivalent amount) and distilled chloroform solvent (10 x the mass of 1 used). To the rapidly stirred mixture is added an excess of thionyl chloride (6 equivalent amounts). The reaction mixture is heated at reflux for 16 hr, after which time excess thionyl chloride is destroyed by the addition of an excess of anhydrous ethanol. The solid product is collected by suction filtration through sintered glass, washed with anhydrous ether and dried under high vacuum. Typical yields of the pure product \( \alpha, \omega \)-dichloride-dicationic salts 3a-c: 85-95%.

\( \alpha, \omega \)-dil-hexaconic acids 4a-c

In a flask fitted with heating oil bath, magnetic stirrer and reflux condenser are placed 2 (2 equivalent amounts) and the appropriate 3 (1 equivalent amount) with solvent acetonitrile (10 x the combined masses of the reagents). The reaction mixture is heated at reflux for 30 hr, after which time the supernatant liquid is decanted and the remaining solid is washed three times with acetonitrile (each time mass equivalent to that used as solvent) and decanted each time. After the final decantation the solid residue is dried under high vacuum. Typical yields of the pure product \( \alpha, \omega \)-dil-hexaconic acids 4a-c: 85-95%.

\( \alpha, \omega \)-dichloride-hexaconic salts 5a-c

In a flask fitted with heating oil bath, magnetic stirrer and reflux condenser are placed 4 (1 equivalent amount) and distilled chloroform solvent (10 x the mass of 4 used). To the rapidly stirred mixture is added an excess (6 equivalent amounts) of thionyl chloride. The reaction mixture is heated at reflux for 16 hr, after which time excess thionyl chloride is destroyed by the addition of an excess of anhydrous ethanol. The solid product is collected by suction filtration through sintered glass, washed with anhydrous ether, and dried under high vacuum. Typical yields of the pure product \( \alpha, \omega \)-dichloride-hexaconic salts 5a-c: 85-95%.

\( \alpha, \omega \)-diol-decasylic acids 6a-c

In a flask fitted with heating oil bath, magnetic stirrer and reflux condenser are placed 2 (2 equivalent amounts) and the appropriate 5 (1 equivalent amount) with solvent acetonitrile (10 x the combined masses of the reagents). The reaction mixture is heated at reflux for 30 hr, after which time the supernatant liquid is decanted and the remaining solid is washed three times with acetonitrile (each time mass equivalent to that used as solvent) and decanted each time. After the final decantation the solid residue is dried under high vacuum. Typical yields of the pure product \( \alpha, \omega \)-diol-decasylic acids 6a-c: 85-95%.

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References

6. Data for compounds 1a-6a: All NMR spectra are measured in D_2O solution. For _1H_ NMR data, spectra are in accord with proposed structures although resolution of signal multiplicity is incomplete. For _13C_ spectra separate signals are observed for all unique carbon sites except for 6a. HETCOR measurements of 6a indicate significant overlap of carbon signals. (Similar _13C_ spectra are obtained for polymeric species produced by heating dabaco with 1a.)

7. Data for compounds 1b-6b: All NMR spectra are measured in D_2O solution. For _1H_ NMR data, spectra are in accord with proposed structures although resolution of signal multiplicity is incomplete. For _13C_ spectra separate signals are observed for all unique carbon sites. 1b: _1H_ NMR: δ 9.68 (m, 4H) 3.67 (m, 8H) 2.18 (m, 8H) 2.18 (m, 8H) 2.18 (m, 8H) 2.18 (m, 8H) 2.18 (m, 8H) 2.18 (m, 8H) 2.18 (m, 8H) 2.18 (m, 8H). 13C NMR: δ 2.13 (m, 4H) 3.67 (m, 8H) 2.18 (m, 8H) 2.18 (m, 8H) 2.18 (m, 8H) 2.18 (m, 8H) 2.18 (m, 8H) 2.18 (m, 8H) 2.18 (m, 8H). 8a: _1H_ NMR: δ 2.00 (m, 2H) 3.67 (m, 8H) 2.18 (m, 8H) 2.18 (m, 8H) 2.18 (m, 8H) 2.18 (m, 8H) 2.18 (m, 8H) 2.18 (m, 8H) 2.18 (m, 8H). 13C NMR: δ 2.13 (m, 4H) 3.67 (m, 8H) 2.18 (m, 8H) 2.18 (m, 8H) 2.18 (m, 8H) 2.18 (m, 8H) 2.18 (m, 8H) 2.18 (m, 8H) 2.18 (m, 8H).
8. Data for compounds 1c-6c: All NMR spectra are measured in D$_2$O solution. For $^1$H NMR data, spectra are in accord with proposed structures although resolution of signal multiplicity is incomplete. For $^{13}$C spectra separate signals are observed for all unique carbon sites except for 5c (18 of 19 unique carbon signals observed) and 6c (22 of 29 unique carbon signals observed). 1c: $^1$H NMR: δ 1.22-1.27 (m, 16H); 1.71-1.72 (m, 4H); 3.41-3.48 (m, 8H); 3.89 (s, 12H). $^{13}$C NMR: δ 23.31; 26.72; 27.02; 29.89; 30.01; 33.05; 52.96; 63.38; 67.17. Calcd. for C$_{32}$H$_{60}$N$_2$O$_5$Cl$_2$: (H$_2$O): C 57.50%, H 10.53%; Found: C 57.18%, H 10.64%. 2c: $^1$H NMR: δ 1.20-1.24 (m, 8H); 1.40-1.42 (m, 2H); 1.63-1.65 (m, 2H); 3.05-3.15 (m, 8H); 3.25-3.29 (m, 6H) 3.48 (s, 2H). $^{13}$C NMR: δ 23.57; 27.34; 27.94; 30.52; 30.63; 33.66; 46.68; 54.53; 64.29; 67.12. Calcd. for C$_{34}$H$_{62}$N$_2$O$_7$Cl$_2$: (H$_2$O): C 58.82%, H 10.58%; Found: C 58.58%, H 10.77%. 3c: $^1$H NMR: δ 1.10-1.32 (m, 16H); 1.60-1.69 (m, 8H); 3.40-3.54 (m, 8H); 3.87 (s, 12H). $^{13}$C NMR: δ 20.67; 24.34; 25.10; 26.84; 27.16; 31.04; 45.07; 50.33; 64.52. Calcd. for C$_{27}$H$_{44}$N$_2$O$_5$Cl$_2$: (H$_2$O): C 50.48%, H 9.43%; Found: C 50.62%, H 9.46%. 4c: $^1$H NMR: δ 1.20-1.34 (m, 32H); 1.43-1.45 (m, 4H); 1.63-1.73 (m, 12H); 3.17-3.22 (m, 16H); 3.36-3.40 (m, 12H); 3.45-3.51 (m, 12H); 3.89 (s, 12H). $^{13}$C NMR: δ 20.24; 20.52; 23.95; 24.19; 24.52; 24.95; 26.70; 27.01; 27.14; 27.25; 30.25; 30.89; 43.25; 44.91; 50.18; 50.90; 60.91; 63.90; 64.37. Calcd. for C$_{30}$H$_{50}$N$_2$O$_7$Cl$_2$: (H$_2$O): C 48.81%, H 10.16%; Found: C 48.48%, H 10.23%. 5c: $^1$H NMR: δ 1.15-1.30 (m, 32H); 1.63-1.75 (m, 16H); 3.36-3.66 (m, 28H); 3.80-3.94 (broad, 24H). $^{13}$C NMR: δ 22.33; 22.41; 22.46; 26.08; 26.19; 26.84; 28.59; 28.86; 28.91; 32.78; 44.96; 46.81; 51.85; 51.95; 52.08; 66.21; 66.27; 67.04. Calcd. for C$_{30}$H$_{60}$N$_2$O$_5$Cl$_2$: (H$_2$O): C 52.63%, H 9.54%; Found: C 52.75%, H 9.41%. 6c: $^1$H NMR: δ 1.16-1.32 (m, 46H); 1.38-1.47 (m, 8H); 1.62-1.75 (m, 18H); 3.31-3.39 (m, 18H); 3.41-3.55 (m, 16H); 3.66-3.72 (m, 16H); 3.75-3.82 (m, 16H); 3.99 (broad, 32H). $^{13}$C NMR: δ 21.68; 21.74; 21.77; 25.16; 25.41; 25.46; 25.56; 26.17; 27.92; 28.17; 28.24; 28.33; 28.45; 31.49; 32.11; 44.29; 46.13; 51.17; 51.41; 62.12; 65.52; 65.61. Calcd. for C$_{36}$H$_{68}$N$_2$O$_{10}$Cl$_2$: (H$_2$O): C 53.02%, H 9.93%; Found: C 52.86%, H 10.07%.