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Formation of Highly Charged Quasi-Molecular Ions of a Polycationic [60]Fullerene Hexakis-Adduct and Their Fragmentation Behavior in the Gas Phase

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A novel polycationic [60]fullerene hexakis-adduct has been synthesized and investigated by electrospray ionization and tandem mass spectrometry. The polycationic ligand system comprises 12 pre-formed positive charges, compensated in the neutral molecule by bromide anions. Stable quasi-molecular cations were obtained through the release of the anionic counter ions covering a charge-state envelope from 3+ to 12+. Collision-induced dissociation experiments revealed three fragmentation pathways. A dominant neutral loss channel leads to daughter ions of the same charge state as the highly charged precursor ion and singly charged daughter ions can be expelled from the polycations through two distinct dissociation channels involving both the neutral and the charge-carrying part of their ligands.

Introduction

The clinical success of gene therapy is highly dependent upon the development of efficient delivery systems.[1–3] In the past decades polycations have gained increasing attention as nonviral gene delivery vectors. The complex (polyplex) formed by the interaction of the polycation with DNA enters the cell/nucleus and releases DNA for gene expression (transfection).[4] Research into the usage of polycationic lipids and polymers as gene delivery systems has been accompanied by investigations into the structural requirements of polycationic fullerene derivatives for efficient transfection.[5] A recent extension to the fullerene-based gene delivery systems in the form of a polycationic [60]fullerene hexakis-adduct has been identified.[6]

We have recently reported on the synthesis of a globular heptafullerene that is the largest well-defined and monodisperse polyelectrolyte known to date.[7] This heptafullerene consists of one central and six peripheral fullerene building blocks each involving a T_h-symmetrical addition pattern. It contains a total of 60 amines as terminating groups. Thus, in slightly acidic conditions the entire architecture carries 60 positive charges. The success in synthesizing such large and highly symmetrical systems is based on our highly regioselective method for generating hexakis-malonates of C_{60} with an octahedral and T_h-symmetric addition pattern.[8–10] To generate an oligocationic fullerene derivative for which the number of positive charges is independent of pH, as needed for transfectional studies, we targeted in this study the synthesis of the dodecapyridinium derivative **1** (Figure 1). Given the need to attach the 12 charges in the last step while being aware of the sensitivity of fullerenes towards nitrogen nucleophiles, we needed a method that would allow the reliable creation of each of the 12 charges.

Synthetic progress towards the production of new fullerene-based nanostructures is connected to the successful application of modern mass spectrometry.[11,12] However, the generation of quasi-molecular ions of certain C_{60} hexakis-adducts has proven difficult. Nierengarten and co-workers encountered several examples[6,13] in which the target molecule was found too unstable for analysis by soft ionization methods, including electrospray ionization (ESI). Problem cases that proved inappropriate for analysis included, for example, hexakis adducts with sugar termini[13] or with pre-formed cations within the ligands.[6] Clearly, the ESI of such systems is expected to lead to multiple charging either through multiple protonation or though the release of counter anions compensating the positive charges of the pre-formed polycationic derivative. Coulombic repulsion may have an additional destabilizing effect in such ions. Also, the analysis by mass spectrometry of multifunction-

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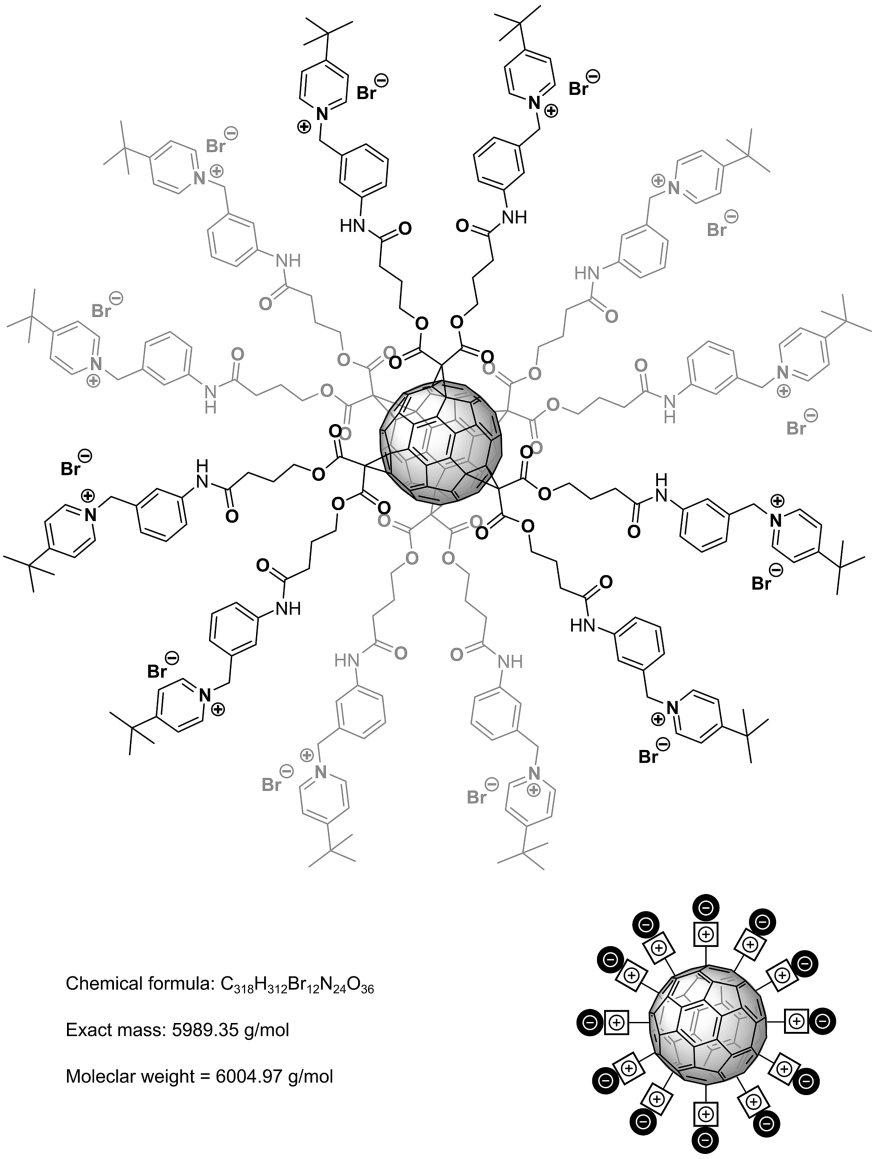


Figure 1. Structure of the polycationic [60]fullerene hexakis-adduct and a simplified version of it in which the pyridinium cations are represented by white squares and the bromide anions by black circles.

alized cationic fullerene adducts (the so-called Prato adducts) has been found too complex for a meaningful analysis.[4]

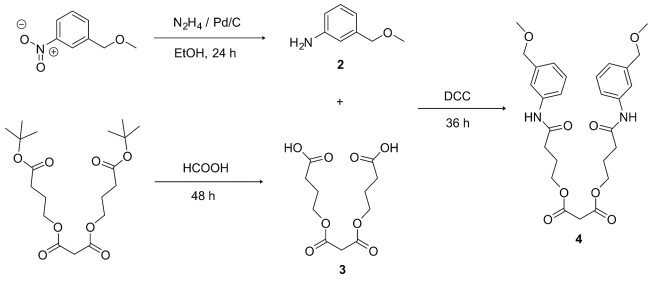
In this paper we report on the successful electrospray formation of stable, multiply charged ions of a pre-formed polycationic hexakis-adduct of C_{60}, namely quasi-molecular ions with 3 to 12 charges. The fragmentation behavior of the molecular polycations has been studied in collision-induced dissociation (CID) experiments.

Figure 1 displays the polycationic fullerene derivative under investigation. The C_{60} core contains six malonate ligands attached in the well-established octahedral addition pattern (hexakis-adduct).[14] Each end of the malonate ligand extends until terminated by cationic pyridinium moieties. The resulting 12 positive charges are compensated by bromide anions. Figure 1 also contains a simplified representation of the compound under study in which the pyridinium cationic parts are represented by white squares and the anionic counter ions by black circles to allow for a quick identification of the charge state of the respective ions.

Results and Discussion

The initial concepts for the synthesis of T_h-symmetrical hexakis-adducts of C_{60} that we previously developed[8–10] and were later modified by other groups[15,16] turned out to be inefficient for the synthesis of the dodeca-cationic target molecule **1**. The quaternization leading to the formation of

the desired pyridinium had to be the final step because chromatographic purification on silica of such highly polar and water-soluble adducts is impossible. For this reason a comparatively nonpolar precursor hexakis-adduct had to be generated. The malonate **4** equipped with two precursor groups that we reported recently[17] (Scheme 1) could be attached in the desired way, however, chromatographic separation from the reaction mixtures obtained by using the previous synthetic protocols[7–10] failed because its surface polarity is very similar to those of side-products such as pentakis-adducts. To circumvent this problem and to simplify the purification we first generated the oxazoline-protected mono-adduct **5** (Scheme 2), which was subsequently converted into the mixed hexakis-adduct **6** following a new procedure that we developed very recently.[18] The chromatographic separation of **6** was then easy to accomplish. The protecting group was subsequently removed upon UV irradiation to form the pentakis-adduct 7[18] and a sixth addend **4** was allowed to bind to the remaining octahedral [6,6]-double bond of **7** by using modified cyclopropanation conditions.[19] The final steps were the conversion of the symmetrical hexakis-adduct **8** into the dodecabromide **9** and the subsequent quaternization with 4-*tert*-butylpyridine.[17]

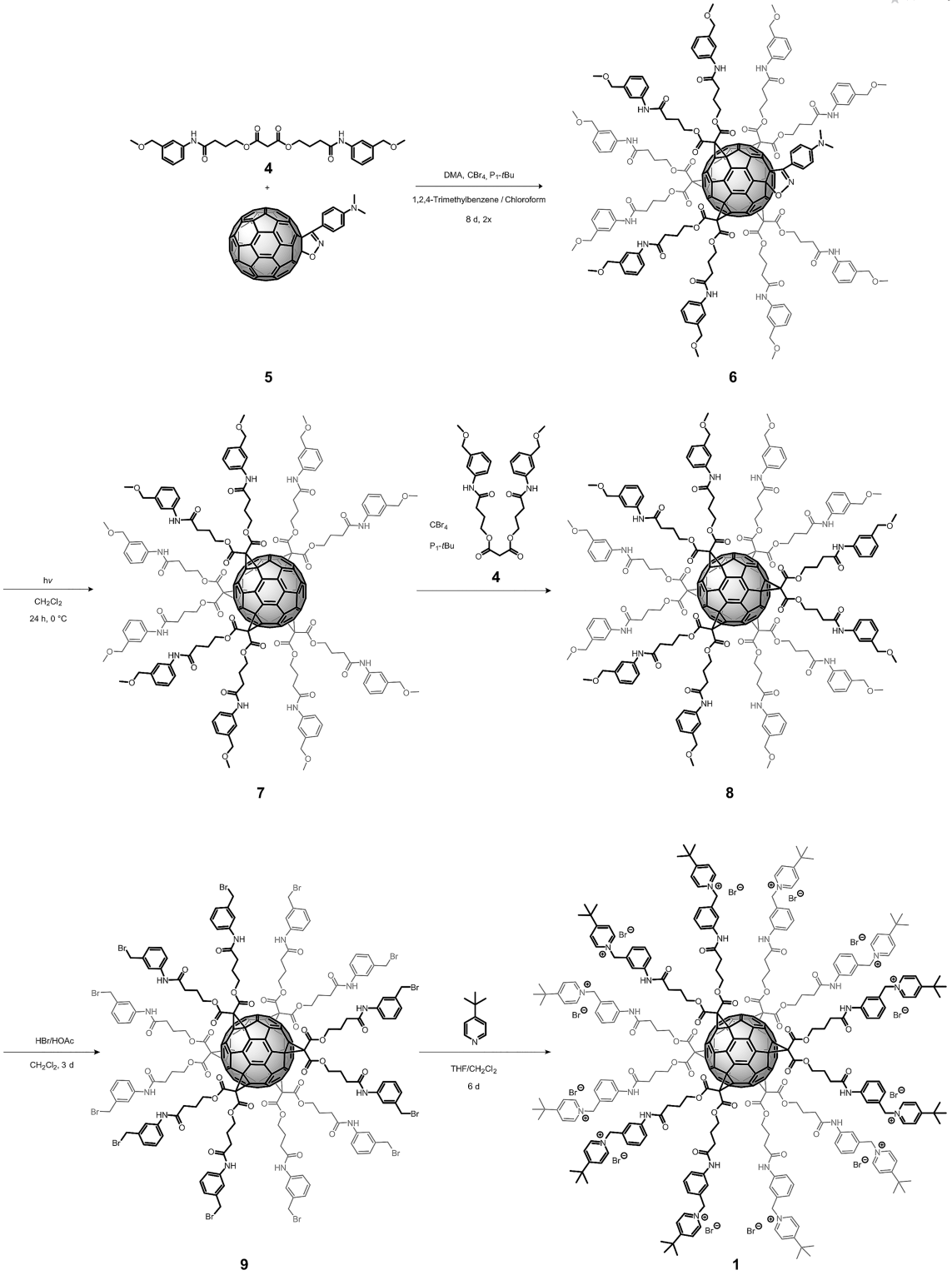


Scheme 1. Synthesis of the malonate ligand precursor **4**.

Figure 2 displays the mass spectrum that was obtained when the title compound was electrospray ionized as a 1.7×10^{-4} mol L^{-1} methanol solution at a capillary entrance voltage of –4500 V. Although a tremendous amount of fragmentation is evident, quasi-molecular ions are clearly observed in enhanced abundances. Through the loss of bromide anions, the corresponding multiply charged cations are produced, covering the charge states of 3+ in the high *m*/*z* region to the 12+ cation in the low *m*/*z* region. The charge states 4+, 5+, 6+, 7+, and 8+ can be observed in abundance, whereas the charge states 3+ and 9+ are low in intensity, and those of 10+ to 12+ show only minor abundance.

The inserts in Figure 2 display enlargements of the quasi-molecular ion region of selected polycations to allow comparison of the recorded (top trace) and simulated signals (bottom trace). The spectrum was recorded with a resolution of approximately 35000 so that the isotope pattern of the signals could be clearly resolved for all the charge states observed. The corresponding mass accuracy was typically slightly better than 1 ppm, which, together with the excellent agreement between recorded and simulated isotope patterns, leaves no doubt regarding the proposed identity of the molecular polycations. The recorded isotope patterns of all the observed quasi-molecular ions can be compared with the simulations in Figure S1 in the Supporting Information and the assignments can be confirmed.

The dissociation behavior of the polycations is discussed in the following. Polycations with the charge states 4+ to 9+ were selected and submitted to collision-induced dissociation (CID). The collisions were performed with N_2 in a collision cell located between the mass-selecting quadrupole (q) and the fragment ion-recording time-of-flight (ToF) part of a qToF mass spectrometer. Figure 3 shows the partial CID mass spectra of the polycations covering the tetracation (top right) to the nona-cation (bottom left). The CID spectra reveal a peculiar pattern in which the number of daughter ion signals corresponds to the number of bromide anions and thus to the number of charge-compensated (neutral) ligands within the precursor ion. That is, the nona-cation with three neutral ligands shows three daughter ions and the tri-cation possessing nine neutral ligands shows nine fragment ion signals, which leads to the conclusion that the fragmentations occur exclusively within the neutral ligands. Moreover, the daughter ions retain the charge state of the dissociating parental polycation. For the CID experiments the particular parent ion was selected by covering all its isotopomers so that the daughter ions would also show the full isotope pattern. The charge state is straightforwardly calculated from the *m*/*z* spacing between the individual isotope peaks of one ion. The procedure is shown in Figure 3 (insert a) for the daughter ion signals of the hexa-cationic parent ion. Also evident is the perfect match of the recorded (top trace) and calculated (bottom trace) isotope patterns with the assumed elemental composition (see below) of a daughter ion that still possesses six positive charges just like its parent ion. The elemental composition of the fragment ion was derived by establishing the mass of the neutral entity that has been expelled from the ion in the dissociation. Figure 3 (insert b) illustrates the procedure by which the mass of the neutral fragment has been established. Within the isotope pattern of two adjacent fragment ions the peaks that would contain the same isotopes have been identified by comparing the overall appearance of both patterns. The mass difference between two such peaks corresponds to the mass of the released neutral fragment. The peak differences between several of the more abundant signals were used to establish an average value of the neutral mass, which was obtained as Δm = 135 Da, which we have assigned to the loss of 4-*tert*-butylpyridine. The fragmentation behavior of the parent polycations is illustrated in Scheme 3. Following the reaction path a, the nitrogen-centered pyridinium cation at the ligand terminus attracts the electron pair that connects the pyridinium cation to the rest of the chain, whereupon it is released as a neutral entity (Δm = 135 Da). The positive charge would be transferred initially to the new terminal carbon atom. That the resulting primary carbenium ion rearranges to a tropylium ion is a speculative assumption that has to be seen in the light of recent investigations into the equilibria of sub-



Scheme 2. Synthesis of the polycationic molecule **1**.

stituted benzylium/tropylium ions.[20,21] The bromide anion would remain within the ionic fragment so that no change in the charge state would result and the reaction could occur as often as there are intact ligands in the ion. Another possibility is a substitution mechanism. The withdrawal of the binding electron pair by the nitrogen (as described previously) can also be compensated by the bond that is formed by the nucleophilic attack of the bromide ion. The result of such a substitution reaction would be the formation of a covalent C–Br bond (Scheme 3, reaction a′). It

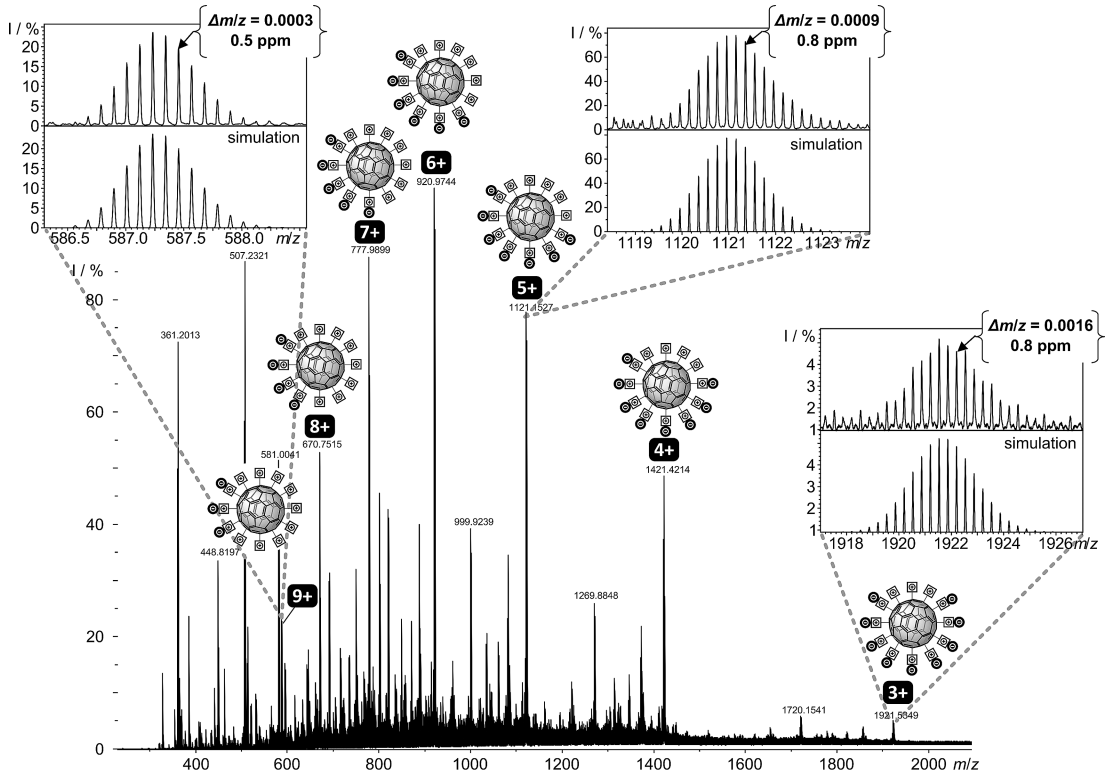
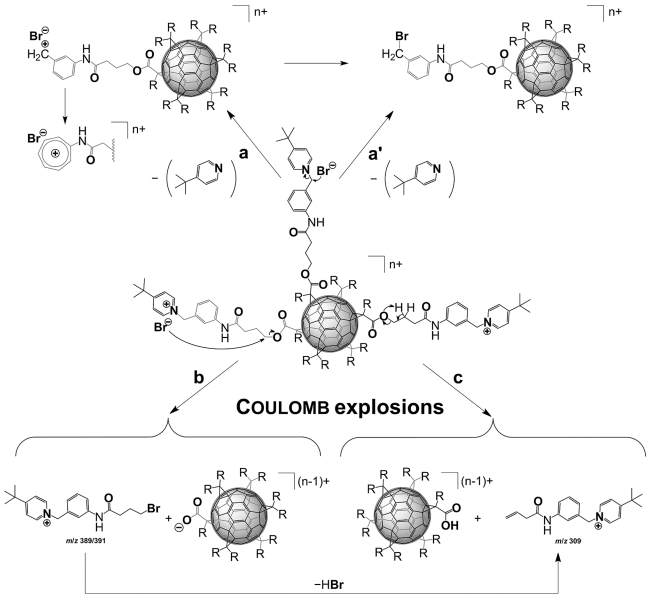


Figure 2. High-resolution positive-ion ESI mass spectrum of the polycationic [60]fullerene hexakis-adduct. The inserts display the recorded (top trace) and simulated (bottom trace) isotope patterns of selected charge states of the quasi-molecular ions, together with the mass accuracy (in ppm) achieved for selected peaks.

should be noted that a second, lower abundant series of ions is observed in the CID spectra (Figure 3). We attributed these ions to the uptake of a residual, gaseous neutral molecule with a mass of 60 Da within the collision cell. This assignment is based on several circumstantial indications. These include 1) a much lower mass accuracy compared with the fragment ions discussed above, 2) the fact that the extent to which these ions are observed differs greatly when different instruments of the same type are employed, and 3) for some ions multiple additions of 60 Da could be identified.

In addition to the loss of the substituted pyridine, two other reactions occurred during CID leading to the detection of singly charged ions at *m*/*z* = 309 and 389/391. The signals are shown in Figure 4, which displays the CID spectra of the charge state 6+ quasi-molecular ion obtained at laboratory collision energies of 150, 180, and 210 eV. Upon increasing the collision energy, the number of pyridines lost increases from one molecule at 150 eV to all six at 210 eV, which underpins the successive nature of these dissociations and that the singly charged ions are formed more abundantly. The 1:1 isotope pattern of the *m*/*z* = 389/391 doublet indicates the presence of one bromine atom. To form the species at *m*/*z* = 389/391, the complete branch of the ligand of the malonate group must be released, including the bromide anion. Pathway b in Scheme 3 illustrates this reaction. Because the bromide formally compensates one positive charge, a second charge must be created during the dissociation for *m*/*z* = 389/391 to appear as a positively



Scheme 3. Illustration of the different fragmentation pathways occurring after collisional activation. Reaction a and a′: the loss of 4-*tert*-butylpyridine; reaction b: the formation of a singly charged ion at *m*/*z* = 389/391; reaction c: the formation of a singly charged ion at *m*/*z* = 309.

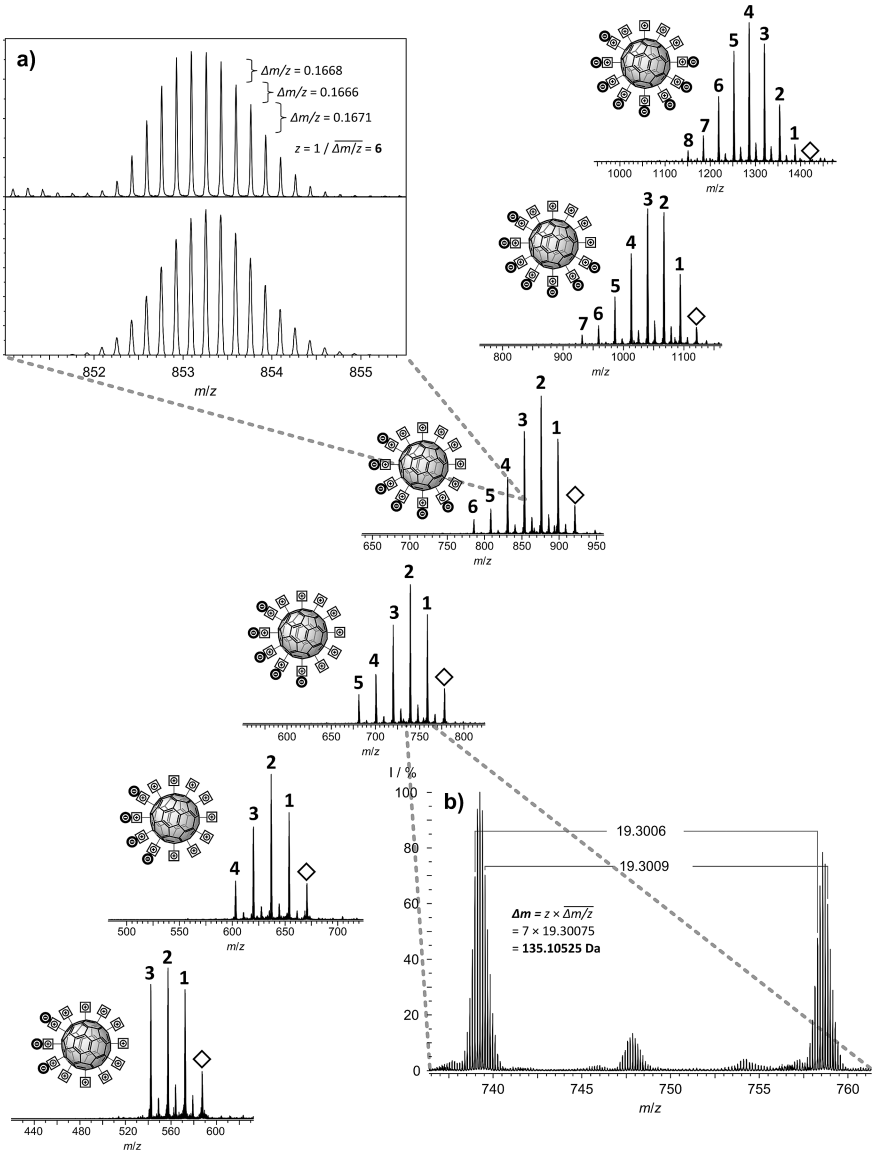


Figure 3. Partial CID mass spectra of the polycationic quasi-molecular ions covering the charge states 4+ (top right) to 9+ (bottom left). a) Fragment ion of the charge state 6+ precursor comparing the recorded isotope pattern (top trace) with the simulation (bottom trace). Establishment of the charge state (z) is also shown. b) Adjacent fragment ion signals obtained from the charge state 7+ precursor. Establishment of the neutral loss is illustrated. The uptake of a neutral with m = 60 Da corresponds to the signals seen between m/z = 747 and 749.

charged fragment ion. This is suggested to occur by a heterolytic cleavage of the O–C bond of the ester functionality of an intact ligand. The resulting carboxylate anion would reduce the charge of the polycationic fullerene fragment by one. Therefore the formation of a singly charged ion at m/z = 389/391 must be accompanied by the production of a complementary multiply charged fragment ion carrying one charge less than the precursor ion. Whether the dissociation mechanism proceeds as suggested cannot be answered by these experiments. The reaction may even be facilitated by the involvement of the bromide anion or proceed through more complex electron movements. The structures of the ions at m/z = 309 and 389/391 shown in Scheme 3 are speculative.

A more likely pathway to the singly charged ion at m/z = 309 proceeds through another unusual decay, this time involving the charged ligand of the polycation, which also undergoes bond cleavage at the ester functionality, however, accompanied by hydrogen transfer to the polycationic fragment ion. Pathway c in Scheme 3 illustrates the reaction. As with the formation of the cation at m/z = 389/391, the formation of the singly charged ion at m/z = 309 must also be accompanied by the formation of the corresponding polycationic fragment ion carrying one charge less than the precursor polycation. Unfortunately, in both cases we have been unable to trace these polycationic fragment ions, which are most probably too low in abundance. Although the singly charged ion at m/z = 309 can be formed from any

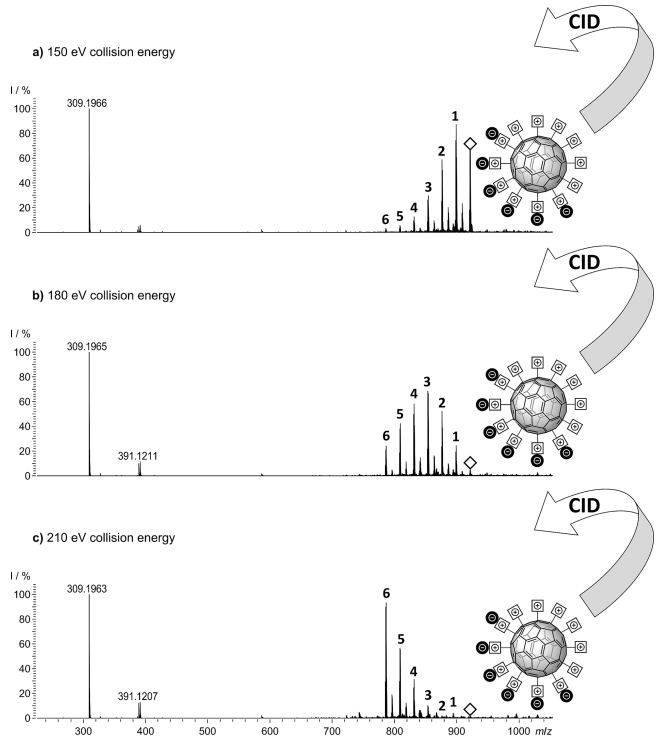


Figure 4. CID mass spectra of the charge state 6+ quasi-molecular ion at laboratory collision energies of a) 150, b) 180, and c) 210 eV.

of the other ions seen in the CID spectrum, the corresponding multiply charged fragment ion of each dissociation will have with each intermediate precursor ion a different mass and thus much lower abundance. Additionally, the greater number of atoms in the multiply charged fragment ions means more isotope peaks. Because the ion abundance correlates with the sum of all isotope intensities, the broader isotope pattern leads to a much lower intensity of each isotope peak.

Conclusions

Electrospray ionization of a novel polycationic [60]fullerene hexakis-adduct produces stable quasi-molecular ions through the loss of its counter anions. Low-energy collision-induced dissociations involve neutral loss and charge permutation reactions in which singly charged fragments are expelled from the polycations.

Experimental Section

Materials and Reagents: All chemicals were purchased from chemical suppliers and used without further purification. CBr_4 was purchased as the unstabilized (water-free) form. Except for toluene, solvents were purified from raw industrial solvents by distillation. Alcohols were distilled from CaO, methylene dichloride and ethyl acetate were distilled from K_2CO_3. For dry conditions, HPLC-grade solvents were used. Toluene was used as HPLC-grade to avoid thiophene impurities.

Instrumentation: TLC: Merck silica gel 60 F254. Detection: UV lamp 254 nm; 1% $KMnO_4$ in 1% KOH. Flash chromatography (FC): Merck silica gel 60 (230–400 mesh, 0.04–0.063 nm, deactivated. NMR spectroscopy: JEOL JNM GX 400, Bruker Avance 300, and Bruker Avance 400 spectrometers. The chemical shifts are given in ppm relative to TMS. The resonance multiplicities are indicated as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). UV/Vis spectroscopy: Varian Cary 5000 spectrophotometer. IR spectroscopy: Bruker Vektor 22 spectrometer with ATR-DiComp-detector. HPLC (preparative): Shimadzu; pump: a single LC-8 A; column: Nucleosil 100–5. To multicycle the substance, the outlet could be switched to the pump inlet while excluding the injector circuit. Detector: SPD-10 A; wavelengths detected: 300, 314 nm.

Electrospray ionization: ESI was performed on methanol solutions of the sample at a concentration of 1.0 g L^{-1} (1.7×10^{-4} mol L^{-1}) employing a syringe pump (KDS 100 pump) at a flow rate of 3 mL min^{-1} at a source temperature of 180 °C (qToF). High-resolution mass spectra were obtained with a large-scale quadrupole/time-of-flight (qToF) instrument (maXis, Bruker) at a mass accuracy of typically just better than 1 ppm in MS^1 experiments. Collision-induced dissociations (CID, MS^2) were conducted in a collision cell located between the mass-selecting quadrupole (q) and the fragment ion-recording time-of-flight (ToF), following mass selection and preceding the high-resolution daughter-ion analysis in the ToF analyzer. Nitrogen was used as the collision gas. The laboratory collision energy was varied between $z \times 10$ and $z \times 40$ eV (z being the charge state number). Regarding the mechanism of ion formation, it appears that the title compound has a rather low degree of dissociation into its ionic components when in solution as the spray and transfer conditions were harsher than under the "normal" conditions used, for example, for the protonation of peptides by ESI. The spray and analyzer were interfaced by a dual funnel system that is capable of transferring ions very gently. We assume that most ions are formed by Br^- loss during the ESI process rather than being already present in solution. Furthermore, for good quality spectra, the sample concentration had to be one to two orders higher compared with solutions of inorganic salts or ionic liquids,[22] which are known to be dissociated in solution.

Synthesis

Compound 2: 3-Nitrobenzyl methyl ether (25 g, 0.1496 mol) was dissolved in EtOH (300 mL). Hydrazine hydrate (150 g, 145 mL, 20 equiv.) was added under nitrogen, followed by Pd/C (10%, 300 mg). After 24 h, the solvent was evaporated and the product purified by simple distillation, yield 99%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.11 (m, 1 H, aniline-5), 6.71 (m, 1 H, aniline-4), 6.66 (s, 1 H, aniline-2), 6.59 (m, 1 H, aniline-6), 4.36 (2 H, CH_2-O), 3.66 (2 H, NH_2), 3.36 (3 H, CH_3-O) ppm. ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 146.5 (1 C, aniline-1), 139.4 (1 C, aniline-3), 129.2 (1 C, aniline-5), 117.8 (1 C, aniline-4), 114.3, 114.2 (2 C, aniline-2,6), 74.6 (1 C, *C*H_2-O), 58.0 (1 C, O-*C*H_3) ppm.

Compound 3: Bis[3-(*tert*-butyloxycarbonyl)propyl] malonate[7] was dissolved in a quadruple volume of 100% formic acid. The mixture was stirred at room temperature for 2 d. After evaporation of the formic acid, traces of formic acid were removed by repeated azeotropic distillation with toluene, and the toluene removed in vacuo, yield quantitative. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.28 (s, 2 H, COO*H*), 4.20 (m, 4 H, CH_2O), 3.36 [2 H, CH_2(COO)$_2$], 2.44 (m, 4 H, CH_2O), 1.99 (m, 4 H, $CH_2CH_2CH_2$) ppm. ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 178.9 (2 C, *C*OOH), 166.3 [2 C,

C(*C*OO)$_2$], 64.4 (2 C, *C*H$_2$-O), 41.5 [1 C, *C*H$_2$(COO)$_2$], 30.4 (2 C, *C*H$_2$COOH), 29.6 (2 C, CH$_2$*C*H$_2$CH$_2$) ppm.

Compound 4: Bis(3-carboxypropyl) malonate (**3**; 50 mmol, 13.635 g, 1 equiv.) and 3-methyoxymethylaniline (**2**; 0.11 mol, 15.1 g, 2.2 equiv.) were dissolved in dichloromethane. After cooling to 0 °C, 1,3-dicyclohexylcarbodiimide (DCC; 45.393 g, 2.2 equiv.) and *N*,*N*-dimethylaminopyridine (10 mmol, 0.252 g, 0.2 equiv.) were added. The reaction mixture was stirred for 36 h. The precipitate was removed and the mixture purified by column chromatography (SiO_2; ethyl acetate/hexanes, 3:1). The solvent was evaporated and traces of ethyl acetate removed in vacuo, yield 30%. ^{1}H NMR (300 MHz, $CDCl_3$, r.t.): δ = 8.04 (s, 2 H, N-*H*), 7.49 (s, 2 H, aniline-2), 7.43 [d, ^3J(H,H) = 9 Hz, 2 H, aniline-6], 7.24 (t, $^3J_{H,H}$ = 9 Hz, 2 H, aniline-5), 7.03 (d, $^3J_{H,H}$ = 9 Hz, 2 H, aniline-4), 4.37 (s, 4 H, C*H*$_2$-O-CH$_3$), 4.23 (m, 4 H, C*H*$_2$-O-C=O), 3.37 [s, 2 H, C*H*$_2$-(C=O)$_2$], 3.33 (s, 6 H, C*H*$_3$), 2.43 (m, 4 H, *H*$_2$CC=O), 2.07 (m, 4 H, CH$_2$C*H*$_2$CH$_2$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, r.t.): δ = 170.5 (2 C, N-*C*=O), 166.8 (2 C, O-*C*=O), 139.1 (2 C, aniline-1), 138.1 (2 C, aniline-3), 129.0 (2 C, aniline-5), 123.4 (2 C, aniline-4), 119.1, 119.0 (4 C, aniline-2,6), 74.3 (2 C, *C*H$_2$-O-CH$_3$), 64.6 (2 C, *C*H$_2$-O-C=O), 58.1 (2 C, *C*H$_3$), 41.6 [1 C, *C*(C=O)$_2$], 33.5 (1 C, *C*H$_2$-C=O), 24.4 (2 C, CH$_2$*C*H$_2$CH$_2$) ppm. FTIR (ATR, r.t.): $\tilde{\nu}$ = 3380–3210 (m), 2990–2800 (m), 1730 (s), 1660 (s), 1610 (m), 1590 (m), 1550 (s), 1490 (m), 1440 (m), 1380 (w), 1330 (m), 1300 (w), 1270 (s), 1190 (s), 1150 (s), 1090 (s), 1030 (s), 960 (w), 920–890 (w), 880 (m), 790 (s), 730 (m), 700 (s), 650–560 (w) 540 (w) cm^{-1}.

Compound 6: Fullerene mono-adduct **5** (200 mg, 225 µmol, 1 equiv.) was dissolved in a dry, degassed mixture of 1,2,4-trimethylbenzene (20 mL) and chloroform (20 mL). 9,10-Dimethylanthracene (DMA; 557 mg, 2.70 mmol, 12 equiv.) was added and the mixture stirred for 4 h. Malonate **4** (2.20 g, 4.28 mmol 19 equiv.) was added to this mixture, followed by the dropwise addition of CBr_4 (895.4 mg, 2.70 mmol, 12 equiv.) and P$_1$-*t*Bu[23] (679 mg, 734 µL, 12 equiv.) in trimethylbenzene/chloroform (1:1). After stirring the biphasic system for 8 d, the mixture was plug-filtered with dichloromethane/THF (1:1), adsorbed onto silica and then washed with dichloromethane/ethyl acetate (1:3), eluted with dichloromethane/THF (1:2), and purified by column chromatography (silica; toluene/dichloromethane/ethyl acetate/ethanol 3.66:2:3.33:0.95) and then multicyclic HPLC (12 cycles per run) on Nucleosil with the same eluent, yield 32%. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.75 (10 H, N-*H*), 7.52 (10 H, *m*-aniline-2), 7.38 (d, $^3J_{H,H}$ = 7 Hz, 10 H, aniline-6), 7.17 (t, $^3J_{H,H}$ = 7 Hz, 10 H, aniline-5), 6.99 (d, $^3J_{H,H}$ = 7 Hz, 10 H, aniline-4), 4.30 (20 H, C*H*$_2$-O-CH$_3$), 4.16 (20 H, C*H*$_2$-O-C=O), 3.28 (30 H, O-C*H*$_3$), 2.36 (20 H, C*H*$_2$-C=O), 1.98 (20 H, CH$_2$C*H*$_2$CH$_2$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.87 (10 C, N-*C*=O), 167.03 (10 C, O-*C*=O), 154.4 (1 C, dimethylaniline-1) 152.7 (1 C, *C*=N), 146.68, 146.64, 146.32, 145.92, 145.8, 145.19, 145.15, 145.09, 144.94, 144.06, 143.44, 143.44, 142.91, 142.00, 141.76, 141.68, 141.25, 141.02, 140.14, 139.49, 139.32, 139.18, 138.98 (48 C, C_{60}-sp^2), 138.8 (10 C, *m*-aniline-1), 138.1 (10 C, *m*-aniline-3), 135.14 (2 C, dimethylaniline-3), 129.4 (1 C, dimethylaniline-4), 128.7 (10 C, *m*-aniline-5), 123.2 (10 C, *m*-aniline-4), 119.3 (2 C, dimethylaniline-2), 119.1 (20 C, *m*-aniline-2,6), 74.2 (10 C, *C*H$_2$-O-CH$_3$), 69.74, 69.69, 69.5, 69.1, 67.8, 67.3 (10 C, C_{60}-sp^2), 66.4, 65.8 (2 C, C_{60}-oxazoline), 65.3 (10 C, *C*H$_2$-O-C=O), 57.9 (10 C, O-*C*H$_3$), 45.4, 44.5, 41.9 [5 C, *C*(C=O)$_2$], 39.8 (2 C, N-*C*H$_3$), 32.9 (10 C, *C*H$_2$-C=O), 24.0 (10 C, CH$_2$*C*H$_2$CH$_2$) ppm. MS (ESI-ToF): *m*/*z* = 1745.6 [M + K]$^+$. FTIR (ATR, r.t.): $\tilde{\nu}$ = 3306 (m), 3151 (w), 3087 (w), 2961 (w), 2961 (w), 2926 (m), 2855 (w), 2822 (w), 2360 (m), 2341 (w), 1742 (m), 1664 (m), 1610 (m), 1596 (w), 1549 (w), 1488 (w), 1442 (w), 1374 (m), 1298 (w), 1260 (s), 1216 (s), 1167 (w), 1088 (s), 1026 (m), 898 (w), 879 (m), 791 (m), 763 (w), 733 (s), 713 (w), 696 (m) cm^{-1}. UV/Vis (CH$_2$Cl$_2$): λ (ε) = 244 (158000), 277 (53000), 283 (50000), 314 (41000), 334 nm (30000 M^{-1} cm^{-1}).

Compound 7: The [5,1]-hexakis-adduct **6** (100 mg, 29 µmol, 1 equiv.) was dissolved in dry dichloromethane (50 mL). Maleic anhydride (85 mg, 30 equiv., 870 µmol) was added and the mixture was degassed thoroughly. The mixture was irradiated with a 500 W UV lamp under cooling at 0 °C for 24 h, followed by plug-filtration (silica; dichloromethane/THF, 1:2) and column chromatography (silica; toluene/dichloromethane/ethyl acetate/ethanol 3.66:2:3.33:0.95). The product is air-sensitive in the presence of light, yield 90%. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.77–8.15 (m, 5 H, N-*H*), 7.51 (s, $^3J_{H,H3}$ = 8 Hz, aniline-2), 7.33 (d, $^3J_{H,H}$ = 8 Hz, aniline-6), 7.22 (t, $^3J_{H,H}$ = 8 Hz, 10 H, aniline-5), 7.03 (t, $^3J_{H,H}$ = 8 Hz, 10 H, aniline-4), 4.36.4.33 (m, 20 H, C*H*$_2$-O-CH$_3$), 4.24 (m, 20 H, C*H*$_2$-O-C=O), 3.33 (s, 30 H, C*H*$_3$), 2.4 (20 H, C*H*$_2$C=O), 2.05 (20 H, CH$_2$C*H*$_2$CH$_2$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.9 (10 C, N-*C*=O), 167.2 (10 C, O-*C*=O), 148.4, 146.8, 145.9, 145.6, 144.7, 144.5, 144.19, 144.17, 143.8, 143.0, 142.0, 149.8, 139.7 (50 C, C_{60}-sp^2), 138.9 (10 C, aniline-1), 138.2 (10 C, aniline-3), 128.9 (10 C, aniline-5), 123.4 (10 C, aniline-4), 119.4, 119.2 (20 C, aniline-2,6), 74.3 (6 C, *C*H$_2$-O-CH$_3$), 69.8, 69.43, 69.37, 69.1 (10 C, C_{60}-sp^3), 65.4 (10 C, *C*H$_2$-O-C=O), 53.4, 51.0 [5 C, *C*(C=O)$_2$], 33.1, (10 C, *C*H$_2$-C=O), 24.1 (10 C, CH$_2$*C*H$_2$CH$_2$) ppm. MS (MALDI-ToF): *m*/*z* = 3307 [M + Na]$^+$. UV/Vis (CH$_2$Cl$_2$): λ (ε) = 277 (48000), 282 (46000), 315 (22000), 341 nm (13000 M^{-1} cm^{-1}).

Compound 8: The pentakis-adduct **7** (37 mg, 11.3 µmol) was dissolved in dry dichloromethane (1 mL). Malonate **4** (200 mg, 389 µmol, 35 equiv.) and CBr_4 (37.0 mg, 113 µmol, 10 equiv.) were added, followed by the dropwise addition of P$_1$-*t*Bu (5.3 mg, 22.5 µmol, 2 equiv.) in dichloromethane (1 mL) over 15 min. The mixture was stirred overnight, plug-filtered (silica; dichloromethane/THF, 1:2), adsorbed on silica, washed with dichloromethane/ethyl acetate (1:3), eluted with dichloromethane/THF (1:2), and isolated in vacuo, yield 93%. ^{1}H NMR (400 MHz, $CDCl_3$, r.t.): δ = 8.66 (s, 12 H, N-*H*), 7.53 (s, 12 H, aniline-2) 7.36 (m, 12 H, aniline-6), 7.17 (m, 12 H, aniline-5), 6.99 (m, 12 H, aniline-4), 4.33 (s, 24 H, C*H*$_2$-O-CH$_3$), 4.26 (m, 24 H, C*H*$_2$-O-C=O), 3 (s, 36 H, C*H*$_3$), 2.36 (m, 24 H, C*H*$_2$CO), 2.02 (m, 24 H, CH$_2$C*H*$_2$CH$_2$) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, r.t.): δ = 171.2 (12 C, N-*C*=O), 164.14 [12 C, *C*(C=O)$_2$], 146.1, 141.6 (48 C, C_{60}-sp^2), 139.4 (12 C, aniline-1), 138.6 (12 C, aniline-3), 129.3 (12 C, aniline-5), 123.9 (12 C, aniline-4), 119.9 (24 C, aniline-2,6), 74.80 (12 C, *C*H$_2$-O-CH$_3$), 69.6 (12 C, C_{60}-sp^3), 66.9 (12 C, H$_2$*C*-O-C=O), 58.6 (12 C, O*C*H$_3$), 46.1 [12 C, *C*(C=O)$_2$], 33.4 (12 C, *C*H$_2$-C=O), 24.5 (12 C, CH$_2$*C*H$_2$CH$_2$) ppm. UV/Vis (CH$_2$Cl$_2$): λ (ε) = 246 (294000), 260 (181000), 278 (101000), 316 (59000), 334 (48000), 378 (12000), 399 nm (8000 M^{-1} cm^{-1}).

Compound 9: The hexakis-adduct **8** (88 mg, 23.1 µmol) was dissolved in dichloromethane (3 mL) and a dry, 48% solution of HBr in acetic acid (3 mL) was added. The mixture was stirred for 3 d. The mixture was poured into water and diluted with dichloromethane. Traces of HBr were removed with solid potassium hydrogen carbonate. After filtration, the solvent and acetic acid were evaporated and the sample was washed with water. After addition of first dichloromethane, then toluene and evaporation three times, the substance was immediately dissolved in dry THF and subjected to the next step, yield 89%. ^{1}H NMR (100.6 MHz, [D$_6$]THF, r.t.): δ = 9.33 (12 H, N-*H*), 7.70 (s, 12 H, aniline-2), 7.53 (m, 12 H, aniline-6), 7.18 (12 H, aniline-5), 7.03 (12 H, aniline-4), 4.46 (s, 24 H, C*H*$_2$Br), 4.34 (m, 24 H, C*H*$_2$O), 2.88 (24 H, C*H*$_2$-C=O), 2.06 (24

H, $CH_2CH_2CH_2$) ppm. ^{13}C NMR (100.6 MHz, [D$_6$]THF, r.t.): δ = 171.5 (12 C, *C*=O-N), 164.5 (12 C, O-*C*=O), 147.1, 142.4 (48 C, C_{60}-sp^2), 141.0 (24 C, aniline-1,3), 130.0 (12 C, aniline-5), 125.0 (12 C, aniline-4), 121.1, (12 C, aniline-6), 120.4 (24 C, aniline-2), 70.5 (12 C, C_{60}-sp^3), 68.5 (12 C, *C*H$_2$-O), 46.9 [6 C, *C*(C=O)$_2$], 34.5 (12 C, *C*H$_2$-Br), 34.0 (12 C, *C*H$_2$-C=O), 26.7 (12 C, $CH_2CH_2CH_2$) ppm. UV/Vis (CH_2Cl_2): λ (ε) = 280 (100), 315 (59), 336 nm (46 a.u.).

Compound 1: The deprotected hexakis-adduct **9** (90 mg, 20 μmol) was dissolved in dry THF (3 mL). 4-*tert*-Butylpyridine (34 mg, 0.2464 μmol, 12 equiv.) and dichloromethane (3 mL) were added and the mixture stirred for 3 d. Dichloromethane (10 mL) was added and the mixture stirred for a further 3 d. The liquid was removed and THF and further 4-*tert*-butylpyridine (34 mg, 0.2464 μmol, 12 equiv.) were added. The supernatant was removed and the sample was washed with dichloromethane and diethyl ether. ^1H NMR (300 MHz, CD_3OD, r.t.): δ = 8.98 (24 H, pyridyl-3), 8.14 (24 H, pyridyl-2), 7.83 (12 H, aniline-2), 7.63 (12 H, aniline-6), 7.34 (12 H, aniline-5), 7.20 (12 H, aniline-4), 5.81 (s, 24 H, C*H*$_2$N$^+$), 5.52 (s, 12 H, N-*H*), 4.4, 4.3 (m, 24 H, C*H*$_2$O), 2.52 (24 H, C*H*$_2$CO), 2.04 (24 H, C*H*$_2$C=O), 1.43 (24 H, $CH_2CH_2CH_2$) ppm. ^{13}C NMR (100.6 MHz, CD_3OD, r.t.): δ = 173.1 (24 C, pyridyl-4, N-*C*=O), 164.7 (12 C, O-*C*=O), 147.0 (24 C, C_{60}-sp^2), 145.2 (24 C, pyridyl-3), 142.5 (24 C, C_{60}-sp^2), 141.0 (12 C, aniline-1), 135.3 (12 C, aniline-3), 131.2 (12 C, aniline-5), 126.9 (12 C, pyridyl-2), 125.3 (12 C, aniline-4), 122.3 (12 C, aniline-2), 121.4 (12 C, aniline-6), 70.5 (12 C, C_{60}-sp^3), 67.92 (12 C, *C*H$_2$O), 64.6 (12 C, Ph-*C*-N$^+$), 47.2 [6 C, *C*(C=O)$_2$], 37.6 (12 C, *C*H$_2$-C=O), 34.2 [12 C, *C*(CH$_3$)$_3$], 30.3 (36 C, *C*H$_3$), 25.5 (12 C, $CH_2CH_2CH_2$) ppm. UV/Vis (MeOH): λ (ε) = 282 (100), 318 (54), 335 nm (43 a.u.).

Supporting Information (see footnote on the first page of this article): UV/Vis, NMR and HRMS spectra of the target compound, and NMR spectra of the precursor compounds.

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[1] D. Luo, W. M. Saltzman, *Nat. Biotechnol.* **2000**, *18*, 33–37.
[2] S. Patil, D. Rhodes, D. Burgess, *AAPS J.* **2005**, *7*, E61–E77.
[3] X. Gao, K.-S. Kim, D. Liu, *AAPS J.* **2007**, *9*, E92–E104.
[4] C. Klumpp, L. Lacerda, O. Chaloin, T. D. Ros, K. Kostarelos, M. Prato, A. Bianco, *Chem. Commun.* **2007**, 3762–3764.
[5] H. Isobe, W. Nakanishi, N. Tomita, S. Jinno, H. Okayama, E. Nakamura, *Chem. Asian J.* **2006**, *1*, 167–175.
[6] D. Sigwalt, M. Holler, J. Iehl, J.-F. Nierengarten, M. Nothisen, E. Morin, J.-S. Remy, *Chem. Commun.* **2011**, *47*, 4640–4642.
[7] P. Witte, F. Hörmann, A. Hirsch, *Chem. Eur. J.* **2009**, *15*, 7423–7433.
[8] I. Lamparth, A. Hirsch, C. Maichle-Mössmer, *Angew. Chem.* **1995**, *107*, 1755–1757.
[9] A. Hirsch, I. Lamparth, H. R. Karfunkel, *Angew. Chem.* **1994**, *106*, 453–455.
[10] A. Hirsch, O. Vostrowsky, *Eur. J. Org. Chem.* **2001**, 829–848.
[11] Y. V. Vasil'ev, O. G. Khvostenko, A. V. Streletskii, O. V. Boltalina, S. G. Kotsiris, T. Drewello, *J. Phys. Chem. A* **2006**, *110*, 5967–5972.
[12] M. P. Barrow, X. Feng, J. I. Wallace, O. V. Boltalina, R. Taylor, P. J. Derrick, T. Drewello, *Chem. Phys. Lett.* **2000**, *330*, 267–274.
[13] J.-F. Nierengarten, J. Iehl, V. Oerthel, M. Holler, B. M. Illescas, A. Munoz, N. Martin, J. Rojo, M. Sanchez-Navarro, S. Cecioni, S. Vidal, K. Buffet, M. Durka, S. P. Vincent, *Chem. Commun.* **2010**, *46*, 3860–3862.
[14] A. Hirsch, I. Lamparth, T. Groesser, H. R. Karfunkel, *J. Am. Chem. Soc.* **1994**, *116*, 9385–9386.
[15] H. Li, A. Kitaygorodskiy, R. A. Carino, Y.-P. Sun, *Org. Lett.* **2005**, *7*, 859–861.
[16] H. Li, S. A. Haque, A. Kitaygorodskiy, M. J. Meziani, M. Torres-Castillo, Y.-P. Sun, *Org. Lett.* **2006**, *8*, 5641–5643.
[17] J.-F. Gnichwitz, R. Marczak, F. Werner, N. Lang, N. Jux, D. M. Guldi, W. Peukert, A. Hirsch, *J. Am. Chem. Soc.* **2010**, *132*, 17910–17920.
[18] F. Hörmann, A. Hirsch, *Chem. Eur. J.* **2013**, *19*, 3188–3197.
[19] X. Camps, A. Hirsch, *J. Chem. Soc. Perkin Trans. 1* **1997**, 1595–1596.
[20] E.-L. Zins, C. Pepe, D. Schröder, *J. Chem. Soc. Faraday Trans.* **2010**, *145*, 157–169.
[21] T. D. Fridgen, J. Troe, A. A. Viggiano, A. J. Midey, S. Williams, T. B. McMahon, *J. Phys. Chem. A* **2004**, *108*, 5600–5609.
[22] J. Li, W. Wei, L. C. Nye, P. S. Schulz, P. Wasserscheid, I. Ivanović-Burmazović, T. Drewello, *Phys. Chem. Chem. Phys.* **2012**, *14*, 5115–5121.
[23] R. Schwesinger, H. Schlemper, *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 1167–1169; *Angew. Chem.* **1987**, *99*, 1212.

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