Polymeric Drug Carriers Functionalized with Pairwise Arranged Hydroxyl and/or Carboxyl Groups for Platinum Chelation

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ABSTRACT: Cancer chemotherapy, alone or in combination with other treatment modalities, has come to play an important part in the fight against malignancies. However, the anticancer drugs in current clinical administration, while efficacious and oftentimes curative against a select number of neoplasias, suffer from a variety of deficiencies, notably severe systemic toxicity and a tendency to elicit drug resistance. These pharmacological shortcomings are eminently in evidence with the outstanding class of platinum drugs as represented by *cis*-diaminedichloroplatinum(II) (cisplatin). The bioreversible binding (conjugating) of a medicinal agent to a water-soluble macromolecular carrier has been recognized as an effective expediency to curtail these deficiencies. In the present communication we describe the synthesis of a special class of polymers featuring hydroxyl and/or carboxyl functionalities designed for use in the construction of square-planar platinum complexes polymer-bound through dihydroxylato, hydroxylatocarboxylato, or dicarboxylato chelation. Accordingly, the polymer structures of this project contain pairs of hydroxyl, hydroxylcarboxyl, or carboxyl groups main chain- or side chain-attached in 1,2-geometry. The target polymers are obtained by a Michael addition type polymerization of bisacrylamide monomers with mono- or diamine comonomers in aqueous medium. Whereas in the first three polymers the hydroxyl and/or carboxyl functionalities are attached directly (**1**) or close (**2**, **3**) to the backbone, the remaining polymers contain these functionalities as terminals on extended spacer segments. The water-soluble polymeric products, purified and fractionated by dialysis and isolated by freeze-drying, will be used as substrates for platinum conjugation in future work. However, their functional proneness to platinum binding is demonstrated in the present project through platination of an exemplifying carrier, providing a water-soluble conjugate with a Pt content of 13.5%. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 96: 10–19, 2005

Key words: polymeric drug carriers; hydroxyl, carboxyl side groups; platinum coordination

INTRODUCTION

For the development of carcinostatic polymeric platinum complexes in which the metal is polymer-bound through tethers of the leaving-group type, we were in need of carrier polymers functionalized with carboxyl and/or hydroxyl side groups capable of coordinating to platinum as carboxylato or hydroxylato ligands.

Platinum complexes, notably those of the cisplatin and carboplatin types, are known to be highly potent antitumor agents, and several representatives of the platinum drug family have been in successful clinical use against a number of malignancies.[1,2] In an effort to overcome the excessive toxicity of these compounds and their notorious tendency to induce drug resistance, platinum complexes have been polymer-bound via biofissionable linking groups, and numerous reports in the literature attest to the improved pharmacological performance of the polymer–drug conjugates so obtained.[3] Thus, cell culture[4–6] and toxicological[7] tests on a variety of polymeric compounds in which carrier-bound amine ligands act as the metal-binding entities, prepared in this laboratory, have indicated quite a rewarding cytotoxic behavior pattern for this type of compound. Platinum conjugates displaying an even more promising pharmacological profile are represented by structures comprising polymeric carriers to which the platinum atom is bound via carboxylato ligands, the latter acting as readily displaceable leaving groups. Developmental work in this field has been (and continues to be) performed in several laboratories, and the more prominent reports were briefly surveyed in recent papers.[8,9]

It has been the specific goal in our laboratory to develop conjugates in which platinum is coordinatively bonded through a chelating ligand system. This

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approach can be functionally realized through construction of carriers containing carboxyl or hydroxyl functionalities arranged in a pair-wise fashion so as to generate small-ring chelates when coordinated to the metal. Carboxylatoplatinum bonds, and hydroxylatoplatinum bonds even more so, are comparatively weak concomitantly with the leaving-group character of these ligands. They are prone to hydrolytic dissociation in aqueous solution and thus may pose problems of premature demetallation of the compounds in biological environments. Chelate rings comprising dicarboxylato-, carboxylatohydroxylato-, or dihydroxylatometal bonding, on the other hand, will benefit from added stability, both kinetic and thermodynamic, and are therefore considered the metal anchoring system of preference.

The synthetic program described in this communication aims at the preparation of carrier polymers functional as platinum chelators. It comprises three polymer types: one of these polymers features the 1,2-dihydroxyl functionality, in another they are characterized by the presence of the 1,2-dicarboxyl substituent system, and pairs of hydroxyl and carboxyl groups are incorporated in still another polymer type.

EXPERIMENTAL

General procedures

Solid-state IR spectra (KBr pellets) were taken over the range 4,000–600 cm^{-1}. ^{1}H-NMR spectra (400 MHz) were taken on D_2O solutions. Chemical shifts, δ, are given in ppm relative to sodium 3-(trimethylsilyl)-2,2,3,3-d_4-propionate; integration error limits ± 12%. Unless stated otherwise, D_2O solutions of polymeric materials were routinely adjusted to pH 10 just prior to scanning to eliminate spurious protonation. A VIRTIS Bench Top 3 freeze-drier operating at −30°C, 0.1 Torr, was used for the lyophilization of aqueous polymer solutions. Dialysis was performed in Spectra/Por 4 membrane tubing (12,000–14,000 molecular mass cut off) and in Spectra/Por 6 wet tubing (25,000 molecular mass cut off), for separation of second polymer fractions also in Spectra/Por 3 (6,000 molecular mass cut off). The operations were conducted against frequently changed batches of magnetically stirred H_2O at specified pH. In selected instances, dialysis was combined with size exclusion chromatography on Sephadex G-10. Polymer samples were dried in a SARTORIUS Thermo Control Infrared Drying System (heating program: 2 × 5 min at 65°C) or in an Abderhalden tube (2 days at 50°C) under reduced pressure.

Reactants and solvents

All amine, diamine, and other reactants and reagents were used as received commercially (Fluka Chemie). The two bisacrylamides, 1,2-dihydroxyethylene-1,2-bisacrylamide (DEBA) and methylenebisacrylamide (MBA), were recrystallized from isopropanol in the presence of 0.5% hydroquinone prior to use. Organic solvents, reagent or laboratory grades, were generally used as received; where required, they were stored over Molecular Sieves 3A. Deionized water was used for all experimental work. *trans*-Cyclohexane-1,2-diaminediaquaplatinum(II) dinitrate (DACH-Pt) was prepared by a method described in the literature.[10]

Key monomers and intermediates

The acryloylaspartic acid educt required for the synthesis of monomer **11** was prepared by N-acryloylation of aspartic acid. To this end, acryloyl chloride, 1.52 mL (18.8 mmol), dissolved in 5 mL of dichloromethane, was added dropwise at ice bath temperature to the vigorously stirred solution of aspartic acid, 2.08 g (15.6 mmol), Na_2CO_3, 1.654 g (15.6 mmol), and hydroquinone inhibitor, 20 mg, in 75 mL of water. The pH was maintained at 8–9 during this operation by simultaneous addition of aqueous 1*M* Na_2CO_3. Stirring was continued for 2 h at 0–5°C and for another 2 h at room temperature. Acidification to pH 3 (HCl) was followed by extraction with several 25-mL portions of ethyl acetate. From the combined extracts, concentrated stepwise by rotatory evaporation and cooled, several fractions of crystallized acryloylaspartic acid, all melting in the range of 158–162°C, were collected in a combined yield of 2.6 g (89%). Crystallization of the final crops was facilitated by the addition of pentane to the rewarmed solution.

^{1}H-NMR, δ/ppm: 6.3–6.2, 2H (CH_2=); 5.8, 1H (CH=); 4.8, 1H (CH, asp); 3.0, 2H (CH_2 asp). Alkalification (pH 10) shifted these signals to 6.1–5.8, 5.45, 4.2, and 2.4–2.2, respectively.

Recrystallized compound, mp 160–162°C, was used in the subsequent step. The acid, 2.01 g (10.7 mmol), together with Na_2CO_3, 1.14 g (10.8 mmol), was dissolved in 1.5 mL of H_2O. Upon the addition of 1,2-diaminopropane, 956 mg (1.10 mL; 12.9 mmol), the mixture was stirred for 3 days at 65°C in an incubator. Volatiles were removed from the solution by rotatory evaporation at 65°C bath temperature, and the residual viscous oil was thoroughly washed with boiling toluene and acetone. This left crude solid sodium salt, which was slurried in 4 mL of MeOH, followed by acidification (HCl) to pH 3. Upon solvent removal under reduced pressure the residue was extracted with 5 mL of boiling MeOH, and the solution was stepwise reduced in volume to allow three fractions of the solute to crystallize out. The process was facilitated in the final step by the addition of small volumes of pentane to the reheated solution, followed by cooling. The fractions, substantially identical by ^{1}H-NMR,

were combined to give 1.9 g (53%) of crystalline **11** · 2HCl, *N*-(4,7-diaza-5-methyl-heptanoyl)aspartic acid (and 6-methyl isomer) in diprotonated form.

^{1}H-NMR, δ/ppm: (expected proton count) 3.8 m, 1H (1H; C*H*CH_3); 3.6–3.2 m, 3.9H (4H; NH_2CH_2); 2.9 m, 1.9H (2H; CH_2CONH); 2.8 m, 1.9H (2H; CH_2COOH); 1.4 m, 3H (3H; CH_3).

On adjustment to pH 10: 4.4 m, 0.9H (1H; C*H*COOH); 3.0 m, 0.9H (1H; C*H*CH_3); 2.8–2.4 m, 7.8H (8H; CH_2); 1.0 m, 3H (3H; CH_3).

Anal. calcd. for $C_{10}H_{21}Cl_2N_3O_5$ (334.19): Cl, 2.18%. Found: 1.89%.

The two key monomers **12**, *N*-(4,8-diaza-octanoyl)-amidoglycolic acid, and **13**, *N*-(4,13-diaza-7,10-dioxa-tridecanoyl)amidoglycolic acid, were synthesized in an analogous fashion from 2-(acrylamido)glycolic acid and the diamines, 1.3-diaminopropane and 1,2-bis(2-aminoethoxy)-ethane, respectively. Thus, for the preparation of **12**, 1,3-diaminopropane, 815 mg (920 μL; 11 mmol), was added to the solution of 2-(acrylamido)-glycolic acid monohydrate, 1.63 g (10 mmol), and Na_2CO_3, 1.06 g (10 mmol), in 1 mL of H_2O. The stirred solution was heated for 3 days at 65°C and worked up as described for **11**, giving the diprotonated **12** · 2HCl in a total yield of 1.23 g (42%) as a crystalline solid.

^{1}H-NMR, δ/ppm: 3.5–3.1 m, 6.7H (6H; CH_2NH_2, CH_2NHCH_2); 2.75 m, 2.1H (2H; CH_2CONH); 2.1 m, 2H (2H; $CH_2CH_2CH_2$).

On adjustment to pH 10: 2.8 t, 1.9H (2H; CH_2CONH); 2.6 m, 7.2H (8H; $CH_2CH_2CH_2$); 2.45 t, 2H (2H; CH_2CH_2CO); 1.6 m, 2H (2H; $CH_2CH_2CH_2$).

Monomer **13** was prepared by adding 1,2-bis(2-aminoethyoxy)ethane, 1.63 g (1.61 mL; 11 mmol), to 2-(acrylamido)glycolic acid hydrate, 1.63 g (10 mmol), and Na_2CO_3, 1.06 g (10 mmol), both predissolved in 1 mL of H_2O. The stirred solution was heated and worked up as described for **11**. The crystalline (diprotonated) **13** · 2HCl was collected in a yield of 1.5 g (41%).

^{1}H-NMR, δ/ppm: 3.8–3.6 m, 8.5H (8H; CH_2OCH_2); 3.4–3.3 m, 5.9H (6H; CH_2NH_2; CH_2NHCH_2); 2.75 t, 2H (2H; CH_2CONH).

On adjustment to pH 10: 3.6 m, 8.8H (8H; CH_2OCH_2); 2.8 t, 2.1H (2H; CH_2CONH); 2.75 s, 4H (4H; CH_2NH_2, OCH_2CH_2NH); 2.5 t, 2H (2H; CH_2CH_2CO).

Polymerizations

Amounts of polymeric compounds are given as base moles, thus referring to the smallest recurring units (polymers **1** to **4**, and **4-Pt**, each normalized to $x = 1$; polymers **5** to **10**, normalized to $y = 1$).

Polymer 1

This was prepared by adding, dropwise, 4,7,10-trioxa-1,13-tridecanediamine, 331 mg (1.5 mmol), dissolved in 2 mL of H_2O, to the stirred solution of 1,2-dihydroxyethylene-1,2-bisacrylamide (DEBA), 330 mg (1.65 mmol), and Na_2CO_3, 53 mg (0.5 mmol), in 8 mL of isopropanol-water (3 : 1) precooled in an ice bath. After saturation with N_2, the solution was stirred in the stoppered flask for 1 day at ice bath temperature, followed by 1 day at ambient temperature and another 2 days at 60°C. Solvent removal by rotatory evaporation left a resinous polymer product, which was thoroughly washed with hot toluene and briefly with acetone for elimination of any unreacted diamine. It was then redissolved in 5 mL of H_2O, and the pH was adjusted to 7.5–8. The solution was chromatographed on a Sephadex G10 size exclusion column (2.0 × 30 cm), followed by dialysis for 2 days in Spectra/Por 6 tubing. Freeze-drying of the retentate and postdrying in the SARTORIUS unit gave 78 mg (12.4%) of water-soluble, solid **1**.

^{1}H-NMR, δ/ppm: 3.7–3.6, 13H (14H; CH_2OCH_2, C*H*OH); 2.8–2.3, 13H (12H; NHCOCH_2, CH_2NHCH_2); 1.6, 4H (4H; $CH_2CH_2CH_2$).

In a parallel experiment conducted as above, however, with the overall reaction period changed to 1 day at 0–5°C and another 4 days at ambient temperature, the ultimate yield of **1** was 8%. Another parallel polymerization run conducted as in the first-described experiment, except that the dialysis step was omitted, gave product polymer in a yield of 536 mg (85%), and the ^{1}H-NMR spectrum proved practically identical to that of the product of the first experiment. This indicates that (1) essentially complete purification was achieved in the chromatographic operation, and (2) the major fractionation step eliminating the bulk of lower-molecular polymer proceeded during dialysis.

Polymer 2

A solution was prepared from methylenebisacrylamide (MBA), 2.47 g (16 mmol), in 4 mL of hot H_2O. Upon the addition of 3-aminopropane-1,2-diol, 729 mg (620 μL; 8 mmol), dissolved in 1 mL of H_2O, the solution, saturated with N_2, was stirred for 1 day at ambient temperature. 3-(*N*,*N*-dimethylamino)propylamine, 817 mg (1.01 mL; 8 mmol), was added. After resaturation with N_2, stirring of the solution was continued for 3 days at 50°C. Major solvent removal by rotating evaporation was followed by the addition of Na_2CO_3, 106 mg (1 mmol), and product precipitation with excess Et_2O–EtOH–hexane (1 : 2 : 1). The resinous precipitate was thoroughly washed with hot toluene and briefly with acetone. It was then redissolved in 25 mL of H_2O. Upon pH adjustment to 7.5–8, the solution was treated as described for the isolation of **1** by size exclusion chromatography, dialysis, and freeze-drying, to give solid, water-soluble **2** in a yield of 810 mg (20.3%).

^{1}H-NMR, δ/ppm: 4.6, 4H (4H; NHCH_2NH); 3.8–3.45, 3.1H (3H; CH(OH)CH_2(OH); 2.8, 8.9H (8H; CH_2CONH); 2.7–2.25, 14.1H (14H; $CH_2N(CH_2)(CH_2)$, CH_2N$(CH_3)_2$); 2.2, 7H (6H; CH_3); 1.6, 2.4H (2H; $CH_2CH_2CH_2$).

Polymer 3

To MBA, 1.542 g (10 mmol, dissolved in 5 mL of hot H_2O and cooled to ambient temperature, was added aspartic acid, 799 mg (6 mmol), and Na_2CO_3, 636 mg (6 mmol). The suspension, saturated with N_2, was stirred in the stoppered flask for 1 day at 50°C in an incubator. 3-(Dimethylamino)propylamine, 613 mg (755 μL; 6 mmol), was added and stirring continued for another 2 days at 50°C. After removing most of the volatiles by rotatory evaporation (50°C bath temperature), Na_2CO_3, 0.1 g, was added, and the product was precipitated with excess Et_2O–EtOH–hexane (1 : 2 : 1), washed with hot toluene and acetone, and redissolved in 20 mL of H_2O. The solution, pH 9, was subjected to size exclusion chromatography for removal of any unreacted aspartic acid as the dissodium salt, and the eluate, after pH adjustment to 7.5, was dialyzed for 2 days in Spectra/Por 6 tubing. For the last 6 h of this operation, the pH of the retentate was lowered to 4 (MeCOOH) and, after several minutes, was raised again to 6 (NH_4OH) for liberation of the carboxyl groups. Freeze-drying of the retentate and postdrying left 694 mg (25.8%) of solid, water-soluble **3**.

^{1}H-NMR, δ/ppm: 4.6, 5.9H (5H; NHCH_2NH); 3.6, 1.1H [1H; CH (asp)]; 2.8, 10.1H (10H; CH_2CONH); 2.7–2.25, 18H [20H; $CH_2N(CH_2)(CH_2)$, CH_2N$(CH_3)_2$, CH_2(asp)]; 1.6, 3H (3H; CH_3).

In other experiments, including those described below, weak signals due to vinyl end groups occasionally emerged at 6.25 and 5.8 ppm.

Polymer 4

A solution was prepared from MBA, 972 mg (6.3 mmol), in 4 mL of hot H_2O. Upon the addition and dissolution of *N*-(4,7-diaza-5-methyl-heptanoyl)aspartic acid dihydrochloride (**11**· 2HCl), 1.17 g (3.5 mmol), and Na_2CO_3, 742 mg (7 mmol), the solution was saturated with N_2 and stirred for 1 day at 55°C in the stoppered flask. 3-(Dimethylamino)propylamine, 327 mg (403 μL; 3.2 mmol), was then added and stirring of the solution, resaturated with N_2, was continued for another 2 days at the same temperature. Removal of most of the solvent at 50°C under reduced pressure and addition of a 53-mg portion (0.5 mmol) of Na_2CO_3 for complete alcalification was followed by polymer precipitation with 15 mL of Et_2O–EtOH–hexane (1 : 2 : 1). The resinous precipitate, thoroughly washed with hot toluene and Me_2CO, was dissolved in 15 mL of H_2O. The pH was adjusted to 7.5–8 (HCl), and the solution was dialyzed for 2 days in Spectra/Por 4 tubing against H_2O (pH 6.8). For the last 6 h of this operation, the inside pH was lowered to 4 (MeCOOH) for liberation of the carboxyl groups and then raised again to 6 (NH_4OH). Frequent exchange of the aqueous outer phase during this period was required for complete elimination of inorganic salt from the retentate, which was then freeze-dried and postdried on the Sartorius unit. The yield of the water-soluble solid polymer was 720 mg (34.0%).

^{1}H-NMR, δ/ppm : 4.6, 4H (4H; NHCH_2NH); 2.8, 9.3H (10H; CH_2CONH); 2.7–2.25, 16H (19H; $CH_2N(CH_2)(CH_2)$, CH_2N$(CH_3)_2$; CHNHCH_2, CH_2(asp)); 1.6, 2H (2H; $CH_2CH_2CH_2$); 1.0, 2.7H (3H; CHCH_3).

Redialysis of the polymer so obtained for 2 days in Spectra/Por 6 tubing and isolation as before provided a higher-molecular fraction (54% recovery, corresponding to a total yield of 18.4%) giving a NMR spectrum virtually identical to that described above.

Polymer 5

To the solution of MBA, 972 mg (6.3 mmol), in 4 mL of hot H_2O was added and dissolved *N*-(4,8-diazaoctanoyl)amidoglycolic acid dihydrochloride (**12**·2HCl), 1.023 g (3.5 mmol), and Na_2CO_3, 557 mg (5.25 mmol). The solution, saturated with N_2, was stirred for 1 day at 55°C, whereupon 3-(dimethylamino)propylamine, 327 mg (403 μL; 3.2 mmol), was added. After brief flushing with N_2, the solution was stirred for another 2 days at the indicated temperature. Workup as in the preceding experiment by precipitation, dialysis in Spectra/Por 4 tubing, and freeze-drying gave 625 mg (31.5%) of solid, water-soluble **5**.

^{1}H-NMR, δ/ppm: 4.6, 3.7H (4H; NHCH_2NH); 2.8, 10.5H (10H; CH_2CONH); 2.6–2.25, 18H (18H; $CH_2N(CH_2)(CH_2)$, CH_2N$(CH_3)_2$, CH_2NHCH_2); 2.2, 6H (6H; CH_3); 1.6, 4H (4H; $CH_2CH_2CH_2$).

Redialysis for 1 day in Spectra/Por 6 tubing and isolation as before gave a higher-molecular fraction with virtually the same NMR spectroscopic data. The recovery was 61%, corresponding to a total yield of 19.2%.

Polymer 6

A reaction was conducted essentially by the procedure of the preceding experiment, but with the following reactants: MBA, 762 mg (4.9 mmol), *N*-(4,13-diaza-7,10-dioxatridecanoyl)amidoglycolic acid dihydrochloride (**13**·2HCl), 1.099 g (3 mmol), Na_2CO_3, 477 mg (4.5 mmol), and 3-(dimethylamino)propylamine, 255 mg (315 μL; 2.5 mmol), in 4 mL of H_2O. After solvent removal and addition of Na_2CO_3, 50 mg, the workup operations involving precipitation, washing, dialysis

in Spectra/Por 4 tubing, and freeze-drying were performed as before. The water-soluble polymer **6** was collected in a yield of 830 mg (48.1%).

^{1}H-NMR, δ/ppm: 4.6, 4.2H (4H; NHCH_2NH); 3.6, 8.8H (8H; CH_2OCH_2); 2.8–2.25, 28H (28H; CH_2CONH, $CH_2N(CH_2)(CH_2)$, CH_2N$(CH_3)_2$, CH_2NHCH_2); 2.2, 6.4H (6H; CH_3); 1.6, 2H (2H; $CH_2CH_2CH_2$).

Redialysis and workup as before provided a higher-molecular fraction with a recovery of 51%, corresponding to an ultimate yield of 24.5%. The NMR data were virtually unchanged.

Polymer 7

This polymer, a variant of **6**, was synthesized as in the preceding experiment, yet in a changed feed ratio. Thus, MBA, 1.158 g (7.5 mmol), was allowed to react with the dihydrochloride of **13**, 549 mg (1.5 mmol), Na_2CO_3, 239 mg (2.25 mmol), and 3-(dimethylamino)-propylamine, 613 mg (755 μL; 6.0 mmol), in 5 mL of H_2O. Conventional workup gave water-soluble solid **7** in a yield of 850 mg (38.5%).

^{1}H-NMR, δ/ppm: 4.6, 9.2H (10H; NHCH_2NH); 3.7, 7.5H (8H; CH_2OCH_2); 2.8–2.25, 62H (64H; CH_2CONH, $CH_2N(CH_2)(CH_2)$, CH_2N$(CH_3)_2$, CH_2NHCH_2); 2.2, 24H (24H; CH_3); 1.6, 8H (8H; $CH_2CH_2CH_2$).

Redialyzed and worked up as before (56% recovery), polymer **7** was isolated in an overall yield of 21.6%. There were no significant changes in the NMR spectrum.

Polymer 8

This polymer was synthesized as described for the preparation of **5**, except that 3-(dimethylamino)propylamine was replaced by the same amount, 3.2 mmol (240 mg; 276 μL) of 2-(methoxy)ethylamine. The water-soluble **8** was obtained in a yield of 537 mg (28.3%).

^{1}H-NMR, δ/ppm: 4.6, 3.9H (4H; NHCH_2NH); 3.6–3.4, 5.1H (5H; CH_2OCH_3); 2.9–2.25, 28H (26H; CH_2CONH, $CH_2N(CH_2)(CH_2)$, CH_2NHCH_2); 1.6, 2H (2H; $CH_2CH_2CH_2$).

Conventional redialysis and workup gave a higher-molecular fraction (60% recovery, corresponding to an overall yield of 17.0%) with an essentially unchanged NMR spectrum.

Polymer 9

For the synthesis of this polymer the preparative procedure leading to **6** was used except that 2-(methoxy)-ethylamine, 188 mg (216 μL; 2.5 mmol), replaced 3-(dimethylamino)propylamine. There was obtained 687 mg (41.4%) of water-soluble, solid **9**.

^{1}H-NMR, δ/ppm: 4.6, 4H (4H; NHCH_2NH); 3.8–3.3, 14H (13H; CH_2OCH_3, CH_2OCH_2); 2.9–2.3, 25.5H (26H; CH_2CONH, $CH_2N(CH_2)(CH_2)$, CH_2NHCH_2).

A higher-molecular fraction was collected (48% recovery, corresponding to an overall yield of 19.9%) by the conventional redialysis and workup procedure.

Polymer 10

The procedure leading to **7** was employed for the preparation of **10**, with 3-(dimethylamino)propylamine replaced by the same amount, 6.0 mmol (451 mg; 517 μL), of 2-(methoxy)ethylamine. This gave 778 mg (38%) of water-soluble, solid **10**.

^{1}H-NMR, δ/ppm: 4.6, 10H (10H; NHCH_2NH); 3.7–3.5, 15.3H (16H; CH_2OCH_3, CH_2OCH_2); 3.3, 13H (12H; CH_3); 2.9–2.3, 56H (56H; CH_2CONH, $CH_2N(CH_2)(CH_2)$, CH_2NHCH_2).

Redialysis and workup as before provided a higher-molecular fraction (recovery 62%) in an overall yield of 23.5%.

Conjugate 4-Pt

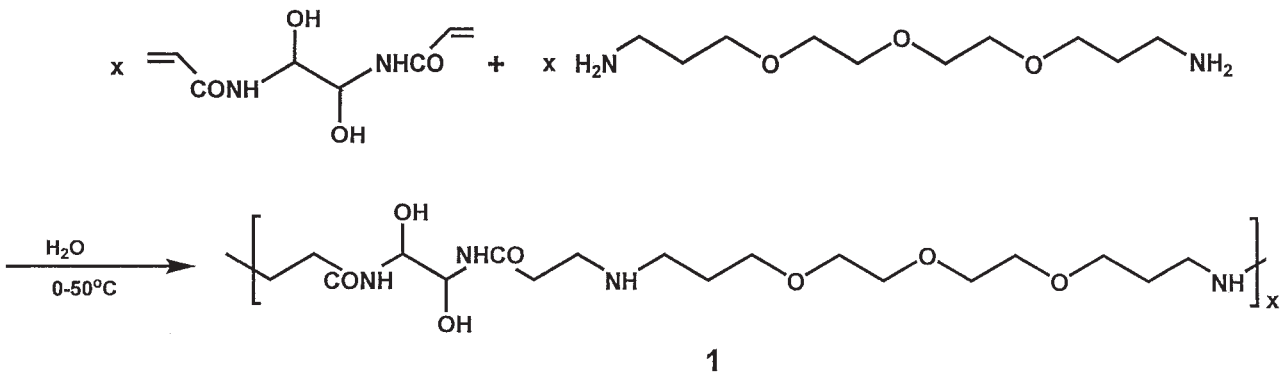
Polymer **4** was platinated by a procedure essentially developed in previous work.[11] A solution was prepared from **4**, 336 mg (0.5 mmol), in 2 mL of H_2O, and N_2 was introduced to saturation. To this was added DACH-Pt, 282 mg (0.6 mmol), dissolved in 2 mL of H_2O. Resaturated with N_2, the solution was stirred in the dark for 3 days at ambient temperature and for a further 12 h at 45°C. The pH was carefully maintained at 6.0–6.5 while the solution was at room temperature, and more closely at 5.5–6.0 during the heating period. The solution, clarified by filtration, was dialyzed for 2 days in Spectra/Por 4 tubing against H_2O at pH 6, and the retentate was freeze-dried to give yellowish conjugate **4-Pt** as a water-soluble solid in a yield of 193 mg (45.1%).

Anal. calcd. for $C_{32.6}H_{60.2}Pt_{0.6}N_{10.2}O_9$ (856.13) (**4-Pt**): Pt, 13.7%. Found : Pt, 13.5%.

RESULTS AND DISCUSSION

The target polymers of this study are polyamidoamines derived from bisacrylamides as the key monomers. The synthesis is based on a Michael addition mechanism proficiently developed by Ferruti et al.[11–13] into a general procedure for polymer formation: it was later used extensively in our laboratory for the preparation of a great variety of polymer structures.[14,15]

Polymerization reactions involving Michael additions are inherently inefficient because, while requiring an aqueous or partially aqueous solvent system for efficacious propagation, the growing



1

Scheme 1

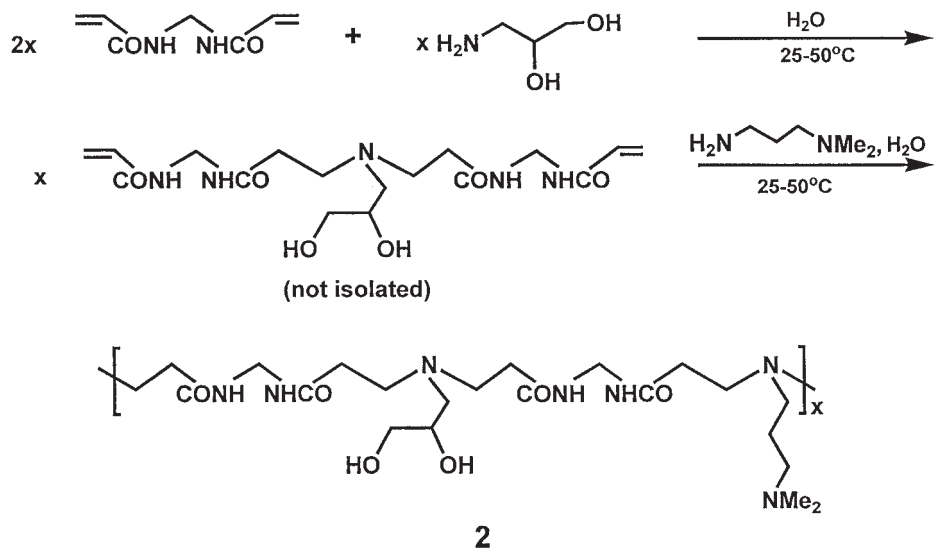
polymer chains are susceptible to hydrolytic fission at the labile amide link constituents of the bisacrylamide monomers. The two reaction sequences, hence, militate against each other, and any polymerization experiment will invariably provide a compromise between propagation and depropagation. As a result, the molecular mass distribution of the polymer products will be unduly wide. To collect polymers in the desired molecular mass range of 20,000 and higher, required for acceptably long extended serum residence times, one has to resort to fractionation techniques cutting off all material substantially below the stated limit. As that material constitutes the bulk of the polymerization product, ultimate yields of the desired fractions, depending critically on the relative hydrolytic stability of the monomers used, are generally quite low (< 25%), yet are accepted in our work as the price to be paid for obtaining material in the proper molecular size.

The first target compound of this investigation, the polyamidoamine **1**, contains pairs of intrachain-type hydroxyl groups (Scheme 1). The rather labile compound, 1,2-dihydroxyethylene-1,2-bisacrylamide (DEBA), served as the key monomer for the synthesis of **1**. The compound was allowed to react with one equivalent of 4,7,10-trioxa-1,13-tridecanediamine in aqueous isopropanol medium over a 4-day period at an ultimate temperature of 50°C. In this macromolecule, the tridecanediamine served as the solubilizing constituent. To ensure monofunctional reactivity of the $-NH_2$ terminals in this diamine, low reactant concentrations (~ 0.2–0.3*M*) were required, and for the first 24 h of the reaction period the temperature was maintained at 0–5°C. The water-soluble product, thoroughly purified and crudely fractionated by aqueous dialysis in membrane tubing with a molecular mass cutoff limit of 25,000, was isolated in the solid state by freeze-drying. Proton magnetic resonance spectroscopy confirmed the proposed structure.

In this polymerization run, as well as in subsequent experiments, the concentrated outer phase collected in the first dialysis operation was redialyzed in membrane tubing with a molecular mass cutoff of 6,000. This afforded a second polymer fraction with yields typically in the range of 25–35%. While of lower molecular mass, these additional fractions (not tabulated or further described) gave ^{1}H-NMR spectra substantially identical to those of the respective main fractions.

Polyamidoamine **2**, characterized by the presence of hydrosolubilizing *tert*-amine side groups in addition to pairs of hydroxyl groups in an extrachain-type fashion, was derived from 3-aminopropane-1,2-diol. Interacting with two equivalents of the key monomer, methylenebisacrylamide (MBA) in aqueous isopropanol (1 day at room temperature), this aminodiol gave an intermediary bisacryloyl adduct. Without isolation, this was treated with one equivalent of 3-(dimethylamino)propylamine for 3 days at ultimately 50°C, thereby converting to the target polymer **2** (Scheme 2). In the propagation sequence of this scheme, contrasting with Scheme 1, the terminal amino groups of the amine coreactants act as difunctional sites, undergoing double addition with resultant formation of *tert*-amine functionality in the main chain. In this constellation, thus, the precautions observed in the preceding experiment are not required. Therefore, considerably higher reactant concentrations (1.5–3*M*) could be used and the initial low-temperature reaction period omitted. The water-soluble product, purified, dialyzed, and isolated in the solid state as above, was routinely characterized.

In an attempt to synthesize a polymer analogous to **2** yet with the dihydroxyl functionality replaced by a dicarboxyl substituent system, experiments were performed in which methylenebisacrylamide was allowed to add to the amino group of aspartic acid (present as the disodium salt), followed by interaction of the bisadduct with 3-(dimethylamino)-

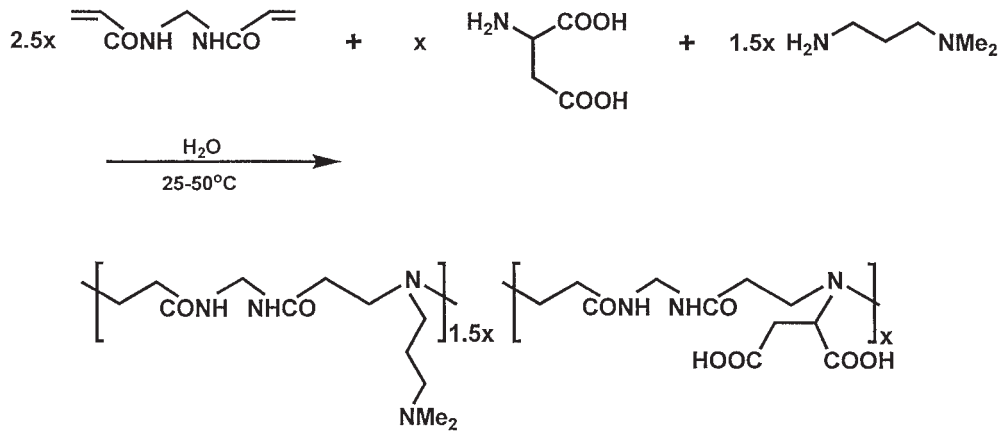


2

Scheme 2

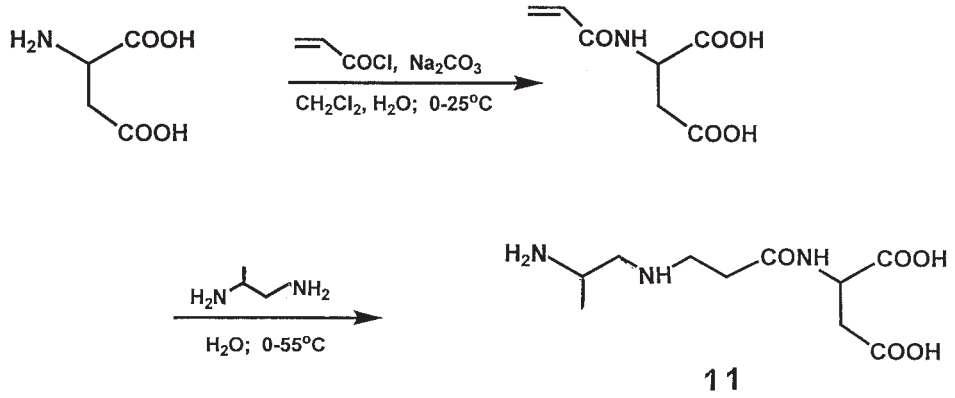
propylamine. As a consequence of the poor nucleophilicity of the amino group in aspartic acid, we expected a comparatively low reactivity of that acid and, therefore, used it in excess over stoichiometry and extended the reaction time of the first step in the sequence. However, despite these modifications, the product polymers were consistently deficient in the aspartic acid component. The experiment described in this communication, leading to a polymer of composition **3**, represents a compromise solution, with 1.5 aminopropyl-containing subunits present in the backbone for every dicarboxyl-functionalized subunit making up the overall main chain (Scheme 3, random distribution of subunits).

The dicarboxyl-functionalized carrier **4** represents a variant of **3**, incorporating the aspartic acid unit as a side chain component, yet with a more extended spacer segment interposed between main chain and dicarboxyl functionality. The biological effects of polymer-bound medicinal agents are frequently dependent on the length of the tether interconnecting carrier and drug. It was thus deemed advantageous to include **4** (as well as the subsequent target polymers **5** to **10**) in this series of polymers with carrier functions. Polymer construction necessitated the two-step presynthesis of the intermediate **11**, *N*-(4,7-diaza-5(6)-methyl-heptanoyl)aspartic acid, from aspartic acid by N-acryloylation with acryloyl chloride, followed by treatment with 1,2-diaminopropane (Scheme 4). The intermediate was now incorporated into a Michael addition polymer by copolymerization with methylenebisacrylamide in



3

Scheme 3



Scheme 4

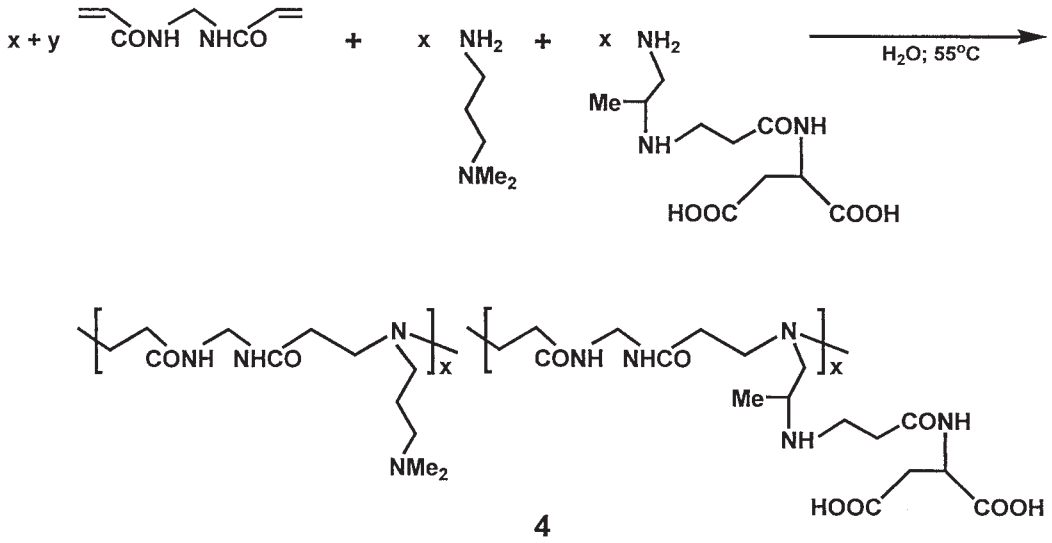
the presence of 3-(dimethylamino)propylamine, again in aqueous medium (Scheme 5), giving water-soluble carrier **4**.

The synthesis of carrier **5** followed that of **4**, except that the glycolic acid derivative **12**, *N*-(4,8-diaza-octanoyl)amidoglycolic acid, replaced **11** as the key monomer (Scheme 7). Compound **12**, previously reported,[9] was synthesized in the present project by a somewhat improved procedure from 1,3-diaminopropane and the commercially available 2-(acrylamido)glycolic acid (Scheme 6).

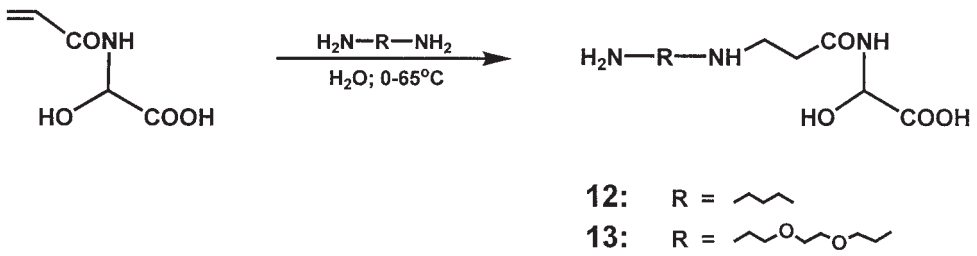
Another monomer, *N*-(8,11-dioxa-4,15-diaza-pentadecanoyl)amidoglycolic acid, **13**, prepared from 1,2-bis(2-aminoethoxy)ethane and 2-(acrylamido)glycolic acid (Scheme 6), served as the comonomer with methylenebisacrylamide and 3-(dimethylamino)propane to give the target polymer **6**. The same reactants employed in a different feed ratio gave rise to the formation of polymer **7** (Scheme 7).

Lastly, for a group of carriers (**8** to **10**) synthetic procedures were analogous to those leading to **5** to **7** except that 2-(methoxy)ethylamine replaced 3-(dimethylamino)propylamine as the comonomer (Scheme 7). The choice of the methoxyethylamine arose from the need to construct carriers possessing the neutral methoxy terminal in the solubilizing unit instead of the basic dimethylamino terminal featured in **2** to **7**. The extent of basicity may critically control the pharmacokinetics (and, hence, the potential accumulation in the affected target cell) of a polymeric drug carrier.[16,17] Polymers **8** to **10** will thus be of interest as key counterparts to those characterized by the basic *tert*-amino side groups.

To demonstrate the functional drug anchoring capabilities of the synthesized carriers, the exemplifying polymer **4** was platinated with the aid of *trans*-cyclohexane-1,2-diaminediaquaplatinum(II) dini-



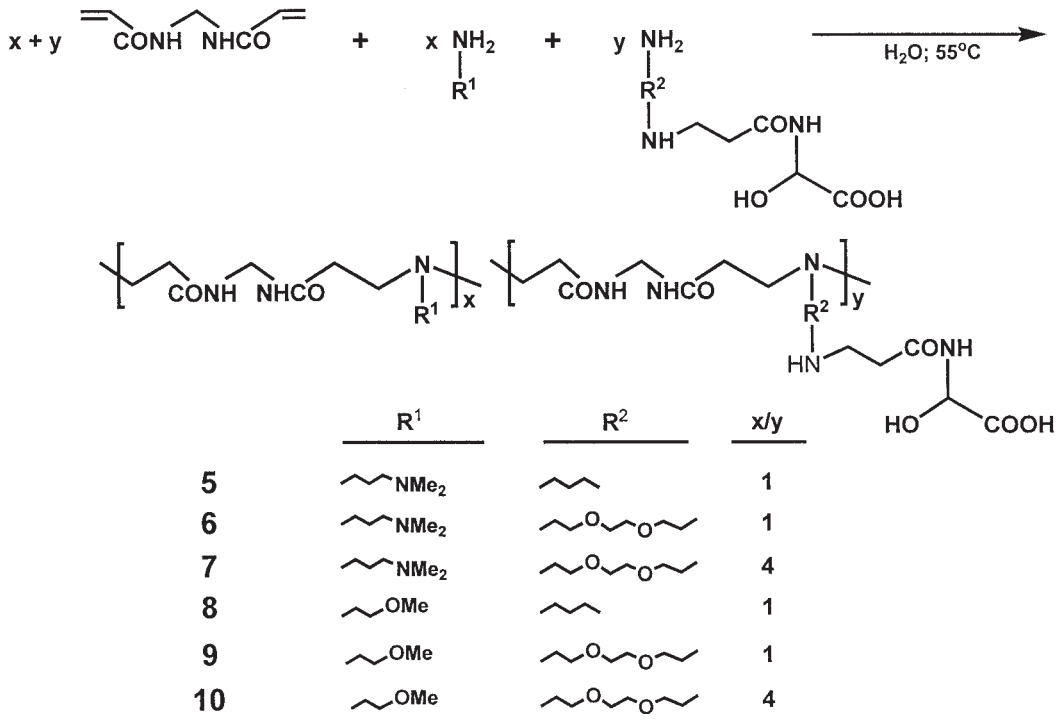
Scheme 5



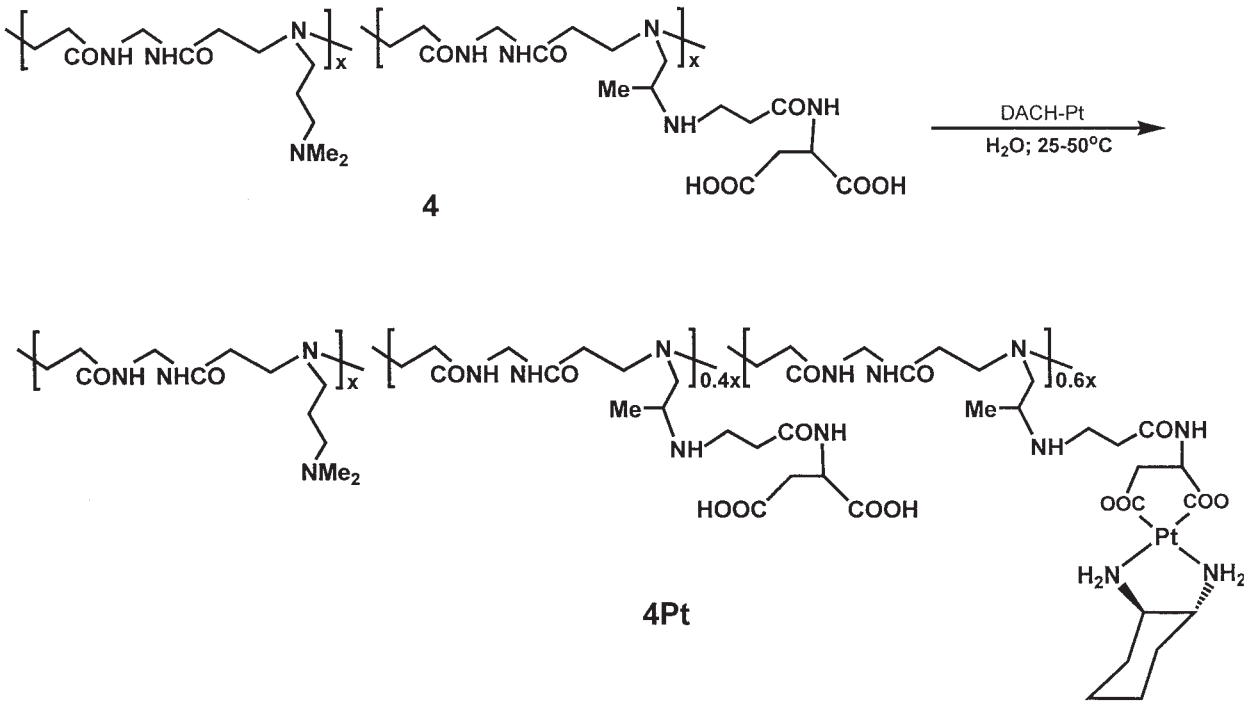
Scheme 6

trate (DACH-Pt). To this end, the carrier was treated in aqueous solution with 1,2 equivalents of the platination agent, converting to a platinum-containing conjugate (Scheme 8). Under the experimental conditions of this experiment, the conjugate's composition accorded with **4-Pt**, with 60% of available ligand pairs coordinated to the metal. The question of O,O- versus O,N-chelation in compounds of the type **4-Pt** is controversial. It has been shown in Appleton's laboratory[18] and elsewhere that a sterically favored amino group as in α-aminoacids, and even in N-acylated α-aminoacids possessing a considerably weaker N-donor, will readily yield a thermodynamically favored 5-membered O,N-chelated platinum complex. Similarly, with aminodicarboxylic acids such as aspartic acid, 2-aminomalonic acid, or iminodiacetic acid, thermodynamically favored 5-membered O,N chelates will ultimately be formed,[18–20] the second carboxylic acid group "sticking out" as free –COOH. In N-acylated aminodicarboxylic acid structures, on the other hand, as in the N-acylated aspartic acid moiety of the carrier **4**, the reaction path is less predictable. While 5-membered ring formation of the O,N-chelation type involving amidic N is feasible, we consider the nitrogen-donor strength of that amidic amino group to be too low in comparison with the competing carboxylate anion to favor such O,N-cyclization. Accordingly, we tentatively ascribe the O,O-chelate structure with dicarboxylato coordination to **4-Pt**.

In compounds featuring the weaker oxygen donors of the hydroxylato type such as **1**, **2**, and **5** to **10**, the metal coordination behavior may well be different. In forthcoming platination studies the question of O,O- versus O,N-chelation involving the various ligand



Scheme 7



Scheme 8

pairs incorporated in the described carriers will be addressed in detail.

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