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Synthesis of 5-Substituted Derivatives of Isophthalic Acid as Non-Polymeric Amphiphilic Coating for Metal Oxide Nanoparticles

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Abstract

In the course of development of novel capping ligands with variable steric factor, which will be used as an organic coating for metal oxide nanoparticles, a base-catalyzed nucleophilic oxirane ring-opening addition reaction between dimethyl 5-hydroxyisophthalate and allyl glycidyl ether was studied. The allyl-terminated 1-1, 1-2 and 1-3 adducts and dihydroxylated derivative of the 1-1 adduct, 5-diglyceroxy isophthalic acid, were synthesized. The latter binds to the surface of 5 nm γ -Fe₂O₃ nanoparticles in reaction with their surfactant-free diethylene glycol colloids.

Keywords

capping ligand; epoxide ring-opening addition; solvent-free synthesis; metal oxide nanoparticles

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Notes

The authors declare no competing financial interest.

ASSOCIATED CONTENT

Supplementary Data. Supplementary data associated with this article can be found in the online version. Synthesis procedures, ESI-MS, 1D and 2D NMR spectra and FT-IR.

INTRODUCTION

Development and study of magnetic nanoparticles for biological and clinical applications remains a field of intensive investigation for the last two decades.¹⁻¹³ It involves a multidisciplinary endeavor and is one of the most challenging research areas in chemistry and materials science. Application of magnetic nanoparticles has been already explored in a wide variety of non-invasive medical treatment and diagnostic procedures, such as cancer therapy for inoperable tumors, gene therapy, blood detoxication, radiation treatment, magnetic resonance imaging (MRI).³⁻¹¹ The performance of these particles as, for example, drug delivery, MRI, hyperthermia or cell tracking agents, depends on their magnetic susceptibility, however, their ability to form stable aqueous colloids, the mobility, and diffusion properties in biological media, largely rely on organic coating. Functional nanoparticles used in clinical research these days are usually coated with hydrophilic biocompatible polymers such as dextrans or poly(ethylene glycol)s. Excessively large macromolecules of these polymers make the nanocomposite unwieldy, and thus limit its mobility and penetration properties. Due to large diamagnetic component, polymers suppress the desired response to an external magnetic field. In addition, they restrict water exchange between superparamagnetic core and biological fluids, which is highly desired for the MRI contrast agent applications.

To address some downfalls associated with polymers, in this work we attempted to develop a non-polymeric organic coating of an adjustable size. The future capping ligand would contain two distinct structural parts: the coordinating head and a bulky substituent with reactive functional group on its end. Coordinating head should be able to act as a polydentate bridging ligand due to several donor atoms in it, which are adequately spaced from one another. Benzenecarboxylic acids offer multiple degrees of freedom, and isophthalic acid was our choice because of its geometry which seemed to be most appealing for the purpose. Selection of the substituent was more challenging as it should have a variable length and solvent affinity properties, and it should be functionalizable at its end. The easiest way to address the size is performing an oligomerization reaction under controlled conditions, and we selected an oxirane ring-opening addition reaction which offers not only an easy control of the condensation, but also amphiphilic properties to the product. Readily available 5-hydroxyisophthalic acid and allyl glycidyl ether were used as an initiator and a monomer respectively, leading to the targeted capping ligand. Its oxygen-rich substituent would provide hydrophilic properties, while allyloxy pendant would offer some freedom for boosting the hydrophobic properties or for conjugating the capping ligand to a biomolecule.

Starting with its dimethyl ester, 5-hydroxyisophthalic acid was functionalized via the phenolic hydroxyl group by a nucleophilic oxirane ring opening addition reaction with allyl glycidyl ether. The chain length was adjusted by changing the stoichiometric ratio of the nucleophile to oxirane monomer. Reported method can be generalized and used for chemical modification of a variety of hydroxyacids if they are not sterically hindered.

RESULTS AND DISCUSSION

Our search for the type of substituents to be attached to iso-phthalate moiety was focused on oxygen-rich carbon chains with reactive side or terminal groups which would be used for the subsequent functionalization followed by the conjugation with biomolecules. Commonly known ether-type structures like in polyethylene glycol or polyglycerol were appealing for us because of their particularly useful set of properties. The structures of the best known polyether types are linear in poly(ethylene oxide) and hyperbranched in polyglycerol¹⁴⁻¹⁸, however we targeted a less-common linear chain glycerol-related structure by using allyl glycidyl ether (AGE) as a convenient monomer with two reactive groups in its molecule, an oxirane ring and an allylic double bond. Cationic¹⁹⁻²¹ and anionic²²⁻²⁴ ring-opening polymerization reactions of this substance are known to yield a linear chain ethylene oxide-type polymer with pendant allyloxymethyl groups. It is also known that polymerization of glycidyl ethers is easily controlled and produces polymers with variable molecular masses and narrow polydispersity.²²⁻²⁴ Based on this information, we expected that an anionic epoxide ring-opening could be controlled by maintaining a desired reagent ratio and reaction conditions, so that a certain number of units, contributing to the chain growth, could be added one at a time. The allyloxy-groups, which remain intact on the chain backbone, might be used for the subsequent functionalization. Several examples of such reactions are known: the thiol-ene coupling (TEC) reactions were used for attaching an amino, carboxy, hydroxy or benzimidazole terminal groups along the polymeric PEG chains;²⁵⁻²⁷ palladium-catalyzed isomerization of allyl into vinyl groups followed by hydrolysis, afforded a linear-chain structured poly(glycidol).²⁸ In order to obtain hydrophilic capping ligands, oxidation of the allyl double bond to 1,2-diol can be carried out in the step following after the ring-opening addition and the ester hydrolysis. This approach can be related to dihydroxylation of poly(allyl glycidyl ether),²⁴ which was utilized in the synthesis of a relatively unusual linear chain-structured polyethylene oxide with glyceroxymethyl groups as branches.

Synthesis of 5-substituted isophthalate esters

A reaction between dimethyl 5-hydroxyisophthalate (HIP) and AGE was studied in solvent-free and solution systems, at different temperatures and at a presence of a base catalyst. It was found that the reaction can be conveniently done without a solvent, but using neat reagents mixed in different ratios. For example, the reaction of HIP with 1.0-1.1 molar equivalents of AGE can be driven to completion at 103-105°C in about 24 hours, and it produces a corresponding ether-ester product HIP-AGE 1- 1 E (1) as a waxy colorless to yellowish substance in > 90% isolated yield (Scheme 1); synthesis details are given in the supplementary data file, pp. 2-3. The identity of this compound was confirmed by ¹H, ¹³C and 2D NMR and ESI-MS (supplementary data, pp. 8-15).

It was found that different batches of HIP showed different reactivity, which was later proven to be associated with the presence or absence of basic impurities. Some of the batches reacted with AGE with no catalyst producing the product (1) in nearly a quantitative yield, while others, which had been determined to be base-free were not reactive at all. In order to determine the optimal conditions which would work for all batches, we studied the HIP-AGE system at presence of a basic catalyst ranging from 10 to 0.1 molar %. The HIP

was first treated with a fixed amount of NaOCH₃ solution in methanol. Then methanol was thoroughly removed in vacuum in order to eliminate the presence of sodium methoxide in the HIP-AGE reaction mixture, and to make sure all sodium is present in the form of phenoxide. This pre-treated HIP readily reacted with AGE in melt producing the adduct (1). The optimal amount of base needed for the HIP pre-treatment was found to be around 0.1 molar %.

A slight excess of AGE (~10 mol.%) was found to be useful in driving this reaction to completion, however, a small amount of HIP-AGE 1-2 E adduct (2) was detected in the product mixture as well. In order to synthesize this and 1-3 E homologs, we studied a reaction of crude (still base-activated) 1-1 ester (1), with an additional equivalent of AGE under the same conditions as in Scheme 1. After the workup, the reaction product was analyzed by GC-MS, and the ratio of (1), (2) and HIP-AGE 1-3 E (3) adducts was found to be 1 : 2.3 : 1.2, respectively. The yield of the desired product can be improved by tuning the reaction conditions, however, we performed a preparative chromatographic separation of the mixture instead and isolated each of the three adducts in pure state. The isolated yields were 0.04, 0.12 and 0.08 g of each 1, 2 and 3, starting with 0.5 g of crude product. The identity of these compounds was confirmed by ¹H, ¹³C and 2D NMR and ESI-MS (supplementary data, pp. 16-33).

Based on proton and C-13 NMR spectra, this reaction is highly regioselective, and HIP attacks the AGE oxirane ring at the less substituted side, which is typical for a base-promoted ring opening. Proton NMR spectra show a splitting pattern which is typical for diastereotopic protons. For adducts with more than one molecule of AGE, carbon spectra reveal duplicated peaks, which is typical for diastereomeric product formation.

Synthesis of 5-substituted isophthalic acids

The ester product (1) was hydrolyzed by heating in ethanol solution with a slight excess (~2.2 equivalents) of NaOH producing disodium salt of HIP-AGE 1-1 A acid (4) isolated as a hygroscopic white crystalline compound. In the subsequent step, this salt was converted into a corresponding acid (5) by acidification of its aqueous solution with sodium hydrosulfate, followed by water evaporation and extraction with MTBE. The acid was achieved in 70-75 % isolated yield and recrystallized from water, producing white needles (hydrate), m.p. 162-165 °C. The HIP-AGE 1-2 A (6) and HIP-AGE 1-3 A (7) were obtained from the corresponding esters in a similar way as waxy substances; synthesis details are given in the supplementary data file, pp. 4-5. The identity of these compounds was confirmed by ¹H, ¹³C and 2D NMR and ESI-MS (supplementary data, pp. 34-65).

Frey *et al* found that under the reaction conditions used for the synthesis of poly(allyl glycidyl ether), a small fraction of allyl terminal groups isomerized into *cis*-prop-1-enyl groups, which was evidenced by ¹H and ¹³C NMR.²⁴ The NMR spectra of 1-1 and 1-2 esters (1) and (2) and acids (5) and (6) obtained in this work, showed clean baseline in the region where the isomerized product would appear, however for 1-3 acid (7) the evidence of isomerization was found in proton NMR spectrum, which showed a weak signal around 4.4 ppm. The ESI(–) mass-spectra of the 1-1 acid (5) showed a minor peak with *m/z* = 255 which can be interpreted as Ar-O-CH₂-CH(OH)-CH₂-OH. This contaminant could have

been formed from hydrolysis of the isomerized propenylic product during the workup. The MS-MS run on $m/z = 255$ peak, confirmed this interpretation. In addition to main peaks of molecular ions $M - H$ (m/z 409.1 and 523.2, respectively), mass-spectra of 1-2 (6) and 1-3 (7) acids contain lower intensity peaks corresponding to “minus allene” (m/z 369.1 and 483.2, respectively) species. Fragmentation of these peaks follows the same path as for the main molecular ions in MS-MS runs. These results indicate that an allyl group isomerization took place in our systems, however to a relatively small extent. Generally, our data are in line with the published work²⁴ except for a small difference in isomerization rate, as yield of the propenylic products was relatively low in our reactions, according to NMR. This difference may be attributed to using sodium as a counter ion in our case *versus* potassium in Frey's work.²⁴ Presumably, sodium forms a less stable intermediate five-membered chelate ring as compared to potassium used in the reference studies. The difference in isomerization activity of sodium and potassium alkoxides was noticed earlier,²⁹ however this comparison could not be considered valid as different alkoxides (potassium *tert*-butoxide and sodium methoxide) were used.

1,2-diol-terminated ligands: synthesis of 5-diglyceroxy isophthalic acid

The convenience of allyl glycidyl ether as a chain-forming substrate is that once attached to the nucleophilic core, it produces a vinyl-terminated derivative which can be further functionalized using various pathways. Oxidation of the terminal double bond into a corresponding 1,2-diol is one of the possible options that introduces more hydrophilic groups into the ligand, so it was studied in this work. Even though permanganate oxidation of terminal alkenes often results in cleavage of the carbon-carbon double bond³⁰, running the reaction with cold basic (NaOH, pH 12) permanganate taken in stoichiometric amount (alkene to $KMnO_4 = 3:2$), appeared to be gentle enough for preparation of desired diol (Scheme 2). The workup procedure consisting of the MnO_2 removal, adjusting the pH to 1, water evaporation, extraction with glyme followed by its evaporation in vacuum, afforded 5-diglyceroxy isophthalic acid (HIP-AGE 1-1 Ao) (8) as a yellowish waxy substance with 55.7 % isolated yield. A portion of crude product was purified by dissolving it in glacial acetic acid, drying with drierite, microfiltration and isolation by CH_2Cl_2 vapor diffusion as a colorless waxy substance. Heating at 70-80°C in vacuum helped to isolate the product as white fluffy powder; synthesis details are given in the supplementary data file, pp. 5-6.

As it was evidenced by NMR and ESI mass-spectra (supplementary data, pp. 66-73), oxidation of the allyl double bond appeared to be highly selective, as the vicinal diol forms under the reaction conditions as a primary product. Further oxidation could be seen on the level of traces in the ESI mass spectra (but not in NMR) by presence of carboxylic acid $Ar-O-CH_2-CH(OH)-CH_2-O-CH_2-COOH$ and its δ -lactone (m/z 313 and 295, respectively).

The NMR and mass-spectra of (8) evidenced a self-esterification side reaction, which most likely occurred when aqueous acidic solutions were heated and evaporated. Line broadening in the 1H NMR spectrum can also be the consequence of a hydrogen bonding-driven aggregation. This would lead to freezing of the certain conformations, slowing the rotation rate and consequently, decreasing T2 relaxation time.

Self-esterification can be avoided if acidic treatment step is excluded from the workup procedure, and the product is isolated in a form of sodium or potassium salt. NMR and ESI mass-spectra of separately prepared Na salt of (8) looked cleaner as compared to the spectra of free acid (supplementary data, pp. 74-87). Proton NMR peaks were narrower and 2D spectra were easier to interpret. The content of ester contaminants resulting from self-esterification, was reduced to traces.

Oxidation and workup protocol described here for the synthesis of acid (8), can be also used for synthesis of the longer chain hydroxylated ligands.

Organic-inorganic nanoparticulate adducts

The ability of the synthesized isophthalate-based capping ligands to bind to metal oxide nanoparticles was tested by reacting 5-diglyceroxy isophthalic acid (8) with 5 nm γ -Fe₂O₃ nanoparticles colloid in surfactant-free diethylene glycol solution, prepared as we reported earlier.³¹ The reaction stoichiometry was calculated based on an assumption that each molecule of ligand will bind to two metal atoms on the nanoparticle's surface. The added amount of capping ligand was based on the known nanoparticles' concentration and their size. In order to calculate the number of metal atoms on each nanoparticle surface, the following formula was used: $F = 4/n^{1/3}$ where F = fraction of atoms, n = total number of atoms per particle. The total number of atoms per particle was, in turn, calculated from the particle diameter, its volume, its mass (density = 4.87 g/cm³), number of mols and formula units per particle.³²

Methanol solution of the capping ligand was added to diethylene glycol γ -Fe₂O₃ colloid under intensive stirring. The resulting homogeneous solution was left at room temperature for 24 hours and examined by the Dynamic Light Scattering (DLS). The hydrodynamic particle size (7.5 nm) remained unchanged, as compared to the original colloid before the capping ligand was added. In order to further characterize the reaction product, we isolated it in a pure form. The colloid was coagulated by addition of equal volume of ethyl acetate, the precipitate was separated from the solution using strong permanent magnet, washed with isopropanol until the drop evaporation test was negative and dried in vacuum. Synthesis details are given in the supplementary data file, p. 7. The IR spectrum of the powdery sample showed features of the coordinated capping ligand (supplementary data, p. 88).

The obtained organic/inorganic adduct tested negative for water solubility or otherwise reactivity at room temperature. This is a typical behavior for the nanoscale powders after they were freed from the surfactant and solvent. In order to further confirm the identity of this product, we performed its high-temperature hydrolysis, followed by separation of the organic and inorganic component on an NMR sample scale. A sample of the adduct (100 mg) was heated with 1 mL of D₂O at 100°C for ~30 min, and then precipitated iron oxide was separated by magnet. The remaining colorless solution was filtered through a 100 nm microfilter. ¹H NMR spectrum of this solution showed presence of the free ligand, which evidenced the hydrolysis and de-ligation reaction of the γ -Fe₂O₃-ligand adduct.

CONCLUSIONS

In a search for novel non-polymeric capping ligands for metal oxide nanoparticles, three 5-substituted derivatives of isophthalic acid with ethylene oxide chain substituents were synthesized by reacting dimethyl 5-hydroxyisophthalate (HIP) with allyl glycidyl ether (AGE) in the presence of a base catalyst. The main step, an anionic epoxide ring-opening reaction, was performed using a solvent-free reaction technique, and products with 1, 2 and 3 molecules of AGE attached to HIP via its formerly phenolic oxygen, were isolated and characterized. HIP-AGE 1-1 adduct can be regarded as a product of the initiation, and the 1-2 and 1-3 adducts as the first two products of the chain propagation steps of an anionic ring-opening polymerization.

An allyl group of the 1-1 adduct was converted into a 1,2-diol by aqueous oxidation, and resulting product (5-diglyceroxy isophthalic acid) can be regarded as the first homolog of polyglycerol-substituted arene with an unusual linear chain structure. This substance was tested as a capping ligand and found to bind to the surface of 5 nm γ -Fe₂O₃ nanoparticles. The ligand-capped nanoparticles form stable colloid in diethylene glycol. The organic/inorganic adduct isolated in a pure powdery form, appears to be stable to hydrolysis at ambient temperatures, but undergoes de-ligation in boiling water. Colloidal chemistry experiments on the longer-chain isophthalate and other benzenecarboxylate capping ligands are in progress and will be reported elsewhere.

The reported method can be extended to the synthesis of O-substituted derivatives of other hydroxyacids of potential applications in biology and medicine. This method is facile and optimized for minimizing waste, and it is therefore consistent with principles of green chemistry.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Scheme 1.

Scheme 2.