Synthesis of Crown Ether-capped 3-(2-O-β-Cyclodextrin)-2-hydroxypropylsilyl Silica Particles for Use as Chiral Stationary Phases in Chromatography

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A series of crown ether-capped 3-(2-O-β-cyclodextrin)-2-hydroxypropylsilyl silica particles have been prepared for use as novel bonded chiral stationary phases (CSP) for liquid chromatography. These materials were prepared using a successive multiple-step liquid-solid phase reaction on the silica gel surface. First, β-cyclodextrin (β-CD) was anchored at its C(2) position (on the larger rim of the β-CD torus) onto the silica surface. This material was then derivatized by treatment with bromoacetyl bromide. The bromoacetate-containing CD-silica was finally treated with four amine-containing crown ethers in acetonitrile in the presence of potassium carbonate. These bonded phases were characterized by means of elemental analysis and Fourier-transform infrared spectroscopy. Since these CSPs have a chiral selector with two recognition sites, *i.e.*, crown ether and substituted β-cyclodextrin, they provide excellent selectivity for the separation of enantiomers and positional isomers. These CSPs exhibit excellent selectivities for a wide variety of chiral separations in ultrahigh pressure capillary liquid chromatography (UHPLC) and capillary electrochromatography (CEC) due to the cooperative functioning of the crown ether and β-CD.

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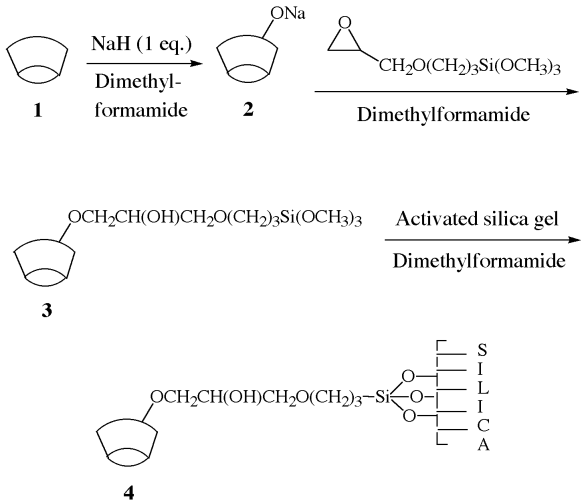
Introduction.

Synthesis and application of new chiral stationary phases with high selectivities are important for the pharmaceutical industry. A large number of the most frequently prescribed drugs contain one or more chiral centers and may exist in two or more enantiomeric forms [1]. In most instances, only one of the enantiomeric forms is therapeutically active, while the other enantiomer is either much less active, inactive, or sometimes even toxic [2]. Therefore, it is important to isolate the enantiomers and to examine each one separately. Many enantiomeric separations for drugs have been accomplished using β-cyclodextrin-type bonded silica particles as stationary phases for liquid chromatography [3].

β-Cyclodextrin (β-CD) has 21 hydroxyl groups available for reaction to anchor it onto silica particles. The seven primary C(6) hydroxyls are on the narrow rim of the CD torus while the 14 secondary C(2) and C(3) hydroxyl groups are on the wide rim of the CD torus. Since the primary hydroxyl groups are more nucleophilic, more basic and less sterically hindered than the secondary hydroxyl groups [4], the former exhibit greater reactivity than the latter [5]. Hence, in β-cyclodextrin-type bonded silica particles, almost all of the β-CDs have been connected with a spacer arm at the primary C(6) hydroxyl position (on the narrow rim of the β-CD torus), or randomly connected at primary or secondary hydroxyl groups [3,6]. We have established a method to synthesize bonded β-CD silica with the β-CD anchored at the C(2) hydroxyl position (on the wide rim of the β-CD torus) [7], leaving the more reactive C(6) hydroxyl groups for further reactions (*e.g.*, reactions with crown ethers).

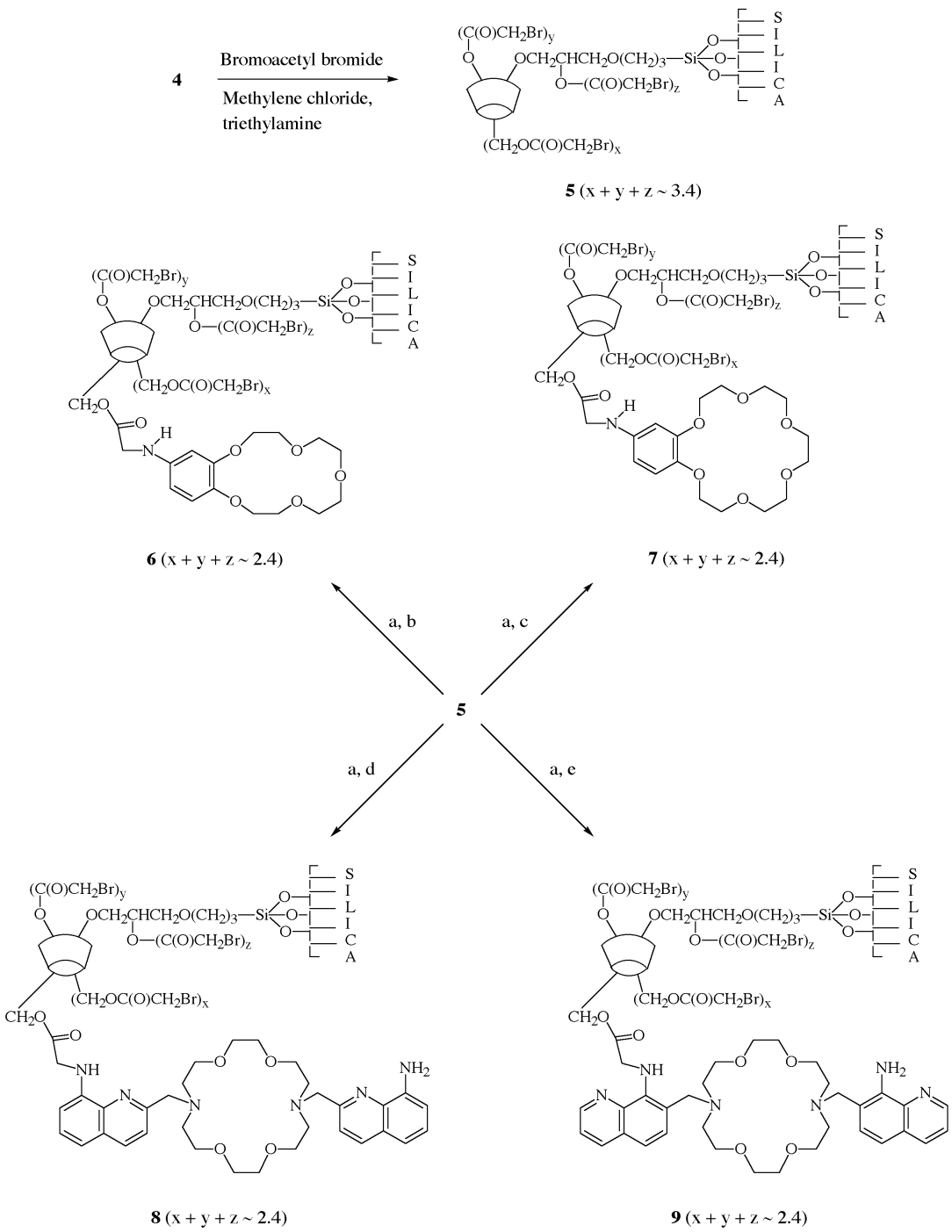
Park and co-workers [8] reported that one drawback in utilizing cyclodextrins was the low binding constants for most guest molecules. Willner and Goren [9] and Park and co-workers [8] reported that diaza-18-crown-6-capped β-CD exhibited high binding constants for several guest molecules due to cooperative functioning of the β-CD and the crown ether. Recently, it was shown that the combination of a crown ether and β-CD as a CE additive sometimes produced better enantioseparations than did either selector alone [10,11]. We have demonstrated

Scheme 1



Synthesis of 3-(2-O-β-cyclodextrin)-2-hydroxypropylsilyl silica particles.

Scheme 2



Syntheses of four crown ether-capped (2-O-β-cyclodextrin) silica materials. a) acetonitrile, potassium carbonate; b) 4'-aminobenzo-15-crown-5; c) 4'-aminobenzo-18-crown-6; d) bis(8-aminoquinolin-2-ylmethyl)diaza-18-crown-6; e) bis(8-aminoquino-lin-7-ylmethyl)diaza-18-crown-6.

excellent enantiomeric selectivities of two new types of crown ether-capped β-CD bonded stationary phases [12,13].

In this paper, we report the synthesis of 4'-aminobenzo-15-crown-5-capped (6) and 4′-aminobenzo-18-crown-6-capped (7) 3-(2-O-β-cyclodextrin)-2-

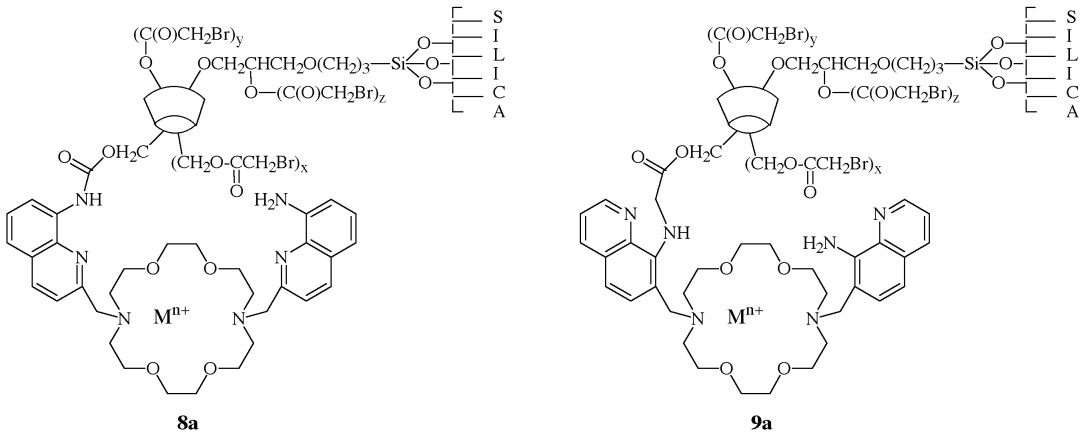


Figure 1. Possible structures of the two bis(8-aminoquinoline)-substituted diaza-18-crown-6-capped β-cyclodextrin-appended gilica gels after inclusion of metal ions.

hydroxypropylsilyl silica particles, and 8-aminoquinoline-containing diaza-18-crown-6-capped 3-(2-O-β-cyclodextrin)-2-hydroxypropylsilyl silica particles (8 and 9). The excellent selectivities of these bonded phases were demonstrated by using them as chiral stationary phases for capillary electrochromatography (CEC) and ultrahigh pressure capillary liquid chromatography (UHPLC).

Results and Discussion.

Selective modification of the primary and secondary hydroxyl groups of β-CD is complicated and difficult to control because of statistical and steric problems. D'Souza and co-workers [14-15] reported a convenient method for the monofunctionalization of cyclodextrins at the C(2) position involving deprotonation of cyclodextrin by

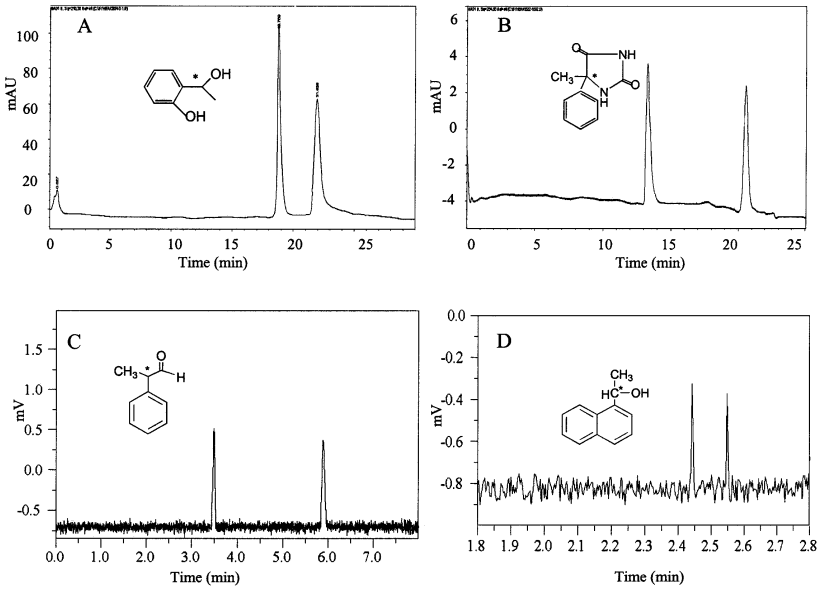


Figure 2. (A) CEC separation of enantiomers of 1-(2-hydroxyphenyl)ethanol using compound **6** as stationary phase. Conditions: 30 cm x 75 µm.d. fused silica column packed with **6** (3 µm, porous); 10 mM Tris buffer (pH = 8.8)/acetonitrile (30:70 v/v), 15 V, 254 nm UV detection. (B) CEC separation of enantiomers of 4-methyl-4-phenylhydantoin using compound **7** as stationary phase. Conditions: 30 cm x 75 µm i.d. fused silica column packed with **7** (3 µm, porous); 10 mM Tris buffer (pH = 8.8)/acetonitrile (40:60 v/v), 20 kV, 210 nm UV detection. (C) UHPLC separation of enantiomers of 2-phenylpropionaldehyde using compound **8** as stationary phase. Conditions: 23 cm x 75 µm i.d. fused silica column packed with **8** (1.5 µm, nonporous); 5 mM phosphate buffer (pH = 7.5)/acetonitrile (80:20 v/v), 8,000 psi column inlet pressure, 215 nm UV detection. (D) UHPLC separation of enantiomers of 1-(1-naphthyl)ethanol using compound **9** as stationary phase. Conditions: 23 cm x 75 µm i.d. fused silica column packed with **9** (1.5 µm, nonporous); 5 mM phosphate buffer (pH = 7.5)/acetonitrile (90:10 v/v), 10,000 psi column inlet pressure, 215 nm UV detection.

sodium hydride followed by nucleophilic attack of the resultant cyclodextrin oxyanion on the desired electrophile reagent. We used this method to anchor the β-CD onto silica particles at the C(2) position using 3-glycidoxypropyltrimethoxysilane to react with the cyclodextrin oxyanion (Scheme 1). Since the C(2) hydroxyl groups of β-CD are the most acidic, and the C(3) hydroxyl groups are more acidic than the C(6) hydroxyl groups [4], the first reaction shown in Scheme 1 for the deprotonation of the hydroxyl group with 1 equivalent of sodium hydride takes place at the β-CD C(2) position [14]. Compound 2, β-CD with the monooxyanion in the C(2) position, was treated with 3-glycidoxypropyltrimethoxysilane to form trimethoxylsilane-containing CD 3. The latter compound was fused onto silica by heating a mixture of 3 and silica in dimethylformamide.

The syntheses of the four crown ether-capped 2-O-β-cyclodextrin silica materials (6-9) are shown in Scheme 2. Glycidoxypropyltrimethoxysilane-containing CD 4 was first treated with bromoacetyl bromide to form CD 5. Due to the fact that the C(6) hydroxyl groups are more reactive than those at C(2) or C(3), we believe that the majority of the bromoacetate units are attached at the C(6) position. Thus, CD5 has β-CD containing a few 6-O-bromoacetate groups and is attached to silica through its 2-O position as shown in Scheme 2. CD 5 was then treated with the appropriate amine-containing crown ethers to form the crown ether-capped CD silica materials [6-9].

Fourier transform infrared spectroscopy (ftir) analysis of the bonded silica particles, after subtraction of the bare silica spectrum, showed a weak broad absorption band at 3400-3600 cm^{-1} that is characteristic of the silanol stretching frequency for bare silica. All of the bonded silica particles (4-9) exhibit peaks at 2850 and 2925 cm^{-1} for the C-H aliphatic CH_2 stretching frequencies and 1150-1170 cm^{-1} for the asymmetric stretches of the ether linkages. In addition, crown ether-capped silica particles 6-9 exhibited bands at 3401 and 3308 cm^{-1} for the N-H stretching frequencies, 1738 cm^{-1} for the C=O stretching frequencies, 1549 and 1505 cm^{-1} for the aromatic C=C stretching frequencies, and 1250 and 950-500 cm^{-1} for the characteristic stretching frequencies of the macrocyclic polyether.

The four crown ether-capped 3-(2-O-β-cyclodextrin)-3-hydroxypropylsilyl silica particles exhibited excellent enantioselectivities when used as chiral stationary phases for CEC or UHPLC applications. After inclusion of a metal ion (*e.g.*, Ni^{2+}, K^+, etc.) from the mobile phase into the diaza-18-crown-6 unit, 8 and 9 became positively charged and the two 8-aminoquinoline side arms of the crown ether moved to the same side of the crown ring (shown in Figure 1), providing two ligand sites for interaction with a solute. Thus, the positively-charged crown ether-capped β-cyclodextrin can participate in various static, dipolar, and host-guest complexation interactions with solutes. This improves chiral recognition and selectivity. Figure 2 shows chromatograms of chiral separations using the four kinds of crown ether-capped 3-(2-O-β-cyclodextrin)-2-hydroxypropylsilyl silica materials as stationary phases for CEC and UHPLC.

In summary, crown ether-capped 3-(2-O-β-cyclodextrin)-2-hydroxypropylsilyl silica particles were synthesized using a convenient successive multiple-step liquid-solid phase reaction on the silica surface. These bonded silica materials have excellent selectivites for chiral separations due to the cooperative functioning of the crown ether and β-CD when used as chiral stationary phases for CEC and UHPLC.

EXPERIMENTAL

A Model FTS165 Bio-Rad (Hercules, CA, USA) ftir spectrometer was used for this study. Elemental analyses were performed with a Perkin Elmer (Norwalk, CT, USA) 2400 series elemental analyzer. CEC was performed using an HP^{3D} CE instrument (Hewlett-Packard, Waldbronn, Germany) equipped with an uv diode-array detector. Capillary liquid chromatography was performed using a home-built UHPLC system [16]. All solvents and starting materials were purchased from commercial sources and purified by standard procedures. 8-Aminoquinoline-2-ylmethyl-substituted diaza-18-crown-6-substituted and 8-aminoquinoline-7-ylmethyl-substituted diaza-18-crown-6 were prepared as reported [17].

Preparation of 3-(2-O-β-Cyclodextrin)-2-hydroxypropylsilyl Silica Particles (**4**) (Scheme 1).

The procedure for preparation of 3-(2-O-β-cyclodextrin)-2-hydroxypropylsilyl silica particles (**4**) has been described in detail [7]. Briefly, 1.14 g (1 mmole) of **1** (β-CD) was dried under vacuum at 120° overnight. After cooling, **1** was dissolved in 30 ml of anhydrous DMF. To the solution, 40 mg of 60% sodium hydride (1 mmole) was added. The mixture was stirred under dry nitrogen at room temperature until the solution became clear. Then, 0.17 ml of 97% 3-glycidoxypropyltrimethoxysilane (0.75 mmole) was added to the solution. This reaction mixture was allowed to react for 6 hours under dry nitrogen at 70°. Then, 1 g of activated spherical silica (porous, 3 μm), that had been dried in vacuum at 120° for 12 hours, was added and the mixture was stirred at 110° for 24 hours. Product **4** was filtered, washed successively with dimethylformamide, water and methanol, purified by Soxhlet extraction with acetone and dried overnight under vacuum at 80°. After cooling, the bonded silica weighed 1.29 g (weight increase of 29%). Elemental analysis for C, 8.77, and H, 2.12, gave a concentration of β-CD anchored onto compound **4** of 181 μmole g^{-1}.

Preparation of Bromoacetate-substituted 2-O-β-Cyclodextrin-2-hydroxypropylsilyl Silica Particles (**5**) (Scheme 2).

Typically, 0.5 g of **4** (dried overnight under vacuum at 90°) was added to 20 ml of anhydrous dichloromethane. One drop of anhydrous triethylamine was added to the mixture, which was then stirred under dry nitrogen in an ice bath while bromoacetyl bromide (56.4 μl) in 10 ml of anhydrous

dichloromethane was dropped into it in an ice bath for over a period of 2 hours. This reaction mixture was then allowed to react for 24 hours under dry nitrogen at room temperature. Product **5** was filtered, washed successively with dichloromethane, acetone, water and methanol, purified by Soxhlet extraction with acetone, and dried overnight under vacuum at 50°. Elemental analysis for C, 10.15, H, 2.09, and Br, 4.81, gave a concentration of bromoacetate groups in compound **5** of 618 μmole g^{-1}. The degree of substitution of bromoacetate was calculated to be 3.4 (x + y + z =3.4 in Scheme 2).

Preparation of Benzo-15-crown-5-capped 3-(2-O-β-Cyclodextrin)-2-hydroxypropylsilyl Silica Particles (**6**) (Scheme 2).

Compound **5** (0.25 g) was added to 20 ml of acetonitrile containing 25 mg of 4'-aminobenzo-15-crown-5 and 6 mg of potassium carbonate. The reaction mixture was refluxed for 24 hours under dry nitrogen. Product **6** was filtered, washed successively with acetonitrile, water, acetone and methanol, purified by Soxhlet extraction with acetone overnight, and dried for 6 hours under vacuum at 50°. Elemental analysis for C, 12.80, H, 1.99, and N, 0.31, gave concentrations of the benzo-15-crown-5 in compound **6** as 165 μmole g^{-1} (carbon content) and 221 μmole g^{-1} (nitrogen content). The average degree of substitution of the benzo-15-crown-5 was calculated to be 1.1 (approximately one β-CD molecule was capped by one crown ether).

Preparation of Benzo-18-crown-6-capped 3-(2-O-β-Cyclodextrin)-2-hydroxypropylsilyl Silica Particles (**7**) (Scheme 2).

Compound **7** was prepared as for **6** above by treating **5** with 4′-aminobenzo-18-crown-6. Elemental analysis for C, 13.21, H, 2.03, N, 0.29, and gave the concentration of benzo-18-crown-6 in compound **7** to be 168 μmole g^{-1} (carbon content) or 207 μmole g^{-1} (nitrogen content). The average degree of substitution of benzo-18-crown-6 was calculated to be 1.0.

Preparation of 8-Aminoquinoline Diaza-18-crown-6-capped 3-(2-O-β-Cyclodextrin)-2-hydroxypropylsilyl Silica Particles (**8** and **9**) (Scheme 2).

Compounds **8** and **9** were prepared as above using nonporous silica (1.5 μm) as starting materials. Since the concentration of silanol groups on the nonporous silica particle surface was less than that for porous silica particles, the concentration of bonded functional groups was, therefore, lower for the nonporous bonded silica. Elemental analysis of **8** showed that the concentration of 8-aminoquinoline-2-ylmethyl diaza-18-crown-6 was 57 μmole g^{-1} (carbon content) or 26 μmole g^{-1} (nitrogen content). The average degree of substitution of 8-aminoquinoline-2-ylmethyl diaza-18-crown-6 was calculated to be 1.39. Elemental analysis of **9** showed that the concentration of 8-aminoquinoline-7-ylmethyl-substituted diaza-18-crown-6 was 29 μmole g^{-1} (carbon content) and 25 μmole g^{-1} (nitrogen content). The average degree of substitution of 8-aminoquinoline-7-ylmethyl-substituted diaza-18-crown-6 was calculated to be 0.92.

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