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A Novel Sustainable Method to Prepare Glutaric Acid from Glucose

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Abstract

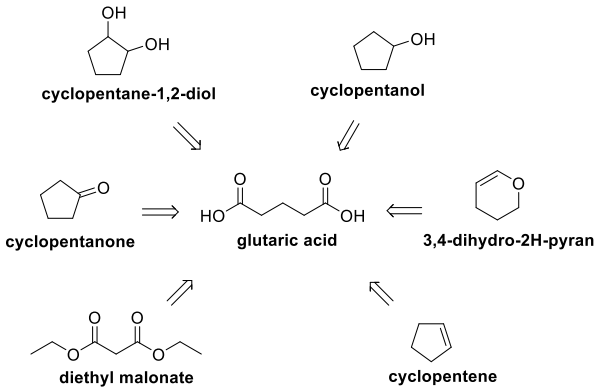
This paper proposes a sustainable method to prepare glutaric acid from glucose via an α-keto glutaric acid derived from glucose by fermentation using Corynebacterium glutamicum GKGA. Glutaric acid was prepared from α-keto glutaric acid via the hydrogenation of a 1,3-dithiolane diamide intermediate as the key step. The combination of biotransformation and chemical transformation of glutaric acid provided an efficient and environment-friendly method for the sustainable synthesis of glutaric acid

Keywords

Glutamic acid;α-Keto glutaric acid; Glucose; Fermertation; Chemical transformation

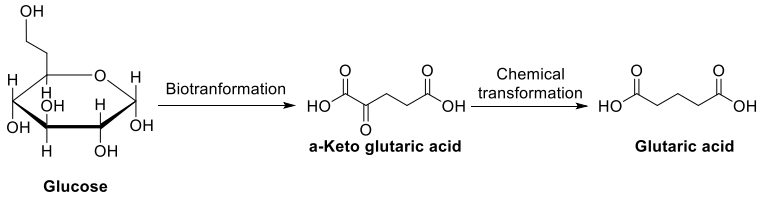
Introduction

Glutaric acid and its derivatives are important bulk chemicals in the industry as they serve as feedstock for polyamides, polyesters, plasticizers and lubricating oils, bactericides, pesticides and liquid crystal materials.[1, 2] Glutaric acid is traditionally obtained as a byproduct in the manufacture of adipic acid; alternatively, it is prepared from cyclopentane-1,2-diol,[3] cyclopentanol,[4] cyclopentanone,[5] 3,4-dihydro-2H-pyran,[6] and diethyl malonate.[7] The former method was limited by the improvement progress to synthesize adipic acid while the latter method is preferable because it neither involves multiple steps nor requires strong oxidation and acidic conditions. In the past thirty years, significant progress has been toward the development of green processes to synthesize glutaric acid via tungsten-catalyzed oxidation of cyclopentene using hydrogen peroxide as the oxidant (Scheme 1).[8-9] However, most of the starting materials for the abovementioned methods are derived from the petrochemical industry, which is undesirable given the increasing concerns on environmental issues and depletion of fossil fuel resources. Hence, there has been much interest in the production of the aforesaid chemicals via the biotransformation of renewable biomass.[10-13] However the productivity for the biosynthesis of Glutaric acid was very low which limited it practical application. Therefore, the development of efficient and sustainable methods to prepare glutaric acid is a pressing need.



Scheme 1: Traditional starting material to synthesize glutaric acid

α-Ketoglutaric acid, an important glutaric acid derivative, is widely used in the pharmaceutical, foodstuff, feedstuff, and fine chemical industries,14-15 and it is manufactured from glucose by fermentation.16 However, to the best of our knowledge, there is no report on the preparation of glutaric acid from α-ketoglutaric acid. In this paper, we report a sustainable method to prepare glutaric acid from glucose via α-ketoglutaric acid (Scheme 2)

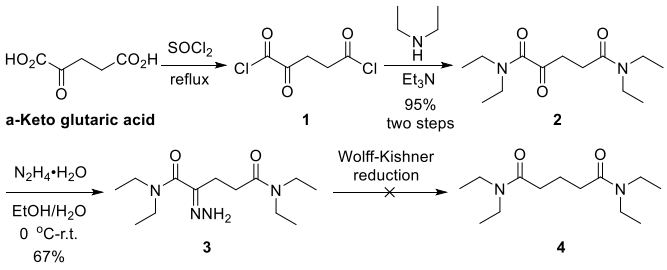


Scheme 2 Preparation of gluttaric acid from glucose

Results and Discussion

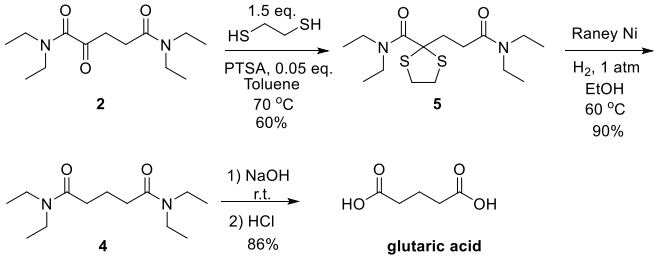
First, α-ketoglutaric acid was prepared from glucose by fermentation. An α-ketoglutaric acid-producing strain, Corynebacterium glutamicum GKGA (△gdh1△gdh2△glt), was constructed from the glutamic acid producer C. glutamicum GKG-047. With the use of a double-phase pH and biotin control strategy12, the titer of α-ketoglutaric acid reached 68.3 g/L. The productivity and yield were 1.54 g/L/h and 0.48 g/g, respectively, in a 30-L fermenter.

Next, we attempted to reduce α-ketoglutaric acid to glutaric by Wolff-Kishner reduction as the key step. Accordingly, α-ketoglutaric acid was treated with sulfurous dichloride to give di-acyl chloride 1, which was transformed into diamide 2 by condensation with diethylamine. Compound 2 was allowed to react with hydrazine hydrate to afford hydrazone 3. With compound 3 in hand, we screened various conditions for the Wolff-Kishner reduction, but the desired product 4 was not obtained because the starting material decomposed under these harsh conditions (Scheme 3).



Scheme 3 Pα-Keto glutaric acid to glutaric by using Wolff-Kishner reduction as key step

An alternative approach was then investigated, i.e., by reducing 2 to 4 through 1,3-dithiolane intermediate 5. First, 2 was treated with ethane-1,2-dithiol in the presence of 4-methylbenzenesulfonic acid (PTSA) as the catalyst in toluene at 70 °C To our delight, the key intermediate 5 was obtained in 60% yield, and it was transformed into 4 by hydrogenation using Raney nickel as the catalyst. Hydrolysis of 4 under basic conditions afforded the target compound in 86% yield (Scheme 4).

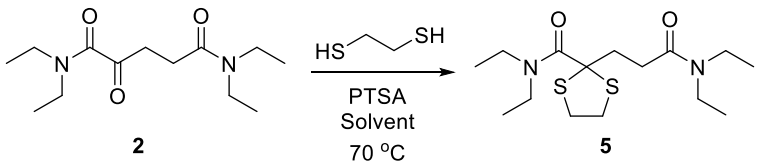


Scheme 4 Transformation of 2 to glutaric acid by using dithiolane as key intermediate

Next, we attempted to optimize ketalization of 2 by using other solvents such as tetrahydrofuran (THF), 1,4-dioxane, hexane, acetonitrile (MeCN), 1,2-dichloroethane (DCE), 1,2-dimethoxyethane (DME) and N,N-dimethylformamide (DMF), (Table 1, Entries 2-8). Among these solvents, MeCN gave the best result (Table 1, Entry 3). Then, the effect of the amounts (equivalents) of ethane-1,2-dithiol and PTSA on the reaction

yield was examined (Entries 9-12). Decreasing the amount of ethane-1,2-dithiol to 1.2 equiv diminished the product yield, and increasing the ethane-1,2-dithiol amount to 1.8 equiv had no notable influence on the yield (Entries 9 and 10). Decreasing the catalyst loading to 0.025 equiv or increasing the loading to 0.1 equiv diminished the yield (Entries 11 and 12). Finally, the effect of the reaction temperature and concentration of 2 was tested. Decreasing the reaction temperature to 60 °C led to a decrease in the yield, and no notable change in the yield was seen when increasing the temperature to 80 °C (Entries 13 and 14). Increasing the concentration of 2 from 0.08 M to 0.4 M diminished the yield (Entry 15). Based on the abovementioned observations, the optimal reaction conditions for the formation of 5 were identified as follows: 2 (0.4 mmol), ethane-1,2-dithiol (0.6 mmol), PTSA (0.02 mmol) in MeCN (5 mL) at 70 °C

Table 1:Optimization of ketalization of 2 by ethane-1,2-dithiol in the presence of PTSA [a]



2 **5**

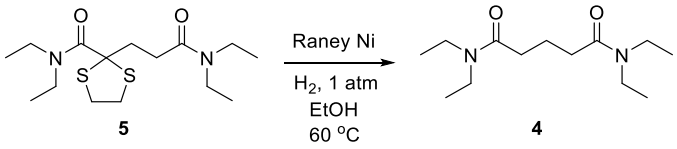
Entry	Solvent	Ethane-1,2-dithiol (eq.)	PTSA(eq.)	Temperature (°C)	Yield (%)
1	Toluene	1.5	0.05	70	60
2	THF	1.5	0.05	70	70
3	1,4-dioxane	1.5	0.05	70	72
4	Hexane	1.5	0.05	70	33
5	MeCN	1.5	0.05	70	77
6	DCE	1.5	0.05	70	58
7	DME	1.5	0.05	70	70
8	DMF	1.5	0.05	70	46
9	MeCN	1.2	0.05	70	70
10	MeCN	1.8	0.05	70	76
11	MeCN	1.5	0.025	70	70
12	MeCN	1.5	0.1	70	75
13	MeCN	1.5	0.05	60	46
14	MeCN	1.5	0.05	80	78
15	MeCN	1.5	0.05	70	70b

[a]Reaction conditions: 2 (0.4 mmol), ethane-1,2-dithiol (0.6 mmol), PTSA (0.02 mmol), solvent (5 mL) at the indicated temperature for 4 h. b Solvent (1 mL) was used.

To make this process more environment-friendly, Raney nickel was recovered from the reaction system and the catalytic ability of the recycled catalyst was tested. The results showed that the catalyst obtained after two rounds of recycling gave the

desired product in over 80% yield. Ethane-1,2-dithiol could also be recycled during the process. When 5.0 g of 2 and 2.75 g of ethane-1,2-dithiol were used as starting materials, 5.02 g of 5 was obtained after ketalization, and 0.78 g of ethane-1,2-dithiol was recovered. When 5.02 g of 5 was reduced to 4, ethane-1,2-dithiol was regenerated and 1.55 g could be recovered, indicating a total recovery rate of 85% after two steps.

Table 2: Reduction of 5 to 4 using recycled Raney nickel catalyst [a]



5 4

Entry	Number of recycles	Catalyst (g)	Recovery rate of the catalyst (%)	Yield (%)
1	0	0.110	-	90
2	1	0.096	95	86
3	2	0.092	84	81

Conclusion

In summary, we developed a sustainable method for glutaric acid synthesis from glucose via α-ketoglutaric acid, which in turn was obtained from the fermentation of glucose by *C. glutamicum* GKGA (titer: 68.3 g/L). Glutaric acid was prepared from α-ketoglutaric acid via Raney nickel hydrogenation of a 1,3-dithiolane diamide intermediate as the key step. During the process, the Raney nickel and 1,2-dithiol could be recovered and recycled. The combination of biotransformation and chemical transformation of glutaric acid thus provided an efficient and environment-friendly method to synthesize glutaric acid in a sustainable manner.

Experimental

1 General methods and material

All solvents were distilled prior to use. For chromatography, 200−300 mesh silica gel was employed. 1H NMR and 13C NMR spectra were recorded at 400 MHz, 100 MHz and 376 MHz respectively. Chemical shifts are reported in ppm using tetramethylsilane as internal standard. HRMS was performed on an FTMS mass instrument.

2 Fermentation section

Corynebacterium glutamicum GKG-047, deposited in China General Microbiological Culture Collection Center (CGMCC No. 5481), was used as the parent strain. The genes were deleted from the chromosome of GKG-047 using the suicide plasmid pK18mobsacB-mediated method.12 The fermentation was performed in a 30-L fermenter (Shanghai BaoXing Bio-Engineering Equipment Co., Ltd.). The fermentation medium was constituted with the following components (per liter): 40 g glucose, 3 g monosodium glutamate, 3 g Na2HPO4, 2 g MgSO4•7H2O, 1.5 g KCl, 10 mg MnSO4•5H2O, 10 mg FeSO4•7H2O, 10 mg ZnSO4•7H2O, 1 mg vitamin B1/B3/B5/B12, 4 μg biotin, 30 mL soybean protein hydrolysate. The pH was controlled around 7.0 with sterile NaOH solution (6.25 mol/L) or 25% NH4OH solution (v/v). During the mid-period of fermentation (16–24 h), a final concentration of 5 μg/L biotin was added sequentially. The temperature was set at 34°C and the dissolved oxygen (DO) was controlled at least 35% in favor of rapid cell growth. After fermetation, the fermentation broth was centrifugated and the supernatant was subjected to micro- and nano- filtration. The liquid was vacuum concentrated and then washed by ethanol to remove the inpurities such as proteins, inorganic salts and glutamic acid. The resulting liquid was sebsequently vacuum concentrated and crystalized. Finally, the crude

product was resolved and recrystalized from ethonol to obtain the pure α-keto glutaric acid (> 98% purity).

3 Chemical transformation section

N1,N1,N5,N5-tetraethyl-2-oxopentanediamide (2) To a round bottom flask was added α-ketoglutaric acid (3.00 g, 20.5 mmol) and thionyl chloride (8.21 g, 69 mmol) and the mixture was heated to reflux for 1 h. After cooled to room temperature, the solvent was removed in vacuo to give a residue which was dissolved in anhydrous tetrahydrofuran (6.00 ml). The mixture was cooled to -10°C, diethylamine (3.74 g, 51.3 mmol) was added dropwised. The mixture was warmed to room temperature and stirred for 1h. Then the reaction mixture was filtered and the filtrate was poured into ice-water and extracted with EtOAc (3×50 mL). The combined organic phase was washed with water (3×50 mL) and saturated brine (50 mL), dried over anhydrous Na2SO4. Solvent was removed in vacuo to give compound 2 (5.0 g, 95%) as a brown oil. 1H NMR (400 MHz, CDCl3) δ 3.39-3.30 (m, 8H), 3.01 (s, 2H), 2.66 (d, J = 11.2Hz, 2H), 1.19-1.06 (m, 12H);13C NMR (100 MHz, CDCl3) δ 200.3, 170.2, 166.9, 42.1, 42.0, 40.4, 39.2, 34.8, 26.6, 14.2, 14.1, 13.0, 12.6.

N1,N1,N5,N5-tetraethyl-2-hydrazonopentanediamide (3) To a round bottom flask was added 2 (1.02 g, 4.0 mmol), Hydrazine hydrochloride (548 mg, 8.0 mmol) and ethanol (5 mL). The mixture was cooled to 0°C and hydrazine hydrate (400 mg, 8.0 mmol) in water (5 mL) was added. The mixture was stirred overnight and extracted EtOAc (3×50 mL). The combined organic phase was washed with saturated brine (50 mL), dried over anhydrous Na2SO4. Solvent was removed in vacuo to give compound 3 (724 mg, 67%) as a brown oil. 1H NMR (400 MHz, CDCl3) δ 6.23 (s, 1H), 5.25 (s, 2H), 3.31-3.47 (m, 12H), 2.76-2.79 (m, 1H), 2.67-2.72 (m, 3H), 2.59-2.63 (m, 2H), 1.09-1.24 (m, 18H);13C NMR (100 MHz, CDCl3) δ 171.6, 170.8, 167.8, 166.3, 148.0, 145.1, 42.1, 41.8, 40.6, 40.2, 38.0, 29.9, 29.1, 28.8, 21.4, 14.3, 14.2, 14.1, 13.0, 12.8; HRMS (ESI) m/z calcd for C13H27N4O2 (M+H)+ 271.2129, found 271.2116.

2-(3-(diethylamino)-3-oxopropyl)-N,N-diethyl-1,3-dithiolane-2-carboxamide (5) To a round bottom flask was added compound 2 (5.00g, 19.50 mmol), 1, 2-Ethanedithiol (2.45 mL, 29.26 mmol), p-Toluenesulfonic acid (0.16 g, 0.90 mmol) and acetonitrile (30mL). The mixture was reacted at 70°C for 4h. Then the reaction mixture was cooled to room temperature. Solvent was removed under vacuo and the residue was diluted with EtOAc (60 mL). The organic phase was washed with water (30 mL) and saturated brine (30 mL), dried over anhydrous Na2SO4 and concentrated to give compound 5 (5.02g, 77%) as palely brown oil. 1H NMR (400 MHz, CDCl3) δ 3.42-3.34 (m, 8H), 3.06 (t, J = 6.0 Hz, 2H), 2.89 (t, J = 6.0 Hz, 2H), 2.86(t, J = 4.0 Hz, 2H), 2.69 (t, J = 4.0 Hz, 2H), 1.24-1.07 (m, 12H). 13C NMR (100 MHz, CDCl3) δ 169.2, 165.9, 41.0, 40.96, 39.4, 38.2, 33.8, 25.6, 13.2, 13.1, 12.1, 11.6; HRMS (ESI) m/z calcd for C15H28N2O2S2Na (M+Na)+ 355.1484, found 355.1495.

N1,N1,N5,N5-tetraethylglutaramide (4) To a round bottom flask was added a suspension Raney Nickel (0.5 g, 8.51 mmol) in ethanol (20 mL) and compound 5 (5.02 g, 15.11 mmol), The mixture was degased and refilled with hydrogen for three time. Then the mixture was heated to 60°C for 8 h under hydrogen (balloon pressure). After cooling to room temperature, the mixture was filtered and the filtrate was concentrated in vacuum to give compound 4 (3.30 g, 90%) as yellow oil. 1H NMR (400 MHz, CDCl3) δ 3.27-3.20 (m, 8H), 2.29 (t, J = 6.0 Hz, 4H), 1.90-1.83 (m, 2H), 1.08-0.98 (m, 12H); 13C NMR (100 MHz, CDCl3) δ 171.8, 41.9, 39.9, 32.2, 21.1, 14.2, 13.0.

Glutaric acid To a round bottom flask was added 4 (3.30 g, 13.6 mmol) and 3M KOH (aq) (20 mL). The reaction mixture was heated to reflux for 12 h. After cooling to room temperature, concentrated hydrochloric acid was added to the reaction mixture to adjust pH to 3. The reaction mixture was extracted with EtOAc (3×50 mL). The aqueous layer was concentrated to give the crude product. The crude product 4 was dissolved in water (15 mL) and magnesium oxide (10 g) was added. The mixture was filtered, the filter cake was washed with concentrated sulfuric acid (5 mL) and the upper layer was evaporated to give compound 4 (1.54 g, 86%) as a white solid. 1H NMR (400 MHz, CDCl3) δ .12.05 (s, 2H), 2.25 (t, J =10 Hz, 4H), 1.75-1.68 (m, 2H); 13C NMR (100 MHz, CDCl3) δ 174.6, 33.2, 20.4.

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