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An Improved Process for Preparation of *S*-Acetyl-L-glutathione

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Supporting Information

ABSTRACT: An efficient one-step synthesis of *S*-acetyl-L-glutathione has been developed in a DMF−TFA mixed solvent with $CoCl_2$ as catalyst. This process not only has the advantages of selective acylation of the glutathione thiol group without involving the free amino but also highlights recycling of the relatively costly solvent TFA, which improves the yield to 91% and quality to 99.7%. The reaction is cost-effective, efficient, and easy to scale-up.

INTRODUCTION

Glutathione (γ-glutamyl-cysteinyl-glycine, GSH), a thiol group-containing tripeptide, serves as an important intracellular water-soluble antioxidant and detoxifying agent.[1] Recently, glutathione has attracted an increasing interest due to the potential therapeutic effects on cancer and AIDS.[2] However, GSH cannot be directly absorbed by cells; it needs to be broken down into amino acids and resynthesized to GSH in cells, and its half-life is very short in serum.[3] In addition, the synthesis process of GSH is often damaged by viral infections. So replenishment of intracellular GSH is still a challenging goal. These obstacles could be overcome by transforming glutathione to its derivatives.[4]

Previous studies have demonstrated that supplementation with a glutathione pro-drug, such as *N*-acetylcysteine (NAC), *S*-acetylcysteine (SAC), *N*-acetylglutathione (*N*-GSH), and *S*-acetylglutathione (*S*-GSH), is more efficient than GSH for intracellular GSH supplementation.[5] However, *N*-acetyl derivatives may have neurotoxic effects on the nerves for patients with glutathione synthetase deficiency.[6] *S*-Acetyl derivatives, such as *S*-acetyl-L-glutathione (*S*-GSH), are promising agents for GSH restoration, which is not only more stable in plasma but also taken up directly by cells and then converted into GSH by intracellular thioesterases.[7]

Quite a few methods for selective acylation of the thiol group of glutathione without involving the free amino group have been reported (Scheme 1). However, these methods have various drawbacks and are not suitable for large-scale applications. For example, the initial method involving exchange reaction of acetyl coenzyme A with glutathione (Scheme 1a) is unsuccessful in isolating product from the reaction mixture.[7] Subsequently, Wieland and Bokelmann[9] have synthesized *S*-GSH using the exchange reaction between *S*-acetylthiophenol and glutathione in 70% yield (Scheme 1b). This method needs at least 10-fold molar *S*-acetylthiophenol under enzyme catalysis. Kielley and co-workers[10] replace *S*-acetylthiophenol with cheap thioacetic acid to prepare *S*-GSH in 40% yield. All of the above-mentioned enzymatic processes are not suitable for industrial scale preparation of *S*-GSH at present, because of the dependence on enzyme and tedious post-treatment. The alternative approach for the synthesis of *S*-GSH, reported by Galzigna,[11] was found to be promising for scale-up (Scheme 1c). The selective acylation of the thiol group of glutathione without involving the free amino group and the adoption of low cost and easily available reagents attracted us to this strategy. Nevertheless, this process would result in poor quality and difficult recycling of the relatively expensive solvent trifluoroacetic acid, owing to the water added to the reaction mixture.

Herein we decided to overcome these difficulties and control the quality of **1** by modifying the method and effectively optimizing the reaction parameters.

RESULTS AND DISCUSSION

In the method reported by Galzigna, byproduct **3** was formed in the reaction (up to 0.78 area %, HPLC) and difficult to be removed during purification, which affected the stability and safety of the *S*-GSH. Therefore, controlling and minimizing the impurity are the key issues in the production of *S*-GSH.[12]

It is reported that *N*-acylglycine derivatives can be induced to undergo intramolecular dehydration in the presence of a dehydrating agent such as *N,N*-dicyclocarbodiimide (DCC) at 0 °C for 2 h or acetic anhydride refluxing 30 min.[13] We attempted to find an efficient catalyst to improve the reaction rate and selectivity and to reduce the byproduct **3**.

It is noted that Zn, ZnO,[14] $CoCl_2$,[15] and $BiCl_3$[16] are active catalysts for the synthesis of some thiol esters with acetyl chloride at room temperature. A large number of experiments were carried out to screen an effective catalyst for the acylation of glutathione with acetyl chloride, and the results are presented in Table 1.

We added acetyl chloride at 0−5 °C in 5 min and performed the reaction at room temperature (20−30 °C). As shown in Table 1, although Zn and ZnO are active catalysts for the acylation of some thiols with acetyl chloride at room temperature, $ZnCl_2$ is not effective in catalyzing acylation of GSH with acetyl chloride (Table 1, entry 2−4). The results indicate that $CoCl_2$ and $BiCl_3$ are very active for the acylation reaction and $CoCl_2$ is more effective than $BiCl_3$. The best result was obtained on treatment of GSH with acetyl chloride in the presence of 0.5% mol $CoCl_2$. The yield of *S*-GSH was 89% in 10 min (Table 1, entry 6). In this case, the impurity content was minimal (0.02%, assay by HPLC).

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Scheme 1. Reported synthetic routes of *S*-GSH

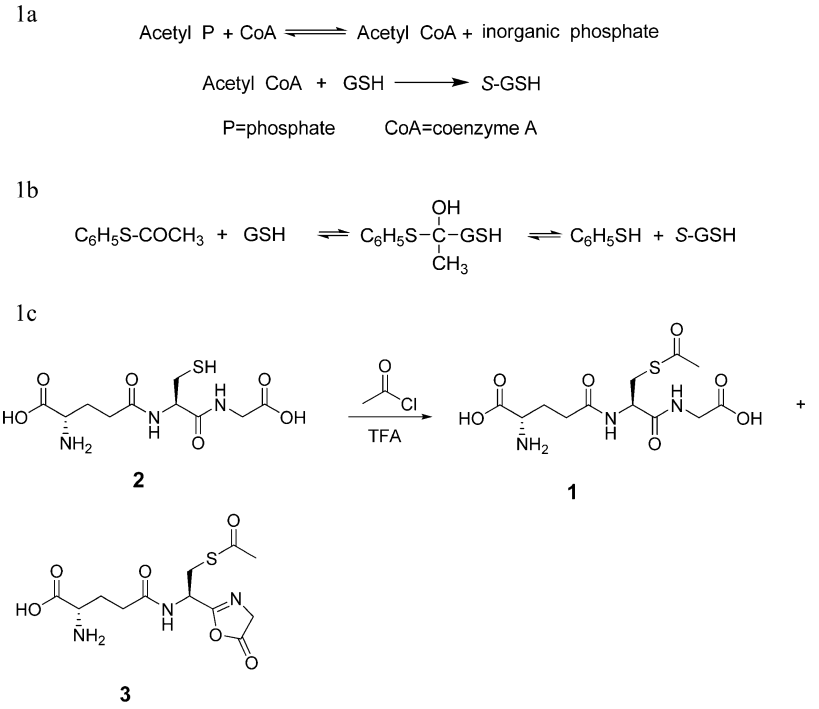

Table 1. Effect of catalyst on the acylation reaction[a]

entry	catalyst	molar (% mol)	time (min)	conversion (%)[b,c]	1[d] (%)	3[b] (area %)
1	none		40	100	80	0.78
2	$ZnCl_2$	1	30	100	81	0.47
3	$ZnCl_2$	5	20	95	76	0.42
4	$ZnCl_2$	10	20	96	77	0.49
5	$CoCl_2$	0.1	20	97	87	0.03
6	$CoCl_2$	0.5	10	100	89	0.02
7	$CoCl_2$	2	5	98	88	0.03
8	$BiCl_3$	0.1	20	100	87	0.07
9	$BiCl_3$	0.5	10	100	86	0.07
10	$BiCl_3$	2	5	95	82	0.06

[a]Reaction conditions: glutathione (10.0 g, 0.0325 mol), TFA (80 mL), acetyl chloride (5.1 g, 0.065 mol). [b]Detected by HPLC. [c]Conversion was calculated from the HPLC area. [d]Isolated yield.

The plausible reaction mechanism for the acetylation of GSH with acetyl chloride using cobalt(II) chloride as catalyst at room temperature is shown in Figure 1.[17]

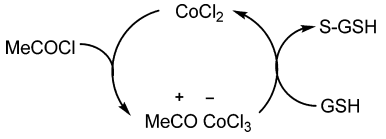

Figure 1. Plausible reaction mechanism for the acetylation reaction with $CoCl_2$ catalyst.

The next focus of our study was to optimize the molar equivalents of the acetyl chloride. We carried out this reaction by using varying amounts of acetyl chloride, and the results are summarized in Table 2. It was found that the yield of compound **1** increased with an increase in amount of acetyl chloride up to 1.1 equiv. At 1.1 equiv, compound **1** was obtained in 89% yield (Table 2, entry 3). A further increase in the amount of acetyl chloride showed no difference in the yield of compound **1**.

Table 2. Effect of the amount of acetyl chloride used[a]

entry	molar (equiv)	time (min)	yield[b] (%)	purity[c] (%)
1	1.00	10	78	99.4
2	1.05	10	81	99.6
3	1.10	10	89	99.7
4	1.15	10	89	99.5
5	1.20	10	89	99.5

[a]Reaction conditions: glutathione (10.0 g, 0.0325 mol), TFA (80 mL), $CoCl_2$ 0.5% mol, addition temperature 0−5 °C, reaction temperature 20−30 °C. [b]Isolated yield. [c]Detected by HPLC.

Trifluoroacetic acid is a relatively expensive reagent. Before proceeding with large-scale synthetic efforts, we turned attention to select a mixed solvent and an appropriate volume ratio. Thus, we screened various solvents to identify the conditions that would lead to good results. The results are summarized in Table 3.

As shown in Table 3, when the volume ratio of DCM to TFA was increased from 1:4 (v/v) to 1:2 (v/v), the yield and purity were not obviously decreased. The mixed solvent of DMF/TFA exhibited even better results until the DMF load up to 50%. However, the reactions proceeded in a mixed solvent of AcOH/TFA (1/2, v/v) or toluene/TFA (1/4, v/v) with only 60% and 40% yields, respectively, and purity was also not satisfactory. We suspected it might be that the trifluoroacetic salt of GSH was soluble in polar aprotic solvents, leading to the reaction proceeding further towards the formation of desired product. Unfortunately, this salt was insoluble in mixed solvent of AcOH/TFA or toluene/TFA, which led to poor yields. Although the mixed solvent of TFA/DCM showed satisfactory results, it was challenging to separate TFA from the recovered solvent in subsequent experiments. For this reason, TFA/DMF (1/1, v/v) was chosen as the ideal reaction mixed solvent rather than TFA/DCM.

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Table 3. Results of acylation reaction carried out in different solvents[a]

entry	solvent	quantity (v/v)	yield (%)[b]	purity (%)[c]
1	DCM/TFA	1/4	88	99.6
2	DCM/TFA	1/3	87.8	99.5
3	DCM/TFA	1/2	87.6	99.5
4	DCM/TFA	1/1	87.1	99.4
5	AcOH/TFA	1/4	87.2	99.3
6	AcOH/TFA	1/3	80.0	99.0
7	AcOH/TFA	1/2	60.0	98.5
8	toluene/TFA	1/5	62	95.2
9	toluene/TFA	1/4	40	86.7
10	DMF/TFA	1/4	90.5	99.7
11	DMF/TFA	1/3	91.0	99.6
12	DMF/TFA	1/2	90.8	99.6
13	DMF/TFA	1/1	91.0	99.7
14	DMF/TFA	2/1	88.4	98.5

[a]Reaction conditions: glutathione (10.0 g, 0.0325 mol), solvent (80 mL), acetyl chloride (2.8 g, 0.036 mol), $CoCl_2$ 0.5% mol, addition temperature 0−5 °C, reaction temperature 20−30 °C. [b]Isolated yield. [c]Detected by HPLC.

As discussed above, we should resolve the issue of recycling of the relatively expensive solvent trifluoroacetic acid. Hence, it became important for us to recover this solvent and establish a recycling procedure in subsequent experiments. First we quenched the reaction with water following Galzigan's work, and the TFA was recycled by atmospheric distillation. However, the recycled TFA was not able to be reused, because the residual water in trifluoroacetic acid was not able to be removed by atmospheric distillation, which would affect the reaction process. Then P_2O_5 was added to the recycled TFA but failed to remove the water. To avoid the water, we decided to use alcohol as the quencher, and excess alcohol was transformed to acetate by addition of acetyl chloride before atmospheric distillation. On the basis of the experimental results, we discovered both methanol and ethanol gave promising results, which are summarized in Table 4. Methyl acetate was easily separated from the recycled TFA because of the big gap between their boiling points, while ethyl acetate was difficult to be separated due to the similar boiling points. So, we chose methanol as the quencher.

Table 4. Effect of quencher on the quenching reaction[a]

entry	quencher	equiv	temp. (°C)	time (min)	yield (%)[b]	purity (%)[c]
1	water	4	Rt	20	90	99.6
2	methanol	4	Rt	20	91	99.5
3	ethanol	4	Rt	20	90	99.6

[a]Reaction conditions: glutathione (10.0 g, 0.0325 mol), solvent (TFA 40 mL and DMF 40 mL), $CoCl_2$ 0.5% mol, acetyl chloride (2.8 g, 0.036 mol), addition temperature 0−5 °C, reaction temperature 20−30 °C. [b]Isolated yield. [c]Detected by HPLC.

With optimized reaction conditions as described above (1.1 equiv of acetyl chloride, addition temperature of 0−5 °C, reaction temperature of 20−30 °C, solvent of TFA/DMF (1:1, v/v), reaction time of 10 min, and quencher of methanol), a few reactions were executed on pilot scale. When completed, the reaction mixture was subjected to vacuum fractional distillation. Fraction 1 mainly contained the unreacted methanol and TFA. Fraction 2 mainly contained DMF.

Acetyl chloride was added dropwise to Fraction 1 at room temperature. After stirring for 1 h at room temperature, atmospheric distillation process was used to purify TFA from Fraction 1. We removed the front fraction (<70 °C) and collected the fraction of 70−75 °C. The collected TFA was analyzed by GC for quality (Table 5). As shown in Table 5, we first attempted to add 1 equiv of acetyl chloride to remove the methanol (Table 5 entry 1). In this case, the purity of TFA was only 97.6%. Then by increasing the amount of acetyl chloride to 2 equiv, we were able to achieve 99.2% purity with the residue of only <0.1% methanol (entry 3). Three experiments were performed using the collected TFA. The results showed that the yield of *S*-GSH was 89%, 90%, and 90%, respectively.

CONCLUSIONS

An improved process for the preparation of *S*-acetyl-L-glutathione (**1**) was developed and optimized. It was found that $CoCl_2$ is an effective catalyst for the acylation of GSH by acetyl chloride in the mixed solvent of TFA/DMF (1:1, v/v) at room temperature. Recycling of solvent TFA could further increase the economic benefits. The current process increased the overall productivity by 6%.

EXPERIMENTAL SECTION

General. Glutathione and other reagents and solvents were obtained from commercial suppliers and used without further purification. ^1H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrometer in DMSO-d_6; chemical shift data were reported in δ (ppm) from the internal standard TMS. The IR spectra were recorded on KBr pellets on a PerkinElmer FTIR (spectrum one) spectrophotometer. The reaction monitoring and purity (area percentage) were analyzed by high-performance liquid chromatography (HPLC) at λ = 210 nm using Inertsil NH_2 column (250 mm × 4.6 mm, 5 μm) at 1.0 mL/min flow. The purity of TFA was analyzed by gas chromatography Agilent 7890A and a HP-FFAP capillary column (capillary dimension = 30 m × 320 μm × 0.25 μm), oven temperature = 60−200 °C, ramp = 15 °C/min, detector heater = 250 °C; N_2 flow = 25 mL/min, H_2 flow = 30 mL/min, and air flow = 400 mL/min.

Preparation of *S*-GSH. Acetyl chloride (1.4 kg, 17.83 mol) was added dropwise to a solution of L-glutathione (5.0 kg, 16.27 mol) in the presence of trifluoroacetic acid (15 L), DMF (15

Table 5. Recovery and recycling of TFA from fraction 1[a]

				content (%)[c]			
entry	fraction 1 (g)	acetyl chloride equiv[a]	temp. (°C)[b]	CH_3OH	methyl acetate	quality of TFA	quantity of TFA (g)
1	700	1	70−75	<1.2	<0.4	>97.6	602
2	700	1.5	70−75	<0.8	<0.4	>98.6	611
3	700	2	70−75	<0.1	<0.1	>99.2	623

[a]Acetyl chloride molar equiv. [b]Collection temperature. [c]Determined by GC.

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L), and $CoCl_2$ (10.56 g, 0.081 mol) at 0−5 °C over a period of 5 min. Thereafter, the temperature was raised to 20−30 °C, and the reaction mixture was stirred for 10 min. The progress of the reaction was monitored by HPLC. After completion, the reaction was quenched by addition of 0.21 L of methanol, and the reaction mixture was stirred for an additional 20 min at room temperature. The TFA and DMF were removed in vacuo, and ethyl acetate (50 L) was added to the resulting oil. The mixture was cooled to 0 °C obtaining a white solid overnight. The crude precipitate was dissolved in 20 L of warm water (40 °C). The obtained solution was further diluted with 60 L of acetone. After cooling in an ice−water bath for about 2 h, the white crystalline material formed was collected, washed with the solvent (3.0 L, acetone/water, 2/1), and dried under vacuum affording *S*-acetyl-L-glutathione (5.20 kg, yield 91.5%).

Chromatographic purity: 99.7% (by HPLC). mp 202−203 °C. $[\alpha]_D^{20} = -16.5$ (1.0 water). ^{1}H NMR (400 Hz, DMSO-d_6): δ (ppm) 8.71 (t, J = 6.0, 1H), 8.47 (d, J = 8.8, 1H), 4.39 (m, 1H), 3.68 (d, J = 6.0, 2H), 3.36 (m, 2H), 3.28 (t, J = 6.4, 2H), 2.93 (m, 1H), 2.31 (s, 3H), 2.28 (m, 2H), 1.90 (m, 1H), 1.83 (m, 1H). ^{13}C NMR (400 Hz, DMSO-d_6): δ (ppm) 196.38, 172.24, 171.35, 170.74, 170.63, 53.48, 52.33, 41.69, 31.82, 31.00, 30.93, 27.21. IR (KBr, cm^{-1}): 3355, 3059, 2956, 2729, 1701, 1677, 2648, 1514, 1432, 1353, 1231, 1132, 963, 636.

Recycling Process for TFA. Acetyl chloride (1.7 kg) was slowly added to fraction 1 (7 kg) at room temperature. Thereafter, the reaction mixture was stirred for 1 h at room temperature. An atmospheric distillation process was used to separate TFA from the Fraction 1. We removed the front cut fraction (<70 °C) and collected the fraction of 70−75 °C (6.3 kg, purity 99.2%, area %).

■ ASSOCIATED CONTENT

Supporting Information

^{1}H, ^{13}C, IR spectrum, and HPLC chromatogram of *S*-acetyl-L-glutathione. GC chromatogram of recovered TFA. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.oprd.5b00081.

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Notes

The authors declare no competing financial interest.

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