Efficient Esterification of Oxidized l-Glutathione and Other Small Peptides

Emily Rose Vogel
*University of Southern Mississippi, emily.vogel@eagles.usm.edu*

William Jackson
*University of Southern Mississippi*

Douglas S. Masterson Dr.
*University of Southern Mississippi, Douglas.Masterson@usm.edu*

---

Follow this and additional works at: [https://aquila.usm.edu/fac_pubs](https://aquila.usm.edu/fac_pubs)

Part of the Chemistry Commons

---

**Recommended Citation**

Vogel, E. R., Jackson, W., Masterson, D. S. (2015). Efficient Esterification of Oxidized l-Glutathione and Other Small Peptides. *Molecules, 20*(6), 10487-10495.

Available at: [https://aquila.usm.edu/fac_pubs/15392](https://aquila.usm.edu/fac_pubs/15392)

---

This Article is brought to you for free and open access by The Aquila Digital Community. It has been accepted for inclusion in Faculty Publications by an authorized administrator of The Aquila Digital Community. For more information, please contact Joshua.Cromwell@usm.edu.
Efficient Esterification of Oxidized L-Glutathione and Other Small Peptides

Emily R. Vogel, William Jackson and Douglas S. Masterson *

Department of Chemistry and Biochemistry, the University of Southern Mississippi, 118 College Drive #5043, Hattiesburg, MS 39406, USA; E-Mails: emily.vogel@eagles.usm.edu (E.R.V.); william.r.jackson@eagles.usm.edu (W.J.)

* Author to whom correspondence should be addressed; E-Mail: douglas.masterson@usm.edu; Tel.: +1-601-266-4714.

Academic Editor: Derek J. McPhee

Received: 17 May 2015 / Accepted: 4 June 2015 / Published: 8 June 2015

Abstract: Oxidized L-glutathione was esterified to the tetra methyl ester using thionyl chloride in methanol solvent. Other alcohols were tested and the reaction progress was monitored via ESI-MS. This procedure proved to be compatible with other small peptides not containing serine and cysteine residues. In contrast to previously reported methods this procedure provided convenient access to esterified peptides requiring no purification, extended reaction times, or complicated reaction setups.

Keywords: esterification; peptide esterification; amino acids; carboxylic acids; esters

1. Introduction

The design and synthesis of novel glutathione analogues are studied extensively for their pharmacological properties in the treatment of a wide range of diseases [1–3]. Reduced glutathione (GSH), γ-L-glutamyl-L-cysteinyl-glycine, is prone to reactivity at the sulphhydryl, terminal amino group, and both carbonyls. Thus, the synthesis of GSH analogues are a synthetic challenge to chemists, and the clever manipulation of protecting groups are needed to prevent unwanted reactions. One approach to synthesize protected GSH analogues is to start with the readily available disulfide dimmer of GSH, L-glutathione (GSSG) 1. GSSG is affordable and the sulphhydryl functional groups are protected in their disulfide form. 1 can be inconveniently converted to 2a by Fischer esterification [4–6] resulting in the
protection of the carboxyl groups. In our hands the reported Fischer esterification protocol required extensive purification and the isolated yield was too low to be of practical value. Although 2a is commercially available the cost is quite high ($1290 per 100 mg) making direct purchase prohibitive in many cases [7]. Additional esterification strategies include utilizing trimethylsilyl chloride in methanol solvent [2,8] and the use of diazomethane to form methyl esters [9,10]. However, the use of diazomethane is toxic, potentially explosive, and requires specialized glassware not readily available in all labs.

Herein, we report a convenient synthesis of 2a using thionyl chloride in methanol solvent as illustrated in Scheme 1. Surprisingly, this method eliminated side reactions, long reaction times, and column purification. Furthermore, we extended the method to other peptides in order to gain an understanding of the compatibility with other protogenic amino acids. The ease with which peptide esters are formed could be an invaluable tool for peptide mass spectrometry [3,11–13] as the protected carboxylic acid enhances signal intensity.

**Scheme 1.** Preparation of oxidized glutathione tetra-alkyl esters (GSSG(OR)₄) from oxidized glutathione (GSSG) using thionyl chloride in alcohol solvent.

### 2. Results and Discussion

The esterification of oxidized glutathione (GSSG) with thionyl chloride was previously reported [14], but studies monitoring the reaction conversion have not been reported. The disposition toward thionyl chloride acylation is likely due to the incompatibilities often associated with the harsh conditions of thionyl chloride which are subject to both side reactions and incomplete formation of product. Therefore, we set out to study the limitations of this method by monitoring the reaction using ESI-MS in the presence of various alcohols using 1 as a substrate.

The reactions were performed on 250 mg of GSSG in 50 mL of anhydrous alcohol. To this solution 2.5 mL of thionyl chloride was added slowly and placed into a refrigerator at 4 °C. At various time intervals a 1.0 mL aliquot was taken, concentrated under reduced pressure, suspended into 1% acetic acid solution (50% methanol/water), and analyzed via ESI-MS. The relative intensities of the m/z for the tetra-ester were plotted as a percentage of ester products as illustrated in Figure 1. The procedure worked well in methanol solvent reaching completion within 16 h. The same experimental conditions were applied using an excess of anhydrous ethanol resulting in complete conversion to 2b in 144 h. In contrast, when the same experimental conditions were applied using an excess of anhydrous isopropyl alcohol only a 14% conversion to 2c was realized along with significant formation of various unidentified side products. We therefore concluded that studying other 2° or even 3° alcohols would likely not be productive.
To further demonstrate the utility of this method the reaction conditions were applied to other small peptides as shown in Table 1. These peptides were chosen based on comparable size to GSSG and the coverage of amino acids present in their sequences. The reactions were performed on a 1.0 µmol scale and immediately concentrated following a 24 h incubation at 4 °C. The peptides were then immediately analyzed via ESI-MS.

Table 1. Peptides subjected to the thionyl chloride esterification with methanol and analyzed via ESI-MS.

| Peptidea | Peptide Sequence | Product Entry | Complete Conversion | Estimated % Conversion |
|----------|------------------|---------------|---------------------|-----------------------|
| L-glutathione oxidized | 2QCG b | 2 | Yes | 100% d |
| L-glutathione reduced | QCG | 3 | No | 26% |
| Fibronectin Analog | GRADSPK | 4 | No | 23% |
| Bradykinin (1–7) | RPPGFSP | 5 | No | 63% |
| Necrofibrin, rat | WTVPTA | 6 | No | 64% |
| [D-Ala2,D-Met5]-Enkephalin | YAGFM | 7 | No | 90% |
| Angiotensin II, human | DRVYIHPF | 8 | Yes | 100% |
| Thymopentin (TP-5) | RKDVY | 9 | Yes | 100% |
| Neurotensin (9–13) | RPYIL | 10 | Yes | 100% |
| [Ile3]-Pressinoic acid | CYIQNC b | 11 | No | 94% |

a Reaction conditions of the peptides were run using 3.44 M thionyl chloride in methanol at 4 °C for 24 h; b Peptide contains a disulfide bridge between the two cysteine residues; c Yields estimated by taking the sum or relative intensities of all product peaks over the total relative intensities observed in ESI-MS in excess of 5% relative abundance; d isolated product yield.

The reactions listed in Table 1 proceeded smoothly under the esterification protocol in most cases. However, the peptides containing serine, fibronectin and bradykinin, appear to have been converted to the esterified alkyl chloride derivative at the serine residue [15–17]. This is evidenced by the m/z corresponding to the molecular formula of the esterified peptide with chlorine replacing the hydroxyl functional group and the signature isotopic ratio of chlorine. These results are not unexpected considering that thionyl chloride is used to transform primary alcohols into alkyl chlorides. However,
we did observe the esterified product containing the unaltered serine residue as a minor constituent in the product mixture. Interestingly, the threonine containing peptide, necrofibrin, underwent smooth conversion to the ester without converting the 2° alcohol into an alkyl chloride. The phenol functional group of tyrosine in [DAla2,DMet5]-Enkephalin, Angiotensin II, Thymopentin (TP-5), and Neurotensin (9–13) did not interfere with the esterification procedure.

The free sulfhydryl of the cysteine residue also proved to be problematic when subjected to the esterification protocol. Reduced L-glutathione was subjected to the reaction conditions producing the desired esterified peptide as a minor constituent. The reaction mixture contained significant quantities of 2 and several other unidentified species by ESI-MS analysis. However, the methionine residue of Enkephalin and the disulfide bond of [Ile3]-Pressinoic acid and GSSG were cleanly converted to their respective esters. In addition, the residues of aspartic acid and glutamic acid were converted to their respective methyl esters as expected. Despite these limitations all of the other protogenic amino acids withstood the reaction conditions and were easily isolated and characterized without the need for extensive purification.

3. Experimental Section

3.1. General Experimental

NMR spectra were acquired on a Bruker 400 MHz NMR in proton decoupled mode. The internal standard used in the NMR experiment was the residual solvent signal for CD$_3$OD. ESI MS was carried out on a ThermoFisher LXQ ESI-Ion trap mass spectrometer using Optima LCMS grade methanol and water from Fisher Scientific. The methanol used for the esterification reactions was distilled from calcium hydride, absolute ethanol was stored over molecular sieves, and 2-propanol distilled from sodium prior to use. L-oxidized glutathione and reduced glutathione were obtained from Sigma-Aldrich. All other peptides were acquired from the American Peptide Company and used as received. Peptides furnished with Certificate of Analysis (COA) contained trace impurities and those masses were provided courtesy of American Peptide Company. Mass spectra and NMR spectra can be found in the supplementary materials section.

3.2. Synthesis of GSSG and GSH Peptides in Alcohol Solutions

3.2.1. Synthesis of Compound 2

In a 125 mL Erlenmeyer flask, 0.250 g of L-oxidized glutathione (0.408 mmol) was added to 50 mL of freshly distilled methanol and capped with a rubber septum. The contents were placed into an ice bath and allowed to cool to 0 °C. The addition of 2.5 mL of thionyl chloride (34.4 mmol) was by syringe, the flask was swirled, and placed into a refrigerator at 4 °C. After 24 h the reaction was concentrated under reduced pressure resulting in 0.272 g of product (100% yield). The product was analyzed via ESI MS. ESI MS of [M + H]$^+$ calculated [C$_{24}$H$_{44}$N$_6$O$_{12}$S$_2$]$^+$, 669.22, found 669.2. $^1$H-NMR (400MHz, CD$_3$OD) $\delta$ = 4.67 (m, 2H), 4.05 (m, 2H), 3.88 (s, 4H), 3.76 (s, 6H), 3.62 (s, 6H), 3.21 (s, 4H), 2.93–2.81 (m, 2H), 2.49 (m, 4H), 2.12 (m, 4H) and $^{13}$C-NMR (100MHz, CD$_3$OD) $\delta$ = 174.3, 173.0, 171.6, 170.7, 54.0, 53.9, 53.7, 52.8, 42.0, 41.4, 32.3, 27.0. Spectra are consistent with published data [18].
3.2.2. Synthesis of Compound 2b

In a 125 mL Erlenmeyer flask, 0.250 g of L-oxidized glutathione (0.408 mmol) was added to 50 mL of absolute ethanol and capped with a rubber septum. The contents were placed into an ice bath and allowed to cool to 0 °C. The addition of 2.5 mL of thionyl chloride (34.4 mmol) was by syringe, the flask was swirled, and placed into a refrigerator at 4 °C. After 24 h the reaction was concentrated under reduced pressure resulting in 0.295 g of product (100% yield). The solid was taken and characterized by ESI-MS. ESI-MS of [M + H]+ calculated [C_{28}H_{44}N_{6}O_{12}S_{2}]^+, 725.28, found 725.25. 1H-NMR (400 MHz, CD_{3}OD) δ = (400 MHz, MeOD) δ 4.78 (dd, J = 9.6, 4.6 Hz, 2H), 4.33 (q, J = 7.1 Hz, 4H), 4.19 (q, J = 7.1 Hz, 4H), 4.13 (m, 2H), 3.97 (d, J = 2.2 Hz, 4H), 3.28 (dd, J = 14.0, 4.6 Hz, 2H), 2.99 (dd, J = 13.9, 9.7 Hz, 2H), 2.61 (t, J = 7.1 Hz, 4H), 2.23 (m, J = 21.7, 14.6, 7.5 Hz, 4H), 1.35 (t, J = 7.1 Hz, 6H), 1.28 (t, J = 7.2 Hz, 6H). 13C-NMR (100MHz, CD_{3}OD) δ = 174.4, 173.0, 171.1, 170.2, 63.4, 62.4, 53.9, 53.7, 42.1, 41.3, 32.6, 27.0, 14.5, 14.43. Spectra are consistent with published data [19].

3.2.3. Synthesis of Compound 2c

In a 125 mL Erlenmeyer flask, 0.250 g of L-oxidized glutathione (0.408 mmol) was added to 50 mL of freshly distilled 2-propanol and capped with a rubber septum. The contents were placed onto an ice bath and allowed to cool to 0 °C. The addition of 2.5 mL of thionyl chloride (34.4 mmol) was by syringe, and the flask was swirled and placed into a refrigerator at 4 °C. After 24 h the reaction was concentrated under reduced pressure. The resulting solid was taken and characterized by ESI-MS. ESI-MS of [M + H]+ calculated [C_{32}H_{57}N_{6}O_{12}S_{2}]^+, 781.35, found 781.25. Expected mass not present in significant quantities so no NMR data was recorded.

3.2.4. Synthesis of Compound 3

In a 125 mL Erlenmeyer flask, 0.250 g of L-reduced glutathione (0.813 mmol) was added to 50 mL of freshly distilled anhydrous methanol and capped with a rubber septum. The contents were placed onto an ice bath and allowed to cool to 0 °C. The addition of 2.5 mL of thionyl chloride (34.4 mmol) was by syringe, and the flask was swirled and placed into a refrigerator at 4 °C. After 24 h the reaction was concentrated under reduced pressure. The resulting solid was taken and characterized by ESI-MS. ESI-MS of [M + H]+ calculated [C_{12}H_{22}N_{3}O_{6}S]^{+}, 336.12, found 336.08. Sample contained multiple impurities so no NMR analysis was performed.

3.3. General Synthesis of Peptides in Methanol Solutions

The conditions for each peptide were scaled down from the GSSG reaction study in methanol based on the number of carboxylic acid groups present in the molecule. The amount of methanol used in the reaction was 756 equivalents of methanol to which 105 equivalents of thionyl chloride was added for each carboxylic acid present in the peptide. This results in a 3.44 M solution of thionyl chloride in methanol. All reactions were performed on 1.0 mg of peptide and incubated at 4 °C for 24 h. At the end of the incubation the crude material was concentrated and analyzed by ESI-MS.
3.3.1. Synthesis of Compound 4

In a dry glass vial fitted with a cap 1.0 mg of Fibronectin (1 µmol) was dissolved into 83.9 µL of anhydrous methanol (2.075 mmol) and placed in an ice bath at 0 °C. To the solution 21.0 µL of thionyl chloride (0.289 mmol) was added slowly via syringe. The vial was shaken and placed into a refrigerator at 4 °C. After 24 h the sample was concentrated under reduced pressure, dissolved into 1.0 mL of 1% acetic acid in a 1:1 solution of methanol:water, and analyzed on the ESI-MS. ESI-MS of [M + H]+ calculated [C31H56N11O11]+, 758.42, found 758.42.

3.3.2. Synthesis of Compound 5

In a dry glass vial fitted with a cap 1.0 mg of Bradykinin (1 µmol) was dissolved into 40.5 µL of anhydrous methanol and placed in an ice bath at 0 °C. To the solution 10.1 µL of thionyl chloride (0.14 mmol) was added slowly via syringe. The vial was shaken and placed into the fridge at 4 °C. After 24 h the sample was concentrated under reduced pressure, dissolved into 1.0 mL of 1% acetic acid in a 1:1 solution of methanol:water, and analyzed on the ESI-MS. ESI-MS of [M + H]+ calculated [C36H55N10O9]+, 771.41, found 771.38.

3.3.3. Synthesis of Compound 6

In a dry glass vial fitted with a cap 1.0 mg of necrofibrin, rat (1 µmol) was dissolved into 45.5 µL of anhydrous methanol (1.12 mmol) and placed in an ice bath at 0 °C. To the solution 11.4 µL of thionyl chloride (0.156 mmol) was added slowly via syringe. The vial was shaken and placed into a refrigerator at 4 °C. After 24 h the sample was concentrated under reduced pressure, dissolved into 1.0 mL of 1% acetic acid in a 1:1 solution of methanol:water, and analyzed on the ESI-MS. ESI-MS of [M + H]+ calculated [C33H50N7O9]+, 688.37; found 688.20.

3.3.4. Synthesis of Compound 7

In a dry glass vial fitted with a cap 1.0 mg of [DAla2,DMet5] Enkephalin (2 µmol) was dissolved into 52.1 µL of anhydrous methanol (1.29 mmol) and placed in an ice bath at 0 °C. To the solution 13.0 µL of thionyl chloride (0.18 mmol) was slowly added via syringe. The vial was shaken and placed into a refrigerator at 4 °C. After 24 h the sample was concentrated under reduced pressure, dissolved into 1.0 mL of 1% acetic acid in a 1:1 solution of methanol:water, and analyzed on the ESI-MS. ESI-MS of [M + H]+ calculated [C29H40N5O7S]+, 602.26, found 602.1.

3.3.5. Synthesis of Compound 8

In a dry glass vial fitted with a cap 1.0 mg of angiotensin II, human (1 µmol) was dissolved into 58.6 µL of anhydrous methanol (1.45 mmol) and placed in an ice bath at 0 °C. To the solution 14.6 µL of thionyl chloride (0.20 mmol) was added slowly via syringe. The vial was shaken and placed into a refrigerator at 4 °C. After 24 h the sample was concentrated under reduced pressure, dissolved into 1.0 mL of 1% acetic acid in a 1:1 solution of methanol:water, and analyzed on the ESI-MS. ESI-MS of [M + H]+ calculated [C52H76N13O12]+, 1074.57, found 1074.6.
3.3.6. Synthesis of Compound 9

In a dry glass vial fitted with a cap 1.0 mg of Thymopentin (TP-5) (1 µmol) was dissolved into 90.1 µL of anhydrous methanol (2.22 mmol) and placed in an ice bath at 0 °C. To the solution 22.5 µL of thionyl chloride (0.289 mmol) was added slowly via syringe. The vial was shaken and placed into the fridge at 4 °C. After 24 h the sample was concentrated under reduced pressure, dissolved into 1.0 mL of 1% acetic acid in a 1:1 solution of methanol:water, and analyzed on the ESI-MS. ESI-MS of [M + H]+ calculated [C_{32}H_{54}N_{9}O_{9}]^+, 708.40, found 708.47. Masses present in starting material from COA: 341(14%), 680 (100%), 1358(8%).

3.3.7. Synthesis of Compound 10

In a dry glass vial fitted with a cap 1.0 mg of Thymopentin (TP-5) (1 µmol) was dissolved into 90.1 µL of anhydrous methanol (2.22 mmol) and placed in an ice bath at 0 °C. To the solution 22.5 µL of thionyl chloride (0.289 mmol) was added slowly via syringe. The vial was shaken and placed into the fridge at 4 °C. After 24 h the sample was concentrated under reduced pressure, dissolved into 1.0 mL of 1% acetic acid in a 1:1 solution of methanol:water, and analyzed on the ESI-MS. ESI-MS of [M + H]+ calculated [C_{32}H_{54}N_{9}O_{9}]^+, 708.40, found 708.47.

3.3.8. Synthesis of Compound 11

In a dry glass vial fitted with a cap 1.0 mg of [Ile3] Pressinoic Acid (1 µmol) was dissolved into 41.3 µL of anhydrous methanol (1.02 mmol) and placed in an ice bath at 0 °C. To the solution 10.3 µL of thionyl chloride (0.14 mmol) was added slowly via syringe. The vial was shaken and placed into a refrigerator at 4 °C. After 24 h the sample was concentrated under reduced pressure, dissolved into 1.0 mL of 1% acetic acid in a 1:1 solution of methanol:water, and analyzed on the ESI-MS. ESI-MS of [M + H]^+ calculated [C_{31}H_{47}N_{8}O_{10}S_{2}]^+, 755.29, found 755.22.

4. Conclusions

We have prepared GSSG methyl esters in high yield requiring no purification or complex reaction protocols. The title compound could also be prepared as the ethyl ester at the expense of increased reaction time. Based on these findings the reaction conditions were applied to other small peptides and found to be highly compatible with many peptide sequences. We believe this procedure will find significant use in the area of peptide modification for both synthetic and analytical purposes.

Supplementary Materials

Supplementary materials can be accessed at: http://www.mdpi.com/1420-3049/20/06/10487/s1.

Acknowledgments

We thank the Department of Chemistry and Biochemistry of the University of Southern Mississippi for continued support of our programs. We also thank the National Science Foundation (NSF-CAREER, MCB-0844478, NSF-GK12 0947944) for financial, fellowship, and instrument support of our programs.
Author Contributions

D.M. conceived and designed the experiments. The experimental work including ESI-MS studies and peptide esterification were carried out by E.R.V.; Analysis was performed by E.R.V.; W.J. and D.S.M. performed preliminary esterification using other stated methods. E.R.V. wrote the paper.

Conflicts of Interest

The authors declare no conflict of interest.

References and Notes

1. Ehrlich, K.; Viirlaid, S.; Mahlapuu, R.; Saar, K.; Kullisaar, T.; Zilmer, M.; Langel, Ü.; Soomets, U. Design, synthesis and properties of novel powerful antioxidants glutathione analogues. Free Radic. Res. 2007, 41, 779–787.
2. Gatterdam, V.; Stoess, T.; Menge, C.; Heckel, A.; Tampé, R. Caged Glutathione–Triggering Protein Interaction by Light. Angew. Chem. Int. Ed. 2012, 51, 3960–3963.
3. Lecchi, P.; Olson, M.; Brancia, F.L. The Role of Esterification on Detection of Protonated and Deprotonated Peptide Ions in Matrix Assisted Laser Desorption/Ionization (MALDI) Mass Spectrometry (MS). J. Am. Soc. Mass Spectrom. 2005, 16, 1269–1274.
4. Falck, J.R.; Sangras, B.; Capdevila, J.H. Preparation of N-tBoc l-glutathione dimethyl and di-tert-butyl esters: Versatile synthetic building blocks. Bioorg. Med. Chem. 2007, 15, 1062–1066.
5. Stepanov, V.M.; Muratova, G.L. Partial esterification of some amino acids and glutathione. Izv. Akad. Nauk SSSR Ser. Khim. 1961, 10, 1677–1680.
6. Thornalley, P.K. Esterification of reduced glutathione. Biochem. J. 1991, 275, 535–539.
7. Aurora Fine Chemicals Catalog. Available online: http://online.aurorafinechemicals.com (accessed on 27 April 2015). Product Number K11.805.561.
8. Li, J.; Sha, Y. A Convenient Synthesis of Amino Acid Methyl Esters. Molecules 2008, 13, 1111–1119.
9. Pozgan, F.; Lukman, K.; Kocevar, M. An efficient synthesis of methyl esters of heterocyclic α,β-didehydro-α-amino acid derivatives. Heterocycles 2010, 82, 543–554.
10. Di Gioia, M.L.; Leggio, A.; le Pera, A.; Liguori, A.; Napoli, A.; Siciliano, C.; Sindona, G. “One-Pot” Methylation of N-Nosyl-α-amino Acid Methyl Esters with Diazomethane and Their Coupling To Prepare N-Methyl Dipeptides. J. Org. Chem. 2003, 68, 7416–7421.
11. Ma, M.; Kutz-Naber, K.K.; Li, L. Methyl Esterification Assisted MALDI FTMS Characterization of the Orcokinin Neuropeptide Family. Anal. Chem. 2007, 79, 673–681.
12. Kim, T.Y.; Brun, Y.V.; Reilly, J.P. Effects of Tryptic Peptide Esterification in MALDI Mass Spectrometry. Anal. Chem. 2005, 77, 4185–4193.
13. Simon, E.S.; Young, M.; Chan, A.; Bao, Z.Q.; Andrews, P.C. Improved enrichment strategies for phosphorylated peptides on titanium dioxide using methyl esterification and pH gradient elution. Anal. Biochem. 2008, 377, 234–242.
14. Su, D.; Ren, X.; You, D.; Li, D.; Mu, Y.; Yan, G.; Zhang, Y.; Luo, Y.; Xue, Y.; Shen, J.; et al. Generation of Three Selenium-Containing Catalytic Antibodies with High Catalytic Efficiency Using a Novel Hapten Design Method. *Arch. Biochem. Biophys.* **2001**, *395*, 177–184.

15. Cerny, C.; Guntz-Dubini, R. Formation of cysteine-S-conjugates in the Maillard reaction of cysteine and xylose. *Food Chem.* **2013**, *141*, 1078–1086.

16. Yamashita, K.; Inoue, K.; Kinoshita, K.; Ueda, Y.; Murao, H. Processes for Producing β-Halogeno-α-amino-carboxylic Acids and S-Phenylcysteine Derivatives and Intermediates Thereof. WO9933785A1, 8 July 1999.

17. Shen, L.; Guan, L.; Liu, F.; Cao, Y.; Zhou, J. Method for Preparing Levetiracetam. CN101550100A, 7 October 2009.

18. Gatterdam, V.; Ramadass, R.; Stoess, T.; Fichte, M.A.H.; Wachtveitl, J.; Heckel, A.; Tampé, R. Three-Dimensional Protein Networks Assembled by Two-Photon Activation. *Angew. Chem. Int. Ed.* **2014**, *53*, 5680–5684.

19. McCulloch, M.W.B.; Coombs, G.S.; Banerjee, N.; Bugni, T.S.; Cannon, K.M.; Harper, M.K.; Veltri, C.A.; Virshup, D.M.; Ireland, C.M. Psammaplin A as a general activator of cell-based signaling assays via HDAC inhibition and studies on some bromotyrosine derivatives. *Bioorg. Med. Chem.* **2009**, *17*, 2189–2198.

*Sample Availability*: Not available.

© 2015 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/4.0/).