Bioorganic & Medicinal Chemistry Letters 19 (2009) 6706–6708

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Solid phase synthesis of acylglycine human metabolites

Rolando Perez-Pineiro [a,*], Ying Wei Dong [a], David S. Wishart [a,b,*]

[a] Department of Biological Sciences, University of Alberta, Edmonton AB, Canada T6G 2E9
[b] Department of Computing Science, University of Alberta, Edmonton AB, Canada T6G 2E9

ARTICLE INFO

Article history:
Received 17 July 2009
Revised 26 September 2009
Accepted 30 September 2009
Available online 3 October 2009

Keywords:
Acylglycines
Human metabolome database
Solid phase synthesis

ABSTRACT

Acylglycines represents a large and important class of human metabolites. They are often used in medicine to identify fatty acid oxidation disorders. A highly efficient solid phase synthesis approach to obtain these clinically important compounds is developed via coupling reaction between glycine-preloaded Wang resin and a set of carboxylic acids. The developed methodology facilitates the preparation of several structurally-diverse acylglycines with high yields and purity.

Metabolomics is a newly emerging field of 'omics' science that uses NMR and mass spectrometry to rapidly identify and quantify large numbers of metabolites from biological materials.[1] One of the continuing challenges in metabolomics is having sufficient quantities of authentic reference compounds to identify or confirm the identity of a putative metabolite. A similar problem also exists in the field of clinical chemistry where the lack of authentic standards for key metabolites often prevents routine testing for certain metabolic disorders. Acylglycines represent an important class of disease-associated metabolites for which a relatively small number of authentic standards are commercially available. Given their importance in both clinical chemistry and in metabolomic studies we have developed an efficient general solid phase synthetic strategy that should greatly enhance the yield, synthetic diversity and ease-of-purification of acylglycines.

Acylglycines **1** possess a common amidoacetic acid moiety and are normally minor metabolites of fatty acids. Elevated levels of certain acylglycines appear in the urine and blood of patients with various fatty acid oxidation disorders. They are normally produced through the action of glycine *N*-acyltransferase which is an enzyme that catalyzes the chemical reaction: acyl-CoA + glycine ↔ CoA + *N*-acylglycine.[2]

Several solution phase synthetic protocols have been described for the synthesis of acylglycines. According to Scheme 1 the amidoacetic functionality could be generated by reaction of acid derivatives with glycine in basic aqueous or organic medium, Scheme 1A.[3,4] Glycine methyl esters have also been reported to afford the desired compounds by reaction with acyl chlorides follow by basic hydrolysis, Scheme 1B.[5] Similarly glycine methyl ester hydrochloride can react with acids and acyl chlorides under different coupling conditions, Scheme 1C.[6,7] In an alternate approach several α,β-unsaturated acylglycines have been recently obtained by acylation of unprotected aminoacids with *N*-(α,β-unsaturated acyl) benzotriazole, Scheme 1D.[8]

Many of the solution phase methodologies described above possess important drawbacks such as long reaction times, poor yields and exhaustive purification protocols. In this context we have foreseen that the use of solid phase strategies[9] to obtain acylglycines could overcome some of the deficiencies observed in previous synthetic protocols.

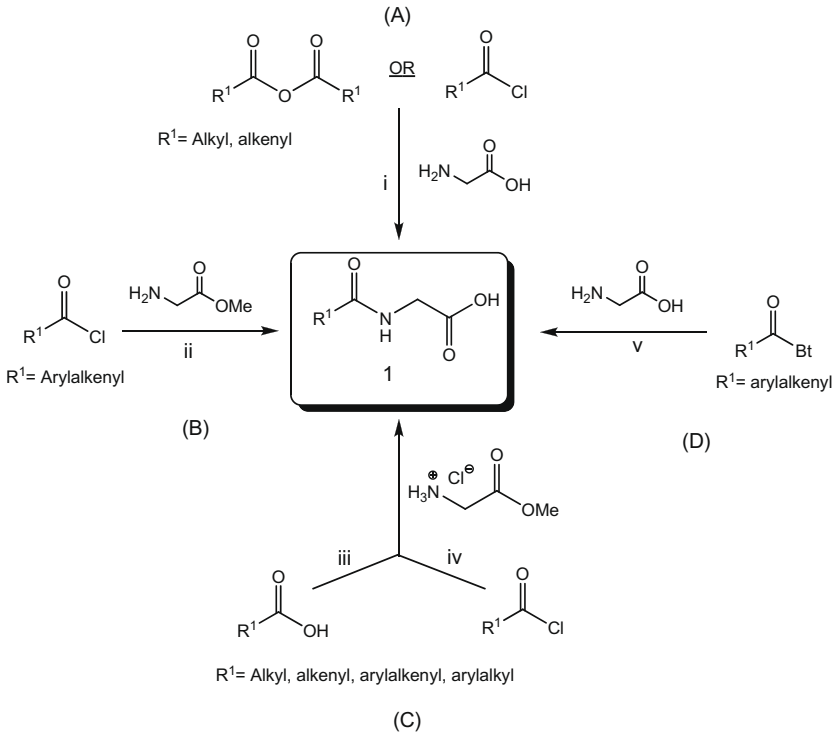
Surprisingly, despite the enormous advancements in the field of solid phase organic synthesis over the past decades the only report about the formation of an amidoacetic functionality on polymeric supports describes the preparation of a series of resin-bound *N*-substituted-*N*-acylglycines as intermediates in the synthesis of ketoimidazoles.[10] In this case the amidoacetic moiety is generated via nucleophilic substitution reaction between previously obtained Wang bromoacetate resin and several amines. With the aim to expand the limited synthetic repertoire of acylglycines currently available we report herein an alternate approach to synthesize this class of compounds. This novel procedure possesses the inherent advantages of solid phase organic synthesis, including simplicity, high yields and easy purification. This strategy has also been successfully implemented to obtain a series of structurally-diverse amidoacetic derivatives.

Our solid phase strategy and the results of the synthesis of the acylglycines are depicted in Scheme 2 and Table 1, respectively.

In the first step Fmoc-Gly-OH is readily attached to Wang resin (1.1 mmol/g) using the symmetrical anhydride protocol.

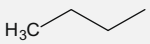
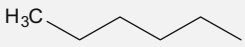
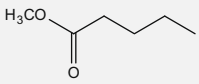
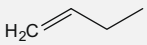
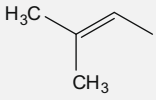
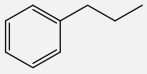
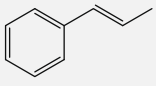
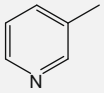
* Corresponding authors. Tel.: +1 780 492 8574; fax: +1 780 492 9234 (R.P.-P.), tel.: +1 780 492 0383; fax: +1 780 492 1071 (D.S.W.).
E-mail addresses: perezpin@ualberta.ca (R. Perez-Pineiro), david.wishart@ualberta.ca (D.S. Wishart).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.09.118



Scheme 1. Solution phase synthesis of acylglycines. Reagents and conditions: (i) NaOH 1 M or Py (anh) 0–4 °C or NaOH 1 M/EtOAc; (ii) TEA/$CHCl_3$, NaOH/MeOH; (iii) EDCI, DMAP/DCM then LiOH/H_2O:TFA; (iv) DIPEA/DCM, LiOH, THF/H_2O/MeOH (8:2:1); (v) DME/H_2O/MeOH.

Table 1
Acylglycine derivatives **1a**–**h** produced via Scheme 2

Compds	HMDB ID #[a]	R^1	Yield[b] (%)
1a	00808		99
1b	00701		87
1c	na		97
1d	00894		90
1e	00459		92
1f	00860		80
1g	11621		83
1h	03269		87

[a] Human metabolome database[12] identification number.
[b] Indicates yield of isolated products.

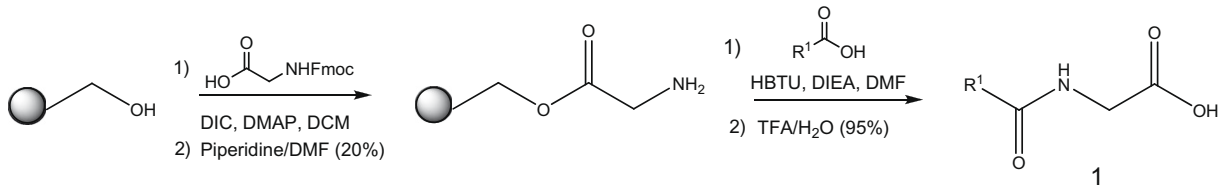
Subsequent capping and spectrophotometric quantification of the Fmoc group revealed an average substitution of 0.8 mmol/g. After deprotection of the amino functionality with piperidine/DMF (20%) the resin is allowed to react with the corresponding acid derivative **1a–h** in the presence of HBTU/DIEA/DMF.[11] In all cases the reaction is completed after 4 h as confirmed by monitoring the presence of unreactive amino groups using the Kaiser test.

Compounds were released from the resin using TFA/H_2O (95:5) and isolated as oily residues. Except for **1c** crystallization from the oil was accomplished using cold ethyl ether or hexane. In most cases the final compounds were obtained in good yields and purity, as confirmed by LCMS and ^1H NMR analysis, so no further purification protocol was required. Minor impurities found in **1f**, **1g** and **1h**, the latter associated with the oxidation of the pyridine ring, could be easily removed by preparative HPLC under isocratic conditions (acetonitrile/water (50:50) with 0.1% formic acid as mobile phase). Since no impurities were detected for the rest of the acylglycines after cleavage from the resin, the differences in yields found among them are related to their partial solubility in the crystallization solvent rather than the presence of reaction byproducts.

Interestingly despite the fact that the synthesis of **1c** was designed to provide the cleavage and the hydrolysis of the methyl ester in a one pot reaction the structural analysis of the final compound revealed the presence of the ester functionality.

One of the most important aspects of the new strategy is its amenability for synthesizing several structurally-diverse acylglycines. In this manner compounds possessing alkyl, alkenyl, arylalkyl, arylalkenyl and heteroaryl moieties could be obtained in good yields and high purity using the same synthetic protocol.

In summary we have described an efficient solid phase synthetic protocol to prepare a wide range of acylglycine derivatives via coupling reaction between glycine-preloaded Wang resin and a set of carboxylic acids. The implemented procedure is compatible with several structurally-diverse acylglycines and it provides the final compounds with high yields and purity so additional purification protocols are not needed. To our knowledge this is the first example of solid phase synthesis of this type of acylglycine



Scheme 2. Solid phase synthesis of acylglycines.

derivatives. Further extension of solid phase methodologies to the synthesis of large numbers of other clinically relevant human metabolites will be the theme of future research.

Acknowledgements

CIHR (Canadian Institutes of health research), Genome Alberta, Alberta Advanced Education and Training (AAET) and NINT. The authors thanks Nikolaus Psychogius and Igor Sinelnikov for helpful hints on NMR and HPLC.

References and notes

1. Wishart, D. S.; Knox, C.; Guo, A. C.; Eisner, R.; Young, N.; Gautam, B.; Hau, D. D.; Psychogios, N.; Dong, E.; Bouatra, S.; Mandal, R.; Sinelnikov, I.; Xia, J.; Jia, L.; Cruz, J. A.; Lim, E.; Sobsey, C. A.; Shrivastava, S.; Huang, P.; Liu, P.; Fang, L.; Peng, J.; Fradette, R.; Cheng, D.; Tzur, D.; Clements, M.; Lewis, A.; De Souza, A.; Zuniga, A.; Dawe, M.; Xiong, Y.; Clive, D.; Greiner, R.; Nazyrova, A.; Shaykhutdinov, R.; Li, L.; Vogel, H. J.; Forsythe, I. *Nucleic Acids Res.* **2009**, *37*, D603.
2. Nandi, D. L.; Lucas, S. V.; Webster, L. T. *J. Biol. Chem.* **1979**, *254*, 7230.
3. Bonham Carter, S. M.; Watson, D. G.; Midgley, J. M.; Logan, R. W. *J. Chromatogr., B.* **1996**, *677*, 29.
4. Geurts, M.; Poupaert, J. H.; Scriba, G. K. E.; Lambert, D. M. *J. Med. Chem.* **1998**, *41*, 24.
5. Moon, S.-J.; Jeon, J. W.; Kim, H.; Suh, M. P.; Suh, J. *J. Am. Chem. Soc.* **2000**, *122*, 7742.
6. http://www.metabolomics.ca/News/sops.htm.
7. Brochu, J.-L.; Prakesch, M.; Enright, G. D.; Leek, D. M.; Arya, P. *J. Comb. Chem.* **2008**, *10*, 405.
8. Katrisky, A. R.; Gyanda, R.; Meher, N. K.; Song, Y. *Heterocycles* **2009**, *77*, 1249.
9. (a) Dörwald, F. Z. *Organic Synthesis on Solid Phase*; Wiley-VCH: Weinheim, 2000. pp 1–497; (b) Bräse, S.; Kirchhoff, J. H.; Köbberling, J. *Tetrahedron* **2003**, *59*, 885.
10. Hamper, B. C.; Jerome, k. D.; Yalamanchili, G.; Walker, D. M.; Chott, R. C.; Mischke, D. A. *Biotechnol. Bioeng.* **2000**, *71*, 28.
11. *General procedure for the synthesis of* **1**: The corresponding acid derivatives (4 equiv) and *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluoro-phosphate (HBTU), (3.8 equiv) are allowed to react in DMF (0.3 M) for 10 min. Then *N*-ethyldiisopropylamine (DIEA), (8 equiv) is added and the resulting solution is mixed with 1 equiv of glycine-preloaded Wang resin (*S* = 0.8 mmol/g), previously swollen in DMF, and the mixture is vigorously shaken for 4 h. The reaction mixture is filtered off and the resin is washed with DMF, DCM, MetOH and dried under vacuum. Dried resin is treated with TFA 95% in water for two hours, filtered off and washed several times with TFA. The filtrate is then concentrated under vacuum to afford the corresponding acylglycines.
12. Wishart, D. S. *Pharmacogenomics* **2007**, *1*, 91.