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A green and expedient synthesis of enantiopure diketopiperazines via enzymatic resolution of unnatural amino acids

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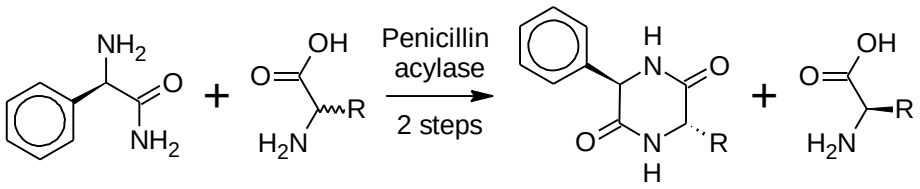
ABSTRACT

Dipeptides comprising a D-phenylglycyl moiety coupled to the L-enantiomer of 2-amino butyric acid, norvaline, norleucine and homocysteine were successfully synthesized from D-phenylglycine amide and the racemate of the corresponding unnatural amino acid. The reaction is catalyzed by an immobilized form of penicillin G acylase in an aqueous medium. The dipeptides were subsequently converted into the corresponding enantiopure diketopiperazines in overall isolated yields of 22% to 33%.

GRAPHICAL ABSTRACT



R Abu (-CH_2CH_3); Nva (-$CH_2CH_2CH_3$); Nle (-$CH_2CH_2CH_2CH_3$); Hcy (-CH_2CH_2SH)

Keywords: Diketopiperazine synthesis; Unnatural amino acids; Kinetic resolution; Penicillin acylase

Piperazine-2,5-diones represent a rich source of biologically interesting compounds.[1] The 2,5-diketopiperazine motif has been the subject of significant attention due to its biological properties.[2,3] These include alteration of blood clotting functions,[2] antitumor,[4] antiviral including HIV-I antagonists,[5,6,7] antifungal,[8] antibacterial,[9] antihyperglycemic,[10] and analgesic,[11] amongst others.[12] Diketopiperazines are now seen as ideal compounds for the rational design of new therapeutic agents.[13,14]

The applicability of these cyclic dipeptides extends beyond this, as they are also known for possessing catalytic activity in the hydrocyanation of aldehydes[15] and in the Strecker synthesis of amino acids.[16,17] In the former case it was shown that several diketopiperazines have enantioselective catalytic activity,[18] with enantiomeric excesses reaching 90%. In addition to this there is great potential displayed by simple peptides in asymmetric catalysis,[19] which has even been considered of prebiotic relevance.[20] The versatile scope of these compounds, together with the recent growth in research developed around them, suggests their full potential is yet to be explored.[21]

Most biologically active diketopiperazines have been obtained from natural sources.[12] One representative example is the case of the (*R*,*R*)-diketopiperazines obtained from marine bacteria. These show inhibitory activity against *Vibrio anguillarum*, a pathogenic bacterium responsible for vibriosis, suffered by wild and farmed bivalves, crustaceans and fishes, which in turn gives rise to severe economic losses, as it is a limitation in the development of aquaculture.[9]

Diketopiperazines can also be synthesized. Although they have frequently been deemed as unwanted side products, research regarding cyclic dipeptides has actually contributed to peptide synthesis in general.[22] This goal could be achieved by solid-phase strategies or solution-phase syntheses. Representative examples of solution-phase syntheses are thermal condensation of a dipeptide ester,[23] coupling of protected amino acids followed by deprotection and condensation,[24,25] a multicomponent approach (Ugi reaction),[26] and an innovative microwave-based procedure has also been reported.[27] Solid-phase strategies are more widely used and have been frequently employed in building libraries of these compounds.[12]

Biocatalysis is today a widely accepted method for the production of chemicals in general and enantiopure compounds in particular.[28-31] Enzyme-catalyzed transformations proceed under mild conditions of temperature and pressure at physiological pH in water as solvent, and are often highly chemo-, regio-, and enantioselective in addition to being atom and step economic.[32] An illustrative example is provided by the industrial synthesis of the artificial sweetener aspartame, a dipeptide, catalyzed by the metalloprotease, thermolysin.[33]

We have previously described a chemo-enzymatic route for the synthesis of some enantiopure diketopiperazines.[34] The key enzymatic step involved coupling of two enantiopure species, namely D-phenylglycine amide and an L-amino acid. This step was catalyzed by an immobilized form (Assemblase 7500®) of penicillin G acylase (penicillin amidohydrolase EC 3.5.1.11) from *Escherichia coli*, which is used in the manufacture of β-lactam antibiotics.[35,36] The resulting dipeptide was subsequently esterified followed by reaction with sodium hydroxide in methanol, to afford the corresponding diketopiperazine (Scheme 1). We have recently broadened the scope of this procedure by testing eleven new enantiopure amino acids, all with chemically functionalized side-chains.[37]

This work describes a further expansion of the scope and synthetic utility of this concise and practical methodology but uses a different approach. This consists of starting with a racemic mixture rather than a pure enantiomer, thereby integrating a kinetic resolution into the procedure. To this end, we have proceeded by ascertaining the viability of this route with substrates that had not been previously tested in this route and could afford interesting products, for example, new compounds or compounds with specific biological activity. [5]

Here we report the results of these studies, in which we tested the racemates of unnatural amino acids as one of the reactants. In addition to fulfilling the above mentioned pre-requisites, these racemic mixtures are significantly less expensive than the corresponding pure enantiomer (Scheme 2).

Dipeptide Syntheses

The enzymatic coupling reaction was performed at pH 9.8, in order to maximize the concentration of reactive amine nucleophile. The acyl donor was added in portions, in order to prevent D-phenylglycine amide induced inactivation, which is known to occur at high concentrations and high pH.[38] Only one coupling product was obtained in all the coupling reactions, consistent with the known selectivity of the enzyme catalyst and demonstrating the feasibility of the starting hypothesis (Table 1).[39]

The dipeptide derived from D-phenylglycine amide and L-norvaline coupling was obtained in pure form according to NMR spectroscopy, with no need for any further purification. The other three linear dipeptides were obtained with minor impurities, though they were easily purified. NMR analysis showed 10-20% impurity for D-phenylglycyl-L-norleucine, 10-11% for D-phenylglycyl-L-homocysteine and not quantifiable for the case of D-phenylglycyl-L-amino-butyric acid. The impurities were identified by NMR as unreacted amino acid in the first three cases and mass spectra indicated traces of D-phenylglycine in the last example. Nevertheless, this was not reflected in the melting points. The isolated yields (Table 1) fit within the window described previously.[34]

Table 1: Isolated yields of step a and steps b and c in the chemo-enzymatic dipeptide synthesis (see Scheme 2) [a]

Dipeptide	Residue	Linear Dipeptide	Diketopiperazine
D-PG-L-Abu	$-CH_2CH_3$	36%	62%
D-PG-L-Nva	$-CH_2CH_2CH_3$	46%	64%
D-PG-L-Nle	$-CH_2CH_2CH_2CH_3$	52%	63%
D-PG-L-Hcy	$-CH_2CH_2SH$	32%	not determined

[a] *Step a* isolated yield was calculated as η=100 x (product/substrate) x 2; *steps b and c* isolated yield was calculated as η=100 x (product/substrate)

Although the trend in our experiments suggests that nucleophiles with more carbon atoms seem to be preferred, the isolated yields depend strongly on solubility. It was previously found that the yield after crystallization is frequently lower than the observed HPLC yield and that this varies with the amino acid residue.[34, 35]

These reactions were carried out on a relatively small scale and were not optimized, suggesting there is ample room for further improvement in yields and product purity. The thiol function showed no hindering effect, as already observed with other chemical functionalities. [34, 35]

Both steps *b* and *c* (Scheme 2) afforded the desired compounds, as was shown previously with similar compounds.[34,35,39] The methyl ester intermediates were not isolated and were used directly in the condensation step, without any further purification or characterization. The final product precipitated from solution. All the expected diketopiperazines were obtained and three of the proposed four were obtained in pure form according to ^{1}H-NMR spectroscopy.

The diketopiperazine containing the L-homocysteine residue could not be dissolved for NMR analysis but was positively identified through mass spectrometry.

In conclusion, we have developed a green, cost-effective and chemo-enzymatic route for the synthesis of enantiopure 2,5-diketopiperazines containing unnatural amino acids, starting from the corresponding racemic amino acids. This was demonstrated in the synthesis of four dipeptides which were subsequently converted into the corresponding 2,5-diketopiperazines, all eight targeted compounds, some of which have known antiviral activity, [5] were isolated and/or identified. This suggests that the approach may be applicable to other cases where the route is already feasible with enantiopure amino acids.

Of the four coupling products, one was obtained pure and three were obtained with minor impurities. Three of the four possible diketopiperazines were obtained in pure state.

The presence of the D-enantiomer of the nucleophilic species did not seem to hinder the formation of the desired enantiopure linear dipeptide, allowing for a synthetic strategy involving a kinetic resolution. The dipeptide, D-PG-L-Abu, and its diketopiperazine were not previously known compounds.

References

1 Li, W.-R.; Yang, J. H.; *J. Comb. Chem.* **2002**, *4*, 106 - 108

2 McCleland, K.; Milne, P. J.; Lucieto, F. R.; Frost, C.; Brauns, S. C.; Van De Venter, M.; Du Plessis, J.; Dyason, K.; *J. Pharm. and Pharmacol.* **2004**, *56*, 1143 - 1153

3 Ressurreição, A. S. M.; Delatouche, R.; Gennare, C.; Piarulli, U.; *Eur. J. Org. Chem.* **2011**, 217 - 228

4 Nicholson, B.; Lloyd, G. K.; Miller, B. R.; Palladino, M. A.; Kiso, Y.; Hayashi, Y; Neuteboom, S. T. C.; *Anticancer Drugs* **2006** *17*, 25 - 31

5 Vanžura, J.; Palát, K.; Čeladník, M.; Hradec, K ; Klimes, J.; František, S.; CS Patent 210383, 1983; *Chem. Abstr*. **1984**; *417326*

6 Sinha, S.; Srivastava, R.; De Clercq, E.; Singh, R. K.; *Nucleosides Nucleotides Nucleic Acids* **2004**, *23*, 1815 - 1824

7 McCombie, S. W.; Tagat, J. R.; Vice, S. F.; Lin, S.-I. ; Steensma, R.; Palani, A.; Neustadt, B. R.; Baroudy. B. M.; Strizki, J. M.; Endres, M.; Cox, K.; Dan, N.; Chou, C.-C. ; *Bioorg. Med. Chem. Lett.* **2003**, *13*, 567 - 571

8 Houston, D. R.; Synstad, B.; Eijsink, V. G. H.; Stark, M. J. R.; Eggleston, I. M.; van Aalten, D.M.F.; *J. Med. Chem.* **2004**, *47*, 5713 - 5720

9 Fdhila, F.; Vásquez, V.; Sánchez, J. L.; Riguera, R.; *J. Nat. Prod.* **2003**, *66*, 1299 - 1301

10 Song, M. K.; Hwang, I. K.; Rosenthal, M. J.; Harris, D. M.; Yamaguchi, D. T.; Yip, I.; Go, V. L. W.; *Exp. Biol. Med.* **2003**, *228*, 1338 - 1345

11 Sasaki, Y.; Akutsu, Y.; Suzuki, K.; Sakurada, S.; Kisara, K.; *Chem. Pharm. Bull.* **1981**, *29*, 3403 - 3406

12 Martins, M. B.; Carvalho, I.; *Tetrahedron* **2007**, *63*, 9923 - 9938.

13 Avendaño, C.; de la Cuesta, E.; *Curr. Org. Synth.* **2009**, *6*, 143 - 168

14 Borthwick, A: D.; Liddle, J.; *Med. Res. Rev.* **2011**, *31*, 576 - 604

15 Tanaka, K.; Atsunori, M.; Inoue, S.; *J. Org. Chem.* **1989**, *55*, 181 - 185

16 Iyer, M. S.; Gigstad, K. M.; Namdev, M. D.; Lipton, M. ; *J. Am. Chem. Soc.* **1996**, *118*, 4910 - 4911

17 Kowalski, J.; Lipton, M. A.; *Tetrahedron Lett.* **1996**, *37*, *33*, 5839 - 5840

18 Jackson, W. R.; Jayatilake, G. S.; Matthews, B. R.; Wilshire, C.; *Aust. J. Chem.* **1988**, *41*, 203 - 213

19 Gruttadauria, M.; Giacalone, F.; Noto, R.; *Adv. Synth. Catal.* **2008**, *351*, 33 - 57

20 Carrea, G.; Colonna S.; Kelly, D. R.; Lazcano, A.; Ottolina, G.; Roberts, S. M. *Trends Biotechnol.* **2005**, *23*, 507 - 513

21 Wennemers, H.; *Chem. Commun.* **2011**, *47*, 12036 - 12041

22 Fischer, P. M.; *J. Pept. Sci.* **2003**, *9*, 9 - 35

23 Kanoh, K.; Kohno, S.; Katada, J.; Takahashi, J.; Uno, I.; Hayashi, Y.; *Bioorg. Med. Chem.* **1999**, *7*, 1451 - 1457

24 Zeng, Y.; Li, Q.; Hanzlik, R. P.; Aubé, J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3034 - 3038

25 Asada, S.; Kobayashi, Y.; Inoue S.; *Macromol. Chem. Phys.* **1985**, *186*, 1755 - 1762.

26 Cho, S.; Keum, G.; Kang, S. B.; Han, S.-Y.; Kim, Y.; *Mol. Divers.* **2003**, *6*, 283 - 286

27 Giraud, M.; Bernad, N.; Martinez, J.; Cavelier, F. *Tetrahedron Lett.* **2001**; *42*, 1895 - 1897

28 Bornscheurer, U. T.; Huisman, G. W.; Kazlauskas, R. J.; Lutz, S.; Moore, J. C.; Robins, K.; *Nature* **2012**; *485*, 185 – 194

29 Sheldon, R. A.; van Pelt, S.; *Chem. Soc. Rev.* **2013**; *42*, 6223 – 6235

30 Huisman, G. W.; Collier, S. J.; *Curr. Opin. Chem. Biol.* **2013**; *17*, 284 – 292

31 Patel, R. N.; *ACS Catal.* **2011**; *1*, 1056 - 1074

32 Sheldon, R. A.; *Chem. Soc. Rev.* **2012**; *41*, 1437 - 1451

33 Cheetham, P. S. J.; Straathof, A. J. J.; Adlercreutz, P. *Applied Biocatalysis* (2nd Edition); Harwood, Amsterdam, 2000; pp 93 - 152.

34 Khimiuk, A. Y.; Korennykh, A. V.; van Langen, L. M.; van Rantwijk, F.; Sheldon, R. A.; Svedas, V. K.; *Tetrahedron: Asymmetry* **2003**; *14*, 3123 – 3128

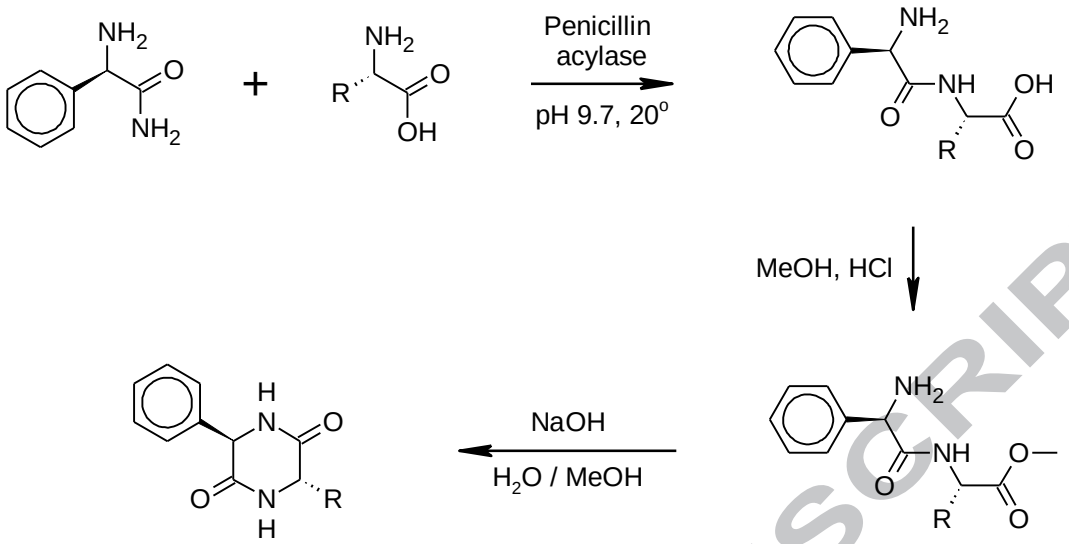
35 Bruggink, A.; Roos, E. C.; de Vroom, E.; *Org. Process Res. Dev.* **1998**; *2*, 128 - 133

36 Kallenberg, A. I.; van Rantwijk, F.; Sheldon, R. A.; *Adv. Synth. Catal.* **2005**; *347*, 905-926

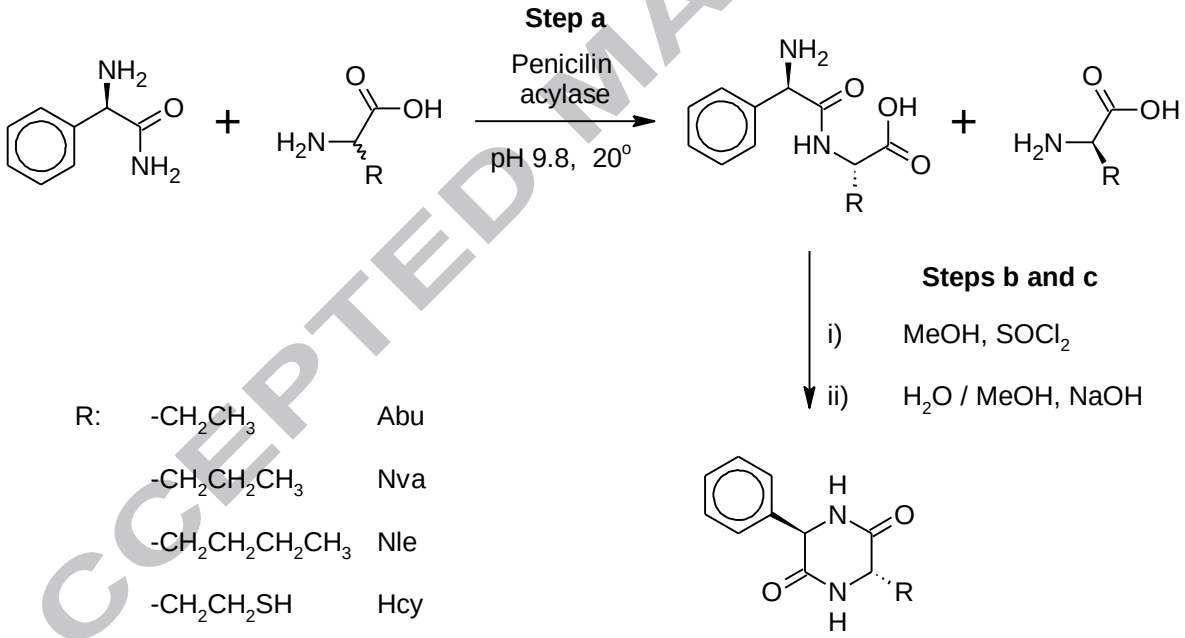
37 Pereira, P. C.; Sheldon, R. A.; Arends, I. W. C. E.; *Tetrahedron: Asymmetry* **2014** DOI 10.1016/j.tetasy.2014.04.005

38 Shcherbakova, T. A.; Korennykh, A. V.; van Langen, L. M.; Sheldon, R. A.; Švedas, V. K.; *J. Mol. Catal. B.: Enzym.* **2004**; *31*, 63 - 65

39 van Langen, L. M.; van Rantwijk, F.; Svedas, V. K.; Sheldon, R. A.; *Tetrahedron: Asymmetry* **2000**; *11*, 1077 – 1083



Scheme 1: Penicillin G acylase catalyzed diketopiperazine synthesis from enantiopure natural amino acids



R:		
	$-CH_2CH_3$	Abu
	$-CH_2CH_2CH_3$	Nva
	$-CH_2CH_2CH_2CH_3$	Nle
	$-CH_2CH_2SH$	Hcy

Scheme 2: Proposed penicillin G acylase catalyzed peptide synthesis with racemic unnatural amino acids