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Short Communication

Convenient syntheses of homopropargylglycine

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Abstract: An improved classic Strecker synthesis was elaborated leading to racemic homopropargylglycine (Hpg) in 61% overall yield, while an asymmetric Strecker reaction produced Hpg and the higher homolog 2-aminohept-6-ynoic acid in significantly higher yields and over 80% ee. Copyright © 2008 European Peptide Society and John Wiley & Sons, Ltd.

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Keywords: 2-amino-5-hexynoic acid; homopropargylglycine; noncanonical amino acids; Strecker reaction

Methods for engineering proteins by an amino acid reservoire beyond the canonical building blocks have advanced substantially in the past years. These are based on site-specific incorporation of noncanonical amino acids into proteins via the nonsense suppression approach [1,2] or by the residue-specific replacement of proteinogenic amino acids with synthetic structurally similar analogs through the use of an auxotrophic bacterial host starved for the natural amino acid and supplemented with the analog [3–5]. The residue-specific method was successfully applied for bioincorporation of various chalcogen and halogenated methionine analogs and most recently of azidohomoalanine (Aha) and homopropargylglycine (Hpg), respectively [6–10]. The latter methionine surrogates contain reactive site chains, which enable specific chemical transformations by the copper catalyzed Huisgen cycloaddition [11,12] without interferences from reactions with any of the natural building blocks. As reduction of Aha to 2,4-diaminobutyric acid at variable extents under the reducing intracellular conditions has been reported [7,13], the Hpg methionine surrogate appears to be the most suitable candidate. Accordingly, a relatively convenient synthesis would be highly recommendable.

Several syntheses of this alkyne-containing amino acid have been reported, but their reproduction, particularly in terms of yields, at least in our hands proved to be difficult. Among these procedures, alkylation of diethyl acetamidomalonate with the appropriate alkyl tosylate followed by decarboxylation and deprotection of the amine function is the most widely used, although the yields are very low (about 10%) [6]. The synthesis from 2-aminohexanoic acid requires harsh conditions and the problem of low yields remains unsolved [14]. An alternative route is based on the retrosynthesis of isotryptophan [15] and asymmetric amino acid synthesis mediated by (*S*)-2-[*N*′-(*N*-benzylprolyl)amino]benzophenone [16]. Hpg was also produced by oxidation of 4-pentynol to the aldehyde under Swern conditions, followed by the Strecker reaction to 2-aminohex-5-yne-1-nitrile, which was hydrolyzed to the racemic Hpg amide. This was enantiomerically resolved by leucine aminopeptidase from *Pseudomonas putida* to produce L-Hpg and D-Hpg amide [17,18].

This Strecker synthesis (Scheme 1) was readily reproduced in our hands yielding racemic Hpg in 61% yield. While hydrolysis of the intermediate nitrile **3** with 6 M HCl was found to produce the 5-chloro contaminant in varying amounts (1–5%), 10% aqueous H_2SO_4 led to a clean conversion of **3** into DL-Hpg (**4**). Attempts of enantioselective hydrolysis of **3** with recombinant nitrilase from *Arabidopsis thaliana* [19,20] (EC 3.5.5.1) failed. Therefore, the racemic amino acid obtained by H_2SO_4 hydrolysis was acetylated with $(Ac)_2O$ and the resulting Ac-DL-Hpg-OH was enantiomerically resolved with kidney acylase I [21] to produce L-Hpg in 69% of the theoretical 50% yield (see Supporting Information). Chiral analysis with the N^{α}-(2,4-dinitro-5-fluorophenyl)-L-alanine amide (FDAA) reagent [22] as well as comparison with an authentic example of L-Hpg-OH (gift from Dr Blaauw, Chiralix B.V.) confirmed the enantiomeric purity of the compound.

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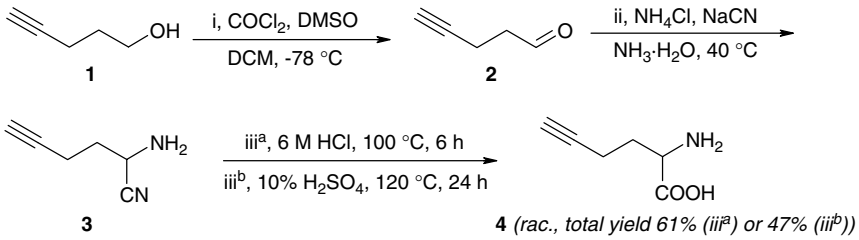
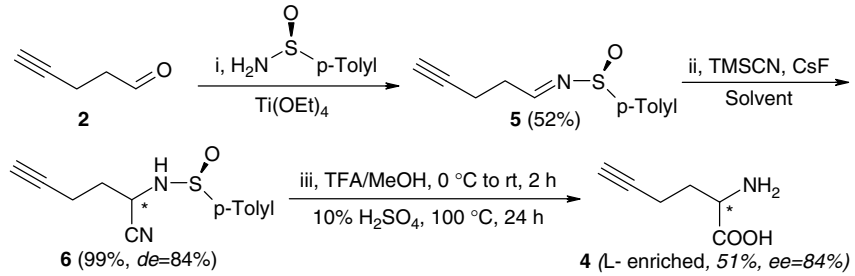


Scheme 1 Strecker synthesis of racemic Hpg.



Scheme 2 Diastereoselective Strecker synthesis of L-Hpg.

Table 1 The effect of solvent in the reaction of TMSCN with sulfinimines

Solvent	T (°C)	de[a] (%)	Yield[b] (%)
THF	Room temperature	75	95
THF	−50	84	99
Hex/THF(3/1)	−50	14	60
Et_2O	−50	68	85
MeOH	−50	22	95
DCM	−50	44	92
AcOEt	−50	83	95
CH_3CN	−50	20	95
toluene	−50	60	58

[a] de value for compound **6**.
[b] Yields of isolated compounds.

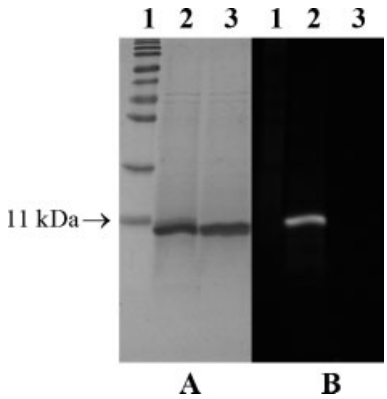


Figure 1 SDS gel analysis of the marker (lane 1), of the reaction products of Hpg[1]-barstar(C40A/C82A/P27A) with 5-azido-fluorescein (lane 2) and of barstar(C40A/C82A/P27A) (lane 3); (A) Coomassie stain and (B) fluorescence.

The diastereoselective Strecker reaction of enolizable aliphatic sulfimines proved to be significantly more efficient [23,24] and is outlined in Scheme 2. Hence a significant effect of the solvent on the reaction of trimethylsilyl cyanide (TMSCN) with sulfimines was observed as shown in Table 1. The intermediate (**6**) was then converted with TFA (2 equiv.) in methanol [25] to the nitrile, which was hydrolyzed with 10% H_2SO_4 at 100 °C. Hpg was isolated in 51% yield with an ee of 84% under optimal conditions. Higher enantiomeric purities are probably prevented by the unsufficient bulkiness of the *n*-butynyl side chain [24]. The superior efficiency of this synthetic route was also confirmed by comparing the two synthetic approaches in the preparation of the higher homolog 2-aminohept-6-ynoic acid (ee of 87%, see Supporting Information).

Since the enantiomeric purity of the supplied amino acid is not required for the expression of protein variants by residue-specific procedures because of the enantiospecificity of the translational machinery, the Hpg obtained by this procedure can directly be applied. When required, acetylation followed by enantiomeric resolution with acylase I [21] yields the desired L-Hpg (see preceding text).

Expression of Hpg-containing proteins using its racemic mixture, the L-enriched preparation, or the acetylated derivatives was studied using a pseudo-wt barstar mutant as model protein [26]. Engineered barstar (C40A/C82A/P27A) is a small bacterial ribonuclease inhibitor [27], which contains only one Met residue at the *N*-terminal position; this is not post-translationally processed by the Met-aminopeptidase because of the presence of a Lys residue in position 2 [28,29]. The protein is expressed in form of inclusion bodies, but can be efficiently refolded [27]. Using the Met-auxotropic strain B834(DE3), we were able to confirm the results of Tirrell *et al.* [10,30]; indeed Hpg is

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very efficiently incorporated into proteins, in the present case into the pseudo-wt barstar mutant. High expression levels were observed by comparing SDS gels of the lysates of parent barstar and the Met/Hpg-barstar variant. Quantitative replacement of Met with Hpg was confirmed by liquid chromatography-mass spectrometry (LC-MS) (expected mass for the parent barstar 10 252 and for the Hpg-variant 10 230; found: 10 230). Its conversion to a fluorescent derivative was readily achieved by reaction with 5-azido-fluorescein in the presence of Cu(I) as catalyst (Figure 1).

Supporting Information

Supporting information may be found in the online version of this article.

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REFERENCES

1. Wang L, Brock A, Herberich B, Schultz PG. Expanding the genetic code of *Escherichia coli*. *Science* 2001; **292**: 498–500.
2. Wang L, Schultz PG. Expanding the genetic code. *Angew. Chem., Int. Ed. Engl.* 2005; **44**: 34–66.
3. Cohen GN, Cowie DB. Biosynthesis by *Escherichia coli* of active altered proteins containing selenium instead of sulphur. *Biochim. Biophys. Acta* 1957; **26**: 252–261.
4. Budisa N, Steipe B, Demange P, Eckerskorn C, Kellermanm J, Huber R. High-level biosynthetic substitution of methionine in proteins by its analogs 20 aminohexanoic acid, selenomethionine, telluromethionine and ethionine in *Escherichia coli*. *Eur. J. Biochem.* 1995; **230**: 788–796.
5. Budisa N, Minks C, Alefelder S, Wenger W, Dong FM, Moroder L, Huber R. Toward the experimental codon reassignment in vivo: protein building with an expanded amino acid repertoire. *FASEB J.* 1999; **13**: 41–51.
6. Van Hest JCM, Kiick KL, Tirrell DA. Efficient incorporation of unsaturated methionine analogues into proteins in vivo. *J. Am. Chem. Soc.* 2000; **122**: 1282–1288.
7. Kiick KL, Saxon E, Tirrell DA, Bertozzi CR. Incorporation of azides into recombinant proteins for chemoselective modification by the Staudinger ligation. *Proc. Natl. Acad. Sci. U.S.A.* 2002; **99**: 19–24.
8. Link AJ, Mock ML, Tirell DA. Non-canonical amino acids in protein engineering. *Curr. Opin. Biotechnol.* 2003; **14**: 603–609.
9. Budisa N. Prolegomena to future experimental efforts on genetic code engineering by expanding its amino acid repertoire. *Angew. Chem., Int. Ed. Engl.* 2004; **43**: 6426–6463.
10. Connor RE, Tirell DA. Non-canonical amino acids in protein polymer design. *J. Macromol. Sci., Part C: Polym. Rev.* 2007; **47**: 9–28.
11. Rostovtsev VV, Green LG, Fokin VV, Sharpless KB. A stepwise Huisgen cycloaddition process: copper(I)-catalyzed regioselective ligation of azides and terminal alkynes. *Angew. Chem., Int. Ed. Engl.* 2002; **41**: 2596–2599.
12. Tornøe CW, Christensen C, Meldal M. Peptidotriazoles on solid phase: [1,2,3]-triazoles by regiospecific copper(I)-catalyzed 1,3-dipolar cycloadditions of terminal alkynes to azides. *J. Org. Chem.* 2002; **67**: 3057–3064.
13. Merkel L, Cheburkin Y, Wiltschi B, Budisa N. In vivo chemoenzymatic control of N-terminal processing in recombinant human epidermal growth factor. *ChemBioChem* 2007; **8**: 2227–2232.
14. Hatanaka SI, Niimura Y, Takishima K, Sugiyama J. (2*R*)-2-Amino-6-hydroxy-4-hexynoic acid, and related amino acids in the fruiting bodies of *amanita miculifera*. *Phytochemistry* 1998; **49**: 573–578.
15. Van Esseveldt BCJ, van Delft FL, Smits JMM, de Gelder R, Schoemaker HE, Rutjesa FPJT. Transition metal-catalyzed synthesis of novel biologically relevant tryptophan analogues. *Adv. Synth. Catal.* 2004; **346**: 823–834.
16. Collet S, Carreaux F, Boucher JL, Pethe S, Lepoivre M, Danion-Bougot R, Danion D. Synthesis and evaluation of ω-borono-α-amino acids as active-site probes of arginase and nitric oxide synthases. *J. Chem. Soc., Perkin Trans. 1* 2000; 177–182.
17. Wolf LB, Tjen KCMF, Rutjes FPJT, Hiemstra H, Schoemaker HE. Pd-Catalyzed cyclization reactions of acetylene-containing α-amino acids. *Tetrahedron Lett.* 1998; **39**: 5081–5084.
18. Wolf LB, Sonke T, Tjen KCMF, Kaptein B, Broxterman QB, Schoemaker HE, Rutjes FPJT. A biocatalytic route to enantiomerically pure unsaturated α-H-α-amino acids. *Adv. Synth. Catal.* 2001; **343**: 662–674.
19. Effenberger F, Oßwald S. (*E*)-Selective hydrolysis of (*E*, *Z*)-α,β-unsaturated nitriles by the recombinant nitrilase AtNIT1 from *Arabidopsis thaliana*. *Tetrahedron: Asymmetry* 2001; **12**: 2581–2587.
20. Effenberger F, Oßwald S. Enantioselective hydrolysis of (*RS*)-2-fluoroarylacetonitriles using nitrilase from *Arabidopsis thaliana*. *Tetrahedron: Asymmetry* 2001; **12**: 279–285.
21. Chenault HK, Dahmer J, Whitesides GM. Kinetic resolution of unnatural and rarely occurring amino acids: enantioselective hydrolysis of N-acyl amino acids catalyzed by acylase I. *J. Am. Chem. Soc.* 1989; **111**: 6354–6364.
22. Adamson JG, Hoang T, Crivici A, Lajoie GA. Use of Marfey's reagent to quantitate racemization upon anchoring of amino acids to solid supports for peptide synthesis. *Anal. Biochem.* 1992; **202**: 210–214.
23. Davis FA, Reddy RE, Portonovo PS. Asymmetric Strecker synthesis using enantiopure sulfinimines: A convenient synthesis of α-amino acids. *Tetrahedron Lett.* 1994; **35**: 9351–9354.
24. Li BF, Yuan K, Zhang MJ, Wu H, Dai LX, Wang QR, Hou XL. Highly diastereoselective Strecker reaction of enolizable aliphatic sulfinimines. *J. Org. Chem.* 2003; **68**: 6264–6267.
25. Lefebvre MI, Evans SA Jr. Studies toward the asymmetric synthesis of -amino phosphonic acids via the addition of phosphites to enantiopure sulfinimines. *J. Org. Chem.* 1997; **62**: 7532–7533.
26. Karnbrock W, Weyher E, Budisa N, Huber R, Moroder L. A new efficient synthesis of acetyltelluro- and acetylselenomethionine and their use in the biosynthesis of heavy-atom protein analogs. *J. Am. Chem. Soc.* 1996; **118**: 913–914.
27. Golbik R, Fischer G, Fersht AR. Folding of barstar C40A/C82A/P27A and catalysis of the peptidyl-prolyl cis/trans isomerization by human cytosolic cyclophilin (Cyp18). *Protein Sci.* 1999; **8**: 1505–1514.
28. Flinta C, Persson B, Jornvall H, Vonheijne G. Sequence determinants of cytosolic N-terminal protein processing. *Eur. J. Biochem.* 1986; **154**: 193–196.
29. Bradshaw RA, Brickey WW, Walker KW. N-Terminal processing: the methionine aminopeptidase and N$^{\alpha}$-acetyl transferase families. *Trends Biochem. Sci.* 1998; **23**: 263–267.
30. Beatty KE, Xie F, Wang Q, Tirrell DA. Selective dye-labeling of newly synthesized proteins in bacterial cells. *J. Am. Chem. Soc.* 2005; **127**: 14150–14151.

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