Published in final edited form as:

Org Lett. 2007 January 4; 9(1): 41–44. doi:10.1021/ol062401a.

Efficient Synthesis of Suitably Protected β -Difluoroalanine and γ -Difluorothreonine from L-Ascorbic Acid

Gongyong Li and Wilfred A. van der Donk*

Roger Adams Laboratory, Department of Chemistry, University of Illinois at Urbana-Champaign, 600 South Mathews Avenue, Urbana, Illinois 61801

Abstract

Fluorinated amino acids are useful building blocks for the preparation of biologically active peptides and peptidomimetics with increased metabolic stability. We report here the synthesis of two fluorinated amino acids, β -difluoroalanine and γ -difluorothreonine as analogs of Ser and Thr, respectively. These compounds were suitably protected for Fmoc-based solid phase peptide synthesis. Once incorporated into peptides, they may serve as alternative substrates or inhibitors of lantibiotic synthetases that post-translationally dehydrate Ser and Thr residues to dehydroalanine and dehydrobutyrine, respectively.

Michael acceptors have been popular functionalities for the design of enzyme inhibitors and active site affinity labels.¹ Dehydroalanines (Dha) and dehydrobutyrines (Dhb) are potential Michael acceptors and are present in a large number of natural products, including the microcystins,² nodularin,³ thiostrepton and other thiopeptides,⁴ and the lantibiotics.⁵ The electrophilicity of dehydroalanine (Dha) is significantly moderated compared to acrylamides due to the inherent enamine functionality in dehydro amino acids. This may explain why natural products containing dehydroalanines often interact with their targets by non-covalent mechanisms⁶ despite the presence of the Michael acceptor. Increasing the electrophilicity of dehydroalanines in natural products or designed inhibitors may lead to the development of powerful tools for mechanistic enzymology or for use in cell biology and signal transduction. Introduction of an electron withdrawing group on the terminal vinyl carbon would provide for the desired increased reactivity. If this functionality also consisted of a good leaving group, the Michael addition could be rendered irreversible by way of an addition-elimination pathway (Scheme 1).⁷ Fluorine-substituted dehydroalanines would be particularly attractive because of the small steric requirements of the fluorine substituent. Moreover, fluorinated amino acids have received much attention due to their potential applications in medicine.^{8,9,10}

In previous studies we have developed synthetic methodology to prepare fluorinated dehydroalanines.¹¹ In this contribution we describe the synthesis of precursor amino acids that are envisioned to be potential substrates for lantibiotic synthetases. This class of enzymes catalyzes the dehydration of Ser and Thr residues in their substrate peptides resulting in formation of Dha and Dhb structures.⁵ With the recent successful in vitro reconstitution of these proteins,¹² they can now be evaluated as potential tools for the construction of more reactive Dha and Dhb analogs. One possibility would be replacement of a Ser in the substrates for dehydration by a difluoroalanine. Enzymatic elimination of one of the fluorines of difluoroalanine^{11b} would then produce a fluoro-Dha (Scheme 2). Alternatively, replacement of the methyl group of Thr with a fluorinated methyl group and subsequent enzymatic dehydration would result in a Dhb analog with increased electrophilicity at the β -carbon.

We report here efficient routes to (2*R*)- β -difluoroalanine (**1**) and (2*S*,3*S*)- γ -difluoro-threonine (**2**) appropriately protected for use in Fmoc-based solid phase peptide synthesis (SPPS). We elected to prepare difluorinated alanine and threonine derivatives as they would be sterically less demanding than the corresponding trifluorinated analogs, and because trifluoroalanine incorporation into peptides is challenging and the products have been reported to have low chemical and configurational stability at physiological pH.¹³ Previous syntheses of difluoroalanine derivatives have been mostly racemic¹⁴ with some asymmetric routes reported.¹⁵

Synthesis of Fmoc- β -Difluoroalanine (**1**)

L-Glyceraldehyde acetonide **3** is commercially available or can be readily prepared from L-ascorbic acid in a large scale in 47% overall yield,¹⁶ or from commercially available 5,6-isopropylidene-L-gulonono-1,4-lactone in one step (56%).¹⁷ The compound was fluorinated with diethylamino sulfurtrifluoride (DAST) to afford compound **4** in 89% yield (Scheme 3).¹⁸ Treatment of **4** with hydrochloric acid in methanol, was followed by selective protection of the primary hydroxyl with a *tert*-butyldimethylsilyl group in 88% yield. The secondary alcohol in product **5** was transformed into azide **6** in 70% yield by reaction with trifluoromethylsulfonic anhydride in pyridine and subsequent treatment with sodium azide. The azide **6** was transformed in 86% yield into Fmoc protected amine **7** by reduction of the azide group to the amine and reaction with FmocCl/NaHCO₃.¹⁹ The TBS group was removed with HF/pyridine to afford Fmoc protected amino alcohol in quantitative yield. Attempts to oxidize the amino alcohol by NaIO₄/RuCl₃ induced partial Fmoc deprotection resulting in isolation of the desired amino acid **1** in only 33%. However, oxidation of the amino alcohol with Jones reagent afforded the target **1** in 81% yield without loss of stereochemical purity.¹⁹ Using L-glyceraldehyde acetonide (**3**) as starting material, Fmoc-difluoroAla-OH (**1**) was obtained in an overall yield of 38%.

Synthesis of Fmoc- γ -Difluoro-Thr(O^{*t*}Bu)-OH (**2**)

Given the successful synthesis of Fmoc- β -difluoro-Ala-OH (**1**) from L-ascorbic acid, we attempted to use the same strategy to prepare Fmoc- γ -difluoro-Thr(O^{*t*}Bu)-OH²⁰ from advanced intermediate **8**, prepared from L-ascorbic acid in three steps in 76% yield.²¹ The hydroxyl group of compound **8** was protected with either a benzyl or *tert*-butyl group to give compounds **9a** and **9b** in 91% and 90% yield, respectively. The benzyl protection group provides more versatility downstream whereas the *tert*-butyl group needs to be installed eventually in the final product since it is the protecting group of choice for alcohols in Fmoc-based SPPS. Compound **9a** was reduced by DIBAL-H and fluorinated with DAST to yield difluoride **10** in 67% yield. Only one diastereomer was detected by ¹H NMR analysis. However, treatment of **9b** did not provide compound **10**,^b presumably because of steric hindrance. The isopropylidene group was then removed from **10** with hydrochloric acid in methanol and the liberated primary alcohol was selectively protected with a *tert*-butyldimethylsilyl group to afford compound **11** in 86% yield (Scheme 4).

The secondary alcohol of compound **11** was converted to the trifluoromethylsulfonate, and the crude product was subsequently reacted with sodium azide providing compound **12** in a yield of 79%. The azide functionality in **12** was hydrogenated in the presence of Pd(OH)₂/C to afford the corresponding amino alcohol, which was reacted with FmocCl/NaHCO₃ to afford compound **13** in 82% yield. It should be noted that using Pd/C as catalyst the azide group in compound **12** could be selectively reduced without debenzylation. Attempts to protect the hydroxyl group of compound **13** with isobutene and catalytic H₂SO₄ provided the desired product in only 21% with products in which the TBS group had been removed making up the mass balance. However, repeated treatment of compound **13** with *t*-BuBr/Ag₂O provided **14**

in an overall yield of 81%. Deprotection of the TBS group was carried out in quantitative yield by using a solution of commercial HF/pyridine in THF after adjustment of the pH to 5 by the addition of pyridine; without the addition of pyridine a complex product mixture was obtained. Finally, subjection of the crude amino alcohol to Jones reagent afforded the final product Fmoc- γ -difluoro-Thr(O^tBu)-OH (**2**) in 73% yield.

In summary, two difluoro amino acids were efficiently synthesized from L-ascorbic acid without loss of stereochemical purity. Given the well-known success of fluorine containing pharmaceuticals,²² including fluoropeptides,²³ the current approach expands the arsenal of fluorinated moieties that can be used in the preparation of bioactive molecules. The fluorinated amino acids will be incorporated into the peptide substrate for lactacin 481 synthetase^{11a} to investigate the enzymatic generation of fluorinated dehydro amino acids within oligopeptides.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

This work was supported by the National Institutes of Health (GM58822). WAV is an Alfred P. Sloan Fellow and Camille Dreyfus Teacher-Scholar. NMR spectra were obtained in the Varian Oxford Instrument Center for Excellence, funded in part by the W. M. Keck Foundation, NIH (S10 RR10444), and NSF (CHE 96-10502).

References

- (a) Walsh C. *Tetrahedron* 1982;38:871. (b) Walsh CT. *Annu. Rev. Biochem* 1984;53:493. [PubMed: 6433782] Silverman, RB. *Mechanism-based Enzyme Inactivation: Chemistry and Enzymology*. Boca Raton, FL: CRC Press; 1988.
- (a) Botes D, Tuinman A, Wessels P, Viljoen C, Kruger H, Williams DH, Santikarn S, Smith R, Hammond SJ. *Chem. Soc. Perkin Trans* 1984;1:2311. (b) Painuly P, Perez R, Fukai T, Shimizu Y. *Tetrahedron Lett* 1988;29:11. (c) Dawson RM. *Toxicon* 1998;36:953. [PubMed: 9690788]
- (a) Namikoshi M, Rinehart KL. *J. Industr. Microbiol* 1996;17:373. (b) Botes D, Wessels P, Kruger H, Runnegar MTC, Santikarn S, Smith R, Barna JCJ, Williams DH. *J. Chem. Soc. Perkin Trans* 1985;1:2747. (b) Rinehart KL, Harada K, Namikoshi M, Chen C, Harvis C, Munro MHG, Blunt J, Mulligan P, Beasley V, Dahlem A, Carmicheal W. *J. Am. Chem. Soc* 1988;110:8557.
- (a) Anderson B, Hodgkin DC, Viswamitra MA. *Nature* 1970;225:233. [PubMed: 5409975] (b) Walker J, Olesker A, Valente L, Rabanal R, Lukacs G. *J. Chem. Soc. Chem. Commun* 1977:706. (c) Bycroft BW, Gowland MS. *J. Chem. Soc. Chem. Commun* 1978:256. (d) Lau RC, Rinehart KL. *J. Antibiot* 1994;47:1466. [PubMed: 7844041]
- Chatterjee C, Paul M, van der Donk WA. *Chem. Rev* 2005;105:633. [PubMed: 15700960]
- Breukink E, de Kruijff B. *Nat. Rev. Drugs Disc* 2006;5:321.
- (a) Wang EA, Walsh C. *Biochemistry* 1981;20:7539. [PubMed: 7034778] (b) Silverman RB, Bichler KA, Leon AJ. *J. Am. Chem. Soc* 1996;118:1253. (b) Silverman RB, Bichler KA, Leon AJ. *J. Am. Chem. Soc* 1996;118:1241. (c) Pan Y, Qiu J, Silverman RB. *J. Med. Chem* 2003;46:5292. [PubMed: 14640537] (d) Berkowitz DB, de la Salud-Bea R, Jahng WJ. *Org. Lett* 2004;6:1821. [PubMed: 15151423]
- For reviews see Soloshonok VA *Enantiocontrolled Synthesis of Fluoro-Organic Compounds* 1999 Chichester Wiley Welch, JT.; Eswarakrishnan, S., editors. *Fluorine in Bioorganic Chemistry*. New York: Wiley; 1991. Kollonitsch, J. *Biochemical Aspects of Fluorine Chemistry*. Filler, R.; Kobayashi, Y., editors. Tokyo and New York: Kodansha Ltd. And Elsevier Biomedical; 1982.
- See for example Metcalf BW, Bey P, Danzin C, Jung MJ, Casara P, Vevert JP. *J. Am. Soc. Chem* 1978;100:2551. (b) Tsushima T, Kawada K. *Tetrahedron Lett* 1985;26:2445. (c) Hart BP, Coward JK. *Tetrahedron Lett* 1993;34:4917. (d) Shi G, Cai WJ. *Org. Chem* 1995;60:6289. (e) Percy JM, Prime ME. *J. Org. Chem* 1998;63:8049. (f) Edwards PD, Andisik DW, Bryant CA, Ewing B, Gomes B,

- Lewis JJ, Rakiewicz D, Steelman G, Strimpler A, Trainor DA, Tuthill PA, Mauger RC, Veale CA, Wildonger RA, Williams JC, Wolanin DJ, Zottola M. *J. Med. Chem* 1997;40:1876. [PubMed: 9191965] (g) Cregge RJ, Durham SL, Farr RA, Gallion SL, Hare CM, Hoffman RV, Janusz MJ, Kim H-O, Koehl JR, Mehdi S, Metz WA, Peet NP, Pelton JT, Schreuder HA, Sunder S, Tardif C. *J. Med. Chem* 1998;41:2461. [PubMed: 9651152]
10. For asymmetric procedures, see for example Guanti G, Banfi L, Narisano E. *Tetrahedron* 1988;44:5553. (b) Baldwin JE, Lynch GP, Schofield CJ. *J. Chem. Soc., Chem. Commun* 1991:736. (c) Kitazume T, Lin JT, Yamazaki T. *Tetrahedron: Asymmetry* 1991;2:235. (d) Von dem Bussche-Huenefeld C, Seebach D. *Chem. Ber* 1992;125:1273. (e) Watanabe H, Hashizume Y, Uneyama K. *Tetrahedron Lett* 1992;33:4333. (f) Sewald N, Seymour LC, Butrger K, Osipov SN, Kolomiets A, Fokin AV. *Tetrahedron: Asymmetry* 1994;5:1051. (g) Soloshonok VA. *ACS Symp. Ser* 1996;639:26–41. (h) Sakai T, Yan F, Kashino S, Uneyama K. *Tetrahedron* 1996;52:233. (i) Sting AR, Seebach D. *Tetrahedron* 1996;52:279. (j) Arnone A, Bravo P, Capelli S, Fronza G, Meille SV, Zanda M. *J. Org. Chem* 1996;61:3375. (k) Soloshonok VA, Kacharov AD, Avilov DV, Ishikawa K, Nagashima N, Hayashi T. *J. Org. Chem* 1997;62:3470. (l) Demir AS, Sesenoglu O, Gercek-Arkin Z. *Tetrahedron: Asymmetry* 2001;12:2309.
11. (a) Zhou H, van der Donk WA. *Org. Lett* 2001;3:593. [PubMed: 11178833] (b) Zhou H, Schmidt DM, Gerlt JA, van der Donk WA. *ChemBioChem* 2003;4:1206. [PubMed: 14613113]
12. (a) Xie L, Miller L, Chatterjee C, Averin O, Kelleher NL, van der Donk WA. *Science* 2004;303:679. [PubMed: 14752162] (b) Li B, Yu J-PJ, Brunzelle JS, Moll GN, van der Donk WA, Nair SK. *Science* 2006;311:1464. [PubMed: 16527981]
13. Hoess E, Rudolph M, Seymour L, Schierlinger C, Burger K. *J. Fluor. Chem* 1993;61:163. and references therein (b) Bordusa F, Dahl C, Jakubke H-D, Burger K, Koks B. *Tetrahedron: Asymmetry* 1999;10:307. (c) Osipov SN, Burger K. *Tetrahedron Lett* 2000;41:5659. (d) Sani M, Bruche L, Chiva G, Fustero S, Piera J, Volonterio A, Zanda M. *Angew. Chem. Int. Ed. Engl* 2003;42:2060. [PubMed: 12746823]
14. (a) Kollonitsch J, Marburg S, Perkins LM. *J. Org. Chem* 1976;41:3107. (b) Tsushima T, Kawada K. *Tetrahedron Lett* 1985;26:2445. (c) D'Orchymont H. *Synthesis* 1993:961. (d) Gerus II, Kolomeitsev AA, Kolycheva MI, Kukhar VP. *J. Fluor. Chem* 2000;105:31.
15. (a) Fustero S, Navarro A, Pina B, Soler JG, Bartolomé A, Asensio A, Simón A, Bravo P, Fronza G, Volonterio A, Zanda M. *Org. Lett* 2001;3:2621. [PubMed: 11506593] (b) Katagiri T, Michiharu M, Matsukawa Y, Kumar JS, Uneyama K. *Tetrahedron: Asymmetry* 2001;12:1303. (c) Abe H, Amii H, Uneyama K. *Org. Lett* 2001;3:313. [PubMed: 11428002]
16. (a) Andres GC, Crawford TC, Bacon BE. *J. Org. Chem* 1981;46:2976. (b) Hubschwerlen C. *Synthesis* 1986:962.
17. Hubschwerlen C, Specklin J-L, Higelin J. *Org. Synth* 1993;72:1.
18. Xu Y, Prestwich GD. *J. Org. Chem* 2002;67:7158. [PubMed: 12354017]
19. Mosher's amide analysis of the free amines **7a** and **1a** both showed an e.r. of 1.00:0.04, see supporting information.
20. Both enantiomers of γ -difluoro Thr have been previously prepared in unprotected form using lipase catalyzed resolutions: Shimizu M, Yokota T, Fujimori K, Fujisawa T. *Tetrahedron: Asymmetry* 1993;4:835.
21. Dahlgren A, Kvarnström I, Vrang L, Hamelink E, Hallberg A, Rosenquist A, Samuelsson B. *Bioorg. & Med. Chem* 2003;11:827. [PubMed: 12614868]
22. (a) Abeles RH, Alston TA. *J. Biol. Chem* 1990;265:16705–16708. [PubMed: 2211585] Filler, R.; Kobayashi, Y.; Yagupolskii, LM. *Organofluorine Compounds in Medicinal Chemistry and Biomedical Applications*. Elsevier; 1993. Ojima, I.; Welch, JT.; McCarthy, JR. *Biomedical Frontiers of Fluorine Chemistry*. American Chemical Society; 1996.
23. Imperiali B. *Adv. Biotechnol. Processes* 1988;10:97–129. [PubMed: 3058149]

Scheme 1.

Scheme 2.

Scheme 3.

Scheme 4.