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

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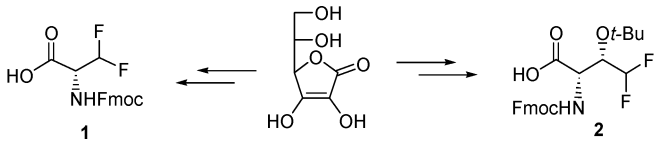
ABSTRACT



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 analogues 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 posttranslationally 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 dehydroamino acids. This may explain why natural products containing dehydroalanines often interact with their targets by noncovalent mechanisms[6] 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

(1) (a) Walsh, C. *Tetrahedron* **1982**, *38*, 871. (b) Walsh, C. T. *Annu. Rev. Biochem.* **1984**, *53*, 493. (c) Silverman, R. B. *Mechanism-based Enzyme Inactivation: Chemistry and Enzymology*; CRC Press: Boca Raton, FL, 1988.

(2) (a) Botes, D.; Tuinman, A.; Wessels, P.; Viljoen, C.; Kruger, H.; Williams, D. H.; Santikarn, S.; Smith, R.; Hammond, S. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2311. (b) Painuly, P.; Perez, R.; Fukai, T.; Shimizu, Y. *Tetrahedron Lett.* **1988**, *29*, 11. (c) Dawson, R. M. *Toxicon* **1998**, *36*, 953.

(3) (a) Namikoshi, M.; Rinehart, K. L. *J. Industr. Microbiol.* **1996**, *17*, 373. (b) Botes, D.; Wessels, P.; Kruger, H.; Runnegar, M. T. C.; Santikarn, S.; Smith, R.; Barna, J. C. J.; Williams, D. H. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2747. (c) Rinehart, K. L.; Harada, K.; Namikoshi, M.; Chen, C.; Harvis, C.; Munro, M. H. G.; Blunt, J.; Mulligan, P.; Beasley, V.; Dahlem, A.; Carmicheal, W. *J. Am. Chem. Soc.* **1988**, *110*, 8557.

(4) (a) Anderson, B.; Hodgkin, D. C.; Viswamitra, M. A. *Nature* **1970**, *225*, 233. (b) Walker, J.; Olesker, A.; Valente, L.; Rabanal, R.; Lukacs, G. *J. Chem. Soc., Chem. Commun.* **1977**, 706. (c) Bycroft, B. W.; Gowland, M. S. *J. Chem. Soc., Chem. Commun.* **1978**, 256. (d) Lau, R. C.; Rinehart, K. L. *J. Antibiot.* **1994**, *47*, 1466.

(5) Chatterjee, C.; Paul, M.; van der Donk, W. A. *Chem. Rev.* **2005**, *105*, 633.

(6) Breukink, E.; de Kruijff, B. *Nat. Rev. Drug Discovery* **2006**, *5*, 321.

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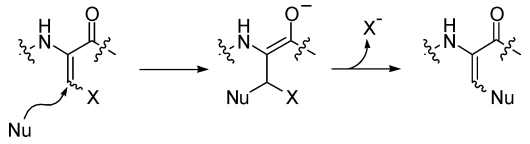
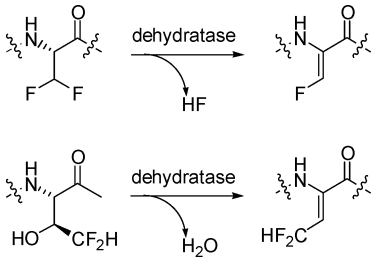
Scheme 1



Scheme 2



much attention due to their potential applications in medicine.[8−10]

In previous studies, we have developed a 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 analogues. One possibility would be the 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 analogue with increased electrophilicity at the β-carbon.

We report here efficient routes to (2*R*)-β-difluoroalanine (**1**) and (2*S*,3*S*)-γ-difluorothreonine (**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 analogues 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 racemic[14] 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-gulono-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

(7) (a) Wang, E. A.; Walsh, C. *Biochemistry* **1981**, *20*, 7539. (b) Silverman, R. B.; Bichler, K. A.; Leon, A. J. *J. Am. Chem. Soc.* **1996**, *118*, 1253. (b) Silverman, R. B.; Bichler, K. A.; Leon, A. J. *J. Am. Chem. Soc.* **1996**, *118*, 1241. (c) Pan, Y.; Qiu, J.; Silverman, R. B. *J. Med. Chem.* **2003**, *46*, 5292. (d) Berkowitz, D. B.; de la Salud-Bea, R.; Jahng, W. J. *Org. Lett.* **2004**, *6*, 1821.

(8) For reviews, see: (a) *Enantiocontrolled Synthesis of Fluoro-Organic Compounds*; Soloshonok, V. A., Ed.; Wiley: Chichester, 1999. (b) *Fluorine in Bioorganic Chemistry*; Welch, J. T., Eswarakrishnan, S. Eds.; Wiley: New York, 1991. (c) Kollonitsch, J. In *Biochemical Aspects of Fluorine Chemistry*; Filler, R., Kobayashi, Y., Eds.; Kodansha Ltd. And Elsevier Biomedical: Tokyo and New York, 1982.

(9) See, for example: (a) Metcalf, B. W.; Bey, P.; Danzin, C.; Jung, M. J.; Casara, P.; Vevert, J. P. *J. Am. Soc. Chem.* **1978**, *100*, 2551. (b) Tsushima, T.; Kawada, K. *Tetrahedron Lett.* **1985**, *26*, 2445. (c) Hart, B. P.; Coward, J. K. *Tetrahedron Lett.* **1993**, *34*, 4917. (d) Shi, G.; Cai, W. *J. Org. Chem.* **1995**, *60*, 6289. (e) Percy, J. M.; Prime, M. E. *J. Org. Chem.* **1998**, *63*, 8049. (f) Edwards, P. D.; Andisik, D. W.; Bryant, C. A.; Ewing, B.; Gomes, B.; Lewis, J. J.; Rakiewicz, D.; Steelman, G.; Strimpler, A.; Trainor, D. A.; Tuthill, P. A.; Mauger, R. C.; Veale, C. A.; Wildonger, R. A.; Williams, J. C.; Wolanin, D. J.; Zottola, M. *J. Med. Chem.* **1997**, *40*, 1876. (g) Cregge, R. J.; Durham, S. L.; Farr, R. A.; Gallion, S. L.; Hare, C. M.; Hoffman, R. V.; Janusz, M. J.; Kim, H.-O.; Koehl, J. R.; Mehdi, S.; Metz, W. A.; Peet, N. P.; Pelton, J. T.; Schreuder, H. A.; Sunder, S.; Tardif, C. *J. Med. Chem.* **1998**, *41*, 2461.

(10) For asymmetric procedures, see, for example: (a) Guanti, G.; Banfi, L.; Narisano, E. *Tetrahedron* **1988**, *44*, 5553. (b) Baldwin, J. E.; Lynch, G. P.; Schofield, C. J. *J. Chem. Soc., Chem. Commun.* **1991**, 736. (c) Kitazume, T.; Lin, J. T.; Yamazaki, T. *Tetrahedron: Asymmetry* **1991**, *2*, 235. (d) Von dem Bussche-Huennefeld, C.; Seebach, D. *Chem. Ber.* **1992**, *125*, 1273. (e) Watanabe, H.; Hashizume, Y.; Uneyama, K. *Tetrahedron Lett.* **1992**, *33*, 4333. (f) Sewald, N.; Seymour, L. C.; Butrger, K.; Osipov, S. N.; Kolomiets, A.; Fokin, A. V. *Tetrahedron: Asymmetry* **1994**, *5*, 1051. (g) Soloshonok, V. A. *ACS Symp. Ser.* **1996**, *639*, 26−41. (h) Sakai, T.; Yan, F.; Kashino, S.; Uneyama, K. *Tetrahedron* **1996**, *52*, 233. (i) Sting, A. R.; Seebach, D. *Tetrahedron* **1996**, *52*, 279. (j) Arnone, A.; Bravo, P.; Capelli, S.; Fronza, G.; Meille, S. V.; Zanda, M. *J. Org. Chem.* **1996**, *61*, 3375. (k) Soloshonok, V. A.; Kacharov, A. D.; Avilov, D. V.; Ishikawa, K.; Nagashima, N.; Hayashi, T. *J. Org. Chem.* **1997**, *62*, 3470. (l) Demir, A. S.; Sesenoglu, O.; Gercek-Arkin, Z. *Tetrahedron: Asymmetry* **2001**, *12*, 2309.

(11) (a) Zhou, H.; van der Donk, W. A. *Org. Lett.* **2001**, *3*, 593. (b) Zhou, H.; Schmidt, D. M.; Gerlt, J. A.; van der Donk, W. A. *Chem. Bio. Chem.* **2003**, *4*, 1206.

(12) (a) Xie, L.; Miller, L.; Chatterjee, C.; Averin, O.; Kelleher, N. L.; van der Donk, W. A. *Science* **2004**, *303*, 679. (b) Li, B.; Yu, J.-P. J.; Brunzelle, J. S.; Moll, G. N.; van der Donk, W. A.; Nair, S. K. *Science* **2006**, *311*, 1464.

(13) (a) Hoess, E.; Rudolph, M.; Seymour, L.; Schierlinger, C.; Burger, K. *J. Fluorine Chem.* **1993**, *61*, 163 and references therein. (b) Bordusa, F.; Dahl, C.; Jakubke, H.-D.; Burger, K.; Koksch, B. *Tetrahedron: Asymmetry* **1999**, *10*, 307. (c) Osipov, S. N.; 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.* **2003**, *42*, 2060.

(14) (a) Kollonitsch, J.; Marburg, S.; Perkins, L. M. *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, I. I.; Kolomeitsev, A. A.; Kolycheva, M. I.; Kukhar, V. P. *J. Fluorine Chem.* **2000**, *105*, 31.

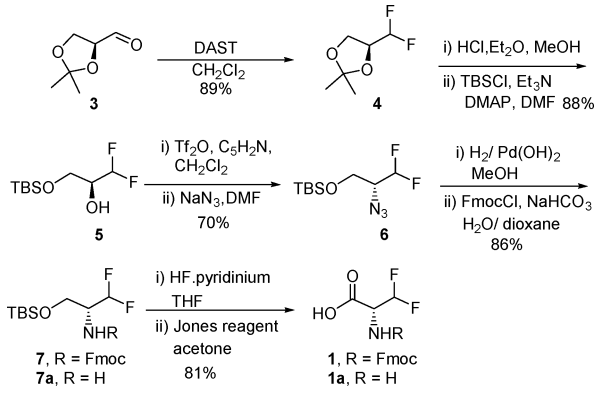
(15) (a) Fustero, S.; Navarro, A.; Pina, B.; Soler, J. G.; Bartolomé, A.; Asensio, A.; Simón, A.; Bravo, P.; Fronza, G.; Volonterio, A.; Zanda, M. *Org. Lett.* **2001**, *3*, 2621. (b) Katagiri, T.; Michiharu, M.; Matsukava, Y.; Kumar, J. S. D.; Uneyama, K. *Tetrahedron: Asymmetry* **2001**, *12*, 1303. (c) Abe, H.; Amii, H.; Uneyama, K. *Org. Lett.* **2001**, *3*, 313.

(16) (a) Andres, G. C.; Crawford, T. C.; Bacon, B. E. *J. Org. Chem.* **1981**, *46*, 2976. (b) Hubschwerlen, C. *Synthesis* **1986**, 962.

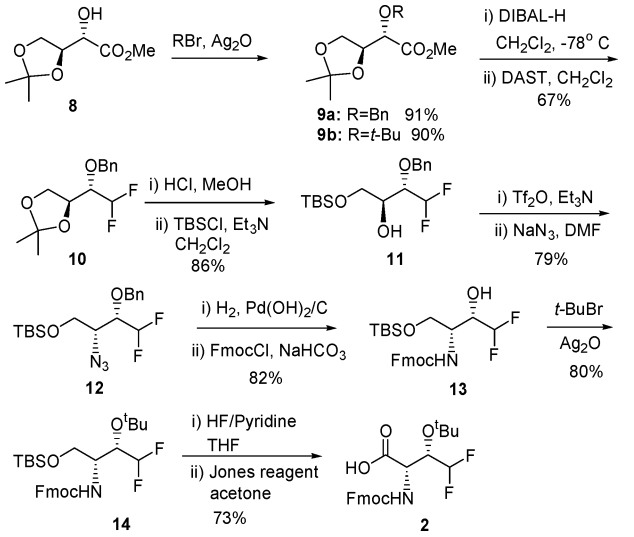
(17) Hubschwerlen, C.; Specklin, J.-L.; Higelin, J. *Org. Synth.* **1993**, *72*, 1.

(18) Xu, Y.; Prestwich, G. D. *J. Org. Chem.* **2002**, *67*, 7158.

Scheme 3



Scheme 4



with FmocCl/$NaHCO_3$.[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 $NaIO_4$/$RuCl_3$ induced partial Fmoc deprotection resulting in isolation of the desired amino acid **1** in only 33% yield. However, oxidation of the amino alcohol with the Jones reagent afforded the target **1** in 81% yield without loss of stereochemical purity.[19] Using L-glyceraldehyde acetonide (**3**) as the starting material, Fmoc-difluoro-Ala-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)-OH[20] 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% yields, respectively. The benzyl protection group provides more versatility downstream, whereas the *tert*-butyl group needs to be installed eventually in the final product because 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 ^{1}H NMR analysis. However, treatment of **9b** did not provide compound **10b**, 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/$NaHCO_3$ to afford compound **13** in 82% yield. It should be noted that using Pd/C as the 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 H_2SO_4 provided the desired product in only 21% yield with products in which the TBS group had been removed making up the mass balance. However, repeated treatment of compound **13** with *t*-BuBr/Ag_2O 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 the Jones reagent afforded the final product Fmoc-γ-difluoro-Thr(O^{t}Bu)-OH (**2**) in 73% yield.

In summary, two difluoroamino acids were efficiently synthesized from L-ascorbic acid. 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 lacticin 481 synthetase[12a] to investigate the enzymatic generation of fluorinated dehydroamino acids within oligopeptides.

Acknowledgment. This work was supported by the National Institutes of Health (GM58822). W.A.V. is an

(19) Mosher's amide analysis of the free amines **7a** and **1a** both showed an er 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.

(22) (a) Abeles, R. H.; Alston, T. A. *J. Biol. Chem.* **1990**, *265*, 16705−16708. (b) Filler, R.; Kobayashi, Y.; Yagupolskii, L. M. *Organofluorine Compounds in Medicinal Chemistry and Biomedical Applications*; Elsevier: New York, 1993. (c) Ojima, I.; Welch, J. T.; McCarthy, J. R. *Biomedical Frontiers of Fluorine Chemistry*; American Chemical Society: Washington DC, 1996.

(23) Imperiali, B. *Adv. Biotechnol. Processes* **1988**, *10*, 97−129.

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Supporting Information Available: Detailed experimental procedures and copies of ^{1}H, ^{13}C, and ^{19}F NMR spectra of all unknown compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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