ORGANIC LETTERS

2001
Vol. 3, No. 4
593−596

Synthesis of 2-Amino-3-fluoroacrylic Acid Containing Peptides

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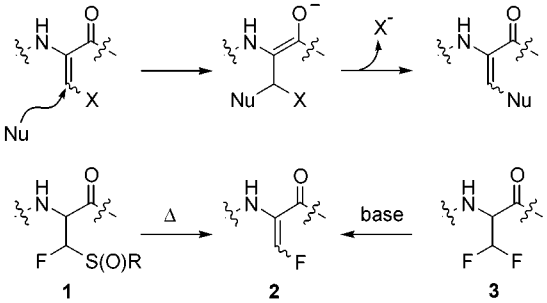
Received December 13, 2000

ABSTRACT

Peptides containing (*E*)- and (*Z*)-3-fluorodehydroalanine have been prepared from serine via a fluoro-Pummerer rearrangement. The resulting electrophilic moieties may be useful affinity labels for the identification of the targets of dehydroamino acid containing natural products that act by covalent mechanisms.

Dehydroalanines are present in a large number of natural products, including the microcystins,[1] nodularin,[2] thiostrepton and other thiopeptides,[3] and the lantibiotics.[4] The precise role of these α,β-unsaturated amides in the biological activities of these compounds is not always well understood. Their function might be strictly structural or they may act as electrophilic entities forming covalent linkages with nucleophilic groups on their targets. Michael acceptors such as the dehydroalanines have been popular functionalities for the design of enzyme inhibitors and active site affinity labels.[5] The reversibility of conjugate additions, however, poses a potential drawback for the permanent attachment of a target to a Michael acceptor, thereby in certain cases precluding identification of the nucleophile. Introduction of a halogen on the terminal vinyl carbon can alleviate this shortcoming since it will lead to an irreversible linkage of the nucleophile to the dehydroalanine (Scheme 1). This strategy has been very successful for instance in the study of pyridoxal phosphate dependent enzymes[5,6] as well as

Scheme 1



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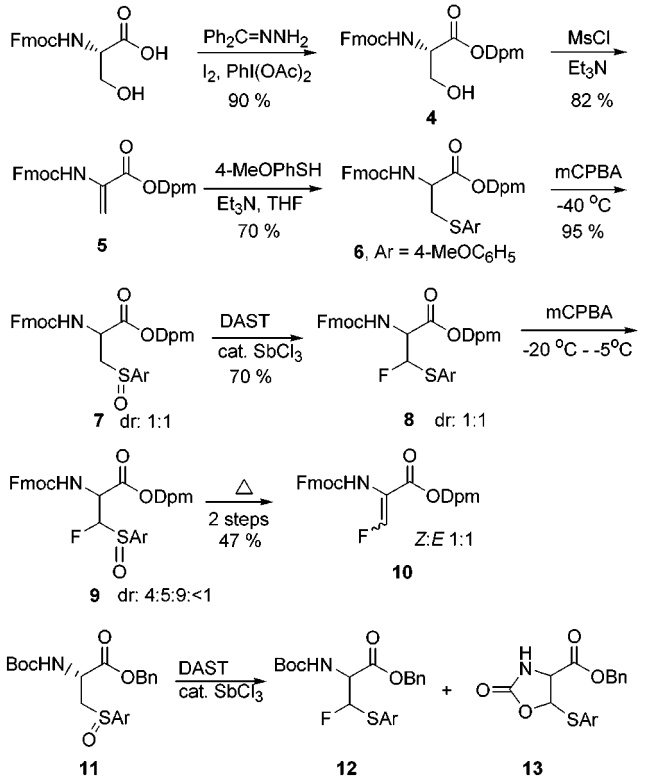
10.1021/ol006997s CCC: $20.00
Published on Web 01/31/2001

D-xylose isomerase,[7] and monoamine oxidase.[8] Fluorine-substituted Michael acceptors have been particularly attractive because of the small steric requirements of the fluorine substituent. To date, no reports have detailed the preparation of fluorine-substituted dehydroalanines in peptides. We report here the synthesis of 3-fluorodehydroalanine-containing peptides **2**.

A number of methods have been reported for the preparation of fluorine-substituted olefins,[9] including elimination of hydrogen fluoride from *gem*-difluorinated compounds,[10] and thermal elimination of α-fluoro-substituted sulfoxides.[11] Thus, fluorine-substituted dehydroalanines might be accessed via precursors **1** or **3** (Scheme 1). Given the strongly basic conditions required for the elimination of hydrogen fluoride from difluoroalanine, which could lead to racemization of other residues in the peptide, we focused our attention on synthon **1**. α-Fluorosulfides can be prepared by the fluoro-Pummerer rearrangement of sulfoxides with diethylaminosulfur trifluoride (DAST),[11] by treatment of sulfides with DAST[12] or Selectfluor,[13] or via the reaction of thioacetals with mercuric fluoride.[14] To evaluate these methods for the preparation of **1**, various *N*-protected serine derivatives were converted to their corresponding sulfides and sulfoxides as outlined for Fmoc-serine in Scheme 2. Fmoc-serine was

Scheme 2



transformed into the diphenylmethyl ester **4**, activated with methylsulfonyl chloride, and treated with base to produce dehydroalanine **5**. Michael addition of 4-methoxybenzenethiol to **5** provided cysteine derivative **6**, which was oxidized with *m*CPBA to the corresponding sulfoxide **7**, producing a 1:1 mixture of diastereomers. The direct fluorination of **6** with either Selectfluor or DAST produced **8** in lower yields than that for the $SbCl_3$-catalyzed[15] fluoro-Pummerer rearrangement of the diastereomeric mixture of sulfoxides **7**. The latter reaction was carried out using the protocols developed in the laboratories of McCarthy[11] and Robins[15] and produced fluorosulfide **8** in good yield as a 1:1 mixture of diastereomers. To address whether the stereochemistry at sulfur in sulfoxide **7** had an influence on the diastereoselectivity of the fluoro-Pummerer reaction, the two diastereomers of **7** were separated by silica gel chromatography and treated in parallel with DAST/$SbCl_3$ in CH_2Cl_2. Compound **8** was obtained with identical diastereomer ratios for both reactions, indicating that the stereochemistry of the reaction is not dependent on the configuration at sulfur of the sulfoxide. This is consistent with the proposed reaction of fluoride anion onto a thiocarbenium intermediate formed upon reaction of the sulfoxide with DAST.[10]

When this synthetic route was followed using Boc as the *N*-protecting group, the reaction of sulfoxide **11** with DAST produced **13** as a major byproduct (30%), which was not formed in appreciable amounts with **7**. Compound **13** is presumably formed by cyclization of the carbamate carbonyl oxygen onto the thiocarbenium intermediate. Similar cyclizations have been utilized intentionally in several laboratories.[16] A number of modifications to the reaction conditions were explored to minimize the formation of this for our purposes undesired byproduct. Attempts to increase the relative rate of the intermolecular reaction with respect to the intramolecular cyclization by increasing the fluoride concentration via addition of CsF or tetrabutylammonium triphenyldifluorosilicate (TBAT) resulted in inhibition of the reaction. Increasing the overall concentration of the reaction mixture did decrease the amount of **13** to <15 %.

Fluorinated cysteine derivative **8** was oxidized with *m*CPBA to yield four diastereomers of the fluorinated

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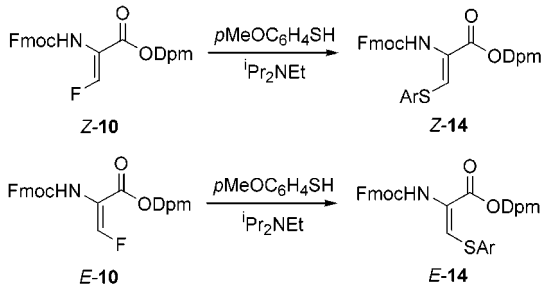
sulfoxide **9**.[17] Subsequent thermolytic elimination in benzene at 80 °C afforded two 3-fluorodehydroalanine isomers in 74% yield. These two isomers were separated by silica gel chromatography, and three approaches were used to determine the olefin geometry of the individual 3-fluorodehydroalanines. First, the ^{13}C NMR spectrum of one of the isomers, assigned to (*Z*)-3-fluorodehydroalanine, displayed a doublet signal for the ester carbonyl carbon with a coupling constant ($^3J_{C-F}$) of 11.6 Hz, whereas the putative (*E*)-isomer showed a singlet. Similar magnetic coupling has been reported for other fluorine-substituted olefins containing fluorine trans to a carbonyl group.[18] In addition, several laboratories have shown for a series of 2-acylaminocrotonates that the chemical shift of the vinyl proton cis to the acylamino group occurs downfield relative to the vinyl proton in the corresponding isomer.[19] The relative chemical shifts of these protons in the two isomers of **10** are consistent with our assignment based on the carbon−fluorine coupling.[20] Finally, ^{19}F-irradiated $^{19}F-^{1}H$ NOE spectroscopy[21] with (*Z*)-3-fluorodehydroalanine showed enhancements of the signals of the vinyl and amide protons as well as aromatic protons on the Fmoc group, while only enhancement of the olefinic proton was detected in (*E*)-3-fluorodehydroalanine.

To evaluate the proposed mechanism of action of fluorine-substituted dehydroalanines as presented in Scheme 1, both isomers of **10** were reacted with *p*-methoxybenzenethiol in the presence of diisopropylethylamine (Scheme 3). Clean

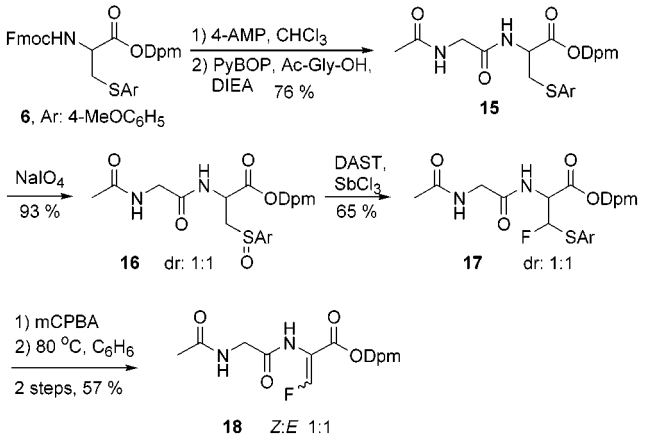
Scheme 3



conversion of *Z*-**10** into the corresponding dehydrocysteine derivative *Z*-**14** was observed, consistent with an addition elimination mechanism. The *E*-isomer of **10** produced predominantly *E*-**14**, along with a small amount of the *Z*-stereoisomer.[22] The stereochemistry of the products was assigned based on the chemical shift of the vinylic proton in comparison with literature values for similar dehydrocysteine derivatives.[23]

The methodology was applied next to the synthesis of a 3-fluorodehydroalanine containing dipeptide as shown in Scheme 4. Dipeptide **15** was synthesized in 76% yield by

Scheme 4



solution-phase techniques[24] from acetyl glycine and **6**. Oxidation of the sulfide to the sulfoxide and treatment with DAST gave the desired product **17** in 65% yield. As has been observed in previous studies on the fluoro-Pummerer reaction,[11,12,25] a small amount (8%) of deoxygenated material (i.e., **15**) was produced in this reaction via an unknown mechanism. Two isomers of the target **18** were obtained after oxidation and thermal elimination at 80 °C. The *E*- and *Z*-isomers were separated by silica gel chromatography and proved stable at room temperature.

In summary, we have developed methodology to prepare fluorine-substituted dehydroalanines and to incorporate these into dipeptides. Given the well-known success of fluorine-containing pharmaceuticals,[26] including fluoropeptides,[27] the current approach expands the arsenal of fluorinated moieties

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(20) The chemical shifts of the vinyl protons of (*E*)-3-fluorodehydroalanine and (*Z*)-3-fluorodehydroalanine are 8.18 ppm ($^2J_{HF}$ = 78.1 Hz) and 7.66 ppm ($^2J_{HF}$ = 72.6 Hz), respectively.

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(22) The formation of some *Z*-**14** from *E*-**10** may either occur during the actual transformation or may be due to isomerization of *E*-**14** catalyzed by *p*-methoxybenzenethiol.

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that can be used in the preparation of bioactive molecules. Efforts to introduce these reactive Michael acceptors into natural products are underway and will be reported in due course.

Acknowledgment. This work was supported by grants from the Burroughs-Wellcome Fund (APP 1920) and the National Institutes of Health (GM58822). W.A.V. thanks 3M for a nontenured faculty grant. We thank Drs. Vera Mainz and Paul Molitor for assistance with the heteronuclear NOE experiments, obtained in the Varian Oxford Instrument Center for Excellence, funded in part by the W. M. Keck Foundation, NIH (PHS 1 S10 RR10444), and NSF (CHE 96-10502), and Prof. Peter Petillo for helpful discussions regarding the stereochemical assignments of **10**.

Supporting Information Available: Experimental procedures for all transformations that produced previously unknown compounds as well as their full spectral characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

OL006997S