Journal of Fluorine Chemistry 131 (2010) 439–445

Contents lists available at ScienceDirect

Journal of Fluorine Chemistry

journal homepage: www.elsevier.com/locate/fluor

Short communication

Separation of fluorinated amino acids and oligopeptides from their non-fluorinated counterparts using high-performance liquid chromatography

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ARTICLE INFO

Article history:
Received 19 October 2009
Received in revised form 3 November 2009
Accepted 30 November 2009
Available online 4 December 2009

Keywords:
Fluorinated amino acids
Fluorinated peptides
Fluorocarbon eluent
Fluorocarbon column
HPLC
Fluorophilicity

ABSTRACT

Chromatographic conditions for the separation of fluorinated amino acids and oligopeptides from their non-fluorinated counterparts were explored. The separation of six pairs of analytes, including both aromatic and aliphatic fluorocarbons, was investigated at various temperatures using both hydrocarbon and fluorocarbon columns and eluents. Our results show that when hydrocarbon eluents are used, fluorocarbon column provides better separation of fluorinated amino acids or oligopeptides from their non-fluorinated counterparts; when fluorocarbon eluents are used, hydrocarbon column provides better separation of fluorinated amino acids or oligopeptides from their non-fluorinated counterparts. These chromatographic behaviors reflect the fluorophilicity possessed by fluorinated amino acids and oligopeptides.

1. Introduction

Although rarely existing in nature [1], organofluorine compounds are finding increasing applications in a wide range of biological and medical sciences, such as biochemistry [2], medicinal chemistry [3], pharmaceutical chemistry [4], green chemistry [5], biotechnology [6], drug delivery [7] and diagnostic imaging [8]. As is the case with any class of organic molecules, separation is an important consideration for organofluorine compounds [9]. Heavily fluorinated molecules have unique partition properties between fluorocarbon solvents and hydrocarbon solvents [10]. This feature has been exploited for the extraction and separation of compounds with multiple fluorine atoms, using either perfluorocarbon solvent extraction [11] or fluorous silica-gel chromatography [12].

The vast majority of fluorinated drugs contain just a few fluorine atoms [3,4]. In this work, we explore the separation of lightly fluorinated amino acids and oligopeptides from their non-fluorinated counterparts using high-performance liquid chromatography (HPLC). This problem arises during our work on developing fluorinated analogs of peptide drugs. We investigated several aspects of the separation of fluorinated amino acids and oligopeptides from their non-fluorinated counterparts, including: aromatic vs. aliphatic fluorocarbons, fluorocarbon (F-) vs. hydrocarbon (H-) columns, fluorocarbon (F-) vs. hydrocarbon (H-) eluents, and temperature. Both fluorocarbon columns and fluorocarbon eluents have been investigated for the separation of lightly fluorinated compounds [13–15]. However, to the best of knowledge, there has been no prior report in which fluorinated eluents, columns and analytes are compared side-by-side with their non-fluorinated counterparts.

We investigated six pairs of analytes, **2**/**1**, **4**/**3**, **6**/**5**, **8**/**7**, **10**/**9** and **12**/**11**, which can be divided into three groups: the aromatic fluorocarbon group ($C_6H_5 \rightarrow C_6H_4F$ substitution), which includes the **2**/**1** and the **4**/**3** pairs; the aliphatic fluorocarbon group ($CH_3 \rightarrow CF_3$ substitution), which includes the **6**/**5** and the **8**/**7** pairs; and the hydrocarbon control group ($H \rightarrow CH_3$ substitution), which includes the **10**/**9** and the **12**/**11** pairs. **8** stems from our effort on making fluorinated analogs of the peptide drug octreotide (Sandostatin®). Fig. 1 shows the structures of the 12 analytes.

For the separation of each pair of analytes, we used an F-column that contains the n-C_8F_{17} group and an H-column that contains the n-C_8H_{17} group. For each column, we used two fluorocarbon eluents, trifluoroethanol (TFE) and hexafluoroisopropanol (HFIP),

Abbreviations: Cys, cysteine; DCM, dichloromethane; DIC, *N*,*N*′-diisopropylcarbodiimide; DIPEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; EtOH, ethanol; F%wt, fluorine weight percentage in an analyte or a solvent; Fmoc, fluorenylmethoxycarbonyl; HFIP, hexafluoroisopropanol; HOBt, *N*-hydroxybenzotriazole; HPLC, high-performance liquid chromatography; ISP, isopropanol; Lys, lysine; MS, mass spectrometry; MW, molecular weight; Nle, norleucine; NMR, nuclear magnetic resonance; Nva, norvaline; Phe, phenylalanine; RPLC, reversed-phase liquid chromatography; tBu, *tert*-butyl; TFA, trifluoroacetic acid; TFE, trifluoroethanol; tfT, trifluorothreonine; Thr^{allo}, *allo*-L-threonine; t_R, retention time; Trp, tryptophan; Trt, trityl; Tyr, tyrosine; δ, chemical shift; Δt_R, retention time difference.

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doi:10.1016/j.jfluchem.2009.11.025

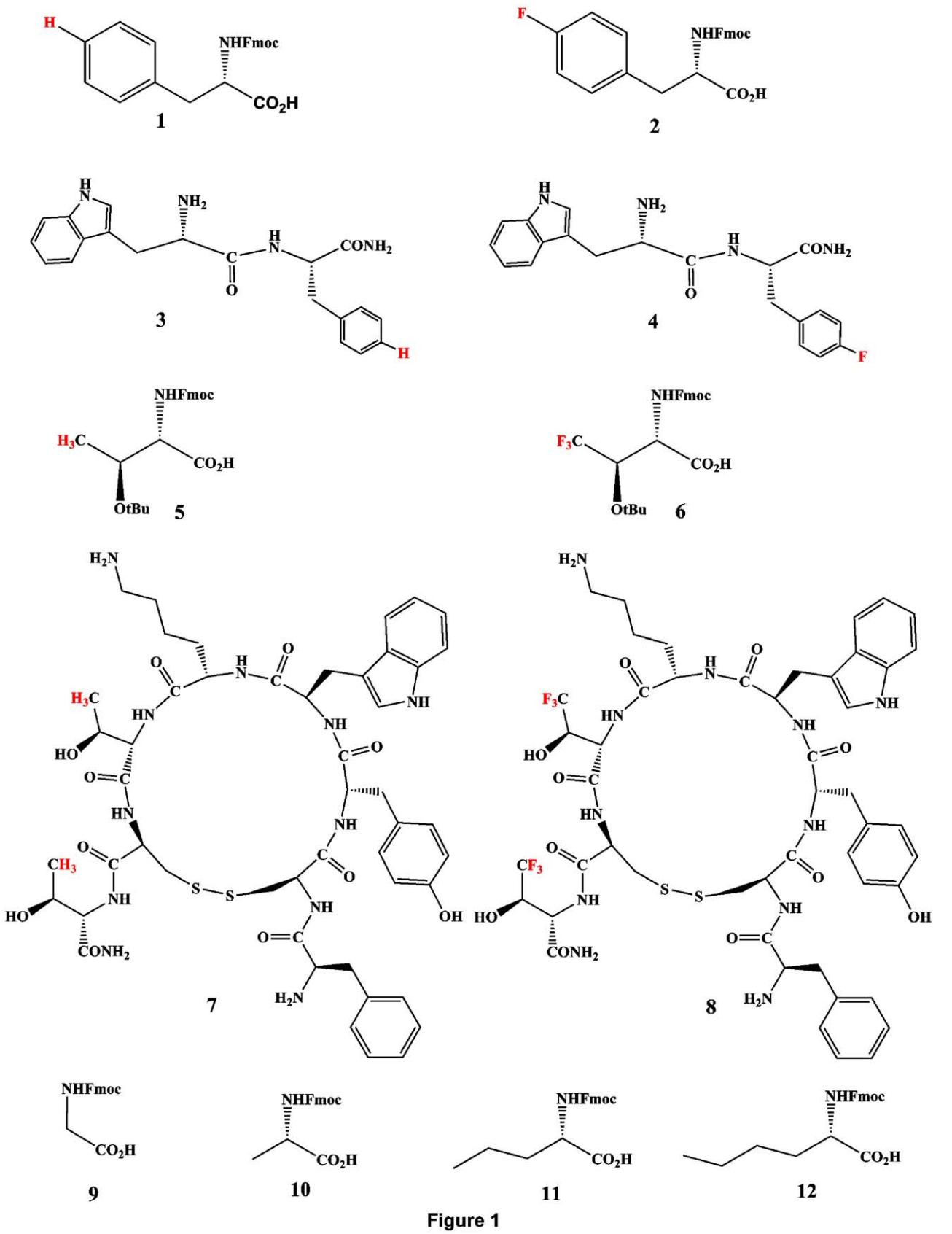

Fig. 1. Structures of analytes. The difference between a fluorinated analyte and its non-fluorinated analyte is highlighted in red. The 12 analytes can be divided into three groups: the $C_6H_5 \rightarrow C_6H_4F$ group (including the **2**/**1** pair and the **4**/**3** pair); the $CH_3 \rightarrow CF_3$ group (including the **6**/**5** pair and the **8**/**7** pair); and the $H \rightarrow CH_3$ group (including the **12**/**11** pair and the **10**/**9** pair). We used protected version of the amino acids because free amino acids were not sufficiently retentive on the HPLC columns we used. Peptide sequences are: Trp-Phe (**3**), Trp-Phe(4-F) (**4**), D-Phe-*c*[Cys-Tyr-D-Trp-Lys-Thr^{allo}-Cys]-Thr^{allo}-amide (**7**) and D-Phe-*c*[Cys-Tyr-D-Trp-Lys-tfT-Cys]-tfT-amide (**8**). **7** and **8** are cyclized through intramolecular disulfide bond, as in octreotide.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)