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Mendeleev Commun., 2021, **31**, 867–868

Mendeleev Communications

Synthesis of a tripeptide biomarker of exposure to sulfur mustard for support of OPCW biomedical proficiency tests

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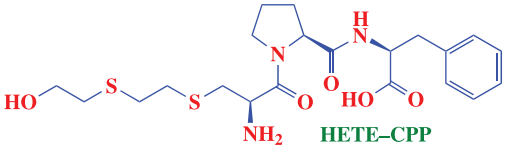
DOI: 10.1016/j.mencom.2021.11.033

***S*-[2-(2-Hydroxyethylthio)ethyl]-Cys-Pro-Phe (HETE–CPP) was synthesized in seven steps as a reference compound for the development of a procedure for its quantification in blood plasma in the OPCW biomedical proficiency tests.**

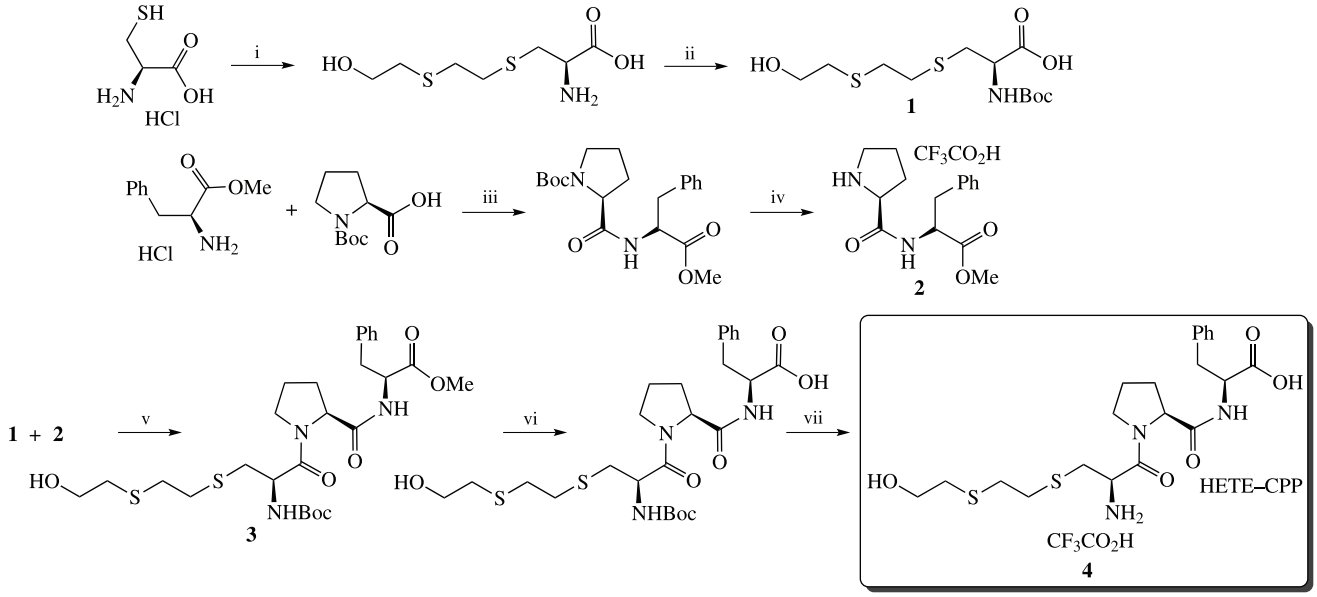


Keywords: OPCW, sulfur mustard, positive control, qualitative and quantitative analysis, collision-induced dissociation, sulfides, peptide synthesis, tripeptides.

The identification and analysis of toxic chemicals and their degradation products is an integral part of activities to ensure fulfillment of Russia's obligations under the Chemical Weapons Convention (CWC).[1] To maintain the readiness of designated laboratories for investigating allegations of the use of chemical weapons incident investigation, the Organization for the Prohibition of Chemical Weapons (OPCW) since 2009 has been regularly conducted confidence building exercises and proficiency tests for biomedical sample analysis.[2] The organizers of the tests prepare and dispatch biological samples (blood plasma and/or urine) spiked with of toxic substances, including sulfur mustard, a vesicant chemical warfare agent listed in Schedule 1A of the CWC.

The covalent adducts with the nitrogenous bases of DNA and with the major blood protein albumin are the most long-term retrospective biomarkers of exposure to sulfur mustard.[3–5] The reaction of sulfur mustard with albumin leads to the formation of an adduct at Cys34 residue, which, after hydrolysis with proteinase K during sample preparation for HPLC–MS analysis, forms modified tripeptide *S*-[2-(2-hydroxyethylthio)ethyl]-Cys-Pro-Phe (HETE–CPP).[6,7]

The synthesis of HETE–CPP has not been reported so far. The target product was prepared from two blocks (Scheme 1). Known[8,9] dipeptide **2**, *viz.* L-Pro-L-Phe-OMe hydrotrifluoroacetate was coupled with compound **1**, *viz.* protected L-cysteine containing 'half sulfur mustard' moiety at the sulfur atom.[10] Compounds **1**, **3**, **4** and the corresponding intermediates from steps iii, iv are new. Consecutive hydrolysis of methyl ester **3** and treatment of the resulting *N*-Boc-protected acid with



Scheme 1 *Reagents and conditions*: i, $Cl(CH_2)_2S(CH_2)_2OH$, MeONa, MeOH, room temperature, 2 h; ii, Boc_2O, $NaHCO_3$, 1,4-dioxane/water, room temperature, 18 h; iii, HBTU, DIPEA, DMF, room temperature, 18 h; iv, TFA, CH_2Cl_2, room temperature, 18 h; v, HBTU, DIPEA, DMF, room temperature, 18 h; vi, LiOH, THF/MeOH/H_2O, room temperature, 18 h; vii, TFA, room temperature, 10 min.

trifluoroacetic acid gave the target product **4**, HETE–CPP, as hydrotrifluoroacetate salt.

The structure of HETE–CPP **4** was confirmed by HRMS data and ^{1}H NMR spectroscopy. The ^{1}H NMR spectrum displays sets of signals characteristic of the structural fragments of *S*-[2-(2-hydroxyethylthio)ethyl]-L-cysteinyl-L-prolyl-L-phenylalanine hydrotrifluoroacetate (see Online Supplementary Materials). The integral intensity of the proton signals corresponds to the number of hydrogen atoms in HETE–CPP. The synthesized peptide HETE–CPP **4** was found to be optically active with $[\alpha]_D^{25}$ –20.04 (*c* 0.634, H_2O). The experimental exact mass of the molecular ion for product **4** being 470.17771 is fully consistent with the theoretical (calculated) value (see Online Supplementary Materials). The collision-induced dissociation mass spectrum of the molecular ion of HETE–CPP (Figure 1) provides evidence for the amino acid sequence in compound **4** as well as the presence of 2-(2-hydroxyethylthio)ethyl moiety at the cysteine residue.

The mass chromatograms of HETE–CPP isolated from protein adducts of sulfur mustard in blood plasma were obtained on a Thermo Fisher Scientific QExactive HPLC–HRMS instrument. Figure 2 shows the MRM mass chromatograms of the synthesized HETE–CPP **4** and that in the plasma sample spiked with sulfur mustard after enzymatic digestion and purification, which demonstrates coincidence of the retention times of the two signals. The deviation of the measured *m/z* values of the characteristic productions with *m/z* 105.03686 and 137.00893 is not larger than 2.5 ppm, and the retention times of the two signals deviate from each other by not more than 0.2 min, which meets the OPCW identification criteria.

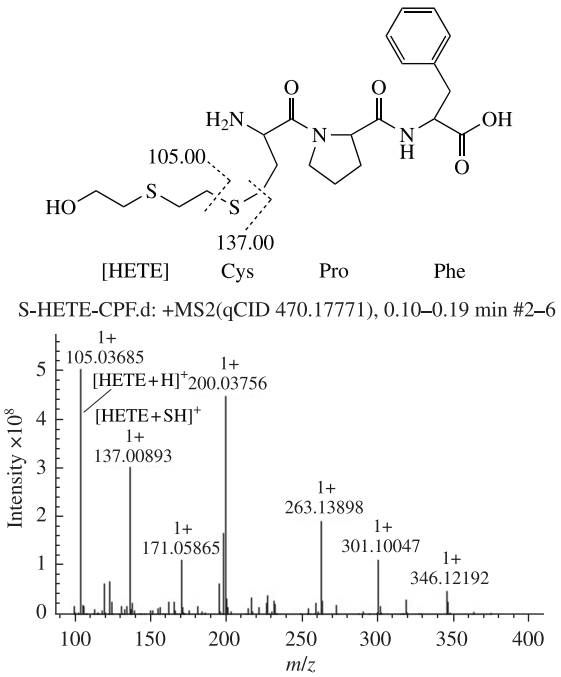


Figure 1 Collision-induced dissociation mass spectrum of the $[M+H]^+$ ion of the synthesized HETE–CPP **4**.

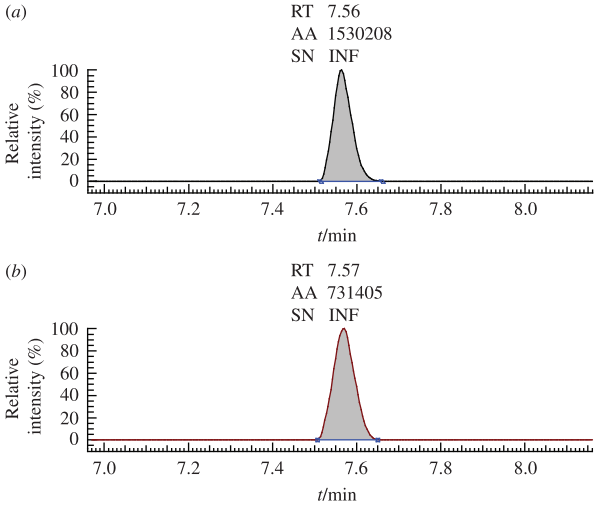


Figure 2 The base peak intensity chromatograms for the characteristic product-ions 105.03686 and 137.00893 obtained by higher energy collision dissociation (HCD) of HETE–CPP (470.1778): (*a*) synthesized compound **4** and (*b*) substance isolated from enzymatically digested and purified plasma sample spiked with sulfur mustard.

The purity of the synthesized compound was higher than 95% (by ^{1}H NMR), with meets the OPCW requirements to reference substances. The synthesized HETE–CPP **4** can be used in the development of the procedures for qualitative and quantitative analysis of sulfur mustard adducts in blood plasma.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.11.033.

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Received: 19th April 2021; Com. 21/6530