Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Adduct of the blistering warfare agent sesquimustard with human serum albumin and its mass spectrometric identification for biomedical verification of exposure

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1. **Table S1** Product ions of single protonated HETETE-CPF

| Structure | Formula | Measured mass | Theoretical mass | Δ [ppm] | Δ [mmu] |
|-----------|---------|---------------|------------------|---------|---------|
| ![Image](image1.png) | C₂₃H₃₆O₅N₃S₃ | 530.1804 | 530.1812 | -1.4 | -0.76 |
| ![Image](image2.png) | C₂₃H₃₄O₄N₃S₃ | 512.1700 | 512.1706 | -1.2 | -0.60 |
| ![Image](image3.png) | C₂₃H₃₁O₄N₂S₃ | 495.1418 | 495.1441 | -4.63 | -2.25 |
| ![Image](image4.png) | C₂₃H₃₂O₃N₃S₃ | 494.1614 | 494.1600 | 2.85 | 1.37 |
| ![Image](image5.png) | C₂₁H₂₇O₄N₂S₃ | 467.1136 | 467.1128 | 1.8 | 0.85 |
| ![Image](image6.png) | C₁₉H₂₆O₄N₃S₂ | 424.1357 | 424.1359 | -0.64 | -0.22 |
| ![Image](image7.png) | C₁₉H₂₃O₄N₂S₂ | 407.1085 | 407.1094 | -2.17 | -0.88 |
Data was extracted from a µLC-ESI MS/HR MS (Orbitrap) run of HETETE-HSA adducts after proteolysis with proteinase K. This reference was produced by incubation of plasma
with Q (100 µM). The corresponding product ion spectrum is shown in Figure 2b. Mass calculation was done using the FreeStyle 1.3 software (Thermo Fisher). Structures represent one possible isomer each.

2. Adding of HETETE-Cys to the GROMOS96 54a7 force field

Output ITP file of Automated Topology Builder (ATB) v.3.0 for HETETE-Cys (Molecule ID 478917)

```
[ moleculetype ]
; Name nrexcl
FMOX 3

[ atoms ]
; nr type resnr resid atom cgnr charge mass
 1 HS14 1 FMOX H17 1 0.339 1.0080
 2 OA 1 FMOX O3 2 -0.479 15.9994
 3 CPos 1 FMOX C9 3 0.525 12.0110
 4 OEqpt 1 FMOX O2 4 -0.561 15.9994
 5 CPos 1 FMOX C8 5 0.240 12.0110
 6 HC 1 FMOX H16 6 0.077 1.0080
 7 NPri 1 FMOX N1 7 -0.861 14.0067
 8 HS14 1 FMOX H18 8 0.358 1.0080
 9 HS14 1 FMOX H19 9 0.358 1.0080
10 CH2 1 FMOX C7 10 0.259 14.0270
11 S 1 FMOX S3 11 -0.453 32.0600
12 CH2 1 FMOX C6 12 0.153 14.0270
13 CH2 1 FMOX C5 13 0.280 14.0270
14 S 1 FMOX S2 14 -0.457 32.0600
15 CH2 1 FMOX C4 15 0.221 14.0270
16 CH2 1 FMOX C3 16 0.219 14.0270
17 S 1 FMOX S1 17 -0.431 32.0600
18 CH2 1 FMOX C2 18 0.204 14.0270
19 CH2 1 FMOX C1 19 0.280 14.0270
20 OAlc 1 FMOX O1 20 -0.632 15.9994
21 HS14 1 FMOX H1 21 0.361 1.0080
; total charge of the molecule: 0.000

[ bonds ]
; ai aj funct c0         c1
 1    4    1    0.1000   1.5780e+07
 2    5    1    0.1340   1.0500e+07
 3    5    2    0.1340   1.0500e+07
 5    6    2    0.1540   7.1500e+06
 5    7    2    0.1540   7.1500e+06
 5   10    2    0.1540   7.1500e+06
 7    8    2    0.1540   7.1500e+06
 7    9    2    0.1540   7.1500e+06
 10   11    2    0.1540   7.1500e+06
 11   12    2    0.1540   7.1500e+06
 12   13    2    0.1540   7.1500e+06
 13   14    2    0.1540   7.1500e+06
 14   15    2    0.1540   7.1500e+06
 15   16    2    0.1540   7.1500e+06
 16   17    2    0.1540   7.1500e+06
 17   18    2    0.1540   7.1500e+06
 18   19    2    0.1540   7.1500e+06
 19   20    2    0.1540   7.1500e+06
 20   21    2    0.1540   7.1500e+06
21   21    2    0.1540   7.1500e+06

[ pairs ]
; ai aj funct ; all 1-4 pairs but the ones excluded in GROMOS itp
 1    4    1
 1    5    1
 2    6    1
 2    7    1
 2    10    1
 3    8    1
 3    9    1
 3    11    1
 4    6    1
 4    7    1
 4    10    1
 5    12    1
 6    8    1
 6    9    1
 6    11    1
 7    11    1
 8    10    1
 9    10    1
10    13    1
11    14    1
```
| ai | aj | ak | funct | angle     | fc     |
|----|----|----|--------|-----------|--------|
| 1  | 2  | 3  | 2      | 104.00    | 490.00 |
| 2  | 3  | 4  | 2      | 124.00    | 730.00 |
| 2  | 3  | 5  | 2      | 115.00    | 610.00 |
| 2  | 3  | 6  | 2      | 104.00    | 490.00 |
| 2  | 3  | 7  | 2      | 108.00    | 465.00 |
| 3  | 5  | 10 | 2      | 111.00    | 530.00 |
| 6  | 5  | 10 | 2      | 107.57    | 484.00 |
| 7  | 5  | 10 | 2      | 115.00    | 610.00 |
| 5  | 7  | 8  | 2      | 109.50    | 425.00 |
| 5  | 7  | 9  | 2      | 109.50    | 425.00 |
| 8  | 7  | 9  | 2      | 106.75    | 503.00 |
| 5  | 10 | 11 | 2      | 113.00    | 545.00 |
| 10 | 11 | 12 | 2      | 100.00    | 475.00 |
| 11 | 12 | 13 | 2      | 113.00    | 545.00 |
| 12 | 13 | 14 | 2      | 113.00    | 545.00 |
| 13 | 14 | 15 | 2      | 100.00    | 475.00 |
| 14 | 15 | 16 | 2      | 113.00    | 545.00 |
| 15 | 16 | 17 | 2      | 113.00    | 545.00 |
| 16 | 17 | 18 | 2      | 100.00    | 475.00 |
| 17 | 18 | 19 | 2      | 113.00    | 545.00 |
| 18 | 19 | 20 | 2      | 111.00    | 530.00 |
| 19 | 20 | 21 | 2      | 109.50    | 450.00 |

Based on this topology output a new entry was generated in the file aminoacids.rtp in the folder of the forcefield for residue CYSX (using methionine as a starting template)
The following entry for CYSX was added to the file aminoacids.hdb to account for hydrogen bonding.

CYSX 2
1  1  H  N  -C  CA
2  2  H8  O7  C6  C5

After constructing the HETETE-moiety using UCSF Chimera the modified residue was named CYSX in the PDB file and the atom names were assigned based on the entry under [atoms] in the aminoacids.rtp file:

CA—CB—SG—CD—CE—S2—C3—C4—S3—C5—C6—O7—H8
3. RMSD fluctuation of MD trajectories

![RMSD fluctuation of MD trajectories](image)

**Fig. S1** apo HSA (200 ns)
Fig. S2 HETETE-HSA- (100 ns) “in solvent”

Fig. S3 HETETE-HSA- (100 ns) “in groove”
4. RMSF of residues over the course of the MD simulation with converged RMSD
   (C-α group used for RMSD calculation, light grey section depicting subdomain IA)

Fig. S4 apo HSA (100 ns – 200 ns)
**Fig. S5** HETETE-HSA “in solvent” (60 ns – 100 ns)

**Fig. S6** HETETE-HSA “in groove” (35 ns – 100 ns)