# Top-down study of $\beta_2$-microglobulin deamidation

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# Supporting Information

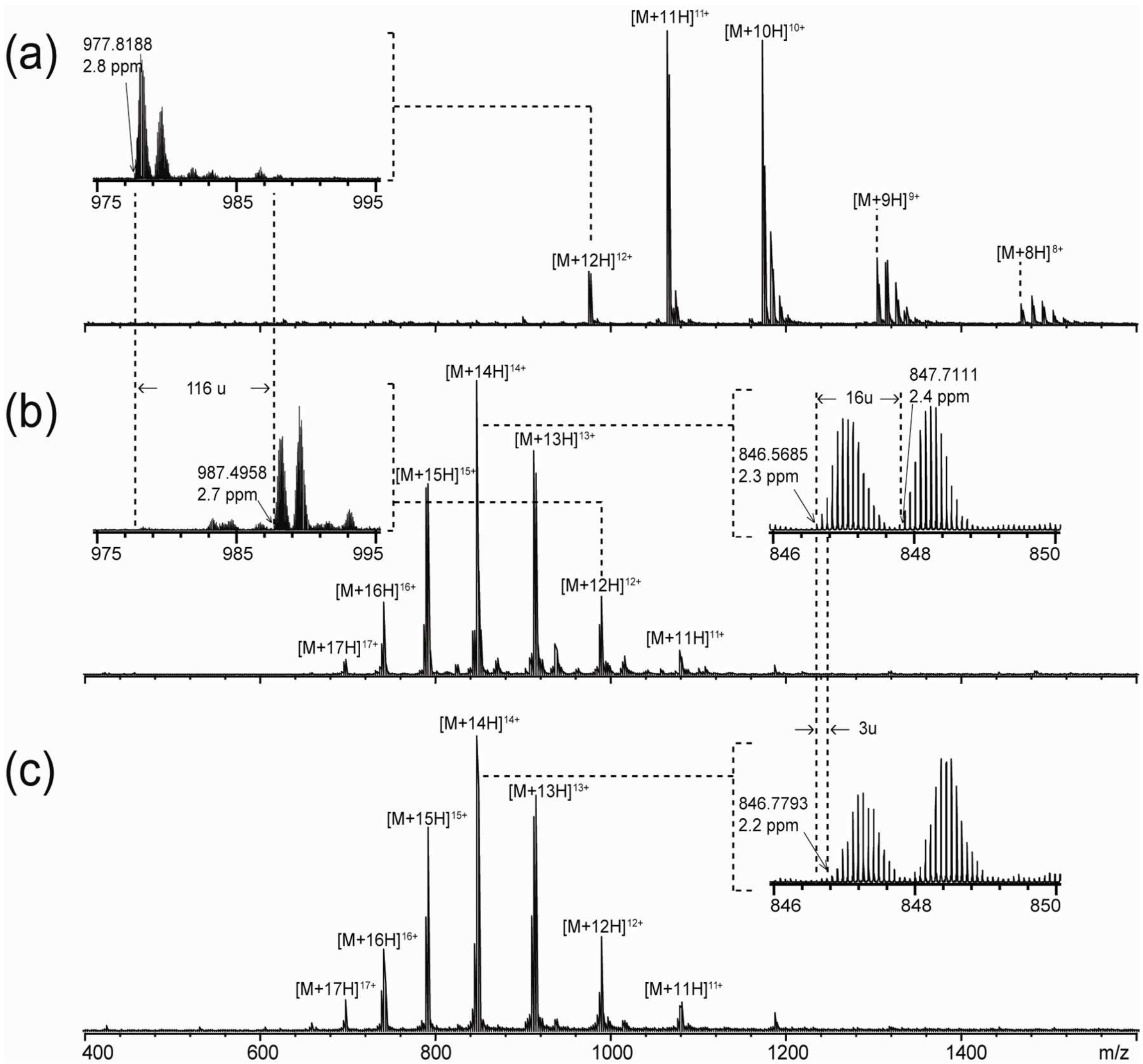


Figure S1. ESI mass spectra of the (a) native, (b) reduced/alkylated, and (c) reduced/alkylated and aged $\beta_2$M. For each spectrum, a selected charge state is enlarged to illustrate the presence of two isotopic clusters (with or without Met oxidation) as well as the mass shifts caused by reductive alkylation and Asn deamidations (insets). All *m/z* values listed are those of the monoisotopic peak as determined by the SNAP$^{TM}$ algorithm.

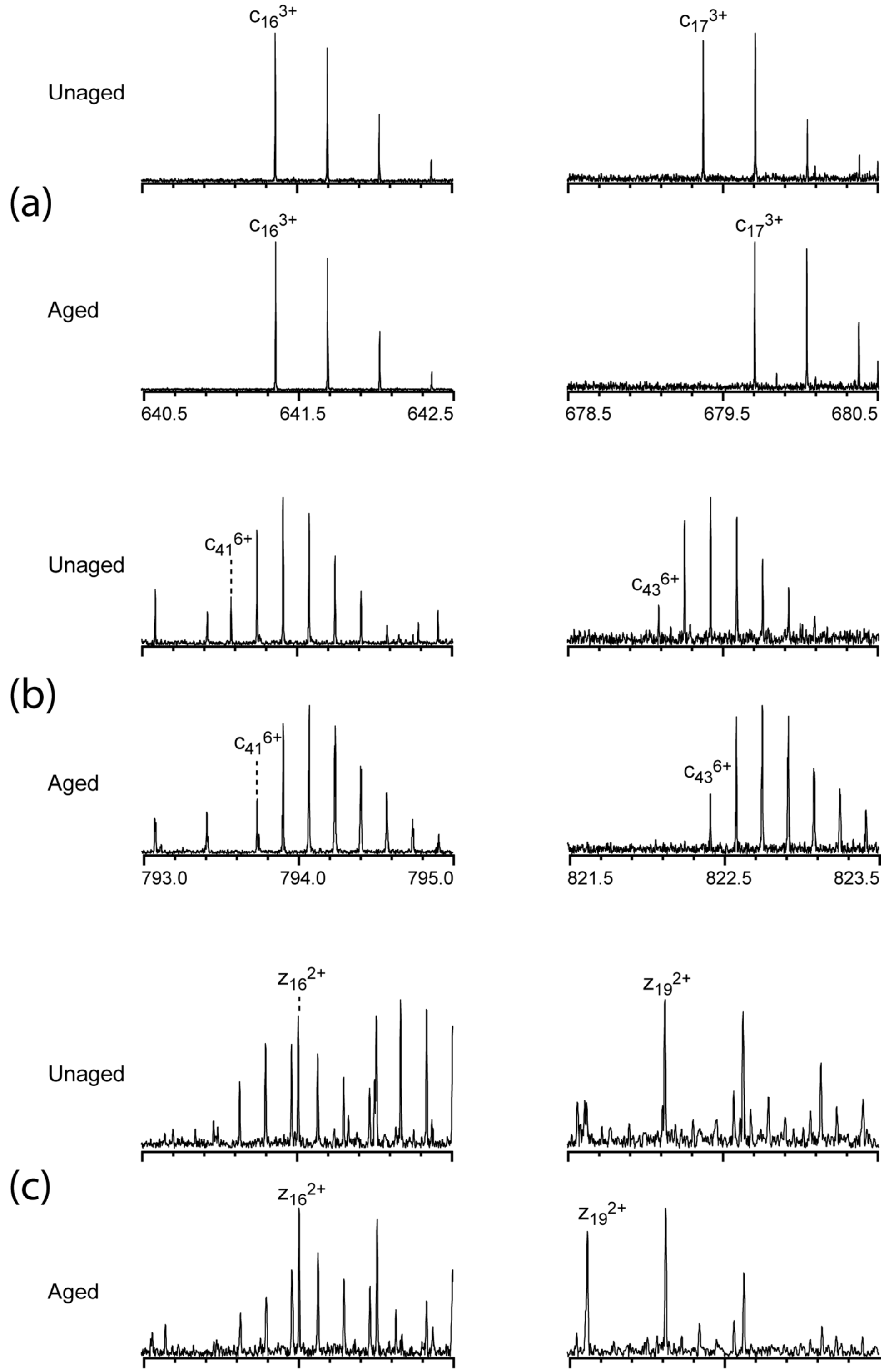


Figure S2. Enlarged regions of the ECD spectra of the unaged and the aged β2M for (a) $c_{16}^{3+}$ and $c_{17}^{3+}$ ions, (b) $c_{41}^{6+}$ and $c_{43}^{6+}$ ions, and (c) $z_{16}^{2+}$ and $z_{19}^{2+}$ ions.

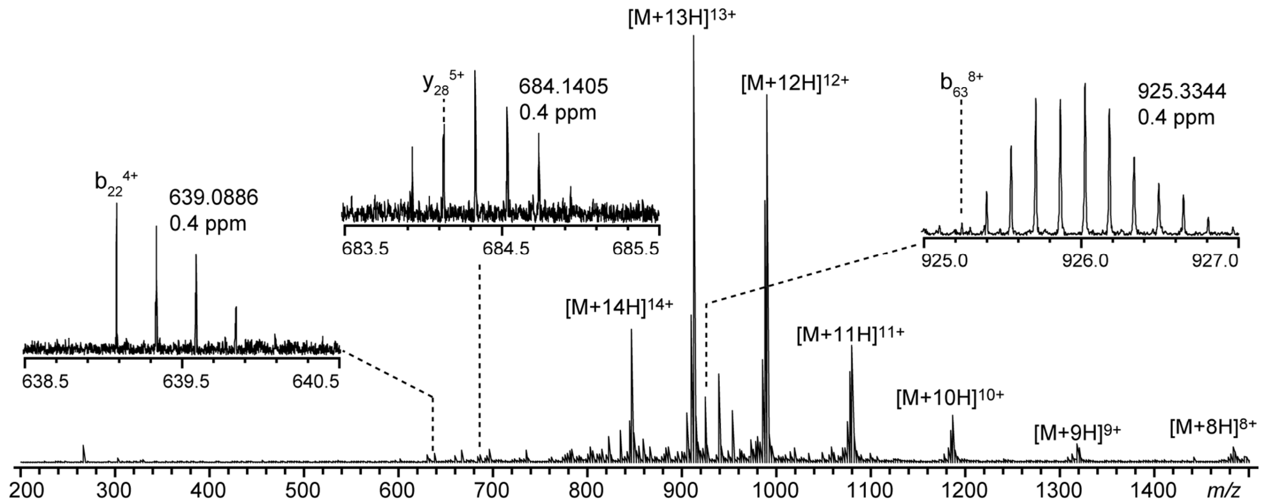


Figure S3. 30 eV NSD spectrum of the aged β2M. Insets show the three isoAsp-containing fragments selected for further ECD analysis.

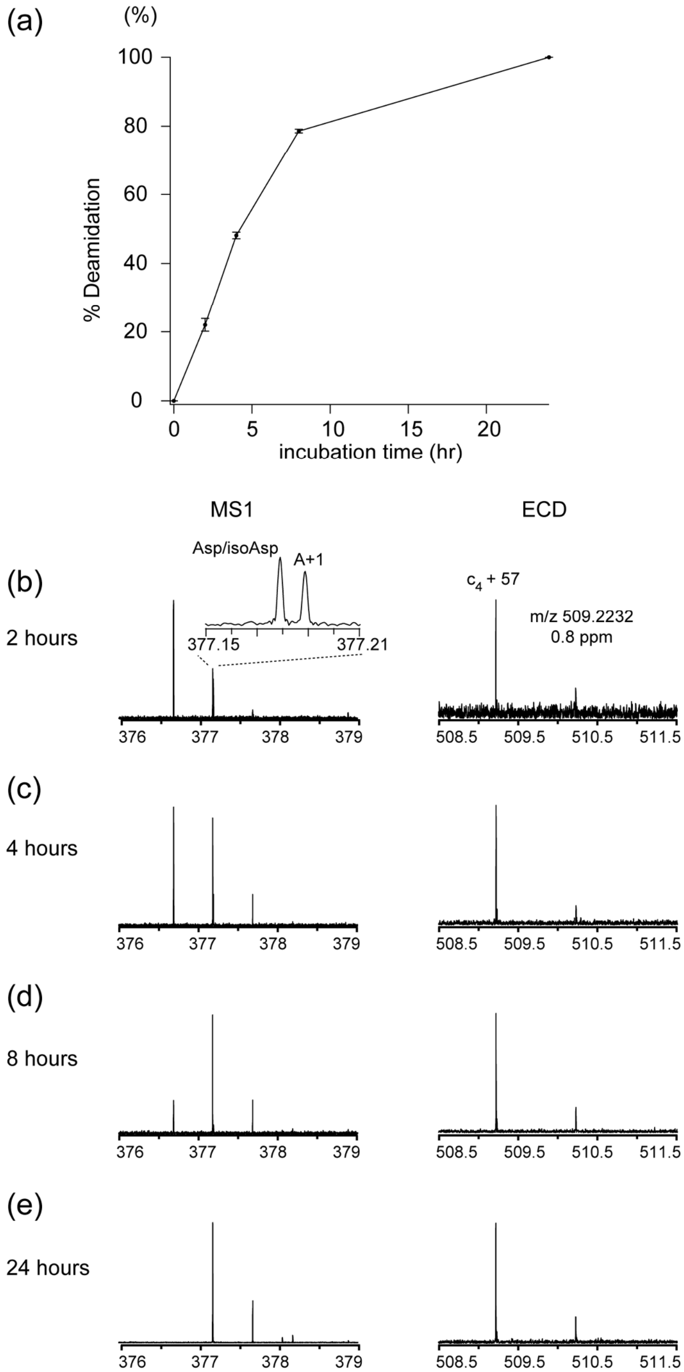


Figure S4. (a) % deamidation of the tryptic peptide HPAENGK as a function of the incubation time. (b-e) The isotopic pattern of this peptide (left column), generated by 2, 4, 8, and 24 hours of digestion, respectively, and the corresponding ECD spectra showing the enlarged region of the $isoAsp_{17}$ diagnostic ion (right column).

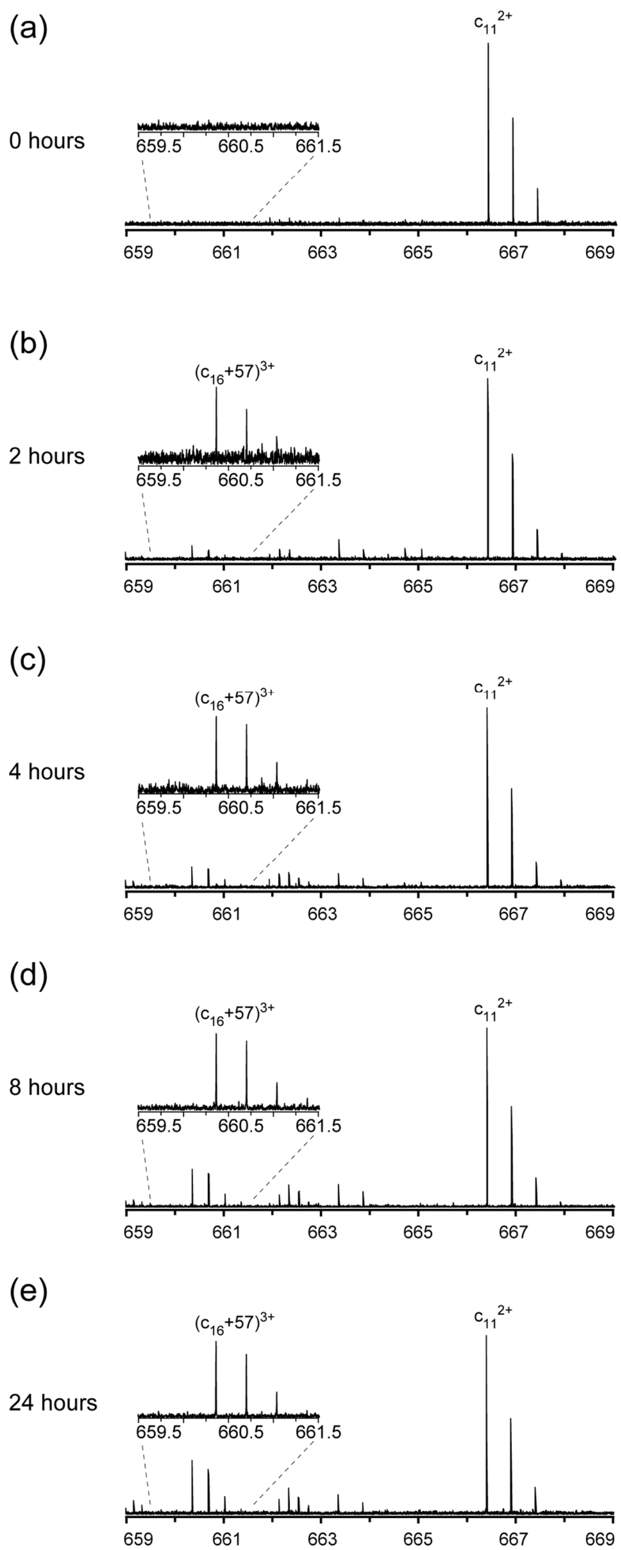


Figure S5. The enlarged isoAsp$_{17}$ diagnostic ion region of the ECD spectra of (a) the unaged β2M (a), and (b-e) β2M after 2, 4, 8, and 24 hours of *in vitro* aging at pH 7.8, 37$^{o}$C.

Table S1. List of all assigned peaks in the ECD spectrum of the $y_{28}^{5+}$ ion and the co-isolated $(a_{24} – NH_3)^{4+}$ ion of the aged β2M. * denotes peaks from the $(a_{24} – NH_3)^{4+}$ ion.

| m/z | Intensity (%) | z | assignment | neutral loss or gain | error (ppm) |
|---|---|---|---|---|---|
| 216.1343 | 0.45 | 1 | $c_2$ | | 0.00 |
| 345.1769 | 0.65 | 1 | $c_3$ | | 0.00 |
| 405.1678 | 0.99 | 1 | $z_3$ | | 0.25 |
| 415.2777 | 0.35 | 1 | $c_3^*$ | | 0.24 |
| 417.6881 | 0.09 | 2 | $z_6$ | | -0.60 |
| 473.2719 | 1.46 | 1 | $c_4$ | | 0.21 |
| 491.8115 | 0.06 | 2 | $c_8^*$ | | -0.41 |
| 520.1948 | 1.28 | 1 | $z_4$ | | 0.38 |
| 541.3457 | 0.10 | 2 | $c_9^*$ | | -0.37 |
| 544.2852 | 0.16 | 1 | $a_5$ | +H | 0.18 |
| 587.8119 | 0.97 | 2 | $z_9$ | | -0.26 |
| 588.2989 | 2.34 | 1 | $c_5$ | | 0.17 |
| 595.8210 | 0.05 | 2 | $y_9$ | | -0.67 |
| 613.3782 | 0.18 | 1 | $c_5^*$ | | 0.33 |
| 622.3734 | 0.04 | 2 | $c_{10}^*$ | -H | -0.48 |
| 622.8775 | 0.36 | 2 | $c_{10}^*$ | | -0.16 |
| 634.2906 | 0.19 | 2 | $c_{10}$ | | -0.16 |
| 641.3623 | 0.08 | 3 | $c_{16}^*$ | | -0.31 |
| 644.3477 | 0.08 | 2 | $y_{10}$ | | -0.16 |
| 646.3445 | 0.05 | 3 | $z_{16}$ | | -0.46 |
| 661.8184 | 0.05 | 2 | $a_{11}$ | +H | 0.53 |
| 665.9993 | 0.17 | 3 | $z_{18}^*$ | | 1.30 |
| 666.3934 | 0.29 | 2 | $c_{11}^*$ | | -0.30 |
| 673.3275 | 0.12 | 1 | $a_6$ | +H | -0.30 |
| 684.0392 | 4.37 | 3 | $a_{18}^*$ | +H | -2.24 |
| 684.1403 | 100.00 | 5 | M | | 0.00 |
| 684.6151 | 19.7 | 4 | $M^*$ | | 0.10 |
| 690.0266 | 0.10 | 3 | $y_{17}$ | | -0.10 |
| 698.7118 | 0.04 | 3 | $c_{18}^*$ | | -0.24 |
| 700.3677 | 1.82 | 2 | $z_{11}$ | | -0.07 |
| 706.2741 | 0.53 | 1 | $z_5$ | | 0.28 |
| 708.3762 | 0.08 | 2 | $y_{11}$ | | -1.27 |
| 717.3416 | 2.41 | 1 | $c_6$ | | 0.28 |
| 722.2928 | 0.12 | 1 | $y_5$ | | 0.28 |
| 741.3383 | 0.77 | 2 | $c_{12}$ | | -0.07 |
| 741.4731 | 0.26 | 1 | $c_6^*$ | | 0.13 |
| 743.8838 | 0.42 | 2 | $z_{12}$ | | 0.07 |
| 744.4440 | 0.31 | 2 | $c_{12}^*$ | | -0.20 |
| 746.8565 | 0.08 | 2 | $z_{14}^*$ | | -0.07 |
| 751.8931 | 0.17 | 2 | $y_{12}$ | | 0.00 |

| | | | | | |
|---|---|---|---|---|---|
| 769.7435 | 0.96 | 3 | $z_{19}$ | | -0.13 |
| 775.0830 | 0.18 | 3 | $y_{19}$ | | -0.22 |
| 776.7224 | 0.06 | 3 | $a_{20}$ | +H | -0.47 |
| 787.8609 | 0.12 | 2 | $a_{13}$ | +H | -0.13 |
| 791.3938 | 1.05 | 3 | $c_{20}$ | | -0.21 |
| 793.1604 | 1.86 | 4 | $c_{26}$ | | -0.91 |
| 800.4257 | 0.75 | 2 | $z_{13}$ | | -0.06 |
| 808.4351 | 0.16 | 2 | $y_{13}$ | | -0.06 |
| 810.9139 | 0.16 | 4 | $a_{27}$ | +H | -0.68 |
| 814.0843 | 0.07 | 3 | $a_{21}$ | | 3.44 |
| 815.3812 | 0.10 | 1 | $z_8^*$ | | 0.49 |
| 817.6589 | 0.04 | 4 | $b_{27}$ | | -2.85 |
| 821.9172 | 2.24 | 4 | $c_{27}$ | | -0.82 |
| 826.6555 | 0.13 | 4 | $z_{27}$ | | -0.76 |
| 828.3890 | 0.06 | 2 | $z_{15}^*$ | | 0.91 |
| 828.4255 | 0.28 | 3 | $y_{20}$ | | -1.49 |
| 829.0883 | 0.57 | 3 | $c_{21}$ | | -0.44 |
| 832.1031 | 0.04 | 3 | $y_{22}^*$ | | -0.52 |
| 834.3694 | 1.17 | 1 | $z_6$ | | 0.60 |
| 836.3948 | 0.11 | 2 | $y_{15}^*$ | | -3.35 |
| 837.3950 | 0.16 | 2 | $a_{14}$ | +H | -0.24 |
| 846.7668 | 0.40 | 3 | $z_{21}$ | | 0.71 |
| 847.4404 | 0.23 | 3 | $a_{22}$ | +H | 0.20 |
| 850.9498 | 0.49 | 2 | $z_{14}$ | | 0.24 |
| 852.1054 | 0.24 | 3 | $y_{21}$ | | -0.43 |
| 858.9590 | 0.23 | 2 | $y_{14}$ | | 0.00 |
| 859.4021 | 0.62 | 2 | $c_{14}$ | | 0.12 |
| 862.1111 | 2.01 | 3 | $c_{22}$ | | -0.43 |
| 869.4490 | 0.10 | 3 | $z_{23}^*$ | | -1.30 |
| 874.7892 | 0.08 | 3 | $y_{23}^*$ | | -0.57 |
| 877.9192 | 0.09 | 2 | $z_{16}^*$ | | -3.70 |
| 880.4049 | 0.70 | 1 | $c_7$ | | 0.23 |
| 887.9188 | 0.07 | 2 | $a_{15}$ | +H | -0.28 |
| 890.1383 | 0.12 | 3 | $a_{23}$ | +H | -0.30 |
| 897.0186 | 0.15 | 2 | $c_{15}^*$ | | 0.06 |
| 900.4838 | 0.43 | 2 | $z_{15}$ | | 0.00 |
| 901.1201 | 1.41 | 3 | $z_{22}$ | | -0.59 |
| 904.8094 | 1.17 | 3 | $c_{23}$ | | -0.44 |
| 906.1243 | 0.31 | 3 | $y_{22}$ | -H | 0.04 |
| 908.4931 | 0.16 | 2 | $y_{15}$ | | -0.11 |
| 909.9259 | 0.87 | 2 | $c_{15}$ | | 0.05 |
| 933.4376 | 0.34 | 1 | $z_7$ | | 0.32 |
| 939.5324 | 0.07 | 2 | $a_{16}^*$ | +H | -0.64 |
| 941.9523 | 0.21 | 2 | $z_{17}^*$ | | 0.58 |
| 944.1341 | 1.25 | 3 | $z_{23}$ | | -0.78 |

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|---|---|---|---|---|---|
| 949.4535 | 0.08 | 1 | $y_7$ | | -2.63 |
| 949.4737 | 0.14 | 3 | $y_{23}$ | | -0.74 |
| 951.4420 | 0.69 | 1 | $c_8$ | | 0.21 |
| 952.1641 | 0.04 | 3 | $a_{24}$ | +H | -0.95 |
| 961.5397 | 0.56 | 2 | $c_{16}^*$ | | -0.16 |
| 966.8355 | 3.20 | 3 | $c_{24}$ | | -0.76 |
| 969.0134 | 0.54 | 2 | $z_{16}$ | | 0.10 |
| 977.0226 | 0.08 | 2 | $y_{16}$ | | -0.05 |
| 982.4764 | 0.86 | 3 | $z_{24}$ | | -0.75 |
| 982.6156 | 0.20 | 1 | $c_8^*$ | | 0.00 |
| 987.8159 | 0.08 | 3 | $y_{24}$ | | -0.84 |
| 987.9762 | 0.06 | 2 | $a_{17}$ | +H | -0.91 |
| 998.4943 | 0.18 | 2 | $z_{18}^*$ | | 0.55 |
| 999.5014 | 0.03 | 3 | $b_{25}$ | | -1.64 |
| 1005.1776 | 1.81 | 3 | $c_{25}$ | | -0.93 |
| 1007.0054 | 0.09 | 2 | $y_{18}^*$ | +H | -1.59 |
| 1009.9838 | 1.98 | 2 | $c_{17}$ | | -0.10 |
| 1019.0532 | 0.12 | 2 | $c_{17}^*$ | | -0.10 |
| 1025.1745 | 0.57 | 3 | $z_{25}$ | | -0.94 |
| 1025.5571 | 0.38 | 2 | $a_{18}^*$ | +H | -0.10 |
| 1026.5268 | 0.68 | 2 | $z_{17}$ | | 0.05 |
| 1030.5143 | 0.18 | 3 | $y_{25}$ | | -0.71 |
| 1034.5361 | 0.07 | 2 | $y_{17}$ | | 0.00 |
| 1042.5387 | 0.05 | 3 | $a_{26}$ | +H | -2.24 |
| 1046.5215 | 0.49 | 1 | $z_8$ | | 0.10 |
| 1047.0616 | 0.03 | 2 | $c_{18}^*$ | -H | 1.39 |
| 1047.5638 | 0.17 | 2 | $c_{18}^*$ | | -0.24 |
| 1052.0075 | 0.04 | 2 | $a_{18}$ | +H | 1.09 |
| 1057.2111 | 0.15 | 3 | $c_{26}$ | | -1.07 |
| 1062.5421 | 0.07 | 2 | $z_{19}^*$ | | 0.80 |
| 1068.1884 | 1.35 | 3 | $z_{26}$ | | -1.19 |
| 1073.5285 | 0.03 | 3 | $y_{26}$ | | -0.68 |
| 1076.0610 | 0.05 | 2 | $z_{18}$ | | 0.05 |
| 1081.6842 | 0.17 | 1 | $c_9^*$ | | 0.18 |
| 1090.2138 | 0.06 | 3 | $b_{27}$ | +H | -1.07 |
| 1101.8713 | 0.31 | 3 | $z_{27}$ | | -0.85 |
| 1111.4728 | 1.14 | 1 | $c_9$ | | 0.27 |
| 1122.5391 | 1.00 | 2 | $c_{19}$ | | -0.45 |
| 1124.8992 | 0.07 | 3 | M | -CO2 | 0.44 |
| 1161.5923 | 0.17 | 2 | $z_{21}^*$ | | 0.73 |
| 1186.5861 | 0.10 | 2 | $c_{20}$ | | -0.80 |
| 1239.6424 | 0.17 | 2 | $z_{22}^*$ | | 0.32 |
| 1243.1285 | 0.07 | 2 | $c_{21}$ | | -0.48 |
| 1244.7476 | 0.15 | 1 | $c_{10}^*$ | | 0.16 |
| 1249.5725 | 0.05 | 1 | $z_{12}^*$ | | 0.24 |

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|---|---|---|---|---|---|
| 1267.5737 | 0.23 | 1 | $c_{10}$ | | 0.08 |
| 1292.6617 | 0.24 | 2 | $c_{22}$ | | -1.24 |
| 1351.6789 | 0.07 | 2 | $z_{22}$ | +H | -1.52 |
| 1356.7089 | 0.06 | 2 | $c_{23}$ | | -1.40 |
| 1366.6421 | 0.69 | 1 | $c_{11}$ | | 0.07 |
| 1416.2027 | 0.03 | 2 | $z_{23}$ | +H | 0.32 |
| 1449.7508 | 0.06 | 2 | $c_{24}$ | | 0.24 |
| 1481.6686 | 0.16 | 1 | $c_{12}$ | | -0.20 |
| 1492.7027 | 0.06 | 1 | $z_{14}^{*}$ | | -1.74 |
| 1507.2614 | 0.04 | 2 | $c_{25}$ | | -1.66 |
| 1602.2851 | 0.07 | 2 | $z_{26}$ | +H | 0.37 |