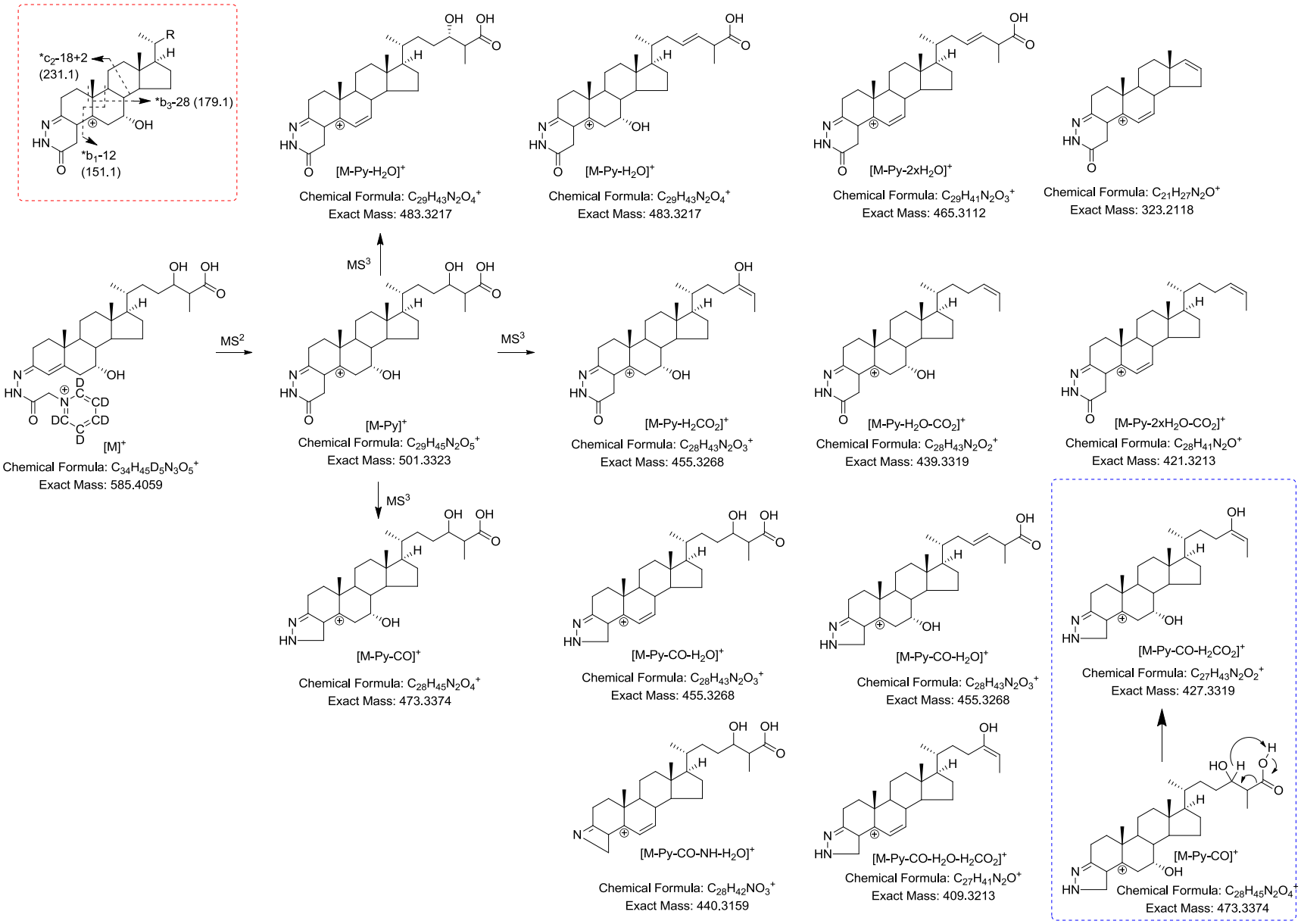


Figure S1. Suggested fragmentation of GP-derivatise CA[4]-7α,24-diol-3-one. Shown in the blue box is the CRF mechanism proposed to give the characteristic fragment-ion at *m/z* 427.3. The inset in the red box shows cleavage in the steroid ring system. Py = pyridine.

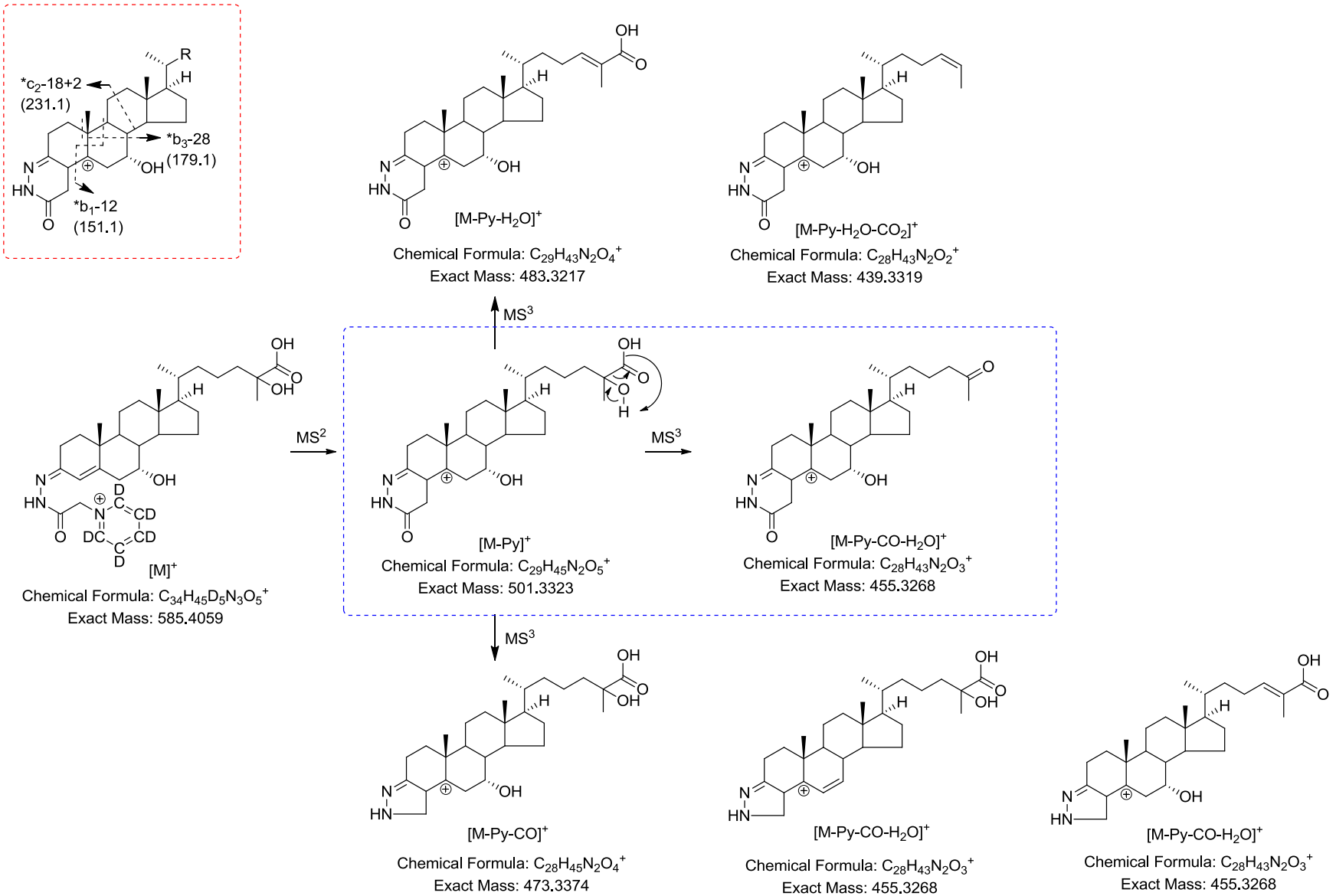


Figure S2. Suggested fragmentation of GP-derivatise CA[4]-7α,25-diol-3-one. Shown in the blue box is the CRF mechanism proposed to give the abundant fragment-ion at *m/z* 455.3. The inset in the red box shows cleavage in the steroid ring system. Py = pyridine.

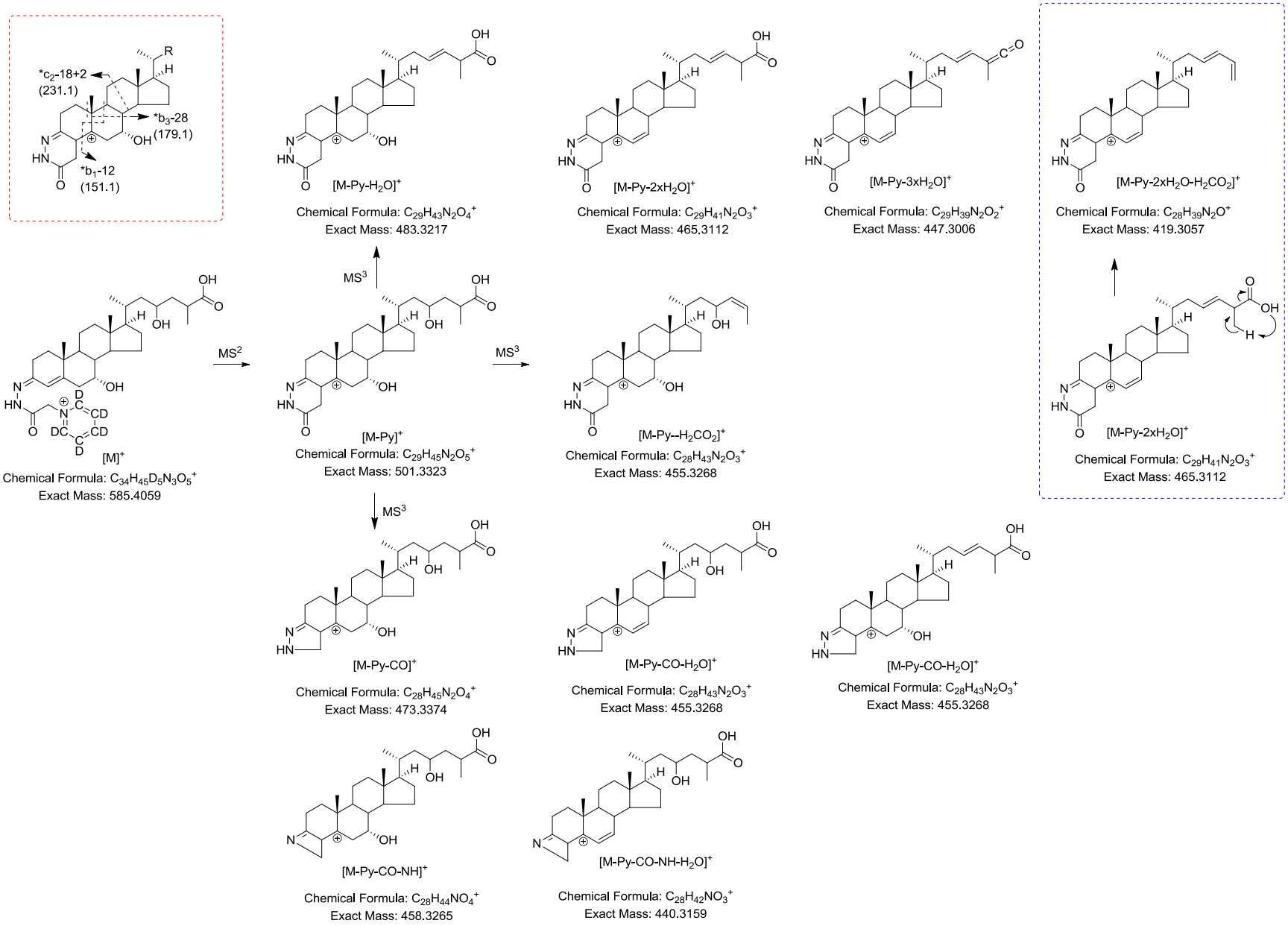


Figure S3. Suggested fragmentation of GP-derivatise CA[4]-7α,x-diol-3-one. The second hydroxy group is drawn at C-23. Shown in the blue box is the CRF mechanism proposed to give the characteristic fragment-ion at *m/z* 419.3. The inset in the red box shows cleavage in the steroid ring system. Py = pyridine.

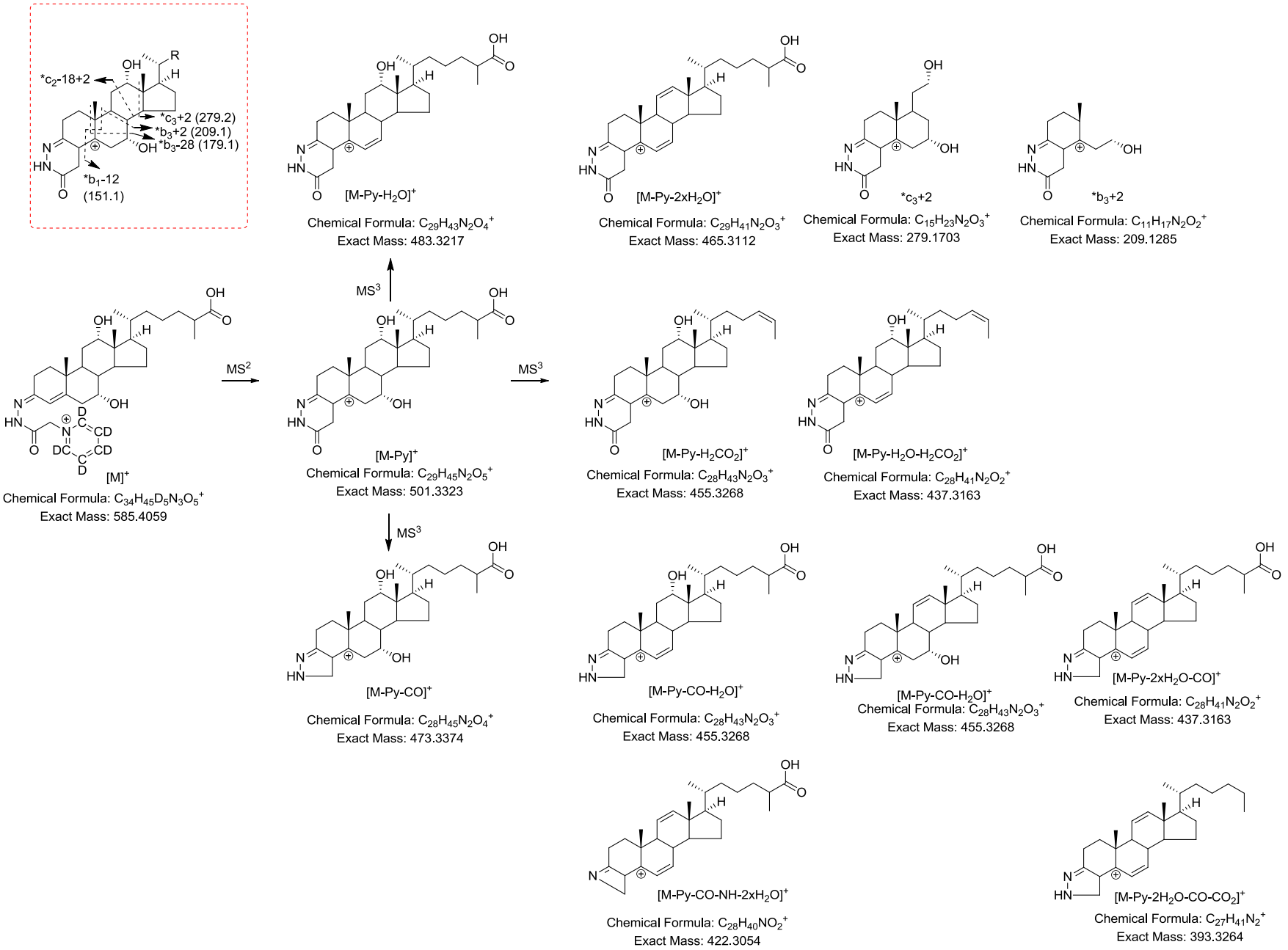


Figure S4. Suggested fragmentation of GP-derivatise CA[4]-7α,12α-diol-3-one. The inset in the red box shows cleavage in the steroid ring system. Py = pyridine.

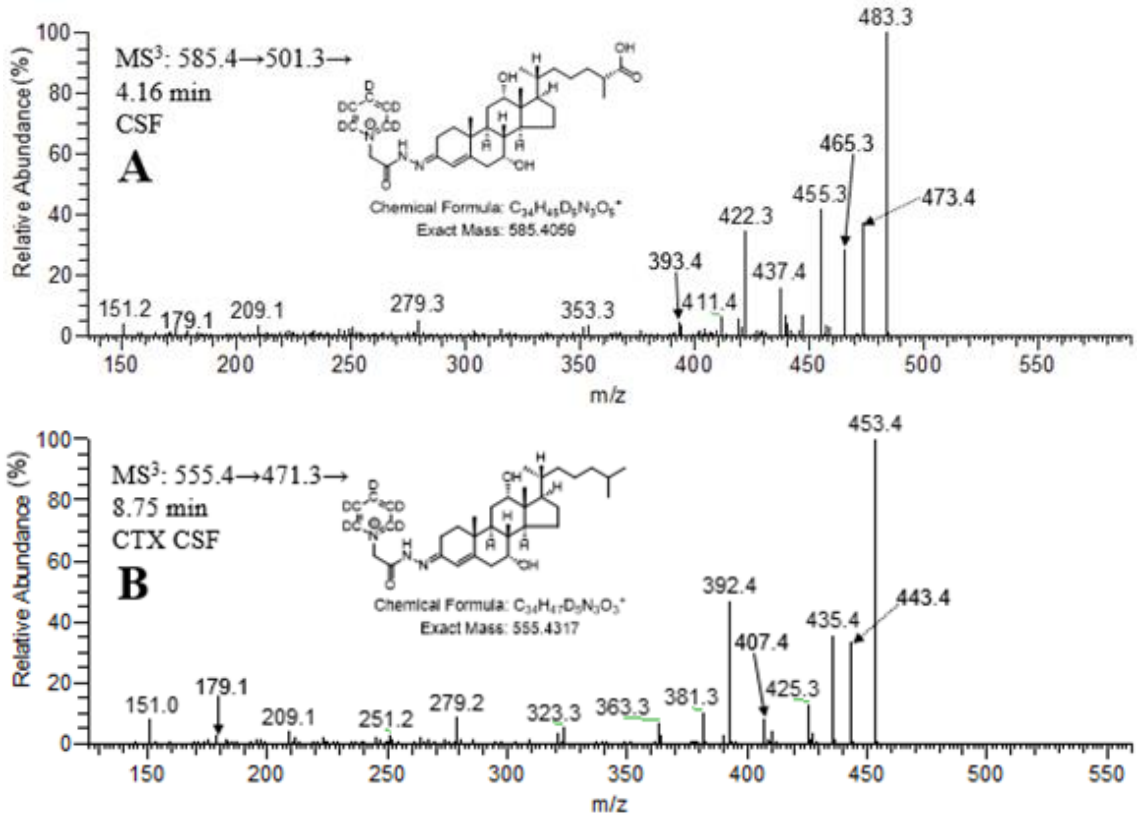
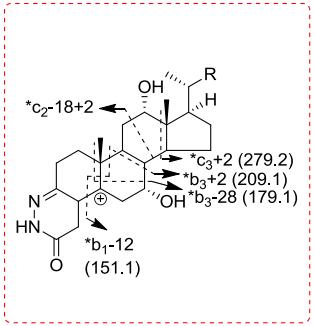
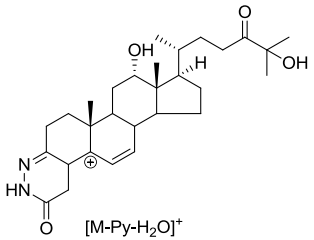
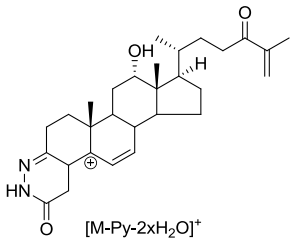


Figure S5. Comparison of the MS^3 ($[M]^+ \rightarrow [M\text{-}Py]^+ \rightarrow$) spectra of (A) CA^4-7α,12α-diol-3-one and (B) C^4-7α,12α-diol-3-one. Both spectra show the steroid-ring fragment ions at *m/z* 151.1, 179.1,209.1 and 279.2. The fragment-ion at *m/z* 422.3 in (A) is displaced by 30 Da in (B), both ions correspond to $[M\text{-}Py\text{-}CO\text{-}NH\text{-}2xH_2O]^+$. Py = pyridine. Data was acquired in the LIT analyser of the Orbitrap Elite hybrid instrument with an accuracy of *m/z* ± 0.1 for most fragment-ions.

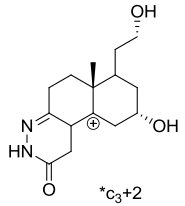




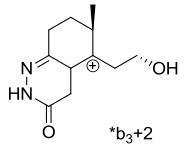
[M-Py-H2O]+
Chemical Formula: C29H43N2O4+
Exact Mass: 483.3217



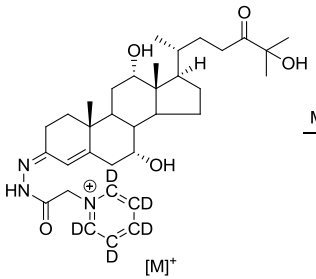
[M-Py-2xH2O]+
Chemical Formula: C29H41N2O3+
Exact Mass: 465.3112



*c3+2
Chemical Formula: C15H23N2O3+
Exact Mass: 279.1703



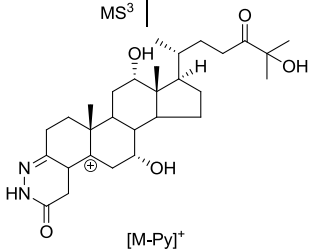
*b3+2
Chemical Formula: C11H17N2O2+
Exact Mass: 209.1285



[M]+
Chemical Formula: C34H45D5N3O5+
Exact Mass: 585.4059

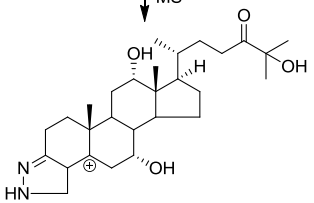
MS2

MS3

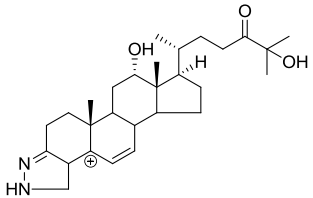


[M-Py]+
Chemical Formula: C29H45N2O5+
Exact Mass: 501.3323

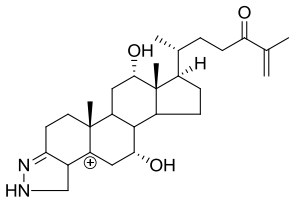
MS3



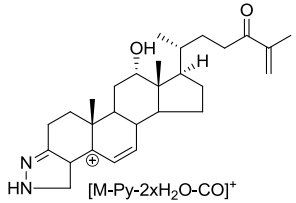
[M-Py-CO]+
Chemical Formula: C28H45N2O4+
Exact Mass: 473.3374



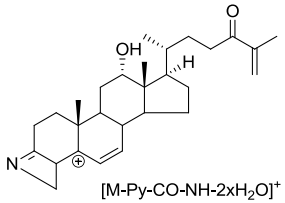
[M-Py-CO-H2O]+
Chemical Formula: C28H43N2O3+
Exact Mass: 455.3268



[M-Py-CO-H2O]+
Chemical Formula: C28H43N2O3+
Exact Mass: 455.3268



[M-Py-2xH2O-CO]+
Chemical Formula: C28H41N2O2+
Exact Mass: 437.3163



[M-Py-CO-NH-2xH2O]+
Chemical Formula: C28H40NO2+
Exact Mass: 422.3054

Figure S6. Suggested fragmentation of GP-derivatise C[4]-7α,12α,25-triol-3,24-dione. The inset in the red box shows cleavage in the steroid ring system. Py = pyridine.

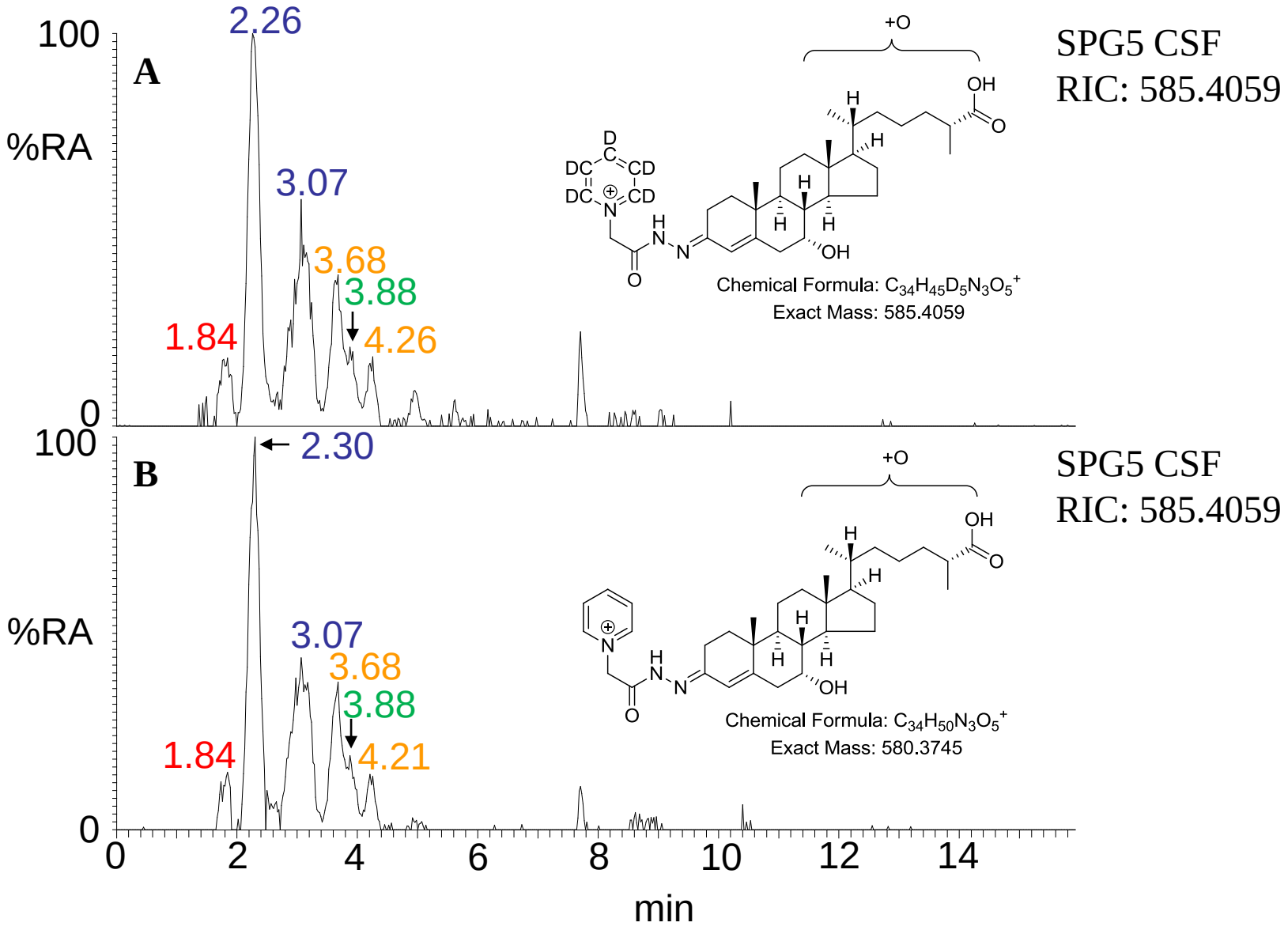


Figure S7A & B. RICs ± 10 ppm from a CSF sample of an SPG5 patient (A) treated with cholesterol oxidase and [$^2H_5$]GP and (B) treated with [$^2H_0$]GP in the absence of cholesterol oxidase. The RICs correspond to $[M]^+$ ions of GP-derivatiseed dihydroxy-3-oxocholest-4-en-26-oic acids.

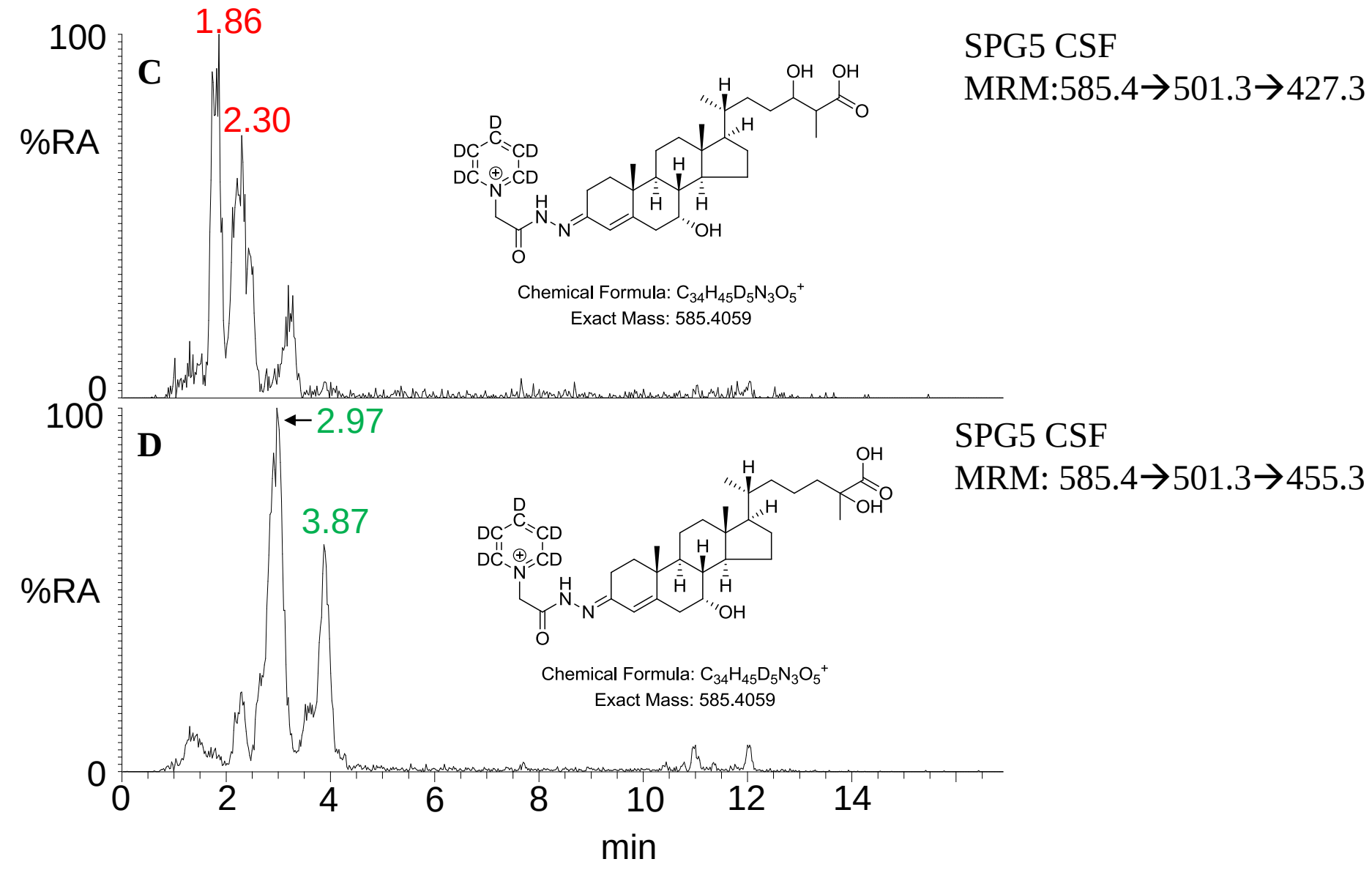


Figure S7C & D. MRM chromatograms from a CSF sample of an SPG5 patient to highlight (C) CA[4]-7α,24-diol-3-one and (D) CA[4]-7α,25-diol-3-one.

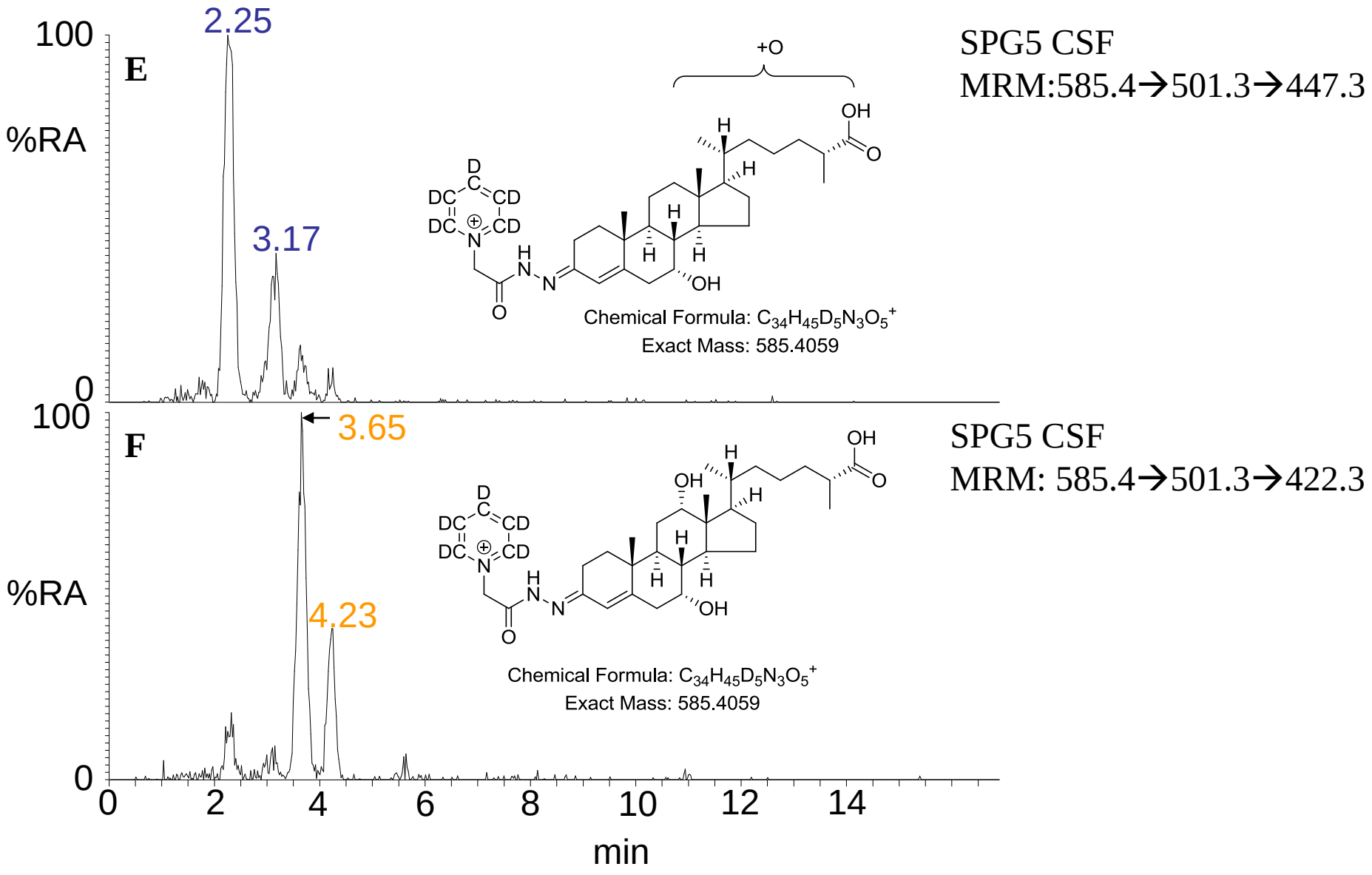


Figure S7E & F. MRM chromatograms from a CSF sample of an SPG5 patient to highlight (E) CA^4-7α,x-diol-3-one and (F) CA^4-7α,12α-diol-3-one .