

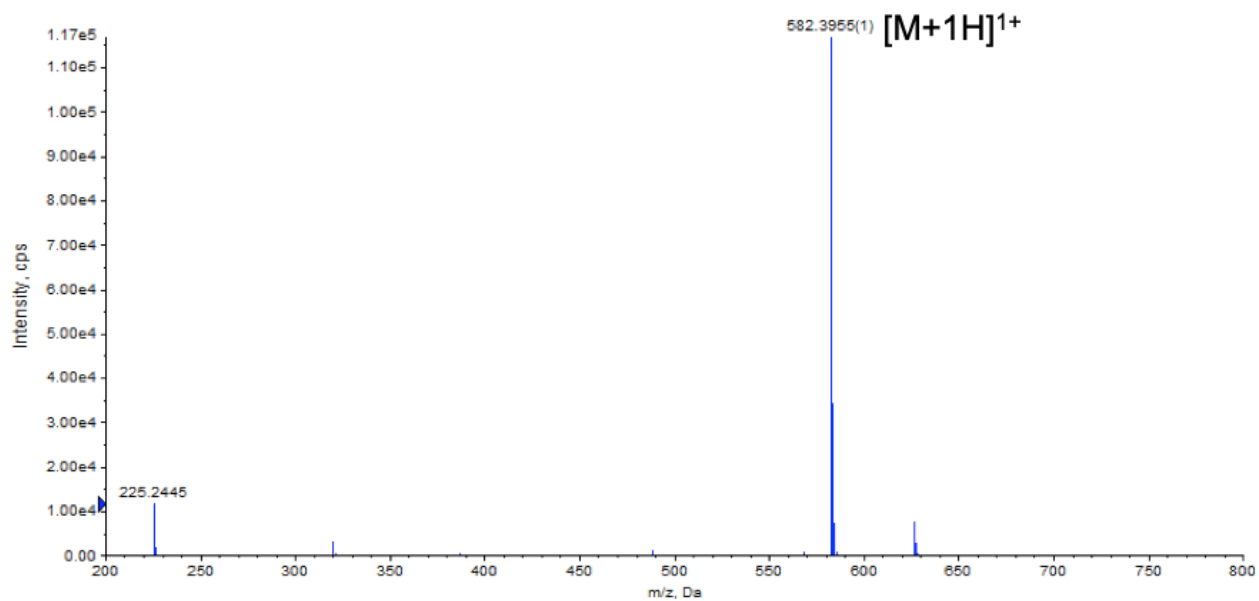
Supporting information

Selective Cyclized α -Melanocyte-Stimulating Hormone Derivative with Multiple *N*-methylations for Melanoma Imaging with Positron Emission Tomography

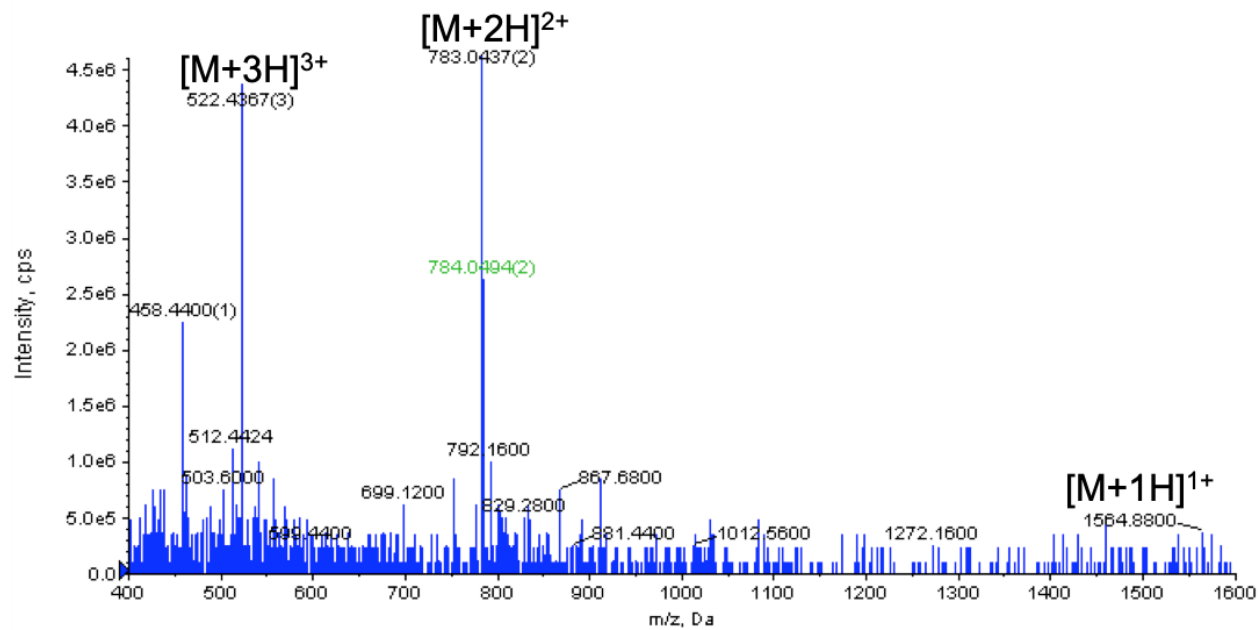
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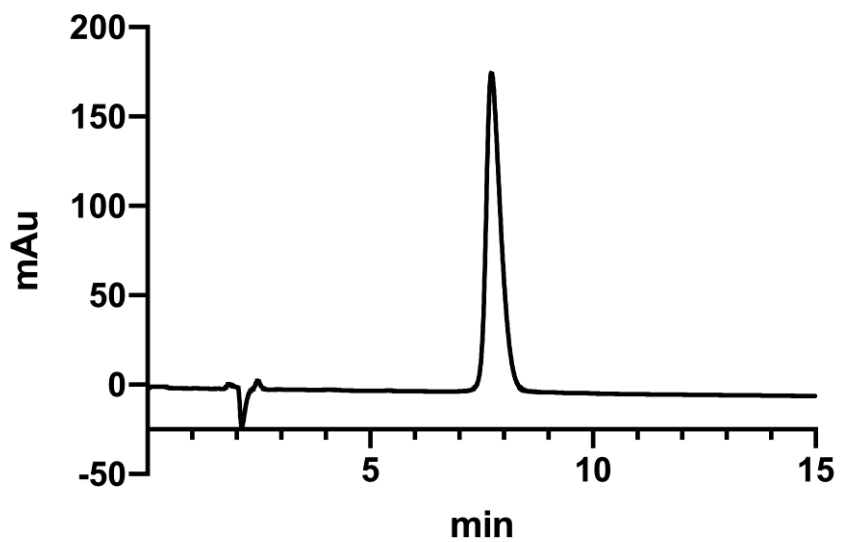


Supplementary Figure S1. Mass spectrometry analysis of an intermediate compound Fmoc-*N*-Me-Trp-*N*-Me-Lys-NH₂. The calculated mass was 582.30 [M+1H]¹⁺, found 582.40. No other non-background peak was observed, which indicated the *N*-methylation reaction on the lys¹⁰ residue was complete.

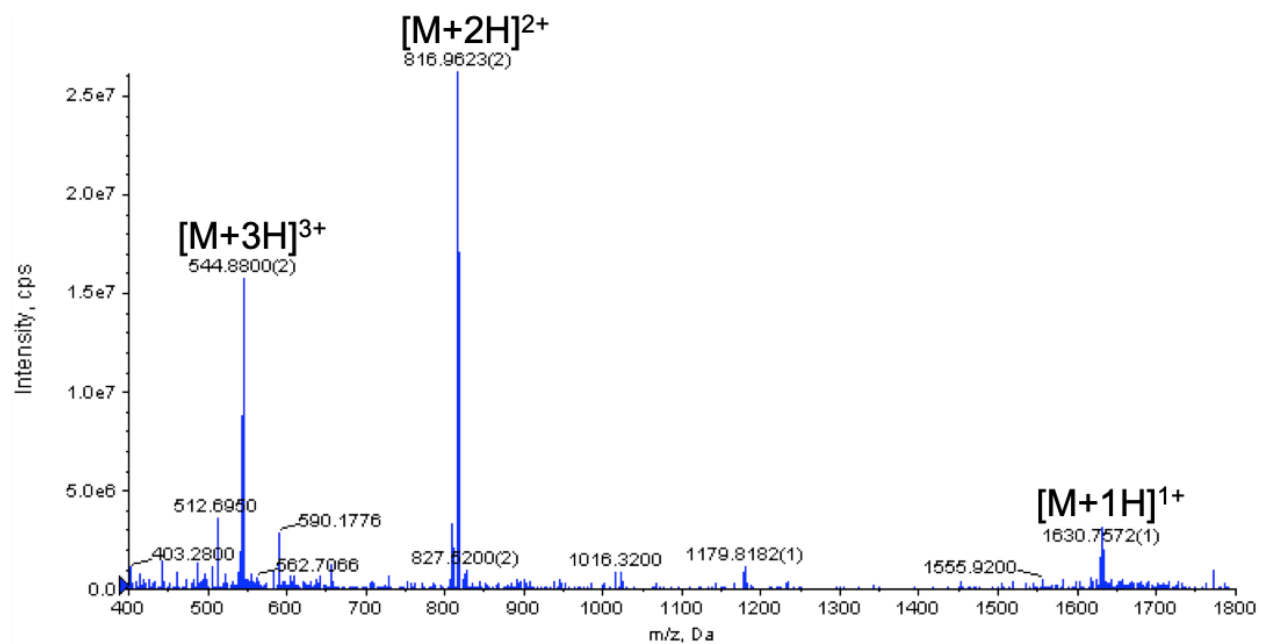


Supplementary Figure S2. Mass spectrometry analysis of CCZ01099. Mass calculated 1564.87

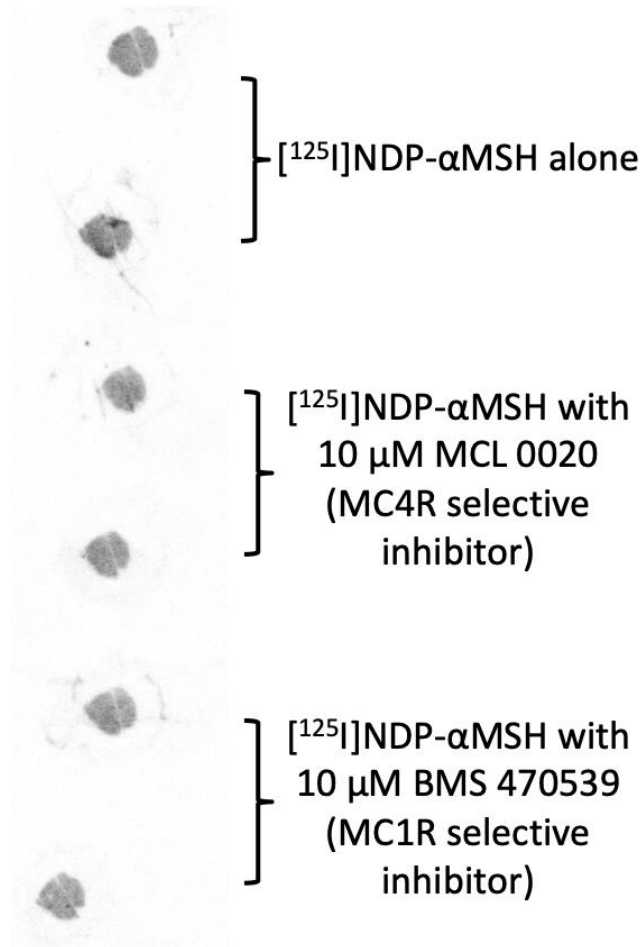
$[M+1H]^{1+}$, found 1564.88.



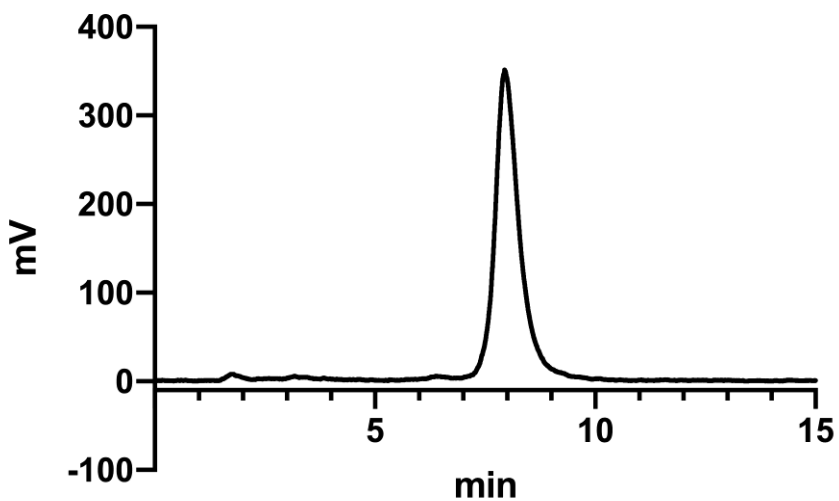
Supplementary Figure S3. Analytical HPLC chromatogram for ^{nat}Ga-CCZ01099 using 21% acetonitrile containing 0.1% TFA at a flow rate of 2 mL/min. The retention time was 7.7 min. Purity was > 99%.



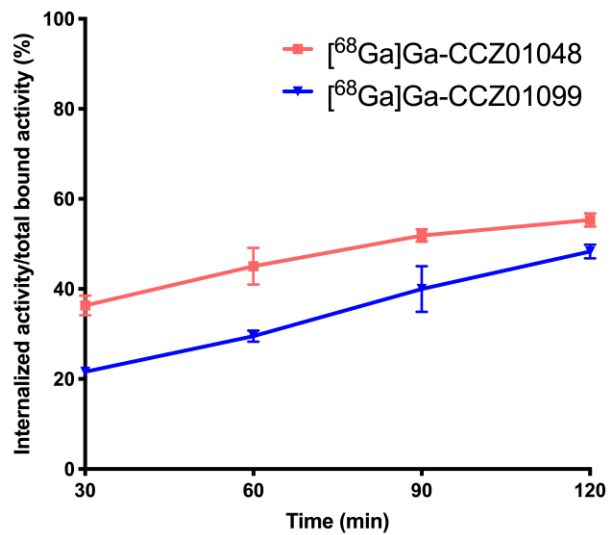
Supplementary Figure S4. Mass spectrometry analysis of non-radioactive gallium-conjugated CCZ01099. Mass calculated 1630.78 [M+1H]¹⁺, found 1630.76.



Supplementary Figure S5. Autoradiography on mouse thyroid tissue incubated with [¹²⁵I]NDP-αMSH without or with MC1R or MC4R inhibitors. Experiments were performed in duplicates.



Supplementary Figure S6. Analytical radio-HPLC chromatogram for [^{68}Ga]Ga-CCZ01099 using 21% acetonitrile containing 0.1% TFA at a flow rate of 2 mL/min. The retention time was 7.9 min. Radiochemical purity was > 99%.



Supplementary Figure S7. Internalization assay of [^{68}Ga]Ga-CCZ01048 (n = 3) and [^{68}Ga]Ga-CCZ01099 (n = 4) on B16-F10 melanoma cell line.