Spatial Localization and Quantitation of Androgens in Mouse Testis by Mass Spectrometry Imaging

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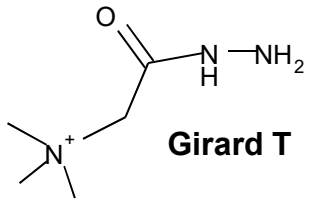
Abstract

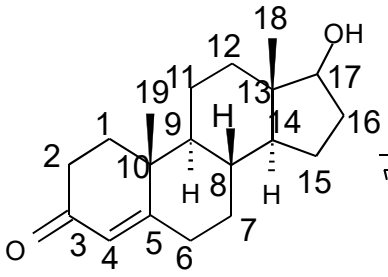
Androgens are essential for male development and reproductive function. Androgens are transported to their site of action as blood-borne endocrine hormones, but can also be produced within tissues to act in intracrine and paracrine fashions. Because of this, circulating concentrations may not accurately reflect the androgenic influence within specific tissue microenvironments. Mass spectrometry imaging permits regional analysis of small molecular species directly from tissue surfaces. However, due to poor ionization and localized ion suppression, steroid hormones are difficult to detect. Here derivatization with Girard T reagent was used to charge-tag testosterone (T) and 5α-dihydrotestosterone (DHT) allowing direct detection of these steroids in mouse testes, in both basal and maximally-stimulated states, and rat prostate. Limits of detection were approximately 0.1 pg for testosterone. Exemplary detection of endogenous steroids was achieved by matrix-assisted laser desorption ionization and either Fourier transform ion cyclotron resonance detection (at 150 μm spatial resolution) or quadrupole-time of flight detection (at 50 μm spatial resolution). Structural confirmation was achieved by collision induced fragmentation following liquid extraction surface analysis and electrospray ionization. This application broadens the scope for derivatization strategies on tissue surfaces to elucidate local endocrine signaling in health and disease.

Supplementary Figure 1 Cobice et al
Steroid Nomenclature and Derivatization Reaction Schemes to form Girard T derivatives of common endogenous androgens.

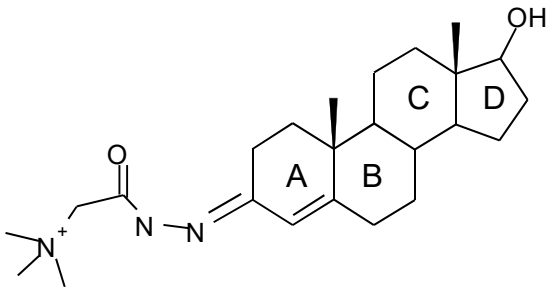


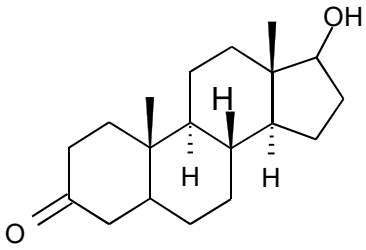
Girard T

Endogenous Steroid ⇌ Girard T Derivative

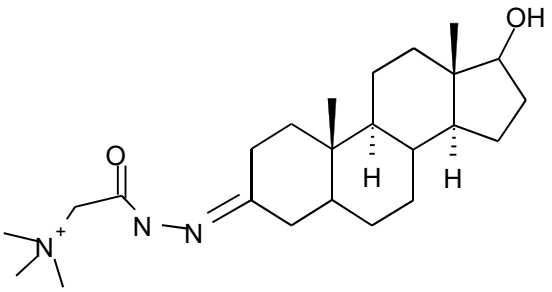


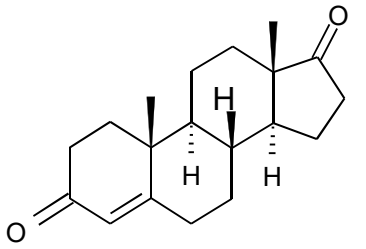
Testosterone



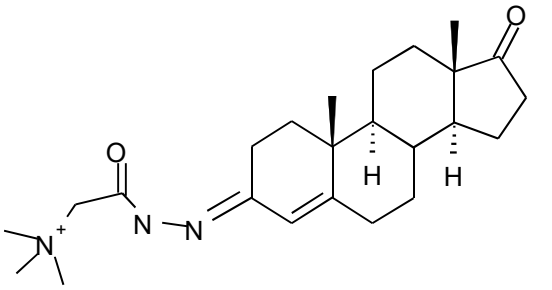


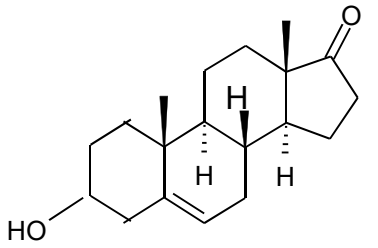
5α-Dihydrotestosterone



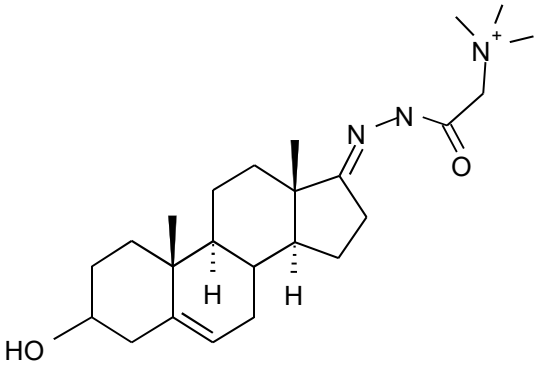


Androstenedione



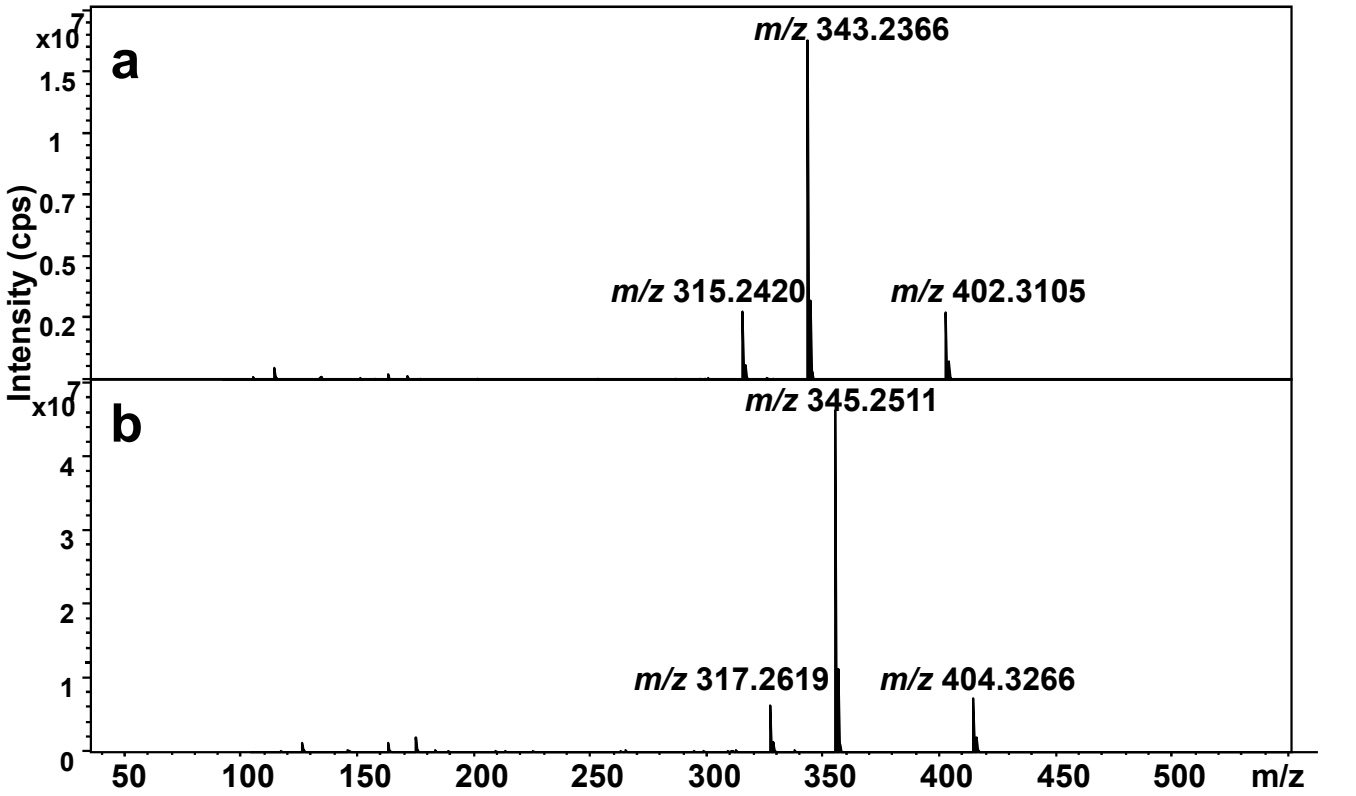


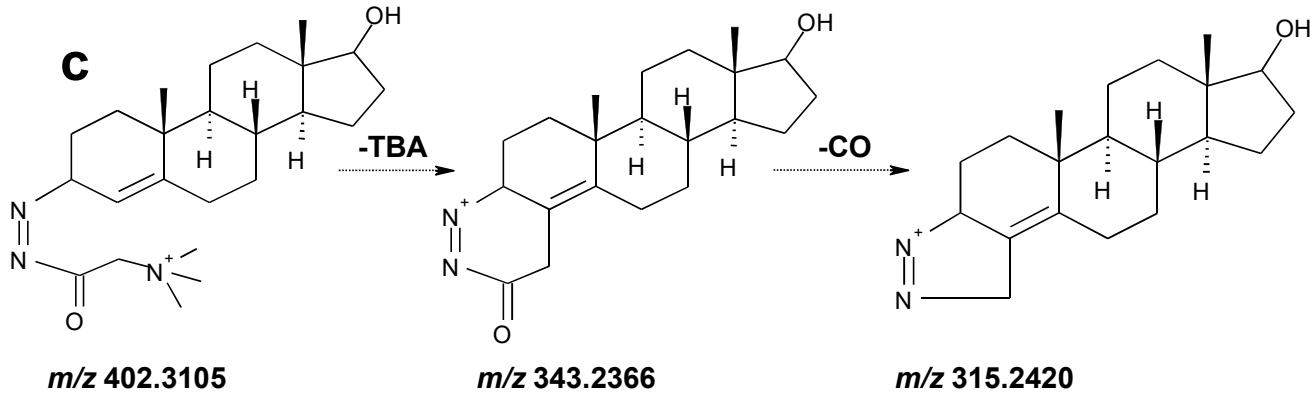
Dehydroepiandrosterone

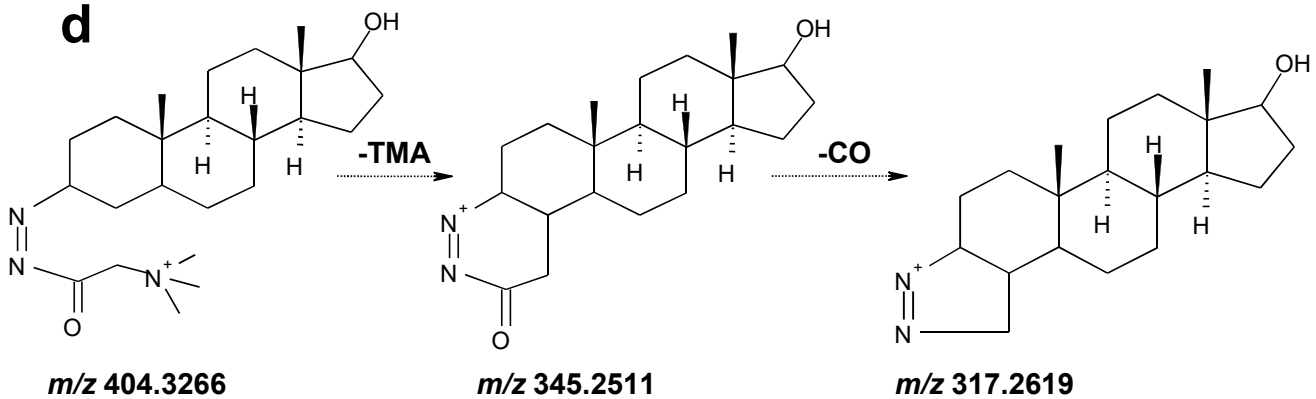


Supplementary Figure 2 Cobice et al

Mass spectra of Girard T-derivative of testosterone and 5α-dihydrotestosterone (DHT) collected following liquid extraction surface analysis with nanoESI-FTICR collision induced dissociation. (**a**) Precursor ions at *m/z* 402.3 Da (isolation window of 0.1 Da) (testosterone) (**b**) Precursor ions at *m/z* 404.33 Da (DHT). Proposed fragmentation patterns for Girard T derivative of **(c)** testosterone **(d)** DHT. Cell isolation was 20 sec and collision energy was set to 32eV. CO: neutral loss of carbon monoxide. TMA: tetramethyl amine moiety.







Supplementary Figure 3 Cobice et al

MALDI-FTICR-MS spectra of a mixture of androstenedione and dehydroepiandrosterone derivatized with Girard T reagent. Derivatized androstenedione at *m/z* 400.2969 Da. Derivatized dehydroepiandrosterone at *m/z* 402.3135 Da. Theoretical spectra are inset. **cps** = counts per second. **(b)** and **(c)** Product ion spectra generated following collision induced dissociation using 32 and 65V respectively; the principle product ion (*m/z* 343) formed by derivatized testosterone was present in both cases.