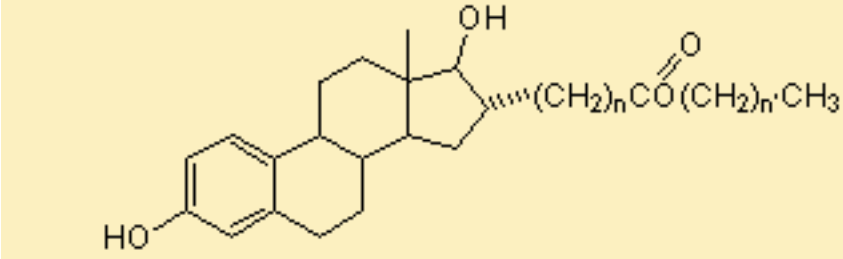
The Design and Synthesis of Steroidal Probes

Our goals are to design and synthesize analogs of steroid hormones as therapeutic agents and probes for research and clinical studies.

Locally Active Estrogen

An important goal of this laboratory is the synthesis of an estrogen that acts solely within the area to which administered, a locally active estrogen. This estrogen is in the family known as "soft drugs" and would be useful for several syndromes and symptoms of estrogen deprivation common to the menopause or through therapeutic intervention by surgery or drugs. One of the well-known effects of estrogen deprivation caused by the menopause is vaginal dyspareunia (atrophy), a disorder which eventually affects a large proportion of all women. Within the first 10 years of menopause, about 40% of all women suffer from dyspareunia It is known to have a severe physical and psychological impact; and to be an important factor in the quality of life for women; it is not only painful but it can dramatically influence a woman's self image, leading to clinical depression. While estrogens can alleviate all of those symptoms, it is usually untreated because of various contraindications as well as the fear of breast and endometrial cancer. Unlike a labile estrogen, even topically administered traditional estrogens are adsorbed through the vaginal mucosa resulting in significant blood levels of estrogens. A local estrogen applied vaginally could solve this prevalent and painful disorder. Additionally, It has been noted that during the estrogen secretory of the menstrual cycle, monkeys are resistant to vaginally administered simian immunodeficiency virus (SIV), a relative of HIV, as are estrogen treated monkeys. The resistance is thought to due to the increased secretions and thickness of the estrogen stimulated vagina, leading to the suggestion that a vaginally applied estrogen could help prevent heterosexual transmission of AIDS. If so, a local estrogen could be very beneficial. Other possible uses of local estrogens include direct administration to bone fractures, topically to skin (there is evidence that the skin is an estrogen target organ and that it contains ER), or as a research probe to localize estrogen effects. Our synthesis of potent but

labile estrogens will produce compounds that will not have systemic action and will be useful for local therapy.



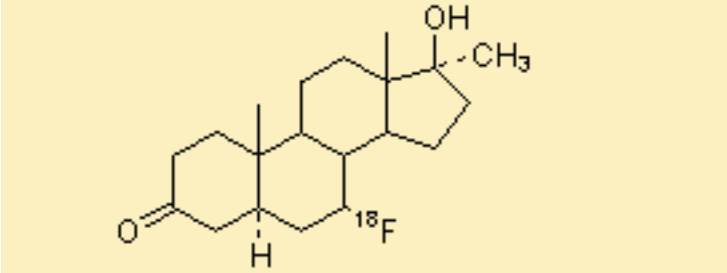
Representative E_2-16α-carboxy esters

Currently, we have synthesized a family of carboxylic analogs of E2 at C-16a (Figure above) and converted these compounds to esters. Many of the esters are biologically active estrogens, while the parent carboxylic acid steroid is inactive. The conversion of the esters into the negatively charged carboxylate is catalyzed by esterases. These enzymes are found in high concentration in all tissues and blood. Thus the E2-esters are rapidly hydrolyzed into the carboxylic acid steroid and inactivated. Thus, the carboxylic acid esters are active only in the tissues to which they are initially applied.

It is our intent to synthesize several families of different esters of estradiol having alkyl groups located in the steroid nucleus at positions where the ER tolerates bulky substituents (7, 11 and 15). They will be tested in structure - activity studies in order to determine the relationship between estrogenic potency, alkyl chain length, position, and rate of hydrolysis of the ester, in order to choose the optimal therapeutic agent. The esters are tested in several different models designed to assess estrogenic potency as well as esterase hydrolysis rates. They include: binding affinity for the estrogen receptor; estrogenic potency in the Ishikawa human endometrial carcinoma cell lines; systemic estrogenic potency in the rat uterine bioassay; local estrogenic potency in the mouse vaginal reductase assay; rate of hydrolysis with rat hepatic microsomal esterases.

Radiochemical Steroidal Probes

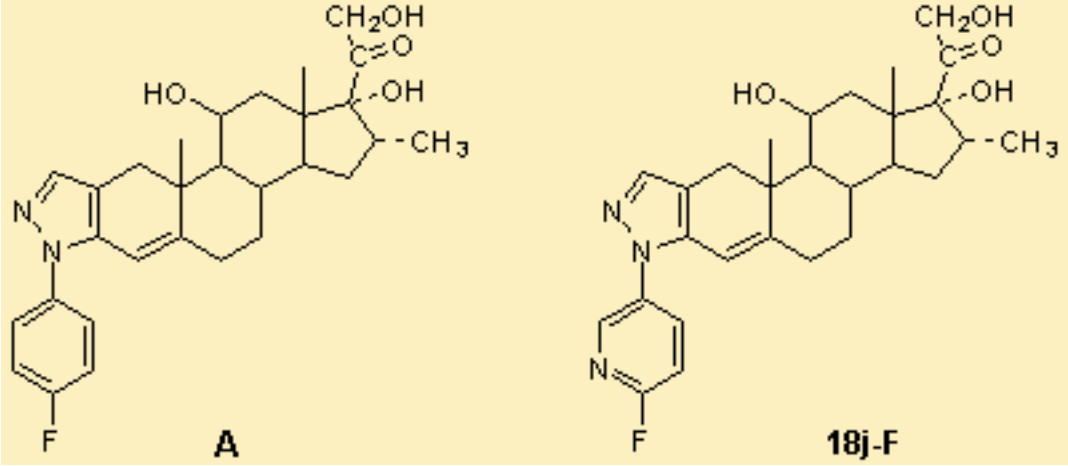
One of the fields that this laboratory is well known for is the design and synthesis of radiolabelled steroid hormones for *in vivo* imaging. Our laboratory synthesized the first steroid hormone labeled with a high energy isotope, an estrogen that could be synthesized with radioiodine. This estrogen, 16a-iodoestradiol, binds with very high affinity to the estrogen receptor and concentrates in estrogen target organs. It could be labeled with 125I for research and analytical studies of breast cancer specimens, or with 123I for for Single Photon Emmission Computerized Tomography (SPECT) imaging. It was used for *in vivo* imaging of breast cancer patients. Currently, an important goal of this laboratory is the synthesis of a radioactive 18F-labelled androgen for Positron Emission Tomography (PET) imaging of prostate cancer. This radiolabeled androgen would allow the detection of prostate tumors and metasteses and allow the non-invasive determination of the effects of surgery and chemotherapy.



7α-^{18}F-17α-methyl-5α-dihydrotestosterone

18F-labeled androgen. We succeeded in synthesizing an 18F-labeled androgen, (7a-18F)fluoro-5a-dihydrotestosterone (Figure above) that binds with high specificity and affinity to the androgen receptor. This radiolabeled androgen localizes in androgen target organs, like the prostate, by an androgen receptor mediated mechanism. It is an analog of the active androgen 5a-dihydrotestosterone, and because of the 17a-methyl group and the 7a-fluoro group it has protection from several catabolic enzymes. Consequently, it has longer biological half life than “natural” androgens which increases both its potency and its uptake into androgen target organs. We are now in pre-clinical testing of this 18F-androgen. Studying its uptake in

several androgen receptor containing tissues *in vivo*, including nude mice with tumors from human prostate, breast and ovarian cancer cell lines. In addition we are determining its uptake into prostate tumors in genetically engineered mice. Such factors as method of administration and “wash-out competition” subtraction are being investigated. PET imaging of androgen sensitive cancers in mice with a micro-PET instrument is also planned.



18F and 123I labeled glucocorticoid. As part of our chemical studies we are attempting to design glucocorticoids that are radiolabeled with 18F or 123I. These corticoids are intended as PET or SPECT agents for imaging the glucocorticoid receptor rich region of the brain, the hippocampus. Dysregulation of hippocampal glucocorticoid receptors is thought to be involved in age and stress related disease as well as neurodegenerative disorders, and such radiolabeled glucocorticoid agents will provide a means to probe the mechanisms that are involved.

In order to synthesize these steroids we have determined that modification (see figure above) of the extra-steroidal ring system in analogs that are based on the potent synthetic corticoid, cortivazol produce compounds that are highly active glucocorticoids. These steroids, such as 18F and 123I labeled , phenyl (such as compound A, above), pyridyl (such as compound 18j-F above) and pyrimidyl N-substituted pyrazolo steroids. The latter 2 families contain N-activated leaving groups in their heterocyclic ring system and are designed for the purpose of isotopic labeling by simple and rapid isotope exchange reactions. Currently, we are synthesizing several different analogs based on

these aromatic and heterocyclic structures. The suitability of the synthetic reactions to radiosynthesis leading to fluorinated and iodinated steroids is evaluated. All of the steroids are assayed for their biological activity: affinity for the glucocorticoid receptor; glucocorticoid action in the induction of alkaline phosphatase in HeLa cells. Suitable steroids are tested for their action *in vivo*, induction of the tyrosine aminotransferase enzyme in rats. The most active steroids are synthesized in radiolabelled form and their concentration in tissues *in vivo* is correlated with glucocorticoid receptor concentration. Finally, the effect of blockers of the multidrug resistance pump, mdr1A P-glycoprotein, is being tested in order to increase uptake of these synthetic steroids into the brain.