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By incorporating a positron-emitting radionuclide into an estrogen receptor ligand, estrogen receptor-positive breast cancer can be visualized with Positron Emission Tomography (PET). In the search for improved estrogen receptor imaging agents, three isomers of 16-methoxyestradiol were synthesized via two synthetic routes, each utilizing methyl hypofluorite. The unusual chemistry of methyl hypofluorite provides a previously unexplored route for functionalizing the 16-position of estradiol and would provide a means of rapidly incorporating carbon-11 into biomolecules. The estrogen receptor binding affinities for these isomers determined these compounds to be ineffective imaging agents for the estrogen receptor. 16α-Methoxyestradiol-17β and 16β-methoxyestradiol-17β, each with the preferred β orientation for the 17-alcohol, were determined to have relative binding affinities of 1.5% and 2.3%, respectively. The stereoisomer with the unfavored α orientation at the 17-position, 16α-methoxyestradiol-17α, exhibited only a 0.5% relative binding affinity for the estrogen receptor. The biological evaluation of these compounds was not pursued further (radiolabeling studies nor animal screening) due to their low binding affinities. Additional studies included reactions of cholesteryl esters with methyl hypofluorite to optimize the reactivity of methyl hypofluorite with steroidal substrates.

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Stephanie D. Jonson 5-28-98

PI - Signature Date

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SECTION 1. GENERAL INTRODUCTION AND BACKGROUND*

1.1. Positron Emission Tomography (PET)

The basis for Positron Emission Tomography (PET) is the following: labeling of a compound with a positron emitting radionuclide; administration of the positron emitting compound to a subject; imaging the subject while the compound distributes over time; and interpretation of the data acquired by applying an appropriate model. Where other imaging modalities provide anatomical information, PET follows a physiological process providing functional information that is valuable in the assessment of disease.

Positron emission occurs in nuclei that are "proton-rich" meaning they contain more protons than neutrons. To balance the number of protons to the number of neutrons, the nucleus converts a proton into a neutron along with the formation of a positron (β^+) and a neutrino (υ). Positrons exhibit similar properties as electrons, but are opposite in charge. When the ejected positron has lost most of its kinetic energy, it combines with an electron from the surrounding matter and undergoes annihilation (Figure 1.1). The mass of the two particles is converted into electromagnetic radiation in the form of two photons. The rest mass of the two particles (1.022 MeV) provides the energy for the two 511 keV photons. Conservation of momentum requires the emission of the two photons to be back-to-back at 180°. A slight deviation of approximately 0.25° is observed due to the initial momentum of the positron/electron pair. There is a small probability during positron annihilation that one, three, or zero annihilation photons will be formed. The probability of the emission of 2-photons versus 3-photons is approximately 372 : 1.[1]

The annihilation photons are detected by two radiation detectors positioned 180° apart and connected in a coincidence circuit. This electronic configuration allows an event to register only if each detector of a coincident pair receives a photon simultaneously or

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* Appears in part in: Jonson, S.D.; Welch, M.J. Pet imaging of breast cancer with fluorine-18 radiolabeled estrogens and progestins. *Q. J. Nucl. Med.* **1998**:41, in press.

nearly simultaneously. The detectors consist of a crystal that fluoresces when exposed to ionizing radiation, coupled to a photomultiplier tube which converts the scintillations into an electronic signal. Scintillation crystals include NaI, CsF, BaF_2, and bismuth germanate.

A PET imaging device consists of a circular array of coincidence-circuited detectors forming one or more rings (Figure 1.2). A "coincident line" is drawn between each pair of detectors receiving a coincidence signal. The radioactivity localized in the patient is positioned along this "coincidence line." The intersection of several of these lines locates the activity in the subject. Some have taken advantage of time-of-flight PET scanners, which differentiate the arrival times of the two annihilation photons at the coincidence detectors. This difference in arrival time provides additional information as to the location of the annihilation event. Complex computer algorithms determine the position of the annihilation and allow images to be viewed in transaxial slices or even a 3-D reconstructed representation.

Figure 1.1. Emission of a positron from the decay of a proton-rich radionuclide followed by annihilation of the positron with an electron in the surrounding matter to produce two photons detected by coincidence circuitry.

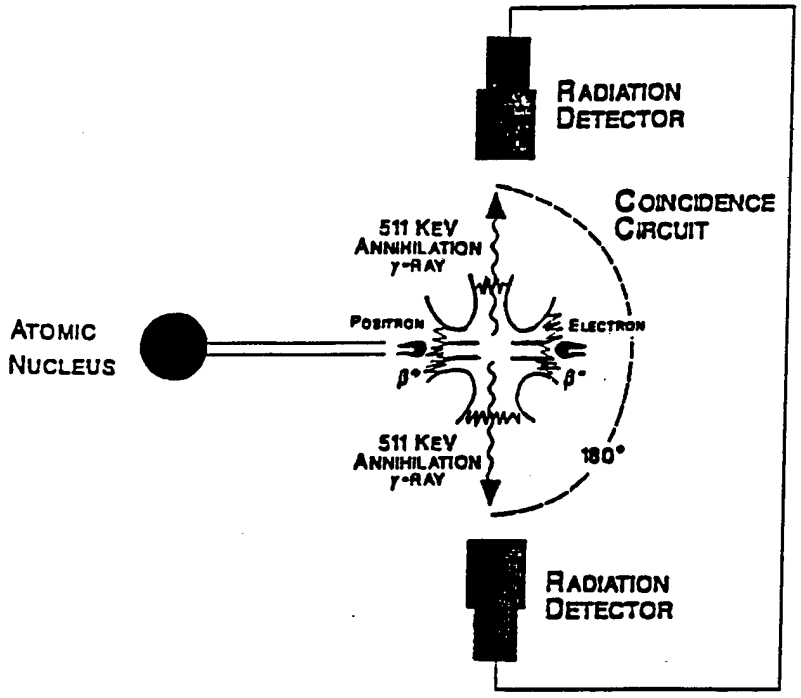
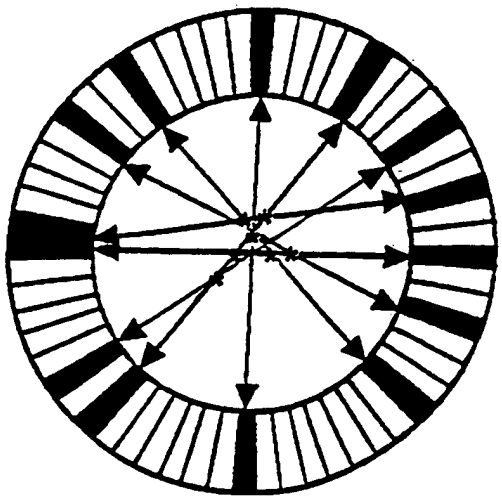


Figure 1.2. A representation of the circular array of radiation detectors found in a PET scanner. The detectors are connected in coincidence, represented as shaded segments separated by 180°, surround the patient and record sets of emitted photons. This information is processed to determine the location of the radioactivity and consequently the tumor site.



1.2. Isotope Production

For biological studies, it is advantageous to incorporate isotopes of elements found naturally occurring in living matter. These include carbon, hydrogen, oxygen, and nitrogen. Each of these elements has a short-lived positron-emitting isotope, except for hydrogen. Fluorine is used as a substitute for hydrogen based on their similar sizes and the good stability of the carbon-fluorine bond. Therefore, carbon-11, oxygen-15, nitrogen-13, and fluorine-18 are the most commonly used radionuclides. The decay attributes and production methods for these isotopes are displayed in Table 1.1. Their short half-lives allow repeat imaging studies and the administration of larger doses of radioactivity without adversely affecting the patient. Due to the short half-lives of these radionuclides, an on-site cyclotron is required for production.

Table 1.1. Decay characteristics of cyclotron produced short-lived positron emitting isotopes of carbon, oxygen, nitrogen, and fluorine.

Isotope	Half-Life (minutes)	% Positron Decay	$E_{\beta+}$ max (MeV)	Cyclotron Production Method
^{11}C	20.4	99.8	0.96	^{14}N (p,α) ^{11}C
^{15}O	2.04	99.9	1.72	^{14}N (d,n) ^{15}O
^{13}N	9.96	100	1.19	^{16}O (p,α) ^{13}N
^{18}F	109.7	96.9	0.64	^{18}O (p,n) ^{18}F

By incorporating positron emitting radionuclides into a biologically active molecule, the *in vivo* utilization and metabolism can be followed over time by PET. The time required for the radiosynthesis of most biomolecules limits the choice of radiolabel to either ^{11}C or ^{18}F, when compared with ^{13}N and ^{15}O. The short half-life of ^{11}C requires rapid synthesis of the imaging agent. Due to the shorter half-life, this isotope is advantageous for repeat imaging studies to follow disease progression of a known tumor site or a

patient's response to therapy, and a compound radiolabeled with ^{11}C will provide a lower radiation dose to the patient as compared to a compound with similar biodistribution and clearance characteristics labeled with ^{18}F. The 110 minute half-life of fluorine-18 allows the imaging agent to be synthesized in multiple steps. This isotope is advantageous for the acquisition of a whole body scan.

The research contained in this final report focuses on the synthesis and work-up of radiopharmaceuticals labeled with carbon-11. Carbon-11 chemistry is limited to a small set of one carbon precursors restricting the synthesis of carbon-11 containing compounds (Figure 1.3).[2] As shown in Table 1.1, carbon-11 is produced by proton bombardment of nitrogen-14. The target is ultra-pure N_2 gas with 0.1-2% O_2. In this manner, carbon-11 is obtained as $[^{11}C]CO_2$, which can be converted to the synthetic precursors shown in Figure 1.3.

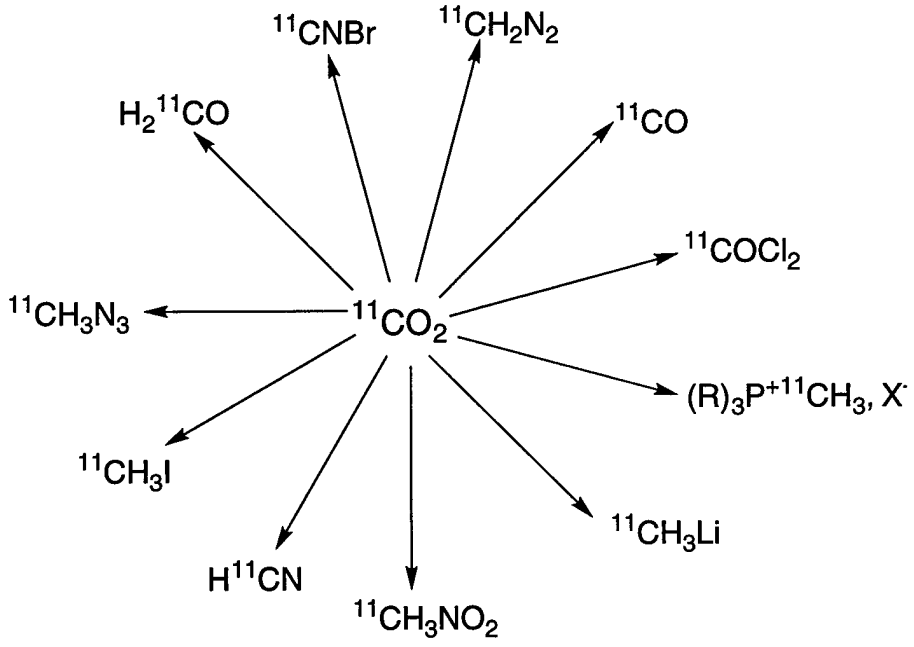


Figure 1.3. ^{11}C one carbon precursors from $[^{11}C]CO_2$.[2]

1.3. Estrogen Receptor-Positive Breast Cancer

In the United States alone, 185,700 new cases of breast cancer will be diagnosed this year.[3] It is the most common form of cancer among women. Imaging techniques which allow detection and the monitoring of disease progression facilitate disease diagnosis, staging, and therapy monitoring. One such imaging modality is PET. Upon injection of a radiopharmaceutical containing a positron emitting isotope, detectors isolate the tumor site as the radiopharmaceutical localizes in the tumor.

Breast cancer biopsies are routinely assayed to evaluate levels of estrogen and progesterone receptors (ER and PR). These *in vitro* measurements indicate the tumor's hormone dependence. Tumors with high concentrations of ER typically respond better to hormonal therapy.[4-6] *In vitro* analysis of ER is limiting as the assay assumes the biopsy to be representative of a homogenous primary tumor. In actuality, the primary tumor is likely to be heterogeneous with respect to ER localization.[4,7] ER concentration in metastases differ from those identified in primary tumors requiring individual biopsies of metastatic tumors to effectively predict overall response to hormonal therapy.[8] Additionally, interlaboratory variability escalates the inaccuracy of *in vitro* assays.[9]

With PET, an *in vivo* analysis of ER status in primary and metastatic breast tumors is obtained. Receptor-positive breast tumors provide an internal targeting system for PET imaging with estrogen positron emitting radiopharmaceuticals. The receptor-mediated radiopharmaceutical uptake into the cancerous tissue provides visualization of ER rich tumors. This receptor-ligand targeting approach is also employed in the treatment of breast cancer by anti-estrogen therapy.

Research among chemists has focused on designing new ligands for the ER in order to improve breast cancer imagery. Characterization of the estrogen receptor (ER) and delineation of signal transduction pathways modulated by the ER are current research topics among molecular biologists and physiologists.[10-17] Research focused toward

understanding the design of the ER provides a broader foundation for treatment and prevention of breast cancer. Data describing the structural requirements for molecules to bind the ER has been determined through contributions from chemists and radiochemists.[18] Their combined efforts joined with expertise from the field of nuclear medicine have successfully achieved imaging of estrogen receptor-positive (ER+) breast cancer with fluorine-18 radiolabeled estrogens.

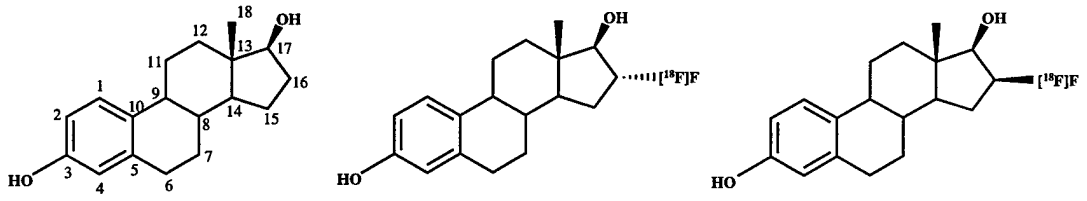
1.4. Design Considerations of Estrogen Receptor Based Imaging Agents

Careful design of an estrogen-receptor ligand is essential in order to image a tumor site. The foundational issue for imaging is resolution: higher accumulation of activity in the target tissue than in surrounding non-target tissues. High resolution imaging provides qualitative information aiding in the detection of tumor sites as well as quantitative information (receptor content) useful in planning therapy regimes. A ligand is designed with high binding affinity for its receptor and low affinity for other receptor systems to affect selective accumulation at the target site. High affinity for the receptor provides enhanced resolution by allowing the radiopharmaceutical to be selectively retained by the target tissue over time.

1.5. Initial Research on Radiolabeled Estrogens with Positron Emitters

The first set of fluorine-18 ER ligands synthesized with assessment of biological activity included two steroidal estradiol derivatives, 16α- and 16β-[^{18}F]fluoroestradiol, and two non-steroidal estrogens, [^{18}F]fluoropentestrol and [^{18}F]fluorohexestrol.[19] The non-steroidal estrogens showed lower uterus/blood and uterus/non-target selectivity ratios than 16α-[^{18}F]fluoroestradiol-17β ([^{18}F]FES) and the fluorohexestrol ligand had high bone uptake attributed to *in vivo* defluorination.

As shown in Figure 1.4, substitution of fluorine-18 into the 16α– (**2**) and 16β– (**3**) position of estradiol was achieved to model the parent estrogen, estradiol-17β (**1**).[19,20] A substitution at the 16 position is in most cases well tolerated with the 16α-epimer having a higher affinity for the ER compared to the 16β-epimer. These two epimers were the first fluorine-18 steroidal estrogens prepared in high specific activity and were evaluated in immature female Sprague-Dawley rats.[19] They showed high uptake into the estrogen-receptor rich uterus and exhibited high selectivity: uterus-to-blood ratios at 1 hr of 39 and 12 for compounds **2** and **3**, respectively. Other fluorine-18 labeled estrogens were prepared and evaluated in animal models, however, the most promising was [^{18}F]FES.[21-23] These initial compounds paved the path to clinical imaging of ER+ breast cancer with fluorine-18 steroidal estrogens.



Estradiol-17β, 1 16α-Fluoroestradiol-17β (FES), **2** 16β-Fluoroestradiol-17β, 3

Figure 1.4. Structures and numbering system for estrogen receptor ligands modeled after the parent steroid estradiol-17β (**1**).

1.6. Clinical PET Imaging With Radiolabeled Estrogens

Favorable biodistribution of [^{18}F]FES in an appropriate tumor bearing animal model predicted this compound to be a good imaging agent for human breast carcinoma.[24] This led to the imaging of human ER+ breast cancer with PET via a fluorine-18 radiolabeled estrogen in 1988.[4] Primary lesions of ER+ breast carcinoma were visualized with

[^{18}F]FES affirming the prospect for development of an *in vivo* technique capable of predicting tumor response to endocrine therapy. This first study by Mintun *et al.* identified PET imaging with radiolabeled estrogens as a useful diagnostic technique based on the following advantages: monitoring recurrent or metastatic lesions without the need for additional biopsy, classification of ER status of individual lesions, and individual patient response predictor for anti-estrogen therapy.[4]

The success of imaging primary ER+ breast carcinomas led to studies aimed at detection of metastatic lesions with [^{18}F]FES. PET was able to identify 53 of 57 individual metastatic lesions in a given study.[5] Three of the 4 lesions not visualized occurred in two patients who received [^{18}F]FES of relatively low specific activity. High specific activity is a necessity when imaging a low capacity receptor system.[25,26] This study by McGuire *et al.* laid the foundation for using PET to assess response to anti-estrogen therapy. Seven patients were imaged with [^{18}F]FES before and after initiation of anti-estrogen therapy with tamoxifen. Reduction in [^{18}F]FES uptake after anti-estrogen therapy was evident in all estrogen dependent lesions. This decrease in uptake reflected a positive response to anti-estrogen therapy in 6 out the 7 patients (positive response = disease improvement for 3 months or longer).

PET was again shown in recent studies by Dehdashti *et al.* and Mortimer *et al.* to be a valuable tool for predicting tumor response to endocrine therapy.[27,28] These two studies compared [^{18}F]FES tumor uptake with 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG) uptake. PET cancer imaging studies routinely employ [^{18}F]FDG as this glucose analog is concentrated in tumor tissues exhibiting an increased rate of glycolysis. Malignant tumors can be differentiated from benign tumors based on [^{18}F]FDG uptake, however, information on ER status was not obtainable with [^{18}F]FDG and a relationship between ER+ tumor uptake of [^{18}F]FDG and [^{18}F]FES was not found.[27] For evaluation of ER status *in vivo*,

[^{18}F]FES has been shown to provide useful quantitative and qualitative ER information not obtainable with [^{18}F]FDG.

Recently, hormone responsive breast cancer patients were found to produce a metabolic "flare reaction" early after initiation of hormone therapy.[29] Women with biopsy confirmed ER+ breast cancer were imaged with [^{18}F]FES and [^{18}F]FDG before institution of tamoxifen therapy and were again imaged with each tracer after 7-10 days. Of the 6 women studied, 3 had lesions in which [^{18}F]FES uptake decreased while [^{18}F]FDG uptake increased as detected in the second set of PET scans. These women were confirmed to be responsive to tamoxifen therapy upon examination 3-7 months later. This "flare reaction" was seen to a lesser extent in 2 of the women as they showed no change in [^{18}F]FDG uptake and a lesser decrease in [^{18}F]FES uptake after 7-10 days on tamoxifen. The disease in these tamoxifen treated women had progressed by a 2 month follow-up examination as the ER+ lesions had not responded to hormone therapy. The tremendous advantage of PET shown in this study by Flanagan *et al.* is the evaluation of tumor responsiveness in as little as 7 days after initiation of tamoxifen.

1.7. Research Objective: Synthesis of New ER Ligands and Application of Methyl Hypofluorite to Complex Steroidal Substrates

Clinical studies utilizing [^{18}F]FES-PET have demonstrated the usefulness of ER imaging agents. The success of [^{18}F]FES has encouraged research efforts to develop superior ligands for the estrogen receptor. Design trends have focused on building ligands with increased receptor affinity and decreased *in vivo* metabolism. Estrogen receptor ligand development encompasses the synthesis of new ligands and the modification of existing ligands to assess their receptor binding affinity and *in vivo* uptake.

As detailed in Section 2, methyl hypofluorite (CH_3OF) was applied to the synthesis of the stereoisomers of 16-methoxyestradiol to investigate the binding affinity of this substituted estradiol. Methyl hypofluorite (CH_3OF) was the first alkyl hypofluorite prepared and has been described as the only source of the novel electrophilic methoxylium ion species "CH_3O^+."[30,31] CH_3OF is generated by passing fluorine gas (20% in Ne) through a solution of methanol and acetonitrile at -40 °C. In as little as 10 min, CH_3OF is formed in 0.10-0.15 M concentration. Reports by Rozen and co-workers showed CH_3OF to react readily with C-C double bonds providing methoxy addition to the more electron-rich carbon and fluorine addition alpha to the methoxy substituted carbon.[32] CH_3OF also reacts with enol ethers to form the corresponding α-methoxy ketones.[31]

We were interested in the chemistry of CH_3OF as a means of preparing a useful one carbon synthon capable of incorporating carbon-11 into biomolecules. In route to this application, [^{11}C]CH_3OF was synthesized in our laboratory, from [^{11}C]CH_3OH, and was used to radiolabel organic substrates.[33] In particular, we desired to synthesize carbon-11 radiolabeled estrogens to image estrogen receptor-positive breast cancer by Positron Emission Tomography (PET).

In order to optimize the reaction conditions of CH_3OF for steroidal substrates, cholesterol derivatives were used as model compounds (as discussed in Section 3). Cholesterol analogs were used because they were easily prepared in high yield. The reactivity of CH_3OF toward double bonds which varied in their degree of substitution was investigated. This reactivity study gave information about solvent requirements necessary to prevent the steroidal substrate from precipitating, purification methods that allowed the isolation of the desired product, and methods for increasing the yield of the desired methoxy-containing product.

SECTION 2. METHYL HYPOFLUORITE IN THE SYNTHESIS OF 16-METHOXYESTRADIOL STEREOISOMERS

(S.D. Jonson, D.A. d'Avignon, J.A. Katzenellenbogen, M.J. Welch, Steroids, **1998**, 63, in-press.)

2.1. INTRODUCTION

Positron Emission Tomography (PET) coupled with radiolabeled estrogens has been used for the diagnostic imaging of estrogen receptors that are present in estrogen receptor-positive (ER+) breast cancer. Currently, the estrogen receptor ligand [^{18}F]-16α-fluoroestradiol-17β ([^{18}F]FES), in conjunction with 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG), is clinically used for breast cancer imaging.[4,5,27] Studies with [^{18}F]FES display the ability of PET to effectively stage breast cancer and monitor therapy response. Several fluorine-18 labeled estrogens have been prepared and biologically evaluated; however, [^{18}F]FES is the only fluorine-labeled ER ligand proven clinically useful.[34-40] The search for improved fluorine-18 estrogens continues, focusing on ligands with decreased *in vivo* metabolism, higher estrogen receptor affinity, and decreased non-specific binding.[36]

While fluorine-18 ($t_{1/2}$ = 110 m) has been the radionuclide of choice for whole body PET imaging and for radiopharmaceutical syntheses requiring multiple steps, ER ligand development has been expanded to the incorporation of carbon-11 ($t_{1/2}$ = 20 m).[41] With a 20 min half-life, carbon-11 radiopharmaceuticals would allow for repeat imaging studies in one sitting to be used to follow disease progression and therapy response of a known tumor site, while providing a lower radiation dose to the patient compared to a fluorine-18 agent. The short half-life of carbon-11, however, requires that the preparation of carbon-11 labeled radiopharmaceuticals be rapid; in addition, the synthesis of carbon-11 containing ER ligands is limited by a small set of precursors commonly available.[2]

Methyl hypofluorite (CH_3OF) has been described as a new carbon-11 synthon, and its high reactivity provides the short reaction times appropriate for rapid incorporation of short-lived isotopes.[33] Methyl hypofluorite, reported as the only source of the novel electrophilic methoxylium ion species "CH_3O^+", is generated by passing F_2 (20% in Ne) through methanol in acetonitrile at -40 °C.[31] The isolation and characterization of CH_3OF and its reactivity toward various alkenes have been reported.[30-32] Enol ethers were found to react rapidly with CH_3OF, forming the corresponding α-methoxy ketones. Previously, compounds of this class were generally prepared by cumbersome multi-step syntheses.[42,43] Thus, application of methyl hypofluorite chemistry to the preparation of novel ER ligands should allow rapid introduction of a methoxy functionality and thereby provide a method for the incorporation of carbon-11.

Figure 2.1. Structures of methoxyestradiol relative to the parent compound estradiol (shown with superimposed steroidal numbering system).

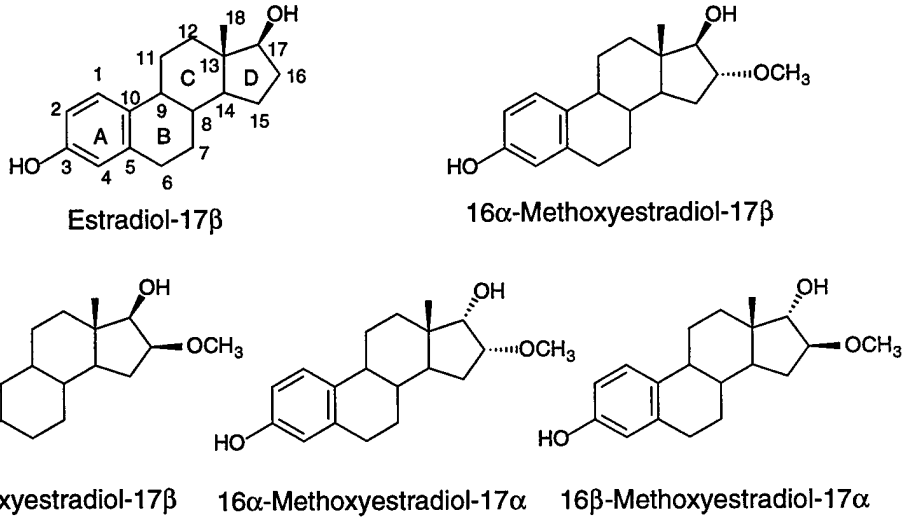


Table 2.1. Relative binding affinities of estrogen receptor ligands substituted at the 16-position (lamb, 0 °C).[44]

Compound	16α	16β	RBA
Estradiol (ES)	--	--	100
Estriol	OH	--	20
16α-Hydroxymethylestradiol*	CH_2OH	--	2.4
16α-FES	F	--	76
16β-FES	--	F	37
16α-Chloroestradiol	Cl	--	100
16α-Bromoestradiol	Br	--	129
16β-Bromoestradiol	--	Br	5.2
16α-Iodoestradiol	I	--	93
16β-Iodoestradiol†	--	I	57

*Reported in reference 45. †Reported in reference 47.

Desiring to utilize the chemistry of CH_3OF to prepare novel ER ligands, we synthesized various 16-methoxyestradiol stereoisomers. Four isomers are possible, since substituents at the 16- and 17-positions can each have the α- or β-orientation (Figure 2.1). Since the ER prefers ligands that have the 17β-OH orientation, 16α-methoxyestradiol-17β and 16β-methoxyestradiol-17β were considered more desirable than the two isomers having the 17α-OH configuration.[44] Fevig et al.[45] synthesized a series of 16α-substituted estradiols to ascertain the ER's tolerance to polarity and steric interference at this site, and Anstead et al.[46] reviewed the structure-affinity correlations of many substituted estrogens for ER. These reports concluded that the receptor can tolerate small, nonpolar substituents at the 16α-position; however, large substituents displayed poor receptor affinity, with affinity decreasing further with large polar substituents. Other binding affinity studies of estrogens, substituted at both the 16α- and 16β-positions with fluorine, bromine, and iodine, revealed a clear preference for 16α- over 16β-substitution (Table 2.1).[44,45,47]

Based on these literature precedents, a methoxy substituent at the 16-position was predicted to be reasonably well tolerated,[44] and the 16α-methoxyestradiol-17β isomer was expected to have the highest binding affinity.

Conditions that were suitable for the reaction of CH_3OF with simple substrates such as the enol acetate of 1-indanone needed to be modified to obtain satisfactory results with the more chemically complex steroidal substrates; specifically, alterations were needed to minimize solubility problems and side product formation.[31] Good results were obtained when the methoxy substituent was introduced by reacting 17-trimethylsilyl enol ether-3-trifloxy (or benzyloxy) estrone with methyl hypofluorite. Deprotection and reduction conditions were varied in order to produce three of the four possible methoxy estradiol stereoisomers: 16α-methoxyestradiol-17β; 16α-methoxyestradiol-17α; and 16β-methoxyestradiol-17β.

^1H NMR resonances in steroids are difficult to assign, due to severe overlap of signals in the aliphatic region, and the need for 2-D (two-dimensional) NMR methods to make complete steroid assignments has been recognized.[48] To confirm the isomeric configurations of the methoxyestradiol compounds, various 2-D correlated NMR techniques were utilized: ^1H-^1H correlated spectroscopy (COSY) methods generally fail for steroids, because the ^1H dispersion is poor, whereas the reasonable ^{13}C dispersion found for many steroids makes identification techniques like ^1H-^{13}C HMQC and HMQC-TOCSY useful, because steroids often have carbons with attached protons on the B, C, and D rings. HMQC-TOCSY provides information in a 2-D format, indicating the correlations between protons and attached carbons belonging to a common spin system.[49] Thus, we found that HMQC experiments correlating ^1H-^{13}C one-bond coupling, combined with the extended coupling identified by HMQC-TOCSY, allowed us to assign all resonances making up the steroid skeleton.

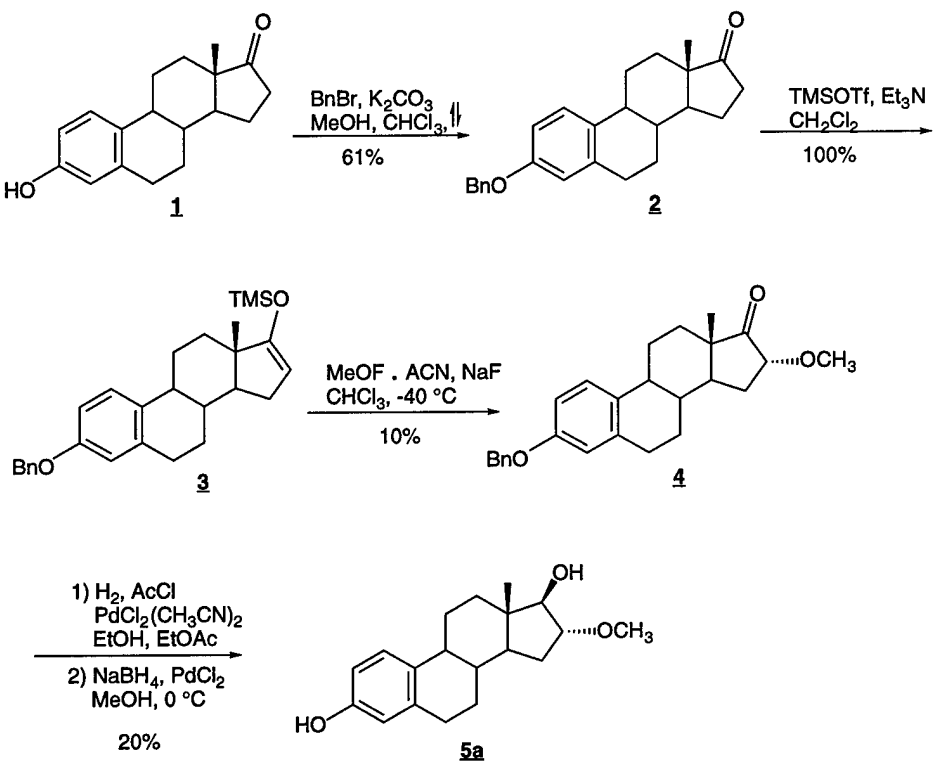
Confirmation of the stereochemistry at the 16- and 17-position was obtained through a NOESY (nuclear Overhauser and exchange spectroscopy) experiment which yielded information about the relative through-space distances between proton atoms. The combination of these NMR techniques was essential for assignment of each isomer's D-ring stereochemistry. Evaluation of the ER binding affinity showed that all three isomers are low affinity ER ligands: Therefore, further biological evaluation was not pursued.

2.2. RESULTS

Synthesis

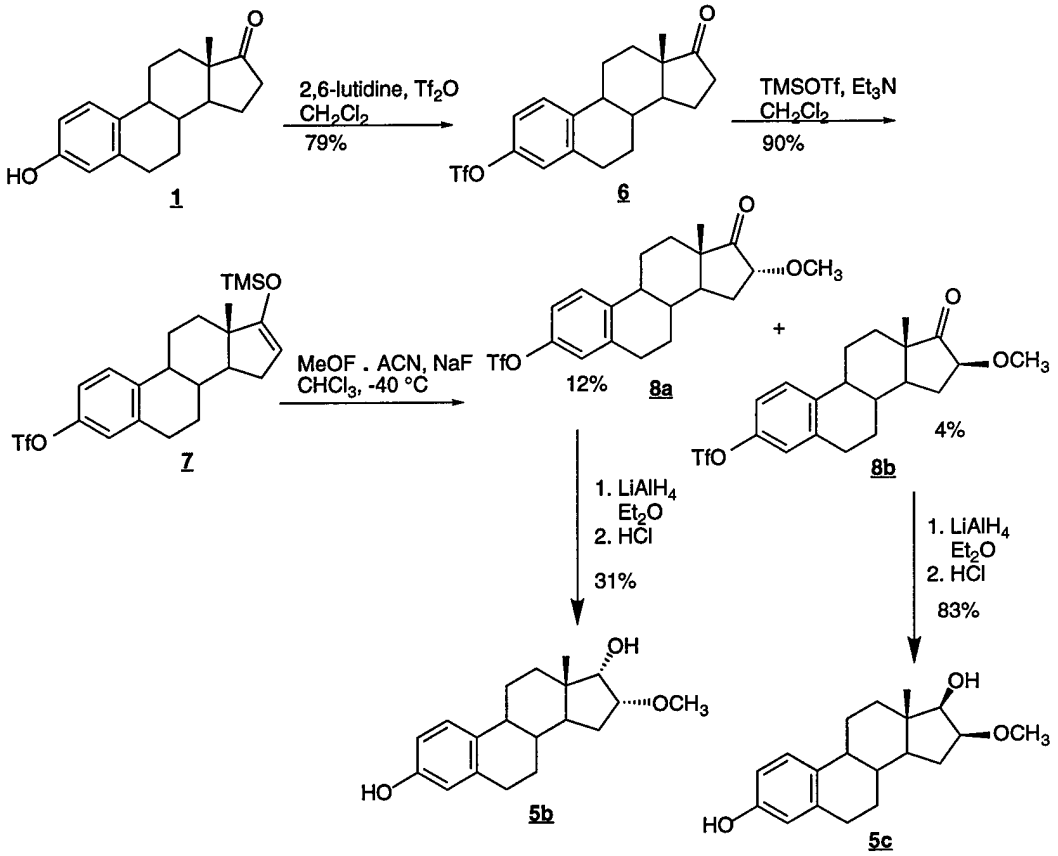
Our synthetic approach to 16α-methoxyestradiol-17β (**5a**) involved reacting the trimethylsilyl enol ether of 3-benzyloxyestrone (**3**) with CH_3OF (Scheme 2.1). This reaction yielded the 16α-methoxy isomer selectively, with minimal to no formation of a 16β-methoxy product. The stereoselectivity of this reaction can be readily ascertained by ^1H NMR: the chemical shift of the 16-H is a doublet at 4.0 ppm in the 16α-methoxy isomer and a triplet at 3.7 ppm in the 16β-methoxy isomer. While the desired methoxy ketone was shown to be produced in 23% yield by ^1H NMR, the isolated yield after column purification was only 10% for this reaction.

Deprotection of 16α-methoxy-3-benzyloxyestrone (**4**) by hydrogenation produced the 16α-methoxyestrone without adversely affecting other functionality on the steroid. NMR analysis showed complete deprotection prior to the reduction. Sodium borohydride reduction in the presence of palladium resulted in the formation of the desired 16α-methoxyestradiol-17β (**5a**) in 20% yield.



Scheme 2.1. Synthesis of 16α-methoxyestradiol-17β (**5a**).

Reacting CH_3OF with the silyl enol ether of 3-trifloxyestrone (**7**) yielded an isomeric mixture of 16α- and 16β-methoxy-3-trifloxyestrone (**8a** and **8b**) in a 3 : 1 ratio, respectively (Scheme 2.2). The yield of methoxy products from the triflate protected precursor increased to 25-37%, as ascertained by ^1H NMR of the crude reaction mixture. Isolation of the 16β-isomer required a two-step purification (silica gravity column chromatography; HPLC) to separate the C-16 epimers. This decreased the yield of the isolated isomers to 16%: 12% and 4% for 16α- and 16β-methoxy-3-trifloxyestrone, respectively.



Scheme 2.2 Synthesis of 16α-methoxyestradiol-17α (**5b**) and 16β-methoxyestradiol-17β (**5c**).

Reduction and deprotection of 16α-methoxy-3-trifloxyestrone (**8a**) with $LiAlH_4$ resulted in formation of the low affinity 17α-OH epimer in 31% yield. Analysis of the remaining products from this reaction failed to show the formation of any of the 16α-methoxyestradiol-17β isomer. Reduction and deprotection of 16β-methoxy-3-trifloxyestrone (**8b**) under the same reaction conditions yielded 16β-methoxyestradiol-17β in 83% yield.

2-D NMR

HMQC and HMQC-TOCSY assignments for compounds **5a**, **5b**, and **5c** confirmed their identity as 16-methoxyestradiols. Representative HMQC and HMQC-TOCSY spectra are shown for **5a** in Figure 2.2 and the assignments are given in Table 2.2. ^{1}H signals for the 16-H and 17-H in **5a** were differentiated by their splitting patterns: doublet for 17-H; multiplet for 16-H. The one-bond ^{1}H-^{13}C correlations resulting from the HMQC were compared with those from HMQC-TOCSY to identify new cross-peaks arising from three-bond ^{1}H-^{1}H couplings.

Table 2.2. ^{1}H-^{13}C HMQC and HMQC-TOCSY assignments for 16α-methoxyestradiol-17β (**5a**).

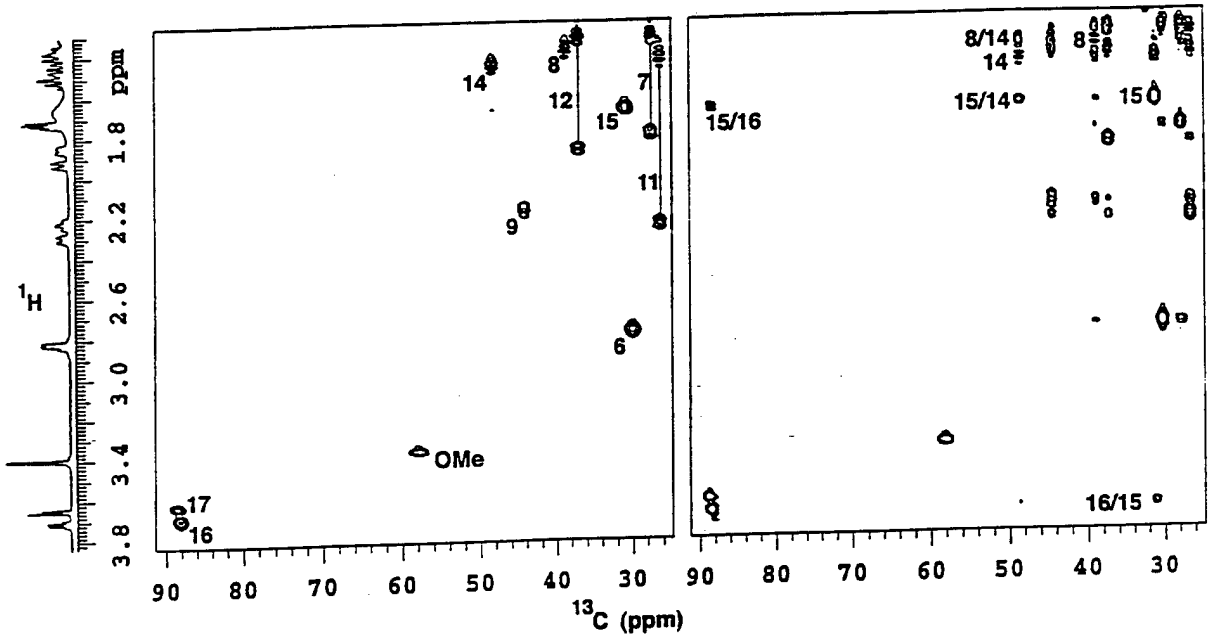
16α-OMe-E2-17β Assignment No.[a]	HMQC ^{1}H Chemical Shift (ppm)	HMQC ^{13}C Chemical Shift (ppm)	HMQC-TOCSY Adjacent Carbon(s) Shift (ppm)
18	0.81	13.0	---
17	3.64	88.2	----
16	3.70	88.0	30.9
15	1.72	30.9	48.4; 88.0
14	1.50	48.4	30.9; 38.8
12	1.35; 1.91	37.0	26.5
11	1.47; 2.29	26.5	37.0
9	2.22	44.2	26.5; 38.8
8	1.43	38.8	27.6; 44.2; 48.4
7	1.36; 1.85	27.6	29.9; 38.8
6	2.82	29.9	27.6
OMe	3.39	57.9	---

[a]Corresponds to the steroidal numbering system shown in Figure 2.1.

The additional cross-peaks in the HMQC-TOCSY contour plot serve to identify three-bond coupled proton partners and thus to identify adjacently bonded carbons (assuming the carbon atoms are protonated). For example, the HMQC-TOCSY contour plot (Figure 2.2) shows additional cross-peaks labeled 15/16 and 16/15 that arise from

three-bond coupling between protons on carbons 15 and 16. With the knowledge of the 16-position carbon and proton assignments, cross-peaks 15/16 and 16/15 guide us to the assignment of position 15. Additional cross-peaks along either the H or C chemical axis for position 15 on the HMQC-TOCSY plot, when compared to the HMQC plot, allowed for the assignment of position 14 resonances. In this fashion, all protonated carbons in the B, C, and D rings were assigned. HMQC-TOCSY signals between the 16- and 17-positions were obscured in **5a**, due to overlapping HMQC signals.

Figure 2.2. Left: ^{1}H-^{13}C HMQC spectrum of 16α-methoxyestradiol-17β (**5a**). Vertical lines indicate cross peaks arising from geminal protons. Numbering refers to the assignment of the carbon position. A high-resolution ^{1}H spectrum is also shown at the far left. Note that the HMQC spectrum differentiates 5 protons in the region of 1.3-1.55 ppm that were indistinguishable by ^{1}H NMR. Right: ^{1}H-^{13}C HMQC-TOCSY spectrum of 16α-methoxyestradiol-17β (**5a**). Signals instrumental in the initial assignment of the spectrum are designated.



Comparison of the NOESY information for the 3 isomers confirmed the stereochemical orientation of the 16- and 17-positions; the results are shown in Table 2.3. The 18β-CH_3 and 14α-H have fixed orientations as assigned for estrone (**1**), and this facilitated the stereochemical assignment of the D-ring. The correlation between the magnitude of the NOESY cross-peak volume integrals and the distance separating the two interacting proton pairs (as determined from energy minimized structures) was quite good. For 16α-methoxyestradiol-17β (**5a**), larger Overhauser enhancement between hydrogen atoms at the 14α- and 17-positions confirmed a same-face orientation. The small interaction seen for the 17α- and 16-hydrogens was highly suggestive that they were on opposing faces. Additional evidence came from the large Overhauser enhancement between the 18β-CH_3 and the 16β-H, implying a same-face orientation.

Table 2.3. NOESY assignments represented as the average relative volume to confirm the stereochemistry of 16-methoxyestradiol isomers.

	NOESY		NOESY		NOESY	
Interacting H Pairs	Volume **5a**	Distance* (Å)	Volume **5b**	Distance* (Å)	Volume **5c**	Distance* (Å)
H_{17} - H_{16}	0.271	3.05	0.825	2.35	0.271	2.36
H_{17} - H_{14}	1.852	2.625	0.058	3.76	---	2.59
H_{17} - OMe	0.376	4.22	0.148	4.13	†	4.41
H_{16} - OMe	1.184	2.35	0.828	2.36	0.440	2.35
18-CH_3 - H_{16}	1.160	2.63	0.808	2.48	0.063	3.87
18-CH_3 - H_{17}	---	3.67	0.833	2.37	0.112	3.67

---A missing value represents that an NOE was not seen for this interaction.
*Structures were built in the modeling program Sybyl with energies minimized. For an interaction involving a methyl or methoxy group, the distance shown is to the nearest proton.
†Represents an obscured interaction by either a cross-peak or an artifact.

A similar comparison of interactions confirmed the stereochemistry of 16α-methoxyestradiol-17α (**5b**). Only a negligible enhancement was seen for the 14α-H with the 17-H, highly suggestive of a 17β-H orientation. A large Overhauser enhancement with the 18β-CH_3 was seen for both the 16- and 17-hydrogens, suggestive that all three substituents are on the same (beta) face. Reaffirming evidence for the 16α- and 17α-hydrogens was the large enhancement between these signals.

The orientation of 16β-methoxyestradiol-17β (**5c**) was confirmed by the weak interaction between the 18β-CH_3 and the 16-hydrogen (opposite face), relative to the large interaction between the 16α- and 17α-hydrogens (same face).

Relative Binding Affinities

The relative binding affinities of the 16-methoxyestradiols for the ER were determined by a competitive radiometric binding assay using lamb uterine ER.[50] The highest RBA for this methoxyestradiol series was 2.3 for **5c** ,while the RBA values for **5a** and **5b** were 1.5 and 0.5, respectively. The isomers of 16-methoxyestradiol all displayed low binding affinity for the ER compared to the natural ligand estradiol.

2.3. DISCUSSION

Synthesis

The methyl hypofluorite reagent allowed the facile incorporation of a methoxy group at the 16-position of the steroid skeleton, and by using two related synthetic routes, we were able to obtain 3 of the 4 possible isomers of 16-methoxyestradiol. This allowed us to evaluate the ability of these ligands to bind to the ER. This study also prompted us to expand the chemistry of CH_3OF from structurally simple to more complex molecules, and the methods we have developed for the synthesis of methoxy substituted estrogens will be applied to the preparation of other steroidal compounds in the future.

In our initial trial reactions with CH_3OF, the direct addition of an enol ether containing substrate dissolved in CH_2Cl_2 was made to the methyl hypofluorite-acetonitrile complex (CH_3OF•ACN) at -40 °C. This procedure yielded a crude mixture of ca. 8 products (detected by TLC), with formation of only minor amounts of the desired product. We noted that a precipitate formed upon substrate addition to CH_3OF•ACN. Further investigation showed that the substrate was insoluble in the ACN/CH_2Cl_2 solvent combination at -40 °C, which presumably caused the precipitation. On the basis of these observations, conditions for substrate addition to CH_3OF•ACN were modified to maintain enol ether solubility, while retaining the reactivity of CH_3OF. Product yields were further increased by changing the substrate solvent to $CHCl_3$, which is more effective in radical scavenging.

The formation of side products, presumed to result from the reaction of substrate with HF formed during the generation of CH_3OF, was decreased by the addition of oven-dried NaF to the CH_3OF•ACN immediately prior to the transfer of this solution to the $CHCl_3$ dissolved substrate. NaF acts as a fluoride ion acceptor, decreasing the acidity of HF through the formation of the HF_2^- ion, thereby reducing its reactivity towards the substrate.[51]

Reduction and deprotection of 16α-methoxy-3-trifloxyestrone with $LiAlH_4$ led to selective formation of 16α-methoxyestradiol-17α (**5b**). This was unexpected because $LiAlH_4$ is used to reduce and deprotect 16α-fluoro-3-trifloxyestrone, furnishing the deprotected 17β-OH and 17α-OH estradiols in a 3 : 1 ratio.[20] Thus, although the combined reduction-deprotection step with $LiAlH_4$ was advantageous in these earlier steroid syntheses, with the 16α-methoxy isomer it did not furnish the desired 17β-OH configuration. Unexpected 17α- and 17β-OH ratios have also been seen in other $LiAlH_4$ reduction/deprotection sequences, such as that of 11β-ethyl-16β-fluoro-3-trifloxyestrone. With the β-face being blocked by both the 11β–ethyl, 16β–fluoro, and 18β-methyl

substituents, hydride attack from the unhindered α-face was expected; however, in this case the attack from the shielded β-face prevailed by 1.6 : 1.[40] By contrast, $LiAlH_4$ reduction of 16β-methoxy-3-trifloxyestrone (**8b**) was not anomalous, giving 16β-methoxyestradiol-17β (**5c**). This configuration was expected, because hydride attack from the β-face of the steroid is blocked simultaneously by the 18β-methyl and 16β-methoxy groups.

Choice of reagent and reaction order determined which stereoisomer was preferentially formed. In order to selectively reduce the protected 16α-methoxyestrone to the 17β-OH, we used sodium borohydride ($NaBH_4$) in the presence of palladium chloride, as this method is known to reduce 16α-hydroxyestrone and 16α-acetoxyestrone selectively to the corresponding 17β-estradiols.[52] Direct application of this procedure to 16α-methoxy-3-trifloxyestrone (**8a**), however, proved unsatisfactory, as it led to the formation of 16α-methoxy-3-deoxyestradiol-17β. It was clear that the triflate protecting group had to be removed prior to ketone reduction, to eliminate deoxygenation at the 3-position. The triflate could be removed with KOH/methanol at 60 °C; however, these base conditions epimerized the 16α-methoxy group, favoring the 16β-methoxy epimer 2 : 1. We avoided these problems by changing the protecting group at the 3-position. When this position was protected as a benzyl ether, it could be rapidly deprotected by hydrogenolysis; subsequent reduction with $NaBH_4$ in the presence of palladium yielded 16α-methoxyestradiol-17β (**5a**).

2-D NMR

Two-dimensional correlative NMR techniques were crucial in the characterization of these isomers. HMQC and HMQC-TOCSY provided a solid means for mapping the steroid structure, through analysis of ^{1}H-^{13}C one-bond and three-bond ^{1}H-^{1}H couplings. The stereochemistry of the isomers was confirmed by analysis of the distance-dependent

nuclear dipole-dipole interactions obtained through NOESY. These NMR techniques lend themselves well to steroid resonance assignments and structural characterization.

Relative Binding Affinities

Estrogens labeled at the 16-position with the electron-withdrawing halogens retain good estrogen receptor binding affinity, suggesting that the productive receptor-ligand interaction is being maintained (Table 2.1).[44] The more polar and electron rich methoxy group at this position, however, does not lead to a favorable receptor interaction. Steric interference of the methoxy group with the ER does not appear to be contributing to the low relative binding affinities (RBA) of these compounds. In the series of 16α-substituted estradiols studied by Fevig et al., substituents larger than the methoxy such as $-CH_2I$, $-CH_2CH{=}CH_2$, and $-CH_2N_3$, are reported to retain good ER binding affinity.[45] However, Fevig et al. reports that 16α-hydroxylmethylestradiol, a structural isomer of 16α-methoxyestradiol-17β (**5a**), has an RBA of only 2.4. This compound is the closest model we have for comparison to the 16α-methoxyestradiol-17β. Interestingly, the calculated partition coefficients for these two compounds, 3.52 and 3.59, respectively, illustrate their closely related lipophilicities. Thus, the low RBA of **5a** is understandable by comparison with the 16-CH_2OH-substituted estradiol with which it shares similar lipophilicity and size. The mechanism responsible for the poor ER binding of these related compounds, however, is not obvious. The determined RBAs for this series of estrogens showed them to be poor receptor binders and, therefore, unsuitable as estrogen receptor imaging agents.

2.4. EXPERIMENTAL SECTION

General. All commercial reagents were used as received from the suppliers unless otherwise noted. HPLC solvents were Optima grade. Fluorine (20% in Ne) was purchased from Acetylene Gas (St. Louis, MO). ***Due to the strong oxidizing and***

corrosive nature of fluorine, appropriate laboratory safety and personnel protective equipment were utilized.[53] 2,6-Lutidine was distilled from barium oxide and stored over molecular sieves. Methylene chloride (CH_2Cl_2) and triethylamine (TEA) were distilled from calcium hydride (CaH_2). Column chromatography was performed using silica gel (60 Å, 230-400 mesh) or basic alumina (40 μm). Thin-layer chromatography (TLC) was performed on UV active 250 μm silica plates visualized with phosphomolybdic acid or potassium permanganate. Melting points are uncorrected. Microanalyses were performed by Galbraith Laboratories.
3-[[(Trifluoromethyl)sulfonyl]oxy]estra-1,3,5(10)-trien-17-one (**6**) was prepared according to the literature.[20] General work-up of organic solutions included drying over $MgSO_4$, filtering, and removing solvent under reduced pressure.

NMR Measurements. All NMR data were recorded at 25 °C on samples dissolved in d-chloroform (concentration: 4-10 mg/600 μL). Routine ^1H, ^{19}F, and ^{13}C spectra were obtained on a Varian Gemini NMR spectrometer at 300, 282, and 75 MHz, respectively, while two-dimensional HMQC, HMQC-TOCSY, and NOESY experiments were obtained using a Varian Unity-Plus instrument operating at 500 MHz. Chemical shifts for ^1H and ^{13}C were referenced to internal tetramethylsilane and ^{19}F was referenced to internal $CFCl_3$. Two-dimensional experiments included ^1H and ^{13}C spectral widths of 5,207 and 19,408 Hz, respectively, with 90° pulse widths of 8 μs (^1H) and 12 μs (^{13}C). In the t_2 dimension, 2,048 complex time points were collected and 600 complex time points in t_1 were employed with zero filling to 2,048 x 2,048 with gaussian weighing in both dimensions prior to Fourier transformation. The NOESY mixing time was 700 ms. For HMQC-TOCSY a 15 ms isotropic mixing period was employed and resulted in strong 3-bond ^1H-^1H correlations, with weak 4-bond interactions also present as an assignment aide. ^{13}C GARP decoupling was used for both HMQC and HMQC-TOCSY.

3-(Benzyloxy)estra-1,3,5(10)-trien-17-one (2). A mixture of 50 mL $CHCl_3$, 25 mL MeOH, and K_2CO_3 (1.23 g, 8.88 mmol) was refluxed under N_2 for 15 min and then added to a solution of **1** (1.2 g, 4.44 mmol) and BnBr (1.06 mL, 8.88 mmol). The reaction was refluxed for 21 hr, cooled to rt, filtered, and filtrate concentrated under reduced pressure. Residue was dissolved in CH_2Cl_2, washed with 1x100 mL 1 N HCl, followed by general work-up. Recrystallization from MeOH yielded **2** as a white solid (0.978 g, 61%). mp 126-128 °C. ^{1}H NMR ($CDCl_3$): δ 0.91 (s, 3H, 18-CH_3), 1.30-2.60 (m, 13H), 2.90 (m, 2H), 5.04 (s, 2H, $PhCH_2OAr$), 6.74 (d, J = 2.7, 1H), 6.79 (dd, J = 8.6, 2.7, 1H), 7.21 (d, J = 8.7, 1H), 7.32-7.45 (m, 5H). HRMS calcd for $C_{25}H_{28}O_2$ (M^+) 360.2089, found 360.2081. Anal. ($C_{25}H_{28}O_2$) C, H.

17-(Trimethylsilyl)oxy-3-(benzyloxy)estra-1,3,5(10),16-tetraene (3). To a solution of **2** (0.770 g, 2.14 mmol) in 15 mL CH_2Cl_2 under N_2 was added Et_3N (1.55 mL, 11.1 mmol, 5.2 eq). The solution was stirred for 20 min prior to addition of TMSOTf (1.24 mL, 8.88 mmol, 4 eq), followed by 30 min of stirring. The reaction mixture was purified directly by pouring onto a basic alumina column that was eluted (CH_2Cl_2/ hexane/Et_3N, 25 : 75 : 1 v/v), followed by general work-up. Product, which coeluted with unreacted starting material under these conditions, was purified by flash column chromatography (EtOAc/hexane, 20 : 80 v/v) on basic alumina to afford **3** as a white solid (0.92g, 100%). mp 105-107 °C. ^{1}H NMR ($CDCl_3$): δ 0.22 (s, 9H); 0.86 (s, 3H); 1.39-2.4 (m, 11H); 2.85-2.91 (m, 2H); 4.52 (m, 1H); 5.03 (s, 2H, $PhCH_2OAr$); 6.73 (d, J = 2.7, 1H); 6.78 (dd, J = 8.4, 2.7, 1H); 7.19 (d, J = 8.7, 1H); 7.31-7.45 (m, 5H). HRMS calcd for $C_{28}H_{36}O_2Si$ (M^+) 432.2485, found 432.2492.

17-(Trimethylsilyl)oxy-3-[[(trifluoromethyl)sulfonyl]oxy]estra-1,3,5(10),16-tetraene (7). Procedure A (adapted from Cazeau[54]). To a flask containing **6** (0.514 g, 1.28 mmol) under N_2, Et_3N (221 μL, 1.59 mmol, 1.24 eq) was added, followed by TMSCl (201 μL, 1.59 mmol, 1.24 eq). The resulting white slurry was stirred while NaI (0.238 g, 1.59 mmol, 1.24 eq) in anhydrous acetonitrile (1.6 mL) was added dropwise. Cold hexane and ice water were added after the solution had been stirred at rt for ca. 66 h. After decantation, the aqueous layer was washed with hexane, and the combined organic extracts were washed thrice with cold saturated sodium bicarbonate, followed by general work-up. Purification by silica flash column chromatography (EtOAc/hexane, 1 : 9 v/v) yielded **7** as a white solid (0.304 g, 50%).

Procedure B. To a solution of **6** (2.53 g, 6.29 mmol) in 40 mL CH_2Cl_2 under N_2 was added Et_3N (1.76 mL, 12.58 mmol, 2 eq). After the solution had been stirred for 20 min and then cooled to 0 °C, TMSOTf (2.44 mL, 12.58 mmol, 2 eq) was added. The ice bath was removed to allow the reaction to warm to rt. The reaction was monitored by TLC (EtOAc/hexane, 23 : 77 v/v) and additional Et_3N (2.0 mL) and TMSOTf (1.5 mL) were added to maximize the yield of the enoxy silane over a reaction time of 3 h. The reaction mixture was purified directly by passage through a plug of basic alumina (CH_2Cl_2/hexane/Et_3N, 25 : 75 : 1 v/v). Solvent was removed under reduced pressure to afford **7** (2.70 g, 90%). mp 84-88 °C. ^{1}H NMR ($CDCl_3$): δ 0.21 (s, 9H); 0.88 (s, 3H); 0.97-2.10 (m, 11H); 2.35-2.40 (m, 2H); 4.55 (m, 1H); 6.72-6.82 (m, 3H). Anal. Calcd for $C_{22}H_{29}O_4F_3SSi$: C, 55.68; H, 6.16. Found: C, 56.12; H, 6.34.

General Procedure For Methyl Hypofluorite (CH_3OF) Reactions. Anhydrous acetonitrile (48 mL) and anhydrous MeOH (2 mL) were added to an N_2 swept flask and cooled to -40 °C (dry ice/acetonitrile bath). The nitrogen flow was stopped, and F_2 (20% in Ne) was bubbled through the solution for 35 min. An aliquot (0.5 mL) of CH_3OF•ACN

was removed and added to a flask containing 25 mL H_2O and KF. The concentration of CH_3OF was determined by titrating the solution with $Na_2S_2O_4$ (equivalence point color change: yellow to colorless). The desired substrate was dissolved in $CHCl_3$ (10 mL) and cooled to 0 °C. NaF (30 mg) was added to the solution of CH_3OF and swirled for 30 sec before the CH_3OF was quickly poured into the substrate flask. The reaction was stirred at 0 °C for 5 min and was then allowed to warm to rt over a 40 min period. The reaction was quenched by the addition of saturated $NaHCO_3$ (250 mL). Separation of the aqueous phase was followed by washing the aqueous extract thrice with $CHCl_3$; combined organic extracts were washed thrice with brine, followed by general work-up.

16α-Methoxy-3-(benzyloxy)estra-1,3,5(10)-triene-17-one (4). The general procedure was followed to generate 6.90 mmol CH_3OF (0.139 M) that was allowed to react with **3** (570 mg, 1.32 mmol). Purification by silica gel gravity column chromatography (hexane/CH_2Cl_2, 30 : 70 v/v) afforded **4** as a white solid (52 mg, 10%). ^1H NMR ($CDCl_3$): δ 0.88 (s, 3H, 18-CH_3); 1.26-2.10 (m, 11H); 2.85-2.95 (m, 2H); 3.52 (s, 3H, -OCH_3); 3.97 (d, J = 7.5, 1H); 5.03 (s, 2H); 6.70-6.81 (m, 2H); 7.19 (d, J = 8.7, 1H), 7.27-7.44 (m, 5H).

16α-Methoxy-3-[[(trifluoromethyl)sulfonyl]oxy]estra-1,3,5(10)-triene-17-one (8a). The general procedure was followed to generate 5.11 mmol CH_3OF (0.105 M) that was allowed to react with **7** (330 mg, 0.695 mmol). Purification by silica gel flash column chromatography (EtOAc/hexane, 15 : 85 v/v) followed by semi-preparative normal phase HPLC (5% isopropanol in CH_2Cl_2/hexane, 6 : 94 v/v) afforded **8a** as a white solid (36 mg, 12%). mp 73-77 °C. ^1H NMR ($CDCl_3$): δ 0.96 (s, 3H, 18-CH_3); 1.25-2.45 (m, 11H); 2.90-2.98 (m, 2H); 3.53 (s, 3H, -OCH_3); 3.98 (d, J = 7.2 Hz, 1H, 16-H); 6.98-

7.06 (m, 2H); 7.34 (d, J = 8.5, 1H). HRMS calculated for $C_{20}H_{23}O_5F_3S$ $(M+H)^+$ 433.1296, found 433.1300.

16β-Methoxy-3-[[(trifluoromethyl)sulfonyl]oxy]estra-1,3,5(10)-triene-17-one (8b). The general procedure was followed to generate 5.11 mmol CH_3OF (0.105 M) that was allowed to react with **7** (330 mg, 0.695 mmol). Purification by silica gel flash column chromatography (EtOAc/hexane, 15 : 85 v/v) followed by semi-preparative normal phase HPLC (5% isopropanol in CH_2Cl_2/hexane, 6 : 94 v/v) afforded **8b** as a white solid (12 mg, 4%). mp 90-94 °C. ^{1}H NMR ($CDCl_3$): δ 1.00 (s, 3H, 18-CH_3); 1.22-2.58 (m, 11H); 2.93-2.98 (m, 2H); 3.54 (s, 3H, -OCH_3); 3.67 (t, J = 8.2 Hz, 1H, 16-H); 7.00-7.05 (m, 2H); 7.34 (d, J = 8.2, 1H). HRMS calculated for $C_{20}H_{23}O_5F_3S$ (M^+) 432.1218, found 432.1208.

16α-Methoxy-estra-1,3,5(10)-triene-3,17β-diol (5a). An aliquot of **4** (17.3 mg, 0.045 mmol) was dissolved in 1 mL EtOAc, and a suspension of 4 mg $PdCl_2(CH_3CH)_2$ and 8 μL EtOH was added. The reaction mixture was stirred under H_2 for 25 min, during which time it progressed through a color change from yellow to clear and colorless. The reaction mixture was diluted with CH_2Cl_2, filtered, and concentrated under reduced pressure. The crude reaction mixture was dissolved in 1 mL EtOAc and passed through a silica gel plug (EtOAc/hexane, 50 : 50 v/v). The procedure was repeated with two additional aliquots of **4**. Analysis by ^{1}H NMR showed complete deprotection. ^{1}H NMR ($CDCl_3$): δ 0.94 (s, 3H, 18-CH_3); 1.95-2.40 (m, 11H); 2.85 (m, 2H); 3.52 (s, 3H, -OCH_3); 3.98 (d, J = 7.4, 1H); 5.05 (b, < 1H, OH); 6.57-6.65 (m, 2H); 7.13 (d, J = 8.1, 1H).

To a solution of an aliquot of deprotected reaction product (13 mg, 0.0433 mmol) in 2 mL anhydrous MeOH was added $PdCl_2$ (15 mg, 0.087 mmol). While stirring under

N_2, the reaction was cooled to 0 °C, $NaBH_4$ was added (9.8 mg, 0.260 mmol), and the reaction mixture was stirred for 4 h. The reaction was filtered into 5% HOAc (8 mL); EtOAc and 1 M $NaHCO_3$ were then added. Organic and aqueous layers were separated, and the aqueous fraction was washed with 3x15 mL EtOAc; combined organic fractions were washed with 3x30 mL H_2O, followed by general work-up. Reduction was carried out on two additional aliquots of deprotected reaction product. Crude reaction products were pooled prior to semi-preparative normal phase HPLC purification (5% isopropanol in CH_2Cl_2/hexane, 30 : 70 v/v) which yielded **5a** as a white solid (8.1 mg, 20% from **4**). mp 95-97 °C. ^{1}H NMR ($CDCl_3$): δ 0.81 (s, 3H, 18-CH_3); 1.30-2.35 (m, 12H); 2.80-2.85 (m, 2H); 3.39 (s, 3H, -OCH_3); 3.64 (d, J = 5.4, 1H, 17-H); 3.68-3.74 (m, 1H, 16-H); 4.50-4.80 (b, < 1H, OH); 6.58-6.68 (m, 2H); 7.17 (d, J = 8.1, 1H).

16-Methoxy-estra-1,3,5(10)-triene-3,17-diol (5b, 5c).

3-Trifloxy-16-methoxyestrone (0.0694 mmol, 30 mg **8a** or 0.0176 mmol, 7.6 mg **8b**) was dissolved in freshly distilled Et_2O (0.013 mmol/mL), stirred under N_2, and cooled to -78 °C (dry ice/isopropanol bath). A 1.0 M $LiAlH_4$/Et_2O solution (0.350 mmol, 350 µL to **8a** or 0.087 mmol, 87 µL to **8b**) was added dropwise over ca. 2 min. The pale yellow reaction was stirred at -78 °C for 25 min and then warmed to rt over 25 min, giving a cloudy white appearance. Addition of 6 N HCl (7.8 mmol, 1.3 mL for **8a** or 1.044 mmol, 0.174 mL for **8b**) quenched the reaction. The aqueous phase was extracted with 1x3 mL Et_2O and 2x3 mL CH_2Cl_2/hexane (50 : 50 v/v). Each organic extract was passed through a $MgSO_4$ plug (2 g) and a 0.22 µm filter. Solvent was removed under reduced pressure. Purification by semi-preparative normal phase HPLC (5% isopropanol in CH_2Cl_2/hexane, 40 : 60 v/v) yielded **5b** (0.022 mmol, 6.6 mg, 31%) or **5c** (0.0175 mmol, 5.3 mg, 83%) as a white solid. **5b:** mp 167-171 °C. ^{1}H NMR ($CDCl_3$): δ 0.71 (s, 3H, 18-CH_3); 1.20-2.40 (m, 12H); 2.78-2.85 (m, 2H); 3.40 (s, 3H, -OCH_3); 3.76 (d, J = 5.1, 1H, 17-

H); 3.99-4.05 (m, 1H, 16-H); 4.68-4.80 (b, < 1H, OH); 6.55-6.68 (m, 2H); 7.16 (d, J = 8.4, 1H). HRMS calculated for $C_{19}H_{26}O_3$ (M^+) 302.1882, found 302.1883. **5c:** mp 173-175 °C. ^{1}H NMR ($CDCl_3$): δ 0.79 (s, 3H, 18-CH_3); 0.95-2.40 (m, 12H); 2.80-2.85 (m, 2H); 3.37 (s, 3H, -OCH_3); 3.49 (d, J = 7.8, 1H, 17-H); 3.73-3.78 (m, 1H, 16-H); 6.55-6.65 (m, 2H); 7.16 (d, J = 8.7, 1H). HRMS calculated for $C_{19}H_{26}O_3$ (M^+) 302.1882, found 302.1881.

SECTION 3. OPTIMIZATION OF METHYL HYPOFLUORITE REACTIONS WITH STEROIDAL SUBSTRATES USING DOUBLE-BOND-CONTAINING CHOLESTERYL ESTERS AS MODEL COMPOUNDS

3.1. INTRODUCTION

Seeking to develop a biologically active carbon-11 radiolabeled estrogen, the target compound 16α-methoxyestradiol-17β was chosen as the substrate for $[^{11}C]CH_3OF$. Prior to the radiolabeling studies, it was important to synthesize the non-radioactive target molecule for evaluation of its estrogen receptor (ER) binding affinity (The synthesis of 16-methoxyestradiol stereoisomers and their receptor binding affinities were discussed in Section 2.). Initial synthetic studies involved reacting CH_3OF with 17-trimethylsilylenolether-3-trifloxyestrone and 17-methylenolether-3-trifloxyestrone. Due to the abundant reactivity of CH_3OF, numerous reaction products formed.

To evaluate the chemistry of CH_3OF with steroidal substrates, a simpler molecule that would react regioselectively with CH_3OF was sought. Cholesterol analogs were chosen as the model compounds for this reactivity study, because of cholesterol's rigid steroid skeleton and internal double bond. The hydroxyl at the 3-position was advantageous for it allowed the synthesis of cholesteryl ester derivatives. By incorporating a double-bond-containing ester linkage at the 3-position of cholesterol, a secondary reactive site for CH_3OF was included. Each cholesterol derivative, therefore, contained two double bonds allowing an investigation as to which one would be more reactive toward CH_3OF. Rozen and co-workers found that the more electron-rich a double bond, the more reactive it should be toward CH_3OF, as the proposed mechanism for CH_3OF addition to olefins proceeds through a carbocation intermediate.[32]

3.2. RESULTS AND DISCUSSION

An initial study reacting cholesteryl acetate (**2**), which contained only the internal double bond, with CH_3OF produced 5-fluoro-6-methoxy cholestanyl acetate in ca. 3% yield after column purification. This low yield was due to solubility problems, the formation of several side products, and losses during purification. Desiring to probe the reactivity of CH_3OF toward an external double bond on the ester linkage of cholesterol in the presence of an internal double bond, various cholesteryl esters containing a secondary double bond (**3**), tertiary double bond (**4**), terminal-tertiary double bond (**5**), and a styrylic double bond (**6**) were synthesized (Figure 3.1). Esterification of cholesterol with the corresponding carboxylic acid proceeded in good yield (ca. 70%) with facile purification by passage through a short plug of silica.

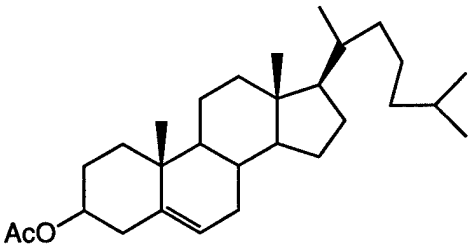
Each cholesteryl ester was reacted with CH_3OF under reaction conditions which progressed toward optimization. The extent and location of CH_3OF incorporation was assessed by ^1H NMR; products were not always isolated. The methoxy signal was evident in the region of 3.2-3.4 ppm, and the presence or absence of the vinylic proton(s) on the internal and external double bond provided the location of methoxy-addition. Integration of the ^1H NMR signal provided an estimate of the reaction yield when products were not able to be isolated in high purity.

Reacting cholesteryl-trans-3-hexenoate (**3**) with CH_3OF resulted in the addition of CH_3OF to the internal double bond (**8**) in low yield as identified by ^1H NMR; no addition of CH_3OF to the external double bond was observed. This was as expected for the internal double bond was more electron-rich than the external secondary double bond. The internal double bond of cholesterol had provided a more stable carbocation intermediate. A solubility problem was noticed during the reaction of CH_3OF and the steroid. Methyl

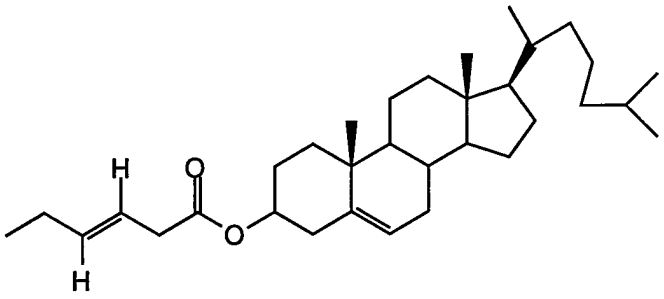
hypofluorite was generated in acetonitrile (ACN) at -40 °C, but the steroidal substrates were not soluble at these conditions.

A ^{1}H NMR of the partially purified reaction mixture of **8** showed starting material in ca. 50% yield; a single methoxy signal at 3.29 ppm represented a methoxy-containing product in 8-15% yield. Integration of the ^{1}H signals for the trans-hydrogens on the ester linkage (multiplet at 5.45-5.68 ppm) showed the external double bond to be ca. 100% intact in the crude material signifying that methoxy addition had occurred selectively on the internal double bond of the cholesterol skeleton.

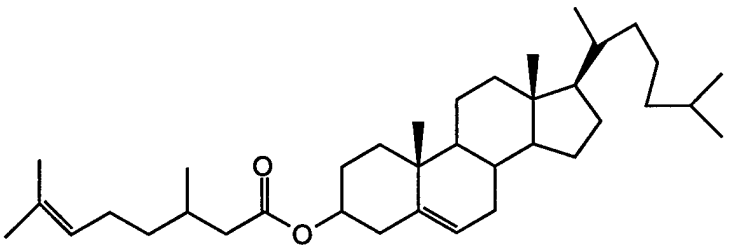
Figure 3.1. Cholesteryl esters synthesized with double-bond-containing external linkages.



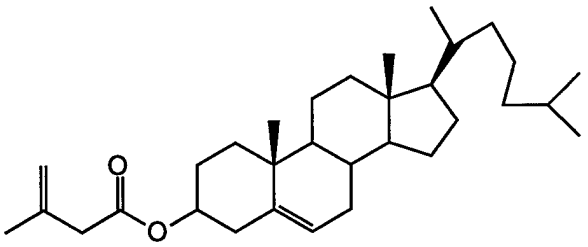
Cholesteryl acetate (**2**)
Only internal db



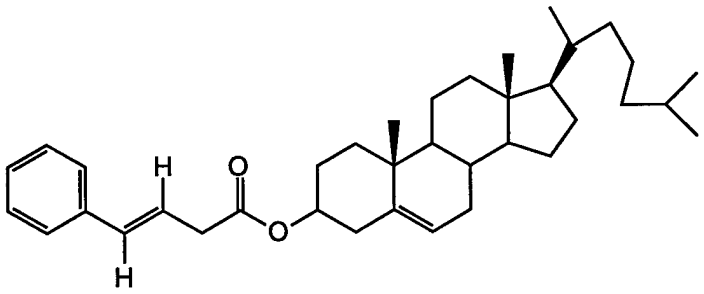
Cholesteryl-trans-3-hexenoate (**3**)
Secondary db on linkage



Cholesteryl citronellate (**4**)
Tertiary db on linkage



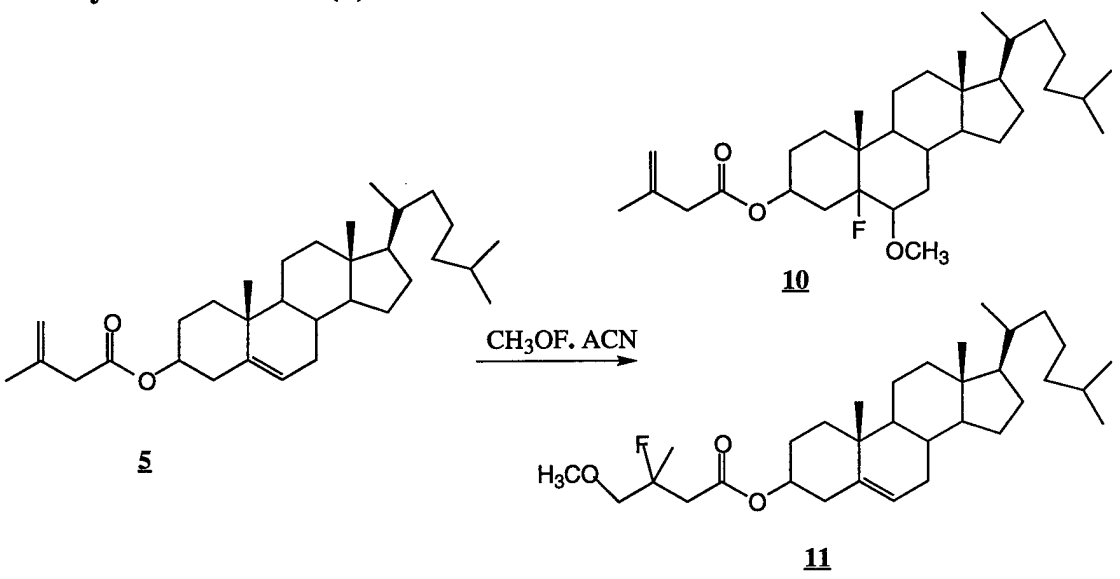
Cholesteryl-3-methyl-3-butenoate (**5**)
Terminal tertiary db on linkage



Cholesteryl-trans-styrylacetate (**6**)
Styrylic db on linkage

Cholesteryl citronellate (**4**) exposed CH_3OF to two tertiary double bonds, however, the internal double bond provided a less hindered environment for the reaction to proceed, as the rigid steroid skeleton provided an unhindered attack path for CH_3OF. 6-Methoxy-5-fluorocholesteryl citronellate (**9**) was purified from much of the reaction side products by silica gel flash column chromatography. Little or no external addition of CH_3OF was observed as assessed by ^1H NMR. Direct addition of the substrate, dissolved in CH_2Cl_2 (ca. 0.08 mmol/mL), to the CH_3OF•ACN resulted in the formation of a precipitate and, therefore, low reaction yield. The precipitate was presumed to be starting material, as the substrate was found to not be soluble in this solvent system at -40 °C and often > 50% of the starting material did not react.

Figure 3.2. Reaction products formed by addition of CH_3OF to cholesteryl 3-methyl-3-butenoate (5).

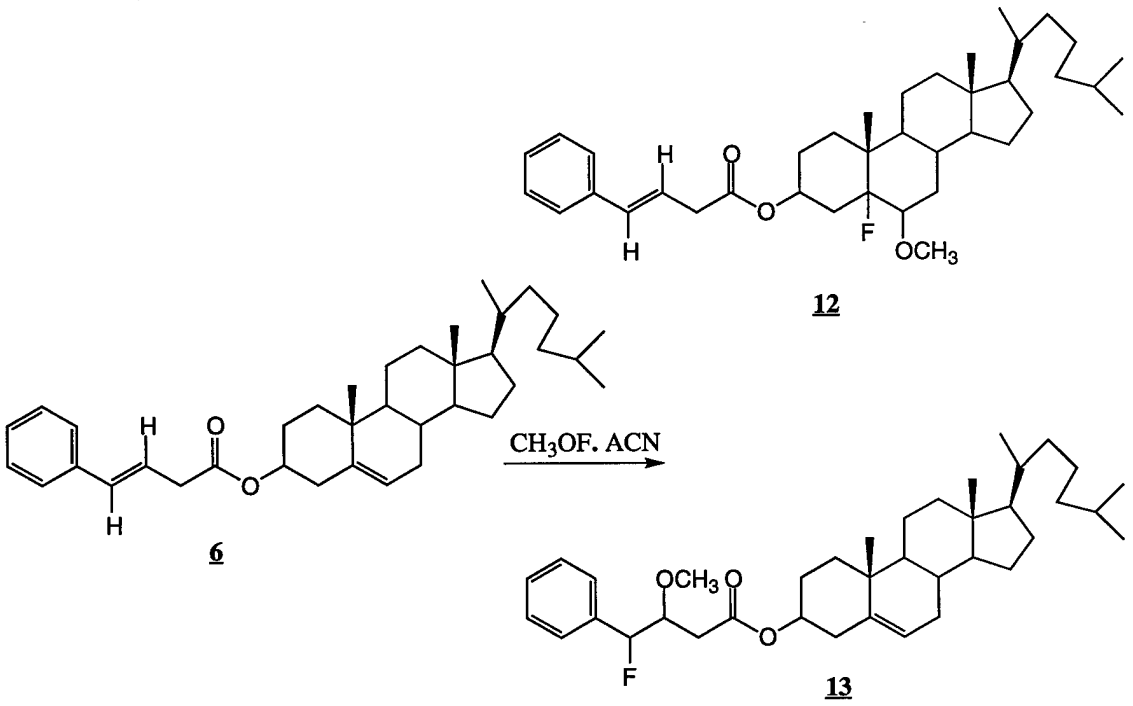


In order to increase the reactivity of the external double bond, a terminal-tertiary double bond was incorporated into the ester linkage. Cholesteryl 3-methyl-3-butenoate (**5**) was synthesized and reacted with CH_3OF. As identified by a ^1H NMR of the crude reaction products, the internal double bond had reacted preferentially over the external double bond by a ratio of 4 : 3, forming products **10** and **11**, respectively, as shown in

Figure 3.2. During the CH_3OF reaction, the substrate was prevented from precipitating by adding the CH_3OF•ACN directly to the flask containing the dissolved substrate in a larger volume of CH_2Cl_2 (0.025 mmol/mL). The yield of methoxy containing products increased when this addition method was instituted.

To increase the reactivity of the ester linkage further, cholesteryl-trans-styrylacetate (**5**) was synthesized as it contains the electron-rich external styrylic double bond. Reacting CH_3OF with **5** showed, by ^1H NMR, preferred addition of CH_3OF to the *external* double bond (**13**) over the *internal* double bond (**12**) by ca. 4 : 1 (Figure 3.3). The CH_3OF•ACN was added to the substrate, dissolved in CH_2Cl_2 (0.04 mmol/mL) and cooled to 0 °C. Of this series of cholesteryl esters, the styrylic double bond proved to be the only external double bond that was more reactive than the internal double bond toward CH_3OF. The UV activity of the styrylic compound was an additional advantage for it would allow the reaction to be monitored by UV detection during HPLC purification.

Figure 3.3. Reaction products formed by addition of CH_3OF to cholesteryl-trans-styrylacetate (6).



3.3. CONCLUSION

This reactivity study gave useful information on the necessary conditions for reacting CH_3OF with steroidal substrates. Initial studies resulted in poor yields due to solubility problems. Steroids are insoluble in acetonitrile, which was the solvent for CH_3OF generation. Modification of the substrate addition step successfully eliminated precipitation of the substrate and led to increased yield of the methoxy-containing products.

An additional problem affecting the yield of the desired product was the observance of several side products. These undesired products are presumed the result of radical reactions with tertiary hydrogens on the steroid skeleton. Further optimization of the reaction conditions involved changing the substrate solvent from CH_2Cl_2 to $CHCl_3$, which is more effective in radical scavenging.

In earlier studies, another undesired product was formed by the addition of HF across the double bond. To further increase the yield of the desired product in more recent studies (as discussed in Section 2), oven-dried NaF was added to the CH_3OF•ACN solution before the addition of the CH_3OF to the substrate. NaF acts as a fluoride ion acceptor, decreasing the acidity of HF through the formation of HF_2^-, thereby, reducing its reactivity towards the substrate.[51] With the addition of NaF, this product was eliminated.

Reactions performed under these optimized conditions had fewer side products and the yield of the methoxy-containing products increased. These optimized conditions were applied to the synthesis of the stereoisomers of 16-methoxyestradiol as discussed in Section 2. Carbon-11 was not incorporated into 16-methoxyestradiol with $[^{11}C]CH_3OF$ due to the compound's low estrogen receptor binding affinity.

3.4. EXPERIMENTAL

General

Mg was obtained from Fisher (40-80 mesh, St. Louis, MO). All commercial reagents were used as received from suppliers unless otherwise noted. Fluorine (20% in Ne) was purchased from Acetylene Gas (St. Louis, MO). ***Due to the strong oxidizing and corrosive nature of fluorine, appropriate laboratory safety and personnel protective equipment were utilized.***[53] THF was distilled from sodium and CH_2Cl_2 was distilled from calcium hydride. Column chromatography was performed using silica gel (60 Å, 230-400 mesh). Thin-layer chromatography (TLC) was performed on 250 μm silica plates (Whatman) visualized with potassium permanganate. ^1H and ^{13}C NMR were obtained on a Gemini-300 spectrometer (Varian Associates, Palo Alto, CA) at 300 and 75 MHz, respectively, and ^{19}F spectra were obtained on a Varian Unity-Plus instrument operating at 282 MHz. Chemical shifts for ^1H and ^{13}C are referenced to internal tetramethylsilane and ^{19}F chemical shifts are referenced to internal $CFCl_3$.

Synthesis

Methyl-3-butenoic acid (1). Mg (0.700 mg, 28.8 mmol, 40-80 mesh) in 25 mL anhydrous THF was cooled to -45 °C under N_2. A crystal of iodine was added, followed by the slow addition (over 1.5 h) of 3-bromo-2-methylpropene (14.8 mmol, 1.49 mL) in THF (17 mL). The reaction was stirred at -45 °C for 30 min and powdered dry ice (ca. 9 g CO_2) was added over 5 min. The reaction was allowed to warm to 0 °C before it was quenched with saturated NH_4Cl (75 mL) and diluted with ether. A mixture of 3.75 mL concentrated HCl over 15 g crushed ice was added to acidify the reaction. The carboxylic acid was extracted from the aqueous phase into ether; the ether phase was extracted with 10% NaOH to remove the carboxylic acid as the sodium salt. The basic extracts were acidified with concentrated HCl and the purified carboxylic acid was again extracted into

ether. Ether layers were dried over $MgSO_4$, filtered, and solvent removed under reduced pressure to give **1** in 23% yield (3.40 mmol). TLC R_f 0.24 (diethyl ether/petroleum ether, 30 : 70). ^1H NMR ($CDCl_3$) δ 1.84 (s, 3), 3.09 (s, 2), 4.93 (d, J = 19.1, 2). ^{13}C ($CDCl_3$) δ 22.40, 43.11, 115.38, 137.90, 177.82.

General Procedure for the Esterification of Cholesterol. Cholesterol (0.5 mmol - 2.5 mmol), the desired acid (1.1 eq), dicyclohexylcarbodiimide (DCC, 1.1 eq), and 4-pyrrolidinopyridine (0.1 eq) were stirred in CH_2Cl_2 (13 µl/mg cholesterol) at rt. When product formation had maximized (ca. 3 hours), as determined by silica TLC (ethyl acetate/ hexane, 5 : 95) the N,N-dicyclohexyl urea was filtered out of the reaction. The filtrate was washed with H_2O, 5% acetic acid, and again with H_2O, followed by drying over $MgSO_4$, filtering, and solvent removal under reduced pressure. The esterified product was purified from starting material by direct loading onto a plug of silica and rinsing with 1 : 1 CH_2Cl_2/hexane.

Cholesteryl-trans-3-hexenoate (3). The general procedure for esterification was followed with 0.517 mmol of cholesterol and 0.569 mmol trans-hexenoic acid to give **3** in 70% yield (0.362 mmol). TLC R_f 0.39 (ethyl acetate/hexane, 5 : 95). ^1H NMR ($CDCl_3$) δ 0.65 (s, 3), 0.83-2.32 (m, 45), 2.97 (d, J = 6.3, 2), 4.55-4.66 (m, 1), 5.35 (d, J = 3.9, 1, C=CH), 5.43-5.65 (m, 2, CH=CH).

Cholesteryl citronellate (4). The general procedure for esterification was followed with 0.517 mmol cholesterol and 0.569 mmol R(+)-citronellic acid to give **4** in 71% yield (0.368 mmol). TLC R_f 0.41 (ethyl acetate/hexane, 5 : 95). ^1H NMR ($CDCl_3$) δ 0.68 (s, 3), 0.86-2.33 (m, 56), 4.55-4.70 (m, 1), 5.09 (t, 1, $(CH_3)_2C$=CH) 5.38 (d, J = 4.2, 1, C=CH).

Cholesteryl 3-methyl-3-butenoate (5). The general procedure for esterification was followed with the exception of using 1.555 mmol cholesterol (1.01 eq), 1.545 mmol methyl-3-butenoic acid (1.0 mmol), and 1.555 mmol DCC (1.01 mmol) to give **5** in 52% yield (0.800 mmol). TLC R_f 0.35 (ethyl acetate/hexane, 5 : 95). ^{1}H NMR ($CDCl_3$) δ 0.68 (s, 3), 0.84-2.07 (m, 41), 2.33 (d, J = 7.8, 2), 3.01 (s, 2, CH_2-CO_2), 4.58-4.70 (m, 1), 4.88 (d, J = 17.4, 2, CH_2=C(CH_3)(CH_2)), 5.38 (d, J = 3.9, 1, CH=C).

Cholesteryl-trans-styrylacetate (6). The general procedure for esterification was followed with 2.58 mmol cholesterol (1.01 eq), 2.56 mmol trans-styrylacetic acid (1.0 eq) and 2.58 mmol DCC (1.01 eq) to give **6** in 71% yield (1.8 mmol). TLC R_f 0.48 (ethyl acetate/hexane, 10 : 90). ^{1}H NMR ($CDCl_3$) δ 0.67 (s, 3), 0.85-2.05 (m, 38), 2.34 (d, J = 7.50, 2), 3.22 (dd, J = 1.23, 7.02; 2, CH_2-CO_2), 4.60-4.73 (m, 1), 5.38 (d, J = 3.90, 1, CH=C), 6.25-6.35 (m, 1), 6.49 (d, J = 15.9, 1), 7.23-7.39 (m, 5, Ph-H).

General Procedure for Methyl Hypofluorite (CH_3OF) Reactions. Anhydrous acetonitrile (ACN, 48 mL) and anhydrous MeOH (2 mL) were added to an N_2 swept flask and cooled to -40 °C (dry ice/acetonitrile bath). The nitrogen flow was stopped, and F_2 (20% in Ne) was bubbled through the solution for 20-35 min. An aliquot (0.5 mL) of CH_3OF•ACN was removed and added to a flask containing 25 mL H_2O and KF. The concentration of CH_3OF was determined by titrating this solution with $Na_2S_2O_4$ (equivalence point color change: yellow to colorless). General work-up refers to the addition of CH_2Cl_2 and separation of the aqueous phase from the organic phase, followed by washing the aqueous extract thrice with CH_2Cl_2; combined organic extracts were washed thrice with brine, dried over $MgSO_4$, filtered, and solvent removed under reduced pressure.

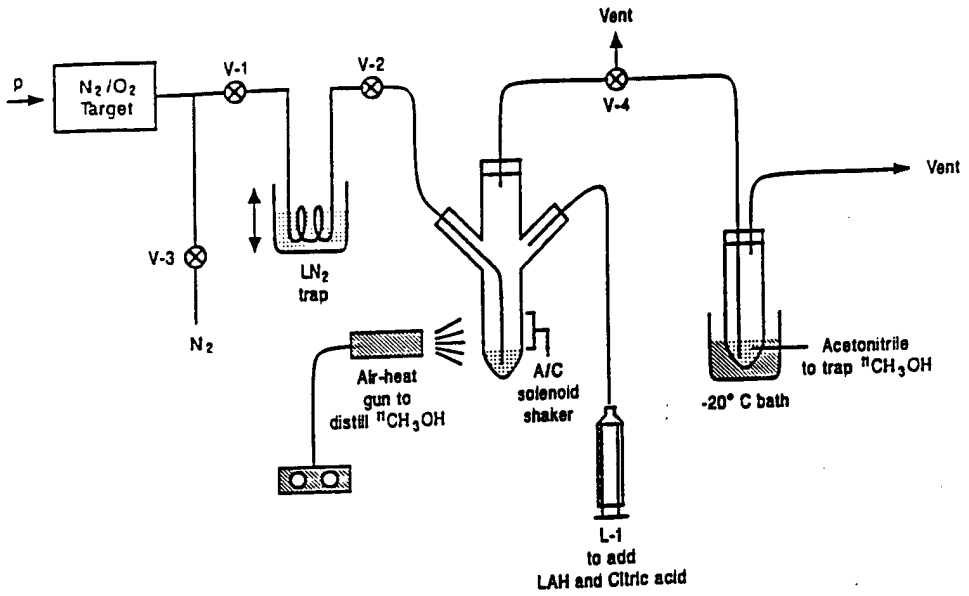
Production of $[^{11}C]CH_3OH$

Synthesis of $[^{11}C]CH_3OF$ requires the availability of anhydrous $[^{11}C]CH_3OH$. Although $[^{11}C]CH_3OH$ is routinely produced by alternative synthetic routes, the stipulation for anhydrous $[^{11}C]CH_3OH$ is met by reducing $[^{11}C]CO_2$ with $LiAlH_4$ in diglyme at -78 °C followed by an anhydrous quench of citric acid in diglyme.[33] The $[^{11}C]CH_3OH$ is trapped in acetonitrile at -20 °C following distillation. The remote system designed in our laboratory for the production of anhydrous $[^{11}C]CH_3OH$ is represented in Scheme 1.

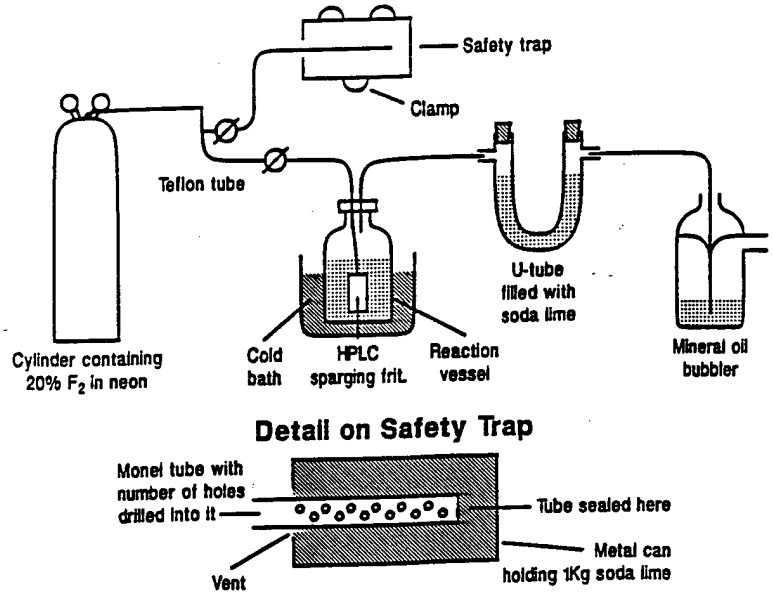
Safe Handling of Fluorine Gas

After CH_3OF (or $[^{11}C]CH_3OF$) is trapped in acetonitrile, it is transferred to a second apparatus designed to safely handle fluorine gas (Scheme 2). The cylinder of F_2 is in-line with a safety trap filled with enough soda lime to contain the release of the full contents of the cylinder if the need arose. During the production of CH_3OF, the safety trap is closed to allow the gas to bubble through the reaction vessel containing $[^{11}C]CH_3OH$ (or CH_3OH) in acetonitrile cooled to -40 °C. The reaction vessel is vented through a U-tube filled with soda lime followed by an oil bubbler, which allows the flow rate of F_2 to be monitored.

Scheme 1. Remote system for the synthesis of anhydrous $[^{11}C]CH_3OH$.



Scheme 2. General apparatus for reactions using F_2.



6-Methoxy-5-fluorocholesteryl acetate (7). The general procedure for generation of CH_3OF was followed with the exception of only adding 1 ml MeOH (F_2 bubbled for 20 min) to yield 3.11 mmol (0.125 M) CH_3OF. Cholesteryl acetate (**2**, 414 mg, 0.96 mmol) was dissolved in CH_2Cl_2 (5 mL) and added to the flask containing CH_3OF•ACN. A white precipitate was noticed upon addition of **2**. The reaction was kept at -40 °C for 15 min and then let warm to rt before it was quenched by the addition of saturated $NaHCO_3$ (200 mL). The white precipitate went into solution as the reaction warmed to rt. General work-up was followed by flash column chromatography (hexane/ethyl acetate, 95 : 5) to yield **7** (14 mg, 0.029 mmol, 3%). Identified by ^1H NMR: δ 3.35 (s, OCH_3), and the absence of the vinylic H at 5.38.

6-Methoxy-5-fluorocholesteryl-trans-3-hexenoate (8). The general procedure for generation of CH_3OF was followed with the exception of the use of 24 mL of anhydrous acetonitrile and 1 mL of anhydrous MeOH. Fluorine gas was bubbled for 15 min to yield 2.29 mmol (0.0996 M) CH_3OF. **3** (104 mg, 0.193 mmol) was dissolved in CH_2Cl_2 (4 mL) and added to the flask containing CH_3OF•ACN. A white precipitate formed upon addition of **3**. The reaction was kept at -40 °C for 15 min and then warmed to rt during which time the precipitate went into solution. The reaction was quenched by the addition of saturated $NaHCO_3$ (100 mL) followed by general work-up. After passing the crude reaction mixture through a short silica column to remove baseline material, as identified on silica TLC, ^1H NMR revealed CH_3OF addition to the internal double bond of cholesterol in ca. 8-15% yield with no observed addition to the external double bond. ^1H NMR ($CDCl_3$) δ 0.68 (s, 3), 0.85-2.05 (m, 44-47), 2.32 (d, J = 7.80, 1), 3.00 (d, J = 5.7, 2), 3.29 (s, < 3, OCH_3), 3.41 (d, < 1), 4.55-4.70 (m, 1), 5.0-5.1 (br, < 1), 5.38 (d, < 1), 5.54-5.68 (m, 2).

6-Methoxy-5-fluorocholesteryl citronellate (9). The general procedure for generation of CH_3OF was followed (F_2 bubbled for 21 min) to yield 4.44 mmol (0.0896 M) CH_3OF. **4** (450 mg, 0.835 mmol) was dissolved in CH_2Cl_2 (10 mL) and added to the flask containing CH_3OF•ACN. A white precipitate formed upon addition of **4**. The reaction was kept at -40 °C for 15 min and then warmed to rt during which time the precipitate went into solution. The reaction was quenched by the addition of saturated $NaHCO_3$ (100 mL) followed by general work-up. Flash column chromatography (methylene chloride/hexane, 40 : 60) only succeeded at partially purifying **9** out of the reaction mixture; these fractions were shown by ^1H NMR to contain **9** in a 33% enrichment. The overall yield of **9** was ca. 4% (19 mg, 0.0337 mmol). Evidence for the addition of CH_3OF to the external double bond on the ester linkage was minimal and suggested little (< 0.5%) to no formation of this product. The fractions were assessed by ^1H NMR for the intensity of the vinylic protons on the external and internal double bonds and then by ^{19}F NMR for the presence of fluorine in the compound.

6-Methoxy-5-fluorocholesteryl 3-methyl-3-butenoate (10) and Cholesteryl 3-fluoro-3-ethoxybutanoate (11). The general procedure for generation of CH_3OF was followed (F_2 bubbled for 45 min) to yield 3.17 mmol (0.0647 M) CH_3OF. **5** (297 mg, 0.634 mmol) was dissolved in CH_2Cl_2 (25 mL) and the CH_3OF•ACN was transferred to the flask containing the substrate. This addition method prevented the formation of a precipitate. After stirring for 15 min, an aliquot was titrated (as describe in the general CH_3OF procedure) to show that no CH_3OF was still present. At this time, the reaction was quenched by the addition of 100 mL saturated $NaHCO_3$ followed by the general work-up. A ^1H NMR of the crude reaction showed addition of CH_3OF to the internal and external double bond of **5** in an approximate 4 : 3 ratio, respectively. Purification by column chromatography (ethyl acetate/petroleum ether, 2 : 98) allowed isolation of fractions

enriched in **10** in 11% yield (36 mg, 0.694 mmol). ^{1}H NMR ($CDCl_3$) δ 0.67 (s, 3), 0.85-2.3 (m, 43), 3.01 (s, > 3, $\mathbf{CH_2}$-CO_2), 3.17 (m, 1), 3.29 (s, 3, CH_3O), 4.87 (d, J = 17.7, > 3, $\mathbf{CH_2}$=C(CH_3)(CH_2)), 5.0-5.15 (m, 1). The product formed by addition of CH_3OF to the external double bond was only isolated at a 55-83% enrichment along with starting material. The unique ^{1}H signals for **11** were: ^{1}H NMR ($CDCl_3$) δ 2.65-2.85 (m, 2), 3.42 (s, 3, CH_3O), 3.50 (s, 1), 3.58 (d, 1), 5.0-5.15 (m, 1).

6-Methoxy-5-fluorocholesteryl-trans-styrylacetate (12) and Cholesteryl-3-methoxy-4-fluoro-4-phenylbutanoate (13). The general procedure for generation of CH_3OF was followed (F_2 bubbled for 31 min) to yield 5.18 mmol (0.1046 M) CH_3OF. **6** (436 mg, 0.821 mmol) was dissolved in CH_2Cl_2 (20 mL) and cooled to 0 °C prior to the addition of the CH_3OF•ACN to the flask containing the substrate. This addition method prevented the formation of a precipitate. After stirring for 5 min the reaction was warmed to rt by removal of the cold bath. At this time, the reaction was quenched by the addition of 100 mL saturated $NaHCO_3$ followed by the general work-up. A ^{1}H NMR of the crude reaction mixture revealed CH_3OF addition to the external double bond as compared to addition to the internal double bond in a 4 : 1 ratio, respectively. Purification by column chromatography (ethyl acetate/petroleum ether, 2 : 98) gave fractions enriched in **12** and **13**. **Unique signals for 12:** ^{1}H NMR ($CDCl_3$) δ 3.28 (s, < 3, OCH_3), 3.42 (d, < 1), absence of vinylic H on double bond of cholesterol skeleton, trans-H on ester linkage still intact (6.25-6.40 (m, 1), 6.5 (d, 1). **Unique signals for 13:** ^{1}H NMR ($CDCl_3$) δ 3.34 (s,3, OCH_3), 3.48 (s, 1), 3.8-4.1 (br, 1), 4.55-4.7 (br, 2), 5.4 (br, 1, C=CH), absence of signal from trans-H on ester linkage of starting material, 7.25-7.40 (m, 5).

SECTION 4. OVERALL CONCLUSIONS

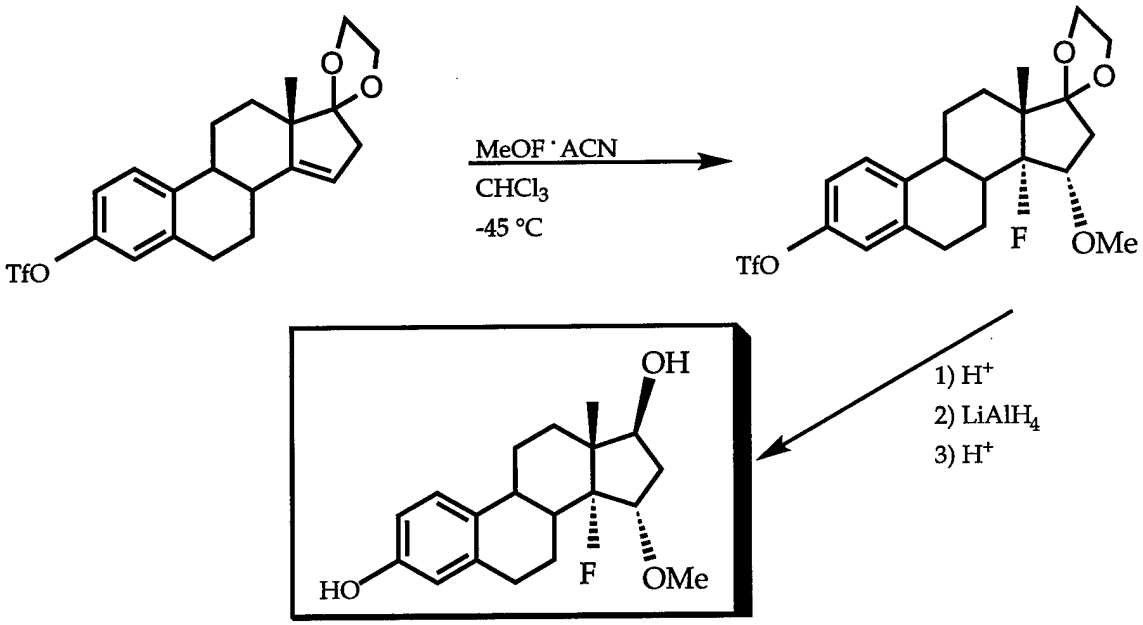
4.1. RESEARCH

The unusual chemistry of methyl hypofluorite provides a previously unexplored route for functionalizing the 16-position of estradiol. Three isomers of 16-methoxyestradiol were prepared via two synthetic routes, each utilizing methyl hypofluorite. The estrogen receptor binding affinity of these compounds was determined, to evaluate their potential as positron emission tomographic (PET) imaging agents targeting estrogen receptor-positive breast cancer. Radiolabeled methyl hypofluorite ($[^{11}C]CH_3OF$) would allow the rapid preparation of novel carbon-11 PET imaging agents. The 17-trimethylsilyl enol ethers of 3-benzyloxy and 3-trifloxyestrone were prepared as substrates to react with methyl hypofluorite. Conditions for the reaction of methyl hypofluorite with simple substrates (cholesterol esters) needed to be optimized to provide reasonable reaction yields with the steroidal substrates. Following introduction of the methoxy substituent at the 16-position, reduction and deprotection conditions were manipulated to yield the various methoxyestradiol isomers. Two-dimensional NMR techniques (HMQC and HMQC-TOCSY) were instrumental in the characterization of the methoxyestradiol isomers. NOESY experiments confirmed the stereochemistry of the 16- and 17-positions. 16α-Methoxyestradiol-17β and 16β-methoxyestradiol-17β, each with the preferred β orientation for the 17-alcohol, were determined to have relative binding affinities of 1.5% and 2.3%, respectively. The stereoisomer with the unfavored α orientation at the 17-position, 16α-methoxyestradiol-17α, exhibited only a 0.5% relative binding affinity for the estrogen receptor. The biological evaluation of these compounds was not pursued further because of their low binding affinities.

We still desire to synthesize carbon-11 radiolabeled ER ligands to probe their imaging potential. The low receptor binding affinity of the 16-methoxyestradiol stereoisomers discounted their use as target compounds (Section 2). To further evaluate

the use of [^{11}C]CH_3OF in the synthesis of radiolabeled steroids, a new target molecule is proposed: 14-fluoro-15-methoxyestradiol. As shown in Scheme 4.1, CH_3OF would react with $\Delta^{14,15}$-protected estrone. It is unknown how the binding affinity to ER would be affected by a methoxy substituent at the 15-position.

Scheme 4.1. Proposed synthesis of 14-fluoro-15-methoxyestradiol-17β using methyl hypofluorite.



4.2. STATEMENT OF WORK

The statement of work that appeared in the original proposal was as follows:

1) Non radioactive compounds will be synthesized using methyl hypofluorite.

2) Non radioactive compounds will be evaluated for ability to bind to receptors.

3) Compounds with high binding affinities will be labeled with carbon-11.

4) Compounds prepared in (3) will be evaluated in animal models.

As described throughout, three stereoisomers of 16-methoxyestradiol were synthesized with the use of methyl hypofluorite and characterized (**1**). The synthesis of these compounds required optimizing the reactivity of methyl hypofluorite with steroids as accomplished using various cholesteryl esters as model compounds (**1**). The isomers of 16-methoxyestradiol were evaluated for their ability to bind the estrogen receptor (**2**). Their resulting low affinity for the estrogen receptor negated the radiolabeling studies (**3**) and animal evaluation (**4**).

My predoctoral fellowship focused on furthering the understanding and aiding the diagnosis of breast cancer. This grant provided me with the resources for training in the field of breast cancer research for which I am sincerely thankful to the Army. My doctorate degree was successfully completed on May 15, however, my participation in breast cancer research will continue.

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List of Abbreviations

ACN	acetonitrile
BnBr	α-bromotoluene
CaH_2	calcium hydride
$CDCl_3$	deuterated chloroform: NMR solvent
$CFCl_3$	fluorotrichloromethane
CH_2Cl_2	methylene chloride
CH_3OF	methyl hypofluorite
CH_3OF•ACN	methyl hypofluorite/acetonitrile complex
$CHCl_3$	chloroform
COSY	correlation spectroscopy
ER	estrogen receptor
ER+	estrogen receptor positive
ES	estradiol: the natural ER ligand
Et_2O	diethyl ether
Et_3N	triethyl amine
EtOAc	ethyl acetate
EtOH	ethanol
F_2	fluorine (gas)
[^{18}F]FDG	2-[^{18}F]fluoro-2-deoxy-D-glucose
[^{18}F]FES	[^{18}F]-16α-fluoroestradiol-17β
H_2	hydrogen (gas)
HCl	hydrochloric acid
HF	hydrofluoric acid
HMQC	heteronuclear multiple quantum coherence
HOAc	acetic acid

HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrum
K_2CO_3	potassium carbonate
KF	potassium fluoride
$LiAlH_4$	lithium aluminum fluoride
MeOF	methyl hypofluorite
MeOH	methanol
$MgSO_4$	magnesium sulfate
N_2	nitrogen (gas)
$Na_2S_2O_4$	sodium thiosulfate
$NaBH_4$	sodium borohydride
NaF	sodium fluoride
$NaHCO_3$	sodium bicarbonate
NaI	sodium iodide
NMR	nuclear magnetic resonance
NOESY	nuclear Overhauser effect spectroscopy
$PdCl_2$	palladium chloride
$PdCl_2(CH_3CH)_2$	palladium chloride diacetonitrile
PET	Positron Emission Tomography
RBA	relative binding affinity
rt	room temperature
TEA	triethyl amine
Tf	trifluormethanesulfonyl (triflyl)
TLC	thin-layer chromatography
TMSCl	chlorotrimethyl silane
TMSOTf	trimethyl silyl triflate

TOCSY	total correlation spectroscopy
UV	ultraviolet

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1. **Jonson, S.D.**, Bonasera, T.A., McCarthy, T.J., Welch, M.J., "Chemistry and Radiochemistry of No-Carrier Added $^{11}CH_3OF$." Presented orally at the 208th National American Chemical Society National Meeting, Washington, D.C., August **1994**. Abstract Number NUCL-42.

2. Flanagan, F.L., Dedashti, F., Mortimer, J.E., Siegel, B.A., **Jonson, S.D.**, Welch, M.J., "PET Assessment of Response to Tamoxifen Therapy in Patients with Metastatic Breast Cancer," J. Nucl. Med., **1996**, 37, 99P (abstract). Presented orally at the Society of Nuclear Medicine 43rd Annual Meeting, Denver, Colorado.

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4. **Jonson, S.D.**, Welch, M.J., "New Approaches to Radiolabeling Estrogens for Imaging Estrogen Receptor Positive Breast Cancer by Positron Emission Tomography (PET)." Platform presentation at the Department of Defense Breast Cancer Research Program Meeting: Era of Hope, Washington, DC, November **1997**. Proceedings Volume I, pp. 225-226.

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PERSONNEL

Stephanie D. Jonson 100% effort