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NEW METHODS FOR RAPID RADIOHALOGEN LABELLING OF ORGANIC MOLECULES

by

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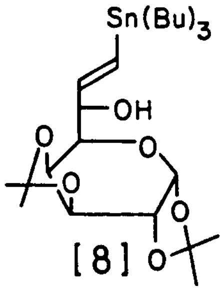
ABSTRACT

The aim of this study was to develop new and rapid procedures for the incorporation of short-lived radiobromine and radioiodine isotopes into organic compounds. This was accomplished by the electrophilic cleavage of tin-carbon bonds with BrCl and ICl generated in-situ from bromide and iodide, respectively, in the presence of an oxidizing agent. The vinyl-tin derivatives, [4], [8], [12], [17], [18] and [19], were prepared by treatment of the corresponding acetylenic compounds with tri-n-butyltin hydride, in the presence of a radical initiator catalyst, in 51-98% yield. Each of the vinyl-tin derivatives, except [17], was brominated in high yield (89-100%) with sodium bromide oxidized by Chloramine-T under acidic conditions. Compound [17] was brominated in 13% yield; competing bromination of the A-ring of [17] was responsible for this low yield. The vinyl-tin precursors [4], [8], [17], [18] and [19] were iodinated with sodium iodide in the presence of acidified Chloramine-T in 88-99% yield, except [17], which was iodinated in 1.9% yield because of facile A-ring iodination.

Compound [18] was subsequently radio-labelled with ^{82}Br, ^{123}I and ^{131}I forming [41], [42] and [43], respectively, using the Chloramine-T method. Compound [41] was isolated in 77-81% radiochemical yield using low specific activity $NH_4{}^{82}Br$. Compounds [42] and [43] were obtained in 82-90% radiochemical yield, in relatively high specific activity. The major side-product formed during the no-carrier-added radioiodinations was the chlorinated derivative [38]. An alternate procedure, based on N-chlorosuccinimide as the oxidant, was developed which gave [41] and [42] in 85% and 62% (no-carrier-added ^{123}I) radiochemical yields, respectively.



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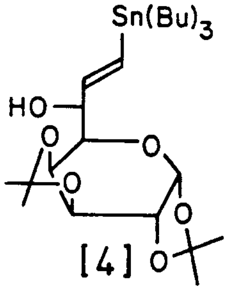

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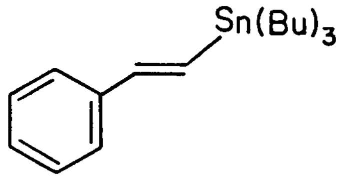

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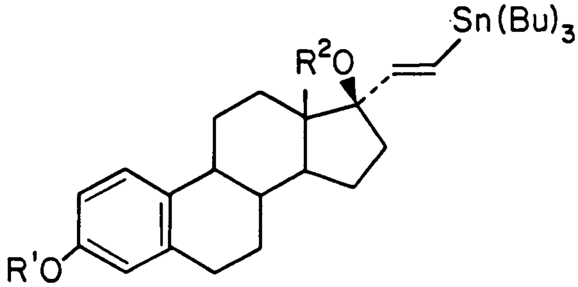
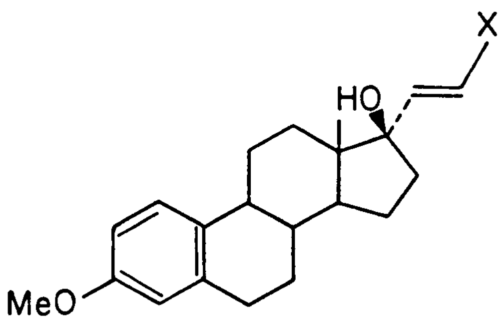


[17] $R^1 = R^2 = H$
[18] $R^1 = Me, R^2 = H$
[19] $R^1 = R^2 = Me$



[41] $X = {}^{82}Br$
[42] $X = {}^{123}I$
[43] $X = {}^{131}I$
[38] $X = Cl$

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CHAPTER I

INTRODUCTION

I.1 Background

During the last fifteen years, medical science has experienced a revolution in the area of medical imaging. Although most of the imaging methods currently in use have existed for a much longer period, it was the widespread use of computers and digital electronics in the 1970's, combined with other technical improvements, that have fuelled this significant growth. The many imaging methods, which now exist, fall into two broad categories, "transmission imaging" and "emission imaging". Transmission imaging can be performed using beams of X-rays, gamma-rays, neutrons or charged particles; in all cases the beam is passed through an object and then observed by a detection system located on the side opposite to the transmitter. In contrast, in emission imaging a radionuclide is administered to the patient and the distribution of the radionuclide is then observed by an external detection system which monitors the emitted gamma-rays. Neither Ultrasound imaging nor the newest imaging technique, Nuclear Magnetic Resonance (NMR) imaging fit easily into the above imaging categories.[1-4]

The general purpose of medical imaging is to provide useful information leading to either an understanding of human biological processes, forming or confirming a diagnosis, or for guiding and monitoring therapy. The information required for these purposes is twofold; first, confirmation of the presence, or absence, of pathology; and second, characterization of the pathology observed. Depending on the imaging method chosen, an image of a brain tumor, for example, may be observed as a change in morphology; this would be seen in a conventional X-ray image. Alternatively, that same brain tumor may be observed by a change in physiological function, such as the glucose metabolism, or blood flow in the brain;[2] either of these physiological functions could be observed with emission imaging using appropriate radiopharmaceuticals.

Emission imaging, which has been developed as part of nuclear medicine, has produced two different tomographic imaging methods which differ in the nature of the radionuclide label used. The first, Single Photon Emission Computed Tomography (SPECT), is based on the use of gamma-emitting radionuclides.[3] The other, which is a more recent development, Positron Emission Tomography (PET), is based on the use of positron-emitting radionuclides.[5] Both methods produce images by similar counting methods. The key difference is the physical properties of the radiation emitted and hence its detection.

To illustrate how these techniques work, the processes involved in a PET measurement will be briefly described. A radiopharmaceutical, labelled with a positron emitting isotope, is administered to the patient. Inside the body, the radioisotope decays by emitting a positron (positive electron). As the positron travels a few millimeters it loses sufficient energy that it can combine with an electron, its antimatter twin, and undergo an annihilation event which produces two gamma-ray photons of 511 KeV each. These travel off, in opposite directions approximately 180° apart (see figure I-1) penetrating

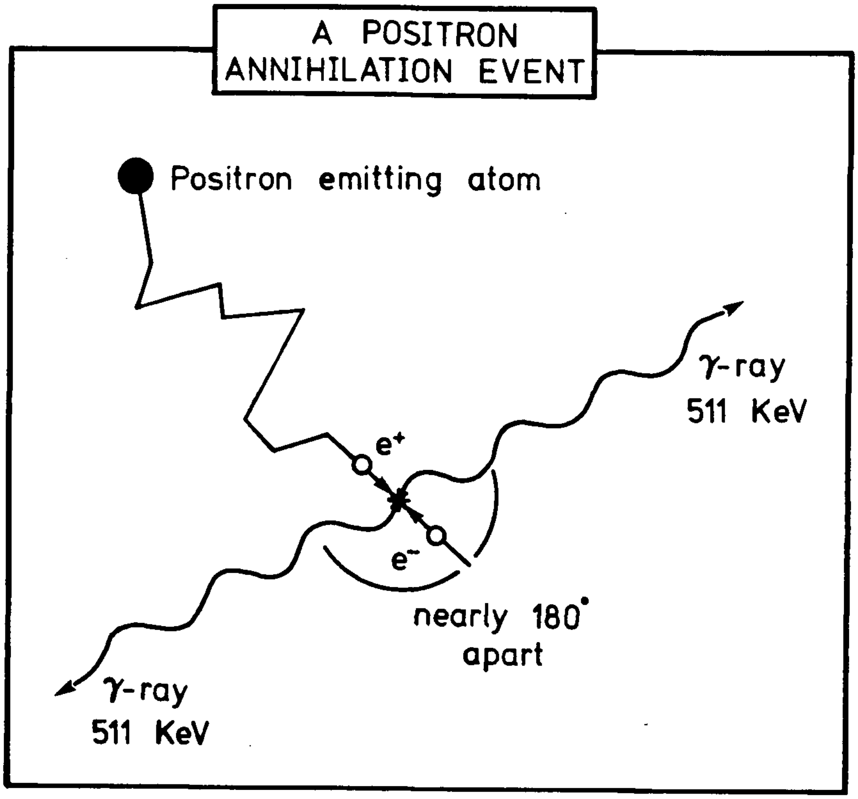


Figure I-1. Decay of positron emitting isotope results in an annihilation event.

the surrounding tissue, and are detected by a circular array of coincidence detectors (see figure I-2a) encircling the body; annihilation events registered by detectors located 180° apart are the only ones recorded. As the radiation counts are accumulated from the selected region of the subject, the radiation intensity at different angles, or projections, about this region are initially recorded as profiles (see figure I-2b). These profiles are then subjected to a mathematical process known as convolution (see figure I-2c) to remove artifacts from the final image. The so-called "filtered profiles" are then back-projected (see figure I-2d) using a second mathematical algorithm applied by computer, to reconstruct an image corresponding to the original

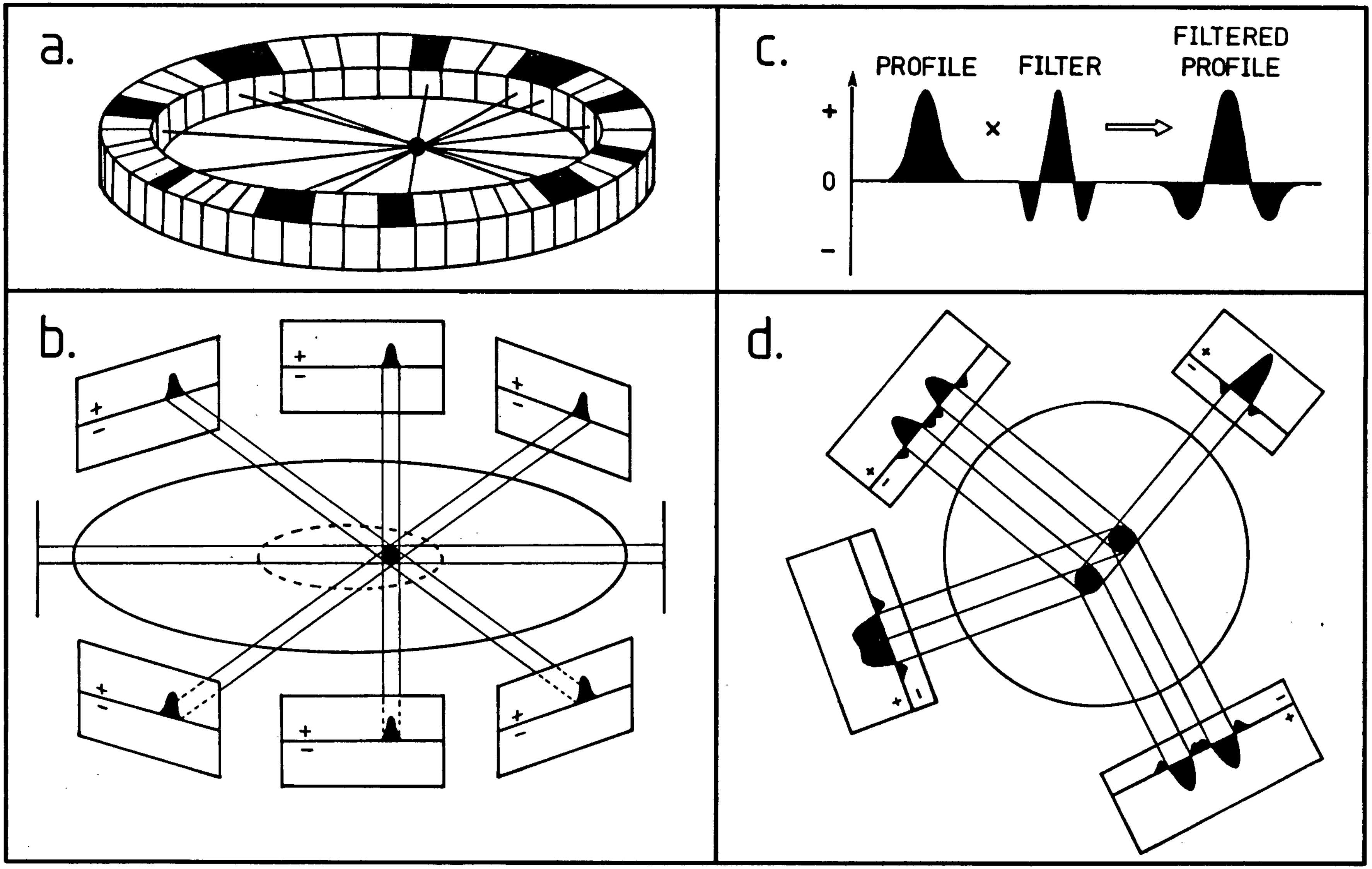


Figure I-2. PET process has four basic steps: (a) detection, (b) data recorded as profiles, (c) profiles are filtered (convolution) and (d) back-projection to form image.

spatial distribution of the radionuclide label; this is finally displayed on a cathode-ray screen as the image.[5,6]

In contrast to PET, in SPECT the random gamma radiation directly emitted from the radioisotope, is recorded by an area imager, usually a gamma scintillation camera. A collimator is used to determine the line of direction which the radiation has travelled; this is in contrast to the line direction obtained from the nearly antiparallel tracks of the photons used in PET. The energy of the emitted photon is determined by a scintillator and photo-multiplier array which can then discriminate against scattered radiation. Thereafter, the image reconstruction is exactly analogous to that used for PET; as the gamma camera systematically scans the subject, it forms a series of profiles at different projections which are mathematically manipulated as described above, to form the image.[2,7]

For both of these methods, the key aspect is the radio-labelled compound itself. It is the function of the radiopharmaceutical upon introduction into the body to become localized in a specific region or organ of interest. Hence, the observation and measurement of the radiation emitted from these areas, over time, provides the means for determination of physiological function. In the simplest situation, a diagnostic test for pathology can be made by comparing the uptake of the radiopharmaceutical with that for the normal organ of interest.[8] One of the cornerstones of modern medicine is the understanding that all clinical symptoms result from biochemical reactions and as a consequence every pathology has an underlying biochemical defect. Therefore, the identification of abnormal biochemical activity associated with a specific pathology is a continuing goal of medicine today, along with the early and direct observation of the abnormality in the afflicted organ.[5] Thus, in this context, the radiopharmaceutical acts as a biochemical probe which enables a physician to directly study various parameters of physiological function such as blood volume, blood flow, oxygen and glucose metabolic rates, drug-receptor interactions, protein synthesis, amino acid transport, tissue pH and the like.[9]

Clearly, the ability to measure the various parameters is dependent on having the appropriate radiopharmaceutical available; certainly this is the key to emission imaging. As a consequence, the ability to produce the wide variety of radionuclide labels and radiopharmaceuticals is probably the most challenging aspect of emission imaging.

I.2 The Problem

The goals of radiopharmaceutical synthesis have many elements in common with traditional synthetic organic chemistry. Both the radiochemist and organic chemist are concerned with developing syntheses which will yield in the most direct manner, the desired compound, in the largest chemical yield possible. In addition, the radiochemist is concerned with obtaining high radiochemical yields. Radiochemical yield is defined as the amount of radioactivity incorporated into the product as a percentage of the initial quantity of radioactivity used. Both the radiochemist and organic chemist require the final compound to be isolated chemically pure. However, radio-labelled compounds must be, in addition, radiochemically pure and free of other radionuclidic impurities.[10] Furthermore, organic compounds produced as pharmaceuticals and formulated for intravenous injection, whether radio-labelled or not, must also be sterile and pyrogen free.[10]

In order to ensure a successful radiopharmaceutical synthesis, several considerations hold which are not common to synthetic organic chemistry. The key aspects that need to be addressed by the radiochemist are as follows: (i) choice of radionuclide, (ii) physical properties of the radionuclide, (iii) source and chemical form of the radionuclide, and (iv) specific activity, stoichiometry, and the "no carrier" problem. These topics will now be discussed in the above order.

The choice of radioisotope that is to be used, whether, for example, it is ^{11}C, ^{18}F or ^{123}I, will determine the possible synthetic routes for incorporating the radioisotope, the likely position of attachment, and the nature and strength of the chemical bond formed. With the radioisotope chosen, the physical properties of the radionuclide will then have a profound influence in the synthesis of the desired radiopharmaceutical. This is chiefly due to the half-life of the radioisotope used. Table I-1 is a partial list of radionuclides used in medicine; the half-lives of these radioisotopes range from a few minutes to a few days. The synthesis time, beginning with the incorporation of the radionuclide, any subsequent chemical modifications (i.e. removal of protecting groups, etc.) through to the final purification of the radiopharmaceutical must be short with respect to the half-life of the radioisotope. Clearly this becomes critical in the use of the very short-lived radionuclides whose half-lives are in the order of minutes. The synthesis time, as an approximation, must be no more than one or two half-lives of the radioisotope used. The actual imaging of the patient must in turn be completed within three or four half-lives of the radioisotope used,[11,12] following commencement of the entire process.

Another physical property of the radionuclides which is of importance is the potential radiation exposure to the radiochemist. The radioisotopes used for in-vivo medical work produce high energy, body-penetrating radiation, that is necessary to enable external detection for medical imaging. Hence,

TABLE I-1. Half-lives of Selected Radionuclides used in Medicine*

Nuclide	Half-life[13]	Decay mode[13]	Application
^{15}O	2.03 min	β^+(99.9%); EC (0.1%)	PET
^{122}I	3.6 min	β^+(77%); EC (23%)	PET
^{38}K	7.6 min	β^+(100%)	PET
^{68}Ga	68.3 min	β^+(90%), EC (10%)	PET
^{75}Br	1.6 h	β^+(75.5%); EC (24.5%)	PET
^{99m}Tc	6.0 h[14]	IT[14]	SPECT
^{123}I	13.02 h	EC (100%)	SPECT
^{111}In	67.2 h	EC (100%)	SPECT
^{201}Tl	73.5 h	EC (100%)	SPECT

*No attempt is made to assign the relative importance of these radionuclides to nuclear medicine.
Decay modes: β^+ = positron emission, EC = electron capture,
IT = internal transition

it is prudent for safety reasons, for one to work with appropriate levels of shielding and use remote operations whenever possible. This need for safety exerts a direct influence on how laboratory manipulations are done, since the danger of radiation exposure from the radioisotopes must always be remembered and guarded against.[15]

The source and chemical form in which a given radioisotope is available has the greatest impact on the development of a practical synthetic strategy. The first problem is the availability of the desired radionuclide. Very few radionuclides for nuclear medicine are available from nuclear reactors; although ^{131}I is a reactor-source radionuclide,[16] most radioisotopes are produced on site or within relatively short travelling distance (time) of the generator, or cyclotron. A number of companies produce compact, "easy to use" cyclotrons for medical research. On-site production is vitally important when any of the very short-lived PET radionuclides are used.[17] A serious concern, therefore, of the radiochemist is to be able to produce, or obtain, the desired radioisotopes in a reliable fashion.

The chemical form of a given radionuclide is the second problem that has to be considered. Radioisotopes as obtained from a cyclotron/generator are usually available in a limited range of chemical forms. Hence, if the chemical form of a radioisotope is not appropriate for the proposed synthesis, it must be converted to the desired reagent form. A good example of this approach is in labelling with ^{11}C. The two readily available chemical forms of ^{11}C, from the cyclotron, are ^{11}C-labelled carbon dioxide and carbon monoxide. These can be converted in turn to other useful reagents.[18] The

$^{11}CO_2$ ^{11}CO	→	$H^{11}CN$, $H^{11}CHO$ $^{11}CH_3OH$, $^{11}CH_3I$
OBTAINED FROM CYCLOTRON		**PREPARED BY ADDITIONAL STEPS**

radioisotope may be also modified to the desired chemical form in-situ during the course of the synthesis. An example of this strategy is in the use of electrophilic iodination to incorporate radioiodine into aromatic structures. All isotopes of radioiodine are initially produced in the iodide form, but the iodide can be converted in-situ to "electrophilic" iodine. This is readily accomplished by a number of oxidizing agents which produce molecular iodine, or some form of hypoiodite.[19]

Nevertheless, the best approach, when possible, is to prepare the radionuclide in the chemical form desired during the radionuclide production. Although the preparation of radioisotopes is dependent on the nuclear reaction used, target design and cyclotron characteristics, the chemical form of the radioisotope will be determined by a number of factors, the most crucial being the chemical composition of the target and the energy deposition in the target.[20]

The last topic of this introduction is the issue of specific activity, stoichiometry and the "no carrier" problem; these three subjects are closely related. Specific activity is defined as the quantity of radioactivity present, generally expressed in curies, per mole of compound. One curie of activity produces 3.70×10^{10} bequerels (disintegrations per second);[21] the maximum specific activity of a radionuclide depends on its half-life (see Table I-2), and is only attainable when no other isotopes of the same element (i.e. carrier) is present; this ideal state is referred to as the "carrier-free" (CF) state. This state can be approached only to within an order of magnitude for some radioisotopes so that some carrier is unavoidably present in most cases. For example, in the production of $H^{11}CN$, the ratio of ^{11}C to ^{12}C is approximately 1:3000. In light of this problem, additional terminology is needed for specifying the extent of dilution present in a

TABLE I-2. Physical Properties of Some Radioisotopes[23]

Nuclide	Half-life	Decay Mode	Maximum Energy (MeV)	Range* (mm)	Maximum Specific Activity (Ci/mol)
^{3}H	12.35 y	β^-(100%)	0.0186	0.0072	2.90×10^{4}
^{14}C	5730 y	β^-(100%)	0.155	0.359	62.4
^{11}C	20.4 min	β^+(99+%)	0.96	4.108	9.22×10^{9}
^{13}N	9.96 min	β^+(100%)	1.19	5.39	1.89×10^{10}
^{18}F	109.7 min	β^+(97%) EC (3%)	0.635	2.39	1.71×10^{9}

* Maximum range in water.

Decay modes: β^- = beta particle emission, β^+ = positron emission
EC = electron capture

radiopharmaceutical product. The "no carrier added" (NCA) state, as applied to an element or compound, means that no carrier of the same element or compound has been added during its preparation. The "carrier added" (CA) state means a known amount of carrier has been added to the element or compound during its preparation.[22]

When dealing with short-lived radiopharmaceuticals near their maximum specific activity, the mass of the product is not detectable by ordinary chemical or spectroscopic means.[22] To illustrate this point, referring to the data presented in Table I-2, the following results can be derived.

$$1\ \mathrm{mCi\ of\ }^{14}C = 9.59 \times 10^{18}\ \mathrm{atoms} = 0.22\ \mathrm{mg}$$

$$1\ \mathrm{mCi\ of\ }^{11}C = 6.53 \times 10^{10}\ \mathrm{atoms} = 1.5\ \mathrm{pg}$$

As a consequence, the production of high specific activity radiopharmaceuticals in the CF or NCA states is highly desirable because the mass of the radiopharmaceuticals are then so small that it is usually below the threshold where any physiological response is invoked,[23] yet there is adequate radioactivity (in the order of 0.1 to 0.5 μCi per gram of tissue in the case of PET instruments) present to be detected with statistical significance. Even highly toxic molecules can be used for studies if adequate specific activities can be achieved.[22]

However, working with small amounts of high specific activity radionuclides leads to two closely related problems, the issue of stoichiometry and the "no carrier" problem. Since NCA radiosyntheses are performed on a very small scale, the amount of impurities present in the reagents and solvents

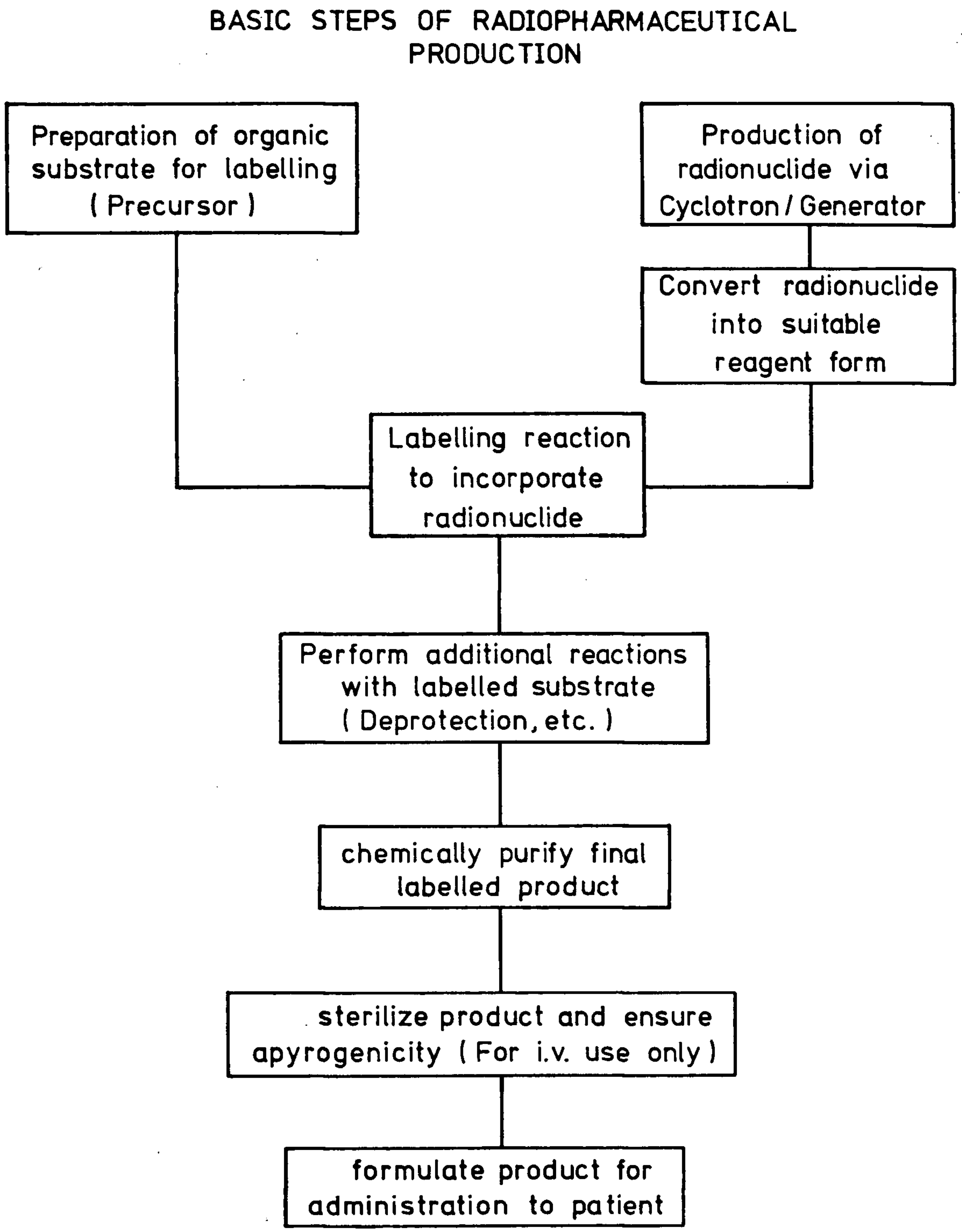


Figure I-3. General scheme for the production of a radiopharmaceutical (this process must be complete within one to two half-lives of the radioisotope used).

used may be comparable to the quantity of the radionuclide used in the synthesis. These impurities may compete with the radionuclide in a given reaction leading to unwanted side-products, or even stopping the formation of the desired compound altogether.[24] One of the impurities present in the synthesis can be carrier, which will lower the specific activity of the product; this is known as the "no carrier" problem.[19,25] To illustrate this problem, a careful study was reported[26] which concerned carrier bromide addition during high specific activity radiobrominations; significant amounts of carrier bromide were found in solvents, reagents and substrates. It was concluded that if high specific activity radiobrominations are desired, all components of the radiobromination reactions had to be analyzed for carrier bromide content (neutron activation analysis was used) before use. The "no carrier" problem also applies to the production of the radionuclide itself. Not all chemical forms of a given radioisotope are available in high specific activities.[17]

Once a successful radiopharmaceutical synthesis has been developed, it still must be implemented into a routine production system.[17] Figure I-3 summarizes the general steps involved in a typical radiosynthesis. As can be seen from this discussion, working with short-lived radio-labelled compounds is a complex research area.

The reason for this lengthy introduction on general background has been to familiarize the reader who is not aware of the purpose and problems of radiopharmaceutical development for nuclear medicine.

I.3 Synthetic Strategy

The aim of the present study was the pursuit of new methods for the incorporation of two radioisotopes of medical interest, namely radiobromine and radioiodine (see Table I-3), into organic compounds. As a consequence of the synthetic considerations discussed above, the following five criteria were set forth.

1) The reaction used to incorporate the radionuclide must be fast and result in high yield.
2) The reaction must be site- and stereospecific.

TABLE 1-3. Medically Useful Radioisotopes of Bromine and Iodine

Nuclide	Half-life[44]	Decay mode[44]	Application
^{75}Br	95 min	β^+(75%),EC(25%)	PET
^{77}Br	57 h	EC	SPECT
^{120}I	1.4 h	EC(54%);β^+(46%)	PET
^{122}I	3.6 min[13]	β^+(77%);EC(23%)[13]	PET
^{123}I	13 h	EC	SPECT
^{131}I	8.04 d[21]	β^- [21]	SPECT

Decay modes: β^- = beta particle emission, β^+ = positron emission
EC = electron capture

3) The reaction must be compatible with high specific activity radiohalogens.

TABLE I-4. Summary of Major Radiohalogenation Methods[19]

Method	Advantages	Disadvantages
Aliphatic nucleophilic exchange	Fast, convenient reaction, high yields, high specific activity possible with non-halogen leaving groups	Medium specific activity with non-isotopic exchange,* racemization possible
Aromatic halogen exchange	Fast reaction, high yield, regiospecific	High temperatures necessary,* harsh reaction conditions, low specific activities
Dediazoniation	Very high specific activities possible, mild reaction conditions with triazene, regiospecific	Oxidative conditions and low pH needed for diazoniation step, variety of side-products, optimization for different substrates complex
Direct electrophilic substitution	Fast, convenient reaction, high yield, high specific activities possible	Oxidation and chlorination side reactions possible, isomeric mixtures formed,* useful only for activated aromatic substrates
Demetallation	Fast reaction, high yields, high specific activity (B, Si, Sn), mild reaction conditions, regiospecific, aliphatic and aromatic substrates	Precursor synthesis required, oxidation and chlorination side reactions depending on reagents
Recoil labelling[21,27]	High specific activities possible, high yields possible for some substrates	Slow reaction times, isomeric mixtures formed*

*Where non-isotopic halogen starting materials, or side-products, separation problems can occur requiring sophisticated chromatography.

4) Protecting groups, if needed, must be rapidly removed after the labelling step.

5) The labelled product must be easily and rapidly purified.

A number of approaches have been reported in the literature[19,21] for radiohalogenation and these are briefly compared in Table I-4. The best approach involves the use of halogenation-demetallation, particularly when contrasted to direct electrophilic substitution at a carbon-hydrogen bond. Due to the polarized nature of a carbon-metal bond, an organometallic compound is sensitive to electrophilic attack. This is important because the electrophilic cleavage of organometallic precursors potentially can be performed under mild conditions with short reaction times. The direction of the bond polarization, in which the metal carries a partial positive charge while the carbon has a partial negative charge, determines that a positively charged electrophilic species will become attached at the metal-carrying carbon; this renders the reaction site specific.

In order to use high specific activity radiobromine and iodine, which are most readily available in the halide form, the radiohalogens must be oxidized to a suitable electrophilic species for use with organometallic compounds. This is no problem, as there are a number of mild oxidizing agents available for this purpose. High pressure liquid chromatography (HPLC) has become the standard, in radio-labelling, for rapid purification of the labelled product. A key feature here is the chromatographic behaviour of the starting organometallic which in general will be quite different from the product, enabling easy separation of excess starting material.[19]

A number of metals (B[28], Si[29,30], Ge[31], Sn[32], Hg[33], Tl[34]) have been used in radiohalogenation - demetallation reactions. Generally their primary application has been in the formation of radio-labelled aromatic structures, with the exception of boron[28] and silicon[30] which have been successfully utilized in preparation of labelled alkyl-halide compounds. Comparatively little has been done to use these metals to produce radio-labelled vinyl-halogen compounds. Although a larger number of biologically interesting molecules exist with aromatic structures, a number of significant compounds having vinyl structures exist.[33,35-37] It was these potential applications which prompted our interest in developing general methods of radiobromination and iodination for the synthesis of vinyl labelled compounds.

Boron, mercury and tin have been the only metals used for radio-labelling vinyl compounds. Kabalka et al.[38] using boron, introduced via hydroboration of acetylenic precursors, have prepared a range of CA ^{125}I-labelled vinyl iodides with radiochemical yields ranging from 41 to 87%; more recently, an iodo-alkenyl fatty acid was prepared with NCA Na^{125}I and Na^{123}I in 38% radiochemical yield.[36] Reaction times were in the order of 30 minutes. The disadvantage of using boron is that it is sensitive to carrier levels, giving the poorest radiochemical yields at the NCA level. Bo-Li.et al[33] used 6-chloromercurycholesterol as a precursor for reaction with low specific activity [^{82}Br]Br_2 in chloroform to produce [^{82}Br]6-bromocholesterol in 46% radiochemical yield (reaction time, 70 min). This strategy is not advantageous owing to the use of labelled molecular bromine which can give only a maximum radiochemical yield of 50%. Hanson et al.[39] used vinyl-tin precursors for ^{125}I-labelling. The tin precursors were prepared by employing bis(tributylstannyl)ethylene,[40] which was transmetallated to a monolithiated analog, and reacted with a ketone functionality, as in the case of estrone. The tin precursor, 17α-E-tributylstannylvinylestadiol prepared from estrone, was initially labelled with low specific activity $[^{125}I]I_2$,[39] but more recently with NCA $Na^{125}I$, using hydrogen peroxide in acetic acid as the oxidant, which resulted in only a 46% radiochemical yield (reaction time, 30 min).[41] Other estradiol derivatives, however, were labelled in excellent radiochemical yields (89-95%).[35] Although that work seems suitable for ^{123}I-labelling, the reaction times are too long for use with either ^{120}I or ^{122}I.

This analysis of the literature suggested to us that vinyl-tin reagents would be the preferred labelling precursors for our own studies. Tin compounds are sufficiently stable to be prepared in bulk and stored, and then used in aliquots as needed.[41] In addition, vinyl-tin precursors can be prepared readily by reduction of the corresponding acetylenic compounds, using tin hydride reducing agents.[42] This affords a potentially more convenient synthesis of vinyl-tin compounds than that of Hanson and co-workers[43] discussed above. Furthermore, given the experience of using aryl-tin precursors for labelling,[32,45] it was anticipated that all five criteria as described earlier could be met with vinyl-tin compounds.

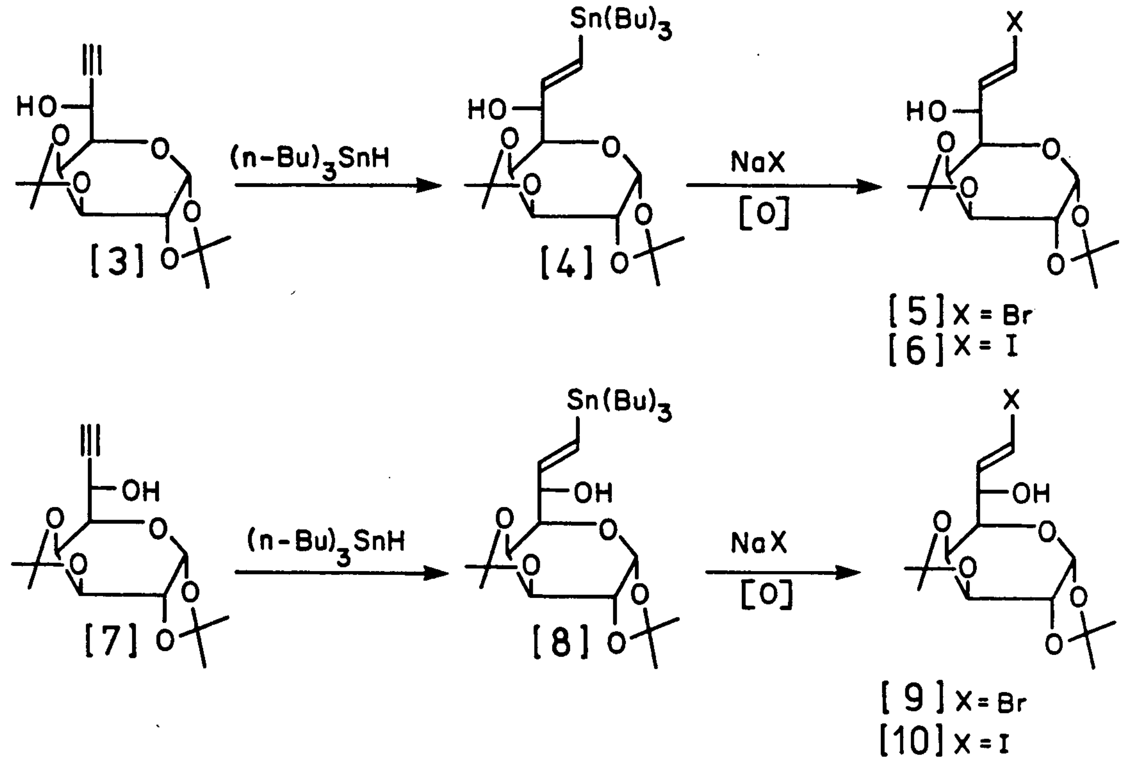
CHAPTER II

DEVELOPMENT OF METHODS FOR RAPID LABELLING WITH NON-RADIOACTIVE BROMINE AND IODINE

II.1 Introduction

Although the intent of this work was to develop methods of labelling with bromine and iodine that would be generally applicable, it was determined that a specific application was needed to evaluate the new methodology. To this end, the synthesis of vinyl-halogen labelled estradiols was chosen. Radio-labelled estradiol derivatives have been shown to be useful for the detection of estrogen-receptor-binding proteins which are present in estrogen-responsive mammary carcinoma (i.e. breast cancer).[46] The vinyl-iodinated estradiol derivatives, 17α-E-iodovinyl-1,3,5(10)-estratriene-3,17β-diol [21] and 3-methoxy-17α-E-iodovinyl-1,3,5(10)-estratriene-17β-ol [23] (see figure II-2) have been labelled with ^{125}I and evaluated in animal models with good results.[35,39] The corresponding bromo-analogues have not been synthesized previously. These estrogen-receptor-seeking radiopharmaceuticals provide an additional challenge in that these must be produced in high specific activities (minimum of 1000 Ci/mmol is deemed necessary) as the concentration of the estrogen receptors in the target tissue is very low (pmole per cc of tissue).[46] Initial development, however, of these new labelling procedures was performed with model compounds.

scheme 1



scheme 2

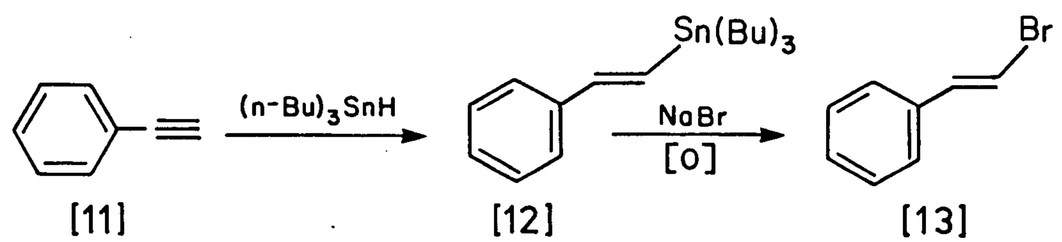


Figure II-1. Synthetic schemes for the preparation of the vinyl-halogenated model compounds [5], [6], [9], [10] and [13].

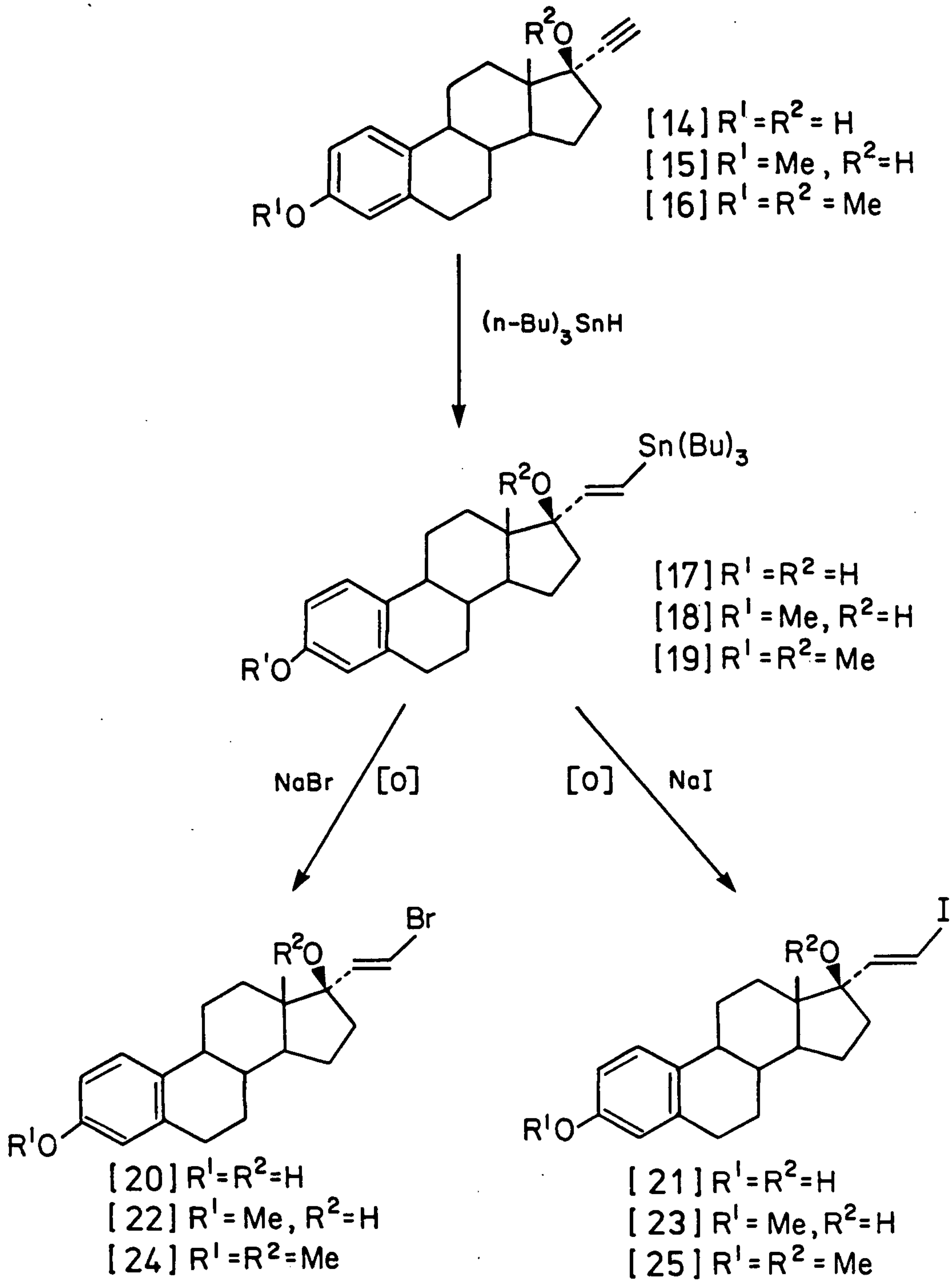


Figure II-2. Synthesis scheme for the preparation of vinyl-halogenated estradiol derivatives.

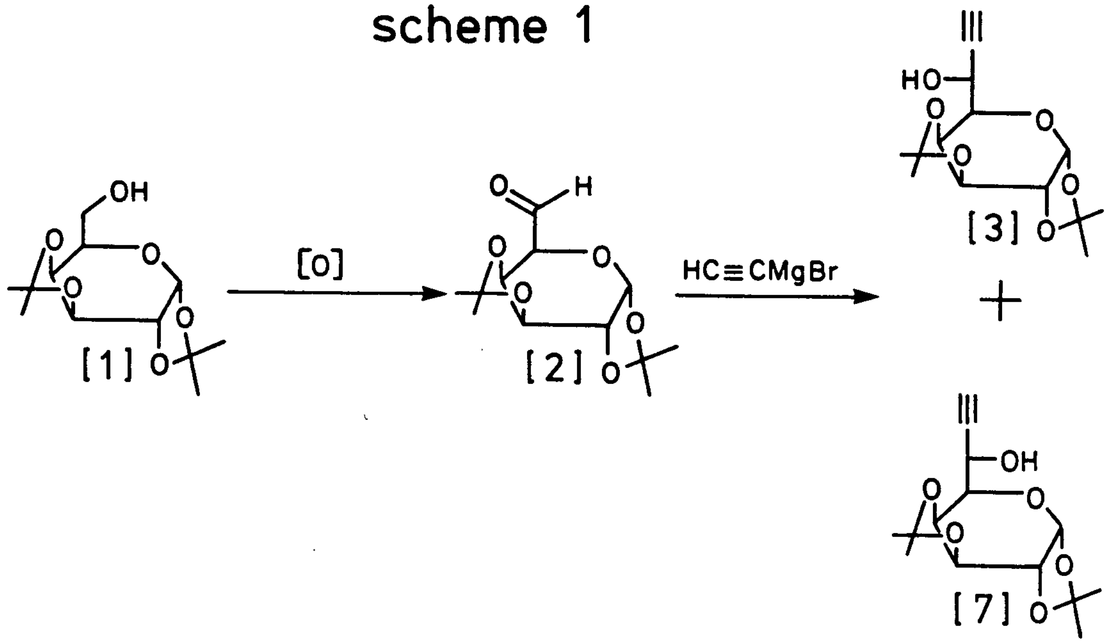
In this chapter, the synthesis of vinyl-tin derivatives will be described. This will be followed by studies in bromination and iodination of the prepared vinyl-tin compounds, using non-radioactive bromine and iodine. The reaction sequences performed are summarized in figures II-1 and II-2. Since the vinyl-tin precursors, along with their corresponding brominated and iodinated products, were in most cases new compounds, these were fully characterized. In this regard, Proton Nuclear Magnetic Resonance (^{1}H nmr) was found to be the most useful characterization tool. The individual steps shown in figures II-1 and II-2 will now be discussed in detail.

II.2 Synthesis of Vinyl-Tin Precursors

In order to prepare the desired vinyl-tin compounds via hydrostannylation, the appropriate acetylenic precursors need to be either purchased commercially or synthesized. For this present study, the acetylenic compounds, phenylacetylene [11] and 17α-ethynyl-1,3,5(10)-estratriene-3,17β-diol [14], were obtained commercially. However, the remaining acetylenic precursors used were prepared using literature methods, as summarized in figure II-3. The acetylenic compounds are known except for 3,17β-dimethoxy-17α-ethynyl-1,3,5(10)-estratriene [16]. This product was readily characterized by ^{1}H nmr. The key features of the spectrum were the presence of the acetylenic proton at δ 2.62 and the two methoxy groups at δ 3.77 and δ 3.42.

The hydrostannylation reaction has been known since the late 1950's.[47] The addition of tri-n-propyltin hydride with a variety of simple acetylenic compounds were reported by van der Kerk and co-workers in 1959.[42] They found

scheme 1



scheme 2

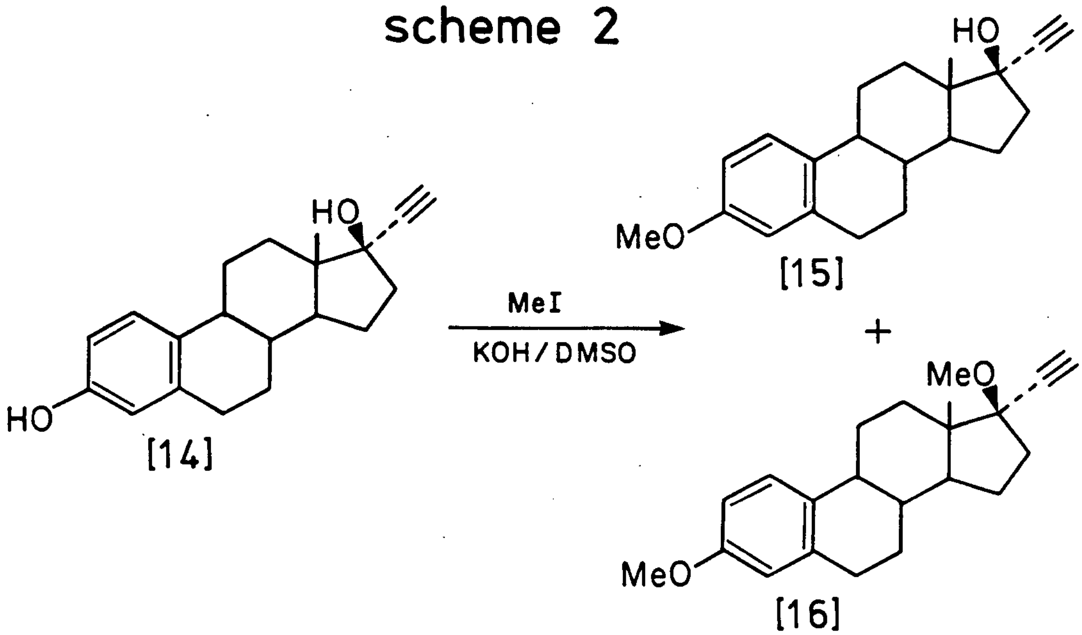


Figure II-3. Synthetic schemes for the preparation of carbohydrate (scheme 1) and estradiol (scheme 2) acetylenic precursors.

that heating a mixture of the tin hydride and acetylenic compound, in the absence of solvent, for several hours, gave the vinyl-tin products in moderate to good yields (34-82%).[42] It also has been reported in the literature that the hydrostannylation reaction can be modified or accelerated by the addition of free radical initiators, ultra-violet radiation, or by the addition of polar solvents.[48]

The most frequently used conditions for hydrostannylation of acetylenes in the recent literature has been to heat the acetylenic compound in neat hydride to about 80-90°, in the presence of AIBN (2,2'-azobis(2-methylpropionitrile)).[49] However, in each of the cases cited, the acetylenic precursors contained only protected hydroxyl groups, whereas 7,8-dideoxy-1,2:3,4-di-O-isopropylidene-D-glycero-α-D-galacto-oct-7-ynopyranose [3] contains an unprotected hydroxyl group and was the first compound in this study to be hydrostannylated. In cases where unprotected hydroxyl groups were present in the acetylenic substrate, such as in [3], the hydrostannylation reaction was performed under thermal addition conditions.[42]

Compound [3] was hydrostannylated in refluxing tetrahydrofuran (THF) with 5.5 equivalents of tri-n-butyltin hydride. Thin layer chromatographic (tlc) analysis showed the reaction had gone to partial completion. Therefore, the reaction was repeated in refluxing 1,4-dioxane (101.5°) and was found to go to completion. Analytical tlc showed a product mixture of 3 components had resulted, in which the compound of highest polarity was strongly dominant. Upon chromatographic workup, the major product was isolated and determined to be (E)-7,8-dideoxy-1,2:3,4-di-O-isopropylidene-8-C-tributylstannyl-D-glycero-α-D-galacto-oct-7-enopyranose [4]. The isolated yield of [4] was 52%.

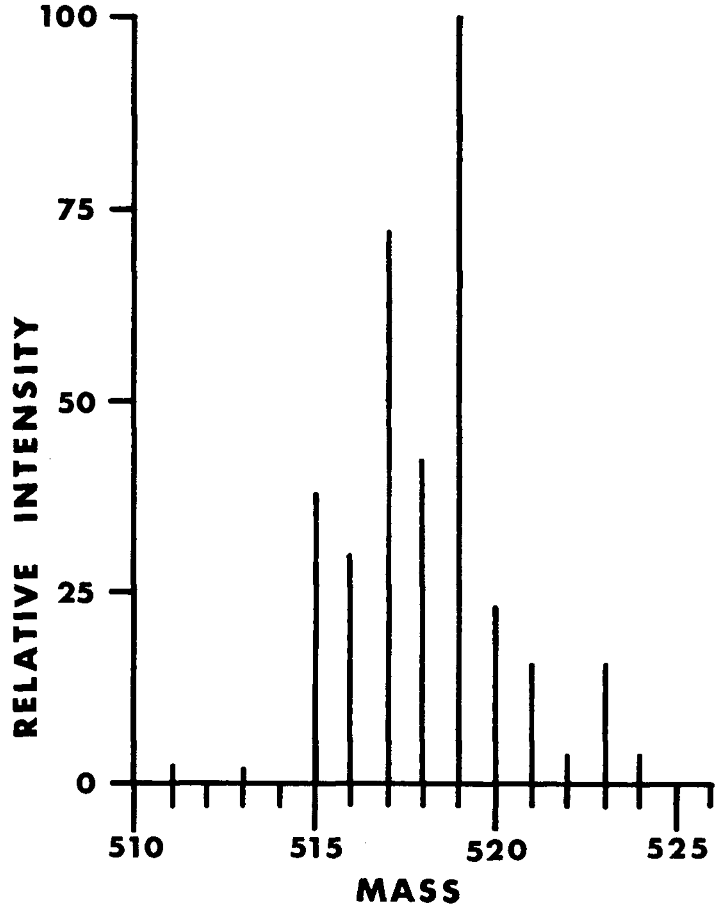


Figure II-4. Simulated mass spectral isotope pattern for the $M^{+}-C_4H_9$ fragment (base peak) of compound [4].

Compound [4] was readily characterized using ^{1}H nmr and mass spectrometry. Tin has ten natural abundance isotopes which confer a distinct mass spectral pattern for tin-containing derivatives. A calculated mass spectral isotope pattern for [4] is shown in figure II-4, which was indeed observed in the mass spectrum of [4]. This confirms the presence of tin in [4]. The ^{1}H nmr exhibited the presence of two vinyl protons at δ 6.18 and δ 6.38. Each resonance was split into a doublet of doublets. The resonance at δ 6.18 was assigned to H-7 due to coupling to H-6 (J = 4.4 Hz), whereas δ 6.38 was assigned to H-8 (J_{6-8} = 1.4 Hz). The vinyl protons were found to be of trans stereochemistry (J_{7-8} = 19.2 Hz). This was further confirmed by the values of

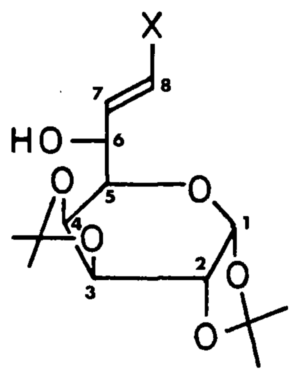


Figure II-5. The numbering scheme of the carbon skeleton of the carbohydrate compounds.

the tin-proton coupling constants,* which were 64 Hz for H-7 and 70 Hz for H-8. It is known that for vinyl-tin compounds $J(Sn\text{-}H)_{trans} > J(Sn\text{-}H)_{gem} > J(Sn\text{-}H)_{cis}$[50,51] so that H-8, which is geminal to the $-Sn(n\text{-}Bu)_3$ group, should have a larger J_{Sn-H} value than H-7, which is cis. This was indeed found to be the case for [4].

Given the results obtained in preparing [4], the same reaction conditions were used to hydrostannylate [14]. However, analytical tlc showed the reaction had seemingly not gone to completion after refluxing overnight. Therefore, the reaction mixture was chromatographed and the "starting material" was recovered after isolation of the product mixture. ^1H nmr analysis of the "starting material" clearly showed this material to be a mixture of [14] and 17α-vinyl-1,3,5(10)-estratriene-3,17β-diol [26] in a ratio of 29:71, respectively (see figure II-6). Compound [26] was isolated in pure form via HPLC, and after recrystallization gave white crystals of mp 169.5-170°, $[\alpha]_D^{25}$ = +58.5° (1,4-dioxane) {lit.[52] mp 148-150°; $[\alpha]_D$= + 57.3° (1,4-dioxane)}.

The product mixture was further purified by silica gel chromatography and the major product, 17α-E-tributylstannylvinyl-1,3,5(10)-estratriene-3,17β-diol [17], was isolated in 20% yield. This low yield was clearly the result of protonolysis of the tin-carbon bond of [17] which resulted in the formation of [26]. Two possible reasons may account for this result. The acetylenic derivative [14] may not be sufficiently anhydrous, thus introducing water into

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*There are three naturally occurring tin isotopes in which the nuclear spin quantum number I=1/2. Since ^{115}Sn is so low in abundance (0.34%), the important nuclei are ^{117}Sn (7.54% abundance) and ^{119}Sn (8.62% abundance).[51] The tin-proton coupling constants are slightly larger for ^{119}Sn than for ^{117}Sn, however, the magnitude of these couplings is always given as the average of the ^{117}Sn and ^{119}Sn values throughout this thesis.

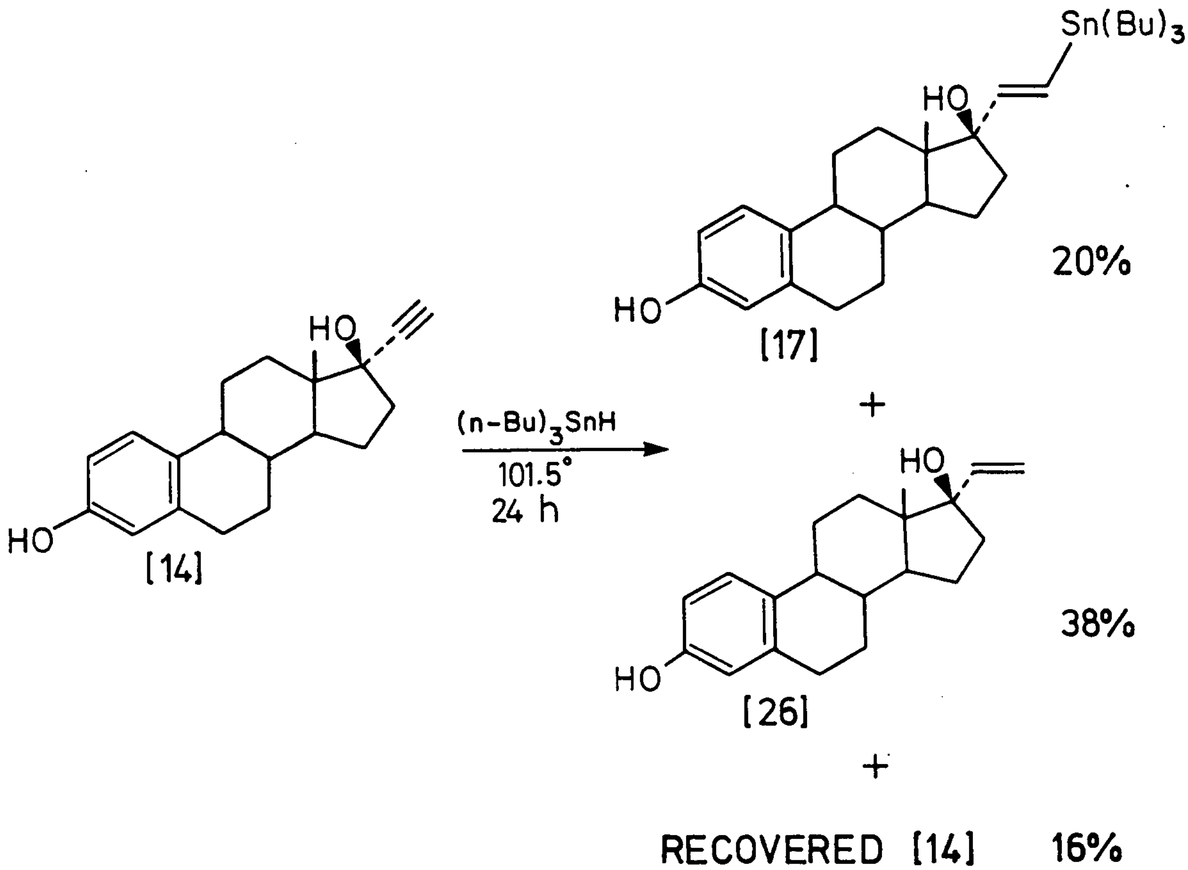


Figure II-6. Hydrostannylation reaction of compound [14], in refluxing 1,4-dioxane, produces a major side-product [26] in addition to the desired product [17].

the reaction mixture which can act as a proton source. Alternatively, the acidic proton from the phenolic hydroxyl group could function in like manner. Whichever the case, the final yield of [17] was disappointing.

Compound [17] was characterized by mass spectrometry and ^1H nmr. The mass spectrum of [17] confirmed the presence of tin as described earlier with [4]. The ^1H nmr (see figure II-8) exhibited the characteristic vinyl resonances as an AB quartet at δ 6.20 and δ 6.07, with a coupling constant of 19.4 Hz. The coupling constant confirms the trans stereochemistry of the

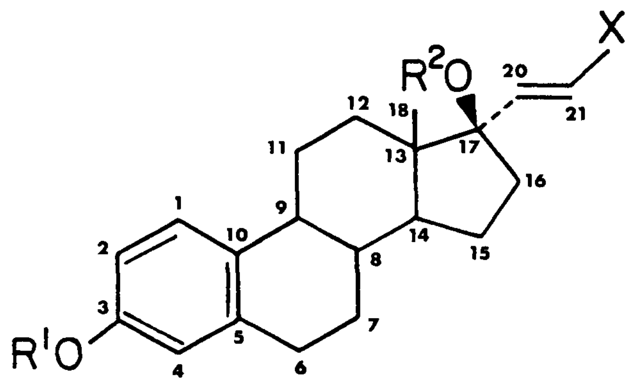


Figure II-7. The numbering scheme of the carbon skeleton of the estradiol compounds.

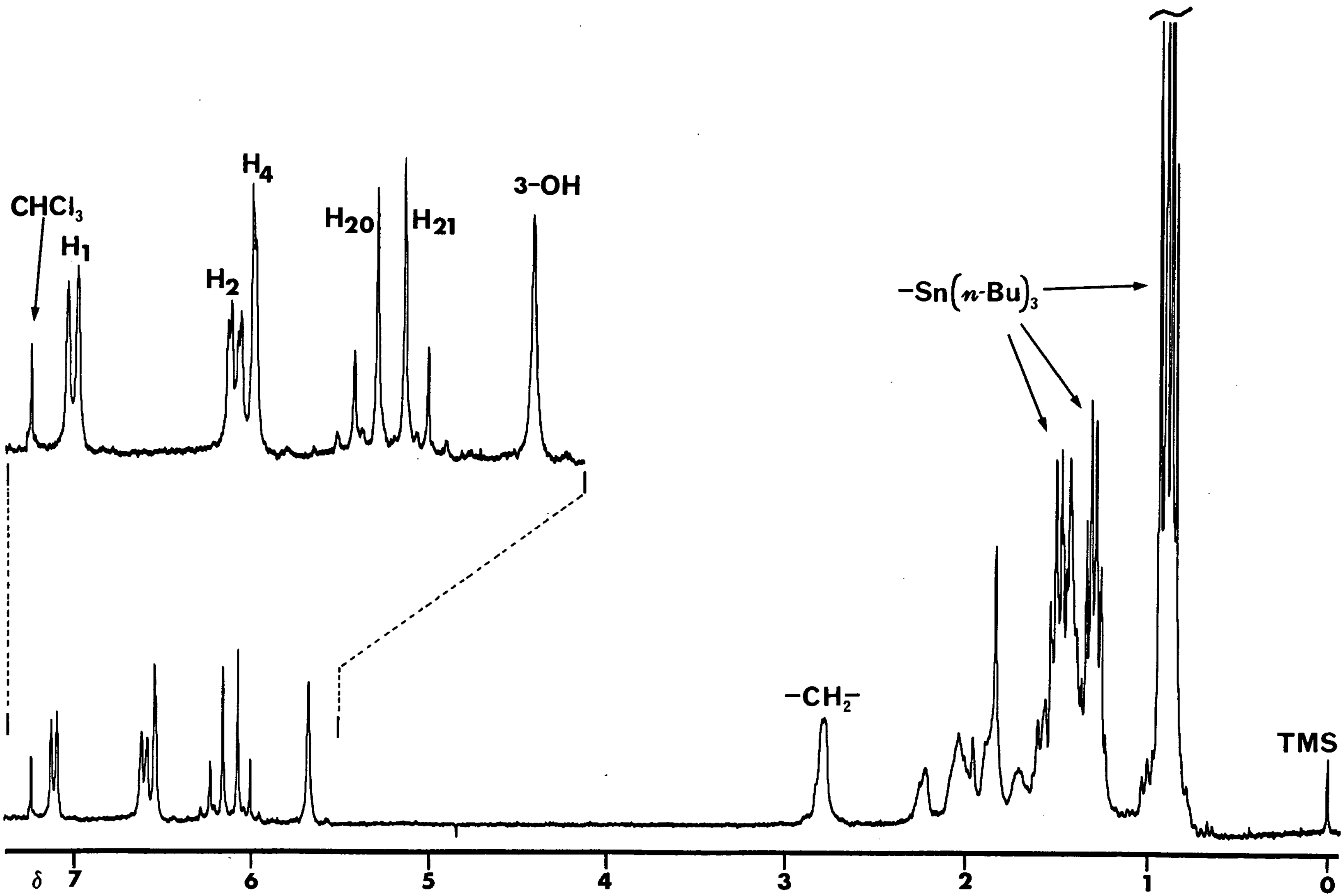


Figure II-8. 270 MHz ^{1}H nmr spectrum of compound [17] in deuteriochloroform.

vinyl protons. Since these vinyl protons were not coupled to other protons in the molecule, assignment was based on the tin-proton coupling constants. The doublet at δ 6.07 had the largest J_{Sn-H} value of 70 Hz and this was assigned as H-21, whereas the other doublet at δ 6.20 had a J_{Sn-H} value of 66 Hz and was assigned as H-20. Hanson and co-workers had reported the preparation of [17] in 1982,[39] and characterization details were reported later in 1984.[43] The ^{1}H nmr data[43] given differs from that obtained in this study, but the field strength of the nmr instrument they used was not reported.

Although the thermal addition of tri-n-butyltin hydride provided the desired vinyl-tin derivatives, [4] and [17], it was not satisfactory in regard to chemical yield and, more importantly, it required laborious chromatography and rechromatography of the isomeric product mixtures to isolate the pure E-isomer. Furthermore, the quantity of tri-n-butyltin hydride used was excessive, and while easily removed, it caused poor chromatography performance during product isolation. Therefore, the thermal addition method was discarded in favour of the AIBN catalyzed procedure recently reported by Jung and Light,[53] and Ensley and co-workers.[54] Both groups reported successful hydrostannylations of a number of acetylenic alcohols and encountered no difficulties with the unprotected hydroxyl groups.

The first acetylenic derivative to be hydrostannylated with the AIBN catalyzed method was 3,17β-dimethoxy-17α-ethynyl-1,3,5(10)-estratriene [16], which has both hydroxyl groups protected. Approximately two equivalents of tri-n-butyltin hydride and a catalytic amount of AIBN was added to [16], and the mixture was heated to 95° overnight. Chromatographic workup yielded 3,17β-dimethoxy-17α-E-tributylstannylvinyl-1,3,5(10)-estratriene [19] in 98% yield. Compound [19] was readily characterized by mass spectrometry and ^{1}H nmr. The spectral properties exhibited by this vinyl-tin derivative were analogous to that of [17], discussed earlier. The chemical shifts and coupling constants of the vinyl protons are summarized in Table II-1.

Based on the above result, the same reaction conditions were employed for the hydrostannylation of [14] and 3-methoxy-17α-ethynyl-1,3,5(10)-estratriene-17β-ol [15]. The results are shown in Table II-2. In the case of [15], very good results were obtained as 3-methoxy-17α-E-tributylstannylvinyl-1,3,5(10)-estratriene-17β-ol [18] was isolated in 87% yield. However, only moderate yields of [17] were obtained. Analytical tlc confirmed that the byproduct [26], discussed previously (see figure II-6), was again present in the reaction mixture of [17]. The problem of tin-carbon bond protonolysis continues to exist under these reaction conditions. It is interesting to note that this problem was not observed in the preparation of the other vinyl-tin derivatives in this study.

Compound [18] has been previously prepared by Franke and Hanson in 1984,[35] but they have yet to publish any characterization data on this compound. Compound [18] was characterized by mass spectrometry and ^{1}H nmr (see Table II-1). The spectral properties exhibited by [18] were analogous to the previous vinyl-tin derivatives discussed earlier.

The other acetylenic substrates in this study, [3], 7,8-dideoxy-1,2:3,4-di-O-isopropylidene-L-glycero-α-D-galacto-oct-7-ynopyranose [7] and [11] were all hydrostannylated using the AIBN catalyzed method. The results are

TABLE II-1. Chemical Shifts (ppm) and Coupling Constants (Hz) of the Vinyl Protons of the Tin Compounds

Compound	H-7	H-8	H-20	H-21	J_{H-H}
[4][a]	6.18 J_{Sn-H}64	6.38 J_{Sn-H}70	-	-	19.2
[8][b]	6.48 J_{Sn-H}65	6.86 J_{Sn-H}76	-	-	19.2
[17][a]	-	-	6.20 J_{Sn-H}66	6.07 J_{Sn-H}70	19.4
[18][a]	-	-	6.20 J_{Sn-H}66	6.06 J_{Sn-H}71	19.3
[19][b]	-	-	6.37 J_{Sn-H}69	6.23 J_{Sn-H}75	19.7

[a] Deuteriochloroform solution
[b] Deuteriobenzene solution

TABLE II-2. Yields of Vinyl-Tin Derivatives[a]

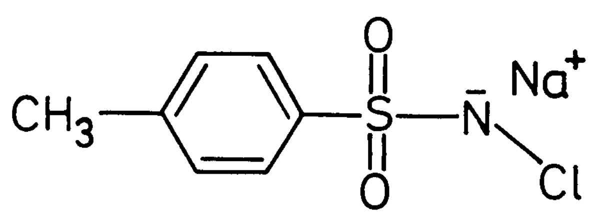
Starting Material	Product	% Yield
[3]	[4]	61 (52)[c]
[7]	[8]	58
[11]	[12]	82[b]
[14]	[17]	51-59 (20)[c]
[15]	[18]	79-87
[16]	[19]	90-98

[a] The products isolated were pure E-isomer.
[b] This product was isolated as an E/Z isomeric mixture (E to Z ratio of 88:12 by ^1H nmr).
[c] These are yields obtained by the reaction of $(n\text{-}Bu)_3SnH$ with starting material (AIBN is not present) in refluxing 1,4-dioxane.

summarized in Table II-2. Surprisingly, the hydrostannylation of [3] and [7] gave lower yields than expected. This is presumably due to a larger quantity of Z-isomer being present. Each of the tin products was characterized, as before, by mass spectrometry and ^{1}H nmr (see Table II-1).

II.3 Bromine Labelling Studies

Our approach to labelling vinyl-tin precursors with bromine involves the direct attack of the tin-carbon bond with a suitable electrophilic brominating species, thus generating the desired product. However, as discussed in the introduction, high specific activity radiobromine and radioiodine are only readily available in the halide form. Therefore, to generate the necessary electrophilic species of bromine, an oxidizing agent must be employed to convert bromide to some species containing Br^+. This oxidation is most conveniently carried out in-situ in the reaction mixture itself, allowing the generated electrophilic brominating species to react with the vinyl-tin precursor present. A number of oxidizing agents have been used for similar purposes,[20,21] but the oxidant chosen for this study is Chloramine-T (the sodium salt of N-chloro-p-toluenesulfonamide) which is shown below.



It is thought under acidic conditions that Chloramine-T generates BrCl from bromide,[19] which then reacts with the vinyl-tin substrate.

This approach was then used for the bromination of each of the vinyl-tin derivatives; [4], (E)-7,8-dideoxy-1,2:3,4-di-O-isopropylidene-8-C-tributyl-stannyl-L-glycero-α-D-galacto-oct-7-enopyranose [8], β-E/Z-tributylstannyl-styrene [12], [17], [18] and [19]. Reaction of the vinyl-tin precursor (see figures II-1 and II-2) with a slight excess of NaBr with two equivalents of Chloramine-T and 1M HCl (saturated with NaCl) in an aqueous-THF mixture at 0°, for one minute, gave the corresponding brominated product. The results are summarized in Table II-3. Very good yields were obtained for each of the brominated products except for 17α-E-bromovinyl-1,3,5(10)-estratriene-3,17β-diol [20] which was isolated in poor yield. Analytical tlc of the reaction mixture of [20] showed the formation of a number of side-products. Upon chromatographic separation, the major side-products were isolated and identified by ^{1}H nmr. These results will be discussed later in detail, but these data basically showed that the aromatic A-ring of [17] underwent concomitant bromination and chlorination in addition to tin-carbon bond cleavage.

A number of general observations were made in connection to the bromination results shown in Table II-3. With the exception of [20], all the reactions resulted in clean conversions from the vinyl-tin precursor to the brominated product. To achieve these results the correct amount of acid to be used in the reaction needed to be determined. If insufficient acid is used, the yield is low because the reaction does not go to completion. Alternatively, if the optimum amount of acid is surpassed, the yield is reduced due to the formation of unidentified by-products. Furthermore, to

TABLE II-3. Yields of Vinyl-brominated Derivatives

Starting Material	Product	% Yield[a]
[4]	[5]	95%
[8]	[9]	89%
[12]	[13]	90%[b]
[17]	[20]	13%
[18]	[22]	100%
[19]	[24]	90%

[a] These were yields obtained after chromatographic isolation.
[b] This yield was determined via HPLC analysis.

TABLE II-4. Chemical Shifts (ppm) and Coupling Constants (Hz) of the Vinyl Protons of the Brominated Compounds

Compound	H-7	H-8	H-20	H-21	J_{H-H}
[5][a]	6.38	6.50	-	-	13.7
[9][b]	6.40	6.63	-	-	13.5
[20][c]	-	-	6.47	6.26	13.5
[22][a]	-	-	6.46	6.29	13.5
[24][a]	-	-	6.31	6.13	13.9

[a] Deuteriochloroform solution
[b] Deuteriobenzene solution
[c] Deuteriochloroform solution with a few drops of Deuteriomethanol added

avoid side-product formation due to radical reactions, the reaction mixture must be shielded from light. It was found that the reaction was complete within one minute. Longer reaction times gave no improvement in yield.

The brominated products listed in Table II-3, except for β-E/Z-bromostyrene [13], were previously unknown compounds. All of these products gave spectral (^{1}H nmr and mass spectrometric) data in full accord with the assigned structures. The presence of bromine was confirmed in the mass spectra of the products, by observation of the characteristic bromine isotope pattern (^{79}Br, 50.5% abundance and ^{81}Br, 49.5% abundance). The ^{1}H nmr data exhibited a marked change in the vinyl proton resonances of the vinyl-brominated products as compared to the starting tin precursors (see Table II-4). A typical ^{1}H nmr spectrum is shown in figure II-9. The coupling constants between the vinyl protons ranged from 13.5 to 13.9 Hz confirming the trans stereochemistry. Although the vinyl protons could be unambiguously assigned for the carbohydrate derivatives ([5], [9]), the vinyl proton resonances of the estradiol derivatives ([20], [22], [24]) could be assigned only tentatively based on calculated chemical shifts. Satisfactory elemental analyses were obtained for each new product except [20]. Compound [20] could only be crystallized (benzene-hexanes) as the solvent adduct, trapping out a half mole of benzene per mole of compound. Attempts from other solvent systems either failed to give crystals or yielded solvent adducts as well. Any attempt to remove the trapped solvent by heating (56°) in vacuo lead to decomposition. However, high resolution mass spectrometry gave a satisfactory molecular weight determination for [20].

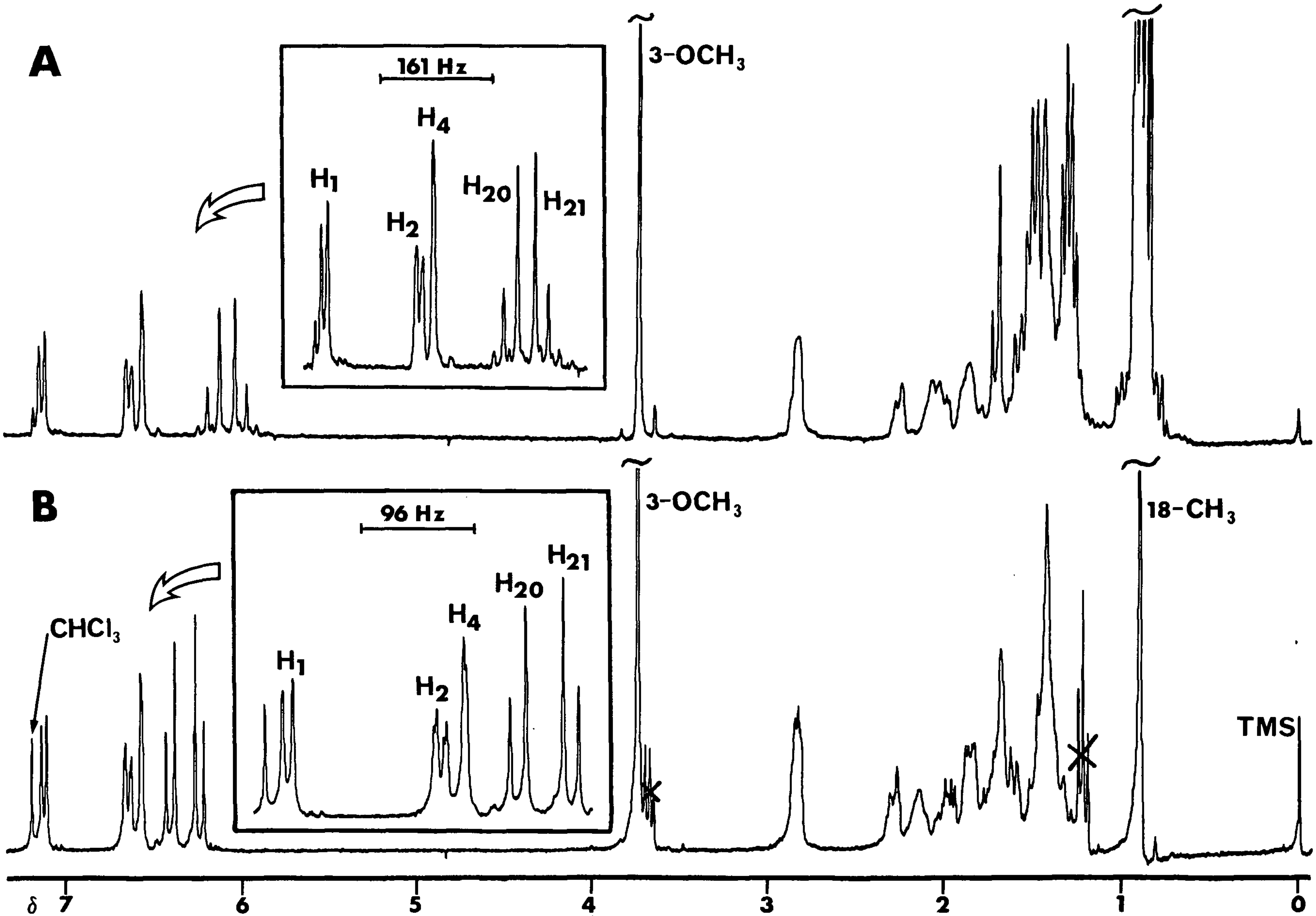


Figure II-9. 270 MHz ^{1}H nmr spectra of the vinyl-tin precursor [18] (spectrum A) and the vinyl-brominated product [22] (spectrum B), obtained in deuteriochloroform.

As was reported earlier, the bromination of [17] gave [20] in poor yield (13%). This directly resulted from side-reactions with the phenolic A-ring of [17]. Phenols are highly activated toward electrophilic aromatic substitution.[55] Therefore, it is no surprise that the A-ring would be capable of reacting with the electrophilic brominating species present.[56] This was evidenced by the side-products obtained in the course of isolation of [20] (see experimental section) and these are shown in figure II-10. The side-products were identified using ^{1}H nmr, assisted in some cases by mass spectrometry. A full characterization of these compounds was not attempted. The ^{1}H nmr assignments were tentatively made, in comparison to other compounds with similar structural features,[57] and are consistent with the assigned structures given in figure II-10.

Other methods were briefly examined to find a more suitable method for bromination of [17]. Compound [17] was treated with 1.1 equivalents of NaBr and N-chlorosuccinimide (NCS) in a THF-methanol mixture at room temperature for 15 minutes. Analytical tlc indicated a mixture of products were obtained that was very similar to that observed in the NaBr/Chloramine-T reaction. Compound [17] was also treated with Br_2 in chloroform at 0°, but likewise gave a mixture of products. Lastly, [17] was allowed to react with an equimolar amount of NaBr in the presence of H_2O_2-acetic acid (buffered with a solution of sodium acetate in acetic acid) for 50 minutes at room temperature. However, analytical tlc showed predominately starting material to be present with very little conversion to product. It is clear that to perform successful brominations at the tin-carbon site requires protection of the

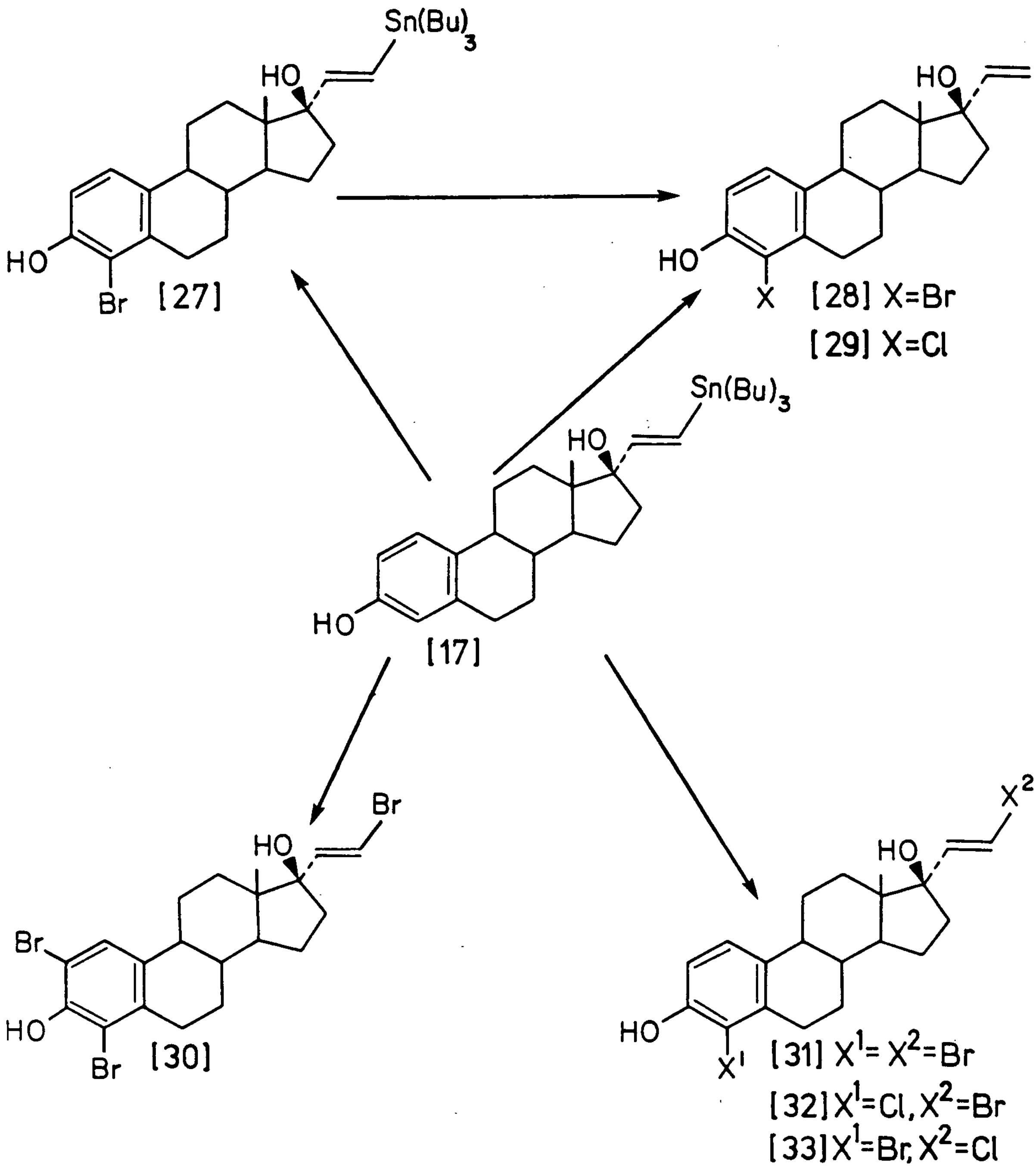


Figure II-10. Side-products obtained during the preparation of compound [20].

phenolic hydroxyl group if the Chloramine-T oxidation method is to be employed.

Concerning the mechanism for the oxidation of NaBr by Chloramine-T, the actual oxidizing species, in aqueous solution, has been assumed to be HOCl which exists in a complex series of equilibria with Chloramine-T (see below).[58] More recent evidence has been claimed to show that at low pH TsNHCl

$$TsN^{-}ClNa^{+} \rightleftharpoons TsN^{-}Cl + Na^{+} \qquad (1)$$

$$TsN^{-}Cl + H^{+} \rightleftharpoons TsNHCl \qquad (2)$$

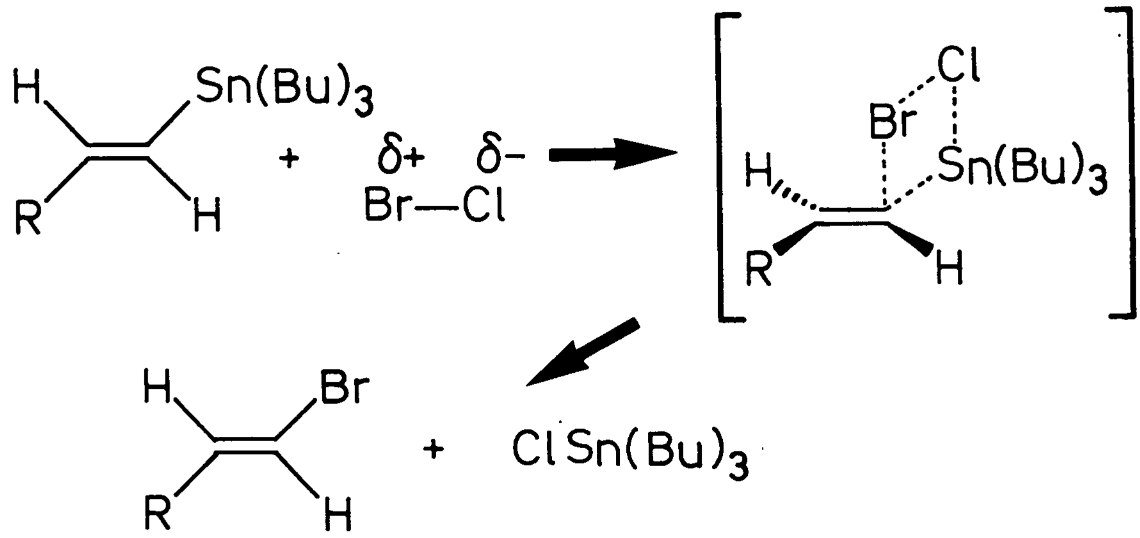
$$2\ TsNHCl \rightleftharpoons TsNCl_2 + TsNH_2 \qquad (3)$$

$$TsNCl_2 + H_2O \rightleftharpoons TsNHCl + HOCl \qquad (4)$$

$$TsNHCl + H_2O \rightleftharpoons TsNH_2 + HOCl \qquad (5)$$

$$\text{where} \quad Ts \equiv CH_3C_6H_4SO_2$$

and $TsNCl_2$ are the actual oxidants, as these are predominate in strongly acidic solution.[58] The oxidant responsible then reacts with NaBr to form some type of electrophilic bromine (Br^+), but the actual complex containing Br^+ varies depending on the pH.[59] At low (<3) pH, the interhalogen species, BrCl,[59] is thought to be formed, which is stabilized at high acid concentrations.[60] The complex H_2OBr^+ has also been suggested.[59] However, at high (>5) pH, N-brominated species, such as TsNClBr, of Chloramine-T are claimed to be the most likely species.[59] If BrCl is the actual brominating species under acidic conditions, the tin-carbon bond cleavage can be rationalized as a concerted reaction proceeding via a four-numbered transition state, like that described for electrophilic substitutions of mercury.[61]



Since BrCl is polarized with bromine carrying a partial positive charge, the bromine atom will become attached to the tin-bearing carbon in a stereospecific fashion as shown above. Unfortunately, little is actually known about these types of reactions.

II.4 Iodine Labelling Studies

The strategy to label vinyl-tin derivatives with iodine is exactly the same as that described for bromine labelling, as discussed in the preceeding section. Chloramine-T is equally suitable for the oxidation of iodide, since iodide is easier to oxidize than bromide. The iodinating species expected to be generated would be ICl, which possesses a more polarized bond than BrCl.[19]

It was found that the conditions for successful iodination could be achieved after modifying the reaction conditions used for bromination. It was observed that the iodinating species was less reactive than the corresponding brominating species. As a result, the reaction needed to be performed at room temperature and the quantity of acid used had to be increased to three equivalents of 1M HCl. Otherwise, the reaction conditions remained the same as those described for bromination in section II.3. The vinyl-tin derivatives [4], [8], [17], [18] and [19] were in turn iodinated (see figures II-1 and II-2) as described above and the results are summarized in Table II-5. Very good yields were obtained for each of the iodinated products except for 17α-E-iodovinyl-1,3,5(10)-estratriene-3,17β-diol [21] which was isolated in very poor yield. As was observed in the bromination of [17], the aromatic A-ring was highly activated toward electrophilic attack,[55] thus A-ring iodination was responsible for the low yield of [21]. In fact, an alternate method was used to prepare [21] in quantity (see third entry in Table II-5), but these results will be described later in detail. The same general observations were made for the iodination results, using the Chloramine-T

TABLE II-5. Yields of Vinyl-Iodinated Derivatives

Starting Material	Product	% Yield[a]
[4]	[6]	92%
[8]	[10]	97%
[17][c]	[21][c]	93%[b,c]
[17]	[21]	1.9%[b]
[18]	[23]	88%
[19]	[25]	99%

[a] These were yields obtained after chromatographic isolation.
[b] These yields were determined via HPLC analysis.
[c] This result was obtained using the method of H_2O_2-acetic acid oxidation of NaI developed by Franke and Hanson.[35]

TABLE II-6. Chemical Shifts (ppm) and Coupling Constants (Hz) of the Vinyl Protons of the Iodinated Compounds

Compound	H-7	H-8	H-20	H-21	J_{H-H}
[6][a]	6.73	6.53	-	-	14.5
[10][b]	6.76	6.68	-	-	14.5
[21][c]	-	-	6.79	6.25	14.4
[23][a]	-	-	6.79	6.29	14.3
[25][b]	-	-	6.67	5.94	14.8

[a] Deuteriochloroform solution
[b] Deuteriobenzene solution
[c] Deuteriochloroform solution with a few drops of Deuteriomethanol added

oxidation of NaI, as was described for the bromination results in section II.3.

The iodinated products listed in Table II-5, except for [21] and 3-methoxy-17α-E-iodovinyl-1,3,5(10)-estratriene-17β-ol [23], were previously unknown compounds. Compound [21] was previously prepared by Hanson and co-workers[39] in 1982 using I_2 cleavage of the vinyl-tin precursor [17], and characterization details were reported later in 1984.[43] The preparation of [21], by a different method, was reported in 1984 by Ferreira and co-workers[62] using the cleavage of vinyl-boronic acids by NaI oxidized by Chloramine-T under neutral conditions. Compound [23] was first synthesized in 1981 by Kabalka and co-workers[63] via ICl cleavage of the corresponding vinyl-boronic acid. Ferreira and co-workers also prepared [23] for their studies.[62]

The iodinated products listed in Table II-5 gave spectral (^{1}H nmr and mass spectrometric) data in full accord with the assigned structures. The ^{1}H nmr exhibited vinyl proton resonances with quite different chemical shifts as compared to either the starting tin precursors or the brominated analogues (see Table II-6). The ^{1}H nmr spectrum of [23] is shown in figure II-11. The coupling constants of the vinyl protons confirmed the trans stereochemistry of the double bond. The vinyl proton resonances of 3,17β-dimethoxy-17α-E-iodovinyl-1,3,5(10)-estratriene [25] were tentatively assigned based on comparison with other known vinyl-iodinated derivatives including [21] and [23]. Satisfactory elemental analyses were obtained for each product listed in Table II-5, except [21]. As was experienced with the vinyl-brominated derivative [20], [21] crystallized with the entrapment of solvent molecules. This was also the experience of Ferreira and co-workers[62] as they initially isolated

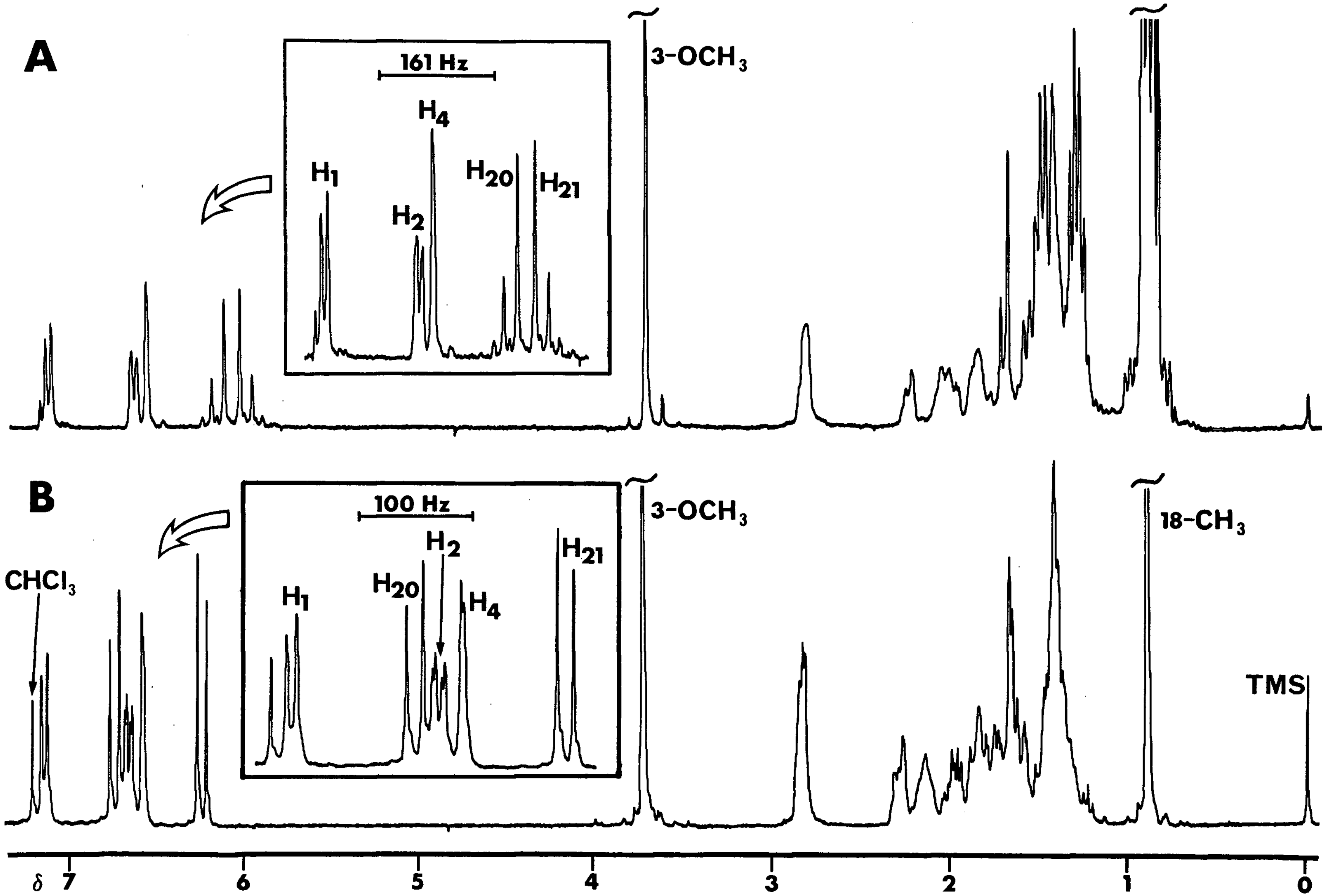


Figure II-11. 270 MHz ^{1}H nmr spectra of the vinyl-tin precursor [18] (spectrum A) and the vinyl-iodinated product [23] (spectrum B), obtained in deuteriochloroform.

[21] as a crystalline adduct with $CHCl_3$. Recrystallization of the $CHCl_3$ adduct from ethanol-water gave [21] as the hydrate.[62] In our case, recrystallization from benzene-hexanes yielded the benzene adduct. Heating (56°) of the product in vacuo led to decomposition. However, high resolution mass spectrometry gave a satisfactory molecular weight determination for [21].

As was reported earlier, the iodination of [17] gave [21] in very poor yield (1.9%). As was discussed for the analogous bromination of [17] earlier in section II.3, the reason for this low yield is the competing electrophilic reactivity of the A-ring. As a result, concurrent A-ring iodination was observed to take place during tin-carbon bond cleavage. This was evidenced by the side-products obtained during isolation of [21] (see experimental section) and these are shown in figure II-12. The side-products were identified by ^1H nmr and mass spectrometry. A full characterization of these compounds was not attempted. The ^1H nmr assignments were readily accomplished by comparison to similar compounds and are consistent with the assigned structures shown in figure II-12. Partial ^1H nmr spectra of some of these side-products are shown in figure II-13.

In order to prepare [21] in sufficient quantity, the radio-labelling procedure developed by Franke and Hanson[35] was adapted and used for non-radioactive iodination. The vinyl-tin derivative was successfully iodinated using this procedure which consisted of reacting a slight excess of NaI in the presence of H_2O_2-acetic acid (buffered with a solution of sodium acetate in acetic acid) for 30 minutes at room temperature. The product [21] was isolated in 77% yield. In this reaction, however, not all the product was

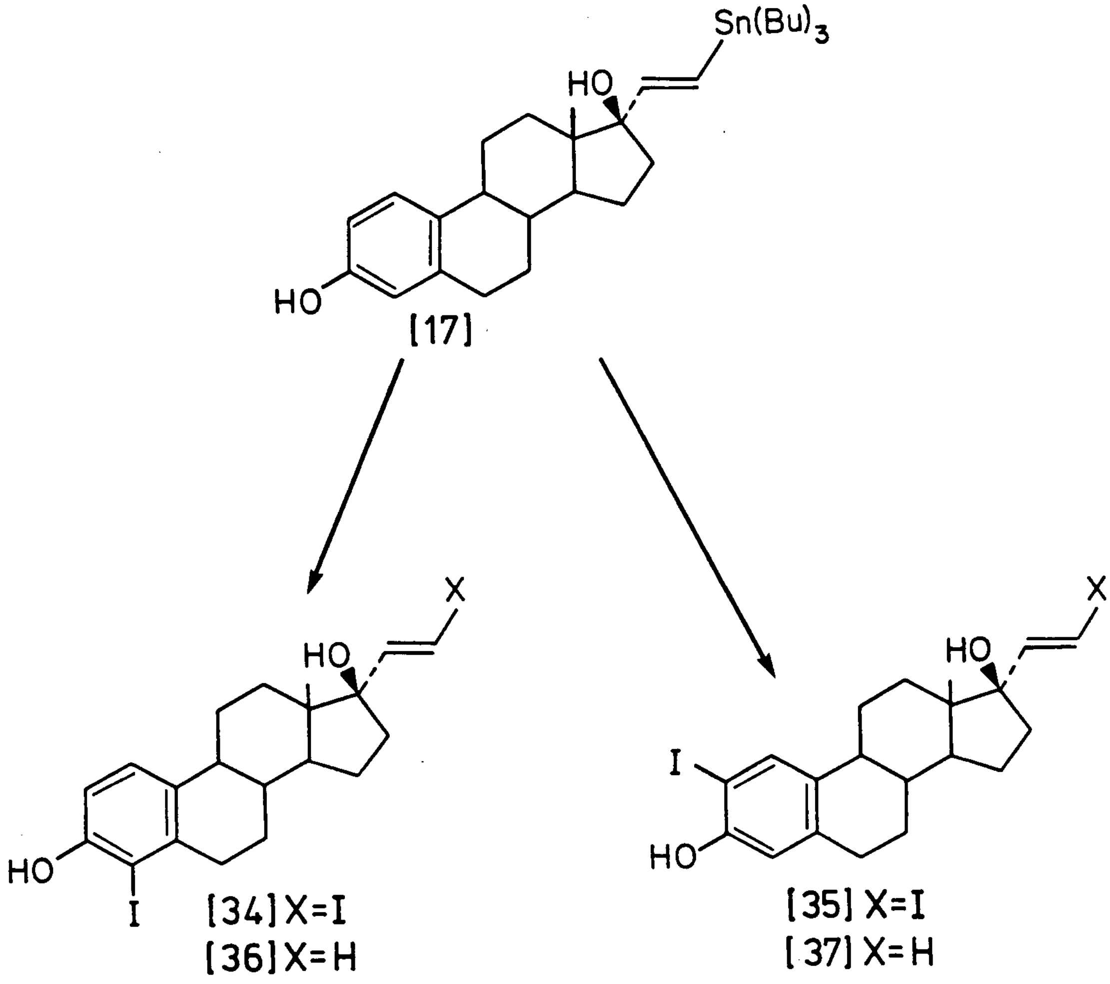


Figure II-12. Side-products obtained during the preparation of compound [21].

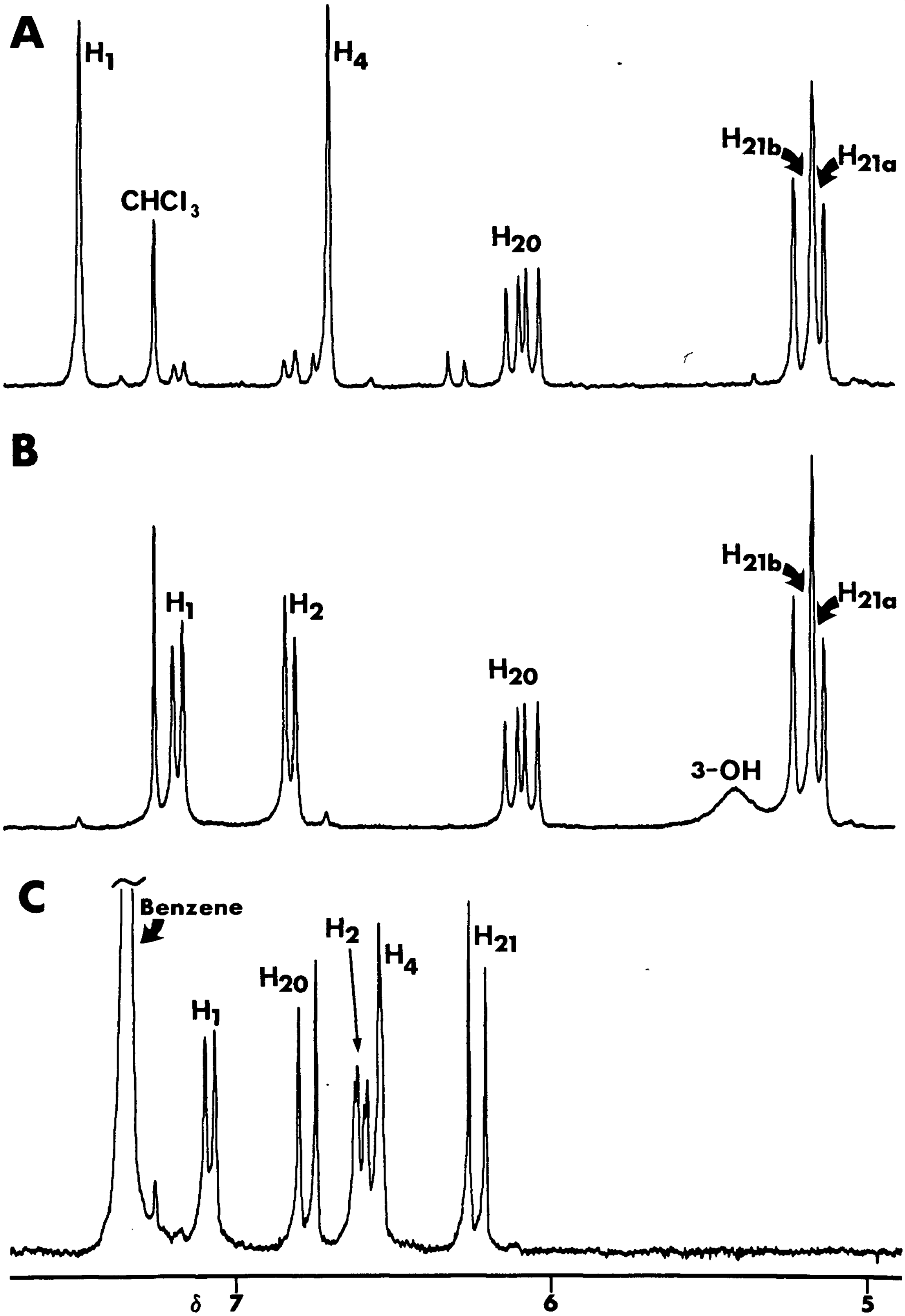


Figure II-13. Partial ^1H nmr spectra of side-products [36] (spectrum A) and [37] (spectrum B), obtained in deuteriochloroform, as compared to compound [21] (spectrum C) in deuteriochloroform with a few drops of deuteriomethanol added.

isolated as efficiently as possible. The analytical tlc clearly showed a quantitative conversion to have resulted. To properly ascertain the chemical yields of this reaction and the NaI/Chloramine-T method, small scale reactions were performed and the reaction mixtures were analyzed by HPLC. The results are presented in Table II-5 (entries 3 and 4). Clearly the NaI/H_2O_2-acetic acid oxidation is superior to NaI/Chloramine-T in this case. Indeed, Franke and Hanson found the NaI/H_2O_2-acetic acid method to be very successful in ^{125}I-labelling, however, they did not report the use of this method for the synthesis of non-radioactive iodinated derivatives.[35]

Mechanistically, the iodination of vinyl-tin derivatives, using the oxidation of NaI via Chloramine-T, can be considered exactly analogous to the corresponding bromination process discussed in section II.3. The only difference is that instead of oxidizing NaBr to BrCl, NaI is oxidized to ICl, under acidic conditions. The same rationalization can then be employed for the reaction of ICl with the vinyl-tin precursor.

CHAPTER III

LABELLING STUDIES WITH RADIOACTIVE BROMINE AND IODINE

III.1 Introduction

In this chapter, radioactive labelling studies with ^{82}Br, ^{123}I and ^{131}I will be described. Rather than label a number of vinyl-tin compounds, the vinyl-tin derivative [18] was used in all the labelling experiments. This approach enabled a more thorough evaluation of the Chloramine-T labelling procedure developed in chapter II, given limited availability of the radionuclides. Since the quantity of the radio-labelled product is so small*, the standard methods of characterization, such as ^{1}H nmr, cannot be used for product identification. Therefore, chromatographic analysis, using non-radioactive analogues as standards, is the best means of product identification available. The chromatographic method best suited for this purpose is HPLC[25]. The radiobromination of [18] will now be discussed in detail, followed by the corresponding radioiodination studies.

III.2 Radiobromination Studies

The radiobromine isotopes, ^{75}Br and ^{77}Br, are the most useful radionuclides of bromine for diagnostic nuclear medicine.[44] Unfortunately,

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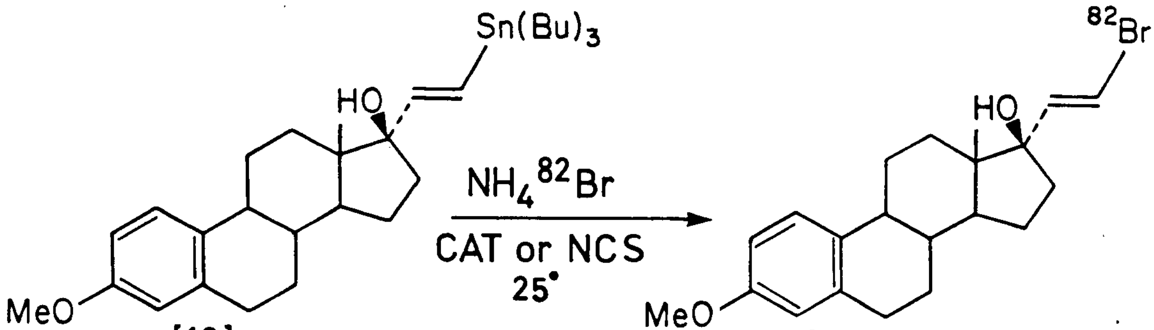
*As was discussed in section I.2, this applies only to short-lived radionuclides possessing high specific activity. Compounds labelled with tritium, for example, can be characterized by standard methods.

^{75}Br and ^{77}Br are not available commercially or from TRIUMF at the present time. As a result, ^{82}Br was chosen for this study as it is commercially available from a number of suppliers. ^{82}Br is a reactor-source radioisotope that decays by β^- emission (half-life is 35.34 hours).[65] The drawback of using ^{82}Br is that it is produced in low specific activity (our batch had a specific activity of ~255 mCi/mmol) from ^{81}Br, which means that a significant amount of ^{81}Br is present as carrier.[66] Therefore, NCA reactions cannot be performed with ^{82}Br.

Compound [18] (4 mg, 6 μmol) was treated with about 120-160 μCi of $NH_4{}^{82}Br$ in the presence of approximately three equivalents of Chloramine-T (CAT), acidified with 1M HCl (saturated with NaCl), in an aqueous-ethanol-THF mixture at room temperature, for one minute, as summarized in figure III-1. After workup, HPLC isolation yielded the radiobrominated product, 3-methoxy-17α-E-(^{82}Br)bromovinyl-1,3,5(10)-estratriene-17β-ol [41], in 77-81% radiochemical yield. A typical radio-HPLC chromatogram, obtained during the isolation of [41], is shown in figure III-2A. The chromatogram indicates a clean conversion from the starting material [18] (peak C) to the desired product [41] (peak B). A small amount of by-product (peak A) is formed and was later identified as 3-methoxy-17α-vinyl-1,3,5(10)-estratriene-17β-ol [39], which will be discussed later. The very large peak, which eluted first, is due to CAT. Two additional features are present in this chromatogram. First, the product [41] registered a significant response from the ultra-violet (uv) detector. This indicates a large quantity of carrier is present, thus the product is of low specific activity. Second, the product, as isolated, was of 100% radiochemical purity since no other radio-labelled side-products are



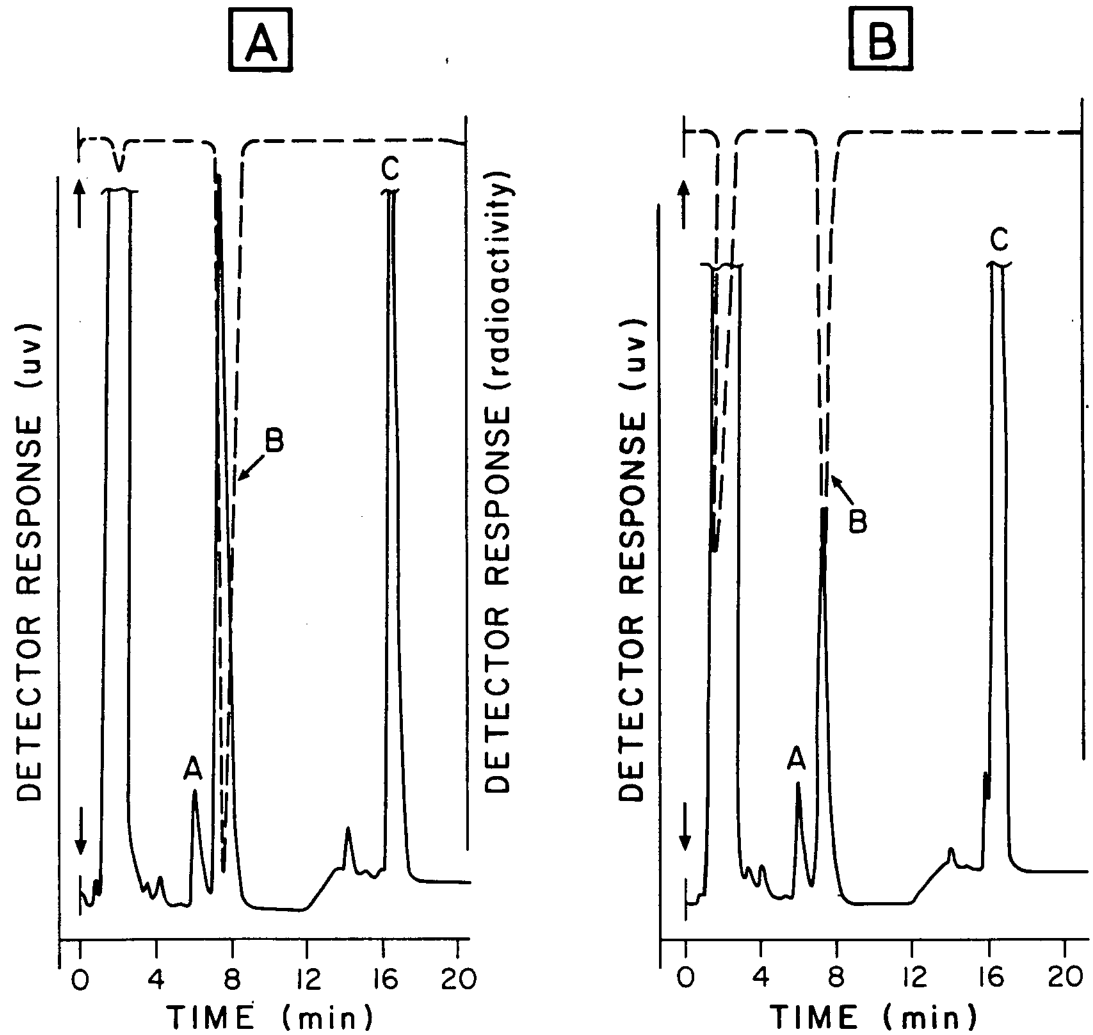
[18]

[41]

Figure III-1. Synthesis of compound [41].

observed (the tiny peak at ~ 2 min retention time in the radiotrace is residual $NH_4{}^{82}Br$) in the radioactive trace. When the radiobromination was performed at 0°, the radiochemical yield decreased to 40%. This can be visibly observed in the radio-HPLC chromatogram for this reaction, shown in figure III-2B.

A number of general observations were made with regard to the radiobromination results. As was found during the non-radioactive bromination studies (see section II.3), the correct quantity of acid needed to be determined quite carefully, as an increase or decrease of 2 μl of 1M HCl solution could change the radiochemical yield by 10-15%. This begins to show the technical problems involved with performing micro-scale radio-labelling reactions. All solutions or materials need to be quantified carefully to obtain reproducible results. The most difficult manipulative problem encountered was the transfer, via syringe, of the reaction mixture to the HPLC instrument.

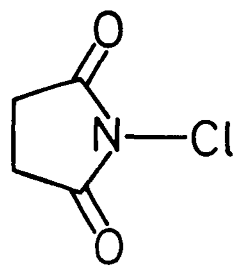


The compounds are: (A, 5.9 min), [39]; (B, 7.4 min), [41]; (C, 16.4 min), [18].
The HPLC conditions are: flow rate = 6 ml/min; solvent program: 0 to 10 min isocratic 85% MeOH/15% H_2O, 10 to 12 min linear gradient from 85% MeOH/15% H_2O to 100% MeOH, 12 to 20 min isocratic 100% MeOH; C-18 reverse phase column; uv detector set at 254 nm.

Figure III-2 HPLC chromatograms obtained during purification of compound [41]. Chromatograms A and B resulted from the radiobromination of [18] with CAT at room temperature and 0°, respectively.

Invariably, a small amount of solution would remain in the glass syringe (1 ml volume) which still contained 10-20 µCi of radioactivity. This amount of radioactivity could represent a loss of 10% radiochemical yield. When disposable plastic syringes were used, the rubber plunger was found to absorb some of the radioactivity, thus making these unsuitable. A completely satisfactory solution was not found.

For comparative purposes, an alternative procedure using NCS (shown



above) as the oxidant, was used for the radiobromination of [18]. Compound [18] was treated with 125 µCi of $NH_4{}^{82}Br$ in the presence of approximately three equivalents of NCS in a THF-methanol mixture, for 30 minutes. HPLC isolation afforded [41] in 85% radiochemical yield. The radio-HPLC chromatogram showed that additional unidentified side-products had formed (observed from uv trace), overlapping partly with the product peak. The radioactivity trace showed the product to be radiochemically pure. The total synthesis time, as measured from the addition of $NH_4{}^{82}Br$ to the HPLC isolation of [41], was 47 minutes. The Chloramine-T procedure required a synthesis time of 17-18 minutes.

NCS is a popular oxidizing agent for bromide and iodide. Although the NCS procedure was not optimized in this case, it does show promise. However, the CAT procedure, based on the short reaction time required and the minimal side-product formation, would seem to be the preferred method. The true test of which method would be best is to compare each method using NCA ^{75}Br or ^{77}Br. This is important as not all labelling procedures, successful with low specific activity radionuclides, will perform well with high specific activity radionuclides.[19]

As was discussed earlier, product identification was performed using radio-HPLC analysis. The vinyl-brominated derivative [22] exhibited the same retention time as the radio-peaks labelled B in figure III-2. This provides strong evidence for the identity of the radio-labelled product as [41]. The side-product (peak A) was thought to be due to either chlorination or protonolysis of the starting vinyl-tin precursor [18]. Since the protonated side-product seemed more likely, a sample of [39] was prepared as summarized in figure III-3, as a standard. Compound [39] was isolated in pure form via silica gel chromatography, and upon recrystallization afforded white crystals of mp 110.5°, $[\alpha]_D^{25}$ = +56.9° (1,4-dioxane){lit.[67] mp 98-100°, $[\alpha]_D^{25}$ = +60° (chloroform)}. Spectral (^1H nmr and mass spectrometric) data were consistent with the assigned structure. The retention time of [39] was found to be the same as the side-product (peak A), thus providing strong evidence for the identification of the side-product as [39].

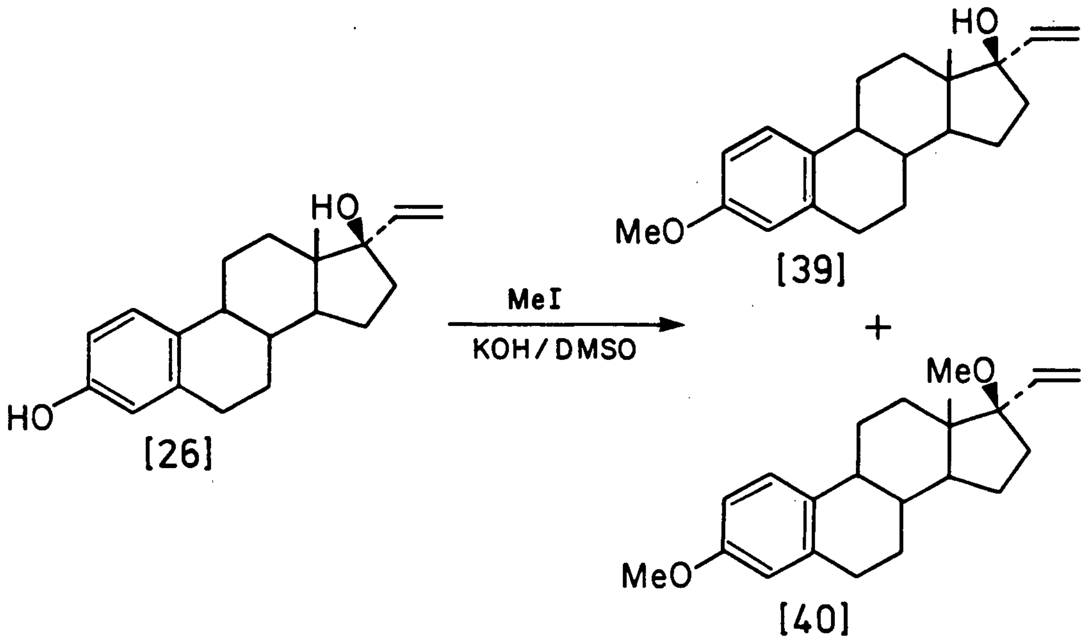


Figure III-3. Synthesis scheme for the preparation of the vinyl derivative [39].

III.3 Radioiodination Studies

The most important radioiodine isotopes, currently in use in diagnostic nuclear medicine, are ^{131}I and ^{123}I. However, ^{131}I is being replaced more and more by ^{123}I as it becomes more widely available.[21] Fortunately, ^{123}I is readily available at TRIUMF through Atomic Energy of Canada Ltd. (AECL) which is a commercial supplier of ^{123}I and other cyclotron produced radionuclides. Therefore, ^{123}I was chosen for this study and its physical properties are summarized in Table I-3. ^{123}I is produced in high specific activity of greater than 1000 Ci/mmol. Some radioiodinations were also performed with ^{131}I.

The ^{125}I-labelled analogue of [23] has been prepared previously by several research groups.[35,38,62] However, neither the ^{123}I- nor ^{131}I-labelled analogues has been synthesized before. It is important to note that methods successful in incorporating ^{125}I have not always worked as well when repeated with high specific activity ^{123}I.[68]

Compound [18] (2 mg, 3 μmol) was allowed to react with between 50 and 270 μCi of NCA $NH_4{}^{123}I$, or $Na^{123}I$, in the presence of approximately two equivalents of CAT, acidified with 1M HCl (saturated with NaCl), in an aqueous-ethanol-THF mixture at room temperature, for one minute (see figure III-4). After workup, HPLC isolation afforded the desired product, 3-methoxy-17α-E-(^{123}I)iodovinyl- 1,3,5(10)-estratriene-17β-ol [42]. The results are summarized in Table III-1. The radioiodination of [18] was also performed, under the same conditions, with NCA $Na^{131}I$ and the corresponding product,

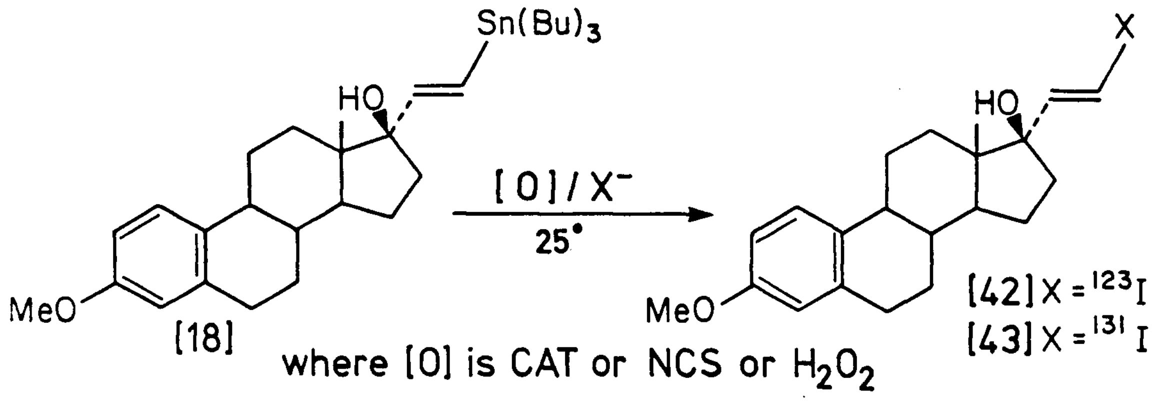
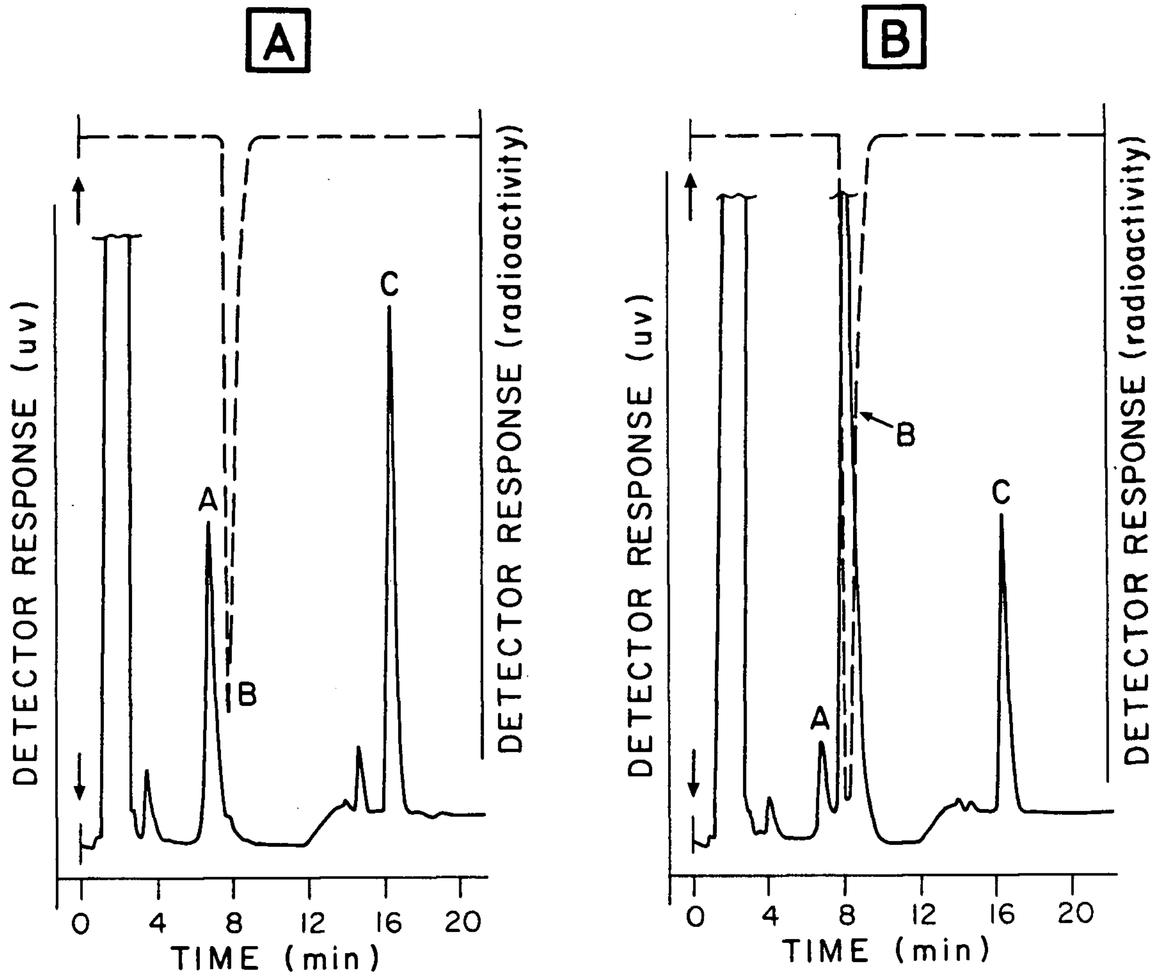


Figure III-4. Synthesis of the radioiodinated compounds [42] and [43].

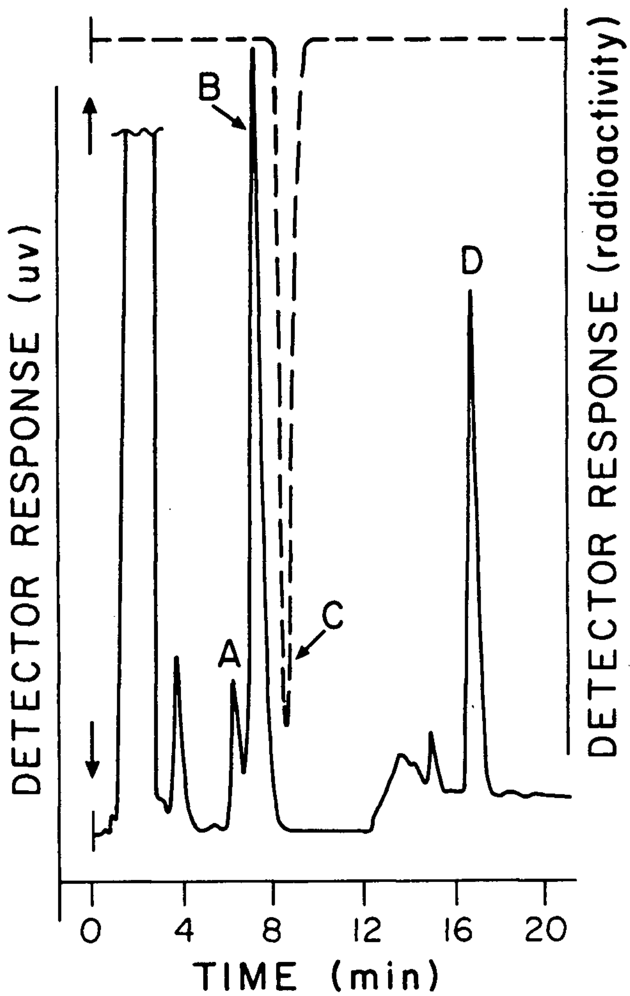
TABLE III-1. Summary of Radioiodination Results

X^-	Oxidant	Reaction Time (min)	Radiochemical Yield (%)
NCA $NH_4{}^{123}I$	CAT	1.0	82-90
NCA $Na^{131}I$	CAT	1.0	82-85
NCA $Na^{123}I$	CAT	1.0	82-88
CA $Na^{123}I$	CAT	1.0	91
NCA $Na^{123}I$	NCS	30	62
CA $Na^{123}I$	NCS	30	85
NCA $Na^{123}I$	H_2O_2-HOAc	30	85
CA $Na^{123}I$	H_2O_2-HOAc	30	81



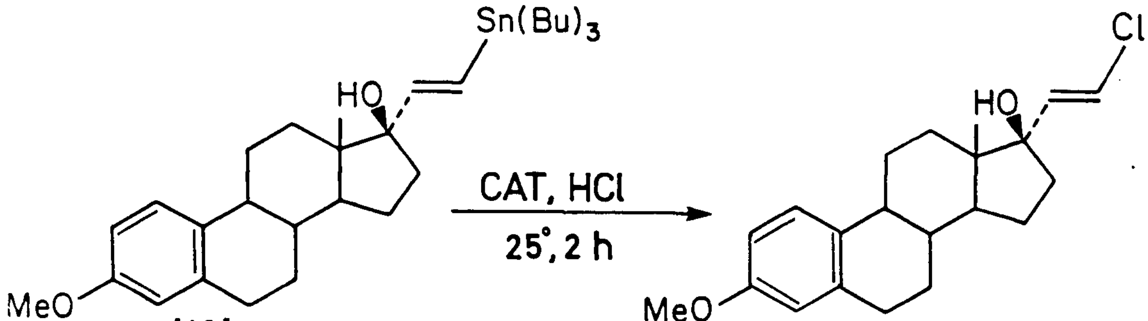
The compounds are: (A, 6.9 min), [38]; (B, 8.1 min), [42]; (C, 16.4 min), [18].
The HPLC conditions are: flow rate = 6 ml/min; solvent program: 0 to 10 min isocratic 85% MeOH/15% H_2O, 10 to 12 min linear gradient from 85% MeOH/15% H_2O to 100% MeOH, 12 to 20 min isocratic 100% MeOH; C-18 reverse phase column; uv detector set at 254 nm.

Figure III-5. HPLC chromatograms obtained during purification of compound [42]. Chromatograms A and B resulted from the radio-iodination of [18] with CAT under NCA and CA conditions, respectively.



The compounds are: (A, 5.9 min), [39]; (B, 6.9 min), [38]; (C, 8.1 min), [43]; (D, 16.4 min), [18].
The HPLC conditions are: flow rate = 6 ml/min; solvent program: 0 to 10 min isocratic 85% MeOH/15% H_2O, 10 to 12 min linear gradient from 85% MeOH/15% H_2O to 100% MeOH, 12 to 20 min isocratic 100% MeOH; C-18 reverse phase column; uv detector set at 254 nm.

Figure III-6. HPLC chromatogram obtained during the purification of compound [43] which resulted from the radioiodination of [18] with CAT under NCA conditions.



[18]

[38]

Figure III-7. Synthesis of compound [38].

3-methoxy-17α-E-(^{131}I)iodovinyl-1,3,5(10)-estratriene-17β-ol [43], was successfully obtained.

The NCA syntheses of [42] and [43] were found to be very successful. Representative radio-HPLC chromatograms are shown in figure III-5A (^{123}I-labelling experiment) and figure III-6 (^{131}I-labelling experiment). The key features of these chromatograms are essentially the same. In figure III-5A the starting material (peak C) formed a single new radioiodinated product [42] (peak B) which exhibited the same chromatographic properties as the vinyl-iodinated derivative [23]. This provides strong evidence for the identity of the radio-peak. However, note the absence of a uv response at the same retention time as [42]. This shows that [42] possesses relatively high specific activity (carrier iodine not detectable), in comparison to the radiobrominated analogue [41] discussed earlier (section III.2).

A major side-product (peak A) was formed in the reaction, and was collected during a number of experiments. This material was analyzed by mass spectrometry. The spectral data indicated the side-product to be 3-methoxy-17α-E-chlorovinyl-1,3,5(10)-estratriene-17β-ol [38] (see figure III-7). Therefore, [38] was prepared by treating [18] with two equivalents of CAT and 1M HCl (saturated with NaCl), in an aqueous-ethanol-THF mixture at room temperature, for two hours. Compound [38] was isolated via silica gel chromatography in 69% yield. Since [38] has not been previously reported, a complete characterization was performed. The ^{1}H nmr spectrum, shown in figure III-8, confirmed the structure of [38]. The vinyl protons (H-20 and H-21) are observed as a two proton singlet at δ 6.20 in deuteriochloroform. However, in deuteriobenzene, an AB quartet is observed with a coupling constant of 13.1 Hz. The low field doublet at δ 6.11 was tentatively assigned to H-21, whereas the high field doublet at δ 6.04 was accordingly assigned to H-20.

The chlorination side-reaction is quite facile, as is shown by the preparation of [38]. The amount of chlorination observed during radioiodination varies from experiment to experiment. The amount of chlorination observed in the radio-HPLC chromatogram shown in figure III-6 was often observed during ^{123}I-labelling studies. The presence of the chlorinated side-product [38] makes for a difficult separation problem. Overlap of [42] with [38],

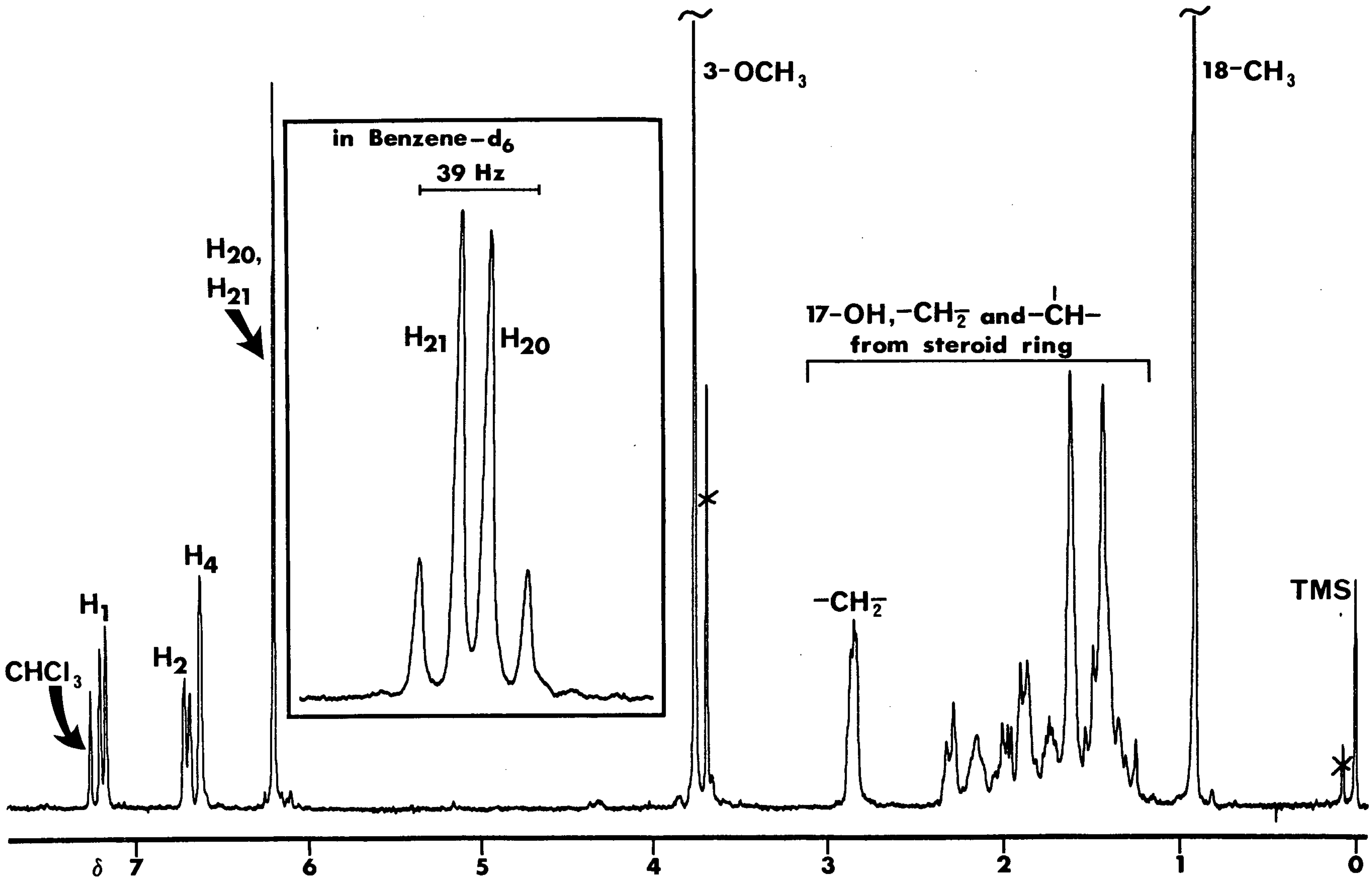
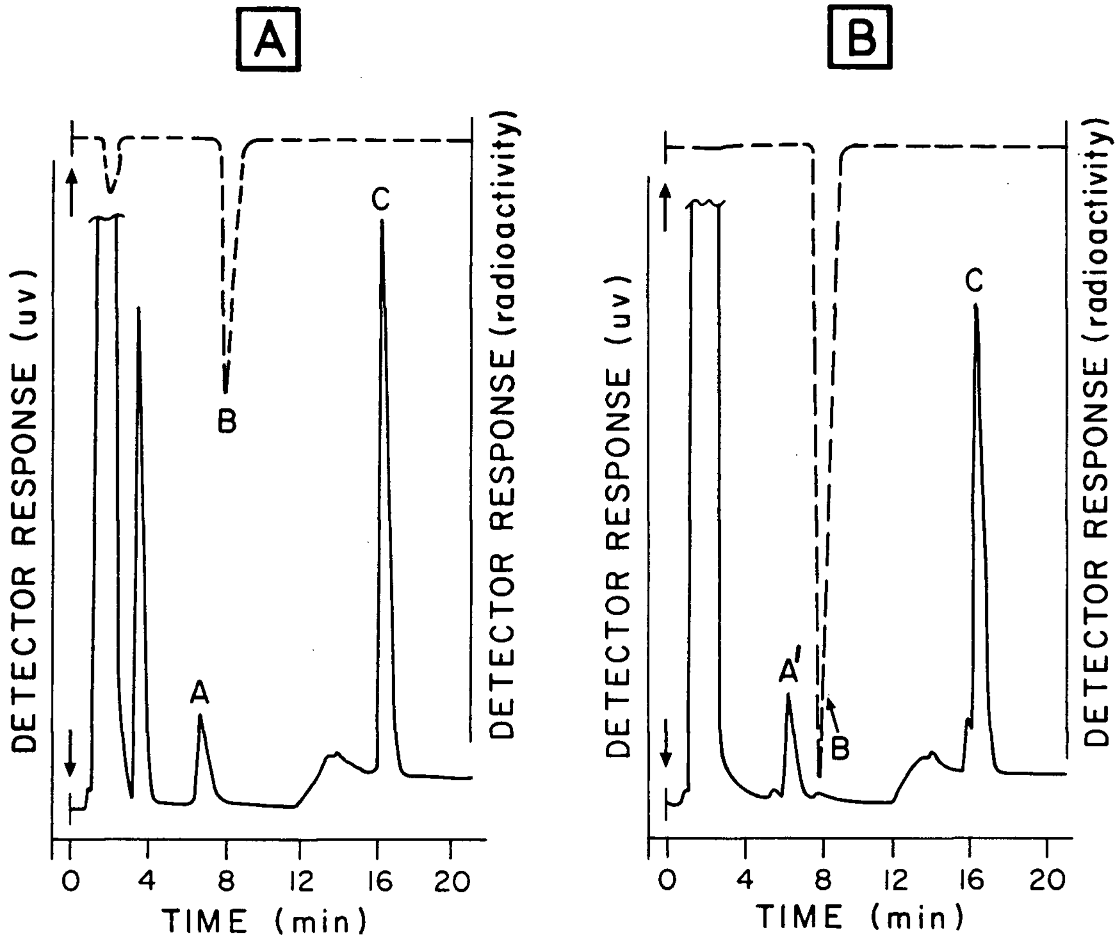


Figure III-8. 270 MHz ^1H nmr spectrum of compound [38] in deuteriochloroform. The insert shows the H-20 and H-21 resonances of [38] in deuteriobenzene.

resulting from band tailing, during product isolation will cause a decrease in the final specific activity of [42] ([38] is functioning as pseudo carrier). This is a very undesirable situation and must be avoided. As was observed during radiobrominations, protonolysis also occurs as a side-reaction (see figure III-6). The identity of peak A was readily confirmed using [39] as a chromatographic standard.

To study the affect of carrier iodide on the radioiodination reaction using CAT as the oxidant, the same procedure was repeated with the addition of 1.6 μmol of NaI as carrier and the results are shown in Table III-1. When performed back to back to NCA radioiodinations, a small increase in radiochemical yield was obtained. The radio-HPLC chromatogram of the CA experiment is shown in figure III-5B. Although a small carrier affect was observed, the CAT procedure nonetheless produced high radiochemical yields with NCA ^{123}I. In addition, the same general observations that were described for the radiobromination studies, using CAT as the oxidant, were observed for the above radioiodination studies.

The use of other oxidizing agents for radioiodination was also investigated. Compound [18] was allowed to react with NCA $Na^{123}I$ in the presence of 1.5 equivalents of NCS in an aqueous-methanol-THF mixture at room temperature, for 30 minutes. HPLC isolation afforded the desired product [42] in 62% radiochemical yield. The radio-HPLC chromatogram of this reaction is shown in figure III-9A. Compound [18] was also successfully radioiodinated with NCA $Na^{123}I$ using the method of Franke and Hanson,[35] based on H_2O_2-acetic acid as the oxidant, in which the ^{125}I-labelled analogue of [42] was obtained in 95% radiochemical yield. Compound [42] was isolated in 85% radiochemical



The compounds are: (A, 6.9 min), [38]; (A', 5.9 min), [39]; (B, 8.1 min), [42]; (C, 16.4 min), [18].
The HPLC conditions are: flow rate = 6 ml/min; solvent program: 0 to 10 min isocratic 85% MeOH/15% H_2O, 10 to 12 min linear gradient from 85% MeOH/15% H_2O to 100% MeOH, 12 to 20 min isocratic 100% MeOH; C-18 reverse phase column; uv detector set at 254 nm.

Figure III-9. HPLC chromatograms obtained during purification of compound [42]. Chromatograms A and B resulted from the radioiodination of [18] using the oxidants NCS and H_2O_2-acetic acid, respectively.

yield, but this result was obtained using a freshly made H_2O_2-acetic acid solution (see experimental section). When the oxidant was prepared 24 hours in advance, as used by Hanson and co-workers,[43] the radiochemical yield was significantly reduced. The radio-HPLC chromatogram of this reaction is shown in figure III-9B. Both the NCS and H_2O_2-acetic acid procedures were repeated in the presence of 1.6 μmol of carrier NaI and the results are summarized in Table III-1.

Compound [42] was successfully synthesized by both the NCS and H_2O_2-acetic acid procedures, although the NCS method gave a lower yield. The radio-HPLC chromatograms indicate that [42] was isolated in 100% radiochemical purity and side-product formation was much reduced over that observed during the CAT procedure. The side-product (peak A', figure III-9A) generated during the NCS procedure was determined to be vinyl-chlorinated derivative [38]. Alternatively, the side-product (peak A, figure III-9B) obtained in the H_2O_2-acetic acid procedure was identified as the vinyl compound [39]. Both methods are compatible with NCA ^{123}I, producing [42] in potentially high specific activity. However, the carrier-added studies seem to indicate that the NCS method is somewhat carrier sensitive as the radiochemical yield was noticeably improved with the addition of NaI. The H_2O_2-acetic acid procedure, in contrast, is seemingly unaffected by carrier. The results suggest that the H_2O_2-acetic acid method is superior to the NCS method for high specific activity radioiodinations. However, for use with short-lived radionuclides of iodine (see Table I-3), the CAT method would seem to be the best choice due to its short reaction time and high radiochemical yields.

The mechanism for the radioiodinations, and radiobrominations, using CAT as the oxidant would be expected to be the same as discussed earlier in chapter II. Nonetheless, a change in kinetic behaviour would be anticipated between the non-radioactive and NCA radioactive iodine labelling experiments.[19] Since the tin-carbon bond cleavage by ICl is thought to be a concerted bimolecular process, a decrease of several orders of magnitude of one of the reacting species should cause a reduction of the reaction rate. This can be observed from the so called carrier effect, in which added NaI produces a greater radiochemical yield due to the increased amount of ICl available for reaction. This problem is usually overcome by using a large excess of starting material and oxidant. Of course, side-reactions can predominate under NCA conditions which may not be normally observed. In the case of NCS, it is thought that ICl is the iodinating species formed.[29] It is interesting to see that a noticeable carrier effect is observed in this procedure. It has been suggested that peracetic acid is the actual oxidizing agent formed from the H_2O_2-acetic acid mixture.[69] From iodide, the peracetic acid probably generates HOI as the iodinating species; this is suggested from the analogous oxidation of bromide by H_2O_2-acetic acid.[69]

III.4 Summary and Conclusions

The purpose of this study was to develop a general methodology to incorporate short-lived radioisotopes of bromine, and iodine, onto the vinyl moiety of an organic compound. This was successfully accomplished by the electrophilic cleavage of tin-carbon bonds with BrCl and ICl generated in-situ from bromide and iodide, respectively, in the presence of an oxidizing agent. This method was found to yield the desired products in high radiochemical yield and purity in short reaction times.

The vinyl-tin precursors were readily prepared via the AIBN catalyzed hydrostannylation of the corresponding acetylenic derivatives, with tri-n-butyltin hydride, in 51-98% yield. The vinyl-tin derivatives [4], [8], [12], [18] and [19], were brominated in high yield (89-100%), with sodium bromide oxidized by Chloramine-T under acidic conditions. Under the same conditions, [17] was brominated in poor yield (13%). This resulted from competing bromination at the A-ring of [17]. The vinyl-tin derivatives [4], [8], [18] and [19], were iodinated with sodium iodide in the presence of acidified Chloramine-T in 88-99% yield, whereas [17] was iodinated in 1.9% yield. The facile electrophilic reactivity of the phenolic A-ring resulted in predominate iodination occurring at the A-ring of [17] rather than at the tin-carbon site. To brominate or iodinate successfully [17] using the Chloramine-T oxidation procedure would require prior protection of the phenolic hydroxyl group, followed by deprotection after the labelling step.

Compound [18] was used for radio-labelling studies with ^{82}Br, ^{123}I and ^{131}I using the Chloramine-T method. The radiobrominated product [41] was isolated in 77-81% radiochemical yield using low specific activity $NH_4{}^{82}Br$. The radioiodinated products [42] and [43] were obtained in 82-90% radiochemical yield, in relatively high specific activity. The major side-product formed during the NCA radioiodinations was the chlorinated derivative [38]. The reaction times needed were short (1 min), thus requiring a synthesis time of approximately 17-18 minutes to obtain the final radio-labelled product after HPLC purification.

An alternative radio-labelling procedure, based on NCS as the oxidant, was developed which gave [41] and [42] in 85% and 62% (NCA ^{123}I) radiochemical yields, respectively. The degree of competing chlorination in this procedure was much reduced over that observed with the Chloramine-T method. However, the NCS method seems more sensitive to carrier levels when performing NCA radioiodinations, and requires longer reaction times in the order of 30 minutes. Therefore, these results suggest that the Chloramine-T based procedure would be best for NCA radiohalogen labelling.

It is appropriate at this stage to suggest some likely extensions of this work. The radiobromination studies should be repeated with high specific activity ^{75}Br or ^{77}Br to ascertain the performance of the Chloramine-T and NCS methods under NCA conditions. It would also be prudent to survey the use of other oxidizing agents which would generate different electrophilic forms of bromine and iodine and compare their behaviour with <u>in-situ</u> generated BrCl and ICl. Lastly, it would be very interesting to determine whether vinyl-tin derivatives could be successfully fluorinated with some form of electrophilic fluorine. This would be very useful for radiofluorinations with ^{18}F.

CHAPTER IV

EXPERIMENTAL

IV.1 General Methods

All solutions were concentrated under reduced pressure with a Büchi rotary evaporator. All melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were determined using a Perkin-Elmer model 241 MC, or model 141, polarimeter.

Thin layer chromatography (tlc) was performed on pre-coated silica gel plates (Baker-flex Silica gel 1B2-F or E. Merck Silica gel 60, No. 5534). Visualization was effected in one of the following manners: (i) by spraying with 30% sulfuric acid in ethanol, and heating, (ii) by spraying with 5% ammonium molybdate dissolved in 10% sulfuric acid in ethanol, and heating, or (iii) with short-wavelength ultra-violet light. Radio-tlc plates were assayed by exposing instant film (Polaroid 545 Land film; 3000 ASA) in contact with the eluted plates. The tlc solvent systems used were very similar to those listed for the column chromatography of the individual compounds. Column chromatography was performed using Silica gel 60 (E. Merck, 70-230 mesh). Flash chromatography was performed using Silica gel 60 (E. Merck, 230-400 mesh) by the method of Still et al.[70]

High pressure liquid chromatography (HPLC) was done with a system consisting of a Spectro-Physics SP 8700 solvent delivery system, a Rheodyne 7126 injector fitted with a 0.5 or 1.0 ml sample loop (1.02 mm id), a C-18 reverse phase Whatman Partisil 10 ODS-3 column (25 cm x 9 mm) with a Waters C-18 guard column, an ISCO (model V^4) variable wavelength U.V. detector operated at 254 nm or 280 nm, and a Spectro-Physics SP 4270 integrator recorder. Radio-HPLC analysis and purification was performed with the same HPLC system fitted with a NaI(Tl) scintillation detector system and a dual channel strip chart recorder. Radioactive samples were assayed by either a Beckman 8000 scintillation counter or in a Capintec well counter (model CRC-543X).

Tetrahydrofuran (THF) was distilled from calcium hydride or sodium benzophenone ketyl.[71] Pyridine was distilled from potassium hydroxide and was stored over 4Å molecular sieves. For HPLC work, singly distilled water and HPLC grade methanol were used, which were filtered (0.45 μm Millipore brand Durapore membrane) before use. All other solvents used were of spectro or reagent grade, and were used without further treatment.

Low resolution mass spectra were recorded with a Varian MAT Atlas CH4-B or a Kratos/AEI MS-50 mass spectrometer. Spectra are quoted as m/z values, while selected ion fragmentations are reported as percentages of the base peak. High resolution mass measurements were determined using a Kratos/AEI MS-50 mass spectrometer. All microanalyses were performed by Mr. P. Borda, Microanalytical Laboratory, University of British Columbia.

IV.2 Nmr Methods and Instrumentation

Proton nmr spectra were measured at room temperature at 270 MHz with a home-built spectrometer based on a Bruker WP-60 console, a Nicolet 1180 computer (32K), a Nicolet 293B pulse programmer, a one Megabyte Diablo disk drive (model 31), and an Oxford Instruments Superconducting solenoid magnet. Data were accumulated in the quadrature detection mode. Prior to Fourier transformation, all the data were multiplied by an exponential line broadening factor (0.29 Hz). Deuterated solvents were obtained from Merck, Sharp and Dohme Canada Ltd. or Stohler Isotope Chemicals, and tetramethylsilane was used as the internal standard.

IV.3 Sources of Materials

Sources for the key chemicals used in the synthesis of compounds in this study are as follows: 1,2:3,4-di-O-isopropylidene-α-D-galactopyranose (Koch-Light Laboratories Ltd.); 17α-ethynylestradiol (Sigma); tri-n-butyltin hydride and N-chlorosuccinimide (Alfa); Phenylacetylene (vacuum distilled prior to use) and 2,2'-azobis(2-methylpropionitrile) referred to as AIBN (Aldrich); Chloramine-T (Eastman and MCB); chromium trioxide (Fisher Scientific Ltd.) was kept dry in vacuo over phosphorus pentaoxide; acetylene, 99.6% pure (Matheson Gas Products Canada). HPLC grade methanol was obtained from Fisher Scientific Ltd. $NH_4{}^{82}Br$ (~255 mCi/mmol) was purchased from New England Nuclear Canada. $NH_4{}^{123}I$ (high specific activity) was donated by Atomic Energy Canada Ltd. (AECL), and $Na^{123}I$ (high specific activity) was donated by Crocker Nuclear Laboratory (University of California, Davis). $Na^{131}I$ (high specific activity) was obtained from Charles E. Frost and Co. (Montreal, Que.).

IV.4 Synthesis of Acetylenic Precursors

Preparation of 1,2:3,4-di-O-isopropylidene-α-D-galacto-hexodialdo-1,5-pyranose [2].[72]

Method A:[73] Under an inert atmosphere (N_2) chromium trioxide (23.05 g, 0.231 mol) was added to dry pyridine (37.1 ml, 0.461 mol) dissolved in CH_2Cl_2 (600 ml). The mixture was stirred for 20 minutes at room temperature to obtain a deep-red solution. With stirring, a solution of 1,2:3,4-di-O-isopropylidene-α-D-galactopyranose [1] (5.0 g, 19.2 mmol) in CH_2Cl_2 (50 ml) was added in one portion to the CrO_3/pyridine solution, giving a dark brown precipitate. The mixture was stirred for an additional 20 minutes at room temperature. The supernatant solution was decanted into ice-cold, saturated, aqueous sodium bicarbonate (650 ml) and the residue was extracted with a small amount of ether which was also added to the sodium bicarbonate solution. The mixture was stirred for a few minutes and the organic layer was separated, washed with water (3x350 ml), and dried over anhydrous magnesium sulfate. The solvent was removed and the resulting dark brown syrup was dried under high vacuum (0.01 torr). Short-path (Kuglerohr) distillation afforded [2] (2.60 g) as a clear, pale yellow syrup in 52% yield. Spectral (^1H nmr) data are consistent with the structure and are in agreement with the literature.[72] The product was stored in vacuo, over phosphorus pentaoxide, for subsequent use.

Method B:[74] Under an inert atmosphere (N_2) chromium trioxide (7.68 g, 76.8 mmol) was added to dry pyridine (12.4 ml, 0.154 mol) dissolved in CH_2Cl_2 (160 ml). The mixture was stirred for 20 minutes at room temperature to obtain a deep-red solution. With stirring, [1] (5.0 g, 19.2 mmol) in CH_2Cl_2 (40 ml) was added in one portion to the CrO_3/pyridine solution. Acetic anhydride (7.25 ml, 76.8 mmol) was added at once and the reaction mixture was stirred for 9 minutes at room temperature. The reaction was quenched with the addition of excess ethanol (4.5 ml, 77 mmol) dissolved in ethyl acetate (25 ml). The mixture was then added to ethyl acetate (250 ml) in which the chromium compounds present were precipitated. The resulting mixture was transferred to the top of a short pad of silica gel (12 cm x 3.5 cm, Mallinckrodt SilicAr CC-4, 60-200 mesh) in ethyl acetate and the product was eluted with ethyl acetate in one fraction. After removal of the solvent, toluene was added to the crude syrup and was evaporated. This was repeated a few times to remove any acetic acid and pyridine present, after which the crude product was dried under high vacuum. Kuglerohr distillation gave [2] (3.35 g) in 68% yield. This product exhibited the same ^{1}H nmr spectrum as the material obtained in method A.

Preparation of 7,8-dideoxy-1,2:3,4-di-O-isopropylidene-D-glycero-α-D-galacto-oct-7-ynopyranose [3] and its L-glycero-α-D-galacto epimer [7].[75]

A solution of ethylmagnesium bromide was prepared in the following manner. Under an N_2 atmosphere, a solution of ethyl bromide (43.8 g, 0.40 mol) in THF (115 ml) was added dropwise to a stirred mixture of magnesium turnings (3.0 g, 0.12 mol) in THF (35 ml) at a rate sufficient to maintain a gentle reflux of the mixture. After the addition of ethyl bromide solution was completed, stirring was continued until the mixture had cooled to room temperature. The resulting mixture had a greenish-brown colouration.

With stirring, acetylene was passed through THF (150 ml) for one hour at room temperature. The prepared solution of ethylmagnesium bromide was in turn added dropwise to the acetylene saturated THF solution, while a continued flow of acetylene was passed through. The resulting solution gradually turned bright red, then finally became dark reddish-brown in colour, and was stirred an additional hour with continued acetylene flow. To the resulting ethynylmagnesium bromide mixture, a solution of [2] (9.45 g, 36.6 mmol) in THF (100 ml) was added dropwise, with stirring, at room temperature. A flow of acetylene was continued throughout the addition of [2] and for a further three hours. The reaction mixture was diluted with ether (500 ml), washed with ice-cold 10% aqueous ammonium chloride (3x300 ml) and water (3×300 ml), and dried over anhydrous magnesium sulfate. Removal of the solvent afforded the crude products [3] and [7] as a dark yellow, crystalline solid (8.80 g) in 85% yield. ^1H nmr analysis of the crude product mixture indicated, by the ratio of the anomeric protons, that [3] and [7] were present in a ratio of 62:38, respectively. Furthermore, the anomeric signal of [2] was completely absent.

Column chromatography of a 1.70 g portion of the crude product mixture was performed on silica gel (150 g) with CH_2Cl_2/hexanes/ether 6:2:1. The first fraction (0.85 g) contained pure [3] as white crystals, mp 130-131° {lit.[75] mp 131.0-131.5°}. Spectral (^1H nmr) data are consistent with the structure and are in agreement with the literature.[75]

The second fraction (0.13 g) obtained consisted of a mixture of [3] and [7]. The final fraction (0.44 g) contained only [7] as white crystals, mp 125-128° {lit.[75] mp 136-137°}. Recrystallization of a sample from ether-hexanes, followed by a further recrystallization from benzene-hexanes gave [7] as white needles, mp 138-139°. Spectral (^1H nmr) data are consistent with the structure and are in agreement with the literature.[75]

The remaining crude product mixture was chromatographed in several portions as described above in order to isolate [3] and [7] in pure form.

Preparation of 3-methoxy-17α-ethynyl-1,3,5(10)-estratriene-17β-ol [15][76] and 3,17β-dimethoxy-17α-ethynyl-1,3,5(10)-estratriene [16].

This procedure was adapted from the literature[77] in which other unrelated compounds were prepared. Powdered potassium hydroxide (1.51 g, 26.9 mmol) was added to DMSO (40 ml). After stirring for five minutes at room temperature, 17α-ethynylestradiol [14] (2.00 g, 6.75 mmol) was added, followed immediately by methyl iodide (1.44 g, 10.1 mmol). Stirring was continued for 25-30 minutes after which the mixture was poured into water (100 ml) and was extracted with dichloromethane (3×100 ml). The combined organic extracts were washed with water (2x100 ml), dried over anhydrous magnesium sulfate and the solvent was removed. Flash chromatography, of the crude mixture, on silica gel (3.5 cm x 15 cm) with hexanes/ether 2:1, was performed. Compound [16] eluted first in one portion (0.67 g), in 31% isolated yield as white needles, mp 142.5-144°, $[\alpha]_D^{25} = +0.8°$ (c=1, $CHCl_3$). Anal. calcd. for $C_{22}H_{28}O_2$: C 81.44, H 8.70, O 9.86; found: C 81.47, H 8.86, O 10.0. Mass spectrum, m/z: 324 (M^+, 100), 309 (M^+-CH_3, 70).

Continued elution afforded [15] (1.40 g) in 67% isolated yield, as white crystals, mp 150.5-151° {lit.[76] mp 150-151.5°}. Spectral (^1H nmr and mass spectra) properties were consistent with the structure.

In order to prepare [16] in higher yield, the following modified procedure was employed. Powdered potassium hydroxide (1.51 g, 26.9 mmol) was added to DMSO (15 ml) and was stirred for five minutes, at room temperature. Compound [14] (1.00 g, 3.37 mmol) was then added, followed by methyl iodide (1.91 g, 13.4 mmol). The mixture was stirred for one hour, and was poured into water (150 ml), then extracted with dichloromethane (3x150 ml). The organic extracts were washed with water (2x75 ml), dried over anhydrous magnesium sulfate and the solvent was removed. Flash chromatography, of the crude mixture, on silica gel (3.5 cm x 15 cm) with hexanes/ether 4:1 yielded 0.90 g (82%) of [16]. Analytical tlc and spectral (^{1}H nmr) properties of this material were identical with those reported earlier.

Further elution with hexanes/ether 1:1 afforded 0.11 g (18%) of [15]. Analytical tlc and spectral (^{1}H nmr) properties of this compound were the same as those obtained previously.

IV.5 Synthesis of Vinyl-Tin Compounds

Hydrostannylation of acetylenic precursors.

Procedure A: Under an N_2 atmosphere, a solution of acetylenic substrate in 1,4-dioxane was prepared. Five equivalents of tri-n-butyltin hydride were then added and the stirred mixture was refluxed (101.5°) overnight under N_2. After the solvent was removed, the crude mixture was chromatographed on a silica gel column to obtain the desired product.

Procedure B:[53,54] Under an N_2 atmosphere, a mixture of acetylenic substrate, a catalytic amount of AIBN and two equivalents of tri-n-butyltin hydride were heated overnight (oil both temperature 95°), with stirring. The product was isolated by chromatography of the crude mixture on silica gel.

General: In all cases, the vinyl-tin derivatives, after chromatography, were thoroughly dried under high vacuum and were stored in vacuo over sodium hydroxide pellets. Although vinyl-tin derivatives are sufficiently air-stable to be easily handled in the open under normal conditions, they will decompose upon exposure to the atmosphere over longer periods of time. Proper storage is therefore vital.

Preparation of (E)-7,8-dideoxy-1,2:3,4-di-O-isopropylidene-8-C-tributylstannyl-D-glycero-α-D-galacto-oct-7-enopyranose [4].[78]

Method A: Compound [3] (390 mg, 1.37 mmol) in 1,4-dioxane (3 ml) was hydrostannylated as outlined in procedure A. Column chromatography of the crude mixture was performed on silica gel (80 g) with CH_2Cl_2/hexanes/ether 6:6:1. The first fraction (390 mg) contained three products in which [4] was dominant, whereas the second fraction (330 mg) was pure [4]. Rechromatography (silica gel; 60 g) of the first fraction gave an additional 80 mg of [4]. Compound [4] was isolated as a colourless syrup in 52% overall yield, $[\alpha]_D^{23}$ = −36.1° (c=1, $CHCl_3$). Anal. calcd. for $C_{26}H_{48}O_6Sn$: C 54.28, H 8.41; found: C 54.21, H 8.52. Mass spectrum, m/z: 561(^{120}Sn: M^+-CH_3, 3), 519 (^{120}Sn: $M^+-C_4H_9$, 100).

Method B: Compound [3] (884 mg, 3.11 mmol) was hydrostannylated as described in procedure B. Column chromatography on silica gel (200 g) with CH_2Cl_2/hexanes/ether 6:6:1 yielded in one fraction 1093 mg (61%) of [4].

Analytical tlc and spectral (^{1}H nmr) properties of this material were identical with those reported earlier.

Preparation of (E)-7,8-dideoxy-1,2:3,4-di-O-isopropylidene-8-C-tributylstannyl-L-glycero-α-D-galacto-oct-7-enopyranose [8].

Compound [7] (1016 mg, 3.57 mmol) was hydrostannylated as outlined in procedure B. Column chromatography on silica gel (200 g) with CH_2Cl_2/hexanes/ether 6:6:1 yielded 1199 mg (58%) of [8] as a colourless syrup, $[\alpha]_D^{24}$ = -37.0° (c=1.3, $CHCl_3$). Anal. calcd. for $C_{26}H_{48}O_6Sn$: C 54.28, H 8.41, O 16.68; found: C 54.57, H 8.47, O 16.55. Mass spectrum, m/z: 561(^{120}Sn: M^+-CH_3, 2), 519(^{120}Sn: M^+-C_4H_9, 100).

Preparation of β-E/Z-tributylstannylstyrene [12].[79]

Phenylacetylene [11] (2.05 g, 20.0 mmol) was hydrostannylated using a modified version of procedure B.[80] The changes were, using tri-n-butyltin hydride (5.52 g, 18.9 mmol) as the limiting reagent, and heating the resulting mixture at 83° (oil bath temperature) for twelve hours. Short-path distillation (air bath temperature; 116-156°, at 0.70 torr) afforded 6.58 g of crude [12]. The crude product was redistilled twice using short-path distillation. Compound [12], in the final distillation (air bath temperature; 117-143°, at 0.60 torr), was isolated as a pale yellow oil (6.09 g) in 82% yield. HPLC analysis of the product on a C-18 column using methanol as the eluant (flow rate 6.0 ml/min; detector set at 280 nm) showed the product to be 98% pure (retention time, 3.96 min). ^{1}H nmr analysis shows the ratio of E-isomer to Z-isomer to be 88:12. The spectral (^{1}H nmr and mass spectra) data are consistent with the structure and are in agreement with the literature.[79] The product was stored under an N_2 atmosphere.

Preparation of 17α-E-tributylstannylvinyl-1,3,5(10)-estratriene - 3,17β-diol [17][43] and 17α-vinyl-1,3,5 (10)-estratriene-3,17β-diol [26].[52]

Method A: Compound [14] (1986 mg, 6.70 mmol) in 1,4-dioxane (10 ml) was hydrostannylated as described in procedure A. Flash chromatography of the crude mixture was performed on silica gel (3.5 cm x 15 cm) with CH_2Cl_2/hexanes/ether 6:2:1. The first fraction contained [17] as the dominant product contaminated with two unidentified byproducts, while further elution isolated a mixture of [14] and [26] (1090 mg) in a ratio of 29:71 as measured from the ^1H nmr spectrum. Therefore, 774 mg of [26] is estimated to be present in the mixture for a yield of 38%. The fraction containing [17] was purified by column chromatography on silica gel (150 g) with CH_2Cl_2/hexanes/ether 6:4:1 which afforded 780 mg of [17] (20% overall isolated yield) as a very viscous syrup, which upon drying overnight in vacuo, crystallized as an off-white solid. An analytical sample was obtained by flash chromatography on silica gel with CH_2Cl_2/hexanes/ether 6:4:1, mp 86-87.5°, $[\alpha]_D^{24}$ = +19.0° (c=1.7, 1,4-dioxane). Anal. calcd. for $C_{32}H_{52}O_2Sn$: C 65.43, H 8.92; found: C 65.60, H 8.78. Mass spectrum, m/z: 531 (^{120}Sn: $M^+-C_4H_9$, 100).

In an attempt to separate [26] from [14] for analysis, column chromatography of a portion of the mixture of [26] and [14] was performed on silica gel with CH_2Cl_2/hexanes/ether 6:4:1. Unfortunately, complete separation was not effected as [14] co-eluted with all fractions containing [26], with the largest percentage of [14] being present in the initial fractions. Therefore, a sample was obtained by combining several of the late fractions which contained the lowest percentage of [14]. This sample was recrystallized from benzene-hexanes and analyzed by HPLC using a C-18 column with methanol/water 75:25 as the eluant (flow rate 6.0 ml/min; detector set at 280 nm). The HPLC analysis showed that a ratio of [14] (retention time, 3.60 min) to [26] (retention time, 5.00 min) of 19:81 was present. As a result, to properly isolate [26], an HPLC separation was carried out by injecting the sample (dissolved in THF) in several portions onto the C-18 column and eluting the product with the same solvent system as described before (flow rate 3.0 ml/min). The collected product was repurified by HPLC again, as described above. The product was recrystallized from benzene-hexanes, which afforded [26] as white crystals, mp 169.5-170°, $[\alpha]_D^{25}$ = +58.5° (c=1, 1,4-dioxane). Anal. calcd. for $C_{20}H_{26}O_2$: C 80.50, H 8.78; found: C 80.54, H 8.72. Mass spectrum, m/z: 298 (M^+, 51), 280 (M^+-H_2O, 15). Exact mass calcd. for $C_{20}H_{26}O_2$: 298.1934; found: 298.1934.

Method B: Compound [14] (1.00 g, 3.37 mmol) was hydrostannylated as outlined in procedure B. Column chromatography on silica gel (100 g) with CH_2Cl_2/hexanes/ether 6:4:1 yielded in one portion 1.17 g (59%) of [17]. Physical and spectral (^1H nmr) properties of this material were identical with those reported earlier. Analytical tlc confirmed the presence of [26] in the reaction mixture, but the compound was not eluted off the column.

Preparation of 3-methoxy-17α-E-tributylstannylvinyl-1,3,5(10)-estratriene-17β-ol [18].[35]

Compound [15] (1.00 g, 3.22 mmol) was hydrostannylated as described in procedure B. Column chromatography on silica gel (200 g) with hexanes/ether 5:1 yielded 1.68 g (87%) of [18] as a colourless oil, $[\alpha]_D^{24}$ = +17.1° (c=1.2, 1,4-dioxane). Anal. calcd. for $C_{33}H_{54}O_2Sn$: C 65.90, H 9.05, O 5.32; found: C 65.83, H 9.00, O 5.28. Mass spectrum, m/z: 602(^{120}Sn: M^+, 0.2), 545(^{120}Sn: $M^+-C_4H_9$, 100).

Preparation of 3,17β-dimethoxy-17α-E-tributylstannylvinyl-1,3,5(10)-estratriene [19].

Compound [16] (1.00 g, 3.08 mmol) was hydrostannylated as described in procedure B. Column chromatography on silica gel (200 g) with hexanes/ether 20:1 afforded, in the first fraction, 0.23 g of [19] contaminated with a minute amount of unidentified byproduct, and in the second fraction, 1.56 g of pure [19]. Compound [19] was isolated in an overall yield of 94% as an oil, which after drying in high vacuum for 15-30 minutes, crystallized as a white solid. The material from the second fraction exhibited mp 50-52.5°, $[\alpha]_D^{24}$= +44.1° (c=1.2, $CHCl_3$). Anal. calcd. for $C_{34}H_{56}O_2Sn$: C 66.35, H 9.17, O 5.20; found: C 66.37, H 9.18, O 5.35. Mass spectrum, m/z: 616(^{120}Sn: M^+, 1), 601(^{120}Sn: M^+-CH_3, 5), 559(^{120}Sn: $M^+-C_4H_9$, 66).

IV.6 Labelling with Non-Radioactive Bromine

Bromination of vinyl-tin precursors; General Procedure.

To a solution of vinyl-tin substrate (0.6-0.9 mmol) dissolved in THF (100 mg of substrate per ml), 1.00 to 1.05 equivalents of sodium bromide in H_2O (0.5 M) was added. The mixture was cooled to 0° and shielded from light. Two equivalents of Chloramine-T in THF/H_2O 1:1 (0.67 M) were added, and then two equivalents of aqueous HCl solution (1M), which had been saturated with sodium chloride, were added in one portion to the mixture. After one minute of stirring at 0°, the mixture was poured into an aqueous sodium acetate solution (1M, 20 ml) and was extracted with ether (3x25 ml). The organic extracts were then washed with water (2x25 ml) and dried over anhydrous magnesium sulfate. The solvent was removed and the product was isolated by silica gel chromatography.

Preparation of (E)-8-C-bromo-7,8-dideoxy-1,2:3,4-di-O-isopropylidene-D-glycero-α-D-galacto-oct-7-enopyranose [5].[78]

Compound [4] (483 mg, 0.84 mmol) was brominated as described in the general procedure using 1.05 equivalents of sodium bromide. Column chromatography on silica gel (100 g) with CH_2Cl_2/hexanes/ether 6:2:1 yielded 291 mg (95%) of [5]. An analytical sample was prepared by recrystallization from ether-hexanes which gave powdery white crystals, mp 81-82°, $[\alpha]_D^{25}$ = -66.5° (c=1.1, $CHCl_3$). Anal. calcd. for $C_{14}H_{21}BrO_6$: C 46.04, H 5.80, Br

21.88; found: C 46.21, H 5.85, Br 21.73. Mass spectrum, m/z: 351(^{81}Br: M^+-CH_3, 3), 349(^{79}Br: M^+-CH_3, 3).

Preparation of (E)-8-C-bromo-7,8-dideoxy-1,2:3,4-di-O-isopropylidene-L-glycero-α-D-galacto-oct-7-enopyranose [9].

Compound [8] (375 mg, 0.65 mmol) was brominated as described in the general procedure using 1.05 equivalents of sodium bromide. Column chromatography on silica gel (100 g) with CH_2Cl_2/hexanes/ether 6:2:1 yielded 211 mg (89%) of [9]. After decolourization with charcoal, followed by recrystallization from ethanol-water, [9] was obtained as white crystals, mp 107-108°, $[\alpha]_D^{25}$ = -70.6° (c=1, $CHCl_3$). Anal. calcd. for $C_{14}H_{21}BrO_6$: C 46.04, H 5.80, Br 21.88; found: C 45.98, H 5.69, Br 21.79. Mass spectrum, m/z: 351(^{81}Br: M^+-CH_3, 4), 349(^{79}Br: M^+-CH_3, 4).

Preparation of β-E/Z-bromostyrene [13].

Compound [12] (102 mg, 260 μmol) was brominated, on small scale, as described in the general procedure using 0.90 equivalents of sodium bromide. Changes in the workup procedure were the use of 5 ml of aqueous sodium acetate (1M), 3 ml of ether for extraction (3x), and 3 ml of water for washing (2x). After solvent removal, the residue was dissolved in a known volume with THF. A standard solution of authentic [13] (Eastman; ratio of E to Z isomers of 87:13 by ^{1}H nmr) was prepared. The product mixture was analyzed by HPLC using the standard solution of [13] as an external standard. The analysis was performed on a C-18 column using methanol/water 75:25 as the eluant (flow rate 6.0 ml/min; detector set at 280 nm). The analysis indicated that 38.4 mg of [13] (retention time, 5.02 min; both isomers co-elute) was present, for a yield of 90% based on the sodium bromide added.

Preparation of 17α-E-bromovinyl-1,3,5(10)-estratriene-3,17β-diol [20]

Compound [17] (448 mg, 0.76 mmol) was brominated as described in the general procedure using 1.05 equivalents of sodium bromide. Column chromatography on silica gel (200 g) with CH_2Cl_2/hexanes/ether 6:4:1 yielded 32 mg of [20] in one portion, followed by 8 mg of a mixture of [20] and [26], in a ratio of 67:33, respectively. Compound [20] was obtained in an overall yield of 13%. An analytical sample was obtained by flash chromatography on silica gel with CH_2Cl_2/hexanes/ether 6:4:1, followed by decolourization with charcoal and recrystallization from benzene-hexanes. Compound [20] was obtained as white needles, mp 87-98°C (dec.), $[\alpha]_D^{25}$ = +11.3° (c=0.9, 1,4-dioxane). Anal. calcd. for $C_{20}H_{25}BrO_2 \cdot 0.5C_6H_6$: C 66.35, H 6.78; found: C 66.26, H 6.76. Mass spectrum, m/z: 378(^{81}Br: M^+, 0.1), 376(^{79}Br: M^+, 0.1); 360(^{81}Br: M^+-H_2O, 0.4), 358(^{79}Br: M^+-H_2O, 0.5), 297(M^+-Br, 0.7). Exact mass calcd. for $C_{20}H_{25}{}^{81}BrO_2$: 378.1018; found: 378.1017. Exact mass calcd. for $C_{20}H_{25}{}^{79}BrO_2$: 376.1038; found 376.1042.

In the course of isolating [20] during column chromatography, a number of other products were isolated and partially characterized (^{1}H nmr and mass spectrometry). The first fraction (83 mg) collected contained a mixture of 4-bromo-17α-E-tributylstannylvinyl-1,3,5(10)-estratriene-3,17β-diol [27] with some unidentified impurities, where [27] was dominant. Compound [27] exhibited the following ^{1}H nmr ($CDCl_3$)δ: 0.71-3.04(several m, 46H, 17-OH, 18-CH_3, -Sn(n-Bu)$_3$ and steroid nucleus), 5.61(s, 1H, 3-OH), 6.06(d, 1H, H-21, J=19.4 Hz), 6.20(d, 1H, H-20, J=19.4 Hz), 6.84(d, 1H, H-2, J=8.5 Hz), 7.17(d, 1H, H-1, J=8.5 Hz). In order to purify [27], flash chromatography on silica gel (2.0 cm × 15 cm) with CH_2Cl_2/hexanes/ether 6:6:1 was performed. However, the sample of [27] had undergone significant decomposition and no improvement in purity was gained. Nonetheless, an interesting by-product of the decomposition, a mixture of 4-bromo-17α-vinyl-1,3,5(10)-estratriene-3,17β-diol [28] and 4-chloro-17α- vinyl-1,3,5(10)-estratriene-3,17β-diol [29], was isolated. The ratio of [28] to [29] was approximated to be 6:4 by ^{1}H nmr. Mass spectroscopic analysis of the mixture gave the following results; mass spectrum of [28], m/z: 378(^{81}Br: M^+, 22), 376(^{79}Br: M^+, 22), 360(^{81}Br: M^+-H_2O, 14), 358(^{79}Br: M^+-H_2O, 16); mass spectrum of [29], m/z: 334(^{37}Cl: M^+, 18), 332(^{35}Cl: M^+, 45). Compound [28] exhibited the following ^{1}H nmr ($CDCl_3$)δ: 0.94(s, 3H, 18-CH_3), 1.21-2.33 (several m, 14H, 17-OH and steroid nucleus), 2.63-3.04(m, 2H, methylene protons), 5.15(d, 1H, H-21a, J_{20-21a}=10.8 Hz), 5.20(d, 1H, H-21b, J_{20-21b} = 17.3 Hz), 5.61(br s, 1H, 3-OH), 6.10(dd, 1H, H-20, J_{20-21a}=10.8 Hz, J_{20-21b}=17.3 Hz), 6.84(d, 1H, H-2, J=8.5 Hz), 7.16(d, 1H, H-1, J=8.5 Hz). Compound [29] exhibited the following ^{1}H nmr ($CDCl_3$)δ: 0.94(s, 3H, 18-CH_3), 1.21-2.33 (several m, 14H, 17-OH and steroid nucleus), 2.63-3.04(m, 2H, methylene protons), 5.15(d, 1H, H-21a, J_{20-21a}=10.8 Hz), 5.20(d, 1H, H-21b, J_{20-21b}=17.3 Hz), 5.61(br s, 1H, 3-OH), 6.10(dd, 1H, H-20, J_{20-21a}= 10.8 Hz, J_{20-21b}=17.3 Hz), 6.84(d, 1H, H-2, J=8.5 Hz), 7.12(d, 1H, H-1, J=8.5 Hz).

The second fraction (18 mg) eluted contained a mixture of 2,4-dibromo-17α-E-bromovinyl-1,3,5(10)-estratriene-3,17β-diol [30] with small amounts of unidentified impurities. Compound [30] exhibited the following ^{1}H nmr ($CDCl_3$)δ: 0.91 (s, 3H, 18-CH_3), 1.07-2.37 (several m, 14H, 17-OH and steroid nucleus), 2.55-2.98 (m, 2H, methylene protons), 5.84(br s, 1H, 3-OH), 6.31 (d, 1H, H-21, J=13.5 Hz), 6.44(d, 1H, H-20, J=13.5 Hz), 7.38(s, 1H, H-1). Unfortunately, [30] decomposed before further purification could be effected.

After continued elution, a third fraction (43 mg) was collected which contained a mixture of three compounds, 4-bromo-17α-E-bromovinyl-1,3,5(10)-estratriene-3,17β-diol [31], 4-chloro-17α-E-bromovinyl-1,3,5(10)-estratriene-3,17β-diol [32] and 4-bromo-17α-E-chlorovinyl-1,3,5(10)-estratriene-3,17β-diol [33]. Small amounts of unidentified impurities were also present. The ratio of [31] to [32] to [33] was approximated to be 51:34:15, respectively, by ^{1}H nmr. To separate the mixture, column chromatography was performed on silica gel (10 g) using hexanes/ether 1:1. However, little separation was achieved as the mixture of compounds co-eluted. The ratio of [31] to [32] to [33] was found to be approximately 62:26:12, respectively, by ^{1}H nmr. Mass spectroscopic analysis of the mixture gave the following results; mass spectrum of [31], m/z: 458(^{81}Br, ^{81}Br: M^+, 2), 456(^{81}Br, ^{79}Br: M^+, 4), 454(^{79}Br, ^{79}Br: M^+, 2), 440(^{81}Br, ^{81}Br: M^+-H_2O, 52), 438(^{81}Br, ^{79}Br: M^+-H_2O, 100), 436(^{79}Br, ^{79}Br: M^+-H_2O, 55); mass spectrum of isomeric mixture of [32] and [33], m/z:

414(^{81}Br, ^{37}Cl: M^+, 0.98), 412(^{79}Br, ^{37}Cl: M^+, 3.7), 410(^{79}Br, ^{35}Cl: M^+, 3.9), 398(^{81}Br, ^{37}Cl: M^+-H_2O, 25), 396(^{79}Br, ^{37}Cl: M^+-H_2O, 88), 394(^{79}Br, ^{35}Cl: M^+-H_2O, 73). Compound [31] exhibited the following ^{1}H nmr ($CDCl_3$)δ: 0.92(s, 3H, 18-CH_3), 1.19-2.42 (several m, 14H, 17-OH and steroid nucleus), 2.59-3.02(m, 2H, methylene protons), 5.59(br s, 1H, 3-OH), 6.30(d, 1H, H-21, J=13.5 Hz), 6.46(d, 1H, H-20, J-13.5 Hz), 6.85(d, 1H, H-2, J=8.6 Hz), 7.16(d, 1H, H-1, J=8.6 Hz). Compound [32] exhibited the following ^{1}H nmr ($CDCl_3$)δ: 0.92(s, 3H, 18-CH_3), 1.19-2.42 (several m, 14H, 17-OH and steroid nucleus), 2.59-3.02(m, 2H, methylene protons), 5.59(br s, 1H, 3-OH), 6.30(d, 1H, H-21, J=13.5 Hz), 6.46(d, 1H, H-20, J=13.5 Hz), 6.85(d, 1H, H-2, H=8.6 Hz), 7.11(d, 1H, H-1, J=8.6 Hz). Compound [33] exhibited the following ^{1}H nmr ($CDCl_3$)δ: 0.92(s, 3H, 18-CH_3), 1.19-2.42 (several m, 14H, 17-OH and steroid nucleus), 2.59-3.02(m, 2H, methylene protons), 5.59(br s, 1H, 3-OH), 6.18(d, 1H, H-20, J=12.8 Hz), 6.21(d, 1H, H-21, J=12.8 Hz), 6.85(d, 1H, H-2, J=8.6 Hz), 7.16(d, 1H, H-1, J=8.6 Hz).

The fourth fraction (25 mg) collected contained a mixture of [28] and [29], but was highly contaminated with unidentified impurities. The ^{1}H nmr spectral properties of [28] and [29] were the same as those observed previously. Lastly, the desired product [20] was isolated as described earlier.

Preparation of 3-methoxy-17α-E-bromovinyl-1,3,5(10)-estratriene-17β-ol [22].

Compound [18] (539 mg, 0.90 mmol) was brominated as outlined in the general procedure using 1.00 equivalent of sodium bromide. Flash chromatography on silica gel (3.5 cm × 15 cm) with hexanes/ether 2:1 yielded 351 mg (100%) of [22]. After crystallization from ethanol-water, [22] was obtained as white crystals, mp 107.5-109°, $[\alpha]_D^{25}$ = +14.5°(c=0.9, 1,4-dioxane). Anal. calcd. for $C_{21}H_{27}BrO_2$: C 64.45, H 6.95, Br 20.42; found: C 64.11, H 7.17, Br. 20.25. Mass spectrum, m/z: 392(^{81}Br: M^+, 10), 390(^{79}Br: M^+, 10), 374(^{81}Br; M^+-H_2O, 99), 372(^{79}Br: M^+-H_2O, 99), 310(M^+-HBr, 100).

Preparation of 3,17β-dimethoxy-17α-E-bromovinyl-1,3,5(10)-estratriene [24].

Compound [19] (507 mg, 0.82 mmol) was brominated as described in the general procedure using 1.02 equivalents of sodium bromide. Column chromatography on silica gel (200 g) with hexanes/ether 24:1 yielded 300 mg (90%) of [24]. After decolourization with charcoal, followed by recrystallization from ethanol, [24] was obtained as white needles, mp 104.5-105°, $[\alpha]_D^{24}$ = +58.2° (c=1, $CHCl_3$). Anal. calcd. for $C_{22}H_{29}BrO_2$: C 65.18, H 7.21, Br 19.71; found: C 65.24, H 7.10, Br 19.55. Mass spectrum, m/z: 406(^{81}Br: M^+, 3), 404(^{79}Br: M^+, 3), 325(M^+-Br, 100).

IV.7 Labelling with Non-radioactive Iodine

Iodination of vinyl-tin precursors; General Procedure

To a solution of vinyl-tin substrate (0.7-0.9 mmol) in THF (100 mg of substrate per ml), shielded from light, was added 1.05 equivalents of sodium iodide in H_2O (1M). Two equivalents of Chloramine-T in THF/H_2O 1:1 (0.67 M) were added, and then three equivalents of aqueous HCl solution (1M), which had been saturated with sodium chloride, were added in one portion to the mixture. The mixture was stirred for one minute at room temperature, then quenched with excess aqueous sodium thiosulfate (1M). The mixture was poured into an aqueous sodium acetate solution (1M, 20 ml) and was extracted with ether (3×25 ml). The organic extracts were then washed with water (2×25 ml) and dried over anhydrous magnesium sulfate. The solvent was removed and the product was isolated by silica gel chromatography.

Preparation of (E)-8-C-iodo-7,8-dideoxy-1,2:3,4-di-O-isopropylidene-D-glycero-α-D-galacto-oct-7-enopyranose [6].

Compound [4] (416 mg, 0.72 mmol) was iodinated as described in the general procedure. Column chromatography on silica gel (100 g) with CH_2Cl_2/hexanes/ether 6:2:1 yielded 273 mg (92%) of [6]. After decolourization with charcoal, followed by recrystallization from ethanol-water, [6] was obtained as white crystals, mp 80-81.5°, $[\alpha]_D^{24}$ = -65.6° (c=1, $CHCl_3$). Anal. calcd. for $C_{14}H_{21}IO_6$: C 40.79, H 5.13, I 30.79; found: C 40.80, H 5.17, I 30.66. Mass spectrum, m/z: 412(M^+, 0.1), 397(M^+-CH_3, 11), 339(3).

Preparation of (E)-8-C-iodo-7,8-dideoxy-1,2:3,4-di-O-isopropylidene-L-glycero-α-D-galacto-oct-7-enopyranose [10].

Compound [8] (402 mg, 0.70 mmol) was iodinated as described in the general procedure. Column chromatography on silica gel (100 g) with CH_2Cl_2/hexanes/ether 6:2:1 yielded 278 mg (97%) of [10]. After decolourization with charcoal, followed by recrystallization from ethanol-water, [10] was obtained as white crystals, mp 119-119.5°, $[\alpha]_D^{25}$ = -64.4° (c=1, $CHCl_3$). Anal. calcd. for $C_{14}H_{21}IO_6$: C 40.79, H 5.13, I 30.79; found: C 40.70, H 5.20, I 30.69. Mass spectrum, m/z: 397(M^+-CH_3, 5), 339(4).

Preparation of 17α-E-iodovinyl-1,3,5(10)-estratriene-3,17β-diol [21][43,62] using hydrogen peroxide/acetic acid as the oxidizing agent.[35]

To a solution of [17] (219 mg, 0.37 mmol) in THF (2.0 ml), shielded from light, was added sodium iodide (59 mg, 0.39 mmol) in water (0.37 ml), a 5% (weight/volume) solution of sodium acetate in glacial acetic acid (0.60 ml) and a solution of 30% hydrogen peroxide in glacial acetic acid (2:1, volume/volume; 0.60 ml). The mixture was stirred for 30 minutes at room temperature. The reaction was terminated with the addition of aqueous sodium thiosulfate (3.6 ml, 0.95 M) and was poured into water (20 ml), which was extracted with ether (3×10 ml). The organic extracts were washed with aqueous saturated sodium bicarbonate (2×15 ml), followed by water (2×15 ml) and finally dried over anhydrous magnesium sulfate. After removal of the solvent, column chromatography on silica gel (50 g) with CH_2Cl_2/hexanes/ether 6:4:1 yielded 121 mg of [21] in one fraction, followed by 51 mg of a mixture of [21] and some unidentified byproducts. The yield of pure [21] was 77%. After decolourization with charcoal, followed by recrystallization (twice) from benzene-hexanes, [21] was obtained as white needles, mp 88-97° (dec.) {lit.[43] mp 93-100° (dec.)}, $[\alpha]_D^{25}$ = -3.8° (c=1, 1,4-dioxane). Anal. calcd. for $C_{20}H_{25}IO_2 \cdot C_6H_6$: C 62.15, H 6.22; found: C 61.81, H 6.35. Mass spectrum, m/z: 424(M^+, 2), 406(M^+-H_2O, 2), 297(M^+-I, 2). Exact mass calcd. for $C_{20}H_{25}IO_2$: 424.0901; found: 424.0902.

In order to compare directly the chemical yield of the above reaction to that of using Chloramine-T as the oxidant (see following preparation of [21]), the above reaction was repeated on a smaller scale using HPLC analysis to quantify the yield of product. To a solution of [17] (61.5 mg, 105 μmol) in THF (0.6 ml), shielded from light, was added sodium iodide (15.6 mg, 104 μmol) in water (0.11 ml), a 5% (weight/volume) solution of sodium acetate in glacial acetic acid (0.15 ml) and a solution of 30% hydrogen peroxide in glacial acetic acid (2:1 volume/volume; 0.15 ml). The mixture was stirred for 30 minutes, at room temperature, and was stopped with the addition of aqueous sodium thiosulfate (0.90 ml, 0.95 M). The reaction mixture was poured into water (5 ml) and extracted with ether (3×3 ml). The organic extracts were washed with aqueous saturated sodium bicarbonate (2×3 ml), then with water (2×3 ml) and were dried over anhydrous magnesium sulfate. After solvent removal, the residue was dissolved in a known volume with THF. A standard solution of authentic [21] (synthesized previously) was prepared. The product mixture was analyzed by HPLC using the standard solution of [21] as an external standard. The analysis was performed on a C-18 column using the following gradient solvent program: from 0 to 10 min , an isocratic mixture of $MeOH/H_2O$ 75:25 was used, followed by a systematic increase to 100% MeOH during 10 to 13 min, and a constant composition of 100% MeOH was maintained from 13 to 20 min. A constant flow rate of 6.0 ml per minute was used and the detector was set at 280 nm. The analysis indicated that 41.2 mg of [21] (retention time, 6.78 min) was present, for a yield of 93%.

Preparation of 17α-E-iodovinyl-1,3,5(10)-estratriene-3,17β-diol [21][43,62] using Chloramine-T as the oxidizing agent.

Compound [17] (104 mg, 176 μmol) was iodinated, on small scale, as described in the general procedure using 1.00 equivalent of sodium iodide and 2 equivalents of aqueous HCl solution. Changes in the workup procedure were the use of 10 ml of aqueous sodium acetate (1M), 6 ml of ether for extraction (3x), and 6 ml of water for washing (2x). After solvent removal, the residue was stored for two months. The reaction mixture was then chromatographed on silica gel (35 g) with CH_2Cl_2/hexanes/ether 6:6:1. The reaction mixture contained several products according to tlc analysis, but only the compounds present in significant amounts were collected. The first fraction contained a mixture in which 2-iodo-17α-E-iodovinyl-1,3,5(10)-estratriene-3,17β-diol [34] was dominant. This mixture was further purified by flash chromatography on silica gel (2 cm × 15 cm) with CH_2Cl_2/hexanes/ether 6:4:1. A mixture (4.1 mg) of [34] with 3 minor, unidentified side-products was obtained and ^1H nmr analysis indicated the ratio of [34] to the three side-products was 67:33. This material exhibited the following properties; mass spectrum, m/z: 550(M^+, 100), 532(M^+-H_2O, 12), 423(M^+-I, 43); ^1H nmr ($CDCl_3$)δ:

0.92(s, 3H, 18-CH_3), 1.25-2.27 (several m, 14H, 17-OH and steroid nucleus), 2.79(m, 2H, methylene protons), 5.12(s, 1H, 3-OH), 6.30(d, 1H, H-21, J=14.4 Hz), 6.72(s, 1H, H-4), 6.77(d, 1H, H-20, J=14.4 Hz), 7.49(s, 1H, H-1).

The second fraction contained a mixture of 4-iodo-17α-E-iodovinyl-1,3,5(10)-estratriene-3,17β-diol [35] and 2-iodo-17α-vinyl-1,3,5(10)-estratriene-3,17β-diol [36]. This mixture was further purified by flash chromatography on silica gel (2 cm × 15 cm) with CH_2Cl_2/hexanes/ether 6:4:1. A mixture (12.5 mg) of [35] and [36] was obtained in a ratio of 12:88, respectively, by ^1H nmr analysis. Mass spectroscopic analysis of the mixture gave the following results; mass spectrum of [35], m/z: 550 (M^+, 3); mass spectrum of [36], m/z: 424(M^+, 100), 406(M^+-H_2O, 54). Compound [35] exhibited the following ^1H nmr ($CDCl_3$)δ: 0.91(s, 3H, 18-CH_3), 1.25-2.23(several m, 14H, 17-OH and steroid nucleus), 2.79(m, 2H, methylene protons), 3-OH not observed, 6.30(d, 1H, H-21, J=14.4 Hz), 6.78(d, 1H, H-20, J-14.4 Hz), 6.83(d, 1H, H-2, J=8.6 Hz), 7.18(d, 1H, H-1, J=8.6 Hz). Compound [36] exhibited the following ^1H nmr ($CDCl_3$)δ: 0.94(s, 3H, 18-CH_3), 1.25-2.23(several m, 14H, 17-OH and steroid nucleus), 2.79(m, 2H, methylene protons), 3-OH not observed, 5.15(d, 1H, H-21a, J_{20-21a}=10.8 Hz), 5.20(d, 1H, H-21b, J_{20-21b}=17.3 Hz), 6.09(dd, 1H, H-20, J_{20-21a}=10.8 Hz, J_{20-21b}=17.3 Hz), 6.71(s, 1H, H-4), 7.50(s, 1H, H-1).

The third fraction contained an isomeric mixture of [36] and 4-iodo-17α-vinyl-1,3,5(10)-estratriene-3,17β-diol [37]. This mixture was further purified by flash chromatography on silica gel (2 cm × 15 cm) with CH_2Cl_2/hexanes/ether 6:4:1. A mixture (6.6 mg) of [36] and [37] was obtained in a ratio of 8:92, respectively, by ^1H nmr analysis. Compound [37] exhibited the following properties; mass spectrum, m/z: 424(M^+, 52), 406(M^+-H_2O, 31); ^1H

nmr ($CDCl_3$)δ: 0.93(s, 3H, 18-CH_3), 1.24-2.33(several m, 14H, 17-OH and steroid nucleus), 2.61-2.88(m, 2H, methylene protons), 5.15(dd, 1H, H-21a, $J_{20-21}a$=10.8 Hz, $J_{20-21}b$=<1Hz), 5.20(dd, 1H, H-21b, $J_{20-21}b$=17.3 Hz, $J_{20-21}b$=<1 Hz), 5.42(br s, 1H, 3-OH), 6.10(dd, 1H, H-20, $J_{20-21}a$=10.8 Hz, $J_{20-21}b$=17.3 Hz), 6.83(d, 1H, H-2, J=8.5 Hz), 7.19(d, 1H, H-1, J=8.5 Hz). Compound [36] exhibited the same ^1H nmr spectrum as obtained earlier.

The final fraction obtained contained the desired product [21] which exhibited the same spectral (^1H nmr) properties as previously reported.

In order to determine the chemical yield of [21] produced in the above reaction, and to compare this value with that obtained using hydrogen peroxide as the oxidant, the above reaction was repeated on a smaller scale using HPLC analysis to quantify the yield. Compound [17] (54.9 mg, 93 μmol) was iodinated, on small scale, as described in the general procedure using 1.00 equivalent of sodium iodide. Changes in the workup procedure were the use of 5 ml of aqueous sodium acetate (1M), 3 ml of ether for extraction (3x), and 3 ml of water for washing (2x). After solvent removal, the residue was dissolved in a known volume with THF. A standard solution of authentic [21] (synthesized previously) was prepared. The product mixture was analyzed by HPLC using the standard solution of [21] as an external standard. The analysis was performed using the same HPLC conditions as described in the previous analysis of [21]. The analysis indicated that 0.76 mg of [21] (retention time, 6.81 min) was present, for a yield of 1.9%.

Preparation of 3-methoxy-17α-E-iodvinyl-1,3,5(10)-estratriene-17β-ol [23].[62]

Compound [18] (472 mg, 0.79 mmol) was iodinated as described in the general procedure. Flash chromatography on silica gel (3.5 cm × 15 cm) with hexanes/ether 2:1 yielded 304 mg (88%) of [23]. After decolourization with charcoal, followed by recrystallization from ethanol-water, [23] was obtained as white crystals, mp 85-91° (dec.), $[\alpha]_D^{25}$ = -2.7° (c=1, 1,4-dioxane). Anal. calcd. for $C_{21}H_{27}IO_2$: C 57.54, H 6.21, I 28.95; found: C 57.68, H 6.15, I 28.90. Mass spectrum, m/z: 438(M^+, 34), 420(M^+-H_2O, 82), 311(M^+-I, 22).

Preparation of 3,17β-dimethoxy-17α-E-iodovinyl-1,3,5(10)-estratriene [25].

Compound [19] (519 mg, 0.84 mmol) was iodinated as outlined in the general procedure except 1.10 equivalents of sodium iodide and 2.20 equivalents of Chloramine-T were used instead. Column chromatography on silica gel (100 g) with hexanes/ether 20:1 yielded 379 mg (99%) of [25]. After decolourization with charcoal, followed by recrystallization from ethanol, [25] was obtained as white needles, mp 65-75° (dec.), $[\alpha]_D^{25}$ = +47.7° (c=1, $CHCl_3$). Anal. calcd. for $C_{22}H_{29}IO_2$: C 58.41, H 6.46, I 28.05; found: C 58.54, H 6.60, I 27.91. Mass spectrum, m/z: 452(M^+, 3), 325(M^+-I, 76).

IV.8 Synthesis of Chromatographic Standards for Radio-labelling

Preparation of 3-methoxy-17α-E-chlorovinyl-1,3,5(10)-estratriene-17β- [38].

To a solution of [18] (206 mg, 0.34 mmol) dissolved in THF (2.0 mmol), shielded from light, was added ethanol (5.0 ml), Chloramine-T (193 mg, 0.69 mmol) in THF/H_2O 1:1 (1.0 ml) and aqueous HCl solution (0.69 ml, 1M), which had been saturated with sodium chloride. The mixture was stirred for 2 hours at room temperature. An excess of aqueous sodium thiosulfate (0.80 ml, 0.95 M) was added to terminate the reaction. The mixture was poured into an aqueous sodium acetate solution (10 ml, 1M) and was extracted with ether (3×10 ml). The organic extracts were then washed with water (2×10 ml) and dried over anhydrous magnesium sulfate. The solvent was removed and [38] was isolated using flash chromatography on silica gel (2.0 cm × 15 cm) with hexanes/ether 4:1 which afforded 81 mg (69%) of [38]. An analytical sample was prepared by column chromatography on silica gel with hexanes/ether 4:1, followed by crystallization from ethanol-water which gave [38] as white needles, mp 47.5-49.5°, $[\alpha]_D^{25}$= +26.0° (c=0.9, 1,4-dioxane). Anal. calcd. for $C_{21}H_{27}ClO_2$: C 72.71, H 7.84, found: C 72.52, H 7.73. Mass spectrum, m/z: 348(^{37}Cl: M^+, 9), 346(^{35}Cl: M^+, 24), 330(^{37}Cl: M^+-H_2O, 20), 328(^{35}Cl: M^+-H_2O, 58), 310(M^+-HCl, 42). Exact mass calcd. for $C_{21}H_{27}{}^{37}ClO_2$: 348.1671; found: 348.1674. Exact mass calcd. for $C_{21}H_{27}{}^{35}ClO_2$: 346.1701; found: 346.1701.

Preparation of 3-methoxy-17α-vinyl-1,3,5(10)-estratriene-17β-ol [39].[67]

This procedure is a modification of the one used in the preparation of [15] and [16]. Powdered potassium hydroxide (0.30 g, 5.4 mmol) was added to DMSO (3 ml) and was stirred for 5 minutes, at room temperature. A mixture of [14] and [26] (200 mg, 0.67 mmol), which was isolated previously (ratio of [14] to [26] was 18:82 by HPLC analysis), was added, followed immediately by methyl iodide (380 mg, 2.68 mmol). Stirring was continued for 60 minutes, then the mixture was poured into water (30 ml) and was extracted with dichloromethane (3×30 ml). The organic extracts were washed with water (3×30 ml), dried over anhydrous magnesium sulfate and the solvent was removed. Flash chromatography of the crude mixture, on silica gel (2 cm × 15 cm) with hexanes/ether 4:1, afforded 90 mg of a mixture of [16] and 3,17β-dimethoxy-17α-vinyl-1,3,5(10)-estratriene [40]. The ratio of [16] (retention time, 2.54 min) to [40] (retention time, 3.10 min) was determined to be 35:65 by HPLC analysis on a C-18 column using methanol as the eluant (flow rate 6.0 ml/min; detector set at 280 nm). This mixture was characterized by ^1H nmr and mass spectrometry. No attempt was made to isolate [40] in a pure form. Mass spectroscopic analysis of the mixture gave the following results; [16] exhibited the same spectral properties as reported earlier; mass spectrum for [40], m/z: 326(M^+, 44).

Further elution with hexanes/ether 1:1 yielded 90 mg of [39], in one portion, for a yield of 52%, based on the initial amount of [26] used. HPLC analysis showed that [39] (retention time, 5.52 min) was the only compound present, with no trace of [15] observed. The HPLC analysis was performed on a

C-18 column with methanol/water 85:15 (flow rate 6.0 ml/min; detector set at 280 nm). After recrystallization from ethanol-water, [39] was obtained as white crystals, mp 110.5°, $[\alpha]_D^{25}$= +56.9° (c=1, 1,4-dioxane). Anal. calcd. for $C_{21}H_{28}O_2$: C 80.73, H 9.03; found: C 80.77, H 9.09. Mass spectrum, m/z: 312(M^+, 100), 294(M^+-H_2O, 8). Exact mass calcd. for $C_{21}H_{28}O_2$: 312.2090; found: 312.2090.

IV.9 Labelling with Radioactive Bromine

General procedures for radiobromination

Procedure A: To a stirred solution of [18] (3.7 mg, 6.2 µmol) in THF (40 µl), shielded from light, was added ethanol (100 µl), $NH_4{}^{82}Br$ (20 µl aqueous stock solution) and 14-18 µl of aqueous HCl solution (1 M) which had been saturated with sodium chloride. Chloramine-T (3.8 mg in 20 µl, THF/H_2O 1:1) was added and the mixture was stirred at either 0° or room temperature for one minute. The reaction was quenched with aqueous sodium thiosulfate (100 µl, 0.1 M).

Procedure B: To a stirred solution of [18] (3.7 mg, 6.2 µmol) in THF (40 µl), shielded from light, was added $NH_4{}^{82}Br$ (20 µl aqueous stock solution). N-Chlorosuccinimide (2.3 mg in 60 µl, THF/MeOH 1:1) was added and the mixture was stirred for 30 minutes at room temperature. The reaction was quenched with aqueous sodium thiosulfate (120 µl, 0.1 M).

General Workup: For both procedures, A and B, the workup and isolation of the radio-labelled product was performed in the same manner. The reaction mixture was withdrawn with a syringe from the reaction vessel. Then the vessel was rinsed with a solvent mixture of THF/H_2O 1:1 (100 µl) three times; each time the solvent rinse was withdrawn into the syringe containing the reaction mixture. The contents of the syringe were injected, in one portion, onto a reverse-phase HPLC column. Elution with the following gradient solvent program afforded the desired radio-labelled product: from 0 to 10 min, an isocratic mixture of MeOH/H_2O 85:15 was used, followed by a systematic increase to 100% MeOH during 10 to 12 min, and a constant composition of 100% MeOH was maintained from 12 to 20 min. A flow rate of 6.0 ml per minute was used and the detector was set at 254 nm. The collected radio-labelled product was immediately assayed to determine the radiochemical yield of the reaction.

Preparation of 3-methoxy-17α-E-(^{82}Br)bromovinyl-1,3,5(10)-estratriene-17β-ol [41].

Method A: Compound [18] was radiobrominated as described in procedure A using 131 µCi of $NH_4{}^{82}Br$ and 14 µl of aqueous HCl solution. The reaction was performed at room temperature. HPLC isolation obtained 105 µCi of [41] (retention time, 7.4 min) for a radiochemical yield of 81%. This material has the same retention time as an authentic cold sample of [22].

Method B: Compound [18] was radiobrominated as outlined in procedure B using 125 µCi of $NH_4{}^{82}Br$. HPLC isolation obtained 106 µCi of [41] for a radiochemical yield of 85%. This material has the same retention time as an authentic cold sample of [22].

IV.10 Labelling with Radioactive Iodine

General procedures for radioiodination.

Procedure A: To a stirred solution of [18] (2 mg, 3.3 μmol) in THF (20 μl), shielded from light, was added ethanol (50 μl), and in the case of carrier added (CA) syntheses, aqueous non-radioactive sodium iodide (5 μl, 0.33 M). This was followed by the addition of 10 μl of aqueous stock solution of either $Na^{131}I$, $Na^{123}I$ or $NH_4{}^{123}I$, then of 5-8 μl of aqueous HCl solution (1 M), which had been saturated with sodium chloride. Chloramine-T (10 μl, 0.67 M in THF/H_2O 1:1) was added and the mixture was stirred for one minute at room temperature. The reaction was quenched with aqueous sodium thiosulfate (63 μl, 0.1 M).

Procedure B: To a stirred solution of [18] (2 mg, 3.3 μmol) in THF (20 μl), shielded from light, was added aqueous non-radioactive sodium iodide (5 μl, 0.33 M), in the case of CA syntheses, and $Na^{123}I$ (10 μl aqueous stock solution). N-Chlorosuccinimide (0.7 mg in 20 μl, THF/MeOH 1:1) was added and the mixture was stirred for 30 minutes at room temperature. The reaction was quenched with aqueous sodium thiosulfate (63 μl, 0.1 M).

Procedure C:[35] To a stirred solution of [18] (2 mg, 3.3 μmol) in THF (20 μl), shielded from light, was added aqueous non-radioactive sodium iodide (5 μl, 0.33 M), in the case of CA syntheses, and $Na^{123}I$ (10 μl aqueous stock solution). To this was added 50 μl of a 5% (weight/volume) solution of sodium acetate in glacial acetic acid and 50 μl of a solution of 30% hydrogen peroxide in glacial acetic acid (2:1, volume/volume). The mixture was stirred for 30 minutes at room temperature and the reaction was stopped with the addition of aqueous sodium thiosulfate (63 μl, 0.1 M).

General Workup: For each procedure, the workup and isolation of the radio-labelled product was conducted in the same fashion. The contents of the reaction vessel were withdrawn with a syringe, after which, the vessel was rinsed with a solvent mixture of THF/H_2O 1:1 (150 μl) [for procedure C, $EtOH/H_2O$ 3:1 (100 μl)] three times; each time the solvent rinse was withdrawn into the syringe containing the reaction mixture. The contents of the syringe were injected, in one portion, onto a reverse-phase HPLC column. Elution of the radio-labelled product was effected using the same gradient solvent program as previously described in the radiobromination procedures. The collected radio-labelled product was then immediately assayed to determine the amount of radioactivity present.

Preparation of 3-methoxy-17α-E-(^{123}I)iodovinyl-1,3,5(10)-estratriene-17β-ol [42]

Method A: Compound [18] (2.2 mg, 3.7 μmol) was radioiodinated as described in procedure A using 256 μCi of $NH_4{}^{123}I$, 6 μl of aqueous HCl solution and 1.9 mg (6.7 μmol) of Chloramine-T. HPLC isolation afforded 221 μCi of [42] (retention time, 8.1 min) for a radiochemical yield of 86%. This material has the same retention time as an authentic cold sample of [23].

Method B: Compound [18] (2.1 mg, 3.5 μmol) was radioiodinated as outlined in procedure B using 74.5 μCi of $Na^{123}I$. HPLC isolation yielded 46.2 μCi of [42] for a radiochemical yield of 62%. This material has the same retention time as an authentic cold sample of [23].

Method C: Compound [18] (2.1 mg, 3.5 μmol) was radiobrominated as described in procedure C using 62.7 μCi of $Na^{123}I$. HPLC isolation obtained 53.5 μCi of [42] for a radiochemical yield of 85%. This material has the same retention time as an authentic cold sample of [23].

Preparation of 3-methoxy-17α-E-(^{131}I)iodovinyl-1,3,5(10)-estratriene-17β-ol [43]

Compound [18] (2.2 mg, 3.7 μmol) was radioiodinated as described in procedure A using 124 μCi of $Na^{131}I$, 7 μl of aqueous HCl solution and 1.9 mg (6.7 μmol) of Chloramine-T. HPLC isolation afforded 105 μCi of [43] (retention time, 8.1 min) for a radiochemical yield of 85%. This material has the same retention time as an authentic cold sample of [23].

IV.11 Nmr Characterization Data

TABLE IV-1. Chemical Shifts (ppm) and Multiplet Splittings (Hz) for the Carbohydrate Derivatives

Compound	H-1	H-2	H-3	H-4	H-5	H-6	H-7	H-8	6-OH	Others
[4][a,g]	5.59	4.32	4.61	4.45	3.68	4.34	6.18	6.38	2.81	1.21-1.55
	(d)	(dd)	(dd)	(dd)	(dd)	(m)	(dd)	(dd)	(d)	(2m, 24H)[c]
	J_{1-2} 5.0	J_{2-3} 2.3	J_{3-4} 8.0	J_{4-5} 1.7	J_{5-6} 6.5	J_{6-7} 4.4	J_{7-8} 19.2	J_{6-8} 1.4	J_{6-OH} 7.0	0.81-0.97
							J_{Sn-H} 64	J_{Sn-H} 70		(m, 15H)[d]
[5][a]	5.57	4.33	4.64	4.44	3.67	~4.35	6.38	6.50	2.82	1.52
	(d)	(dd)	(dd)	(dd)	(dd)	(m)	(dd)	(dd)	(d)	1.48
	J_{1-2} 5.1	J_{2-3} 2.4	J_{3-4} 8.0	J_{4-5} 1.8	J_{5-6} 6.9	J_{6-7} 5.1	J_{7-8} 13.7	J_{6-8} 1.0	J_{6-OH} 6.6	1.36
										1.34
										(4s, ea. 3H)[e]
[6][a]	5.56	4.33	4.63	4.43	3.66	~4.31	6.73	6.53	2.92	1.52
	(d)	(dd)	(dd)	(dd)	(dd)	(m)	(dd)	(dd)	(d)	1.48
	J_{1-2} 5.0	J_{2-3} 2.4	J_{3-4} 7.9	J_{4-5} 1.9	J_{5-6} 6.9	J_{6-7} 5.1	J_{7-8} 14.5	J_{6-8} 1.4	J_{6-OH} 6.6	1.36
										1.33
										(4s, ea. 3H)[e]

TABLE IV-1. Continued

Compound	H-1	H-2	H-3	H-4	H-5	H-6	H-7	H-8	6-OH	Others
[8][b,g]	5.50 (d)	4.18 (dd)	4.50 (dd)	4.30 (dd)	3.82 (dd)	4.73 (m)	6.48 (dd)	6.86 (dd)	3.07 (s)	1.24-1.71 (2m, 18H)[f]
	J_{1-2} 4.9	J_{2-3} 2.3	J_{3-4} 7.9	J_{4-5} 1.5	J_{5-6} 7.6	J_{6-7} 5.1	J_{7-8} 19.2	J_{6-8} 1.0		1.17
							J_{Sn-H} 65	J_{Sn-H} 76		1.04
										(2s, ea. 3H)[e]
										0.86-1.01 (m, 15 H)[d]
[9][b]	5.39 (d)	4.09 (dd)	4.29 (dd)	3.68 (dd)	3.56 (dd)	4.40 (m)	6.40 (dd)	6.63 (dd)	2.77 (br s)	1.36
										1.33
	J_{1-2} 5.0	J_{2-3} 2.3	J_{3-4} 7.9	J_{4-5} 1.7	J_{5-6} 8.0	J_{6-7} 5.2	J_{7-8} 13.5	J_{6-8} 1.4		1.03
										1.01
										(4s, ea. 3H)[e]
[10][b]	5.39 (d)	4.10 (dd)	4.30 (dd)	3.71 (dd)	3.56 (dd)	4.38 (m)	6.76 (dd)	6.68 (d)	2.83 (br s)	1.36
										1.33
	J_{1-2} 4.9	J_{2-3} 2.3	J_{3-4} 7.9	J_{4-5} 1.7	J_{5-6} 7.9	J_{6-7} 4.4	J_{7-8} 14.5			1.03
										1.01
										(4s, ea. 3H)[e]

[a] Deuteriochloroform solution, [b] Deuteriobenzene solution
[c] 2 x $C(CH_3)_2$ and $-Sn(CH_2\underline{CH_2}\underline{CH_2}CH_3)_3$, [d] $-Sn(\underline{CH_2}CH_2CH_2\underline{CH_3})_3$, [e] $-\underline{CH_3}$ from $C(CH_3)_2$
[f] 1 x $C(CH_3)_2$ and $-Sn(CH_2\underline{CH_2}\underline{CH_2}CH_3)_3$, [g] J_{Sn-H} values are an average of the ^{117}Sn and ^{119}Sn values.

TABLE IV-2. Chemical Shifts (ppm) and Multiplet Splittings (Hz) for the Steroidal Derivatives

Compound	H-1	H-2	H-4	H-20	H-21	3-OH	3-OCH_3	17-OCH_3	18-CH_3	Others
[17][a,d]	7.13 (d) J_{1-2} 8.8	6.62 (dd) J_{2-4} 2.9	6.57 (d)	6.20 (d) J_{20-21} 19.4 J_{Sn-H} 66	6.07 (d) J_{Sn-H} 70	5.30 (s)	-	-	over- lapped	2.80 (m, 2H)[e] 1.20-2.37 (several m, 26H)[h] 0.81-0.97 (m, 18H)[j]
[20][c]	7.10 (d) J_{1-2} 8.4	6.62 (dd) J_{2-4} 2.5	6.56 (d)	6.47 (d) J_{20-21} 13.5	6.26 (d)	not observed	-	-	0.91 (s)	2.82 (m, 2H)[e] 1.24-2.34 (several m, 14H)[f]
[21][c]	7.10 (d) J_{1-2} 8.4	6.62 (dd) J_{2-4} 2.5	6.56 (d)	6.79 (d) J_{20-21} 14.4	6.25 (d)	not observed	-	-	0.91 (s)	2.82 (m, 2H)[e] 1.24-2.37 (several m, 14H)[f]

TABLE IV-2. Continued

Compound	H-1	H-2	H-4	H-20	H-21	3-OH	3-OCH_3	17-OCH_3	18-CH_3	Others
[26][a]	7.14 (d) J_{1-2} 8.2	6.62 (dd) J_{2-4} 2.8	6.56 (d)	6.11 (dd) J_{20-21a} 10.8	H-21a 5.15 (dd) $J_{21a-21b}$ 1.2 H-21b 5.20 (dd) J_{20-21b} 17.3	4.68 (br s)	-	-	0.95 (s)	2.80 (m, 2H)[e] 1.24-2.37 (several m, 14H)[f]
[18][a,d]	7.19 (d) J_{1-2} 8.5	6.69 (d)	6.62 (s)	6.20 (d) J_{20-21} 19.3 J_{Sn-H} 66	6.06 (d) J_{Sn-H} 71	-	3.76 (s)	-	over- lapped	2.84 (m, 2H)[e] 1.20-2.31 (several m, 26H)[h] 0.82-0.97 (m, 18H)[j]

TABLE IV-2. Continued

Compound	H-1	H-2	H-4	H-20	H-21	3-OH	$3\text{-}OCH_3$	$17\text{-}OCH_3$	$18\text{-}CH_3$	Others
[22][a]	7.18 (d)	6.71 (dd)	6.63 (d)	6.46 (d)	6.29 (d)	-	3.77 (s)	-	0.92 (s)	2.86 (m, 2H)[e]
	J_{1-2} 8.6	J_{2-4} 2.5		J_{20-21} 13.5						1.23-2.35 (several m, 14H)[f]
[23][a]	7.18 (d)	6.70 (dd)	6.63 (d)	6.79 (d)	6.29 (d)	-	3.77 (s)	-	0.92 (s)	2.85 (m, 2H)[e]
	J_{1-2} 8.6	J_{2-4} 2.6		J_{20-21} 14.3						1.23-2.38 (several m, 14H)[f]
[38][a]	7.19 (d)	6.71 (dd)	6.63 (d)	6.20 (s)	6.20 (s)	-	3.77 (s)	-	0.92 (s)	2.86 (m, 2H)[e]
	J_{1-2} 8.5	J_{2-4} 2.6								1.20-2.37
				6.04[b] (d)	6.11[b] (d)					(several m, 14H)[f]
				J_{20-21} 13.1						

TABLE IV-2. Continued

Compound	H-1	H-2	H-4	H-20	H-21	3-OH	$3\text{-}OCH_3$	$17\text{-}OCH_3$	$18\text{-}CH_3$	Others
[39][a]	7.19 (d) J_{1-2} 8.6	6.70 (dd) J_{2-4} 2.6	6.63 (d)	6.11 (dd) J_{20-21a} 10.8	H-21a 5.15 (d) H-21b 5.20 (d) J_{20-21b} 17.3	-	3.77 (s)	-	0.94 (s)	2.86 (m, 2H)[e] 1.24-2.34 (several m, 14H)[f]
[16][a]	7.21 (d) J_{1-2} 8.6	6.71 (dd) J_{2-4} 2.7	6.63 (d)	-	2.62 (s)	-	3.77 (s)	3.42 (s)	0.88 (s)	2.86 (m, 2H)[e] 1.47-2.39 (several m, 13H)[g]
[19][b,d]	~7.16 (d) J_{1-2} 8.6	6.75 (dd) J_{2-4} 2.6	6.68 (d)	6.37 (d) J_{20-21} 19.7 J_{Sn-H} 69	6.23 (d) J_{Sn-H} 75	-	3.39 (s)	3.23 (s)	1.12 (s)	2.63-2.86 (m, 2H)[e] 1.21-2.29 (several m, 25H)[i] 1.01 (m, 6H)[k] 0.93 (t, 9H)[l] J=7.3

TABLE IV-2. Continued

Compound	H-1	H-2	H-4	H-20	H-21	3-OH	3-OCH$_3$	17-OCH$_3$	18-CH$_3$	Others
[24][a]	7.17 (d) J_{1-2} 8.6	6.69 (dd) J_{2-4} 2.6	6.62 (d)	6.31 (d) J_{20-21} 13.9	6.13 (d)	-	3.77 (s)	3.19 (s)	0.90 (s)	2.83 (m, 2H)[e] 1.23-2.35 (several m, 13H)[g]
[25][b]	7.10 (d) J_{1-2} 8.7	6.78 (dd) J_{2-4} 2.6	6.68 (d)	6.67 (d) J_{20-21} 14.8	5.94 (d)	-	3.42 (s)	2.95 (s)	0.90 (s)	2.70 (m, 2H)[e] 1.02-2.08 (several m, 13H)[g]
[40][a]	7.16 (d) J_{1-2} 8.4	~6.68 (d)	6.61 (s)	5.92 (dd) J_{20-21a} 10.9	H-21a 5.35 (d) H-21b 5.12 (d) J_{20-21b} 17.6	-	3.75 (s)	3.16 (s)	0.91 (s)	2.83 (m, 2H)[e] 1.22-2.37 (several m, 13H)[g]

[a] Deuteriochloroform solution, [b] Deuteriobenzene solution, [c] Deuteriochloroform solution with a few drops of Deuteriomethanol added.

[d] J_{Sn-H} values are an average of the ^{117}Sn and ^{119}Sn values.

[e] Methylene protons of steroid nucleus, [f] 17-OH and steroid nucleus, [g] Steroid nucleus

[h] 17-OH, -Sn(CH$_2$<u>CH$_2$</u><u>CH$_2$</u>CH$_3$)$_3$ and steroid nucleus, [i] -Sn(CH$_2$<u>CH$_2$</u><u>CH$_2$</u>CH$_3$)$_3$ and steroid nucleus

[j] 18-CH$_3$ and -Sn(<u>CH$_2$</u>CH$_2$CH$_2$<u>CH$_3$</u>)$_3$, [k] -Sn(<u>CH$_2$</u>CH$_2$CH$_2$CH$_3$)$_3$, [l] -Sn(CH$_2$CH$_2$CH$_2$<u>CH$_3$</u>)$_3$

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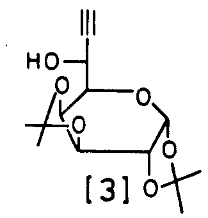
APPENDIX

Selected Structural Formulae



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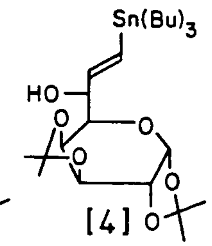

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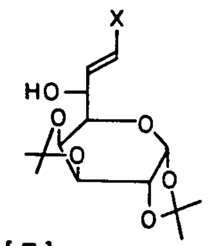

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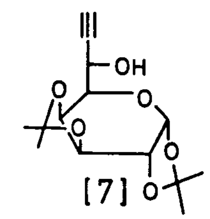
[6] X = I



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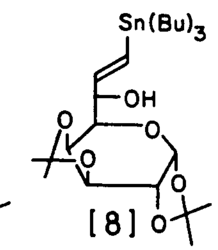

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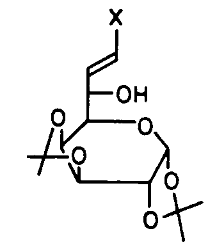

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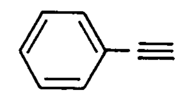

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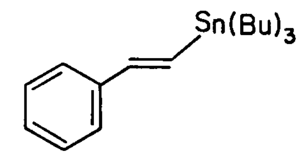

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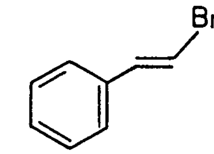

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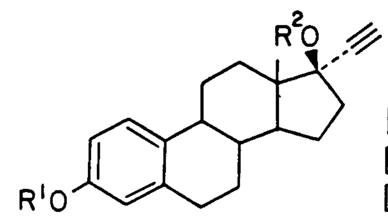
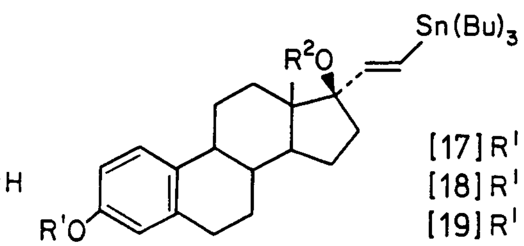
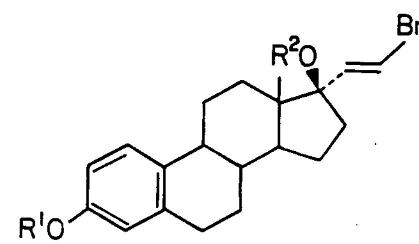


[14] $R^1 = R^2 = H$
[15] $R^1 = Me$, $R^2 = H$
[16] $R^1 = R^2 = Me$



[17] $R^1 = R^2 = H$
[18] $R^1 = Me$, $R^2 = H$
[19] $R^1 = R^2 = Me$



[20] $R^1 = R^2 = H$
[22] $R^1 = Me$, $R^2 = H$
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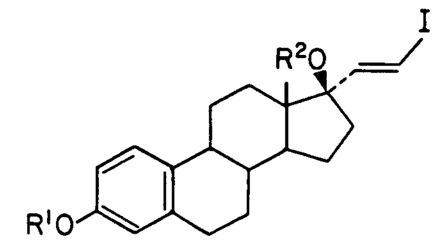
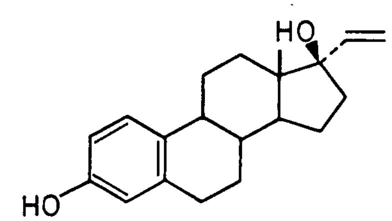


[21] $R^1 = R^2 = H$
[23] $R^1 = Me$, $R^2 = H$
[25] $R^1 = R^2 = Me$



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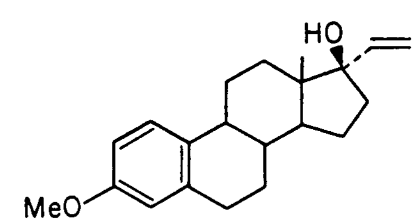

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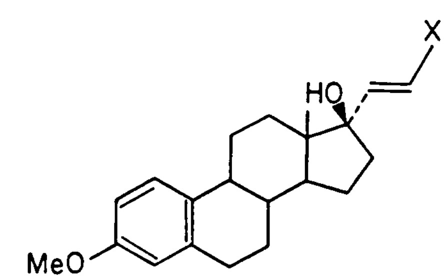

[41] $X = {}^{82}Br$
[42] $X = {}^{123}I$
[43] $X = {}^{131}I$
[38] $X = Cl$