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SYNTHESIS AND NMR SPECTROSCOPY OF TRIPEPTIDE DERIVED BIOMOLECULES FOR SITE SPECIFIC RADIOPHARMACEUTICALS

By

JOHN FITZMAURICE VALLIANT, B.Sc.

A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Doctor of Philosophy

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THE SYNTHESIS OF SITE-SPECIFIC RADIOPHARMACEUTICALS

DOCTOR OF PHILOSOPHY (1997)
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McMASTER UNIVERSITY
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AUTHOR: John Fitzmaurice Valliant, B.Sc. (McMaster University)

SUPERVISOR: Professor Russell Arthur Bell
Professor Colin James Lyne Lock

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Abstract

This thesis describes a bifunctional approach to the development of new, site-specific radioimaging agents. The approach taken involved covalently linking a chelate, which binds strongly to ^{99m}Tc (the most commonly used radionuclide in diagnostic medicine), to a drug, usually through a spacer chain. The biological molecule, which has a well defined receptor site in the body, is then expected to guide the labelled chelate to that receptor.

The initial stages of the work entailed improving the synthesis of the diamidodithiol (DADT) chelate. This chelant is of particular interest because its technetium complex is known to be stable *in vivo*. Tripeptide analogues of the DADT chelate were then synthesised by the use of standard peptide coupling chemistry. The peptides were of the type mercaptoacetic acid-X-L-cysteine, where X can be any amino acid. Several variations on X (Gly, Phe, His, Ile, Ser, Met, Tyr) were synthesised to provide a series of chelates with varying solubility, coordination chemistry and sites of derivatization. The rhenium complexes of two peptides, Tr-S-Mer-L-His-S-Bn-L-Cys-OMe and Tr-S-Mer-L-Ile-S-Bn-L-Cys-OMe were prepared and characterized by NMR and the former by X-ray crystallography and NMR spectroscopy. The reaction of rhenium with the histidine containing peptide resulted in the formation of two diastereomers while the rhenium complex of the second chelant formed only one isomer because of the steric hindrance

from the isoleucine side chain.

The second phase of this work involved coupling the chelants to biomolecules. The DADT chelant was coupled to tamoxifen, a drug used in the treatment of hormone-dependent breast cancer. A total synthesis was required to conjugate the chelant to an appropriate site on tamoxifen. The desired chelate-tamoxifen species was prepared in 18 steps in 7% overall yield. The 4-hydroxy analogue of tamoxifen, with a linker arm for conjugation to a chelate was also prepared.

Further development towards cancer imaging agents was accomplished by conjugating one of the tripeptide chelants, Tr-S-Mer-L-Ser-S-Bn-L-Cys, to the alkylating agent chlorambucil. The product of the 10 step synthesis (30% overall yield) was characterized by several high resolution NMR techniques.

Acknowledgements

I would like to thank my family: Jim, Margo, Jamie, Julie-Anne, Mim (and Kelly), for all their support throughout my life as a student. Without their encouragement and love I would never be where I am today.

To Professor Russell Bell- who, from the first day that I passed through the lab door, treated me as much as a colleague as a student- I owe so much. Without his guidance and friendship I could never have accomplished what I did. I shall miss those Thursday beers where we discussed many things including chemistry and wine (more the latter than the former).

I also would like to thank Dr. Alan Guest for his friendship and his advice. Alan and I started at essentially the same time and though we differ in age there was never a time when that was noticeable. We shared many laughs and nearly as many beers and I shall miss spending breaks together sorting out the problems of the world.

I cannot leave out the support I received from my friends, I would like to thank my friends: from Kingston, Randy, Kenny, John and Kelly; from my undergraduate days Pat, Casey, Pippa, Ralph, Jessica; and from more recently Tanya, Scott, and Melanie. I would also like to thank Laura for putting up with me during the writing of this document. Laura, you have made this last year very special and I look forward to the years ahead of us.

There are several people that I would like to thank in the department who helped me along the way. Their advice and friendship aided me in my pursuits. I would like to

thank Professor B.E. McCarry, Professor M.J. McGlinchey, Professor P. Harrison, John Thornback, Mike Malott, Don Hughes, Brian Sayer, Richard Smith, Faj Ramelan and Carol Dada.

Finally, the most difficult of the acknowledgement is to Professor Colin Lock. The reason that I chose this project was because of his enthusiasm and his zest for life. He was a kind man, who gave me nothing but support and encouragement. I will miss that cheery, confident smile. Cheers C.J.L.

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List of Abbreviations and Symbols

AcOH	Acetic acid
ACQ	Acquire
AN	Acetonitrile
Bn	Benzyl
Boc	Butoxycarbonyl
bs	Broad signal (^{1}H NMR)
^{13}C NMR	C-13 Nuclear Magnetic Resonance Spectroscopy
CI	Chemical Ionization Mass Spectrometry
COSY	Correlation Spectroscopy
Cys	Cysteine
d	Doublet (^{1}H NMR)
dd	Doublet of Doublets (^{1}H NMR)
DADS	Diamido Disulfide
DADT	Diamido Dithiol
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DIPEA	Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	N,N Dimethylformamide
DW	Distilled water
E	Entgegen
EDAC	Ethyl-3-(3-dimethylamino)-propylcarbodiimide hydrochloride
EI	Electron Impact Mass Spectrometry
ER	Estrogen receptor
ES	Electrospray Mass Spectrometry
EtOH	Ethanol
Gly	Glycine
His	Histidine
HPLC	High Performance Liquid Chromatography
^{1}H NMR	Proton Nuclear Magnetic Resonance Spectroscopy
HMBC,	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum coherence
Hr/hrs/hr	Hours
HSQC	Heteronuclear single quantum coherence

IR	Infrared Spectroscopy
Ile	Isoleucine
m	Multiplet (^{1}H NMR)
Me	Methyl
MeOH	Methanol
Mer	Mercaptoacetic acid
Met	Methionine
mp	Melting point
MS	Mass Spectrometry
NOE	Nuclear Overhauser Effect
NOESY	Nuclear Overhauser Effect Spectroscopy
PET	Positron Emission Tomography
Pet ether	Low boiling petroleum ether
Phe	Phenylalanine
PPh_3	Triphenylphosphine
ppm	Parts per million
PTC	Phase Transfer Catalyst
s	Singlet (^{1}H NMR)
Ser	Serine
SPECT	Single Photon Emission Computed Tomography
t	Triplet (^{1}H NMR)
TBAF	Tetrabutylammonium fluoride
TBS	*t*-Butyldimethylsilyl
TBSCl	*t*-Butyldimethylsilyl chloride
TES	Triethylsilane
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TOCSY	Total correlation spectroscopy
TsOH	*P*-Toluenesulfonic acid
Tr	Trityl
Tyr	Tyrosine
Z	Zusammen

Chapter 1

Introduction and Rationale

Because of the development of medical imaging, physicians no longer need to rely solely on their intuition and the patient's description of pain, which can be misleading, to formulate a diagnosis. Modern medicine routinely uses imaging techniques as a tool to indicate damaged tissue or organs.

1.1 Objectives

The long term goal of the present project is to synthesise compounds for use in either imaging (based on ^{99m}Tc) or treatment (based on ^{186}Re) of estrogen-dependent breast cancer. Bifunctional radiopharmaceuticals were to be synthesised by covalently linking chelates to biomolecules which are known to interact with tumour cells.

A second objective was to develop new technetium and rhenium chelates. The synthetic approach towards the chelates should allow for facile interchanging of synthon units (amino acids) so that compounds with differing donor atoms, solubility and sites of derivatization could be synthesised. The coordination chemistry of these chelates towards either technetium or rhenium would then be evaluated.

1.2 Anatomical imaging techniques

X-ray imaging, which is the most common type of imaging, produces images based on the difference in absorption of X-rays between hard and soft tissue[1]. Computed tomography (CT), which is an advanced form of X-ray, involves projecting a fan-shaped x-ray beam through a slice of the body. In order to obtain enough information to produce a full image, the X-ray beam is rotated around the section of interest and the radiation that has penetrated the body is measured by an array of detectors[1]. The scan data are then converted to a digital image by computer.

Ultrasound detection, which uses frequencies over 20 000 Hz, produces images by observing reflections, or echos, of sound waves from structural interfaces within the body[1]. Reflected pulses, or echoes are detected by a transducer, which converts the sound wave into electrical pulses that are amplified and displayed as an image. The amplitude and the time it takes the reflected sound wave to return to the transducer provide structural information.

Magnetic resonance imaging (MRI) is based on the fact that protons, and other nuclei with non zero nuclear spin, possess a nuclear magnetic moment, and therefore interact with an external field to produce several nuclear spin energy states[2]. For MRI, the magnetic fields required to produce a transition between two states (^{1}H atoms) are on the

---

[1]Sprawls Jr., P., Physical Principles of Medical Imaging, Aspen Publishers, Inc. Rockville, Maryland, **1987**.

[2]Morris, P.G., Nuclear Magnetic Resonance Imaging in Medicine and Biology, Clarendon Press, Oxford, **1986**.

order of 0.002 to 2 Tesla; therefore, the energy required is in the radio frequency region, 0.08 to 80 MHz. The proton nuclei of tissue water molecules are responsible for the signal imaged by MRI; therefore damage to soft tissue can be detected. MRI is a complement to X-ray analysis because it does not image bone matter, as skeletal tissue contains little water. Magnetic resonance imaging is advantageous in that it utilizes non-ionizing radiation and modest magnetic fields while providing excellent spatial resolution (1-3 mm) [*vide infra*].

1.3 Radio imaging

Single photon emission computed tomography (SPECT) and positron emission tomography (PET) generate images by detecting *gamma* (*vide infra*) rays. In the case of SPECT the gamma rays are directly emitted by a radioactive nucleus. The electromagnetic energy emitted from the radioactive nuclei, which is highly penetrating, is detected by a scintillation camera[1]. A collimator is used to localize the radiation on the detector as the radiation is randomly emitted. The detector consists of a thallium-doped sodium iodide crystal that emits a flash of light when struck by a *gamma* ray. The light flashes are amplified using photomultipliers and are counted as electrical pulses. A three-dimensional image is obtained by mounting the scintillation camera on a rotating gantry, recording multiple images at different angles of the patient and reconstructing the activity distribution.

Imaging based on radionuclides has two main advantages over the aforementioned

techniques: superior resolution and the ability to image function as well as structure. The resolution of an imaging technique is defined as the ability of the system to distinguish or separate objects that are close together. **Figure 1-I** clearly shows the superiority of imaging techniques based on radionuclides at resolving anatomical details.

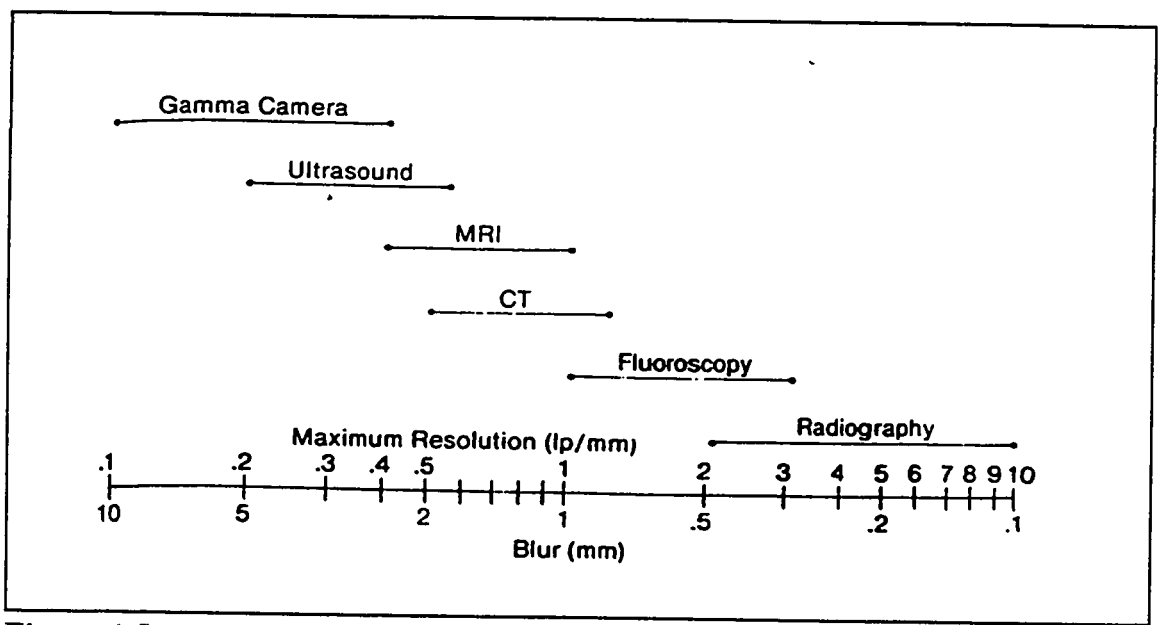

Figure 1-I: Range of blur values and visibility of detail obtained with various imaging techniques[2].

Radio imaging techniques can be used to determine how an organ or collection of tissues is functioning. For example, if a patient inhales a radioactive gas such as ^{133}Xe, then the distribution of radioactive nuclei within the lung can be used as an indicator of regional air flow. This distribution can be mapped by forming an image of the *gamma* rays emitted by xenon.

1.4 Nuclear Decay

Gamma rays, the source of information in SPECT and PET, are one of three types of nuclear decay; *alpha* particles, which are physically identical to helium nuclei, have energies between 2 and 10 MeV[3]. Nuclei decaying by an *alpha* emission yield daughter isotopes that have an atomic number of two units less, while the mass decreases by four units. *Beta* disintegrations are classified as either β^+ or β^- particles. In the case of nuclei which emit β^- particles, a neutron is transformed into a proton, a β^- particle and an antineutrino. In the case of a β^+ decay, a proton is transformed into a neutron, a positron (β^+ particle) and a neutrino. *Gamma* rays are electromagnetic radiations which have frequencies higher than those of X-rays. *Gamma* emissions frequently accompany *beta* decay; the mass and charge of the *gamma* emitting nucleus remains unchanged.

The cost of positron emitting nuclei (a β^+ decay) such as ^{11}C ($t_{1/2}$= 20 minutes), ^{13}N ($t_{1/2}$=10 minutes) or ^{18}F ($t_{1/2}$=110 minutes) is quite high, therefore, the number of hospitals which have PET facilities is limited. The radionuclides used in SPECT are substantially less expensive and as a result radio imaging can be used as a "routine" diagnostic technique.

1.5 ^{99m}Technetium

The three most common radionuclides used in SPECT imaging are ^{123}I ($t_{½}$ = 13

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[3]De Soete, D.; Gijbels, R.; Hoste, J. Neutron Activation Analysis, P.J. Elving, I.M. Kolthoff (Eds.), Wiley-Interscience, England, **1972**, P163-165.

hours), ^{201}Tl ($t_{½}$ = 73.5 hours) and ^{99m}Tc ($t_{½}$= 6 hours)[4]. Eighty percent of radiopharmaceuticals used in clinics are labelled with ^{99m}Tc because of its ideal nuclear properties, relatively low cost of production and diverse chemistry[4]. The half life of ^{99m}Tc is long enough to isolate the radionuclide from its parent source, to carry out the labelling and to perform the *in vivo* measurement without significant loss of radioactivity. The energy of the *gamma* ray (140 keV) is sufficient to study organs deep within the body with minimal radiation exposure to the patient. The decay product is a pure *beta* emitting nucleus (^{99}Tc, E_{max}= 0.29 MeV, $t_{½}$= 2.1 x 10^5 years) which does not contribute noticeably to the overall radiation exposure[4].

^{99m}Tc is a decay product of ^{99}Mo which is prepared by irradiating natural molybdenum with thermal neutrons or as a decay product of ^{235}U fission[5] (**Figure 1-II**). ^{99}Mo decays with an 87% probability to ^{99m}Tc which almost quantitatively converts to the ground state of ^{99}Tc; only 4 x 10^{-3} % undergoes β^- emission to the stable ^{99}Ru isotope. ^{99m}Tc is separated from ^{99}Mo by ion exchange chromatography (acidic aluminum oxide). ^{99}Mo, as molybdate ($^{99}MoO_4^{2-}$) is loaded at the top of the column while ^{99m}Tc as pertechnetate ($^{99m}TcO_4^-$) is eluted with 0.15M NaCl[6].

---

[4]Schwochau, K. *Angew. Chem. Int. Ed. Engl.* **1994**, 33, 2258-2267.

[5]Browne, E. Firestone, R.B. *Tables of Radioactive Isotopes,* V.S. Shirley (Ed.), Wiley, New York, **1986.**

[6]Tucker, W.D.; Greene, M.W.; Weiss, A.J.; Murrenhoff, A.P. *Trans. Am. Nucl. Soc.*, **1958**, 1, 160.

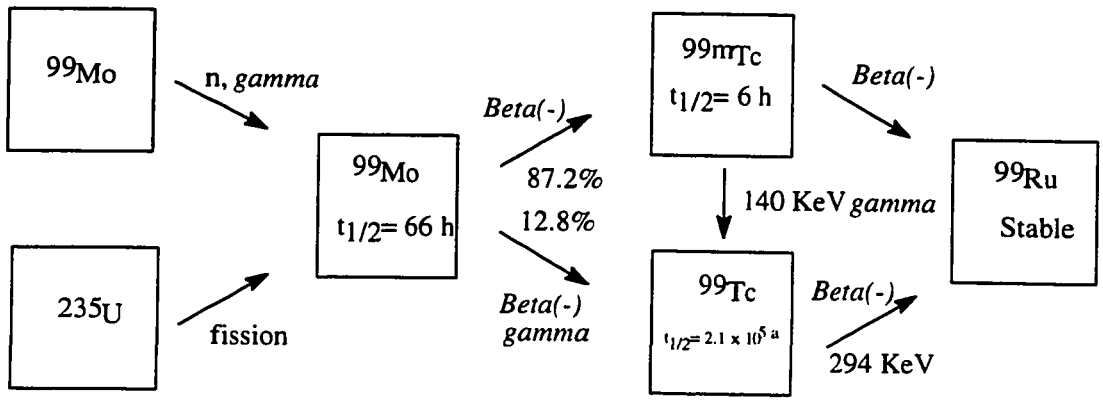


Figure 1-II: Formation and Decay of ^{99m}Tc and ^{99}Tc

1.6 Technetium Chemistry

Technetium exhibits a diverse chemistry which rivals that of molybdenum and ruthenium. Compounds have been characterized in oxidation states ranging from -I(d^8) to VII(d^0). Tetraoxotechnetate(VII) is the most thermodynamically stable compound of technetium in contrast to tetraoxomanganate(VII)[4]. The standard electrode potential in acidic media for TcO_4^-/TcO_2 is only +0.738V but +1.695V for MnO_4^-/MnO_2[4]. If TcO_4^- is reduced in the absence of a complexing ligand (or in the presence of a weakly complexing ligand) the sparingly soluble TcO_2 hydrate is the predominant product.

Nitrogen based coordinating ligands and halides bind to technetium in most

oxidation states[7]. Conversely, oxygen typically binds to technetium in high oxidation states only (V-VII) while sulfur and phosphorus tend to bind to intermediate oxidation states (II-V).

Tc(V) has proven to be the most suitable oxidation state for the synthesis of radiopharmaceuticals because most Tc(V) compounds are well-defined monomeric species which are sufficiently stable *in vivo* to obtain an image[6]. The chemistry of Tc(V) is dominated by the oxo species "TcO^{3+}". The reason for the formation and stability of the oxo species is that the high formal charge on technetium(V) is reduced by the oxygen double bond and, in addition the oxygen atom can back-donate 2p electron density into the Tc d orbitals. The length of the Tc-O bond and the number of technetium oxygen bonds are governed by the ability of the coordinating ligands to donate electron density to the metal[8]; as a result oxotechnetium(V) species are categorized based on their cores (**Figure 1-III**). Negatively charged ligands favour mono oxo species, neutral ligands, such as amines, or those with efficient π back bonding favour the bisoxo core (TcO_2^+) while the third type of oxo core contains a $[Tc_2O_3]^{4+}$ unit[7].

In the mono oxo species, the metal typically lies above the equatorial plane of the

---

[7]Spies, H.; Johannsen, B. Topics in Current Chemistry , #176, *Technetium(V) Chemistry Relevant to Nuclear Medicine*, p79-121 **1996.**

[8]Deutsch, E.; Libson, K.; Jurisson, S.; Lindoy, L.F. *Progr. Inorg. Chem.* **1993**, 30, 75.

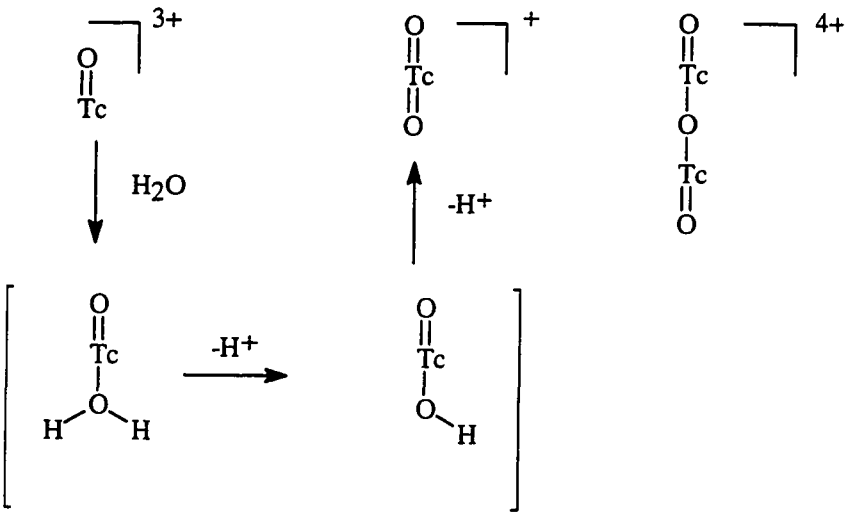


Figure 1-III: Oxotechnetium cores

four basal ligand atoms and the oxo group is at the apex[9]. The Tc-O bond length correlates with the displacement of the technetium atom from the basal plane towards the oxygen at the apical position[7].

Species containing the dioxo core are generally six-coordinate ($[MO_2L_4]$) and adopt an octahedral geometry[10]. The average Tc-O and Tc-L bond lengths in pentacoordinate compounds are shorter than hexacoordinate compounds because of the difference in steric hindrance[11].

The $[Tc_2O_3]^{4+}$ core involves the oxo group of one metal coordinating to another

---

[9]Bandoli, G; Mazzi, U; Roncari, E.; Deutsch, E. *Coord. Chem. Rev.* 77, 275.

[10]Fackler, P.H.; Kastner, M.E.; Clarke, M.J. *Inorg. Chem.* **1984**, 23, 3968.

[11]Melnick, M.; Van Lier, J.E. *Coord. Chem. Rev.* **1987**, 77, 275.

technetium center, thus generating a nearly linear oxygen bridged structure. Examples of this type of structure are derived from tetradentate Shiff base ligands[12] and bidentate neutral thio-ether ligands[13].

1.7 Rhenium and 99-Technetium

Because of the radiation hazard posed by using large quantities of ^{99m}Tc, ^{99}Tc is used to develop the chemistry of new radiopharmaceuticals. For example, 110μg of $Na^{99m}TcO_4$ has more activity (270 Ci) than 50 mg of $Na^{99}TcO_4$ (0.45 mCi)[4]. Milligram quantities of ^{99}Tc can be handled safely in a well ventilated fumehood; glass is sufficient to stop the low energy β^- emission[14].

As a result of the lanthanide contraction, the chemistry of technetium(V) and rhenium(V) are similar; therefore synthesis of rhenium analogues of radio imaging agents is a convenient method of studying the chemistry on the milligram scale. In addition, ^{186}Re is an attractive isotope for therapeutic radiopharmaceuticals[7]. ^{99m}Tc and ^{186}Re can be considered a matched pair for imaging and therapy[7].

---

[12]Tisato, F.; Refosco, F.; Mazzi, U.; Bandoli, G.; Domella, A. *Inorg. Chim. Acta.* **1989**, 164, 127.

[13]Pietzsch, H.J.; Spies, H.; Leinbnitz, P.; Reck, G.; Berger, J.; Jacobi, R. *Polyhedron*, **1993**, 12, 187.

[14]Schwochau, K. *Chem.-Ztg.* **1978**, 102, 329-337.

1.8 Preparation of Radio imaging Agents

For imaging and functional testing of organs in humans, about 30 mCi (1110 MBq) of ^{99m}Tc are required; this corresponds to about 6 ng[4]. A common procedure for labelling involves the use of a "kit" which contains pertechnetate, the species to be labelled and a reducing agent. Labelling should occur immediately after elution of $^{99m}TcO_4^-$ in a solvent which is suitable for injection. The labelling reaction must be rapid (less than one half life) and occur in high radiochemical yield (>90%).

1.9 Technetium Based Radiopharmaceuticals

Currently there are two main types of Tc-based radiopharmaceuticals: *Tc-essential* radiopharmaceuticals, which are simple technetium compounds whose biodistribution is determined by the intrinsic nature of the substance, and *Tc-tagged* compounds which are large biomolecules that have been derivatized with ^{99m}Tc. Selected examples of Tc-essential radiopharmaceuticals are shown in **Figure 1-IV**; they include the brain imaging agent TcO-d,l- HM-PaO[15], a hexakisisonitrile Tc(I) cation (Tc-HEXAMIBI)[16], a cardiac imaging agent and a kidney imaging agent based on dimercaptosuccinic acid (DMSA)[17].

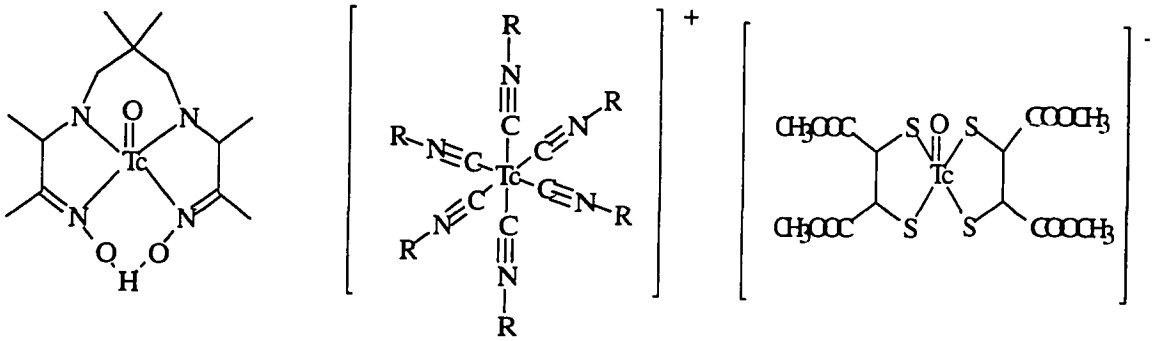
---

[15]Sharp, P.F.; Smith, F.W.; Gemmell, H.G.; Lyall, D.; Evans, N.T.S.; Gvozdanovic, J.; Davidson, D.A.; Tyrrell, R.D.; Pickett, R.D. Neirinckx, J., *J. Nucl. Med.*, **1986**, 27, 171-177.

[16]Piwnica-Worms, D.; Kronauge, J.F.; Holman, B.L.; Davison, A.; Jones, A.G., *Invest. Radiol.* **1989**, 24, 25-29.

[17]Taylor Jr., A.; Eshima, D.; Fritzberg, A.R.; Christian, P.E.; Kasina, S.; *J. Nucl. Med.*, **1986**, 27, 795-803.

Tc Essential Radiopharmaceuticals



TcO-d,l-HM-PaO (brain)

Tc-HEXAMIBI, (Heart)

Tc-DMSA (Kidneys)

Tc Tagged Radiopharmaceuticals

Tagged Compound	Organ Imaged	Application
Macroaggregated human serum albumin (MAA)	Lungs	Blood perfusion analysis
Sulfur colloid	Liver, gall bladder	gall bladder infection, liver function, tumors

Figure 1-IV: Examples of Tc essential and Tc tagged radiopharmaceuticals

Included in the figure are two types of Tc-tagged compounds, Tc-sulfur colloid, which is used for liver and gall bladder imaging and Tc labelled macroaggregated human serum albumin, which is used in lung imaging. The discovery of a site-specific Tc-essential radiopharmaceutical is usually the result of serendipity while the nature and location of

metal binding in Tc-tagged compounds is usually unknown and in consequence this can affect the natural biodistribution of the parent molecule in unforeseen ways.

1.10 Bifunctional Approach to Radiopharmaceuticals

Our approach to the synthesis of site specific radiopharmaceuticals is a combination of the two approaches presented in section **1.9**; a small chelating group is covalently linked to a biologically important molecule, usually through a spacer chain. The biological molecule, which has a well defined receptor in the body, is used to guide the Tc-chelate complex to that receptor. In order for this approach to be successful, the chelant group must be bound to the biological molecule at a position distant from the binding site, and one hopes that the chelated metal is sufficiently far away that it does not interfere markedly with the binding of the biomolecule to the receptor.

For example, earlier work resulted in the preparation of a potential heart imaging agent by derivatizing the cardiac glycosides digitoxin[18] and digitoxigenin[19] (**Figure 1-V**). Both molecules bind to ATPase which is found in high concentration in heart muscle. Because the steroid interacts with the enzyme through the aglycone ring, the spacer chain and chelate were attached at the C-3 hydroxyl of digitoxigenin and at a sugar hydroxyl of digitoxin; two positions that should not have affected the substrates binding. Both compounds were tested in live canine models and both compounds demonstrated

---

[18]R.A. Bell, C.J.L. Lock, Z.Wang, *Unpublished results.*

[19]Maharaj, R. *Ph.D. Thesis*, McMaster University, 1993.

significant cardiac uptake.

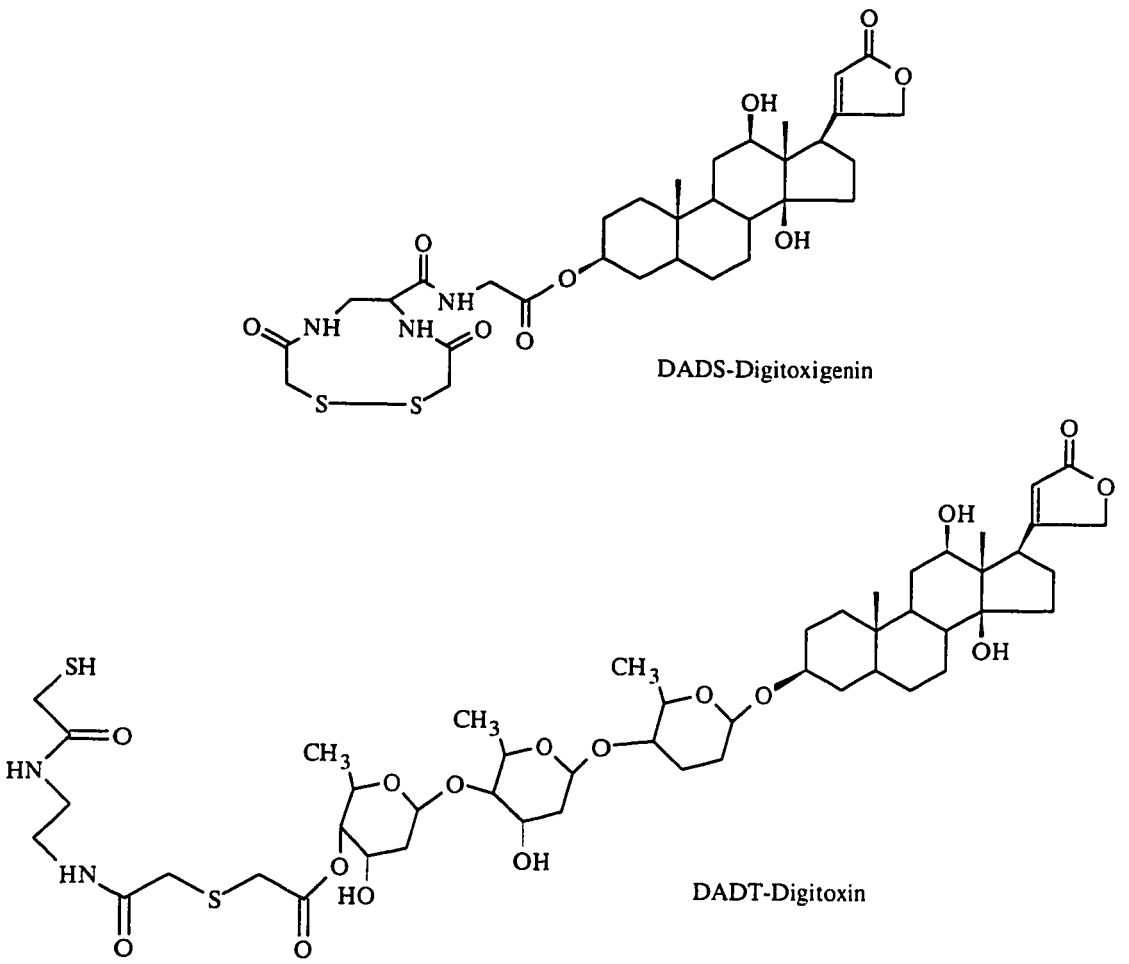


Figure 1-V: Digitoxigenin and digitoxin derived radiopharmaceuticals

Chapter 2

The Diamido Dithiol (DADT) Chelate

N_2S_2 Chelates

2.1 Technetium complexes of chelates which contain two amine and two sulfur donor atoms (N_2S_2) have been shown to have *in vivo* stability sufficient to develop effective radio imaging agents[1]. Three main types of N_2S_2 chelates are recorded in the literature (**Figure 2-I**); the bis aminoethanethiol (BAT) ligand, which forms a neutral technetium complex, the monoamine-monoamide (MAMA) which also forms a neutral Tc complex and the diamidodithiol (DADT) (also known as diamidodisulfide (DADS)) chelate which forms an anionic technetium complex.

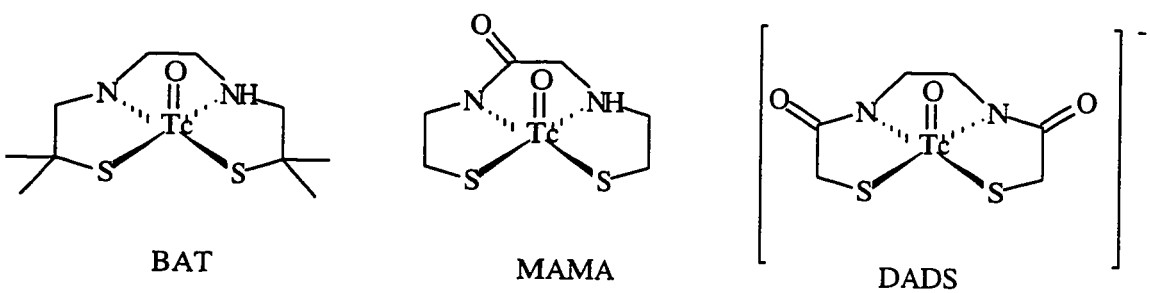


Figure 2-I: N_2S_2-Tc Chelants

[1]Baidoo, K.E.; Lever, S.Z. *Bioconj. Chem.* **1990**, 1, 132.

The BAT ligand, first reported by Corbin *et al.*[2] consists of two amine donor atoms and two thiols. Burns *et al.*[3] showed that upon reaction with technetium, one of the amines of the chelate deprotonated, which resulted in a neutral Tc(V) species. There are several publications concerning derivatization of the chelate by substitution at one of the amines by either alkylation or acylation. For example, the quinuclidinyl benzilate (QNB) analogue shown in **Figure 2-II** exhibits an *in vivo* affinity for neuroreceptors[4]. However, because of the low reactivity of the amine functional groups, vigorous conditions were required during synthesis; this has limited further development of BAT based bifunctional imaging agents.

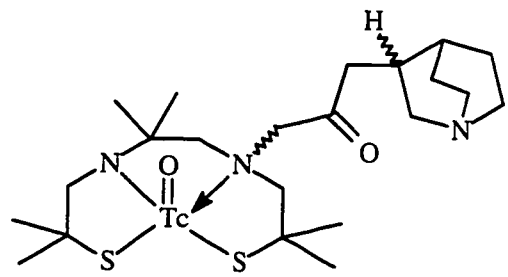


Figure 2-II: A Tc(BAT) quinuclidinyl benzilate analogue

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[2]Corbin, J.L.; Work, D.E. *J. Org. Chem.* **1976**, 41, 489.

[3]Burns, H.D.; Dannals, R.F.; Dannals, T.E. *J. Label. Comp.*, **1981**, 18, 54.

[4]Lever, S.Z.; Baidoo, K.E.; Mahmood, A.; Matsumura, K.; Scheffel, U.; Wagner, H.N. Jr. *Nucl. Med. Biol.* **1994**, 21, 157.

The MAMA chelate[5] is a combination of the DADT (*vide infra*) and BAT chelates as it contains an amide, an amine and two thiol groups. The amide, being more acidic than an amine, deprotonates upon coordination to technetium (or rhenium) maintaining the overall neutrality of the complex. O'Neil *et al.*[6] reported the derivatization of the basic MAMA chelate with a benzyl group (MAMA') which was used as a test compound to investigate the conditions required to combine the chelate with a progestin (**Figure 2-III**). Upon coordination with Tc and Re, the MAMA' chelate resulted in the formation of two diastereomers, the stereogenic centers being the metal and the substituted amine, the major product being the syn isomer.

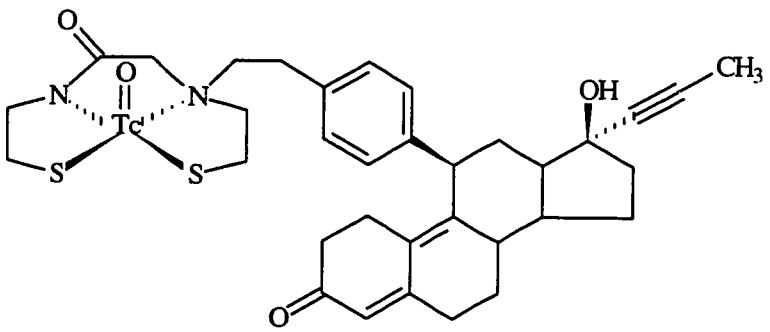

Figure 2-III: Progestin derivatized with a Tc-MAMA chelate

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[5]Bandoli, G.; Nicolini, M.; Mazzi, U.; Refosco, F. *J. Chem. Soc. Dalton Trans.* **1984**, 2505

[6]O'Neil, J.P.;Wilson, S.R.; Katzenellenbogen, J.A. *Inorg. Chem.*, **1994**, 33, 319-323.

The amine group of the MAMA chelate was similar to that of the BAT chelate in showing poor nucleophilic reactivity. Complete alkylation of the amine with a primary bromide was found to require heating at 100°C for a week[7]; a primary mesylate was more effective but the reaction time was again exceedingly long. These substitution conditions are not suitable for coupling MAMA to acid or thermally sensitive biomolecules.

From our earlier work on cardiac glycosides[8], the DADT chelant was of particular interest because of its stability and rapid binding of technetium. The stability characteristic is important because the imaging agent must stay intact *in vivo* for a significant period of time to obtain a reasonable image. The chelate's rapid binding of technetium is a desired trait because of the six hour half-life of ^{99m}Tc which requires a relatively short preparation time for the radio labelled complex.

The square based pyramidal structure of the Tc-DADT complex is a result of the electronic structure of technetium and the steric hindrance about the ligand[9]. The technetium is in the +5 oxidation state and the most stable form for the d^2 system is the square based pyramidal structure. Distortions in the base of the pyramid are a result of steric interactions.

Bell *et al.*[10] have published an approach to the synthesis of the DADT chelate,

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[7]R.A. Bell, C.J.L. Lock and Z. Wang, *unpublished results*

[8]R.A. Bell, C.J.L. Lock, J.F. Valliant and Z. Wang, *unpublished results*

[9]Brenner, D.; Davison, A.; Lister-James, J.; Jones, A.G. *Inorg. Chem.* **1984**, 23, 3793.

[10]Capretta, A.; Maharajh, R.B.; Bell, R.A., *Carbohydrate Research*, **1995**, 267, 49.

which contained a free carboxylate moiety. During an attempt to repeat the synthesis a number of problems arose: several steps in the synthesis were not robust; yields were inconsistent as was the purity of the products. By altering some of the reagents and reaction conditions but with the use of the same basic approach, the yields and purity of the final product were greatly improved .

2.2 Synthetic Strategies For DADT

Because of the radioactivity, the final step in the development of a bifunctional imaging agent, must be the addition of the radio label (^{99m}Tc) to an active form of the chelate. In our case the chelant can be either a dithiol (DADT) or a disulfide (DADS). The latter is used when the biomolecule is acid sensitive; the former is used in all other cases because yields of the intramolecular disulfide formation step were consistently low. Coupling of the biomolecule to the chelate can be accomplished by either esterification (O-linkage) or amide formation (NH linkage). As a result of its greater stability towards *in vivo* hydrolysis, the amide bond is preferable to an ester linkage, but because of our synthetic methodologies, formation of an amide was not always possible.

The retrosynthetic analysis of the ligand is shown in **Figure 2-IV**. The sequence involved the condensation of suitably protected 2,3-diaminopropionic acid (synthon **A**) with an appropriately activated thioglycolic acid derivative (synthon **B**) prior to the addition of an optional glycine spacer chain (synthon **C**). The acid of the diamine and the thiols were protected with orthogonal protecting groups (methyl ester and triphenylmethyl

respectively) such that deprotection, for the purpose of derivatization, would not cause any unwanted side reactions.

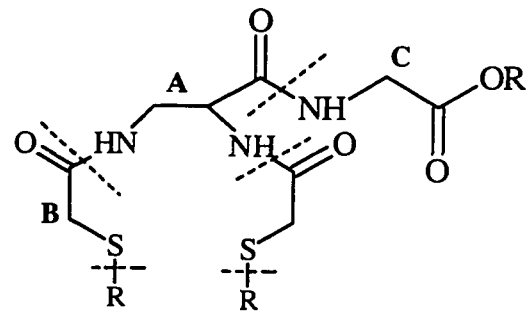

Figure 2-IV: Retrosynthetic analysis of **2.9**

2.3 Synthesis of DADT

The conversion of (±)2,3-diaminopropionic acid to its methyl ester (**2.2**) was initially performed by a standard HCl catalysed Fisher esterification (**Figure 2-V**). The yields of this particular reaction were excellent (98%) but the product, a very insoluble salt, was frequently contaminated with starting material. Once the gaseous HCl was replaced with *p*-toluenesulfonic acid, the yield of the reaction remained the same but the product purity increased. The formation of compound **2.2** could be followed by normal phase silica TLC using ninhydrin as the indicator. The ^{1}H NMR of the purified product exhibited a singlet at 3.85 ppm which corresponds to the methyl ester protons, while in the ^{13}C NMR spectrum, the signal which corresponds to the carbonyl carbon in the product had moved

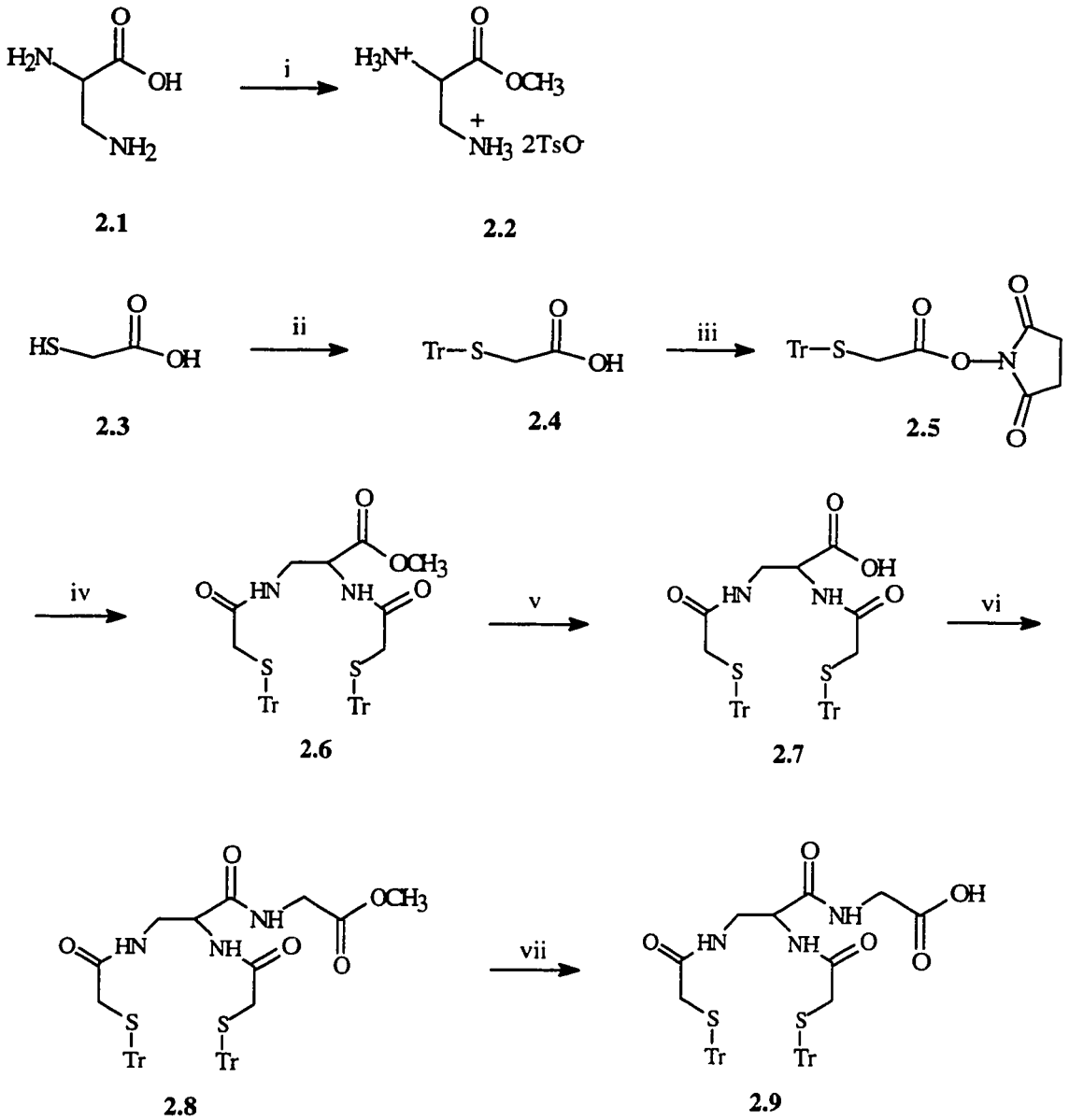

Figure 2-V: I) *p*-TsOH, MeOH, Δ ii) TrOH, BF_3-Et_2O, CH_2Cl_2, AcOH iii) EDAC, N-hydroxysuccinimide iv) NEt_3, **2.2**, Δ v) NaOH(aq), THF vi) glycine methylester hydrochloride, EDAC, NEt_3 vii) NaOH(aq), THF.

downfield when compared to the starting material.

The synthesis of **2.4** was accomplished by modification of the method of Brenner *et al*[11]. Reaction of triphenylcarbinol with boron trifluoride etherate results in the formation of a triphenylmethyl cation (**Figure 2-VI**) which rapidly reacts with the thiol of mercaptoacetic acid to generate the desired compound. The synthetic method was improved by changing the solvent in which the reaction was performed. Methylene chloride was added as a co-solvent, and the reaction was performed at room temperature instead of the literature recommendation of 80°C; as a result, there was a significant

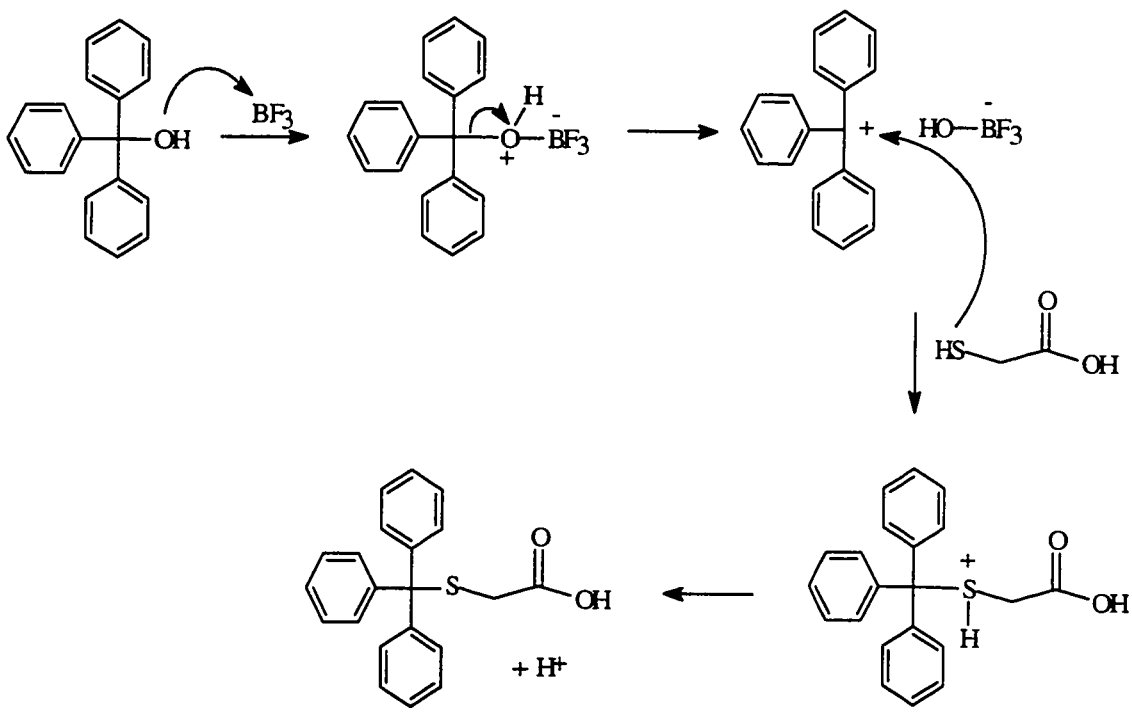

Figure 2-VI: Proposed mechanism for the formation of **2.4**

[11]Brenner, D.; Davison, A.; Lister-Jones, J.; Jones, A.G., *Inorg. Chem.* **1984**, 23, 3793.

improvement in yield (87% versus the previous 65%) of the product.

The use of N-hydroxysuccinimides in amide synthesis improves yields by eliminating the problems of hydrolysis and rearrangement which are frequently encountered when forming amides with the use of carbodiimides. An example of this is the reported activation of **2.4** by converting it to an N-hydroxysuccinimido ester *via* DCC coupling[12]. **Figure 2-VII** presents the accepted mechanism of carbodiimide type couplings. The carboxylic acid reacts with the highly electrophilic carbon of the carbodiimide *via* a six membered transition state which rapidly converts to a mixed

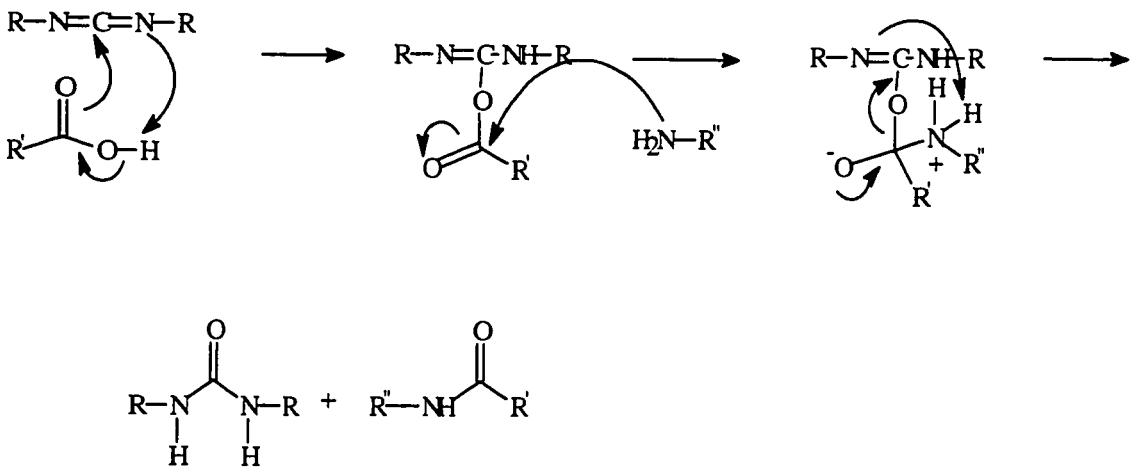

Figure 2-VII: Proposed mechanism of peptide bond formation

[12]Maharajh, R. *Ph.D. Thesis*, McMaster University, 1993.

anhydride. The mixed anhydride intermediate can then react with a nucleophile to form the species of interest. The limitation of this method is that if the nucleophile is poor, rearrangement can occur *via* an internal nucleophilic attack by the adjacent imine nitrogen of the mixed anhydride to give an acylurea which is an unreactive byproduct.

The major problem that arose with the use of DCC as the N-hydroxysuccinimido coupling agent was contamination of the ester **2.5** with dicyclohexylurea. To overcome this, the coupling agent was changed to 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC), a water soluble carbodiimide. EDAC and its corresponding hydrolysis product (the urea) were soluble in acetonitrile while compound **2.5** was not; as a result, compound **2.5** precipitated from the acetonitrile solution in excellent yield (85%) in a short period of time (2 hours). When synthesised using DCC, the ^{1}H NMR of **2.5** routinely exhibited extra peaks between 1.12 and 1.90 ppm. These peaks, which were present after several recrystallizations, were ascribed to residual dicyclohexylurea. **Figure 2-VIII** is the ^{1}H NMR spectrum of the material prepared with the use of EDAC. The downfield multiplets (≈7.25 ppm) were assigned to the aromatic protons of the trityl group while the signals at 3.091 ppm and 2.644 ppm correspond to the mercaptoacetic acid methylene and the two N-hydroxysuccinimido methylenes respectively. Development of a simple route to high purity samples of **2.5** greatly simplified the remainder of the synthesis.

Coupling of **2.2** and **2.5** in the presence of diisopropylethylamine or triethylamine, was followed by disappearance of the ninhydrin active spot on a silica TLC plate. An excess of the succinimide was required to isolate pure product because, if there was any

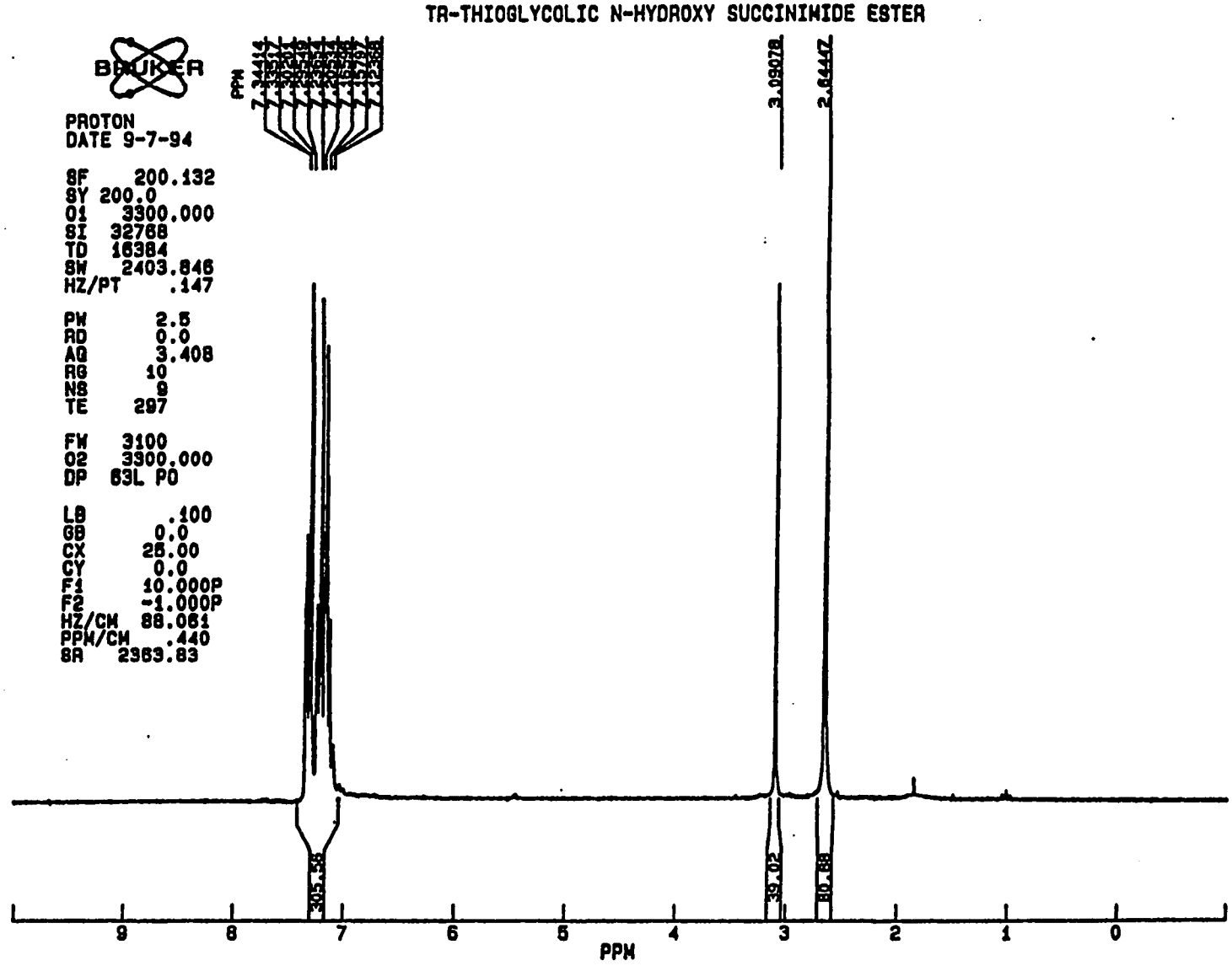

Figure 2-VIII: ^{1}H NMR spectrum of compound **2.5** (200 MHz)

mono-acylated material, it co-crystallized with the product during purification. The reaction was performed at elevated temperatures because of the low reactivity of the α amine group, which is caused by the electron-withdrawing nature and steric hindrance of the ester function. Note however, that diacylation did occur at room temperature when the reaction was allowed to proceed for long time periods, *e.g.* 48 hours. Direct coupling of the diamine and the acid **2.4** with the use of a carbodiimide was not attempted because both elevated temperatures and long reaction times tend to lead to O-to-N acyl transfer.

The acid **2.7** was synthesised by saponification of the methyl ester with sodium or potassium hydroxide. Because the starting material was a racemate, epimerisation of the α proton on the diamine was not a concern. If it had been, however, potassium carbonate and methanol could have been used as a milder hydrolysis method. It was reported that the carbonyl group of **2.7** was unreactive towards oxygen nucleophiles under mild coupling conditions (DCC/DMAP)[13]. The low reactivity was probably a result of steric crowding around the carbonyl, induced by the molecule's preferred conformations. These are a consequence of hydrogen bonding between the acid and the amide groups. The steric hindrance can be reduced by extending the acid functionality away from the chelate and this was accomplished by the addition of a glycine residue.

Coupling glycine methyl ester hydrochloride to **2.7** was accomplished by using EDAC in the presence of a tertiary base. The use of a mixed anhydride coupling was necessary because N-hydroxysuccinimide was not a good enough nucleophile to generate a

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[13]Bell, R.A. *Personal Communication* (1993).

reasonable amount of an active intermediate. The reaction was easily followed by TLC (UV-vis indicator). Furthermore, the formation of the product was indicated in the ^1H NMR spectrum of the crude reaction mixture by the appearance of an additional downfield multiplet at 7.44 ppm. This multiplet corresponded with the new amide NH. Hydrolysis of the ester to give the desired acid **2.9** was again accomplished with sodium or potassium hydroxide. The characterization and purity of the final product, an amorphous solid, was determined by comparing high resolution NMR spectra, melting point and thin layer chromatographic data with those of an authentic sample.

2.4 Experimental Section

*2,3-Diaminopropionic acid methyl ester p-toluenesulfonic acid salt (**2.2**)*

To 2.0 g (14.28 mmol) of (±) 2,3-diaminopropionic acid monhydrochloride suspended in 60 mL of MeOH, p-toluenesulfonic acid was added (10.82 g, 56.9 mmol). After the solution was heated at reflux for 24 hours it was evaporated to dryness at reduced pressure and the remaining solid was washed with 200 mL of diethyl ether rendering the methyl ester as colourless crystals. Yield: 6.5g, 99%; mp: 200^{0}C (decomp.); ^1H NMR (CD_3OD) [200 MHz]: δ 7.719 (d, J = 8.3, 2H, H-*ortho*), 7.251 (d, 2H, H-*meta*), 5.003 (bs, NH), 4.494 (m, 1H, CH), 3.853 (s, 3H, OCH_3), 3.529 (m, 2H, CH_2), 2.354 (s, 3H, $PhCH_3$); ^{13}C NMR (CD_3OD) [50 MHz]: δ 167.99 (COOMe), 143.00 (C-*para*), 141.99 (C-*ipso*), 129.91 (C-*meta*), 126.90 (C-*ortho*), 54.55 (CH), 51.14 (OCH_3), 39.51 (CH_2), 21.32 ($Ph\text{-}CH_3$).

2,3-Diaminopropionic acid methyl ester dihydrochloride

To 1.03 g (7.34 mmol) of (±)2,3-diaminopropionic acid monohydrochloride, suspended in 200 mL methanol and cooled in ice, HCl gas was added for 30 minutes. The solution was heated to reflux for 48 hours with rapid stirring. On cooling the solution was filtered and the filtrate concentrated at reduced pressure to give the methyl ester as a colourless solid; the yield was 698 mg (50%). The compound showed: mp: 152-155°C, lit.152-155°C[12]; ^{1}H NMR (DMSO-d6) [200 MHz]: 9.00 (bs, 6H, NH), 4.42 (m, 1H, CH_2), 3.80 (s, 3H, OCH_3), 3.36 (m, 2H, CH); ^{13}C NMR (DMSO-d6) [50 MHz]: 167.48 (COOMe), 51.46 (OCH_3), 50.17 (CH), 38.42 (CH_2).

2-(Triphenylmethylthio)ethanoic acid (**2.4**)

Triphenylcarbinol (24 g, 92.3 mmol) and mercaptoethanoic acid (8.5 g, 92.4 mmol) were dissolved in dichloromethane (50 mL) and glacial acetic acid (50 mL). A deep red solution was formed when boron trifluoride etherate (16 mL, 130 mmol) was added. The solution was stirred at room temperature for 1 hour, during which a precipitate formed. The dichloromethane was removed *in vacuo* and water (100 mL) was added to the residue. The product was collected by filtration, washed with water (3x100 mL), acetonitrile (50 mL) and cold diethyl ether (20 mL). The product was recrystallized from benzene. Yield: 26.68 g, 87%; mp: 155-157°C, lit. 155-157°C[10]; TLC: R_f = 0.33 (10:90 v:v CH_3OH/CH_2Cl_2); ^{1}H NMR [200MHz] ($CDCl_3$): δ 7.25 (m, 15H, aryl), 2.90 (s, 2H, S-CH_2); ^{13}C NMR [50 MHz] ($CDCl_3$): δ 175.77 (COOH), 144.09-128.05 (C-aryl), 67.44

(CPh_3), 34.67 ($S\text{-}CH_2$).

2-(Triphenylmethylthio)ethanoic acid N-hydroxysuccinimide ester via DCC coupling (**2.5**)

To 5.01 g of **2.4** (15 mmol) in dry DME (38 mL), 1.72 g of N-hydroxysuccinimide (15 mmol) was added. The solution was cooled to 0°C and DCC was added (3.20 g, 15.5 mol). The coupling agent was added slowly, so that the temperature did not rise above 5°C. The solution was stirred for one hour and subsequently refrigerated overnight. The heterogenous solution was filtered and the residue washed with DCM (50 mL). The filtrate was dried over sodium sulfate and then the solvent was removed under reduced pressure leaving a colourless semi-solid. The mixture was recrystallized from either ethyl acetate or THF leaving the title compound as a colourless solid. Yield: 3.2 g, 49%; mp: 206.0-207.5°C; $R_f = 0.46$ (2:98 v:v CH_3OH/CH_2Cl_2); ^1H NMR [200 MHz] ($CDCl_3$): δ7.17 (m, Haryl), 3.08 (s, 2H, SCH_2) 2.47 (s, 4H, $NC(O)CH_2$); ^{13}C NMR [50 MHz] ($CDCl_3$): δ165.0 (NC(O)), 163.00 (COON), 143.49 (C-*ipso*), 129.42 (C-*ortho*), 128.17 (C-*meta*), 127.08 (C-*para*), 67.98 (Ph_3C), 31.35 (SCH_2), 25.45 ($NC(O)\mathbf{CH_2}$).

2-(Triphenylmethylthio)ethanoic Acid N-hydroxysuccinimide ester via EDAC (**2.5**)

N-Hydroxysuccinimide (1.72 g, 15 mmol) was added to 2-(triphenylmethyl)-(thio)ethanoic acid (5.01 g, 15 mmol) in acetonitrile (20 mL). When ethyl-3-(3-dimethylamino)-propylcarbodiimide hydrochloride (EDAC-HCl) (3.16 g, 16.5 mmol) was added to the mixture, the solution became transparent after five minutes; shortly thereafter

a precipitate was seen. After the suspension was stirred for two hours the precipitate was collected by filtration. The colourless solid (3.88 g, 60%) was washed with cold acetonitrile (15 mL). After cooling the filtrate to 4°C overnight an additional crop of product was isolated by filtration (1.62 g, 25%). The compound showed: mp: 183-185°C, lit. 178.5-179.5°C[10]; TLC: R_f = 0.46 (2:98 v:v CH_3OH/CH_2Cl_2); ^1H NMR [200MHz] ($CDCl_3$): δ 7.17 (m, 15H, H-aryl), 3.08 (s, 2H, SCH_2) 2.47 (s, 4H, ($\mathbf{CH_2}C(O)$); ^{13}C NMR [50MHz] ($CDCl_3$): δ 165.0 ($CH_2\mathbf{C}(O)$), 163.00 ($SCH_2\mathbf{C}OON$), 143.49 (C-*ipso*), 129.42 (C-*ortho*), 128.17 (C-*meta*), 127.08 (C-*para*), 67.98 ($\mathbf{C}Ph_3$), 31.35 (SCH_2) 25.45 ($\mathbf{CH_2}C(O)$).

Methyl 2,3-bis(triphenylmethylthioacetylamino)propanoate (**2.6**)

To 3.62 g (8.40mmol) of **2.5** dissolved in 50 mL DCM, 1.92 g (4.16 mmol) of **2.2** was added. To the rapidly stirred mixture 1.34 mL of DIPEA (7.69 mmol) was added. The mixture was then heated to reflux for 8 hours. The solution was cooled and extracted with 1M HCl (2 x 10 mL), 1M $NaHCO_3$ (2 x 10 mL) and distilled water (DW) (3 x 15 mL). The solution was then evaporated to dryness at reduced pressure and the solid was washed with DW (200 mL), MeOH (5 mL) and ether (5 mL). The colourless solid was recrystallized from acetone and subsequently dried *in vacuo* to give 2.2 g (78%) of **2.6**. The compound showed: mp: 68-70°C, lit. 66-70°C[12]; TLC: R_f =0.44 (2% MeOH/DCM); ^1H NMR ($CDCl_3$) [200 MHz]: δ 7.324-7.075 (m, 17H, H-aryl), 6.708 (d, J = 6.7, 1H, **CHNH**), 6.094 (m, 1H, $CH_2\mathbf{NH}$), 4.064 (m, 1H, CH), 3.087 (m, 2H, $CH\mathbf{CH_2}$), 2.934 (s,

2H, CH_2); ^{13}C NMR ($CDCl_3$) [50 MHz]: δ 170.06 (**C**OOMe), 168.55, 168.89 (C(O)NH), 143.87 (C-*ipso*), 129.50 (C-*ortho*), 128.09 (C-*meta*), 126.98 (C-*para*), 67.71 (Ph_3C), 52.72 (C-2 and C-12), 41.24 (CH**C**H_2), 35.93 (CH_2), 35.72 (SCH_2).

2,3-Bis(triphenylmethylthioacetylamino)propanoic acid (**2.7**)

To 1.0 g (1.33 mmol) of **2.6** in a 1:1 mixture of THF and water (80 mL), 100 mg (2.5 mmol) of NaOH was added. After the mixture was heated to reflux for three hours under an atmosphere of nitrogen, the solution was acidified to pH 3.9 using 6M HCl, and concentrated to 30 mL under reduced pressure. The precipitate which formed was collected by filtration and the colourless solid washed with DW (100 mL) and sparingly with ether (5 mL). The filtrate was concentrated and diluted with 15 mL of MeOH and refrigerated overnight. The resulting solid was collected by filtration and washed again with DW and ether as above, yielding an additional crop of the title compound. Yield 900 mg, (97%); mp: 214-215°C, lit. 206-207.5°C[12]; TLC: R_f = 0.22 (10%MeOH/DCM); ^1H NMR (CD_3OD) [200 MHz]: δ 7.643 (m, 15H, Haryl), 4.590 (m, 1H, CH), 4.063 (m, 2H, **C**H_2CH), 3.269 (m, 2H, $TrSCH_2$); ^{13}C NMR (CD_3OD) [50 MHz]: δ171.21 (C-COOH), 168.90, 168.55 (C(O)NH), 143.87 (C-*ipso*), 129.50 (C-*ortho*), 128.09 (C-*meta*), 126.98 (C-*para*), 65.88 (Ph_3C), 52.05 (CH), 43.96 (**C**H_2CH), 35.85, 32.33 ($TrSCH_2$).

Methyl N-{N', N''-bis[2-(triphenylmethyl)(thio)ethanoyl]-2,3-diaminopropanoyl} glycinate (**2.8**)

Compound **2.7** (800 mg, 1.09 mmol) was dissolved in DCM (15 mL) along with glycine methyl ester hydrochloride (149.5 mg, 1.20 mmol) and triethylamine (1 mL). EDAC-HCl was then added (230.4 mg, 1.20 mmol) and the solution was stirred for 36 hours before it was extracted with 0.1N HCl (2 x 10 mL) and distilled water (2 x 10 mL). The organic layer was evapourated and the product isolated by radial chromatography (CH_2Cl_2/ MeOH). Yield (850 mg, 97%); mp: 168-171°C, lit. 168-171°C[12]; TLC: $R_f = 0.30$ (98% DCM:2% MeOH); ^{1}H NMR ($CDCl_3$) [500 MHz]: δ7.16-7.44 (m, overlap, H-aryl, amide, **NH**CH_2), 7.09 (d, J= 5.8, 1H, **NH**CH), 6.53 (t, J= 6.3, 1H, **NH**CH_2CH), 3.99 (m, 1H, CH_2**CH**), 3.92 (m, 2H, **CH**$_2$C(O)OCH_3), 3.70 (s, 3H, OCH_3), 3.27 (m, 1H, **CH**$_2$CH), 3.03 (m, 5H, SCH_2, **CH**$_2$CH); ^{13}C NMR ($CDCl_3$) [126 MHz] δ170.13, 169.80, 169.69, 169.35 (C(O)), 149.93 (C-*ipso*), 126.98-129.56 (C-aryl), 67.72 (CPh_3), 54.75 (OCH_3), 52.31 (CH_2**CH**), 41.18 (**CH**$_2$CH), 36.06, 35.80 (SCH_2); MS (NH_3^+ DCI) m/z(RI%): 808[M+1](20), 322(10), 262(38), 243(100)[Tr^+].

N-{N', N''-bis[2-(triphenylmethyl)thioethanoyl]-2,3-diaminopropanoyl} glycine (**2.9**)

Compound **2.8** (1.19 g, 1.47 mmol) was suspended in absolute ethanol (25 mL) and sodium hydroxide (2.9 mL, 1M) was added. After 12 hours the solution was acidified to pH= 2.9 with HCl (6M), and the ethanol evaporated under reduced pressure. The milky suspension was diluted with brine (10 mL) and then extracted with chloroform (3 x 50 mL). The organic layers were combined, evaporated to dryness and the product isolated by radial chromatography (chloroform/methanol); the yield was 1.06 g (91%). The compound,

an off white solid, showed: mp: 120-121°C, lit. 118.5-121°C[12]; TLC: R_f= 0.13 (90% DCM:10% MeOH); ^{1}H NMR (CD_3COCD_3) [500 MHz]: δ7.38-7.17 (m, 29H, H-aryl), 4.25 (m, 1H, CH_2**CH**), 3.89 (m, 2H, **CH_2**COOH), 3.31 (m, 2H, **CH_2**CH), 2.92, 2.89 (s, 4H, SCH_2); ^{13}C NMR (CD_3COCD_3) [126 MHz]: δ169.87, 168.78, 169.26, 167. 24 (C(O)), 143.31 (C-*ipso*), 128.51-125.83 (C-aryl), 66.0 (CPh_3), 52.70 (CH_2**CH**), 40.24 (**CH_2**COOH), 39.55 (**CH_2**CH), 35.11 (SCH_2).

Chapter 3

Tamoxifen

3.1 Rationale

Breast cancer is the most common form of cancer; 183 000 cases were reported in the United States in 1993 and in the same year 46 000 deaths were associated with patients who had breast cancer[1]. One in every three new cancers in women is breast cancer and it is the second leading cause of death among all forms of cancer. An imaging agent which could detect tumours in their infancy would be invaluable because early detection of cancerous tumours is crucial if chemotherapy is to be effective[2]. By taking advantage of the excellent resolution of SPECT and PET it may be possible to detect extremely small tumours- much smaller than those detected by mammography. Breast cancer imaging using radio nuclides would have the added advantage that it would reduce the physical discomfort felt by the patients who undergo mammograms.

3.2 Estrogen and Breast Cancer

In 1974 a study was completed which showed that approximately 60% of patients

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[1]Berg, J.W.; Hutter, R.V. P., *Cancer*, **1995**, 1, 75.

[2]Carter, C.L.; Allen, G.; Henderson, D.E. *Cancer*, **1989**, 63, 181.

with estrogen receptor positive breast tumours responded to endocrine therapy, whereas less than 10% of patients with estrogen receptor negative tumours responded[3]. This is related to the discovery that estrogen receptors are found in varying concentrations in breast tumours. It is generally believed that estrogen can directly stimulate the growth of breast cancer; therefore treatment with antiestrogens that block the estrogen receptor (ER) is a logical approach to reducing tumour growth.

3.3 Antiestrogens

Diethylstilbestrol was one of the first potent antiestrogens discovered[4]. Further research led to the discovery that triphenylethylene derivatives were also antiestrogenic. Triphenylethylene derivatives enccloclomiphene, nafoxidine and tamoxifen, which were originally designed as postcoital contraceptives, were all tested in phase I and phase II clinical trials as antiestrogens but only tamoxifen (**Figure 3-I**) had a low incidence of side effects[5].

The mechanism of action of tamoxifen, Z-1-*p*-(2-dimethylaminoethoxy)phenyl)-1,2-diphenylbut-1-ene) is thought to be displacement of the growth promoting hormone estradiol from its protein receptor[6]. The Z isomer of tamoxifen has antiestrogenic activity

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[3]Borgna, J.L.; Coezy, E.; Rochefort, H., *Biochem. Pharmacol.* **1982**, 31, 3187-3191.

[4]Dodds, E. C.; Goldberg, L.; Lawson, W.; Robinson, R., *Nature*, **1938**, 141, 247-248.

[5]Legha, S.S.; Carter, S.K. *Cancer Treat. Rev.* **1976**, 3, 205-216.

[6]Jordan, V.C. *Pharmacological Reviews*, Vol. 36, No. 4, p245-276 (1984).

while the E-isomer is estrogenic. Studies toward the synthesis of tamoxifen focus on developing diastereoselective routes towards the Z-isomer.

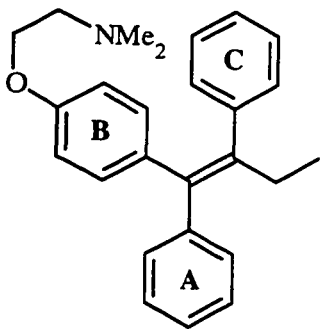

Figure 3-I: Z-Tamoxifen

3.4 Radio labelled Tamoxifen Compounds

^{131}I derivatives of E and Z tamoxifen have been synthesised and their respective biodistributions in mice and humans evaluated[7]. Hunter *et al.*[7] found that the biodistribution of [^{131}I]-E tamoxifen (**Figure 3-II**) in mice which contained cancer tumours demonstrated significant radioactivity in the tumours and the uterus. There was, however, insufficient uptake in human cancer patients to develop a commercial imaging agent. The location of the iodine label and the loss of iodine due to the relatively weak carbon iodine

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[7]Strickland, L.A.; Ponce, Y.Z.; Hunter, D.H.; Zabel, P.L.; Powe, G.M.; Driedger, A.A.; Chamberlain, M.J.; *Drug Design and Delivery*, **1990**, Vol. 6, pp 195-212.

bond could explain the insufficient tumour uptake in human breast cancer tumours. One method of overcoming these problems is to covalently attach a chelate, whose radionuclide complex is known to be stable *in vivo*, at a site at which substituents do not tend to affect the biodistribution of the parent molecule.

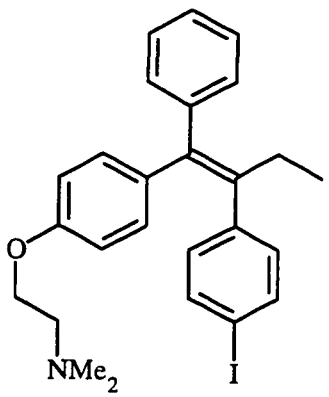

Figure 3-II: An ^{131}I derivative of tamoxifen

3.5 Design Rationale

Substitution on ring B of tamoxifen is not feasible because data from binding assays of tamoxifen derivatives suggest that ring B derivatives alter tamoxifen's pharmacological activity[2]. From the aforementioned binding studies of tamoxifen derivatives, ring A appears to be a more feasible location for derivatization. While studying the atropisomers of tamoxifen (which differ in the wind of the helix created by the

propeller-like arrangement of the aromatic rings), McCague *et al.*[8] synthesised trans-2-methyl-tamoxifen. This compound, where the methyl substituent was in the ortho position of ring A, had an estrogen receptor binding affinity identical to that of tamoxifen[8]. From this, we concluded that the *ortho* position of ring A would be the logical site to attach a chelant, through a spacer chain.

3.6 Retrosynthetic Analysis

Two approaches to the synthesis of **I** (**Figure 3-III**) were used; approach **A** involved the total synthesis of the tamoxifen derivative before coupling to the chelate, while strategy **B** entailed coupling of a chloro-tamoxifen species (**XII**) to the chelate prior to the addition of the dimethylamine moiety. In approach **A**, the first disconnection was the amide bond between the chelate and the tamoxifen species **III**. The amine **III** originated from the azide-substituted fatty acid derivative of **VI**, the synthesis of which was based on the approach of McCague *et al.*[8] Compound **VI** was generated by a nucleophilic substitution, followed by a base catalysed elimination, of aryl anion **VIII** on ketone **VII**. The ketone arose from a Friedel-Crafts acylation of 2-phenylbutyric acid **IX** on phenol **X**. With the exception of **VII** and **X**, all intermediates in the synthesis of **I** have not been reported in the literature.

In approach **B**, chloro compound **XI** was constructed prior to addition of the

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[8]McCague, R.; Jarman, M.; Leung, On-Tai.; Foster, A.B.; Leclercq, G.; Stoessel, S.; *J. Steroid Biochem.* **1988**, Vol. 31, No. 4B, 545-547.

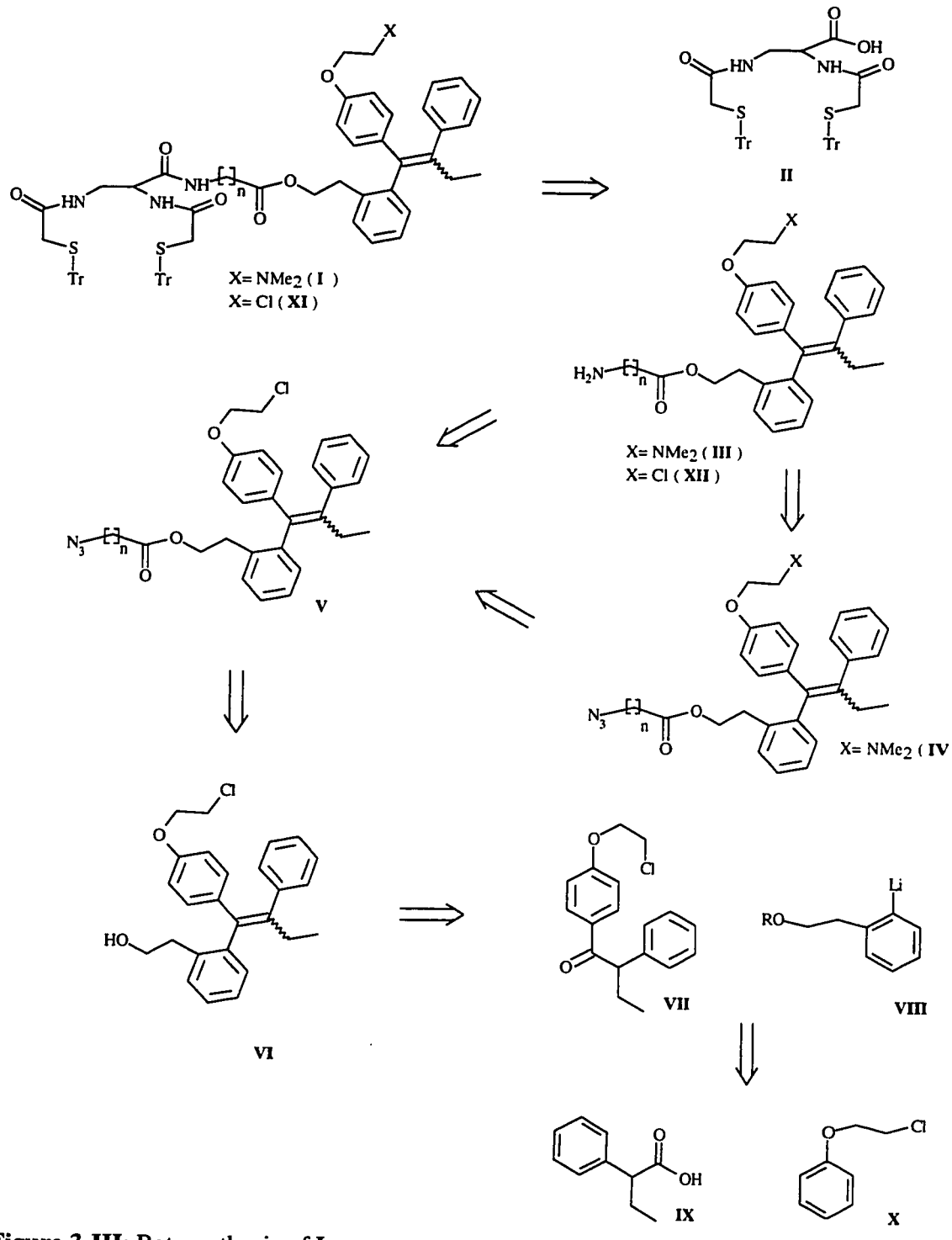

Figure 3-III: Retrosythesis of I

dimethylamino moiety. This approach proved more successful because it facilitated isolation of the final two intermediates, **XI** and **XII**.

*3.7 Synthesis of **I***

The synthesis began by following the literature procedure for the monosubstitution of 1,2-dichloroethane by phenolate anion under phase transfer (PTC) conditions[9] (**Figure 3-IV**). Sodium hydroxide deprotonated phenol which, in the presence of dimethyldioctadecylammonium bromide, reacted with the organic phase (an excess of 1,2-dichloroethane) to give the desired compound in good yield (76%). The chloro-ether was subjected to Friedel-Crafts acylation[10] by use of either (±)-2-phenylbutyric acid and trifluoroacetic anhydride (TFAA), or (±)-2-phenylbutyric acid and aluminum trichloride in carbon disulfide to give **3.2** in 79% and 57% yields respectively.

The acylation took place regioselectively as a result of the electronic and steric influence of the phenoxy derivative. The oxygen substituent activated the ortho and para positions to electrophilic acylation (**Figure 3-V**) because the cation generated by the addition of the acylium ion (from the addition of TFAA) was stabilized by delocalization of the charge over several canonical forms. Evidence from ^{1}H and ^{13}C NMR confirmed that the acylation product was entirely *para* substituted as a result of steric hindrance of

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[9]McCague, R. *J. Chem Miniprint*, **1986**, 771-790.

[10]F.R. Jensen and G. Goldman; Friedel-Crafts and Related Reactions, vol. II, G. Olah (ed.), Wiley Interscience, New York, 1964, Chapter XXXVI.

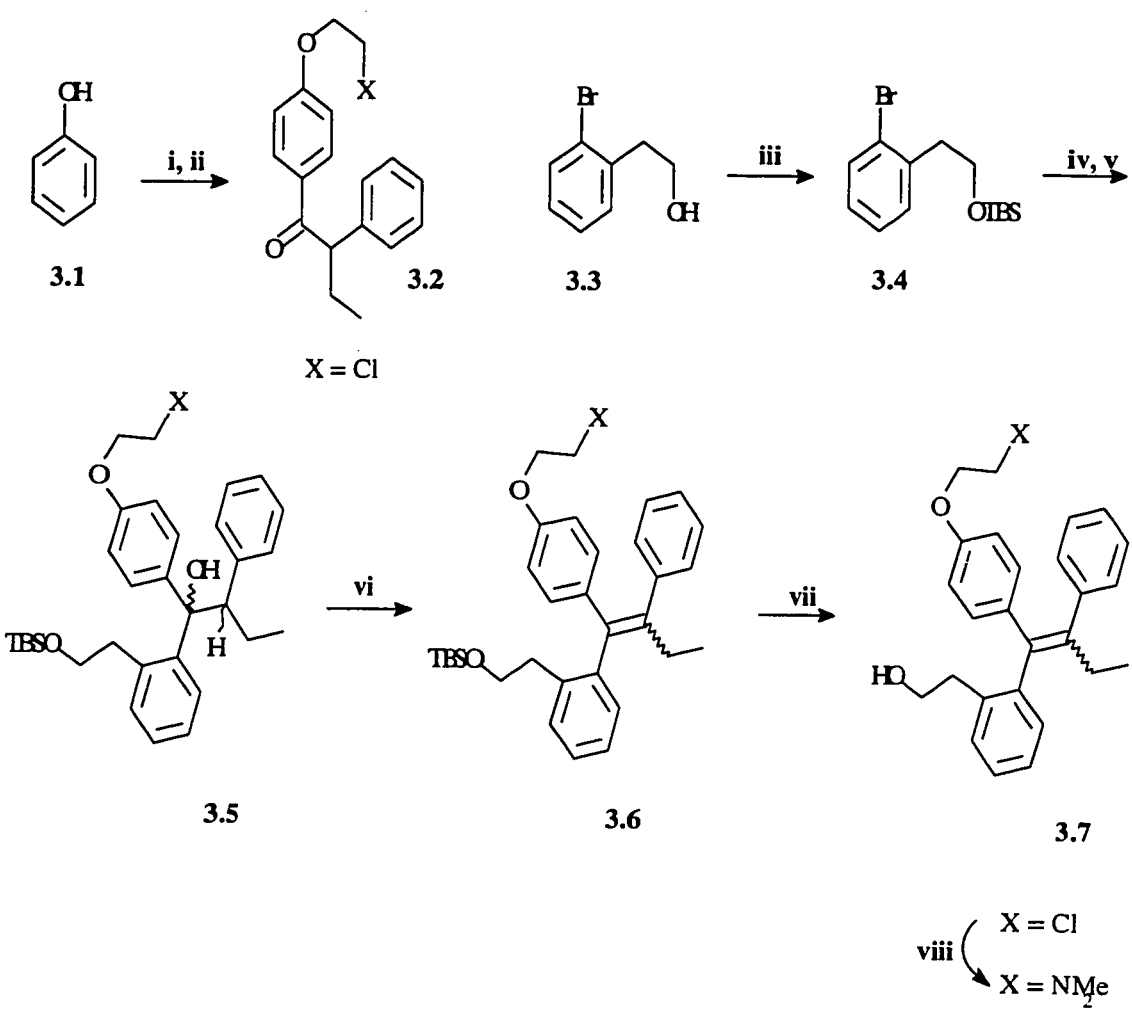

Figure 3-IV: I) 1,2-dichloroethane, NaOH, PTC ii) 2-phenylbutyric acid, TFAA iii) TBSCl, imidazole iv) *n*BuLi, -78°C v) **3.2**, THF vi) $(COCl)_2$, pyridine, -10°C vii) TBAF viii) $HNMe_2$, EtOH, Δ.

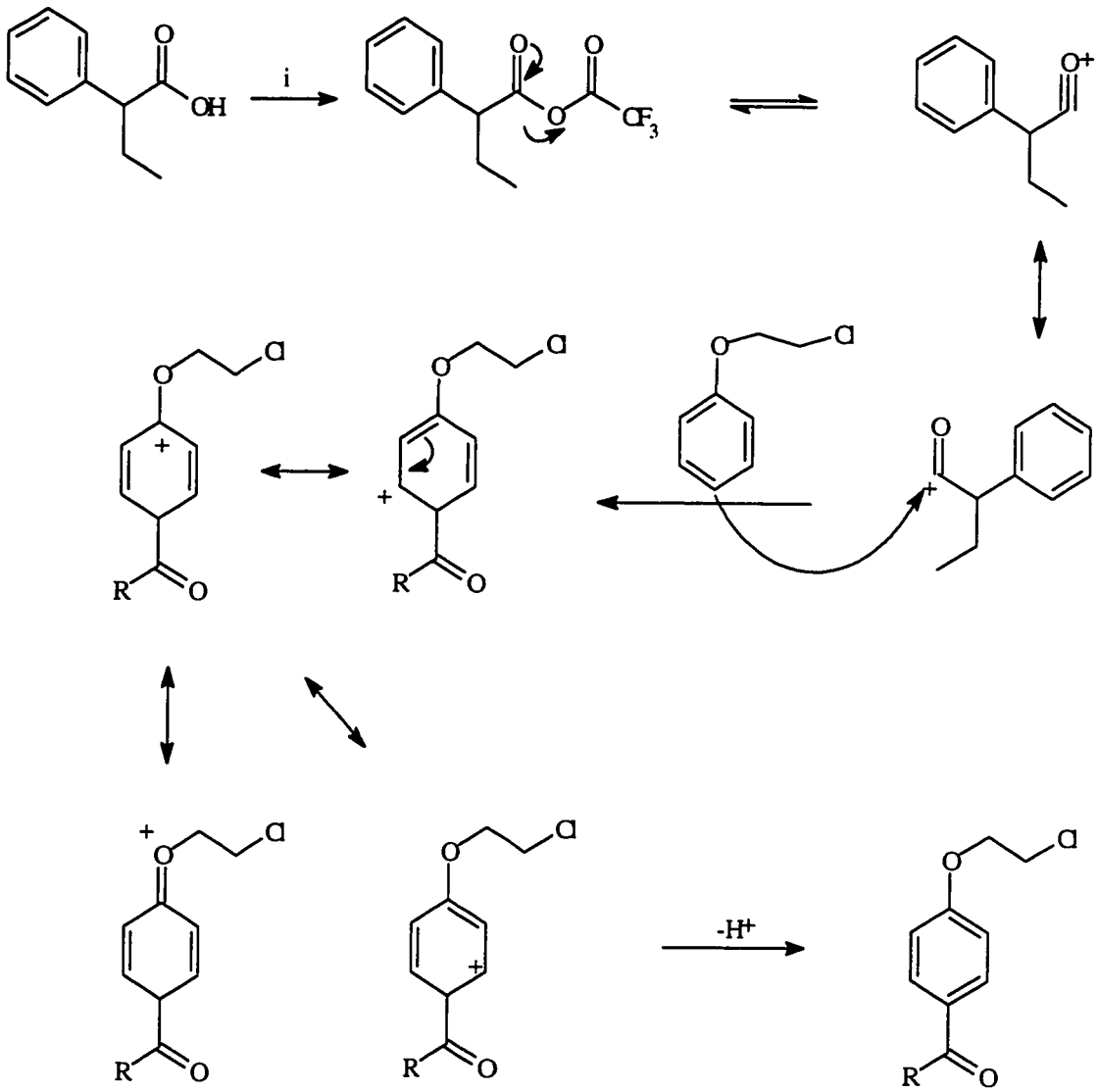

Figure 3-V: Mechanisim of a TFAA induced Friedel Crafts acylation-i) TFAA

the chloro ethyl group to ortho substitution.

The A ring containing part of the linker arm for the chelate was then added in the form of a *tert*-butyldimethylsilyl ether[11] of 2-bromophenethyl alcohol, the nucleophile being generated by metal halogen exchange. The anion reacted with the ketone **3.2** to generate two diastereomeric alcohols, **3.5**, which were not normally isolated but converted directly to the alkenes **3.6** by reaction with thionyl chloride and pyridine at -10^{0}C. These milder elimination conditions were used because the literature procedure employing concentrated HCl in ethanol caused the loss of the silyl protecting group and subsequent elimination of the resultant alcohol. The probable mechanism for elimination (**Figure 3-VI**) involved formation of the chlorosulfite intermediate which, rather than form the chloride, eliminated to form the alkene in the presence of pyridine.

The next step in the synthesis was the removal of the TBS protecting group of **3.6** by the use of tetrabutylammonium fluoride in THF[11], which occurred in excellent yield (96%).

Cram's rules[12] were used to predict which isomer of **3.6** should predominate. The rules are based on a model for determining the tendency of a system to generate asymmetric induction in ketones upon nucleophilic substitution. Cram's rules assume a kinetically controlled reaction (non-equilibrating and non-catalytic) for asymmetric 1,2-addition to aldehydes and ketones. The three groups attached to the chiral center are R_S

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[11]Corey, E.J; Venkateswarlu, A. *J. Am.Chem. Soc.* **1972**, 94, 6190.

[12]Cram, D.J.; Abd Elhafez, F.A. *J. Am. Chem. Soc.* **1952**, 74, 5828.

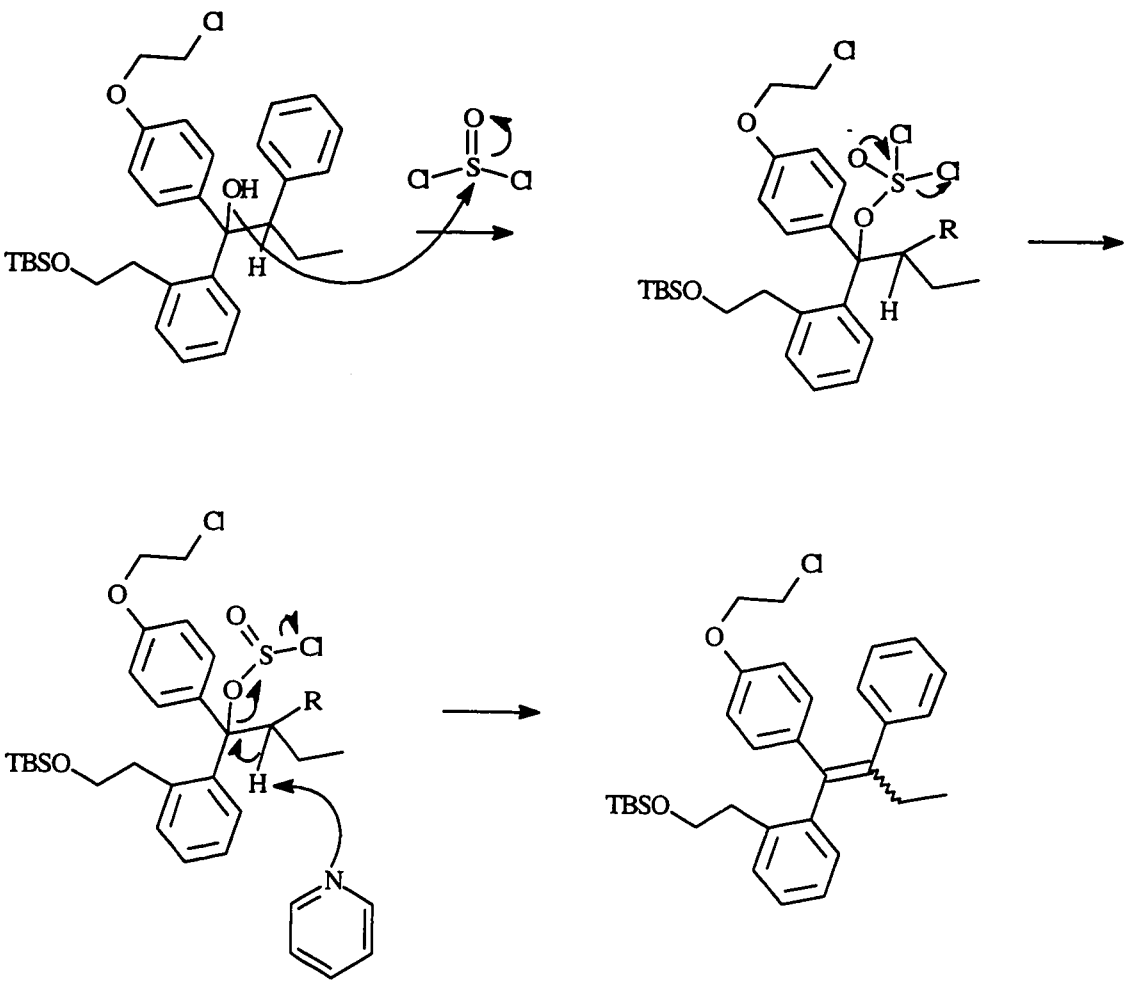

Figure 3-VI: Mechanism of a thionyl chloride/pyridine elimination of compound **3.5**

(small substituent), R_M (medium substituent) and R_L (large substituent). Cram's model is shown as a Newman projection (**Figure 3-VII**) and presumes a predominant rotamer in which the large substituent (R_L) is syn to the R^1 group attached to the carbonyl. The nucleophile is delivered from the less sterically hindered face (over the smallest

substituent) to give the major diastereomer. Cram's rules predict that compound **3.5** should be predominately *erythro,* which, under E_2 conditions, should form the desired E-alkene.

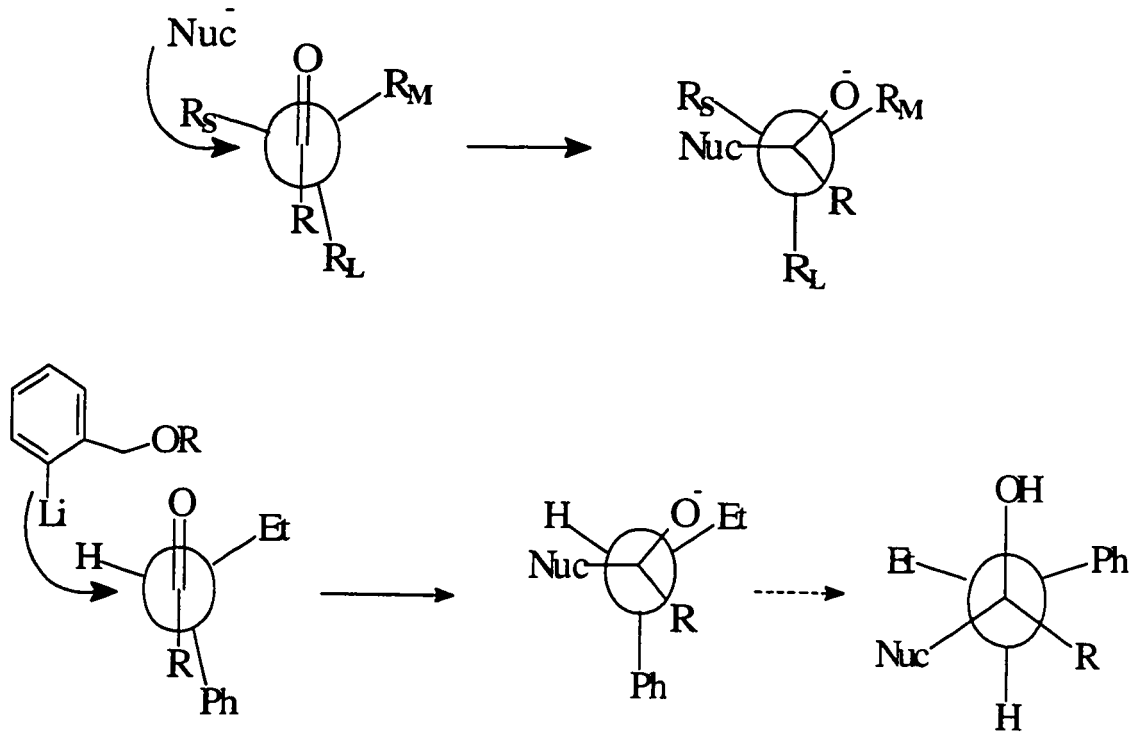

Figure 3-VII: Asymmetric induction by nucleophilic substitution on ketones (Cram's Rules).

The chemical shifts of the proton (**Figure 3-VIII**) and carbon atoms of each diastereomer of **3.7**, at 200 MHz and 50 MHz respectively, were significantly different and

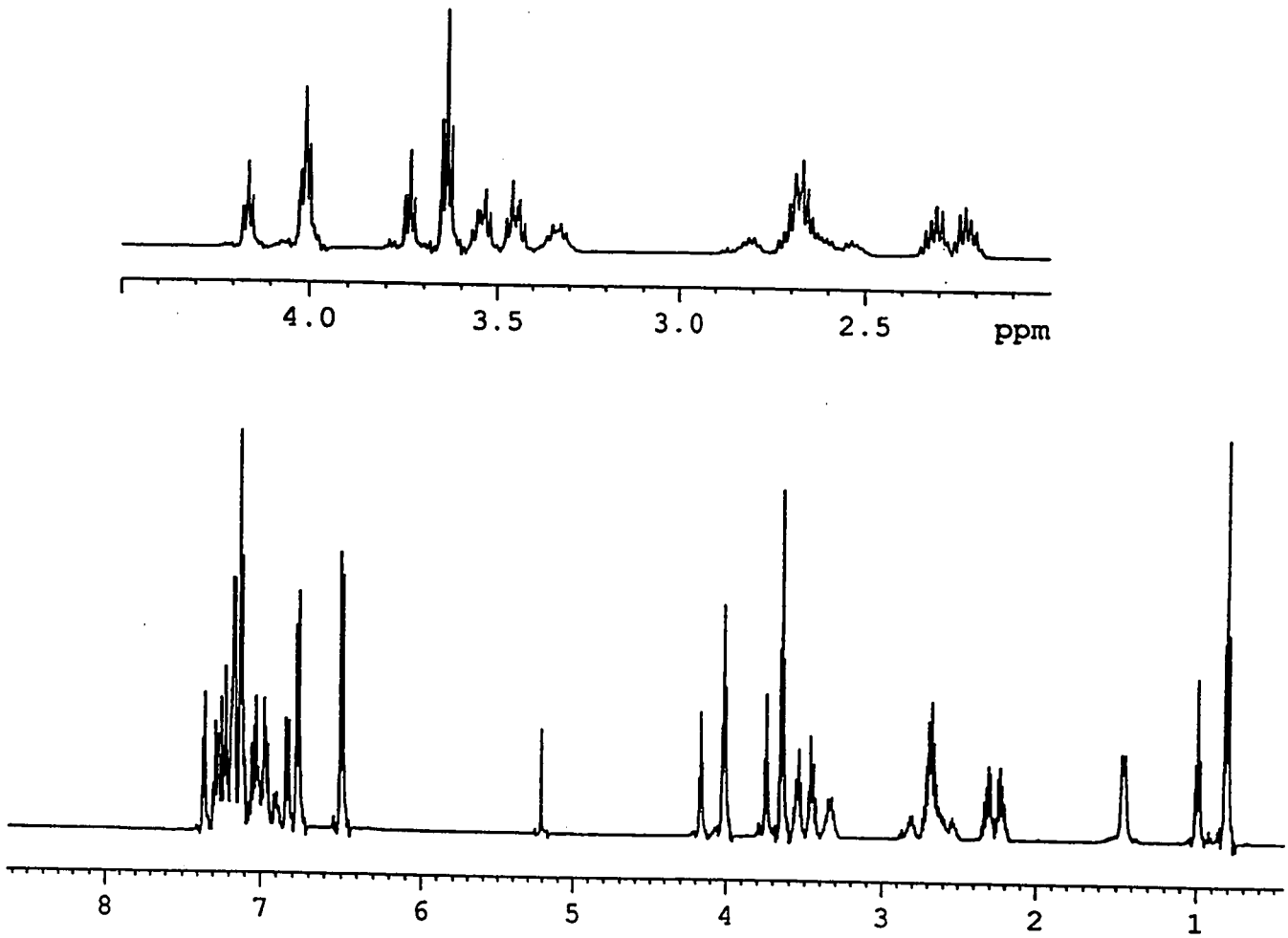

Figure 3-VIII: ^{1}H NMR spectrum of compound **3.7** [500 MHz]

each isomer could be distinguished readily. However, to assign which isomer was the E and which was the Z, a two-dimensional nuclear Overhauser effect[13] (2-D NOE) experiment (NOESY) was used. From the ^1H spectrum, the pairs of doublets at 6.81 and 6.50 ppm were assigned to the AB system of ring B. The downfield doublet at 6.81 exhibited an NOE correlation to signals in the aromatic region but showed no correlation to the methylene of the ethyl group of the alkene. This confirmed that these signals belonged to the E-isomer. The COSY spectrum was then used to assign the remainder of the signals belonging to that isomer and it was concluded that the major isomer was in fact the E-isomer in a ratio of approximately 5:1.

After the chlorine was substituted with dimethylamine, attempts were made to couple the alcohol **3.7** to the DADT chelant without success; it was obvious by thin layer chromatography that no reaction was taking place. An attempt to convert the phenethyl alcohol to an azide was also ineffective and it was decided therefore to extend the linker arm further away from the triaryl alkene.

Fatty acid derivatives were selected for the spacer chain, because the length of the spacer can be altered easily as there are numerous fatty acid derivatives which are commercially available. Once the general synthetic route was developed, varying lengths of spacers can be tested until the optimum length associated with high binding efficiency is found. In the present synthesis (**Figure 3-IX**), bromododecanoic acid was converted to the azide prior to reaction with thionyl chloride. The acid chloride was then converted to an

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[13]Bodenhausen, G; Kogler, H.; Ernst, R.R. *J. Magn. Reson.* **1984**, 58, 370.

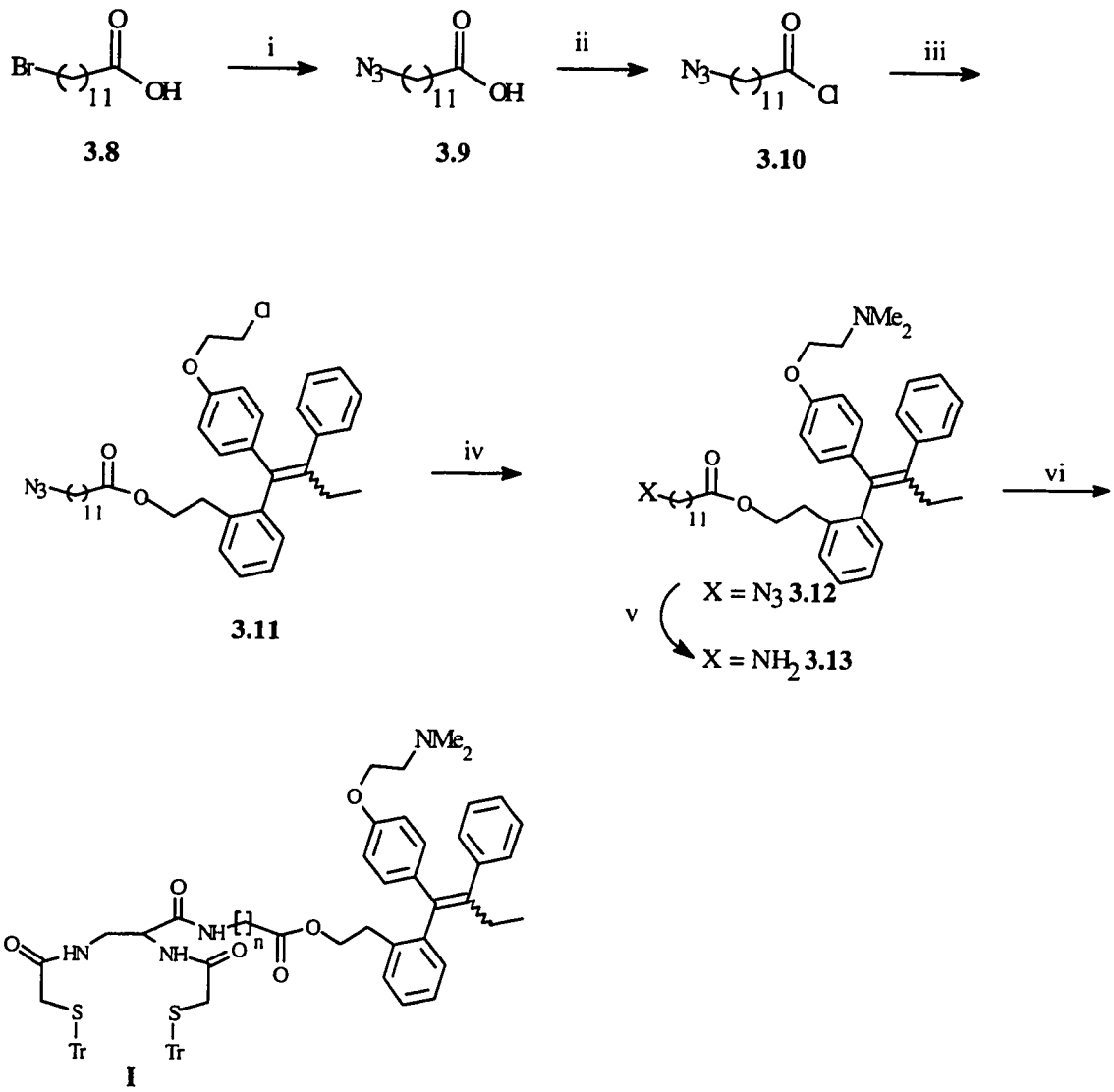

Figure 3-IX: i) NaN_3, DMF, Δ ii) $SOCl_2$, DCM iii) **3.7**, CH_2Cl_2, Δ iv) $HNMe_2$, EtOH, Δ v) PPh_3, H_2O vi) DADS-COOH, EDAC.

ester by reaction with alcohol **3.7**. An unexpected advantage of using the long chain fatty acid was that small quantities of each isomer of **3.11** could be separated by radial chromatography. Spectrum **a** (**Figure 3-X**) shows a fraction which contained pure E-isomer while spectrum **b** is of an enriched fraction containing a 1:5 E:Z ratio. In spectrum **a**, the doublets at 6.76 and 6.51 ppm are the protons on ring B of the E-isomer. The equivalent pair of protons in the Z isomer, shown in spectrum **b**, move to a lower field as a result of the lack of the C-ring anisotropy. Another set of signals used to determine the isomer ratios was the triplet at 0.810 ppm in spectrum **a**, assigned to the homoallylic methyl group, and the corresponding triplet at 0.960 ppm in the Z-isomer. This triplet shifted downfield in the Z- isomer, again because of a change in magnetic anisotropy. During the development of the synthetic methodology towards **I**, a relatively large quantity of **3.11** was required and for practical purposes the non-enriched, 5:1 E:Z material was used. Should the results of the biodistribution studies show promise, the isomers of **3.11** can be separated and pure E-**I** and Z-**I** synthesised.

In approach **A**, the azido chloro species **3.11** was converted to the dimethylamino species **3.12** by reaction with dimethylamine in ethanol at an elevated temperature. If extensive reaction times were used (greater than 4 days) a minor byproduct occurred as a result of cleavage of the ester bond generating the phenethyl alcohol species **3.7**. The ester cleavage was most likely caused by ethoxide which was generated by the deprotonation of ethanol by dimethylamine.

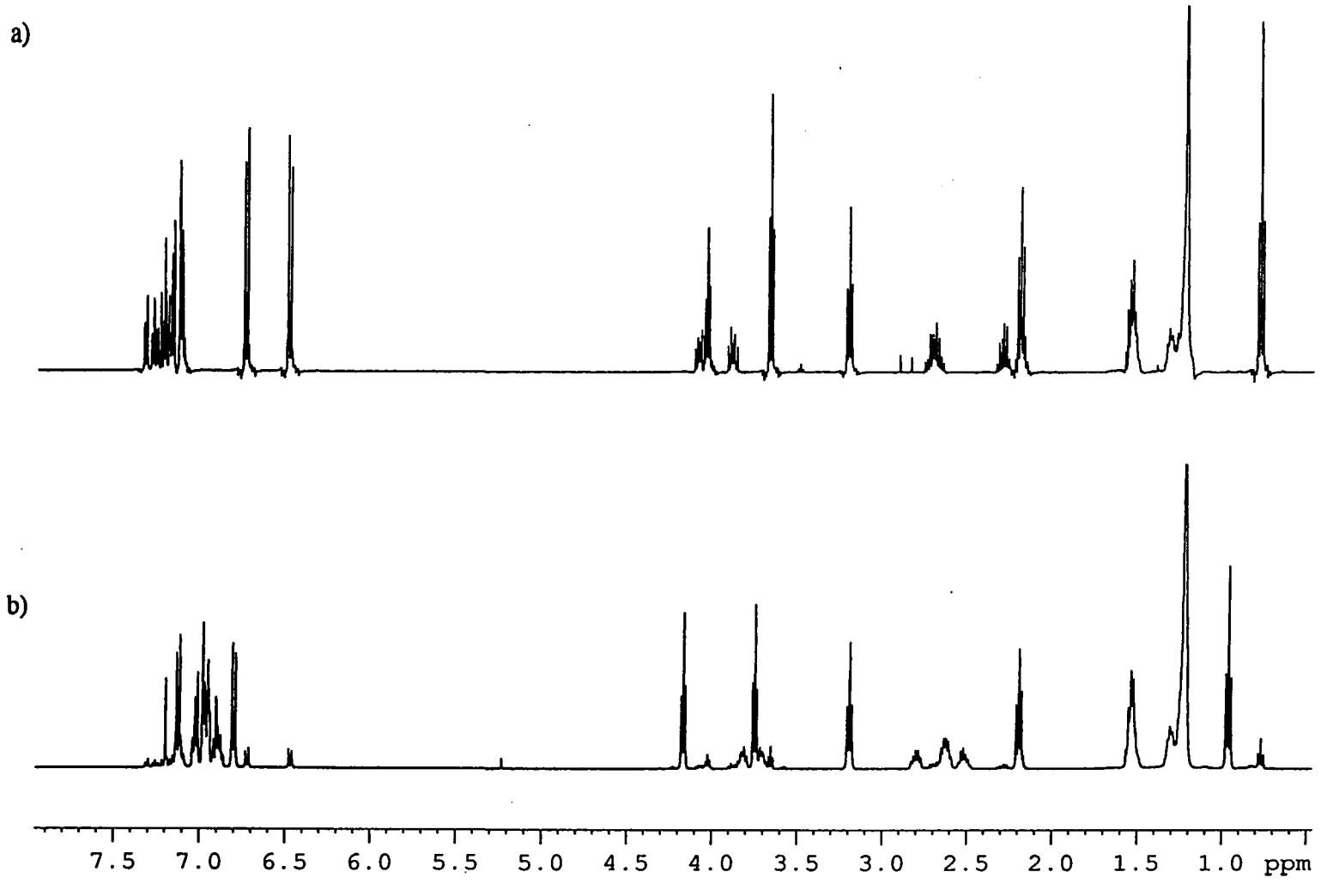

Figure 3–X: Proton NMR spectrum a) E–3.11 b) 1:5 E:Z 3.11 [500 MHz]

The azide **3.12** was reduced by triphenylphosphine[14] because other methods (lithium aluminum hydride and catalytic hydrogenation) would have caused reduction of the ester and alkene groups respectively. The mechanism of the phosphine- induced reduction is shown in **Figure 3-XI**. The attack on the azide by triphenylphosphine results in a phosphotriazene intermediate which quickly loses nitrogen to yield the isolable iminophosphorane[15]. In the presence of water, this ylid hydrolyzes to give an amine and triphenylphosphine oxide. Note that, for reasons that are not well understood, this reaction must be run under concentrated conditions; reactions performed under dilute conditions result in exceedingly low yields[15].

Reduction appeared to occur quantitatively; however, isolation of the resulting amine was difficult and only a low yield of product was isolated (25%). The amine was immediately coupled to the acid of the DADT chelate by the use of EDAC[16] in apparently good yield as judged by thin layer chromatography. Again, however, purification of the product from impurities proved difficult and required radial chromatography and a low yield of pure product was isolated (26%).

In an attempt to improve the yields in the synthesis a strategy **B** was developed. The azide **3.11** was reduced, again by the use triphenylphosphine (**Figure 3-XII**).

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[14]Staudinger, H.; Meyer, J., *Helv. Chim. Acta.*, **1919**, 2, 635; Vaultier, M.; Knouzi, N.; Carrie, R. *Tetrahedron Lett.*, **1983**, 24, 763.

[15]Alfredo Capretta, *Ph.D. Thesis*. McMaster University (1992).

[16]Sheehan, J.C.; Ledis, S.L. *J. Am. Chem. Soc.*, **1973**, *95*, 875.

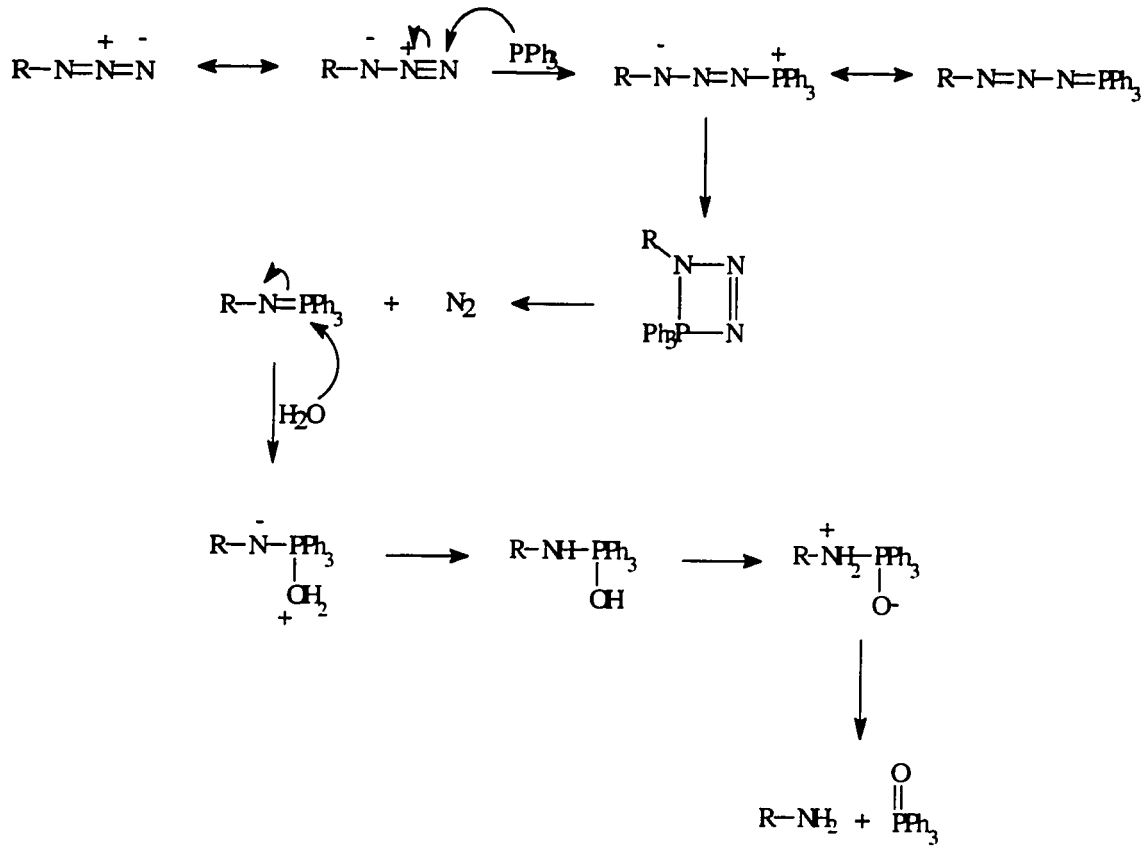

Figure 3-XI: Mechanism of azide reduction by triphenylphosphine

Formation of the phosphonium salt from the alkyl chloride fragment was not a concern because the reduction was carried out at room temperature. The resulting amine **3.14** was coupled to the chelate in good yield (76%) and replacement of the chloro substituent with dimethylamine also occurred in good yield (60%). There was no evidence of decomposition of the ester or amide bonds so long as the reaction times were kept to a

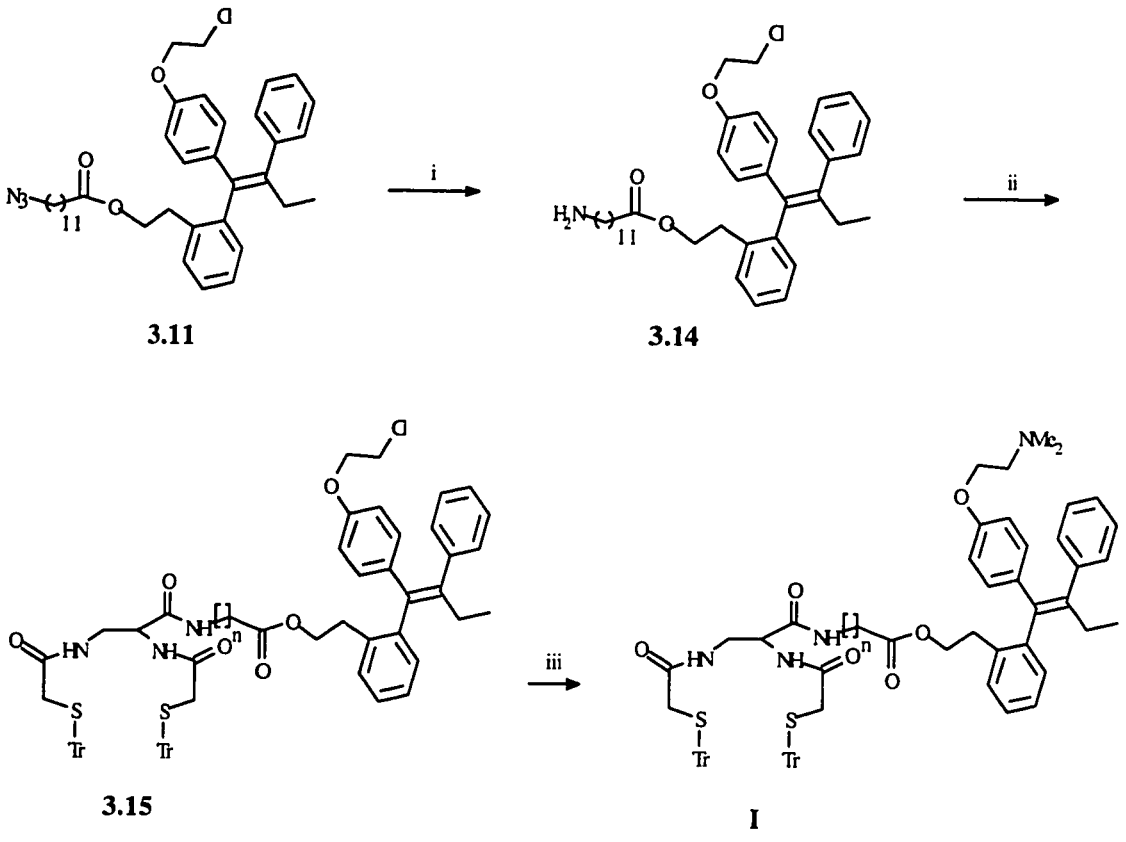

Figure 3-XII: i) PPh_3, H_2O ii) DADS-COOH, EDAC iii) $HNMe_2$, EtOH, Δ

reasonable length (20-24 hours).

The addition of dimethylamine to **3.14** was observed in the ^1H NMR spectrum as two singlets at 2.325 and 2.267 ppm, corresponding to the two isomers. There was also a significant shift of the methylene protons adjacent to the dimethylamine moiety; upon substitution, the protons shifted upfield by approximately 1 ppm. The ^{13}C NMR spectrum contained no ambiguities; the resonance associated with the N-methyl moiety was

observed at 45.80 ppm. The methylene protons adjacent to the N-methyl moiety shifted downfield upon substitution of the chloro group from 41.99 ppm to 58.18 ppm.

Using approaches **A** and **B**, compound **I** was synthesised in 18 steps in 0.8% and 7% yield, respectively. This work is an example of a total synthesis of a biomolecule-chelate complex for the purpose of developing a site-specific radioimaging agent. Testing of compound **I** in tumour models remains for future work.

3.8 Experimental Section

(2-Chloroethoxy)benzene

The procedure described by McCague was used[9]. Phenol (28.2 g, 300 mmol), dimethyldioctadecylammonium bromide (1.8 g, 3.0 mmol), sodium hydroxide (24.0 g, 600 mmol) in distilled water (225 mL) and 1,2- dichloroethane (225 mL) were heated to reflux together for 48 hours. The organic phase was separated, dried with magnesium sulfate, and concentrated under reduced pressure to produce a translucent yellow oil. This crude product was subsequently purified by vacuum distillation to give the title compound as a colourless oil (35.6 g, 76%). The compound showed: b.p.: 100-102°C @ 12 mmHg; ^{1}H NMR ($CDCl_3$) [200 MHz]: δ 7.30 (m, 5H, ArH), 4.23 (t, 2H, OCH_2), 3.81 (t, 2H, J= 5.9, CH_2Cl); ^{13}C NMR ($CDCl_3$) [50 MHz]: δ159.8 (C- *ipso*), 131.0 (C-*meta*), 122.5 (C-*para*), 118.3 (C-*ortho*), 67.86 (OCH_2), 41.86 (CH_2Cl).

1-[4-(2-Chloroethoxy)phenyl]-2-phenyl-1-butanone (**3.2**)

The procedure developed by McCague was used[9]. 1-Chloro-2-phenoxyethane (21.8 g, 140 mmol), 2-phenylbutyric acid (20.2 g, 120 mmol), and trifluoroacetic anhydride (27.3 g, 130 mmol) were mixed and stirred magnetically at 20°C for 70 hours. The reaction mixture was then poured slowly into distilled water (150 mL) to give a pink solid which was collected and recrystallized from ethanol to give colourless crystals (28.8 g, 79%). The compound showed: m.p.: 65-70 °C; TLC R_f= 0.79 (100% DCM); ^{1}H NMR ($CDCl_3$) [200 MHz]: δ7.98-6.85 (m, 9H, ArH), 4.39 (t, 1H, J= 7.3, ArCH), 4.24 (t, 2H, OCH_2), 3.80 (t, 2H, J= 5.8, CH_2Cl), 2.02 (m, 2H, **CH_2CH_3**), 0.89 (t, 3H, J= 7.4, CH_3); ^{13}C NMR ($CDCl_3$) [50 MHz]: δ198.07 (C(O)), 162.11-107.38 (C-aryl), 67.53 (**CH**$PhCH_2CH_3$), 55.17 (OCH_2), 41.39 (CH_2Cl), 26.48 (CH_2), 12.15 (CH_3).

2-(2-Bromophenyl)-O-(t-butyldimethylsilyl) ethan-2-ol (**3.3**)

The procedure developed by Corey was used[11]. 2-Bromophenethyl alcohol (2.0 g, 9.95 mmol) and imidazole (1.02 g, 14.9 mmol) were dissolved in DCM (30 mL) under nitrogen and cooled over ice before *tert*-butyldimethylsilyl chloride (2.25 g, 14.9 mmol) was added. After 8 hours the reaction mixture was filtered and the residue washed with dichloromethane (25 mL). The filtrate was extracted with distilled water (3 x 30 mL), concentrated to 1 mL and passed down a silica column (petroleum ether/ DCM). The first band to elute from the column was collected, evaporated to dryness and dried under high vacuum until the odour of the silicon starting material could no longer be detected (2-3 hours). The product, a colourless oil (2.6 g, 83%) showed: TLC R_f= 0.66 (50:50 v/v

dichloromethane / hexanes); ^{1}H NMR ($CDCl_3$) [200 MHz]: δ7.58-6.99 (m, 4H, H-aryl), 3.85 (t, 2H, J= 8.8, CH_2O), 2.99 (t, 2H, $PhCH_2$), 0.90 (s, 9H, $SiC(CH_3)_3$), 0.03 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$) [50 MHz] δ 138.29 (C-2), 131.7, 132.6 (C-3, C-6), 127.8, 127.1, (C-4, C-5), 124.56 (C-*ipso* ie. C-1), 62.40 (CH_2O), 53.27 ($SiC(CH_3)_3$), 39.59 ($PhCH_2$), 25.89 ($SiC(\mathbf{CH_3})_3$), 18.22 ($Si(CH_3)_2$); MS [NH_3 DCI] m/z(RI%): 334(100)[M + $2H^+$ + NH_3], 332(89)[M + NH_3], 331(20)[M + NH_3 - H^+], 317(100)[M + $2H^+$], 315(90)[M].

1-[4-(2-Chloroethoxy)phenyl]-1-[2-(O-(t-butyldimethylsilyl)ethyl)phenyl]-2-phenyl butan-1-ol (**3.5**)

Compound **3.4** (2.02 g, 6.41 mmol) was dissolved in 5 mL of dry THF in a flame dried flask under an argon atmosphere and cooled to -78°C. When *n*-butyllithium (4.0 mL, 1.6M solution in hexanes) was added *via* syringe the solution became heterogeneous; it was left to stir for an additional 20 minutes. After the addition of compound **3.2** (1.09 g, 3.6 mmol in 5 mL dry THF) the solution cleared. The solution was allowed to gradually warm to room temperature and after twelve hours the reaction was complete. Distilled water (50 mL) was added slowly and the solution extracted with ether (3 x 50 mL). The organic layers were combined, dried over sodium sulfate, filtered and evaporated to dryness. The resulting oil was used without further purification or characterization.

1-[4-(2-Chloroethoxy)phenyl]-1-[2-(O-(t-butyldimethylsilyl) ethyl)phenyl]-2-phenyl but-1-ene (**3.6**)

Compound **3.5** was dissolved in freshly distilled pyridine (10 mL) and cooled to -10°C; thionyl chloride (788 μL, 10.8 mmol) was added and the mixture stirred under argon for three hours. The yellow/orange coloured solution was diluted with distilled water (20 mL) and extracted with diethyl ether (4 x 40 mL). The organic layers were combined and evaporated to dryness. The product was isolated by radial chromatography (10:1 low boiling petroleum ether/ether). The compound, a yellow oil (1.18 g, 64%) showed: TLC R_f = 0.72 (10:90 v/v ether/ petroleum ether); ^{1}H NMR ($CDCl_3$) [200 MHz]: δ7.102 (m, H-aryl), 6.500 (d, 2H, H-*ortho* ring B), 4.189 (t, J= 3.9, 2H, Z-isomer O**C**$\mathbf{H_2}$$CH_2Cl$), 4.042 (t, J= 3.8, 2H, E-isomer O**C**$\mathbf{H_2}$$CH_2Cl$), 3.910 (m, 1H, $SiOCH_2$), 3.769 (t, J= 3.9, 2H, Z-isomer CH_2Cl), 3.672 (t, J= 4.0, 2H, E-isomer CH_2Cl), 3.449 (m, $TBSOCH_2$), 2.867 (t, J= 3.6, 2H, E-isomer CH_2Ph), 2.787 (m, 1H, E-isomer **C**$\mathbf{H_2}$$CH_3$), 2.686 (t, J= 4.9, 2H, E-isomer CH_2Ph), 2.560 (m, 1H, Z-isomer **C**$\mathbf{H_2}$$CH_3$), 2.276 (m, 2H, Z-isomer **C**$\mathbf{H_2}$$CH_3$), 1.007 (t, J= 4.9, CH_3), .814 (m, $SiC(CH_3)_3$), 0.02 (s, $Si-CH_3$); ^{13}C NMR ($CDCl_3$) [50 MHz] δ156.77, 156.06, 142.77, 142.53, 142.16, 141.89, 141.74, 137.55, 136.95, 135.97, 134.77, 131.71, 131.55, 130.65, 130.42, 130.01, 129.73, 129.46, 128.96, 128.26, 127.97, 127.47, 126.95, 126.20, 126.0, 125.36, 120.05, 117.0, 114.18, 113.505 (C-aryl), 67.93 (Z-isomer O**C**$\mathbf{H_2}$$CH_2Cl$), 67.70 (E-isomer O**C**$\mathbf{H_2}$$CH_2Cl$), 63.43 (E-isomer $SiOCH_2$), 63.20 (Z-isomer $SiOCH_2$), 41.76 (CH_2Cl), 36.72 (E-isomer CH_2Ph), 36.43 (Z-isomer CH_2Ph), 29.51 (**C**$\mathbf{H_2}$$CH_3$), 25.93 ($SiC(CH_3)_3$), 18.31 ($SiCH_3$), 13.80 (Z-isomer CH_2**C**$\mathbf{H_3}$), 12.93 (E-isomer

$CH_2\mathbf{CH_3}$); MS [NH_3 DCI] m/z(RI%): 538(100)[M + NH_3], 520(80)[M], 406(85)[M-TBS+1].

1-[4-(2-Chloroethoxy)phenyl]-1-[2-(hydroxyethyl)phenyl]-2-phenyl but-1-ene (**3.7**)

The procedure developed by Corey was used[11]. To compound **3.6** (800 mg, 1.54 mmol) in 10 mL of THF, tetrabutylammonium fluoride (5.07 mL, 1.0 M solution in THF) was added. The reaction was allowed to stir for 24 hours before dilution with distilled water (20 mL) and extraction with diethyl ether (4 x 25 mL). The organic layer was concentrated to a volume of 2 mL and the product isolated by radial chromatography (hexanes/DCM). The title compound (600 mg, 96%), an oil, showed: TLC R_f = 0.15 (100% DCM); ^{1}H NMR ($CDCl_3$) [200 MHz]: δ7.20 (m, H-aryl), 6.520 (d, 2H, H-*ortho* ring B), 4.143 (t, J= 5.9, Z-isomer, $O\mathbf{CH_2}CH_2Cl$), 3.996 (t, J= 5.9, 2H, E-isomer $O\mathbf{CH_2}CH_2Cl$), 3.725 (t, J= 5.8, 2H, Z-isomer CH_2Cl), 3.629 (t, J= 5.9, 2H, E-isomer CH_2Cl), 3.425 (m, E/Z, $\mathbf{CH_2}OH$), 2.711, 2.200 (m, overlap, CH_2Ph, $\mathbf{CH_2}CH_3$), 0.954 (t, J= 7.5, 3H, Z-isomer CH_3), 0.769 (t, J= 7.4, 3H, E-isomer CH_3); ^{13}C NMR ($CDCl_3$) [50 MHz] δ156.10, 156.06, 142.98, 142.69, 142.05, 141.70, 136.65, 136.37, 136.15, 135.86, 134.60, 132.01, 131.50, 130.35, 130.24, 129.65, 128.87, 127.98, 127.52, 127.22, 126.50, 126.26, 126.06, 125.64, 114.24, 113.52 (C-aryl), 67.93 (Z-isomer $O\mathbf{CH_2}CH_2Cl$), 67.67 (E-isomer $O\mathbf{CH_2}CH_2Cl$), 62.63 (E-isomer CH_2OH), 62.41 (Z-isomer CH_2OH), 41.79 (CH_2Cl), 36.47 (E-isomer $PhCH_2$), 36.14 (Z-isomer $PhCH_2$), 29.49 (E-isomer $\mathbf{CH_2}CH_3$), 28.04 (Z-isomer $\mathbf{CH_2}CH_3$), 13.77 (Z-isomer CH_3), 12.92 (E-isomer CH_3).

12-Azido-dodecanoic acid (**3.9**)

Sodium azide (4.7 g, 72.3 mmol) was added to a solution of 12-bromododecanoic acid (2.0 g, 7.16 mmol) in DMF (45 mL). After heating at 80°C for 12 hours, the reaction mixture was cooled to room temperature and poured into distilled water (100 mL). The solution was extracted with ether (2 x 70 mL) and the organic fractions pooled and evaporated *in vacuo*. The resulting yellow oil was diluted with a 3:1 mixture of water/brine (10 mL) and cooled in the fridge overnight. The resulting colourless precipitate was filtered, washed with cold water (30 mL) and dried in air. The compound (1.56 g, 90%) showed:^{1}H NMR ($CDCl_3$) [200 MHz] δ3.320 (t, J= 6.8, 2H, CH_2-N_3), 2.313 (t, J= 7.4, 2H, **CH_2**COOH), 1.570 (m, 4H, CH_2), 1.257 (m, 18H, CH_2); ^{13}C NMR ($CDCl_3$) [50 MHz] 180.04 (COOH), 51.45 (CH_2-N_3), 34.15 (**CH_2**COOH), 29.40, 29.18, 29.09, 29.02, 28.8, 26.67, 24.68 (nCH_2); MS(-ES) m/z(RI%): 241.4(18)[M], 240.4(100)[M-H].

1-[4-(2-Chloroethoxy)phenyl]-1-[2-[(12-azidododecanoyl)oxyethyl)phenyl]-2-phenyl but-1-ene (**3.11**)

12-Azido-dodecanoic acid (500 mg, 2.07 mmol) was dissolved in thionyl chloride (8 mL) and heated to reflux under nitrogen for 1 hour. Residual thionyl chloride was evaporated *in vacuo* and the residue dissolved in dry DCM (25 mL), which was subsequently evaporated. The oily residue was redissolved in dry DCM (20 mL) and compound **3.7** (766 mg, 1.89 mmol) in 5 mL of dry DCM was added. The solution was heated to reflux for 12 hours before the slow addition of 10% Na_2CO_3 (20 mL). The

solution was extracted with chloroform (2 x 50 mL) and the organic layers were pooled, concentrated to 2 mL and the product, a yellow oil (766 mg, 64%) isolated by radial chromatography (Pet ether/ether). The compound showed: MS (HRDEI): obs: 629.33695, calc: 629.3384;

E-Isomer: ^{1}H NMR ($CDCl_3$) [500 MHz] δ 7.280 (m, H-aryl), 6.764 (d, J= 9.3, 2H, H-*meta*- ring B), 6.512 (d, 2H, H-*ortho*- ring B), 4.117 (m, 1H, O**C**$H_2$$CH_2$Ph), 4.063 (m, 2H, O**C**$H_2$$CH_2$Cl), 3.914 (m, 1H, O**C**$H_2$$CH_2$Ph), 3.694 (t, 2H, J= 6.0, CH_2Cl), 3.233 (t, 2H, CH_2N_3), 2.734 (m, 2H, OCH$_2$**C**H_2Ph), 2.325 (m, 1H, **C**$H_2$$CH_3$), 2.224 (t, J= 6.2, 2H, CH_2C(O)), 2.211 (m, 1H, **C**$H_2$$CH_3$), 1.564, 1.344, 1.245 (m, CH_2), 0.810 (t, J= 7.4, 3H, CH_3); ^{13}C NMR ($CDCl_3$) [125 MHz]: δ173.61 (COOR), 156.13, 142.68, 142.28, 141.76, 136.50, 135.82, 134.48, 131.53, 130.28, 130.20, 129.70, 128.00, 127.20, 126.44, 126.28, 113.60 (C-aryl), 67.78 (O**C**$H_2$$CH_2$Cl), 63.71 (O**C**$H_2$$CH_2$Ph), 51.48 (**C**$H_2N_3$), 41.78 ($CH_2$Cl), 34.32 (**C**$H_2$OC(O)), 32.48 OCH$_2$**C**$H_2$Ph), 29.42, 29.38, 29.23, 29.10 (CH_2), 28.82 (**C**$H_2$$CH_3$), 26.69, 24.94 ($CH_2$), 12.90 ($CH_3$).

Z-Isomer: ^{1}H NMR ($CDCl_3$) [500 MHz] δ 7.00 (m, H-aryl), 4.166 (t, J= 6.0, 2H, O**C**$H_2$$CH_2$Cl), 3.816 (m, O**C**$H_2$$CH_2$Ph), 3.746 (t, J= 6.0, 2H, O**C**$H_2$$CH_2$Cl), 3.652 (m, O**C**$H_2$$CH_2$Ph), 3.191 (t, J= 7.0, 2H, CH_2N_3), 2.801, 2.620, 2.553 (m, overlap, OCH$_2$**C**H_2Ph and **C**$H_2$$CH_3$), 2.192 (t, J= 7.6, 2H, CH_2C(O)), 1.511, 1.288 (m, CH_2), 0.960 (t, J= 7.4, 3H, CH_3); ^{13}C NMR ($CDCl_3$) [125 MHz] 173.60 (COOR), 142.92, 142.01, 137.18, 135.65, 131.95, 130.39, 129.71, 128.95, 127.54, 126.52, 126.12, 125.80, 114.35 (C-aryl), 68.05 (O**C**$H_2$$CH_2$Cl), 63.72 (O**C**$H_2$$CH_2$Ph), 51.48 ($CH_2N_3$), 41.83

(CH_2Cl), 34.33 (**CH_2**OC(O)), 32.05 (OCH_2**CH_2**Ph), 29.45, 29.25, 29.13, 28.82 (CH_2), 26.70 (**CH_2**CH_3), 24.95 (CH_2), 13.79 (CH_3).

1-[4-(2-Dimethylaminoethoxy)phenyl]-1-{2-[(12-azidododecanoyl)oxyethyl]phenyl}-2-phenyl but-1-ene (**3.12**)

The procedure developed by McCague was used[9]. In a round bottom flask fitted with a dry ice condenser, compound **3.11** (150 mg, 0.235 mmol) was dissolved in dimethylamine (20 mL, 5.6 M solution in ethanol) and the mixture was heated to reflux for 2 days. The solution was evaporated to dryness, the residue dissolved in DCM (2 mL) and the title compound isolated by centrifugal chromatography ($CHCl_3$/ MeOH). The title compound, a yellow oil (76 mg, 50%) showed: ^{1}H NMR ($CDCl_3$) [500 MHz] δ 7.298 (m, H-aryl), 6.934 (d, J=8.9, 2H, *H-meta* ring A), 6.512 (d, 2H, H-*ortho*, ring A), 4.110 (m, O**CH_2**CH_2Ph), 3.916 (overlap, t, J= 6.0, O**CH_2**$CH_2$$NMe_2$, and m, O**$CH_2$**$CH_2$Ph), 3.242 (t, J= 6.8, 2H, CH_2N_3), 2.713 (m, OCH_2**CH_2**Ph), 2.644 (t, J= 5.7, 2H, **CH_2**NMe_2), 2.210 (m, ovelap, **CH_2**CH_3, **CH_2**COOR, NCH_3), 1.331 (m, $(CH_2)_n$), 0.813 (t, J= 7.2, 3H, CH_2**CH_3**); ^{13}C NMR ($CDCl_3$) [125 MHz] δ 173.59 (COOR), 156.69, 142.72, 141.88, 141.74, 136.57, 135.76, 133.68, 131.33, 130.21, 130.11, 129.65, 128.89, 127.90, 127.46, 127.07, 126.34, 126.14, 114.05 (C-aryl), 65.52 (O**CH_2**$CH_2$$NMe_2$), 63.67 (O**$CH_2$**$CH_2$Ph), 58.13 ($CH_2N$), 51.38 ($CH_2N_3$), 45.73 ($NCH_3$), 34.25 (**$CH_2$**COOR), 32.42 (O$CH_2$**$CH_2$**Ph), 29.37, 29.18, 29.05 ($(CH_2)_n$), 28.75 (**CH_2**CH_3), 26.63, 24.88 ($(CH_2)_n$), 12.90 (CH_2**CH_3**); MS (HRDEI): obs: 638.4190, calc: 638.4196.

1-[4-(2-Dimethylaminoethoxy)phenyl]-1-{2-[(12-aminododecanoyl)oxyethyl]phenyl]-2-phenyl but-1-ene (**3.13**)

The procedure developed by Vaultier *et al.* was used[13]. To compound **3.12** (69 mg, 0.108 mmol) in THF (1 mL), triphenylphosphine (29 mg, 0.110 mmol) followed by distilled waster (3.0 μL) were added. The reaction was allowed to stir for 24 hours whereupon an additional aliquot of triphenylphosphine (29 mg, .110 mmol) and water (3.0 μL) were added. After an additional 24 hours the solution was evaporated to dryness, diluted with chloroform (1 mL) and the title compound isolated by centrifugal chromatography (16 mg, 25 %). The compound showed: ^{1}H NMR ($CDCl_3$) [500 MHz] δ 7.230 (m, H-aryl), 6.691 (d, J= 8.8, 2H, *H-meta* ring A), 6.488 (d, 2H, H-*ortho*, ring A), 4.036 (m, $O\mathbf{CH_2}CH_2Ph$), 3.832 (overlap, t, J= 5.8, $O\mathbf{CH_2}CH_2NMe_2$, and m, $O\mathbf{CH_2}CH_2Ph$), 2.637 (m, overlap, CH_2NH_2, $OCH_2\mathbf{CH_2}Ph$, $\mathbf{CH_2}NMe_2$), 2.197 (m, $\mathbf{CH_2}CH_3$, $\mathbf{CH_2}COOR$, NCH_3), 1.492, 1.353, 1.182 (m, $(CH_2)_n$), 0.743 (t, J= 7.2, 3H, $CH_2\mathbf{CH_3}$); ^{13}C NMR ($CDCl_3$) [125 MHz] δ 173.64 (COOR), 156.73, 142.72, 141.87, 141.76, 136.58 135.77, 133.66, 131.33, 130.22, 130.22, 130.11, 129.66, 128.25, 127.91, 127.07, 126.34, 126.14, 113.34 (C-aryl), 65.60 ($O\mathbf{CH_2}CH_2NMe_2$), 63.68 ($O\mathbf{CH_2}CH_2Ph$), 58.18 (CH_2N), 45.78 (NCH_3), 42.08 (H_2NCH_2), 34.27 ($\mathbf{CH_2}COOR$), 33.69 ($(CH_2)_n$), 32.42 ($OCH_2\mathbf{CH_2}Ph$) 29.44, 29.21($(CH_2)_n$), 29.06. ($\mathbf{CH_2}CH_3$), 26.82, 24.89 ($(CH_2)_n$), 12.91 ($CH_2\mathbf{CH_3}$); MS [NH_3-DCI] m/z(RI%): 613(100)[M+1], 541(14)[M-$CH_2CH_2NMe_2$].

1-[4-(2-Dimethylaminoethoxy)phenyl]-1-{2-[(12-((2,3-Bistriphenylmethylthioacetyl-amino) propanamido)dodecanoyloxy)ethyl]phenyl}-2-phenyl but-1-ene (**I**)

Compound **3.13** (16 mg, 0.026 mmol) was dissolved in DCM (10 mL) and triethylamine was added (1 mL), followed by compound **2.7** (20 mg, 0.027 mmol). To this well stirred solution EDAC (19 mg, 0.099 mmol)was added slowly. The reaction mixture was stirred for 48 hours before dilution with DCM (10 mL) and extraction with brine (3 x 10 mL). The organic phase was concentrated to 1 mL and an impure fraction of the title compound isolated by radial chromatography ($CHCl_3$/ MeOH). This fraction was concentrated to 0.5 mL and a pure sample of the title compound isolated by preparative TLC (silica). The title compound was a yellow oil (9 mg, 26%).

1-[4-(2-Chloroethoxy)phenyl]-1-{2-[(12-aminododecanoyl)oxyethyl]phenyl}-2-phenyl but-1-ene (**3.14**)

The procedure developed by Vaultier *et al.* was used[14]. Triphenylphosphine (326 mg, 1.24 mmol) was added to a solution of compound **3.11** (710 mg, 1.13 mmol) in THF (2 mL) and water (50 μL). The solution was stirred (protected from light) for 12 hours before concentration of the solvent under reduced pressure. The oily residue was dissolved in chloroform (2 mL) and the product, a yellowish oil (450 mg, 66%) was isolated by radial chromatography ($CHCl_3$/ MeOH) and used immediately after isolation.

1-[4-(2-Chloroethoxy)phenyl]-1-[2-((12-((2,3-Bistriphenylmethylthioacetylamino) propanamide)dodecanoyl)oxyethyl)phenyl]-2-phenyl but-1-ene (**3.15**)

To a dichloromethane solution (10 mL) of compound **3.14** (180 mg, 0.298 mmol) and compound **2.7** (264 mg, 0.36 mmol), EDAC (70 mg, 0.36 mmol) and triethylamine (1 mL) were added. The mixture was allowed to stir for 48 hours whereupon the solvent was evaporated at reduced pressure, the oily residue dissolved in chloroform (2 mL) and the product isolated by radial chromatography (hexanes/chloroform). The compound, a yellow oil (300 mg, 76%) showed: ^{1}H NMR ($CDCl_3$) [500 MHz] δ 7.250 (m, H-aryl), 6.755 (d, J= 8.9, 2H, *H-meta* ring A), 6.491 (d, 2H, H-*ortho*, ring A), 4.150 (t, J= 5.8, 2H, Z-$\mathbf{OCH_2}CH_2Cl$), 4.118 (m, overlap, $\mathbf{OCH_2}CH_2Ph$), 4.028 (t, J= 3.9, 2H, E-$\mathbf{OCH_2}CH_2Cl$), 3.771 (m, overlap, $\mathbf{CH}CH_2$ and $\mathbf{OCH_2}CH_2Ph$) 3.761 (t, J= 4.2, Z-$OCH_2\mathbf{CH_2}Cl$), 3.665 (t, J= 5.8, 2H, E- $OCH_2\mathbf{CH_2}Cl$), 3.181 (m, 2H, $CH\mathbf{CH_2}$), 2.971 (s, 4H, CH_2S), 2.718 (m, overlap, E/Z $OCH_2\mathbf{CH_2}Ph$), 2.246 (m, overlap, E/Z $\mathbf{CH_2}CH_3$), 2.179 (t, J= 5.6, 2H, $\mathbf{CH_2}COOR$), 1.534, 1.385, 1.200 (m, $(CH_2)_n$), 0.988 (t, J= 7.5, 3H, Z-$CH_2\mathbf{CH_3}$), 0.793 (t, J= 7.3, 3H, $CH_2\mathbf{CH_3}$); ^{13}C NMR ($CDCl_3$) [125 MHz] δ 173.54 (ester C(O)), 169.71, 169.08, 168.88 (amide C(O)), 156.00, 143.81, 142.78, 142.56, 142.15, 141.63, 136.39, 135.69, 134.32, 131.43, 130.21, 130.08, 129.60, 129.42, 129.35, 128.85, 128.02, 127.45, 127.12, 126.90, 126.36, 126.19, 125.71, 114.22, 113.46, 83.14 (CPh_3), 67.65 (Z-$\mathbf{OCH_2}CH_2Cl$), 67.62 (E-$\mathbf{OCH_2}CH_2Cl$), 63.61 ($\mathbf{OCH_2}CH_2Ph$), 51.11 ($\mathbf{CH}CH_2$), 41.99 (Z-CH_2Cl), 41.76 (E-CH_2Cl), 39.55 ($CH\mathbf{CH_2}$), 36.04, 35.74 (SCH_2), 34.22 ($\mathbf{CH_2}COOR$), 32.38 ($OCH_2\mathbf{CH_2}Ph$), 30.81, 29.42, 29.18, 26.79 ($(CH_2)_n$), 29.03 ($\mathbf{CH_2}CH_3$), 26.79, 24.86

($(CH_2)_n$), 13.05 (Z-$CH_2\mathbf{CH_3}$); 12.85 (E-$CH_2\mathbf{CH_3}$).

1-[4-(2-Dimethylaminoethoxy)phenyl]-1-{2-[(12-((2,3-Bistriphenylmethylthioacetyl-amino) propanamide)dodecanoyl)oxyethyl]phenyl}-2-phenyl but-1-ene (**I**)

The procedure developed by McCague was used[9]. A sample of 300 mg (0.227 mmol) of crude **3.15** was dissolved in a 5.6M solution of dimethylamine in ethanol (5 mL) in a round bottom flask that was fitted with a dry ice condenser. The solution was heated to reflux for 24 hours when, after cooling, the solvent was evaporated *in vacuo*. The product, a dark oily semi-solid (181 mg, 60 %) was isolated by radial chromatography (chloroform/methanol). The compound showed:

TLC: R_f= 0.36 (10% MeOH/DCM); Anal: Expected C, 76.3% H, 7.1% N, 4.1% Obs. C, 74.3% H, 7.4% N, 4.9%; ^{1}H NMR ($CDCl_3$) [500 MHz] δ 7.250 (m, H-aryl), 6.735 (d, J= 8.9, H, *H-meta* ring A), 6.505 (d, 2H, H-*ortho*, ring A), 4.122 (m,overlap, $O\mathbf{CH_2}CH_2Ph$,$\mathbf{CH}CH_2$), 4.055 (m, Z-$O\mathbf{CH_2}CH_2N$), 3.926 (m, overlap, $O\mathbf{CH_2}CH_2Ph$), 3.896 (t, J= 5.9, E-$O\mathbf{CH_2}CH_2N$), 3.194 (m, $CH\mathbf{CH_2}$), 2.985 (s, 4H, CH_2S), 2.718 (m, overlap, Z-$OCH_2\mathbf{CH_2}N$ and $OCH_2\mathbf{CH_2}Ph$), 2.625 (t, J= 5.8, 2H, E-$OCH_2\mathbf{CH_2}N$), 2.325 (s, Z-NCH_3), 2.267 (E-NCH_3), 2.211 (m, overlap, E/Z $\mathbf{CH_2}CH_3$, $\mathbf{CH_2}COOR$), 1.540, 1.407, 1.213 (m, $(CH_2)_n$), 0.994 (t, J= 7.4, 3H, Z= $CH_2\mathbf{CH_3}$), 0.797 (t, J= 7.5, 3H, $CH_2\mathbf{CH_3}$); ^{13}C NMR ($CDCl_3$) [125 MHz] δ 173.63 (ester C(O)), 169.86, 169.21, 168.96 (amide C(O)), 156.73, 143.87, 142.75, 141.92, 136.60, 135.82, 133.72, 131.37, 130.26, 129.69, 129.50, 129.42, 128.94, 128.11, 126.98, 126.38, 126.19, 114.10, 113.39, (C-aryl), 67.73 (CPh_3),

65.57 ($O\mathbf{C}H_2CH_2N$), 63.70 ($O\mathbf{C}H_2CH_2Ph$), 58.18 (CH_2N), 54.55 ($NHCH_2$), 45.80 (NCH_3), 42.07 ($\mathbf{C}HCH_2$), 39.64 ($CH\mathbf{C}H_2$), 36.10, 35.79 (SCH_2), 34.31 ($\mathbf{C}H_2COOR$), 32.45 ($OCH_2\mathbf{C}H_2Ph$), 29.48, 29.26 ($(CH_2)_n$), 29.03 ($\mathbf{C}H_2CH_3$), 26.86, 24.94 ($(CH_2)_n$), 13.05 ($Z\text{-}CH_2\mathbf{C}H_3$); 12.95 ($E\text{-}CH_2\mathbf{C}H_3$).

Chapter 4

4-Hydroxytamoxifen

4.1 Introduction

4-Hydroxytamoxifen (**Figure 4-I**) has a relative binding affinity for the estrogen receptor which is nearly identical to that of the natural substrate[1]. The activities of E and Z-4-hydroxytamoxifen were evaluated and both were found to be antiestrogenic[2]. Using the synthetic approach developed in Chapter **3**, a Z-4-hydroxytamoxifen derivative, which has

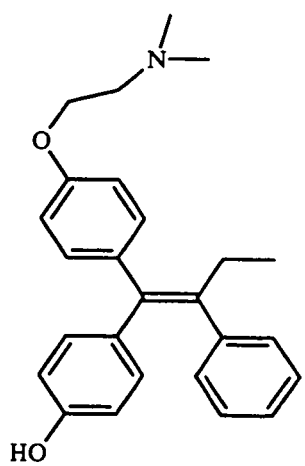

Figure 4-I: 4-Hydroxytamoxifen

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[1]Jordon, V.C.; Collins, M.M.; Rowsby, L.; Prestwich, G. *J. Endocrinol.* **1977**, 75, 305-316.

[2]Jordon, V.C; Haldemann, B.; Allen, K.E.; *Endocrinology*, **1981**, 108, 1353-1361.

the potential to be coupled to a chelate, was synthesised.

4.2 Retrosynthesis

The overall synthetic approach was analogous to that used in Chapter **3**. Synthon **A** (**Figure 4-II**) was added *via* nucleophilic substitution on a ketone, which in turn was synthesised by Friedel-Crafts acylation reaction of 2-phenylbutyric acid on a phenol derivative (synthon **B**). The chelant linker arm (synthon **C**) was synthesised in the early stages of the synthesis by a Heck reaction of a 3-halophenol with 4-pentyn-1-ol. All intermediates in the synthesis of **4.16**, with the exception of **4.9** have not been reported in the literature.

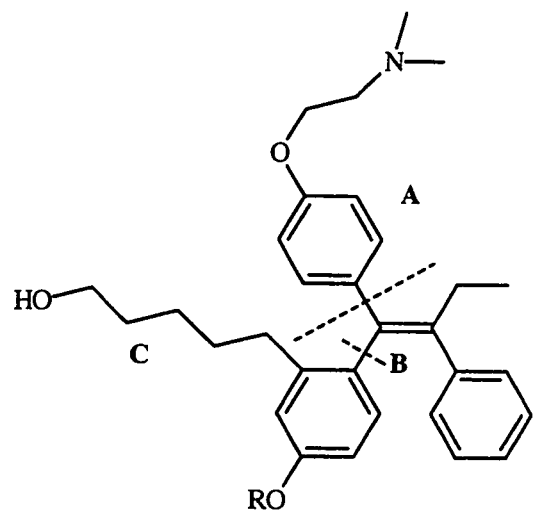

Figure 4-II: Retrosynthetic analysis of **4.16**

4.3 Synthesis of ***4.16***

The linker arm portion of the molecule was synthesised from 4-pentyn-1-ol. Compound **4.1** was protected as a pivalate ester by reaction with trimethylacetyl chloride in the presence of pyridine[3] (**Figure 4-III**). The purification of **4.2** proved to be more of a challenge than expected: column chromatography along with vacuum distillation were required to isolate a pure sample. The purity of the sample was established by gas chromatography (FID detector) and NMR spectroscopy.

Triisopropylsilyl chloride (TIPS-Cl), in the presence of imidazole[4], reacted with compound **4.3** to form compound **4.4** in good yield (95%). One of the uses of the bulky silyl protecting group was to increase the solubility of the iodo (or bromo) phenol in organic solvents. Its main function however, was as a directing group in a subsequent Friedel-Crafts acylation (*vide infra*).

The coupling of **4.2** and **4.4**, which was accomplished by use of the Heck reaction[5], was initially catalysed by tetrakis(triphenylphosphine)palladium(0); however, poor yields were obtained because of the catalyst's tendency to decompose and become inactive. The literature reported the use of bis(triphenylphosphine)palladium(II) chloride in the presence of triphenylphosphine and a copper(I) salt in place of tetrakis(triphenylphosphine)

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[3]Robins, M.J.; Hawrelak, S.D.; Kanai, T.; Siefert, J.-M.; Mengel, R. *J. Org. Chem.* **1979**, 44, 1317.

[4]Kendall, P.M.; Johnson, J.V.; Cook, C.E., *J. Org. Chem.*, **1979**, 44, 1421.

[5]Heck, R.F. Palladium Reagents in Organic Synthesis. (London: Academic Press, Inc., 1985) p. 299

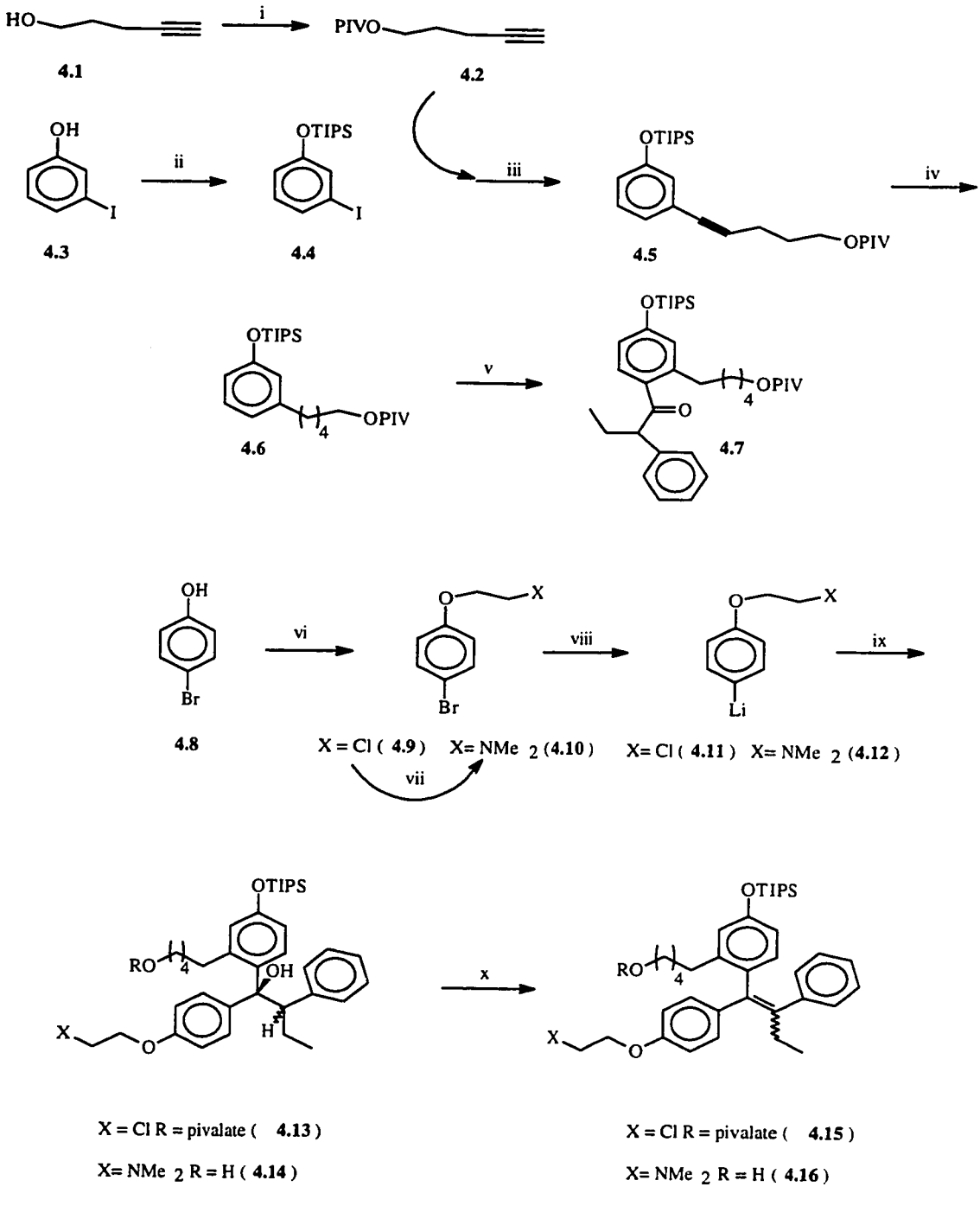

Figure 4-III: i) piv-Cl, pyridine, ii) TIPS-Cl, imidazole iii) $PdCl_2$, $HNEt_2$, PPh_3, CuI iv) H_2, 10% Pd/C v) (±)-2-phenylbutyric acid, TFAA vi) 1,2-dichloroethane, NaOH, PTC vii) $HNMe_2$, EtOH, Δ viii) *n*-BuLi, THF, -78°C ix) compound **4.7** x) $SOCl_2$, pyridine.

palladium(0) to accomplish similar alkynyl-aryl couplings[6]. A minor modification of this method was used to improve yields of compound **4.5**. The use of palladium(II) chloride, in the presence of triphenylphosphine and copper iodide as the catalyst resulted in the formation of **4.5** in improved yields (80% vs 50%) in less time (6 hours vs 48 hours). An added advantage of using palladium(II) chloride is that it was significantly less expensive than tetrakis(triphenylphosphine) palladium(0).

Reduction of **4.5** by hydrogenation was necessary to avoid any potential side reactions during the subsequent Friedel-Crafts acylation. Using 10% palladium on carbon and 35 psi of hydrogen, the reduction occurred in nearly quantitative yield.

Reaction of 2-phenylbutyric acid in the presence of TFAA with compound **4.6** resulted in the formation of compound **4.7** after 4 days. The steric bulk of the TIPS group prevented any ortho acylation and decomposition of the TIPS group by trifluoroacetic acid, a byproduct of the acylation, was not observed.

Ring A was incorporation was achieved by reacting the lithium salt of a 4-bromophenol derivative with compound **4.7**. Initially, the chloro species **4.9**, which was synthesised from *p*-bromophenol, was used as the nucleophile to generate the diastereomeric alcohols **4.13**, which were subsequently converted to the corresponding alkenes. Cram's rules (see Chapter **3**) predicted that the 'like' (1R, 2R) form of the intermediate alcohol would be the predominant product. Assuming an E_2 elimination with

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[6]Takahasi, S.; Kuroyama, Y.; Sonogashira, K.; Hagihara, N., *Synthesis*, **1980**, 627.

pyridine/thionyl chloride at -10°C, the Z-alkene should be the major product and. When the product **4.15** was isolated, integration of the NMR signals in the alkenes showed there was approximately a 3:1 Z:E ratio. The chemical shifts of the methylene units on the chloroethoxy side chain were different for each isomer and were used as an indicator of isomer ratios. The yield of **4.15** was poor (5%) which was most likely a result of decomposition of the alkyl chloride **4.9** in the highly basic reaction mixture.

To overcome the low yields of **4.15**, the dimethylamine compound **4.10** was synthesised and its lithium salt used as the nucleophile. After addition of the lithium salt of **4.10**, TLC indicated that all the starting material had been consumed within 4 hours. The product of the reaction was converted directly to the alkene with thionyl chloride in the presence of pyridine and the mass spectrum of the product indicated that the alkene had formed; however, it appeared that the pivalate group had been cleaved. Cleavage of the protecting group was a result of nucleophilic attack of the lithium anion on the ester group and the increased nucleophilicity of the anion was presumably a result of ion pair separation induced by the dimethylamine substituent; most likely in an intermolecular manner. The excess anion used in the reaction was sufficiently reactive to overcome the steric repulsion of the neighbouring methyl groups of the ester. The yield of the two steps combined was 60%.

The ^{1}H NMR of **4.16** indicated that the Z:E ratio was now greater than 9:1. If the diastereomeric alcohols **4.14** eliminated by an E2 type mechanism then the major product would have been the Z-isomer but, if the system formed an intermediate cation (*ie.* E_1

like), then the Z:E selectivity would be reduced. The dimethylamine substituent, which is more basic than the solvent, possibly becomes involved in the elimination process and influences the system to proceed by a more E_2 like mechanism rather than a unimolecular process. It must be stressed that the 3:1 Z:E ratio obtained from the chloro compound **4.9** may not truly reflect the real preference of the nucleophile's attack on ketone **4.7** because only a 5% yield of alkenes was isolated. It is quite possible that the high Z:E ratio found for **4.16** is closer to the real preference of a phenyl nucleophile for **4.7**.

4.4 Future Work

Attempts to couple compound **4.16** to the DADT-chelant, which contained a glycine spacer, were unsuccessful despite the alcohol group being primary. Future work would involve converting the alcohol **4.16** to an amine, which would facilitate coupling to the DADT chelant. Future work must also determine the relative binding constant of final product so as to ensure that the addition of the linker arm has not altered the relative binding affinity (RBA) of the parent molecule.

4.5 Experimental Section

4-Pentynyl-trimethylacetate (4.2)

The procedure developed by Robins *et al.* was used[3]. 4-Pentyne-1-ol (1.1 mL, 11.9 mmol) was dissolved in dry pyridine (10 mL) under argon and cooled to -10°C. Trimethylacetyl chloride (2.93 mL, 23.7 mmol) was added dropwise over five minutes.

After 24 hours the solution was diluted with DW (30 mL) and extracted with ether (3 x 30 mL). The organic layers were pooled and concentrated to dryness. The title compound was purified by silica gel chromatography followed by distillation at reduced pressure. The compound showed: GLC[7]: 1 Peak 5.86 minutes, Area % (100); ^{1}H NMR ($CDCl_3$): δ3.947 (t, J= 7.4, 2H, OCH_2), 2.077 (m, 2H, CCH_2), 1.80 (m, 1H, CH), 1.656 (m, 2H, CH_2), 0.997 (s, 9H, CH_3) [200.132MHz]; ^{13}C NMR ($CDCl_3$): δ177.64 (C(O)), 82.50 (HC**C**), 68.78 (H**C**C), 62.39 (OCH_2), 38.30 ($\mathbf{C}(CH_3)_3$), 27.3 ($CH_2\mathbf{C}H_2CH_2$), 26.78 ($C(\mathbf{C}H_3)_3$), 14.78($\mathbf{C}H_2CC$) [50.32 MHz]; MS: (NH_3-DCI) m/z(RI%): 204(87)[M+2NH_3+2], 186(100)[M+NH_3+1], 169(35)[M+1].

O-Triisopropylsilyl-3-bromophenol

The procedure developed by Kendall *et al.* was used[4]. 3-Bromophenol (10g, 57.8 mmol) and imidazole (3.95g, 58 mmol) were dissolved in dry DCM (50 mL). The solution was cooled to -5°C before the addition of triisopropylsilyl chloride (11.2 mL). The reaction was allowed to warm to room temperature and stirred for 16 hours. The solution was extracted with DW (2 x 20 mL) and 0.1M NaOH (4 x 30 mL). The organic layer was dried using sodium sulfate and evaporated to dryness *in vacuo* leaving a colourless oil (15g, 79%). The compound showed: TLC: 0.56 (100% Pet ether); ^{1}H NMR ($CDCl_3$):δ7.213(m, 4H, H-aryl), 1.262 (m, CH and CH_3) [200.132MHz]; ^{13}C NMR ($CDCl_3$): δ156.89 (C-6),

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[7]HP5890A GLC: 25M LoopOven Temp: 246 Oven Max:250 Init. Temp. 50Init. Time:1.00min fin. time:25 min Ramp: 10.00

130.29 (C-2), 124.14, 123.28 (C-3, C-5), 122.48 (C-1), 118.52 (C-4) [50.32MHz]. MS (DEI) m/z(RI%): 328(18)[M-1].

O-Triisopropylsilyl-3-iodophenol (4.4)

m-Iodophenol (10.0 g, 45.5 mmol) and imidazole (3.09 g, 45.5 mmol) were dissolved in dry DCM (50 mL) and the mixture cooled to -10°C under nitrogen. Through a septum, triisopropylsilyl chloride was added (9.0 mL, 43.3 mmol) dropwise. Within five minutes a colourless precipitate formed; the solution was stirred for a total of 16 hours. The heterogenous mixture was filtered through a fritted glass funnel (fine) and the filtrate extracted with 1M HCl (20 mL) and 0.5M NaOH (2 x 20 mL). The organic layer was dried over sodium sulfate before concentration under reduced pressure leaving a colourless oil which had a distinctive odour (15.5g, 95%). The compound showed: TLC: R_f= 0.68 (100% Pet ether); ^{1}H NMR($CDCl_3$):δ7.38 (m, 2H, H-aryl), 7.00 (m, 2H, H-aryl), 1.40 (m, 1H, CH), 1.30 (m, 6H, CH_3) [200 MHz]; ^{13}C NMR($CDCl_3$): δ156.7 (TipsO-C, C-*meta*1), 130.58 (C-*meta*2), 130.13 (C-*ortho*2), 129.21 (C-*ortho1*), 119.2 (C-*para*), 94.1 (C-*ipso*), 17.87 (CH_3), 12.6 (CH) [50.32 MHz].

5-(3-O-Triisopropylsilylphenol)-pent-4-ynltrimethylacetate (**4.5**)-Method I

The procedure developed by Just and Singh was used[8]. The Compound **4.2** (1.5 g, 8.93 mmol) and **4.4** (3.20 g, 7.48 mmol) were dissolved in freshly distilled diethylamine

[8]Just, G.; Singh, R. *Tetrahedron Lett.*, **1987**, 28, 5981.

(30 mL). The solution was stirred under dry nitrogen, protected from light and refluxed for two days after the addition of tetrakis(triphenylphosphine)palladium(0) (2 mol%, 0.17 mmol, 197 mg). After the solution was diluted with saturated ammonium chloride and extracted with ether (2 x 100 mL), the organic extracts were combined and evaporated to dryness giving a brown oil. The title compound was isolated by silica gel chromatography (pet ether / ether). The compound showed: ^{1}H NMR ($CDCl_3$): δ 7.220 (m, 4H, H-aryl), 4.300 (t, J= 8.8, 2H, $CH_2CH_2\mathbf{CH_2}O$), 2.588 (t, J= 5.0, 2H, $PhCCCH_2$), 2.051 (m, 2H, $CH_2\mathbf{CH_2}CH_2$), 1.401 (m, $\mathbf{CH}(CH_3)_2$, 1.311 (s, 9H, $C(\mathbf{CH_3})_3$, 1.200 (s, 6H, $CH(\mathbf{CH_3})_2$ [200.132MHz]; ^{13}C NMR ($CDCl_3$) δ 177.41 (ester C(O)), 155.38, 124.53, 124.47, 124.25, 122.47, 119.24 (C-aryl), 87.83 (PhC**C**), 80.4 (Ph**C**C), 62.56 ($CH_2CH_2\mathbf{CH_2}O$), 27.58 ($CH_2\mathbf{CH_2}CH_2$), 26.78 ($CH(\mathbf{CH_3})_2$), 17.53 ($C(\mathbf{CH_3})_3$), 15.80 ($PhCCCH_2$), 12.30 ($\mathbf{CH}(CH_3)_2$) [50.32MHz]; MS (HRSDEI): obs: 416.2732, calc: 416.2747.

5-(3-O-Triisopropylsilylphenol)-pent-4-ynltrimethylacetate (**4.5**)-Method II

Compound **4.2** (1.7 g, 10.01 mmol) and compound **4.4** (1.8 g, 4.8 mmol) were dissolved in freshly distilled diethylamine under nitrogen. To the well stirred solution, palladium(II) chloride (45 mg, 0.255 mmol) and triphenylphosphine (133 mg, 0.507 mmol) were added followed by copper(I) iodide (24 mg, 0.126 mmol). The solution was heated to reflux for six hours whereupon the solvent was evaporated *in vacuo* and the title compound, a colourless oil (1.60 g, 80%), isolated by radial chromatography (Pet ether/ ether).

5-(3-O-Triisopropylsilylphenol)-pentyltrimethylacetate (4.6)

Compound **4.5** (609mg, 1.46 mmol) was dissolved in methanol in a hydrogenation vessel. Slowly, 61 mg (2% by mass) of 10% palladium on activated carbon was added to the methanol solution. The vessel was placed in a hydrogenation apparatus and shaken at 35 psi for 24 hours. The heterogenous reaction mixture was filtered through a fritted glass funnel and washed with ether (50 mL). The organic layer was evaporated to dryness *in vacuo* leaving a light yellow oil (615 mg, >99%). The compound showed: ^{1}H NMR ($CDCl_3$): δ7.110, 6.702 (m, H-aryl), 4.002 (t, J= 7.8, 2H, CH_2O), 2.502 (t, J= 7.4, 2H, $PhCH_2$), 1.611 (m, overlap, CH_2), 1.293 (s, 9H, $C(\mathbf{CH_3})_3$), 1.111 (m, overlap, SiCH, $CH(\mathbf{CH_3})_2$, CH_2) [200.132MHz]; ^{13}C NMR ($CDCl_3$): δ178.20 (ester C(O)), 155.93, 143.80, 128.97, 121.08, 119.95, 117.09 (C-aryl), 64.36 (CH_2O), 38.64 (CR_3), 35.59 ($PhCH_2$), 30.83 ($PhCH_2\mathbf{CH_2}$), 28.43 ($\mathbf{CH_2}CH_2O$), 27.13 ($C(\mathbf{CH_3})_3$), 25.42 ($PhCH_2CH_2\mathbf{CH_2}$), 17.88 ($CH(\mathbf{CH_3})_2$, 12.63 ($\mathbf{CH}(CH_3)_2$ [50.32MHz]; MS (HRSDEI): obs:420.3058, calc:420.3060.

5-[3-(O-Triisopropylsilyl)-5-(2-phenyl-1-butanoyl)phenol]pentyltrimethylacetate (4.7)

The procedure developed by McCague was used[9]. A mixture of compound **4.6** (615 mg, 1.46 mmol) and (±)-2-phenylbutyric acid (265 mg, 1.61 mmol) were dissolved in trifluoroacetic anhydride (219 μL, 1.55 mmol) under argon. After 4 days, the reaction was diluted with ether (25 mL) and extracted with 10% $NaHCO_3$ (2 x 15 mL) *caution:*

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[9]*Res. M* McCague, R. *J. Chem iniprint*, **1986**, 771-790.

pressure. The organic layer was concentrated to 2 mL and the title compound, a yellow oil (698 mg, 85%) isolated by radial chromatography (pet ether-ether). The compound showed: TLC: R_f= 0.52 (10%Et_2O/Pet ether); ^1H NMR($CDCl_3$): δ 8.000, 7.610, 6.988 (m, H-aryl), 4.610 (t, J= 7.2, 1H, CHC(O)), 4.381 (t, J= 7.0, 2H, CH_2O), 2.998 (m, 2H, $PhCH_2$), 2.610 (m, 1H, $\mathbf{CH_2}CH_3$), 2.111 (m, 1H, $\mathbf{CH_2}CH_3$), 1.888 (m, overlap, CH_2), 1.498 (m, overlap, CH_2, $CH(CH_3)_2$, $C(CH_3)_3$, $CH_2\mathbf{CH_3}$); ^{13}C NMR ($CDCl_3$): δ179.11 (ester C(O)), 145.83, 139.67, 131.52, 130.78, 128.60, 128.27, 126.80, 122.27, 116.52 (C-aryl), 64.38 (CH_2O), 58.27 (CHPh), 38.70 ($\mathbf{C}R_3$), 34.17 ($PhCH_2$), 31.63, 27.16, 22.36 (CH_2), 17.81 ($C(\mathbf{CH_3})_3$), 14.01 ($CH(\mathbf{CH_3})_2$), 12.71 (SiCH), 12.33 ($CH_2\mathbf{CH_3}$); MS(HRSDEI): obs: 567.3860, calc: 567.3870.

2-Chloro-[4-bromophenoxy] ethane (**4.9**)

The procedure developed by McCague was used[9]. 4-Bromophenol (25.95 g, 150 mmol), dimethyldioctadecylammonium bromide (900 mg, 1.5 mmol) and NaOH (12.0 g, 300 mmol) in DW (113 mL) and 1,2-dichloroethane (113 mL) were refluxed together for 48 hours. The reaction was cooled to room temperature, and the two layers separated. The organic phase was evaporated to dryness *in vacuo* and the compound isolated by silica column chromatography (pet ether-ether) and vacuum distillation. The compound, a thick oil showed: ^1H NMR($CDCl_3$): δ 7.369 (d, J= 6.8, 2H, H-*meta*), 6.796 (d, 2H, H-*ortho*), 4.154 (t, J= 5.7, 2H, OCH_2), 3.776 (t, 2H, CH_2Cl); ^{13}C NMR ($CDCl_3$): δ 157.22, 132.29, 116.44, 113.49 (C-aryl), 68.14 (OCH_2), 41.73 (CH_2Cl); MS (HRDEI): obs: 233.9446,

calc: 233.9447.

*2-Dimethylamino-(4-bromophenoxy) ethane (**4.10**)*

The procedure developed by McCague was used[9]. Compound **4.9** (3.6 g, 15.38 mmol) was dissolved in dimethylamine (20 mL, 33% w/v solution in ethanol) in a round bottom flask equipped with a dry ice condenser. With rapid stirring the reaction was heated to reflux for 48 hours wherupon the solvent was evaporated *in vacuo* and the oily residue dissolved in 1:1 MeOH / AN (5 mL) and cooled to -10^{0}C overnight. The resulting solution was filtered and the title compound purified by column chromatography (pet ether-ether). The compound, a thick oil showed: ^{1}H NMR($CDCl_3$): δ 7.222 (d, J= 6.8, 2H, H-*meta*), 6.661 (d, 2H, H-*ortho*), 3.852 (t, J= 5.8, 2H, OCH_2), 2.555 (t, 2H, CH_2N), 2.190 (s, 6H, NCH_3); ^{13}C NMR ($CDCl_3$): δ 157.35, 131.57, 115.77, 112.20 (C-aryl), 65.60 (OCH_2), 57.55 (CH_2N), 45.30 (NCH_3).

1-[4-(2-Chloroethoxy)phenyl]-1-[3-(5-pentyltrimetylacetate)-O-triisopropylsilylphenol]-2-phenyl butan-1-ol (**4.13**)

Compound **4.9** (290 mg, 1.23 mmol) was dissolved in freshly distilled THF (5 mL) in a flamed 2-neck round bottom flask under an argon atmosphere and cooled to -78°C. *n*-Butyllithium (800μL, 1.6M solution in hexanes) was added slowly and after complete addition the reaction was stirred for twenty minutes. Compound **4.7** (698 mg, 1.23 mmol) was dissolved in dry THF under argon and cooled to -78°C. The anion of **4.9** was added to

the solution of **4.7** over a five minute period. The reaction mixture was allowed to warm up gradually over 20 hours. Saturated ammonium chloride was added slowly (10 mL) and the solution extracted with ether (3 x 20 mL). The organic fractions were pooled and dried over sodium sulfate prior to evaporation. The resulting yellow oil was used without further purification or analysis.

E/Z-1-[4-(2-Chloroethoxy)phenyl]-1-[3-(5-pentyltrimetylacetate)-O-triisopropylsilylphenol]-2-phenyl but-1-ene (**4.15**)

The entire sample of crude **4.13** was dissolved in pyridine (10 mL), cooled to -10°C whereupon thionyl chloride was added (90 μL). After two hours, the pyridine was removed *in vacuo* and the product isolated by radial chromatography (pet ether/ether/DCM). The product, a yellow oil (43 mg, 5%) showed: ^{1}H NMR ($CDCl_3$) [200 MHz]: δ7.211, 6.814, 6.442 (m, H-aryl), 4.211 (t, 2H, E-O**C**$H_2$$CH_2Cl$), 4.083 (t, 2H, Z-O**C**$H_2$$CH_2Cl$), 3.957 (t, 2H, CH_2OPiv), 3.791 (t, 2H, E-OCH_2**C**H_2Cl), 3.699 (t, 2H, Z-OCH_2**C**H_2Cl), 2.222 (m, 2H, **C**H_2CH_3), 1.911 (m, 2H, CH_2Ph), 1.200 (m, overlap, $C(CH_3)_3$), $CH(CH_3)_2$, CH_2**C**H_3), 0.891 (t, 3H, CH_2**C**H_3); MS (HRDEI): obs. 704.4019, calc. 704.4027.

1-[4-(2-Dimethylaminoethoxy)phenyl]-1-[3-(pentyn-5-ol)-O-triisopropylsilylphenol]-2-phenylbutan-1-ol (***4.14***)

Compound **4.10** (1.11 g, 4.55 mmol) was dissolved in freshly distilled THF (15 mL) in a flame dried 2-neck round bottom flask under an argon atmosphere and cooled to

-78°C. *n*-Butyllithium (1.35 mL, 1.6M solution in hexanes) was added slowly and after complete addition, the reaction mixture was stirred for 20 minutes. Compound **4.7** (611 mg, 1.08 mmol) was dissolved in dry THF (5 mL) under argon and cooled to -78°C. The anion of **4.9** was added to the solution of **4.7** over a five minute period. The reaction mixture was allowed to warm up gradually over 4 hours before the addition of water (50 mL). The resulting solution was extracted with ether (3 x 50 mL) and the organic extracts pooled, dried over sodium sulfate and evaporated to dryness *in vacuo*. The residue, a yellow oil, was used without further purification or analysis.

E/Z-1-[4-(2-Dimethylaminoethoxy)phenyl]-1-[3-(5-pentyltrimetylacetate)-O-triisopropylsilylphenol]-2-phenyl but-1-ene (**4.16**)

Compound **4.14** was dissolved in DCM (20 mL) and pyridine (2 mL) and cooled to -10°C before the addition of thionyl chloride (500 μL). The reaction was stirred for 4 hours prior to evaporation of the solvent *in vacuo*. The title compound, a yellow oil (408 mg, 60%) was isolated by radial chromatography (DCM, MeOH). The compound showed: ^{1}H NMR ($CDCl_3$): δ 7.396-6.321 (m, H-aryl), 4.036 (t, J= 4.7, 2H, Z-O**CH$_2$**CH$_2$N), 3.891 (t, J= 5.2, 2H, E-O**CH$_2$**CH$_2$N), 3.405 (m, 2H, E-CH$_2$OH), 3.344 (t, J= 7.0, 2H, Z-HOCH$_2$), 2.710 (t, J= 5.8, 2H, Z-CH$_2$N), 2.615 (t, J= 6.8, 2H, E-CH$_2$N), 2.724 (m, overlap, NCH$_3$, E/Z-**CH$_2$**CH$_3$), 1.312-0.722 (m, overlap, E/Z-CH$_2$**CH$_3$**, CH(CH$_3$)$_2$, CH$_2$); ^{13}C NMR ($CDCl_3$): δ 158.68, 157.25, 154.42, 138.84, 136.88, 130.33, 130.16, 129.64, 129.27, 128.90, 128.83, 127.43, 127.34, 126.28, 122.27, 120.65, 115.52, 114.48, 113.20 (C-aryl),

80.02 ($O\mathbf{CH_2}CH_2N$), 66.39 (CH_2OH), 65.73 (CH_2OH), 58.21 (CH_2N), 58.13 (CH_2N), 45.86 (NCH_3), 45.69 (NCH_3), 33.90, 32.69, 31.22 , 26.18 (CH_2), 25.71 ($\mathbf{CH_2}CH_3$), 25.16 (CH_2), 17.83 ($CH(\mathbf{CH_3})_2$), 12.54 ($\mathbf{CH}(CH_3)_2$, 12.39 ($CH_2\mathbf{CH_3}$) [125.77 MHz]; MS (EI) m/z(RI%): 629(82)[M]; 528(100).

Chapter 5

Amino Acid Based Chelates

5.1 Introduction

Poor solubility is one of the greatest problems encountered while testing radio imaging agents. Compounds are either too hydrophobic to perform accurate binding assays *in vitro* or insufficiently lipophilic to gain access to the site of interest *in vivo*. Development of a new chelating system whose solubility could be "tuned" to a desired lipophilicity remains a primary goal in radiopharmaceutical research; this goal can be accomplished by using amino acids as the chelate's synthon units. Several chelating systems are clinically utilized as radiopharmaceuticals and, of these compounds, two examples which have amino acids as synthon units are shown in **Figure 5-I**. ^{99m}Tc-L,L-ethylenecysteine dimer (^{99m}Tc-L,L ECD)[1] is a neutral Tc(V) diester compound which is used as a brain imaging agent. After the lipophilic Tc(V) compound crosses the blood brain barrier (BBB), one of the ester groups is hydrolysed and the resulting anionic species is sufficiently retained to obtain an image. The other imaging compound, ^{99m}Tc-MAG_3, is a renal imaging agent which forms an anionic Tc(V) complex[2].

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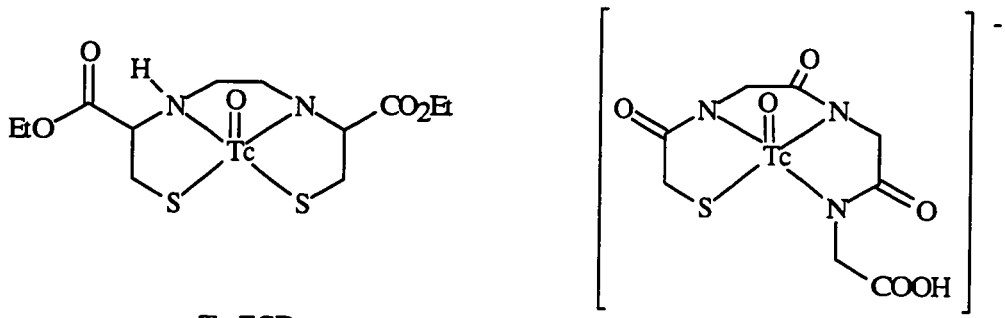
[1]Walovitch, R.C.; Hill, T.C.; Garrity, S.T. *et al.*, *J. Nucl. Med*, **1989**, *30*, 1892.

[2]Fritzberg, A.R.; Kasina, S; Eshima, D; Johnson, D.L., *J. Nucl. Med.*,**1986**, *27*, 111.



Tc-ECD Tc-MAG3

Figure 5-I: Amino acid based technetium chelates

There is a report in the literature of a Cys-Thr-Cys unit which was incorporated within a larger peptide for use in thrombus imaging[3]. The arrangement of the cysteine peptides was designed to mimic the DADT chelating system. The extent of binding of the labelled peptide to the thrombin receptor was not sufficient to develop a clinical imaging agent but the uptake of technetium into the chelate was sufficient to warrant the use of the chelating system in other bifunctional imaging compounds.

By improving on the Cys-Thr-Cys design, bifunctional chelates were synthesised such that the solubility, coordination chemistry and site of derivatization was altered simply by changing the central amino acid.

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[3]Knight, L.C.; Radcliffe, R.; Maurer, A.H.; Rodwell, J.D.; Alvarez, V.L., *J. Nucl. Med.*, **1994**, *35*, 282-288.

5.2 Chelate Design

The tripeptide, mercaptoacetic acid-X-cysteine (where X= any amino acid) is a more accurate mimic of the DADT type of chelate because upon metal coordination, there would be three- 5 membered rings as opposed to two- 5 and one- 6 membered rings in the Cys-X-Cys chelate (**Figure 5-II**). The formation of three- 5 membered rings is important in terms of the metal complexes' *in vivo* stability. Replacement of one of the cysteine residues in Cys-X-Cys with mercaptoacetic acid had the added advantage that it simplified the synthesis of the chelate by avoiding the need for an additional protecting group for the second cysteine amine.

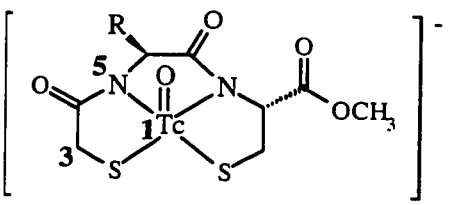

Figure 5-II

The solubility of a chelate based on the Mer-X-Cys design can be changed simply by selecting a different amino acid for X. This type of flexibility is not possible for the DADT, BAT or MAMA type of chelates. Extensive synthesis would be required to provide as many different derivatives for these chelates as could be easily, and economically prepared by the use of amino acids as synthons. For example, hydrophobic peptides were made in this work by incorporating isoleucine or phenylalanine into the tripeptide while

hydrophilic peptides were synthesised by including histidine or serine (**Figure 5-III**). Intermediate solubilities were also possible by the use of tyrosine, methionine or glycine as the central X amino acid.

5.3 Retrosynthesis

The first disconnection in the synthesis of chelates for use in radio imaging is the step in which the metal is incorporated into the chelate. In the case of the Mer-X-Cys peptides, the active form of the chelates had one free thiol and one S-benzyl thioether. It was proposed that upon reaction with technetium or rhenium, the four donor atoms of the chelate would bind and there would be subsequent cleavage of the benzyl group to produce a DADT type coordination complex.

There were two possible approaches to the synthesis of the chelates themselves. In the first approach, a dipeptide, consisting of mercaptoacetic acid and the central amino acid of interest was coupled to cysteine. The dipeptide was synthesised by coupling an active ester of mercaptoacetic acid to a suitably protected amino acid. The second approach involved the coupling of mercaptoacetic acid to a dipeptide unit of the central amino acid and cysteine. Examples of each approach are given below.

5.4 Synthesis of Tr-S-Mer-L-Phe-S-Bn-L-Cys-OMe

The synthesis of Tr-S-Mer-L-Phe-S-Bn-L-Cys-OMe is presented as an example of

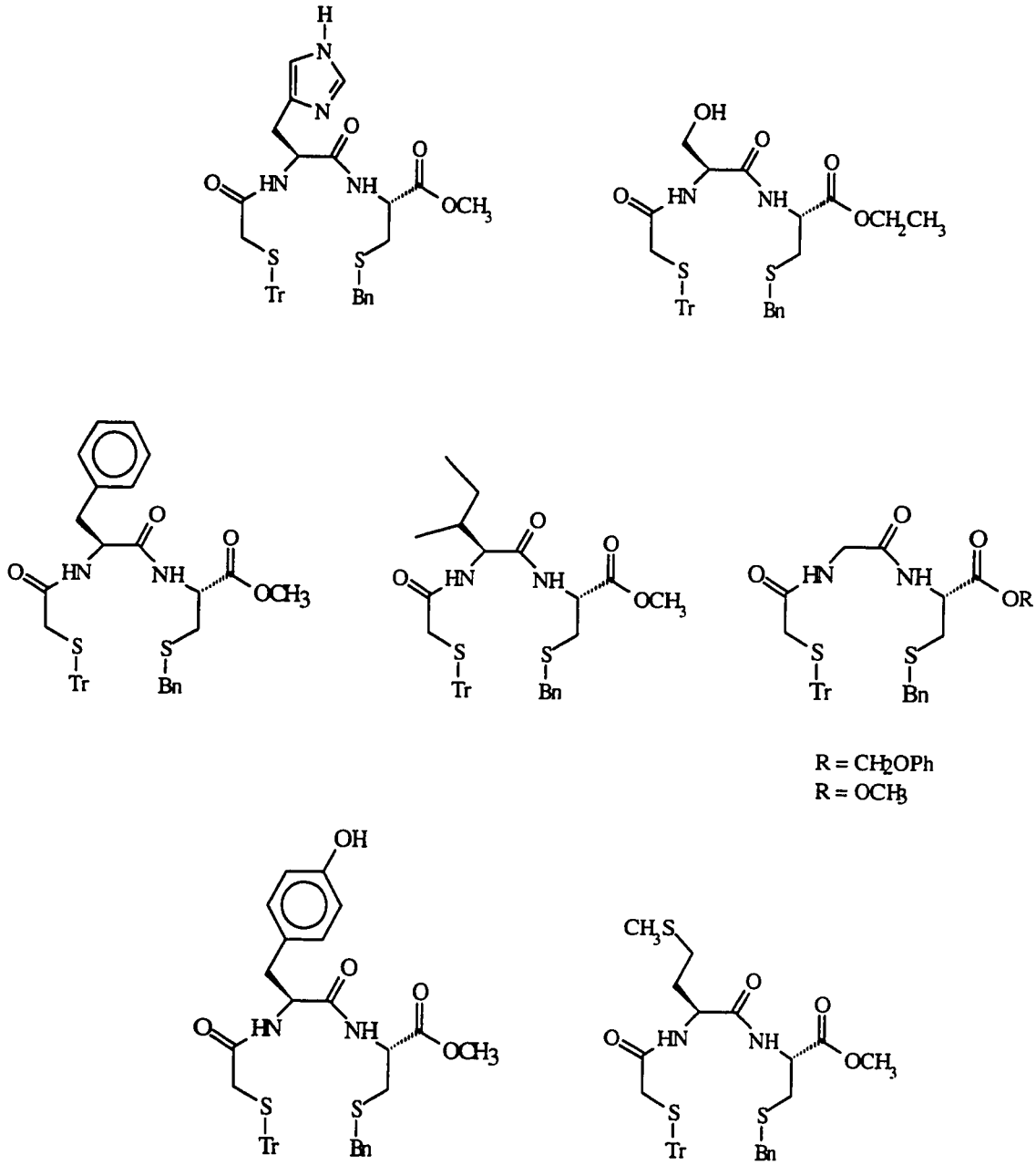

Figure 5-III: Tr-S-Mer-X-S-Bn-L-Cys-OR chelates

method I and an outline is shown in **Figure 5-IV**. L-Phenylalanine ethyl ester hydrochloride, **5.1** was extracted with 10% sodium carbonate in order to deprotonate the amine, a step which was found necessary for efficient coupling. This approach, as opposed to the addition of a tertiary amine base during the reaction, resulted in good yields of the dipeptide **5.2** (69%) in reasonably short time periods. The extraction approach was practical with phenylalanine because the free amine of phenylalanine ethyl ester was appreciably soluble in mildly polar organic solvents such as CH_2Cl_2 or dimethoxyethane. The use of the ethyl ester as a protecting group was found to be necessary because direct coupling of L-phenylalanine with compound **2.5** was not possible because of the poor solubility of phenylalanine in organic solvents. Alternatively, attempts at coupling the free

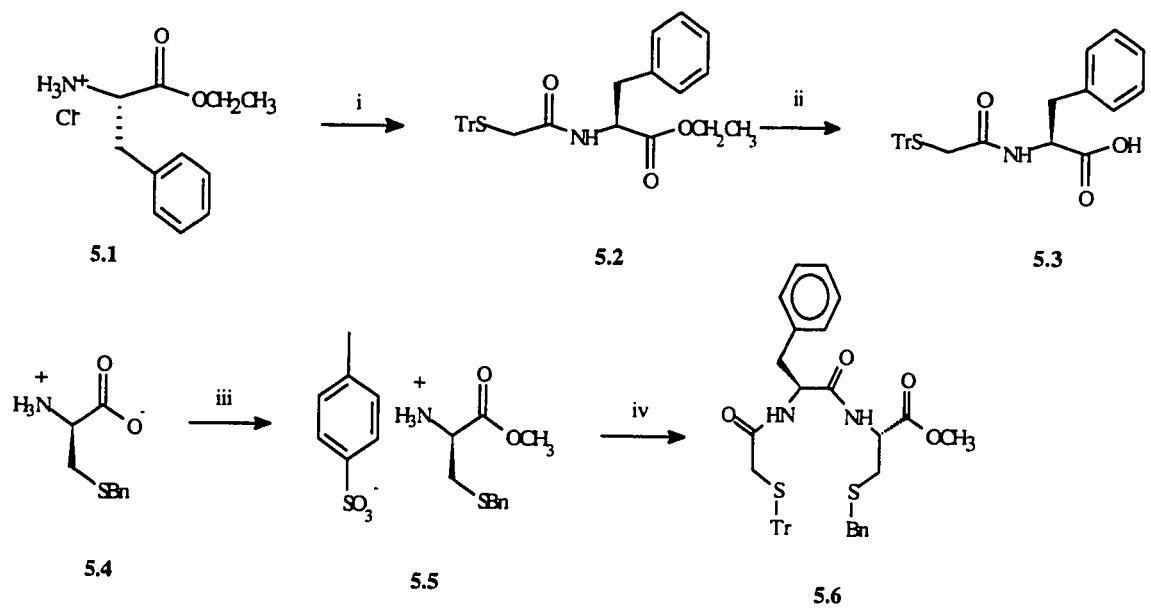

Figure 5-IV: i) Compound **2.4**, EDAC ii) NaOH, THF iii) *p*-TsOH, MeOH, Δ iv) a) 10% Na_2CO_3, DCM b) Compound **5.3**, EDAC.

amine of phenylalanine to the N-hydroxysuccinimido ester of mercaptoacetic acid also resulted in poor yields of the dipeptide. These difficulties were overcome by coupling 2-(triphenylmethylthio)ethanoic acid (Chapter **2**, compound **2.4**) with L-phenylalanine ethyl ester in the presence of ethyl-3-(3-dimethylamino)-propylcarbodiimide hydrochloride (EDAC). The use of the carbodiimide resulted in the formation of the desired compound in good yield (69%). The improved yield may be ascribed to the N-hydroxysuccinimido ester being less reactive than the mixed anhydride intermediate that formed when the carbodiimide was used. The steric influence of the phenyl side chain may have increased the activation energy barrier sufficiently that the succinimide was not a reactive enough acylating agent.

The next step was the hydrolysis of the ethyl ester which was accomplished by the use of aqueous sodium hydroxide in tetrahydrofuran. The reaction was monitored by thin layer chromatography which indicated when all the starting material (R_f= 0.33, 100% $CHCl_3$) had been converted to the acid **5.3** (R_f= 0.20, 10% MeOH/DCM).

Commercially available S-benzyl-L-cysteine was subjected to a Fisher esterification with methanol using *p*-toluenesulfonic acid as catalyst. The use of the organic acid, as in chapter **2** for compound **2.2**, facilitated the dissolution of the ester salt in organic solvents which in turn allowed for the isolation of the free amine of S-benzyl-L-cysteine methyl ester by carbonate extraction. The free amine, which if heated would polymerize, was coupled to the dipeptide **5.3** immediately after isolation. Removal of trace amounts of water prior to coupling by the use of anhydrous sodium sulfate was crucial in obtaining

good yields of the tripeptide. The reaction was monitored by the use of thin layer chromatography and the presence of the phenyl substituent facilitated the isolation of compound **5.6** by radial chromatography. The overall yield of the synthesis was 25% for the five steps.

5.5 NMR of Tr-S-Mer-L-Phe-S-Bn-L-Cys-OMe

Detailed NMR spectra of all chelants prepared were collected and analysed in detail. In order to ensure accurate assignments of the ^{1}H and ^{13}C resonances, 2-D NMR experiments were used including ^{1}H-^{1}H correlation spectroscopy (COSY) and heteronuclear single quantum coherence spectroscopy (HSQC).

Tr-S-Mer-L-Phe-S-Bn-L-Cys-OMe, **5.6**, is a typical example and its spectral assignments are discussed in detail. The ^{1}H spectrum (500 MHz) of compound **5.6** is shown in **Figure 5-V**. The multiplet at 7.30 ppm is an overlap between the triphenylmethyl and benzyl proton resonances and those of the aryl ring of phenylalanine. The two doublets at 6.64 and 6.36 ppm are the two amide proton resonances. In dry deuterochloroform these amide protons do not exchange but will do so in a protic solvent such as deuteromethanol. The multiplets at 4.70 and 4.45 ppm are the *alpha* proton resonanes of the two amino acids. The multiplets are the X part of an ABX spin system; the AB parts of the spin systems are at 2.91 and 2.75 ppm.

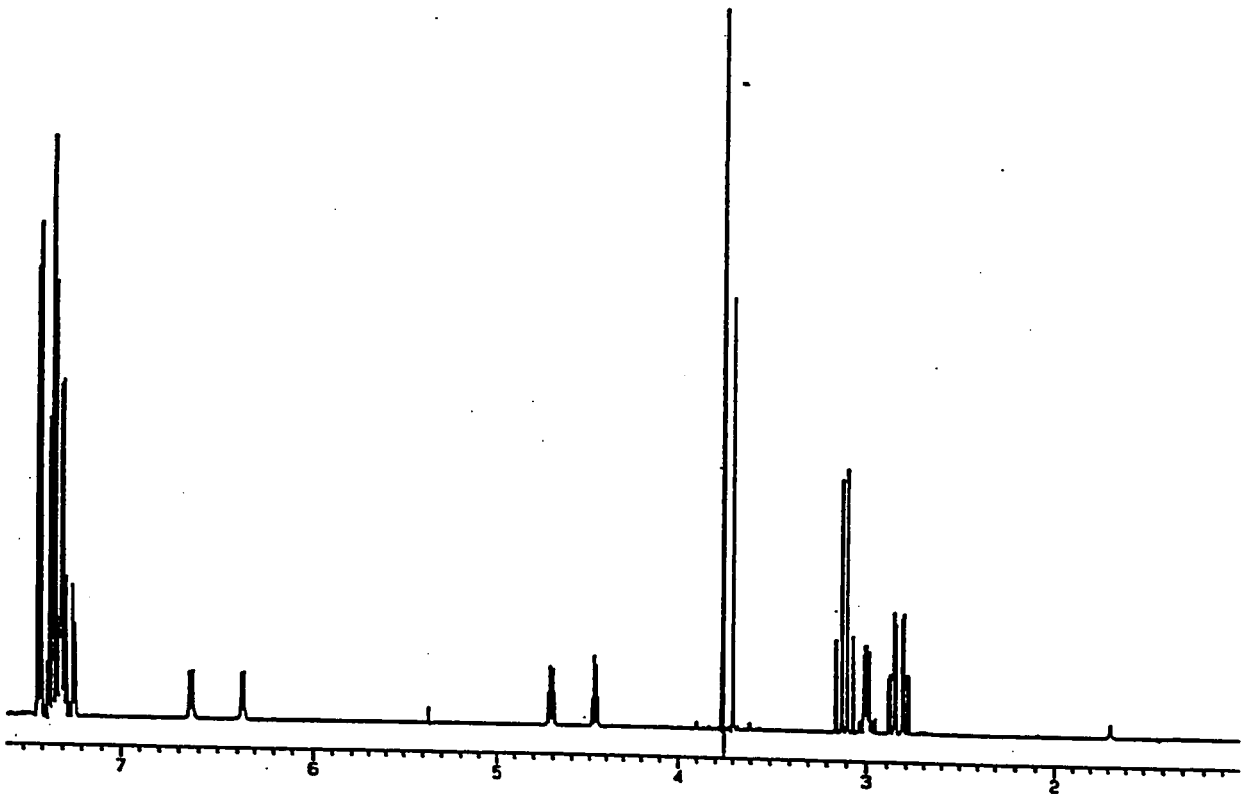

Figure 5-V: ^{1}H NMR Spectrum of Tr-S-Mer-L-Phe-S-Bn-L-Cys-OMe

The remaining multiplet was assigned to the methylene group of the mercaptoacetic acid fragment; it was an AB spin system with a coupling constant of -15.7 Hz.

The COSY spectrum (**Figure 5-VI**) indicated that the downfield amide proton correlated to the upfield *alpha* proton which, based on its chemical shift was assigned to phenylalanine. Knowing the *alpha* proton shift facilitated the assignment of the *beta* protons either by comparing coupling constants or by use of the COSY spectrum. The Phe *beta* protons were assigned to the multiplet at 2.91 ppm while the Cys *beta* protons were assigned to the multiplet at 2.75 ppm.

Once the COSY spectrum had been used to assign the proton resonances, the HSQC experiment (**Figure 5-VII**) was used to assign the ^{13}C signals. The resonance at 54.63 ppm exhibited a HSQC correlation to the signal which was ascribed to the phenylalanine *alpha* proton; the cysteine *alpha* carbon was upfield at 51.75 ppm. Upfield from the Cys *alpha* carbon signal was that of the methyl ester which correlated to the ^{1}H signal at 3.66 ppm. The ^{13}C signal associated with the *beta* carbon of phenylalanine (37.79 ppm) was downfield compared to the cysteine *beta* carbon (33.16 ppm). The remaining two signals were those of the S-benzyl aliphatic protons (36.48 ppm) and of the methylene of mercaptoacetic acid (36.17 ppm).

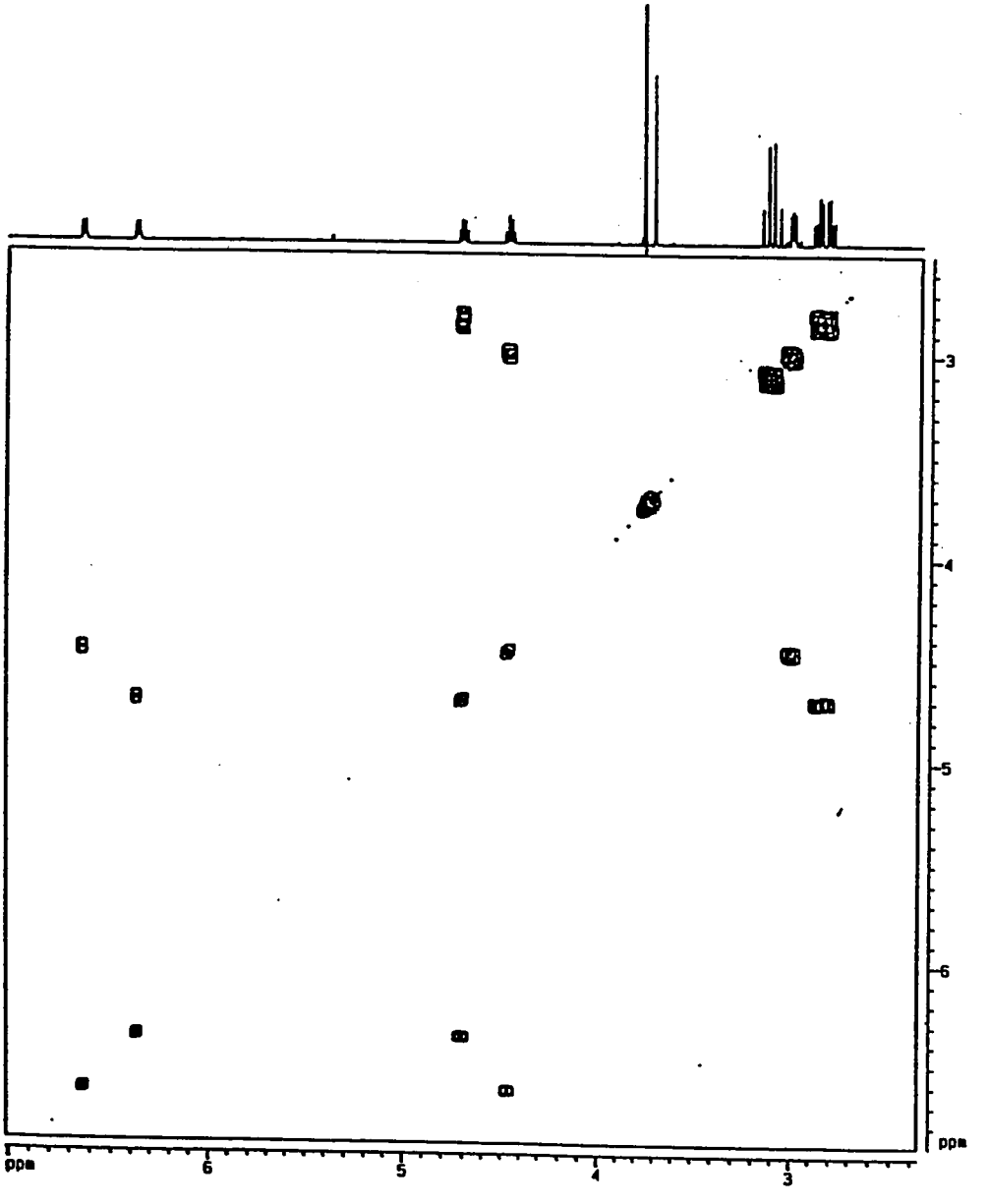

Figure 5-VI: COSY Spectrum of Tr-S-Mer-L-Phe-S-Bn-L-Cys-OMe

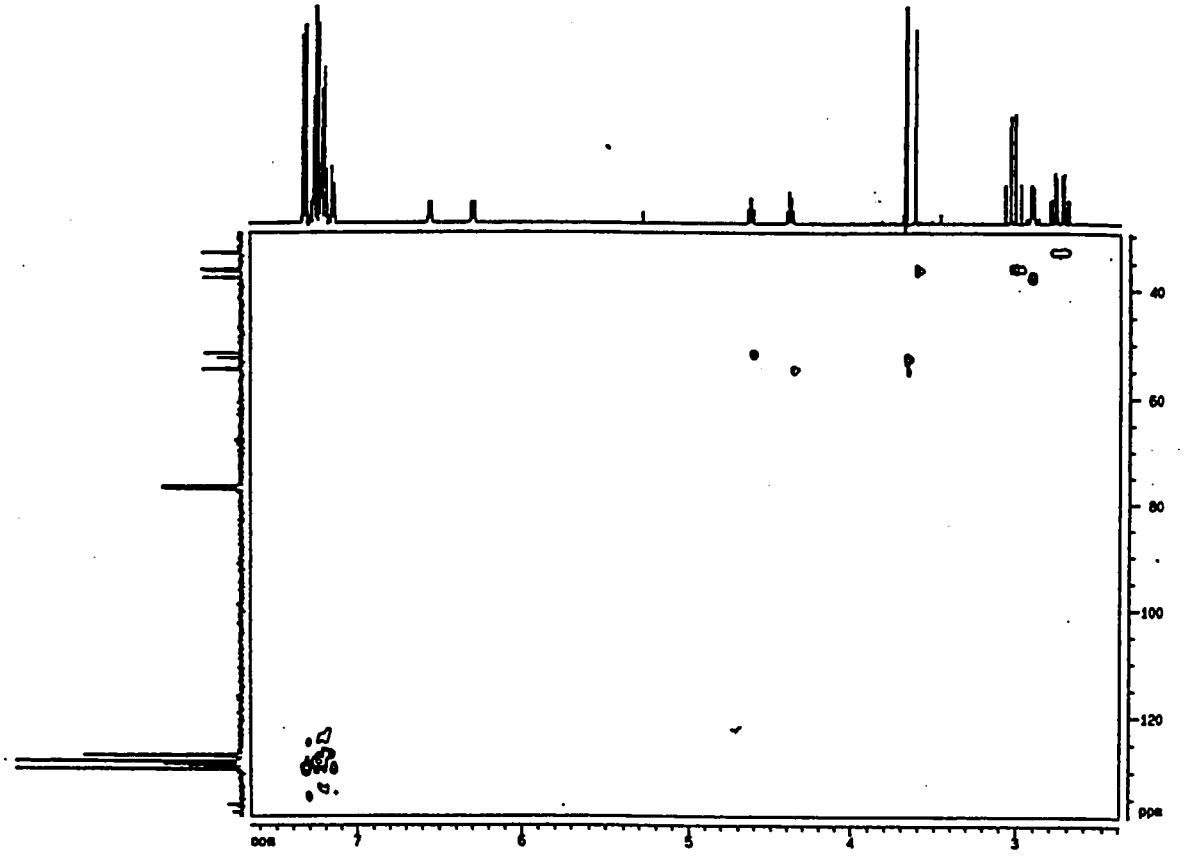

Figure 5-VII: HSQC spectrum of Tr-S-Mer-L-Phe-S-Bn-L-Cys-OMe

5.6 Synthesis of Tr-S-Mer-L-His-S-Bn-L-Cys-OMe

The synthesis of Tr-S-Mer-L-His-S-Bn-L-Cys-OMe is presented as an example of method II and an outline is shown in **Figure 5-VIII**. Because S-benzyl-L-cysteine was stable to acid, the alternative synthetic strategy was possible. S-Benzyl-L-cysteine methyl ester p-toluenesulfonic acid salt was deprotonated prior to coupling to N-t-butoxycarbonyl-L-histidine; the condensation with **5.7** occurred in good yield (72%). Conversion of the Boc carbamate **5.8** to the amine was accomplished by the use of trifluoroacetic acid and

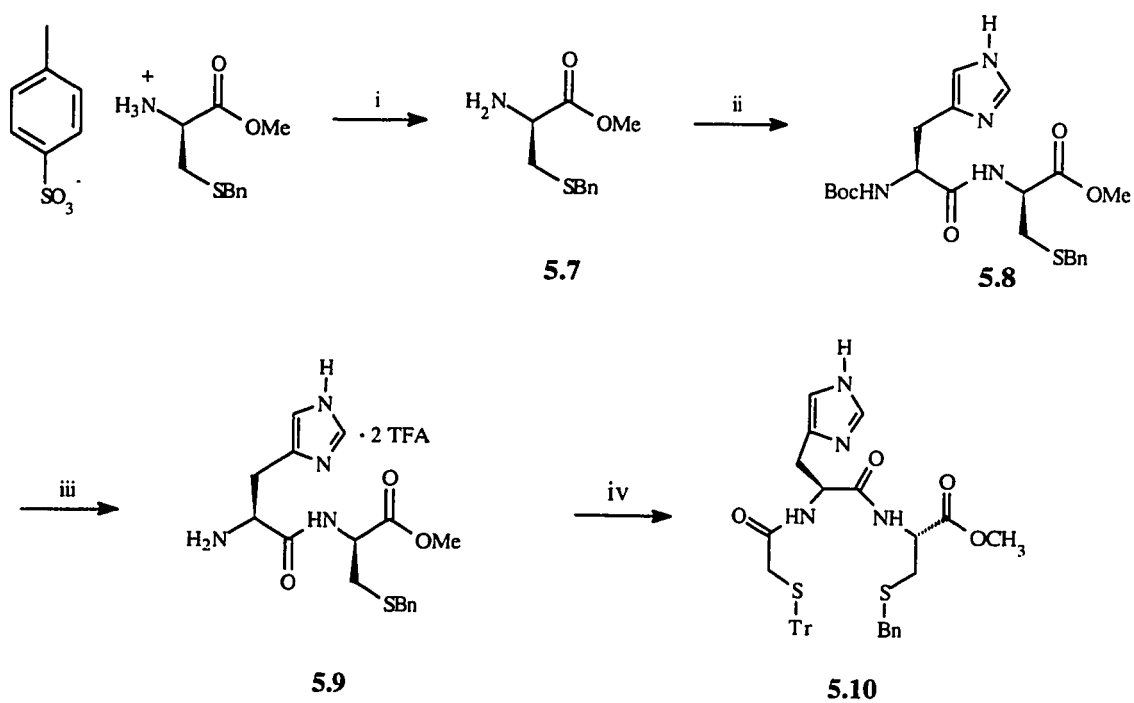

Figure 5-VIII: i) 10% Na_2CO_3/ DCM ii) N-t-Boc-L-Histidine, EDAC iii) TFA, TES iv) Compound **2.5**

triethylsilane[4]. The proposed mechanism for this reaction (**Figure 5-IX**) involves protonation of the carbamate followed by formation of the carbamic acid and t-butyl cation, which subsequently eliminates to form isobutylene. The carbamic acid, which is unstable, decomposes to form carbon dioxide and the desired amine. Triethylsilane enhances the reaction by acting as a hydride donor to the *t*-butyl cation immediately after its formation and preventing it from undergoing other undesirable reactions. Because the deprotection was performed in TFA, which is an excellent solvent for acid-stable amino acids, the product of the deprotection was the ditrifluoroacetate salt. This was confirmed by observing the appropriate TFA peaks in the ^{13}C NMR spectrum (see experimental methods section). An excess of triethylamine or diisopropylethylamine was used to liberate the amine during the coupling of **5.9** to the N-hydroxysuccinimido ester **2.5**. The overall yield of the synthesis was 24% in 5 steps.

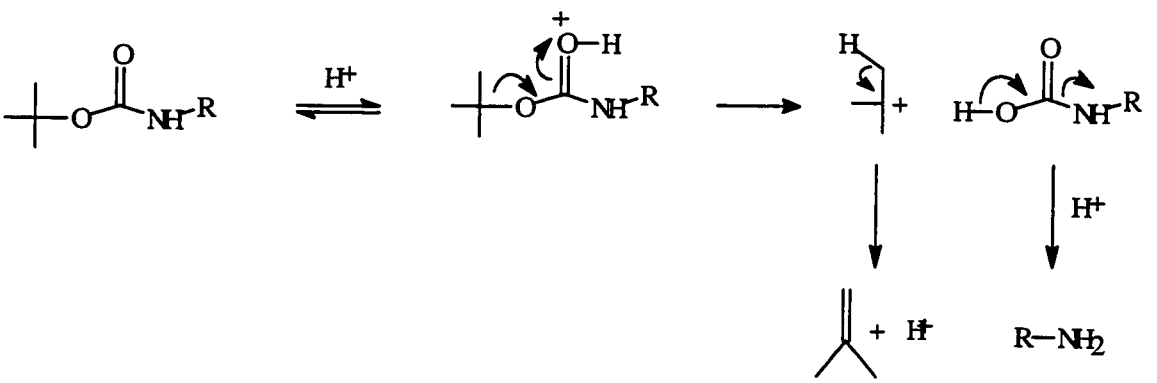

Figure 5-IX: Mechanism of Boc deprotection

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[4] Brenner, D.; Davison, A.; Lister-James, J.; Jones, A.G., *Inorg. Chem.*, **1984**, *23*, 3793.

5.7 Mass Spectrometry of Tr-S-Mer-L-His-S-Bn-L-Cys-OH

Conventional mass spectrometry (chemical ionization or electron impact) for trityl protected compounds is not useful because the spectra are overwhelmed with the signal from the triphenylmethyl cation. Electrospray mass spectrometry (ES-MS) can be used for trityl protected peptides so long as they contain an acidic or basic residue.

Compound **5.10** was de-esterified by the use of 10% Na_2CO_3 in methanol in order to provide a site for derivatization. The positive ES-MS spectrum of the free acid, in the presence of 0.1% trifluoroacetic acid exhibited the M+1 peak (m/z= 665.4) (**Figure 5-X**) which confirmed that the ester had been hydrolysed. The other main peak in the spectrum (m/z= 243.4) was ascribed to triphenylmethyl cation that was generated in the mass spectrometer. High resolution ES is not possible with available instrumentation so all chelates were subjected to elemental analysis to confirm their composition.

5.8 Synthesis of Re-Mer-L-His-L-Cys-OMe

Compound **5.10** was deprotected (TFA, Et_3SiH) to give the free thiol, **5.11**, which was reacted with $ReOCl_3(PPh_3)_2$, a common starting material for the synthesis of rhenium(V) complexes (**Figure 5-XI**)[5]. Sodium acetate was included as a buffer in the preparative reaction because the metal displaces two amide protons during formation of the complex.

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[5]Lock, C.J.L.; Wilkinson, G., *Chem. and Indust.*, **1962**, *40*.

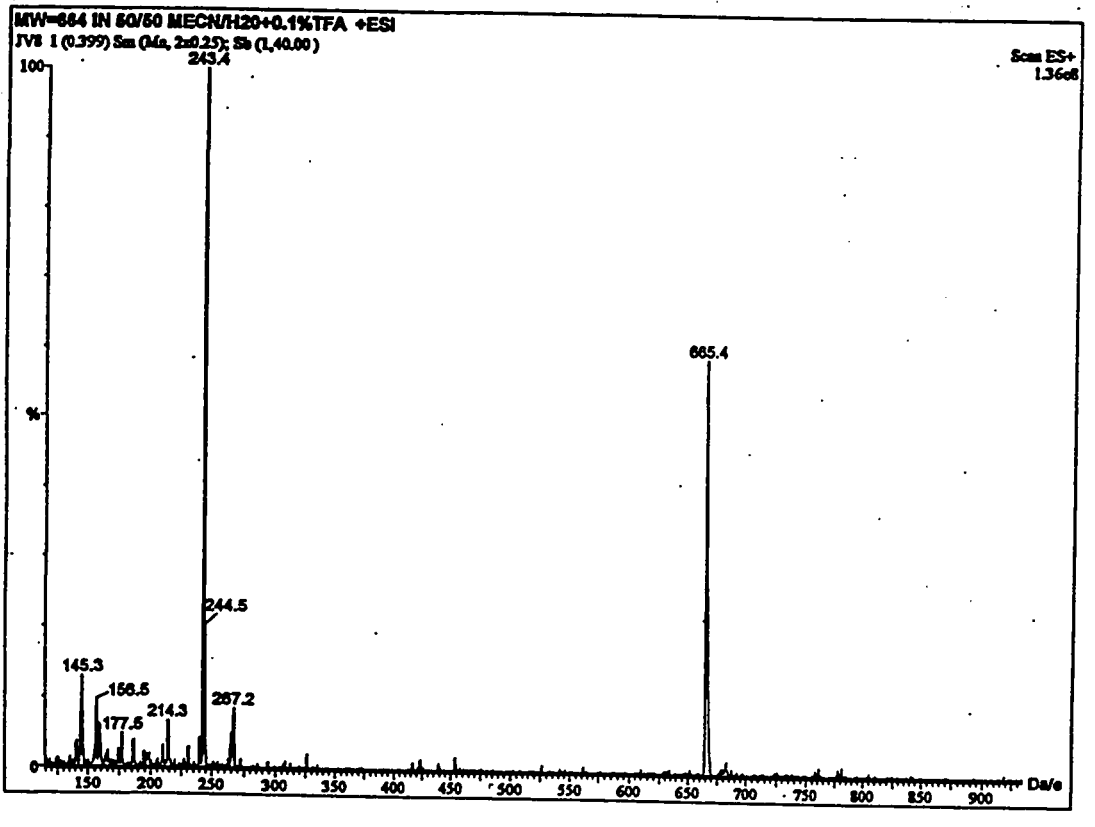
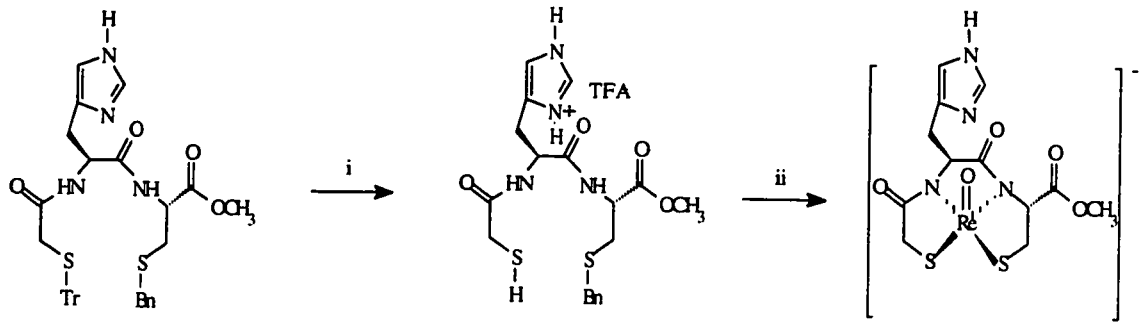
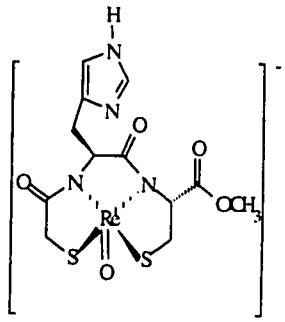


Figure 5-X: ES-MS of Tr-S-Mer-L-His-S-Bn-L-Cys-OH



5.9 **5.25a**



5.25b

Silica thin layer chromatography of the MeOH/THF soluble fraction of the reaction mixture indicated the presence of a orange/red compound which moved on the plate in 10% methanol in DCM. Electrospray mass spectrometry indicated the presence of a high mass compound which contained an isotope ratio similar to that for rhenium. Attempts at purification of this apparent rhenium compound by silica gel chromatography or silica preparative plate chromatography were unsuccessful as the products were always contaminated with residual triphenylphosphine or triphenylphosphine oxide. Reverse phase HPLC was however successful in isolating the desired compound and **Figure 5-XII** shows a typical chromatogram where the two largest peaks had absorbances in the visible region;

these were collected and examined spectroscopically.

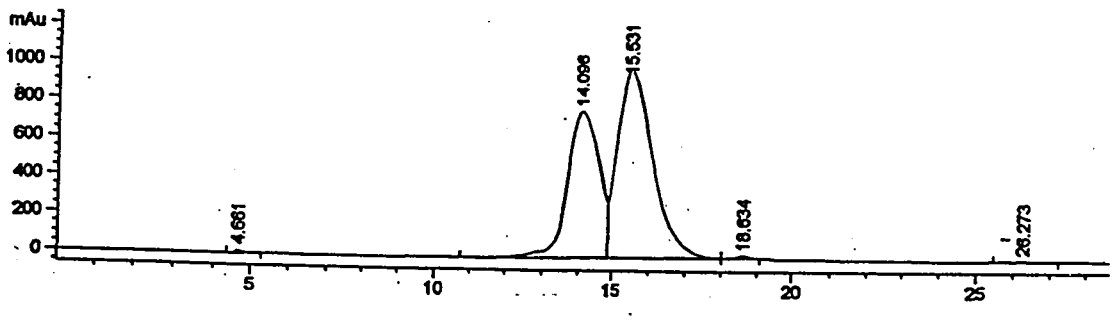


Figure 5-XII: HPLC chromatogram of **5.25**

For both HPLC fractions, the electrospray mass spectrum (**Figure 5-XIII**) indicated the presence of an anion (m/z= 543/545), which confirmed that the S-benzyl protecting group had been cleaved upon coordination. Thin layer chromatography of the samples (10% DCM/ CH_3OH) on a silica plate indicated only one product when UV was used as the detector.

Expansions of the aliphatic region of the ^1H NMR of the two fractions are shown in **Figure 5-XIV**. The spectrum of fraction 1, which was the first fraction to elute from the HPLC, exhibited two downfield multiplets at 5.09 ppm and 4.81 ppm which were

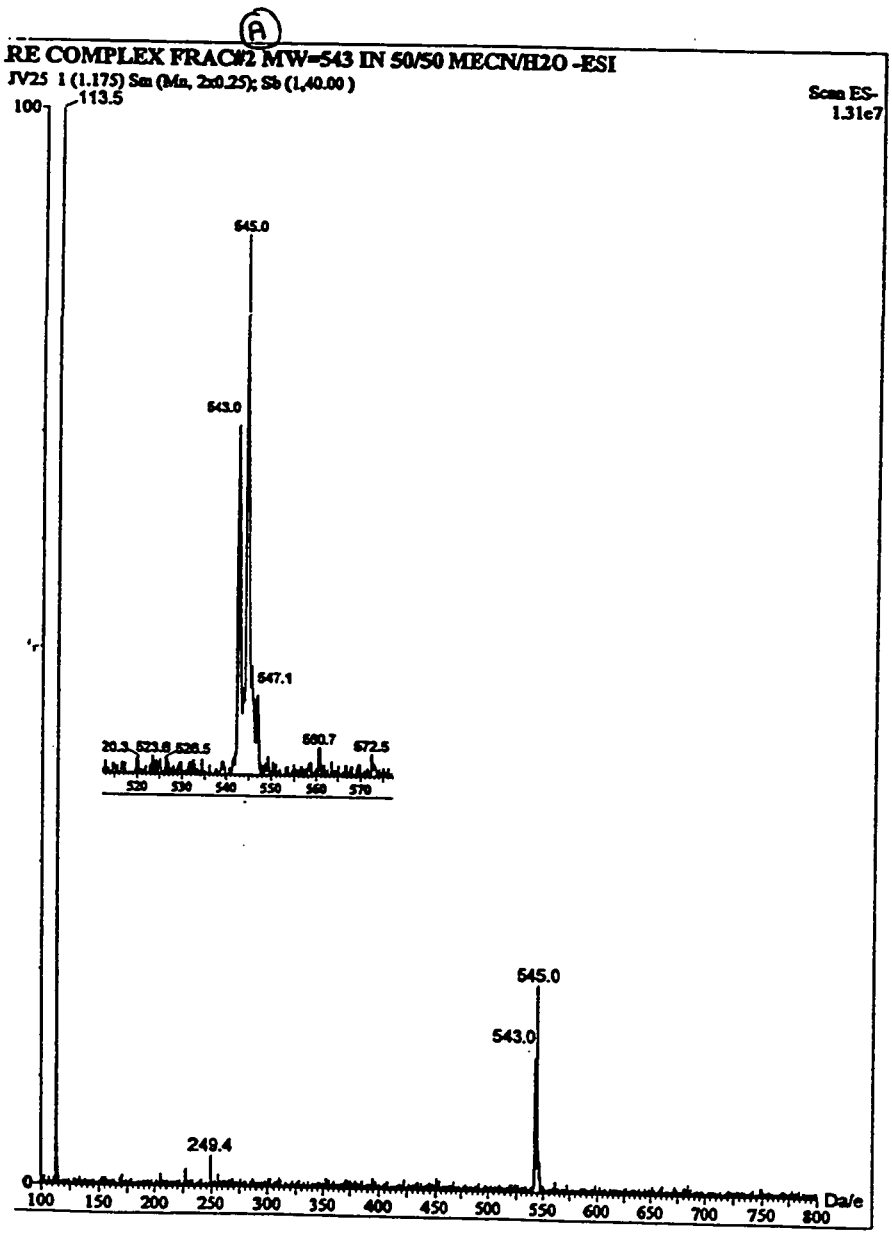


Figure 5-XIII: ES-MS of compound **5.25**

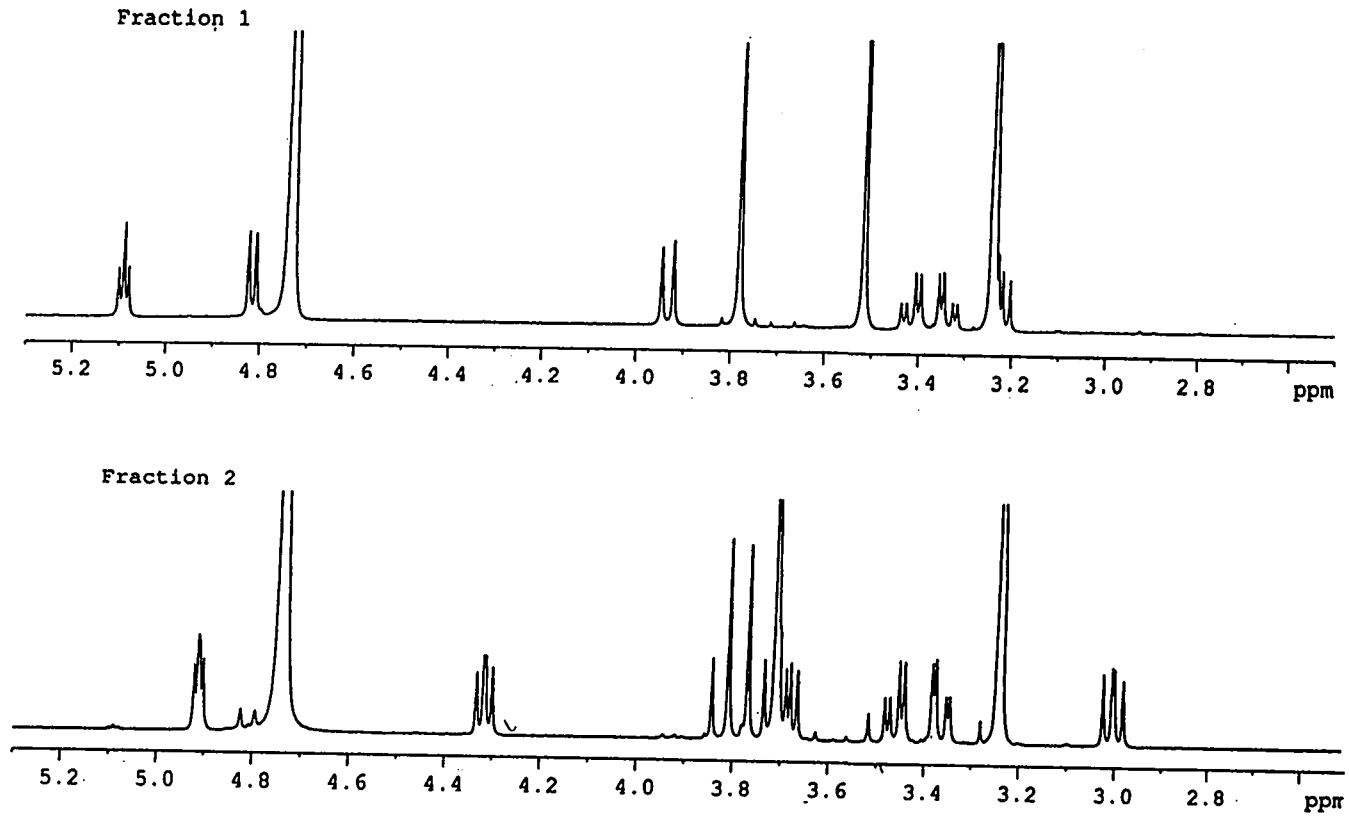
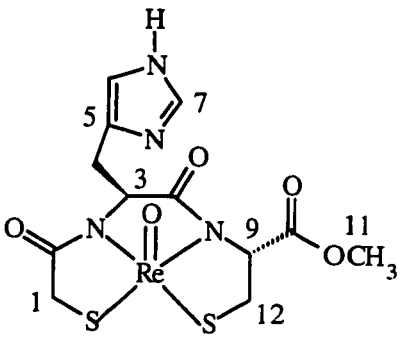


Figure 5-XIV: Expansion of proton NMR of Re-Mer-L-His-L-Cys-OMe

assigned as the *alpha* protons of the amino acids. The multiplet at 5.09 ppm, which is the X part of an ABX spin system, exhibited a correlation to the multiplet at 3.38 ppm. Because the AB part of the spin system did not exhibit any unusual chemical shift changes, which are usually observed for protons within the chelate ring upon complexation of rhenium, it was assigned to the *beta* protons of histidine. The doublet at 4.81 ppm was assigned as the cysteine *alpha* proton; one of the corresponding cysteine *beta* protons was also a doublet. Because the Cys *beta* protons were in the ring of the chelate, they were affected by the magnetic anisotropy induced by the rhenium which explains why they differed in chemical shift by approximately 0.8 ppm. The splitting pattern of the Cys spin system indicated that one of the *beta* protons was nearly at right angles to the Cys *alpha* proton thereby giving a coupling constant value of zero.

The cysteine *alpha* proton in fraction 2 was a doublet of doublets (δ= 4.32 ppm, J=10.1 and 7.2 Hz). Because the coupling constants between the *alpha* and both *beta* protons were non-zero, the angles between the *alpha* and *beta* protons must be different than those in the compound from fraction 1. The corresponding Cys *beta* protons were at 3.68 and 3.00 ppm. The His *alpha* proton was also a doublet of doublets (δ= 4.91 ppm, J= 5.1 and 3.8 Hz); its corresponding *beta* protons exhibited a coupling pattern very similar to that of fraction 1. Other than the coupling constant values for the cysteine spin system there were only minor differences, usually small changes in chemical shifts, between the ^{1}H spectra of fractions 1 and 2 (**Figure 5-XV**).



^{1}H NMR		
Proton	Chemical Shift (δ)-Frac 1	Chemical Shift (δ)-Frac 2
H-1	3.827, 3.752	3.792, 3.752
H-3	5.093	4.914
H-4	3.376	3.362
H-6	7.156	7.301
H-7	8.567	8.558
H-9	4.812	4.317
H-11	3.519	3.712
H-12	3.928, 3.243	3.680, 3.004

Figure 5-XV: A comparison of the chemical shifts in the ^{1}H NMR spectrum [500 MHz] of compounds **5.25a** and **b**.

The most distinguishing feature of the ^{13}C NMR spectra of both fractions was the change in the chemical shift of the carbon atoms of the amide groups. The significant downfield shift stems from the fact that upon coordination, the nitrogen atom of the amide bond becomes less effective at back- donating electron density to the carbonyl group. Between the two isomers, there were only minor differences in ^{13}C chemical shifts (**Figure 5-XVI**).

The data from the NMR, HPLC and ES-MS suggested that the two fractions were diastereomers. The rhenium is a stereogenic center and its associated oxygen atom can be on the same or opposite side as the *alpha* protons of the Cys and His amino acids. From the peak heights in the HPLC there were approximately equal amounts of each isomer suggesting that they must be roughly equal in ground state energy. These results agree with those of another rhenium chelate complex, Re-N,N-dimethylaminoglycine-L-serine-L-cysteine-(L-glycinamide) which was also isolated as equal amounts of two diastereomers[6].

Single crystals from the first fraction of compound **5.25**, were obtained by slow evaporation of a saturated methanol solution and these proved suitable for X-ray crystallographic analysis. The structure (**Figure 5-XVII**), a typical square pyramidal arrangement, contained the imidazole ring on the same side as the rhenium oxygen (the syn isomer). The complex was a zwitterion with the metal having a charge of -1 and the counter ion being the protonated imidazole ring. The Re-O (1.684(5) Å), Re-N (2.003(5)

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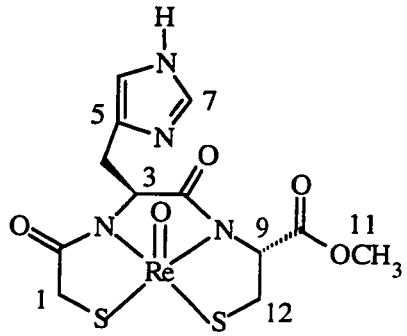
[6]Bell, R.A.; Bennett, S.; Fauconnier, T.; Thornback, J.; Valliant, J.; Wong, E. *Unpublished results*.



^{13}C NMR		
Carbon	Chemical Shift (δ)-Frac 1	Chemical Shift (δ)-Frac 2
C-1	40.05	39.45
C-3	67.12	66.37
C-4	26.95	27.00
C-5	119.6	128.16
C-6	117.15	118.11
C-7	133.13	133.94
C-9	67.45	68.36
C-11	51.20	51.26
C-12	47.60	46.18

Figure 5-XVI: Chemical shifts of the aromatic and aliphatic region of the ^{13}C NMR spectrum [125 MHz] of compound **5.25** for both fraction 1 and 2.

and 2.015(5) Å) and Re-S (2.292(2) and 2.310(2) Å) distances were all within expected values. The torsion angles between the *alpha* and *beta* protons of cysteine were 24.6° for $H_9C_9C_{12}H_{12a}$ and -93.1° $H_9C_9C_{12}H_{12b}$ and were entirely consistent with the coupling constants observed in the ^1H NMR for structure **5.25a**. HPLC fraction 1 was thus structure **5.25a** and fraction 2 can be assigned to structure **5.25b**. Full data on the crystal structure can be found in appendix **II**.

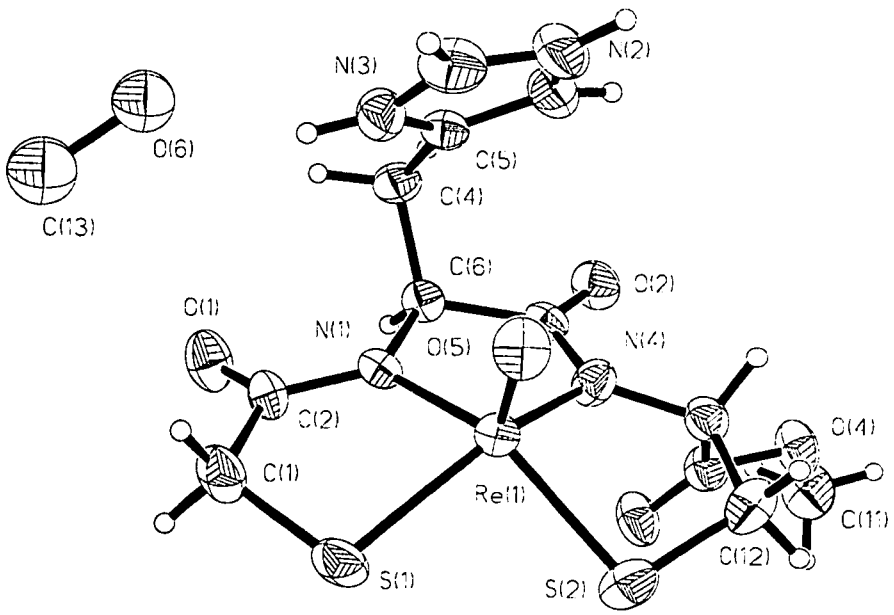


Figure 5-XVII: Structure of compound **5.25a** and a methanol of crystallization (50% thermal probability ellipsoids)

5.9 Reaction of Mer-L-Ile-S-Bn-L-Cys-OMe with $ReOCl_3(PPh_3)_2$

A Newman projection along the α and β-carbon atoms of histidine in compound **5.25a** (the syn isomer) showed that the β protons of histidine interact with the two amide carbonyl oxygens. It was postulated that if the steric bulk of one of the β substituents were increased, then the energy of the resulting gauche interaction would force the molecule to adopt the anti isomer preferentially.

The tripeptide, Tr-S-Mer-L-Ile-S-Bn-L-Cys, **5.15**, which contains a methyl substituent at the β position, was reacted with $ReOCl_3(PPh_3)_2$ in a similar fashion to that for Tr-S-Mer-L-His-S-Bn-L-Cys-OMe, **5.9**. The tripeptide was detritylated and the complex formed by heating in refluxing methanol in the presence of sodium acetate. The time required for the reaction mixture to change colour was significantly longer than that for the Mer-L-His-L-Cys-OMe tripeptide (12 hours vs 2 hours). After a total reaction time of 16 hours the reddish/orange solution was evaporated to dryness, diluted with methanol and the resulting coloured solution applied to a preparative TLC plate. Only one coloured band was observed moving on the TLC plate; it was separated and the coloured compound collected by extraction with methanol.

Electrospray mass spectrometry of the coloured band indicated a different product than that obtained from the histidine tripeptide (**5.9**). The ES-MS results suggested that the product still contained the benzyl protecting group but that the methyl ester had been cleaved from the chelate (**Figure 5-XVIII**).

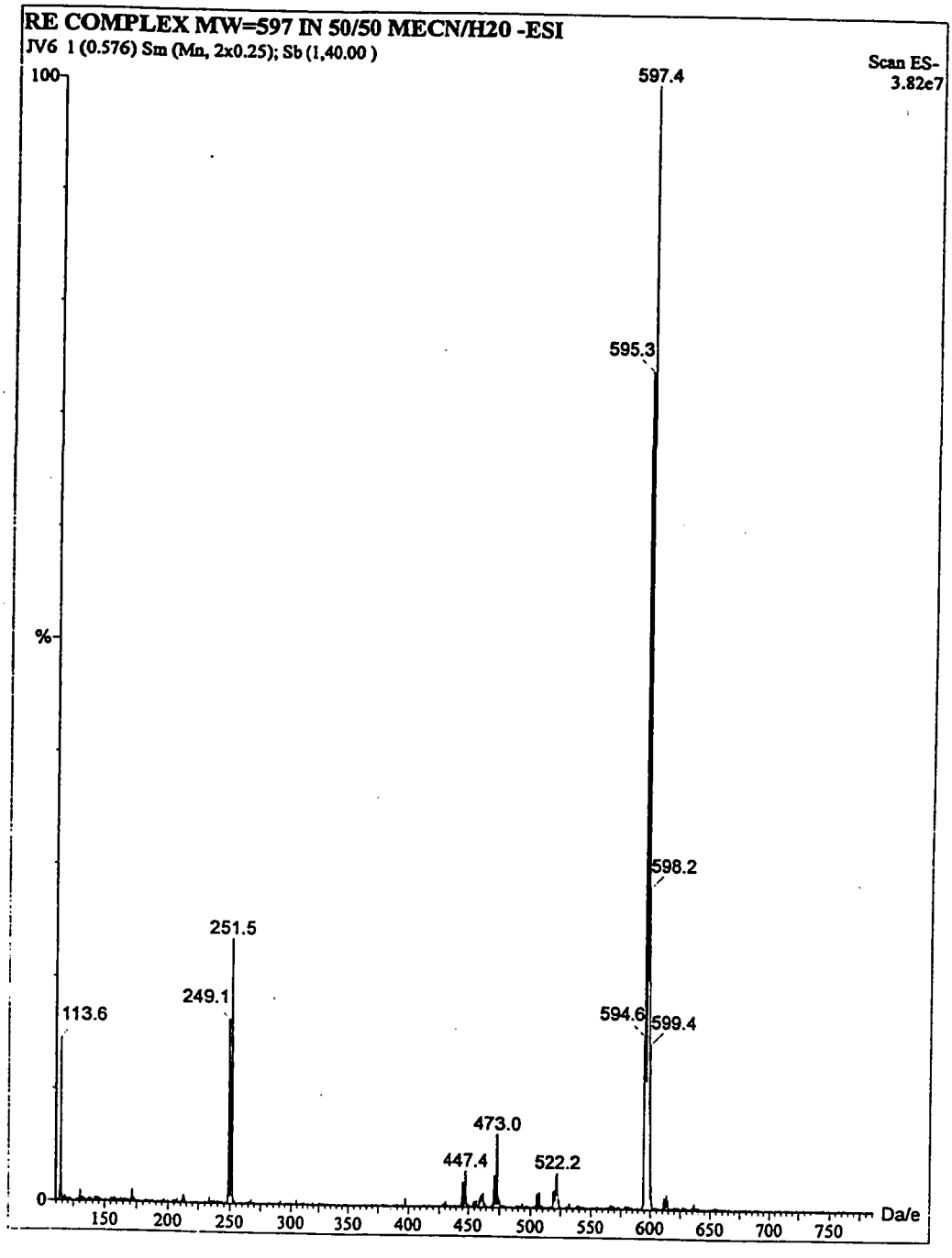


Figure 5-XVIII: Electrospray mass spectrum of compound **5.26**

The NMR spectra supported the ES-MS results in that aromatic signals corresponding to the benzyl group were observed in both the ^{1}H and ^{13}C spectra. The most distinguishing feature of the ^{1}H NMR spectrum however, was the presence of only two amino acid *alpha* proton signals. The isoleucine *alpha* proton was a doublet (J= 5.6 Hz) at 4.28 ppm while the cysteine *alpha* proton was upfield at 4.36 ppm. The splitting pattern (doublet of doublets) and chemical shift were similar to the pattern and chemical shift values observed for the cysteine *alpha* CH in the second fraction of Re-Mer-L-His-L-Cys-OMe, which was assigned structure **5.25b**, the anti isomer. There were two AB systems which overlapped at 3.78 and 3.67 ppm and these signals were assigned to the S-benzyl and the mercaptoacetic acid methylene groups respectively. The *beta* protons of cysteine differed in chemical shift by approximately 0.2 ppm, as noted above for **5.25b**; the difference in chemical shift was a result of anisotropy induced by the metal center. There was only a slight downfield shift of protons on the isoleucine side chain compared to the free ligand which suggested that the isoleucine side chain is extending away from the metal and its magnetic effects. The structure of the Re-Mer-L-Ile-L-Cys complex was thus assigned as the anti isomer as in **5.25b**.

5.10 Complex Formation

A proposed mechanism for the formation of the rhenium metal complexes for the tripeptides, **5.9** and **5.15** involves the thiol group, which is the most nucleophilic donor group, reacting with the metal center and displacing a leaving group (a phosphine, or a

chloride). The next step would involve coordination of the amides which most likely occurs through the carbonyl oxygens prior to deprotonation of the amide, and then rearrangement of the rhenium from oxygen to nitrogen. After coordination of the benzyl thioether group, the large δ^+ charge on the sulfur weakens the C-S bond and enables a nucleophile (such as chloride or acetate) to react at the benzyl methylene and give a debenzylated anionic rhenium complex.

The long reaction times were a result of the poor solubility and low reactivity of the rhenium starting material. When a small scale room temperature reaction was performed using [tetrabutylammonium][oxotetrachlororhenium(V)], which is a very reactive and soluble rhenium starting material, with Mer-Ser-S-Bn-L-Cys-OEt, the colour of the solution changed immediately without addition of base. The electrospray mass spectrum of the crude material indicated the presence of an anion with an analogous structure to the Re-histidine chelate compound, **5.25**.

Cleavage of the ester bond can be ascribed to the larger concentration of sodium acetate used in the isoleucine reaction as compared to the histidine reaction (20 equivalents vs 10 equivalents). Such acetolyses of methyl esters have been observed previously in chelate formation reactions[7] and it is plausible that the metal ion is acting as a Lewis acid to enhance the rate of these normally slow processes.

Cleavage of the benzyl group in the histidine chelate, **5.9** but not in the isoleucine chelate **5.15** suggested that the bulky isoleucine residue hinders attack of a nucleophile on

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[7]Bell, R.A.; Valliant, J.F. *Unpublished results*.

the methylene of the benzyl group. In order for the isoleucine side chain to hinder nucleophilic attack, the benzyl group must adopt (a) conformation(s) in which the methylene group is *syn* to the isoleucine side chain and the phenyl group hinders attack from the outside face. It is conceivable that the reaction could be an S_N1 process. However, if the mechanism occurs through the formation of the benzyl cation, one would expect deprotection to occur at similar rates in both cases.

The formation of only one isomer of **5.26** suggests that the steric bulk at the β position of the central amino acid causes an increase in a gauche interaction which is sufficiently high in energy to ensure that the anti diastereomer is the only species formed in solution. The use of hindered side chains in the central amino acid may conceivably be used to facilitate the diastereoselective synthesis of chiral rhenium(V) and technetium(V) chelate complexes.

5.11 Experimental Section

Tr-S-Mer-L-Phe-OEt (**5.2**)

L-Phenylalanine ethyl ester hydrochloride (3.73 g, 16.4 mmol) was dissolved in dichloromethane (50 mL) and extracted with 10% sodium carbonate (25 mL). The aqueous layer was back extracted with dichloromethane (2 x 25 mL) and the organic layers pooled, dried over sodium sulfate and evaporated to dryness. After dilution with dichloromethane (40 mL), compound **2.5** (5.0 g, 15.0 mmol) and EDAC (3.16 g, 16.4 mmol) were added. The mixture was stirred for 24 hours under nitrogen and protected from light prior to

extraction with 1M sodium bicarbonate (2 x 25 mL), 0.1M HCl (2 x 25 mL) and distilled water (2 x 25 mL). The organic layer was concentrated, dissolved in methanol (10 mL) and cooled to -10°C for 16 hours. The solution was filtered twice leaving a colourless solid (3.25 g, 43 %); further cooling of the filtrate (-10°C) yielded a second crop of the title compound (2.0 g, 26%). The compound showed: mp: 90-91°C; TLC: R_f = 0.33 (100% $CHCl_3$); ^1H NMR ($CDCl_3$) [200 MHz]:δ7.24 (m, H-aryl), 6.470 (d, J = 7.2, NH), 4.462 (m, 1H, CH), 4.023 (q, J= 7.1, OCH_2), 2.941 (s, 2H, SCH_2), 2.890 (m, 2H, CH_2Ph), 1.093 (t, 3H, CH_3); ^{13}C NMR ($CDCl_3$) [50 MHz]: δ170.87 (COOR), 167.58 (amide C(O)), 143.85 (Tr-*ipso*), 135.64 (Phe-ipso), 129.42 (trityl-*ortho*), 129.20 (Phe-*ortho*), 128.36 (Phe-*meta*), 127.97 (trityl-*meta*), 126.92 (trityl-*para*), 67.69 (CPh_3), 61.25 ($O\mathbf{CH_2}CH_3$), 53.44 (Phe-αCH), 37.72 ($CH\mathbf{CH_2}$), 36.06 ($TrSCH_2$), 13.95 ($OCH_2\mathbf{CH_3}$).

Tr-S-Mer-L-Phe-OH (5.3)

Compound **5.2** (3.25 g, 6.38 mmol) was dissolved in a THF / water (30 mL, 40:10 v/v) mixture. With rapid stirring under nitrogen and protected from light, sodium hydroxide (289 mg, 7.22 mmol) was added to the solution. After 9 hours, the pH was adjusted to 3.9 with 6M HCl, the THF evaporated, the remaining solution diluted with water (10 mL) and extracted with dichloromethane (3 x 30 mL). The organic layers were pooled and evaporated leaving **5.3** as a colourless crystalline solid (2.78 g, 91%). The compound showed: m.p.: 58-60°C; TLC: R_f= 0.2 (10% MeOH/ DCM); ^1H NMR ($CDCl_3$) [200MHz]: δ7.230 (m, H-aryl), 4.280 (m, 1H, Phe-αCH), 3.395 (s, 2H, SCH_2), 2.835 (m,

2H, $CHCH_2$); ^{13}C NMR ($CDCl_3$) [50 MHz]: δ173.65 (COOH), 169.10 (amide C(O)), 143.80 (Tr-*ipso*), 135.30 (Phe-*ipso*), 129.44 (trityl-*ortho*), 129.34 (Phe-*ortho*), 128.70 (Phe-*meta*), 128.15 (trityl-*meta*), 127.34 (Phe-*para*), 127.14 (trityl-*para*), 68.00 (CPh_3), 53.73 (Phe-αCH), 36.82 ($CHCH_2$), 35.84 ($TrSCH_2$) (OCH_2CH_3).

S-Bn-L-Cys-OMe-p-TsOH (5.5)

S-Benzyl-L-cysteine (5.0 g, 23.7 mmol) was dissolved in methanol (150 mL) and *p*-toluenesulfonic acid was added (18 g, 104 mmol). The mixture was heated to reflux for 48 hours whereupon the solution was evaporated to dryness and the product isolated by recrystallizing the resulting solid from diethylether. Compound **5.5**, a colourless solid (9.3 g, 98%) showed: m.p.: 78-79°C; ^1H NMR (CD_3OD) [200MHz]: δ7.688, 7.245 (m, 9H, H-aryl), 5.176 (s, 2H, SCH_2Ph), 4.149 (m, 1H, $CHCH_2$), 3.779 (s, 3H, OCH_3), 2.926 (m, 2H, $CHCH_2$), 2.353 (s, 3H, Ph-CH_3); ^{13}C NMR: ($CDCl_3$) [50MHz]: δ169.37 (ester C(O)), 142.78, 141.86, 138.43 , 129.99, 129.79, 129.54, 128.31, 126.82, 53.80 ($CHCH_2$), 53.15 (OCH_3), 36.78 (SCH_2Ph), 31.94 ($CHCH_2$), 21.35 (Ph-CH_3).

Tr-S-Mer-L-Phe-S-Bn-L-Cys-OMe (5.6)

Compound **5.5** (908 mg, 2.29 mmol) was dissolved in DCM (30 mL) and extracted with 10% Na_2CO_3 (20 mL). The organic layer was collected, dried over sodium sulfate and evaporated to dryness. To the resulting oil, 30 mL of freshly distilled DCM was added followed by compound **5.3** (1.0 g, 2.08 mmol) and DMAP (12 mg). To this solution,

EDAC (440 mg, 2.29 mmol) in DCM (20 mL) was added dropwise over five minutes. The solution was allowed to stir for 20 hours before extraction with 1 M sodium bicarbonate (2 x 20 mL), 0.1M HCl (2 x 20 mL) and distilled water (2 x 20 mL). The DCM solution was dried, evaporated to dryness and compound **5.6** isolated by use of radial chromatography (DCM/ chloroform) and finally recrystallized from methanol. The compound, a colourless crystalline solid showed: m.p.: 132-133 °C; TLC: R_f= 0.32 (2% MeOH/DCM); ^{1}H NMR ($CDCl_3$) [500 MHz]: δ7.27 (m, H-aryl), 6.55 (d, J= 7.3, 1H, Phe-NH), 6.29 (d, J= 7.6, 1H, Cys-NH), 4.62 (m, 1H, Cys-αCH), 4.38 (m, 1H, Phe-αCH), 3.66 (s, 3H, OCH_3), 3.61 (s, 2H, SCH_2Ph), 3.05 (AB, J= 15.7, 1H, Tr-SCH_2), 2.99 (AB, 1H, Tr-SCH_2), 2.91 (ABX, J_{AB} = -13.9, J_{AX}= 6.35, $J_{BX=}$ 4.4, 2H, CH**C**$\mathbf{H_2}$Ph), 2.75 (ABX, J_{AB} = -13.5, J_{AX}= 5.0, $J_{BX=}$ 6.0, 2H, CH**C**$\mathbf{H_2}$SBn); ^{13}C NMR ($CDCl_3$) [125.77 MHz]: δ170.45 (ester-C(O)), 170.10 (Phe-NHC(O)), 168.23 (Cys-NHC(O)), 143.97 (trityl-*ipso*), 137.55, 136.20, 129.52 (trityl-*ortho*), 54.63 (Phe-αCH), 52.52 (OCH_3), 51.75-(Cys-αCH), 37.79 (CH**C**$\mathbf{H_2}$Ph), 36.48 (SCH_2Ph), 36.17 (Tr-SCH_2), 33.16 (CH**C**$\mathbf{H_2}$SBn); Analysis: (C, H, N):Obs: C 72.01 H 5.47 N 4.00 %, Calc: C 71.5 H 5.81 N 4.07 %.

N-t-Boc-L-His-S-Bn-L-Cys-OMe (**5.7**)

Compound **5.5** (7.07g, 17.8 mmol) was suspended in DCM (65 mL) and extracted with 10% Na_2CO_3 (50 mL). The organic layer was separated, dried over sodium sulfate and evaporated to dryness. The oily residue was dissolved in DCM (40 mL) and N-*t*-Boc-L-histidine (5g, 19.6 mmol) was added followed by EDAC (3.76g, 19.58 mmol) and

diisopropylethylamine (1.5 mL). The mixture was stirred under a slow flow of nitrogen and protected from light. After 48 hours the solution was extracted with 0.1M HCl (2 x 20 mL), 1M $NaHCO_3$ (2 x 20 mL) and distilled water (2 x 10 mL). The organic layer was concentrated to dryness and the residue dissolved into DCM (2 mL). The title compound, a colourless crystalline solid (6.0 g, 72 %), was isolated by radial chromatography (MeOH/ DCM). The compound showed: m.p.: 58-60 °C; TLC: R_f= 0.52 (10% MeOH/DCM); ^1H NMR ($CDCl_3$) [200MHz]: δ8.230 (m, 1H, amide-NH), 7.480 (d, J= 5.3, 1H, NH**CH**N), 7.270 (m, H-aryl), 6.78 (d, C**CH**N), 6.047 (t, J= 7.5, 1H, Boc-NH), 4.680 (m, 1H, Cys-αCH), 4.436 (m,1H, His-αCH), 3.652 (m, overlap, OCH_3, SCH_2Ph), 3.069 (m, 2H, His-CH_2), 2.771 (m, 2H, CH_2SBn), 1.401 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) [50 MHz]: δ172.02 (ester-C(O)), 170.95 (amide-C(O)), 155.52 (carbamate-C(O)), 137.44, 137.26, 135.07, 128.75, 128.38, 127.05 (C-aryl), 80.04 ($\mathbf{C}(CH_3)_3$), 54.18 (His-CH), 52.42 (OCH_3), 51.75 (Cys-CH), 36.19 ($\mathbf{SCH_2}Ph$), 32.81 ($\mathbf{CH_2}SBn$), 29.15 (CH_2-imidazole), 28.12 ($C(\mathbf{CH_3})_3$); MS (HRSDEI): calc: 439.2283, obs: 439.2267.

L-His-S-Bn-L-Cys-OMe ditrifluoroacetate salt (**5.8**)

Compound **5.7** (1.0g, 2.28 mmol) was dissolved in TFA (5 mL) with rapid stirring. Triethylsilane was added dropwise until the colour of the solution discharged. The solution was stirred for an additional hour before the TFA was removed *in vacuo*. The product was used without furthur purification.

Tr-S-Mer-L-His-S-Bn-L-Cys-OMe (5.9)

To compound **5.8** (2.28 mmol) in DCM (30 mL), compound **2.5** (1.1g, 2.5 mmol) and triethyamine (2.5 mL) were added. The solution was stirred for 36 hours before extraction with brine (3 x 15 mL). The organic layer was concentrated to 2 mL and the product isolated by radial chromatography ($CHCl_3$/MeOH). The product, a crystalline solid (1.0 g, 65 %) showed: m.p.: 56-59°C; ^{1}H NMR (CD_3OD) [200MHz]: δ7.513 (s, 1H, N**C**H**N**H), 7.292 (m, H-aryl), 6.809 (s, 1H, C**C**H**N**), 4.584 (m, 1H, His-αCH), 4.473 (m, 1H, Cys-αCH), 3.716 (s, 2H, SCH_2Ph), 3.689 (s, 3H, OCH_3), 2.954 (s, $TrSCH_2$), 2.804 (m, 4H, $CHCH_2$); ^{13}C NMR ($CDCl_3$) [50MHz]: δ172.93, 172.29 (amide C(O)), 170.777 (ester C(O)), 145.51 (Tr-*ipso*), 135.55 (NH**C**N), 130.70 (Tr-*ortho*), 130.08 (HC-imidazole), 129.49 (CH_2C), 129.04 (Tr-*meta*), 127.99 (Tr-*ortho*), 68.37 (Ph_3C), 54.68(His-αCH), 53.39 (OCH_3), 52.93 (Cys-αCH), 37.11 (SCH_2Ph), 36.90 ($TrSCH_2$), 33.41($CHCH_2$).

S-Bn-L-Cys-OBn p-TsOH salt (5.10)

To S-benzyl-L-cysteine (5.0 g, 23.7 mmol) and benzyl alcohol (20 mL, 193 mmol) in CCl_4 (40 mL), p-toluenesulfonic acid (5.0 g, 26.31 mmol) was added and an azeotropic distillation performed. Approximately 50 mL aliquots of CCl_4 were added until the boiling point of the distillate reached 77°C. The remaining carbon tetrachloride was distilled off and the resulting orange/red solution was poured into a mixture of ether (200 mL) and DCM (20 mL). The mixture was stirred for two hours at 0°C where upon a colourless

precipitate formed. The precipitate was collected by filtration and washed with ether (30 mL) to give **5.10** as a colourless solid (11g, 50%). The compund showed: m.p.: 136-138 °C; ^{1}H NMR (CD_3OD) [200MHz]: δ7.667-7.146 (H-aryl), 5.100 (s, 2H, OCH_2Ph), 4.149 (m, 1H,CH), 3.683 (s, 2H, SCH_2Ph), 3.253 (s, 3H, $PhCH_3$). 2.868 (m,2H, CH_2S); MS: m/z(RI%): 302(10)[M], 180(15)[M-SBn], 133(100)[M-p-TsOH].

*N-t-Boc-Gly-S-Bn-L-Cys-OBn (**5.11**)*

S-Benzyl-L-cysteine benzyl ester p-toluenesulfonate salt (5.91 g, 12.5 mmol) was dissolved in DCM (50 mL) and extracted with 10% Na_2CO_3 (25 mL) until all the solid material had dissolved. The organic layer was dried over sodium sulfate, decanted, evaporated to dryness and diluted with freshly distilled DCM (30 mL). To this solution, N-t-Boc glycine (2.0 g, 11.3 mmol) followed by EDAC (2.4 g, 12.5 mmol) were added. The mixture was stirred at room temperature for 48 hours and then extracted with distilled water (1 x 30 mL). The mixture was evaporated to dryness and the residue purified by centrifugal chromatography by the use of DCM/chloroform eluent. The major impurity eluted with DCM while the product, a yellow oil, eluted in chloroform (3.91 g, 76%). Compound **5.11** showed: TLC: R_f= 0.68 (2% MeOH/DCM); ^{1}H NMR ($CDCl_3$) [200MHz]: δ7.216 (H-aryl), 5.508 (m,1H, *Boc*-NH), 5.079 (s, 2H, OCH_2Ph), 4.780 (m, 1H, NH**CH**), 3.765 (m, 2H,NH**CH$_2$**), 3.585 (s, 2H, SCH_2Ph), 2.817-2.765 (m, 2H, CH_2S), 1.385 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) [50MHz]: δ170.32 (COOBn), 169.45 (amide-C(O)), 155.80 (Boc-C(O)), 137.45, 134.86, 128.79, 128.40, 128.13, 127.04 (C-aryl), 80.04

(**C**$(CH_3)_3$), 67.34 (OCH_2Ph), 53.33 ($NHCH_2$), 51.61 (NHCH), 36.34 (SCH_2Ph), 33.10 ($CH\mathbf{CH_2}S$), 28.15 ($C(\mathbf{CH_3})_3$); MS (HRSDEI): Calc. 459.1938, Obs. 459.2043.

Gly-S-Bn-L-Cys-OBn trifluoroacetate salt (**5.12**)

N-t-Boc-Glycine-S-benzyl-L-cysteine benzyl ester, **5.11** (1.72 g, 3.74 mmol) was dissolved in trifluoroacetic acid (6 mL) and to this solution, triethylsilane was added dropwise until the yellow colour discharged. The solution was allowed to stir for three hours before the TFA was removed under reduced pressure. The oily residue was used without furthur analysis or purification.

Tr-S-Mer-Gly-S-Bn-L-Cys-OBn (5.13)

A solution of glycine-S-benzyl-L-cysteine benzyl ester trifluoroacetate salt, (**5.12**, 3.74 mmol) was dissolved in DCM (30 mL) and extracted with 10% Na_2CO_3 (1 x 20 mL). The organic layer was dried over sodium sulfate, filtered and evaporated to dryness. The oily residue was dissolved in DCM (30 mL) and compound **2.5** added (1.37 g, 3.1 mmol) along with triethylamine (3 mL). The reaction mixture was stirred for 24 hours under nitrogen before extraction with 0.1M HCl (20 mL), 1M $NaHCO_3$ (20 mL) and DW (30 mL). The product, a hydroscopic semi-solid was purified by radial chromatography ($CHCl_3$) to give 1.8 g (84%) of **5.13**.. The compound showed: m.p.: 40-43 °C; TLC: R_f= 0.53 (2% MeOH/DCM); ^{1}H NMR ($CDCl_3$) [200MHz]: δ7.45 (H-aryl), 7.052 (d, J= 7.8, 1H, **NH**CH), 6.681 (t, J= 4.8, 1H, $\mathbf{NH}CH_2$), 5.14 (s, 2H, $NH\mathbf{CH_2}$), 4.78 (m, 1H,

CHCH_2S), 3.659 (s, 2H, OCH_2Ph), 6.631 (s, 2H, SCH_2Ph), 2.829 (ABX, J_{AX}= 5.4 , J_{BX}= 5.7 , J_{AB}= 11.4, 2H, CH**CH_2**S); ^{13}C NMR ($CDCl_3$) [50MHz]: δ170.136 (COOBn), 168.47, 168.18 (amide C(O)), 143.77, 137.42, 134.89, 129.35, 128.78, 128.44, 128.15, 128.02, 127.07, 126.87 (C-aryl), 76.37 ($C(Ph)_3$), 67.61 ($NHCH_2$), 67.32 (OCH_2Ph), 51.69 (NHCH), 36.30 (SCH_2Ph), 35.53 (Tr-SCH_2), 33.0 (CH**CH_2**S); Analysis: (C, H, N): Obs: C 68.75 H 5.14 N 3.92 %, Calc: C 71.2 H 5.6 N 4.15 %.

*N-t-Boc-Ile-S-Bn-L-Cys-OMe (**5.14**)*

Compound **5.5** (3.96 g, 9.99 mmol) was dissolved in DCM (50 mL) and extracted with 10% Na_2CO_3 (35 mL). The organic layer was collected, dried over sodium sulfate and evaporated to dryness. After dilution with DCM (30 mL), N-t-Boc-L-isoleucine hemi-hydrate (2.0 g, 8.33 mmol) followed by EDAC (2.6 g, 13.54 mmol) were added. The mixture was stirred for 48 hours whereupon it was extracted with distilled water (1 x 30 mL) and subsequently evaporated to dryness. The resulting oil was dissolved in methanol (5 mL) and cooled to -10°C for 12 hours. The precipitate, a colourless solid (2.0 g, 55 %) was filtered and air dried, and the filtrate cooled for an additional 12 hours which resulted in a second crop of the product (600 mg, 16%). Compound **5.14** showed: m.p.: 118-119°C; TLC: R_f= 0.42 (2% MeOH/DCM); ^1H NMR ($CDCl_3$) [200MHz]: δ7.22 (s, 5H, H-aryl), 6.572 (d, J= 7.5, 1H, amide-NH), 4.990 (d, J= 8.4, 1H, carbamate-NH), 4.726 (m, 1H, Cys-CH), 3.941 (m, 1H, Ile α-CH), 3.675 (s, 3H, OCH_3), 3.646 (s, 2H, SCH_2Ph), 2.818 (m, J_{AX}= 5.0, J_{BX}= 3.8, J_{AB}=-9.8, 2H, CH**CH_2**S), 1.814 (m, 1H, Ile β-CH), 1.442 (m, 1H,

Ile-β-CH), 1.380 (s, 9H, $C(CH_3)_3$), 1.087 (m, 2H, $CH(CH_3)\mathbf{CH_2}CH_3$), 0.887 (d, J= 6.7, $CH(\mathbf{CH_3})CH_2CH_3$); ^{13}C NMR ($CDCl_3$) [50MHz]: δ171.39 (ester-C(O)), 170.90 (amide-C(O)), 155.60 (carbamate-C(O)), 137.59 (C-*ipso*), 128.92 (C-*ortho*), 128.58 (C-*meta*), 127.25 (C-*para*), 79.96 ($\mathbf{C}(CH_3)_3$), 59.21 (Ile-CH), 52.55 (OCH_3), 51.54 (Cys-CH), 37.37 (Ile β-CH), 36.58 ($S\mathbf{CH_2}Ph$), 33.28 (CH_2SBn), 28.28 ($C(\mathbf{CH_3})_3$), 24.70 ($CH(CH_3)\mathbf{CH_2}CH_3$), 15.48 ($CH(\mathbf{CH_3})CH_2CH_3$) 11.49 ($CH(CH_3)CH_2\mathbf{CH_3}$); MS (HRSDEI): calc: 439.2283, obs: 439.2267.

*Tr-S-Mer-L-Ile-S-Bn-L-Cys-OMe (**5.15**)*

To compound **5.14** (1.45 g, 3.3 mmol) in TFA (3 mL), triethylsilane was added until the yellow colour discharged (0.5 mL). The solution was stirred for one hour whereupon it was evaporated to dryness, dissolved in DCM (30 mL), and evaporated to dryness again. The solution was dissolved in DCM (30 mL), and extracted with 10% sodium carbonate (1 x 20 mL) then dried with sodium sulfate. The solution, after filtering, was evaporated to dryness and the resultant yellow oil dissolved in DCM (35 mL). To this solution, compound **2.5** (1.29 g, 3.0 mmol) and triethylamine (1.5 mL) were added. The reaction mixture was stirred for 48 hours under nitrogen and protected from light whereupon it was extracted with 0.1 N HCl (1 x 30 mL) and DW (2 x 25 mL). The organic layer was concentrated to 1 mL and the product, an oily semi-solid (1.3g, 68%) isolated by radial chromatography (DCM/MeOH). Compound **5.15** showed: ^1H NMR ($CDCl_3$) [200MHz]: δ7.33 (m, 20H, H-aryl), 6.786 (d, J= 7.2, 1H, Cys-amide-NH), 6.626 (d, J= 8.3, 1H, Ile-

amide-NH), 4.677 (m, 1H, Cys-αCH), 4.308 (m, 1H, Ile-αCH), 3.662 (s, 3H, OCH_3), 3.613 (s, 2H, SCH_2Ph), 3.014 (AB, J= 16.02,Tr-SCH_2), 2.973 (AB, Tr-SCH_2), 2.799 (m, 2H, CH**CH_2**SBn), 1.712 (m, 1H, **CH**$(CH_3)CH_2CH_3$), 1.451 (m, $CH(CH_3)$**CH_2**CH_3), 1.020 (m, $CH(CH_3)$**CH_2**CH_3) 0.836 (m, $CH($**CH_3**$)CH_2CH_3$), (q, J= 3.2, $CH(CH_3)CH_2$**CH_3**); ^{13}C NMR ($CDCl_3$) [50MHz]: δ170.80, 170.48, 168.13 (C(O)), 144.08 (trityl-*ipso*), 137.60-(benzyl-*ipso*), 129.57 (trityl-*ortho*), 128.93 (benzyl-*ortho*), 128.62 (benzyl-*meta*), 128.13 (trityl-*meta*), 127.04 (benzyl-*para*), 126.77 (trityl-*para*),67.98 (CPh_3), 57.71 (Ile-αCH), 52.52 (OCH_3), 51.68 (Cys-αCH), 37.45 (SCH_2Ph), 36.56 (Tr-SCH_2), 33.04 (CH**CH_2**SBn), 24.96 (**CH**$(CH_3)CH_2CH_3$), 15.09 ($CH(CH_3)$**CH_2**CH_3), 11.40 ($CH($**CH_3**$)CH_2CH_3$), 6.60 ($CH(CH_3)CH_2$**CH_3**); Analysis: (C, H, N): Obs: C 70.34 H 6.45 N 4.34 %, Calc: C 69.7 H 6.4 N 4.28 %.

*Tr-S-Mer-L-His-OMe (**5.16**)*

L-Histidine methyl ester dihydrochloride (624 mg, 2.59 mmol) and DIPEA (480 μL) were added to compound **2.5** (740 mg, 1.72 mmol) in DCM (30 mL). The mixture was stirred for 24 hours under nitrogen whereupon it was extracted with DW (5 x 30 mL). The organic layer was separated and evaporated to dryness leaving **5.16** as a colourless crystalline solid (520 mg, 56%). The compound showed: m.p.: 62-65°C; TLC: R_f= 0.41 (10%MeOH/DCM); ^1H NMR ($CDCl_3$) [200MHz]: δ8.490 (s, 1H, NC**H**NH), 7.290 (m, H-aryl), 7.200 (s, 1H,CC**H**N), 4.810 (s, 3H, OCH_3), 4.191 (m, 1H, His-αCH), 3.040 (m, 2H, TrSCH_2), 3.010 (m, 2H, CH**CH_2**); ^{13}C NMR ($CDCl_3$) [50MHz]: δ175.28 (ester C(O)),

170.57 (amide C(O)), 145.51 (NCHNH), 134.64 (trityl-*ipso*), 132.26 ($CHCH_2\mathbf{C}$), 130.79 (trityl-*meta*), 129.10 (trityl-*meta*), 128.08 (trityl-*para*), 118.15 (**C**CHN), 68.64 (CPh_3), 55.09 (His-αCH), 53.12 (OCH_3), 37.18 ($TrSCH_2$), 29.08 ($CH\mathbf{CH_2}$).

Tr-S-Mer-L-His-OH (5.17)

To **5.16** (442 mg, 0.911 mmol) in a 1:1 THF/DW solution (15 mL), NaOH (36.4 mg, 0.911 mmol) was added. The mixture was stirred under N_2 for 4 hours prior to acidification to pH 3.9 with 6N HCl. The THF was removed under reduced pressure and the remaining heterogenous mixture diluted with DW (15 mL). The aqueous suspension was extracted with DCM (3 x 30 mL) and the extracts combined and subsequently evaporated to dryness. The product, a colourless, crystalline material (375 mg, 87%) showed: m.p.: 130-132°C; TLC: R_f= 0.21 (35%MeOH/DCM); ^{1}H NMR ($CDCl_3$) [200MHz]: δ7.290 (m, H-aryl), 7.200 (s, 1H,C**C**H**N**), 4.190 (m, 1H, His-αCH), 3.040 (m, 2H, $TrSCH_2$), 3.001 (m, 2H, $CH\mathbf{CH_2}$); ^{13}C NMR ($CDCl_3$) [50MHz]: δ175.28 (ester C(O)), 170.57 (amide C(O)), 145.51 (NCHNH), 134.64 (trityl-*ipso*), 132.26 ($CHCH_2\mathbf{C}$), 130.79 (trityl-*meta*), 129.10 (trityl-*meta*), 128.08 (trityl-*para*), 118.15 (**C**CHN), 68.64 (CPh_3), 55.09 (His-αCH), 53.12 (OCH_3), 37.18 ($TrSCH_2$), 29.08 ($CH\mathbf{CH_2}$).

Tr-S-Mer-L-His-S-Bn-L-Cys-OBn (**5.18**)

Compound **5.10** (703 mg, 2.33 mmol) was dissolved in DCM (25 mL) and extracted with 10% Na_2CO_3 (25 mL) until all the solid material dissolved. The organic

layer was dried over sodium sulfate, filtered, evaporated to dryness and diluted with freshly distilled DCM (30 mL). To this solution, compound **5.17** (1.0 g, 2.12 mmol) and triethylamine (2 mL) were added followed by EDAC (450 mg, 2.33 mmol). After stirring overnight, the solution was extracted with 0.1 M HCl (2 x 15 mL), 1.0 M $NaHCO_3$ (2 x 15 mL) and DW (3 x 15 mL). The organic layer was evaporated and the title compound, a colourless crystalline solid (400 mg, 45%) isolated by radial chromatography (MeOH/DCM). Compound **5.18** showed: ^{1}H NMR ($CDCl_3$) [200MHz]: δ7.770 (m, 1H, H-15), 7.305 (m, H-aryl), 6.665 (s, 1H, H-12), 5.121 (s, 2H, H-24), 4.649 (m, 1H, H-16), 4.339 (m, 1H, H-10), 3.598 (s, 2H, H-18), 3.023 (s, 2H, H-6), 12.77 (m, overlapp, H-12, H-13); ^{13}C NMR ($CDCl_3$) [50MHz]: δ171.6 (C-23), 170.14 (C-14), 168.69 (C-7), 143.97 (C-4), 137.54-127.03 (C-aryl), 67.76 (C-5), 67.55 (C-4), 52.84 (C-9), 52.18 (C-16), 36.42 (C-18), 36.21 (C-17), 32.83 (C-10), 28.87 (C-6); MS: (+NH_3-DCI):m/z(RI%) 755(15)[M+1], 631(100)[M-1-benzyl].

N-t-Boc-L-Tyr-S-Bn-L-Cys-OMe (5.19)

To a DCM solution (40ml) of **5.5** (1.78 g, 4.49 mmol), 30 mL of 10% Na_2CO_3 was added. The mixture was shaken until everything had dissolved. The aqueous layer was back extracted with DCM (2 x 40 mL) and the organic layers combined and dried over sodium sulfate. The organic extracts were evaporated to dryness and the resulting yellow oil was dissolved in DCM (50 mL) before the addition of N-t-Boc-L-tyrosine (1.0g, 3.74 mmol) and EDAC (862 mg, 4.49 mmol). After the reaction mixture was stirred for 48

hours under nitrogen and protected from light, it was extracted with DW (1 x 30 mL), concentrated to 2 mL and the product purified by radial chromatography (chloroform/ methanol). Compound **5.19**, an oily semi-solid (1.43g, 83%), showed: TLC: R_f= 0.36 (10% MeOH/DCM); ^{1}H NMR ($CDCl_3$) [200MHz]: δ7.257(s, 5H, H-aryl), 6.980 (d, J= 8.1, 2H, H-*meta*), 6.725 (d, 2H, H-*ortho*), 5.353 (m, 1H, Boc-NH), 4.724 (m, 1H, Cys-αCH), 4.365 (m, 1H, Tyr-αCH), 3.630 (s, overlap, OCH_3, SCH_2Bn), 2.836 (m, overlap, CH_2Ar), 1.369 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) [50MHz]: δ171.64 (ester-C(O)), 170.41 (amide-C(O)), 155.18 (Boc-C(O) and Tyr-*ipso*), 137.17 (Bn-*ipso*), 129.97, 128.56, 128.17, 126.92, 126.82 (C-aryl), 115.28 (Tyr-*ortho*), 80.01 ($\mathbf{C}(CH_3)_3$), 55.45 (Tyr-αCH), 52.26 (OCH_3), 51.44 (Cys-αCH), 37.18 (Tyr-CH_2), 35.93 (SCH_2Ph), 32.63 ($CH\mathbf{CH_2}S$), 27.86 ($C(\mathbf{CH_3})_3$); MS (HRSDEI): calc: 488.1977, obs: 488.1981.

L-Tyr-S-Bn-L-Cys-OMe trifluoroactetate salt (**5.20**)

Compound **5.19** (715 mg, 1.46 mmol) was dissolved in trifluoroacetic acid (6 mL) and with rapid stirring, triethylsilane was added dropwise until the colour of the solution discharged. After 1.5 hours, the solution was evaporated to dryness and the residue dissolved in DCM (10 mL) and again evaporated to dryness. This compound was used without furthur purification.

Tr-S-Mer-L-Tyr-S-Bn-L-Cys-OMe (5.21)

A DCM solution (20 mL) of compound **5.20** (1.46 mmol) was extracted with 10%

Na_2CO_3 (30 mL), dried over sodium sulfate then evaporated to dryness. The oily residue was dissolved in DCM (20 mL) and compound **2.5** was added (695 mg, 1.61 mmol). After the solution was stirred for 24 hours, under nitrogen and protected from light, it was extracted with DW (20 mL). The organic layer was concentrated to 2 mL and the material purified by radial chromatography (hexanes/chloroform) to give **5.21** as a colourless solid. The compound showed: m.p.: 98-99°C; TLC: R_f= 0.46 (10% DCM/MeOH); ^{1}H NMR ($CDCl_3$) [200MHz]: δ7.30 (m, H-aryl), 6.938 (d, J= 8.4, 2H, Tyr-H-*meta*), 6.756 (d, J=7.3, 1H, amide-NH), 6.644 (d, Tyr-H-*ortho*), 6.584 (d, J= 6.4, amide-NH), 4.623 (m, 1H, Cys-αCH), 4.370 (m, 1H, Tyr-αCH), 3.621 (s, 3H, OCH_3), 3.596 (s, 2H, SCH_2Ph), 3.043 (s, 2H, SCH_2), 2.730 (m, CH_2Ar); ^{13}C NMR ($CDCl_3$) [50MHz]: δ170.41, 170.40, 168.59 (C(O)), 155.43 (Tyr-*ipso*), 143.76 (Tr-*ipso*), 137.34 (Bn-*ipso*), 130.27, 129.374, 128.79, 128.47, 128.04, 127.12, 126.96 (C-aryl), 115.56 (Tyr-C-*ortho*), 77.20 (CPh_3), 54.76 (Tyr-αCH), 52.54 (OCH_3), 51.73 (Cys-αCH), 37.29 (Tyr-CH_2), 36.23 (SCH_2Ph), 35.96 (SCH_2), 32.82 (CH_2SBn); Analysis:(C, H, N): Obs: C 70.21 H 5.51 N 3.75 %, Calc: C 69.9 H 5.7 N 3.9.

N-t-Boc-L-Met-S-Bn-L-Cys-OMe (5.22)

To S-Benzyl-L-cysteine methyl ester p-TsOH salt (2.93 g, 7.38 mmol) was dissolved in DCM (50 mL) and extracted with 10% Na_2CO_3 (50 mL). The organic layer was separated and dried over sodium sulfate. N-t-Boc-L-Methionine (1.65 g, 6.63 mmol) was added, followed by EDAC (1.42 g, 7.40 mmol). The solution was allowed to stir for

24 hours before extraction with 0.1M HCl (2 x 20 mL), 1M $NaHCO_3$ (2 x 20 mL) and DW (2 x 20 mL). The organic layer was dried over sodium sulfate and then evaporated to dryness leaving **5.22** as a colourless crystalline solid (2.79g, 87%). The compound showed: m.p.: 78-79°C; ^{1}H NMR ($CDCl_3$) [200MHz]: δ7.280 (m, H-aryl, amide-NH), 5.521 (d, J= 6.7, 1H, Boc-NH), 4.735 (m, 1H, Cys-αCH), 4.320 (m, 1H, Met-αCH), 3.679 (s, 2H, SCH_2Ph), 3.669 (s, 3H, OCH_3), 2.815 (m, 2H, $\mathbf{CH_2}SCH_3$), 2.538 (m, 2H, CH_2SBn), 2.035 (s, 3H, SCH_3), 2.023 (m, 2H,$\mathbf{CH_2}CH_2SCH_3$), 1.392 (s, 9H, $C(\mathbf{CH_3})_3$); ^{13}C NMR ($CDCl_3$) [50 MHz]: δ171.41 (ester-C(O)), 170.63 (amide-C(O)), 155.15 (Boc-C(O)), 137.33 (benzyl-*ipso*)), 128.65 (benzyl-*ortho*), 128.24 (benzyl-*meta*), 126.90 (benzyl-*para*), 79.60 ($\mathbf{C}(CH_3)_3$), 53.07 (Cys-αCH), 52.24 (OCH_3), 51.41 (Met-αCH), 36.15 (SCH_2Ph), 32.76 (CH_2SBn), 31.54 ($\mathbf{CH_2}CH_2SCH_3$), 29.72 ($\mathbf{CH_2}SCH_3$), 28.01 ($C(\mathbf{CH_3})_3$), 15.08 (SCH_3); MS (HRSDEI): calc: 457.18405, obs: 457.18355.

L-Met-S-Bn-L-Cys-OMe trifluoroacetate salt (**5.23**)

Compound **5.22** (910 mg, 2.00 mmol) was dissolved in TFA (5 mL) and Et_3SiH was added dropwise until the colour discharged. The reaction mixture was stirred for 4 hours prior to removal of the solvent under reduced pressure. The resulting semi-solid was placed under high vaccum until the odour of TFA could no longer be detected.

*Tr-S-Mer-L-Met-S-Bn-L-Cys-OMe (**5.24**)*

The oily semi-solid **5.23** (2.00 mmol) was suspended in DCM (25 mL) and

extracted with 10% Na_2CO_3 (15 mL). The aqueous layer was back extracted with DCM (2 x 15 mL) and the organic layers combined, dried over sodium sulfate and evaporated to dryness. The residue, an oil, was dissolved in DCM (20 mL) and triethylamine was added (1 mL) followed by compound **2.5** (782 mg, 1.81 mmol). The reaction mixture was allowed to stir for 48 hours under nitrogen prior to extraction with 0.1M HCl (2 x 10 mL), 1M $NaHCO_3$ (1 x 10 mL) and distilled water (2 x 20 mL). The organic layer was concentrated to 1 mL and the product isolated by radial chromatography ($CHCl_3$/MeOH). The product **5.24**, an oily semi solid (900 mg, 74 %) showed: ^{1}H NMR ($CDCl_3$) [300MHz]: δ7.300 (m,H-aryl), 6.748 (d, J= 7.7, 1H, amide-NH), 4.669 (m, 1H, Cys-αCH), 4.472 (m, 1H, Met-αCH), 3.646 (s, 3H, OCH_3), 3.625 (s, 2H, SCH_2Ph), 3.077 (AB, J= 15.4, 1H, $TrSCH_2$), 3.012 (AB, 1H, $TrSCH_2$), 2.786 (m, 2H,CH_2SBn), 2.432 (m, 2H, $CH_2\mathbf{CH_2}S$), 2.034 (s, 3H, SCH_3), 1.953 (m, 1H, $\mathbf{CH_2}CH_2S$), 1.802 (m, 1H, $\mathbf{CH_2}CH_2S$); ^{13}C NMR ($CDCl_3$) [75.47 MHz]: δ170.47 (ester-C(O)), 170.36 (amide-C(O)), 167.97 (amide-C(O)), 143.77, 137.29, 129.28, 128.69, 128.34, 128.15, 127.90, 127. 01, 126.81(C-aryl), 67.63 (CPh_3), 53.33 (Met-αCH), 52.38 (Cys-αCH), 51.58 (OCH_3), 36.31 (SCH_2Ph), 32.76 ($CH_2\mathbf{CH_2}S$), 31.36 (CH_2SBn), 29.53 ($TrSCH_2$), 14.91 ($\mathbf{CH_2}CH_2S$) .

Re-Mer-L-His-L-Cys-OMe (**5.25a, 5.25b**)

Compound **5.9** (103 mg, 0.152 mmol) was dissolved in TFA (5 mL) with rapid stirring while triethylsilane was added added dropwise until the colour discharged. The solvent was removed *in vacuo* and the residue dissolved in MeOH (20 mL) and evaporated

to dryness. The residue was dissolved in a 1:1 THF/MeOH solution (10 mL) and freshly prepared 1M sodium acetate added (1.5 mL) followed by $ReOCl_3(PPh_3)_2$ (139 mg, 0.167 mmol). The reaction mixture was heated to reflux for 12 hours whereupon it was cooled, filtered and evaporated to dryness. The residue was dissolved in acetonitrile (500 μL) and filtered through a plug of glass wool. The dark red homogenous solution was concentrated to approximately half its volume. The material was purified by reverse phase HPLC using a Vydac 201HS10110 semi-preparative column (9.4 x 250 mm). The conditions used for purification were developed with the use of an analytical column and involved using a partial gradient from 10%-25% AN/H_2O over a twenty minute period. Non-polar reaction products were washed off the column after isolation of the two desired species with the use of 70-90% AN/H_2O. Fraction 1, **5.25a** which was obtained as a orange semi-solid showed: MS(-ES) m/z (RI%): 543.1/545.1 (23/39%)[M]; ^{1}H NMR (CD_3OD) [500 MHz]: δ8.567 (s, 1H, H-7), 7.156 (s, 1H, H-6), 5.093 (t, J= 5.2, 1H, H-3), 4.812 (d, J= 7.3, 1H, H-9), 3.935 (d, J= 12.0, 1H, H-12A), 3.827 (AB, J= 8.2, 1H, H-1A), 3.752 (AB, 1H, H-1B), 3.519 (s, 3H, H-11), 3.376 (m, 2H, H-4), 3.243 (m, 1H, H-12B); ^{13}C NMR (CD_3OD) [125.77 MHz]: δ200.001, 194.75 (amide C(O)), 171.88 (ester C(O)), 133.13 (C-7), 119.6 (C-5), 117.15 (C-6), 67.45 (C-9), 67.12 (C-3), 51.20 (C-11), 47.06 (C-12), 40.05 (C-1), 26.95 (C-4); Fraction 2, **5.25b**, obtained as an orange semi-solid showed: ^{1}H NMR (CD_3OD) [500 MHz] δ8.558 (s, 1H, H-7), 7.301 (s, 1H, H-6), 4.914 (dd, 1H, J= 5.1 and 3.76, H-3), 4.317 (dd, J= 10.1 and 7.15, 1H, H-9), 3.792 (AB, J= 17.0, 1H, H-1A), 3.752 (AB, 1H, H-1B), 3.712 (s, 3H, H-11), 3.680 (m, 1H, H-12A), 3.362 (m, 2H, H-4), 3.004

(m, 1H, H-12B); ^{13}C NMR (CD_3OD) [125.77 MHz]: δ193.95 (C-2), 191.03 (C-8), 173.75 (C-10), 133.94 (C-7), 128.16 (C-5), 118.11 (C-6), 68.36 (C-9), 66.37 (C-3), 51.26 (C-11), 46.18 (C-12), 39.45 (C-1), 27.00 (C-4).

Re-Mer-L-Ile-S-Bn-L-Cys-OH (5.26)

Compound **5.15** (103 mg, 0.157 mmol) was dissolved in TFA (5 mL) with stirring and TES added dropwise until the colour discharged. The solvent was removed *in vacuo* and the residue dissolved in MeOH (20 mL) and evaporated to dryness. The residue was dissolved in a 1:1 THF/MeOH solution (10 mL) and freshly prepared 1M sodium acetate added (3.0 mL) followed by $ReOCl_3(PPh_3)_2$ (144 mg, 0.173 mmol). The reaction mixture was heated to reflux for 16 hours whereupon it was diluted with DW (20 mL) and the organic solvent evaporated. The aqueous suspension was extracted with DCM (3 x 20 mL). The aqueous layer was separated, evaporated to dryness and the residue dissolved in methanol (1.5 mL) whereupon it was filtered through a plug of glass wool. The solution was evaporated to half its volume and the sample purified by preparative plate chromatography (silica, 10% MeOH/DCM). Compound **5.26** was obtained as an orange oil and showed: MS(-ES) m/z (RI%): 598(22)[^{187}M+1], 597(100)[^{187}M], 595(60)[^{185}M]; ^{1}H NMR (CD_3CN) [500 MHz]: δ7.255 (m, 5H, H-aryl), 4.739 (d, J= 5.6, 1H, Ile-αCH), 4.360 (dd, J= 4.3, J= 3.2, 1H, Cys-αCH), 3.790 (AB, J= 12.6, 1H, SCH_2Ph), 3.781 (AB, J= 16.3, 1H, $TrSCH_2$), 3.685 (AB, 1H, SCH_2Ph), 3.680 (AB, 1H, $TrSCH_2$), 3.282 (m, 1H, $CHCH_2SBn$), 3.103 (m, 1H, $CHCH_2SBn$), 1.956 (m, 1H, $CH(CH_3)$), 1.620 (m, 1H,

$CHCH_2CH_3$), 1.003 (d, J= 6.9, 3H, $CH(CH_3)$), 0.862 (t, J= 7.5, 2H, $CHCH_2CH_3$); ^{13}C NMR (CD_3CN) [125.77 MHz]: δ193.38 (COOH), 191.45 (amide C(O)), 190.52 (amide C(O)), 138.91 (Bn-*ipso*), 128.94 (Bn-*ortho*), 128.36 (Bn-*meta*), 125.67 (Bn-*para*), 69.13, 59.57, 48.84, 40.82, 39.24, 38.70, 37.28, 35.31, 26.26, 14.95, 11.21.

Chapter 6

Chlorambucil

6.1 Introduction

In a further effort to develop compounds that could potentially image cancer, a tripeptide from chapter **5** was covalently linked to the alkylating agent chlorambucil (**Figure 6-I**).

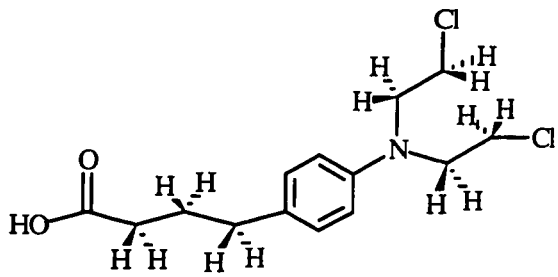

Figure 6-I: Chlorambucil

Alkylating agents usually produce their clinical effect by binding covalently to nucleophilic functional groups found in the body[1]. The mechanism of action for chlorambucil involves formation of an azirinium ion, which is susceptible to nucleophilic substitution. The positively charged azirinium ion is reported[1] to react with water,

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[1]Knock, F.E, Anticancer Agents., Kugelmass, I.N. (Ed.), C.C. Thomas Publisher, Springfield, Illinois, U.S.A., 1967.

mercaptans or amines and *in vivo* it reacts with the nitrogen atoms in purine bases.

Chlorambucil, 4-[bis(2-chloroethyl)amino]benzene butanoic acid, otherwise known as CB-1348 or Leukeron™, was developed at the Chester Beatty Research Institute in England in 1953. Its initial use was to treat lung cancer where life expectancy of terminal lung cancer patients were found to increase upon administration of the drug. Now chlorambucil is used for the treatment of lymphomas, chronic lymphatic leukemia, ovarian cancer, Hodgkins disease, and testicular carcinoma[1]. Chlorambucil exerts its clinical effect, which is similar to most types of nitrogen mustards, by alkylating the N7 atom of guanine in the DNA of rapidly growing cells, thereby inhibiting further growth of the cell. The selectivity of chlorambucil is not perfect and as a result there are serious side effects which include nausea, vomiting, dermatitis, and hepatic toxicity. However, the toxic side effects are not a concern in medical imaging because the quantity of drug administered during imaging is well below the amount required to induce a pharmacological response.

6.2 Chlorambucil Conjugates

Mehta *et al.*[2] reported the synthesis of a porphyrin-chlorambucil adduct for use in photodynamic therapy (PDT). The authors reasoned that this type of compound could act as both a conventional chemotherapeutic agent and a light-switched PDT agent. ω-Hydroxyalkoxy porphyrins were reacted with the acid chloride of chlorambucil in the

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[2]G. Mehta, T. Sambaiah, B.G. Maiya, M. Sirish and A. Dattagupta, *Tetrahedron Lett.*, **1994**, *35*, 4201.

presence of pyridine in good yield (60-70%). Amide formation between the porphyrin and chlorambucil was also possible *via* a mixed anhydride approach. Results of *in vitro* studies with the chlorambucil-porphyrin complex showed increased damage to DNA than occurred with chlorambucil alone; an *in vivo* model and clinical studies are pending.

6.3 Retrosynthesis of Chlorambucil-Tripeptide Conjugate

As noted in chapter **5**, the central amino acid of the Mer-X-Cys tripeptides can not only be used to affect the solubility of an imaging agent but can also be used as a site of derivatization. A tripeptide, Tr-S-Mer-L-Ser-S-Bn-L-Cys-OEt was coupled to chlorambucil *via* an ester linkage. The first method used to try to synthesize **6.10** consisted of making the tripeptide fragment and attaching chlorambucil subsequently. This led, however, to an inseparable mixture, despite the coupling method used. The successful approach entailed coupling the protected dipeptide N-*t*-Boc-L-Ser-S-Bn-L-Cys-OEt (fragment **A**, **Figure 6-II**) to chlorambucil (fragment **B**) before the addition of the mercaptoethanoic acid segment (fragment **C**).

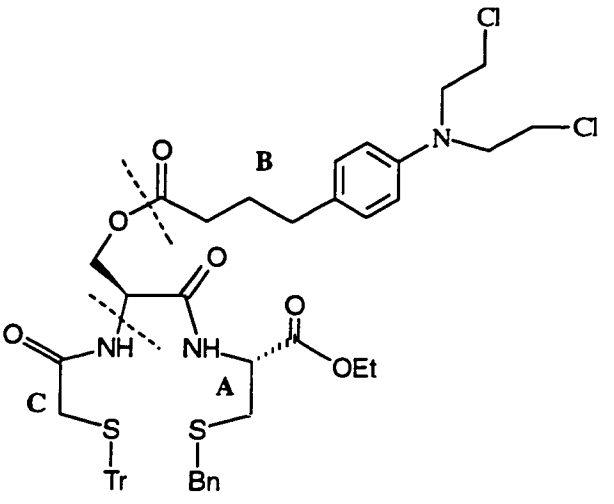


Figure 6-II: Synthon units of compound **6.10**

6.4 Synthetic Considerations

Chlorambucil is unstable and in the presence of light or moisture decomposes readily. All reactions were performed in the absence of light, under nitrogen and under anhydrous conditions. Chlorambucil is susceptible to decomposition by reactive nucleophiles or high concentrations of base; therefore, the use of hydroxide ion or large concentrations of triethylamine was avoided throughout the synthesis.

*6.5 Attempted Synthesis of **6.10** via the Tripeptide Approach*

The tripeptide Tr-S-Mer-L-Ser-S-Bn-L-Cys-OEt, **6.6**, (**Figure 6-III**) was synthesised by use of method **B** in chapter **5**. The ethyl ester of S-benzyl-L-cysteine was

coupled to N-*t*-Boc-L-serine in good yield (80%) by the use of EDAC to give the dipeptide **6.4**. Because the free amine of cysteine was utilized during the coupling, protection of the hydroxyl group of serine was not required as the superior nucleophilicity of the amine over the alcohol resulted in preferential formation of the amide. Deprotection of the carbamate to afford **6.5**, was again accomplished by the use of TFA and TES. In the presence of triethylamine, coupling of the amine **6.5** to the N-hydroxysuccinimide **2.5** occurred in good yield (71%) and gave the ethyl ester **6.6** as a colourless oil. The overall yield of the synthesis was 45%. When the synthesis was repeated with S-benzyl-L-cysteine methyl ester, good yields were also obtained, but the final product was a crystalline solid rather than a colourless oil.

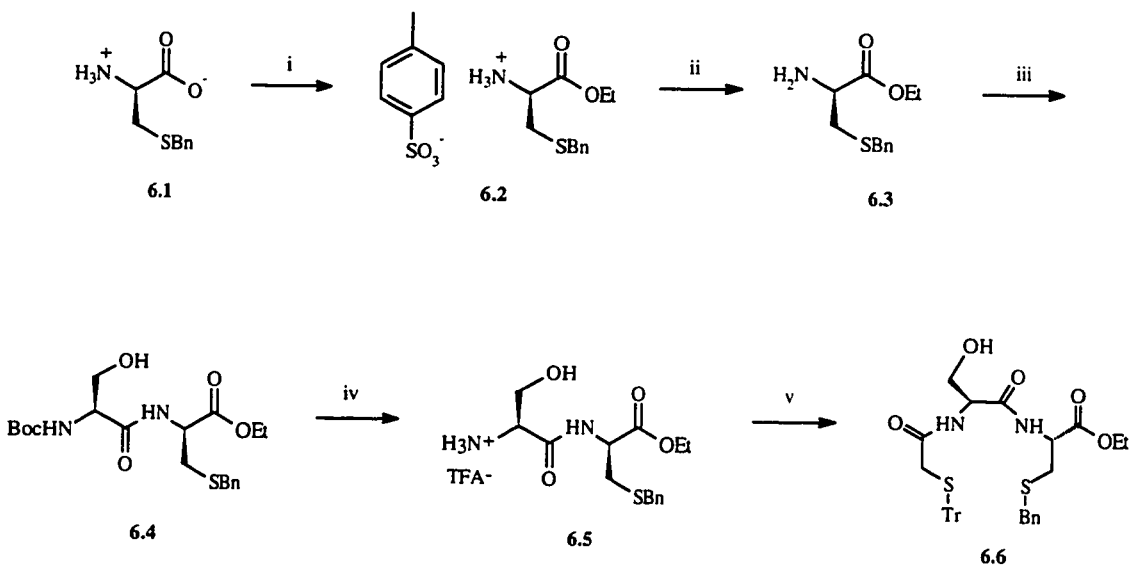


Figure 6-III: i) *p*-TsOH, EtOH, Δ ii) 10 % Na_2CO_3, CH_2Cl_2 iii) N-*t*-Boc-L-serine, EDAC iv) TFA, TES v) Compound **2.5**, NEt_3

As mentioned earlier, coupling of the serine OH group in the tripeptide to chlorambucil was unsuccessful. The use of mixed anhydrides or acid chlorides of chlorambucil in a variety of solvents resulted in poor yields of the desired ester product. A possible explanation for the poor yields is the steric hindrance at the OH group caused by other substituents within the tripeptide. This hypothesis suggested that a different synthetic strategy was required where some of the steric hindrance was removed.

6.6 Synthesis of ***6.10*** *via a Chlorambucil-dipeptide*

Because chlorambucil is stable to trifluoroacetic acid it was found possible to couple it to the dipeptide **6.4** prior to removal of the carbamate and addition of the bulky S-triphenyl ethanoic acid derivative (**2.4**). The dipeptide **6.4** (**Figure 6-IV**) was coupled to chlorambucil by using EDAC and 4-dimethylaminopyridine (DMAP) to give the ester **6.7** in good yield (71%). Removal of the carbamate on the dipeptide-chlorambucil adduct was accomplished with use of trifluoroacetic acid and triethylsilane and with no apparent loss of the chlorambucil fragment. The product, **6.8**, after extraction with aqueous sodium carbonate solution, was coupled to **2.5** and gave the tripeptide-chlorambucil adduct **6.10**, in modest yield (50%).

6.7 NMR Spectroscopy of ***6.10***

The Bruker DRX-500 spectrometer, with its gradient capability, allowed the acquisition of two dimensional spectra in remarkably short time periods. The gradient-

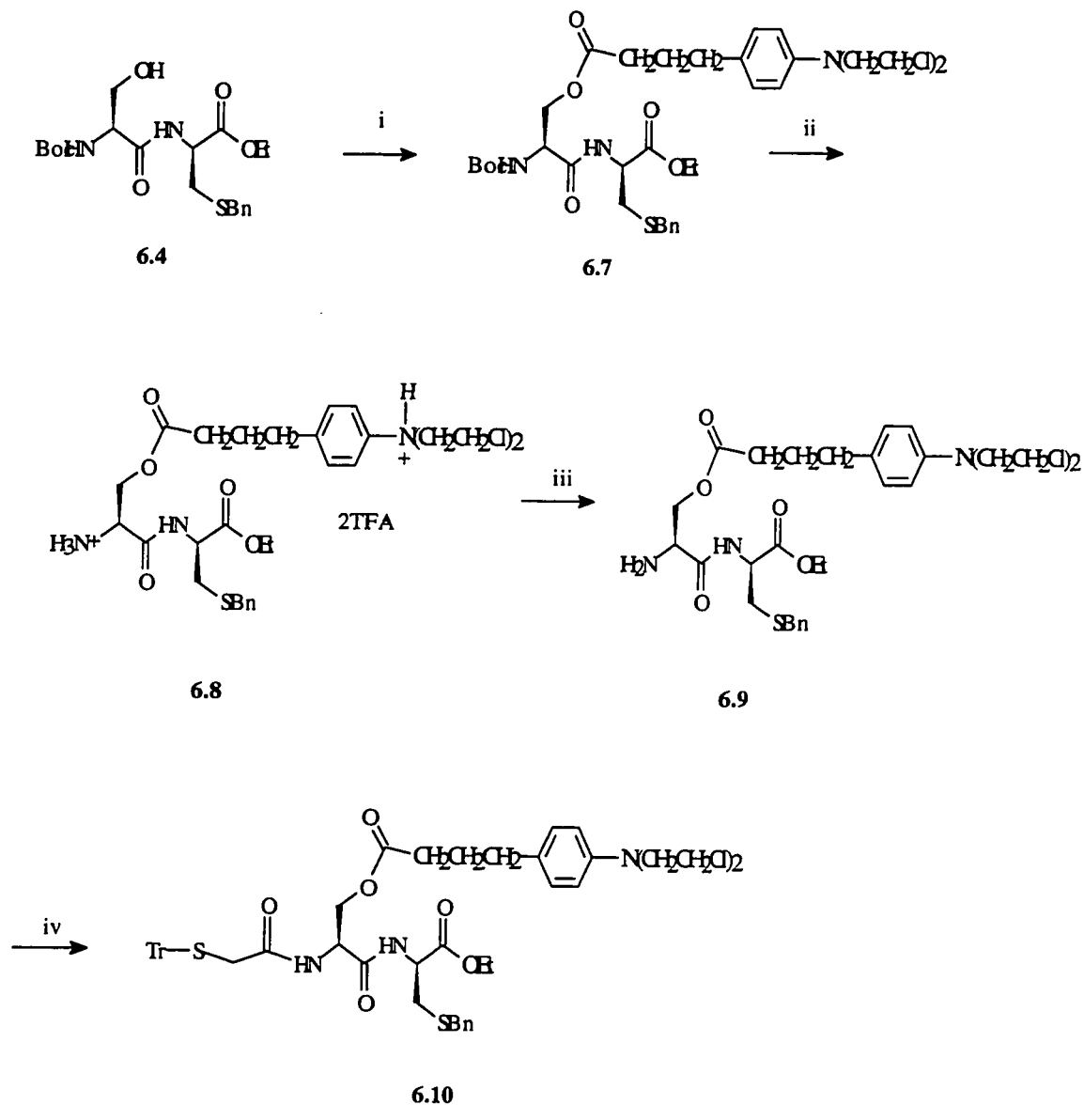


Figure 6-IV: i) Chlorambucil, 4-DMAP, EDAC ii) TFA, TES iii) 10% Na_2CO_3 iv) compound **2.5**.

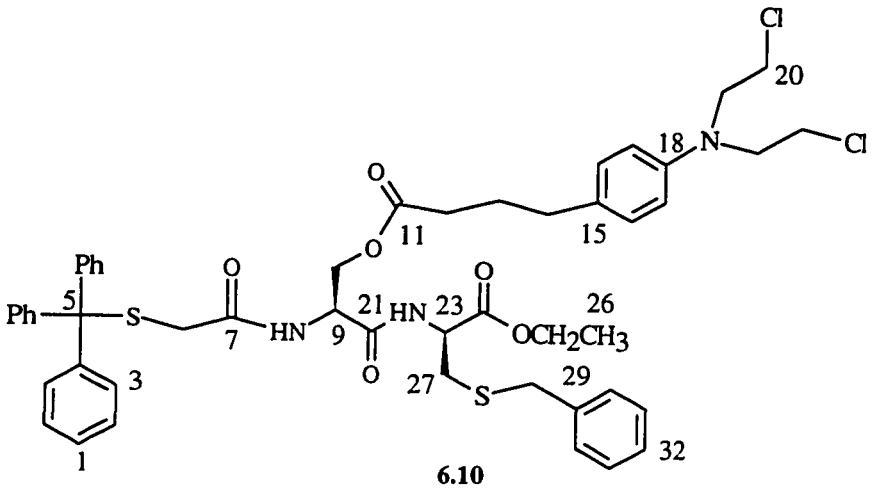
COSY and HSQC spectra for compound **6.10** were each acquired in 5.5 minutes on approximately 15 mg of sample. The majority of the aliphatic ^{1}H and ^{13}C signals of **6.10** were assigned (**Figure 6-V and 6-VI**) by the aforementioned two dimensional techniques and these were consistent with assignments made for the synthetic precursors. There remained, however, several assignments in both the proton and carbon NMR spectra of **6.10** that could not be made with absolute certainty. Consequently, Heteronuclear Multiple Bond Correlation (HMBC) and Heteronuclear Multiple Quantum Coherence-Total Correlation Spectroscopy (HMQC-TOCSY) experiments were performed (**Figure 6-VII and 6-VIII**).

The use of the HMBC pulse sequence with a low pass J filter allowed proton resonances to be correlated with neighbouring carbon atom resonances through spin coupling interactions of $^{2}J_{H\text{-}C}$ and $^{3}J_{H\text{-}C}$ (**Figure 6-VII**) . The initial use of the HMBC experiment was to assign the four carbonyl signals which corresponded to the two amide and two ester groups. The ethyl ester carbonyl peak (C-24) was assigned by correlation of the quartet of the methylene group of the ester (4.15 ppm) with the carbon atom signal at 169.9 ppm. The amide carbonyl signals were assigned by the observation of a two bond correlation with the adjacent α proton signal. The α proton chemical shifts had been assigned previously to the appropriate amino acid by use of the COSY experiment. The remaining carbonyl signal belonged to the ester between the chlorambucil unit and the serine hydroxyl group. The HMBC experiment also facilitated the assignment of all of the carbon atom signals in the aromatic systems as well as confirming the assignments of H-6,

Chemical Shift, δ	Proton	J(Hz)
7.390-7.180	Haryl	
7.016	H-16	$^3J_{16,17}$=8.8
6.761	H-8	$^3J_{8,9}$=7.0
6.679	H-22	$^3J_{22,23}$=7.6
6.581	H-17	
4.663	H-23	
4.382	H-9	
4.146	H-25	
4.151	H-10_A*	
4.009	H-10_B	$^3J_{AX}$=4.9 $^3J_{BX}$=6.4 $^2J_{AB}$=-11.2
3.646	H-28	
3.678-3.563	H-19,H-20	
3.117	H-6_A	$^2J_{6A,6B}$=-15.9
3.065	H-6_B	
2.872	H-27_A	
2.814	H-27_B	$^3J_{AX}$=4.9 $^3J_{BX}$=5.7 $^2J_{AB}$=-13.9
2.491	H-14	$^3J_{13,14}$=7.8
2.299	H-12	$^3J_{12,13}$=7.5
1.848	H-13	
1.225	H-26	$^3J_{25,26}$=7.1

* In the case of diastereotopic pairs of protons, the symbols A and B refer to the downfield and upfield signals, respectively, where these could be resolved.

Figure 6-V: The proton N.M.R. assignments for ethyl N-triphenylmethylthioethanoyl-O-{4'-[4"-(1"-bis(2"'-chloroethyl)amino)phenyl]butanoyl}-L-seryl-S-benzyl-L-cysteine, **6.10**.



6.10

Chemical Shift(ppm)	Carbon atom	Chemical Shift(ppm)	Carbon atom
173.0	C-11	170.0	C-24
168.5	C-7	168.2	C-21
144.4	C-18	143.9	C-4
137.5	C-29	130.5	C-15
129.6	C-16	129.5	C-3
128.9	C-30	128.6	C-31
128.2	C-2	127.3	C-32
127.1	C-1	112.2	C-17
67.9	C-5	63.1	C-10
61.9	C-25	53.6	C-20
52.0	C-9	51.9	C-23
40.5	C-19	36.6	C-28
36.0	C-6	33.9	C-14
33.2	C-27, C-12	26.4	C-13
14.1	C-26		

Figure 6-VI: The carbon-13 N.M.R. assignments for ethyl N-triphenylmethylthioethanoyl-O-{4'-[4"-(1"-bis(2"'-chloroethyl)amino)phenyl]butanoyl}-L-seryl-S-benzyl-L-cysteine, **6.10**.

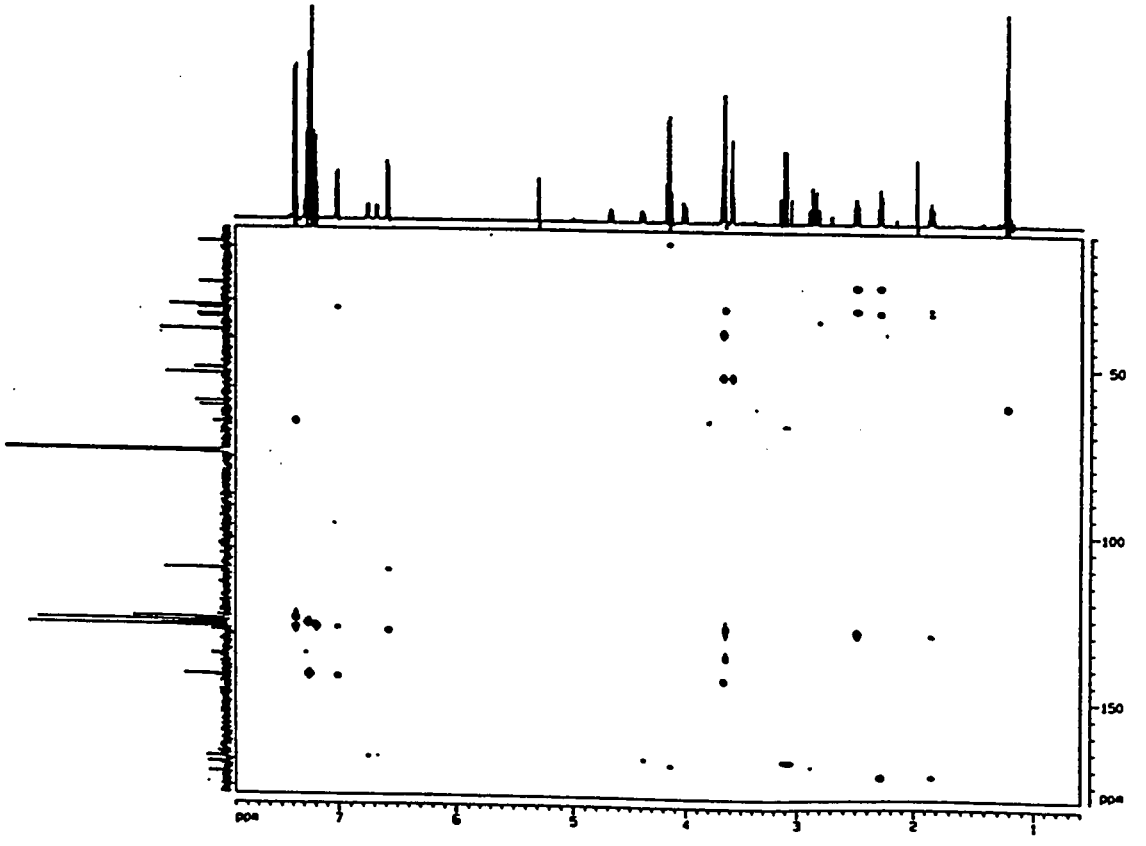


Figure 6-VII: HMBC Spectrum of **6.10**

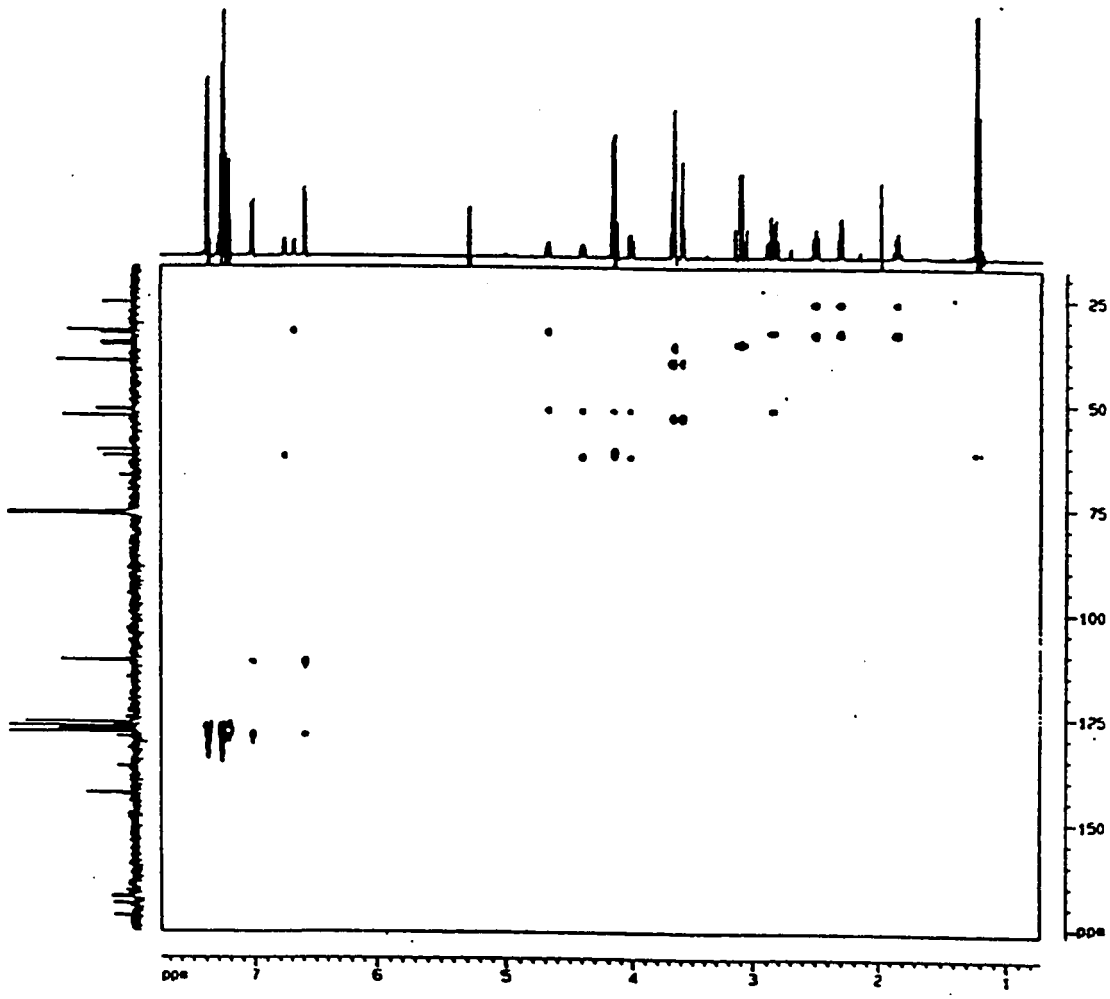


Figure 6-VIII: HMQC-TOCSY Spectrum of **6.10**

H-12 and H-19.

The HMQC-TOCSY experiment (**Figure 6-VIII**) was used to corroborate the ^{1}H and ^{13}C assignments. For example, the proton resonance at 4.38 ppm was assigned to the resonance of the α proton of serine (H-9). In the HMQC-TOCSY experiment, H-9 exhibited a HMQC correlation to C-9, the carbon to which it is directly bound. As a result of the TOCSY portion of the pulse sequence, H-9 also showed correlation with H-8 and H-10, the amide and β protons of serine respectively. The proton signal assigned as H-10 also correlated to its directly bound carbon atom. As a result, all the proton and carbon atom signals within the serine portion of the molecule could be assigned. The results of the HMQC-TOCSY experiment were consistent with the assignment made in **Figures 6-V** and **6-VI**.

The one- dimensional NOE difference spectra and the two- dimensional NOESY spectra of **6.10** in $CDCl_3$ were entirely consistent with the assignments presented above. The presence of strong NOE's between H-9 and H-22, and H-8 and H-6, and the complete absence of NOE's between H-9 and H-6, and H-9 and H-23, showed the anticipated preponderance of the Z geometric isomers of the two amide groups. Otherwise, the molecule appeared to be relatively flexible with no particularly demanding conformational preferences. Thus the ortho protons on each of the aromatic rings showed NOE's to side chain protons that were on carbon atoms one, two, and three bonds removed from the ring, but there was no evidence for NOE's over longer distances. Likewise, there were no

observable NOE's between one set of aromatic protons and another that might have arisen from any possible stacking of the aromatic rings.

In summary, the synthesis of **6.10** was completed in ten steps with 30% overall yield. A combination of two- dimensional NMR techniques was used to assign completely the proton and carbon atom spectra. As a result of the spectrometer's gradient capability, the entire collection of spectra, ^{1}H, ^{13}C, COSY, HMQC, HMBC, and HMQC-TOCSY were collected within 6 hours on a moderately dilute sample (15 mg/mL). The use of compound **6.10** as a reagent for the early detection of breast cancer remains a goal of future work.

6.8 Experimental Section

Ethyl S-benzyl-L-cysteine p-toluenesulphonate (**6.1**)

p-Toluenesulphonic acid (10.8 g, 56.8 mmol) was added to S-benzyl-L-cysteine (3.0 g, 4.2 mmol) in absolute ethanol (100 mL) and the mixture heated to reflux for 48 hours. The solution was then evaporated to dryness and diethylether (100 mL) was added. The resulting colourless precipitate was collected by filtration and washed with ether (200 mL). Yield of **6.1**: 4.67 g, 80%; mp:131-134°C; ^{1}H NMR [200MHz] (CD_3OD): δ7.299 (m, 9H, H-aryl), 4.155 (q, 2H, $O\mathbf{CH_2}CH_3$), 4.102 (m, 1H, H_2N-**CH**), 3.752 (s, 2H, $S\mathbf{CH_2}Ph$), 2.886 (m, 2H, CH-$\mathbf{CH_2}S$), 2.319 (s, 3H, CH_3-Ph), 1.232 (t, 3H, $OCH_2\mathbf{CH_3}$); ^{13}C NMR [50.3MHz] (CD_3OD): δ 169.12 ($\mathbf{C}O_2Et$), 143.15 (C-SO_3H (pTsOH-*para* C) 141.90

(Bn-*ipso* C), 138.61 (pTsOH-*ipso* C), 130.16 (pTsOH-*meta* C), 129.85 (Bn-*ortho* C), 129.66 (pTsOH-*ortho*), 128.42 (Bn-*para* C), 126.95 (Bn-*meta* C), 63.86 ($O\mathbf{CH_2}CH_3$), 53.29 (H_2N-**CH**), 36.79 ($\mathbf{SCH_2}Ph$), 32.06 (pTsOH-$\mathbf{CH_3}$), 21.31 ($CH\mathbf{CH_2}S$), 14.30 ($OCH_2\mathbf{CH_3}$).

Ethyl N-t-butoxycarbonyl-L-seryl-S-benzyl-L-cysteine (**6.4**).

Aqueous sodium carbonate (40 mL of 10%) was added to a suspension of **6.1** (5.0 g, 12.2 mmol) in DCM (80 mL). The mixture was shaken until everything dissolved. The aqueous layer was back extracted with DCM (2x40 mL) and the organic layers combined and dried over anhydrous sodium sulphate. The organic layers were combined and evaporated to dryness. The resulting yellow oil was then diluted with DCM (50 mL). N-*t*-butoxycarbonyl-L-serine (2.27 g, 11.1 mmol) and EDAC-HCl (2.33 g, 12.2 mmol) were added to this solution. The solution was stirred under nitrogen and protected from light for 16 hours. The solution was extracted with 1M HCl (2 x 20 mL), 1M $NaHCO_3$ (2 x 20 mL) and distilled water (2 x 20 mL). The organic layer was evaporated to dryness and the resulting solid recrystallized from acetonitrile. Yield of **6.4**: 3.7 g, 80%; mp: 78-79°C; TLC: R_f = 0.58 (10:90 v:v CH_3OH/CH_2Cl_2); ^{1}H NMR [200MHz] ($CDCl_3$): δ 7.308 (s, 5H, H-aryl), 5.485 (d, J=7.2, 1H, amide NH), 4.740 (m, 1H, $\mathbf{CH}CH_2S$), 4.155 (q, 2H, $O\mathbf{CH_2}CH_3$), 4.066 (m, 1H, $\mathbf{CH}CH_2OH$), 3.688 (s, 2H, $S\mathbf{CH_2}Ph$), 3.650 (m, 2H, $CH\mathbf{CH_2}OH$), 2.851 (m, 2H, $CH\mathbf{CH_2}S$), 1.751 (bs, OH), 1.436 (s, 9H, $C(CH_3)_3$), 1.243 (t, J=7.12, 3H, $OCH_2\mathbf{CH_3}$); ^{13}C NMR [50MHz] ($CDCl_3$): δ171.18 (**C**OOEt), 170.54 (amide

C(O)), 155.78 (carbamate C(O)), 137.42 (C-*ipso*), 128.86 (C-*ortho*), 128.03 (C-*meta*), 127.17 (C-*para*), 80.36 (**C**tBu), 62.89 ($O\mathbf{C}H_2CH_3$), 61.91 (CH_2OH), 55.34 ($\mathbf{C}HCH_2S$), 51.74 ($\mathbf{C}HCH_2OH$), 36.27 (SCH_2Ph), 32.97 ($CH\mathbf{C}H_2S$), 28.19 ($C(\mathbf{C}H_3)_3$), 13.95 ($OCH_2\mathbf{C}H_3$); MS: (NH_3-DCI) m/z (RI%) 444 (15, M+1+NH_3), 427 (100, M+1), 327 (30, M+1-Boc).

*N-t-Boc-L-Ser-O-Chlorambucil-S-Bn-L-Cys-OEt (**6.5**)*

TES was added (1.5 mL) to compound **6.4** (1.90 g, 4.4 mmol) in TFA (3.5 mL). After two hours the solution was evapourated to dryness and diluted with DCM (80 mL). The solution was extracted with 10% Na_2CO_3 (40 mL), followed by extraction of the aqueous layer with DCM (2 x 40 mL). The organic layers were combined, dried over sodium sulfate and evapourated to dryness. The compound was used without further purification.

*S-Trityl-thioglycolic acid-L-serine-S-benzyl-L-cysteine ethyl ester (**6.6**)*

To a DCM solution (40 mL) of compound **6.5** (4.4 mmol), triethylamine (3.0 mL) was added followed by **2.4** (2.1 g, 4.84 mmol). The reaction was stirred for 24 hours whereupon it was extracted with 0.1 M HCl (2 x 10 mL), 1.0 M $NaHCO_3$ and brine (2 x 20 mL). The organic layer was evaporated and the product, a yellow oil (2.0 g, 71 %) was isolated by radial chromatography (DCM, MeOH). Compound **6.6** showed: ^{1}H NMR ($CDCl_3$) [300 MHz]: 7.311 (m, H-aryl), 6.995 (d, J= 6.5, 1H, amide-NH), 4.644 (m, 1H,

cys-αCH), 4.147 (m, overlap, ser-αCH, OCH_2CH_3), 3.804 (m, 1H, CH_2OH), 3.651 (S, 2H, SCH_2Ph), 3.390 (m, CH_2OH), 3.110 (AB, J= 15.9, $TrSCH_2$), 3.040 (AB, $TrSCH_2$), 2.806 (ABX, J_{AB}= 9.2, J_{AX}= 3.3, J_{BX}= 4.5 $CHCH_2SBn$), 1.211 (t, J= 4.8, 3H, OCH_2CH_3); ^{13}C NMR [75.47 MHz] ($CDCl_3$): δ 170.48 (ester-C(O)), 170.40, 169.15 (amide C(O)), 143.90 (trityl-*ipso*), 137.46 (benzyl-*ipso*), 129.52 (trityl-*ortho*), 129.50, (benzyl-*ortho*), 128.62 (benzyl-*meta*), 128.13 (trityl-*meta*), 127.31 (benzyl-*para*), 127.02 (trityl-*para*), 67.53 (CPh_3), 62.53 (OCH_2CH_3), 62.03 (CH_2OH), 54.46 (ser-αCH), 51.89 (cys-αCH), 36.46 (SCH_2Ph), 35.95 ($TrSCH_2$), 32.89 (CH_2SBn), 14.03 (OCH_2CH_3) [75.47 MHz].

Ethyl N-t-butoxycarbonyl-O-[4'-[4"-(1"-bis(2"'-chloroethyl)amino)phenyl]butanoyl]-L-seryl-S-benzyl-L-cysteine (**6.7**)

A solution of EDAC (100 mg, 0.52 mmol) and DMAP (6 mg, 10 mol %) in DCM (5mL) was added to **6.4** (200 mg, 0.47 mmol) and chlorambucil (136 mg, 0.45 mmol) in dry DCM (15 mL). The reaction was stirred under a nitrogen atmosphere and protected from the light for 6 hours. The solution was then extracted with 1M HCl (2 x 10 mL), 1M $NaHCO_3$ (2 x 10 mL) and distilled water (2 x 10 mL). The organic layer was concentrated and the product purified by chromatography (1% CH_3OH in CH_2Cl_2) to yield **6.7** a colourless oil (237 mg, 71%). The compound showed: TLC: R_f = 0.80 (5:200 v:v CH3OH/CH_2Cl_2); ^1H NMR [200MHz] ($CDCl_3$): δ 7.269 (s, 5H, H-aryl), 6.997 (d, 2H, aniline meta), 6.997 (d, 1H, amide NH), 6.584 (d, J=8.8, 2H, aniline-ortho), 5.210 (m, J=7.2, 1H, Boc-NH), 4.710 (m, 1H, $CHCH_2O$), 4.431 (m, 3H, CH-CH_2O and $CHCH_2S$),

4.154 (q, 2H, OCH_2CH_3), 3.659 (s, 2H, SCH_2Ph), 3.609 (m, 8H, CH_2CH_2Cl), 2.893 (m, 2H, J_{AX}=4.9, J_{BX}=5.7, $^2J_{AB}$=-19.6, CH_2S), 2.497 (t, 2H, J=7.8, CH_2Ph), 2.307 (t, J=7.7, 2H, $C(O)CH_2$), 1.852 (m, 2H, $CH_2CH_2CH_2$), 1.434 (s, 9H, $C(CH_3)_3$), 1.233 (t, J=7.16, 3H, OCH_2CH_3); ^{13}C NMR [50MHz]: δ 173.18 ($C(O)CH_2CH_2CH_2$), 170.12 ($C(O)CH_2CH_3$), 168.96 (amide-C(O)), 154.37 (Boc-C(O)), 144.26 (aniline-*ipso*), 137.52 (aniline-*para*), 130.33 (benzyl-*ipso*), 129.60 (benzyl-*ortho*), 128.85 (benzyl-*meta*), 128.52 (aniline-*meta*), 127.21 (benzyl-*para*), 112.08 (aniline-*ortho*), 80.60 ($C(CH_3)_3$), 63.79 ($CH_2OC(O)$), 61.85 (OCH_2CH_3), 53.49 (CH_2Cl), 51.79 ($CHCH_2S$ and $CHCH_2O$), 40.43 (NCH_2), 36.47 (SCH_2Ph), 33.80 (CH_2Ph), 33.21 ($C(O)CH_2$ and $CHCH_2S$), 28.18 ($C(CH_3)_3$), 26.41 ($CH_2CH_2CH_2$), 14.00 (OCH_2CH_3); MS (HRDEI): Obs: 711.2526, Calc: 711.2540.

Ethyl N-triphenylmethylthioethanoyl-O-{4'-[4"-(1"-bis(2"'-chloroethyl)amino) phenyl]butanoyl}-L-seryl-S-benzyl-L-cysteine (**6.10**).

Triethylsilane was added dropwise to a solution of **6.7** (50 mg, 0.070 mmol) in trifluoroacetic acid, (TFA, 5 mL), until the yellow solution became colourless. The reaction mixture, which was protected from light, was allowed to stir for 2 hours before the TFA was removed *in vacuo*. The resulting oil was diluted with dry DCM (20 mL) and the reaction mixture extracted with aqueous 10% Na_2CO_3 (10 mL). The aqueous layer was back extracted with DCM (2 x 10 mL) and the organic layers combined and evaporated to dryness. The resulting oil was diluted to 10 mL with DCM and compound **2.5** (28 mg, 0.064 mmol) was added together with freshly distilled diisopropylethylamine (9.1 mg,

0.070 mmol). The reaction mixture was stirred under nitrogen and protected from light for 24 hours. After removal of volatile solvents by evaporation *in vacuo* the product was isolated by radial chromatography (1% CH_3OH in CH_2Cl_2) to yield **6.10** as a colourless oil (33 mg, 50%). The compound showed: TLC: R_f= 0.71 (2:98 v:v CH_3OH/CH_2Cl_2). Elemental Analysis: Calc: C 64.7 H 5.9 N 4.5%; Obs: C 65.2 H 6.1 N 4.6%.

Chapter 7

N_3S Chelates

7.1 Introduction and Rationale

Chelates which contain two amides, one amine and one thiol are of particular interest in nuclear medicine because they are reported to form stable Tc(V) and Re(V) complexes[1]. The most clinically utilized N_3S chelate is the MAG_3 chelate which, as mentioned earlier, is used in renal imaging. On coordination to the metal center, the donor atoms are deprotonated, forming an anionic complex (**Figure 5-I**). The geometry of the complex was, as expected, a square based pyramid, with the oxo group occupying the apical position. The carboxylate residue was not coordinated and it is postulated that this pendant arm is crucial for the compound's uptake into the kidneys. MAG_3 chelates have also been used in the bifunctional approach to the development of radiopharmaceuticals. For example, a peptide bond between the MAG_3 chelate and interleukin 2 (IL2) was prepared through the unbound carboxylate[2].

For medical imaging purposes, each MAG_3 kit contains 1 mg of N-[N[benzoylthio(acetyl)]glycyl]glycyl]glycine, 0.02-0.2 mg of stannous chloride dihydrate,

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[1]Fritzberg, A.R.; Kasina, S; Eshima, D; Johnson, D.L., **1986**, *J. Nucl. Med. 27*, 111.

[2]Mather, S.H.; Ellison, D., *J. Nucl. Med.*, **1994**, *38*, 481.

40 mg sodium tartrate dihydrate and 20 mg lactose monohydrate. The preparation of the labelled species involves the adding of $^{99m}TcO_4^-$ and then heating the mixture for five minutes (to cleave the benzoate ester). The success of MAG_3 as a technetium chelate suggested that other N_3S tripeptide donors could be prepared and by modification of the approach to the tripeptides synthesised in chapter **5**, a new N_3S analogue was synthesised.

7.2 Chelate Design

There are numerous examples of a metal coordinated by the imidazole group of histidine, both in biomolecules and small molecules prepared in the laboratory[3]. N_3S type chelates were synthesised in the present work by replacing cysteine in the N_2S_2 type chelates synthesised in chapter **5** with histidine. Chelates with the general formula, mercaptoacetic acid-X-histidine (where X= any amino acid), can be prepared by the use of standard peptide coupling techniques and the general synthetic approach was analogous to that used in chapter **5**. The protected tripeptide can be synthesised from a dipeptide of the amino acid of interest and histidine (**Figure 7-I**, fragment **A**), which in turn would be synthesised from the amino acids by carbodiimide or N-hydroxysuccinimido couplings. One chelate, Tr-S-Mer-O-Bn-L-Ser-L-His-OMe was synthesised in this way. The use of the protected O-benzyl-L-serine resulted in the formation of a hydrophobic tripeptide and

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[3]Wu, F.-J.; Kurtz, Jr., D.M.; Hagen, K.S.; Nyman, D.; Debrunner, P.G.; Vankai, V.A.; *Inorg. Chem.*, **1990**, 29, 5174-5183. Brown, R.S.; Salmon, D.; Curtis, N.J.; Kusuma, S., *J. Am. Chem. Soc.*, **1982**, 104, 11. Cowan, J.A. *Inorganic Biochemistry; An Introduction*, VCH, Weinheim, **1993**.

after deprotection (hydrogenation), the peptide would be hydrophilic in nature.

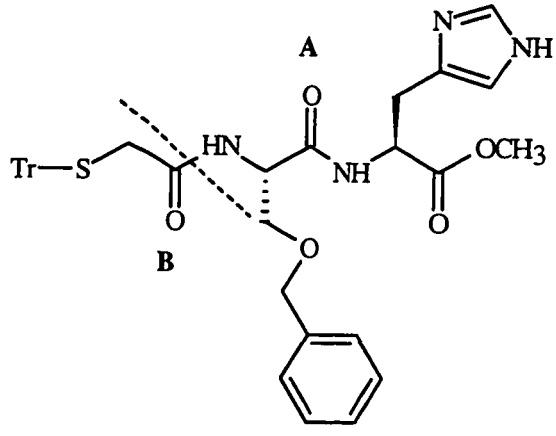


Figure 7-I: Synthon units of compound **7.4**

7.3 Synthesis of Tr-S-Mer-O-Bn-L-Ser-L-His-OMe

The initial step in the preparation of the title compound was the coupling of L-histidine methyl ester dihydrochloride and the N-hydroxysuccinimido ester of N-t-Boc-L-serine (**Figure 7-II**). Because the free amine of histidine was in equilibrium with its hydrochloride salt, the hydroxyl group of serine had to be protected to prevent the alcohol group from acting as a nucleophile. The N-hydroxysuccinimido ester of O-benzyl-L-serine (**7.1**) was commercially available (BACHEM) and, in the presence of a tertiary amine base, was coupled to histidine in reasonable yield (66%). The presence of the benzyl group facilitated monitoring of the reaction by TLC (UV indicator) and isolation of the product (**7.2**) by radial chromatography. Excess histidine was used during the coupling so that upon completion of the reaction the major impurities would be the hydrolysed EDAC and

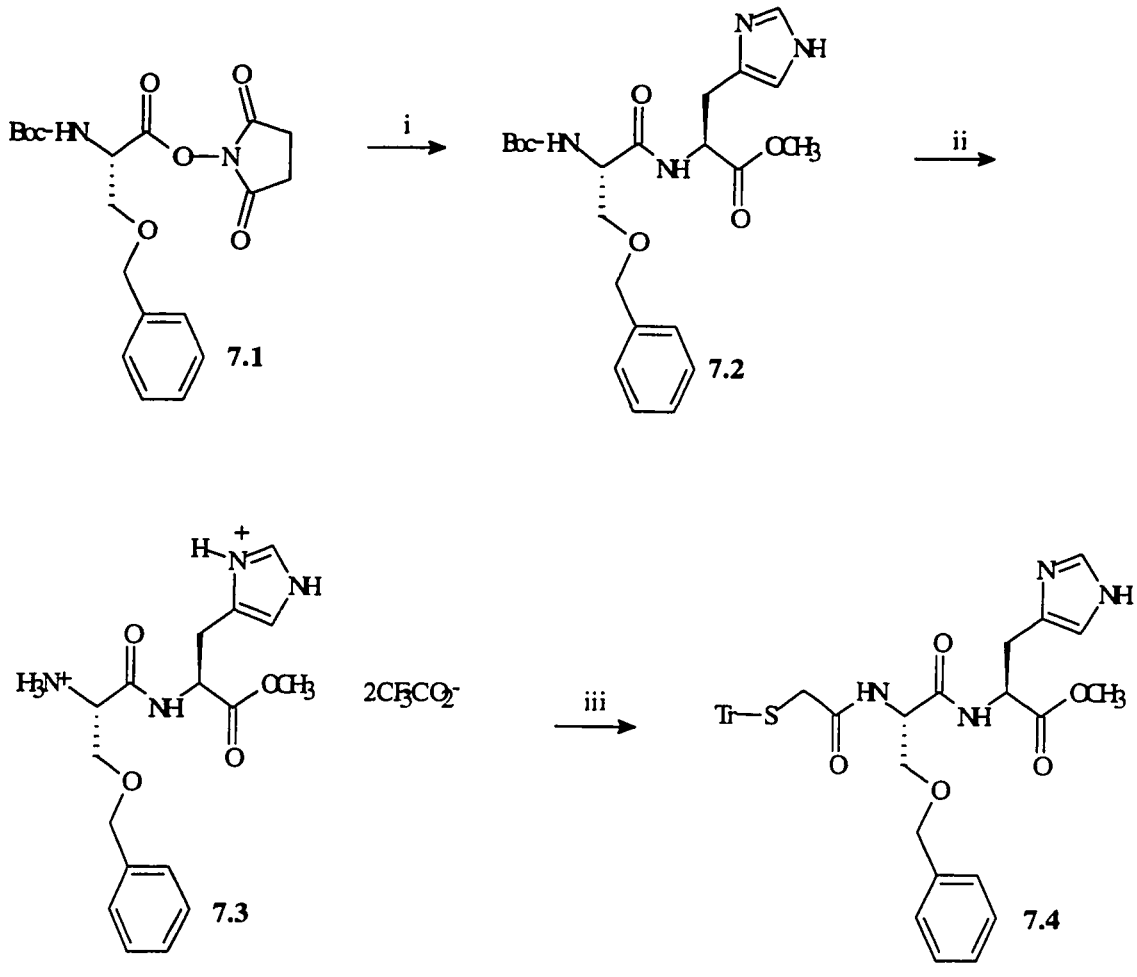


Figure 7-II: i) L-histidine methyl ester dihydrochloride, NEt_3 ii) TFA, TES iii) Compound **2.5**, NEt_3.

surplus histidine, both of which could be removed by extraction with aqueous acid; minor impurities were removed finally by radial chromatography. The ^1H NMR of **7.2** confirmed the formation of the desired amide bond because of the appearance of a doublet at 7.92 ppm (J= 7.5 Hz), which was assigned to the amide proton between histidine and serine.

The carbamate proton was, as expected, upfield at 5.84 ppm (d, J= 7.2 Hz). There were three signals in the carbonyl region of the ^{13}C NMR spectrum. The ester and amide carbonyl signals were at 170.95 and 170.18 ppm respectively, while the shielded carbamate carbonyl was at 155.34 ppm. There were no ambiguous assignments in either the ^{1}H or ^{13}C spectra.

The dipeptide **7.2** was deprotected by the use of TFA/TES, which resulted in the formation of the ditrifluoroacetate salt (**7.3**). The reaction was completed shortly after the addition of triethylsilane; this was confirmed by ^{1}H NMR and thin layer chromatography using ninhydrin as the indicating solution. The addition of a large excess of base to **7.3** in the presence of compound **2.5** resulted in the formation of compound **7.4** in 40% overall yield.

The ^{1}H NMR spectrum of compound **7.4** showed a downfield singlet which corresponded to one of the protons on the imidazole residue; the other imidazole methine proton was upfield at 6.73 ppm and the complex multiplet at 7.30 ppm was associated with the trityl protecting group. The alpha protons of the two amino acids resonated at 4.67 (His) and 4.21 ppm (Ser), and the related *beta* protons were found at 3.06 and 3.45 ppm respectively. The remaining resonances were associated with the methyl ester (3.65 ppm), the O-benzyl methylene group (4.45 ppm) and the methylene group of mercaptoacetic acid (2.98 ppm).

7.4 Synthesis of Re-Mer-O-Bn-L-Ser-L-His-OMe

The trityl protecting group of compound **7.4** was removed by use of TFA and TES in a manner analogous to that described in chapter **5**, for compound **5.10** (**Figure 7-III**). The free thiol, **7.5**, was reacted with $ReOCl_3(PPh_3)_2$ in methanol solvent in the presence of

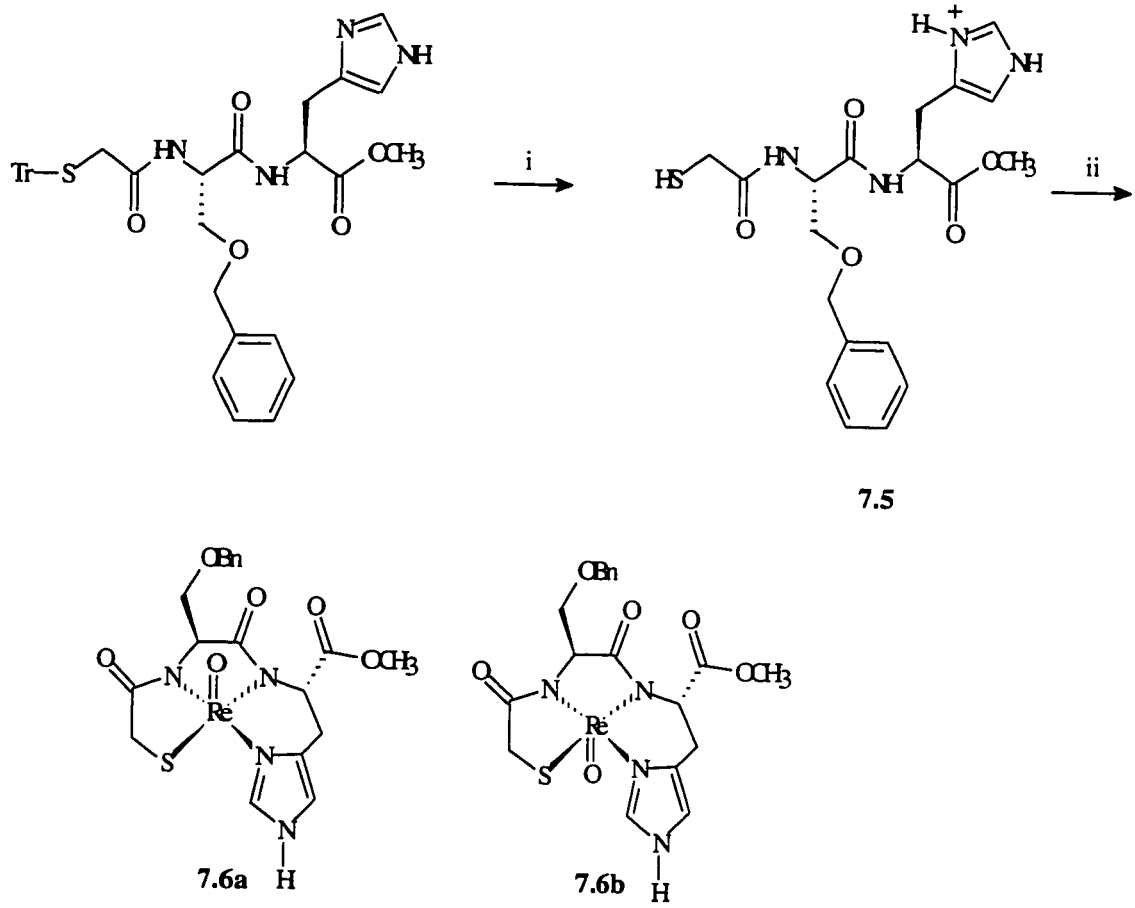


Figure 7-III: i) TFA, TES ii) $ReOCl_3(PPh_3)_2$, MeOH, THF, Δ

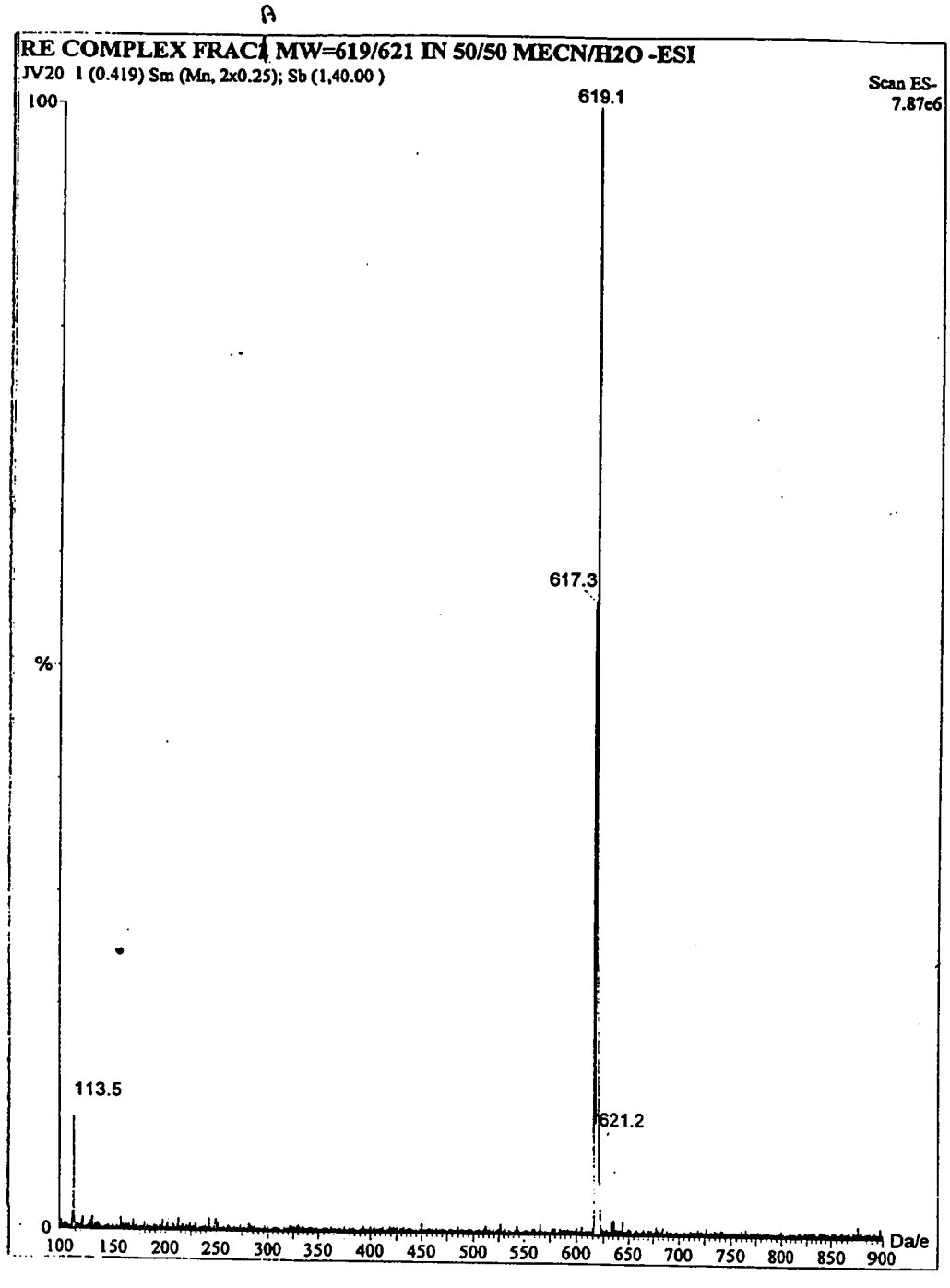
a sodium acetate buffer. The reaction mixture showed a distinct colour change on heating from yellow/green to orange, and the crude product, when examined by HPLC showed a fairly complex mixture. There were, however, two large peaks with absorbances in the visible region; these were isolated and examined spectroscopically.

7.5 Spectroscopic Studies of ***7.6***

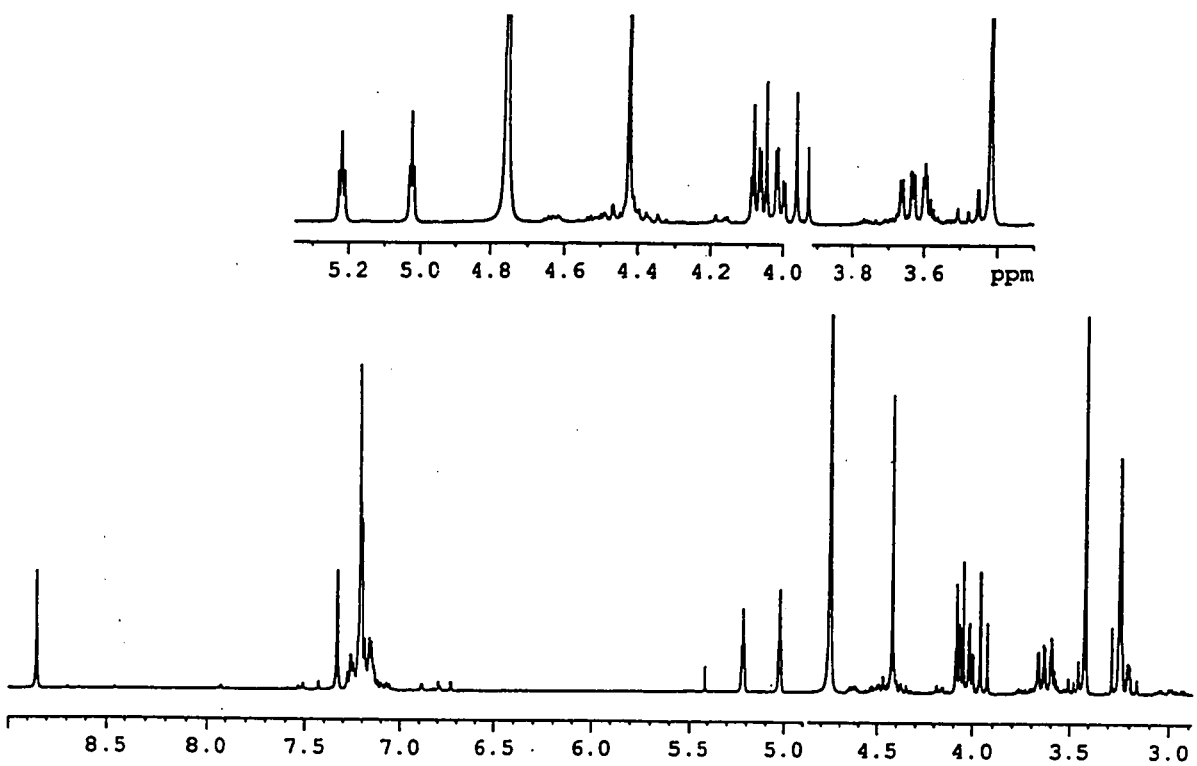
The reaction of $ReOCl_3(PPh_3)_2$ with tripeptide **7.5** was expected to form at least two major diastereomers, **7.6a** and **7.6b** by analogy with the N_2S_2 tripeptide, Mer-His-Cys, **5.25**, studies that are discussed in chapter **5**. The compounds giving rise to the two most intense peaks in the HPLC chromatogram of the crude reaction mixture were examined in detail on the basis of the working hypothesis that they corresponded to the two diastereomers **7.6a** and **7.6b**.

The highest mass peak in the ES-MS (**Figure 7-IV**) (m/z= 617/619) of each of the two main fractions obtained from HPLC corresponded to the molecular weight of **7.6** after losing one proton. The loss of the proton was most likely from the imidazole residue because upon coordination the pK_a of the amine proton would be lowered.

The ^1H NMR of the second fraction (**Figure 7-V**) (elution time= 15.1 minutes), which, based on HPLC peak heights, was the major product, exhibited only one signal for each of the two *alpha* protons. As noted in chapter **5**, this confirmed the presence of only one isomer. There were some residual signals at the baseline level in the spectrum that were ascribed to small amounts of the other isomer that contaminated the sample. The



7-IV: ES-MS of compound **7.6**

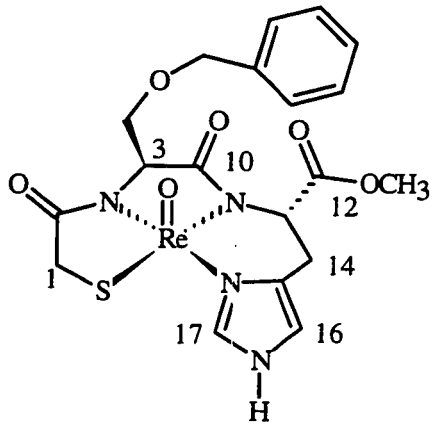


7-V: ^{1}H NMR of fraction 2, compound **7.6**

alpha protons, which were found at 5.219 and 5.025 ppm, had similar coupling constants (3.9 and 3.1 Hz respectively).

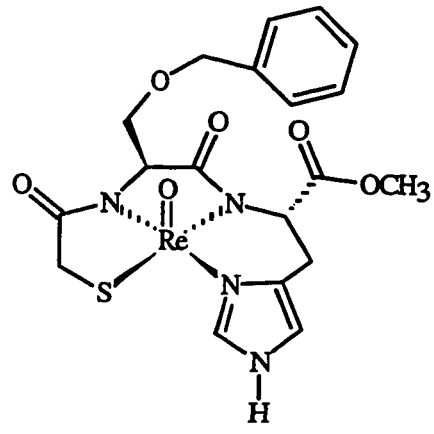
Upon coordination, the chemical shift of the histidine protons (H-17 and H-16, **Figure 7-VI**) moved to lower field. This is consistent with the idea that the metal removes electron density from the ring, thereby changing the pK_a of the amine proton. The *beta* protons of histidine were diastereotopic and because of the magnetic anisotropy caused by the metal, they had significantly different chemical shifts. Upon coordination, the two amide carbons, which were similar in chemical shift, moved downfield in a manner similar to the Re-N_2S_2 complexes (192.74 and 192.64 ppm) (**Figure 7-VII**). Attempts were made, without success, to determine which of the two possible stereoisomers was contained in fraction 2 by comparison of NMR chemical shifts to that of other serine- containing rhenium chelates whose structure has been determined by X-ray crystallography. To determine the absolute structure, future work should focus on isolating single crystals for use in X-ray diffraction studies.

The ^1H NMR spectrum of fraction 1 contained peaks which matched those found in the ^1H NMR of fraction 2 in addition to several other multiplets. The presence of fraction 2 in fraction 1, which was confirmed by analytical HPLC, was unexpected because during preparative fractionation, extreme care was taken to avoid contaminating fraction 1 with fraction 2. There was evidence in the ^1H NMR of fraction 1 however, which suggested that the other isomer was present. There was a multiplet at 5.113 ppm which is



^{1}H NMR		^{13}C NMR (aliphatic region)	
Proton	Chemical Shift (δ) ppm	Carbon	Chemical Shift (δ) ppm
8.854	H-17	73.04	C-5
7.330	H-16	70.71	C-4
7.202	H-aryl	65.37	C-11
5.219	H-3	57.59	C-3
5.025	H-11	51.33	C-13
4.412	H-5	38.88	C-1
4.068	H-1A	30.50	C-14
4.048	H-4		
3.994	H-1B		
3.628	H-14A		
3.246	H-14B		

Figure 7-VI: Summary of ^{1}H NMR and ^{13}C NMR (aliphatic region) assignments for compound **7.6**



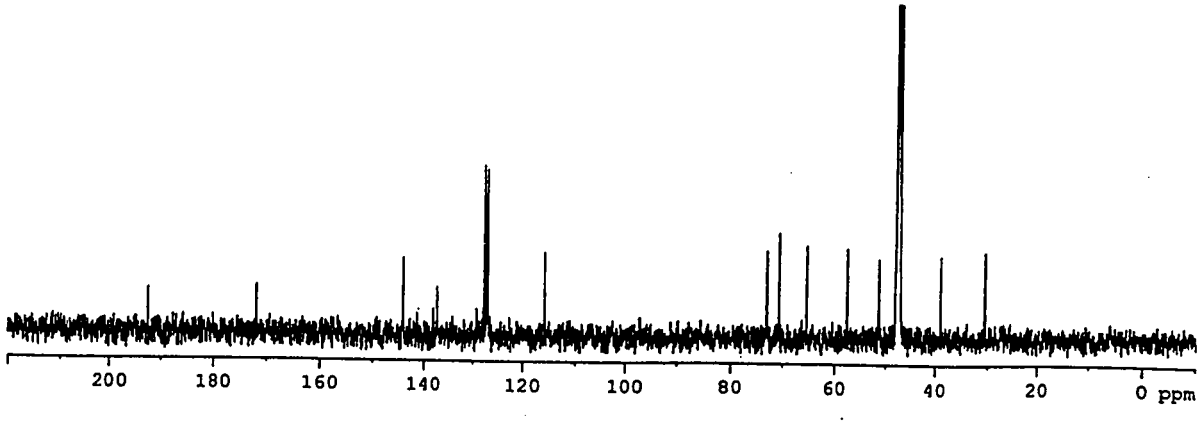


Figure 7-VII: ^{13}C NMR Spectrum [125 MHz] of Re-Mer-OBn-L-Ser-L-His-OMe

most likely the alpha proton of one of the amino acids of the other isomer of **7.6**. In addition, there was another set of imidazole singlets at different chemical shifts to those found in fraction 2. There were also additional AB parts of ABX spin systems between 4.3 and 3.0 ppm which had different chemical shifts to those of the compound found in fraction 2. Though the ^{1}H NMR spectrum was complex, along with the electrospray results there is sufficient evidence to propose that fraction 1 contained some of the other isomer of **7.6**. The formation of a complex reaction mixture could potentially be explained by isomerization of the rhenium complex upon removal of the eluent (water/ methanol/ acetonitrile) at elevated temperatures after isolation by HPLC. Under acidic conditions or elevated temperatures, the amine donor can equilibrate between being bound to the metal or being protonated and leaving a vacant coordination site (**Figure 7-VIII**). The vacant coordination site can be occupied by a water molecule which could result in the formation of the trans-dioxo system which would convert back to the monooxo system in the presence of acid or heat or be converted to some other species (resulting in the impurities seen in the NMR of fraction 1). If decomposition of the chelate complex does not occur, then water can eliminate from either side of the chelate resulting in the formation of both diastereomers. The second fraction did not decompose or isomerize; therefore it must be stable to the conditions used to remove the solvent which suggests that it is more thermodynamically stable than the isomer associated with the first fraction. This type of

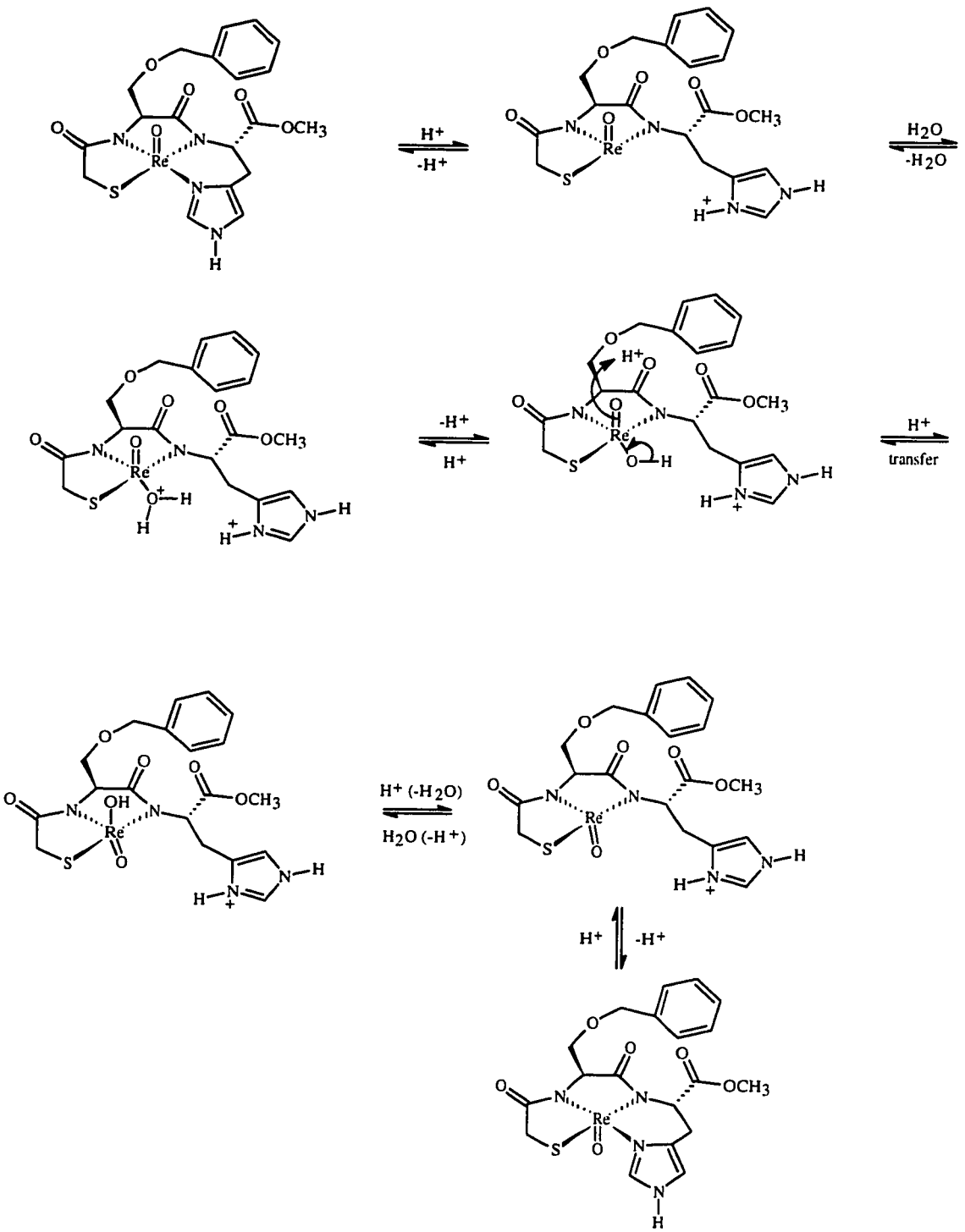


Figure 7-VIII: Proposed mechanism of isomerization of compound **7.6**

isomerization has been observed in another Re-N_3S complex[4].

In summary, one of the two isomers of **7.6** was isolated and characterized by ^1H and ^{13}C NMR spectroscopy, HPLC and electrospray mass spectrometry. The other isomer, which appears to convert to the more stable isomer upon heating was not isolated in a pure form. Future work should focus on isolating the less stable isomer and determining the amount of energy required to induce isomerization. If the rate of isomerization is slow, the proposed mechanism of interconversion can be scrutinized by NMR spectroscopy.

7.6 Experimental Section

N-t-Boc-O-Bn-L-Ser-L-His-OMe (**7.2**)

To N-t-butoxy-O-benzyl-L-serine-N-hydroxysuccinimido ester (2.0 g, 5.10 mmol) and L-histidine methyl ester dihydrochloride (1.84 g, 7.65 mmol) in DCM (30 mL), diisopropylethylamine (3 mL) was added. The reaction was stirred at room temperature for 16 hours before extraction with 0.1M HCl (30mL), 1M $NaHCO_3$ (30 mL) and DW (4 x 30ml). The organic layer was concentrated (1 mL) and the title compound, a colourless crystalline solid, isolated by radial chromatography (DCM/MeOH) (1.5 g, 66%). Compound **7.2** showed: m.p.: 58-60°C; ^1H NMR: ($CDCl_3$) [200MHz]: δ 7.916 (d, J = 7.5, 1H, amide NH), 7.401 (s, 1H, **NCH**NH), 7.208 (s, H-aryl), 6.710 (s, 1H, imidazole CH), 5.835 (d, J= 7.2, 1H, Boc-NH), 4.731 (m, 1H, His-CH), 4.432 (s, 2H, O**C**$\mathbf{H_2}$Ph), 4.420

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[4]Bell, R.A.; Bennett, S.; Fauconnier, T.; Thornback, J.; Valliant, J.; Wong, E. *Unpublished results.*

(m, 1H, Ser-CH), 3.741 (m, 2H,CH**CH$_2$**OBn), 3.546 (s, 3H, OCH_3), 3.057 (m, 2H,CH**CH$_2$**imidazole), 1.359(s, 9H, C(**CH$_3$**)$_3$);^{13}C NMR: ($CDCl_3$) [50MHz]: δ 170.95 (ester C(O)), 170.18 (amide C(O)), 155.3 (Boc-C(O)), 137.08 (**NCH**NH), 134.69(benzyl-ipso), 131.52 (imidazole- ipso), 128.02 (benzyl-ortho), 127.36 (benzyl-para), 127.16 (benzyl-meta), 117.26 (imidazole CH), 79.76 (**C**(CH$_3$)$_3$), 72.88 (O**CH$_2$**Ph), 69.65 (CH**CH$_2$**OBn), 54.04 (His-CH), 52.36 (Ser-CH), 51.93 (OCH_3), 28.29 (CH**CH$_2$**imidazole), 27.87 (C(**CH$_3$**)$_3$).

O-Benzyl-L-Ser-L-His-OMe ditrifluoroacetate salt (**7.3**)

To compound **7.2** (875 mg, 1.96 mmol) in TFA (4 mL), triethylsilane was added drop-wise until the yellow colour discharged. The solution was stirred at room temperature for 2 hours prior to evapouration to dryness. Methanol was added (40 mL) and subsequently evaporated, leaving a yellow oil. The compound showed: ^{1}H NMR (CD_3CN) [200 MHz]: δ9.011(d, J=5.1, 1H, amide NH), 8.816 (s, 1H, N**CH**N), 7.255 (s, 1H, N**CH**C), 7.178 (s, H-aryl), 4.592 (m, 1H, His αCH), 4.332 (s, 2H, O**CH$_2$**Ph), 3.996 (m, 1H, Ser αCH), 3.639 (m, 2H, CH_2OBn), 3.477 (s, 3H, OCH_3), 2.999 (m, 2H, CH_2-imidazole) [200MHz]; ^{13}C NMR (CD_3CN) [50 MHz]: δ169.88 (ester-C(O)), 166.37 (amide-C(O)), 158.57 (TFA-C(O)), 137.06 (N**CH**N), 133.57 (**C**NHCH), 128.23 (*C-ipso*), 127.74 (C-*ortho*), 127.17 (C-*meta*), 116.86 (C-*para*), 72.11 (O**CH$_2$**Ph), 67.84 (CH_2OBn), 54.24 (OCH_3), 51.95 (Ser αCH), 51.17 (His αCH), 25.46 (CH_2-imidazole).

Tr-S-Mer-O-Bn-L-Ser-L-His-OMe (**7.4**)

The oil from **7.3** was dissolved in DCM (40 mL) and **3** (931 mg, .907 mmol) was added along with DIPEA (1 mL). After refluxing for 5 hours, the solution was extracted with 1M HCl (30 mL), 1M $NaHCO_3$ (30 mL) and DW (4 x 30 mL). The organic phase was concentrated to 1 mL and the product isolated by radial chromatography (MeOH/ DCM). The product, a crystalline solid (350 mg, 59%), showed: ^{1}H NMR ($CDCl_3$) [200 MHz] δ 7.840 (d, J= 7.1, 1H, amide NH), 7.555 (s, 1H, N**CH**NH), 7.300 (m, H-aryl) 7.017 (d, 1H, amide NH), 6.733 (s, 1H, N**CH**C), 4.666 (m, 1H, His αCH), 4.448 (s, 1H, O**CH$_2$**Ph), 4.207 (m, 1H, Ser αCH), 3.648 (s, 3H, OCH_3), 3.453 (m, 2H, CH_2OBn), 3.057 (m, 2H, CH_2-imidazole), 2.977 (s, 2H, TrSCH_2); ^{13}C NMR ($CDCl_3$) [50MHz] δ170.71 (ester-C(O)), 169.44 (amide C(O)), 169.14 (amide CO)), 144.4-118.28 (C-aryl), 73.35 (**C**Ph_3), 68.94 (O**CH$_2$**Ph C-10), 67.79 (CH_2OBn), 66.27 (Ser αCH), 53.67 (His αCH), 52.66 (OCH_3), 36.0 (TrSCH_2), 27.16 (CH_2-imidazole).

Re-Mer-O-Bn-L-Ser-L-His-OMe (7.6)

Compound **7.4** (100 mg, 0.151 mmol) was dissolved in TFA (4 mL) and triethylsilane was added dropwise until the colour discharged, whereupon the solution was stirred for one hour. After evaporation of the solvent, the solution was diluted with methanol (20 mL) and evaporated to dryness to remove any traces of TFA. To the residue in methanol (10 mL) sodium acetate (1M, 2.0 mL) followed by $ReOCl_3(PPh_3)_2$ (138 mg, 0.166 mmol) were added and the mixture heated to reflux. After 30 minutes, THF (5 mL)

was added to the reaction mixture. The colour of the solution changed from yellow/green to orange; after 55 minutes the reaction was cooled to room temperature, filtered and evaporated to dryness. The coloured solution was diluted with methanol (1 mL) and acetonitrile (1 mL), cooled in the freezer for one hour, filtered and evaporated to dryness.

The remaining sample was dissolved in methanol (350 μL) and filtered through glass wool. The product was purified by reverse phase HPLC using a Vydac 201HS10110 semi-prep column (9.4 x 250 mm). The conditions, developed with the use of an analytical column, involved using a partial gradient from 10%-25% AN/H_2O over a twenty minute period. Non-polar reaction products were washed off the column after isolation of the desired species with the use of 70-90% AN/H_2O. The flow rate was 4.0 mL/min. The detector was set at 320 nm and 254 nm. The fraction 1 was collected from 11.8-13.8 minutes while fraction 2 was colleted from 14.7 to 16.3 minutes. Fraction 2: ^{1}H NMR (CD_3OD) [500 MHz]: δ8.854 (H-17), 7.330 (H-16), 7.202 (H-aryl), 5.219 (H-3), 5.025 (H-11), 4.412 (H-5), 4.068 (H-1A), 4.048 (H-4), 3.994 (H-1B), 3.628 (H-14A), 3.246 (H-14B); ^{13}C NMR (CD_3OD) [125.77 MHz]: δ192.72 (amide C(O)), 192.65 (amide C(O)), 171.90 (ester C(O)), 144.30 (Benzyl-*ipso*), 138.20 (imidazole CH), 137.38 (imidazole CH), 128.84 (imidzaole C), 127.76 (benzyl-*ortho*), 127.36 (benzyl-*meta*), 115.91 (benzyl-*para*), 73.04 (C-5), 70.71 (C-4), 65.37 (C-11), 57.59 (C-3), 51.33 (C-13), 38.88 (C-1), 30.50 (C-14); MS (-ES) m/z (RI%): 619.1 (100)[^{187}M-1H$^+$], 617.2 (52)[^{185}M-1H$^+$].

Chapter 8

Conclusions

A series of chelates were synthesised which contained amino acids as the synthon units. The N_2S_2 type chelates, which were of the general form Mer-X-Cys (where X= an amino acid) chelated rhenium in a manner similar to the previously reported DADT chelate. The structure of one diastereomer of one rhenium tripeptide complex (Re-Mer-L-His-L-Cys-OMe) was determined by NMR spectroscopy and X-ray crystallography.

By changing the amount of steric hindrance at the *beta* position of the central amino acid, the ratio of the two diastereomers which formed on chelation could be altered. The reaction of rhenium with Mer-L-Ile-S-Bn-L-Cys was unique in that it gave predominantly one isomer.

The rhenium complex of one N_3S chelate, Mer-O-Bn-L-Ser-L-His-OMe, was synthesised and one of the two possible diastereomers isolated by HPLC. Initial attempts at isolating the other diastereomer resulted in a complex mixture and the hypothesis that the two diastereomers were, at elevated temperatures, isomerizing.

Potential breast cancer imaging agents were synthesised by coupling a tamoxifen derivative and chlorambucil to the DADT and Tr-S-Mer-L-Ser-S-Bn-L-Cys-OEt chelates respectively. The products were characterized by 1-D and 2-D NMR spectroscopy. Future work should focus on determining the biodistribution of the ^{99m}Tc complexes of compound **6.10** and **3-I**.

Appendix I

Experimental Methods

Analytical TLC was performed on silica gel 60-F_{254} (Merck) plates with detection by long wavelength ultra violet light unless specified otherwise. Chromatography was performed with use of either a chromatotron (Harrison Research Model 7924T) that used a 4 mm plate (EM Science silica gel 60 PF_{254} containing gypsum) or silica gel column chromatography (200-400 mesh). The mobile phase consisted of a gradient which starts off with 100% of the less polar solvent moving to 100% of the other solvent. For example; (DCM/MeOH), the gradient would begin with 100% DCM and then small quantities of methanol would be added until the desired compound eluted. All commercial reagents were used as supplied. Solvents were distilled, under nitrogen, from calcium hydride. Nitrogen was dried by passing it through calcium sulphate. All reactions were protected from light and carried out under a slow flow of nitrogen unless stated otherwise. Solvents were evaporated with a rotary evaporator (20 mmHg) at elevated temperatures (30-50°C). Melting points were recorded on a Gallenkamp capillary tube melting point apparatus.

Selected NMR spectra were recorded on a Bruker Avance DRX-500 spectrometer. Proton spectra were acquired at 500.130 MHz with a 5 mm broadband inverse probe with triple axis gradient capability. Spectra were obtained in 8 scans in 32K data points over a 4.006 kHz spectral width (4.096 s acquisition time). Sample temperature was maintained at 30 °C by a Bruker Eurotherm variable temperature unit. Gaussian multiplication (line

broadening: -1.5 Hz, Gaussian broadening: 0.2) was used to process the free induction decay (FID) which was zero-filled to 64K before Fourier transformation. Coupling constants (J) were reported in Hz.

Proton COSY two dimensional NMR spectra were recorded in the absolute value mode with the pulse sequence 90° - t_1 - 45° - ACQ and included pulsed field gradients for coherence selection. Spectra were acquired in 1 scan for each of the 256 FIDs that contained 2K data points in F2 over the previously mentioned spectral width. The ^1H 90° pulse width was 6.6 μs. A 1.0 s relaxation delay was employed between acquisitions. Zero-filling in F1 produced a 1K x 1K data matrix with a digital resolution of 3.91 Hz/point in both dimensions. During two dimensional Fourier transformation a sine-bell squared window function was applied to both dimensions. The transformed data were then symmetrized.

Carbon-13 NMR spectra were recorded at 125.758 MHz with a 5 mm broadband inverse probe with triple axis gradient capability. The spectra were acquired over a 28.986 kHz spectral width in 32K data points (0.557 s acquisition time). The ^{13}C pulse width was 4.0 μs (30° flip angle). A relaxation delay of 0.5 s was used. Exponential multiplication (line broadening: 4.0 Hz) was used to process the FID which was zero-filled to 64K before Fourier transformation.

Inverse detected ^1H - ^{13}C two dimensional chemical shift correlation spectra were acquired in the phase sensitive mode and used the pulsed field gradient version of the HSQC pulse sequence. The FID's in the F2 (^1H) dimension were recorded over a 3.655

kHz spectral width in 1K data points. The 128 FID's in the F1 (^{13}C) dimension were obtained over a 21.368 kHz spectral width. Each FID was acquired in 2 scans. The fixed delays during the pulse sequence were a 1.0 s relaxation delay and a polarization transfer delay of 1.786 ms.. The 90° ^{1}H pulse was 6.6 μs while the ^{13}C 90° pulse was 11.6 μs. The data were processed with a sine-bell squared window function shifted by π/2 in both dimensions and linear prediction to 256 data points in F1 followed by zero-filling to 1K.

The pulsed field gradient version of the HMBC pulse sequence was used to acquire the inverse detected ^{1}H - ^{13}C two dimensional chemical shift correlation spectra through two- and three-bond coupling interactions in the absolute value mode. The FID's in the F2 (^{1}H) dimension were recorded over a 3.655 kHz spectral width in 1K data points. The 128 FID's in the F1 (^{13}C) dimension were obtained over a 21.368 kHz spectral width. Each FID was acquired in 2 scans. The fixed delays during the pulse sequence were a 1.0 s relaxation delay, a 3.3 ms delay for the low pass J-filter and 0.08 s delay to allow evolution of the long-range coupling. The 90° ^{1}H pulse was 6.6 μs while the ^{13}C 90° pulse was 11.6 μs. The data were processed with a sine-bell window function in both dimensions and linear prediction to 256 data points in F1 followed by zero-filling to 1K.

The HMQC-TOCSY spectra were acquired in the phase-sensitive mode. The FID's in the F2 (^{1}H) dimension were recorded over a 4.006 kHz spectral width in 1K data points. The 128 FID's in the F1 (^{13}C) dimension were obtained over a 21.368 kHz spectral width. Each FID was acquired in 32 scans. The fixed delays during the pulse sequence were a 1.0 s relaxation delay, a 0.3 s delay between the BIRD pulse and HMQC pulse

sequence and 3.571 ms for polarization transfer. The TOCSY spin lock was 100 ms. The 90° ^{1}H pulse was 6.6 μs while the ^{13}C 90° pulse was 11.6 μs. The ^{1}H spin lock 90° pulse width was 27.0 μs. The data were processed with a sine-bell squared window function shifted by $\pi/2$ in both dimensions and linear prediction to 256 data points in F1 followed by zero-filling to 1K.

Proton-proton NOE difference spectra were obtained by subtraction of a control FID from an on-resonance FID. The decoupler in the control FID irradiated a position in the spectrum where there was no proton signal. The on-resonance FID was obtained while the proton of interest was selectively saturated. In both cases the same decoupler power and duration of saturation (5.0 s) were used. This saturation period also served as the relaxation delay for both the control and on-resonance FIDs. The decoupler was gated off during acquisition. Eight scans were aquired for both the control and on-resonance FIDs was repeated four times for a total of 32 scans for the final difference spectrum. A 90° ^{1}H pulse width of 6.6 μs was used. The FID's were processed with exponential multiplication (line broadening: 4.0 Hz) and were zero filled to 64K during Fourier transformation. The sample was not degassed.

Two dimensional NOESY spectra were acquired in the phase-sensitive mode with use of the pulse sequence: 90°- t_1- 90°-τ- 90°- ACQ. Phase-sensitive data were obtained with time proportional phase incrementation (TPPI). The mixing time τ was 0.8 s. In the F2 dimension 2K data points were used during the acquisition of the 256 FIDs. Each FID was acquired in 32 scans over 4.006 KHz spectral width using a 1.0 s relaxation delay.

Zero filling in the F1 dimension produced a 1K x 1K data matrix after 2-D Fourier transformation of the phase-sensitive data. This resulted in an F2 digital resolution of 3.91 Hz/point. During the 2-D Fourier transform a sine-bell window function shifted by $\pi/2$ was applied to both dimensions. The transformed data were not symmetrized.

Compounds studied by NMR were dissolved in the appropriate deuterated solvents (Isotec, Inc.) to a concentration of approximately 15.0 mg mL^{-1} whenever possible. Chemical shifts are reported in ppm relative to TMS. The residual solvent signals were used as internal references for the ^{1}H and ^{13}C spectra, respectively.

All other NMR spectra were recorded on a Bruker AC-200 spectrometer. Proton spectra were acquired at 200.133 MHz with a 5 mm dual frequency probe. Spectra were obtained in 8 scans in 16K data points over a 2.403 KHz spectral width (3.408 s acquisition time). Spectra were acquired at ambient probe temperature. The free induction decay (FID) was processed with exponential multiplication (line broadening: 0.1 Hz) and was zero-filled to 32K before Fourier transformation.

Carbon-13 NMR spectra were recorded at 50.323 MHz with the 5 mm QNP probe. The spectra were acquired over a 12.195 kHz spectral width in 16K data points (0.672 s acquisition time). The ^{13}C pulse width was 1.5 μs (42° flip angle). A 0.5 s relaxation delay was used. The FIDs were processed with exponential multiplication (line broadening: 3.0 Hz) and zero-filled to 32K before Fourier transformation.

Infrared spectra were recorded on a Bio Rad FTS-40 Fourier transform spectrometer. Solid samples were prepared in Nujol or as KBr pellets in the region of

4000-400 cm^{-1}.

Chemical ionization (CI), with ammonia as the reagent gas and electron impact (EI) mass spectra were recorded on a VG Analytical ZAB-E double focusing mass spectrometer. Typical experimental conditions were: mass resolution 1000, electron energy 70 eV, source temperature 200°C, source pressure 2 x 10^{-6} mbar for EI and 4 x 10^{-5} mbar for CI. Mass spectra were recorded as percent intensity versus mass/charge ratio.

Electrospray ionization mass spectrometry was performed with 50/50 CH_3CN/ H_2O as the mobile phase at a flow arte of 15 μL per minute, with the use of a Brownlee Microgradient syringe pump. Samples were dissolved in 50/50 CH_3CN/ H_2O with an addition of 1 drop of 0.1% ammonium hydroxide for samples to be analysed in the negative mode, or 1 drop of 0.1% TFA for samples that were to be analyzed in the positive mode. Full scan ESMS experiments were performed with a Fisons Platform quadrupole instrument.

X-ray crystallographic data for **5.25a** were collected from a single crystal sample, which was mounted on a glass fiber. Data were collected using a P4 Siemens diffractometer, equipped with a Siemens SMART 1K Charge-Coupled Device (CCD) Area Detector (using the program SMART[1]) and a rotating anode using graphite-monochromated Mo-Kα radiation (λ= 0.71073 Å). The crystal-to-detector distance was 3.991 cm, and the data collection was carried out in 512 x 512 pixel node, utilizing 2 x 2

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[1]SMART (1996), Release 4.05; Siemens Energy and Automation Inc., Madison, WI 53719.

pixel binning. The initial unit cell parameters were determined by a least-squares fit of the angular settings of strong reflections[2], coolected by a 4.5 dgree scan in 15 frames over three different parts of reciprocal space (45 frames total). One complete hemisphere of data was collected, to better tha 0.8Å resolution. Upon completion of the data collection, the first 50 frames were recollected in order to improve the decay corrections analysis (if required). Processing was carried out by the use of the program SAINT[3], which applied Lorentz and polarization corrections to three-dimensionally integrated diffraction spots. The program SADABS[4] was utilized for the scaling of diffraction data, the application of a decay correction, and an emprical absorption correction based on redundant reflections. The structure was solved using the direct methods procedure in the Siemens SHELXTL program library[5], and refined by full-matrix least squares methods with anisotropic thermal parameters for all non-hydrogen atoms.

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[2]To determine the number of reflections, consult the .p4p file associated with the data set.

[3]SAINT (1996), Release 4.05; Siemens Energy and Automation Inc., Madison, WI 53719.

[4]Sheldrick, G.M. SADABS (Siemens Area Detector Absorption Correction) (1996).

[5]Sheldrick, G.M. Siemens SHELXTL (1994), Version 5.03; Siemens Crystallographic Research, Madison, WI 53719.

Appendix II

Table 1. Crystal data and structure refinement for **5.25**

Identification code	jv97
Empirical formula	C26 H30 N8 O12 Re2 S4
Formula weight	1147.22
Temperature	300(2) K
Wavelength	0.71073 A
Crystal system	Monoclinic
Space group	P2(1)
Unit cell dimensions	a = 10.9805(3) A alpha = 90 deg. b = 8.1596(2) A beta = 106.0710(10) deg. c = 11.1007(3) A gamma = 90 deg.
Volume, Z	955.71(4) A^3, 1
Density (calculated)	1.993 Mg/m^3
Absorption coefficient	6.612 mm^-1
F(000)	552
Crystal size	.07 x .3 x .7 mm
Theta range for data collection	1.91 to 26.34 deg.
Limiting indices	-13<=h<=13, -5<=k<=10, -13<=l<=13
Reflections collected	7724
Independent reflections	3278 [R(int) = 0.0223]
Absorption correction	None

Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3278 / 1 / 225
Goodness-of-fit on F^2	0.756
Final R indices [I>2sigma(I)]	R1 = 0.0292, wR2 = 0.0781
R indices (all data)	R1 = 0.0300, wR2 = 0.0801
Absolute structure parameter	-0.002(10)
Largest diff. peak and hole	1.275 and -1.766 e.A^-3

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3) for **5.25**. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
Re(1)	-4224(1)	-2456(1)	-2212(1)	25(1)
S(1)	-5066(2)	-959(2)	-868(2)	40(1)
S(2)	-5439(2)	-1011(3)	-3887(2)	43(1)
O(2)	-827(4)	-2274(11)	-3131(5)	40(1)
C(6)	-1413(4)	-2330(16)	-1175(4)	27(1)
N(4)	-2948(5)	-2265(10)	-3207(4)	33(1)
C(8)	-1694(5)	-2286(10)	-2603(5)	25(1)
N(1)	-2606(4)	-2096(6)	-862(4)	26(1)
O(5)	-4737(5)	-4404(7)	-2242(4)	40(1)
O(1)	-1537(5)	-1203(8)	1092(5)	45(1)
C(5)	-1464(5)	-5406(9)	-1043(5)	29(1)
C(4)	-708(5)	-3894(9)	-626(6)	33(1)
C(9)	-3254(6)	-1920(8)	-4568(6)	32(1)
C(6)	-1675(8)	-6286(11)	-2109(7)	44(2)
C(1)	-3785(7)	-1234(12)	580(7)	42(2)
C(2)	-2527(6)	-1492(9)	313(5)	31(1)
C(12)	-4704(8)	-1712(11)	-5090(7)	41(2)
N(2)	-2525(5)	-7510(12)	-2067(5)	49(1)
C(7)	-2837(6)	-7385(14)	-1010(6)	44(1)
O(3)	-2439(5)	858(7)	-4185(4)	44(1)
O(4)	-2303(5)	-505(7)	-5894(4)	42(1)
N(3)	-2213(5)	-6127(8)	-374(5)	38(1)
C(10)	-2609(6)	-359(9)	-4832(5)	34(1)
C(11)	-1737(9)	931(12)	-6322(7)	51(2)
C(13)	-1515(9)	-4422(14)	3088(9)	66(2)
O(6)	-1375(6)	-5551(8)	2165(5)	55(1)

Table 3. Bond lengths [A] and angles [deg] for **5.25**.

Re(1)-O(5)	1.684(5)
Re(1)-N(1)	2.003(5)
Re(1)-N(4)	2.015(5)
Re(1)-S(2)	2.292(2)
Re(1)-S(1)	2.310(2)
S(1)-C(1)	1.833(8)
S(2)-C(12)	1.833(8)
O(2)-C(8)	1.248(6)
C(6)-N(1)	1.458(6)
C(6)-C(8)	1.529(6)
C(6)-C(4)	1.530(13)
N(4)-C(8)	1.355(7)
N(4)-C(9)	1.481(7)
N(1)-C(2)	1.374(7)
O(1)-C(2)	1.211(8)
C(5)-C(6)	1.349(9)
C(5)-N(3)	1.382(8)
C(5)-C(4)	1.487(10)
C(9)-C(10)	1.525(9)
C(9)-C(12)	1.546(10)
C(6)-N(2)	1.377(12)
C(1)-C(2)	1.505(9)
N(2)-C(7)	1.314(9)
C(7)-N(3)	1.324(11)
O(3)-C(10)	1.209(8)
O(4)-C(10)	1.318(7)
O(4)-C(11)	1.466(9)
C(13)-O(6)	1.418(12)
O(5)-Re(1)-N(1)	112.1(2)
O(5)-Re(1)-N(4)	110.0(3)
N(1)-Re(1)-N(4)	78.0(2)
O(5)-Re(1)-S(2)	110.7(2)
N(1)-Re(1)-S(2)	136.7(2)
N(4)-Re(1)-S(2)	81.5(2)
O(5)-Re(1)-S(1)	108.7(2)
N(1)-Re(1)-S(1)	82.53(14)
N(4)-Re(1)-S(1)	140.9(2)
S(2)-Re(1)-S(1)	90.25(7)
C(1)-S(1)-Re(1)	98.9(2)

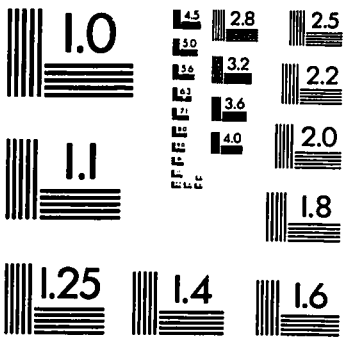
C(12)-S(2)-Re(1)	98.9(3)
N(1)-C(6)-C(8)	107.9(4)
N(1)-C(6)-C(4)	113.8(7)
C(8)-C(6)-C(4)	111.5(7)
C(8)-N(4)-C(9)	114.8(4)
C(8)-N(4)-Re(1)	119.6(3)
C(9)-N(4)-Re(1)	125.3(4)
O(2)-C(8)-N(4)	124.8(5)
O(2)-C(8)-C(6)	121.7(5)
N(4)-C(8)-C(6)	113.5(4)
C(2)-N(1)-C(6)	116.6(5)
C(2)-N(1)-Re(1)	124.9(4)
C(6)-N(1)-Re(1)	118.1(3)
C(6)-C(5)-N(3)	105.2(6)
C(6)-C(5)-C(4)	131.3(6)
N(3)-C(5)-C(4)	123.3(5)
C(5)-C(4)-C(6)	113.0(5)
N(4)-C(9)-C(10)	111.6(5)
N(4)-C(9)-C(12)	108.8(5)
C(10)-C(9)-C(12)	108.8(6)
C(5)-C(6)-N(2)	108.0(6)
C(2)-C(1)-S(1)	111.6(5)
O(1)-C(2)-N(1)	123.8(6)
O(1)-C(2)-C(1)	121.6(6)
N(1)-C(2)-C(1)	114.6(5)
C(9)-C(12)-S(2)	112.1(5)
C(7)-N(2)-C(6)	108.7(7)
N(2)-C(7)-N(3)	108.2(7)
C(10)-O(4)-C(11)	117.0(6)
C(7)-N(3)-C(5)	109.9(5)
O(3)-C(10)-O(4)	124.7(6)
O(3)-C(10)-C(9)	125.1(6)
O(4)-C(10)-C(9)	110.1(6)

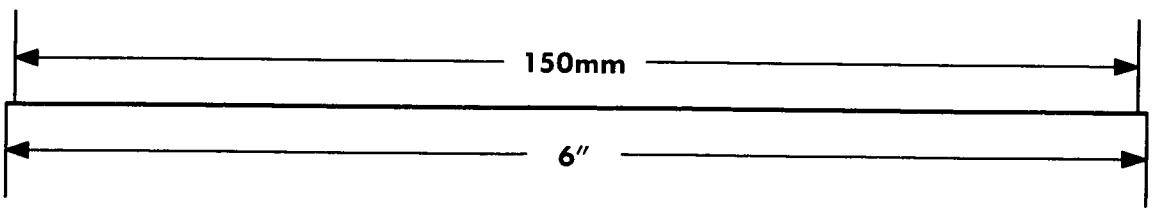
Table 4. Anisotropic displacement parameters (A^2 x 10^3) for **5.25**.
The anisotropic displacement factor exponent takes the form:
-2 pi^2 [h^2 a*^2 U11 + ... + 2 h k a* b* U12]

	U11	U22	U33	U23	U13	U12
Re(1)	22(1)	26(1)	29(1)	0(1)	9(1)	-3(1)
S(1)	31(1)	47(1)	47(1)	-6(1)	19(1)	8(1)
S(2)	32(1)	50(1)	45(1)	11(1)	8(1)	6(1)
O(2)	29(2)	53(4)	41(2)	-4(4)	17(1)	4(3)
C(6)	23(2)	30(3)	29(2)	-2(4)	9(2)	-3(4)
N(4)	36(2)	37(3)	27(2)	6(3)	10(2)	1(3)
C(8)	30(2)	18(3)	33(2)	-1(3)	17(2)	-2(3)
N(1)	27(2)	24(4)	31(2)	-3(2)	12(2)	1(2)
O(5)	37(3)	42(3)	41(2)	-2(2)	12(2)	-12(2)
O(1)	40(2)	58(4)	38(2)	-15(2)	11(2)	-12(3)
C(5)	27(3)	30(4)	31(3)	0(2)	8(2)	6(2)
C(4)	23(3)	37(4)	36(3)	1(3)	5(2)	7(3)
C(9)	39(3)	27(3)	31(3)	-3(2)	11(2)	-2(2)
C(6)	59(4)	37(4)	39(3)	-3(3)	21(3)	3(4)
C(1)	38(4)	56(5)	40(3)	-5(3)	23(3)	1(4)
C(2)	33(3)	35(4)	27(3)	-4(2)	14(2)	-4(3)
C(12)	41(4)	45(4)	34(3)	8(3)	3(3)	-4(3)
N(2)	54(3)	40(4)	45(2)	-14(4)	4(2)	3(5)
C(7)	49(3)	30(3)	56(3)	3(5)	17(2)	-8(5)
O(3)	66(3)	32(3)	38(2)	-7(2)	21(2)	-10(2)
O(4)	64(3)	37(3)	33(2)	-2(2)	26(2)	-13(2)
N(3)	49(3)	34(3)	33(2)	1(2)	15(2)	-2(3)
C(10)	38(3)	34(4)	32(3)	2(3)	12(2)	-2(3)
C(11)	63(5)	51(5)	48(4)	8(4)	33(4)	-9(4)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (A^2 x 10^3) for **5.25**.

	x	y	z	U(eq)
H(6A)	-864(4)	-1396(16)	-837(4)	32
H(7A)	72(5)	-3963(9)	-871(6)	39
H(7B)	-485(5)	-3832(9)	282(6)	39
H(16A)	-2977(6)	-2844(8)	-4990(6)	39
H(9A)	-1309(8)	-6099(11)	-2759(7)	52
H(2A)	-3736(7)	-274(12)	1106(7)	51
H(2B)	-3973(7)	-2172(12)	1034(7)	51
H(21A)	-5077(8)	-2752(11)	-5425(7)	50
H(21B)	-4882(8)	-926(11)	-5772(7)	50
H(10A)	-2805(5)	-8237(12)	-2637(5)	58
H(11A)	-3399(6)	-8063(14)	-753(6)	53
H(12A)	-2267(5)	-5805(8)	347(5)	45
H(20A)	-1556(9)	680(12)	-7100(7)	76
H(20B)	-965(9)	1218(12)	-5704(7)	76
H(20C)	-2318(9)	1834(12)	-6443(7)	76





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