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Enantioselective Receptors for Anionic Species

by

Fionn Christopher Hurley

A Thesis presented to the University of Dublin for the Degree of Doctor of Philosophy.

Department of Chemistry,
University of Dublin,
Trinity College.

October 2000

Declaration

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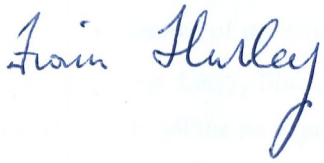

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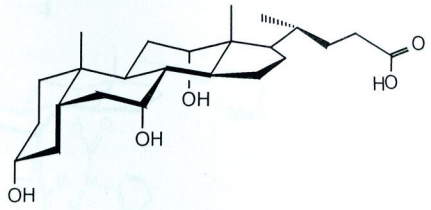
Thanks for the last two years Jules. Hang in there, you're nearly finished!

Last, but not least, a big thanks to Oisín and Mairéad for their kind hospitality and use of computer facilities when writing this thesis. It is unlikely that this thesis would ever have been completed without them.

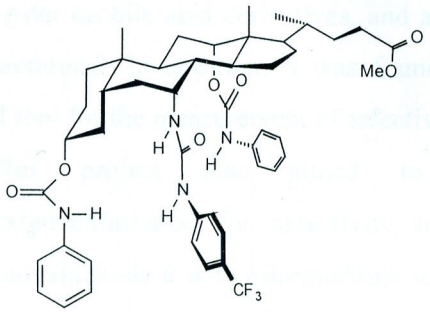
Summary

This project aimed to design, synthesise and test neutral, tripodal steroid receptors based on cholic acid **1** that were capable of binding to carboxylates (especially *N*-acyl-α-aminoacid carboxylates), and differentiating between their enantiomers.



1

It was envisioned that this binding would principally be through multiple hydrogen-bonding interactions between the steroid receptor and the guest molecule, with a urea moiety on the steroid providing the main binding site for the guest carboxylate. Thus, receptor **2** was synthesised in 7 steps from cholic acid, and in an overall yield of *ca.* 23%.



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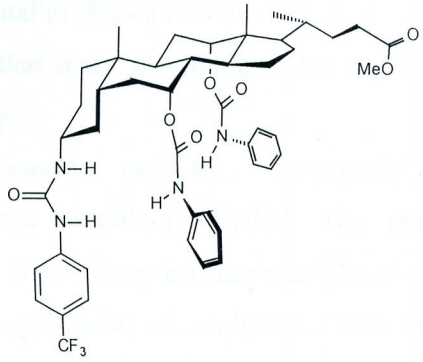


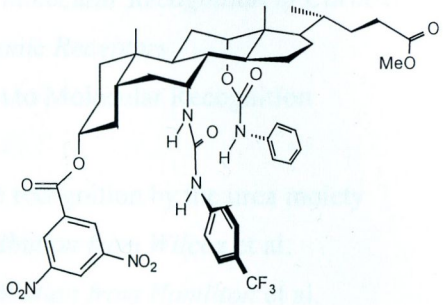
3

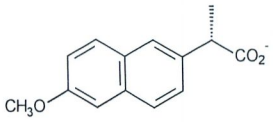
The key step in this synthesis was the reductive amination of the 7 ketone to give the amino group as the 7α-epimer only. This allowed for the synthesis of a 7α-NHBOC derivative in five synthetic steps from **1** (overall yield of *ca.* 47 %) which represents a significant improvement on the literature yield (*ca.* 34 % from methyl cholate). This improved synthesis was carried out on a large laboratory scale (starting from 52 g) without the need for chromatography.

2 was tested for selectivity in extractions of tetrabutylammonium *N*-acetyl-phenylalaninate from an aqueous phase into chloroform. The analyses of these extractions were carried out using ^{1}H nmr and chiral HPLC. Chiral HPLC proved to be the better technique for this analysis. By modifying the conditions, it was possible to obtain good selectivity (84 % e.e. in favour of the L-enantiomer) using **2** in these extractions. **3**, which had already been synthesised before in our laboratory, was resynthesised, and tested under the same conditions as **2**. **3** also gave good selectivity (74 % e.e. in favour of the L-enantiomer) in these extractions. Receptor **4** was synthesised in 10 steps from **1** (overall

yield *ca.* 7 %) for the purposes of recognising Naproxenate **5**. However, preliminary extractions of racemic Naproxenate from an aqueous phase into chloroform using **4**, generated only a small selectivity (6.6 % in favour of the *S*-enantiomer).

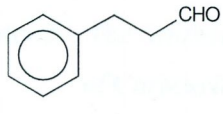
4 **5**

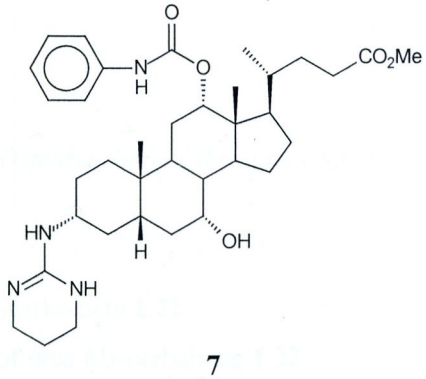


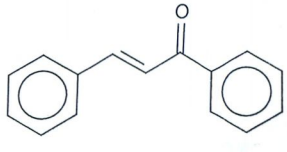


As part of the foregoing work, synthetic investigations were carried out on the formation of steroid carbamates, a procedure was developed for the synthesis of a sterically hindered ester on bile acid derivatives, and a total of fifteen new steroids were synthesised and characterised. In addition, it was found that mass spectrometry can be used as an analytical tool for the measurement of selectivity.

This project also aimed to screen molecules (especially steroid guanidines/guanidiniums) for selectivity in the nitroaldol reaction. The reaction of hydrocinnamaldehyde **6** with nitromethane was chosen as a test reaction. Eleven molecules were screened for selectivity in this reaction using a variety of conditions. Chiral HPLC was used to analyse the selectivity generated. The results of this screening show that of those molecules studied, only a guanidine or a combination of guanidinium and base generates any selectivity in this reaction. Guanidine **7** gave the highest selectivity (26 % e.e.).







6 **7** **8**

7 also gave a similar selectivity (26 % e.e.) in the conjugate addition of nitromethane to chalcone **8**. For selectivity purposes, it was found to be important to use THF as the solvent in all of these reactions, and to use only a slight excess of nitromethane to **6** or **8**. **7** is now being used as a lead compound in the combinatorial development of new molecular catalysts.

Contents

Abbreviations

ACE	Acetyl Choline Esterase
BINOL	Binaphthol
BOC	*Tert*-butyloxycarbonyl
$(BOC)_2O$	Di-*tert*-butyldicarbonate
Cbz	Benzyloxycarbonyl
CDI	1,1'-Carbonyldiimidazole
DIPEA	Diisopropylethylamine
DCC	1,3-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DMAP	4-(Dimethylamino)pyridine
DMF	*N*, *N*-dimethylformamide
DMPU	1,3-Dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone
DMSO	Dimethylsulfoxide
ES-MS	Electrospray Mass Spectrometry
HMPA	Hexamethylphosphoramide
HPLC	High performance liquid chromatography
MOM	Methoxymethyl
NBA	*N*-bromoacetamide
NBS	*N*-bromosuccinimide
nOe	nuclear Overhauser effect
nmr	nuclear magnetic resonance
PTFE	Polytetrafluoroethylene
r.t.	room temperature
TBA	Tetrabutylammonium
TBD	1,5,7-Triazabicyclo[4.4.0]dec-5-ene
TBDMSCl	*Tert*-butyldimethylsilylchloride
TEA	Triethylamine
THF	Tetrahydrofuran
tlc	thin layer chromatography
TMG	1,1,3,3-Tetramethylguanidine
TMSCl	Trimethylsilylchloride (Chlorotrimethylsilane)
Ts	Tosyl (*para*-toluenesulfonyl)
UV	Ultraviolet

Chapter 1

The Molecular Recognition of Carboxylates by Electrically Neutral Organic Receptors

1.1 Introduction to Molecular Recognition

Molecular recognition is the branch of chemistry concerned with the non-covalent interactions between molecules. Molecular receptors, the molecules engaged in molecular recognition, are "able to bind selectively ionic or molecular substrates (or both) by means of various intermolecular interactions, leading to an assembly of two or more species, a supermolecule".[1.1] The ability of a molecular receptor (host) to specifically recognise a certain substrate (guest) over other substrates is based in thermodynamics - a larger difference in binding free energy results in higher recognition. In order for binding between host and guest to occur, several factors are important. There must be steric and interactional complementarity between the receptor and substrate, that is, the receptor and substrate must be able to fit together and possess complementary binding sites. Multiple interaction sites between the host molecule and the guest are necessary because of the weakness of non-covalent interactions compared to covalent bonds. Finally, strong interaction between the receptor and guest are necessary. Efficient molecular recognition requires the combination of all these factors.[1.1]

Much of the work already done in the field of molecular recognition has focused on the elucidation of complementary binding motifs, and the measurement and comparison of their strength. In the present day, the challenge for molecular recognition focuses on the incorporation of known specific binding sites into more complex artificial receptors, and the application of these receptors to molecular transport, resolution of racemates, asymmetric catalysis, biomimesis, and nanoscale technology.

1.2 Preamble

Although the chemical literature contains many examples of receptors that bind to cations, the design and synthesis of receptors that bind to anions is an area which has not been fully explored. Within this particular field, the recognition of carboxylates has generated much interest because of the relatively recent discovery of specific moieties (e.g. guanidinium, (thio)urea, 2-amidopyridine) which bind to this functionality, and because of the ubiquity of the carboxylate group in chemical and biological systems.

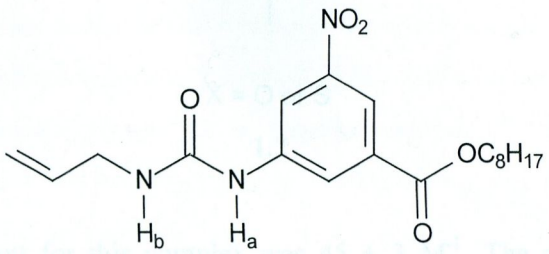
Of the various carboxylate-binding moieties, ureas are particularly useful because of their ease of synthesis and handling. Their electroneutrality can also be advantageous, especially in "phase-transfer" applications where it is important that the receptors should locate in an organic phase. The main aim of this project was to explore the potential of a number of functionalised steroidal ureas as enantioselective receptors for carboxylates, specifically for anions derived from *N*-acyl-α-aminoacids.

This chapter outlines the important contributions from the chemical literature that demonstrate the ability of the urea group to form complementary hydrogen bonds to carboxylates. This is followed by some examples of the application of uncharged organic molecules in the recognition of *N*-protected aminoacid carboxylates. Following this, a more detailed description of the aims of the project is given.

1.3 Carboxylate recognition by the urea moiety

1.3.1 Contribution from Wilcox et al.

Wilcox *et al.* found that the urea **1.1** was an excellent host for forming ion pairs with carboxylates in chloroform.[1,2] Addition of one equivalent of tetrabutylammonium benzoate ($TBA^+PhCO_2^-$) to a 2.0 mM solution of **1.1** in $CDCl_3$ resulted in large downfield shifts of the NH resonances of the urea in the ^1H nmr spectrum [$\Delta\delta(NH)_a$ 4.99 ppm, $\Delta\delta(NH)_b$ 3.80 ppm].

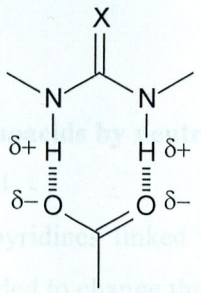


1.1

The authors say that this implies that a hydrogen-bonded complex has been formed. The authors measured the strength of this association using UV/vis spectrophotometry: titration of a solution of **1.1** (100 μM) in chloroform with increasing concentrations of $TBA^+PhCO_2^-$ (25-500 μM) gave a series of absorption spectra. By observing the extinction coefficients at a fixed wavelength (361 nm) as a function of $TBA^+PhCO_2^-$ concentration, the authors were able to calculate the association constant (K_a) as $2.7 \pm 0.8 \times 10^4$ M^{-1}. The authors conclude by suggesting that the control of these types of interactions can be used to increase the selectivity of chemical transformations.

1.3.2 Contribution from Hamilton et al.

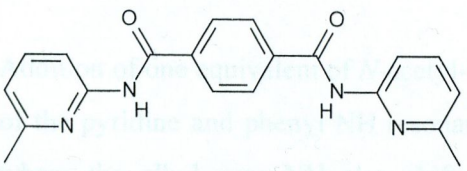
Hamilton *et al.* investigated the possibility of using hydrogen-bonding receptors that function in solvents which compete with the guest for H-donor sites.[1.3] To test the hypothesis that urea groups could form hydrogen bonds in competitive solvents, the authors added tetramethylammonium acetate ($TMA^+CH_3CO_2^-$) to a 10 mM solution of 1,3-dimethylurea in DMSO-d_6. This gave downfield shifts of the urea NH resonances (> 1 ppm) in the ^1H nmr spectrum, which the authors suggest is consistent with the formation of the bidentate hydrogen-bonded complex **1.2**.



X = O or S

1.2

The association constant for this complex was 45 ± 3 M^{-1}. The corresponding thiourea derivative gave an association constant of 3.4 ± 0.7 x 10^2 M^{-1}. The authors attribute the increase stability of this complex to the more acidic nature of the protons on the thiourea. Based on their work with *bis*(2-amidopyridine) **1.3** which formed a complex with glutaric acid in 5% THF/$CDCl_3$ (K_a = 6.4 ± 1.4 x 10^2 M^{-1}), and the preliminary results described above, the authors decided to modify **1.3** to incorporate two urea groups, and to test this molecule, **1.4**, for the ability to bind glutarate in DMSO.[1.4]



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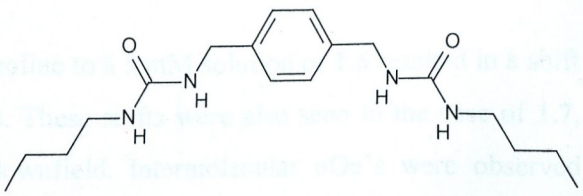
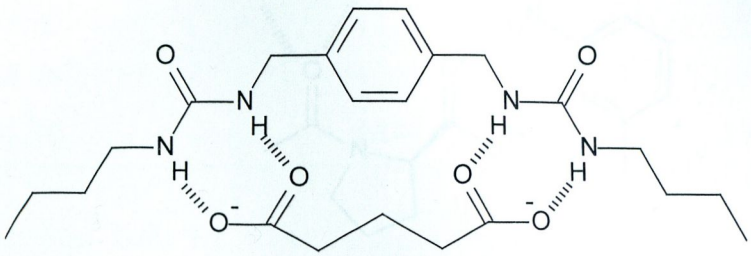


1.4

Thus they found that unlike **1.3**, **1.4** bound to the *bis*-tetrabutylammonium salt of glutaric acid in DMSO-d_6 with a K_a of 6.4 ± 0.4 x 10^2 M^{-1}. They proposed that the complex **1.5** was formed between these two species based chiefly on the downfield shifts of the urea NH

resonances (> 1 ppm) in the ^{1}H nmr spectrum and intramolecular nOe's between the receptor aryl and substrate CH_2 resonances.

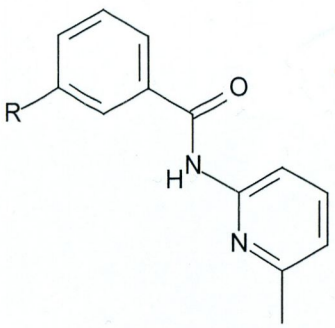


1.5

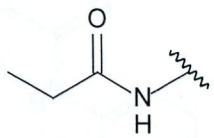
1.4 Recognition of *N*-protected aminoacids by neutral organic receptors

1.4.1 Contribution from Hamilton et al.

Having shown that 2-amidopyridines linked to an isophthalic spacer can bind to carboxylic acids, Hamilton *et al.* decided to change the spacer unit to a 3-aminobenzoic acid unit, which upon derivatisation, would provide a cavity that could form hydrogen bonds to both the acid and amide groups of a terminal peptide.[1.5] Thus they synthesised amidopyridines **1.6** and **1.7** containing an amide and a urea group respectively. They also synthesised **1.8** to function as a control.

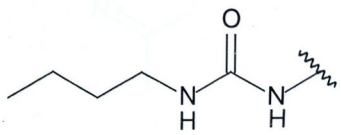


1.6: R =

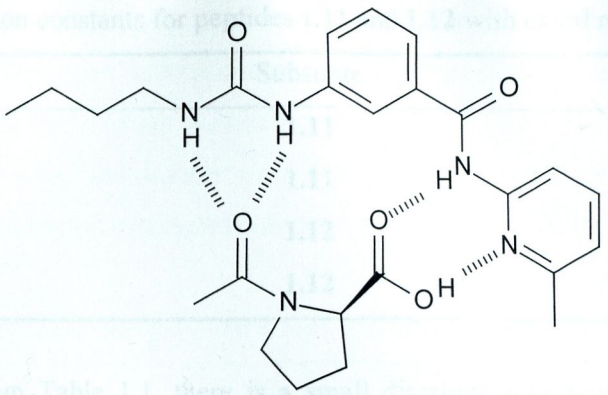


1.8: R = H

1.7: R =

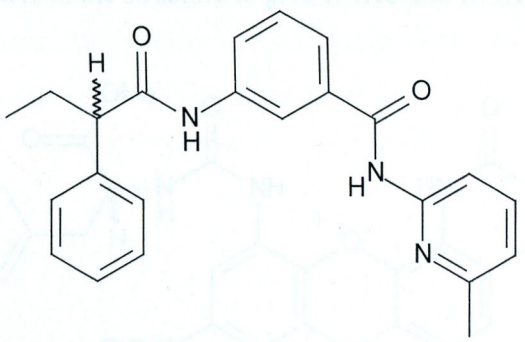


Addition of one equivalent of *N*-acetyl-L-proline to a 5 mM solution of **1.6** resulted in a shift of the pyridine and phenyl NH resonances. These shifts were also seen in the case of **1.7**, where the alkyl urea NH also shifted downfield. Intermolecular nOe's were observed between the aminoacid α-H and both the pyridine methyl group and the phenyl-2H, suggesting that this substrate formed complex **1.9** with this type of receptor.



1.9

Nmr titrations of receptors **1.8**, **1.6**, and **1.7** with *N*-acetyl-L-proline gave association constants of 50, 410 and 2600 M^{-1} respectively, showing that K_a increases with increasing numbers of hydrogen bonds. In the case of the complex formed between **1.6** and *N*-butyryl-D-valine, the acyl group of the aminoacid derivative appeared close to the alkyl chain on the receptor. Using this information, Hamilton and co-workers synthesised the chiral receptors (*R*)-**1.10** and (*S*)-**1.10**, and measured their association constants with the peptide **1.11**, and the ACE inhibitor captopril **1.12**. The results are shown in Table 1.1.



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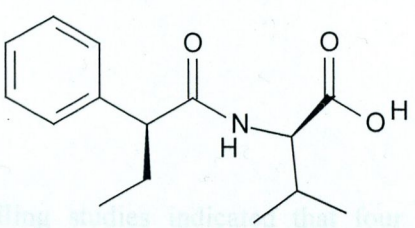

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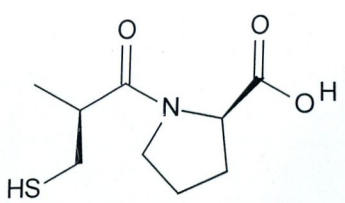


1.12

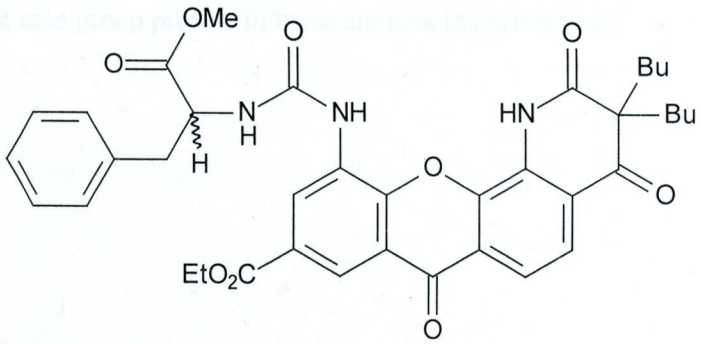
Table 1.1. Association constants for peptides **1.11** and **1.12** with chiral receptor **1.10**.

Receptor	Substrate	K_a (M^{-1})
(*R*)-**1.10**	**1.11**	680
(*S*)-**1.10**	**1.11**	350
(*R*)-**1.10**	**1.12**	500
(*S*)-**1.10**	**1.12**	210

As seen from Table 1.1, there is a small discrimination between the association constants obtained using (*R*)-**1.10** and (*S*)-**1.10**, with the (*R*)-enantiomer preferred by both substrates by *ca.* 2:1. In both substrates, resonances due to the two diasteromeric complexes can in seen when racemic **1.10** is added to the optically pure peptide. The authors summarise their work by saying that simple synthetic receptors can form strong complexes with acylaminoacid carboxylates and that the contribution from individual hydrogen bonds in these complexes can be readily assessed.

1.4.2 Contribution from Morán et al.

Morán *et al.* prepared compound **1.13** for the purposes of recognising *N*-Cbz-aminoacid derivatives.[1,6] Chirality was introduced by incorporating L-phenylalanine or D-phenylalanine methyl esters in the structure to give L-**1.13** and D-**1.13** respectively.



1.13

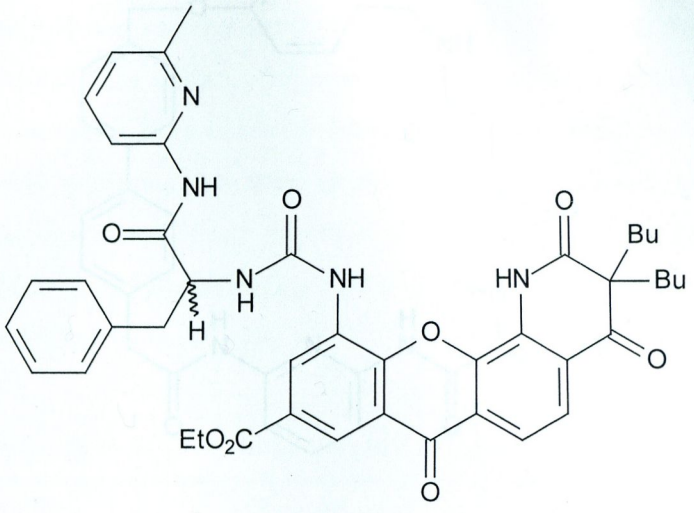
Modelling studies indicated that four hydrogen bonds should be formed between the receptor and substrate in these cases. Nmr titrations carried out in $CDCl_3$ between **1.13** and *N*-Cbz-glycine, *N*-Cbz-L-alanine and *N*-Cbz-L-phenylalanine showed that the glycine derivative had the highest association constant, presumably due to steric interference between **1.13** and the sidechains of the other guest compounds. The complexes formed between L-**1.13** and the two chiral aminoacid derivatives showed double the stability of

those formed using D-**1.13**, but the titration curves did not fit the experimental points very well. Further experimentation showed that the receptor was partly dimerised in chloroform, and that the dimerisation constant was of the same order of magnitude as the association constants with the guests. The authors used this data to generate a new set of curves, and from these, a set of corrected associated constants was calculated (Table 1.2).

Table 1.2. Association constants between receptor **1.13** and *N*-Cbz-(L)-aminoacids

Substrate	K_a with (L)-**1.13** (M^{-1})	K_a with (D)-**1.13** (M^{-1})
N-Cbz-L-alanine	8.7×10^3	5.8×10^3
N-Cbz-L-phenylalanine	5.9×10^3	4.4×10^3
N-Cbz-L-serine	1.5×10^4	5.0×10^3

Further modelling studies indicated that *N*-Cbz-L-serine could form an additional hydrogen bond from its hydroxyl group to the methyl ester carbonyl of the host. The chiral recognition in this case was higher than that of the other aminoacid derivatives studied (Table 1.2). *N*-Cbz-L-aspartic acid and *N*-Cbz-L-glutamic acid were also studied. In these cases, complexes formed with receptor **1.13** proved difficult to quantify due to low association, and titration curves which did not correspond to 1:1 binding. In an attempt to overcome these problems, an amidopyridine unit was incorporated into the receptor to form hydrogen bonds to the second carboxylic acid group present in these aminoacid derivatives.

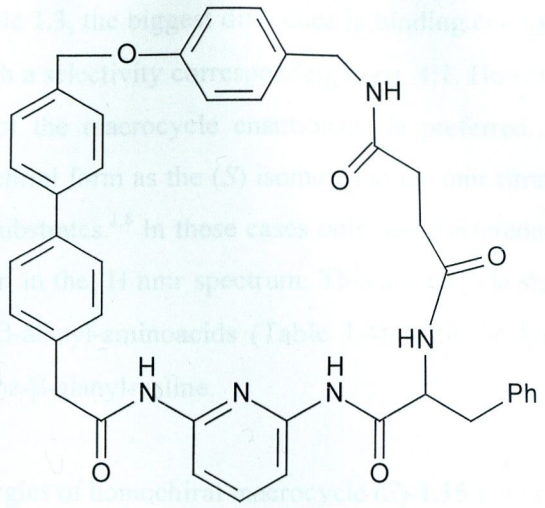


1.14

Nmr titrations showed that the association constant between the new receptor, **1.14**, and *N*-Cbz-L-aspartic acid were higher than with receptor **1.13**, but signal broadening meant that accurate values of K_a could not be reported. The authors suggest that values of between 6 x 10^4 M^{-1} and 2 x 10^{-5} M^{-1} are reasonable in these cases.

1.4.3 Contribution from Kilburn et al.

Kilburn *et al.* developed a series of neutral macrocyclic receptors to recognise and bind to the carboxylic acid or carboxylate terminus of *N*-protected aminoacids and peptides.[1.7-1.11] The macrocycle **1.15** was obtained in racemic form, which the authors believe occurred during the formation of one of the amide bonds to the 2-aminopyridine.[1.7]



1.15

Addition of homochiral peptide substrates to **1.15** gave two distinctive diastereomeric signals in the ^{1}H nmr spectrum in $CDCl_3$, but these were well separated, and could be followed in titration experiments. The binding energies between **1.15** and substrates were calculated after titration experiments were carried out (Table 1.3).

Table 1.3. Binding energies for **1.15** and substrates in $CDCl_3$

Substrate	$-\Delta G_{assoc}$ (kJ mol^{-1})
Phenylacetic acid	11.5
N-Cbz-glycine	14.4
N-Cbz-β-alanine	12.9
N-Cbz-L-alanine	15.8/15.2
N-Cbz-D-alanine	15.6/15.2
N-Cbz-L-phenylalanine	14.2/13.7
N-Boc-L-alanine	14.2/13.7
N-Cbz-L-alanyl-L-alanine	16.0/14.6
N-Cbz-β-alanyl-L-alanine	19.4/16.2

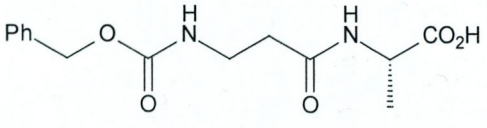
The two values quoted refer to the two diastereomeric complexes formed

As seen in Table 1.3, the biggest difference in binding energy occurred with *N*-Cbz-β-alanyl-L-alanine, with a selectivity corresponding to *ca.* 4:1. However, it is not possible to tell from this which of the macrocycle enantiomers is preferred. Macrocycle **1.15** was resynthesised in homochiral form as the (*S*) isomer, and the nmr titrations were extended to include other peptide substrates.[1.8] In these cases only one diastereomeric complex between host and guest was seen in the ^{1}H nmr spectrum. This macrocycle showed selectivity in the recognition of *N*-Cbz-β-alanyl-aminoacids (Table 1.4), with the highest selectivity (68% e.e.) occurring for *N*-Cbz-β-alanyl-valine.

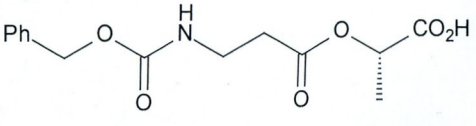
Table 1.4. Binding energies of homochiral macrocycle (*S*)-**1.15** with peptide substrates.

Substrate	$-\Delta G_{assoc}$ (kJ mol^{-1})	Substrate	$-\Delta G_{assoc}$ (kJ mol^{-1})
N-Cbz-L-alanyl-L-alanine	18.4 ± 0.7	*N*-Cbz-β-alanyl-D-phenylalanine	14.0 ± 0.5
N-Cbz-D-alanyl-D-alanine	16.5 ± 0.3	*N*-Cbz-β-alanyl-L-valine	16.8 ± 0.6
N-Cbz-β-alanyl-L-alanine	19.9 ± 0.7	*N*-Cbz-β-alanyl-D-valine	12.7 ± 0.2
N-Cbz-β-alanyl-D-alanine	16.6 ± 0.7	*N*-Cbz-β-alanyl-L-lactate	13.1 ± 0.3
N-Cbz-β-alanyl-L-phenylalanine	16.2 ± 0.4	*N*-Cbz-β-alanyl-D-lactate	11.2 ± 0.2

Interestingly, replacement of the amide NH in *N*-Cbz-β-alanyl-L-alanine with that of an oxygen atom (*N*-Cbz-β-alanyl-L-lactate - see below), resulted in substantially lower binding. This suggests that this NH participates in a key hydrogen bonding interaction with this receptor.

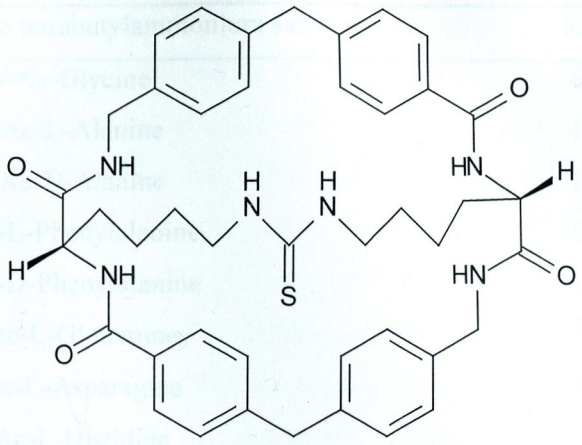


N-Cbz-β-alanyl-L-alanine



N-Cbz-β-alanyl-L-lactate

Kilburn and co-workers also synthesised macrocycle **1.16** which incorporated a thiourea group for carboxylate recognition at the base of the cavity, two rigid spacer groups at the rim of the cavity, and amide groups at the rim of the cavity to provide additional hydrogen bonding sites for the substrate.[1.9]



1.16

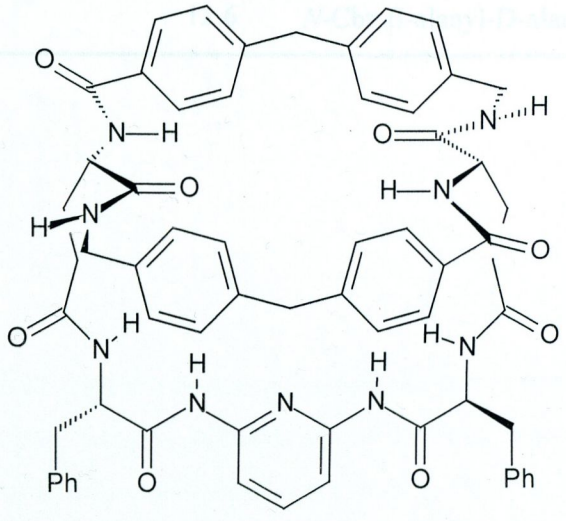
Initially, binding studies were carried out using macrocycle **1.16** and tetrabutylammonium *N*-acetyl-aminoacid carboxylates in $CDCl_3$. However, the authors were unable to determine the association of host and guest using this method because the NH resonances followed in the titration experiments shifted into the aromatic region of the spectrum. Instead, Kilburn *et al.* determined the binding constants using a single extraction method: the tetrabutylammonium salts were partitioned between water and chloroform in the presence of **1.16** and in the absence of **1.16**. Aliquots from each layer were taken, and the quantity of salt present in each aliquot was determined by ^1H nmr relative to a known quantity of dioxane. The partition and extraction coefficients were then determined, and from these values the association constants calculated (Table 1.5). Each extraction was carried out five times to obtain consistent results.

Table 1.5. Association constants for receptor **1.16** and *N*-acetyl-aminoacid carboxylates

Substrate (as the tetrabutylammonium salt)	K_a/mol^{-1}
N-Ac-Glycine	68,600
N-Ac-L-Alanine	16,900
N-Ac-D-Alanine	14,600
N-Ac-L-Phenylalanine	22,000
N-Ac-D-Phenylalanine	13,300
N-Ac-L-Glutamine	11,100
N-Ac-L-Asparagine	9,600
N-Ac-L-Histidine	5,800
N^{α}-Ac-L-Lysine	130,000

Although receptor **1.16** binds *N*-acetyl-aminoacid carboxylates, the selectivity between enantiomers is not high. Evidence from the ^1H nmr spectrum suggested that the enantiomers of *N*-Ac-alanine and *N*-Ac-phenylalanine were bound in two different environments. Subsequently, it was discovered that the L-enantiomers were bound inside the cavity of **1.16** with the acetyl amide bond adopting a *cis* conformation, while the D-enantiomers were bound to the outside of the cavity.[1.10] In each case, the predominant interaction was that of carboxylate with thiourea.

Based on their work with **1.16**, Kilburn *et al.* synthesised the larger C_2-symmetric macrocycle **1.17**, which incorporates the rigid spacer units found in **1.16**, but features the amidopyridine unit as a binding site for carboxylic acids.[1.11]



1.17

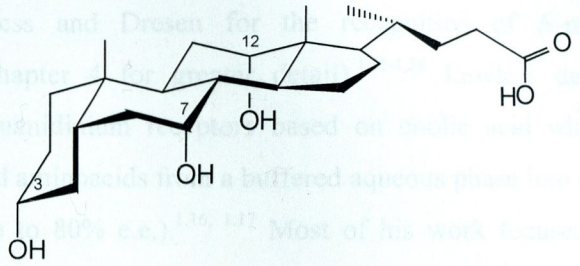
In this case, the macrocycle was obtained optically pure. Nmr studies showed that the pyridine unit faced into the cavity, unlike another similar receptor.[1.12] Binding studies were carried out in $CDCl_3$ between **1.17** and various *N*-protected aminoacids (Table 1.6). Some selectivity was observed for the D-enantiomers of alanine and phenylalanine, but the opposite selectivity was seen for the valine and serine substrates. In the case of D-phenylalanine substrates, decreasing the size of the protecting group resulted in stronger binding. Of the dipeptide substrates studied, only those of the β-alanyl series showed stronger binding than the single aminoacid residues, with the D-enantiomer preferred over the L. To conclude, the authors say that the binding is dominated by the carboxylic acid-amidopyridine interaction, although the selectivities observed must arise from other binding interactions.

Table 1.6. Binding energies of *N*-protected aminoacids and peptides with **1.17**.

Substrate	$-\Delta G_{assoc}$ (kJ mol^{-1})	Substrate	$-\Delta G_{assoc}$ (kJ mol^{-1})
N-Cbz-glycine	16.3	*N*-Boc-D-valine	10.7
N-Cbz-β-alanine	16.5	*N*-Boc-L-serine	17.6
N-Cbz-L-alanine	15.6	*N*-Boc-D-serine	14.8
N-Cbz-D-alanine	17.6	*N*-Cbz-glycinyl-L-serine	19.2
N-Boc-L-phenylalanine	14.6	*N*-Cbz-glycinyl-D-serine	15.4
N-Boc-D-phenylalanine	15.8	*N*-Cbz-L-alanyl-L-alanine	14.6
N-Cbz-D-phenylalanine	17.6	*N*-Cbz-D-alanyl-D-alanine	17.7
N-Ac-D-phenylalanine	19.5	*N*-Cbz-β-alanyl-L-alanine	19.1
N-Boc-L-valine	12.6	*N*-Cbz-β-alanyl-D-alanine	22.8

1.5 Aims of this Project

This project aimed to design, synthesise and test neutral, tripodal steroid receptors based on cholic acid **1.18** that were capable of binding to carboxylates (especially *N*-acyl-α-aminoacid carboxylates), and differentiating between their enantiomers.



1.18

Cholic acid **1.18** has many features suitable for use in molecular recognition. It has a rigid, concave shape with three well-spaced hydroxy groups (positions 3, 7 and 12) which are arranged on the same side of the steroid (α), and which have the potential for further individual transformations due to their steric and electronic differences. These allow cholic acid to be used as a "scaffold", from which three pendant "legs" (A, B, and C) can be hung (Figure 1.1).

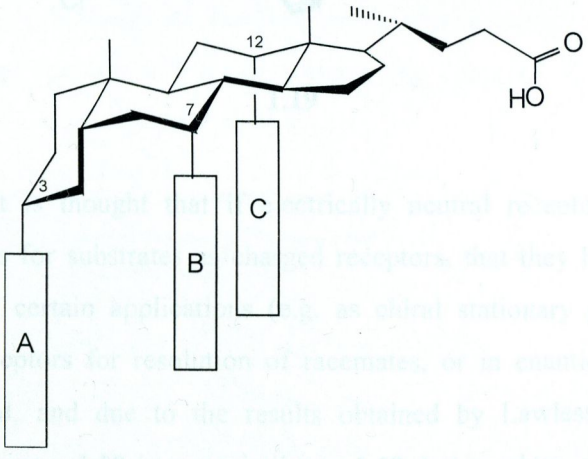
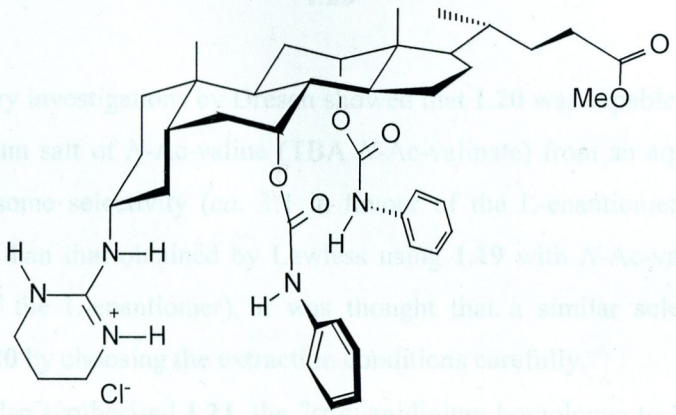


Figure 1.1

This tripodal arrangement creates a cavity underneath the steroid backbone that is ideal for small molecules to fit into. Suitable derivatisation of the three hydroxyls (3α-OH, 7α-OH, and 12α-OH), and/or of their amine homologues (3α-NH_2, 7α-NH_2, and 12α-NH_2), allows for the possibility of recognising different substrates. The potential for creating unique binding sites, along with it's low cost, ready availability, inherent chirality, and well known

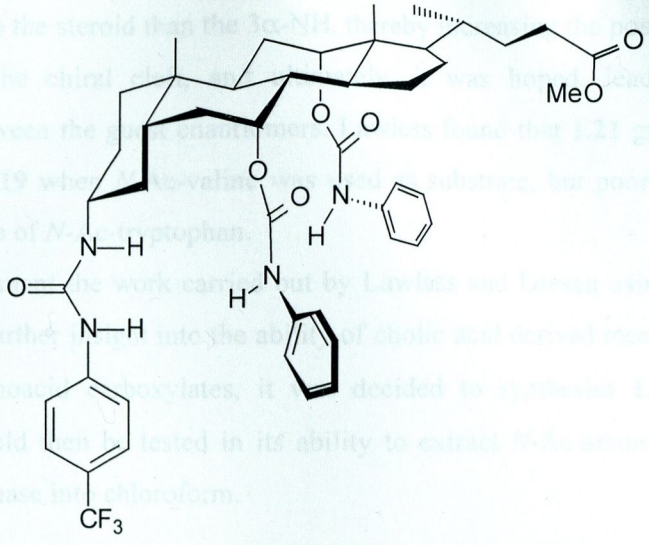
chemistry, make cholic acid an ideal structural framework for the synthesis of tripodal molecular receptors.

Cholic acid has already been successfully exploited within our laboratory to make receptors for carbohydrates, simple anions such as halides, and more recently, *N*-protected-α-aminoacid carboxylates.[1.13-1.16] Of direct relevance to this project are the receptors developed by Lawless and Dresen for the recognition of *N*-protected-α-aminoacid carboxylates (see Chapter 4 for greater detail).[1.16-1.18] Lawless developed a series of positively charged guanidinium receptors based on cholic acid which were capable of extracting *N*-protected aminoacids from a buffered aqueous phase into chloroform with good enantioselectivity (up to 80% e.e.).[1.16, 1.17] Most of his work focused on the synthesis of steroids similar to **1.19**, with the guanidinium moiety at the 3α position.



1.19

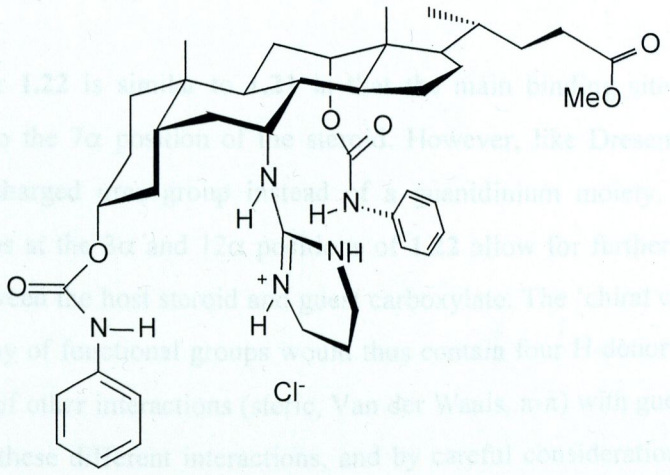
In general, it is thought that if electrically neutral receptors exhibit the same recognition properties for substrates as charged receptors, that they have advantages over charged receptors in certain applications (e.g. as chiral stationary phases in HPLC, as membrane-bound receptors for resolution of racemates, or in enantioselective transport). Bearing this in mind, and due to the results obtained by Lawless with **1.19**, Dresen synthesised **1.20**. Receptor **1.20** is very similar to **1.19**, but employs an electrically neutral 3α-urea group as a carboxylate binding site, instead of the positively charged 3α-guanidinium group used by Lawless.



1.20

Preliminary investigations by Dresen showed that **1.20** was capable of extracting the tetrabutylammonium salt of *N*-Ac-valine (TBA *N*-Ac-valinate) from an aqueous phase into chloroform with some selectivity (*ca.* 3:1 in favour of the L-enantiomer). Although this selectivity is less than that obtained by Lawless using **1.19** with *N*-Ac-valine as substrate (7:1 in favour of the L-enantiomer), it was thought that a similar selectivity could be achieved using **1.20** by choosing the extraction conditions carefully.

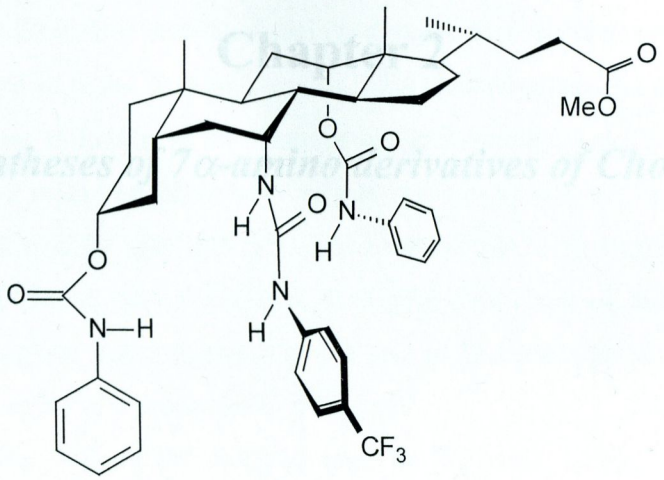
Lawless also synthesised **1.21**, the 7α-guanidinium homologue to **1.19**, and tested it in extractions of *N*-protected-aminoacid carboxylates (see Chapter 4, Table 4.1).



1.21

In this case, molecular modelling studies had shown that the 7α-NH was more constrained to point underneath the steroid than the 3α-NH, thereby increasing the possibility of binding of the guest in the chiral cleft, and ultimately, it was hoped, leading to increased discrimination between the guest enantiomers. Lawless found that **1.21** gave a very similar result to that of **1.19** when *N*-Ac-valine was used as substrate, but poorer selectivity was obtained in the case of *N*-Ac-tryptophan.

To complement the work carried out by Lawless and Dresen using receptors **1.19-1.21**, and to gain further insight into the ability of cholic acid derived receptors to recognise *N*-protected-α-aminoacid carboxylates, it was decided to synthesise **1.22** in this work. Receptor **1.22** would then be tested in its ability to extract *N*-Ac-aminoacid carboxylates from an aqueous phase into chloroform.



1.22

Receptor **1.22** is similar to **1.21** in that the main binding site for carboxylate is incorporated into the 7α position of the steroid. However, like Dresen's receptor **1.20**, it employs an uncharged urea group instead of a guanidinium moiety. Like **1.21**, phenyl carbamate groups at the 3α and 12α positions of **1.22** allow for further hydrogen bonding interactions between the host steroid and guest carboxylate. The 'chiral cleft' obtained from this tripodal array of functional groups would thus contain four H-donor groups, but would also be capable of other interactions (steric, Van der Waals, π-π) with guest molecules. By a combination of these different interactions, and by careful consideration of the extraction conditions, it was hoped that **1.22** would recognise only one enantiomer of *N*-acetyl-aminoacid carboxylates, and selectively extract this enantiomer from an aqueous buffered phase into chloroform.

Chapter 2

The Syntheses of 7α-amino derivatives of Cholic Acid

2.1 Preamble

The incorporation of a nitrogen atom into the 7α-position of cholic acid was central to this project. An amine in this position would allow for the creation of a urea group, which it was felt would be ideal for forming complementary hydrogen bonds with carboxylates (see Chapter 1). This intermediate could then be further elaborated to give the desired receptor **1.22**, and other receptors (see Chapter 3).

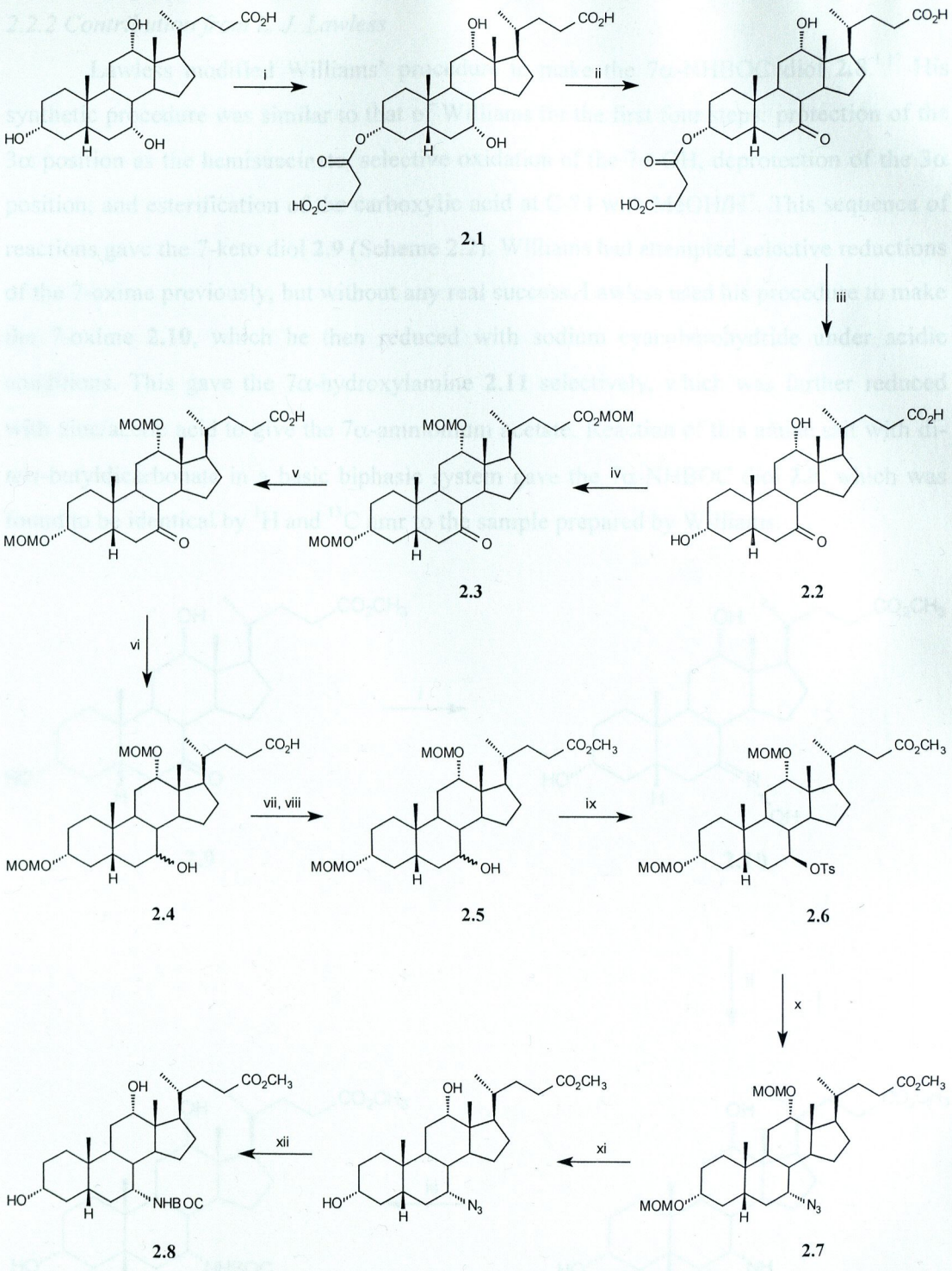
It was necessary to find a synthetic procedure by which an amine could be inserted into the 7α position. Several different routes had been used previously to synthesise this type of intermediate. R. P. Williams had synthesised a BOC protected amine in this position in twelve steps and *ca.* 20% yield, with the key step being azide displacement of the 7β-tosylate.[2.1] L. J. Lawless improved upon this synthesis, and obtained the same 7α-NHBOC diol in seven steps and *ca.* 30% yield.[1.17] The key step in this synthesis was the reduction of the 7-oxime to the 7α-hydroxylamine. Kasal *et al.* reported a five-step procedure leading to a BOC protected amine in the 7α-position in *ca.* 34% yield (from methyl cholate).[2.2] The key step was a reductive amination of the 7-ketone to the 7α-amine. Kasal's procedure had an advantage over the other two procedures in that the 3α-position of the steroid remained protected. This allowed for easy differentiation between the 3α and 12α positions in later synthetic steps. Due to the shorter synthesis, the higher yield obtained, and the possibility of carrying out independent transformations on the 3α and 12α positions at an advanced stage, it was decided to use Kasal's procedure in this work.

This chapter outlines the schemes used by Williams, Lawless and Kasal in the synthesis of 7α-NHBOC derivatives of cholic acid. Following this, the work done to improve upon Kasal's route and to develop it to a larger synthetic scale is shown.

2.2 The syntheses of 7α-NHBOC derivatives of cholic acid

2.2.1 Contribution from R. P. Williams

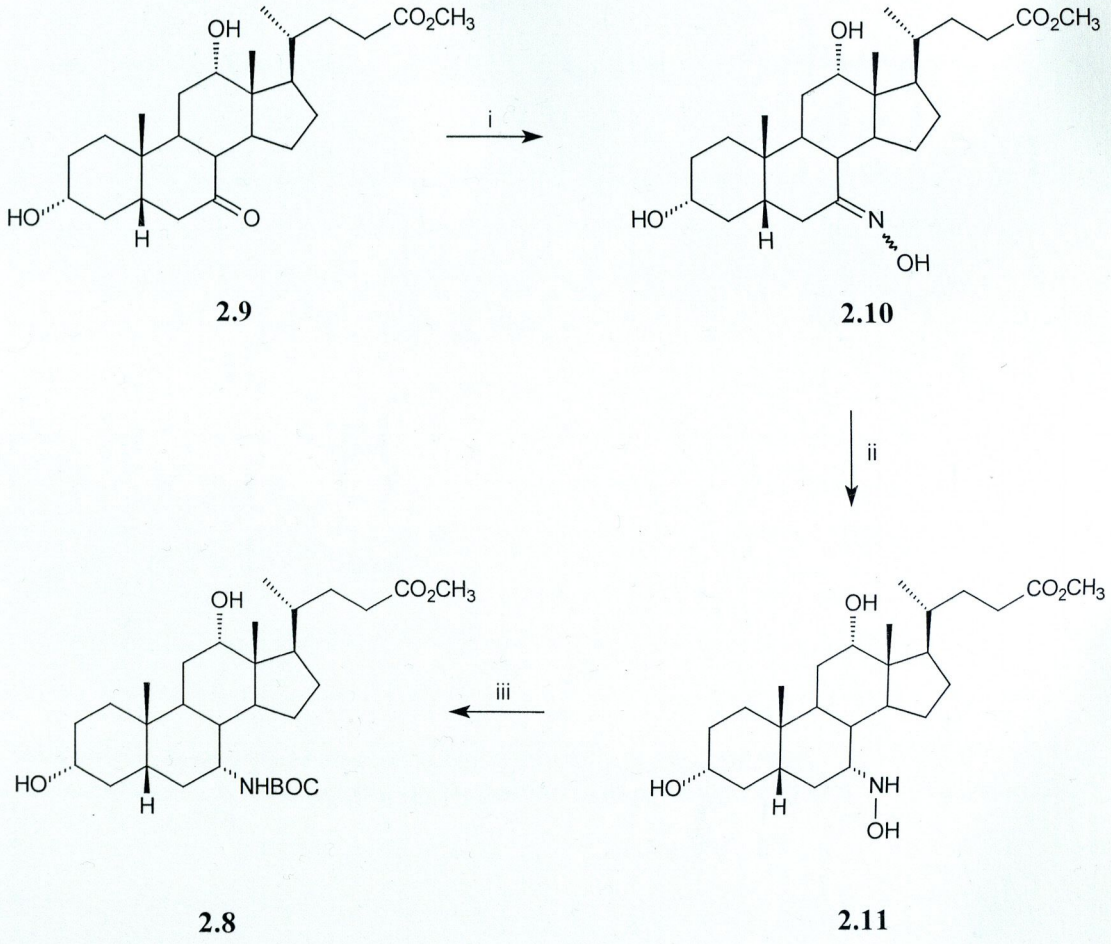
Williams protected the 3α-position of cholic acid as the hemisuccinate **2.1**, and selectively oxidised the 7α-OH with *N*-bromosuccinimide in aqueous $NaHCO_3$ (Scheme 2.1).[2.1] Subsequent deprotection of the 3α position gave the 7-keto diol **2.2**. Global protection with dimethoxymethane gave **2.3**, the MOM ester of which could be cleaved selectively with 5% KOH in MeOH. Dissolving metal reduction of the 7-ketone with potassium in *tert*-amylalcohol gave predominantly the 7β-OH **2.4** (7α:7β - 3:97). Formation of the caesium salt, followed by methylation with MeI in DMF afforded the methyl ester **2.5**. Treatment of **2.5** with TsCl in pyridine gave the 7β-tosylate **2.6**. S_N2 displacement of the 7β-tosylate with azide in DMPU gave the 7α-azide **2.7**, which was deprotected and converted to the 7α-NHBOC derivative **2.8**.



Scheme 2.1 *(Reagents, conditions, and yields)*: i, succinic anhydride, pyridine, 1,1,1-trichloroethane, reflux, 3 h, 95%; ii, NBS, sat. aq. $NaHCO_3$, 70°C, 18 h; iii, 5% NaOH in MeOH, reflux, 3 h, 77% from **2.1**; iv, $CH_2(OMe)_2$, P_2O_5, $CHCl_3$, 1 h, r.t.; v, 5% KOH in MeOH, 2 h, reflux, 91% from **2.2**; vi, K, *tert*-amylalcohol, reflux, 1 h, 93%; vii, $CsCO_3$, $MeOH/H_2O$; viii, MeI, DMF, 93%; ix, TsCl, pyridine, r.t., 24 h, 71%; x, NaN_3, DMPU, 70°C, 36 h, 71%; xi, CH_3COCl, MeOH, reflux, 3 h, 91%; xii, H_2, PtO_2, $(BOC)_2O$, toluene, 4 d, 78%.

2.2.2 Contribution from L. J. Lawless

Lawless modified Williams' procedure to make the 7α-NHBOC diol **2.8**.[1.17] His synthetic procedure was similar to that of Williams for the first four steps: protection of the 3α position as the hemisuccinate, selective oxidation of the 7α-OH, deprotection of the 3α position, and esterification of the carboxylic acid at C-24 with $MeOH/H^+$. This sequence of reactions gave the 7-keto diol **2.9** (Scheme 2.2). Williams had attempted selective reductions of the 7-oxime previously, but without any real success. Lawless used his procedure to make the 7-oxime **2.10**, which he then reduced with sodium cyanoborohydride under acidic conditions. This gave the 7α-hydroxylamine **2.11** selectively, which was further reduced with zinc/acetic acid to give the 7α-ammonium acetate. Reaction of this amine salt with di-*tert*-butyldicarbonate in a basic biphasic system gave the 7α-NHBOC diol **2.8**, which was found to be identical by ^1H and ^{13}C nmr to the sample prepared by Williams.



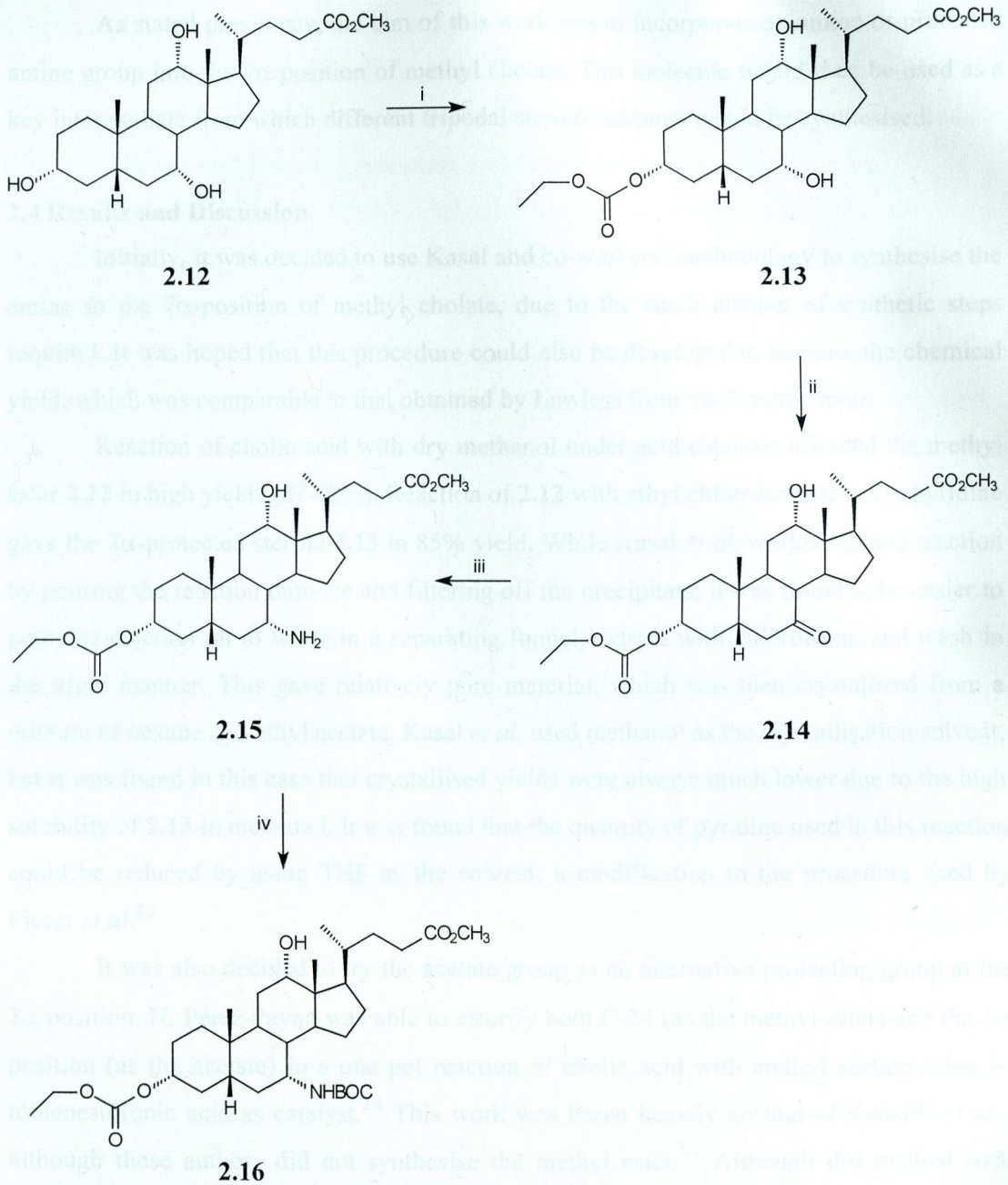
2.9 **2.10**

2.8 **2.11**

Scheme 2.2 *(Reagents, conditions and yields)*: i, $NaOAc.3H_2O$, aq. $NH_2OH.HCl$, MeOH, reflux, 3 h, 71%; ii, crude **2.10**, $NaCNBH_3$, MeOH, pH ~2.5 with CH_3CO_2H, r.t., 24 h, 68%; iii, Zn dust, CH_3CO_2H, r.t., 24 h, then sat. aq. $NaHCO_3$, $(BOC)_2O$, THF, r.t., 3 d, 86%.

2.2.3 Contribution from Kasal et al.

Kasal and co-workers' synthetic strategy to the 7-ketone was similar to that of Williams and Lawless (Scheme 2.3).[2.2] Esterification of cholic acid with $MeOH/H^+$ gave methyl cholate **2.12**, which was selectively protected at the 3α position with ethyl chloroformate in pyridine to give the ethyl carbonate **2.13**. Selective oxidation of the 7α-OH with *N*-bromoacetamide in aqueous acetone gave the 7-ketone **2.14**. Reductive amination of the 7-ketone with ammonium acetate and sodium cyanoborohydride in MeOH gave the desired 7α-amine **2.15**. This amine was then protected with di-*tert*-butyldicarbonate in THF/water under basic conditions to give the 7α-NHBOC derivative **2.16**.



2.12 **2.13**

2.15 **2.14**

2.16

Scheme 2.3 *(Reagents, conditions and yields)*: i, EtOCOCl, pyridine, 0°C, 45 min., 85%; ii, NBA, CH_3CO_2H, Acetone/H_2O, r.t., 1 h, 81%; iii, $NH_4CH_3CO_2$, $NaCNBH_3$, MeOH, 50°C, 18 h, 66%; iv, $(BOC)_2O$, aq. KOH, THF, r.t., 30 min., 76%.

2.3 Aim of this work

As stated previously, the aim of this work was to incorporate an amine or protected amine group into the 7α position of methyl cholate. This molecule would then be used as a key intermediate from which different tripodal steroid receptors would be synthesised.

2.4 Results and Discussion

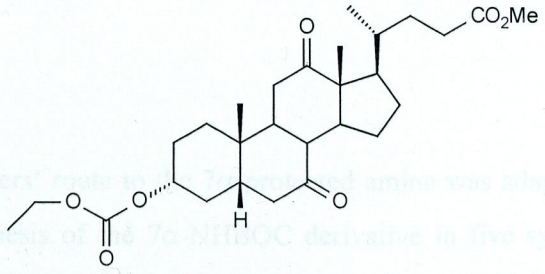
Initially, it was decided to use Kasal and co-workers' methodology to synthesise the amine in the 7α-position of methyl cholate, due to the small number of synthetic steps required. It was hoped that this procedure could also be developed to increase the chemical yield, which was comparable to that obtained by Lawless from his 7-oxime route.

Reaction of cholic acid with dry methanol under acid catalysis afforded the methyl ester **2.12** in high yields (87-92%). Reaction of **2.12** with ethyl chloroformate in dry pyridine gave the 3α-protected steroid **2.13** in 85% yield. While Kasal *et al.* worked up this reaction by pouring the reaction onto ice and filtering off the precipitate, it was found to be easier to pour the reaction on to water in a separating funnel, extract with chloroform, and wash in the usual manner. This gave relatively pure material, which was then crystallised from a mixture of hexane and ethyl acetate. Kasal *et al.* used methanol as the crystallisation solvent, but it was found in this case that crystallised yields were always much lower due to the high solubility of **2.13** in methanol. It was found that the quantity of pyridine used in this reaction could be reduced by using THF as the solvent, a modification to the procedure used by Fieser *et al.*[2.3]

It was also decided to try the acetate group as an alternative protecting group at the 3α position. N. Pérez-Payan was able to esterify both C-24 (as the methyl ester) and the 3α position (as the acetate) in a one pot reaction of cholic acid with methyl acetate using *p*-toluenesulfonic acid as catalyst.[2.4] This work was based heavily on that of Kuhajda *et al.*, although these authors did not synthesise the methyl ester.[2.5] Although this method was attractive because it allowed two protections in one step, it was not problem free. Reaction of cholic acid with methyl acetate under identical conditions to that of Pérez-Payan, gave the crude product in 90% yield. However, ^{1}H nmr and tlc identified methyl cholate as an impurity of *ca.* 10% in the crude product. Tlc of this crude product using Kuhajda's solvent system (chloroform/acetic acid/acetone 12:1:7) showed four spots (R_f's 0.78, 0.67, 0.56, 0.26). Crystallisation of the crude product from methanol or hexane/ethyl acetate mixtures gave no improvement in purity. Crystallisation from methanol/water proved slightly more effective, but did not give pure material. Pure material could be obtained by flash chromatography using chloroform/acetone (9:1) as eluent. However, this was not ideal because it limited the quantities of material available at the start of the synthetic sequence.

Therefore, it was decided to oxidise the 7α-OH of the crude product to the 7-ketone (see below) and try to purify at this stage. Again, it proved difficult to purify the crude product from this reaction. It was thought that the presence of the ethyl carbonate group on the steroid was more favourable for the purpose of crystallisation at this stage and at later stages, so the acetate protection procedure was abandoned.

Returning to Scheme 2.3, selective oxidation of the 7α-OH was not as straightforward as Kasal and co-workers reported. Oxidation of **2.13** using Kasal's conditions (NBA, acetic acid, aqueous acetone) gave a mixture of 7-ketone **2.14** and small amounts of the 7, 12-diketone **2.17**. These steroids co-crystallised in methanol (the solvent recommended by Kasal *et al.*), and proved difficult to separate by flash chromatography because of their similar polarities. **2.17** was formed in the reaction mixture even after five minutes at room temperature (tlc). Addition of more acetic acid resulted in an acceleration of the reaction, but little difference in the proportion of **2.14** to **2.17** was observable by tlc. Other procedures from the chemical literature were explored to selectively oxidise the 7α-OH.[2.6-2.8] Reaction of **2.13** with potassium chromate in a sodium acetate/acetic acid buffer yielded a complex mixture by tlc, which included equal proportions of **2.14** and **2.17**. Slow addition of potassium chromate in a non-buffered solution gave identical results. Reaction of either cholic acid or methyl cholate with NBS in aqueous acetone gave a mixture of products, which were not separated. Oxidation of 3α-protected steroid **2.13** with NBS in aqueous acetone resulted in a mixture of **2.14** and **2.17** in approximately equal proportions by tlc.



2.17

It was clear from these experiments that a 3α-protected steroid should be used, despite literature precedents for the selective oxidation of the 7α-OH in the presence of the 3α-OH.[2.6, 2.7] *N*-bromoacetamide also seemed to be the oxidant of choice. It was decided therefore to modify Kasal's conditions to obtain the desired steroid. After several attempts, it was found that the 7-ketone **2.14** could be obtained by a combination of carrying out the

reaction at lower temperature, followed by crystallisation from hexane/ethyl acetate mixtures. Using this method, pure **2.14** was obtained in 70% yield.

After **2.14** was successfully isolated, the reductive amination to give **2.15** was attempted using Kasal's conditions. This method was successful in giving the 7α-amine selectively (see later). However, it was found that the work-up conditions of Kasal (toluene as work-up solvent) were not the optimum. By changing the work-up solvent to chloroform, and with appropriate washing, **2.15** or **2.15.HCl** was obtained in virtually quantitative yield. **2.15** or **2.15.HCl** was protected with di-*tert*-butyldicarbonate using aqueous KOH/THF as the biphasic solvent system. Again, it was necessary to modify Kasal's conditions to increase the yield and purity of the NHBOC derivative **2.16**. By reducing the quantity of di-*tert*-butyldicarbonate in the reaction from 1.67 equivalents (relative to the steroid) to 1.05 equivalents, the crude material became much cleaner, and easier to crystallise. Thus, crystallisation of the crude product from a mixture of hexane/ethyl acetate gave **2.16** in 88% yield.

The ^{1}H nmr spectrum of **2.16** was identical to that obtained by Kasal *et al.* In addition, the NH and the three identical methyl groups of the *tert*-butylcarbamate in the ^{1}H nmr spectrum appeared at the same chemical shift as those obtained by Williams, who introduced the NHBOC group into this position *via* S_N2 displacement of the 7β-tosylate with azide ion. This lends credence to Kasal's interpretation that the amine occupied the axial (α) position. Furthermore, a tripodal receptor synthesised from this intermediate (see later) and from a 7α-NHBOC intermediate obtained from L. J. Lawless (*via* the 7-oxime) were identical by ^{1}H and ^{13}C nmr, and had identical R_f's on tlc when run in several different solvent systems.

2.5 Summary

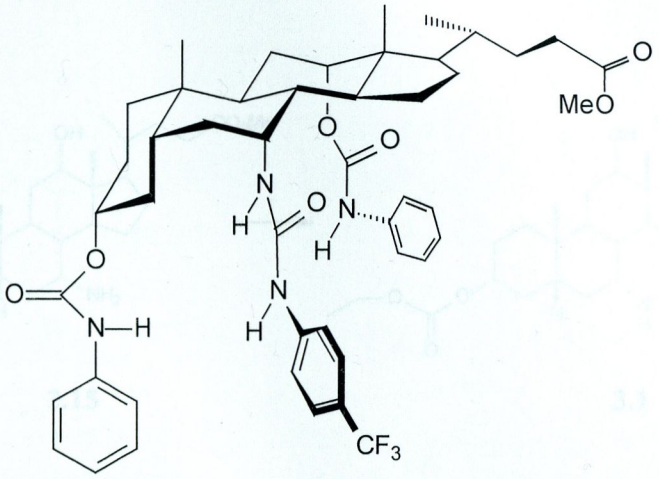
Kasal and co-workers' route to the 7α-protected amine was adapted and improved. This allowed for the synthesis of the 7α-NHBOC derivative in five synthetic steps from cholic acid, and in an overall yield of *ca.* 47 %. This represents a significant improvement on the authors' overall yield (*ca.* 34 % from methyl cholate). This improved synthesis was carried out on a large laboratory scale (starting from 52 g) without the need for chromatography.

Chapter 3

The Syntheses of Tripodal Steroid Receptors for the Molecular Recognition of Carboxylates

3.1 Preamble

Having synthesised the amine **2.15** and the protected amine **2.16** (Chapter 2), it was necessary to derivatise the steroid further to incorporate functional groups suitable for recognition of carboxylates. As previously stated, the initial goal was the synthesis of the tripodal receptor **1.22**.



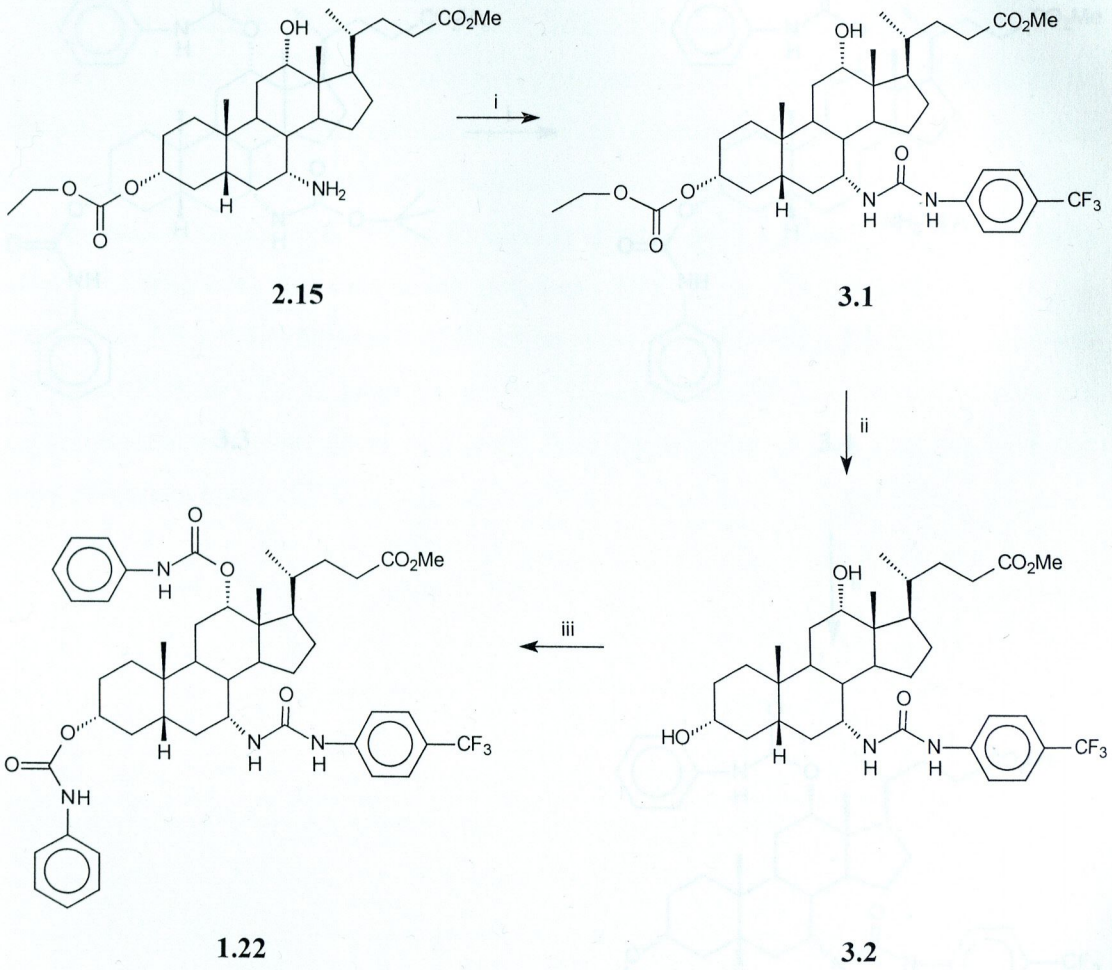
1.22

However, the synthetic strategy towards **1.22** was designed to be flexible, so that a range of steroid receptors could be synthesised from a common intermediate. This chapter outlines the synthesis of **1.22** and related receptors, the synthetic investigations into carbamate formation on the steroid, and the synthesis of a sterically hindered ester in the 3α position of bile acid derivatives.

3.2 The synthesis of urea *bis*-carbamate 1.22

It was decided to try to synthesise **1.22** from the amine **2.15**, rather than use unnecessary amine protection/deprotection steps. This strategy worked well (Scheme 3.1). Reaction of the amine **2.15** with α,α,α-trifluoro-*p*-tolylisocyanate in dry DCM gave the urea **3.1** in good yield (79-87%). **3.1** could be purified by chromatography, or by trituration with hexane/ethyl acetate mixtures (see Experimental). Deprotection of the 3α-ethyl carbonate group using the conditions of Kasal *et al.* gave the urea diol **3.2** in reasonable yield (58%).[2.2] It was found that this deprotection step could be carried out using a solution of sodium methoxide in methanol without affecting the urea or methyl ester groups present. This method afforded **3.2** in higher yield (85%), and in shorter time (< 1 h). Finally, reaction of **3.2** with an excess of phenyl isocyanate gave the urea *bis*-carbamate **1.22** in 56.7% yield. Thus, **1.22** was obtained in seven steps from cholic acid, and in an overall yield of 23.5%.

The relatively long reaction time required in the last step of this synthetic sequence, and the moderate yield obtained, reflects two things: (i) the hindered nature of the 12α-OH, augmented by the presence of groups already attached to the 3α and 7α positions of the steroid, and (ii) the difficulty in purifying the steroid after an excess of isocyanate was employed in the reaction. Unfortunately, these two points proved to be recurring problems in the synthesis of these types of steroid receptors (see later).

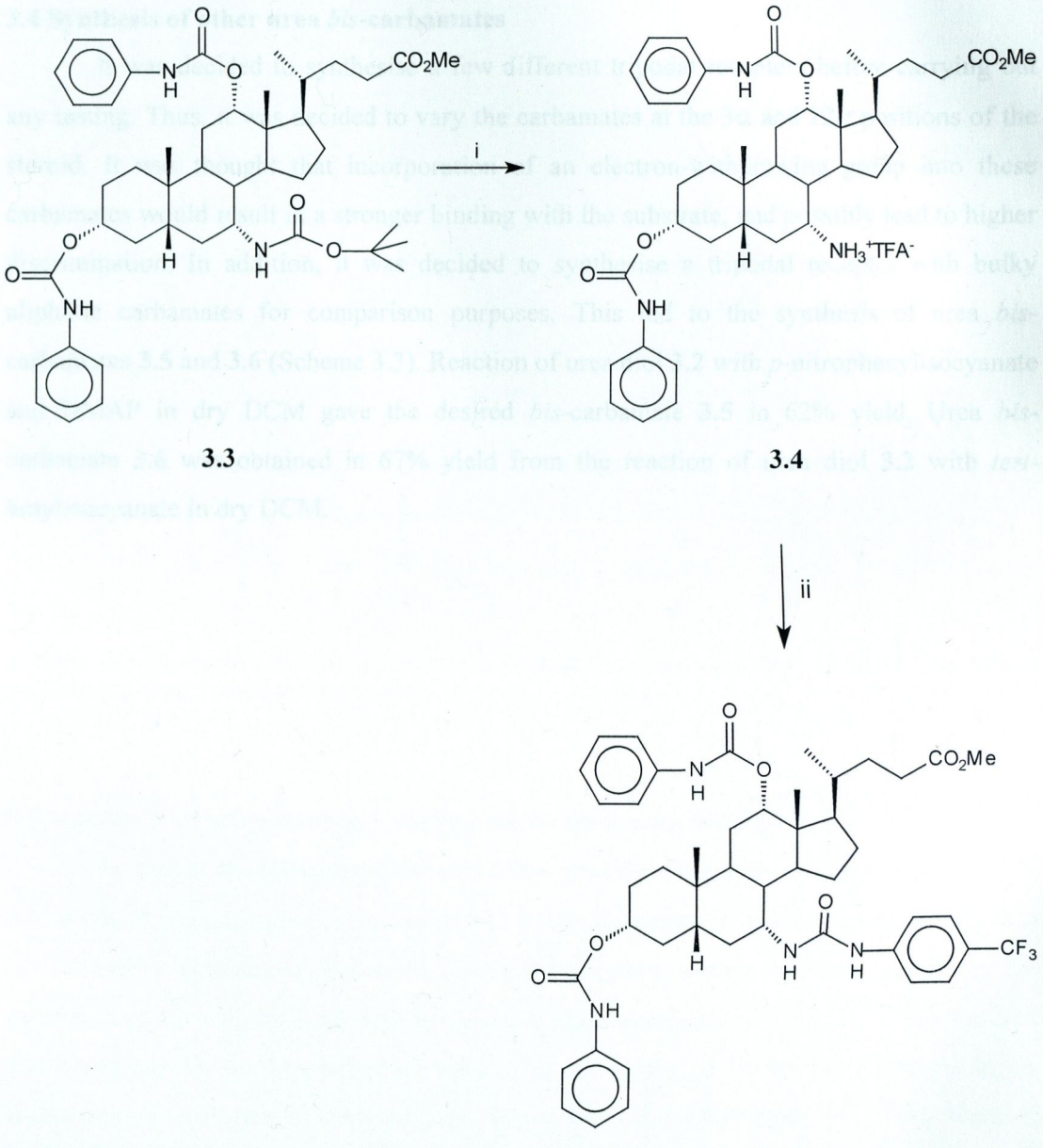


2.15 **3.1** **3.2** **1.22**

Scheme 3.1 *(Reagents, conditions and yields)*: i, α,α,α-trifluoro-*p*-tolylisocyanate, Et_3N, DCM, r.t., 1.5 h, 86%; ii, 2M NaOMe in MeOH, r.t., 1 h, 85%, or 10% KOH in MeOH, r.t., 21 h, then 50°C, 3 h, 58%; iii, PhNCO, cat. TMS-Cl, $CHCl_3$, r.t., 6 d, then 4 h, reflux, 56.7%.

3.3 An alternative synthesis of urea *bis*-carbamate 1.22

An alternative synthesis of urea *bis*-carbamate **1.22** was also carried out. Crude 7α-NHBOC *bis*-carbamate **3.3** was obtained from L. Lawless and deprotected to give the amine **3.4**, which was purified by chromatography. Reaction of **3.4** with α,α,α-trifluoro-*p*-tolylisocyanate in dry DCM gave the urea *bis*-carbamate **1.22** (Scheme 3.2).



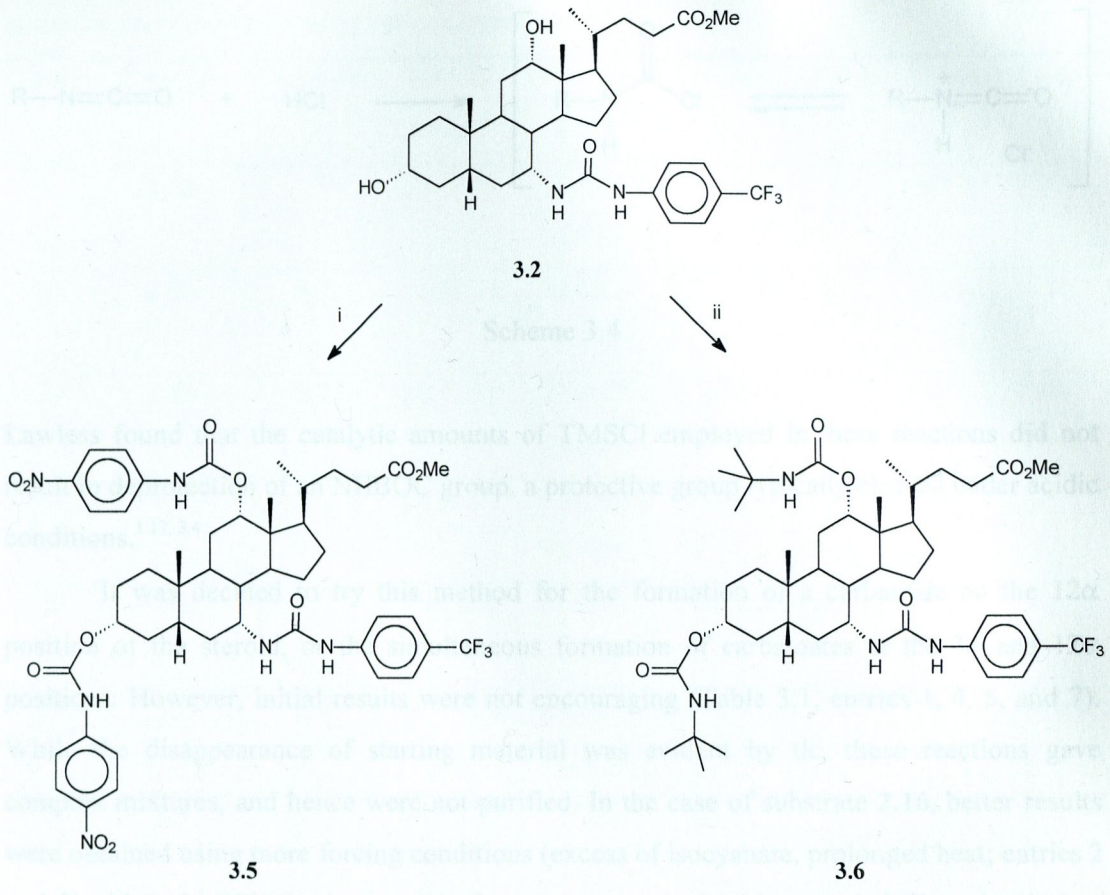
3.3 **3.4** **1.22**

Scheme 3.2 *(Reagents, conditions and yields)*: i, 25% TFA in DCM, r.t., 2.5 h; ii, α,α,α-trifluoro-*p*-tolylisocyanate, Et_3N, DCM, r.t., 30 min., 86%.

This synthesis allowed for further proof of the stereochemistry at C-7 of the steroid. Lawless had synthesised the 7α-NHBOC *via* his 7-oxime route (see Chapter 2). The urea *bis*-carbamate synthesised from this intermediate possessed identical ^{1}H and ^{13}C nmr spectra to that of **1.22** synthesised from the reductive amination route (see Chapter 2). These two molecules also possessed the same R_f's on tlc [0.20 DCM/MeOH (49:1), 0.43 hexane/ EtOAc (1:1), 0.45 $CHCl_3$/acetone (9:1)], and when co-spotted on tlc, ran together.

3.4 Synthesis of other urea *bis*-carbamates

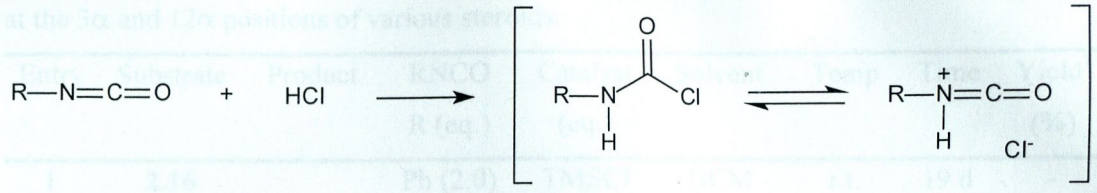
It was decided to synthesise a few different tripodal receptors before carrying out any testing. Thus, it was decided to vary the carbamates at the 3α and 12α positions of the steroid. It was thought that incorporation of an electron-withdrawing group into these carbamates would result in a stronger binding with the substrate, and possibly lead to higher discrimination. In addition, it was decided to synthesise a tripodal receptor with bulky aliphatic carbamates for comparison purposes. This led to the synthesis of urea *bis*-carbamates **3.5** and **3.6** (Scheme 3.3). Reaction of urea diol **3.2** with *p*-nitrophenylisocyanate and DMAP in dry DCM gave the desired *bis*-carbamate **3.5** in 62% yield. Urea *bis*-carbamate **3.6** was obtained in 67% yield from the reaction of urea diol **3.2** with *tert*-butylisocyanate in dry DCM.



Scheme 3.3 *(Reagents, conditions and yields)*: i, *p*-nitrophenylisocyanate, DMAP, DCM, r.t., 2 d, 62%; ii, *tert*-butylisocyanate, TMSCl, DCM, 9 d, 67%.

3.5 Synthetic Investigations into carbamate formation on steroids

As part of the foregoing syntheses of the urea *bis*-carbamate receptors **1.22**, **3.5**, and **3.6**, synthetic investigations were carried out on the formation of steroid carbamates. Within our laboratory, trimethylsilyl chloride (TMSCl) has typically been used as a catalyst for the formation of carbamates from aryl isocyanates and steroid alcohols.[1.17, 3.1, 3.2] This method was based heavily on the method of Vaultier *et al.*, who used 5% HCl as a catalyst in the formation of carbamates from tertiary isocyanates and alkylalcohols.[3.3] This reaction proceeded smoothly at room temperature in dichloromethane. Vaultier and co-workers found that commercial TMSCl gave essentially the same result as HCl, and suggested that this was either a result of the presence of HCl in the unpurified TMSCl, or the formation of HCl in the reaction mixture from the reaction of TMSCl with the alcohol. It is assumed in either case that the isocyanate is activated towards attack by formation of the carbamoyl chloride intermediate (Scheme 3.4).



Scheme 3.4

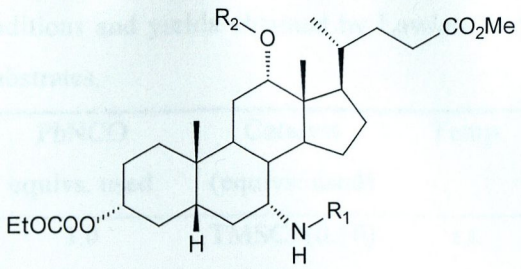
Lawless found that the catalytic amounts of TMSCl employed in these reactions did not result in deprotection of an NHBOC group, a protective group typically cleaved under acidic conditions.[1.17, 3.4]

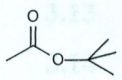
It was decided to try this method for the formation of a carbamate on the 12α position of the steroid, or the simultaneous formation of carbamates at the 3α and 12α positions. However, initial results were not encouraging (Table 3.1, entries 1, 4, 5, and 7). While the disappearance of starting material was evident by tlc, these reactions gave complex mixtures, and hence were not purified. In the case of substrate **2.16**, better results were obtained using more forcing conditions (excess of isocyanate, prolonged heat; entries 2 and 3) although difficult chromatography was necessary in this case to isolate the desired product.

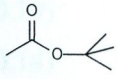
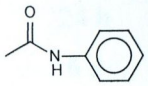
Table 3.1. Conditions used for carbamate formation at the 12α position, or (simultaneously) at the 3α and 12α positions of various steroids.

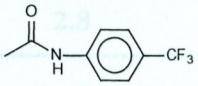
Entry	Substrate	Product	RNCO R (eq.)	Catalyst (eq.)	Solvent	Temp	Time	Yield[1] (%)
1	**2.16**		Ph (2.0)	TMSCl (0.10)	DCM	r.t.	19 d	-
2	**2.16**	**3.7**	Ph (2.2)	TMSCl (0.20)	1, 2-DCE	40°C	62 h	26
3	**2.16**	**3.7**	Ph (3.3)	TMSCl (0.30)	1, 2-DCE	40°C	90 h	34
4	**3.1**		Ph (2.0)	TMSCl (0.42)	DCM	r.t.	25 d	-
5	**3.1**		Ph (3.0)	TMSCl (0.22)	1, 2-DCE	reflux	13 d	-
6	**3.1**	**3.8**	*p*-NO_2Ph (3.0)	DMAP (2.0)	DCM	r.t.	2 d	44
7	**3.9**		*p*-CF_3Ph (3.0)	TMSCl (0.16)	DCM	r.t.	93 h	-
8	**3.10**		aniline (2.0)		DCM	r.t.	7 d	no rxn
9	**2.8**		Ph (2.1)	CuCl (2.0)	DMF	r.t.	4 h	-
10	**2.8**		Ph (6.3)	DMAP (4.0)	DCM	r.t.	32 h	-
11	**2.8**		CDI (2.0) + aniline (2.0)		DCM	r.t.	6 d	no rxn
12	**3.2**	**1.22**	Ph (7.5)	TMSCl (0.3)	$CHCl_3$	r.t.	6 d	57
13	**3.2**	**3.5**	*p*-NO_2Ph (6.0)	DMAP (4.0)	DCM	r.t.	2 d	62
14	**3.2**	**3.6**	*tert*-butyl (5.0)	TMSCl (0.6)	DCM	r.t.	9 d	67

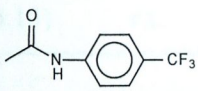
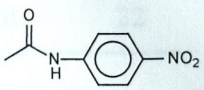
[1]where no yield is given, the reaction gave a complex mixture on tlc and was not purified.

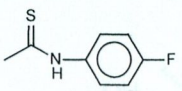


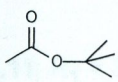
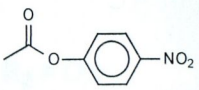
2.16: R_1 =  ; R_2 = H

3.7: R_1 =  ; R_2 = 

3.1: R_1 =  ; R_2 = H

3.8: R_1 =  ; R_2 = 

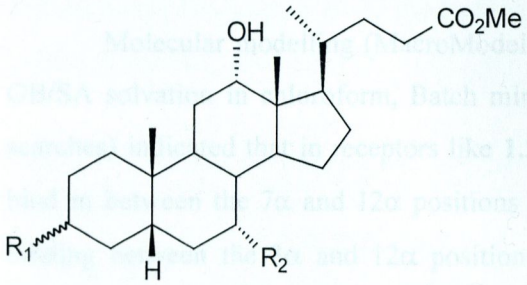
3.9: R_1 =  ; R_2 = H

3.10: R_1 =  ; R_2 = 

Other methods were tried (entries 6, 8-11, and 13) to introduce carbamates into the 12α position or the 3α and 12α positions, but with the exception of DMAP as catalyst, these were not successful.[3.5-3.7] In particular, it seems that the use of DMAP as catalyst (entries 6, 10, 13) is more effective when isocyanates bearing electron-withdrawing groups are employed in this reaction, presumably due to the increased susceptibility of these substrates to undergo nucleophilic attack. It is also suggested that in general, the hindered nature of the 12α hydroxyl makes it difficult to introduce a carbamate into this position, and that this difficulty is exacerbated by bulky groups in the 7α position of the steroid. This is borne out by the results obtained by Lawless (Table 3.2), who obtained good yields of carbamate at the 12α position when the 7α-position was unsubstituted (entries 1 and 2), but decreased yields as the substituent on the 7α position increased in size (entries 3-5).[1.17]

Table 3.2. Reaction conditions and yields obtained by Lawless in the formation of phenyl carbamates on steroid substrates.

Entry	Substrate	PhNCO equivs. used	Catalyst (equivs. used)	Temp.	Time	Yield (%)
1	**3.11**	3.0	TMSCl (0.10)	r.t.	24 h	78
2	**3.12**	3.0	HCl	reflux	3 d	83
3	**3.13**	1.5	TMSCl (0.10)	r.t.	24 h	53
4	**3.14**	2.0	TMSCl (0.14)	r.t.	6 d	57
5	**2.8**	2.0	TMSCl (0.15)	r.t.	2 d	22



3.11: R_1 = 3α–N_3 ; R_2 = OH

3.12: R_1 = 3α–HN(C=NH$_2^+$)NH Cl^-; R_2 = OH

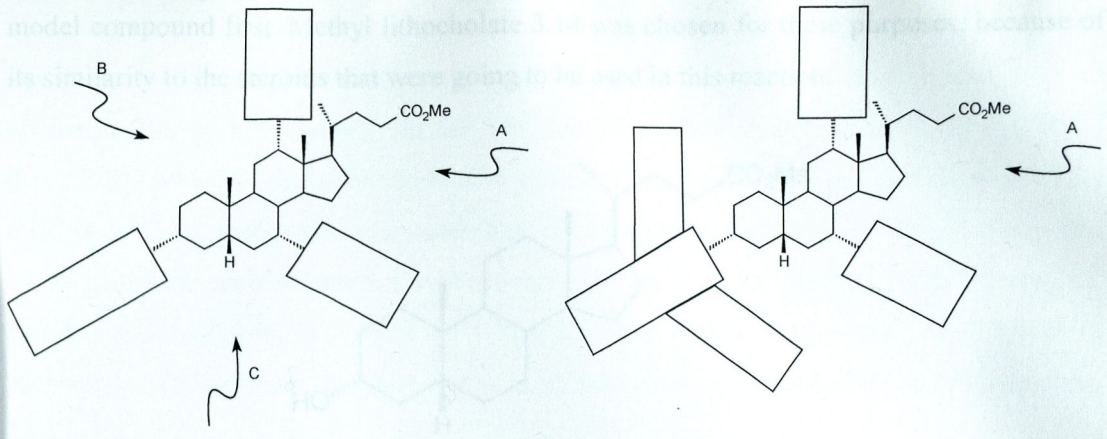
3.13: R_1 = 3β-HCO_2 ; R_2 = CH_3CO_2

3.14: R_1 = 3α-CH_3CO_2 ; R_2 = CH_3CO_2

2.8: R_1 = 3α-OH ; R_2 = NHBOC

3.6 Synthesis of a sterically hindered ester in the 3α-position of bile acid derivatives

While urea *bis*-carbamates such as **1.22** were proposed to be good receptors for *N*-protected aminoacid carboxylates, it was thought that the introduction of a sterically hindered ester into the 3α-position of the steroid would act as a 'steric blockade', decreasing the ability of the guest to find different binding orientations with the receptor (Scheme 3.5). It was thought that forcing the guest compound to choose one particular binding site could be desirable for high recognition.



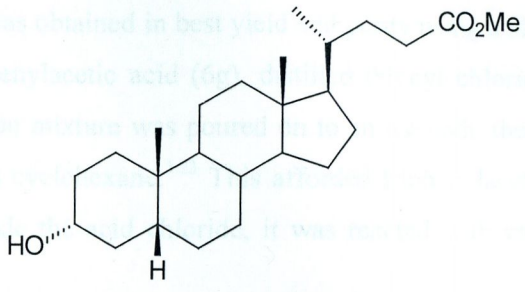
Scheme 3.5

Molecular modelling (MacroModel version 5.0, AMBER and/or MM2 forcefields, GB/SA solvation in chloroform, Batch minimisations and/or Monte Carlo conformational searches) indicated that in receptors like **1.22**, non-planar carboxylates were most likely to bind in between the 7α and 12α positions of the steroid (Scheme 3.5 - direction A), with binding between the 3α and 12α positions contributing to a lesser extent (direction B). Binding between the 3α and 7α positions (direction C) was never observed.

It was decided to use the triphenylacetate group as a blocking group at the 3α position because of its unique propeller shape. Molecular modelling studies indicated that this sterically hindered ester would effectively close off the gap between the 3α and 12α positions of the steroid, leaving guests only one direction of approach for binding (direction A). In some studies it was also noticed that one of the phenyl rings of the triphenylacetate could orientate itself to stick into the binding cavity underneath the steroid. While it was acknowledged that this could lead to a disruption of the binding of substrates, it was thought that the weaker binding enantiomer would be disrupted to a greater extent, and thus this conformation of the triphenylacetate could actually improve recognition of the guest.

A comprehensive literature search on triphenylacetic acid and its esters revealed that the cyclohexyl ester had never been prepared. Typically, esters of triphenylacetic acid were formed by reacting a large excess of the alcohol with triphenylacetyl chloride, or by reacting the carboxylate of triphenylacetic acid with a reactive species such as a diazoalkane, alkyl halide, or trialkyloxonium salt.[3.8-3.18] As it was desired to introduce this group into the steroid at a reasonably advanced stage in the synthesis (i.e. after the 7α-NHBOC had been made), none of these methods were suitable. Instead, it was decided initially to try to couple the acid directly with a steroid alcohol using different methods.[3.7, 3.19, 3.20] Rather than waste material which had taken six synthetic steps to make, it was decided to try out reactions on a

model compound first. Methyl lithocholate **3.14** was chosen for these purposes, because of its similarity to the steroids that were going to be used in this reaction.



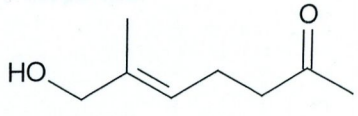
3.14

Unfortunately however, none of the methods using the acid and alcohol gave any of the 3α-triphenylacetate (Table 3.3).

Table 3.3. Attempted conditions for the coupling of triphenylacetic acid with methyl lithocholate.

Substrate	Conditions	Reference
3.14	Triphenylacetic acid, DCC, DMAP, DCM, r.t., 19 h.	3.19
3.14	Triphenylacetic acid, DCC, DMAP, Et_3N, DCM, 40 h.	
3.14	Triphenylacetic acid, CDI, THF, r.t., 24 h, then reflux, 44 h.	3.7
3.14	Triphenylacetic acid, TMSCl, THF, reflux, 18 h.	3.20

It had been noticed from the literature search that triphenylacetyl chloride had been employed in the synthesis of triphenylacetate esters. In particular, there were two reports of triphenylacetates being formed from primary alcohols and triphenylacetyl chloride, albeit in low yields.[3.21, 3.22] For example, Kelly *et al.* reacted the hydroxyketone **3.15** with triphenylacetyl chloride in dry benzene/pyridine, and obtained the corresponding triphenylacetate in 35% yield.[3.22]



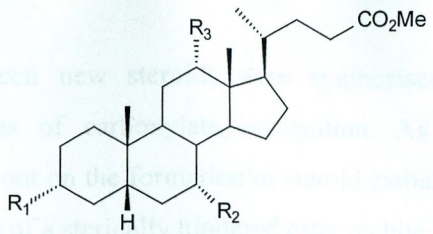
3.15

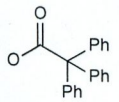
As initial investigations into the direct coupling of the acid with **3.14** had not shown any promise, it was decided not to pursue this line of inquiry. Instead, it was tried to synthesise the triphenylacetate *via* the acid chloride. After several attempts, it was found that the acid chloride was obtained in best yield and purity using a slight modification of the method of Zook [triphenylacetic acid (6g), distilled thionyl chloride (25mL), reflux, 4h], where the crude reaction mixture was poured on to an ice bath, the precipitate filtered off, and recrystallised from cyclohexane.[3.23] This afforded triphenylacetyl chloride (TPACl) in 79% yield. Having made the acid chloride, it was reacted with various steroid substrates (Table 3.4).

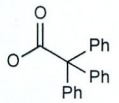
Table 3.4. Reaction conditions for the formation of 3α-triphenylacetates from triphenylacetylchloride and steroid alcohols.

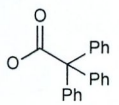
Substrate	TPACl (equivs)	Solvent[1]	DMAP (equivs)	Temp. (°C)	Time	Conc. (M)	Product	Yield[2] (%)
3.14	2.0	Tol/Pyr 3:1	0.47	r.t.	7 d	0.25	**3.16**	25
3.14	1.3	THF	0.15	r.t.	5 d	0.26		-
3.14	4.2	Tol/Pyr 3:1	-	80	2 d	0.17		n.p.
2.12	4.0	Tol/Pyr 7:3	-	80	25 h	0.12	**3.17**	46
2.8	3.0	Tol/Pyr 2:1	-	80	2 h	0.13	**3.18**	17
2.8	4.0	Pyridine	0.75	r.t., 3 d, then 50°C, 1 d		0.22		n.p.
2.8	5.0	Tol/Pyr 7:3	-	80	2 d	0.13	**3.18**	30
3.19	5.0	Tol/Pyr 1:1	-	80	18.5 h	0.11	**3.20**	25

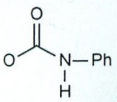
[1]Tol = Toluene; Pyr = Pyridine. [2] n.p. = not purified.

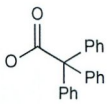
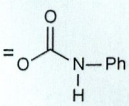


3.16: R_1 =  ; R_2 = H ; R_3 = H

3.17: R_1 =  ; R_2 = OH ; R_3 = OH

3.18: R_1 =  ; R_2 = NHBOC ; R_3 = OH

3.19: R_1 = OH ; R_2 = NHBOC ; R_3 = 

3.20: R_1 =  ; R_2 = NHBOC ; R_3 = 

As can be seen from Table 3.4, this reaction proved to be rather sluggish, with high temperatures and an excess of the acid chloride usually required to obtain the triphenylacetate. As expected, steroids containing several hydroxyl groups (**2.8** and **2.12**) reacted selectively at the less hindered 3α-position. In all cases, a non-polar, UV active by-product was formed in the reaction mixture, which displayed resonances in the aromatic region of the ^{1}H nmr spectrum. In the case of substrates containing an NHBOC group (**2.8** and **3.19**), other steroid by-products appeared in the reaction mixture. Whilst these were not fully identified, it appeared that partial deprotection of the BOC group had occurred in these cases, suggesting that this protective group should be changed in future before carrying out this reaction, or different reaction conditions should be chosen for these substrates.

Although substrates **2.8** and **3.19** gave the triphenylacetate successfully, these were not further elaborated to give the 7α-urea derivatives. In the case of **2.8**, it was decided that the introduction of a phenyl carbamate in the 12α position would prove almost impossible with the presence of the sterically hindered triphenylacetate in the 3α-position. In the case of **3.19**, it was assumed that the deprotection of the BOC group and subsequent formation of the urea was trivial, but this was not carried out because of the small quantities of material available.

3.7 Summary

In summary, thirteen new steroids were synthesised, including three steroid receptors for the purposes of carboxylate recognition. As part of these syntheses, investigations were carried out on the formation of steroid carbamates, and a procedure was developed for the formation of a sterically hindered ester on bile acid derivatives.

Chapter 4

Enantioselective Extraction Experiments and their Analyses

4.1 Preamble

The development of efficient and general methods for separating enantiomers is a continuing challenge in organic chemistry. The biological activity of a drug often depends upon its chirality, so that while one enantiomer of a drug is therapeutic, the other enantiomer may be biologically inactive, or even toxic. As a result, the view currently held is that only the active enantiomer should be present in new pharmaceuticals.

One approach to the separation of enantiomers is to make synthetic receptors that are designed to bind preferentially only one of the enantiomers of a chiral compound. The receptor can then be used to extract selectively the preferred enantiomer from an aqueous phase into an organic phase, leaving the undesired enantiomer behind, and thus separating the two antipodes. Alternatively, the receptor can be covalently bound to silica gel and used as a novel chiral stationary phase (CSP) that can separate enantiomers chromatographically. In this work, it was decided to use a single-extraction methodology to measure the selectivity of the steroid receptors that were synthesised. In this method, the racemic guest is initially present in the aqueous phase, and the chiral receptor in the organic phase. By stirring these two phases together, it was hoped that the chiral receptor would selectively extract only one of the guest enantiomers from the aqueous phase into the organic phase.

This chapter outlines the single-extraction procedures previously used within our laboratory which are of direct relevance to this project, and the adaptation of these extraction procedures in this work to a new procedure suitable for uncharged receptors. The subsequent analyses of these extractions by ^{1}H nmr, chiral HPLC, and mass spectrometry are then discussed.

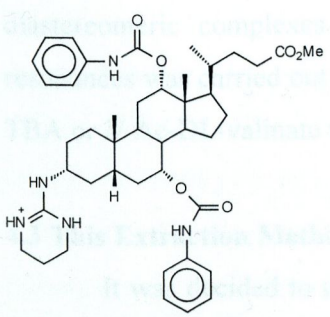
4.2 Single extraction methodologies in the enantioselective recognition of *N*-protected-aminoacid carboxylates.

4.2.1 Contribution from Lawrence Lawless

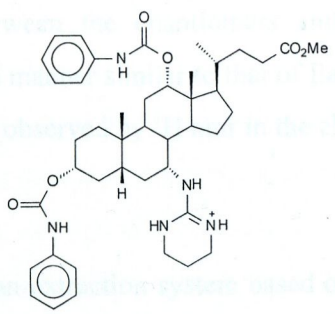
Lawless employed a single extraction method to measure the selectivity of positively charged guanidinium receptors in the recognition of *N*-protected aminoacid carboxylates.[1.17] After a few attempts, he found that the best conditions were obtained using a receptor concentration of 6.4 mM in the organic phase (chloroform), and a guest concentration of 7.7 mM in the aqueous phase [0.1 M KH_2PO_4 / K_2HPO_4 buffer (pH 7.4)]. Using this method, Lawless obtained good enantioselectivities (up to 80% e.e.) and efficiencies (up to 93% extraction) with different steroid receptors. The most important results are summarised in Table 4.1.

Table 4.1. Summary of the most important extraction results obtained by Lawless using guanidinium receptors **4.1H**$^+$, **4.2H**$^+$, and **4.3H**$^+$.

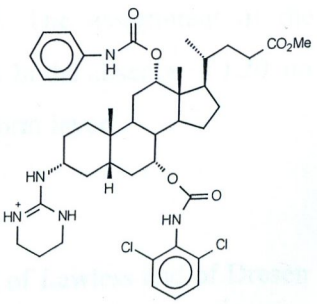
Receptor	Substrate (as the carboxylate)	Ratio (L:D) Extracted	Enantiomeric Excess (%)	Substrate Extracted
4.1H$^+$	*N*-Ac-DL-alanine	7:1	75	52%
4.1H$^+$	*N*-Ac-DL-phenylalanine	7:1	75	87%
4.1H$^+$	*N*-Ac-DL-valine	7:1	75	71%
4.1H$^+$	*N*-Ac-DL-tryptophan	7:1	75	83%
4.2H$^+$	*N*-Ac-DL-valine	13:2	73	50%
4.2H$^+$	*N*-Ac-DL-tryptophan	9:2	63	75%
4.2H$^+$	*N*-BOC-DL-histidine	4:1	60	51%
4.2H$^+$	*N*-BOC-DL-valine	3:1	50	92%
4.2H$^+$	*N*-Cbz-DL-alanine	3:1	50	92%
4.3H$^+$	*N*-Ac-DL-phenylalanine	9:1	80	93%
4.3H$^+$	*N*-Ac-DL-methionine	9:1	80	93%
4.3H$^+$	*N*-Ac-DL-valine	9:1	80	89%
4.3H$^+$	*N*-Ac-DL-alanine	6:1	71	76%
4.3H$^+$	*N*-Ac-DL-tryptophan	6:1	71	92%
4.3H$^+$	*N*-Ac-DL-proline	4:1	60	74%
4.3H$^+$	*N*-BOC-DL-histidine	4:1	60	46%



4.1H$^+$



4.2H$^+$



4.3H$^+$

As can be seen from Table 4.1, the best results obtained were when receptor **4.3H**$^+$ was used in these extractions. Interestingly, receptor **4.2H**$^+$, the 7α-guanidinium homologue to **4.1H**$^+$, displayed slightly poorer selectivity in these extractions to that of **4.1H**$^+$. The measurement of the L:D ratios was carried out using ^{1}H nmr: each of the enantiomers gave rise to discrete resonances due to the formation of diastereomeric complexes with the

receptor. By integrating these resonances, it was possible to obtain a direct comparison of how much of each of the enantiomers had been extracted, relative to one another. The assignment of the resonances was carried out by repeating the extraction using each of the single enantiomers instead of the racemic substrate.

4.2.2 Contribution from Stephan Dresen

Dresen tested the 3α-urea receptor **1.20** in extraction experiments with *N*-Ac-valine.[1.18] This procedure was based on that of Lawless (i.e. a chloroform and phosphate buffer extraction system). However, the experiment differed in that a cation was required to co-extract with the carboxylate, due to the electrical neutrality of the receptor. Initially, the ability of this receptor to extract the potassium salt of *N*-Ac-DL-valine was investigated, but ^{1}H nmr revealed that virtually none of the carboxylate was extracted. After several attempts, it was found that by changing the cation to the more lipophilic tetrabutylammonium cation (TBA), and by including a reservoir of *N*-Ac-valine in the aqueous phase, extraction of TBA *N*-Ac-valinate occurred. Thus, performing the extraction using 3 equivalents of TBA *N*-Ac-DL-valinate (formed *via* *N*-Ac-valine, TBACl and NaOH) and 10 equivalents of *N*-Ac-DL-valine in the aqueous phase and 1 equivalent of **1.20** (6 mM) in the organic phase, led to extraction of *N*-Ac-valine with an L:D ratio of *ca.* 4:1 (62% e.e.). When this extraction was repeated using 6 equivalents of TBA *N*-Ac-DL-valinate instead of 3 equivalents, this selectivity dropped to *ca.* 3:1 (48% e.e.) in favour of the L enantiomer. These measurements of the L:D ratio were carried out by integrating the resonances of the α-CH of valine in the ^{1}H nmr spectrum (L at 4.47 ppm, D at 4.31 ppm) which were split due to the formation of diastereomeric complexes between the enantiomers and **1.20**. The assignment of the resonances was carried out in a manner similar to that of Lawless. In the absence of **1.20**, no TBA or *N*-Ac-DL-valinate was observed by ^{1}H nmr in the chloroform layer.

4.3 This Extraction Method

It was decided to use an extraction system based on that of Lawless and of Dresen (see above), because of the results they obtained. Thus, chloroform was chosen as the organic phase, and the aqueous phase was chosen to be KH_2PO_4 / K_2HPO_4 buffer (0.1 M, pH 7.4). Initially, it was decided to use *N*-Ac-valine as the substrate, but this was changed to *N*-Ac-phenylalanine in subsequent extractions (see later). The principal steroid receptor to be tested in this work was **1.22**, but Dresen's receptor **1.20** was re-synthesised using an advanced intermediate obtained from Lawless (see Experimental), so that comparisons could be drawn between these two receptors. As these steroid receptors are electrically neutral, it was necessary to use a lipophilic cation (tetrabutylammonium cation) to ion-pair

with the carboxylate in these extraction experiments (cf. Dresen's method). Similar to the extraction procedure of Dresen, it was thought that a reservoir of carboxylate in the aqueous phase would be desirable for high selectivity. However, unlike Dresen's method, only one equivalent of lipophilic cation relative to receptor was to be employed to minimise the likelihood of background extraction of (unbound) racemic TBA carboxylate. The salt was to be formed *in situ* from TBAOH and the *N*-protected aminoacid (Dresen had some problems with extraction of chloride in his method, so it was decided to use TBAOH rather than TBACl). Combining these ideas, a typical extraction procedure was used as follows: a solution of NaOH (54 mM) and TBAOH (6 mM) was made up in phosphate buffer (KH_2PO_4 / K_2HPO_4, 0.1 M, pH 7.4). 1 mL of this solution was pipetted into a clean, dry sample tube. 1 mL of a solution of *N*-Ac-DL-aminoacid (60 mM) in the same phosphate buffer was added to the solution in the sample tube *via* pipette. This formed 1 mole equivalent (6×10^{-3} mmol) of TBA *N*-Ac-aminoacid carboxylate and 9 mole equivalents (54×10^{-3} mmol) of sodium *N*-Ac-aminoacid carboxylate *in situ* (relative to the steroid receptor) in the aqueous phase (total volume: 2 mL). The pH of this aqueous phase was 7.4. A solution of steroid receptor (3 mM, 2 mL, 6×10^{-3} mmol - but see Section 4.5) in purified chloroform was pipetted into the sample tube, and the biphasic mixture stirred vigorously for 5 minutes. The two layers were allowed settle for a further 5 minutes. The aqueous layer was mostly removed via Pasteur pipette, and the resulting wet organic layer filtered through hydrophobic filter paper or a PTFE syringe filter (Gellman Acrodisc, 0.45μm) into a round bottomed flask. The chloroform was removed under reduced pressure, and the residue redissolved in a solvent suitable for the analysis technique used.

4.4 Analyses of Extractions by ^{1}H nmr

Due to the success of Lawless and Dresen in using ^{1}H nmr as a means to measure the selectivity of their receptors, it was decided initially to employ this method in this work. It was also decided to try an extraction using Dresen's conditions to provide a direct comparison of the selectivity of the 7α-urea receptor **1.22** with it's 3α-homologue, **1.20**. Thus, an extraction of TBA *N*-Ac-DL-valinate was carried out with **1.22** using the method of Dresen. ^{1}H nmr analysis of the extract showed large downfield shifts (2-3 ppm) of the steroid NH's, indicative of complex formation. In addition, the α-CH of the valinate shifted from 3.75 ppm to *ca.* 4.4 ppm and split into two resonances (L at 4.34 ppm, D at 4.44 ppm), providing further evidence that **1.22** was complexing with the valinate. These resonances were identifed by repeating the extraction with single enantiomers instead of the racemate (cf. Lawless and Dresen). However, the resonance due to the D-enantiomer (4.40-4.45 ppm) was obscured by the broad resonance of the 3β-H (4.41-4.52 ppm) of the steroid, therefore

the L:D ratio could not be determined directly. All other substrate resonances of use in determining this ratio (i.e. acetyl CH_3, NH) were also obscured by the steroid. It was thought that by adding a small amount of a more polar solvent to the nmr sample, that it might be possible to shift either the CH resonances or the 3β-H resonance, so that an accurate integration of the CH resonances could be obtained. While addition of 5% or 10% acetone-d_6 to the nmr sample ($CDCl_3$) shifted both the 3β-H resonance and the resonance due to the L-enantiomer, the D-enantiomer remained obscured (Figure 4.1).

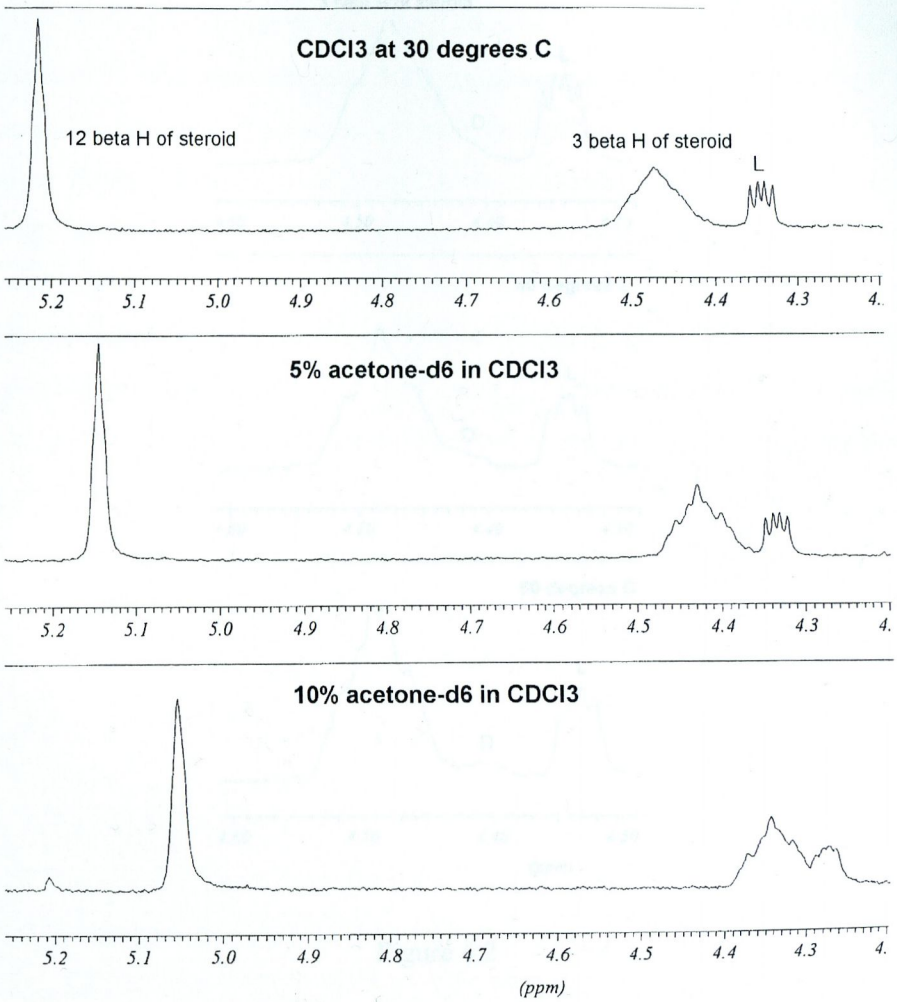


Figure 4.1

Variable temperature experiments were then conducted in $CDCl_3$, in an attempt to see the resonance due to the D-enantiomer. This proved more successful. As the temperature was increased from 30°C to 40°C and 50°C, the resonance due to the D-enantiomer gradually shifted away from the 3β-H resonance, which in turn sharpened slightly (Figure 4.2).

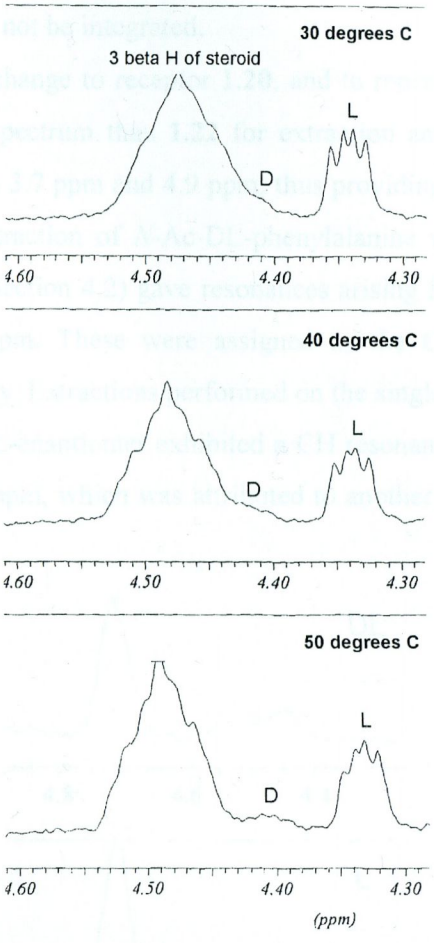


Figure 4.2

This allowed the resonance due to the D-enantiomer to be seen more clearly, but it could not be integrated accurately due to poor signal to noise ratio. As it was considered unwise to allow a sample in $CDCl_3$ to remain at this temperature for a long period of time within the nmr machine, this approach was discontinued. However, it is reasonable to assume that from the ^1H spectrum of the extract at 50°C, that **1.22** displayed a selectivity of *ca.* 3 or 4:1 in favour of the L enantiomer. Variable temperature nmr experiments of the extract in benzene-d_6 did not result in any clearer indication of this selectivity.

It was decided to change the substrate from *N*-Ac-DL-valine to *N*-Ac-DL-phenylalanine because of the difficulties described above, and to try an extraction using the method described in Section 4.2. It was hoped that the change in substrate would give resonances in an area of the ^{1}H nmr spectrum that was not obscured by the steroid. When this extraction was performed, shifts due to both the substrate and receptor resonances were observed in the ^{1}H nmr of the extract. However, this time the resonances of the two diastereomeric complexes formed between the two enantiomers and **1.22** were not well-separated, and hence could not be integrated.

It was decided to change to receptor **1.20**, and to repeat this extraction. **1.20** has a more favourable ^{1}H nmr spectrum than **1.22** for extraction analyses, in that there are no steroid resonances between 3.7 ppm and 4.9 ppm, thus providing a 'clear window' for guest resonances to fit into. Extraction of *N*-Ac-DL-phenylalanine with **1.20** using the typical procedure for extraction (Section 4.2) gave resonances arising from the guest between 4.4-4.8 ppm and 5.75-6.15 ppm. These were assigned as the CH and NH resonances of phenylalaninate respectively. Extractions performed on the single enantiomers instead of the racemate showed that the L-enantiomer exhibited a CH resonance at 4.71 ppm and a much smaller resonance at 4.52 ppm, which was attributed to another bound conformation of the guest (Figure 4.3).

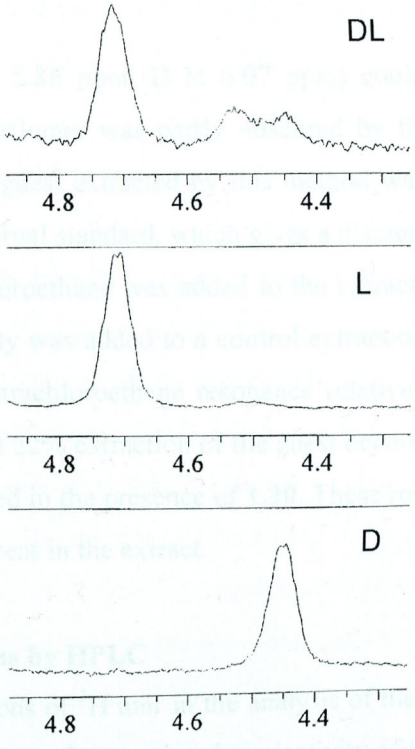


Figure 4.3

The D-enantiomer exhibited only one CH resonance at 4.45 ppm. Integration of these three resonances (4.71 ppm, 4.52 ppm, 4.45 ppm) gave ratios of 100:37:33 respectively, or *ca.* 4:1 (61% e.e.) in favour of the L-enantiomer (Figure 4.4).

Group	Peak	Start (ppm/Hz)	End (ppm/Hz)	Area	Area %
1		4.62 1847.1	4.36 1742.7	1.8376E+007	100.00
	1	4.62 1847.1	4.48 1793.9	9.7672E+006	53.15
	2	4.48 1793.9	4.36 1742.7	8.6091E+006	46.85

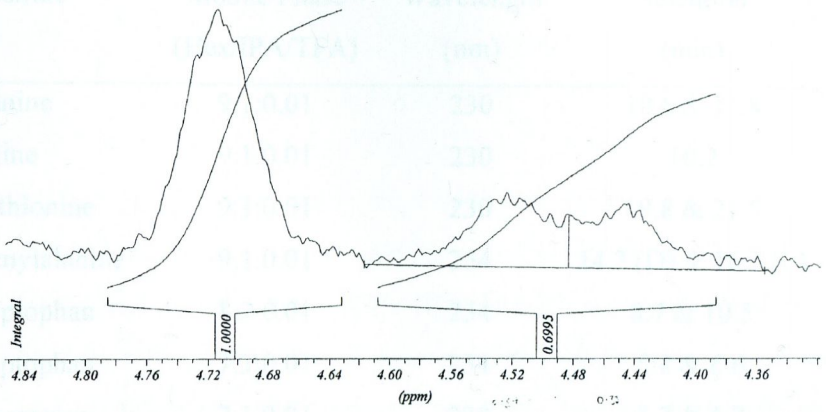


Figure 4.4

The NH resonances (L at 5.86 ppm, D at 6.07 ppm) could also be compared, but the resonance from the D-enantiomer was partly obscured by the 'mid-point' of the ^{1}H nmr spectrum. The quantity of guest extracted by this method was calculated by using 1,1,2,2-tetrachloroethane as an internal standard, which gives a discrete singlet at 6.0 ppm. A known quantity of 1,1,2,2-tetrachloroethane was added to the extraction carried out using racemic guest, and the same quantity was added to a control extraction performed in the absence of **1.20**. By integrating the tetrachloroethane resonance relative to the CH resonances of the guest, it was calculated that 22% extraction of the guest occurred in the absence of **1.20**, and that 63% extraction occurred in the presence of **1.20**. These results raised the possibility that unbound substrate was present in the extract.

4.5 Analyses of Extractions by HPLC

Due to the limitations of ^{1}H nmr in the analysis of these extractions, it was decided to try chiral HPLC as a means of measuring the selectivity of these extractions. Thus, it was necessary to see if a chiral column (Daicel Chiralpak AD) could separate the enantiomers of *N*-protected aminoacids. The results are listed in Table 4.2. While initially there were some

reservations about running *N*-protected aminoacids in normal phase mode (the conditions recommended by the manufacturers) for solubility reasons, it was found that at the concentration level used (1-3 mg / mL) that this did not pose a significant problem. Furthermore, the use of *iso*-propanol as injection solvent did not seem to show any adverse effects on the analysis.

Table 4.2. Resolution of *N*-protected-aminoacids in normal phase mode on a Chiralpak AD column (4.6 mm I.D. x 250 mm) with guard column (4.6 mm I.D. x 50 mm).

Substrate	Mobile Phase (Hex/IPA/TFA)	Wavelength (nm)	Retention (min)	Selectivity (α)
N-Ac-Alanine	9:1:0.01	230	10.6 & 11.8	1.18
N-Ac-Valine	9:1:0.01	230	10.2	0
N-Ac-Methionine	9:1:0.01	230	19.8 & 21.5	1.11
N-Ac-Phenylalanine	9:1:0.01	254	14.2 (D) & 16.7 (L)	1.23
N-Ac-Tryptophan	8:2:0.01	254	8.7 & 10.5	1.61
N-Ac-Tryptophan	7:3:0.01	254	5.8 & 6.6	1.35
N-Ac-Asparagine	7:3:0.01	230	4.7 & 6.8	1.5[1]
N-Cbz-Alanine	9:1:0.01	254	15.4	0
N-Cbz-Valine	9:1:0.01	254	19.1	0
N-Cbz-Serine	9:1:0.01	254	21.5 & 28.4	1.38

All flow rates are 1.0 mL/min. Injection solvent is *iso*-propanol. Substrate concentration is 1-3 mg/mL. [1]This is exaggerated because of fronting of the first peak.

As can be seen from Table 4.2, this chiral column was most effective at separating the enantiomers of *N*-Ac-phenylalanine, *N*-Ac-tryptophan, and *N*-Cbz-serine. Due to the work already done with *N*-Ac-phenylalanine in extractions, it was decided to use this substrate again, and to analyse the result by chiral HPLC to see if the result concurred with that obtained using ^{1}H nmr. Therefore, each of the single enantiomers were injected on HPLC to see which of the enantiomers eluted first (Table 4.2).

Thus, an extraction of *N*-Ac-DL-phenylalanine with **1.20** was carried out in the manner described above. HPLC of the extract using the conditions described in Table 4.2 for *N*-Ac-phenylalanine analysis, gave four peaks in the area of interest, at retention times of 12.7, 13.6, 14.8 and 16.2 minutes (Figure 4.5). These retention times differ from those quoted in Table 4.2 because a different HPLC instrument was used for these analyses.

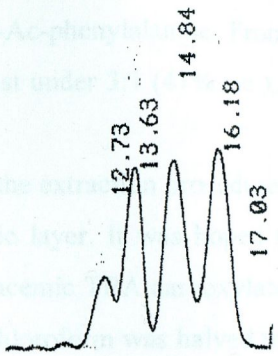


Figure 4.5

These peaks had peak height ratios of 1.05:2.75:2.9:3.05 (the peaks were partially overlapping, so the integration proved unreliable). In an attempt to identify which of these peaks were belonging to the guest, *N*-Ac-L-phenylalanine was co-injected with the extract. This resulted in an enlargement of the third peak of this series only (now appearing at 14.3 minutes - in normal phase HPLC peaks can show appreciable shifts due to small concentration differences in the mobile phase), showing that this peak can be attributed to the L-enantiomer (Figure 4.6).

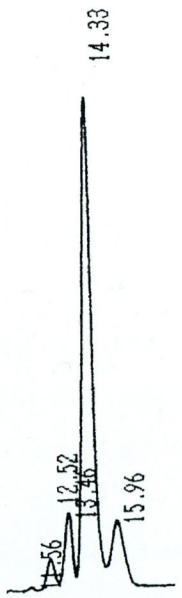
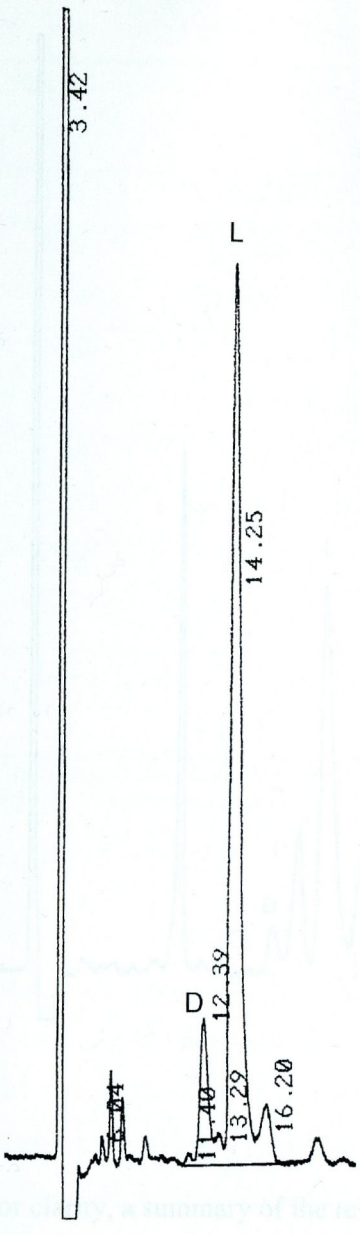


Figure 4.6

Subsequently, it was shown through a similar experiment that the first peak in the series corresponded to the D-enantiomer. Although the other two peaks were not identified, they did not seem to belong to the *N*-Ac-phenylalanine. From the peak height ratios, it can be calculated that the L:D ratio is just under 3:1 (47% e.e.), lower than that obtained using ^{1}H nmr as the analysis technique.

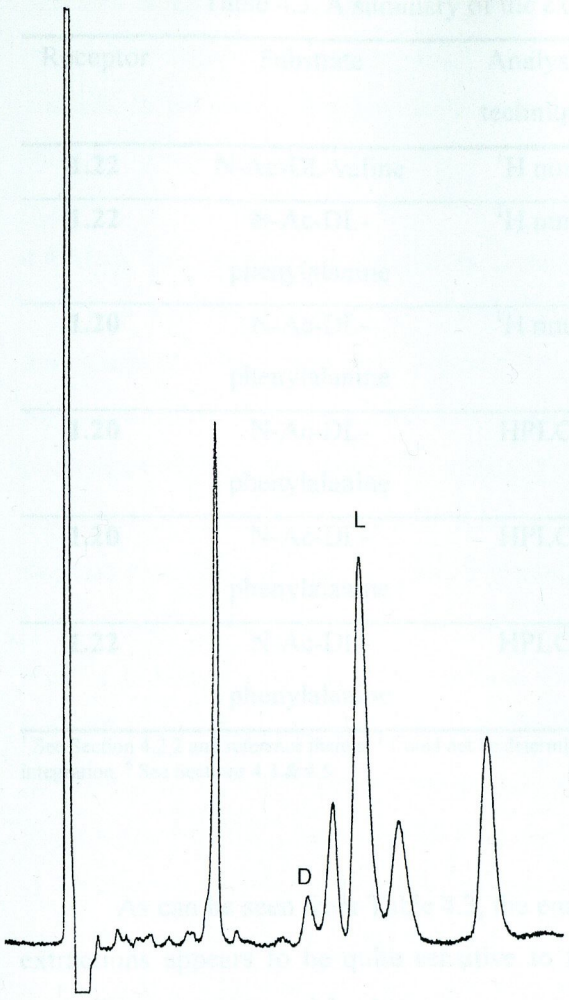
It was decided to change the extraction procedure slightly, by decreasing the volume of chloroform used in the organic layer. It was hoped that this change would result in a lower background extraction of racemic TBA carboxylate, and hence increase the measured selectivity. Thus, the volume of chloroform was halved to 1 mL from 2 mL, but the absolute quantity of receptor was not changed. This represents a net increase in concentration of the receptor in the chloroform layer from 3 mM to 6 mM, a similar concentration to that used by Lawless and Dresen in their extractions. The aqueous phase was not changed. It was decided to try the *N*-Ac-DL-phenylalanine extraction again under these conditions using **1.20** as the receptor. In this case, HPLC analysis of the extract again revealed 4 peaks in the area of interest (retention times 12-17 min.), but the two peaks identified as that of the guest enantiomers (at retention times 12.4 & 14.3 min.) appeared to be greater in size than the other two peaks. Moreover, this time a greater difference in peak size between the L and D enantiomers was evident. Integration of these peaks gave a L:D ratio of *ca.* 7:1 (74% e.e.), with the peak height ratios giving good agreement at 72% e.e. (Figure 4.7).



	PEAK#	AREA%	RT	AREA	BC
	1	51.453	3.42	1700145	01
	2	30.765	4.65	1016545	01
D	3	2.112	12.39	69793	02
L	4	14.575	14.25	481589	02
	5	1.095	16.2	36175	03
	TOTAL	100.		3304247	

Figure 4.7

Having found that HPLC provided a means of determining selectivity in these extractions, it was decided to return to using receptor **1.22** in extractions. Previously, extractions carried out using **1.22** had proven difficult to analyse by ^{1}H nmr. Thus, an extraction (new procedure – extraction method 2 in Table 4.3) was carried out with **1.22** using *N*-Ac-DL-phenylalanine as substrate. HPLC analysis of this extract showed four peaks as previously described (Figure 4.8). In this case, integration of the peaks corresponding to the L and D enantiomers of the guest (retention times 12.6 & 14.6 min.) gave a L:D ratio of *ca.* 12:1 (84% e.e.) with the peak height ratios appearing at slightly less (77% e.e.).



	PEAK#	AREA%	RT	AREA	BC
	1	50.991	3.41	1039968	01
	2	13.778	4.56	281000	02
	3	3.733	4.67	76131	03
	4	0.318	6.2	6492	03
	5	6.038	9.06	123142	03
D	6	0.928	12.59	18931	02
	7	3.098	13.57	63181	02
L	8	11.226	14.59	228950	02
	9	4.037	16.1	82332	03
	10	5.853	19.53	119367	01

Figure 4.8

For clarity, a summary of the results of the extractions carried out is given in Table 4.3.

Table 4.3. A summary of the extraction results from this work.

Receptor	Substrate	Analysis technique	L:D ratio	Enantiomeric Excess (%)	Extraction Method
1.22	N-Ac-DL-valine	^{1}H nmr	3-4:1	50-60	Dresen[1]
1.22	N-Ac-DL-phenylalanine	^{1}H nmr	-[2]	-	1[3]
1.20	N-Ac-DL-phenylalanine	^{1}H nmr	*ca.* 4:1	61	1
1.20	N-Ac-DL-phenylalanine	HPLC	*ca.* 3:1	47[4]	1
1.20	N-Ac-DL-phenylalanine	HPLC	*ca.* 7:1	74[5]	2[6]
1.22	N-Ac-DL-phenylalanine	HPLC	*ca.* 12:1	84[5]	2

[1] See Section 4.2.2 and reference therein. [2] Could not be determined. [3] See Section 4.3. [4] From peak height ratio. [5] From integration. [6] See Sections 4.3 & 4.5.

As can be seen from Table 4.3, the enantiomeric excesses obtained in these types of extractions appears to be quite sensitive to the conditions used. It is thought that using method 1, some unbound (and hence racemic) TBA carboxylate is present in the extract, and that the presence of this lowers the measured selectivity. By decreasing the amount of chloroform used, the measured selectivity increases. This suggests that less unbound TBA carboxylate is being extracted into the organic layer. It will be important in the future to optimise these conditions to ensure that no extraction of unbound TBA carboxylate occurs.

It is interesting to note that the result obtained with the neutral 3α-urea **1.20** using *N*-Ac-phenylalanine as substrate, compares favourably with the result Lawless obtained with his positively charged 3α-guanidinium receptor, **4.1H**$^+$, albeit under a different set of conditions. Unfortunately, Lawless did not perform an extraction with *N*-Ac-phenylalanine using his 7α-guanidinium receptor **4.2H**$^+$ to compare with the result obtained with **1.22**.

Although it was possible to measure the selectivity of these extractions using the currently available chiral HPLC column (Daicel Chiralpak AD) running in normal phase mode, this method did have the disadvantage of long analysis times (*ca.* 2-2½ hours per run). This was due to a slow-running peak, which was presumed to be the steroid receptor. In order to avoid this in future, it is suggested that a suitable sample preparation be used (e.g. solid-phase extraction) before injection into the HPLC, or that a chiral column that

operates in the reverse phase mode be employed. If the latter case is chosen, it may be possible to separate substrates which cannot be resolved using the Daicel Chiralpak AD column. In the future, it will also be important to validate the results of these extractions using a different analytical technique.

4.6 Mass Spectrometry as an analytical tool for the measurement of selectivity

It was decided to try electrospray mass spectrometry as an analytical tool for the measurement of selectivity, and to attempt to apply the method to the analysis of extractions. Mass spectrometry has significant advantages over both ^{1}H nmr and HPLC in this respect because of its increased sensitivity, which allows for the possibility of measuring very small quantities of material (typically 10^{-8}-10^{-9} g for ES-MS with a TOF analyser). Moreover, because each chemical species gives a discrete peak corresponding to its mass/charge ratio (*m/z*, *z* usually 1 for small molecules), it was hoped that any undesired species present in the mixture to be analysed would not interfere with the ability to measure a peak at the mass of interest. In this work, the idea was to deuterate L-phenylalanine with an acetyl-d_3 group to form *N*-Ac-d_3-L-phenylalanine, and to make a pseudoracemate (i.e. a 1:1 mixture) between this enantiomer and its non-deuterated antipode, *N*-Ac-D-phenylalanine. This would result in a difference of three units in the mass spectrum between these two species, thus allowing the mass spectrum to 'see' each enantiomer. Integration of these peaks would then allow for a comparison of how much of each pseudoenantiomer was present, and hence the selectivity could be calculated.

In order to test this hypothesis, it was necessary first of all to optimise the parameters under which the mass spectrum was obtained. TBA *N*-Ac-DL-phenylalaninate was chosen as the test substrate. The electrospray mass spectrometer was operated in negative mode so that the negatively charged carboxylate could be detected. After many attempts, the optimum conditions for detecting *N*-Ac-phenylalaninate were found (Table 4.4). The most important parameters for this detection were found to be the flow rate of the mobile phase, and to a lesser extent, the sample cone voltage of the spectrometer.

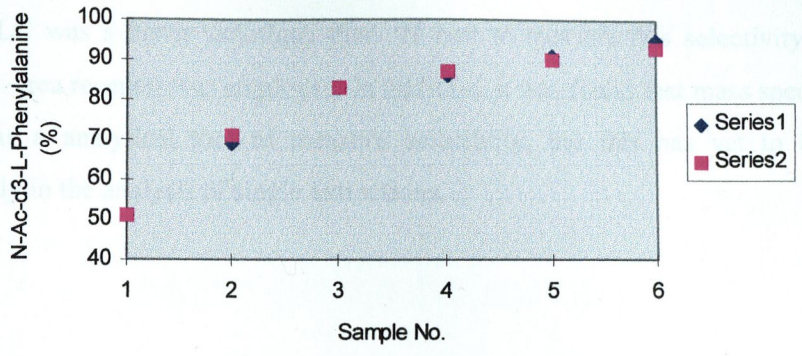
Table 4.4. Optimum conditions for the detection of *N*-Ac-phenylalaninate in ES-MS.

Mode	negative
Capillary Voltage	3,500 V
Sample Cone Voltage	32 V
Extraction Cone Voltage	5 V
Source Temperature	100°C
Desolvation Temperature	120°C
Mobile Phase	MeOH
Flow Rate	0.020 mL/min.
Injection Solvent	MeOH
Injection Volume	50μL[1]
Sample Concentration	2.0ng/μL

[1] Sample loop is 25μL, so only half this amount goes into the spectrometer.

Using the conditions described in Table 4.4, it was possible to detect a peak at *m/z* 206 for *N*-Ac-phenylalaninate (the corresponding cation, TBA, was easily detected at *m/z* 242 in the positive mode). When a sample of *N*-Ac-d_3-phenylalanine was injected, a peak at *m/z* 209 was observed. Having been able to detect the two pseudoenantiomers individually by mass spectrometry, it was decided to make up a series of solutions in which the ratio of the two pseudoenantiomers varied by a known amount. It was hoped that it would be possible to determine this ratio using mass spectrometry. Thus, a series of solutions containing *N*-Ac-d_3-L-phenylalanine and *N*-Ac-D-phenylalanine in different percentages were made up and injected into the mass spectrometer. The two peaks at *m/z* 206 and *m/z* 209 were integrated (8 integrations were performed per peak and an average was taken), and these values compared with those of theory. The results are shown in Graph 4.1.

Graph 4.1. Graph of theoretical (Series 1) versus observed (Series 2) percentages of N-Ac-d3-L-phenylalanine in different samples as measured by ES-MS.



As can be seen in Graph 4.1, the percentages obtained from the integration of the two peaks at *m/z* 206 and *m/z* 209 agree well with those of theory. This shows that mass spectrometry can be used to measure different ratios of pseudoenantiomers, and hence determine selectivity. It was decided to attempt to apply this method to the extraction system already described. Thus, an extraction was carried out in the usual manner, except that a pseudoracemate of *N*-Ac-d_3-L-phenylalanine and *N*-Ac-D-phenylalanine was used instead of the usual *N*-Ac-DL-phenylalanine. Unfortunately however, no meaningful result could be interpreted from the mass spectrum that was acquired from this extraction. It is not clear why this was the case, but it is hypothesised that the presence of a large concentration of acid (relative to the sample) in the injection port of the mass spectrometer made it impossible to detect the substrate in negative mode (the mass spectrometer was regularly cleaned with a formic acid and methanol solution). Due to time constraints, it was not possible to develop this method any further. However, it is hoped that this technique will be more successful in the future, and that it's sensitivity can be exploited to determine the selectivity of a combinatorial library of receptors.

4.7 Summary

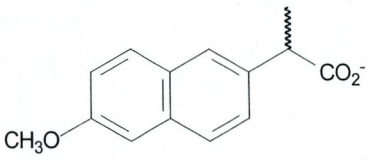
In summary, a new extraction procedure based on that of Lawless and Dresen was developed. The best result obtained was in the extraction of TBA *N*-Ac-phenylalaninate with receptor **1.22**, where an enantiomeric excess of 84% was achieved. It was found that chiral HPLC was a better technique than ^{1}H nmr to measure this selectivity, particularly when a 7α-urea receptor was employed. In addition, it was found that mass spectrometry can be used as a analytical tool to measure selectivity, but this has yet to be employed successfully in the analysis of single extractions.

Chapter 5

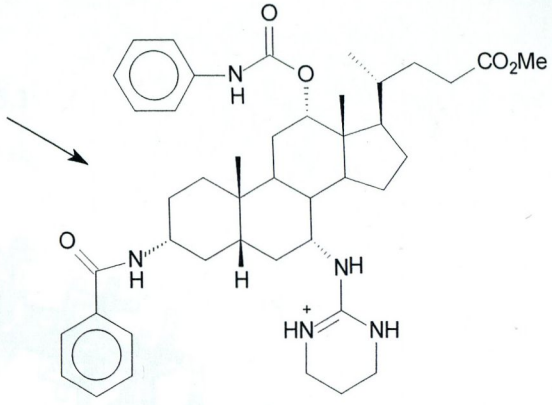
The Design, Synthesis, and Testing of a Steroid Receptor for the Molecular Recognition of Naproxenate

5.1 Preamble

Although this work focused principally on the synthesis of receptors for the recognition of *N*-acetyl-α-aminoacid carboxylates, it was decided to make a receptor specifically for Naproxenate **5.1**. Computer based molecular modelling studies (MacroModel version 5.0, AMBER forcefield, GB/SA solvation in chloroform, Batch minimisation), suggested that receptor **5.2** has a number of features suitable for the recognition of Naproxenate (see Figure 5.1). In particular, it can be seen from the modelling that the relatively planar Naproxenate can slide in between the functional groups at the 3α and 12α positions of the steroid (direction of arrow). The main interaction between the steroid and guest molecule is the stabilisation of the carboxylate group of **5.1** by the guanidinium group at the 7α position and the amide NH at the 3α position of **5.2**. However, in the case of the *S*-enantiomer of **5.1**, this hydrogen-bonding interaction is complemented by an aromatic stacking interaction between the phenyl ring of the 3α-amide and the naphthalene group of the Naproxenate (Figure 5.1). This stabilisation is not seen in the case of the *R*-enantiomer (Figure 5.2).



5.1



5.2

From this modelling, it can also be seen that the NH of the 12α-carbamate does not participate in hydrogen bonding with the guest carboxylate. However, this group does seem to fulfil a steric requirement, in that it limits the binding conformations of the guest. The molecular modelling suggests that this particular array of groups on the steroid is more favourable for binding to the *S*-enantiomer of **5.1** than the *R*-enantiomer. This is due to the aromatic stacking interaction already described, and because of unfavourable steric interactions occurring between the methyl group of the *R*-enantiomer and the steroid backbone. This suggests that a rationally designed receptor, such as **5.2**, could differentiate significantly between the enantiomers of **5.1**.

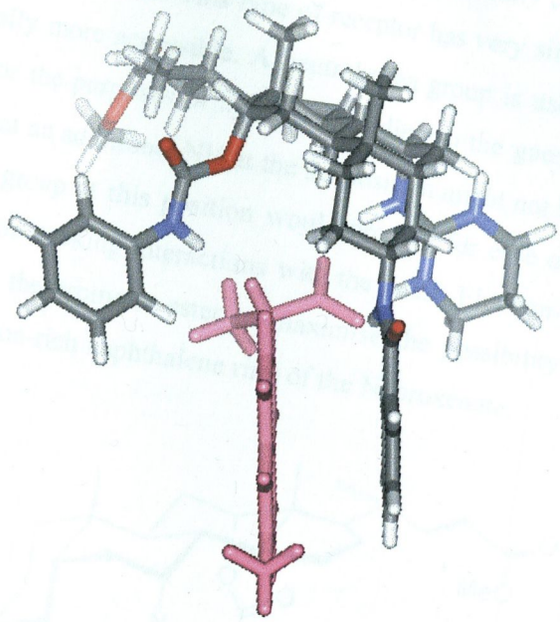


Figure 5.1

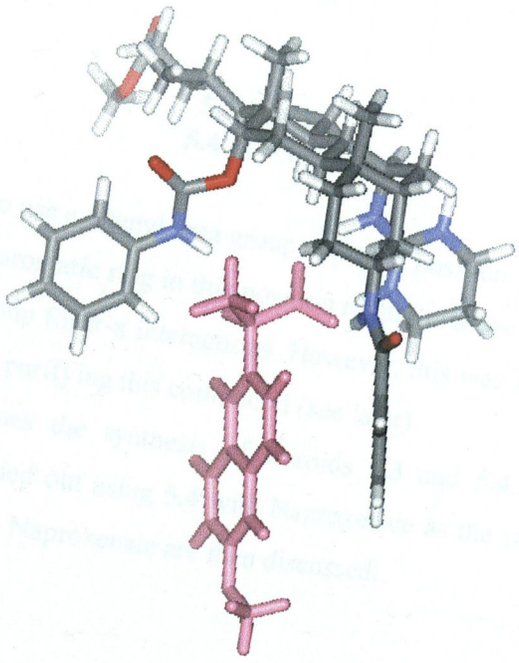
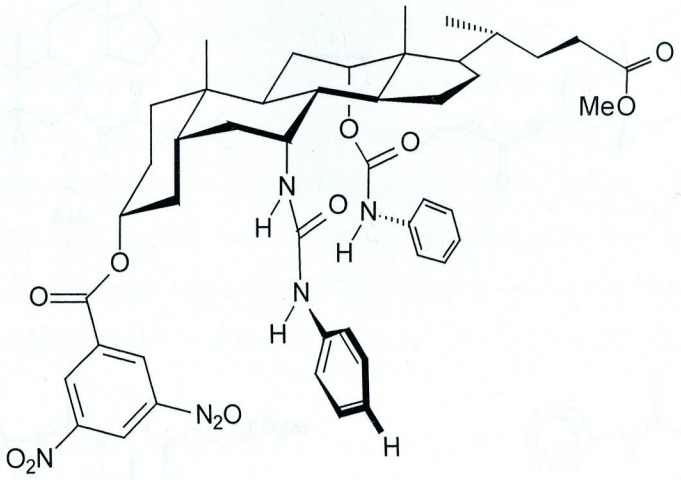


Figure 5.2

Due to the ready availability of steroids such as **2.8** and **2.16**, and because the focus of this work was on the recognition of carboxylates with neutrally charged receptors, it was decided to synthesise receptor **5.3**. This type of receptor has very similar features to that of **5.2**, but is synthetically more accessible. A neutral urea group is used in **5.3** in place of a guanidinium group for the purposes of hydrogen bonding to the guest carboxylate. At this time it was thought that an additional NH at the 3α position might not be necessary. Instead, formation of an ester group at this position would provide for ease of synthesis, and still provide a framework for stacking interactions with the guest. Electron-withdrawing groups were incorporated into the aromatic ester to maximise the possibility of this interaction occurring with the electron-rich naphthalene ring of the Naproxenate.



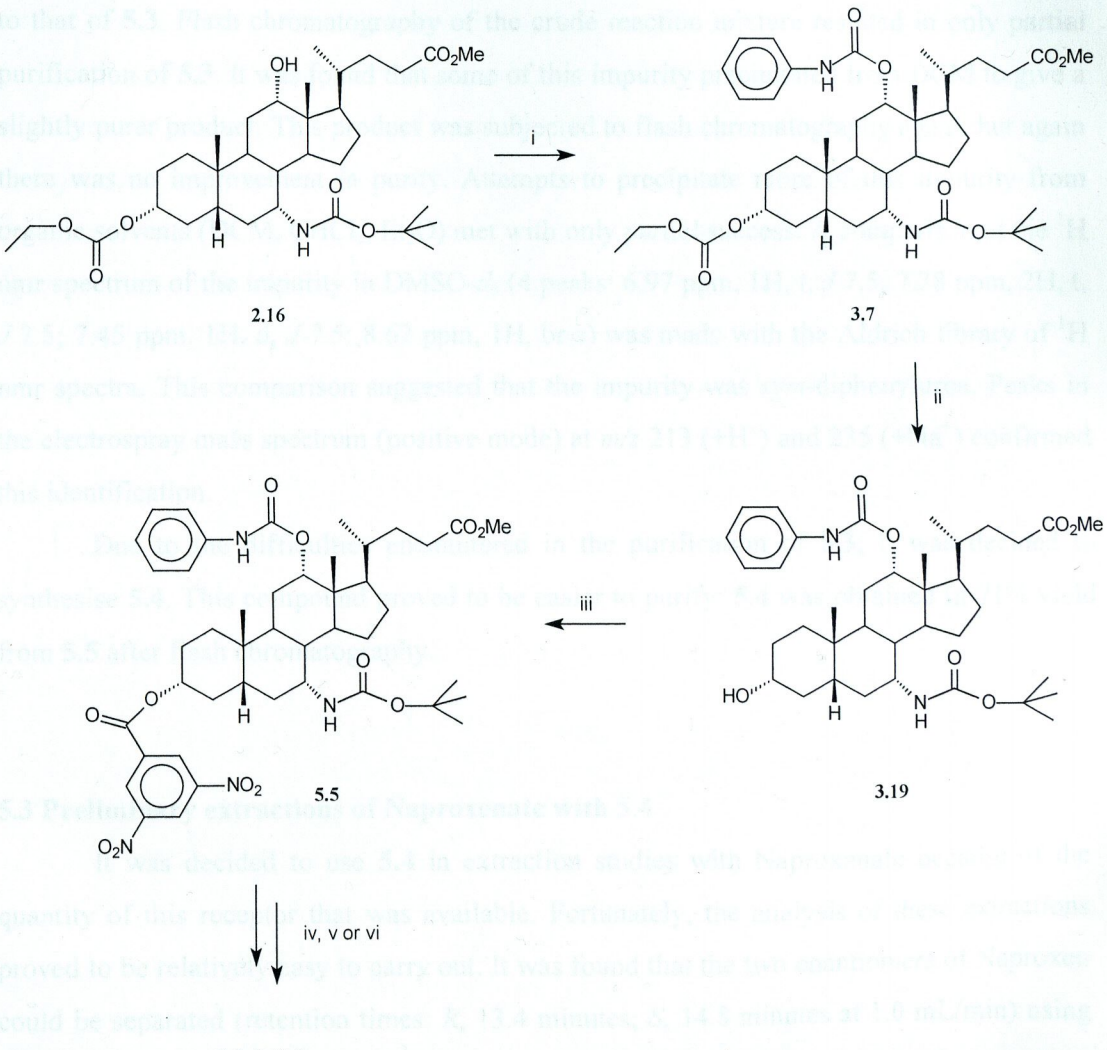
5.3: R = H.
5.4: R = CF_3.

Initially, it was decided to use a phenyl urea group at the 7α position, because it was thought that an electron deficient aromatic ring in this position might 'confuse' the Naproxenate into choosing this aromatic group for π-π interactions. However, this was subsequently changed because of the difficulty in purifying this compound (see later).

This chapter outlines the synthesis of steroids **5.3** and **5.4**, and describes the preliminary extractions carried out using **5.4** with Naproxenate as the substrate. Molecular modelling studies of **5.4** with Naproxenate are then discussed.

5.2 Synthesis of receptors for Naproxenate

Initial attempts to selectively esterify the 3α position of 7α-NHBOC diol **2.8** with 3,5-dinitrobenzoyl chloride were not very successful, giving mixtures of the 3α and 12α esters. After a few attempts, this approach was abandoned in favour of the use of the 3α-protected steroid **2.16** (Scheme 5.1 - see Chapter 2 for the synthesis of **2.16**). Thus, reaction of **2.16** with phenylisocyanate in 1,2-dichloroethane gave the 12α-phenyl carbamate **3.7** in 34% yield (See Section 3.5).



Scheme 5.1. (*Reagents, conditions, and yields*): i, PhNCO, TMSCl, 1,2-dichloroethane, 40°C, 90 h, 34%; ii, 2M NaOMe in MeOH, r.t., 1 h, 79%; iii, 3, 5-dinitrobenzoyl chloride, pyridine, r.t., 3 h, 75%, iv, TFA, DCM, r.t., 2 h; v, Et_3N, PhNCO, DCM; vi, DIPEA, Et_3N, α,α,α-trifluoro-*p*-tolylisocyanate, r.t., 4 d, 71% from **5.5**.

Deprotection of the 3α-ethyl carbonate group of **3.7** was best achieved with a solution of sodium methoxide in methanol. When this reaction mixture was acidified with mineral acid, a small amount of BOC deprotection resulted. It was found that a solution of ammonium chloride in methanol could be employed instead of mineral acid without adversely affecting either the BOC or methyl ester groups. The 3α-alcohol of **3.19** was esterified with 3,5-dinitrobenzoyl chloride in pyridine to give **5.5** in 75% yield. Removal of the BOC group with trifluoroacetic acid in DCM, followed by reaction of the amine with phenylisocyanate gave the 7α-phenyl urea derivative, **5.3**. This particular compound proved exceedingly difficult to purify, due to the presence of a UV-active impurity with similar R_f to that of **5.3**. Flash chromatography of the crude reaction mixture resulted in only partial purification of **5.3**. It was found that some of this impurity precipitated from DCM to give a slightly purer product. This product was subjected to flash chromatography again, but again there was no improvement in purity. Attempts to precipitate more of this impurity from organic solvents (DCM, $CHCl_3$, Et_2O) met with only partial success. A comparison of the ^1H nmr spectrum of the impurity in DMSO-d_6 (4 peaks: 6.97 ppm, 1H, t, *J* 7.5; 7.28 ppm, 2H, t, *J* 7.5; 7.45 ppm, 1H, d, *J* 7.5; 8.62 ppm, 1H, br s) was made with the Aldrich library of ^1H nmr spectra. This comparison suggested that the impurity was *sym*-diphenylurea. Peaks in the electrospray mass spectrum (positive mode) at *m/z* 213 ($+H^+$) and 235 ($+Na^+$) confirmed this identification.

Due to the difficulties encountered in the purification of **5.3**, it was decided to synthesise **5.4**. This compound proved to be easier to purify: **5.4** was obtained in 71% yield from **5.5** after flash chromatography.

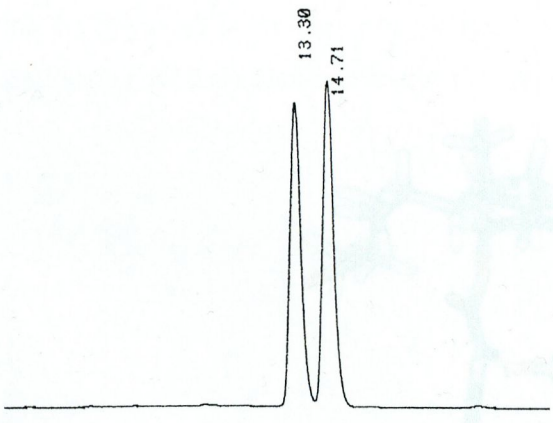
5.3 Preliminary extractions of Naproxenate with 5.4

It was decided to use **5.4** in extraction studies with Naproxenate because of the quantity of this receptor that was available. Fortunately, the analysis of these extractions proved to be relatively easy to carry out. It was found that the two enantiomers of Naproxen could be separated (retention times: *R*, 13.4 minutes; *S*, 14.8 minutes at 1.0 mL/min) using the Daicel Chiralpak column that was previously employed for the analysis of extractions. This technique was sensitive enough to detect 0.9 % of the *R*-enantiomer in a sample of "pure" (*S*)-Naproxen.

The extractions of Naproxenate were carried out using a method similar to that employed for the extractions of *N*-acetyl-α-aminoacid carboxylates with steroid receptors (see Chapter 4). Racemic Naproxen (13.8 mg) was dissolved in a solution (1 mL) of NaOH (54 mM) and TBAOH (6 mM) in phosphate buffer (KH_2PO_4 / K_2HPO_4, pH 7.4, 0.1 M) and

pure phosphate buffer (1 mL) with the aid of a little heating. The solution was allowed cool to room temperature (20.5°C), and a solution of **5.4** (5.5 mg) in HPLC grade $CHCl_3$ (1 mL) was added *via* pipette. The sample tube containing the solution of **5.4** was rinsed quickly with $CHCl_3$ (1 mL), and this was also added to the extraction viol. A bright fluorescent yellow colour was visible in the chloroform layer when both layers were stirred together. The biphasic mixture was stirred vigorously for five minutes, then allowed stand for five minutes. The aqueous layer was mostly removed using a Pasteur pipette, and the coloured $CHCl_3$ layer filtered through a syringe filter (Gellman Acrodisc, PTFE, 0.2 μm) which had been preconditioned by flushing with $CHCl_3$ (6 mL). The $CHCl_3$ layer was concentrated *in vacuo*, and diluted for HPLC analysis using the mobile phase solvent.

Unfortunately, analysis of this extraction using HPLC showed that only a small enantiomeric excess had been generated (3.2 % in favour of the *S*-enantiomer). It was thought that a lot of the reservoir of Naproxenate in the aqueous phase had come across into the chloroform layer during this extraction, and that this had resulted in reduced selectivity. In an effort to increase the selectivity, the quantity of Naproxen used was decreased from 13.8 mg (10 equivalents) to 1.6 mg (1.15 equivalents), and the extraction repeated. This resulted in a slight increase of the enantiomeric excess to 6.6 % in favour of the *S*-enantiomer (Figure 5.3).



PEAK#	AREA%	RT	AREA BC	%	e.e. (%)
1	46.681	13.3	5848393 02	46.7	
2	53.319	14.71	6679952 03	53.3	6.6
TOTAL	100.		12528345		

Figure 5.3

Attempts to perform an extraction using a saturated sodium bicarbonate solution as the aqueous phase failed because of the insolubility of Naproxen in this medium.

5.4 Molecular modelling studies of 5.4 with Naproxenate

Computer based molecular modelling studies using MacroModel (Version 5.0) on a Silicon Graphics workstation were carried out between **5.4** and the two enantiomers of Naproxenate. Two successive minimisations (500 iterations) were carried out individually on the receptor and guest molecules, followed by a Monte Carlo conformational search (1000 iterations) on the complex formed between **5.4** and each enantiomer. The AMBER forcefield was employed in these studies, with Polak-Ribière Conjugate Gradient minimisation, and GB/SA solvation in chloroform.[5.1, 5.2]

In the case of the *S*-enantiomer, the Monte Carlo conformational search revealed 172 unique conformations, with only 3 minimised with good convergence. The lowest energy conformer (- 488.17 kJ/mol^{-1}) is shown in Figure 5.4. As can be seen from Figure 5.4, the carboxylate is hydrogen bonding to the NHs of the 7α-urea group, and the NH of the 12α-carbamate, which is a different binding motif to that observed in the modelling studies with **5.2**. In addition to the expected π-π interaction of the naphthalene ring of Naproxen with the electron deficient aromatic ester, it can also be seen that there is another stacking interaction with the phenyl ring of the 12α-phenyl carbamate. This effectively forms a 'sandwich' of Naproxenate between the phenyl rings of the functional groups at the 3α and 12α positions of **5.4**. This additional stacking interaction was not observed in the case of **5.2**.

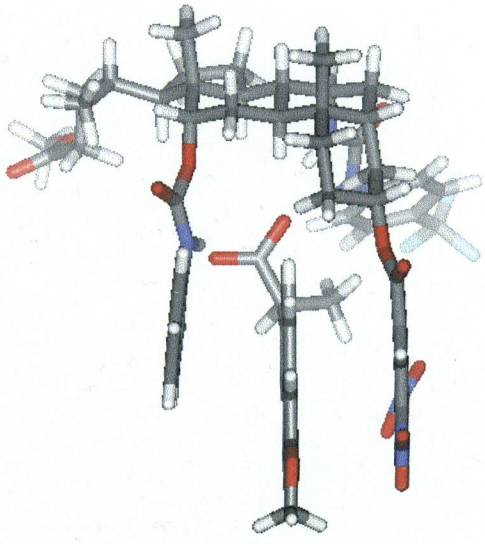


Figure 5.4

In the case of the *R*-enantiomer, the Monte Carlo conformational search revealed 174 unique conformations, with 9 minimised with good convergence. Of these 9 conformations, only 6 showed the stacking interaction between the Naproxenate and the 3α-ester (including the lowest energy conformer at – 486.88 kJ/mol^{-1}), and only 2 of these 6 showed the additional stacking interaction with the 12α-phenyl carbamate. Three of the 9 minimised conformations showed stacking with the phenyl group of the 7α-urea instead of with the 3α-ester, suggesting that in the modelling at least, that there is some confusion over which site this stacking interaction should occur.

5.5 Conclusion

Comparisons can be made between the results obtained from the extraction experiment and those obtained using molecular modelling. Neither of the two phases in the extraction experiment is coloured at the concentration used (the receptor is a pale yellow solid), yet when mixed, a very bright yellow colour is observed in the chloroform layer only. This lends credence to the formation of a π-π interaction between **5.4** and **5.1**, as seen in the molecular modelling. In the molecular modelling, the lowest energy conformation (- 486.88 kJ/mol^{-1}) of the *R*-enantiomer of **5.1** with **5.4** is very similar in energy to the complex formed between **5.4** and the *S*-enantiomer of **5.1** (- 488.17 kJ/mol^{-1}). This suggests that there should be very little selectivity for either Naproxenate enantiomer using **5.4**. This is borne out in the extraction experiments, where only *ca.* 6 % e.e. was obtained. It is hoped that this selectivity can be improved in the future by the use of another steroid receptor, such as **5.2**, and/or by optimising the extraction conditions.

Chapter 6

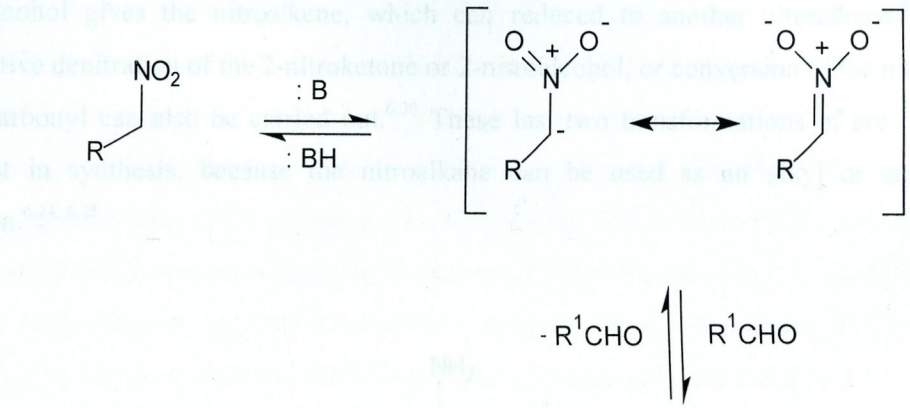
An Introduction to Selectivity in the Nitroaldol Reaction

6.1 Preamble

This chapter describes the nitroaldol reaction and its use in carbon-carbon bond formation. Following a general introduction, this chapter reviews the more important chemical literature in the area, focusing on the means by which selectivity can be introduced into the reaction.

6.2 Introduction to the Nitroaldol reaction

The nitroaldol reaction, discovered by Henry in 1895, is a classical carbon-carbon bond forming reaction involving the coupling of a carbonyl compound (typically an aldehyde) with a nitroalkane to give a vicinal nitroalcohol (Scheme 6.1).[6.1]



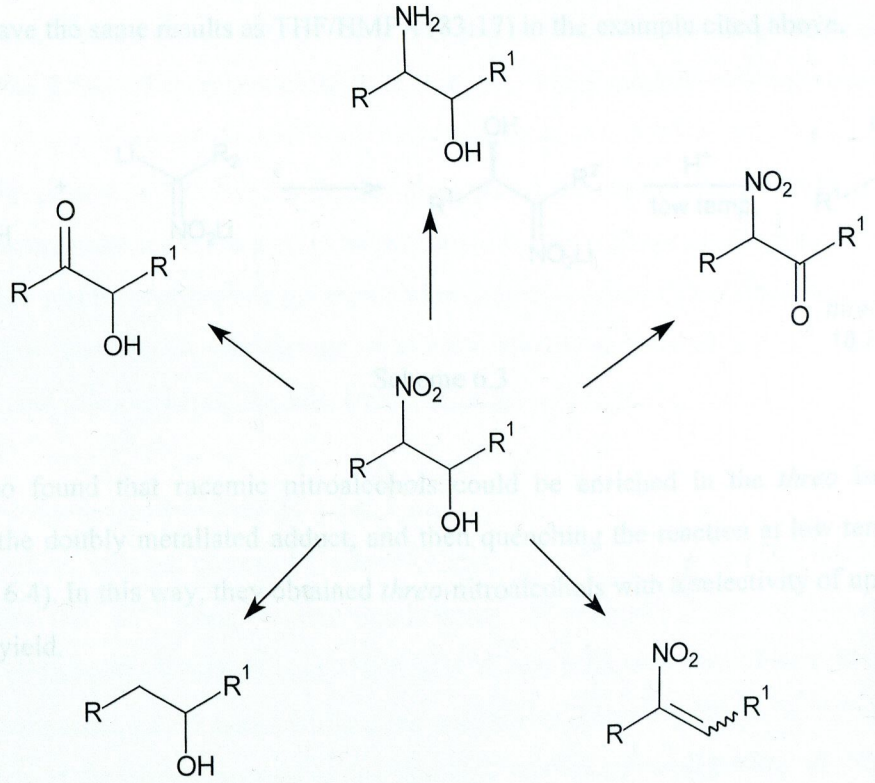
Scheme 6.1

Deprotonation of the nitroalkane (pK_a 10.2) by a suitable base gives rise to a nitronate anion, which is stabilised by resonance delocalisation of the negative charge over the oxygen atoms of the nitro group. The nitronate anion then undergoes nucleophilic addition to the carbonyl of an aldehyde, which yields the 2-nitroalcohol upon protonation.

Typically, the nitroaldol reaction is carried out using only a catalytic amount of base. Alkali metal hydroxides, alkoxides, carbonates, bicarbonates, calcium and barium hydroxides and magnesium and aluminium ethoxides have been used as bases in this reaction, as well as primary and tertiary amines, ammonium acetate, fluoride, anion-exchange resins and chromatographic alumina.[6.2, 6.3] More recently, the nitroaldol reaction

has been carried out using Amberlyst A-21, sodium hydroxide under phase transfer conditions, powdered KOH in a solvent-free reaction, and by using guanidines.[6.4-6.8] The nitroaldol reaction can be problematic if the concentration of the aldehyde is not kept to a minimum, as side products from the aldol condensation or Cannizzaro reaction can occur. Base-catalysed elimination of water from the 2-nitroalchohol to give the α-nitroalkene can also occur, especially when the double bond can conjugate with an aromatic ring (i.e. when an aryl aldehyde is used).

The usefulness of the nitroaldol reaction as a carbon chain lengthening process lies in the wealth of transformations that can be carried out on the nitroalcohol product (Scheme 6.2). For example, reduction of the nitro group leads to a 2-aminoalcohol, while oxidation of the alcohol moiety gives the corresponding 2-nitroketone.[6.9-6.19] Dehydration of the nitroalcohol gives the nitroalkene, which can reduced to another nitroalkane.[6.2, 6.21-6.23] Reductive denitration of the 2-nitroketone or 2-nitroalcohol, or conversion of the nitro group to a carbonyl can also be carried out.[6.20] These last two transformations of are particular interest in synthesis, because the nitroalkane can be used as an alkyl or acyl anion synthon.[6.24, 6.25]



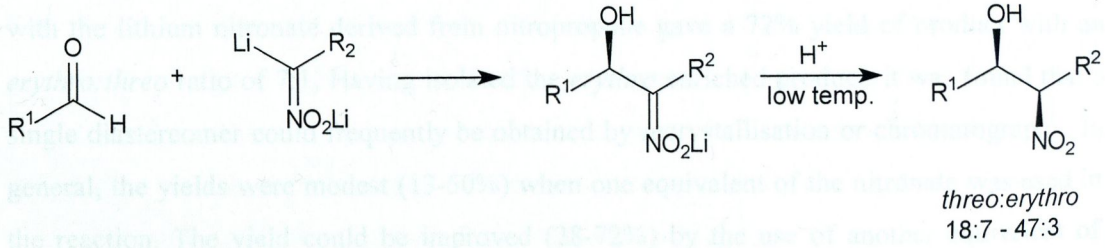
Scheme 6.2

6.3 The Diastereoselective Nitroaldol Reaction

While the chemical literature contains many methods of carrying out the classical nitroaldol reaction, the number of entries concerned with introducing diastereoselectivity into this reaction is few by comparison. The following pages contain the important contributions of the chemical literature to date in this area.

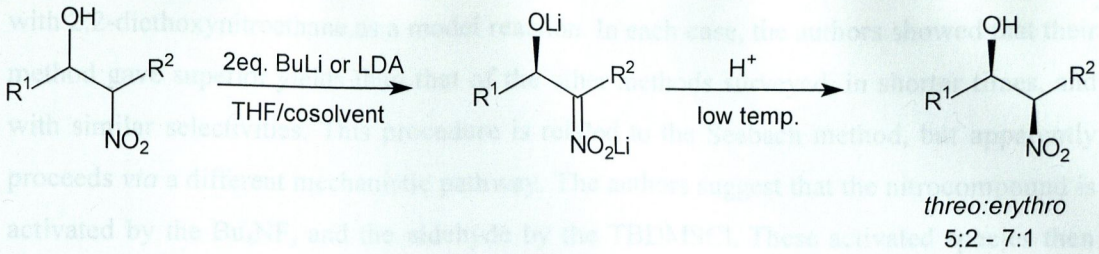
6.3.1 Contribution by Seebach et al.

During the course of their work on silyl-protected vicinal nitroalcohols, Seebach *et al.* discovered that some of the derivatives that they isolated were diastereomerically enriched.[6.14] For example, when acetic acid was used to quench the reaction of doubly deprotonated nitroalkanes with aldehydes at low temperature, they isolated *threo*-nitroalcohols with up to 87% d.e. (Scheme 6.3). They noted that the degree of selectivity depended on the amount of hexamethylphosphoramide (HMPA) that they used as co-solvent with THF in the protonation step. For example, protonation of the adduct derived from doubly deprotonated nitroethane and benzaldehyde gave a 1:1 mixture of diastereomers when no HMPA was used as co-solvent. However, when THF/HMPA (83:17) was employed as solvent in the protonation step, the *threo*/*erythro* ratio increased to 9:1. They found that DMPU could be used in place of HMPA, if required. Thus a co-solvent of THF/DMPU (75:25) gave the same results as THF/HMPA (83:17) in the example cited above.



Scheme 6.3

They also found that racemic nitroalcohols could be enriched in the *threo* isomer, by forming the doubly metallated adduct, and then quenching the reaction at low temperature (Scheme 6.4). In this way, they obtained *threo*-nitroalcohols with a selectivity of up to 7:1 in *ca.* 50% yield.



Scheme 6.4

Seebach *et al.* also found that low-temperature protonation of the mono-lithiated nitronate derived from TBDMS-protected nitroaldols gave *erythro*-nitroalcohols with very high selectivity (82-95% d.e.), even if the *erythro* content in the original silyl-protected nitroaldol was less than 50%. In addition, they prepared *erythro*-silylnitroalcohols in *ca.* 60% yield and very high selectivity (75-95% d.e.) from the direct reaction of silyl nitronates with aldehydes. In this case, very precise experimental conditions are required for reproducible diastereoselectivity.

6.3.2 Contribution from Barrett et al.

Barrett and co-workers found that lithium nitronates reacted with aldehydes in the presence of isopropoxytitanium trichloride to give β-nitroalcohols with *erythro* selectivity (up to 47:5).[6.26] This system proved to be better for aromatic aldehydes bearing electron-withdrawing groups than any other type of aldehyde. Thus, reaction of *p*-nitrobenzaldehyde with the lithium nitronate derived from nitropropane gave a 72% yield of product with an *erythro:threo* ratio of 7:1. Having isolated the *erythro* enriched product, it was found that a single diastereomer could frequently be obtained by recrystallisation or chromatography. In general, the yields were modest (13-50%) when one equivalent of the nitronate was used in the reaction. The yield could be improved (28-72%) by the use of another equivalent of nitronate, and in some cases, the selectivity increased with this extra equivalent.

6.3.3 Contribution from Gómez-Sánchez et al.

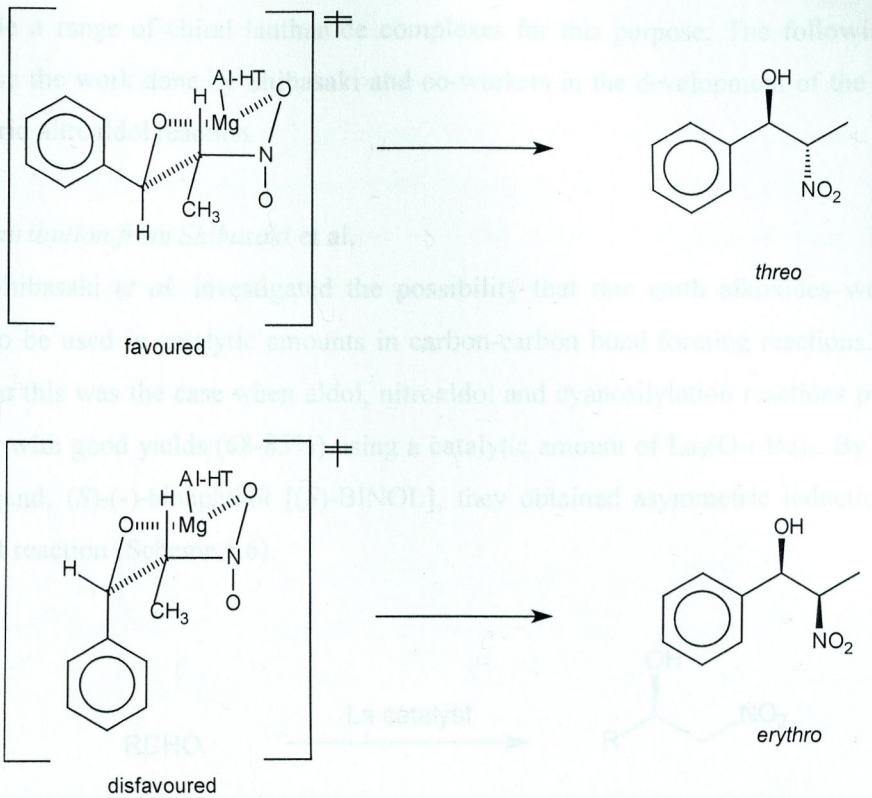
Gómez-Sánchez obtained β-nitroalcohols in good yields (41-96%), but only small selectivities (*ca.* 2:1) from the one pot reaction of aldehydes with nitroalkanes in the presence of Et_3N, $Bu_4NF.3H_2O$, and TBDMSCl.[6.27] A typical reaction was carried out at 0°C or room temperature for 2 hours without the need for an inert atmosphere. By increasing the proportions of the nitrocompound or $Bu_4NF.3H_2O$ relative to the aldehyde, the yields were improved and the reaction time reduced to 5 minutes, but at the expense of selectivity. Replacing TBDMSCl with TMSCl decreased the yields. The authors compared their work directly with some of the other literature methods by choosing the reaction of benzaldehyde

with 2,2-diethoxynitroethane as a model reaction. In each case, the authors showed that their method gave superior yields than that of the other methods surveyed, in shorter times, and with similar selectivities. This procedure is related to the Seebach method, but apparently proceeds *via* a different mechanistic pathway. The authors suggest that the nitrocompound is activated by the Bu_4NF, and the aldehyde by the TBDMSCl. These activated species then merge in a complex intermediate, the hydrolysis of which gives the β-nitroalcohol. Previous work tends to confirm these activations.[6.28, 6.29]

6.3.4 Contribution by Sudalai et al.

Sudalai *et al.* found that hydrotalcites can be used as basic heterogeneous catalysts in the nitroaldol reaction.[6.30] Hydrotalcites are a family of compounds represented by the general formula $[M(II)_{1-x}M(III)_x(OH)_2]^{x+}$ $[(A^{n-})_{x/n}Y.H_2O]^{x-}$ where M(II) is a divalent metal cation, M(III) is a trivalent cation, A^{n-} is an interlayer anion, and the value of x is in the range 0.1 – 0.33. Thermal calcination of these materials leads to non-stoichiometric, mixed metal oxides that possess a high surface area. Sudalai and co-workers found that when they refluxed 3-nitrobenzaldehyde (2 mmol) and nitroethane (2 mmol) together in THF with hydrotalcite (20% w/w compared to the aldehyde) for 6 hours under an atmosphere of nitrogen, they obtained nitroalkanols in high yields (up to 95%). Of the various hydrotalcites tried, they found that the hydrotalcites obtained from magnesium and aluminium gave the best yields, and that these yields also depended on the ration of Mg to Al in the hydrotalcite. Thus, Mg-Al (3:1) gave the best results. The authors attribute the increased activity of this catalyst to its higher surface area (176 m^2g^{-1}) compared to that of the other hydrotalcites (125-154 m^2g^{-1}). This catalyst was suitable for both primary and secondary nitroalkanes, and dehydration to the corresponding nitroalkenes was not observed, even when aromatic aldehydes were used. Furthermore, they were able to successfully reuse the catalyst by filtration and subsequent activation at 450°C, without any loss of activity. In some cases, this Mg-Al hydrotalcite showed moderate to very high selectivity for the *threo* isomer (5:4 – 100:0) with good yields (41-95%), while in others, no selectivity was seen. The best results were obtained in the reaction of nitroethane with aromatic aldehydes containing electron-withdrawing groups. For example, reaction of nitroethane with *p*-nitrobenzaldehyde gave only the *threo* isomer in 84% yield. Decreased selectivity was seen if nitromethane or nitropropane was used, and in the case of aliphatic aldehydes no selectivity was seen. The authors suggest that in the case of nitroethane and benzaldehyde, the *threo* selectivity can be rationalised in terms of a chairlike transition state, where the oxygen of the aldehyde and one of the oxygens of the nitronate are co-ordinated with Mg on the hydrotalcite (Scheme 6.5). In this model, the transition state that is favoured (and which leads to the *threo* isomer)

is the one in which the phenyl group of the aldehyde and the methyl group of the nitronate are both equatorial.



Scheme 6.5

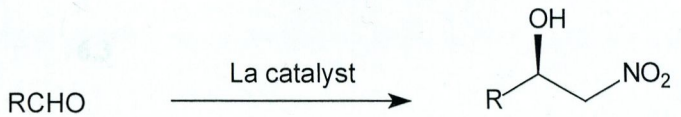
To conclude, the authors say that this catalyst is a practical alternative to homogeneous bases in the nitroaldol reaction in view of its high catalytic activity, its easy separation from the reaction mixture, and its possibility of reuse.

6.4 The Catalytic Asymmetric Nitroaldol Reaction

The most important entries in the chemical literature concerned with the introduction of enantioselectivity into the nitroaldol reaction come from Shibasaki *et al.*, who made a range of chiral lanthanide complexes for this purpose. The following pages summarise the work done by Shibasaki and co-workers in the development of the catalytic asymmetric nitroaldol reaction.

6.4.1 Contribution from Shibasaki et al.

Shibasaki *et al.* investigated the possibility that rare earth alkoxides were basic enough to be used in catalytic amounts in carbon-carbon bond forming reactions.[6.31] They found that this was the case when aldol, nitroaldol and cyanosilylation reactions proceeded smoothly with good yields (68-85%) using a catalytic amount of $La_3(O\text{-}t\text{-}Bu)_9$. By adding a chiral ligand, (*S*)-(-)-binaphthol [(*S*)-BINOL], they obtained asymmetric induction in the nitroaldol reaction (Scheme 6.6).

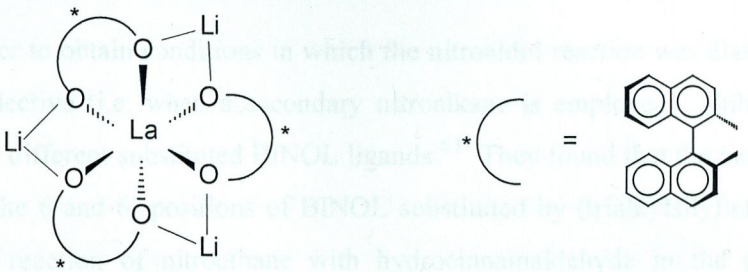


6.1a: R = $PhCH_2CH_2$ — **6.2**a: 79% (73% e.e.)
6.1b: R = *iso*-propyl — **6.2**b: 80% (85% e.e.)
6.1c: R = cyclohexyl — **6.2**c: 91% (90% e.e.)

Scheme 6.6

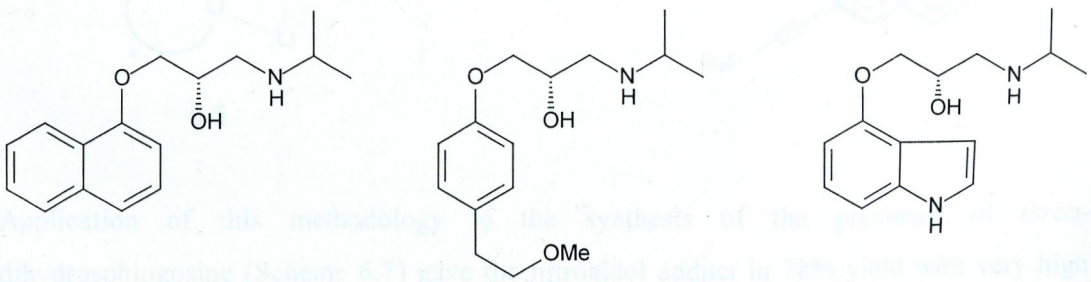
They found that the addition of LiCl and water was essential to achieving these selectivities, and that the presence of either of these species increased the rate of the reaction. Later, they investigated the effect of water on the enantioselectivity of the reaction between nitromethane and hexanal in the presence of their (*S*)-BINOL-La complex.[6.32] Thus, no selectivity was observed in the absence of water, while selectivity increased to a maximum when 10 molar equivalents of water were used, and decreased again when more than this amount was employed. Based on these results, the authors suggest that co-ordination of water to the optically active La complex modifies its asymmetric environment. The authors also developed a more practical method for the synthesis of their catalyst by starting from inexpensive $LaCl_3.7H_2O$ instead of the air and moisture sensitive $La_3(O\text{-}t\text{-}Bu)_9$. This catalyst is stable at room temperature, and does not lose its activity even after several months.

In order to elucidate the structure of the Lanthanum catalyst, Shibasaki *et al.* carried out mass spectrometry using the laser desorption/ionisation time of flight (LDI-TOF) method.[6.33] In both positive and negative modes, a peak in the mass spectrum was observed which could be accounted for by a mixed lanthanum-lithium complex. Other rare earth (Pr, Nd, Eu) complexes with BINOL showed similar peak patterns. Although the authors were unable to grow crystals of their Lanthanum catalyst, they were able to crystallise similar complexes by substituting another rare earth metal (Pr, Nd, Eu) for La, and Na for Li. By analogy from the crystal structure, and with the information obtained from the mass spectrum, they concluded that their Lanthanum catalyst had the structure **6.7**.



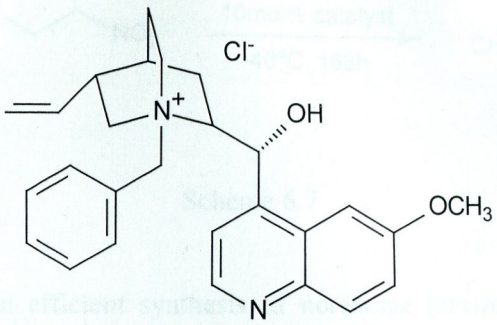
6.3

Shibasaki and co-workers applied this catalytic asymmetric nitroaldol reaction to the synthesis of the β-blockers, (*S*)-propranolol, (*S*)-metoprolol, and (*S*)-pindolol, which have similar structures.[6.34-6.36]



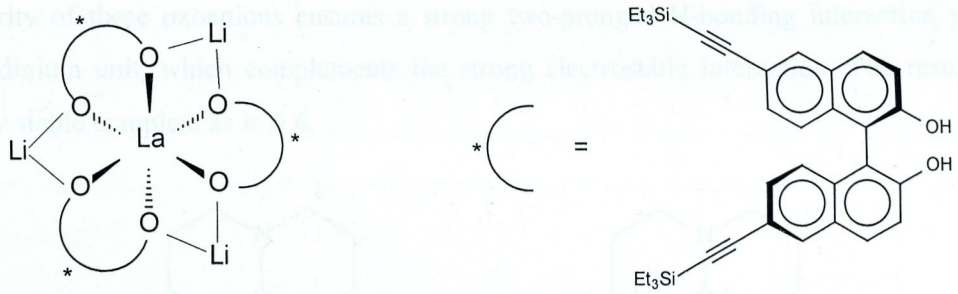
(*S*)-Propranolol (*S*)-Metoprolol (*S*)-Pindolol

In the case of the nitroaldol precursor to (*S*)-propranolol, the authors also tested the combination of quaternary chinchona alkaloids and an inorganic base for comparison with their La-BINOL complex.[6.34] These were not as effective as the complex, however. For example, reaction of α-naphthoxyacetaldehyde with nitromethane in the presence of a catalytic amount of *N*-benzylquininium chloride **6.4**, gave the nitroalcohol in 41% yield and only 23% e.e.



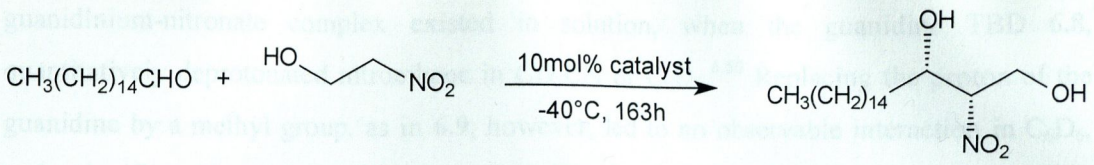
6.4

In order to obtain conditions in which the nitroaldol reaction was diastereoselective and enantioselective (i.e. when a secondary nitroalkane is employed), Shibasaki and co-workers tested different substituted BINOL ligands.[6.37] They found that the best catalyst was the one with the 6 and 6' positions of BINOL substituted by (trialkylsilyl)ethynyl groups. For example, reaction of nitroethane with hydrocinnamaldehyde in the presence of a catalytic amount of **6.8**, gave the *threo* nitroaldol adduct in 70% yield (78% d.e., 93% e.e.).



6.5

Application of this methodology to the synthesis of the precursor of *threo*-dihydrosphingosine (Scheme 6.7) gave the nitroaldol adduct in 78% yield with very high enantioselectivity (97% e.e.) and diasteroselectivity (82% d.e.).



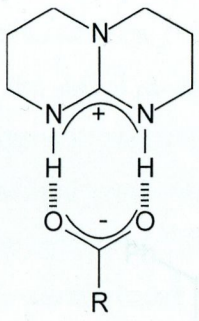
Scheme 6.7

They also carried out an efficient synthesis of norstatine (*erythro*-3-amino-2-hydroxy-4-phenylbutanoic acid), a component of the HIV protease inhibitors KNI-227 and KNI-272, using similar methodology.[6.38]

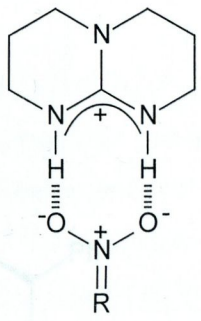
Shibasaki *et al.* have also successfully applied their chiral rare earth alkoxides to catalytic asymmetric aldol, hydrophosphonylation, Michael, tandem Michael-aldol and epoxidation reactions.[6.39-6.46]

6.5 Molecular recognition of the nitronate anion by the guanidinium group

The ability of the guanidinium moiety to bond to oxoanions such as carboxylate, phosphate and nitrate has become a powerful tool of the molecular recognition chemist in the attempt to mimic natural biological recognition using abiotic receptors.[6.47-6.49] The planarity of these oxoanions ensures a strong two-pronged H-bonding interaction with a guanidinium unit, which complements the strong electrostatic interaction. The result is a highly stable complex, as in **6.6**.



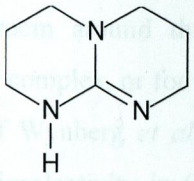
6.6



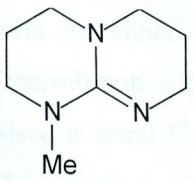
6.7

Davis *et al.* and independently, Wynberg *et al.*, were the first to recognise that the planar anion derived from deprotonation of a nitroalkane, a nitronate, should be expected to bond to a guanidinium unit in an analogous fashion to that of a carboxylate, **6.7**.[6.50, 6.51] Both of these authors hypothesised that this type of complex could be exploited in enantioselective catalytic C-C bond forming reactions. Davis *et al.* showed that the

guanidinium-nitronate complex existed in solution, when the guanidine TBD **6.8**, quantitatively deprotonated nitroethane in CD_3CN or C_6D_6.[6.50] Replacing the proton of the guanidine by a methyl group, as in **6.9**, however, led to no observable interaction in C_6D_6, and a significant decrease in nitroethane deprotonation in CD_3CN.



6.8

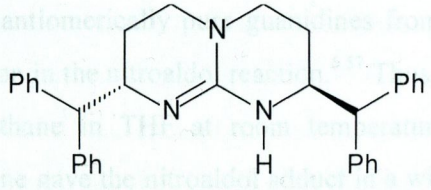


6.9

Wynberg *et al.* obtained a crystal structure of **6.8** with phenylnitromethane, proving this type of complexation occurred in the solid state, while independently and at a similar time, Davis *et al.* obtained a crystal structure of the same substrate with the amidinium analogue of **6.8**.

6.6 The development of chiral guanidine and amidine bases for use in enantioselective catalysis of nitroaldol and related reactions

Following the discovery that the cyclic guanidine TBD deprotonates nitroalkanes in non-polar solvents, Davis *et al.* decided to synthesise a chiral variant of TBD (and its amidinium analogue) to test for enantioselectivity in the nitroaldol and related reactions.[6.52-6.55] They chose to synthesise **6.10,** a C_2-symmetric guanidine with two bulky diphenylmethyl substituents.

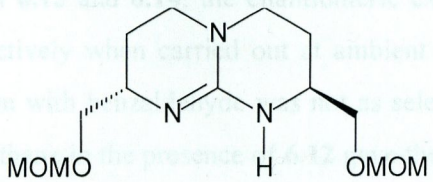


6.10

It was thought that the incorporation of these bulky groups into the guanidine would create a hindered chiral environment conducive to the asymmetric recognition of substrates or transition states. This was borne out by molecular modelling studies of **6.10H**$^+$, which clearly showed that the two diphenylmethyl substituents severely hindered the area around the two NH groups.[6.52] Unfortunately, despite a considerable effort to synthesise **6.10**, no

evidence of nitronate formation was evident when **6.10** was exposed to solutions of nitromethane or nitroethane in C_6D_6 or CD_3CN.[6.54] It was presumed by the authors that some amount of proton transfer occurred, as a number of conjugate additions of nitroalkanes to α, β-unsaturated ketones were catalysed by **6.10**. However, the enantiomeric excesses in these reactions were low (9-12%). Davis *et al.* suggest that these disappointing results stem from leaving insufficient room around the guanidine NHs for either the formation of the guanidinium-nitronate complex, or for the reaction of the substrate with the complex.

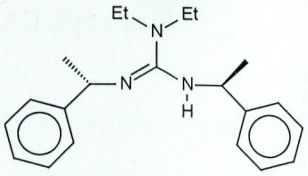
Van Aken (of Wynberg *et al.*) also synthesised a chiral C_2-symmetric guanidine base to test for enantioselectivity in C-C bond forming reactions of nitroalkanes.[6.56] After some effort, the guanidine **6.11** was synthesised in eight steps and 11% yield from asparagine, but with an optical purity of at most 44%.



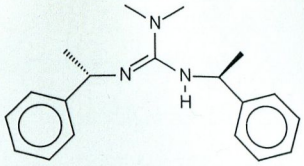
6.11

Van Aken tested **6.11** for catalytic activity in the conjugate addition of nitroethane to methylvinylketone and other substrates. While **6.11** exhibited catalytic activity for this reaction, its activity was somewhat slower than the parent guanidine, TBD. No selectivity was observed in these reactions, however.

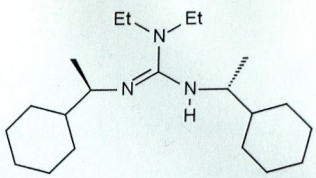
Following the initial suggestions of Davis *et al.* and Wynberg *et al.* that chiral guanidines had potential applications in enantioselective C-C bond-forming reactions, Nájera *et al.* synthesised enantiomerically pure guanidines from chiral amines, and tested them for asymmetric induction in the nitroaldol reaction.[6.57] Thus, reaction of *iso*-pentanal or benzaldehyde with nitromethane in THF at room temperature in the presence of 0.1 equivalents of chiral guanidine gave the nitroaldol adduct in a wide range of yields (6-85%) and low selectivity (0-26% e.e.). The best results were obtained with the C_2-symmetric guanidines **6.12**, **6.13** and **6.14**.



6.12



6.13



6.14

Reaction of *iso*-pentanal with nitromethane in the presence of **6.12**, gave the β-nitroalcohol in 26% e.e. and in 85% yield. The enantiomeric excess was improved by conducting the reaction at lower temperatures - at -45°C the e.e. for the same reaction increased to 34%, while at -65°C the e.e. increased to 54%. These increases in enantiomeric excess were at the expense of chemical yield, however, which dropped to 40% and 33% respectively. In the case of **6.13** and **6.14**, the enantiomeric excesses in the same reaction were 17% and 18% respectively when carried out at ambient temperature, with yields of 72% and 75%. The reaction with benzaldehyde was not as selective, however. Reaction of benzaldehyde with nitromethane in the presence of **6.12** gave the adduct with 13% e.e. when carried out at room temperature, and 33% e.e. when carried out at -65°C. Other non-C_2 symmetric guanidines gave virtually no selectivity in these reactions. The enantiomeric excesses in these reactions were measured by hydrogenating the nitroalcohols to aminoalcohols with palladium on carbon, and then either measuring the optical rotation, or forming a diasteromeric salt with (*R*)-*O*-(4-chloro-2-methylphenyl)lactic acid and integrating the two different sets of peaks in the ^{1}H nmr spectrum. The authors investigated the influence of solvent and amount of guanidine present in these reactions. They found that when the reaction was carried out in ethereal solvents, the optical yields were highest. Reducing the amount of guanidine present in the reaction to 0.05 equivalents (compared to aldehyde) reduced the optical yield, as did increasing the amount to 0.2 equivalents. In each case, the guanidine could be recovered from the reaction mixture and used without any loss of activity. Using guanidine **6.12**, the authors applied this methodology to a synthesis of enantiomerically enriched (*R*)-propranolol, which they obtained in four steps in 26% e.e., but with only 2.7% overall yield.

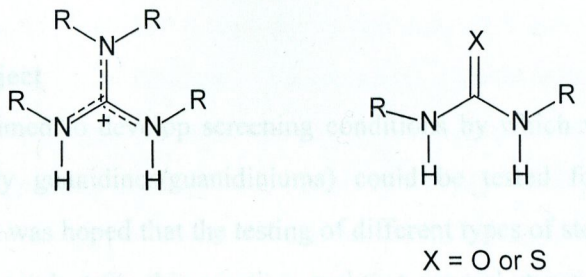
Chapter 7

The Screening of Steroid Receptors as Enantioselective Catalysts in the Nitroaldol Reaction

7.1 Preamble

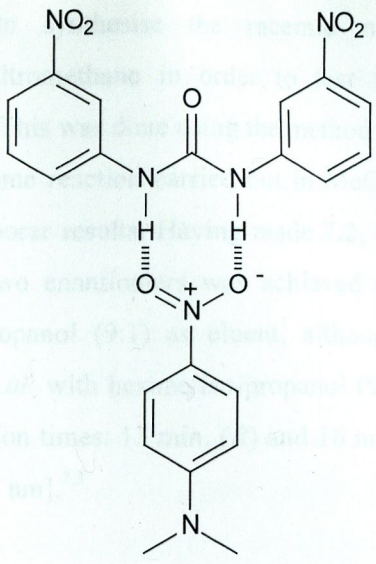
While it is known from the literature that chiral guanidines can influence the selectivity of the nitroaldol reaction (see Chapter 6), other chiral molecules with similar H-donor properties could, in theory, also influence the selectivity in this reaction. In the latter case, a base would have to be present in the reaction mixture - this could be either a free base, or a basic site incorporated into the chiral molecule. It is envisaged that the base would deprotonate the nitroalkane to give the nitronate, and this would form a complex with the chiral molecule, analogous to the guanidinium-nitronate complex. Addition of this nitronate to an aldehyde would hopefully lead to an enantiomerically enriched vicinal nitroalcohol.

An obvious choice for incorporation into a chiral molecule would be a urea or thiourea group, because of the similarity in the spatial arrangement of its H-donor groups to that of a guanidinium (Scheme 7.1).



Scheme 7.1

Ureas have also been shown to bond to oxoanions. This has been demonstrated by Wilcox *et al.* who showed that arylureas bound very strongly to sulfonates, phosphates and carboxylates in $CDCl_3$.[1,2] Etter *et al.* were able to grow co-crystals of diarylureas with a wide range of guest molecules.[7.1] For example, a 1,3-*bis*(*m*-nitrophenyl)urea displayed a complementary H-bonding pattern with *N,N*-dimethyl-*p*-nitroaniline (Scheme 7.2).



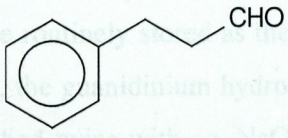
Scheme 7.2

7.2 Aims of this project

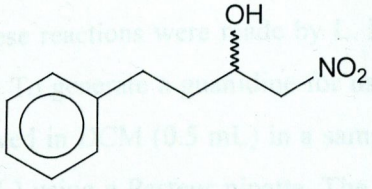
This work aimed to develop screening conditions by which various steroid-based molecules (especially guanidines/guanidiniums) could be tested for selectivity in the nitroaldol reaction. It was hoped that the testing of different types of steroid would provide a lead compound as a catalyst for this reaction, and that general structural information from this lead compound could be used to develop new, improved catalysts in the future.

7.3 Development of a test system

In order to screen steroids for selectivity in the nitroaldol reaction, it was necessary to develop a test system which would give relatively clean conversion of starting material to desired product, and which could be analysed directly (e.g. by chiral HPLC), without the need for product derivatisation. Hydrocinnamaldehyde **7.1** was eventually chosen as the test substrate in these nitroaldol reactions. Although an aliphatic aldehyde, it possesses a phenyl group that makes it suitable for UV detection (tlc and HPLC). Previous work in the literature using this substrate also allowed for a direct comparison of our results.



7.1



7.2

It was necessary to synthesise the racemic nitroaldol product **7.2** from hydrocinnamaldehyde and nitromethane in order to test for a separation of the two enantiomers by chiral HPLC. This was done using the method of Wollenberg *et al.* using 5% KF in *iso*-propanol.[7.2] The same reaction carried out in MeOH, EtOH, toluene, DCM and THF was slower and gave poorer results. Having made **7.2**, it was tested on chiral HPLC. Baseline separation of the two enantiomers was achieved using a Daicel Chiralcel OD column using heptane/*iso*-propanol (9:1) as eluent, although the Daicel Chiralpak AD column used by Shibasaki *et al.* with hexane/*iso*-propanol (9:1) as eluent was found to be more effective [typical retention times: 13 min. (*R*) and 16 min. (*S*) using a flow rate of 1.0 mL/min., and detection at 254 nm].[7.3]

7.4 General reaction conditions

Two general conditions were found for carrying out the nitroaldol reaction in the presence of the steroids (Methods A and B). In the case of a guanidinium or non-basic molecule (Method A), nitromethane (1.5 equivs.), triethylamine (1 equiv.) and hydrocinnamaldehyde (0.1 mmol, 1 equiv.) were added, in this order, to a solution of the steroid (0.1 equiv.) in solvent (THF, DCM or toluene; 130 μL or 300 μL – see later). In the case of a guanidine (see below) or other basic molecule (Method B), nitromethane (1.5 equivs.), and hydrocinnamaldehyde (1 equiv.) were added to a solution of the steroid (0.1 equiv.) in solvent (THF, DCM or toluene; 130 μL or 300 μL – see later). In each case, the reaction was monitored by tlc, and when the nitroaldol product was indicated, an aliquot (25-100 μL) was taken, the exact amount depending on the amount of product deemed to be formed by tlc. The volatiles were evaporated, and the remaining residue loaded on to a silica plug in a Pasteur pipette, and eluted with hexane/ethyl acetate mixtures (usually 2 or 3:1) to remove the guanidinium and any residual triethylamine (other non-basic steroids are not, in general, removed by this technique because of a similarity in polarity on tlc- see later). Evaporation of this solvent, and redissolution in HPLC grade *iso*-propanol (0.5-1.0 mL depending on the quantity of material - the concentration should be 1-3 mg/mL), followed by filtration through a 0.45 μm PTFE syringe filter prepared the sample for analysis on HPLC.

The steroid-based guanidiniums used in these reactions were made by L. Lawless, and were routinely stored as the hydrochloride salt. To generate a guanidine for use in this reaction, the guanidinium hydrochloride was dissolved in DCM (0.5 mL) in a sample tube, and washed twice with aq. NaOH (1 M; 2 x 0.5 mL) using a Pasteur pipette. The aqueous layer was removed, and the organic layer decanted into another sample tube and dried (Na_2SO_4). The solution of guanidine was carefully decanted into another sample tube and

the solvent removed *in vacuo*. This method gives approximately 80% of the free base, as judged by tlc. In these cases, a slight excess of guanidinium was used so that 80% deprotonation would give 10 mol % of the guanidine for use in the nitroaldol reaction.

Due to only limited quantities of these steroids being available, the reaction was of necessity carried out on a very small scale (typically 5-8 mg of steroid used per reaction). From this point of view, it was considered that initially at least the chemical yield of the reaction was not as important as the optical yield generated. Thus all reactions were monitored carefully by tlc, and tested directly for enantiomerically enriched product by HPLC when tlc indicated that some product had formed. It should be noted that except in the case of guanidine **7.3** we have no measurement of conversion of aldehyde to nitroalcohol, and therefore cannot guarantee that any of these reactions proceeded beyond 10% conversion, i.e. that the concentration of product exceeded that of catalyst at the end of the reaction. This is particularly relevant to the nitroaldol reactions carried out using non-guanidine containing compounds or tripodal guanidinium receptors which were observed to be quite sluggish by tlc. By contrast, the relatively non-hindered guanidiniums **7.3H**$^+$ and **3.12H**$^+$ clearly showed a preponderance of product by tlc after overnight reaction, indicating that in these cases the reaction was essentially complete.

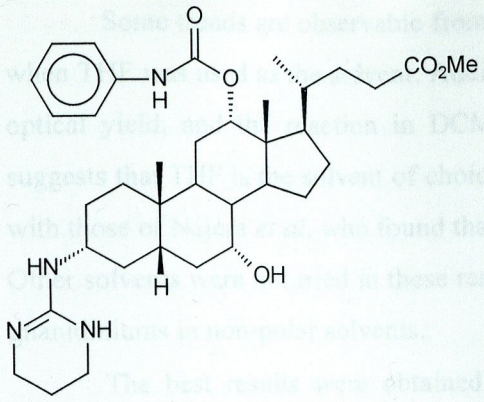
7.5 Results and Discussion

The results of the screening of steroids containing guanidinium groups in the reaction of hydrocinnamaldehyde with nitromethane are detailed in Table 7.1. The steroids used are shown in Scheme 7.3.

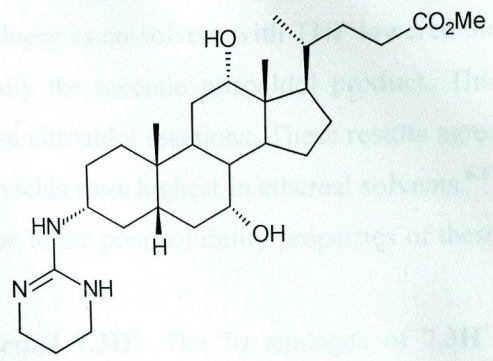
Table 7.1. Results of the screening of steroid-based guanidiniums in the reaction of hydrocinnamaldehyde with nitromethane.

Guanidinium catalyst	Mol (%) Used	Solvent	e.e. (%)	(*R* / *S*)	Base used	Time of testing
7.3H$^+$	10	THF	21	*R*	Et_3N	2.5 h
7.3H$^+$	10	Toluene/THF (5:3)	13	*R*	Et_3N	22.5 h*
7.3H$^+$	11	DCM	0	-	Et_3N	2.5 h
3.12H$^+$	9	DCM	0	-	Et_3N	29.5 h
7.4H$^+$	11	THF	14	*R*	DIPEA	48.5 h
7.4H$^+$	10	DCM	0	-	DIPEA	40 min.
7.5H$^+$	9	THF	4	*S*	Et_3N	1.5 h
4.2H$^+$	11	THF	0	-	Et_3N	5 h

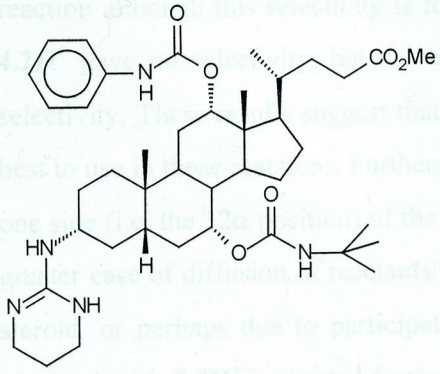
* Reaction carried out under dilute conditions due to poor solubility of this catalyst in toluene



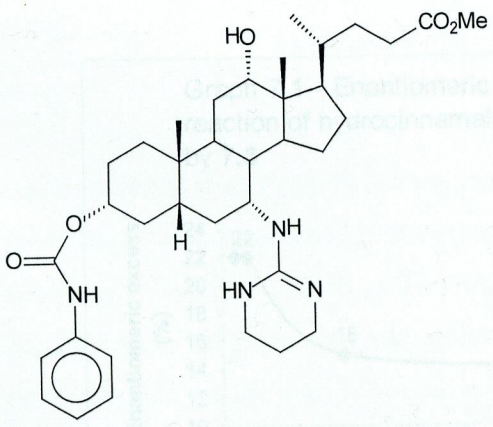
7.3



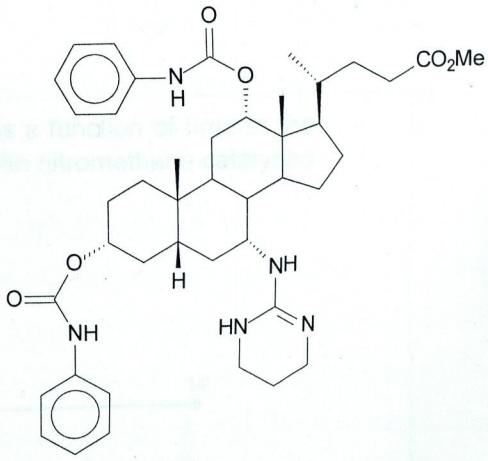
3.12



7.4



7.5



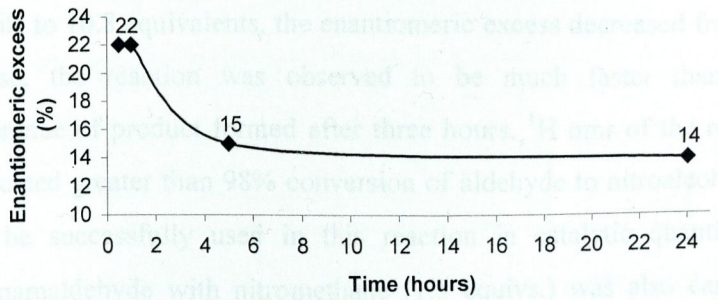
4.2

Scheme 7.3

Some trends are observable from Table 7.1. Enantiomeric enrichment only occurred when THF was used as the solvent. Addition of toluene as co-solvent with THF lowered the optical yield, and the reaction in DCM gave only the racemic nitroaldol product. This suggests that THF is the solvent of choice for these nitroaldol reactions. These results agree with those of Nájera *et al.* who found that optical yields were highest in ethereal solvents.[6,57] Other solvents were not tried in these reactions due to the poor solubility properties of these guanidiniums in non-polar solvents.

The best results were obtained using steroid **7.3H**$^+$. The 7α analogue of **7.3H**$^+$, **7.5H**$^+$, i.e. the steroid in which the functional groups at the 3α, 7α, and 12α positions have been rotated anti-clockwise by one position, shows only very slight selectivity in this reaction although this selectivity is for the opposite isomer. The trisubstituted guanidinium **4.2H**$^+$ gave no selectivity, but the other trisubstituted guanidinium **7.4H**$^+$ did give some selectivity. These results suggest that a guanidinium at the 3α position of the steroid is the best to use in these reactions. Furthermore, it is tentatively suggested that a steroid with only one side (i.e. the 12α position) of the molecule blocked off is the best to use, perhaps due to greater ease of diffusion of reactants/products into and out of the chiral cleft underneath the steroid, or perhaps due to participation of the 7α-OH in the transition state. The results obtained with **7.3H**$^+$ prompted further investigation to see if the guanidine **7.3** gave similar results in this reaction. Thus, the same reaction was carried out (Method B) using the guanidine, and the enantiomeric excess measured over a 24 h period using HPLC (Graph 7.1).

Graph 7.1 - Enantiomeric excess as a function of time in the reaction of hydrocinnamaldehyde with nitromethane catalysed by **7.3**

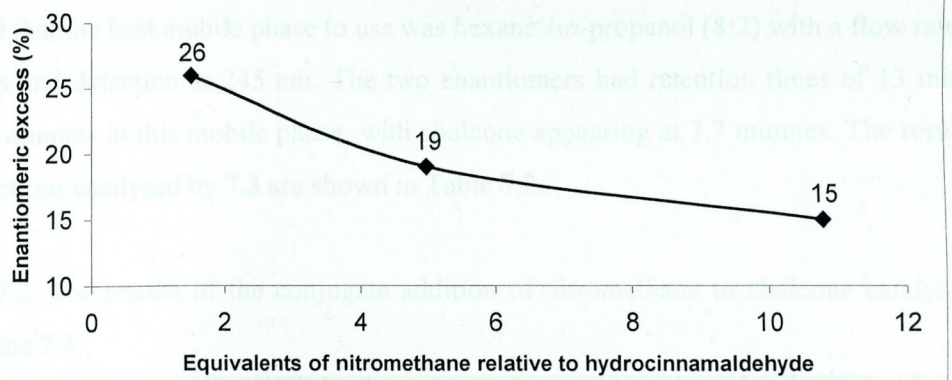


As illustrated in Graph 7.1, the enantiomeric excess in this reaction decreased gradually from an initial value of 22% after 30 minutes, to 14% after 24 hours. It was considered that the concentration of the guanidine in the reaction mixture (*ca.* 0.08 M) facilitated partial

racemisation of the product. To prevent this decrease in optical yield over time, it was decided to increase the amount of solvent, thus decreasing the contact of the nitroalcohol with the guanidine after the product was formed initially in the reaction. The reaction was repeated, but this time the amount of THF used as solvent was increased from 130 μL to 300 μL, giving a decrease in guanidine concentration from 77 mM to 33 mM. HPLC analysis of this reaction showed that under these conditions the enantiomeric excess remained constant at 26% even after 120 hours. The slight difference between the two enantiomeric excesses (22% and 26%) is attributed to the different quantities of guanidine employed (*ca.* 7 mol % and 9 mol % respectively).

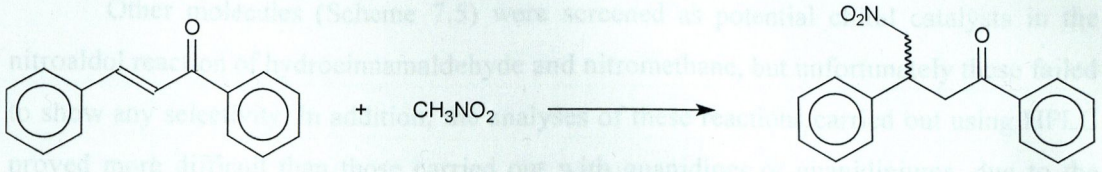
To investigate further the parameters of this reaction catalysed by **7.3**, the quantity of nitromethane used in the reaction was studied (Graph 7.2).

Graph 7.2 - Effect of increasing nitromethane concentration on enantiomeric excess in the reaction of hydrocinnamaldehyde with nitromethane catalysed by **7.3**



As the number of equivalents (relative to aldehyde) of nitromethane was increased from 1.5 equivalents to 10.8 equivalents, the enantiomeric excess decreased from 26% to 15%. In the latter case, the reaction was observed to be much faster than the former, with a preponderance of product formed after three hours. ^{1}H nmr of the reaction mixture at this time indicated greater than 98% conversion of aldehyde to nitroalcohol, demonstrating that **7.3** can be successfully used in this reaction in catalytic quantities. The reaction of hydrocinnamaldehyde with nitromethane (1.5 equivs.) was also carried out using **7.5** as catalyst. In this case, the enantiomeric excess was 6% in favour of the *S*-isomer, which is the opposite selectivity than that observed for **7.3**.

Due to the selectivity observed in the nitroaldol reaction with **7.3** as catalyst, it was decided to test **7.3** for selectivity in the conjugate addition of nitromethane to chalcone (Scheme 7.4).



Scheme 7.4

It was necessary to make the racemic product to test for a separation on chiral HPLC. This was achieved using TMG as the catalyst in DCM. Aliquots were taken from the reaction mixture, processed in a similar manner to that of the nitroaldol reaction, and injected on HPLC. Fortunately, the Daicel Chiralpak AD column used for analysis in the nitroaldol reactions also separated the two enantiomers formed in this reaction. Several attempts showed that the best mobile phase to use was hexane/*iso*-propanol (8:2) with a flow rate of 1 mL/min and detection at 245 nm. The two enantiomers had retention times of 13 minutes and 17 minutes in this mobile phase, with chalcone appearing at 7.7 minutes. The results of the reactions catalysed by **7.3** are shown in Table 7.2.

Table 7.2. The results of the conjugate addition of nitromethane to chalcone catalysed by guanidine **7.3**.

Equivalents of **7.3** used (mol %)	Solvent	Volume of Solvent	Equivalents of nitromethane used	Temperature (°C)	Enantiomeric excess (%)	Time of testing
10	THF	300 μL	1.5	r.t.	26	30 min.
10	DCM	300 μL	1.5	r.t.	15	30 min.
10	DCM	300 μL	1.5	-80	12	1 h

Table 7.2 shows that guanidine **7.3** also exhibits selectivity in the conjugate addition of nitromethane to chalcone. Although the enantiomeric excess in this reaction was measured, the absolute configuration of the preferred enantiomer was not discovered. The highest selectivity (26%) was obtained using THF as solvent, but the reaction was cleaner

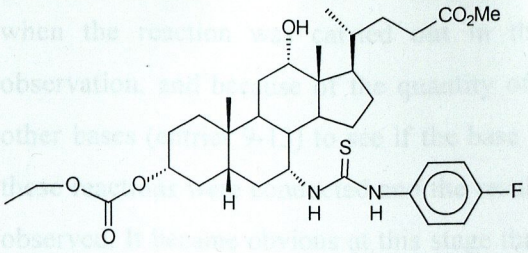
when carried out in DCM, perhaps due to competitive nitroaldol reaction in the former solvent. When the reaction was carried out at low temperature, the enantiomeric excess seemed to fall slightly, although this value is probably within the error range for HPLC analysis.

Other molecules (Scheme 7.5) were screened as potential chiral catalysts in the nitroaldol reaction of hydrocinnamaldehyde and nitromethane, but unfortunately these failed to show any selectivity. In addition, the analyses of these reactions carried out using HPLC proved more difficult than those carried out with guanidines or guanidiniums, due to the similarity in R_f between the nitroalcohol and the molecules studied. This meant that it was almost impossible to remove these molecules from the sample to be analysed, and hence the chromatograms appeared complicated. The conditions that were used in these reactions are shown in Table 7.3.

Table 7.3. Conditions for the screening of chiral non-guanidine containing compounds in the reaction of hydrocinnamaldehyde with nitromethane.

Entry	Molecule †	Mol. (%) Used	Solvent	Volume of solvent used	Equivs. of CH_3NO_2 used	Base used	Time of testing	Notes
1	**3.9**	10	THF	130 μL	1.4	Et_3N	5 h	
2	**3.9**	10	Toluene	130 μL	1.4	Et_3N	5 h	
3	**3.9**	10	DCM	130 μL	1.4	Et_3N	2.5 h	
4	**7.6**[1]	8	THF	130 μL	1.4	Et_3N	1 h	
5	**7.7**[2]	10	THF	300 μL	10	Et_3N	27 h	
6	**7.8**[3]	10	THF	300 μL	10	Et_3N	24 h	
7	**7.8**	10	THF	650 μL	10	Et_3N		
8	**7.8**	100	THF	650 μL	10	Et_3N		
9	**7.8**	10	THF	300 μL	10	TMG[6]	37 min.	1eq. of TMG used
10	**7.8**	10	THF	300 μL	10	TMG	40 min.	0.1eq. of TMG used
11	**7.8**	10	THF	300 μL	10	Pyr.[7]	4 h	Not enough product formed
12	**7.8**	10	THF	300 μL	10	Quin.[8]	1 h	1eq. of base used
13	**7.8**	10	THF	300 μL	10	DIPEA[9]	1.5 h	Not enough product formed
14	**7.9**[4]	10	THF	130 μL	1.4	-	-	No product formed after 49h
15	**7.9**	10	THF	300 μL	10	-	-	No product formed
16	**7.9**	10	DCM	300 μL	10	-	-	No product formed
17	**7.10**[5]	10	THF	300 μL	10	-	-	Only a trace of product formed

†Molecules kindly donated for testing by: [1] S. Broderick, [2] L. Lawless, [3] N. Pérez Payan, [4] U. Piarulli of the University of Milan, [5] J. Barry. Bases used: [6] 1,1,3,3-tetramethylguanidine. [7] pyridine. [8] quinuclidine. [9] diisopropylethylamine.



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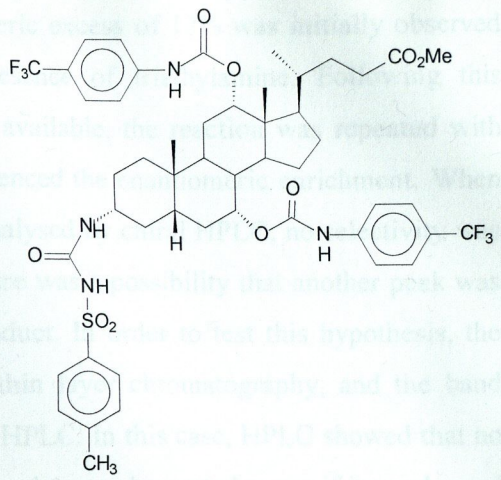
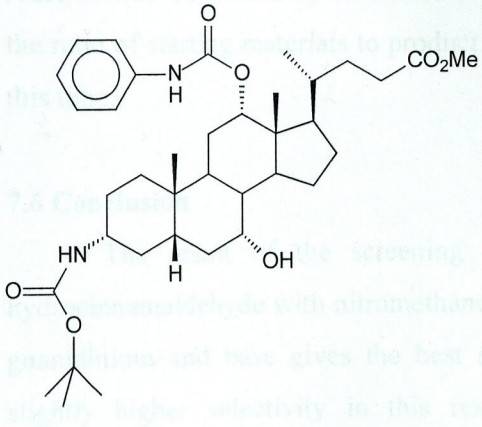
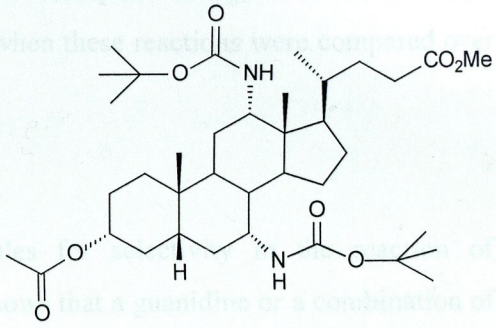


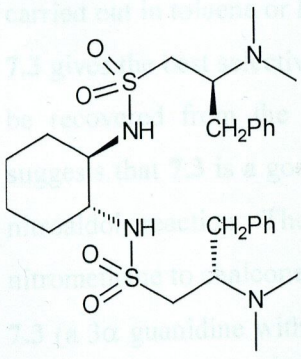
7.8



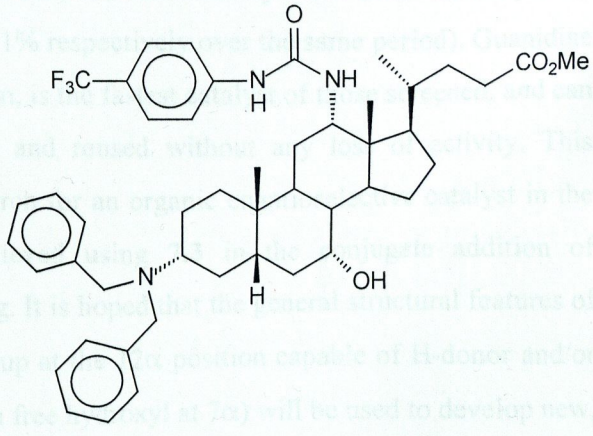
7.9



7.10



7.11



7.12

In the case of **7.8** (entry 6), an enantiomeric excess of 17% was initially observed when the reaction was carried out in the presence of triethylamine. Following this observation, and because of the quantity of **7.8** available, the reaction was repeated with other bases (entries 9-13) to see if the base influenced the enantiomeric enrichment. When these reactions were conducted and the results analysed by chiral HPLC, no selectivity was observed. It became obvious at this stage that there was a possibility that another peak was co-eluting on HPLC with the *R*-nitroalcohol product. In order to test this hypothesis, the reaction mixture was separated by preparative thin layer chromatography, and the band corresponding to the nitroalcohol tested again on HPLC. In this case, HPLC showed that no selectivity had occurred in the reaction. This molecule was also tested to see if it accelerated the nitroaldol reaction. To this end, three reactions were set up in parallel using identical conditions except for the quantities of **7.8** in each one (0 mol %, 10 mol %, 100 mol %). The reaction was monitored by tlc over the course of 50 hours, but no significant difference in the ratio of starting materials to product occurred when these reactions were compared over this time.

7.6 Conclusion

The result of the screening of molecules for selectivity in the reaction of hydrocinnamaldehyde with nitromethane clearly shows that a guanidine or a combination of guanidinium and base gives the best selectivity. Of these two cases, the former shows slightly higher selectivity in this reaction than the latter, presumably because of a background racemic reaction (catalysed by triethylamine) that occurs in the latter. ^{1}H nmr showed that this background racemic reaction gives 11% conversion of aldehyde to nitroalcohol after 24 h when conducted in THF, and substantially more when the reaction is carried out in toluene or DCM (65% and 91% respectively over the same period). Guanidine **7.3** gives the best selectivity in this reaction, is the fastest catalyst of those screened, and can be recovered from the reaction mixture and reused without any loss of activity. This suggests that **7.3** is a good lead in the search for an organic enantioselective catalyst in the nitroaldol reaction. The selectivity achieved using **7.3** in the conjugate addition of nitromethane to chalcone is also promising. It is hoped that the general structural features of **7.3** (a 3α guanidine with a functional group at the 12α position capable of H-donor and/or steric interactions with the substrate, and a free hydroxyl at 7α) will be used to develop new, improved catalysts not only for these reactions, but also for any reaction which can be catalysed by base.

Chapter 8

Experimental

Experimental

General

^{1}H nmr spectra (400MHz) and ^{13}C nmr (100MHz) spectra were measured using a Bruker DPX-400 spectrometer for solutions in $CDCl_3$, with residual $CHCl_3$ (δ_H 7.26) or $CDCl_3$ (δ_C 77.0) as internal reference peaks. *J* values are given in Hz. Carbon atoms were assigned using the DEPT technique and/or by comparison with similar steroids. Melting points were carried out on a Gallenkamp digital melting point apparatus and are uncorrected. FT-IR spectra were measured using a Perkin-Elmer FT-IR PARAGON 1000 spectrometer or a Mattson Genie FT-IR spectrometer using sodium chloride plates. Tlc was carried out using Merck Kieselgel 60 F_{254} plates, with visualisation of compounds by UV fluoresence and/or phosphomolybdic acid dip or by charring over a Bunsen burner. Flash chromatography was carried out using Merck Kieselgel 60 0.040-0.063nm (230-400 mesh). Elemental analyses were carried out in the Microanalytical Department, Department of Chemistry, University College Dublin. All solvents were fractionally distilled before use, and when necessary, dried using standard techniques.[8.1] Cholic acid was a gift from Freedom Chemical Diamalt GmbH and used without further purification. All solvents were fractionally distilled before use. Technical grade hydrocinnamaldehyde was obtained from Aldrich Chemical Co. and fractionally distilled before use.

Methyl 7α, 12α-*bis*(phenylcarbamoyloxy)-3α-{3-[4-(trifluoromethyl)phenyl]ureylene}-5β-cholan-24-oate 1.20

Dry triethylamine (63 μL, 0.452 mmol, 1.1 equivs.) and α,α,α-trifluoro-*p*-tolylisocyanate (65 μL, 0.455 mmol, 1.1 equivs.) were syringed into a solution of methyl 3α-amino-7α, 12α-*bis*(phenylcarbamoyloxy)-5β-cholan-24-oate (272 mg, 0.412 mmol) in dry DCM (3 mL) under Argon. The solution was stirred at room temperature overnight (22 h), then concentrated *in vacuo*. Purification by flash chromatography using gradient elution (hexane/ethyl acetate 3:1, 2:1, 1:1, 0:1) gave **1.20** as a white foam (253 mg, 72 %), R_f 0.14 [hexane/EtOAc (2:1)], mp 163-164°C; δ_H(400MHz; $CDCl_3$) 0.81 (3H, s, 18-CH_3), 0.93 (3H, d, *J* 6.5, 21-CH_3), 0.97 (3H, s, 19-CH_3), 1.62 (s, H_2O), 3.26 (1H, br m, 3β-H), 3.64 (3H, s, CO_2CH_3), 5.01 (1H, br m, 7β-H), 5.16 (1H, br m, 12β-H), 6.22 (1H, br s, NH), 6.84 (1H, br s, NH), 6.88 (1H, br s, NH), 7.0-7.6 (ArH), [lit.[1.18] δ_H(300MHz) 0.76, 0.86, 0.89, 3.09, 3.62, 4.96, 5.10, 6.72, 7.0-7.5]; δ_C(100MHz; $CDCl_3$) 11.7 (CH_3), 17.1 (CH_3), 22.1 (CH_3), 22.5 (CH_2), 25.1 (CH_2), 26.7 (CH_2), 27.3 (CH_2), 28.5 (CH), 30.3 (CH_2), 30.5 (CH_2), 31.0 (CH_2), 33.9 (C), 34.3 (CH), 35.1 (CH_2), 35.2 (CH_2), 37.6 (CH), 41.0 (CH), 43.1 (CH), 44.9 (C), 47.1 (CH), 51.0 (CO_2CH_3), 75.9 (CH), 76.0 (CH), 118.2 (ArCH), 118.5 (ArCH), 122.9 (ArCH), 123.1 (ArCH), 125.8 (ArCH), 128.6 (ArCH), 128.7 (ArCH), 137.6 (C), 141.7 (C), 152.6 (C), 152.8 (C), 174.2 (CO_2CH_3).

Methyl 3α, 12α-*bis*(phenylcarbamoyloxy)-7α-{3-[4-(trifluoromethyl)phenyl]ureylene}-5β-cholan-24-oate 1.22

*From urea diol **3.2**:*

Phenyl isocyanate (89 μL, 0.52 mmol, 2.5 equivs.) and chlorotrimethylsilane (4 μL, 0.032 mmol, 0.1 equivs.) were added to a solution of **3.2** (200 mg, 0.329 mmol) in dry $CHCl_3$ (2 mL) at room temperature under Argon. Two further portions of phenyl isocyanate (2 x 89 μL; total: 7.5 equivs.) and chlorotrimethylsilane (2 x 4 μL; total: 0.3 equivs.) were added after 2 days and after 6 days. After the last addition, the reaction was refluxed for 4 h. The reaction was then cooled to room temperature and water (2 mL) added. The biphasic mixture was stirred vigorously for 30 min., then poured onto aq. HCl (1M; 15 mL) and extracted into $CHCl_3$ (3 x 10 mL). The organic layer was kept, washed with aq. HCl (1M; 2 x 15 mL), water (2 x 15 mL), aq. $NaHCO_3$ (4 %; 2 x 15 mL) and water (2 x 15 mL), dried (Na_2SO_4), and concentrated *in vacuo* to give an oil (542 mg). Flash chromatography of this oil with hexane/ethyl acetate (4:1 - 1:1) gave **1.22** as a white solid (164 mg, 56.7 %), R_f 0.43 [hexane/EtOAc (1:1)], mp 174-175°C; (Found: C, 65.42; H, 6.72; N, 6.36. $C_{47}H_{57}F_3N_4O_7.H_2O$ requires C, 65.26; H, 6.87; N, 6.48); ν_{max}(film from $CDCl_3$/cm^{-1}) 3433

(NH), 3354 (NH), 1726 (overlapping CO); δ_H(400MHz; $CDCl_3$) 0.79 (3H, s, 18-CH_3), 0.91 (3H, d, *J* 6.5, 21-CH_3), 0.97 (3H, s, 19-CH_3), 1.79 (s, H_2O), 3.65 (3H, s, CO_2CH_3), 3.92 (1H, m, 7β-H), 4.47 (1H, br m, 3β-H), 4.93 (1H, br d, N*H*CONHAr), 5.14 (1H, m, 12β-H), 6.53 (1H, s, NH), 6.81 (1H, br s, NH), 6.97 (1H, br s, NH), 7.04 [1H, sept, *J* 3, 3α -(*p*-ArH)], 7.11 [1H, t, *J* 7.5, 12α-(*o*-ArH)], 7.28 [m, 3α-(*o*- & *p*-ArH)], 7.35 [2H, t, *J* 7.5, 12α-(*m*-ArH)], 7.46 [2H, d, *J* 8, 12α-(*o*-ArH)], 7.53 (4H, s, 7α-ArH); δ_C(100MHz; $CDCl_3$) 11.9 (18-CH_3) 17.1 (19-CH_3), 22.0 (21-CH_3), 22.4 (CH_2), 25.3 (CH_2), 26.5 (CH_2), 26.6 (CH_2), 28.5 (CH), 30.3 (CH_2), 30.5 (CH_2), 31.7 (CH_2), 34.1 (CH), 34.2 (CH_2), 35.2 (CH_2), 36.4 (CH), 40.5 (CH), 43.7 (CH), 44.9 (C), 46.5 (CH), 47.0 (CH), 51.1 (CO_2CH_3), 75.8 (CH), 76.0 (CH), 117.9 (ArCH), 118.6 (ArCH), 123.2 (ArCH), 123.3 (ArCH), 123.8 (q, *J* 270, CF_3), 123.9 (q, *J* 33, CCF_3), 125.7 (ArCH), 128.6 (ArCH), 137.1 (PhCNH), 137.6 (PhCNH), 142.1 (ArCNH), 152.8 ($NHCO_2$), 153 ($NHCO_2$), 153.8 (NHCONH), 174.5 (CO_2CH_3). Some of the 3α-monocarbamate was also isolated (33 mg).

From 7α-NHBOC bis*(phenylcarbamate) derivative **3.3**:*

Crude **3.3** (1.00g) was dissolved in dry DCM (6 mL) and trifluoroacetic acid (2 mL) at room temperature under Argon. The reaction was stirred for 2.5 h, and then concentrated *in vacuo*. Flash chromatography of the crude product using gradient elution [DCM/methanolic ammonia (99:1 – 96:4)] gave **3.4** as a white foam, (395 mg, 46 %). δ_H(400MHz; $CDCl_3$) 0.80 (3H, s, 18-CH_3), 0.93 (3H, d, *J* 6.5, 21-CH_3), 0.97 (3H, s, 19-CH_3), 3.22 (1H, m, 7β-H), 3.66 (3H, s, CO_2CH_3), 4.55 (1H, m, 3β-H), 5.16 (1H, m, 12β-H), 6.49 (1H, s, NH), 7.0 (br s, NH), 7.0-7.1 (m, 2 x *p*-ArH), 7.2-7.4 (m, *m*- & *o*-ArH), 7.48 (2H, d, *J* 8, *o*-ArH).

α, α, α-Trifluoro-*p*-tolylisocyanate (63 μL, 0.44 mmol, 1.1 equivs.) and dry triethylamine (61 μL, 0.44 mmol, 1.1 equivs.) were added to a solution of **3.4** (256 mg, 0.398 mmol) in dry DCM (4 mL) at room temperature under Argon. The reaction was stirred for 30 min., then concentrated *in vacuo*. Flash chromatography eluting with hexane/ethyl acetate (2:1) gave **1.22** as a white solid (290 mg, 86 %), which was identical by nmr and tlc to **1.22** obtained from the urea diol **3.2**.

Methyl 3α, 7α, 12α-trihydroxy-5β-cholan-24-oate 2.12

These procedures are modified from that of Fieser *et al.*[2.7]

Method A:

Cholic acid (51.9 g, 127 mmol) and conc. H_2SO_4 (5 mL) were stirred together in dry methanol (300 mL) at room temperature for 18.5 h under a balloon of Argon. Some methyl cholate had precipitated after this time. The methanol was removed under reduced pressure, and the residue redissolved in DCM (300 mL). The solution in DCM was washed with $NaHCO_3$ (4 %; 2 x 100 mL) and water (1 x 200 mL, 1 x 100 mL) with a further addition of DCM (100 mL) after the base washes and the first aqueous wash. The organic layer (approx. 500 mL) was dried ($MgSO_4$), and the solvent removed *in vacuo* to give methyl cholate (47.7 g, 89 %), R_f 0.25 (EtOAc), mp 154-155°C (lit.[2.7] 156-157°C); δ_H(400MHz; $CDCl_3$) 0.69 (3H, s, 18-CH_3), 0.90 (3H, s, 19-CH_3), 0.98 (3H, d, *J* 3, 21-CH_3), 3.45 (1H, m, 3β-H), 3.67 (3H, s, CO_2CH_3), 3.83 (1H, m, 7β-H), 3.98 (1H, m, 12β-H).

Method B:

Cholic acid (19.967 g, 48.86 mmol) was dissolved in dry methanol (200 mL) with warming under a hot tap. Conc. H_2SO_4 (2 mL) was added, and the reaction was stirred at room temperature for 16 h under a balloon of Argon. After this time, the stirring was stopped, and **2.12** spontaneously crystallised over the course of the next 2 hours. The remaining solution was decanted from the crystals and added to the same volume of water. A precipitate occurred upon this addition. The precipitate was filtered off at the pump, sucked dry, and allowed dry further in air overnight to give **2.12** (18.834 g, 91 %).

Methyl 3α-ethoxycarbonyloxy-7α, 12α-dihydroxy-5β-cholan-24-oate 2.13

Method A:

This procedure is modified from that of Kasal *et al.*[2.2]

Ethyl chloroformate (6.0 mL, 63 mmol, 2.1 equivs.) was syringed gradually into a stirred solution of **2.12** (12.678 g, 30.0 mmol) in dry pyridine (40 mL) at 0°C. A white precipitate formed upon this addition. The mixture was kept at 0-1°C for 4 h, then poured onto water (100 mL) and extracted into $CHCl_3$ (3 x 80 mL). The organic extract was washed with water, 2M HCl and water (3 x 100 mL each), dried ($MgSO_4$), and the solvent removed *in vacuo* to yield **2.13** as a white crystalline solid (12.720 g, 85 %), R_f 0.21 [hexane/EtOAc (2:1)], mp 175-176°C (hexane/EtOAc) (lit.,[2.7] 176-177°C); ν_{max}(film from $CHCl_3$)/cm^{-1} 3456 (OH), 1736 (overlapping CO); δ_H(400MHz; $CDCl_3$) 0.63 (3H, s, 18-CH_3), 0.84 (3H, s, 19-

CH_3), 0.91 (3H, d, *J* 6, 21-CH_3), 1.22 (3H, t, *J* 7, CH_3CH_2O), 3.60 (3H, s, CO_2CH_3), 3.78 (1H, m, 7β-H), 3.91 (1H, m, 12β-H), 4.09 (2H, q, *J* 7, CH_3CH_2O), 4.37 (1H, m, 3β-H).

Method B:

This procedure is modified from that of Fieser *et al.*[2.3]

Ethyl chloroformate (2.0 mL, 21 mmol, 4.4 equivs.) was added dropwise *via* syringe to a solution of **2.12** (2.00 g, 4.73 mmol) in a mixture of dry pyridine (1.6 mL, 19.8 mmol, 4.2 equivs.) and dry THF (10 mL). Occasional cooling was necessary with this addition. When all the ethyl chloroformate was added, a reasonably vigorous evolution of gas occurred. Some precipitation occurred at this stage. After 30 min., the solvent was removed under reduced pressure, and the residue redissolved in DCM (100 mL). This solution was washed with aq. HCl (2M; 2 x 50 mL), water (2 x 50 mL), dried ($MgSO_4$) and concentrated *in vacuo* to yield a white foam (2.121 g) which contained an impurity of *ca.* 10 % by tlc. Recrystallisation of the crude material (1.776 g) from hexane/ethyl acetate gave **2.13** as a white solid (1.401 g, 79 %).

Methyl 3α-ethoxycarbonyloxy-12α-hydroxy-7-keto-5β-cholan-24-oate 2.14

This procedure is modified from that of Kasal *et al.*[2.2]

Water (150 mL), *N*-bromoacetamide (8.38 g, 60.7 mmol, 1.50 equivs.) and acetic acid (20 mL) were added to a solution of **2.13** (20.0 g, 40.4 mmol) in acetone (600 mL) at 0°C. The reaction mixture became orange in colour over the course of 15 min. The reaction was stirred at 0°-1°C for 1 h (a small amount of precipitation was evident after this time), then allowed to warm to room temperature. The reaction was maintained at this temperature for 2.75 h, then poured onto aq. $NaHCO_3$ (4 %; 400 mL). The product was extracted into $CHCl_3$ (2 x 200 mL), washed with water (2 x 200 mL) and dried ($MgSO_4$). Removal of the solvent *in vacuo* yielded a slightly wet white solid that smelled of acetic acid. This residual acid was removed by azeotroping with toluene, and the toluene removed with DCM. Crystallisation from ethyl acetate gave **2.14** as fine white crystals (8.92 g). A further two crops of crystals were obtained from the mother liquor using ethyl acetate/hexane (5.11 g; total yield 14.03 g, 70.5 %), R_f 0.26 [toluene/Et_2O (3:1)], mp 178-180°C (hexane/ethyl acetate) (lit.[2.2] 183-185°C); ν_{max}(film from $CHCl_3$)/cm^{-1} 3530 (OH), 1732 (overlapping CO); δ_H(400MHz; $CDCl_3$) 0.72 (3H, s, 18-CH_3), 1.01 (3H, d, *J* 6, 21-CH_3), 1.23 (3H, s, 19-CH_3), 1.33 (3H, t, *J* 7, CH_3CH_2O), 3.71 (3H, s, CO_2CH_3), 4.03 (1H, m, 12β-H), 4.19 (2H, q, *J* 7, CH_3CH_2O), 4.57 (1H, m, 3β-H); δ_C(100MHz; $CDCl_3$) 12.7 (18-CH_3), 14.1 (CH_3CH_2O), 17.4 (19-CH_3),

22.6 (21-CH_3), 24.1 (CH_2), 25.7 (CH_2), 27.4 (CH_2), 29.1 (CH_2), 30.8 (CH_2), 31.0 (CH_2), 32.9 (CH_2), 33.6, 34.5, 34.7, 35.8, 40.6, 45.0 (CH_2), 45.6, 46.4, 46.5, 49.3, 51.4, 63.6 (CH_2), 71.9, 154.4 (O*C*OO), 174.4 (*C*O_2CH_3), 210.6 (CH_2*C*OCH_2).

Methyl 7α-amino-3α-ethoxycarbonyloxy-12α-hydroxy-5β-cholan-24-oate 2.15

This procedure is modified from that of Kasal *et al.*[2.2]

Methanol (300 mL) was added to a mixture of **2.14** (13.00 g, 26.39 mmol), ammonium acetate (16.27 g, 211.1 mmol, 8.0 equivs.) and sodium cyanoborohydride (3.88 g, 61.75 mmol, 2.34 equivs.). The mixture went into solution over the course of 1 h as the reaction was heated to 50°C. The reaction was maintained at 50°C for 18.5 h, then brine (150 mL) was added and the reaction stirred for 30 min. The reaction mixture was diluted with water (200 mL), extracted into DCM (5 x 100 mL), washed with aq. $NaHCO_3$ (4 %; 1 x 200 mL, 2 x 150 mL) and water (1 x 200 mL, 2 x 150 mL), dried (Na_2SO_4), and concentrated *in vacuo* to give **2.15** as a fine white powder (12.24 g, 94 %), R_f 0.39 [DCM/MeOH (9:1)]; δ_H(400MHz; $CDCl_3$) 0.70 (3H, s, 18-CH_3), 0.93 (3H, s, 19-CH_3), 0.99 (3H, d, *J* 6.5, 21-CH_3), 1.30 (t, *J* 7, C*H*$_3$$CH_2O$), 3.09 (1H, m, 7β-H), 3.67 (3H, s, CO_2CH_3), 4.14 (1H, m, 12β-H), 4.16 (2H, q, *J* 7, CH_3C*H*$_2$O), 4.43 (1H, m, 3β-H).

Methyl 7α-ammonium-3α-ethoxycarbonyloxy-12α-hydroxy-5β-cholan-24-oate chloride 2.15.HCl

This procedure is modified from that of Kasal *et al.*[2.2]

Methanol (110 mL) was added to a mixture of **2.14** (4.71 g, 9.56 mmol), ammonium acetate (5.897 g, 76.5 mmol, 8.0 equivs.) and sodium cyanoborohydride (1.407 g, 22.4 mmol, 2.34 equivs). The mixture was warmed to 50°C and maintained at that temperature for 16.5 h. Brine (50 mL) was then added, and after evolution of gas, the reaction mixture was diluted with water (150 mL), and extracted into DCM (3 x 100 mL). The organic layer was washed with aq. $NaHCO_3$ (4 %; 2 x 100 mL), water (2 x 100 mL), and aq. HCl (1M; 2 x 100 mL), and dried (Na_2SO_4). Removal of the solvent under reduced pressure yielded the *title compound* as a white solid (4.997 g, 98.6 %), R_f 0.27 [DCM/MeOH (9:1)] which was used directly in subsequent reactions.

Methyl 7α-*tert*-butoxycarbonylamino-3α-ethoxycarbonyloxy-12α-hydroxy-5β-cholan-24-oate 2.16

This procedure is modified from that of Kasal *et al.*[2.2]

2.15.HCl (5.00 g, 10.1 mmol) and di-*tert*-butyldicarbonate (2.30 g, 10.5 mmol, 1.04 equivs.) were stirred together in a biphasic mixture of THF (75 mL) and aq. KOH (10 % w/v; 600 mg in 6 mL, 10.7 mmol, 1.06 equivs.) at room temperature. After 23 h, aq. citric acid (10 % w/v; 25 mL) was added, and the mixture stirred for a further 0.5 h, then transferred to a separating funnel using diethyl ether (200 mL) and water (30 mL). The organic layer was kept, washed with water (3 x 50 mL), dried ($MgSO_4$), and concentrated *in vacuo* to yield **2.16** as a white foam (6.22 g). Recrystallisation from hexane/ethyl acetate (2:1; 75 mL) gave large white crystals (3.83 g). A second crop (1.47 g) was obtained from the mother liquor of these crystals. Total yield (5.30 g, 88 %), R_f 0.30 [hexane/ethyl acetate (2:1)], mp 179-180°C (lit.[2.2] 181-182°C); ν_{max} (film from $CHCl_3$)/cm^{-1} 3454 (NH & OH), 1735 (CO), 1702 (CO); δ_H(400MHz; $CDCl_3$) 0.70 (3H, s, 18-CH_3), 0.95 (3H, s, 19-CH_3), 0.98 (3H, d, *J* 6, 21-CH_3), 1.30 (t, CH_3CH_2O), 1.44 [s, $C(CH_3)_3$], 3.68 (4H, s, CO_2CH_3 & 7β-H), 3.99 (1H, m, 12β-H), 4.17 (2H, q, *J* 7, CH_3CH_2O), 4.43 (1H, br m, 3β-H), 4.82 (1H, br m, NH); δ_C(100MHz; $CDCl_3$) 12.2 (18-CH_3), 13.8 (19-CH_3), 16.9 (21-CH_3), 22.2 (CH_3), 22.4 (CH_2), 26.2 (CH_2), 27.6 (CH_2), 28.0 (CH), 28.1 [$C(CH_3)_3$], 30.4 (CH_2), 30.9 (CH_2), 31.3 (CH_2), 34.1 (CH_2), 34.2 (C), 34.5 (CH_2), 34.7 (CH), 36.6 (CH), 40.8 (CH), 42.2 (CH), 46.1 (C), 46.8 (CH), 46.9 (CH), 51.2 (CH_3), 63.1 (CH_2), 72.2 (CH), 77.3 (CH), 79 (C), 154.1 (OCOO), 155.0 (NHCOO), 174.6 (CO_2CH_3).

Methyl 3α-ethoxycarbonyloxy-12α-hydroxy-7α-{3-[4-(trifluoromethyl)phenyl]ureylene}-5β-cholan-24-oate 3.1

Method A:

Dry triethylamine (120 μL, 0.861 mmol, 2.13 equivs.) and α,α,α-trifluoro-*p*-tolylisocyanate (69 μL, 0.486 mmol, 1.2 equivs.) were added to a solution of **2.15.HCl** (200 mg, 0.405 mmol) at room temperature under Argon. After 1.5 h, the volatiles were removed under reduced pressure. The remaining oil was purified by flash chromatography using gradient elution (hexane/ethyl acetate 3:1, 3:2, 0:1) to yield **3.1** as a white solid (238 mg, 86 %), R_f 0.25 [hexane/EtOAc (3:2)], mp 253-254°C; (Found: C, 63.22; H, 7.49; N, 3.99. $C_{36}H_{51}F_3N_2O_7$ requires C, 63.51; H, 7.55; N, 4.11); ν_{max}(film from $CHCl_3$)/cm^{-1} 3388 (OH), 1734 (overlapping CO), 1696 (urea CO); δ_H(400MHz; $CDCl_3$) 0.71 (3H, s, 18-CH_3), 0.99 (6H, 19-CH_3 & 21-CH_3), 1.28 (3H, t, *J* 7, CH_3CH_2O), 3.69 (3H, s, CO_2CH_3), 3.95 (1H, m,

7β-H), 4.05 (1H, m, 12β-H), 4.16 (2H, q, *J* 7, CH_3CH_2O), 4.46 (1H, br m, 3β-H), 5.12 (1H, br d, N*H*CONHAr), 6.78 (1H, s, NHCON*H*Ar), 7.52 (4H, ArH); δ_C(100MHz; $CDCl_3$) 12.6 (18-CH_3), 14.2 (CH_3CH_2O), 17.4 (19-CH_3), 22.5 (21-CH_3), 23.0 (CH_2), 26.8 (CH_2), 27.2 (CH_2), 28.1 (CH), 28.4 (CH_2), 30.9 (CH_2), 31.2 (CH_2), 32.1 (CH_2), 34.6 (C), 34.7 (CH_2), 35.0 (CH), 35.2 (CH_2), 37.1 (CH), 41.2 (CH), 42.6 (CH), 46.5 (C), 47.0 (C_7H), 47.4 (CH), 51.7 (CO_2CH_3), 63.7 (CH_3CH_2O), 72.8 ($C_{12}H$), 77.8 (C_3H), 118.3 (ArCH), 124.3 (q, *J* 32, Ar*C*CF_3), 124.6 (q, *J* 270, Ar*C*F_3), 126.2 (ArCH), 142.6 (Ar*C*NH), 153.8 (NH*C*ONH), 154.6 (O*C*OO), 175.0 (CO_2CH_3).

Method B:

Dry triethylamine (0.55 mL, 3.94 mmol, 2.04 equivs.) and α,α,α-trifluoro-*p*-tolylisocyanate (0.33 mL, 2.31 mmol, 1.2 equivs.) were added to a solution of **2.15.HCl** (1.023 g, 1.93 mmol) in dry DCM (100 mL) at room temperature under Argon. After 30 min., the reaction was concentrated *in vacuo*. Trituration with a small amount of hexane and ethyl acetate gave the *title compound* (955 mg, 73 %) which contained two small (< 5 %) impurities by tlc.

Methyl 3α, 12α-dihydroxy-7α-{3-[4-(trifluoromethyl)phenyl]ureylene}-5β-cholan-24-oate 3.2

Using the method of Kasal et al.[2.2] (*K_2CO_3 as base)*

Aqueous K_2CO_3 (10 % w/v; 2 mL) was added to a solution of **3.1** (940 mg, 1.38 mmol) in MeOH (50 mL). The mixture was stirred at room temperature for 21 h, then at 50°C for 3 h. The reaction was cooled to room temperature and the MeOH removed under reduced pressure. The crude product (780 mg) was dissolved in $CHCl_3$ and washed with 1M HCl, followed by brine. The organic layer was dried (Na_2SO_4) and the solvent removed *in vacuo*. Flash chromatography eluting with chloroform/acetone (4:1) gave **3.2** as a white solid (489 mg, 58 %), R_f 0.13 [$CHCl_3$/acetone (4:1)], mp 176-177°C; (Found: C, 63.52; H, 7.70; N, 4.47. $C_{33}H_{47}F_3N_2O_5.H_2O$ requires C, 63.24; H, 7.88; N, 4.47); ν_{max}(film from DCM)/cm^{-1} 3410 (OH), 1691 (CO); δ_H(400MHz; $CDCl_3$) 0.71 (3H, s, 18-CH_3), 0.96 (6H, d, *J* 6.5, 21-CH_3 and s, 19-CH_3), 3.5-3.8 (4H, s, CO_2CH_3 and br m, 3β-H), 3.91 (1H, m, 7β-H), 4.11 (1H, m, 12β-H), 6.14 (1H, d, *J* 8, N*H*CONHAr), 7.12 (1H, s, NHCON*H*Ar), 7.35 (AB, *J* 9, ArH); δ_C(100MHz; $CDCl_3$) 12.0 (18-CH_3), 17.1 (19-CH_3), 21.8 (21-CH_3), 22.7 (CH_2) 26.8 (CH_2), 27.1 (CH), 27.2 (CH_2), 30.0 (CH_2), 30.3 (CH_2), 30.6 (CH_2), 31.9 (CH_2), 34.4 (CH_2), 34.6 (CH), 36.9 (CH), 38.2 (CH_2), 40.9 (CH), 41.8 (CH), 45.8, 46.3 (CH), 46.9 (CH), 51.1

(CO_2CH_3), 71.7 (CH), 73.3 (CH), 76.7 (CH), 116.8 (ArCH), 125.5 (ArCH), 142.2 (ArCNH), 153.8 (NHCONH), 174.2 (CO_2CH_3).

Using NaOMe as base:

3.1 (300 mg, 0.441 mmol) was dissolved in a solution of sodium methoxide in methanol (2M; 4 mL) over the course of 5 minutes at room temperature under Argon. The reaction was stirred for 1 h, then acidified with conc. H_2SO_4. The crude mixture was partitioned between brine and ethyl acetate. The ethyl acetate layer was washed with water, dried ($MgSO_4$) and solvent removed *in vacuo* to give **3.2** (228 mg, 85 %).

Methyl 3α, 12α-*bis*[(4-nitrophenyl)carbamoyloxy]-7α-{3-[4-(trifluoromethyl)phenyl]ureylene}-5β-cholan-24-oate 3.5

Dry DCM (10 mL) was syringed into a mixture of **3.2** (130 mg, 0.214 mmol), DMAP (104 mg, 0.851 mmol, 4.0 equivs.) and *p*-nitrophenylisocyanate (212 mg, 1.29 mmol, 6.0 equivs.) under Argon. The resulting solution was stirred at room temperature for 2 days, then morpholine (75 μL, 0.85 mmol, 4 equivs.) was added. The reaction was stirred for a further 0.5 h, then diluted with DCM (100 mL) and washed with aq. HCl (1M; 50 mL). The organic layer was kept, filtered and the filtrate concentrated *in vacuo* to give a yellow solid (287 mg). Flash chromatography using gradient elution (chloroform/acetone 100:0, 19:1, 9:1) gave **3.5** (125 mg, 62 %), R_f 0.27 [$CHCl_3$/acetone (19:1)], mp 232-234°C; (Found: C, 59.13; H, 5.91; N, 8.70. $C_{47}H_{55}F_3N_6O_{11}.H_2O$ requires C, 59.11; H, 6.02; N, 8.80); ν_{max}(film from DCM/cm^{-1}) 3415 (NH), 1738 (CO), 1701 (CO); δ_H(400MHz; $CDCl_3$) 0.84 (s, 18-CH_3), 0.88 (d, *J* 6, 21-CH_3), 1.06 (s, 19-CH_3), 3.62 (3H, s, CO_2CH_3), 4.09 (1H, m, 7β-H), 4.41 (1H, br m, 3β-H), 5.10 (1H, m, 12β-H), 5.55 (1H, d, *J* 7.5, N*H*CONHAr), 6.49 (1H, s, NH), 7.12 (2H, d, *J* 9, ArH), 7.37 (2H, d, *J* 9, ArH), 7.47 (1H, s, NH), 7.53 (4H, AB, *J* 8.5, ArH), 7.61 (1H, s, NH), 7.98 (4H, AB, *J* 4.5, ArH); δ_C(100MHz; $CDCl_3$) 11.7 (18-CH_3), 17.0 (19-CH_3), 22.0 (21-CH_3), 22.5 (CH_2), 25.1 (CH_2), 26.2 (CH_2), 26.8 (CH_2), 28.4 (CH), 30.2 (CH_2), 30.4 (CH_2), 31.9 (CH_2), 34.1 (CH_2), 34.2 (CH), 34.4 (C), 34.5 (CH_2), 36.4 (CH), 40.7 (CH), 43.6 (CH), 44.9 (C), 46.3 (CH), 46.8 (CH), 51.0 (CO_2CH_3), 77 (CH), 77.6 (CH), 116.9 (ArCH), 117.0 (ArCH), 117.3 (ArCH), 123 (q, *J* 33, CCF_3), 124 (q, *J* 270, CF_3), 124.6 (ArCH), 124.7 (ArCH), 125.6 (ArCH), 141.1 (ArCNH), 141.6 (ArCNH), 142.4 (ArCNH), 144.4 ($ArCNO_2$), 144.5 ($ArCNO_2$), 151.1 ($NHCO_2$), 152.0 $NHCO_2$), 153.8 (NHCONH), 174.6 (CO_2CH_3). Some of the 3α-monocarbamate was also isolated (21 mg).

Methyl 3α, 12α-*bis*[(*N*-*tert*-butylcarbamoyl)oxy]-7α-{3-[4-(trifluoromethyl)phenyl]ureylene}-5β-cholan-24-oate 3.6

Tert-butylisocyanate (6 μL, 7 x 10^{-3} mmol, 2.7 equivs.) and chlorotrimethylsilane (1 μL, 8 x 10^{-3} mmol, 0.3 equivs.) were added to a solution of **3.2** (16 mg, 2.6 x 10^{-2} mmol) in dry DCM (2 mL) under Argon. The solution was stirred at room temperature for three days, at which time tlc indicated the presence of starting material only. Further quantities of *tert*-butylisocyanate (5 μL) and chlorotrimethylsilane (1 μL) were then added, and the reaction stirred for another six days. After this time, the reaction was concentrated *in vacuo*, equal volumes of water and DCM (10 mL) were added, and the reaction stirred for 48 h, then partitioned between DCM and brine. The organic layer was washed with water, dried (Na_2SO_4), and the solvent removed under reduced pressure to yield an oil which contained some destroyed isocyanate as a partly crystalline solid. A little chloroform was added to dissolve the oil, and this solution was decanted from the solid material. Evaporation of this solution *in vacuo* yielded a white solid (12 mg). Flash chromatography of this solid eluting with hexane/ethyl acetate (3:2) gave **3.6** (8 mg, 67 %); δ_H(400MHz, $CDCl_3$) 0.76 (3H, s, 18-CH_3), 0.89 (3H, d, *J* 6.5, 21-CH_3), 0.96 (3H, s, 19-CH_3), 1.33 [s, $C(CH_3)$], 1.37 [s, $C(CH_3)$], 3.67 (3H, s, CO_2CH_3), 3.92 (1H, br m, 7β-H), 4.39 (1H, m, 3β-H), 4.52 (1H, br m, 12β-H), 4.68 (1H, s, NH), 4.76 (1H, br d, N*H*CONHAr), 4.97 (1H, br s, NH), 6.49 (1H, s, NHCON*H*Ar), 7.55 (4H, AB, *J* 9, ArH); δ_C(100MHz, $CDCl_3$) 12.5, 17.5, 22.7, 22.9 (CH_2), 25.9 (CH_2), 27.1 (CH_2), 27.2 (CH_2), 29.0, 29.1 [$C(CH_3)$], 29.2 [$C(CH_3)$], 30.8 (CH_2), 31.0 (CH_2), 32.2 (CH_2), 34.61, 34.64, 34.8 (CH_2), 36.2, 36.9, 41.3, 44.4, 45.4 (C), 47.3, 47.5, 50.38 (C), 50.43 (C), 51.5, 74.3, 75.0, 118.7 (ArCH), 126.3 (ArCH), 134.4 (C), 142.3 (C), 153.8 (C), 154.5 (C), 174.6 (CO_2CH_3).

Methyl 7α-*tert*-butoxycarbonylamino-3α-ethoxycarbonyloxy-12α-phenylcarbamoyloxy-5β-cholan-24-oate 3.7

Phenyl isocyanate (0.40 mL, 3.68 mmol, 2.2 equivs.) and chlorotrimethylsilane (43 μL, 0.34 mmol, 0.2 equivs.) were syringed into a solution of **2.16** (1.00 g) in dry 1,2-dichloroethane (10 mL) at room temperature under Argon. The reaction was warmed to 40°C and stirred at this temperature for 66 h, at which time tlc indicated the presence of **2.16**. Further quantities of phenylisocyanate (0.20 mL, 1.84 mmol, 1.1 equivs.) and chlorotrimethylsilane (25 μL, 0.20 mmol, 0.12 equivs.) were added at this time. The reaction was stirred at 40°C for another 24 h then concentrated *in vacuo*. Flash chromatography eluting with chloroform/acetone (49:1) yielded **3.7** as a white foam (407 mg, 34 %), R_f 0.32

[$CHCl_3$/acetone (49:1)], mp 106-108°C; (Found: C, 67.00; H, 8.41; N, 3.95. $C_{40}H_{60}N_2O_9$ requires C, 67.39; H, 8.48; N, 3.93); ν_{max}(film from DCM/cm^{-1}) 3427 (NH), 1732 (overlapping CO); δ_H(400MHz; $CDCl_3$) 0.79 (3H, s, 18-CH_3), 0.92 (3H, d, *J* 6, 21-CH_3), 0.96 (3H, s, 19-CH_3), 1.30 (t, *J* 7, CH_3CH_2O), 1.51 [s, $C(CH_3)_3$], 3.66 (3H, s, CO_2CH_3), 3.73 (1H, br s, 7β-H), 4.17 (2H, q, *J* 7, CH_3CH_2O), 4.44 (1H, br m, 3β-H), 4.57 (1H, br s, NHBOC), 5.15 (1H, s, 12β-H), 6.76 (1H, br s, $ArNHCO_2$), 7.11 (1H, t, *J* 7, *p*-ArH), 7.36 (2H, t, *J* 7.5, *m*-ArH), 7.47 (2H, br d, *o*-ArH); δ_C(100MHz; $CDCl_3$) 12.0 (CH_3), 13.7 (CH_3), 17.2 (CH_3), 22.2 (CH_3), 22.3 (CH_2), 25.5 (CH_2), 26.2 (CH_2), 26.7 (CH_2), 28.1 (CH_3), 30.3 (CH_2), 30.6 (CH_2), 31.3 (CH_2), 34.0 (CH_2), 34.3 (CH), 34.6 (CH_2), 36.2 (CH), 40.7 (CH), 44.0 (C), 45 (C), 47.1 (CH), 51.0 (CH_3), 63.2 (CH_2), 77.1 (CH), 128.6 (ArCH), 174.0 (CO_2CH_3).

Methyl 3α-ethoxycarbonyloxy-12α-(4-nitrophenyl)carbamoyloxy-7α-{3-[4-(trifluoromethyl)phenyl]ureylene}-5β-cholan-24-oate 3.8

Dry DCM (10 mL) was syringed into a mixture of DMAP (179 mg, 1.47 mmol, 2.0 equivs.), *p*-nitrophenylisocyanate (361 mg, 2.20 mmol, 3.0 equivs.) and **3.1** (500 mg, 0.734 mmol) at room temperature under Argon. The reaction was stirred for two days, then concentrated *in vacuo*. The crude product was only partially purified by flash chromatography using chloroform as eluent. Another column using chloroform/acetone (49:1) as eluent yielded **3.8** (393 mg, 44 %); δ_H(400MHz, $CDCl_3$) 0.81 (3H, s, 18-CH_3), 0.87 (3H, d, *J* 6.5, 21-CH_3), 1.15 (3H, t, *J* 7, CH_3CH_2O), 3.63 (3H, s, CO_2CH_3), 4.09 (3H, q, *J* 7, CH_3CH_2O & m, 7β-H), 4.49 (1H, br m, 3β-H), 5.05 (1H, br m, 12β-H), 5.34 (1H, br d, NHCONHAr), 7.15 (1H, br s, NH), 7.46(3H, d, *J* 9, 12α-ArH & s, NH), 7.57 (4H, AB, *J* 8.5, 7α-ArH), 8.17 (2H, d, *J* 9, 12α-ArH).

Methyl 3α-ethoxycarbonyloxy-7α-[3-(4-fluorophenyl)thioureylene]-12α-hydroxy-5β-cholan-24-oate 3.9

Dry DCM (25 mL) and triethylamine (120 μL, 0.86 mmol, 2.1 equivs.) were syringed into a mixture of **2.15** (200 mg, 0.41 mmol) and 4-fluorophenylisothiocyanate (78 mg, 0.51 mmol, 1.3 equivs.) at room temperature under Argon. The reaction was allowed stir overnight, then concentrated *in vacuo* to yield a white solid (288 mg). Flash chromatography of this solid eluting with hexane/ethyl acetate (3:2) yielded **3.9** as a colourless oil, which became a glassy solid after several hours under high vacuum, (225 mg, 86 %), mp 98-102°C; ν_{max}(film from

$CHCl_3$)/cm^{-1} 3388 (NH & OH), 1731 (CO); δ_H(400MHz, $CDCl_3$) 0.68 (3H, s, 18-CH_3), 0.93 (3H, s, 19-CH_3), 0.97 (3H, d, *J* 6, 21-CH_3), 1.36 (t, *J* 7, CH_3CH_2O), 3.71 (3H, s, CO_2CH_3), 3.96 (1H, br m, 12β-H), 4.20 (2H, q, *J* 7, CH_3CH_2O), 4.24 (2H, 3β-H & 7β-H), 5.96 (1H, d, *J* 8.5, NHCSNHAr), 7.21 (ArH), 7.43 (1H, s, NHCSNHAr); δ_C(100MHz, $CDCl_3$) 12.4 (CH_3), 14.3 (CH_3), 17.4 (CH_3), 22.6 (CH_3), 23.1 (CH_2), 26.5 (CH_2), 27.2 (CH_2), 28.3 (CH), 28.4 (CH_2), 30.7 (CH_2), 30.9 (CH_2), 31.2 (CH_2), 34.2 (C), 34.4 (CH_2), 35.1 (CH), 35.4 (CH_2), 37.2 (CH), 40.8 (CH), 42.8 (CH), 46.4 (C), 47.4 (CH), 51.5 (CH_3), 51.9, 63.6 (CH_2), 72.1 (CH), 117.3, 117.5, 129.2, 129.3, 131.5 (C), 154.5 (OCO_2), 160.9 (C), 163.3 (C), 174.5 (CO_2CH_3), 179.8 (C).

Methyl 7α-*tert*-butoxycarbonylamino-3α-ethoxycarbonyloxy-12α-(4-nitrophenyl)carbonyloxy-5β-cholan-24-oate 3.10

A solution of *p*-nitrophenylchloroformate (535 mg, 2.65 mmol, 2.05 equivs.) in dry THF (2 mL) was cannulated into a solution of **2.16** (767 mg, 1.29 mmol) in dry THF (10 mL) at room temperature under Argon. The solution was stirred for five minutes before dry pyridine (0.2 mL, 2.5 mmol, 1.9 equivs.) was added dropwise. A white precipitate occurred, but no significant rise in temperature was noticed. The reaction was allowed stir at room temperature for 49 h, then the precipitate was filtered off, and the filtrate diluted with Et_2O (50 mL). The Et_2O layer was washed with aq. Na_2CO_3 (2M; 2 x 25 mL), and water (6 x 25 mL), dried ($MgSO_4$), and concentrated *in vacuo* to give an off-white foam (1.036 g) which contained a large UV active impurity. Flash chromatography of the crude product eluting with DCM gave **3.10** as a white foam (376 mg, 38 %), R_f 0.33 [hexane/EtOAc (2:1)]; δ_H(400MHz, $CDCl_3$) 0.82 (3H, s, 18-CH_3), 0.93 (3H, d, *J* 7, 21-CH_3), 0.99 (3H, s, 19-CH_3), 1.33 (t, *J* 7, CH_3CH_2O), 1.48 [s, $C(CH_3)$], 3.68 (3H, s, CO_2CH_3), 3.76 (1H, br m, 7β-H), 4.21 (2H, q, *J* 7, CH_3CH_2O), 4.49 (1H, br m, 3β-H), 4.61 (1H, br m, NH), 5.11 (1H, br m, 12β-H), 7.44 (2H, d, *J* 9, ArH), 8.32 (2H, d, *J* 9, ArH).

Triphenylacetyl chloride

This procedure is modified from that of Zook *et al.*[3.23]

Triphenylacetic acid (6.03 g, 20.9 mmol) and distilled thionyl chloride (25 mL) were refluxed (85°C) together for 4 h, allowed cool to room temperature, then poured on to an ice/water mixture (*ca.* 150 mL) and the resulting precipitate filtered. The filtered solid was dissolved in DCM (*ca.* 60 mL), dried ($MgSO_4$), and concentrated *in vacuo* to give an off-

white solid (6.10 g). Crystallisation overnight from cyclohexane yielded the *title compound* as off-white crystals (5.06 g, 79 %), R_f 0.69 [Hexane/EtOAc (5:1)]; ν_{max}(film from DCM/cm^{-1}) 1773 (CO), 1601, 1492, 1447, 1035, 1017, 999; δ_H(400MHz, DMSO-d_6) 7.0-7.4 (m, ArH); δ_C(100MHz, DMSO-d_6) 66.9 (Ph_3C), 126.6 (*p*-ArC), 127.6 (*m*-ArC), 129.9 (*o*-ArC), 143.2 (ArC), 174.2 (*C*OCl).

Methyl 3α-triphenylmethylcarbonyloxy-5β-lithocholan-24-oate 3.16

Dry pyridine (1 mL) was syringed into a solution of methyl lithocholate (391 mg, 1.00 mmol) and triphenylacetylchloride (369 mg, 1.20 mmol, 1.20 equivs.) in dry toluene (3 mL) at room temperature under Argon. A pale yellow colour resulted. A small amount of precipitation was observed in the reaction flask after 1 h. After 20 h, DMAP (18 mg, 0.147 mmol) and toluene (1 mL) were added, and the reaction stirred for another 5 days. Further quantities of triphenylacetylchloride (96 mg, 0.31 mmol), DMAP (39 mg, 0.319 mmol) and pyridine (1 mL) were added at this time. The reaction was stirred for another day, and more triphenylacetylchloride (153 mg, 0.50 mmol; total 2.0 equivs.) added. The following day, the precipitate was filtered off. The filtrate was diluted with Et_2O (50 mL) and washed with aq. Na_2CO_3 (2M; 2 x 25 mL), aq. CH_3CO_2H (2M; 2 x 25 mL), H_2O (2 x 25 mL), dried ($MgSO_4$), and concentrated *in vacuo*. Flash chromatography eluting with hexane/ethyl acetate (6:1) gave **3.16** as a white foam (162 mg, 25 %), R_f 0.39 [hexane/EtOAc (6:1)], mp 72-73°C; (Found: C, 82.15; H, 8.34. $C_{45}H_{56}O_4$ requires C, 81.78; H, 8.54); ν_{max}(film from DCM/cm^{-1}) 1726 (CO); δ_H(400MHz; $CDCl_3$) 0.66 (3H, s, 18-CH_3), 0.93 (6H, s, 19-CH_3 and d, *J* 6.5, 21-CH_3), 3.69 (3H, s, CO_2CH_3), 4.92 (1H, m, 3β-H), 7.0-7.2 (ArH); δ_C(100MHz; $CDCl_3$) 11.6 (CH_3), 17.8 (CH_3), 20.4 (CH_2), 22.9 (CH_3), 23.7 (CH_2), 25.85 (CH_2), 25.93 (CH_2), 26.6 (CH_2), 27.7 (CH_2), 30.58 (CH_2), 30.62 (CH_2), 31.4 (CH_2), 34.2 (C), 34.6 (CH_2), 34.9 (CH), 35.4 (CH), 39.6 (CH_2), 39.9 (CH), 41.6 (CH), 42.3 (C), 48.3 (C), 51.0 (CH_3), 55.5 (CH), 55.8 (CH), 75.7 (CH), 126.3 (CH), 126.6 (CH), 127.1 (CH), 127.5 (CH), 129.6 (CH), 129.9 (CH), 142.7 (C), 172.6 (Ph_3CCO_2), 174.3 (CO_2CH_3), 188.5 (C).

Methyl 3α-triphenylmethylcarbonyloxy-5β-cholan-24-oate 3.17

Dry pyridine (3 mL) was syringed into a mixture of **2.12** (500 mg, 1.18 mmol), triphenylacetylchloride (1.45 g, 4.73 mmol, 4.0 equivs.) and dry toluene (7 mL) at room temperature under Argon. The reaction mixture went into solution upon this addition. The reaction was heated at 80°C under an atmosphere of Argon for 1 h, after which time some

precipitation was visible in the flask. The reaction was maintained at 80°C for another 24 h, then allowed cool to room temperature and the precipitate filtered off. The filtrate was diluted with Et_2O (50 mL), washed with aq. Na_2CO_3 (2M; 2 x 25 mL), aq. CH_3CO_2H (2M; 2 x 25 mL) and water (2 x 25 mL), and dried ($MgSO_4$). Some crystallisation (120 mg) of an aromatic impurity occurred from this Et_2O solution. These were filtered off and the filtrate concentrated *in vacuo* to give an oil. The oil was redissolved in a minimum of Et_2O and a small quantity of hexane added. A solid (707 mg) precipitated. Flash chromatography of this solid eluting with chloroform gave **3.17** (378 mg, 46 %), R_f 0.27 ($CHCl_3$); δ_H(400MHz, $CDCl_3$), 0.70 (3H, s, 18-CH_3), 0.90 (3H, s, 19-CH_3), 1.00 (3H, d, *J* 6, 21-CH_3), 3.69 (3H, s, CO_2CH_3), 3.84 (1H, br m, 7β-H), 3.94 (1H, br m, 12β-H), 4.76 (1H, br m, 3β-H), 7.15-7.35 (ArH).

Methyl 7α-*tert*-butoxycarbonylamino-12α-hydroxy-3α-triphenylmethylcarbonyloxy-5β-cholan-24-oate 3.18

Dry pyridine (1.5 mL) was syringed into a mixture of NHBOC diol (350 mg, 0.671 mmol), triphenylacetylchloride (1.03 g, 3.35 mmol, 5.0 equivs.) and dry toluene (3.5 mL) at room temperature under Argon. The mixture gradually went into solution as the reaction was heated to 80°C. The reaction was stirred at 80°C for 2 days, then allowed cool to room temperature and diluted with Et_2O (20 mL). Some precipitation occurred upon this addition. This precipitate was filtered off, and the filtrate transferred to a separating funnel with Et_2O (30 mL). The Et_2O layer was washed with aq. HCl (1M; 2 x 25 mL), aq. Na_2CO_3 (2M; 2 x 25 mL) and H_2O (2 x 25 mL), dried (Na_2SO_4), and concentrated *in vacuo* to yield a sticky peach coloured foam (1.01 g). Flash chromatography of this foam eluting with DCM/MeOH (99:1) resulted in the purification of the compound appearing at R_f 0.14 in this solvent system (424 mg). Further tlc analysis in other solvent systems [toluene/Et_2O (6:1), hexane/EtOAc (3:1), toluene/EtOAc (8:1)] revealed that this single spot consisted of three compounds. Flash chromatography of this mixture eluting with toluene/dichloromethane/ethyl acetate (7:2:1) yielded **3.18** as a white powder (163 mg, 30 %), R_f 0.27 [toluene/DCM/EtOAc (7:2:1)], mp 216-218°C; (Found: C, 75.56; H, 8.25; N, 1.80. $C_{50}H_{65}NO_7$ requires C, 75.82; H, 8.27; N, 1.77); ν_{max}(film from DCM/cm^{-1}) 3473 (OH), 1730 (CO), 1695 (CO); δ_H(400MHz; $CDCl_3$) 0.70 (3H, s, 18-CH_3), 0.94 (3H, s, 19-CH_3), 0.99 (3H, d, *J* 6, 21-CH_3), 1.46 [s, C(CH_3)], 3.67 (4H, s, CO_2CH_3 and s, 7β-H), 4.01 (1H, m, 12β-H), 4.66 (1H, br d, NH), 4.76 (1H, br m , 3β-H), 7.2-7.3 (ArH); δ_C(100MHz; $CDCl_3$) 12.2 (18-CH_3), 16.9 (19-CH_3), 22.4 (21-CH_3), 26.2 (CH_2), 26.8 (CH_2), 28.1 (CH), 28.2

(CH_3), 30.4 (CH_2), 30.7 (CH_2), 31.2 (CH_2), 34.2 (C), 34.3 (CH_2), 34.6 (CH), 36.5 (CH), 41.0 (CH), 42.4 (CH), 46.1 (C), 46.8 (CH), 46.9 (CH), 51.1 (CH_3), 66.9 (C), 72.3 (CH), 75.4 (CH), 78.5 (C), 126.3 (ArCH), 127.2 (ArCH), 129.9 (ArCH), 142.6 (C), 155.0 (C), 172.6 (Ph_3CCO_2), 174.3 (CO_2CH_3).

Methyl 7α-*tert*-butoxycarbonylamino-3α-hydroxy-12α-phenylcarbamoyloxy-5β-cholan-24-oate 3.19

3.7 (260 mg, 0.365 mmol) was dissolved in a solution of sodium methoxide in methanol (2M; 2 mL, 4 mmol, 11 equivs.) at room temperature under Argon. The reaction was stirred for 55 min., then acidified with aq. NH_4Cl (500 mg in 3 mL, 9.3 mmol, 25.5 equivs.). Some precipitation occurred. The reaction mixture was transferred to a separating funnel and extracted with DCM (3 x 5 mL). The combined organic layers were diluted to twice their volume and washed with half saturated brine (2 x 10 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give **3.19** as a white foam (185 mg, 79 %), R_f 0.23 [toluene/EtOAc (2:1)], mp 115-118°C (lit.[1.17] 116-123°C); ν_{max}(film from DCM/cm^{-1}) 3340 (OH), 1724 (overlapping CO); δ_H(400MHz; $CDCl_3$) 0.79 (3H, s, 18-CH_3), 0.94 (6H, d, *J* 6.5, 21-CH_3 and s, 19-CH_3), 1.51 [s, C(CH_3)], 1.61 (s, H_2O), 3.47 (1H, br m, 3β-H), 3.66 (3H, s, CO_2CH_3), 3.74 (1H, m, 7β-H), 4.68 (1H, br s, N*H*BOC), 5.16 (1H, m, 12β-H), 6.82 (1H, br s, ArN*H*CO_2), 7.11 (1H, br t, *p*-ArH), 7.37 (2H, br t, *m*-ArH), 7.47 (2H, br d, *o*-ArH), (lit.[1.17] 0.77, 0.90, 0.93, 1.49, -, 3.45, 3.64, 3.71, 4.64, 5.16, 6.82, 7.11, 7.37, 7.47); δ_C(100MHz; $CDCl_3$) 12.0 (18-CH_3), 17.2 (19-CH_3), 22.3 (21-CH_3), 22.3 (CH_2), 25.5 (CH_2), 26.7 (CH_2), 28.1 (CH_3), 28.8 (CH?), 30.1 (CH_2), 30.3 (CH_2), 30.6 (CH_2), 31.5 (CH_2?), 34.0 (C), 34.3 (CH), 34.4 (CH_2), 36.2 (CH), 38.8 (CH_2), 40.9 (CH), 44.0 (CH), 44.9 (C), 46.8 (CH_3), 47.1 (CH), 51.0 (CH_3), 71.2 (CH), 78.9 (C), 118.4, 123.1, 128.6 (CH), 137.6 (C), 152.6, 154.4, 154.9, 174.1 (CO_2CH_3).

Methyl 7α-*tert*-butoxycarbonylamino-12α-phenylcarbamoyloxy-3α-triphenylmethylcarbonyloxy-5β-cholan-24-oate 3.20

3.19 (142 mg, 0.22 mmol) and triphenylacetylchloride (340 mg, 1.11 mmol, 5.0 equivs.) were dissolved in dry toluene (1 mL) and dry pyridine (1 mL) at room temperature under Argon. The reaction was heated to 80°C over the course of 30 min., and maintained at that temperature for 18.5 h, then cooled to room temperature and diluted to *ca.* 25 mL with Et_2O. The Et_2O layer was washed with aq. HCl (1M; 2 x 10 mL), H_2O (2 x 10 mL), aq. Na_2CO_3 (2M; 2 x 10 mL) and H_2O (2 x 10 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give an

off-white foam (343 mg). The crude product was dissolved in a minimum of toluene/ethyl acetate (14:1, *ca.* 1 mL) and some crystallisation of an impurity occurred. These crystals (133 mg) were filtered off. Flash chromatography of the filtrate eluting with toluene/ethyl acetate (14:1) gave two steroidal products, which had R_f 0.28 and 0.13 in the same solvent system. The fractions eluting at lower R_f contained **3.20** (50 mg, 25 %), R_f 0.13 [toluene/EtOAc (14:1)], mp 186-187°C; (Found C, 73.88; H, 7.64; N, 3.01. $C_{57}H_{70}N_2O_8.H_2O$ requires C, 73.68; H, 7.81; N, 3.01); ν_{max}(film from DCM/cm^{-1}) 3427 (NH), 1724 (CO); δ_H(400MHz; $CDCl_3$) 0.77(3H, s, 18-CH_3), 0.93 (6H, s, 19-CH_3 and d, *J* 6.5, 21-CH_3), 1.52 [s, C(CH_3)], 1.60 (s, H_2O), 3.66 (4H, s, CO_2CH_3 and 7β-H), 4.6 (1H, br s, N*H*BOC), 4.77 (1H, br m, 3β-H), 5.12 (1H, m, 12β-H), 6.85 (1H, br s, $NHCO_2$), 7.0-7.5 (ArH); δ_C(100MHz; $CDCl_3$) 11.9 (18-CH_3), 17.2 (19-Ch_2), 22.3 (CH_2), 22.3 (CH_3), 25.5 (CH_2), 26.0 (CH_2), 26.7 (CH_2), 28.1 (CH_3), 30.4 (CH_2), 30.6 (CH_2), 31.2 (CH_2), 34.1 (CH_2), 34.2 (CH_2), 34.3 (CH), 36.2 (CH), 40.9 (CH), 44.9 (C), 47.1 (CH), 51.0 (CO_2CH_3), 66.9 (C), 75.3 (CH), 124.8 (ArCH), 126.3 (ArCH), 127.2 (ArCH), 127.8 (ArCH), 128.6 (ArCH), 129.8 (ArCH), 142.6 (C), 172.4 (Ph_3CCO), 174.1 (CO_2CH_3).

Methyl 3α-(3, 5-dinitrophenyl)carbonyloxy-12α-phenylcarbamoyloxy-7α-{3-[4-(trifluoromethyl)phenyl]ureylene}-5β-cholan-24-oate 5.4

Trifluoroacetic acid (0.5 mL) was syringed into a solution of **5.5** (153 mg, 0.183 mmol) in dry DCM (1.5 mL) at room temperature under Argon. The solution was stirred for 1.5 h, then concentrated *in vacuo* to yield the ammonium trifluoroacetate as a light yellow powder (167 mg). DIPEA (30 μL, 0.17 mmol, 2.2 equivs.) and α,α,α-trifluoro-*p*-tolylisocyanate (13 μL, 0.091 mmol, 1.15 equivs.) were syringed into a solution of this ammonium trifluoroacetate (67 mg, 0.079 mmol) in dry DCM (1 mL) at room temperature under Argon. The reaction was stirred for 21 h, then more DIPEA (30 μL) was added. The reaction was stirred for another 4 days, at which time tlc indicated the presence of starting material. Triethylamine (0.1 mL) and α,α,α-trifluoro-*p*-tolylisocyanate (10 μL) were added, and the reaction stirred for another 22 h, then concentrated *in vacuo* to yield an oil. Flash chromatography of this oil using gradient elution [DCM/MeOH (100:0 – 98:2)] yielded a light yellow solid (62 mg) which was judged to be 90 % pure by tlc. Another column eluting with chloroform/acetone (24:1) yielded **5.4** as a light yellow solid (52 mg, 71 %), R_f 0.23 [$CHCl_3$/acetone (24:1)], mp 157-160°C; (Found: C, 59.29; H, 5.77; N, 7.18. $C_{47}H_{54}F_3N_5O_{11}.2H_2O$ requires C, 58.93; H, 6.10; N, 7.31); ν_{max}(film from DCM/cm^{-1}) 1727 (CO), 1697 (CO); δ_H(400MHz; $CDCl_3$) 0.84 (3H, s, 18-CH_3), 0.93 (3H, d, *J* 6.5, 21-CH_3),

1.07 (3H, s, 19-CH_3), 1.58 (s, H_2O), 3.65 (3H, s, CO_2CH_3), 3.92 (1H, m, 7β-H), 4.72 (1H, br s, N*H*CONHAr), 4.95 (1H, m, 3β-H), 5.18 (1H, m, 12β-H), 6.44 (1H, br s, NH), 6.88 (1H, br s, NH), 7.12 (1H, t, *J* 7, *p*-ArH), 7.39 (2H, t, *J* 8, *m*-ArH), 7.50 (2H, d, *J* 8, *o*-ArH), 7.55 (4H, s, ArH), 9.11 (2H, d, *J* 2.5, ArC*H*CNO_2), 9.21 [1H, t, *J* 2, $ArCH(CNO_2)_2$]; δ_C(100MHz; $CDCl_3$) 12.0 (CH_3), 17.1 (CH_3), 22.29 (CH_3), 22.34 (CH_2), 25.5 (CH_2), 26.5 (CH_2), 26.6 (CH_2), 28.8 (CH), 30.3 (CH_2), 30.5 (CH_2), 31.4 (CH_2), 34.1 (CH_2), 34.2 (CH), 34.8 (CH_2), 36.3 (CH), 40.8 (CH), 43.9 (CH), 45.0 (C), 47.0 (CH), 47.1 (CH), 51.1 (CH_3), 75.9 (CH), 76.6 (CH), 118.7 (CH), 121.8 (CH), 123.3 (CH), 123.7 (q, *J* 270, CF_3), 124.4 (q, *J* 33, *C*CF_3), 125.8 (CH), 128.7 (CH), 128.8 (CH), 131.5 (C), 133.8 (C), 141.6 (C), 148.1 (C), 152.5 (C), 153.4 (C), 161.3 (C), 174.3 (*C*O_2CH_3).

Methyl 7α-*tert*-butoxycarbonylamino-3α-(3, 5-dinitrophenyl)carbonyloxy-12α-phenylcarbamoyloxy-5β-cholan-24-oate 5.5

3.19 (263 mg, 0.410 mmol) and 3, 5-dinitrobenzoylchloride (95 mg, 0.410 mmol, 1.0 equivs.) were dissolved in dry pyridine (3 mL) at room temperature under Argon. The reaction was stirred for 3 h, then concentrated *in vacuo*, using toluene to azeotrope residual pyridine. Flash chromatography eluting with toluene/ethyl acetate (8:1) gave **5.5** as a light brown solid (225 mg, 75 %), R_f 0.28 [toluene/EtOAc (8:1)], mp 120-123°C; (Found: C, 63.36; H, 6.99; N, 6.41. $C_{44}H_{58}N_4O_{12}$ requires C, 63.29; H, 7.00; N, 6.71); ν_{max}(film from DCM/cm^{-1}) 1726 (CO); δ_H(400MHz; $CDCl_3$) 0.80 (3H, s, 18-CH_3), 0.91 (3H, br d, 21-CH_3), 1.00 (3H, s, 19-CH_3), 1.49 [s, $C(CH_3)_3$], 3.64 (3H, s, CO_2CH_3), 3.71 (1H, m, 7β-H), 4.62 (1H, br s, N*H*BOC), 4.89 (1H, br m, 3β-H), 5.16 (1H, m, 12β-H), 6.89 (1H, br s, ArN*H*CO_2), 7.08 (1H, br m, *o*-ArH), 7.34 (2H, br m, *m*-ArH), 7.47 (2H, br m, *o*-ArH), 9.11 (2H, s, ArC*H*NO_2), 9.20 [1H, s, ArC*H*$(CNO_2)_2$]; δ_C(100MHz; $CDCl_3$) 12.0 (18-CH_3), 17.1 (19-CH_3), 22.2 (CH_2), 22.3 (CH_3), 25.6 (CH_2), 26.5 (CH_2), 26.7 (CH_2), 28.1 (CH_3), 30.3 (CH_2), 30.5 (CH_2), 34.0 (CH_2), 34.1 (C), 34.3 (CH), 34.6 (CH_2), 36.2 (CH), 40.9 (CH), 44.9 (C), 47.2 (CH), 51.0 (CH_3), 121.8 (ArCH), 128.7 (ArCH), 128.9 (ArCH), 133.9 (C), 148.2 (C), 174.1 (CO_2CH_3).

2-hydroxy-4-phenylnitrobutane 7.2

Using the method of Wollenberg et al.[7.2]

Hydrocinnamaldehyde (275 μL, 2.01 mmol) was added dropwise to a solution of nitromethane (245 μL, 3.56 mmol, 1.77 equivs.) and aq. KF (5.8 mg in 100μL) in *iso*-propanol (2 mL), and the reaction stirred at room temperature for 54 h, then concentrated *in vacuo* to yield an oil. Trituration of this oil with hexane gave a white solid. This solid was taken up in DCM (20 mL), washed with water (2 x 10 mL), dried (Na_2SO_4), and the solvent removed under reduced pressure to yield an off-white solid (323 mg). Two recrystallisations from hexane/ethyl acetate yielded the *title compound* as a white solid (200 mg, 51 %), R_f 0.32 [hexane/EtOAc (2:1)], mp 82-83°C; ν_{max}(film from $CHCl_3$/cm^{-1}) 3388 (OH), 1556 (NO_2 *anti*); δ_H(400MHz; $CDCl_3$) 1.86 (2H, m, PhCH_2), 2.68 (1H, d, *J* 4.5, OH), 2.82 (2H, m, CH_2CHOH), 4.33 (1H, br m, C*H*OH), 4.42 (2H, m, CH_2NO_2), 7.29 (5H, m, ArH); δ_C(100MHz, $CDCl_3$) 30.9 (Ph*C*H_2), 34.7 (*C*H_2CHOH), 67.4 (*C*HOH), 80.1 (*C*HNO_2), 125.9 (ArCH), 128.0 (ArCH), 128.2 (ArCH), 140.2 (ArC).

Chapter 9

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