Approaches Towards the Solid Phase Synthesis of Small Molecule Libraries

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This thesis is submitted in part fulfilment of the requirement for the degree of Doctor of Philosophy at the University of Edinburgh. Unless otherwise stated, the work is original, and has not been previously submitted, in whole or in part, for any degree at this, or any other university.

To all my family - glad I made it!

Abstract

The increased appreciation of combinatorial synthesis as a valuable tool in the drug discovery process has led to the recent escalation in research into solid phase synthesis techniques. The use of multiple parallel solid phase syntheses has been described as an efficient method of lead compound optimisation. This method is explored for the generation of analogues of a substituted acrylic acid, identified from random screening, in order to increase receptor affinity. The optimised solution phase synthesis of the lead compound *via* a Horner-Wadsworth-Emmons reaction is reported. The synthetic route is then described for the solid phase synthesis of a simple analogue. Attempts have been made towards the multiple parallel solid phase syntheses of a range of substrates.

Alternative methods for facile product purification as alternatives to covalent binding to a polymeric resin have recently been researched. The affinity of tetrabenzo [*a,c,g,i*]fluorene (Tbf) for charcoal has already been described as a useful method for the purification of peptides and proteins, and this property has been harnessed, for the first time, for application to general organic synthesis. An efficient solution-solid phase synthesis of the quinolone antibacterial, Ciprofloxacin, is described, in which charcoal was used to purify Tbf-bound intermediates in a *pseudo* solid phase purification step.

Acknowledgements

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Finally, I would like to thank Martin Andrews, Craig Jamieson and Jennie Patterson for proof-reading this thesis, and to all those in Lab 29, past and present, for whom the *Old Bell* still tolls loudly.

Abbreviations

Ac	acyl
Ar	aryl
APCi	atmospheric pressure chemical ionisation
Boc	*t*-butoxycarbonyl
BHA	benzhydrylamine
Bpoc	1-methyl-1-(4-biphenyl)ethoxycarbonyl
Bn	benzyl
Bu	butyl
br.	broad
CAN	ceric ammonium nitrate
Cbz	benzyloxycarbonyl
Cy	cyclohexyl
d	doublet (nmr)
DABCO	1,4-diazabicyclo[2.2.2]octane
dba	dibenzylideneacetone
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	dicyclohexylcarbodiimide
DCE	dichloroethane
DCM	dichloromethane
DEAD	diethyl azodicarboxylate
DHP	dihydropyran
DIBAL-H	diisobutylaluminium hydride
DIC	diisopropylcarbodiimide
DIEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DME	dimethoxyethane
DMF	*N,N*-dimethylformamide
DMS	dimethylsuphide
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
DVB	divinylbenzene

ECGC	electron capture gas chromatography
EI	electron impact
eq	equivalents
ES	electrospray
Et	ethyl
FAB	fast atom bombardment
Fmoc	fluoren-9-ylmethoxycarbonyl
FTIR	Fourier Transform Infra Red
h	hours
HOBt	1-hydroxybenzotriazole
HPLC	high pressure liquid chromatography
HRMS	high resolution mass spectrometry
HWE	Horner-Wadsworth Emmons
Hz	Hertz
i	*iso*
In	indolyl
IPA	*iso*-propylalcohol
IR	infra red
LDA	lithium diisopropylamide
m	*meta*
MAS	magic angle spinning
MCP	mono chemotactic protein
Me	methyl
MEM	β-methoxyethoxymethyl
MHz	megahertz
MOM	methoxymethyl
m.p.	melting point
Ms	mesyl
MS	mass spectrometry
MW	molecular weight
n	normal
NaHMDS	sodium hexamethyldisilazide
NMO	*N*-methylmorpholine *N*-oxide

nm	nanometres
NMP	*N*-methylpyrrolidinone
nmr	nuclear magnetic resonance
NOE	Nuclear Overhauser Effect
p	*para*
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGC	porous graphitised carbon
Ph	phenyl
pip	piperidine
PMB	*p*-methoxybenzyl
Pr	propyl
PS	polystyrene
py	pyridyl
pyBOP	benzotriazole-1-yloxy-*tris*-pyridinophosphonium hexafluorophosphate
q	quartet (nmr)
q	*quaternary*
REACAP	resin activation/capture
Rf	retention factor
RF	radio frequency
RP	reverse phase
s	singlet (nmr)
SAR	structure activity relationship
SASRIN	super acid sensitive resin
sept.	septet (nmr)
SPOS	solid phase organic synthesis
t	triplet (nmr)
t	*tertiary*
Tbf	tetrabenzo[*a,c,g,i*]fluorene
TBAF	tetra-*n*-butylammonium fluoride
TBAH	tetra-*n*-butylammonium hydroxide
TBAI	tetra-*n*-butylammonium iodide
TBS	*t*-butyldimethylsilyl

^{t}Bu	*t*-butyl
Tf	triflate
TFA	trifluoroacetic acid
TFMSA	trifluoromethanesulphonic acid
THF	tetrahydrofuran
THP	tetrahydropyranyl
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TMEDA	*N*, *N*, *N'*, *N'*-tetramethylethylenediamine
TMG	1,1,3,3-tetramethylguanidine
TMS	tetramethylsilane
TPAP	tetrapropylammonium perruthenate
uv	ultraviolet

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Chapter 1: Introduction

1.1 Overview

Synthetic organic chemistry is a science which has a huge variety of applications. Research in this field concentrates on many areas: the development of new synthetic methodologies; the adaptation of existing organic reactions to asymmetry; the application of organic transformations to the synthesis of natural products. Additionally, there is overlap of organic chemistry with other disciplines, such as the investigation of transition metal complexes, the use of enzymes to afford desired molecular transformations, or in the optimisation of the properties of materials and fuel efficiency.

The synthetic chemist has been highly innovative in developing his field and the invention employed is seemingly endless. However, demonstration of the *practical application* of an area of research is often detailed in terms of leading towards a compound which occurs naturally in plants or animals, or especially one which exhibits a human therapeutic effect. It is in covering the overlap of chemistry with biology that synthetic organic chemistry is perceived to be of the greatest benefit and hence much research is geared towards obtaining these objectives.

The pharmaceutical industry is substantially driven by synthetic organic chemistry, and *vice versa*. Potential drug candidates must be discovered, and this has traditionally involved the individual synthesis of many thousands of molecules by medicinal chemists. A lead compound which exhibits suitable biological activity may then be structurally altered to have the optimum possible activity and selectivity. The pharmaceutical business, as well as providing undoubted benefit to the treatment of disease, is a multi-billion dollar business and so relies heavily on the innovation shown by chemists to sustain a steady flow of new drug compounds. It is not surprising, therefore, that publications of research in organic chemistry often include possible applications to the pharmaceutical industry.

The entire process of lead compound generation and optimisation can take several years to accomplish and is likely to require the synthesis of thousands of compounds. A procedure which makes this stage of drug development more efficient will therefore be highly beneficial in terms of time and cost. The synthesis of many compounds in an automated fashion during these early stages is a much more attractive proposition than synthesising compounds individually. It is in this area that combinatorial chemistry[1] can provide an easier method of generating the numbers of compounds which require testing for early stage drug development.

Combinatorial techniques generally involve the use of solid phase organic synthesis (SPOS).[2] The advantages of synthesising compounds on an insoluble polymeric support greatly increases the ability of chemists to handle large numbers of compounds simultaneously, and has also opened the door for research in a wide variety of other areas.

1.2 Solid Phase Synthesis

The use of an insoluble solid support for organic reactions was first successfully performed by Merrifield[3] in 1963, who synthesised a tetrapeptide by attaching the *C*-terminus of the first amino acid in the sequence to a chloromethylated copolymer of styrene (PS) and divinylbenzene (DVB). The polymer effectively acted as a carboxyl protecting group, allowing the remaining amino acids to be coupled sequentially until the desired peptide was synthesised (*Figure 1.1*). Merrifield demonstrated that purification of the growing peptide chain could be achieved after the coupling of each amino acid in a straightforward manner. The insolubility of the solid support (resin) meant that purification was accomplished by simple filtration, with impurities and excess reagents removed in the filtrate.

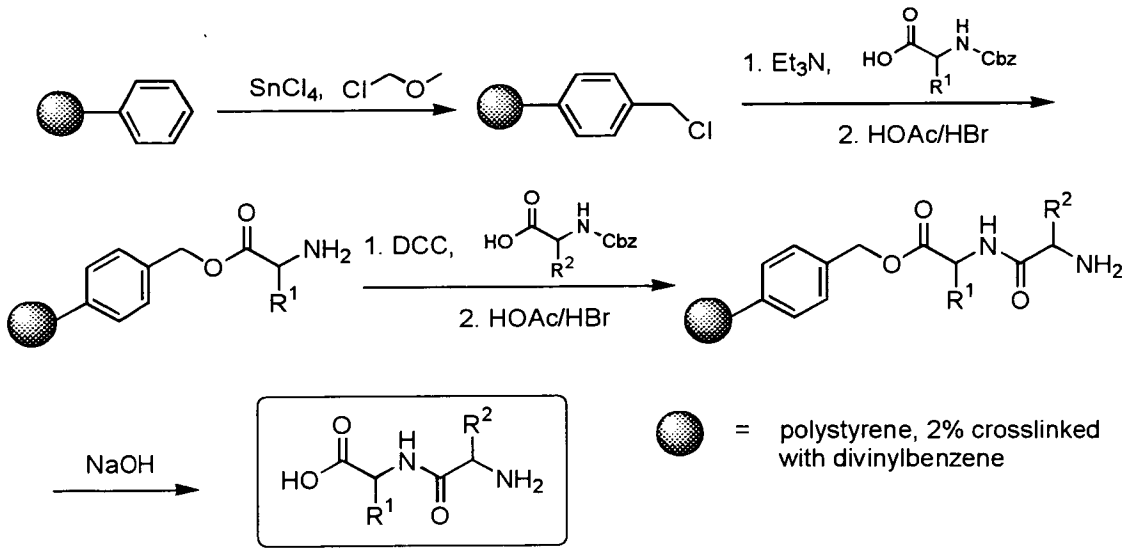


Figure 1.1: *Merrifield's Solid Phase Peptide Synthesis.*

Merrifield envisaged that the purification advantages of synthesis on a resin would ultimately lend itself to automation. Indeed, soon afterwards, he published details of an automatic peptide synthesiser.[4] Its efficiency was demonstrated by the synthesis of the nonapeptide, bradykinin, which was synthesised in greater yield and in less time than possible by manual synthesis.[5] Automation has since enabled the chemical synthesis of many peptides which otherwise would have been inaccessible by manual means, such as ubiquitin,[6] MCP-1[7] and interleukin-3.[8] More generally, the technique has been employed successfully in the synthesis of a number of other polypeptides,[9] oligonucleotides[10] and oligosaccharides.[11]

1.2.1 The Solid Support

The principle of SPOS lies in the binding of an organic molecule to an insoluble support *via* a suitable spacer or linker group. The PS-DVB resin used by Merrifield is still the polymer of choice for many researchers. This type of resin has proved to be highly robust and its properties can be altered depending on the degree of cross-linking (usually 1-5%).

One reason that the PS-DVB support has proved so popular is because of its favourable swelling properties - particularly in non-polar aprotic solvents such as

dichloromethane (DCM) and toluene. The ability of a resin to swell in solvent is one of its most important properties, as only when the resin is highly swollen do the functional sites in the resin beads become available for reaction with reagents dissolved in the solvent. Investigations have shown that >99% of the functionalised sites are situated within the interior of a polymer bead,[12] and so high solvation levels are crucial for efficient reactions.

With virtually all of the early developments in solid phase techniques being focused on polypeptide synthesis, larger and larger peptides and proteins were soon synthesised. Problems were observed in the synthesis of these long chain proteins and it was thought that the hydrophobic nature of polystyrene was a cause of this. Furthermore, the cross-linked nature of the polymer means that steric effects are likely to impinge on the couplings. To counteract this, and to increase reaction rates and yields, a polystyrene based polymer containing a large hydrophilic polyethylene glycol (PEG) unit was devised[13] (*Figure 1.2*).

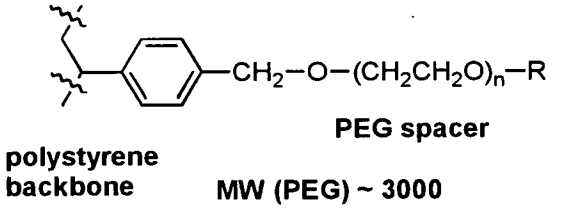


Figure 1.2: *The TentaGel Polymer Support.*

The PEG spacer group of the new polymer (TentaGel), was optimal at a molecular weight of approximately 3000, and so constituted around 70% of the overall polymer matrix. This meant that the PEG group had a major influence over the overall properties of the polymer and its greater mobility was designed to create an environment which was more "solution-like" for the growing peptide chain. The swelling properties of TentaGel resins have been found to be more uniform than PS-DVB resins, and are favourable over a range polar and non-polar solvents[13] (*Table 1.1*).

Solvent	PS-1%DVB	PS-PEG (MW_{PEG}=3300)
water	-	2.5
methanol	0.95	2.5
acetonitrile	2.0	3.0
DMF	3.5	3.2
toluene	5.3	3.1
DCM	5.2	3.0

Table 1.1: *Swelling Ratios of PS-DVB and PS-PEG in Various Solvents.*

Increased reaction rates of amino acid coupling reactions indicated that the PEG group was indeed creating a favourable environment. As well as the PEG having greater hydrophilicity than PS-DVB polymers, the TentaGel resins tend to be more monodisperse *ie.* the beads are of a uniform size. Commercially available PS-DVB resin beads have large differences in diameter, and overall reaction time is governed by the swelling of the largest of these.[13] TentaGel, consisting of more uniform, and smaller particles overcomes this problem.

One disadvantage of TentaGel, however, is its comparatively low level of functionalisation, or loading. Typical loading levels for PS-DVB resins are approximately 1.0mmol/g, whilst TentaGel is in the range 0.2-0.3mmol/g. This is due to the difficulty in preventing both ends of the PEG group from binding with the PS backbone during synthesis, particularly for long chains (MW>800). This has been overcome in one case where a short PEG unit (MW~200, loading~1.8mmol/g) has been appended to PS and has been used in the synthesis of quinazoline-2,4-diones.[14]

Recent comparisons have been carried out between TentaGel and PS-DVB supports in general organic synthesis.[12,15] Yan investigated a series of reactions including oxidation, ester formation, hydrazone formation and amide formation/ring opening. TPAP/NMO[16] oxidation was found to proceed faster on TentaGel than on PS-DVB. This supports the argument that the "solution-like" and more polar properties of TentaGel create a more favourable environment for the TPAP salt to react. However, that TentaGel will always increase reaction rates compared to PS resins is not supported by the evidence from amide forming/ring opening reactions

(*Figure 1.3*). In these reactions, the reaction rate is almost twenty times faster on PS-DVB resin than on TentaGel.

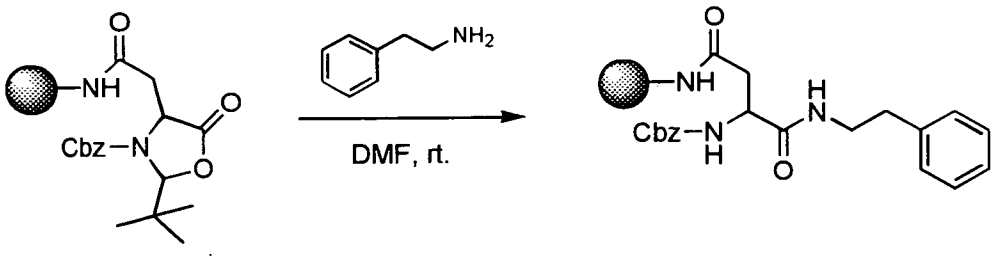


Figure 1.3: *Ring-opening Reaction on PS-DVB and TentaGel Supports.*

With much drug research focusing on asymmetric synthesis, Burgess has compared two different PS-DVB resins (Merrifield [chloromethylated] and Wang functionalised[17]) with TentaGel in enantioselective alkylation reactions[15] (*Figure 1.4*). Of these, the Wang PS-DVB (see *Section 1.2.2*) resin gave the highest yields and enantiomeric excesses whilst the TentaGel supported substrates gave reduced yields of product over time. Similar experiments performed with a slightly different auxiliary on Merrifield resin[18] also showed the applicability of PS-resins to asymmetric synthesis.

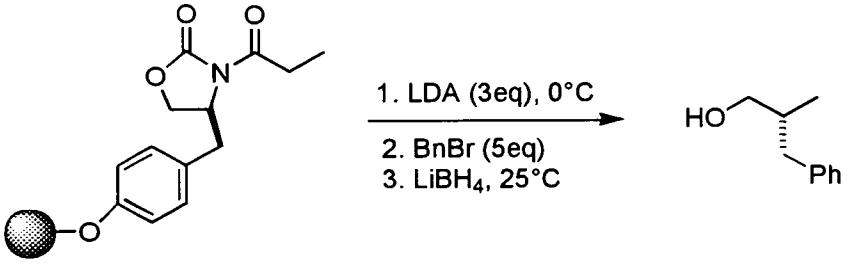


Figure 1.4: *Asymmetric Alkylation Reactions on PS-DVB and TentaGel Supports.*

Overall, Yan suggests[12] that no single polymer type favours all reactions, and that any swollen resin bead (the weight of which is up to 80% solvent) behaves exactly like another solvent, with the rate of reaction being affected by the polymeric matrix much like the effect of different solvents on reaction rate.[19]

Perhaps of more consequence than the nature of the polymer backbone itself regarding SPOS is the degree and nature of functionalisation which the polymer presents to reagents in solution.

1.2.2 Functionalising the Solid Support

In order that organic molecules can be bound to supports, the polymer must possess suitable functionality. This can involve either direct functionalisation of the polymer itself,[20] or more commonly, the polymer is derivatised with a suitable linker or spacer group. In either case, binding the organic compound to the resin must proceed in a straightforward manner. The bond with the resin must be stable to the proposed reaction conditions, and cleavage of the product from the resin on completion of the synthesis must proceed under conditions which will not degrade the compound. For example, Merrifield[3] employed orthogonal acid and base conditions to cleave amino protecting groups and resin products respectively (see *Figure 1.1*).

Early examples of functionalised polymers[20] included phosphine, trityl and boronic acid derivatives, but one of the first true linker systems was devised by Wang[17] and is still one of the most popular PS-DVB resins used today (*Figure 1.5*). It is readily synthesised from chloromethylated Merrifield resin, and products are cleaved with 95% TFA.

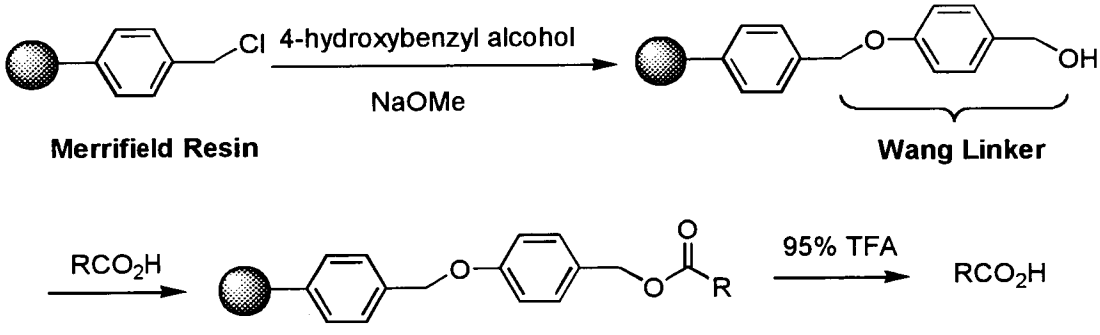


Figure 1.5: *Synthesis and Cleavage Conditions of Wang Resin.*

As virtually all organic chemistry using polymeric supports was in polypeptide synthesis, most of the linker systems developed were for immobilising carboxylic acids. A representative sample of other such linkers is illustrated in *Figure 1.6*.

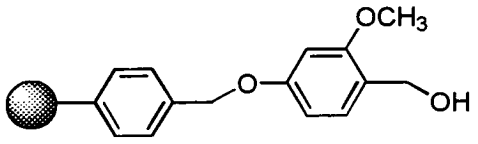
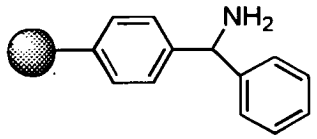
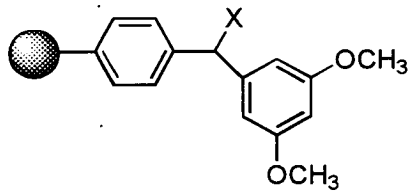
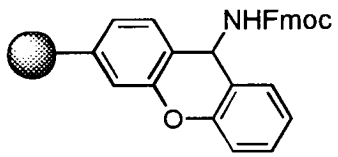
Linker	Comments
	SASRIN[21] linker; cleaved with 1% TFA
	Benzhydrylamine[22] (BHA); cleaved with TFMSA forming amides.
	Rink[23] acid (X=OH) cleaved with AcOH/DCM forming acids; or amide (X=NHFmoc) cleaved with TFA/DCM forming amides.
	Sieber[24] amide; cleaved with 1%TFA/DCM forming amides.

Figure 1.6: *Representative Examples of Linkers Used to Bind Carboxylic Acids to Resin.*

With emphasis growing in the area of non-peptide synthesis, linkers capable of binding other functional groups have been developed (*Figure 1.7*). These include the tritylchloride[25] linker, **1**, which is capable of binding nucleophiles, and Ellman's dihydropyranyl (DHP), **2**, linker for anchoring alcohols.[26]

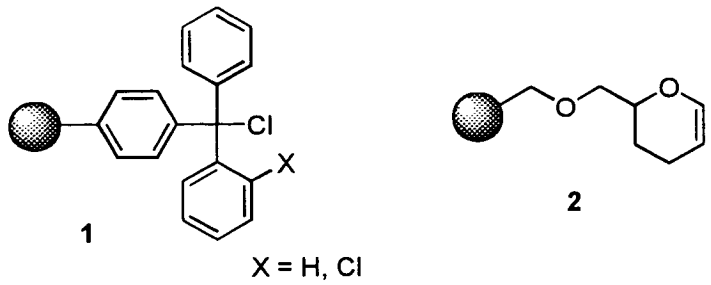


Figure 1.7: *Examples of Other Linkers Used in SPOS.*

The acid sensitivity of the trityl chloride linker is both useful and a disadvantage in SPOS. Mild acid cleavage means that acid-sensitive molecular fragments are likely to remain unaffected during the cleavage step, but likewise, resin cleavage may occur at unwanted steps in the reaction scheme. Ellman's DHP linker, **2**, effectively acts as a hydroxy protecting group and has been used in Janda's synthesis of prostaglandin E_2 methyl ester.[27] Many different linkers for SPOS are now widely commercially available,[28] but research continues into tailoring linker technology to cater for different requirements.

1.2.3 Safety-Catch Linkers

One of the most important aspects of linker technology is the question of lability. It is crucial that the linker gives quantitative release of the organic molecule from the resin on completion of the reaction sequence. However, the conditions for cleavage should be mild enough so as not to cause degradation of the released molecule. To assist in successfully achieving these aims, the "safety-catch" principle was exploited by Kenner.[29] This technique involves the labilisation of an otherwise stable bond at the appropriate moment by a specific chemical modification (*Figure 1.8*). A sulphonamide linker on PS-DVB used in peptide synthesis, was stable to base as well as the TFA conditions used for the *N*-terminal *t*-butyloxycarbonyl (Boc) protecting group cleavage of **3**. After *N*-methylation using diazomethane, **4** was cleaved rapidly with hydroxide or nucleophilic amines.

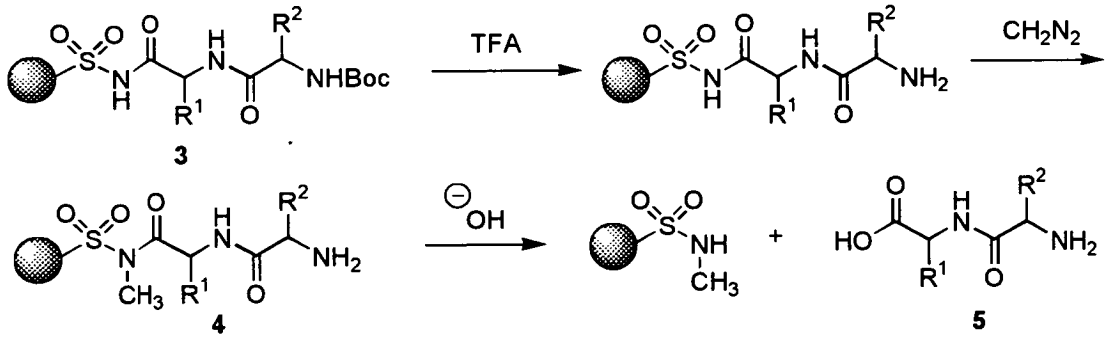


Figure 1.8: *Peptide Synthesis Using Kenner's Safety-catch Linker.*

Ellman developed this system,[30] **6**, and concentrated on using nucleophilic amines to initiate cleavage of amides (*Figure 1.9*). Carboxylic acids were anchored to the linker, which was then activated by cyanomethylation to give **7**. Cleavage with primary or secondary aliphatic/aromatic amines gave amides in near quantitative yields, without the need for excess amine. Indeed, when an equimolar mixture of five amines was added, equal quantities of the corresponding cleaved amides were obtained.

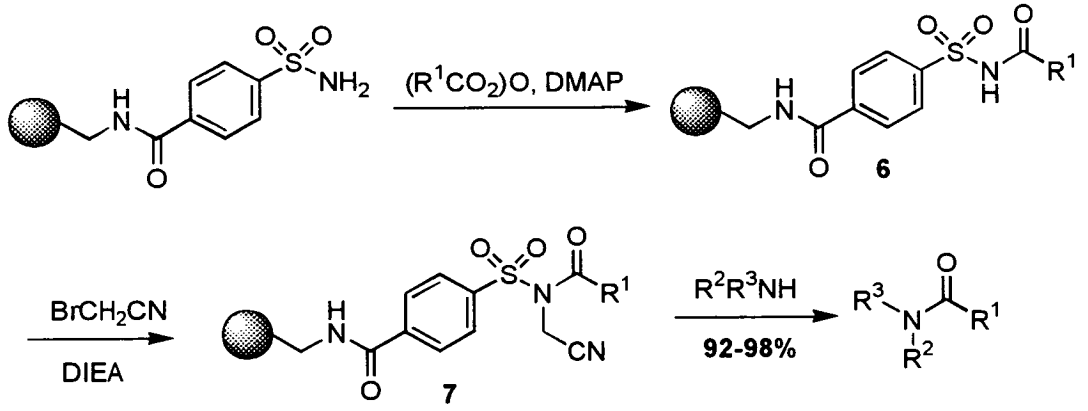


Figure 1.9: *Ellman's Safety-catch Sulphonamide Linker with Amine Cleavage.*

Problems were experienced in the cyanomethylation step using substrates possessing electronegative α-substituents. An aliphatic variant of the linker was found to be more activated giving comparable amide yields upon amine cleavage.

Recently, other variations of the safety-catch linker have been devised by Hulme,[31] forming acids or methyl esters by cleavage with hydroxide and methoxide respectively.

1.2.4 Traceless Linkers

In all of the cases so far discussed, appropriate (polar) functionality must be present in a molecule to allow binding to a resin. Invariably, the same, or slightly modified functionality is recovered upon cleavage. Often, strongly polar groups can have a detrimental effect on the pharmacokinetics or bioavailability of a drug substance, and so much interest has evolved in so-called "traceless" linkers. Using this technique, compounds can be synthesised by solid phase techniques with no trace of the attachment point.

The first traceless linkers used silicon-aryl bonds, but these generally relied on harsh cleavage conditions (*eg* HF) to permit cleavage of the silicon-aromatic carbon bond.[32,33] A silyl linker, **8**, which can be cleaved under milder conditions was developed by Hone[34] (*Figure 1.10*). Isoxazole, **9**, was synthesised in good overall yield using this improved linker.

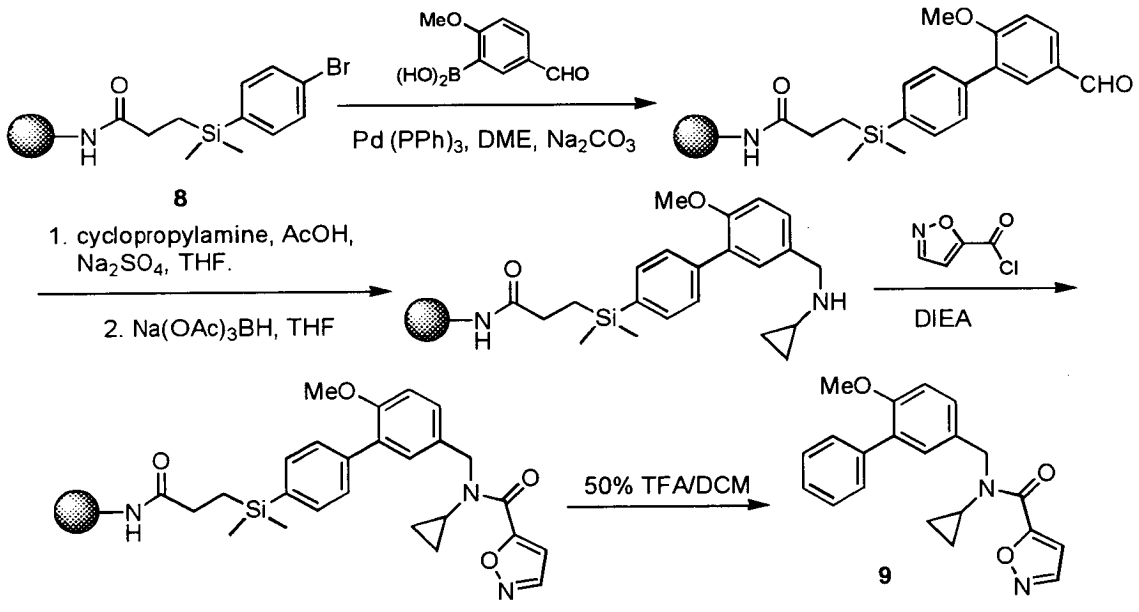


Figure1.10: *Hone's Traceless Silyl Linker.*

As with earlier linkers of this sort,[32,33] binding of the organic substrate to the linker was limited to aromatic compounds *via* the Stille or Suzuki coupling reactions. However, the milder cleavage conditions (presumably afforded by the presence of the amide functionality) makes Hone's linker more generally applicable.

Traceless linkers have proved a challenging target for several research groups. Silyl linkers have been used for the synthesis of dienes,[35] whilst a related strategy has been developed by Gallop[36] using a rhodium catalysed 1,3-dipolar cycloaddition reaction to form furans. A novel hydrazide linker developed by Lowe[37] (cleaved with base and catalytic copper(II) acetate), has also recently been published.

A traceless method of generating amines has been devised by Morphy,[38,39] which has the added advantage of being able to recycle the polymer. REM (REgenerated Michael Addition) Resin, **10**, is amine-functionalised *via* Michael addition. The tertiary amine is then quaternised, and cleavage afforded by DIEA in a Hoffman elimination (*Figure 1.11*). An improved vinyl sulphone linker[40] has been shown to be more base/nucleophile stable and so should be more adaptable to a wider range of chemistry.

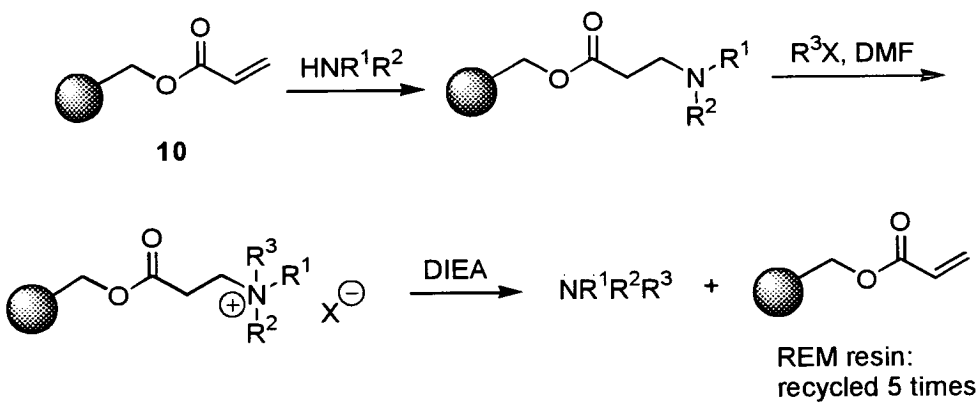


Figure 1.11: *Traceless Synthesis of Amines Using Recyclable REM Resin.*

Amines have also been the target of work by Willson.[41] Based on the work by Patel,[42] he has designed an acid-sensitive SASRIN-based aldehyde resin, AMEBA (Acid sensitive MEthoxy BenzAldehyde), and managed to derivatise amines forming sulphonamides, amides, ureas and carbamates (*Figure 1.12*). The linker also acts in a safety-catch manner, as before derivatisation, resin-bound amine, **11**, remains

uncleaved in 95% TFA. However, after derivatisation, the final products may be cleaved in only 5% TFA/DCM. This difference in cleavage labilities led to very high product purities.

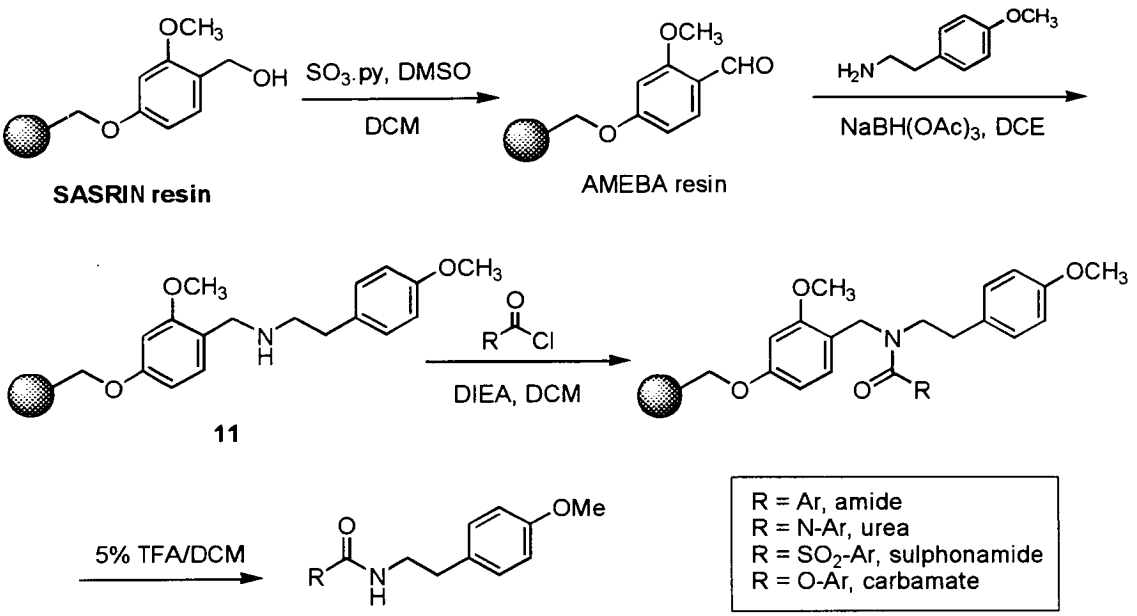


Figure 1.12: *Traceless AMEBA Resin.*

1.2.5 Other Linker Types

One group of linkers which does not require chemical cleavage are the photolabile linkers.[43] Although not as commonly used because of long cleavage times and generally poorer chemical stability, some recent developments have been made in terms of substrate compatibility and cleavage conditions. The linkers generally have a nitro group on an aromatic ring, *ortho-* to the site of binding to the substrate (*Figure 1.13*).

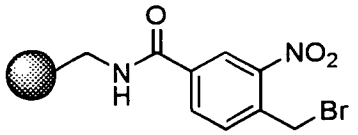
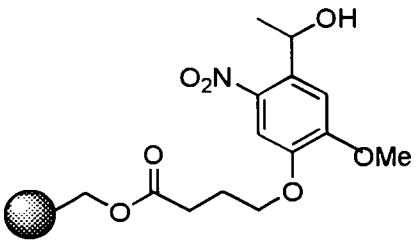
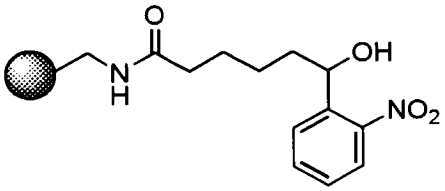
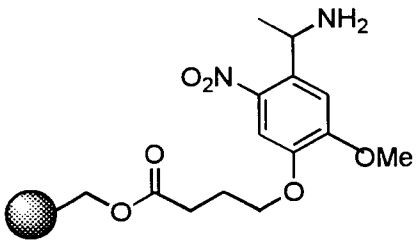
Photolabile Linker	Comments
	Original photolabile linker[44] used for amino-acid synthesis; cleaved at 350nm, 18-24h.
	Carboxylic acid synthesis;[45] cleaved at 354nm, 24h, 92%.
	Carbohydrate synthesis;[46] cleaved at 365nm, 15h, synthesis 43% over 4 steps.
	Amide synthesis;[47] cleaved at 365nm, 3h, >90% (1-2h for single resin beads).

Figure 1.13: *Structure and Cleavage Properties of Photolabile Linkers.*

Dendrimers have been synthesised on resin by Bradley[48] on both polystyrene and PEG-based polymers. This was particularly useful for resin-loading enhancement as third generation amine dendrimers increased the resin loading up to 2.8mmol/g. Bradley was able to use this resin to synthesise a dipeptide, and selectively cleave this without removing any of the dendrimeric linker.

Silicon-based linkers have previously been discussed for their use as traceless linkers, but they have also been investigated for their labiltity to fluoride. They have been employed with some success in peptide synthesis by Ramage[49] and Chao,[50] with cleavage afforded by tetrabutlyammonium fluoride (TBAF). More recently, the concept has been adapted for small molecule synthesis by Flitsch and Turner,[51] and

several substrates have been cleaved from a silicon based linker using TBAF or CsF. A silyl-based linker which possesses the unique properties of traceless tethering with mild fluoride cleavage was designed by Ellman and used in the synthesis of tricyclic pyridine derivatives.[52] The mild cleavage conditions are clearly an advantage, but unlike other traceless silyl linkers,[31-33] the silicon group is part of a genuine resin bound linker, rather than bound to the resin as part of the substrate structure (see *Figure 1.10*).

Flitsch and Turner have also developed a linker for immobilising alcohols which can be cleaved with the enzyme, penicillin amidase, or mild acid (10% TFA).[53] Although the enzyme cleavage conditions are relatively inefficient (50% cleavage), cleavage in mild acid is more favourable than the DHP-derived linker of Ellman[26] for immobilising acid labile substrates such as carbohydrates.

1.2.6 Analysis of Compounds on Resin

The analysis and characterisation of organic compounds bound to resin has proved a great challenge. Such analysis is important for determining the level of loading of the linker group on resin, and for monitoring a synthesis as it proceeds. Cleavage of material from the resin then analysis is wasteful, but direct, on-resin analysis is often difficult due to interference from the polymer backbone. Elemental analysis is occasionally useful for compounds with an appropriate atom content (*eg* N, Cl, Br), but infra-red (IR) and nuclear magnetic resonance (nmr) techniques have now been adapted to maximise their potential for on-resin analysis.

IR spectroscopy on polymer-bound compounds has been used with some success, particularly for identifying diagnostic stretches such as carbonyl.[12] Samples are usually prepared by swelling the resin in a small quantity of DCM and then crushed between two NaCl plates. Although much of the resultant spectrum is due to the polymer backbone, the presence of many functional groups can be confirmed by this method. A disadvantage, however, is that the technique is destructive. A non-destructive method is Difuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS)

which has been used by Sofia[54] in a high throughput method of analysing resin-bound sugars .

IR spectroscopy has also been used successfully in investigating the site isolation/interaction effects of polymers. It was originally argued that PS-DVB resins had a very rigid structure, in which the functionalised sites were completely isolated (hyperentropic effect). The mono-protection by resin of symmetrical diols[55] and dialdehydes[56] was provided as evidence for the site isolation effect, and IR spectroscopy has been used to investigate this further. Yan[57] has produced IR evidence which supports previous findings[58] that polymer-bound alcohols exhibited two -OH bands, corresponding to isolated (sharp) and hydrogen-bonded (broad) molecules. This indicated that site isolation and interaction effects were occurring. Furthermore, sterically hindered alcohols exhibited a stronger isolation band and weaker H-bonded band.

Evidence that the functional sites of resins are mobile and not rigidly fixed comes from the monoprotection of diacid chlorides of various chain length[57] (*Figure 1.14*). Strong ester stretches compared to acid chloride stretches indicated that double binding had occurred and so significant site interaction behaviour must exist (although the presence of any acid chloride stretch must indicate that there is some site isolation effect). The chain length made no difference to the relative band intensities suggesting that dynamic movement of the polymer led to consistent levels of site interaction.

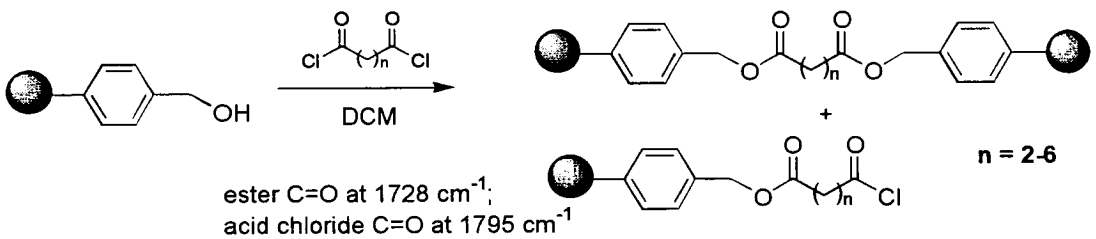


Figure 1.14: *Polymer Site Isolation Effect Determination by IR Spectroscopy.*

Recently, nmr techniques have progressed a great deal in terms of analysing resin-bound compounds. Gel-phase nmr (usually ^{13}C), where the resin is swollen in an appropriate deuterated solvent and the usual pulse sequence applied, generally produces spectra which have broad aromatic and aliphatic signals due to the

polymeric backbone. The technique is not very sensitive, and usually requires thousands of scans before a reasonable spectrum is produced. However, despite signal-broadening, some aspects of the linker or resin-bound compounds can be observed. Perhaps more successful applications of the gel-phase method have been in obtaining ^{31}P spectra[59] and ^{19}F spectra[60] where appropriate.

Compared with PS-DVB resins, TentaGel resins have been shown to give better gel-phase nmr results.[13] This is due to the PEG units in the swollen state having long relaxation times, and so the PS signals collapse altogether, leaving a single sharp PEG resonance at δ 70ppm.

More recent advances in the quality of resin nmr spectra have been due to the technique of Magic Angle Spinning (MAS). This is a method which uses a specially designed probe which is aligned to the sample in a manner which compensates for the magnetic irregularities responsible for line-broadening in gel-phase spectra of polystyrene.[61] MAS nmr has allowed a vast improvement in the quality of spectra obtaining good quality ^{13}C and ^{1}H spectra.[62,63] The technique has also extended to correlation spectroscopy with coupling constants being measured by ^{1}H-^{1}H spectra[64] and even single-bead ^{13}C-^{1}H spectra have been successfully analysed.[65]

1.2.7 Applications of Solid Phase Synthesis

1.2.7.1 The "Fish-hook" Principle

In its simplest sense, a resin can be thought of as an insoluble protecting group in organic synthesis. Many of the early uses of resins in general organic synthesis involved the polymer in exactly that role, and Leznoff[66] has reported many such examples. He was particularly interested in the monoprotection of symmetrical bifunctional compounds, and recognised insoluble polymers as a way of doing this efficiently. Leznoff originally monoprotected symmetrical diols[55,67] using what he termed the "fish-hook" principle[66,68]: the polymer acts as a fish-hook to "catch" a portion from the "sea" of excess diol. He was then able to protect the free hydroxyl as the trityl,[67(a)] THP[67(b)] and acetate[55(d)] derivatives, cleave from the resin and pursue syntheses as required. This proved to be a more efficient process than the solution

phase equivalent. For example, solution phase trityl protection formed unreacted diol, mono- and diprotected diols, and trityl alcohol which led to major separation problems.

Monofunctional protection of symmetrical bifunctional compounds was developed to include the complete monoprotection of dialdehydes[56] and diacidchlorides,[69] and the partial diprotection of amines[70] and dihydroxy aromatics.[71] Both monoprotected diols and aldehydes have been used in natural product synthesis, leading to insect sex attractants[55(a-c)] and unsymmetrical carotenoids[56 (c)] (*Figure 1.15*).

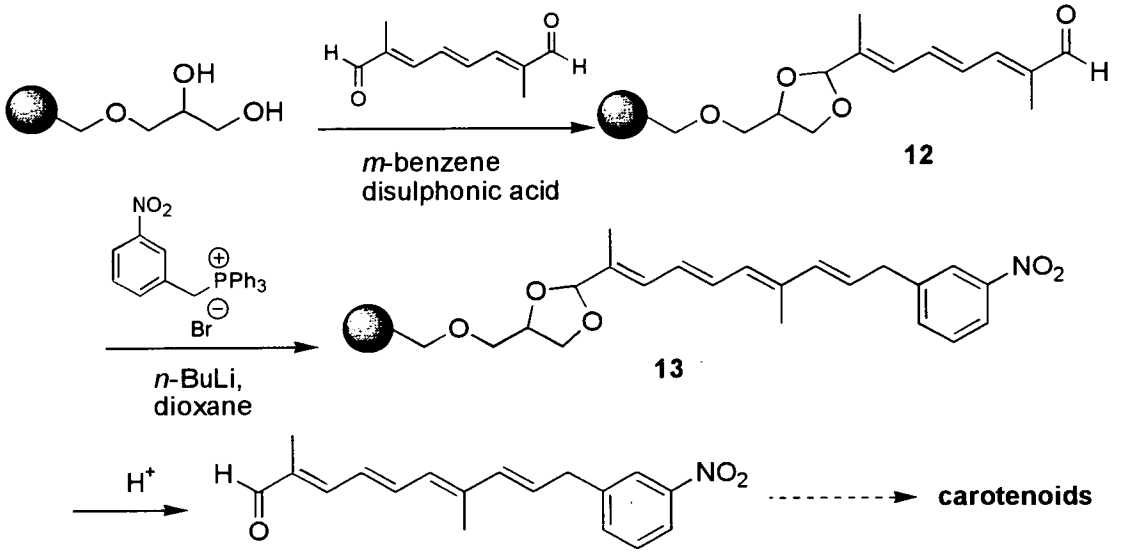


Figure 1.15: *Leznoff's Synthesis of Carotenoids from Monoprotected Dialdehydes.*

Solution syntheses of carotenoids have met with the problematic reaction of symmetrical aldehydes with two different Wittig reagents, either in one pot or in a serial manner. Both methods are low yielding, and are contaminated with symmetrical products. Leznoff's synthesis allows the complete monoprotection of the relevant dialdehyde using a resin bound diol, and reacting the corresponding acetal, **12**, with the appropriate ylide. Acetal cleavage of **13** leads efficiently to intermediates which can easily be transformed to the carotenoid series.

The fish-hook principle has been more demandingly employed to extract desired components from a compound mixture also containing staring materials and reagents.[72] Using this method, Fréchet[72(b)] trapped a *cis*-cyclohexyl diol, **14**, with a

boronic acid functionalised polymer (*Figure 1.16*). On completion of the binding, the resin-bound diol, **15**, was then easily removed from the crude mixture containing several products, by filtration. Washing the resin with water released the diol back into solution.

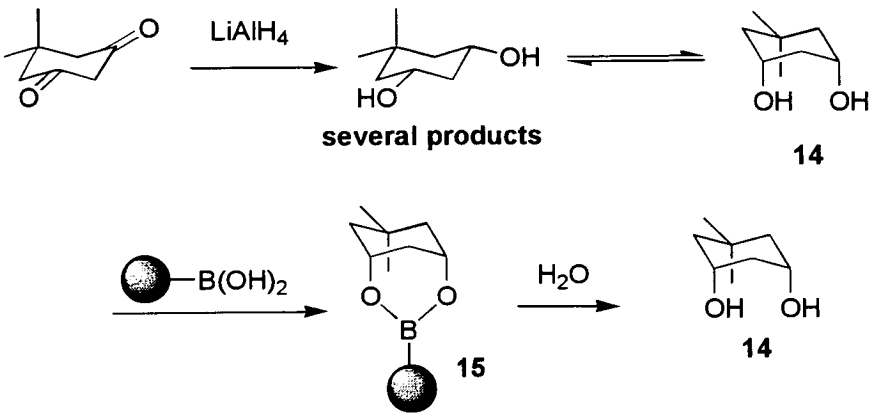


Figure 1.16: *Fish-hook Purification of Cyclohexyl cis-Diol.*

1.2.7.2 Resin Capture and Scavenge Techniques

Related to the fish-hook method of purification are the methods of resin capture and resin scavenging. Resin capture[73] is a term which has been used to describe, like the fish-hook technique, the removal of the desired product at the end of a solution synthesis by binding to resin, making it available for further treatment. Scavenging is a complementary technique, and involves the use of an insoluble polymer to remove excess reagent, by-products and starting materials, leaving the desired compound in solution.

Aminomethylated resin has proved very popular as a scavenger for the removal of excess isocyanates[74] acid chlorides, chloroformates and sulphonyl chlorides.[75] Such techniques have been used to extract products from Ugi four-component condensations,[73] and another interesting example where by-products and excess reagents were tagged to make them more labile to attack from amine resin. Munoz devised the Resin Activation/Capture (REACAP)[76] procedure, where only those resin bound products which have reacted as required, will participate in future steps. By

this method, a series of substituted dihydro-4-pyridones were synthesised (*Figure 1.17*).

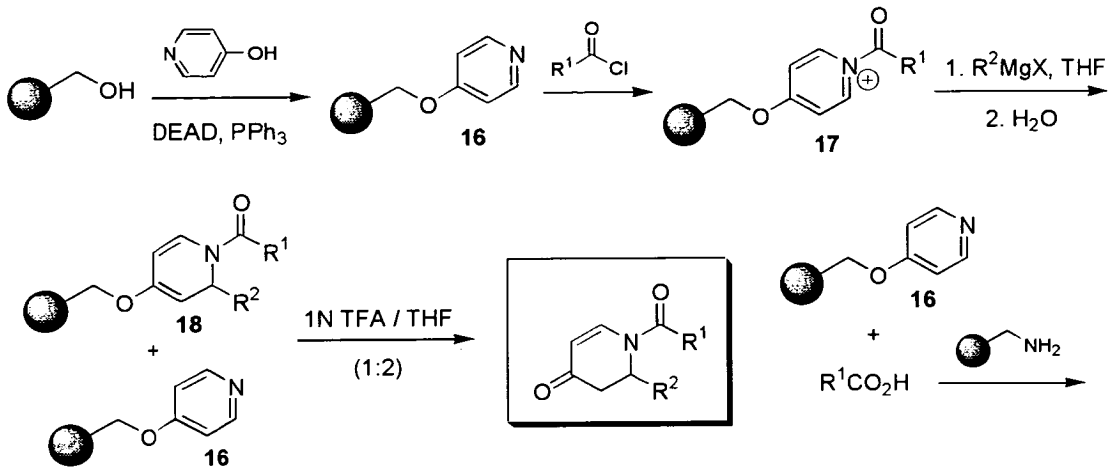


Figure 1.18: *REACAP Approach Towards Dihydro-4-pyridones.*

Resin-bound hydroxy-pyridine, **16** was acylated and the resulting product reduced with a Grignard reagent to form enol ether, **18**. Only acylated material reacts, and the resin is contaminated with starting material, **16**, reformed by hydrolysis of **17** in the aqueous work-up. Resin cleavage of **18** is selective and the carboxylic acid formed due to incomplete hydrolysis of **17** is removed by aminomethylated polymeric scavenger apparently without activation.

1.2.7.3 Functionalised Polymers as Mechanistic Probes

The use of functionalised polymers as a probe into reaction mechanisms and intermediates has been investigated by Rebek[77] (*Figure 1.18*). Polymers functionalised by two different, but potentially interactive, functional groups are combined in the same solvent with a potential catalysing reagent. Virtually all of the functional sites are held within the core of the resin beads and so will only react with each other *via* the dissolved catalyst. Rebek proved this by showing that imidazole is required for acyl transfer reactions of this type. Furthermore, a control experiment showed that the reaction proceeded smoothly when the resins were physically separated by a sintered barrier.

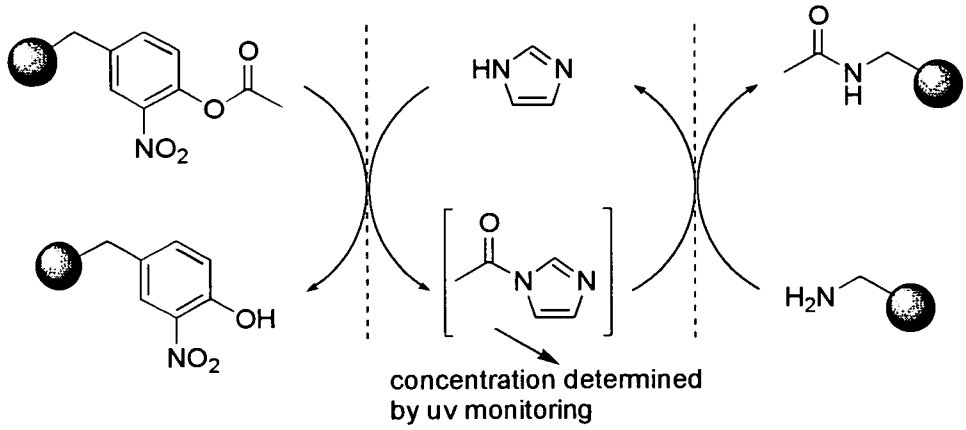


Figure 1.18: *Rebek's Three-Phase Test for Identifying Reaction Intermediates.*

1.2.7.4 Polymer Supported Reagents

One aspect of SPOS which has recently received increased attention is the use of solid supported reagents.[78] Substrates are transformed in solution by reagents which are bound covalently to the resin, and so are easily removed on completion of the reaction. The method is particularly advantageous, as no cleavage steps are required and the technique is applicable for reactions where recycling of the reagent is an advantage.

Many examples of standard chemistry being performed by solid supported reagents exist, such as amide bond formation which has been facilitated by the polymer bound coupling reagents, HOBt[79] and carbodiimide.[80] Polymer-bound reagents can bring other advantages, too. For example, efficient Swern oxidations have been performed on PEG-bound sulphoxide,[81] **20** (*Figure 1.19*). The sulphide, **19**, formed on completion of the reaction, unlike the solution phase analogue, is odourless and can be recycled efficiently to the sulphoxide, **20**, using sodium periodate. PEG has the advantage over PS-DVB polymers that it is soluble in most organic and aqueous solvents, but is insoluble in ether. This enables the chemistry to be performed efficiently in solution, but straightforward recovery of the reagent is afforded by precipitation from ether (see Section 1.4.3), leaving clean, oxidised product in solution.

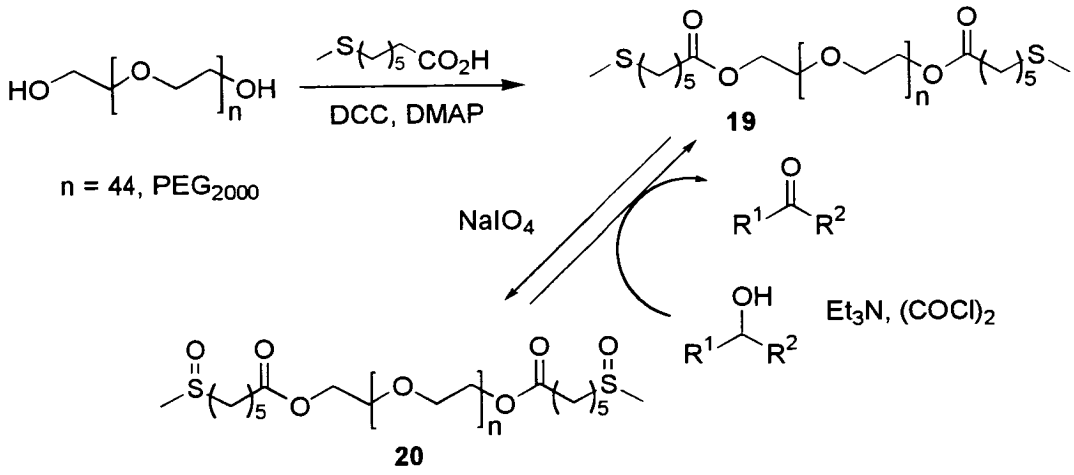


Figure 1.19: *Swern Oxidation Using PEG-Supported Sulphoxide.*

With much research focussing today on asymmetric synthesis, chiral auxiliaries have been considered prime candidates for binding to a solid support. These have included a "C_2 symmetric" resin-bound pyrrolidine auxiliary,[82] and the more widely investigated chiral Evans oxazolidinones.[15,18,83] Allin[18] and Burgess[15] have both successfully used PS-bound auxiliaries in alkylation reactions (see *Figure 1.4*) and Abell[83] has employed an auxilliary in conjugate addition and aldol reactions (*Figure 1.20*). Importantly, efficient regeneration of the auxiliary is achieved.

Other polymer-bound reagents have also been used by Ley with great success. His group has modified the oxidation reagent, TPAP[16] for use on solid support in the oxidation of alcohols to aldehydes and ketones[84] and hydroxylamines to nitrones.[85] Additionally, he has further transformed the aldehydes formed upon oxidation by using other polymer supported reagents in aldol,[86] reductive amination[87] and Wittig[88] reactions, in the first examples of clean, multi-step synthesis using polymer-bound reagents to purify each synthetic intermediate (*Figure 1.21*).

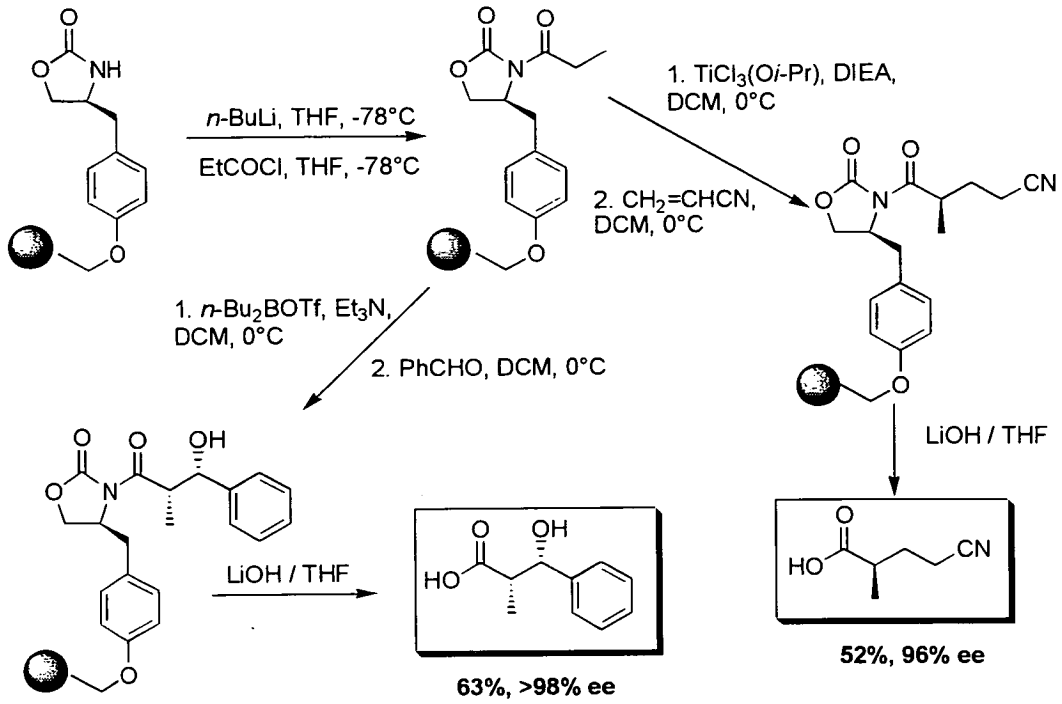


Figure 1.20: *Resin-Supported Auxiliaries in Aldol and Michael Addition Reactions.*

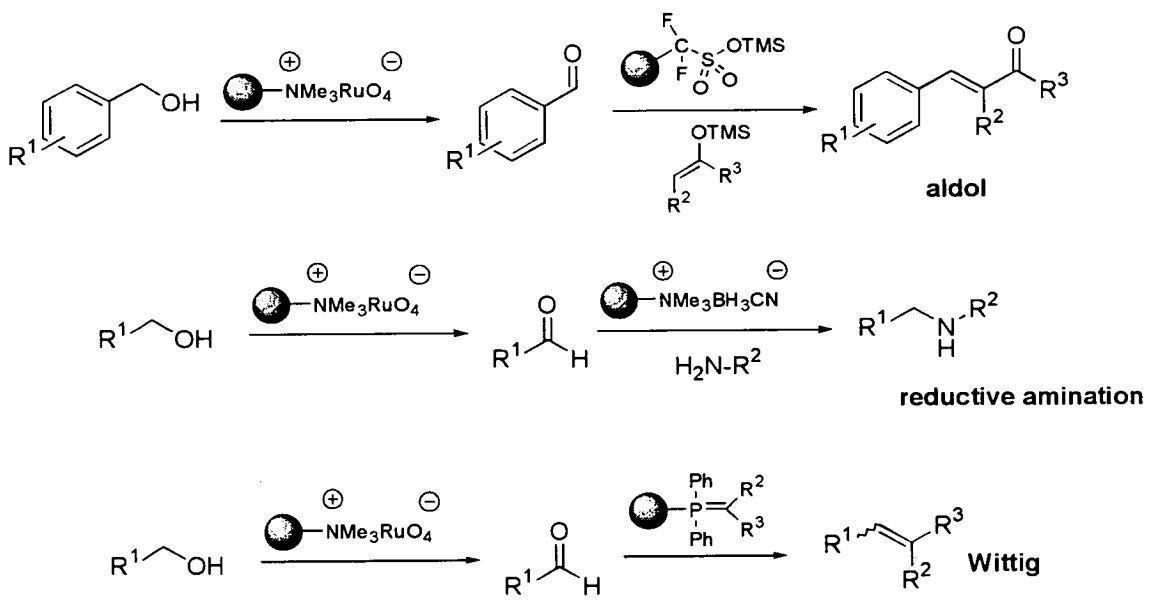


Figure: 1.21: *Multi-Step Synthesis Using Polymer-Bound Reagents.*

1.2.7.5 Synthesis of Heterocyclic Molecules

The examples of the use of functionalised resin in organic syntheses so far discussed have generally focused on specific uses of the resin in functional group binding or one-step transformation process. These techniques have clear benefits in purifying products in organic synthesis. However, the transformation of a single-step benefit into the serial transformation of resin-bound compounds in the multi-step synthesis of low molecular weight compounds is the truest test of the advances which SPOS offers. Using greatly increased purification rates to synthesise intermediates towards classes of "drug-like" or "privileged" structures will offer the greatest assistance to the pharmaceutical industry.

One of the first such compounds to be synthesised entirely on the solid support was a small series of benzodiazepines[89] (*Figure 1.22)*. This synthesis involved the isolation of two resin-bound synthetic intermediates and a concomitant cyclisation/cleavage step driven efficiently by the site-isolation effect to prevent intermolecular interactions.

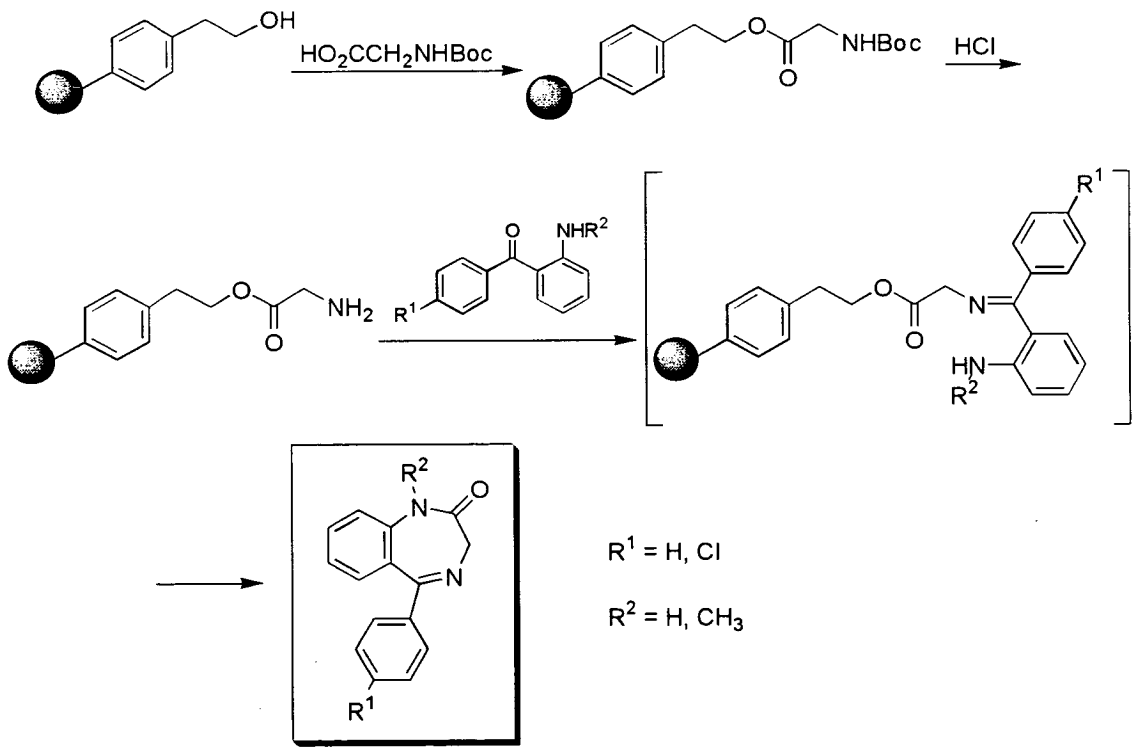


Figure 1.22: *Benzodiazepine Synthesis by Multi-Step SPOS.*

In recent years, there have been a very large number of examples of multi-step, solid-supported syntheses of small molecules in the literature.[2(b),(c)] Some examples are used to illustrate the ever-expanding range of specific reactions on support, whilst others are geared towards the synthesis of specific heterocyclic target molecules. Oxidation, reduction, addition, substitution, condensation, cycloaddition and organometallic reactions have all been illustrated in such a manner. However, the main driving force behind this research has been its applicability to combinatorial synthesis.

1.3 Combinatorial Chemistry

Drug discovery is traditionally initiated by the random screening of large numbers of compounds against a specific receptor target. This "lead generation" process may identify a compound which exhibits some degree of receptor affinity, and analogues of this compound can then be individually synthesised and assayed to determine the compound which achieves the maximum possible effect ("lead optimisation"). Alternatively, studies of the receptor site in question can also be carried out in order to determine a likely molecular structure which will bind most effectively. Such information may be used to identify the pharmacophore of high affinity compounds, thus aiding drug discovery by a more rational design process than mass screening.

Whether for specifically designed structure activity relationship experiments or the random synthesis of many compounds, combinatorial chemistry is becoming an increasingly useful tool in the drug discovery process. In a definitive sense, combinatorial chemistry involves the synthesis of mixtures of compounds consisting of all the possible incorporated components or building blocks. The term is also used to describe (automated) multiple parallel synthesis of individual compounds. Both methods have the same goal - to synthesise large numbers of compounds for biological screening as quickly as possible. The generally accepted ideal is that the drug discovery process is enhanced by faster compound synthesis, and to a certain extent can it be thought of as a "numbers game," *ie.* more compounds synthesised should furnish more potent compounds. Coupling high throughput screening assays

with combinatorial methods using optimised synthetic routes, a chemist can potentially generate biological results on many thousands of compounds every week, compared to the hundred or so a single chemist could synthesise in a year.

1.3.1 The Synthesis of Compound Mixtures

The simultaneous synthesis of large numbers of compounds relies on robust, reproducible chemistry and quick purification methods. Whilst the first factor is almost always likely to depend to some extent on substrate variation, SPOS can consistently provide a very quick method of purifying compounds which is also amenable to automation. Additionally, efficient purification procedures enable reactions to be driven to completion by using excess reagents. The recent development of new methodologies compatible with SPOS has recently become a major research area, and this has greatly contributed to the whole combinatorial paradigm.

Much of the initial research into combinatorial processes was carried out using peptides. Reliable solid phase techniques existed for the chemical synthesis of proteins and the accessible pool of the twenty naturally occurring amino acids provides an adequate source for variation *eg* a library of pentapeptides constituting each natural amino acid at every position will produce a library of 20^5 = 3.2 million compounds. Mixture generation by adding equimolar quantities of reagents to a single substrate is likely to have varying success due to differing reactivities of the reagents, and so a much more reliable technique was devised by Furka.[90] This is known as the "split and mix" technique, and is illustrated below for the synthesis of nine dimers formed from three constituent amino acids (*Figure 1.23*).

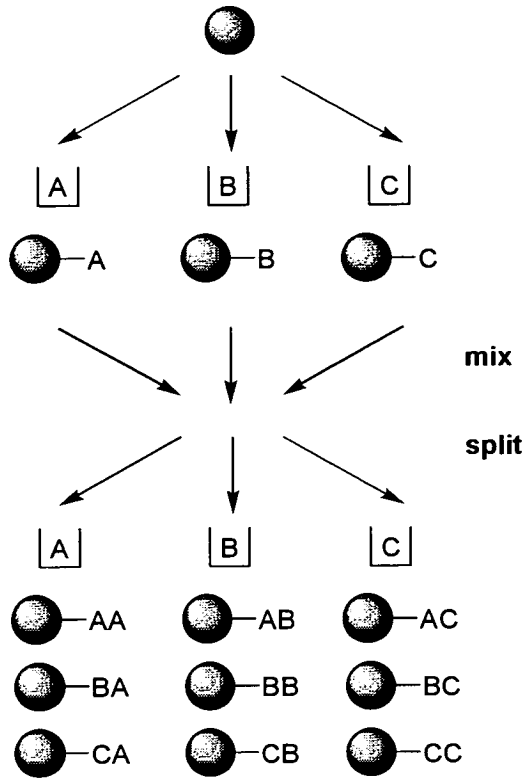


Figure 1.23: *Furka's Split and Mix Method of Peptide Library Generation.*

The functionalised resin is split into three portions or pools containing different amino acids, A, B and C. The resin is then recombined and is once more split into three pools, again containing the constituent amino acids. The resulting nine compounds have been formed using six coupling reactions over two steps, and can be recombined for analysis or kept as three separate sublibraries.

The potential number of peptides which can be synthesised in this manner is extremely high. However, it is recognised that peptides themselves have limited therapeutic value due to their poor bioavailability, and so attention has shifted recently to adapting library methodology for the generation of small molecules. The split and mix technique of library generation has recently been used to generate mixtures of low molecular weight molecules, such as 4-thiazolidinones,[91] pyridinium salts[92] and mercaptoacyl prolines.[93] The 4-thiazolidinone library (*Figure 1.24*) was synthesised using 5 Fmoc-protected amino acids (AA, R^3) on TentaGel resin. These were pooled together and separated into five equal portions and each reacted with a different aldehyde (R^1). Recombining, mixing and splitting again into five pools, followed by

reaction of each with a mercaptoacid (R^2), led to the final cyclised products. These were cleaved from the resin forming the corresponding acids (X=O) or amides (X=NH). A portion of each of the carboxylic acids was esterified giving a total of three libraries. Each library consisted of a total of 125 (5×5×5) members as 540 (6×10×9) diasteroisomers.

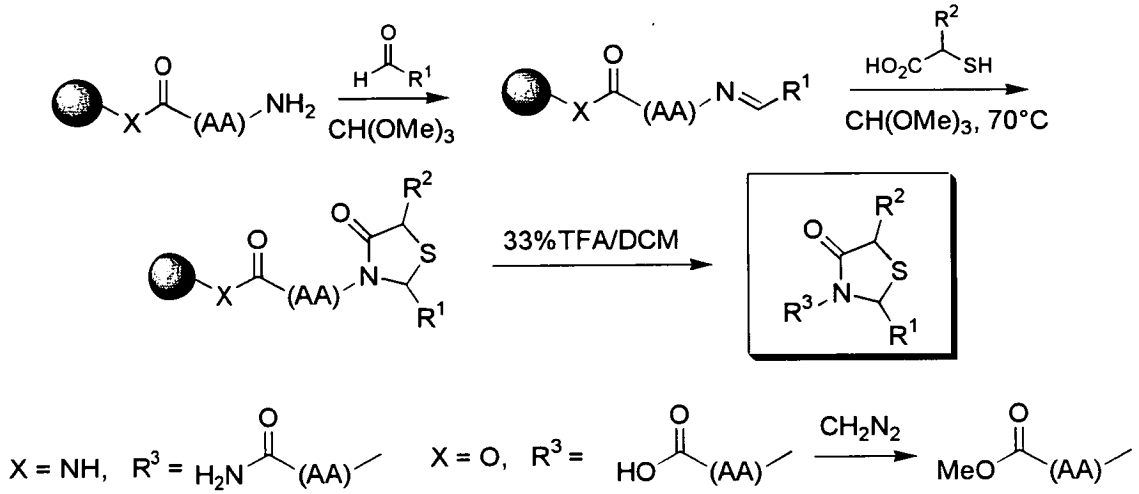


Figure 1.24: *4-Thiazolidinone Libraries by The Split and Mix Method.*

Each of the libraries was tested for binding affinity to the COX-1 receptor which is associated with inflammation mediation. Active compounds were identified by a variation of the iterative technique developed by Houghten[94] for peptides. On completion of the synthesis of each library, cleavage was completed on the five separate pools corresponding to each of the different mercaptoacids used, and the biological assay conducted separately on each of the five mixtures. As the R^2 position in each case was known from the corresponding mercaptoacid, assay results identified the best substituent in this class; in this case butyl (*Figure 1.25*). The library was resynthesised, keeping the sublibraries separate after the imine formation, and reacting each mixture with the butyl mercaptoacid, before resin-cleavage. In this second round of assays, two aspects of the product structure are known (R^1 and R^2) and the mixture containing the best R^1 substituent identified. The final iteration involved the individual synthesis of the five compounds with varying amino acid (R^3) substituents and fixed R^1 and R^2 groups. This enabled the identification of the most active compound.

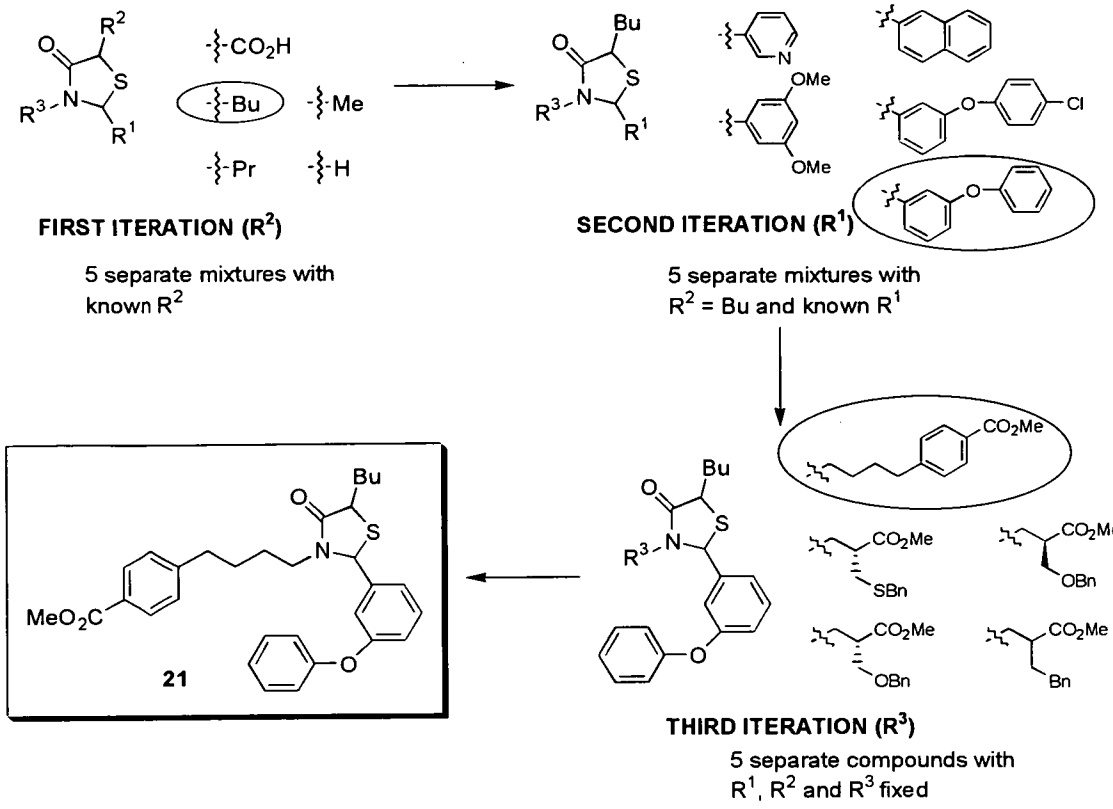


Figure 1.25: *Iterative Approach Towards Lead Identification.*

The iterative method of identifying active compounds has been shown to be effective, as the resultant compound, **21**, is very similar to a known COX-1 inhibitor (ethyl rather than methyl ester). The process also allows for product enrichment as fewer compounds are being synthesised during each iteration. However, resynthesis is time consuming and wasteful of resources. One method of overcoming these problems was devised by Janda,[95] who devised a technique known as recursive deconvolution. This method includes the removal and archiving of resin samples at each step prior to mixing in the normal library generation procedure. (*Figure 1.26*).

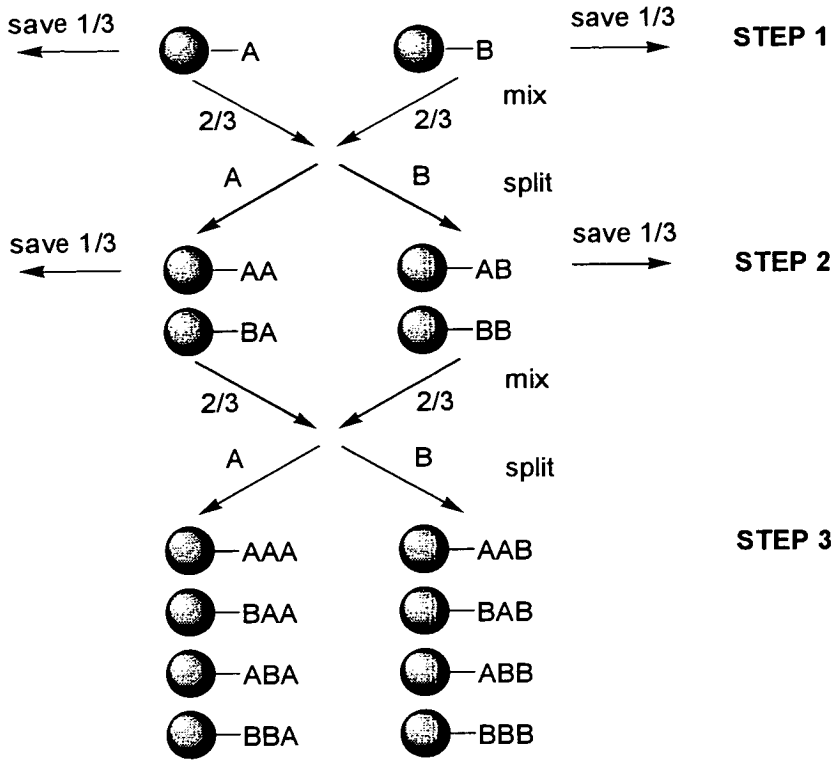


Figure 1.26: *Recursive Deconvolution Method of Active Compound Identification.*

Biological testing on the two pools from Step 3 may indicate that the active compound has building block A in the third position. The two saved pools from Step 2 are kept separate and reacted only with A to form (AAA, BAA) and (ABA, BBA). Since each pool contains only two species instead of four, a two-fold enrichment has been achieved. The process is continued until only one product is synthesised and the active sequence determined. Using this method, Janda identified a known β-endorphin antibody epitope, H_2N-Tyr-Gly-Gly-Phe-Leu-CO_2H from a library of its constituent amino acids. This technique essentially minimises the amount of resynthesis that is required for active compound identification.

Other methods of active compound identification have been dependent on the design of the library synthesis. Houghten developed the positional scanning method[96] to overcome the time-consuming iterative techniques. The method involves the synthesis of multiple sublibraries, where a single position is fixed. For example, a hexapeptide library synthesised from 18 constituent amino acids will consist of 108 (6×18) sublibraries, with one position containing a fixed amino acid and the remaining

positions randomised. The most active compound is present only in those six sublibraries with the corresponding fixed-position amino acid and so will show the "hit". From this information, the complete sequence can be determined. However, there is no product enrichment process, and so the signal to noise ratio in assays is not improved.

A technique known as orthogonal combinatorial chemistry has been reported by Tartar,[97] for the synthesis of 15,625 tripeptides. The technique involves the synthesis of two libraries consisting of the same compounds, but designed in a manner such that the sublibrary mixtures are different, hence active compounds will have different spatial addresses and can be identified.

One approach, known as the "one-bead-one-compound" method, was first recognised by Lam,[98] and has been used in active compound identification. For these purposes, a resin bead can be thought of as an individual reactor, and since all the functional sites on the bead have identical synthetic histories in a split-and-mix library synthesis, a single bead will furnish only one compound. Reversible, colourimetric biological analysis can be performed directly on the resin bead, which can then be isolated and the protein identified by microsequencing.

A method of partial release of products from resin has been used by Gallop,[99] for solution-based assays in a multi-well format (*Figure 1.27*). In a tiered release process, compounds are joined to the resin with a photocleavable linker. Limited periods of irradiation release only 50% of the compound into solution and wells containing active compounds are processed further by splitting the resin into smaller portions. Further irradiation releases more compound from the bead, and the process is continued until assays on a single bead per well format are performed, and the structure determined.

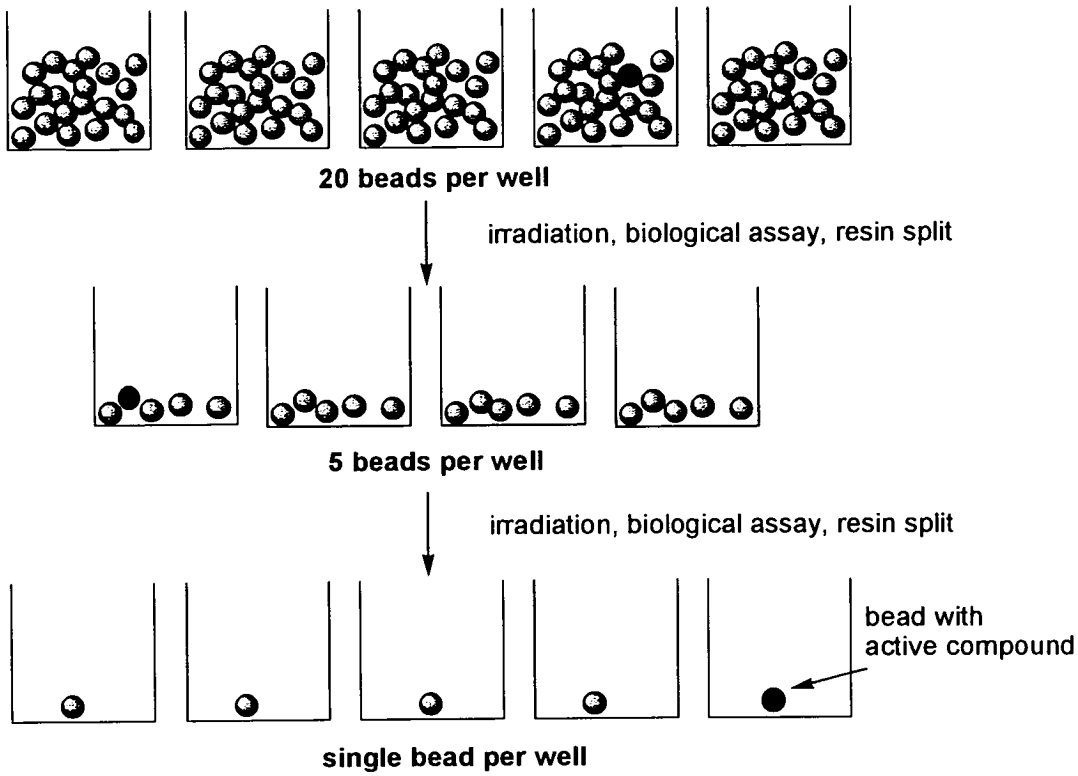


Figure 1.27: *Tiered Compound Release and Single Bead Isolation.*

Iterative methods of product identification are cumbersome and time-consuming processes. A much more efficient technique would enable direct identification of active compounds once the biological assay has been completed. Methods of appending an identification label to the resin beads are used in "encoded" or "tagged" combinatorial libraries.[100] Various types of tags have been used, but every method requires a procedure where the tags can be efficiently joined to the resin, are stable to the synthetic conditions, easily (orthogonally) cleaved from the resin and identified in an unambiguous manner. The detection method usually needs to have high sensitivity, as the tags are generally present in much lower quantities than the parent compound.

Chemical tags in the form of nucleotides have been used as tags for peptide libraries.[101] In what is effectively parallel combinatorial synthesis, the coupling of an amino acid is followed by an identifying nucleotide sequence. Biological testing identifies beads with compounds exhibiting interesting properties, and the "genetic" sequence is then cleaved. The sequence, once amplified by PCR and identified, is unique to one peptide sequence and so the active structure is revealed.

Small organic molecules have been used by Still and Ohlmeyer as tags in combinatorial chemistry.[102] These have been used in the synthesis of both peptide[102(a)] and small molecule[102(b)] libraries, to identify active compounds from solution based assays. Substrates were loaded onto the resin, followed by the appropriate combination of tagging molecules *via* an orthogonal linking strategy. The tagging molecules were designed such that they could be separated by electron capture gas chromatography (*Figure1.28*).

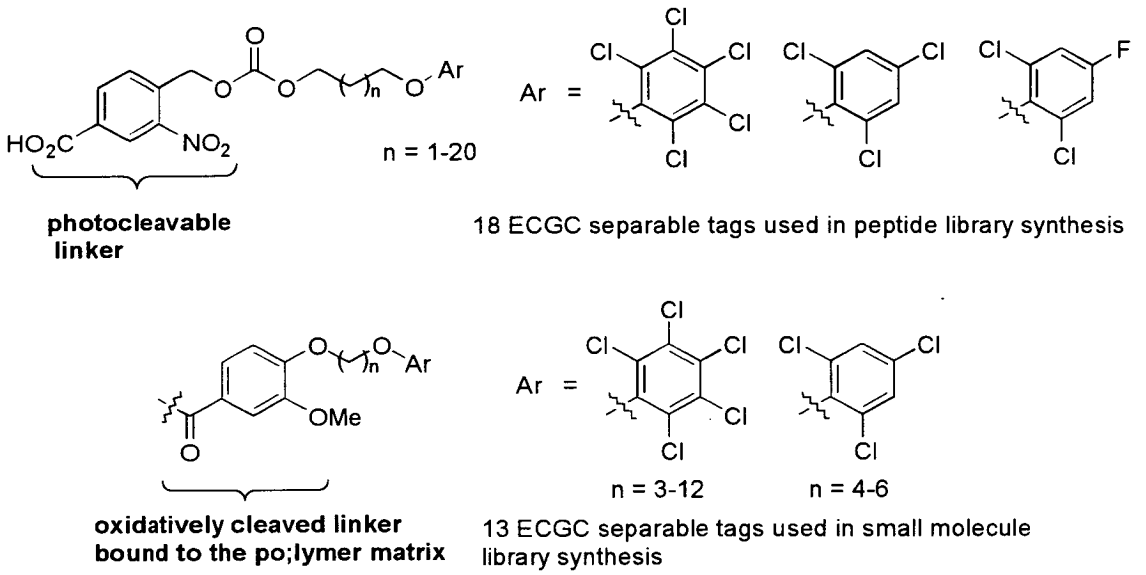


Figure 1.28: *Small Molecule Tags Used in Combinatorial Synthesis.*

After each synthetic step, tags were bound to the resin in a binary encoding system according to which substrate had previously been used. Orthogonal cleavage of the tags from "active" beads, followed by analysis by ECGC, enabled identification of the active compound from the combination of tags detected.

Tagging methodology has proved to be a challenging area in combinatorial synthesis and non-chemical methods, including laser-etching onto ceramic plates supporting functionalised polystyrene,[103] and a method using colour encoded glass beads and vial caps[104] have been reported. One of the most successful chemical or non-chemical tagging methods has been the radio frequency (RF) encoded methods of Nicolaou[105] and Armstrong.[106] These methods involve the containment of resin in porous microreactors, each containing a RF encodable chip (*Figure 1.29*). Split and

mix synthesis is performed as normal, with each pool subjected to a unique RF pulse. Each reactor has a different synthetic history at the end of the library synthesis, and this is reflected in the RF sequence held by the microchip. This information can be retrieved, and so the compound structure identified. The method has recently been used in the synthesis of a 400-member taxoid library.[107] The potential exists for information such as reaction temperature or solvent conditions to be stored on the chip as well.

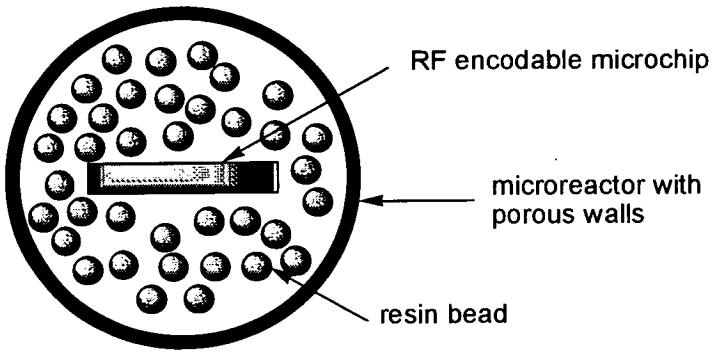


Figure 1.29: *Microreactor Containing RF Encodable Chip.*

The combinatorial synthesis of compound mixtures, coupled with effective deconvolution strategies, has proved a very efficient method of synthesising and identifying many thousands of compounds for biological testing. Split and mix synthesis will readily assist the lead identification process by generating large numbers of compounds in a short time. However, the technique is not well suited for quick structure activity relationship (SAR) determination or lead optimisation, as generally only those compounds which exhibit strong biological activity are identified. The best method of completing these studies is by synthesising individual compounds, and so multiple parallel synthesis techniques have also been developed.

1.3.2 Multiple Parallel Techniques

Although the concept of synthesising analogues of a given template structure for the purpose of biological testing is not new, the rapid generation of analogues has only recently become possible due to developments using solid phase methodologies. As with the generation of compound mixtures, published research into parallel synthesis has centred on classes of compounds which consist of "privileged" templates. For example, pyridine derivatives are common heterocyclic systems in pharmaceutical agents, and have been the subject of solid phase synthesis by Gordeev.[108] He has synthesised 16 pyridines in a parallel manner using Knovenagel and Hantsch condensations (*Figure 1.30*), and 9 dihydropyridines[109] using similar methodology.

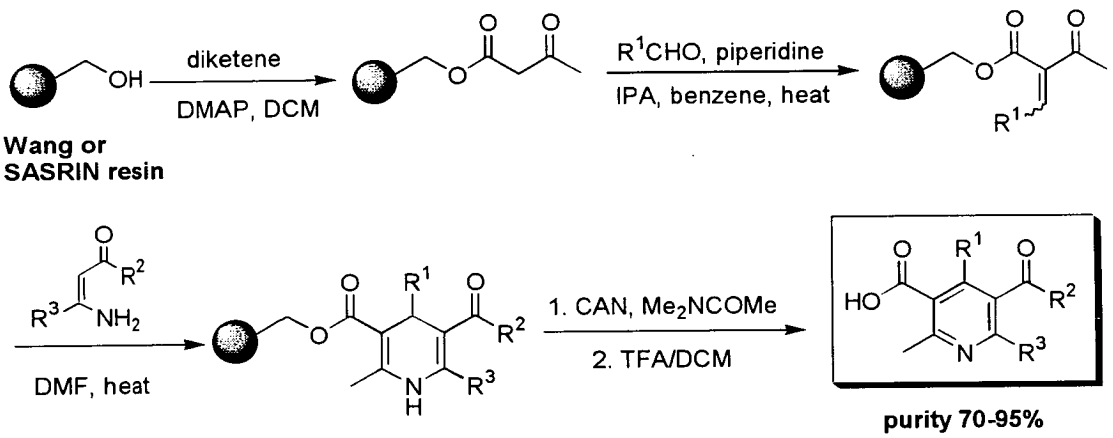


Figure 1.30: *Parallel Solid Phase Synthesis of Pyridines.*

1,4-Benzodiazepine derivatives have also proved popular substrates for SPOS, and have been synthesised by Ellman.[110] The original library consisted of 192 compounds, but an improved synthesis employing a Stille coupling reaction[110(b)] led to the parallel generation of 11,200 compounds[110(c)] (*Figure 1.31*).

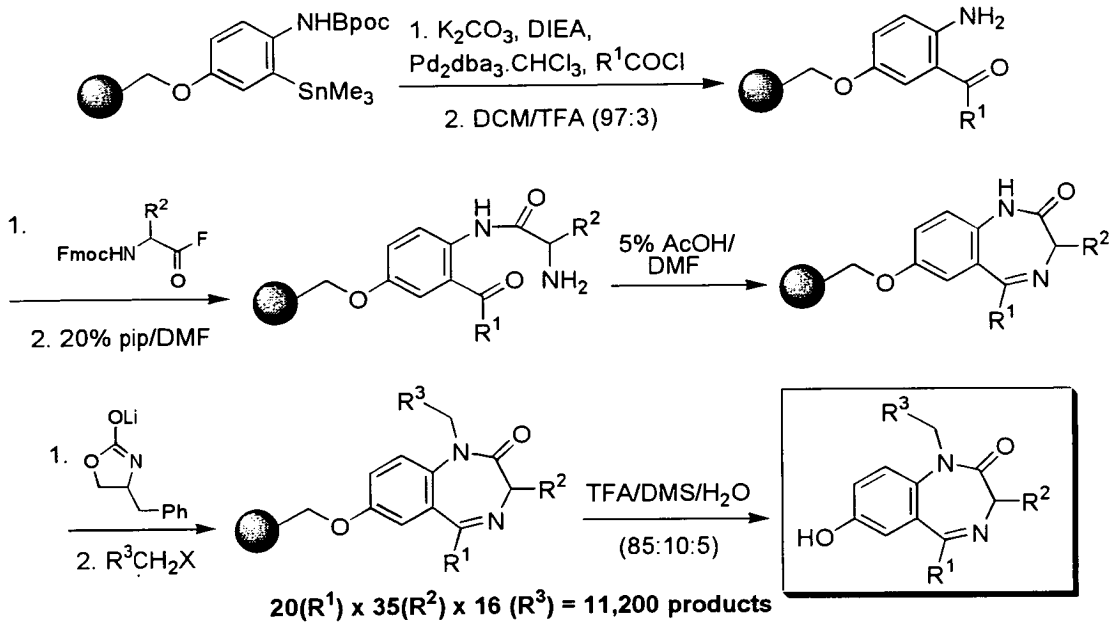


Figure 1.31: *Parallel Synthesis of 1,4-Benzodiazepines via Stille Coupling Reaction.*

The construction of the 1,4-benzodiazepine libraries has been carried out using specially designed equipment for solid phase synthesis. The first equipment was designed for the synthesis of arrays of peptides by Geyson.[111] The equipment consists of polyethylene pins coated with appropriate functionalised resin, and are spaced to fit in a 96-well microtitre plate which houses the solvents and reagents. This equipment has been used directly by Ellman in his benzodiazepine synthesis and adapted for the synthesis of 1,320 (2,508 diastereoisomers) 1,4-benzodiazepine-2,5-diones.[112]

Equipment designed for the solid phase synthesis of compound arrays is often of a proprietary nature, although some methodologies, for example, Geyson's Chiron mimotope assembly[111,113] described above is commercially available. The "teabag" approach is another which has been reported by Houghten[114] for the parallel synthesis of peptides. In this method, porous polypropylene bags containing resin are labelled according to the reagents used. The teabags can be combined for common steps such as washing and deprotection, and active products identified from the label.

One of the early leaders in the automated multiple parallel synthesis using solid phase methods, was the Diversomer™ technique devised by Parke-Davis.[115] The apparatus consists of sintered pins in an addressed array (4×2 or 4×10). The resin (100-800mg) is enclosed in the fritted pin, and placed in a block containing reaction

wells with solvent and reagents (*Figure 1.32*). The entire system is enclosed in a manifold capable of ensuring inert reaction conditions and temperature control, and a gasket allows for needle injections. Reagent and solvent addition is handled automatically by a liquid handling robot, allowing for a completely automated synthesis protocol.

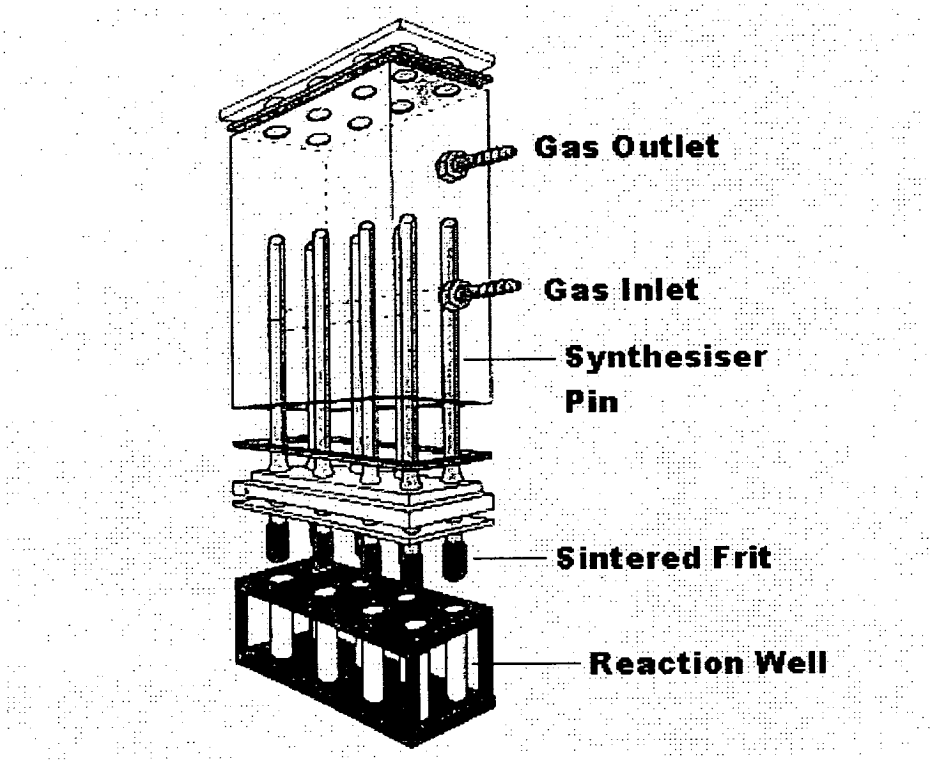


Figure 1.32: *Diversomer™ Apparatus for Multiple Parallel SPOS.*

The Diversomer™ technique has been reported by Parke-Davis for the synthesis of benzodiazepines,[115(a)] hydantoins,[115(a)] benzisothiazolones,[115(b)] and cyclic dinucleotides,[115(b)] and the synthesis of a series of quinolone antibiotics was conducted at Edinburgh University[116] (*Figure 1.33*). The multistep solid phase synthesis followed by chromatographic purification to remove resin impurities yielded products in high purity.

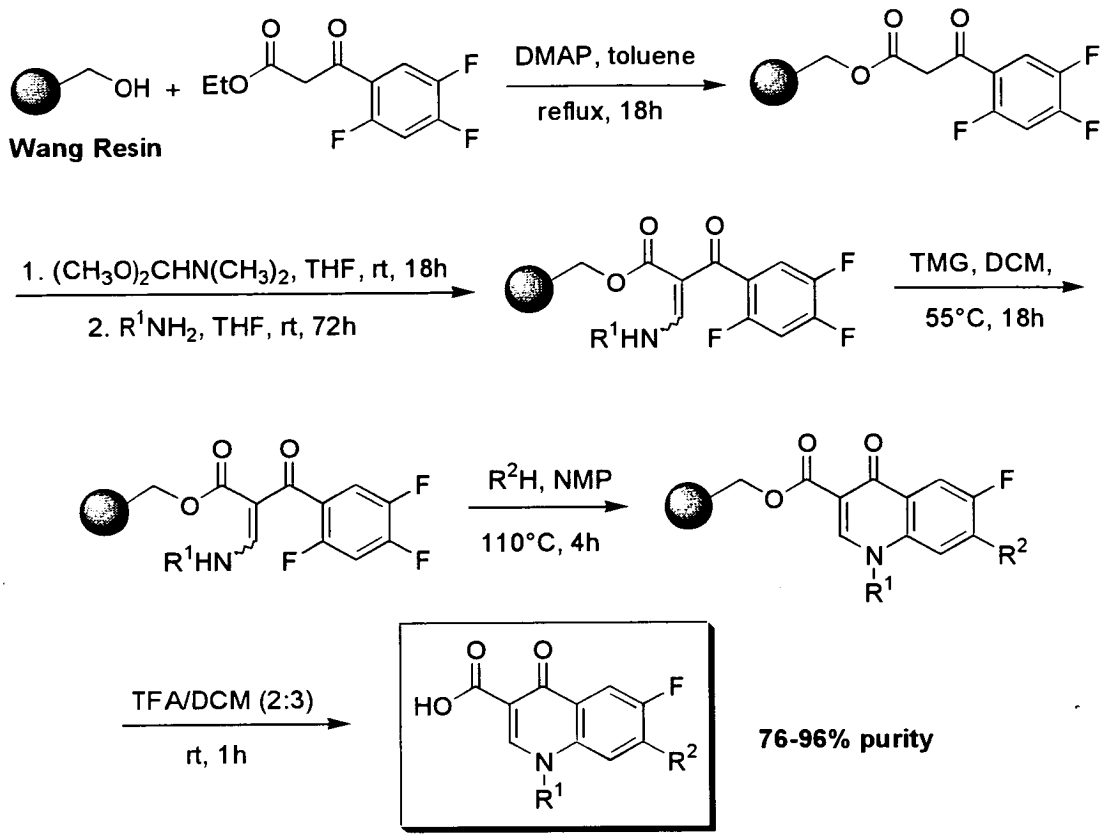


Figure 1.33: *Diversomer Synthesis of Quinolone Antibiotics.*

Methods of automation have been amongst the most recent advances in solid phase combinatorial techniques. While research continues to be published illustrating the uses of SPOS in heterocyclic synthesis, interest has only recently been switched to other viable methods of cleanly generating compounds with biological interest.

1.4 Library Synthesis by Techniques other than SPOS

All of the examples of combinatorial techniques so far described have used SPOS to access high numbers of chemical entities. The undoubted advantage which the technique has in the purification of resin-bound materials has meant that it has largely been the method of choice. However, alternative methods have been developed, and may, in the future, prove to be more popular than SPOS.

The main reason for searching for alternative methods is the fact that the use of polymers often restricts the conditions which can be used to complete a synthesis:

solvents must be chosen to allow good swelling of the resin; reaction temperature is limited to avoid resin breakdown; reaction times are usually longer, as reaction kinetics are generally slower; compound analysis of resin-bound compounds is problematic. Therefore, an ideal system would allow the chemistry to be performed in solution phase, but with the benefits which phase separation afforded by SPOS gives product purification.

1.4.1 Fluorous Phase Synthesis

For several decades, the general immiscibility of highly fluorinated compounds with aqueous and hydrocarbon solvents has been known.[117] However, this observation was only recently developed to assist in organic synthesis. Zhu[118] used the specific solvating power of perfluorinated solvents in biphasic transesterification reactions to drive them to completion. Hovarth and Rabai[119] used modified phosphine catalysts containing a highly fluorinated 'ponytail' to hydroformylate olefins. Liquid phase extraction partitioned the catalyst into the fluorous phase allowing easy separation and recycling.

Fluorous phase chemistry has been more powerfully utilised by Curran[120,121] in multiple parallel synthesis. Organic substrates are made fluorous by attaching a highly fluorinated tag. Reactions are then performed as normal and products purified by liquid-liquid extractions. Reagents and impurities partition into the aqueous or organic phases, whilst the desired product exists in the fluorinated layer. The tag can then be detached, and the organic product isolated (*Figure 1.34*).

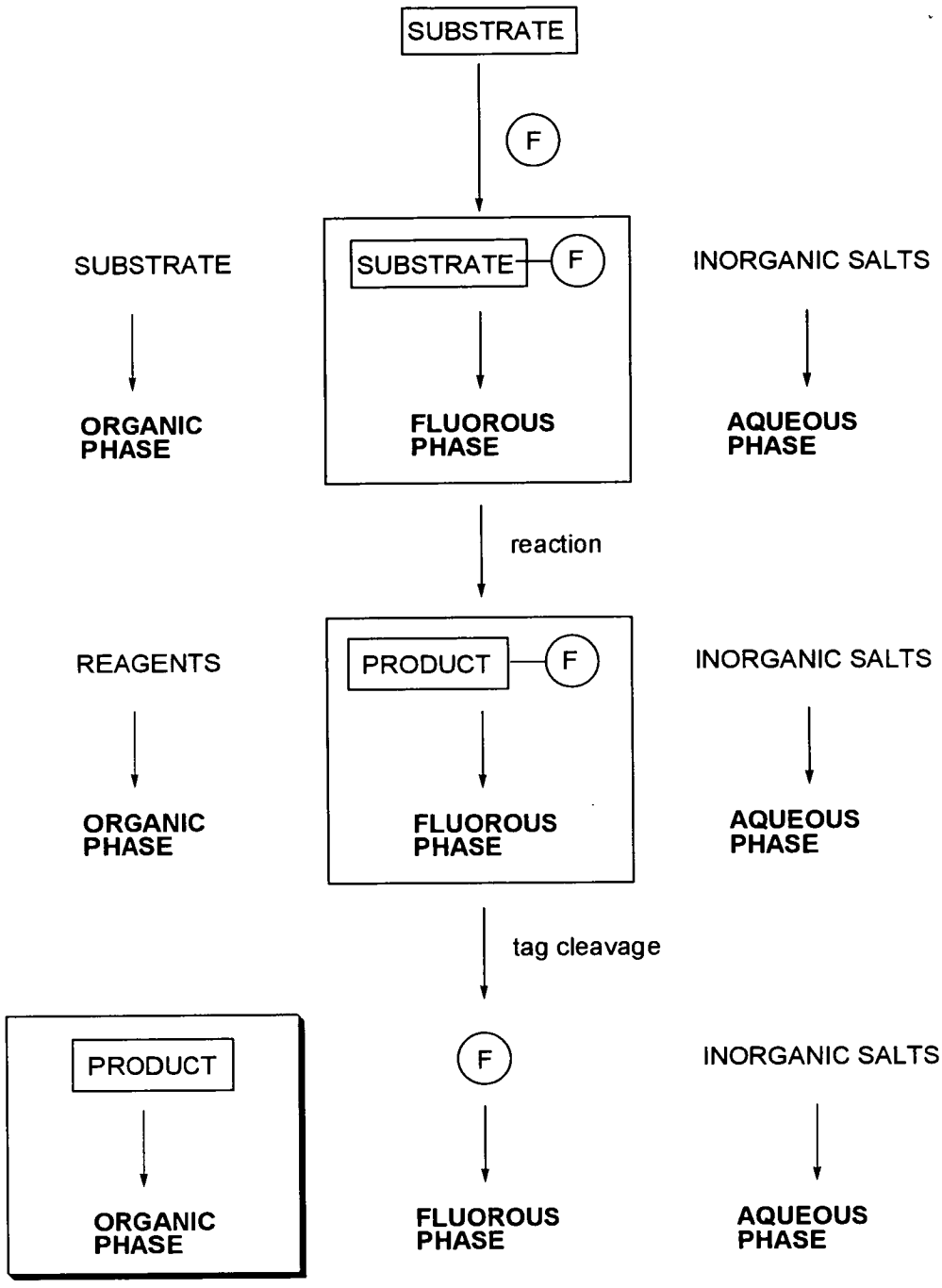


Figure 1.34: *Principle of Fluorous Phase Extraction Technique.*

Fluorous phase extraction has been used by Curran in the parallel synthesis of a small number of isoxazoles and isoxazolines.[120] Allyl or propargyl alcohols were 'protected' with a highly fluorinated silyl group (*Figure 1.35*). Isolation of the fluorinated compound using a three-phase system (H_2O top, DCM middle and FC-

72[122] bottom), followed by reaction with a range of nitrile oxides produced a small library of compounds in overall yields of 73-99% after tag removal.

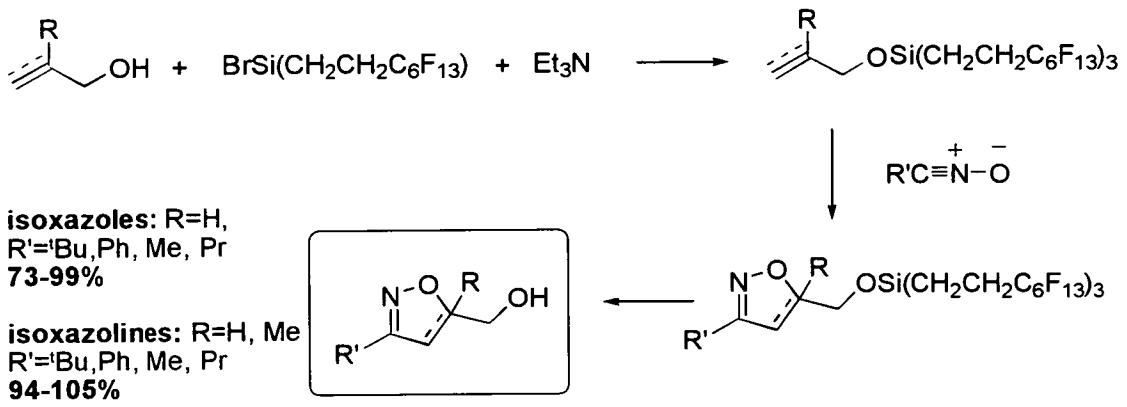


Figure 1.35: *Fluorous Synthesis of Isoxazol(in)es.*

Curran has also employed the fluorous extraction technique[121] in Stille coupling reactions. A highly fluorinated tin species was recycled after aryl coupling, using a three phase system similar to that used in the cycloaddition reaction shown above. Inorganic salts partitioned to the upper aqueous layer, coupled products to the organic layer, and the recyclable tin halide in the bottom fluorous phase (*Figure 1.36*). One drawback of this reaction, however, was the required chromatographic separation of symmetrical biaryl coupled product observed in some cases.

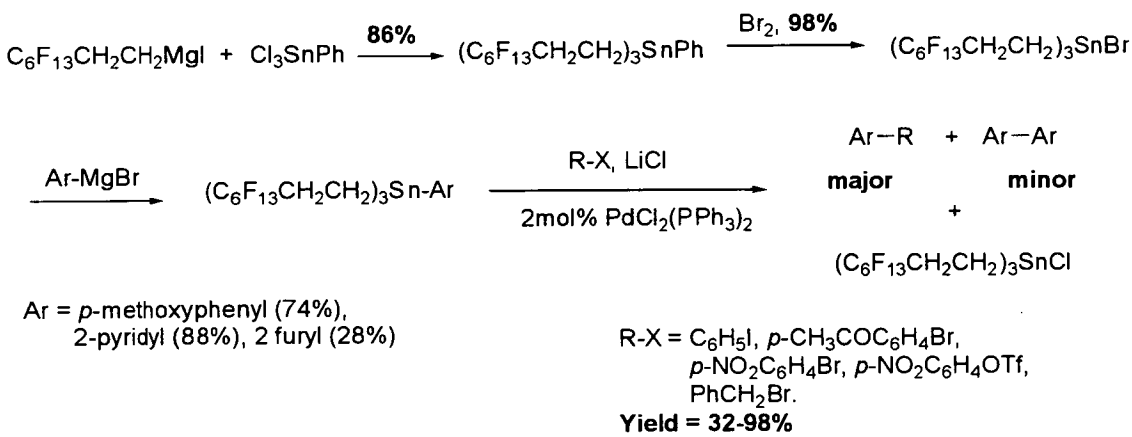


Figure 1.36: *Fluorous Stille Coupling Reactions.*

Curran recognises the limitations of fluorous techniques, however; by-products containing the fluorinated label will also be partitioned to the fluorous layer. This would contaminate products in the case of the isoxazole synthesis, or the recycled tin halide in the case of Stille couplings. However, this is really no different from resin-bound impurities occurring from incomplete reactions, and so it is clear that great advantages are obtained by allowing traditional solution phase synthesis to be coupled with a non-chromatographic purification step.

1.4.2 Solution Phase Methods

One obvious way to overcome the problems experienced when using polymeric supports is to use traditional solution phase methods to synthesise compound libraries. Clearly, conducting reactions in solution allows the enormous diversity of chemical reactions to be utilised to the full, but the task of purifying the products (whether single entities or mixtures) is the main stumbling-block. Thus, systems must be designed to permit straightforward purification by quick liquid-liquid or liquid-solid extractions.

Although solution phase library synthesis is a relatively unexplored area of research, a few examples of the method have been reported. Boger *et al* have designed reaction sequences which use acid/base washes or filtration to purify intermediate products.[123] An anhydride template, **22**, was used to synthesise a series of triamides[123(a)] entirely in the solution phase in good yield and purity (*Figure 1.37*). The parallel synthesis of a 27 member library (3×3×3) was reported using manual acid-base washings. Suitable quantities of material (5-60mg) were obtained in high purity (>90%). Furthermore, the method was reported to be amenable with nucleophiles other than amines (alcohols, thiols) and also the use of batch solid-phase extraction methods in the purification step.

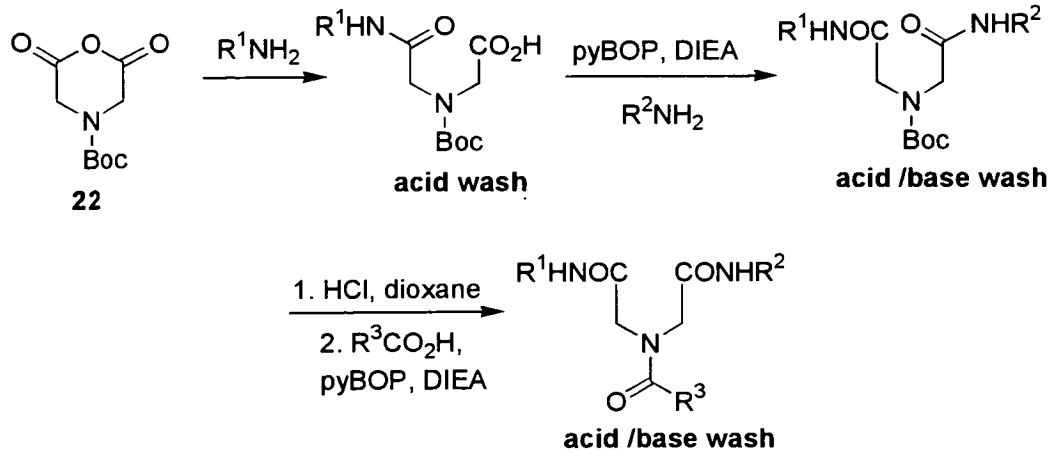


Figure 1.37: *Solution Phase Synthesis of Triamide Library.*

The anhydride template, **22**, was explored in more complicated split and mix metathesis reactions.[124] Using this method, up to 600 olefins (including *E/Z* isomers) were initially synthesised in a series of arbitrarily designed libraries, using 12 amines and 4 ω-alkene carboxylic acids (*Figure 1.38*).

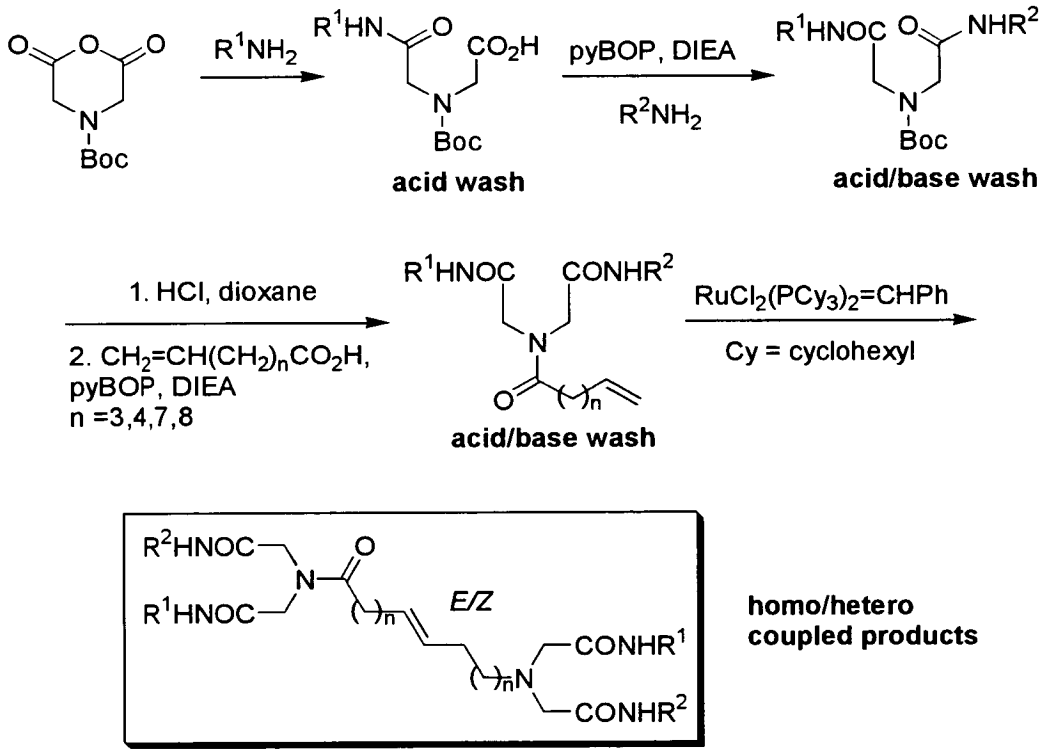


Figure 1.38: *Olefin Libraries in Solution Phase.*

As with the triamide libraries, intermediate products were purified by liquid-liquid extraction techniques, yielding highly pure compounds free of contamination by starting materials and reagents. Products from the olefin metathesis reactions were purified by chromatography, taking advantage of a system which allowed straightforward separation of the desired dimers (either as single entities or designed mixtures) from the catalyst and related impurities. Using similar reaction protocols a series of sub-libraries totalling in excess of 100,000 related alkene derivatives was also synthesised.

Solution phase synthesis has also been used to synthesise mixtures of carbamates[125] and amides/esters.[126] Each was synthesised in an indexed format - a technique which involves the synthesis of a number of sublibraries. For example, the synthesis of 54 carbamates[125] from nine alcohols ($A_{1\text{-}9}$) and six isocyanates ($I_{1\text{-}6}$) was accomplished in fifteen sublibraries of the format $A_1 \times I_{1\text{-}6}$, $A_2 \times I_{1\text{-}6}$, ..., $A_9 \times I_{1\text{-}6}$, $A_{1\text{-}9} \times I_1$, ..., $A_{1\text{-}9} \times I_6$. By this method, each compound is synthesised twice in different sublibraries, and so the identification of possible "hits" after biological assay becomes more straightforward. However, neither of these indexed examples address the problem of generalised purification of compounds in solution phase libraries.

1.4.3 Soluble Polymers

The obvious attraction of combining solution phase synthesis with filtration to facilitate product purification has been elegantly harnessed by Janda *et al.*[127] He used the soluble, linear homopolymer polyethylene glycol monomethyl ether (MeO-PEG-OH) as a support for the synthesis of peptides and non-peptide molecules. PEG-polymers (and hence any components bound to them) are soluble in a wide range of solvents, but are insoluble in ether. Using this feature to purify PEG-bound intermediates, a small series of sulphonamides was synthesised (*Figure 1.39*).

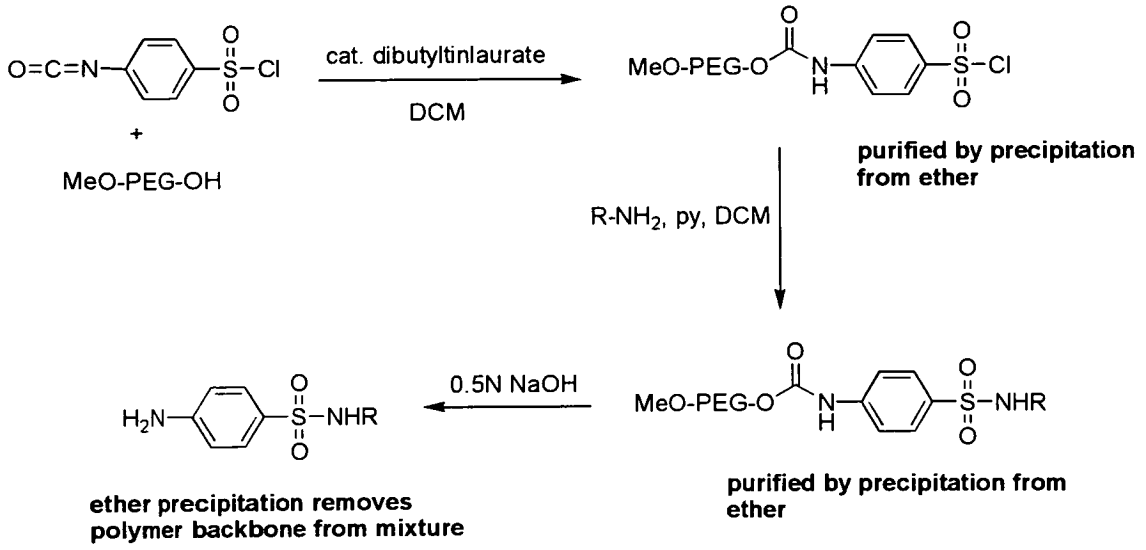


Figure 1.39: *Sulphonamide Synthesis Using Janda's Soluble PEG Polymer.*

The technique was enhanced by Janda to embrace traceless linker methodology.[128] Whereas the above example leaves amino functionality, a traceless system requires that effectively only a new C-H bond is formed upon cleavage of the organic fragment from the polymer. This requires a suitable linker group as well as appropriate cleavage conditions (*Figure 1.40*).[128(c)]

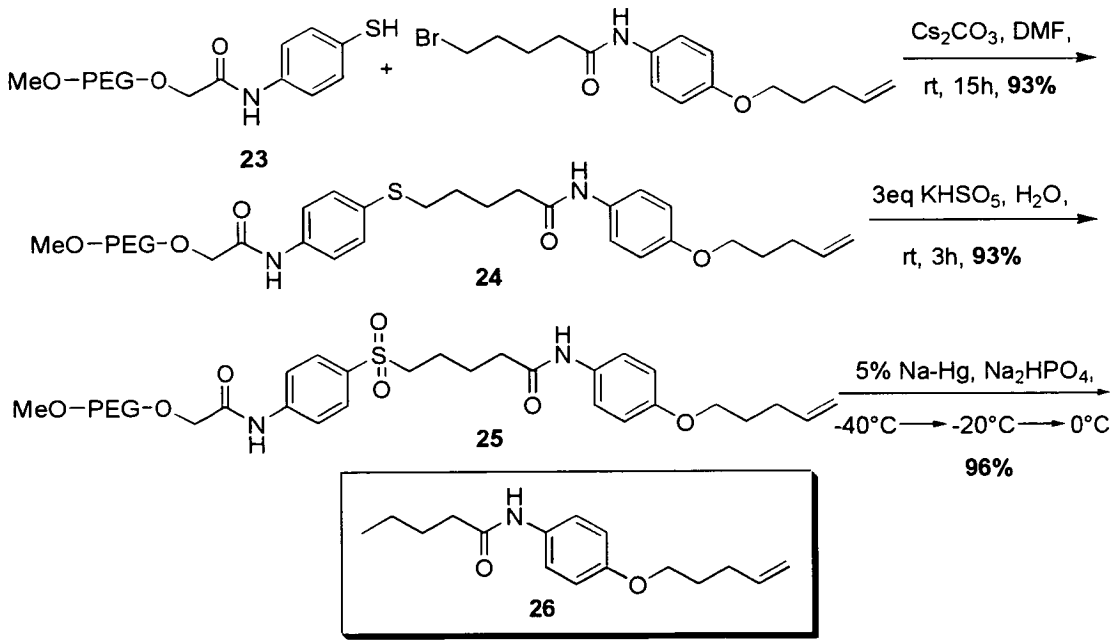


Figure 1.40: *Traceless Sulphide Linker to Soluble Polymers.*

The PEG-bound linker group, **23** is bound to the organic fragment to form PEG-thioether, **24**. This, along with the PEG-bound sulphone, **25**, is purified by precipitation from ether in the normal way. The linker is activated by oxidation of the sulphide to the sulphone and the traceless removal of the PEG moiety achieved by reduction using Na/Hg to form **26**. Although this scheme does not incorporate any synthetic transformations on the organic fragment, the aim of traceless linking has been adequately demonstrated.

Janda has also recently adapted the soluble PEG polymer for use with the "fish-hook" principle[66,68] which involves using the polymer to extract the desired component from a reaction mixture. The other components are removed and the desired molecule then released from the polymer. This technique was used to purify the β-adrenergic receptor, propanolol.[129] The method takes advantage of the known reversible binding of the β-amino alcohol subunit to boranes (*Figure 1.41*) to selectively "hook" propanolol from the previously unpurified reaction mixture.

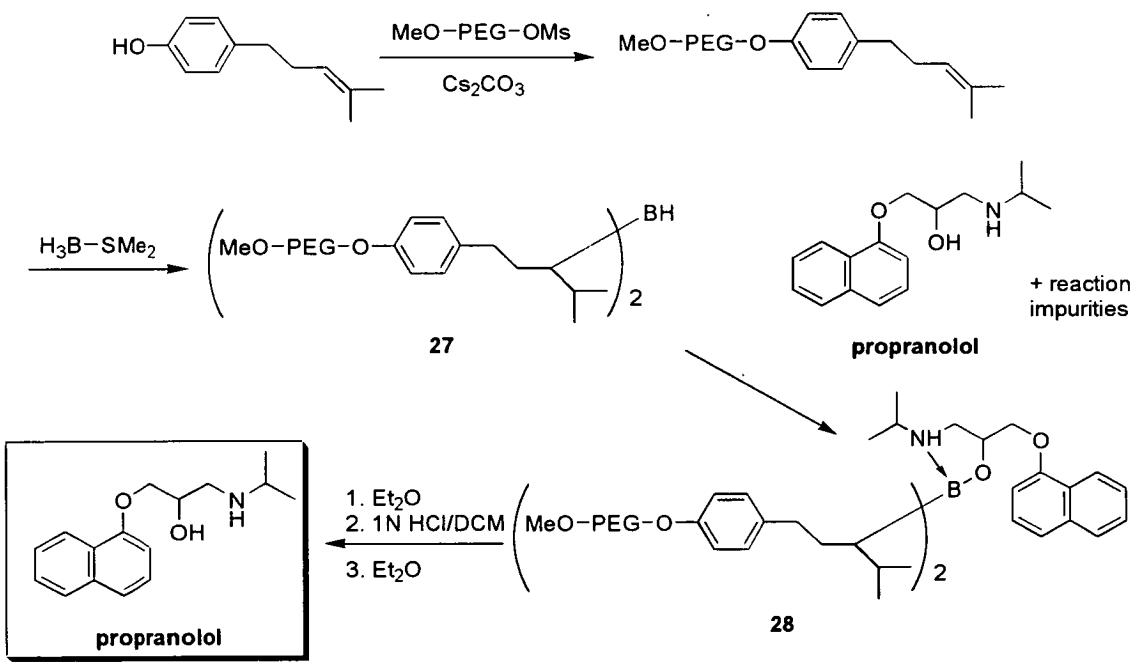


Figure 1.41: *Purification of Propanolol by the "Fish-hook" Principle.*

Propanolol was synthesised in solution without purification of intermediates and then selectively bound to the PEG-anchored borane, **27**. Ether precipitation then

extracted the polymer-bound species, **28,** from the reaction mixture. Propanolol was released from the polymer by aqueous acid, and the spent polymer removed by precipitation using ether. Propanolol was then isolated in 58% overall yield and 92% purity. Using a similar protocol, the synthesis of a series of 20 β-amino alcohols was completed in 27-100% yield and >88% purity.

1.5 The Impact of Combinatorial Chemistry

There is no doubt that combinatorial chemistry has changed the role of medicinal chemists in the drug discovery process. With human genomics projects expected to lead to the identification of many new therapeutic targets, processes which quickly generate compounds which have high affinity for these potential new targets, are likely to be highly rewarded. High throughput screening methods designed to automate the routine synthesis and biological screening of many thousands of compounds have fuelled interest in the solid phase synthesis techniques required for the rapid synthesis and purification of such large numbers of compounds.

Whilst the common belief exists that testing increased numbers of compounds should furnish more compounds with increased potency, discussion prevails as to what sort of preparation, prior to library generation, should be carried out. Issues such as the merits of optimising solution phase chemistry prior to solid phase library synthesis are frequently argued.

The type and quantity of compounds which should be synthesised is often disputed and much emphasis has been placed on the choice of members for any given compound library - the issue of compound "diversity" remains a moot point. Although it is a common belief that library members should be as varied as possible, the combined effect of the parameters which govern how different compounds are from each other (shape, molecular weight, hydrophobicity, bioavailability, etc.) are rarely agreed.

The biological testing of compounds as mixtures has also raised questions as to the reliability of producing "real" results. For example, an active mixture may simply be the accumulative effect of moderately active compounds, rather than one single

compound. Consequently, libraries of compound mixtures require robust design to remove "false" positives and negatives.

With much of the development in combinatorial techniques in the pharmaceutical industry being of a proprietary nature, it is difficult to know how successful combinatorial techniques have been in genuinely assisting the drug discovery process. With development periods and clinical trials remaining unavoidable stages, it will be some years before drugs which have been discovered by true combinatorial methods will be marketed.

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Chapter 2: Solid Phase Synthesis of Acrylic Acids

2.1 Synthetic Routes to Acrylic Acids

Acrylic acids have been a structural template of interest at Parke-Davis since the identification of **29** from a random screen. This indole derivative was found to be an antagonist of a receptor in an area of therapeutic interest at micromolar concentrations, and was considered to be a candidate for further investigation by the Diversomer™ technique (see *Section 1.3.2*). This would determine the structure activity relationship (SAR) and possibly enable lead optimisation.

For successful generation of analogues, a general synthetic route to acrylic acids compatible with solid phase techniques required development. The reported synthesis of **29**[1] was conducted as part of a series of analogues using Wittig chemistry (*Figure 2.1*). In this synthesis, phosphorane, **30** was condensed with gramine, **31**, to form substituted ylide, **32**. Wittig reaction with aldehyde, **33**, followed by ester hydrolysis, afforded the desired acrylic acid, **29**.

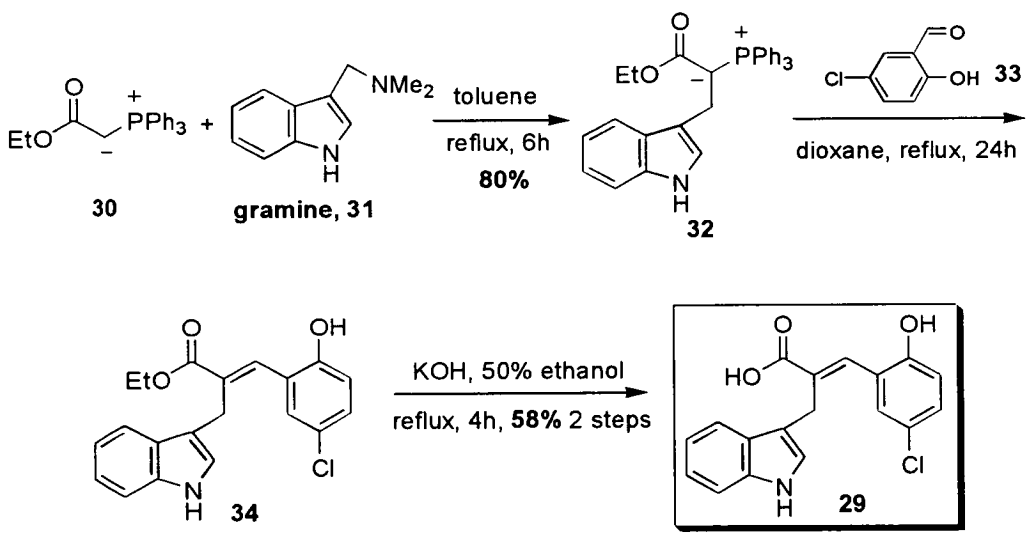


Figure 2.1: *Original Solution Phase Synthesis of Acrylic Acid, 29.*

The geometry of the alkene was originally tentatively assigned as *E*-, due to the uv absorbance of the product, which had a maximum absorbance of the same order of magnitude as *trans*-cinnamic acid. Crystal structure data has since confirmed this assignment.[2]

The Wittig reaction has been successfully carried out by solid phase synthesis on many occasions. Fréchet[3] and Leznoff[4] were the first to investigate the reaction, and were able to reproduce solution phase results. Both methods of the reaction have been performed, *ie* a resin bound aldehyde with the ylide in solution,[3,4(c)] and the "reverse" Wittig, which has the ylide bound to the solid support and excess aldehyde in solution.[4(a),(b),(d)] One other method of performing Wittig reactions which is exclusively available *via* SPOS, is the use of phosphine functionalised resins.[5] The ylide is built upon a resin-bound phosphine, and the reaction is then performed in the normal manner. This method has the added advantage that the phosphine oxide by-product formed on completion of the reaction remains bound to the resin, and so does not interfere with product purification. The various methods of performing the Wittig reaction using SPOS are shown in *Figure 2.2*.

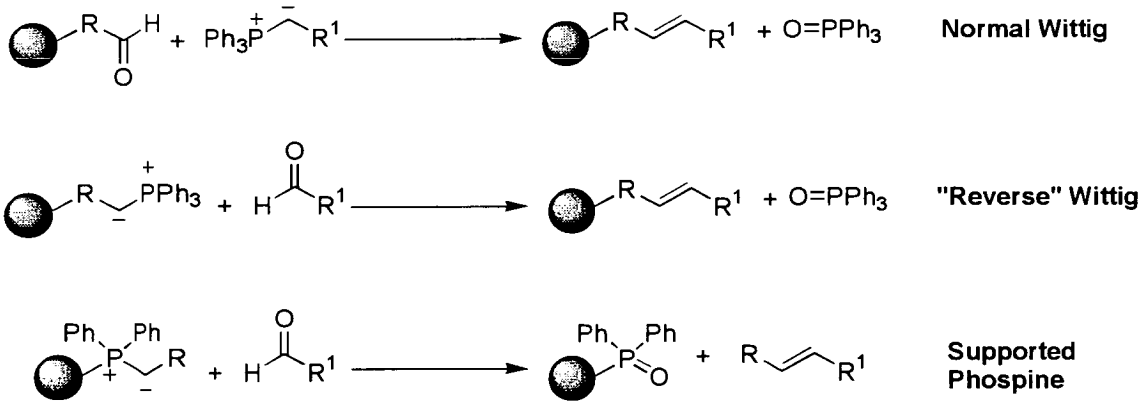


Figure 2.2: *Methods for Performing the Wittig Reaction on Solid Support.*

Two of the above methods are viable for the synthesis of **29** on solid phase. The "reverse" Wittig reaction could be carried out by binding the carboxyl group to Wang resin and forming the ylide with triphenylphosphine. This could then be condensed with gramine to form the resin-bound ylide, **35** and Wittig reaction with aldehyde **33**

would form the resin bound acrylic ester, **36**, which could be cleaved under standard conditions to form the desired acrylic acid. (*Figure 2.3*). The only disadvantage of this method is that insoluble triphenylphosphine oxide will be formed, which would be relatively difficult to separate from the resin and so purification problems would exist.

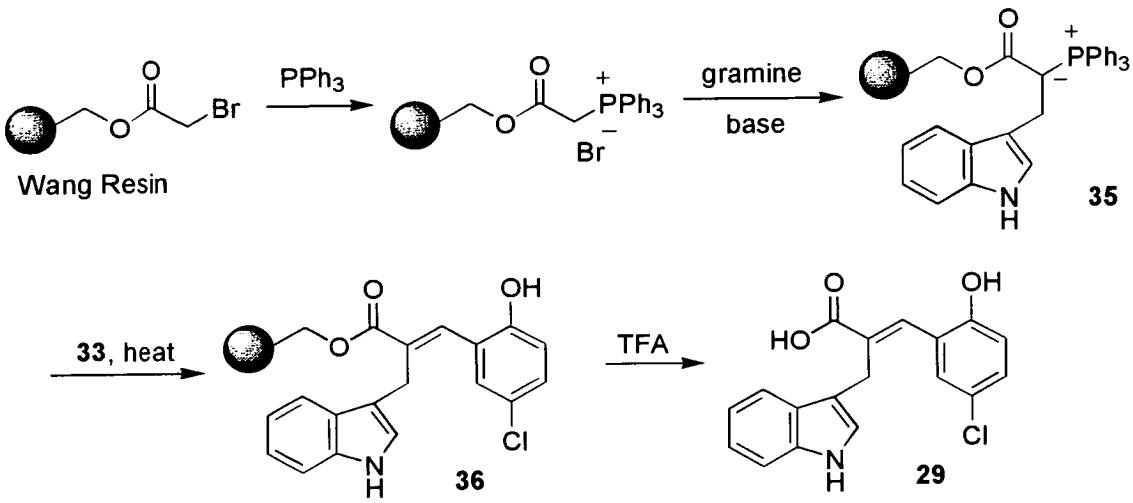


Figure 2.3: *SPOS of Acrylic Acids Using the Reverse Wittig Reaction.*

The second possible route involves the formation of a phosphine functionalised resin, **37**, and forming the ylide, **38** (*Figure 2.4*), for reaction in the "reverse" Wittig.

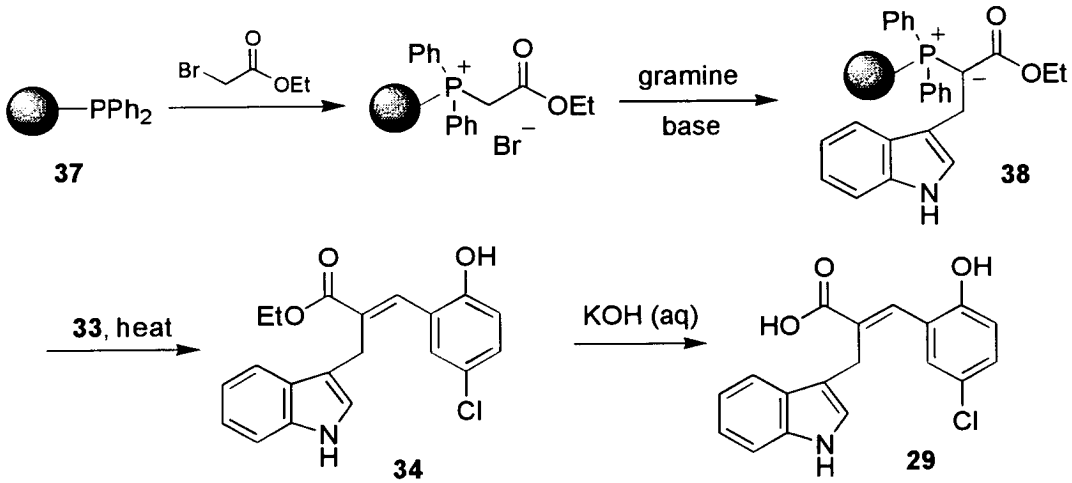


Figure 2.4: *Acrylic Acid Synthesis Using Resin-bound Phosphine.*

The use of the resin-bound phosphine has the advantage that the triphenylphosphine oxide by-product formed during the Wittig reaction remains bound

to the resin, and so does not interfere with product purification. However, ester hydrolysis of **34,** and ultimately other library members, would have to be conducted in solution phase. The normal Wittig reaction is not likely to lend itself to library generation, as the multi-step solution phase synthesis of a range of functionalised ylides would be required beforehand.

As neither form of the described Wittig reactions is ideal, two alternative possibilities were considered. These involve elimination and Horner-Wadsworth-Emmons[6,7] (HWE) reactions respectively to furnish the acrylic acid functionality. The elimination route was chosen (*Figure 2.5*), as a reliable route to key β-ketoester intermediates had been previously established[8] and used successfully by MacDonald and Ramage[9] in the solid phase synthesis of quinolones. Furthermore a quantity of a suitable aryl β-ketoester was available from related work (see *Section 3.3*) and could be used in model studies. Therefore, resin bound β-ketoester, **39**, after alkylation, could be selectively reduced to form hydroxy-ester, **40**. Dehydration followed by resin cleavage would furnish the desired acrylic acid, **29**.

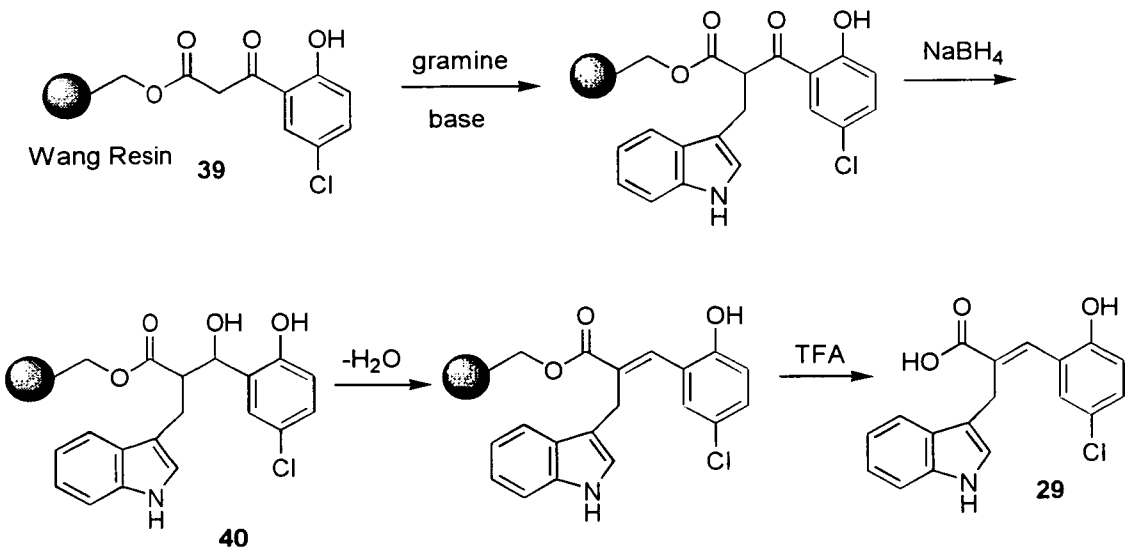


Figure 2.5: *Proposed Elimination Route to Acrylic Acid, 29.*

The HWE reaction has been little explored on solid phase, compared to the related Wittig reaction. It has been investigated using combinations of mild bases[10] and reactions have been followed using gel phase ^{31}P nmr[10] and magic angle spinning nmr

techniques.[11] The HWE reaction involves the reaction of phosphonate esters with aldehydes and ketones, rather than phosphonium ylides in the Wittig reaction. This results in the HWE reaction exhibiting several advantages over the Wittig reaction. For example, phosphonate carbanions are known to be more nucleophilic than phosphonium ylides,[6(b)] and so will generally react with a wider range of aldehydes and ketones under milder conditions. Alkylation of phosphonates is more readily achieved than ylides,[6(b)] and so more highly substituted olefin products can be accessed. Finally, and of greater consequence in SPOS, the phosphate by-product formed during the reaction is water soluble, and so will not inhibit olefin purification. Therefore, resin-bound phosphonate, **41**, could be alkylated to give indole phosphonate, **42**. HWE reaction with salicylaldehyde, **33**, furnishing olefin, **36**, followed by resin cleavage would yield the desired acrylic acid, **29** (*Figure2.6*).

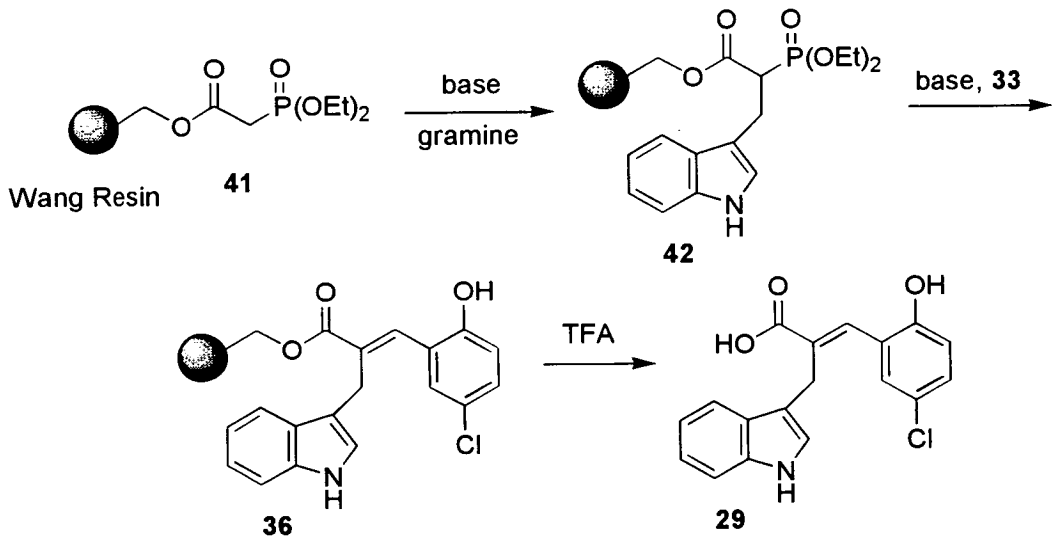


Figure 2.6: *HWE Route to Acrylic Acid, 29.*

The elimination route and HWE route were both considered as viable alternatives to the Wittig reaction in achieving the solid phase synthesis of **29** and ultimately a library of acrylic acid analogues. However, due to potential problems of following reactions and analysing compounds on solid support, the optimisation of each synthesis in the solution phase was first attempted. It was hoped that by using reagents and solvents which would be compatible with solid phase methods, any problems could be quickly overcome, and alternatives sought if necessary.

2.2 Model Studies (1): Elimination Route

The elimination route to acrylic acids was investigated using the β-ketoester, **43**, which was available as a precursor of investigations into the synthesis of the quinolone antibacterial, Ciprofloxacin (see *Section 3.3*). The retrosynthesis for the corresponding methylindole acrylic acid, **44**, is shown below (*Figure 2.7*).

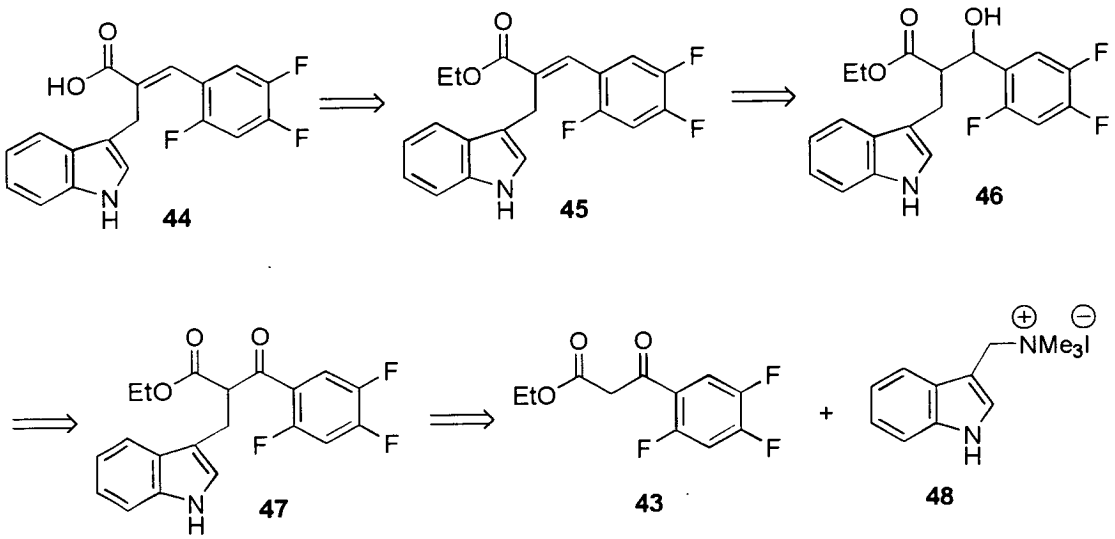


Figure 2.7: *Retrosynthesis of Model Acrylic Acid, 44.*

The synthesis is composed of well established chemistry; the only potential drawback being a lack of geometric control in the formation of the olefin. However, the synthesis, separation and biological assay of both *E*- and *Z*- isomers would perhaps generate greater understanding of the receptor site in question. One key synthetic point was the choice of gramine methiodide, **48**, rather than unquaternised gramine, **31**, as the alkylating agent as used in the original synthesis of **29**.[1] This was because the methiodide salt has been known to alkylate compounds with active methylene groups under milder conditions than gramine alone.[12]

The synthesis of gramine methiodide was carried out as reported by Geissman,[13] with the slow addition of gramine to neat methyl iodide (*Figure 2.8*). This contrasts with the methods reported by Brown[14] and Snyder,[12] who added gramine to an excess of methyl iodide in ethanol. However, Geissman, and later Melhado,[15] proved

that the latter method showed considerable levels of contamination by a *bis*-indole methiodide salt.

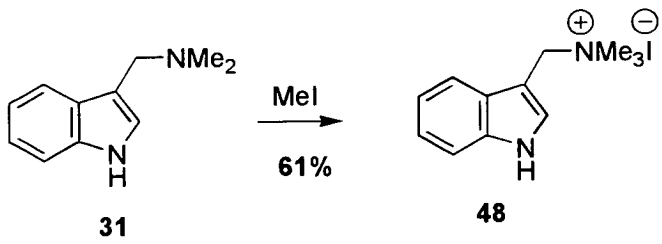


Figure 2.8: *Synthesis of Gramine Methiodide.*

Gramine methiodide was prepared in 61% yield, and although the procedure required a lengthy washing and crystallisation protocol, multigram quantities could be made. Nmr spectroscopy indicated that none of the *bis*-indole product was present in the purified samples by comparison with reported data,[15] regarding the chemical shift of the methylene and methyl protons.

Alkylation of β-ketoester, **43**, with gramine methiodide was attempted using a number of amine bases compatible with solid phase chemistry. Use of the bases DBU, DABCO and TMG was not successful, giving a mixture of products as well as unreacted starting material. Reaction with triethylamine, however, proceeded smoothly and the desired alkylated β-ketoester, **47**, was formed cleanly in 72% yield (*Figure 2.9*).†

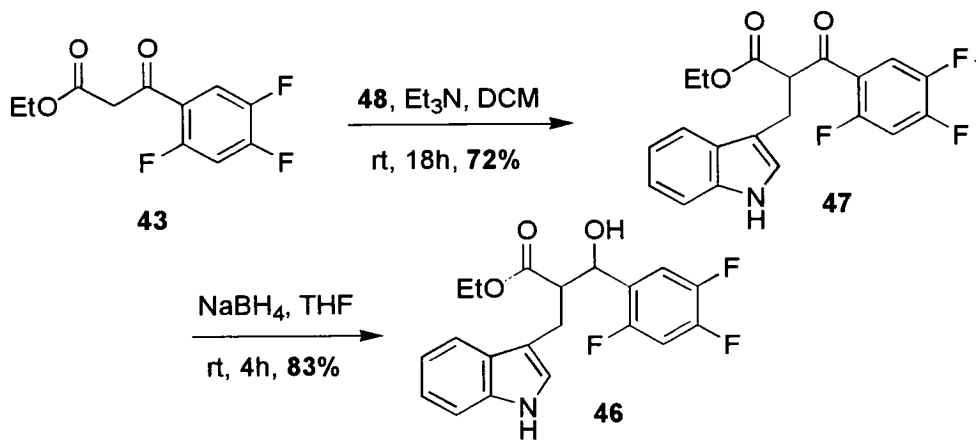


Figure 2.9: *Alkylation and Selective Reduction to form Hydroxyester, 46.*

† For full details of the synthesis of β-ketoester, **43** see *Figure 3.6.*

Although many reagents exist for the direct dehydration of alcohols,[16] none proved satisfactory in this case. Treatment of **46** with DMSO at 130°C gave only recovered starting material, whilst reaction with $POCl_3$ in pyridine[17] gave an isomeric mixture of products in low (~30%) yield. This product mixture was thought to contain both *E*- and *Z*- olefins, as no conformational control was expected from the elimination reaction. The isomers were separated by exhaustive chromatography, and characterised by ^1H nmr and mass spectrometry. It was clear that the products were not the expected olefins and were subsequently identified as the diastereomeric chloride, **49** (*Figure 2.10*).

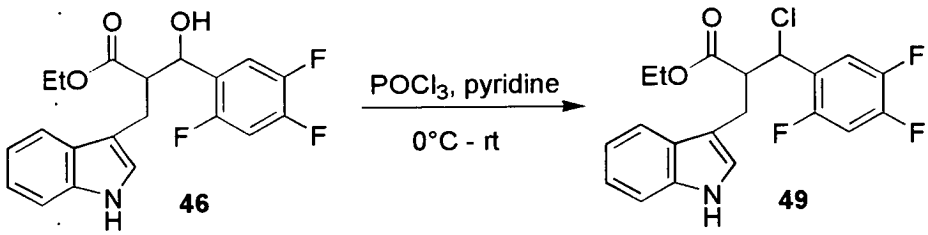
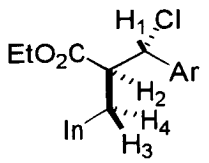
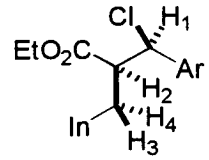


Figure 2.10: *Action of $POCl_3$ on Hydroxyester to form Chloride, 49.*

The assignment of the relative configuration of the two diastereoisomers was not unequivocally clear, but certain information gained from the ^1H nmr spectrum of each isomer indicated a possible solution (*Figure 2.11*). The resonances for proton H_1 suggest that that of **isomer 1** has an *anti*- relationship with H_2 due to the large coupling constant of 9.7Hz. The corresponding coupling constant for **isomer 2** is 5.9Hz which indicates a *syn*- relationship is more likely.



Isomer 1, 49A



Isomer 2, 49B

δ = 5.41ppm (1H, d, $^3J_{HH}$ = 9.7Hz, H_1)
δ = 3.46ppm (1H, m, H_2)
δ = 3.03ppm (1H, dd, $^3J_{HH}$ = 14.2, 10.2Hz, H_3)
δ = 2.74ppm (1H, dd, $^3J_{HH}$ = 14.2, 4.8Hz, H_4)

δ = 5.09ppm (1H, d, ^3J = 5.9Hz, H_1)
δ = 3.60ppm (1H, m, H_2)
δ = 3.40ppm (1H, dd, $^3J_{HH}$ = 14.5, 8.6Hz, H_3)
δ = 3.17ppm (1H, dd, $^3J_{HH}$ = 14.5, 6.4Hz, H_4)

Figure 2.11: *Assignment of Chloride Diastereoisomers by ^1H NMR.*

Methods of activating the hydroxyl functionality to the elimination process by transformation into a better leaving group were attempted, but synthesis of the corresponding acetate, tosylate and triflate of **46** were unsuccessful. One method which did furnish identifiable products was that of Furstner[18] who reported the elimination of a secondary alcohol using methanesulphonyl chloride and a large excess of DMAP. Treatment of **46** under these conditions yielded two products, which were identified as chloride, **49B**, and mesylate, **50** (*Figure 2.12*). Coupling constants in the ^{1}H nmr spectrum of **50** led to the tentative assignment of the *anti-* isomer. These reaction conditions, however, gave unreproducible product mixtures, and at no time were olefinic products observed.

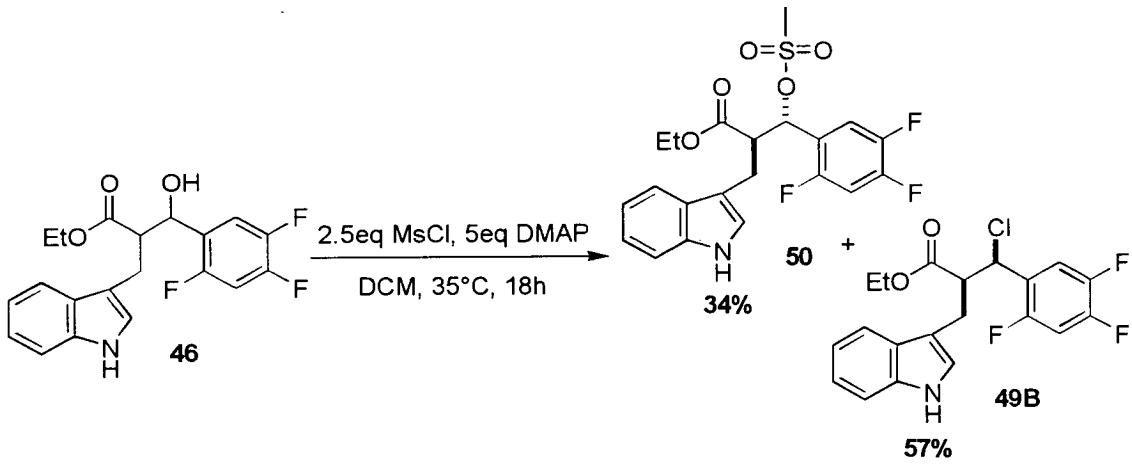


Figure 2.12: *Attempted Alkene Formation via Active Mesylate.*

The reasons behind such difficulties in olefin formation were not obvious, especially as elimination would be expected to be driven by the conjugation of the eliminated product. Furthermore, with the hydroxyl group in the benzylic position, any subsequent carbocation formed would be highly stabilised. It may be that the acidic indole *NH-* is being deprotonated under basin conditions, causing side reactions to occur, and that *NH*-protection may be required. Another possible explanation may be that the compound may be restricted in forming the anti-periplanar arrangement between the H and OH required for elimination to result. This may be represented by the four Newman projections of hydroxyester, **46** shown in *Figure 2.13*.

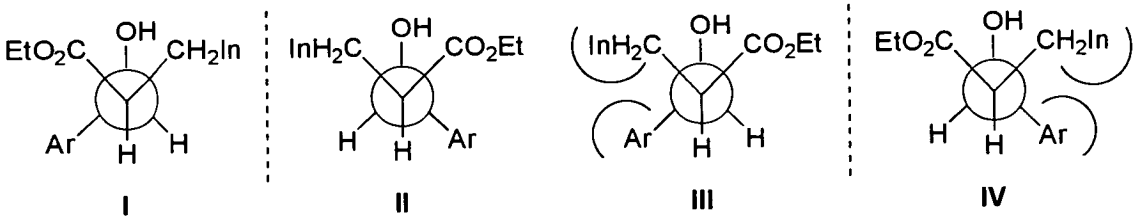


Figure 2.13: *Newman Projections of the Diastereoisomers of β-Hydroxyester, 46.*

It is possible that steric hindrance between the trifluoro aromatic (Ar) and the methylindolyl (CH_2In) group would prevent isomers **III** and **IV** from aligning in the required fashion. Similarly, π-stacking between the aromatic groups, may make the overall structure more rigid, preventing the correct geometric arrangement from arising.

An alternative method for elimination reactions uses the Mitsunobu reagents triphenylphosphine and diethyl azodicarboxylate (DEAD). The synthesis of dehydroaminoacids has been performed under such conditions[19] and the method was attempted to drive the dehydration of **46**. The reaction did yield alkene, **45**, as a single geometric isomer, but in only 26% yield (*Figure 2.14*).

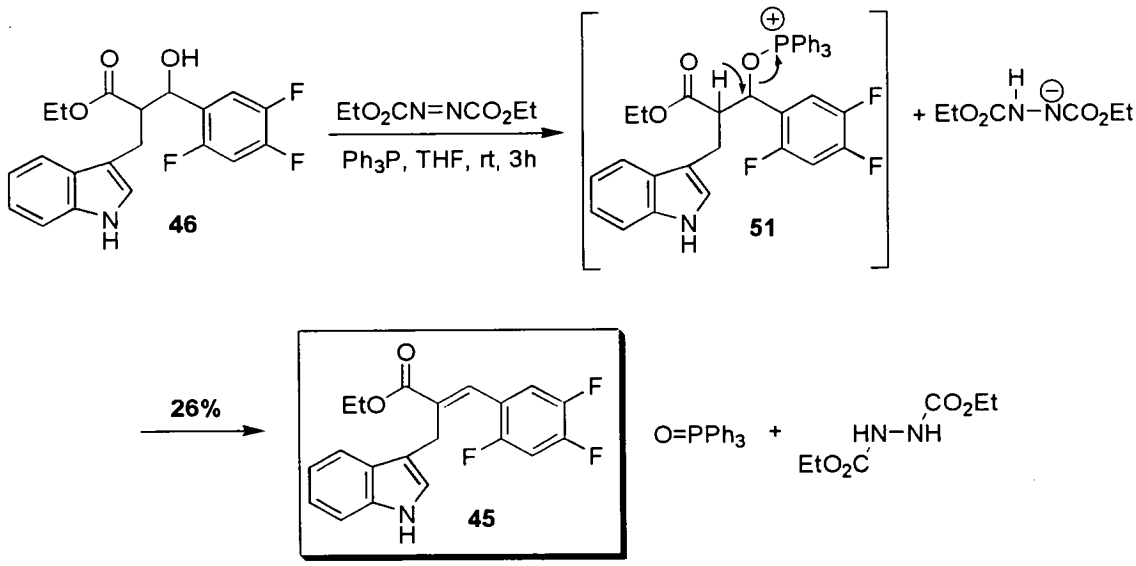


Figure 2.14: *Elimination Reaction Using Mitsunobu Conditions.*

Why Mitsunobu conditions should form the olefin over the other activation methods described is unclear. It can only be reasoned that formation of insoluble

triphenylphosphine oxide formed on completion of the reaction drives the process more than the equivalent eliminations using other activation methods. This is perhaps due to the charged nature of the intermediate, **51**. The crude product was a complicated mixture, and isolation of only one geometric isomer of the alkene (unassigned, but shown as *E*-) should not suggest any inherent selectivity of the reaction. More effort would be required in order to investigate the Mitsunobu conditions further.

In parallel studies, methods of forming the correctly functionalised aryl β-ketoester for the ultimate synthesis of the lead acrylic acid, **29**, were investigated. The acid chloride, **52**, of 5-chlorosalicylic acid, **53**, was synthesised in good yield *via* the sodium salt, using a procedure reported by Shakirov *et al.*[20] The corresponding β-ketoester, **54**, could not be isolated from the complex product mixture obtained on treatment with standard conditions[8] (*Figure 2.15*).

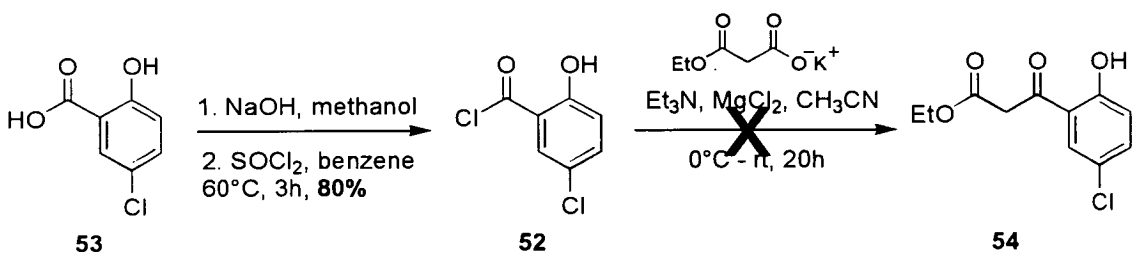


Figure 2.15: *Attempted Formation of β-Ketoester, 54*

The mixture of products observed from the above reaction is possibly due to the deprotonation of **52** by excess triethylamine. It is likely that this may be overcome by protecting the phenol of **53** prior to acid chloride formation. It may also be true that protection of the indole *NH*- of β-ketoester, **47**, may ultimately lead to easier elimination reactions, and so whilst there are many possible ways of exploring further the β-ketoester route towards **29**, it was clear that the general procedure was becoming more complex. More fundamentally, the need to individually synthesise a range of many β-ketoesters in order to explore the effect of alternative aromatic substituents on biological effect, would be inefficient and inappropriate for rapid, solid

phase, library generation methods. It was therefore decided to investigate the proposed HWE route for the generation of acrylic acids.

2.3 Model Studies (2): The Horner-Wadsworth-Emmons Route

As in the β-ketoester route, the synthesis of acrylic acid, **29**, *via* the HWE reaction was first attempted in the solution phase. Experience from results of investigations into the failed β-ketoester route led to optimised alkylation reactions, as well as a comprehensive study of phenolic protecting groups. Although it was recognised that efforts should be made to synthesise the lead compound, there was also a concern that this indole substrate was not representative of potential library members in terms of chemical stability. Therefore, a simpler benzyl derivative was also investigated. Furthermore, the outcome of the HWE reaction in terms of alkene geometry was unknown, and detailed solution phase analysis was expected to be required. The general retrosynthesis for the acrylic acids is shown in *Figure 2.16*.

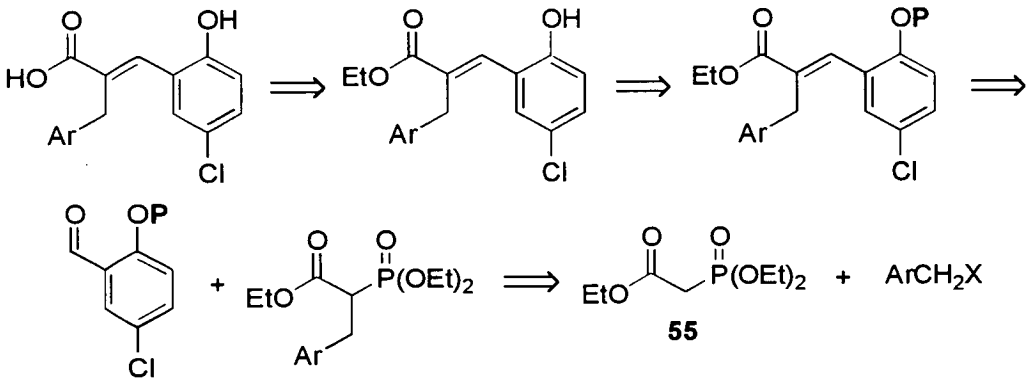


Figure 2.16: *General Retrosynthesis of Acrylic Acids via the HWE.*

The first key step, therefore, was to optimise conditions for the alkylation of triethyl phosphonoacetate, **55**.

2.3.1 Phosphonate Alkylation

Triethyl phosphonoacetate, **55**, was synthesised in a straightforward manner, using triethyl phosphite and ethyl bromoacetate by the Arbuzov reaction,[21] in 90% yield. Alkylation of the phosphonate ester was attempted using gramine methiodide under the same conditions as for the β-ketoester alkylation step (see *Figure 2.9*). Unfortunately, this led only to the recovery of starting material.

Conclusions from investigations into the elimination reaction indicated that protection of the indole *NH* may assist in controlling reactions of these substrates. Furthermore, Padwa[22] has reported the straightforward quaternisation of *NH*-protected gramine using a less time consuming and more atom-efficient procedure than the method of Geissman.[13] Therefore, 1-triisopropylsilylgramine, **56**, was synthesised according to a procedure described by Iwao,[23] and the protected species quaternised forming **57**. Alkylation of the phosphonate ester was then attempted using a range of bases (*Figure 2.17*). Pleasingly, the indole-protection and quaternisation reactions could be carried out almost quantitatively. Indeed, the complete transformation of gramine to **57** could be performed without isolation of the protected intermediate, in a yield in excess of 90%.

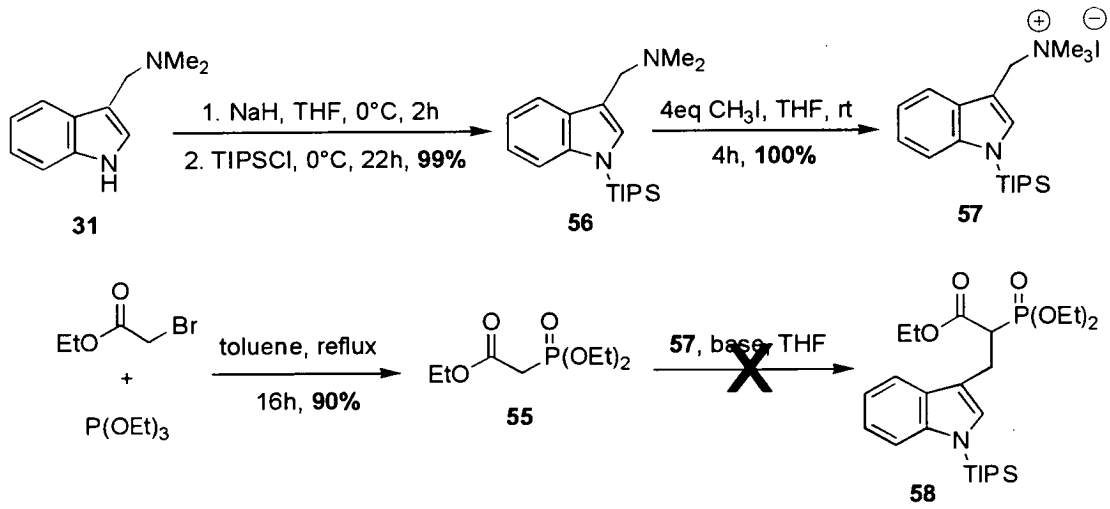


Figure 2.17: *Gramine Protection and Attempted Phosphonate Ester Alkylation.*

Alkylation of **55** using **57**, however, did not proceed and furnished either starting materials or a mixture of unidentified products. The range of conditions tried included

a series of solid phase compatible bases, as well as others which would have been expected to work well in solution phase (*Table 2.1*).

	Reaction Conditions	Observed Result
1	3eq Et_3N, THF, rt, 16h, then 40°C, 24h	recovered starting material
2	1eq TMG, CH_3CN, rt, 19h	mixture
3	2eq ^tBuOK, THF, rt, 15h	unidentified product
4	5eq NaH, THF/CH_3CN	unidentified product
5	1.6eq. NaH, TBAI (cat.) THF/CH_3CN, rt, 26h	mixture
6	1.5eq Na, ethanol, rt, 90mins	mixture

Table 2.1: *Conditions Used for Attempted Alkylation of Attempted Alkylation of Phosphonate Ester.*

Using the same conditions as for the β-ketoester alkylation (entry 1), only starting materials were recovered. Some conditions gave complicated mixtures of products (entries 2, 5, 6), whilst others (entries 3 and 4) gave a product, which although exhibiting some features of the desired product, ^1H nmr indicated that it contained too many aromatic protons, and could not be purified sufficiently for satisfactory characterisation.

To investigate if it was the TIPS group which was affecting reactivity, the alkylation of β-ketoester, **43**, was attempted using the same conditions used with the unprotected gramine salt (see *Figure 2.9*). Therefore, **43** was stirred in dry THF with TIPS-gramine methiodide and triethylamine. Even after the addition of large excesses of base and prolonged reaction times, the reaction did not go to completion. In fact, the compounds isolated were unreacted starting material (41%) and the *NH*-deprotected β-ketoester, **47** (44%) (*Figure 2.18).*

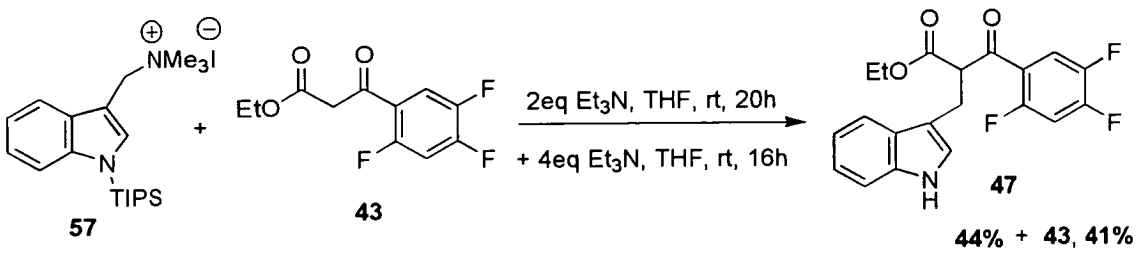
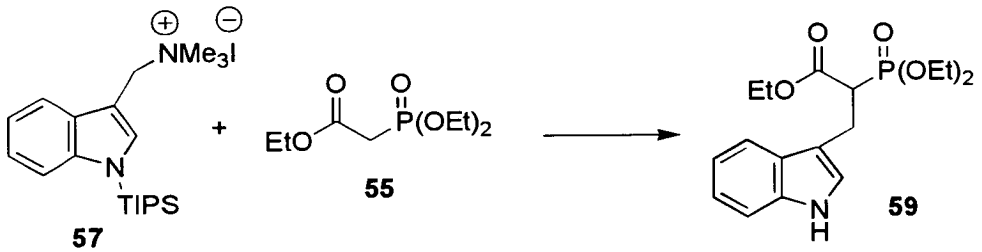


Figure 2.18: *β-Ketoester Alkylation from TIPS-Gramine Methiodide.*

Clearly, the base must be causing TIPS-deprotection before alkylation can proceed, and so the mechanism of alkylation is unlikely to be straightforward nucleophilic substitution of methiodide by the enolate of **43**. Therefore, some method of *in situ* TIPS removal, followed by nucleophilic attack, would be required in order to achieve alkylation of the phosphonate ester, **55**. As silyl groups are commonly removed by treatment with the fluoride anion, it was expected that pretreatment of **57** with tetrabutylammonium fluoride (TBAF) followed by reaction with **55** in base would successfully lead to alkylated phosphonate, **59** (*Figure 2.19*).



	Reaction Conditions	Product Yield
1	**(1)** 55, 1.3eq TBAF, THF, 1h **(2)** 55, Et_3N, rt, 5h	0%
2	**(1)** 55/57, 1eq Et_3N, THF, rt, 10mins **(2)** 1eq TBAF, rt, 1h	49%
3	55/57, 1eq TBAF, THF, rt, 1h	79% (crude)
4	55/57, 1eq TBAF, THF, 0°C, 30mins - rt, 60mins	67% (crude)
5	55/57, 2.2eq TBAF, THF, rt, 1h	92%

Figure 2.19: *Alkylation of Phosphonate Ester with in situ TIPS Deprotection.*

It was apparent from this series of experiments, that treatment of the phosphonate ester, **55**, with triethylamine (entries 1 and 2) prevented a clean reaction from occurring. Indeed, somewhat surprisingly, treatment of the reactants with TBAF alone was found to be the most effective method of alkylation. However, when only 1 equivalent of reagent was employed, impurities were formed which could not be separated from the desired product (entries 3 and 4), and by far the cleanest and most efficient method was to use over 2 equivalents (entry 5) of TBAF. These conditions led to smooth alkylation forming **59** in excellent 92% yield. Importantly, this procedure should be applicable to methods of solid phase synthesis.

The mechanism of alkylation is almost certainly initiated by fluoride cleavage of the TIPS group of **57**, and concomitant elimination of trimethylamine forming the highly reactive intermediate, **60**. Deprotonation of **55** by the amine or by the excess TBAF, followed by addition to **60** forms the indole-phosphonate, **59** (*Figure 2.20*). The intermediate, **60**, has been postulated by Melhado[15] for being involved in the formation of *bis*-indole methiodide salts in the formation of gramine methiodide (see *Figure 2.8*), and is almost certainly the reactive intermediate in the β-ketoester alkylation (see *Figure 2.9*).

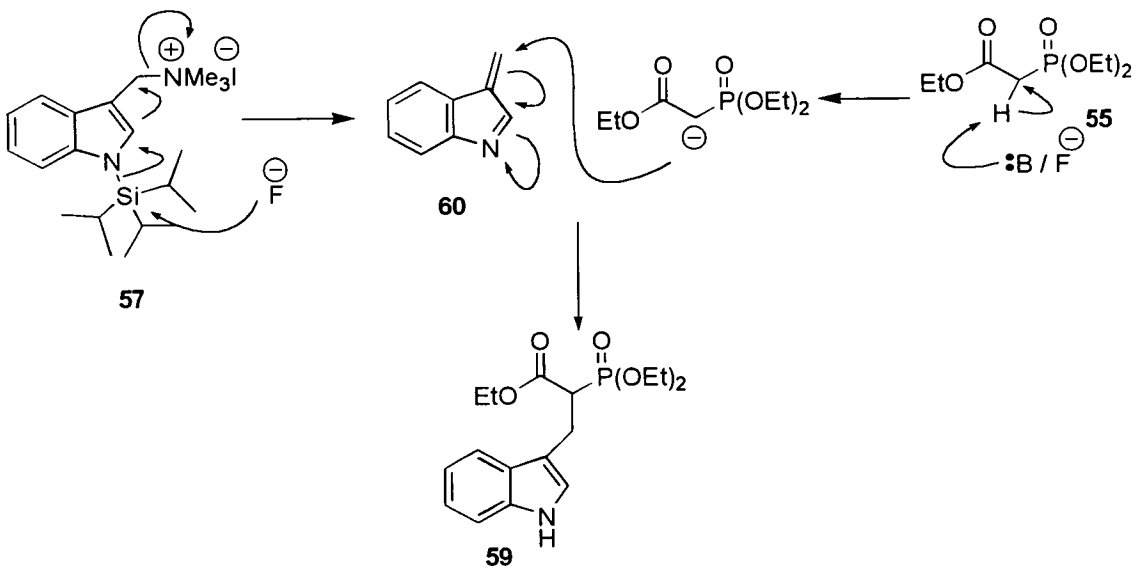


Figure 2.20: *Mechanism for Synthesis of Indole-phosphonate, 59.*

Clearly, the above method of alkylating phosphonates is only useful for the gramine-based substrate, and is not generally applicable to substrates of the type $ArCH_2X$. Therefore, a suitable, general method of forming benzyl-phosphonate, **61** was sought. Literature precedent for the synthesis and subsequent use of **61** in the HWE reaction has been reported by Martinez[24] and Roques.[25] Both groups report the highly efficient alkylation of phosphonate ester, **55**, with benzyl bromide, using sodium hydride. As the insoluble nature of sodium hydride renders it unsuitable for solid phase synthesis, and amine bases have already been shown not to give alkylation, the base, sodium hexamethyldisilazide (NaHMDS), was used (*Figure 2.21*).

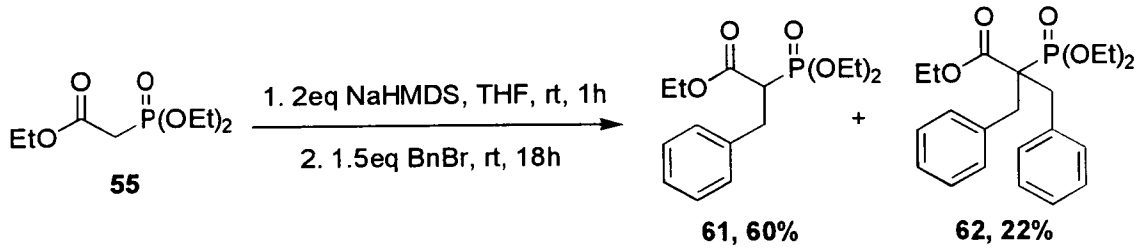


Figure 2.21: *Synthesis of Benzyl-phosphonate, **61** with bis-Benzyl By-product, **62**.*

NaHMDS is a strong, non-nucleophilic base which is soluble in THF. Alkylation of **55** was attempted using this base and benzyl bromide, and the reaction was surprisingly found to give a mixture of compounds in a consistent ratio. These were identified as the desired benzyl phosphonate, **61** and the *bis*-benzyl product, **62**. They could not be separated by column chromatography and so were isolated by preparative HPLC (*Figure 2.22*).

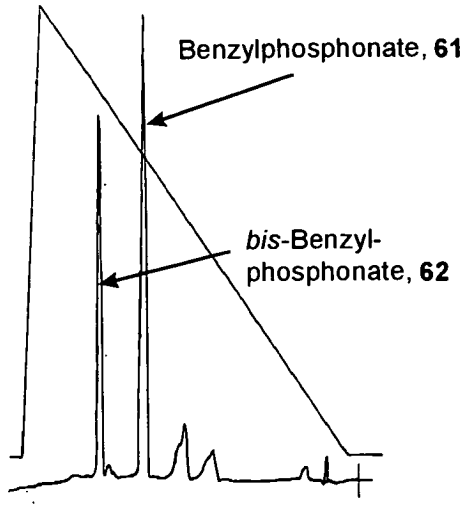


Figure 2.22: *Analytical HPLC Trace of the Crude Mixture of* ***61*** *and* ***62****.*

The products were formed in a consistent ratio of approximately 2.7:1 as determined by ^{1}H nmr of the crude mixture, and this product ratio was found to be independent of the concentration of base or benzyl bromide. This would suggest that the second deprotonation was not a result of excess base, but, rather due to self-deprotonation by the enolate, **63** (*Figure 2.23*). The newly formed enolate, **64**, would then over-alkylate, forming **62**.

Figure 2.23: *Self-deprotonation of Benzyl-phosphonate,* ***61****.*

At this stage, it was unclear whether the corresponding alkylation reaction would be more or less likely to produce the *bis*-benzyl product when performed on the solid phase. It may be expected that the excess reactions used to drive solid phase reactions to completion would encourage a greater proportion of *bis*-benzyl product, However, if the self-deprotonation theory is correct, then this is less likely to occur on resin due to the site isolation effect (see *Section 1.2.6*) observed with functionalised polymers.

Although the presence of the *bis*-benzyl phosphonate is undesirable, it will play no further part in the synthesis, as the next step, a HWE reaction, requires deprotonation of the active methine in order to proceed. It was expected that unreacted **62** could be easily separated from alkene products by chromatography.

2.3.2 Phenol Protecting Groups

Previous discussion of the elimination route to acrylic acids, illustrated problems with the synthesis of the key β-ketoester intermediate, **54**. This is thought to be due to intramolecular hydrogen bonding of the salicylic acid, **53**, affecting its reactivity and so impeding attack from nucleophiles. Furthermore, the nucleophilicity of the phenol itself may cause side reactions. These effects will also be observed in the salicylaldehyde, **33**, required for the HWE reaction. The expectation that phenol protection would be required to allow the HWE reaction to proceed smoothly was confirmed by the reaction of indole-phosphonate, **59**, with **33** (*Figure 2.24*). The only identifiable product from the reaction was approximately 50% recovered phosphonate, despite complete consumption of aldehyde.

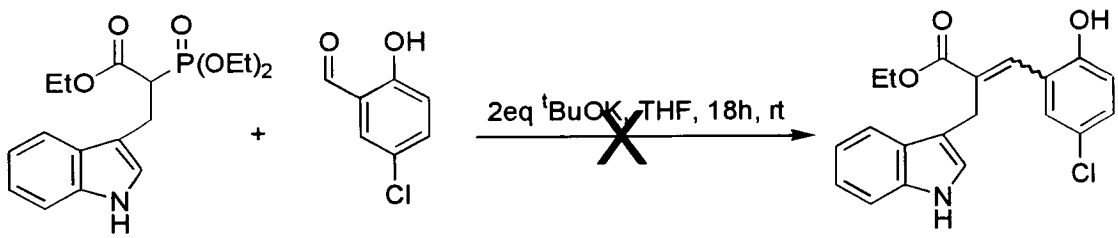


Figure 2.24: *Attempted HWE Synthesis of Hydroxy-olefin, 65.*

For a protecting group strategy to be successful, several factors must be considered. The general requirements of straightforward protecting group incorporation and removal, and stability to reaction conditions, are important, as with all syntheses using protecting groups. However, of most importance was the cleavage strategy which would enable the most efficient overall synthesis. Since the last step in solid phase synthesis is the final product cleavage from the resin, the most efficient

protecting group strategy would employ a group which cleaved under identical conditions. Therefore, since Wang resin - cleaved with TFA - was the initial resin of choice for the synthesis of acrylic acids (see *Figure 2.6*), TFA labile protecting groups would be the most effective. Examples of protecting groups which fall into this category are *para*-methoxybenzyl (PMB),[26] methoxyethoxymethyl (MEM)[27] and methoxymethyl (MOM).[28] Attempts were made to protect **33** with each of these groups (*Figure 2.25*).

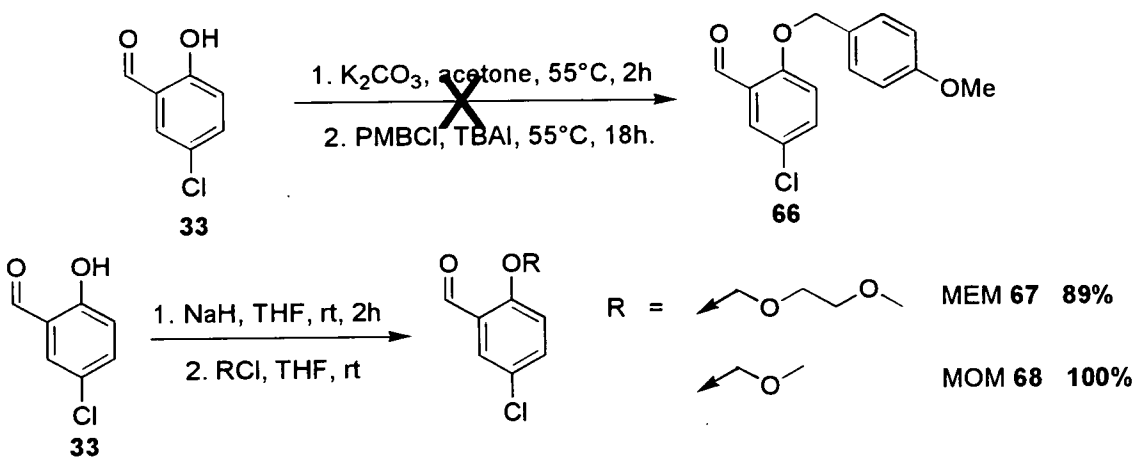


Figure 2.25: *Synthesis of TFA-Cleavable Phenol Protecting Groups.*

Despite several attempts, the PMB-phenol ether, **33**, could not be formed. Generally a complicated mixture of products was obtained, and on only one occasion was the desired ether observed by ^{1}H nmr, in very low yield (2%). More success was achieved in forming the MEM and MOM ethers, **68** and **69** respectively. These were both formed in very good yields, of 89% and 100% respectively.

Other protecting groups were also synthesised, in case alternatives to the TFA-cleavable groups were required. The *t*-butyldimethylsilyl (TBS), **70**, and acetate, **71**, derivatives were chosen for their mild cleavage protocols by TBAF and saponification respectively. This would enable straightforward cleavage, either prior to or subsequently after resin cleavage. The methyl ether of **33** was synthesised and considered a viable option as its sterically undemanding nature may enhance the efficiency of the HWE reaction. Aryl-methyl ethers can be cleaved under a variety of

conditions, including trimethylsilyl iodide[29] or boron tribromide.[30] The synthesis of these alternative protected phenols is shown in *Figure 2.26*.

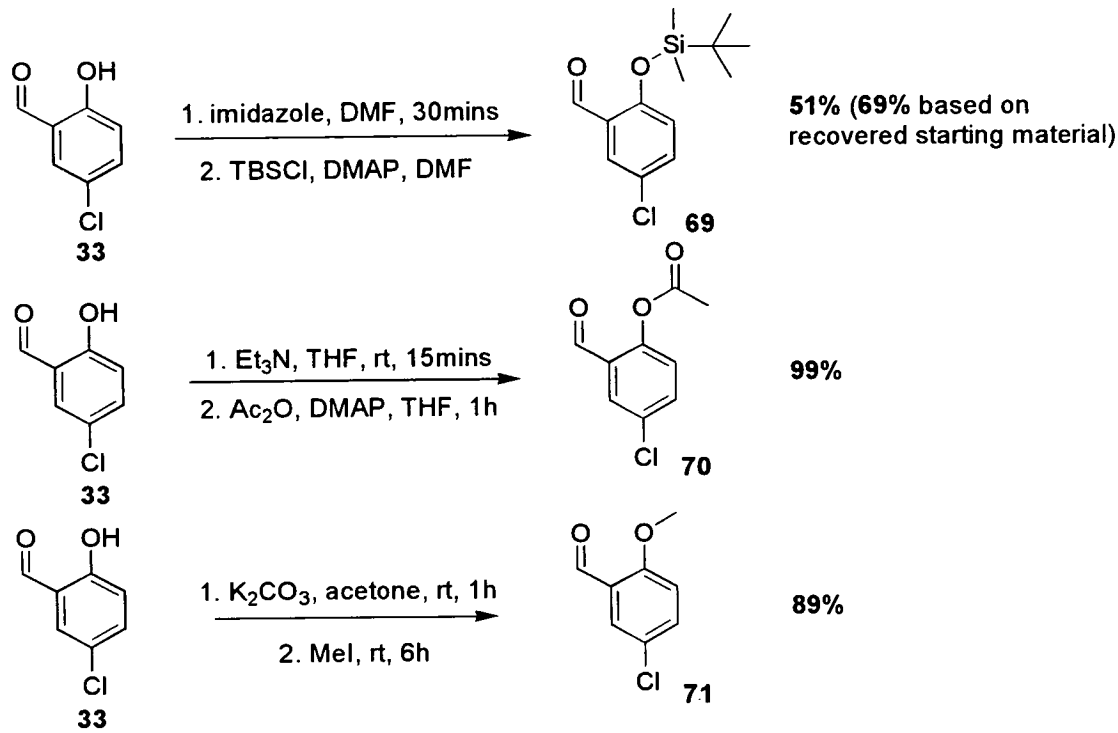


Figure 2.26: *The Synthesis of Alternative Phenol Protecting Groups.*

The cleavage conditions required for each protecting group were not tested on the above substrates, but rather, their suitability for the HWE reaction was investigated by reaction with the indole-phosphonate, **59**, or benzyl-phosphonate, **61**.

2.3.3 Horner-Wadsworth-Emmons Reactions

Generally, strong bases such as *n*-butyllithium, sodium hydride and potassium *t*-butoxide have been employed in the HWE reaction.[7(b)] Of these, *n*-butyllithium and sodium hydride were expected to be unsuitable for use in solid phase synthesis, and so initial experiments were carried out using potassium *t*-butoxide as base. (Milder bases such as DBU, triethylamine or diisopropylamine have been reported for use in the HWE reaction in combination with lithium or magnesium salts,[31] but these too were reasoned to be unsuitable for resin techniques.) The base used in the successful

benzylation of the phosphonate ester, NaHMDS, was also investigated for use in the HWE reaction.

The indole phosphonate, **59**, was used in the HWE reaction with each of the five protected aldehydes **67-71** (*Figure 2.27*). The effect of protecting the indole *NH* using the TIPS group was also investigated. Although this requires additional synthetic steps, it was hoped that the HWE reaction would proceed more smoothly and that the protected indole would be more stable to future phenol protecting group cleavage conditions.

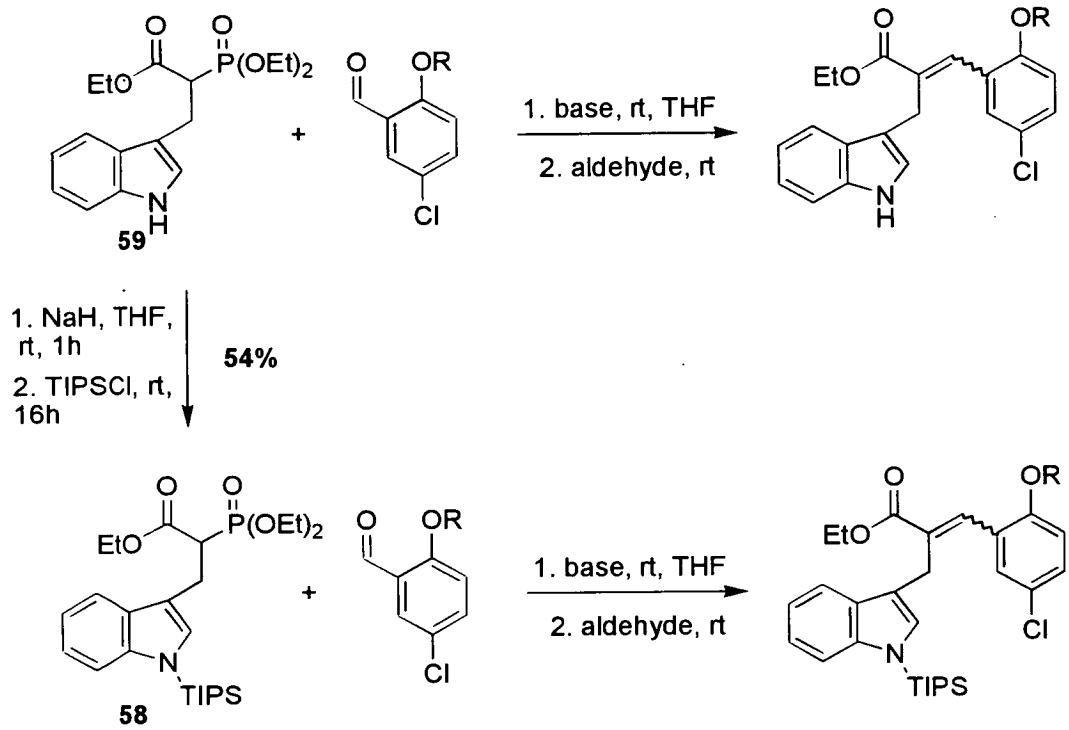


Figure 2.27: *HWE Reactions Performed on Protected Salicylaldehydes.*

The results show that the MEM and methyl ether protecting groups were the most suitable for this sequence of HWE reactions (*Table 2.2*). The MEM-olefin, **72**, was synthesised efficiently using both KO^{t}Bu and NaHMDS as base (entries 1 and 3), and this was a more efficient reaction than that with the MOM-salicylaldehyde (entry 2). Reactions involving the methoxy-salicylaldehyde, **71**, were only successful when the stoichiometric ratio of aldehyde to excess base was greater than 1 (entries 7-9), as otherwise the aldehyde was being consumed by the base (possibly through directed

metalation). Neither the TBS nor acetate protected aldehdyes reacted to give olefins (entries 4 and 6). Both groups are susceptible to migration *via* the adjacent carbonyl group.[32] Indeed the product from entry 4 was a TBS protected indole derivative. Disappointingly, only starting materials were recovered when this substrate was used with the TIPS protected indole, **58** (entry 5). This double protection strategy did, however, help to enhance reaction with **58**, reducing the reaction time to only 2 hours (entry 9).

Entry	Phosphonate	Conditions and Product Yield	Isomeric Ratio
1	**59**	2eq KO^{t}Bu, 1eq **67**, 20h, **77%** (**72**)	2.2:1
2	**59**	2eq KO^{t}Bu, 1eq **68**, 20h, **48%** (**73**)	2.2:1
3	**59**	1eq NaHMDS, 1.2eq **67**, 20h, **88%** (**72**)	2.2:1
4	**59**	2.2eq KO^{t}Bu, 1.1eq **69**, 20mins, **0%**	-
5	**73**	1.2eq KO^{t}Bu, 1eq **69**, 36h, **0%**	-
6	**59**	2.1eq KO^{t}Bu, 1eq **70**, 20h, **0%**	-
7	**59**	2.1eq KO^{t}Bu, 1.1eq **71**, 20h, **0%**	-
8	**59**	1.2eq NaHMDS, 1.5eq **71**, 16h, **63%** (**74**)	1.8:1
9	**73**	1.1eq KO^{t}Bu, 1.1eq **71**, 2h, **96%** (**75**)	7:2

Table 2.2: *HWE Reactions Between Indole-phosphonates and Protected Salicylaldehydes.*

All of the above olefins were obtained as a mixture of *E*- and *Z*- isomers. Although the absolute geometry could not be determined from the data available, it was clear that the major isomer was consistent in all cases from ^{1}H nmr spectra. The chemical shift of the alkene proton of the major alkene product in each mixture was observed at δ~7.8ppm as a singlet. Ratios were calculated from the integrals of the ethyl group alkoxy protons of the two isomers. The successfully synthesised alkenes are shown in *Figure 2.28.*

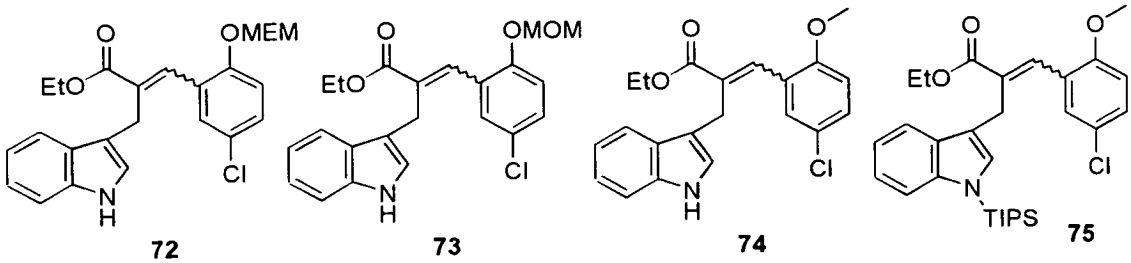


Figure 2.28: *Indole-olefins Synthesised by the HWE Reaction.*

The HWE reaction was also attempted using the benzyl phosphonate, **61**, with both the MEM- and methoxy- protected aldehydes (*Figure 2.29*), as these had previously been shown to be the most successfully employed substrates.

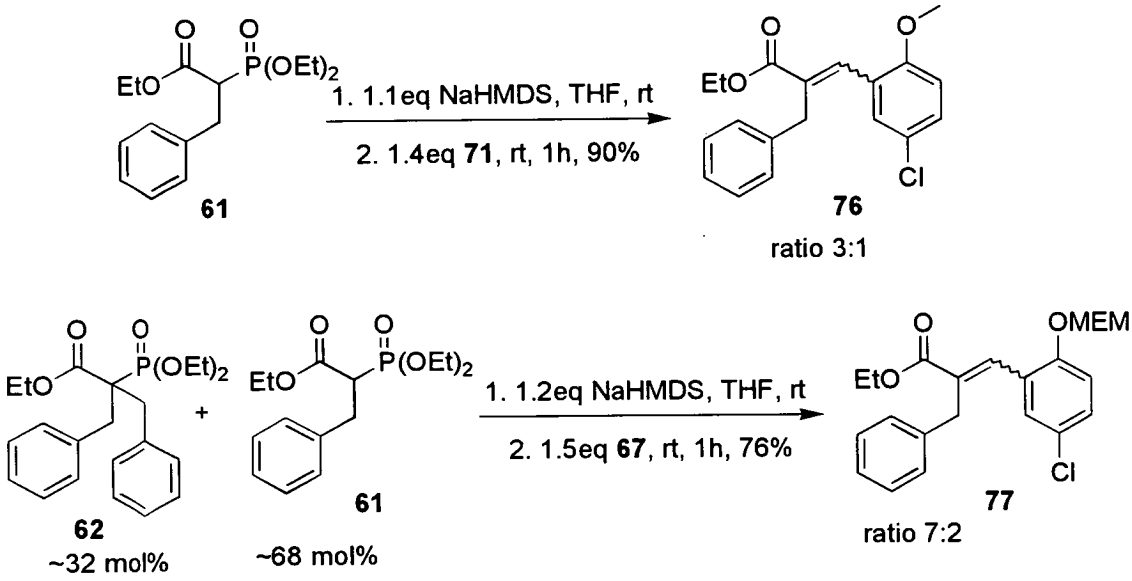


Figure 2.29: *HWE Reactions With Benzyl Phosphonate and Protected Salicylaldehydes.*

The yields of alkenes **76** and **77** were highly satisfactory. As with the indole substrates, the alkenes were formed as a mixture of *E*- and *Z*- isomers, but with slightly greater selectivity. Also, as predicted, the mixture of mono and *bis*-alkylated phosphonates, **61** and **62** reacted cleanly as expected with MEM aldehyde, **67**, furnishing the corresponding alkene mixture in an efficient 76% yield, based on starting material composition.

2.3.4 Protecting Group Cleavage

With reliable synthetic routes to several alkenes in hand, methods of efficient protecting group cleavage were examined. Synthetically, the most efficient route would be the TFA cleavage of the MEM group from alkenes **72** and 77 (*Figure 2.30*), as concomitant cleavage of substrates from Wang resin could also be achieved under these conditions. The MOM-ether was disregarded as a potential protecting group due to the much less efficient synthesis than the MEM analogue.

The indole substrate, **72**, proved to be unsuitable for protecting group cleavage using TFA, and only a complicated mixture of products from a dark-coloured residue was obtained. Although the presence of a yellow fluorescence indicated that ionised phenol may be present in the reaction mixture, no individual products could be identified. Even reducing the concentration of TFA to 1% led only to a complicated mixture (over 18 hours), and recovered starting material. Other methods of MEM-cleavage reported by Corey,[33] including treatment with zinc bromide or titanium tetrachloride, led only to the formation of mixtures of unidentified products.

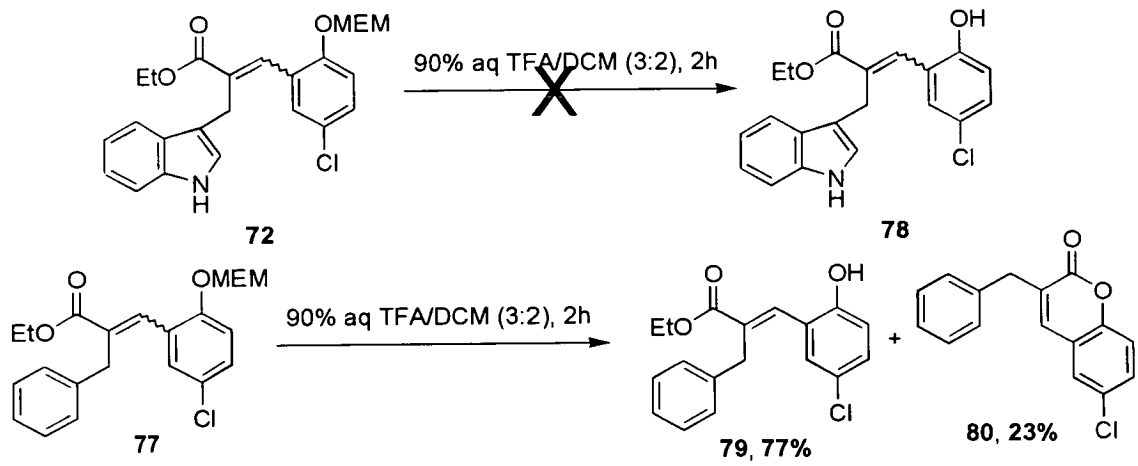


Figure 2.30: *Attempted TFA-cleavage MEM-protected Alkenes.*

The likelihood that efficient cleavage of the MEM group of **72** was being hindered by the indole moiety was supported by the reaction of 77 under the same conditions. Complete consumption of starting material furnished olefin **79** and coumarin **80**. This was achieved quantitatively, *with the ratio of products exactly corresponding to that*

of the starting olefin mixture. It would reasonable to expect that the *Z*-olefin would be more likely to form the coumarin, and so the major isomer of the products obtained from the HWE reactions was likely to be the *E*-isomer, as expected from activated phosphonates of this type.[34]

Assignment of the alkene geometry was confirmed from results of experiments into conditions promoting the cleavage of the methyl ethers, **75** and **76**. Use of sufficient quantities of BBr_3[30] led to the products shown in (*Figure 2.31*).

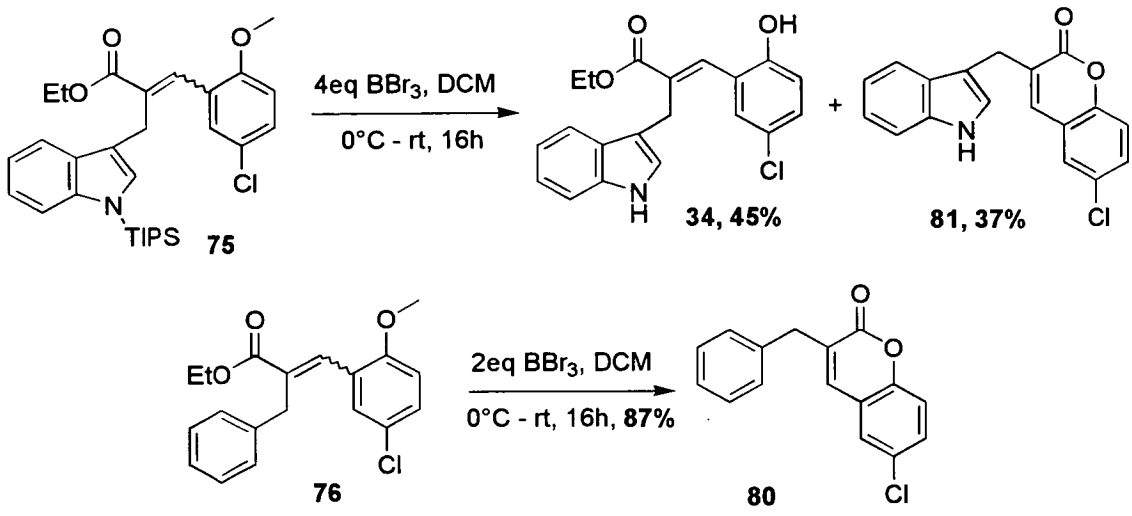
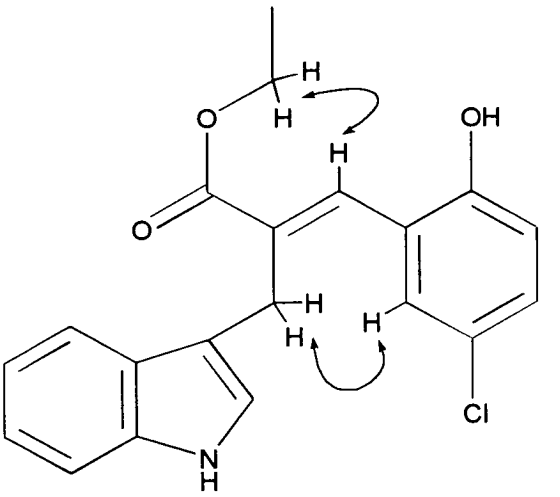


Figure 2.31: *Methyl Ether Cleavage Using BBr_3.*

Using trivalent boron, there should only be a requirement to use one-third of a molar equivalent to permit cleavage to occur. However, subjecting **75** to this quantity of BBr_3 merely served to cleave the TIPS group, with the vast majority of recovered material being the fully protected starting material. Indeed, only when 4 molar equivalents of reagent were used, was there seen to be full consumption of starting material, forming phenol, **34**, as a single geometric isomer, and coumarin **81**. However, this reaction was not reproducible, and often gave the coumarin as the only identifiable product. Unfortunately, sufficient material was not obtained to pursue this route towards **29**.

The geometry of the alkene double bond of **34** was the same as the major isomer of **75** by ^1H nmr, and was assigned *E*- as for **79**. This was proved by accumulating more detailed nmr data, including through-space coupling by Nuclear Overhauser Effect

(NOE) experiments (*Figure 2.32*). This confirms the rationale described for the product ratio obtained upon MEM- cleavage of benzyl acrylate, **77**. Interestingly, the elimination product, **45**, obtained under Mitsunobu conditions (see *Figure 2.14*), also possessed the olefinic proton at δ~7.8ppm, confirming the assignment of *E*-geometry.



34

Figure 2.32: *Methylindolylacrylate 34, with H-Atoms Exhibiting Strong Through-space Coupling.*

The relatively large quantity of the coumarins, **80** and **81**, formed during methoxy-cleavage, served to suggest that BBr_3 catalyses alkene isomerisation. Additionally, it is likely, given that no other products were observed by thin layer chromatography (TLC) over the time-scale of the reaction, that the isomerisation process is faster than methoxy cleavage.

Although formation of coumarins, **80** and **81**, was unexpected, it was clear that base-hydrolysis of these would furnish the corresponding acrylic acids. Survey of the literature indicated that *Z*-cinnamate salts would spontaneously cyclise on acidification,[35] but that under certain conditions, isomerisation of the double bond would lead to the isolation of *E*-cinnamates (*Figure 2.33*). Methods reported have included ethanolic sodium hydroxide solution in strong light,[35] aqueous sodium hydroxide with mercury (II) oxide[36] or stirring with sodium ethoxide at 70°C.[37]

However, in the case of benzyl coumarin, **80**, hydrolysis was unsuccessful and only starting material was recovered.

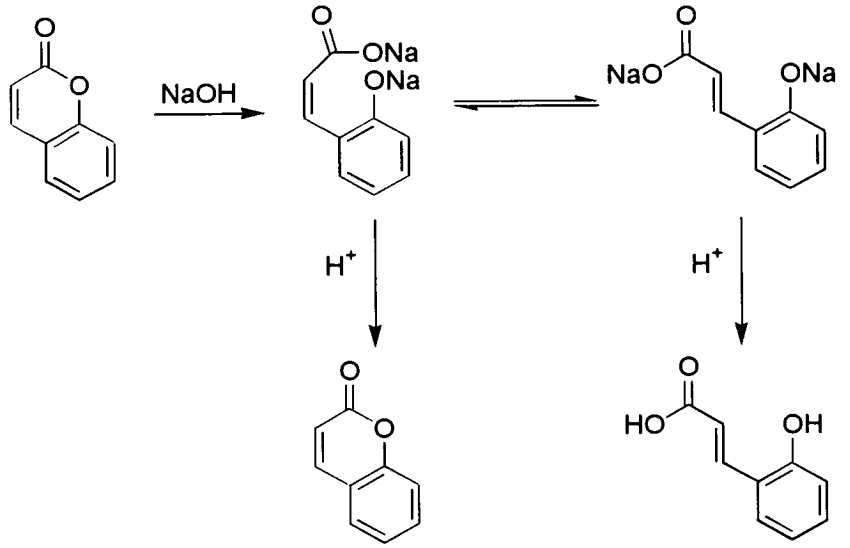
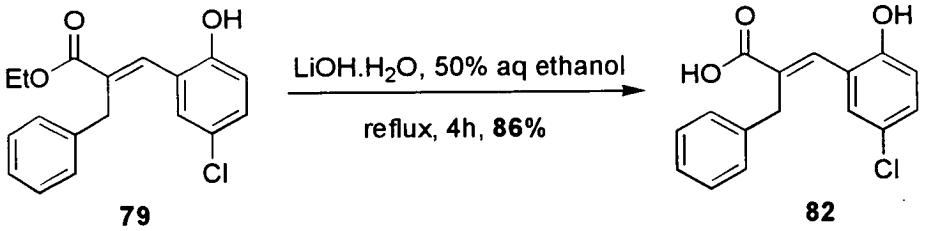


Figure 2.33: *Coumarin Hydrolysis and Isomerisation.*

With a reliable and efficient method of cleaving the MEM- group from the benzyl acrylate, 77, in hand, straightforward ester hydrolysis was performed in order to synthesise the target acrylic acid, **82**. This step would not be required when cleaving products from resin, but served to gain full characterisation of **82** for comparison purposes.



79 **82**

Figure 2:34: *Ester Hydrolysis of Benzyl-acrylate, 79.*

The efficient solution phase synthesis of acrylic acid, **82**, has been achieved under conditions compatible with solid phase synthesis. As with all synthetic routes for potential compound library generation, the conditions chosen were expected to be compatible with a wide range of substrates and, additionally, the phenol deprotection

required no extra synthetic steps, thus having no detraction from the generality of the route. Attempts were therefore ready to be made at the solid phase synthesis of **82**.

Although, disappointingly, the indole acrylic acid, **29**, could not be synthesised in solution phase using the methods described, it was envisaged that the indole building block would be suitable for library members where no deprotection steps are required. Indoles have successfully been cleaved from Wang resin using 50% TFA.[38]

2.3.5 Completed Solution Phase Synthesis of Indole Acrylic Acid.

Although a general route had been devised towards acrylic acids which would be amenable to solid phase techniques, there remained the goal of synthesising the original lead compound, **29**. Previous protecting group strategies had been incompatible due to the nature of the conditions required for cleavage and clearly, phenol protecting groups which could be cleaved under milder conditions were more appropriate.

The tetrahydopyranyl (THP) group is a hydroxyl protecting group which is readily cleaved in relatively mild conditions using oxalic acid.[39] Although considered as one of a range of possible phenol protecting groups, it was initially disregarded due to the lengthy, low-yielding reported synthesis of protected salicylaldehyde systems.[40] Furthermore, the direct protection of 5-chlorosalicylaldehyde yielded only starting materials. However, recently, the synthesis of THP-aldehyde, **84** has been reported by *ortho*-formylation of the phenol protected precursor.[41]

HWE reaction between **84** and **59** efficiently led to the mixture of alkenes, **85**. This reaction was the most selective of all the HWE reactions carried out, and yielded the isomeric alkenes in the geometric ration of 5:1 (*E:Z*). THP-deprotection, followed by ester hydrolysis, led to the completed synthesis of **29** (*Figure 2.35*). This product was submitted for biological screening for comparison with the originally synthesised lead compound, and was found to be active at similar micromolar concentrations.

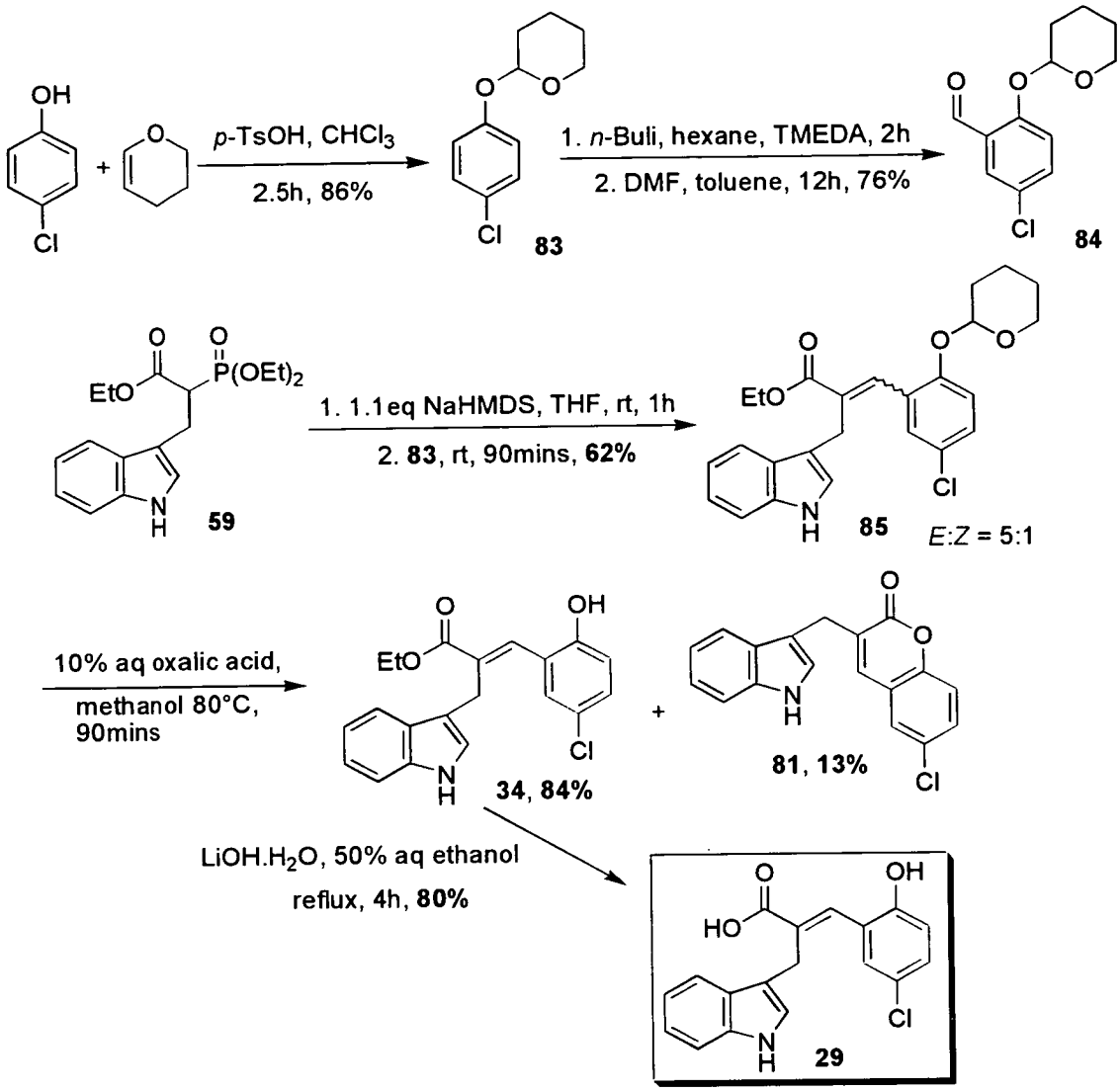


Figure 2.35: *Completed Synthesis of Methylindolylacrylic Acid, 29.*

2.4 Model Studies (3): Solid Phase Synthesis

Before initiating the synthesis of a library of acrylic acids, the solution phase protocol for the synthesis of the benzyl derivative, **82**, was transferred to the solid phase to confirm the suitability of the optimised conditions. Of key interest and concern was that the alkylation step would yield the desired mono-phosphonate and not the *bis*-alkyl phosphonate as the major product.

The support chosen to allow concomitant protecting group and resin cleavage, was Wang resin. Two possible methods for loading triethylphosphonoacetate onto Wang

resin were explored (*Figure 2.36*). The direct method of transesterification[42] is reported to occur solely at the carboxylic functionality and should efficiently form resin-bound phosphonate, **41**. However, this method presents no obvious way of determining the loading of the phosphonate on the resin; a synthetic method of performing the Arbuzov reaction on resin-bound bromoacetate, **86**, could overcome this. The quantity of bromine could then be determined by combustion analysis, and hence the degree of loading could be calculated. (A recent publication by Nouvet[43] *et al* has described the efficient formation of bromoacetylated Wang resin, **86**, under Mitsunobu conditions, claiming greater efficiency than the DIC/DMAP method described below.)

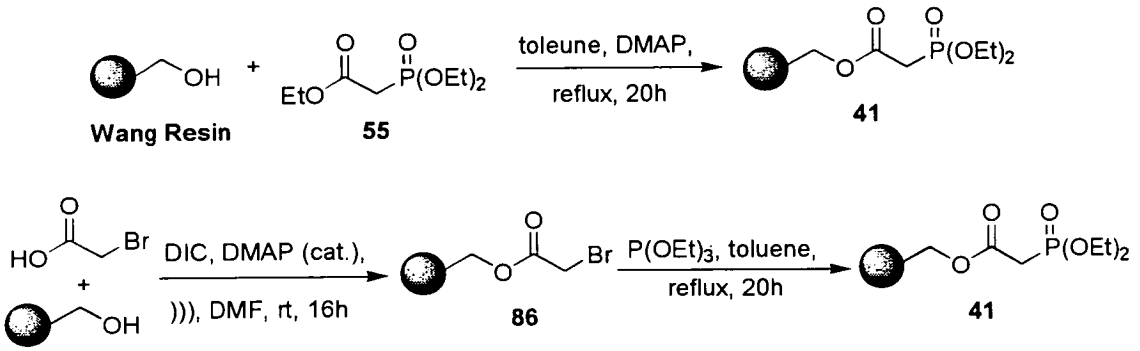


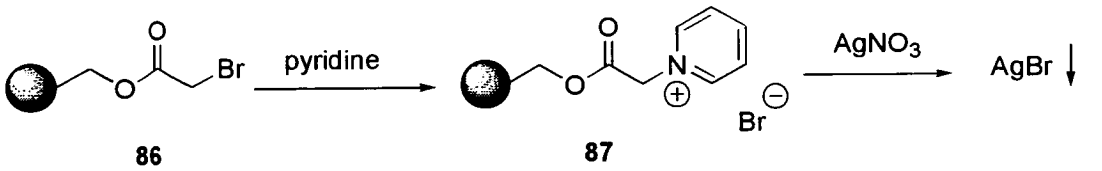
Figure 2.36: *Two Alternative Methods of Synthesising Wang-phosphonate, 41.*

Combustion analysis of **86** indicated a composition of 5.33% (±0.6%) bromine, which corresponds to a loading level of 0.67mmol/g (±0.07mmol/g). Although this degree of loading is sufficient in order to obtain significant quantity of products upon cleavage, the relatively large error in these results prompted alternative methods of loading determination to be sought.

A photometric method of loading determination involved reaction of the free sites of the resin with *p*-nitrophenolchloroformate, and then cleaving to form *p*-nitrophenol. A known quantity of this was then measured photometrically against a known standard. However, this method was found to give poor reproducibility, and so was deemed to be unsuitable.

A general method for the quantitative determination of bromine is the Volhard titration.[44] Quaternisation of pyridine with **86** forms the pyridinium salt species, **87**,

which itself forms a precipitate of silver bromide upon addition of silver nitrate (*Figure 2.37*). Excess silver nitrate is determined by titration with ammonium thiocyanate using ferric alum as indicator and the quantity consumed is a measure of the concentration of salt present and hence the loading of **86**.



86 **87**

Figure 2.37: *Sample Preparation for the Volhard Titration.*

Trial titrations on the salt formed from ethylbromoacetate and pyridine illustrated that the technique was accurate and reproducible. However, titration with the resin bound pyridinium salt, **87**, failed to give any result, indicating that either the pyridinium salt did not form on the resin, or, more likely, that the silver nitrate could not react with the resin-bound substrate. The loading of bromoacetate onto the resin was therefore accepted as 0.67mmol/g as calculated by combustion analysis.

The solid phase synthesis of the benzylacrylic acid, **82**, was completed as shown below in *Figure 2.38*. Analysis of each of the intermediates was performed directly on resin by IR and gel phase ^{13}C and ^{31}P nmr, which despite giving poorly resolved spectra, gave sufficient qualitative data for identification. The most important tool for qualitative analysis was the phosphorus nmr, as this was used particularly to monitor the success of the alkylation and HWE reactions.

The crucial alkylation reaction to form resin-bound benzylphosphonate, **88**, was approached very carefully in order to determine whether or not the *bis*-alkylated product was formed. Although solid phase synthesis usually operates most efficiently when excess reagents are used to drive reactions to completion, the alkylation reaction proceeded best when only 1.1 molar equivalent of base was used with 2.5 equivalents of benzyl bromide. This furnished a single product by ^{31}P nmr, which was ultimately used to furnish **82**. When 2 and 4 molar equivalents of base and benzyl bromide respectively were used, a different single product was formed which did not yield **82** upon resin cleavage. The ^{13}C nmr spectrum of the product from the HWE,

89, reaction indicated from characteristic resonances of the MEM group, as well as an absence of the phosphonate methyl carbon, that the desired alkene had formed. However, the ^{31}P nmr showed that some phosphonate remained, and so the reaction had not gone to completion.

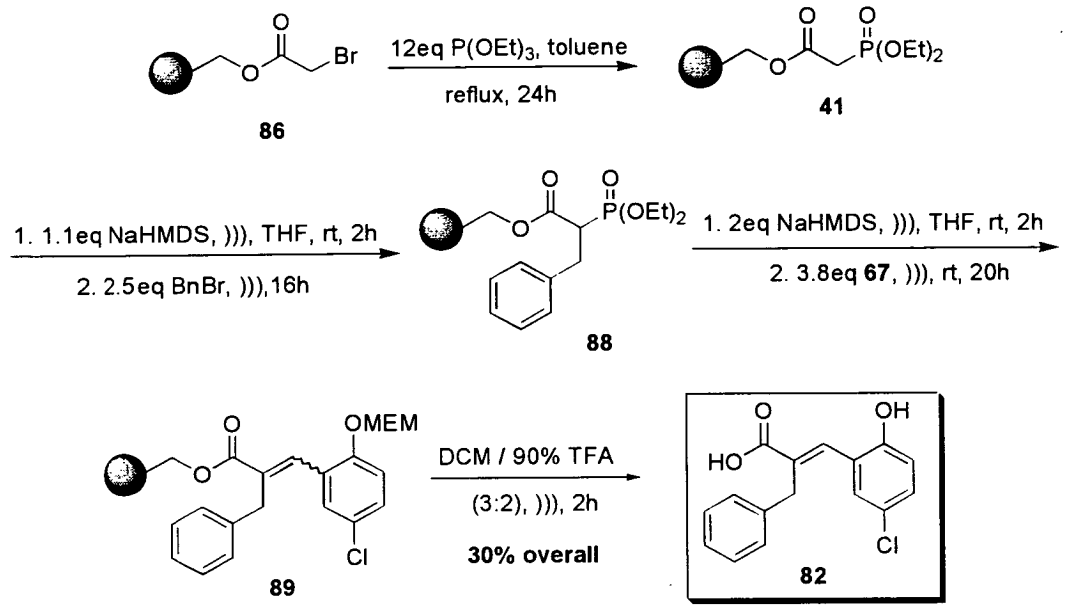
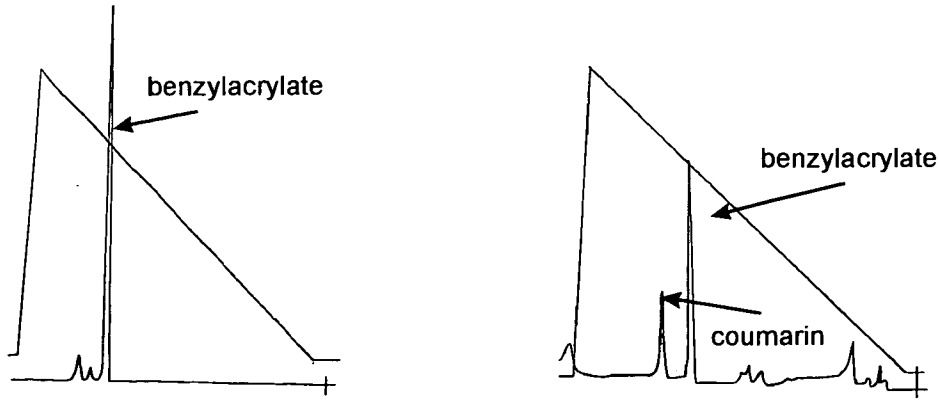


Figure 2.38: *Solid Phase Synthesis of Benzylacrylic Acid, 82.*

Cleavage of **89** from the resin was carried out using 90% aqueous TFA in DCM. HPLC of the cleavage mixture indicated that two main products were present (*Figure 2.39*). The retention time of these two compounds identified them as the desired benzyl acrylic acid, **82**, and the corresponding coumarin, **80**. Purification by preparative HPLC, and analysis by mass spectrometry, confirmed that the major product was indeed **82**. As expected, the conditions used to cleave the products from the resin had also to cleave the MEM-protecting group. Therefore, **82** could be accessed on the solid phase using only four chemical steps, including cleavage.



Pure **82** after Solution Phase Synthesis

Crude **82** after Solid Phase Synthesis

Figure 2.39: *HPLC Traces of Benzylacrylate, 82.*

Following the successful solid phase synthesis of benzylacrylic acid, **82**, the protocol was used to in the parallel, solid phase synthesis of a library of acrylic acids.

2.5 Multiple Parallel Synthesis of Acrylic Acids

The parallel synthesis of acrylic acids was attempted using the 4×2 array Diversomer apparatus, illustrated in *Figure 1.32*. This equipment allows the user to handle up to 100mg of resin per sample in one block, under inert atmospheric conditions if required. Unlike larger models, liquid handling is not automated using this equipment, and so the manual addition of reagents, as well as resin washing between steps, was required.

It was envisaged that 40 compounds in all would be synthesised, as five sublibraries of eight compounds each. The targets were chosen from a pool of 10 aromatic alkyl halides, including the gramine derivative, **48**, and 10 benzaldehydes, including the salicylaldehyde derivative, **33** (*Figure 2.40*). These were arbitrarily chosen to cover a range of substitution patterns, and possible effects on polarity and electronic parameters.

Included in the 40 compounds chosen for synthesis, were all the possible combinations of **91** where Ar was fixed as methylindolyl (from **48**), and Ar' fixed as *p*-chlorophenol (from **33**). This was to enable easy comparison of biological data, and

if possible, generate SAR data. The remainder of the 40 analogues were then to be chosen at random from the other 18 substrates.

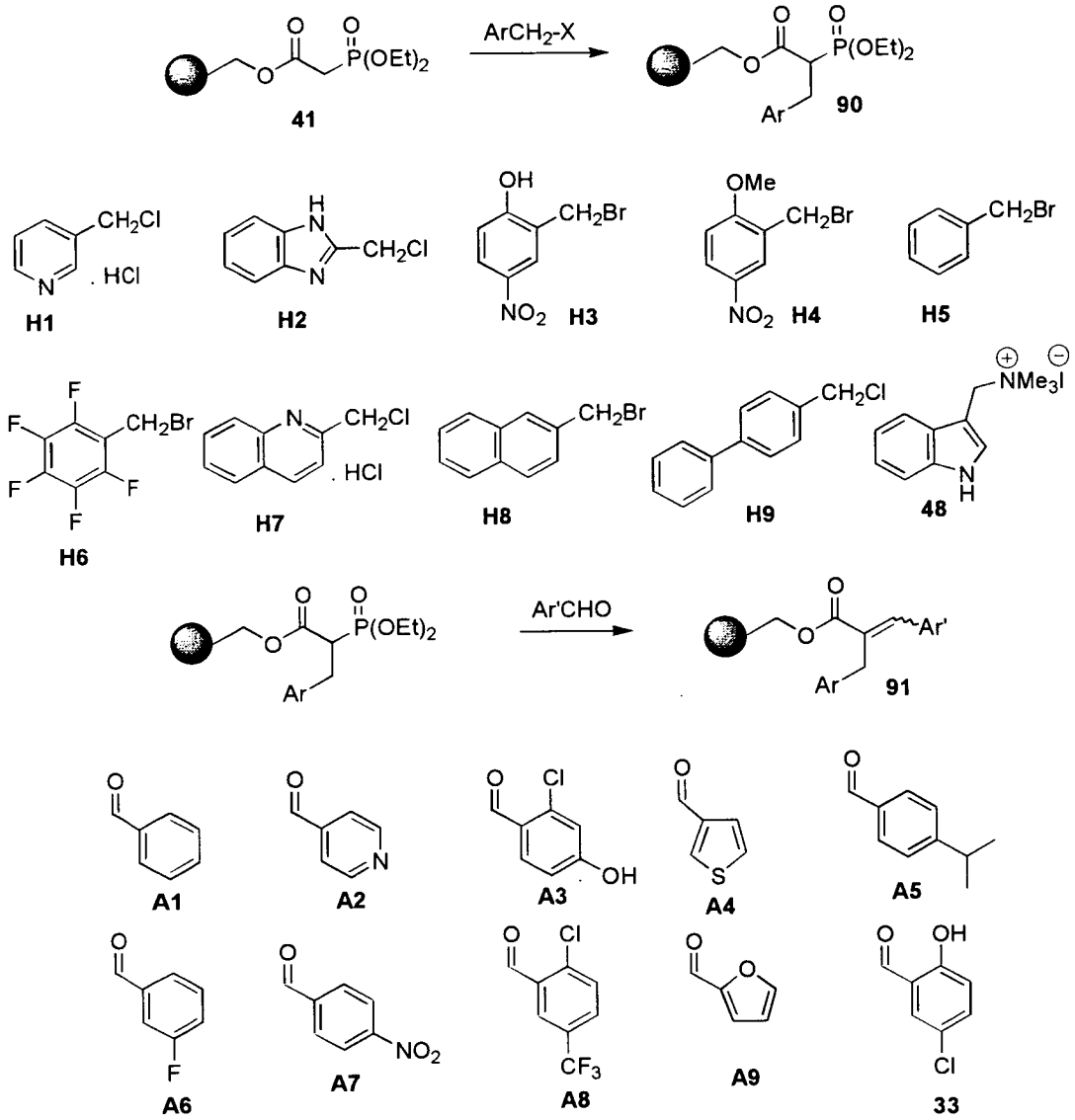


Figure 2.40: *Proposed Substrates for the Acrylic Acid Library.*

2.5.1 Library 1: Indole Sublibrary

The first sublibrary synthesised was that in which the final compounds each possessed the methylindolyl group and where the acrylic acids were derived from the aldehydes, **A1-A8**. Although the solid phase synthesis protocol had not been developed from the initial indole-based lead compound, **29**, it was hoped that the

same problems encountered during the solution phase syntheses of **29** would not be met in this solid phase library synthesis. Crucially, the aldehydes chosen did not possess the problematic *ortho*-phenol group, and so cleavage of the final products using TFA were not thought to be too harsh. A series of indoles have previously been synthesised on Wang resin, and cleaved using 50% TFA/DCM.[38]

The indole-phosphonate, **42**, was synthesised in one batch, using the previously optimised alkylation procedure. The resin was then split into portions of approximately 100mg and the HWE reaction performed with each of the aldehydes **A1-A8**, and cleavage of the products from resin carried out with TFA (*Figure 2.41*).

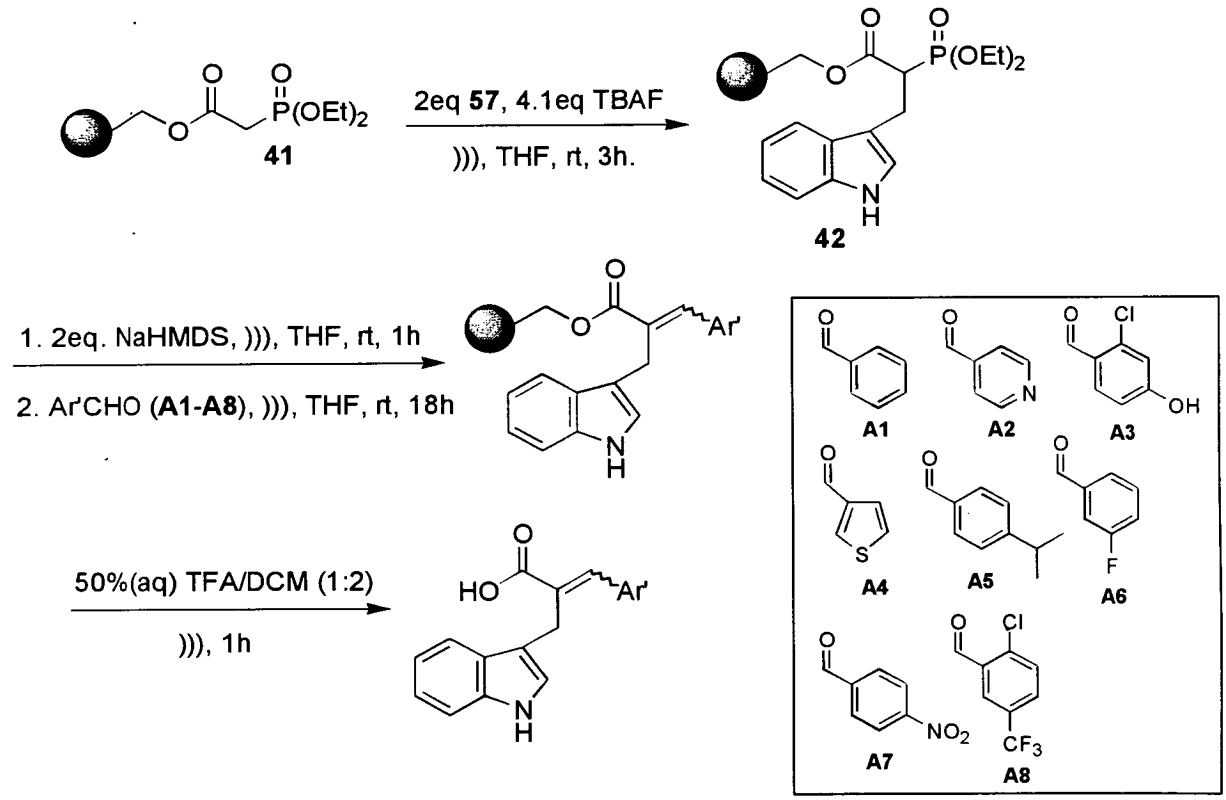


Figure 2.41: *Synthesis of Methylindolylacrylic Acids in Library 1.*

After cleavage from the resin, the crude mixture of each compound was analysed by HPLC. Unfortunately, each sample showed a slight pink colouration, which, in previous experiments, has been associated with indole decomposition. Each cleavage mixture was washed with a small quantity of 0.5N NaOH solution in an attempt to remove excess TFA, and prevent further decomposition. A sample HPLC trace of the

cleavage mixture from the reaction of **42** with aldehyde, **A5**, is shown in *Figure 2.42*. The trace shows that two main products were formed; the trace was typical of that obtained for all of the samples, and the marked peaks were isolated by preparative HPLC in each case.

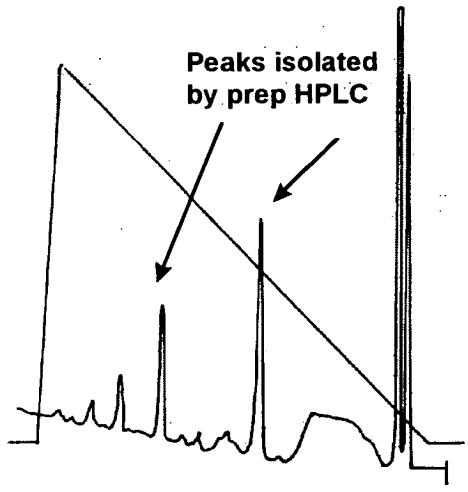


Figure 2.42: *HPLC Trace of Cleavage Mixture from Indole Library 1.*

The fact that two main products was observed was to be expected, as these could correspond to the *E*- and *Z*- isomers of the product acrylic acid. However, mass spectrometry (MS) on each of the samples, was inconclusive, and gave no idication as to the identity of the isolated products. The fact that each of the HPLC chromatograms was very similar, and that the MS profiles of the samples was very similar, indicated that the same products, common to all of the samples, was being isolated - perhaps a common synthetic intermediate. However, the mass of the unknown compound did not correspond to the most likely of these - the carboxylic acid which would form on resin-cleavage of unreacted phosphonate, **42**.

The unsuccessful conclusion to the synthesis of methylindolylacrylic acids, although disappointing, may still have been due to resin cleavage conditions which were too harsh for this range of substrate. It was hoped that other substrates would be inert to TFA, and present no obstacles to successful synthesis.

2.5.2 Library 2: Chlorophenol Sublibrary

The second 4×2 sub-library attempted, involved a range of phosphonates formed from reaction of **41** with halides **H1-H8**, each then reacted with the MEM-protected salicylaldehyde, **67** (*Figure 2.43*). Concomitant resin and protecting group cleavage, using TFA, as illustrated by the solid phase synthesis of the benzylacrylic acid, **82**, was expected to yield a library of eight chlorophenolacrylic acids.

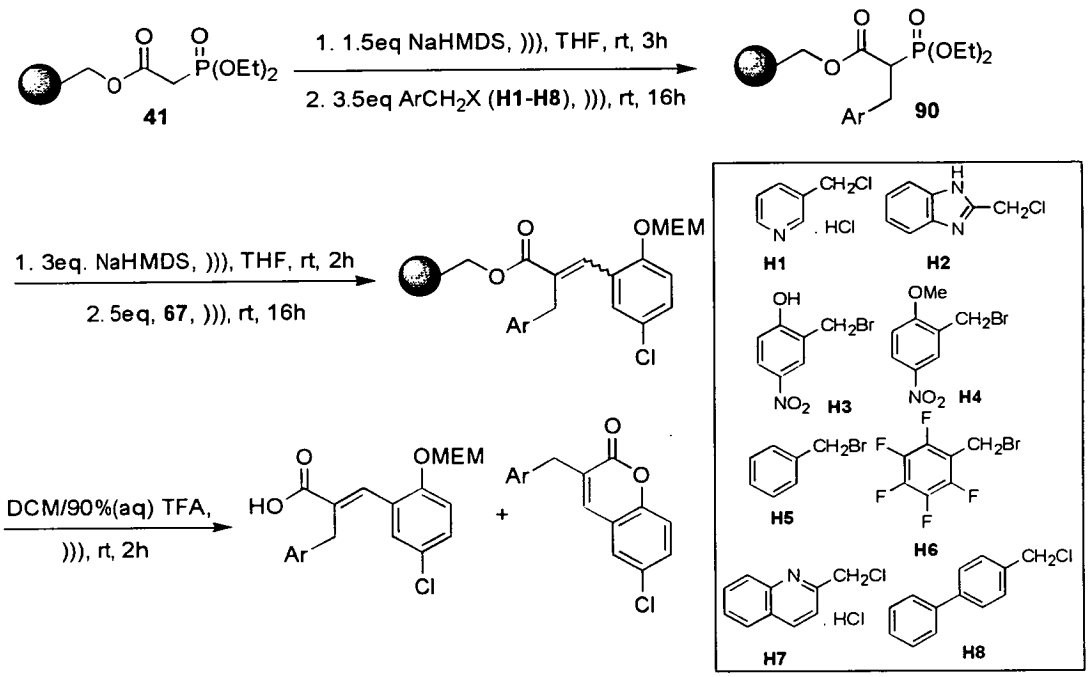


Figure 2.43: *Synthesis of Chlorophenol Library 2.*

As expected, HPLC analysis of the crude reaction mixture indicated the presence of two compounds, which could be attributed to the acid and coumarin products (*Figure 2.44*). However, there was very little variation in the retention time of corresponding peaks between samples, and MS analysis after preparative HPLC, again gave identical profiles.

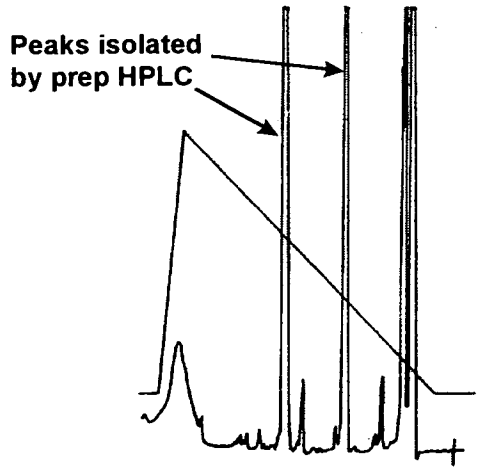


Figure 2.44: *HPLC Trace of Cleavage Mixture from Chlorophenol Library 2.*

As with the methylindolyl library, the products of the chlorophenol library could not be identified. No acrylic acid products were observed, and disappointingly, the benzylacrylic acid, **82**, previously synthesised in isolation by SPOS (*Section 2.4*), and which should have been isolated from the well used to react **H5**, was not observed.

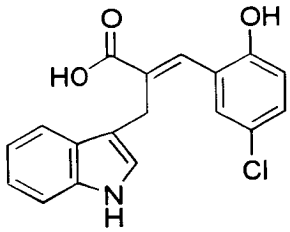
2.6 Conclusions

The efficient solution phase synthesis of acrylic acids, **29** and **82**, *via* the HWE reaction, has been made possible by the thorough examination of suitable phenolic protecting groups. Reaction conditions have been shown to be viable with the solid phase synthesis, and isolation, of benzylacrylic acid, **82**. Progression towards synthesising arrays of acrylic acid analogues on Wang resin using Diversomer apparatus, however, has not been achieved.

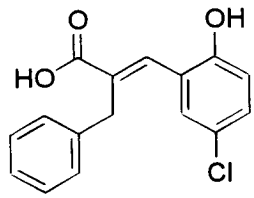
The reasons behind the unsuccessful parallel array synthesis are unclear, although several possibilities exist. Phosphonate ester alkylation has been shown to furnish an unavoidable mixture of products using standard methods in the solution phase, although this did not appear to be carried through to trials on the solid phase, the reaction may be causing similar problems. More analysis of the reaction on resin may be required. The design of the Diversomer equipment itself may have influenced the passage of reactions, as difficulty was experienced in maintaining an inert atmosphere.

Questionable diffusion of reagents through the sintered pin, may also have reduced reaction efficiency, thus requiring greatly increased reaction times.

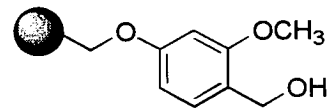
With the successful solution phase synthesis of the methylindolylacrylic acid, **29**, achieved using THP-protected phenol, different linker strategies may assist the solid phase synthesis. Since the THP group is relatively acid labile, a more acid-sensitive linker, such as SASRIN, may be more suitable, preventing the acid decomposition of products. Biological testing of the affinity of **29** and **82** to the receptor site of interest confirmed that **29** synthesised by the HWE route was of comparable reactivity to the previously tested material. No significant affinity was observed with the benzyl analogue, **82**, indicating the information which structural variation can provide on receptor substrate.



29



82



SASRIN resin

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Chapter 3: Charcoal as an Alternative Solid Support

3.1 Tetrabenzo[*a,c,g,i*]fluorene

Recently, solution-based methods of compound library synthesis have begun to be investigated as an alternative to using SPOS.[1] Synthetic limitations encountered when using polymeric supports have led to alternative methods of synthesis which maintain straightforward purification protocols (see *Section 1.4*).

Ramage has recently reported the use of tetrabenzo[*a,c,g,i*]fluorene (Tbf), **92**, as a pendant group in the purification of peptides and proteins[2] and DNA.[3] This polyaromatic group, which is substituted for Fmoc as the *N*-terminus protecting group of the completed protein sequence, possesses several properties which assist in the purification of such synthetic molecules. These include the hydrophobicity of the group, as well as its characteristic uv absorbance at 364nm, greatly assist in target compound identification and isolation from deletion and truncation sequences by preparative HPLC. However, the property of greatest potential use in general organic synthesis, is the affinity which Tbf has for charcoal or porous graphitised carbon (PGC) (*Figure 3.1*). In protein purification, the target Tbf-bound sequence, is selectively adsorbed onto the carbon surface, contaminant sequences removed in the filtrate, and the desired protein cleaved from the Tbf anchor group back into solution.

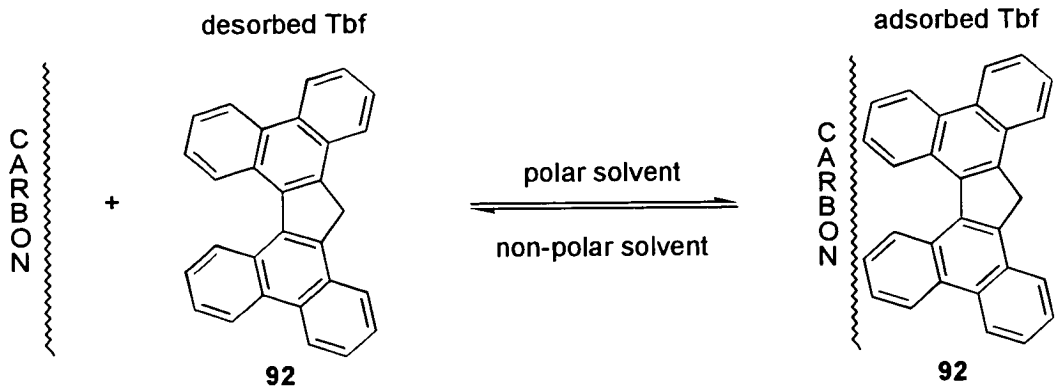


Figure 3.1: *Adsorption-Desorption Equilibrium of Dissolved Tbf With Carbon.*

The adsorption of hydrophobic compounds onto carbon is a well known equilibrium process.[4] It has been observed that polar solvents and lower temperatures aid the equilibrium towards adsorption, whilst non-polar solvents and elevated temperatures assist desorption and solubilisation. It was proposed that the adsorption process could be used as a purification aid in general organic synthesis.

Organic compounds, covalently bound to Tbf *via* a suitable linker group, could be reacted using traditional solution phase chemistry, and the product purified by exploiting the affinity of the Tbf group for carbon, with non-Tbf-bound compounds removed in the filtrate. As no covalent bonds are formed with the support, this may be described as a *pseudo*-solid phase purification step, and has all the associated purification advantages with SPOS. Recovery of Tbf-bound product can be achieved by simple desorption from the carbon, and the compound is then ready for the next synthetic step.

Ciprofloxacin, **93**, was selected as a suitable target for establishing a purification protocol using Tbf-anchored compounds. This quinolone antibacterial has been the subject of more conventional SPOS,[5] and it was hoped that the synthesis could be adapted to the solution phase by binding a precursor to a suitable Tbf-anchor group, and purifying the synthetic intermediates by carbon purification (*Figure 3.2*).

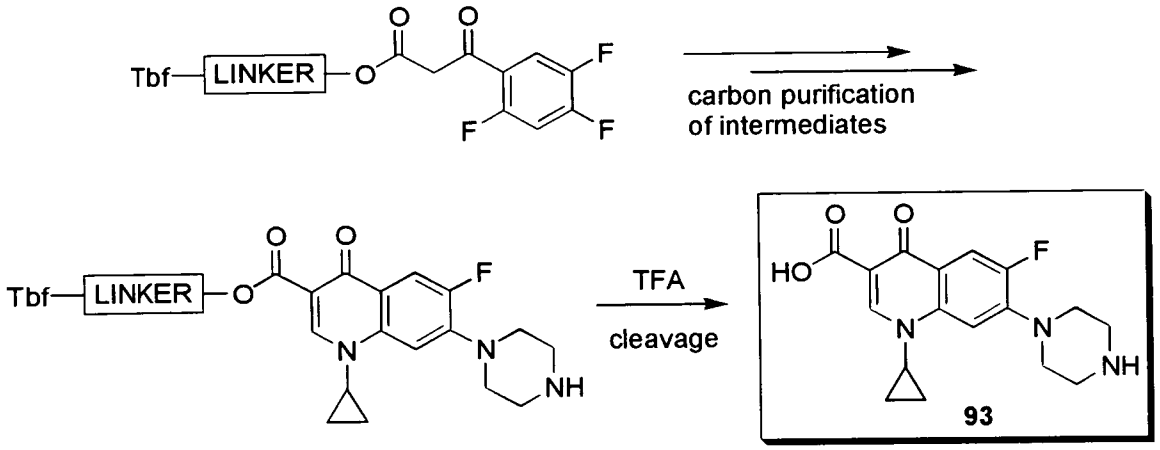


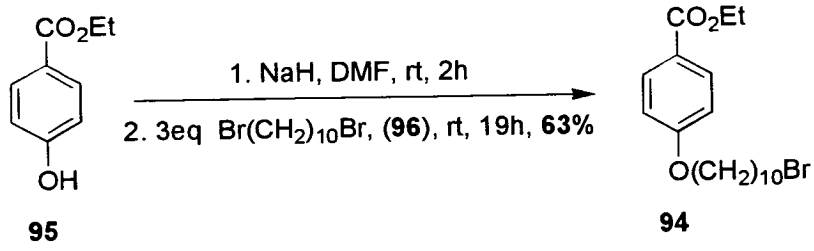
Figure 3.2: *Synthesis of Ciprofloxacin by Carbon Purification of Intermediates.*

3.2 Synthesis of the Tbf-Linker Group

The success of a Tbf-based anchor group for tethering to the quinolone precursor as a potential purification aid, depends greatly on the design of the linker group. As with linkers in solid phase synthesis, the group must possess several qualities which are key to its role: appropriate functionality must be present in order that bonds may be formed with both Tbf and the quinolone backbone; the group must be stable to the proposed reaction conditions; the linker must provide sufficient separation of the Tbf and heterocyclic fragments so that the bulky Tbf group in no way sterically-hinders reactions; the group must cleave from the heterocyclic moiety under specifically defined conditions once all transformations have been completed.

Ramage and Dutton[6] have previously examined a number of Tbf-anchored chiral amines and their use as a stationary phase in the HPLC resolution of chiral alcohols and protected Fmoc amino acids. The resolution was tested using amines tethered to Tbf *via* alkyl linkers of 3, 6 and 10 carbons in length, and suitable methods for their synthesis determined. These findings were considered when choosing the construction of a suitable linker group for the quinolone synthesis.

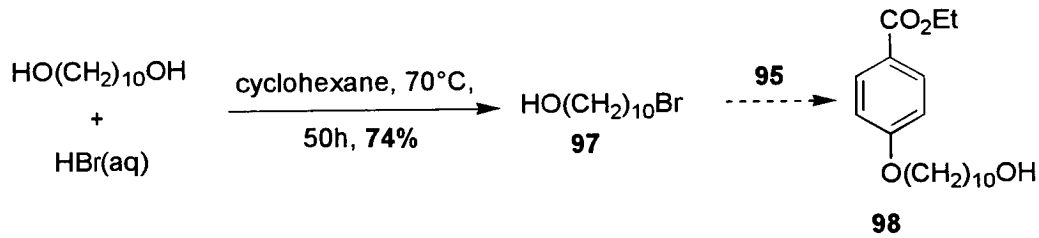
It was envisaged that ethyl 4-(10-bromodecyloxy)benzoate, **94**, would meet all the required criteria for a suitable linker group. The ten carbon alkyl chain would provide adequate separation of the Tbf group and organic fragment, and reduction of the ester functionality to the alcohol would ultimately provide a suitable point of anchorage for the quinolone backbone. Finally, the *para*-alkoxy functionality would aid cleavage from the final quinolone product in a manner similar to Wang resin (see *Figure 1.5*). Bromoester, **94**, was synthesised from ethyl 4-hydroxybenzoate, **95**, and 1,10-dibromodecane, **96**, in 63% yield (*Figure 3.3*) using a Williamson ether synthesis. The moderate yield was due to the excess of **96** required to encourage, as far as possible, only mono-etherification of the symmetrical substrate, and which made product purification less efficient. Unavoidable di-etherification also contributed to a lowering of the yield of **94**.



95 94

Figure 3.3: *Synthesis of Linker Group, 94.*

It is possible that, by altering the functionality of **96** to form an unsymmetrical compound, the requirement for a large excess of reagent would be diminished. One possible method could be to synthesise 10-bromodecan-1-ol, **97** and react this with **95**. The synthesis of **97** has been achieved by Tomohiro[7] *et al,* from decan-1,10-diol reacting with hydrobromic acid and continuous extraction using cyclohexane, in 74 % yield without further purification (*Figure 3.4*). However, the hydroxester, **100**, would require further functional group modification before alkylation by Tbf would be possible.



98

Figure 3.4: *Synthesis of 10-Bromodecan-1-ol, 97.*

With suitable quantities of the linker group, **94**, in hand, coupling to Tbf using Dutton`s method[6] was attempted. Tbf itself has received relatively little attention in the literature, but two syntheses of the polyaromatic have been reported by Martin.[8] However, both of these have contained harsh, low-yielding steps, which were impractical for large scale preparation. The much more straightforward synthesis developed by Ramage and Wahl[3] was used to synthesise the 8*bH*- analogue of Tbf, **99**, in multi-gram quantities over only two steps (*Figure 3.5*). Two molar equivalents of the Grignard reagent of 9-bromophenanthrene, **100**, were reacted with methylformate to form the *bis*-phenanthryl methanol product, **101**. Elimination and

cyclisation using TFA formed **99** in good overall yield. The symmetrical polyaromatic, Tbf, could be formed by treatment of **99** with base, but this was not required for future synthetic steps.

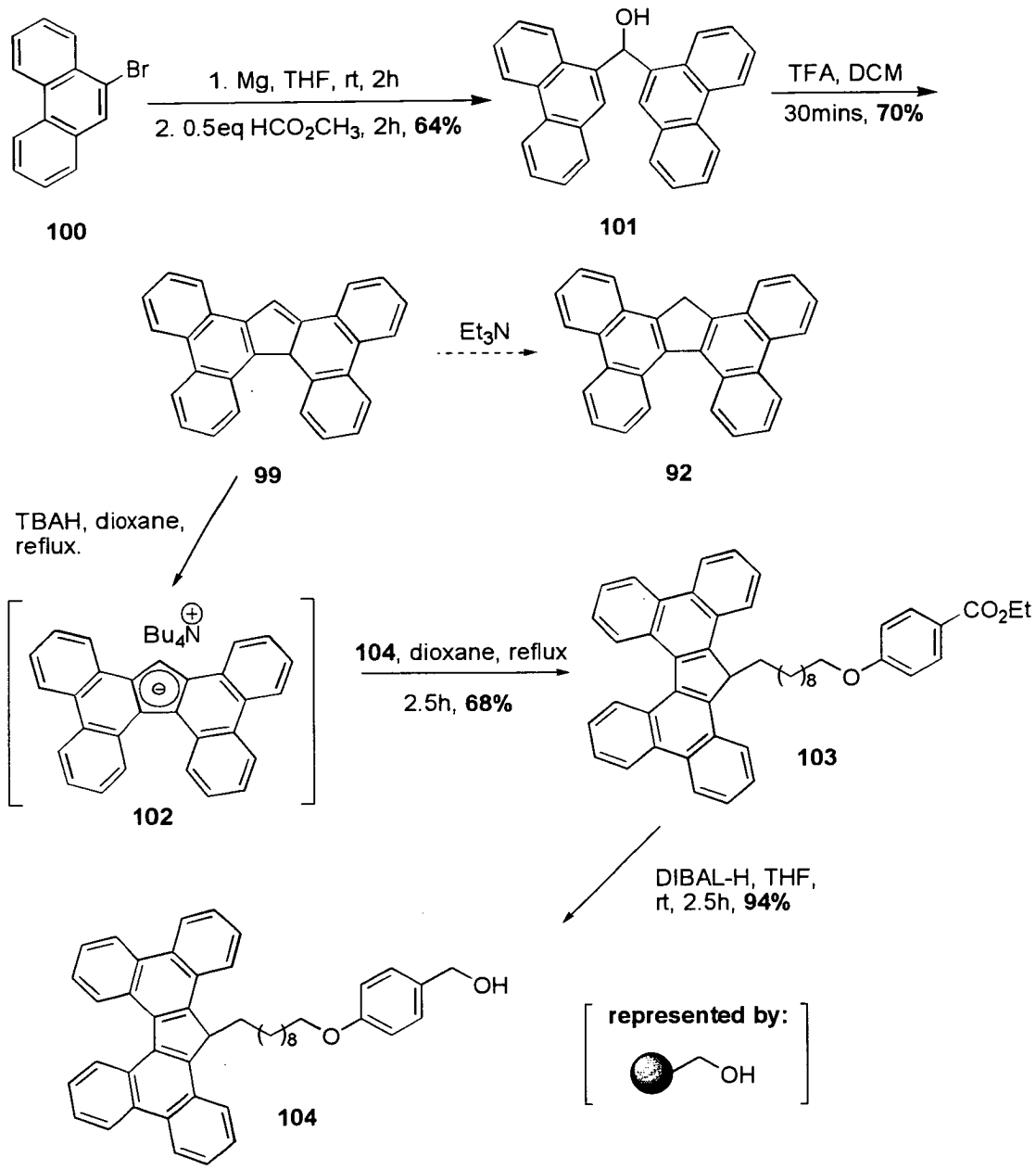


Figure. 3.5: *Synthesis and Alkylation of Tbf.*

Coupling of the bromoester, **94**, to Tbf was achieved by first forming the tetrabutylammonium salt of 8*bH*-Tbf, **102**, using tetrabutylammonium hydroxide (TBAH) under anaerobic conditions. The bright yellow salt, was then isolated by

filtration and reacted immediately with **94**, to form Tbf-ester, **103**, in a good overall yield of 68%. The final anchor group, Tbf-alcohol, **104**, was synthesised by reduction of **103** using diisobutylaluminium hydride (DIBAL-H). The anchor group was efficiently synthesised in multi-gram quantities and was suitable for direct binding to the precursor of Ciprofloxacin.

3.3 Solution Phase Synthesis of Tbf-Ciprofloxacin

Before any experiments could be performed to investigate the properties which influence the adsorption of Tbf-bound compounds onto charcoal, the suitability of the Tbf-anchor group to organic synthesis was examined. It is clear that the affinity of Tbf for carbon would only be a successful aid to the purification of organic compounds if the bulky, hydrophobic group had no detrimental effect on the solution phase synthesis.

The quinolone precursor, β-ketoester, **43**, was synthesised in very high yield using acid chloride, **105** and potassium ethylmalonate in a synthesis described by Wemple[9] (*Figure3.6*). The methodology was developed in order that a variety of β-ketoesters may be synthesised as precursors for quinolone antibacterials. The method employed the mild base, triethylamine and used anhydrous magnesium chloride to stabilise the enolate intermediate.

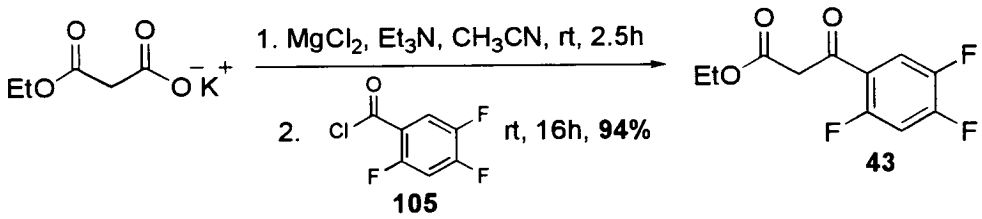


Figure 3.6: *Synthesis of β-Ketoester, 43.*

The β-ketoester, **43**, existed as keto-enol tautomers in the ratio, easily observed by ^{1}H nmr, in a ratio of 2.7:1 (*Figure 3.7*). The enol protons at (δ 12.69ppm, integral 0.27 and δ 5.81ppm, integral 0.27) for **106**, were easily distinguished from the methylene protons (δ 3.92ppm, integral 1.46) of **43**.

Figure 3.7: *Observed Keto-enol Tautomerism of β-Ketoester, 43.*

Coupling of the quinolone precursor, **43**, with the Tbf anchor group, **104**, was performed in a transesterification reaction using catalytic DMAP in refluxing toluene[10] (*Figure 3.8*). The product, Tbf-ketoester, **107**, exhibited the same degree of keto-enol tautomerism as **43**.

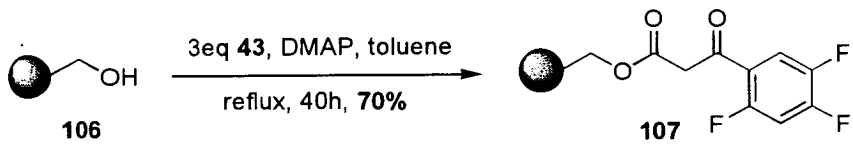


Figure 3.8: *Synthesis of Tbf-bound Quinolone Backbone.*

The quinolone template was synthesised from the backbone of **107**, using a variation of the synthetic procedure described by MacDonald and Ramage[5] (*Figure 3.9*). It was hoped that the method would be tolerant to direct transposition to the Tbf-bound analogues, but this was found not to be the case and a degree optimisation was required.

The quinolone template was built upon the backbone using Meerwein's reagent, dimethylformamide diacetal.[11] Both the diethyl and the dimethyl acetals (shown) were found to work equally efficiently in forming enamide **108**, and *in situ* transamination with cyclopropylamine, followed by cyclisation in one pot using TMG, led to the Tbf-bound quinolone, **109** in an excellent overall yield of 68%. The final synthesis of Tbf-Ciprofloxacin was achieved in a good yield of 76% by nucleophilic aromatic substitution with piperazine.

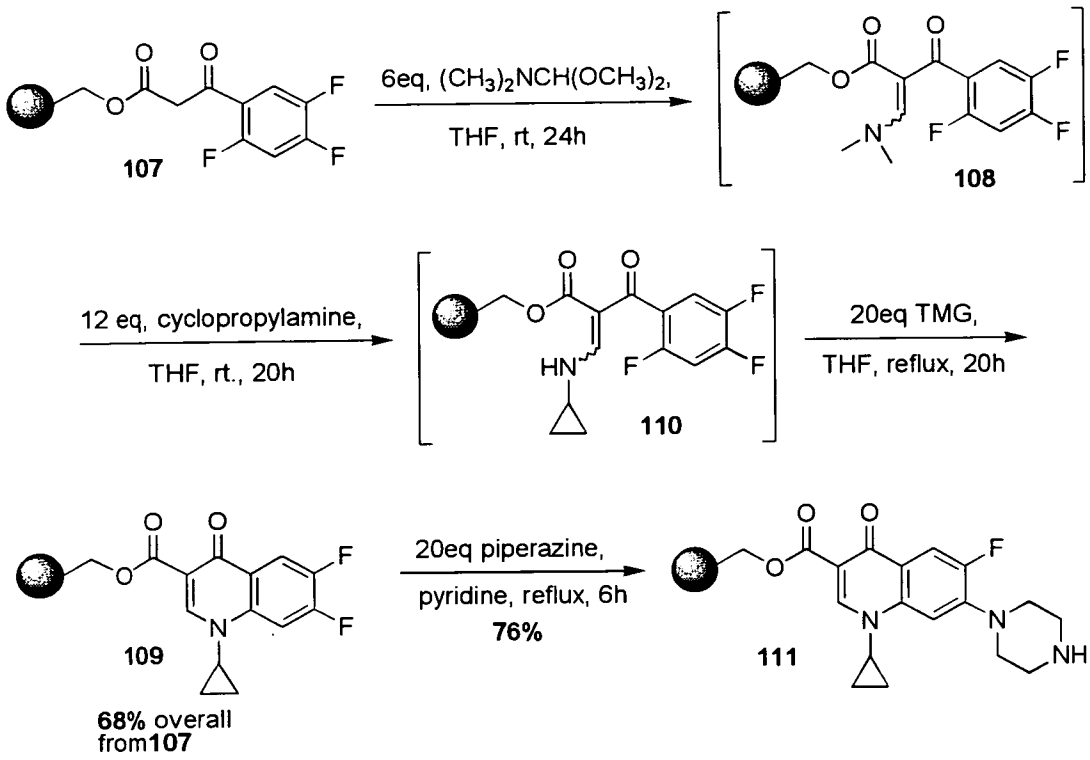


Figure 3.9: *Synthesis of Tbf-bound Ciprofloxacin.*

The one-pot synthesis of **109** was not the preferred method in the original synthesis of Ciprofloxacin, but it was found to be the most efficient route for the Tbf-bound substrates (*Table 3.1*). Enamide, **110**, could be isolated, but consistently poor recovery after flash chromatography meant that product was obtained in, at best, a moderate 69% yield (entries 1 and 2). Cyclisation of **110** using pure material, led to **109** in only 55% (38% overall, entry 3), whilst cyclisation of crude material (entry 4) led to recovery of the quinolone in only 18% overall yield. The one-pot method was clearly the most efficient method overall, both in terms of yield and time-scale (entries 5 and 6), leading to recovery of **109** in an excellent overall yield of 68%.

Starting Material	Reaction Conditions	(Product) Yield
(1) **107**	7.5eq DMF-diethylacetal, THF, rt, 20h; 2.7eq cyclopropylamine, THF, rt, 72h	**(110) 41%**
(2) **107**	8eq DMF-diethylacetal, THF, rt, 18h; 10eq cyclopropylamine, THF, rt, 4h	**(110) 69%**
(3) **110**	20eq TMG, DCM, reflux, 20h	**(109) 55%**
(4) **107**	5.6eq DMF-diethylacetal, THF, rt, 24h; 2.5eq cyclopropylamine, THF, rt, 72h; 19eq TMG, DCM, reflux, 24h	**(109) 12%**
(5) **107**	5eq DMF-diethylacetal, THF, rt, 3h; 4.7eq cyclopropylamine, THF, rt, 20h; 10eq TMG, THF, reflux, 18h	**(109) 18%**
(6) **107**	6eq DMF-diethylacetal, THF, rt, 22h; 8eq cyclopropylamine, THF, rt, 24h; 20eq TMG, THF, reflux, 18h	**(109) 68%**

Table 3.1: *Optimisation of the Synthesis of Tbf-Quinolone,* ***109****.*

The completed synthesis of Tbf-Ciprofloxacin, **113**, has shown that solution phase synthesis can be performed efficiently on compounds bound to the hydrophobic, and sterically demanding Tbf anchor group. Before attempting to use the anchor group to help purify the intermediates towards Ciprofloxacin, a greater understanding of the affinity which Tbf has for charcoal and PGC, as well as the factors which influence it, was required.

3.4 Interaction of Tbf-derivatives with Carbon

The use of carbon as a solid support in organic reactions may be employed in two ways. The reaction may be carried out in solution phase as normal, then at the end of the reaction sequence, charcoal added to adsorb all Tbf-derivatives. Alternatively, the Tbf-substrate may be pre-adsorbed onto carbon and the residue suspended in solvent. The Tbf-compound may then interact with dissolved reagents by two possible

mechanisms. Firstly, the Tbf-derivative may remain tightly adsorbed to the carbon, allowing only distant functional groups to remain in solution - much like the action of resins in SPOS. Reagents in solution are then free to react with these groups. Secondly, an equilibrium may exist, whereby some of the Tbf-derivative exists entirely in solution where it may react with reagents and re-adsorb onto carbon. Over a period of time, all of the substrate may react during the adsorption - desorption equilibrium.

Initial studies into the adsorption process, investigated the adsorption of pure Tbf-derivatives onto carbon. Factors investigated, included which solvents promoted adsorption and desorption, and how much carbon was required in order to achieve complete adsorption. Also investigated was the difference between using charcoal and porous graphitised carbon (PGC) as the adsorbent. Several compounds were adsorbed onto carbon using a variety of conditions (*Table 3.2*).

Compound	Adsorbent	Solvent	Adsorption (%)	Loading (mmol/g C)
(1) Tbf-alcohol **104**	charcoal	DCM	92	0.02
(2) Tbf-ketoester **107**	charcoal	DCM	97	0.03
(3) Tbf-ketoester **107**	charcoal	DCM/MeOH (3:2)	92	0.10
(4) Tbf-ketoester **107**	PGC	DCM/MeOH (3:2)	95	0.03
(5) Tbf-quinolone **109**	charcoal	DCM/MeOH (3:2)	90	0.10

Table 3.2: *Adsorption of Tbf-derivatives onto Carbon.*

The compound of interest was dissolved in the appropriate solvent, adsorbent added, then the suspension stirred in an ice bath (lower temperatures generally assist the adsorption of hydrophobic compounds[4]). Measurement of the uv spectrum of the supernatant was recorded after a period of time, with particular attention paid to the

λ_{max} at 364nm - the Tbf chromophore. More adsorbent was added and stirring continued as required until the uv absorbance at 364nm was zero, indicating that no compound remained in solution. All samples were adsorbed within one hour of stirring.

The results show that near quantitative adsorption (≥90%) of Tbf-bound compounds can be achieved under any solvent conditions. However, the quantity of carbon required to achieve these high levels of adsorption was variable. This is represented by the "Loading" column in *Table 3.2* which quantifies the amount of compound adsorbed per gram of carbon. The figure is not meant to signify any level of functionalisation in the way that resin loadings are described, but merely indicates the efficiency of the adsorption process under the conditions described. For example, comparison of entries 2 and 3, shows that more than three times the quantity of charcoal was required to adsorb fully, the same quantity of Tbf-ketoester. This efficiency difference was due solely to the nature of the solvent - the polar solvent used in entry 3 permits greater efficiency of adsorption. Indeed, a DCM/methanol mixture of 3:2 was the optimum solvent for adsorption, enabling solubilisation of the Tbf-derivatives, but with relatively high polarity to encourage adsorption. This finding was supported by the similarly efficient adsorption of Tbf-quinolone, **109**, under the same conditions (entry 5). Direct comparison of charcoal and PGC as adsorbent (entries 3 and 4), showed that charcoal was almost four times as efficient as PGC.

With a reasonable understanding of the factors affecting the adsorption of Tbf compounds onto carbon, the conditions best afforded the desorption of the compounds back into solution were examined. The main factor expected to affect desorption was the nature of the solvent, and so several, including toluene, dioxane and THF were investigated. The charcoal residue was stirred in solvent at 40°C and the uv spectrum of the supernatant recorded at twenty minute intervals. If the uv spectrum of the supernatant indicated that desorption was occurring, the process was repeated with fresh portions of solvent until no further desorption was observed (*Table 3.3*).

Compound	Loading (mmol/mg C)	Solvent	Desorption by UV (%)
(1) Tbf-alcohol **104**	0.02	toluene	19
(2) Tbf-alcohol **104**	0.02	dioxane	0
(3) Tbf-ketoester **107**	0.03	toluene	27
(4) Tbf-ketoester **107**	0.03	dioxane	0
(5) Tbf-ketoester **107**	0.10	toluene	85
(6) Tbf-ketoester **107**	0.10	THF	10
(7) Tbf-quinolone **109**	0.10	toluene	18

Table 3.3: *Desorption of Tbf-derivatives From Charcoal.*

The superimposed uv spectra of Tbf-ketoester, **107**, after charcoal adsorption and desorption are shown in *Figure 3.10.*

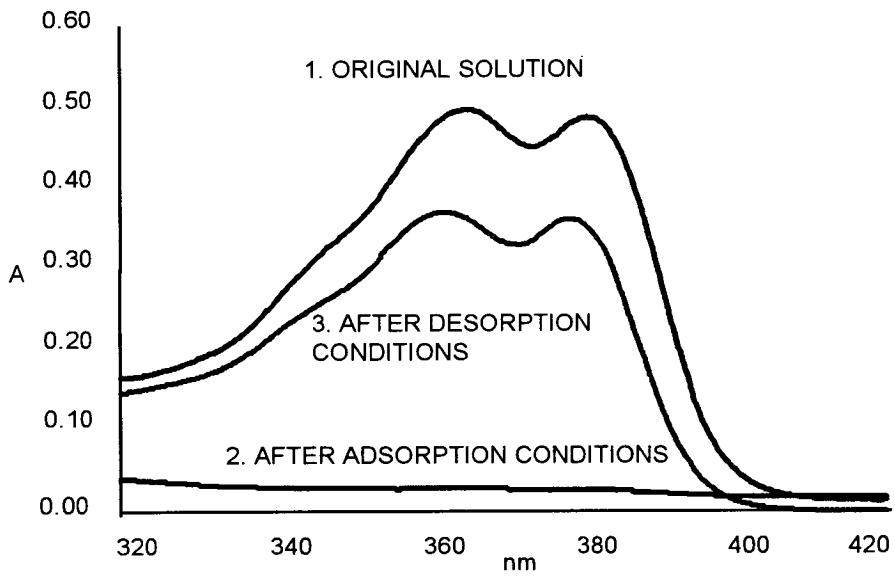


Figure 3.10: *Uv Spectra of Tbf-Ketoester During Charcoal Adsorption and Desorption.*

Two main conclusions may be drawn from these results. Firstly, toluene is easily the most suitable solvent for desorbing Tbf-derivatives from carbon. This possibly gives a clue as to the nature of the binding of the Tbf to the charcoal surface. Since

other hydrophobic solvents do not permit such high levels of desorption as toluene, binding to charcoal is probably related to substrate planarity as well as hydrophobicity. This means that planar toluene is much a more effective solvent for desorption than dioxane, which has similar hydrophobicity.[12]

The second conclusion which may be drawn from the reported data, is that the degree of desorption is dependent on the loading level of compound on the charcoal. The greater the loading (*ie.* the more concentrated the Tbf is on the charcoal surface), the greater the observed desorption. This implies that the charcoal surface has a non-homogeneous nature, and that some sites are more tightly binding than others. In situations where the adsorption is "dilute" (low loading), more of the Tbf is situated in tightly binding sites, and so is less easily removed.

3.5 Organic Reactions Using Carbon as Solid Support

With a good understanding of the conditions which influence the interaction of Tbf-derivatives with charcoal developed, the synthesis of Tbf-Ciprofloxacin, **113**, was repeated, but with the charcoal purification of each synthetic intermediate. Reactions were performed in solution as normal, and upon completion, charcoal was added to selectively adsorb Tbf-bound products from the crude reaction mixture. The option of pre-adsorbing substrates onto charcoal prior to heterogeneous reaction was rejected on the grounds that reaction monitoring would be more difficult, and unwanted charcoal-reagent interactions could not be avoided.

The Tbf-bound intermediates, **107** and **109** were purified using the charcoal protocol (*Figure 3.11*). On completion of the reaction, solvent was removed, and the crude residue was dissolved in the polar solvent system which affords efficient adsorption to charcoal. An appropriate quantity of charcoal was added to the solution such that quantitative adsorption would give the optimum loading of compound of 0.1mmol/g charcoal, and the suspension stirred in an ice bath. If TLC indicated that not all of the product had adsorbed, then more methanol and charcoal was added until this was achieved. Adsorbed compounds were recovered by stirring the charcoal

residue in warm toluene. All material was found to be recovered after three washes with fresh solvent.

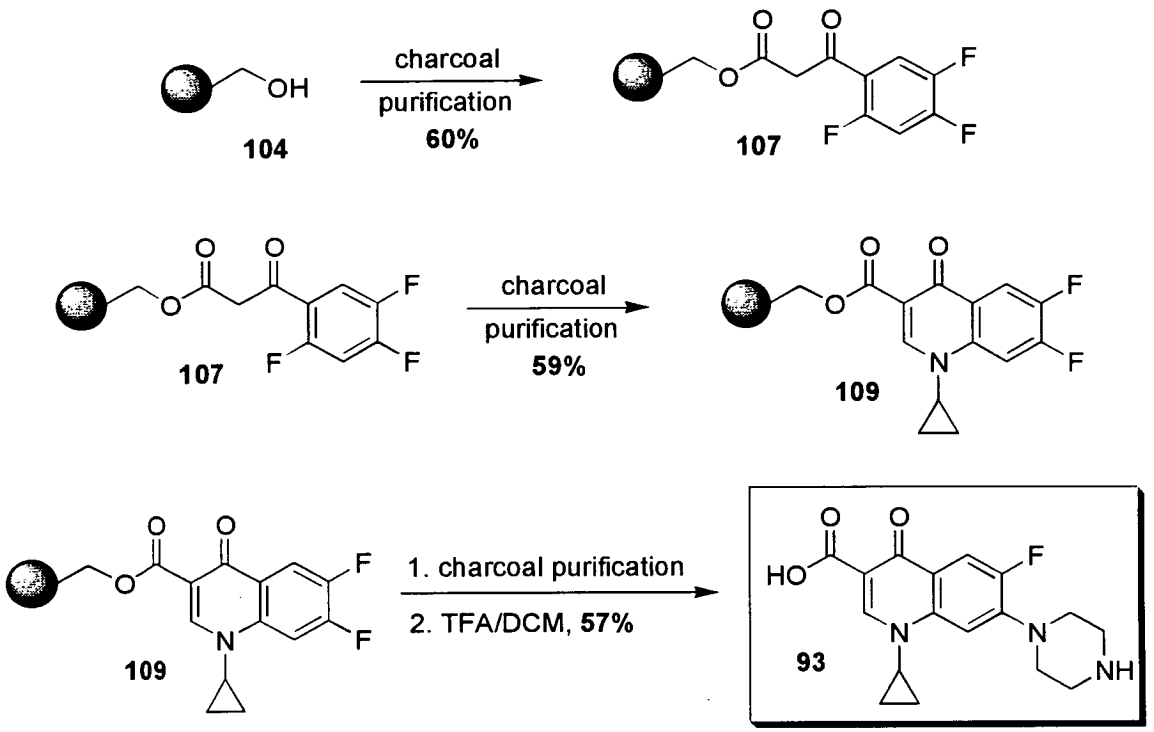


Figure 3.11: *Synthesis of Ciprofloxacin by Charcoal Purification of Intermediates.*

As with any phase separation purification protocol, any unreacted starting material or by-products, which have the same affinity for the alternative phase as the desired product, will also be isolated with the product. This is observed in methods such as SPOS and fluorous phase synthesis, and is no different with this charcoal method of purification. Therefore, *solely to compare reaction efficiency with the solution phase method*, chromatography was performed to purify the products obtained in the toluene extracts. Thus, Tbf-ketoester, **107**, was isolated in 60% yield and Tbf-quinolone, **109**, recovered in 59% yield. These compare very favourably with the yields obtained from solution phase synthesis and traditional work-up and purification procedures (70%, **107**, and 68% **109**). More importantly, TLC indicated that toluene extracts contained only Tbf-bound compounds and so the excess reagents used in these reactions did not interfere with the purification process.

The final synthesis of Ciprofloxacin, **93**, was achieved by adsorbing the crude Tbf-Ciprofloxacin, **111**, onto charcoal in the normal manner. However, rather than treating the charcoal residue with toluene, the product was cleaved from the Tbf anchor using appropriate conditions determined by the linker design. Therefore, upon treatment of the residue with TFA, the anchor remained bound to the charcoal, and Ciprofloxacin was released into solution (along with any other Tbf-bound compounds). The product obtained from this procedure was analysed by HPLC, and compared to a known sample of Ciprofloxacin (*Figure 3.12*).

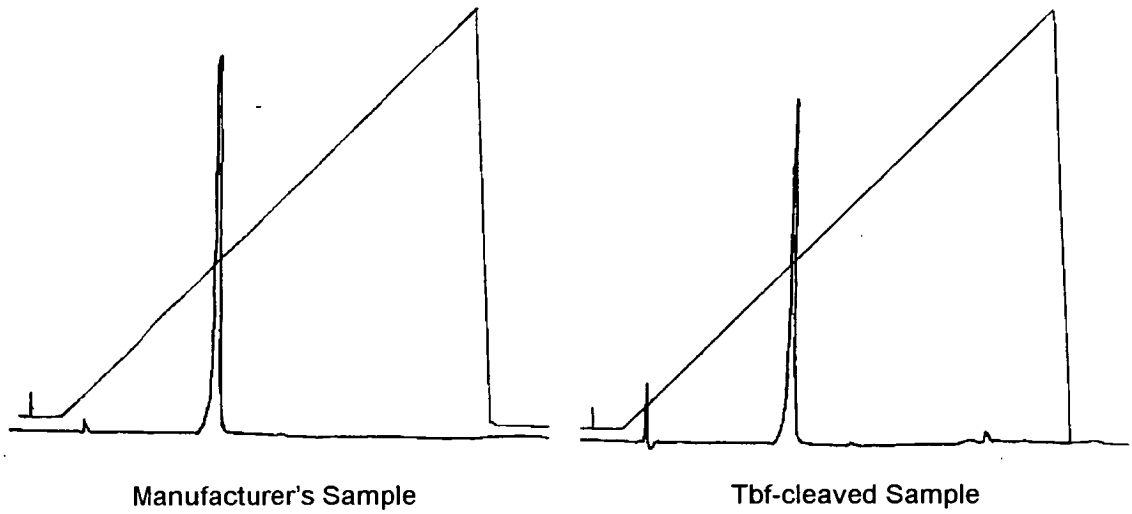


Figure 3.12: *HPLC Traces of Ciprofloxacin.*

The HPLC trace of the Tbf-cleaved sample of Ciprofloxacin demonstrates exactly how powerful a tool the charcoal purification tool is. As well as confirming the identity of the major product to be Ciprofloxacin, the levels of impurities are extremely low and so no further purification was required.

3.6 Conclusions

The successful synthesis of Ciprofloxacin, **93** using Tbf-bound intermediates has demonstrated the applicability of an alternative purification strategy in organic synthesis. Validation of an efficient synthesis of the target molecule was carried out using substrates bound to the hydrophobic Tbf group *via* a specifically designed linker. A reproducible protocol of recovering Tbf-derivatives, efficiently adsorbed onto charcoal, led to the efficient purification of such compounds from crude reaction mixtures.

The results obtained illustrate how Tbf can be used as an anchor group for organic molecules to assist in their purification. The method enables all the advantages of traditional solution phase synthesis to be available, but couples this with the ease of purification which few techniques can fulfil. The methodology, therefore, could make a valuable contribution to the field of combinatorial chemistry and parallel array synthesis.

Future studies using electron microscopy could be carried out to determine the exact nature of the binding of Tbf to charcoal, and perhaps identify surfaces which may offer even more efficient and selective binding. The anchoring process could then be used in the multiple parallel syntheses of suitable substrates.

3.7 References

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Chapter 4: Experimental

4.1 Reagents and Instrumentation

All chemicals and reagents were obtained from Aldrich chemical company, or Fisher Acros Organics and used without further purification unless otherwise stated. Solvents used in reactions were freshly distilled before use: THF over sodium and benzophenone as indicator; toluene over sodium; DCM over calcium hydride; and acetonitrile over calcium hydride. NaHMDS solutions in THF were titrated against pyreneacetic acid (yellow to purple), and butyllithium titrated against pivaloyl *o*-toluamide (colourless to yellow) prior to use.

All reactions were performed using oven or flame-dried glassware, under an atmosphere of dry nitrogen, unless otherwise stated. Thin layer chromatography (TLC) was performed on aluminium sheets coated with silica gel (Kieselgel 60 F254), and compounds were visualised using uv light at 254nm, potassium permanganate solution, or ammonium molybdate solution. Flash chromatography was carried out using silica gel 60 230-400 mesh.

Melting points were determined in open capillaries using a Buchi 510 melting point apparatus. Nuclear magnetic resonance (nmr) spectroscopy was performed on 250MHz Bruker AC 250, 200MHz Bruker WP 200, or a 600MHz Varian Inova, using tetramethylsilane (TMS) as external standard. Fourier Transform Infra Red (FTIR) Spectroscopy was performed on a Bio-Rad FTS-7 spectrometer. Ultraviolet-visible (uv-vis) spectra were recorded on a Perkin Elmer Lambda 11. Fast atom bombardment (FAB) mass spectrometry was performed on a Kratos MS50 and electron impact (EI) mass spectrometry performed on a Kratos MS902. Atmospheric pressure chemical ionisation (APCi) and electrospray (ES) mass spectrometry were both performed using a Micromass Platform II with Mass Lynx 2.3 Build 5 software. Elemental analyses were performed on Carlo Erba 1106 or Perkin Elmer 2400 instruments.

Multiple parallel solid phase synthesis was performed using Diversomer™ 4×2 array, manual synthesis kit. The resin used was copoly(styrene-1%-divinylbenzene)-*p*-benzyloxybenzyl alcohol resin with 1.07mmol/g loading, supplied by Bachem.

High Pressure Liquid Chromatography (HPLC) was performed using a Gilson modular system: 305 pump and programmer, 306 pump, 805 solvent delivery system, 811C mixer, and a 118 uv-vis detector monitoring at 220nm. Reverse phase, C8 columns were used at 1ml/min (analytical) and 5ml/min (preparative) flow rates. The solvents used were 0.1%TFA/water (A) and 0.1%TFA/acetonitrile (B) using two programmed gradients:

Gradient 1: 0 min 10%B; 2 mins 10%B; 32 mins 90%B; 34 mins 10%B

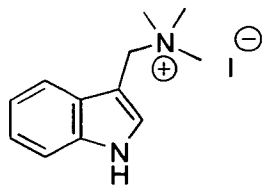
Gradient 2: 0 min 18%B; 2 mins 18%B; 23 mins 75%B; 25 mins 18%B

All compounds synthesised reported satisfactory analytical data, including elemental analysis, accurate mass, or nominal mass with observed fragmentation patterns as appropriate. Elemental analysis of tetrabenzo[*a,c,g,i*]fluorene compounds generally proved unsatisfactory, due to the high percentage of carbon present in these molecules, leading to incomplete combustion, hence inaccurate results.

4.2 Acrylic Acids by Elimination Route

Preparation of Gramine Methiodide, 48.[1]

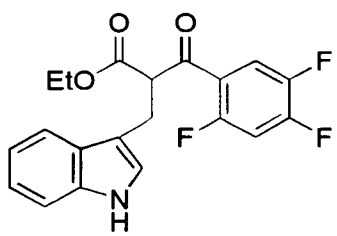
To freshly distilled methyl iodide (15mL) was added powdered gramine, **31** (0.88g, 5.06mmol), over a period of 1 hour and the mixture stirred for a further 2 hours at room temperature. The mixture was then stored overnight at 4°C, and the precipitate formed, isolated by filtration. This was dissolved in the minimum volume of hot methanol (~8mL), and the precipitate formed on cooling then isolated and discarded (tetramethylammonium iodide). The methanolic filtrate was combined with benzene (120mL), and stored at 4°C for 48h. The precipitate was isolated by filtration and washed with water (5×50mL). The combined aqueous washes were concentrated to 5-10mL and cooled to 4°C overnight. The desired product was collected as a white solid (0.97g, 3.07mmol, 61%).



Rf 0.34 ($CHCl_3$/methanol/acetic acid, 9:1:0.5). **m.p.** 165-168°C (lit. 168-169°C[1]). **FTIR ν_{max}/cm^{-1} (Nujol)** 3201 (NH); 2916, 2851 (CH); 1613, 1539 (C=C). **λ_{max}/nm (ethanol, ε/dm^{3}mol^{-1}cm^{-1})** 286 (8130), 276 (9590), 272 (9590). **^{1}Hnmr (DMSO-d_6, 250MHz, δ/ppm)** 11.65 (1H, s, NH); 7.84-7.80 (1H, m, Ar H); 7.69, (1H, d, $^3J_{HH}$=2.7Hz, Ar H); 7.50-7.46 (1H, m, Ar H); 7.22-7.10 (2H, m, Ar H); 4.68 (2H, s, CH_2), 3.03 (9H, s, $N(CH_3)_3$). **^{13}C{^{1}H}nmr (DMSO-d_6, 63MHz, δ/ppm)** 136.2, 127.8, 101.9 (*q* Ar C); 130.4, 122.0, 120.2, 118.6, 112.3 (Ar CH); 60.7 (CH_2); 51.3 (CH_3). **HRMS (FAB, M$^+$/z)** $C_{12}H_{17}N_2$ (M^+) requires 189.1392, found 189.1385.

Preparation of Ethyl 2-(3-Methylindolyl)-3-(2,4,5-trifluorophenyl)-3-oxopropionate, 47.

To ethyl 3-(2,4,5-trifluorophenyl)-3-oxopropionate, **43** (363.5mg, 1.48mmol), in acetonitrile (28mL) was added gramine methiodide, **48** (465.5mg, 1.48mmol). Triethylamine (265.9mg, 2.63mmol) was added *via* graduated pipette and the reaction mixture stirred at room temperature for 24hours. The mixture was then filtered through a pad of kieselguhr and solvent removed under reduced pressure. The crude residue was purified by flash chromatography (DCM) to yield ethyl 2-(3-methylindolyl)-3-(2,4,5-trifluorophenyl)-3-oxopropionate, **47**, as a pale yellow film (397.1mg, 1.06mmol, 72%).

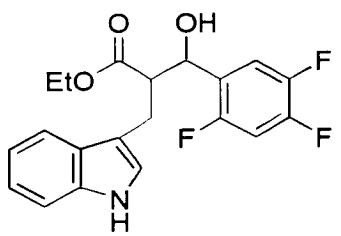


Rf 0.39 (DCM). **FTIR ν_{max}/cm^{-1} (DCM)** 3465 (NH), 3062 (Ar CH); 2984 (CH); 1739 (ester C=O); 1692 (ketone C=O); 1623, 1511 (C=C). **λ_{max}/nm (DCM, ε/dm^{3}mol^{-1}cm^{-1})** 282 (9380), 229 (20300). **^{1}Hnmr (CDCl$_3$, 250MHz, δ/ppm)** 8.04 (1H, s, NH); 7.70-7.57 (2H, m, Ar H); 7.35-6.86 (5H, m, Ar H); 4.62 (1H, t, $^3J_{HH}$=6.8Hz, CH); 5.15 (2H, q, $^3J_{HH}$=7.1Hz, OCH_2); 3.60-3.37 (2H, m, diastereotopic CH_2); 1.16 (3H, t, $^3J_{HH}$=7.1Hz, CH_3). **^{13}C{^{1}H}nmr (CDCl$_3$, 63MHz, δ/ppm)** 191.0 (ketone C=O); 169.2 (ester C=O); 158.9, 155.0, 151.3, 148.8, 145.1, 138.2 (Ar CF); 135.9, 126.9, 121.5, 111.0 (*q* Ar C); 123.0, 121.9, 119.4, 118.3, 111.0 (indole Ar CH); 118.6, 106.4 (Ar CH);

61.4 (OCH_2); 58.4 (CH); 24.1 (CH_2); 13.8 (CH_3). **HRMS (FAB, M^+/z)** $C_{20}H_{16}F_3NO_3$ (M^+) requires 375.1082; found 375.1086.

Preparation of Ethyl 2-(3-Methylindolyl)-3-(2,4,5-trifluorophenyl)-3-hydroxypropionate, 46.

To ethyl 2-(3-methylindolyl)-3-(2,4,5-trifluoro-phenyl)-3-oxo-propionate, **47** (304.2mg, 0.81mmol), in THF (35mL) was added sodium borohydride (61.3mg, 1.61mmol), and the suspension was stirred room temperature for 4 hours. After this time, the mixture was cooled in an ice bath, and carefully quenched with HCl (0.5N, 20 ml). The organic products were extracted with DCM (30ml), washed with water (30ml), and dried over sodium sulphate. Solvent was removed under reduced pressure to yield a yellow residue. This was purified by flash chromatography (ethyl acetate/hexane, 2:3) to yield ethyl 2-(3-methylindolyl)-3-(2,4,5-trifluoro-phenyl)-3-hydroxypropionate, **46**, as a pale yellow film (252.1mg, 0.67mmol, 83%). Spectra were consistent with a diastereomeric ratio of 1:1.

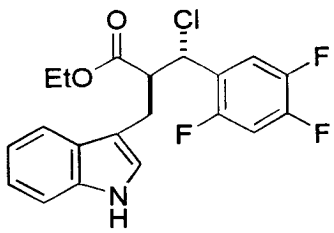


Rf 0.39 (ethyl acetate/hexane 2:3). **FTIR ν_{max}/cm^{-1} (DCM)** 3603 (OH); 3479 (NH); 3032, 3012 (Ar CH); 2929, 2857 (CH); 1706 (ester C=O), 1632, 1513 (C=C); **λ_{max}/nm (DCM, ε/dm^{3}mol^{-1}cm^{-1})** 290 (4110), 273 (6230), 230 (11840), 227 (8950). **^{1}Hnmr ($CDCl_3$, 250MHz, δ/ppm)** 8.06, 7.97 (1H, both br. s, NH); 7.58-6.79 (7H, m, Ar H); 5.31, 5.13 (1H, both s, CHOH); 3.97, 3.95 (2H, both q, $^3J_{HH}$=7.1Hz, OCH_2); 3.82, 3.54 (1H, both br. s, OH, D_2O exchange); 3.31-2.95 (3H, m, CH and CH_2); 0.98, 0.96 (3H, both t, $^3J_{HH}$=7.1Hz). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz, δ/ppm)** 174.9, 174.7 (C=O); 156.1, 152.3, 151.4, 148.9, 147.2, 144.8, (Ar C-F); 136.1, 136.0, 127.1, 126.3, 124.8, 112.5, 111.9 (*q* Ar C); 122.3, 122.1, 122.0, 121.9, 119.4, 119.2, 118.5, 118.3, 111.0, 110.9 (Ar CH); 116.3, 115.6, 105.5 104.8 (Ar CH); 67.7, 67.5 (CHOH); 60.8 (OCH_2); 51.5, 51.0 (CH); 25.2, 22.1 (CH_2); 13.7, 13.6 (CH_3). **MS (FAB, M^+/z)** $C_{20}H_{18}F_3NO_3$ (M^+) requires 377; found 377 (10%), 286 (10%), 170 (21%), 130 (100%).

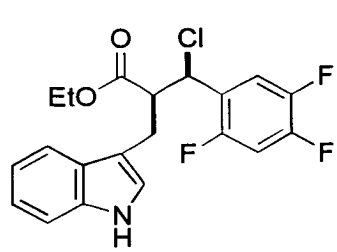
Attempted Preparation of Ethyl 2-(3-Methylindolyl)-3-(2,4,5-trifluorophenyl)-acrylate, 45.

(i) Using Phosphorus Oxychloride[2]

Ethyl 2-(3-methylindolyl)-3-(2,4,5-trifluoro-phenyl)-3-hydroxypropionate, **46** (40.6mg, 0.11mmol), was stirred in dry pyridine (5mL) at 0°C. $POCl_3$ (83.5mg, 0.55mmol) was added dropwise, and the mixture was then stirred at room temperature for 20 hours. The reaction was then quenched with careful addition of water and organic products extracted with ether (2×20mL). The combined extracts were washed with brine (20mL) and water (20mL), dried over sodium sulphate and solvent remove under reduced pressure to yield a pale yellow residue. Two close running products were obtained after passing the residue through a silica plug. These were separated by flash chromatography (DCM) and subsequently identified as diastereoisomers of ethyl 2-(3-methylindolyl)-3-chloro-3-(2,4,5-trifluorophenyl)-propionate, **49** (combined 12.0mg, 0.030mmol, 27%).



Rf (49A) 0.63 (DCM). **FTIR ν_{max}/cm^{-1} (DCM)** 3468 (NH); 3052 (Ar CH); 2987, 2929, 2856 (CH); 1732 (ester C=O), 1631, 1521 (C=C). **^{1}Hnmr ($CDCl_3$, 200MHz, δ/ppm)** 7.98 (1H, br. s, NH); 7.43-6.90 (7H, m, Ar H); 5.41 (1H, d, $^3J_{HH}$=9.7Hz, benzyl CH); 4.03 (2H, q, $^3J_{HH}$=7.2Hz, OCH_2); 3.52-3.40 (1H, m, CH); 3.03 (1H, dd, $^3J_{HH}$=14.2, 4.8Hz diastereotopic H); 2.74 (1H, dd, $^3J_{HH}$=14.2, 10.2Hz diastereotopic H); 1.03 (3H, both t, $^3J_{HH}$=7.2Hz). **MS (FAB, M^+/z)** $C_{20}H_{17}ClF_3NO_3$ (M^+) requires 395; found 395 (5%), 359 (4%), 286 (10%), 148 (16%), 130 (100%).

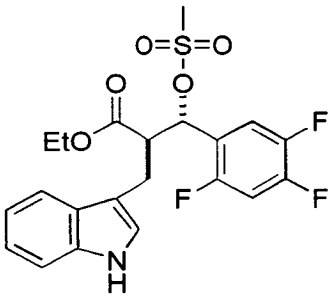


Rf (49B) 0.70 (DCM). **FTIR ν_{max}/cm^{-1} (DCM)** 3458 (NH); 3049 (Ar CH); 2986, 2930, 2859 (CH); 1729 (ester C=O), 1630, 1520 (C=C). **^{1}Hnmr ($CDCl_3$, 200MHz, δ/ppm)** 7.79 (1H, br. s, NH); 7.53-6.83 (7H, m, Ar H); 5.09 (1H, d, $^3J_{HH}$=5.9Hz, benzyl CH); 4.22 (2H,

q, $^3J_{HH}$=7.2Hz, OCH_2); 3.65-3.55 (1H, m, CH); 3.37 (1H, dd, $^3J_{HH}$=14.7, 8.6Hz diastereotopic H); 3.18 (1H, dd, $^3J_{HH}$=14.7, 6.4Hz diastereotopic H); 1.29 (3H, both t, $^3J_{HH}$=7.2Hz).

(ii) Using DMAP/Methanesulphonyl chloride[3]

Ethyl 2-(3-methylindolyl)-3-(2,4,5-trifluoro-phenyl)-3-hydroxy-propionate, **46** (49.0mg, 0.13mmol), was stirred in DCM (2mL) at room temperature, and a mixture of methanesulphonyl chloride (37.0mg, 0.323mmol) and DMAP (79.7mg, 0.65mmol) in DCM (4mL) was added to the mixture stirred at 35°C for 18 hours. The mixture was then cooled to room temperature and quenched with saturated ammonium chloride solution (20mL). Organic products were extracted with DCM (2×20mL) and the combined extracts washed with water (20mL), dried over sodium sulphate, and solvent removed under reduced pressure to yield a pale yellow oil. Purification by flash chromatography (DCM) yielded ethyl 2-(3-methylindolyl)-3-chloro-3-(2,4,5-trifluorophenyl)propionate, **49B** (29.2mg, 0.074mmol, 57%), and ethyl 2-(3-methylindolyl)-3-methanesulphonyl-3-(2,4,5-trifluorophenyl)propionate, **50** (19.8mg, 0.044mmol 34%).

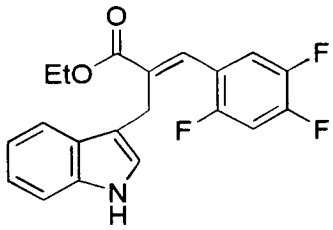


Rf (DCM) 0.37. **^{1}Hnmr ($CDCl_3$, 250MHz, δ/ppm)** 8.02 (1H, br. s, NH); 7.48-6.89 (7H, m, Ar H); 6.03 (1H, d, $^3J_{HH}$=7.7 Hz, benzyl H); 3.84 (2H, q, $^3J_{HH}$=7.0Hz. OCH_2); 3.46-3.28 (3H, m, CH/CH_2); 2.93 (3H, s, SO_2CH_3); 0.91 (3H, t, $^3J_{HH}$=7.0Hz). **MS (FAB, M^+/z)** $C_{21}H_{20}F_3NO_5S$ (M^+) requires 455, found 455 (12%), 359 (18%), 285 (40%), 230 (27%), 185 (100%), 130 (49%).

(iii) Mitsunobu Conditions[4]

To ethyl 2-(3-methylindolyl)-3-(2,4,5-trifluoro-phenyl)-3-hydroxypropionate, **46** (86.7mg, 0.23mmol) and triphenylphosphine (88.9mg, 0.33mmol) in THF (10mL) at room temperature, was added diethyl azodicarboxylate (56.0mg, 0.32mmol), and the mixture was then stirred at room temperature for 3 hours. Solvent was then removed under reduced pressure, and the residue diluted with benzene (15mL) and filtered

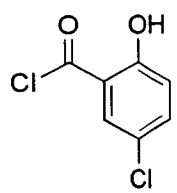
through a small pad of silica gel, washing with toluene (30mL). Solvent was removed under reduced pressure, and the crude residue was purified by flash chromatography (DCM) yielding ethyl 2-(3-methylindolyl)-3-(2,4,5-trifluorophenyl)acrylate, **45** (21.2mg, 0.059mmol, 26%) as a single (undefined, but depicted *E*) geometric isomer.



Rf (DCM) 0.69. **^{1}Hnmr ($CDCl_3$, 250MHz, δ/ppm)** 7.94 (1H, br. s, NH); 7.68 (1H, s, Ar H); 7.29 (1H, d, $^3J_{HH}$=8.1Hz); 7.21-6.87 (5H, m, Ar H); 6.84 (1H, s, Ar H); 4.15 (2H, q, $^3J_{HH}$=7.3Hz. OCH_2); 3.86 (2H, s, CH_2); 1.18 (3H, t, $^3J_{HH}$=7.3Hz). **MS (FAB, M^+/z)** $C_{20}H_{16}F_3NO_2$ (M^+) requires 359, found 359 (100%), 285 (89%), 185 (82%), 157 (41%), 130 (41%).

Preparation of 5-Chloro-2-hydroxybenzoyl Chloride, 52[5]

5-Chlorosalicylic acid, **53** (5.1g, 0.03mol), was stirred in methanol (40ml) and to the solution was added 2 drops of phenolphthalein indicator. Sodium hydroxide solution (2N) was added to the methanolic solution until a permanent pink colour formed. HCl solution (2N) was then added dropwise until the pink colour just disappeared. The solvent was then removed under reduced pressure, and the residue dried under vacuum 40°C (mp>300°C). The salt was suspended in dry benzene (80ml), and heated to 60-70°C. Thionyl chloride (3.9g, 0.033mol) was added slowly, and heating continued for 3h. The reaction mixture was then cooled to room temperature, and the insoluble material removed by centrifugation. The precipitate was washed with fresh benzene (30ml), and the supernatants combined. Solvent was removed under reduced pressure to yield 5-chloro-2-hydroxy-benzoyl chloride, **52**, as a white solid (4.54g, 0.024mmol, 80%).



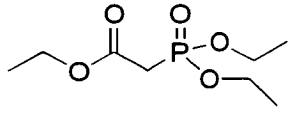
Rf 0.48 ($CHCl_3$/methanol, 9:1). **m.p.** 53-55°C (lit.[6] 56.5-57°C). **FTIR ν_{max}/cm^{-1} (bromoform)** 3706 (OH); 3021 (Ar CH); 1690 (C=O); 1609, 1566 (C=C). **λ_{max}/nm (DCM), ε/dm^{3}mol^{-1}cm^{-1})** 346 (4140), 257 (11500), 229 (14600). **^{1}Hnmr ($CDCl_3$, 200MHz, δ/ppm)** 9.63 (1H, br. s, OH); 8.03 (1H, d, $^3J_{HH}$=2.5Hz, Ar H); 7.52 (1H, dd, $^3J_{HH}$=9.0, 2.5Hz, Ar H); 6.99 (1H, d, $^3J_{HH}$=9.0Hz, Ar H). **^{13}C{^{1}H}nmr ($CDCl_3$,**

63MHz, δ/ppm) 172.8 (C=O); 159.9, 125.0, 117.8 (*q* Ar C); 138.2, 132.5, 119.7 (Ar CH). **HRMS (EI, M^+/z)** $C_7H_4Cl_2O_2$ (M^+) requires 189.9588, found 189.9594.

4.3 Synthesis of Alkylated Phosphonates

Preparation of Triethyl Phosphonoacetate, 55.[7]

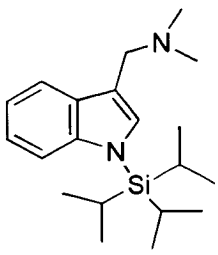
Ethyl bromoacetate (10.0g, 0.060mol) was heated at reflux in toluene (30mL). Triethylphosphite (10.0g, 0.060mol) was added *via* syringe, and reflux continued for 18 hours. The reaction mixture was cooled to room temperature, and solvent removed under reduced pressure to leave a yellow oil. This was purified by distillation (74°C, 0.05mmHg; lit. 142°C, 9mmHg[7]) to yield triethyl phosphonoacetate, **55**, as a colourless oil (12.1g, 0.054mol, 90%).



Rf 0.72 ($CHCl_3$/methanol, 9:1). **FTIR ν_{max}/cm^{-1} (liquid film)** 2985 (CH); 1738 (C=O); 1271 (P=O); 1026 (P-O). **^{1}Hnmr ($CDCl_3$, 250MHz, δ/ppm)** 4.18-4.05 (6H, m, 3×OCH_2); 2.89 (2H, d, $^2J_{PH}$=21.6Hz, CH_2); 1.28 (6H, dt, $^3J_{HH}$=7.1Hz, $^4J_{PH}$=0.5Hz, 2×$POCH_2\underline{CH_3}$); 1.22 (3H, t, $^3J_{HH}$=7.1Hz, CH_3). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz, δ/ppm)** 165.6 (d, $^2J_{PC}$=6Hz, C=O); 62.5 (d, $^2J_{PC}$=6.2Hz, $POCH_2$); 61.3 (OCH_2); 34.1, (d, $^1J_{PC}$=124Hz, CH_2); 16.1, (d, $^3J_{PC}$=6.3Hz, CH_3); 13.8 (CH_3). **MS (EI, M^+/z)** $C_8H_{17}O_5P$ (M^+) requires 224.0814; found 224.0810.

Preparation of 1-Triisopropylsilylgramine, 56.[8]

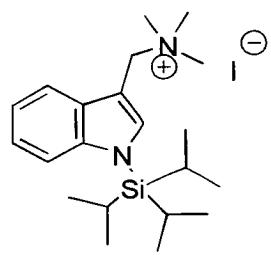
Sodium hydride (80% dispersion in oils, 1.0g, 33mmol) was THF (8mL) at 0°C. Gramine, **31** (1.0g, 5.75mmol), was added as a solution in THF (5mL) and the suspension stirred at 0°C for 3 hours. Triisopropylsilyl chloride was added dropwise, and the resultant mixture stirred at 0°C under argon for 18 hours. The suspension was then carefully quenched with water (30mL) and organics extracted with ether (2×30mL). The combined extracts were washed with water (2×40mL), dried over sodium sulphate, and solvent removed under reduced pressure to yield a yellow oil. This was purified by flash chromatography to yield 1-triisopropylsilylgramine, **56**, as a pale yellow oil (1.89g, 5.7mmol, 99%).



Rf 0.49 ($CHCl_3$/methanol, 9:1). **FTIR ν_{max}/cm^{-1} (liquid film)** 3046 (Ar CH); 2944, 2866 (CH); 2763 (N-CH_3); 1540, 1458. **λ_{max}/nm (ethanol, ε/dm^{3}mol^{-1}cm^{-1})** 290 (4250), 279 (5820), 273 (5980), 223 (27800). **^{1}Hnmr ($CDCl_3$, 250MHz, δ/ppm)** 7.69-7.65 (1H, m, Ar H); 7.50-7.46 (1H, m, Ar H); 7.15-7.09 (3H, m, Ar H); 3.65 (2H, s, CH_2); 2.28 (6H, s, 2×NCH_3); 1.71 (3H, sept., $^3J_{HH}$=7.6Hz, 3×CH); 1.14 (18H, d, $^3J_{HH}$=7.6Hz, 6×CH_3). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz, δ/ppm)** 141.1, 131.2, 114.7 (*q* Ar C); 136.5, 130.4, 121.2, 119.4, 118.9 (Ar CH); 54.5 (CH_2); 45.1 (2×NCH_3); 18.0 (6×CH_3); 12.7 (3×CH). **HRMS (FAB, M$^+$/z)** $C_{20}H_{35}N_2Si$ (MH^+) requires 331.2564, found 331.2564.

Preparation of 1-Triisopropylsilylgraminemethiodide, 57[9]

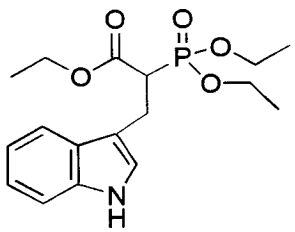
Methyl iodide (1.7g, 12mmol) was added dropwise to a solution of 1-triisopropylsilylgramine, **56** (0.99g, 3.0mmol), in THF (20mL) and the resultant mixture stirred for 3 hours at room temperature. The precipitate formed was filtered, and recrystallised from DCM/ether to form 1-triisopropylsilylgramine-methiodide, **57**, as a white powder (1.42g, 3.0mmol, 100%).



Rf 0.22 ($CHCl_3$/methanol, 9:1). **m.p.** 187-191°C. **FTIR ν_{max}/cm^{-1} (DCM)** 3051 (Ar CH); 2953, 2868 (CH); 1606, 1543 (C=C). **λ_{max}/nm (ethanol, ε/dm^{3}mol^{-1}cm^{-1})** 288 (5020), 270 (8410). **^{1}Hnmr ($CDCl_3$, 250MHz, δ/ppm)** 7.89-7.85 (1H, m, Ar H); 7.78 (1H, s, Ar H); 7.51-7.47 (1H, m, Ar H); 7.17-7.13 (2H, m, Ar H); 5.14 (2H, s, CH_2); 3.40 (9H, s, 3×CH_3); 1.70 (3H, sept., $^3J_{HH}$=7.6Hz, 3×CH); 1.06 (18H, d, $^3J_{HH}$=7.5Hz, 6×CH_3). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz, δ/ppm)** 140.9, 130.2, 104.5 (*q* Ar C); 136.9, 122.4, 121.3, 118.9, 114.3 (Ar CH); 62.0 (CH_2); 52.5 (3×NCH_3); 17.8 (6×CH_3); 12.4 (3×CH). **HRMS (FAB, M$^+$/z)** $C_{21}H_{37}N_2Si$ (M^+) requires 345.2726, found 345.2725.

Preparation of Triethyl 2-(3-Methlylindolyl)phosphonoacetate, 59.

Triethyl phosphonoacetate, **55** (2.08g, 9.28mmol), and TIPS-graminemethiodide, **57** (4.38g, 9.28mmol), were stirred in THF (80mL) at 0°C. Tetrabutylammonium-fluoride (1.0M in THF, 20mL, 20mmol) was added in one portion, and stirring continued for 1 hour. The mixture was quenched with saturated ammonium chloride solution (60mL) and organic products extracted with ether (2×50mL). The combined organics were washed with saturated sodium thiosulphate (50mL) and water (50mL) and dried over sodium sulphate. Solvent was removed under reduced pressure and the residue purified by flash chromatography (ethyl acetate/hexane, 1:1 to ethyl acetate/hexane, 5:1) to yield triethyl 2-(3-methlylindolyl)phosphonoacetate, **59**, as a colourless oil (3.02g, 8.54mmol, 92%).

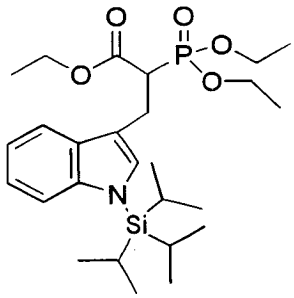


Rf 0.24 (ethyl acetate/hexane, 3:1). **FTIR ν_{max}/cm^{-1} (liquid film)** 3264 (NH); 3058 (Ar CH); 2980, 2931 (CH); 1734 (C=O); 1239 (P=O); 1023 (P-O). **λ_{max}/nm (DCM, ε/dm^{3}mol^{-1}cm^{-1})** 290 (3740), 280 (4600), 274 (4490). **^{1}Hnmr ($CDCl_3$, 250MHz, δ/ppm)** 8.22 (1h, br. s, NH); 7.61-7.57 (1H, m, ArH); 7.35-7.31 (1H, m, Ar H); 7.20-7.00 (3H, m, Ar H); 4.25-4.01 (6H, m, 3×OCH_2); 3.46-3.28 (3H, m, CH/CH_2); 1.39-1.30 (6H, m, 2×CH_3); 1.11 (3H, t, $^3J_{HH}$=7.1Hz, CH_3). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz, δ/ppm)** 168.9 (C=O); 136.1, 126.7 (*q* Ar); 112.1 (d, $^3J_{PC}$=17Hz, *q* Ar); 122.3, 121.7, 119.0, 118.2, 111.1 (Ar CH); 62.7 (d, $^2J_{PC}$=6.6Hz, $POCH_2$); 61.1 (OCH_2); 46.7 (CH, d, $^1J_{PC}$=128Hz, CH); 22.7 (d, $^2J_{PC}$=4.1Hz, CH_2); 16.1 (d, $^3J_{PC}$=6.0Hz, $POCH_2\underline{C}H_3$); 13.7 (CH_3). **MS (FAB, M^+/z)** $C_{17}H_{25}NO_5P$ (MH^+) requires 354.1470; found 354.1466.

Preparation of Triethyl 2-(1-Triisopropylsilyl-3-methlylindolyl)phosphono-acetate, 58.

Triethyl 2-(3-methlylindolyl)posphonoacetate, **59** (572.1mg, 1.62mmol), was stirred in THF (10mL) at room temperature. Sodium hydride (60% dispersion in oils, 80.2mg, 2.01mmol) was added as a suspension in THF (4mL) *via* syringe and the resultant mixture stirred for 1 hour. Triisopropylsilyl chloride (360mg, 1.87mmol) was added and the mixture then stirred for 16 hours. The reaction was then quenched by

careful addition of water (30mL), and organic products were extracted with ether (2×40mL). The combined extracts were washed with water (50mL), dried over sodium sulphate, and solvent removed under reduced pressure. The residue was purified by flash chromatography (ethyl acetate/hexane, 2:1) to yield triethyl-2-(1-triisopropylsilyl-3-methlylindolyl)-phosphonoacetate, **58**, as a pale yellow oil (442.0mg, 0.867mmol, 54%).

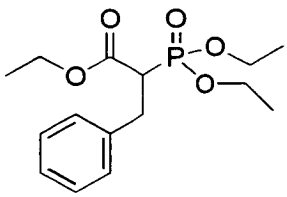


Rf 0.49 (ethyl acetate/hexane, 2:1). **FTIR ν_{max}/cm^{-1} (DCM)** 3054 (Ar CH); 2985, 293 (CH); 1730 (C=O); 1248 (P=O); 1023 (P-O). **^{1}Hnmr ($CDCl_3$, 250MHz, δ/ppm)** 7.59-7.55 (1H, m, ArH); 7.48-7.43 (1H, m, Ar H); 7.15-7.10 (2H, m, Ar H); 7.04 (1H, s, ArH); 4.29-4.12 (4H, m, 2×OCH_2); 4.10-3.96 (2H, m, OCH_2); 3.44-3.29 (3H, m, CH/CH_2); 1.66 (sept., 3H, $^3J_{HH}$=7.3Hz, 3×CH); 1.36 (6H, dt, $^3J_{HH}$=7.0Hz, $^4J_{PH}$=2.9Hz, 2×$POCH_2C\underline{H_3}$); 1.10 (18H, d, $^3J_{HH}$=7.3Hz, 6×CH_3); 1.06 (3H, t, $^3J_{HH}$=7.0Hz, CH_3). **$^{13}C\{^1H\}$nmr ($CDCl_3$, 63MHz, δ/ppm)** 168.9 (d, $^2J_{PC}$=4.4Hz); 141.0, 130.2 (q Ar); 114.4 (d, $^3J_{PC}$=17.9Hz, q Ar); 129.0, 121.3, 119.3, 118.2, 113.8 (Ar H); 62.9, 62.5 (both d, $^2J_{PC}$=6.7, 7.0Hz, $POCH_2$), 61.0 (OCH_2); 46.5 (CH, d, $^1J_{PC}$=128Hz, CH); 22.7 (d, $^2J_{PC}$=3.7Hz, CH_2); 17.9 (6×CH_3); 16.3, 16.2 (both d, $^3J_{PC}$=2.0, 2.4Hz, $POCH_2\underline{C}H_3$) 13.8 (CH_3); 12.6 (3×CH). **HRMS (FAB, M^+/z)** $C_{26}H_{45}NO_5PSi$ (MH^+) requires 510.2805; found 510.2806.

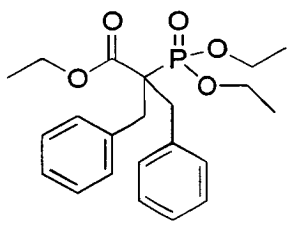
Preparation of Triethyl 2-benzylphosphonoacetate, 61.

Triethyl phosphonoacetate, **55** (502.7mg, 2.24mmol), was stirred in THF (10mL) at room temperature. Sodium hexamethyldisilazide (NaHMDS, 1.0M in THF, 2.7mL, 2.7mmol) was added, and the solution stirred for 1.5 hours. Benzyl bromide (544mg, 3.28mmol) was added, and stirring continued for 20 hours, after which time, a white precipitate had formed. The mixture was quenched with water (20mL), and organic products were extracted with ethyl acetate (2×25mL). The combined extracts were dried over sodium sulphate and solvent removed under reduced pressure. The residue was purified by flash chromatography (ethyl acetate/hexane, 3:1) to yield a mixture of co-eluting products (620.5mg). These were separated by preparative HPLC (Gradient

A), and identified as the desired compound triethyl-2-benzyl-phosphonoacetate, **61** (420.1mg, 1.34mmol, 60%) and 2,2-dibenzyl-triethylphosphonoacetate, **62** (200.4mg, 0.50mmol, 22%).



Rf 61[10] 0.36 (ethyl acetate/hexane, 3:1). **HPLC Retention Time (Gradient 1)** 21.5 minutes. **FTIR ν_{max}/cm^{-1} (DCM)** 3026 (Ar CH); 2981, 2931, 2909 (CH); 1730 (C=O); 1603, 1494 (C=C); 1027 (P-O). **λ_{max}/nm (DCM, ε/dm^{3}mol^{-1}cm^{-1})** 226 (4600). **^{1}Hnmr (CDCl$_3$, 250MHz, δ/ppm)** 7.27-7.13 (5H, m, ArH); 4.21-4.09 (4H, m, 2×POCH$_2$); 4.11-4.02 (2H, m, -OCH$_2$); 3.31-3.14 (3H, m, CH/CH$_2$); 1.32 (6H, t, $^3J_{HH}$=7.1Hz, 2×POCH$_2$C<u>H$_3$</u>); 1.11 (3H, t, $^3J_{HH}$=7.1Hz, CH$_3$). **^{13}C{^{1}H}nmr (CDCl$_3$, 63MHz, δ/ppm)** 168.3 (d, $^2J_{PC}$=4.8Hz, C=O); 138.3 (d, $^3J_{PC}$= 15.9Hz, *q* Ar); 128.4, 128.3, 126.5 (Ar CH); 62.7, 62.6 (both d, $^2J_{PC}$=6.5, 6.8Hz, OCH$_2$), 61.1 (OCH$_2$); 47.4 (d, $^1J_{PC}$=129Hz, CH); 32.6 (d, $^2J_{PC}$=4.5Hz, CH$_2$); 16.2, 16.1 (both d, $^3J_{PC}$=1.8, 2.3Hz, POCH$_2$<u>C</u>H$_3$) 13.8 (CH$_3$). **^{31}Pnmr (CDCl$_3$, 101MHz, δ/ppm)** 22.6. **MS (EI, M$^+$/z)** $C_{15}H_{23}O_5P$ (M$^+$) requires 314.1283; found 314.1285.

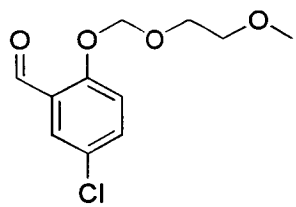


Rf 62 0.36 (ethyl acetate/hexane, 3:1). **HPLC Retention Time (Gradient 1)** 25.6 minutes. **FTIR ν_{max}/cm^{-1} (DCM)** 3034 (Ar CH); 2982, 2931, 2909 (CH); 1726 (C=O); 1603, 1494 (C=C); 1055 (P-O). **λ_{max}/nm (DCM, ε/dm^{3}mol^{-1}cm^{-1})** 275 (5010). **^{1}Hnmr (CDCl$_3$, 250MHz, δ/ppm)** 7.29-7.17 (10H, m, ArH); 4.21 (2H, q, $^3J_{HH}$=7.1Hz, OCH$_2$); 4.03-3.80 (4H, m, 2×POCH$_2$); 3.28 (2H, s, CH$_2$); 3.22 (2H, d, $^3J_{PH}$=2.5Hz, CH$_2$); 1.24 (3H, t, $^3J_{HH}$=7.1Hz, CH$_3$); 1.11 (6H, t, $^3J_{HH}$=7.1Hz, 2×POCH$_2$C<u>H$_3$</u>); **^{13}C{^{1}H}nmr (CDCl$_3$, 63MHz, δ/ppm)** 170.5 (d, $^2J_{PC}$=1.4Hz, C=O); 136.3 (d, $^3J_{PC}$= 7.8Hz, *q* Ar); 130.7, 127.6, 126.5 (Ar CH); 62.1 (d, $^2J_{PC}$=7.3Hz, POCH$_2$), 61.2 (OCH$_2$); 55.2 (d, $^1J_{PC}$=141Hz, *q* C); 39.8 (d, $^2J_{PC}$=2.9Hz, CH$_2$); 16.0 (d, $^3J_{PC}$=6.4, 2.3Hz, POCH$_2$<u>C</u>H$_3$) 13.8 (CH$_3$). **^{31}Pnmr (CDCl$_3$, 101MHz, δ/ppm)** 24.8. **MS (EI, M$^+$/z)** $C_{22}H_{29}O_5P$ (M$^+$) requires 404.1753; found 404.1749.

4.4 Synthesis of Phenol-protected 5-Chlorosalicylaldehydes.

Preparation of β-Methoxyethoxymethoxy-5-chlorosalicylaldehyde, 67.

Sodium hydride (60% dispersion in oils, 0.57g, 14mmol) was suspended in THF (25mL) at 0°C. 5-Chlorosalicylaldehyde, **33**, (2.0g, 13mmol) was then added dropwise as a solution in THF (5mL), and the suspension immediately turned bright yellow. The mixture was stirred at room temperature for 1 hour. β-Methoxythethoxymethylchloride (1.7g, 14mmol) was added, and the resultant mixture stirred at room temperature for 2 hours, by which time the yellow colour had disappeared. The mixture was then quenched at 0°C by addition of HCl (1N, 40mL). Organic products were extracted with DCM (2×50mL), washed with water (2×50mL) and dried over sodium sulphate. Solvent was removed under reduced pressure forming a pale yellow oil, which was purified by flash chromatography (ethyl acetate/hexane, 2:1) yielding β-methoxy-ethoxymethoxy-5-chloro-salicylaldehyde, **67**, as a pale yellow oil (2.78g, 11.4mmol, 89%).

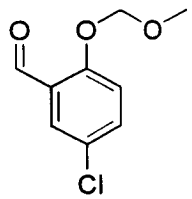


Rf 0.65 (ethyl acetate/hexane, 3:2). **FTIR ν_{max}/ cm^{-1} (liquid film)** 3073 (Ar CH); 2905, 2880 (CH); 1682 (C=O); 1596, 1479 (C=C); 1105 (C-O). **λ_{max}/nm (DCM, ε/dm^{3}mol^{-1}cm^{-1})** 325 (3450), 250 (8780), 228 (8270). **^{1}Hnmr ($CDCl_3$, 250MHz, δ/ppm)** 10.39 (1H, s, CHO); 7.75 (1H, d, $^3J_{HH}$=2.8Hz, Ar H); 7.44 (1H, dd, $^3J_{HH}$=8.8, 2.8Hz); 7.22 (1H, d, $^3J_{HH}$=8.8Hz); 5.36 (2H, s, OCH_2O); 3.85-3.82 and 3.55-3.52 (both 2H, m, OCH_2); 3.34 (3H, s, OCH_3). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz, δ/ppm)** 188.2 (C=O); 158.0, 127.5, 126.2 (*q* Ar C); 135.3, 127.7, 116.8 (Ar CH); 93.7 (OCH_2O); 71.3, 68.2 (OCH_2); 58.9 (OCH_3). **HRMS (EI, M$^+$/z)** $C_{11}H_{13}O_4$ (M^+) requires 244.0502, found 244.0507.

Preparation of Methoxymethoxy-5-chlorosalicylaldehyde, 68.

Sodium hydride (80% dispersion in oils, 45.9mg, 1.53mmol) was suspended in THF (5mL) at room temperature. 5-Chlorosalicylaldehyde, **33** (52.6mg, 0.34mmol) was added dropwise as a solution in THF (5mL), and the suspension immediately turned bright yellow. The mixture was stirred at room temperature for 30 minutes. Methoxymethylchloride (84.8mg, 1.05mmol) was added, and the resultant mixture

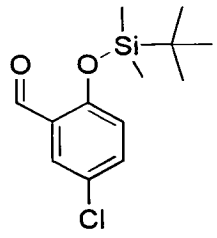
stirred at room temperature for 20 minutes, by which time the yellow colour had disappeared. The mixture was quenched at 0°C by addition of water (10mL). The organic products were then extracted with DCM (2×15mL), washed with water (2×15mL) and dried over sodium sulphate. Solvent was removed under reduced pressure forming a pale yellow oil, which was purified by column chromatography to yield methoxymethoxy-5-chloro-salicylaldehyde, **68**, as a pale yellow oil (72.8mg, 0.36mmol, 100%).



Rf 0.34 (DCM/hexane, 1:1). **FTIR ν_{max}/ cm^{-1} (liquid film)** 3070 (Ar CH); 2956, 2869 (CH); 1681 (C=O); 1595, 1478 (C=C); 1123 (C-O). **λ_{max}/nm (ethanol, ε/dm^{3}mol^{-1}cm^{-1})** 321 (1830), 249 (4970). **^{1}Hnmr ($CDCl_3$, 250MHz, δ/ppm)**[11] 10.42 (1H, s, CHO); 7.79 (1H, d, $^3J_{HH}$=2.8Hz, Ar H); 7.47 (1H, dd, $^3J_{HH}$=9.0, 2.8Hz); 7.19 (1H, d, $^3J_{HH}$=9.0Hz); 5.29 (2H, s, OCH_2O); 3.52 (3H, s, OCH_3). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz, δ/ppm)** 188.3 (C=O); 158.0, 127.5, 126.2 (*q* Ar C); 135.3, 127.7, 116.7 (Ar CH); 94.7 (OCH_2O); 56.4 (CH_3). **MS (EI, M$^+$/z)** $C_9H_9ClO_3$ (M^+) requires 200.0240, found 200.0236.

Preparation of 1-*t*-Butyldimethylsilyloxy-4-chloro-2-benzaldehyde, 69.

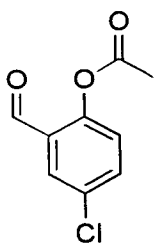
5-Chlorosalicylaldehyde, **33** (365.9mg, 2.34mmol), was stirred with imidazole (939.6mg, 13.8mmol) in DMF (10mL) at room temperature, forming a yellow solution. Meanwhile, *t*-butyldimethylsilyl chloride (TBSCl, 701.6mg, 4.66mmol) was sonicated with DMAP (10mol%) in DMF (4mL). After 30 mins, the TBSCl solution was added dropwise to the yellow reaction mixture. Stirring was continued for 20 hours, after which time the mixture was diluted with ether (20mL) and quenched with HCl (1N, 20mL). The organic layer was separated and the aqueous layer washed with more ether (2×20mL) and the combined organic extracts washed with 5% LiCl solution (40mL). The organic layer was then dried over sodium sulphate and solvent removed under reduced pressure. The crude residue was purified by flash chromatography (hexane/DCM, 2:1), to yield 1-*tert*-butyldimethylsilyloxy-4-chloro-2-benzaldehyde, **69**, as a white crystalline solid (326.1mg, 1.20mmol, 51%). Starting material (94.4mg, 0.602mmol, 26%) was also recovered.



Rf 0.42 (DCM/hexane, 1:1). **m.p.** 65-67°C. **FTIR ν_{max}/ cm^{-1} (DCM)** 3063 (Ar CH); 2953, 2862 (CH); 1684 (C=O); 1595, 1543, 1474 (C=C). **λ_{max}/nm (ethanol, ε/dm^{3}mol^{-1}cm^{-1})** 325 (3710), 248 (9190). **^{1}Hnmr ($CDCl_3$, 250MHz, δ/ppm)** 10.35 (1H, s, CHO); 7.72 (1H, dd, $^3J_{HH}$=2.8, 0.4Hz, ArH); 7.37 (1H, dd, $^3J_{HH}$=8.8, 2.8Hz, ArH); 6.81 (1H, dd, $^3J_{HH}$=8.8, 0.4Hz. ArH); 0.99 (9H, s, ^{t}Bu); 0.25 (6H, s, 2×CH_3). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz, δ/ppm)** 190.6 (C=O); 157.2, 127.8, 126.9 (*q* Ar C); 135.2, 127.7, 121.6 (Ar CH); 25.4 (3×CH_3); 18.2 (*q* C); -4.5 (CH_3). **MS (FAB, M$^+$/z)** $C_{13}H_{20}ClO_2Si$ (MH^+) requires 271.0921, found 271.0923.

Preparation of 5-Chloro-2-acetylbenzaldehyde, 70.

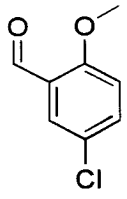
5-Chlorosalicylaldehyde, **33** (262.2mg, 1.67mmol), was stirred in THF (10mL) at room temperature and triethylamine (190mg, 1.88mmol) added, forming a yellow solution. A solution of acetic anhydride (390mg, 3.82mmol) in THF (2mL), was treated with DMAP (10mol%) for 15 minutes before slow addition to the yellow solution *via* a cannula. The reaction mixture turned colourless on complete addition of the anhydride solution and stirring was continued for a further 90 minutes. The mixture quenched with HCl (1N, 20mL) and the organic products were extracted with ether (3×25mL). The combined organics were washed with sodium bicarbonate solution (25mL), and dried over sodium sulphate. Solvent was removed under reduced pressure to yield 4-chloro-2-acetylbenzaldehyde, **70**, as a white crystalline solid (330.7mg, 1.66mmol, 99%).



Rf 0.32 (DCM/hexane, 1:1). **m.p.** 56-58°C. **FTIR ν_{max}/ cm^{-1} (liquid film)** 3066 (Ar CH); 2988, 2857 (CH); 1769 (CO_2CH_3); 1692 (C=O); 1596, 1575, 1475 (C=C). **λ_{max}/nm (ethanol, ε/dm^{3}mol^{-1}cm^{-1})** 301 (1830), 245 (8860). **^{1}Hnmr ($CDCl_3$, 250MHz, δ/ppm)** 10.00 (1H, s, CHO); 7.78 (1H, d, $^3J_{HH}$=2.6Hz, Ar H); 7.52 (1H, dd, $^3J_{HH}$=8.7, 2.6Hz, Ar H); 7.10 (1H, d, $^3J_{HH}$=8.7Hz, Ar H); 2.33 (3H, s, CH_3). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz, δ/ppm)** 187.1 (C=O); 168.8 ($COCH_3$); 149.8, 132.0, 128.8 (*q* Ar C); 134.8, 130.1, 124.8 (Ar CH); 20.5 (CH_3). **MS (FAB, M$^+$/z)** $C_9H_7ClO_3$ (M^+) requires 198.0084; found 198.0088.

Preparation of 5-Chloro-2-methoxybenzaldehyde, 71[12].

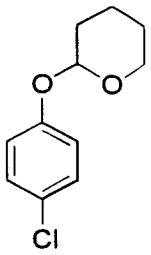
5-Chlorosalicylaldehyde, **33** (2.60g, 16.6mmol), was dissolved in acetone (pre-distilled over potassium carbonate, 100mL) at room temperature. Potassium carbonate (9.10g, 65.9mmol) was added, and a yellow suspension formed immediately. The mixture was stirred for 1 hour, after which time methyl iodide (19.2g, 135mmol) was added. The resultant mixture was stirred for 6 hours, then filtered through a small pad of kieselguhr and washed with acetone. The filtrate was concentrated, and the residue was suspended in DCM (40mL) and filtered through a small pad of silica. The filtrate was concentrated to yield 5-chloro-2-methoxybenzaldehyde, **71**, as a pale yellow solid (2.53g, 14.8mmol, 89%).



Rf 0.47 (DCM). **m.p.** 78-80°C (lit.[12] 80-81°C). **FTIR ν_{max}/ cm^{-1} (DCM)** 3015 (Ar CH); 2942, 2874, 2845 (CH); 1680 (C=O); 1595, 1484 (Ar C=C); 1127 (C-O). **λ_{max}/nm (ethanol, ε/dm^{3}mol^{-1}cm^{-1})** 331 (4570), 249 (9320), 223 (21000). **^{1}Hnmr ($CDCl_3$, 250MHz, δ/ppm)**. 10.36 (1H, s, CHO); 7.73 (1H, d, $^3J_{HH}$=2.8Hz, ArH); 7.45 (1H, dd, $^3J_{HH}$=8.9, 2.9Hz, ArH); 6.92 (1H, d, $^3J_{HH}$=8.9Hz, ArH); 3.90 (3H, s, OCH_3). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz, δ/ppm)** 188.3 (C=O); 160.1, 126.1, 125.4 (*q* Ar); 135.2, 127.8, 113.1 (Ar CH); 55.8 (OCH_3). **HRMS (EI), M$^+$/z** $C_8H_7ClO_2$ (M^+) requires 170.0138; found 170.0135. **Elemental Analysis** $C_7H_8ClO_2$ requires 56.32% C, 4.14% H; found 56.40% C, 4.05% H.

Preparation of Tetrahydro-2-(4-chlorophenoxy)-2*H*-pyran, 83[13].

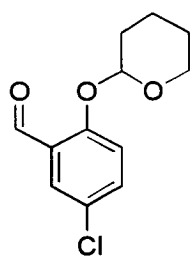
4-Chlorophenol (25.1g, 0.195mol) and dihydropyran (33.5g, 0.398g) were stirred in dry chloroform at room temperature. *para*-Toluenesulphonic acid (10mg) was added, and the solution stirred for 2.5 hours (heat evolved). Chloroform was removed under vacuum and the crude product diluted with ether (100mL) and washed with sodium hydroxide (1N, 3×50mL). The ether solution was then dried over sodium sulphate, and solvent removed under reduced pressure. The resultant yellow oil was distilled over sodium hydroxide pellets (95-98°C, 0.7mmHg) to give a white crystalline solid. This was recrystallised from hexane to yield tetrahydro-2-(4-chlorophenoxy)-2*H*-pyran, **83**, as a white crystalline solid (35.6g, 0.167mol, 86%).



Rf 0.80 (DCM/hexane, 3:2). **m.p.** 48-50°C (lit. 48-49°C[13]). **FTIR ν_{max}/ cm^{-1} (DCM)** 3060 (Ar CH); 2948, 2873, 2854 (CH); 1596, 1489 (C=C); 1120 (C-O). **λ_{max}/nm (ethanol, ε/dm^{3}mol^{-1}cm^{-1})** 286 (1250), 279 (1560), 226 (12200). **^{1}Hnmr ($CDCl_3$, 250MHz, δ/ppm)**. 7.22 and 6.98 (both 2H, m, Ar H); 5.36 (1H, t, $^3J_{HH}$=3.2Hz, OCHO); 3.91-3.82 and 3.63-3.55 (both 1H, m, OCH_2); 2.05-1.89 (1H, alkyl H); 1.87-1.81 (2H, m, alkyl H); 1.71-1.55 (3H, m, alkyl H). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz, δ/ppm)** 155.5, 126.2 (*q* Ar); 129.0, 117.6 (Ar CH); 96.3 (OCHO); 61.8 (OCH_2); 30.0, 24.9, 18.5 (CH_2). **HRMS (EI), M$^+$/z** $C_{11}H_{13}ClO_2$ (M^+) requires 212.0603, found 212.0604. **Elemental Analysis** $C_{11}H_{13}ClO_2$ requires 62.12% C, 6.16% H; found 62.14% C, 6.16% H.

Preparation of 5-Chloro-2-(tetrahydro-2*H*-pyran-2yloxy)-benzaldehyde, 84.[13]

To *n*-butyllithium (2.4M in hexanes, 6.8mL, 16.3mmLol) at O°C was added TMEDA (2.5mL, 16.6mmol) dropwise, and the mixture stirred for 30 minutes. Tetrahydro-2-(4-chlorophenoxy)-2*H*-pyran, **83** (3.17g, 14.9mmol), was then added dropwise over a period of 20 minutes as a solution in THF (4mL). Stirring was continued for 2 hours after which time a white precipitate had formed. DMF (4.0mL, 52mmol) was then added as a solution in dry toluene (5mL), the precipitate cleared, and stirring was continued for 12h. The mixture was then poured onto iced HCl (1N, 100mL), and organics were extracted with ether (2×100mL), washed with brine (100mL), and dried over sodium sulphate. Solvent was removed under reduced pressure, and the crude residue was purified by flash chromatography to yield a pale yellow solid. This was recrystallised from hexane to yield 5-chloro-2-(tetrahydro-2*H*-pyran-2yloxy)-benzaldehyde, **84**, as a pale yellow solid (2.73g, 11.4mmol, 76%).



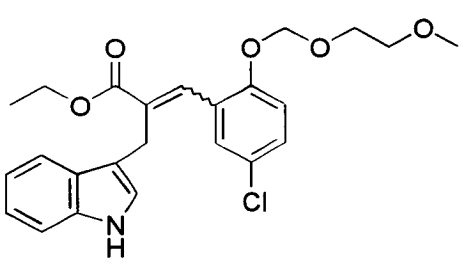
Rf 0.66 (DCM/hexane, 3:2). **m.p.** 54-56°C. **FTIR ν_{max}/ cm^{-1} (DCM)** 3060 (Ar CH); 2949, 2874 (CH); 1683 (C=O); 1595, 1475 (C=C); 1120 (C-O). **λ_{max}/nm (ethanol, ε/dm^{3}mol^{-1}cm^{-1})** 325 (3760), 249 (9440). **^{1}Hnmr ($CDCl_3$, 250MHz, δ/ppm)**. 10.42 (1H, s, CHO); 7.76 (1H, d, $^3J_{HH}$=2.8Hz, ArH); 7.40 (1H, dd, $^3J_{HH}$=9.0, 2.7Hz, ArH); 7.17 (1H, d, $^3J_{HH}$=9.0Hz, Ar H); 5.50 (1H, t, $^3J_{HH}$=3.7Hz, OCHO);

3.83-3.75 and 3.65-3.59 (both 1H, m, OCH_2); 1.95-1.87 (3H, alkyl H); 1.75-1.59 (3H, m, alkyl H). **$^{13}C\{^1H\}$nmr ($CDCl_3$, 63MHz, δ/ppm)** 188.2 (C=O); 157.7, 126.9, 126.0 (*q* Ar); 135.1, 127.2, 117.0 (Ar CH); 96.5 (OCHO); 61.9 (OCH_2); 29.7, 24.7, 18.2 (CH_2). **HRMS (EI), M^+/z** $C_{12}H_{13}ClO_3$ requires 240.0553, found 240.0565. **Elemental Analysis** $C_{12}H_{13}ClO_3$ requires 59.88% C, 5.44% H; found 59.90% C, 5.25% H.

4.5 Horner-Wadsworth-Emmons Reactions

Preparation of Ethyl 2-(3-Methylindolyl)-3-(2-β-methoxyethoxymethoxy-5-chlorophenyl)acrylate, 72.

To triethyl 2-(3-methlylindolyl)phosphonoacetate, **59** (483.6mg, 1.37mmol), in THF (30mL) at room temperature, was added NaHMDS (1.08M in THF, 1.29mL, 1.39mmol), slowly *via* syringe, and the resultant solution stirred for 30 minutes. 2-β-Methoxyethoxymethoxy-5-chlorosalicylaldehyde, **67** (410.8mg, 0.211mmol), was added as a solution in THF (5mL) *via* syringe, and the mixture then stirred for 16 hours. The reaction was quenched with HCl (1N, 50mL), and organic products extracted with ether (2×50mL). The combined extracts were dried over sodium sulphate and solvent was removed under reduced pressure. The crude residue was purified by flash chromatography (ethyl acetate/hexane, 1:2 to 1:1) to yield ethyl [2-(3-methylindolyl)-3-(2-β-methoxyethoxymethoxy-5-chlorophenyl)]acrylate, **72**, as a pale yellow oil (535.8mg, 1.21mmol, 88%). Spectra were consistent with an isomeric mixture in a 2.2:1 ratio.

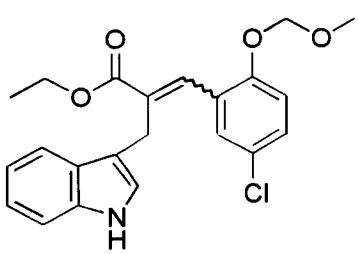


Rf 0.62 (ethyl acetate/hexane, 1:1). **FTIR ν_{max}/ cm^{-1} (liquid film)** 3382 (NH); 3058 (Ar CH); 2980, 2931 (CH); 1705 (C=O); 1622, 1592, 1479 (C=C); 1097 (C-O). **λ_{max}/nm (ethanol, ε/$dm^3mol^{-1}cm^{-1}$)** 289 (9100) 265 (15000). **^{1}Hnmr ($CDCl_3$, 250MHz, δ/ppm)** 8.14 and 8.06 (1H, both br. s, NH); 7.82-6.73 (9H, m, Ar/olefin H); 5.23 and 5.15 (2H, both s, OCH_2O); 4.21 and 4.03 (2H, both q, $^3J_{HH}$=7.1Hz, OCH_2); 3.91 (2H, s, allyl CH_2); 3.77-3.73 and 3.68-3.65

(2H, both m, MEM OCH_2); 3.53-3.49 and 3.47-3.43 (2H, both m, MEM OCH_2); 3.36 and 3.33 (3H, both s, OCH_3) 1.23 and 1.01 (3H, both t, $^3J_{HH}$=7.1Hz, CH_3). **$^{13}C\{^1H\}$nmr ($CDCl_3$, 63MHz, δ/ppm)** 168.6, 168.1 (C=O); 153.8, 152.8, 136.3, 136.1, 133.1, 128.4, 127.2, 127.0, 126.9, 126.5, 126.2, 113.7, 112.3 (*q* Ar/olefin C); 133.8, 129.4, 129.2, 129.1, 128.3, 122.8, 121.9, 121.8, 121.7, 119.2, 119.1, 118.8, 115.7, 111.0, 110.8 (Ar/olefin CH); 93.7 (OCH_2O); 71.3, 67.7 (MEM OCH_2); 60.8, 60.4 (OCH_2); 30.6, 23.4 (allyl CH_2); 14.0 (CH_3). **MS (FAB, M^+/z)** $C_{24}H_{26}ClNO_5$ (M^+) requires 443, found 443 (82%), 368 (52%), 294 (30%), 193 (37%), 154 (96%).

Preparation of Ethyl 2-(3-Methylindolyl)-3-(2-methoxymethoxy-5-chlorophenyl)acrylate, 73.

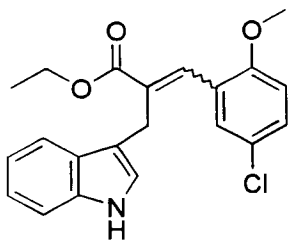
To triethyl 2-(3-methlylindolyl)phosphonoacetate, **59** (38.3mg, 0.108mmol), in THF (5mL) at room temperature, was added potassium *tert*-butoxide (1.0M in THF, 0.22mL, 0.22mmol), slowly *via* syringe, and the resultant solution stirred for 30 minutes. 2-Methoxymethoxy-5-chlorosalicylaldehyde, **68** (21.5mg, 0.107mmol), was added as a solution in THF (2mL) *via* syringe, and the mixture then stirred for 16 hours. The reaction was quenched with HCl (1N, 20mL), and organic products extracted with ether (2×20mL). The combined extracts were dried over sodium sulphate and solvent was removed under reduced pressure. The crude residue was purified by flash chromatography (ethyl acetate/hexane, 1:1) to yield ethyl 2-(3-methylindolyl)-3-(2-methoxymethoxy-5-chlorophenyl)acrylate, **73**, as a pale yellow oil (20.5mg, 0.070mmol, 48%). Spectra were consistent with an isomeric mixture in a 2.2:1 ratio.



Rf 0.62 (ethyl acetate/hexane, 1:1). **FTIR ν_{max}/ cm^{-1} ($CHCl_3$)** 3478 (NH); 3036 (Ar CH); 2959, 2929, 2868 (CH); 1705 (C=O); 1635, 1594, 1481 (C=C); 1091 (C-O). **^{1}Hnmr ($CDCl_3$, 200MHz, δ/ppm)** 8.06 and 7.99 (1H, both br. s, NH); 7.84-6.73 (9H, m, Ar/olefin H); 5.15 and 5.06 (2H, both s, OCH_2O); 4.21 and 4.03 (2H, both q, $^3J_{HH}$=7.0Hz, OCH_2); 3.93 (2H, s, allyl CH_2); 3.43 and 3.35 (2H, both s, OCH_3); 1.05 and 0.87 (3H, both t, $^3J_{HH}$=7.0Hz, CH_3).

Preparation of Ethyl 2-(3-Methylindolyl)-3-(2-methoxy-5-chlorophenyl)acrylate, 74.

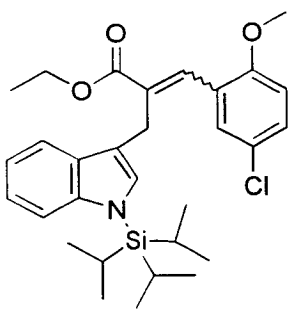
To 2-(3-methlylindolyl)-triethylphosphonoacetate, **59** (108.6mg, 0.307mmol), in THF (6mL) at room temperature, was added NaHMDS (1.0M in THF, 0.38mL, 0.38mmol), slowly *via* syringe, and the resultant solution stirred for 1 hour. 5-Chloro-2-methoxybenzaldehyde, **71** (76.8mg, 0.450mmol), was added as a solution in THF (2mL) *via* syringe, and the mixture then stirred for 2 hours. The reaction was quenched with HCl (1N, 20mL), and organic products extracted with ether (2×25mL). The combined extracts were dried over sodium sulphate and solvent was removed under reduced pressure. The crude residue was purified by flash chromatography (ethyl acetate/hexane, 1:1) to yield ethyl 2-(3-methylindolyl)-3-(2-methoxy-5-chlorophenyl)acrylate, **74**, as a pale yellow oil (70.9mg, 0.192mmol, 63%). Spectra were consistent with an isomeric mixture in a 1.8:1 ratio.



Rf 0.52 (ethyl acetate/hexane, 1:1). **FTIR ν_{max}/ cm^{-1} (DCM)** 3467 (NH) 3041 (Ar CH); 2985, 2935 (CH); 1706 (C=O); 1603, 1484 (C=C); 1091 (C-O). **λ_{max}/nm (ethanol, ε/dm^{3}mol^{-1}cm^{-1})** 267 (15100). **^{1}Hnmr ($CDCl_3$, 250MHz, δ/ppm)** 8.19 and 8.13 (1H, both br. s, NH); 7.88-6.70 (9H, m, Ar/olefin H); 4.21 and 4.06 (2H, both q, $^3J_{HH}$=7.1Hz, OCH_2); 3.94 and 3.92 (2H, both s, allyl CH_2); 3.81 and 3.73 (3H, both s, OCH_3); 1.24 and 1.3 (3H, both t, J=7.1Hz, CH_3). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz, δ/ppm)**; 168.8, 168.1 (C=O); 156.0, 155.1, 136.2, 136.1, 134.7, 132.8, 127.2, 126.9, 126.0, 125.1, 124.7, 113.6, 112.1 (*q* Ar/olefin C); 133.7, 129.3, 129.1, 128.6, 128.2, 122.8, 121.7, 119.1, 119.0, 119.0, 118.7, 111.5, 111.2, 111.0, 111.9 (Ar/olefin CH); 60.8, 60.4 (OCH_2); 55.6, 55.5 (OCH_3); 30.6, 23.4 (allyl CH_2); 14.0, 13.5 (CH_3). **HRMS (FAB, M$^+$/z)** $C_{21}H_{20}ClNO_3$ (M^+) requires 369.1132; found 369.1135.

Preparation of Ethyl 2-(1-Triisopropylsilyl-3-methylindolyl)-3-(2-methoxy-5-chlorophenyl)acrylate, 75.

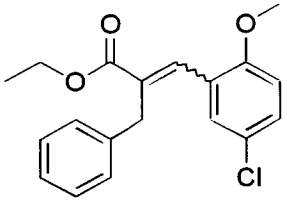
To triethyl 2-(1-triisopropylsilyl-3-methlylindolyl)phosphonoacetate, **58** (176.8mg, 0.347mmol), in THF (8mL) at room temperature, was added potassium *tert*-butoxide (1.0M in THF, 0.39mL, 0.39mmol), slowly *via* syringe, and the resultant solution stirred for 2 hours. 5-Chloro-2-methoxybenzaldehyde, **71** (64.2mg, 0.376mmol), was added as a solution in THF (2mL) *via* syringe, and the mixture then stirred for 2 hours. The reaction was quenched with HCl (1N, 20mL), and organic products extracted with ether (2×25mL). The combined extracts were dried over sodium sulphate and solvent was removed under reduced pressure. The crude residue was purified by flash chromatography (ethyl acetate/hexane, 1:2 to 1:1) to ethyl 2-(1-triisopropylsilyl-3-methylindole)-3-(2-methoxy-5-chlorophenyl)acrylate, **75**, as a pale yellow oil (176.0mg, 0.334mmol, 96%). Spectra were consistent with an isomeric mixture in a 7:2 ratio.



Rf 0.88 (ethyl acetate/hexane, 1:1). **FTIR ν_{max}/ cm^{-1} (DCM)** 3058 (Ar CH); 2985, 2949, 2868 (CH); 1705 (C=O); 1638, 1595, 1484 (C=C). **λ_{max}/nm (ethanol, ε/dm^{3}mol^{-1} cm^{-1})** 314 (4920), 291 (7710), 267 (13500). **^{1}Hnmr ($CDCl_3$, 250MHz, δ/ppm)** 7.85-6.69 (9H, m, Ar/olefin H). 4.14 and 4.03 (2H, both q, $^3J_{HH}$=7.1Hz, OCH_2); 3.91 (2H, s, allyl CH_2); 3.82 and 3.72 (3H, both s, OCH_3); 1.64 (3H, sept. $^3J_{HH}$=7.7Hz, 3×CH); 1.17-1.02 (21H, m, TIPS 6×CH_3/CH_3). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz, δ/ppm)** 168.9, 168.1 (C=O), 156.0, 155.2, 141.3, 134.9, 132.7, 132.3, 130.5, 129.8, 127.3, 126.1, 1125.2, 124.7, 113.9 (*q* Ar/olefin C); 133.7, 130.8, 129.3, 129.1, 128.8, 128.7, 128.3, 128.2, 121.3, 119.3, 119.1, 118.9, 118.6, 115.6, 113.8, 113.7, 111.5, 111.3 (Ar/olefin CH); 60.6, 60.3 (OCH_2); 55.7, 55.5 (OCH_3); 23.6, 22.9 (allyl CH_2); 18.0, 17.6 (6×CH_3); 14.1, 13.6 (3×CH), 12.6, 12.1 (CH_3). **MS (FAB, M$^+$/z)** $C_{30}H_{40}ClNO_3Si$ (M^+) requires 525.2466, found 525.2485.

Preparation of Ethyl 2-Phenyl-3-(2-methoxy-5-chlorophenyl)acrylate, 76.

To triethyl 2-benzylphosphonoacetate, **61**, (208.1mg, 0.660mmol), in THF (15mL) at room temperature, was added NaHMDS (1.1M in THF, 0.73mL, 0.80mmol), slowly *via* syringe, and the resultant solution stirred for 1 hour. 5-Chloro-2-methoxybenzaldehyde, **71** (174.3mg, 1.02mmol), was added as a solution in THF (2mL) *via* syringe, and the mixture then stirred for 2 hours. The reaction was quenched with HCl (1N, 20mL), and organic products extracted with ether (2×25mL). The combined extracts were dried over sodium sulphate and solvent was removed under reduced pressure. The crude residue was purified by flash chromatography (DCM/hexane, 2:1) to yield ethyl 2-phenyl-3-(2-methoxy-5-chlorophenyl)acrylate, **76**, as a pale yellow oil (196.7mg, 0.595mmol, 90%). Spectra were consistent with an isomeric mixture in a 3:1 ratio.

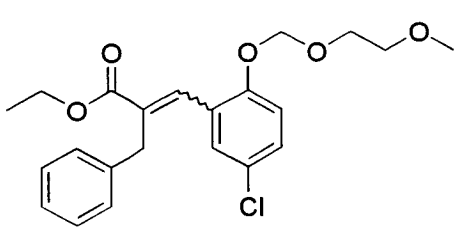


Rf 0.42 (DCM/hexane, 2:1). **FTIR ν_{max}/ cm^{-1} (DCM)** 3061 (Ar CH); 2987, 2938, 2907 (CH); 1705 (C=O); 1636, 1595, 1484 (C=C); 1092 (C-O). **λ_{max}/nm (ethanol, ε/dm^{3}mol^{-1} cm^{-1})** 315 (5150), 264 (10500), 227 (19100). **^{1}Hnmr ($CDCl_3$, 250MHz, δ/ppm)** 7.90 and 6.72 (1H, both s, olefin H) 7.31-7.10 (8H, m, Ar H); 4.19 and 4.02 (2H, both q, $^3J_{HH}$=7.1Hz, OCH_2); 3.84 (2H, s, benzyl CH_2); 3.82 and 3.78 (3H, both s, OCH_3); 1.23 and 1.01 (3H, both t, $^3J_{HH}$=7.1Hz, 6×CH_3). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz, δ/ppm)**; 168.2, 167.6 (C=O); 156.0, 155.2, 139.5, 138.2, 132.5, 125.0 (*q* Ar/olefin C); 135.9, 134.9, 130.3, 129.4, 129.2, 129.0, 128.8, 128.4, 128.3, 128.3, 127.9, 126.3, 125.9, 111.6, 111.3 (Ar/olefin CH); 60.8, 60.4 (OCH_2); 55.6, 55.5 (OCH_3); 41.0, 33.2 (benzyl CH_2); 14.0, 13.5 (CH_3). **HRMS (EI, M$^+$/z)** $C_{19}H_{19}ClO_3$ (M^+) requires 330.1023; found 330.1014.

Preparation of Ethyl 2-Benzyl-3-(2-β-Methoxyethoxymethoxy-5-chlorophenyl)acrylate, 77.

To triethyl 2-benzylphosphonoacetate, **61**, (68mol% by ^{1}Hnmr 416.3mg, 0.900mmol) in THF (15mL) at room temperature, was added NaHMDS (1.1M in THF, 1.0mL, 1.0mmol), slowly *via* syringe, and the resultant solution stirred for 2 hours. 5-Chloro-2-β-methoxyethoxymethoxybenzaldehyde, **67** (336.9mg, 1.38mmol)

was added as a solution in THF (4mL) *via* syringe, and the mixture then stirred for 2 hours. The reaction was quenched with HCl (1N, 25mL), and organic products extracted with ether (2×25mL). The combined extracts were dried over sodium sulphate and solvent was removed under reduced pressure. The crude residue was purified by flash chromatography (DCM/hexane, 2:1) to yield ethyl 2-benzyl-3-(2-β-methoxyethoxymethoxy-5-chlorophenyl)acrylate, **77**, as a pale yellow oil (276.2mg, 0.682mmol, 76%). Spectra were consistent with an isomeric mixture in a 7:2 ratio.

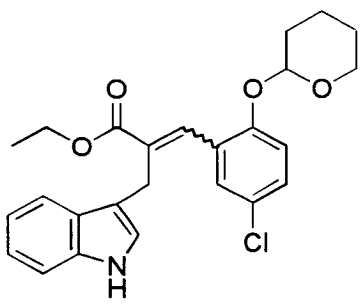


Rf 0.74 (ethyl acetate/hexane, 2:1). **FTIR ν_{max}/ cm^{-1} (DCM)** 3063 (Ar CH); 2929, 2895, 2821 (CH); 1706 (C=O); 1636, 1601, 1481 (C=C); 1098 (C-O). **λ_{max}/nm (ethanol, ε/dm^{3}mol^{-1}cm^{-1})** 308 (4250), 262 (10100), 229 (18600). **^{1}Hnmr ($CDCl_3$, 250MHz, δ/ppm)** 7.86 and 6.71 (1H, both s, olefin H) 7.35-7.02 (8H, m, Ar H); 5.25 and 5.22 (2H, both s, OCH_2O); 4.19 and 4.00 (2H, both q, $^3J_{HH}$=7.1Hz, OCH_2); 3.80-3.74 and 3.54-3.49 (both 2H, m, OCH_2); 3.82 (2H, s, benzyl CH_2); 3.36 and 3.35 (3H, both s, OCH_3); 1.21 and 0.99 (3H, both t, $^3J_{HH}$=7.1Hz, CH_3). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz, δ/ppm)**; 168.1, 167.6 (C=O); 153.8, 152.9, 139.4, 138.1, 132.7, 126.7 (*q* Ar/olefin C); 135.1, 130.4, 129.4, 129.2, 129.0, 128.8, 128.5, 128.3, 128.3, 127.9, 126.3, 125.9, 115.7 (Ar/olefin CH); 93.8 and 93.6 (OCH_2O); 71.3, 67.7 (MEM-OCH_2); 60.8, 60.4 (OCH_2); 58.9 (OCH_3); 40.9, 33.2 (benzyl CH_2); 14.0, 13.5 (CH_3). **HRMS (FAB, M$^+$/z)** $C_{22}H_{26}ClO_5$ (M^+) requires 405.1468; found 405.1484.

Preparation of Ethyl 2-(3-Methylindolyl)-3-(2-tetrahydro-2*H*-pyran-2yloxy)-5-chlorophenyl)acrylate, 85.

To triethyl 2-(3-methlylindolyl)phosphonoacetate, **59** (502.7mg, 1.42mmol), in THF (8mL) at room temperature, was added NaHMDS (1.0M in THF, 1.9mL, 1.6mmol), slowly *via* syringe, and the resultant solution stirred for 1.5 hours. 5-Chloro-2-(tetrahydro-2*H*-pyran-2yloxy)-benzaldehyde, **84** (643.7mg, 2.67mmol), was added as a solution in THF (3mL) *via* syringe, and the mixture then stirred for 2 hours. The reaction was quenched with HCl (1N, 20mL), and organic products

extracted with ether (2×25mL). The combined extracts were dried over sodium sulphate and solvent was removed under reduced pressure. The crude residue was purified by flash chromatography (DCM/hexane, 2:1 to DCM) to yield ethyl 2-(3-methylindole)-3-(2-tetrahydro-2*H*-pyran-2yloxy)-5-chlorophenyl)acrylate, **85**, as a pale yellow oil (388.3mg, 0.883mmol, 62%). Spectra were consistent with an isomeric mixture in a 5:1 ratio.



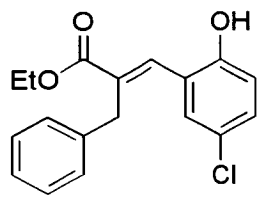
Rf 0.25 (DCM/hexane, 2:1). **FTIR ν_{max}/ cm^{-1} (DCM)** 3467 (NH); 3064 (Ar CH); 2947, 2861 (CH); 1705 (C=O); 1621, 1590, 1479 (C=C), 1093 (C-O). **λ_{max}/nm (ethanol, ε/dm^{3}mol^{-1}cm^{-1})** 313 (5280), 266 (15300), 222 (52800). **^{1}Hnmr ($CDCl_3$, 250MHz, δ/ppm)** 8.16 and 8.06 (1H, both s, NH); 7.96 and 6.77 (1H, both s, olefin H) 7.51-6.91 (8H, m, Ar H); 5.42 and 5.30 (1H, both t, $^3J_{HH}$=2.9Hz OCHO); 4.22 and 4.08 (2H, both q, $^3J_{HH}$=7.1Hz, OCH_2); 3.97 and 3.92 (2H, s, allyl CH_2); 3.86-3.74 and 3.62-3.56 (both 1H, m, diastereotopic OCH_2); 2.00-1.82 (3H, m, alkyl CH); 1.73-1.56 (3H, m, alkyl CH) 1.24 and 1.04 (3H, both t, $^3J_{HH}$=7.1Hz). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz, δ/ppm)** ***[Major Isomer]*** 168.2, (C=O); 153.4, 136.3, 132.6, 126.9, 126.8, 126.1, 113.7 (*q* Ar/olefin C); 133.8, 129.4, 129.0, 121.8, 121.7, 119.1, 118.8, 116.0, 115.7 (Ar/olefin CH); 96.4 (OCHO); 61.8, 60.7 (OCH_2); 30.0, 24.9, 18.3 (alkyl CH_2); 23.4 (allyl CH_2);14.0 (CH_3). **HRMS (FAB, M$^+$/z)** $C_{25}H_{26}ClNO_4$ (M^+) requires 439.1550; found 439.1556. **Elemental Analysis** $C_{25}H_{26}ClNO_4$ requires 68.25% C, 5.96% H, 3.18% N; found 68.25% C, 5.89% H, 2.98% N.

4.6 Protecting Group Cleavages

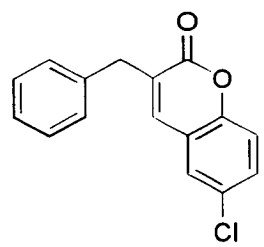
MEM-ether Cleavage of Ethyl 2-Benzyl-3-(2-β-Methoxyethoxymethoxy-5-chlorophenyl)acrylate, 77.

To ethyl 2-benzyl-3-(2-β-methoxyethoxymethoxy-5-chlorophenyl)acrylate, 77 (alkene ratio, 7:2, 404.9mg, 0.540mmol), in dry DCM (9mL) at room temperature, was added TFA (90% aqueous, 6mL), and the mixture stirred for 2 hour. The mixture

was then diluted with water (15mL), and the organic products were extracted with DCM (2×20mL). The combined extracts were washed with water (3×20mL), dried over sodium sulphate, and solvent removed under reduced pressure. The crude residue purified by flash chromatography (DCM), to yield to yield two products. These were identified as 6-chloro-3-benzylcoumarin, **80** (32.8mg, 0.121mmol, 23%) and ethyl 2-benzyl-3-(2-hydroxy-5-chlorophenyl)-*E*-acrylate, **79** (127.3mg, 0.402mmol, 77%).



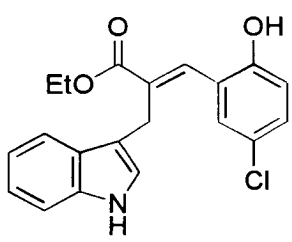
Rf (79) 0.18 (DCM). **FTIR ν_{max}/ cm^{-1} (DCM)** 3564 (OH); 3330 (br OH); 3065, 3028 (Ar CH); 2986, 2931, 2868 (CH); 1718 (C=O); 1635, 1600, 1480 (C=C). **λ_{max}/nm (ethanol, ε/dm^{3}mol^{-1}cm^{-1})** 313 (4650), 262 (9510), 229 (18200). **^{1}Hnmr ($CDCl_3$, 250MHz, δ/ppm)** 7.90 (1h, s, olefin CH); 7.29-7.10 (7H, m, Ar H); 6.91 (1H, br. s, OH); 6.78 (1H, d, $^3J_{HH}$=8.5Hz, Ar H); 4.20 (2H, q, $^3J_{HH}$=7.1Hz, OCH_2) 3.86 (2H, s, benzyl CH_2); 1.21 (3H, t, $^3J_{HH}$=7.1Hz). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz, δ/ppm)** 168.4 (C=O); 152.8, 139.0, 133.3, 124.8, 123.7 (*q* Ar/olefin C); 134.8, 129.7, 128.7, 128.3, 127.9, 126.1, 117.0 (Ar/olefin CH); 61.3 (OCH_2); 33.3 (benzyl CH_2); 13.9 9CH_3). **HRMS (FAB, M$^+$/z)** $C_{18}H_{17}ClO_3$ (M^+) requires 316.0866; found 316.0872.



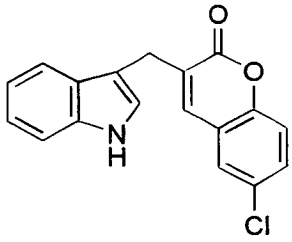
Rf (80) 0.56 (DCM). **mp** 138-140°C (lit.[14] 140-142°C) **FTIR ν_{max}/ cm^{-1} (DCM)** 3050 (Ar CH); 2984, 2921 (CH); 1717 (C=O); 1604, 1570, 1481 (C=C). **λ_{max}/nm (ethanol, ε/dm^{3}mol^{-1}cm^{-1})** 320 (5170), 269 (1330), 265 (11800), 262 (12100). **^{1}Hnmr ($CDCl_3$, 250MHz, δ/ppm)** 7.41-7.16 (9H, m, Ar H); 3.88 (2H, d, $^4J_{HH}$=0.9Hz). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz, δ/ppm)** 160.9 (C=O); 151.2, 137.0, 130.7, 129.3, 120.3 (*q* Ar C); 137.8, 130.5, 129.3, 128.7, 128.2, 126.8, 126.5, 117.7 (Ar CH); 36.4 (CH_2). **HRMS (FAB, M$^+$/z)** $C_{16}H_{12}ClO_2$ (MH^+) requires 271.0526; found 271.0529.

Attempted Methylether Cleavage of Ethyl 2-(1-Triisopropylsilyl-3-methylindolyl)-3-(2-methoxy-5-chlorophenyl)acrylate, 75.

To ethyl 2-(1-triisopropylsilyl-3-methylindolyl)-3-(2-methoxy-5-chlorophenyl)-acrylate, **75** (alkene ratio, 7:2, 79.5mg, 0.15mmol), in dry DCM (4mL) at 0°C, was added boron tribromide (1.0M in DCM, 0.6mL, 0.6mmol), and the mixture stirred for 1 hour. The reaction was then stirred at room temperature for 16 hours, followed by quenching with the careful addition of water (15mL). The organic products were extracted with DCM (2×20mL), and the combined extracts were washed with water (20mL) and dried over sodium sulphate. Solvent was removed under reduced pressure and the crude residue purified by flash chromatography (DCM - ethyl acetate/hexane, 3:1). Two compounds were isolated, which were identified as ethyl 2-(3-methylindole)-3-(2-hydroxy-5-chlorophenyl)-*E*-acrylate, **34** (23.9mg, 0.067mmol, 45%) and 6-chloro-3-(3-methylindolyl)coumarin, **81** (17.4mg, 0.056mmol, 37%).



Rf (34) 0.54 (ethyl acetate/hexane, 1:1). **FTIR ν_{max}/ cm^{-1} (DCM)** 3566 (OH); 3467 (NH) 3300 (br. OH) 3063 (Ar CH); 2982, 2957, 2932, 2860 (CH); 1706 (C=O); 1602, 1481 (C=C). **λ_{max}/nm (ethanol, ε/dm^{3}mol^{-1}cm^{-1})** 323 (5560), 269 (17100). **^{1}Hnmr ($CDCl_3$, 600MHz, δ/ppm)** 8.07 (1H, br. s, NH) 7.81 (1H, s, olefin H, NOE with δ 4.34ppm) 7.53 (1H, d, $^3J_{HH}$=7.9Hz Ar H); 7.45 (1H, d, $^3J_{HH}$=8.0Hz, Ar H) 7.33 (1H, s, Ar H, NOE with δ 4.02ppm); 7.29 (2H, m, Ar H); 7.20 (1H, m, Ar H) 7.04 (1H, m, Ar H); 6.94 (1H, d, $^3J_{HH}$=8.6Hz); 5.29 (1H, br. s, OH); 4.34 (2H, q, $^3J_{HH}$=7.1Hz, OCH_2, NOE with δ 7.81ppm); 4.02 (2H, s, allyl CH_2, NOE with δ 7.33ppm); 1.37 (3H, t, $^3J_{HH}$=7.1Hz). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz, δ/ppm)** 168.5 (C=O); 153.1, 136.1, 133.6, 126.9, 124.6, 124.0, 113.4 (*q* Ar/olefin C); 133.4, 129.5, 128.9, 121.8, 121.8, 119.20 118.7, 116.9, 110.9 (Ar/olefin CH); 61.0 (OCH_2); 23.4 (allyl CH_2); 14.0 (CH_3). **MS (FAB, M$^+$/z)** $C_{20}H_{19}ClNO_3$ (MH^+) requires 356.1054; found 356.1047.



Rf (81) 0.54 (DCM). **FTIR ν_{max}/ cm^{-1} (DCM)** 3466 (NH); 3063 (Ar CH); 2929, 2865 (CH); 1726 (C=O); 1604, 1570, 1481 (C=C). **λ_{max}/nm (ethanol, ε/dm^{3}mol^{-1}cm^{-1})** 320 (1390), 273 (3450). **^{1}Hnmr (DMSO-d$_6$, 250MHz, δ/ppm)** 11.00 (1H, br. s, NH) 7.70 (1H, s, Ar H); 7.61 (1H, s, Ar H); 7.56-7.36 (4H, m, Ar H); 7.26 (1H, d, $^3J_{HH}$=2.2Hz, Ar H); 7.11-6.93 (2H, m, Ar H); 3.91 (1H, s, 1CH$_2$). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz, δ/ppm)** 160.6 (C=O); 151.2, 136.5, 130.0, 128.3, 120.8, 110.0 (*q* Ar C); 137.7, 130.5, 127.1, 124.4, 121.2, 118.7, 118.5, 117.9, 111.7 (Ar CH); 26.0 (CH$_2$). **HRMS (FAB, M$^+$/z)** $C_{18}H_{13}ClNO_2$ (MH$^+$) requires 310.0635; found 310.0638.

Attempted Methylether Cleavage of Ethyl 2-Benzyl-3-(2-methoxy-5-chlorophenyl)acrylate, 77.

To ethyl 2-benzyl-3-(2-methoxy-5-chlorophenyl)acrylate, **77** (alkene ratio, 7:2, 299.1mg, 0.904mmol), in dry DCM (10mL) at 0°C, was added boron tribromide (1.0M in DCM, 1.8mL, 1.8mmol), and the mixture stirred for 1 hour. The reaction was then stirred at room temperature for 16 hours, followed by quenching with the careful addition of water (15mL). The organic products were extracted with DCM (2×20mL), and the combined extracts were washed with water (20mL) and dried over sodium sulphate. Solvent was removed under reduced pressure and the crude residue purified by flash chromatography (DCM), to yield 6-chloro-3-benzylcoumarin, **80** (212.6mg, 0.785mmol, 87%).

THP-ether Cleavage of Ethyl 2-(3-Methylindolyl)-3-[(2-tetrahydro-2*H*-pyran-2yloxy)-5-chlorophenyl]acrylate, 85.

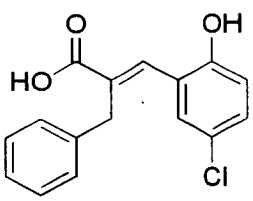
Ethyl 2-(3-methylindolyl)-3-[(2-tetrahydro-2*H*-pyran-2-yloxy)-5-chlorophenyl]-acrylate, **85** (alkene ratio, 5:1, 263.0mg, 0.598mmol), was stirred in methanol (6mL) at room temperature. Oxalic acid (10% aqueous, 4ml, 4.4mmol) was added and the resultant mixture stirred at 80°C for 90mins. The mixture was cooled then methanol removed under reduced pressure. The residue was diluted with water (10mL) then organic products extracted with ether (2×20mL), washed with saturated sodiumbicarbonate solution (20mL) and water (20mL) and solvent removed under

reduced pressure. The residue was purified by flash chromatography (ethyl acetate/hexane, 1:1) to yield ethyl 2-(3-methylindole)-3-(2-hydroxy-5-chlorophenyl)-*E*-acrylate, **34** (179.8mg, 0.505mmol, 84%) and 6-chloro-3-(3-methylindolyl)coumarin, **81** (23.9mg, 0.077mmol, 13%).

4.7 Carboxylic Ester Hydrolysis

Preparation of 2-Benzyl-3-(2-hydroxy-5-chlorophenyl)-*E*-acrylic acid, 82.

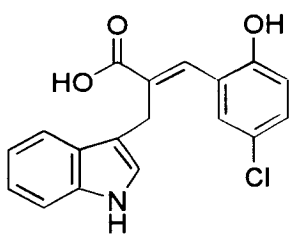
To ethyl 2-benzyl-3-(2-hydroxy-5-chlorophenyl)-*E*-acrylate, **79** (155.2mg, 0.490mmol), in 50% aqueous ethanol (8mL) was added lithium hydroxide monohydrate (103.6mg, 2.47mmol). The mixture was heated at reflux for 4 hours, then cooled to room temperature and the ethanol was removed under reduced pressure. Organic products were extracted with ether (10mL) from the basic solution which was then acidified forming a white precipitate. This was removed by filtration to yield 2-benzyl-3-(2-hydroxy-5-chlorophenyl)-*E*-acrylic acid, **82** (122.1mg, 0.423mmol, 86%).



Rf 0.45 (DCM/methanol, 9:1). **RP-HPLC (Gradient 1)** 21.9 minutes. **m.p.** 204-207°C. **FTIR ν_{max}/ cm^{-1} (KBr)** 3448 (OH); 3088 (Ar CH); 2990, 2922, (CH); 2690, 2550 (CO_2H); 1670 (C=O); 1618, 1560, 1495 (C=C). **λ_{max}/nm (ethanol, ε/dm^{3}mol^{-1}cm^{-1})** 319 (5770), 263 (11400). **^{1}Hnmr (CD_3OD, 250MHz, δ/ppm)** 8.09 (1H, s, olefin H); 7.38-7.19 (7H, m, Ar H); 6.90 (1H, ddd, $^3J_{HH}$=9.0, 1.3, 1.3Hz, Ar H); 5.03 (br. s, CD_3OH); 3.93 (2H, s, benzyl CH_2). **^{13}C{^{1}H}nmr (CD_3OD, 63MHz, δ/ppm)** 169.5 (C=O); 154.0, 139.1, 130.4, 123.6, 122.9 (*q* Ar/olefin C); 135.0, 128.9, 127.8, 127.6, 127.0, 125.2, 115.8 (Ar/olefin CH); 32.2 (benzyl CH_2). **MS (FAB, M$^+$/z)** $C_{16}H_{14}ClO_3$ (MH^+) requires 289.0632; found 289.0634. **MS (APCi, +20V)** $C_{16}H_{14}ClO_3$ (MH^+) requires 289 found 271 (MH^+ -H_2O).

Preparation of 2-(3-Methylindolyl)-3-(2-hydroxy-5-chlorophenyl)acrylic Acid, 29.

To ethyl 2-(3-methylindole)-3-(2-hydroxy-5-chlorophenyl)-*E*-acrylate, **34** (191.6mg, 0.542mmol), in 50% aqueous ethanol (10mL) was added lithium hydroxide monohydrate (120.0mg, 2.86mmol). The mixture was heated at reflux for 4 hours, then cooled to room temperature and the ethanol was removed under reduced pressure. Organic products were extracted with ether (15mL) from the basic solution which was then acidified forming a white precipitate. This was removed by filtration to yield 2-methylindolyl-3-(2-hydroxy-5-chlorophenyl)-*E*-acrylic acid, **29** (142.7mg, 0.436mmol, 80%).



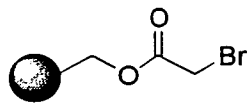
Rf 0.37 (DCM/methanol, 9:1). **m.p.** 200-201°C (lit.[15] 202-205°C). **FTIR ν_{max}/ cm^{-1} (KBr)** 3442 (NH/OH); 3053 (Ar CH); 2989, 2918 (CH); 2625, 2550 (CO_2H); 1664 (C=O); 1603, 1570, 1486 (C=C). **λ_{max}/nm (ethanol, ε/dm^{3}mol^{-1}cm^{-1})** 319 (4630), 265 (11500). **^{1}Hnmr (CD_3OD, 250MHz, δ/ppm)** 8.04 (1H, s, olefin H); 7.55 (1H, d, $^3J_{HH}$=7.0Hz, Ar H); 7.42 (1H, d, $^3J_{HH}$=8.0Hz, Ar H); 7.34 (1H, d, $^3J_{HH}$=2.6Hz, Ar H); 7.22-704 (4H, m, Ar H); 6.91 (1H, d, $^3J_{HH}$=8.7Hz, Ar H); 4.00 (2H, s, allyl CH_2). **^{13}C{^{1}H}nmr (CD_3OD, 63MHz, δ/ppm)** 162.6 (C=O); 146.5, 128.7, 124.0, 118.9, 116.2, 115.5, 104.6 (*q* Ar/olefin C); 125.9, 121.3, 120.7, 113.6, 113.0, 110.2, 110.0, 108.2, 102.7 (Ar/olefin CH); 15.2 (allyl CH_2). **MS (FAB, M$^+$/z)** $C_{18}H_{14}ClNO_3$ (M^+) requires 327.0662; found 327.0667. **Elemental Analysis** $C_{18}H_{14}ClNO_3$ requires 65.96% C, 4.31% H, 4.27% N; found 65.80% C, 4.44% H, 3.99% N.

4.8 Solid Phase Synthesis of 2-Benzyl-3-(2-hydroxy-5-chlorophenyl)acrylic Acid.

Synthesis of Copoly(styrene-1%-divinylbenzene)-*p*-benzyloxybenzyl bromo-acetate, 86.

To bromoacetic acid (0.52g, 3.7mmol) in DMF (15mL), was added diisopropylcarbodiimide (0.24g, 1.9mmol) and the mixture sonicated under argon for

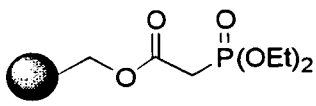
1 hour. A catalytic quantity of DMAP followed by copoly(styrene-1%-divinylbenzene)-*p*-benzyloxybenzyl alcohol resin (0.84g, 1.07mmol/g, 0.90mmol), was added to the mixture and sonication was then continued for 18 hours. The resin was then filtered, washed with copious quantities of DMF, DCM/methanol (1:1), THF and DCM, and then dried in a vacuum dessicator for 2 hours to yield copoly(styrene-1%-divinylbenzene)-*p*-benzyloxybenzyl bromoacetate, (0.86g), **86**, as a pale yellow solid.



FTIR ν_{max}/cm^{-1}(DCM) 1734 (C=O). **Elemental Analysis** 1.07mmol/g Br requires 7.67%, found 5.33% (±0.6%), hence resin loading is 0.67mmol/g (±0.07mmol/g).

Synthesis of Copoly(styrene-1%-divinylbenzene)-*p*-benzyloxybenzyldiethyl Phosphonoacetate, 41.

To copoly(styrene-1%-divinylbenzene)-*p*-benzyloxybenzyl bromoacetate, **86** (0.69g, 0.67mmol/g, 0.46mmol), in toluene (10mL), was added triethylphosphite (1.0g, 5.8mmol), and the mixture was heated at reflux for 24 hours. The resin was then filtered, washed with copious quantities of DMF, DCM/methanol (1:1), THF and DCM, and then dried in a vacuum dessicator for 2 hours to yield copoly(styrene-1%-divinylbenzene)-*p*-benzyloxybenzyldiethyl phosphonoacetate (0.68g, 0.64mmol/g), **41**, as a pale yellow solid.

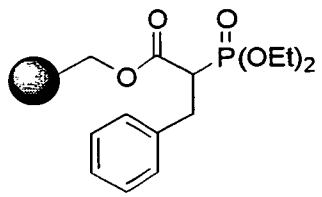


FTIR ν_{max}/cm^{-1} (DCM) 1735 (C=O). **Gel Phase $^{13}C\{^1H\}$nmr (CD_2Cl_2, 63MHz, δ/ppm)** 158.8 (C=O); 67.0, 62.6 (OCH_2); 34.2 (d, $^1J_{PC}$=133Hz, CH_2); 16.2 (CH_3). **Gel Phase ^{31}Pnmr (CD_2Cl_2, MHz, δ)** 20.2ppm. **Elemental Analysis** no Br present.

Synthesis of Copoly(styrene-1%-divinylbenzene)-*p*-benzyloxybenzyldiethyl 2-Benzyl-phosphonoacetate, 88.

To copoly(styrene-1%-divinylbenzene)-*p*-benzyloxybenzyldiethyl phosphono-acetate, **41**, (0.68g, 0.64mmol/g, 0.44mmol), in THF (10mL), was added NaHMDS (1.1M in THF, 0.43mL, 0.47mmol), and the mixture sonicated for 1 hour. Benzylbromide (0.19g, 1.1mmol) was added as a solution in THF (2mL) and sonication was then continued for 20 hours. The resin was filtered, washed with

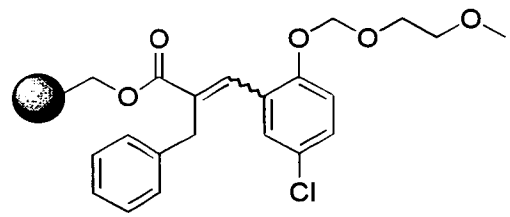
copious quantities of DMF, DCM/methanol (1:1), THF and DCM, and then dried in a vacuum dessicator for 2 hours to yield copoly(styrene-1%-divinylbenzene)-*p*-benzyloxybenzyldiethyl 2-benzyl-phosphonoacetate (0.68g, 0.60mmol/g) **88**, as a pale yellow solid.



FTIR ν_{max}/cm^{-1} (DCM) 1732 (C=O). **Gel Phase $^{13}C\{^1H\}$nmr (CD_2Cl_2, 63MHz, δ/ppm)** 67.0, 63.1 (OCH_2); 36.4 (benzyl CH_2); 16.4 (CH_3). **Gel Phase ^{31}Pnmr (CD_2Cl_2, MHz, δ)** 5.2ppm.

Synthesis of Copoly(styrene-1%-divinylbenzene)-*p*-benzyloxybenzyl 2-Benzyl-3-(2-β-Methoxyethoxymethoxy-5-chlorophenyl)acrylate, 89.

To copoly(styrene-1%-divinylbenzene)-*p*-benzyloxybenzyldiethyl 2-benzyl-phosphonoacetate, **88** (0.60mmol/g, 0.33g, 20mmol), in THF (5mL), was added NaHMDS (1.0M in THF, 0.40mL, 0.40mmol) and the mixture was then sonicated for 1 hour. β-Methoxyethoxymethoxy-5-chlorosalicylaldehyde (0.19g, 0.76mmol), **67**, as added as a solution in THF (2mL) and sonication was then continued for 20 hours. The resin was filtered, washed with copious quantities of DMF, DCM/methanol (1:1), THF and DCM, and then dried in a vacuum dessicator for 2 hours to yield copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 2-benzyl-3-(2-β-methoxy-ethoxymethoxy-5-chlorophenyl)acrylate, (0.33g, 0.57mmol/g) **89**, as a pale yellow solid.



FTIR ν_{max}/ cm^{-1} (DCM) 1734 (C=O). **Gel Phase $^{13}C\{^1H\}$nmr (CD_2Cl_2, 63MHz, δ/ppm)** 190.2 (C=O); 93.6 (OCH_2O); 71.2, 67.7, 65.9 (OCH_2); 61.8 (OCH_3); 33.4 (CH_2). **Gel Phase ^{31}Pnmr (CD_2Cl_2, MHz, δ)** 5.2ppm.

Resin Cleavage to form 2-Benzyl-3-(2-hydroxy-5-chlorophenyl)acrylic acid, 82.

To sonicating copoly(styrene-1%-divinylbenzene)-*p*-benzyloxybenzyl 2-benzyl-3-(2-β-methoxyethoxymethoxy-5-chlorophenyl)acrylate, **89** (0.10g, 0.57mmol/g), in DCM (3mL) was added TFA (90% aqueous, 2mL) dropwise. The mixture was sonicated for 2 hours. After which time, the resin was filtered, and washed with DCM (5mL). TFA was removed from the filtrate by washing with NaOH (1N, 3mL), and concentration of the resin products gave a residue which was dissolved in 0.1% TFA/acetonitrile. Preparative HPLC (Gradient 1) yielded 2-benzyl-3-(2-hydroxy-5-chlorophenyl)-acrylic acid, **82**, as a cream coloured solid (4.8mg, 0.017mmol, 30% overall, based on resin loading). **RP-HPLC (Gradient 1)** 21.5 minutes **MS (APCi, +20V)** $C_{16}H_{14}ClO_3$ (MH^+) requires 289 found 271 (MH^+ -H_2O).

4.9 4×2 Array Multiple Parallel Synthesis of Acrylic Acids

General Resin Washing Procedure

Resin portions were washed using the following protocol, allowing drainage by gravity between each solvent portion: DMF(4×2mL), DCM/methanol (4×2mL), THF (4×2mL), DCM (4×2mL).

General Procedure for the Horner-Wadsworth Emmons Reaction

To each Diversomer™ pin containing alkylated copoly(styrene-1%-divinylbenzene)-*p*-benzyloxybenzyldiethyl phosphonoacetate swollen in THF (4mL), was added NaHMDS (1.0M in THF). More THF (2mL) was added, and the entire reactor block sonicated for 1 hour. Each aromatic alkyl halide was added to an individual pin as a solution in THF (2mL), and sonication was continued for 18 hours. The resins were then washed using the general washing procedure, and dried in a vacuum dessicator for 2 hours.

General Procedure for Resin Cleavage

To each Diveromer™ pin containing resin from the HWE reaction, swollen in DCM, was added aqueous TFA. The entire reactor block was sonicated for 2 hours and the resins were then washed with DCM (2×2mL), with the filtrates from

individual pins combined. TFA was removed from each filtrate by washing with NaOH (0.5N, 2mL), and concentration of the resin products gave a residue which was dissolved in 0.1% TFA/acetonitrile prior to purification by preparative HPLC. The isolated resin products were analysed by chemical ionisation or fast atom bombardment mass spectrometry.

4.9.1 Library 1: 4×2 Array of Methylindolylacrylic Acids

Synthesis of Copoly(styrene-1%-divinylbenzene)-*p*-benzyloxybenzyldiethyl 2-(3-Methylindolyly)phosphonoacetate, 43.

To copoly(styrene-1%-divinylbenzene)-*p*-benzyloxybenzyldiethyl phosphonoacetate, **41** (0.95g, 0.64mmol/g, 0.61mmol), in THF (20mL), was added 1-triisopropylsilylgramine-methiodide, **5** (0.71g, 1.51mmol), and the mixture sonicated. Tetrabutylammoniumfluoride (1.0M in THF, 2.1mL, 3.1mmol) was added and sonication continued for 3 hours. The resin was then filtered, washed with copious quantities of DMF, DCM/methanol (1:1), THF and DCM, and then dried in a vacuum dessicator for 2 hours to yield copoly(styrene-1%-divinylbenzene)-*p*-benzyloxybenzyldiethyl 2-(3-methylindolyly)phosphonoacetate, **42** as a pale yellow solid (0.95g, 0.59mmol/g). The resin was then used immediately in the 4×2 array synthesis.

Horner Wadsworth Emmons Reaction

The general procedure was followed using approximately 100mg of copoly(styrene-1%-divinylbenzene)-*p*-benzyloxybenzyldiethyl 2-(3-methylindolyl)-phosphonoacetate, **42**, in each Diversomer™ pin, 0.12mmol of NaHMDS, and approximately 0.25mmol of aldehyde in the following array addresses: **A1** benzaldehyde (27mg, 0.26mmol); **A2** 3-pyridinecarrboxaldehyde (28mg, 0.27mmol); **A3** 3-chloro-4-hydroxybenzaldehyde (39mg, 0.25mmol); **A4** 3-thiophene-carboxaldehyde (27mg, 0.24mmol); **B1** 4-isopropylbenzaldehyde (38mg, 0.26mmol); **B2** 3-fluorobenzaldehyde (31mg, 0.25mmol); **B3** 4-nitrobenzaldehyde (36mg, 0.23mmol); **B4** 2-chloro-5-(trifluoromethyl)benzaldehyde (50mg, 0.24mmol).

Resin Cleavage

The general procedure for resin cleavage was followed for the resin in each of the eight Diversomer™ pins. Two products were isolated from each sample by preparative HPLC using Gradient 1: **A1** (14.0mins, 23.4mins); **A2** (14.0mins, 23.4mins); **A3** (13.3mins, 23.8mins); **A4** (13.6mins, 23.8mins); **B1** (15.5mins, 23.4mins); **B2** (14.4mins, 23.4mins); **B3** (13.4mins, 23.8mins); **B4** (14.8mins, 23.4mins). No positive product identification was obtained by APCiMS or FABMS.

4.9.2 Library 2: 4×2 Array of *p*-Chlorophenolacrylic acids

Phosphonate Alkylation Reaction

To each Diversomer™ pin containing copoly(styrene-1%-divinylbenzene)-*p*-benzyloxybenzyldiethyl phosphono-acetate, **41** (100mg, 0.64mmol/g, 0.064mmol), swollen in THF (4mL), was added NaHMDS (1.0M in THF, 0.1mL, 0.1mmol), and the entire reactor block sonicated for 3 hours. Aromatic alkyl halides were added as a solution in THF (1mL) in the following spatial addresses (to those samples purchased as hydrochloride salts, the appropriate quantity of NaHMDS was added prior to addition to the pins): **A1** benzyl bromide (46mg, 0.27mmol); **A2** 3-picolyl chloride hydrochloride (40mg, 0.24mmol); **A3** 2-(chloromethyl)benzimidazole (42mg, 0.25mmol); **A4** 2-hydroxy-5-nitrobenzyl bromide (53mg, 0.23mmol); **B1** 2-methoxy-5-nitrobenzyl bromide (57mg, 0.23mmol); **B2** α-bromo-2,3,4,5,6-pentafluorotoluene (61mg, 0.23mmol); **B3** 2-chloromethylquinoline hydrochloride (53mg, 0.25mmol); 4-(chloromethyl)-biphenyl **B4** (49mg, 0.24mmol). Sonication was continued for 16 hours, then the resins were washed using the general washing procedure, and dried in a vacuum dessicator for 2 hours.

Horner Wadsworth Emmons Reaction

The general procedure was followed, treating each of the dry resins from the phosphonate alkylation reaction with NaHMDS (0.2mL, 0.2mmol) and 2-β-methoxyethoxymethoxy-5-chlorosalicylaldehyde, **67** (79mg, 0.32mmol).

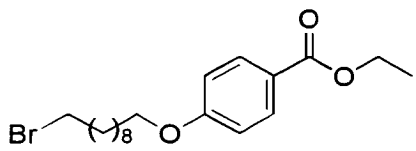
Resin Cleavage

The general procedure for resin cleavage was followed for the resin in each of the eight Diversomer™ pins. Two products were isolated from each sample by preparative HPLC using Gradient 2: **A1** (9.4mins, 14.5mins); **A2** (9.7mins, 14.5mins); **A3** (9.0mins, 14.5mins); **A4** (10.1mins, 14.9mins); **B1** (9.7mins, 14.5mins); **B2** (8.6mins, 14.5mins); **B3** (9.7mins, 14.5mins); **B4** (9.7mins, 14.5mins). No positive product identification was obtained by APCiMS.

4.10 Synthesis of Tbf-bound Ciprofloxacin

Preparation of Ethyl 4-(10-Bromodecyloxy)benzoate, 94.

To sodium hydride (1.55g, 61mmol) in DMF (150mL), was added ethyl-4-hydroxybenzoate, **95** (5.06g, 30mmol), as a solution in DMF (30mL). The reaction mixture was then stirred at room temperature for 2 hours, before adding 1,10-dibromodecane, **96** (30.84g, 103mmol), as a solution in DMF (50mL) *via* syringe. The reaction mixture was then stirred at room temperature for 19 hours. The reaction was quenched with water (200mL) and the organic products extracted with ether (4×150mL), dried over $MgSO_4$ and solvent removed under reduced pressure to form a colourless oil. This was purified by flash chromatography (DCM/hexane, 1:1 to DCM/hexane, 3:1) forming ethyl 4-(10-bromodecyloxy)benzoate, **94** (7.38g, 19.1mmol, 63%), as a white crystalline solid, which was recrystallised from ethanol.

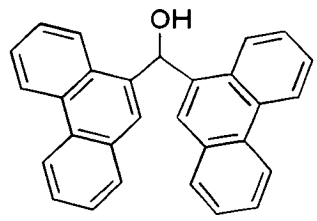


Rf 0.65 (DCM). **m.p.** 41-42°C. **FTIR, ν_{max}/cm^{-1} (bromoform)** 3023 (Ar CH); 2927, 2855 (CH); 1708 (C=O); 1605, 1509 (C=C); 1105 (C-O). **λ_{max}/nm (DCM, ε/dm^{3}mol^{-1}cm^{-1})** 257 (24600). **^{1}Hnmr ($CDCl_3$, 250MHz), δ/ppm** 7.96 and 6.87(both 2H, m, ArH); 4.32 (2H, q, $^3J_{HH}$=7.1Hz, $OC\underline{H}_2CH_3$); 3.97 (2H, t, $^3J_{HH}$=6.5Hz, $OC\underline{H}_2CH_2$); 3.38 (2H, t, $^3J_{HH}$=6.9Hz, CH_2Br); 1.80 (4H, m, $OCH_2C\underline{H}_2$ and $C\underline{H}_2CH_2Br$); 1.42-1.27 (12H, m, 6×CH_2); 1.35 (3H, t, $^3J_{HH}$=7.1Hz, CH_3). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz), δ/ppm** 166.2 (C=O); 152.6 and 122.4 (*q* Ar C); 131.3 and 113.8 (Ar CH); 67.9 and 60.4 (OCH_2); 33.9, 32.6, 29.2, 29.1, 29.0, 28.9, 28.5, 28.0, 25.8 (CH_2); 14.2 (CH_3). **HRMS (FAB), M$^+$/z** $C_{19}H_{30}BrO_3$ (MH^+) requires

385.1378, found 385.1374. **Elemental Analysis** $C_{19}H_{29}BrO_3$ requires 59.22% C, 7.59% H, 20.74% Br; found 58.92% C, 7.45% H, 20.87% Br.

Preparation of *Bis*-(phenanthren-9-yl)methanol, 101[16]

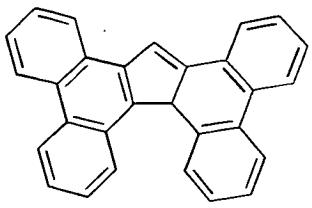
To magnesium turnings (4.7g, 0.196mol) and a crystal of iodine, in a three-necked flask fitted with a dropping funnel and condenser, was added 9-bromophenanthrene (50.6g, 0.197mol), as a solution in THF (80mL), dropwise, over a period of twenty minutes. Heat evolved and the reaction mixture turned into a green liquid. After 2.5 hours, methylformate (5.95g, 0.099mol) was added as a solution in THF (25mL) over a period of 20 minutes. The reaction mixture turned yellow/brown and was stirred at room temperature for a further 2 hours. The reaction mixture was then poured onto HCl (2M, 100mL), forming a white precipitate. Excess magnesium was destroyed with more HCl, and the white precipitate was filtered, washed with ether (200mL) and collected as bis-(phenanthren-9-yl)methanol, **101** (24.5g, 0.063mol, 64%).



Rf 0.50 ($CHCl_3$). **m.p.** 236-238°C (Lit. 238-239°C[16]). **FTIR, ν_{max}/cm^{-1} (bromoform)** 3466 (OH), 3018 (Ar CH), 1603, 1492 (C=C). **^{1}Hnmr ($CDCl_3$, 250MHz), δ/ppm** 8.79-8.67 (4H, m, Ar H); 8.13-8.10 (2H, m, Ar H); 7.80-7.50 (12H, m, Ar H); 7.31 (1H, s, CHOH); 2.76 (1H, s, OH). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz), δ/ppm** 136.3, 131.2, 130.8, 130.3, 130.0 (*q* Ar C); 129.0, 126.9, 126.8, 126.6, 126.4, 126.0, 124.3, 123.1, 122.3 (Ar CH); 69.6 (CHOH). **HRMS (FAB), M^+/z** $C_{29}H_{20}O$ (M^+) requires 384.1514; found 384.1498.

Preparation of *8bH*-Tetrabenzo[*a,c,g,i*]fluorene, 99[16]

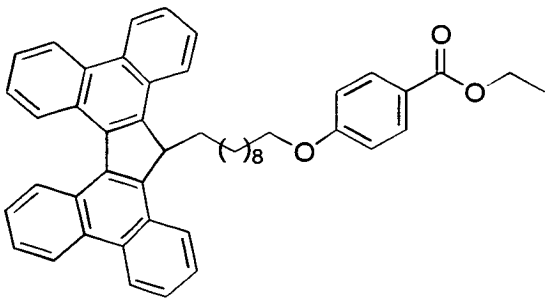
To *bis*-(phenanthren-9-yl)methanol, **101** (24.5g, 63mmol), in DCM (100mL), was added TFA (40mL), dropwise, over a period of 10 minutes. The yellow suspension formed was stirred at room temperature for 20 minutes after which time solvent was removed under reduced pressure. The resulting yellow solid was reconcentrated five times from DCM (100mL), and the residue was then washed with ether, yielding *8bH*-tetrabenzo[*a,c,g,i*]fluorene, **99**, as a bright yellow solid (16.4g, 45mmol, 70%).



Rf 0.84 ($CHCl_3$/methanol, 9:1). **m.p.** 276-278°C (Lit. 280-282°C[16]). **FTIR,** ν_{max}**/cm**$^{-1}$ 3019 (Ar CH); 1608, 1495 (C=C). λ_{max}**/nm (DCM,** ε**/dm**^{3}**mol**$^{-1}$**cm**$^{-1}$**)** 374 (11700), 358 (9880), 301 (31800), 254 (62200). **^{1}Hnmr ($CDCl_3$, 250MHz), δ/ppm** 8.81-8.76 (2H. m. Ar H); 8.28-7.08 (15H, m, Ar H); 5.39 (1H, s, CH). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz), δ/ppm** 148.3, 141.3, 137.2, 135.2, 134.0, 133.2, 130.7, 130.3, 129.3, 127.5, (*q* Ar C); 128.3, 128.2, 127.1, 126.9, 126.9, 126.6, 126.5, 126.3, 126.2, 125.8, 125.1, 124.9, 124.6, 123.6, 123.5, 123.2, 121.2, (Ar CH); 53.1 (CH). **HRMS (FAB), M$^+$/z** $C_{29}H_{18}$ requires 366.1409, found 366.1396.

Preparation of Ethyl 4-[10-(17'-Tetrabenzo[*a,c,g,i*]fluorenyl)decyloxy]benzoate, 103.[17]

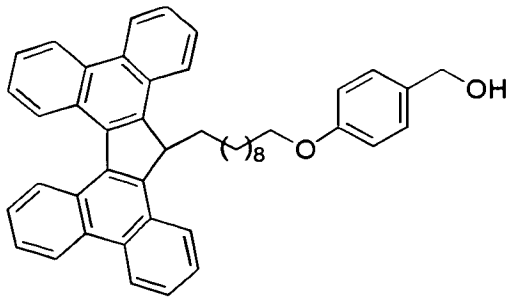
To *8bH*-Tetrabenzo[*a,c,g,i*]fluorene, **99** (2.08g, 5.68mmol), in degassed dioxane 80mL) heated at reflux, was added tetrabutyl-ammonium hydroxide (40% w/w in H_2O, 3.50g, 5.41mmol) as a solution in degassed dioxane (20mL) *via* syringe, immediately forming a yellow precipitate. This was filtered under nitrogen and washed with warm dioxane (100mL) and ether (100mL). The salt was resuspended in dioxane (100mL) and to it added ethyl 4-(10-bromodecyloxy)benzoate, **94** (2.08g, 5.40mmol), and the mixture heated to reflux for 2 hours. Solvent was removed from the darkened solution, forming a residue which was dissolved in ether (50mL), and the supernatant removed to leave a yellow slurry which was washed with ether (2×30mL). The combined extracts were dried over magnesium sulphate, and solvent removed under reduced pressure, forming a residue which was purified by flash chromatography using (DCM/hexane, 1:1). The product was isolated as a pale yellow airy solid which was triturated from ether, to yield ethyl 4-[10-(17'-tetrabenzo[*a,c,g,i*]-fluorenyl)decyloxy]benzoate, **103**, as a pale yellow powder (2.46g, 3.67mmol, 68%).



Rf 0.71 (DCM). **m.p.** 122-124°C. **FTIR, ν_{max}/cm^{-1} (bromoform)** 3072 (Ar CH); 2922, 2850 (CH); 1702 (C=O); 1605, 1505 (C=C); 1105 (C-O). **λ_{max}/nm (DCM, ε/dm^{3}mol^{-1}cm^{-1})** 381 (20700), 365 (21000), 301(45800), 256 (86900). **^{1}Hnmr ($CDCl_3$, 200MHz), δ/ppm** 8.81 and 8.68 (both 6H, m, Ar H); 8.23-8.18 (2H, m, Ar H); 8.06-7.98 (2H, m, ArH); 7.72-7.58 (8H, m, Ar H); 6.93-6.82 (2H, m, Ar H); 4.92 (1H, t, $^3J_{HH}$=4.2Hz, fluorenyl CH); 4.39 (2H, q, $^3J_{HH}$=7.1Hz, $OC\underline{H}_2CH_3$); 3.86 (2H, t, $^3J_{HH}$=6.5Hz, OCH_2); 2.60-2.52 (2H, m, $OCH_2C\underline{H}_2$); 1.76-1.53 (2H, m, CH_2); 1.23-0.71 (12H, m, 6×CH_2); 1.42 (3H, t, $^3J_{HH}$=7.1Hz, CH_3); 0.32-0.28 (2H, m, CH_2). **^{13}C{^{1}H}nmr ($CDCl_3$, 50MHz), δ/ppm** 166.3 (C=O); 162.7, 122.4 (*q* Ar C); 131.3, 113.8 (Ar CH); 144.2, 136.6, 131.1, 130.2, 128.6, 127.9 (*q* Tbf Ar C); 127.3, 126.6, 125.7, 125.4, 124.8, 124.3, 123.3, 123.3 (Tbf Ar CH); 67.9, 60.4 (OCH_2), 47.0 (fluorenyl C); 33.4, 29.2, 28.9, 28.8, 28.6, 25.5, 22.0 (CH_2); 14.2 (CH_3). **HRMS (FAB), M$^+$/z** $C_{48}H_{46}O_3$ (M^+) requires 670.3447, found 670.3447.

Preparation 4-[10-(17'-Tetrabenzo[a,c,g,i]fluorenyl)decyloxy]benzyl Alcohol, 104.

To ethyl 4-[10-(17'-tetrabenzo[a,c,g,i]fluorenyl)decyloxy]benzoate, **105** (2.03g, 3.03mmol), in THF (40mL) was added diisobutyl aluminium hydride (1.0M in THF, 12.0mL, 12.0mmol) *via* syringe and the resulting mixture stirred at room temperature for 2 hours. The reaction was quenched at 0°C with the careful addition of HCl (2M, 40mL) and the organic products were extracted with ethyl acetate (3×50mL), washed with saturated Na_2CO_3 (40mL) and water (40mL) and dried over $MgSO_4$. Solvent was removed under reduced pressure to yield 4-[10-(17'-tetrabenzo[a,c,g,i]fluorenyl)-decyloxy]benzyl alcohol, **104**, as a yellow solid (1.7765g, 2.82mmol, 94%).



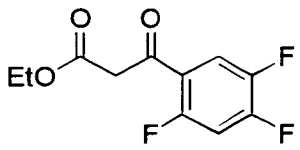
Rf 0.35 (DCM). **m.p.** 64-66°C. **FTIR, ν_{max}/cm^{-1} (bromoform)** 3460 (OH); 3016 (Ar CH); 2920, 2853 (CH); 1609, 1583, 1510 (C=C); 1142 (C-O). **λ_{max}/nm (DCM, ε/dm^{3}mol^{-1}cm^{-1})** 381 (18400), 365 (18800), 301 (41400), 254 (70700). **^{1}Hnmr ($CDCl_3$, 250MHz), δ/ppm** 8.82-8.77 (4H, m, Tbf Ar H); 8.77-8.69 (2H, m, Tbf ArH); 8.24-8.20 (2H, m, Tbf Ar H); 7.72-7.60 (8H, m, Tbf Ar H); 7.27-7.24 (2H, m, Ar H); 6.86-6.83 (2H, m, Ar H); 4.93 (1H, t, $^3J_{HH}$=4.3Hz, fluorenyl CH); 4.59 (1H, s, $C\underline{H_2}OH$); 3.81 (2H, t, $^3J_{HH}$=6.5Hz, $OC\underline{H_2}$); 2.61-2.56 (2H, m, $OCH_2C\underline{H_2}$); 1.87 (1H, s, OH); 1.64-1.58 (2H, m, CH_2); 1.30-0.72 (12H, m, 6×CH_2); 0.42-0.30 (2H, m, CH_2). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz) δ/ppm** 158.5, 132.6 (*q* Ar C); 128.4, 114.2 (Ar CH); 144.2, 136.6, 131.0, 130.2, 128.6, 127.8 (*q* Tbf Ar C); 127.3, 126.6, 125.7, 125.4, 124.8, 124.3, 123.3, 123.3 (Tbf Ar CH); 67.7 (OCH_2); 64.8 (CH_2OH); 47.0 (fluorenyl C); 33.3, 29.2, 29.0, 28.9, 28.6, 25.6, 22.0 (CH_2). **HRMS (FAB), M$^+$/z** $C_{46}H_{44}O_2$ (M^+) requires 628.3341, found 628.3339.

Preparation of Ethyl 3-(2,4,5-Trifluorophenyl)-3-oxopropionate, 43[18]

Magnesium chloride and potassium ethyl malonate were dried over silica in a vacuum dessicator overnight prior to use.

To potassium ethyl malonate (3.66g, 21.5mmol) in freshly distilled acetonitrile (70mL) at 10-15°C was added anhydrous magnesium chloride (2.44g, 25.7mmol) and triethylamine (2.05g, 20.3mmol). The mixture was then stirred at room temperature for 2.5 hours, followed by cooling the resultant white slurry to 0°C, and subsequent addition of 2,4,5-trifluorobenzoyl chloride (2.00g, 10.3mmol) over a period of fifteen minutes, followed by more triethylamine (0.23g, 2.3mmol). The mixture was then stirred at room temperature for 16 hours. Acetonitrile was removed under reduced pressure and toluene (30mL) added. The mixture was reconcentrated and further toluene (60mL) added. HCl (1.5M, 40mL) was cautiously added, ensuring that the temperature did not exceed 25°C, and the aqueous layer separated. The organic fraction was washed with 1.5M HCl (2×25mL) and water (2×25mL), dried over

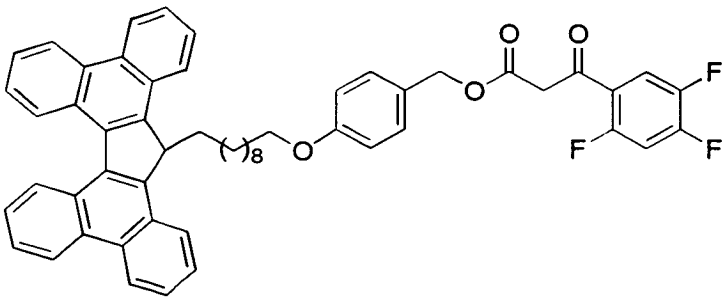
$MgSO_4$, and solvent removed under reduced pressure to yield ethyl 3-(2,4,5-trifluorophenyl)-3-oxo-propionate, **43**, as a pale orange solid (2.35g, 9.55mmol, 94%).



Rf 0.55 (DCM). **m.p.** 57-59°C. **FTIR, ν_{max}/cm^{-1} (bromoform)** 3018 (Ar CH); 2990 (CH); 1735 (ester C=O); 1687 (C=O); 1622, 1514 (C=C). **λ_{max}/nm (DCM, ε/dm^{3}mol^{-1}cm^{-1})** 295 (4781), 281 (3962), 240 (5191). **^{1}Hnmr ($CDCl_3$, 250MHz), δ/ppm** [12.69 (s), 5.81 (s) and 3.92 (d, $^3J_{HH}$=3.9Hz)] (2H, keto-enol tautomers); 7.74 (1H, m, Ar H); 6.98 (1H, m, Ar H); 4.24 and 4.17 (2H, both q, $^3J_{HH}$=7.1Hz); 1.31 and 1.24 (3H, both t, $^3J_{HH}$=7.1Hz). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz), δ/ppm** 187.8 (C=O); 173.0 (HC=C); 166.9 (CO_2Et); 163.7, 159.8, 155.9, 151.8, 149.0, 145.3, 120.9 (*q* Ar C); 118.7, 106.6 (Ar CH); 92.9 (HC=C); 61.4 and 60.6 (OCH_2); 49.4 (CH_2); 14.0 (CH_3). **^{19}Fnmr ($CDCl_3$, 235MHz) δ/ppm**; -110.6 and -111.7 (m, Ar F); -123.5 and -129.0 (m, Ar F); -140.5 and -141.7 (m, Ar F). **HRMS (EI), M$^+$/z** $C_{11}H_9F_3O_3$ (M^+) requires 246.0504; found 246.0514. **Elemental Analysis** $C_{11}H_9F_3O_3$ requires 53.67% C, 3.68% H; found 53.97% C, 3.77% H.

Preparation of 4-[10-(17'-Tetrabenzo[*a,c,g,i*]fluorenyl)decyloxy]benzyl 3-(2,4,5-Trifluorophenyl)-3-oxopropionate, 107.

To 4-[10-(17'-Tetrabenzo[a,c,g,i]-fluorenyl)decyloxy]benzyl alcohol, **104** (508.8mg, 0.81mmol), in toluene (40mL), was added DMAP (30mg, 0.25mmol), and ethyl 3-(2,4,5-trifluorophenyl)-3-oxopropionate, **43** (532.0mg, 2.16mmol), and the mixture heated to reflux for 40 hours. The reaction was then cooled to room temperature and filtered through a small pad of silica. The filtrate was washed with saturated ammonium chloride solution (20mL) and brine (20mL) and the organic phase dried over $MgSO_4$. Solvent was removed under reduced pressure to yield an orange oil, which was purified by flash chromatography (DCM), to yield 4-[10-(17'-tetrabenzo-[*a,c,g,i*]fluorenyl)decyloxy]benzyl 3-(2,4,5-trifluorophenyl)-3-oxo-propionate, **107**, as a yellow solid (470.0mg, 0.57mmol, 70%).

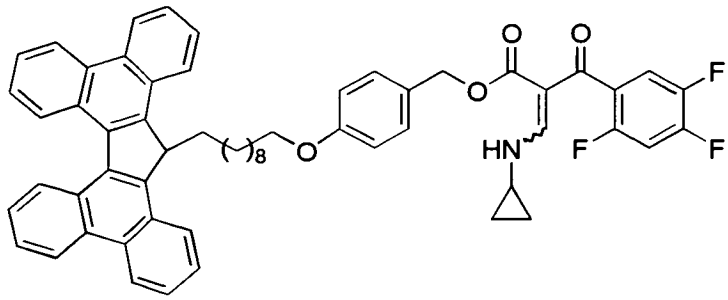


Rf 0.53 (DCM). **m.p.** 58-60°C. **FTIR** ν_{max}/cm^{-1} **($CHCl_3$)** 3089, 3062 (Ar CH); 2933, 2853 (CH); 1737 (CO_2Ar); 1690 (C=O); 1620, 1514 (C=C). λ_{max}**/nm (DCM,** $\varepsilon/dm^3mol^{-1}cm^{-1}$**)** 381 (4950), 365 (4950), 301 (14000), 288 (13200), 254 (22700).^{1}**Hnmr ($CDCl_3$, 250MHz),** δ**/ppm** [12.69 (s), 5.86 (s) and 3.95 (d, $^3J_{HH}$=3.9Hz)] (2H, keto-enol tautomers); 8.82-8.76 (4H, m, Ar H); 8.72-8.68 (2H, m, Ar H); 8.25-8.22 (2H, m, Ar- H); 7.82-7.59 (9H, m, Ar H); 7.33-7.22 (2H, m, Ar H); 6.95-6.80 (3H, m, Ar H); 5.13 (2H, s, OCH_2Ar); 4.97 (1H, t, $^3J_{HH}$=4.3Hz, fluorenyl H); 3.83 (2H, t, $^3J_{HH}$=6.6Hz, OCH_2); 2.64-2.55 (2H, m, $OCH_2C\underline{H}_2$); 1.67-1.56 (2H, m, CH_2); 1.30-0.73 (12H, m, 6×CH_2); 0.42-0.34 (2H, m, CH_2). ^{13}**C{^{1}H}nmr ($CDCl_3$, 63MHz),** δ**/ppm** 187.6 (C=O); 172.8 (enol HC=$\underline{C}$); 166.7 (CO_2Ar); 159.2, 126.8 (*q* Ar C); 163.7, 159.8, 155.9, 151.8, 149.0, 145.3, 120.9 (*q* Ar C [C-F]); 144.2, 136.6, 131.1, 130.2, 128.7, 127.9 (Tbf *q* Ar C); 130.0, 114.3 (Ar CH); 118.4, 106.5 (Ar CH [C-F]); 127.3, 126.6, 125.7, 125.5, 124.9, 124.3, 123.4, 123.3 (Tbf Ar CH); 92.9 (H$\underline{C}$=C); 67.8 (OCH_2); 67.0 and 66.2 (OCH_2Ar); 49.4 (OC$\underline{C}H_2$CO); 47.0 (fluorenyl C); 33.4, 29.2, 29.0, 28.9, 28.6, 25.6, 22.0 (CH_2). **HRMS (FAB), M^+/z** $C_{55}H_{47}F_3O_4$ (M^+) requires 828.3426; found 828.3424. **Elemental Analysis** $C_{55}H_{47}F_3O_4$ requires 79.69% C, 5.71% H; found 79.59% C, 5.82% H.

Preparation of 4-[10-(17'-Tetrabenzo[a,c,g,i]fluorenyl)decyloxy]benzyl 3-Cyclopropylamino-2-(2,4,5-trifluorobenzoyl)acrylate, 110.

To 4-[10-(17'-tetrabenzo-[*a,c,g,i*]fluorenyl)decyloxy]benzyl 3-(2,4,5-trifluorophenyl)-3-oxopropionate, **107** (51.7mg, 0.062mmol) in THF(2mL), was added *N,N*-dimethylformamide dimethyl acetal (60.0mg, 0.50mmol) as a solution in THF (1mL) *via* syringe, and the mixture was stirred at room temperature for 18 hours. Cyclopropylamine (35.4mg, 0.62mmol) was added as a solution in THF (1mL) and the mixture stirred at room temperature for 4 hours. The reaction was quenched by the addition of saturated ammonium chloride solution (20mL) and then filtered

through a small pad of silica. Organic products were extracted with ethyl acetate (2×20mL), dried over $MgSO_4$ and solvent removed under reduced pressure to yield a dark yellow oil. This was purified by flash chromatography (DCM) to yield 4-[10-(17'-tetrabenzo[a,c,g,i]fluorenyl)decyloxy]benzyl 3-cyclopropylamino-2-(2,4,5-trifluorobenzoyl)acrylate, **110**, as a pale yellow solid (38.9mg, 0.043mmol, 69%). Spectra were consistent with an isomeric ratio of 8:3.



Rf 0.44 (DCM). **m.p.** 70-72°C. **FTIR, ν_{max}/cm^{-1} ($CHCl_3$)** 3082 (Ar CH); 2930, 2854 (CH); 1738 (CO_2Ar); 1677 (C=O) 1623, 1561 1511 (C=C). **λ_{max}/nm (DCM, ε/dm^{3}mol^{-1}cm^{-1})** 381 (17900), 364 (189), 316 (21700), 301 (56100), 289 (44800), 254 (81100), 239 (69300). **^{1}Hnmr ($CDCl_3$, 250MHz), δ/ppm** 10.82 and 9.43 (1H, both d, $^3J_{HH}$=11.8Hz, NH); 8.83-8.78 (4H, m, Ar H); 8.71-8.66 (2H, m, Ar H); 8.28-8.23 (3H, m, Ar H); 7.74-7.59 (8H, m, Ar H); 7.09-6.61 (6H, m, Ar H); 5.05 (1H, t, $^3J_{HH}$=4.4Hz, fluorenyl H); 4.95 and 4.88 (2H, both s, OCH_2Ar); 3.83 (2H, t, $^3J_{HH}$=6.6Hz, OCH_2); 2.94-2.91 (1H, m, N-CH); 2.65-2.51 (2H, m, $OCH_2\underline{CH_2}$); 1.68-1.60 (2H, m, CH_2); 1.26-1.19 (4H, m, 2×CH_2); 1.06-0.74 (14H, m, 7×CH_2); 0.38-0.34 (2H, m, CH_2). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz), δ/ppm** 168.0 (CO_2Ar); 166.4 (C=O); 160.8 ($\underline{C}H$=C); 159.0, 127.4 (*q* Ar C); 155.9, 152.3, 148.4, 117.1, (*q* Ar C [C-F]); 144.3, 136.7, 131.1, 130.3, 128.7, 127.9, (Tbf *q* Ar C); 129.9, 114.1 (Ar CH); 116.3, 105.0 (Ar CH [C-F]); 127.4, 127.0, 125.8, 125.5, 124.9, 124.4, 123.4, (Tbf Ar CH); 101.4 (CH=$\underline{C}$); 67.8 (OCH_2); 65.7 and 65.5 (OCH_2Ar); 47.1 (fluorenyl C); 33.4, 29.3, 29.1, 29.0, 28.6, 25.7, 22.0 (CH_2); 30.4 and 30.0 (N-CH); 6.42 ($CH\underline{C}H_2\underline{C}H_2$). **^{19}Fnmr ($CDCl_3$, 235MHz) δ/ppm** -116.4 and -117.2 (Ar F); -131.7, -132.3 (m, Ar F); 143.5 and 143.7 (m, Ar F). **HRMS (FAB) M$^+$/z** $C_{59}H_{52}F_3NO_4$ (M^+) requires 895.3848; found 895.3816.

Preparation of 4-[10-(17'-Tetrabenzo[a,c,g,i]fluorenyl)decyloxy]benzyl 1-Cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxo-3-quinoline Carboxylate, 109.

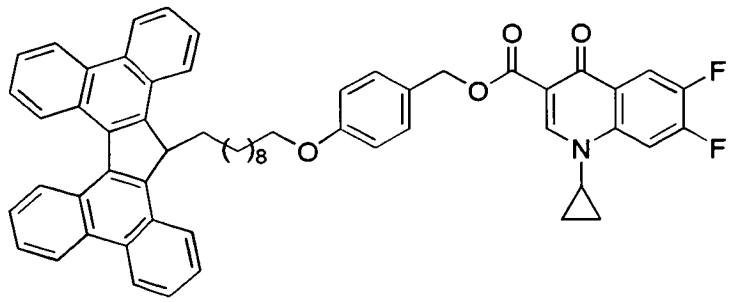
*1. From 4-[10-(17'-Tetrabenzo[a,c,g,i]fluorenyl)decyloxy]benzyl 3-Cyclopropylamino-2-(2,4,5-trifluorobenzoyl)acrylate, **110**.*

To 4-[10-(17'-Tetrabenzo[*a,c,g,i*]-fluorenyl)decyloxy]benzyl 3-cyclopropylamino-2-(2,4,5-trifluorobenzoyl)acrylate, **110** (80.7mg, 0.09mmol), in DCM (8mL), was added 1,1,3,3-tetramethylguanidine (207.5mg, 1.80mmol), and the reaction mixture was heated at reflux for 20 hours. The mixture was then cooled to room temperature, quenched with water (20mL), and the aqueous layer separated and washed with DCM (2×20mL). The combined extracts were washed with 2M HCl (20mL), saturated sodium carbonate solution (20mL), water (20mL) and brine (20mL), dried over $MgSO_4$, and solvent removed under reduced pressure to yield an orange oil. This was purified by flash chromatography (ethyl acetate/hexane, 3:1) to yield 4-[10-(17'-tetrabenzo[a,c,g,i]fluorenyl)decyloxy]benzyl 1-cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxo-3-quinoline carboxylate, **109**, as a yellow solid (43.4mg, 0.049mmol, 55%).

*2. From 4-[10-(17'-Tetrabenzo-[a,c,g,i]fluorenyl)decyloxy]benzyl 3-(2,4,5-trifluorophenyl)-3-oxopropionate,**107**.*

To 4-[10-(17'-tetrabenzo[*a,c,g,i*]fluorenyl)decyloxy]benzyl 3-(2,4,5-trifluorophenyl)-3-oxopropionate, **107** (200.4mg, 0.242mmol), in THF (15mL), was added *N,N*-dimethylformamide diethyl acetal (214.6mg, 1.46mmol), and the mixture was stirred at room temperature for 22 hours. Cyclopropylamine (110.4mg, 1.93mmol) was added as a solution in THF (2mL) and the resultant mixture was stirred at room temperature for 24 hours. TMG (550.8mg, 4.79mmol) was added as a solution in THF (2mL) and the reaction mixture heated at reflux for 18 hours. The solution was cooled to room temperature and quenched with saturated ammonium chloride solution (20mL), then filtered through a pad of silica. Organic products were extracted with ethyl acetate (40mL), washed with brine (30mL), dried over $MgSO_4$, and solvent removed under reduced pressure to yield a dark oil. This was purified by flash chromatography (ethyl acetate/hexane, 3:1) to yield 4-[10-(17'-

tetrabenzo[a,c,g,i]fluorenyl)decyloxy]benzyl 1-cyclo-propyl-6,7-difluoro-1,4-dihydro-4-oxo-3-quinoline carboxylate, **109**, as a yellow solid (143.5mg, 0.164mmol, 68%).

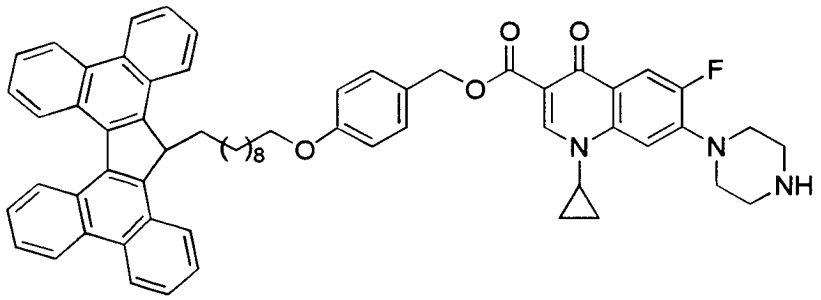


Rf 0.65 (ethyl acetate/hexane, 3:1). **m.p.** 71-73°C. **FTIR, ν_{max}/cm^{-1}** 3071 (Ar CH); 2987, 2932, 2854 (CH); 1726 (CO_2Ar); 1690 (C=O); 1643, 1513, 1494 (C=C). **λ_{max}/nm (DCM, ε/dm^{3}mol^{-1}cm^{-1})** 380 (7370), 364 (7830), 331 (9210), 317 (8290), 301 (22100), 289 (19300), 257 (39200), 238 (33200). **^{1}Hnmr ($CDCl_3$, 250MHz), δ/ppm** 8.80-8.75 (4H, m, Ar H); 8.68-8.64 (2H, m, Ar H); 8.41 (1H, s, unsat. H); 8.26-8.15 (4H, m, Ar H); 7.71-7.56 (9H, m, Ar H); 7.41-7.38 (2H, m, Ar H); 6.86-6.82 (2H, m, Ar H); 5.03 (1H, t, $^3J_{HH}$=4.1Hz, fluorenyl H); 4.92 (2H, s, OCH_2Ar); 3.83 (2H, t, $^3J_{HH}$=6.5Hz, OCH_2); 3.27-3.21 (1H, m, N-CH); 2.62-2.56 (2H, m, $OCH_2C\underline{H}_2$); 1.71-1.55 (4H, m, 2×CH_2); 1.35-1.11 (4H, m, 2×CH_2); 1.04-0.72 (12H, m, 6×CH_2); 0.36-0.32 (2H, m, CH_2). **^{13}C{^{1}H}nmr ($CDCl_3$, 63MHz), δ/ppm** 172.4 (CO_2Ar); 164.8 (C=O); 158.9, 127.9, (*q* Ar C); 148.7 ($\underline{C}H$=C); 155.7, 150.8, 146.5, 137.3, (*q* Ar C [C-F]); 144.2, 136.6, 131.1, 130.2, 128.6, 127.8, (Tbf *q* Ar C); 129.9, 114.3 (Ar CH); 127.3, 126.7, 125.7, 125.5, 124.9, 124.3, 123.4, 123.3 (Tbf Ar CH); 115.3, 105.4 (Ar CH [C-F]); 110.5 (-CH=$\underline{C}$); 67.7 (OCH_2); 66.2 (OCH_2Ar); 47.1 (fluorenyl C); 34.5 (N-CH); 33.3, 29.1, 29.0, 28.9, 28.5, 25.6, 21.9 (CH_2); 7.9 ($CH\underline{C}H_2\underline{C}H_2$). **^{19}Fnmr ($CDCl_3$, 235MHz) δ/ppm** -127.6 (m, Ar F); 139.1 (m, Ar F). **HRMS (FAB), M$^+$/z** $C_{59}H_{52}F_2NO_4$ (MH^+) requires 876.3864; found 876.3860.

Preparation of 4-[10-(17'-Tetrabenzo[a,c,g,i]fluorenyl)decyloxy]benzyl 1-Cyclo-propyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline Carboxylate, 111.

To 4-[10-(17'-tetrabenzo[a,c,g,i]fluorenyl)decyloxy]benzyl 1-cyclo-propyl-6,7-difluoro-1,4-dihydro-4-oxo-3-quinoline carboxylate, **109**, (49.6mg, 0.057mmol) in pyridine (distilled over sodium, 8ml), was added piperazine (recrystallised from ethanol, 99.1mg, 1.15mmol) and the mixture heated at reflux for 6 hours. The mixture

was then cooled to room temperature, and quenched with saturated ammonium chloride solution (15mL) and organic products extracted with DCM (2x20mL). The combined extracts were washed with water (25mL), dried over sodium sulphate, and solvent removed under reduced pressure. The crude residue was purified by flash chromatography ($CHCl_3$/methanol, 9:1) to yield 4-[10-(17'-tetrabenzo[a,c,g,i]-fluorenyl)decyloxy]benzyl 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylate, **111**, as a yellow solid (40.0mg, 0.043mmol, 76%).



Rf 0.24 ($CHCl_3$/methanol, 9:1). **FTIR, ν_{max}/cm^{-1}** 3679 (NH); 3074 (Ar CH); 2927, 2852 (CH); 1724 (CO_2Ar); 1689 (C=O); 1619, 1523, 1494 (C=C). **λ_{max}/nm (DCM, ε/dm^{3}mol^{-1}cm^{-1})** 380 (11400), 364 (11400), 336 (15800), 323 (14400), 302 (36600), 290 (41200), 281 (33900), 254 (60400). **^{1}Hnmr ($CDCl_3$, 250MHz), δ/ppm** 8.81-8.76 (4H, m, Ar H); 8.68-8.65 (2H, m, Ar H); 8.42 (1H, s, unsat. H); 8.27-8.24 (2H, m, Ar H); 8.00 (1H, d, $^3J_{HH}$=13.3Hz, Ar H); 7.72-7.57 (8H, m, Ar H); 7.44-7.40 (2H, m, Ar H); 7.15 (1H, d, $^3J_{HH}$=7.1Hz, Ar H); 6.86-6.83 (2H, m, Ar H); 5.29 (2H, s, OCH_2Ar); 5.05 (1H, t, $^3J_{HH}$=4.4Hz, fluorenyl H); 3.84 (2H, t, $^3J_{HH}$=6.5Hz, OCH_2); 3.28-3.23 (1H, m, N-CH); 3.19-3.15 (4H, m, 2×CH_2); 3.06-3.03 (4H, m, 2×CH_2); 2.62-2.56 (2H, m, OCH_2CH_2); 1.66-1.57 (2H, m, CH_2); 1.35-0.72 (18H, m, 9×CH_2); 0.36-0.30 (2H, m, CH_2). **^{19}Fnmr ($CDCl_3$, 235MHz) δ/ppm** -123.9 (s, Ar F). **HRMS (FAB), M$^+$/z** $C_{63}H_{61}FN_3O_4$ (M^+) requires 942.4646; found 942.4649.

4.11 Adsorption of Tbf Derivatives onto Charcoal

General Procedure

A solution of the Tbf-derivative was prepared in 25mL of the appropriate solvent ($c = 5\times10^{-5}M$), and the uv-visible spectrum of the solution measured in the region 320-420nm. The adsorbent was added and the suspension stirred in an ice bath for twenty minutes. The uv-visible spectrum of the supernatant was measured, and more adsorbent added if necessary with stirring, until the uv-visible spectrum indicated that no compound remained in solution ($\lambda_{max} \sim 0$).

Desorption was monitored by stirring the carbon residue in fresh solvent (25mL) for periods of 20 minutes at 40°C. Uv-visible spectra of the supernatant were recorded until no more desorption was detected.

4-[10-(17'-Tetrabenzo[a,c,g,i]fluorenyl)decyloxy]benzyl Alcohol, 104

The general procedure was followed to adsorb **104** (0.55mg, 88mmol) onto charcoal (40mg) using DCM as solvent. The uv-visible spectrum indicated an adsorbance of 92% (0.02mmol/g carbon). Attempted desorption with dioxane and toluene, and monitoring by uv-visible spectroscopy, indicated a desorption of 0% and 19% respectively

4-[10-(17'-Tetrabenzo[*a,c,g,i*]fluorenyl)decyloxy]benzyl 3-(2,4,5-Trifluoro-phenyl)-3-oxopropionate, 107.

1. The general procedure was followed to adsorb **107** (0.54mg, 0.66mmol) onto charcoal (21.3mg) using DCM as solvent. The uv-visible spectrum indicated an adsorbance of 97% (0.03mol/g carbon). Attempted desorption with dioxane and toluene, and monitoring by uv-visible spectroscopy, indicated a desorption of 0% and 27% respectively.

2. The general procedure was followed to adsorb **107** (0.52mg, 0.63mmol) onto charcoal (5.8mg) using DCM/methanol (3:2). The uv-visible spectrum indicated an adsorbance of 92% (0.1mol/mg carbon). Attempted desorption using THF, and monitoring by uv-visible spectroscopy, indicated a desorption of 10%. Re-adsorption

to the same charcoal, followed by desorption using toluene and monitoring by uv-visible spectroscopy, indicated a desorption of 85%.

3. The general procedure was followed to adsorb **107** (0.7mg, 845nmol) onto PGC (28.9mg) using DCM/methanol (3:2) as solvent. The uv-visible spectrum indicated an adsorbance of 95% (0.03mmol/mg carbon loading). Desorption was not attempted.

4-[10-(17'-Tetrabenzo[a,c,g,i]fluorenyl)decyloxy]benzyl 1-Cyclo-propyl-6,7-difluoro-1,4-dihydro-4-oxo-3-quinoline Carboxylate, 109.

1. The general procedure was followed to adsorb **109** (1.4mg, 1.6mmol) onto charcoal (14.2mg) using DCM/methanol (3:2) as solvent. Uv-visible spectrum indicated an adsorbance of 90% (0.10mol/g carbon). Desorption was attempted using toluene as the solvent. The maximum level of desorption by uv spectoscopy was 18%.

4.12 Synthesis of Ciprofloxacin by Charcoal Purification of Intermediates.

General Charcoal Purification Protocol

To the crude product residue in DCM (15mL) at 0°C, was added methanol and charcoal (prewashed with toluene) in portions, until TLC indicated that all of the desired product had been adsorbed. The charcoal was isolated by centrifugation and stirred in portions of toluene (40mL) at 40°C, until TLC indicated that no more desorption was occurring. Product identity was confirmed by uv-vis spectroscopy.

Preparation of 4-[10-(17'-Tetrabenzo[*a,c,g,i*]fluorenyl)decyloxy]benzyl 3-(2,4,5-trifluorophenyl)-3-oxopropionate, 107.

To 4-[10-(17'-tetrabenzo[a,c,g,i]-fluorenyl)decyloxy]benzyl alcohol, **104** (44.9mg, 0.071mmol), in toluene (8mL), was added DMAP (3mg, 0.025mmol), and ethyl 3-(2,4,5-trifluorophenyl)-3-oxopropionate, **43** (46.2mg, 0.19mmol), and the mixture heated to reflux for 20 hours. The reaction was then cooled to room temperature and solvent removed under reduced pressure. The crude residue was treated with the general charcoal purification protocol using 45mL methanol, and 510mg charcoal,

yielding a yellow oil, which was further purified by flash chromatography (DCM) to yield 4-[10-(17'-tetrabenzo-[*a,c,g,i*]fluorenyl)-decyloxy]benzyl 3-(2,4,5-trifluorophenyl)-3-oxopropionate, **107**, as a yellow solid (35.2mg, 0.043mmol, 70%). **Rf** 0.53 (DCM) λ_{max}**/nm (DCM,** ε**/dm^{3}mol^{-1}cm^{-1})** 381 (4500), 365 (4500), 301 (12700), 288 (11300), 254 (20500).

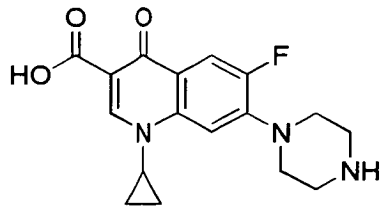
Preparation of 4-[10-(17'-Tetrabenzo[a,c,g,i]fluorenyl)decyloxy]benzyl 1-Cyclo-propyl-6,7-difluoro-1,4-dihydro-4-oxo-3-quinoline Carboxylate, 109.

To 4-[10-(17'-tetrabenzo[*a,c,g,i*]fluorenyl)decyloxy]benzyl 3-(2,4,5-trifluorophenyl)-oxopropionate, **107** (50.1mg, 0.061mmol), in THF (8mL), was added *N,N*-dimethylformamide diethyl acetal (53.6mg, 0.365mmol), and the mixture was stirred at room temperature for 24 hours. Cyclopropylamine (28.4mg, 1.93mmol) was added as a solution in THF (1mL) and the resultant mixture was stirred at room temperature for 22 hours. TMG (138mg, 1.20mmol) was added as a solution in THF (1mL) and the reaction mixture heated at reflux for 20 hours. The solution was cooled to room temperature and solvent removed under reduced pressure. The crude residue was treated with the general charcoal purification protocol using 40mL methanol, and 400mg charcoal, yielding a yellow oil, which was further purified by flash chromatography (ethyl acetate/hexane, 3:1) to yield 4-[10-(17'-tetrabenzo-[*a,c,g,i*]fluorenyl)-decyloxy]benzyl 1-cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxo-3-quinoline carboxylate, **109**, as a yellow solid (31.4mg, 0.036mmol, 59%). **Rf** 0.68 (ethyl acetate/hexane, 3:1), λ_{max}**/nm (DCM,** ε**/dm^{3}mol^{-1}cm^{-1})** 380 (6650), 364 (7000), 331 (8350), 317 (7400), 301 (20400), 289 (17300), 257 (3520), 238 (30100).

Preparation of 1-Cyclo-propyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline Carboxylic Acid, Ciprofloxacin, 93.

To 4-[10-(17'-tetrabenzo[a,c,g,i]fluorenyl)decyloxy]benzyl 1-cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxo-3-quinoline carboxylate, **109**, (75.2mg, 0.086mmol) in pyridine, was added piperazine (147.5mg, 1.72mmol) and the mixture heated at reflux for 6 hours. The mixture was then cooled to room temperature, and adsorbed onto charcoal (860mg) using 40mL methanol as described in the general procedure. The charcoal residue was treated with DCM/TFA/H_2O (18:11:1mL) at room temperature

for 15 minutes, the charcoal removed by centrifugation, and the product was precipitated by the addition of cold ether, to yield 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid, **93**, as a yellow solid (16.3mg, 0.049mmol, 57%).



RP-HPLC (Gradient 1) 18.1mins **m.p.** 228-230°C (dec.). **λ_{max}/nm (ethanol, ε/dm^{3}mol^{-1}cm^{-1})** 283 (24600), 254 (7300). **^{1}Hnmr (CD_3OD, 250MHz), δ/ppm** 8.95 (1H, s, unsat. H); 8.13 (1H, d, $^3J_{HH}$=13.0Hz, Ar H); 7.76 (1H, d, $^3J_{HH}$=6.9Hz, Ar H); 3.92-3.87 (1H, m, N-CH); 3.71-3.67 (4H, m, 2×CH_2); 3.55-3.53 (4H, m, 2×CH_2); 1.52-1.49 (1H, m, CH_2); 1.37-1.33 (1H, m, CH_2). **^{19}Fnmr (CD_3OD, 235MHz) δ/ppm** -73.3 (s, Ar F). **MS (ESI +35V), M$^+$/z** $C_{17}H_{18}FN_3O_3$ (MH^+) requires 332; found 332.

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Courses Attended

Organic Research Seminars, University of Edinburgh, 1995-1998

Current Awareness in Organic Chemistry, University of Edinburgh, 25th-28th May, 1998 and 26th-30th May 1997.

RSC Perkin Division, Scottish Meeting, University of Strathclyde, 1997; University of Edinburgh, 1996; University of Glasgow, 1995.

SCI Graduate Symposium, University of Edinburgh, 1998; University of Aberdeen, 1997; University of St. Andrews, 1996.

New Combinatorial Chemistry, London, 25th February 1997.

Combinatorial Approaches to Chemistry and Biology, Churchill College, Cambridge, 27th-30th July 1997.

Solid Phase Synthesis and Combinatorial Libraries, 5th International Symposium, Imperial College, London, 2nd-6th September 1997.

RSC Organic Chemistry Postgraduate Industry Tour, Cambridge 5th-7th October 1997.

Zeneca Pharmaceutical Medicinal Chemistry Workshop, January 1998.

Presentations and Awards

Publication: *The Use of Tetrabenzo[a,c,g,i]fluorene as an Anchor Group for the Solid/Solution Phase Synthesis of Ciprofloxacin.* A. M. Hay, S H.-Dewitt, A. A. MacDonald and R. Ramage, *Tetrahedron Lett.* **1998**, *39*, 8721

SCI Graduate Symposium, University of Edinburgh, 1998.

Organic Research Seminars, University of Edinburgh, April 1997.

Young Chemists Award at the Solid Phase Synthesis and Combinatorial Libraries, 5th International Symposium, Imperial College, London, 2nd-6th September 1997.

Use of Tetrabenzo[*a,c,g,i*]fluorene as an Anchor Group for the Solid/Solution Phase Synthesis of Ciprofloxacin.®

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Abstract: The affinity of tetrabenzo[*a,c,g,i*]fluorene for charcoal has been applied successfully to provide an alternative to existing solid phase synthesis methodology. In a synthesis of the quinolone antibacterial, Ciprofloxacin,® traditional solution phase synthesis has been coupled with efficient *pseudo*-solid phase purification.

Keywords: Affinity Purification; Solid/Solution Phase; Quinolone Antibiotics

With the advent of combinatorial synthesis [1], the focus of much academic and industrial research has been directed towards solid phase organic synthesis (SPOS) [2]. Conducting chemistry on molecules covalently bound to an insoluble polymeric resin has accessed large numbers of individual compounds for the potential use in drug lead discovery and optimisation. However, an inherent problem with the use of polymers in synthesis is their questionable compatibility with many reagents and reaction conditions. We sought to design a system which would combine the versatility of solution phase synthesis (homogeneous phase) with the ease of purification afforded by SPOS (heterogeneous phase).

The affinity of tetrabenzo[*a,c,g,i*]fluorene (Tbf), **1**, for charcoal has been utilised in the purification of peptides and proteins [3] and DNA [4] at Edinburgh. In general, the choice of solvent will influence the adsorption-desorption equilibrium [5] (Figure 1). It was proposed that a similar protocol be developed for general organic synthesis.

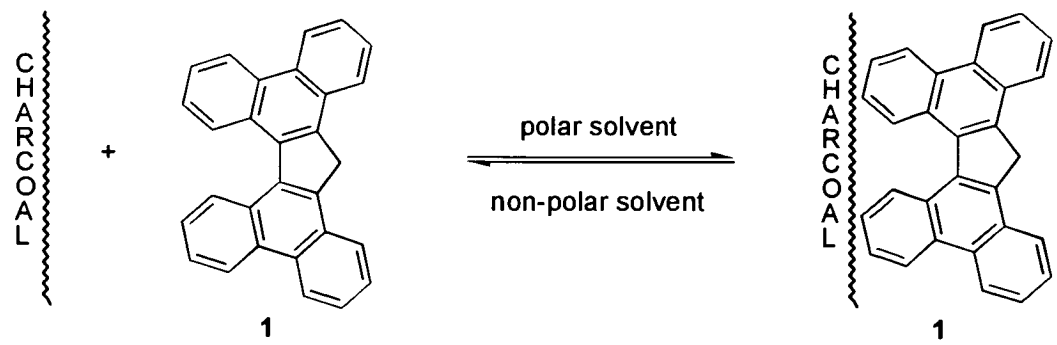
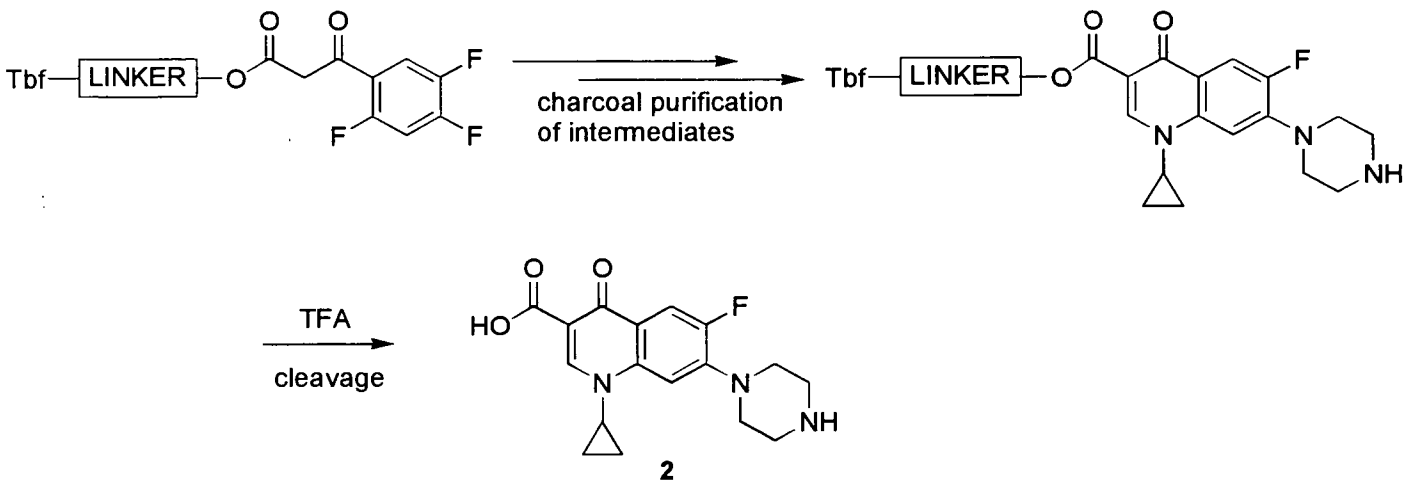


Figure 1.

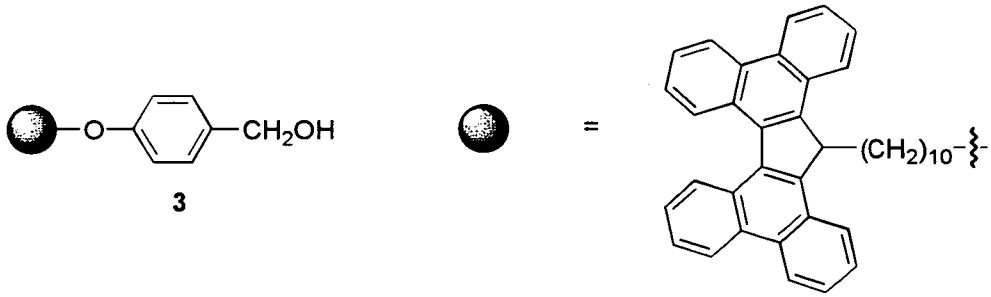
Organic compounds, covalently bound to Tbf *via* a suitable linker group could be reacted using traditional solution phase chemistry and the product purified by exploiting the affinity of the Tbf group for charcoal. Non-Tbf-bound compounds can be removed in the filtrate. As no covalent bonds are formed with the support, this may be described as a *pseudo*-solid phase purification step, and recovery of Tbf-bound product can be achieved by simple desorption from the carbon. The compound is then ready for the next synthetic step. Methodology was first developed (Figure 2) incorporating the Tbf-group for the solution synthesis of the antibacterial agent Ciprofloxacin,® **2**, which has been the subject of more conventional SPOS [6].



2

Figure 2.

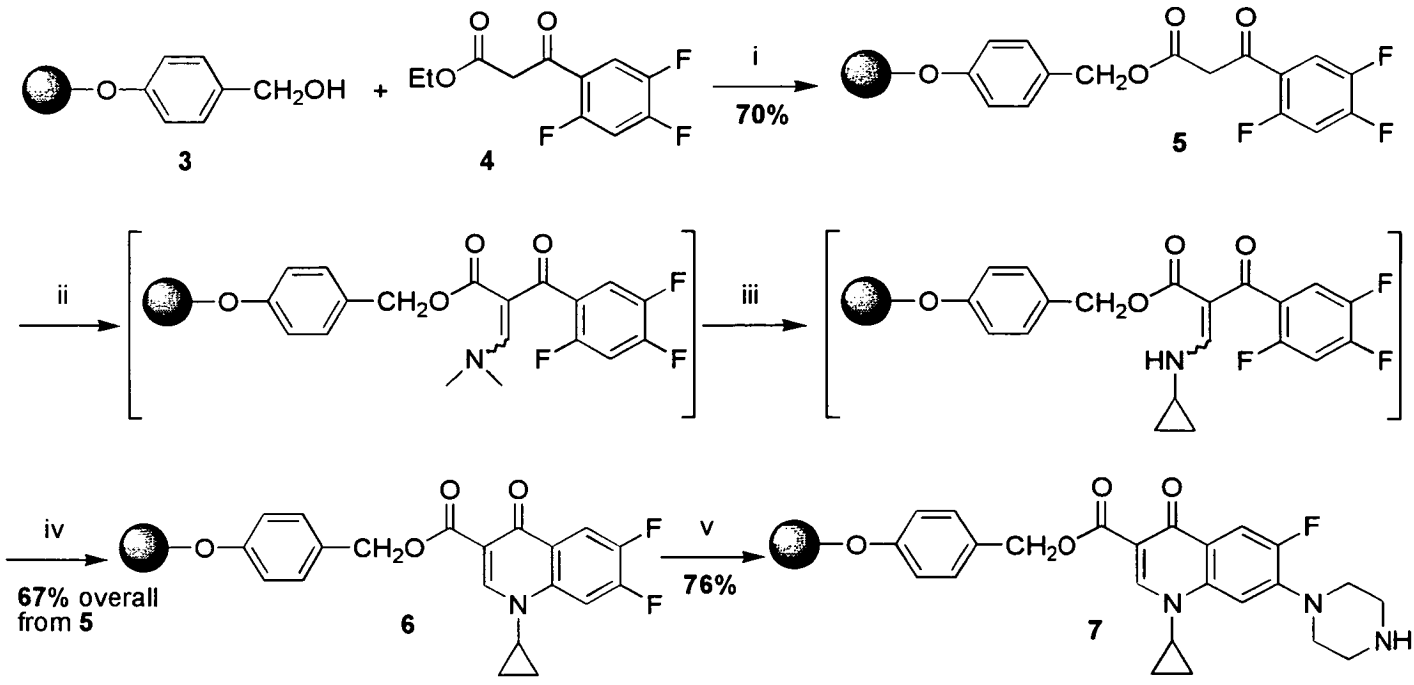
Successful synthesis of a suitable anchor group, **3** (Figure 3), was achieved in good yield following an established procedure [7]. The linker group was designed to possess suitable benzyl alcohol functionality to permit binding to a precursor in the quinolone synthesis and also include *para*-alkoxy functionality to allow TFA-cleavage of the final product from the anchor system in a manner analogous to Wang resin [8].



3

Figure 3.

Tbf-bound Ciprofloxacin,® **7**, was synthesised in solution phase with traditional work-up and chromatographic purification in order to optimise reaction conditions (Figure 4). The quinolone precursor, β-ketoester, **4**, was synthesised [9] and bound to the anchor group, **3**, *via* a transesterification reaction. One-pot enamide formation [10], transamination and base cyclisation, yielded the Tbf-bound quinolone, **6**. Synthesis of **7** was completed by nucleophilic aromatic substitution with piperazine.



(i) cat. DMAP, toluene, reflux, 40h; (ii) $(CH_3)_2NCH(OCH_3)_2$, 6eq., THF, rt., 24h; (iii) cyclopropylamine, 12eq., THF, rt., 20h; (iv) tetramethylguanidine, THF, 20eq., reflux, 20h; (v) piperazine, pyridine, reflux, 6h.

Figure 4.

Extensive studies of the factors influencing the adsorption-desorption equilibrium of pure Tbf-bound compounds with charcoal developed those conditions which yielded the greatest recovery of material. Analysis by ultraviolet (UV) spectrometry (364nm) indicated that adsorption of the substrate onto charcoal was quantitative using DCM/methanol (3:2) as the polar solvent. Isolating the carbon residue by centrifugation and stirring in toluene at 40°C afforded desorption of Tbf-bound compounds with a recovery in excess of 80% by UV (Figure 5).

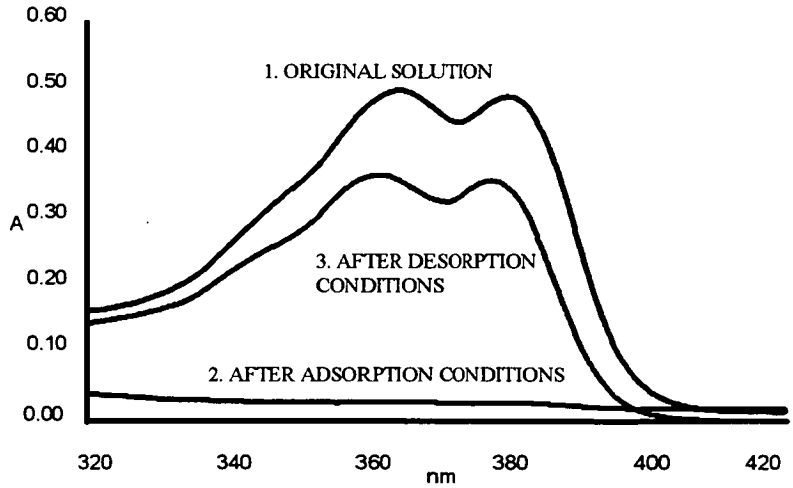


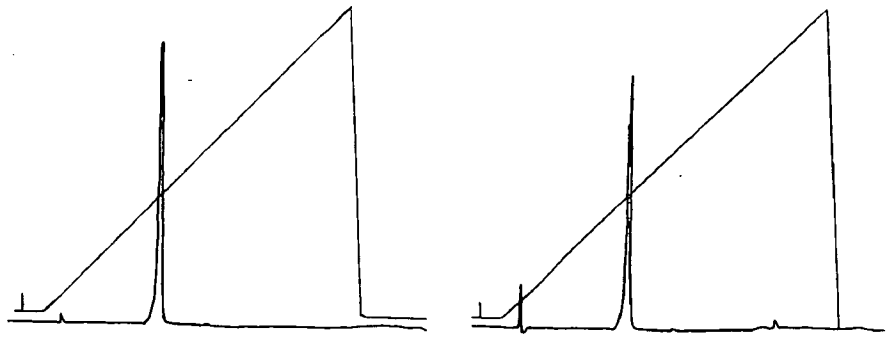
Figure 5

Of crucial importance to the success of this procedure was the quantity of charcoal used for adsorption. It was observed that addition of charcoal in excess of 1g/0.1mmol Tbf-compound reduced the recovery of material after the desorption step. It was reasoned that this may be due to undesired adsorption processes occurring as larger quantities of charcoal were added. Recovery of Tbf-bound material was enhanced by pre-washing the charcoal with toluene, possibly preventing excessive adsorption occurring.

The reproducible protocol for recovering pure Tbf-compound adsorbed onto charcoal was tested as a purification method by trials on crude samples of compounds **5** and **6**. Solution phase synthesis of these compounds was performed as normal, but instead of traditional work-up and purification, the crude residue was subjected to the described charcoal protocol. The excellent selectivity of the procedure was confirmed by TLC which indicated that only Tbf-compounds were recovered and no other reagents were present in the toluene

washings. In order to compare directly the efficiency of the process with traditional methods, chromatography was performed to isolate the desired compound (*e.g.* from unreacted starting material). The new purification protocol compared highly favourably with traditional methods and furnished **5** and **6** in 60% and 59% respectively (70% and 67% by normal work-up and chromatography).

The synthesis and isolation of Ciprofloxacin® **2**, was completed using a similar procedure. After adsorption of **7** onto charcoal from the crude mixture, cleavage of the quinolone from the Tbf-anchor group with 90% aqueous TFA in DCM yielded the desired product in 57% yield from **6**. This was observed to be >95 % pure by reverse phase HPLC (Figure 6).



Manufacturer's sample *Tbf-cleaved sample*

Figure 6.

The successful synthesis of Ciprofloxacin® **2**, has shown that traditional solution phase synthesis can be performed efficiently on molecules covalently bound to a Tbf anchor group. More importantly, however, the high affinity of Tbf for charcoal has enabled a *pseudo*-solid phase purification protocol of organic molecules to be developed. This has allowed us to take maximum advantage of solution phase chemistry whilst also capturing the undoubted benefits which heterogeneous purification methods afford. Furthermore, it is envisaged that this technique will make a valuable contribution to the multiple parallel synthesis of arrays of compounds.

Acknowledgements: We thank Parke-Davis for financial support throughout this research and Dr. R. M. Cresswell for his continued interest.

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The Use of Tetrabenzo[*a,c,g,i*]fluorene Towards the Synthesis of Ciprofloxacin.

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Introduction

Combinatorial chemistry has led to a recent explosion in the development of Solid Phase Organic Synthesis (SPOS).[1] Performing chemistry on molecules covalently bound to an insoluble polymeric resin has enabled the parallel synthesis of large numbers of compounds for potential use in the drug discovery process. However, an inherent problem with the use of polymers in organic synthesis is their sensitivity to many reagents and conditions. A more ideal system would combine the versatility of solution phase synthesis with the ease of purification afforded by solid phase synthesis.

Tetrabenzo[*a,c,g,i*]fluorene

The affinity of tetrabenzo[*a,c,g,i*]fluorene (Tbf) for charcoal has been utilized in the purification of peptides and proteins at Edinburgh.[2] It was envisaged that that this property could be harnessed in general synthesis for the purification of organic molecules (*Fig. 1*).

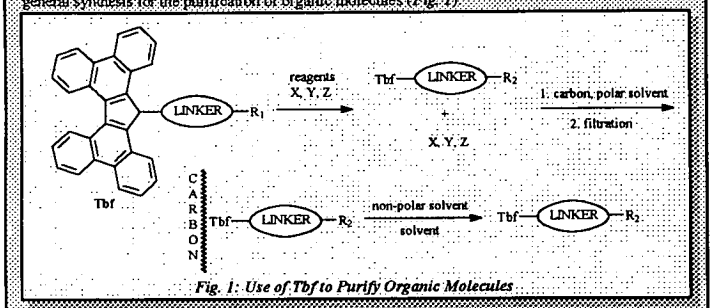
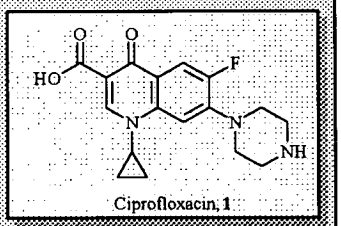


Fig. 1: Use of Tbf to Purify Organic Molecules

An organic fragment bound to Tbf by a suitable linker group could be subjected to traditional solution phase chemistry. The Tbf-bound product is then selectively adsorbed onto carbon in a *pseudo*-solid phase purification step. Impurities and excess reagents are removed in the supernatant, and the product, still bound to Tbf, desorbed using a non-polar solvent.

Target Molecule

The quinolone antibacterial, Ciprofloxacin, **1**, was chosen as a suitable target molecule.[3] Once bound to an appropriate Tbf-anchor group and the solution phase synthesis optimised, the synthesis could then be repeated using charcoal to purify intermediates.



Ciprofloxacin, **1**

Synthesis

Linker Group

A suitable linker group, **2**, was synthesised and bound to Tbf (*Fig. 2*). The Tbf-alcohol possessed suitable functionality to allow binding to the quinolone precursor, a 10-carbon alkyl chain to separate Tbf from the organic moiety and *p*-alkoxy functionality to allow TFA cleavage of the organic fragment from the anchor system in a manner analogous to Wang resin.[4]

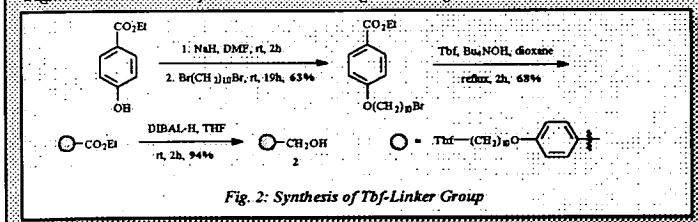
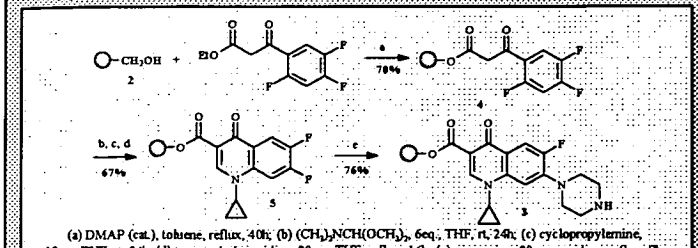


Fig. 2: Synthesis of Tbf-Linker Group

Ciprofloxacin

Synthesis of Tbf-bound Ciprofloxacin, **3**, was first performed in solution phase using traditional chromatographic purification. The quinolone backbone was synthesised by transesterification to form β-ketoester, **4** (*Fig. 3*), in 70% yield. The following three steps were then completed in one pot. Alkylation with dimethyl formamide dimethyl acetal,[5] followed by transamination and cyclisation yielded Tbf-quinolone, **5**, in an excellent overall yield of 67%. Nucleophilic aromatic substitution with piperazine in 76% yield completed the synthesis of **3**.



(a) DMAP (cat.), toluene, reflux, 40h; (b) $(CH_3)_2NCH(OCH_3)_2$, 6eq., THF, rt, 24h; (c) cyclopropylamine, 12eq., THF, rt, 24h; (d) tetramethylguanidine, 20eq., THF, reflux, 16h; (e) piperazine, 20eq., pyridine, reflux, 8h.

Fig. 3: Solution Phase Synthesis of Tbf-Ciprofloxacin

Charcoal Purification

Before using charcoal as a method of purifying intermediate Tbf-bound compounds, factors affecting the adsorption-desorption equilibrium were investigated. Uv spectroscopy was used to monitor the adsorption and desorption of Tbf-ketoester, **4** (*Fig. 4*). Adsorption was most efficiently achieved by stirring in a DCM/methanol (3:2) suspension of charcoal at room temperature. Heating the filtered charcoal residue in toluene at 40°C efficiently desorbed **4** into solution. High levels of material were obtained after 3-4 washes.

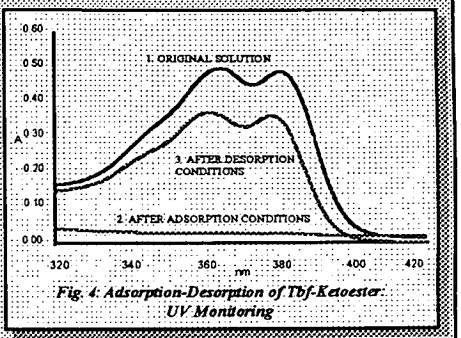


Fig. 4: Adsorption-Desorption of Tbf-Ketoester: UV Monitoring

Purification of Synthetic Intermediates

Optimised solution phase synthesis was repeated, but instead of normal work-up and chromatographic purification of intermediates, solvent was removed and replaced by the DCM/methanol mixture. Charcoal was added in portions until all the desired Tbf-compound had adsorbed (*Fig. 5*), and the carbon residue was then washed successively with toluene until no further material was desorbed.

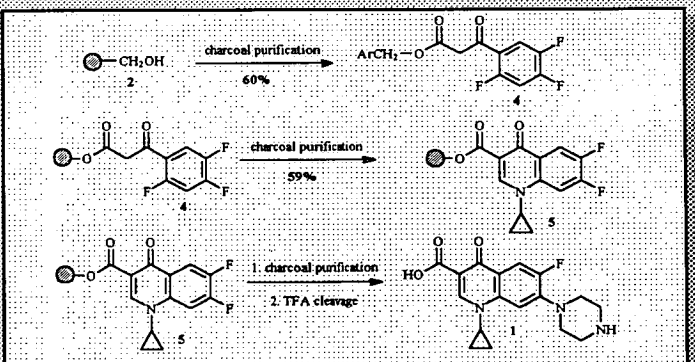


Fig. 5: Reactions Using Charcoal Purification

The final step in the synthesis of Ciprofloxacin performed with some variation to the previous ones. Rather than subjecting the adsorbed Tbf product, **3**, to warm toluene, the charcoal residue was stirred in a DCM / 95% aqueous TFA solution. To our delight, the product obtained in solution was found to co-elute by HPLC with a genuine sample of **1** and was >95 % pure (*Fig. 6*).

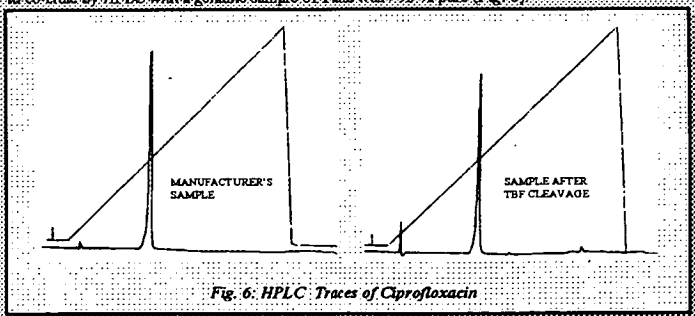


Fig. 6: HPLC Traces of Ciprofloxacin

Conclusions

This synthesis of Ciprofloxacin has illustrated the viability of performing organic chemistry on molecules bound to a Tbf anchor group. However, more importantly, the protocol involving charcoal has been observed to selectively purify such compounds. This may have important implications in the generation of compound libraries - versatile solution phase synthesis followed by efficient purification of intermediates.

References and Notes

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