Synthesis of Quinolone Antibiotics by DIVERSOMER™ Technology

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Gu m'athair agus mo mathair
le moran goal.

Aideachadh

Bu chaomh leam taing a thoirt gu an T-Ollamh Robert Ramage, FRS, airson na faoidean, an comhairle agus an cuideachadh aige tron na tri bliadhann a chaidh seachad anns an oilthigh Dhun-Eideann. Tha mi airson taing mor a thoirt gu a Dtr. Sheila Hobbs DeWitt, cha ni a mhain an cuideachadh, comhairle agus faoidean aice, ach a toil-inntinn a bi a obair leatha. Gu Parke-Davis Pharmaceutical Res. Co. (Ann Arbor, Michigan, USA) airson a taic airgead, agus taic airson a rannsaich seo.

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"A man would make but a very sorry chemist if he attended to that department of human knowledge alone. If your wish is to become really a man of science and not merely a petty experimentalist, I should advise you to apply to every branch of natural philosophy".

Mary Wollstonecraft Shelley

(Frankenstein)

1818

Abstract

The generation of chemical diversity by the parallel synthesis of potential drug candidates has been demonstrated by Parke-Davis' DIVERSOMER™ Technology. This technology combines solid phase organic synthesis, (SPOS), miniaturization, automation, integration and custom equipment to generate "libraries" of discrete compounds.

The research programme involved a detailed analysis of the synthesis of the quinolones, a well-known class of antibacterial agents of which over 6000 compounds are known to date. A solution phase synthesis was developed which was compatible with solid phase reaction conditions and also amenable to parallel SPOS protocols. Results of these studies and the parallel synthesis, isolation, purification and analysis of a quinolone library will be discussed.

Additionally, an alternative "solid support" for the SPOS of quinolones will be discussed. Construction of the quinolones with a tetrabenzo[*a,c,g,i*]fluorene, (Tbf) handle enables controlled adsorption onto and off porous graphitised carbon (PGC). The Tbf / PGC system also has demonstrated utility for automated, parallel purification strategies.

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ABBREVIATIONS

AcOH	acetic acid
α	alpha
Å	Ångströms
ATP	adenosine triphosphate
β	beta
b	broad
t-$BuNH_2$	*tert*-butylamine
t-BuOH	*tert*-butanol
C	carbon
CBI	chemical and biological information
CDI	carbonyl diimidazole
CCl_4	carbon tetrachloride
$CDCl_3$	chloroform-d
$CHBr_3$	bromoform
$CHCl_3$	chloroform
CH_3CN	acetonitrile
CHN	carbon, hydrogen, nitrogen
CPG	Contolled Porous Glass
d	doublet
4-DMAP	4-Dimethylaminopyridine
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCE	dichloroethane
DCM	dichloromethane
DIBAL	diisobutylaluminium hydride
DIC	diisopropylcarbodiimide
DMF	dimethylformamide
DMFA	N,N-dimethylformamide dimethyl acetal
DMSO	dimethyl sulphoxide
DIMSYL Na	sodium salt of DMSO
DNA	deoxyribonucleic acid
DVB	divinylbenzene
EC-1	escherichia coli vogel
EI	electron impact
Et	ethyl
Ether	diethyl ether
Et_3N	triethylamine
EtOAc	ethyl acetate
EtOH	ethanol
HOCt	ethyl 1-hydroxy-1H-1,2,3-triazole-4-carboxylate
HRMS	high resolution mass spectroscopy
F	fluorine
FAB	fast atom bombardment

Fmoc	9-fluorenylmethoxycarbonyl
γ	gamma
g	gramme
H	hydrogen
HCl	hydrochloric acid
HF	hydrogen fluoride
H_2O	water
h	hours
HPLC	high performance liquid chromatography
H_2SO_4	sulphuric acid
IR	infrared
KBr	potassium bromide
KO*t*Bu	potassium *tert*-butoxide
LCAA	long chain alkylamine
LDA	lithium diisopropylamide
M	molar
m	multiplet
MAS	magic angle spinning
max	maximum
Me	methyl
MeOH	methanol
mg	milligramme
$MgCl_2$	magnesium chloride
$MgSO_4$	magnesium sulphate
MHz	megahertz
mmol	millimoles
m.p.	melting point
MS	mass spectroscopy
NaH	sodium hydride
nm	nanometers
NMP	1-methyl-2-pyrrolidinone
NMR	nuclear magnetic resonance
NR	no reaction
P	product
PA-7	pseudomonas aeruginosa UI-18
PPA	polyphosphoric acid
ppm	parts per million
PS-PEG	polystyrene-polyethyleneglygol
py	pyridine
q	quartet
®	registered trademark
R_f	retention factor
RNA	ribonucleic acid
RSP	robotic sample processor
R1S1	reaction one-step one
R2S1	reaction two-step one
R2S2	reaction two-step two

R3S1	reaction three-step one
R4S1	reaction four-step one
R5S1	reaction five-step one
s	singlet
SPE	solid phase extraction
SPOS	solid phase organic synthesis
SPS	solid phase synthesis
SPPS	solid phase peptide synthesis
t	triplet
TFA	trifluoroacetic acid
TFA-d	trifluoroacetic acid-d
THF	tetrahydrofuran
TLC	thin layer chromatography
TM	trade mark
TMG	1,1,3,3-tetramethylguanidine
TMS	tetramethylsilane
TMSBr	trimethylbromosilane
UTI	urinary tract infections
UV	ultraviolet
v/v	volume / volume
w	weak

CHAPTER 1: INTRODUCTION

• 1.1. Molecular Diversity and Combinatorial Chemistry

A key step in any drug development program is the identification of a lead compound suitable for clinical trials. Increasing competitiveness in the pharmaceutical market and escalating costs for discovery efforts have placed extreme demands on the pharmaceutical industry. The search for new drugs has generally relied on traditional medicinal chemistry approaches which are both time consuming and costly. The discovery and development of a new drug takes on average 12 years, at an estimated cost of $357 million.[1] Recently, advances in high throughput screening and laboratory automation have resulted in the synthesis of medicinal compounds becoming a rate limiting step in the drug discovery process, (see fig 1.1).[2]

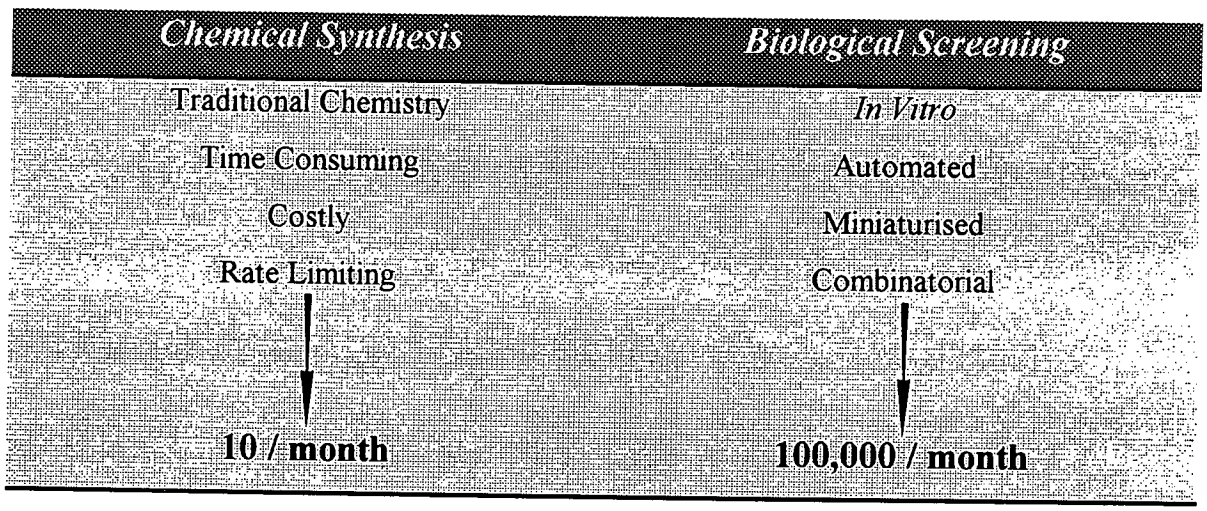
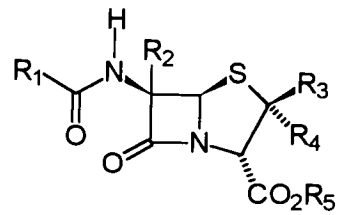


Fig. 1.1. Molecular Diversity: The Need

Traditional methods for the generation and evaluation of large numbers of compounds have relied heavily on natural products, fermentation broths, marine organisms,[3] and recombinant[4] and chemically synthesised[5] peptides. The advent of combinatorial chemistry has provided a means for the preparation of hundreds, or even thousands, of diverse chemical libraries at a fraction of the normal cost and time.

A recent phenomenon, combinatorial chemistry has resulted in the successful preparation of peptide[6] and oligonucleotide[7] based libraries. This new field of chemistry has also been expanded to small organic molecules such as the benzodiazepines[8,9] and hydantoins.[8] Consistent with this trend, most of the top selling drugs on the market are low molecular weight, heterocyclic compounds.

As an illustration of the power of combinatorial chemistry, the synthesis of a penicillin antibiotic containing five sites of diversity could provide over 3.2 million compounds,[2] (fig 1.2). This application of combinatorial chemistry should considerably shorten the drug discovery process, provided that methods are available for deconvolution of such large numbers of compounds.



Variations at Each R Position	*No. of Compounds*
2	$2^5 = 32$
5	$5^5 = 3125$
20	$20^5 = 3.2$ Million!

Fig. 1.2. Synthesis & Optimisation Example: Penicillin Analogues

It has been said that, "the power to synthetically create and evaluate huge numbers of known and future pharmacophores is unprecedented and suggests that combinatorial technologies may rapidly intersect and ultimately shortcut the traditional path of medicinal chemistry".[10] The promise of combinatorial chemistry has yet to be realised. However, the potential is evident and worthy of pursuit.

• 1.2. Solid Phase Synthesis, (SPS)

Historically solid phase chemistry has focused on the preparation of biopolymers such as peptides by the use of a few well characterised chemical transformations to generate repetitive structural backbones. In comparison, small organic molecules are generated from a large repertoire of diverse chemical reactions. For example, a β-lactam antibiotic may be synthesised *via* eight different reactions, while a pentapeptide is generated in eight steps but using only two different reactions. The limited number of chemical transformations developed on solid supports has therefore restricted the number of non-oligomeric compounds synthesised, despite the inherent advantages of this method.

The concept of performing reactions on an insoluble polymeric support or "immobilised media" was enunciated by R.B Merrifield[11] in 1963 and later by Letsinger and Kornet in 1964.[12] Today Solid Phase Peptide Synthesis (SPPS) is a straightforward yet elegant contribution to the art of chemical synthesis. The actual chemistry used is based on synthesising a peptide in a repetitive, sequential manner on a solid support (resin), from attachment of the first amino acid to the addition of the final residue. During the synthesis, the peptide-resin heterogeneous mixture remains in the same reaction vessel until the final product is cleaved and released into solution. The simplicity of the methodology has led to semi-automated systems.[13]

Since Merrifield's pioneering work, solid phase chemistry has progressed significantly and now dominates peptide chemistry. Oligonucleotide[14] and oligosaccharide[15] chemistry have similarly adopted solid phase approaches, and most present day combinatorial chemistry approaches have also embraced this methodology.

The multiple, simultaneous synthesis of peptide libraries was pioneered by Mario Geysen *et al.* in 1984.[16] Based on Merrifield's SPPS methods, the group at Australian Coselco Mimotopes Pty. successfully synthesised over 200 peptides a day. The initial utility of the peptide libraries was epitope mapping. To enable this, the peptides were left attached to the polystyrene rods and exposed to soluble antibodies. However, "the rate limiting factor for their work was the testing and evaluation" of

the library mixture.[17] Ironically, the rate limiting step in the drug discovery process today is the synthesis of compounds for both lead generation and lead optimisation.[2] Today, advances in molecular biology and automation have driven combinatorial chemistry as an efficient method for the synthesis and the supply of compounds for testing. In turn, this has renewed the interest of the chemical community in organic synthesis on solid supports.[18,19]

The advantages of solid phase synthesis over traditional solution based methods were clearly demonstrated with the synthesis of a tetrapeptide by Merrifield.[11] Excess reagents are readily tolerated by the solid support, reactions generally show favorable kinetics and can be driven to completion, product isolation is improved by washing away excess reagents from the solid support, and no purification of reaction intermediates is required. Thus, SPPS has now developed into a multi-million dollar business. In general, these advances have resulted in the current day methodology for combinatorial chemistry heavily relying on solid phase techniques.

Furthermore, the utility of solid supports as a tool for the chemists and biologists is evident by the interdisciplinary nature and possible applications in the fields of biochemistry, organic synthesis, catalysis and organometallic chemistry.[20]

• 1.3. Solid Supports

1.3.1. Polystyrene Supports

A variety of polymers have been described for solid phase synthesis, yet the literature related to polymeric supports is dominated by functionalised cross-linked polystyrene.[21] The most probable reasons for this are:[22]

i. Widely available and easily functionalised.

ii. The chemistry has been extensively studied.

iii. The notable success of early workers using this resin in the field of SPPS.

Polystyrene, (**2**) has generally been prepared by copolymerisation of styrene, (**1**). In order to create a cross-linked structure, styrene is polymerised in the presence

of a small amount of a divinyl compound such as *p*-divinylbenzene (DVB), (**3**) to provide an insoluble styrene polymer, (**4**), (as shown in fig.1.3).

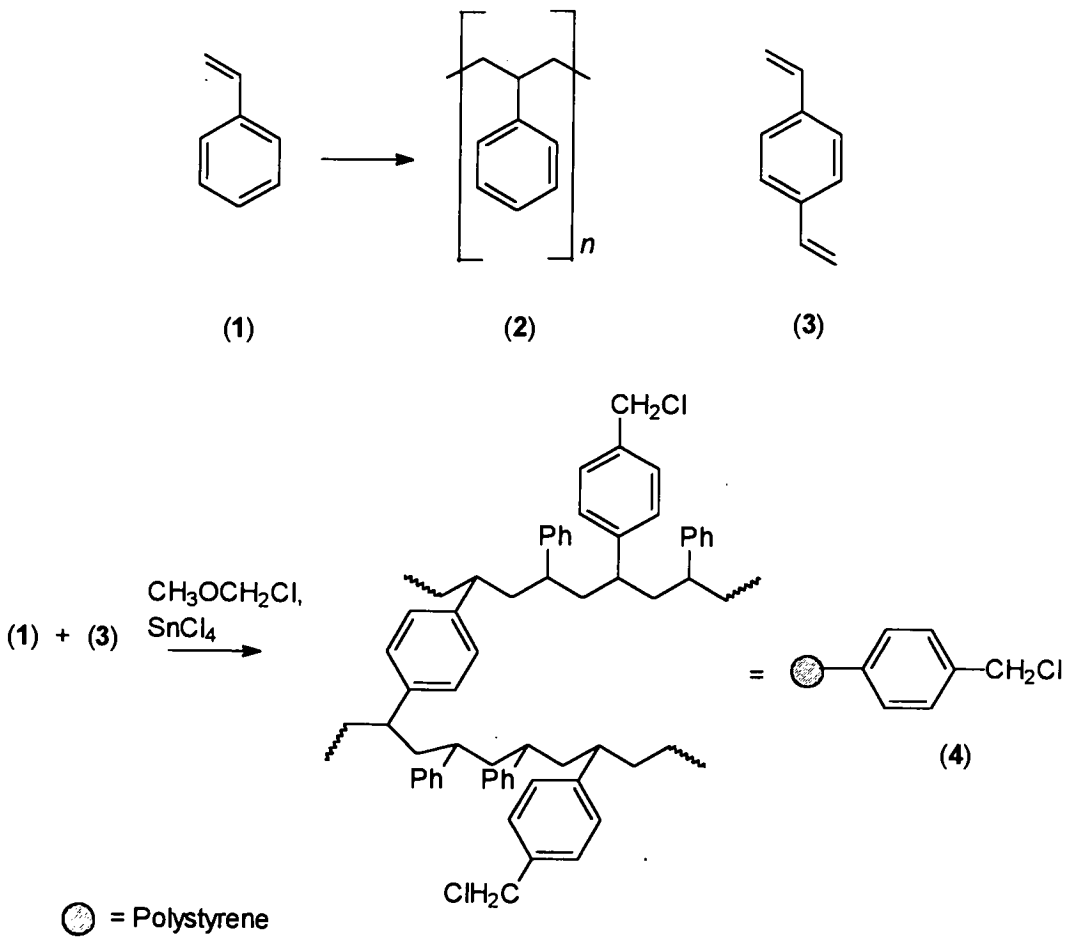


Fig. 1.3. Preparation of Merrifield Resin

Copoly(styrene-1%-divinylbenzene)chloromethylated resin, (**4**) was the solid support chosen by Merrifield. More commonly referred to as Merrifield resin, the polymer served as the prototype for the standard approach associated with his name.[23]

The structure and morphology of polystyrene generally depends on the degree of cross-linking incorporated into the polymeric network.[24] The resin originally used by Merrifield contained 2% DVB,[25] but most commercial resins typically contain 1% DVB cross-linking.[26] This has arisen directly as a consequence of the swelling properties of the resin. Once suspended in a compatible solvent such as

dichloromethane (DCM) the polymeric chain forms a gel. With 1% DVB present, such gels have a greater degree of swelling, are generally porous and allow good molecular mobility. Crosslinking greater than 1% DVB limits porosity which in turn limits resin swelling, while resins with crosslinking lower than 1% are soft and cannot be filtered.[28] Within this there are macroscopic (particle size/shape, chemical/thermal stability) and microenvironmental (degree of functionality, loading) considerations.[27]

Solvent selection is a critical component in both solution phase and solid phase chemistry. Polystyrene derivatised resins are largely hydrophobic in nature and good swelling is obtained in nonpolar, aprotic solvents. On swelling the resin, the compact network of the support opens out and exposes functional groups for enhanced reaction kinetics. However, polar solvents such as water and methanol are to be avoided for the opposite reasons, (see Table 1.1).[29]

Table 1.1. Solvent Swelling Capacity[29]

Solvent	*Swollen Resin (ml/g)*	*Solvent*	*Swollen Resin (ml/g)*
H_2O	--	Dioxane	5.0
MeOH	1.5	THF	5.1
EtOH	1.8	Pyridine	5.1
$PhCH_2OH$	2.0	DCM	5.2
DMSO	2.0	$CHCl_3$	5.9
AcOH	2.1	CCl_4	5.3
Ether	2.4	Benzene	5.5
DMF	3.5	Hexane	1.8
EtOAc	3.7		

The influence of solvents was clearly recognised in the early development of SPPS. Apart from swelling properties, the solid support must be inert to all the solvents and reagents used during the multi-step synthesis, apart from the required appropriate functionality. The uniformity of the resin beads is also critical to the quality of solid phase reactions. Inhomogeneity in the density of crosslinking is a

major problem in the polymerisation of styrene,[30] resulting in subsequent variable degrees of functionality.

The degree of loading (substitution) measured in (mmol/g) must also be considered. A general problem in SPPS is poor or incomplete amino acid coupling. This can vary with distance from the resin and the sequence of the growing peptide chain; however high resin substitution can lead to incomplete couplings.[31] For SPOS, high substitution (0.7-1.0 mmol/g) is more favoured since it involves the generation of low molecular weight compounds; however, it can lead to intermolecular aggregation within the polymeric network.[32]

The chemical modification of cross-linked polystyrene has provided an attractive route to functionalisation. Chloromethylation and lithiation have been the most frequently used methods of introducing functionalisation into the polymeric backbone, (fig. 1.4).[33] While chloromethylation provides an electrophilic centre within the polymer, lithiation is complementary by introducing a nucleophilic site.

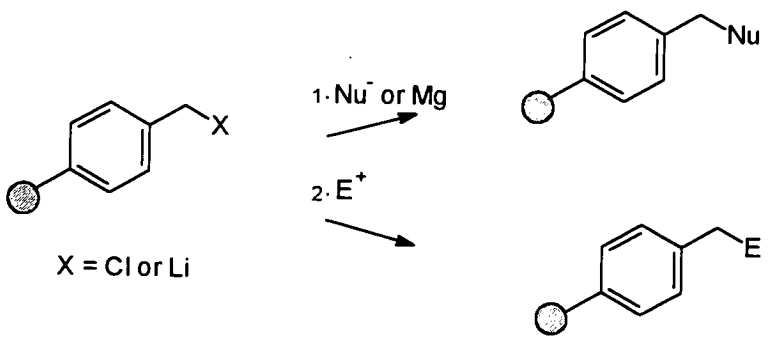


Fig. 1.4. Functionalisation of Polystyrene

The traditional route pursued by Merrifield was chloromethylation; however, over the years chemical modification of Merrifield resin has greatly increased the number of polymeric supports on the market.[27] The introduction of Wang (*p*-benzyloxybenzyl alcohol) resin[34] in 1973 by Wang replaced Merrifield resin as the resin of choice for Fmoc based SPPS.[35] The synthesis and cleavage of Wang resin, (**5**) is illustrated in (fig. 1.5).

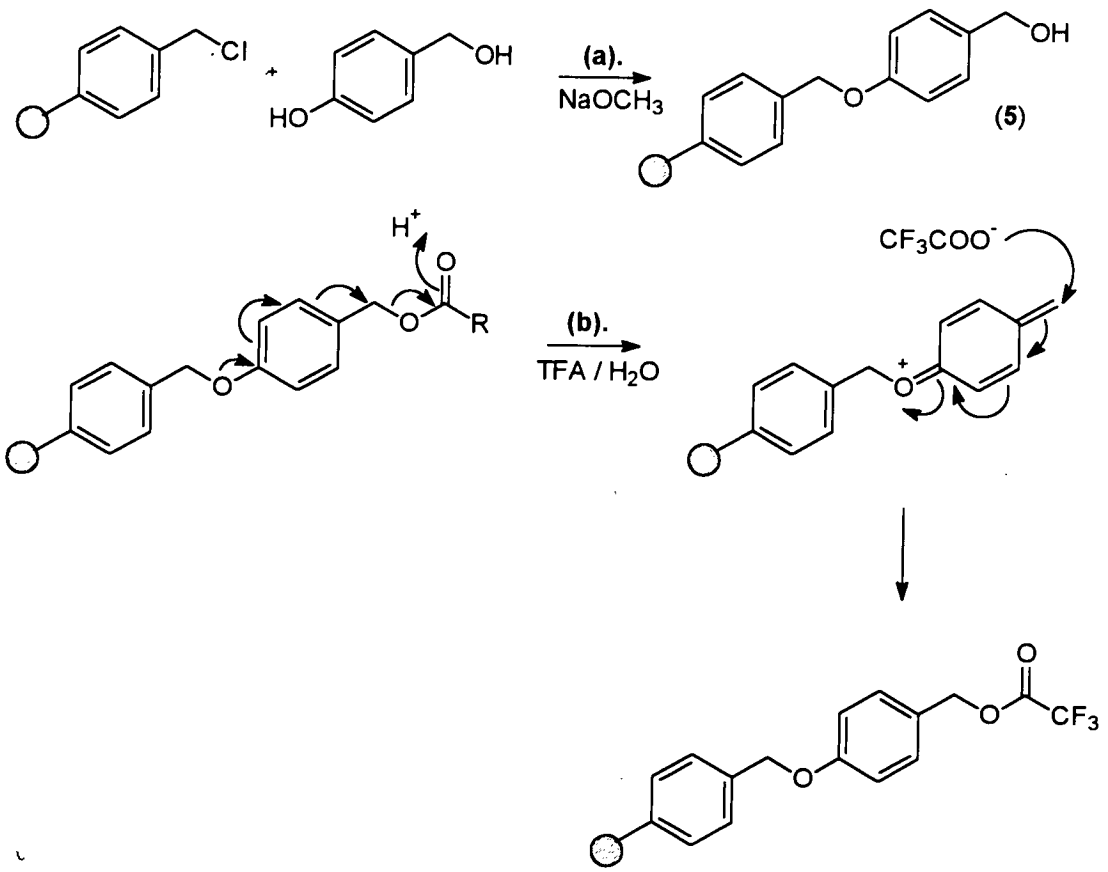


Fig. 1.5. a. Preparation of Wang Resin

b. Cleavage of Wang Resin

In principle, a wide variety of synthetic methods exist for the modification of polystyrene resin. For SPOS, polystyrene supports have proved the most successful both as a solid support and analytical tool. Aside from Merrifield resin, other supports include aminomethylated resin,[36] (**6**), 10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-one resin,[37] (**7**), and 2-chloro-triphenylmethyl chloride resin,[38] (**8**), (see fig. 1.6).

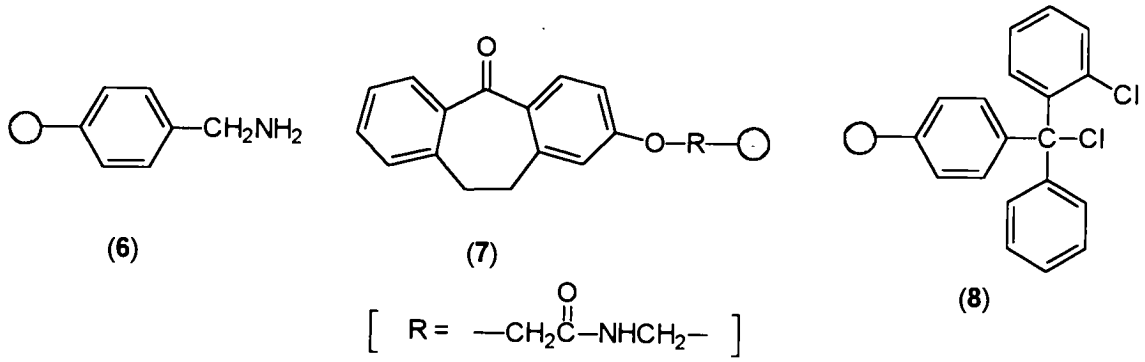


Fig. 1.6. Representative Polystyrene Supports

1.3.2 Tentagel® Supports.

Polystyrene-polyethylene glycol (PS-PEG™) graft copolymers, (**9**), more commonly referred to as Tentagel®, are a family of polystyrene derivatised solid supports.[39] Compared to traditional polystyrene, Tentagel® resins are advantageous for SPS, particularly for peptide and oligonucleotide chemistries.[40,41]

For the preparation of Tentagel®, polystyrene is derivatised with a polyethylene glycol (PEG) spacer between the polystyrene backbone and the attachment point of the synthetic peptide[42] (fig. 1.7). The PEG spacer is believed to match the hydrophilic nature of the growing peptide chain enabling more efficient syntheses of long and complex peptides. As with traditional polystyrene resins, the terminus of the resin can be functionalised with various R groups.

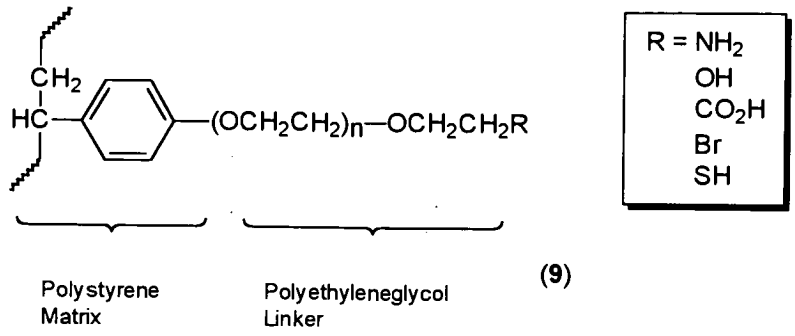


Fig.1.7. General Structure of Tentagel® Supports

While the swelling properties of polystyrene supports play a key role in reaction conditions, Tentagel® resins are easily solvated in both polar and aprotic solvents with the swelling ratio in water and DCM nearly equal,[43] (Table 1.2). Subsequently, Tentagel® beads are more suitable for continuous flow synthesis.

Table 1.2. Comparative Swelling Properties

Solvent	*Polystyrene (ml/g)*	*Tentagel® (ml/g)*
DCM	5.2	3.0
THF	5.5	3.4
CH_3CN	1.4	3.0
MeOH	0.95	2.5
H_2O	--	2.5

With the advent of combinatorial chemistry there has been renewed interest in Tentagel® resin as an alternative solid support to polystyrene. Chapman and Rano report the use of Tentagel® in the solid phase synthesis of aryl ethers[44] while Dorff and Hauske use it as a new matrix specific linker.[45] The key attraction of Tentagel® supports is that they provide an environment that closely resembles solution phase chemistry conditions. Recently, Tentagel® supports have been particularly useful as an

analytical tool. Bayer *et al.*[46] and Gallop *et al.*[47] both report that PEG grafted resins exhibit unique physical and nuclear magnetic resonance (NMR) properties.

The use of ^{13}C NMR to visualise compounds bound to a solid support has been well documented.[48-50] However, typical ^{13}C NMR experiments involving polystyrene resins require good swelling and thousands of transients. Often, differential relaxation rates in the resin-bound products gives poorly resolved spectra. The incorporation of large amounts of PEG in Tentagel® results in highly swollen states. This in turn generally reduces the extensive changes in size and solvation properties which are normally encountered with standard polystyrene supports. Additionally, ^{13}C NMR spectra of polystyrene supports are complicated by broad aromatic and aliphatic resonances. The spectra obtained from Tentagel® supports provide enhanced spectral resolution because the polystyrene matrix is not visible in the ^{13}C NMR. Functional groups such as carbonyls are easily recognised and the PEG spacer appears as a single resonance peak at 70 ppm.[47]

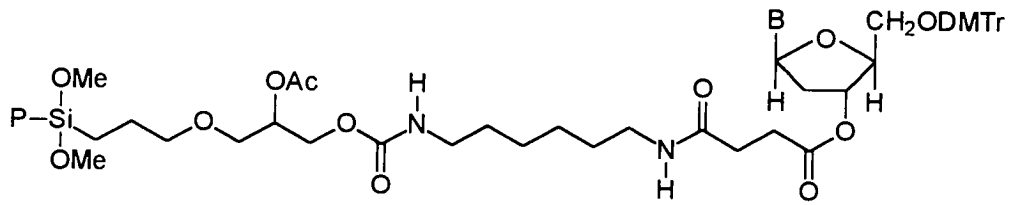
NMR studies of Tentagel® supports have also been extended to ^{1}H spectra. Fitch *et al.*[51] demonstrated the use of magic-angle spinning (MAS) for line narrowing in the ^{1}H NMR of Tentagel®-bound substrates. The detection of two diasteroisomers of Tentagel®-bound norborane-2-carboxylic acid has also been successfully demonstrated using MAS ^{13}C-^{1}H correlated NMR.[52]

The utility of Tentagel® resins as alternatives to polystyrene resins is attractive; however, Tentagel® resins offer low loading, typically 0.15-0.30 mmol/g.[53] While this may be of use in oligomer synthesis, higher loading is desired in the solid phase synthesis of small organic compounds. Furthermore, Tentagel® supports are mechanically unstable to magnetic stirring and sonication.[54] This subsequently limits the utility of the resin to certain reactions and automation techniques exemplified by the DIVERSOMER™ technology.

1.3.3 Controlled Pore Glass Supports.

Controlled Pore Glass (CPG), unlike polystyrene derivatised supports, does not have a microporous structure and morphology, but instead consists of an inorganic, rigid silicate matrix uniformly milled with screened particles of almost pure silica that are honeycombed with pores of controlled size.[55] CPG is currently the accepted solid support for automated deoxyribonucleic acid (DNA) synthesis.[56] CPG has also been applied to the synthesis of small peptides.[57]

For use in solid phase DNA synthesis, the beads of CPG are derivatised with a long chain alkylamine (LCAA) resulting in a primary amine loading of about 100 μmol/g, (fig. 1.8).[55] This can then be reacted directly with 5'-O-dimethyloxytrityl deoxyribonucleoside-3'-O-succinates to give the general structure, (**10**).



(**10**)

Fig. 1.8. Linkage of DNA to LCAA/CPG

In contrast to polystyrene derivatised solid supports, the inorganic nature of CPG makes it a non-swelling support. Thus, while peptide synthesis is frequently impacted by differential amino acid coupling efficiencies, the 4 bases adenine, guanine, thymine and cytosine (A, G, C, T) in DNA synthesis give almost equivalent rates of coupling.[58] The excellent mechanical properties of CPG also makes it a good candidate for continuous flow systems, with oligonucleotides greater than 180 bases in length being capable of synthesis.[59] Problems associated with CPG are generally leaching or decomposition of silicates. Fitzpatrick *et al.*[60] attempted to eliminate this problem by synthesising DNA sequences on a hydrophilic membrane support.

Despite its success in DNA synthesis, and to a limited extent in SPPS, the use of CPG as a solid support has not been generally applied to SPOS. As with Tentagel

supports, CPG provides low loading. On average, nucleoside loadings are 25-40 μmol/g.[55]

1.3.4 Polydimethylacrylamide-Kieselguhr Supports.

In the early 1970s, R.C. Sheppard and co-workers[61] prepared an alternative support to the original Merrifield resin, based on the argument that the polymeric support would be more desirable if it was chemically analogous to the attached peptide chain. This led to the introduction of polyamide supports and "Fmoc polyamide synthesis".[62] More commonly known as PepSyn™,[63] the polyamide support, (**14**) is derived from the copolymerisation of bis(acrylamido)ethane, (**11**), dimethylacrylamide, (**12**), and acryloylsarcosine methyl ester, (**13**), (fig. 1.9), with properties the reverse of polystyrene based resins.[61]

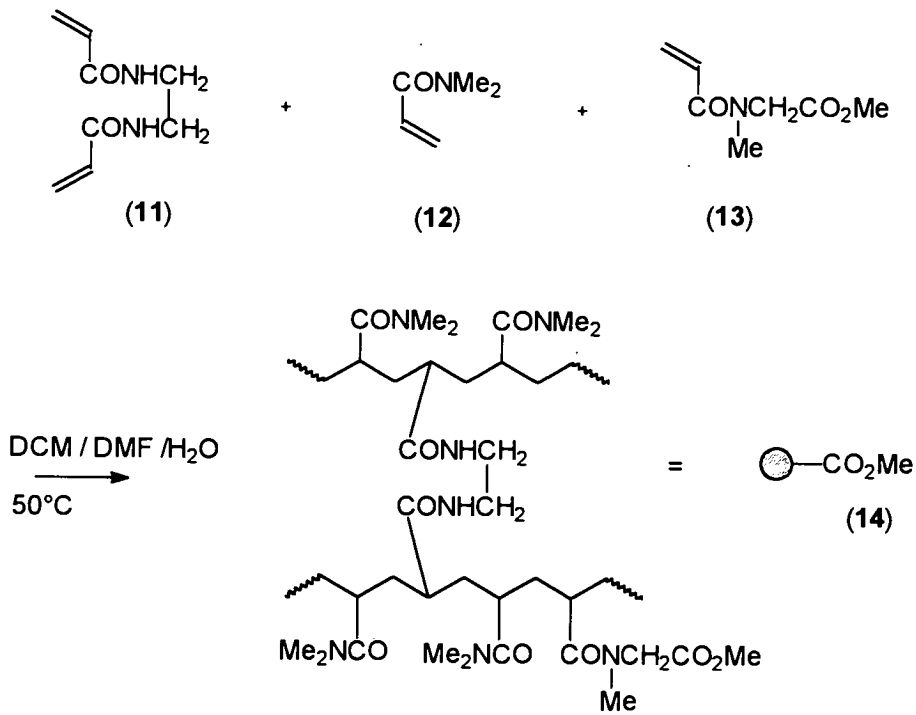


Fig. 1.9. Preparation of PepSyn™

Although, generally used for the solid phase synthesis of small peptides, PepSyn™ supports are not applicable to continuous flow synthesis. However, polyacrylamides are more hydrophilic than polystyrene, therefore reactions can be carried out in polar media, making PepSyn™ an ideal support for SPOS. PepSyn™ also gives moderate loading, typically 0.3 mmol/g.[64]

In order to create a more free-flowing support, Sheppard and co-workers,[65] in 1986 introduced polydimethylacrylamide-Kieselguhr (PepSynK™) supports. The structure of PepSynK™ is a composite of cross-linked polydimethylacrylamide gel which is polymerised within the pores of inert, fabricated macroporous Kieselguhr beads. Kieselguhr is a low density, highly permeable inorganic matrix and is more polar than standard polystyrene supports. The resulting polymer mixture has pores several thousand Ångstroms in diameter, creating an organic gel of high porosity allowing rapid permeation of solvents and reagents. However, loading is low, typically 0.1 mmol/g, therefore limiting its use in SPOS.[27]

1.3.5 Alternative Polymeric Supports

Polystyrene derivatised solid supports have largely dominated peptide chemistry and SPOS, while CPG has been the accepted support for DNA synthesis. Over the years several groups have studied alternative polymeric supports in order to increase the diversity of reactions carried out on a solid support.

In 1972, Koster and Heyns published two papers reporting the use of silica[66] and Sephadex LH 20[67] as alternative polymeric supports in DNA synthesis. These supports are highly hydrophilic in nature, and at the time, were a suitable alternative to polystyrene in order to avoid the problems encountered with the high polarity of the phosphodiester bond. Reactions involving Sephadex LH 20 could also be followed by infrared, (IR) spectroscopy, making it a useful support in organic synthesis.

In the late 1980's, simultaneous multiple peptide synthesis was reported by several groups. Geysen's PEPSCAN method[R] ("pin-method") synthesised peptides on the tip of polyacrylate grafted polyethylene rods. Later Koster *et al.*[68] and Frank *et*

al.[69,70] report the use of membranes and cellulose paper discs respectively. Unlike the covalent attachment to polystyrene beads, synthesis on membrane and cellulose sheets is based on absorption onto the solid support. Both supports can be functionalised and the absorbed peptides can be used immobilised on the support in a solid phase binding assay, or can be desorbed for use in solution.[71] The supports are mechanically stable and amenable to miniaturisation, and automated operation. Despite the non-swelling nature of these supports, their application to SPOS is limited by their low loading, 10-20 μmol/g and the use of solvents of high volatility which are not acceptable. Furthermore, cellulose rapidly disintegrates in strong acid.[72] Table 1.3. summarises some properties of the different solid supports discussed in this chapter.

Table 1.3. Summary of Comparative Solid Supports

Solid Support	*Loading < 0.1mmol/g*	*Loading > 0.1mmol/g*	*Analytical IR*	*Analytical ^{1}H NMR*	*Analytical ^{13}C NMR*	*Rigidity, Chemical*	*Rigidity, Mechanical*
Polystyrene	++	++	++	-	+	+	++
Tentagel®	++	+/-	-	+	++	+	–
CPG	++	-	-			+	++
PepSyn™	++	+	+		+	+	+
PepSynK™	++	-	+		-	+	+
Silica Gel	++	+/-				+	+/-
Cellulose	+	-				–	++
Sephadex LH 20	+	-	++			+	++

++ Excellent, + Good, +/- Moderate, - Poor.

• 1.4. Representative Solid Phase Reactions

While peptide chemistry has branched into its own field of science, the general application of insoluble polymer supports to organic synthesis has received scattered attention.[73,74] As early as 1969, Merrifield, recognised the potential of polymeric supports in organic synthesis, "a gold mine awaits the discovery by organic chemists".[75] Despite this, the subsequent 20 years saw limited research into this area, either as a result of limited resources, or due to no interest in SPS. After all, solution phase chemistry was well established therefore there was a lack of impetus for change.

1.4.1. Polymer Supports in Organic Synthesis

Most of the reactions to generate non-oligomeric compounds on polymeric supports have used polystyrene derivatised resins largely due to their advantages as discussed in the previous section.[22,23] One of the earliest to report the use of insoluble polymers as polymeric reagents in organic synthesis was Takagi in 1967.[76] The reaction involved the formation of an insoluble peracid reagent for use in epoxidation of olefins. Thus, the product of the reaction can be obtained by evaporation of the solvent, while the acid by-product remains attached to the solid support.

Following this, Shambhu and Digenis[77] reported the use of polymeric acetylating reagents. Using Letsinger's "popcorn" polymer,[12] insoluble anhydrides were prepared which on subsequent treatment with aniline or ethanol gave resin-bound benzanilide and ethyl benzoate, respectively.

The use of polymer-bound ylides has been well documented.[78-80] Ylides are generally highly reactive; however, once attached to a solid support they are easier to handle compared to the analogous, classical solution reagents. Furthermore, by attaching the Wittig reagent to an insoluble support, residual triphenylphosphine oxide remaining in the reaction mixture can simply be filtered off from the product.

For the solid phase Wittig reaction, (fig. 1.10), the resin-bound Wittig reagent, **(16)** is prepared by copolymerisation of styrene, **(1)**, DVB, **(3)** and *p*-

styryldiphenyphosphine, (**15**). Addition of an alkyl halide followed by base treatment provides the resin-bound ylide reagent, (**17**). Subsequent treatment with an appropriate aldehyde or ketone releases the desired olefin (**19**) into solution and the resin-bound triphenylphosphine oxide, (**18**), which can be recycled back to (**16**).

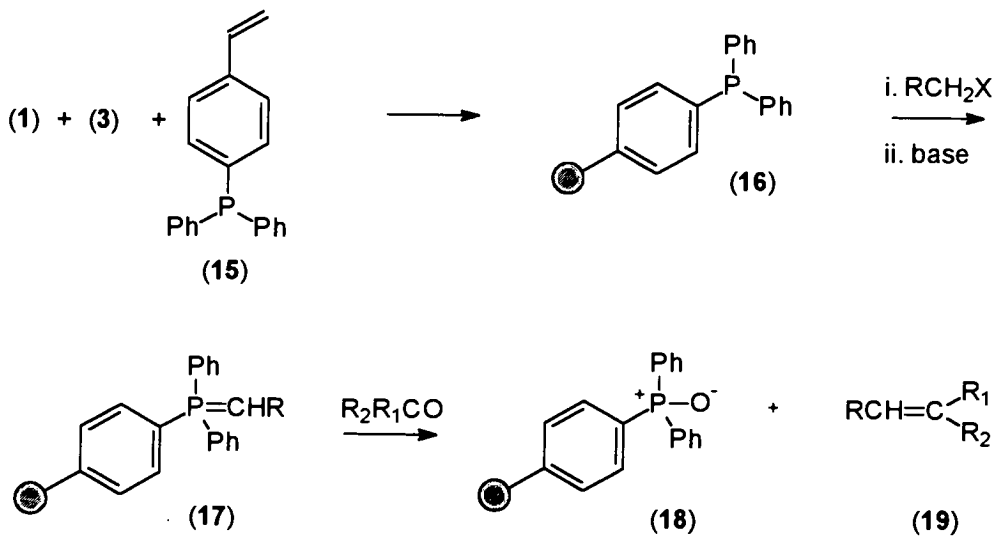


Fig. 1.10. Polymeric Wittig Reagent

The solution phase synthesis of cyclic compounds has long been a synthetic problem in organic chemistry with yields often low and, in many cases, the products isolated as mixtures.[81] One example of this is the Dieckmann cyclisation of di-esters, (fig. 1.11).[82] In solution phase, the cyclisation of a benzyl triethylmethyl diester yields a mixture of keto-esters, which are inseparable by chromatographic methods. Crowley and Rapoport[82] showed that by executing the reaction on a solid support not only did they obtain higher yields, but that during cyclisation of the resin-bound ester, (**20**), the mixture of products, (**21**) and, (**22**) could be easily separated by filtration of the resin with all the by-products liberated into solution.

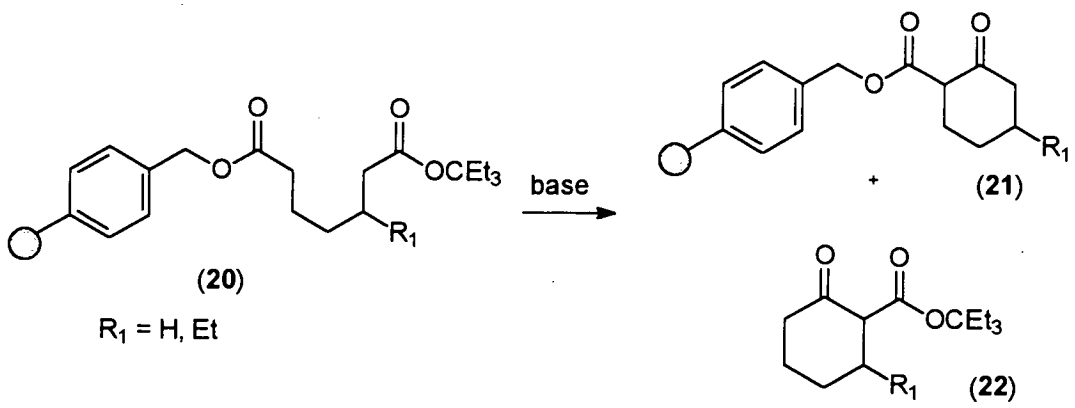


Fig. 1.11. Solid Phase Dieckmann Cyclisation

In many synthetic schemes, it is often desired that only one functional group reacts. However, the direct reaction of one equivalent of a bifunctional compound and one equivalent of reagent often gives a mixture of unreacted starting materials, over-reacted products and the desired monoreacted product. Protecting groups can be used, but selective protection of one functional group of a symmetrical bifunctional compound can be difficult. Leznoff and Wong[83,84] were the first to demonstrate that an insoluble polymer support can be used as a selective blocking group for completely symmetrical diols and dials by "site separation", (fig. 1.12).

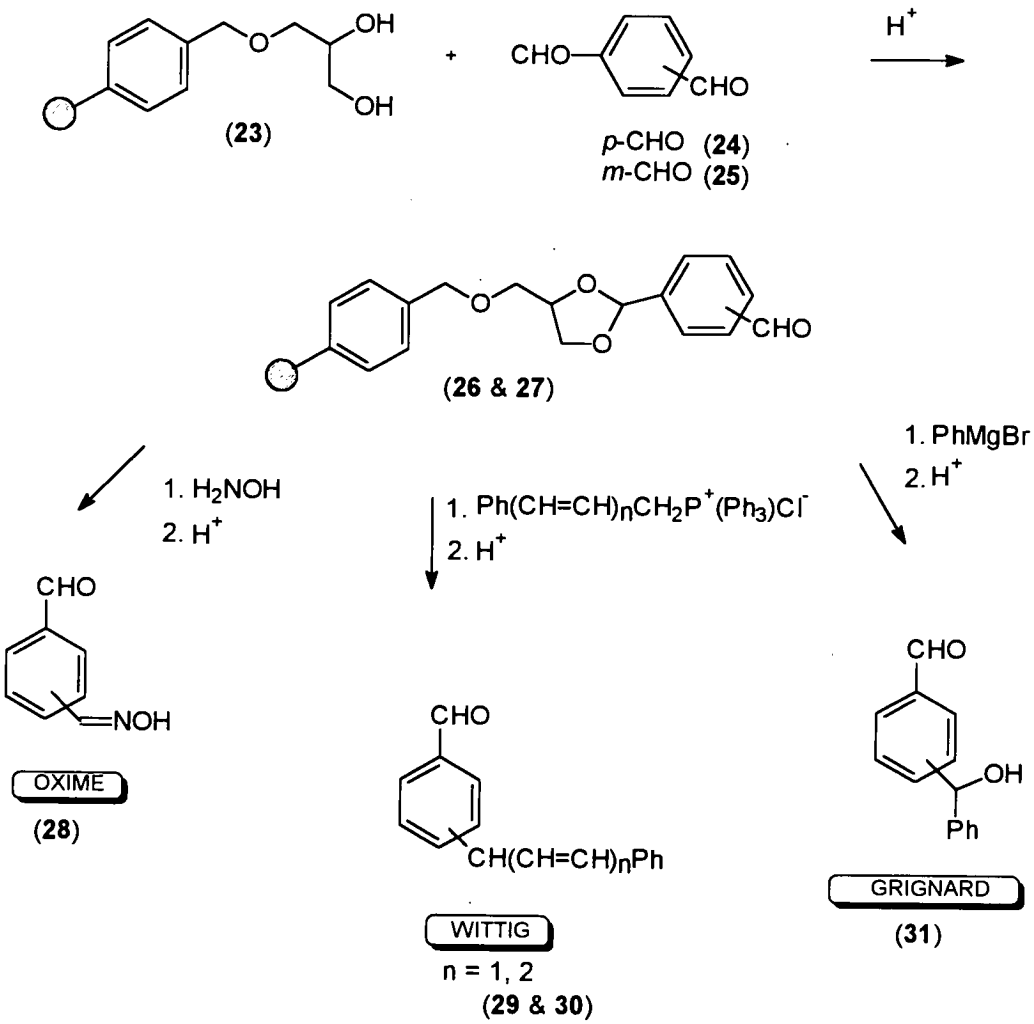


Fig. 1.12. Solid Phase Monoprotection

Initially, Merrifield resin, (**4**) was reacted with the sodium salt of 2,2-dimethyl-1,3-dioxolane-4-methanol in excess 2,2-dimethyl-1,3-dioxolane-4-methanol as the solvent.[85] Acid hydrolysis gave the resin-bound diol, (**23**) containing a vicinal diol functional group which was subsequently treated with terephthaldehyde, (**24**), or isophthalaldehyde, (**25**) to generate the resin-bound acetals, (**26**) and (**27**), respectively containing a free aldehyde group. The resulting products were then used to prepare oximes, (**28**), alkenes, (**29**) and (**30**) *via* a solid phase Wittig reaction, and for the preparation of the Grignard products, (**31**). In each case the resin-bound diol, (**23**) is regenerated for subsequent use. While classical solution phase preparations of

these products can lead to mixtures which are often difficult to purify, the primary advantage of using resin (**23**) is shown in the high yields and selectivity of the solid phase reactions. However, resin-bound acetals are limited to use in reactions that only employ basic conditions.

Similar selective functionalisation of polyhydroxy alcohols was reported by Frecht and Nuyens in 1975.[86] Later that year Leznoff and Fyles[87] report the use of solid supports for the protection of symmetrical diols in the synthesis of insect pheromones.

It is clear from this early work that Leznoff, like Merrifield, was well aware of the key advantages of SPS versus traditional solution phase chemistry. However, in contrast to Merrifield, Leznoff also demonstrated the problems, and to a certain extent the limitations, of SPOS.

Incomplete coupling or reaction completion was a problem. While this issue has largely been eliminated in peptide synthesis, our work has shown this to be a problem in the present SPOS methodology. The stability of the polymer supports was a common problem, however this point has been confronted over the years, with new, more stable, non-degradable polymers prepared offering wider general use.[88] The limitations of compatible solvents similarly restricted the chemistry attempted on solid supports. A long term problem has been the difficulty of determining the course and extent of a chemical reaction on a polymer support.[89-91]

IR spectroscopy of insoluble resin-bound products and / or cleavage of the resin-bound products from the insoluble polymer after each reaction have been the main methods of following reactions on insoluble supports. More recently ^{13}C gel phase NMR[48-50] and ^{1}H NMR[51] have further aided the identification of resin-bound intermediates and products. However, IR and NMR spectroscopy are not quantitative and in many cases the complex spectrum of the resin tends to obscure the spectral features of the attached moiety. Quantitative results can be obtained from cleavage of the resin-bound intermediates, but this can be time consuming and in many cases the intermediates are not stable to the cleavage conditions.

In 1976, Crowley and Rapoport[74] published a paper, where they further review the problems discussed by Leznoff regarding "non-peptide solid phase

chemistry whose resolution is required if the process is to mature from publishable novelty to fundamental methodology". Hyperentropic Efficacy[82] was the main topic of discussion. This is predominantly concerned with studying possible conditions of high dilution on an insoluble support and intra-resin reactions.[91] To a certain extent the paper is obscure and in conclusion the authors presumed that SPOS would remain a "laboratory curiosity" in the years to come.

This did not deter Leznoff and co-workers who yet again published a series of papers reporting new solid phase organic chemistry.[92-94] As with previous work, the key attraction of solid supported reactions versus solution phase chemistry was the ease of product workup and isolation by simple filtration. Leznoff called this the "Fish-hook" principle,[93] whereby the fish is the desired product which has been "fished" out of the reaction mixture, while the hook is analogous to the insoluble polymeric support.

Four main areas of multistep organic synthesis were studied on a solid support, with the chemistry largely concentrated on the selective monoprotection of difunctional compounds.

The insect sex attractants of Lepidoptera, (**36**) have the general formula shown below, (fig. 1.13)[91] and are particularly useful candidates to prepare on a solid support since the initial reaction requires monoprotection of the symmetrical diol. The use of trityl chloride resin, (**32**), which reacted selectively with primary hydroxyl groups versus secondary and tertiary groups,[95] was attractive due to the ease of cleavage of the trityl ether in mildly acidic medium. Three independent routes were successfully attempted using (**32**) and a series of three long-chain diols. The first route pursued was an alkyne approach,[92] (fig. 1.13). Following monoprotection of the diols and monomesylation of the free hydroxyl, the resin-bound monomesylates (**33**) were stirred in the desired alkyne mixture overnight to generate the resin-bound alkyne derivatives, (**34**). Acidic cleavage gave the recovered trityl resin, (**32**) and the acetylenic alcohols, (**35**) which were reduced and acetylated to yield a mixture of *cis* and *trans* isomers of the Lepidoptera family, (**36**).

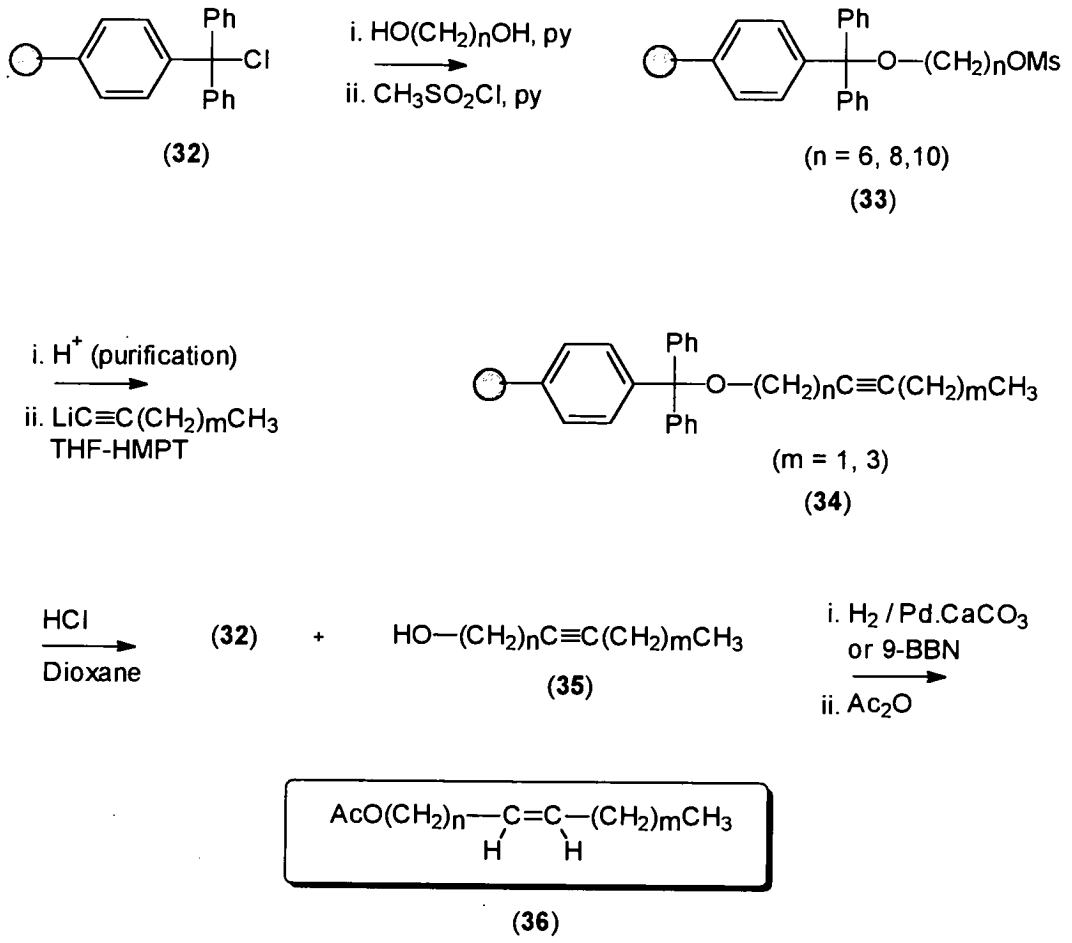


Fig. 1.13. Alkyne Route

In order to achieve higher stereoselectivity of the final products, (**36**), a solid phase "normal" Wittig reaction[92] (fig. 1.14) was attempted by condensation of Wittig reagents, (**38**) with the resin-bound aldehydes, (**37**) to yield the resin-bound olefins, (**39**). They also attempted a "reverse" Wittig reaction[92] (fig. 1.14) of a resin-bound Wittig reagent, (**41**) with a range of aliphatic aldehydes, (**42**). In this case, the resin-bound monomesylate, (**40**) was treated with molten triphenylphosphine at 100°C to give the corresponding resin-bound phosphonium salt, (**41**). As before, acid hydrolysis of the product from the Wittig reaction gave the recovered polymer, (**32**) and alcohol which on acetylation yielded the product, (**36**). Again comparable yields with solution phase methods were obtained with 91.2% stereoselectivity for the *cis* isomer reported.

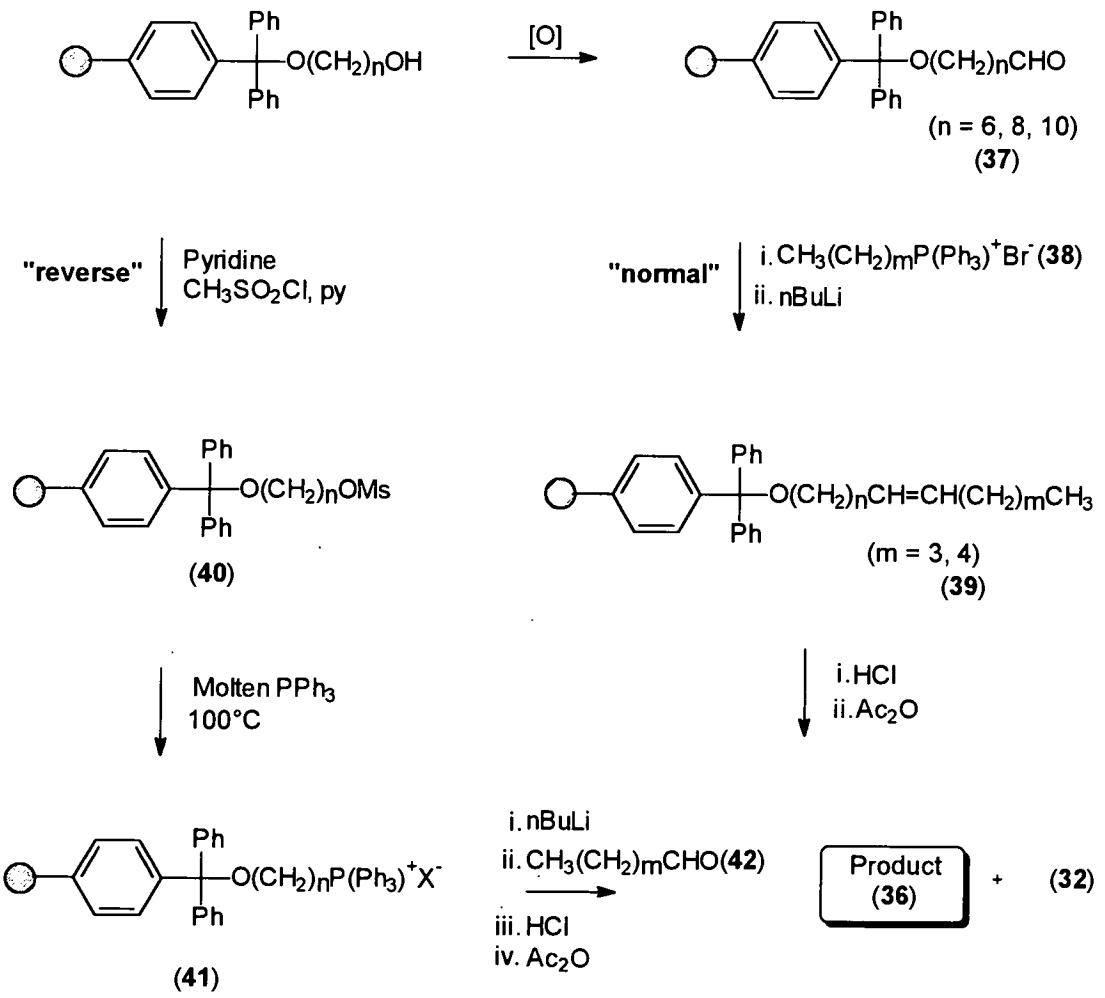


Fig. 1.14. "Normal" Wittig and "Reverse" Wittig Reaction

The monoprotection reaction was also used in the synthesis of helicenes via resin-bound terephthaldehyde, however overall yields were very low. Macrocyclic formation of the lycoramine-like alkaloid skeleton has also been attempted by solid phase approaches. The solid phase synthesis of the 1,4-benzodiazepinone tranquillisers, (**44**) date back to 1974. The synthesis critically depends on site isolation of the resin-bound imine, (**43**) for a cyclisation step analogous to Patchornik's synthesis of cyclic peptides[96] and is particularly aided by the unlikely probability of intermolecular dimerisations, (fig. 1.15). More recent methods have reported the solid phase synthesis of a library of benzodiazepines,[8,9,97] (see section 1.4.2).

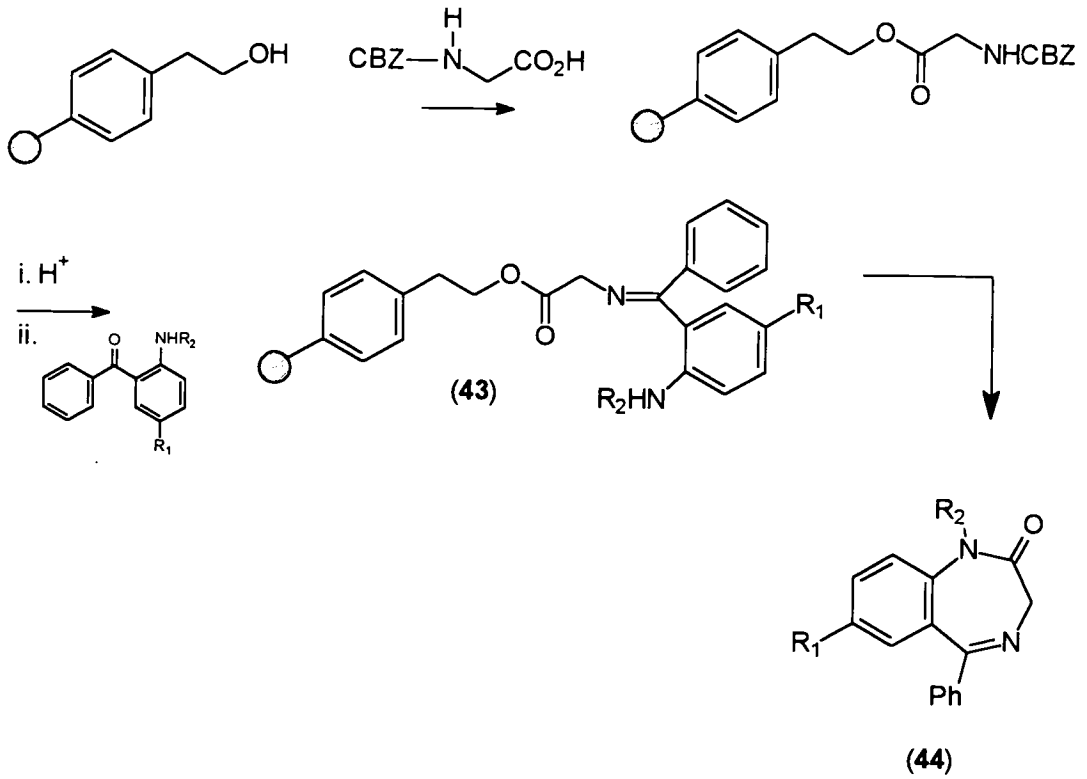


Fig. 1.15. SPOS of 1,4-Benzodiazepinone Tranquillisers

1.4.2. Combinatorial Chemistry

While real practical advances have been made in SPOS, it has essentially lain dormant since Leznoff's work in the late 1970s. More recently it has generally been accepted that SPS facilitates the assembly of combinatorial libraries.

Combinatorial chemistry is a synthetic strategy by which large, diverse libraries of compounds can theoretically be created.[98] Combinatorial chemistry has added to the pool of compound sources available at present in the pharmaceutical industry, (fig. 1.16). Many believe that combinatorial chemistry will revolutionise the drug discovery process by cutting the time and cost of finding suitable drug candidates.[2]

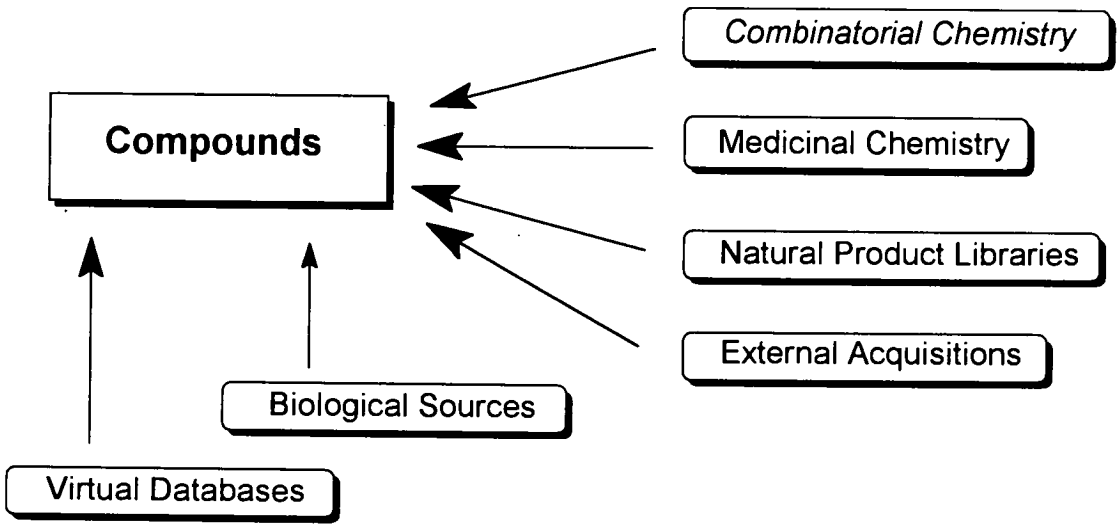


Fig. 1.16. Compound Sources

To date, combinatorial strategies have primarily concentrated on libraries of peptides, and this area has been extensively reviewed.[6,10,18] Other methods for the synthesis of oligomeric libraries include, peptoids, carbamates, vinylogous amides, pyrrolinones and peptidyl phosphonates, as shown in (fig. 1.17).[99]

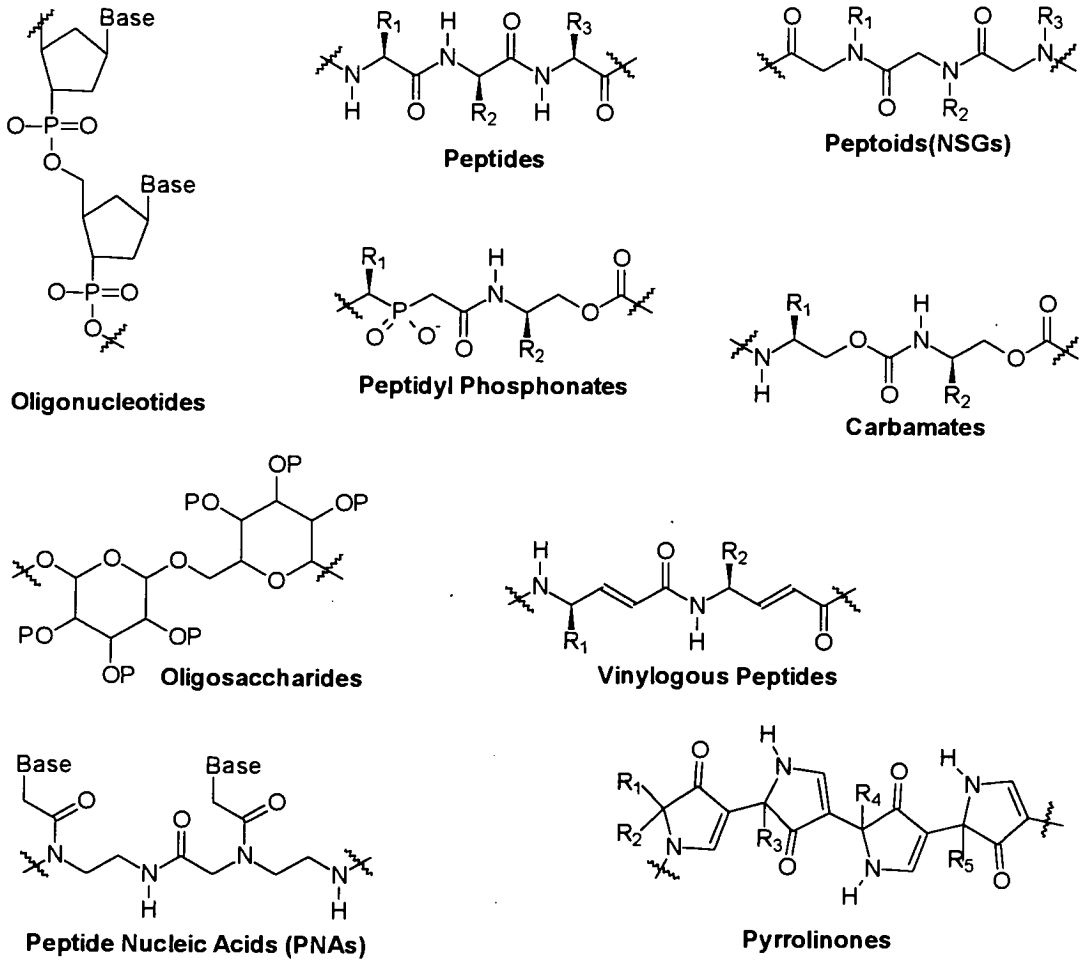


Fig 1.17. Current Oligomeric Libraries

For a long time peptides have served as models for the more traditional, small molecule drugs. However, due to poor bio-availability and pharmokinetic parameters, peptides have limited use as suitable drug candidates. Alternatively, small molecule libraries, with molecular weight < 500 amu, such as the benzodiazepines, β-lactams and imidazoles are typically heterocyclic in nature, and are ideal drug candidates. Fig. 1.18 generalises the pros and cons of both oligomeric and non-ologimeric libraries, while (fig. 1.19) demonstrates the current molecular diversity approaches and the number of compounds which can be generated.[19]

	Advantages	Disadvantages
Oligomeric	Ease of Synthesis	Limited Building Blocks
	Easily Automated	Repetitive Backbone
	Precedence	Limited Bioavailability
Non-Oligomeric	Direct Drug Candidates	Difficult to Synthesis
	Unlimited Building Blocks	Automation???
	Novel Templates	Lack of Precedence

Fig. 1.18. Oligomeric vs Non-Oligomeric Libraries

At present, combinatorial chemistry, in conjunction with SPOS, is at a critical and delicate stage in the drug discovery process. Without doubt, combinatorial chemistry offers medicinal chemistry the possibility to expand the scope of structures that may be screened for biological activity. The main objectives of combinatorial chemistry are two-fold;

❶. **Lead Generation**

or random screening. A lead compound is identified in the absence of any structural information.

❷. **Lead Optimisation**

or direct screening. The objective in this case is to evaluate and establish SAR around a known biologically active pharmacophore.

Both paradigms are aimed at preparing large, structurally diverse libraries of low molecular weight compounds. A common feature with each case has been the renaissance of SPOS.

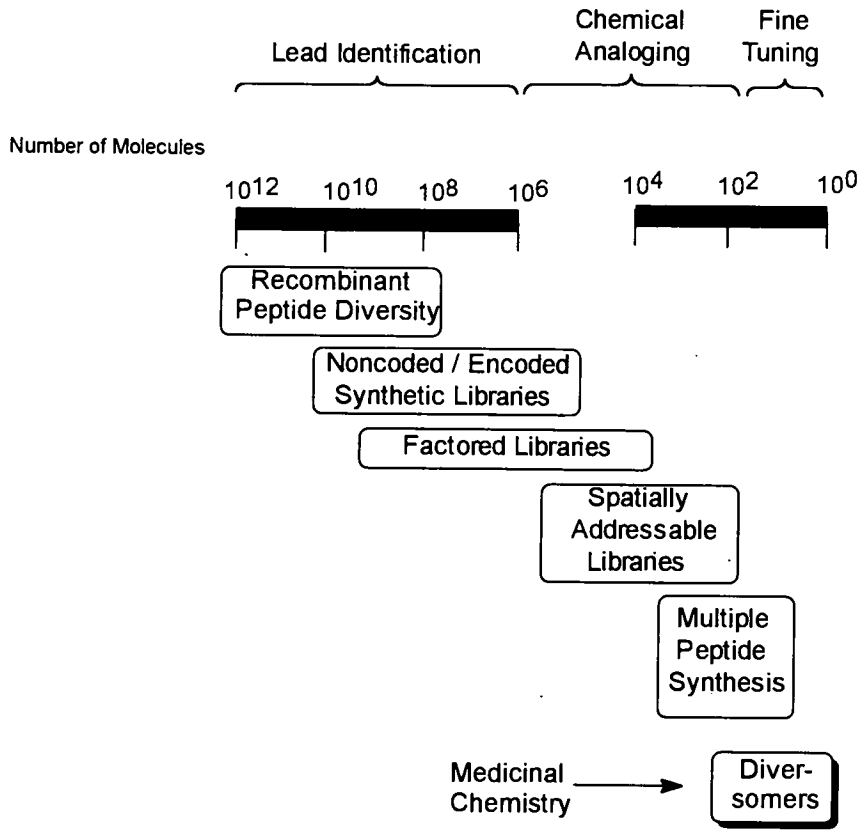


Fig. 1.19. Molecular Diversity Approaches

In comparison with SPPS, the chemistry of small molecules on a solid support is not fully understood, yet a number of non-peptide libraries have been prepared, most notably the 1,4-benzodiazepines which is a common pharmacophore found in many drugs, such as Valium®. Ellman and Bunin[9] laid the groundwork for the construction of a small molecule library of 1,4-benzodiazepines utilising solid phase methodology, (fig. 1.20).

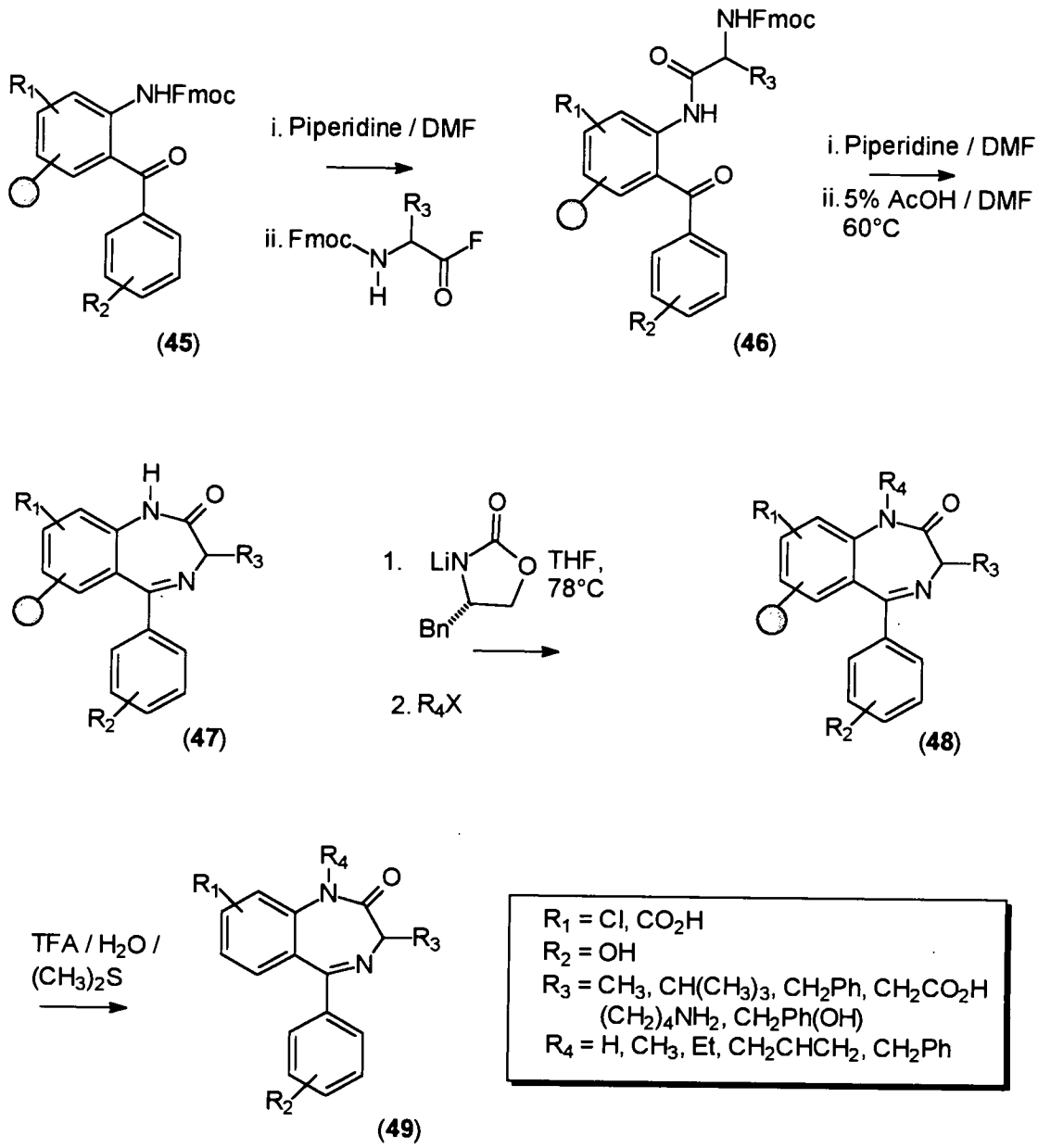


Fig. 1.20. Ellman et al. SPOS of Benzodiazepines

A library of 8 benzodiazepines were synthesised from three major components: aminobenzophenones, amino acids and alkylating agents. Initially, the 2-aminobenzophenone hydroxy or carboxy derivatives were attached to the polystyrene solid support via an acid-cleavable linker (4-hydroxymethylphenoxyacetic acid) to yield the resin-bound starting material, (**45**). Deprotection of the Fmoc group, was followed by coupling (**45**) to an α-N-Fmoc amino acid fluoride. A second Fmoc

deprotection of the intermediate, (**46**) and treatment with 5% acetic acid in DMF provided the resin-bound cyclised lactam, (**47**). Subsequent alkylation introduced the final site of diversity and acid cleavage of the resin-bound benzodiazepines, (**48**) generated 8 pure compounds, (**49**) in high overall yield based on HPLC data. No structure activity relationships (SARs) of the products was carried out. More recently, Ellman and co-workers have reported the synthesis and biological testing of a library of 192 1,4-benzodiazepines.[97]

Independently, DeWitt *et al.*,[8] at Parke Davis reported the preparation of an array of 40 benzodiazepines, (**53**), (fig. 1.21) simultaneously but separately by reacting each of five amino acid resins, (**50**), with eight 2-amino benzophenone imines, (**51**).

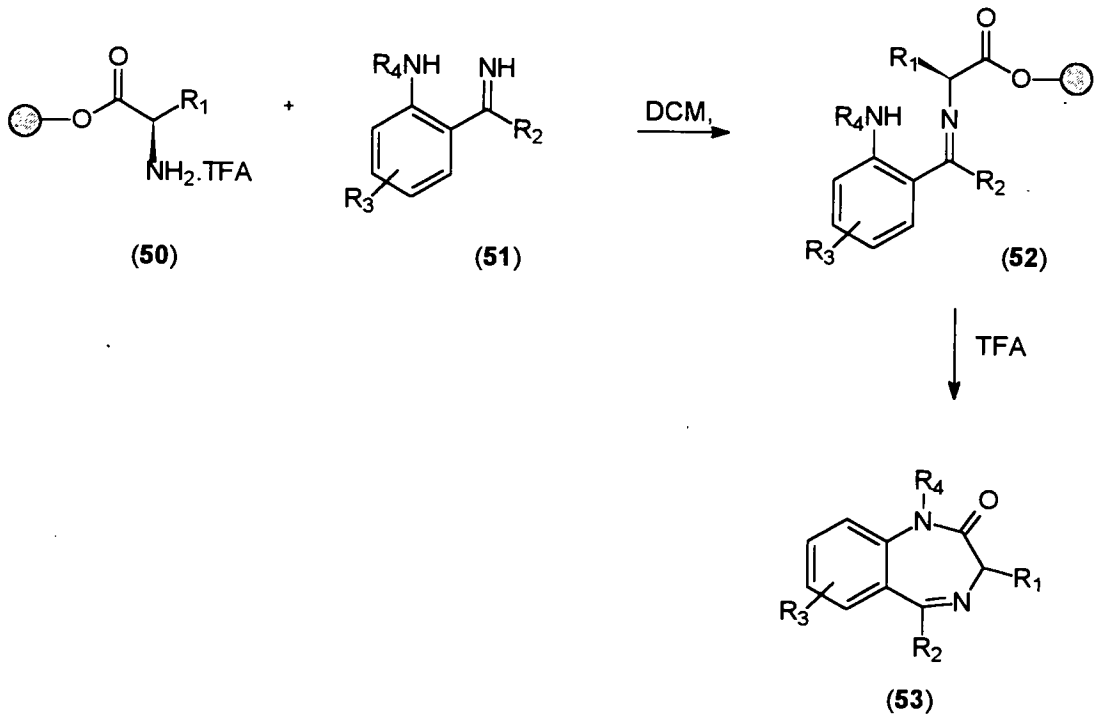
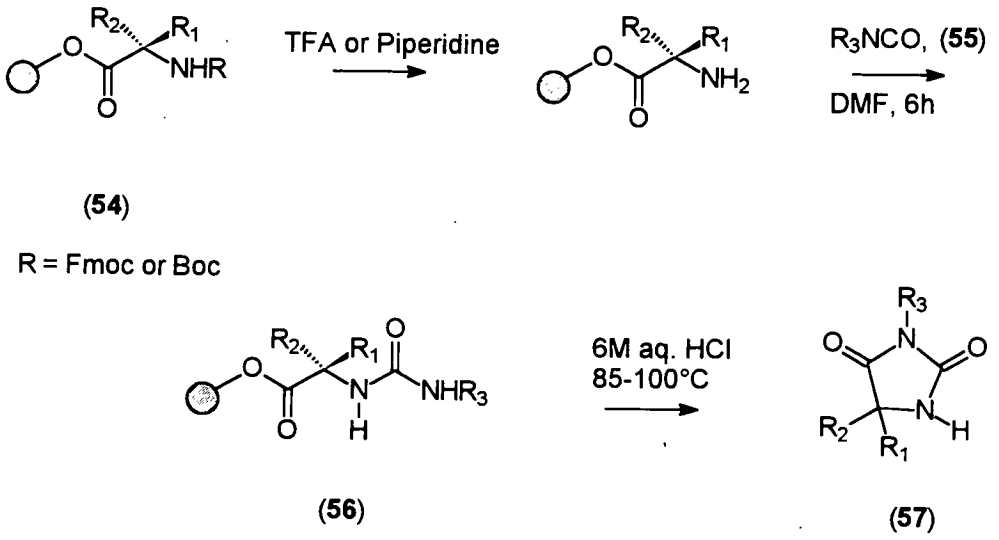


Fig. 1.21. DeWitt et al. SPOS of Benzodiazepines

Unlike Ellman's approach,[97] the Parke-Davis route required no prior introduction of functionality for resin attachment. Additionally, the process was fully automated with an apparatus unique to the solid phase synthesis of small molecules which they term DIVERSOMERS™. A further advantage of the DeWitt route is that the

benzodiazepines are directly released into solution by acid cyclisation of the resin-bound products, (**52**). This should result in unreacted products or side-reagents remaining attached to the solid support with the final products cleaved in greater purity.

DeWitt *et al.*[8] have also described the solid phase synthesis of an array of 40 hydantoins, (**57**), (fig. 1.22), utilising the DIVERSOMER™ technology. The hydantoins form a primary nucleus in the anti-epileptic drugs Phenytoin® and Dilantoin®, and the aldose reductase inhibitor Sorbinil®.[100] The solid phase synthesis involved treatment of five groups of resin-bound amino acids, (**54**) with a series of isocyanates, (**55**), after Fmoc or Boc deprotection. The resulting resin-bound ureas, (**56**) were subsequently cleaved by acid induced cyclisation to provide 40 separate hydantoins, (**57**) in solution.



(**54**)

R = Fmoc or Boc

(**56**)

(**57**)

Fig. 1.22. SPOS of Hydantoins

Both sets of 40 benzodiazepine and hydantoin libraries were characterised by ^{1}H NMR and MS, and analysed for biological activity. Another unique feature of the DeWitt approach has been the use of ^{13}C gel-phase NMR to monitor reaction progress of the resin-bound intermediates.

Not surprisingly, polymer-supported synthesis has not been restricted to the benzodiazepines and hydantoins. Other areas of research by the Parke-Davis group

have involved the generation of benzoisothiazolone[101] and substituted amide libraries.[102] Ellman and co-workers have covered a wider range of compounds including the solid phase synthesis of prostaglandins,[103] 1,4-benzodiazepine-2,5-diones,[104] aspartic acid protease inhibitors[105] and β–turn mimetics.[106]

Kurth and co-workers[107] have reported the multi-step solid phase synthesis of 2,5-disubstituted tetrahydrofurans, (**61**) which are important structural moieties in many polyether antibiotics.[108] The reaction involved a tandem 1,3-dipolar cycloaddition/electrophilic cyclisation sequence. In solution phase, the complex nature of the reaction frequently results in the undesired *bis*-1,3-dipolar cycloaddition product.

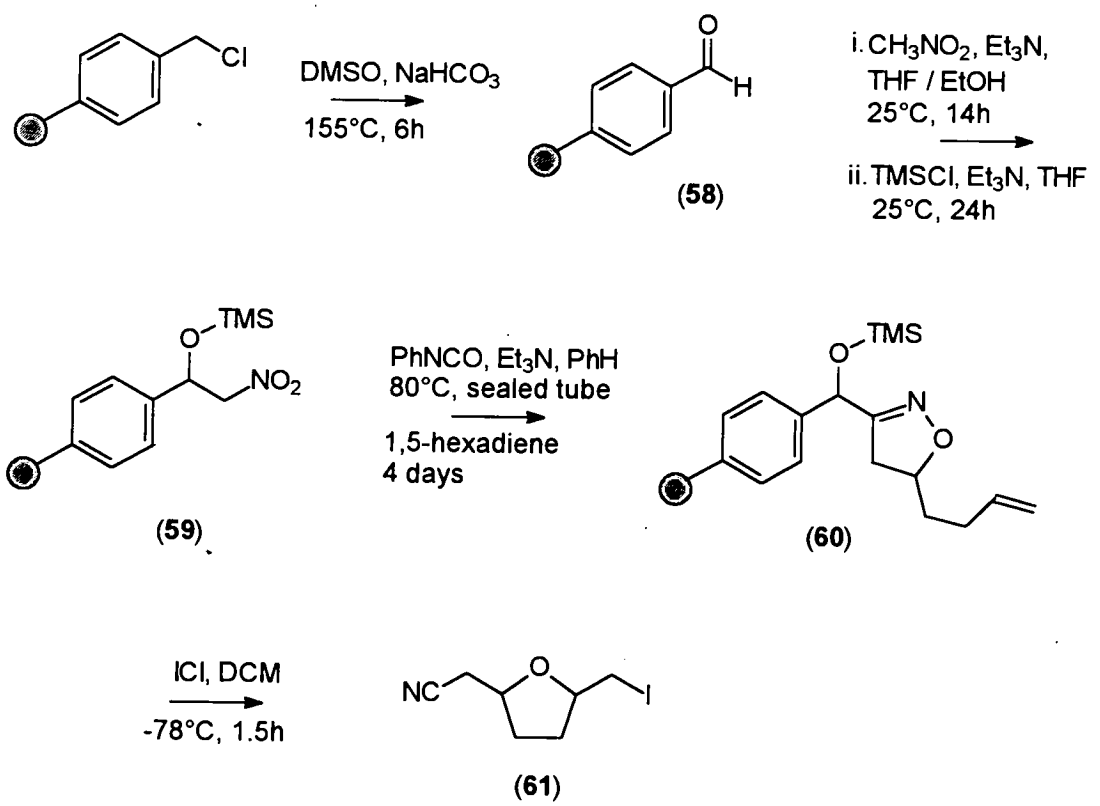


Fig. 1.23. SPOS of 2,5-Disubstituted Tetrahydrofurans

As an approach to solving this, they attached a nitromethane group to the resin-bound aldehyde, (**58**) via the Henry reaction. After protection of the hydroxyl group as the TMS ether, phenylisocyanate mediated dehydration of, (**59**) and 1,3-

dipolar cycloaddition with 1,5-hexadiene provided resin-bound isoxazoline, (**60**). As with the benzodiazepine library generated by the group at Parke-Davis, the final stage in the synthesis involved direct release of the target cyclic ethers, (**61**) in solution following iodocyclisation. The resin-bound aldehyde, (**58**) was also regenerated in the same step. The authors do not report the preparation of a library of compounds, however the diversity of reactions and conditions involved in the synthesis demonstrates the versatility of SPOS.

In a later paper Kurth and co-workers[109] report the preparation of a library of 27 water soluble antioxidants, (**64**), (fig. 1.24). Surprisingly, and in comparison with the Ellman and DeWitt approaches they adopted a "split and mix synthesis" which is commonly used for the generation of peptide libraries.

The first step involved split-vessel esterification of sodium acetate, methoxyacetate and hydrocinnamate with Merrifield resin, (**4**) to introduce the first site of diversity, (R_1). The resulting resin-bound products, (**62**) were then mixed and partitioned into nine separate flasks. Following enolate formation and aldol condensation with seven aryl aldehydes ($R_2R'_2CO$), and two aryl ketones ($R_2R'_2CO$), a library of 27 resin-bound propanediol analogues, (**63**), were prepared. Subsequent reduction with diisobutylaluminum hydride liberated the 27 antioxidants, (**64**) as a mixture. Despite the fact that a mixture was obtained, a deconvolution assay was set up in solution. The results indicated that three trimethoxy analogues gave comparable antioxidative efficiency, with (**64a**) identified as the lead compound.

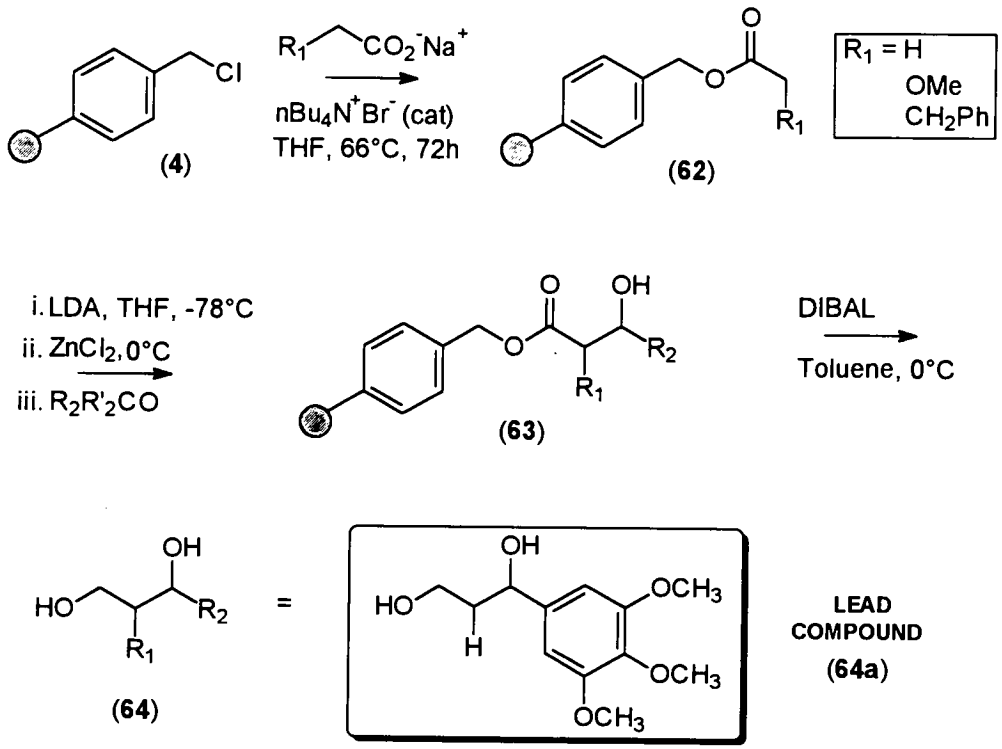


Fig. 1.24. SPOS of Antioxidants

More recent work, by Gallop *et al.* at Affymax have reported the SPOS of a library of the potent ACE inhibitor, Captopril.[110] All these examples have successfully demonstrated the renewed interest in solid phase chemistry. However, construction of a combinatorial library of a class of therapeutic agents critically depends on the availability of general and high-yielding strategies for synthesising these compounds on a solid support.

Many groups have adopted this approach in their research either in the development of new solid supports[111-114] or new resin-bound reactions. The formation of solid phase carbon-carbon bonds has received considerable attention; after all such bonds are of great importance in the construction of small organic compounds. Typical reactions have included enolate alkylation,[115] palladium-mediated Suzuki cross-coupling,[115,116] Heck arylation,[117-119] the Stille reaction[120] and the Mitsunobu reaction.[121]

1.4.3. Concluding Remarks

The concept of combinatorial chemistry is at an early stage, and represents a major advance in medicinal chemistry/drug discovery and development. Judging by the number of review articles, papers, scientific meetings and new companies dealing with combinatorial chemistry, this has resulted in one of the fastest growing research areas in chemistry today. Combinatorial technologies are also important in food and agricultural chemistry, immunology, molecular biology, polymer science and inorganic synthesis.

• 1.5. DIVERSOMER™ Technology.

The production of large populations of molecules or libraries involves a complex interplay between classical organic synthesis techniques, rational drug design strategies, and, robotic and scientific information management. An on-going priority in the field of combinatorial chemistry is the possibility of, and need for, automation of library generation, data handling and combinatorial screening of final products.

While large numbers of oligomeric compounds such as peptides and oligonucleotides can be rapidly synthesised, few methods have been developed for the preparation of small organic compounds. The potential for small molecule diversity is vast and a key element in this equation is automation and technology for both lead optimisation and lead generation.

By combining solid phase chemistry, organic synthesis and automation, Parke Davis Pharmaceuticals (Ann Arbor, MI)[2,8] have created a unique apparatus capable of generating "libraries" of non-oligomeric compounds which they term DIVERSOMERS™;

❶ Multiple, Simultaneous Synthesis

❷ 40 Potential Drug Candidates

❸ Multiple Components

- Solid Phase Chemistry
- Organic Synthesis
- Miniaturisation
- Robotics
- Electronic data handling
- Unique Apparatus

The DIVERSOMER™ technology was conceptualised as a method for the multiple, simultaneous synthesis of organic compounds. The library, or array, of compounds is synthesised in a parallel array (8 or 40) on a solid support (resin). The

whole process is fully automated, from initial addition of the resin to the final submission of the soluble products for biological testing.

Using the DIVERSOMER™ technology, DeWitt *et al.*[8] have simultaneously synthesised and characterised several unique arrays of compounds, including 40 benzodiazepines and 40 hydantoins, (fig. 1.21 and fig. 1.22), respectively. The libraries generated incorporated 3 sites and 4 sites of diversity respectively, yielding 2-14 mg (9-63% yield) of the desired benzodiazepines and 0.3-11.5 mg (4-81% yield) of the desired hydantoins.

Planning.

The generation of 40 discrete compounds involves a complex system of data collection, manipulation, analysis and calculations. The DIVERSOMER™ technology has implemented several Microsoft® Excel spreadsheets as a method for library design and data control.

Apparatus.

A diagrammatic representation of the DIVERSOMER™ apparatus is shown in (fig. 1.25).[8] The apparatus design employs gas dispersion tubes, referred to as "PINS", with fritted glass filters which physically contain the resin, allowing efficient mixing between reactants in the reservoir wells and resin in the frit. Additionally, it aids in the separation of resin-bound intermediates from excess reagents, solvents and by-products. The upper portion of the PINS serve as condensers when a chilled atmosphere of gas is circulated into the manifold allowing for refluxing conditions. Inert atmospheric conditions can also be applied via the manifold. The holder block serves to secure the PINS and allows the array to be handled as a single unit. Teflon gaskets on either side of the holder block act as sealants. The reaction block (2 x 4, or 4 x 10) containing disposable glass vials (wells) acts to accommodate the PINS. Separate reaction wells are necessary to allow individual reactions to be executed and monitored, while at the same time maintaining the integrity of filtrates, intermediates and final products at each point in the array. The reservoir block can be successfully

heated or cooled as required. Addition of reagents or removal of samples during reaction cycles is achieved via the gasket layer at the top of the manifold.

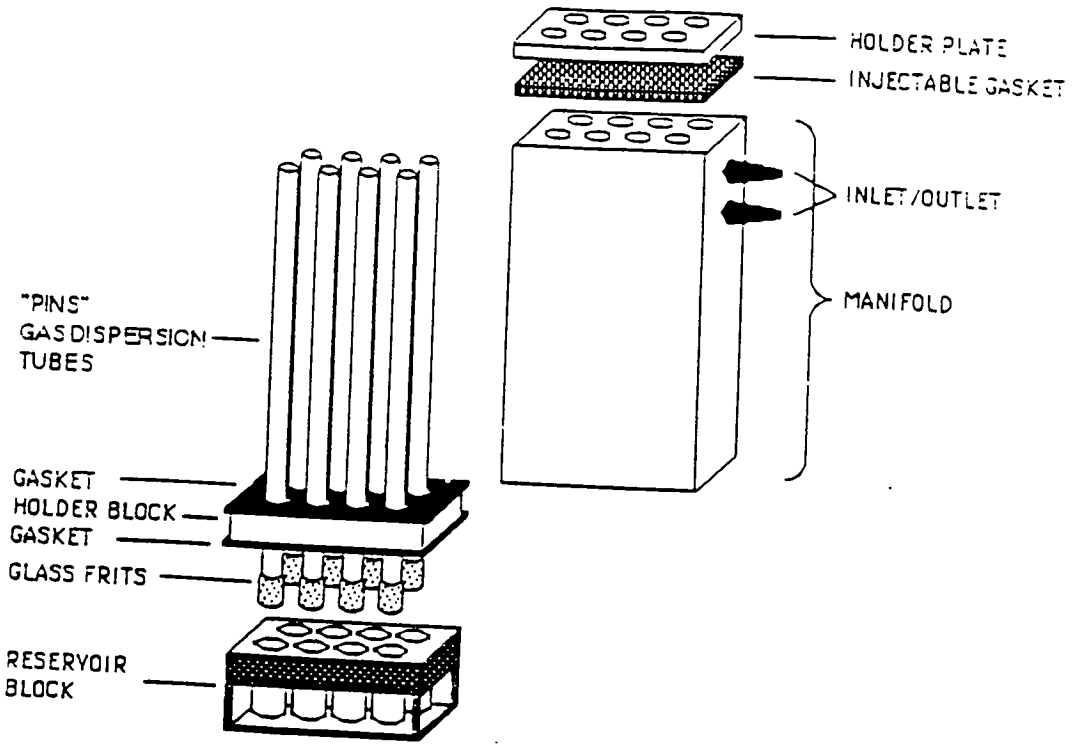


Fig. 1.25. DIVERSOMER™ Apparatus

Once complete, the DIVERSOMER™ apparatus can be agitated by sonication or in some cases rotational platform shaking. Heating and cooling of the apparatus can be carried out in either water, a mixture of water/anti-freeze, or silicone oil depending on the temperature of reaction.

Automation.

A novel method for the rapid generation of non-oligomeric molecules has been designed. However the multiple, parallel synthesis of 40 discrete samples over a number of steps employing several reagents is both physically and mentally challenging. The DIVERSOMER™ technology has eliminated this problem by utilising a Tecan™ 5032 Robotic Sample Processor (RSP). A typical protocol for

library generation involves resin loading, reaction cycles, reaction monitoring, wash cycles, isolation & purification and final product handling.

At present Parke Davis' technology is the only method whereby full automation for the generation of small molecule libraries has been successfully demonstrated. In combinatorial chemistry terms, the actual number of compounds produced in a single array (40) utilising the DIVERSOMER™ technology is significantly smaller than the current methods for generating peptide libraries (10^5-10^7 peptides). However, the technology offers increased chemical and structural diversity. Undoubtedly this unique technology has the leading edge for small molecule combinatorial synthesis by automation and integration.

• 1.6. QUINOLONE Antibacterials and related Compounds: Mode of Action & Biological Activity.

The synthesis of DNA and ribonucleic acid (RNA) is essential to all living cells. The formation of nucleic acids can be blocked by a variety of means.[122-124] The quinolone antibiotics inhibit the replication of DNA in bacteria, without immediately affecting RNA or protein synthesis in sensitive cells.[125] The mode of action of the quinolones is believed to be different from other antibiotics, which generally operate at the bacterial cell wall or at the ribosome.[126] Studies have shown that the primary physiological target of the quinolones within the bacterial cell is DNA gyrase, a bacterial type II topoisomerase which is not present in eukaryotic cells.[127,128]

DNA gyrase is an essential enzyme required for DNA replication and transcription.[129] The DNA bacterial chromosome is compact, however DNA replication and recombination require that the DNA strands separate and unwind the double helix. One protein that controls this process is DNA gyrase.[130] This enzyme introduces negative supercoils into DNA by binding covalently to specific sequences of the DNA, cleaving the two DNA strands, passing one under the other and resealing the strands. ATP hydrolysis plays a key part in this supercoiling, knotting-unknotting and catenation-decatenation process of the DNA gyrase.[131]

The quinolones, like other mammalian topoisomerase II inhibitors appear to stabilise the transient DNA fragments cleaved by DNA gyrase in the initial gate-opening step of the DNA supercoiling process, (see fig. 1.26).[132] Consequently, this leads to inhibition of bacterial growth with the overall end point of the reaction being bacterial cell death, with the DNA gyrase being converted into a cellular poison.[133] Clearly the selectivity of the quinolones for bacterial DNA gyrase over mammalian topoisomerase II is vital to the process since both these enzymes are closely related. More recently, the quinolones have been shown to have *in vitro* activity against *M. tuberculosis*[134] and *in vivo* antineoplastic activity.[135]

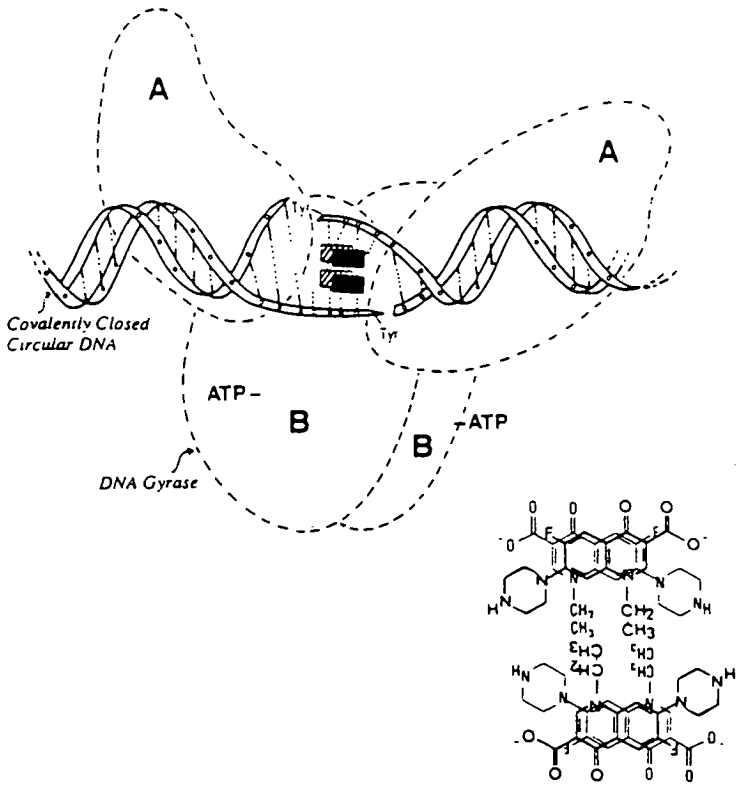


Fig. 1.26. Cellular Action

The therapeutic utility of the quinolones as effective antibiotics is well demonstrated by their annual world-wide sales; Ciprofloxacin® alone recorded $1.3 billion sales in 1994.[136] In addition to being prescribed for urinary tract infections (UTI) such as cystitis, the quinolones have other clinical uses, such as treatment for endocarditis, osteomyelitis and chronic prostatis.[137] Compared to the frequent development of resistance to earlier quinolones and other classes of antibiotics, the fluoroquinolones are active against both Gram negative and Gram positive pathogens. However, side effects such as nausea, vomiting and diarrhoea are still common.[138]

CHAPTER 2: RESULTS AND DISCUSSION

• 2.1. QUINOLONES & 1,8-NAPHTHYRIDINONES; General Synthetic Routes

Since the early 20th century many antibiotics derived from microorganisms or by synthetic means have been discovered as effective drugs in the anti-infective chemotherapy field.[135] The discovery of penicillin by Fleming led to one of the first antibiotic substance to find use in man in the 1940s.[140] Antibiotics such as the β-lactams, macrolides, aminoglycosides and tetracyclines are generally obtained from the chemical modification of the naturally isolated substrates[141] whereas the more recent quinolone and 1,8-naphthyridinone antibacterial agents are purely of synthetic origin.

The identification of the first lead structure of this family dates back to the late 1950's where the drug was discovered solely by serendipity.[142] The first member of this group to be synthesised was nalidixic acid, (**69**), [1,4-dihydro-1-ethyl-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid] in 1962, by Lesher *et al.*[143] The original synthetic route used to prepare nalidixic acid is illustrated in (fig. 2.1).

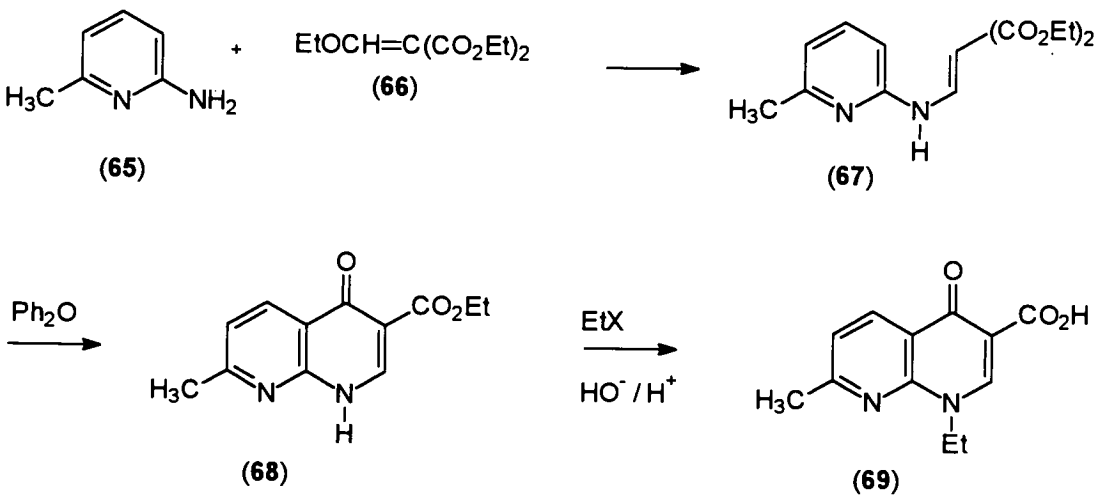


Fig. 2.1. Synthesis of Nalidixic acid

In the initial step, 6-methyl-2-aminopyridine, (**65**) was condensed with diethyl ethoxymethylenemalonate, (**66**) to yield the anilinomethylenemalonate derivative,

(**67**). Cyclisation was achieved by heating in Dowtherm A (Gould-Jacobs reaction), or by Friedel-Crafts catalyst[144] to yield the oxonaphthyridine-3-carboxylate, (**68**). Alkylation followed by hydrolysis give nalidixic acid, (**69**).[145]

Several variations of the Gould-Jacobs reaction were introduced in subsequently. In 1971, Agui *et al.*[146] replaced the traditional cyclisation conditions by the use of polyphosphoric acid (PPA), while in 1978 Mitscher *et al.*[147] reported the preparation of the naphthyridone ester by reaction of isatoic anhydride with sodio ethyl formylacetate. In another approach, the naphthyridones were prepared by a Dieckmann cyclisation step[148] followed by oxidation and hydrolysis, (fig. 2.2).

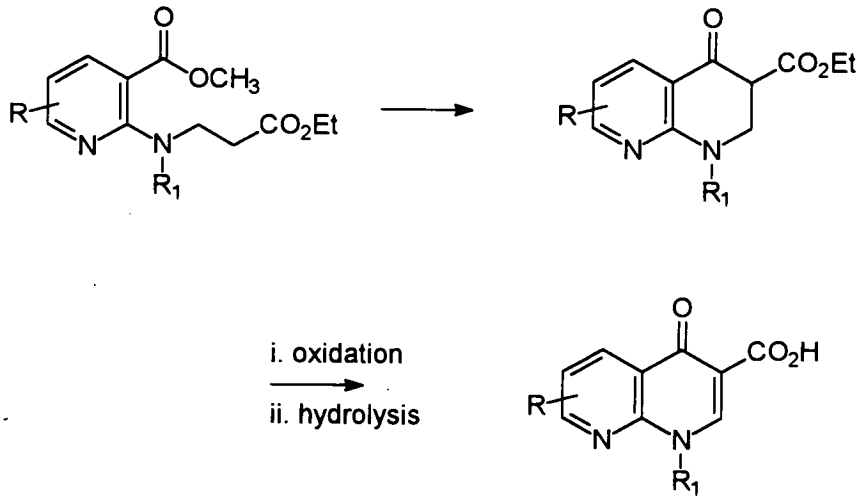
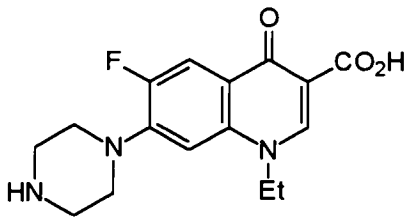


Fig. 2.2. Dieckmann Cyclisation

Since the clinical introduction of nalidixic acid, numerous other derivatives have been prepared in the search for improved antibacterial activity. The early 1980s saw major developments in this field, most notably with the introduction of Norfloxacin, (**70**), [1,4-dihydro-1-ethyl-6-fluoro-4-oxo-7-(1-piperazinyl)-quinoline-3-carboxylic acid], (fig. 2.3).[149]



(70)

Fig. 2.3. Structure of Norfloxacin

Norfloxacin was the first of many quinolones with a fluorine atom substituted at the C-6 position and a piperazine moiety at C-7. Additional variations at N-1 were also introduced. It also represented the first significant increase in antibacterial activity covering both Gram-positive and Gram-negative micro-organisms.[150] However, the synthetic methodology, the Gould-Jacobs reaction and related methods normally used for preparing the quinolones proved to be unsuccessful for the synthesis of the new quinolones, generally referred to as the fluoroquinolones.[151]

An efficient and regiospecific general synthetic method was quickly reported by Grohe *et al.*,[152] and by Chu *et al.*[153] Called the cycloaracylation process, the reaction involved an intramolecular nucleophilic displacement cyclisation step as shown in (fig. 2.4).

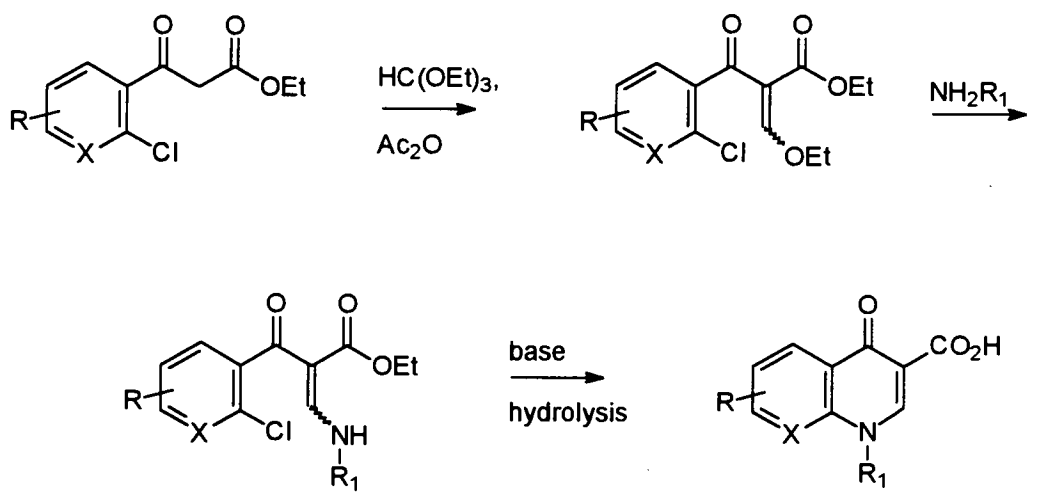


Fig. 2.4. Cycloaracylation Process

The synthesis of the quinolones has accelerated at an unprecedented rate in recent years. To date, the cycloaracylation process has remained the method of choice for the synthesis of the quinolones. More recent studies have introduced a Vilsmeier type approach,[154,155] (fig. 2.5).

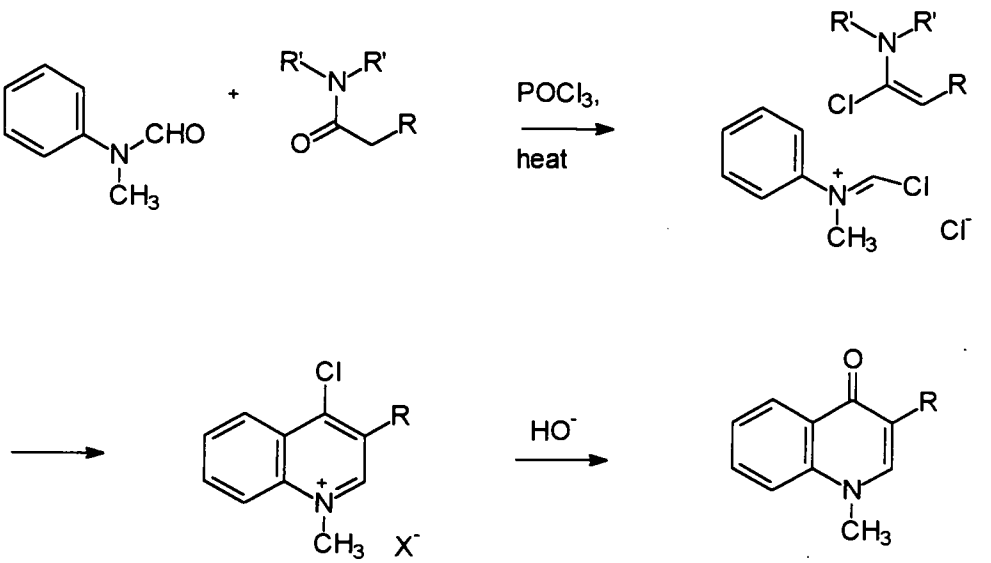


Fig. 2.5. Vilsmeier Approach to the Quinolones

In 1984, Smith[156] devised a general nomenclature and numbering system for the arylated fused 4-pyridine anti-bacterials. The general body consists of a 1-substituted-1,4-dihydro-4-oxopyridine-3-carboxylic acid moiety (A) combined with an aromatic or heteroaromatic ring (B), nalidixic acid being the prototype of the naphthyridine class, while Norfloxacin represents the quinolone family. Fig. 2.6 shows a diagrammatical representation of the most favourable structural variations around the molecule. It is obvious that the antibacterial activity, in addition to the bicyclic heteroaromatic system also depends on the nature of the peripheral substituents and their spatial relationship.

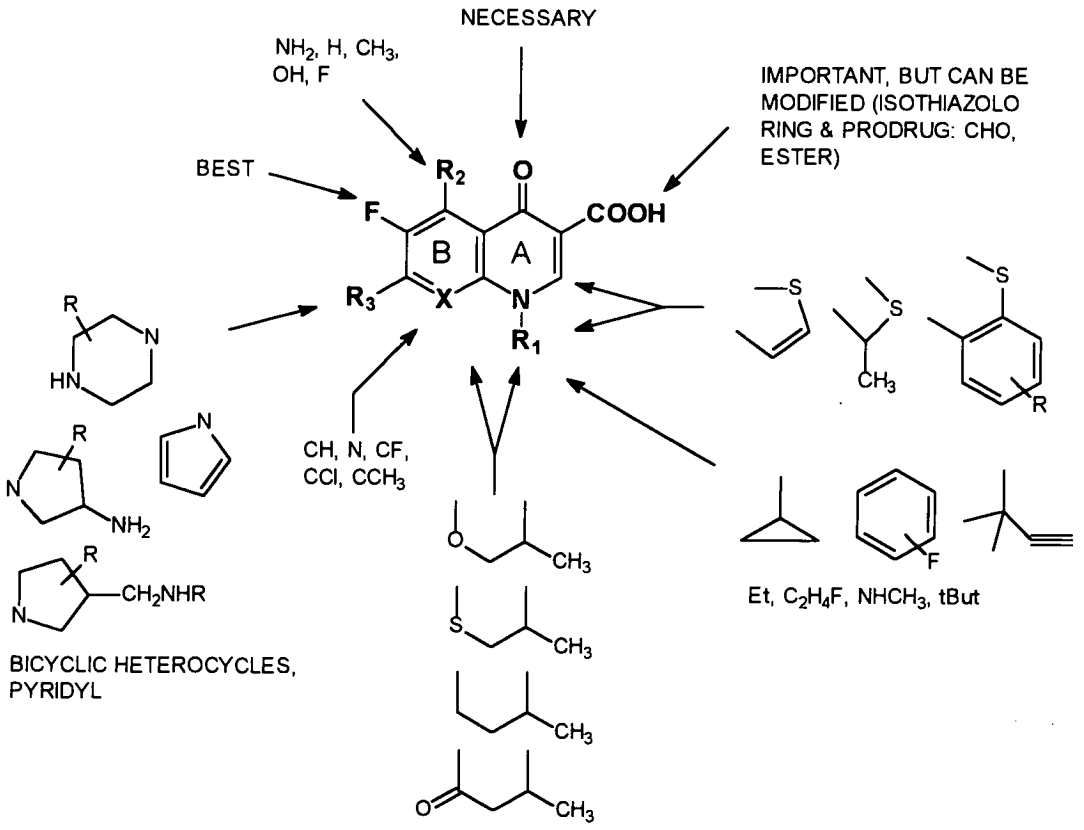


Fig. 2.6. Structural Variations of the Quinolones

Extensive research into both the chemistry and biology of the quinolones has been carried out by Schentag *et al.*,[157] Chu & Fernandes[158] and Mitscher *et al.*[159] It has generally been accepted that the preferred substitutions involve the utilization of fluorine at C-6, the piperazinyl group at C-7 and the ethyl, cyclopropyl, or fluorophenyl groups at N-1. Several tricyclic analogues which contain a three atom bridge connecting the vicinal positions of the quinolones have also been reported.[160-163]

• 2.2. SPOS of the Quinolones

2.2.1. Project Aims

The quinolones represent a class of highly potent, broad-spectrum antibacterial agents (see section 1.5). Over 6000 novel quinolone compounds have been synthesised worldwide to date.[164] However, this represents only a fraction of the total potential diversity for this class of compounds. For example, (fig. 2.7) showing three sites of diversity can lead to the generation of over 9000 quinolones.

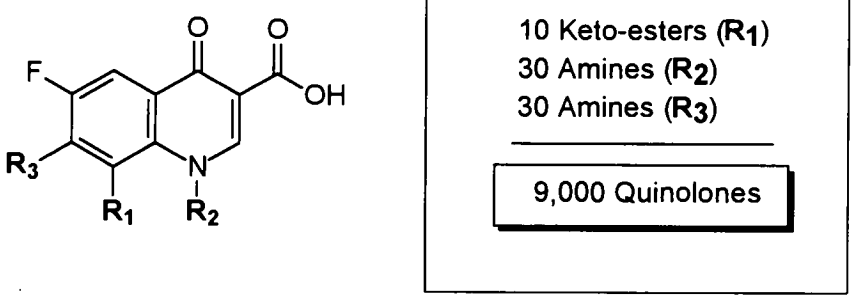


Fig. 2.7. Quinolones: Potential Diversity

The synthesis of the quinolones involves a complex series of reactions, and our initial objective was validation and optimisation of a solution phase route which would also be amenable to SPOS. Typically, reaction conditions were designed to exploit the advantages of SPOS while circumventing the disadvantages of the polystyrene solid support. For the development of the solution phase chemistry, and its compatibility to a solid phase route, several criteria are vital to any synthetic pathway, as highlighted below;

❶ Identify **Compound Targets**.

❷ Design and Validate **Solution Phase Chemistry** Amenable to Solid Phase Chemistry.

❸ Validate and Optimize **Solid Phase Chemistry**.

❹ **Select Diversity** for Building Blocks.

❺ Design **Reaction Format**.

❻ Develop **Automated** Methods for **Synthesis**.

❼ Develop **Automated** Methods for **Purification**.

❽ Execute **Synthetic Process**.

This section will discuss the aspects involved in this work; from resin functionalisation for the attachment of the quinolones to a solid support, through the development of the solution phase chemistry, to the SPOS of the quinolones and, finally the generation of a library of 40 quinolones utilising the DIVERSOMER™ technology.

2.2.2. Resin Functionalisation of Ring (B)

With few exceptions, the quinolone antibacterials of this type are generally prepared by the cycloaracylation process[152,153] discussed in the previous section. In order to generate a library of the quinolones using the DIVERSOMER™ technology, we implemented several studies of the possibilities for attachment of the quinolone moiety to a solid support, preferably polystyrene derivatised resins.

Our initial studies investigated resin attachment *via* ring (B) of the quinolones. Ring (B) is usually aromatic or heteroaromatic in nature, with a range of subtituents introduced at various sites, (fig. 2.6). Particulary notable in this regard are a fluorine atom at C-6, and a five or six membered nitrogen-linked heterocycle at C-7. Among the many modifications investigated at the C-7 position, the 1-piperazinyl group and related derivatives have long been the group of choice, offering excellent antibacterial activity.[165,166]

In 1992, Segawa *et al.*,[163] and later in 1993, Laborde *et al.*,[167] separately reported the preparation of the quinolones starting from a range of piperazinyl derivatives. Both routes were viable possibilities for our studies of SPOS with attachment at ring (B).

An aminomethyl derivatised polystyrene resin[168] developed in our laboritories was used in an attempt to prepare resin-bound piperazine, (fig. 2.8). The resin, 2-copoly(styrene-1%-divinylbenzene)methylaminocarboxy-methoxydibenzocyclohepta-dien-5-one, (7) was initially suspended in a solution of DCM containing excess piperazine. Following the addition of a Lewis acid, titanium(IV) chloride, it was hoped that the resin-bound imine salt, (**71**) would be formed and subsequently reduced with lithium borohydride.

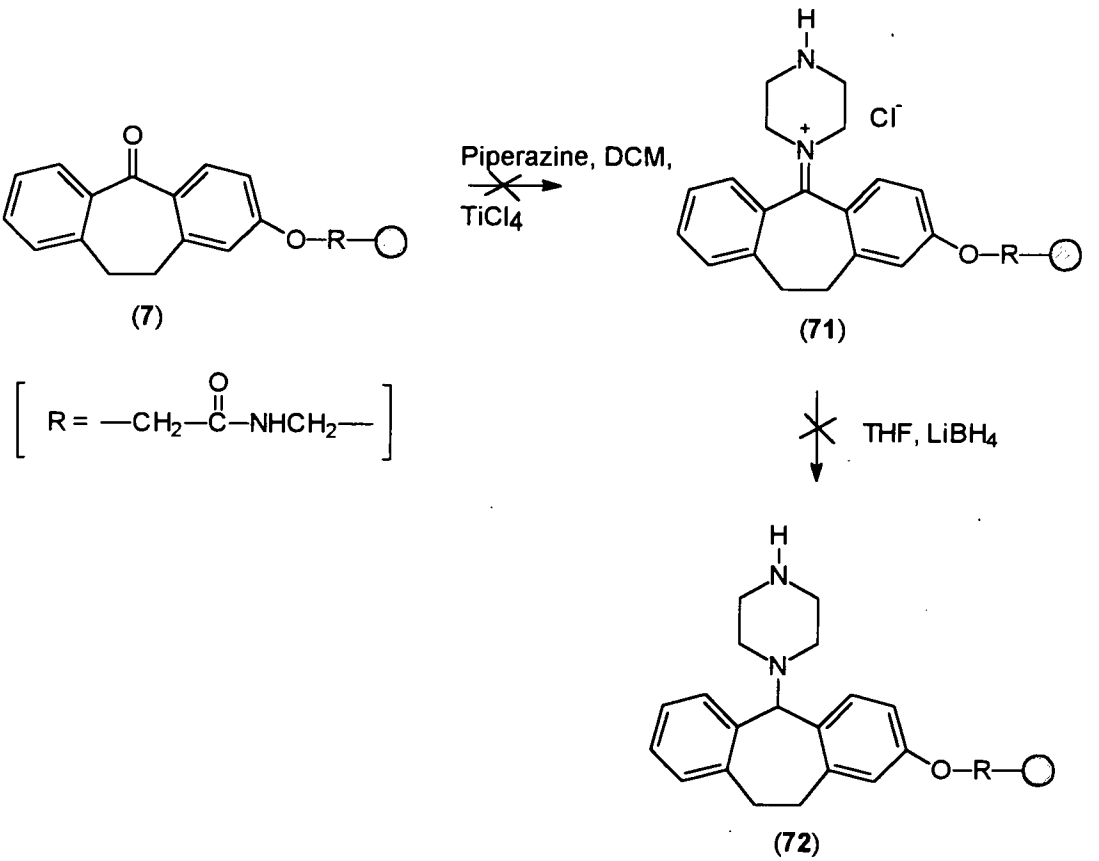


Fig. 2.8. Attempted Synthesis of Resin-bound Piperazine

However, analysis of the resin-bound piperazine product, 2-copoly(styrene-1%-divinylbenzene)methylaminocarboxymethoxy-5-(9-piperazinyl)dibenzocyclohepta-dien-5-one, (**72**) by an isatin test give a negative result, indicating that no reaction had occurred. An analogous reaction with resin-bound heptylamine give a positive isatin test.[169] Additionally, both IR and ^{13}C gel phase NMR of the resin-bound piperazine

products confirmed the failure of the reaction, with only the starting material, (7) identified.

Following these results, we continued the solid phase studies of alternative resins and routes for the generation of resin-bound ring (B) derivatives. Again we concentrated on the piperazine moiety as a viable attachment point. In order to prepare the resin-bound piperazine,[170,171] (**73**), chloromethylated resin, (**4**) was suspended in a solution of toluene containing piperazine, and heated to 70°C for 18 hours, (fig. 2.9).

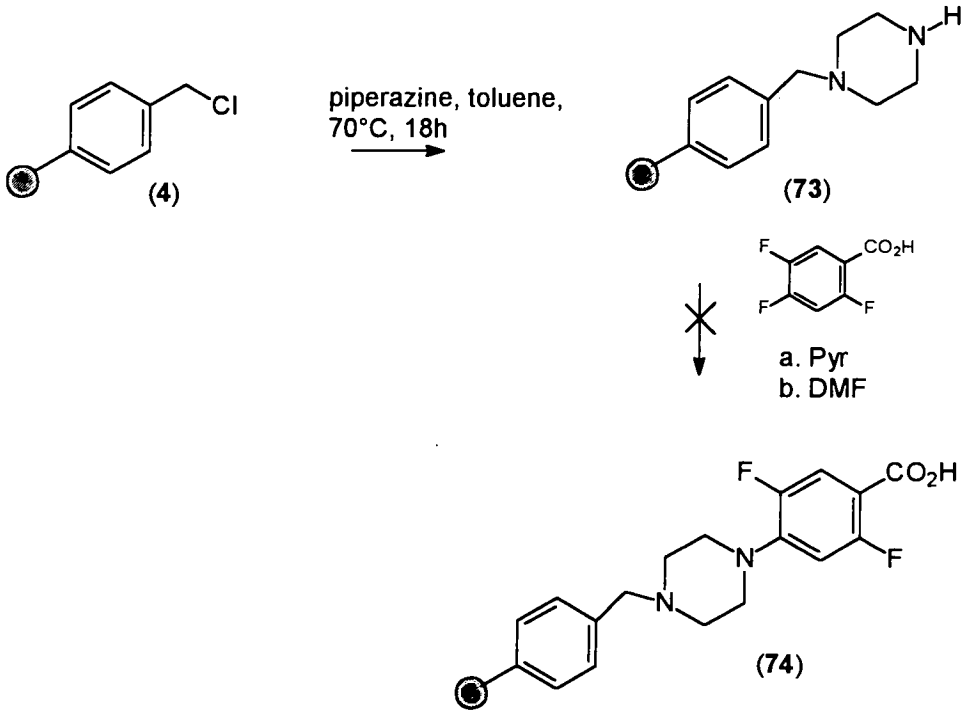


Fig. 2.9. Synthesis of Resin-bound Piperazine

Analysis of the resin-bound piperazine intermediate, copoly(styrene-1%-divinylbenzene)piperazinyl, (**73**) by ^{13}C gel phase NMR confirmed formation of the desired product. However, subsequent to the nucleophilic aromatic substitution reaction with 2,4,5-trifluorobenzoic acid in pyridine, no carbonyl stretching band was seen in the IR spectrum. The arylation reaction was later repeated in DMF to give a strong band at 1683 cm^{-1}, corresponding to the resin-bound product, (**74**) but the ^{13}C NMR spectrum obtained was very weak.

These results offered a potential solid phase route for the generation of the quinolones *via* an attachment point on ring (B). Additionally, an analogous reaction to that reported by Laborde *et al.*[167] for the quinolones could be attempted, as shown in (fig. 2.10).

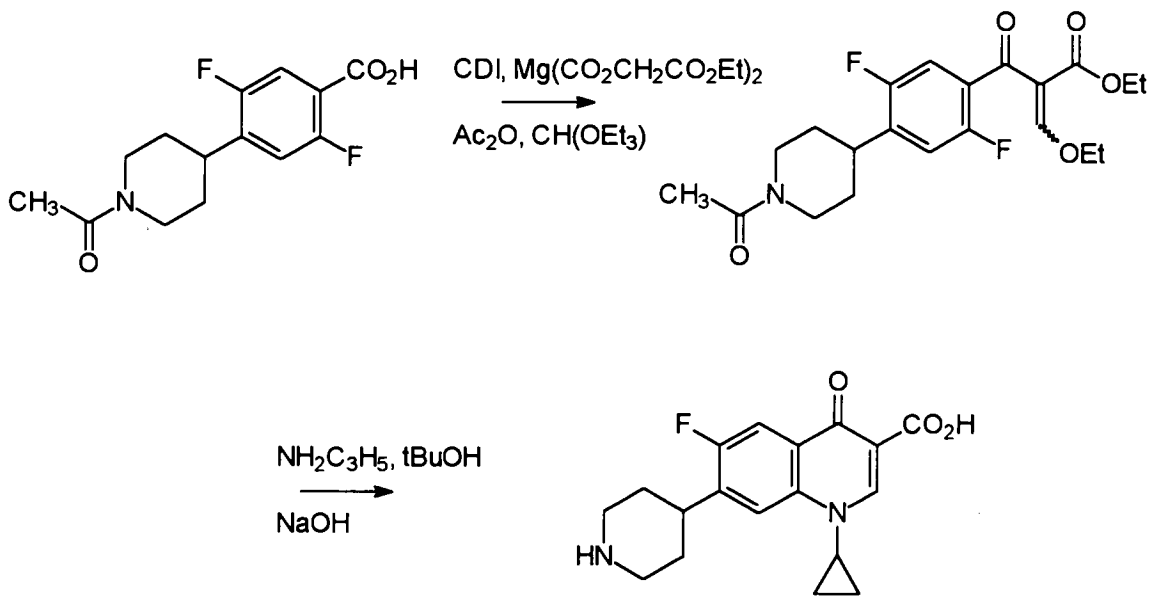


Fig. 2.10. Synthesis of Quinolones via Ring (B)

However, the attachment of a solid support to the piperazine moiety at C-7 limits the overall diversity generated at this active site of the quinolone ring. This in turn would restrict the number of quinolones generated using the DIVERSOMER™ technology. An additional problem could be the final cleavage of the resin-bound quinolone from the solid support.

Recently, Dankwardt *et al.*[172] have reported the SPOS of aryl and benzyl piperazines. Many active therapuetics contain the piperazinyl moeity, and this work introduces a possible method for the generation of piperazinyl based libraries. Their work also illustrates some new solid phase chemistry, increasing the number and type of reactions now possible on polystyrene based solid supports.

2.2.3. Resin Functionalisation of Ring (A)

The synthetic methodology for the generation of the quinolones has been extensively discussed in section 2.1. Following the limited utility of the resin-bound ring (B) derivatives as potential linkers in the SPOS of the quinolones, we concentrated our studies on the more traditional Gould-Jacobs reaction and cycloaracylation process. With both cases, we studied a possible resin attachment point to the quinolone structure *via* ring (A).

For the synthesis of Norfloxacin, we employed the Gould-Jacobs reaction sequence (fig. 2.11). A key attraction of this route for the solid phase synthesis of the quinolones *via* ring (A) was the possibility of selective attachment of diethyl ethoxymethylenemalonate, (**66**), to a solid support.

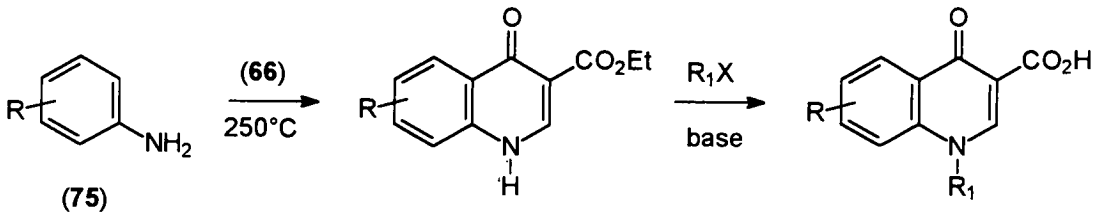


Fig. 2.11. Gould Jacobs Reaction

The reaction is also of interest in terms of library generation, whereby a variety of substituted anilines, (**75**), can be employed; however, introduction of the first site of diversity (R_1) at position one is limited to groups that undergo S_N2 alkylation or amination. Consequently, this would limit the overall diversity of groups introduced at R_1 and therefore does not meet with the requirements of the DIVERSOMER™ technology.

Another limitation and concern with the Gould-Jacobs reaction was the high temperature cyclisation step. While this is easily achieved in solution, our work has shown that high temperature reactions are generally not favoured by polystyrene solid supports, with resin bead breakdown or polymerisation a frequent problem.

Thus, as an alternative approach to the SPOS of the quinolones and considering the disadvantages of the above route, the cycloaracylation process was attractive since it provided a direct pathway for attachment of the β-keto ester, (77) to a functionalised polystyrene solid support. In order to provide the corresponding β-keto enamide intermediates, (79), two separate routes are possible, (fig. 2.12).[152,173] As with the Gould-Jacobs route, several problems were of concern with the cycloaracylation for application to solid phase methodology.

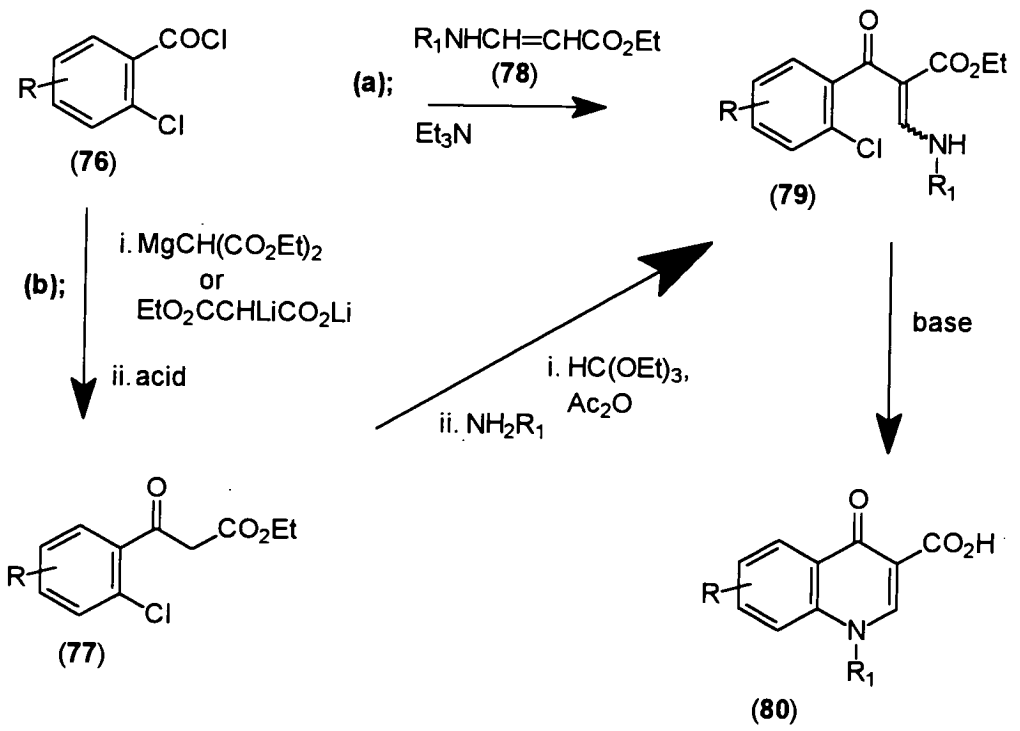


Fig. 2.12. Cycloaracylation Process

Pursuing the reaction *via* route (a) would allow the direct attachment of the enamine starting material, (78), to a solid support, however for each R_1 group introduced, the starting materials would require prior preparation in solution phase. Again this would not meet with the requirements of the DIVERSOMER™ technology. Both routes, (a) and (b) have the advantage of providing an array of R_1 substituents at position one which would be otherwise unattainable using the S_N2 methodology described above. This in turn will increase the total number of molecules

generated in any library. However, a potential drawback of the cycloaracylation process is the availability of the starting benzoic acid derivatives, (**76**), relative to the anilines, (**75**), in the Gould Jacobs reaction.

For the SPOS of the quinolones, we ultimately pursued route (b) of the cycloaracylation process. Our main concern at this stage was the preparation of the resin-bound β-keto ester intermediate. In the original synthetic plan for a DIVERSOMER™ approach to the quinolones the reaction involved the generation of the resin-bound β-keto ester *via* the β-keto acid.[174] Subsequently, we set out to prepare the β-keto acids, (fig. 2.13).[175]

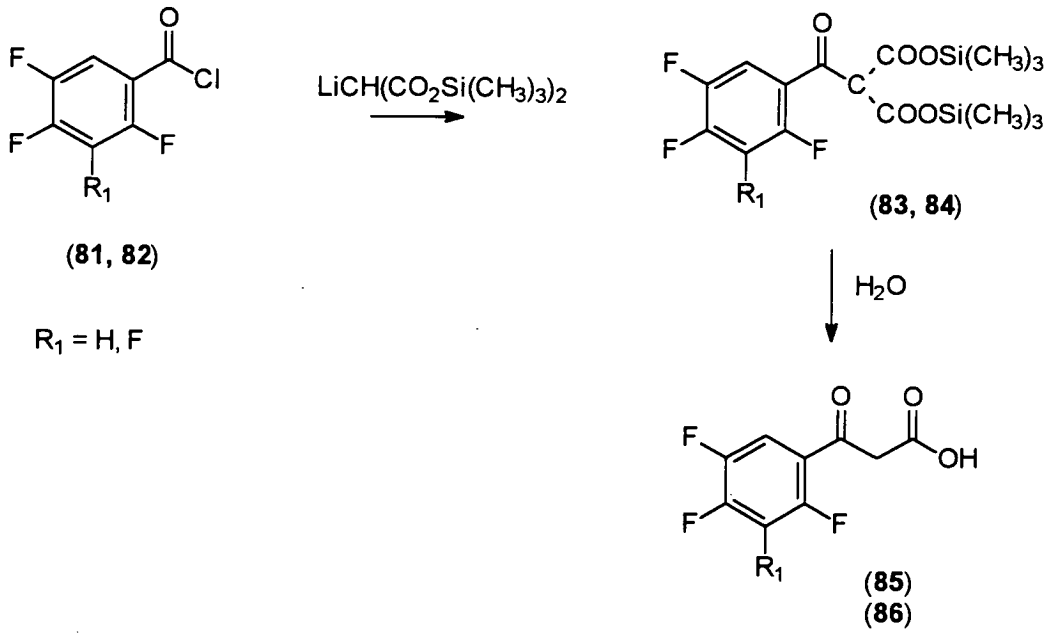


Fig. 2.13. Preparation of β-Keto Acids

The acid chlorides, (**81**, and **82**), (R_1 = H, F) were suspended in a reaction mixture containing the mono anion of bis[trimethylsilyl] malonate. Subsequent treatment with water led to hydrolysis and decarboxylation of the intermediate triacyl compounds, (**83**, and **84**), to yield the desired β-keto acids, (**85**, and **86**), as white crystalline powders. On repeating the reaction, yields of greater than 90% were

obtained when an excess of bis[trimethylsilyl] malonate was employed. The acids were found to exist in keto-enol tautomerism (3:1).

In order to prepare the corresponding resin-bound β-keto esters we concentrated our efforts on a number of methods for activating the carboxylic acid functional groups. SPPS has for a long time used acid chlorides[176] and acid azides[177] as methods of coupling the first amino acid residue to the polystyrene resin. Preliminary work had shown that the acid chloride of monoethyl malonate had successfully coupled to hydroxybenzyl resin; however such results were not reproducible with the activated β-keto acids, (fig. 2.14).

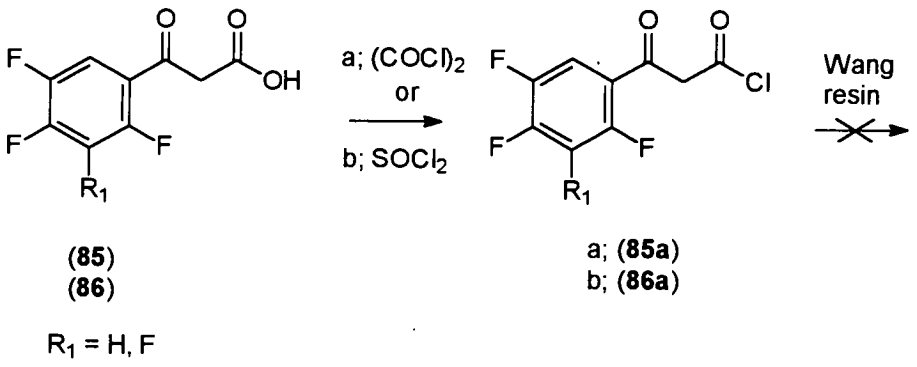


Fig. 2.14. Acid Chloride Activation

Two separate routes were employed for the preparation of the acid chlorides; (a); oxalyl chloride and (b); thionyl chloride, with the corresponding products, (**85a**) and (**86a**), confirmed by TLC and IR spectroscopy. On coupling the acid chlorides to Wang resin, the product from the oxalyl chloride reaction gave a black resin, while the thionyl chloride product give a pale orange coloured resin identified as unreacted Wang resin.

Following this and analogous to SPPS, we attempted activation of the carboxylic acids by the more favoured methods of mixed anhydrides[178] and active esters.[179] A pre-formed symmetrical anhydride, (**87**), was prepared of each of the β-keto acids, (**85**, and **86**) by activation of the carboxyl group using three equivalents of DIC and six equivalents of the β-keto acids in DMF.[180] Following this, the respective

solutions were added to Wang resin pre-swollen in DMF. As an alternative to the mixed anhydride approach, ethyl-1-hydroxy-1H-1,2,3-triazole-4-carboxylate, (HOCt)[181,182] was added to a solution of DIC containing the β-keto acids. The resulting active esters, (**88**), were then added to a solution of Wang resin, (fig. 2.15).

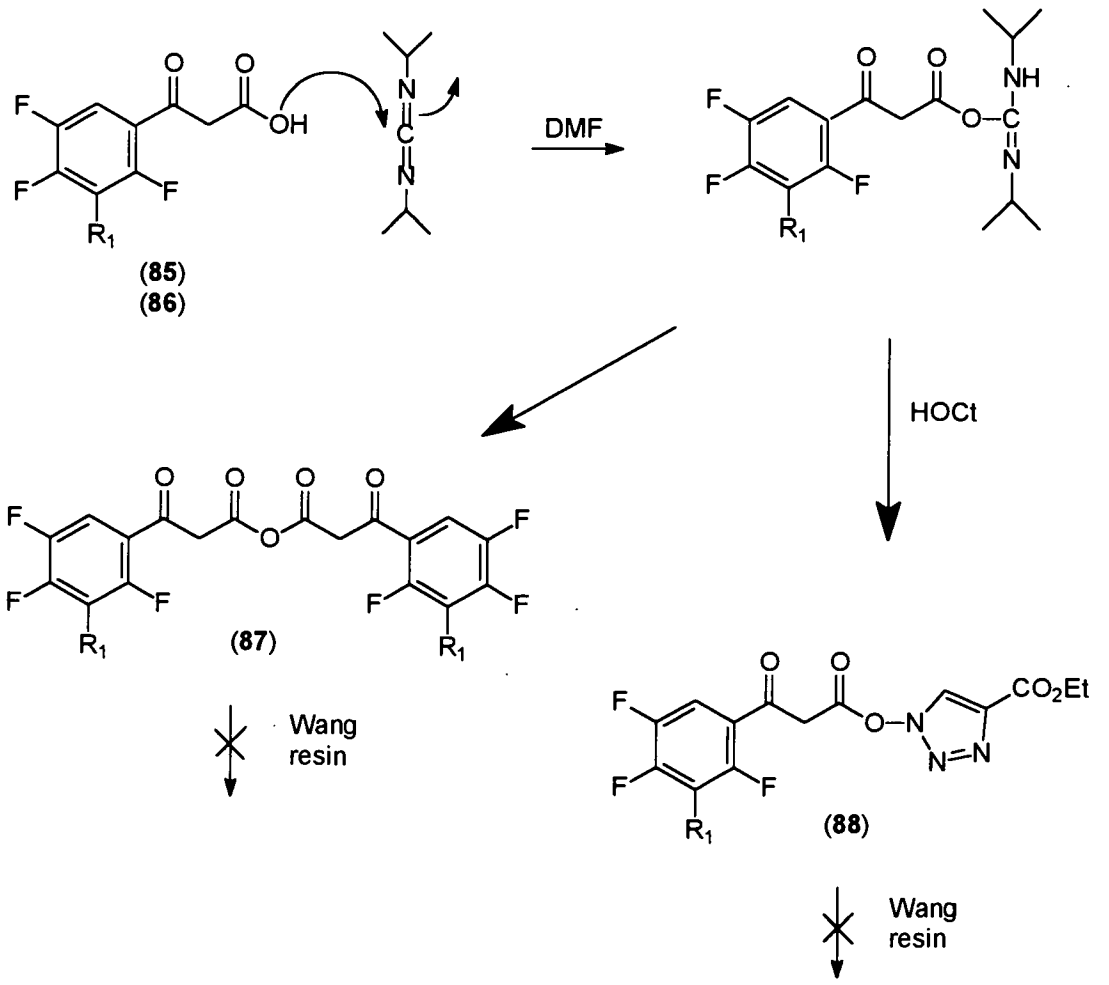


Fig. 2.15. Mixed Anhydride and Active Ester Activation

IR studies suggested the addition of the activated acids to Wang resin; however, as with the products from the acid chloride routes, ^{13}C gel phase NMR confirmed no reaction to have taken place with all the products identified as unreacted Wang resin. The sensitivity of β-keto acids to hydrolysis and decarboxylation[183] is the most likely cause of failure of all four reactions.

The solution phase synthesis of the quinolones *via* the cycloaracylation process involves the preparation of the β-keto ester, (77), as shown in (fig. 2.12). Following the unsuccessful solid phase activation of the β-keto acids, we subsequently prepared the β-keto esters 2,4,5-trifluorobenzoylacetic acid ethyl ester, (**97**), and 2,3,4,5-tetrafluorobenzoylacetic acid ethyl ester, (**98**), (fig.2.19)[184] and studied methods for the generation of the corresponding resin-bound β-keto esters.[185-188] We initially used diethyl malonate and di-*t*-butyl malonate as the prototype in our studies. After an extensive study of various reagents and reaction conditions, transesterification of the β-keto esters to Wang resin, (**5**), was achieved by heating in toluene with a catalytic amount of DMAP, at 110°C, for 72 hours,[188] to generate the resin-bound β-keto esters. On repeating the reaction with 2,4,5-trifluorobenzoylacetic acid ethyl ester, and 2,3,4,5-tetrafluorobenzoylacetic acid ethyl ester, the corresponding resin-bound β-keto esters, (**89**, and **90**), were successfully identified by ^{13}C gel phase NMR, (fig 2.16).

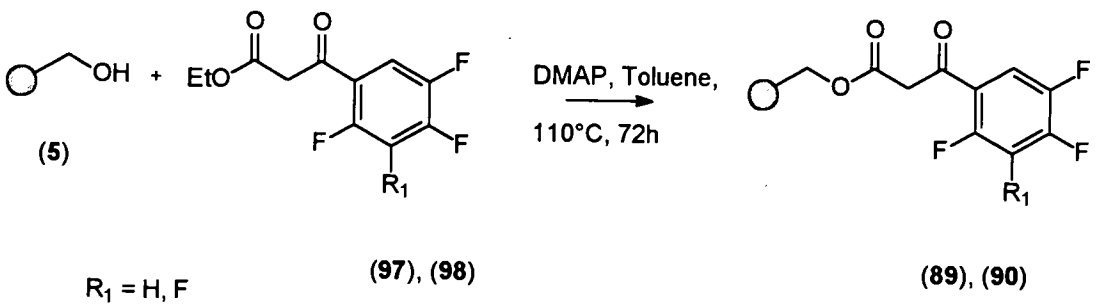


Fig. 2.16. Transesterification Reaction

• 2.3. Development of Solution Phase Chemistry

For the solution phase synthesis of the quinolones, Ciprofloxacin® (**91**), [1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-quinoline-3-carboxylic acid], and the related compound, 1-cycloproryl-6,8-difluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-quinoline-3-carboxylic acid were used as the prototypes for our research. The traditional synthesis of Ciprofloxacin®, as carried out by the group at Parke-Davis,[189] and other groups[153,164,190] has generally used the cycloaracylation process, (fig. 2:17).

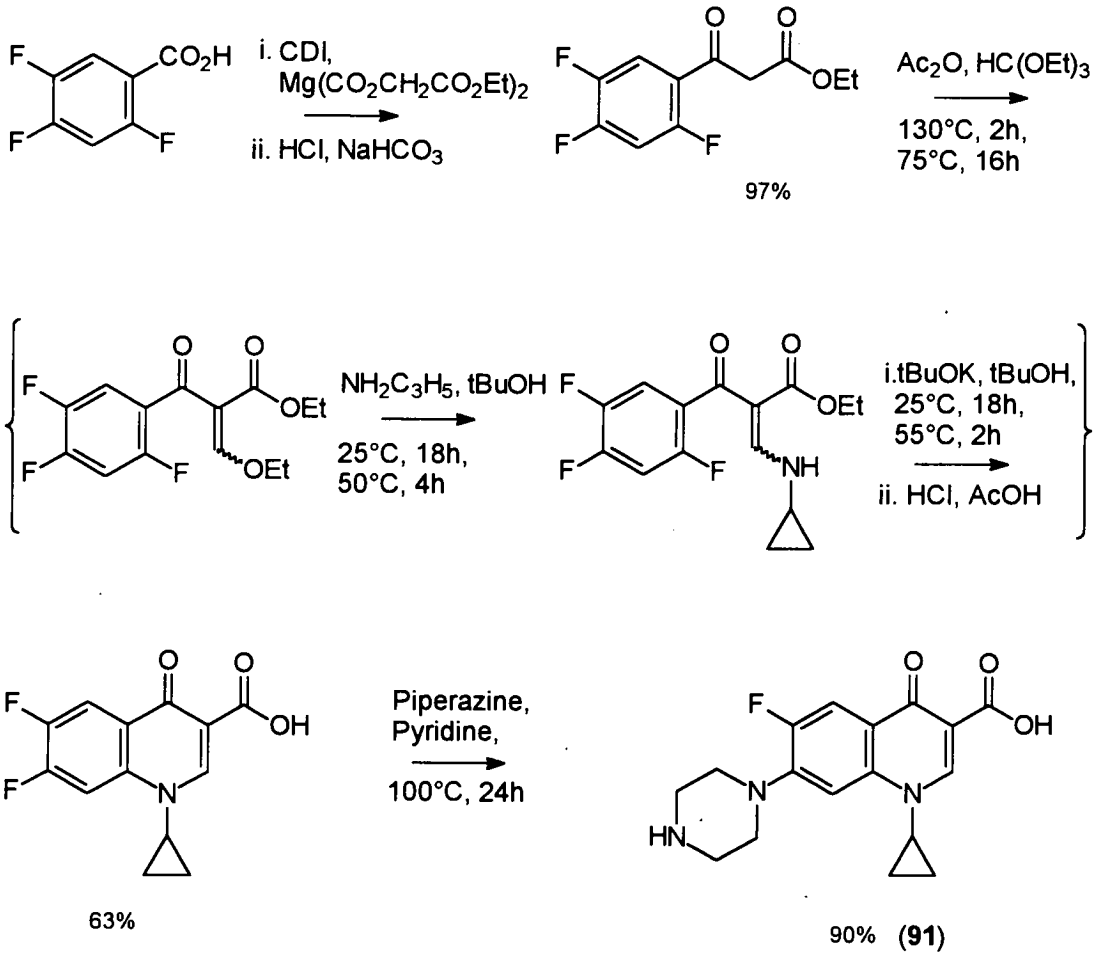


Fig. 2.17. Parke-Davis Solution Phase Synthesis of Ciprofloxacin®

With a point of resin attachment *via* the ester functionality at the C-3 position of the quinolone structure now established, we attempted the Parke-Davis synthetic

route for the solid phase synthesis of Ciprofloxacin®, (fig. 2.18). Transesterification of the β-keto ester, (**97**), to Wang resin, (**5**), generated the corresponding resin-bound β-keto ester, copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl2,4,5-trifluorobenzoylacetate,[188] (**89**), starting material. Treatment of (**89**), with triethyl orthoformate in acetic anhydride at 130°C for 2 hours followed by 16 hours at 75°C gave the one carbon homologue enol ether intermediate, copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl-(2-ethoxymethylene)-2,4,5-trifluorobenzoylacetate, (**92**).

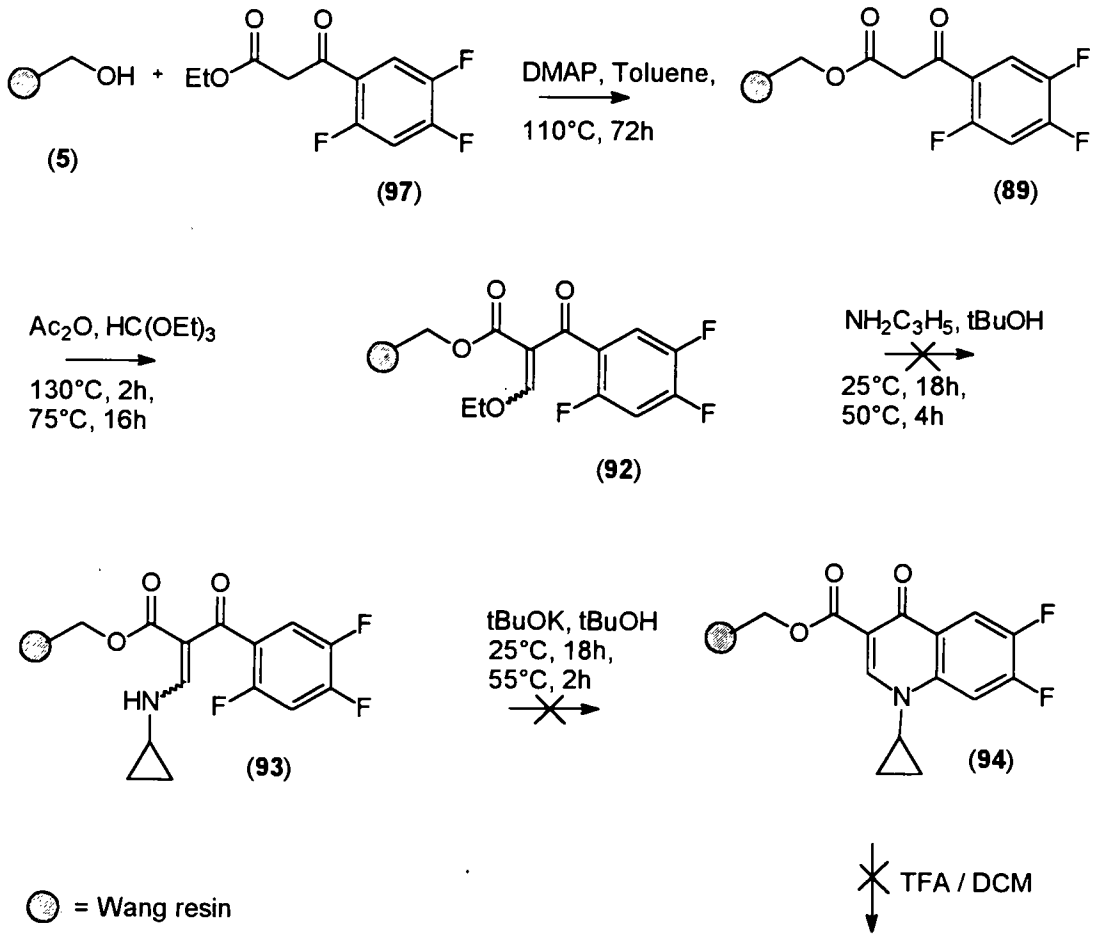


Fig. 2.18. Preliminary SPOS Studies

Subsequent displacement of ethyl ether in the resin-bound enol ether was attempted by the addition of cyclopropylamine in a solution of *t*-butanol. Following

18 hours at room temperature, the resin mixture was then heated to 50°C for a period of 4 hours. As an attempt to induce cyclisation, the predicted resin-bound β-keto enamide, copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 2-(2,4,5-trifluoro benzoyl)-3-cyclopropylaminoacrylate, (**93**) was resuspended in a slurry of potassium *t*-butoxide and *t*-butanol and stirred for 18 hours at room temperature followed by a further 2 hours at 55°C. However, attempted acid cleavage of the expected resin-bound product, copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 6,7-difluoro-4-oxo-1-cyclo-propylamino-quinoline-3-carboxylate, (**94**) confirmed no cyclisation to have taken place.

In a second attempt to prepare resin-bound Ciprofloxacin®, the resin-bound enol ether, (**92**) and enamide, (**93**) intermediates were each isolated and analysed by ^{13}C gel phase NMR. While the enol ether product was successfully identified, the "enamide intermediate" was clearly not the desired product, with the ^{13}C NMR being identical to that of the enol ether, (**92**) starting material.

This attempt was instructive in two major issues. Firstly, it highlighted the problems of using high temperatures and poor resin swelling solvents in SPOS. Secondly, it showed the importance of solution phase development of the synthetic scheme for compatibility with solid phase methodology. In light of these results, we subsequently carried out an extensive re-evaluation and optimisation study for the solution phase chemistry for the quinolones. Particular interest was focused on the use of low to ambient temperatures and non-polar aprotic solvents which swell the resin well. Such conditions are important for the development of solid phase methodology, yet both limit the reaction conditions and reagents which can be used in the solid phase synthesis of the quinolones.

2.3.1. Optimisation of Enol Ether and Enamide Chemistry

Again our studies concentrated on the cycloaracylation process using the two β-keto ester starting materials 2,4,5-trifluorobenzoylacetic acid ethyl ester, (**97**) and 2,3,4,5-tetrafluorobenzoylacetic acid ethyl ester, (**98**). For the preparation of the β-

keto esters, 2,4,5-trifluorobenzoic acid, (**95**), and the related compound, 2,3,4,5-tetrafluorobenzoic acid, (**96**) were treated with thionyl chloride to give the corresponding acid chlorides, which were directly treated with potassium ethyl malonate, anhydrous magnesium chloride and triethylamine in acetonitrile to afford, (**97**), and (**98**), respectively, (fig. 2.19).[184] On treatment of the β-keto esters with triethyl orthoformate and acetic anhydride at 130°C for 3 hours, followed by a further 10 hours at 80°C the enol ether intermediates, ethyl (2-ethoxymethylene)-2,4,5-trifluorobenzoylacetate, (**99**), and ethyl (2-ethoxymethylene)-2,3,4,5-tetrafluorobenzoylacetate, (**100**) were isolated. While this reaction gives good results in solution phase, the poor resin swelling nature of acetic anhydride was of obvious concern to us, as was the high temperature of reaction. However, the reaction was repeated and analysis of the products after only 3 hours at 130°C gave the enol ether intermediates, (**99**, and **100**) in 95 and 96% yields respectively. Both products were present as a mixture of *E* and *Z* isomers (3:1).

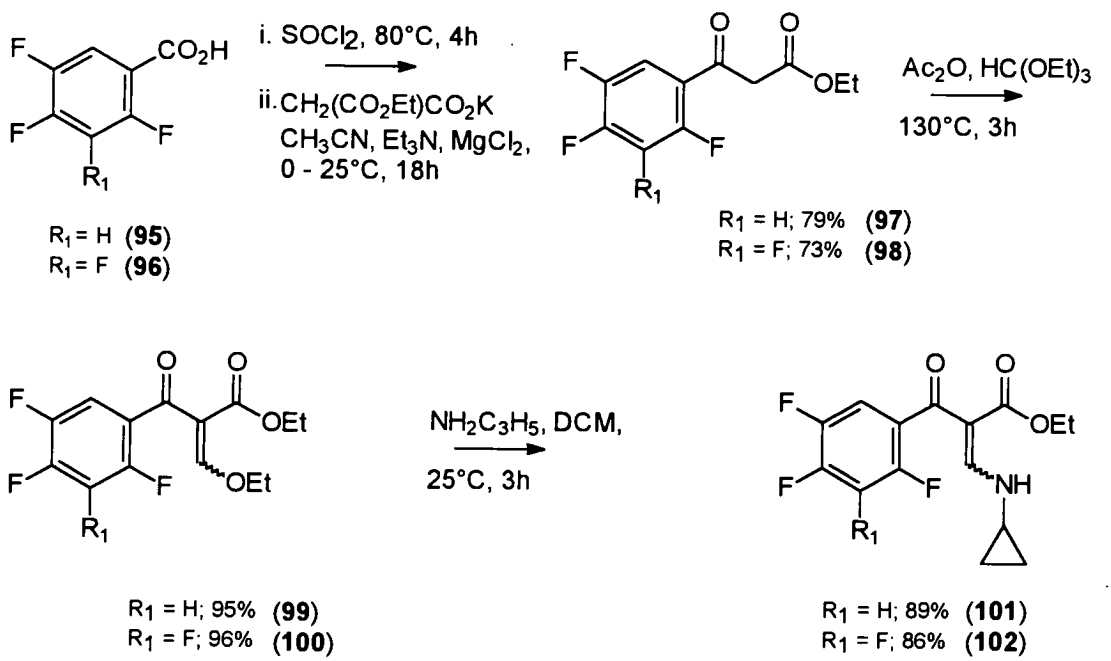


Fig. 2.19. Solution Phase Optimisation of Enol Ether and Enamide Intermediates

Preparation of the corresponding β-keto enamides, however, proved more problematic. Solid phase studies had shown the failure of the reaction was probably due to the poor resin swelling properties of *t*-butanol. Subsequently we attempted the displacement reaction with a suspension of cyclopropylamine in DCM. After only 3 hours, at 25°C, the reaction had gone to completion, and the resulting products; ethyl 3-cyclopropylamino-2-(2,4,5-trifluorobenzoyl)acrylate, (**101**), and ethyl 3-cyclopropylamino-2-(2,3,4,5-tetrafluorobenzoyl)acrylate, (**102**) were isolated in 89 and 86% yields, respectively. Again the products were present as a mixture of *E* and *Z* isomers (3:1). For an analogous reaction in the solid phase, the replacement of *t*-butanol with DCM should give improved resin swelling; hence a larger surface area of reactive beads is exposed which can undergo reaction. Also, the reaction is carried out at ambient temperatures rather than at 50°C, which would further aid the solid phase reaction.

However, despite the successful solid phase synthesis of the enol ether intermediates and the re-evaluated solution phase reaction, there was still concern with the poor resin swelling nature of acetic anhydride and the elevated temperature used in the reaction. This led us to find an alternative route which would be more favoured when applied to SPOS.

In the synthesis of the quinolones, the β-keto esters, have an active methylene bridge situated between the dicarbonyl moiety. Such methylene units are known to react with formamide acetals under mild conditions.[191] They are also known to be useful in the synthesis of enamines.[192,193] Recently, Wentland *et al.* [194] have reported the preparation of β-keto enamide intermediates, *via* an analogous reaction to that shown in (fig. 2.20). For our work, the enamide intermediate, (**103**), was prepared.

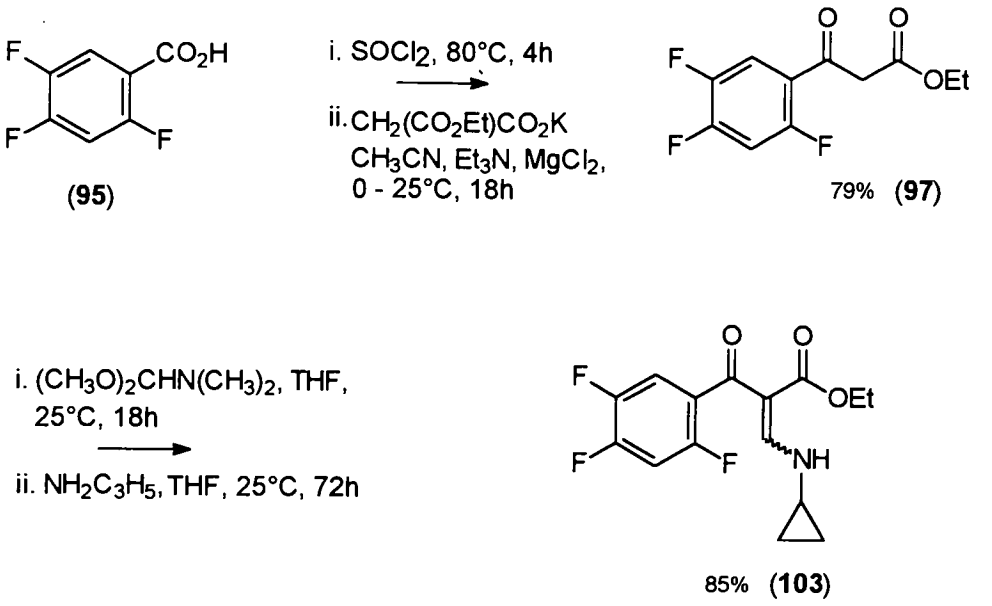


Fig. 2.20. Alternative Enamide Synthesis

Initially, the β-keto ester, (**97**) was dissolved in a solution of anhydrous THF and dimethylformamide dimethyl acetal added. Following agitation for 18 hours at room temperature, 2 equivalents of cyclopropylamine was added and the reaction mixture was stirred for a further 72 hours, at 25°C, with the resulting enamine identified as the desired product, (**103**), which was present as a mixture of *E* and *Z* isomers in 85 % yield.

Compared to the traditional synthetic route shown in (fig. 2.19), the alternative enamide method introduces more compatible conditions for the analogous solid phase methodology. The reaction is carried out at ambient temperatures, thus avoiding problems inherent in the use of elevated temperatures with a solid support. Furthermore, the use of THF instead of acetic anhydride gives improved swelling of the polystyrene solid support and therefore enhances the reaction kinetics. This has subsequently been confirmed by an analogous reaction with the resin-bound β-keto ester, (see section 2.4).

2.3.2. Optimisation of Base Cyclisation

Re-evaluation of the solution phase synthesis has successfully optimised the first three steps in the synthesis of the quinolones using reaction conditions which are compatible with SPOS. It was also necessary to re-evaluate the solution phase chemistry of the base cyclisation step, which to date has proved the most problematic. Generally, the most favourable conditions for ring closure are the use of potassium *t*-butoxide in a solution of either *t*-butanol or DMSO.[195] Early solid phase studies confirmed the failure of both these reaction conditions when the reaction was attempted using the traditional synthetic methodology. On replacement of *t*-butanol with DMSO, it was hoped that the good swelling properties of DMSO would improve the solid phase reaction. Also, the use of a slurry of potassium *t*-butoxide in DMSO yields a more basic system than in *t*-butanol;[196] however, both *t*-butanol and DMSO mixtures give heterogeneous slurries which are not suitable for solid phase methodology.

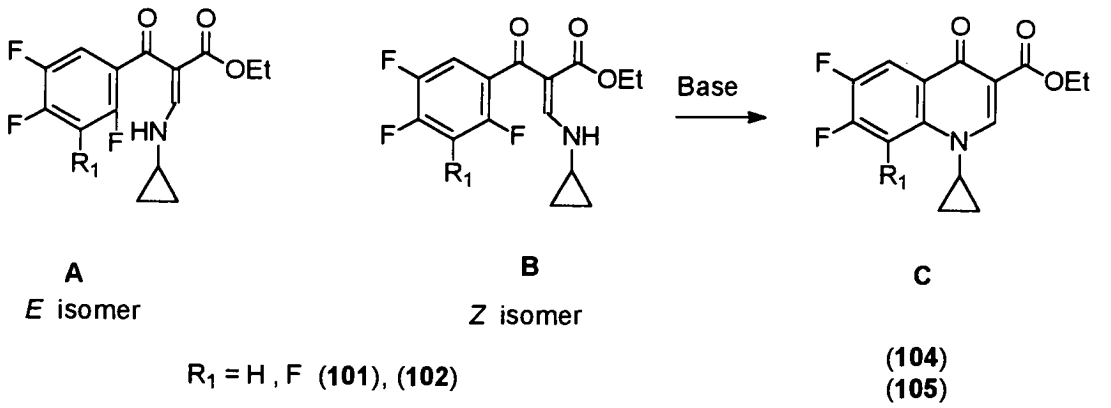


Fig. 2.21. Base Cyclisation

A systematic study of various bases and reaction conditions, seventeen in total, was executed whilst focusing on conditions compatible with SPOS, (see table 2.1). Cyclisation of, **(101)**, and concomitant elimination of hydrogen fluoride (HF) proved dependent upon equilibration of the *E* and *Z* isomers of the enamide

intermediates, (fig. 2.21). Incomplete cyclisation was monitored by NMR studies, which indicated a mixture of two products; namely, the desired quinolone, ethyl 1-cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylate, (**104**) and the *Z* isomer, (B), obtained in a (3:1) ratio respectively. In an analogous reaction, cyclisation of ethyl 3-cyclopropylamino-2-(2,3,4,5-tetrafluorobenzoyl)acrylate, (**102**) gave similar results. For solution phase chemistry, a mixture of products can easily be separated by chromatographic methods, but incomplete cyclisation of the resin-bound enamine is not satisfactory as this would lead to the build-up of resin-bound impurities.

Table 2.1. Optimisation of Base Cyclisation Studies

(See Fig. 2.21)

Entry	Conditions	A	B	C
1	**KOtBu / tBuOH, 25°C, 16h + 55°C, 2h**			✓
2	KOtBu / THF, 25°C, 16h + 55°C, 2h	✓	✓	✓
3	KOtBu / THF, 50°C, 16h	✓	✓	
4	KOtBu / DMSO, 25°C, 16h + 55°C, 2h		✓	✓
5	Et_3N / DMSO, 25°C, 16h	✓	✓	
6	Et_3N / DCM, reflux, 16h	✓	✓	
7	DBU / DCM, reflux, 16h		✓	✓
8	DBU / DCM, reflux, 24h		✓	✓
9	DBU / $CHCl_3$, reflux, 16h	✓	✓	✓
10	DBU / THF, 25°C, 16h	✓	✓	
11	DIMSYL Na / DMSO, 70°C, 16h	✓	✓	
12	DIMSYL Na / DMSO, 25°C, 16h	✓	✓	✓
13	TMG / DCM, 25°C, 16h		✓	✓
14	**TMG / DCM, 55°C, 16h**			✓
15	TMG / THF, 25°C, 16h	✓	✓	✓
16	**NaH / THF, 66°C, 4h**			✓
17	LDA / THF, -78° to 25°C, 16h	✓	✓	

Ultimately, two of the seventeen reactions studied in solution phase were suitable for later evaluation on a solid phase support. The organic base, 1,1,3,3-tetramethylguanidine (TMG) in DCM at reflux for 16 hours, (entry 14), gave the pure cyclised quinolones in 80% and 76% yields respectively. Several studies have also been carried out using TMG at reduced temperatures and timescales, with a mixture of products, both cyclised quinolone and *Z* isomer observed. Although complete formation of the quinolone ring system proceeded with sodium hydride in THF at reflux for 4 hours, (entry 16), the physical manipulation of reactions in parallel using sodium hydride would prove unmanageable.

In a similar reaction, the solution phase synthesis of ethyl 1-[4-fluorophenyl]-6,7,8-trifluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylate, (**107**) was successfully carried out, which further confirmed the efficiency of the cyclisation step, (fig. 2.22). Due to the strong electron withdrawing power of the 4-fluorophenyl group in the enamide, ethyl 3-[4-fluoroanilino]-2-(2,3,4,5-tetrafluorobenzoyl)acrylate, (**106**) there was concern that such an electronegative effect would reduce the basicity of the nitrogen and hence that the TMG / DCM reaction mixture would not induce cyclisation. Nonetheless, the desired product was isolated in 85% yield as a bright orange powder.

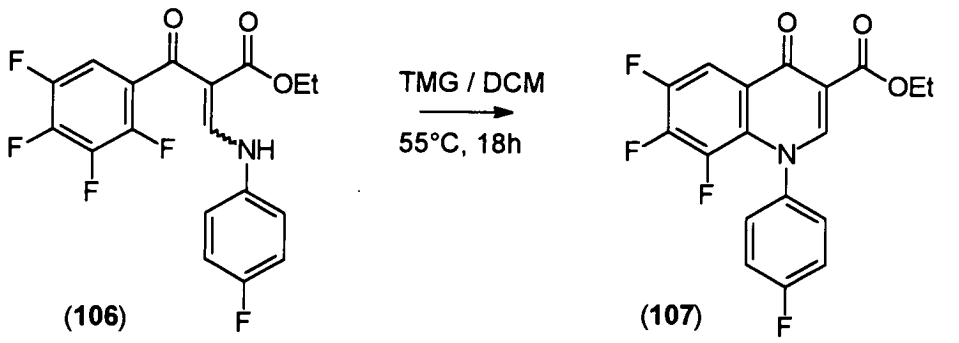


Fig. 2.22. Synthesis of Ethyl 1-(4-Fluorophenyl)-6,7,8-trifluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylate

2.3.3. X-ray analysis of the Quinolones

The quinolones, (**104**) and (**105**) were analysed by the standard techniques. Furthermore, confirmatory X-ray studies were also performed following crystallisation of the products from chloroform. These crystal structures have not been previously reported either in Chemical Abstracts or in the Crystallographic database[197] and analysis of the esters shows that the molecules are planar with the cyclopropyl ring at approximately 45° to the plane of the molecule. An interesting feature is the complexation of ethyl 1-cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylate, (**104**), with chloroform, at a distance of 3.0Å between fluorine at position seven of the quinolone ring, and chlorine at position two in chloroform, (table 2.2 and fig. 2.23a). This is clearly not present in the related molecule, ethyl 1-cyclopropyl-6,7,8-trifluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylate, (**105**), (table 2.3 and fig. 2.24a). On the other hand, the stacking arrangement of both crystals, reveals that the molecules form an antiparallel alignment with adjacent molecules, (fig. 2.23b, and fig 2.24b). It is widely held that this is analogous to the conformation adopted by the corresponding acids in the bacterial cell, as shown in (fig. 1.26).[132]

Table 2.2. Crystallographic Information of the Ethyl 1-Cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylate / Chloroform Complex, (104)

(See Figs. 2.23a & b)

Empirical formula	$C_{16}H_{14}Cl_3F_2NO_3$
Formula weight	412.63
Crystal system	Orthorhombic
Crystal size	0.82 x 0.31 x 0.23 [mm]
Cell dimensions a [Å]	6.7432 (8)
Cell dimensions b [Å]	14.760 (3)
Cell dimensions c [Å]	17.595 (3)
Cell dimensions α [°]	90.00
Cell dimensions β [°]	90.00
Cell dimensions γ [°]	90.00
Density	1.565 [g/cm^3]
Z	4

Table 2.3. Crystallographic Information of Ethyl 1-Cyclopropyl-6,7,8-trifluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylate, (105)

(See Figs. 2.24a & b)

Empirical formula	$C_{15}H_{12}F_3NO_3$
Formula weight	311.26
Crystal system	triclinic
Crystal size	0.45 x 0.15 x 0.10 [mm]
Cell dimensions a [Å]	7.830 (3)
Cell dimensions b [Å]	9.601 (5)
Cell dimensions c [Å]	10.169 (5)
Cell dimensions α [°]	62.92 (3)
Cell dimensions β [°]	82.17 (3)
Cell dimensions γ [°]	89.23 (3)
Density	1.535[g/cm^3]
Z	2

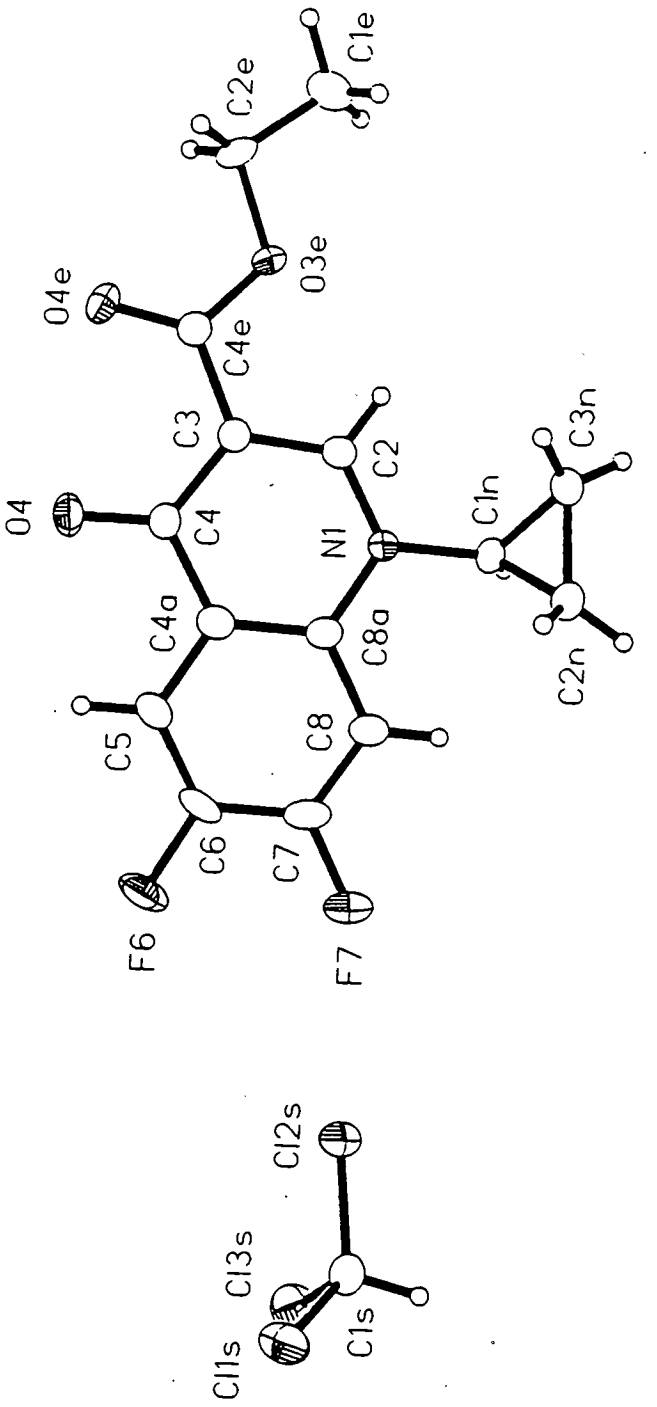


Fig. 2.23a. X-ray Structure of the Ethyl 1-Cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylate / Chloroform Complex

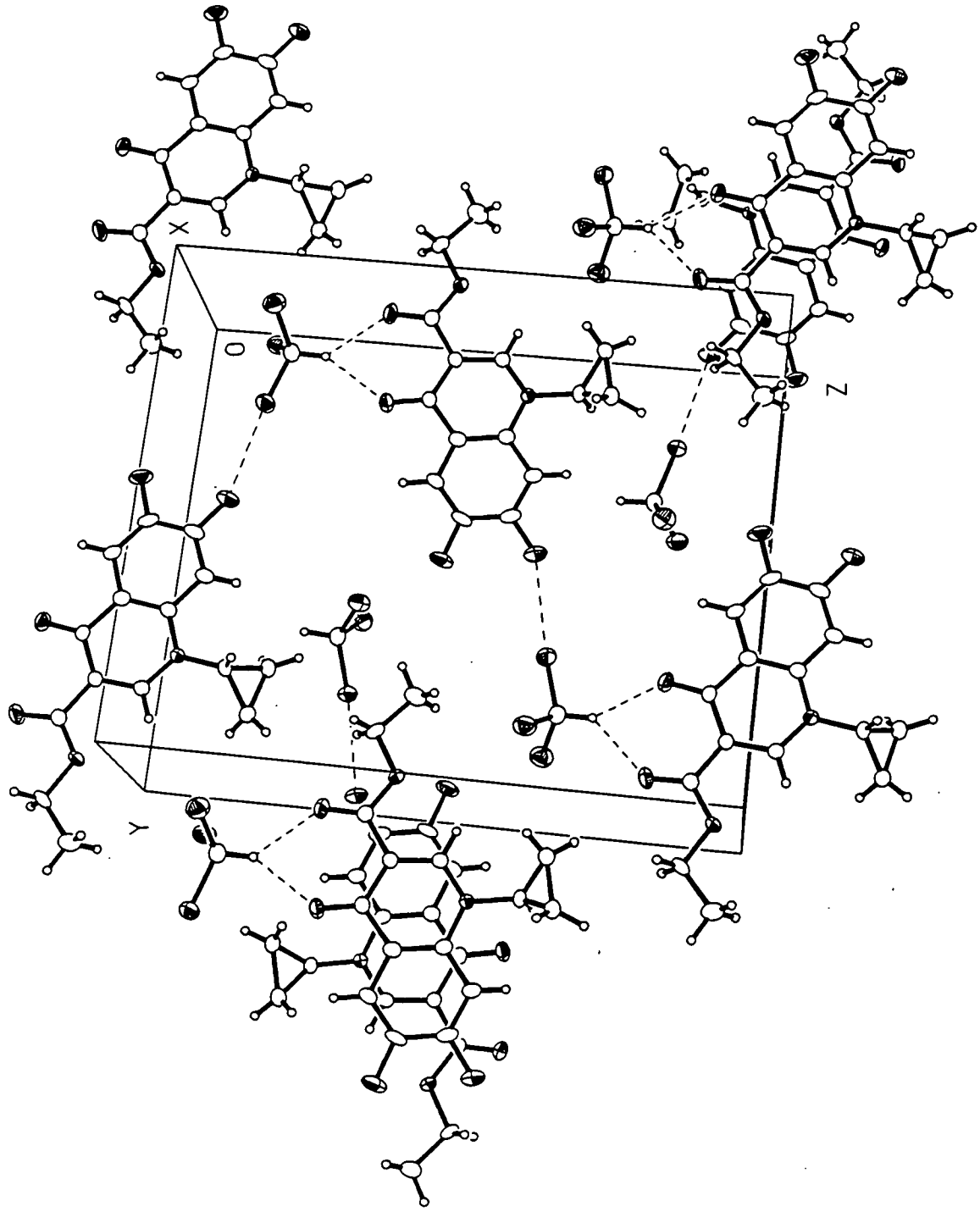


Fig. 2.23b. X-ray Stacking Arrangement of the Ethyl 1-Cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylate / Chloroform Complex

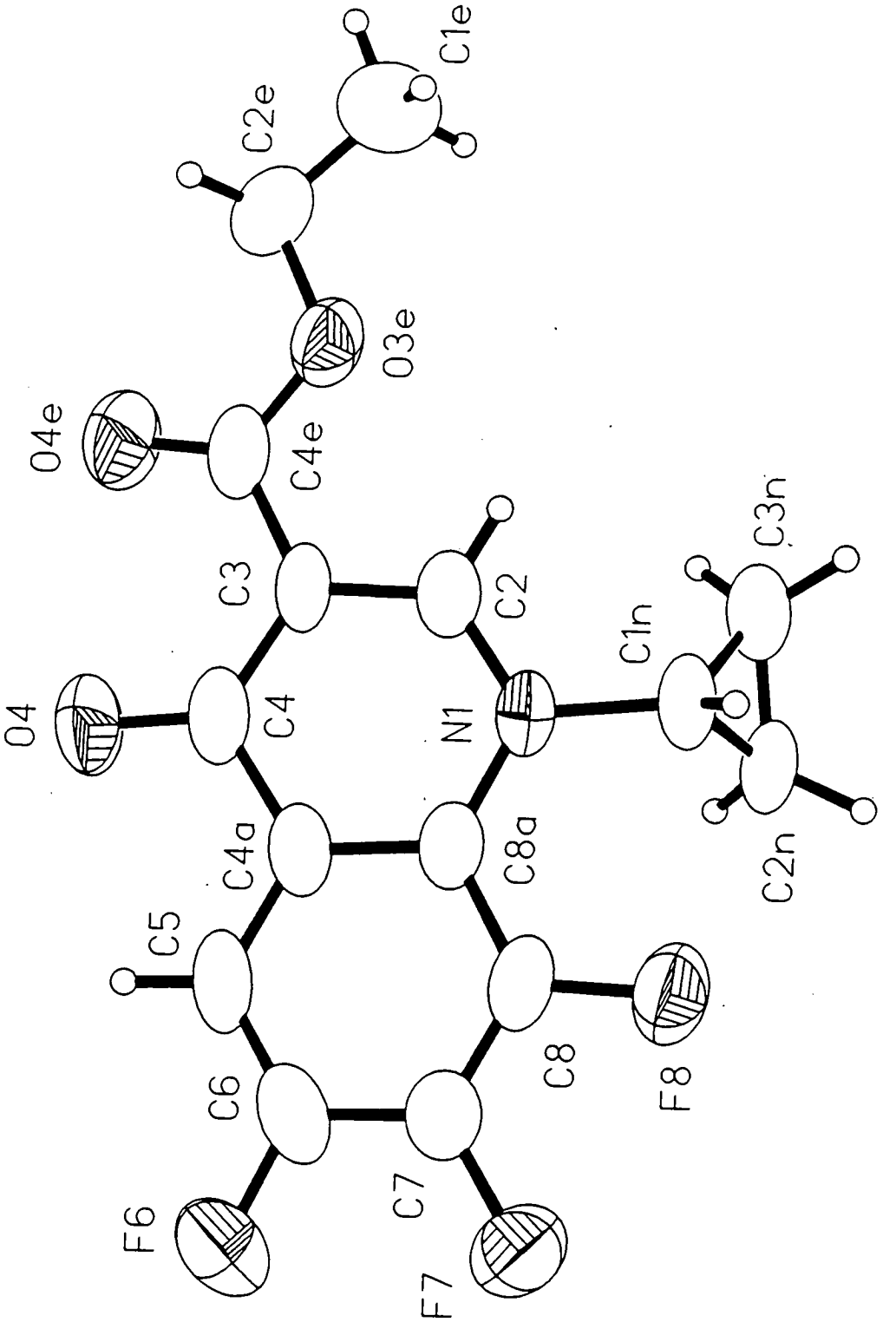


Fig. 2.24a. X-ray Structure of Ethyl 1-Cyclopropyl-6,7,8-trifluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylate

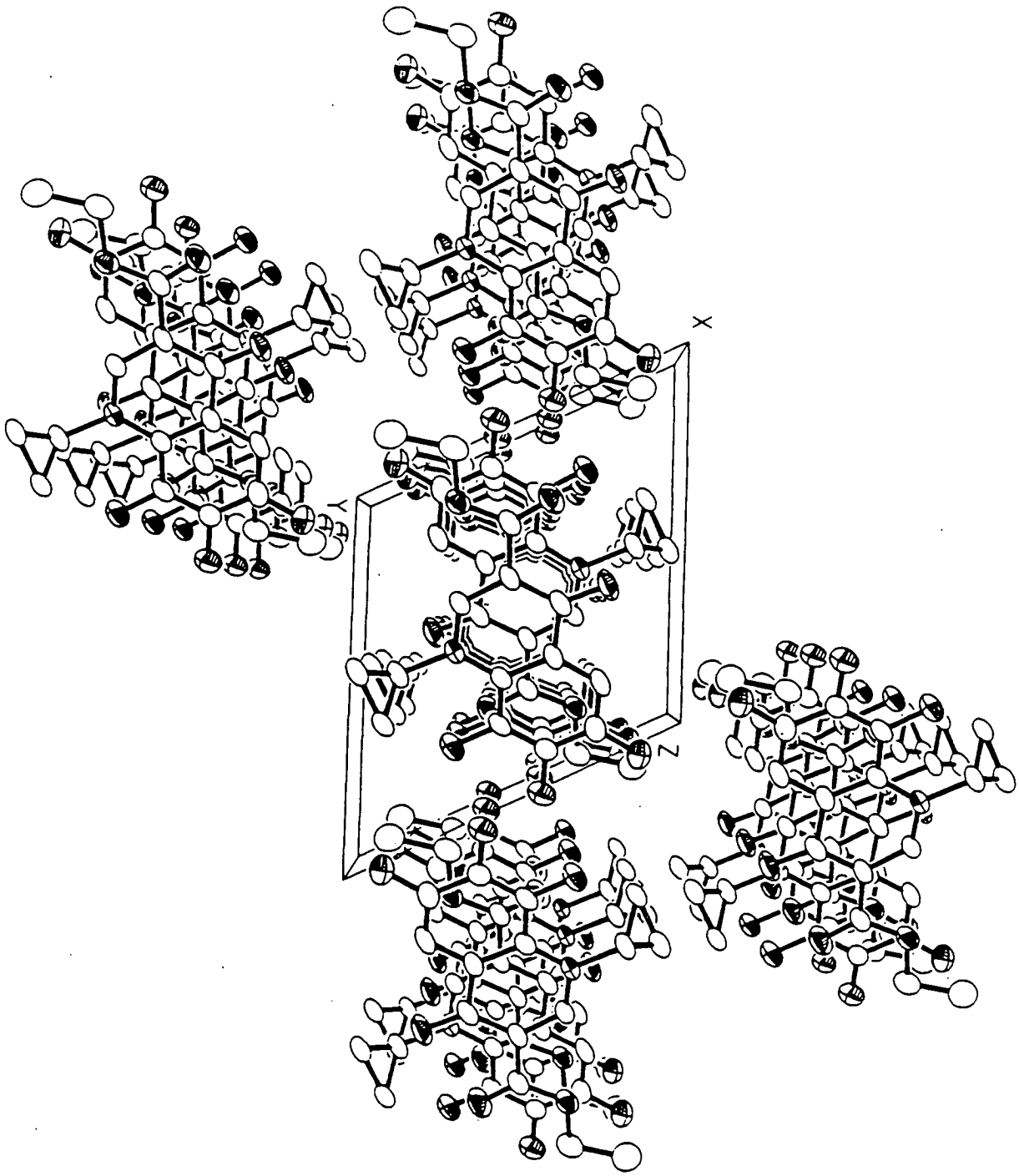


Fig. 2.24b. X-ray Stacking Arrangement of Ethyl 1-Cyclopropyl-6,7,8-trifluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylate

2.3.4. Optimisation of Arylation

In the traditional solution phase cycloaracylation route, the final step in the preparation of the quinolones involves nucleophilic aromatic substitution in the presence of the carboxylic acid moiety at C-3. However, in our SPOS route, this reaction must occur in the presence of the resin-bound ester, (fig. 2.25).

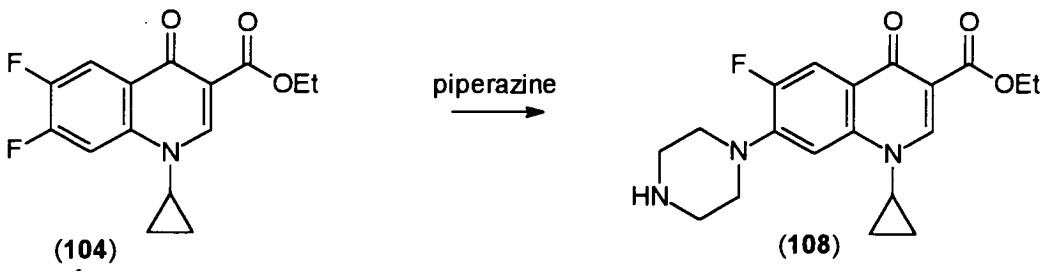


Fig. 2.25. Optimisation of Aromatic Nucleophilic Substitution

Therefore an extensive study of the solution phase chemistry was undertaken to elucidate reaction conditions compatible to SPOS, (table 2.4). In total, 7 reactions were attempted. The desired product, ethyl 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-quinoline-3-carboxylate, (**108**), was obtained by refluxing the ester, (**104**) with piperazine in pyridine for 48 hours (entry 6). However, the long reaction time was of concern, and it was found that heating the quinolone in 1-methyl-2-pyrrolidinone (NMP) for only 4 hours, (entry 7), gave the product in 89 % yield. Later work confirmed the reaction to have gone to completion after only 2 hours. Additionally, no amide product in either case was observed. Base hydrolysis of (**108**) in aqueous sodium hydroxide afforded the required product, Ciprofloxacin®.

Table 2.4. Aromatic Nucleophilic Substitution Studies

(See Fig. 2.25)

Entry	Conditions	Results
1	DMF, 110°C, 3h	NR
2	DMF, 25°C, 16h	NR
3	Dioxane, 100°C, 24h	NR
4	DCM / TMG, 55°C, 60h	NR
5	DMSO, 150°C, 20h	NR
6	**Pyridine, 115°C, 48h**	**108**
7	**NMP, 110°C, 4h**	**108**

• 2.4. SPOS of Ciprofloxacin®

The previous sections have discussed the development of the solution phase chemistry for the preparation of the quinolones, and its compatibility to solid phase chemistry. Polystyrene derivatised Wang resin has been successfully used as the solid support for the SPOS of the quinolones, however Tentagel® resins were also investigated as possible solid supports. Tentagel® resins are generally known to exhibit good swelling properties in both aqueous and organic solvents,[43] and thus can be useful for carrying out reactions which are limited to aqueous conditions. However, our work has shown that Tentagel® resins are mechanically unstable to both sonication and magnetic agitation. This was confirmed by transesterification of 2,4,5-trifluorobenzoylacetic acid ethyl ester, (**97**), onto Tentagel® resin, whereby analysis of the resin-bound product by ^{13}C NMR revealed breakdown of the Tentagel® solid support.

The development of a new route to the quinolones has not been a trivial task. Our early work proved unsatisfactory for the solid phase preparation of the quinolones and illustrated the need for re-evaluation of the traditional synthetic methodology in order to attempt such reactions on a polystyrene solid support. With a possible resin linkage obtained via ring (A), and the solution phase methodology fully optimised for the synthesis of the quinolones, we attempted the solid phase synthesis of Ciprofloxacin®, (fig. 2.26) and some related quinolones, (fig. 2.27).

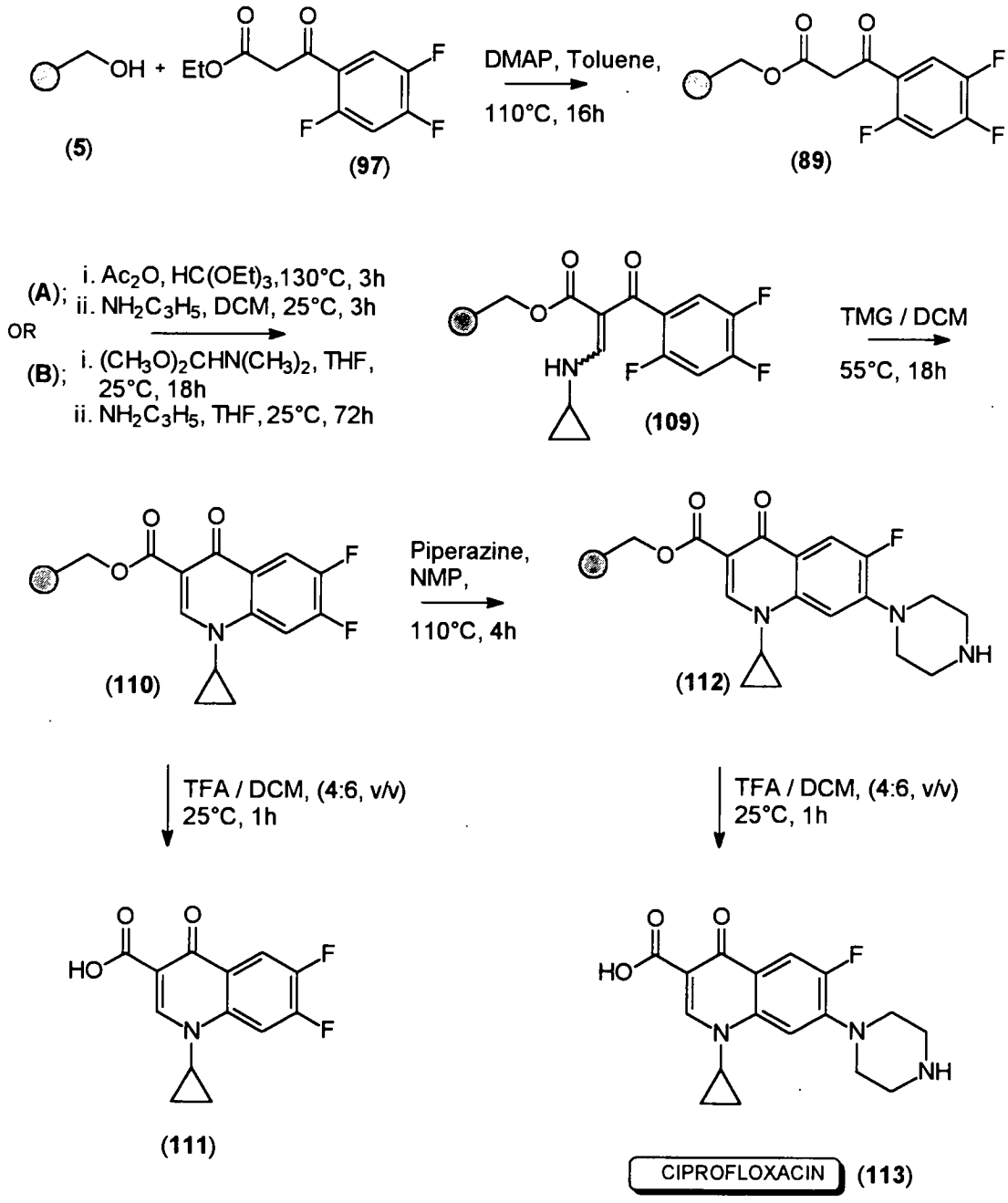


Fig. 2.26. Solid Phase Synthesis of Ciprofloxacin®

Transesterification was carried out as discussed in section 1. The reaction required refluxing at 110°C, for 72 hours, however this caused some concern regarding the possibility of resin bead breakdown. Subsequently, we studied different solvents and reaction conditions, (Table 2.5), but on analysis of the resin-bound products, only unreacted Wang resin, (**5**) was identified. This led us to return to the

original toluene solution and study the reaction over the given timescale by ^{13}C gel phase NMR. To our surprise the product, copoly(styrene-1%-divinylbenzene)*p*-benzyl 2,4,5-trifluorobenzoylacetate, (**89**), had formed after only 16 hours, and thus eliminated any fears we had with regard to resin bead breakdown at the elevated temperature of 110°C. Using the DIVERSOMER™ apparatus, a (4 x 1) array-transesterification reaction was set up using Wang resin and each of the following β-keto esters: diethyl malonate,di-*t*-butyl malonate, 2,4,5-trifluorobenzoylacetic acid ethyl ester, and 2,3,4,5-tetrafluorobenzoylacetic acid ethyl ester.

Table 2.5. Optimisation of Transesterification Conditions

Entry	Transesterification Conditions	Results
1	DMF / DMAP, 154°C, 72h	NR
2	Pyridine, 110°C, 72h	NR
3	Dioxane, 100°C, 72h	NR
4	**Toluene / DMAP, 110°C, 72h**	**Product**
5	Benzene / DMAP, 80°C, 72h	NR
6	Pentan-3-one / DMAP, 110°C, 72h	NR
7	**Toluene / DMAP, 110°C, 16h**	**Product**

The resin-bound enamide, copoly(styrene-1%-divinylbenzene)*p*-benzyl 2-(2,4,5-trifluorobenzoyl)-3-cyclopropylaminoacrylate, (**109**), was prepared by two alternative routes. Route (B) involved activation of the resin-bound β-keto ester, (**89**), with dimethylformamide dimethyl acetal *in situ*, followed by the addition of an excess of cyclopropylamine.[194] The good swelling properties of THF, and the ambient temperatures used, both proved successful in the reaction in comparison to the conditions used in route (A), (fig. 2.26).

Cyclisation of, (**109**), in TMG / DCM at reflux, for 18 hours provided the resin-bound quinolone, copoly(styrene-1%-divinylbenzene)*p*-benzyl 6,7-difluoro-1-cyclopropyl-quinoline-3-carboxylate, (**110**). In addition to ^{13}C NMR analysis, further

confirmation of the reaction was achieved by treatment of (**110**) with 40% TFA in DCM and analysis of the filtrates by both ^{1}H NMR and MS. Post-cleavage purification was carried out by silica gel chromatography to liberate the product, 1-cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid, (**111**), in approximately 56% yield.

The remaining resin-bound cyclised quinolone, (**110**) was subsequently arylated with a solution of piperazine in NMP. After 4 hours at 110°C, the presence of resin-bound Ciprofloxacin®, (**112**) was confirmed by ^{13}C NMR. Treatment of (**112**) under resin cleavage conditions confirmed the isolated product as Ciprofloxacin®, (**113**). Following purification by chromatography, Ciprofloxacin® was isolated in 61% yield. The purity was quantitatively determined to be >95 % by ^{1}H NMR in combination with acetonitrile as an internal standard (ISTD).

Additionally, the following quinolones were prepared using the methodology outlined in (fig.2.26). The resulting resin-bound quinolones were characterised by gel phase ^{13}C NMR. Analysis of the cleaved products are pending, (fig. 2.27).

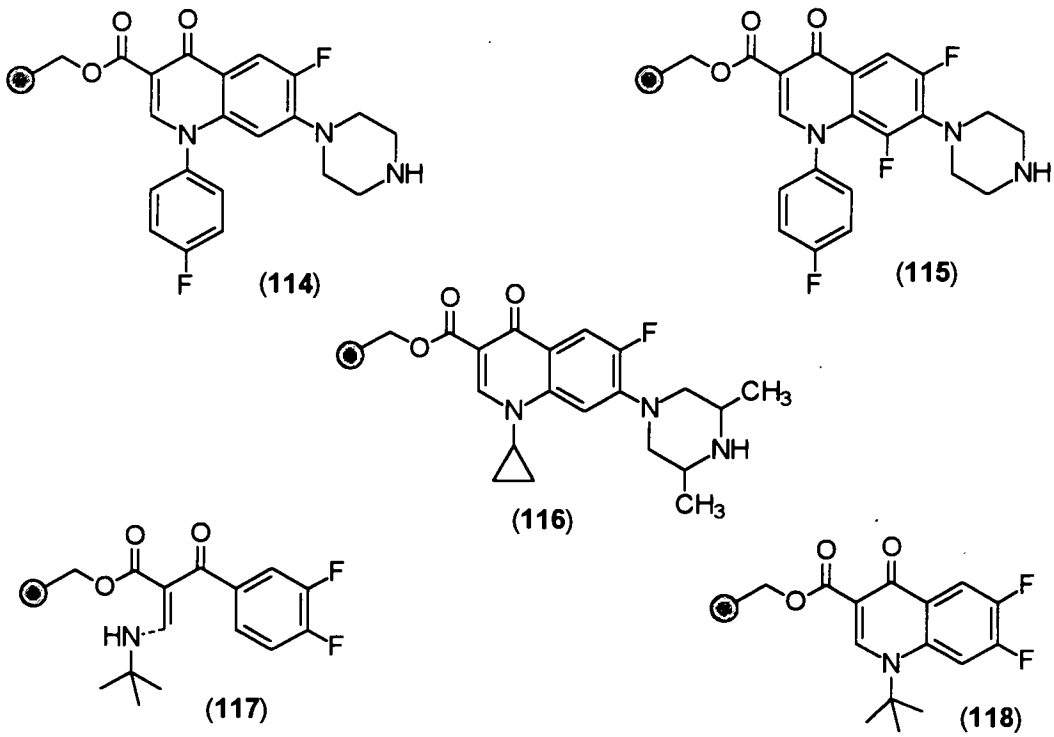


Fig. 2.27. SPOS of Quinolones and Related Compounds

2.4.1. Cleavage Studies

The powerful electron donating *p*-alkoxy substituent of Wang resin[34] enhances the acid senstivity of the ester linkage in the resin-bound synthesis of the quinolones. Early workers in the field of SPS used a combination of IR spectroscopy and cleavage of the resin-bound products for characterisation of the solid phase products. Both these methods are still in use and proved highly efficient for the SPOS of the quinolones. However, during the synthesis of the quinolones, acid cleavage of the resin-bound β-keto ester, enol ether and enamide intermediates proved to be unsuccessful due to the acid sensitivity of the products. Subsequently, ^{13}C gel phase NMR played a key role in the analysis of all resin-bound products in the quinolone synthesis. In addition to ^{13}C NMR analysis of the resin-bound quinolones, both these products were successfully cleaved by acid and characterised. In fact, the quinolones are soluble only in TFA.

Table 2.6. Acid Cleavage Studies to Produce Ciprofloxacin®

Entry	Cleavage Conditions	Results
1	TFA / H_2O, (95%), 25°C, 18h	Product
2	TFA / H_2O, (70%), 25°C, 2h	Product
3	TFA / H_2O, (50%), 25°C, 2h	Product
4	TFA / DCM, (50%), 25°C, 16h	Product
5	**TFA / DCM, (40%), 25°C, 1h**	**Product**
6	IM TMSBr / TFA, 0°C, 0.25h	NR

Table 2.6 illustrates a range of acid cleavage conditions investigated in order to optimise the product yield of the cleaved Ciprofloxacin®. Our early work used concentrations of 95% TFA, however, analysis obtained after cleavage of the solution revealed a large percentage of impurities present, as well as the desired product. Later we reduced the actual concentration of the acid, whilst at the same time replacing water with DCM, to improve resin swelling during the cleavage stage. The optimium

conditions were finally obtained with 40% TFA in DCM for only one hour, at room temperature, (entry 5). Further attempts utilized 1M TMSBr in TFA at 0°C, for 15 minutes, (entry 6). However this route did not afford any of the desired quinolone.

While the TFA cleavage conditions gave promising results, there were still residual impurities present in the ^{1}H NMR spectrum of the cleaved quinolones, even with concentrations as low as 40% TFA. Initially, we concluded these had arisen from the cleavage of some resin-bound intermediates formed during the synthesis of the quinolones. However, none of the impurities could be identified by TLC and UV studies, thus suggesting that they were not derived from resin-bound intermediates.

Subsequently, we investigated the possibility that the impurities arose from the Wang resin. In an attempt to eliminate these possible resin impurities, Wang resin was pre-treated with the cleavage conditions (40% TFA in DCM) prior to synthetic use, (fig. 2.28). Analysis of the treated Wang resin by IR confirmed that esterification in the presence of TFA had occurred. When we subsequently attempted to use the pre-treated resin for the SPOS of the quinolones, the reaction proved unsuccessful.

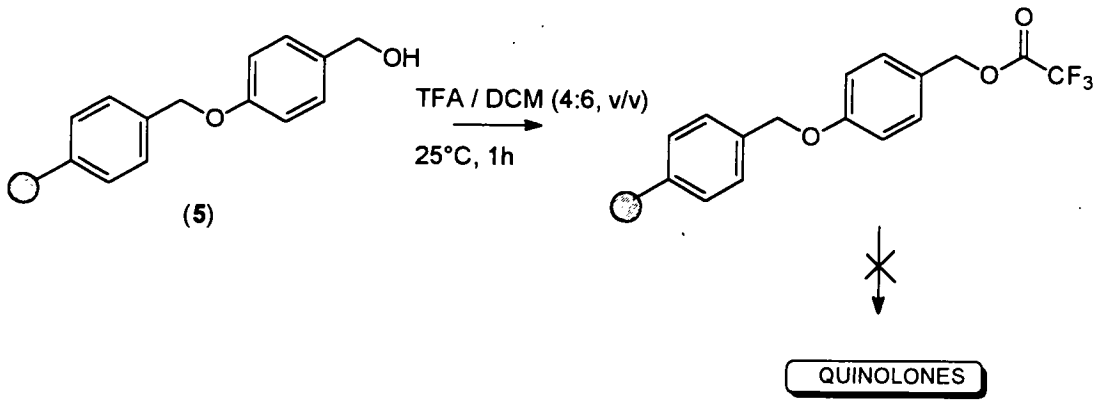


Fig. 2.28. Pre-treatment of Wang Resin with the Cleavage Conditions

The acidic filtrate from the pre-treated Wang resin was also examined. Following concentration of the sample, the resulting oil was submitted for ^{1}H NMR analysis, which confirmed that the impurities in the cleaved Ciprofloxacin® sample came solely from the Wang resin. In another study, three commercial samples of Wang resin, (0.6 - 0.9 mmol/g, BaChem, Fluka, and NovaBioChem), were treated with the above acidic medium for one hour, at room temperature. As with the

previous example, the BaChem sample gave the same impurities. However, the samples from both Fluka and NovaBioChem revealed DMF as the impurity. As a further possible method of eliminating resin impurities, Wang resin was sonicated in a solution of dioxane for one hour at 25°C. Again, several common impurities were observed on analysis of the filtrate.

The problem of resin impurities is undoubtedly fundamental in the development of any SPOS reaction. While our work has only touched on this issue, future work for the development of SPOS protocols should include controlled experiments designed to isolate and quantify resin degradation products and impurities.

2.4.2. Biological Results

No medicinal chemistry project is complete without the testing of potential drug candidates for biological activity. This is also true for any set of compounds synthesised using the DIVERSOMER™ technology. For our studies, which involved the SPOS of four separate samples of Ciprofloxacin®, the resin cleaved samples were assayed and the results compared with an authentic sample of Ciprofloxacin®, (Lot 5), (Table 2.7).

Table 2.7. Biological Results of Ciprofloxacin®

Lot	*Sample*	*EC-1 (μg/ml)*	*PA-7 (μg/ml)*
1	Solid Phase	0.2	1.6
2	Solid Phase	**0.1**	**0.2**
3	Solid Phase	0.3	1.1
4	Solid Phase	0.2	3.1
5	Solution Phase	0.025	0.1

All four samples were prepared in individual round-bottomed flasks and confirmed as crude products by ^{1}H NMR and / or MS, with the major impurities related to resin by-products and not intermediates in the quinolone synthesis, as discussed above.

The results for all four samples prepared using SPOS methodology clearly do not give the same biological activity as the solution phase sample, although Lot 2 is consistent with known results. It must also be noted that from the literature Ciprofloxacin® gives widely varying results, ranging from 0.2 to 0.8 μg/ml for PA-7. While these results were initially disappointing, they demonstrated four mains considerations for the SPOS of the quinolone:

i; the need for purification;
ii; the need for quantification of contained product;
iii; the confirmation of product identity in combination with NMR / MS;
iv; the need to correlate light and temperature sensitivity with product purities.

The results obtained from our research have resulted in a new synthetic route to the quinolone antibacterial agents. The successful re-evaluation of the solution phase chemistry has demonstrated the need for reagents and reaction conditions which are compatibile with SPOS. Resin impurities have been and continue to be a major problem in the purification of the final products.

• 2.5. Synthesis of Quinolone Antibiotics by DIVERSOMER™ technology

Following the successful SPOS of Ciprofloxacin® and some related quinolones, the DIVERSOMER™ technology employing robotic automation and proprietary equipment was implemented with the SPOS route in (fig. 2.26) for the preparation of a library of 40 quinolones. The generation of a DIVERSOMER™ library involves a complex set of methodologies that range from planning the synthetic reaction scheme, to building compound libraries and finally interpreting the results. All of these methods require the use of Microsoft® Excel Spreadsheets which help in data handling and tracking for the generation and storage of large volumes of information. One such system has been developed by MDL Information Systems Inc, which has helped in this crucial planning stage of the library design, by providing a series of databases for new structure creation, storage, and the ability to manage large volumes of data associated with the library generation.

For the parallel synthesis of 40 quinolones incorporating three sites of diversity, (R_1, R_2 and R_3) over a six step synthesis, the information generated and required can be both immense, and mentally draining, in terms of the amount of paperwork produced. However, with the use of Project Library, a "master" spreadsheet to designate and organise the final compounds which are generated in the DIVERSOMER™ array was set up as shown in (fig. 2.29). Initially, a single generic structure for the quinolone library was built up, followed by the thirteen building blocks associated with the library generation. In addition, to the building blocks, other reagents in the synthetic scheme are fed into the programme for later use in the reaction sequence handling. Following this, the library of 40 quinolones was automatically formatted, with any errors in the manipulation easily identified by Project Library.

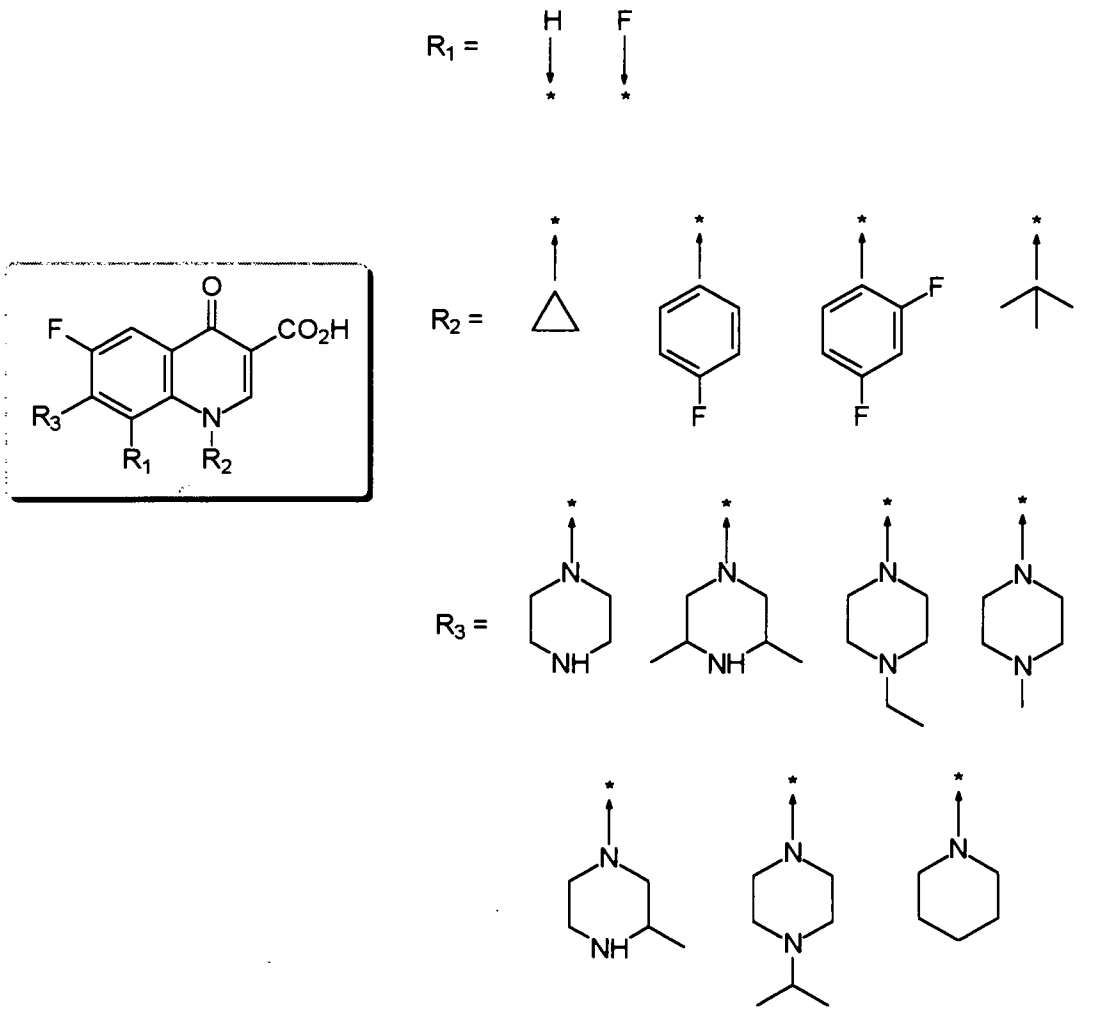


Fig. 2.29. Quinolone Generic Structure and Library

Subsequently, for each discrete structure generated in the library, (1-40), the molecular formula and weight are automatically calculated, with only the notebook number, type and product identifier being fed into the programme manually, (fig. 2.30). Additional spreadsheets are set up for calculating reaction yields, reaction conditions and reagent concentrations. Furthermore, the Chemical and Biological Information (CBI) file can be accessed easily, and for the 40 quinolones generated in the array, 17 were identified as new to the Parke-Davis archive, (Table 2.8).

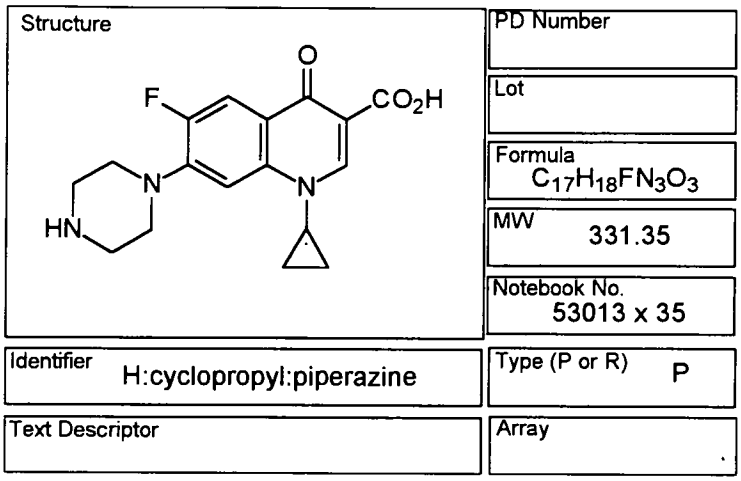


Fig 2.30. Library Information Programme

The reaction scheme for the synthesis of 40 quinolones, outlined in (fig. 2.31), involved the use of a 40 (4 x 10) array format labelled, A1-J4, corresponding to quinolones 1 to 40 respectively. The first site of diversity (R_1) was introduced during the transesterification reaction (R1S1). In 12 of the 40 reactions, R_1 = H, (A1-C4) and in the remaining 28, R_1 = F, (D1-J4). Activation of all 40 resin bound β-keto esters with dimethylformamide diemethyl acetal, (R2S1) was followed by the *in situ* addition of four separate amines (R_2); (R2S2), cyclopropylamine, 4-fluoroaniline, 2,4,-difluoroaniline, and *t*-butylamine, in the arrangement (A1-J1) to (A4-J4) respectively. Following TMG cyclisation (R3S1), of all 40 resin-bound enamides, the final site of diversity (R_3), was introduced by arylation (R4S1) with a range of seven piperazinyl derivatives; (A1-C4) and (D1-F4) were each suspended in a solution of piperazine, 2,6-dimethylpiperazine and 1-ethylpiperazine respectively, while the remaining four reagents; 1-methylpiperazine, 2-methylpiperazine, 1-isopropylpiperazine and piperidine were added to the reaction wells (G1-J4) respectively. Finally all 40 of the resin-bound quinolones were cleaved (R5S1) in 40% TFA to generate 40 discrete, soluble, crude compounds which were identified by TLC.

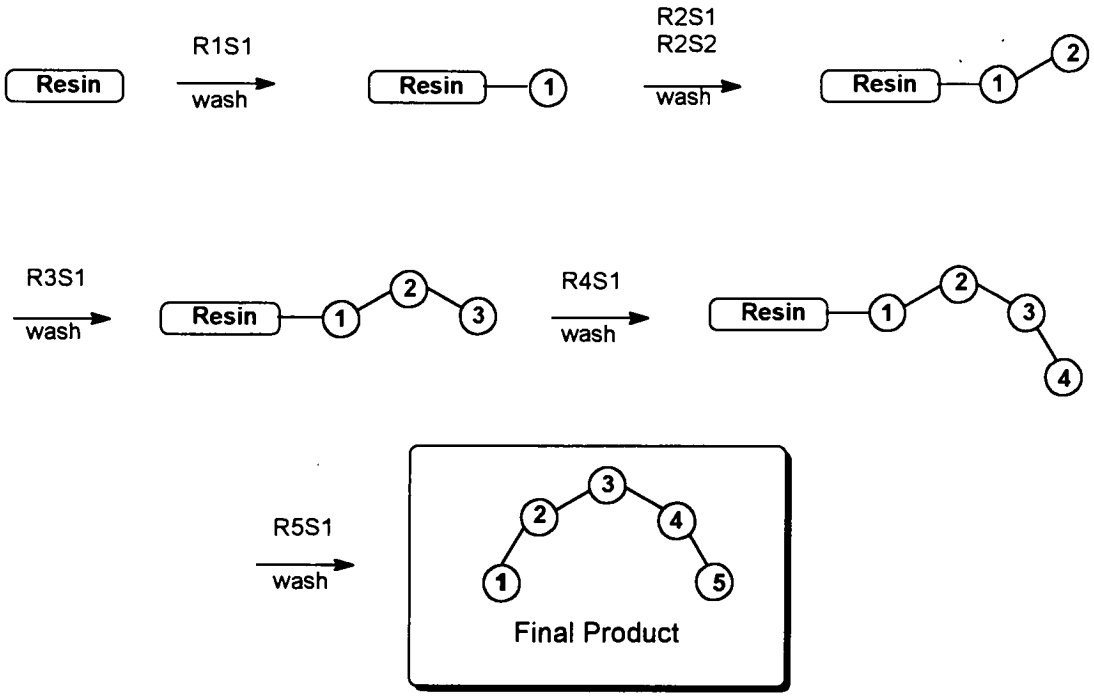


Fig. 2.31. Reaction Scheme Format

In an attempt to remove resin by-products, automated parallel purification, by gradient elution of pre-packed chromatography cartridges, and solid phase extraction (SPE) methods were developed and implemented. In total 10 sets of fractions (400 samples) were analysed by automated TLC methods, combined robotically and concentrated to afford an average of 26% yield (9.2 mg), based upon loading of commercial polystyrene Wang resin. Two of the samples, B1 and J3 were contaminated with residual *t*-butylamine from the eluting solvent mixture.

Table 2.8. Quinolone Product Yields

Well	R_1	R_2	R_3	Yield mg	Yield %
A1	H	cyclopropyl	piperazine	17.0	61
A2	H	4-fluorophenyl	piperazine	5.0	15
A3	H	2,4-difluorophenyl	piperazine	6.1	18
A4	H	t-butyl	piperazine	7.2	25
B1	H	cyclopropyl	2,6-dimethylpiperazine	27.1	90*
B2	H	4-fluorophenyl	2,6-dimethylpiperazine	8.2	24
B3	H	2,4-difluorophenyl	2,6-dimethylpiperazine	2.7	7
B4	H	t-butyl	2,6-dimethylpiperazine	3.0	10
C1	H	cyclopropyl	1-ethylpiperazine	18.7	62

#C2	H	4-fluorophenyl	1-ethylpiperazine	4.3	12
C3	H	2,4-difluorophenyl	1-ethylpiperazine	6.1	17
C4	H	t-butyl	1-ethylpiperazine	4.9	16
D1	F	cyclopropyl	piperazine	7.5	23
D2	F	4-fluorophenyl	piperazine	12.5	33
D3	F	2,4-difluorophenyl	piperazine	7.2	18
#D4	F	t-butyl	piperazine	6.5	19
E1	F	cyclopropyl	2,6-dimethylpiperazine	9.6	27
#E2	F	4-fluorophenyl	2,6-dimethylpiperazine	8.1	20
E3	F	2,4-difluorophenyl	2,6-dimethylpiperazine	9.7	23
#E4	F	t-butyl	2,6-dimethylpiperazine	8.4	23
F1	F	cyclopropyl	1-ethylpiperazine	14.3	41
#F2	F	4-fluorophenyl	1-ethylpiperazine	8.4	21
F3	F	2,4-difluorophenyl	1-ethylpiperazine	8.6	21
#F4	F	t-butyl	1-ethylpiperazine	6.4	17
G1	F	cyclopropyl	1-methylpiperazine	8.1	24
#G2	F	4-fluorophenyl	1-methylpiperazine	7.6	20
G3	F	2,4-difluorophenyl	1-methylpiperazine	8.4	21
#G4	F	t-butyl	1-methylpiperazine	8.5	24
H1	F	cyclopropyl	2-methylpiperazine	6.9	20
#H2	F	4-fluorophenyl	2-methylpiperazine	4.2	11
H3	F	2,4-difluorophenyl	2-methylpiperazine	7.8	19
#H4	F	t-butyl	2-methylpiperazine	8.8	25
I1	F	cyclopropyl	isopropylpiperazine	6.8	19
#I2	F	4-fluorophenyl	1-isopropylpiperazine	10.1	24
#I3	F	2,4-difluorophenyl	1-isopropylpiperazine	3.6	8
#I4	F	t-butyl	1-isopropylpiperazine	8.7	23
#J1	F	cyclopropyl	piperidine	10.0	31
#J2	F	4-fluorophenyl	piperidine	11.3	30
#J3	F	2,4-difluorophenyl	piperidine	30.4	78*
#J4	F	t-butyl	piperidine	6.4	19
		Average		**9.2**	**26**

* residual t-$BuNH_2$

new quinolones.

Several random samples from the library of 40 were analysed by ^1H NMR, however additional impurities were shown to be present, which were later identified as polypropylene by-products from the SPE cartridges. A second attempt to purification of the quinolones was attempted, using 5 ml pipette columns, automatically loaded with silica. However additional problems were encountered, such as disintegration of the viton o-rings, resulting in contamination of the fractions,

(fig. 2.32). Analysis of the products by both ^{1}H NMR and MS spectroscopy confirmed decomposition of the products.

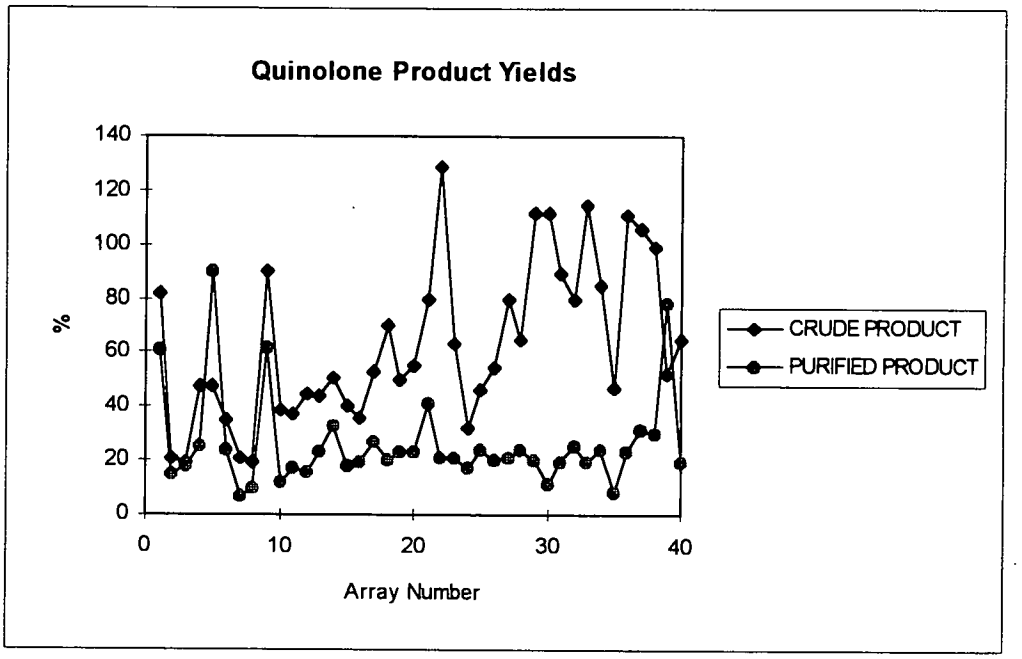


Fig. 2.32. Qunilone Product Yields

These results were discouraging, however, our work in comparison to our competitors has been unique in stressing the utility of parallel purification and quantitative purity assessment. Indeed we are the only ones employing any purification techniques.

• 2.6. Conclusion and Future work

The chemistry of the quinolones involves a complex set of reactions. Our work has been the first to successfully demonstrate the SPOS of the quinolone antibiotics. Additionally, we have illustrated the utility of the DIVERSOMER™ technology to carry out 240 reactions in the parallel synthesis of 40 quinolones. The problem of final product purification has clearly been of great importance in our work. Subsequently, the 40 quinolones will be resynthesised.

• 3.1. Alternative Support: Tbf Derivatives and PGC

3.1.1. Introduction

Merrifield's pioneering SPPS methodology, which was based on the sequential addition of N^{α}-protected amino acid residues to an insoluble polymeric support, revolutionised peptide synthesis. This greatly simplified the task of peptide synthesis and, in particular, eliminated conventional methods for purification of intermediates. However, one of the main obstacles in SPPS is the difficulty of purification of the final product due to the accumulation of truncated peptides on the resin.[198] Several methods of separation have utilised antibody affinity based interactions;[199-201] however these methods can prove costly. Instead most research groups have predominantly concentrated on chromatographic separations using ion-exchange columns and reverse phase-high performance liquid chromatography (RP-HPLC).[168]

In 1986, Knox *et al.*[202] published a paper reporting the use of porous graphitised carbon (PGC) as a stationary phase for use in HPLC, giving results comparable to those using traditional bonded silica gels. More importantly, PGC was shown to have strong hydrophobic adsorption with unique selectivity, particularly to aromatic systems.[203]

PGC has a porous two-dimensional graphite structure, and has a large surface area (ca. 150 m^2/g) available for affinity binding.[202] Preparation of the material involves polymerising a phenol-hexamine mixture within the pores of silica gel, followed by pyrolysing the resin in an atmosphere of nitrogen and then heating to an excess of 2000°C. The resulting amorphous glassy carbon is black in colour, and is chemically and physically stable.

In order to exploit the hydrophobic interaction which PGC exhibited for large, flat molecules, Ramage & Raphy [204,205] set out to design a new, planar, aromatic system for the purification of peptides.

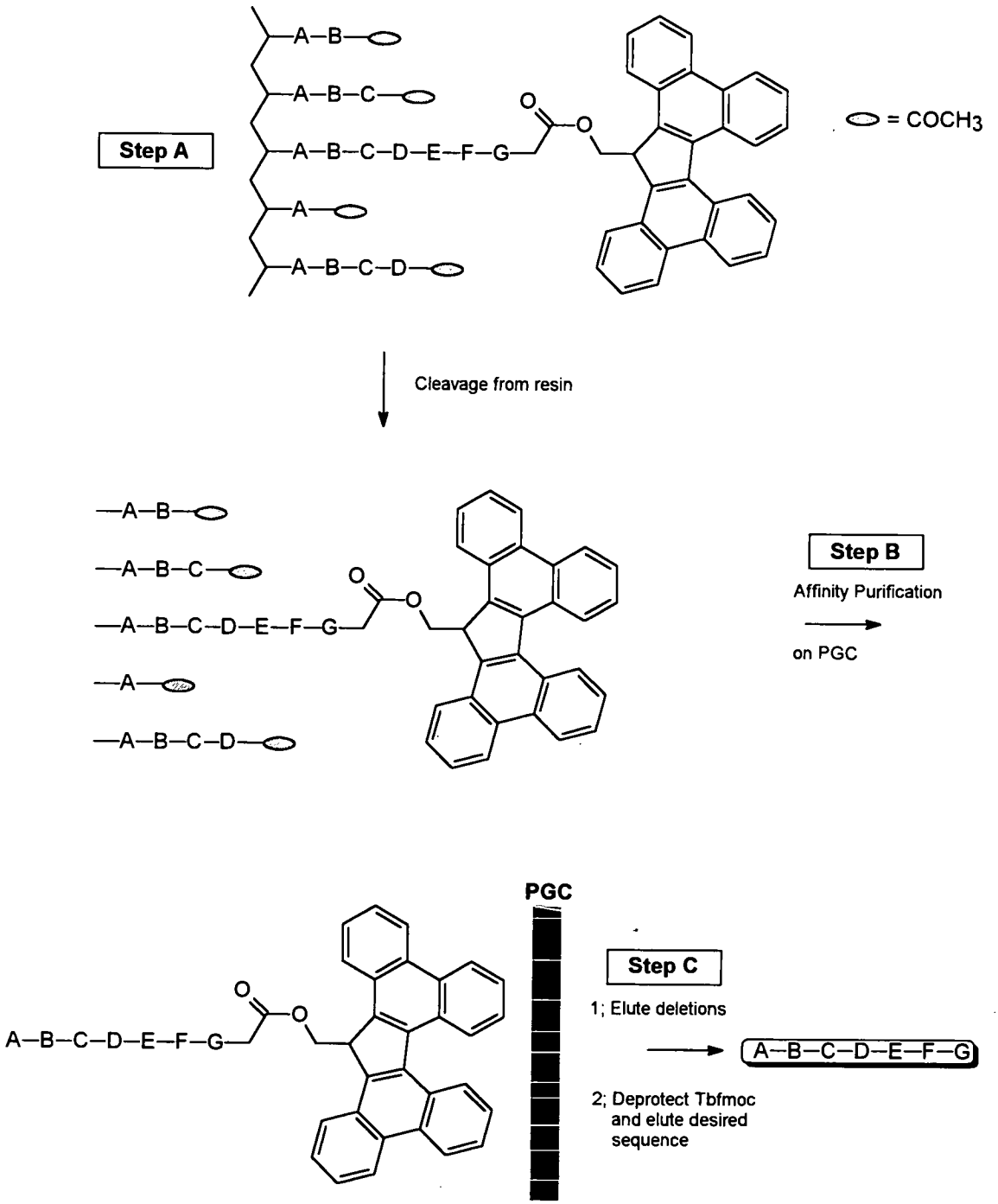
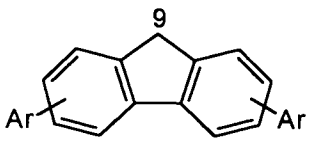


Fig. 3.1. Affinity Purification of Tbfmoc Peptide on PGC[206]

Their concept was to tag the N-terminus of a peptide chain with a suitable aromatic derivatised protecting group, as shown in (fig. 3.1), (step A). Subsequently,

the tag should be selectively adsorbed on PGC, (step B). Truncated peptides could then be easily removed by washing, and the final pure peptide obtained by deprotection and elution from PGC, (step C).

Their general concept centred on the utility of benzo-fused fluorenes, (**119**), (fig. 3.2), as the basis for the required protecting group.[207,208] While such polycyclic aromatic compounds containing fluorene have been extensively described in the literature, derivatives of tetrabenzo[*a,c,g,i*]fluorene, (Tbf), (**123**) have received little attention. In 1960, Martin *et al.*[209,210] reported the first synthesis of tetrabenzo[*a,c,g,i*]fluorene. During the next 30 years, the chemistry of Tbf remained untouched until 1988 when Ramage & Raphy[205] synthesised three derivatives of Tbf as potential intermediates in the synthesis of N^{α}-(17-tetrabenzo[*a,c,g,i*]fluorenyl)-17-methyoxycarbonyl, (Tbfmoc) amino acids and peptides.



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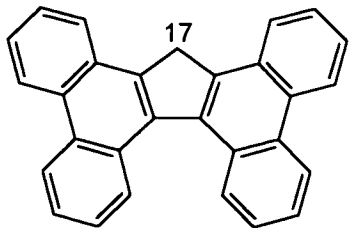


(123)

Fig. 3.2. Benzofluorenes and Tetrabenzo[a,c,g,i]fluorene

In addition to the strong fluorescent properties of tetrabenzo[*a,c,g,i*]fluorene, it also exhibited strong hydrophobic binding to PGC. Both of these properties could be exploited in peptide and oligonucleotide synthesis. Indeed Ramage, Irving & Brown[211] and Ramage & Wahl[212] have successfully investigated tetrabenzo[*a,c,g,i*]fluorene derivatives for the design of a highly hydrophobic N^{α}-amino protecting group and a 5'-hydroxyl protecting group for DNA synthesis respectively. It was found to be useful in the final purification step either by selective affinity binding to PGC, or as a hydrophobic chromatographic label to allow HPLC-

based purification. More recently, work in our laboratories has used Tbfmoc and its affinity binding to PGC for the purification of a library of 27 endothelin antagonists.[213]

A key element in the synthesis of appropriate protecting groups has been the reactivity of the methylene bridge at position 17 of the tetrabenzo[*a,c,g,i*]fluorene molecule. The hydrogen atoms are highly acidic and analogous to those of the methylene bridge at position 9 of fluorene. The acidity in both systems is attributed to the high stability of the resonance stabilised cyclopentadienyl anion generated under basic conditions.

With these unique properties in mind, Ramage[214] in 1991 proposed to the BioOrganic chemistry group at Parke-Davis the possibility of utilising the PGC / Tbf affinity interaction for the generation of a library of DIVERSOMERS™. It was predicted that by replacing the standard resin supports with tetrabenzo[*a,c,g,i*]fluorene derivatives, which have strong adsorption onto PGC, it should be possible to prepare pure compounds analogous to the methods demonstrated by peptide and oligonucleotide chemistry, (fig. 3.3). Thus, solution phase chemistry could be adopted but at the same time the advantages of solid phase methodology could be fully exploited.

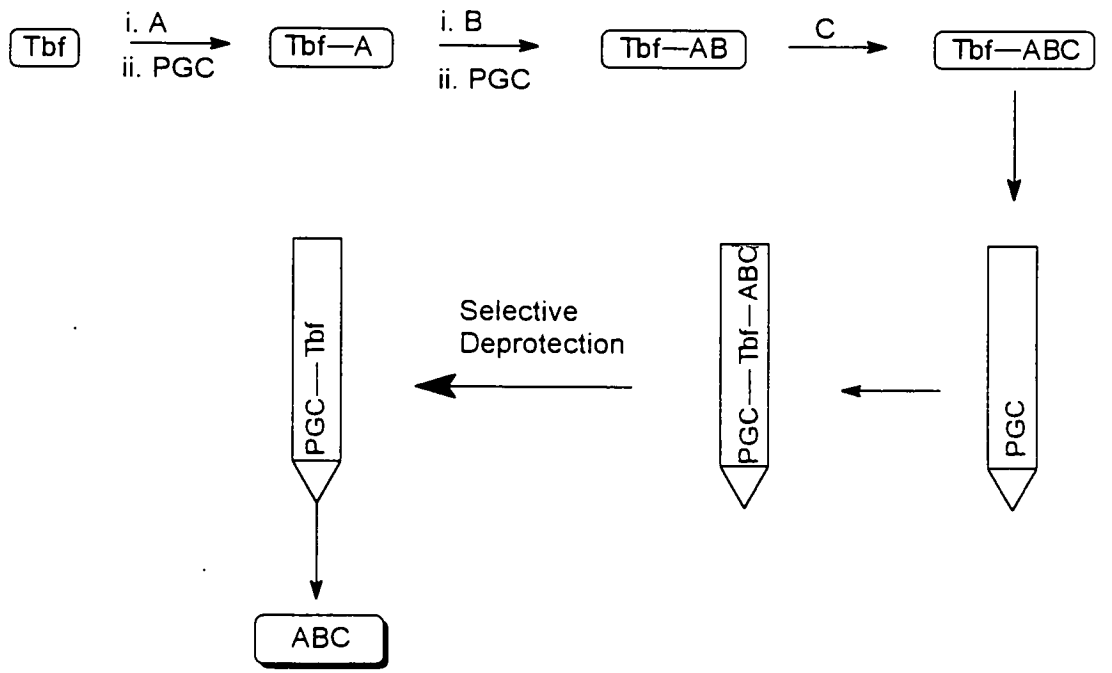


Fig. 3.3. Alternative Support: PGC / Tbf.

3.1.2. Tbf derivatives / Quinolones.

For our studies, the preparation of Ciprofloxacin® attached to a Tbf derivative was attempted in order to investigate the use of PGC as a method for library purification of the final product in the quinolone synthesis. The original synthesis of tetrabenzo[*a,c,g,i*]fluorene described by De Ridder & Martin[209] involved a series of reactions requiring harsh conditions, and gave a poor overall yield of less than 12% of the final product. In a second synthetic route, Martin *et al.*[210] prepared several derivatives *via* the Wagner-Meerwein rearrangement of the fluorene system. As with the first route, poor yields were reported making it somewhat difficult for a large scale synthesis. Inspired by Martin's work, Ramage & Raphy[204] exploited a procedure developed by Hopkinson *et al.*[215] for the synthesis of benzofluorenes via the rearrangement of α-alkoxycarbonyldiarylmethyl cations. However, low yields were obtained with several chromatographic separations required in the synthesis.

In light of these results, it was thought that an analogous reaction for the synthesis of 9-fluorenylmethanol as described by Carpino[216] could also be applied for the preparation of 17-tetrabenzo[*a,c,g,i*]fluoreneylmethanol *via* tetrabenzo-[*a,c,g,i*]fluorene.

In the reaction,[217] (fig. 3.4), the Grignard reagent derived from 9-bromophenanthrene, (**120**) was generated in anhydrous THF. Following the addition of 0.5 equivalents methyl formate, the alcohol, (*bis*-phenanthren-9-yl)methanol, (**121**) was isolated in 80% yield. A [2+2] cyclisation of the *bis* alcohol occurred upon treatment with TFA in DCM, to give the cyclised fluorescent Tbf compound, (**122**) in 90% yield. Previous attempts to generate tetrabenzo[*a,c,g,i*]fluorene *via* cyclisation of the alcohol in polyphosphoric acid,[205] concentrated sulphuric acid,[218] aluminium chloride[219] and using high temperatures[217] all proved problematic.

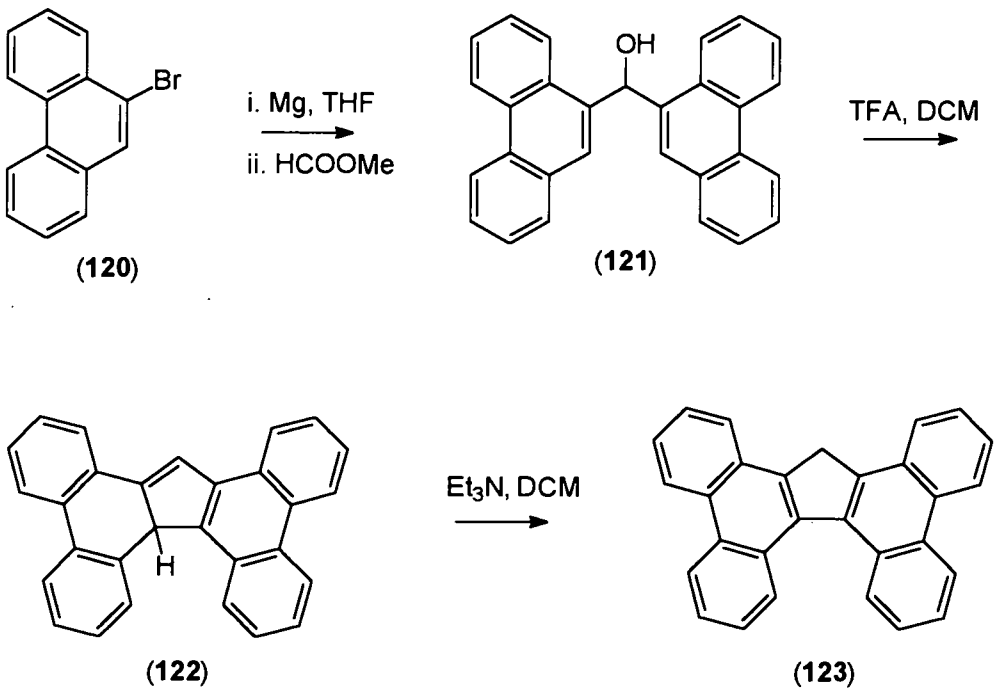


Fig. 3.4. Synthesis of Tetrabenzo[a,c,g,i]fluorene

While initial studies predicted the isolated product to be (**123**), analysis of the cyclised material by ^{1}H NMR showed subtle differences from the literature compound. A more complex aromatic splitting pattern was observed than would normally be expected for a symmetrical structure such as, (**123**). Additionally, only one proton signal at δ 5.42 ppm was observed, whereas structure (**123**) would require two. There were also slight differences between the UV spectrum and the published spectra.[209]

Based on earlier work, Ramage *et al.*[204,211,212] predicted that the polycyclic aromatic tetrabenzo[*a,c,g,i*]fluorene (**123**) was formed through a deprotonation and reprotonation isomerisation reaction, catalysed by base. The resulting isomeric products are generally referred to as 8*bH*-tetrabenzo[*a,c,g,i*]fluorene, (**122**), and 17-tetrabenzo[*a,c,g,i*]fluorene, (**123**), respectively. Further confirmation of the structure of 17-tetrabenzo[*a,c,g,i*]fluorene was obtained by X-ray analysis which revealed a molecule, symmetrical about the C2 axis, with a slight twist between the two phenanthrene moieties indicating non-planarity in the molecule, in the crystalline

state.[217] The ring system is mobile in solution as exhibited by NMR which indicates a symmetrical molecule.

Treatment of, (**122**) with *n*-butyllithium at -78°C in THF, followed by the addition of allyl bromide gave the alkene derivative, 1-(17'-tetrabenzo[*a,c,g,i*]fluorenyl)prop-2-ene, (**124**) in 70% yield. The terminal alkene was subsequently hydroborated using 9-BBN in anhydrous THF, followed by the addition of sodium hydroxide and hydrogen peroxide to generate the primary alcohol, 3-(17'-tetrabenzo[*a,c,g,i*]fluorenyl)propanol, (**125**) in 57% yield, (fig. 3.5).

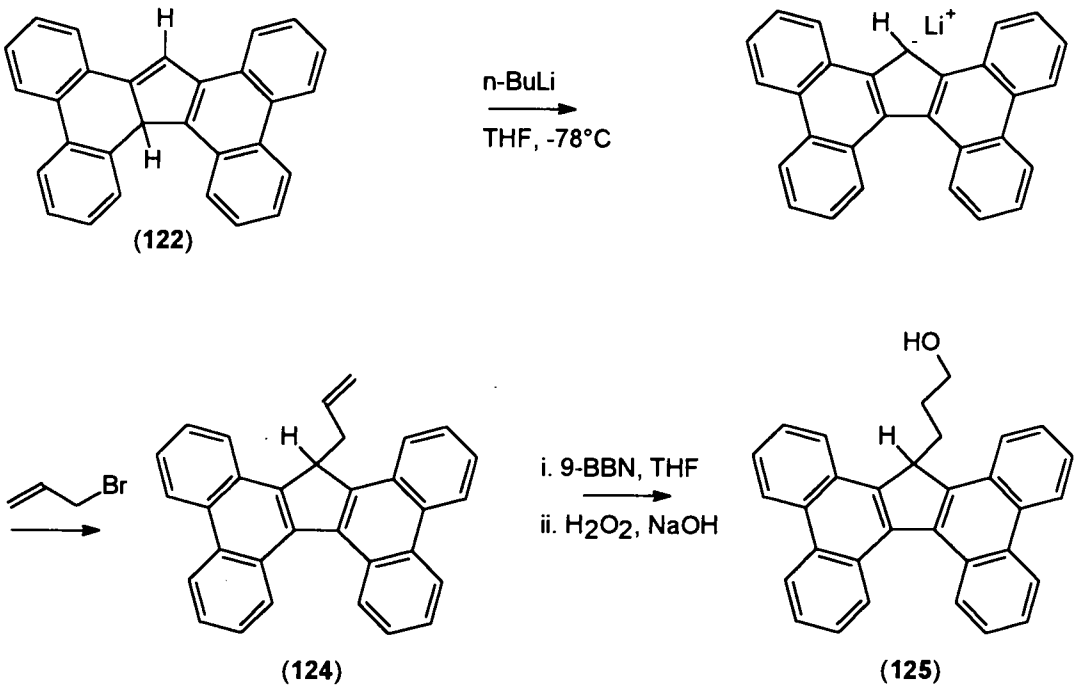


Fig. 3.5. Synthesis of 3-(17'-Tetrabenzo[a,c,g,i]fluorenyl)propanol

In order to synthesise the quinolones, we predicted that the preparation of the alcohol, (**125**), would provide us with a linker for the attachment of the quinolone structure to the Tbf moiety via the transesterification reaction discussed in chapter 2. Initial studies involved transesterification of 3-(17'-tetrabenzo[*a,c,g,i*]fluorenyl)-propanol, (**125**) and three equivalents of ethyl 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-quinolone-3-carboxylate, (**108**) in the presence of a catalytic

amount of DMAP, (fig. 3.6). The reaction mixture was heated to reflux in toluene and monitored by TLC. After 72 hours, analysis of the reaction mixture confirmed that only the starting materials were present, with no product formed. This result confirmed previous evidence reported in the literature[188] which concluded that only enolizable β-keto esters undergo transesterification under the conditions shown.

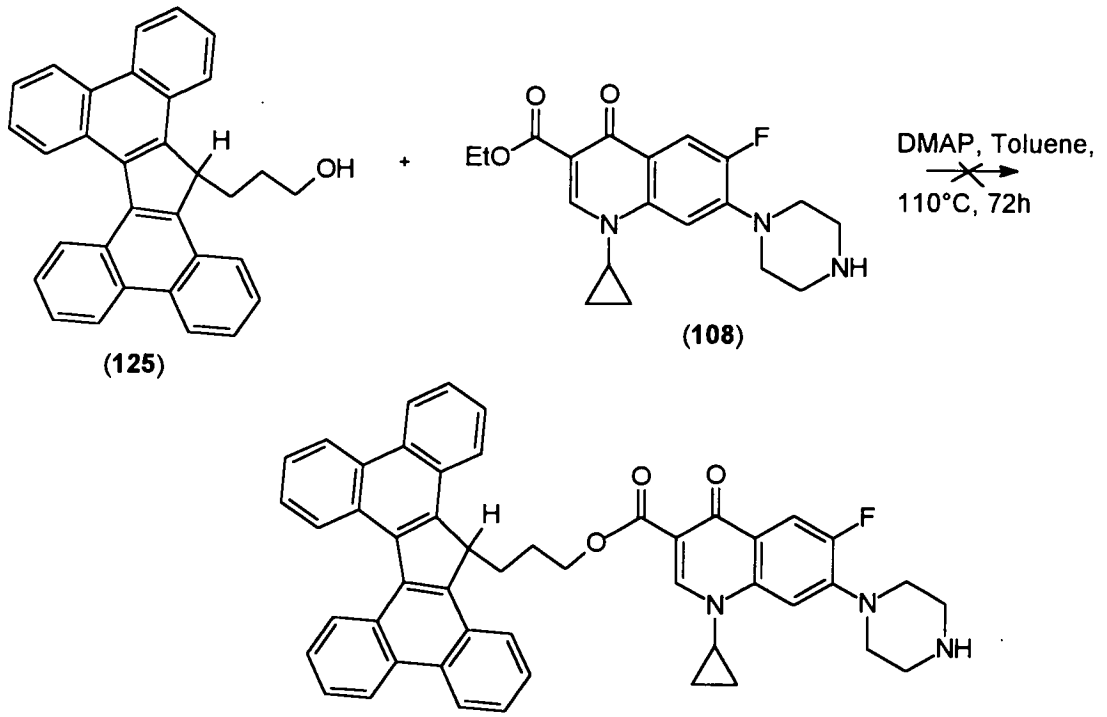


Fig. 3.6. Attempted Transesterification Synthesis

Rather than find an alternative transesterification route, we decided to pursue the route shown above *via* the cycloaracylation process for the quinolones, as discussed in the previous chapter. Preliminary experiments were carried out on transesterification of 3-(17'-tetrabenzo[*a,c,g,i*]fluorenyl)propanol, (**125**) with diethyl malonate or di-*t*-butyl malonate, to confirm the Tbf-bound β-keto esters. As predicted, in an analogous reaction, the transesterification product of 2,4,5-trifluorobenzoylacetic acid ethyl ester, (**97**) with (**125**) under the same conditions gave

the Tbf-bound β-keto ester, 3-(17'-tetrabenzo[*a,c,g,i*]fluorenyl)propyl 2,4,5-trifluorobenzoylacetate, (**126**) in 56% yield after purification, (fig. 3.7). The product was shown to exist in keto-enol (3:1) tautomerism.

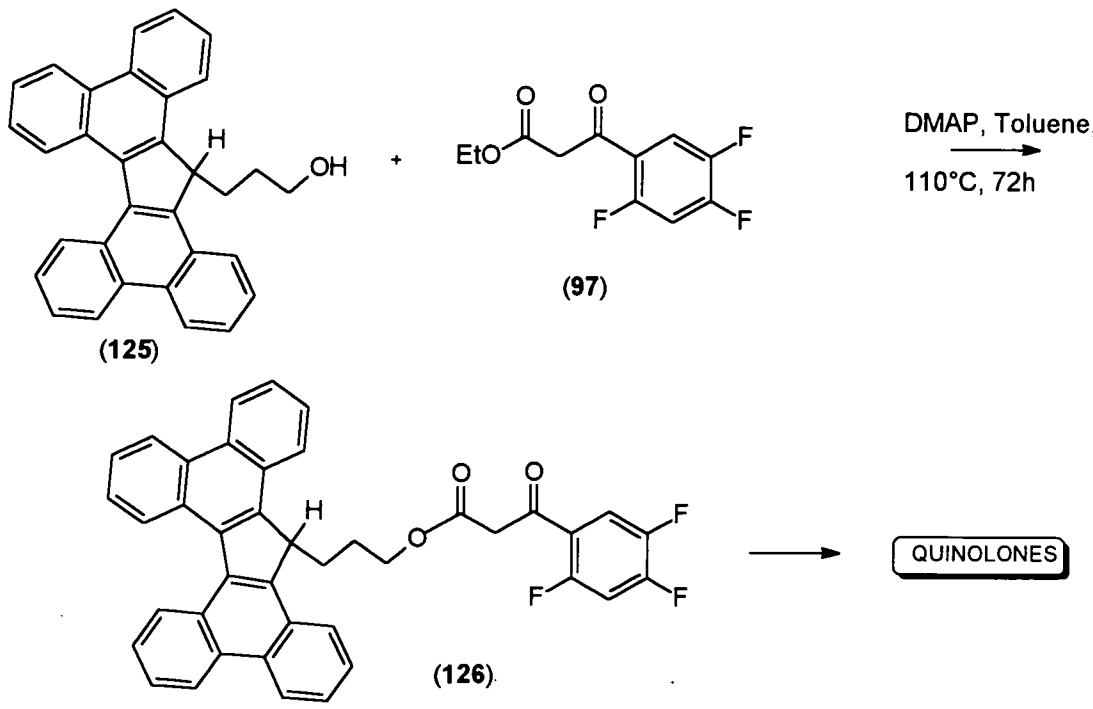


Fig. 3.7. Synthesis of 3-(17'-Tetrabenzo[a,c,g,i]fluorenyl)propyl 2,4,5-trifluorobenzoylacetate

With the primary synthetic intermediate of the quinolones now attached to the Tbf moiety, the binding affinity of (**126**) to PGC was studied prior to any further synthetic work in the quinolone synthesis. Some UV studies were carried out, see (fig. 3.8). Initially, (**126**) was dissolved in DCM and the UV spectrum measured for the presence of the Tbf chromophore in the range 400-350 nm. Following the addition of PGC (40 mg)[220], the Tbf / PGC mixture was agitated and centrifuged. UV analysis of the supernatant confirmed 80% adsorption of (**126**) onto PGC. This was later increased to 90% adsorption by the addition of methanol.

The hydrophobic interaction of 3-(17'-tetrabenzo[*a,c,g,i*]fluorenyl)propyl 2,4,5-trifluorobenzoylacetate with PGC results in a solid phase environment

analogous to that of the resin-bound β-keto ester in (fig. 2.20), (chapter 2). However, rather than filtering the resulting Tbf-PGC complex (**126**), impurities and by-products from the reaction mixture were removed in the supernatant following centrifugation. A further advantage of this methodology is that the equilibrium can be easily reversed by the addition of alternative solvents or by heating of the reaction mixture. In our studies, toluene was added to the Tbf-PGC complex and following analysis of the supernatant, 72% desorption of (**126**) from PGC was observed.

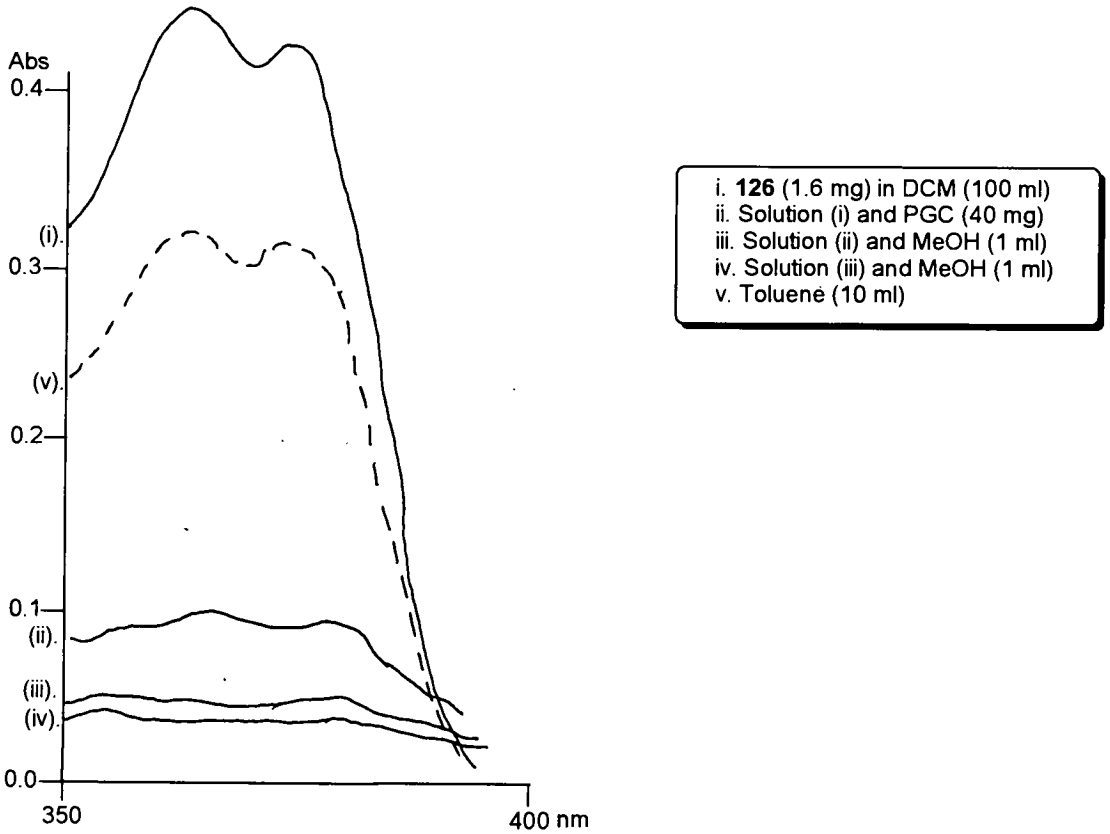
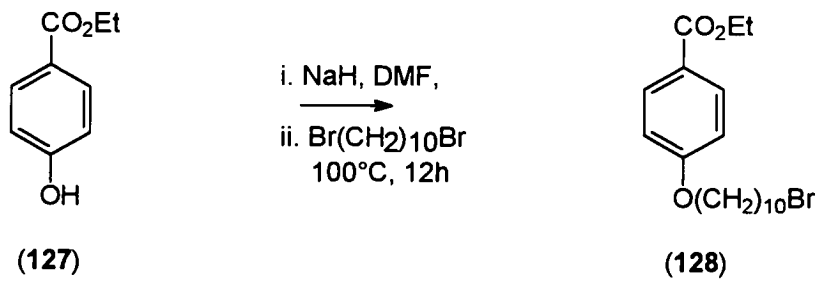


Fig 3.8. UV studies of 3-(17'-Tetrabenzo[a,c,g,i]fluorenyl)propyl 2,4,5-trifluorobenzoylacetate

3.1.3. Alternative Linker

As mentioned earlier, a major advantage in the synthesis of tetrabenzo[*a,c,g,i*]fluorene is the reactivity of the methylene bridge at C-17, which can be selectively targetted for the introduction of various functional groups.

Despite the fact that 3-(17'-tetrabenzo[*a,c,g,i*]fluorenyl)propyl 2,4,5-trifluorobenzoylacetate was envisaged as a suitable reagent for the synthesis of the quinolones, we decided to further increase the distance between the Tbf moiety and the quinolone β-keto ester starting material. The reasons for this were two-fold; 3-(17'-tetrabenzo[*a,c,g,i*]fluorenyl)propanol, (**125**) only has a three carbon linker between the C-17 position of the Tbf moiety and the hydroxyl position of the chain terminus, which we predicted might result in some steric interaction between the planar Tbf rings and the attached quinolone. Secondly, cleavage of the Tbf-bound quinolone product was also of some concern in the synthesis design. The use of polystyrene derivatised solid supports has demonstrated the success of substituting hydroxymethyl resin with Wang resin, whereby the powerful donating *p*-alkoxy moiety aids in the acid cleavage of the final product, (fig, 1.5). (chapter 1). Thus, by the introduction of an analogous ether linkage into the tetrabenzo[*a,c,g,i*]fluorene chain, the acid sensitivity of the ester linkage between the Tbf derivative and the quinolone unit could be substantially enhanced. In order to achieve both these requirements, it was decided to introduce an acid sensitive linker, *via* the preparation of the ether product, (**128**), (fig. 3.9).



(127) **(128)**

Fig. 3.9. Synthesis of Ethyl 4-(10-bromodecyloxy)benzoate

The linker, ethyl-4-(10-bromodecyloxy)benzoate, (**128**) was prepared by a Williamson ether type synthesis. Ethyl 4-hydroxybenzoate, (**127**) was deprotonated in a solution of sodium hydride and DMF, to generate the sodium salt *in situ*. Following the addition of 1,10-dibromodecane, the solution was heated to 100°C for 12 hours. The product, (**128**), was isolated in 54% yield after purification.

In order to attach the ten carbon ether chain of (**128**) to the C-17 position of tetrabenzo[*a,c,g,i*]fluorene, (**122**), deprotonation of the latter was carried out by the addition of tetrabutylammonium hydroxide in degassed dioxane.[221] On heating the mixture to reflux, a bright yellow salt was precipitated and isolated under nitrogen by filtration. The solid was subsequently resuspended in dioxane, ethyl 4-(10-bromodecyloxy)benzoate added and the mixture refluxed for 2.5 hours. Extraction and purification of the resulting brown oil gave the alkylated product, ethyl 4-[10-(17'-tetrabenzo[*a,c,g,i*]fluorenyl)decyloxy]benzoate, (**129**), in 63% yield, (fig. 3.10).

Subsequent reduction of the ester, (**129**) to the corresponding benzyl alcohol was carried out at room temperature using DIBAL in THF with the product, 4-[10-(17'-tetrabenzo[*a,c,g,i*]fluorenyl)decloxy]benzyl alcohol, (**130**) being isolated in 88% yield. Previous work by Ramage & Wahl had demonstrated an analogous reaction for the reduction of 4-(17'tetrabenzo[*a,c,g,i*]fluorenylmethyl)benzoate.

In order to generate the Tbf-bound quinolones, transesterification of (**130**) and 2,4,5-trifluorobenzoylacetic acid ethyl ester, (**97**) was attempted and the resulting β-keto ester, 4-[10-(17'-tetrabenzo[*a,c,g,i*]fluorenyl)decloxy]benzyl 2,4,5-trifluorobenzoylacetate, (**131**) isolated as a keto-enol (3:1) tautomeric mixture in 38% yield. The product, (**131**) was subsequently studied by UV analysis and its affinity to PGC was investigated, (fig. 3.11).

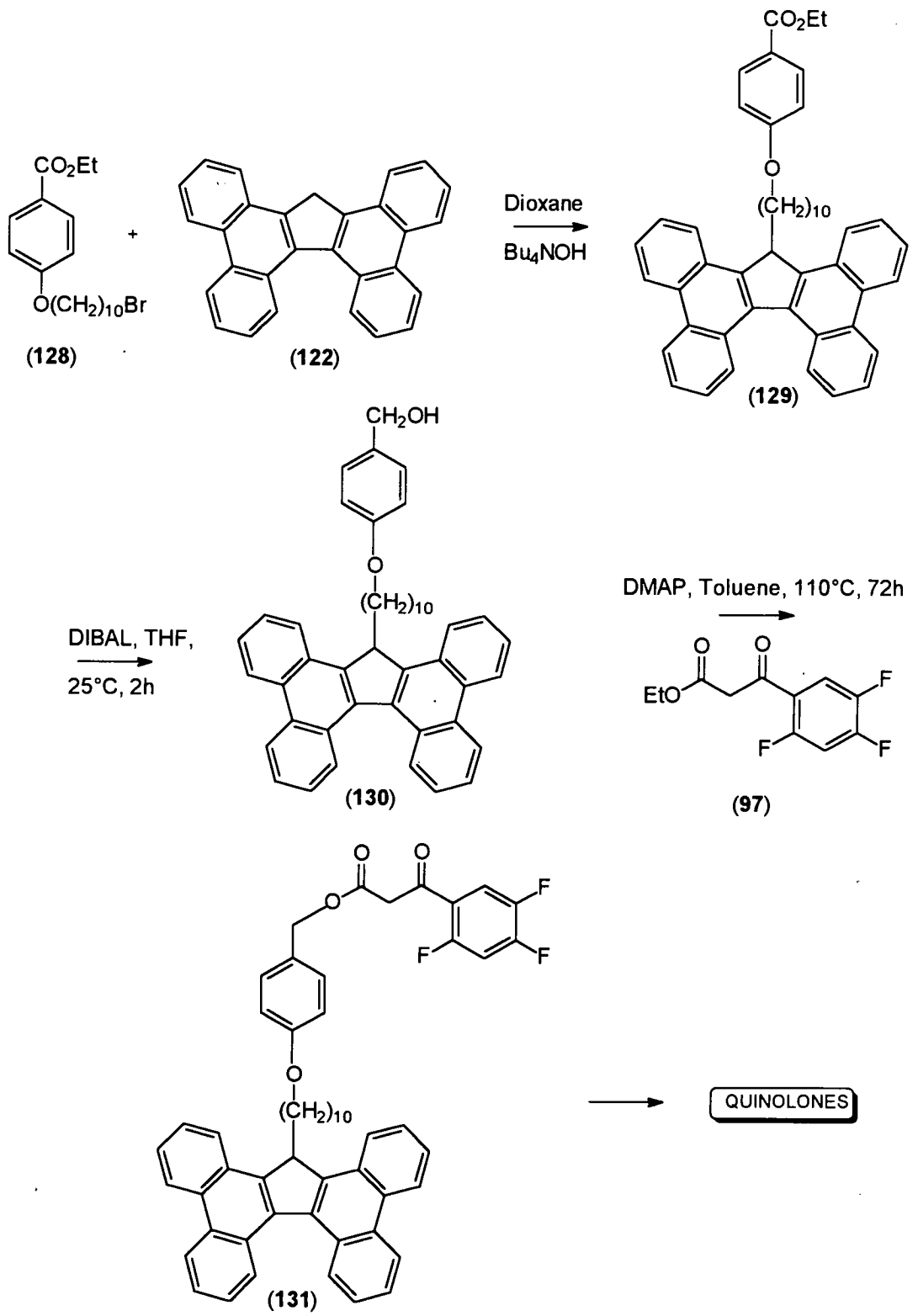


Fig. 3.10. Synthesis of 4-[10-(17'-Tetrabenzo[a,c,g,i]fluorenyl)decloxy]benzyl 2,4,5 trifluorobenzoylacetate

Initially, (**131**) was dissolved in DCM and the UV spectrum recorded over the normal range. Following the addition of PGC (44 mg) and analysis of the supernatant, 7% of the Tbf derivative remained in solution after the addition of methanol. In comparison to the previous example, this represented an increase of 13% adsorption to PGC. Desorption studies were carried out to further investigate the properties of the new linker. While 3-(17'tetrabenzo[a,c,g,i]fluorenyl)propanol resulted in a 72% desorption profile, the new linker "released" only 36% of the product in toluene. This caused some concern and consequently we decided to heat the Tbf-PGC toluene mixture. After sonication at 40°C for twenty minutes, a total of 72% desorption was recorded, analogous to the results obtained for Tbf-propanol. Further heating and agitation revealed no additional desorption of the product from PGC.

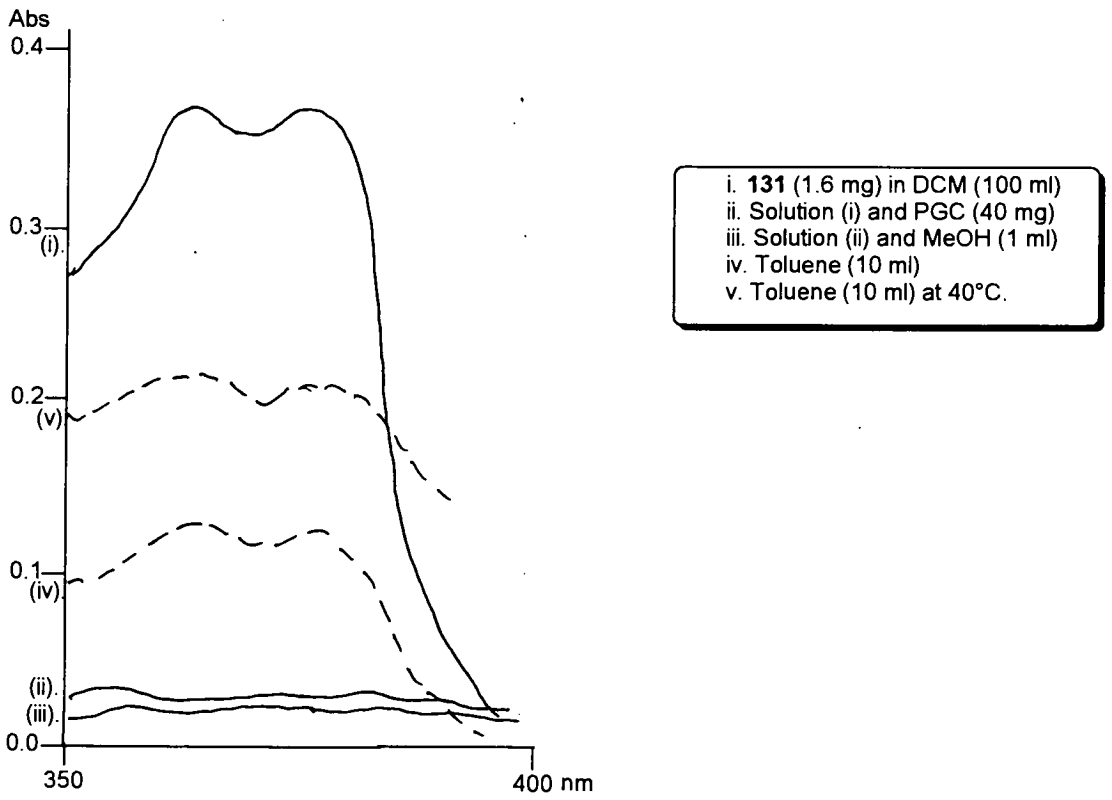


Fig. 3.11. UV studies of 4-(10-(17'-Tetrabenzo[a,c,g,i]fluorenyl)decloxy)benzyl 2,4,5-trifluorobenzoylacetate

3.1.4. Future Work

The preparation of the Tbf quinolones has been limited to the initial transesterification reaction. Preliminary work on the enol ether derivatives of the Tbf-β-keto esters looks promising. The introduction of an alternative linker, ethyl 4-(10-bromodecyloxy)benzoate, between the Tbf moiety and the quinolone structure has given interesting results. The UV studies of both Tbf derivatised β-keto esters have successfully demonstrated the affinity of the quinolone backbone for PGC. Furthermore, this work illustrates the advantages of using an "alternative" solid support which would allow standard solution phase chemistry to be used, while at the same time being able to exploit the advantages of solid phase chemistry. Future work will continue with the preparation of the quinolones, thus demonstrating the utility of PGC for the purification of small molecule libraries.

CHAPTER 4:EXPERIMENTAL

• 4.1. Techniques and Instrumentation

Functionalised resins for solid phase synthesis were purchased from BACHEM, Switzerland, unless otherwise stated. The resins were used as supplied. Other chemicals were obtained from commercial sources such as Aldrich and Lancaster and used without further purification. Melting points were determined in open capillaries using a Buchi 510 melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was performed on plastic or aluminium sheets precoated with silica gel (Kieselgel 60 F254) in the following solvent systems;

A	chloroform
B	9:1 chloroform / methanol
C	8:2 hexane / ethyl acetate
D	9:1:0.5 chloroform / methanol / acetic acid
E	9:1:0.5 acetonitrile / triethylamine / water
F	18:2:1 acetonitrile / tributylamine / water
G	3:1:1 propanol / ammonia / water

Flash chromatography was performed using silica gel 60 230-400 mesh (wet flash) or silica gel 60 5-40 μm (dry flash). Compounds were visualised using suitable combinations of ultra violet absorption at 254 and 365 nm, iodine vapour, methanolic sulphuric acid, Mary's reagent, (4,4'-bis(dimethylamino)diphenylcarbinol), and ninhydrin. Infrared spectra were recorded on a Bio-Rad FTS-7 spectrometer in bromoform solution or KBr discs. Ultra violet spectra were recorded on a Varian Cary 210 double beam spectrophotometer. Fast atom bombardment mass spectra (FAB MS) were recorded on a Kratos MS50TC, and electron impact mass spectra (EI MS) on a Kratos 902MS. Elemental analyses were carried out on Carlo Erba 1106 or Perkin Elmer 2400 instruments. Proton NMR spectra were recorded on either Brucker WP 80 (80 MHz), WP 200 (200 MHz), or AC 250 (250 MHz) spectrometers in the solvent indicated relative to tetramethylsilane (TMS) as the external standard. Carbon-13 NMR spectra were recorded on either Brucker WP 200

(50 MHz), AC 250 (250 MHz) or WH 360 (90 MHz) machines in the solvents indicated relative to TMS as the external standard. Gel phase carbon-13 NMR spectra were recorded on either Brucker WP 200 (50 MHz) or AC 250 (250 MHz) machine. The resins were swollen in $CDCl_3$. Fluorine-19 NMR spectra were recorded on a AC (235 MHz). Single crystal X-ray structure determination was performed on a Stoe Stadi-4, four circle diffractometer, graphite monochromated (Cu-Ka radiation, 1=1.54184Å). All solvents used were of analytical grade, or were distilled before use. The following solvents were dried when required using the reagents indicated: benzene (sodium wire), dichloromethane (calcium hydride), diethyl ether (sodium wire), THF (sodium/benzophenone indicator), toluene (sodium wire).

Elemental analysis on tetrabenzo[*a,c,g,i*]fluorene derivatives.

Microanalysis results on tetrabenzo[*a,c,g,i*]fluorene derivatives have generally been unsatisfactory. This has been attributed to the large number of quaternary carbons present in the system.[168] Increased oxygen concentrations and the addition of vanadium pentoxide have given improved combustion.

Automation - DIVERSOMER™ apparatus.

Information management was accomplished with Microsoft Excel® spreadsheets, Parke-Davis proprietary database and MDL® ISIS / Base, ISIS / Draw and Project Library™ software packages on Gateway 2000 4DX2-66V and Macintosh Iici Personal Computers. All liquid sample handling in the DIVERSOMER™ approach was achieved using a Tecan™ 5032 Robotic Sample Processor (RSP). Reaction agitation for the arrays was accomplished with Ney 250 ultrasonic baths coupled with Brinkmann RM6 Lauda temperature controllers. The heat exchange fluid used in the temperature controller was a ratio of 70% ethylene glycol / 30% water (v/v). The ultrasound bath fluid was water at ambient temperatures, and Dow Corning 550

silicone fluid at high temperatures. Final products were purified using Varian Mega-Bond Elut pre-packed 12 ml cartridges. Normal phase cartridges were packed with 2 grams of silica gel. Concentration of the final product solutions was achieved by applying vacuum in a centrifuge, SAVANT Instruments SC110 SpeedVac™. Drying of the final products with vacuum was done in a NAPCO model 5831 vacuum oven. Proton NMR spectra and carbon NMR spectra were recorded on either Varian Unity 400 (400 MHz), or Varian UnityPlus 400 (400 MHz) spectrometers, in the solvents indicated relative to TMS as an internal standard. Electrosray mass spectra (ES MS) were recorded on a Finnigan MAT 900 Q Mass Spectrometer.

• 4.2. Synthetic Procedures

Copoly(styrene-1%-divinylbenzene)piperazine, (73).

Copoly(styrene-1%-divinylbenzene)chloromethyl resin (0.20 g, 0.7 mmol / g), was suspended in toluene (100 ml). Piperazine (0.47 g, 55 mmol) was added and the resin mixture heated to 70°C, under an atmosphere of nitrogen, overnight. After 16 hours, the resin was filtered off and washed with copious amounts of dioxane, dioxane:water, toluene, methanol, water, THF and DCM. The product was dried *in vacuo* to yield a cream coloured resin identified as the *title compound*, (0.20 g). **δC** (50 MHz, $CDCl_3$), 145, 133-125 (polystyrene), 63, (polystyrene), 54 (w, CH_2's), 46 (CH_2's), 40 (polystyrene) ppm.

2,4,5-Trifluorobenzoylacetic acid, (85).

A 1.6M solution (13 ml) of butyllithium in hexane was added over 10 minutes to a stirred solution of *bis*[trimethylsilyl] malonate (4.96 g, 20 mmol) in dry ether (20 ml), under an atmosphere of nitrogen, at -60°C. The mixture was then allowed to warm to 0°C and a solution of 2,4,5-trifluorobenzoyl chloride (1 g, 5.1 mmol), in dry ether (20 ml) was added in one portion. The mixture was stirred for another 10 minutes, at 0°C, and then shaken thoroughly with cold 5% aq. sodium hydrogen carbonate (100 ml) for 10 minutes. The aqueous layer was acidified to pH 1-2 with cold 4N H_2SO_4 and extracted with ether, (3 x 50ml). The extract was dried over $MgSO_4$ and evaporated *in vacuo* to give the β–keto acid as a white crystalline powder. (1.1 g, 96 %); **m.p.** 104-105°C; **TLC,** Rf(C) 0.26; **C,H,** found C, 48.10; H, 2.29, $C_9H_5F_3O_3$ requires C, 48.16; H, 2.29 %; $\nu_{max.}$ ($CHBr_3$ mull) 3500-3000 (b, OH), 1726 (C=O), 1712 (C=O) 1598 (aromatic) cm^{-1}; **δH** (60 MHz, $CDCl_3$), 8.9-8.4 (m, 1H, aromatic), 8.0-

7.5 (m, 1H, aromatic), 3.9 (d, CH_2) ppm; **m/z** (FAB) 219 (M+), HRMS(FAB) found 219.02691, $C_9H_5F_3O_3$ requires 219.02690.

2,3,4,5-Tetrafluorobenzoylacetic acid, (86).

A 1.6M solution (6.5 ml) of butyllithium in hexane was added over 10 minutes to a stirred solution of *bis*[trimethylsilyl] malonate (2.48 g, 10 mmol) in dry ether (20 ml), under an atmosphere of nitrogen, at -60°C. The mixture was then allowed to warm to 0°C and a solution of 2,4,5-trifluorobenzoyl chloride (1 g, 5.0 mmol), in dry ether (10 ml) was added in one portion. The mixture was stirred for another 10 minutes, at 0°C, and then shaken thoroughly with cold 5% aq. sodium hydrogen carbonate (50 ml) for 10 minutes. The aqueous layer was acidified to pH 1-2 with cold 4N H_2SO_4 and extracted with ether (4 x 30ml). The extract was dried over $MgSO_4$ and evaporated *in vacuo* to give the β–keto acid as a white crystalline powder. (0.90 g, 77%); **m.p** 111-113°C; **TLC,** Rf (C) 0.24; **C,H,** found: C 45.76; H, 1.96, $C_9H_4F_4O_4$ requires C, 45.76; H, 1.69 %; ν_{max} ($CHBr_3$ mull) 3500-3000 (b, OH), 1739 (C=O), 1712 (C=O), 1599 (aromatic) cm^{-1}; **δH** (200 MHz, $CDCl_3$ / DMSO), 7.65-7.50 (m, 1H, aromatic), [12.3 (s), 5.92 (s), 4.03 (d), ^3J = 3.8 Hz] 2H, ppm; **m/z** (FAB) 237 (M+), HRMS(FAB) found 237.01749, $C_9H_4F_4O_3$ requires 237.01747.

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl-monoethyl malonate.

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl alcohol, (Wang) resin (0.20 g, 0.8 mmol) was suspended in toluene (15 ml). A catalytic amount of 4-DMAP and diethyl malonate (0.33 g, 2.1 mmol) was added. The reaction mixture was allowed to stir, for 3 days, under a nitrogen atmosphere. After 72 hours, the resin was filtered off and washed with copious amounts of toluene, DMF, and finally DCM to yield a pale

cream coloured resin (0.20 g). ν_{max} (KBr disc) 1750 (C=O), 1736 (C=O) cm^{-1}. δC (60 MHz, $CDCl_3$), 145, 133-125 (polystyrene), 114 (polystyrene), 69 (*p*-benzyloxy CH_2), 65 (benzyl CH_2), 61 ($CO\underline{C}H_2CH_3$), 41 ($CO\underline{C}H_2CO$), 14 ($COCH_2\underline{C}H_3$) ppm.

2,4,5-Trifluorobenzoylacetic acid ethyl ester, (97).

Potassium ethyl malonate (10.2 g, 60 mmol) was stirred in MeCN (90 ml) under a nitrogen atmosphere at 10-15°C. To this mixture was added Et_3N (6.35 g, 62 mmol) followed by $MgCl_2$ (6.6 g, 70 mmol) and stirring continued at 20-25°C for 2.5h. The resulting slurry was recooled to 0°C and 2,4,5-trifluorobenzoyl chloride (5 g, 26 mmol) added dropwise over a period of 25 minutes, followed by the addition of more Et_3N (0.58 g, 5.7 mmol). The mixture was allowed to stir overnight at room temperature and the next day concentrated under vacuum to remove MeCN. Toluene (50 ml) was added and the resulting mixture reconcentrated under vacuum. More toluene (60 ml) was added and the mixture cooled to 10-15°C. Aqueous HCl (13 %, 50 ml) was added cautiously while keeping the temperature below 25°C. The aqueous layer was separated and the organic layer washed with 12% aq. HCl (2 x 30 ml) followed by H_2O (2 x 40 ml). After drying over $MgSO_4$ the organic layer was concentrated under vacuum to give the title compound as a white coloured product, (5.04 g, 79 %). **m.p.** 57-59°C (lit.[184] 55-57°C); **TLC**, R_f (A) 0.35, R_f (B) 0.62; ν_{max} ($CHBr_3$ mull) 2986, 2933 (aromatic st.), 1733 (C=O), 1707 (C=O) cm^{-1}; λ_{max} (DCM) 296 (ε = 11885 $dm^3mol^{-1}cm^{-1}$), 280 11270 nm; **C,H,** found C 53.36; H 3.90; $C_{11}H_9F_3O_3$ requires C 53.60; H 3.66 %; **δH** (200MHz, $CDCl_3$), 7.74 (m, 1H, aromatic), 7.04 (m, 1H, aromatic), 4.26 (m, 2H), [13.21 (s), 5.84 (s), 3.93 (d,J=4)], 2H, 4.32-4.16 (m, 2H, ^3J = 7.14 Hz, $CO_2\underline{C}H_2CH_3$), 1.35-1.15 (m, 3H, ^3J = 7.12 Hz, $CO_2CH_2\underline{C}H_3$) ppm; δC (50MHz, $CDCl_3$), 187 (C=O), 173 (CO_2Et), 166 (CO_2Et), 163, 159, 155, 151, 149, 145 (quaternary aromatic C's), 118 (aromatic CH), 107

(aromatic CH), 61 ($CO\underline{C}H_2CH_3$), 49 ($CO\underline{C}H_2CO$), 13.8 ($COCH2\underline{C}H_3$) ppm; **δF** (235 MHz, $CDCl_3$), -110 (m, aromatic F), -111 (m, aromatic F), -123 (m, aromatic F), -128 (m, aromatic F), -140 (m, aromatic F), -141 (m, aromatic F) ppm; **m/z** (EI); 247 (M+), 201, 159; HRMS (EI), found 247.05820, $C_{11}H_{10}F_3O_3$ requires 247.05820.

2,3,4,5-Tetrafluorobenzoylacetic acid ethyl ester, (98).

Potassium ethyl malonate (3.84 g, 22 mmol) was stirred in MeCN (34 ml) under a nitrogen atmosphere at 10-15°C. To this mixture was added Et_3N (2.25 g, 21 mmol) followed by $MgCl_2$ (2.50 g, 27 mmol) and stirring continued at 20-25°C for 2.5h. The resulting slurry was recooled to 0°C and 2,3,4,5-tetrafluorobenzoyl chloride (2.5 g, 11 mmol) added dropwise over a period of 25 minutes, followed by the addition of more Et_3N. The mixture was allowed to stir overnight at room temperature and the next day concentrated under vacuum to remove MeCN. Toluene (20 ml) was added and the resulting mixture reconcentrated under vacuum. More toluene (30 ml) was added and the mixture cooled to 10-15°C. Aqueous HCl (13 %, 25 ml) was added cautiously while keeping the temperature below 25°C. The aqueous layer was separated and the organic layer washed with 12% aq. HCl (2 x 15ml) followed by H_2O (2 x 20 ml). After drying over $MgSO_4$ the organic layer was evaporated *in vacuo* to give the title compound as a pale orange low melting solid. The product was recrystallized from hexane, (2.11 g, 73 %). **m.p.** 59-61°C, (Lit.[184] 63-64°C); **TLC**, R_f (A) 0.24, R_f (B) 0.60; **C,H,** found: C, 49.89; H, 2.91; $C_{11}H_8F_4O_3$ requires C, 50.00; H, 3.03 %; ν_{max} ($CHBr_3$ mull) 2984, 2937 (Ar st.), 1739 (C=O), 1712 (C=O) cm^{-1}; **λmax** (DCM) 286 (ε = 26402 $dm^3mol^{-1}cm^{-1}$), 241 (6270) nm; **δH** (250MHz, $CDCl_3$), 7.67-7.51 (m,1H, aromatic), [12.7 (s), (OH), 5.82 (s), 3.96 (d), ^3J=3.8Hz,] (2H), 4.32-4.16 (m, 2H, ^3J = 7.11 Hz, $CO_2\underline{C}H_2CH_3$), 1.36-1.22 (m, 3H, ^3J = 7.15 Hz, $CO_2CH_2\underline{C}H_3$) ppm; **δC** (50 MHz, $CDCl_3$), 186 (C=O), 172 (CO_2Et), 166

(CO_2Et), 162, 146, 145, 143, 141, 138 (quaternary aromatic C's), 111 (aromatic CH), 93 (C=CH), 61 ($CO\underline{C}H_2CH_3$), 49 ($CO\underline{C}H_2CO$), 13 ($COCH_2\underline{C}H_3$) ppm; **m/z** (FAB) 265 (M+), 219, 177; HRMS (FAB), found 265.04878, $C_9H_5F_4O_3$ requires 265.04877.

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 2,4,5-trifluorobenzoyl-acetate, (89).

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl alcohol resin (1.0 g, 0.96 mmol / g) was suspended in toluene (30 ml). A catalytic amount of 4-DMAP and 2,4,5-trifluorobenzoylacetic acid ethyl ester (1.24 g, 5.0 mmol) were added and the reaction mixture heated to 110°C under an atmosphere of nitrogen, overnight. After 18 hours, the resin was cooled and filtered off, then washed with copious amounts of the following solvents; toluene, DMF (x 2), methanol, water, THF and chloroform. The *title compound* was obtained as an orange product (1.0 g). ν_{max} (KBr disc), 1751 (C=O), 1735 (C=O) cm^{-1}; δC (60 MHz, $CDCl_3$), 145, 133-125 (polystyrene), 117 (aromatic CH), 114 (polystyrene), 106 (aromatic CH), 70 (*p*-benzyloxy CH_2), 64 (benzyl CH_2), 49 ($CO\underline{C}H_2CO$) ppm.

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 2,3,4,5-tetrafluoro-benzoylacetate, (90).

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl alcohol resin (1.0 g, 0.96 mmol / g) was suspended in toluene (30 ml). A catalytic amount of 4-DMAP and 2,3,4,5-tetrafluorobenzoylacetic acid ethyl ester (1.39 g, 5.0 mmol) were added and the reaction mixture heated to 110°C under an atmosphere of nitrogen, overnight. After 18 hours, the resin was cooled and filtered off, then washed with copious

amounts of the following solvents; toluene, DMF (x 2), methanol, water, THF and chloroform. The *title compound* was obtained as an orange product (1.0 g). ν_{max} (KBr disc), 1751 (C=O), 1735 (C=O) cm^{-1}; δC (60 MHz, $CDCl_3$), 145, 133-125 (polystyrene), 117 (aromatic CH), 114 (polystyrene), 69 (*p*-benzyloxy CH_2), 65 (benzyl CH_2), 48 ($CO\underline{C}H_2CO$) ppm.

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl (2-ethoxymethylene)-2,4,5-trifluorobenzoylacetate, (92).

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 2,4,5-trifluorobenzoylacetate (0.20 g, 0.96 mmol / g) was suspended in a solution of acetic anhydride (10 ml) and triethyl orthoformate (2.40 ml, 7.25 mmol). The resin mixture was heated to 130°C for 4 hours. After cooling, the resin was filtered off and washed with copious amounts of DMF (x 2), ethanol, chloroform and DCM. On drying, the *title compound* was isolated as a dark brown product (0.195 g); δC (60 MHz, $CDCl_3$), 145, 133-125 (polystyrene), 118 (aromatic CH), 114 (polystyrene), 111 (aromatic CH), 69 (*p*-benzyloxy CH_2), 66 (benzyl CH_2), 58 ($O\underline{C}H_2CH_3$), 18 ($OCH_2\underline{C}H_3$) ppm.

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 2-(2,4,5-trifluorobenzoyl) -3-cyclopropylaminoacrylate, (109), route (A).

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 2,4,5-trifluorobenzoylacetate enol ether, (0.17 g, 0.96 mmol / g) was suspended in DCM (20 ml). Cyclopropylamine (1.00 ml, 14.7 mmol) was added and the mixture was stirred, under an atmosphere of nitrogen, at 25°C. After 3 hours, the resin was filtered and washed with copious amounts of the following solvents; DMF (x 2), methanol, water, THF and chloroform. The *title compound* was dried *in vacuo* to yield an orange coloured

product (0.20g). δC (60 MHz, $CDCl_3$), 145, 133-125 (polystyrene), 118 (aromatic-CH), 114 (polystyrene), 105 (aromatic-CH), 69 (*p*-benzyloxy CH_2), 66 (benzyl CH_2), 30 (CH), 6 (CH_2CH_2) ppm.

Ethyl (2-ethoxymethylene)-2,4,5-Trifluorobenzoylacetate, (99).

Triethyl orthoformate (1.21 ml, 7.25 mmol), was added to a solution of 2,4,5-trifluorobenzoylacetic acid ethyl ester (1 g, 4.0 mmol) in acetic anhydride (2.11 ml, 22.35 mmol). The solution was heated at 130°C for 3 hours and, on cooling, evaporated *in vacuo* under reduced pressure to yield a pale orange mobile oil, (1.17 g, 95 %).**TLC**, R_f (A) 0.24, R_f (B) 0.60; ν_{max} ($CHBr_3$ mull) 3064, 2986, 2939, 2900 (aromatic st.), 1713 (C=O), 1668 (C=O), 1626 (aromatic) cm^{-1}; λ_{max} (DCM) 290 (ε = 8636 $dm^{-3}mol^{-1}cm^{-1}$), 240 (12121) nm; δ**H** (250 MHz, $CDCl_3$), 7.64 (s, 1H, C=C), 7.59-7.49 (m, 1H, aromatic), 6.90-6.79 (m, 1H, aromatic), 4.14-4.08 (q, 2H, ^3J = 7.12 Hz, $CO\underline{C}H_2CH_3$), 4.07-3.99 (q, 2H, ^3J = 7.11 Hz, $O\underline{C}H_2CH_3$), 1.37-1.14 (t, 3H, ^3J = 7.10 Hz, $COCH_2\underline{C}H_3$), 1.12-0.98 (t, 3H, ^3J = 7.11 Hz, $COCH_2\underline{C}H_3$) ppm; δ**C** (50 MHz, $CDCl_3$), 186 (C=O), 184 (CO_2Et), 167, 163 (C=CH), 158, 154, 150, 148, 144 (quaternary aromatic C's), 124 (C=C), 118 (aromatic CH), 112 (C=C), 105 (aromatic CH), 72 ($CO\underline{C}H_2CH_3$), 60 ($O\underline{C}H_2CH_3$), 14 ($COCH_2\underline{C}H_3$), 13 ($OCH_2\underline{C}H_3$) ppm; δ**F** (235 MHz, $CDCl_3$), -113 (m, aromatic F), -126 (m, aromatic F), -142 (m, aromatic F) ppm; **m/z** (EI); 302(M+), 257, 200, 159; HRMS (EI), found 302.07625 requires $C_{14}H_{13}F_3O_4$ requires 302.07659.

Ethyl (2-ethoxymethylene)-2,3,4,5-Tetrafluorobenzoylacetate, (100).

Triethyl orthoformate (1.21 ml, 7.25 mmol), was added to a solution of 2,3,4,5-tetrafluorobenzoylacetic acid ethyl ester (1.16 g, 4.4 mmol) in acetic anhydride (2.11

ml, 22.35 mmol). The solution was heated at 130°C for 4 hours and, on cooling, evaporated *in vacuo* to yield a pale orange mobile oil, (1.36 g, 96 %).**TLC**, R_f (A) 0.24, R_f (B) 0.60; ν_{max} ($CHBr_3$ mull) 3064, 2986, 2939, 2900 (aromatic st.), 1713 (C=O), 1668 (C=O), 1626 (aromatic) cm^{-1}; **δH** (250 MHz, $CDCl_3$), 7.67 (s, 1H, C=C), 7.44-7.36 (m, 1H, aromatic), 4.19-4.16 (q, 2H, ^3J = 7.11 Hz, $CO\underline{C}H_2CH_3$), 4.14-4.06 (q, 2H, ^3J = 7.16 Hz, $O\underline{C}H_2CH_3$), 1.38-1.18 (t, 3H, ^3J = 7.12 Hz, $COCH_2\underline{C}H_3$), 1.17-1.07 (t, 3H, ^3J = 7.16 Hz, $OCH_2\underline{C}H_3$) ppm; **δC** (50 MHz, $CDCl_3$), 185 (C=O), 183 (CO_2Et), 168 (C=CH) 164 (C=CH), 148, 144, 142, 141, 138 (quaternary aromatic C's), 123 (C=C), 111 (aromatic CH), 72 ($CO\underline{C}H_2CH_3$), 60 ($O\underline{C}H_2CH_3$), 14 ($COCH_2\underline{C}H_3$), 13 ($OCH_2\underline{C}H_3$) ppm; **δF** (235 MHz, $CDCl_3$), -138.0 - -138.8 (m, aromatic F x 2), -149 (m, aromatic F), -155 (m, aromatic F) ppm; **m/z** (EI); 320(M+), 275, 247, 159; HRMS (EI), found 320.06701, $C_{14}H_{12}F_4O_4$ requires 320.06717.

Ethyl 3-cyclopropylamino-2-(2,4,5-trifluorobenzoyl)acrylate, (101).

The mobile oil, ethyl (2-ethoxymethylene)-2,4,5-trifluorobenzoylacetate (1.17 g, 3.9 mmol), was suspended in DCM (15 ml) and cyclopropylamine (0.413 g, 7.25 mmol) added. The solution was stirred at 25°C for 3 hours, under an atmosphere of nitrogen. After evaporation *in vacuo*, the residue was dried to yield a pale green coloured solid, (1.09 g, 89 %); **m.p.** 52-54 °C; **TLC**, R_f (B) 0.59, R_f (D) 0.75; **C,H,N,** found: C, 57.33; H, 4.44; N, 4.27; $C_{15}H_{14}F_3NO_3$ requires C, 57.50, H, 4.47; N, 4.47 %; ν_{max} ($CHBr_3$ mull), 3280 (NH), 3232 (NH), 3110, 2939, 2907 (aromatic st.), 1693 (C=O), 1630 (C=O) cm^{-1}; λ_{max} (DCM) 318 (ε = 10658 $dm^3mol^{-1}cm^{-1}$) 248 (15360) nm; **δH** (250 MHz, $CDCl_3$), 10.86 (d, NH), 9.37 (NH), 8.20 (m, 1H, C=C), 7.24-7.10 (m, 1H, aromatic), 6.89-6.78 (m, 1H, aromatic), 4.03-3.98 (q, 2H, ^3J = 7.14 Hz, $CO\underline{C}H_2CH_3$), 3.00-2.90 (m, 1H, CH), 1.07-1.02 (t, 3H, ^3J = 7.13 Hz, $COCH_2\underline{C}H_3$), 0.93-0.72 (m, 4H; CH_2CH_2) ppm; **δC** (50 MHz, $CDCl_3$), 187 (C=0), 185 (C=O), 167 (CO_2Et), 160 (C=CH), 159 (C=CH), 156, 152, 148, 144 (quaternary aromatic C's), 127 (C=C),

117 (aromatic CH), 104 (aromatic CH), 59 ($CO\underline{C}H_2CH_3$), 30 (CH), 13 ($COCH_2\underline{C}H_3$), 5.9 (CH_2CH_2) ppm. **δF** (235 MHz, $CDCl_3$), -116 (m, aromatic F), -132 (m, aromatic F), -143 (m, aromatic F) ppm. **m/z** (EI); 313(M+), 267, 238; HRMS (EI), found 313.09316, $C_{15}H_{14}F_3NO_3$ requires 313.09258.

Ethyl 3-cyclopropylamino-2-(2,3,4,5-tetrafluorobenzoyl)acrylate, (102).

The mobile oil, ethyl (2-ethoxymetylene)-2,3,4,5-tetrafluorobenzoylacetate (1.11 g, 3.5 mmol), was suspended in DCM (20 ml) and cyclopropylamine (0.413 g, 7.25 mmol) added. The solution was stirred at 25°C for 3 hours, under an atmosphere of nitrogen. After evaporation *in vacuo* the residue was dried to yield a pale orange coloured solid, (0.95 g, 86 %); **m.p.** 59-61 °C; **TLC**, R_f (B) 0.58, R_f (D) 0.71; **C,H,N,** found: C, 53.93; H, 3.99; N, 4.15; $C_{15}H_{13}NF_4O_3$ requires C, 54.28; H, 3.92; N, 4.23 %; ν_{max} ($CHBr_3$ mull), 3225 (NH), 3169 (NH), 3110, 2939, (aromatic st.), 1695 (C=O), 1632 (C=O) cm^{-1}; λ_{max} (DCM) 314 (ε = 16544 $dm^{-3}mol^{-1}cm^{-1}$) nm; **δH** (250 MHz, $CDCl_3$), 10.90 (d, NH), 9.54 (NH), 8.23 (m, 1H, C=C), 6.99-6.86 (m, 1H, aromatic), 4.08-3.92 (q, 2H, ^3J = 7.13 Hz, $CO\underline{C}H_2CH_3$), 3.00-2.90 (m, 1H, CH), 1.11-1.07 (t, 3H, ^3J = 7.11 Hz, $COCH_2\underline{C}H_3$), 1.03-0.70 (m, 4H, CH_2CH_2) ppm; **δC** (50 MHz, $CDCl_3$), 187 (C=O), 167 (CO_2Et), 160 (C=CH), 149, 146, 144, 141, 138 (quaternary aromatic C's), 124 (C=C), 109 (aromatic CH), 59 ($O\underline{C}H_2CH_3$), 30 (CH), 13 ($OCH_2\underline{C}H_3$), 6 (CH_2CH_2) ppm; **δF** (235 MHz, $CDCl_3$), -139 (m, aromatic F), -141 (m, aromatic F), -155 (m, aromatic F), -159 (m, aromatic F) ppm; **m/z** (EI); 331 (M+), 285, 177; HRMS (EI), found 331.08253, $C_{15}H_{13}F_4NO_3$ requires 331.08316.

Ethyl 3-cyclopropylamino-2-(2,4,5-trifluorobenzoyl)acrylate, (103).

A solution of 2,4,5-trifluorobenzoylacetic acid ethyl ester (0.50 g, 2.0 mmol) and dimethylformamide dimethyl acetal (0.3 ml, 2.2 mmol) in dry THF (13 ml) was stirred overnight, at 25°C, under an atmosphere of nitrogen. Cyclopropylamine (0.3 ml, 4.3 mmol) was added dropwise, and stirring at 25°C was continued for a further 72 hours. After 3 days, the solution was evaporated *in vacuo* and dried to yield a damp orange solid as the *title compound*, (0.53 g, 85 %); **TLC**, R_f (B) 0.58, R_f (D) 0.74; ν_{max} ($CHBr_3$ mull); 3279 (NH), 3232 (NH), 3019 (aromatic st.), 1693 (C=O), 1630 cm^{-1}; **δH** (250 MHz, $CDCl_3$), 10.80 (d, NH), 9.30 (s, NH), 8.13 (m, 1H, C=CH), 7.26-7.06 (m, 1H, aromatic), 6.82-6.71 (m, 1H, aromatic), 4.09-3.89 (q, 2H, ^3J = 7.13 Hz, $CO\underline{C}H_2CH_3$), 2.91-2.82 (m, 1H, CH), 1.13-1.10 (t, 3H, ^3J = 7.10 Hz, $COCH_2\underline{C}H_3$), 1.05-0.86 (m, 4H, CH_2CH_2) ppm; **δF** (235 MHz, $CDCl_3$), -116 (m, aromatic F), -132 (m, aromatic F), -143 (m, aromatic F) ppm; **m/z** (EI), 314 (M+), 267, 159; HRMS (EI), found 313.09229, $C_{15}H_{14}F_3NO_3$ requires 313.09258.

Ethyl 1-cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylate, (104).

Ethyl 3-cyclopropylamino-2-(2,4,5-trifluorobenzoyl)acrylate (0.100 g, 0.32 mmol) was dissolved in DCM (20 ml). TMG (0.86 ml, 6.4 mmol) was added and the solution heated to reflux under nitrogen. After 20 hours, the solution was cooled and water (20 ml) added. The aqueous layer was separated and the organic layer washed with 2M HCl (2 x 20 ml), water (2 x 20 ml) and brine (2 x 30 ml). After drying over $MgSO_4$ the solution was evaporated *in vacuo* to give the *title compound* as a white solid, (0.75 g, 80 %); **m.p.** 202-205°C; **TLC**, R_f (B) 0.40, R_f (D) 0.63; **C,H,N,** found C, 61.29; H, 4.84; N, 4.28; $C_{15}H_{13}F_2NO_3$ requires C, 61.43; H, 4.44; N, 4.78 %; ν_{max} ($CHBr_3$ mull); 3010 (aromatic st.), 1729 (C=O), 1691 (C=O), 1623 cm^{-1}. λ_{max} (DCM) 330 (ε = 12682 $dm^3mol^{-1}cm^{-1}$), 316 (11707), 256 (16585), 248 (13658), 239 (11219)

nm; δH (250 MHz, $CDCl_3$), 8.5 (s, 1H, C=C), 8.26-8.18 (m, 1H, aromatic), 7.74-7.67 (m, 1H, aromatic), 4.4-4.3 (q, 2H, ^3J = 7.13 Hz, $CO\underline{C}H_2CH_3$), 3.4 (s, 1H, CH), 1.41-1.30 (t, 3H, ^3J = 7.08 Hz, $COCH_2\underline{C}H_3$), 1.17-1.10 (m, 4H, CH_2CH_2) ppm; **δC** (50 MHz, $CDCl_3$), 171 (C=O), 164 (C=O), 150 (C=CH), 146, 141, 139, 127, 124 (quaternary aromatic C's), 117 (aromatic CH), 108 (aromatic CH), 60 ($CO\underline{C}H_2CH_3$), 38 (CH), 14 $COCH_2\underline{C}H_3$), 8.8 (CH_2CH_2) ppm; **δF** (235 MHz, $CDCl_3$) -127 (aromatic F), -139 (aromatic F) ppm. **m/z** (EI), 293 (M+), 248, 221; HRMS (EI), found 293.08834, $C_{15}H_{13}F_2NO_3$ requires 293.08635 .

Ethyl 1-cyclopropyl-6,7,8-trifluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylate, (105).

Ethyl 3-cyclopropylamino-2-(2,3,4,5-tetrafluorobenzoyl)acrylate (0.15 g, 0.45 mmol) was dissolved in DCM (25 ml). TMG (1.21 ml, 9.0 mmol) was added and the solution heated to reflux under nitrogen. After 20 hours, the solution was cooled and water (20 ml) added. The aqueous layer was separated and the organic layer washed with 2M HCl (2 x 20 ml), water (2 x 20 ml) and brine (2 x 30 ml). After drying over $MgSO_4$ the solution was evaporated *in vacuo* to give the *title compound* as a pale yellow solid, (0.118 g, 76 %); **m.p.** 166-169°C; **TLC**, R_f (B) 0.48, R_f (D) 0.67; **C,H,N,** found C, 58.05; H, 4.10; N, 4.35; $C_{15}H_{12}F_3NO_3$ requires C, 57.88; H, 3.85; N, 4.50 %; ν_{max} ($CHBr_3$ mull); 3019 (aromatic st.), 1729 (C=O), 1693 (C=O), 1623 cm^{-1}. λ_{max} (DCM) 328 (ε = 11333 $dm^{-3}mol^{-1}cm^{-1}$), 316 (12888), 256 (11111), 248 (13333), 240 (12444) nm; δH (250 MHz, $CDCl_3$), 8.5 (s, 1H, C=C), 8.00-7.99 (m, 1H, aromatic), 4.38-4.29 (q, 2H, ^3J = 7.13 Hz, $CO\underline{C}H_2CH_3$), 3.87 (s, 1H, CH), 1.39-1.33 (t, 3H, ^3J = 7.08 Hz, $COCH_2\underline{C}H_3$), 1.27-1.15 (m, 4H, CH_2CH_2) ppm; **δC** (50 MHz, $CDCl_3$), 171 (C=O), 164 (C=O), 150 (C=CH), 146, 145, 141, 139, 127, 124 (quaternary aromatic C's), 108 (aromatic CH), 60 ($CO\underline{C}H_2CH_3$), 38 (CH), 14 $COCH_2\underline{C}H_3$), 8.8 (CH_2CH_2) ppm; **δF** (235 MHz, $CDCl_3$) -135 (aromatic F), -139

(aromatic F), -150 (aromatic F) ppm. **m/z** (EI), 311 (M+), 266; HRMS (EI), found 311.07771, $C_{15}H_{12}F_3NO_3$ requires 311.07693.

Ethyl 3-(4-fluoroanilino)-2-(2,3,4,5-tetrafluorobenzoyl)acrylate, (106).

The mobile oil, ethyl (2-ethyoxymethylene)-2,3,4,5-tetrafluorobenzoylacetate (0.20 g, 0.63 mmol), was suspended in DCM (10 ml) and 4-fluoroaniline (0.145 g, 1.31 mmol) added. The solution was stirred at 25°C for 3 hours, under an atmosphere of nitrogen. After evaporation *in vacuo* the residue was dried to yield a pale cream coloured solid, (0.21 g, 82 %); **m.p.** 88-90 °C; **TLC**, R_f (A) 0.18, R_f (B) 0.61; **C,H,N,** found: C, 55.92; H, 3.57; N, 3.80; $C_{18}H_{12}F_5NO_3$ requires C, 56.10; H, 3.12; N, 3.63 %; ν_{max} ($CHBr_3$ mull), 3071, 2987, (aromatic st.), 1698 (C=O), 1631 (C=O) cm^{-1}; λ_{max} (DCM) 348 (ε = 19789 $dm^3mol^{-1}cm^{-1}$), 240 (9243) nm; **δH** (250 MHz, $CDCl_3$), 12.5 (d, NH), 11.1 (NH), 8.47 (m, 1H, C=C), 7.24-6.97 (m, 5H, aromatic), 4.12-4.03 (m, 2H, J=7.08 Hz, $CO\underline{C}H_2CH_3$), 1.11-1.07 (m, 3H, J=7.04 Hz, $COCH_2\underline{C}H_3$), ppm; **δC** (50 MHz, $CDCl_3$), 171 (C=O), 165 (CO), 153 (C=CH), 162, 158, 148, 145, 143, 126 (quaternary aromatic C's), 134 (C=C), 119, 116, 109 (aromatic CH's), 103 (quaternary aromatic C), 59 ($O\underline{C}H_2CH_3$), 13 ($OCH_2\underline{C}H_3$) ppm; **δF** (235 MHz, $CDCl_3$), -115 (s, aromatic F), -139 (m, aromatic F), -140 (m, aromatic F), -153 (m, aromatic F), -156 (m, aromatic F), ppm; **m/z** (EI); 385 (M), 311, HRMS (EI), found 385.7348, $C_{18}H_{12}F_5NO_3$ requires 385.07373.

Ethyl 1-(4-fluorophenyl)-6,7,8-trifluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylate, (107).

Ethyl 3-(4-fluoroanilino)-2-(2,3,4,5-tetrafluorobenzoyl)acrylate (0.100 g, 0.26 mmol) was dissolved in DCM (15 ml). TMG (0.70 ml, 5.2 mmol) was added and the solution

heated to reflux under nitrogen. After 20 hours, the solution was cooled and water (20 ml) added. The aqueous layer was separated and the organic layer washed with 2M HCl (2 x 20 ml), water (2 x 20 ml) and brine (2 x 30 ml). After drying over $MgSO_4$ the solution was evaporated *in vacuo* to give the *title compound* as a bright orange solid, (0.82 g, 86 %); **m.p.** 216-218°C; **TLC**, R_f (B) 0.40, R_f (D) 0.63; **C,H,N,** found C, 58.98; H, 3.35; N, 3.78; $C_{18}H_{11}F_4NO_3$ requires C, 59.17; H, 3.01 ; 3.83 N, %; ν_{max} ($CHBr_3$ mull); 3077, 2932 (aromatic st.), 1729 (C=O), 1698 (C=O), 1623 cm^{-1}. λ_{max} (DCM) 330 (ε = 12682 $dm^{-3}mol^{-1}cm^{-1}$), 314 (16794), 295 (12778), 248 (11938), nm; **δH** (250 MHz, $CDCl_3$), 8.32 (s, 1H, C=C), 8.16-8.08 (m, 1H, aromatic), 7.750-7.45 (m, 2H, aromatic), 7.28-7.21 (m, 2H, aromatic), 4.4-4.3 (q, 2H, J=7.04 Hz, $CO\underline{C}H_2CH_3$), 1.46-1.24 (t, 3H, J=7.05 Hz, $COCH_2\underline{C}H_3$) ppm; **δC** (60 MHz, $CDCl_3$), 171 (C=O), 165 (C=O), 150 (C=CH), 164, 160, 146, 143, 141, 124 (quaternary aromatic C's), 138 (C=C), 127, 117, 109 (aromatic CH's), 61 ($CO\underline{C}H_2CH_3$), 14 $COCH_2\underline{C}H_3$) ppm; **δF** (235 MHz, $CDCl_3$) -110 (s, aromatic F), -134 (m, aromatic F), -138 (m, aromatic F), -150 (m, aromatic F) ppm. **m/z** (EI), 365 (M), 293 HRMS (EI), found 365.06638, $C_{18}H_{11}F_4NO_3$ requires 365.09751.

Ethyl 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-quinoline-3-carboxylate, (108).

Ethyl 1-cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylate (0.100 g, 0.34 mmol) was dissolved in NMP (15 ml). Piperazine (0.246 g, 2.86 mmol) was added and the reaction mixture was heated to 110°C under an atmosphere of nitrogen. After 4 hours, the solution was cooled and evaporated *in vacuo* to yield a pale yellow/orange solid. The crude product was washed with water (20 ml) and extracted with chloroform (2 x 20 ml). Finally the chloroform extracts were dried over $MgSO_4$ and evaporated *in vacuo* to yield the *title compound* as a yellow solid. (0.107 g, 89 %); **m.p.** 176-178°C; **TLC**, R_f (D) 0.21, R_f (F) 0.57; **C,H,N,** found C, 62.32; H, 5.94; N, 10.12; $C_{19}H_{22}FN_3O_3$ requires C, 63.50; H, 6.12; N, 11.69. ν_{max}

($CHBr_3$ mull), 3397 (NH st), 3018, 2965 (aromatic st.), 1720 (C=O), 1675 (C=O), 1620 (aromatic) cm^{-1}; **λmax** (DCM), 335 (ε = 10538 $dm^{-3}mol^{-1}cm^{-1}$), 322 (9417), 280 (28699) nm; **δH** (250 MHz, $CDCl_3$), 8.48 (s, 1H, C=CH), 8.0-7.94 (d, 1H, aromatic), 7.22 (s, 1H, aromatic), 4.39-4.30 (q, 2H, ^3J = 7.13 Hz, $CO\underline{C}H_2CH_3$), 3.42 (m, 1H, CH), 3.39-3.08 (m, 8H, CH_2's), 2.39 (b, 1H, NH), 1.40-1.25 (t, 3H, ^3J = 7.08 Hz, $COCH_2\underline{C}H_3$), 1.19-1.12 (m, 4H, CH_2CH_2) ppm; **δC** (50 MHz, $CDCl_3$), 173 (C=O), 165 (CO_2Et), 150 (C=CH), 155, 147, 144, 137, 122 (quaternary C's), 113, 109 (aromatic CH), 60 ($CO\underline{C}H_2CH_3$), 50, 45 (CH_2's), 34 (CH), 14 ($COCH_2\underline{C}H_3$), 7.9 (CH_2CH_2) ppm. **δF** (235 MHz, $CDCl_3$), -123 (s, aromatic F) ppm; **m/z** (EI), 359 (M+), 317, 287; HRMS (EI), found 359.16499, $C_{19}H_{22}FN_3O_3$ requires 359.16452.

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 2-(2,4,5-trifluorobenzoyl) -3-cyclopropylacrylate, (109), route (B).

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 2,4,5-trifluorobenzoylacetate (0.40 g, 0.96 mmol / g) was suspended in dry THF (20 ml). Dimethylformamide dimethyl acetal, (2.55 ml, 19.2 mmol) was added and the mixture was stirred under an atmosphere of nitrogen at 25°C, overnight. After 18 hours, cyclopropylamine (2.60 ml, 38.4 mmol) was added and the mixture was stirred for a further 72-80 hours at 25°C. The resin was then filtered and washed with copious amounts of the following solvents; DMF (x 2), methanol, water, THF, DMF and chloroform. The *title compound* was obtained as an orange coloured product (0.37g). **δC** (60 MHz, $CDCl_3$), 145, 133-125 (polystyrene), 118 (aromatic CH), 114, (polystyrene), 105 (aromatic CH), 69 (*p*-benzyloxy CH_2), 65 (benzyl CH_2), 30 (CH), 6 (CH_2CH_2) ppm.

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 6,7-difluoro-4-oxo-1-cyclopropyl-quinoline-3-carboxylate, (110).

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 2-(2,4,5-trifluorobenzoyl)-3-cyclopropylacrylate (0.20 g, 0.96 mmol / g) was suspended in DCM (20 ml). TMG (4.81 ml, 38.2 mmol) was added and the reaction mixture was heated to reflux, under an atmosphere of nitrogen, overnight. After 18 hours, the resin was cooled and filtered off, then washed with copious amounts of DMF (x 2), methanol, water, THF, DMF and chloroform. The *title compound* was dried *in vacuo* to yield a pale brown/orange product, (0.20 g). **δC** (60 MHz, $CDCl_3$), 145, 133-125 (polystyrene), 118 (aromatic CH), 114 (polystyrene), 105 (aromatic CH), 69 (*p*-benzyl CH_2), 66 (benzyl CH_2), 34 (CH), 7 (CH_2CH_2) ppm.

1-Cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid, (111).

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 6,7-difluoro-4-oxo-1-cyclo-propyl-quinoline-3-carboxylate (0.20 g, 0.96 mmol / g) was suspended in a solution of TFA/DCM (4:6, v/v, 10 ml) and stirred under an atmosphere of nitrogen at 25°C. After 1 hour, the resin was filtered off and washed with TFA followed by DCM. The filtrate was evaporated *in vacuo* to yield a pale yellow/brown oil. The residue was purified by short column chromatography on silica gel with $CHCl_3$ / MeOH / AcOH; 90 : 8 : 2. The collected fractions were evaporated to give a pale brown oil as the *title compound*, (16.5 mg, 56 %). **δH** (250 MHz, TFA-d), 9.12 (s, 1H, C=CH), 8.17-8.11 (m, 2H, aromatic), 8.05 (s, 1H, aromatic), 3.81 (s, 1H, CH), 1.38-1.13 (m, 4H, CH_2CH_2) ppm. **m/z** (EI); 265 (M), 221; HRMS (EI), found 265.05682, $C_{13}H_{19}F_2NO_2$ requires 265.05505.

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 6-fluoro-7-piperazinyl-4-oxo-1-cyclopropyl-quinoline-3-carboxylate, (112).

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 6,7-difluoro-4-oxo-1-cyclo propyl-quinoline-3-carboxylate (0.20 g, 0.96 mmol / g) was suspended in NMP (20 ml). Piperazine (2.00 g, 23.2 mmol) was added and the reaction mixture was heated to reflux under an atmosphere of nitrogen. After 4 hours, the resin was cooled, filtered off and washed with copious amounts of DMF (x 2), methanol, water, THF and chloroform. The yellow/orange coloured resin was dried *in vacuo* to yield the *title compound* (0.190 g). **δC** (60 MHz, $CDCl_3$), 145, 133-125 (polystyrene), 118 (aromatic CH), 114 (polystyrene), 106 (aromatic CH), 69 (p-benzyloxy CH_2), 64 (benzyl CH_2), 54, 50 (CH_2's), 35 (CH), 7.8 (CH_2CH_2) ppm.

1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-quinoline-3-carboxylic acid, Ciprofloxacin®, (113).

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 6-fluoro-7-piperazinyl-4-oxo-1-cyclopropyl-quinoline-3-carboxylate (0.10 g, 0.96 mmol / g) was suspended in a solution of TFA / DCM (4:6, v/v, 10 ml) and stirred under an atmosphere of nitrogen at 25°C. After 1 hour, the resin was filtered off and washed with TFA followed by DCM. The filtrate was evaporated *in vacuo* to yield a pale yellow/brown oil. The residue was purified by short column chromatography on silica gel (varian mega bond elute S1) with CH_3CN / H_2O / $tBuNH_2$; 18 : 2 : 1. The collected fractions were evaporated *in vacuo* to yield a pale orange solid as the *title compound*, Ciprofloxacin®, (0.017 g, 61 %). **δH** (400 MHz, TFA-d), 9.4 (s, 1H, C=CH), 8.28 (d, 1H, aromatic), 8.05 (s, 1H, aromatic), 4.20 (s, 1H, CH), 4.09-3.80 (m, 8H, CH2's), 1.31-1.04 (m, 4H, CH_2CH_2) ppm.

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 6-fluoro-7-(1-piperazinyl) -4-oxo-1-(4-fluoroanilino)-quinoline-3-carboxylate, (114).

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 2,4,5-trifluorobenzoylacetate (0.20 g, 0.96 mmol / g) was suspended in dry THF (15 ml). Dimethylformamide dimethyl acetal (1.27 ml, 19.2 mmol) was added and the mixture was stirred under an atmosphere of dry nitrogen, at 25°C, overnight. After 18 hours, 4-fluoroaniline (ml, 38.4 mmol) was added and the mixture was stirred for a further 72-80 hours, at 25°C. The resin was then filtered and washed with copious amounts of the following solvents; DMF (x 2), methanol, water, THF, DMF and chloroform. The resulting resin-bound enamide was suspended in a solution of TMG (4.81 ml, 38.2 mmol), in DCM (20 ml). To affect cyclization, the mixture was heated to reflux, under an atmosphere of nitrogen, overnight. After 18 hours, the resin was cooled and filtered off, then washed with copious amounts of DMF (x 2), methanol, water, THF, DMF and chloroform. The resin-bound quinolone was then suspended in NMP (20 ml) and piperazine (2.00 g, 23.2 mmol) added. The mixture was heated to 110°C, under an atmosphere of nitrogen, for 4 hours. On cooling, the resin was filtered off and washed with copious amounts of DMF (x 2), methanol, water, THF and chloroform. The *title compound* was dried *in vacuo* to yield a brown/orange product, (0.19 g). δC (250 MHz, $CDCl_3$), 145, 133-125 (polystyrene), 118 (aromatic CH), 114 (polystyrene), 105 (aromatic CH), 69 (*p*-benzyl CH_2), 66 (benzyl CH_2), 54, 50 (w, CH_2's) ppm.

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 6,8-difluoro-7-(1-piperazinyl)-4-oxo-1-(4-fluoroanilino)-quinoline-3-carboxylate, (115).

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 2,3,4,5-tetrafluorobenzoyl acetate (0.20 g, 0.96 mmol / g) was suspended in dry THF (15 ml).

Dimethylformamide dimethyl acetal (1.27 ml, 19.2 mmol) was added and the mixture was stirred under an atmosphere of dry nitrogen, at 25°C, overnight. After 18 hours, 4-fluoroaniline (ml, 38.4 mmol) was added and the mixture was stirred for a further 72-80 hours, at 25°C. The resin was then filtered and washed with copious amounts of the following solvents; DMF (x 2), methanol, water, THF, DMF and chloroform. The resulting resin-bound enamide was suspended in a solution of TMG (4.81 ml, 38.2 mmol), in DCM (20 ml). To affect cyclization, the mixture was heated to reflux, under an atmosphere of nitrogen, overnight. After 18 hours, the resin was cooled and filtered off, then washed with copious amounts of DMF (x 2), methanol, water, THF, DMF and chloroform. The resin-bound quinolone was then suspended in NMP (20 ml) and piperazine (2.00 g, 23.2 mmol) added. The mixture was heated to 110°C, under an atmosphere of nitrogen, for 4 hours. On cooling, the resin was filtered off and washed with copious amounts of DMF (x 2), methanol, water, THF and chloroform. The *title compound* was dried *in vacuo* to yield a brown/orange product, (0.19 g). δC (250 MHz, $CDCl_3$), 145, 133-125 (polystyrene), 118 (aromatic CH), 114 (polystyrene), 69 (*p*-benzyl CH_2), 66 (benzyl CH_2), 54, 50 (CH_2's) ppm.

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 6-fluoro-7-(2,6-dimethyl)piperazinyl-4-oxo-1-cyclopropyl-quinoline-3-carboxylate, (116).

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl-6,7-difluoro-4-oxo-1-cyclopropyl-3-quinoline carboxylate (0.20 g, 0.96 mmol / g) was suspended in NMP (20 ml). 2,6-Dimethylpiperazine (2.64 g, 23.2 mmol) was added and the reaction mixture was heated to reflux, under an atmosphere of nitrogen. After 4 hours, the resin was cooled, filtered off and washed with copious amounts of DMF (x 2), methanol, water, THF and chloroform. The orange coloured resin was dried *in vacuo* to yield the *title compound* (0.20 g). δC (60 MHz, $CDCl_3$), 145, 133-125 (polystyrene), 118 (aromatic CH), 114 (polystyrene), 109 (aromatic CH), 69 (*p*-benzyl CH_2), 66 (benzyl CH_2), 55, 52 (w,CH_2's), 36 (w, CH), 18 (CH_3's), 7 (CH_2CH_2) ppm.

1-Cyclopropyl-1,4-dihydro-6-fluoro-7-(2,6-dimethyl)piperazinyl-quinoline-3-carboxylic acid, (116a).

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl-6-fluoro-7-[2,6-dimethyl]-piperazinyl-4-oxo-1-cyclopropyl-3-quinoline carboxylate (0.20 g, 0.96 mmol / g) was suspended in a solution of TFA / DCM (4:6, v/v, 10 ml) and stirred under an atmosphere of nitrogen at 25°C. After 1 hour, the resin was filtered off and washed with TFA followed by DCM. The filtrate was evaporated *in vacuo* to yield a pale yellow/brown oil. The residue was purified by short column chromatography on silica gel with TFA / DCM. The collected fractions were evaporated *in vacuo* to yield a pale orange solid as the *title compound*, (0.020 g, %). **δH** (250 MHz, TFA-d), 9.29 (s, 1H, C=CH), 8.23 (b, 2H, aromatic), 4.18-3.90 (m, 7H, CH2's), 3.51-3.20 (b, 1H, NH), 1.76-1.25 (m, 7H) ppm.

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 2-(2,3,4,5-tetrafluoro-benzoyl)-3-*tert.*butylaminoacrylate, (117).

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl-2,3,4,5-tetrafluorobenzoyl acetate (0.20 g, 0.96 mmol / g) was suspended in THF (20 ml). Dimethylformamide dimethyl acetal (1.27 ml, 19.2 mmol) was added and the mixture was stirred under an atmosphere of nitrogen, at 25°C, overnight. After 18 hours, *tert.*butylamine (4.0 ml, 38.4 mmol) was added and the mixture was stirred for a further 72-80 hours, at 25°C. The resin was then filtered off and washed with copious amounts of the following solvents; DMF (x 2), methanol, water, THF, DMF and chloroform. The *title compound* was obtained as an orange coloured product (0.20 g). **δC** (60 MHz, $CDCl_3$), 145, 133-125 (polystyrene), 118 (aromatic CH), 114 (polystyrene), 69 (*p*-benzyl CH_2), 66 (benzyl CH_2), 29 (CH_3's) ppm.

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 6,7,8-trifluoro-4-oxo-1-*tert.*butyl-quinoline-3-carboxylate, (118).

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 2-(2,3,4,5-tetrafluorobenzoyl)-3-*tert.*butylaminoacrylate (0.20 g, 0.96 mmol / g) was suspended in DCM (20 ml). TMG (4.81 ml, 38.2 mmol) was added and the reaction mixture was heated to reflux, under an atmosphere of nitrogen, overnight. After 18 hours, the resin was cooled, filtered off, then washed with copious amonts of DMF (x 2), methanol, water, THF, DMF and chloroform. The *title compound* was dried *in vacuo* to yield a pale brown/orange product (0.20 g). δC (60 MHz, $CDCl_3$), 145, 133-125 (polystyrene), 118 (aromatic CH), 114 (polystyrene), 69 (*p*-benzyl CH_2), 66 (benzyl CH_2), 31 (CH_3's) ppm.

(4 x 1) Array- transesterification.

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl alcohol resin (0.10 g, 0.80 mmol / g) was measured manually into 4 seperate PINS (A1-A4). Each resin was suspended in toluene (4 ml). A catalytic amount of 4-DMAP was added to each PIN and the apparatus sonicated at room temperature for 30 minutes. After this time each of the following esters was added to the PINS (A1-A4) respectively; di-*t*-butyl malonate (0.34 g, 2.0 mmol), diethyl malonate (0.33 g, 2.0 mmol), 2,4,5-trifluorobenzoylacetic acid ethyl ester (0.19 g, 0.8 mmol), and 2,3,4,5-tetrafluorobenzoylacetic acid ethyl ester (0.21 g, 0.8 mmol). The apparatus was heated to reflux, for 3 days, under an atmosphere of chilled nitrogen. After 72 hours, each PIN was washed with copious amounts of toluene, DMF, dioxane:water, ether, and finally DCM. The PINS containing the resin samples were dried, *in vacuo*, overnight to yield the following products, (0.10 g);

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl *t*-butyl acetate; ν_{max} (KBr disc); 1751 (C=O), 1736 (C=O), cm^{-1}; δC (60MHz, $CDCl_3$), 145, 133-125 (polystyrene), 114 (polystyrene), 69 (*p*-benzyloxy CH_2), 65 (benzyl CH_2), 42 ($CO\underline{C}H_2CO$), 27 ($COC(CH_3)_3$) ppm.

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl ethyl acetate; ν_{max} (KBr disc) 1750 (C=O), 1736 (C=O), cm^{-1}; δC (60MHz, $CDCl_3$), 168 (C=O), 145, 133-125 (polystyrene), 114 (polystyrene), 69 (*p*-benzyloxy CH_2), 65 (benzyl CH_2), 61 ($CO\underline{C}H_2CH_3$), 41 ($CO\underline{C}H_2CO$), 14 ($COCH_2\underline{C}H_3$) ppm.

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 2,4,5-trifluorobenzoylacetate; ν_{max} (KBr disc) 1751 (C=O), 1735 (C=O), cm^{-1}; δC (60 MHz, $CDCl_3$), 145, 133-125, (polystyrene), 119 (aromatic CH), 114 (polystyrene), 107 (aromatic CH), 70 (*p*-benzyl CH_2), 64 (benzyl CH_2), 49 ($CO\underline{C}H_2CO$) ppm.

Copoly(styrene-1%-divinylbenzene)*p*-benzyloxybenzyl 2,3,4,5-tetrafluorobenzoyl-acetate; ν_{max} (KBr disc) 1751 (C=O), 1734 (C=O), cm^{-1}; δC (60MHz, $CDCl_3$), 145, 133-125 (polystyrene), 117 (aromatic CH), 114 (polystyrene), 69 (*p*-benzyloxy CH_2), 65 (benzyl CH_2), 48 ($CO\underline{C}H_2CO$) ppm.

Loading of resin to DIVERSOMER™ Apparatus.

p-Benzyloxybenzyl alcohol resin (1.0 g, 0.84 mmol / g) was suspended in chloroform (50 ml), while an additional lot of *p*-benzyloxybenzyl alcohol resin (3.5 g, 0.93 mmol / g) was suspended in chloroform (100 ml). Using the Tecan™ Robotic Sample Processor, (RSP), 5 ml (5 x 1 ml) of the first resin slurry was dispensed into each of the PINS, (A1-C4), and 5 ml (5 x 1 ml) of the second resin was dispensed into each of the PINS, (D1-J4). The holder block containing the 40 PINS, (A1-J4) was drained by gravity. The resins in the PINS were then extensively rinsed with toluene (3 x 2 ml) using the Tecan™ RSP.

40 Unit Quinolone array-R1S1 (transesterification).

A solution of 4-DMAP (1.02 g, 8.36 mmol) in toluene (135 ml) was prepared. 2,4,5-Trifluorobenzoylacetic acid ethyl ester, X (1.82 g, 7.4 mmol) and 2,3,4,5-tetrafluorobenzoylacetic acid ethyl ester, Y (5.88 g, 22.19 mmol) were dissolved in toluene (30 & 70 ml respectively). The 4-DMAP solution was dispensed (1 x 3 ml) into each of the 40 reaction wells, (A1-J4) using the Tecan™ RSP. The β-keto esters, X & Y were dispensed, (1 x 2 ml) into the reaction wells (A1-C4) and (D1-J4) respectively using the Tecan™ RSP. To form the resin bound β-ketoesters, the PINS containing the resins were submerged in the corresponding reaction wells. The manifold was attached and the apparatus was agitated in an ultrasound bath, at 110°C, for 18 hours, while an atmosphere of chilled nitrogen was maintained. After 18 hours, the ultrasound bath and apparatus were cooled to 25°C. The PINS were drained and washed repeatedly by dispensing fresh solvent directly to the resins in the PINS robotically; DMF (4 x 2 ml), methanol (4 x 2 ml), water (4 x2 ml), THF (4 x 2 ml), and DCM (6 x 2 ml). After each wash, the PINS were allowed to drain by gravity. The filtrates were collected in a common reservoir and monitored by TLC. The holder block and PINS containing the resin-bound products were dried *in vacuo* overnight.

40 Unit Quinolone array-R2S1 (DMFA-activation).

DMFA (25.87 g, 217 mmol) was suspended in dry THF (200 ml). Using the Tecan™ RSP, the dimethylformamide dimethyl acetal solution was dispensed, (1 x 4 ml) into clean reaction wells (A1-J4). The PINS containing the resin-bound β-keto esters (A1-J4) were submerged in the reaction wells, the manifold was attached and the apparatus was agitated by ultrasound, for 18 hours, at 25°C, under an atmosphere of nitrogen.

40 Unit Quinolone array-R2S2 (amine displacement)

After 18 hours, each of the following amines were dissolved in dry THF and dispensed, (1 x 1 ml) directly into the appropriate PINS using the Tecan™ RSP; while maintaining an anhydrous environment at the Tecan™ workstation with Argon: cyclopropyl amine (6.32 g, 110 mmol) to PINS (A1-J1), 4-fluoroaniline (12.16 g, 110 mmol) to PINS (A2-J2), 2,4-difluoroaniline (14.13 g, 110 mmol) to PINS (A3-J3) and tert.butylamine (8.00 g, 110 mmol) to PINS (A4-J4). The apparatus was agitated by ultrasound, at 25°C, under an atmosphere of nitrogen. After 72 hours, the PINS were drained and washed repeatedly by dispensing fresh solvent directly to the resins in the PINS robotically; DMF (4 x 2 ml), methanol (4 x 2 ml), water (4 x 2 ml), THF (4 x 2 ml), and DCM (6 x 2 ml). After each wash, the PINS were allowed to drain by gravity. The filtrates were collected in a common reservoir and monitored by TLC. The holder block and PINS containing the resin-bound products were dried *in vacuo* for 5 hours.

40 Unit Quinolone array-R3S1 (cyclisation).

A solution of TMG (101 g, 878 mmol) in DCE (50 ml) and DCM (100 ml) was robotically dispensed, (1 x 5 ml) into 40 reaction wells (A1-J4). To affect cyclization of the resin-bound enamides, the PINS were submerged in the reaction wells, the manifold was attached and the apparatus was agitated by ultrasound, at 55°C, under an atmosphere of chilled nitrogen. After 18 hours, the ultrasound bath and apparatus were cooled to 25°C. The PINS were drained and washed repeatedly by dispensing fresh solvent directly to the resins in the PINS robotically; DMF (4 x 2 ml), methanol (4 x 2 ml), water (4 x 2 ml), THF (4 x 2 ml), and DCM (6 x 2 ml). After each wash, the PINS were allowed to drain by gravity. The filtrates were collected in a common

reservoir and monitored by TLC. The holder block and PINS containing the resin-bound products were dried *in vacuo* for 2-3 hours.

40 Unit Quinolone array-R4S1 (arylation).

Solutions of piperazinyl derivatives were prepared in NMP and dispensed into the appropriate reaction wells using the Tecan™ RSP; piperazine (7.83 g, 91 mmol) to wells (A1-A4) and (D1-D4), 2,6-dimethylpiperazine (10.39 g, 91 mmol) to wells (B1-B4) and (E1-E4), 1-ethylpiperazine (11.85 g, 91 mmol) to wells (C1-C4) and (F1-F4), 1-methylpiperazine (6.34 g, 55 mmol) to wells (G1-G4), 2-methylpiperazine (5.46 g, 55 mmol) to wells (H1-H4), 1-isopropylpiperazine (1.50 g, 12 mmol) to wells (I1-I4), and piperidine (4.64 g, 55 mmol) to wells (J1-J4). The PINS, containing the resin-bound quinolones were submerged in the reaction wells. The manifold was attached and the apparatus agitated by ultrasound, at 110°C, for 4 hours, under an atmosphere of chilled nitrogen, for 4 hours. On cooling, the PINS were drained and washed repeatedly by dispensing fresh solvent directly to the resins in the PINS robotically; DMF (4 x 2 ml), methanol (4 x 2 ml), water (4 x 2 ml), THF (4 x 2 ml), and DCM (6 x 2 ml). After each wash, the PINS were allowed to drain by gravity. The filtrates were collected in a common reservoir and monitored by TLC. The holder block and PINS containing the resin-bound products were dried *in vacuo* overnight.

40 Unit Quinolone array-R5S1 (acid cleavage).

To effect cleavage the quinolones from the solid support, the PINS were submerged in the reaction wells containing a solution of TFA/DCM (4:6, v/v, 5 ml aliquots). The apparatus was assembled and agitated by ultrasound, at 25°C, for 1 hour. The PINS were removed from the reaction wells and drained by gravity into the corresponding

wells containing the dissolved products. Repetitive washing of the solid support to thoroughly extract the products was carried out The PINS were submerged in reaction wells containing TFA/DCM (4:6, v/v) and DCM (5 ml aliquots) sequentially, agitated by ultrasound, at 25°C, for 15 minutes and drained by gravity between each extraction cycle. All the filtrate extracts were robotically combined and analysed by TLC, R_f (D) 0.27. Concentration of the crude final product solutions was achieved by a positive nitrogen flow through a 40-channel manifold.

40 Unit Quinolone array-Purification.

Following concentration, the crude products were manually weighed. The 40 quinolones (A1-J4) were purified by gravity chromatography on silica gel utilizing SPE cartridges, (Varian Mega bond elute S1). Intially, a holder rack containing 40 SPE cartridges was placed on the Tecan™ workstation. The cartridges were preconditioned with CH_3CN / H_2O / $tBuNH_2$; 18:2:1 with the Tecan™ RSP. The products were dissolved in the minimum amount of TFA and preloaded (0.5 ml) on the cartridges. The products were eluted robotically with CH_3CN / H_2O / tBuNH$_2$: 18:2:1. In total, ten racks were collected and using theTecan™ RSP each of the filtrates from the ten elutes were analysed by TLC. Appropriate fractions, racks D, E & F were combined robotically and transferred to Speedvac tared vials. Concentration of the final product solutions was achieved by applying vacuum in a speedvac at low temperature, overnight. The pure products were obtained as damp pale brown solids, weighing between 3.6-27.1 mg, corresponding to 8-90 % yield .

***Bis*-(phenanthren-9-yl)methanol, (121).**

A solution of 9-bromophenanthrene (100 g, 39.0 mmol) in dry THF (100 ml) was slowly added to magnesium turnings (9.36 g, 39.0 mmol) under an atmosphere of

nitrogen. A crystal of iodine was added to initiate the exothermic reaction. After 2 hours stirring, a thick green jelly formed and a solution of methyl formate (12 ml, 19.0 mmol) in dry THF (25 ml) was added over a period of 20 minutes. After 2 hours under reflux, iced 2M HCl (100 ml) was added and a white solid precipitated. The precipitate was filtered and washed with water (2 x 50 ml) and ether (2 x 50 ml) to give a white solid (60 g, 80 %) as the *title compound.* **m.p.** 237-240°C, (Lit.[217] 238-239°C); **TLC**, R_f (A) 0.13, R_f (B) 0.67; **C,H,** found C, 90.77; H, 5.29; $C_{29}H_{20}O$ requires C, 90.02, H, 5.20; ν_{max} ($CHBr_3$ mull) 3408 (OH), 3100 (aromatic st.), 1628, 1610, 1500 (aromatic C=C) cm^{-1}; **δH** (200 MHz, CD_3SOCD_3), 8.86-8.74 (d, 2H, ^3J = 8.4 Hz, aromatic), 8.79-8.76 (d, 2H, ^3J = 8.2 Hz, aromatic), 8.17-8.15 (d, 2H, ^3J = 8.05 Hz, aromatic), 7.80-7.51 (m, 12H, aromatic), 7.20 (d, 1H, ^3J = 6.1 Hz, CH), 2.5 (s, 1H, OH) ppm. **δC** (50 MHz, CD_3SOCD_3), 137.8, 130.9, 130.2, 130.1, 129.7 (quaternary aromatic C's), 128.7, 126.8, 126.4, 125.6, 124.6, 123.3 122.6, (aromatic CH), 68.1 (CHOH) ppm; **m/z** (FAB) 384 (M+), 367, 205; HRMS (FAB), found 384.15146, $C_{29}H_{20}0$ requires 384.15141.

***8bH*-Tetrabenzo[*a,c,g,i*]fluorene, (122).**

Bis-(phenanthren-9-yl)methanol (59 g, 15.0 mmol) was suspended in DCM (200 ml) and trifluoroacetic acid (75 ml) added. The reaction was monitored by the brief appearance of a blue colour. After stirring for 30 minutes the yellow material was evaporated to dryness. TFA was removed by successive evaporation of freshly added DCM (5 x 100 ml). The resulting residue was suspended in ether and filtered to yield a yellow solid, (44 g, 89 %). **m.p.** 279-280°C (Lit[217] 280-282°C); **TLC**, R_f (A) 0.65, R_f (B) 0.77; **C,H,** found C, 95.43; H, 4.96; $C_{29}H_{18}$ requires C, 95.34, H, 4.66; ν_{max} ($CHBr_3$ mull) 1638, 1606 (aromatic) cm^{-1}. λ_{max} (DCM) 375 (ε = 11105 $dm^{-3}mol^{-1}cm^{-1}$, 326 (4644), 253 (58575) nm; **δH** (200 MHz, $CDCl_3$), 8.82-8.68 (m, 2H, aromatic),

8.22-7.07 (m, 15H, aromatic), 5.40 (s, 2H, CH) ppm. **m/z** (FAB) 365 (M); HRMS (FAB), 365.13303, $C_{29}H_{18}$ requires 365.13356.

1-(17'-Tetrabenzo[*a,c,g,i*]fluorenyl)prop-2-ene, (124).

To a suspension of *8bH*-tetrabenzo[*a,c,g,i*]fluorene, (43 g, 12.0 mmol) in dry THF (200 ml) at -78°C, n-butyllithium (1.6M in hexane, 80 ml, 13.0 mmol, 1.1equiv) was added and the orange solution stirred for 1 hour, under an atmosphere of nitrogen. Then allyl bromide (14.4 g, 12.0 mmol, 1equiv) was added dropwise and the solution stirred for a further 1 hour at -78°C. On warming to room temperature, the solution was left stirring for a further 2 hours after which a saturated solution of ammonium chloride (200 ml) was added followed by DCM (2 x 200 ml). The organic layer was separated, dried over magnesium sulphate and evaporated *in vacuo* to give a dark yellow solid (33.8 g, 70 %). **m.p.** 114-116°C, (Lit.[217] 118-120°C); **TLC**, R_f (A) 0.62; **C,H,** found C, 91.46; H, 5.13; $C_{32}H_{22}$ requires C, 94.58, H, 5.41; ν_{max} ($CHBr_3$ mull), 3070 (aromatic st.), 2980, 2900, 2850 (alkyl st.), 1638 (C=C), 1609, 1500 (aromatic) cm^{-1}; λ_{max} 380 (DCM) (ε = 22315 $dm^3mol^{-1}cm^{-1}$), 364 (23119), 301 (51286), 295 (31222), 253 (80386) nm; **δH** (200 MHz, $CDCl_3$), 8.80-8.66 (m, 6H, aromatic), 8.21-8.19 (m, 2H, aromatic), 7.72-7.57 (m, 8H, aromatic), 4.95 (s, 1H, Tbf CH), 4.80-4.71 (m, 1H, CH), 4.43-4.24 (m, 2H, CH_2 3-propene), 3.32-3.29 (dd, 2H, CH_2 1-propene) ppm; **δC** (50 MHz, $CDCl_3$), 143.5, 136.7, 131.2, 130.3, 128.5, 127.6 (quaternary aromatic C's), 131.9, 127.6, 125.7, 125.5, 124.8, 124.3, 124.1, 123.4, (aromatic CH's), 116 (=CH_2), 46.7 (C-17), 37.6 (CH_2) ppm; **m/z** (FAB) 406 (M), 365; HRMS (FAB), found 406.17214, $C_{32}H_{22}$ requires 406.17215.

3-(17'-Tetrabenzo[*a,c,g,i*]fluorenyl)propanol, (125).

A solution of 1-(17-tetrabenzo[*a,c,g,i*]fluorenyl)prop-2-ene, (10 g, 24.6 mmol) in dry THF (66 ml) was cooled to <20°C under nitrogen. 9-BBN (59 ml, 0.5M in THF, 1.2 equiv) was added and the solution stirred for 3 hours. After 3 hours, aqueous sodium hydroxide (1.67 g in 17 ml water) was added followed by hydrogen peroxide (3.3 ml, 30% in water, 1.2 equiv) and the temperature rose to ca. 45°C. The reaction mixture was stirred, at 60°C, for 1 hour and then cooled to room temperature. Fresh hydrogen peroxide (1 equiv) was added and the solution left to stir overnight. The product was extracted with DCM / methanol (9:1, 200 ml), washed with water, aqueous sodium hydrogen carbonate and dried over magnesium sulphate. After evaporation of the solvents, the residue was recrystallised from THF / $CHCl_3$ to give a white solid (4.01 g, 40 %). **m.p.** 187-189°C, (Lit.[217] 188-189°C); **TLC,** R_f (A) 0.14; **C,H,** found C, 82.00; H, 5.30; $C_{32}H_{24}O$ requires C, 90.56, H, 5.66 ν_{max} ($CHBr_3$ mull) 3317 (OH), 3010 (aromatic st.), 2950, 2860 (alkyl st.), 1604, 1500 (aromatic) cm^{-1}; λ_{max} 380 (DCM) ($\varepsilon = 20400 dm^3 mol^{-1} cm^{-1}$), 365 (20900), 295 (26600), 254 (36280) nm; **δH** (200 MHz, $CDCl_3$), 8.95-8.91 (m, 4H, aromatic), 8.57-8.54 (d, 2H, ^3J = 7.9 Hz, aromatic), 8.35-8.33 (d, 2H, ^3J = 7.9 Hz, aromatic), 7.78-7.65 (m, 8H, aromatic), 5.31 (s, 1H, Tbf CH), 3.98 (t, 1H, ^3J = 5.1 Hz, OH), 2.84-2.79 (m, 2H, CH_2), 2.58-2.50 (m, 2H, CH_2), 0.32-0.28 (m, 2H, CH_2) ppm; **δC** (50 MHz, $CDCl_3$), 144.4, 135.6, 130.7, 129.7, 128.0 (quaternary aromtic C's), 158.3, 127.8, 127.1, 126.5, 126.3, 125.9, 125.3, 124.5, 123.9, 123.6 (aromatic CH's), 60.4 ($\underline{C}H_2OH$), 46.1 (C-17), 29.9, 25.6 (CH_2) ppm; **m/z** (FAB) 424 (M), 407, 365; HRMS (FAB), found 424.18273, $C_{32}H_{24}O$ requires 424.18270.

3-(17'-Tetrabenzo[*a,c,g,i*]fluorenyl)propyl 2,4,5-trifluorobenzoylacetate, (126).

3-(17'-Tetrabenzo[*a,c,g,i*]fluorenyl)propanol (0.120 g, 0.28 mmol) was dissolved in toluene (20 ml) and a catalytic amount of 4-DMAP added. To this solution 2,4,5-trifluorobenzoylacetic acid ethyl ester (0.20 g, 0.84 mmol, 3 equiv.) was added and the mixture refluxed, under an atmosphere of nitrogen. After 72 hours the mixture was cooled in an ice/water bath and quenched with a saturated solution of ammonium chloride (10 ml). The product was extracted with toluene (4 x 50 ml), dried over $MgSO_4$ and concentrated *in vacuo* to give a pale brown oil. The product was purified by dry flash chromatography (9:1, MeOH / $CHCl_3$) to yield the *title compound* as a pale brown solid (0.049 g, 56 %). **m.p.** 152-153°C; **TLC**, R_f (C) 0.20, R_f (B) 0.69;.**C,H,** found: C, 75.18, H, 6.12; $C_{41}H_{27}F_3O_3$ requires C, 78.84, H, 4.32 %; ν_{max}($CHBr_3$ mull) 2950, 2890 (CH st.), 1735 (C=O), 1691 (C=O), 1625, 1511 (aromatic) cm^{-1}. λ_{max} (DCM) 380 (ε = 16375 $dm^3mol^{-1}cm^{-1}$), 364 (17187), 301 (51250), 288 (43750) nm; **δH** (250 MHz, $CDCl_3$), 8.81-8.64 (m, 6H, aromatic), 8.25-8.20 (m, 2H, aromatic), 7.73-7.57 (m, 9H, aromatic), 6.95-6.74 (m, 1H, aromatic), 5.07 (t, 1H, Tbf CH), [12.35 (s), 5.36 (s), 3.54 (d, ^3J = 3.87 Hz)] 2H, 3.61 (t, 2H, CH_2), 2.76-2.65 (m, 2H, CH_2), 0.75-0.61 (m, 2H, CH_2) ppm; **δC** (50 MHz, $CDCl_3$), 187.4 (C=O), 166.0 (C=O), 143.2, 136.9, 131.2, 130.3, 128.4, 127.8, (quaternary C's), 127.3, 126.8, 125.9, 125.7, 125.0, 124.1, 123.4, (aromatic CH's), 118.2 (aromatic CH), 106 (aromatic), 65.7 (CH_2O), 49.0 ($CO\underline{C}H_2CO$), 46.3 (C-17), 29.6 (CH_2), 21.5 (CH_2) ppm; **m/z** (FAB) 624 (M), 424, 365; HRMS (FAB), found 624.19130, $C_{41}H_{27}F_3O_3$ requires 624.19121.

Ethyl 4-(10-bromodecyloxy)benzoate, (128).

Ethyl 4-hydroxybenzoate (5 g, 30 mmol) was dissolved in dry DMF (100 ml) and sodium hydride (2.0 g, 80 mmol) slowly added. After 2 hours stirring, at room

temperature, a white solid precipitated and a solution of 1,10-dibromodecane (30.0 g, 100 mmol) in dry DMF (50 ml) was added. After heating to 100°C the reaction was stirred for another 12 hours. On cooling, the product was extracted with ether (3 x 150 ml), washed with water (3 x 150 ml), dried over magnesium sulphate and evaporated to dryness to give an off white solid. The crude product was purified by dry flash chromatography (20 % DCM in heptane - 100 % DCM) to yield a white solid (6.2 g, 54 %) as the *title compound.* **m.p.** 35-37°C; **C,H,** found: C 58.90; H, 7.92 $C_{19}H_{29}BrO_3$ requires C, 59.22; H, 7.53 %; **TLC**, R_F (A) 0.77; ν_{max} ($CHBr_3$ mull) 2930, 2854 (CH st), 1707 (C=O), 1605, 1508 (aromatic) cm^{-1}; λ_{max} (DCM) 256 (ε = 4817 $dm^3mol^{-1}cm^{-1}$) nm; **δH** (200 MHz, $CDCl_3$), 7.96 (m, 2H, aromatic), 6.87 (m, 2H, aromatic), 4.32 (q, 2H, ^3J = 7.14 Hz, $CO_2CH_2CH_3$), 3.97 (t, 2H, ^3J = 6.49 Hz, 2 x H-1,alkyl), 3.38 (t, 2H, ^3J = 6.83, alkyl), 1.90-1.66 (m, 4H, alkyl), 1.39-1.29 (m, 15H, alkyl) ppm; **δC** (50 MHz, $CDCl_3$), 166.2 (C=O), 162.7, 131.5, 122.5, 113.8 (aromatic), 67.9 (alkyl), 60.45 ($CO_2\underline{C}H_2CH_3$), 33.9 - 25.82 (alkyl), 14.26 ($CO_2CH_2\underline{C}H_3$) ppm; **m/z** (FAB) 388 (M+2), 386 (M+), 166; HRMS (FAB), found 385.13607, $C_{19}H_{29}O_3Br$ requires 385.13783.

Ethyl 4-(10-(17'-Tetrabenzo[*a,c,g,i*]fluorenyl)decyloxy)benzoate, (129).

17-Tetrabenzo[*a,c,g,i*]fluorene (5.0 g, 14.0 mmol) was suspended in a degassed solution of dioxane (100 ml). The reaction mixture was heated to reflux, under an atmosphere of nitrogen and a solution of tetrabutylammonium hydroxide (10 ml) in degassed dioxane (30 ml) added whereby a yellow precipitate formed. The solid was separated by filtration under nitrogen, washed with warm dioxane (2 x 50 ml) and ether (2 x 50 ml). The resulting bright yellow solid was resuspended in dioxane (250 ml) and ethyl 4-(10-bromodecyloxy)benzoate (4.76 g, 12.0 mmol) added. The mixture was refluxed under an atmosphere of nitrogen for 2.5 hours until the precipitate dissolved. After removal of all volatiles *in vacuo,* a dark green oil was formed and the

product extracted with ether, (2 x 100 ml). The crude brown solid was then purified by dry flash chromatography (5 % ether in hexane - 100 % DCM) to yield a beige solid (5.70 g, 63 %) as the *title compound.* **m.p.** 61-63°C; **C,H,.** found C, 84.61, H, 8.18, $C_{48}H_{46}O_3$ requires C, 85.97, H, 6.86 %; **TLC** R_f (B) 0.60; ν_{max} ($CHBr_3$ mull) 3067 (aromatic st), 2930, 2851 (CH st), 1701 (C=O), 1605, 1507 (aromatic); λ_{max} (DCM) 380 (ε = 19287 $dm^3mol^{-1}cm^{-1}$), 370 (20126), 301 (46961), 288 (38575), 255 (101469) nm; **δH** (200 MHz, $CDCl_3$), 8.81-8.76 (m, 4H, aromatic), 8.70-7.67 (m, 2H, aromatic), 8.01-7.95 (m, 2H, aromatic), 7.72-7.58 (m, 8H, aromatic), 6.87-6.82 (m, 2H, aromatic), 5.00 (t, 1H, ^3J = 4.39 Hz, Tbf H), 4.40-4.31 (q, 2H, ^3J = 7.12 Hz, $CO_2\underline{C}H_2CH_3$), 3.85 (t, 2H, ^3J = 6.57 Hz, alkyl), 2.63-2.55 (m, 2H, alkyl), 1.67-1.56 (m, 2H, alkyl, 1.39 (t, 3H, ^3J = 7.12 Hz, $CO_2CH_2\underline{C}H_3$), 1.28-0.72 (m, 14H, alkyl) ppm. **δC** (50 MHz, $CDCl_3$), 166.2 (C=O), 162.9 (aromatic CH), 131.3 (aromatic CH), 144.2, 136.6, 131.2, 130.2, 128.9, 127.9 (quaternary aromatic C's), 126.0, 125.72, 125.5, 124.8, 124.3, 123.3, 122.4 (aromatic CH's), 113.4 (aromatic CH), 67.9 ($CO_2\underline{C}H_2CH_3$), 47.07 (C-17), 33.36-25.67 (alkyl) ppm; **m/z** (FAB) 670 (M), 365; HRMS(FAB), found 670.34480, $C_{48}H_{46}O_3$ requires 670.34470.

4-(10-(17'-Tetrabenzo[*a,c,g,i*]fluorenyl)decloxy)benzyl alcohol, (130).

Ethyl 4-(10-(17'-tetrabenzo[*a,c,g,i*]fluorenyl)decyloxy)benzoate (1.0 g, 1.5 mmol)) was dissolved in dry THF (20 ml) and diisobutylaluminium hydride (4 ml, 4.0 mmol, 1.0M in DCM) was added. After 2.5 hours at room temperature the solution was poured into 2M HCl (30 ml). The product was extracted with ethyl acetate (2 x 50 ml), washed with aqueous sodium hydrogen carbonate (20 ml), water (2 x 50 ml) and dried over magnesium sulphate. Evaporation *in vacuo* give a pale yellow solid (0.73 g, 77 %) as the *title compound.* **m.p.** 69-71°C; **TLC** R_f (B) 0.44; **C,H,** found: C, 88.40; H, 7.64; $C_{46}H_{44}O_2$ requires C, 87.89; H, 7.00 %; ν_{max} ($CHBr_3$ mull) 3593,

3433 (OH st.), 2927, 2852 (alkyl st.), 1609, 1511 (aromatic) cm^{-1}; λ_{max} (DCM) 380 (ε = 17088 $dm^3mol^{-1}cm^{-1}$), 364 (17722), 301 (40506), 289 (35443), 252 (62658) nm; **δH** (250 MHz, $CDCl_3$), 8.82-8.67 (m, 6H, aromatic), 8.26-8.21 (m, 2H, aromatic), 7.82-7.53 (m, 8H, aromatic), 7.28-7.21 (m, 2H, aromatic), 6.90-6.8 (m, 2H, aromatic), 5.01 (t, 1H, ^3J = 4.33 Hz, C-17), 4.60 (s, 2H, CH_2OH), 3.96-3.82 (t, 2H, ^3J = 6.55 Hz, alkyl), 2.64-2.55 (m, 2H, alkyl), 2.39 (b, 1H, OH), 1.66-1.52 (m,,2H, alkyl), 1.28-0.35 (m, 14H, alkyl) ppm; **δC** (50 MHz, $CDCl_3$), 158.6 (quaternary aromatic C), 144.2, 136.6, 132.5, 131.1, 130.2, 127.9 (quaternary aromatic C's), 128.6, 128.4, 127.3, 126.6, 125.7, 125.4, 124.8, 124.3 (aromatic CH's), 123.3, 114.3 (aromatic CH's), 67.8 ($\underline{C}H_2OH$), 64.9 (CH_2O), 47.0 (C-17), 33.3-22.0 (alkyl CH's) ppm; **m/z** (FAB) 629 (M+1), 522, 365; HRMS (FAB), found 628.33580 $C_{46}H_{44}O_2$ requires 628.33413.

4-(10-(17'-Tetrabenzo[*a,c,g,i*]fluorenyl)decyloxy)benzyl 2,4,5-trifluorobenzoyl acetate, (131).

4-(10-(17'-Tetrabenzo[*a,c,g,i*]fluorenyl)decyloxy)benzyl alcohol (0.60 g, 1 mmol) was dissolved in toluene (15 ml) and a catalytic amount of DMAP added. 2,4,5-Trifluorobenzoyl acetic acid ethyl ester (0.70 g, 3 mmol, 3 equiv.) was added and the reaction mixture refluxed, under an atmosphere of nitrogen. After 72 hours, the mixture was cooled in an ice/water bath and quenched with a saturated solution of ammonium chloride (20 ml). The product was extracted with toluene (4 x 70 ml), dried over magnesium sulphate and concentrated *in vacuo* to give a pale brown oil. The product was purified by dry flash chromatography (MeOH / $CHCl_3$, 9:1) to yield the *title compound* as a dark brown solid. (0.21 g, 26 %); **m.p.** 63-65°C; **TLC**, R_f (A) 0.39, R_f (B) 0.71; **C,H,** found C, 77.36, H, 6.48, $C_{55}H_{47}F_3O_4$ requires C, 79.71; H, 5.67 %; ν_{max} ($CHBr_3$ mull) 2927, 2854 (CH st. alkyl), 1733 (C=O), 1688 (C=O), 1622, 1511 (aromatic) cm^{-1}; λ_{max} (DCM) 380 (ε = 17355 $dm^3mol^{-1}cm^{-1}$), 364

(18181), 300 (45454), 289 (42148), 263 (76859), 247 (66942) nm. **δH** (250 MHz, $CDCl_3$), 8.83-8.66 (m, 6H, aromatic), 8.29-8.25 (m, 2H, aromatic), 7.77-7.58 (m, 9H, aromatic), 7.31-7.19 (m, 2H, aromatic), 6.98-6.78 (m, 3H, aromatic), 5.07 (t, 1H, ^3J = 4.41 Hz, C-17), [12.6 (s), 5.84 (s), 3.94 (d, ^3J = 3.8 Hz)] 2H, 3.88 (m, 2H, alkyl), 2.64-2.58 (m, 2H, alkyl), 1.67-1.56 (m, 2H, alkyl), 1.28-0.74 (m, 12H, alkyl), 0.39-0.36 (m, 2H, alkyl) ppm. **δC** (50 MHz, $CDCl_3$), 185.0 (C=O), 167.1 (C=O), 159.2, 144.2, 136.6, 131.1, 128.7, 128.4 (quaternary aromatic C's), 130.2, 130.0, 127.9, 127.3, 126.6, 125.7, 125.5, 124.9, 123.3 (aromatic CH's), 118.7, 114.3, 106.1 (aromatic CH's), 67.8 ($\underline{C}H_2O$), 64.9 (CH_2, alkyl), 49.4 ($CO\underline{C}H_2CO$), 47.1 (C-17), 33.4 (CH_2, alkyl), 29.2 (CH_2, alkyl), 28.9 (CH_2, alkyl), 25.6 (CH_2, alkyl), 22.0 (CH_2, alkyl) ppm; **m/z** (FAB) 829 (M + 1), 722, 364; HRMS (FAB), found 828.34400, $C_{55}H_{47}F_3O_4$ requires 828.34265.

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Courses Attended

Organic Research Seminars, University of Edinburgh, various speakers, 1992-95.

NMR Spectroscopy, Drs I. Sadler & J. Parkinson, University of Edinburgh, 1993.

Royal Society of Chemistry, Perkin Division, Scottish Meeting, various speakers, Edinburgh 1992, Aberdeen 1993.

"Medicinal Chemistry", Prof. R. Baker and colleagues, Merck Sharp & Dohme, Terlings Park, UK. 1993-95.

"Chemical Development in the Pharmaceutical Industry", various speakers, SmithKline Beecham, UK. 1993-94.

"Biomolecule Libraries-Synthesis & Selection", various speakers, London, 17th February 1994.

Combinatorial Synthesis Symposium, University of Exeter, England, various speakers, 20th-21st July 1995.

Solid Phase Synthesis & Combinatorial Chemical Libraries, 4th International Symposium, Edinburgh, Scotland, various speakers, 12th-16th September 1995.

German Language Course, Institute of Applied Language Studies, University of Edinburgh, Jan 1993 - Dec 1994.