synthetic potential of this type of resin is currently under investigation.

Conclusion

The potential of solid-phase organic chemistry is impressive and intriguing possibilities are rapidly unfolding in the development of combinatorial techniques, in the creation of synthetic sequences and reaction types, and in the discovery of novel resins and linkers. It is a privilege to participate in this unfolding.

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Consideration of Solid-Phase Synthesis with Reference to Quinolone Antibiotics

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Abstract. The general utility of solid-phase synthesis for creating libraries of compounds will be discussed with particular reference paid to the development of a solid-phase organic synthetic route to quinolones. Using the DIVERSOMER® technology a library of quinolones have been prepared, purified and analyzed. Additionally, the issue of resin impurities and by-products will be discussed.

1. Introduction

1.1. *Development of Solid-Phase Synthesis*

The concept of solid-phase synthesis (SPS) was introduced by *R.B. Merrifield* in 1963 with the synthesis of a tetrapeptide [1]. Since then, solid-phase peptide synthesis (SPPS) has rapidly progressed allowing for the automated synthesis of biologically active peptides of over a hundred amino acids. Oligonucleotide [2] and oligosaccharide [3] chemistry have similarly adopted solid-phase approaches. More recently, the synthesis of combinatorial compound libraries has utilized the methodology for solid-phase organic synthesis (SPOS) of small molecules [4]. Consistent with this trend, most of the top selling drugs on the market today are low-molecular weight (< 700 g/mol), heterocyclic based compounds [5].

1.2. *Advantages of Solid-Phase Synthesis*

The advantages of SPS over traditional solution-based methods are clearly evident. Most notably, excess reagents are readily tolerated by the solid support, which typically increases reaction kinetics, and drives reactions to completion. No purification of reaction intermediates is required, with product isolation improved by washing away excess reagents from the solid support. In many cases resin-bound intermediates are generally more stable and easier to handle than the corresponding solution-phase analogues. Additionally, site isolation allows for selective attachment of a bifunctional compound to a solid support leaving the second active site free for further activation and derivatization. Finally, as successfully demonstrated by peptide and oligonucleotide chemistry, SPS is amenable to automation.

1.3. *Solid Supports*

A variety of polymers have been described for SPS, yet the literature related to polymeric supports is dominated by functionalized cross-linked polystyrene-divinylbenzene [6]. SPPS has largely used polystyrene-based solid supports for the synthesis of peptides and proteins. However, cross-linked polystyrene is highly hydrophobic and good resin swelling is only obtained in non-polar, aprotic solvents such as dichloromethane (DCM) and *N,N*-dimethylformamide (DMF), as demonstrated by *Merrifield* resin **1** (*Fig. 1*).

Polystyrene-poly(ethylene glycol) (PS-PEG™) graft coploymers have also been used in SPS [7]. More commonly referred to as Tentagel® **2** (*Fig. 1*) this polystyrene-based resin has a PEG spacer between the dense polystyrene network and the linker [8]. This creates a more hydrophilic solid support that provides an environment that more closely resembles solution-phase chemistries. Subsequently, *Tentagel*® resins are easily solvated in both polar and

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non-polar solvents with the swelling properties in water and DCM nearly equal [9]. Despite these advantages, *Tentagel®* resins offer low loading, typically 0.15–0.30 mmol/g (polystyrene : 0.5–1.2 mmol/g). Furthermore, *Tentagel®* supports are mechanically unstable, rendering agitation by magnetic stirring or sonication incompatible [10]. This subsequently limits the utility of the solid support to certain reaction conditions and automation techniques. A wide variety of other supports have also been used in SPS, such as silica [11], cotton [12], cellulose [13], CPG [14] and *Sephadex LH 20* [15].

In addition to solid supports, one other consideration in SPS is the nature of the linker which is bound firmly to the resin and also the synthetic target system. This latter union must serve two purposes; the first being that cleavage of the completed synthetic target(s) must be accomplished under tightly controlled conditions compatible with the product(s) and, secondly, that the linker function should afford the required functionality of the product(s).

To this end, we have designed a family of acid-labile linkers [16] based upon the chemistry of the dibenzocycloheptadien-5-one system shown below (*Scheme 1*), which was studied by *Pless* [17] exactly twenty years ago. This resin-bound ketone intermediate **3** can be transformed easily, as required, into a family of seven linkers.

An example of the application is illustrated by the formation of the resin-bound peptide thiol **4** for the subsequent use in chemical ligation of peptides (*Scheme 2*) [18].

1.4. *Compound Libraries*

With renewed interest in SPS as a method to generate compounds on a solid support, the chemistry community has quickly adopted this approach to synthesize libraries of compounds either as mixtures or single compounds. This has been demonstrated by the preparation of oligomeric libraries consisting of peptides, peptoids (NSGs), peptidyl phosphonates, vinylogous peptides, carbamates, peptide nucleic acids (PNAs), pyrrolinones, oligosaccharides and oligonucleotides [19]. While these libraries have obvious utility for increased molecular diversity, some of the compounds such as peptides offer poor bioavailability and pharmacokinetic parameters. However, it must be noted that oligomeric compounds have successfully served as ideal models for the more traditional, small-molecule drugs.

Solid-phase approaches to non-oligomeric, small-molecule libraries have also received considerable attention. Some of the compounds synthesized have included the benzodiazepines [20][21], hydantoins [21], β-lactams [22] and thiazolidines [23]. In comparison to oligomeric based libraries, the synthesis of small molecules allows for the generation of compounds from a more diverse range of chemical reactions and building blocks, and is compatible with both solution-phase and solid-phase synthesis. Furthermore, as proven by the synthesis of peptide-based libraries, this approach is also amenable to automation.

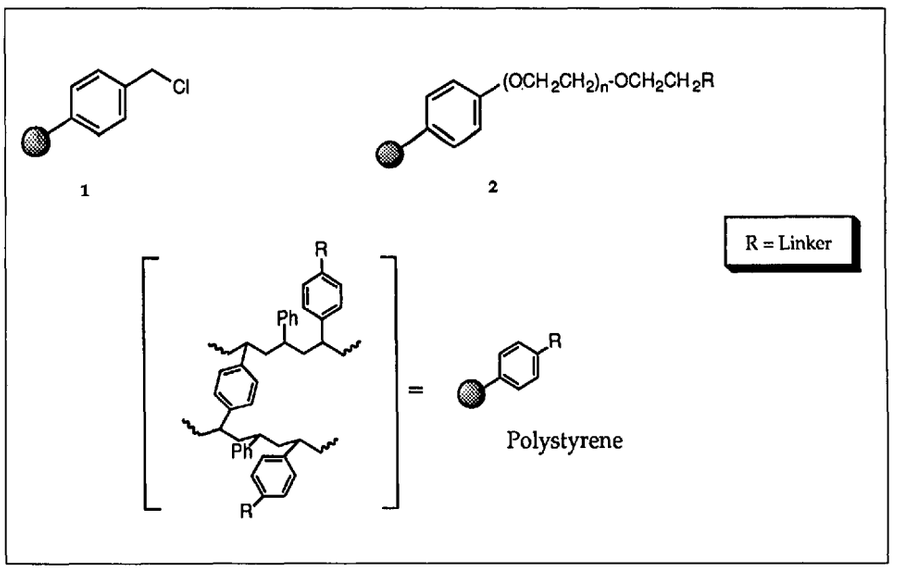
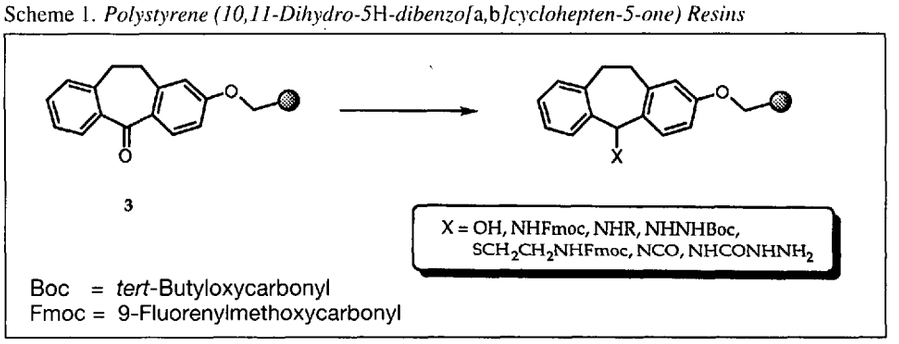
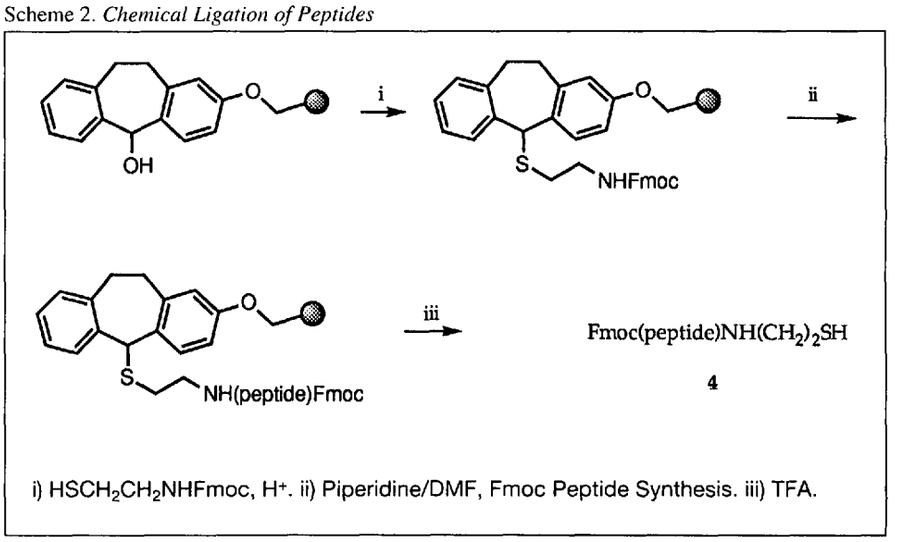


Fig. 1. *Structure of polystyrene (Merrifield) resin and* Tentagel®

Scheme 1. *Polystyrene (10,11-Dihydro-5H-dibenzo[a,b]cyclohepten-5-one) Resins*



Scheme 2. *Chemical Ligation of Peptides*



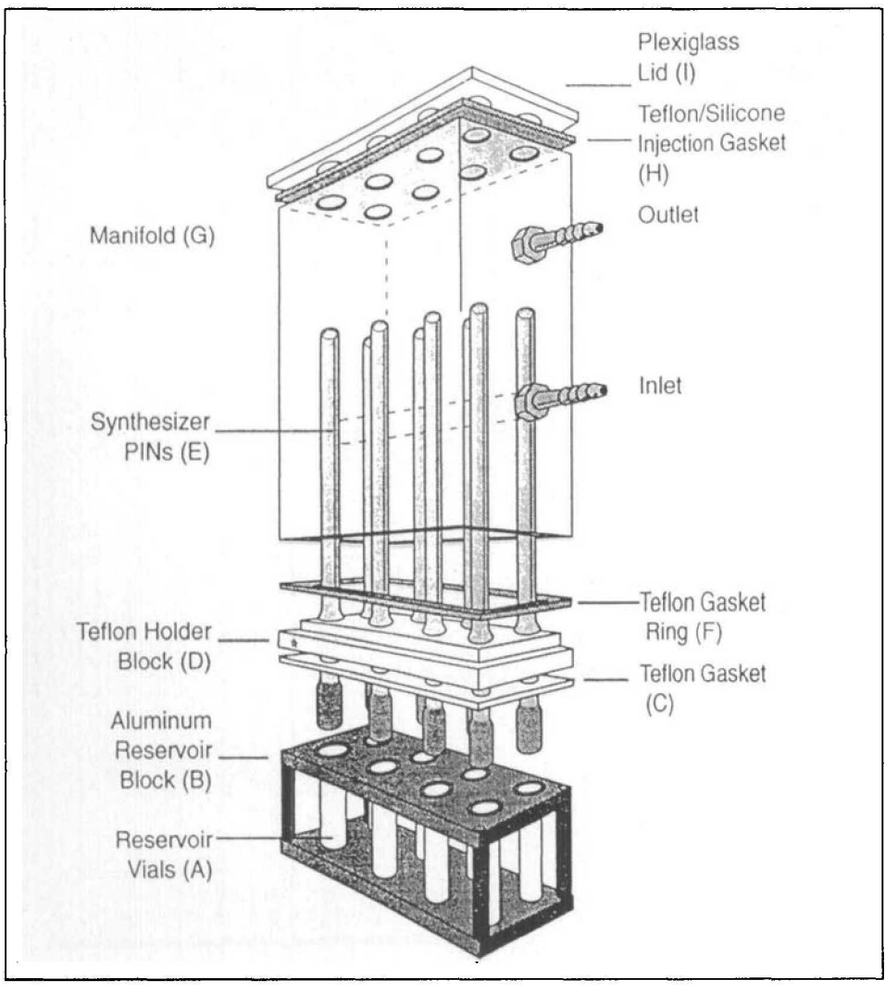
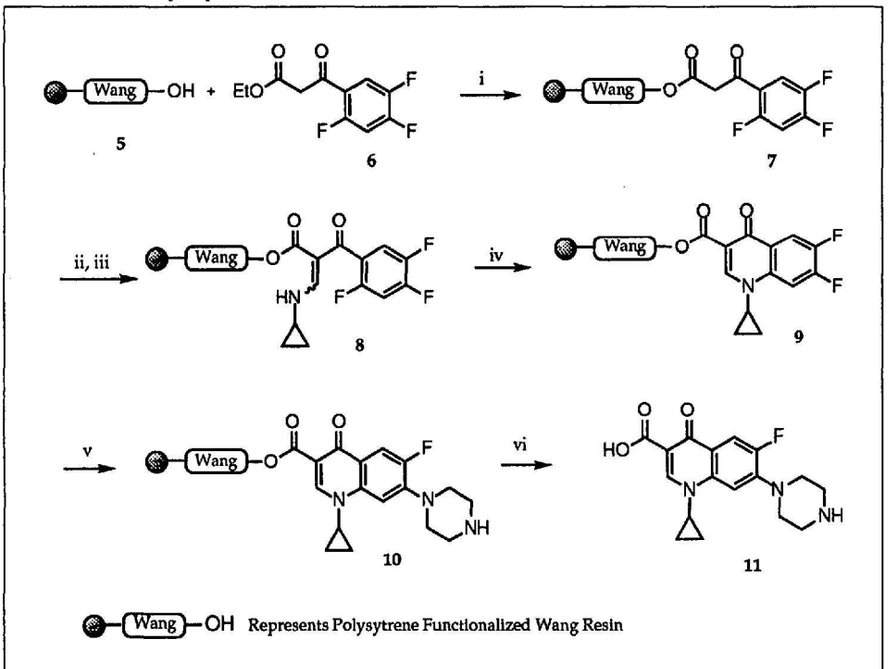


Fig. 2. *DIVERSOMER® 8-PIN synthesizer* (reproduced with permission from *Chemglass, Inc.* Original publication: The *Chemglass* 8-PIN Synthesizer Manual, Fig. 1)

Scheme 3. *SPOS of* Ciprofloxacin®



i) Toluene, DMAP, 110°, 18 h. ii) $(CH_3O)_2CHN(CH_3)_2$, THF, 25°, 18 h. iii) $NH_2C_3H_5$, THF, 25°, 72 h. iv) TMG, DCM, 55°, 18 h. v) Piperazine, NMP, 110°, 4 h. vi) 40% (*v/v*) TFA/DCM, 25°, 1 h.

2. A Solid-Phase Approach to the Quinolones

In order to test the feasibility of sequential SPOS stages, we studied a solid-phase approach to the quinolones which represent a class of highly potent, broad-spectrum antibiotics [24]. Moreover, using the DIVERSOMER® technology we synthesized a library of the quinolones in a semi-automated fashion, using the DIVERSOMER® synthesizer (*Fig. 2*) and a liquid-handling robot.

The DIVERSOMER® method initiated at *Parke-Davis* Pharmaceuticals, Ann Arbor, Michigan, allows for the parallel synthesis of eight or forty compounds in milligram quantities, by solution-phase or solid-phase, and by a manual or automated method [25]. This technology has successfully introduced a new element to automated synthesis, as well as demonstrated new SPOS chemistries.

Prior to using the DIVERSOMER® technology for the synthesis of a library of the quinolones or indeed any other pharmacophore, alternative synthetic strategies must be investigated. For the SPOS of the quinolones, polystyrene-functionalized *p*-benzyloxybenzyl alcohol (*Wang*) resin **5** (*Scheme 3*) was selected as the solid support to accommodate all the reactions necessary for the quinolone synthesis. The traditional solution-phase route was re-engineered with reaction conditions designed to exploit the advantages of solid-phase synthesis while at the same time circumventing the disadvantages of the polystyrene solid support. For example, the use of resin swelling solvents and low to ambient temperatures.

Ciprofloxacin® was studied as the model compound. Following the successful re-design of the solution-phase chemistry, analogous conditions were attempted on *Wang* resin which provided a direct pathway for attachment *via* ester linkage of the *β*-keto ester **6** as shown in (*Scheme 3*). Transesterification was achieved by heating **5** and **6** in toluene with a catalytic amount of *N,N*-dimethylaminopyridine (DMAP) at 110° for 18 h, to generate the resin-bound *β*-keto ester **7**.

Preparation of the resin-bound enamide **8** was achieved by activation of **7** with dimethylformamide dimethyl acetal followed by *in situ* addition of cyclopropylamine at 25°, for 18 and 72 h, respectively. In contrast to the solution derived enamide, which was unstable and required handling under anhydrous conditions, the resin-bound enamide, **8**, was stored on the bench for several weeks without any evidence of decomposition. Cyclization of **8**

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in a solution of tetramethylguanidine (TMG) and DCM at 55° for 18 h provided the corresponding resin-bound quinolone **9**.

In the final step of the synthesis, **9** was reacted with a solution of piperazine in *N*-methylpyrrolidinone (NMP) at 110° for 4 h, to form the resin-bound *Ciprofloxacin®* **10**. Treatment of **10** with 40% (*v/v*) trifluoroacetic acid (TFA) in DCM at 25° for 1 h confirmed the cleaved product as crude Ciprofloxacin® **11**.

Initially, the chemistry was attempted in a round-bottomed flask equipped with a magnetic stir-bar. Subsequently, the same reaction conditions were repeated in a single DIVERSOMER® 'PIN' employing ultrasound agitation. Both experiments yielded the desired compound, *Ciprofloxacin®* **11** which was identified as the crude product by ^{1}H-NMR and MS.

For the synthesis of a library of quinolones, continued studies focused on selecting a diversity of reagents (building blocks). The six-step quinolone synthesis was then expanded for the preparation of a library of 40 compounds using the 40-unit DIVERSOMER® synthesizer (*model 40–100*) in combination with automation. Acid cleavage and semi-automated purification by solid-phase extraction (SPE) methods afforded the quinolones in average yield of 26%. Despite the fact that the quinolones are highly ultraviolet (UV) active and easily identified by conventional thin-layer chromatography (TLC) methods, they have limited solubility, and are best solubilized in concentrated TFA. While purification by SPE eliminated resin impurities and by-products from the reaction pathway, the products were further contaminated by degradation of the silica gel and leaching of plasticizers from the polypropylene cartridges used during purification.

As a result of the difficulties with the above purification protocol, alternative methods were studied. Ultimately, reverse phase high-performance liquid chromatography (RP-HPLC) methods yielded pure quinolone products. Although this is a serial, labor-intensive process, it was justified by the outcome of the high-quality products. This was later proven with the synthesis of a sub-library of eight quinolones (*Fig. 3*). Using a DIVERSOMER® synthesizer compatible with up to 800 mg of resin and eight reactions (*model 8–800*), 500 mg of functionalized *Wang* resin afforded sufficient quantities of product to purify and fully characterize the library. The corresponding products were characterized by both ^{1}H-NMR and MS analysis. This library has subsequently been submitted for full biological testing, and the results will be disclosed in due course. *Fig. 4* illustrates the ^{1}H-NMR of *Ciprofloxacin®* prepared by both the solution-phase (*a*) and solid-phase (*b*) routes, demonstrating the excellent correlation in product profiles.

3. Problems with Polystyrene Solid Supports

In the solution-phase approach to the quinolones all reactions were monitored by conventional analytical methods such as UV, TLC, IR, NMR and MS. However, these useful analytical tools are generally

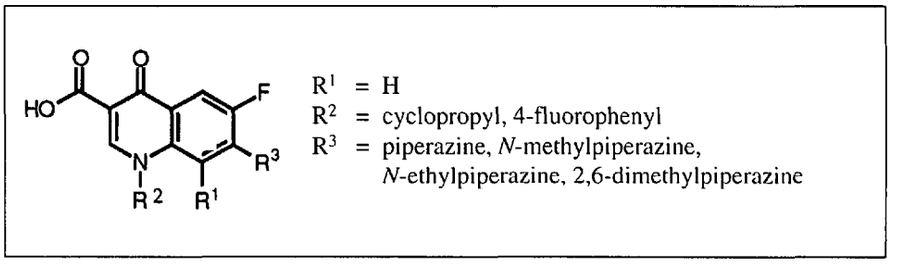

Fig. 3. *Large-scale quinolone 8-unit array*

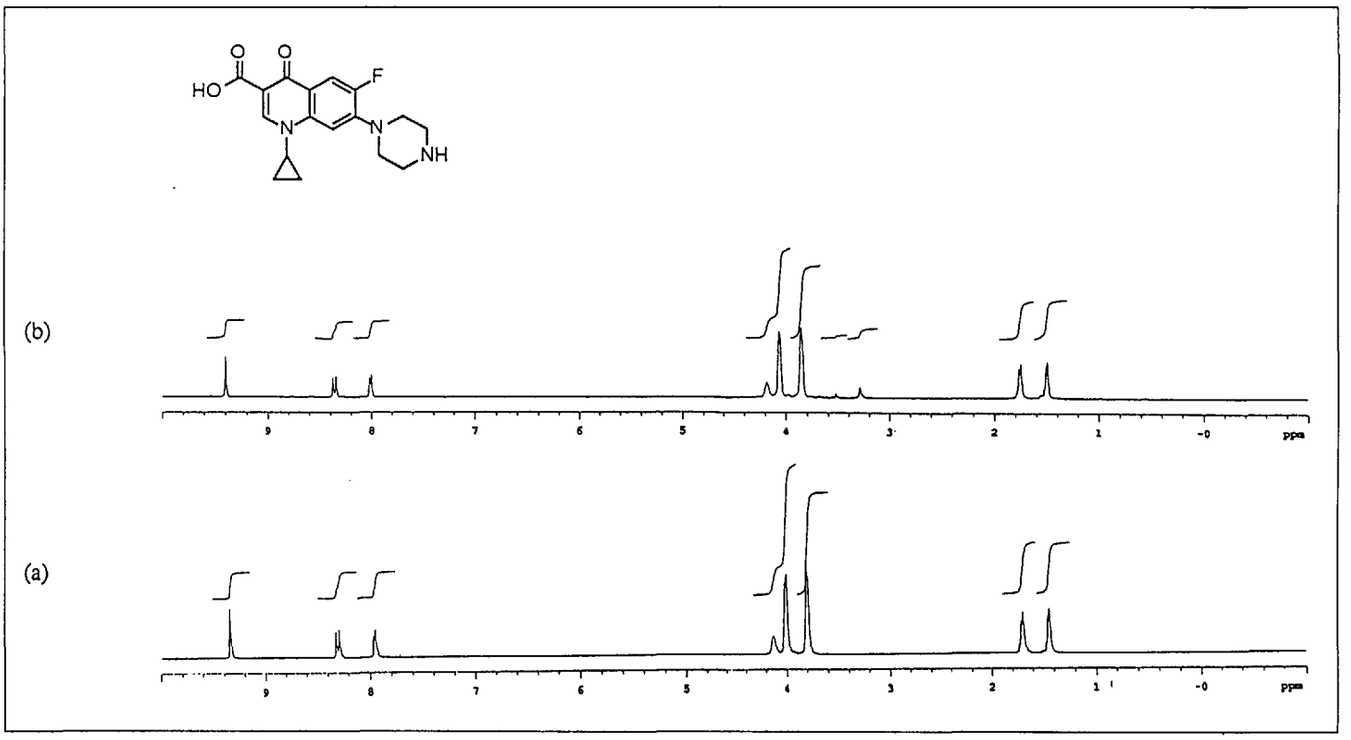

Fig. 4. *^{1}H-NMR of* Ciprofloxacin® *samples,* a) *solution phase and* b) *solid phase*

not applicable to monitoring SPOS reactions. To date, IR and ^{13}C-NMR have predominantly been used to identify compounds bound to a solid support. Recently, the introduction of magic angle spinning (MAS) ^{1}H-NMR [26] and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) MS [27] have enabled alternative methods of resin-based analysis.

Although the SPOS of quinolones represents one of the longest and most difficult SPOS routes to be reported, our work has also focused on providing high-quality products to enable full characterization and meaningful biological results. This rationale is intended to avoid false negatives within compound libraries which could ultimately misrepresent the SAR of a series, and therefore miss a viable lead.

The advantages of SPOS are clearly obvious to the user, however the selection and use of polystyrene resins in SPOS is frequently impacted by the presence of entrapped impurities such as solvents, or by-products from copolymerization of the resins. These entrapped impurities can affect the SPOS sequence if the impurity reacts with, or competes with the reagents in the first step of the SPOS route. Furthermore, impurities can effectively lower the resin loading and therefore affect the determination of product yields and reaction monitoring. However, analysis of resins, or resin-bound adducts, by ^{13}C gel-phase NMR, is useful for identifying these impurities prior to the execution of a SPOS route.

The problems associated with entrapped resin impurities are best overcome by repetitive washing with a broad range of solvents followed by drying prior to the execution of a synthesis. Unfortunately, the impurity profile of the resins is inconsistent among commercial suppliers and also varies from batch to batch [28], therefore analysis and/or washing of every resin prior to synthetic use is recommended.

While pre-washing improves the quality of the resins prior to SPOS, requisite cleavage conditions result in impurities in the final products. Thus, we looked at pre-treatment of polystyrene-functionalized resins with representative cleavage conditions prior to synthetic use. Pre-treatment was however unsuccessful in improving product profiles. Furthermore, the nature of these by-products was elusive and varied dependent upon the functionalized resin and cleavage conditions used.

Since adequate procedures to eliminate resin by-products employing resin-based methods were not identified, product-based purification methods were explored. A post-cleavage solution-based approach, allowed us to exploit automated SPE, two-phase extractions and HPLC. Currently, these methods are being developed further to provide products of high quality for mass screening and hence negate the use of compound mixtures or deconvolution strategies.

4. Concluding Remarks

In the last few years the pharmaceutical sector has invested millions of dollars into new technologies for the efficient synthesis and identification of lead compounds. Combinatorial chemistry and automated synthesis as enabling technologies have begun to contribute to this drug discovery effort.

The results herein demonstrate a new SPOS route to the quinolones. The problem of resin impurities is fundamental in the development of any SPOS reaction. With the continued development of SPOS including new solid supports and linkers the issue of resin quality and degradation should be overcome. This work has also proven the utility of the DIVERSOMER® technology to a wide range of reagents and reaction conditions that are routinely used in organic synthesis. Furthermore, the technology allows for the synthesis of 8 or 40 compounds in milligram quantities for full characterization and multiple assays.

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CONFERENCE REPORTS

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Nouvelle Société Suisse de Chimie
New Swiss Chemical Society

Spring Assembly 1996 (14 March 1996)

organized by the Section of Analytical Chemistry (SACh)

University of Fribourg, Institute of Chemistry

'Chiral Separations'

Why did we choose this topic: 'Chiral Separations'?

The technological and methodological progress in analytical chemistry is growing at such a fast rate that there is a need for all scientists working in this field to keep abreast with the latest development. This rapid and constant progress in the development of analytical sciences stems directly from the stringent demands for rationalisation of quality control, the need to solve new problems and demands for the development of new technologies.

So the aim of this Symposium was to inform all scientists from academic as well as industries and research institutions about the recent progress in the area of chiral separations.

We know that chirality is essential to all living organisms at the molecular level. Consequently, the preparation and study of pure enantiomers is of paramount importance in a great many disciplines lying at the interface between chemistry and biology. In addition to the well recognized significance of stereochemistry in the pharmaceutical, agrochemical, environmental, flavor and fragrance fields, the study of enantiomers is of a crucial importance in many disciplines in particular biosynthetic studies, quality control of products of natural origin and biotechnology. But this cannot be done without analytical chemistry interested in the two fundamental steps: separation of chiral compounds and enantiomer ratio determination.

So, for this purpose we have invited seven outstanding specialists in this topic. Three of them came from overseas. They were Prof. *Wainer* coming from Canada, Prof. *Pirkle* and Prof. *Armstrong* coming from USA. All of them are internationally reknowned in this field. Our invitees from Europe are also eminent in this field. They were Prof. *Lindner* from Austria, Dr. *Francotte*, Prof. *Veuthey* and, last but not least, a women, Dr. *Meyer* from Switzerland.

Prof. W. Haerdi

The Use of Computational Chemistry and Molecular Modelling to Describe and Predict Enantioselective Separations

Irving W. Wainer, McGill University, Montreal, Quebec

Quantitative structure-retention relationships (QSRR) is an approach to the analysis of chromatographic data which can be used to predict and describe solute-stationary phase interactions. These studies require a set of quantitatively comparable retention data (dependent variable) for a sufficiently large set of solutes and a set of quantities (independent variables) reflecting structural features of these solutes. Through the use of chemometric computational techniques, retention parameters are characterized in terms of various combinations of solute descriptors or in terms of systematic knowledge extracted from these descriptors. The QSRR analysis can then be used to guide molecular modelling studies to give a comprehensive view of solute-stationary phase interactions.

When the stationary phase contains a chiral selector and the solutes are enantiomeric, the QSRR/molecular modelling can provide an insight into the chiral recognition mechanism as well as predict enantioselective separations. This experimental strategy has been illustrated by the results from a study which investigated the enantioselective separation of a series of 45 α-alkyl arylcarboxylic acids on a chiral sta-