1

General Overview

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1.1 Introduction, background and pivotal discoveries

In 1963, Bruce Merrifield described the synthesis of short peptides making use of a new concept in which a terminal amino acid was first covalently joined to, or immobilized onto, a solid support and subsequently reacted sequentially with other amino acids to essentially 'grow' a desired (tetra)peptide (Figure 1.1).[1] This monumental work would usher in a new paradigm within organic synthesis that was markedly different from previous traditional approaches and would change synthetic chemistry in striking ways. Prior to Merrifield's insightful work, which would garner him a Nobel Prize, organic synthesis, as a discipline, was often relegated to cumbersome and inefficient solution-based reactions that necessitated precise stoichiometry and laborious purifications.

In the early 1990s, several research groups presented findings that solidified this exciting concept within organic synthesis. The Ellman laboratory reported on an expedient solid-phase method for the synthesis of 1,4-benzodiazepines.[2–4] In this work, three components, namely immobilized 2-aminophenones, aminoacids and alkylating agents, were reacted in a combinatorial manner to generate a small library of these therapeutic compounds (Figure 1.2). The reaction sequence was highly efficient, tolerant of functional group diversity and devoid of racemization. Collectively, this work demonstrated that versatile organic reactions could be conducted with one reactant immobilized on a solid support, and that this methodology allowed the different modules or components that make up a target molecule to be chemically joined in a combinatorial fashion, which could offer advantages to traditional linear synthesis.

Researchers at Parke-Davis also developed a similar approach to non-peptide chemical diversity with the introduction of their 'diversomer' technology.[5, 6] In this seminal work, a series of structurally-related compounds were synthesized in a multiple, simultaneous manner by making and using structurally diverse building blocks which were immobilized on an insoluble polystyrene-based solid support. The potential

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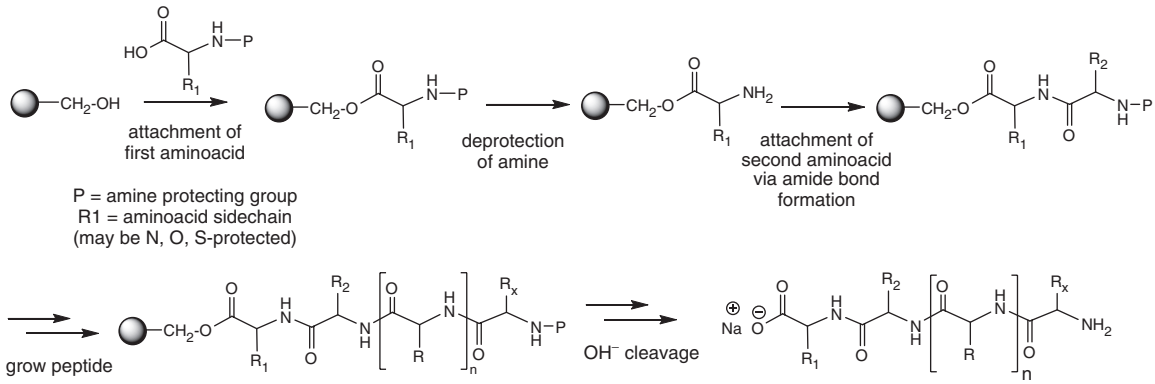

Figure 1.1 *Representation of Merrifield solid-phase synthesis*

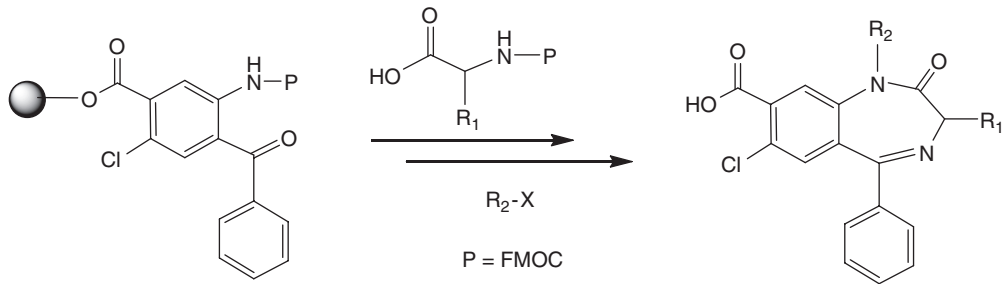

Figure 1.2 *Representation of Ellman's solid-phase synthesis of 1,4-benzodiazepines*

applicability to medicinal chemistry was enticing from the outset, as collections of pharmacologically-privileged structures (hydantoins (Figure 1.3) and benzodiazepines) were synthesized. An innovative feature of this work was the use of a cyclization reaction to release hydantoin final products from the solid support. This general strategy of cyclative mechanism-based cleavage has found much use and is discussed in detail in Chapter 4.

In retrospect, the Merrifield synthesis opened up seemingly countless possibilities in its heyday, as peptides and proteins, often inaccessible or at best available only in small quantities through heroic traditional chemical transformations, would eventually lie within reach through the expansion and automation of this technology. So, too, would this become the case for the Parke-Davis 'diversomer' technology and the many useful modifications that would follow.[7–15]

The Parke-Davis group (Sheila DeWitt, John Kiely, Mike Pavia, Walter Moos, Donna Reynolds Cody, Tony Czarnik and colleagues) introduced apparatus, built upon materials often found in an organic chemistry laboratory, in which to carry out 'diversomer' technology.[16–18] This was a key event because other groups swiftly constructed their own homemade versions in which to conduct parallel solid-phase synthesis. The 'diversomer' technology, arguably the underpinnings of non-peptide solid-phase organic synthesis, became widely available to the chemist and would lead to many creative applications.

Solid-phase technology has reshaped the landscape of organic synthesis and thus it is useful to provide some general definitions at the outset and to draw some contrasts. Traditional organic synthesis, a tremendously powerful science, relies upon establishing a usually homogenous solution phase to dissolve and

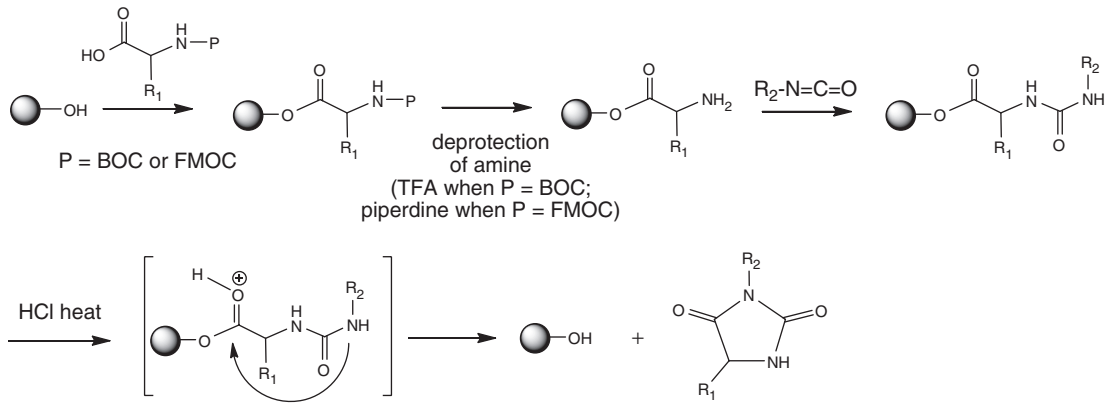

Figure 1.3 *Solid-phase synthesis of hydantoins using 'Diversomer' technology*

liberate reactants, so that they may collide with each other with proper orientation and sufficient energy to allow for new bond formation at the expense of weaker or less favorable chemical bonds. In contrast, a solid-phase synthesis invokes that at least one reactant exists and remains as a undissolved component in the reaction medium, thus inferring that a heterogeneous mixture is present. While every practicing chemist at one time or another may debate the homogeneity of a given solution-based reaction, it is important to realize that solid-phase chemistry intentionally makes use of the interaction of two (or more) components that exist in different phase states – one being that of a solid and the other(s) in solution or liquid form. The term solid support refers to an inert insoluble macromolecule (also called a resin) to which a much smaller organic molecule or moiety is or can be attached. This molecular fragment or moiety, that joins the two, is termed a linking group (or linker unit) because it will serve as an atomic or molecular bridge between the solid support and the starting material of the synthesis, which is referred to as the 'organic substrate'. The organic substrate is usually very similar, perhaps even identical, to any starting material that might be destined for 'traditional' solution-based synthesis. The starting material should be a carefully selected molecule that can be subjected to a series of reactions that transform its functional groups or reactive substituents, to provide the desired target molecule(s). However, as shall be seen, making a given starting material or organic substrate amenable to solid-phase technology can often require that additional functionality be present or be installed. The 'organic substrate' terminology places this component within the conglomeration that constitutes solid-phase synthesis. It is the 'organic substrate' that is of interest to the synthetic chemist, for this component will be subjected to subsequent chemical transformations to make the targeted molecules.

Returning to the topic of this textbook, the linker group is a critical structural element in this interplay of inert polymeric solid support and low molecular weight organic substrate because it dictates how the two will be joined and under what conditions the two can be eventually separated. The development of linker groups has followed many varied approaches, mostly to accommodate the chemical transformations needed to convert any given organic substrate into desired product(s), but also to push the limits of solid-phase technology. The reader will learn of the many strategies that have evolved and will encounter specific examples that illustrate the utility of linker groups in solid-phase organic synthesis. The material presented herein purposely does not cover polypeptide or oligonucleotide solid-phase synthesis, which have been tremendously successful predecessors to this broad area of science, but will rather focus upon what may be viewed as solid-phase synthesis of 'small organic' molecules. There will be an effort to showcase synthetic versatility by providing specific examples that will be rich in diverse functional group chemistry.

As background to such strategies and at the simplest level, most organic molecules, particularly those that can be used for medicinal, biological or other industry-based applications, contain heteroatoms and more complex arrays of heteroatoms referred to as functional groups, in addition to carbon and hydrogen atoms. Synthetic organic chemists have long recognized that such heteroatoms and functional groups can, in essence, mark viable (dis)connection points from which smaller molecular pieces (fragments, precursors) can be joined together to build a target molecule. Indeed, chemists have been trained to look for such features when devising a synthetic pathway, a strategy pioneered by the legendary R. B. Woodward and others.

Historically, and without solid-phase techniques, a typical 'traditional' synthesis involves a sequence of reactions that transform a starting material and various selected reagents into a desired final product. In each reaction, a starting material is dissolved in an inert solvent as is the reactant, and reaction of the two occurs in a (usually) homogenous solution. In some cases, the reactivity of one component may require that key functional groups are made inert at various stages through the use of protecting group chemistry. Separately, in other scenarios, one component may be used in (molar) excess in order to increase the efficiency of the transformation by 'driving the reaction to the right'. Regardless, side reactions occur between the starting material and the reagent, or with impurities or side products that form under the reaction conditions. Therefore, the reaction mixture must be subjected to purification and this is often accomplished by quenching the reaction and conducting some (work-up) extraction procedure to concentrate impure final product, which is then further purified by chromatographic techniques. The isolation and purification of a molecular entity from a solution-based reaction mixture can often be laborious and require much more effort than conducting the chemical reaction itself.

In contrast, solid-phase synthesis entails the immobilization of a starting material onto a polymeric solid support (resin) that, if chosen properly, is inert to reagents and subsequent reaction conditions. Solid-phase techniques can be optimized to offer advantages compared to traditional solution chemistry. Since the resin does not dissolve in solvent, the organic substrate (starting material) can be exposed to solutions containing large excesses of reagent to drive the reaction to completion. Relatively straightforward filtering and washing techniques can often be used to remove impurities and circumvent chromatographic purifications. Additionally, the use of scavenger resins to remove unwanted excess starting materials and/or by-products can be effectively employed in some instances. Figure 1.4 is a representation of solid-phase synthesis in which the components or building blocks or diversity elements (terms often used interchangeably) are depicted by the differently shaped symbols; the solid support and linker group are represented by the gray circle and oval, respectively. The X substituent is used generically to indicate the point at which the organic substrate (or starting material) is joined to the linker group. The focus of this book is on the role of this attachment within the linker group and the chemistry used to attach and liberate small-molecule products from the solid support.

Referring back to Merrifield protein synthesis, the amide linkage marks a logical (dis)connection point from which smaller pieces (amino acids) can be joined together to build a desired peptide sequence. An advantage of the Merrifield methodology is that amino acid protecting group chemistry can be accomplished through immobilization onto the solid support. Subsequent functional group activation and amide bond formation allows for the (poly)peptide to be built one amino acid at a time. Thus, the attachment of the organic substrate (amino acid or peptide) to a resin can serve as a means not only to protect a key functional group, but to also provide a 'directionality' from which to assemble the peptide. While the original work grew the peptide from the C-terminus towards the N-terminus, it is important to realize that peptides can be assembled in the 'opposite direction', namely by attaching the N-terminus to a solid support and adding amino acids in the opposite order.

In designing ligands for proteins, the underlying principle is that receptors, enzymes and channels recognize certain structural elements, such as molecular shape, size, lipophilicity/hydrophilicity and charge. Proteins, as diverse as they are as a family, however, are composed of a basis set of only about 20 common

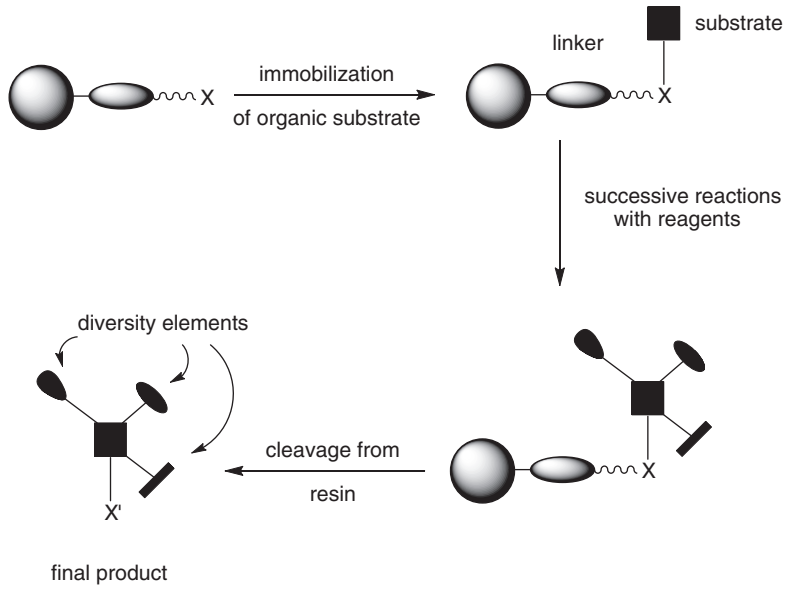

Figure 1.4 *Representation of solid-phase organic synthesis (SPOS)*

amino acids. Therefore, a large number of unique monomers or building blocks is apparently not essential for structural and functional diversity, but rather it is the 'space' which key amino acids can occupy once attached to a peptide backbone or a unique structural motif or scaffold that is critical. In fact, despite being comprised of only one type of chemical reaction (amide bond formation) and a limited set of building blocks (amino acids), highly specific peptidic ligands for many proteins are well known, whether endogenously formed or synthesized using some variation of the Merrifield technology.

Using solid-phase chemistry to synthesize non-peptidic organic molecules should provide markedly greater diversity since, theoretically, the vast repertoire of organic reactions should be amenable to the technology and, therefore, not limited solely to amide bond formation. It is this nearly unbound opportunity to build compound libraries by applying nearly *all* organic reactions and this 'sampling of vast chemical space' that holds the promise of discovering new small-molecule drugs. The number of building blocks should be nearly without boundary given the countless ways carbon-based molecules exist and react through the rich universe of functional groups.

But even more striking is the ability to construct organic molecules in a combinatorial manner. Very often, synthetic organic chemistry has been primarily inspired by very specific but narrow endeavors, such as syntheses of complex natural products or the construction of hand-crafted molecules to drive pharmacological evaluation and drug discovery. One powerful opportunity afforded by combinatorial chemistry was the ability to 'sample' molecular diversity and conformational space effectively by incorporating many structural variations during the course of synthetic sequences used to make compound libraries. An ideal compound library is diverse so as to include members that span a spectrum of shape, size and lipophilicity in order to probe for binding to a protein of interest. It is desirable to introduce multiple elements of diversity when possible at each step of the synthesis. In practical terms, a well conceived strategy makes use of versatile chemical transformations that not only afford structurally diverse compounds of high purity, but also can serve as source of novel intermediates that can be further elaborated to prepare additional libraries, preferably prior to liberation from the solid support. This is the 'libraries from libraries' approach that is a well-known strategy to optimize the molecular diversity one obtains in any given synthetic sequence.

The idea of generating large number of molecules simultaneously was certainly not new given the groundbreaking Houghten[19] syntheses of thousands of peptides. However, having the capability to assemble molecules in a modular fashion with the control afforded by immobilization onto a solid support made such processes feasible. The technology would be used to generate a single compound per well (from a single resin – see Figure 1.4) or, in contrast, so-called 'split and pool' techniques (Figure 1.5) would be used to rapidly expand diversity. In split and pool synthesis, individual resins each containing a different organic substrate are pooled together and then subjected to solid-phase synthesis. Pools resulting from each transformation can be further pooled to generate large numbers of distinct molecules within a few reiterations (reactions).

Thus some labs chose to use this approach and generate large numbers of molecules in a parallel fashion, so giving rise to massive compound libraries. These features allow for the synthesis of hundreds or even thousands of compounds in the time it typically took to make a handful or dozen of molecules using conventional linear syntheses. This prospect was eagerly welcomed as high-throughput screening methods were routinely coming online as the industry focused on increasing R&D productivity.

An exceptional example was reported by the Schreiber group in which the synthesis of over 3000 spirooxindoles was achieved (Figure 1.6).[20] A key feature of this work was the use of a three-component reaction to install multiple elements of diversity in a single step. (Multicomponent reaction on a solid phase will be discussed later.) Remarkably, despite the complexity of the chemistry used to assemble the final products, it was determined that more than 80% of the compounds in the library had a purity of greater than

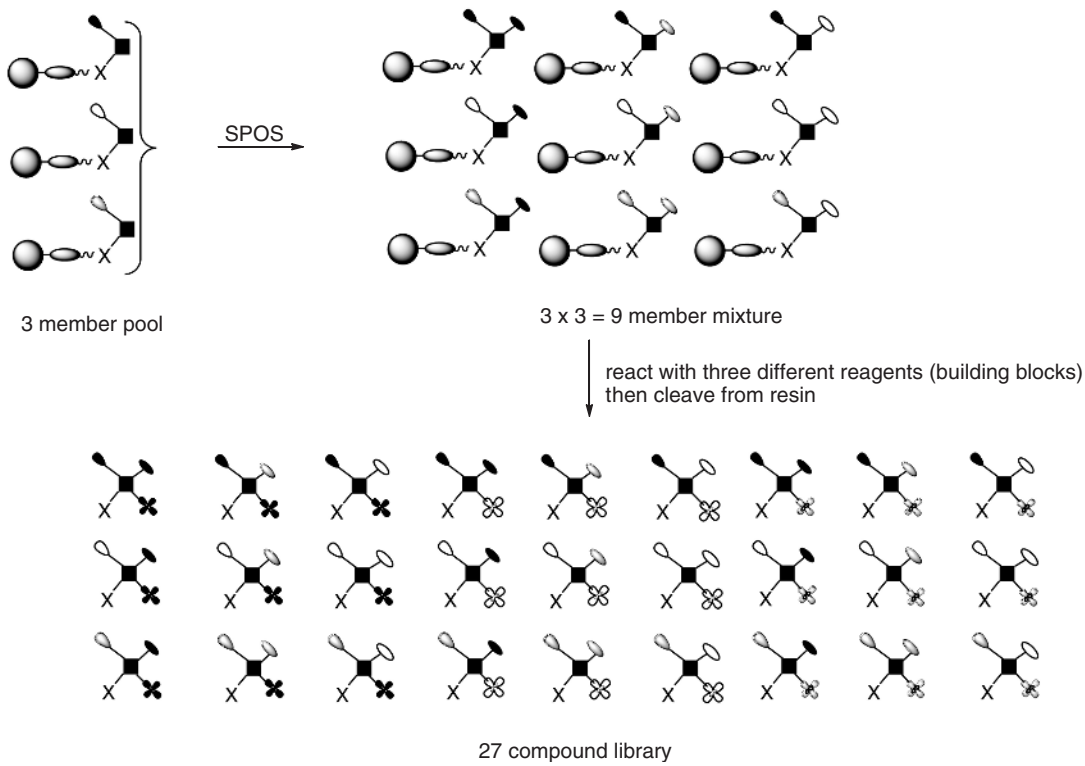

Figure 1.5 *Representation of 'split and pool' methodology*

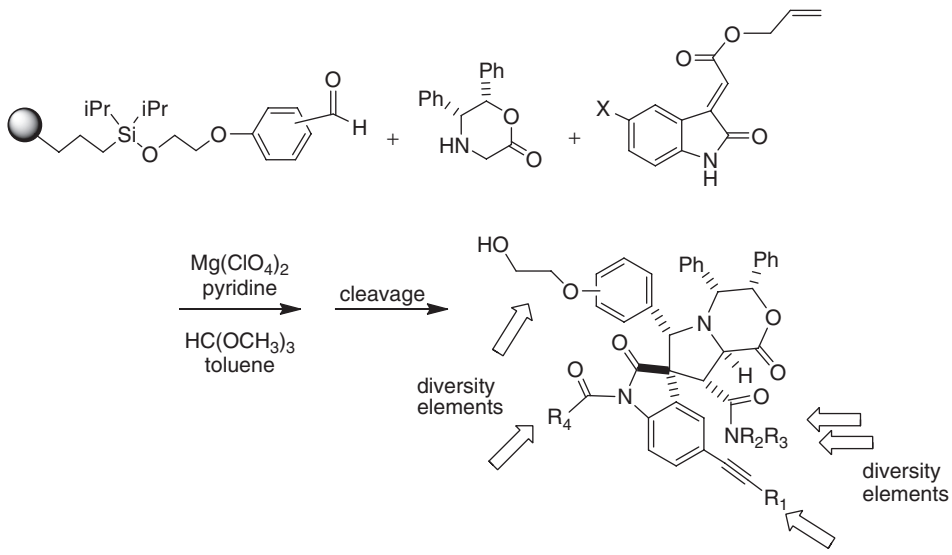

Figure 1.6 *Schreiber's split and pool three-component reaction to generate a three thousand member spirooxindole library*

80%. The Schreiber work sets a noteworthy precedence for future applications of combinatorial pool and spilt applications in the context of synthesizing libraries of challenging, high functionalized and complex small molecules.

1.2 Fundamentals of conducting solid-phase organic chemistry

1.2.1 Apparatus

There is a host of apparatus available to conduct solid-phase organic synthesis and the purpose herein is not to try to define each variation, but rather speak to the purpose and principles. The critical features for any are, firstly, the ability to mix the heterogeneous reaction (resin-bound organic substrate and reagent solution) and, secondly, to provide a means to separate solid resin from solution, preferably in a way that allows for washing of the resin. A variety of simple mixing techniques are applied, including devices to shake or invert the vessel or mechanical stirrers to agitate the mixtures or, lastly, gas bubblers to confine resin or physically move resin within the solution. Thus, in its simplest form, resins containing organic substrate can be exposed to reagent in solution in a conventional glass vessel with stirring and then the resin can be merely filtered off by some means (e.g., glass frit). The use of vacuum pressure can be applied to draw washing solutions over and through resins and to aid in drying of the resin. Many variations of this theme have been developed, from simple glass cylinders, a glass frit and a stockcock (Figure 1.7).

More sophisticated approaches have been developed. Organic substrates can be enveloped in polymeric 'bags' that are permeable to solutions of reagents in order to carry out chemical transformations. After the diversity components are attached, the 'bags' can be removed and exposed to washing solutions to remove excess reagent and by-products. The final products can then be removed from the 'tea bag'. The Houghten laboratory first developed this powerful methodology for the synthesis of peptides[19] but the methodology has been extended to a variety of small molecules.[21]

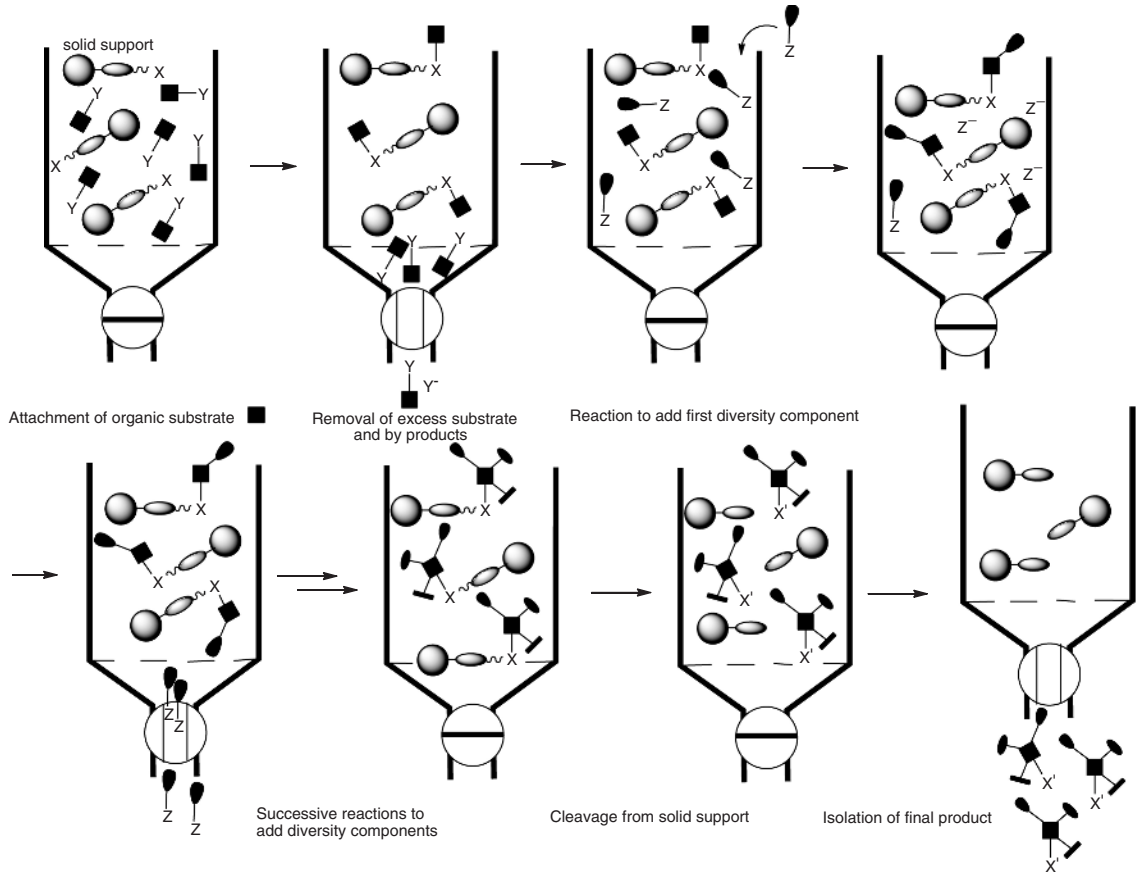

Figure 1.7 *Principle operations of solid-phase organic synthesis*

1.2.2 Typical solid supports

Solid supports comprise a polymeric resin that has been chemically derivatized to incorporate a functional group within the matrix that is able to undergo reaction with small organic molecules. The polymer or resin itself can be made from a number of materials. Not surprisingly, the criteria for selecting a resin revolves around its chemical and physical properties. Firstly, the material must be inert to the contemplated reaction conditions and reagents. Since the mass of the resin far exceeds that of the organic substrate, even minor competing reactions or degradation is usually problematic. In addition, the material needs to swell sufficiently in solvent to expose a large enough surface area to allow the chemical reactions to occur. Additionally, the resin should suspend well in the desired solutions to allow for efficient reaction and washing. Perhaps most importantly, the resin needs to be amenable to functionalization, so organic substrates can be covalently attached and so sequential transformations are therefore possible. The extent to which a functional group has been incorporated onto a resin is quantified and presented as a theoretical loading value, typically given in millimoles per gram.

The most common solid supports are derived from polymeric polystyrene and polyethylene glycol. Often these polymers are cross-linked with additives (divinylbenzene for example) to impart desired physical characteristics (size, swelling). Copolymers are also employed as solids supports as with the Tentagel^{TM}

family of resins, which are low cross-linked polystyrene matrices upon which polyethylene glycol is grafted. Polystyrene resins, typically of 50–400 mesh size with loading values of typically 0.5–1.5 mmol/g, have been extensively functionalized to include many common and versatile organic functional groups. Some resins are functionalized with nucleophilic moieties such as alcohols and amines whereas others contain electrophilic centers (e.g., α,β-unsaturated ketone, carbonates, etc.). More about how moieties are used to form linker groups is given in subsequent chapters. The functional groups themselves can be incorporated onto a resin directly, or via some inert tether (Table 1.1 below shows representative examples). Furthermore, these moieties can be obtained in a protected form or activated towards subsequent reaction in many cases.

Table 1.1 *Resin derivatization; attachment of versatile functional groups*

Functional group	Moiety presented to organic substrate
Alcohol	~~~CH_2-**OH**
	~~$C_6H_4CH_2OC_6H_4CH_2$-**OH**
	~~C_6H_4CH(**OH**) (C_6H_5)
	~~$C_6H_4C(C_6H_5)_2$-**OH**
Aldehyde	~~~C_6H_4-**CHO**
	~~$C_6H_4CH_2O((CH_3O)_2C_6H_2)$-**CHO**
	~~$CH_2CH_2[OCH_2CH_2]_5NHC(O)(CH_2)_4$-**CHO**
(Acetal)	~~~$C_6H_4CH_2CH_2NHC(O)(CH_2)_4$-**CH(OEt)$_2$**
Alkenyl	~~~C_6H_4-O-C(O)-**CH = CH$_2$**
	~~$C_6H_4CH_2OCH_2CH_2$-SO_2-**CH = CH$_2$**
	~~C_6H_4-**CH = CH$_2$**
Amine	~~~CH_2-**NH$_2$**
	~~C_6H_4-CH_2-**NH$_2$**
	~~$C_6H_4CH_2OC_6H_4CH_2$**NH$_2$**
	~~-CH_2-**N(CH$_3$)$_2$**
	~~$C_6H_4C(Ph)_2$-OC(O) $CH_2C_6H_4CH((CH_3O)_2C_6H_3)$-**NH$_2$**
Amine (benzyl)	~~~$C_6H_4CH_2$-**NH**-$CH(CH_3)CH_2Ph$
Amine (sulfonyl)	~~~NHC(O)-C_6H_4**SO$_2$**-**NH$_2$**
Amidine	~~~$C_6H_4CH_2OC_6H_4$-**N = CH-N(CH$_3$)$_2$**
Carbonates	~~~**OC(O)O**C_6H_4-NO_2
Carboxylic acid	~~~C_6H_4-**COOH**
	~~$C_6H_4CH_2$-**COOH**
	~~$C_6H_4CH_2NHC(O)CH_2CH_2$-**COOH**
	~~CH_2CH_2 $OCH_2CH_2NHC(O)CH_2CH_2$-**COOH**
Halogenated	~~~**CH$_2$-Cl**
	~~$C_6H_4CH_2OC_6H_4$-**CH$_2$-Cl**
	~~C_6H_4-**I**
Hydroxylamine	~~~$C_6H_4CH_2OC_6H_4CH_2$-**NH-OH**

Examples (as will be seen in later chapters) include the common fmoc ((*9H*-fluoren-9-ylmethoxycarbonyl)) derivatives (not shown) used to cap amines, esters to activate carboxylic groups and *para*-nitrocarbonates used to activate carbonyl centers.

1.2.3 Fluorous supports

The development of fluorous chemistry, led by the Curran research group, has hugely impacted solid-phase organic synthesis as well as traditional solution-based chemistry.[22] Highly fluorinated organic molecules, particularly perfluorinated hydrocarbons, possess unique physical properties (e.g., high degree of hydrophobicity) that have been described as 'orthogonal' to traditional (non-fluorinated) 'organic' and 'aqueous' phases. Thus, fluorous solvents can be used to solubilize fluorous reactants and react with an organic substrate. The fluorous product can be separated from the reaction media by several methods. Liquid–liquid extraction is a straightforward procedure to isolate fluorous products from non-fluorinated by-products by using solvents with high fluorine content to separate product from non-fluorous materials. Such separations closely resemble aqueous quench, work-up and extraction protocols commonly used in organic synthesis.

Separately, fluorous tags can be used to covalently modify organic molecules so as to make the physical and chromatographic properties of the tagged molecule similar to fluorohydrocarbons, and thus allow for fluorous separation and purification. Ideally, the fluorous tag can subsequently be removed to yield the final product. Lastly, chromatographic stationary phases can be made fluorous and used to effect separation and purification, much as in the same way silica gel is routinely used with non-fluorinated solvents.[23]

Following these advances, highly fluorinated or perfluorinated hydrocarbons have also been functionalized and immobilized on a solid support to serve as linker groups (Chapter 21). All non-fluorous materials (unreacted reagents for example) are then readily removed by simple washing techniques and the final product is then cleaved from its fluorous host. Resin-bound fluorinated alkylsulfonate esters, for example, aryltriflates, can undergo palladium catalyzed transfer hydrogenolysis as a means to induce traceless cleavage or, conversely, undergo Suzuki coupling to arylate upon cleavage (Figure 1.8).[24, 25]

1.2.4 Linker strategies[26, 27]

To accommodate the burgeoning traditional chemistry that would be adapted to solid-phase organic synthesis, the composition and fate of the so-called linker group garnered great interest for several reasons. Since attachment of the organic substrate constitutes the first chemical reaction within the synthesis, the linker group needs to form a covalent bond with the organic substrate readily and efficiently. Ideally, this

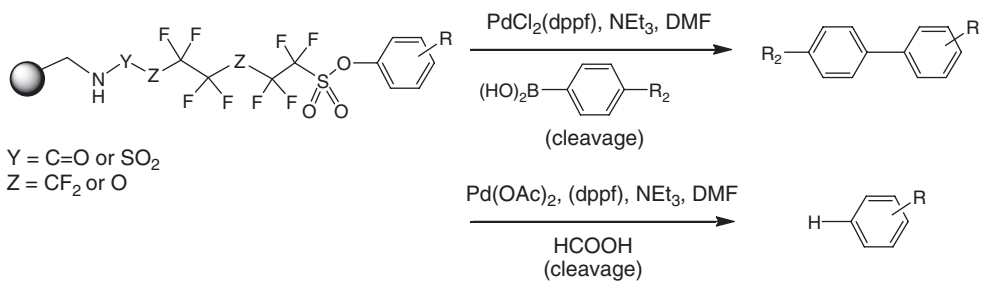

Figure 1.8 *Examples of fluorous linker groups*

process will result in a high degree of 'loading' of the organic substrate onto the resin. In cases where this does not happen, additional procedures are often needed to cap remaining active sites within the resin in order to carry out subsequent chemistry as desired. Since the linkage needs to remain intact for the duration of the synthesis, the optimal linker needs to be inert to a wide range of reaction conditions and reagents. Moreover, the linker group often needs to provide some 'molecular' distance from the macromolecular solid support, so as to allow for exposure of the organic substrate to the solution-based reagent(s). While this factor is difficult to ascertain empirically, an optimum linker group will allow for the organic substrate to dispose and orientate itself within the solvent, so as to allow for an efficient reaction with reagent and preferably an acceptable (rapid) rate of reaction. Lastly, once fully synthesized, it is vital that the organic substrate can be cleaved from the solid support with ease. Thus, specialized reaction conditions have been developed depending upon the specific linking moiety, in order to liberate pure final products. Notice the requirements of the successful linker group – it has to be robustly attached to the solid support for the duration of the synthesis yet able to react with the organic substrate, inert towards subsequent chemical transformations and, lastly, under a separate set of conditions, able to release the organic substrate.

The linking groups themselves can be divided into categories based upon their structural impact on the organic substrate molecule (Figure 1.9). Many linkers rely upon a reactive functional group that forms a robust functional group upon capturing the organic substrate. In such cases, cleavage from the resin revolves upon a reaction of that functional group and this process (e.g., hydrolysis) often leaves a heteroatom bonded to the organic substrate at site of cleavage. This heteroatom (or functional group) can be 'traced back' to the precise site of attachment to the linker group and, hence, to the solid support. Such traditional linkers can be especially valuable if they are used to install a key heteroatom or functional group at some desired

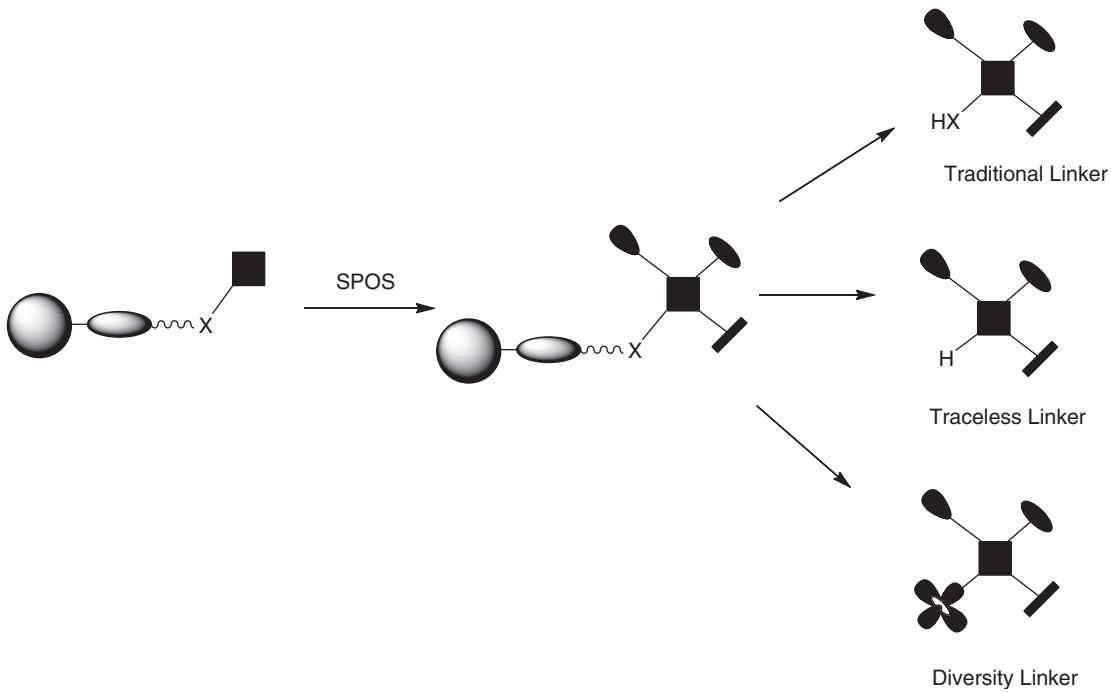

Figure 1.9 *Depiction of different linker group strategies. (Reproduced with permission from Eur. J. Org. Chem* ***2006****, 2251–2267. Copyright Wiley-VCH Verlag GmbH & Co. KGaA.)*

position (e.g., a structural element needed for some pharmacological activity). However, too often, this is not the case and one settles for an extraneous, if not undesirable, functional group uniformly installed at the same position across a compound library.

For these reasons, a great amount of effort went into developing so-called 'traceless' linkers, in which a hydrogen atom replaces the heteroatom or functional group that covalently joins the organic substrate to the solid support. In doing so, there is no tell-tale sign that the organic molecule was joined to a solid support and, more importantly, there is no extraneous heteroatom or functional group that needs to be addressed (e.g., removed via additional chemical transformations). Traceless linker groups removed a major shortcoming with the early groups. After all, a chemist carrying out solution-based synthesis would rarely find reason to choose a more complex starting material containing superfluous heteroatoms; to be relegated to do otherwise in order to conduct solid-phase synthesis diminishes the application and usefulness of the technology.

The last category of linker groups is those that structurally alter the original attachment point of the organic substrate during the process of cleavage. This strategy has been another major advance to the field as a whole because it allows for an additional element of diversity to be introduced into the compound library, and at the very last step of solid-phase synthesis, namely upon cleavage. Diversity-based (or oriented) linker groups contain functional groups (or heteroatoms) that are specifically chosen for their latent reactivity towards cleavage conditions (e.g., a nucleophile) and their ability to undergo an efficient chemical transformation as a means to induce cleavage. This may be accomplished in a number of ways, including either the addition of a nucleophilic component or an electrophilic component to react with the linking group and liberate a modified organic substrate. The key distinction compared to the other types of linker groups is that diversity-oriented linkers afford liberated molecules that do not have the original heteroatom or functionality present at the site of attachment, nor are the final products 'traceless' (i.e., have a hydrogen atom at the site of attachment). The majority of linker groups discussed in this book will deal with diversity-oriented linker groups which, as will become apparent, have been cleverly conceived and crafted to install even more diversity into compound libraries.

Hundreds of linkers that have been developed, many are variations on a common theme; only a few will be highlighted here to illustrate key concepts about the technology as a whole. The reader is encouraged to consult the subsequent chapters, in which specific linking groups are discussed in detail. The early linker units, not surprisingly, followed chemistry developed for Merrifield peptide synthesis (and oligonucleotide synthesis) and attached the organic substrate to the solid support through the covalent reaction of a polar functional group with a nucleophilic or electrophilic site within the resin. Alcohols, amines and carboxylic acids serve as common linker groups. For example, resin-bound benzyl alcohol readily reacts with acids (as activated esters) to form ester linkages, or with isocyanates to form carbamate linkages. After synthetic modification, the organic product can be cleaved using rather strongly acidic conditions (Merrifield cleavage originally used hydrofluoric acid) or by the reaction of strong nucleophile such as hydroxide (Figure 1.10). Additionally, amides are accessible from ester-linked substrates by the addition of nucleophilic amines.

Unfortunately other functional groups can be labile under such harsh conditions and thus milder conditions have since been developed. The Wang resin makes use of a *para*-oxygen atom within the benzylic alcohol linker to stabilize the resultant cation and allow for cleavage under much milder conditions. Thus, Wang resin can first be activated by conversion to its *para*-nitrobenzylcarbamate to which nucleophilic amines readily add. N-methylated amine products are then accessible by a reductive cleavage from the resin while des-methylated analogs can be obtained via mild acid cleavage (Figure 1.11).[28] In a similar way, benzhydryl amines and amides, such as the Rink linker, can be used to make amides and ureas with mild cleavage from the resin (Figure 1.11).[29]

Mild base-induced cleavages, to compare to the use of strong nucleophiles, have also been developed and one such method has led to a specialized regenerative Michael acceptor (REM) resin that contains an

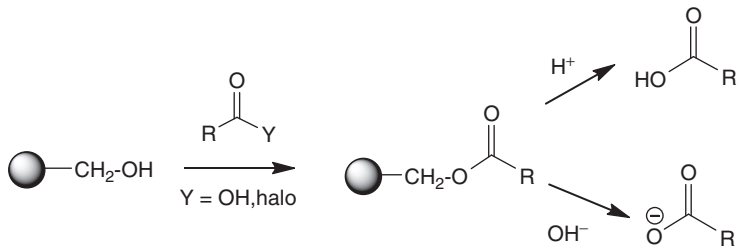

Figure 1.10 *Cleavage via acid or by the reaction of a nucleophile*

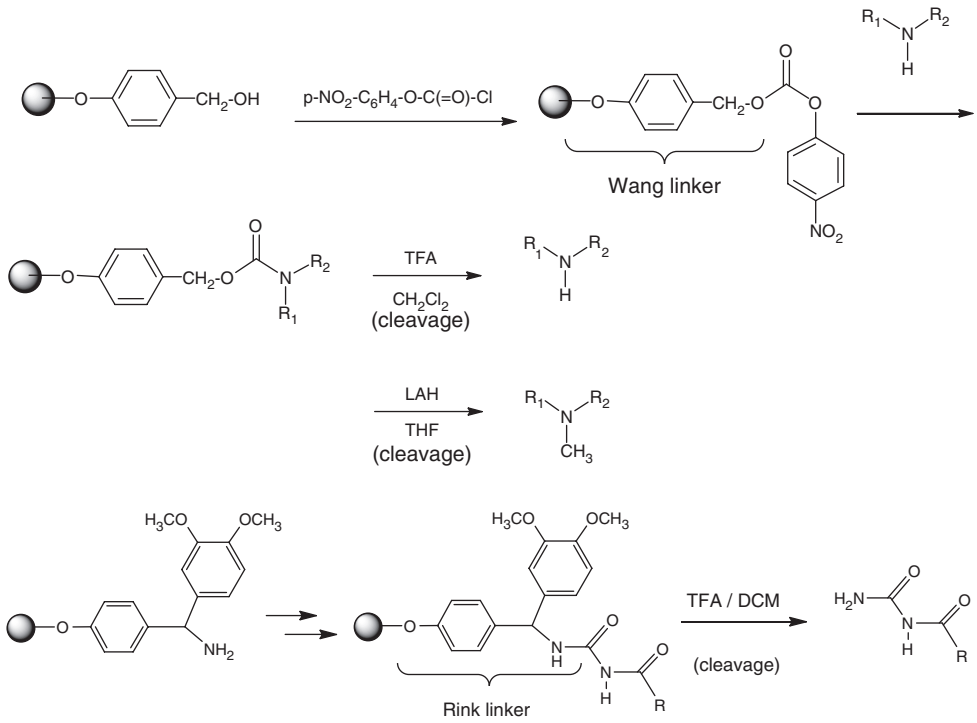

Figure 1.11 *Wang and Rink linker groups allow for mild acid cleavage*

acrylate group to readily accept a nucleophilic organic substrate via a Michael addition.[30] After subsequent transformations, the final product is cleavage by the action of amine base which promotes a Hoffmann elimination reaction (Figure 1.12). In this case, it is the basicity of the added amine, not its nucleophilicity, that is responsible for cleavage.

A major limitation of using strong acids or bases, or many nucleophiles, to induce cleavage of organic substrate joined to the resin via simple carboxylic acids derivatization, such as esters, is their lability. Ideally, a linker group will release an organic substrate only as called upon, despite the myriad of reaction conditions it may encounter throughout its synthetic lifetime. The development of a so-called 'safety catch' linker group introduced the concept of making use of a robust sulfonamide linker group that could be activated towards cleavage as desired, through simple and specific N-alkylation (Figure 1.13).[31, 32]

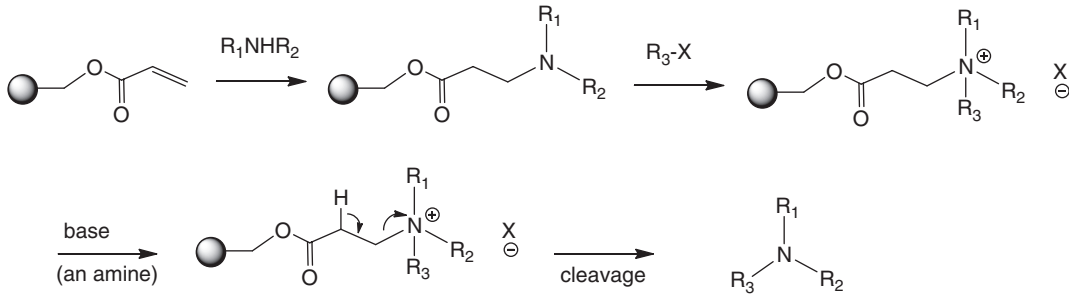

Figure 1.12 *Cleavage by a base inducing an elimination reaction*

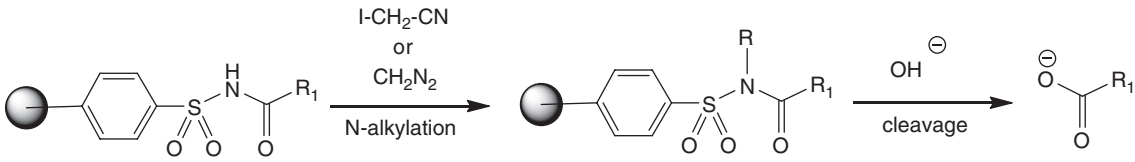

Figure 1.13 *A sulfonamide 'safety-catch' linker group*

This linker group is inert to most nucleophiles, including hydroxide, and this feature greatly expands the types of nucleophilic transformations that can be accomplished on the organic substrate without causing premature release.

In addition to perfecting cleavage methodology through the use of functional group cleavage (ester, sulfonamides, etc. as shown above), a great deal of attention was to develop chemistry that would remove all atomic evidence that an organic substrate, a small molecule, was indeed ever attached to a resin. Towards this, the first traceless linker to be developed and widely used makes use of the ability of silicon to undergo ipso substitution, by which hydrogen can be introduced in place of the heteroatom or functional group that originally bonded the organic substrate. The ability to introduce a hydrogen atom makes the linker traceless; the final product contains no tell-tale sign or signature that allows the deduction of which position of the organic substrate was bonded to the linker group, and therefore to the solid support. The prototypic traceless linker was pioneered by Ellman and again applied initially to the synthesis of benzodiazepines (Figure 1.14).[33]

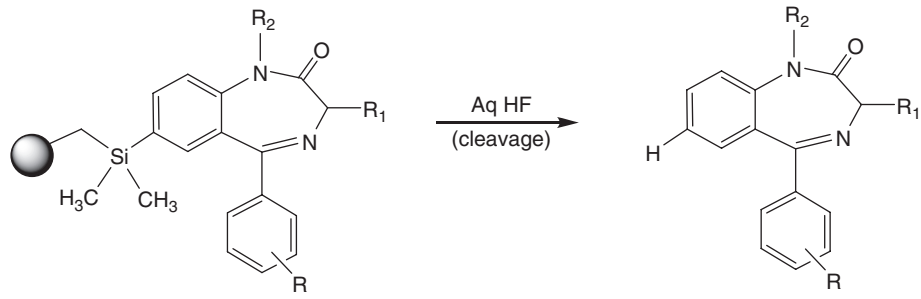

Figure 1.14 *Traceless cleavage using a silicon-based linker group*

Diversity-oriented (or diversity-generating) linker groups introduce another element of diversity to the organic substrate upon the cleavage process. There are numerous examples of this approach, which will be covered extensively in Part 2 of this book. Some are very direct examples in which an atom, often an electrophile, mimics hydrogen by interacting with an organometallic atom within the linker group, and induces a cleavage similar to the traceless cleavage previously discussed. Thus, germanium-based linking groups react with electrophilic halogens to directly substitute bromine, chlorine or iodine upon releasing the substrate from resin. Germanium, like silicon, undergoes ipso substitution via a β-stabilized carbocation, allowing for a halogen to be introduced (Figure 1.15).[34]

Very innovative linker groups have been developed based upon the unique properties of other elements and so almost all non-metal and many organometallic elements have found their way into solid-phase organic synthesis (SPOS). One striking example is the use of phosphonates to firstly serve as a linker group and then, secondly, to allow for arylation via palladium catalyzed Suzuki chemistry upon cleavage (Figure 1.16).[35]

The examples provided above capture some of the breadth of chemistry that has been developed in the context of linker groups. The early work was based primarily on carboxylic acid functionality to attach and then cleave the organic substrate. As milder cleavage methods were sought, specialized variants, such as benzylic alcohols and amines, were immobilized and developed as linkers. Efforts to design linker groups that were traceless ensued, along with the recognition that linker groups themselves could be used as synthetic handles to install additional diversity. Collectively, these major advances allowed for remarkable expansion of the technology as is illustrated in subsequent chapters.

1.2.5 Challenges

Despite the many advantages that solid-phase organic chemistry can afford, there remain significant challenges. Macromolecular resins and polymers remain limited in the amount, or number of substrate

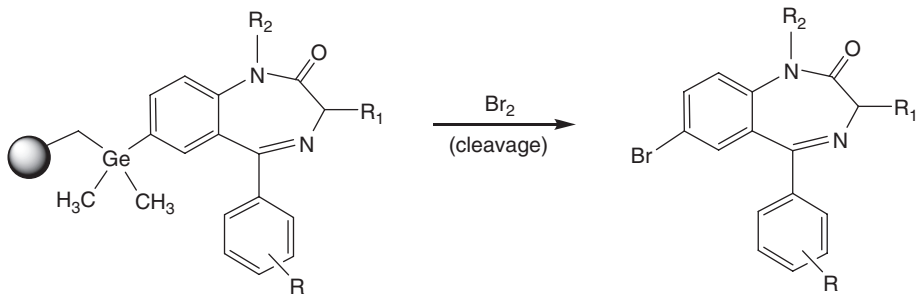

Figure 1.15 *Cleavage via an electrophile: modification of a traceless approach to install a diversity element (halogen) directly*

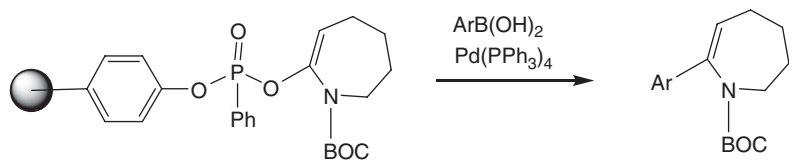

Figure 1.16 *Phosphonate-based linker groups (arylation as a diversity element)*

molecules that can be accommodated onto the solid support. First of all, even under optimal loading conditions, the amount of organic substrate per volume or weight, can pale in comparison to the traditional homogeneous solution-based chemistry (depending upon concentrations of the latter). Secondly solid-phase synthesis, in typically yielding milligram to gram quantities, has been relegated to basic research applications such as stocking compound collections or driving *in vitro* structure–activity relationship (SAR), rather than finding widespread use in scale-up or process operations. Additionally, even highly efficient loading reactions that covalently attach the organic substrate to the solid support, will leave reactive sites on the resin intact. These moieties can then undergo chemical transformations that can result in the formation of significant quantities of side products. For example, a resin bearing free hydroxyl groups can readily accept organic substrates via formation of an ester or ether bond but any remaining unreacted free hydroxyl groups can then act as nucleophiles themselves towards subsequent reagents (e.g., electrophiles). Conversely, while most resins are remarkably inert, the scope of chemical reactions for any given solid-phase supported organic substrate can be limited by side reactions that either cleave the substrate or covalently modify the resin itself. Since the bulk of material in any solid-phase synthesis comes from the solid-support, even minor side reactions to the macromolecule can be problematic in either consuming a reagent or by altering the physical or chemical properties of the resin.

Similarly, applying solid-phase organic synthesis in a combinatorial manner is not without limitations. The concept of conducting multiple simultaneous chemical reactions infers that all reactions will proceed with similar efficiency and, usually, at similar rates. These assumptions are necessary when generating large number of compounds unless there is a willingness to check and monitor each and every substrate and its chemical reactions. If one goal of generating a library is to install a high level of diversity, as is often the case, then structurally diverse building blocks (reagents and organic substrates) are required. The selection of building blocks may include members with wide variations in molecular size, sterics, electronic character around a key functional group, polarity and lipophilicity. Such a rich medley of molecular participants will present differing reactivities and solubilities, which can be challenging in terms of efficient production, isolation and purification of desired target molecules.

While many of the issues raised above are addressable to some degree, one fundamental intangible, central to the very heart of combinatorial chemistry, is subject to near constant debate: What constitutes diversity? While there have been attempts to define ‘diversity’ in terms of ‘chemical space’, it is often the pharmacological or biological activity that generates interest in a molecule. Such interactions, with enzymes, proteins, channels, receptors, and so on, most likely involve conformational changes, pockets of solvation and lipophilic interactions and so the way in which a chemist may view a molecule (e.g., with this backbone ‘extended’ and its substituents neatly disposed in two dimensions) may be quite different from how a molecule actually ‘works’ with respect to ‘activity’. Even from a pure chemical perspective, ‘diversity’ may be in the ‘eye of the beholder’ because the sum of individual molecular fragments, however delineated, does not accurately describe a given molecule as a whole.

1.2.6 Linker groups

This textbook is dedicated to describing linker strategies in solid-phase organic synthesis. After this introduction, the Part Two of the book covers traditional linker units, namely those which leave a molecular (or atomic) footprint on the final products that can be traced back to the site of attachment to the linker group. Chapter 2, *Electrophile Cleavage Linker Units* by Michio Kurosu (Colorado State University, USA) and Chapter 3, *Nucleophile Cleavable Linker Units* by Andrea Porcheddu and Giampaolo Giacomelli (University of Sassari, Italy), discuss these large categories of linker groups and build upon a few of the landmark examples that were briefly illustrated in this chapter (Figures 1.1, 1.10–1.12). Such linkers clearly originated from Merrifield peptide synthesis and were instrumental in moving solid-phase technology ahead from

peptide assembly into the realm of pure organic synthesis. Chapter 4, *Cyclative Cleavage as a Solid-Phase Strategy* by A. Ganessan (University of Southampton, UK), describes cyclization cleavage methodology, which has been a very creative undertaking, often giving rise to interesting heterocyclic compound libraries (one example is provided in Figure 1.3 in this chapter). Chapter 5, *Photolabile Linker Units* by Christian Bochet and Sébastien Mercier (University of Fribourg, Switzerland), introduces the topic of mild photolytic cleavage from the solid support, typically using rather straightforward irradiation reactions. However, one specific challenge to such linker groups arises from shadowing by the polymeric support, which can slow photolysis. Chapter 6, *Safety-Catch Linker Units* by Sylvain Lebreton and Marcel Patek (Sanofi Aventis, USA and France), covers a family of linkers that require activation through a chemical transformation prior to cleavage (Figure 1.13). This approach has been a breakthrough in circumventing side reactions often encountered with cleavages that require harsh conditions. Chapter 7, *Enzyme Cleavable Linker Units* by Mallesham Bejugam (University of Cambridge, UK) and Sabine Flitsch (University of Manchester, UK), introduces a rather new, tailored concept by describing linker units that are cleaved by enzymatic processes.

The evolution of traceless and multifunctional linker groups is covered extensively in Part Three of the book, which makes up the bulk of this book. Chapter 8, *Introduction to Diversity-Oriented Synthesis* by Richard Spandl, Gemma Thomas, Monica Diaz-Gavilan, Kieron O'Connell and David Spring (University of Cambridge, UK), discusses how linker groups can be designed to participate in well known contemporary organic reactions thereby enabling a diversity element to be introduced during the cleavage process. The adoption of such solution-phase chemistry to solid-phase technology in this context has been extraordinarily powerful as subsequent chapters reveal.

Chapter 9, *T1 and T2 – Versatile Triazene Linker Groups* by Kerstin Knepper and Robert Ziegert (Woergl, Austria), Chapter 10, *Hydrazone Linker Units* by Rysard Lazny (University of Bialystok, Poland), and Chapter 11, *Benzotriazole Linker Units* by Daniel Whelligan (Institute of Cancer Research, UK), cover linkers that revolve around nitrogen chemistry in describing triazene-based linker groups and hydrazone/benzotriazole variants, respectively. The remaining chapters turn to linker groups that use certain chemical properties that are unique to specific elements. Thus, Chapter 12, *Diversity Cleavage Strategies from Phosphorous Linkers* by Patrick Steel and Tom Woods (University of Durham, UK), illustrates Wittig and Horner–Emmons type chemistry as well as palladium mediated aryl couplings, which are extremely valuable in adding complex diversity units (e.g., Figure 1.16). Chapter 13, *Sulfur Linker Units* by Peter Scott (University of Michigan, USA), showcases various thiol-derived linkers that can be oxidatively activated for cleavage and the subsequent installation of an additional diversity unit. Intriguing organometallic-based linker groups include selenium and tellurium congeners (Chapter 14, *Selenium and Tellurium Linker Units* by Tracy Yuen Sze But and Patrick Toy (University of Hong Kong, Hong Kong)). There have been a number of cleavage methods that make use of free radical chemistry (from sulfur, oxygen and selenium linker units) and this topic is covered in Chapter 15, *Sulfur, Oxygen, Selenium and Tellurium Linker Units Cleaved by Radical Processes* by Guiditta Guazzelli, Marc Miller and David Procter (University of Manchester, UK). Chapter 16, *Silicon and Germanium Linker Units* by Alan Spivey (Imperial College, UK) and Chris Diaper (NAEJA Pharmaceutical Inc., Canada), discusses the use of these organometallic-centered groups to act as either traceless linkers, or as linkers that are readily cleaved by a simple halogen, respectively. Other rarer organometallic linker units are discussed in Chapter 17, *Boron and Stannane Linker Units*, and Chapter 18, *Bismuth Linker Units* (Peter Scott, University of Michigan, USA), which address these rather rare linkers, highlighting Stille/Suzuki-type couplings and bismuth cross-coupling reactions, respectively. Transition metal variants such as chromium-based linkers are discussed in Chapter 19, *Transition Metal Carbonyl Linker Units* by Sue Gibson and Amol Walke (Imperial College, UK). Finally in this section, Chapter 20, *Linkers releasing Olefins or Cycloolefins by Ring Closing* by Jan H. van Maarseveen (Universiteit of Amsterdam, The Netherlands), turns to the linkers with carbon–carbon unsaturated bonds and the use of metathesis chemistry as a powerful means to attach and cleave organic substrates.

Part Four of the book, *Alternative Linker Strategies*, contains the final chapters discussing emerging uses of solid-phase synthesis. Chapter 21, *Fluorous Linker Units* by Wei Zhang (Fluorous Technologies, USA), is devoted to a discussion on alternative linker strategies including fluorous technologies which is a rapidly expanding area. Chapter 22 by Brian Hockley, Peter Scott and Michael Kilbourn (University of Michigan, USA) showcases the emerging use of solid-phase synthesis in radiochemistry and the process of producing radiopharmaceuticals. Lastly, Part Five, *Linker Selection Tables*, provides comprehensive and extremely useful information that the reader can turn to when designing and using linker groups in solid-phase chemistry.

1.3 Concluding comments

In its infancy (mid-1990s), the lure of solid-phase synthesis, coupled with combinatorial techniques, was irresistible. Large pharmaceutical companies, selling perhaps at best dozens of drugs looked in dissatisfaction at their meager corporate compound libraries that numbered in the mere tens of thousands. In a desire to discover new drug leads, it was easy to fathom how many compounds were historically needed to obtain one clinical candidate (estimates run from 3000 to 10,000), yet alone a successful, revenue-generating pharmaceutical product. Therefore, it was assumed or could even be calculated that having the ability to synthesize (and screen) many tens or hundreds of thousands of compounds would give better lead compounds and therefore more marketable drugs quicker (by some measure). Remember, at the time, earlier biological break-throughs, such as polymerase chain reaction (PCR) and other molecular biology advances (cloning), were well in hand and in widespread use and, thus, biology was 'outpacing' chemistry. Chemists, as well as others in the industry, were eager to embrace and drive this new chemistry technology to new heights.

Along the way, the combi-chemists (as they were rightfully or wrongly termed) accomplished so much as they redefined productivity and structural diversity on some level, but as many have witnessed, more is not always better. Having the capability and capacity does not alone drive progress in research. Science must be built upon previous learnings and discoveries and is not subject to calculated odds. Thus, while the field of chemistry has benefitted enormously from solid-phase synthesis and combinatorial techniques, the industries that use and pay for chemistry innovation in terms of bringing products to market, have been left perhaps somewhat disappointed (i.e., pharmaceutical houses). The translation of the technology to the marketplace has yet to be realized because the expectations from the outset were misplaced. More meaningful expectations need to be set within the context of delivering to 'the bottom line' within the pharmaceutical sectors.

Few, if any technologies within the field of synthetic organic chemistry have generated more promise and potential than combinatorial chemistry. Looking back upon the past decade or two, and under the cloud of stumbling productivity within the pharmaceutical industry, it may be time to ask what this amazing technology is best used for, rather than using its power to plays odds against the discovery of a drug. Indeed, pharmaceutical sciences have advanced in wonderful ways due to solid-phase organic synthesis and combinatorial chemistry. The staggering numbers of compounds that have been synthesized attests to this fact. The increasing ways in which complex synthetic chemistry can be made more efficient, or even automated, are evident. Furthermore, analytical techniques, such as magic-angle NMR, tagging and deconvolution processes, which were once reserved for the few, are now widely used and appreciated by many in the field.

In the future, a continuation of expansion of the solid-phase synthesis/combinatorial chemistry universe will be seen because there are significant frontiers that have yet to be surmounted. Moreover, further refinement in scope will be seen, so as to better translate the chemistry from the resin in the vessel to the biology in the well on the plate, or even into the animal.

1.4 Personal perspective and testimony: solid-phase Mannich chemistry

In our labs at Johnson & Johnson, we became interested in developing small compound libraries that could be loosely targeted towards G-protein coupled receptors (GPCRs). The general premise was to install lipophilicity to allow for penetration within the transmembrane region while maintaining requisite charge site(s) to interact with conversed aspartic acid residues within specific members of the family. We desired a way to rapidly expand 'diversity' and to 'dial in' shape to our molecules and envisioned additional chemical transformations that could be carried out late in the synthetic route so as to add yet another element of diversity. With these items in mind, we turned to multicomponent reaction systems because multiple elements of diversity can be introduced in a single transformation. The Mannich reaction (Figure 1.17) is a classic three-component system in which 'hydrogen active' substrates react with imine species that arise from condensation of an amine with an aldehyde. The application of Mannich chemistry to resin-bound substrates had been meager despite the general utility of this reaction in traditional solution-based organic synthesis.[36] The Kobayashi group recognized that silyl enol ethers can serve as the 'hydrogen active' component and applied this variation to solid-phase technology.[37] Resin-bound silyl enol ethers reacted with imines that were pre-formed via the reaction of amines with aldehydes in the presence of a Lewis acid catalyst and a dehydrating agent. However, terminal alkynes, in the presence of a copper(I) salt, can also behave as the 'hydrogen active' Mannich coupling partner and without the need to pre-form the imine species.[38] From this finding, we decided to explore solid-phase Mannich chemistry and in order to fully use the power of this reaction, with respect to generating highly diversified compound libraries, we sought to separately immobilize each component onto a solid support.

We went on to demonstrate that solid-phase Mannich reactions of aldehydes, amines and alkynes indeed occur smoothly and efficiently, and is not hampered by the heterogeneity of the reaction (Figure 1.18).[39–43] This multicomponent strategy is powerful for several reasons.[44] Any single one of the components can be immobilized on an appropriate solid support, which makes the methodology very versatile from a synthetic perspective. Structurally-diverse compound libraries can be readily prepared due to the numerous amines, aldehydes and alkynes that are either commercially available or easily synthesized. The alkyne moiety itself presents an opportunity for further synthetic elaboration and provides a site for the introduction of another element of diversity. Finally, many functional groups are tolerant to the experimental conditions. In our studies, the solid-phase Mannich reactions are very efficient with isolated products typically being of high purity (>90%).

The resultant Mannich adducts contain a carbon–carbon triple bond, which is a particularly attractive feature. Alkynes are among the most versatile functional groups in organic chemistry[45] and can serve as a synthetic handle for a myriad of additional manipulations. Furthermore, simple modifications to alkynes greatly alter their shape and flexibility, changing considerably the molecular scaffold thus allowing for more conformational space to be accessed. Whereas carbon–carbon triple bonds are rigid and linear, *cis*- and *trans*-alkenes possess characteristic cupped or extended arrays, respectively, and the corresponding saturated alkane is a floppy tether. In addition to reductions, other synthetic modifications of carbon–carbon

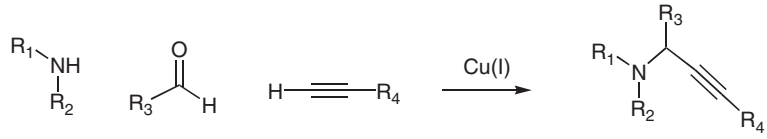

Figure 1.17 *A Mannich reaction*

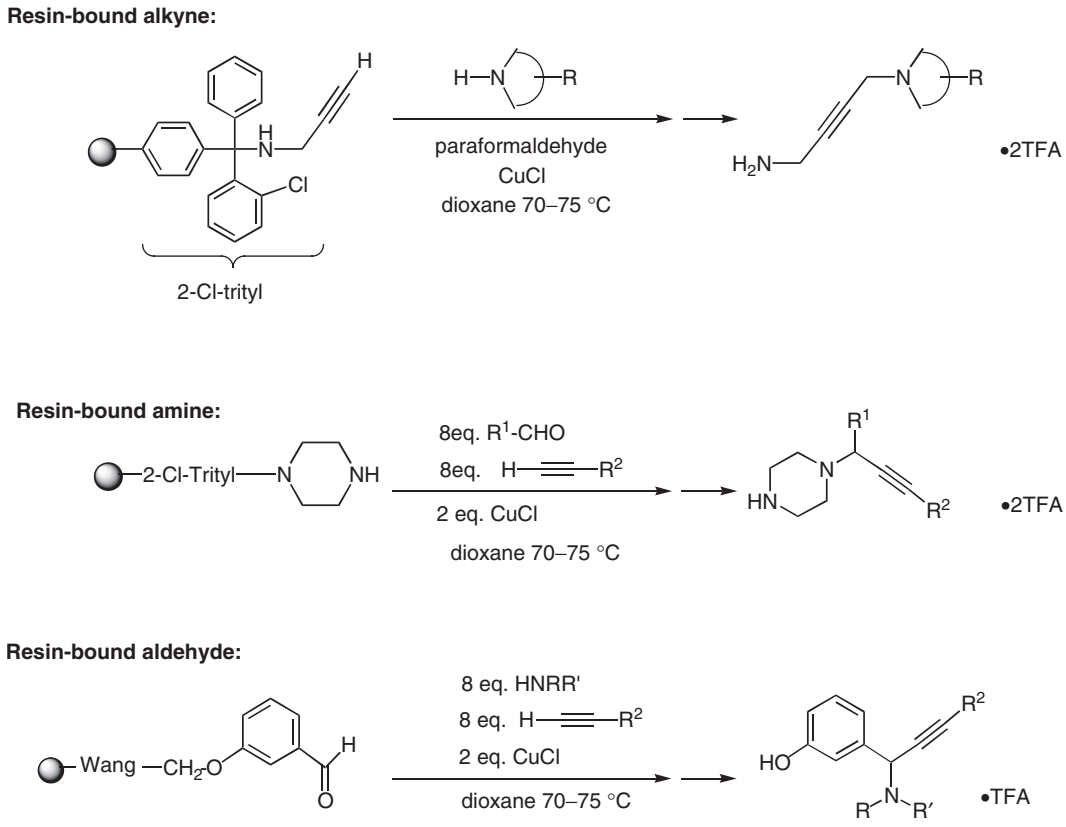

Figure 1.18 *Solid-phase Mannich reactions*

triple bonds are well-known and include hydrometalation[46] and cycloaddition[47] chemistry. Such heterocyclic systems are widely found within biologically active substances with medicinal, veterinary, and agricultural application. By developing and using this chemistry, we were able to synthesize hundreds of novel compounds and rapidly found submicromolar ligands for a variety of GPCRs thus validating our approach.

This project was not a main focus of our laboratories and two superb chemists, Mark A. Youngman and James J. McNally conducted this work literally 'working in the back of the hood'. The solid-phase technology outpaced the number of compounds made by other members of the group, again demonstrating the power of solid-phase synthesis and combinatorial approaches.

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