16 Combinatorial and parallel synthesis

Combinatorial and parallel synthesis have become established tools in drug discovery and drug development, allowing the use of a defined reaction route to produce a large number of compounds in a short period of time. The full set of compounds produced in this way is called a **library.** Reactions are usually carried out on small scale, so that the process can be automated or semi-automated, allowing reactions to be carried out in several reaction vessels at the same time and under identical conditions, but using different reagents for each vessel. The compact nature of the apparatus means that the process can be carried out within a normal fume cupboard.

16.1 Combinatorial and parallel synthesis in medicinal chemistry projects

In the past, medicinal chemistry involved the identification of a lead compound having a useful activity which was then modified to develop a clinically useful drug. Identification of the molecular target for the drug, and the mechanism by which it worked often took many years to establish. Today, most medicinal chemistry projects start with an identifiable target, and the emphasis is on discovering a lead compound that will interact with this target. This reversal of priorities came about as a result of the human genome project and the proteomic revolution that followed. Once the genome was mapped, a vast number of previously unknown proteins were identified, all of which could be considered as potential drug targets. Pharmaceutical companies were faced with the problem of identifying the function of each target and finding a lead compound to interact with it. Before the advent of combinatorial chemistry and parallel synthesis, the need to find a lead compound was becoming the limiting factor in the whole process. Now, with the aid of these techniques, research groups can rapidly synthesize and screen thousands of structures in order to find new lead compounds, identify structure–activity relationships, and find analogues with good activity and minimal side effects (Fig. 16.1).

The procedures used in combinatorial synthesis are designed to produce mixtures of different compounds within each reaction vessel, whereas those used in parallel synthesis produce a single product in each vessel. In general, parallel synthesis is favoured since it is easier to identify the structures that are synthesized. However, there is still scope for combinatorial chemistry in finding lead compounds, especially since this procedure can generate significantly more structures in a set period of time, thus increasing the chances of finding a lead compound. In the procedure, mixtures of compounds are deliberately produced in each reaction vessel, allowing chemists to produce thousands and even millions of novel structures in the time that they would take to synthesize a few dozen by conventional means. This method of synthesis goes against the grain of conventional organic synthesis where chemists set out to produce a single identifiable structure which can be purified and characterized. The

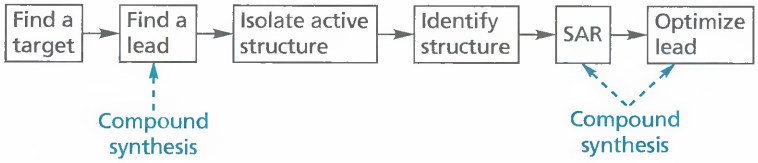


FIGURE 16.1 The stages in drug development requiring synthesis of large numbers of compounds (SAR = structure–activity relationships).

structures in each reaction vessel of a mixed combinatorial synthesis are not separated and purified, but are tested for biological activity as a whole. If there is no activity, then there is no need to continue studies on that mixture, and it is stored. If activity *is* observed, then one or more components in the mixture are active, although false positives can sometimes be an issue (section 12.3.5). Overall, there is an economy of effort, as a negative result for a mixture of 100 compounds saves the effort of synthesizing, purifying, and identifying each component of that mixture. On the other hand, identifying the active component of an active mixture is not straightforward.

In a sense, a mixed combinatorial synthesis can be looked upon as the synthetic equivalent of nature's chemical pool. Through evolution, nature has produced a huge number and variety of chemical structures, some of which are biologically active. Traditional medicinal chemistry dips into that pool to pick out the **active principles** and develop them. A mixed combinatorial synthesis produces pools of purely synthetic structures that we can dip into for active compounds. The diversity of structures from the natural pool is far greater than that likely to be achieved by combinatorial synthesis, but isolating, purifying and identifying new agents from natural sources is a relatively slow process, and there is no guarantee that a lead compound will be discovered against a specific drug target. The advantage of combinatorial chemistry is the fact that it produces new compounds faster than those derived from natural sources, and can produce a diversity not found in the traditional banks of synthetic compounds held by pharmaceutical companies.

A few words of caution should be made here with regards to negative assays. There is always the possibility that a combinatorial mixture does not contain all the structures expected. This can happen if some of the starting materials or intermediates in the synthesis do not react as expected. A negative assay would then lead to the conclusion that these compounds are inactive, even though they are not present. This could mean that an active compound is missed. Assays might also be affected adversely if the individual components of a mixture interact with each other or have conflicting activities.

16.2 Solid phase techniques

Although some combinatorial experiments have been performed in solution, most have been achieved using solid phase techniques, where the reaction is carried out on a solid support such as a resin bead. There are several advantages to this:

- A range of different starting materials can be bound to separate beads. The beads can then be mixed together such that all the starting materials can be treated with another reagent in a single experiment. The starting materials and products are still physically distinct, as they are bound to separate beads. In most cases, mixing all the starting materials together in solution chemistry is a recipe for disaster, with polymerizations and side reactions producing a tarry mess.
- Since the starting materials and products are bound to a solid support, excess reagents or unbound by-products can easily be removed by washing the resin.
- Large excesses of reagents can be used to drive the reactions to completion (greater than 99%) because of the ease with which excess reagent can be removed.
- Intermediates in a reaction sequence are bound to the bead and do not need to be purified.
- The individual beads can be separated at the end of the experiment to give individual products.
- The polymeric support can be regenerated and reused if appropriate cleavage conditions and suitable anchor/linker groups are chosen (see later).
- Automation is possible.

Solid phase synthesis was pioneered by Merrifield for the synthesis of peptides, and most of the early work carried out on combinatorial synthesis was performed on peptides. Peptides have serious disadvantages as oral drugs (section 14.8.2), however, and so a large amount of research has been carried out to extend solid phase synthetic methods to the synthesis of small non-peptide molecules. The essential requirements for solid phase synthesis are:

- a cross-linked insoluble polymeric support which is inert to the synthetic conditions (e.g. a resin bead)
- an anchor or linker covalently linked to the resin—the anchor has a reactive functional group such that substrates can be attached to it
- a bond linking the substrate to the linker, which will be stable to the reaction conditions used in the synthesis
- a means of cleaving the product or the intermediates from the linker
- chemical protecting groups for functional groups not involved in the synthetic route.

16.2.1 The solid support

The earliest form of resin used by Merrifield was polystyrene beads, where the styrene is partially crosslinked with 1% divinylbenzene. The beads are derivatized with a chloromethyl group (the anchor/linker) to which amino acids can be coupled via an ester group (Fig. 16.2). This ester group is stable to the reaction conditions used in peptide

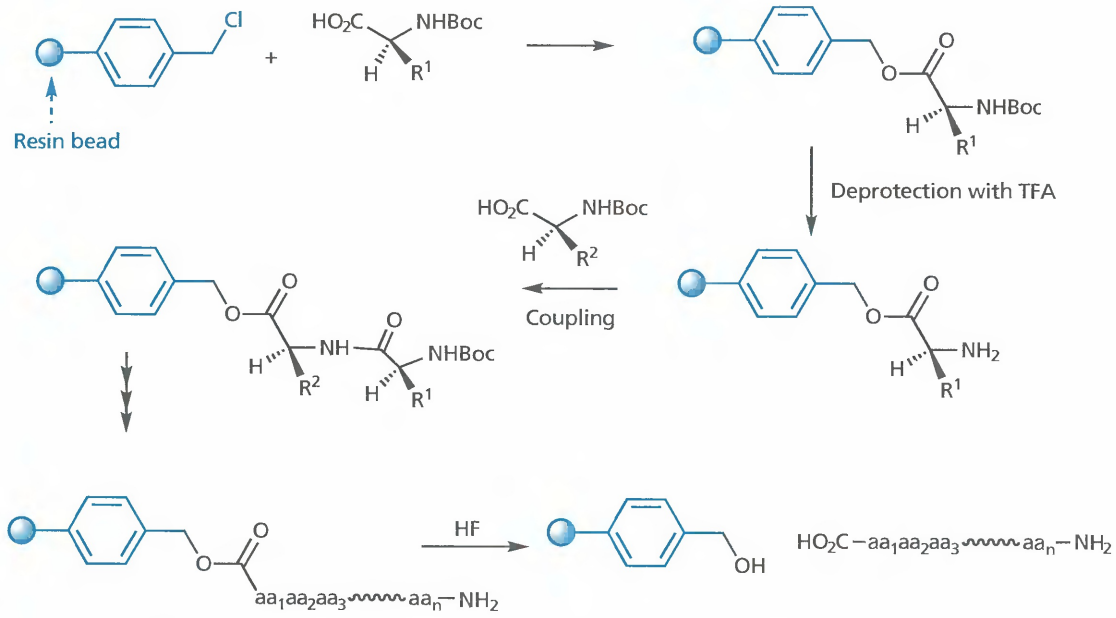


FIGURE 16.2 Peptide synthesis on a solid support (Boc = tert-butyloxycarbonyl = t-BuO-CO; TFA = trifluoroacetic acid).

synthesis, but can be cleaved at the end of the synthesis using vigorous acidic conditions (hydrofluoric acid).

One disadvantage of polystyrene beads is the fact that they are hydrophobic and the growing peptide chain is hydrophilic. As a result, the growing peptide chain is not solvated and often folds in on itself, forming internal hydrogen bonds. This in turn hinders access of further amino acids to the exposed end of the growing chain. To address this, more polar solid phases were developed such as **Sheppard's polyamide resin**. Other resins have been developed to be more suitable for the combinatorial synthesis of non-peptides. For example, **Tentagel resin** is 80% polyethylene glycol grafted to cross-linked polystyrene, and provides an environment similar to ether or tetrahydrofuran. Regardless of the polymer that is used, the bead should be capable of swelling in solvent, yet remain stable. Swelling is important because most of the reactions involved in solid phase synthesis take place in the interior of the bead rather than on the surface. It is wrong to think of resin beads as being like miniature marbles with an impenetrable surface. Each bead is a polymer and swelling involves unfolding of the polymer chains such that solvent and reagents can move between the chains into the heart of the polymer (Fig. 16.3).

Although beads are the common shape for the solid support, a range of other shapes such as pins have been designed to maximize the surface area available for reaction, and hence maximize the amount of compound linked to the solid support. Functionalized glass surfaces have also been used and are suitable for oligonucleotide synthesis.

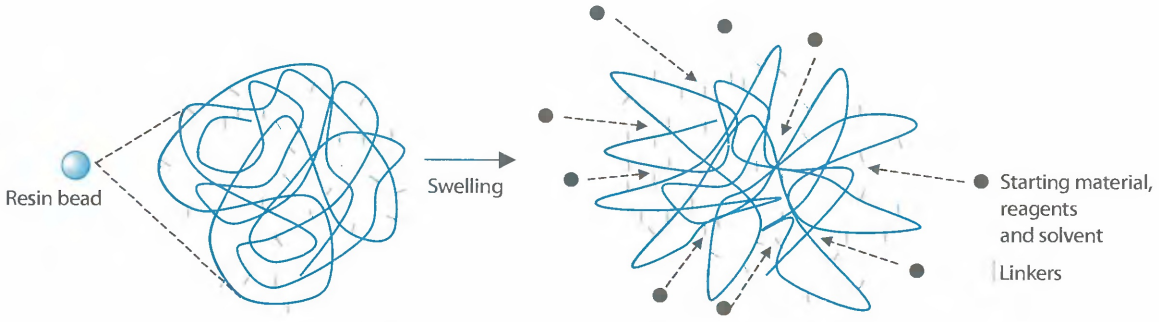


FIGURE 16.3 Swelling of a resin bead allowing access of reagents and solvent.

16.2.2 The anchor/linker

The anchor/linker is a molecular unit covalently attached to the polymer chain making up the solid support. It contains a reactive functional group with which the starting material in the proposed synthesis can react and hence become attached to the resin. The resulting link must be stable to the reaction conditions used throughout the synthesis, but be easily cleaved to release the final compound once the synthesis is complete (Fig. 16.4). Since the linkers are distributed along the length of the polymer chain, most of them will be in the interior of the polymer bead, emphasizing the importance of the bead swelling if the starting material is to reach them.

Different linkers are used depending on:

- the functional group that will be present on the starting material
- the functional group that is desired on the final product once it is released.

Resins having different linkers are given different names. For example, the **Wang resin** has a linker which is suitable for the attachment and release of carboxylic acids, whereas the **Rink resin** is suitable for the attachment of carboxylic acids and the release of carboxamides (the linkage point is circled in Fig. 16.5). The dihydropyran-derivatized resin is suitable for the attachment and release of alcohols.

The **Barlos resin** contains a trityl linker and is suitable for the attachment and release of carboxylic acids.

The Wang resin can be used in peptide synthesis whereby an *N*-protected amino acid is linked to the resin by means of an ester link. This ester link remains stable to coupling and deprotection steps in the peptide synthesis, and can then be cleaved using trifluoroacetic acid (TFA) to release the final peptide from the bead (Fig. 16.6). One problem with the Wang resin is that the first amino acid linked to the resin is prone to racemization. The Barlos resin was designed to avoid this problem and is used in peptide synthesis in the same way. The final product can be cleaved under very mild conditions due to the high stability of trityl cations (e.g. HOAc/TFE/CH_2Cl_2 or TFA/CH_2Cl_2). Molecules can also be linked to the resin by means of an alcohol group.

Starting materials with a carboxylic acid (RCO_2H) can be linked to the Rink resin via an amide link. Once the reaction sequence is complete, treatment with TFA releases the product with a primary amide group, rather than the original carboxylic acid ($R'CONH_2$; Fig. 16.7).

Primary and secondary alcohols (ROH) can be linked to a dihydropyran-functionalized resin. Linking the alcohol is done in the presence of pyridinium 4-toluenesulfonate (PPts) in dichloromethane. Once the reaction sequence has been completed, cleavage can be carried out using TFA (Fig. 16.8).

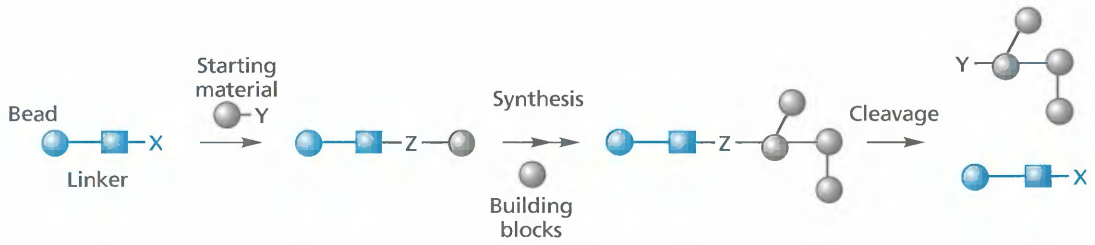


FIGURE 16.4 The principles of an anchor/linker. X, Y, Z are functional groups.

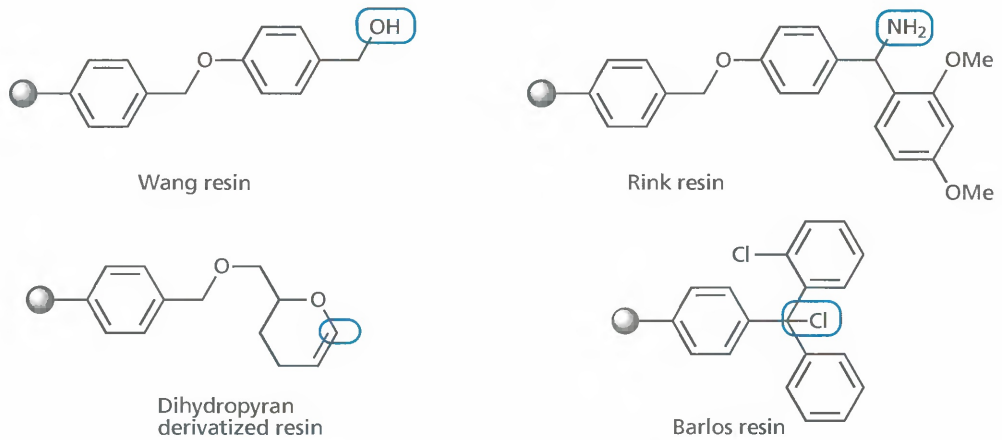


FIGURE 16.5 Types of resin with the linkage point circled.

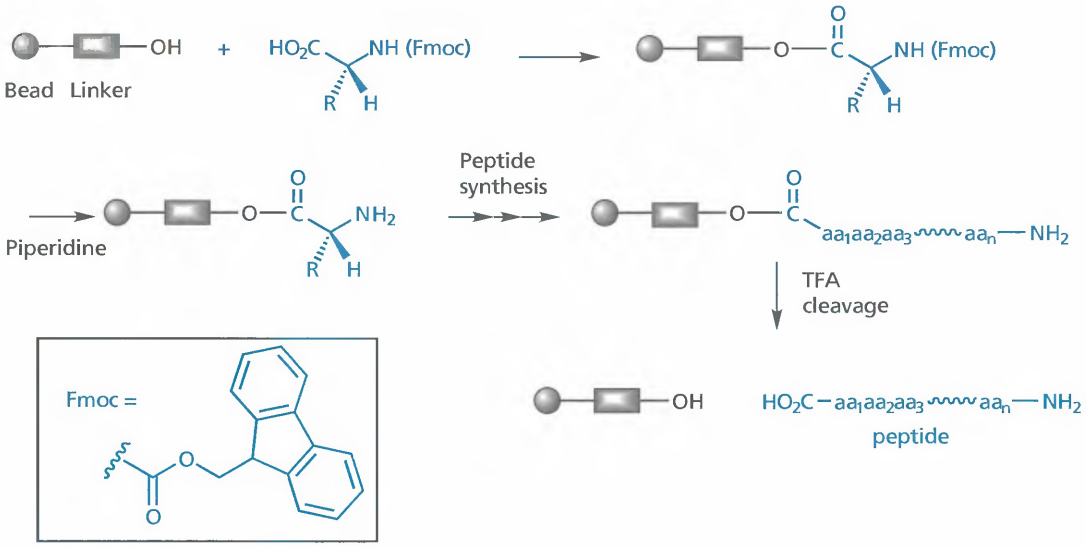


FIGURE 16.6 Peptide synthesis with a Wang resin—the structure of the linker is shown in Fig. 16.5.

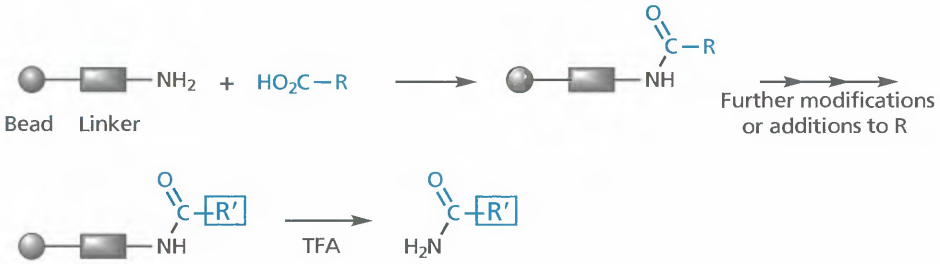


FIGURE 16.7 Solid phase synthesis with a Rink resin (R contains functional groups which allows further modifications of the molecule to give R'). The structure of the linker is shown in Fig. 16.5.

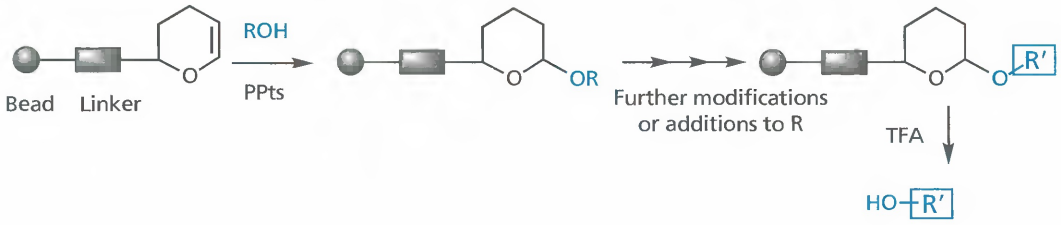


FIGURE 16.8 Solid phase synthesis with a dihydropyran-functionalized resin (R contains functional groups which allows further modifications of the molecule to give R').

16.2.3 Protecting groups and synthetic strategy

When a molecule is being constructed by solid phase synthesis, it is important to protect any reactive functional groups that are not meant to react during the reaction sequence. Otherwise, undesirable side reactions will occur. The selection of suitable protecting groups is extremely important. They should be stable to the reaction conditions involved in the synthesis, but be capable of being removed in high yield under mild conditions once the synthesis is complete. As far as peptide synthesis is concerned, two main protecting group strategies are used.

16.2.3.1 Boc/benzyl protection strategy

This strategy is suitable for the Merrifield resin as illustrated in Fig. 16.2. The *N*-terminus of each amino acid used in

the synthesis is protected by a *tert*- or *t*-butyloxycarbonyl (Boc) group. Once each amino acid has been added to the growing peptide chain, its Boc group is removed with TFA to free up the amino group and the next protected amino acid can be coupled on to the chain. The bond which connects the growing peptide chain to the linker is stable to TFA and remains unaffected by the synthesis. However, this bond is susceptible to strong acid and once the synthesis is complete, hydrofluoric acid is used to release the peptide. Functional groups on the amino acid residues also have to be protected during the synthesis, which means that the protecting groups have to be stable to TFA. Benzyl-type protecting groups fit the bill. They are stable to TFA but susceptible to hydrofluoric acid, and so treatment with hydrofluoric acid releases the final peptide and deprotects the residues at the same time. One major disadvantage with this procedure is the need to use hydrofluoric acid. This is a particularly nasty chemical which dissolves glass, so expensive Teflon equipment has to be used. The harsh conditions can also result in peptide decomposition in some cases. Finally, there is a serious health risk and it is important to ensure that no hydrofluoric acid gets on the skin. It is reputed that a garage worker once decided to take advantage of the glass-dissolving properties of hydrofluoric acid, by soaking a rag with it and removing scratches from a car windscreen. Unfortunately, his hands had to be surgically removed as well.

16.2.3.2 Fmoc/*t*-Bu strategy

The alternative Fmoc/*t*-Bu strategy involves milder conditions and is partly illustrated in Fig. 16.6 with a Wang resin. The 9-fluorenylmethoxycarbonyl (Fmoc) group is an alternative protecting group for the terminal amino group and can be removed using a mild base such as piperidine. Functional groups on amino acid residues can be protected with a *t*-butyl group which can be removed by TFA. Since totally different reaction conditions are involved in removing the Fmoc group and the *t*-butyl group, one basic and one acidic, the protecting group strategy is defined as being orthogonal. The link to the Wang resin is also susceptible to TFA and avoids the need to use hydrofluoric acid. Thus, the peptide can be cleaved from the resin, and the functional groups on the residues are deprotected at the same time.

KEY POINTS

- Combinatorial synthesis and parallel synthesis have proved valuable in producing compounds for lead discovery, structure–activity relationships and drug optimization.
- Parallel combinatorial synthesis involves the synthesis of a different compound in each reaction vial and is useful in all aspects of medicinal chemistry where synthesis is required.
- Combinatorial synthesis involves the synthesis of mixtures of compounds in each reaction vial and is useful in discovering lead compounds.
- Solid phase synthesis has several advantages. Intermediates do not need to be isolated or purified. Reactants and reagents can be used in excess to drive the reaction to completion. Impurities and excess reagents or reactants are easily removed. Different compounds are linked to different solid phase surfaces such that they are physically separated, allowing them to undergo reactions and work-up procedures in the same reaction vessel.
- The solid support consists of a polymeric surface and a linker molecule, which allows a starting material to be covalently linked to the support.
- Different linkers are used depending on the functional group that is available on the starting material, and the functional group that is desired on the product.

16.3 The mix and split method in combinatorial synthesis

A mixed combinatorial synthesis is designed to produce a mixture of products in each reaction vessel, starting with a wide range of starting materials and reagents. This does not mean that all possible starting materials are thrown together in the one reaction flask. If this was done, a black tarry mess would result. The synthesis is designed such that molecular structures are synthesized on solid supports such as beads. Each bead may contain a large number of such molecules, but all the molecules on that bead are identical—'the one-bead-one-compound concept'. Beads with different structures can be mixed together in a single vial such that the molecules attached to the beads undergo the same reaction. In this way, each vial contains a mixture of structures, but each structure is physically distinct from another since it is attached to a different bead. Planning has to go into designing a combinatorial synthesis to minimize the effort involved and to maximize the number of different structures obtained. The strategy of **mix and split** is a crucial part of this.

As an example, suppose we wish to synthesize all the possible dipeptides of five different amino acids. Using orthodox chemistry, we would synthesize these one at a time. There are 25 possible dipeptides, and so we would have to carry out 25 separate experiments (Fig. 16.9).

Using a mix and split strategy, the same products can be obtained with far less effort (Fig. 16.10). First of all,

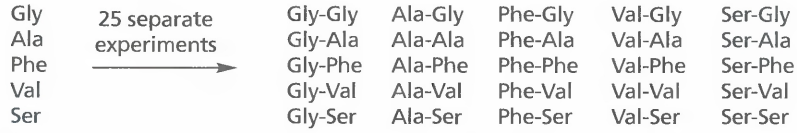


FIGURE 16.9 The possible dipeptides that can be synthesized from five different amino acids.

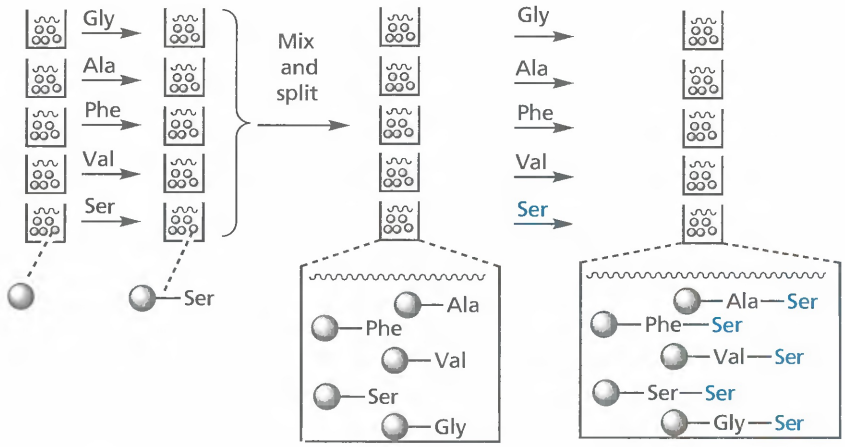


FIGURE 16.10 Synthesis of five different dipeptides using the mix and split strategy.

the beads are split between five reaction vials. The first amino acid is attached to the beads, using a different amino acid for each vial. The beads from all five flasks are collected, mixed together, then split back into the five vials. This means that each vial now has the same mixture. The second amino acid is now coupled, with a different amino acid being used for each vial. Each vial now contains five different dipeptides, with no one vial containing the same dipeptide. Each of the five mixtures can now be tested for activity. If the results are positive, the emphasis is on identifying which of the dipeptides is active. If there is no activity present, the mixture can be ignored and stored.

In studies such as these, one can generate large numbers of mixtures, many of which are inactive. However, these mixtures are not discarded. Although they may not contain a lead compound on this particular occasion, they may provide the necessary lead compound for a different target in medicinal chemistry. Therefore, all the mixtures (both active and inactive) resulting from a combinatorial synthesis are stored and are referred to as **combinatorial or compound libraries.** The example above produced 25 compounds in 5 mixtures. However, combinatorial synthesis can be used to produce several thousand compounds.

16.4 Structure determination of the active compound(s)

The direct structural determination of components in a compound mixture is no easy task, but advances have been made in obtaining interpretable mass, NMR, Raman, infrared and ultraviolet spectra on products attached to a single resin bead. Peptides can be sequenced while still attached to the bead. Each 100 μm bead contains about 100 pmole of peptide, which is enough for microsequencing. With non-peptides, the structural determination of an active compound can be achieved by deconvolution methods (see OUP website). Alternatively, **tagging** procedures can be used during the synthesis.

16.4.1 Tagging

In this process, two molecules are built up on the same bead. One of these is the intended structure; the other is a molecular tag (usually a peptide or oligonucleotide) which will act as a code for each step of the synthesis. For this to work, the bead must have a multiple linker capable of linking both the target structure and the molecular tag. A starting material is added to one part of the linker, and

an encoding amino acid or nucleotide to another part. After each subsequent stage of the combinatorial synthesis, an amino acid or nucleotide is added to the growing tag to indicate what reagent was used. One example of a multiple linker is called the **Safety-Catch Acid-Labile Linker** (**SCAL**) (Fig. 16.11), which includes lysine and tryptophan. Both these amino acids have a free amino group.

The target structure is constructed on the amino group of the tryptophan moiety, and after each stage of the synthesis, a tagging amino acid is built on to the amino groups of the lysine moiety. Figure 16.12 illustrates the strategy for a synthesis involving three reagents, so that by the end of the process there is a tripeptide tag where each amino acid defines the identity of the variable groups R, R′, and R″ in the target structure.

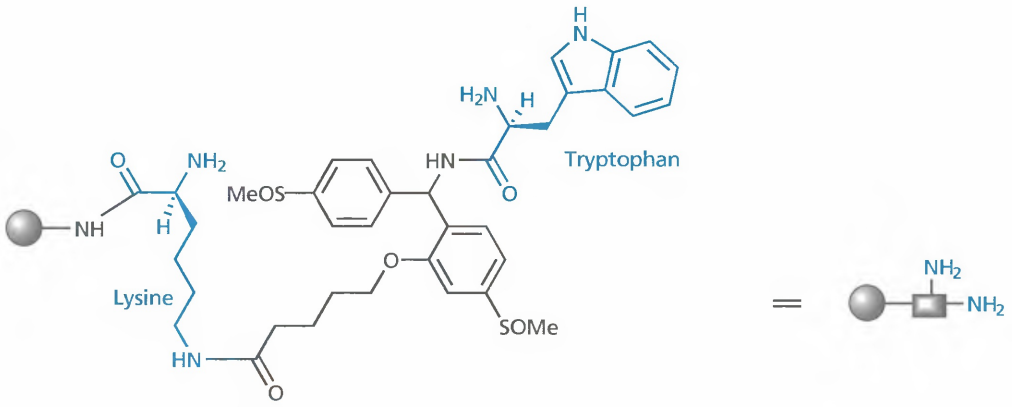


FIGURE 16.11 SCAL (Safety-Catch Acid-Labile Linker).

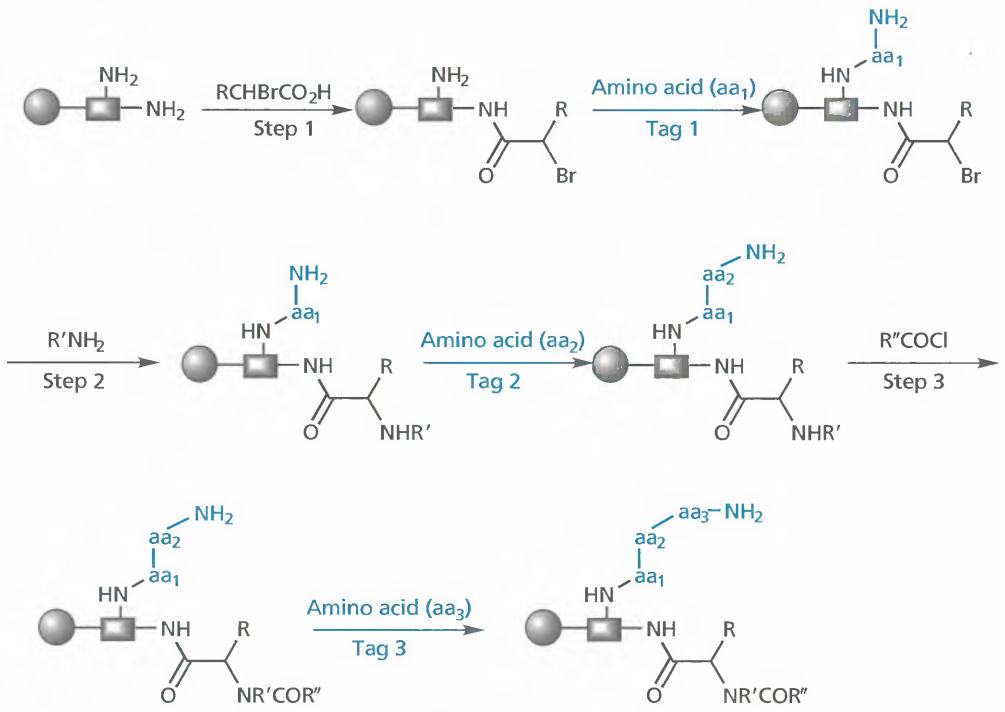


FIGURE 16.12 Tagging a bead to identify the structure being synthesized. Note that the reaction sequence has been simplified here to illustrate the principle of tagging. Amino acids are *N*-protected when coupled and the protecting group is removed before the next coupling. Coupling agents are also present. An orthogonal protection strategy is also required to distinguish between the amino groups of the safety-catch acid-labile linker.

The non-peptide target structure can be cleaved by reducing the two sulfoxide groups in the safety catch linker, then treating with acid. Under these conditions, the tripeptide sequence remains attached to the bead and can be sequenced on the bead to identify the structure of the compound which was released.

The same strategy can be used with an oligonucleotide as the tagging molecule. The oligonucleotide can be amplified by replication and the code read by DNA sequencing.

There are drawbacks to tagging processes; they are time consuming and require elaborate instrumentation. Building the coding structure itself also adds extra restraints on the protection strategies that can be employed and may impose limitations on the reactions that can be used. In the case of oligonucleotides, their inherent instability can prove a problem. Another possible problem with tagging is the possibility of an unexpected reaction taking place, resulting in a different structure from that expected. Nevertheless, the tagging procedure is still valid since it identifies the starting materials and the reaction conditions, and when these are repeated on larger scale, any unusual reactions would be discovered.

These tagging methods require the use of a specific molecular tag to represent each reagent used in the synthesis. Moreover, the resultant molecular tag has to be sequenced at the end of the synthesis. A more efficient method of tagging and identifying the final product is to use some form of encryption or 'bar code'. For example, it is possible to identify which one of seven possible reagents has been used in the first stage of a synthesis with the use of only three molecular labels (A–C). This is achieved by adding different combinations of the three tags to set up a triplet code on the bead. Thus, adding just one of the tags (A, B, or C) will allow the identification of three of the reagents. Adding two of the tags at the same time allows the identification of another three reagents, and adding all three tags at the same time allows the identification of a seventh reagent. The presence (1) or absence (0) of the tag forms a triplet code; the presence of a single molecular tag (A, B, or C) gives the triplet codes (100, 010, and 001), the presence of two different tags is indicated by another three triplet codes (110, 101, 011), and the presence of all three tags is represented by 111. The tags are linked to the bead by means of a photocleavable bond, so irradiating the bead releases all the tags. These can then be passed through a gas chromatograph and identified by their retention time.

Three different molecular tags could now be used to represent seven reagents in the second stage, and so on. All the tags used to represent the second reagent would have longer retention times than the tags used to represent the first reagent. Similarly, all subsequent tags would have longer retention times. Once the synthesis is complete, all the tags are released simultaneously and passed through the gas chromatograph as before. The 'bar code' is then read from the chromatograph in one go, not only identifying the reagents used but the order in which they were used (Fig. 16.13).

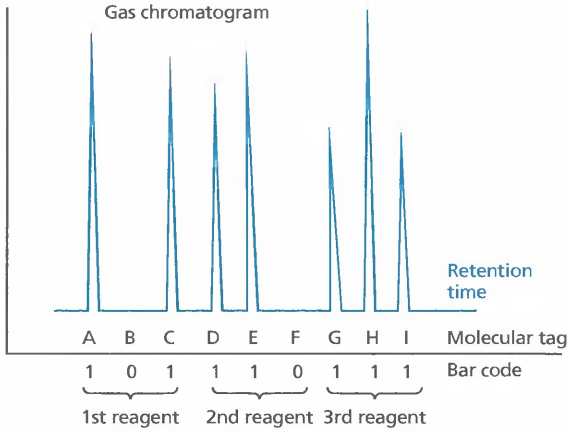


FIGURE 16.13 Identification of reagents and order of use by bar coding.

16.4.2 Photolithography

Photolithography is a technique which permits miniaturization and spatial resolution such that specific products are synthesized on a plate of immobilized solid support. In the synthesis of peptides, the solid support surface contains an amino group protected by the photolabile **nitroveratryloxycarbonyl** (**NVOC**) protecting group (Fig. 16.14). Using a mask, part of the surface is exposed to light resulting in deprotection of the exposed region. The plate is then treated with a protected amino acid and the coupling reaction takes place only on the region of the plate which has been deprotected. The plate is then washed to remove excess amino acid. The process can be repeated on a different region using a different mask, and so different peptide chains can be built on different parts of the plate; the sequences are known from the record of masks used.

Incubation of the plate with a protein receptor can then be carried out to detect active compounds which bind to the binding site of the receptor. A convenient method to assess such interactions is to incubate the plate with a fluorescently tagged receptor. Only those regions of the plate which contain active compounds will bind to the receptor and fluoresce. The fluorescence intensity can be measured using fluorescence microscopy and is a measure of the affinity of the compound for the receptor. Alternatively, testing can be carried out such that active compounds are detected by radioactivity or chemiluminescence.

The photodeprotection described above can be achieved in high resolution. At a 20-μm resolution, plates can be prepared with 250 000 separate compounds per square centimetre.

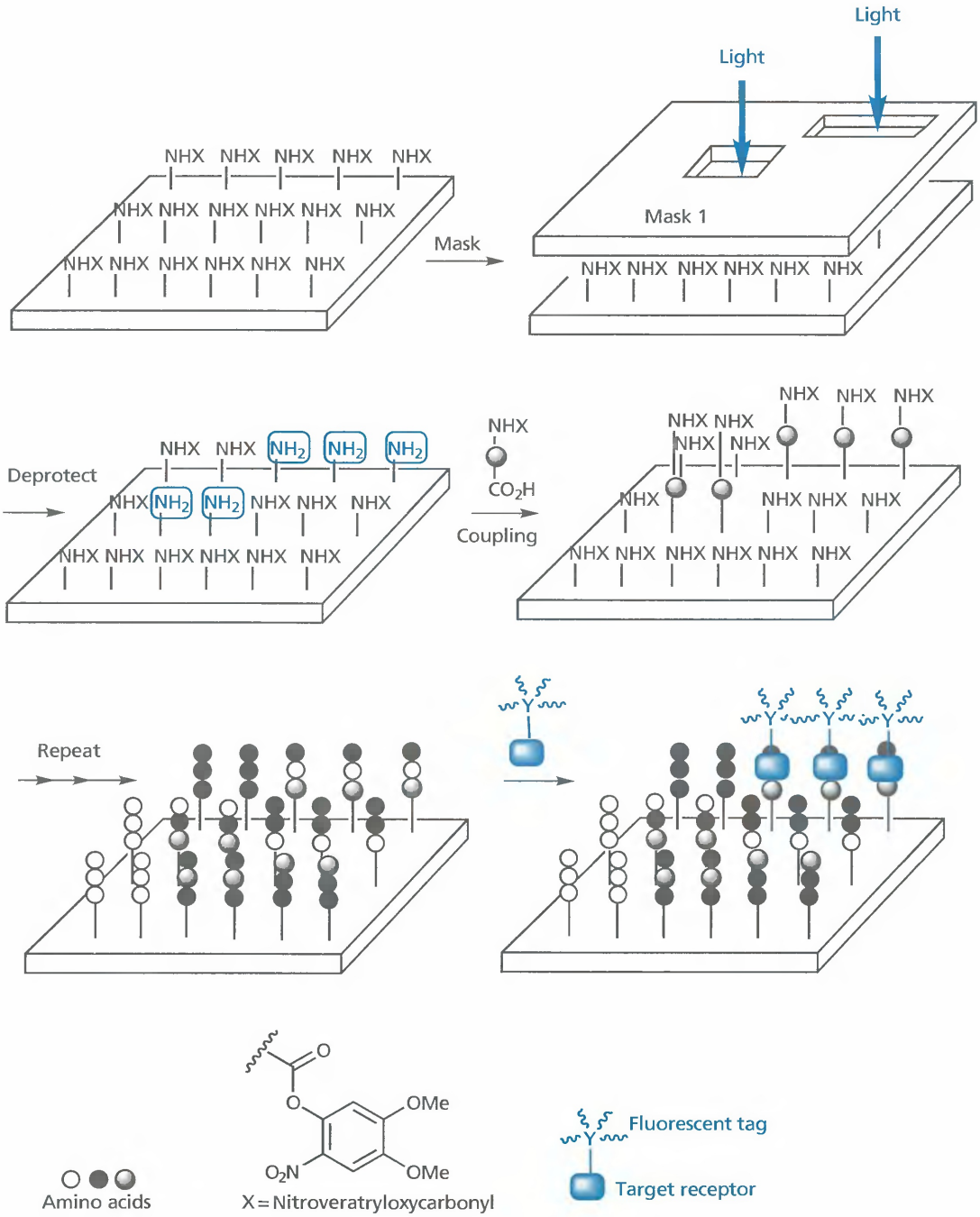


FIGURE 16.14 Photolithography.

16.5 Examples of combinatorial synthesis

Combinatorial chemistry has proved its worth in throwing up new lead compounds in a variety of fields. Much of the early work in combinatorial chemistry was carried out on peptides since the solid phase procedures had already been developed for that field. This resulted in the discovery of new HIV-protease inhibitors, antimicrobial agents, opioid receptor ligands, and aspartic acid protease inhibitors. However peptides are not ideal drug candidates, as they usually have poor oral bio-availability (section 14.8.2).

The first move away from peptides was to use the same peptide coupling procedures, but with non-natural amino acids. Peptides could also be modified once they were built, by reactions such as *N*-methylation. Peptides have

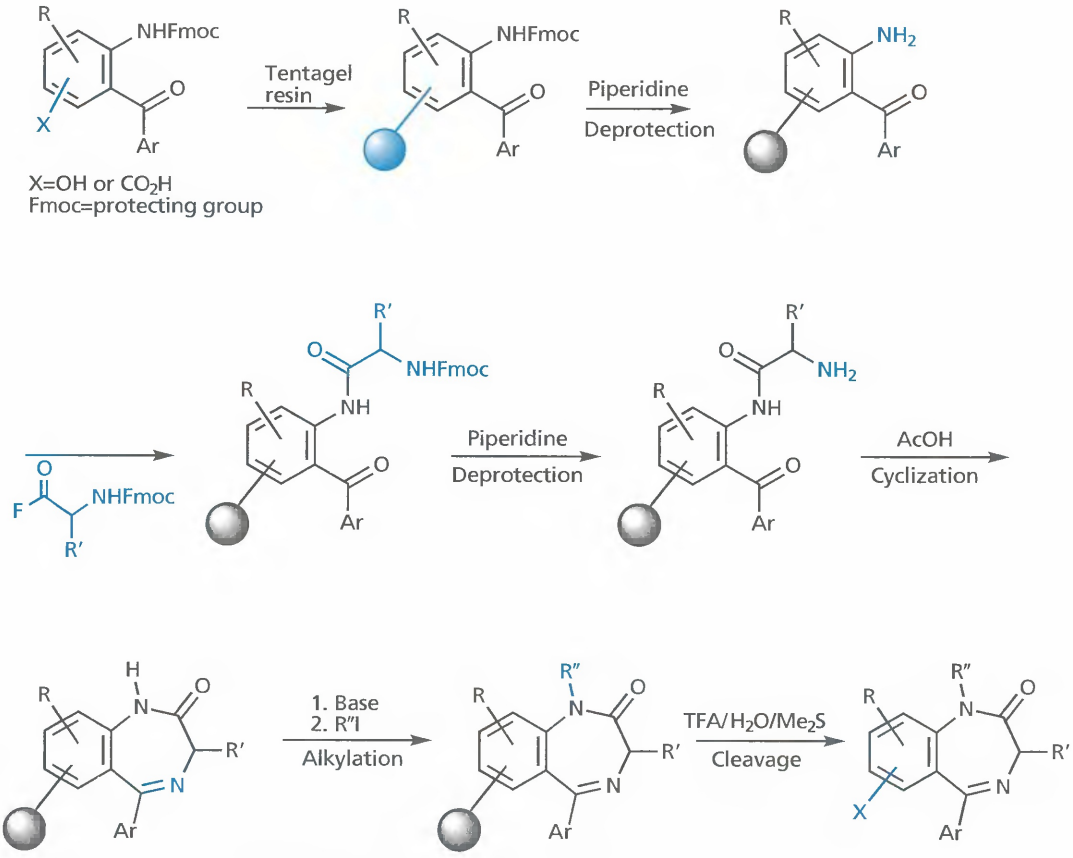


FIGURE 16.15 Solid phase synthesis of 1,4-benzodiazepines.

also been built linking *N*-substituted glycine units to produce structures known as **peptoids** where the side chain is attached to the nitrogen rather than the α-carbon. Some of these have been shown to be ligands for various important receptors and show increased metabolic stability.

A disadvantage with all the above structures is the fact that they are linear, flexible molecules linked together by a regular molecular backbone. The real interest in combinatorial chemistry began when it became possible to produce heterocyclic combinatorial libraries. Heterocycles are less susceptible to metabolism, and have better pharmacokinetic properties. They are more rigid, and diversity is possible by varying the substituents around the heterocyclic 'core'. One of the earliest examples of a heterocyclic solid phase synthesis was that of 1,4-benzodiazepines (Fig. 16.15). This was a good synthesis, as three distinct units were involved which could be varied. The final product has five variable substituents, two of which can be positionally varied on the aromatic ring.

A limitation with this synthesis was the need to have a phenol or carboxylic acid group present to allow attachment to the solid support. This meant that the final product retained this functional group when released. Later, an alternative heterocyclic synthesis was devised which avoided this problem (Fig. 16.16). A selection of amino acids was linked to resin beads through the carboxylic acid

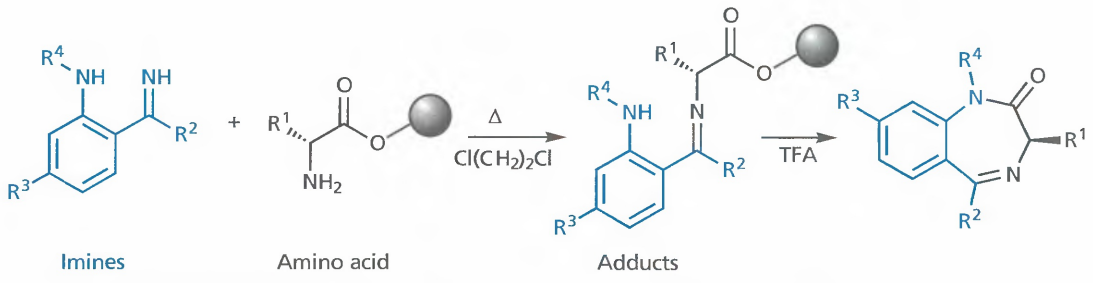


FIGURE 16.16 Alternative benzodiazepine synthesis involving a cyclo-release strategy.

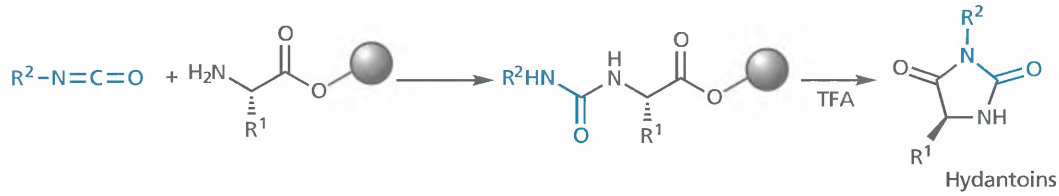


FIGURE 16.17 Synthesis of hydantoins.

group. Reaction with a variety of imines gave the adducts shown. Treatment with TFA released the adducts which then cyclized to give the final products. The advantage of this synthesis lies in the fact that the functional group released from the resin takes part in the final cyclization and does not remain as an extra, and possibly redundant group.

A similar strategy was employed for the synthesis of hydantoins (Fig. 16.17) and a large variety of heterocyclic compounds have now been synthesized by combinatorial methods.

The range of reactions which can be carried out on solid phase has also been extended: most common reactions are now feasible, including moisture sensitive and organometallic reactions. For example, aldol condensations, DIBAL reductions, Wittig reactions, LDA reductions, Heck couplings, Stille couplings, and Mitsunobu reactions are all possible. Automated or semi-automated synthesizers can cope with 6, 12, 42, 96 or 144 reaction vials depending on the instrument and the size of the reaction tubes used. The addition of solvent, starting materials and reagents can be carried out automatically using syringes. Automated work up procedures such as the removal of solvent, washing and liquid–liquid separations are also possible. Reactions can be stirred and carried out under inert atmospheres, and the reactions can be heated or cooled as required.

16.6 Dynamic combinatorial synthesis

Dynamic combinatorial synthesis is an exciting development which has been used as an alternative to the classic mix and split combinatorial syntheses in the search for new lead compounds. The aim of dynamic combinatorial synthesis is to synthesize all the different compounds in one flask at the same time, screen them *in situ* as they are being formed, and thus identify the most active compound in a much faster time period (Box 16.1). How can this be achieved? There are several important principles which are followed.

- The best way of screening the compounds is to have the desired target present in the reaction flask along with the building blocks. This means that any active compounds can bind to the target as soon as they are formed. The trick is then to identify which of the products are binding.
- The reactions involved should be reversible. If this is the case, a huge variety of products are constantly being formed in the flask then breaking back down into their constituent building blocks. The advantage of this may not seem obvious, but it allows the possibility of 'amplification' where the active compound is present to a greater extent than the other possible products. By having the target present, active compounds become bound and are effectively removed from the equilibrium mixture. The equilibrium is now disturbed such that more of the active product is formed. Thus, the target serves not only to screen for active compounds, but to amplify them as well.
- In order to identify the active compounds, it is necessary to 'freeze' the equilibrium reaction such that it no longer takes place. This can be done by carrying out a further reaction, which converts all the equilibrium products into stable compounds that cannot revert back to starting materials.

A simple example of dynamic combinatorial synthesis involved the reversible formation of imines from aldehydes and primary amines (Fig. 16.18). A total of three aldehydes

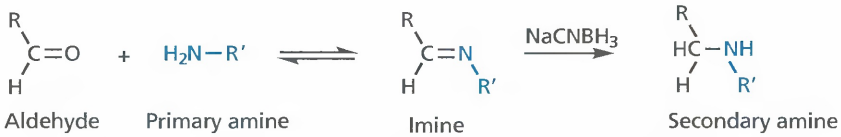


FIGURE 16.18 Example of dynamic combinatorial synthesis.

BOX 16.1 Dynamic combinatorial synthesis of vancomycin dimers

Vancomycin is an antibiotic that works because it masks the building blocks required for bacterial cell wall synthesis (section 19.5.5.2). Binding takes place specifically between the antibiotic and a peptide sequence (L-Lys-D-Ala-D-Ala), which is present in the building block. It is also known that this binding promotes dimerization of the vancomycin-target complex, which suggests that covalently linked vancomycin dimers might be more effective antibacterial agents than vancomycin itself. A dynamic combinatorial synthesis was carried out to synthesize a variety of different vancomycin dimers covalently linked by bridges of different lengths. The vancomycin monomers used had been modified such that they contained long-chain alkyl substituents with double bonds at the end. Reaction between the double bonds in the presence of a catalyst then led to bridge formation through a reaction known as olefin metathesis (Fig. 1).

The tripeptide target was present to accelerate the rate of bridge formation and to promote formation of vancomycin dimers having the ideal bridge length. As shown in Fig. 2, the vancomycin monomers bind the tripeptide which encourages the self-assembly of non-covalently linked dimers. Once formed, those dimers having the correct length of substituent are more likely to react together to form the covalent bridge (Fig. 2).

Having established the optimum length of bridge, another experiment was carried out on eight vancomycin monomers that had the correct length of 'tether' but varied slightly in their structure. The mixture of 36 possible products was analysed by mass spectrometry to indicate the relative proportion of each dimer formed. Eleven of the 36 compounds were then synthesized separately, and it was found that their antibacterial activity matched their level of amplification (i.e. the compounds present in greater quantities had the greater activity.

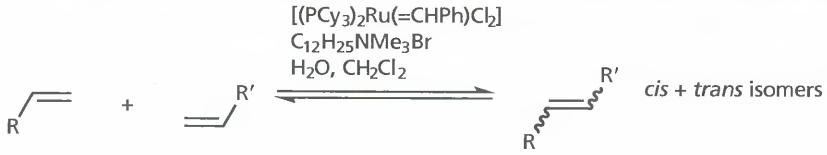


FIGURE 1 Olefin metathesis.

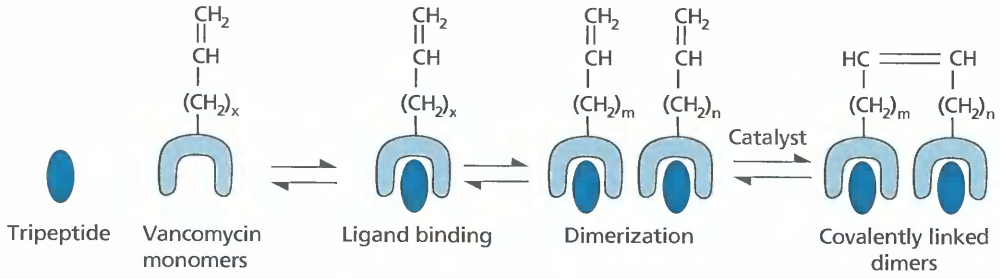


FIGURE 2 Formation of covalently linked dimers.

and four amines were used in the study (Fig. 16.19), allowing the possibility of 12 different imines in the equilibrium mixture.

The building blocks were mixed together with the target enzyme **carbonic anhydrase** and allowed to interact. After a suitable period of time, sodium cyanoborohydride was added to reduce all the imines present to secondary amines so that they could be identified (Fig. 16.18). The mixture was separated by reverse-phase HPLC, allowing each product to be quantified and identified. These results were compared with those obtained when the experiment was carried out in the absence of carbonic anhydrase, making it possible to identify which products had been amplified. In this experiment, the sulfonamide shown in Fig. 16.20 was significantly amplified, which demonstrated that the corresponding imine was an active compound.

The above example illustrates a simple case involving one reaction and two sets of building blocks, but it is feasible to have more complex situations. For

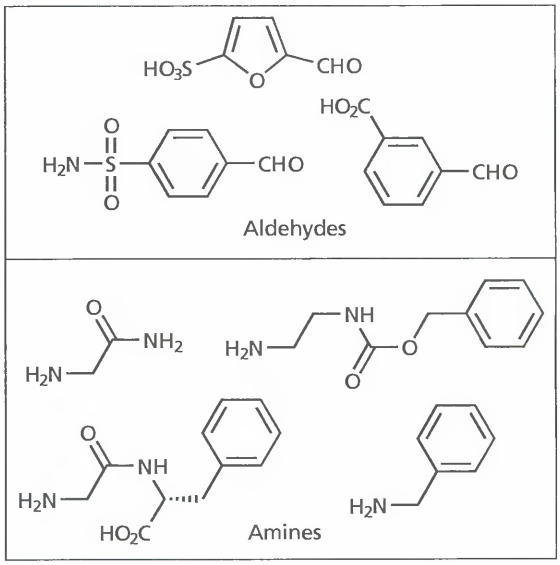


FIGURE 16.19 Aldehyde and amine building blocks used in the dynamic combinatorial synthesis of imines.

example, a molecule with two or more functional groups could be present to act as a scaffold on to which various substituents could be added from the building blocks available (Fig. 16.21). The use of a central scaffold has another benefit; it helps the amplification process. If the number of scaffold molecules present is equal to the number of target molecules, then the number of products formed cannot be greater than the number of targets available. If any of these products binds to the target, the effect on the equilibrium will be greater than if there were more products than targets available.

There are certain limitations to dynamic combinatorial chemistry.

- Conditions must be chosen such that the target does not react chemically with any of the building blocks, or is unstable under the reaction conditions used.
- The target is normally in an aqueous environment, so the reactions have to be carried out in aqueous solution.
- The reactions themselves have to undergo fast equilibration rates to allow the possibility of amplification.
- It is important to avoid using some building blocks that are more likely to react than others, as this would bias the equilibrium towards particular products and confuse the identification of the amplified product.

16.7 Planning and designing a combinatorial synthesis

16.7.1 'Spider-like' scaffolds

In order to find a new lead compound from combinatorial synthesis, we need to generate a large number of different compounds but we also need to ensure that these compounds are as structurally diverse as possible. This may not seem very likely if we are restricted to using

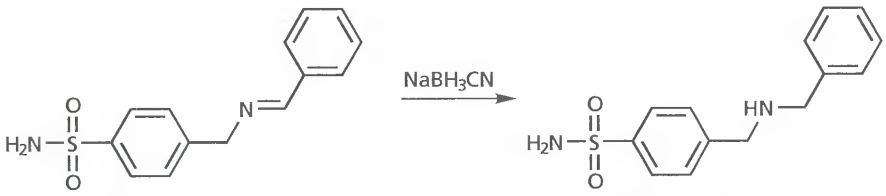


FIGURE 16.20 Amplified imine and the amine obtained from reduction.

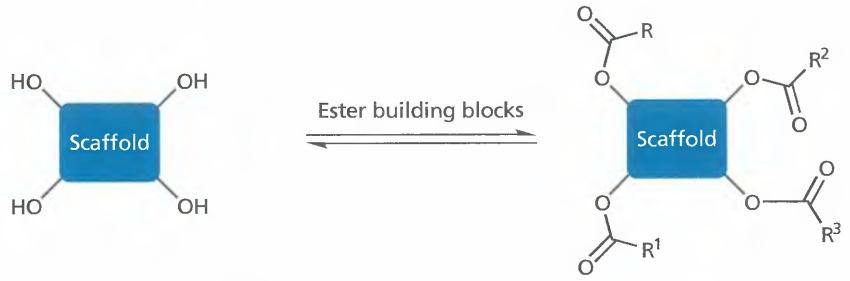


FIGURE 16.21 Use of a scaffold molecule.

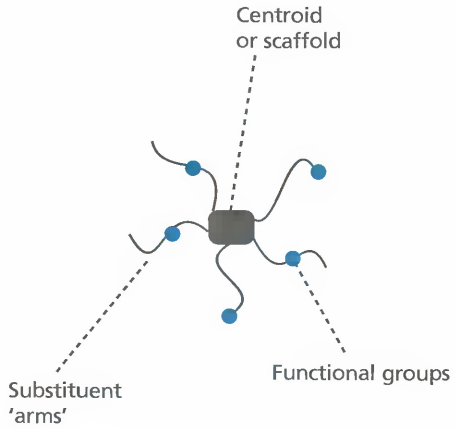


FIGURE 16.22 'Spider-like' molecule.

a single reaction sequence, but if we are careful in the type of molecule we synthesize and the method by which we synthesize it, then such diversity *is* possible. In general, it is best to synthesize 'spider-like' molecules, so called because they consist of a central body (called the **centroid** or **scaffold**) from which various 'arms' (substituents) radiate (Fig. 16.22). These arms contain different functional groups which are used to probe a binding site for binding regions once the spider-like molecule has entered (Fig. 16.23). The chances of success are greater if the 'arms' are evenly spread around the scaffold, as this allows a more thorough exploration of the three-dimensional space (**conformational space**) around the molecule. The molecules made in the synthesis are planned in advance to ensure that they contain different functional groups on their arms, placed at different distances from the central scaffold.

16.7.2 Designing 'drug-like' molecules

The 'spider-like' approach increases the chances of finding a lead compound which will interact with a target binding site, but it is also worth remembering that compounds with good binding interactions do not necessarily make good medicines. There are also the pharmacokinetic issues to be taken into account (chapter 11) and so it is worthwhile introducing certain restrictions to the types of molecule that will be produced, in order to increase the chance that the lead compound will be orally active. In general, the chances of oral activity are increased if the structure obeys Lipinski's rule of five or Veber's parameters (section 11.2). In addition, groups such as esters which are liable to be easily metabolized should be avoided. Finally, scaffolds or substituents likely to result in toxic compounds should be avoided; for example alkylating groups or aromatic nitro groups.

16.7.3 Scaffolds

Most scaffolds are synthesized by the synthetic route used for the combinatorial synthesis, and the synthesis determines the number and variety of substituents which can be attached to that scaffold. The ideal scaffold should be small, in order to allow a wide variation of substituents (Box 16.2). It should also have its substituents widely dispersed round its structure (spider-like) rather than restricted to one part of the structure (tadpole-like) if the conformational space around it is to be fully explored (Fig. 16.24). Finally, the synthesis should allow each of the substituents to be varied independently of each other.

Scaffolds can be flexible (e.g. a peptide backbone) or rigid (a cyclic system). They may contain groups that are capable of forming useful bonding interactions with the binding site, or they may not. Some scaffolds are already common in medicinal chemistry (e.g. benzodiazepine, hydantoin, tetrahydroisoquinoline, and benzenesulfonamide) and are associated with a diverse range of activities. Such scaffolds are termed '**privileged**' scaffolds.

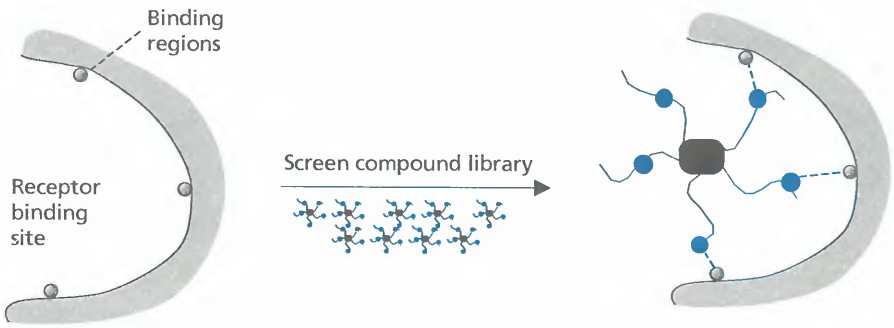


FIGURE 16.23 Probing for an interaction.

BOX 16.2 Examples of scaffolds

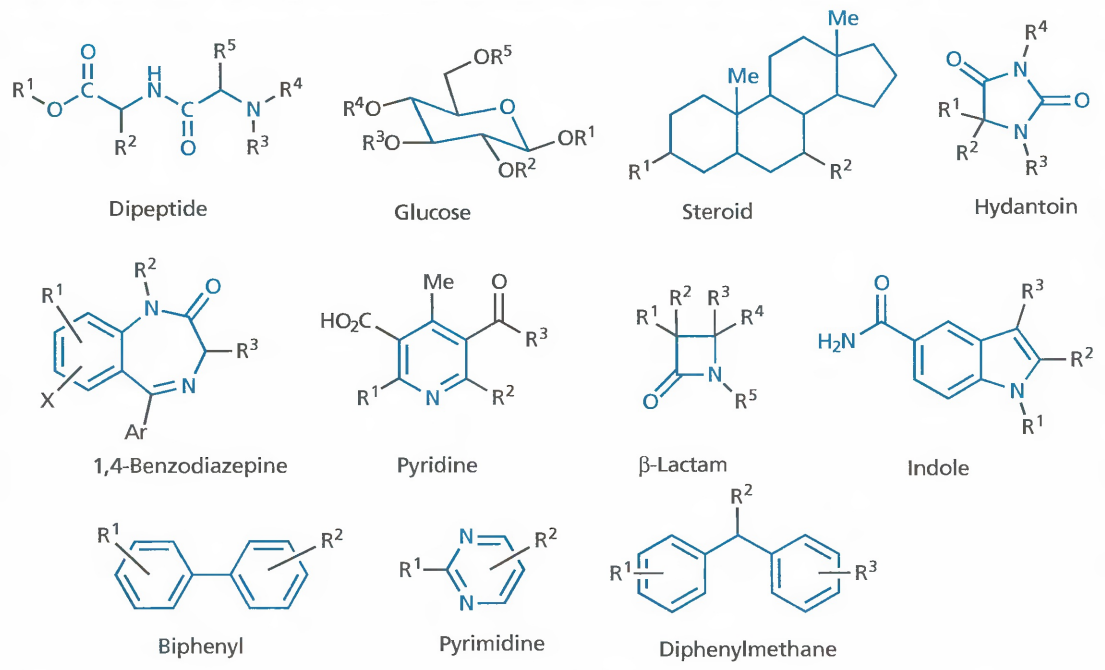
Benzodiazepines, hydantoins, β-lactams and pyridines are examples of extremely good scaffolds. They all have small molecular weights and there are various synthetic routes available which produce the substitution patterns required to fully explore the conformational space about them. For example, it is possible to synthesize benzodiazepines such that there are variable substituents round the whole structure.

Peptide scaffolds are flexible scaffolds which have the capacity to form hydrogen bonds with target binding sites. They are easy to synthesize and a large variety of different substituents is possible by using the amino acid building blocks. Further substitution is possible on the terminal amino and the carboxylic acid functions. The substituents are widely distributed along the peptide chain allowing a good exploration of conformational space. If we consider Lipinski's rule of five, the peptide scaffold should ideally be restricted to dipeptides and tripeptides in order to keep the molecular weight below 500. It is interesting to note that the antihypertensive agents **captopril** and **enalapril** are dipeptide-like and are orally active, whereas larger peptides such as the **enkephalins** are not orally active. Oral activity has also been a problem with those HIV protease inhibitors having molecular weights over 500 (section 20.7.4).

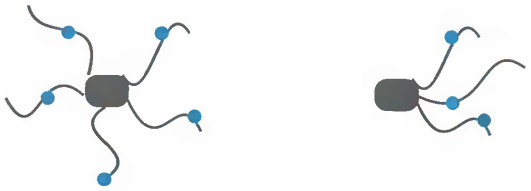
Some of the scaffolds shown below have various disadvantages. Although **glucose** has a small molecular weight and the possibility of five variable substituents, it contains multiple hydroxyl groups. Attaching different substituents to similar groups usually requires complex protection and deprotection strategies. Nevertheless, the potential of sugar-based drugs is so great that a lot of progress has been made in developing combinatorial syntheses based on sugar scaffolds.

Steroids might appear attractive as scaffolds. However, the molecular weight of the steroid skeleton itself (314) limits the size of the substituents that can be added if we wish to keep the overall molecular weight below 500. Furthermore, there are relatively few positions where substituents can be easily attached. This limits the conformational space that can be explored around the steroid scaffold.

The indole scaffold shown suffers the disadvantage in having its variable substituents located in the same region of the molecule, preventing a full exploration of conformational space (i.e. it is a 'tadpole-like' scaffold).



Examples of scaffolds.



'Spider' scaffold with dispersed substituents

'Tadpole' scaffold with restricted substituents

FIGURE 16.24 Dispersed and restricted substituents.

16.7.4 Substituent variation

The variety of substituents chosen in a combinatorial synthesis depends on their availability and the diversity required. This would include such considerations as structure, size, shape, lipophilicity, dipole moment, electrostatic charge, and functional groups present. It is usually best to identify which of these factors should be diversified before commencing the synthesis.

16.7.5 Designing compound libraries for lead optimization

When using combinatorial synthesis to optimize a known lead compound, the variations planned should take into account several factors such as the biological and physical properties of the compound, its binding interactions with the target, and the potential problems of particular substituents. For example, if the binding interactions of a target receptor with its usual ligand are known, this knowledge can be used to determine what size of compounds would be best synthesizing, the types of functional groups that ought to be present and their relative positions. For example, if the target was a zinc-containing protease (e.g. angiotensin-converting enzyme), a library of compounds containing a carboxylic acid or thiol group would be relevant.

16.7.6 Computer-designed libraries

It has been claimed that half of all known drugs involve only 32 scaffolds. Furthermore, it has been stated that a relatively small number of moieties account for the large majority of side chains in known drugs. This may imply that it is possible to define 'drug-like molecules' and use computer software programs to design more focused combinatorial compound libraries. Descriptors used in this approach include log *P*, molecular weight, number of hydrogen bond donors, number of hydrogen bond acceptors, number of rotatable bonds, aromatic density, the degree of branching in the structure, and the presence or absence of specific functional groups. One can also choose to filter out compounds that do not obey the rules mentioned in section 16.7.2. Computer programs can also be used to identify the structures which should be synthesized in order to maximize the number of different pharmacophores produced (section 17.16).

16.8 Testing for activity

We shall now look in more detail at how the products from combinatorial synthesis are tested for biological activity.

16.8.1 High throughput screening

Because combinatorial synthesis produces a large quantity of structures in a very short period of time, biological testing has to be carried out quickly and automatically. The process is known as high throughput screening (HTS) and was developed before combinatorial synthesis. Indeed, the existence of HTS was one of the pressures to develop combinatorial synthesis. Since biological testing was so rapid and efficient, the pharmaceutical companies soon ran out of novel structures to test, and the synthesis of new structures became the limiting factor in the whole process of drug discovery. Combinatorial synthesis solved that problem, and the number of new compounds synthesized each year has increased dramatically. In fact, there are now so many compounds being produced that the focus is on making HTS even more efficient. Traditionally, compounds are automatically tested and analysed on a plate containing 96 small wells each with a capacity of 0.1 ml. There is now a move to use test plates of similar size but containing 1536 wells, where the test volumes are only 1–10 µl. Moreover, methods such as fluorescence and chemiluminescence are being developed which will allow the simultaneous identification of active wells. Further miniaturization of open systems is unlikely, because of the problems of evaporation involving small volumes less than 1 µl. However, miniaturization using closed systems is on the horizon. The next major advance will be in the science of **microfluidics**, which involves the manipulation of tiny volumes of liquids in confined space. Microfluidic circuits on a chip can be used to control fluids electronically, allowing separation of an analytical sample using capillary electrophoresis. Companies are now developing machines that combine ultra-small-scale synthesis (section 16.9.5) and miniaturized analysis. A single 10 × 10 cm silicon wafer can be microfabricated to support 10^5 separate syntheses/bioassays on a nanolitre scale!

16.8.2 Screening 'on bead' or 'off bead'

Sometimes structures can be tested for biological activity when they are still attached to the solid phase. 'On bead' screening assays involve interactions with targets which are tagged with an enzyme, fluorescent probe, radionuclide, or chromophore. A positive interaction results in a recognizable effect such as fluorescence or a colour change. These screening assays are rapid, and 10^8 beads can be readily screened. Active beads can then be picked out by micromanipulation and the structure of the active compound determined.

A false negative might be obtained if the solid phase sterically interferes with the assay. If such interference is suspected, it is better to release the drug from the solid phase before testing. This avoids the uncertainty of false negatives. On the other hand, there are cases where the compounds released prove to be insoluble in the test assay and give a negative result, whereas they give a positive result when attached to the bead.

KEY POINTS

- Most combinatorial syntheses are carried out using automated or semi-automated synthesizers.
- The mix and split method allows the efficient synthesis of large numbers of compounds with a minimum number of operations.
- The compounds synthesized in a combinatorial synthesis are stored as combinatorial libraries.
- Tagging involves the construction of a tagging molecule on the same solid support as the target molecule. Tagging molecules are normally peptides or oligonucleotides. After each stage of the target synthesis, the peptide or oligonucleotide is extended and the amino acid or nucleotide used defines the reactant or reagent used in that stage.
- Photolithography is a technique involving a solid support surface containing functional groups protected by photolabile groups. Masks are used to reveal defined areas of the plate to light, thus removing the protecting groups and allowing a reactant to be linked to the solid support. A record of the masks used determines what reactions have been carried out at different regions of the plate.
- Combinatorial synthesis has been used for the synthesis of peptides, peptoids and heterocyclic structures. Most organic reactions are feasible.
- Dynamic combinatorial chemistry involves the equilibrium formation of a mixture of compounds in the presence of a target. Binding of a product with the target amplifies that product in the equilibrium mixture.
- A scaffold is the core structure of a molecule round which variations are possible through the use of different substituents.
- Spider-like scaffolds allow substituent variation around the whole molecule, making it possible to explore all the conformational space around the scaffold. This increases the possibility of finding a lead compound that will bind to a target binding site.
- Lipinski's rule of five can be used when planning combinatorial libraries to increase the chances of identifying an orally active lead compound.
- A privileged scaffold is one that is commonly present in known drugs.
- Computer software is available to assist in the planning of combinatorial libraries.
- High-throughput screening allows the automated analysis of large numbers of samples for their biological activity against defined targets. The analysis requires only small quantities of each sample.
- Screening can be carried out on compounds attached to resin beads, or on compounds that have been released into solution.

16.9 Parallel synthesis

In parallel synthesis, a reaction is carried out in a series of wells such that each well contains a single product. This method is a 'quality rather than quantity' approach and is often used for focused lead optimization studies. For parallel synthesis to be fast and efficient, it is necessary to remove or simplify the bottlenecks associated with classical organic synthesis. These include laborious work-ups, extractions, solvent evaporations and purifications. A typical medicinal chemist may synthesize one or two new entities a week using the classical approaches to organic synthesis. With parallel synthesis, that same researcher can synthesize a dozen or more pure molecules, thus increasing the synthetic output, and speeding up the lead optimization process. Parallel synthesis can be carried out on solid phase, and we have already seen the advantages of solid phase synthesis (section 16.2). However, parallel syntheses can also be carried out in solution and in this section we focus on methods that make solution phase organic synthesis (SPOS) more efficient.

This can be illustrated by considering the synthesis of an amide, which typically involves the reaction of a carboxylic acid with an amine in the presence of a coupling reagent such as dicyclohexylcarbodiimide (DCC) (Fig. 16.25). Conventionally, a work up procedure has to be carried out once the reaction is complete. This involves washing the organic solution with aqueous acid in order to remove unreacted amine. Once the aqueous

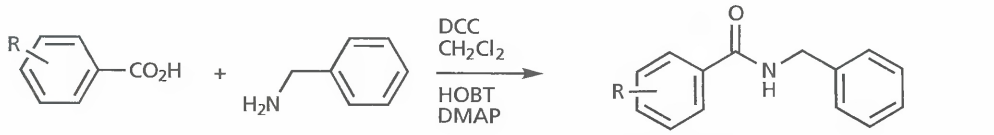


FIGURE 16.25 Coupling reaction of a carboxylic acid and an amine to give an amide library (DCC = dicyclohexylcarbodiimide, DMAP = dimethylaminopyridine, HOBT = hydroxybenztriazole).

and organic layers have been separated, the organic layer is washed with an aqueous base in order to remove unreacted acid. The organic and basic layer are separated, and then the organic layer is treated with a drying agent such as magnesium sulfate. The drying agent is filtered off and then the solvent is removed to afford the crude amide. Purification then has to be carried out by crystallisation or chromatography. In order to synthesize a small 12-component amide library by reacting different carboxylic acids with the same amine, one would have to repeat all of these steps and this would prove both time consuming and equipment intensive.

Equipment miniaturization for parallel synthesis means that it is possible to house a mini-parallel synthesis laboratory in a fume cupboard for each chemist (Fig. 16.26). Small footprint work stations often enable one to perform up to 24 reactions followed by 24 simultaneous evaporations on a normal heater stirrer unit. Multiple parallel or sequential automated chromatography units can facilitate purification, and microwave reactors can dramatically speed up reaction times. In this way, all 12 amides in our library can be made at the same time in parallel. A variety of useful techniques can also be used to minimize the work-up procedures as described below.

16.9.1 Solid phase extraction

Solid phase extraction (SPE) can be used to avoid the 'hassle' of carrying out liquid–liquid extractions to remove acidic or basic compounds or impurities. For example, the twelve crude amides that have been prepared can be taken up from their wells at the same time using a multichannel pipettor and applied to a battery of silica columns. An acidic column (SCX column) removes basic

FIGURE 16.26 Laboratory stations for microwave assisted organic reactions. CEM Explorer-24, kindly supplied by CEM Corporation, NC (left).

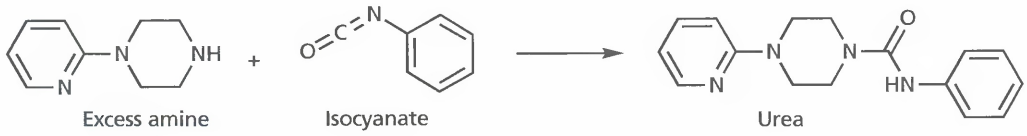


FIGURE 16.27 Reaction of an isocyanate with excess amine to produce a urea.

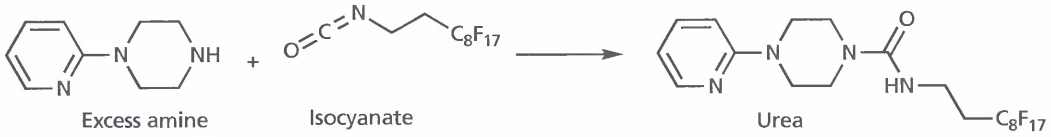


FIGURE 16.28 Removal of excess amine by reaction with a fluorinated isocyanate.

impurities, while a basic column (SAX column) removes acid impurities. Once the solutions have passed through the columns, the solvents are concentrated in parallel to yield essentially pure amide.

Another method of removing excess amine from a reaction is to use fluorous solid phase extraction (F-SPE). This consists of silica columns where the silica has been linked to alkyl chains containing a large number of fluoro-substituents. The highly fluorinated silica has a high affinity for fluorinated molecules and can be used to separate fluorinated compounds from non-fluorinated compounds. For example, consider the reaction shown in Fig. 16.27 where an isocyanate is treated with an amine to give a urea product. The amine is used in excess in order to drive the reaction to completion, but the amine left over has to be removed. In order to do this, a fluorinated isocyanate is added which reacts with the excess amine to produce a fluorinated urea (Fig. 16.28). The crude solution is passed through an F-SPE column which acts as a scavenger resin to retain the highly fluorinated urea, and allow the desired unfluorinated urea product to pass through.

Sometimes an aqueous work-up cannot be avoided. For example, an aqueous work up is required following a Grignard reaction which means that the aqueous and organic phase have to be separated. Fortunately, there are efficient methods of carrying out such separations in parallel.

One such method is to use a Lollipop Phase Separator. A pin is inserted into a mixture of the two phases, and the mixture is rapidly cooled in a dry ice/acetone bath at −78°C. The aqueous phase freezes onto the pin to form a 'lollipop'. The pin and its lollipop can then be removed from the reaction vial, leaving the organic phase behind. Up to 96 such separations may be performed in parallel with specially designed units.

Another method is to use phase separation columns, which can be used to separate a dense chlorinated organic layer from an aqueous phase. The lower organic layer passes through a hydrophobic frit by gravity, whereas the upper aqueous layer is retained on the frit. It is important not to apply pressure; otherwise the aqueous phase may be forced through the frit as well.

16.9.2 Use of resins in solution phase organic synthesis (SPOS)

By carrying out a parallel synthesis in solution, it is easy to monitor the reaction by ^{1}H NMR spectroscopy or by thin layer chromatography. Work up procedures can be greatly simplified by the use of a variety of resins. Since resins are solid supported, there is little interaction between different types, allowing a variety of resins to be used in the same reaction. Thus, it is common to have a reaction cocktail which includes nucleophilic and electrophilic resins, or acidic and basic resins without any problems arising.

Reactions are carried out such that one of the reagents—usually the cheaper and more readily available—is used in excess in order to drive the reaction to completion (Fig. 16.29). The crude reaction mixture will comprise the product AB and excess starting material A. The crude mixture is treated with a solid-supported scavenger resin that is capable of reacting with the excess reagent (A). As a result, excess reagent becomes attached to the resin and can be removed by filtering the resin. Removal of the solvent then leaves the pure product (AB).

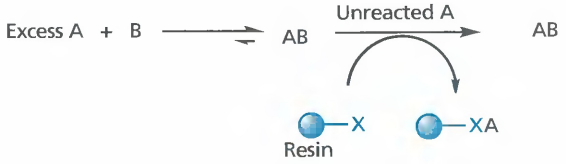


FIGURE 16.29 The use of scavenger resins in solution phase organic synthesis.

16.9.3 Reagents attached to solid support: catch and release

It is possible to attach a reagent to a solid support. This has the advantage that the reagent or its by-product can be easily removed at the end of the reaction. For example, the coupling agent used for amide synthesis can be attached to a resin instead of being present in solution (Fig. 16.30). The reaction involves a carboxylic acid starting material reacting with the coupling reagent to form an intermediate which is still linked to the resin. Thus, the carboxylic acid is taken out of solution—the 'catch' phase. The resin-bound intermediate now reacts with the amine, and the amide product is released back into solution. The urea by-product which is formed remains bound to the resin and is easily removed when the resin is finally filtered. Acidic and basic resins can also be added to remove reagents and excess starting materials as described above.

The formation of a sulfonamide library shown in Fig. 16.31 makes use of a variety of different resins. The reaction involves an amine being treated with an excess of a sulfonyl chloride. A basic catalyst is required for the reaction and triethylamine is normally used in a conventional synthesis. However, this is quite a smelly, volatile compound and would have to be removed once the reaction was complete. Instead of triethylamine, a resin-bound base such as morpholine (PS-morpholine) can be used.

Following the reaction, nucleophilic and electrophilic scavenger resins are added. The nucleophilic resin PS-trisamine reacts with excess sulfonyl chloride to remove it from solution, while the electrophilic resin PS-isocyanate removes unreacted amine (Fig. 16.32). Filtration, to remove the resins, leaves the pure sulphonamide in solution.

Solid supported reagents can be used in a variety of very common synthetic reactions. For example a solid supported borohydride can be used to reduce carbonyl groups (Fig. 16.33). In some reactions, it is also possible to reduce

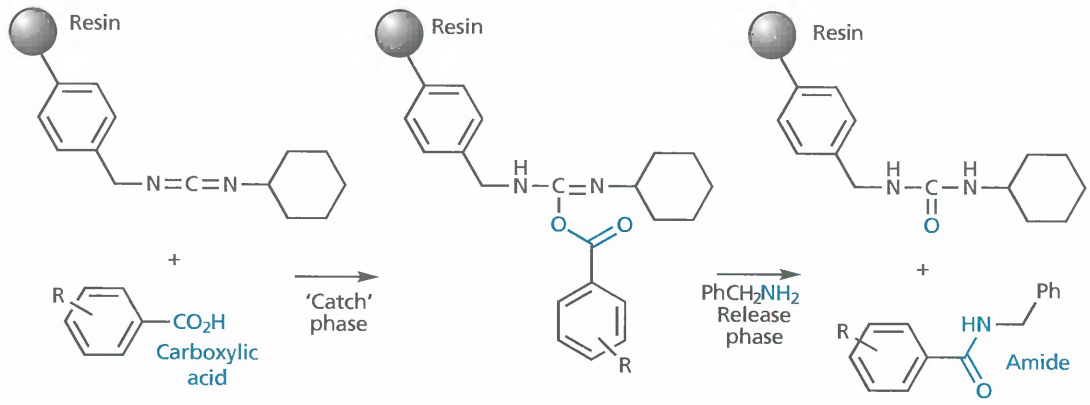


FIGURE 16.30 'Catch and release' during a coupling reaction.

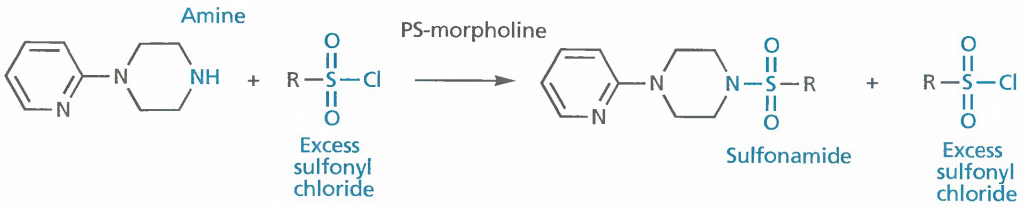


FIGURE 16.31 Formation of a sulfonamide library.

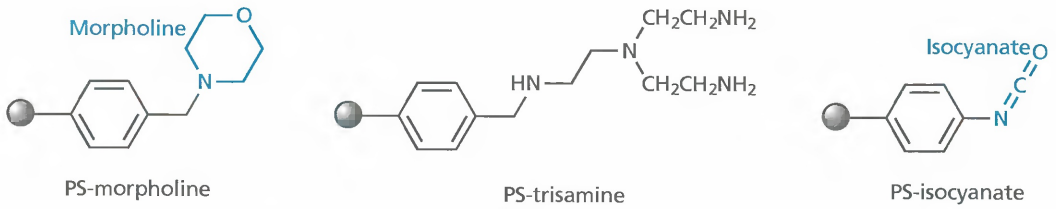


FIGURE 16.32 PS-morpholine, PS-trisamine and PS-isocyanate.

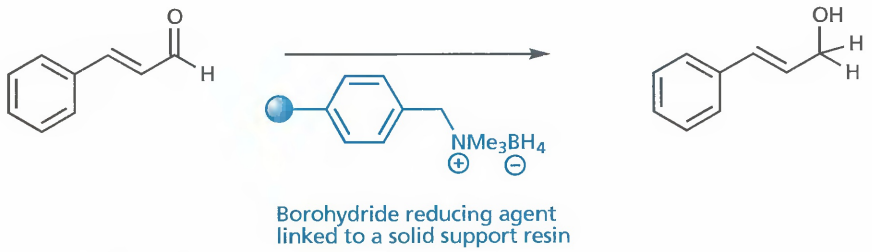


FIGURE 16.33 Reduction of an aldehyde with a solid-supported borohydride reagent.

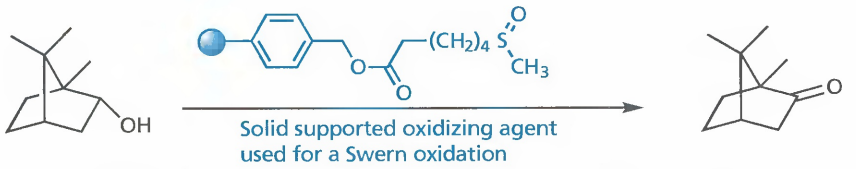


FIGURE 16.34 Swern oxidation using a solid supported reagent.

the toxicity and odour of reagents and their by-products. For example, the normal Swern oxidation involves the formation of dimethylsulfide as a by-product—a compound that has a pungent cabbage odour! This is avoided by using a solid supported reagent instead (Fig. 16.34).

16.9.4 Microwave technology

Drug discovery is a very expensive process and microwave assisted organic synthesis (MAOS) is proving to be a very useful tool for accelerating syntheses and making the process more efficient. There are many examples of thermal reactions that take several hours to complete using heaters or oil baths, but which are carried out in minutes using microwave conditions. There is a much greater efficiency of energy transfer using microwave technology which accounts for the faster reaction times. Moreover, yields can sometimes be dramatically improved with less decomposition and side reactions. Specially designed microwave units are now commonly employed in library syntheses (Fig. 16.26). Examples of reactions that have been carried out using microwave technology include the formation of amides from acids and amines without the need for coupling agents (Fig. 16.35), metal-catalysed Suzuki couplings which can be performed even on usually unreactive aryl chlorides (Fig. 16.36), and metal-mediated reductions and aminations (Fig. 16.37). The reduction shown in Fig. 16.37

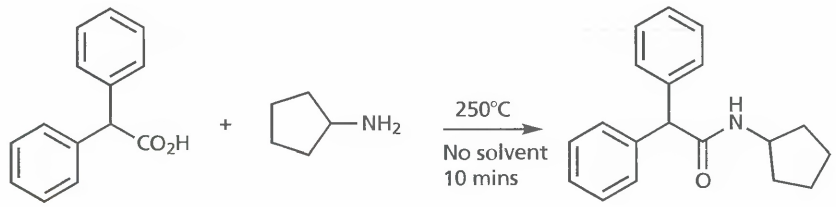


FIGURE 16.35 Amide formation using microwave technology.

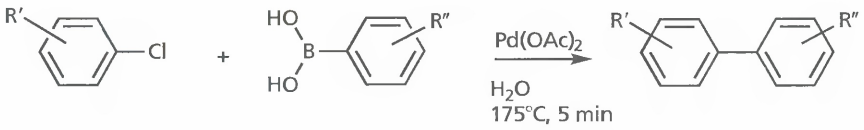


FIGURE 16.36 A Suzuki coupling carried out under microwave conditions.

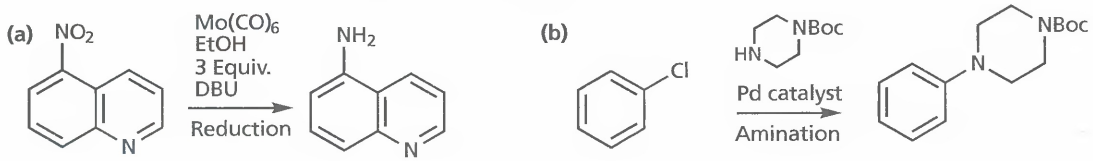


FIGURE 16.37 Microwave-assisted transition metal-mediated reactions. (**a**) Reductions and (**b**) aminations.

took 24 hours using conventional heating, and only 15 minutes using microwave heating.

16.9.5 Microfluidics in parallel synthesis

The science of **microfluidics** involves the manipulation of tiny volumes of liquids in a confined space. Companies are devising microreactors that can be used to carry out parallel syntheses on microchips (Fig. 16.38) using a continuous flow of reactants in microfluidic channels. The channels are designed such that various reactants are mixed and reacted as they flow through the microchip. Several reactions have already been successfully carried out on microscale and it is found that many reaction times are shortened from hours to minutes. Some reactions take place in higher yield and with less side products. It is also possible to control the temperature of each reaction extremely accurately. Another advantage of microreactors is the potential to handle a vast number of parallel reactions on microchips. The channels through each chip can be fabricated to allow all possible mixing combinations of the various reactants, either on separate microchips or on a three-dimensional microchip. The example in Fig 16.38 is a simple illustration of how a microreactor system could be set up to react a library of two structures (A and B) with another library of two structures (C and D).

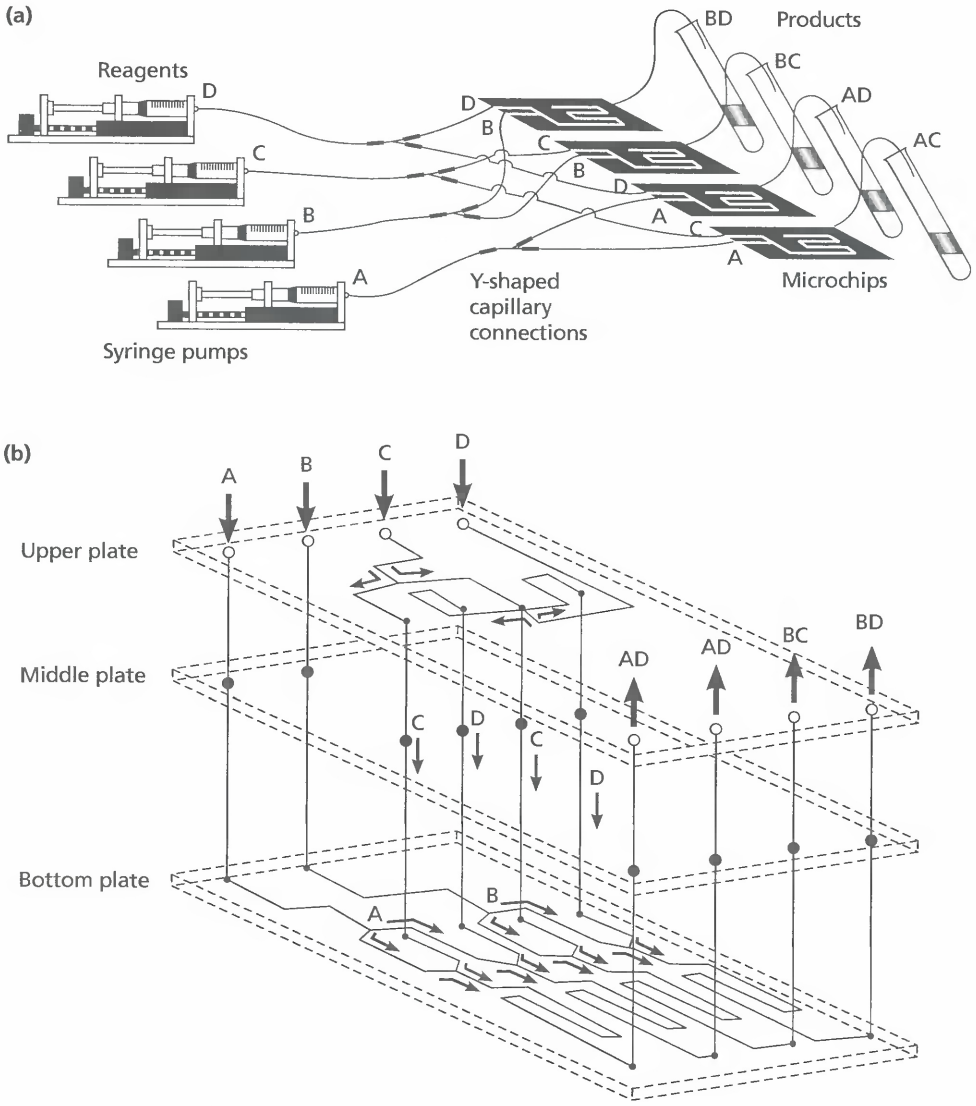


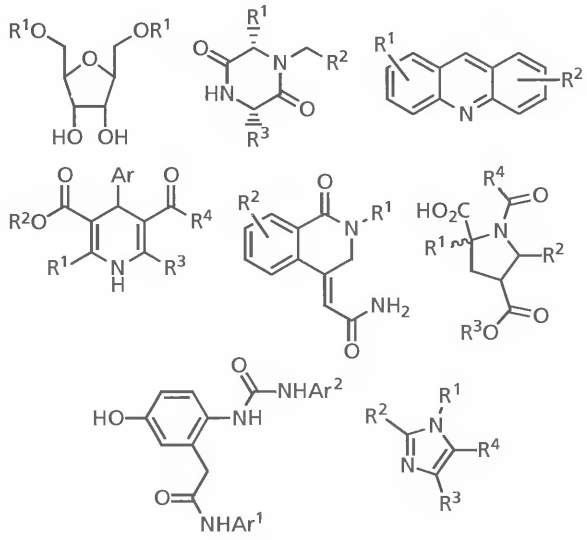
FIGURE 16.38 Parallel synthesis on a microchip. Parallel synthesis of four products using **(a)** four separate two-dimensional microchips, and **(b)** a three-dimensional microchip. (Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Drug Discovery (5, 210–18) 2006)

KEY POINTS

- In parallel synthesis, a reaction or series of reactions is carried out on a series of wells to produce a range of analogues. Each reaction well contains a single product.
- Parallel synthesis can be carried out on solid phase or in solution.
- Parallel synthesis allows the synthesis of a large number of easily identifiable analogues that can be quickly and easily tested, speeding up the optimization process.
- Solid phase extraction is often used in parallel synthesis for work up. It involves the use of columns to remove impurities and excess reagents.
- An aqueous phase can be separated from an organic phase using phase separation columns or by freezing the aqueous phase onto a solid surface.
- Catch and release strategies involve reagents which are linked to a solid support. Reactants are taken out of solution when they react with the reagent, and are then released when a subsequent reaction takes place.
- Solid supported reagents are easily removed at the end of a reaction. The potential toxicity of the reagent or its byproduct is reduced when attached to a solid support.
- Microwave technology allows several reactions to be carried out in a much quicker time period than by conventional heating.

QUESTIONS

1. Identify three stages of the drug discovery, design, and development process where combinatorial chemistry or parallel synthesis is of importance.
2. A pharmaceutical laboratory wishes to synthesize all the possible dipeptides containing the amino acids tyrosine, lysine, phenylalanine, and leucine. Identify the number of possible dipeptides and explain how the lab would carry this out using combinatorial techniques.
3. What particular precautions have to be taken with the amino acids tyrosine and lysine in the above synthesis?
4. Identify the advantages and disadvantages of the following structures as scaffolds.



5. You wish to carry out the combinatorial synthesis shown in Fig. 16.12 using bar coding techniques rather than the conventional tagging scheme shown in the figure. You have nine molecules suitable for tagging purposes (A–I), seven bromo acids (B1–B7), seven amines (A1–A7), and seven acid chlorides (C1–C7). Construct a suitable coding system for the synthesis.
6. Based on your coding scheme from Question 5, what product is present on the bead if the released tags resulted in the gas chromatograph shown in Fig. 16.13.

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Titles for general further reading are listed on p. 725, see also articles in Journal of Combinatorial Chemistry.