

Part One Introduction

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The (Classic Concept of) Solid Support

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1.1 Introduction

The use of a solid support in a chemical process can be traced back to the birth of chromatography. However, it was at the beginning of the 1960s with the publication by the Nobel Prize winner R.B. Merrifield of his seminal paper “Solid phase peptide synthesis. I. The synthesis of a tetrapeptide” [1] that solid-supported or solid-phase chemistry was truly born. The idea of using a solid support as protecting group (carboxylic-protecting group in the case of the Merrifield tetrapeptide) was rapidly and enthusiastically adapted by Letsinger to the synthesis of oligonucleotides (protection of the exocyclic amino function of the 5'-O-trityldeoxycytidine) [2]. Although at the end of the 1960s Merrifield himself predicted that: “. . . it seems quite clear that a gold mine is awaiting the organic chemist who would look to solid supports for controlling and directing his synthetic reactions” [3], only a few examples of the extension of the solid supported protecting group concept to other areas of synthetic organic chemistry can be found in the literature at that time. These examples involved the monoalkylation and monoacylation of carboxylic acids [4, 5], the synthesis of aldehydes and ketones [6] and the use of resin-bound dienes or dienophiles to trap reactive intermediates [7]. A new milestone in this long journey was reached at the beginning of the 1990s when Ellman published the synthesis of 1,4-benzodiazepine derivatives on a solid phase [8]. This achievement can be considered the advent of small molecule combinatorial chemistry strategies for drug discovery. This field had its roots in the work of Houghten [9] and Lam [10], who examined the solid-phase synthesis of peptide libraries. In contrast to what happens in other disciplines, where knowledge and technology transfer from academia to industry is slow, in this case the pharmaceutical companies set up their own facilities and departments and sponsored the establishment of small- and medium-sized companies dedicated to this branch of chemistry.

Simultaneously, numerous academic groups started and even reoriented their activities towards the implantation of solid-phase strategies in their laboratories. Although progress towards fulfillment of the initial great expectations has slowed in recent years, solid-supported chemistry is now a very useful tool in all modern organic chemistry laboratories [11] and, of course, is the method of choice for both research and industrial synthesis of peptides [12] and oligonucleotides [13].

The solid support in synthetic chemistry has amplified its range of applications. Thus, from the protecting group concept applied for the synthesis of peptides, oligonucleotides, oligosaccharides and other biomolecules, it has evolved to solid-supported reagents and resins as scavengers for high-throughput purification.

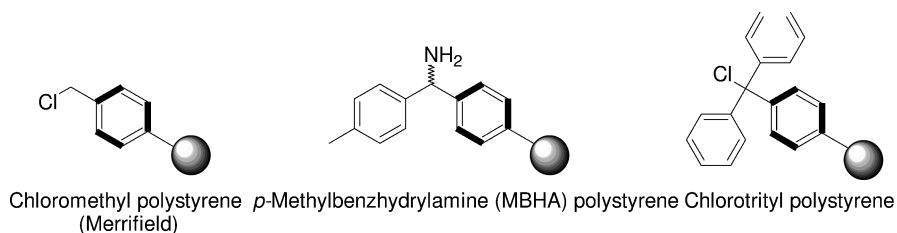
Usually, chemical processes take longer in solid-phase mode than in solution. Furthermore, the solid support should be considered analogous to a co-solvent [14]. Thus, this support is an integral part of the process and very often each new support requires optimization. Consequently, the translation of organic processes from solution- to solid-phase commonly calls for some work to optimize the overall process. In general, reactions that tolerate excesses of reagent can be transferred well to the solid phase. In contrast, those reactions that require stoichiometric amounts of reagents rarely work well because the solid-phase approach is based on the use of large excesses of reagents to drive the processes to completion [15].

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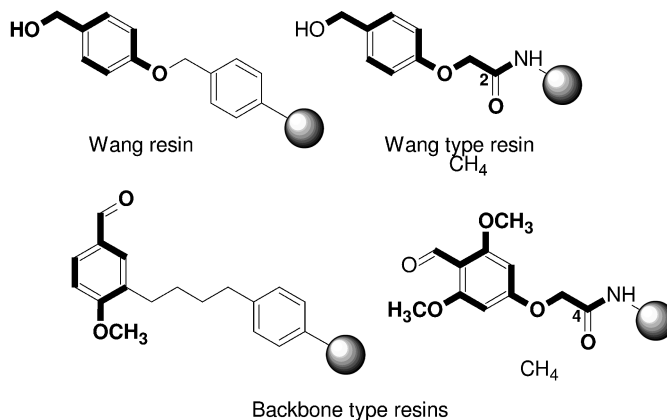
Linkers/Handles

There is a tendency to confuse the terms “solid support/resin” and “handle/linker” [16]. As pointed out by Bradley and coworkers, resins/solid supports are inert matrices, which are passive to chemistry [17]. In contrast, linkers/handles are immobilized protecting groups [16, 17], which can be classified into two types, integral and non-integral [17]. Integral linkers/handles are characterized by the fact that part of the solid support forms part or all of the linker/handle, as shown in some polystyrene-based linkers. Non-integral linkers/handles are attached permanently to the solid support (Figure 1.1). In all cases, the linker/handle should contain a functional group that will link it temporarily to a growing molecule. The final step in all solid-phase synthetic processes is the release of the final compound.

Although non-integrated linkers/handles can be attached to the solid support through an ether (Wang resin) or even a C–C bond, the most convenient strategy is through an amide bond. Thus, the carboxylic acid-containing linker/handle is prepared and characterized in solution and then incorporated into an amino solid support. In this strategy, the amide that links the linker/handle to the solid support should be totally stable to all synthetic processes, including the final treatment that will detach the target compound from the solid support. Very often the linker/handle is attached to a MBHA resin, commonly used for the preparation of peptide amides using a *tert*-butoxycarbonyl (Boc)/benzyl strategy. In this case, the bond



A. Integral linkers/handles. In bold, the part of the solid support that forms part of the linker



B. Non-integral linkers/handles. In bold, the linker/handle

Figure 1.1 Examples of integral (a) and non-integral (b) linkers/handles. Adapted from reference [16, 17].

formed between the linker/handle and the BHA resin is not totally stable to the acid conditions (TFA–scavengers–CH₂Cl₂ mixtures at 25–60 °C) and therefore the carbocation-containing linker is detached from the solid support. This can add impurities to the final crude or, what is even more damaging, can cause back-alkylation of the target compound [18, 19]. To overcome this side-reaction, the use of aminobenzyl polystyrene or aminoalkyl resins, which form a more acid stable bond, is recommended [19].

A similar undesired cleavage can take place with (poly)alkoxybenzyl [Wang, backbone amide linker (BAL), Rink-type resins] [19, 20] (Figure 1.1B).

Thus in peptide synthesis, Tsikaris *et al.* [21] have described the incorporation of the *p*-hydroxybenzyl moiety cleaved from the Wang resin into the N- of the C-terminal amide of a peptide during TFA cleavage (Figure 1.2). Similarly, Martinez *et al.* [22] have reported the alkylation of the indol ring of Trp-containing peptides by the *p*-hydroxybenzyl moiety. Furthermore, Stanger and Krchnak have demonstrated the formation of O-(4-hydroxy)benzyl derivatives [23]. The use of the Wang resin for the solid-phase preparation of small molecules has led to the introduction

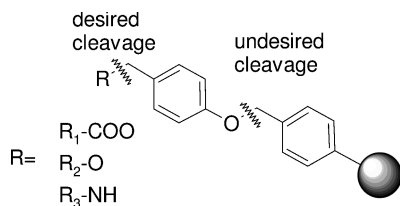


Figure 1.2 Dual cleavage on a Wang resin as an example of this side-reaction in other (poly)alkoxybenzyl resins. Adapted from reference [20].

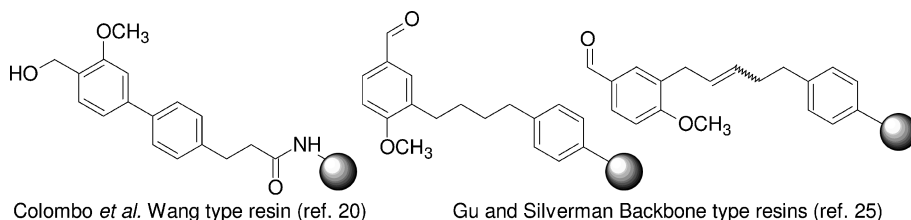


Figure 1.3 Non-acid degradable linkers.

of impurities due to the undesired cleavage from the resin (no cleavage at the benzyl position) or from a back-alkylation of the *p*-hydroxybenzyl cation in the case of furopyridine and furoquinoline target derivatives [24]. To overcome these problems, two resins have been developed based on the activation of the benzyl position by a MeO group, a non-cleavable electron-donating group, in either the ortho or para position. Thus, Gu and Silverman [25] incorporated the precursor of their backbone linker to the resin through a metal-catalyzed coupling reaction, and Colombo *et al.* incorporated the precursor of their Wang type resin through an amide bond (Figure 1.3) [20].

The use of non-integral linkers/handles is usually more recommendable because they provide control and flexibility for the synthetic process [26]. Thus, (i) any functionalized solid support can be used; (ii) when the linker/handle is attached through an amide bond, the quality of the starting resin can be assured by controlling the purity of the initial linker/handle; (iii) loadings can be easily fine-tuned; (iv) it allows the introduction of internal reference amino acids (IRaas) [27–29] between the linker/handle and the solid support, which can facilitate monitoring of the synthetic process; and (v) when the first building block (BB) is incorporated to the linker/handle through a more demanding ester bond, it can be attached to the linker/handle in solution and, after characterization and, if required, purification, incorporated to the solid support (preformed linker/handle, Figure 1.4) [29–30].

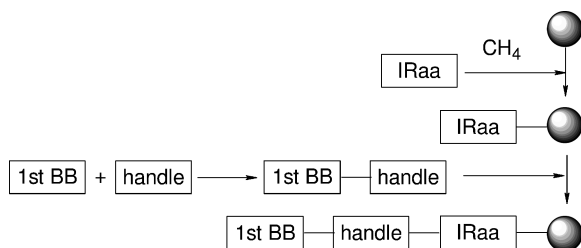


Figure 1.4 Optimized solid-phase strategy using a preformed linker/handle and IRaa.

1.3

Solid Supports

An optimal solid support should (i) be mechanically robust; (ii) be stable to variation in temperatures; (iii) have reagent-accessible sites; (iv) show acceptable loadings; (v) present acceptable bead sizes, if applicable, when required, facilitating filtration; (vi) be stable in diverse media, and in the case of being used in biochemical assays (vii) show biocompatibility and swelling in aqueous buffers.

In contrast to most chemical reagents, which are usually of the same quality regardless of the supplier, the source of solid support is extremely important. Thus, distinct solid supports from different manufacturers or even from the same one, but from separate batches, may differ in performance. Consequently, the choice of the solid support source can have a significant influence on the result of the chemical process. If the large-scale synthesis of a molecule or the production of a library is preceded by an optimization step, both steps should be carried out with the same batch of solid support.

Several classifications of solid supports have been proposed on the basis of their physical properties [31–32]. However, the solid supports most widely used today can be classified into just two groups: gel type and modified surface type.

1.3.1

Gel-Type Support

These types of supports are the most commonly used. The polymer network is flexible and can expand or exclude solvent to accommodate the growing molecule within the gel. Thus, the chemistry occurs within the well-solvated gel that contains mobile and reagent-accessible chains [33]. Three types of gel resin are commercially available:

1.3.1.1 Polystyrene (PS) Resins

These are, without doubt, the most widely used solid supports. PS systems used for the synthesis of peptides and small molecules consist of 1% cross-linked hydrophobic resins obtained by suspension polymerization from styrene and divinylbenzene. For other uses, PS with 2% cross-linking, which is mechanically more stable than those with less cross-linking, is also employed. This 2% PS was used

in the early years of solid-phase peptide synthesis (SPPS). However, due to problems observed during the synthesis of difficult sequences, the cross-linking of this resin was then reduced to 1%. Although full derivatization of PS would give a substitution of approximately 10 mmol g^{-1} , practical loadings can vary from 0.3 to 3 mmol g^{-1} . For SPPS, the loading should be kept at around 0.5 mmol g^{-1} to ensure a successful synthetic outcome [34]. PS as hydrophobic beads swell well in non-polar solvents like toluene or CH_2Cl_2 [35]. However, these systems can also be used in combination with other more polar solvents such as *N,N*-dimethylformamide (DMF), dioxane, and tetrahydrofuran. In SPPS, the use of acetonitrile is incompatible with a good quality final product [36].

1.3.1.2 Poly(Ethylene Glycol)–Polystyrene (PEG-PS) Resins

Based on the early work of Mutter [37], the first commercially available PEG-PS resins were developed independently in the mid-1980s by Zalipsky, Albericio, and Barany (PEG-PS) [38–39] and Bayer and Rapp (TentaGel) [40–41]. These systems can be synthesized either by reaction of preformed oligoxyethylenes with aminomethylated polystyrene beads (PEG-PS) or by graft polymerization on polystyrene beads (TentaGel).

The first PEG-PS resin was developed with the idea of combining a hydrophobic PS core with hydrophilic PEG chains on the same support. Furthermore, in some of the aforementioned PEG-PS resins, the PEG unit might act as a spacer, separating the starting point of the solid-phase synthesis from the PS core [40]. This early hypothesis has not been corroborated in any other formulations of PEG-PS resins and it is, therefore, possible to conclude that the environment effect of the PEG is far more critical than any spacer effect [15, 42]. Given the unique conformational flexibility of PEG chains, PEG-PS resins are compatible with both polar and non-polar solvents [43]. The high degree of swelling provides the beads with a firmer and flow-stable character and makes these resins physically stable in flow systems. The content of PEG varies significantly between distinct resins and therefore their swelling properties can differ markedly. Furthermore, several of these systems can lose PEG during treatment with TFA. PEG-PS resins usually show lower loadings ($0.15\text{--}10.3 \text{ mmol g}^{-1}$) than PS ones.

1.3.1.3 Hydrophilic PEG-Based Resins

These resins have their roots in the polyacrylamide resins introduced in the 1980s for SPPS using the fluorenylmethoxycarbonyl (Fmoc)-*tert*-butyl (tBu) strategy. They were developed following the concept that the insoluble support and peptide backbone should have comparable polarities [44].

Kempe and Barany [45] developed the CLEAR family, which is based on the copolymerization of branched PEG-containing cross-linkers such as trimethylolpropane ethoxylate triacrylate, which contain various ethylene oxide units, with amino-functionalized monomers such as allylamine or 2-aminoethylmethacrylate. These amino groups constitute the starting points for the solid-phase synthesis. The loadings of these solid supports are affected by the amount of functionalized monomer used for polymerization. Typical loadings are in the range

0.1–0.3 mmol g⁻¹. The CLEAR family supports swell in a broad range of hydrophobic and hydrophilic solvents and has excellent physical and mechanical properties for both batch-wise and continuous-flow systems.

Meldal designed a series of PEG-based resins, first of all combining them with a small amount of PS or polyamide and, finally, using neat PEG [31]. The first member of this family, and the most widely used, was the PEGA resin [46], obtained by inverse suspension radical polymerization of various sizes of linear bis- and branched tris-2-aminopropyl-PEG samples with acryloyl chloride. The uniform beads swelled in all solvents, ranging from toluene to aqueous buffers. To prevent the presence of secondary amide bonds, which can interfere with reactions involving carbon and carbenium ions, the POEPS, a new family of PEG-based resins, was developed by partial derivatization of linear PEG with vinylphenylmethyl chloride or vinylphenylpropyl chloride, followed by inverse suspension radical polymerization [47–48]. The methyl version of this resin is not stable to Lewis acids because of the benzylic linkage between the PEG and the PS. In addition, the preparation of vinylphenylpropyl chloride is not straightforward—even the presence of a small amount of PS in the polymer leads to a slight decrease in the favorable swelling observed for PEGA resins in polar solvents. Alternatively, polyoxyethylene cross-linked polyoxypropylene (POEPOP), which contains only ether bonds, was developed from a polymerization of PEG that was partially derivatized with chloromethyloxirane [49]. Although the POEPOP resin is mechanically robust, shows relatively high loading (primary and secondary alcohols) and good performance for organic transformations, the presence of secondary ether bonds implies that this solid support is not totally stable to strong Lewis acids [50]. To overcome this problem, the SPOCC resin, in which all ether bonds and functional alcohol groups are primary, was developed [51].

In a parallel manner, Côté [52] developed the ChemMatrix resin, a total PEG-based resin consisting of primary ether bonds. Because of its highly cross-linked matrix, ChemMatrix has greater mechanical stability than other PEG resins. This resin swells well in all of the most common solvents and is, therefore, useful for a broad range of organic chemistries. ChemMatrix resin performs extremely well compared to PS resins in the solid-phase synthesis of hydrophobic, highly structured, poly-Arg peptide, β -amyloid (1-42), RANTES – a complex aggregated chemokine – and HIV protease [53–56], showing that the presence of PFG chains impairs the aggregation of the growing peptide chain, facilitating the solid-phase synthesis of complex peptides. Furthermore, ChemMatrix is convenient for the synthesis of oligonucleotides and oligonucleotide peptide conjugates and oligonucleotide hybrids by Cu⁺-catalyzed cycloaddition reactions [57].

The compatibility of all these PEG-based resins with aqueous buffers allows their use for biochemical applications such as on-resin screening of chemical libraries and in the development of affinity chromatography [58–61]. All these families of PEG-based resins, except POEPS, are free of aromatic rings. This feature makes these solid supports highly suitable for a broad range of applications where such rings can react with reagents or/and jeopardize the solid-phase NMR control of the reactions [62].

Regarding the functional site distribution in these gel-type supports, there is some controversy. Thus, using fluorescence optical analysis, McAlpine and Schreiber determined that for PS and TentaGel resins there is a higher effective concentration of functional sites at the surface relative to the macroporous resin ArgoPore and controlled pore glass (CPG) [63–64]. In contrast, Bradley and coworkers [65], using confocal Raman and fluorescence microscopy, showed that for both resins there is a uniform distribution of reactive sites throughout the beads but that the spatial distribution of reacted sites depends on the polymer type, with a fine balance between reaction and diffusion rate. On PS resin beads, there is a uniform distribution of sites, with the reaction rate being slower than diffusion. However, on TentaGel this is not the case because the reaction is diffusion-controlled. These findings were confirmed by Rademann and coworkers for PS resin, using confocal and non-confocal fluorescence microscopy as well as by FT-IR microscopy [66].

1.3.2

Modified Surface Type Supports

Although numerous materials have been used for surface functionalization for solid-phase synthesis [15, 31, 32], the most common, which are commercially available, are membranes and pellicular solid supports, where a mobile polymer is grafted to a rigid and inert plastic.

1.3.2.1 Cellulose Membranes

These systems are the basis of the SPOT concept, a highly parallel and technically simple arrangement that was first developed by Frank [67–69]. SPOT is very flexible and inexpensive compared to other multiple solid-phase procedures, especially regarding miniaturization and array geometries. Cellulose paper was first used for the solid-phase synthesis of oligonucleotides [70] and peptides [71]. Cellulose, usually Whatman 50 or 540, can be easily modified by O-acylation with protected amino acids to form amino acid esters [72]. However, although these functionalized membranes have been applied for peptide synthesis and screening of peptide libraries, their use in a broader range of chemical reactions is jeopardized by several factors. The lability of the ester bond limits their use. To circumvent this problem, the hydroxy functions of cellulose have been alkylated with epoxides containing a protected amino function [73]. However, the preparation of the N-protected epoxypropylamines is laborious and therefore this method is impractical for the functionalization of cellulose membranes. Optimized methods have been developed that involve the incubation of cellulose membranes with epibromohydrin in the presence of perchloric acid, followed by reaction with 4,7,10-trioxo-1,13-tridecanediamine or diaminopropane [74–75]. Furthermore, chemical degradation of cellulose can occur under the conditions used for more conventional organic reactions [68]. For instance, the glycosidic bonds of cellulose can be labile under nucleophilic and acidic conditions. Finally, the large number of hydroxy functionalities can cause instability and interfere with subsequent reactions.

1.3.2.2 Polyolefinic Membranes

Polyalkanes (polyethylene and polypropylene) and their fluorinated derivatives [poly(vinylidene fluoride) and polytetrafluoroethylene (PTFE)] have been successfully used for solid-phase synthesis in reactors [76–78] as well as in the SPOT technique [68]. Polypropylene membranes are highly stable, show low expansion in the presence of most common solvents, and display acceptable loadings. Functionalization can be performed by photo-induced graft copolymerization with functional acrylates by the following sequence: (i) coating with photo-initiator (benzophenone) and selective UV-excitation, causing H-abstraction and radical formation; (ii) application of an acrylate monomer solution, resulting in addition of radical sites to the double bond of the monomer; and (iii) free radical polymerization. If acrylic acid and its methyl ester are used as monomers, the final functionalization in the form of amine groups can be achieved by amidation with 4,7,10-trioxa-1,13-tridecanediamine after activation of the carboxylic acids with SOCl_2 , $(\text{COCl})_2$, PCl_3 or PCl_5 .

1.3.2.3 Pellicular Solid Supports

In these systems a mobile polymer is grafted onto a rigid and inert plastic. This idea was first developed by Geysen and initiated the “Multipin concept” [79, 80]. This design has several handling advantages in that it can be made to take any particular shape/form and can be adapted to any desired array. Furthermore, the size of the inert plastic, together with the efficiency of the grafting, will determine the amount of product that can be synthesized. Thus, unlike gel-type supports, it is the surface area and not the volume that determines the loading capacity. A further advantage of this kind of support is the consistency of the kinetics between grafted supports of different shapes and sizes. Such a correlation is not possible with gel-type supports because the rate of diffusion changes with size and, therefore, the reaction rates are modified. However, comparison studies carried out in our laboratory between pellicular supports and PS resins have demonstrated that kinetic reactions are usually slower with the pellicular solid supports.

The original pin support was poly(acrylic acid) grafted onto inert polyethylene, where the carboxylic acid moieties were capped with mono Boc-protected ethylenediamine [79]. PS and the extremely hydrophilic polyhydroxyethyl methacrylate and poly(methacrylic acid/dimethylacrylamide) were also grafted on [81]. The last grafted support in the “Multipin concept” is the so-called Lanterns [80], where the actual solid support is PS, which can be functionalized in a similar way to PS gel-type supports.

In conclusion, the concept of solid-supported chemistry has been extended from the preparation of peptides and other biomolecules to any organic molecule. PS- and PEG-based resins are the most widely used; however, syntheses can be carried out in any solid support. The development of new solid supports and linkers/handles is crucial to fulfill the new requirements of modern drug discovery programs.

Acknowledgments

The work carried out in the laboratory of the authors was partially supported by CICYT (CTQ2006-03794/BQU), the *Generalitat de Catalunya* (2005SGR 00662), ISCIII (CIBER, nanomedicine 0074), the Institute for Research in Biomedicine, and the Barcelona Science Park.

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