Linkers and Cleavage Strategies in Solid-Phase Organic Synthesis and Combinatorial Chemistry

Fabrice Guillier,[†] David Orain,[‡] and Mark Bradley*[,‡]

Department of Chemistry, Southampton University, Highfield, Southampton SO17 1BJ, U.K., and Alanex Division of Agouron Pharmaceuticals, A Werner Lambert Company, 3550 General Atomic Courts, San Diego, California 92121

Received September 30, 1999

Contents

I. Introduction

The massive increase in the number of papers describing the use of polymeric supports in organic synthesis over the past decade is a vivid demonstration of its impact in the chemical community. Few other changes in synthetic chemistry methodology have displayed such a growing passion or had such a profound influence on the way synthetic chemistry

* To whom correspondence should be addressed.
[†] Agouron Pharmaceuticals.
[‡] Southampton University.

Fabrice Guillier was born in 1968 in the city of Saint Denis, France. He received his diplome d'Ingénieur chimiste in 1992 from the National Institute of Applied Sciences (INSA Rouen) after studying chemical engineering and organic chemistry. In 1996 he completed his Ph.D. thesis on natural product synthesis using metalation and cross-coupling reactions in the laboratories of Professor Guy Quéguiner (URA1429-IRCOF–Rouen-France). These methodologies using palladium and lithium derivatives were suitable for the synthesis of polyheterocyclic marine alkaloids of the pyridoacridine family. He spent then one year as a postdoctoral fellow at Southampton University, working with Professor Jeremy Kilburn (Department of Chemistry, University of Southampton) studying the synthesis and supramolecular interactions of guanidinium-based tweezer receptors of C-terminal tripeptides. From 1997 to 1999 he joined the research group of Professor Mark Bradley at the University of Southampton, where he conducted research on the development of new methods for screening on solid supports and the synthesis of libraries of polyamines as inhibitors of *trypanothione reductase* and substituted pyrimidines for *kinases* inhibition. Currently he is developing new reactions for lead discovery using solution-phase parallel synthesis at Alanex Division of Agouron Pharmaceuticals (San Diego, CA) as a research fellow.

David Orain was born in 1971 in Rennes, France. He studied chemistry at the University of Rennes and received his DEA (Masters) degree in 1994 under the supervision of Dr. Paul Mosset at ENSCR (School of Chemistry at Rennes) where he studied the synthesis of α,α'-bifunctionnalized heterocycles. Then he moved downstairs at ENSCR and worked with Dr. J. C. Guillemin's group during his Ph.D. studies. His research involved the use of zirconium organometallic complexes for solution and support chemistries. He was awarded his Ph.D. degree in December 1998. In January 1999, he crossed the Channel and joined Professor Mark Bradley's group (Department Chemistry, Southampton University) as a postdoctoral researcher. His current research focuses on the development of a new safety-catch linker for amine release under biological conditions and new direct screening processes of a library of *trypanothione reductase* inhibitors.

is carried out. The advantages gained by this methodology are striking, with four main factors contributing to the popularity of the technique. (i) *The ease*

Mark Bradley was born in the United Kingdom in 1962. He received his first degree from the University of Oxford in 1986 and his DPhil degree from the same institution three years later under the supervision of Professor Sir Jack Baldwin in the area of Penicillin biosynthesis. In 1989 he moved to the United States to work with Professor Chris Walsh at Harvard Medical School. He then returned to the United Kingdom and to Southampton University in 1992 as a Royal Society University Research Fellow. In 1997 he was made a Professor of Combinatorial Chemistry and has published in excess of 50 papers in the combinatorial area. In January 2000 he became director of the Combinatorial Centre of Excellence now housed in Southampton. His research interests span across the whole area of combinatorial high-throughput synthesis, screening, and analysis from an academic viewpoint. This includes interests in solid-phase and small-molecule synthesis as well combinatorial activities in the area of catalysts, dendrimers, fluorophores, and polymers. He has interests in the area of enzyme inhibition including work in the area of proteases, antibacterial agents, and antiparastics.

washing the resin, thus allowing many simple automated procedures to be developed. (ii) *The elimination of purification steps en route.* For each step of a multiple-step synthesis, the only purification needed is a resin-washing step. Only the final product of cleavage needs to be purified. (iii) *In a solid-phase synthesis, high concentrations of reagents can be used to drive reactions to completion.* (iv) *The straightforward nature of parallel solid-phase synthesis.*

However, for a solid-phase synthesis to be practical, several important issues need to be addressed, including the correct choice of solid support and the mode of attachment and cleavage of materials from the resin matrix. Efficiency in anchoring and removing a small organic molecule from the polymeric resin relies on the correct choice of the linker group. This key fragment is crucial in planning a synthetic strategy. However, if the objective of a single or parallel solid-phase organic synthesis is to produce one or several defined products upon release, the correct choice of an adequate linker system can enable further goals to be attained. Multidetachable linkers allow the preparation of different products depending on the cleavage conditions selected. Partial release can be useful for monitoring reactions or screening mixtures for deconvolution. Structural elucidation of hits from mixtures of products is another application of linkers in combinatorial chemistry, allowing "tags" of a variety of forms to be incorporated and read.

II. Solid Supports for Organic Synthesis

field first used a chloromethylated-nitrated copolymer of styrene and divinylbenzene (DVB).[1] Resins now used in solid-phase organic synthesis have changed little since this time. These insoluble supports have a gel-type structure which readily allows penetration of reagents and solvents into the beads to sites where chemistry is taking place. A compromise has been found between moderately cross-linked resins (5% DVB) which are very stable but do not swell particularly well, thus reducing site access and low cross-linked resins where mechanical stability becomes an issue. A general consensus now seems to have been reached, and typical supports used for solid-phase synthesis consist of polystyrene with a 1−2% DVB cross-linking. The three dominant polystyrene supports currently in use are the following. (i) *Chloromethylpolystyrene*. Originally prepared by resin postderivatization using chloromethylmethyl ether and $SnCl_4$, it has been more recently prepared by copolymerization using chloromethylstyrene/styrene/DVB mixtures. This core resin is used widely for the attachment of linkers by ether formation. (ii) *Hydroxymethylpolystyrene*. Prepared from Merrifield resin by esterification with potassium acetate followed by saponification or reduction of the ester.[2] (iii) *Aminomethylpolystyrene*. Mitchell[3] prepared this resin either by potassium phthalimide substitution of the Merrifield resin followed by hydrazinolysis or by direct aminomethylation of the polystyrene resin. Aminomethyl resin allows a multitude of spacers/linkers to be appended to the resin by amide bonds, which are stable under strongly acidic conditions. This still provides one of the main workhorse resins of today.

A few other materials are used but polystyrene resin dominates; among the other materials used, TentaGel resin (TG) and ArgoGel (AG), both polystyrene/DVB−poly(ethylene glycol) graft copolymers (PS−PEG), developed by Bayer,[4] are the most favored. They have specific uses, such as when polar solvents are needed or when distancing from the resin core becomes necessary. Crowns/Pins (CP) are another kind of support used in solid-phase synthesis and consist of a radiation-grafted polyethylene/polypropylene support.[5] Kieselguhr/polyacrylamide-based resins (KPA)[6] and controlled-pore glass (CPG)[7] are used in continuous flow SPPS and oligonucleotide synthesis but are usually avoided by the synthetic chemist. PEGA,[8] a poly(ethylene glycol)/dimethylacrylamide copolymer, is a very polar material which confers unparalleled swelling properties in water and possesses a flexible interior enabling access for a variety of large macromolecules such as enzymes; however, low mechanical stability makes handling difficult and expense precludes large-scale use.

III. Linker and Linker Attachment

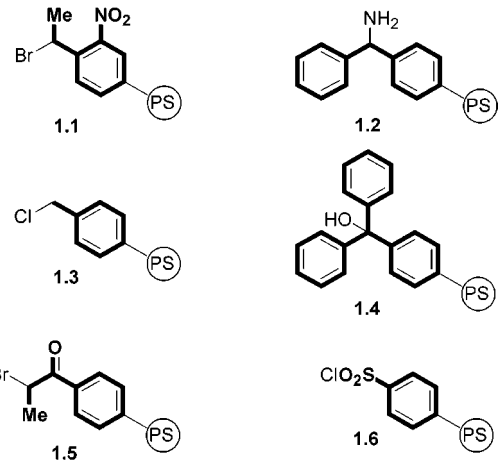
The attachment point of the linker to the solid support or spacer should be chemically stable during the synthesis and cleavage, and as for any solution-phase protecting group, yields for its loading and cleavage should be as quantitative as possible. In protecting group for solid-phase synthesis. A great number of linkers (more than 200) have been developed over the past 15 years in order to allow many multistep organic syntheses to be performed and the use of a broad range of reagents, allowing cleavages in a very selective manner (see refs 9−15 for reviews). Although a linker should ideally enable a selective cleavage to take place under a defined set of conditions, these conditions are in reality not only dependent on the linker but also on the compound attached to the linker, the spacer, and importantly on the resin type, its loading, and bead size. Thus, smaller beads have a much greater efficiency of cleavage under photolysis conditions. Reduced cross-linking dramatically enhances the rate of cleavage from solid supports under acidic conditions.[16] Lack of resin preswelling in CH_2Cl_2 prior to cleavage with TFA can also cause reduction in yields.[16] Many different parameters are thus involved in the cleavage of compounds from the solid support and not just the linker needs to be considered.

A. Linker Types

In this review, to aid clarification and to avoid inaccuracies, there will be a clear distinction made between resins and linkers: Resins will be considered as an inert matrix, passive to chemistry. Linkers will be considered simply as immobilized protecting groups and will be classified into one of two types: (i) *Integral linkers* in which part of the solid support core forms part or all of the linker and (ii) *Nonintegral (or grafted) linkers* in which the linker is attached to the resin core. A linker which has been prepared in solution will be defined as a *unloaded linker*.

Many examples of integral linkers exist (Scheme 1), and certainly they were very popular in the early

Scheme 1



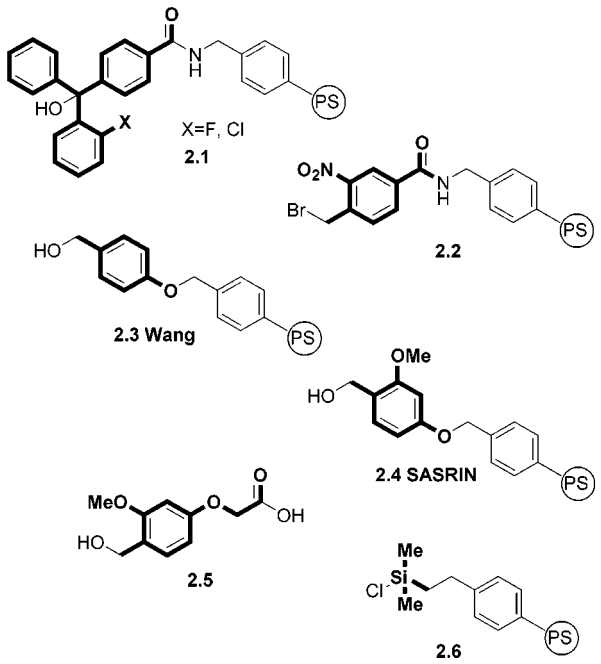
days of solid-phase synthesis. Thus, linkers such as the *o*-nitro-(α-methyl)bromobenzyl linker **1.1** prepared by Pillai[17] is a classic example of an integral linker. Its preparation was realized by functionalizing polystyrene/DVB resin with acetyl chloride/$AlCl_3$, reducing the resulting ketone and bromination of the resulting alcohol. The nitro group was then incorpo-

pared by Friedel−Crafts acylation of polystyrene with benzoyl chloride.[18] The resulting benzophenone derivative was transformed into the desired product by either reduction of an oxime, ammonolysis of the bromo derivative, or reductive amination with ammonium formate. The original chloromethylated polystyrene resin **1.3** used by Merrifield can in many respects be considered as an integral linker. This allowed Merrifield to anchor *N*-protected amino acids onto solid supports by formation of immobilized benzyl esters. The trityl linker **1.4** was developed by Leznoff[19] by lithiation of polystyrene and reaction with benzophenone and by Fréchet[20,21] by treatment of benzophenone-based polystyrene with phenylmagnesium bromide. Another light-cleavable bromine-derivatized linker **1.5** was obtained by functionalization of 2% polystyrene/DVB with 2-bromopropionyl chloride/$AlCl_3$ under Friedel−Crafts conditions.[22] Two percent cross-linked benzene sulfonyl chloride **1.6** was prepared from Dowex 50W ion-exchange resin (−SO_3H).[23]

The disadvantage with any integral linker is the control of synthesis, taking place as it does directly on the resin, with the whole range of steric and electronic effects having an influence over the synthetic outcome. The exact degree of loading and functionalization can be hard to control.

The majority of linkers used in solid-phase synthesis are thus of the nonintegral type (Scheme 2).

Scheme 2

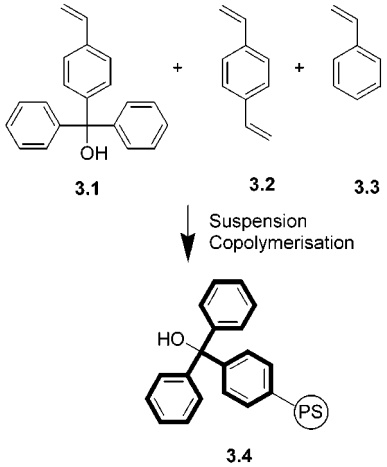


These can be loaded onto the resin and then derivatized or preloaded prior to attachment. This resin attachment is generally realized in one of three ways: (i) *Ethers*, (ii) *Amides*, and (iii) *C−C bonds*.

Thus, derivatives of the trityl linker **2.1** have been prepared in the nonintegral manner by attachment of 4-carboxy derivatives[24] through an amide bond. A light-cleavable linker *o*-nitrobenzyl (ONB) **2.2** was contrast to the integral linkers described above. The *p*-alkoxybenzyl alcohol Wang linker **2.3** was initially prepared by reacting 4-hydroxybenzyl alcohol with Merrifield resin in the presence of sodium methoxide.[26] The Sasrin linker **2.4** was first described by Mergler[27,28] and was initially anchored onto the resin by etherification. Sheppard[29,30] prepared the unloaded linker **2.5**, allowing attachment to an aminomethylpolystyrene resin. Dimethylsilyl chloride groups have also been attached to the polystyrene core through an ethylene bridge to give **2.6** by hydrosilylation of (vinyl)polystyrene.[31] Here again the resin core is not an integral part of the linker.

Linkers that are copolymerized into resin beads can be either of the integral or nonintegral type. Those which are not part of the polymer core can be considered as nonintegral (or grafted) in nature. Scheme 3 shows the preparation of a trityl (integral) linker **3.4** by suspension copolymerization of monomer **3.1**, DVB **3.2**, and styrene **3.3**.[32]

Scheme 3

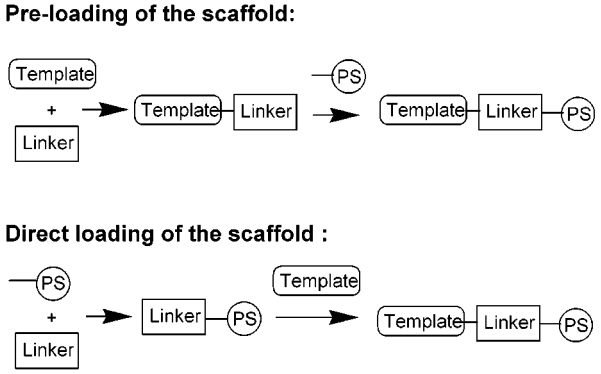


B. Scaffold Preloading and Direct Loading

The choice of which method, ***preloading*** or ***direct loading*** of the scaffold onto a nonintegral (or grafted) linker, is the most suitable for use in solid-phase synthesis is not clear-cut. The first method usually ensures much higher loading levels and that only purified materials are coupled onto the solid support and also reduces the number of solid-phase steps. The second method is usually less efficient since excess materials are often used in the coupling step, a problem if valuable scaffolds are being used, but is faster since no solution steps or purifications are needed (Scheme 4).

In addition, if all the derivatized sites on the loaded linker are not reacted, then undesirable side reactions can take place. There are certainly cases where linkers attached to resins have not been added cleanly and have given rise to numerous side reactions and impure products. Thus, the attachment of 4-hydroxybenzyl alcohol onto Merrifield resin to form the Wang linker using the original procedure described had to be improved in order to limit the side

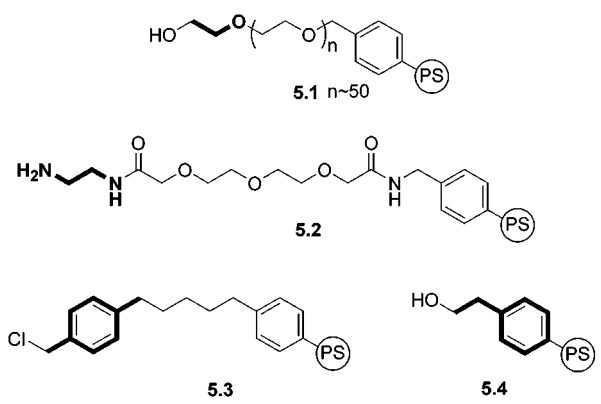
Scheme 4



C. Spacers

A group can be attached to the solid support to act as a spacer unit. The spacer has a number of roles. Principally, it acts to distance chemistry from the solid support and tailors the swelling properties of the resin materials to give more "solution-like" properties and better solvent compatibility. Typical examples of spacers are PEG chains (as in PS−PEG-based resins such as TentaGel **5.1** or resin **5.2**[34]) or alkyl chains as shown in **5.3**[35] (Scheme 5). Spacers

Scheme 5

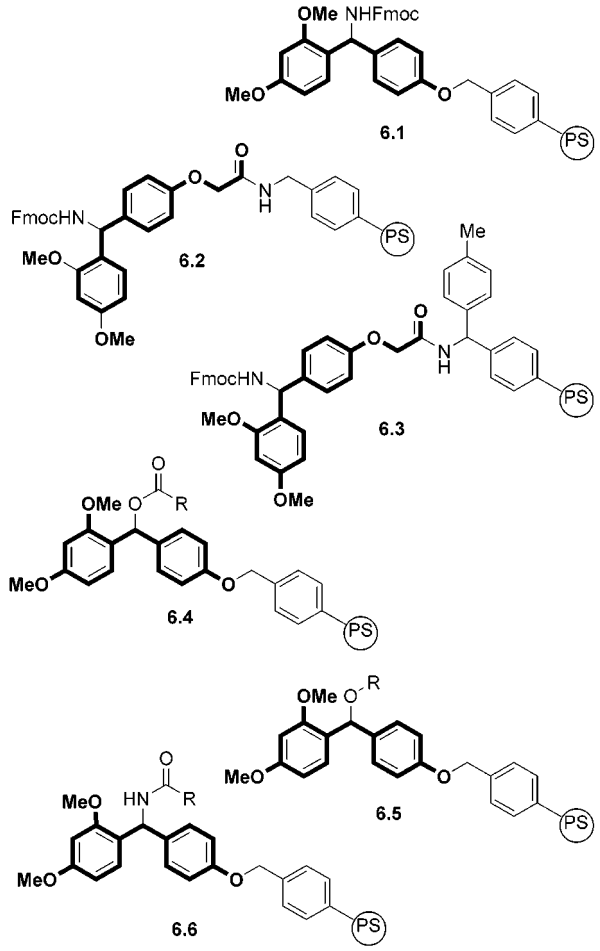


can therefore alter the cleavage properties of the linker, affecting resin swelling as well as complicating electronic effects. The extra methylene unit present in **5.4** compared to the classical hydroxymethylpolystyrene resin confers crucial properties such as acid stability,[36,37] although possibly sensitivity to β-elimination once loaded.

D. Linker Attachment

Although the core structure of the linker may remain unchanged, the group placed between the linker and the support can modify the cleavage conditions and also alter the degree of linker cleavage as well. An example has been illustrated with the Rink resin **6.1**.[38] High concentrations of TFA can sometimes cleave some of the Rink linker from the polystyrene support and introduce colored impurities into the cleaved product. The Rink amide AM **6.2** (RAM) and Rink amide MBHA **6.3** are much more stable to TFA (Scheme 6) (these constructs are made

Scheme 6



E. Leaving Groups and Scavengers

The Rink linker may be employed to attach a range of different functional groups to a common solid support, for example acids, amides, amines, etc. However, each functionality can be cleaved only under specific conditions. Thus, 0.1% TFA in CH_2Cl_2 will release acids[38] from linker **6.4** while 5% TFA in CH_2Cl_2 is needed to cleave alcohols from **6.5** and amides[40] from **6.6** (Scheme 6). Since the dimethoxybenzhydryl cation is generated in all of these cases, labilities must depend on the ease of protonation of the attached scaffold as well as its leaving ability. Thus, clearly, linkers are only one factor in determining "cleavability".

Another important factor, which can impede cleavage, is the reversibility of the reaction. This is certainly well-documented in solid-phase peptide synthesis (SPPS) where linker cation alkylation can be a serious problem, especially with peptides containing cysteines (thiols) or tryptophans (indoles), with the extent of alkylation being directly related to the proximity of specific residues to the linker. This is presumably also a problem in solid-phase organic synthesis (SPOS), although less well-documented with poor yields usually attributed to other factors. In the peptide area, scavengers are often used in order to trap the cationic linker species and prevent

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.