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Tools for efficient high-throughput synthesis

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Here, we detail the major developments in methods and techniques that are applicable to high-throughput synthesis that have evolved over the past five years, with an emphasis on the combination of microwave-based synthesis with techniques such as polymer-assisted purification and immobilized reagents and catalysts. Other aspects, such as automation, miniaturization and flow-based synthesis, are also presented as approaches that can be used for the rapid discovery and optimization of small organic molecules.

Introduction

High-throughput chemical synthesis has been a major field of interest and activity over the past decade because of its direct application to the drug discovery process (enhancing efforts in lead generation, optimization and development), and its applicability to the discovery of a wide variety of new materials (ranging from novel fuel-cell catalysts to new liquid crystal-based materials) [1]. Here, we present an overview of the main developments in high-throughput organic synthesis that have occurred over the past five years and focus on the development of several innovative tools and enabling techniques [2]. Specific emphasis is placed on the combination of microwave-based synthesis with techniques such as polymer-assisted purification and immobilized reagents and catalysts. However, other evolving areas, such as flow synthesis, including the recent innovation of continuous-flow hydrogenation, automation and miniaturization, are presented as systems that, in specific situations, will have a major role in the future of chemical synthesis.

Supported chemistry and work-up strategies

Since the first report on solid-phase synthesis by Merrifield in 1963 [3], this technique has shown its tremendous power with respect to the rapid synthesis of a variety of oligomers (i.e. peptides and DNA). Indeed, Merrifield's revolutionary developments in solid-phase synthesis offered perhaps the first insights into high-throughput synthesis by the transformation of a laborious solution approach (to prepare peptides) to a solid-phase variant that took hours rather than weeks. To illustrate Merrifield's achievements, none of the massive recent sequencing efforts (e.g. Ref. [4]) would have been possible without the ability to prepare synthetic DNA oligomers or primers, which is a direct result of solid-phase synthesis. Numerous traditional solution-phase protocols have been adapted to a solid-phase format and, in a particularly innovative approach, Houghten developed 'volatilizable' supports for the high-throughput synthesis of peptides (Figure 1a) [5]. In this approach, silica gel is functionalized initially using a 'volatilizable' linker before being used for traditional solid-phase synthesis of peptides. Treatment with hydrofluoric acid causes both the linker and support (SiF_4) to become volatile, and the remaining residue is the desired peptide.

Another area of supported chemistry that has gained interest is polymer-assisted solution-phase synthesis [6]. Although used for many years, it is only relatively recently that this has been used with such vigour in high-throughput organic synthesis [7]; this can be attributed to the major advantages of limiting the need for purification when using such supported materials. There are now many reports of this approach in high-throughput synthesis, including the synthesis of natural products [8]. One of the major advantages of polymer-assisted organic synthesis techniques is the cleanness of the work-up steps; in most cases, pure final product is recovered from reaction mixtures by simple filtration, but the ability to use mass action without purification is also important. This is possible because of the heterogeneous nature of supported reactants. However, this property is also the reason for some of the

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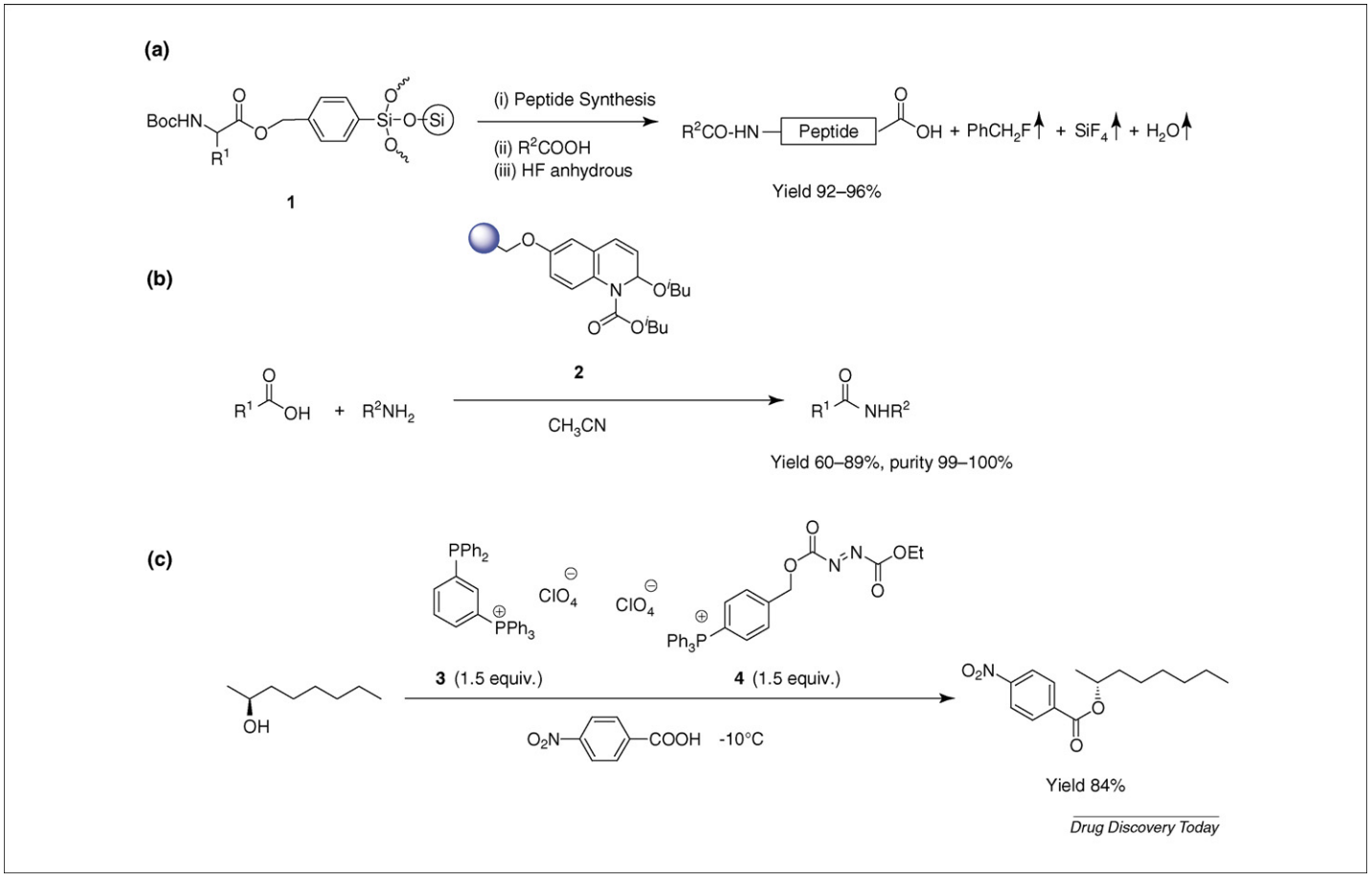

FIGURE 1

Supported chemistry. **(a)** Synthesis of *N*-protected peptides on a 'volatilizable' support **1**. The N-terminal Boc group was removed with trifluoroacetic acid (TFA) and protected amino acids were coupled using standard peptide synthesis chemistry (step i) to afford the support-bound peptide, that was subsequently N-acylated (step ii). In step iii the silica portion was converted to volatile SiF4 and the linker became benzyl fluoride which is also volatile by treatment with hydrofluoric acid (HF) [5]. **(b)** Polymer-assisted solution phase synthesis of amides using (PS-IIDQ) **2** [12]. **(c)** Mitsunobu reactions using 'ionic liquid tagged' diethyl azodicarboxylate (DEAD) **3** and phosphine **4** [13].

limitations of these techniques, such as compatibility issues (i.e. swelling and stability) and the practical problems of handling small amounts of beads in a high-throughput manner. Sealing the supported reactant in a defined space offers the possibility of automated, high-throughput purification, therefore, solid-phase extraction cartridges [9] and 'resin plugs' [10] are valuable tools. Many new supported reagents have been developed and applied, and many protocols for parallel synthesis that use supported reagents and scavengers have been developed. A recent example in the area of supported reagents is application of the Fukuyama reaction with the alkylation of primary amines using supported PS-thiophenol and Amberlite IRA-67 (free base) to cleave the activating sulphonamide, which avoids chromatographic purification steps [11]. Recently, Valeur *et al*. reported the synthesis and application of polymer-supported 2-isobutoxy-1-isobutoxycarbonyl-1,2-dihydroquinoline (PS-IIDQ) as a coupling reagent that does not require pre-activation (Figure 1b) [12]. PS-IIDQ is more efficient for general amide-bond formation than are many classic agents [e.g. *N,N,N′,N′*-tetramethyl-*O*-(7-azabenzotriazol-1-yl)-uronium hexafluorophosphate (HATU); phosphoric acid bis(2-oxooxazolidide) chloride (BOP-Cl); and (Benzotriazol-1-yloxy)-tripyrrolidinophosphonium hexafluorophosphate (PyBOP)]. Moreover, the supported reagent could be regenerated, yielding recycled polymer-supported IIDQ with a coupling efficiency similar to the original material.

Another approach, reported by Poupon *et al.*, was the development of 'ionic liquid tagged' reagents, such as triphenylphosphine and DEAD (Figure 1c) [13]. These 'tagged reagents' have been utilized in Corey-Fuchs reactions and, for example, in Mitsunobu chemistry, with all excess reagents and by-products removed by precipitation with ether.

In the past few years, much effort has been dedicated to developing several solid-supported catalysts. For example, Wang *et al.* [14] reported an efficient protocol for Suzuki coupling reactions using FibreCat (Pd immobilized on a 'fibre glass/wool type material'), which gives excellent conversions and offers ease of handling. Other recent approaches involve a microencapsulation-type process, where a variety of metals have been enveloped physically by thin films of polymer. This approach was first reported by Kobayashi, who encapsulated both osmium tetroxide and various Pd catalysts using several polymers including poly[4-(phenoxyethoxymethyl)styrene-*co*-styrene] (PEM) (Figure 2a) [15]. PEM-microencapsulated osmium has been used in the asymmetric dihydroxylations of olefins to give diols in good yields and high enantiomeric excess without contamination of the product with osmium tetroxide. The encapsulation also enables multiple re-use,

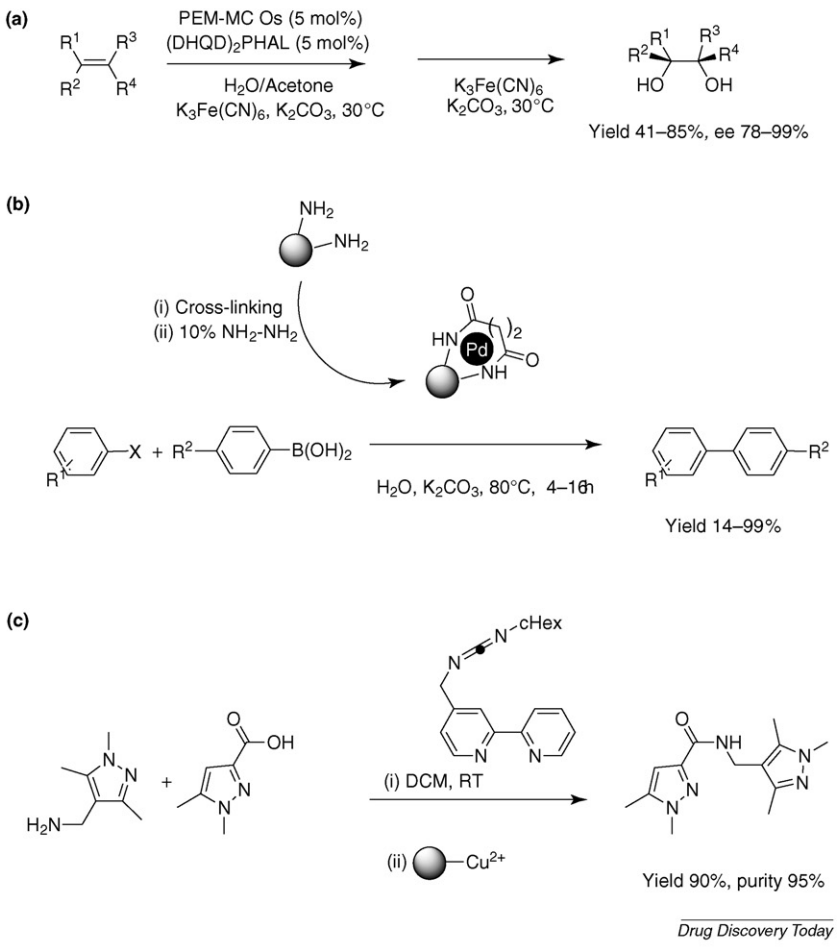

FIGURE 2

Polymer assisted solution phase synthesis. **(a)** Solution phase synthesis of asymmetric diols using encapsulated osmium **5** [15]. **(b)** Synthesis of biaryls using entangled palladium **7**. The resin-captured palladium **7** was prepared by treating a mixture of aminomethylated TentaGel resin **6** with palladium acetate (step i), the resin was subsequently filtered, treated with hydrazine hydrate to give Pd(0) (step ii), and then cross-linked. In step iii the entangled palladium **7** was employed in Suzuki couplings between a range of aryl bromides and aryl boronic acids [17]. **(c)** Scavenging using polymer-supported copper **9**. A bipyridine-modified carbodiimide **8** was employed in the first step to allow the coupling between the acid and the amine, the removal of the urea by-product was accomplished using the immobilised copper(II) **9** which has high affinity for the bipyridine moiety (step ii) [19].

flow-based synthesis and removes the toxicity issues associated with handling osmium tetroxide. A more recent example was reported by Baxendale *et al.* [16], who have successfully applied a microencapsulated palladium catalyst (Pd EnCat^{TM}) in a polyurea matrix to various Suzuki cross-coupling reactions under microwave irradiation in continuous-flow applications, furnishing multigram quantities of material. Cho *et al.* [17] have developed another approach in which the metal is not encapsulated, but is either entrapped or entangled within an existing polymer network such as aminomethylated Tentagel resin (Figure 2b). The 'trapped' palladium has been employed in Suzuki cross-coupling reactions using water as solvent and recycled many times without either loss of activity or evidence of leaching. Polymer-supported metals have also been used for scavenging [18]. Thus, Siu *et al.* [19] have developed work by others and reported a carbodiimide tagged with a 4,4′-bis(hydroxymethyl)-2,2′-bipyridine moiety that could be trapped on an immobilized Cu(II) resin (Figure 2c), in some respects this is analogous to fluorous tagging in that the 'bipyridine tag' can be used to manipulate and control the location of the chemical reagent. Fluorous separation methods continue to be used [20] because of the wide range of fluorous reagents, solvents and solid-phase extractors that are available [21]. Zhang *et al.* [22], for example, have reported the synthesis of piperazinedione-fused tricyclic compounds via a fluorous multicomponent reaction and fluorous solid-phase extraction to facilitate reaction and separation processes. In another article, Fustero *et al.* [23] reported the application of fluorous-(trimethylsilyl)ethanol (^{F}TMSE) as a new reagent for carboxylic acid tagging and protection in peptide synthesis.

High performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC) are the approaches that are applied widely to library purifications. A recent comparison of the two techniques did not demonstrate a clear supremacy between the two [24], whereas literature reports indicate that the combination of SFC and mass spectrometry (MS) has advantages in purification. Maftouh *et al.* [25] have achieved rapid enantiomer resolution of many compounds using SFC. The strategy has been evaluated using a set of 40, marketed, chemically diverse drugs,

with a success rate of 98%. Other approaches that have been reported to simplify reaction work-up include an innovative method for efficient liquid-liquid extraction that involves top layer organic extraction [26].

Microreactors and flow-based synthesis

Miniaturization is one of the most important trends in all fields of science. Decreasing the size of normal laboratory reactors can increase efficiency and safety with control of reaction conditions, including temperature, mixing and time [27]. Microreactors usually consist of a network of miniaturized channels from 10 μm to 300 μm in diameter etched into a flat surface called a 'chip', although larger diameter channels (up to 1 mm) offer a number of advantages (reduced blockage, greater availability etc.). Linked to the chip are different reservoirs that contain chemical reagents that are pushed inside the channel network by either a high-pressure pump or electroosmotic flow; by controlling the flow-rate, it is possible to define reaction times and the mixing of reagents [28]. Many examples of reactions carried out using microreactors have shown how these new devices can be excellent tools for synthesis

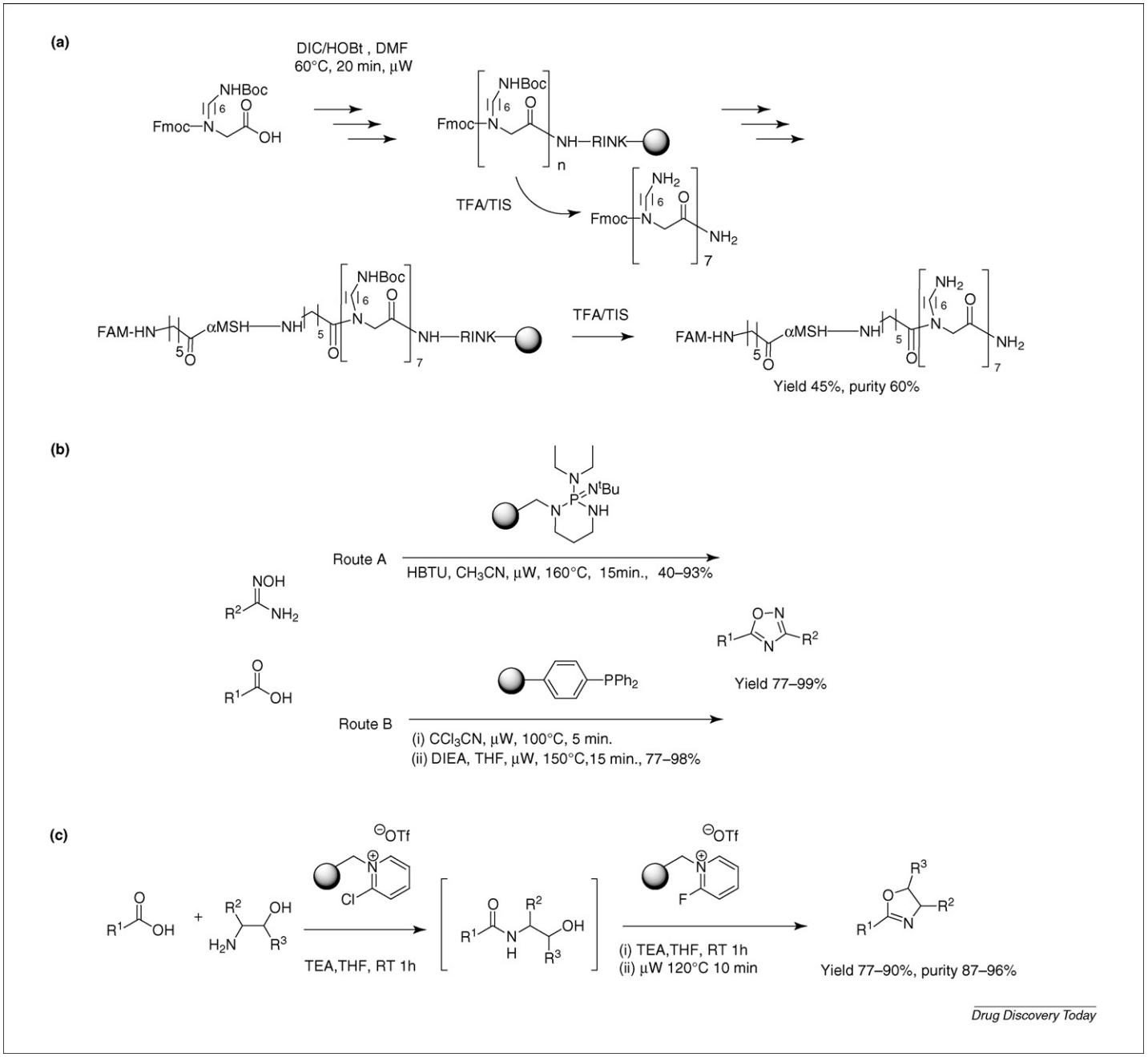

FIGURE 3

Application of microwave-assisted chemistry. **(a)** Solid-phase synthesis of a dye labelled peptide (α-MSH) conjugated to a heptapeptoid cell delivery agent **10** under microwave heating conditions [41]. **(b)** Microwave assisted synthesis of 1,3,4-oxadiazoles using polymer supported 2-tert-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (PS-BEMP) **11** and polymer supported triphenylphosphine **12** [43]. **(c)** Microwave assisted synthesis of 2,4,5-trisubstituted oxazolines using polymer-supported 2-chloro-*N*-pyridinium triflate **13** and polymersupported 2-chloro-*N*-pyridinium triflate **14** [44].

[29]. In most cases, products are obtained with high purities and yields, with short reaction times and in sufficient quantities to achieve full spectroscopic characterization [30–32]. Perhaps one of the most significant advances in this area, and one that is applicable to most practising organic chemists, is the, so-called, H-cube. Developed by Thales Nanotechnology (http://thalesnano.com), this device enables in-line hydrogenation via incorporation of hydrogen catalysts in a HPLC-type 'system' and flow through of reagents and *in situ* generated hydrogen, importantly using standard HPLC type tubing. Recently, Jones *et al.* [33] have combined this reactor with a liquid handler to generate an automated high-throughput hydrogenation system. The beauty of this system is its ease of use and general applicability, removing much of the risk and paraphernalia that is associated traditionally with laboratory-scale hydrogenation. In another development, Baxendale *et al.* [34] report the serial application of the Syrris AFRICA® mirofluidic system followed by the H-Cube® in the preparation of the natural product (±)-oxomaritidine.

Microwave heating and organic synthesis

Since the first report of microwave-assisted organic synthesis by the groups of Gedye [35] and Giguere [36], this technique has received considerable interest as a tool for accelerating and controlling reactions, and increasing yields. With the introduction of controlled, precise microwave reactors, it has been used in laboratories as an alternative to oil baths in an increasingly wide range of organic transformations [37–39]. The advantage of microwave technology in terms of heating can be applied to high-throughput techniques, such as solid-phase synthesis and polymer-assisted solution-phase synthesis.

Solid supports and microwave heating

The combination of solid phase organic synthesis and microwave technology has emerged as a powerful tool in recent years. Peptides have been synthesized almost exclusively on solid supports at room temperature, although there are a few reports regarding the advantages of solid-phase peptide synthesis using microwave irradiation. Recently, Bacsa *et al.* [40] have reported the solid-phase synthesis of a nona-peptide under microwave conditions in which the alternation of short pulses of microwave irradiation with cooling of the reaction vessel enabled the synthesis of peptides in high purity compared to standard conditions. Fara *et al.* [41] described the synthesis of fluorescence resonance energy transfer (FRET)-peptides and FRET-peptoids using *N,N′*-diisopropylcarbodiimide/1-Hydroxybenzotriazole as the coupling reagents under microwave irradiation; the method labels peptides with a variety of fluorophores and quenchers in high yields and with purities of >90% (Figure 3a).

Supported reagents and scavengers and microwave heating

The combination of microwave chemistry and polymer-assisted solution-phase synthesis has attracted much interest and been used in many chemistry transformations over the years [42]. Wang *et al.* [43] described the synthesis of 1,3,4-oxadiazoles using two different solid-supported reagents, PS-BEMP and PS-PPh_3, to give the desired cyclized products with >98% purity following a simple filtration to remove the solid-supported reagents (Figure 3b). Crosignani *et al.* [44] reported the development of a polymer-supported 2-chloro-*N*-pyridinium triflate (Mukaiyama reagent) (Figure 3c), which has been used, for example, in the synthesis of 2,4,5-trisubstitute-2-oxazolines.

Microwave-assisted, continuous-flow organic synthesis

Microwave technology has been combined with continuous-flow synthesis to avoid the use of traditional heating systems [45], and it can be applied to automated pathways to enable multigram reaction scale-up. Comer *et al.* [46] have developed a microreactor system that consists of a glass microreactor block placed in a microwave cavity and connected to an external syringe pump via Microtight™ fittings. Capillary tubes coated with Pd films [47] (200–1200 μm) were used in Suzuki-Miyaura and Heck reactions, giving 100% conversions after 4–5 min at 200 °C. Microwave irradiation is important to achieve the rate enhancements and to mix the reagents in the capillary channel. Using this kind of device, parallel synthesis could be performed by simultaneously pumping reagents through the inlets into the mixing chamber.

Conclusions

Polymer-assisted solution-phase synthesis and solid-phase synthesis have demonstrated their worth as important tools for high-throughput synthesis over the past decade, with the simplification of work-up procedures and automation responsible for their success. Here, we have discussed how these two tools has been combined successfully with microwave technology (instead of traditional heating systems) to create several new technology platforms. A new trend is the combined use of flow reactors and support catalysts, with miniaturization offering many advantages in terms of productivity, safety and cost. Microreactors can be interfaced easily with microwave and analytical-detection techniques, and these new hybrid techniques offer much, as high throughput synthesis techniques continue to evolve and mature.

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