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Review

Solid-phase extraction for combinatorial libraries

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Abstract

Solid-phase extraction (SPE) has during the last three years emerged as a convenient method for the purification of compound libraries prepared by solution synthesis. The widespread use of SPE in combinatorial chemistry can be explained by straightforward SPE method development facilitated by the availability of numerous commercial SPE resins. High-speed automated SPE is readily accomplished by taking advantage of commercial laboratory robot systems. The present review summarizes and discusses advancements made in the use of different SPE resins and molecule tagging techniques for optimization of ion-exchange, reversed-phase, normal-phase and fluorous-phase SPE in combinatorial chemistry.

Keywords: Review; Solid-phase extraction; Combinatorial chemistry; Synthesis

Contents

1. Introduction

The development of combinatorial chemistry [1–3] has had a tremendous impact on the drug discovery process. The use of combinatorial techniques is today well-established and most, if not all, pharmaceutical companies, as well as many academic research groups, apply combinatorial techniques to their work. Combinatorial chemistry is a technique not only useful in drug discovery processes, but also attractive to all disciplines of chemistry where large numbers of compounds are desirable. In recent years combinatorial techniques have spread to research involving for example reaction condition optimization, material science, and discovery of catalyst or catalyst ligands.

The Nobel-prize rewarding solid-phase peptide synthesis introduced by Merrifield [4] provided a tool necessary for the development of combinatorial

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chemical synthesis; rapid and simple purification by means of filtration. As a consequence, the first compound libraries made were large peptide libraries [1] sometimes containing more than a million members. It was soon realized that combinatorial chemistry would be a valuable technique for preparing non-peptide and non-oligomeric libraries of any potentially biologically active molecules and reports on combinatorial solid-phase synthesis of such libraries appeared in 1992 [5,6].

However, the application of traditional solution-phase organic reactions onto solid-phase is not straightforward. Different reaction rates, yields and stereo- and regiochemical outcomes are commonly encountered when a solution organic synthesis is transferred onto solid-phase. Furthermore, heterogeneous reagents or catalysts are less efficient in combination with solid-phase chemistry, rendering the development of solid-phase combinatorial organic synthesis a rather time-consuming task. Consequently, methods for performing combinatorial/parallel synthesis in solution were highly desirable, because;

1. Solution-phase combinatorial chemistry can take advantage of a wealth of organic reaction that has already been carefully optimized and very little time has to be spent on finding and optimizing reaction conditions.
2. Reaction scale is not limited by bead loading capacity.
3. Solution-phase synthesis does not require complementary functionalized substrates (i.e., orthogonally cleavable linkers attached to a solid support). Linker introduction, resin attachment and cleavage introduce additional reaction steps.
4. Solution-phase reaction monitoring is straightforward [thin-layer chromatography (TLC) gas chromatography (GC), high-performance liquid chromatography (HPLC), etc.] as compared to solid-phase reaction monitoring.
5. Purification of solution-phase synthesis intermediates is possible, which implies that each step in a sequence does not have to be high-yielding in order to give a pure final product. (Solid-phase chemistry relies on that each synthetic step is high-yielding, since by-products accumulate during a multi-step sequence. For example, a four-step solid-phase sequence giving 97% yield in each step, gives a final product of 89% purity with the consequence that HPLC purification is necessary in many cases).
6. Solution-phase allows convergent combinatorial synthesis.

However, a serious drawback of solution-phase combinatorial synthesis, as compared to solid-phase, is that it leaves the chemist without a simple and standardized high-throughput purification method. Consequently, several different novel and innovative methods for rapid and simple parallel purification of solution-phase combinatorial libraries have been suggested, including resin capture [7,8], scavenger resins [9], liquid-phase organic synthesis [10], liquid–liquid extraction [11–18], and solid-phase extraction (SPE). The present review discusses advancements made in the use of solid-phase extraction as means for purification of synthetic compound libraries.

SPE is particularly suited for purification of solution-phase combinatorial libraries, because solution-phase combinatorial synthesis together with SPE purification, combines advantages of solid-phase with those of solution-phase chemistry. The actual reaction is performed in solution and the product (or excess of reagent) is attached to a solid phase *after* completion of the reaction via a *non-covalent* and *reversible* interaction allowing purification by simple washing of the resin. Furthermore, commercial equipment for automation and robotization is available from several suppliers and the market offers, apart from bulk resins, ready-to-use SPE resins in various formats, such as pre-packed columns, cartridges, disks or pads.

The combinatorial reaction and SPE protocol can be designed according to two principles; either the desired product or by-products and excess unreacted reagent are extracted onto the resin (Fig. 1). Reports concerning both approaches are common and are discussed in the present review. Furthermore, different formats for performing SPE for combinatorial chemistry have been described: (i) addition of a SPE resin to the crude reaction mixture, followed by stirring and filtration and (ii) charging the crude reaction mixture onto SPE columns or cartridges, followed by washing and elution of products. The latter format has been used in automated SPE using laboratory robotics, which is of particular interest in

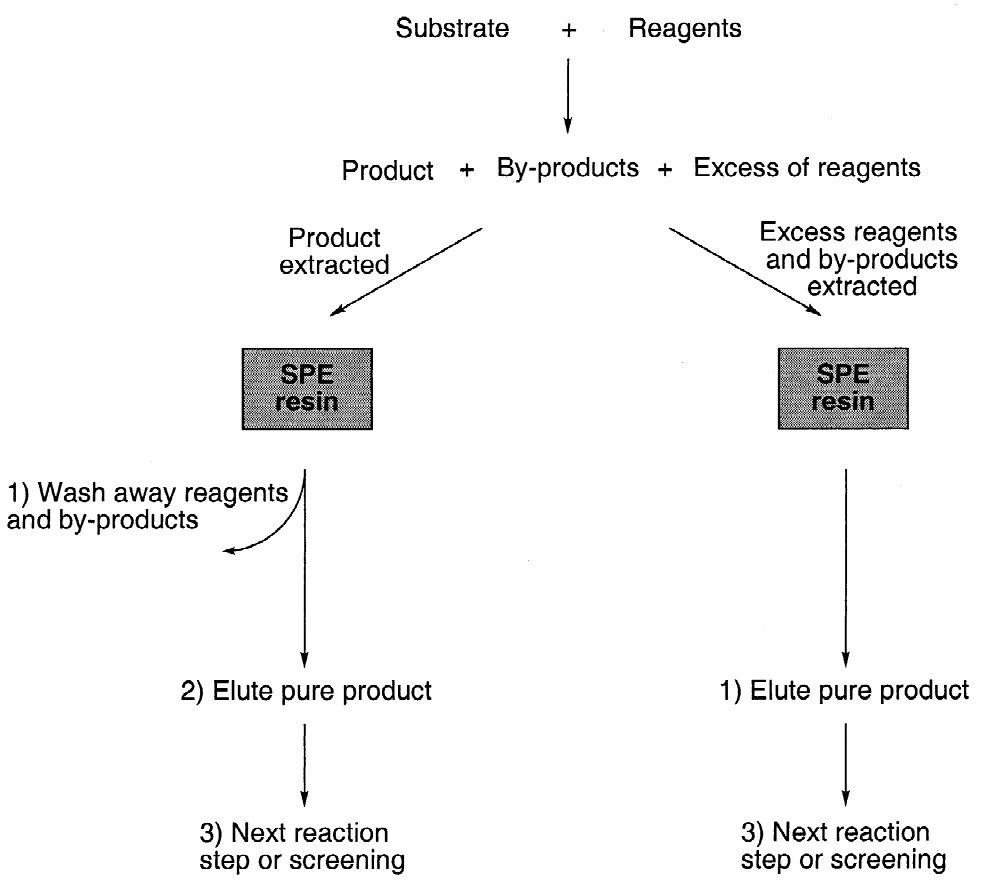
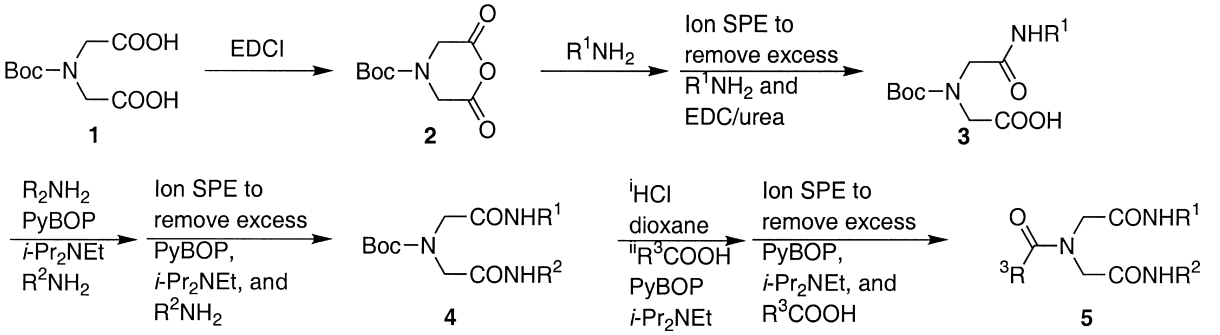


Fig. 1. Solid-phase extraction in combinatorial chemistry and parallel synthesis can be designed to extract reagents and/or by-products, as well as to extract the desired products.

combinatorial chemical synthesis where many reactions are handled simultaneously.

2. Ion-exchange SPE

Ion-exchange SPE has found widespread use as purification method in solution-phase combinatorial chemistry. It has been used to remove ionic reagents or by-products, as well as to selectively extract ionic products out of crude mixtures. Boger and co-workers have in a series of reports on solution-phase synthesis [11–18] convincingly demonstrated that acid–base liquid–liquid extractions and ion-exchange SPE purification are powerful purification techniques for parallel synthesis. In an early report [12], libraries of dipeptidomimetics were prepared via a three-step sequence involving (i) opening of *N-tert*-butoxycarbonyl (Boc)-protected iminodiacetic anhydride **2** with an amine (R^1NH_2) to generate a carboxylic acid **3**, (ii) followed by PyBOP (benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate)-mediated peptide bond formation with a second amine (R^2NH_2), and finally (iii) Boc removal and a PyBOP-promoted peptide bond formation with a third amine component (R^3NH_2) to give the library **5** (Scheme 1). Following each of the two first reactions, removal of excess of amines and the amino-functionalized carbodiimide (EDC) was accomplished by ion-exchange SPE. The third reaction left neutral products **5**, which also could be purified from excess of amine and carboxylic acid



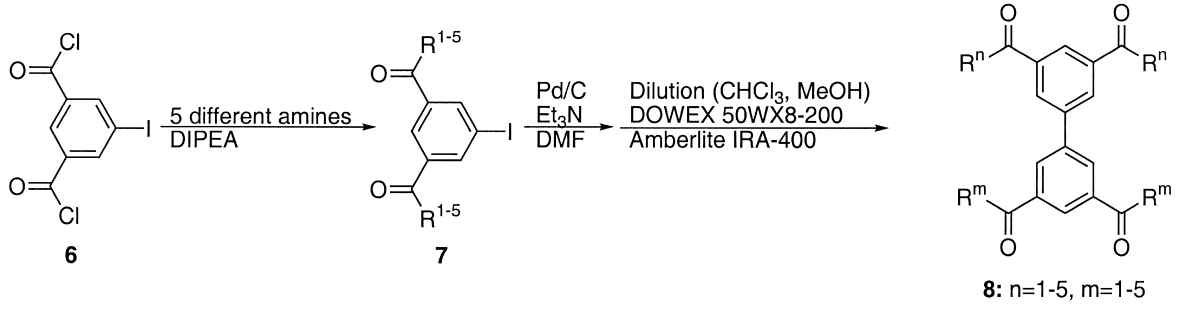
Scheme 1.

using ion-exchange SPE. Each library member was isolated in 30–150 mg amount and in purities of >90%.

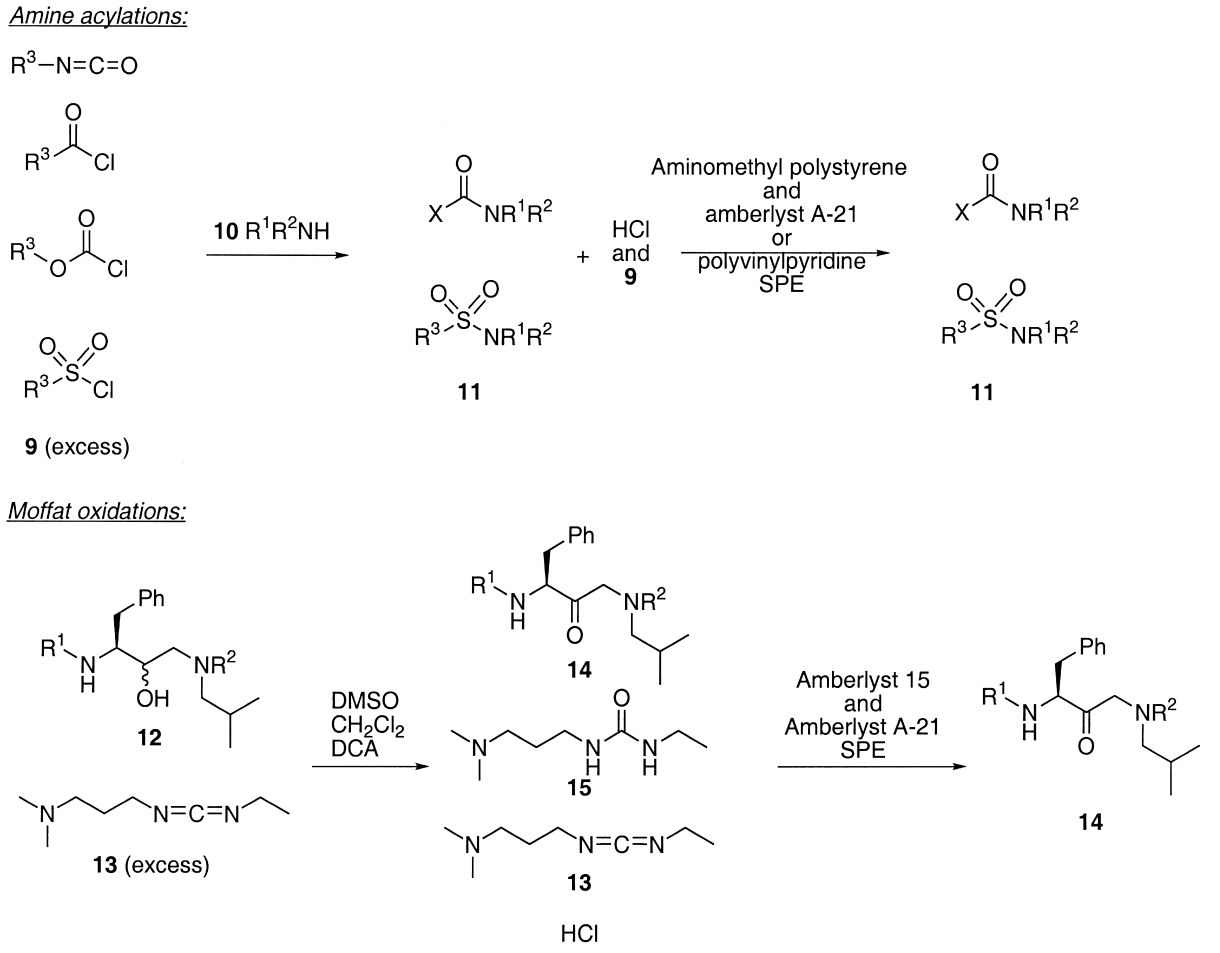
The research group of Boger later extended this methodology to prepare libraries of di-, tri- and tetramers of iminodiacetic acid diamides by PyBrOP/*i*-Pr_2NEt-promoted cross-linking of various iminodiacetic acid diamides with excesses of dicarboxylic acids [16]. It was found that removing diisopropylethylamine and excess of starting amines from the crude reaction mixtures was better done using acidic ion-exchange SPE (Dowex 50W-X8-400) than using conventional acid–base liquid–liquid extraction (especially in cases involving more hydrophilic products).

In a more recent publication, Boger et al. turned their attention towards solution synthesis of biaryl libraries by Pd/C catalyzed coupling of iodoarenes (Scheme 2) [18]. Reaction of the diacid dichloride **6** with five different amines furnished five pure diamides **7** after liquid–liquid extractive work-up. The diamides were coupled in the presence of Pd/C to afford a mixture of 15 biaryls **8**. Crude reaction mixtures containing **8** were diluted with chloroform and methanol and strongly acidic Dowex 50W-X8-200 and strongly basic Amberlite IRA-400 resins were added in order to remove triethylamine and hydrogen iodide, respectively. Filtration and concentration furnished pure biaryls **8** (32 mg) in 75–95% yields. Purification by means of liquid–liquid extraction or filtration through a pad of silica proved less general than SPE. It is noteworthy that two ion-exchange resins having chemically incompatible functionalities, the strongly acidic (Dowex 50W-X8-200) and the strongly basic (Amberlite IRA-400), could be used simultaneously. Physical isolation of the different reactive functionalities of the two resins prevented reaction between them.

Procedures for removing by-products and excess reagents from parallel amine acylations, Moffat oxidations and alkyllithium/Grignard additions to aldehydes have been detailed by Flynn et al. [19] (Scheme 3). The hydrochloric acid formed in reactions of amines **10** (0.1 mmol) with acyl chlorides,



Scheme 2.



Scheme 3.

chloroformates, or sulfonyl chlorides **9**, was removed by using Amberlyst A-21 or polyvinylpyridine and unreacted excess of acylating reagent was covalently scavenged with aminomethyl polystyrene, which generated a 12-member library (**11**) in 50–100% individual yields and >95% purities.

The methodology was then further extended to Moffat oxidations of hydroxyethylamines (Scheme 3). The oxidation urea by-product **15**, as well as unreacted excess of EDC **13**, were tagged with a tertiary amine functionality and could be removed using SPE with the sulfonic acid resin Amberlyst 15, while HCl was removed with the tertiary amine resin Amberlyst A-21 to give the ketones **14**. Again, two

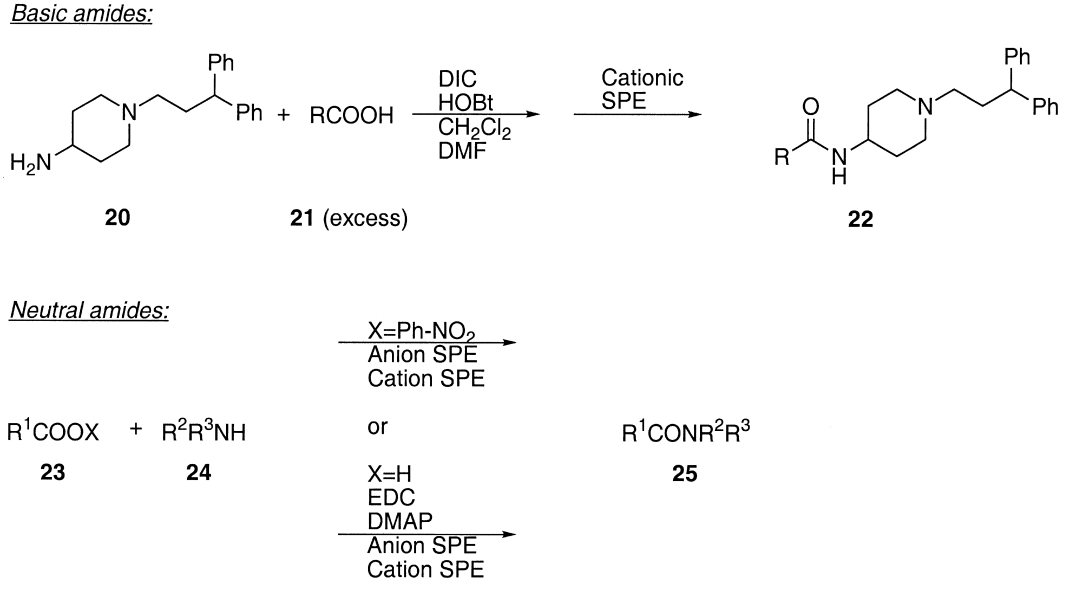
resins carrying chemically incompatible functionalities could be used to simultaneously remove a tertiary amine and HCl.

In yet another example, Flynn's group showed that SPE is a valuable technique for purifying compound libraries prepared by using organometallic chemistry (Scheme 3). Following the addition of excess of butyllithium or allylmagnesium chloride **16** to aldehydes **17**, a carboxylic acid-functionalized resin, Amberlite IRC-50S, was added to protonate excess organometallic reagent **16** and alkoxide products **18**. Filtration and concentration afforded alcohols **19** in 75–97% yields and >95% purities on a 0.5 mmol scale.

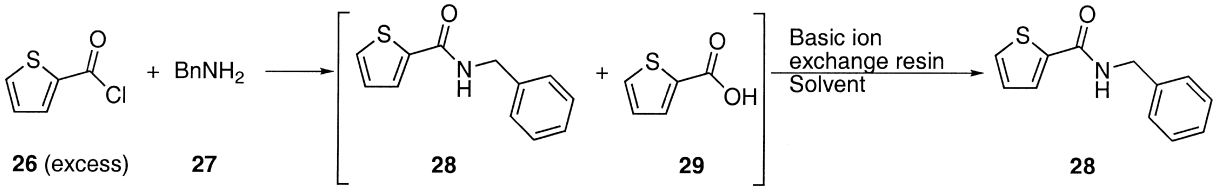
Similar approaches have been disclosed by the same group for removing excess reagents and by-products from syntheses of heterocyclic carboxamides [20]. Furthermore, tetrafluorophthalic anhydride has been suggested as a reagent to convert amines of low reactivity (thus being difficult to extract by acidic ion-exchange resin) into carboxylic acids that are readily extracted with a polyamine ion-exchange resin [21].

In the early reports concerning ion-exchange SPE described above, by-products and/or excess reagents were extracted from the crude product mixtures. Examples of extracting the library *product* in a fully automated setup using robotics (Zymark Benchmate Robotic Workstation) and ion-exchange SPE for purification of compound libraries were disclosed by Lawrence et al. [22]. In a first example, the diamine **20** was acylated with excess of carboxylic acids **21** in the presence of DIC (diisopropylcarbodiimide) and HOBt (1-hydroxybenzotriazole) to ensure complete consumption of **20** (Scheme 4). The crude product mixtures, containing the amides **22** as the only cationic components, were applied onto sulfonic acid strong cation-exchange (SCX) (Varian) SPE cartridges, which then were washed with methanol and 0.1 *M* ammonia in methanol to remove unreacted excess of **21** and urea by-product. Pure amides **22** (88–98%, HPLC) were subsequently eluted in high yields (70–95%) with 1 *M* ammonia in methanol.

Lawrence et al. further developed their automated protocol to perform 25–100 reactions in parallel to obtain more than 150 neutral amides on 25–300 mg scale (Scheme 4). Reaction of *p*-nitrophenyl esters **23** with amines **24**, left the product amides **25**, unreacted excess of amines **24**, and *p*-nitrophenol as the only components in the crude mixture. Excess of **24** and *p*-nitrophenol were extracted by sequential automated SPE with strong anion-exchange (SAX) cartridges (Varian), followed by cationic SCX cartridges, furnishing amides **25** in yields of 63–89%



Scheme 4.

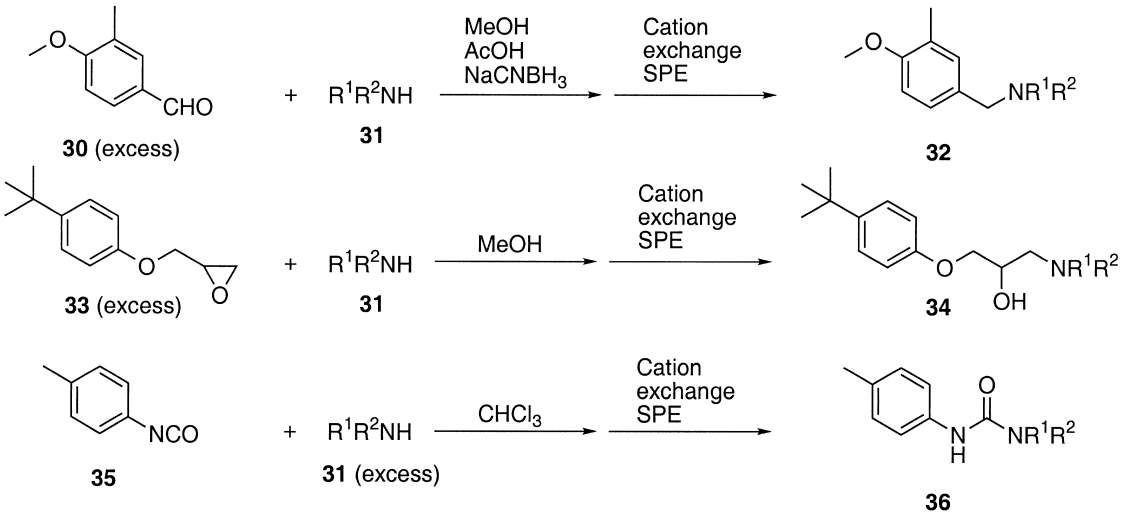


Scheme 5.

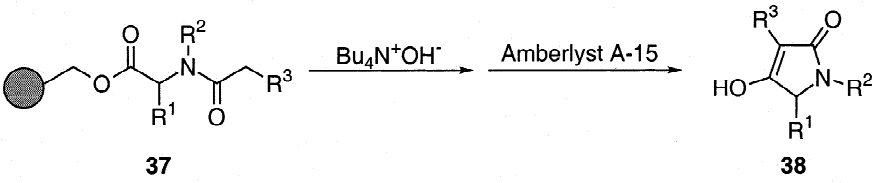
and purities of 92–96% (HPLC). Alternatively, carboxylic acids **23** were activated with the amino-functionalized EDC and DMAP (4-dimethylamino-pyridine) to promote reaction with amines **24**. Automated SPE with SCX cartridges efficiently removed excess **24** and EDC, as well as DMAP and the urea by-product derived from EDC to give amides **25** in 53–94% yields and 89–99% purities (HPLC).

Gayo and Suto compared the efficiency of nine different basic ion-exchange resins in extracting the carboxylic acid by-product **29** formed in solution-phase reactions between an excess 2-thiophene carbonyl chloride **26** with benzylamine **27** [23] (Scheme 5). The extraction conditions were combinatorially optimized in 96-well format by varying the ion-exchange resin, the solvent (EtOAc, CH_2Cl_2 and THF), and the addition order of the resin (before or after reaction). The purity of the product **28** was >95% when resins were used to remove the acid **29** from the reaction mixture, whereas the purity of **28** averaged 61% when no resin was used. Furthermore, it was concluded that addition of the weakly basic Amberlite IRA-68 prior to reactions performed in EtOAc provided the highest yield and purity of the amides **28**. The optimized conditions were subsequently validated by synthesis of a nine-member amide/ester library in yields of 84–100% and purities of 98–99%.

Fully automated ion-exchange SPE procedures on a Hamilton Microlab 2200 robot for purification of parallel reductive aminations of an aldehyde (**30**→**32**), opening of an epoxide by amines (**33**→**34**), and amine acylations with an isocyanate (**35**→**36**) have been published [24] (Scheme 6). Reaction conditions were designed to ensure that only the products or excess reagents were left as the only ionizable components after completion of the reactions. Excess aldehyde **30** was used in reductive aminations and excess epoxide **33** was used in epoxide openings to leave the amines **32** and **34** as



Scheme 6.



Scheme 7.

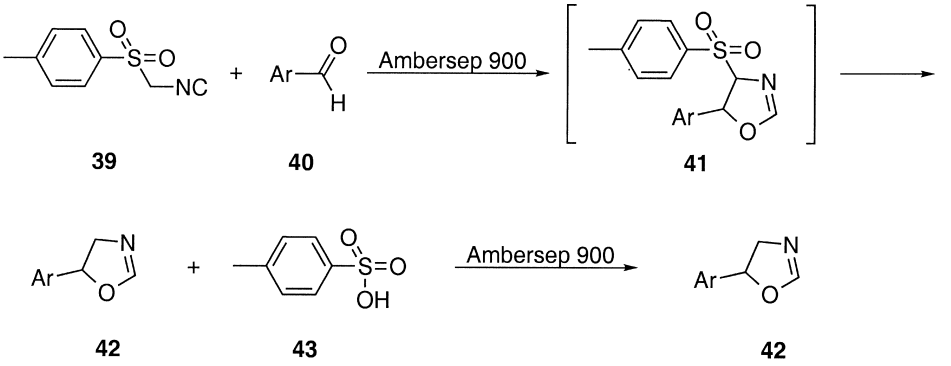
the only ionizable entities, while excess of amines **31** was used in isocyanate acylations. Following completion of reactions, the ionizable products or unreacted reagent excess were extracted by automated SPE on cationic SCX (Varian) cartridges. In protocols where products (**32** and **34**) were extracted by the SCX resin, elution with 2 *M* ammonia in methanol was necessary to give the final library. The products **32**, **34** and **36** were thus obtained in yields of 71–92% in purities of 80–99% (HPLC) on 30 mg scale. A similar purification protocol was later applied to the parallel synthesis of 48 ethanolamines in large amounts (up to 50 mg) and high purities (75–93%) via opening of epoxides with neopentyl amines [25].

Deegan et al. have found that using DDQ (2,3-dichloro-5,6-dicyanobenzoquinone) for oxidative cleavage of alcohols attached to Wang resins gave higher yields than simple TFA treatment. Excess DDQ present in the crude product mixture was reduced to DDQH (2,3-dichloro-5,6-dicyanohydroquinone) using a solid-supported ascorbic acid resin prior to removal of DDQH by SPE on a Amberlyst A-26 (HCO_3^-) resin [26].

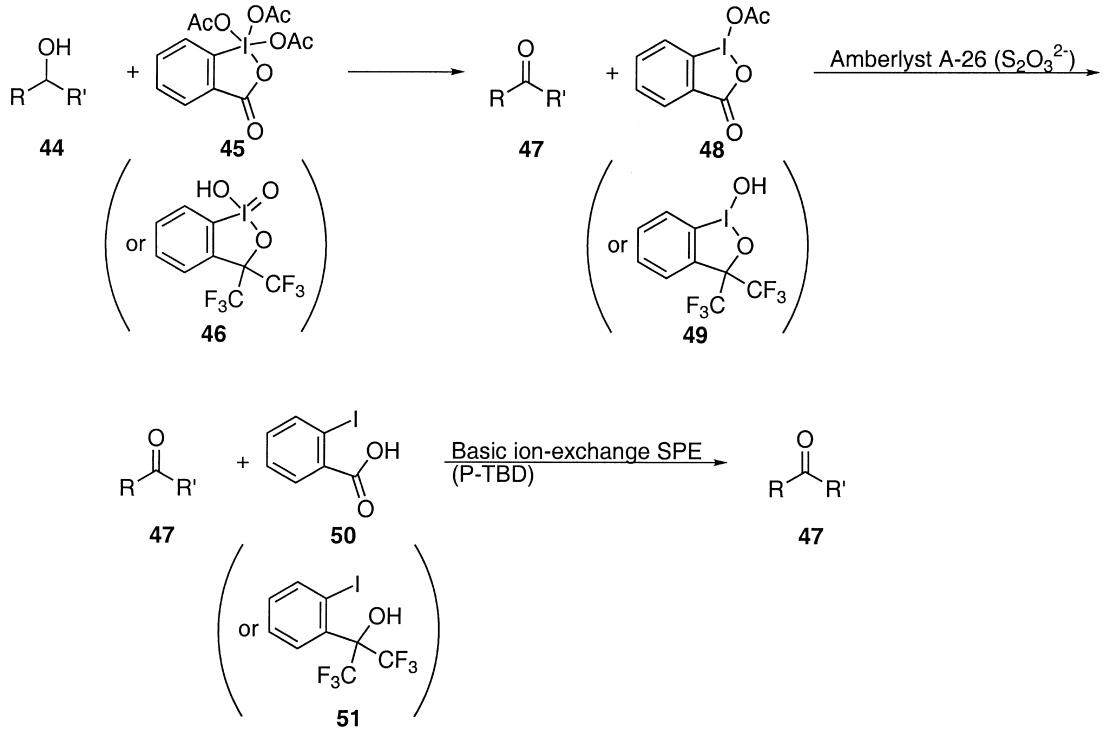
Synthesis of the tetramic acid library **38** was accomplished by tetrabutylammonium hydroxide treatment of *N*-acyl amino acid attached to a Wang resin **37** [27] (Scheme 7). The use of tetrabutylammonium hydroxide as the base was preferable, since it was readily extracted using the acidic Amberlyst A-15 resin, leaving the products **38** in 60–96% purity (HPLC).

The basic Ambersep 900 resin served a dual function in the solution-phase parallel synthesis of oxazoles **42** via addition of *p*-tolylsulfonylmethyl isocyanide **39** to aromatic aldehydes **40** [28] (Scheme 8). The basic resin first catalysed the addition reaction, then extracted the sulfinic acid by-product **43** from the crude reaction mixture. Filtration yielded 5-aryloxazoles **42** in 54–85% (57–94% pure, HPLC) on >0.1 mmol scale.

Recently, Parlow et al. disclosed the combined use of a solid-phase reagent and ion-exchange SPE for purification of solution-phase parallel periodinane oxidation reactions [29]. Following oxidation of an alcohol **44** with Dess–Martin periodinane **45** or Grieco–Dess–Martin periodinane **46**, a thiosulfate resin derived from Amberlyst A-26 was added to



Scheme 8.



Scheme 9.

reduce the reagent by-products **48** and **49** to I(I) species **50** or **51** (Scheme 9). The I(I) species **50** and **51** carry acidic protons and could subsequently be extracted from the reaction mixture using basic ion-exchange resins [preferably a P-TBD (1,5,7-triazabicyclo[4.4.0]dec-5-ene) resin] to give pure ketones and aldehydes **47** (>80% purity) in good yields (65–98%) on >10 mg scale.

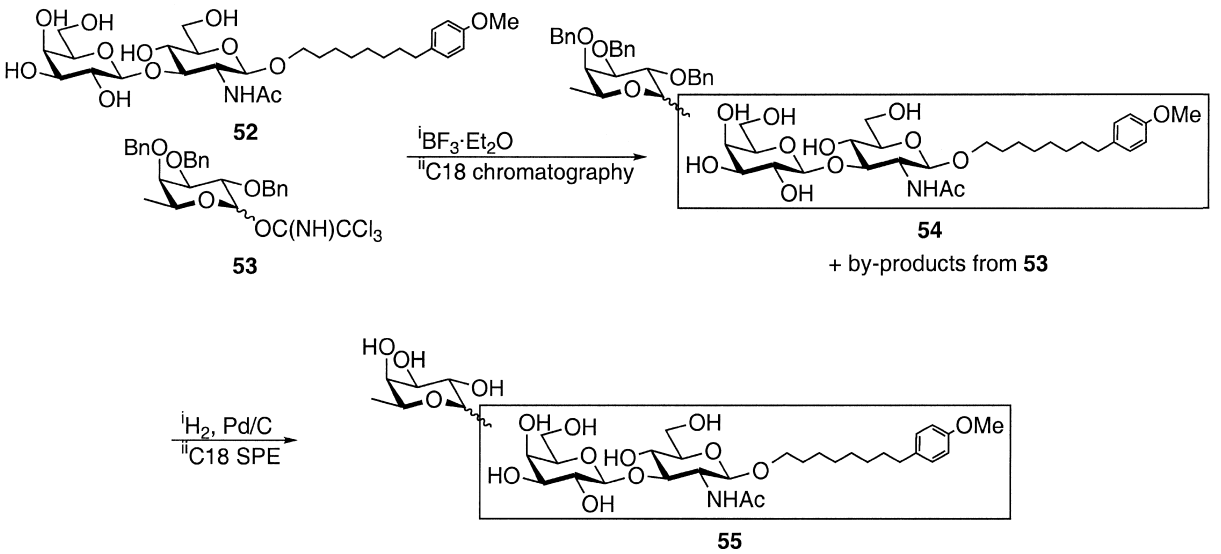
Finally, solid-supported organic bases have been used as catalysts for the parallel synthesis of aryl ethers from phenols and alkyl halides [30,31]. The solid-supported organic bases served a dual function in the aryl ether syntheses described, since they activate the phenol for nucleophilic attack on alkyl halides, as well as extract by-products (hydrogen halides) from the crude products.

3. Reversed-phase SPE

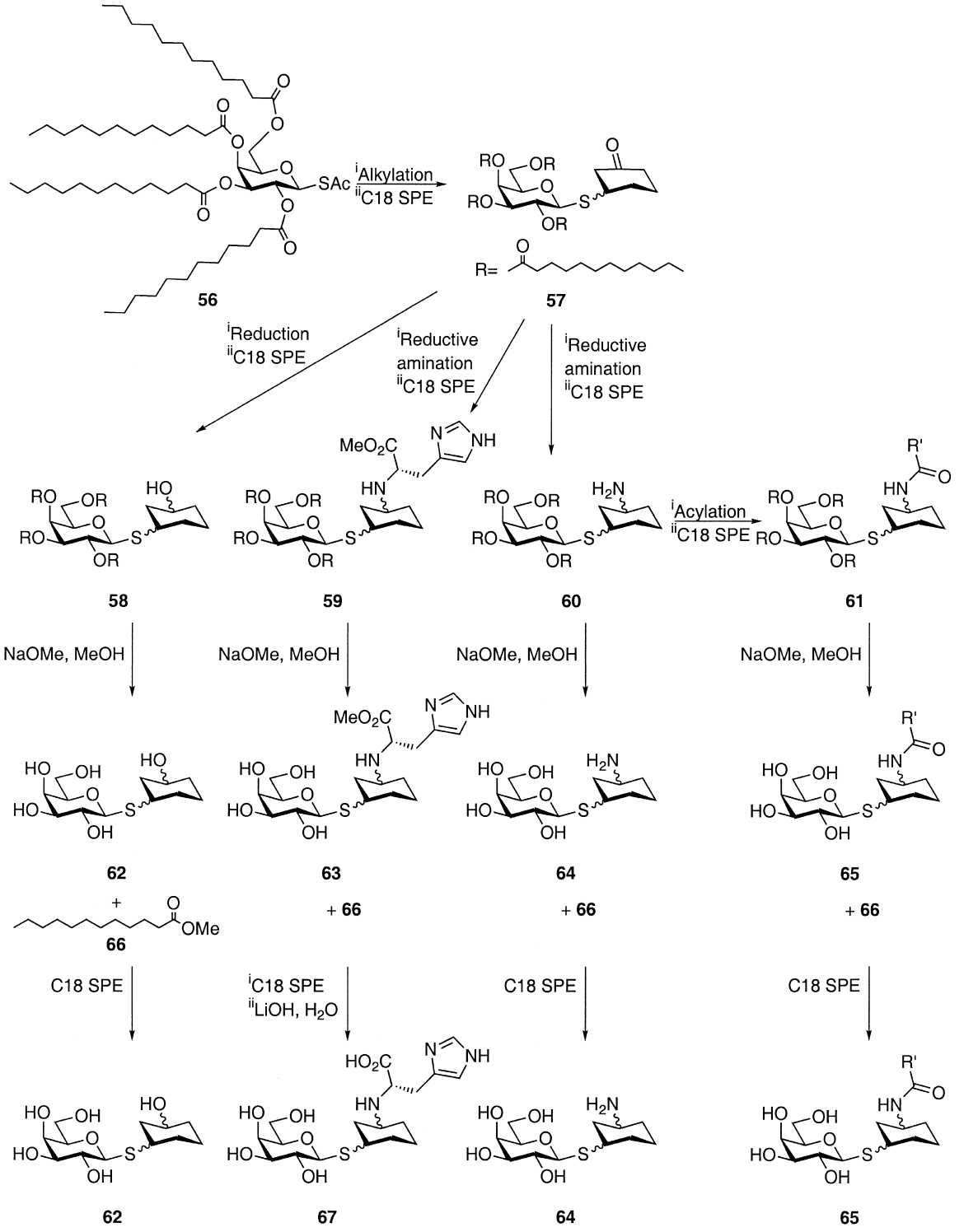
The use of reversed-phase SPE in combinatorial chemistry has only been sparingly reported, which is somewhat surprising since the efficiency of reversed SPE in purification/isolation of compounds from complex mixtures is generally appreciated. However, hydrophobicity is generally not a property linked to the intrinsic nature of reactions and the substrate or reagents must be hydrophobic in themselves or *hydrophobic tags* have to be introduced. [This is in contrast to the examples on ion-exchange SPE described above, where many reactions involve components ionic by nature (reagents, by-products, or products) that could be selectively extracted with ion-exchange resins. Consequently, ion-exchange SPE rapidly and quite naturally established itself as a technique for high-speed purification of solution-phase parallel synthesis]. Three requirements on a hydrophobic tag have to be fulfilled: (1) the tag has to be hydrophobic enough to ensure efficient solid-phase extraction independent of the reaction conditions. (2) The tag has to be chemically inert under the reaction conditions used. (3) The tag has to be easily removed from the final product after being cleaved off.

The use of hydrophobic tags in order to simplify purification of compound libraries was reported by Hindsgaul and co-workers in their syntheses of carbohydrate libraries via random glycosylations [32,33]. One example is depicted in Scheme 10, where glycosylation of an unprotected disaccharide acceptor **52**, permanently tagged with a hydrophobic aglycon (8-*p*-methoxyphenyloctyl), with the trichloroacetimidate donor **53** gave a mixture of all possible trisaccharides **54**. Reversed-phase chromatography was used to remove unreacted **52** and minor amounts of tetrasaccharides. Hydrogenolysis of benzyl ethers afforded the desired trisaccharide library **55** containing only by-products derived from the donor **53**. The library members **55** were present as 8-*p*-methoxyphenyloctyl glycosides, which permitted their selective extraction from aqueous solution onto cartridges containing a C_{18} resin. By-products derived from the donor **53** did not carry any hydrophobic tag and could be washed away with water, followed by elution of the pure trisaccharide library **55** with methanol. The hydrophobic tag used in this work was left attached to the final trisaccharide products **55**.

Nilsson and co-workers later reported a procedure involving the use of temporary hydrophobic tags for high-speed purification of parallel synthesis of thioglycosides [34,35]. Saccharide hydroxyl groups were used as attachment sites for hydrophobic tags by simply protecting them as lauric acid esters (Scheme 11). Thus, the 2,3,4,6-tetra-*O*-lauroylated thioacetate **56** was in situ activated with diethylamine or piperidine in parallel Michael addition reactions and nucleophilic substitutions of α-chloro ketones using excess of electrophilic reagent. The synthetic procedures are exemplified with selected library members in Scheme 11. Crude reaction mixtures were concentrated, then dissolved in methanol, and applied onto Waters Sep-Pak Plus C_{18} cartridges or a pad of C_{18} resin. Washing of the C_{18} resin with methanol removed unreacted excess of diethylamine and electrophile, as well as acetamide by-product, whereafter up to 0.5 g of pure (>90%) keto-functionalized thioglycosides **57** could be recovered in high yields (71–98%) by elution with *n*-pentane. The ketones **57** were subjected to a second set of reactions in parallel involving reductions to alcohols **58**, reductive aminations with amino acid esters to *N*-alkylated amino acids **59**, and reductive aminations to primary amines **60**. The primary amines were further acylated to give thioglycosides amides **61**. In this work, the hydrophobic tags had to be removed in order to obtain a final library for screening against biological targets. Cleavage of the hydrophobic tag/protecting group was accomplished with Zemplén transesterfications



Scheme 10.


SAc
iAlkylation
iiC18 SPE
56
57
R=
iReduction
iiC18 SPE
iReductive amination
iiC18 SPE
iReductive amination
iiC18 SPE
iAcylation
iiC18 SPE
58
59
60
61
NaOMe, MeOH
NaOMe, MeOH
NaOMe, MeOH
NaOMe, MeOH
62
63
64
65
+ 66
+ 66
+ 66
66
C18 SPE
iC18 SPE
iiLiOH, H2O
C18 SPE
C18 SPE
62
67
64
65

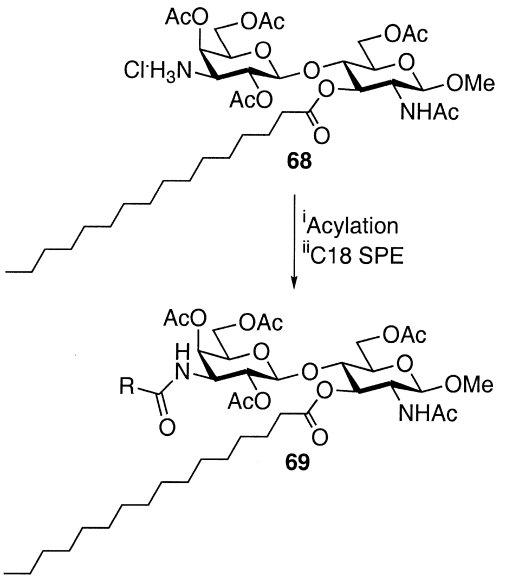
Scheme 11.

to give the alcohols **62**, *N*-alkylated amino acid esters **63**, amines **64**, and amides **65**. The Zemplén transesterfications produced methyl laurate **66** as a tag-derived by-product, which could be conveniently extracted onto C_{18} resin from an aqueous solution leaving the final library members **62**, **64**, **65** and **67** in purities of 70–99%.

Tagging of carbohydrate hydroxyl groups to enable C_{18} SPE purification has been successfully used for other monosaccharide-based thioacetates carrying three or four lauric acid ester tags [36]. It should be noted that tagging of the saccharide hydroxyl groups using the shorter octanoates was not sufficient to allow extraction onto C_{18} cartridges.

A similar approach was recently reported for the synthesis of *N*-acetyl lactosamine derivatives [37]. The 3′-amino *N*-acetyl lactosamine derivative **68** carrying a stearic ester as a hydrophobic tag and protecting group on HO-3 was used for solution synthesis of 3′-amido *N*-acetyl lactosamine derivatives **69** (Scheme 12). The stearic acid tag allowed efficient solid-phase extraction of **69** from 70% methanol using Waters Sep-Pak Plus C_{18} cartridges. Washing of the C_{18} cartridges with 70% methanol removed excess acylation reagents and by-products. Compounds **69** could subsequently be recovered in pure form by elution with 90% methanol.

A phenyl SPE resin has been described for purification of a library of aryl β-D-xylopyranosides **71** [38]. Glycosylation of aromatic alcohols were performed using a large excess of the xylosyl trichloroacetimidate **70** to ensure complete consumption of the aromatic alcohol (Scheme 13). Methanolic sodium methoxide was added when the xylosylation was judged complete by TLC analysis, which yielded a mixture consisting of the desired xylosides **71**, xylose **72**, as well as boric acid derived

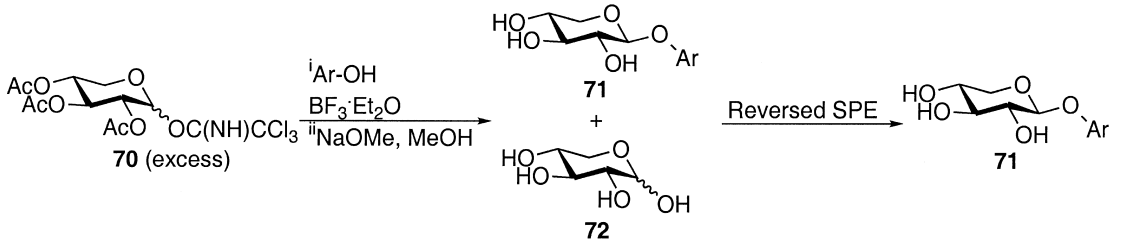


Scheme 12.

by-products. The xylosides **71**, carrying hydrophobic aromatic aglycons, could be isolated via SPE onto a phenyl resin from an aqueous solution of the crude mixture. Washing with water left only the xylosides **71** on the phenyl SPE resin and subsequent elution with methanol yielded pure compounds.

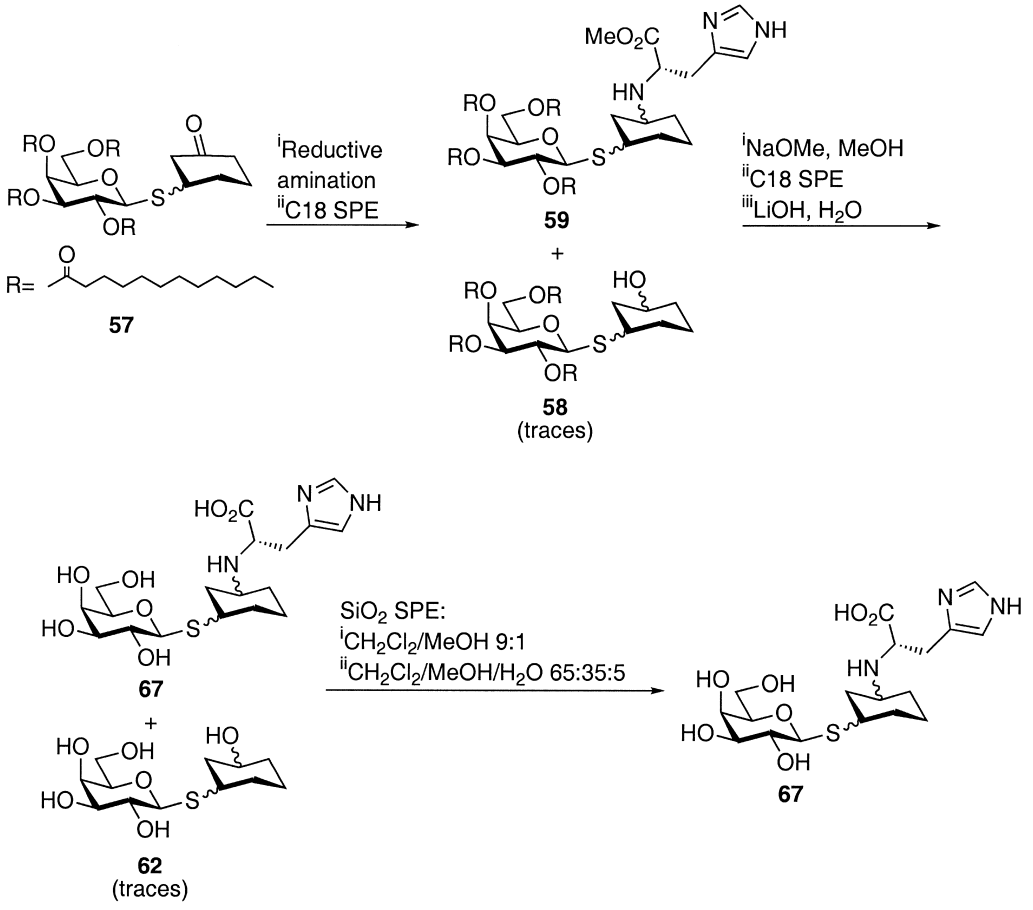
4. Normal-phase SPE

Normal-phase SPE has great potential as a purification technique for solution-phase combinatorial chemistry, provided that products and by-products display significantly different polarities. A solvent is chosen for crude mixtures so that products (or reagents and by-products) are completely retarded

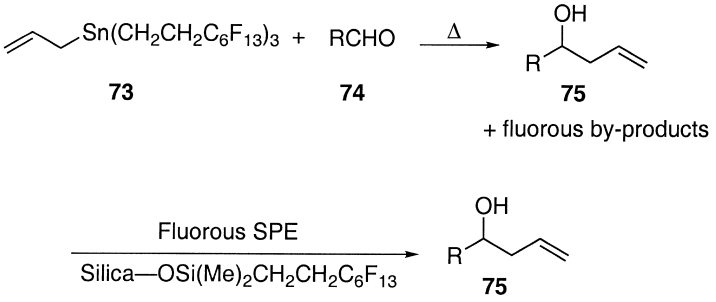


Scheme 13.

on, for example, a SiO_2 SPE cartridge. Washing to remove reagents and by-products, followed by elution with a more polar solvent gives the desired products (or vice versa). An advantageous feature of normal-phase SPE is that numerous solvents or solvent-mixtures are compatible with SiO_2 and normal-phase SPE can thus be easily fine-tuned by simply varying the solvents. Nilsson et al. described the use of normal-phase SPE for the purification of a library of *N*-alkylated L-amino acids [34]. Reductive amination of various L-amino acid esters with carbohydrate-derived ketones, reversed-phase SPE and deblocking, as reviewed above (Scheme 11), gave the *N*-alkylated L-amino acids. However, the reductive amination reactions in some cases produced the corresponding alcohols as by-products (via direct reduction of the ketones) and the final *N*-alkylated L-amino acid products were contaminated with small amounts of alcohols, as exemplified by the synthesis of the library member **67** in Scheme 14. Fortunately, alcohols (e.g., **62**) could be removed with normal-phase SPE, since they were devoid of charges and consequently less polar. The *N*-alkylated L-amino acids containing traces of the corresponding alcohols were applied onto Sep-Pak Plus LongBody SiO_2 cartridges, washed with dichloromethane–methanol (9:1) to remove the alcohols (e.g., **62**), followed by elution with dichloromethane–methanol–water (65:35:5) to furnish pure *N*-alkylated L-amino acids (e.g., **67**).



Scheme 14.



Scheme 15.

5. Fluorous-phase SPE

Curran et al. have suggested the use of fluorous SPE (FSPE) for the purification of fluorous combinatorial libraries [39]. Fluorous SPE requires that either starting material or reagents are tagged with polyfluorinated hydrocarbon chain(s), which allows selective extraction of tagged species from an organic solution onto a fluorous solid-phase (e.g., fluorous reversed-phase silica gel). Although not yet fully described for combinatorial chemistry, the principle of fluorous SPE was convincingly demonstrated with thermal allylations of aldehydes **74** with fluorous allylstannane **73** (Scheme 15). The crude product mixtures containing the sought-after alcohols **75** and fluorous by-products were dissolved in acetonitrile and charged onto a fluorous SPE column, which was eluted with acetonitrile to yield the alcohols **75** in 76–100% purity. Quite interestingly, reversed-phase C_{18} silica was less efficient in extracting fluorous by-products from the crude reaction mixtures.

6. Conclusions

SPE is today a well-established technique for purification of compound libraries. Ion-exchange SPE in particular has reached a high level of sophistication and has become more or less a routine technique for purification of solution-phase parallel reactions in many laboratories. A reason for ion-exchange SPE immediately becoming embraced by combinatorial chemists is probably that various resins are commercially available, as well as equipment for automatization and robotization, thus making the time necessary for developing a high-speed purification protocol relatively short. The relatively limited number of publications on the subject does probably not reflect the widespread use of ion-exchange SPE in combinatorial chemistry today.

Nevertheless, there is still a demand for novel SPE techniques for purification of solution-phase combinatorial libraries, such as the reversed- and fluorous-phase techniques, which do not rely on that reactions produce ionizable products, reagents, or by-products. The development of novel SPE phases and resins having higher loading capacities, improved selectivities, and better swelling/wettability properties, as well as development of novel transient molecule tags for more selective and high-yielding extractions, are highly desirable and may very well be described in the near future. Finally, the demand for high-speed preparation of large numbers of compounds will certainly continue to fuel the design of more efficient automated synthesis/SPE handling systems, such as the recently described extraction plates capable of performing 96 parallel SPE in combination with 96-well plates [40,41].

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References

[1] M.A. Gallop, R.W. Barrett, W.J. Dower, S.P.A. Fodor, E.M. Gordon, J. Med. Chem. 37 (1994) 1233.

[2] N.K. Terrett, M. Gardner, D.W. Gordon, R.J. Kobylecki, J. Steele, Tetrahedron 51 (1995) 8135.
[3] J.A. Ellman, L.A. Thompson, Chem. Rev. 96 (1996) 555.
[4] R.B. Merrifield, J. Am. Chem. Soc. 85 (1963) 2149.
[5] B.A. Bunin, J.A. Ellman, J. Am. Chem. Soc. 114 (1992) 10997.
[6] S.H. Dewitt, J.S. Kiely, C.J. Stankovic, M.C. Schroeder, D.M.R. Cody, M.R. Pavia, Proc. Natl. Acad. Sci. USA 90 (1993) 6909.
[7] K.A. Keating, R.W. Armstrong, J. Am. Chem. Soc. 118 (1996) 2574.
[8] S.D. Brown, R.W. Armstrong, J. Am. Chem. Soc. 118 (1996) 6331.
[9] R.J. Booth, J.C. Hodges, Acc. Chem. Res. 32 (1999) 18.
[10] C.W. Harwig, D.J. Gravert, K.D. Janda, Chemtracts 12 (1999) 1.
[11] D.L. Boger, C.M. Tarby, P.L. Myers, L.H. Caporale, J. Am. Chem. Soc. 118 (1996) 2109.
[12] S. Cheng, D.D. Comer, J.P. Williams, P.L. Myers, D.L. Boger, J. Am. Chem. Soc. 118 (1996) 2567.
[13] S. Cheng, C.M. Tarby, D.D. Comer, J.P. Williams, L.H. Caporale, P.L. Myers, D.L. Boger, Bioorg. Med. Chem. 4 (1996) 727.
[14] D.L. Boger, W. Chai, R.S. Ozer, C.-M. Andersson, Bioorg. Med. Chem. Lett. 7 (1997) 463.
[15] D.L. Boger, R.S. Ozer, C.-M. Andersson, Bioorg. Med. Chem. Lett. 7 (1997) 1903.
[16] D.L. Boger, J. Goldberg, W. Jiang, W. Chai, P. Ducray, J.K. Lee, R.S. Ozer, C.-M. Andersson, Bioorg. Med. Chem. 6 (1998) 1347.
[17] D.L. Boger, W. Chai, Q. Jin, J. Am. Chem. Soc. 120 (1998) 7220.
[18] D.L. Boger, J. Goldberg, C.-M. Andersson, J. Org. Chem. 64 (1999) 2422.
[19] D.L. Flynn, J.Z. Crich, R.V. Devraj, S.L. Hockerman, J.J. Parlow, M.S. South, S. Woodard, J. Am. Chem. Soc. 119 (1997) 4874.
[20] J.J. Parlow, D.A. Mischke, S.S. Woodard, J. Org. Chem. 62 (1997) 5908.
[21] J.J. Parlow, W. Naing, M.S. South, D.L. Flynn, Tetrahedron Lett. 38 (1997) 7959.
[22] R.M. Lawrence, S.A. Biller, O.M. Fryszman, M.A. Poss, Synthesis, (1997) 553.
[23] L.M. Gayo, M.J. Suto, Tetrahedron Lett. 38 (1997) 513.
[24] M.G. Siegel, P.J. Hahn, B.A. Dressman, J.E. Fritz, J.R. Grunwell, S.W. Kaldor, Tetrahedron Lett. 38 (1997) 3357.
[25] A.J. Shuker, M.G. Siegel, D.P. Matthews, L.O. Weigel, Tetrahedron Lett. 38 (1997) 6149.
[26] T.L. Deegan, O.W. Gooding, S. Baudart, J.A. Porco Jr., Tetrahedron Lett. 38 (1997) 4973.
[27] B.A. Kulkarni, A. Ganesan, Tetrahedron Lett. 39 (1998) 4369.
[28] B.A. Kulkarni, A. Ganesan, Tetrahedron Lett. 40 (1999) 5637.
[29] J.J. Parlow, B.L. Case, M.S. South, Tetrahedron 55 (1999) 6785.
[30] J.J. Parlow, Tetrahedron Lett. 37 (1996) 5257.
[31] W. Xu, R. Mohan, M.M. Morrisey, Tetrahedron Lett. 38 (1997) 7337.
[32] O. Kanie, F. Barresi, Y. Ding, J. Labbe, A. Otter, L.S. Forsberg, B. Ernst, O. Hindsgaul, Angew. Chem. Int. Ed. 34 (1995) 2720.
[33] Y. Ding, J. Labbe, O. Kanie, O. Hindsgaul, Bioorg. Med. Chem. 4 (1996) 683.
[34] U.J. Nilsson, E.J.-L. Fournier, O. Hindsgaul, Bioorg. Med. Chem. 6 (1998) 1563.
[35] U.J. Nilsson, E.J.-L. Fournier, E.J. Fryz, O. Hindsgaul, Bioorg. Med. Chem. Submitted for publication.
[36] U.J. Nilsson, O. Hindsgaul, unpublished results.
[37] P. Sörme, H. Leffler, U.J. Nilsson, presented at ESOC 11, Gothenburg, 1999, abstract P167.
[38] N. Falk, G. Magnusson, presented at ESOC 11, Gothenburg, 1999, abstract P42.
[39] D.P. Curran, S. Hadida, M. He, J. Org. Chem. 62 (1997) 6714.
[40] J.P. Allanson, R.A. Biddlecomb, A.E. Jones, S. Pleasance, Rapid Commun. Mass. Spectrom. 10 (1996) 811.
[41] B. Kaye, W.J. Herron, P.V. Macrae, S. Robinson, R.V. Stopher, W. Wild, Anal. Chem. 58 (1996) 1658.