Solid-Phase Synthesis in the Twenty-First Century

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Abstract: Solid-phase synthesis is a powerful tool for achieving high-throughput chemistry. This review discusses recent diverse examples from my group: the solid-phase synthesis of unsymmetrical guanidines, polymer-supported versions of cyclooctadiene and 9-BBN, a triflate-like linker, the synthesis of tetrahydro-β-carbolines by the acyliminium Pictet-Spengler reaction, and a total synthesis of the antimycobacterial cyclic depsipeptide natural product kahalalide A.

Keywords: Solid-phase synthesis, combinatorial chemistry, guanidines, polymer-supported reagents, traceless cleavage, alkaloids, Pictet-Spengler reaction, depsipeptides.

It is now half a century since R. B. Merrifield introduced the Nobel prizewinning concept of peptide synthesis on an immobilized support. Peptide synthesis is inherently lengthy, consisting of a repetitive cycle of deprotection, monomer activation, and coupling to the growing chain. Merrifield's innovation enabled this reiteration to be performed without the purification of each intermediate, while large reagent excesses helped drive reactions to completion. Since only three functional group interconversions are involved, rather than more challenging carbon-carbon bond formation or cyclization reactions, these were optimized to the point of being virtually quantitative, and the entire process was successfully automated.

Until the last decade, the organic chemistry community largely ignored solid-phase synthesis outside the peptide and nucleotide realm. By then, high-throughput screening by the pharmaceutical industry had created a need for rapid compound synthesis. Solid-phase methods were intensively examined for the synthesis of diverse small molecules, and this rejuvenation has continued into the present time. It is a testament to Merrifield's scientific acumen that the lightly crosslinked polystyrene resins he pioneered remain by far the most popular support for solid-phase synthesis. Indeed, Merrifield-type resins were used in each of the following twenty-first century examples [1, 2] from my group.

SOLID-PHASE GUANIDINE SYNTHESIS

For some time, we have been interested [3] in the synthesis of unsymmetrical trisubstituted guanidines. This motif occurs in biologically active compounds of both natural and synthetic origin, such as the MRSA-active antibiotic TAN-1057. In our first generation solid-phase synthesis [4] (Scheme **1**), graduate student Peishan Lin started with amino acids linked to either the Wang or the Rink amide linker. The amine was condensed with *p*-nitrophenyl chloroformate, which functioned as a phosgene equivalent, followed by reaction of the resulting carbamate by *S*-methylisothiourea. While this might be accompanied by overaddition to give 2:1 adducts in solution-phase, the step proceeded uneventfully in solid-phase. The remaining nitrogen was acylated, followed by mercury(II) promoted displacement of the thiomethyl group to yield the guanidine. In solution-phase, careful removal of mercury(II) salts would be needed, whereas in solid-phase the usual washing of the resin suffices. Finally, acidic cleavage furnishes the guanidines bearing an alkyl, an acyl, and a carbamoyl substituent. Overall, this modular process is straightforward, and Peishan prepared several hundred guanidines, which were then assayed for antimicrobial activity.

Recently, graduate student Paul Boguszewski devised a solid-phase route to guanidines bearing three different alkyl substituents. This synthesis [5] (Scheme **2**) illustrates the principle of resin capture as a means for purification. Unsymmetrical carbodiimides were prepared in solution-phase by the Staudinger (aza-Wittig) condensation between iminophosphoranes and isocyanates. These reactions were readily monitored by IR spectroscopy, and a slight excess of the iminophosphorane was used to drive the reaction forward. Afterwards, the crude carbodiimide was extracted with hexanes, leaving behind any polar reagents and byproducts. The crude carbodiimide was then reacted with secondary amine resins, which were obtained by reductive alkylation of the SASRIN linker. Capture of the carbodiimide afforded the resin-bound guanidine, which was then released upon acidic cleavage. Initially, the guanidine is in the form of its trifluoroacetate salt, and Paul came up with a parallel processing method for generating the free base. The salt is captured by a sulfonic acid ion-exchange solid-phase extraction (SPE) cartridge, and the free base then eluted by methanolic ammonia. Overall, we have built up the trisubstituted guanidine in a modular fashion from three readily diversified building blocks- organic azides, isocyanates, and amines.

POLYMER-SUPPORTED REAGENTS: IMMOBILIZED VERSIONS OF CYCLOOCTADIENE AND 9-BBN

In the twenty-first century, the boundaries between solid and solution-phase synthesis have blurred and there is no longer a sharp distinction between the two. An increasing number of hybrid examples that combine elements of both approaches are becoming popular. In the previous example, for instance, the resin-bound amine served as a reagent and a purification aid in fishing out the desired carbodiimide from

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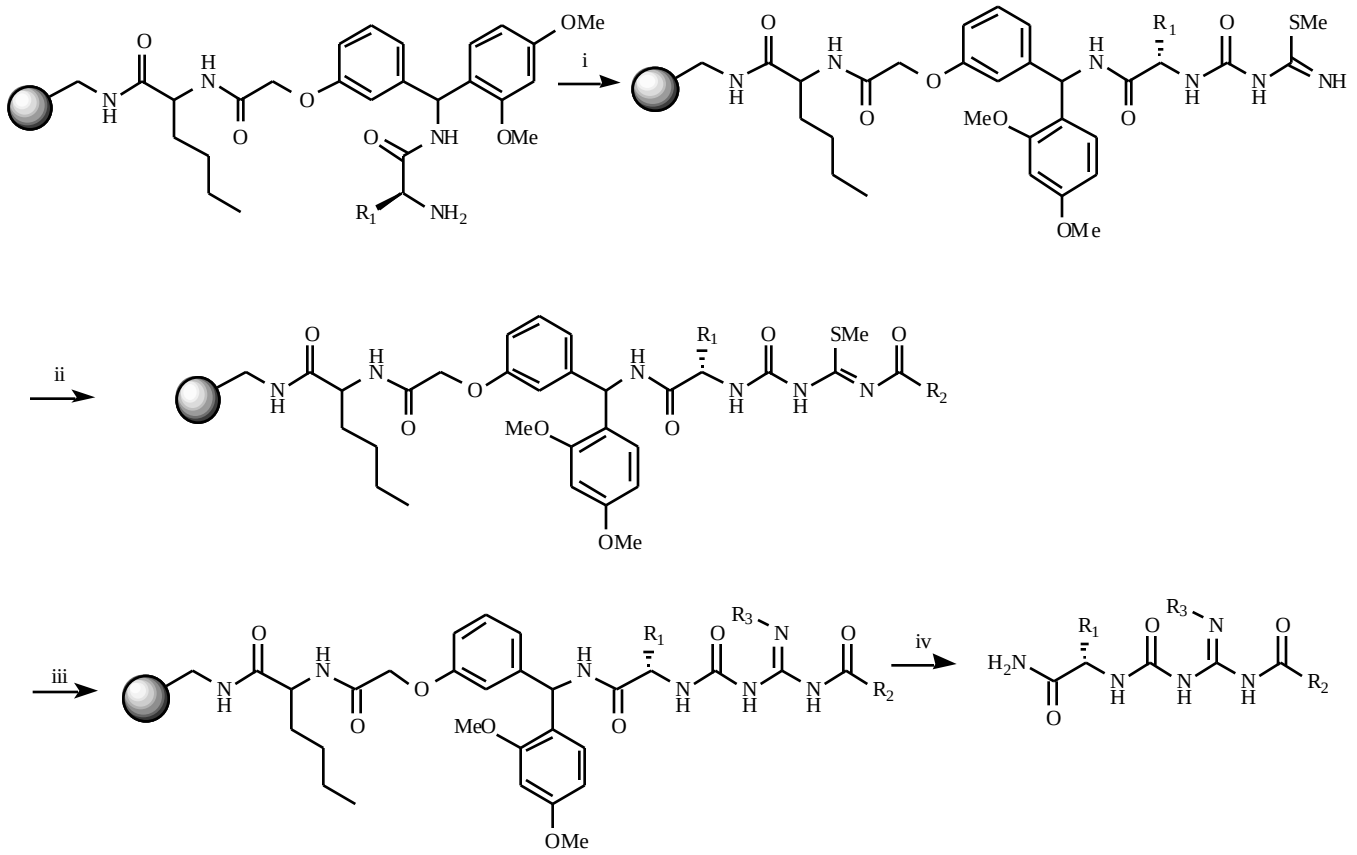
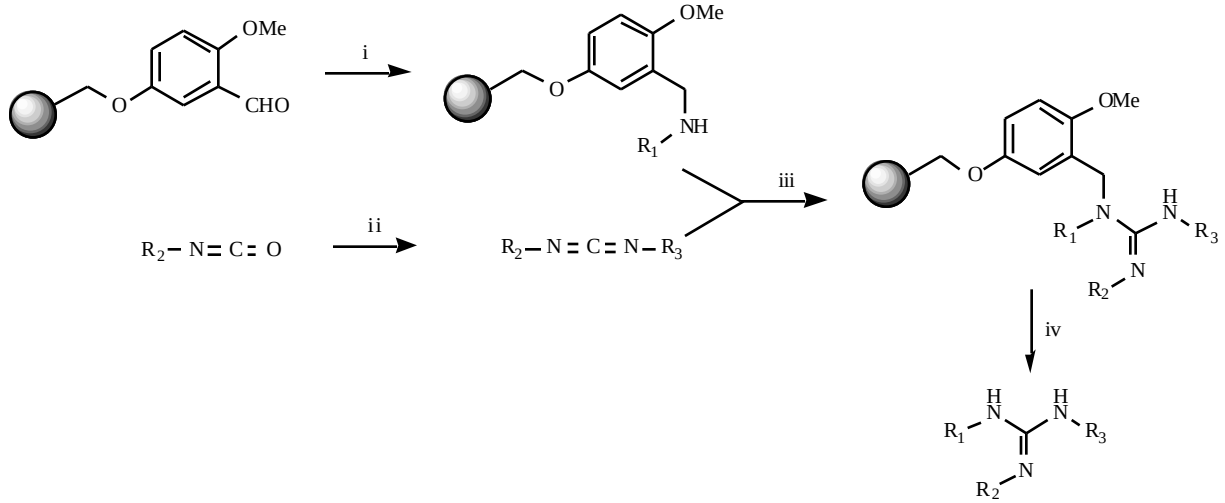


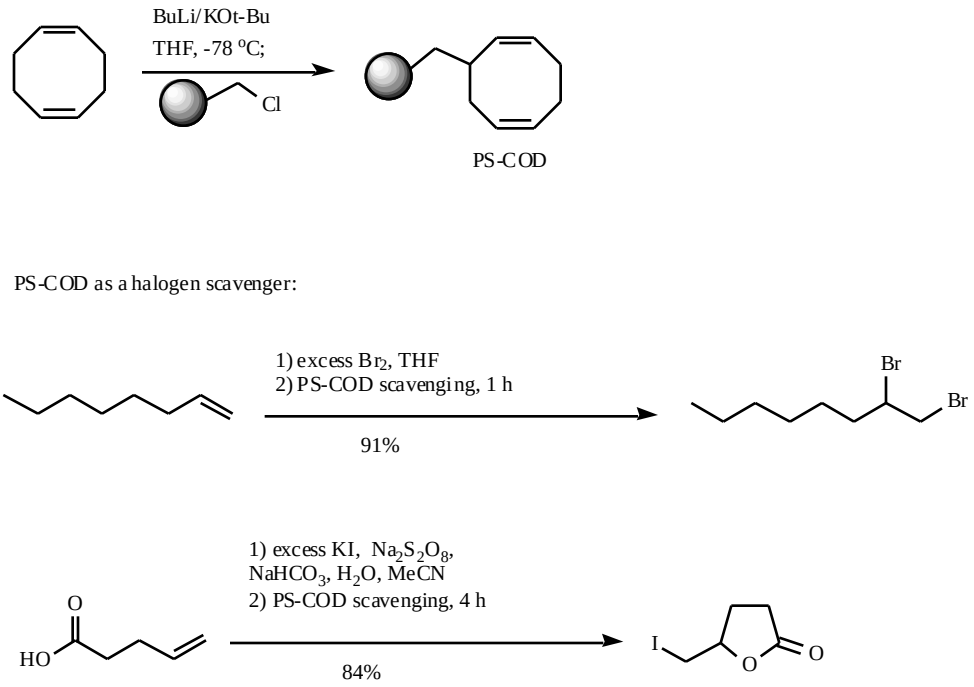
Scheme 1. A solid-phase synthesis of guanidines bearing an acyl and carbamoyl substitutent. Reagents and conditions: i) a) *p*-NO_2-C_6H_4OCOCl, py, CH_2Cl_2; b) H_2N-C(SMe)=NH, Et_3N, DMF; ii) R_2COCl, Et_3N, CH_2Cl_2; iii) R_3NH_2, $HgCl_2$, Et_3N, DMF; iv) TFA.

a crude reaction mixture. Two of the most important advantages of solid-phase synthesis are the ability to use large reagent excesses to improve yields, followed by rapid removal of these reagents and byproducts by simple filtration of the resin beads. By reversing the immobilized partner, the same strategies are now being applied to solution-phase parallel synthesis. In one approach, the reagent is used in excess in an immobilized form, thus facilitating phase separation from the product afterwards. In a second approach, all reagents are in solution with one being employed in large excess. Afterwards, a resin-bound scavenger that reacts with the excess reagent is added.

Ideally, immobilized reagents and scavengers should be prepared from inexpensive starting materials in a scalable manner that permits preparation on a multigram scale. Synthetic flexibility, whereby the same intermediate is a precursor to more than one resin, is highly desirable. The



Scheme 2. Solid-phase synthesis of unsymmetrical trialkylsubstituted guanidines. Reagents and conditions: i) R_1-NH_2, $NaBH(OAc)_3$, AcOH/NMP; ii) R_3-N=PPh_3, CH_2Cl_2; iii) resin capture, i-Pr_2NEt, CH_2Cl_2; iv) a) 30% TFA, H_2O/ CH_2Cl_2,; b) SPE purification.

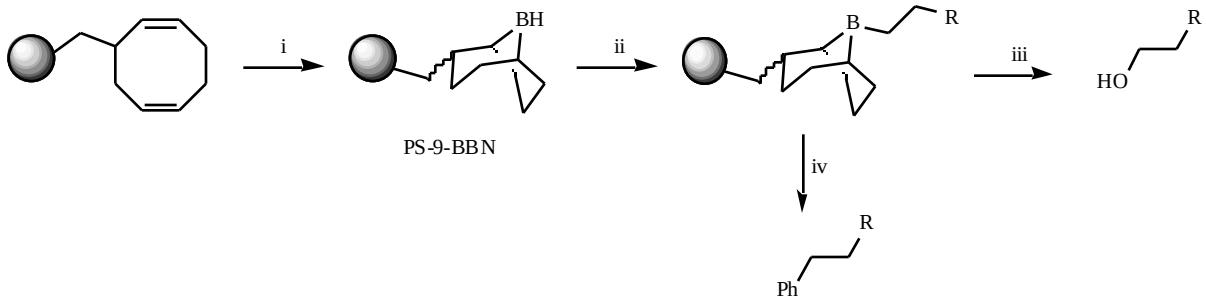


Scheme 3. Synthesis and applications of PS-COD.

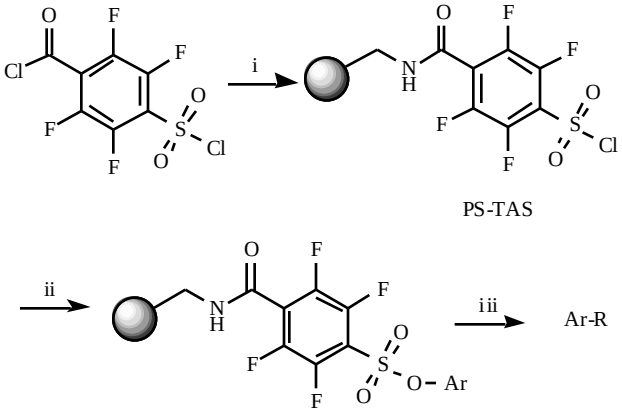
final loading should be high (significantly greater than 1 mmol/g), and the performance of these reagents not compromised compared to their solution-phase counterparts. Although a wide variety of immobilized reagents are commercially available and the number of new ones being reported in the literature is steadily rising, many fail one or more of the above criteria.

During his Ph.D. studies, Jefferson Revell designed a polymer-bound version of 1,5-cyclooctadiene and 9-BBN that illustrates these features. Devising a means of attaching 1,5-cyclooctadiene to a resin appears straightforward, but was in fact rather challenging. Several approaches that initially appeared promising did not withstand the stringent requirements that we had set ourselves for practicality. The final solution turned out to be surprisingly simple with hindsight. 1,5-Cylooctadiene was lithiated under LICKOR (BuLi/KOt-Bu) conditions [6], and the allylic anion reacted with high-loading Merrifield resin. This one step procedure from inexpensive reagents provided PS-COD with a loading of 3.85 mmol/g, as determined by residual chloride analysis. With this high concentration of diene functionality, PS-COD turns out to be an efficient halogen scavenger. Jefferson successfully carried out [7] solution-phase brominations with excess bromine and an iodolactonization in which the workup consists of simply stirring with PS-COD to remove the excess halogen (Scheme **3**).

The ability to prepare more than one reagent from a common resin makes economic and scientific sense. Treatment of PS-COD with borane resulted in an immobilized version of the popular hydroborating agent, 9-BBN. We demonstrated that PS-9-BBN functions very well in the hydroboration/oxidation of terminal alkenes to primary alcohols. By employing the resin in excess, yields were typically 80% or higher. When 9-BBN is used in such reactions in solution-phase, the product alcohol needs to be separated from the cyclooctanediol coming from the reagent. With PS-9-BBN, this byproduct remains on the resin, and simple filtration is all that is needed. Jefferson also carried out preliminary investigations [8] on the B-alkyl Suzuki cross-coupling reaction of the intermediate hydroboration product with arylboronic acids. Our initial yields have been disappointing, although it is likely that they can be significantly improved by optimization of reaction conditions (Scheme **4**).



Scheme 4. Synthesis and applications of PS-9-BBN. Reagents and conditions: i) BH_3, THF; ii) R-CH=CH_2, THF; iii) aq. H_2O_2, Bu_4NOH, THF; iv) $PhB(OH)_2$, cat. Pd(0), DMF.

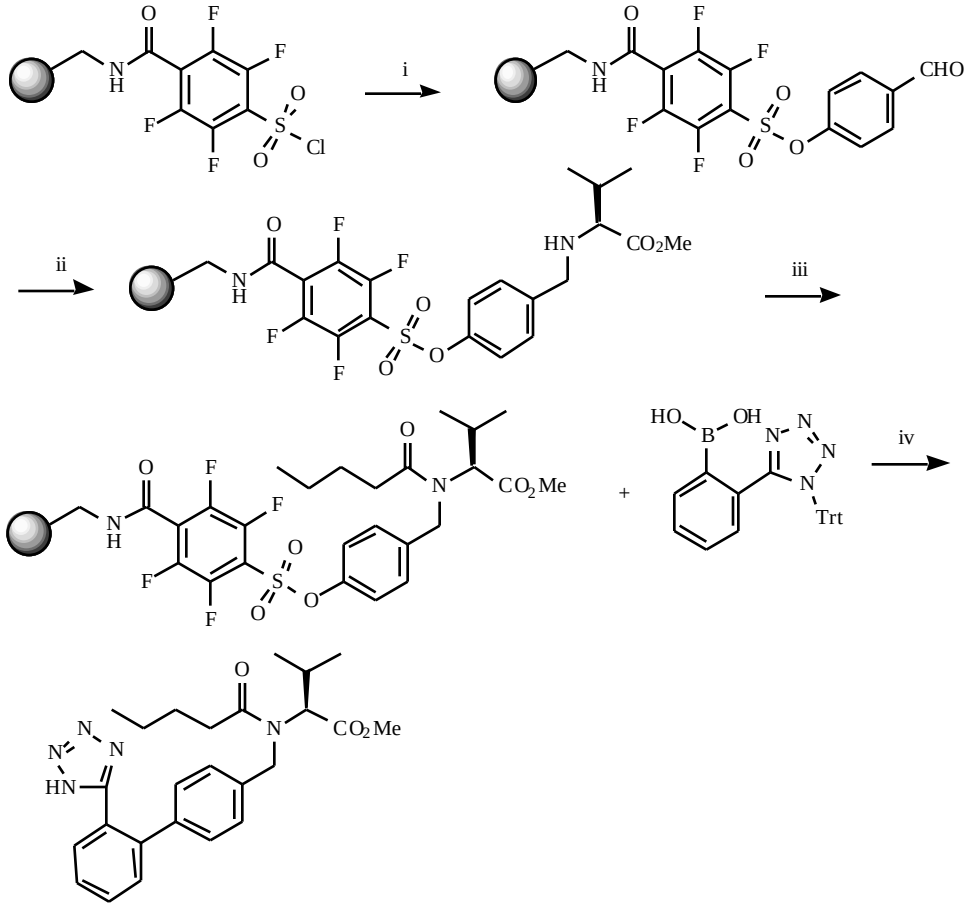


Scheme 5. Synthesis of PS-TAS linker and its applications. Reagents and conditions: i) Aminomethylpolystyrene; ii) ArOH, i-Pr_2NEt, CH_2Cl_2; iii) cat. $Pd(OAc)_2$, dppp, HCOOH, Et_3N, DMF (for R=H); cat. $PdCl_2(dppf)$, R-$B(OH)_2$, Et_3N, DMF (for R=aryl); cat. $PdCl_2(dppf)$, CH_2=CH-CO_2Me, Et_3N, Bu_4NI, DMF (for R=CH=CH-CO_2Me).

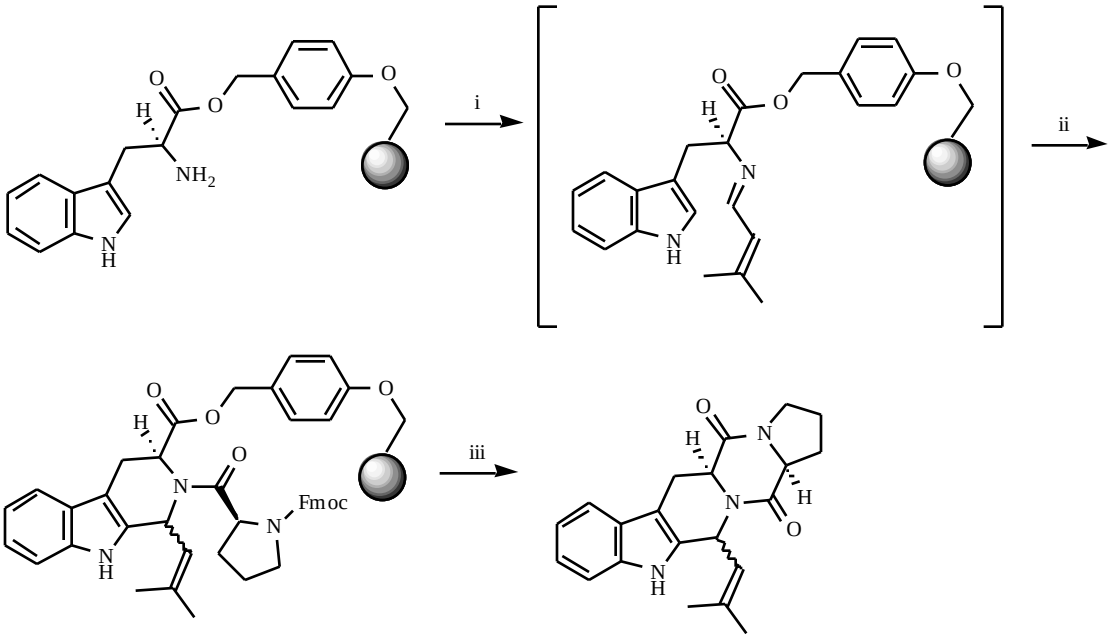
A TRIFLATE-LIKE SOLID-PHASE MULTIFUNCTIONAL LINKER

In solid-phase combinatorial synthesis, the final transformation is cleavage from the resin. Usually, this merely serves to release the product rather than a means of increasing diversity. With 'multifunctional' linkers, on the other hand, a family of products is released depending upon the cleavage cocktail. During his highly productive Ph.D., Jefferson Revell designed a linker that serves as a resin-bound triflate. Phenols can be attached to the linker, followed by further transformations. Finally, reaction with Pd(0) cleaves the substrate to give an arylpalladium intermediate. This can either be reduced under transfer hydrogenation conditions, in what amounts to a 'traceless' synthesis, or cross-coupled by familiar name reactions such as the Suzuki and Heck. This concept was first demonstrated [9] by Chris Holmes at Affymax, with a 'nonaflate'-like linker. While Holmes' proprietary linker works very nicely, its preparation takes a number of steps and begins from a relatively expensive starting material. As the size of the linker is quite large, the final loading is also modest.

Jefferson's linker is prepared from a known bis acid chloride, obtained on a multigram scale in three steps from inexpensive pentafluorobenzoic acid. Reaction with aminomethylpolystyrene generates the linker that we have christened PS-TAS (Scheme **5**). Jefferson showed [10] that phenols attached to PS-TAS behave like triflates, and participate in Pd(0)-catalyzed cleavage to either the hydrocarbon (by transfer hydrogenation) or cross-coupled products. Similar results were independently obtained [11] by Andrew Cammidge's group at the University of East Anglia in Norwich, UK.



Scheme 6. Synthesis of Valsartan methyl ester using the PS-TAS linker. Reagents and conditions: i) p-HO-C_6H_4-CHO, i-Pr_2NEt, CH_2Cl_2; ii) L-Val-OMe, $NaBH(OAc)_3$, AcOH/ DMF; iii) valeryl chloride, Et_3N, CH_2Cl_2; iv) cat. $Pd(OAc)_2$, Xantphos, DME.

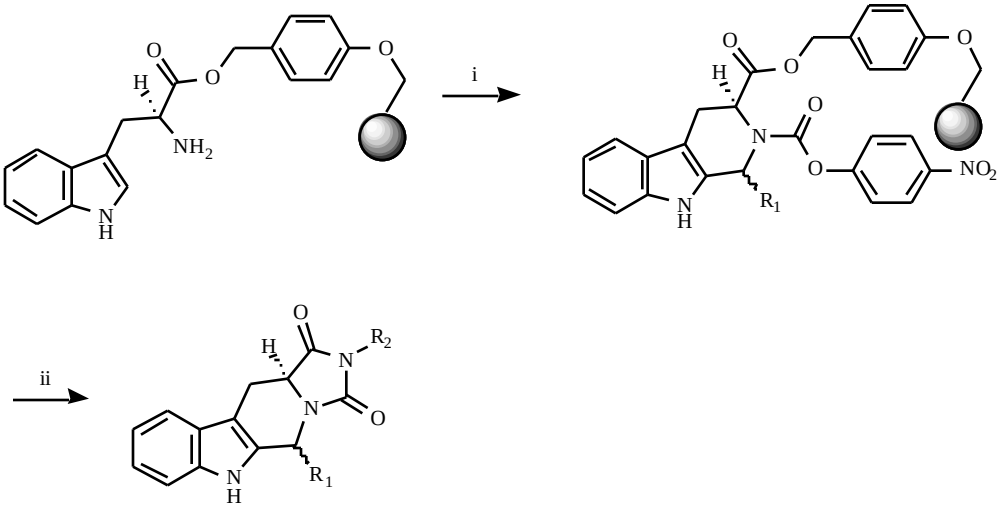


Scheme 7. Solid-phase total synthesis of demethoxyfumitremorgin C. By variation of the aldehyde and amino acid in steps i and ii, the method was applied to the synthesis of analogues. Reagents and conditions: i) senecialdehyde, $HC(OMe)_3$; ii) Fmoc-L-Pro-Cl, py, CH_2Cl_2; iii) piperidine, CH_2Cl_2.

We then wished to demonstrate the utility of the linker in multistep sequences, and targeted a solid-phase synthesis of the drug Valsartan, an angiotensin receptor antagonist from Novartis. The route begins (Scheme **6**) with the attachment of 4-hydroxybenzaldehyde to PS-TAS. In subsequent steps, the aldehyde is reductively aminated with valine methyl ester, and the resulting secondary amine acylated. The final reaction is a Suzuki cross-coupling with a boronic acid, releasing Valsartan methyl ester in an overall yield of 39% after trityl deprotection. Thus, the drug is assembled from four discrete entities (aromatic aldehyde, amino acid, carboxylic acid, and boronic acid), which can be independently varied to generate a family of analogues.

SOLID-PHASE *N*-ACYLIMINIUM PICTET-SPENGLER REACTIONS

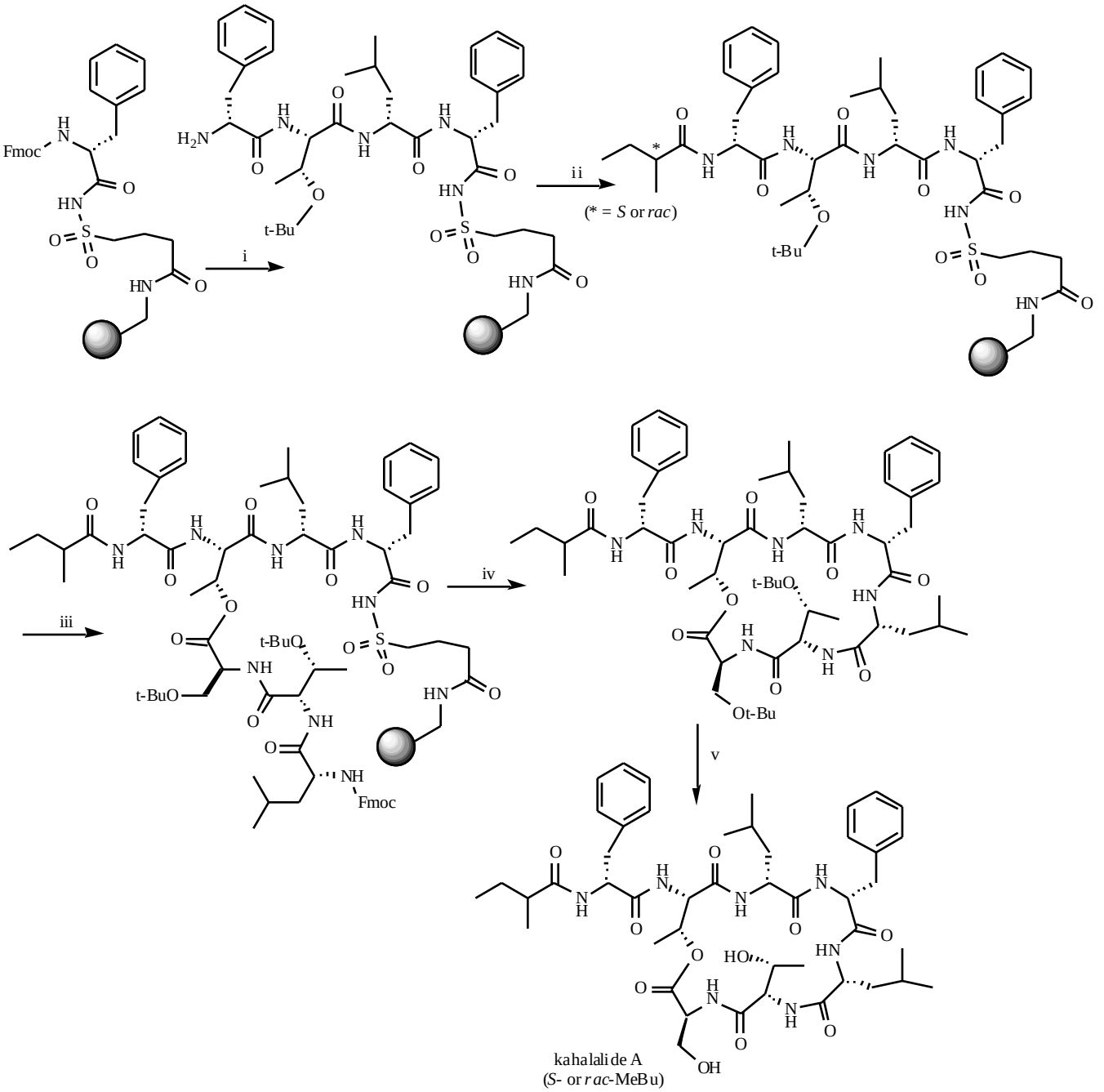
Several years ago, we embarked on a program to achieve the total synthesis of the fumitremorgin group of fungal alkaloids by a route amenable to the preparation of analogues. These alkaloids have recently attracted attention as potential leads for anticancer therapy, being inhibitors of mammalian cell cycle progression as well as reversing multidrug resistance mediated by the breast cancer resistance protein (BCRP). An extremely talented postdoctoral associate, Haishan Wang, successfully completed the total synthesis of demethoxyfumitremorgin C [12] (the most active of the alkaloids) and spirotryprostatin B [13] by



Scheme 8. Solid phase synthesis of tetrahydro-β-carboline-hydantoins. Reactions and conditions: i) a) R_1-CHO, $HC(OMe)_3$; ii) p-NO_2-C_6H_4OCOCl, py, DMAP, CH_2Cl_2; ii) R-NH_2, Et_3N, DMF.

traditional solution-phase methods. The key step in both syntheses was an *N*-acyliminium variant of the venerable Pictet-Spengler reaction. Compared to the conventional protic acid catalyzed iminium Pictet-Spengler process, the *N*-acyliminium version facilitates the reaction with normally recalcitrant α,β-unsaturated imines. Haishan Wang translated this process to solid-phase by immobilizing tryptophan on the Wang resin (Scheme **7**), and a number of unnatural analogues of the natural products were accessed in this manner and evaluated [14] for biological activity. After the solid-phase Pictet-Spengler reaction, removal of the Fmoc protecting group unmasks a free amine that undergoes intramolecular reaction to form a diketopiperazine and release the desired product into solution. We have employed similar cyclative cleavages [15] in many solid-phase reaction sequences. Since only the desired intermediate can participate in cyclization, this strategy ensures a high purity of the final product uncontaminated by other failed intermediates over the course of the synthesis.

Recently, postdoctoral associate Dominique Bonnet modified [16] the *N*-acyliminium Pictet-Spengler reaction so that the final cyclative cleavage produces a hydantoin



Scheme 9. A solid-phase total synthesis of the cyclic depsipeptide natural product kahalalide A. Reactions and conditions: i) Fmoc(Ot-Bu) peptide coupling, DIC, HOBt, DMF; ii) (*S*)- or *rac*-methylbutyric acid, DIC, HOBt, DMF; iii) Fmoc(Ot-Bu) peptide coupling, DIC, HOBt, DMF; iv) a) 20% piperidine/DMF; b) Trt-Cl, i-Pr_2NEt; c) ICH_2CN, i-Pr_2NEt; d) 5% TFA/CH_2Cl_2; i-Pr_2NEt; v) TFA/*i*-Pr_3SiH/H_2O.

skeleton rather than the diketopiperazine ring present in the natural products. In this version (Scheme **8**), *p*-nitrophenyl chloroformate is used as the imine acylating agent, leading to a tetrahydro-β-carboline where the ring nitrogen is functionalized as a carbamate. The *p*-nitrophenyl group is then displaced by a amine, and the resulting urea undergoes cyclative cleavage to the hydantoin upon heating.

SOLID-PHASE TOTAL SYNTHESIS OF KAHALALIDE A

Cyclic peptide natural products offer some unique opportunities for combinatorial synthesis. Many such compounds are known with high biological activity. Once a synthetic route is established, the availability of a wide variety of amino acids enables the discovery of structure-activity relationships by systematic alterations in the side-chains of the peptide backbone. At the same time, the cyclic structure provides useful conformational constraints, often enhancing potency as well as pharmacokinetic properties compared to linear peptides.

Kahalalide A was isolated [17] by Professor Scheuer's group in Hawaii from the marine mollusk *Elysia rufescens* and its algal diet *Bryopsis* sp. It is the simplest of a family of cyclic peptides identified from this extract, and showed [18] activity against *Mycobacterium tuberculosis*, inhibiting 83% of bacterial growth at 12.5 μg/mL. Kahalalide A does not contain obviously reactive functional groups and it is not cytotoxic to various tumor cell lines, suggesting a selective antibacterial target. For these reasons, we believed that kahalalide A would be an attractive lead for total synthesis.

Although she had no prior experience in solid-phase synthesis, Line Bourel, a visiting scientist from the University of Lille, accepted the challenge of working out a route to kahalalide A. Our solid-phase strategy employed the Kenner 'safety-catch' linker, which was recently shown [19] to be suitable for cyclic peptide synthesis. The Kenner linker is stable to acidic and nucleophilic conditions, until 'safety-catch' activation by alkylation of the acylsulfonamide. These virtues make the linker eminently suitable for the present purpose. Line began (Scheme **9**) by attachment of Fmoc-D-Phe to the commercially available sulfonamide resin. Sequential peptide coupling then provided a linear tetrapeptide. At this stage, the amine terminus needs to be capped with methylbutyric acid. In the original structure elucidation, the absolute configuration of this acid was not determined. Unfortunately, the acid is commercially available in only the racemic form or as the (*S*)-enantiomer. Line carried out the total synthesis [20] twice with either of these acids. The racemate would ultimately result in a 50-50 mixture of kahalalide A and its epimer at the methylbutyrate. The second synthesis would yield a single diastereomer, with the methylbutyrate having (*S*) configuration. At the outset, we did not know whether this would be the natural product, or its diastereomer.

Further peptide coupling after addition of the methylbutyric acid eventually led to a linear heptapeptide. Sulfonamide alkylation with iodoacetonitrile activated the safety-catch linker, and amine deprotection then resulted in macrocyclative cleavage of the desired depsipeptide into solution. Acidic cleavage of the *t*-butyl ethers completed the total synthesis. In collaboration with Mark Hamann's group, who were involved in the initial structure elucidation of kahalalide A, careful comparison of the ^{1}H NMR with that of the naturally isolated material led to the stereochemistry of the 2-methylbutyrate residue being established as (*S*). Thus, Line's choice of methylbutyric acid enantiomer, dictated by practical reasons, was fortuitously shown to be the correct choice. With the kahalalide A total synthesis completed, Line prepared a number of additional analogues. The results so far confirm that the stereochemistry of the methylbutyrate unit is not important for antimycobacterial activity. Replacement by a longer achiral hexanoyl moiety in fact resulted in a twofold improvement of activity over the natural product.

CONCLUSIONS

In the short space of fifty years, solid-phase organic synthesis on polystyrene beads has become established as a powerful alternative to classical solution-phase synthesis. A plethora of different chemistries has been adapted to these conditions, and it is fair to say that it is rare to find organic reactions that completely fail on solid-phase. This versatility does not mean that solid-phase synthesis is an end in itself, and its power is best utilized when it offers complementary advantages to solution-phase methods.

The examples discussed in this mini-review highlight some of the attractive features of solid-phase synthesis. The acylcarbamoylguanidine library was prepared in a four-step sequence that would need significant purification of intermediates if carried out in solution-phase. The trialkylguanidine library benefited from resin capture to isolate the desired material from a crude reaction mixture. The polymer-supported reagent PS-COD is easily and inexpensively prepared, and shown to be a halogen scavenger. Furthermore, it is readily converted to PS-9-BBN, an immobilized reagent that has the same hydroboration properties as the more familiar 9-BBN in solution-phase. The new PS-TAS solid-phase linker is a conduit for a variety of Pd(0) catalyzed reactions for resin cleavage, as illustrated by the synthesis of the drug Valsartan. In the total synthesis field, solid-phase methods are being applied to a variety of structural classes. The short entry to fumitremorgin alkaloids and related analogues is a noteworthy example, while the total synthesis of kahalalide A harkens back to the original reasons for the invention of solid-phase chemistry for peptide bond formation.

Merrifield's simple concept has withstood the test of time remarkably well. Although solid-phase synthesis has already reached a sophisticated level of maturity, the next fifty years will undoubtedly see further evolution and improvement in the process.

ABBREVIATIONS

BBN = 9-borabicyclo[3.3.1]nonane

COD = 1,5-cyclooctadiene

DIC = diisopropyl carbodiimide

Fmoc = 9-fluorenylmethoxycarbonyl

HOBt = hydroxybenzotriazole

LICKOR = alkyllithium, potassium alkoxide

MRSA = methicillin resistant *Staphylococcus aureus*

PS = polystyrene

SASRIN = super acid sensitive resin

TAS = tetrafluoroarylsulfonate

Trt = trityl

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