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Solid Phase Chemical Technologies for Combinatorial Chemistry

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Abstract This article describes the exploration of synthetic methodologies on solid phase for combinatorial chemistry. Examples will cover various cyclisation strategies that include; the intramolecular Heck cyclisation leading to the formation of oxindoles, the radical cyclisation mechanism in the synthesis of furans, and a stereoselective cyclisation to form oxopiperazines. J. Cell. Biochem. Suppl. 37: 28–33, 2001. © 2002 Wiley-Liss, Inc.

Key words: synthesis; resin; cyclisation; radical; Heck

The 1990s have seen the beginnings of a transformation of how synthetic organic chemists prepare compounds for discovery chemistry. The major driving force for change has been the need to accelerate and improve the process of drug discovery, although other fields such as material science and catalyst discovery have also seen changes in approach. A significant part of this advancement has been lead, and continues to be lead by novel chemical technologies on insoluble solid supports (the solid phase). The synthesis of peptides, and nucleic acids on solid phase is well-understood, established and automated. However, the more generalised synthesis of organic molecules on solid phase is a fertile ground for chemical exploration and challenges. In this article, I describe three solid phase synthetic methodologies that have been studied in my group each involving the synthesis of heterocyclic molecules via a distinct cyclisation chemistry.

Intramolecular Heck Reaction

The Heck reaction [Cortese et al., 1978] has proven to be a popular and successful strategy for the formation of carbon–carbon bonds on solid phase [Yu et al., 1994]. In particular, the intramolecular Heck reaction is a useful synthetic method for the formation of five, six or seven membered rings fused to aromatic rings. Examples of important classes of molecules prepared by the intramolecular Heck on solid phase include the synthesis of indoles [Yun and Mohan, 1996], isoquinolines [Goff and Zuckermann, 1995], and the synthesis of oxindoles [Arumugam et al., 1997], which is described below. Oxindoles are known to possess interesting biological properties, which include anti-rheumatics, auxin activity in addition to inhibitors of mandelonitrile lyase and protein tyrosine kinases. Therefore, they represent an interesting class of molecular template for a solid phase synthesis amenable for combinatorial variation.

The synthesis was based on first generating the immobilised iodoaniline (**4**) by the route shown in Figure 1. Alkylation of 3-iodo-4-nitrophenol (**1**) with ethylbromoacetate followed by hydrolysis afforded the acid (**2**), which was immobilised on Rink amide resin to give the nitro derivative (**3**), which could be reduced with $SnCl_2$ to form (**4**). The most challenging of these reactions proved to be the Sn(II) reduction, which has been somewhat variable for the reduction of nitroaromatic molecules on solid phase by a number of groups pursuing other targets. An alternative approach would be to directly load an *N*-Boc protected 3-iodo-4-aminophenol [Gordon and Balasubramanian, 2001], which would circumvent the need for reduction of the nitro group on solid phase.

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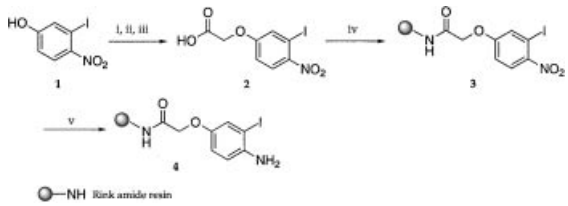

Fig. 1. (i) Ethylbromoacetate, K_2CO_3, DMF (95%); (ii) 1 N NaOH, MeOH, reflux; (iii) Conc. HCl (90%); (iv) Deprotected Rink amide resin, DIC, DMAP, NMM, DMF (quantitative); (v) $SnCl_2.2H_2O$, DMF, (70%).

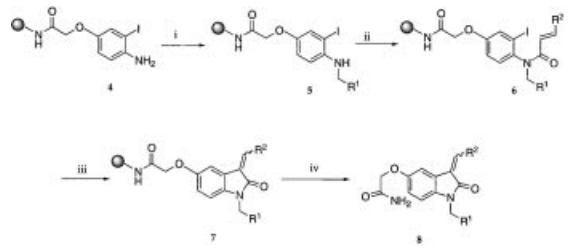

Fig. 2. Yields refer to the case, where R^1 = cyclohexyl and $R^2 = CH_3$. (i) R^1CHO, DCM, sonnicate; $NaBH(OAc)_3$, DCM, (95%); (ii) $R^2CH{=}CHCOCl$, DIPEA, DMAP, DMF, (90%); (iii) $Pd(OAc)_2$, Ag_2CO_3, PPh_3, DMF, (88%); (iv) 25% TFA, DCM, (92%).

The synthetic route from iodoaniline to oxindole is shown in Figure 2. The steps comprise reductive alkylation of aniline (**4**) with an aldehyde to give resin-bound secondary amine (**5**), which is then acylated with an α,β-unsaturated acyl chloride to give tertiary amide (**6**). The intramolecular Heck cyclisation of (**6**) to give oxindole (**8**) proceeds in 90% yield for the case (R^1 = cyclohexyl, R^2 = methyl). The proof of concept for expansion of this synthesis was carried out using three commercially available aldehydes and three α,β-unsaturated acid chlorides to generate the nine oxindoles (**8a–8i**) shown in Table I in respectable yields and variable purities after cleavage.

Further elaboration of such oxindoles was also demonstrated by the efficient 1,4-conjugate addition of soft nucleophiles, e.g., thiophenol, benzylmercaptan or diethyl malonate, onto the resin-bound oxindole (**7**) ($R^1 = CH_2CH(CH_3)_2$ R_2 = Ph)[Arumugam et al., 1997]. This is a robust example of the application of the intramolecular Heck cyclisation on solid phase, which could be expanded into a broader combinatorial synthesis.

Radical Cyclisation on Solid Phase

The application of carbon radical chemistry to solid phase organic synthesis presents intriguing possibilities for differences and potential advantages over the same chemistries carried out in a solution phase reaction. We setout to explore carbon radical chemistry on solid phase using the intramolecular radical cyclisation to form furans as a model reaction.[Routledge et al., 1997] Indeed, the intramolecular radical cyclisation has proved to be a valuable chemical reaction in the solution phase synthesis of carbocyclic and heterocyclic rings systems [Curran, 1988].

Figure 3 shows a reaction sequence for a model radical cyclisation in which radical initiator AIBN leads to the generation of a tributyl tin radical, which abstracts the bromine atom from substrate (**9**) to generate the aryl radical (**10**). Intramolecular radical cyclisation leads to formation of the alkyl radical (**11**), which can then abstract a hydrogen atom

TABLE I. Synthesis of Oxindole Analogues

Entry	R^1	R^2	Yield[a]	Purity[b]	E:Z[c]
8a	H	CH_3	91	65	3:1
8b	H	Ph	92	76	5.5:1
8c	H	H	65	10	—
8d	$CH_2C_6H_{11}$	CH_3	92	70	2.7:1
8e	$CH_2C_6H_{11}$	Ph	90	71	5.8:1
8f	$CH_2C_6H_{11}$	H	70	17	—
8g	$CH_2CH(CH_3)_2$	CH_3	90	82	3:1
8h	$CH_2CH(CH_3)_2$	Ph	90	70	5.9:1
8i	$CH_2CH(CH_3)_2$	H	75	16	—

[a]% mass recovered based on initial loading of resin.
[b]% Purity refers to the mixture of isomers and was determined by C-18 reverse phase HPLC (20–80% CH_3CN in H_2O containing 0.1% TFA), monitored at 254 nm using a UV detector and by a SEDEX Evaporative Light Scattering Detector.
[c](E):(Z) ratio was determined from NOSEY spectra and HPLC chromatograms. All compounds were characterised by ^1H NMR spectroscopy and by mass spectrometry.

from tributyl tin hydride both generating dihydrobenzofuran product and also propagating the tributyl tin radical for another round of radical cyclisation. The precursor (**9**) for solid phase dihydrobenzofuran synthesis was prepared on polystyrene resin via an ester linkage to the carboxy resin [Routledge et al., 1997]. The cyclisation reaction was carried out under standard conditions using < 1 mol equivalent ($\sim$5 mol %) of AIBN initiator and an excess (20–25-fold) of Bu_3SnH in toluene and methanol. The reaction was monitored by cleavage of the substrate/product, and showed that substrate turnover was low. Interestingly, increasing the quantity of AIBN lead to higher turnover with > 1 mol equivalent of AIBN being required for complete turnover of substrate. This suggested that the radical propagation during each reaction cycle was unsuccessful. The analogous cyclisation reaction was carried out using the precursor (**9**), but using Tentagel resin. In this case, complete cyclisation was observed using excess Bu_3SnH, but only 6 mol% of AIBN. These observations suggested a clear advantage provided by the polyethylene glycol matrix of Tentagel as compared to polystyrene for this reaction. A possible explanation might well be competitive hydrogen atom abstraction by the benzylic positions of polystyrene resin that does not occur as easily for Tentagel. A second possibility could be more favourable partitioning of Bu_3SnH into the matrix of Tentagel as compared to polystyrene. This is one of a series of clearly documented cases in the literature that demonstrate the potential importance of the resin matrix for solid phase synthetic chemistry. Prior to the synthesis, a potential concern was that carbon radicals are sufficiently reactive to be diverted by alternative pathways resulting in reduction of the aryl halide or intermolecular transfer of the bromine atom. However, only a single major product was obtained by this solid phase route.

These observations were exploited to demonstrate the solid phase synthesis of furans (Fig. 4). Precursors (**12**) were generated by coupling 2-butyne-1,4-diol to carboxy Tentagel, then treatment of commercially available alkenes under iodoetherification conditions [Routledge et al., 1997]. The radical cyclisation was carried out using 20–25 equivalents of Bu_3SnH and 5 mol% of AIBN to give the alkylidene tetrahydrofurans shown in Figure 4. It is noteworthy that cyclisation of an alkyl radical to a triple bond is about four orders of magnitude slower than the corresponding cyclisation of an aryl radical to a double bond (Fig. 3) [Beckwith and Schiesser, 1985]. Therefore, one might expect a greater tendancy to form byproducts in the presence of Bu_3SnH [Routledge and Weavers, 1993]. However, no byproducts were detected on the solid phase cyclisation. Indeed the solution phase reaction did show evidence of side reactions, for example the analogous solution phase synthesis

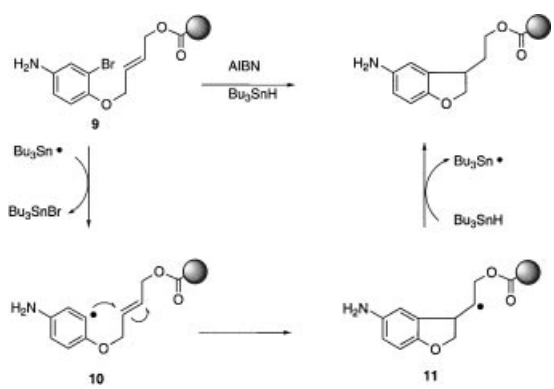

Fig. 3. Radical cyclisation leading to formation of dihydrobenzofuran product.

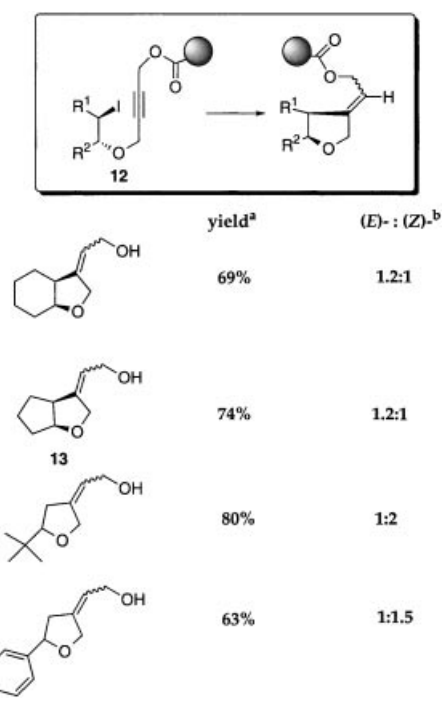

Fig. 4. Preparation of furans by solid phase radical cyclisation. [a]Isolated yield based on loading of the corresponding iodoacetylenic ether. [b]Determined by ^{1}H NMR spectroscopy.

of (**13**) gave a mixture of the desired product and the corresponding iodo alkylidene tetrahydrofuran product which has presumably resulted from iodine atom transfer [Routledge et al., 1997]. We attribute this significant difference to site isolation of the resin bound substrate radical, which would prevent the intermolecular iodine atom transfer in the solid phase case. A further feature that is noteworthy with this chemistry is that the excess tin residues could be removed from the resin with relative ease by cycles of washing and filtering, as compared to a more challenging purification that is normally required in solution phase. This was the first reported radical cyclisation on solid phase and it has been followed up by several other reports on resin-bound radical cyclisations [e.g. see Du and Armstrong, 1997; Miyabe et al., 2001].

Stereoselective Cyclisation

The oxopiperazines are an important class of constrained peptide mimics that constitute a significant number of bioactive compounds that target protein receptors in particular, for example Leu-Enkaphalin analogs, cholecystokinin receptor antagonists, and neurokinin-2 receptor ligands. Diketopiperazines have been observed as a byproduct during solid phase peptide synthesis in cases where an ester linker can be cleaved with cyclisation by attack of the deprotected *N*-terminus. This has been exploited in a synthesis of diketopiperazine libraries on solid phase [Gordon and Steele, 1995]. We planned an oxopiperazine synthesis based on analogous chemistry except generating a pseudodipeptide of lower oxidation level by the reductive alkylation of an *N*-protected α-amino aldehyde onto a resin-bound amino acid (Fig. 5). The pseudodipeptide (**14**) can be *N*-functionalised for example by acylation. Deprotection, acid cleavage, and overnight reflux then results in complete cyclisation to form oxopiperazine (**15**) in reasonable yields. The α-amino aldehyde is configurationally unstable to the reductive alkylation conditions employed, which leads to the formation of a mixture of diastereoisomers. Otherwise this synthetic route is robust and valid for a wide range of substituents (R^1, R^2, R^3). Figure 6 shows a potential variation if one acylates the protected pseudodipeptide with α-chloroacetyl chloride to generate the intermediate (**16**), which can now undergo one of two possible cyclisations. If the protecting group is Boc, one can cleave/deprotect and cyclise to generate a "type I" oxopiperazine akin to (**15**). Alternatively, it should be possible to use Fmoc chemistry and carry out a deprotection of (**16**) with mild base followed by intramolecular S_N2 chemistry to form a "type II" oxopiperazine on-resin (**17**). Resin-bound type II oxopiperazines can be further *N*-functionalised on resin to generate 1,4,5-substituted oxopiperazines.

To test the on-bead cyclisation approach shown in Figure 6 (L) isoleucine (S-configuration) was loaded onto Tentagel using a Wang linker and the pseudo dipeptide was generated by reductive alkylation of Fmoc-protected phenylalinal (S-configuration) onto the deprotected amino acid. Acylation with chloroacetyl chloride gave the intermediate (**18**) (Fig. 7). In accordance with expectations, (**19**) was in fact a mixture of diatereoisomers owing to some epimerisation of the phenylalinal derived stereogenic center during reductive alkylation. A rapid removal of Fmoc using piperidine, was

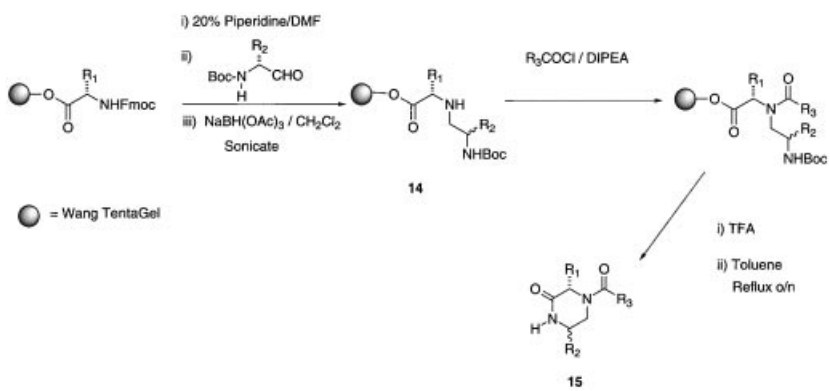

Fig. 5. Synthesis of oxopiperazines on solid phase.

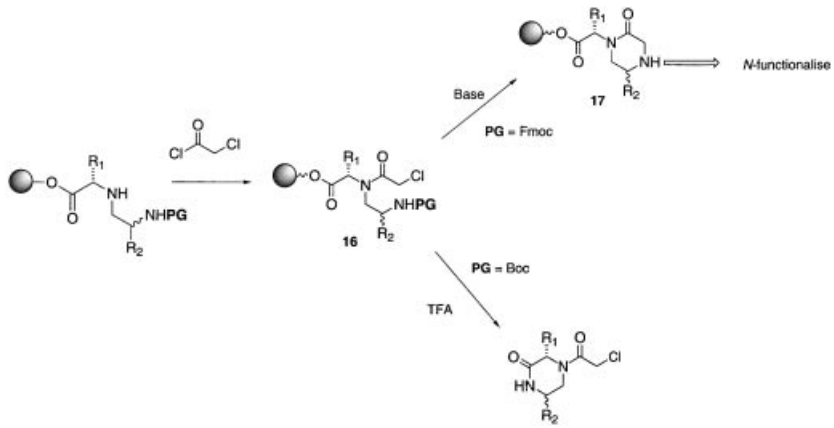

Fig. 6. Alternative cyclisation pathways leading to different oxopiperazines.

followed by a slower DIPEA promoted S_N2 intramolecular cyclisation to generate the type II oxopiperazine (**19**) in 35% yield. (**19**) was characterised by LC–MS, NMR spectroscopy and by X-ray crystallography of the *N*-toluene sulphonyl derivative. Surprisingly, (**19**) was found to be a single pure diastereoisomer of (S, R) configuration as shown (Fig. 7), in spite of being formed from a disatereoisomeric precursor. A mechanistic rationale for this observation was provided by analysis of the residual solution after the DIPEA promoted cyclisation. The residues off resin contained significant quantities of type I oxopiperazine (**20**) comprising major and minor diastereoisomers by LC–MS analysis, which we have presumed to be the (2S, 5S) and (2S, 5R), respectively. It appears that upon deprotection of the *N*-terminus of intermediate (**18**), one diastereoisomer (S, S) preferentially undergoes cyclisation and premature cleavage to generate the type I oxopiperazine (**20**), whilst the other diastereoisomer (S, R) cleaves relatively slowly, but prefers to undergo on-bead cyclisation to form a type II oxopiperazine (**19**). The apparent kinetic preferences for cyclisation by each of the diastereoisomers are illustrated in Figure 8. This mechanistic hypothesis is supported by the observation that when repeating the reaction sequence with (L) Isoleucine (S-configuration) and Fmoc-protected phenylalinal (R-configuration), the yield of oxopiperazine (**19**) increases from 35–75%. This is presumably due to incomplete epimerisation of the R-configuration stereocenter derived from phenylalinal which leads to the (S, R) diastereoisomer as the major intermediate, which favours on-bead cyclisation (Path B, Fig. 8). This is the first example we are aware of, of a "self-purifying" reaction pathway that results from stereoselective cyclisation on solid phase. The full details of this stereoselective cyclisation with more examples will be reported in due course [Khan et al., 2001].

Future Prospects

This article has discussed three examples of cyclisation chemistries that have been studied on solid phase. Such studies contribute to the

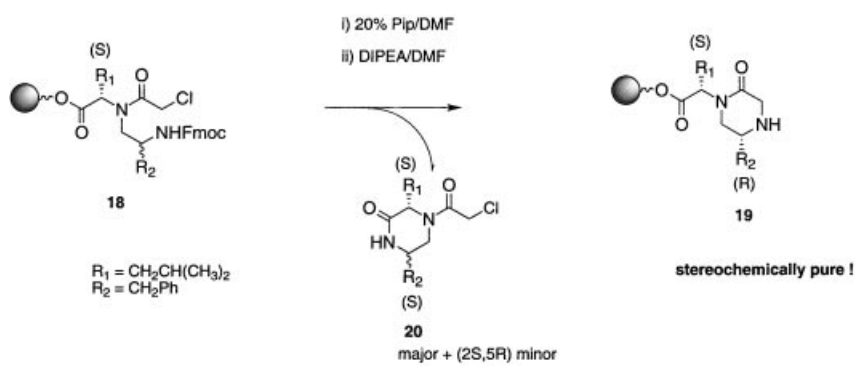

Fig. 7. Stereoselective on-bead cyclisation.

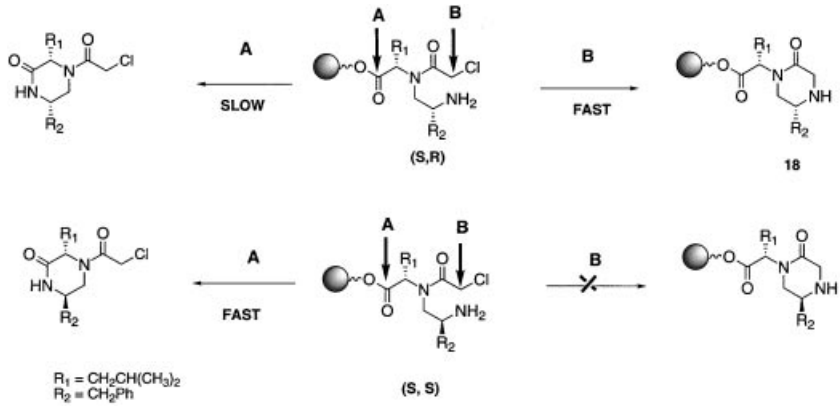

Fig. 8. Kinetic preference for cyclisation.

proof of concept of new chemical technologies that provide an ever broadening toolkit for chemists to generate compounds of potential interest for discovery chemistry. It is noteworthy that new directions in the field, now more broadly referred to as "high speed chemistry", include technological advances that exploit solid phase catalysts and reagents, and resin capture as approaches to assist *solution* phase synthetic organic chemistry. These advances should be seen as complementary to the continuing developments in the field of solid phase organic synthesis and together such technologies will provide a set of tools to assist and enable discovery chemistry in the future.

REFERENCES

Arumugam V, Routledge A, Abell C, Balasubramanian S. 1997. Synthesis of 2-oxindole derivatives via the intramolecular Heck reaction on solid support. Tetrahedron Lett 38:6473–6476.

Beckwith ALJ, Schiesser CH. 1985. Regio- and stereoselectivity of alkenyl radical ring closure: A theoretical study. Tetrahedron 41:3925–3941.

Cortese NA, Ziegler CB, Hrnjez BJ, Heck RF. 1978. Palladium-catalysed synthesis of 2-quinolone derivatives from 2-indoanilines. J Org Chem 43:2952–2958.

Curran DP. 1988. The design and application of free radical chain reactions in organic synthesis. Part 2. Synthesis 489–513.

Du XH, Armstrong RW. 1997. Synthesis of benzofuran derivatives on solid support via SmI_2-mediated radical cyclization. J Org Chem 62:5678–5679.

Goff DA, Zuckermann RN. 1995. Solid-phase synthesis of highly substituted peptoid 1(2*H*)-isoquinolinones. J Org Chem 60:5748–5749.

Gordon KH, Balasubramanian S. 2001. Exploring a benzyloxyaniline linker utilizing cerium ammonium nitrate (CAN) as a cleavage reagent: Solid-phase synthesis on *N*-unsubstituted-lactams and secondary amides. Org Lett 3:53–56.

Gordon DW, Steele J. 1995. Reductive alkylation on a solid-phase—Synthesis of a piperazinedione combinatorial library. Biorg Med Chem Lett 5:47–50.

Khan N, Cano M, Balasubramanian S. 2001 submitted.

Miyabe H, Fujii K, Tanaka H, Naito T. 2001. Solid-phase tandem radical addition-cyclisation reaction of oxime ethers. Chem Commun 9:831–832.

Routledge A, Weavers RT. 1993. Synthesis of iodoalkylidene lactones from alkenes. Tetrahedron 49:4229–4252.

Routledge A, Abell C, Balasubramanian S. 1997. An investigation into solid-phase radical chemistry—Synthesis of furan rings. Synlett 61–62.

Yu KL, Deshpande MS, Vyas DM. 1994. Heck reactions in solid-phase synthesis. Tetahedron Lett 35:8919–8922.

Yun W, Mohan R. 1996. Heck reaction on solid support: Synthesis of indole analogs. Tetrahedron Lett 37:7189–7192.