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Exploring a chemical encoding strategy for combinatorial synthesis using Friedel–Crafts alkylation

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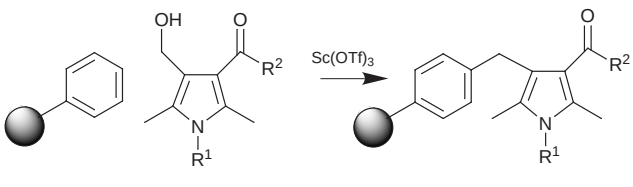
The use of scandium(III) triflate and ytterbium(III) triflate-catalysed Friedel–Crafts alkylation to insert a set of hydroxymethyl pyrrole amide tags (1b–i) on to polystyrene resins under mild conditions and the encoding of a split and mix peptide library is demonstrated.

Combinatorial approaches to molecular problem solving are the subject of great conceptual and experimental exploration. The approach is dependent on the ability to rapidly generate and screen large and diverse libraries.[1,2] The split and mix synthesis method,[3] which generates 'one-bead-one-compound' libraries, is a powerful approach, although the identification of active compounds remains a technical issue. A variety of deconvolution protocols exist,[1,4] of which the chemical tag-based encoding strategy is an inexpensive and general method that can be employed without the need for specialised equipment.[5,6] Key issues associated with chemical tagging include: the need for convenient, orthogonal chemistry for the attachment of tags to resin beads without disruption of the library molecules; the design of tags that are sufficiently robust to withstand a wide range of chemistry; and a simple cleavage and sensitive analysis strategy for decoding.

We have explored the use of Lewis acid-catalysed Friedel–Crafts alkylation to insert tags directly into the aromatic rings of polystyrene or other electron rich aromatic rings incorporated into the polymer. Friedel–Crafts chemistry has previously been used to functionalise polystyrene resins.[7,8] Having studied a variety of hydroxymethyl aromatics, the pyrrole derivative **1a** (R^1 = H, R^2 = OMe) was found to have promising reactivity with toluene (used as a resin mimic), reacting in 80% yield after 12 h at room temperature using a scandium catalyst (Scheme 1).[9] Scandium(III) triflate has particular advantages as the choice of Lewis acid since it is relatively insensitive to water, catalyses alkylations with alcohols[10] and its use has been demonstrated for solid phase chemistry.[11]

A mixture of polar and non-polar solvents was essential to retain both resin swelling and solubility of the scandium triflate. Since only small amounts of each tag need be incorporated into a bead (typically 1% of the resin capacity equating to about 0.05% of the available aromatic rings), the model solution phase reaction used 100 equiv. of toluene. Therefore in these reactions, the tags were present at relatively low concentrations. For acceptable reaction rates (completion in < 1 day), the concentration of Lewis acid required was at millimolar levels (which often equates to several equivalents compared to the tags).

Owing to the high reactivity of the pyrollic tag molecule with toluene, more stable amide derivatives were also prepared starting from the fully substituted methyl 4-formyl-1,2,5-trimethylpyrrole-3-carboxylate **2** (Scheme 2). Pyrrole ester **2** was hydrolysed to the free acid, then coupled to a variety of secondary amines. Reduction of the formyl group with $NaBH_4$ on alumina gave the corresponding alcohols **1b–i** in overall yields of 54–90% and these were found to be stable at −20 °C for at least six months. Once attached to the resin loss of the encoding tag is unlikely as the amide linkage is chemically robust, although in principle a linkage of any choice could be utilised in this methodology.



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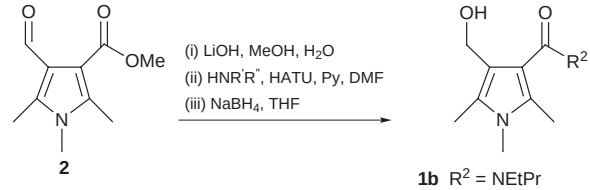
Scheme 1

Scandium-catalysed reaction of alcohol **1c** with toluene at room temperature was essentially complete in 2 days to give 74% of the desired product **3** and 23% of side product **4** (Table 1). Reaction of **1c** with the more reactive 1,3-dimethoxybenzene (DMB, 100 equiv.) resulted in a substantially improved reaction giving **5** in almost quantitative yield in only a few minutes. In the absence of an aromatic substrate, **1c** was shown to react slowly with itself in the presence of $ScOTf_3$ to form a dimer **4**, most likely by self-condensation and subsequent deformylation. These solution phase studies suggested that a resin containing a nucleophile more reactive than toluene would be less prone to side reactions.

The kinetics of pyrrole amide **1c** reacting with various polystyrene resins was followed by observing the loss of **1c** from the reaction mixture as compared to a control with no resin present. The observed reaction rate of amide **1c** with chloromethylpolystyrene **6** (Aldrich, 200–400 mesh) was comparable to the model studies with toluene (Table 1) under similar conditions. Reaction of **1c** with the more reactive, acetylated Wang resin **7** (Novabiochem, 200–400 mesh) gave the expected rate improvement leading to a half-life of 1.5 h as compared to 10.5 h for **6**. These reactivity studies are consistent with the observations made for the model reactions of **1c** with toluene and DMB and suggests that the resin environment does not impede the desired chemistry.

Chloromethylpolystyrene (Polymer Labs, 50–60 mesh, 1.8 mmol g^{-1}) was reacted with 3-methoxyphenol and subsequently 4-hydroxymethylphenol, to give the modified Wang resin **8**, which could be used for a library synthesis. However, some degree of cleavage of the Wang linker was observed when resin **8**, loaded with Fmoc-alanine, was treated with 10 mM



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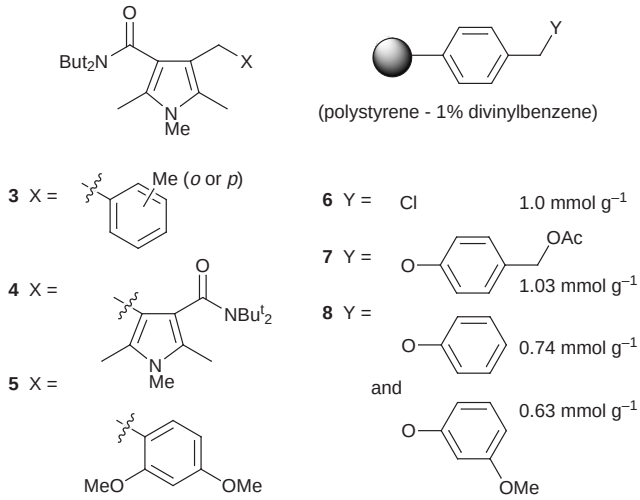
1b R^2 = NEtPr
c R^2 = NBu_2
d R^2 = $N(C_5H_{11})_2$
e R^2 = $N(C_6H_{13})_2$
f R^2 = $NMeC_{12}H_{25}$
g R^2 = $N(C_8H_{17})_2$
h R^2 = $N(C_{10}H_{21})_2$
i R^2 = $N(C_{12}H_{25})_2$

Scheme 2



scandium triflate (6.2% cleavage in 3 h). It was found that ytterbium triflate, which has similar properties to scandium triflate, [12] was more compatible with Wang resin, giving only 0.9% cleavage of the linker in 3 h.

To demonstrate that this technology can be applied to the encoding of a combinatorial library, a split and mix[3] tripeptide library (*step 1*, ala, gly, leu; *step 2*, val, met, phe; *step 3*, trp, ser, ser-lys) was synthesised on resin **8** and encoded with alcohols **1d**–**1i**. Three batches of resin **8** were coupled to an amino acid using benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) chemistry, the resin was capped with Ac_2O after each step and subsequently reacted with the appropriate encoding pyrroles **1d**–**i**, at 30 μmol g^{-1} of resin (approx. 300 pmol per bead, 3% compared to library loading) in 15 mM ytterbium triflate in 2 : 1 $ClCH_2CH_2Cl$–$MeNO_2$ for 16 h. The beads were then mixed and split and the Fmoc group removed ready for the next round of synthesis. The peptides, from single beads, were cleaved in TFA (95%), then the beads were subjected to hydrolysis with 5.6 M HCl to cleave the secondary amines from the encoding tags. The hydrolysate was dried *in vacuo* then reacted with dansyl chloride in the presence of Na_2CO_3 and analysed by fluorescence HPLC.

The only limitation revealed in this experiment was that no incorporation of the pyrroles was seen to beads that had incorporated methionine, presumably due to *S*-alkylation of the pyrrole tags. However, no other amino acid in the library appeared to interfere with the Friedel–Crafts encoding chemistry (notably phenylalanine and tryptophan). A number of beads were decoded and Fig. 1 shows a typical analysis of a single bead. The HPLC trace unambiguously identified the peptide from the encoding tags and the identity of the peptide was confirmed by electrospray mass spectrometry. The overall efficiency for incorporation, cleavage and detection for tags **1d**–**i** was 20 ± 10% based on a dansylated standard solution of the amines analysed under the same conditions.

Table 1 Friedel-Crafts reactions of **1c**[a]

Substrate (equiv.)	$ScOTf_3$/ mM	Reaction time/h	Unreacted **1c**[b] (%)	$t_{1/2}$/h for **1c**[c]	Yield (%)
None	10	24	63	> 48	**4** 37
None	20	48	31	17	**4** 69[d]
Toluene (100)	20	48	3	8	**3** 74[d] **4** 23[d]
DMB(40)	10	24	1	< 0.2	**5** 99[d]
Resin **6** (5)[e]	10	24	34	10.5	—
Resin **7** (4)[e]	10	24	2	1.5	—

[a] Reactions were carried out at room temperature in 2:1 $ClCH_2CH_2Cl$–$MeNO_2$ with 1 equiv. of **1c**. [b] Based on HPLC-ELSD analysis of the reaction mixture. [c] Half-lives measured in a more comprehensive study of the kinetics. [d] Products isolated and characterised by ^1H, ^{13}C NMR and FT-IR spectroscopy and high resolution mass spectrometry. [e] Compared to the loading.

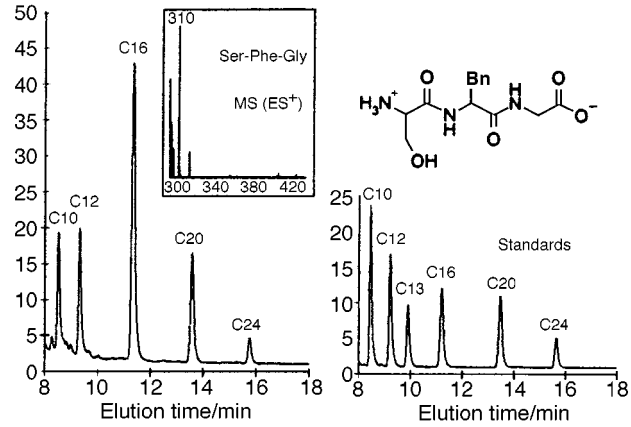


Fig. 1 Analysis of a bead: (*a*) mass spectrum of the cleaved peptide and (*b*) the HPLC trace of the cleaved tags (reverse phase, H_2O–MeCN, Novapak phenyl 1.5 × 150 mm column, excitation/emission at 340/500 nm).

These studies show that Friedel–Crafts insertion of such tags may be employed in a chemical encoding strategy. In this first instance, the methodology has been exemplified using secondary amine tags which were based on published work by the Affymax group,[5] however the pyrrole/Friedel–Crafts chemistry should also be suitable as a carrier for attachement of other tag moieties (*e.g.* MS tags) to a resin bead. In comparison with existing chemical encoding methods,[5,6] orthogonal protecting groups are not required and the tags are not randomly inserted but are directed into the resin backbone. Furthermore, it has been demonstrated that the insertion kinetics is tunable by doping the resin with electron-rich aromatics. The further application of this chemistry to the encoding of compound libraries is currently being carried out to evaluate its broader compatibility with other resins and chemistries.

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