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Preparation of a Novel Resin Derived from Ion-Exchange Resin and its Evaluation in Peptide- and Benzodiazepine Synthesis

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ABSTRACT The preparation of an extremely cheap and novel resin synthesised from a commercially available ion exchange resin is described. The free carboxylic acid group of the resin was converted into a methylester. Subsequent reduction of this ester to the corresponding alcohol has been carried out in one step, by using a solution of DIBAH as a reducing agent. The application of this resin for solid phase combinatorial synthesis has been evaluated by synthesizing 2 representative peptides and 2 selected 1,4-benzodiazepines.

KEYWORDS: solid phase chemistry, novel resin, ester linkage, DIBAH reduction, ion-exchange resin.

INTRODUCTION

Parallel multi-step solid phase combinatorial synthesis (SPCS)[1] relies on the availability of suitable resins for the desired chemical reaction. A great deal of effort has been invested in the development of novel linkers,[2] in which the template molecule is connected to the resin and then cleaved from the solid support after the reaction sequence is carried out. We have to bear in mind that the ester linkage is still the most common and reliable linkage, using an activated free carboxylic acid and a solid supported hydroxy group for its formation.

Historically, the Merrifield resin was the most important resin, being widely used for combinatorial peptide synthesis. It was also the starting point for small molecule combinatorial synthesis.[3] Its good swelling properties and ease of preparation enhance its importance and popularity.

In a remarkable paper,[4] the synthesis of peptides on cellulose was reported by attaching the peptides *via* an ester linkage to the solid support. The most recent development in resin technology is the SynPhase crown, in which the resin is grafted onto a polystyrene carrier and the chemistry is carried out on the surface of this crown.

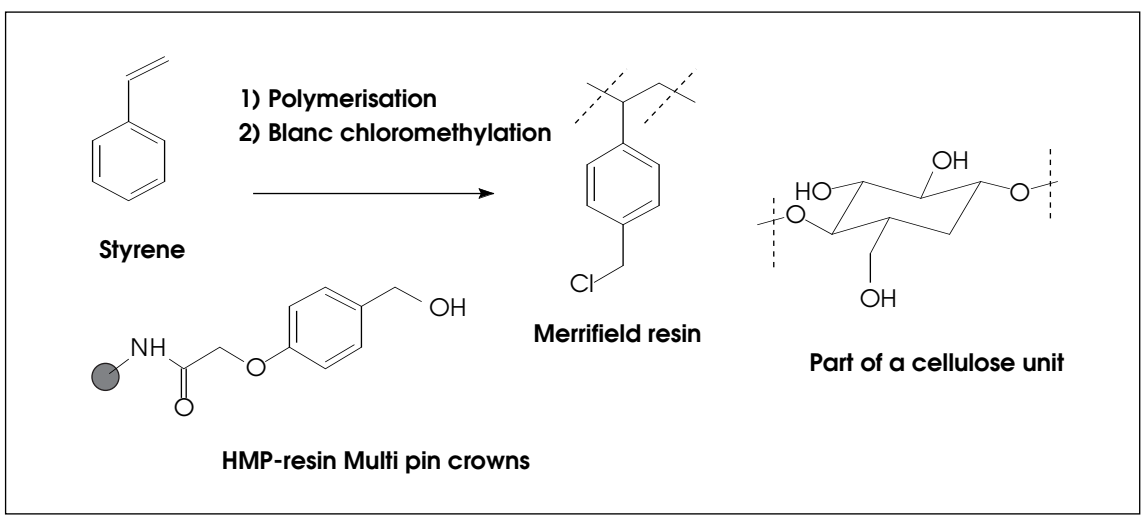


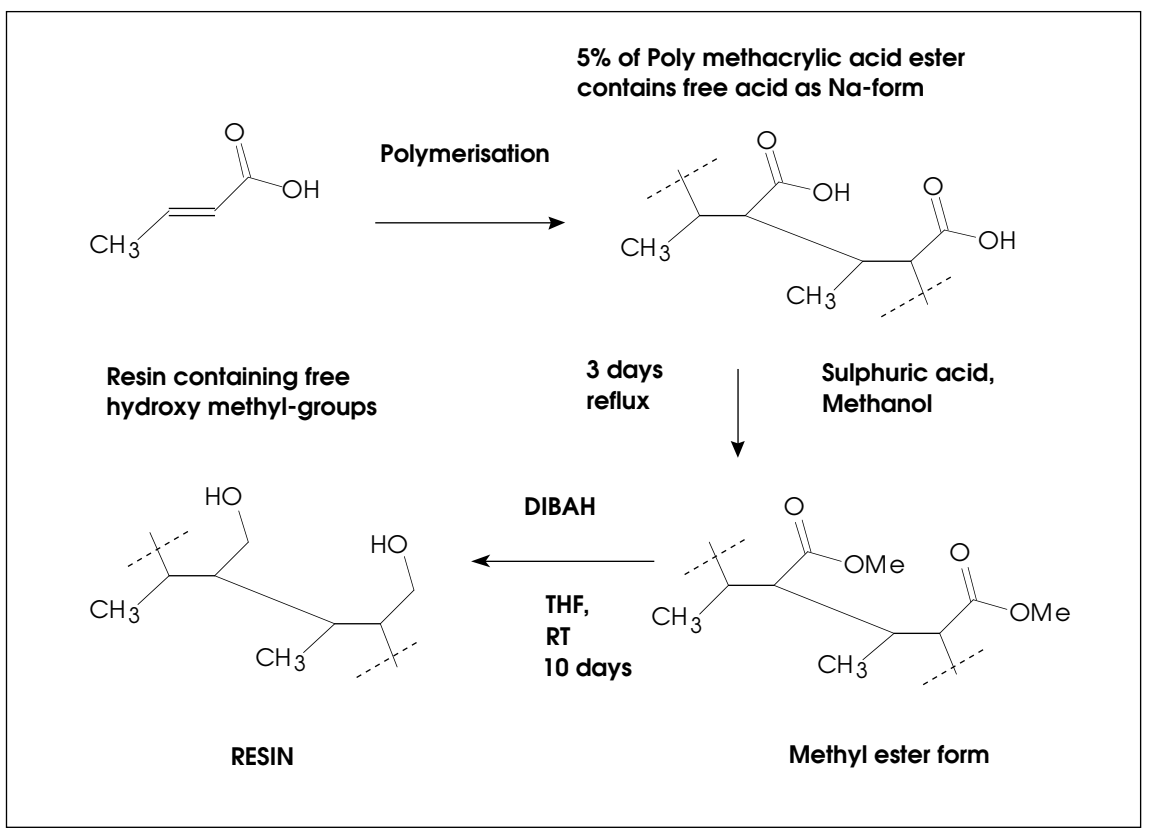
Fig 1.

In this paper we report the preparation of a novel resin derived from a commercially available ion exchange resin, and its application to solid phase synthesis, enabling an economic solid phase synthesis for the first time. Our new resin, derived from ion exchange resin, has an extremely low price and is easy to handle in solid phase synthesis[5] due to its suitable bead size. Filtration is feasible without the need for any special peptide flasks containing a fritt in the bottom of the flask. All the washing steps for purification are fast and very efficient. Two different chemical approaches were applied to chemically evaluate this new resin, by synthesizing a dipeptide and a 1,4-benzodiazepine as an example of a small organic molecule.[6]

A variety of bead sizes are available and we have selected Mesh 50 bead size which enables extremely easy handling. Washing, filtration and dispensing are all easy and comparable to the recently developed SynPhase crowns.

Commercially available resins include anion exchange resins, which contain a quaternary ammonium ion and two types of cation exchange resins. The cation exchange resins contain either the sulfonic acid functionality, as strongly acidic cation exchange resin, or the carboxylic acid functionality as mild acidic cation exchange resin. A cation exchange resin containing the carboxylic acid functionality, as the reactive group, was selected as the starting material for our development of the novel solid support. Attempts to derivitise other resins were unsuccessful or resulted in a lower loading.

Commercially available Amberlite IRC-50, a weak acidic cation exchanger, containing a carboxylic acid functionality on polymethacrylic matrix was esterified with an excess of methanol and a catalytic amount of sulphuric acid. Even though only a small percentage of the resin, which is usually about 5% of the methacrylic acid ester, is present in form of the free carboxylic acid, the initial esterification is essential to achieve the highest possible loading. In the initial esterification, all functionality of the surface, suitable for subsequent chemical reactions, were converted into a defined end group, the methylester, and not a mixture of ester and free acid functionality. After repeated washings with THF the



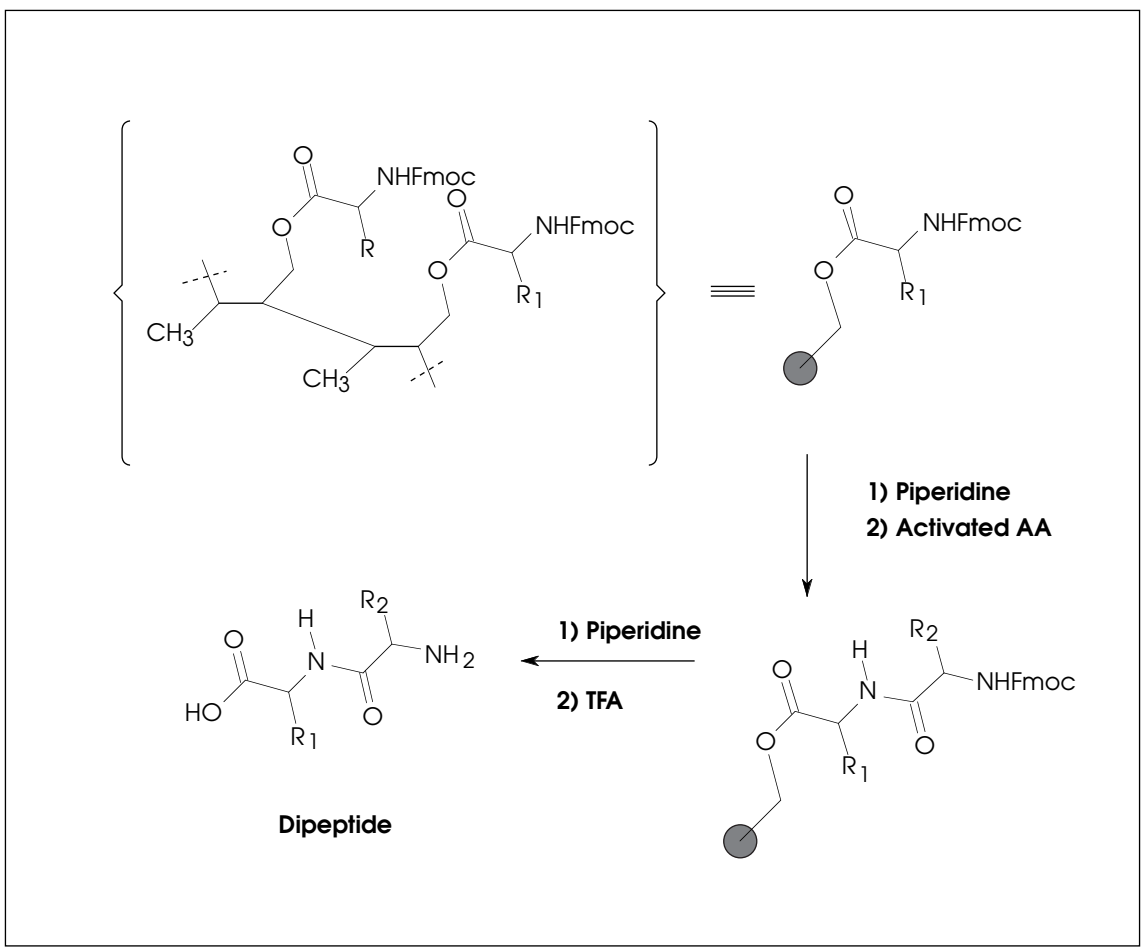
Scheme 1 .
Preparation of the novel resin, derived from the cation-exchange resin Amberlite IRC-50.

methylester was reduced by using a solution of DIBAH in THF at ambient temperature over a period of 10 days. For work up, repeated washings with methanol and a trace of TFA were applied followed by dry methanol and THF in order to remove traces of the acid. Additionally, very large and very small beads were also removed in this washing cycle, resulting in a resin with a defined range of bead size. Drying of the resin in a vacuum oven decreased the loading dramatically and must be avoided. The resin can be kept in a freezer without losing the initial chemical loading over a period of some months. Analogue to the SynPhase crowns, the functional group interconversion cannot be monitored directly on the beads by standard IR spectroscopy. Therefore, the beads were grinded to a fine powder on which the progress of the reduction, from the ester group to the alcohol, could be monitored by using standard FT-IR spectroscopy in a form of a KBr disk or a suspension in chloroform.

The syntheses of two selected peptides[7] and two representative 1,4-benzodiazepines[8] are outlined in Scheme 2 and Scheme 3, respectively, demonstrating generally the practical use of this new resin.

The synthesis of the dipeptides is shown in scheme 2. The first amino acid which was either phenylalanine or tryptophan was suspended in a mixture of DMF/DCM (1:4). DIC (disosopropylcarbodiimide) was then added to convert the amino acid to its reactive form. The resin bearing the hydroxy group was reacted with the activated amino acid forming the desired ester linkage at ambient temperature over night. This coupling step was repeated at least once. The first step that both syntheses have in common was the coupling of the amino acid to the resin *via* an ester linkage.

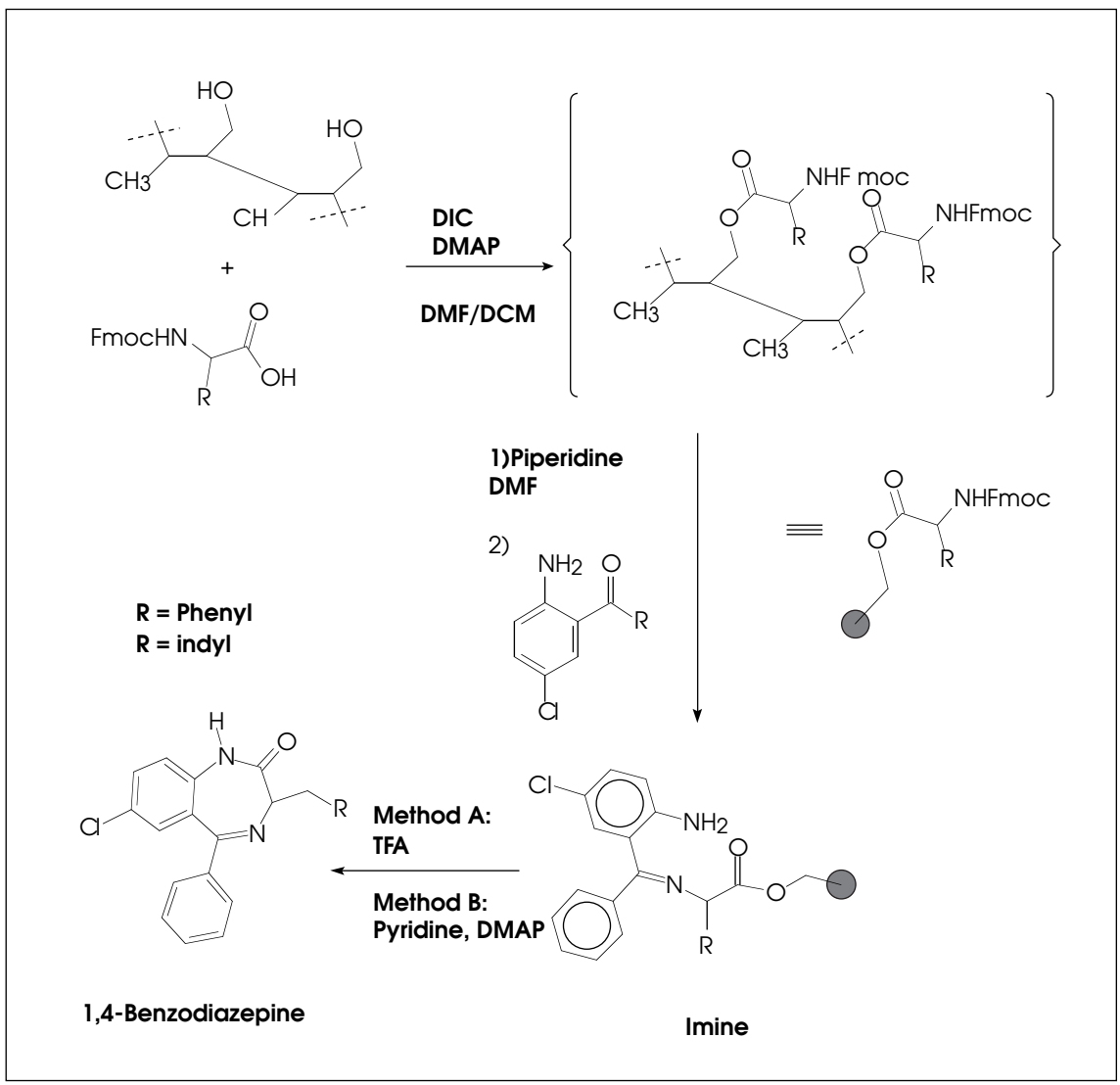
The Fmoc protecting group was removed with piperidine and the free amino acid attached to the resin was washed at least three times with a mixture



Scheme 2 .
Preparation of dipeptides.

of DMF/DCM. A solution of the next DIC-activated amino acid was added and the formation of the dipeptide proceeded at room temperature over night. A 20% solution of piperidine in DMF was used to remove the Fmoc protecting group and the desired dipeptide was cleaved from the resin with trifluoroacetic acid in DCM at RT over a period of hours. The crude material was purified by preparative TLC to determine the exact yield.

The synthesis of 1,4-benzodiazepines, as a typical example of a small non-peptide organic molecule, is illustrated in Scheme 3. The benzodiazepine synthesis had the first step, the coupling of the amino acid to the resin, with the peptide synthesis in common. The loading based on this first coupling step was calculated in mmol per gramm on the weight difference of the dried resin before and after this first step. The Fmoc protecting group was cleaved with piperidine and after repeated washings with DCM/DMF, the resin was reacted with a concentrated solution (20%) of p-chloroamino-benzophenone in dichloroethane at 60°C over a period of 2 days. The reaction of the resin bound imine with TFA or pyridine with a catalytic amount of DMAP gave the desired 1,4-benzodiazepines.[9] These were purified further by preparative TLC.



Scheme 3 .
Second chemical evaluation of the solid support: Synthesis of 1,4-benzo-diazepines.

Table 1

Resin	Weight / g	Loading / mmol	Price /g for r esin	Price / mmol compound
Amberlite **(A)**	1	0.08	3.6 p	45 p
HMP-crown **(C)**	1	0.05	£ 17.0	£ 340
Wang -resin **(W)**	1	0.60	£ 6.6	£ 11

In Table 1 the loading for one gramm of the resin, derived from Amberlite [A], the HMP-crown [C] and one commercially available Wang resin [W] are compared. It was shown that the newly synthesised resin (Ambelite) had a slightly higher loading than the recently developed HMP SynPhase crown, if the loading is calculated per weight of the solid support. Considering the cost for the resins, resin [A] derived from Amberlite is 24 times cheaper than the comparable Wang resin [W] and is much easier to handle because of its ideal bead size. It is 755 times less costly than the SynPhase crowns [C]. The data, given in Table 1, are based on the price for the resin material only and exclude labour costs and chemicals used to modify the resin.

In Table 2 the yields for 4 compounds, two peptides **entry 1-6** and two 3-substituted 1,4-benzodiazepines **entry 7-12** are given which were prepared on the three resins [A], [C] and [W]. Generally, the SynPhase crowns exhibit the highest chemical yields for both synthetic pathways, followed by the commercially available Wang resin [W] and the newly developed solid phase support [A].

Currently, the solid phase approach can only be used to provide small quantities of compounds for screening proposes. This is because of the enormous costs of preparing compounds by the solid phase approach. Exploring this approach further, there is the opportunity to carry out a technical synthesis on a larger scale, using this inexpensive, readily available solid support. In a technical synthesis the solid phase reaction could be driven to completion by using a large excess of the reagents, providing the desired products in very good yields. After the reaction was carried out, the reagents could be recycled and thus reducing chemical wastes to a minimum.

Table 2

Entry	Compounds	Resins	Loading [10] mmol/g	Yield [11] %
1	Phe-Gly	A	0.08	52
2		C	0.05	78
3		W	0.60	72
4	Trp-Phe	A	0.08	55
5		C	0.05	72
6		W	0.60	76
7	BDZ, R=Phe	A	0.08	43
8		C	0.05	62
9		W	0.60	59
10	BDZ, R=indol	A	0.08	39
11		C	0.05	58
12		W	0.60	61

[A] *Amberlite IRC-50*, methacrylic acid matrix, weakly acetic ion exchanger, carboxylic acid functionality; 1 kg £36

[C] 4-(hydroxymethyl)phenoxyacetamido handle multipin crown, 100 crowns ?340, 10 micomol, Chiron Mimotopes Ltd. crown weight: 190 mg, 0.095 mmol

[W] Wang resin (p-benzyloxy)benzyl alcohol resin 5g 355, 100 g £660; novabiochem.

In conclusion, a novel resin was synthesized, derived from the readily available Amberlite in 2 steps, an esterfication and a reduction with DIBAH. The bead size enables simplified handling comparable to most recently developed SynPhase crown technology. The utility of the newly derived resin was proved by synthesizing two peptides and two 1,4-benzodiazepines as typical representatives of small organic molecules and is applicable to combinatorial solid phase synthesis.

Notes and References

Entry 7-9: 3(S)-Benzyl-7-chloro-5-phenyl-1,3-dihydro-benzo[e-1,4]diazepin-2-one: APCI + m/s: m/z = 361; IR (KBr disc) υ_{max} = 3341, 3205, 3058, 2933, 1706, 1476, 1320, 1234, 1091, 736, 699 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.58 (d, J = 7Hz , 2H, , 2 H, $C_3HC\underline{H}_2$), 3.79 (t, J = 7Hz, 1H, $C_3\underline{H}$), 7.13 (d, J = 9Hz, 1H), 7.2 - 7.5 (m, 12H, arom. H), 9.45 ppm (br s, 1 H, $N_1\underline{H}$); ^{13}C-NMR ($CDCl_3$) δ 36.8 (C-1`), 61.2 (C-3), 121.9, 125.8, 128.3, 128.6, 129.0, 129.5, 129.6, 130.6, 130.8, 131.4, 137.3, 138.8, 139.4, 164.8 (C-5), 171.8 (C-2).

Entry 10-12: 7-Chloro-3(S)-(1*H*-indol-3ylmethyl)-5-phenyl-1,3-dihydro-benzo[e-1,4]diazepin-2-one: APCI + m/s: m/z = 400; IR (KBr disc) υ_{max} = 3411, 3220, 2921, 1677, 1600, 1481, 1322, 1226, 1095, 829, 742 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.68 (t, J = 9 Hz, 1 H, $C_3\underline{H}$), 3.82 (d, J = 8 Hz, 2 H, $C_3HC\underline{H}_2$), 7.0-7.7 (m, 13 H, arom. H), 8.05 (br s, 1 H, indole $N\underline{H}$), 8.68 ppm (br s, 1 H, $N_1\underline{H}$); ^{13}C-NMR ($CDCl_3$) δ 36.8 (C-1`), 61.2 (C-3), 112.1, 120.5, 121.7, 121.9, 122.8, 125.8, 128.3, 128.6, 129.0, 129.5, 129.6, 130.6, 130.8, 131.4, 136.5, 137.3, 138.8, 139.4, 164.8 (C-5), 171.8 (C-2).

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10. in mmol per g based on weight difference between resin and resin + Fmoc-amino acid.
11. isolated yield of pure compound purified by preparative TLC over all steps of the reaction sequence.