

# Investigation of Parameters that Affect Resin Swelling in Green Solvents

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The influence of various physical and chemical factors on the swelling of polystyrene and PEG based resins in greener organic solvents has been systematically investigated. In general, chemical factors: the nature of the functionality/linker and the degree of loading were found to have a far larger influence on the swelling of the resins than physical parameters such as bead size. The results are interpreted in terms of Hansen

solubility parameters for the solvents and there is evidence that some solvents interact with the polymeric core of a resin whilst others interact with the functionality. The results are extended to a study of the changes in resin swelling observed during both deprotection and chain elongation reactions during solid phase peptide synthesis.

## 1. Introduction

By far the most important source of waste in the pharmaceuticals, agrochemicals and fine chemicals sectors of the chemicals industry is solvent which can account for up to 90% of the total chemical mass used in batch reactions.<sup>[1]</sup> In addition to their detrimental effect on the *E* factor<sup>[2]</sup> for a reaction, widely used conventional solvents are increasingly being found to be highly toxic. Examples include the polar aprotic solvents dimethylformamide (DMF), dimethylacetamide (DMA) and *N*-methyl-2-pyrrolidone (NMP) all of which are reprotoxic,<sup>[3]</sup> resulting in their classification as substances of highest concern under the EU REACH regulations.<sup>[4]</sup> The chlorinated solvent, 1,2-dichloroethane, is a known carcinogen and is classified as a substance of very high concern.<sup>[5]</sup> As a result, there is extensive interest in the development of replacements for conventional solvents which have low toxicity and are sustainably sourced.<sup>[6]</sup>

There is currently considerable interest in making solid-phase organic synthesis (SPOS) and especially solid phase peptide synthesis (SPPS) more sustainable.<sup>[7]</sup> However, SPOS is traditionally very dependent on the highly toxic polar aprotic and chlorinated solvents as the resins typically used in SPOS swell in these solvents.<sup>[8,9,10]</sup> This was apparent in our previous work where propylene carbonate (**S1**) was used instead of conventional solvents in SPPS.<sup>[11]</sup> It totally failed to swell cross-


linked polystyrene based resins such as the widely used Merrifield resin (cross-linked chloromethyl polystyrene), so its use in SPPS was restricted to the considerably more expensive polyethylene glycol (PEG) based ChemMatrix resin.


As a result of this limitation, we investigated<sup>[12]</sup> the ability of 25 green solvents to swell nine commercially available resins used in SPOS and developed a model using the Hansen solubility parameters in practice software package<sup>[13]</sup> to predict resin swelling in a given solvent. This model was experimentally validated by using it to predict solvents for SPOS of a multicomponent Ugi condensation carried out on both cross-linked polystyrene and PEG-based resins. The model showed that the interaction between a solvent and resin was a complex process and that for binary solvent systems, resin swelling would be expected to vary non-linearly with solvent composition. This was experimentally validated and confirmed that a resin occupies a region of HSP space rather than a single point.<sup>[14]</sup>

SPOS resins (even if uniformly functionalised<sup>[15]</sup>) are not a homogeneous polymer; rather they consist of linear polymer chains, joined together by cross-links and partially functionalised to facilitate the attachment of organic compounds. As a result, their 3D-structure better resembles that of an enzyme, with regions that will interact very differently with a given solvent or solvent mixture, rather than that of a homogeneous linear polymer. This could explain the complex variation in swelling behaviour observed for SPOS resins, especially as the swelling could also be influenced by physical parameters such as bead size and degree of cross-linking.

There have only been limited previous studies on aspects of resin swelling in conventional solvents and no systematic study of the effect of multiple resin parameters.<sup>[16]</sup> Sarin *et al.* investigated how the swelling of Merrifield resin in dichloromethane and DMF varied during the construction of pseudo-peptides containing up to 60 monomer units and found that swelling increased as the length of the pseudo-peptide chain increased.<sup>[17]</sup> Related work by Rodionov *et al.* subsequently confirmed this trend and provided a theoretical basis for it.<sup>[18]</sup>

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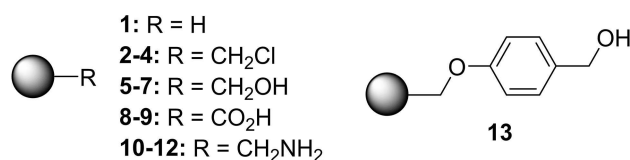
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Rana *et al.* studied the swelling of Merrifield resin with degrees of cross-linking between 0.3 and 6% in 15 solvents and three solvent mixtures.<sup>[19]</sup> The highest swelling was observed for the least cross-linked resins in chlorinated solvents. Nakaie *et al.* investigated the synthesis of the decapeptide salmon-LHRH on polystyrene based methylbenzhydrylamino resins and measured the swelling of each intermediate peptide.<sup>[20]</sup> The synthesis was conducted in three solvents (dichloromethane, DMF and dimethylsulfoxide) with resin loadings of both 0.3 and 2.6 mmol g<sup>-1</sup>. No major dependence of resin swelling on loading was observed. Therefore, we initiated a study to systematically investigate the effect of various parameters of commercially available resins on the swelling of the resin in greener solvents (as defined by the GSK green solvent guide<sup>[21]</sup>) with the aim of gaining an understanding of which factors influence resin swelling and their relative importance. In this paper we report the results of this study.

## 2. Results and Discussion

### 2.1. Cross-Linked Polystyrene Based Resins

Cross-linked polystyrene based resins were selected as the basis for this study as a range of resins with different functionalities (handles) and physical parameters are commercially available. Throughout this project, all measurements were made on the same batch of a resin to avoid potential issues associated with



**Figure 1.** Structures of the cross-linked polystyrene based resins used in this study. As commonly accepted, the sphere represents cross-linked polystyrene.

Table 1. Solvents S1–S15 used in this work and their Hansen solubility parameters.				
Solvent number	Solvent name	$\delta_D^{[a]}$	$\delta_P^{[a]}$	$\delta_H^{[a]}$
S1	Propylene carbonate	20.0	18.0	4.1
S2	Isopropyl acetate	14.9	4.5	8.2
S3	2-Methyltetrahydrofuran	16.9	5.0	4.3
S4	Methanol	14.7	12.3	22.3
S5	1-Heptanol	16.0	5.3	11.7
S6	1,2-Dihydroxyethane	17.0	11.0	26.0
S7	Ethanol	15.8	8.8	19.4
S8	2-Propanol	15.8	6.1	16.4
S9	1-Pentanol	15.9	5.9	13.9
S10	1-Propanol	16.0	6.8	17.4
S11	1-Butanol	16.0	5.7	15.8
S12	2-Butanone	16.0	9.0	5.1
S13	Anisole	17.8	4.4	6.9
S14	Acetone	15.5	10.4	7.0
S15	Cyclopentyl methyl ether	16.7	4.3	4.3

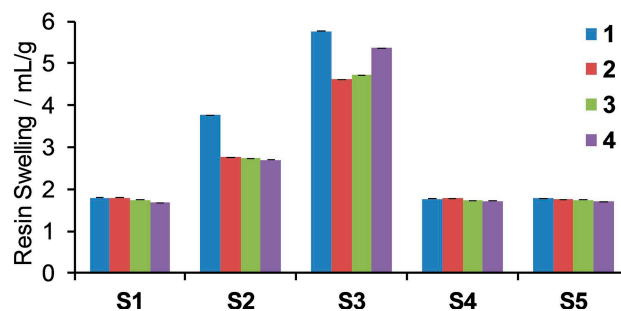
[a] HSPs obtained from reference 13;  $\delta_D$  = dispersion energy,  $\delta_P$  = dipolar energy and  $\delta_H$  = hydrogen bonding energy.

batch to batch variability of resin cross-linking and extent of functionalisation.<sup>[22]</sup> The first resin selected for this study was commercially available, unfunctionalised, 1% cross-linked polystyrene 1 (Figure 1) with a bead size of 37–75  $\mu\text{m}$  (200–400 mesh). This was chosen to provide baseline data to which results obtained on functionalised resins could be compared.

Based on our previous work,<sup>[12,14]</sup> five greener solvents S1–S5 (Table 1) were chosen as a basis set to study the swelling of the resins in this work, supplemented by additional solvents S6–S15 as appropriate on a case by case basis. Propylene carbonate, 2-methyltetrahydrofuran and isopropyl acetate (S1–S3) are all aprotic solvents but with a range of different polarities and hydrogen bond accepting abilities, reflected in their differing HSPs. Methanol and 1-heptanol (S4, S5) are both polar protic solvents, but with very different dipolar ( $\delta_P$ ) and hydrogen bonding ( $\delta_H$ ) energies within their HSPs.<sup>[13]</sup> Together, the five solvents cover a range of each of the HSPs. All five solvents are included in the latest version of the GSK green solvents guide<sup>[21]</sup> with S1 and S5 being the greenest carbonate and alcohol solvent respectively. S2 is in the green category for esters, whilst S3 and S4 receive yellow ratings (there are no green ethers in the guide).

The swelling of resins in each solvent was determined by measuring the increase in volume occupied by a resin sample held in a syringe on addition of the appropriate solvent according to the method of Griffith *et al.*<sup>[23]</sup> All resin swelling experiments were carried out in triplicate and the average value calculated along with error bars based on the standard deviations obtained for each solvent. For use in solid phase synthesis, a solvent which swells a given resin by at least 4.0 mL g<sup>-1</sup> is considered to be a good solvent; one which swells the resin by 2.0–4.0 mL g<sup>-1</sup> a moderate solvent and if the swelling is less than 2.0 mL g<sup>-1</sup>, then the solvent is considered a poor solvent.

Figure 2 shows the swelling data obtained for 1% cross-linked polystyrene 1 in each of the five solvents. Only S3 is a good solvent for this resin, S2 is a moderate solvent, though with a resin swelling of 3.8 mL g<sup>-1</sup>, it is close to the good solvent borderline. The other three solvents all swell resin 1 by just 1.8 mL g<sup>-1</sup> and are poor solvents. Table 1 shows that S2, S3 and S5 have very similar values of  $\delta_P$  (dipolar energy) and  $\delta_D$  (dispersion energy), but differ significantly in their  $\delta_H$  (hydrogen bonding energy) values with resin swelling increasing when  $\delta_H$  is less than eight. S1 also has a very low  $\delta_H$  value (4.1), but its  $\delta_P$



**Figure 2.** Swelling of resins 1–4 in solvents S1–S5.

is much higher than those of **S2**, **S3** and **S5** (18.0 versus 4.5–5.3). This suggests that good swelling of resin 1 requires a solvent with values of  $\delta_p$  and  $\delta_H$  below six and eight respectively. To further investigate this correlation, the swelling of resin 1 in anisole (**S13**) and cyclopentyl methyl ether (**S15**) was investigated. Based on their HSPs, both these solvents would be expected to be good solvents for resin 1 and this was found to be the case as they gave swellings of 6.8 and 6.5 mLg<sup>-1</sup> respectively.

Having obtained baseline data on resin 1, the swelling of three commercially available chloromethyl functionalised cross-linked polystyrene resins (**2–4**) was studied. Resins **2–4** all had similar reported loadings of 0.9–1.2 mmolg<sup>-1</sup>, but differed in their bead sizes and degrees of cross-linking as detailed in Table 2, with resin 4 having the same bead size and degree of cross-linking as unfunctionalised resin 1. The swelling data for resins **2–4** is also shown in Figure 2 and it is apparent that the three resins show very similar swelling in each solvent. Thus, bead size and degree of cross-linking do not appear to influence the resin swelling, at least within the rather narrow range that could be studied using commercially available resins. Rana *et al.* have previously shown that the swelling of chloromethyl functionalised cross-linked polystyrene (Merrifield resin) does decrease as the degree of cross-linking increases over a significantly wider range of 0.3–6.0%.<sup>[19]</sup> In a recent paper we mapped out the high resin swelling area of Merrifield resin in HSP space using binary solvent mixtures.<sup>[14]</sup> This work showed that a good solvent for swelling Merrifield resin should have  $\delta_H$  as low as possible,  $\delta_p < 10$  and  $\delta_D$  in the range of 16–20. Of the five solvents **S1–S5**, only **S3** meets all of these requirements. Figure 2 also shows that the introduction of ca. 1 mmolg<sup>-1</sup> of chloromethyl groups (equivalent to the resin containing just 3.6% chlorine), reduces the resin swelling in both **S2** and **S3** by ca. 1 mLg<sup>-1</sup>. Thus, whilst **S3** is still a good solvent for swelling resins **2–4**, **S2** is only a moderate solvent for these resins as opposed to a borderline good solvent for resin 2. This is a clear indication that even relatively small changes to the chemical nature of a resin can have a significant impact on the swelling of the resin beads. In terms of SPOS, the swelling of a resin-reactant conjugate may be very different before and after a

chemical reaction that changes a functional group within the reactant.

To further investigate the effect on resin swelling of changes to a resin-bound functional group, commercial resins **5–7** all of which contain a hydroxymethyl group were studied. All of resins **5–7** were specified as being based on 1% cross-linked polystyrene, but they differed in bead size and degree of loading as detailed in Table 2. The swelling of these resins in solvents **S1–S5** is shown in Figure 3. Once again, **S1** and **S5** were poor solvents for all three hydroxymethyl functionalised resins. However, some interesting results were obtained for the other three solvents. Methanol (**S4**) is a poor solvent for resins **5** and **6**, but a moderate solvent for resin **7**. This is likely to be a consequence of resin **7** having the highest loading of hydroxymethyl groups (3.1 mmolg<sup>-1</sup>); which corresponds to OH groups making up 5.1% of the resin mass compared to 3.4% for resin **6** and 1.7% for resin **5**. In contrast, isopropyl acetate (**S2**) is a moderate solvent for all three hydroxymethyl functionalised resins, but swells resin **7** less well than resins **5** and **6**. Resin **7** swells to a very similar extent in both **S4** (2.8 mLg<sup>-1</sup>) and **S2** (3.1 mLg<sup>-1</sup>), despite the very different values of the  $\delta_p$  and  $\delta_H$  HSPs for these two solvents. All of resins **1–7** swell to a very similar amount in **S2** (2.7–3.8 mLg<sup>-1</sup>), suggesting that this solvent is predominantly interacting with the polystyrene backbone of the resin (consistent with its low value of  $\delta_p$  and relatively low value of  $\delta_H$ ). In contrast, **S4** appears to interact predominantly with the hydroxyl groups (as expected given its very high value of  $\delta_H$ ) and so can only show any swelling of the most highly loaded hydroxymethyl resin.

**S3** was a good solvent for swelling all of resins **5–7**, but the best swelling was observed for resin **6** with the intermediate loading. The swelling of this resin in **S3** (6.8 mLg<sup>-1</sup>) surpasses that of unfunctionalised cross-linked polystyrene **1** (5.8 mLg<sup>-1</sup>) or chloromethyl functionalised resins **2–4** (4.6–5.4 mLg<sup>-1</sup>) in the same solvent. In contrast, the swelling of resin **5** (5.7 mLg<sup>-1</sup>) with the lowest loading closely resembles that of unfunctionalised cross-linked polystyrene, whilst the swelling of resin **7** (4.8 mLg<sup>-1</sup>) with the highest hydroxymethyl loading resembles that of chloromethyl functionalised resins **2** and **3**. These trends cannot be explained on the basis of a single HSP, but rather probably reflect the fact that introducing some hydroxyl groups onto the resin causes favourable dispersion and dipolar interactions with the solvent, but when too many hydroxyl groups are present this becomes unfavourable due to the low

Table 2. Physical parameters of resins 2–13.

Resin	Bead size [μm]	Bead size [mesh]	Loading [mmolg <sup>-1</sup> ]	Cross-linking [%]
2	75–150	100–200	1.2	1
3	75–150	100–200	0.9	2
4	37–75	200–400	1.2	1
5	75–150	100–200	1.1	1
6	75–150	100–200	2.0 <sup>[a]</sup>	1
7	150–210	75–100	3.1	1
8	75–150	100–200	1.2	1
9	75–150	100–200	2.6	1
10	75–150	100–200	0.4	1
11	170–225	70–90	0.8–1.0	1
12	75–150	100–200	1.4	1
13	37–75	200–400	0.6–1.0	1

[a] Hydroxymethyl Paramax resin

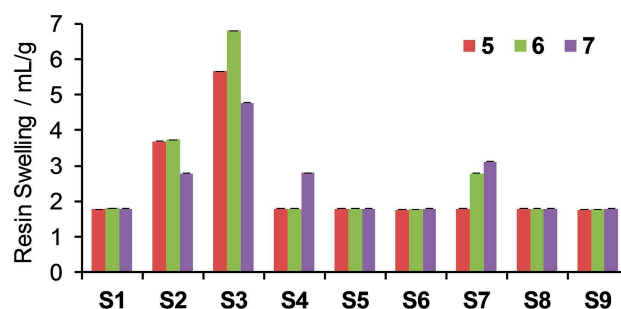


Figure 3. Swelling of resins 5–7 in solvents **S1–S9**.

hydrogen bond energy of **S3** (Table 1). This again illustrates how the swelling of a functionalised resin in a particular solvent system can be influenced by a complex interplay of multiple factors.

Hydroxyl groups are particularly important in SPPS as threonine residues are usually introduced with the hydroxyl group unprotected. In addition, many reactions in SPOS involve a nucleophilic addition to a carbonyl compound and can generate alcohol containing products. Therefore, the swelling of resins **5–7** in a wider range of alcohol solvents was investigated. Initially, four additional solvents **S6–S9** were studied with each of resins **5–7** (Figure 3). 1,2-Dihydroxyethane (**S6**) has a higher  $\delta_H$  than methanol (**S4**) (Table 1) and was a poor solvent for all of resins **5–7**. In contrast, ethanol (**S7**) has a lower  $\delta_H$  than **S4** (Table 1) and was a moderate solvent for resins **6** and **7**, whilst still being a poor solvent for resin **5** which has the lowest loading of hydroxyl groups. Solvents **S8** and **S9** have lower  $\delta_H$  values than **S7** (Table 1) and were poor solvents for all of resins **5–7**. In view of the above results with **S4** and **S7**, a systematic study was undertaken using 1-alcohols **S4**, **S5**, **S7**, **S9–S11** as solvents. In the case of resin **5**, all these solvents gave the same swelling ( $1.8 \text{ mLg}^{-1}$ ), indicating that they were all poor solvents for this low loaded resin. The results for resins **6** and **7** are shown in Figure 4.

Resin **6** with a hydroxymethyl loading of  $2.0 \text{ mmolg}^{-1}$  was found to give moderate swelling ( $2.8 \text{ mLg}^{-1}$ ) in both ethanol (**S7**) and 1-propanol (**S10**) and poor swelling ( $1.8 \text{ mLg}^{-1}$ ) in all the other alcoholic solvents. The contrast between the swelling of this resin in 1-propanol (**S10**) (Figure 4) and 2-propanol (**S8**) (Figure 3) is particularly notable as the only significant difference between the HSPs for these two solvents is their  $\delta_H$  values (17.4 and 16.4 respectively, Table 1). Combining this with the ethanol HSP data suggests that for resin **6** to experience at least moderate swelling, an alcoholic solvent should have a  $\delta_H$  value between 17 and 20. Resin **7** with a hydroxymethyl loading of  $3.1 \text{ mmolg}^{-1}$  was found to give moderate swelling ( $2.8–3.1 \text{ mLg}^{-1}$ ) in all of the lower molecular weight alcohols, but poor swelling ( $1.8 \text{ mLg}^{-1}$ ) in 1-pentanol (**S9**) and 1-heptanol (**S5**). This suggests that for resin **7** to experience at least moderate swelling, a primary alcoholic solvent can have a wider range of  $\delta_H$  values (15–23). Taken together, the swelling of resins **5–7** in various solvents again clearly indicate that even

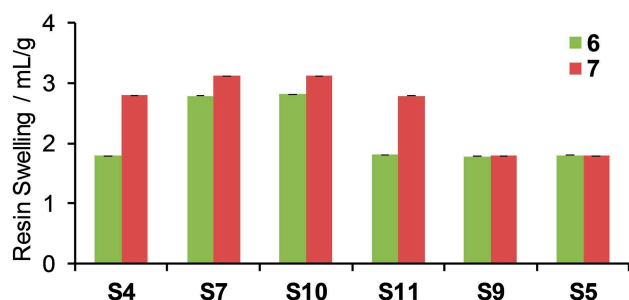


Figure 4. Swelling of resins **6** and **7** in primary alcohols **S4**, **S5**, **S7** and **S9–S11**.

small and subtle changes to the structure of a resin can have a significant effect on the ability of a particular solvent to swell it.

To further investigate the influence of hydrogen bonding functionality on the swelling of a resin, carboxylic acid functionalised resins **8** and **9** were studied. These resins have identical physical parameters and differed only in their loadings (Table 2). Resin **8** has a loading of  $1.2 \text{ mmolg}^{-1}$  (corresponding to carboxylic acid groups constituting 5.4% of the resin) whilst resin **9** has a loading of  $2.6 \text{ mmolg}^{-1}$  (corresponding to carboxylic acid groups constituting 11.7% of the resin). The swelling of these solvents in the standard set of solvents (**S1–S5**) as well as alcohols **S7** and **S9–S11** is shown in Figure 5.

It is apparent from Figure 5 that resins **8** and **9** closely mirror hydroxymethyl resins **5** and **7** in their swelling behaviour. Thus, hydroxymethyl resin **5** (with  $1.1 \text{ mmolg}^{-1}$  loading) and carboxylic acid functionalised resin **8** (with  $1.2 \text{ mmolg}^{-1}$  loading) both showed swelling of less than  $2 \text{ mLg}^{-1}$  in propylene carbonate and all six alcohols. Both resins did however show good swelling ( $5.7–7.9 \text{ mLg}^{-1}$ ) in 2-methyltetrahydrofuran **S3** and good or close to good swelling ( $3.7–4.7 \text{ mLg}^{-1}$ ) in isopropyl acetate **S2**. In contrast, both resins **7** and **9** (with loadings of  $3.1$  and  $2.6 \text{ mmolg}^{-1}$  respectively) showed good swelling ( $4.8$  and  $5.7 \text{ mLg}^{-1}$  respectively) in **S3**, moderate swelling ( $2.8–3.1 \text{ mLg}^{-1}$  and  $2.8–3.8 \text{ mLg}^{-1}$  respectively) in **S2**, **S4**, **S7**, **S10** and **S11** and poor swelling ( $1.5–1.8 \text{ mLg}^{-1}$ ) in **S1**, **S5** and **S9**. The main difference between the swelling of resins **7** and **9** is that resin **9** swells more in ethanol (**S7**) and 1-propanol (**S10**) ( $3.8 \text{ mLg}^{-1}$  rather than  $3.1 \text{ mLg}^{-1}$ ) and as such approaches the high swelling borderline. These results strongly suggest that the swelling of resins **5–9** in alcoholic solvents is determined by interactions between the solvent and the hydroxyl containing functionality, rather than interactions between the solvent and the resin backbone.

The final group of functionalised cross-linked polystyrene based resins included in this study was aminomethyl resins **10–12**. These three resins have similar physical properties (Table 2), and loadings of  $0.4–1.4 \text{ mmolg}^{-1}$ . The swelling of these three resins in solvents **S1–S5** is shown in Figure 6. Only 2-methyltetrahydrofuran **S3** gave good swelling of any of these three resins, and in this solvent the swelling of resins **10–12** decreased as the loading increased, with resin **12** giving only moderate swelling. Isopropyl acetate **S2** gave moderate swelling for all three resins, but again the resin with the lowest loading (**10**) gave the highest swelling. The other three solvents

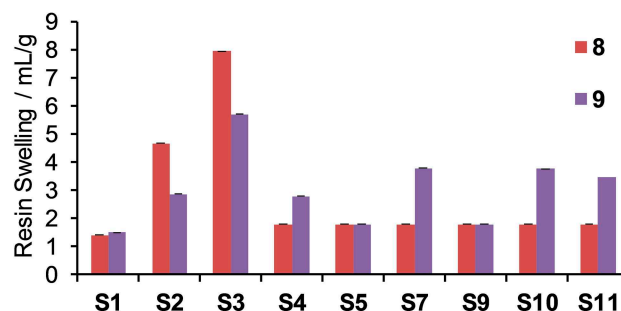


Figure 5. Swelling of resins **8** and **9** in solvents **S1–S5**, **S7** and **S9–S11**.

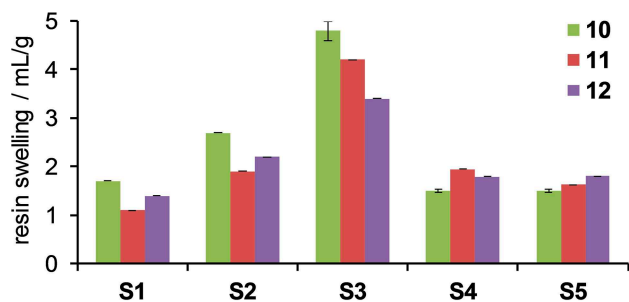


Figure 6. Swelling of resins 10–12 in solvents S1–S5.

gave low swelling for all three resins. For comparison with resins 5–9, the swelling of resins 10 and 12 in primary alcohols was also investigated, but poor swelling ( $1.8 \text{ mLg}^{-1}$ ) was observed in all of solvents S4, S5, S7 and S9–S11. It seems likely that this is due to the low loading of these aminomethyl cross-linked polystyrene resin as the hydroxymethyl and carboxylic acid functionalised resins only showed moderate swelling when the loading of the functional group was at least  $2 \text{ mmolg}^{-1}$ .

Resins 2–12 all possess a simple functional group (or handle) attached to the cross-linked polystyrene. For solid phase synthesis, a linker is usually attached to this handle to facilitate cleavage of the final product from the resin. One of the best known examples of this is the Wang resin<sup>[24]</sup> 13 which also introduces hydroxyl functionalities onto cross-linked polystyrene. We have previously reported<sup>[12]</sup> the swelling of resin 13 (see Table 2 for physical parameters) in 25 green solvents including S1–S5. Figure 7 compares the swelling of resins 1, 4, 5, 8, 11 and 13, all of which are based on 1% cross-linked polystyrene, have a loading of about  $1 \text{ mmolg}^{-1}$  and have similar bead sizes. It is apparent from Figure 7 that in the three low swelling solvents (S1, S4 and S5) the swelling of the resin is not influenced by the functionality attached to the resin. However, the four resins do show some variability in swelling in isopropyl acetate S2 ( $2.7\text{--}4.7 \text{ mLg}^{-1}$ ) and 2-methyltetrahydrofuran S3 ( $4.2\text{--}7.9 \text{ mLg}^{-1}$ ), thus showing that the handle and linker can influence the swelling of a resin.

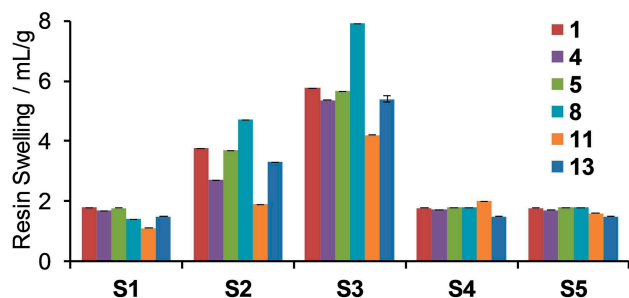


Figure 7. Comparison of the swelling of resins 1, 4, 5, 8, 11 and 13.

## 2.2. ChemMatrix Resins

To provide a comparison with cross-linked polystyrene based resins, the swelling of a totally different type of resin was investigated. ChemMatrix<sup>[25]</sup> is a polyethylene glycol (PEG) based resin which consists of aminomethyl terminated PEG chains, cross-linked with methylene groups. Compared to cross-linked polystyrene based resins, it is known to exhibit good swelling in a much wider range of solvents, but far fewer functionalised ChemMatrix resins are commercially available. The cross-linking of ChemMatrix resin cannot readily be varied and commercial material has a bead size of  $150\text{--}400 \mu\text{m}$  (35–100 mesh, wet sieved).

Figure 8 shows the ChemMatrix based resins used in this study. Initially, the swelling of commercially available aminomethyl resin 14 with a loading of  $0.5\text{--}0.6 \text{ mmolg}^{-1}$  was investigated in solvents S1–S5 and the results are shown in Figure 9. This provides baseline data with which to compare more functionalised ChemMatrix resins. Only isopropyl acetate (S2) and 2-methyltetrahydrofuran (S3) were not good solvents for the swelling of resin 14, both giving moderate swelling of  $3.0\text{--}3.8 \text{ mLg}^{-1}$ . This contrasts markedly with the results obtained for polystyrene based resins 1–13 where solvents S3 and S2 were the solvents that gave the highest swelling of the resins.

Two additional, commercially available ChemMatrix resins 15 and 16 were studied next. These were both derived from the aminomethyl ChemMatrix resin by incorporation of Wang and HMPB linkers respectively and in both cases the aminomethyl functionality of resin 14 was converted into a benzylic alcohol. The swelling of these resins in solvents S1–S5 is also shown in

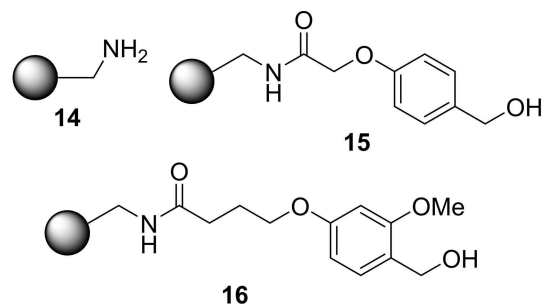


Figure 8. Structures of the ChemMatrix based resins. As commonly accepted, the sphere represents the cross-linked PEG.

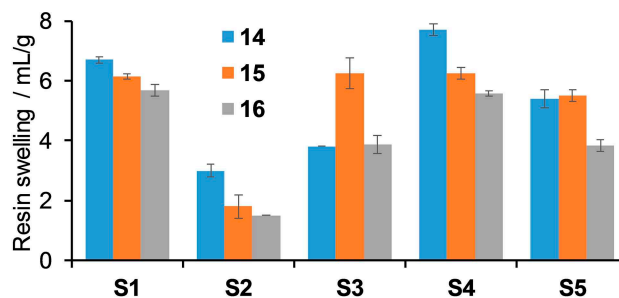


Figure 9. Swelling of resins 14–16 in solvents S1–S5.

Figure 9. It is apparent from Figure 9 that whilst resins 15 and 16 have essentially identical swelling in S1, S2 and S4, resin 16 with a HMPB linker swells to a significantly lower extent than resin 15 in both S3 and S5. Thus, whilst resin 15 swells to  $>4 \text{ mLg}^{-1}$  in all the solvents except S2, resin 16 is only moderately swollen ( $3.8\text{--}3.9 \text{ mLg}^{-1}$ ) in S3 and S5 and displays low swelling in S2. With the exception of solvent S3, the general trend in Figure 9 is that the ChemMatrix resins with a linker attached (15 and 16) swell less well than ChemMatrix resin 14. This is consistent with the linkers introducing aromaticity into the resin-linker conjugate and thus making the conjugate more polystyrene like. Notably in this respect, 2-methyltetrahydrofuran S3 is the best solvent for swelling polystyrene based resins 1–13 (Figure 7).

### 2.3. Swelling of Cross-Linked Polystyrene Based Resins During Peptide Synthesis

Having investigated the influence of various physical and chemical parameters within resins, handles and linkers on the swelling of the resin in a range of solvents, it was of interest to extend this to a study of how the structure of a growing peptide chain influences the resin swelling. Structural factors that change during SPPS and which could influence resin swelling include the nature of the amino acids, the presence of protecting groups and the length of the peptide chain. Initially this work was again carried out on polystyrene resins as it had been possible to obtain more data on the influence of the physical and chemical properties of the resins, handles and linkers for this system.

To investigate the effect of protecting groups and amino acid sidechains, two tripeptides were assembled directly onto aminomethyl cross-linked polystyrene which had a loading of  $0.8 \text{ mmolg}^{-1}$ . The swelling of the resin-tripeptide conjugate was studied both before and after removal of one or both protecting groups, giving a total of six polystyrene supported tripeptide sequences (17–22) as shown in Figure 10. Tripeptides 17 and 18 contain only unfunctionalised aromatic and aliphatic amino acids. These were expected to give a peptide sequence with similar electronic properties to the polystyrene backbone

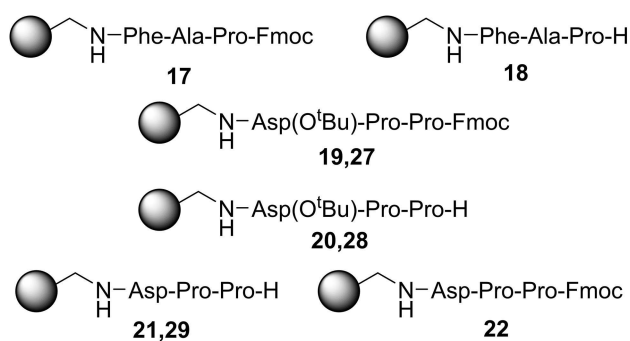


Figure 10. Structures of resin supported tripeptides 17–22 and 27–29. The sphere represents the cross-linked polystyrene for 17–22 and cross-linked PEG for 27–29.

of the resin, especially for protected tripeptide 17 which possesses a large, aromatic Fmoc protecting group. In contrast, tripeptides 19–22 contain only aliphatic amino acids and include an aspartic acid residue, the sidechain of which (when deprotected as in tripeptides 21 and 22) would provide additional hydrogen bonding capabilities.

The swelling of resin-peptide conjugates 17–22 was investigated in seven solvents and the results, along with those for aminomethyl cross-linked polystyrene resin 11, are shown in Figures 11 and 12. Five of the solvents (S1–S5) were the same as those used for the resin swelling study. However, as only S2 and S3 had shown any significant swelling of polystyrene based resins, 2-butanone (S12) and anisole (S13) were also included in this study. 2-Butanone has a yellow rating in the latest version of the GSK green solvents guide,<sup>[21]</sup> whilst anisole is the greenest aromatic solvent in the guide. HSP parameters for these two solvents are included in Table 1. They both have a  $\delta_{\text{H}}$  which is between the  $\delta_{\text{H}}$  values for S2 and S3, but S12 has a  $\delta_{\text{P}}$  value which is significantly higher than those of S2 and S3 whilst S13 has a  $\delta_{\text{P}}$  value which is lower than either S2 or S3.

For peptide sequences 17 and 18 (Figure 11), it is clear that S1, S2, S4 and S5 are all poor solvents for swelling aminomethyl functionalised resin 11, and this does not change when the protected or deprotected tripeptide is attached to the resin. It should be noted that the contribution of even a tripeptide to the resin-peptide conjugate is not negligible, and the mass of the Fmoc-peptide within resin-peptide conjugate 17 is about half the mass of the polystyrene resin, so that the loading drops from  $0.8 \text{ mmolg}^{-1}$  for the aminomethyl polystyrene to  $0.5 \text{ mmolg}^{-1}$  for conjugate 17 (in conjugate 18 the peptide constitutes about a fifth the total mass, so the loading is  $0.6 \text{ mmolg}^{-1}$ ). The other three solvents (S3, S12 and S13) are all

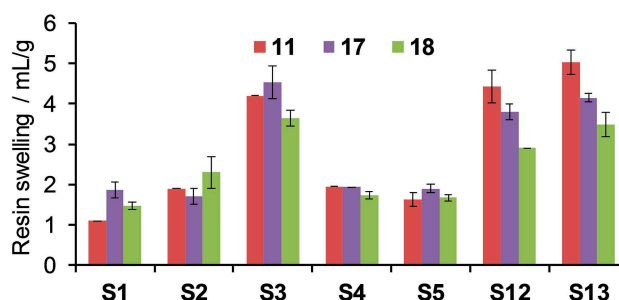


Figure 11. Swelling of resins 11, 17 and 18 in S1–S5 and S12, S13.

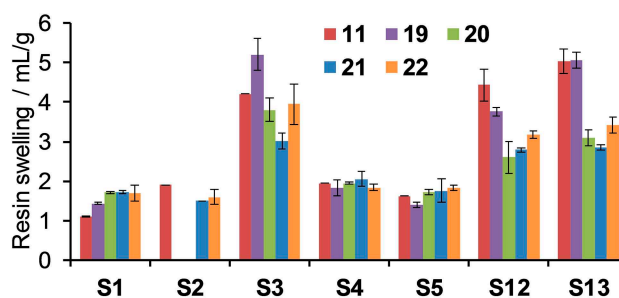
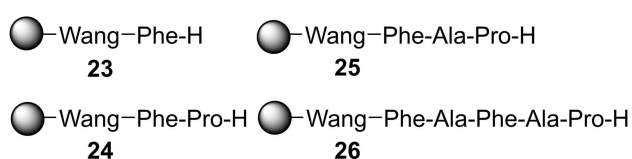


Figure 12. Swelling of resins 11 and 19–22 in S1–S5 and S12, S13.<sup>[26]</sup>

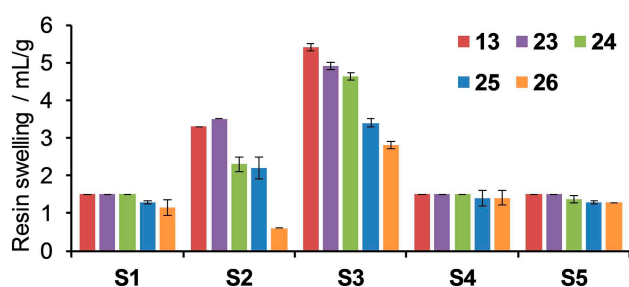
good solvents for swelling the aminomethyl functionalised cross-linked polystyrene resin, giving swellings greater than  $4 \text{ mLg}^{-1}$ . After construction of the protected tripeptide to give resin conjugate **17**, no significant change in resin swelling is observed in **S3**, but a reduction in swelling is seen in both **S12** and **S13**. However, despite this reduction in swelling, **S3** and **S13** remain good swelling solvents for conjugate **17** and **S12** is a borderline good solvent ( $3.8 \text{ mLg}^{-1}$ ). The most significant change in swelling in these three solvents occurs after removal of the Fmoc protecting group to give resin-peptide conjugate **18** as in all cases there is a significant drop in the swelling of the resin and all three solvents only give moderate swelling ( $2.9\text{--}3.6 \text{ mLg}^{-1}$ ).

A similar trend was seen for peptide-resin conjugates **19–22**, all based on the resin-Asp–Pro–Pro sequence. Once again, very poor swelling of all of these species was observed in **S1**, **S2**, **S4** and **S5** (Figure 12). Compared to the parent aminomethyl resin **11**, fully protected peptide sequence **19** swells to a greater extent in **S3**, has the same swelling in **S13** and swells to a lesser extent in **S12**. Removal of either the Fmoc or *tert*-butyl protecting group (to give **20** and **22** respectively), results in a significant and similar reduction in swelling in **S3**, **S12** and **S13**, giving moderate swelling ( $2.6\text{--}3.9 \text{ mLg}^{-1}$ ) in each case. Removal of both protecting groups to give deprotected peptide-resin conjugate **21** results in only a small or no further reduction in resin swelling in these three solvents and moderate swelling ( $2.8\text{--}3.0 \text{ mLg}^{-1}$ ) is still observed.

The swelling results obtained with peptide-resin conjugates **17–22** clearly indicate that the large, aromatic and aliphatic protecting groups used in SPPS are advantageous for resin swelling and have clear implications for SPPS where peptide couplings and Fmoc deprotections alternate throughout the synthesis, which will result in significant and oscillating changes in resin swelling.



**Figure 13.** Structures of cross-linked polystyrene-Wang supported peptides **23–26**. As commonly accepted, the sphere represents the cross-linked polystyrene. For the structure of the Wang linker, see Figure 1.



**Figure 14.** Swelling of resins **13** and **23–26** in solvents **S1–S5**.

To investigate the effect of peptide chain length on resin swelling, deprotected peptide sequences **23–26** (Figure 13) were assembled on polystyrene-Wang resin, starting from commercially available cross-linked polystyrene-Wang-Phe–Fmoc with a bead size of  $75\text{--}150 \mu\text{m}$  ( $100\text{--}200$  mesh) and a loading of  $0.7 \text{ mmolg}^{-1}$  (after Fmoc deprotection). The swelling of compounds **23–26** and of the parent cross-linked polystyrene-Wang resin **13** (with a loading of  $0.8 \text{ mmolg}^{-1}$ ) in **S1–S5** is shown in Figure 14. Only **S2** and **S3** showed anything other than poor swelling for this series of resin-peptide conjugates. **S3** is a good solvent for swelling Wang-resin **13** ( $5.3 \text{ mLg}^{-1}$ ), but the resin swelling gradually drops as the length of the attached peptide chain increases. Attachment of a single amino acid (**23**) or dipeptide (**24**) still gives resin-peptide conjugates with good swelling, but there is a significant drop in swelling (to  $3.3 \text{ mLg}^{-1}$ ) when a tripeptide (**25**) is attached and the resin swelling remains moderate ( $2.8 \text{ mLg}^{-1}$ ) when a pentapeptide (**26**) is attached. **S2** is a moderate solvent for swelling Wang-resin **13** ( $3.3 \text{ mLg}^{-1}$ ) and a similar level of swelling is observed if a single amino acid is attached (**23**). However, attachment of a di- or tripeptide (**24** and **25**) results in a significant decrease in swelling to  $2.2\text{--}2.3 \text{ mLg}^{-1}$ . Attachment of a pentapeptide (**26**) results in a further substantial reduction in swelling to just  $0.6 \text{ mLg}^{-1}$ .

On-going from compound **23** to **26**, the resin loading drops from  $0.70 \text{ mmolg}^{-1}$  to  $0.55 \text{ mmolg}^{-1}$  and the amino acid content of the peptide-resin conjugate increases from 10% to 29%. Correspondingly, the amount of amide bonds in the peptide-resin conjugate increases from 0% (for **23**) to 10% (for **26**). This increase in amide bond character may be responsible for the decrease in resin swelling as we have previously shown<sup>[12]</sup> that the lysine polyamide based resin SpheriTide does not swell well in a range of green solvents including **S2** and **S3**.

In view of the poor swelling of cross-linked polystyrene based resins in most of the green solvents included in this study, further studies on the effect of peptide parameters on resin swelling were carried out using ChemMatrix supported peptides and are discussed in the next section. However, we recently reported that binary mixtures of solvents could give better swelling of resins (including polystyrene-Wang resin) than either individual solvent.<sup>[14]</sup> As part of that work, it was shown that a 1:9 (v/v) **S1**: ethyl acetate solvent system was more effective for the solid phase synthesis of tripeptide H–Leu–Ala–Phe–OH on polystyrene-Wang resin than use of either **S1** or ethyl acetate alone. Therefore, the swelling of the resin-peptide conjugate in a 1:9 (v/v) **S1**: ethyl acetate mixture at each stage of the synthesis was measured and the results are shown in Figure 15. It is apparent from Figure 15 that there is far less variability in the resin swelling as peptide synthesis proceeds in this solvent system ( $3.2\text{--}3.9 \text{ mLg}^{-1}$  for all amino acid containing resins) than in the single solvents shown in Figures 11, 12 and 14. It is also notable that there are no significant differences in resin swelling between Fmoc protected (purple) and Fmoc deprotected (green) peptides in marked contrast to the results seen in Figures 11 and 12. Polystyrene-Wang-resin could be recovered after cleavage of the tripeptide and there was only a small drop in its swelling in

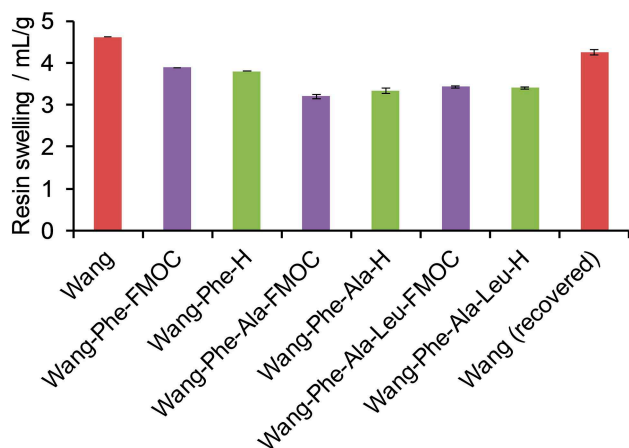


Figure 15. Swelling of polystyrene-Wang-peptide resins in 1:9 (v/v) S1:EtOAc.

this solvent system compared to the starting polystyrene-Wang-resin ( $4.6\text{--}4.3\text{ mLg}^{-1}$ , red bars). These results suggest that one reason why the 1:9 (v/v) S1: ethyl acetate solvent system is so effective for SPPS is that it gives consistent resin swelling throughout each stage of the synthesis.

#### 2.4. Swelling of ChemMatrix Based Resins During Peptide Synthesis

Since ChemMatrix resins generally swell to a far greater extent and in a wider range of solvents than cross-linked polystyrene, it was felt that more informative data would be obtained for peptide sequences on this resin. The first study was carried out on resin-peptide conjugates 27–29 (Figure 10) to provide a direct comparison with cross-linked polystyrene conjugates 19–21 (see Figures 10 and 12). Conjugates 27–29 were synthesised starting from ChemMatrix resin 14 (bead size of  $150\text{--}400\ \mu\text{m}$ ).

Figure 16 shows the resin swelling results obtained for resin 14 and resin-peptide conjugates 27–29 in solvents S1–S5, acetone (S14) and cyclopentyl methyl ether (S15). S14 and S15 both receive yellow ratings in the latest GSK solvent guide<sup>[21]</sup> and were included in this study to allow the influence of HSPs on resin swelling to be investigated in more detail. S14 has almost identical  $\delta_D$  and  $\delta_H$  values to S2 (Table 1), but has a

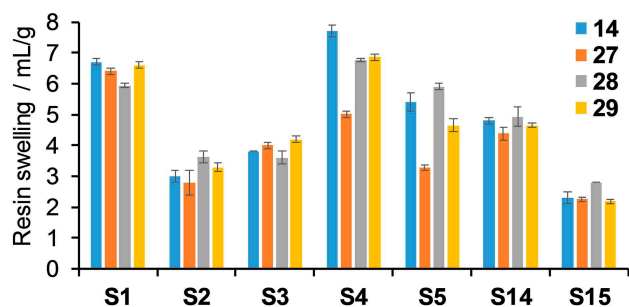


Figure 16. Swelling of resins 14 and 27–29 in solvents S1–S5, S14 and S15.

much larger  $\delta_p$  ( $10.4$  versus  $4.5$ ). S15 has almost identical HSPs to S3, with just a slightly lower  $\delta_p$  ( $4.3$  versus  $5.0$ ).

The most notable feature of Figure 16 is the relatively low swelling of fully protected resin-peptide conjugate 27 in both alcoholic solvents relative to both the starting resin 14 and the Fmoc-deprotected peptide-resin conjugates 28 and 29. In the case of 1-heptanol (S5), this reduces the swelling of conjugate 27 to just  $3.3\text{ mLg}^{-1}$ , so S5 is only a moderate solvent for this species whilst it is a good solvent for resins 14, 28 and 29. This is consistent with the alcoholic solvents interacting to a large extent with free amines in resins 14, 28 and 29. In contrast, the five aprotic solvents included in Figure 16 all show very little variation in resin swelling between resins 14, 27–29. The swelling of resins 14 and 27–29 in aprotic solvents correlates very well with the  $\delta_p$  value of the solvent. Thus, S15 ( $\delta_p = 4.3$ ) gives swellings of just  $2.2\text{--}2.8\text{ mLg}^{-1}$ , S2 ( $\delta_p = 4.5$ ) gives slightly higher swellings of  $2.5\text{--}2.6\text{ mLg}^{-1}$ , S3 ( $\delta_p = 5.0$ ) gives borderline good swellings of  $3.7\text{--}4.4\text{ mLg}^{-1}$ , S14 ( $\delta_p = 10.4$ ) always gives good swellings ( $4.4\text{--}4.9\text{ mLg}^{-1}$ ) and S1 with the highest  $\delta_p$  value ( $18.0$ ) gives the best swellings ( $5.9\text{--}6.6\text{ mLg}^{-1}$ ).

To investigate if a longer peptide sequence would have more of an effect on the swelling of ChemMatrix-peptide conjugates, the Asp–Pro–Pro sequence was duplicated to give peptide-resin conjugates 30–32 (Figure 17) and the swelling of these conjugates in the same seven solvents used for conjugates 27–29 is shown in Figure 18.

Compared to the results obtained with tripeptide-conjugates 27–29 (Figure 16), the most apparent difference in

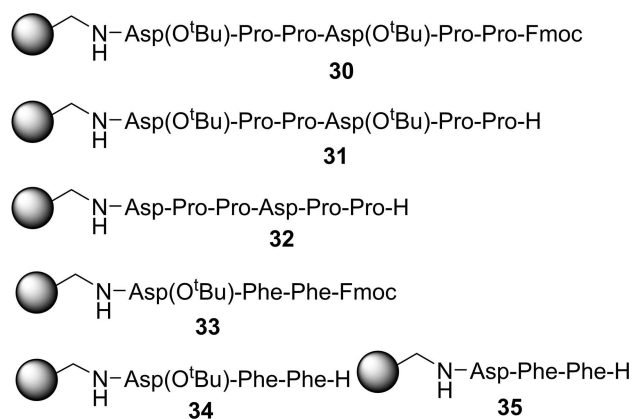


Figure 17. Structures of ChemMatrix supported tripeptides 30–35. As commonly accepted, the sphere represents the ChemMatrix resin.

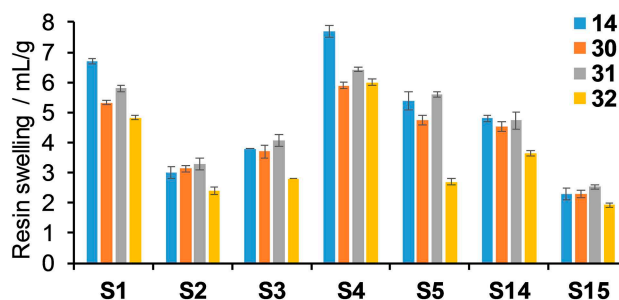


Figure 18. Swelling of resins 14 and 30–32 in solvents S1–S5, S14 and S15.



Figure 18 is the disappearance of the decrease in swelling of the fully protected peptide in alcoholic solvents. This is most likely due to the lower contribution to the overall mass of the resin-peptide conjugate made by the Fmoc group in resin 30 compared to that in resin 27. In conjugate 27, the protected peptide constitutes 27% of the total mass with the Fmoc group within the peptide being responsible for 10% of the total mass. In contrast, in conjugate 30, the longer peptide constitutes 37% of the total mass, but the Fmoc group is only responsible for 8.5% of this mass.<sup>[27]</sup> The most pronounced effect observed for peptide-resin conjugates 30–32 is that the fully deprotected hexapeptide 32 generally swells less well in both protic and aprotic solvents than the corresponding fully (30) and partially (31) protected peptides. This effect is particularly notable in S3, S5 and S14 which are good swelling solvents for the partially protected peptide-resin conjugate 31, but only moderate swelling solvents for fully deprotected conjugate 32. This effect may be related to the relatively high contribution that the fully deprotected peptide makes to the mass of peptide-resin 32 (28%) compared to 31% for the partially protected peptide within conjugate 31 and 37% for the fully protected peptide within conjugate 30. For conjugates 27–29 the contributions of the peptides to the overall mass are significantly lower (27, 20 and 16% respectively) which may explain why a similar trend was not seen for these shorter peptide-resin conjugates. Once again, the swelling of resin-peptide conjugates 30–32 in aprotic solvents correlates with the  $\delta_p$  HSP, with a higher value of  $\delta_p$  giving a higher degree of swelling.

The peptide sequences in peptide resin conjugates 27–32 all contain exclusively aliphatic amino acids. However, the results obtained with these conjugates suggested that the presence or absence of an aromatic Fmoc-protecting group could affect the resin swelling in some solvents. Therefore, peptide-resin conjugates 33–35 (Figure 17), all of which contain the Asp–Phe–Phe tripeptide sequence were prepared and the swelling of these conjugates in the same seven solvents used for conjugates 27–32 is shown in Figure 19.

In the five aprotic solvents, there was very little difference between the swelling of peptide-resin conjugates based on the Asp–Pro–Pro (27–29) or Asp–Phe–Phe (33–35) sequences (Figures 16 and 19). In the protic solvents (S4, S5) however, peptide-resin conjugates 33–35 swell to a significantly lower extent than conjugates 27–29. In 1-heptanol (S5), this effect is so pronounced that whilst S5 is a good solvent for swelling

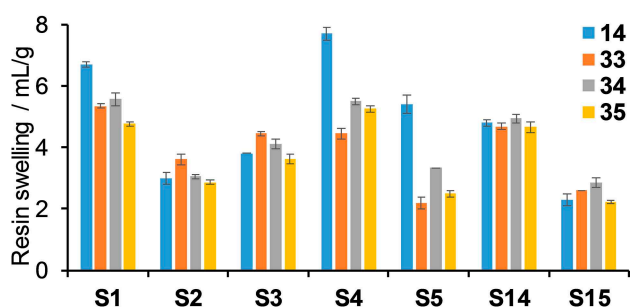


Figure 19. Swelling of resins 14 and 33–35 in solvents S1–S5, S14 and S15.

ChemMatrix resin 14, it is only a moderate solvent for swelling fully protected, partially deprotected and fully deprotected resin-peptide conjugates 33–35.

Finally, the swelling of resin-peptide conjugates 36–39 (Figure 20) was studied to investigate any change in swelling as the peptide chain increases in length and makes an increasing contribution to the overall mass of the peptide-resin conjugate. Conjugates 36–39 are analogous to cross-linked-polystyrene-Wang-conjugates 23–26 (Figure 13), though the base resin and linker are both different. The swelling of these conjugates in the same solvents S1–S5 is shown in Figure 21.

A clear trend is apparent in Figure 21, for all solvents except S2 (for which the swelling is always poor (1.5–2.0 mLg<sup>-1</sup>)), resin-swelling decreases as the length of the attached peptide increases. Thus, whilst ChemMatrix-HMPB resin 16 swells well (>5.5 mLg<sup>-1</sup>) in methanol and propylene carbonate, resin-linker-pentapeptide conjugate 39 swells only moderately (2.8 mLg<sup>-1</sup>) in these two solvents. 2-Methyltetrahydrofuran and 1-heptanol are borderline good solvents for swelling 16 (3.8–3.9 mLg<sup>-1</sup>), but are poor solvents for conjugate 39 (1.4–1.7 mLg<sup>-1</sup>). These results clearly indicate that resin swelling can vary significantly during peptide synthesis as the peptide chain increases in length and becomes an increasingly significant contributor to the overall mass of the resin-linker-peptide conjugate. The percentage of amino acid/peptide in resins 36–39 increases from 9% in 36 to 14% in 37, 18% in 38 and 24% in resin 39.

### 3. Conclusions

We have investigated the influence of various chemical and physical parameters on the swelling of solid-phase synthesis resins in 15 different solvents. Chemical parameters: the nature of functionality attached to the resin and the loading were



Figure 20. Structures of ChemMatrix-HMPB supported peptides 36–39. As commonly accepted, the sphere represents the ChemMatrix resin. For the structure of the HMPB linker, see Figure 8.

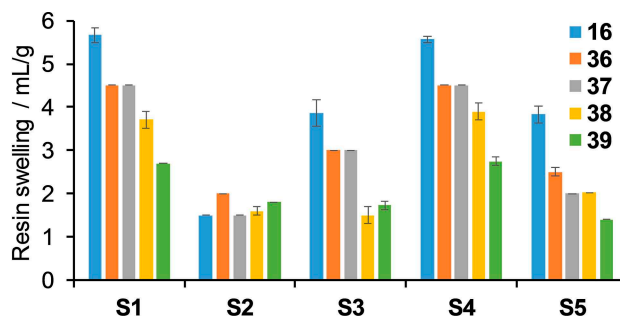


Figure 21. Swelling of resins 16 and 36–39 in solvents S1–S5.

shown to have a far greater impact on the swelling behaviour than physical parameters such as bead size and degree of cross-linking. The influence of loading is shown particularly well by polystyrene-based resins 5–9 with primary alcohol or carboxylic acid functionalities. Amongst these, the higher loading resins (6, 7 and 9) show swelling in some alcoholic solvents unlike the lower loading resins (5 and 8). The effect of functionality is shown on both polystyrene and ChemMatrix resins by analysis of peptides of differing chain lengths and differing degrees of deprotection. In general, as amide bonds make an increasing contribution to the resin-peptide conjugate, the observed swelling decreases.

These results are important not just to solid-phase peptide synthesis, but to solid-phase organic synthesis in general as the chemical functionality is likely to change during each step of the synthesis. Previously,<sup>[14]</sup> we have shown that a mixed solvent system of 1:9 propylene carbonate: ethyl acetate gives excellent results for solid-phase peptide synthesis on Merrifield-Wang resin and in this work we have shown that this correlates with the swelling of the intermediate resin-peptide conjugates not varying significantly in this solvent system. This illustrates that by judicious choice of solvent system it is possible to overcome problems associated with changing chemical functionality during solid-phase organic synthesis.

## Experimental Section

Commercially available resins, solvents and reagents were used as received. 2-MeTHF was stabilised with 250 ppm of butylated hydroxytoluene (BHT). Low and high resolution electrospray ionisation (ESI) mass spectra were recorded on a Bruker micrOTOF time-of-flight mass spectrometer in tandem with an Agilent 1200 series LC system.

**General Procedure for Determination of Resin Swelling.** Resin (74–141 mg) was transferred to a 2 mL syringe fitted with a polypropylene fritted disc with a void volume of 0.15 mL. Solvent (2 mL) was added and the syringe agitated for 1 h at room temperature. Excess solvent was removed by compressing the syringe piston before slowly withdrawing the piston and allowing the resin to return to its maximum volume. The volume was recorded and the degree of swelling calculated from the formula:

$$\text{Swelling (mL g}^{-1}\text{)} = \frac{\text{(measured volume} - \text{void volume)}}{\text{(mass of resin)}}$$

Each resin was analysed in triplicate with the average value and standard deviation from the average being used to determine the resin swelling value and error bars respectively.

**General Procedure for Kaiser Test.** Stock solutions of reagents A–C were prepared as follows:

Reagent A : 0.001 M KCN solution (2 mL) diluted in pyridine (98 mL).

Reagent B : Ninhydrin (1.0 g) was dissolved in 1-butanol (20 mL).

Reagent C : Phenol (40 g) was dissolved in 1-butanol (20 mL).

A few beads of resin were treated with 5 drops of each of reagents A–C in a test tube. The resulting mixture was heated to 120 °C for 5 min. The presence of free resin-bound primary amines was signified by a colour change from pale yellow to dark blue/purple.

**General Procedure for Chloranil Test.** Stock solutions of reagents A and B were prepared as follows:

Reagent A : Acetaldehyde (1 mL) was added to DMF (49 mL)

Reagent B : p-Chloraniline (1 g) was dissolved in DMF (49 mL)

A few beads of resin were treated with 3 drops of each of reagents A and B in a test tube. The resulting mixture was left at room temperature for 5 min. The presence of free resin-bound secondary amines was signified by a colour change from pale yellow to dark green.

**General Procedure for SPPS.** SPPS was carried out in a 6 mL filtration tube fitted with a polypropylene frit. Resin was first washed with CH<sub>2</sub>Cl<sub>2</sub> (3 × 5 mL) and DMF (3 × 5 mL) and then swollen in DMF (2 mL) for 1 h. Coupling reactions were carried out by dissolving a Fmoc-amino acid (3.0 equiv.), HBTU (3.0 equiv.), HOBt (3.0 equiv.) and diisopropylethylamine (6.0 equiv.) in DMF (2.1 mL). After stirring for 3 min., this activated amino acid solution was added to the resin and agitated for 1 h. Coupling reactions were performed at room temperature and carried out in duplicate. Following each coupling reaction, the resin was washed using CH<sub>2</sub>Cl<sub>2</sub> (3 × 5 mL) and DMF (3 × 5 mL). Fmoc-deprotections were carried out using a freshly prepared solution of piperidine (3 mL of 20% (v/v) in DMF) and performed in duplicate (10 min followed by 20 min agitation). The resin was then washed with CH<sub>2</sub>Cl<sub>2</sub> (3 × 5 mL) and DMF (3 × 5 mL). The success of the deprotection was determined using either the Kaiser or chloranil colorimetric test. Once the required peptide was synthesised and deprotected, the resin was washed with CH<sub>2</sub>Cl<sub>2</sub> (3 × 5 mL) and DMF (3 × 5 mL) and dried under reduced pressure. If a Wang or HMPB linker was attached between the resin and peptide, a small amount of peptide was then cleaved from the resin (20 mg) using a mixture of TFA: TIPS:H<sub>2</sub>O (1 mL, 95:2.5:2.5) with agitation for 1 h. at ambient temperature. The resin was removed by filtration and the filtrate evaporated to dryness. The residue was analysed by electrospray mass spectrometry to confirm the success of the peptide synthesis.

Resin 24 gave H–Pro–Phe–OH. MS(ESI) m/z 263 [MH<sup>+</sup>, 100]; HRMS (ESI) found 263.1387, calculated for C<sub>14</sub>H<sub>19</sub>N<sub>2</sub>O<sub>3</sub> MH<sup>+</sup> 263.1390.

Resin 25 gave H–Pro–Ala–Phe–OH. MS(ESI) m/z 334 [MH<sup>+</sup>, 100]; HRMS(ESI) found 334.1755, calculated for C<sub>17</sub>H<sub>24</sub>N<sub>3</sub>O<sub>4</sub> MH<sup>+</sup> 334.1761.

Resin 26 gave H–Pro–Ala–Phe–Ala–Phe–OH. MS(ESI) m/z 552 [MH<sup>+</sup>, 100], 574 [(M+Na)<sup>+</sup>, 10]; HRMS(ESI) found 552.2817, calculated for C<sub>29</sub>H<sub>38</sub>N<sub>5</sub>O<sub>6</sub> MH<sup>+</sup> 552.2817.

Resin 37 gave H–Pro–Phe–OH. MS(ESI) m/z 263 [MH<sup>+</sup>, 100], 285 [(M+Na)<sup>+</sup>, 16]; HRMS(ESI) found 285.1205, calculated for C<sub>14</sub>H<sub>19</sub>N<sub>2</sub>NaO<sub>3</sub> (M+Na)<sup>+</sup>.

Resin 38 gave H–Pro–Ala–Phe–OH. MS(ESI) m/z 334 [MH<sup>+</sup>, 100]; HRMS(ESI) found 334.1735, calculated for C<sub>17</sub>H<sub>24</sub>N<sub>3</sub>O<sub>4</sub> MH<sup>+</sup> 334.1761.

Resin 39 gave H–Pro–Ala–Phe–Ala–Phe–OH. MS(ESI) m/z 552 [MH<sup>+</sup>, 100]; HRMS(ESI) found 552.2829, calculated for C<sub>29</sub>H<sub>38</sub>N<sub>5</sub>O<sub>6</sub> MH<sup>+</sup> 552.2817.

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**Keywords:** polystyrene · solvent · swelling · ChemMatrix · peptide

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