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Solution phase synthesis of β-peptides using micro reactors

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Abstract—The synthesis of β-peptides has been successfully performed using a borosilicate glass micro reactor, in which a network of channels has been produced using a photolithographic and wet etching method. The reagents were mobilised by electroosmotic flow (EOF). The micro reactor was initially evaluated using a carbodiimide coupling reaction to form a dipeptide. The methodology has been extended such that the peptides may also be produced via the pentafluorophenyl ester derivatives of amino acids. It was found that performing the pentafluorophenyl ester reactions in the micro reactor resulted in an increase in the reaction efficiency over the traditional batch method. We postulate that the enhancement in rate of reaction is an electrochemical phenomenon, due to the reaction being performed in an electric field, which is unique to micro reactor systems. It has also been demonstrated that selective deprotection of the resultant dipeptides can be achieved. This approach has been used in the synthesis of a tripeptide. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

A micro reactor is generally defined as a series of interconnecting channels (10–300 μm in diameter), formed in a planar surface, in which small quantities of reagents are manipulated. Thus reagents can be brought together in a specific sequence, mixed and allowed to react for a specified time in a controlled region of the reactor. During the past 10 years, there has been a rapid growth in the development of micro-Total Analytical Systems (μTAS)[1–8] which exploit electroosmotic flow (EOF).[9–12] The development of micro reactor devices for chemical synthesis based on complimentary technology is less common. However, recent research has shown that Suzuki,[13] Wittig,[14,15] diazo coupling reactions[16] and enamines[17] may be performed using micro chemical systems. In addition, we have demonstrated that peptide bonds may be prepared using such technology.[18] The reactions performed in micro reactors have recently been reviewed by Haswell and co-workers.[19]

Micro reactors are advantageous because they allow the rapid optimisation of reactions and reduce exposure to hazardous chemicals. They also have the ability to generate reagents in situ, allowing the preparation and subsequent reaction of highly reactive or toxic intermediates. The ability to scale out the synthesis allows large quantities of chemicals to be prepared and it has been calculated that 1 kg of material could be produced in 24 h using 1000 micro reactors in parallel.[20] In addition, because EOF may be used to mobilise the solvents and reagents around the micro reactor manifold, electrophoretic separation would enable isolation of pure products.[21]

To illustrate the principles of EOF, one can consider a micro channel fabricated from a material having negatively charged functional groups on the surface. If a liquid, displaying some degree of dissociation, is brought into contact with the surface, positive counter ions will form a double layer such that the positively charged ions are attracted to the negatively charged surface. Application of an electric field causes this layer to move towards the negative electrode, thus causing the bulk liquid to move within the channel (Fig. 1). Electroosmotic flow is not just restricted to aqueous systems and a range of organic solvents can be mobilised using the technique. A major advantage of EOF is that it gives a high degree of spacial and temporal control to reactants mixing under a diffusive regime. This high level of fluidic control and mixing has been attributed to the reactions[13–19] reported in the literature. Electroosmotic flow also has the advantage that the direction and magnitude of the flow may be readily changed by altering the applied voltage, where the flow rates increase

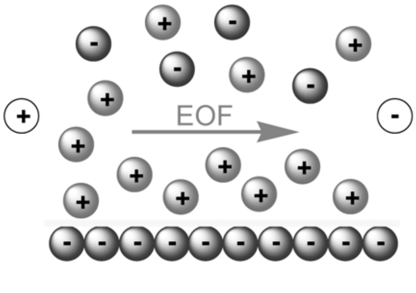

Figure 1. Principle of electroosmotic flow.

Keywords: β-peptides; electroosmotic flow; dipeptides.

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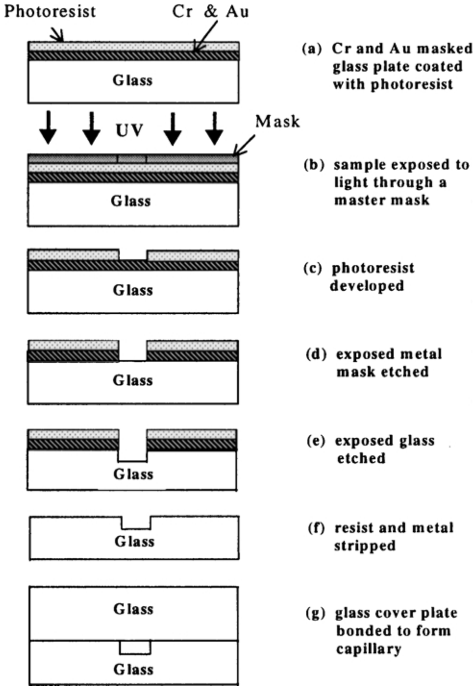


Figure 2. Sequence of processes in photolithographic fabrication.

with voltage. The flow rates, which are in the order of μl min^{-1}, are reproducible using EOF. In addition, the pumping technique has no mechanical or moving parts, hence it is highly suitable for operation in miniaturised systems. An automated power supply with multiple electrodes can support several pumping channels. Importantly, when using EOF, plugs of fluid are transported without significant hydrodynamic dispersion.

Following the discovery by Merrifield in 1963,[22] peptides have been commonly prepared via solid supported techniques. Solid phase peptide synthesis (SPPS) is based on the addition of a protected amino acid residue to an insoluble polymeric support. The acid-labile Boc group[23] and base-labile Fmoc group[24] have been commonly used for *N*-protection. After removal of the protecting group the next protected amino acid may be added using either a coupling reagent or a pre-activated amino acid derivative. If this dipeptide is the desired product, it may be cleaved from the polymer support.[25,26] If a longer peptide is required additional amino acids can be added by repeating further coupling reactions. Solid phase peptide synthesis is advantageous because of the ease of product purification. In addition the process is readily automated and has become common in the combinatorial synthesis of peptides. Solid phase peptide synthesis however, has the disadvantage that an expensive polymer support is required. Extra steps are also added to the synthesis as a result of initially linking the amino acid to the support and finally having to remove the peptide from the polymer. In this paper, a micro reactor has been used to prepare peptides using solution phase chemistry and the results are compared with traditional solid phase procedures.

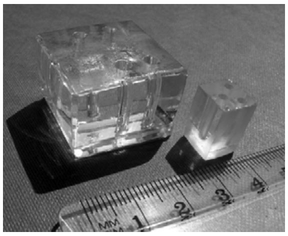


Figure 3. A borosilicate glass micro reactor.

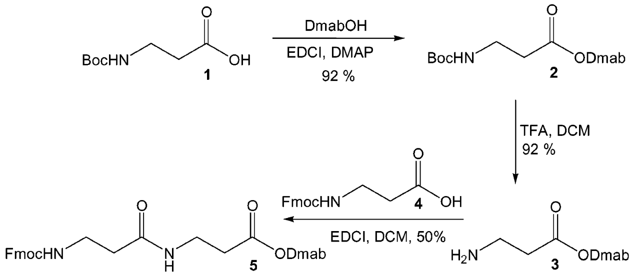
2. Fabrication and use of the micro reactors

A number of materials such as silicon, quartz, glass, metals and polymers have been used to construct micro reactors. Glass and certain polymers have been particularly useful because of their physical properties and chemical inertness. These substances allow the mobilisation of organic solvents and reagents using a number of pumping mechanisms such as hydrodynamic pumping and electroosmotic flow. A range of fabrication methods such as photolithography, hot embossing, powder blasting, injection moulding and laser micro forming alone, or in combination, have been reported in the literature.[19,27,28]

The micro reactor devices used in this work were prepared using the popular technique of photolithography and wet etching to produce channels in a glass micro reactor (Fig. 2).[29] Firstly, a thin layer of metal, such as chromium, is deposited onto the surface of a borosilicate glass plate. This controls the degree of undercutting during the wet etching process. A layer of positive photoresist is then spin coated on top of the chromium to a depth of 0.5–2.0 μm. The pattern of the required network of interconnecting channels is subsequently transferred to the photoresist layer using photolithography. After exposure, the photoresist is developed and removed, together with the chromium layer, to reveal the areas of glass to be etched. The plate is then heated to allow the volatiles to evaporate, before performing the chemical etch. The channels are etched using a mixture of 1% HF and 5% NH_4F in water at 65°C, resulting in an etch rate of 0.3–0.5 μm min^{-1}. A glass top block, with pre-drilled holes that act as reservoirs for reagents, is then aligned with the channel geometry and thermally bonded to the base plate, producing an all glass device (Fig. 3). Microporous silica frits[30] were placed in the channels to prevent hydrodynamic flow occurring.

3. Results and discussion

We selected to demonstrate the multi-step synthesis of peptides derived from β-amino acids for two reasons. Firstly, the simplest β-amino acids lack chiral centres, hence we avoid potential problems with racemisation and the generation of diastereomeric mixtures, which would complicate the analysis of the products in our initial studies. In addition, β-peptides have attracted much interest due to their structural[31,32] and biological properties.[33] In particular, their stability to degradation by peptidases[34,35] makes them potentially superior to the drugs derived from α-amino acids. Both solution and solid phase synthetic methods for these have been developed which would serve to benchmark the micro reactor synthesis.[36,37]



Scheme 1. Synthesis of a standard dipeptide derivative.

In the first instance, a one-step reaction was considered in which an *N*-protected β-amino acid was reacted with an *O*-protected β-amino acid, to prepare the protected β-dipeptide. To enable the methodology to be applicable to the synthesis of more complex peptides, the use of orthogonal protecting groups was clearly required. As in SPPS, the base-labile Fmoc protecting group[24] was selected for *N*-protection while the Dmab ester[38] was chosen for protection of the carboxylic acid. Importantly, both protecting groups may be removed under mild conditions, since electroosmotic flow is retarded if the pH of the reaction is less than 3.[9–12] We have already reported the application of micro reactors in the formation of peptide bonds,[18] but here we give full experimental details for all procedures and also exemplify more complex examples.

Commercially available Boc-β-alanine **1** was protected as the Dmab ester using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and 4-dimethylamino pyridine (DMAP), to give the ester **2** in 92% yield (Scheme 1). Treatment of **2** with trifluoroacetic acid furnished the desired amine **3** in 92% yield, which was subsequently reacted with Fmoc-β-alanine **4** via a carbodiimide coupling reaction, to give a synthetic sample of the target dipeptide **5** in 50% yield. Addition of a catalytic amount of DMAP resulted in appreciable quantities of Fmoc deprotection, hence DMAP was omitted in subsequent coupling reactions when the Fmoc protecting group was present.

Having prepared dipeptide **5**, it represented a synthetic target for preparation within the micro reactor. Prior to synthesis, the micro reactor channels were primed with anhydrous *N*,*N*-dimethylformamide (DMF) to remove any air and moisture from the channels and the microporous silica frits. A standard solution of Fmoc-β-alanine **4** (50 μl, 0.1 M) in anhydrous DMF was added to reservoir A, a solution of EDCI (50 μl, 0.1 M) in DMF was placed in reservoir B and a solution of amine **3** (50 μl, 0.1 M) in DMF was placed in reservoir C (Fig. 4). Anhydrous DMF (40 μl) was placed in reservoir D, which was used to collect the products of the reaction. Platinum electrodes were placed in each of the reservoirs (A, B and C positive, D ground) and an external voltage was applied to the channels inducing electroosmotic flow of the reagents. The reactions were conducted at room temperature for a period of 20 min, in order to acquire sufficient volume of product to determine the conversion of the reaction by HPLC. Product conversions were based on the amount of carboxylic acid **4** remaining in the sample.

When stoichiometric quantities of the reagents were used only ca. 10% conversion to dipeptide **5** was achieved when a voltage of 700 V was applied to each of the reagents (A, B and C). By lowering the applied voltages to 500 V, hence reducing the flow rates and increasing the residence time of the reaction, no significant increase in conversion was observed. However, by using 2 equiv. of EDCI (0.2 M solution) an increase in conversion to ca. 20% was observed. By applying a stopped flow technique (2.5 s injection length with stopped flow for 5 s) thus further increasing the residence time of the reaction, the conversion was further increased to approximately 50%. Since the efficiency of the reaction appeared to be greatly dependent on the number of equivalents of EDCI used, we wished to further investigate the effect of carbodiimide concentration on the reaction, however we found that EDCI was insoluble in DMF above 0.2 M concentrations. In further experiments dicyclohexylcarbodiimide (DCC) was used as the coupling reagent as it was considerably more soluble in DMF. Using 5 equiv. of DCC (0.5 M solution in reservoir B) a conversion of 93% to dipeptide **5** was obtained, using the stopped flow regime described above. This represented a typical yield for a classic carbodiimide coupling reaction in SPPS.

We wished to demonstrate that alternative methods used in peptide synthesis, could also be applied to the synthesis within the micro reactors. Another common method utilised in peptide bond formation involves the reaction of a pre-activated amino acid derivative, such as a pentafluorophenyl ester, with an amine.[39,40] Fmoc-β-alanine **4** was activated as pentafluorophenyl ester **6** via an EDCI coupling reaction, to give the product in 90% yield (Scheme 2). Subsequent treatment of ester **6** with amine **3** afforded dipeptide **5** in 46% yield in a bulk reaction, using DMF as solvent. The yield did not increase even when prolonged reaction times were employed.

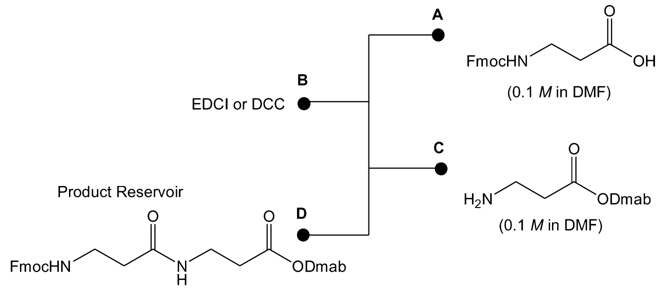
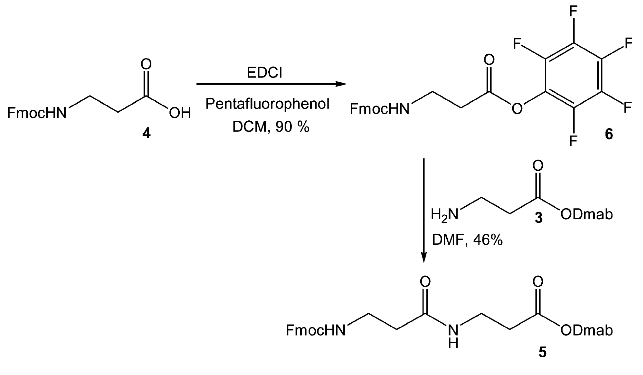
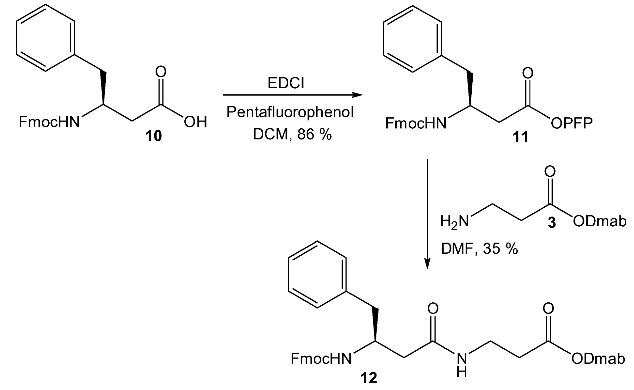


Figure 4. Schematic of the micro reactor used in carbodiimide coupling reactions.



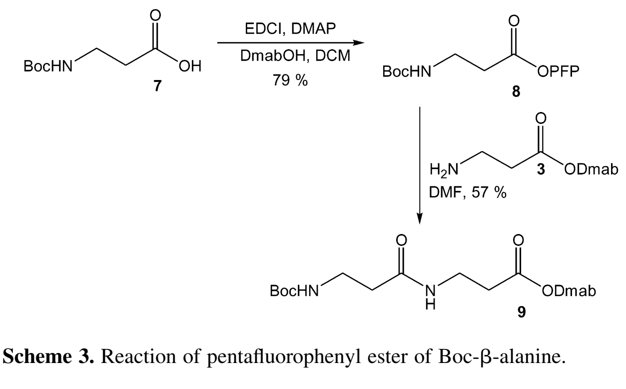
Scheme 2. Preparation and reaction of pentafluorophenyl esters of Fmoc protected amino acids.



Scheme 4. Reaction of PFP ester of Fmoc-L-β-homophenylalanine.

Having prepared dipeptide **5** via the alternative pre-activated strategy, we wished to investigate if the reaction could be performed within a micro reactor. A standard solution of the pentafluorophenyl ester of Fmoc-β-alanine **6** (50 μl, 0.1 M) in anhydrous DMF was added to reservoir A and a solution of amine **3** (50 μl, 0.1 M) was placed in reservoir B. Anhydrous DMF (40 μl) was placed in reservoir D, which was used to collect the products of the reaction. It was found that using continuous flow of both reagents, where the ester **6** was maintained at 700 V and the amine **3** was maintained at 600 V, dipeptide **5** was produced quantitatively in 20 min. Product conversions were based on the amount of pentafluorophenyl ester **6** remaining in the sample. This represented a significant increase in yield compared with solution phase synthesis, where lengthy reaction times are also required.

We were also interested to determine the effect of the protecting group on the reaction. Thus, Boc-β-alanine **7** was activated as pentafluorophenyl ester **8** via an EDCI coupling reaction, to give the product in 79% yield. The pentafluorophenyl ester **8** was reacted with amine **3** in DMF as solvent to give the dipeptide **9** in 57% yield (Scheme 3). Subsequently, the reaction between the pentafluorophenyl ester **8** of Boc-β-alanine and amine **3** was also investigated in the micro reactor. In this case, when the reagents were mixed using continuous flow, with both reagents maintained at 700 V, again quantitative conversion to dipeptide **9** was observed. The fact that pentafluorophenyl ester **8** needed to be maintained at a higher potential, in order to obtain a quantitative conversion, demonstrates that the different pentafluorophenyl esters **6** and **8** have different electroosmotic mobilities within the micro reactor channels.

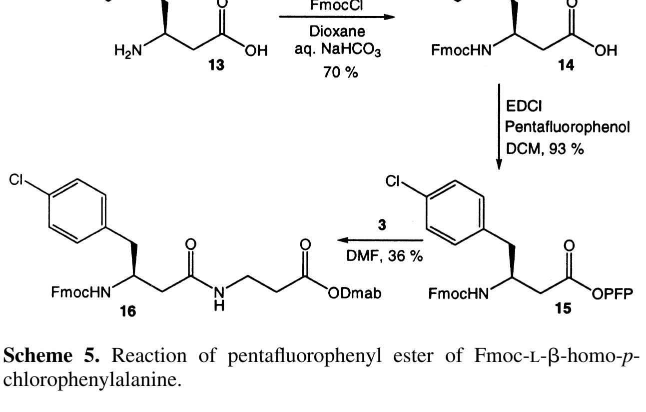


Scheme 3. Reaction of pentafluorophenyl ester of Boc-β-alanine.

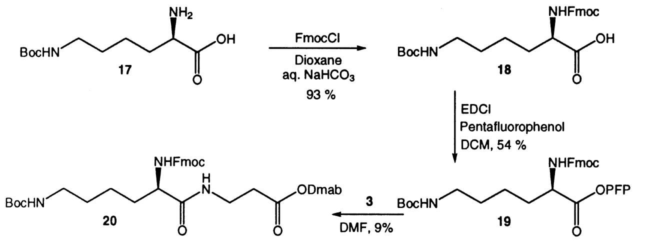
In order to demonstrate the generality of the technique we wished to demonstrate that a selection of peptides could be prepared. Thus, commercially available Fmoc-L-β-homophenylalanine **10** was converted into pentafluorophenyl ester **11** in 86% yield, under standard conditions. This was reacted with amine **3** to prepare a synthetic sample of dipeptide **12** in 35% yield (Scheme 4).

The reaction was subsequently investigated in the micro reactor. A solution of the pentafluorophenyl ester **11** in anhydrous DMF was added to reservoir A, a solution of amine **3** was placed in reservoir B and anhydrous DMF was placed in reservoir D. It was found that using continuous flow of both reagents, where the ester **11** was maintained at 900 V and the amine **3** was maintained at 600 V, dipeptide **12** was produced quantitatively in 20 min. As before this represented a significant increase in yield compared with bulk reaction.

Similarly L-β-homo-*p*-chlorophenylalanine **13** was reacted with 9-fluorenylmethyl chloroformate[41] to effect *N*-protection affording carboxylic acid **14**, followed by an EDCI coupling reaction with pentafluorophenol to give the corresponding pentafluorophenyl ester **15**. The ester **15** was reacted with amine **3** in DMF, to prepare a synthetic sample of dipeptide **16** in 36% yield (Scheme 5).



Scheme 5. Reaction of pentafluorophenyl ester of Fmoc-L-β-homo-*p*-chlorophenylalanine.



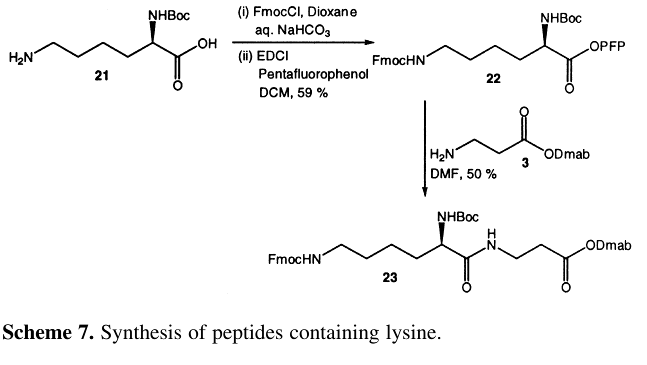
Scheme 6. Synthesis of peptides containing lysine.

The reaction was then transferred to the micro reactor. As in the previous example, it was found that using continuous flow of both reagents, where the ester **15** was maintained at 900 V and the amine **3** was maintained at 600 V, dipeptide **16** was produced quantitatively in 20 min.

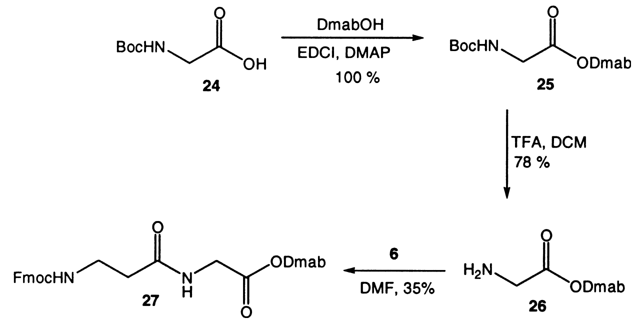
Having demonstrated the success of the technique using simple β-amino acids, we wished to perform the synthesis of more complex peptides. We selected to prepare peptides containing lysine, which contains two amine functionalities, which require orthogonal protection. Thus, commercially available *N*ε-Boc-L-lysine **17** was converted into pentafluorophenyl ester **19**, under standard conditions (Scheme 6). This was reacted with amine **3** to prepare a synthetic sample of dipeptide **20** in a disappointing 9% isolated yield.

The reaction was further investigated in the micro reactor. A solution of the pentafluorophenyl ester **19** in anhydrous DMF was added to reservoir A, a solution of amine **3** was placed in reservoir B and anhydrous DMF was placed in reservoir D. It was found that using continuous flow of both reagents, where the ester **19** was maintained at 1000 V and the amine **3** was maintained at 600 V, dipeptide **20** was produced quantitatively in 20 min.

Similarly *N*α-Boc-L-lysine **21** was converted into pentafluorophenyl ester **22**, in 59% yield (Scheme 7). This was reacted with amine **3** to prepare a synthetic sample of dipeptide **23** in 50% yield. The reaction was then transferred to the micro reactor. As in the previous example, it was found that using continuous flow of the reagents, where the ester **22** was maintained at 1000 V and the amine **3** was maintained at 600 V, dipeptide **23** was produced quantitatively. As expected, this result demonstrates that both pentafluorophenyl esters of lysine **19** and **22** have the same electrophoretic mobility within the channels of the micro reactor. Fmoc deprotection of the peptides **20** and **23** would prepare different amines, which could be used in further reactions.



Scheme 7. Synthesis of peptides containing lysine.



Scheme 8. Preparation of peptides containing glycine.

We were interested to observe that all reactions between pentafluorophenyl esters and amine **3** appeared to be faster in the micro reactor than when performed in a bulk reaction. However, bulk reactions were generally performed at much higher concentrations than micro reactions. In order to make comparison between rates we monitored the conversion of pentafluorophenyl ester **11** into peptide **12** at 0.05 M concentration, the same as used in the micro reaction studies (Scheme 8). Fig. 5 shows how conversion increases with time, the graph shows that after 20 min, the length of a micro reaction, only about 5% conversion to dipeptide was observed in the bulk reaction. Even after 400 h (ca. 16 days) only 70% conversion was attained. This result clearly shows that the rate of reactions is considerably enhanced when the reactions are conducted in a micro reactor. We postulate that the enhancement in rate of reaction is an electrochemical phenomenon, due to the reaction being performed in an electric field.

Having prepared numerous examples of peptides prepared from the Dmab ester of β-alanine **3**, we wished to demonstrate that other amines may be used in the synthesis of peptides. Thus, commercially available Boc-glycine **24** was protected as the Dmab ester via an EDCI/DMAP coupling reaction to give the ester **25** in 100% yield in a bulk reaction (Scheme 8). Treatment of **25** with trifluoroacetic acid furnished the desired amine **26** in 78% yield. This was subsequently reacted with pentafluorophenyl ester **6** to give a synthetic sample of the dipeptide **27** in 35% yield.

The reaction was subsequently investigated in the micro reactor. A solution of the pentafluorophenyl ester **6** in anhydrous DMF was added to reservoir A, a solution of amine **26** was placed in reservoir B and anhydrous DMF was placed in reservoir D, which was used to collect the

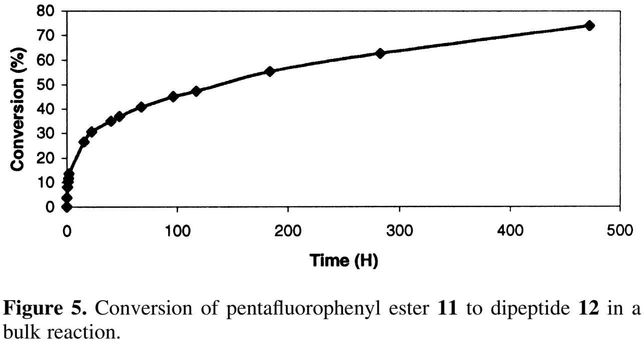
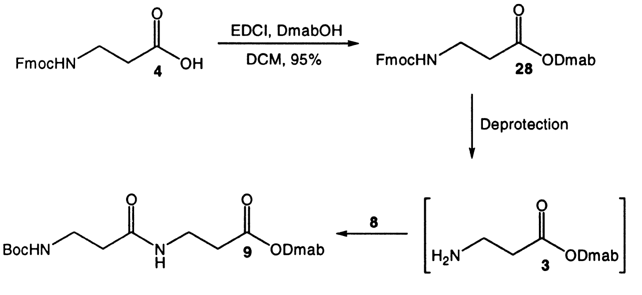


Figure 5. Conversion of pentafluorophenyl ester **11** to dipeptide **12** in a bulk reaction.



Scheme 9. Multi-step peptide synthesis.

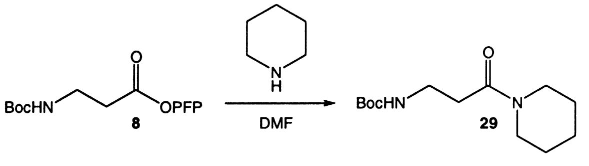
products of the reaction. It was found that using continuous flow of reagents, where both were maintained at 800 V, dipeptide **27** was produced quantitatively in 20 min.

Having extensively demonstrated that peptide bonds could be successfully formed when using a micro reactor, we wished to show that we could extend the methodology to the preparation of longer chain peptides. Consequently, we needed to be able to conduct deprotection reactions and subsequently perform further peptide bond forming reactions. Fmoc-β-alanine **4** was converted into the Dmab ester **28**, in a bulk reaction, using standard conditions (Scheme 9). It was proposed to convert ester **28** into amine **3** by deprotection of the Fmoc group and subsequently react the amine 'in situ' within the micro reactor with pentafluorophenyl ester **8**, to give the dipeptide **9**.

Several methods for the deprotection of the Fmoc group are reported in the literature, the most common method being treatment with 20% piperidine in DMF.[42,43] The advantage of using piperidine in the reaction, is that the piperidine subsequently reacts with the dibenzofulvene, to form the DMF soluble piperidine adduct. This is advantageous, as precipitation or polymerisation of the dibenzofulvene within the micro reactor could cause blockage of the channels. Preliminary experiments revealed that treatment of **28**, with 10 equiv. of piperidine in DMF using continuous flow within the micro reactor, resulted in 60–70% deprotection over a 20 min period, to give amine **3**.

Subsequently, a standard solution of the Dmab ester of Fmoc-β-alanine **28** (50 μl, 0.1 M) in anhydrous DMF was added to reservoir A, a solution of piperidine (50 μl, 1.0 M) was placed in reservoir B and a solution of pentafluorophenyl ester **8** (50 μl, 0.1 M) was placed in reservoir C, in an attempt to prepare dipeptide **9** using this multi-step approach. Anhydrous DMF (40 μl) was placed in reservoir D, to collect the products of the reaction. The HPLC of the reaction mixture showed that Fmoc deprotection had occurred, however no dipeptide **9** was evident.

It was postulated that the excess piperidine was reacting with the pentafluorophenyl ester. This was confirmed from a batch reaction, in which piperidine was reacted with the pentafluorophenyl ester of Boc-β-alanine **8** (Scheme 10). The only product isolated from the reaction was amide **29**, resulting from the nucleophilic attack of piperidine on the ester.

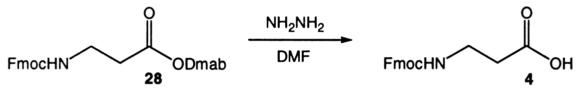


Scheme 10. Reaction of piperidine with pentafluorophenyl esters.

As a result, an alternative method of Fmoc deprotection was required that would not cause the aforementioned problem. Using the micro reactor, the Dmab ester of Fmoc-β-alanine **28** was reacted with 1 equiv. of 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU)[44,45] to give the free amine **3** which was then reacted with the pentafluorophenylester of Boc-β-alanine **8**, in an attempt to form the dipeptide **9**.

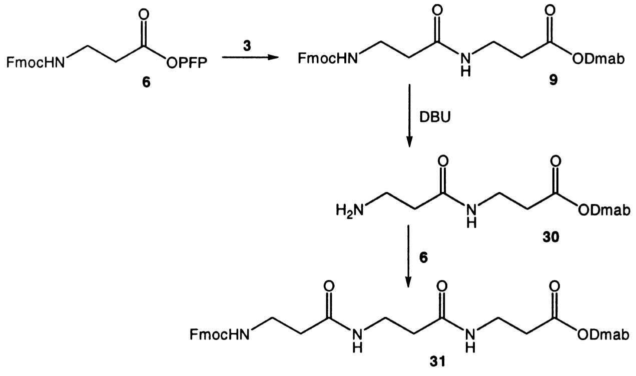
In this case, when the reagents were mixed using continuous flow, with the reagents maintained at 700 V, product **9** was observed in typically 25% yield. Surprisingly, when the reagents were mobilised using a stopped flow technique, the yield of product was actually reduced. By comparing the flows of each reagent at this stage we were able to optimise the reaction. The Dmab ester of Fmoc-β-alanine **28** was maintained at 750 V while reacted with DBU at 800 V. The deprotected amine was further reacted, using continuous flow, with the pentafluorophenyl ester of Boc-β-alanine **8**, maintained at 700 V, to give a conversion of 96%, based on the amount of Dmab ester **28** present at the end of the reaction. Importantly we have shown that deprotection may be achieved in micro reactions using just 1 equiv. of DBU, compared with 2% DBU used in SPPS.[44,45]

Having shown that more complex peptides could be produced by removal of the *N*-protecting group we wished to determine if we could remove the Dmab protecting group using hydrazine.[38] Hence, a solution of the Dmab ester of Fmoc-β-alanine **28** (50 μl, 0.1 M) in anhydrous DMF was added to reservoir A, a solution of hydrazine (50 μl, 0.1 M) was placed in reservoir B and anhydrous DMF (40 μl) was placed in reservoir D. Using continuous flow of both reagents maintained at 700 V, quantitative deprotection was observed to give carboxylic acid **4** (Scheme 11). Again deprotection was achieved in the micro reactions using just 1 equiv. of hydrazine, compared with 2% solutions used in SPPS.[38] This suggests that reactions conducted within micro reactors are more molecule efficient.



Scheme 11. Removal of the Dmab protecting group.

Having successfully demonstrated that peptide bonds could be formed and that the protecting groups could be selectively removed, we wished to show that we could use the methodology in the preparation of tripeptides within micro reactor devices. Our initial target was the tripeptide of β-alanine **31**. We selected to initially investigate the synthesis of dipeptide **9** followed by the selective deprotection of the Fmoc group and subsequent reaction of amine **30** with another equivalent of pentafluorophenyl ester **6** (Scheme 12).



Scheme 12. Tripeptide synthesis.

A standard solution of the pentafluorophenyl ester of Fmoc-β-alanine **6** (50 μl, 0.1 M) in anhydrous DMF was added to reservoir A and a solution of amine **3** (50 μl, 0.1 M) was placed in reservoir B, such that dipeptide **9** could be prepared in the channel of the micro reactor (Fig. 6). A solution of DBU (50 μl, 0.1 M) was placed in reservoir C to effect Fmoc deprotection and a second equivalent of pentafluorophenyl ester **6** was added to reservoir D. Anhydrous DMF (40 μl) was placed in reservoir E, which was used to collect the products of the reaction. Using continuous flow of all reagents where reservoir A was maintained at 1000 V, reservoir B was at 1000 V, reservoir C was at 400 V and reservoir D was at 700 V, tripeptide **31** was prepared in 30% overall conversion, for the three step synthesis. Further work is currently underway to purify the tripeptide by electrophoretic separation.

4. Conclusion

We have demonstrated the application of micro reactors to perform multi-step synthesis, potentially allowing the high throughput synthesis of peptides. It has been demonstrated that peptide bonds may be prepared in high yield by either a carbodiimide coupling reaction or from pre-activated derivatives such as pentafluorophenyl esters. It was found that performing these reactions in the micro reactor resulted in an increase in the reaction efficiency over the traditional batch method. We propose that this enhancement in rate is an electrochemical phenomenon, due to the reaction being performed in an electric field. It has also been demonstrated that selective deprotection of the resultant dipeptides can be achieved. The Fmoc group was successfully deprotected using 1 equiv. of DBU while the Dmab ester was removed by treatment with 1 equiv. of hydrazine. After deprotection further peptide bond forming reactions were performed, resulting in the synthesis of longer chain peptides. We have successfully used this approach in the synthesis of tripeptide **31**. Further studies are currently underway in our laboratories to increase the overall conversion of the multi-step reactions and to purify the peptides by electrophoretic separation.

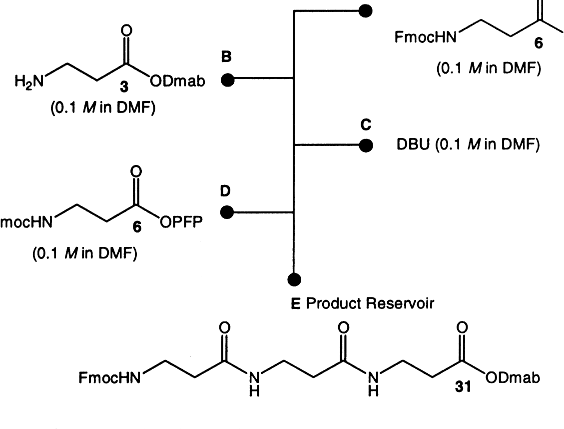


Figure 6. Schematic of the micro reactor used to prepare tripeptide.

5. Experimental

5.1. Micro reactions

Platinum electrodes were placed in each of the reservoirs of the micro reactor and an external voltage was applied to the channels inducing electroosmotic flow of the reagents. The power supply was manufactured by Kingfield Electronics (Sheffield, UK) and was controlled using LabVIEW™ software written by Dr X. Zhang (University of Hull).

The reactions were conducted at room temperature for a period of 20 min, in order to acquire sufficient volume of product to determine the conversion of the reaction. Reaction products were determined by HPLC via comparison with retention times and spectra with those obtained from synthetic standards. Analysis was achieved by high performance liquid chromatography (Jupiter C_{18} 10 μm, 4.6×250 mm, mobile phase composition: 0.1% trifluoroacetic acid in water and 0.1% trifluoroacetic acid in acetonitrile, using a gradient system of 30% aqueous to 70% aqueous over 20 min, with a flow rate of 2.5 ml min^{-1} at room temperature). Product conversions were based on the amount of carboxylic acid or pentafluorophenyl ester remaining in the sample.

5.2. Bulk reactions

All solvents were purchased as anhydrous solutions over molecular sieves from Fluka. Reagents were purchased from Aldrich and used as supplied. Amino acids were obtained from Bachem (UK) and Peptech. Column chromatography was carried out using Fluka silica gel 60 as the solid support. Compounds were eluted using various mixtures of ethyl acetate, hexane and methanol. Thin layer chromatography was performed using Merck Kieselgel 60 HF_{254} aluminium backed TLC plates with various mixtures of ethyl acetate and hexane as the eluent. Visualisation of the plates was carried out by either exposure to short wave ultra violet light or by development in aqueous potassium permanganate (0.5%) and sodium hydrogen carbonate (2.5%) solution, followed by heating with a hot air gun.

Nuclear magnetic resonance (NMR) spectra were recorded as solutions in deuteriochloroform, unless otherwise stated, using tetramethylsilane (TMS) as internal standard. The spectra were recorded on Jeol GX270 or GX400 spectrometers. The chemical shift values for all spectra are given in parts per million with coupling constants in Hertz. Mass spectra were recorded on a Thermo-Finnigan LCQ, using electrospray ionisation. Infrared spectra were recorded in the range 4000–600 cm^{-1} using a Perkin Elmer Paragon 1000 FT-IR spectrometer and peaks are reported (ν_{max}) in wavenumbers (cm^{-1}). Liquid samples were obtained as thin films and solid samples were dissolved in chloroform, on sodium chloride discs. Melting points were measured on a Gallenkamp melting point apparatus using open capillary tubes and are uncorrected. Elemental combustion analyses were measured using a Fisons Carlo Erba EA1108 analyser.

5.2.1. Boc-β-alanine-ODmab 2. A solution of EDCI (1.27 g, 6.62 mmol) and DMAP (72 mg, 0.59 mmol) in DCM (100 ml) was added to a stirred solution of Boc-β-alanine **1** (1.10 g, 5.81 mmol) and DmabOH (1.92 g, 5.83 mmol) in DCM (100 ml) at room temperature under nitrogen. After 24 h, the solvent was washed with dilute hydrochloric acid (50 ml) and the aqueous layer was further extracted with DCM (2×50 ml). The combined organic extracts were dried over magnesium sulphate and concentrated in vacuo to give a solid, which was purified by column chromatography. Elution with 20% ethyl acetate in hexane gave *Boc-β-alanine-ODmab* **2** (2.67 g, 92%) as a white solid; mp 78–80°C (from DCM/hexane); (Found C, 67.03; H, 7.79; N, 5.36. $C_{28}H_{40}N_2O_6$ requires C, 67.20; H, 8.00; N, 5.60%); ν_{max} (cm^{-1}) 3453, 3360, 2940, 1734, 1708, 1641, 1454 and 754; δ_H (399.6 MHz) 0.78 (6H, d, J=6.6 Hz, $CH(CH_3)_2$), 1.08 (6H, s, $C(CH_3)_2$), 1.43 (9H, s, $C(CH_3)_3$), 1.84 (1H, m, $CH(CH_3)_2$), 2.40 (2H, s, $CH_2C(CH_3)_2$), 2.49 (2H, s, $CH_2C(CH_3)_2$), 2.61 (2H, t, J=6.1 Hz, CH_2CO_2), 3.01 (2H, br d, J=6.1 Hz, $CH_2CH(CH_3)_2$), 3.43 (2H, dt, J=6.1 and 6.1 Hz, $NHCH_2$), 5.01 (1H, m, $NHCH_2$), 5.17 (2H, s, CO_2CH_2), 7.14 (2H, d, J=8.4 Hz, ArH), 7.41 (2H, d, J=8.4 Hz, ArH) and 15.30 (1H, s, ArNH); δ_C (100.4 MHz) 22.6, 28.3, 29.5, 30.0, 34.6, 36.1, 38.3, 52.3, 53.8, 65.5, 79.4, 100.5, 107.8, 126.7, 129.0, 135.3, 136.9, 155.7, 172.2, 176.4, 196.3 and 200.2; m/z 501 (M^++1).

5.2.2. β-Alanine-ODmab 3. Trifluoroacetic acid (20 ml) was added to a solution of Boc-β-alanine-ODmab **2** (1.28 g, 2.56 mmol) in DCM (5 ml). After stirring for 30 min, the reaction mixture was concentrated in vacuo to remove the excess trifluoroacetic acid. The residue was dissolved in DCM (50 ml) and washed with saturated sodium hydrogen carbonate solution (50 ml). The aqueous solution was further extracted with DCM (2×50 ml) and the combined organic extracts were dried over magnesium sulphate and concentrated in vacuo to give a solid which was recrystallised using DCM/Et_2O/hexane to give *β-alanine-ODmab* (as the TFA salt) **3** (1.21 g, 92%) as a pale yellow solid; mp 162–164°C; (Found C, 58.39; H, 6.50; N, 5.29. $C_{25}H_{33}N_2O_6F_3$ requires C, 58.37; H, 6.42; N, 5.45%); ν_{max} (cm^{-1}) 3451, 3251, 2960, 1730, 1672, 1648, 1450, 1195 and 755; δ_H (399.6 MHz) 0.77 (6H, d, J=6.6 Hz, $CH(CH_3)_2$), 1.08 (6H, s, $C(CH_3)_2$), 1.84 (1H, m, $CH(CH_3)_2$), 2.40 (2H, s, $CH_2C(CH_3)_2$), 2.49 (2H, s, $CH_2C(CH_3)_2$), 2.86 (2H, t, J= 6.4 Hz, CH_2CO_2), 3.00 (2H, br d, J=5.1 Hz, $CH_2CH(CH_3)_2$), 3.26 (2H, t, J=6.6 Hz, NH_2CH_2), 5.01 (1H, m, $NHCH_2$), 5.17 (2H, s, CO_2CH_2), 7.14 (2H, d, J=8.4 Hz, ArH), 7.41 (2H, d, J=8.4 Hz, ArH), 8.42 (2H, br s, NH_2) and 15.31 (1H, s, ArNH); δ_C (100.4 MHz) 22.6, 28.2, 29.5, 30.0, 31.3, 35.3, 38.3, 52.3, 53.8, 66.1, 107.8, 126.7, 129.2, 134.8, 137.0, 171.0, 176.4, 196.3 and 200.2; m/z 401 (M^++1).

5.2.3. Fmoc-β-alanine-β-alanine-ODmab 5. A solution of EDCI (91 mg, 0.47 mmol) in DCM (10 ml) was added to a stirred solution of Fmoc-β-alanine **4** (94 mg, 0.30 mmol) and amine **3** (130 mg, 0.33 mmol) in DCM (10 ml) at room temperature under nitrogen. After 24 h, the solvent was washed with dilute hydrochloric acid (20 ml) and the aqueous layer was further extracted with DCM (2×50 ml). The combined organic extracts were dried over magnesium sulphate and concentrated in vacuo to give an oil which was purified by column chromatography. Elution with ethyl acetate gave *Fmoc-β-alanine-β-alanine-ODmab* **5** (105 mg, 50%) as a colourless gum; δ_H (399.6 MHz) 0.77 (6H, d, J=6.8 Hz, $CH(CH_3)_2$), 1.08 (6H, s, $C(CH_3)_2$), 1.84 (1H, m, $CH(CH_3)_2$), 2.39 (2H, m, CH_2CO_2), 2.40 (2H, s, $CH_2C(CH_3)_2$), 2.49 (2H, s, $CH_2C(CH_3)_2$), 2.61 (2H, t, J= 6.4 Hz, CH_2CO), 3.00 (2H, m, $CH_2CH(CH_3)_2$), 3.48 (2H, m, $NHCH_2$), 3.56 (2H, m, $NHCH_2$), 4.20 (1H, t, J=6.8 Hz, $CHCH_2O$), 4.37 (2H, d, J=6.8 Hz, $CHCH_2O$), 5.30 (2H, s, CO_2CH_2), 5.52 (1H, m, NH), 6.17 (1H, br t, NH), 7.12 (2H, d, J=8.0 Hz, ArH), 7.30 (2H, t, J=7.6 Hz, ArH), 7.39 (2H, t, J=7.1 Hz, ArH), 7.40 (2H, d, J=8.0 Hz, ArH), 7.59 (2H, d, J=7.8 Hz, ArH), 7.76 (2H, d, J=7.8 Hz, ArH) and 15.29 (1H, s, ArNH); δ_C (100.4 MHz) 22.6, 28.2, 29.6, 30.0, 31.9, 34.0, 34.9, 38.4, 47.2, 52.3, 53.7, 64.5, 65.8, 66.7, 107.8, 120.0, 125.1, 126.7, 127.1, 127.7, 129.1, 135.1, 137.0, 141.3, 143.9, 155.9, 172.1, 172.3, 176.5, 196.5 and 200.3; m/z 694 (M^++1).

5.2.4. Fmoc-β-alanine-OPFP 6. A solution of EDCI (1.32 g, 6.88 mmol) in DCM (50 ml) was added to a stirred solution of Fmoc-β-alanine **4** (2.06 g, 6.62 mmol) and pentafluorophenol (1.22 g, 6.63 mmol) in DCM (100 ml) at room temperature under nitrogen. After 24 h, the solvent was washed with dilute hydrochloric acid (50 ml) and the aqueous layer was further extracted with DCM (2×50 ml). The combined organic extracts were dried over magnesium sulphate and concentrated in vacuo to give a solid which was which was purified by column chromatography. Elution with 20% ethyl acetate in gave *Fmoc-β-alanine-OPFP* **6** (2.85 g, 90%) as a white solid; mp 112–114°C (from EtOAc/hexane); (Found C, 60.06; H, 3.09; N, 2.67. $C_{24}H_{16}NO_4F_5$ requires C, 60.38; H, 3.35; N, 2.94%); ν_{max} (cm^{-1}) 3422, 1787, 1696, 1518, 1002 and 758; δ_H (399.6 MHz) 2.94 (2H, t, J=6.1 Hz, CH_2CO_2), 3.60 (2H, dt, J=5.8 and 6.1 Hz, $NHCH_2$), 4.22 (1H, t, J=6.8 Hz, $CHCH_2O$), 4.42 (2H, d, J=6.8 Hz, $CHCH_2O$), 5.22 (1H, m, NH), 7.31 (2H, t, J=7.3 Hz, ArH), 7.40 (2H, t, J= 7.3 Hz, ArH), 7.58 (2H, d, J=6.8 Hz, ArH) and 7.76 (2H, d, J=7.5 Hz, ArH); δ_C (100.4 MHz) 33.8, 36.4, 47.2, 66.9, 120.0, 125.0, 127.0, 127.7, 136.6 (m, CF), 138.4 (m, CF), 139.1 (m, CF), 139.8 (m, CF), 140.8 (m, CF), 141.3, 142.2 (m, CF), 143.7, 156.3 and 168.3; m/z 478 (M^++1).

5.2.5. Fmoc-β-alanine-β-alanine-ODmab 5. A solution of pentafluorophenyl ester **6** (181 mg, 0.38 mmol) and amine **3** (161 mg, 0.40 mmol) in DMF (2 ml) was stirred for 24 h. The DMF was removed in vacuo, the residue was dissolved in DCM (50 ml) and washed with dilute hydrochloric acid (20 ml). The aqueous layer was further extracted with DCM (2×50 ml) and the combined organic extracts were dried over magnesium sulphate and concentrated in vacuo to give a yellow oil which was purified by column chromatography. Elution with ethyl acetate gave *Fmoc-β-alanine-β-alanine-ODmab* **5** (121 mg, 46%) as a colourless oil. Spectroscopic data as reported.

5.2.6. Boc-β-alanine-OPFP 8. A solution of EDCI (2.35 g, 12.26 mmol) and DMAP (60 mg, 0.49 mmol) in DCM (100 ml) was added to a stirred solution of Boc-β-alanine **7** (2.28 g, 12.05 mmol) and pentafluorophenol (2.25 g, 12.22 mmol) in DCM (100 ml) at room temperature under nitrogen. After 24 h, the solvent was washed with dilute hydrochloric acid (50 ml) and the aqueous layer was further extracted with DCM (2×50 ml). The combined organic extracts were dried over magnesium sulphate and concentrated in vacuo to give a solid which was recrystallised using DCM and hexane to give *Boc-β-alanine-OPFP* **8** (3.37 g, 79%) as a white solid; mp 56–57°C; (Found C, 47.27; H, 3.86; N, 3.61. $C_{14}H_{14}NO_4F_5$ requires C, 47.32; H, 3.94; N, 3.94%); ν_{max} (cm^{-1}) 3374, 2989, 1797, 1691, 1518, 1097, 988 and 756; δ_H (399.6 MHz) 1.45 (9H, s, $(CH_3)_3CO$), 2.93 (2H, t, J=6.1 Hz, CH_2CO_2), 3.53 (2H, dt, J=6.1 and 6.1 Hz, $NHCH_2$) and 4.98 (1H, m, NH); δ_C (100.4 MHz) 28.4, 34.0, 36.0, 79.9, 136.6 (m, CF), 138.3 (m, CF), 139.2 (m, CF), 139.8 (m, CF), 140.8 (m, CF), 142.3 (m, CF), 155.8 and 168.5; m/z 356 (M^++1).

5.2.7. Boc-β-alanine-β-alanine-ODmab 9. A solution of pentafluorophenyl ester **8** (142 mg, 0.40 mmol) and amine **3** (176 mg, 0.44 mmol) in DMF (2 ml) was stirred for 48 h. The DMF was removed in vacuo, the residue was dissolved in DCM (50 ml) and washed with dilute hydrochloric acid (20 ml). The aqueous layer was further extracted with DCM (2×30 ml) and the combined organic extracts were dried over magnesium sulphate and concentrated in vacuo to give a yellow oil which was purified by column chromatography. Elution with ethyl acetate gave *Boc-β-alanine-β-alanine-ODmab* **9** (129 mg, 57%) as a colourless oil; δ_H (399.6 MHz) 0.78 (6H, d, J=6.8 Hz, $CH(CH_3)_2$), 1.08 (6H, s, $C(CH_3)_2$), 1.43 (9H, s, $C(CH_3)_3$), 1.85 (1H, m, $CH(CH_3)_2$), 2.38 (2H, t, J=6.1 Hz, CH_2CO_2), 2.45 (4H, s, $2\times CH_2C(CH_3)_2$), 2.63 (2H, t, J=6.1 Hz, CH_2CO), 3.00 (2H, br d, J=6.1 Hz, $CH_2CH(CH_3)_2$), 3.38 (2H, m, $NHCH_2$), 3.56 (2H, dt, J=6.1 and 6.1 Hz, $NHCH_2$), 5.01 (1H, m, $NHCH_2$), 5.14 (1H, br s, NH), 5.17 (2H, s, CO_2CH_2), 6.21 (1H, br s, NH), 7.14 (2H, d, J=8.3 Hz, ArH), 7.41 (2H, d, J=8.3 Hz, ArH) and 15.30 (1H, s, ArNH); δ_C (100.4 MHz) 22.6, 28.2, 28.4, 29.5, 30.0, 34.0, 34.8, 36.3, 38.4, 53.0, 54.9, 65.7, 79.4, 107.8, 126.7, 129.1, 135.1, 137.0, 156.0, 171.4, 172.2, 176.4, 196.3 and 200.2; m/z 572 (M^++1).

5.2.8. Fmoc-L-β-homophenylalanine-OPFP 11. A solution of EDCI (0.37 g, 1.96 mmol) in DCM (50 ml) was added to a stirred solution of Fmoc-L-β-homophenylalanine **10** (0.43 g, 1.24 mmol) and pentafluorophenol (0.35 g, 1.91 mmol) in DCM (50 ml) at room temperature under nitrogen. After 24 h, the solvent was washed with dilute hydrochloric acid (50 ml) and the aqueous layer was further extracted with DCM (2×50 ml). The combined organic extracts were dried over magnesium sulphate and concentrated in vacuo to give a solid which purified by column chromatography. Elution with 20% ethyl aceate in hexane gave *Fmoc-L-β-homophenylalanine-OPFP* **11** (0.55 g, 86%) as a white solid; mp 140–142°C (DCM/hexane); ν_{max} (cm^{-1}) 3326, 1782, 1691, 1518, 993 and 754; δ_H (399.6 MHz) 2.83–3.05 (4H, m, CH_2CO_2 and CH_2Ph), 4.20 (1H, t, J=6.8 Hz, $CHCH_2O$), 4.36–4.42 (3H, m, $CHCH_2O$ and $CHNH$), 5.13 (1H, d, J=8.8 Hz, NH), 7.19–7.33 (7H, m, ArH), 7.40 (2H, t, J=7.3 Hz, ArH), 7.55 (2H, d, J=7.3 Hz, ArH) and 7.76 (2H, d, J=7.3 Hz, ArH); δ_C (100.4 MHz) 36.8, 39.7, 47.1, 49.2, 66.8, 120.0, 125.0, 127.0, 127.1, 127.7, 128.8, 129.3, 136.6 (m, CF), 138.4 (m, CF), 136.8, 139.1 (m, CF), 139.8 (m, CF), 140.8 (m, CF), 141.3, 142.2 (m, CF), 143.8, 155.5 and 167.5; m/z 567 (M^++1).

5.2.9. Fmoc-L-β-homophenylalanine-β-alanine-ODmab 12. A solution of pentafluorophenyl ester **11** (171 mg, 0.30 mmol) and amine **3** (125 mg, 0.31 mmol) in DMF (2 ml) was stirred for 24 h. The DMF was removed in vacuo, the residue was dissolved in DCM (50 ml) and washed with dilute hydrochloric acid (20 ml). The aqueous layer was further extracted with DCM (2×50 ml) and the combined organic extracts were dried over magnesium sulphate and concentrated in vacuo to give a yellow oil which was purified by column chromatography. Elution with ethyl acetate gave *Fmoc-L-β-homophenylalanine-β-alanine-ODmab* **12** (83 mg, 35%) as a cream solid; mp 116–118°C (DCM/hexane); ν_{max} (cm^{-1}) 3432, 3316, 2951, 1734, 1686, 1556, 1445, 1166 and 756; δ_H (399.6 MHz) 0.76 (6H, d, J=6.8 Hz, $CH(CH_3)_2$), 1.08 (6H, s, $C(CH_3)_2$), 1.83 (1H, m, $CH(CH_3)_2$), 2.40 (2H, s, $CH_2C(CH_3)_2$), 2.49 (2H, s, $CH_2C(CH_3)_2$), 2.61 (2H, br t, CH_2CO_2), 2.87–2.99 (6H, m, $CH_2CH(CH_3)_2$, CH_2CO_2 and CH_2Ph), 3.54 (2H, m, CH_2NH), 4.18 (5H, t, J=7.0 Hz, $CHCH_2O$), 4.28–4.39 (2H, m, $CHCH_2O$ and $CHNH$), 5.12 (2H, s, CO_2CH_2), 5.84 (1H, br d, J=8.4 Hz, NH), 6.28 (1H, m, NH), 7.09 (2H, d, J=7.3 Hz, ArH), 7.18–7.57 (13H, m, ArH), 7.76 (2H, d, J=7.3 Hz, ArH) and 15.28 (1H, s, ArNH); δ_C (100.4 MHz) 22.6, 28.2, 29.5, 30.0, 34.0, 34.9, 36.5, 38.3, 40.2, 47.2, 52.3, 53.7, 65.7, 66.7, 107.8, 120.0, 125.1, 126.7, 127.1, 127.7, 128.6, 129.1, 129.3, 135.0, 136.8, 137.8, 141.3, 143.9, 155.9, 162.6, 170.9, 172.2, 176.4, 196.4 and 200.2; m/z 784 (M^++1).

5.2.10. Fmoc-L-β-homo-*p*-chlorophenylalanine 14. A solution of 9-fluorenylmethyl chloroformate (0.37 g, 1.45 mmol) in dioxane (30 ml) was added to a stirred solution of L-β-homo-*p*-chlorophenylalanine **13** (0.30 g, 1.22 mmol) in saturated aqueous sodium hydrogen carbonate (50 ml). After stirring for 24 h the mixture was concentrated in vacuo and acidified using dilute hydrochloric acid (50 ml) and extracted using dichloromethane (3×50 ml). The combined organic extracts were dried over magnesium sulphate and concentrated in vacuo to give a solid which was recrystallised from DCM/hexane to give *Fmoc-L-β-homo-p-chlorophenylalanine* **14** (0.37 g, 70%) as a white solid; mp 176–178°C; (Found C, 68.90; H, 5.16; N, 3.14. $C_{25}H_{22}NO_4Cl$ requires C, 68.97; H, 5.06; N, 3.22%); ν_{max} (cm^{-1}) 3326, 1682, 1652, 1537 and 737; δ_H (DMSO, 399.6 MHz) 2.47 (2H, d, J=6.1 Hz, CH_2CO_2), 2.84 (2H, d, J=6.6 Hz, CH_2Ph), 4.12–4.36 (4H, m, $CHCH_2O$ and NH), 4.42 (2H, d, J=5.6 Hz, $CHCH_2O$), 6.63 (1H, d, J= 8.0 Hz, NH), 7.15 (2H, d, J=8.1 Hz, ArH), 7.21 (2H, d, J=8.1 Hz, ArH), 7.31 (2H, dt, J=7.3 and 3.7 Hz, ArH), 7.40 (2H, t, J=7.3 Hz, ArH), 7.58 (2H, m, ArH) and 7.76

(2H, d, J=7.6 Hz, ArH); δ_C (100.4 MHz) 29.4, 38.4, 47.1, 49.4, 65.8, 119.8, 125.1, 127.0, 127.6, 128.2, 130.8, 131.7, 136.9, 141.0, 143.8, 155.6 and 173.0; m/z 436 (M^++1).

5.2.11. Fmoc-L-β-homo-*p*-chlorophenylalanine-OPFP 15. A solution of EDCI (0.20 g, 1.07 mmol) in DCM (20 ml) was added to a stirred solution of Fmoc-L-β-homo-*p*-chlorophenylalanine **14** (0.31 g, 0.71 mmol) and pentafluorophenol (0.14 g, 0.75 mmol) in DCM (20 ml) at room temperature under nitrogen. After 24 h, the solvent was washed with dilute hydrochloric acid (50 ml) and the aqueous layer was further extracted with DCM (2×50 ml). The combined organic extracts were dried over magnesium sulphate and concentrated in vacuo to give a solid which was recrystallised using DCM and hexane to give *Fmoc-L-β-homo-p-chlorophenylalanine-OPFP* **15** (0.40 g, 93%) as a white solid; mp 160–162°C; ν_{max} (cm^{-1}) 3322, 1777, 1687, 1520, 991, 758 and 741; δ_H (399.6 MHz) 2.86–3.01 (4H, m, CH_2CO_2 and CH_2Ph), 4.20 (1H, t, J=5.6 Hz, C*H*CH_2O), 4.30 (3H, m, C*H*NH), 4.42 (2H, d, J=5.6 Hz, CHC*H*$_2$O), 5.09 (1H, d, J=9.0 Hz, NH), 7.12 (2H, d, J= 7.6 Hz, ArH), 7.26–7.32 (4H, m, ArH), 7.40 (2H, t, J= 7.3 Hz, ArH), 7.54 (2H, d, J=7.3 Hz, ArH) and 7.76 (2H, d, J=7.6 Hz, ArH); δ_C (100.4 MHz) 36.8, 39.1, 47.2, 49.2, 66.8, 120.0, 125.0, 127.1, 127.8, 129.0, 130.6, 133.0, 135.3, 136.6 (m, CF), 138.4 (m, CF), 139.1 (m, CF), 139.8 (m, CF), 140.8 (m, CF), 141.4, 142.2 (m, CF), 143.8, 155.5 and 167.4; m/z 602 (M^++1).

5.2.12. Fmoc-L-β-homo-*p*-chlorophenylalanine-β-alanine-ODmab 16. A solution of pentafluorophenyl ester **15** (130 mg, 0.21 mmol) and amine **3** (102 mg, 0.26 mmol) in DMF (2 ml) was stirred for 24 h. The DMF was removed in vacuo, the residue was dissolved in DCM (50 ml) and washed with dilute hydrochloric acid (20 ml). The aqueous layer was further extracted with DCM (2×50 ml) and the combined organic extracts were dried over magnesium sulphate and concentrated in vacuo to give a yellow oil which was purified by column chromatography. Elution with ethyl acetate gave *Fmoc-L-β-homo-p-chlorophenylalanine-β-alanine-ODmab* **16** (64 mg, 36%) as a white solid; mp 152–156°C (DCM/hexane); ν_{max} (cm^{-1}) 3451, 3297, 2951, 1734, 1681 and 753; δ_H (399.6 MHz) 0.76 (6H, d, J=6.7 Hz, $CH(CH_3)_2$), 1.08 (6H, s, $C(CH_3)_2$), 1.83 (1H, m, C*H*$(CH_3)_2$), 2.40 (2H, s, C*H*$_2C(CH_3)_2$), 2.49 (2H, s, C*H*$_2C(CH_3)_2$), 2.60 (2H, br t, CH_2CO_2), 2.78–3.00 (6H, m, C*H*$_2CH(CH_3)_2$, CH_2CO_2 and CH_2Ph), 3.54 (2H, m, C*H*$_2$NH), 4.18 (5H, t, J=6.7 Hz, CHC*H*$_2$O), 4.30–4.39 (2H, m, C*H*CH_2O and C*H*NH), 5.13 (2H, s, CO_2CH_2), 5.80 (1H, br d, J=8.2 Hz, NH), 6.24 (1H, m, NH), 7.11 (2H, d, J=7.6 Hz, ArH), 7.22–7.56 (12H, m, ArH), 7.76 (2H, d, J=7.6 Hz, ArH) and 15.29 (1H, s, ArNH); δ_C (100.4 MHz) 22.6, 28.3, 29.6, 30.0, 34.0, 34.9, 36.5, 38.4, 39.4, 47.2, 52.3, 53.8, 65.8, 66.6, 107.8, 120.0, 125.1, 126.7, 127.1, 127.7, 128.7, 129.1, 130.6, 132.5, 135.0, 136.4, 137.0, 141.3, 143.9, 155.9, 162.6, 170.7, 172.2, 176.4, 196.4 and 200.3; m/z 818 (M^++1).

5.2.13. *N*ε-Boc-*N*α-Fmoc-L-lysine 18. A solution of 9-fluorenylmethyl chloroformate (0.52 g, 2.02 mmol) in dioxane (50 ml) was added to a stirred solution of *N*ε-Boc-L-lysine **17** (0.45 g, 1.83 mmol) in saturated aqueous sodium hydrogen carbonate (50 ml). After stirring for 24 h the mixture was concentrated in vacuo and acidified using dilute hydrochloric acid (100 ml) and extracted using dichloromethane (3×100 ml). The combined organic extracts were dried over magnesium sulphate and concentrated in vacuo to give a solid which was recrystallised from DCM/hexane to afford *Nε-Boc-Nα-Fmoc-L-lysine* **18** (0.80 g, 93%) as a white solid; mp 44–46°C (from DCM/hexane); ν_{max} (cm^{-1}) 3422, 3336, 2970, 1705, 1648, 1516 and 757; δ_H (DMSO, 399.6 MHz) 1.25–1.41 (4H, m, CH_2CH_2), 1.36 (9H, s, $(CH_3)_3CO$), 1.51–1.68 (2H, m, CH_2), 2.89 (2H, m, C*H*$_2$NH), 3.89 (1H, m, C*H*CO_2H), 4.21 (1H, t, J=6.1 Hz, C*H*CH_2O), 4.27 (1H, d, J=6.1 Hz, CHC*H*$_2$O), 6.77 (1H, t, J=5.6 Hz, N*H*CH_2), 7.31 (2H, t, J=7.3 Hz, ArH), 7.41 (2H, t, J=7.3 Hz, ArH), 7.72 (2H, d, J=7.6 Hz, ArH), 7.88 (2H, d, J=7.3 Hz, ArH) and 12.56 (1H, br s, CO_2H); m/z 467 (M^+−1).

5.2.14. *N*ε-Boc-*N*α-Fmoc-L-lysine-OPFP 19. A solution of EDCI (0.32 g, 1.68 mmol) in DCM (30 ml) was added to a stirred solution of *N*ε-Boc-*N*α-Fmoc-L-lysine **18** (0.74 g, 1.58 mmol) and pentafluorophenol (0.29 g, 1.60 mmol) in DCM (50 ml) at room temperature under nitrogen. After 24 h, the solvent was washed with dilute hydrochloric acid (50 ml) and the aqueous layer was further extracted with DCM (2×50 ml). The combined organic extracts were dried over magnesium sulphate and concentrated in vacuo to give a solid, which was purified by column chromatography. Elution with 20% ethyl acetate in hexane gave *Nε-Boc-Nα-Fmoc-L-lysine-OPFP* **19** (0.54 g, 54%) as a white solid; mp 100–102°C (from DCM/hexane); (Found C, 60.30; H, 4.81; N, 4.25. $C_{32}H_{31}N_2O_6F_5$ requires C, 60.57; H, 4.89; N, 4.42%); ν_{max} (cm^{-1}) 3432, 3345, 2900, 1787, 1681, 1518, 993 and 753; δ_H (399.6 MHz) 1.28–1.59 (4H, m, CH_2CH_2), 1.44 (9H, s, $(CH_3)_3CO$), 1.91–2.09 (2H, m, CH_2), 3.16 (2H, m, C*H*$_2$NH), 4.24 (1H, t, J=6.8 Hz, C*H*CH_2O), 4.40–4.72 (4H, m, CHC*H*$_2$O, $CHCO_2$ and NH), 5.58 (1H, d, J=7.8 Hz, N*H*), 7.31 (2H, t, J=7.3 Hz, ArH), 7.40 (2H, t, J=7.3 Hz, ArH), 7.61 (2H, d, J=7.3 Hz, ArH) and 7.77 (2H, d, J=7.6 Hz, ArH); δ_C (100.4 MHz) 22.1, 28.4, 29.7, 31.4, 39.6, 47.1, 53.7, 67.2, 79.4, 120.0, 125.0, 127.1, 127.7, 136.6 (m, CF), 138.4 (m, CF), 139.1 (m, CF), 139.7 (m, CF), 141.0 (m, CF), 141.3, 142.3 (m, CF), 143.7, 155.9, 156.3 and 168.8; m/z 635 (M^++1).

5.2.15. *N*ε-Boc-*N*α-Fmoc-L-lysine-β-Aalanine-ODmab 20. A solution of carboxylic acid **18** (142 mg, 0.30 mmol), amine **3** (131 mg, 0.33 mmol) and EDCI (95 mg, 0.50 mmol) in DCM (20 ml) was stirred under nitrogen. After 24 h, the solvent was washed with dilute hydrochloric acid (50 ml) and the aqueous layer was further extracted with DCM (2×50 ml). The combined organic extracts were dried over magnesium sulphate and concentrated in vacuo to give an oil which was purified by column chromatography. Elution with ethyl acetate in gave *Nε-Boc-Nα-Fmoc-L-lysine-β-alanine-ODmab* **20** (23 mg, 9%) as a colourless gum; ν_{max} (cm^{-1}) 3432, 3336, 2951, 1667, 1551, 1450, 1166 and 748; δ_H (399.6 MHz) 0.77 (6H, d, J=6.4 Hz, $CH(CH_3)_2$), 1.08 (6H, s, $C(CH_3)_2$), 1.24–1.54 (6H, m, $CH_2CH_2CH_2$), 1.43 (9H, s, $(CH_3)_3CO$), 1.83 (1H, m, C*H*$(CH_3)_2$), 2.40 (2H, s, C*H*$_2C(CH_3)_2$), 2.49 (2H, s, C*H*$_2C(CH_3)_2$), 2.61 (2H, t, J=5.6 Hz, CH_2CO_2), 2.99 (2H, br d, J=5.3 Hz, $CH_2CH(CH_3)_2$), 3.10 (2H, m, CH_2NH), 3.55 (2H, m, CH_2NH), 4.21 (2H, t, J=6.8 Hz, $CHCH_2O$), 4.39 (2H, m, $CHCH_2O$ and $CHCH$), 4.69 (1H, m, NH), 5.09 (2H, s, CO_2CH_2), 5.56 (1H, m, NH), 6.70 (1H, m, NH), 7.09 (2H, d, J=8.4 Hz, ArH), 7.31 (2H, dt, J=7.3 and 1.1 Hz, ArH), 7.33 (2H, t, J=8.4 Hz, ArH), 7.40 (2H, t, J=7.3 Hz, ArH), 7.59 (2H, d, J=7.3 Hz, ArH), 7.76 (2H, d, J=8.4 Hz, ArH) and 15.28 (1H, s, ArNH); δ_C (100.4 MHz) 22.6, 28.3, 24.4, 29.6, 29.7, 30.0, 32.0, 33.9, 35.0, 38.4, 39.8, 47.2, 52.3, 53.8, 54.9, 60.4, 65.7, 67.1, 79.3, 107.8, 120.0, 125.1, 126.7, 127.1, 127.8, 129.1, 135.1, 136.9, 141.3, 143.8, 156.3, 171.8, 172.1, 176.5, 196.5 and 200.2; *m*/*z* 851 (M^++1).

5.2.16. *N*α-Boc-*N*ε-Fmoc-L-lysine-OPFP 22. A solution of 9-fluorenylmethyl chloroformate (0.62 g, 2.40 mmol) in dioxane (30 ml) was added to a stirred solution of *N*α-Boc-L-lysine **21** (0.50 g, 2.05 mmol) in saturated aqueous sodium hydrogen carbonate (30 ml). After stirring for 24 h the mixture was concentrated in vacuo and acidified using dilute hydrochloric acid and extracted using dichloromethane (3×50 ml). The combined organic extracts were dried over magnesium sulphate and concentrated in vacuo to give an oil which was then reacted with EDCI (0.55 g, 2.86 mmol) and pentafluorophenol (0.41 g, 2.22 mmol) in DCM (100 ml). After 24 h, the solvent was washed with dilute hydrochloric acid (50 ml) and the aqueous layer was further extracted with DCM (2×50 ml). The combined organic extracts were dried over magnesium sulphate and concentrated in vacuo to give a solid, which was purified by column chromatography. Elution with 20% ethyl acetate in hexane gave *Nα-Boc-Nε-Fmoc-L-lysine-OPFP* **22** (0.76 g, 59%) as a white solid; mp 146–148°C (from DCM/hexane); (Found C, 60.80; H, 5.19; N, 4.62. $C_{32}H_{31}N_2O_6F_5$ requires C, 60.57; H, 4.89; N, 4.42%); ν_{max} (cm^{-1}) 3413, 3336, 1787, 1686, 1518, 998 and 758; δ_H (399.6 MHz) 1.25–1.65 (4H, m, CH_2CH_2), 1.44 (9H, s, $(CH_3)_3CO$), 1.83–2.00 (2H, m, CH_2), 3.22 (2H, m, CH_2NH), 4.20 (1H, t, J=6.8 Hz, $CHCH_2O$), 4.37–4.83 (4H, m, $CHCH_2O$, $CHCO_2$ and NH), 5.58 (1H, d, J=7.8 Hz, NH), 7.30 (2H, t, J=7.3 Hz, ArH), 7.39 (2H, t, J=7.3 Hz, ArH), 7.58 (2H, d, J=7.3 Hz, ArH) and 7.76 (2H, d, J=7.3 Hz, ArH); δ_C (100.4 MHz) 22.3, 28.3, 29.6, 31.8, 40.4, 47.4, 53.4, 66.7, 77.4, 120.0, 125.1, 127.1, 127.7, 136.7 (m, CF), 138.4 (m, CF), 139.2 (m, CF), 139.9 (m, CF), 141.0 (m, CF), 141.4, 142.3 (m, CF), 144.0, 155.3, 156.7 and 169.2; *m*/*z* 635 (M^++1).

5.2.17. *N*α-Boc-*N*ε-Fmoc-L-lysine-β-alanine-ODmab 23. A solution of pentafluorophenyl ester **22** (184 mg, 0.293 mmol) and amine **3** (139 mg, 0.35 mmol) in DMF (2 ml) was stirred for 24 h. The DMF was removed in vacuo, the residue was dissolved in DCM (50 ml) and washed with dilute hydrochloric acid (20 ml). The aqueous layer was further extracted with DCM (2×50 ml) and the combined organic extracts were dried over magnesium sulphate and concentrated in vacuo to give a yellow oil which was purified by column chromatography. Elution with ethyl acetate gave *Nα-Boc-Nε-Fmoc-L-lysine-β-alanine-ODmab* **23** (125 mg, 50%) as a pale yellow oil; ν_{max} (cm^{-1}) 3393, 2951, 1710, 1648, 1547, 1243, 1162 and 757; δ_H (399.6 MHz) 0.77 (6H, d, J=6.7 Hz, $CH(CH_3)_2$), 1.07 (6H, s, $C(CH_3)_2$), 1.34–1.63 (6H, m, $CH_2CH_2CH_2$), 1.42 (9H, s, $(CH_3)_3CO$), 1.83 (1H, m, $CH(CH_3)_2$), 2.40 (2H, s, $CH_2C(CH_3)_2$), 2.49 (2H, s, $CH_2C(CH_3)_2$), 2.61 (2H, t, J=5.6 Hz, CH_2CO_2), 3.00 (2H, br d, J=5.3 Hz, $CH_2CH(CH_3)_2$), 3.18 (2H, m, CH_2NH), 3.55 (2H, m, CH_2NH), 4.02 (1H, m, NH), 4.20 (1H, m, $CHCH_2O$), 4.39 (2H, m, $CHCH_2O$), 5.09 (1H, m, NH), 5.18 (1H, m, NH), 5.30 (2H, s, CO_2CH_2), 6.75 (1H, m, NH), 7.12 (2H, d, J=8.1 Hz, ArH), 7.30 (2H, dt, J=7.3 and 0.9 Hz, ArH), 7.39 (4H, t, J=8.4 Hz, ArH), 7.59 (2H, d, J=7.3 Hz, ArH), 7.76 (2H, d, J=8.1 Hz, ArH) and 15.29 (1H, s, ArNH); δ_C (100.4 MHz) 22.6, 28.2, 24.3, 29.4, 29.5, 30.0, 31.9, 33.9, 34.9, 38.3, 40.3, 47.2, 52.3, 53.4, 53.7, 60.4, 65.6, 66.6, 80.1, 107.8, 119.9, 125.0, 126.7, 127.0, 127.7, 129.1, 135.2, 136.9, 141.3, 143.9, 156.6, 172.0, 172.2, 176.4, 196.4 and 200.2; *m*/*z* 851 (M^++1).

5.2.18. Boc-glycine-ODmab 25. A solution of EDCI (0.95 g, 4.96 mmol) and DMAP (62 mg, 0.51 mmol) in DCM (50 ml) was added to a stirred solution of Boc-glycine **24** (0.82 g, 4.68 mmol) and DmabOH (1.55 g, 4.70 mmol) in DCM (100 ml) at room temperature under nitrogen. After 24 h, the solvent was washed with dilute hydrochloric acid (50 ml) and the aqueous layer was further extracted with DCM (2×50 ml). The combined organic extracts were dried over magnesium sulphate and concentrated in vacuo to give a solid which was purified by column chromatography. Elution with 20% ethyl acetate in hexane gave *Boc-glycine-ODmab* **25** (2.30 g, 100%) as a white solid; mp 50–52°C (from DCM/hexane); (Found C, 66.52; H, 7.97; N, 5.49. $C_{27}H_{38}N_2O_6$ requires C, 66.67; H, 7.82; N, 5.76%); ν_{max} (cm^{-1}) 3441, 3364, 2951, 1749, 1710, 1633, 1551 and 754; δ_H (399.6 MHz) 0.78 (6H, d, J=6.6 Hz, $CH(CH_3)_2$), 1.08 (6H, s, $C(CH_3)_2$), 1.45 (9H, s, $C(CH_3)_3$), 1.84 (1H, m, $CH(CH_3)_2$), 2.40 (2H, s, $CH_2C(CH_3)_2$), 2.49 (2H, s, $CH_2C(CH_3)_2$), 3.00 (2H, br d, J=6.1 Hz, $CH_2CH(CH_3)_2$), 3.99 (2H, d, J=5.6 Hz, $NHCH_2$), 5.07 (1H, m, $NHCH_2$), 5.21 (2H, s, CO_2CH_2), 7.14 (2H, d, J=8.3 Hz, ArH), 7.41 (2H, d, J=8.3 Hz, ArH) and 15.30 (1H, s, ArNH); δ_C (100.4 MHz) 22.5, 28.2, 29.5, 30.0, 38.3, 42.4, 52.2, 53.7, 66.1, 80.1, 107.8, 126.7, 129.1, 134.9, 137.0, 155.7, 170.2, 176.4, 196.3 and 200.2; *m*/*z* 487 (M^++1).

5.2.19. Glycine-ODmab 26. Trifluoroacetic acid (20 ml) was added to a solution of Boc-glycine-ODmab **25** (0.43 g, 0.89 mmol) in DCM (10 ml). After stirring for 30 min, the reaction mixture was concentrated in vacuo to remove the excess trifluoroacetic acid. The residue was dissolved in DCM (50 ml) and washed with saturated sodium hydrogen carbonate solution (50 ml). The aqueous solution was further extracted with DCM (2×50 ml) and the combined organic extracts were dried over magnesium sulphate and concentrated in vacuo to give a solid which was recrystallised using DCM and hexane to give *glycine-ODmab* **26** (0.35 g, 78%) as a pale yellow solid; mp 132–136°C; (Found C, 57.51; H, 6.49; N, 5.53. $C_{24}H_{31}N_2O_6F_3$ requires C, 57.60; H, 6.20; N, 5.60%); ν_{max} (cm^{-1}) 3430, 2958, 1755, 1669, 1558 and 754; δ_H (DMSO, 399.6 MHz) 0.68 (6H, d, J=6.8 Hz, $CH(CH_3)_2$), 0.99 (6H, s, $C(CH_3)_2$), 1.70 (1H, m, $CH(CH_3)_2$), 2.38 (4H, s, $2\times CH_2C(CH_3)_2$), 2.96 (2H, br d, J=6.1 Hz, $CH_2CH(CH_3)_2$), 3.92 (4H, br s, NH_2CH_2), 5.28 (2H, s, CO_2CH_2), 7.33 (2H, d, J=8.6 Hz, ArH), 7.51 (2H, d, J=8.6 Hz, ArH) and 15.28 (1H, s, ArNH); δ_C (100.4 MHz) 22.3, 27.8, 28.8, 29.7, 37.3, 66.1, 107.1, 126.4, 129.1, 134.7, 136.5, 167.6, 175.0, 196.3 and 200.2; *m*/*z* 387 (M^++1).

5.2.20. Fmoc-β-alanine-glycine-ODmab 27. A solution of pentafluorophenyl ester **8** (144 mg, 0.30 mmol) and amine **3** (50 mg, 0.10 mmol) in DMF (1 ml) was stirred for 48 h. The DMF was removed in vacuo, the residue was dissolved in DCM (50 ml) and washed with dilute hydrochloric acid (20 ml). The aqueous layer was further extracted with DCM (2×30 ml) and the combined organic extracts were dried over magnesium sulphate and concentrated in vacuo to give a yellow oil which was purified by column chromatography. Elution with ethyl acetate gave *Fmoc-β-alanine-glycine-ODmab* **27** (24 mg, 35%) as a colourless oil; δ_H (399.6 MHz) 0.69 (6H, d, J=6.5 Hz, $\mathrm{CH(C}H_2\mathrm{)}_2$), 1.00 (6H, s, $\mathrm{C(CH_3)_2}$), 1.76 (1H, m, $\mathrm{C}H\mathrm{(CH_3)_2}$), 2.32 (2H, m, $\mathrm{CH_2CO}$), 2.41 (2H, s, $\mathrm{C}H_2\mathrm{C(CH_3)_2}$), 2.50 (2H, s, $\mathrm{C}H_2\mathrm{C(CH_3)_2}$), 2.45 (2H, m, $\mathrm{CH_2CO}$), 2.89 (2H, d, J=5.8 Hz, $\mathrm{C}H_2\mathrm{CH(CH_3)_2}$), 3.42 (2H, m, $\mathrm{NHC}H_2$), 4.12 (1H, t, J=6.8 Hz, $\mathrm{C}H\mathrm{CH_2O}$), 4.27 (2H, d, J=6.8 Hz, $\mathrm{CHC}H_2\mathrm{O}$), 5.30 (2H, s, $\mathrm{CO_2CH_2}$), 5.47 (1H, m, NH), 5.70 (1H, br t, NH), 7.04 (2H, d, J=8.4 Hz, ArH), 7.32 (2H, t, J=7.3 Hz, ArH), 7.31 (2H, t, J=7.3 Hz, ArH), 7.51 (2H, d, J=7.3 Hz, ArH), 7.67 (2H, d, J=7.6 Hz, ArH), 7.93 (2H, s, ArH) and 15.21 (1H, s, ArNH); δ_C (100.4 MHz) 22.6, 28.3, 29.6, 30.1, 32.0, 34.3, 35.4, 38.4, 47.3, 52.3, 53.8, 66.3, 66.7, 107.8, 120.0, 125.2, 126.8, 127.1, 127.7, 129.3, 134.8, 137.1, 141.3, 144.0, 156.6, 171.6, 172.1, 176.5, 196.6 and 200.3; *m/z* 680 ($\mathrm{M^+}$+1).

5.2.21. Fmoc-β-alanine-ODmab 28. A solution of EDCI (0.41 g, 2.15 mmol) in DCM (20 ml) was added to a stirred solution of Fmoc-β-alanine **4** (0.55 g, 1.78 mmol) and DmabOH (0.59 g, 1.80 mmol) in DCM (100 ml) at room temperature under nitrogen. After 24 h, the solvent was washed with dilute hydrochloric acid (50 ml) and the aqueous layer was further extracted with DCM (2×50 ml). The combined organic extracts were dried over magnesium sulphate and concentrated in vacuo to give a solid which was purified by column chromatography. Elution with 20% ethyl acetate in hexane gave *Fmoc-β-alanine-ODmab* **28** (1.05 g, 95%) as a white solid; mp 44–46°C (from DCM/hexane); (Found C, 73.61; H, 7.04; N, 4.33. $\mathrm{C_{38}H_{42}N_2O_6}$ requires C, 73.31; H, 6.75; N, 4.50%); ν_max ($\mathrm{cm^{-1}}$) 3451, 3365, 2951, 1720, 1638, 1450 and 756; δ_H (399.6 MHz) 0.77 (6H, d, J=6.1 Hz, $\mathrm{CH(C}H_3\mathrm{)_2}$), 1.08 (6H, s, $\mathrm{C(CH_3)_2}$), 1.84 (1H, m, $\mathrm{C}H\mathrm{(CH_3)_2}$), 2.40 (2H, s, $\mathrm{C}H_2\mathrm{C(CH_3)_2}$), 2.49 (2H, s, $\mathrm{C}H_2\mathrm{C(CH_3)_2}$), 2.64 (2H, t, J=6.1 Hz, $\mathrm{CH_2CO_2}$), 3.00 (2H, br d, J=5.9 Hz, $\mathrm{C}H_2\mathrm{CH(CH_3)_2}$), 3.51 (2H, dt, J=6.1 and 5.6 Hz, $\mathrm{NHC}H_2$), 4.20 (1H, t, J=7.1 Hz, $\mathrm{C}H\mathrm{CH_2O}$), 4.40 (2H, d, J=7.1 Hz, $\mathrm{CHC}H_2\mathrm{O}$), 5.17 (2H, s, $\mathrm{CO_2CH_2}$), 5.29 (1H, m, NH), 7.12 (2H, d, J=8.1 Hz, ArH), 7.30 (2H, dt, J=7.6 and 1.0 Hz, ArH), 7.39 (2H, t, J=7.1 Hz, ArH), 7.40 (2H, d, J=8.1 Hz, ArH), 7.59 (2H, d, J=7.6 Hz, ArH), 7.76 (2H, d, J=7.6 Hz, ArH) and 15.30 (1H, s, ArNH); δ_C (100.4 MHz) 22.6, 28.3, 29.6, 30.2, 34.4, 36.5, 38.3, 47.2, 52.3, 53.8, 65.7, 66.7, 107.8, 120.0, 125.0, 126.7, 127.0, 127.7, 129.0, 135.2, 137.0, 141.3, 143.9, 156.3, 172.1, 176.4, 196.4 and 200.2; *m/z* 623 ($\mathrm{M^+}$+1).

5.2.22. Boc-β-alanine-piperidine 29. Piperidine (20 μl, 0.20 mmol) was added to a stirred solution of Boc-β-alanine-OPFP **8** (33 mg, 0.09 mmol) in DMF (0.5 ml) under nitrogen. After 10 min the reaction was concentrated in vacuo and the residue was diluted with DCM (50 ml) and washed with dilute sodium hydroxide (20 ml) and dilute hydrochloric acid (20 ml). The DCM was dried over magnesium sulphate and concentrated in vacuo to give *Boc-β-alanine-piperidine* **29** (20 mg, 83%) as a yellow oil; δ_H (399.6 MHz) 1.42 (9H, s, $\mathrm{(CH_3)_3CO}$), 1.51–1.67 (6H, m, $\mathrm{CH_2(C}H_2\mathrm{)_3}$), 2.49 (2H, t, J=5.6 Hz, $\mathrm{CH_2CO}$), 3.36 (2H, t, J=5.6 Hz, $\mathrm{CH_2N}$), 3.42 (2H, dt, J=5.8 and 5.6 Hz, $\mathrm{NHC}H_2$), 3.55 (2H, t, J=5.6 Hz, $\mathrm{CH_2N}$) and 5.35 (1H, m, NH); δ_C (100.4 MHz) 24.5, 25.6, 26.4, 28.4, 33.3, 36.4, 42.6, 46.4, 79.0, 156.1 and 169.8; *m/z* 257 ($\mathrm{M^+}$+1).

5.2.23. Fmoc-β-alanine-β-alanine-β-alanine-ODmab 31. A solution of 9-fluorenylmethyl chloroformate (0.39 g, 1.92 mmol) in dioxane (10 ml) was added to a stirred solution of β-ALA-β-ALA (175 mg, 1.09 mmol) in saturated aqueous sodium hydrogen carbonate (10 ml). After stirring for 24 h the mixture was concentrated in vacuo and acidified using dilute hydrochloric acid and extracted using dichloromethane (3×50 ml). The combined organic extracts were dried over magnesium sulphate and concentrated in vacuo to give an oil which was then reacted with EDCI (0.41 g, 2.13 mmol) and pentafluorophenol (0.42 g, 2.29 mmol) in DCM (50 ml). After 24 h, the solvent was washed with dilute hydrochloric acid (50 ml) and the aqueous layer was further extracted with DCM (2×50 ml). The combined organic extracts were dried over magnesium sulphate and concentrated in vacuo to give a solid, which was used without further purification. The pentafluorophenyl ester was then added to amine **3** (48 mg, 0.12 mmol) in DMF (1 ml) and the reaction was stirred for 48 h. The DMF was removed in vacuo, the residue was dissolved in DCM (50 ml) and washed with dilute hydrochloric acid (20 ml). The aqueous layer was further extracted with DCM (2×30 ml) and the combined organic extracts were dried over magnesium sulphate and concentrated in vacuo to give a yellow oil which was purified by column chromatography. Elution with 5% methanol in ethyl acetate gave *Fmoc-β-alanine-β-alanine-β-alanine-ODmab* **31** (21 mg, 3% over three step synthesis) as a pale yellow gum; δ_H (399.6 MHz) 0.76 (6H, d, J=6.7 Hz, $\mathrm{CH(C}H_3\mathrm{)_2}$), 1.07 (6H, s, $\mathrm{C(CH_3)_2}$), 1.83 (1H, m, $\mathrm{C}H\mathrm{(CH_3)_2}$), 2.39 (2H, t, J=5.9 Hz, $\mathrm{CH_2CO}$), 2.39 (2H, s, $\mathrm{C}H_2\mathrm{C(CH_3)_2}$), 2.49 (4H, br s, $\mathrm{C}H_2\mathrm{C(CH_3)_2}$ and $\mathrm{CH_2CO}$), 2.56 (2H, t, J=5.9 Hz, $\mathrm{CH_2CO}$), 2.99 (2H, d, J=4.5 Hz, $\mathrm{C}H_2\mathrm{CH(CH_3)_2}$), 3.43–3.52 (6H, m, 3×$\mathrm{NHC}H_2$), 4.19 (1H, t, J=7.0 Hz, $\mathrm{C}H\mathrm{CH_2O}$), 4.35 (2H, d, J=7.0 Hz, $\mathrm{CHC}H_2\mathrm{O}$), 5.11 (2H, s, $\mathrm{CO_2CH_2}$), 5.72 (1H, br t, J=5.6 Hz, NH), 6.50 (1H, m, NH), 6.70 (1H, br t, NH), 7.11 (2H, d, J=8.2 Hz, ArH), 7.29–7.40 (6H, m, ArH), 7.58 (2H, d, J=7.6 Hz, ArH), 7.75 (2H, d, J=7.6 Hz, ArH) and 15.27 (1H, s, ArNH); δ_C (100.4 MHz) 22.6, 28.2, 29.6, 30.0, 34.0, 35.0, 35.5, 36.1, 37.2, 38.4, 47.2, 52.3, 53.7, 60.4, 65.8, 66.8, 107.8, 120.0, 125.1, 126.7, 127.1, 127.7, 129.1, 135.1, 137.0, 141.3, 143.9, 156.7, 171.8, 172.4, 176.5, 196.5 and 200.3; *m/z* 765 ($\mathrm{M^+}$+1).

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