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The Peptide Synthesis

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The synthesis of peptides by amide bond formation between suitably protected amino acids is a fundamental part of the drug discovery process. However, the required coupling and deprotection reactions are routinely carried out in dichloromethane and DMF, both of which have serious toxicity concerns and generate waste solvent which constitutes the vast majority of the waste generated during peptide synthesis. In this work, propylene carbonate has been shown to be a green polar aprotic solvent which can be used to replace dichloromethane and DMF in both solution- and solid-phase peptide synthesis. Solution-phase chemistry was carried out with Boc/benzyl protecting groups to the tetrapeptide stage, no epimerisation occurred during these syntheses and chemical yields for both coupling and deprotection reactions in propylene carbonate were at least comparable to those obtained in conventional solvents. Solid-phase peptide synthesis was carried out using Fmoc protected amino acids on a ChemMatrix resin and was used to prepare the biologically relevant nonapeptide Bradykinin with comparable purity to a sample prepared in DMF.

Introduction

Peptides are central compounds in the pharmaceutical industry, providing both final medications and lead compounds for the preparation of peptidomimetic or non-peptidic pharmaceuticals.¹ Therapeutic peptides are recognised as being extremely specific in their binding to *in vivo* targets, resulting in them often being highly potent and very selective, whilst having very few negative side effects.^{2,3} They are therefore expected to play a major role in the future of drug discovery.⁴ Currently there are more than 60 peptide based medicines approved for use by the US Food & Drug Administration, around 140 peptide drugs currently in clinical trials and over 500 in preclinical trials.^{2,5} Consequently, the peptide medicines market is currently worth an estimated US\$ 14.1 billion, and is predicted to grow to around US\$ 25.4 billion by 2018.² As such, there is a resurgence of interest in the synthesis of synthetic peptides.

The synthesis of peptides by amide bond formation between partially protected amino acid derivatives is however, one of the most wasteful and least green chemical processes.⁶ Numerous auxiliary reagents in the form of protecting groups and coupling agents are required,⁷ giving the process low atom efficiency. Additionally, peptide synthesis is almost universally carried out in toxic solvents such as DMF or DMF/dichloromethane mixtures.⁸ These issues are amplified in solid-phase peptide synthesis^{8,9} where large excesses of reagents are used and resins are washed multiple times with toxic solvents. The use of polar aprotic solvents such as DMF or

NMP is a particular concern as these solvents are known to be reprotoxic,¹⁰ cause environmental problems when present in waste¹¹ and are two of the six chemicals of highest concern under EU RECh (Registration, Evaluation and Authorisation of Chemicals) regulations.¹² As a result, green solvent selection guides have repeatedly highlighted the need to replace DMF with a greener alternative in all chemical processes.^{13,14}

As for most chemical transformations,¹¹ by far the largest source of waste in either solution- or solid-phase peptide synthesis is the solvent, but only a few attempts have previously been made to avoid the use of DMF. Acetonitrile^{15,16} and THF¹⁶ have been used as replacements for DMF in peptide bond formations and acetonitrile has also been used as a solvent for Fmoc deprotections in solid-phase peptide synthesis,¹⁵ but neither of these are considered to be green solvents.^{13,14} Recently, the use of 2-MeTHF as a solvent for coupling reactions during solid-phase peptide synthesis was reported though the Fmoc deprotection steps were still performed in DMF.¹⁷ 2-MeTHF is a sustainable but not very green solvent due to concerns about its effect on health, safety and the environment.^{13,14} Whilst this paper was being written, the use of a mixture of 2-MeTHF and ethyl acetate for both coupling and Fmoc deprotection steps was reported.¹⁸ However, in some cases elevated temperatures were required and the production of mixed solvent waste is undesirable as it complicates solvent recycling especially when the solvents have virtually identical boiling points (77 and 78 °C for ethyl acetate and 2-MeTHF respectively). There are also a few reports of use of water as a solvent for solid-phase peptide bond formations using coupling agents¹⁹ or enzymes²⁰ and of solvent free non-resin supported peptide synthesis by ball-milling.²¹ However, the development of a green solvent for both solution- and solid-phase peptide synthesis remains an unsolved challenge.

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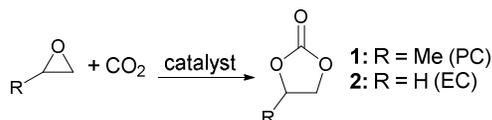
Electronic Supplementary Information (ESI) available: [characterising data including copies of NMR spectra and HPLC traces]. See DOI: 10.1039/x0xx00000x

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Table 1: Physical properties of polar aprotic solvents

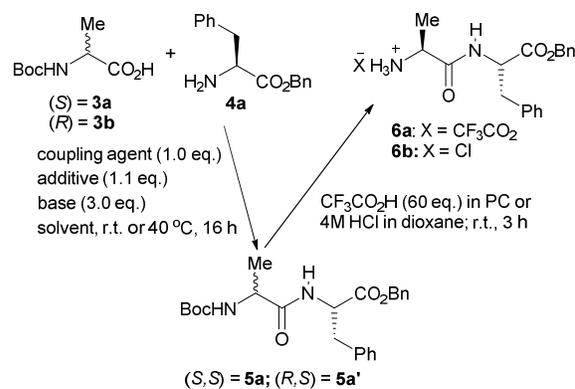
| Solvent | Bp (Mp) °C | Dipole moment (D) | Dielectric constant |
|---------|------------|-------------------|---------------------|
| DMF | 153 | 3.8 | 38 |
| NMP | 204 | 4.1 | 32 |
| MeCN | 82 | 3.4 | 38 |
| DMSO | 189 | 3.9 | 47 |
| PC 1 | 242 | 4.9 | 65 |
| EC 2 | 248 (36) | 4.9 | 90 |

**Scheme 1:** Synthesis of cyclic carbonates.

Cyclic carbonates, especially propylene carbonate (PC) **1** and ethylene carbonate (EC) **2** are remarkably polar aprotic compounds with dipole moments and dielectric constants higher than those of conventional polar aprotic solvents²² (Table 1). Unlike conventional polar aprotic compounds, they contain only carbon, hydrogen and oxygen and so generate no NO_x or SO_x on incineration and PC is known to be non-toxic and is used in topically applied medical products²³ and cosmetics.²⁴ PC is also less expensive than DMF²⁵ and unlike DMF is not significantly hygroscopic.²⁶ Cyclic carbonates, including PC and EC, can be prepared by the 100% atom economical reaction between epoxides and carbon dioxide (Scheme 1),²⁷ a reaction which is currently attracting considerable attention due to its ability to utilize waste carbon dioxide in chemical synthesis.²⁸ Cyclic carbonates including PC and EC are already produced commercially²⁹ and have a number of applications, including as electrolytes in lithium ion batteries.³⁰ PC was ranked as the greenest solvent in the latest green solvents guide¹⁴ and has previously been used as a solvent for cycloaddition reactions³¹ and for metal catalysed^{24,32} and organocatalysed³³ transformations. Therefore we decided to investigate the use of PC and EC as solvents for both coupling and deprotection steps in both solution- and solid-phase peptide synthesis using a range of common protecting groups and coupling agents.

Results and Discussion

To investigate the feasibility of peptide synthesis in cyclic carbonate solvents, the known³⁴ coupling reaction between Boc-alanine **3a** and phenylalanine benzyl ester **4a** was studied (Scheme 2) and the results are presented in Table 2. Entry 1 gives the literature conditions³⁴ using a conventional dichloromethane and DMF solvent system. Entry 2 shows that changing the coupling agent, additive and base to give a less costly and homogeneous reaction whilst still using the conventional solvent system results in a 10% reduction in the yield of dipeptide **5a**. However, as shown in entry 3, simply changing the solvent to PC virtually restored the yield of dipeptide **5a** to the literature value and use of EC as solvent

**Scheme 2:** Synthesis of dipeptides **5-6a,b** in PC.**Table 2:** Synthesis of dipeptide **5a**^a

| Entry | Coupling agent / additive | T (°C) | Base | Solvent | Yield (%) |
|-------|---------------------------|--------|----------------------------------|--------------------------------------|------------------|
| 1 | EDC / HOAt | 20 | NaHCO ₃ | CH ₂ Cl ₂ :DMF | 86 ³⁴ |
| 2 | DCC / PFP | 20 | Et ₃ N | CH ₂ Cl ₂ :DMF | 76 |
| 3 | DCC / PFP | 20 | Et ₃ N | PC | 83 |
| 4 | DCC / PFP | 40 | Et ₃ N | EC | 96 |
| 5 | EDC / HOBt | 20 | ^t Pr ₂ EtN | PC | 93 |
| 6 | EDC / HOBt | 20 | ^t Pr ₂ EtN | CH ₂ Cl ₂ :DMF | 81 |
| 7 | EDC / HOBt | 20 | ^t Pr ₂ EtN | DMF | 83 |
| 8 | EDC / HOBt | 70 | ^t Pr ₂ EtN | DMF | 78 |
| 9 | EDC / HOBt | 70 | ^t Pr ₂ EtN | PC | 82 |

a) ^tEDC = *N*-Ethyl-*N*-(3-dimethylaminopropyl)carbodiimide; DCC = *N,N*-Dicyclohexylcarbodiimide; HOAt = 1-Hydroxy-7-azabenzotriazole; HOBt = Hydroxybenzotriazole; PFP = Pentafluorophenol.

(entry 4) gave a significantly higher yield of **5a** than that obtained in the conventional solvent system. However, whilst other reactions have been successfully carried out in EC at ambient temperature,³³ this peptide coupling had to be carried out at 40 °C to keep the reaction mixture liquid. Therefore, the coupling reaction in PC was further optimised (entry 5) and this allowed the literature yield of dipeptide **5a** to be exceeded. To allow a direct comparison between PC and conventional solvent systems to be made, the use of EDC, HOBt and diisopropylethylamine in a dichloromethane and DMF mixed solvent and in just DMF was also carried out (entries 6 and 7). In both cases the yield of peptide **5a** was inferior to that obtained in PC.

It is becoming increasingly common to carry out peptide synthesis at elevated temperatures, often with microwave heating.³⁵ Therefore, the synthesis of peptide **5a** in both DMF and PC was also carried out at 70 °C in a microwave reactor (entries 8 and 9). In both cases high yields of peptide **5a** were again obtained. No evidence for reaction of amine **4a** with the propylene carbonate was seen in these reactions even though there is literature precedent for the reaction of amines with cyclic carbonates.³⁶ A control experiment was therefore carried out in which amine **4a** was dissolved in PC and heated to 70 °C in a microwave reactor in the absence of any other reagents. Even under these conditions, only 1% reaction

between the amine and solvent was detected. Thus, reaction of amino acid derivatives with PC does not occur to any significant extent under the reaction conditions used for peptide synthesis, even at 70 °C and cannot compete with peptide synthesis.

Activated amino acid derivatives generated during peptide synthesis are prone to racemisation.⁸ To demonstrate that no such racemisation occurs when the coupling reactions are carried out in PC, diastereomeric dipeptide **5a'** was prepared from Boc-(*R*)-alanine **3a** and ester **4a** in 87% yield using the conditions of Table 1, entry 5. HPLC analysis of dipeptides **5a** and **5a'** as well as a 1:1 mixture of the two dipeptides confirmed that they had stereochemical purities of greater than 99:1 even when the peptide synthesis was carried out at 70 °C.³⁷

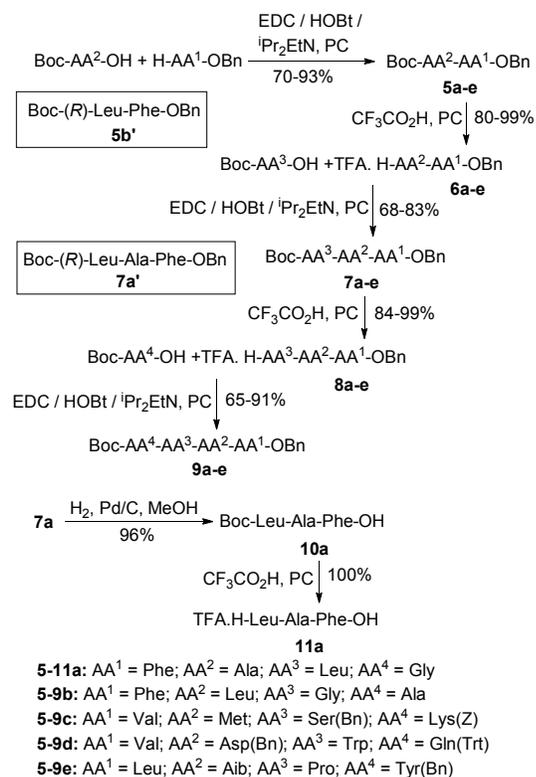
The other key step for solution-phase peptide synthesis is selective removal of the *N*-terminal Boc protecting group to allow subsequent chain elongation. This is usually achieved by use of a strong acid such as hydrogen chloride or trifluoroacetic acid in solvents such as 1,4-dioxane, dichloromethane, THF, acetonitrile or DMF. Therefore, the use of PC as solvent for Boc deprotection reactions was also investigated. Initially, hydrogen chloride was generated in PC and used to convert fully protected dipeptide **5a** into the hydrochloride salt of Ala-Phe-OBn in 76% yield. However, as a solution of hydrogen chloride in PC is not commercially available, it was more convenient to use trifluoroacetic acid in PC and this converted fully protected dipeptide **5a** into trifluoroacetate salt **6a** in 99% yield. In contrast, use of 4M

hydrogen chloride in dioxane gave the corresponding chloride salt **6b** in just 93% yield. Thus, both coupling and deprotection reactions could be carried out in PC in higher yield than the yields obtained using DMF, dichloromethane and dioxane.

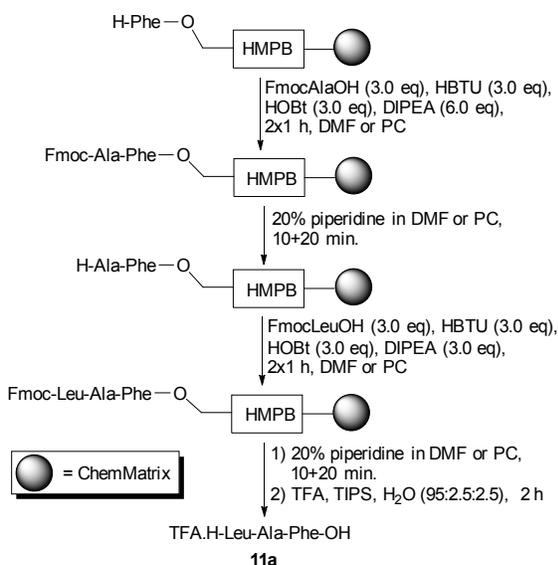
To illustrate the generality of this process, five fully protected tetrapeptides **9a-e** were prepared as shown in Scheme 3. Peptide sequences **5-9a,b** contain only unhindered and unfunctionalised amino acids. However, sequences **5-9c-e** were chosen to include the sterically hindered amino acids valine and aminoisobutyric acid (Aib), functionalised amino acids with their functional groups unprotected (methionine and tryptophan) and functionalised amino acids with their functional groups protected using standard protecting groups (serine, lysine, aspartic acid and glutamine). All five tetrapeptides were successfully prepared using PC as the only solvent. To further prove that no racemisation of the activated amino acids occurs during peptide synthesis in PC, diastereomeric dipeptide **5b'** and tripeptide **7a'** were prepared using Boc-(*R*)-leucine and HPLC analysis of the diastereomers **5b/5b'** and **7a/7a'** again showed stereochemical purities of greater than 99:1.³⁷ Finally, to show that the methodology was compatible with the palladium on carbon used to remove benzyl based protecting groups, tripeptide **7a** was subjected to hydrogenolysis to give acid **10a** in 96% yield and then treated with trifluoroacetic acid in PC to give fully deprotected tripeptide **11a** in quantitative yield.

Having demonstrated that PC could be used as a solvent for solution-phase peptide synthesis, its application to solid-phase peptide synthesis was investigated. This presented a new challenge: the solvent must swell the resin upon which the peptide is being constructed. We have recently reported a study of the swelling of various resins used for solid-phase synthesis in over 20 green solvents including PC and EC.³⁸ This showed that polystyrene based resins such as 2% cross linked polystyrene (Merrifield resin³⁹) did not swell adequately in PC. However, a cross-linked polyethylene glycol (ChemMatrix resin⁴⁰) did swell (to 6.0 mL g⁻¹) in PC which is well above the 4.0 mL g⁻¹ required for a resin to be considered as well swollen.⁴¹ If a resin swells too much in a particular solvent, this can also be a problem for solid-phase peptide synthesis as it can cause blockages and result in over-filling of resin holding vessels. It is thus advantageous that whilst PC swelled ChemMatrix resin to 6.0 mL g⁻¹ this is less than the 7.6–7.8 mL g⁻¹ swelling observed in DMF and NMP.³⁸ Hence, all solid-phase peptide synthesis was carried out on ChemMatrix resin.

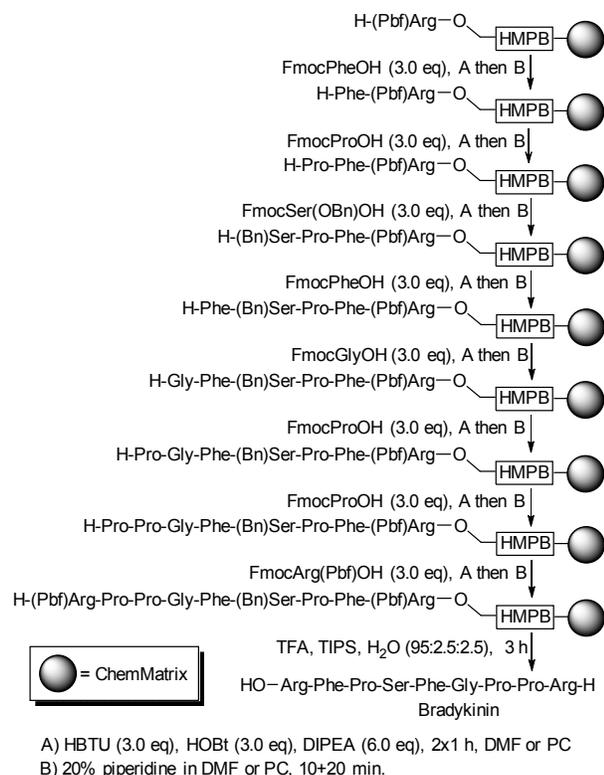
The synthesis of the tripeptide TFA.H-Leu-Ala-Phe-OH **11a** was chosen as a test sequence and its solid-phase preparation was undertaken in both DMF and PC (Scheme 4). The synthesis started with commercially available ChemMatrix resin preloaded with an acid-labile HMPB (4-(4-hydroxymethyl-3-methoxyphenoxy)butyric acid) linker⁴² and a phenylalanine residue with a free amino group. Each coupling reaction was carried out using three equivalents each of an Fmoc-protected amino acid, HBTU⁴³ (3-[Bis(dimethylamino)methylumyl]-3*H*-benzotriazol-1-oxide hexafluorophosphate) and HOBt and six equivalents of diisopropylethylamine. At the end of each coupling and deprotection reaction, the resin was only washed



Scheme 3: Synthesis of tetrapeptides in PC.

Scheme 4: Solid-phase synthesis of tetrapeptide **11a**.

with the solvent being used for the synthesis rather than with a range of solvents (usually including DMF, dichloromethane and methanol) to ensure that the use of non-green solvents was completely avoided. The tripeptide was cleaved from the resin by treatment with trifluoroacetic acid in the presence of TIPS (triisopropylsilane) and water as cation scavengers,⁴⁴ then precipitated from and triturated with diethyl ether. Analysis of



Scheme 5: Solid-phase synthesis of bradykinin in PC.

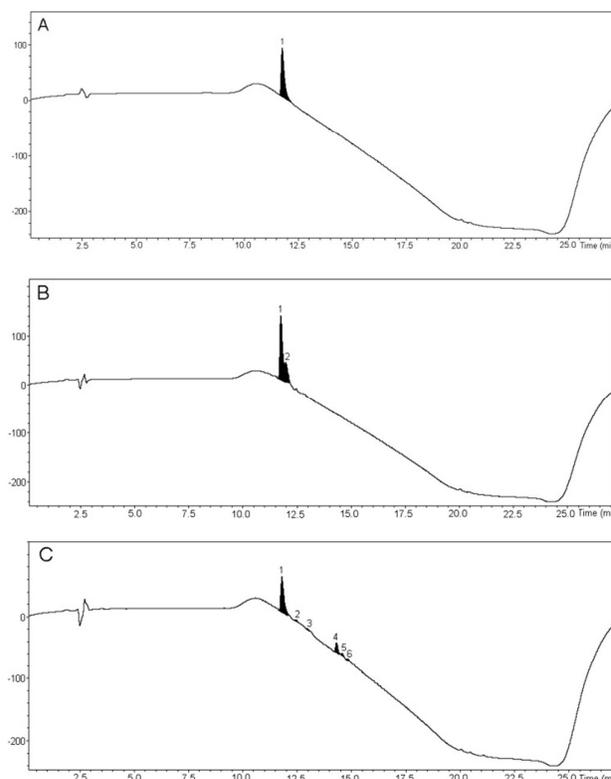


Figure 1: HPLC-UV traces of bradykinin. A: commercial sample, B: sample prepared in DMF, C: sample prepared in PC. Chromatograms were eluted with a gradient from 1:9 MeCN:H₂O to 1:1 MeCN:H₂O.³⁷

both samples by ¹H NMR spectroscopy showed that TFA.H-Leu-Ala-Phe-OH **11a** had been successfully prepared in both solvents.

Having demonstrated that PC could be used as a solvent for solid-phase peptide synthesis, the synthesis of a biologically relevant peptide was undertaken. The naturally occurring nonapeptide bradykinin (H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH) which is a vasodilator the concentration of which is increased by ACE-inhibitors⁴⁵ was selected as a suitable test as it is of moderate length and contains a mixture of functionalised and unfunctionalised amino acids. The synthesis of bradykinin starting from H-Arg-HMPB-ChemMatrix was carried out using the coupling and deprotection reactions given in Scheme 5.³⁷

The cleaved peptide was precipitated into cold diethyl ether, triturated and lyophilised. To allow a comparison with conventional methodology to be undertaken, the synthesis was also carried out in DMF and both synthetic samples were compared with commercially available bradykinin by HPLC-UV-HRMS (Figure 1).³⁷ The bradykinin samples prepared in PC and DMF were found to have purities of 77 and 79% respectively, showing that use of PC as solvent had no significant detrimental effect on the synthesis. The sample prepared in DMF (Figure 1B) contained a single impurity peak which overlapped with the bradykinin peak and so would be difficult to separate by preparative HPLC. Analysis of the mass spectrum of this impurity peak showed that it was actually a

mixture of the premature chain termination sequences H-Ser-Pro-Phe-Arg-OH and H-Pro-Phe-Arg-OH. In contrast, this impurity was not present in the sample prepared in PC (Figure 1C) and the impurity peaks that were present were well separated from the bradykinin peak, thus facilitating purification by preparative HPLC if desired.

Experimental

Details of instrumentation are given in the supplementary information.

General procedure for peptide coupling reactions in PC

To a suspension of an *N*-Boc-amino acid (1.0 eq.) and an amino acid or peptide benzyl ester (1.0 eq.) in PC (5 mL mmol⁻¹), at 0 °C, was added a solution of HOBt (1.1 eq.) and ¹Pr₂EtN (3.0 eq.) in a minimal quantity of PC. EDC (1.1 eq.) was added dropwise and the reaction mixture was allowed to stir at room temperature for 16h. The reaction mixture was then diluted using EtOAc (50 mL) and washed with 1M HCl_{aq} (3 × 25 mL), saturated Na₂CO₃ (3 × 25 mL) and H₂O (3 × 25 mL). The organic layer was dried (MgSO₄), filtered and concentrated in vacuo. Any residual PC was removed via short path distillation. Purification details for each peptide and characterising data are given in the supplementary information.

General procedure for Boc deprotections in PC

An *N*-Boc-peptide benzyl ester (1.0 eq.) was dissolved in a minimum amount of PC and trifluoroacetic acid (60 eq.) was added. The reaction mixture was allowed to stir for 3h. at room temperature before being concentrated in vacuo. Any residual PC was removed via short path distillation. Characterising data for each deprotected peptide are given in the supplementary information.

Synthesis of Boc-Ala-Phe-OBn 5a in PC in a microwave reactor

N-Boc-Alanine (189 mg, 1.0 mmol, 1.0 eq), phenylalanine benzyl ester (292 mg, 1.0 mmol, 1.0 eq) and HOBt (149 mg, 1.0 mmol, 1.1 eq) were combined in a G10 microwave vial. PC (2 mL) and ¹Pr₂EtN (523 μL, 3.0 mmol, 6.0 eq) were then added. The vial was cooled to 0 °C and EDC (195 μL, 1.1 mmol, 1.1 eq) was added dropwise. The reaction mixture was then heated at 70 °C for 3 hours using an Anton Parr Monowave 300 microwave reactor. The reaction was then diluted with EtOAc (50 mL) and washed with 1M HCl (50 mL), sat. Na₂CO₃ (50 mL) and brine (50 mL). The organic fraction was dried (MgSO₄), filtered and concentrated *in vacuo*. Residual PC was removed *via* short path distillation. The residue was purified using flash column chromatography (40:60, EtOAc:petroleum ether) to give *N*-Boc-Ala-Phe-OBn as a white solid (352 mg, 82%).

Procedure for Boc deprotection of dipeptide 5a using HCl in PC

Boc-Ala-Phe-OBn 5a (50 mg, 0.117 mmol) was dissolved in PC (2.34 mL). MeOH (0.40 mL, 9.8 mmol) was added and the solution cooled to 0 °C. Acetyl chloride (0.67 mL, 9.36 mmol)

was added dropwise and the solution allowed to stir at room temperature for 2h. Then, PC was removed by short path distillation. The residue was suspended in Et₂O and stirred for 5 minutes before being filtered to give HCl.Ala-Ph-OBn as a white solid (32.4 mg, 76%).

General procedure for solid-phase peptide synthesis in PC

The synthesis of peptides on a solid-phase was carried out in a 5 mL syringe fitted with a polypropylene frit. Preloaded ChemMatrix-HMPB resin (200 mg, 0.6 mmol g⁻¹) was first washed with PC (3 × 5mL) and then swollen in PC (3.5 mL) for 1h. In a separate vial, an Fmoc-amino acid (0.36 mmol, 3.0 eq.), HBTU (137 mg, 0.36 mmol, 3.0 eq.), HOBt (49 mg, 0.36 mmol, 3.0 eq.) and DIPEA (125 μL, 0.72 mmol, 6.0 eq.) were combined in PC (3.6 mL). After 3 min., this preactivated amino acid solution was added to the resin. Coupling reactions were performed for 1h. at ambient temperature and carried out in duplicate. Following each coupling reaction the resin was washed using PC (3 × 5mL). Fmoc-deprotections were performed in duplicate (10 min followed by 20 min) using a freshly prepared solution of 20% (v/v) piperidine in PC (10–12 mL g⁻¹). Following deprotection the resin was washed using PC (3 × 5mL). Once the full sequence was constructed and the final Fmoc-deprotection had been performed, the resin was dried under reduced pressure. The peptide was then cleaved from the resin using TFA:TIPS:H₂O (95:2.5:2.5) (8–10 mL g⁻¹) for 2h. at ambient temperature. The resin was removed by filtration and the peptide precipitated into cold Et₂O, triturated and lyophilised. Characterising data for the peptides prepared in this way are given in the supplementary information.

Conclusions

Propylene carbonate **1** has been shown to be a green replacement for reprotoxic amide based solvents which are widely used in peptide synthesis. Both solution- and solid-phase peptide synthesis can be carried out in propylene carbonate using acid and base labile amine protecting groups respectively. No significant racemisation of the activated amino acids occurs in propylene carbonate and the viability of solid-phase peptide synthesis in propylene carbonate was demonstrated by the synthesis of the nonapeptide bradykinin.

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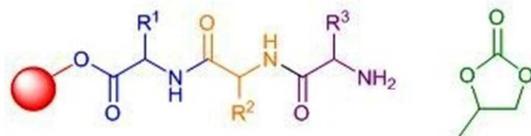
Notes and references

- 1 a) Y. Liu and T. Liu, *Curr. Med. Chem.*, 2012, **19**, 3982–3999; b) S. Lohan and G. S. Bisht, *Mini-Rev. Med. Chem.*, 2013, **13**, 1073–1088; c) J. Vabeno, B. E. Haug and M. M. Rosenkilde, *Future Med. Chem.*, 2015, **7**,

- 1261–1283; d) R. Domalaon, G. G. Zhanel and F. Schweizer, *Curr. Topic. Med. Chem.*, 2016, **16**, 141–155.
- 2 K. Fosgerau and T. Hoffmann, *Drug Discov. Today*, 2015, **20**, 122–128.
- 3 a) A. A. Kaspar and J. M. Reichert, *Drug Discov. Today*, 2013, **18**, 807–817; b) J. P. Tam, *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 5409–5413.
- 4 a) D. Goodwin, P. Simerska and I. Toth, *Curr. Med. Chem.*, 2012, **19**, 4451–4461; b) D. J. Craik, D. P. Fairlie, S. Liras and D. Price, *Chem. Biol. Drug Des.*, 2013, **81**, 136–147.
- 5 F. Albericio and H. G. Kruger, *Futur. Med. Chem.*, 2012, **4**, 1527–1531.
- 6 For examples of previous work on improving the efficiency of peptide synthesis using flow reactors see: a) O. Flögel, J. D. C. Codée, D. Seebach and P. H. Seeberger, *Angew. Chem. Int. Ed.*, 2006, **45**, 7000–7003; b) M. D. Simon, P. L. Heider, A. Adamo, A. A. Vinogradov, S. K. Mong, X. Li, T. Berger, R. L. Policarpo, C. Zhang, Y. Zou, X. Liao, A. M. Spokoiny, K. F. Jensen and B. L. Pentelute, *ChemBioChem*, 2014, **15**, 713–720; c) I. M. Mándity, B. Olasz, S. B. Ötvös and F. Fülöp, *ChemSusChem*, 2014, **7**, 3172–3176.
- 7 a) F. Albericio, *Biopolymers*, 2000, **55**, 123–239; b) S.-Y. Han and Y.-A. Kim, *Tetrahedron*, 2004, **60**, 2447–2467; c) A. El-Faham and F. Albericio, *Chem. Rev.*, 2011, **111**, 6557–6602.
- 8 *Methods of Organic Synthesis: Synthesis of Peptides and Peptidomimetics Vol. E22a* (Eds.: M. Goodman, A. Felix, L. Moroder and C. Toniolo), Thieme, Stuttgart, 2004.
- 9 a) E. Atherton and R. C. Sheppard in *Solid phase peptide synthesis a practical approach* OUP, Oxford, 1989; b) I. Coin, M. Beyermann and M. Bienert, *Nature Protocols* **2007**, **2**, 3247–3256.
- 10 G. L. Kennedy, *Critical Rev. Toxicology*, 2012, **42**, 793–826.
- 11 D. J. C. Constable, C. Jimenez-Gonzalez, R. K. Henderson, *Org. Process Res. Dev.*, 2007, **11**, 133–137.
- 12 Regulation (EC) 1907/2006 of the European Parliament and of the Council of 18 December 2006 Concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH); Candidate List of Substances of Very High Concern for Authorisation, <http://echa.europa.eu/candidate-list-table> (accessed July 20, 2016).
- 13 D. Prat, J. Hayler and A. Wells, *Green Chem.*, 2014, **16**, 4546–4551.
- 14 C. M. Alder, J. D. Hayler, R. K. Henderson, A. M. Redman, L. Shukla, L. E. Shuster and H. F. Sneddon, *Green Chem.*, 2016, **18**, 3879–3890.
- 15 G. A. Acosta, M. del Fresno, M. Paradis-Bas, M. Rigau-DeLlobet, S. Côté, M. Royo and F. Albericio, *J. Pept. Sci.* 2009, **15**, 629–633.
- 16 Y. E. Jad, G. A. Acosta, S. N. Khattab, B. G. de la Torre, T. Govender, H. G. Kruger, A. El-Faham and F. Albericio, *Org. Biomol. Chem.*, 2015, **13**, 2393–2398.
- 17 Y. E. Jad, G. A. Acosta, S. N. Khattab, B. G. de la Torre, T. Govender, H. G. Kruger, A. El-Faham and F. Albericio, *Amino Acids*, 2016, **48**, 419–426.
- 18 Y. E. Jad, G. A. Acosta, T. Govender, H. G. Kruger, A. El-Faham, B. G. de la Torre and F. Albericio, *ACS Sust. Chem. Eng.*, 2016, **4**, 6809–6814.
- 19 a) A. S. Galanis, F. Albericio and M. Grøtli, *Org. Lett.*, 2009, **11**, 4488–4491; b) K. Hojo, A. Hara, H. Kitai, M. Onishi, H. Ichikawa, Y. Fukumori and K. Kawasaki, *Chem. Central J.*, 2011, **5**, 49; c) R. De Marco, A. Tolomelli, A. Greco and L. Gentilucci, *ACS Sustainable Chem. Eng.*, 2013, **1**, 566–569; d) K. Hojo, N. Shinozaki, K. Hidaka, Y. Tsuda, Y. Fukumori, H. Ichikawa and J. D. Wade, *Amino Acids*, 2014, **46**, 2347–2354.
- 20 a) R. V. Ulijn, B. Baragaña, P. J. Halling and S. L. Flitsch, *J. Am. Chem. Soc.*, 2002, **124**, 10988–10989; b) R. Haddoub, M. Dauner, F. A. Stefanowicz, V. Barattini, N. Laurent and S. L. Flitsch, *Org. Biomol. Chem.*, 2009, **7**, 665–670; Water enzyme
- 21 a) V. Declerck, P. Nun, J. Martinez and F. Lamaty, *Angew. Chem. Int. Ed.*, 2009, **48**, 9318–9321; b) J. Bonnamour, T.-X. Métro, J. Martinez and F. Lamaty, *Green Chem.*, 2013, **15**, 1116–1120
- 22 a) R. Payne and I. E. Theodorou, *J. Phys. Chem.*, 1972, **76**, 2892–2900; b) L. B. Silva and L. C. G. Freitas, *J. Mol. Struct.: THEOCHEM*, 2007, **806**, 23–34.
- 23 J. H. Clements, *Ind. Eng. Chem. Res.*, 2003, **42**, 663–674.
- 24 B. Schöffner, F. Schöffner, S. P. Verevkin and A. Börner, *Chem. Rev.*, 2010, **110**, 4554–4581.
- 25 All work in this paper used 99% grade solvents and 2016 prices from Thermo Fisher Scientific were £9.5 and £17.4 for 250g of PC and DMF respectively.
- 26 T. Fujinaga and K. Izutsu, *Pure Applied Chem.*, 1971, **27**, 273–280.
- 27 a) M. North, *Arkovic*, 2012, part (i), 610–628; b) C. Martín, G. Fiorani and A. W. Kleij, *ACS Catal.*, 2015, **5**, 1353–1370; c) A. C. Kathalikkattil, R. Babu, J. Tharun, R. Roshan and D.-W. Park, *Catal. Surv. Asia*, 2015, **19**, 223–235; d) B. Yu and L.-N. He, *ChemSusChem*, 2015, **8**, 52–62; e) M. Cokoja, M. E. Wilhelm, M. H. Anthofer, W. A. Herrmann and F. E. Kühn, *ChemSusChem*, 2015, **8**, 2436–2454; f) B.-H. Xu, J.-Q. Wang, J. Sun, Y. Huang, J.-P. Zhang, X.-P. Zhang and S.-J. Zhang, *Green Chem.*, 2015, **17**, 108–122; g) V. D'Elia, J. D. A. Pelletier and J. -M. Basset, *ChemCatChem*, 2015, **7**, 1906–1917; h) J. W. Comerford, I. D. V. Ingram, M. North and X. Wu, *Green Chem.*, 2015, **17**, 1966–1987.
- 28 a) I. S. Metcalfe, M. North, R. Pasquale and A. Thursfield, *Energy Environ. Sci.*, 2010, **3**, 212–215; b) M. North, B. Wang and C. Young, *Energy Environ. Sci.*, 2011, **4**, 4163–4170; c) A. Barthel, Y. Saih, M. Gimenez, J. D. A. Pelletier, F. E. Kühn, V. D'Elia and J. -M. Basset, *Green Chem.*, 2016, **18**, 3116–3123.
- 29 a) W. J. Poppel, *Ind. Eng. Chem.*, 1958, **50**, 767–770; b) M. Yoshida and M. Ihara, *Chem. Eur. J.*, 2004, **10**, 2886–2893.
- 30 a) K. Xu, *Chem. Rev.*, 2004, **104**, 4303–4417; b) S. S. Zhang, *J. Power Sources*, 2006, **162**, 1379–1394; c) V. Etacheri, R. Marom, R. Elazari, G. Salitra and D. Aurbach, *Energy Environ. Sci.*, 2011, **4**, 3243–326; d) B. Scrosati, J. Hassoun and Y.-K. Sun, *Energy Environ. Sci.*, 2011, **4**, 3287–3295.
- 31 a) C. E. P. Galvis and V. V. Kouznetsov, *Org. Biomol. Chem.*, 2013, **11**, 7372–7386; b) J. S. B. Forero, E. M. D. Carvalho, J. J. Junior and F. M. Silva, *Curr. Org. Synth.*, 2015, 102–107.
- 32 a) M. North and R. Pasquale, *Angew. Chem. Int. Ed.*, 2009, **48**, 2946–2948; b) M. North and M. Omedes-Pujol, *Tetrahedron Lett.*, 2009, **50**, 4452–4454; c) P. Lenden, P. M. Ylioja, C. González-Rodríguez, D. A. Entwistle and M. C. Willis, *Green Chem.*, 2011, **13**, 1980–1982; d) B. Yu, Z.-F. Diao, C.-X. Guo, C.-L. Zhong, L.-N. He, Y.-N. Zhao, Q.-W. Song, A.-H. Liu and J.-Q. Wang, *Green Chem.*, 2013, **15**, 2401–2407; e) B. Pieber and C. O. Kappe, *Green Chem.*, 2013, **15**, 320–324; f) H. L. Parker, J. Sherwood, A. J. Hunt and J. H. Clark, *ACS Sustainable Chem. Eng.*, 2014, **2**, 1739–1742; g) P.

- Neubert, S. Fuchs and A. Behr, *Green Chem.*, 2015, **17**, 4045–4052.
- 33 a) M. North, F. Pizzato and P. Villuendas, *ChemSusChem*, 2009, **2**, 862–865; b) W. Clegg, R. W. Harrington, M. North, F. Pizzato and P. Villuendas, *Tetrahedron Asymmetry*, 2010, **21**, 1262–1271; c) M. North and P. Villuendas, *Org. Lett.*, 2010, **12**, 2378–2381.
- 34 J. Nam, D. Shin, Y. Rew and D. L. Boger, *J. Am. Chem. Soc.*, 2007, **129**, 8747–8755.
- 35 a) B. Bacsa, K. Horváti, S. Bösze, F. Andrae and C. O. Kappe, *J. Org. Chem.*, 2008, **73**, 7532–7542; b) B. Bacsa, S. Bösze and C. O. Kappe, *J. Org. Chem.*, 2010, **75**, 2103–2106.
- 36 S. Sopeña, V. Laserna, W. Guo, E. Martin, E. C. Escudero-Adán and A. W. Kleij, *Adv. Synth. Catal.*, 2016, **358**, 2172–2178.
- 37 Data given in supporting information.
- 38 S. Lawrenson, M. North, F. Peigneguy and A. Routledge, *Green Chem.*, 2017, **19**, 952–962.
- 39 R. B. Merrifield, *J. Am. Chem. Soc.*, 1963, **85**, 2149–2154.
- 40 F. García-Martín, M. Quintanar-Audelo, Y. García-Ramos, L. J. Cruz, C. Gravel, R. Furic, S. Côté, J. Tulla-Puche and F. Albericio, *J. Comb. Chem.*, 2006, **8**, 213–220.
- 41 R. Santini, M. C. Griffith and M. Qi, *Tetrahedron Lett.*, 1998, **39**, 8951–8954.
- 42 B. Riniker, A. Flörsheimer, H. Fretz, P. Sieber and B. Kamber, *Tetrahedron*, 1993, **49**, 9307–9320.
- 43 R. Knorr, A. Trzeciak, W. Bannwarth and D. Gillessen, *Tetrahedron Lett.*, 1989, **30**, 1927–1930.
- 44 D. A. Pearson, M. Blanchette, M. L. Baker and C. A. Guindon, *Tetrahedron Lett.*, 1989, **30**, 2739–2742.
- 45 M. Perez, A. Adam and G. Molinaro, *J. Clinical Basic Cardiology*, 2001, **4**, 39–46.

Graphical Abstract



HO–Arg–Phe–Pro–Ser–Phe–Gly–Pro–Pro–Arg–H
Bradykinin

Both couplings and deprotections for solution and solid-phase peptide synthesis can be carried out in polar aprotic solvent propylene carbonate.