Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron Letters
Tetrahedron Letters 47 (2006) 1011–1014

Microwave-assisted coupling with DIC/HOBt for the synthesis of difficult peptoids and fluorescently labelled peptides—a gentle heat goes a long way

Mario A. Fara, Juan José Díaz-Mochón and Mark Bradley*

School of Chemistry, University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ, United Kingdom

Received 7 October 2005; revised 14 November 2005; accepted 24 November 2005
Available online 20 December 2005

Abstract—Mild thermal effects (Arrhenius based) achieved with microwave heating proved to be highly successful in enabling rapid and efficient secondary amine couplings and the labelling of peptides with a variety of fluorophores and quenchers in high yields and purities with just traditional, yet robust, HOBt/DIC chemistry.

Peptide synthesis was revolutionized when solid phase chemistry was introduced by Merrifield back in 1963[1] and since this time peptides have been almost exclusively synthesized on solid supports. Along with changes in protection group strategies and solid supports, many efforts have focussed on developing more efficient coupling reagents compared to the original carbodiimde strategies originally introduced, and over the past 10 years coupling reagents and additives such as HOAt, HATU, HBTU, PyBOP and PyBroP[2] have all been introduced with the aim of increasing yields and reducing side reactions.[3]

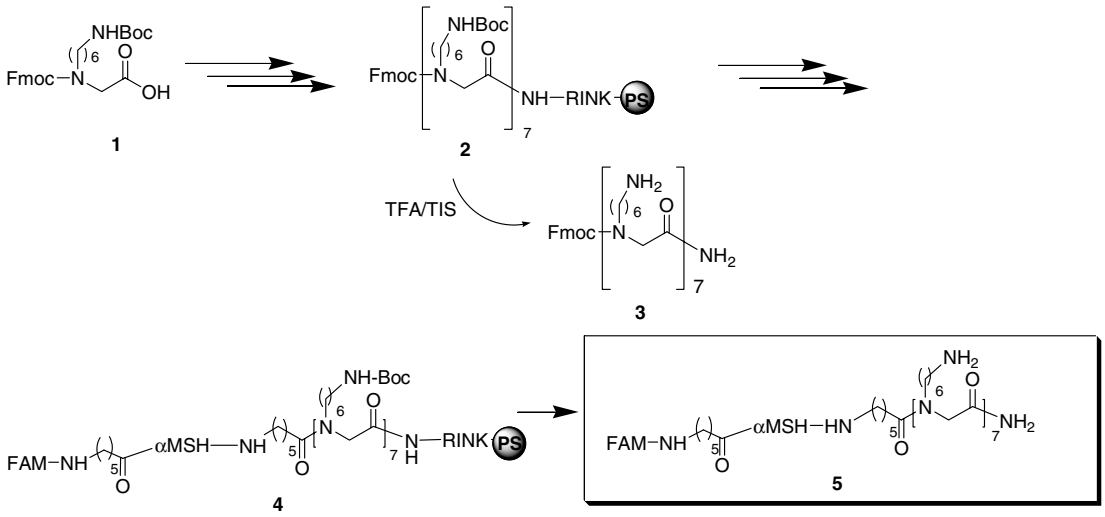
Recently, the synthesis and evaluation of a series of cell penetrating polycationic peptoids[4] were reported, using *N*-[6-(*tert*-butyloxycarbonyl)aminohexyl]-*N*-Fmoc-glycine (monomer **1)** as a building block. This synthesis was not trivial due to the nature of the building blocks, which necessitated multiple couplings to secondary amines, a well-known complication in peptide chemistry. These issues were alleviated to some degree by the use of PyBroP, but even this coupling reagent gave truncated polycationic sequences under standard coupling conditions. This was of concern as it was necessary for our studies on cellular delivery to conjugate the cell penetrating peptoids to a variety of payloads, including a 13-mer peptide (αMSH) and a 15-mer antisense PNA, significantly increasing the difficulty of synthesis.

In 1992, Wang described the use of a single-mode microwave as an excellent heating source to reduce the reaction time needed to achieve difficult peptide couplings,[5] and several different groups have since reported the synthesis of peptides using the Wang protocol, dramatically reducing reaction times but with the use of expensive and exotic coupling agents such as HBTU, PyBOP and HATU.[6]

We decided to investigate the use of microwave heating to accelerate peptide couplings, exploring the use of standard and robust DIC/HOBt coupling protocols, taking into account that the Fmoc group is not stable to high temperatures under many typical coupling conditions. In particular, we wanted to see if under microwave conditions the coupling of secondary amines could be carried out rapidly and in high yields. The most efficient reaction conditions with complete couplings of the secondary amine proved to be microwave irradiation at 60 °C for 20 min[7] using three equivalents of monomer **1**, DIC and HOBt in DMF at a concentration of 0.1 M, allowing the preparation of *hepta*-peptoid **3** in a single day and with very high purity (98%) (Scheme 1, Fig. 1) following cleavage from resin **2**. This represented a significant improvement on the previous PyBroP coupling methods, which required three equivalents of monomer **1** and a 4 h coupling time and gave a less pure product.

* Corresponding author. Tel.: +44 (0)131 6504280; e-mail: Mark.Bradley@ed.ac.uk

0040-4039/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2005.11.127



Scheme 1. Synthesis of fluorescently labelled α-MSH-*hepta*-peptoid conjugate (α-MSH: SYSMEHFRWGKPV). Reagents and conditions; *couplings:* acids (3 equiv), DIC (3 equiv) and HOBt (3 equiv) in DMF at 0.1 M; microwave irradiation at 60 °C for 20 min. *Fmoc deprotection*: 20% piperidine in DMF (2 × 5 min) at room temperature. FAM = 5(6)-carboxyfluorescein; PS = polystyrene.

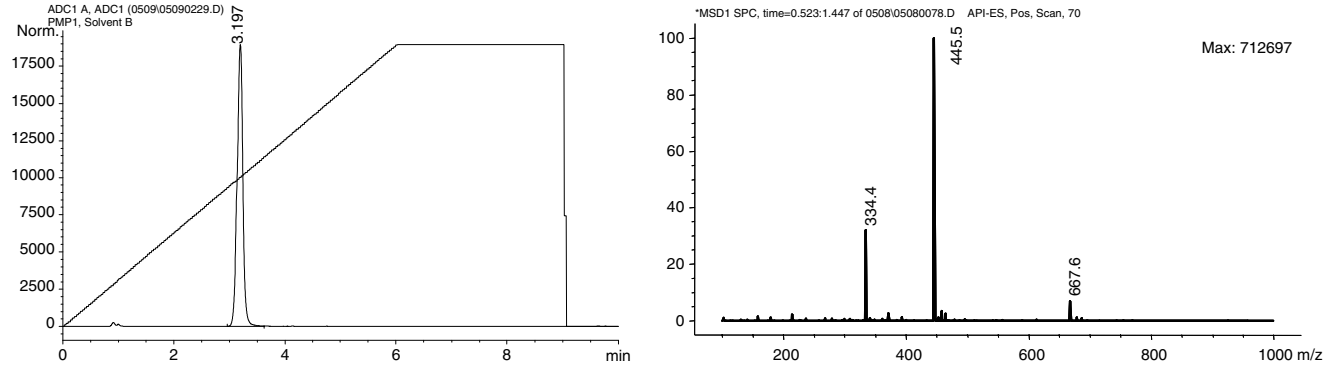


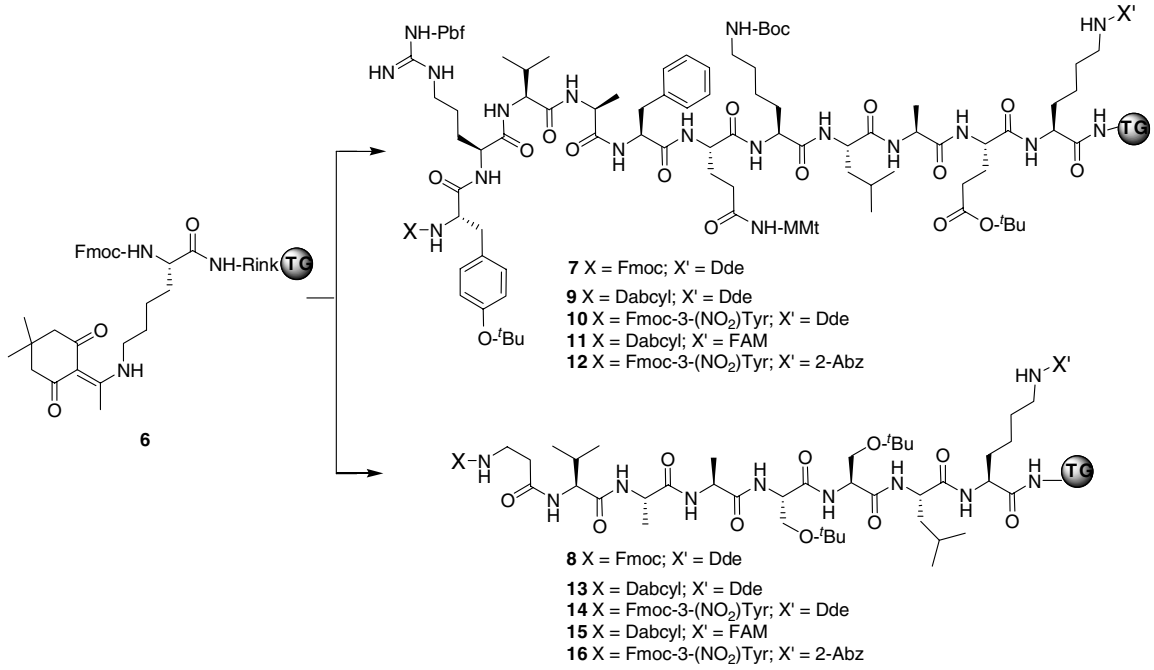
Figure 1. Analysis of the crude Fmoc-hepta-peptoid obtained using DIC/HOBt/chemistry at 60 °C (microwave irradiation); left panel: HPLC trace (98% purity according to ELS[8] detection; MeOH gradient is shown); right panel: MS spectra (ES^+) (calcd for $C_{71}H_{125}N_{15}O_9$ 1332.84, mass found *m/z*: 667.6 $(M+2H)^{2+}$, 445.5 $(M+3H)^{3+}$, 334.4 $(M+4H)^{4+}$).

Resin **2** underwent additional modification allowing the attachment to the peptoid of a fluorescein labelled αMSH peptide following additional Fmoc chemistry with microwave heating. Prior to resin cleavage and concomitant side chain deprotection,[9] resin **4** was treated with 20% piperidine in DMF in order to cleave any fluorophore dimers (esters formed between the phenols and carboxyls of fluorescein)[10] before isolating the fluorescein-αMSH-peptoid **5** (Scheme 1). Using these conditions, the αMSH conjugate **5** was obtained, again with a considerable reduction in reaction time and much higher purity (60%) and yield (45%) compared to the synthesis at room temperature with PyBroP (35% purity with an overall yield of 20%), with reduced levels of all reagents. It was also observed that the usually difficult labelling with 5(6)-carboxyfluorescein (FAM) was straightforward.

To evaluate these conditions further, the microwave-assisted synthesis of a number of derivatized peptides, which have proved problematic in the group, were targeted and included, for example, the preparation of FRET-based peptides, which are widely used probes to study protease activity, and which contain both a fluorescence donor and quencher separated by a peptide sequence. From a synthetic point of view, the labelling of these peptides can be challenging due to the lack of reactivity of some of the fluorophores and quenchers used.

A deca- and a hexa-peptide were synthesized as potential substrates for the protease ADAM33,[11] a relatively unknown enzyme involved in asthmatic processes.[12] The synthesis began from TentaGel resin **6** using an Fmoc/Bu^t approach. A set of different coupling agents were evaluated (PyBOP, HBTU and HATU) but DIC/HOBt chemistry was highly successful, requiring just 2 min of pre-activation prior to addition to the pre-swollen resin and heating at 60 °C for just 10 min.[7] This temperature seems to be crucial as higher temperatures led to less pure compounds, while cleavage from resins **7** and **8** showed outstanding purity (97%) (Scheme 2).

Resins **7** and **8** were split into two different pools in order to label with two different FRET couples [5(6)-carboxyfluorescein/dabcyl and 2-aminobenzoic acid/



Scheme 2. Synthesis of peptides **7**–**16** on TentaGel resin.[7]

3-nitro-tyrosine].[13] After Fmoc deprotection, resins **7** and **8** were coupled to dabcyl-OH using the HOBt/DIC protocol for 10 min to give resins **9** and **13**. The same procedure was followed to couple Fmoc-3-nitro-tyrosine to give resins **10** and **14**, with Kaiser tests demonstrating the complete coupling of both acids to the solid supports. The Dde groups were removed using $NH_2OH{\cdot}HCl$/imidazole in NMP[14] prior to coupling to the side chain of lysine either 5(6)-carboxyfluorescein to give resins **11** and **15** or 2-*tert*-butyloxycarbonyl-amino-benzoic acid to give resins **12** and **16**. Again complete couplings were achieved in just 10 min using DIC/HOBt.

X-YRVAFQKLAEK(X')-NH_2	**X-*β*AVAASSLK(X')-NH_2**
17 X = Dabcyl; X' = FAM	**19** X = Dabcyl; X' = FAM
18 X = 3-Nitro-Tyr; X' = 2-Abz	**20** X = 3-Nitro-Tyr; X'= 2-Abz

Figure 2. FRET-based peptides **17**–**20**.[15] βA = β-Alanine.

Prior to cleavage of the peptides from the solid supports, resins **11** and **15** were treated with 20% piperidine in DMF to cleave the fluorophore esters[10] and for resins **12** and **16** to remove the Fmoc groups. Peptides were finally cleaved with a mixture of TFA:TIS:DCM to give rise to peptides **17**–**20** (Fig. 2). Purities of the crude peptides were over 90% (e.g., Fig. 3) giving an average isolated yield of 75%.

Mild thermal effects (Arrhenius based) using microwave heating have proven to be highly successful in enabling rapid and efficient secondary amine couplings to generate a poly-cationic peptoid and its conjugation to a 13 mer peptide (αMSH). Furthermore, microwave irradiation was very successful in allowing the labelling of peptides with a variety of fluorophores and quenchers in high yields and purities with just traditional, simple, yet robust HOBt/DIC chemistry. The heating effects may enhance resin swelling and peptide chain accessibility, but the enhancement is, we believe, simply thermal in nature. Given such efficiency, the question can be

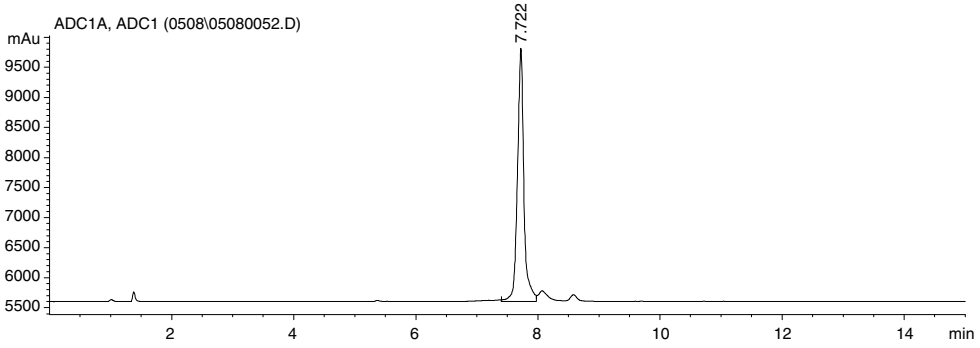


Figure 3. HPLC trace of crude deca-peptide **17** (90% purity according to ELS detection).

asked as to whether the more exotic coupling agents are necessary in solid phase peptide synthesis.

Acknowledgements

M.B. and J.J.D. are grateful to the BBSRC and EPSRC for funding. We thank Biotage for use of the SmithSyntheziser.

References and notes

1. Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149–2154.
2. DIC: *N*,*N*′-Di*iso*propylcarbodiimide; HOBt: *N*-Hydroxybenzotriazole; HOAt: 1-Hydroxy-7-azabenzotriazole; HATU: 2-(7-Aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HBTU: 2-(1*H*-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; PyBOP: Benzotriazole-1-yl-oxy-tris-pyrrolidinophosphonium hexafluorophosphate; PyBroP: Bromo-tris-pyrrolidino-phosphonium hexafluorophosphate.
3. Albericio, F. *Curr. Opin. Chem. Biol.* **2004**, *8*, 211–221, and references cited therein.
4. Peretto, I.; Sanchez-Martin, R. M.; Wang, X. H.; Ellard, J.; Mittoo, S.; Bradley, M. *Chem. Commun.* **2003**, 2312–2313.
5. Yu, H. M.; Chen, S. T.; Wang, K. T. *J. Org. Chem.* **1992**, *57*, 4781–4784.
6. (a) Campiglia, P.; Gomez-Monterrey, I.; Longobardo, L.; Lama, T.; Novellino, E.; Grieco, P. *Tetrahedron Lett.* **2004**, *45*, 1453–1456; (b) Murray, J. K.; Gellman, S. H. *Org. Lett.* **2005**, *7*, 1517–1520; (c) Erdelyi, M.; Gogoll, A. *Synthesis* **2002**, 1592–1597; (d) Matsushita, T.; Hinou, H.; Kurogochi, M.; Shimizu, H.; Mishimura, S. I. *Org. Lett.* **2005**, *7*, 877–880; (e) Murray, J. K.; Farooqi, B.; Sadowsky, J. D.; Scalf, M.; Freund, W. A.; Smith, L. M.; Chen, J.; Gellman, S. H. *J. Am. Chem. Soc.* **2005**, *127*, 13271–13280.
7. To solutions of acids (0.1 M, 3 equiv) in DMF were added HOBt (3 equiv) and DIC (3 equiv) and the mixtures were allowed to stand for 2 min before adding to pre-swollen resins in 5 mL microwave vessels (purchased from Biotage). The vessels were capped and subjected to microwave irradiation to keep the temperature constant at 60 °C for 20 min (10 min for AA couplings) with magnetic stirring using a single-mode microwave from Biotage (SmithSynthesizer). Resins were then washed with DMF, DCM, MeOH, Et_2O (3×10 ml for each solvent) with Chloranil or Kaiser tests carried out before Fmoc deprotection (20% piperidine in DMF (2×5 min)).
8. An Evaporative Light Scattering (PL-ELS) detector was used in-line with conventional UV/vis detection and attached to an Agilent HP1100 Chemstation for HPLC analysis.
9. Cleavage mixture: 81.5% TFA, 5% thioanisole, 5% phenol, 5% water, 2.5% EDT (1,2 ethanedithiol), 1% TIS (triisopropylsilane). Analysis by MALDI-TOF MS following precipitation with ice cold ether centrifugation gave a molecular ion, which agreed with the proposed structure **5**; calcd for $C_{164}H_{251}N_{37}O_{33}S$, 3301.04 (average mass); mass found *m*/*z*: 3302.53 $[M+H]^+$.
10. Fischer, R.; Mader, O.; Jung, G.; Brock, R. *Bioconjugate Chem.* **2003**, *14*, 653–660.
11. Zou, J.; Zhang, R.; Zhu, F.; Liu, J.; Madison, V.; Umland, S. P. *Biochemistry* **2005**, *44*, 4247–4256.
12. Haitchi, H. M.; Powell, R. M.; Shaw, T. J.; Howarth, P. H.; Wilson, S. J.; Wilson, D. I.; Holgate, S. T.; Davies, D. E. *Am. J. Resp. Crit. Care* **2005**, *175*, 958–965.
13. (a) Yaron, A.; Carmel, A.; Katchalski-Katzir, E. *Anal. Biochem.* **1979**, *95*, 228–235; (b) Matayoshi, E. D.; Wang, G. T.; Krafft, G. A.; Erickson, J. *Science* **1990**, *247*, 954–958; (c) Mittoo, M.; Sundstrom, L. E.; Bradley, M. *Anal. Biochem.* **2003**, *319*, 234–238.
14. (a) Diaz-Mochon, J. J.; Bialy, L.; Bradley, M. *Org. Lett.* **2004**, *6*, 1127–1129; (b) Diaz-Mochon, J. J.; Bialy, L.; Keinicke, L.; Bradley, M. *Chem. Commun.* **2005**, *11*, 1384–1386; (c) Diaz-Mochon, J. J.; Bialy, L.; Watson, J.; Sanchez-Martin, R. M.; Bradley, M. *Chem. Commun.* **2005**, *26*, 3316–3318; (d) Bialy, L.; Diaz-Mochon, J. J.; Specker, E.; Keinicke, L.; Bradley, M. *Tetrahedron* **2005**, *61*, 8295–8305.
15. Analysis by MALDI-TOF MS, following precipitation with ice cold ether, centrifugation and purification by semi-preparative HPLC gave molecular ions which agreed, in all cases, with the proposed structures. MALDI-TOF MS analysis: **17** $C_{99}H_{125}N_{21}O_{22}$, 1961.1 (average mass), mass found *m*/*z*: 1962.3 $[M+H]^+$; **18** $C_{79}H_{115}N_{21}O_{20}$, 1678.8 (average mass), mass found *m*/*z*: 1679.1 $[M+H]^+$; **19** $C_{68}H_{83}N_{13}O_{17}$, 1353.6, mass found *m*/*z*: 1376.5 $[M+Na]^+$; **20** $C_{48}H_{73}N_{13}O_{15}$, 1071.5, mass found *m*/*z*: 1072.6 $[M+H]^+$.