

Solid-phase synthesis and screening of a library of C-terminal arginine peptide aldehydes against Murray Valley encephalitis virus protease

Nicholas J. Ede,^{a*} Jeffrey Hill,^b Joma K. Joy,^b Anne-Marie Ede^a and Merran L. Koppens^a

Murray Valley encephalitis virus is a member of the flavivirus group, a large family of single-stranded RNA viruses, which cause serious disease in all regions of the world. Unfortunately, no suitable antivirals are available, and there are commercial vaccines for only three flaviviruses. The solid-phase synthesis of a library of 400 C-terminal arginine peptide aldehydes and their screening against Murray Valley encephalitis virus protease are demonstrated. The library was utilised to elucidate several tripeptide sequences that can be used as inhibitors in further SAR studies. Copyright © 2012 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: Murray Valley encephalitis virus (MVEV); peptide aldehyde; protease inhibitors; solid-phase peptide synthesis; SynPhase lanterns

Introduction

C-terminal peptide aldehydes are important tools for studying serine and cysteine proteases [1–3]. Proteases are involved in the regulation of a wide variety of essential physiological processes, and their dysregulation has been implicated in a number of disorders including cardiovascular disease, rheumatoid arthritis, Alzheimer's disease and cancer, to name only a few. Hence, proteases and their substrates are increasingly viewed as valuable drug targets in disease treatment. We have previously developed a robust and reliable synthetic method for the solid-phase synthesis of peptide aldehyde protease inhibitors [4]. This method was used to synthesise a small library of aldehydes and used to probe the proteolytic sites of hepatitis C virus polyprotein.

Murray Valley encephalitis virus (MVEV) is a member of the flavivirus group, a large family of single-stranded RNA viruses, which cause serious disease in all regions of the world. Unfortunately, no suitable antivirals are available, and there are commercial vaccines for only three flaviviruses [5]. MVEV has been isolated from mosquitoes in Australia and Papua New Guinea and, like West Nile virus, is grouped in the Japanese encephalitis serocomplex [6]. Its genome encodes a large polyprotein that is processed by both host proteinases and a virally encoded serine proteinase, non-structural protein 3 (NS3). NS3, an essential viral enzyme, requires another virally encoded protein cofactor, NS2B, for proteolytic activity [7].

Because the protease is likely to be essential for viral replication, it is an attractive target for inhibitor development. This paper outlines our synthetic method and strategy for the solid-phase synthesis of a 400 member tripeptide library of C-terminal arginine peptide aldehydes resulting in a (20 × 20 × 1) combinatorial library (P3, P2 = 20 natural AAs, P1 is fixed as arginine).

Arginine is a preferred P1 cleavage site amino acid for many serine and cysteine proteases such as trypsin and thrombin [1], dengue virus NS3 protease [8] and MVEV.

Materials and Methods

Peptides were synthesised on SynPhaseTM-PA D Series lanterns (loading 15 μmol/lantern), available from Mimotopes Pty Ltd (Melbourne, Australia). These supports had been radiation grafted with methacrylic acid/dimethylacrylamide (polyamide) and functionalized with Fmoc-Gly. Fmoc-L-α-amino acids were peptide synthesis grade and purchased from Genzyme Pharmaceuticals (Leistal, Switzerland). HOBt was purchased from GL Biochem (Shanghai, China). THF was Sure-Seal grade and was obtained from Sigma-Aldrich (Sydney, Australia). DMF was Guaranteed Reagent (GR) grade and was obtained from Merck (Melbourne, Australia). It was distilled over ninhydrin before further use. DCM, MeOH, triethylamine and piperidine were GR grade and were obtained from Merck. Acetonitrile was HPLC grade and was obtained from Merck. Water was Milli-Q grade (Merck Millipore, Melbourne, Australia). TFA was BiogradeTM and was obtained from Halocarbon (River Edge, NJ, USA). DIPEA was

* Correspondence to: Nicholas Ede, Consegna Group Ltd, 7/21 Northumberland St, Collingwood 3066, Victoria, Australia. E-mail: nede@consegna.com

^a Mimotopes Pty Ltd, 11 Duerdin St Clayton 3168 Victoria, Australia

^b Experimental Therapeutics Centre (ETC), A*STAR, 31 Biopolis Way Nanos Level 3 Singapore, 138669

obtained from Fluka (Sydney, Australia). DIC was obtained from Sigma-Aldrich.

Mass spectral analysis was performed on a Perkin Elmer Sciex API III (Waltham, MA, USA) ion spray mass spectrometer. The data were processed by software developed at Mimotopes Pty. Ltd. [9].

Synthesis of the protected amino acid aldehyde Fmoc-Arg(Pbf)-H was achieved via reduction of the corresponding *N,O*-dimethylhydroxamate with LiAlH_4 as previously described [4].

Preparation of Support-Bound Threonine Aldehyde Linker 1

To a glass bottle (500 ml), 700 SynPhaseTM lanterns (loading 15 μmol /lantern) derivatised with Fmoc-Gly-OH were added. The Fmoc group was removed with 20% piperidine/DMF (400 ml) for 30 min. The lanterns were washed (all washes with lanterns were 5 min) with DMF (2 \times 400 ml), MeOH (2 \times 400 ml) and air dried. To the bottle containing the lanterns, a solution of Fmoc-Thr-OH (13.64 g, 40 mmol), DIC (6.08 ml, 40 mmol) and HOBt (6.12 g, 40 mmol) in DMF (400 ml), 0.1 M or 6–7 equivalents, was added. The reaction was allowed to proceed for 2 h at room temperature after which the reaction mixture was drained of solution, and the lanterns were washed with DMF (2 \times 400 ml). To the washed lanterns, a solution of 20% piperidine in DMF (400 ml) was added, and the reaction was allowed to proceed for 30 min at room temperature. The reaction mixture was drained of solution, and the lanterns were washed with DMF (3 \times 400 ml), MeOH (2 \times 400 ml) and air dried to yield lanterns derivatised with threonine aldehyde linker 1.

Synthesis of Support-Bound Fmoc-Arg(Pbf)-H Oxazolidine Aldehyde Linker 2

Lanterns (500) 1 were placed into sealable glass bottle. The aldehyde Fmoc-Arg(Pbf)-H (15.48 g, 24.46 mmol) was dissolved in a 1% DIPEA/MeOH solution (250 ml) to give a 0.1-M solution. This solution was added to the lanterns and the mixture heated at 60 °C for 2 h (with occasional swirling). The reaction mixture was drained of solution and the lanterns washed with MeOH (3 \times 250 ml). The yield of attachment of Fmoc-Arg(Pbf)-H to the threonine linker can be determined spectrophotometrically by cleaving the Fmoc protecting group with 20% piperidine in DMF and measuring absorbance at 301 nm. A single lantern was treated with 20% piperidine/DMF (10 ml) for 30 min at 20 °C, and the absorbance of the solution was determined at 301 nm after diluting the deprotection solution 1:10 in 20% piperidine/DMF and using the following equation: loading (nmol/crown) = (Abs/0.0078) \times 110. The loading was determined to be 47% (7.0 μmol).

Peptide Synthesis Protocol

The lanterns (400 for library and 10 for controls) were subjected to Fmoc peptide synthesis using the following conditions. All reactions utilising lanterns require 0.5 ml of solution per lantern, either when reacted in bulk or in the microtitre plate format. (i) Fmoc deprotection: 20% piperidine in DMF for 30 min, followed by washing with DMF (3 \times 3 min) and DCM (2 \times 3 min). (ii) Coupling conditions: Fmoc-protected amino acid, DIC, HOBt in DMF (0.1 M), 2 h at 25 °C. Following all couplings, the lanterns were washed with DMF (2 \times 3 min), DCM (2 \times 3 min) and air dried. Acetylation of the N-terminus was achieved with acetic anhydride:DIPEA:DMF (ratio 3:1:96 v:v:v) at 4 °C for 5 min only. The lanterns were washed with DMF (2 \times 30 s), DMF (2 \times 3 min),

DCM (2 \times 3 min) and air dried. (iii) Side-chain deprotection and cleavage: each lantern was treated with TFA (0.5 ml per lantern) for 30 min after which the lanterns were washed with DCM (2 \times 3 min) and air dried. The peptide aldehydes were cleaved with TFA:MeCN:H₂O (0.1:60:40, v:v:v) (2.5 ml per lantern) at 60 °C for 30 min. Each lantern was removed and the cleavage solution lyophilized to give peptide aldehydes as white solids.

Protease Assay

The inhibition of MVEV protease by different acetyl-tripeptide-aldehyde inhibitors was performed in a reaction buffer with 10-mM Tris-HCl, pH 8.0, 1-mM CHAPS (3-[(3-Cholamidopropyl)dimethylammonio]-1-propane sulfonate) and 30% glycerol. 40 nM of the enzyme and varying concentrations of the inhibitor were pre-incubated in a reaction buffer for 1 h at 25 °C before the reaction was initiated by the addition of the fluorogenic peptide substrate Pyr-Arg-Thr-Lys-Arg-AMC (Bachem [Bubendorf, Sweden], Switzerland) to a final concentration of 40 μM . The components of the reaction were mixed with a 5-s shake, and the progress of the reaction was monitored continuously at 37 °C by measuring the increase in fluorescence (λ_{ex} = 355 nm and λ_{em} = 460 nm) every 45 s for 1 h on a SpectraMax Gemini XS plate reader (Molecular Devices, Sunnyvale, CA, USA). The % inhibition was calculated by comparing it with wells with no inhibitor.

Measurement of IC₅₀ Inhibition Constants

The 23 peptides identified as potential acetyl-tripeptide-aldehyde inhibitors against MVEV were re-synthesised, and a 10-mM stock was prepared. Varying concentrations of the inhibitor were incubated with 40 nM of the enzyme in 10-mM Tris-HCl, pH 8.0, 1-mM CHAPS and 30% glycerol buffer for 1 h at 25 °C. The reaction was initiated by the addition of the fluorogenic peptide substrate Pyr-Arg-Thr-Lys-Arg-AMC (Bachem) to a final concentration of 40 μM . The components of the reaction were mixed with a 5-s shake, and the progress of the reaction was monitored continuously at 37 °C by measuring the increase in fluorescence (λ_{ex} = 355 nm and λ_{em} = 460 nm) every 45 s for 1 h on a SpectraMax Gemini XS plate reader. The IC₅₀ was derived by fitting the initial velocity against the log (inhibitor) with sigmoidal dose response curve in GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA).

Results and Discussion

The solid-phase synthesis of peptide aldehydes via the oxazolidine linker [4] is one of a number of reported methods, including the semicarbazone [10], the Weinreb amide [11], the ozonolysis [12,13], the thiazolidine [14], the backbone amide linker [15], synthesis via reduction of peptide thioesters [16] and oxidation of support-bound peptide alcohols [17]. The synthesis of peptide aldehydes has recently been reviewed [18]. The oxazolidine linker satisfies the following criteria for a general linker for the immobilisation of aldehyde functionality: (i) it is low cost and easy to construct, (ii) it is chemically stable (long shelf life), (iii) it does not require preformation (i.e. the aldehyde couples directly to the solid phase), (iv) aldehyde attachment is generic and (v) it cleaves under mild conditions that leave no residue upon evaporation, allowing biological screening without further purification. The linker has been used to synthesise several peptide aldehydes including fellutamide B [19] and a library of reversible SARS coronavirus main protease inhibitors [20].

Table 2. As-synthesised mass spectrum of those inhibitors with an IC₅₀ result under 15 μM

Peptide inhibitor	IC ₅₀ (μM)	Mass spectrum
Ac-CKR-H	8.5 ± 1.4	<p>MIMOTOPES</p> <p>Mass Spectrometry Sample : PS2010601067.R2</p> <p>Ac-CKR-H</p> <p>Percentage of Total Ion Count</p> <p>Molecular Weight (Dalton)</p>
Ac-IWR-H	9.68 ± 2.2	<p>MIMOTOPES</p> <p>Mass Spectrometry Sample : PS2010601168.R2</p> <p>Ac-IWR-H</p> <p>Percentage of Total Ion Count</p> <p>Molecular Weight (Dalton)</p>
Ac-KKR-H	4.56 ± 1.6	<p>MIMOTOPES</p> <p>Mass Spectrometry Sample : PS2010601257.R2</p> <p>Ac-KKR-H</p> <p>Percentage of Total Ion Count</p> <p>Molecular Weight (Dalton)</p>

Table 2. (Continued)

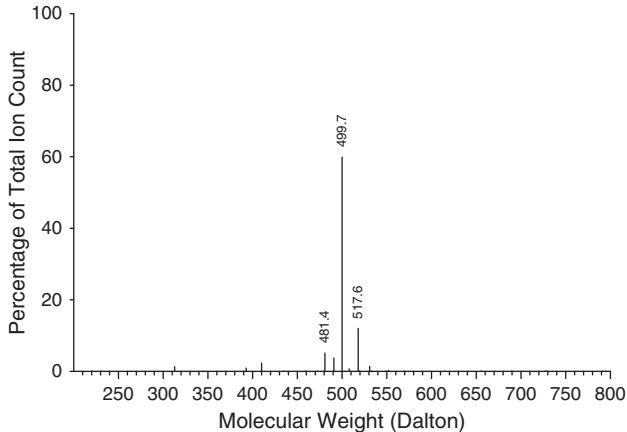
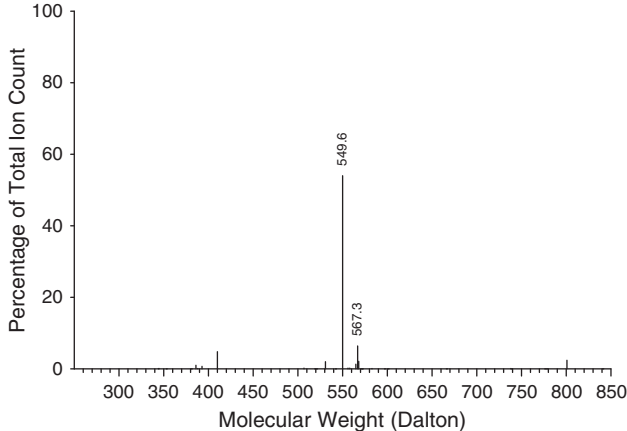
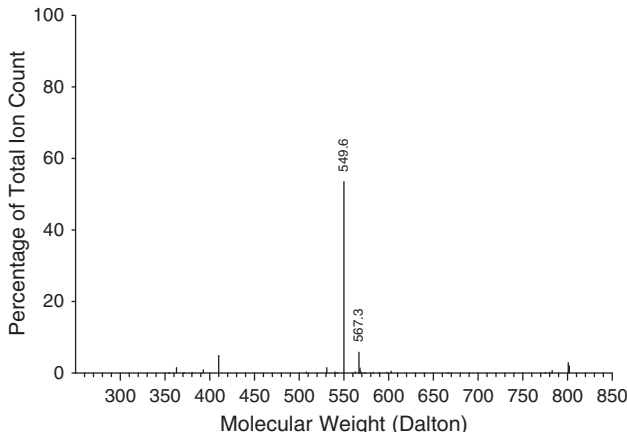
Peptide inhibitor	IC ₅₀ (μM)	Mass spectrum
Ac-LWR-H	8.8 ± 1.4	MIMOTOPES Mass Spectrometry Sample : PS2010601260.R2 Ac-LWR-H  <p>Percentage of Total Ion Count</p> <p>Molecular Weight (Dalton)</p>
Ac-YWR-H	9.7 ± 2.8	MIMOTOPES Mass Spectrometry Sample : PS2010601456.R2 Ac-YWR-H  <p>Percentage of Total Ion Count</p> <p>Molecular Weight (Dalton)</p>
Ac-WYR-H	10.90 ± 4.46	MIMOTOPES Mass Spectrometry Sample : PS2010601462.R2 Ac-WYR-H  <p>Percentage of Total Ion Count</p> <p>Molecular Weight (Dalton)</p>

Table 2. (Continued)

Peptide inhibitor	IC ₅₀ (μM)	Mass spectrum
Ac-WRR-H	10.58 ± 1.24	<p>MIMOTOPES</p> <p>Mass Spectrometry Sample : PS2010601422.R2</p> <p>Ac-WRR-H</p> <p>Percentage of Total Ion Count</p> <p>Molecular Weight (Dalton)</p>
Ac-WLR-H	12.17 ± 3.12	<p>MIMOTOPES</p> <p>Mass Spectrometry Sample : PS2010601461.R2</p> <p>Ac-WLR-H</p> <p>Percentage of Total Ion Count</p> <p>Molecular Weight (Dalton)</p>
Ac-CWR-H	12.53 ± 2.4	<p>MIMOTOPES</p> <p>Mass Spectrometry Sample : PS2010601068.R2</p> <p>Ac-CWR-H</p> <p>Percentage of Total Ion Count</p> <p>Molecular Weight (Dalton)</p>

subsequent removal of residual compounds such as TFA, organic solvents and scavengers before mild cleavage of the peptide aldehyde 4 using aqueous TFA (0.1%), containing acetonitrile as a cosolvent. The two-step cleavage conditions allow for a shorter (30 min) TFA treatment because there is no concentration of product with no evidence of Pbf protecting group remaining on cleaved peptides. The mild cleavage conditions allowed for direct testing of all 400 unpurified peptide aldehydes in a protease assay.

Library Design Strategy

To demonstrate the powerful utility of this synthesis system, we chose to lock in the P1 amino acid, selected from a challenging but more importantly, a P1 residue that is common to many serine and cysteine proteases. Arginine is particularly well suited to this design criterion because it is the preferred substrate P1 cleavage amino acid for a number of proteases. To prepare for library synthesis, we synthesised the peptide aldehyde leupeptin as a control sequence. Leupeptin is a naturally occurring microbial peptide aldehyde derived from the culture filtrates of different streptomycetes. Importantly, all C-terminal arginine peptide aldehydes partially exist in the hydrated form as demonstrated by the $[M + H + H_2O]^+$ peak in all MS run (Table 2). Lewis and Wolfenden [22] demonstrated this originally in their work on the active site of papain, whereas Gancsik *et al.* [23] extended this work using ^{13}C NMR spectroscopy. The hydrated form also exists in N-terminal aldehydes as demonstrated by Rademann, Meldal and Bock [24] and Groth and Meldal [25]. Hence, in aqueous solutions, peptide aldehydes exist as an equilibrium state between the aldehyde and hydrated forms. For the library synthesis, we synthesised an extra two copies of leupeptin on each block as internal controls.

The modular nature of SynPhase lanterns allows for their use in traditional solution-phase reaction vessels without the need for filtration, as is required with resins. This can be extended to the 'split and mix' technique if the lanterns are tagged with, e.g. coloured tags (SynPhase cogs and spindles) or radio frequency tags (Transtems). Both array and reaction vessel strategies can be used in combination to simplify the synthesis process. At the completion or during the synthesis, the SynPhase lanterns are sorted into the highly convenient 8×12 matrix format for multiple synthesis and cleavage of the target compounds. Hence, the following strategy was used to synthesise the 400-member peptide aldehyde library resulting in a $(20 \times 20 \times 1)$ combinatorial library (P3, P2 = 20 natural AAs, P1 is fixed as arginine).

Step 1: Attach Fmoc-Arg(Pbf)-H to 400 lanterns in a single reaction vessel (P1 position).

Step 2: Divide 400 lanterns into sets of 20 and place in 20 reaction vessels for coupling 20 natural Fmoc-protected amino acids (P2 position).

Step 3: Array 'dipeptide' aldehyde lanterns onto reaction backing plates and couple 20 natural Fmoc amino acids in microtitre plate format (P3 position).

Step 4: Acetylate the library by immersing plates in baths containing capping solution.

Step 5: Cleave 400 peptide aldehydes by first immersing backing plates with lanterns into baths containing TFA for concomitant side-chain protecting group removal, and after several washes, cleave from the solid support into microtitre plates containing pre-weighed tubes.

Step 6: Freeze dry in parallel, analyse and weigh, and screen in biological assay.

By utilising a strategy of positioning, the lanterns in five blocks of 8×10 (80 peptide aldehydes per block), the synthesis of the library becomes straightforward. After the second amino acid is coupled (step 2), the lanterns are arrayed on backing plates so as to orchestrate the third amino acid coupling in two side-by-side rows of 10 wells, using a multi-channel pipette to dispense the activated amino acids.

The synthesis of the Fmoc-Arg(Pbf)-H followed the same protocol reported earlier [4]. Synthesis of the protected amino acid aldehydes was achieved via reduction of the corresponding *N,O*-dimethylhydroxamate with LiAlH_4 by adapting the method of Fehrentz and Castro [26]. The yield for attachment of Fmoc-Arg(Pbf)-H was 50% determined by quantitative Fmoc test. Standard peptide synthesis methodologies were used for the synthesis of 400 peptide aldehydes. Although there is potential to acylate the oxazolidine nitrogen during peptide synthesis, it is relatively unreactive [27], and no acylation occurs with DIC/HOBt activation. Acetylation with acetic anhydride is performed at 4°C for 5 min to avoid reaction with the oxazolidine nitrogen.

The 400 isolated peptide aldehydes were weighed and analysed by mass spectrometry individually. The average mass obtained was 1.8 mg with a standard deviation of 0.2 mg ($4.1 \mu\text{mol}$, SD $0.5 \mu\text{mol}$) that represents an average yield (based on an initial loading of Fmoc-Arg(Pbf)-H of $7 \mu\text{mol}$) of 60%. All 400 peptide aldehydes showed target molecule peak by MS (Table 2 for representative MS). The mild cleavage conditions allowed for direct testing of unpurified peptide aldehyde library in a protease assay.

The 400 aldehydes were screened against the NS2B-NS3 domain of MVEV using a protease assay (Figure 1). Among the 400 aldehyde peptide inhibitors tested at $10\text{-}\mu\text{M}$ concentration, 23 peptides showed $>50\%$ inhibition at $10.0 \mu\text{M}$. The remaining stock with these 23 peptides was further tested at 5, 1.0 and $0.1 \mu\text{M}$. The inhibition (%) for these peptides is represented in Figure 1. The acetyl-tripeptide-aldehyde inhibitors such as CKR and IWR showed inhibition even at $1.0 \mu\text{M}$.

The 23 peptides identified as potential acetyl-tripeptide-aldehyde inhibitors against MVEV were re-synthesised and IC_{50} inhibition constants obtained (Table 1). The compounds were tested in duplicate with a goodness of fit (R^2) between 0.94 and 0.98. Table 2 presents the as-synthesised mass spectrum of those inhibitors with an IC_{50} result under $15 \mu\text{M}$.

Conclusion

A 400-member library of C-terminal arginine peptide aldehydes was prepared by the straightforward approach described here and was demonstrated to be an effective tool in expediting rapid SAR analysis of serine proteases, such as MVEV.

References

- 1 Otto H-H, Schirmeister T. Cysteine proteases and their inhibitors. *Chem. Rev.* 1997; **97**: 133–171.
- 2 Malcolm BA, Lowe C, Shechosky S, McKay RT, Yang CC, Shah VJ, Simon RJ, Vederas JC, Santi DV. Peptide aldehyde inhibitors of hepatitis A virus 3C proteinase. *Biochemistry* 1995; **34**: 8172–8179.
- 3 Scheidt KA, Roush WR, McKerrow JH, Selzer PM, Hansell E, Rosenthal PJ. Structure-based design, synthesis and evaluation of conformationally constrained cysteine protease inhibitors. *Bioorg. Med. Chem.* 1998; **6**: 2477–2494.

- 4 Ede NJ, Eagle SN, Wickham G, Bray AM, Warne B, Shoemaker K, Rosenberg S. Solid phase synthesis of peptide aldehyde protease inhibitors. Probing the proteolytic sites of hepatitis C virus polyprotein. *J. Peptide Sci.* 2000; **6**: 11–18.
- 5 Ray D, Shi PY. Recent advances in flavivirus antiviral drug discovery and vaccine development. *Recent Pat. Anti-Infect. Drug Discov.* 2006; **1**: 45–55.
- 6 Burrow JN, Whelan PI, Kilburn CJ, Fisher DA, Currie BJ, Smith DW. Australian encephalitis in the Northern Territory: clinical and epidemiological features, 1987–1996. *Aust. N. Z. J. Med.* 1998; **28**: 590–596.
- 7 Joy JK, Mee NF, Kuan WL, Perlyn KZ, Wen TS, Hill J. Biochemical characterisation of Murray Valley encephalitis virus proteinase. *FEBS Letts.* 2010; **584**: 3149–3152.
- 8 Yin Z, Patel SJ, Wang W-L, Chan W-L, Ranga Rao KR, Wang G, Ngew X, Patel V, Beer D, Knox JE, Ling Ma N, Ehrhardt C, Pheng Lim S, Vasudevan SG, Keller TH. Peptide inhibitors of dengue virus NS3 protease. Part 2: SAR study of tetrapeptide aldehyde inhibitors. *Bioorg. Med. Chem. Letts.* 2006; **16**: 40–43.
- 9 Smart SS, Mason TJ, Bennell PS, Maeji NJ, Geysen HM. High-throughput purity estimation and characterization of synthetic peptides by electrospray mass spectrometry. *Int. J. Peptide Protein Res.* 1996; **47**: 47–55.
- 10 Murphy AM, Dagnino R, Jr., Vallar PL, Trippe AJ, Sherman SL, Lumpkin RH, Tamura SY, Webb TR. Automated synthesis of peptide C-terminal aldehydes. *J. Am. Chem. Soc.* 1992; **114**: 3156–3157.
- 11 Fehrentz J-A, Paris M, Heitz A, Velek J, Winternitz F, Martinez J. Solid phase synthesis of C-terminal peptide aldehydes. *J. Org. Chem.* 1997; **62**: 6792–6796.
- 12 Pothion C, Paris M, Heitz A, Rocheblave L, Rouch F, Fehrentz J-A, Martinez J. Use of ozonolysis in the synthesis of C-terminal peptide aldehydes on solid support. *Tetrahedron Lett.* 1997; **44**: 7749–7752.
- 13 Hall BJ, Sutherland JD. A practical method for the combinatorial synthesis of peptide aldehydes. *Tetrahedron Letts.* 1998; **39**: 6593–6596.
- 14 Galeotti N, Giraud M, Jouin P. Solid phase synthesis of peptidyl aldehydes from C-terminal thiazolidinyl peptides. *Letts. Pept. Sci.* 1997; **4**: 437–440.
- 15 Jensen KJ, Alsina J, Songster MF, Vagner J, Albericio F, Barany G. Backbone amide linker (BAL) strategy for solid-phase synthesis of C-terminal-modified and cyclic peptides. *J. Am. Chem. Soc.* 1998; **120**: 5441–5452.
- 16 Wyslouch-Cieszyńska A, Tam PT. *Peptides: Frontiers of Peptide Science, Proceedings of the Fifteenth American Peptide Symposium*, Tam PJ, Kaumaya PTP (eds). Kluwer Academic Publishers: The Netherlands, Dordrecht, 1999; 263–264.
- 17 Page P, Bradley M, Walters I, Teague S. Solid phase synthesis of tyrosine peptide aldehydes. Analogues of (S)-MAPI. *J. Org. Chem.* 1999; **64**: 794–799.
- 18 Moulin A, Martinez J, Fehrentz J-A. Synthesis of peptide aldehydes. *J. Peptide Sci.* 2007; **13**: 1–15.
- 19 Schneekloth JS, Jr., Sanders JL, Hines J, Crews CM. Neurotrophic peptide aldehydes: solid phase synthesis of fellutamide B and a simplified analog. *Bioorg. Med. Chem. Lett.* 2006; **16**: 3855–3858.
- 20 Al-Gharabli SI, Ali Shah ST, Weik S, Schmidt MF, Mesters JR, Kuhn D, Klebe G, Hilgenfeld R, Rademann J. An efficient method for the synthesis of peptide aldehyde libraries employed in the discovery of reversible SARS coronavirus main protease (SARS-CoV Mpro) inhibitors. *Chem. Bio. Chem.* 2006; **7**: 1048–1055.
- 21 Ede NJ. Multiple parallel synthesis of peptides on SynPhase™ grafted supports. *J. Immunol. Methods* 2002; **267**: 3–11.
- 22 Lewis CA, Jr., Wolfenden R. Thiohemiacetal formation by inhibitory aldehydes at the active site of papain. *Biochemistry* 1977; **16**: 4890–4895.
- 23 Gamcsik MP, Malthouse JPG, Primrose WU, Mackenzie NE, Boyd ASF, Russell RA, Scott AI. Structure and stereochemistry of tetrahedral inhibitor complexes of papain by direct NMR observation. *J. Am. Chem. Soc.* 1983; **105**: 6324–6325.
- 24 Rademann J, Meldal M, Bock K. Solid-phase synthesis of peptide isosteres by nucleophilic reactions with N-terminal peptide aldehydes on a polar support tailored for solid-phase organic chemistry. *Chem. Eur. J.* 1999; **5**: 1218–1225.
- 25 Groth T, Meldal M. Synthesis of aldehyde building blocks protected as acid labile *N*-Boc *N,O*-acetals: toward combinatorial solid phase synthesis of novel peptide isosteres. *J. Comb. Chem.* 2001; **3**: 34–44.
- 26 Fehrentz J-A, Castro B. An efficient synthesis of optically active α -(*t*-butoxycarbonylamino)-aldehydes from α -amino acids. *Synthesis* 1983; 676–678.
- 27 Ede NJ, Bray AM. A simple linker for the attachment of aldehydes to the solid phase. Application to solid phase synthesis by the Multipin™ method. *Tetrahedron Lett.* 1997; **38**: 7119–7122.