Rapid Communication

Journal of PeptideScience

Received: 1 August 2008 Revised: 5 September 2008 Accepted: 18 September 2008 Published online in Wiley Interscience: 1 December 2008

(www.interscience.com) DOI 10.1002/psc.1080

Morpholinone mediated oxazolone-free *C*-terminus amide coupling permitting a convergent strategy for peptide synthesis

Laurence M. Harwood,[a]* Simon J. Mountford[a] and Ran Yan[b]

3-Substituted-5-phenylmorpholinones have been demonstrated to act as *N*-protected *C*-terminus activated α-amino acids capable of undergoing solution phase *N*-terminus peptide extension following standard coupling procedures. The *N*-acylated morpholinones do not undergo epimerisation of the stereocentre of the *C*-terminus amino acid residue as oxazolone formation is sterically prevented, although *C*-terminus peptide coupling is still possible. This convergent approach to peptide synthesis is exemplified by the preparation of L-ala-L-ala-L-ala and L-ala-D-ala-L-ala. **Copyright © 2008 European Peptide Society and John Wiley & Sons, Ltd.**

Supporting information may be found in the online version of this article

Keywords: morpholinone; *C*-terminus; synthesis; oxazalone

Peptide synthesis continues to evolve, with new techniques, protecting groups and activating groups, developed for both solution and solid phase synthesis, being reported [1]. However, one of the prevailing drawbacks of any peptide synthesis is that construction of the sequence is generally limited to extension at the *N*-terminus in a linear manner. It is well established that, with attempts at *C*-terminus extension of a peptide chain, comes a substantial risk of epimerisation of the *C*-activated acylamino acid residue via oxazolone formation [2]. The iterative nature of peptide synthesis means that even low levels of epimerisation at the activated *C*-terminus would lead to complex inseparable mixtures during the preparation of even moderate length peptides.

Addressing this problem to some extent, the backbone amide linker (BAL) anchoring method for peptide synthesis described by Albericio has allowed *C*-terminal modifications to be achieved relatively free from racemisation and diketopiperazine formation [3–5]. The method involves immobilising the *C*-carboxyl protected penultimate residue (via preformed handles or on-resin reductive amination), selective removal of the *C*-carboxyl protecting group, activation of the *C*-carboxyl group and coupling of the desired *C*-terminal residue, acylation of the BAL anchored amine, peptide elongation in the conventional manner and finally cleavage from the support. Furthermore, Albericio has also showed the possibility of $N \rightarrow C$ (reverse) solid phase peptide synthesis by firstly immobilising an allyl protected amino acid through its α-amino group to the acid-labile 2-Cl-trityl resin and using $Cu(OBt)_2$/DIPCDI or HATU/DIEA as a coupling method [6].

Morpholinone-based chiral relay systems for the preparation of optically active amino acids have been a major research focus of our group over many years [7–9]. We herein report a novel approach for the convergent synthesis (*N*- and *C*-terminus extension) of peptides using these morpholinone templates as key structural motifs.

Previously published X-ray structure data from our laboratory have shown that 5-phenylmorpholinones with the secondary amine unsubstituted (as exemplified by compound **1,** Scheme 1) adopt a flattened chair conformation with the *C*-5 phenyl group adopting an equatorial conformation [10], while the *N*-acylated derivatives adopt a boat conformation with the *C*-5 phenyl axial [11–13]. On inspection, we noted that the *N*-acyl substituent on the nitrogen is prevented from intramolecular attack at the lactone carbonyl to form an oxazolone. Moreover, in previous studies it has been demonstrated that the non-acylated morpholinone ring is sensitive to nucleophilic attack resulting in opening of the lactone [14]. We therefore reasoned that, if this lactone reactivity extended to the *N*-acylated derivatives, these would have the potential to act as *C*-terminus activated dipeptides circumventing the problem of oxazolone formation and attendant epimerisation of the activated amino acid residue.

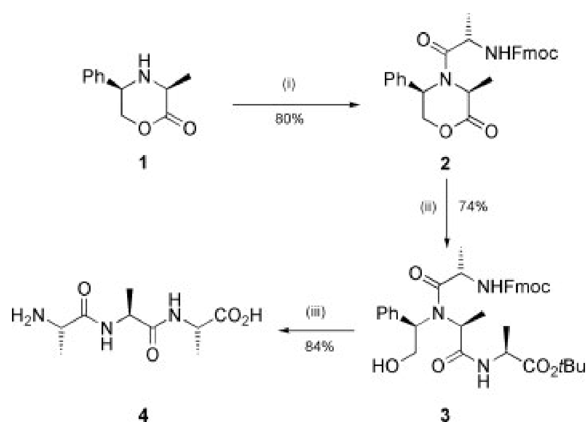
The morpholin-2-one templates required for the peptide synthesis can be prepared via synthetically complementary pathways. Routes described by Caplar and Sunjic [15] and Dellaria and Santarsiero [16] require access to the *N*-terminus protected amino acid or the amino alcohol; whereas two routes developed within our group permit access to (3*S*)- and (3*R*)- configured morpholinones which correspond to templated L and D amino acids respectively, not necessarily limited to the proteinogenic series [14,17,18].

* *Correspondence to: Laurence M. Harwood, Department of Chemistry, University of Reading, Whiteknights, Reading RG6 6AD, UK. E-mail: l.m.harwood@reading.ac.uk*

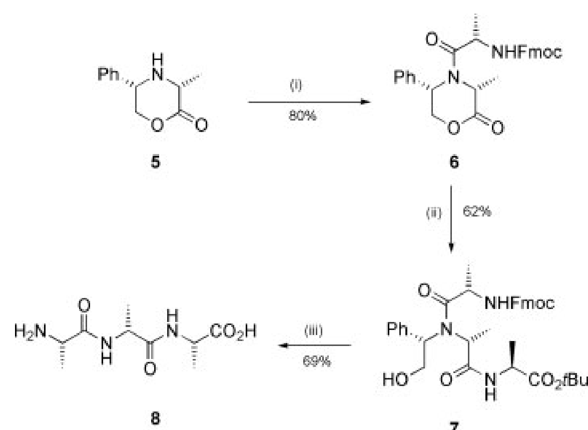
a *Department of Chemistry, University of Reading, Whiteknights, Reading RG6 6AD, UK*

b *School of Chemistry, Bedson Building, University of Newcastle upon Tyne, Newcastle upon Tyne, NE1 7RU, UK*

Copyright © 2008 European Peptide Society and John Wiley & Sons, Ltd.



Scheme 1. (i) *N*-Fmoc-L-alanine acid chloride, Na_2CO_3, CH_2Cl_2, 1 h; (ii) *t*-butyl-L-alanate, $Al(CH_3)_3$, CH_2Cl_2, 24 h; (iii) Li, liq. NH_3, *t*-BuOH, THF, $-78\,^{\circ}$C, 15 min.



Scheme 2. (i) *N*-Fmoc-L-alanine acid chloride, Na_2CO_3, CH_2Cl_2, 1h; (ii) *t*-butyl-L-alanate, $Al(CH_3)_3$, CH_2Cl_2, 24 h; (iii) Li, liq. NH_3, *t*-BuOH, THF, $-78\,^{\circ}$C, 15 min.

In an initial study, the (3*S*,5*R*)-morpholinone template **1** was *N*-acetylated as a model for a *C*-terminus activated dipeptide and the adduct heated in DMSO-d_6 at 120 °C for 3 weeks (see supporting information). After this period, examination of the ^{1}H NMR spectrum showed no evidence for the *N*-acetyl morpholinone epimerising at the *C*-3 position (detection down to at least 0.5% by comparison with intensities of the ^{13}C satellites of the acetyl and methyl resonances). This evidence, that the morpholinone ring can efficiently prevent the formation of the oxazolone, supported our proposal that *C*-terminus extension of morpholinone based dipeptides without racemisation at the *C*-3 position could be possible.

A variety of *N*-acylation methods has been developed for use in peptide synthesis; in particular, the activation of the acid by carbodiimides [19], phosphonium reagents [20], uronium reagents [21] or the use of the corresponding acid fluorides [22,23] acid azides [24] or acid chlorides [25].

Our initial attempts at acylating the morpholinone template **1** with carbodiimide activation, *N*-*t*-Boc-L-alanine acid fluoride or azide and *N*-*t*-Boc-L-alanine with the phosphonium salt PyBrop failed and our attention turned to the use of amino acid chlorides.

Owing to their high reactivity, acid chlorides are useful species in sterically demanding amide bond formations although their application in peptide chemistry was initially limited due to unfavourable conditions for their generation and their high susceptibility to racemisation [26]. However, with the development of the Fmoc protecting group, Carpino described a method for preparing Fmoc amino acid chlorides under mild conditions which led to high yielding peptide coupling with low rates of racemisation [25] whilst Meldal has reported the use of *N*-azido amino acid chlorides [27].

Gratifyingly, following an adaptation of Carpino's methodology, *N*-terminus peptide extension was successfully carried out by vigorously stirring a mixture of the morpholinone **1** and *N*-Fmoc-L-alanine acid chloride with a suspension of Na_2CO_3 in dichloromethane to give the coupled product **2** as homogeneous material in 80% yield after 2 h at room temperature (Scheme 1).

After several unsuccessful attempts at ring opening using carboxyl-protected amino acids, aminolysis of the template was achieved using Weinreb's modified dimethylaluminium amide method [28]. The desired dimethylaluminium amide was generated by treating 3 equivalents of *t*-butyl-L-alanate with 3.5 equivalents of trimethylaluminium under a nitrogen atmosphere. The dipeptide adduct **2** in anhydrous dichloromethane was added and the reaction was completed after 24 h to give **3** in 74% yield. Despite the air and moisture sensitivity of trimethylaluminium, the coupling was operationally simple and conversion occurred cleanly.

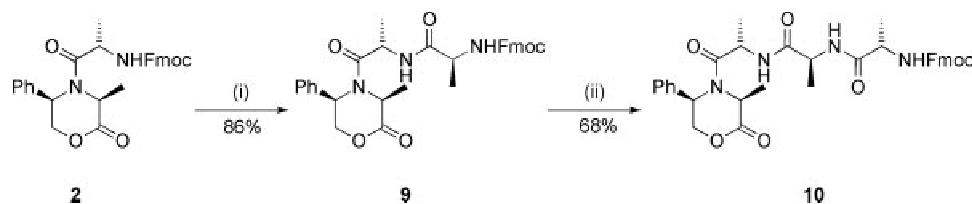
Global removal of the *t*-butyl and Fmoc protecting groups and the *N*-benzyl substituent from the tripeptide precursor **3** was achieved by the use of lithium in liquid ammonia with *t*-butanol present as a proton source. The pure material was obtained after acidic ion-exchange chromatography (DOWEX 200) and flash chromatography on silica. The synthetic tri-L-alanine **4** obtained gave spectroscopic data and a specific rotation corresponding to those of a commercial sample. Omission of the *t*-butanol from the reaction mixture led to approximately 6% of an epimer.

The sequence was repeated with (3*R*,5*S*)-morpholinone **5**, corresponding to templated D-alanine (Scheme 2). Coupling with *N*-Fmoc-L-alanine acid chloride furnished the dipeptide adduct **6** in 80% yield, *C*-terminus extension as before gave the tripeptide adduct **7** in 62% yield and global deprotection and purification as before furnished pure L-D-L-trialanine **8** in 69% yield. This product was different to the epimerised material obtained in the previous sequence in the absence of *t*-butanol and so we conclude that epimerisation in that instance had occurred at the *C*-terminus residue.

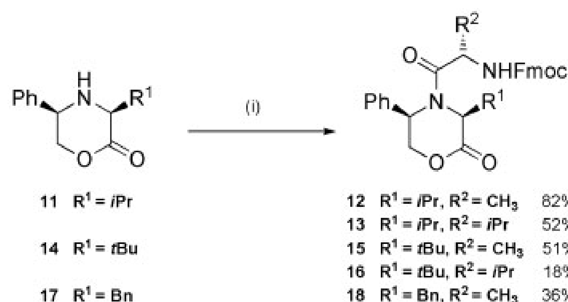
The dipeptide derivative **2** was further *N*-terminus extended (Scheme 3) by removal of the Fmoc group using DBU (10 mol%) in THF, and coupling Fmoc-L-alanine (1.2 equiv.) using bromotripyrrolidinophosphonium hexafluorophosphate (1.2 equiv.) and *N*,*N*-diisopropylethylamine (1.1 equiv.). This tripeptide derivative **9** was formed within 18 h in 86% yield. Repeating this protocol, a further Fmoc-L-alanine was coupled onto derivative **9** and the subsequent tetrapeptide derivative **10** was isolated in a yield of 68%. It was found that 0.5 equivalent of DBU was necessary to remove the Fmoc group at this stage.

To determine the scope of the *N*-terminus extension of the morpholinone template, the sterically hindered 3*S*-*iso*-propyl-5*R*-phenyl morpholinone **11**, corresponding to templated L-valine, was prepared and *N*-terminus extended with *N*-Fmoc-L-alanine acid chloride (Scheme 4). The *N*-extended product **12** was obtained after 1 h in 82% yield. Furthermore, the more sterically disfavoured *N*-Fmoc-L-valine acid chloride was also coupled to the (3*S*)-*iso*-propyl morpholinone template **11** using the same method and the adduct **13** was obtained in a 52% yield after 6 h. Only a single diastereoisomer was found in

 Copyright © 2008 European Peptide Society and John Wiley & Sons, Ltd.



Scheme 3. (i) 10 mol% DBU, THF, 5h; DIPEA, PyBrop, Fmoc-L-alanine, THF, 18 h; (ii) 50 mol% DBU, THF, 5h; DIPEA, PyBrop, Fmoc-L-alanine, THF, 18 h.



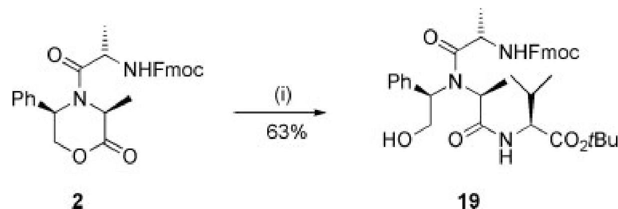
Scheme 4. (i) *N*-Fmoc-L-alanine acid chloride, Na_2CO_3, CH_2Cl_2, 1–48 h.

high temperature ^{1}H NMR spectroscopy studies in each case showing that our morpholinone based peptide synthesis strategy appears to be free from epimerisation even in sterically hindered cases.

To test the effect of steric bulk at the *C*-3 position to the limit, the *N*-terminus extension of *C*-3 *tert*-butyl morpholinone **14**, corresponding to templated L-*tert*-leucine, was carried out (Scheme 4). The template was firstly reacted with *N*-Fmoc-L-alanine acid chloride to give the coupled product **15** in 51% yield after 18 h. Secondly the template was reacted with the more sterically hindered *N*-Fmoc-L-valine acid chloride. This reaction was significantly slower, taking 48 h, and the desired dipeptide derivative **16** was obtained only in an 18% yield.

Phenylalanine is among the more readily racemised α-amino acids; therefore the templated analogue 3*S*-benzyl-5*R*-phenyl morpholinone **17** was synthesised (Scheme 4). *N*-terminus extension of the template with *N*-Fmoc-L-alanine using the previously described coupling conditions gave the product **18** in a 36% isolated yield after 4 h. Two conformers of this product (*ca* 1 : 1 ratio) can be observed in the ^{1}H NMR spectrum at ambient temperature, however, these two sets of signals coalesce at 100 °C, indicating the desired adduct **18** and about 3% of the contaminant, possibly the *C*-3 diastereoisomer.

The effect of steric factors on the ring opening process was tested by using the more hindered *t*-butyl-L-valinate for the *C*-terminus extension of template **2** (Scheme 5). The reaction proceeded at room temperature in 24 h and the 63% yield of **19** obtained (*cf* 74% for the analogous *t*-butyl-L-alanate extension) suggests that the degree of substitution on the free amine does not have a large steric influence over the ring opening process.



Scheme 5. (i) *t*-butyl-L-valinate, $Al(CH_3)_3$, CH_2Cl_2, 24 h.

In conclusion, this methodology allows preparation of peptides containing proteinogenic and non-proteinogenic amino acid components using a morpholinone template as the key reactive motif. The novel peptide coupling protocol described herein, comprising *ab initio* construction of the initial amino acid residue, *N*-terminus peptide extension using standard solution peptide coupling procedures, *C*-terminus extension of the dipeptide derivative without epimerisation and global deprotection, permitted the synthesis of tri-L-alanine and L-D-L-trialanine in good overall yields and excellent purity. This proof of concept study demonstrates a strategy that makes experimentally amenable convergent peptide synthesis, a feasible prospect for the first time and extensions of this work to permit *C*-terminus coupling of longer peptides and development of a solid phase variant will be reported in due course.

Experimental Procedures

Procedure for the Synthesis of Tri-L-alanine 4

To a vigorously stirred mixture of (3*S*,5*R*)-3-methyl-5-phenyl-3,4,5,6-tetrahydro-2*H*-1,4-oxazin-2-one **1** (600 mg, 3.14 mmol), Na_2CO_3 (1.70 g, 15.70 mmol, 5.0 equiv.) in dichloromethane (40 ml) was added *N*-Fmoc-L-alanine acid chloride (1.26 g, 3.84 mmol, 1.2 equiv.) in dichloromethane (10 ml) dropwise over 5 min. The resulting mixture was stirred for 2 h. Filtration through a short pad of Celite and the removal of solvent from the filtrate *in vacuo* gave the crude material which was purified by flash column chromatography on silica, eluting with petrol and diethyl ether (1 : 4) to furnish **2** as colourless needles (1.21 g, 80%), m.p: 89–90 °C; $[\alpha]_D^{20}$ −13.1 (*c* 1.06 in $CHCl_3$); IR (KBr): $\nu = 3321, 2983, 1741, 1718, 1654$ cm^{-1}; ^{1}H NMR (250 MHz, DMSO-*d*): $\delta = 7.91–7.27$ (m, 13H), 5.50 (bs, 1H), 5.07 (bs, 1H), 4.90 (bs, 1H), 4.79–4.76 (m, 1H), 4.61–4.49 (m, 2H), 4.26 (m, 1H), 4.19 (m, 2H), 1.28 (d, $J = 4$ Hz, 3H), 1.10 (d, $J = 4$ Hz, 3H); ^{13}C NMR (62.5 MHz, DMSO-*d*): $\delta = 172.0$, 170.0, 156.1, 144.2, 141.1, 136.2, 128.9, 128.3, 127.9, 127.4, 126.7, 125.7, 120.4, 66.0, 65.3, 53.0, 50.7, 47.8, 46.9, 18.6, 17.8; HRMS (CI): calcd for $C_{29}H_{29}N_2O_5$: 485.2076 $[M + H]^+$; found, 485.2060.

Trimethyl aluminium (1.87 ml, 3.75 mmol, 2M in hexane, 3.5 equiv.) to a solution of L-alanine *t*-butyl ester (466 mg, 3.21 mmol, 3.0 equiv.) in anhydrous dichloromethane (30 ml) was added under an atmosphere of nitrogen. After 15 min, (3*S*,5*R*)-*N*-[*N*-Fmoc-(*S*)-alanyl]-3-methyl-5-phenyl-3,4,5,6,-tetrahydro-2*H*-1,4-oxazin-2-one **2** (520 mg, 1.07 mmol) in anhydrous dichloromethane (10 ml) was added. The resulting solution was stirred at room temperature for 24 h. The reaction was quenched by the addition of water (10 ml) and the organic phase was then washed with saturated copper sulfate (20 ml). The aqueous phase was extracted with diethyl ether (3 × 20 ml) and the combined organic extracts were washed with brine (50 ml) and dried over $MgSO_4$. The solvents were removed *in vacuo* and the crude product was

 Copyright © 2008 European Peptide Society and John Wiley & Sons, Ltd.

purified by flash column chromatography on silica, eluting with diethyl ether and dichloromethane (4 : 1) to furnish **3** as colourless needles (497 mg, 74%), m.p: 69–71 °C; $[\alpha]_D^{20}$ −41.2 (*c* 1.08 in $CHCl_3$); IR (KBr): ν = 3409, 2979, 1733, 1718, 1656 cm^{-1}; ^1H NMR (250 MHz, DMSO-*d*) δ = 7.96–7.34 (m, 13H), 7.90 (d, *J* = 7.4 Hz, 1H), 6.10 (s, 1H), 5.41–5.33 (m, 1H), 4.86 (q, *J* = 6.8 Hz, 1H), 4.40–4.24 (m, 3H), 4.16–4.07 (m, 2H), 3.79 (q, *J* = 7.0 Hz, 1H), 3.63(q, *J* = 6.7 Hz, 1H), 1.44 (d, *J* = 6.7 Hz, 3H), 1.40 (d, *J* = 6.8 Hz, 3H), 1.27 (s, 9H), 0.90 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (62.5 MHz, DMSO-*d*) δ = 173.5, 171.3, 170.3, 156.6, 144.1, 141.1, 137.6, 129.1, 128.7, 128.0, 127.6, 125.6, 121.7, 120.5, 80.2, 66.2, 60.9, 60.2, 48.7, 47.4, 46.9, 27.8, 17.4, 17.1, 14.9; HRMS (CI): calcd for $C_{36}H_{44}N_3O_7$: 630.3179 $[M + H]^+$; found, 630.3174.

To a solution of *N*-Fmoc-(*S*)-alanyl-*N*-((1*R*)-phenyl-2-hydroxylethyl)-(*S*)-alanyl-(*S*)-alanine *t*-butyl ester **3** (235 mg, 0.37 mmol) and *tert*-butanol (0.12 ml, 1.20 mmol, 3.0 eq.) in liquid ammonia (15 ml) and anhydrous tetrahydrofuran (10 ml) was added lithium (23 mg, 3.70 mmol, 10.0 eq.) at −78 °C under an atmosphere of nitrogen. The resulting solution was stirred until the blue colour disappeared and then allowed to warm to room temperature to evaporate off the liquid ammonia. A mixture of water (15 ml) and diethyl ether (10 ml) was added and the aqueous phase was extracted with diethyl ether (3 × 10 ml). The water was removed *in vacuo* and the crude product was purified first by acidic ion-exchange chromatography and then by flash column chromatography on silica, eluting with methanol and water (7 : 3) to furnish the **4** as colourless needles (71 mg, 84%), $[\alpha]_D^{20}$ −72.8 (*c* 1.01 in H_2O), (commercial sample ex Sigma $[\alpha]_D^{20}$ −73.2 (*c* 1.02 in H_2O)); IR (KBr): ν = 3347, 3276, 2985, 1645, 1592, 1531 cm^{-1}; ^1H NMR (250 MHz, D_2O): δ = 4.53 (q, *J* = 7.3 Hz, 1H), 4.03 (q, *J* = 7.1 Hz, 2H), 1.23 (d, *J* = 7.1 Hz, 3H), 1.09 (d, *J* = 7.1 Hz, 3H), 1.02 (d, *J* = 7.3 Hz, 3H); ^{13}C NMR (62.5 MHz, D_2O): δ = 180.1, 173.9, 170.9, 51.3, 50.1, 49.2, 17.7, 16.9, 16.7; HRMS (CI): calcd for $C_9H_{17}N_3O_4$: 231.1219 $[M]^+$; found, 231.1209.

Supporting information

Supporting information may be found in the online version of this article.

Acknowledgements

We thank the Overseas Research Students Award Scheme (RY), HEFCE Higher Education Innovation Fund (SJM) and the University of Reading for financial support.

References

1. Benoiton NL. *Chemistry of Peptide Synthesis*. C.R.C Press: Boca Raton, FL, 2005.
2. Jones J. *Amino Acid and Peptide Synthesis*. Oxford University Press: New York, 2002.
3. Alsina J, Yokum TS, Albericio F, Barany G. A modified backbone amide linker (BAL) solid-phase peptide synthesis strategy accommodating prolyl, *N*-alkylamino acyl, or histidyl derivatives at the *C*-terminus. *Tetrahedron Lett.* 2000; **41**: 7277.
4. Alsina J, Yokum TS, Albericio F, Barany G. Backbone amide linker (BAL) strategy for *N*α-9-fluorenylmethoxycarbonyl (Fmoc) solid-phase synthesis of unprotected peptide *p*-nitroanilides and thioesters. *J. Org. Chem.* 1999; **64**: 8761.
5. Jensen KJ, Alsina J, Songster MF, Vagner J, Albericio F, Barany G. Backbone Amide Linker Strategy for Solid-Phase Synthesis of *C*-Terminal-Modified and Cyclic Peptides. *J. Am. Chem. Soc.* 1998; **120**: 5441–5452.
6. Thieriet N, Guibe F, Albericio F. Solid-phase peptide synthesis in the reverse (*N* → *C*) direction. *Org. Lett.* 2000; **2**: 1815.
7. Anslow AS, Harwood LM, Lilley IA. Synthesis of α-phenyl proline derivatives via 1,3-dipolar cycloaddition of chiral stabilized azomethine ylids. *Tetrahedron: Asymmetry* 1995; **6**: 2465.
8. Alker D, Harwood LM, Williams CE. Cycloadditions of aromatic imines to enantiomerically pure stabilized azomethine ylids: construction of threo (2*S*,3*R*)-3-aryl-2,3-diamino acids. *Tetrahedron Lett.* 1995; **39**: 475.
9. Aldous DJ, Drew MGB, Draffin WN, Hamelin EM-N, Harwood LM, Thurairatnam S. 1,3-Dipolar cycloadditions to unsymmetrical ketone-derived chiral stabilized azomethine ylides: Strategies for the synthesis of highly substituted amino acids. *Synthesis* 2005; **19**: 3271.
10. Harwood LM, Tyler SNG, Anslow AS, MacGilp ID, Drew MGB. Synthesis of enantiomerically pure α-amino acids via chemo- and diastereoselective alkylation of (5*S*)-5-phenyl-5,6-dihydro-2*H*-1,4-oxazin-2-one. *Tetrahedron: Asymmetry* 1997; **8**: 4007.
11. Vigneron JP, Kagan H, Horeau A. Synthèse asymètrique de l'acide aspartique optiquement pur. *Tetrahedron Lett.* 1968; **54**: 5681.
12. Vigneron JP, Kagan H, Horeau A. Stereospecific asymmetric synthesis. Quantitative preparation of optically pure aspartic acid. *Bull. Soc. Chim. Fr.* 1972; **10**: 3836.
13. Drew MGB, Harwood LM, Park G, Price DW, Tyler SNG, Park CR, Cho SG. An ab initio and X-ray study of morpholinone derivatives: the intermolecular 1,3-dipolar cycloaddition of a derived ylide with maleimide. *Tetrahedron* 2001; **57**: 5641.
14. Alker D, Hamblett G, Harwood LM, Robertson SM, Watkin DJ, Williams CE. Application of enantiopure templated azomethine ylids to β-hydroxy-α-amino acid synthesis. *Tetrahedron* 1998; **54**: 6089.
15. Caplar V, Lisini A, Kajfez F, Sunjic V. Synthesis of the 2,3-Dihydro-6*H*-1,4-oxazin-2-ones chiral at C(3) and asymmetric induction in hydrogenation of the azomethine bond. *J. Org. Chem.* 1978; **43**: 1355.
16. Dellaria JF, Santarsiero BD. Stereoselective alkylation of chiral glycine enolate synthons. The enantioselective synthesis of α-amino acid derivatives. *Tetrahedron Lett.* 1988; **29**: 6079.
17. Cox GG, Harwood LM. Synthesis of homochiral α-amino acids by reductive amination of α-ketoacids via 3-substituted-5-phenyl-3,4-didehydromorpholin-2-ones: Synthesis of (*S*)- and (*R*)-2-aminobutanoic acid. *Tetrahedron: Asymmetry* 1994; **5**: 1669.
18. Harwood LM, Vines KJ, Drew MGB. Synthesis of homochiral α-substituted alanine derivatives by diastereocontrolled alkylation of (5*R*)-5-phenyl-3-methyl-3,4-dehydromorpholinones. *Synlett* 1996; **11**: 1051.
19. Sheehan JC, Hess GP. A new method of forming peptide bonds. *J. Am. Chem. Soc.* 1995; **77**: 1067.
20. Coste J, Nguyen DL, Castro B. PyBOP: a new peptide coupling reagent devoid of toxic byproduct. *Tetrahedron Lett.* 1990; **31**: 205.
21. Dourtoglou V, Ziegler JC, Gross B. *O*-Benzotriazolyl-*N*,*N*-tetramethyluronium hexafluorophosphate: a new and effective reagent for peptide coupling. *Tetrahedron Lett.* 1978; **15**: 1269.
22. Carpino LA, Sadat-Aalaee D, Chao HG, DeSelms RH. [(9-Fluorenylmethyl)oxy] carbonyl (FMOC) amino acid fluorides. Convienient new peptide coupling reagents applicable to the FMOC/*tert*-butyl strategy for solution and solid-phase syntheses. *J. Am. Chem. Soc.* 1990; **112**: 9651.
23. Carpino LA, Mansour ESME, Sadat-Aalaee D. *tert*-Butyloxycarbonyl and benzyloxycarbonyl amino acid fluorides. *New, stable rapid-acting acylating agents for peptide synthesis. J. Org. Chem.* 1991; **56**: 2611.
24. Babu VVS, Ananda K, Vasanthakumar GR. (Fluoren-9-ylmethoxy) carbonyl (Fmoc) amino acid azides: Synthesis, isolation, characterisation, stability and application to synthesis of peptides. *J. Chem. Soc., Perkin Trans. 1* 2000; 4328.
25. Carpino LA, Cohen BJ, Stephens KE, Sadat-Aalaee SY, Tien JH, Langridge DC. (Fluoren-9-ylmethoxy)carbonyl (Fmoc) amino acid chlorides. Synthesis, characterization, and application to the rapid synthesis of short peptide segments. *J. Org. Chem.* 1986; **51**: 3732.
26. Jones JH. Analysis, synthesis and biology. In *The Peptides*, Gross J, Meienhofer J (eds). Academic Press: New York, 1979.
27. Meldal M, Juliano MA, Jansson AM. Azido acids in a novel method of solid-phase peptide synthesis. *Tetrahedron Lett.* 1997; **38**: 2531.
28. Basha A, Lipton M, Weinreb SM. A mild, general method for conversion of esters to amides. *Tetrahedron Lett.* 1977; **18**: 4171.

 Copyright © 2008 European Peptide Society and John Wiley & Sons, Ltd.