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Tetrahedron Letters
Tetrahedron Letters 46 (2005) 3319–3322

A new efficient enantioselective synthesis of malonylphenylalanyl and malonylmethylphenylalanyl derivatives suitable for solid phase peptide synthesis

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Received 9 February 2005; revised 15 March 2005; accepted 16 March 2005
Available online 5 April 2005

Abstract—A new synthesis of enantiomerically pure malonylphenylalanyl and malonylmethylphenylalanyl derivatives was developed in which the corresponding prochiral enamides were treated by asymmetric hydrogenation using the Rh(I)-(*S*,*S*)-Me-DuPHOS system. These unnatural amino acids were suitably protected and can be used in solid phase peptide synthesis.

Many of the signalling pathways and regulatory systems in eukaryotic cells are controlled by phosphorylation and dephosphorylation of tyrosine residues in proteins. Phosphotyrosyl residue (pY) and surrounding amino acids serve as high affinity and specific binding sites for intracellular proteins interacting via Src-homology 2 (SH2) or phosphotyrosyl-binding (PTB) domains. Due to the critical function of signalling transduction in normal cell growth, differentiation and activation, dysfunction of these signal pathways can participate in the etiology of human neoplastic diseases.[1] The important role of pY residues in protein tyrosine kinase (PTK)-dependent signal transduction has generated great interest in synthesising stable non-hydrolysable phosphotyrosine mimics in the design of peptide-based signalling transduction inhibitors. As part of our program to develop inhibitors of SH2 domains of Grb family, we required enzymatically stable, non-hydrolysable analogues of phosphotyrosine. In particular we were interested in *para*-malonylphenylalanine (Pmf), which is the most potent non-phosphorus-containing pY mimetic reported against Grb2 SH2 domains.[2] Pmf-containing compounds show potent inhibition (IC_{50} = 8 nM) of Grb2 SH2 domain binding in ELISA assays and almost completely inhibit Met PTK-dependent hepatocyte growth factor-induced cell migration at 3 nM concentration in cell-based assays.[3]

The synthesis of Fmoc-protected Pmf was previously reported by Gao and Burke,[4] using the method of William.[5] This compound has also been synthesised in our laboratory by another pathway, using a camphor sultam as chiral auxiliary (not published). However, such a synthetic pathway is rather lengthy and has a low yield. Recently, it was shown that Rh complexes bearing DuPHOS ligand are extremely effective catalysts in enantioselective hydrogenation of a variety of prochiral unsaturated substrates including enamide esters with an ee up to 99%,[6] providing a new enantioselective route to α-aminoacids. In this letter we report a new short enantioselective synthesis of *para*-malonylphenylalanine (Pmf), *para*-malonylmethylphenylalanine (Pmmf) in which the amino group was protected with a widely used protecting group such as Fmoc, Boc or Cbz (Schemes 1 and 2). Such derivatives can be incorporated into peptides by solid phase synthesis.

As shown in Scheme 1, the differently protected Pmfs were conveniently achieved in a four-step synthesis starting from 4-bromobenzaldehyde diethyl acetal by using the strategy of asymmetric catalytic hydrogenation of the corresponding unsaturated derivatives.

The palladium catalysed cross-coupling reaction[7] of 4-bromobenzaldehyde diethyl acetal with di-*tert*-butyl malonate was performed in anhydrous dioxane at

Keywords: Enantioselective synthesis; Phosphotyrosine mimetics; SH2 domain.
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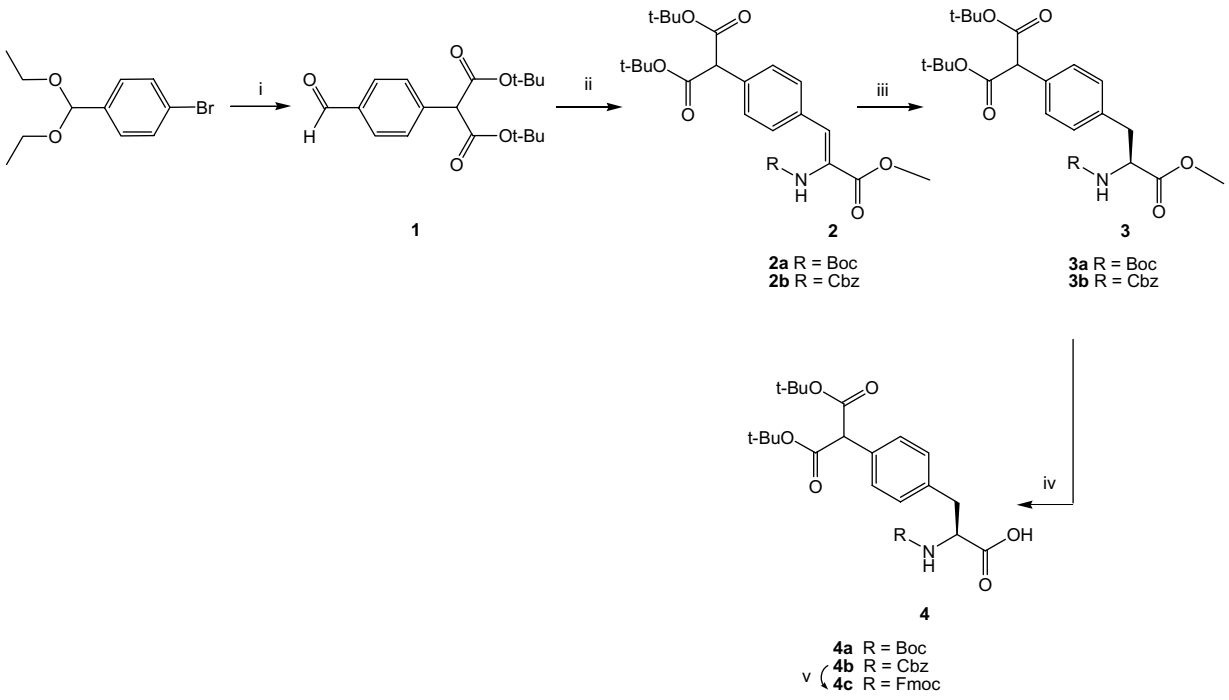
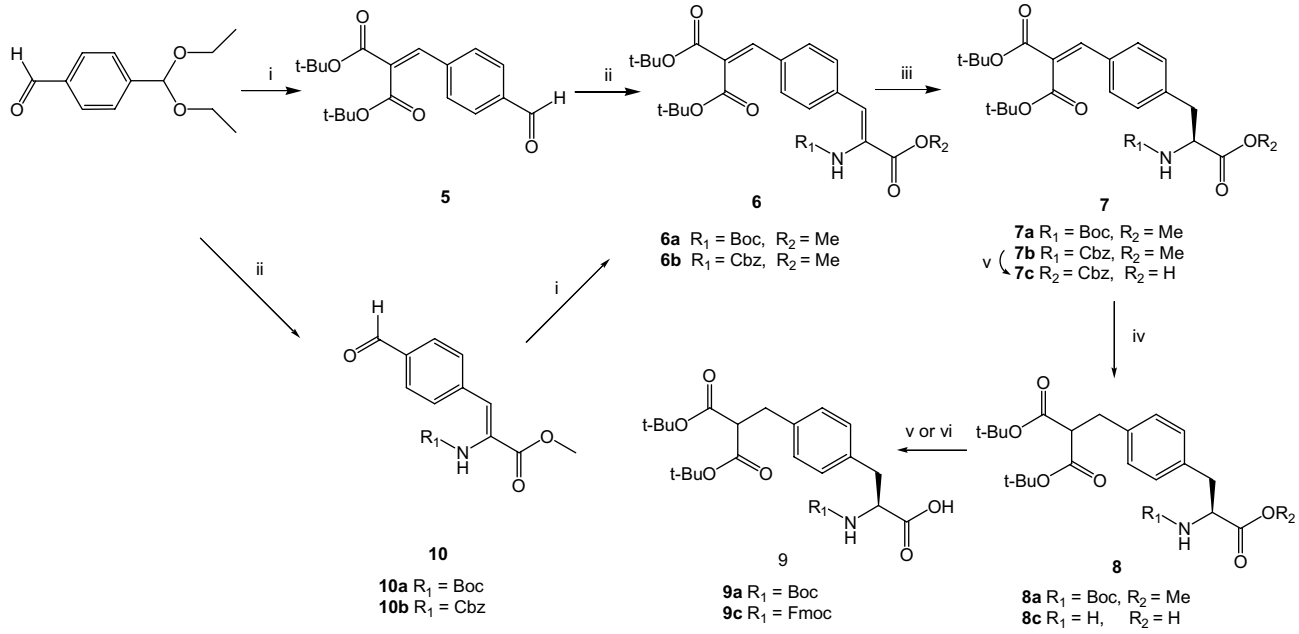
doi:10.1016/j.tetlet.2005.03.105



Scheme 1. Asymmetric synthesis of *N*-protected *p*-malonylphenylalanine. Reagents and conditions: (i) *t*butyl malonate/Pd(dba)$_2$/P(*t*Bu)$_3$/KO*t*Bu; citric acid 10%; (ii) methyl 2-(*N*-Boc-amino)-2-dimethylphosphonylacetate or methyl 2-(*N*-Cbz-amino)-2-dimethylphosphonylacetate/TMG; (iii) H_2 10 atm/Rh(COD)$_2$OTf/(*S*,*S*) Me-DuPhOS; (iv) Na_2CO_3/MeOH; (v) H_2/Pd–C; FmocOSu/Na_2CO_3.



Scheme 2. Asymmetric synthesis of *N*-protected *p*-malonylmethylphenylalaninyl derivative. Reagents and conditions: (i) *t*butyl malonate/$TiCl_4$/pyridine; (ii) methyl 2-(*N*-Boc-amino)-2-dimethylphosphonylacetate or methyl 2-(*N*-Cbz-amino)-2-dimethylphosphonylacetate/TMG; (iii) H_2 10 atm/Rh(COD)$_2$OTf/(*S*,*S*) Me-DuPhOS; (iv) H_2/Pd–C; (v) Na_2CO_3/MeOH; (vi) FmocOSu/Na_2CO_3.

80 °C for 6 h, using P(*t*Bu)$_3$ as ligand and KO*t*Bu as base.

Aldehyde **1** was obtained in 75% yield, after treatment with 10% citric acid. A Horner–Emmons (HE) type olefination of the resulting aldehyde **1** in anhydrous THF proceeded smoothly with methyl 2-(*N*-Boc-amino)-2-dimethylphosphonylacetate or methyl 2-(*N*-Cbz-amino)-2-dimethylphosphonylacetate using tetramethylguanidine (TMG) as base at −78 °C, followed by warming up to ambient temperature overnight. The (*Z*)-enamido esters **2a**–**b** were formed in yields of 76%

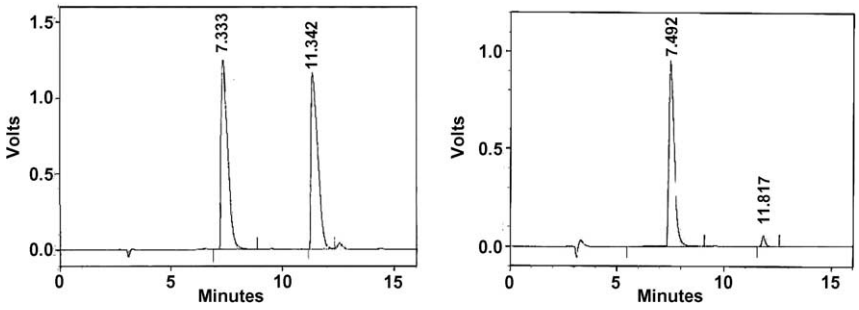


Figure 1. Reverse phase HPLC chromatograms of crude dipeptides **13** (left) and **14** (right), using a linear gradient: 10–25% B/15 min (A: 0.1% TFA in water; B: 70% acetonitrile in aqueous solution with 0.09% TFA) on a Vydac C-18 column (5 μm, 4.6 × 250 mm) at the flow 1 mL/min.

and 79%, respectively. The configuration was based on the fact that didehydroamino acid derivatives with an *E*-configuration have been reported to display NOE effects between the olefinic CH and NH protons.[8] No such effects were observed for **2a**–**b**. Asymmetric hydrogenation of **2a**–**b** using Burk's catalytic Rh(I)-(*S*,*S*)-Me-DuPHOS system[6] (0.1 mol %) in deoxygenated MeOH under reaction conditions of 10 atm H_2 at 25 °C for 24 h afforded the fully protected L-Pmf derivatives **3a**–**b** in 94% and 96% yields, respectively. The absolute configurations were assigned as S based on the selectivity of the (*S*,*S*)-Me-DuPHOS ligand.[6,9] The synthesis of **4a** was initially attempted by standard saponification procedures, but was unsuccessful due to the concomitant partial deprotection of the malonyl *tert*-butyl ester. Thus in order to avoid such a hydrolysis, compounds **3a**–**b** were treated in mild conditions (Na_2CO_3/MeOH, 25 °C, 16 h) to provide the desired acids **4a**–**b**[10,11] in yields of 86% and 89%. Finally, Pd-catalysed hydrogenolytic removal of Cbz in **4b** in MeOH followed by treatment with *N*-Fmoc succinimide (Fmoc-OSu) in dioxane–water (1:1) using Na_2CO_3 as base afforded the N-Fmoc form **4c**,[12] in yield of 93% by two steps.

The new Pmmf derivatives were prepared following a similar synthetic pathway which is outlined in Scheme 2.

The commercially available 4-(diethoxymethyl)-benzaldehyde was converted into α,β-unsaturated di-*tert*-butyl malonate **5** by a mild Knoevenagel condensation with 1 equiv of di-*tert*-butyl malonate in the presence of 1 equiv of $TiCl_4$ and 1 equiv of pyridine in anhydrous THF at 0 °C, followed by warming up to ambient temperature for 4 h in yield of 82%. A HE olefination of aldehyde group in **5** with methyl 2-(*N*-Boc-amino)-2-dimethylphosphonylacetate or methyl 2-(*N*-Cbz-amino)-2-dimethylphosphonylacetate in anhydrous THF, using TMG as base gave (*Z*)-enamido esters **6a**–**b** in yields of 80% and 75%, respectively. Alternatively, these compounds could be obtained by a HE olefination of 4-(diethoxymethyl)benzaldehyde, followed by Knoevenagel condensation. Subsequent generation of **8a** was easily achieved by hydrogenation of **6a** in the presence of Rh(I)-(*S*,*S*)-Me-DuPHOS catalyst (0.1 mol %) and by Pd-catalysed hydrogenation in yield of 93%. The carboxylic methyl ester **8a** was saponified in mild conditions (Na_2CO_3/MeOH) to give the corresponding acid **9a**[13] in yield of 87%. Hydrogenation of **6b** in the presence of Rh(I)-(*S*,*S*)-Me-DuPHOS catalyst (0.1 mol %) afforded **7b**, which was subsequently saponified in MeOH, using Na_2CO_3 as base to generate the acid **7c**[14] with a yield of 83% by two steps. The latter was finally hydrogenated on palladium and protected using Fmoc-OSu to give the Fmoc derivative **9c**[15] in yield of 92%.

In order to verify the enantiomeric purity of Pmf and Pmmf, dipeptides H-(D,L)-Pmf-(L)-Ala-OH **11** and H-(L)-Pmf-(L)-Ala-OH **12** or H-(D,L)-Pmmf-(L)-Ala-OH **13** and H-(L)-Pmmf-(L)-Ala-OH **14** were prepared by DCC, HOBt, NMP coupling and TFA deprotection, using *tert*-butyl alaninate with Boc-(L)-Pmf **4a** or Boc-(L)-Pmmf **9a** or their racemic mixtures. The latter compounds Boc-(D,L)-Pmf and Boc-(D,L)-Pmmf were obtained by treatment of **2a** or **6a** in MeOH using Pd-catalysed hydrogenation followed by mild hydrolysis. Comparison of HPLC profiles of **13** and **14** (Fig. 1) showed a very high enantiomeric purity (>96%). This was also observed by 1H NMR spectroscopy (DMSO-*d*6). Thus, in **13**, the NH (Ala) gave two doublets at 8.82 and 8.65 ppm, and the CH3β (Ala) group two doublets at 1.33 ppm and 1.13 ppm. In **14**, the NH (Ala) appeared as a single peak at 8.82 ppm and the CH_3β (Ala) group led to a doublet at 1.33 ppm. Finally, the similar results were also observed for the peptides **11** and **12**.

In conclusion, we have developed a very rapid synthesis of optically pure malonylphenylalanyl and malonylmethylphenylalanyl derivatives. The applicability of these unnatural amino acids to solid phase peptide synthesis is now in progress in our laboratory.

Acknowledgements

We thank the Ligue Nationale contre le Cancer (Comité de Paris) for financial support.

References and notes

1. Edwarss, D. R. *Trends Pharmacol. Sci.* **1994**, *15*, 239–244.
2. Gao, Y.; Luo, J.; Yao, Z. J.; Zou, H.; Kelly, J.; Voigt, J. H.; Yang, D.; Burke, T. R., Jr. *J. Med. Chem.* **2000**, *43*, 911–920.
3. Atabey, N.; Yao, Z. J.; Breckenridge, D.; Soon, L.; Soriano, J. V.; Burke, T. R.; Bottaro, D. P., Jr. *J. Biol. Chem.* **2001**, *276*, 14308–14314.

4. Gao, Y.; Burke, T. R., Jr. *Synlett* **2000**, *1*, 134–136.
5. William, R. M.; Im, M. N. *J. Am. Chem. Soc.* **1991**, *113*, 9276–9286.
6. Burk, M. J.; Feaster, J. E.; Nugent, W. A.; Harlow, R. L. *J. Am. Chem. Soc.* **1993**, *115*, 10125–10138.
7. Beare, N. A.; Hartwig, J. F. *J. Org. Chem.* **2002**, *67*, 541–555.
8. Shimohigashi, Y.; Nitz, T. J.; Stammer, C. H.; Inubushi, T. *Tetrahedron Lett.* **1982**, *23*, 3235–3323.
9. RajanBabu, T. V.; Radetich, B.; You, K. K.; Ayers, T. A.; Casalnuovo, A. L. *J. Org. Chem.* **1999**, *64*, 3429–3447.
10. Compound **4a**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.41 (s, 27H, $3\times t$Bu), 2.78–3.10 (m, 2H, $CH_2\beta$), 4.41 (s, 1H, $CH(CO_2tBu)_2$), 4.52 (t, 1H, CHα), 5.34 (s, 1H, NH), 7.13 (s, 4H, aromatic). MS(ESI): 502.4 $(M+Na)^+$, 518.5 $(M+K)^+$. $[\alpha]_D^{20}$ +13.6 (*c* 1, $CHCl_3$).
11. Compound **4b**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.49 (s, 18H, $2\times t$Bu), 3.18 (d, 2H, $CH_2\beta$), 4.47 (s, 1H, $CH(CO_2tBu)_2$), 4.72 (t, 1H, CHα), 5.15 (s, 2H, CH_2Ph), 5.44 (s, 1H, NH), 7.18–7.57 (m, 9H, aromatic). MS(ESI): 536.6 $(M+Na)^+$, 552.5 $(M+K)^+$. $[\alpha]_D^{20}$ +17.7 (*c* 1, $CHCl_3$).
12. Compound **4c**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.49 (s, 18H, $2\times t$Bu), 3.20 (d, 2H, $CH_2\beta$), 4.10–4.55 (m, 4H, CH_2–CH, $CH(CO_2tBu)_2$), 4.72 (t, 1H, CHα), 5.25 (s, 1H, NH), 7.16–7.81 (m, 12H, aromatic). HRMS(ESI): calcd for $C_{35}H_{39}NO_8Na$: 624.2573 $(M+Na)^+$. Found: 624.2581. $[\alpha]_D^{20}$ +24.2 (*c* 1, $CHCl_3$).
13. Compound **9a**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.42 (s, 27H, $3\times t$Bu), 3.0–3.3 (m, 4H, $CH_2\beta$, CH_2), 3.52 (t, 1H, $CH(CO_2tBu)_2$), 4.42 (m, 1H, CHα), 5.06 (s, 1H, NH), 7.13 (s, 4H, aromatic). MS(ESI): 516.6 $(M+Na)^+$, 532.6 $(M+K)^+$. $[\alpha]_D^{20}$ 11.9 (*c* 1, $CHCl_3$).
14. Compound **7c**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.54 (s, 18H, $2\times t$Bu), 3.22 (m, 2H, $CH_2\beta$), 4.70 (m, 1H, CHα); 5.12 (s, 2H, CH_2Ph), 5.25 (s, 1H, NH), 7.1–7.4 (m, 9H, aromatic), 7.53 (s, 1H, =CH). MS(ESI): 548.6 $(M+Na)^+$, 564.5 $(M+K)^+$. $[\alpha]_D^{20}$ +26.5 (*c* 1, $CHCl_3$).
15. Compound **9c**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.42 (s, 18H, $2\times t$Bu), 3.14–3.44 (m, 4H, $CH_2\beta$, CH_2), 3.50 (s, 1H, $CH(CO_2tBu)_2$), 4.21–4.46 (m, 3H, CH_2–CH), 4.68 (t, 1H, CHα), 5.20 (s, 1H, NH), 7.16–7.81 (m, 12H, aromatic). HRMS(ESI): calcd for $C_{36}H_{41}NO_8Na$: 638.2730 $(M+Na)^+$. Found: 638.2929. $[\alpha]_D^{20}$ +15.2 (*c* 1, $CHCl_3$).