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A one-step synthesis of N^{α}-Fmoc-4-*O*-[*O*′,*O*″-di-*tert*-butyl-2-(2-fluoromalonyl)]-L-tyrosine from commercially available starting material

Sang Uk Kang and Terrence R. Burke, Jr.*

Laboratory of Medicinal Chemistry, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Frederick, MD 21702, USA

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Abstract—A one-step high yield synthesis from commercially available starting material is reported for the novel phosphotyrosyl mimetic, N^{α}-Fmoc-4-*O*-[*O*′,*O*″-di-*tert*-butyl-2-(2-fluoromalonyl)]-L-tyrosine. The conditions employed for this transformation may also be applicable for the direct electrophilic fluorination of other N^{α}-Fmoc-protected amino acids.
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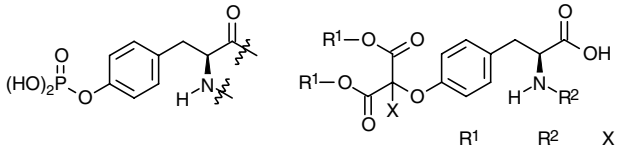
1. Introduction

Central roles played by phosphotyrosyl (pTyr) residues (**1**) in signal transduction associated with diseases such as diabetes and cancers, have made the development of pTyr mimetics an important area of study.[1–3] Among a large number of analogues reported to date, *O*-malonyl-tyrosine[4] (OMT, **2**, Fig. 1) has found application in signalling antagonists directed against Src homology 2 (SH2) domains,[5–8] phosphotyrosine binding domains (PTBs)[9] and more particularly, against protein–tyrosine phosphatases (PTPs).[10–17] In further work based on molecular modelling studies, the corresponding fluoro-*O*-malonyl-tyrosine (FOMT, **3**) was prepared and shown to exhibit 10-fold higher affinity than OMT when incorporated in place of pTyr in a PTP 1B-directed substrate peptide.[18] Subsequently, the X-ray crystal structure of a low-nanomolar affinity FOMT-containing peptide[19] bound to the PTP 1B protein clarified the manner in which the FOMT residue interacts within the PTP catalytic site.[20] More recent uses of FOMT in peptides directed against other PTPs, including the *Yersinia pestis* YopH[16] and purple acid phosphatases,[17] have continued to validate the utility of FOMT. However, unlike OMT, which is commercially avialable in its N^{α}-Fmoc-4-*O*-(*O*′,*O*″-di-*tert*-butyl)-L-tyrosine form [N^{α}-Fmoc (^{t}BuO)$_2$-OMT-OH, **4**],[21] to date the corresponding protected FOMT analogue, N^{α}-Fmoc-4-*O*-[*O*′,*O*″-di-*tert*-butyl-2-(2-fluoromalonyl)]-L-tyrosine, [N^{α}-Fmoc (^{t}BuO)$_2$-FOMT-OH, **5**] has been available only by multi-step synthesis.[18] In light of this, work reported herein was undertaken to effect a direct conversion of commercially available protected-OMT **4** to the desired FOMT analogue **5**.



		R1	R2	X
1 pTyr Residue				
2	OMT	H	H	H
3	FOMT	H	H	F
4		^{t}Bu	Fmoc	H
5		^{t}Bu	Fmoc	F

Figure 1. Structures of pTyr and malonyl-based mimetics.

The original preparation of **5** employed electrophilic fluorination of the N^{α}-Fmoc (^{t}BuO)$_2$-OMT methyl ester **6**, followed by LiOH-catalyzed hydrolysis of the resulting **8**. This procedure was noteworthy by its maintenance of base-labile N^{α}-Fmoc-protection in the presence of two strongly alkaline reagents.[18] The choice

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* Corresponding author. Tel.: +1 301 846 5906; fax: +1 301 846 6033; e-mail: tburke@helix.nih.gov

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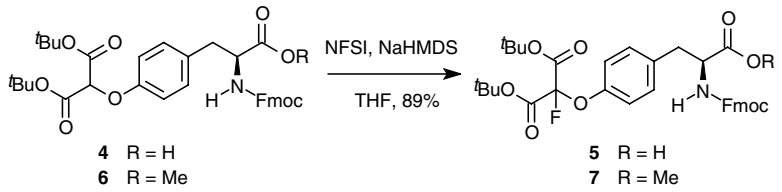
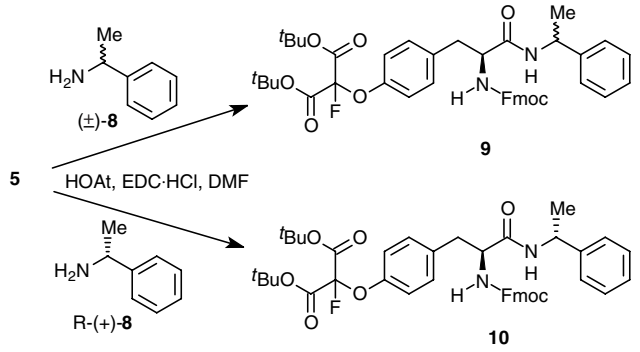


Scheme 1. Approaches towards electrophilic flourination leading to FOMT analogues.

of methyl ester **6** as the fluorination substrate was predicated on its availability as an intermediate in the synthesis of N^{α}-Fmoc (^{t}BuO)$_2$-OMT-OH (**4**). A similar approach starting from commercially available **4** would require a three-step protocol of esterification to **6** followed by electrophilic flourination to **7**, then demethylation to **5** (Scheme 1). Given the cost of starting **4** (approximately \$1800/g),[21] circumvention of such a multi-step scenario by elimination of the carboxylic acid protection/deprotection would be desirable. Indeed, it was found that treatment of **4** with *N*-fluorobenzenesulfonimide (NFSI) in the presence of slightly greater than 2 equiv of sodium bis(trimethylsilyl)amide (NaHMDS) at carefully controlled temperature, followed by quenching with 1 N HCl provided the desired **5** cleanly in 89% (Scheme 1).

The enantiomeric purity of **5** was determined by coupling with (±)-α-methylbenzylamine [(±)-**8**] and with *R*-(+)-α-methylbenzylamine [(±)-**8**] to yield the corresponding diastereomeric amides **9** and **10**, respectively (Scheme 2). Comparison of ^{1}H NMR of **9** and **10** in $CDCl_3$ indicated a clearly resolved multiplet in **9** at δ 7.03–6.98 corresponding to the five benzylamide five aryl protons of the (*S*,*S*) diastereomer, which were absent in **10**. In benzene-d_6 better resolution was achieved for several diastereomeric protons, including those arising from certain aryl protons, the amide proton and the methyl protons. Within the limits of detection, no enantiomeric contamination of **5** was evidient.

The facile and direct conversion of **4** to **5** makes N^{α}-Fmoc (^{t}BuO)$_2$-FOMT-OH readily available for use in signal transduction-related studies. Of equal importance, the conditions reported herein may also be applicable for the direct electrophilic fluorination of other N^{α}-Fmoc-protected amino acids.



Scheme 2. Determination of enantiomeric purity.

2. Experimental

2.1. Synthesis of N^{α}-Fmoc (^{t}BuO)$_2$-FOMT-OH (5)

To a stirred solution of N^{α}-Fmoc (^{t}BuO)$_2$-OMT-OH[21] (500 mg, 0.81 mmol) in anhydrous THF (10 mL) at −78 °C under argon was added NaHMDS (1.7 mL, 1.70 mmol) dropwise. After stirring at −78 °C (15 min) a solution of NFSI (307 mg, 0.97 mmol) in THF (5 mL) was added dropwise and the mixture was stirred at −78 °C (1 h). The reaction was quenched (2.5 mL of 1 N HCl at −78 °C) then subjected to extraction (H_2O/EtOAc). The organic extract was dried ($MgSO_4$) and purified by silica gel flash column chromatography (CH_2Cl_2:MeOH, 20:1 followed by CH_2Cl_2:MeOH, 10:1 then CH_2Cl_2:MeOH, 5:1) to provide N^{α}-Fmoc (^{t}BuO)$_2$-FOMT-OH (460 mg, 89% yield) as an oil.[22] ^{1}H NMR ($CDCl_3$) δ 8.92 (1H, br s), 7.75 (2H, d, J = 7.4 Hz), 7.54 (2H, d, J = 7.2 Hz), 7.39 (2H, t, J = 7.2 Hz), 7.30 (2H, t, J = 7.3 Hz), 7.11 (2H, d, J = 8.0 Hz), 7.04 (2H, d, J = 8.2 Hz), 5.21 (1H, d, J = 7.4 Hz), 4.62 (m, 1H), 4.48–4.35 (2H, m), 4.18 (1H, t, J = 6.4 Hz), 3.17–3.02 (2H, m), 1.39 (18H, s). ^{13}C NMR (400 MHz, $CDCl_3$) δ 175.6, 161.4, 161.0, 155.8, 152.7, 143.6. 141.3, 132.3, 130.3, 127.8, 127.1, 125.0, 120.2, 120.0, 105.2, 102.8, 84.6, 67.0, 54.6, 47.1, 37.5, 27.5.

2.2. Enantiomeric purity determination for 5: synthesis of amides 9 and 10

To a solution of **5** (30 mg, 0.047 mmol) in anhydrous DMF (0.4 mL) was added EDCI·HCl (8 mg, 0.061 mmol) followed by HOAt (0.5 M in DMF, 0.1 mL, 0.047 mmol). The mixture was stirred for 10 min at room temperature, then either (±)-α-methylbenzylamine [(±)-**8**] or *R*-(+)-α-methylbenzylamine [(+)-**8**] (8 mg, 0.066 mmol) was added dropwise. The mixture was stirred overnight then purified directly by column chromatography to yield the corresponding amides **9** (7.9 mg, 23% yield) and **10** (6.4 mg, 18% yield).

For **9**: FABMS *m*/*z* 739 (MH^+); ^{1}H NMR ($CDCl_3$, a mixture of two diasteromers) δ 7. 76 (4H, d, J = 7.0 Hz, overlapping), 7.54 (4H, m, overlapping), 7.40 (4H, t, J = 7.4 Hz, overlapping), 7.33–7.21 (10H, m, overlapping), 7.17–7.12 (7H, m, overlapping), 7.03–6.98 (5H, m, (*S*,*S*)), 5.81 (1H, br s), 5.76 (1H, br s), 5.35 (2H, br s, overlapping), 4.99 (2H, m, overlapping), 4.41 (4H, m, overlapping), 4.29 (2H, m, overlapping), 4.18 (2H, m, overlapping), 3.10–2.92 (4H, m, overlapping), 1.41 (39H, m), 1.30 (3H, broad doublet, J = 5.1 Hz (*S*, *R*)).

^{1}H NMR (C_6D_6, a mixture of two diasteromers) δ 7.49 (4H, d, J = 7.4 Hz, overlapping), 7.34 (4H, m, overlapping), 7.17–6.87 (22H, m, overlapping), 6.73 (2H, broad doublet, (S,R)), 6.61 (broad doublet, (S,S)), 5.53 (2H, broad m, overlapping), 5.27 (1H, broad doublet, (FmocN*H*, S,S)), 5.09 1H, broad doublet, (FmocN*H*, (S,R)), 4.98 (2H, m, overlapping), 4.33–4.15 (6H, m, overlapping), 3.90 (2H, m, overlapping), 2.75–2.54 (4H, m, overlapping), 1.17 (36H, s, overlapping), 1.01 (3H, d, J = 6.6 Hz, (S,S)), 0.97 (3H, d, J = 6.8 Hz, (S,R)).

For **10**: FABMS m/z 739 (MH^+); ^{1}H NMR ($CDCl_3$, (S,R)) δ 7.76 (2H, d, J = 7.3 Hz), 7.54 (2H, d, J = 7.3 Hz), 7.40 (2H, t, J = 7.3 Hz), 7.33–7.21 (6H, m), 7.17–7.12 (5H, m), 5.76 (1H, br s), 5.33 (1H, br s), 4.98 (1H, m), 4.39 (2H, m), 4.28 (1H, m), 4.18 (1H, t, J = 6.3 Hz), 3.10 (1H, m), 2.94 (1H, m), 1.42 (19H, s), 1.30 (3H, d, J = 6.1 Hz).

^{1}H NMR (C_6D_6) δ 7.49 (2H, d, J = 7.4 Hz), 7.32 (2H, m), 7.17–6.88 (11H, m), 6.72 (2H, broad doublet), 5.45 (1 H, br s), 4.99 (2H, m), 4.28 (2H, broad doublet), 4.12 (1H, m), 3.88 (1 H, broad triplet), 2.75 (1H, m), 2.63 (1H, m), 1.18 (9H, s), 1.17 (9 H, s), 0.97 (3H, d, J = 6.8 Hz).

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