Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 3943–3946

New syntheses of tetrazolylmethylphenylalanine and *O*-malonyltyrosine as pTyr mimetics for the design of STAT3 dimerization inhibitors

Jennifer Dourlat, Bruno Valentin, Wang-Qing Liu and Christiane Garbay*

Université Paris Descartes, UFR Biomédicale, Laboratoire de Pharmacochimie Moléculaire et Cellulaire, Paris F-75006, France
INSERM, U648, Paris F-75006, France

Received 11 April 2007; revised 26 April 2007; accepted 28 April 2007
Available online 3 May 2007

Abstract—Investigation within the pTyr-binding pocket of the STAT3 SH2 domain led us to develop a novel synthesis of two pTyr mimetics, L-tetrazolylmethylphenylalanine (L-Tmp) and L-*O*-malonyltyrosine (L-OMT), that were next incorporated in a high affinity ligand of STAT3 SH2 domain. Biological evaluation of peptidomimetics on STAT3 dimerization identified L-OMT as the first non-phosphorus pTyr mimetic so far reported against STAT3 SH2 domain, harboring an activity similar to that of the Pmp-containing reference peptidomimetic.

Signal Transducer and Activator of Transcription 3 is a latent cytoplasmic transcription factor that plays a key role in cancer, by regulating as a dimer the expression of anti-apoptotic or pro-survival genes.[1] In response to extracellular stimuli, STAT3 is recruited to phosphorylated receptors via its SH2 domain. It then becomes phosphorylated on Tyr^{705} by kinases such as JAK or Src, or as a result of the intrinsic tyrosine kinase activity of the receptor. STAT3 then dimerizes through reciprocal interaction between the SH2 (Src Homology 2) domain of one monomer and the pTyr residue of a second one.

To design small inhibitors of STAT3 dimerization, a powerful strategy consists in using phosphotyrosine-based peptidomimetics targeted to the SH2 domain of STAT3.[2]

Although a pTyr residue is essential in recognition and potent binding by SH2 domain, its hydrolytic lability to cellular phosphatases and poor cell penetration due to is dianionic charge limit its use for inhibitor design.

Accordingly, pTyr mimetics have been developed to address these drawbacks while retaining recognition within the pTyr-binding pocket.[3,4] These include phosphonic or acidic non-phosphorus-based phenylalanine compounds. While in vivo efficiency of phosphonate derivatives seems to depend on the mode of cell delivery, the latter compounds may offer alternative approaches by facilitating cell penetration.[5]

Among them, carboxylic groups or its more lipophilic bioisostere tetrazole have been used to mimic acidic phosphate group.[6–8]

Although phosphopeptidomimetic inhibitors of STAT3 dimerization have been reported,[9–11] to date, only one study examines the use of non-hydrolyzable pTyr mimetics.[9] In this paper, pTyr moiety was replaced either by L-4-(phosphonomethyl)phenylalanine (Pmp), L-4-(carboxyl)phenylalanine (Cpa) or L-4-(tetrazol-5-yl)-phenylalanine (Tpa) in a high affinity ligand of the STAT3 SH2 domain, Ac-pYLPQTV.[9] Pmp was found to be the most potent mimetic, although being 40-fold less active than its phosphate analog.

We surmised that the loss of activity of Cpa and Tpa peptides relative to their pTyr analog was due to their shorter acidic side chains and thus decided to synthesize pTyr mimetics bearing longer side chains. This paper describes the novel synthesis of two pTyr mimetics, L-4-((tetrazol-5-yl)methyl)phenylalanine (Tmp) and L-(*O*-malonyl)tyrosine (OMT) and their further biological

Keywords: STAT3; pTyr mimetics; Inhibitors; SH2 domain; Cancer.
* Corresponding author. Tel.: +33 142 864 080; fax: +33 142 864 082; e-mail: christiane.garbay@univ-paris5.fr

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.04.107

evaluation on STAT3 dimerization, when incorporated in the Ac-pYLPQTV sequence.

Tilley et al. previously reported a synthesis of racemic Tmp **5**, that is not well suited for our peptidomimetic design, since it leads to two diastereoisomers.[12]

Therefore, we developed an enantioselective synthesis of Nα-protected L-tetrazolylmethylphenylalanine **5**, based on a procedure developed by Oppolzer, which allows the obtention of α-amino acids with excellent enantiomeric purities (>99.5% e.e.) (Scheme 1).[13] Chiral auxiliary **1** was alkylated by 4-(cyanomethyl)-benzylbromide in the presence of BuLi to afford compound **2** in 60% yield. Imine function was then hydrolyzed by treatment with 0.5 N HCl in THF, followed by N-acetylation with acetic anhydride to give compound **3** in 95% yield.

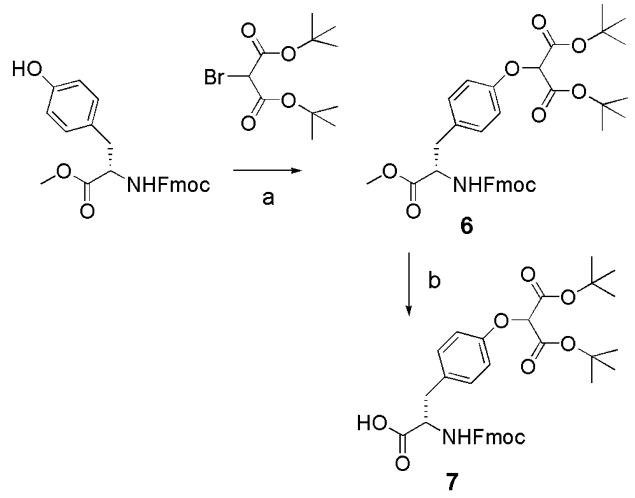
Removal of the sultam group was achieved by hydrolysis with ice-cold 0.2 N LiOH in THF to provide acetyl-L-4-(cyanomethyl)-phenylalanine **4** in 98% yield.

The nitrile group was finally converted to the corresponding tetrazolyl function, by reaction with azidotrimethylstannane in dry toluene under reflux to give final compound **5** in 40% yield.[14]

The synthesis of L-*O*-(2-malonyl)tyrosine (OMT) **7**, suitably protected for solid-phase peptide synthesis with Fmoc chemistry, was first reported by Burke et al. with the use of di-*tert*-butyl diazomalonate, which is a toxic and potentially explosive derivative.[15,16]

We describe here a shorter and safer synthesis using di-*tert*-butyl bromomalonate (Scheme 2).

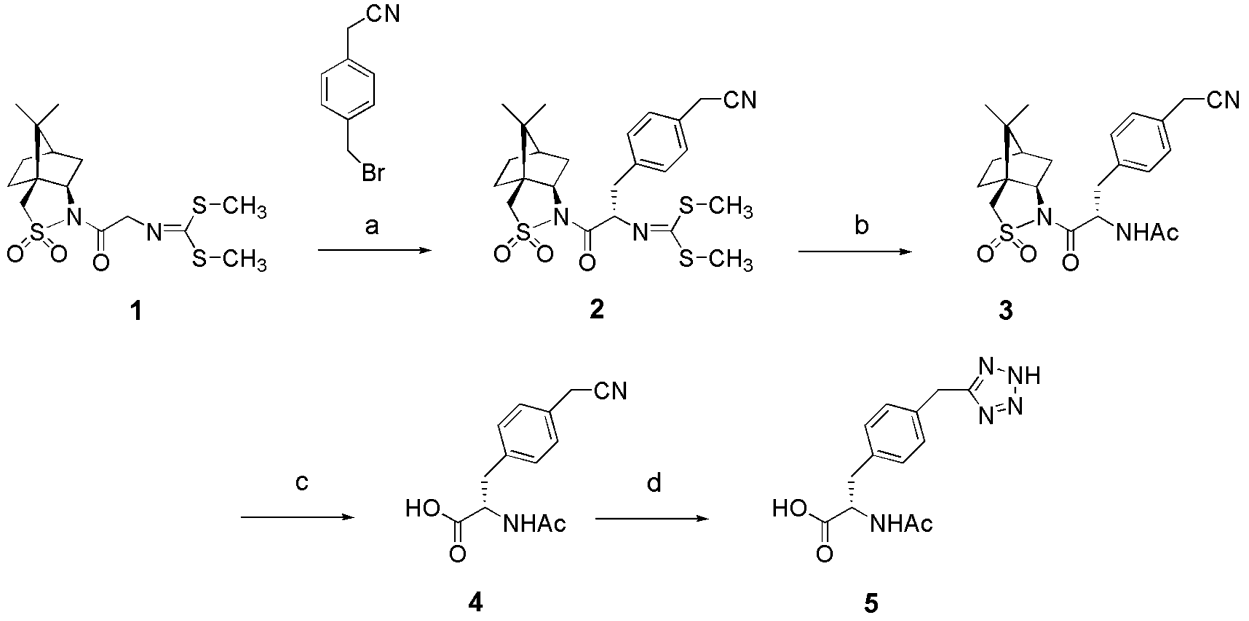
Fmoc-L-Tyr-OMe was alkylated by di-*tert*-butyl bromomalonate[17,18] in the presence of NaH to give the malonyl adduct **6** in 70% yield.[19,20] Selective methyl ester hydrolysis was then accomplished using ice-cold 0.2 N LiOH in THF, without removal of the base-labile Fmoc group, to give final compound **7** in 98% yield.[15]



Scheme 2. Reagents and conditions: (a) NaH, THF, −40 °C to rt; (b) LiOH 0.2 N, THF, 0 °C.

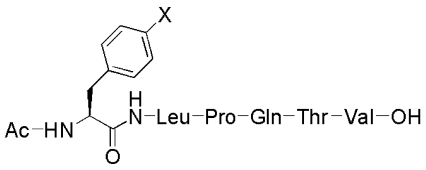
Structural data of final compounds **5** and **7** are given in note 21.

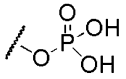
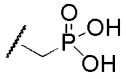
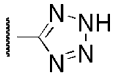
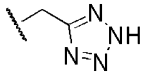
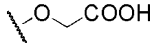
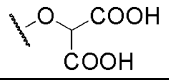
To investigate the structural requirements within the pTyr-binding pocket, three other pTyr mimetics, including Tpa, Pmp, and L-*O*-(carboxymethyl)phenylalanine,[14,23,24] were also prepared and introduced in the Ac-pYLPQTV sequence (Table 1). Peptide synthesis was performed on solid phase on HMP resin by Fmoc chemistry.[22]



Scheme 1. Reagents and conditions: (a) BuLi 2.5 M in hexane, HMPA, THF, −78 °C to rt; (b) i—0.5 N HCl in THF, rt; ii—acetic anhydride; (c) LiOH 0.2 N, THF, 0 °C; (d) Me_3SnN_3, toluene, reflux.

Table 1. Disruption of STAT3 dimerization and DNA-binding by peptides



Compound	X:	IC_{50}[a] (μM)
8[b]		9 ± 0.6
9[b]		138 ± 29
10[b]		>800
11		>1000
12		>1000
13		108 ± 21

[a] IC_{50} values expressed with standard error.
[b] See Ref. 9.

In vitro evaluation on STAT3 dimerization was carried out through pre-incubation of peptides with nuclear extracts containing STAT3 dimer for 30 min at room temperature, before incubation in an ELISA plate pre-coated with oligonucleotides. This test assesses the ability of compounds to inhibit STAT3 DNA-binding. It thus indirectly reflects their ability to inhibit STAT3 dimerization. Results are reported in Table 1.

These data indicate that substituting the phosphate group with either anionic tetrazole groups (peptides **10** and **11**) or a monocarboxylic group (peptide **12**) strongly impairs inhibition. These results agree with those obtained by McMurray et al. for Tpa and Cpa[9] and suggest that a monoacidic moiety may be not sufficient for STAT3 SH2 binding, regardless of acid chain length.

In fact, it is well established that STAT3-pTyr pocket is mainly constituted by the side chains of the two basic residues Lys591 and Arg609, as well as by those of Ser 611 and Ser 613.[25] It is thus conceivable that one additional negative charge may be required for effective binding.

Indeed, introduction of an additional carboxyl group in the geminal position of peptide **12** led to a compound exhibiting moderate activity (IC_{50} = 108 μM for peptide **13**), which is slightly better than that of the Pmp-containing reference peptidomimetic **9** (IC_{50} = 138 μM).

Noteworthy, **9** was found in our ELISA only 17-fold less potent than its phosphate analog **8**. This result differs from previous experiments by McMurray et al., where it was found 40-fold less active in an EMSA (Electrophoretic mobility shift assay).[9]

Thus, our results strongly suggest that a diacidic function is required for efficient binding to the STAT3-SH2 domain, as is the case for malonate and phosphonate groups.

In fact, numerous pTyr mimetics have been described in the literature, but their potencies depend on the protein type.[3,4]

For instance, Pmp was found to be the most potent mimetic against Grb2 SH2 domain,[26] while *O*-malonate was found more potent against Src and SH-PTP2 SH2 domains.[15] In the case of the STAT3 SH2 domain, our data revealed that both anionic functions could be used as phosphate alternatives for the future design of non-peptidic STAT3 dimerization inhibitors.

In conclusion, our work has resulted in the novel syntheses of two pTyr mimetics, namely L-4-((tetrazol-5-yl)methyl)phenylalanine (L-Tmp) and L-*O*-malonyltyrosine (L-OMT). More importantly, their incorporation into a high affinity ligand yielded OMT as the first non-phosphorus-containing pTyr mimetic so far reported against the STAT3 SH2 domain.

Acknowledgments

This work was supported by the 'Ligue Nationale contre le Cancer', Equipe Labellisée 2006. Jennifer Dourlat benefited from a grant by the 'Ligue Nationale contre le Cancer'. We thank Dr. N. Gresh for manuscript reading.

References and notes

1. Yu, H.; Jove, R. *Nat. Rev. Cancer* **2004**, *4*, 97.
2. Turkson, J.; Ryan, D.; Kim, J. S.; Zhang, Y.; Chen, Z.; Haura, E.; Laudano, A.; Sebti, S.; Hamilton, A. D.; Jove, R. *J. Biol. Chem.* **2001**, *276*, 45443.
3. Burke, T. R., Jr.; Yao, Z. J.; Liu, D. G.; Voigt, J.; Gao, Y. *Biopolymers* **2001**, *60*, 32.
4. Burke, T. R., Jr.; Lee, K. *Acc. Chem. Res.* **2003**, *36*, 426.
5. Yao, Z. J.; King, C. R.; Cao, T.; Kelley, J.; Milne, G. W.; Voigt, J. H.; Burke, T. R., Jr. *J. Med. Chem.* **1999**, *42*, 25.
6. Herr, R. J. *Bioorg. Med. Chem.* **2002**, *10*, 3379.
7. Burke, T. R., Jr.; Luo, J.; Yao, Z. J.; Gao, Y.; Zhao, H.; Milne, G. W.; Guo, R.; Voigt, J. H.; King, C. R.; Yang, D. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 347.

8. Beaulieu, P. L.; Cameron, D. R.; Ferland, J. M.; Gauthier, J.; Ghiro, E.; Gillard, J.; Gorys, V.; Poirier, M.; Rancourt, J.; Wernic, D.; Llinas-Brunet, M.; Betageri, R.; Cardozo, M.; Hickey, E. R.; Ingraham, R.; Jakes, S.; Kabcenell, A.; Kirrane, T.; Lukas, S.; Patel, U.; Proudfoot, J.; Sharma, R.; Tong, L.; Moss, N. *J. Med. Chem.* **1999**, *42*, 1757.
9. Ren, Z.; Cabell, L. A.; Schaefer, T. S.; McMurray, J. S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 633.
10. Turkson, J.; Kim, J. S.; Zhang, S.; Yuan, J.; Huang, M.; Glenn, M.; Haura, E.; Sebti, S.; Hamilton, A. D.; Jove, R. *Mol. Cancer Ther.* **2004**, *3*, 261.
11. Coleman, D. R. T.; Ren, Z.; Mandal, P. K.; Cameron, A. G.; Dyer, G. A.; Muranjan, S.; Campbell, M.; Chen, X.; McMurray, J. S. *J. Med. Chem.* **2005**, *48*, 6661.
12. Tilley, J. W.; Danho, W.; Lovey, K.; Wagner, R.; Swistok, J.; Makofske, R.; Michalewsky, J.; Triscari, J.; Nelson, D.; Weatherford, S. *J. Med. Chem.* **1991**, *34*, 1125.
13. Oppolzer, W.; Moretti, R.; Thomi, S. *Tetrahedron Lett.* **1989**, *30*, 6009.
14. McMurray, J. S.; Khabashesku, O.; Birtwistle, J. S.; Wang, W. *Tetrahedron Lett.* **2000**, *41*, 6555.
15. Ye, B.; Akamatsu, M.; Shoelson, S. E.; Wolf, G.; Giorgetti-Peraldi, S.; Yan, X.; Roller, P. P.; Burke, T. R., Jr. *J. Med. Chem.* **1995**, *38*, 4270.
16. Ledon, H. J. *Org. Synth.* **1988**, *6*, 414.
17. House, H. O.; Czuba, L. J.; Gall, M.; Olmstead, H. D. *J. Org. Chem.* **1969**, *34*, 2324.
18. Reuss, R. H.; Hassner, A. *J. Org. Chem.* **1974**, *39*, 1785.
19. Sarges, R.; Hank, R. F.; Blake, J. F.; Bordner, J.; Bussolotti, D. L.; Hargrove, D. M.; Treadway, J. L.; Gibbs, E. M. *J. Med. Chem.* **1996**, *39*, 4783.
20. Larsen, S. D.; Barf, T.; Liljebris, C.; May, P. D.; Ogg, D.; O'Sullivan, T. J.; Palazuk, B. J.; Schostarez, H. J.; Stevens, F. C.; Bleasdale, J. E. *J. Med. Chem.* **2002**, *45*, 598.
21. Compound **5**: ^{1}H (NMR, 250 MHz, (DMSO-d_6): δ (ppm) 2.02 (s, 3H, CH_3), 3.14 (m, 1H, βCH_a), 3.26 (m, 1H, βCH_b), 4.32 (s, 2H), 4.91 (m, 1H, αCH), 6.27 (d, 1H, NH), 7.19 (d, 2H, J = 7.5 Hz), 7.27 (d, 2H, J = 7.5 Hz). MS (ESI) *m*/*z* calcd: 289.1 Found: 312.1 $[M+Na]^+$.
    Compound **7**: ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.51 (s, 18H), 3.12 (m, 2H, βCH), 4.45 (m, 4H, Fmoc CH, Fmoc CH_2, αCH), 5.18 (s, 1H), 5.26 (d, 1H, NH), 6.91 (d, 2H, J = 7.5 Hz), 7.07 (d, 2H, J = 7.5 Hz), 7.33–7.46 (m, 4H), 7.58 (d, 2H, J = 7.5 Hz), 7.78 (d, 2H, J = 7.5 Hz). MS (ESI) *m*/*z* calcd: 617.2 Found: 640.3 $[M+Na]^+$.
22. Synthesis of the core peptide LPQTV was carried out by solid phase on HMP resin on an A433 synthesizer (Applied Biosystems) using the standard Fmoc chemistry protocol. HBTU/HOBt/DIPEA were used as coupling agents. Phosphotyrosine was introduced with its phosphate group diprotected by methyldiphenylsilyl(ethyl) moiety. N-Fmoc or *N*-acetyl-protected phenylalanine derivatives were coupled manually using either coupling agents HBTU/HOBt/DIPEA or HATU/HOAt/DIPEA in the case of tetrazolyl derivatives. In the case of N-Fmoc protected peptides, final N-acetylation was achieved, after Fmoc-deprotection, by treatment with excess acetic anhydride in NMP.
    Resin cleavage and peptide side-chain deprotection were achieved by treatment with TFA/TIPS/H_2O 95:2.5:2.5 in volume. Peptides were purified by reverse-phase HPLC and gave the correct mass by electrospray mass spectrometry.
    MS (ESI) *m*/*z*:
    Peptide **8**: calcd: 841.4 Found: 842.4 $[M+H]^+$.
    Peptide **9**: calcd: 839.4 Found: 840.3 $[M+H]^+$.
    Peptide **10**: calcd: 813.4 Found:812.4 $[M-H]^-$.
    Peptide **11**: calcd: 827.4 Found: 828.4 $[M+H]^+$.
    Peptide **12**: calcd: 819.9 Found: 820.6$[M+H]^+$.
    Peptide **13**: calcd: 863.4 Found: 864.3 $[M+H]^+$.
23. Liu, W. Q.; Roques, B. P.; Garbay-Jaureguiberry, C. *Tetrahedron: Asym.* **1995**, *6*, 647.
24. Burke, T. R., Jr.; Yao, Z. J.; Zhao, H.; Milne, G. W.; Wu, L.; Zhang, Z. Y.; Voigt, J. *Tetrahedron* **1998**, *54*, 9981.
25. Becker, S.; Groner, B.; Muller, C. W. *Nature* **1998**, *394*, 145.
26. Yao, Z. J.; King, C. R.; Cao, T.; Kelley, J.; Milne, G. W.; Voigt, J. H.; Burke, T. R., Jr. *J. Med. Chem.* **1999**, *42*, 25.