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Design and Synthesis of a β-Amino Phosphotyrosyl Mimetic Suitably Protected for Peptide Synthesis

Kyeong Lee,[a] Manchao Zhang,[b] Dajun Yang[b] and Terrence R. Burke, Jr.[a,*]

[a]*Laboratory of Medicinal Chemistry, Center for Cancer Research, NCI-Frederick, National Institutes of Health, Frederick, MD 21702, USA*

[b]*Department of Hematology/Oncology, University of Michigan, Ann Arbor, MI 48109, USA*

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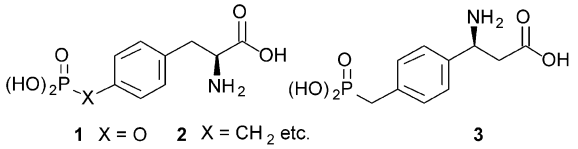
Abstract—Mimetics of phosphotyrosine (pTyr) such as phosphonomethylphenylalanine (Pmp) have traditionally retained α-amino functionality. However, β-amino acids represent isomeric variants, which may exhibit properties that are distinct from the parent. Reported herein is the first β-amino pTyr mimetic (Pmp_{β}) bearing protection suitable for peptide synthesis. Preparation of Pmp_{β} was accomplished enantioselectively in 43% overall yield from commercially available 4-vinylbenzyl chloride.

Physiochemical properties of peptides can be highly dependent on solution conformations. For this reason amino acid analogues are often employed in order to induce desired bioactive backbone or side-chain geometries.[1,2] Beta-amino acids are isomeric variants of α-amino acids which can serve as useful building blocks due to their ability to induce turn structures either when used exclusively,[3,4] or when used in combination with α-amino acids.[5] Phosphotyrosyl residues (pTyr, **1**) provide critical recognition motifs in a variety of signal transduction pathways, including those associated with diseases such as cancers.[6] Phosphotyrosyl-dependent binding phenomena mediated by Src homology 2 (SH2) and phosphotyrosyl-binding (PTB) domains are key to several of these processes,[7,8] and disruption of these interactions may afford new therapeutic approaches.[9] Accordingly, a number of phosphatase-stable pTyr mimetics have been developed for such purposes.[10,11]

In spite of the fact that critical binding functionality can originate from the amino-proximal region of the pTyr residue[12–14] and that bend conformations can be preferred by both SH2 domains and PTB domains,[15,16] most pTyr mimetics have retained the natural α-amino functionality of parent pTyr. An example of such a pTyr mimetic is provided by phosphonomethylphenylalanine (Pmp, **2**, Fig. 1). Since in a variety of contexts, β-amino variants may provide useful alternatives to traditional α-amino pTyr mimetics, β-amino phosphonomethylphenylalanine (Pmp_{β}, **3**) was designed as the first example of a β-amino pTyr mimetic (Fig. 1). Presented herein is the synthesis of **3** bearing amino protection suitable for Boc-based peptide synthesis, as well as its incorporation into a model tripeptide.

Asymmetric syntheses of β-amino acids have been achieved by several methods,[17] including addition of nitrogen nucleophiles to α,β unsaturated esters, enolate additions to imines and hydrogenation of β-enamino esters. Our initial attempts at the asymmetric synthesis of β-amino-containing **7** utilizing conjugate addition onto Evan's auxillary-containing **4** of either protected amines or azide, failed (Fig. 2). Subsequently, Mannich-type reaction of chiral enolate **6** with imine **5** derived from a variety of amines, including trimethylsilylamine and *p*-methoxybenzylamine, also failed due to instability of the imine species. Recently, *tert*-butanesulfinamide



1 X = O **2** X = CH_2 etc. **3**

Figure 1. Structures of pTyr (**1**), representative α-amino pTyr mimetic Pmp (**2**) and a new β-amino pTyr mimetic Pmp_{β} (**3**).

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

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(**11**, Scheme 1)[18] has been employed in a variety of contexts, including the synthesis of imines, which can be converted to β-amino acids.[19,20] Sulfinylimines derived from **11** are chemically stable and contain chirality appropriate for induction of asymmetric addition by achiral nucleophiles. In addition, after creation of a new stereogenic center, the *tert*-butanesulfinyl auxiliary can be removed easily by acid treatment or utilized as an acid-labile amine-protecting group. Accordingly, (*R*)-(−)-*tert*-butanesulfinylamide **11** was employed for the efficient synthesis of protected β-amino pTyr mimetic **14** (Scheme 1). Preparation of requisite arylaldehyde **10** began by reaction of commercially available 4-vinylbenzyl chloride (**8**) with lithiated di-benzylphosphite in the presence of tetra-*n*-butylammonium iodide as a catalyst. Resulting phosphonate **9** provided **10**, following oxidative cleavage of the vinyl group (66% yield from **8**).

Of note, although aldehydes of type **10** have previously been prepared as versatile intermediates for a variety of pTyr mimetics,[21–23] the current synthetic approach is significantly improved relative to these previous methods.[20,22] Transformation of **10** to chiral aldimine **12** was achieved by refluxing with sulfinylamide **11** in the presence of Ti(*O*-Pr^i)$_2$ (79% yield from **10**).[24] Treatment of **12** with the sodium enolate of methyl acetate, which was generated from NaHMDS in ether at −78° C, failed to give the desired product **12**.[25] Alternatively, using the titanium enolate (formed by transmetallation of lithium methylacetate using 2.3 equiv of ClTi(*O*-Pr^i)$_3$ in THF),

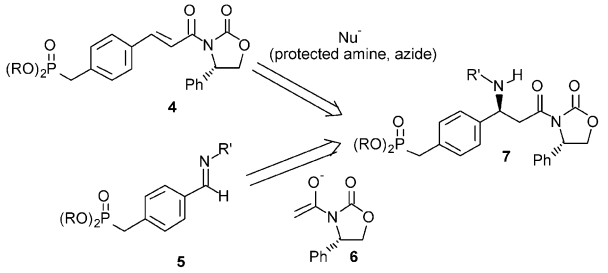
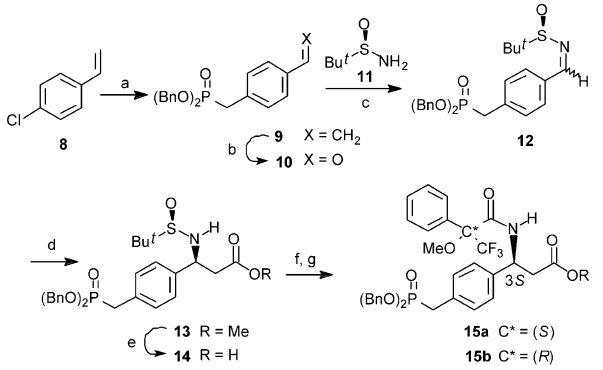


Figure 2. Unsuccessful approaches toward asymmetric introduction of β-amino functionality.



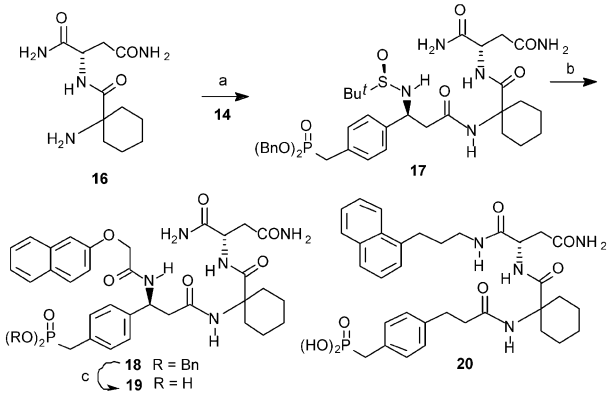
Scheme 1. (a) HP(O)(OBn)$_2$, *n*-BuLi, TBAI, −78–C to rt; (b) (i) cat. OsO_4, NMO, THF, water, (ii) $NaIO_4$, THF/H_2O, 1:1; (c) Ti(OiPr)$_4$, reflux; (d) methyl acetate, *n*-BuLi, THF, then ClTi(OiPr)$_3$; (e) 1 M LiOH, THF/MeOH, 1:1, rt; (f) 4 N HCl/dioxane, MeOH, rt; (g) (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetiyl chloride for **15a**, (*R*)-(−)-α-methoxy-α-(trifluoro-methyl)phenylacetiyl chloride for **15b**.

according to the method of Tang et al.[19] gave **13** in 83% yield without β-lactam formation. Diastereoselectivity of the reaction was determined by the method of Mosher.[26] In summary, amino-deprotection of **13** by treatment with 4 N HCl in dioxane followed by acylation using either (*S*)-(−)- or (*R*)-(+)-α-methoxy-α-trifluoromethylphenyl acetyl chloride, yielded diastereomeric Mosher-amides **15a** and **15b**, respectively. Characterization of these derivatives by both proton and fluorine NMR indicated high diastereoselectivity (d.e. of >90% for the (3*S*) isomer). Finally, saponification of **13** provided title compound **14** in quantitative yield.

In order to validate the suitability of analogue **14** for the synthesis of β-amino pTyr mimetic-containing peptides, compound **20**[27] (Scheme 2) was chosen as a model system, since it had previously been shown to function as a Grb2 SH2 domain-binding ligand (IC_{50} value of 2 μM). Using a functionalized variant of **20**, earlier modeling studies had highlighted the hydrophobic binding interactions of the carboxy-terminal naphthylpropyl group with a hydrophobic region of the Grb2 protein formed by residues Lys109 and Leu111.[28]

As shown in Figure 3 (Panel A), hydrophobic interactions could also reasonably be expected in the binding of **20**. Accordingly, tripeptide **19** was designed utilizing β-amino p-Tyr mimetic **3**, wherein a similar type of hydrophobic interaction was envisioned by translocating the naphthyl group from the carboxy-terminal to the amino-terminal position (Fig. 3, Panel B). A close correspondence of naphthyl-functionality binding could potentially be achieved, as shown in Panel C.

Synthesis of **19** commenced with the preparation of dipeptide **16**, which was obtained in 90% yield by coupling the HOBt-active ester of *N*-Fmoc-1-aminocyclohexanecarboxylic acid with Asn-amide•HCl in the presence of *i*Pr$_2$NEt, followed by standard *N*-Fmoc deprotection using piperidine (Scheme 2). Introduction of *N*-Bu^{t}S(O)-protected **14** was then accomplished using HOBt active ester coupling (HOBt/diisopropyl-carbodiimide), to provide **17** in 57% yield. Attempted removal of *N*-Bu^{t}S(O)-protection using TFA failed, however, treatment with 4N HCl/dioxane in MeOH



Scheme 2. (a) HOBt, DIPCDI, DMF; (b) (i) 4 N HCl/dioxane, MeOH, (ii) 2-naphthyloxyacetic acid, HOBt, DIPCDI, DMF; (c) H_2, 10% Pd-C, MeOH, 40 psi.

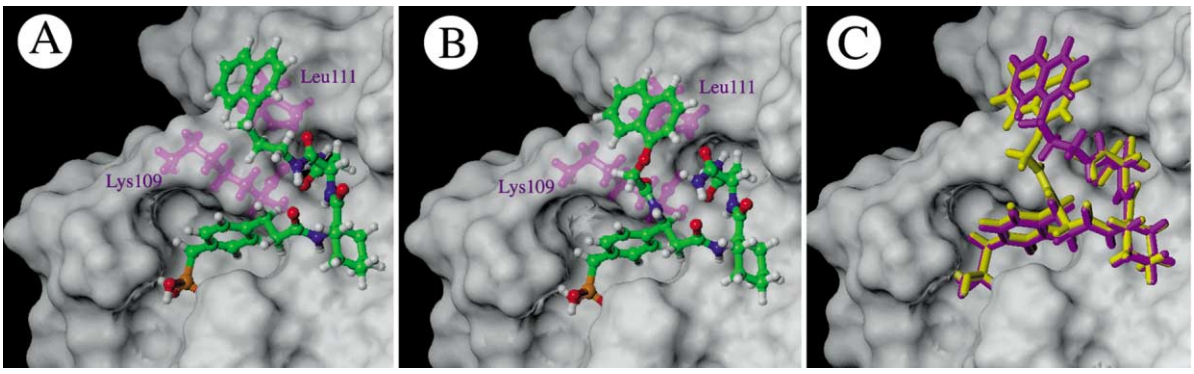


Figure 3. Simulated binding of **20** (panel A) and **19** (Panel B) to the Grb2 SH2 domain comparing potential interaction of naphthyl units with a

gave the β-amino analogue quantitatively, which was then acylated using commercially available 2-naphthyloxyacetic of **18**, using 10% palladium on activated carbon in MeOH at 40 psi, 2 h, and purification by reverse phase HPLC, led to β-amino pTyr-containing **19** (81% yield). Although in an ELISA-based Grb2 SH2 domain assay,[29] **19** exhibited significantly less affinity than parent **20** (data not reported), the original intent of demonstrating the suitability of **14** for peptide synthesis was successfully achieved.

In conclusion, reported herein is the design and efficient synthesis of the first reported β-amino pTyr mimetic, bearing protection suitable for peptide synthesis. This new amino acid analogue may expand the range of peptide structures available for investigators studying signal transduction processes.

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