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# α-DICARBONYLS AS *"NON-CHARGED"* ARGININE-DIRECTED AFFINITY LABELS. NOVEL SYNTHETIC ROUTES TO α-DICARBONYL ANALOGS OF THE PP60$^{c\text{-}src}$ SH2 DOMAIN-TARGETED PHOSPHOPEPTIDE Ac-TYR($OPO_3H_2$)-GLU-GLU-ILE-GLU

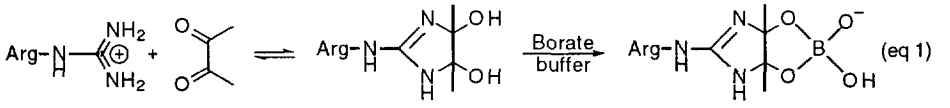
Mukund M. Mehrotra*§ , Daniel D. Sternbach, Marc Rodriguez† , Paul Charifson, and Judd Berman

*Glaxo Wellcome Research, Five Moore Drive, Research Triangle Park, N.C. 27709*

**Abstract:** The phosphopeptide **1** is a potent inhibitor of pp60$^{c\text{-}src}$ SH2 domain mediated phosphoprotein interactions ($IC_{50} \leq 0.5$ μM), but lacks cell permeability. The syntheses of its less charged analogs **2** and **3** are described, in which the arginine-binding phosphate group has been substituted with uncharged α-dicarbonyl moieties. The chemistry described here may be of general use for the synthesis of other α-dicarbonyl compounds.


Arginine residues, serving the function of an anion recognition site, are found in the binding region of a large number of enzymes and signaling proteins. Such proteins that employ arginyl residues to interact with negatively charged phosphate or carboxylate moieties of substrates or cofactors are ubiquitous.[1] These include the cytosolic tyrosine kinases (e.g., pp60$^{c\text{-}src}$) whose SH2 (src homology-2) domains recognize short phosphotyrosine containing peptide sequences (e.g., **1**).[2]

It has been proposed[3] that the lower p$K_a$ value of arginyl residues of anion-binding sites due to the strong positive electrical potential leads to their hyperreactivity toward α-dicarbonyl compounds. This permits their facile detection by α-dicarbonyl reagents (e.g., 1,2-cyclohexanedione, phenylglyoxal, 2,3-butanedione, etc.) that form a reversible dihydroxyimidazoline adduct. Subsequent trapping of these adducts as their cyclic boronic acid derivatives allows for this affinity labeling (eq 1).

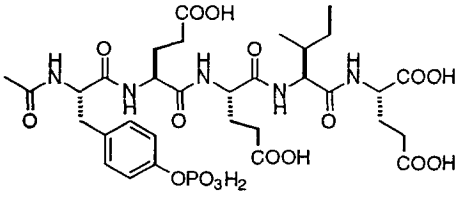


(eq 1)

A report describing the synthesis of a purine nucleoside phosphorylase (PNP) inhibitor, in which an α-dicarbonyl moiety successfully served the function of an arginine trap,[4] led us to target the synthesis of such analogs of **1**. Herein, we report the synthesis of compounds **2** and **3** in which an α-dicarbonyl moiety substitutes for the phosphate group that has been observed to bind with $Arg^{178}/Arg^{158}$ of the SH2 domain of pp60$^{c\text{-}src}$ in X-ray crystal structures.[2,5] The feasibility of this approach and the optimal number of methylene units between the phenyl ring and the alpha dicarbonyl functionality was determined by molecular modeling (**Figure 1**).

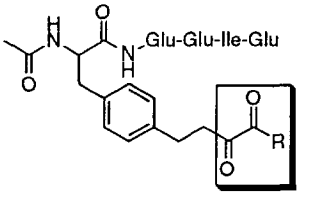
§ Current address: COR Therapeutics Inc., 256 East Grand Ave., Suite 80, South San Francisco, CA 94080
† Current address: Neosystems, 7 Rue de Boulogne, 67100 Strasbourg, France.

Besides circumventing the problem of lability to phosphatases, these analogs by virtue of being less charged would be expected to have enhanced cell penetration characteristics which is deemed to be an absolute requirement for an intracellular target like $pp60^{c\text{-}src}$.




(1)

Ac-Tyr($OPO_3H_2$)-Glu-Glu-Ile-Glu ($IC_{50}$ ≤ 0.5 μM)




(2) R = $CH_3$, (3) R = H

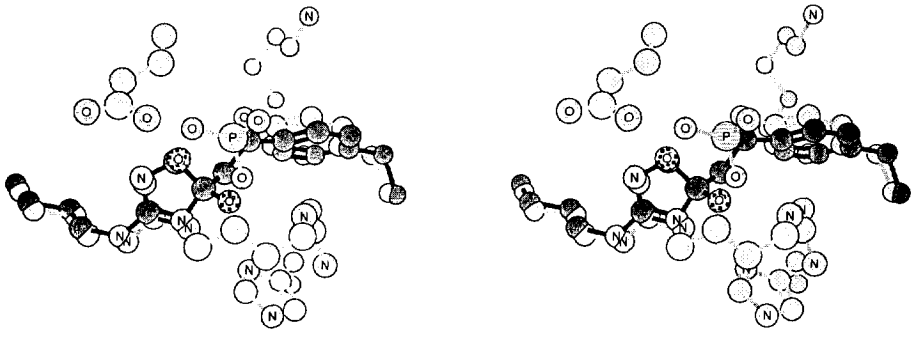


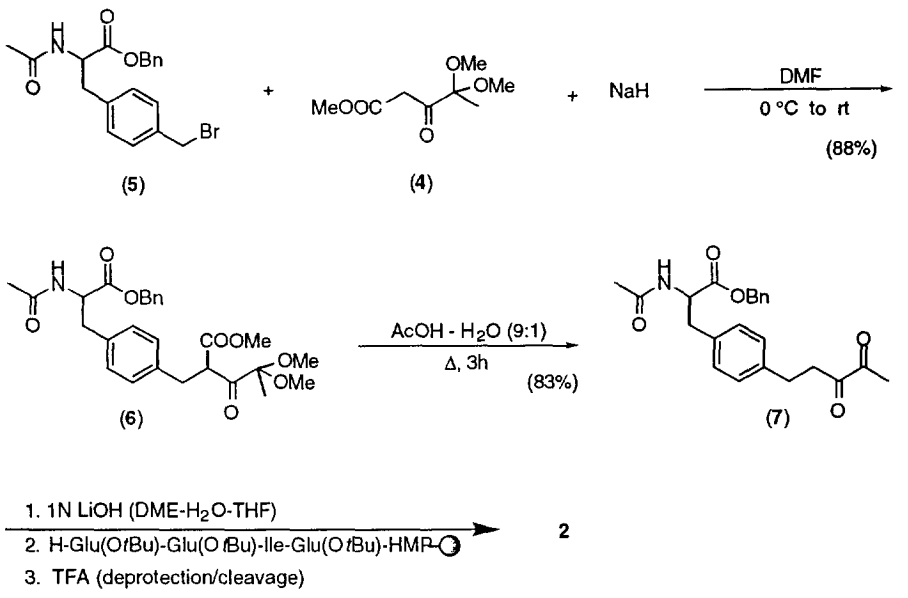
**Figure 1.** A depiction of the proposed arginine adduct superimposed on the phosphotyrosine of **1.** The crystal coordinates of **1** bound in the $pp60^{c\text{-}src}$ SH2 were used as a starting point for the molecular modeling and minimization. The adduct has dark bonds while the phosphotyrosine along with some key residues of the binding site are shown with light bonds.

The synthetic strategy involves as the key step the coupling of the bromomethyl phenylalanyl derivative **5** with α-dicarbonyl synthons **4** and **8**. The acute reactivity of the α-dicarbonyl functionality dictates that during the planned synthetic operations one of the carbonyls be in a protected form.

For the synthesis of **2** (Scheme 1), the sodium salt of methyl 4,4-dimethoxy-3-oxovalerate (**4**) (NaH, DMF, 0 °C) was treated with the benzylic bromide **5**[6] to yield **6**. Interestingly, the treatment of **6** with AcOH:$H_2O$ (9:1) at 120 °C yielded directly **7**, by bringing about deacetalization, hydrolysis of the methyl ester, followed by decarboxylation of the resulting β-keto-acid. Such a facile hydrolysis of the carbomethoxy group can be rationalized by taking into consideration the nucleophilic assistance of the dimethylacetal functionality. Ester **7** was then saponified using 1 N LiOH (1:1 DME-$H_2O$ + ca. 10% THF) to yield the free acid,[7] which was coupled

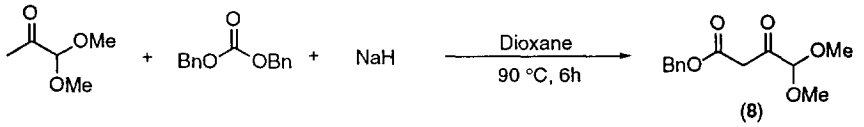
with the tetrapeptide on solid support {H-Glu(O*t*Bu)-Glu(O*t*Bu)-Ile-Glu(O*t*Bu)-HMP-resin} under the standard peptide synthesis protocol (2.0 equiv BOP, 4.0 equiv Hunig's base (DIPEA), 2.0 equiv monomer, and 30 min coupling time)[8] to yield **2**.

**Scheme 1**




For the synthesis of **3**, the requisite precursor to the α-keto aldehyde functionality **8** was synthesized from pyruvic aldehyde dimethylacetal, sodium hydride, and dibenzyl carbonate, in accordance with the general procedure of Corey *et al.* for the synthesis of β-ketoesters (Scheme 2).[9]

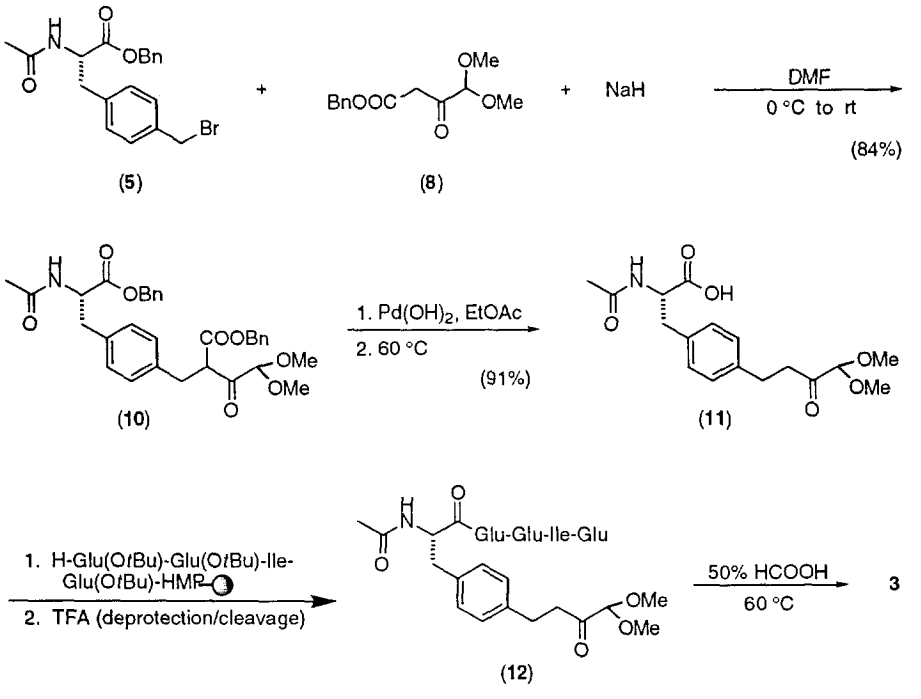
**Scheme 2**




The coupling of **8** with the benzylic bromide **5** was done exactly as described above for the synthesis of **6**, and **10** was isolated in 84% yield. The debenzylation of **10** using $Pd(OH)_2/C$ under 1 atm of $H_2$, followed by heating the crude β-keto acid to ca. 60 °C brought about decarboxylation to yield **11**, which was then coupled with the tetrapeptide as described before to give rise to **12**. Interestingly, the dimethylacetal protecting group did

not come off under the TFA treatment conditions employed during the peptide coupling protocol for the deprotection of amino acid side chains. However, under more harsh conditions, involving treatment of the pentapeptide with 50% HCOOH at 60 °C, this deacetalization could be effected to yield **3** (61%) as its hydrate (Scheme 3).[10]

**Scheme 3**




In an ELISA, which measures the activity of the test compounds to inhibit the binding of human pp60$^{c\text{-}src}$ SH3-SH2 domain to autophosphorylated EGFR, compounds **2** and **3** were 520 and 370 times, respectively, less potent than **1**.[2] While this binding is significantly weaker than that observed with phosphate or other doubly charged phosphate mimics, it represents the best binding of all the neutral and singly charged tyrosine replacements in this pentapeptide series.

In conclusion, the synthesis described here may be of general use for the synthesis of other α-dicarbonyl compounds that could serve as selective traps for arginine residues in proteins.

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6. The benzylic bromide **5** was synthesized using the standard acetoacetic ester synthesis protocol for the synthesis of α-amino acids (Carey, F. A. *Advanced Organic Chemistry* **1992**, Chapter 27). Thus, α-bromo p-toluic acid was reduced with $BH_3$.THF (2.50 equiv) in THF (0 °C to rt, 94%) to give 4-hydroxymethyl benzylbromide. This was condensed with 1.2 equiv of the sodium salt of diethyl acetamidomalonate (sodium ethoxide, EtOH) at rt. (84%). Treatment with 1 N NaOH (1.0 equiv, rt), followed by heating to ca. 130-40 °C brought about decarboxylation of the malonic acid half-ethyl ester to yield N-acetyl 4-hydroxymethyl phenylalanine ethyl ester. The ester exchange from ethyl to benzyl was achieved by saponifying with 2 N NaOH (EtOH, $H_2O$), followed by treating with benzyl bromide (1.1 equiv) and DBU (1.1 equiv) in $CH_3CN$ at reflux (92%). Finally, this benzylic alcohol was converted to **5** by reaction with $Ph_3P.Br_2$ (1.1 equiv) in $CH_3CN$ at rt (87%). $^1$H NMR (**5**) (DMSO-$d_6$ δ) 8.20(d, 1H), 7.25(m, 9H), 5.06(AB quartet, $J$ =12.5 Hz), 4.66(s, 2H), 4.48(m, 1H), 3.0 (dd, 1H), 2.88 (dd, 1H), 1.78 (s, 3H).

7. Attempted hydrogenolysis of **7** led also to reduction of one of the carbonyl groups.

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9. Corey, E. J.; Mitra, R. B.; Uda, H. *J. Amer. Chem. Soc.* **1964**, *86*, 485. The procedure for the synthesis of **8** was adapted from this paper. See also, Krapcho, A. P.; Diamanti, J. *Org. Synth. coll.* **1973**, **5** 198.

(**8**): A mixture of dibenzyl carbonate (50.0 g, 206.38 mMol, 6.1 equiv), and NaH (60% in oil, 2.70 g, 67.5 mMol, 2.0 equiv) in 1,4-dioxane (70 mL) was heated to ca. 90 °C. Then a solution of pyruvic aldehyde dimethylacetal (4.0 g, 33.86 mMol) in dioxane (25 mL) was added *via* a syringe pump over ca. 6 h. The brown mixture was stirred at this temperature for another 2 h. The reaction was quenched at 0 °C by the addition of AcOH:water, and diluted with EtOAc/brine. The aqueous layer was extracted with EtOAc. Evaporation of the organic extract followed by flash column chromatography (30% EtOAc/Hexane) provided pure **9** (7.43 g, 87%). $^1$H NMR ($CDCl_3$, δ) 7.38 (s, 5H), 5.18 (s, 2H), 4.56 (s, 1H), 3.62 (s, 2H), 3.38 (s, 6H). Peaks corresponding to enol form (ca. 25%) are also observed (δ 5.50, 5.20, 4.82). MS(FAB): *m/z* (253.11, $MH^+$), 221.02 ($MH^+$-$CH_3OH$).

10. All new compounds gave satisfactory spectral and analytical data, including amino acid analysis whereever applicable.

(**6**): Methyl 4,4-dimethoxy-3-oxovalerate (**4**) (1.10 mL, 6.42 mMol) in DMF (2.0 mL) was added to NaH (60% in oil, 240 mg, 6.0 mMol) in DMF (3.0 mL) at 0 °C. After 1 h, a solution of **5** (2.0 g, 5.12 mMol) in DMF (5.0 mL) was added and the mixture stirred at rt overnight. The reaction was quenched with water/brine, and extracted with EtOAc. Evaporation of the EtOAc extract, followed by purification by flash column chromatography (65% EtOAc/hexane) afforded pure **6** (2.25 g, 88%). $^1$H NMR (DMSO-$d_6$ δ) 8.35 (d, 1H), 7.30 (m, 5H), 7.04 (s, 5H), 5.10 (dd, *J* = 18.8, 12.7 Hz, 2H), 4.42 (m, 1H), 4.18 (t, *J* =7.5Hz, 1H), 3.30 (s, 3H), 3.02 (s, 3H), 2.95 (s, 3H), 2.90 (m, 4H), 1.76 (s, 3H), 1.10 (s, 3H). MS (FAB): *m/z* 500.2 ($MH^+$), 468.2 ($MH^+$-$CH_3OH$).

(**7**): $^1$H NMR ($CDCl_3$ δ) 7.36 (m, 5H), 7.02 (d, *J* =8.50 Hz, 1H), 6.90 (d, *J* =8.50 Hz, 1H), 5.90 (d, 1H), 5.16 (dd, *J* =19, 12 Hz, 1H), 4.90 (m, 1H), 3.02 (m, 4H), 2.84 (t, *J* =6.8 Hz, 2H), 2.32 (s, 3H), 1.98 (s, 3H). MS (FAB) *m/z* 396.27 ($MH^+$).

(**10**): $^1$H NMR ($CDCl_3$ δ) 7.32 (m, 10H), 7.02 (d, *J* = 8.5 Hz, 2H), 6.83 (dd, *J* = 8.5, 1.5 Hz, 2H), 5.83 (d, 1H), 5.12 (m, 4H), 4.90 (m, 1H), 4.48 (s, 1H), 4.16 (m, 1H), 3.21 (s, 3H), 3.19 (s, 3H), 3.10 (m, 4H), 1.98 (s, 3H). MS (FAB) *m/z* 562.40 ($MH^+$), 530.35 ($MH^+$-$CH_3OH$).

(**11**): $^1$H NMR ($CDCl_3$ δ) 7.12 (d, *J* =8 Hz, 1H), 7.08 (d, *J* = 8 Hz, 1H), 5.96 (d, 1H), 4.82 (dd, *J* =14, 7 Hz, 1H), 4.44 (s, 1H), 3.38 (s, 6H), 3.14 (two dd, 4H), 2.88 (s, 2H), 1.98 (s, 3H). MS (FAB) *m/z* 338.31 ($MH^+$).

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