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Synthesis of a conformationally constrained tyrosine–glycine dipeptide mimetic: design of a potential substrate of Syk kinase

Paolo Ruzza,* Andrea Calderan, Alessio Osler, Stefano Elardo and Gianfranco Borin

CNR-Biopolymer Research Center, via F. Marzolo, 1-35131 Padova, Italy

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Abstract—The synthesis of a conformationally restricted tyrosine–glycine dipeptide mimetic and its insertion into an octapeptide is described.

A critical area for the design of biological active peptides is the three dimensional structure of the side chain moieties. As illustrated in Fig. 1, each side chain χ^1 torsional angle can assume three low energy staggered conformations: *gauche* (−), *gauche* (+), and *trans*. Yet the orientation of the side chain group of an L-amino acid residue with respect to the peptide backbone will be dramatically different: for *gauche* (−), the side chain points towards the N-terminus of the peptide chain; for *trans*, the side chain points towards the C-terminus, and for *gauche* (+) the side chain points on the peptide backbone.[1] Clearly, in short peptides, different dispositions of side chain groups in terms of χ^1 space can provide a remarkable diversity of chemical surfaces, even considering a single peptide backbone template (α-helix, β-turn, …).

Phosphorylation is one of the major post-traslational modifications that regulate many cellular processes such as cell cycle, growth and differentiation.[2] In particular, protein tyrosine kinases (PTKs) are enzymes involved, by the phosphorylation of specific tyrosine residues, in the transmission of mitogenic signals and in the regulation of cell cycle.[3] During the course of a program to develop useful competitive inhibitors of these enzymes, we found that the replacement of the phosphorylatable tyrosine with its Htc (1,2,3,4-tetrahydro-7-hydroxyisoquinoline-3-carboxilic acid) constrained analog in some of their peptide-substrates, strongly inhibits the phosphorylation by Lyn and *c*-Fgr, two kinases of Src-family. On the contrary, Syk can phosphorylate Htc-analog, albeit with a lower efficiency with respect to the native sequence. Htc analog has only two allowed low-energy side chain orientations about the C^{α}–C^{β} bond: the *gauche* (−) or the *gauche* (+).

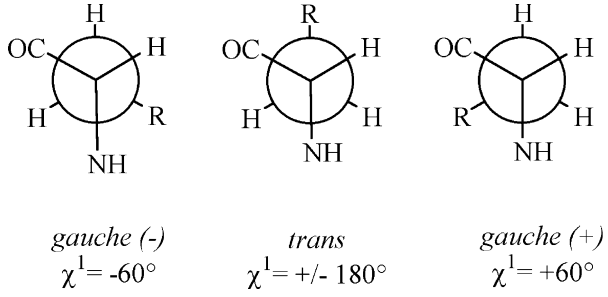

Figure 1. Newman projections of the three staggered χ^1 rotamers in L-amino acids.

In order to correlate the side chain orientation of the phosphorylatable tyrosine residue, present into short peptide sequences, with the efficiency and the selectivity of the analogs as substrates for Src and Syk/Zap70 PTK families we fixed the side chain of Tyr into the *trans* conformation by using an aminobenzazepine-type structure. Indeed 4-amino-2-benzazepin-3-ones have proven very useful for studying the biological active conformations of peptides.[4] In this structure, the side chain of tyrosine is fixed in the *gauche* (+) or the *trans* conformer by cyclizing the aromatic ring onto the nitrogen of the succeeding amino acid.

In this paper, we report the synthesis of a conformationally constrained tyrosine–glycine dipeptide mimetic (Hba-Gly, **5**) and its insertion into a peptide corresponding to a modified sequence of a phosphorylation domain of the haematopoietic lineage-specific protein HS1. The key point of this synthetic route is the

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* Corresponding author. Tel.: +39.049.8275258; fax: 39.049.8275239; e-mail: paolo.ruzza@unipd.it

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conversion of the oxazolidinone **3** into the benzazepine derivative **4**. In our procedure (Scheme 1) the phenolic moiety of the tyrosine residue was protected as benzyl derivative and the Hba-Gly analog was precisely performed using TFMSA, adapting the method described by Flynn and de Laszlo for the corresponding phenylalanine–glycine dipeptide.[5,6] In a previous work, Casimir et al.[7] reported that in the same conditions this TFMSA treatment led to a mixture of by-products. Therefore, they preferred to use $SnCl_4$ as Lewis acid catalyst, and the 2,6-dichloro benzyl group for the tyrosine hydroxy protection, to obtain the corresponding acid stable 2,6-dichloro benzyl benzazepine derivative.

The synthetic pathway is in succession shortly described. Condensation of Pht-Tyr(OBzl)-OH **1** with glycine *tert*-butyl ester utilizing the DCCI/HOSu reagent provided the corresponding dipeptide in 93% yield. The Pht-Tyr(Bzl)-Gly-OH dipeptide **2** was obtained in quantitative yield by treatment of compound **1** with 50% TFA in DCM. Starting from this dipeptide, the oxazolidinone **3** was formed by condensation of **2** with paraformaldehyde, in presence of *p*-toluensulfonic acid, in toluene refluxed in a Dean–Stark apparatus (84% yield). The preparation of the oxazolidinone **3** starting from the dipeptide Pht-Tyr-Gly-OH, unprotected to the phenolic moiety, does not succeed due to a polymerization reaction to a phenol–formaldehyde polymer.[8] On the other hand, when the phenolic hydroxyl group of tyrosine was protected as its benzyloxycarbonyl ester or 2,6-dichloro benzyl ether, treatment of **3** with TFMSA gave only recovered starting material for more than 80% (our experiments). To our satisfaction, the treatment of oxazolidinone **3**, under acidic conditions of Pictet–Spengler reaction (17% TFMSA in CH_2Cl_2),[9] provided the desired benzazepine structure **4** in 70–75% overall yield. In this condition the benzyl group was removed by the treatment with TFMSA. This is very advantageous for our purpose, since the obtained compound may be used in Fmoc/Boc peptide synthesis. Dephthaloylation of **4** by treatment with hydrazine hydrate in ethanol afforded the dipeptide mimetic in 70% yield. The corresponding Fmoc derivative **5** was obtained by treatment with Fmoc-OSu in water–dioxane solution (75% yield).

The utility of dipeptide mimetics as tools to probe the binding requirements of peptides of interest is dependent on their compatibility with standard peptide coupling and deprotection methods. The Hba-Gly mimetic replaced the phosphorylatable tyrosine residue into the synthetic peptide Glu-Asp-Asp-Glu-Tyr-Glu-Glu-Val, a modified sequence of the phosphorylation domain of the HS1 protein.[10] The Fmoc derivative **6** was incorporated into the sequence using the classical solid Fast-Fmoc/HBTU protocol,[11] and the corresponding peptide was obtained in good yield after HPLC purification.[12] The obtained peptide possessed a phosphorylation efficiency comparable to the L-Htc containing peptide. In addition, the NMR data of the Hba-Gly containing octapeptide ($^3J_{\alpha\beta}$ and NH-$CH^{\beta}CH^{\beta'}$, CH^{α}-$CH^{\beta}CH^{\beta'}$ NOE) are consistent with a $C^{\alpha}HC^{\beta}H_2$ moiety in a *trans* conformation, for the tyrosine analog.[13]

In conclusion, dipeptide mimetic **5** is a versatile conformationally constrained intermediate which can be incorporated into peptides to help to define the important determinants for selective phosphorylation by protein tyrosine kinases.

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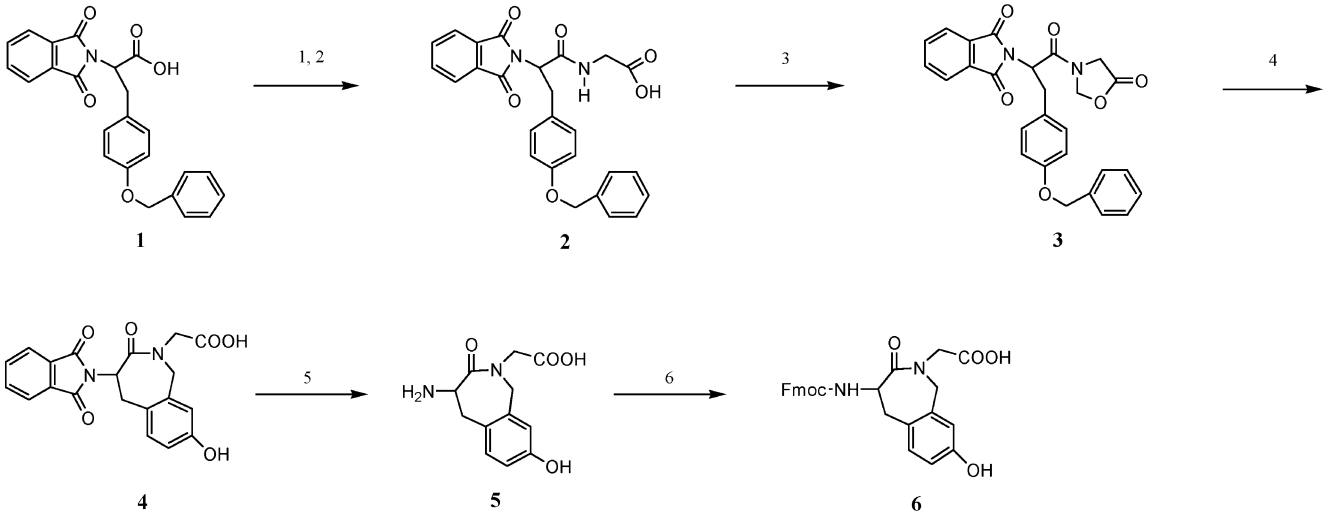

Scheme 1. *Reagents and conditions*: (1) H-Gly-OtBu (1 equiv.), DCCI (1 equiv.), HOSu (1 equiv.), TEA (1 equiv.), CH_2Cl_2, 93%; (2) 50% TFA in CH_2Cl_2, 99%; (3) $(CH_2O)n$ (3×10 equiv.), *p*Tos-OH (0.1 equiv.), toluene, reflux, 7 h, 84%; (4) 17% TFMSA in CH_2Cl_2, rt, overnight in N_2 atm, 70–75%; (5) NH_2–$NH_2 \cdot H_2O$, EtOH, reflux, 2 h, 70%; (6) Fmoc-OSu (2 equiv.), Na_2CO_3 (1 equiv.), water–dioxane 1:1, rt, overnight, 75%.

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References

1. Hruby, V. J.; Li, G.; Haskett-Luevano, C.; Shenderovich, M. *Biopolymers (Peptide Sci.)* **1997**, *43*, 219–266.
2. Marks, F. *Protein Phosphorylation*; VCH: Weinheim, 1996.
3. Hunter, T. *Cell* **1997**, *88*, 333–346.
4. Tourwé, D.; Verschueren, K.; Frycia, A.; Davis, P.; Porreca, F.; Hruby, V. J.; Toth, G.; Jaspers, H.; Verheyden, P.; Van Binst, G. *Biopolymers* **1996**, *38*, 1–12.
5. Flynn, G. A.; Burkholder, T. P.; Huber, E. W.; Bey, P. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 309–312.
6. de Laszlo, S. E.; Bush, B. L.; Doyle, J. J.; Greenlee, W. J.; Hangauer, D. G.; Halgren, T. A.; Lynch, R. J.; Schorn, T. W.; Siegl, P. K. *J. Med. Chem.* **1992**, *35*, 833–846.
7. Casimir, J. R.; Tourwé, D.; Iterbeke, K.; Giuchard, G.; Briand, J. P. *J. Org. Chem.* **2000**, *65*, 6487–6492.
8. Vert, M. *Eur. Polym. J.* **1972**, *8*, 513–524.
9. Yokoyama, A.; Ohwada, T.; Shudo, K. *J. Org. Chem.* **1999**, *64*, 611–617.
10. Ruzza, P.; Calderan, A.; Donella Deana, A.; Cesaro, L.; Elardo, S.; Pinna, L. A.; Borin, G. In *Peptides*: *The Wave of the Future*; Lebl, M.; Houghten, R. A., Eds.; San Diego: American Peptide Society, 2001; pp. 982–983.
11. Applied Biosystems, Inc., Model 431A Peptide Synthesizer User's Manual, Applied Biosystems Inc., Foster City, CA, **1989**.
12. ESI-MS $m/z=1095$ $[MH]^+$; t_r (min) 25.6 min. (Elution conditions: eluent A, 0.05% TFA in water; eluent B, 0.05% TFA in 9:1 v/v acetonitrile–water; column Jupiter C18, 10 μ, 250×4.6 mm; flow rate 1 ml/min; detection at 216 and 275 nm; isocratic elution at 5% B for 3 min and then linear gradient from 5 to 30% B in 40 min). Amino acid ratios in acidic hydrolyzate: Asp 1.92 (2), Glu 4.07 (4), Val 0.98 (1), Hba-Gly 0.95 (1).
13. Characteristic data for the key compounds. Compound **2**: ^{1}H NMR (DMSO-d_6): δ (ppm) 7.81–7.55 (m, arom. Pht); 7.35 (m, arom. Bzl); 7.2–6.95 (d, arom. Tyr); 5.06 (s, CH_2 Bzl); 4.65 (m, $C_\alpha H$); 3.85 ($C_\alpha H$ Gly); 3.18–2.8 (dd, $C_\beta H$). Compound **3**: ^{1}H NMR (DMSO-d_6): δ (ppm) 7.83 (m, arom. Pht); 7.35 (m, arom. Bzl); 7.06–6.81 (d, arom. Tyr); 5.55–5.30 (d, CH_2); 5.15 (m, $C_\alpha H$ Tyr); 4.98 (m, CH_2 Bzl); 4.15–4.08 (dd, $C_\alpha H_2$ Gly); 3.3 (m, $C_\beta H_2$). Compound **4**: ^{1}H NMR (DMSO-d_6): δ (ppm) 9.40 (s, OH); 7.90 (m, arom. Pht); 7.04 (d, arom.); 6.72 (s, arom.); 6.68 (d, arom.); 5.20 (m, $C_\alpha H$ Tyr); 4.81–4.48 (dd, $C_\varepsilon H$); 4.22–4.03 (dd, $C_\alpha H$ Gly); 3.16–3.12 (dd, $C_\beta H$). Compound **5**: ^{1}H NMR (DMSO-d_6): δ (ppm) 9.75 (s, OH); 7.52 (d, arom.); 7.38 (d, arom.); 6.62 (s, arom.); 5.07, 4.04 (d, $C_\varepsilon H$); 4.81 (m, $C_\alpha H$ Hba); 4.27, 3.98 (d, $C_\alpha H$ Gly); 2.69, 2.83 (dd, $C_\beta H$). Compound **6**: ^{1}H NMR (DMSO-d_6): δ (ppm) 9.3 (s, OH); 7.45 (m, NH); 7.25–7.9 (m, arom. Fmoc); 6.92 (d, arom.); 6.64 (m, arom.); 6.58 (s, arom.); 5.08 (m, $C_\alpha H$ Tyr); 5.10–4.02 (d, $C_\varepsilon H$); 4.32 (m, CH2 Fmoc); 4.25 (m, CH Fmoc); 4.20–3.90 ($C_\alpha H$ Gly); 3.06–2.86 (m, $C_\beta H$); ESIMS $m/z=473.2$ $[MH]^+$.