Pergamon

Tetrahedron Letters 39 (1998) 7393–7396

TETRAHEDRON LETTERS

First Aromatic Electrophilic Iodination Reaction on the Solid-Phase: Iodination of Bioactive Peptides

Gemma Arsequell,[†] Gemma Espuña,[†] Gregorio Valencia,[*,†] José Barluenga,[*,‡] Raquel Pérez Carlón,[‡] and José M. González,[‡]

[†]*Unit for Glycoconjugate Chemistry, C.I.D.-C.S.I.C., Jordi Girona 18-26, E-08034 Barcelona (Spain)*

[‡]*Instituto Universitario de Química Organometálica "Enrique Moles"-Unidad Asociada al C.S.I.C., Universidad de Oviedo E-33071, Oviedo (Spain)*

Received 7 May 1998; accepted 27 July 1998

Abstract

Direct iodination of Tyr residues of peptides anchored on solid supports was accomplished, for the first time, by aromatic electrophilic attack of iodonium ions provided by the IPy_2BF_4 reagent. Compatibility studies of the iodination with routine solid-phase synthesis protocols are reported.

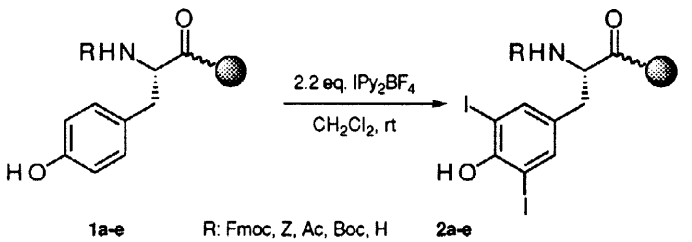
Keywords: Amino acids and derivatives; Halogenation; Peptides and polypeptides; Supported reagents/reactions.

Solid phase organic synthesis (SPOS), driven by the interest and the requirements of new synthetic trends in medicinal chemistry,[1] such as the ones related to combinatorial chemistry techniques,[2] is a major focus of ongoing research efforts.[3] However, an inherent problem with the use of polymers in synthesis is their questionable compatibility to many reagents and reaction conditions; extending organic transformations to solid-phase variants is not always a trivial pursuit. The best example of the vast effort required to substantiate an original idea by providing a reliable solid phase methodology is the Merrifield´s concept of peptide synthesis.[4] Herein, we describe for the first time successful examples of direct aromatic iodination on the solid phase (Scheme 1).[5]

The procedure makes use of the bis(pyridine) iodonium (I) tetrafluoroborate (IPy_2BF_4)[6] reagent which has not been previously applied to SPOS. The reaction proceeds instantaneously and quantitatively at room temperature in open atmosphere on tyrosine derivatives, and peptides containing them, without affecting oxidation sensitive functional groups present in the same molecule. Therefore, this is also the first example of bioactive peptide iodination on a solid support. First, we tested a single amino acid anchored onto a resin to determine a set of proper experimental conditions to carry out the iodination. Preliminary studies in solution showed that diiododerivatives of Tyr

0040-4039/98/$ - see front matter
PII: S0040-4039(98)01604-9

could be prepared quantitatively by using a 2.2:1 molar ratio of IPy_2BF_4 to peptide, therefore, we selected this stoichiometry to prepare first the diiodinated species. A Rink amide resin was chosen because of the simplicity of anchoring amino-acids to the resin through an amide linkage. Then, the coupling of commercially available Tyr derivatives containing different amino-protecting groups, such as 9-fluorenylmethoxycarbonyl (Fmoc), benzyloxycabonyl (Z), *N*-Acetyl Tyrosine and *tert*-butoxycarbonyl (Boc) **1a-d** were assayed using standard solid-phase protocols.

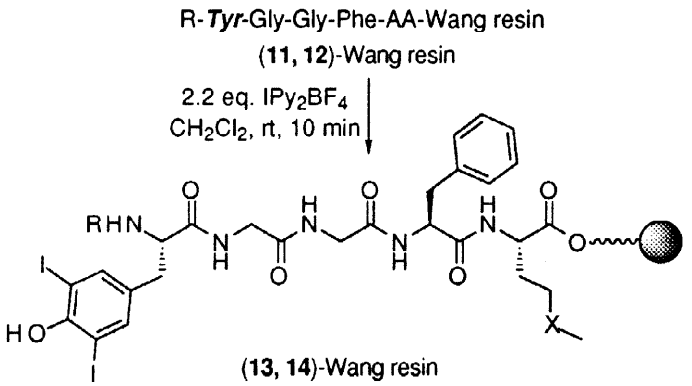


Scheme 1

Iodination[7] occurred almost instantly as checked by withdrawing a resin aliquot which was subsequently analysed by HPLC and MALDI-TOF-MS (matrix-assisted laser desorption/ionization).[8] A single peak corresponding to the diiodinated Tyr derivative **2a-d** with no traces of starting material was always observed. The nature of the amino protecting group on the anchored amino-acid did not affect the iodination yield, all being stable to the iodination conditions. The iodination reaction is also compatible with the presence of a free amino-group (Scheme 1) on the *N*-terminal amino-acid. Thus, deprotection with piperidine of the Fmoc-Tyr-Rink resin followed by iodination gave a positive Kaiser test on the peptidyl resin **2e** which quantitatively afforded diiodinated Tyr amide after cleavage.

A second set of iodination experiments was performed with a dipeptide model to check the influence of both chain elongation and the nature of the linker. In this case a pre-derivatized Wang resin for the immobilization of carboxylic acids was selected. Thus, by coupling to an Fmoc-Leu-Wang resin different Tyr derivatives such as Fmoc-Tyr-OH, Z-Tyr-OH, and Ac-Tyr-OH, the resin bound dipeptides Fmoc-Tyr-Leu-OH, Z-Tyr-Leu-OH and Ac-Tyr-Leu-OH, **3a-c** were obtained. After an iodination step, compounds **4a-c** showed an iodination trend similar to that of the single amino-acid derivatives. Diiodination was also quantitative with the Fmoc-Tyr-Leu-Wang resin after removal of the Fmoc group furnishing compound **4e**. No influence of the linker nature could be observed. We tested the compatibility of this method with the presence of oxidation sensitive side chains such as Met and two types of experiments were performed. Different peptidyl resins were prepared by coupling the commercially available Fmoc-Met-OH, Fmoc-Met(O)-OH and Fmoc-Met(O_2)-OH to a Rink amide resin. Fmoc-Tyr-OH was coupled to each of the three peptidyl resins **5-7** and iodination was performed after removal of the Fmoc group to yield three different resin-bound

peptides: H_2N-Tyr(I,I)-Met-NH_2 **8**, H_2N-Tyr(I,I)-Met(O)-NH_2 **9** and H_2N-Tyr(I,I)-Met(O_2)-NH_2 **10**. On the H_2N-Tyr(I,I)-Met-NH_2 sample no traces of the other two oxidised reference peptides could be detected by RP-HPLC. Moreover, the stability of the Fmoc-Met-Wang resin towards the iodination conditions was confirmed by RP-HPLC and ^{1}H-NMR analyses of the reaction product, after reductive cleavage, taking as reference commercial samples of FmocMetOH, sulfoxide (FmocMet(O)OH) and sulfone (FmocMet(O_2)OH) derivatives. Finally, to prove the versatility of the proposed method, the solid-phase synthesis and iodination of two pain-related pentapeptides, enkephalin analogues, (Met-Enk **11** and Leu-Enk **12**), which contain other aromatic side-chain residues such as Phe, and oxidation sensitive amino acids such as Met, has been undertaken.[9] After preparing the tetrapeptides, two different Tyr derivatives having Fmoc and Z protecting groups (compounds series **a** and **b**, respectively) were coupled. The Fmoc peptidyl resins were treated with piperidine to afford the final free amino terminal resins (series **e**). Iodination on the solid-phase was applied to the six peptidyl resins following identical experimental conditions (Scheme 2). The same iodination pattern as in the case of amino acids or dipeptides was observed.



R: Fmoc	**AA**: Met	X: S	di-I-Met-Enk,**13a**
R: Z	**AA**: Met	X: S	di-I-Met-Enk,**13b**
R: H	**AA**: Met	X: S	di-I-Met-Enk,**13e**
R: Fmoc	**AA**: Leu	X: $(CH_3)CH$	di-I-Leu-Enk,**14a**
R: Z	**AA**: Leu	X: $(CH_3)CH$	di-I-Leu-Enk,**14b**
R:H	**AA**: Leu	X: $(CH_3)CH$	di-I-Leu-Enk,**14e**

Scheme 2

As further evidence for the identification of the diiodinated pentapeptides **13** and **14**, the preparation of these diiodinated enkephalins was also performed using a diiodinated Tyr building block such as FmocTyr(3',5'-di-I)OH (commercially available). Solid-phase stepwise synthesis of the diiodinated enkephalins yielded products with identical chromatographic and spectroscopic data. This alternative synthesis also shows that following the global and convergent iodination strategy proposed here, the target peptides could be readily obtained with no need of using costly intermediates.

In summary, IPy_2BF_4 reagent has been successfully used for first time in SPOS. In particular, the global solid-phase iodination strategy proposed here allows quantitative

preparation of diiodinated Tyr containing peptides.[10] The iodination is selective for Tyr residues *vs* Phe and gives no oxidation by-products of the Met residue. The very mild reaction conditions and the stability of the reagent make the IPy_2BF_4 a good candidate as a reagent for automated synthesis. To the best of our knowledge this is the first time that iodination on solid support by an electrophilic aromatic substitution reaction has been described. Work is in progress in our labs to apply this method to the solid phase synthesis of iodinated intermediates[11] that may be of interest for combinatorial chemistry applications.

Acknowledgements. This research was supported by DGICYT (Grant PB92-1005). Fellowship to R.P.-C. (DGICYT) is gratefully acknowledged. The authors thank Prof. R.A. Dwek and Dr. D. Harvey for allowing us to run MALDI-TOF-MS at the Glycobiology Institute, Department of Biochemistry, University of Oxford, UK.

1. For applications of SPOS techniques to boost production of new lead compounds generation and optimization in drug discovery see, for instance: a) Madden, D.; Krchnák, V.; Lebl, M. *Pespect. Drug Discovery Des.* **1994**, *2*, 269-285; b) Gallop, M. A.; Barret, R. W.; Dower, W. J.; Fodor, S. P. A.; Gordon, E. M. *J. Med. Chem.* **1994**, *37*, 1233-1251; c) Gordon, E. M.; Barret, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. *J. Med. Chem.* **1994**, *37*, 1385-1401; d) Ecker, D. J.; Crooke, S. T. *Biotechnology* **1995**, *13*, 351-360.
2. a) Jung, G. *Combinatorial Peptide and non Peptide Libraries*, VCH: Weinheim, Germany, 1996. For recent reviews on combinatorial libraries and molecular diversity: b) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, 96, 555-600; c) Petsko, G. A. *Nature* **1996**, *384*, 1 (Supplement to issue 6604); d) Balkenhohl, F.; von dem Bussche-Hünnefeld, C.; Lansky, A.; Zechel, C. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2288-2337; e) Wilson, S. R.; Czarnik, A. W. *Combinatorial Chemistry. Synthesis and Applications*, Wiley: New York, 1997; f) Lam, K. S.; Lebl, M.; Krchnák, V. *Chem. Rev.* **1997**, *97*, 411-448; g) Nefzi, A.; Ostresh, J. M.; Houghten, R. A. *Chem. Rev.* **1997**, *97*, 449-472.
3. For reviews on recent advances in SPOS: a) Früchtel, J. S.; Jung, G. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 17-42; b) Hermkens, P. H. H.; Ottenheijm, H. C. J.; Rees, D. C. *Tetrahedron* **1996**, *52*, 4527-4554; c) Hermkens, P. H. H.; Ottenheijm, H. C. J.; Rees, D. C. *Tetrahedron* **1997**, *57*, 5643-5678.
4. a) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149-2154.
5. Iodination is a particularly difficult electrophilic aromatic substitution process, a major class of reactions that remain undeveloped for covalently attached small molecules to polymeric resins, see above quoted reference 3b.
6. For previous work on solution, on the use of the solid, stable in air, IPy_2BF_4 reagent as a mild iodinating agent towards arenes at room temperature, including amino acids and peptide derivatives, see: a) Barluenga, J.; González, J.M.; García-Martín, M.A.; Campos, P.J.; Asensio, G. *J. Org. Chem* **1993**, *58*, 2058-2060; b) Barluenga, J.; González, J.M.; García-Martín, M.A.; Campos, P.J. *Tetrahedron Lett.* **1993**, *34*, 3893-3896; c) Barluenga, J.; García-Martin, M.A.; González, J.M.; Clapés, P.; Valencia, G. *Chem. Commun.* **1996**, 1505-1506.
7. Typically, the IPy_2BF_4 reagent (2.2 equiv) is dissolved in CH_2Cl_2 and added to the resin (1 equiv) suspended in CH_2Cl_2 in a peptide synthesis flask. The resin is shaked for 10 min and the excess of reagent filtered, washed with CH_2Cl_2, MeOH and CH_2Cl_2 again several times, and dried under vacuum.
8. Egner, B.J.; Langley, G.J.; Bradley, M. *J. Org. Chem.* **1995**, *60*, 2652-2653
9. All the peptides prepared in this paper were further characterized by RP-HPLC, amino acid analyses, mass spectrometry (MALDI-TOF-MS, ES-MS) and ^{1}H-NMR.
10. Useful, either as precursors of tritiated compounds for radioimmunoassays or to prepare radioiodinated peptides, *i.e.* by exchange reaction: Breslav, M.; McKinney, A.; Becker, J.M.; Naider, F. *Anal. Biochem.* **1996**, *239*, 213-217.
11. Solid-phase Heck reaction: a) Yu, K.L.; Deshpande, M.S.; Vyas, D. M. *Tetrahedron Lett.* **1994**, *35*, 8919-8922; b) Beaver, K.A.; Siegmund, A.C.; Spear, K.L. *Tetrahedron Lett.* **1996**, *37*, 1145-1148. Solid-phase Stille reaction: c) Deshpande, M.S. *Tetrahedron Lett.* **1994**, *35*, 5613-5614. Solid-phase Suzuki reaction: d) Frenette, R.; Friesen, R.W. *Tetrahedron Lett.* **1994**, *35*, 9177-9180.