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Control of the iodination reaction on activated aromatic residues in peptides†

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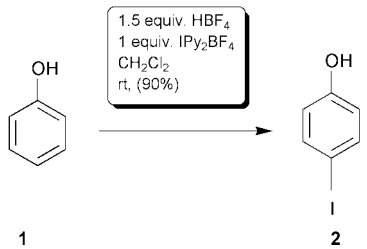
By using a slight molar excess of the IPy_2BF_4 reagent in an acid containing medium, activated aromatic residues on Tyr derivatives and peptides selectively react yielding, as major products, the monoiododerivatives; this level of reaction control cannot be achieved by other iodinating methods.

The methods available for either labeling complex biomolecules throughout radioiodination[1] or extending the reactivity of organic molecules by iodination[2] share similar problems. For most applications of iodinated aromatic compounds, monoiodination will be ideal, but known iodinating methods usually yield mixtures of mono-, diiodinated and unreacted species.[3] Accordingly, efficient control of the iodination of phenol-like compounds[4] at room temperature, that would result in a practical approach to monoiodinate target Tyr containing peptides still remains to be developed. The iodonium reagent IPy_2BF_4 that we are currently investigating[5] is very effective at iodinating aromatic functions of both simple organic molecules[6] and more complex polyfunctional peptides.[7] The performance of IPy_2BF_4 is dependent on factors such as reagent to substrate stoichiometry, the solvent and the presence of acid.[8]

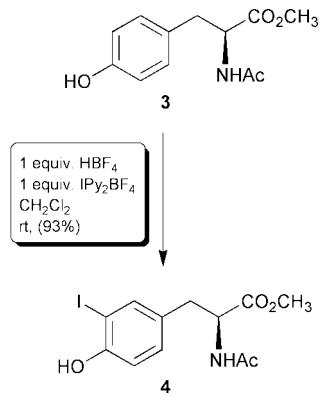
In searching for conditions for the selective and direct preparation of monoiodinated Tyr derivatives as new building blocks for use in combinatorial chemistry and Tyr containing peptides, starting from **1** we have established conditions for its regioselective conversion to **2** at rt, as outlined in Scheme 1. Thus, mixing HBF_4 with **1** in CH_2Cl_2 prior to the addition of stoichiometric amounts of IPy_2BF_4 results in the formation of **2** in 90% yield (the presence of minor amounts of the 2-regioisomer was noticed by ^1H NMR analysis of the crude reaction mixture). Next, we explored the iodination of **3** under the same conditions and **4** was obtained in 93% isolated yield (Scheme 2).

Then, we examined the reaction conditions shown in Table 1 on the protected Tyr derivative Fmoc-Tyr-OH **5**. As expected, if no acid was present, the sole reaction product was the diiodinated derivative when an excess of IPy_2BF_4 was added to a CH_3CN solution of Fmoc-Tyr-OH. Under the same conditions, stoichiometric amounts of the reagent produced mixtures of mono-, diioiodinated and unreacted species. However, addition of an equimolar quantity of HBF_4, in CH_3CN or CH_2Cl_2, or 10% TFA in CH_2Cl_2, and a slight excess of the reagent, the monoiodinated building block Fmoc-Tyr(3′-I)-OH **6** was the exclusive reaction product (Scheme 3).

For comparison to the traditional Chloramine T iodinating method,[9] Fmoc-Tyr-OH was reacted in CH_3CN with excess of both NaI and Chloramine T and the diiodinated derivative was produced. However, in conditions involving stoichiometric amounts of the iodinating reagents,[9c] low yields of Fmoc-Tyr(3′-I)-OH were obtained.



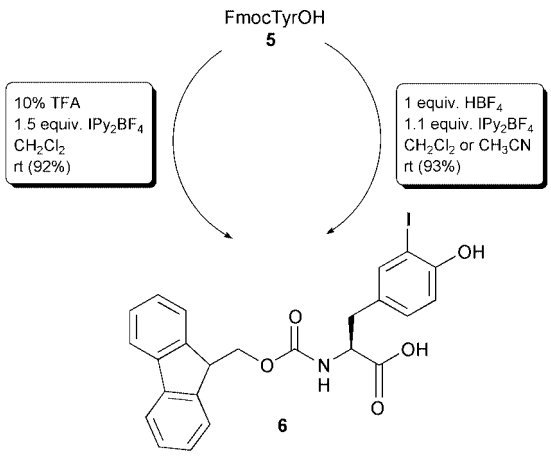
Scheme 1



Scheme 2

Table 1 Conditions assayed for the monoiodination of Fmoc-Tyr-OH

Entry	IPy_2BF_4 (equiv.)	Acid	Solvent
1	2.2	—	CH_3CN
2	1.1	—	CH_3CN
3	1.1	1 equiv. HBF_4	CH_3CN
4	1.1	1 equiv. HBF_4	CH_2Cl_2
5	1.5	10% TFA	CH_2Cl_2



Scheme 3

† Electronic supplementary information (ESI) available: experimental and characterization data for compounds **4**, **6**, **7**–**13**. See http://www.rsc.org/suppdata/cc/b0/b003064n/

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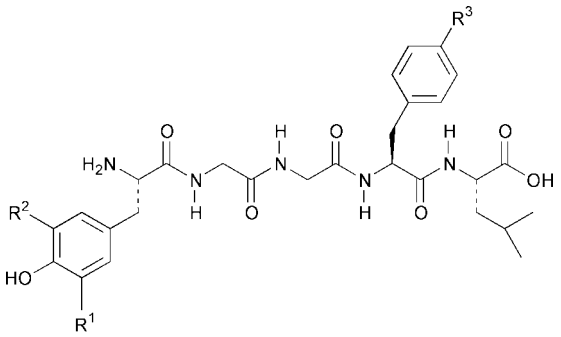
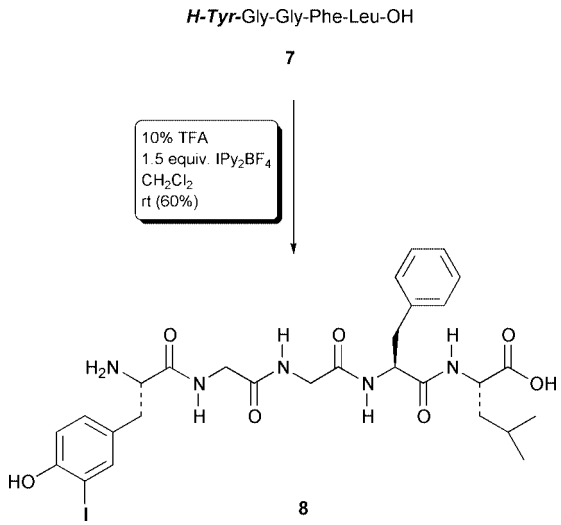


R^1 = H	R^2 = H	R^3 = H	**7** Leu-Enk
R^1 = I	R^2 = H	R^3 = H	**8** [**Tyr(I)**]-I-Leu-Enk
R^1 = H	R^2 = H	R^3 = I	**9** [**Phe(*p*-I)**]-I-Leu-Enk
R^1 = I	R^2 = I	R^3 = H	**10** [**Tyr(I,I)**]-di-I-Leu-Enk

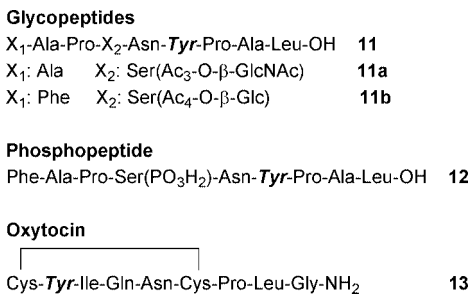
Scheme 4

The potential of this method was also explored on different peptides. The opioid pentapeptide (Leu-enkephalin: H-Tyr-Gly-Gly-Phe-Leu-OH **7**) provided a simple model, showing both Tyr and Phe residues with aromatic side chains that may react towards aromatic electrophilic iodination with our reagent in acidic conditions. To facilitate the identification of the possible iodination products, a series of reference peptides were synthesized from commercially available building blocks. Thus, peptides **8**, **9** and **10** (Scheme 4) were prepared and characterized by RP-HPLC, MALDI-TOF-MS and ^{1}H-NMR. Reaction of **7** in 10% TFA in CH_2Cl_2, which are conditions frequently applied in peptide chemistry, with 1.5 equiv. IPy_2BF_4, afforded the Tyr monoiodinated species **8** as a major product (Scheme 5). The monoiodinated derivative, with an approximate conversion of 60%, was isolated by RP-HPLC and was further characterized by MALDI-TOF-MS and ^{1}H-NMR. After HPLC and NMR data comparison with **8**, **9** and **10** (Scheme 4), no trace of iodination on Phe residues could be observed, which confirmed the selectivity of the reagent towards Tyr side chains.



Scheme 5

These reaction conditions were also successfully tested on biologically active peptides with acid labile post-translational modifications such as in the case of glyco- and phosphopeptides,[10] **11** and **12**, (containing *O*-glycosyl and phosphoseryl residues, respectively) (Scheme 6). This method may also be of potential application on larger and water-soluble peptides and proteins; preliminary evidence was gathered after assaying these iodination conditions on **13** in aqueous solution. Thus, oxytocin, which has only one Tyr residue in its sequence



Scheme 6

(Scheme 6), was reacted in 10% TFA aqueous media with 1.3 equiv. IPy_2BF_4 and the monoiodinated derivative was observed as a major product with 50% conversion. HPLC and MALDI-TOF-MS analysis confirmed the identity of this product, which holds its disulfide bridge in place.

In summary, a method for controlling iodination reactions on activated aromatic residues of peptides is proposed for the first time. The procedure can be carried out in aqueous media and reaction conditions are mild enough for application to acid-labile glyco- and phosphopeptides. Work is in progress to extend these procedures to protein chemistry.

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