Iodination

Regioselective Postsynthetic Modification of Phenylalanine Side Chains of Peptides Leading to Uncommon *ortho*-Iodinated Analogues**

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Interest in therapeutic drugs derived from natural peptides has been steadily increasing as diversity in peptides can be easily implemented by well-established combinatorial

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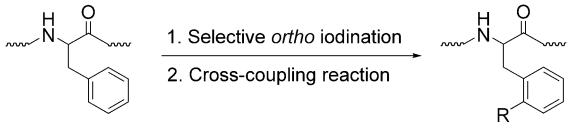
chemistry techniques[1] and because effective ways to improve their bioavailability and resistance to metabolic breakdown by peptidomimetic approaches were found.[2] Among the different strategies to facilitate entry to new conformationally restricted peptidomimetic structures and novel ways of diversity generation, selective and efficient manipulation methods of either the backbone[3] or the various side-chain functions[4] of already preformed peptide sequences are being investigated. These postsynthetic modifications of peptides avoid the use of preformed amino acid building blocks and a linear synthesis for every analogue, which has been the basis of many structure–activity relationship (SAR) studies in peptide chemistry.[5] Thus, these newer drug-discovery approaches depart from a single presynthesized peptide sequence that can be conveniently modified (by applying different types of reactions or the same reaction with different substrates) to yield a series of homologous derivatives in a parallel fashion.

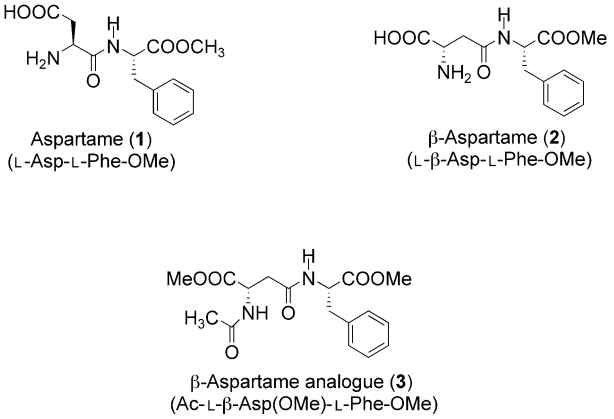
Among the known postsynthetic modifications that could be applied to implement these techniques, the Suzuki, Heck, and Stille type of reactions[6] are especially interesting because Pd^0 catalysis does not compromise peptide stability and is compatible with the multifunctional character of peptides.[7] A common feature among these methods is the need for mainly brominated and iodinated aromatic substrates. To date, general reaction conditions for aromatic electrophilic halogenations of amino acid derivatives and other simple organic molecules are too harsh and result in oxidation and degradation when applied to peptides. This is the main reason for the limited number of methods available for the halogenation of aromatic side chains of presynthesized peptides.[8] In the case of iodination, electrophilic aromatic reagents and milder conditions are only known for the more activated phenolic moiety of Tyr residues, which can be performed, for instance, by the chloramine T method[9] or by the use of the mild and selective iodinating reagent, bis(pyridine) iodonium tetrafluoroborate (IPy_2BF_4),[10] as reported by our group.[11] These iodination methods have never been adapted for the non-activated aromatic side chains of Phe in peptides and direct iodination reactions of such presynthesized peptides have yet to be described. Alternatively, current synthetic methods for halogenated peptides make use of suitable halogenated amino acid building blocks in stepwise linear synthesis protocols. However, the scope of these procedures is restricted by the cost and the number of commercially available halogenated isomeric derivatives of Tyr and Phe. In fact, *ortho*-halogenated Phe derivatives are not commercially available and are also not easy to prepare, as direct electrophilic substitutions always render the *para* derivatives as main reaction products.

Herein we report innovative protocols for the use of IPy_2BF_4 as the first solution for the direct halogenation of phenylalanine-containing peptide sequences and the unusual *ortho* selectivity accompanying this iodination reaction. Furthermore, by overcoming the limitations mentioned above, these results could provide a solution to establish a powerful postsynthetic modification of such peptides with known Pd^0 cross-coupling reactions (Scheme 1).



Scheme 1. Postsynthetic modification of a phenylalanine residue in peptides by sequential iodination/cross-coupling reactions.

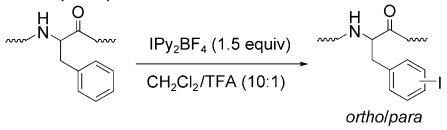
To search for suitable solvents, reaction conditions, peptide/iodonium stoichiometries, and potential acid catalysts, the well-known and readily available dipeptide sweetener aspartame[12] (**1**) and two closely related analogues were selected (Scheme 2). The choice was made to check for possible effects on the iodination reaction of the relative positions of the functional groups in the proximity of the aryl group of Phe.



Scheme 2. Dipeptide models.

We searched for good reaction media that included dichloromethane as the solvent. Trifluoroacetic acid, a broadly used reagent in peptide synthesis, proved to be very valuable as it not only solubilized the peptides, but also provided the required acidic conditions to activate the iodonium reagent. Under these original conditions (Table 1), the reagent is highly effective, and produces the monoiodinated peptide derivatives in short reaction times and preserves peptide integrity. Analysis of crude reaction

Table 1: Monoiodination of model dipeptides promoted by IPy_2BF_4 in the presence of TFA (10%) in CH_2Cl_2.



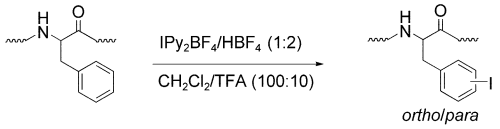
Dipeptide	*ortho*/*para*[a]	*t* [h]	Conversion [%][b]
1	3:1	0.5	87
2	3:1	0.5	91
3	14:1	0.5	90

[a] Determined by NMR spectroscopic analysis. [b] Conversion determined by HPLC.

products by ^{1}H NMR spectroscopy and analytical HPLC showed that mixtures of *ortho*/*para*-iodinated regioisomers (3:1) were obtained as the only reaction products of **1** and **2**. In contrast, the protected analogue **3** gave a 14:1 ratio (Table 1). These results were confirmed by isolation of each isomer by semipreparative HPLC and characterization by ^{1}H and ^{13}C NMR spectroscopy and MALDI-TOF MS techniques. This *ortho* selectivity had not been observed previously in aromatic iodination reactions of simple arene compounds,[13] or even in different protected derivatives of phenylalanine.[11a]

The attractiveness of such a simple entry to the elusive *ortho*-iodo isomer of a phenylalanine derivative prompted us to search for more regioselective reaction conditions. We found that other iodonium-activating acids typically used with IPy_2BF_4 improved the selectivity. The best results were found when we used the same relative amount of TFA (10%) and added the iodonium reagent along with 2 equivalents of HBF_4. Quantitative conversions were observed and the *ortho* isomer was favored over the *para* congener (20:1 in the case of **2**). Most interestingly, the ratio was concentration-dependent: the *ortho*/*para* ratio increased substantially when the reaction was diluted tenfold (Table 2). The proportion of iodonium to HBF_4 (1:2) was very critical to secure high regioselectivity, but did not influence the conversion.[14]

Table 2: Monoiodination of model dipeptides promoted by IPy_2BF_4 in the presence of TFA (10%) in CH_2Cl_2 and HBF_4 (2 equiv).



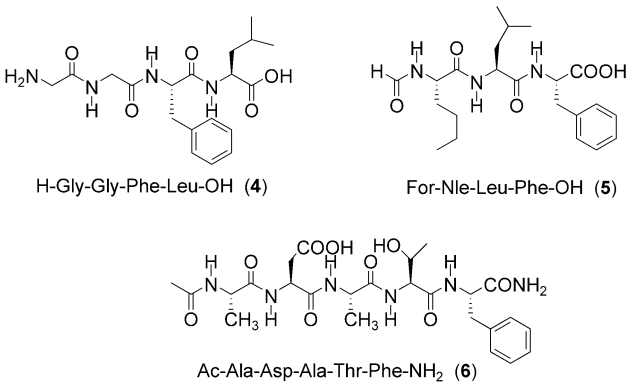
Peptide	*ortho*/*para*[a]	*t* [h]	Conversion [%][b]
1	20:1	0.5	100
2	40:1	0.5	100
3	23:1	0.5	100

[a] Determined by NMR spectroscopic analysis. [b] Conversion determined by HPLC.

The *ortho*/*para* ratio obtained from **2** is twice that obtained from the closely related peptide **1** and from the diprotected analogue **3** (Table 2). This is a good indication of the influence of the structural features surrounding Phe side chains on the regioselectivity of this process.

As mentioned above, *ortho*-iodo derivatives of Phe-containing peptides such as those described herein cannot be prepared by direct iodination or by conventional stepwise peptide elongation methods. Therefore, only *para* analogues are reported in the literature.[15] Furthermore, the potential of running the method on a preparative scale has also been explored. Similar results were observed when the iodination reaction of **2** was performed on a 1-gram scale under the reactions conditions outlined in Table 2 (same ratio, 86% yield).

Because of these encouraging results, three Phe-containing peptides were chosen to test the reaction (Scheme 3): a) **4** is a free tetrapeptide used as an opioid peptide precursor,[16]



Scheme 3. Biologically activity peptides. Nle = norleucine.

b) **5** is a tripeptide that exhibits chemotactic properties,[17] and c) **6** was chosen as a model pentapeptide sequence. All three oligopeptides were synthesized by conventional Fmoc solid-phase protocols. Remarkably, the results observed with these oligopeptides correspond well to those discussed above for the dipeptides (Table 3). In all cases, peptide integrity was preserved, very good conversions were observed, and the peptides yielded mixtures of iodinated isomers as the main reaction products. Again, addition of HBF_4 (method B) was very significant in increasing the *ortho*/*para* ratios. The reaction has proved successful for peptides presenting a single Phe residue. More electron-rich arenes, such as those present in the side chains of Tyr, Trp, and His, are iodinated under these conditions.

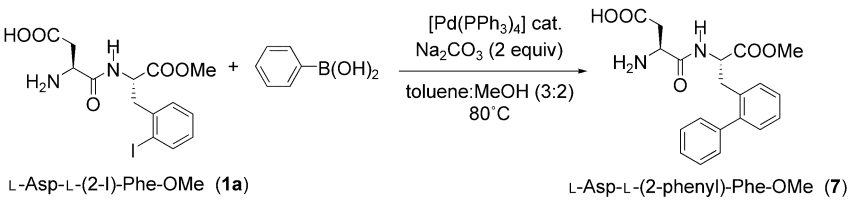
Table 3: Monoiodination of biologically active peptides.

Peptides	Method[a]	*ortho*/*para*[b]	*t* [h]	Conversion [%][c]
5	A	4:1	0.5	100
5	B	7:1	3	100
4	A	3:1	0.5	84
4	B	13:1	3	100
6	A	1:1	0.5	84
6	B	8:1	0.5	90

[a] Method A: 100 mg in CH_2Cl_2/TFA (10:1); IPy_2BF_4 (1.5 equiv). Method B: 100 mg in CH_2Cl_2/TFA (100:10); IPy_2BF_4/HBF_4 (1:2 equiv). [b] Determined by NMR spectroscopic analysis. [c] Conversion based on HPLC.

To illustrate possible applications of these iodinated peptide intermediates, a Pd^0-catalyzed Suzuki-type biphenyl synthesis was attempted (Scheme 4).[7e] The unsually iodinated *ortho* isomer **1a** of aspartame was treated with a simple phenyl boronic acid to yield the constrained *ortho*-phenyl aspartame derivative **7** in good conversion.[18]

In conclusion, a new peptide postsynthetic modification is described. The method provides the first iodination protocol for aromatic side chains of Phe-containing peptides. The procedure is effected by acid-triggered aromatic electrophilic substitution with IPy_2BF_4 to provide *ortho*/*para*-monoiodinated mixtures of peptide analogues mostly quantitatively while preserving peptide integrity. The reaction is sequence- and acid-dependent, a feature that can be used to access the otherwise unavailable *ortho*-iodinated Phe derivatives



Scheme 4. Suzuki cross-coupling reaction of L-Asp-L-(2-I)-Phe-OMe.

through a highly regioselective procedure. We foresee that this new method will find wide applications in Pd^0 chemistry, particularly for the introduction of novel pharmacologically active functionalities into the Phe side chains of peptides, while preparing large arrays of analogues for SAR studies.

Experimental Section

Typical procedures for the synthesis of β-iodoaspartame derivatives:

Method A: The peptide (100 mg, 0.34 mmol) was dissolved in a mixture of CH_2Cl_2 (10 mL) and TFA (1 mL). IPy_2BF_4 (0.51 mmol) was added (the solution turned dark pink). The reaction mixture was stirred for 0.5 h at room temperature. The solvent was removed in vacuo. The pyridinium salt formed in the reaction was removed by filtration through a short column of silica gel (eluent MeOH/TFA 10:1). The resulting solid was washed with diethyl ether and further purified by reversed-phase HPLC.

Method B: The peptide (100 mg, 0.34 mmol) was dissolved in a mixture of CH_2Cl_2 (100 mL) and TFA (10 mL). HBF_4 (0.75 mmol) was added, followed by addition of IPy_2BF_4 (0.37 mmol) (the solution turned dark pink). The mixture was stirred at room temperature for 0.5 h. The reaction was worked up as described above.

RP-HPLC: C18 (Lichrosorb, 250 × 4 mm). Solvents: A = 0.1% TFA in H_2O, B = 0.1% TFA in CH_3CN. Detection: $\lambda = 214$ nm. Elution gradient for aspartame and its derivatives: from 80 to 20% of A in 25 min.

L-β-Asp-L-(2-I)-Phe-OMe: ^{1}H NMR (400 MHz, CD_3OD): $\delta =$ 2.80 (dd, $^3J(H,H) = 9.3$ Hz, $^2J(H,H) = 8.0$ Hz, 1H), 2.99 (dd, $^2J(H,H) = 8.0$ Hz, $^3J(H,H) = 3.6$ Hz, 1H), 3.11 (dd, $^3J(H,H) =$ 13.9 Hz, $^2J(H,H) = 9.5$ Hz, 1H), 3.35 (dd, $^3J(H,H) = 6.1$ Hz, $^3J(H,H) = 5.9$ Hz, 1H), 3.70 (s, 3H), 4.13 (dd, $^3J(H,H) = 9.3$ Hz, $^3J(H,H) = 3.6$ Hz, 1H), 4.82 (dd, $^2J(H,H) = 9.5$ Hz, $^3J(H,H) = 5.9$ Hz, 1H), 6.97 (t, $^3J(H,H) = 7.7$ Hz, 1H), 7.24 (d, $^3J(H,H) = 7.5$ Hz, 1H), 7.32 (t, $^3J(H,H) = 7.5$ Hz, 1H), 7.86 ppm (d, $^3J(H,H) = 7.7$ Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 36.1$ (CH_2), 42.7 (CH_2), 50.9 (CH), 53.0 (OCH_3), 54.0 (CH), 101.0 (C-I), 129.6 (CH), 130.1 (CH), 131.9 (CH), 140.6 (C), 141.0 (CH), 169.4 (CO), 172.6 (CO), 172.9 ppm (CO); MALDI-TOF MS: m/z: 442.8 $[M+Na]^+$ (calculated 443.2); retention time): 10.83 min.

L-β-Asp-L-(4-I)-Phe-OMe: ^{1}H NMR (400 MHz, CD_3OD): $\delta =$ 2.69 (dd, $^3J(H,H) = 9.2$ Hz, $^2J(H,H) = 7.9$ Hz, 1H), 2.92 (m, 2H), 3.21 (dd, $^3J(H,H) = 4.0$ Hz, $^3J(H,H) = 5.0$ Hz, 1H), 3.72 (s, 3H), 4.08 (dd, $^2J(H,H) = 9.5$ Hz, $^3J(H,H) = 4.0$ Hz, 1H), 4.71 (dd, $^2J(H,H) = 9.5$ Hz, $^3J(H,H) = 5.0$ Hz, 1H), 7.03 (d, $^3J(H,H) = 8.4$ Hz, 2H), 7.65 ppm (d, $^3J(H,H) = 8.4$ Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 36.8$ (CH_2), 37.5 (CH_2), 51.3(CH), 53.0 (OCH_3), 55.2 (CH), 93.0 (C-I), 132.3 (2CH), 137.9 (C), 138.9 (2CH), 169.7 (CO), 172.7 (CO), 174.0 ppm (CO); MALDI-TOF MS: m/z: 442.8 $[M+Na]^+$ (calculated 443.2); retention time: 11.99 min

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