A Novel Approach for Iodolabelling Synthetic Peptides

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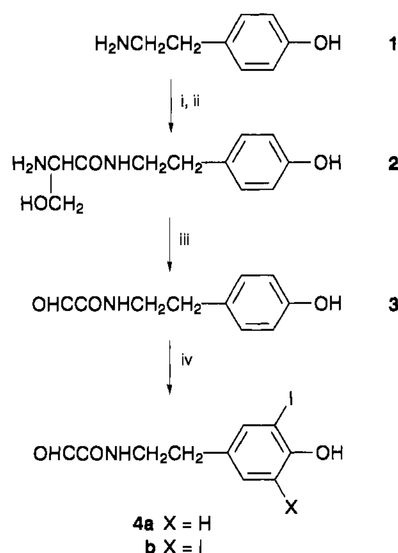
A novel, highly specific iodolabelling reagent, glyoxylyltyramide **3**, and its iodo-substituted derivatives are synthesized and used for the specific labelling of a linker modified Leu–enkephalin analogue.

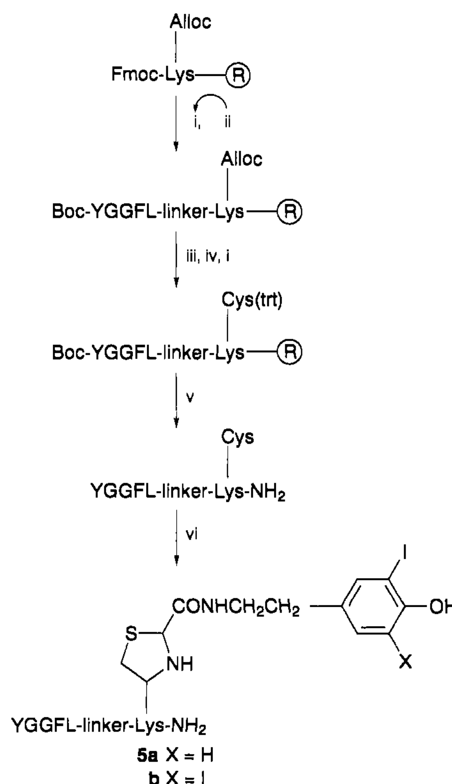
Radioiodolabelled analogues of biologically active peptides and macromolecules are widely use in biochemical investigations, *e.g.* receptor studies, affinity labelling and immunochemistry. Common methods of radioiodination of proteins and peptides include: (*a*) direct electrophilic substitution of iodine on tyrosine or histidine side chains in the presence of a variety of oxidants,[1] and (*b*) indirect iodination using prelabelled reagents such as the Bolton–Hunter reagent [*N*-succinimidyl-3-(4′-hydroxy-3′-[^{125}I]iodophenyl)propionate][2] and the maleimido-based iodination reagent.[3] It is well known that the direct methods often lead to complex mixtures, and the oxidizing reagents are often harmful to peptides and proteins.[4] The most popular indirect iodination approach, the Bolton–Hunter method, is often non-specific, as there are usually numerous primary amino groups in proteins and peptides. The maleimido-based reagents offer more site-specific labelling, mainly due to the lower abundance of cysteine residues in many proteins and peptides. However, when the labelling is caried out in excess of maleimido-based reagents, some of the primary amino groups and the imidazole side chains may also be derivatized.[5] In this communication we report a novel approach in iodolabelling synthetic peptides with a highly specific, and indirect glyoxylyl-based iodolabelling reagent, glyoxylyltyramide **3**.

The synthesis of glyoxylyltyramide and its iodinated derivatives is illustrated in Scheme 1. Acylation of tyramine **1** with Boc-protected serine in the presence of HOBT† and DCC gave the Boc-protected seryltyramide (white solid, 83% yield), which was then deprotected quantitatively to seryltyramide **2** (white solid) with 85% aqueous TFA. Quantitative oxidation of **2** with sodium periodate in phosphate buffer (0.1 mol dm^{-3}, pH = 7) gave glyoxylyltyramide **3** (white solid), which was quantitatively converted to either monoiodo- or diiodo-substituted glyoxylyltyramide **4a** and **4b**‡ using the conventional chloramine T method.[1]



Scheme 1 *Reagents*: i, Boc-Ser-OH, HOBT, DDC; ii, 85% aqueous TFA; iii, $NaIO_4$; iv, Chloramine T, NaI

It has been reported that glyoxylyl group specifically reacts with a weak base such as 1,2-amino thiol to give a thiazolidine ring, and unprotected side chains of lysine, arginine and other amino acids are unaffected.[6] Therefore, the novel glyoxylyl-based iodolabelling reagent (**4a** or **4b**) could be utilized to specifically iodolabel a peptide with a *N*-terminal cysteine or a side chain attached cysteine, leaving the side chains of other amino acids intact. As an example, a leucine–enkephalin analogue (YGGFL-linker-Lys(Cys)-NH_2§) was iodinated by chemical ligation, Scheme 2. The chemical ligation was conducted in acetate buffer (0.02 mol dm^{-3}, pH = 4.2) containing 0.02 mol dm^{-3} EDTA. The enkephalin analogue was quantitatively modified after treating with 2 equiv. of **4b**¶ the product was purified by preparative HPLC and characterized by ion spray MS (MH^+ requires *m*/*z* 1515.4, observed 1515.8). Finally, it is important to note that, in addition to its high specificity, the new reagent can be readily synthesized in bulk quantities. The stability of this compound is also superior to both the Bolton–Hunter reagents and the maleimido-based reagents, because it is stable in both acidic and basic conditions. Furthermore, since this glyoxylyl-based ligation method is gentle and specific for *N*-terminal cysteine, it is possible, in principle, to extend this approach by cloning and expressing a protein with *N*-terminal cysteine as the sole site of specific radioiodination.



Scheme 2 *Regents*: i, 20% piperidine in DMF; ii, Fmoc-amino acids, HOBT, BOP, DIEA; iii, $(Ph_3P)_4Pd$; iv, Fmoc-Cys(trt)-OH, HOBT, BOP, DIAE; v, TFA (85%), EDT (10%), H_2O (2.5%), phenol (2.5%); vi, glyoxylyl-3,5-diiodotyramide **4b**. Ⓡ = Rink amide resin.

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Footnotes

† Amino acids and peptides are abbreviated following rules of the IUPAC–IUB Commission Biochemical Nomenclature in *J. Biol. Chem.*, 1972, **247**, 977. In addition: Fmoc = 9-fluorenylmethyloxycarbonyl, HOBT = 1-hydroxybenzotriazole, BOP = benzotriazol-1-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate, EDT = ethanedithione, DIEA = diisopropylethylamine and Alloc = Allyloxycarbonyl.

‡ The yield of **4b** *vs.* **4a** depends on the amount in excess of NaI and Chloramine T used in the iodination.

§ The synthesis of FMOC-linker-OH will be reported elsewhere. Linker = $NH(CH_2)_3O(CH_2CH_2O)_2(CH_2)_3NHCOCH_2CH_2CO$.

¶ In a control experiment under the same conditions, no modification was observed for a C-terminal cysteine-containing peptide: wGeyidvk-linker-Cys-NH_2§; the lower case single letter in the peptide represent D-amino acids, the studies regarding this peptide will be reported elsewhere.

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