New Single-Step Radioiodination Technique for Peptides: Cu(I)-Catalyzed Nucleophilic Nonisotopic Displacement Reaction. Synthesis of Radioiodinated Deltorphin and Dermorphin Analogues

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Received March 27, 1995 (Revised Manuscript Received August 14, 1995)

The most common technique for radioiodination of peptides is the oxidative radioiodination, using Na*I in the presence of an oxidant, chloramine T, Iodogen, or the lactoperoxidase enzymatic system. Under these conditions, electrophilic hydrogen–iodine exchange readily occurs on activated aromatic rings, such as the phenolic moiety of tyrosine and the imidazole ring in histidine.[1] This reaction, however, has some drawbacks: (i) the method is limited to those amino acids containing activated aromatic rings; (ii) when more than one amino acid bearing an activated aromatic ring (e.g., two Tyr residues) are present, selective iodination is a problem; and (iii) radioiodination of an amino acid residue, embedded in the bioactive sequence of the peptide, can be detrimental to the biological profile. Various examples have been mentioned[2] where incorporation of a (radio)iodine results in loss of biological activity or receptor selectivity. In the δ-opioid receptor antagonist H-Tyr-Tic-Phe-Phe-OH (TIPP), iodination of Tyr at the 3′-position turns the peptide into an agonist.[3] Some of these problems can be avoided by using conjugates, containing an activated aromatic ring (e.g., Bolton–Hunter reagent);[1,4] these small molecules, which are already radioiodinated, are linked to the peptide via the N-terminus or a NH_2 side chain. The use of conjugates can also result in loss of biological activity.

The need for an alternative radioiodination technique for peptides which would be applicable to other aromatic rings (i.e., Phe), therefore, has emerged. Radioiodination via exchange, nucleophilic as well as electrophilic, of an aryl substituent, meets this need. The large benefit of this method is the complete regioselectivity, determined by the position of the original substituent on the aromatic ring. Thus, theoretically, any position on the aromatic moiety can be radioiodinated.

Electrophilic exchange occurs via radioiododemetalation ($-SnR_3$, $-BR_2$, $-SiR_3$): the preparation of the organometallic precursors, mostly a multistep synthesis, is not readily applicable to peptides using standard methodology.[5] Moreover, in the course of the radioiododemetalation reaction, Tyr and His residues may also be radioiodinated. The alternative, which consists of a radioiododestannylation reaction and subsequent coupling of Boc-4′-radioiodophenylalanine tetrafluorophenyl ester, requires that the peptide synthesis is performed with the radioactive amino acid.[6]

The nucleophilic exchange method is very common in the synthesis of labeled pharmaceuticals containing aromatic rings.[1] Summarized, the nucleophilic exchange can occur via: (i) Br/*I exchange in solution (mostly catalyzed by Cu-salts); (ii) Br/*I exchange in the (pseudo)-melt; and (iii) exchange via diazonium salts (Cu-catalyzed). The nucleophilic labeling method, however, has been rarely used for the radioiodination of peptides.[2,7] The two-step procedure involves diazonium salt formation of a [4′-NH_2-Phe]precursor peptide followed by copper-catalyzed substitution using Na*I with typical radiochemical yields after HPLC purification of 25–35%.[2] The selectivity of the diazotization reaction in the presence of other amine functions is not documented.

One of us recently[8] reported a new approach on the nucleophilic exchange of an arylbromide substituent, based on the use of Cu(I) in reducing and acidic conditions, and the benefits of this method as compared to the earlier methods[1]. At the moment, this Cu(I)-catalyzed nucleophilic nonisotopic displacement reaction is a well-established single-step procedure for the preparation of various *I-labeled pharmaceuticals in high radiochemical yields (>70%, labeling yield, i.e., the amount of incorporated radiolabel, >90%).[8] We now report the application of this procedure for the radioiodination of peptides (Scheme 1). This procedure uses a [4′-Br-Phe]precursor peptide **1** which is converted to the corresponding [4′-*I-Phe]labeled peptide **2**.

The peptides chosen for this study are the opioids [D-Ala^2,Glu^4]deltorphin and dermorphin.[9] Radioiodination of the former has already been reported starting from the [4′-NH_2-Phe]precursor by radioiododediazonization resulting in a radiochemical yield of 30–35%.[2,10]

The [4′-Br-Phe]precursor peptides **3** and **4** were prepared by standard solid phase peptide synthesis[11] and were purified by reversed phase HPLC (Tables 1 and 2).

When [D-Ala^2,4′-Br-Phe^3,Glu^4]deltorphin **3** was radioiodinated using Cu(I) catalysis in a 20% aqueous acetic acid solution (method 1),[12] the labeling yield of peptidic material was 69–79%. The labeling yield was determined via reversed-phase HPLC which showed, however, the presence of two labeled peptides as well as two corresponding unlabeled peptides, both in a peak surface ratio of 1:2. The minor compounds were identical with the starting peptide **3** and with the labeled [D-Ala^2,4′-^{131}I-Phe^3,Glu^4]deltorphin **5**. The latter was identified by

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(1) (1) Dewanjee, M. K. In *Radioiodination: Theory, Practice, and Biomedical Applications*; Kluwer Academic Publishers: Boston/Dordrecht/London, 1992.
(2) Sharma, S. D.; Toth, G.; Hruby, V. J. *J. Org. Chem.* **1991**, *56*, 4981.
(3) Schiller, P. W.; Nguyen, T. M.-D.; Weltrowska, G.; Wilkes, B. C.; Marsden, B. J.; Schmidt, R.; Lemieux, C.; Chung, N. N. In *Peptides, Chemistry, Structure and Biology*; Hodges, R. S., Smith, J. A., Eds.; Escom: Leiden, 1994; pp 483–486.
(4) Wilbur, D. S. *Bioconjugate Chem.* **1992**, *3*, 433.
(5) Kabalka, G. W.; Varma, R. S. *Tetrahedron* **1989**, *45*, 6601.
(6) Wilbur, D. C.; HamLin, D. K.; Srivastava, R. R.; Burns, H. D. *Bioconjugate Chem.* **1993**, *4*, 574.
(7) Bossé, R.; Escher, E. In *Peptides 1990*; Giralt, E., Andreu, D., Eds.; Escom: Leiden, 1991; pp 632–634.
(8) Mertens, J.; Gysemans, M. In *New Trends in Radiopharmaceutical Synthesis, Quality Assurance and Regulatory Control*; Emran, A. M., Ed.; Plenum Press: New York, 1991; pp 53–65. This paper also discusses the mechanism of this reaction.
(9) Schiller, P. W. *Prog. Med. Chem.* **1991**, *28*, 301.
(10) Fang, L.; Knapp, R. J.; Matsunaga, T.; Weber, S. J.; Davis, T.; Hruby, V. J.; Yamamura, H. I. *Life Sci.* **1992**, *51*, PL189.
(11) Stewart, J. M.; Young, J. D. *Solid Phase Peptide Synthesis*, 2nd ed.; Pierce Chemical Company: Rockford, IL, 1984.
(12) For solubility reasons, the peptides are best dissolved in AcOH prior to addition of the stock solution.

0022-3263/95/1960-8324$09.00/0

Scheme 1. Radioiodination of a Phenylalanine Residue in a Peptide by Cu(I)-Catalyzed Nonisotopic Substitution

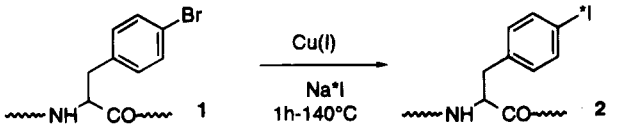


Table 1. Sequence of the Synthesized Peptides

H-Tyr-D-Ala-4′-Br-Phe-Glu-Val-Val-Gly-NH_2	**3**
H-Tyr-D-Ala-4′-Br-Phe-Gly-Tyr-Pro-Ser-NH_2	**4**
H-Tyr-D-Ala-4′-I-Phe-Glu-Val-Val-Gly-NH_2	**5**
H-Tyr-D-Ala-4′-Br-Phe-Glu-Val-Val-Gly-OH	**6**
H-Tyr-D-Ala-4′-Br-Phe-Gly-Tyr-Pro-Ser-OH	**9**

Table 2. Analytical Data of the Synthesized Peptides

	HPLC retention times (min)		FAB-MS data	
peptide	Vydac[a]	LiChroSpher[b]	obsd	calcd
3	7.11	3.70	860/862	860/862
4	13.22	5.74	880/882	880/882
5	8.61	4.4[c]	908	908
6	8.28	4.45	861/863	861/863
7	*d*	5.4[c]	*d*	
8	*d*	7.4[c]	*d*	
9	15.89	6.89	881/883	881/883
10	*d*	8.8[c]	*d*	

[a] Analytical C-18 column (Vydac 218TP54, 1.5 mL/min flow rate); isocratic elution; deltorphin analogues: water/acetonitrile 75/25, 0.1% TFA; dermorphin analogues: water/acetonitrile 60/40, 0.1% TFA. [b] Analytical LiChroSpher column (Merck 50943, 1 mL/min flow rate); isocratic elution; deltorphin analogues: water/acetonitrile 66.5/33.5, 0.1% TFA; dermorphin analogues: water/acetonitrile 45/55, 0.1% TFA. [c] Retention time of the radioiodopeptide. [d] Not determined.

separate peptide synthesis of the "cold" standard, starting from 4′-I-Phe. The major peptides were identified as the corresponding C-terminal carboxylic acids **6** and its labeled analogue **7**. Evidently, in the acidic conditions of the labeling reaction rapid hydrolysis of the C-terminal amide occurs. No hydrolysis of peptide bonds was observed, however, since no peaks of peptide fragments are observed in the HPLC chromatogram. Various experiments were performed to minimize this side reaction (lower reaction temperature, shorter reaction time, DMF/water solvent system). In all cases, however, the generation of the carboxylic acids **6** and **7** was almost linear with the labeling yield. This can seriously hinder the isolation of the labeled amide. Indeed, since the precursor is in large excess, and the acid elutes just before the iodinated amide (Table 2, **5** and **6**), an efficient separation with a minimum of tailing has to be found (Vydac versus LiChroSpher). Submitting peptide amide **3** to the acidic reaction conditions for 5 h (no copper catalyst, no Na*I) resulted in quantitative conversion into the C-terminal acid **6**. During this reaction no racemization is observed, as determined by chiral GC-MS.[13] When the acidity of the labeling medium is decreased by using 70% aqueous ethanol as a solvent (method 2)[14] no deamidation is observed; unfortunately, the labeling yield is lowered to 28%.

The rate of hydrolysis of the C-terminal amide appears to be dependent on the C-terminal amino acid. The labeling reaction of [4′-Br-Phe^3]dermorphin **4** using method 1 results in a labeling yield of 64%, with a amide (starting peptide **4** and labeled **8**) to hydrolyzed compounds (**9** and labeled **10**) ratio of 3:1.

The radioiodination was also performed on the deltorphin–acid analogue **6**, as it also has an interesting biological profile.[15] Application of method 1 yields the radioiodinated peptide **7** with a labeling yield of 49–55% and a radiochemical yield of 29% after HPLC purification using a Vydac C-18 semipreparative column.

A major drawback of the direct radioiodination is undesired oxidation of tryptophan or methionine residues. Under the conditions of method 1, both amino acids are recovered quantitatively, as judged by HPLC amino acid analysis after Fmoc derivatization.

In conclusion, the Cu(I)-catalyzed nucleophilic nonisotopic substitution reaction combines an easy preparation of the [4′-Br-Phe]precursor peptides with high labeling yields. The major limitation is the hydrolysis reaction of a C-terminal peptide–amide, the extent of which appears to be dependent on the C-terminal amino acid. For peptide **3**, lowering the acidity of the reaction medium avoids this side reaction but unfortunately decreases the labeling yield to 28%. In comparison with the iododediazonization method,[2,10] for which the preparation of the precursor requires extra steps, the radiochemical yield is only slightly lower. Oxidative damage to tryptophan or methionine is not observed. Therefore, this new method may have an application domain for peptides which is complementary to the previously described ones.

Materials and Methods

Analytical HPLC of the "cold" peptides was carried on a Spectra Physics system (P4000) using a C-18 column (Vydac 218TP54, 4.6 × 250 mm,1.5 mL/min flow rate). Semipreparative HPLC was carried on a Gilson system (equipped with a Gilson 712 HPLC system controller) using a C-18 column (Vydac 218TP152022, 22 × 250 mm, 20 mL/min flow rate). The HPLC setup (analytical and semipreparative) for the radioiodinated peptides is reported.[16] A LiChroSpher 100 RP-18 (5 μm) analytical column (Merck 50943, LiChroCART 125–4, 4 × 125 mm, 1 mL/min flow rate) and a C-18 semipreparative column (Vydac 218TP510, 10 × 250 mm, 6 mL/min flow rate) were used.

A Na^{131}I solution (Nordion, specific activity = 6.13 × 10^{-8} mmol/mCi) containing 5 mCi in 25 μL 0.01 N NaOH(aq), was diluted with 75 μL of water. From this solution 100 μCi (method 1 and 2) was taken using a syringe and added to the reaction mixture through the septum.

Solid Phase Peptide Synthesis. The peptides **3**, **4**, **6**, and the "cold" standard **5** were prepared by solid phase peptide synthesis.[11] The main-chain amino group was protected with a Boc, the side chain of Glu with a cyclohexyl, the side chain of Tyr (N-terminal Tyr was used unprotected) with a 2-Br-Cbz, and the side chain of Ser with a Bn. Peptides were assembled using the following cycle: (i) deprotection with a TFA solution; (ii) neutralization with base; and (iii) coupling of the following Boc-protected amino-acid using *N,N*-diisopropylcarbodiimide/1-hydroxybenzotriazole. The peptide was cleaved from the resin with HF and the crude peptide was purified by reversed phase HPLC. Analytical data are collected in Table 2.

Radiolabeling Experiment, Method 1. A stock solution, containing 1 mg of $SnSO_4$ (4.7 × 10^{-3} mmol), 25 mg 2,5-dihydroxybenzoic acid (gentisic acid) (1.6 × 10^{-1} mmol), 35 mg of citric acid monohydrate (1.7 × 10^{-1} mmol) in 2 mL of water, and a Cu^{2+}-solution, containing 32.5 mg of $CuSO_4 \cdot 5H_2O$ in 10 mL of water (0.013 M), were prepared. Both solutions were sonicated to dissolve all the material. To 1 mg of [4′-Br-Phe]-precursor peptide (10^{-3} mmol) in 100 μL of AcOH were added 400 μL of stock solution and 60 μL of Cu^{2+} solution, respectively.

(13) Peter, A.; Laus, G.; Tourwé, D.; Gerlo, E.; Van Binst, G. *Peptide Res.* **1993**, *6*, 48. this paper contains all the necessary experimental information for the GC-MS study.
(14) Mertens, J. Unpublished results.
(15) Salvadori, S.; Marastoni, M.; Balboni, G.; Borea, P. A.; Morari, M.; Tomatis, R. *J. Med. Chem.* **1991**, *34*, 1656.
(16) Mertens, J.; Gysemans, M.; Bossuyt-Piron, C.; Thomas, M. *J. Lab. Compds. Radiopharm.* **1990**, *28*, 731.

The reaction vial (1-mL Pierce conical reaction vial) was tightly closed by a septum (Chrompack 12 mm Septum Blue). After sonification until all the peptide was dissolved (vortex, if necessary), the reaction mixture was flushed with N_2 for 5 min and ± 100 μCi of $Na^{131}I$ (6×10^{-9} mmol) was added. The reaction vial was heated during 1 h at 140 °C. An aliqout (10 μL) was taken and diluted with 190 μL of eluent for control by reversed phase HPLC.

Radiolabeling Experiment, Method 2. A stock solution, containing 40 mg of gentisic acid (2.6×10^{-1} mmol) in 4 mL of ethanol/water (70/30, g/g), and a Cu^2 -solution, containing 32.5 mg of $CuSO_4 \cdot 5H_2O$ in 10 mL of water (0.013 M), were prepared. Both solutions were sonicated to dissolve all the material. To a solution of 1 mg of [4′-Br-Phe]precursor peptide (10^{-3} mmol) in 100 μL of ethanol was added 400 μL stock solution and 90 μL of Cu^{2+} solution, respectively. Reaction conditions were as described in method 1. A 10 μL aliquot was analyzed by reversed phase HPLC.

Purification of the Labeled Peptide. The reaction mixture was diluted with 1.5 mL of HPLC eluent, filtered over a Millipore HV filter (0.45 μm), and purified by semipreparative reversed phase HPLC (one injection, isocratic elution, water/acetonitrile/methanol 72/20/8, 0.1% TFA as eluent). The radioiodinated peptide was collected and analyzed by reversed phase HPLC which confirmed the radiochemical and chemical purity (>99%). The overall radiochemical yield after HPLC purification was determined using a γ counter (Vinten, Isocal II, radionuclide assay calibrator).

Acknowledgment. This work was supported by a grant from F.G.W.O. (3.0131.93). The authors thank G. Laus and W. Van Den Nest for the GC–MS study.

JO950599Z

Additions and Corrections

Vol. 59, 1994

Barbara A. Schweitzer and Eric T. Kool*. Aromatic Nonpolar Nucleosides as Hydrophobic Isosteres of Pyrimidine and Purine Nucleosides.

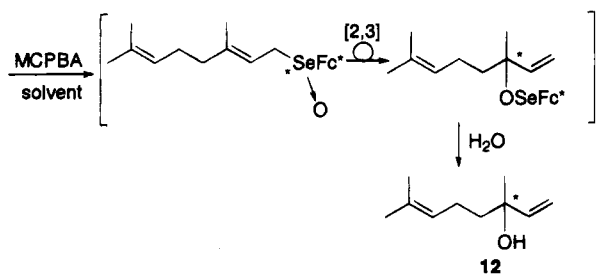
Page 7238. Recent X-ray crystallographic and ^{1}H-NOE data indicate that the stereochemistry of **1** and **2** were incorrectly assigned. Both **1** and **2** as synthesized are in fact α-rather than β-anomers. Compound **3** is correct as shown. The original assignments were made on the basis of correlation with a published proton NMR spectrum of a related phenyl nucleoside and also by correlation of H-1′ coupling constants to known α- and β-nucleosides. We now know, however, that these coupling patterns are generally reversed for aromatic C-deoxynucleosides as compared to N-nucleosides. A complete description of this unexpected finding, and synthesis of the β-anomers, will be published elsewhere. The primary conclusions of the paper, involving the proposed use of nonpolar aromatics as nucleoside isosteres, still stand.

JO9540211

Vol. 60, 1995

Yoshiaki Nishibayashi, Jai Deo Singh, Shin-ichi Fukuzawa, and Sakae Uemura*. [*S*,*R*;*S*,*R*]-Bis[2-[1-(dimethylamino)ethyl]ferrocenyl] Diselenides and Their Application to Asymmetric Selenoxide Elimination and [2,3]Sigmatropic Rearrangement.

Page 4116. The bottom line of Scheme 3 should be as shown below.



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JO954022T

Mark Lautens,* Patrick H. M. Delanghe, Jane B. Goh, and C. H. Zhang. Studies in the Transmetalation of Cyclopropyl, Vinyl, and Epoxy Stannanes.

Page 4214, eq 3. The reaction described in eq 3 (ref 32c) incorrectly attributes this result to Nozaki and coworkers. In fact, ref 32d should be added with the following citation:

32. (d) Tanaka, K.; Minami, K.; Funaki, I.; Suzuki, H. *Tetrahedron Lett.* **1990**, *31*, 2727.

I would like to thank Professor Tanaka for bringing this error to our attention.

JO954023L

Tara J. Sprules and Jean-Francois Lavallée*. Unexpected Contrasteric Alkylation Leading to a Model for Five-Membered Ring Enolate Alkylation: Short Stereoselective Synthesis of (±)-Acetomycin.

Page 5041, column 1, line 5, should read ...center at the β-position of these enolates....

Page 5043, column 2, last line, should read ...the $\Delta G^{\ddagger}_{A\beta}$ and $\Delta G^{\ddagger}_{B\alpha}$ are unequally increased....

JO954024D

Hitomi Suzuki,* Tohru Nakamura, Tohru Sakaguchi, and Kohji Ohta*. A Convenient Synthesis of Functionalized Dibenzotellurophenes and Related Compounds via the Intramolecular Telluro Coupling Reaction. The Positive Effect of Heavy Chalcogen Atoms on the Molecular Hyperpolarizability of a Captodative Conjugation System.

Page 5275, Table 1, R^3 of biphenyl **2c** should be Cl.

JO9540256