Trifluoroacetyl-HYNIC Peptides: Synthesis and ^{99m}Tc Radiolabeling

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Fmoc-lys(HYNIC-Boc)-OH, a precursor for solid-phase synthesis of ^{99m}Tc-labeled peptides, was synthesized efficiently without HPLC purification. HPLC-ESMS showed that deprotection and decoupling of peptide from the resin with trifluoroacetic acid gave initially HYNIC-peptide, which was trifluoroacetylated upon prolonged incubation. The trifluoroacetyl-HYNIC group was hydrolyzed during ^{99m}Tc labeling, rendering deprotection unnecessary. Trifluoroacetyl-HYNIC peptide was ^{99m}Tc-labeled as efficiently, producing the same product, as HYNIC-peptide. These modifications enhance the versatility of HYNIC for ^{99m}Tc peptide labeling.

Introduction

The 6-hydrazinonicotinyl group, known as HYNIC[a], is an attractive bifunctional coupling agent for preparing ^{99m}Tc-labeled peptides and proteins for medical imaging.[1–10] Conventionally, HYNIC is coupled non-site-specifically with amine groups of lysine side chains and N-terminal residues using an activated ester form of Boc-protected HYNIC **1a**, such as the Boc-HYNIC-*N*-hydroxysuccinimide ester **1d** (Scheme 1). This strategy cannot discriminate between different lysine residues and gives non-site-specific labeling. Recently, Greenland et al. demonstrated a "single amino acid chelator" strategy in which the protected amino acid Fmoc-lys(HYNIC-Boc)-OH **2a** is incorporated into peptides during solid-phase peptide synthesis (SPPS).[11] This strategy permits site-specific labeling and combinatorial synthesis, but still presents some difficulties that hinder routine use of HYNIC for ^{99m} Tc labeling: the amino acid chelator **2a** used as a building block requires purification by preparative HPLC, and the HYNIC group protection and deprotection methods are not fully compatible with SPPS conditions. The prevailing method for deprotection of the Boc-HYNIC group and decoupling the peptide from the resin, using trifluoroacetic acid (TFA),[7–9] is unsatisfactory due to the formation of the trifluoroacetyl-HYNIC derivative (e.g., **3c**, see Scheme 2) as a byproduct. An alternative deprotection method using hydrogen bromide in acetic acid afforded lower yields.[9,12,13]

The work described here was undertaken to arrive at a set of convenient and robust deprotection and radiolabeling strategies for use with HYNIC-conjugated peptides and to characterize the trifluoroacetyl-HYNIC derivative as either a useful protected derivative or an unwanted byproduct. The methods are demonstrated using a small peptide radiopharmaceutical, "nanogastrin". Nanogastrin is a truncated form of gastrin comprising the C-terminal domain with the sequence NH_2-Lys-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe$CONH_2$ (see Scheme 2). It was chosen for this work because of its potential applications in imaging tumors that express cholecystokinin type 2 (CCK-2) receptors.[14–19] A ^{99m}Tc-labeled nanogastrin analog incorporating a HYNIC moiety at the N-terminus has been shown to have good receptor binding affinity and serum stability and is a good candidate for in vivo evaluation as an imaging agent.[20] We also report an optimized synthesis of the important precursor Fmoc-lys-(HYNIC-Boc)-OH **2a**,[11] which eliminates the need for HPLC purification.

Results

Synthesis of Fmoc-lys(HYNIC-Boc)-OH 2a. In the literature procedure for the synthesis of technetium-binding amino acid **2a**, a 3:1 molar ratio of the NHS active ester **1d** to Fmoc-lysine is used (Scheme 1), followed by preparative HPLC.[11] However, using a molar ratio of 1.2:1, **2a** was isolated pure in 80–95% yield simply by precipitation from the reaction mixture and washing with water. Spectroscopy and RP HPLC[21] revealed no evidence for the byproduct previously reported[11] that could result from an additional Fmoc-lysine coupling with **2a**.

Synthesis of Nanogastrin Analogs 3a, 3b, and 3c. Synthesis of resin-bound peptide **3a** (Scheme 2) by conventional Fmoc-SPPS, with Fmoc-Lys-HYNIC-Boc **2a** as the final amino acid added, followed by Boc deprotection and decoupling from the resin with TFA/EDT/TIS, produced two soluble peptide fractions separable by HPLC[21] (Figure 1) and identified by ES-MS as **3b** and its trifluoroacetylated form **3c** (Scheme 2). Similar difficulties were encountered during the synthesis of HYNIC-linked sCT.[11] Further investigation of the Boc deprotection of HYNIC was therefore necessary to monitor formation of the free HYNIC peptide and its trifluoroacetylation and to determine whether hydrolysis of the trifluoroacetylhydrazine group is a prerequisite for ^{99m}Tc radiolabeling and, if so, under what conditions it could be achieved.

Fmoc-lys(HYNIC-Boc)-OH **2a** was chosen as a solution-phase model to study the reaction of HYNIC-Boc groups during treatment with TFA in the presence of TIS and EDT (to mimic

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[a] Abbreviations: Boc, *tert*-butyloxycarbonyl; Cbz, benzyloxycarbonyl; DMSO, *N,N*-dimethylsulfoxide; EDDA, 1,2-ethylenediamine-*N,N'*-diacetic acid; EDT, 1,2-ethanedithiol; Fmoc, 9-fluorenylmethoxy-carbonyl;" HPLC, high-pressure liquid chromatography; HYNIC, hydrazinonicotinamido; ITLC, instant thin layer chromatography; MS, mass spectrometry; *m*/*z*, mass to charge ratio; NHS, *N*-hydroxysuccinamide; RP, reverse phase; RT, room temperature; sCT, salmon calcitonin; SPPS, solid-phase peptide synthesis; TFA, trifluoroacetic acid; TIS, triisopropylsilane.

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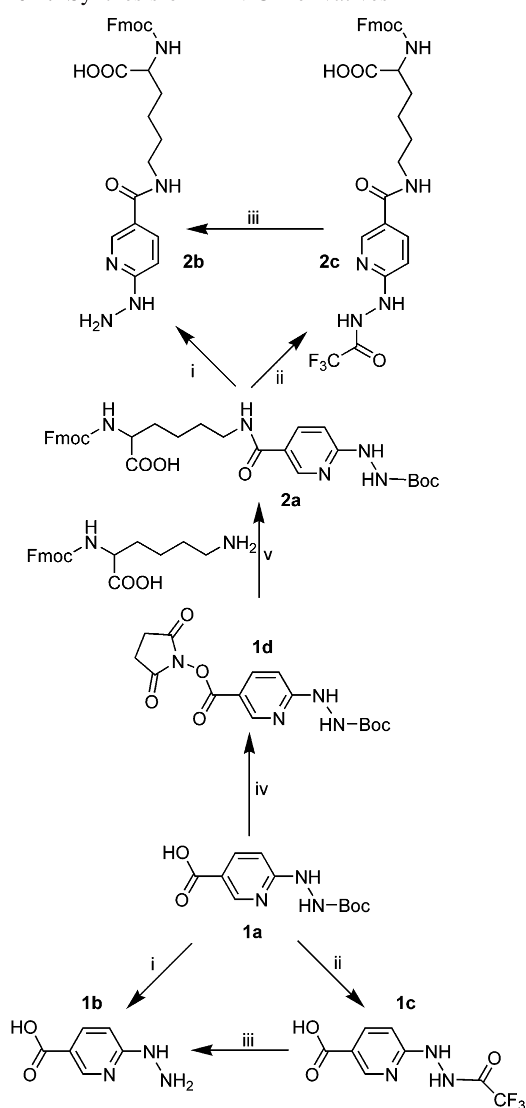
Scheme 1. Synthesis of HYNIC Derivatives[a]



[a] Reagents and conditions: (i) 5 M HCl, RT, 3−5 h, 82% for **1b** and 75% for **2b**; (ii) concd TFA, RT, 15 h; (iii) 1 M HCl, 1 h (88% for **1b** and 85% for **2b**) or 0.1 M NaOH, 30 min; (iv) ref 1; (v) DMSO, 15 h, then H_2O, 50 °C, 1 h, 80−95%.

the SPPS reaction conditions) and in their absence. Samples taken at intervals and analyzed by positive mode RP HPLC-MS (electrospray) showed no significant differences with and without TIS/EDT.[21] Removal of the *tert*-butyloxycarbonyl group to form **2b** was fast: after 10 min, the yield of **2b** (*m/z* 504, $[M + H]^+$) was 84%, and less than 5% of **2a** (*m/z* 604 for $[M + H]^+$) remained (determined from HPLC profile), while Fmoc-lysine-trifluoroacetyl-HYNIC **2c** (*m/z* 600, $[M + H]^+$) was already formed in more than 10% yield. Conversion of **2b** into **2c** continued thereafter, and the transformation to **2c** was almost complete by 50 h (Figure 2). Thus, a short reaction time of 10 min gives **2b** as the desired major product, while a 50 h incubation time yields predominantly the trifluoroacetyl derivative **2c**.

These findings were then applied to optimize synthesis of the HYNIC-Nanogastrin nonapeptide **3b**. Ten minutes treatment of resin bound peptide **3a** with TFA[21] afforded HYNIC-nanogastrin peptide **3b** as the major decoupled product (**3b**:**3c** ratio 20:1, determined from HPLC profile; *m/z* 1281, $[M + H]^+$ for **3b** and 1376, $[M + H]^+$ for trifluoroacetyl-HYNIC-nanogastrin **3c**), but 75% of peptide was still attached to the resin. By 30 min, the decoupling yield had reached 50% and the ratio of **3b** to **3c** in the decoupled product had decreased to 6:1. By 2 h, the peptide was completely detached from the resin, with the **3b** to **3c** ratio of 1.5:1, and after 24 h, trifluoroacetyl-HYNIC-nanogastrin **3c** was the almost exclusive product. An optimized reaction time of 7 h afforded trifluoroacetyl-HYNIC-nanogastrin **3c** in approximately 95% yield. Thus, essentially homogeneous trifluoroacetyl-HYNIC-nanogastrin **3c** can be produced quantitatively by extending the incubation time during the resin-decoupling step. We therefore investigated methods for efficient removal of the trifluoroacetyl group (and indeed whether this is actually necessary for effective ^{99m}Tc labeling), as well as alternative Boc deprotection methods that do not involve TFA.

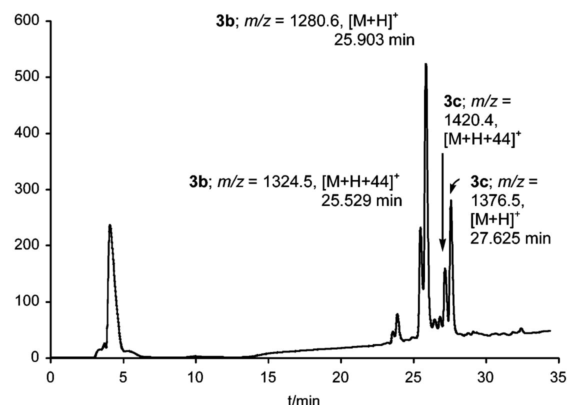


Figure 1. RP HPLC profile of the crude nanogastrin mixture after incubation with TFA for 2 h. The peak with a retention time of 4.5 min is acetic acid. Peaks with retention times of 25.5 and 27.2 min, respectively, correspond to the presence of the CO_2 moiety on the Trp residue, as a result of Boc-deprotection during SPPS. Both peaks subsequently disappear upon freeze-drying under high vacuum.

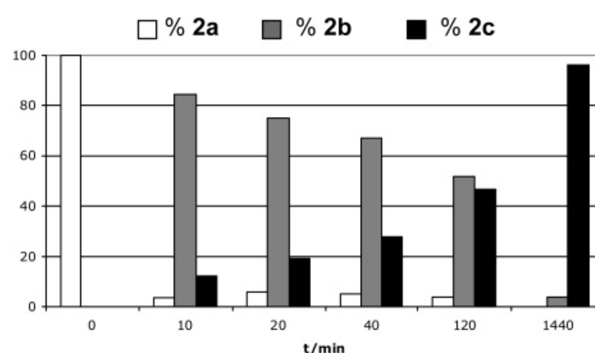
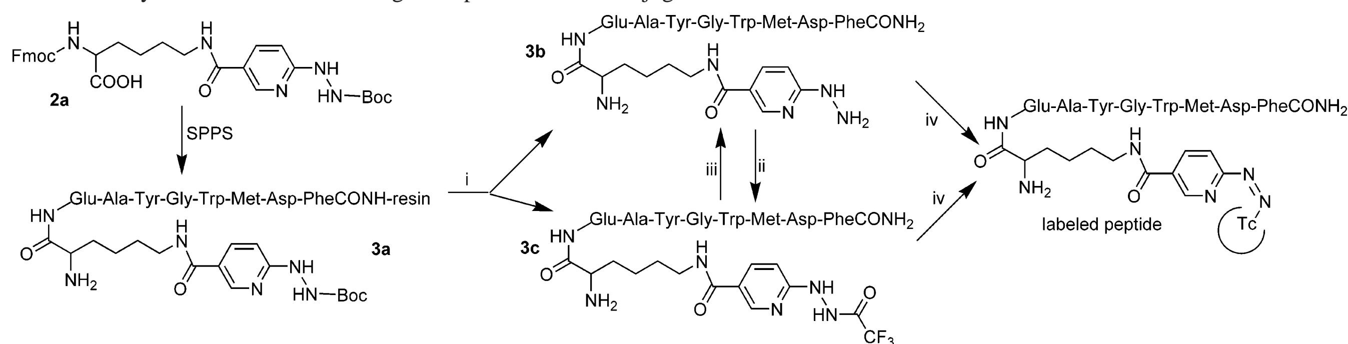


Figure 2. Time course study for the transformation of Fmoc-lys-(HYNIC-Boc)-OH **2a** (white) with TFA in the presence of TIS and EDT to give first **2b** (gray) then **2c** (black). Similar results were obtained in the absence of TIS and EDT.

Methods for Removal of Boc and TFA Groups from HYNIC. Overnight treatment of the Boc-protected compounds **1a** and **2a** with 5 M hydrochloric acid affords the deprotected products **1b** and **2b** in 75 and 82% isolated yield, respectively (Scheme 1). This method may find application when TFA is not essential. However, in the present context, TFA is needed to decouple the peptide from the resin and to deprotect other residues, therefore, efficient methods for hydrolyzing the trifluoroacetyl-HYNIC group were also sought. Treatment of **1c** and **2c** with 1 M hydrochloric acid for 1 h furnished the free HYNIC derivatives **1b** and **2b** in 88 and 85% yield, respectively. Base hydrolysis was also efficacious: treatment of **3c** with 0.1 M NaOH for 30 min at room temperature afforded **3b** quantitatively (as determined by RP-HPLC-MS).

Scheme 2. Synthesis and Radiolabeling of Peptide-HYNIC Conjugates[a]



[a] Reagents and conditions: (i) concd TFA, TIS, EDT, RT, 30 min; (ii) concd TFA, TIS, EDT, RT, 7 h; (iii) 1 M HCl, 1 h or 0.1 M NaOH, 30 min; (iv) aq. tricine, $^{99m}TcO_4^-$, $SnCl_2$, 95 °C, 30 min.

^{99m}Tc Radiolabeling of HYNIC-Nanogastrin 3b. A process of optimization of the ^{99m}Tc-radiolabeling of the nontrifluoroacetylated HYNIC-peptide **3b** by varying the reagent concentration, pH, reaction temperature, and time led to the labeling procedures described in the Experimental Section, which gave high labeling efficiency, as determined using HPLC (>95% labeled peptide) and thin layer chromatography (<0.1% ^{99m}Tc colloid, <5% pertechnetate and labeled coligands). The use of EDDA and tricine as coligand in an exchange labeling approach gave a chromatogram[21] with a single dominant radioactive peak at 20.8 min, while combined use of tricine and nicotinic acid gave a dominant radioactive peak at 21.1 min, with an additional small peak (20.3 min) corresponding to a ^{99m}Tc peptide−tricine complex.[21] The use of tricine alone as coligand shows two main peaks with retention times of 20.0 and 20.3 min, respectively.[21]

^{99m}Tc Radiolabeling of Trifluoroacetyl-HYNIC Derivatives. The conditions identified above for removal of trifluoroacetyl groups from the HYNIC derivatives are mild, raising the possibility that typical conditions under which HYNIC-derivatized peptides are labeled with ^{99m}Tc will also release the free HYNIC group from trifluoroacetyl-HYNIC groups, thus allowing convenient ^{99m}Tc-radiolabeling in one step. Trial radiolabeling experiments were therefore performed using tricine as coligand with the three trifluoroacetyl-HYNIC compounds (**1c**, **2c**, and **3c**) to hand, compared to their free hydrazine-containing analogs **1b**, **2b**, and **3b**. Using the labeling conditions described above, the labeled HYNIC-conjugated peptides **3b** and **3c** gave virtually identical radiochemical yield/purity and radiochromatograms.[21] Radiochromatograms of radiolabeled HYNIC (**1b**) and trifluoroacetyl-HYNIC (**1c**), respectively, were also identical to one another, each showing four peaks with the same set of retention times (though different relative abundances).[21] Similarly, when Fmoc-lysine-HYNIC **2b** and its corresponding TFA-derivative **2c** were radiolabeled, both chromatograms showed two peaks with the same two elution times, possibly corresponding to isomeric complexes.[21] Hence, using the same radiolabeling conditions, all three trifluoroacetyl-HYNIC compounds can be labeled as efficiently (radiochemical purity of >80%, >99.8%, and >99.8%, respectively) as their free HYNIC counterparts without any prior hydrolysis step.

Discussion

The simplified, more efficient synthesis of the important Tc-binding amino acid **2a** described here eliminates the need for chromatographic purification, facilitating the wider adoption of the "single amino acid chelator" strategy. The remainder of this work addresses the problem that the organohydrazine group is not entirely compatible with the conditions of solid-phase peptide synthesis, because treatment of HYNIC-Boc-derivatized peptides with TFA (the conventional methodology[7,22] both for decoupling from the support resin and for removing the Boc protecting group) gives rise to trifluoroacetyl-HYNIC, as well as the requisite free HYNIC compounds.[8,9,11] Trifluoroacetyl hydrazine derivatives are not extensively described in the literature. The two trifluoroacetyl-HYNIC compounds reported to date, each characterized only by mass spectrometry, are 6-trifluoroacetylhydrazinopyridine-3-[*N*-(2-diethylaminoethyl)]-carboxamide[8] and trifluoroacetyl-HYNIC-sCT.[11] A similar compound, *N*-2-pyridyl-*N′*-trifluoroacetylhydrazine, was synthesized by reaction of 2-pyridylhydrazine with trifluoracetic anhydride.[23] Methods for removal of the trifluoroacetate group prior to radiolabeling have not been reported previously.

The results described here show that the formation of trifluoroacetyl-HYNIC derivative during deprotection/decoupling is not a barrier to production of pure peptides for labeling with ^{99m}Tc. If TFA treatment is not required to remove other protecting groups, formation of trifluoroacetyl-HYNIC can be avoided by using 5 M hydrochloric acid to remove the Boc protecting group. During SPPS, if TFA decoupling or deprotection of other residues is essential, then HYNIC trifluoroacetylation can be minimized by keeping the TFA incubation time to a minimum, although a large fraction of the peptide remains resin-bound. In light of the above results, it is better to prepare the fully trifluoroacetylated form in high yield by extending the TFA incubation time. The trifluoroacetyl group can then be removed readily using 1 M hydrochloric acid for 1 h or 0.1 M NaOH for 0.5 h, although this will normally be unnecessary because we have also shown that the hydrolysis is efficiently achieved under typical ^{99m}Tc labeling conditions.

Hydrolysis under labeling conditions is a fortuitous attribute of the trifluoroacetyl-HYNIC group, making it a convenient masked form of HYNIC. The trifluoroacetyl group has been shown to offer protection of HYNIC under the oxidative conditions required to form disulfide bonds in synthetic peptides.[11] If this protection can be shown to be effective against other undesirable side reactions that occur during synthesis, storage, and manipulation of HYNIC-peptides, the trifluoroacetyl-HYNIC group may offer a very versatile and convenient protected form of HYNIC. This would be of value because current protection strategies remain problematic. For example, Rajopadhye et al. reported low yields of free HYNIC-peptides obtained from Boc-deprotection using TFA.[9] Use of alternative groups, benzyloxycarbonyl (Cbz) and Fmoc,[9] and an alternative deprotection method for Boc[9,12,13] (using hydrogen bromide/acetic acid) also resulted in relatively low yields. Harris et al. coupled a series of hydrazone-protected succinimidyl HYNIC derivatives to a presynthesised peptide before radiolabeling. The hydrazone afforded protection of HYNIC against reaction with

aldehyde and ketone impurities during storage and, like the trifluoroacetyl derivative, was readily radiolabeled without an additional deprotection step.[22] Therefore, work is now in progress to evaluate the utility of trifluoroacetyl as a protecting group in comparison with hydrazones of HYNIC derived from aldehydes.

Conclusion

Use of the trifluoroacetyl-HYNIC group reported above will significantly enhance the flexibility and versatility of the HYNIC approach to peptide labeling. Importantly for the efficient development of peptide radiopharmaceuticals, trifluoroacetyl-HYNIC derivatives such as **1c**, **2c**, and **3c** can be used directly for ^{99m}Tc radiolabeling with high efficiency without need of an additional hydrolysis step to free the HYNIC functionality. Indeed, we would now advocate use of HYNIC-peptides routinely in their trifluoroacetylated form for kit-based ^{99m}Tc labeling, thus turning the trifluoroacetylation of HYNIC from an undesirable side reaction during peptide synthesis[7-9] into a positive advantage.

Experimental Section

NMR spectra were recorded on a 270 MHz JEOL GSX 270 FT spectrometer (^{1}H, 270 MHz; ^{13}C, 67.5 MHz; ^{19}F, 254.1 MHz). ^{1}H NMR spectra were referenced to the residual solvent signals $(CD_3)_2SO$ and CD_3OD (δ 2.50 and 3.31 ppm, respectively, downfield from Me_4Si). ^{13}C NMR spectra were referenced to solvent signals $(CD_3)_2SO$ (δ 39.50) and CD_3OD (δ 49.00). ^{19}F NMR spectra were referenced to $CFCl_3$. IR spectra were recorded on a Thermo Nicolet Corporation Avatar 360 FT-IR spectrometer, with samples prepared as KBr discs. Mass spectra and accurate mass (HRMS) measurements were recorded on a VG ProSpec or VG ZabSpec spectrometer (EI) and Micromass LCT TOF spectrometer (ES; Mass Spectrometry Facility, School of Chemistry, University of Birmingham, U.K.). Electrospray ionization mass spectra (ES-MS) were obtained with a Finnigan Mat LCQ ion trap mass spectrometer coupled to a Hewlett-Packard 1100 HPLC system for LCMS. Solid-phase peptide synthesis of the nanogastrin peptides was performed using conventional Fmoc chemistry as previously described[11] (see Supporting Information). Solvents used were of Analar quality and purchased from the following suppliers: triisopropylsilane, Acros Organics, U.K.; ethanedithiol, ethylenediamine-*N*,*N*'-diacetic acid, and nicotinic acid, Aldrich Chemicals, U.K.; *N*-*R*-Fmoc-Lys ("Fmoc-lysine"), Novabiochem, U.K.; trifluoroacetic acid and tricine, Sigma, U.K.; concentrated and 1 M hydrochloric acid, BDH Chemicals Ltd., U.K. $Na^{99m}TcO_4$ was eluted from a ^{99}Mo/^{99m}Tc generator (Mallinckrodt, Petten, The Netherlands) with 0.9% saline.

Synthesis of HYNIC Compounds and Trifluoroacetyl-HYNIC Derivatives. Trifluoroacetylhydrazinonicotinic Acid (1c): A solution of Boc-hydrazinonicotinic acid **1a**[1] (250 mg, 0.988 mmol) in TFA (5 mL) was stirred at room temperature for 15 h and then evaporated under reduced pressure. The crude cream solid obtained was then stirred in ether (25 mL) and filtered off, washed further with ether, and dried under high vacuum to afford **1c** as a pale yellow powder (118 mg, 78% yield). Analytical and spectroscopic data are given as Supporting Information.

N^{α}-(Fmoc)-N^{ϵ}-(t-butoxyhydrazinonicotinyl)-lysine (2a): To a solution of Boc-HYNIC-NHS ester **1d**[1] (1 g, 2.88 mmol) in DMSO (80 mL) was added Fmoc-lysine (878 mg, 2.40 mmol) in small batches while stirring at room temperature. A clear yellow solution was obtained after 1 h. After 15 h, water (150 mL) was added with stirring for 1 h at 40 °C to generate a white precipitate, which was filtered and washed with warm water (2 × 30 mL) to afford **2a** as a white solid (1.60 g, 92%). Analytical and spectroscopic data are given as Supporting Information.

N^{α}-(Fmoc)-N^{ϵ}-(hydrazinonicotinyl)-lysine (2b): The desired product was prepared in a similar manner to **1b**[21] using **2a** as starting material instead of **1a** (145 mg, 75% yield). It was also prepared in a similar manner to **1b** using Fmoc-lysine-HYNIC-TFA **2c** as starting material instead of **1c**[21] (164 mg, 85% yield). Analytical and spectroscopic data are given as Supporting Information.

N^{α}-(Fmoc)-N^{ϵ}-(trifluoroacetylhydrazinonicotinyl)-lysine (2c): The desired product was prepared in a similar manner to **1c** using **2a** as starting material instead of **1a** (122 mg, 80% yield). Analytical and spectroscopic data are given as Supporting Information.

Time Course Investigation of the Incubation of Fmoc-lys-(HYNIC-Boc)-OH 2a with TFA: To a concentrated TFA solution (2 mL, containing 2 μL of triisopropylsilane and 7 μL of ethanedithiol, where applicable) was added **2a** (50 mg, 83 μmol), while stirring at room temperature. Samples in the amount of 20 μL were taken at intervals[21] and diluted in 480 μL of DMSO/water (1:1; v/v) before analyzing by RP HPLC-MS (positive mode electrospray, Finnigan Mat LCQ ion trap mass spectrometer coupled to a Hewlett-Packard 1100 HPLC system running HPLC method 1).[21]

Time Course Investigation of the Incubation of Resin-Bound HYNIC-Nanogastrin 3a with TFA: Three batches of 73 mg of peptide-bound resin were used for each experiment. One was treated with TFA for 10 min, washed (filtrate washings were collected and analyzed separately), and dried before being subjected to TFA hydrolysis again for a further 20 min. The other two batches were treated separately for 2 and 24 h, respectively. The crude peptide washed from the resin was then analyzed by RP HPLC-MS, as described above for the experiment with **2a**.

Optimized Method for HYNIC-Nanogastrin 3b: The peptide was released from the resin and side chains deprotected by treatment with 95% TFA, 2.5% H_2O, and 2.5% TIS for 30 min and isolated by precipitation in ice-cold diethyl ether, centrifuging and washing twice with ice-cold diethyl ether. The pellet was then dissolved in water and recovered by freeze-drying. HYNIC-nanogastrin **3b** was further purified by RP HPLC, using the Hewlett-Packard instrument running HPLC Method 2.[21] Peptide **3b** and the trifluoroacetyl-HYNIC by product **3c** were eluted at 22.11 and 23.78 min, respectively, and were obtained in 85% and 15% relative yield, respectively (50% overall yield); elution time, 22.11 min; *m/z* (ES$^+$), 1303 (15%, $[M + Na]^+$) and 1281 (100, $[M + H]^+$).

HYNIC-nanogastrin **3b** was also prepared by adding trifluoroacetyl-HYNIC-nanogastrin **3c** (0.3 mg, 0.5 μmol) to 1 mL of aqueous 0.1 M NaOH. The solution was stirred at room temperature for 30 min, then brought to neutral pH by dropwise addition of 0.5 M aqueous HCl. The resulting solution was freeze-dried, and the residue was purified by RP HPLC (as described above) to afford **3b** quantitatively.

Optimized Method for Trifluoroacetyl-HYNIC-Nanogastrin 3c: Peptide **3c** was prepared in a similar manner to **3b**, except that treatment of the resin-bound peptide with 95% TFA, 2.5% H_2O, and 2.5% TIS lasted 7 h instead of 30 min; elution time, 23.78 min; *m/z* (ES$^+$), 1398 (20%, $[M + Na]^+$) and 1376 (100, $[M + H]^+$).

^{99m}Tc Radiolabeling of HYNIC-Nanogastrin Conjugates and HYNIC Derivatives. Tris(hydroxymethyl)methylglycine(tricine) as Coligand. In a screw top 2.5 mL polypropylene Corning vial, 10 μg of **1b**, **1c**, **2b**, **2c**, **3b**, or **3c** in water was incubated with 0.5 mL of tricine solution (100 mg/mL in water), 0.5 mL of $^{99m}TcO_4^-$ solution (>200 MBq), and 10 μL of stannous chloride dihydrate solution (3 mg/mL in ethanol) for 30 min at 95 °C.

Tricine/Ethylenediaminediacetic Acid (EDDA) as Coligand. In a screw top 2.5 mL polypropylene Corning vial, 10 μg of **3b** in water was incubated with 0.25 mL of tricine solution (20 mg/mL in 0.3 M sodium dihydrogen phosphate), 0.25 mL of EDDA solution (10 mg/mL in 0.1 M NaOH), final pH 6.0, 0.5 mL of $^{99m}TcO_4^-$ solution (>200 MBq), and 10 μL of stannous chloride dihydrate solution (3 mg/mL in ethanol) for 30 min at 95 °C.

Tricine/Nicotinic Acid as Coligand. In a screw top 2.5 mL polypropylene Corning vial, 10 μg of **3b** in water was incubated with 0.4 mL of tricine solution (100 mg/mL in water), 0.1 mL of nicotinic acid (90 mg/mL in water), 0.5 mL of $^{99m}TcO_4^-$ solution (>200 MBq), and 10 μL of stannous chloride dihydrate solution (3 mg/mL in ethanol) for 30 min at 95 °C.

Radioanalytical Methods. Radiolabeled peptides and HYNIC derivatives were analyzed by HPLC using a Beckman System Gold running 24 karat proprietary software and a Beckman 168 UV detector in series with a GABI radioactivity monitor (Raytest). The system was running HPLC method 3.[21] ITLC was performed on silica gel (ITLC-SG, Gelman Sciences, Ann Arbor, MI), with saline as eluent, for detection of ^{99m}Tc-pertechnetate and ^{99m}Tc-labeled coligands and 50% acetonitrile−water solution for determination of ^{99m}Tc colloid.

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Supporting Information Available: RP HPLC-MS profile of purified Fmoc-lys(HYNIC-Boc)-OH **2**; table illustrating the time course study of the incubation of Fmoc-lys(HYNIC-Boc)-OH **2a** with TFA in the presence/absence of a catalytic amount of TIS and EDT; preparation and analytical data for HYNIC derivatives **1b**, **1c**, **2a**, **2b**, and **2c**; solid-phase peptide synthesis methods, chromatograms to show purity of peptides **3b** and **3c**, and radiochromatograms comparing labeling of HYNIC conjugates and their trifluoracoetylated derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

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