Trifluoroacetyl-HYNIC peptides: Synthesis and ^{99m}Tc radiolabeling

M. Bashir-Uddin Surfraz[a], Robert King, Stephen J. Mather, Stefano C.G. Biagini and Philip J. Blower

Supporting information

Page S1

Contents:

S2 **Figure A**. HPLCanalysis of purified **2a**

S3 **Table A.** Time course study of the incubation of Fmoc-lysine-HYNIC-Boc **2a** with TFA in presence/absence of catalytic amount of TIS and EDT

S4 **Table B.** Time course data for peptide cleavage from resin (**3a**) to give free HYNIC-nanogastrin **3b** and trifluoroacetylated HYNIC-nanogastrin **3c**

S5 **Figure B: a)** HPLC chromatogram for the ^{99m}Tc labeling of nanogastrin-HYNIC **3a** with EDDA, tricine/nicotinic acid, and tricine as co-ligands

S6 **Figure C:** Radiochromatograms for the ^{99m}Tc labeling of HYNIC **1b** and ^{99m}Tc labeling of trifluoroacetylHYNIC **1c**

S7 **Figure D:** HPLC radiochromatograms for the ^{99m}Tc labeling of Fmoc-lysine-HYNIC **2b**, Fmoc-lysine-HYNIC-TFA **2c**, and nanogastrin-HYNIC-TFA **3c**, all with tricine as co-ligand.

S8 **Figure E.** HPLC radiochromatograms for the ^{99m}Tc labeling of nanogastrin-HYNIC **3b** and nanogastrin-HYNIC-TFA **3c** with tricine as co-ligand, under identical labeling conditions.

S9 Preparation and analytical data for *Hydrazinonicotinic acid (1b)*

S10 Table of analytical data for *Trifluoroacetylhydrazinonicotinic acid (**1c**)*

S11 Analytical data for N^{α}-(Fmoc)-N^{ε}-(t-butoxyhydrazinonicotinyl)-lysine (**2a**)

S12 Analytical data for N^{α}-(Fmoc)-N^{ε}-(hydrazinonicotinyl)-lysine (**2b**)

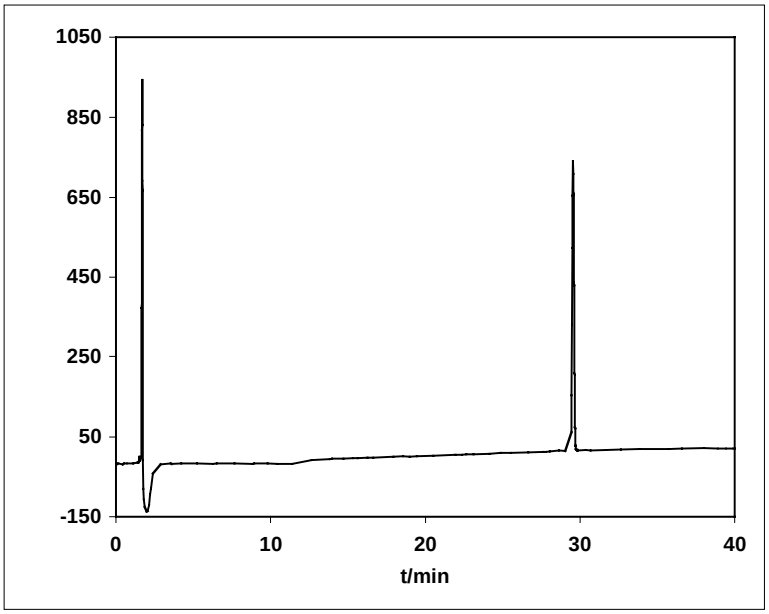
S13 Analytical data for N^{α}-(Fmoc)-N^{ε}-(trifluoroacetylhydrazinonicotinyl)-lysine (**2c**)

S14 Experimental details for solid phase peptide synthesis

S15 **Figure F.** HPLC analysis of purity for peptide **3b**

S16 **Figure G.** HPLC analysis of purity for peptide **3c**

S17 HPLC methods



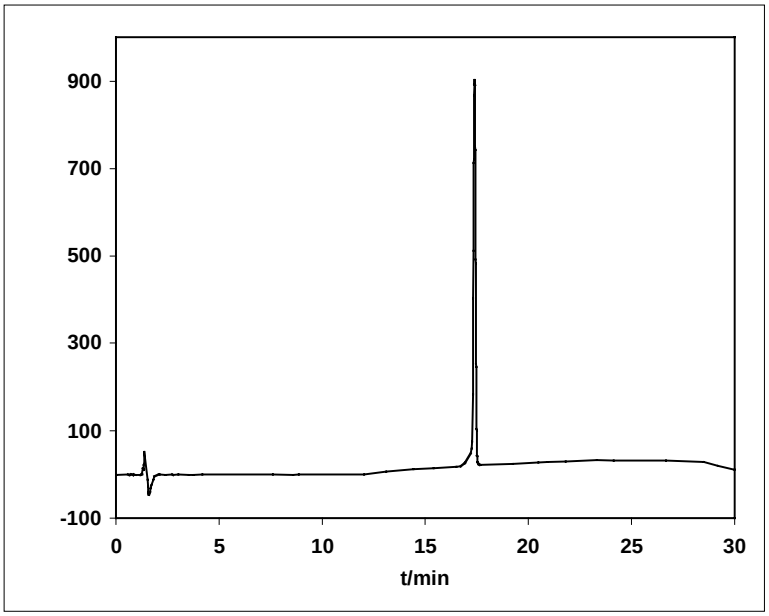


Figure A: top: RP HPLC of Fmoc-lysine-HYNIC-Boc **2a** using Method 4 (see p. S16); wavelength 214 nm; percentage purity: 100%; retention time: 29.6 min. Bottom: RP HPLC of Fmoc-lysine-HYNIC-Boc **2a** using Method 5 (see p. S16); wavelength 214 nm; percentage purity: 100%; retention time: 17.4 min.

Table A. Time course study of the incubation of Fmoc-lysine-HYNIC-Boc **2a** with TFA in presence/absence of catalytic amount of TIS and EDT

t/min	% of **2a** (*)	% of **2b** (*)	% of **2c** (*)
0	100 (100)	0 (0)	0 (0)
10	3.6 (4.0)	84.4 (79.5)	12.0 (16.5)
20	6.0 (3.2)	74.9 (75.7)	19.1 (21.1)
40	5.1 (0)	67.3 (73.8)	27.6 (26.2)
120	4.1 (0)	51.6 (54.8)	46.3 (45.2)
1440	0 (0)	14.9 (12.1)	85.1 (87.9)
3000	0 (0)	3.9 (4.2)	96.1 (95.8)

(*) Data obtained in the absence of TIS and EDT

Table B. Time course data for peptide cleavage from resin (**3a**) to give free HYNIC-nanogastrin **3b** and trifluoroacetylated HYNIC-nanogastrin **3c**

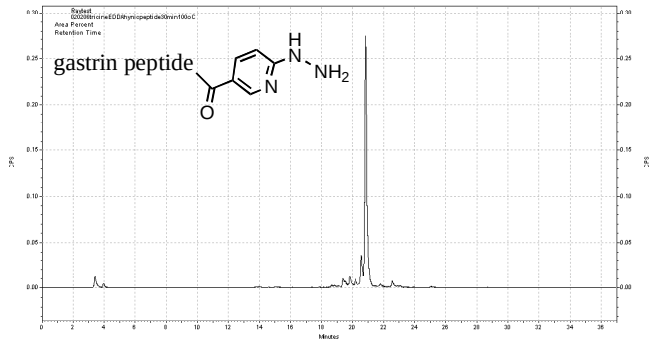
t/min	% of **3b**	% **3c**	overall estimated % decoupling yield
10	95.2	4.8	24.3
30[*]	84.6	15.4	49.1[**]
120	61.4	38.6	quantitative
1440	5.6	94.4	87.0

[*]The resin recovered from the 10 min incubation was incubated once more with TFA for an extra 20 min.

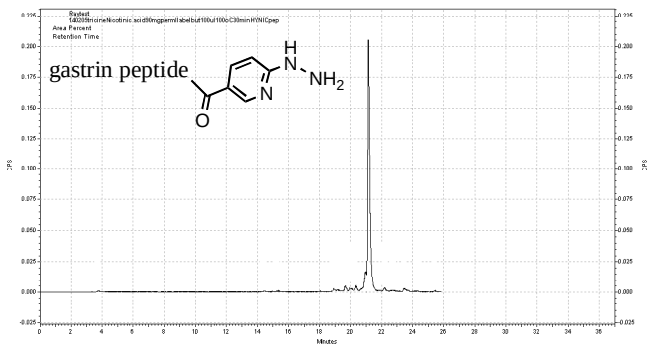
[**]Cumulative.

Figure B: a) HPLC chromatogram for the ^{99m}Tc labeling of nanogastrin-HYNIC **3a** with EDDA as co-ligand; **b)** HPLC chromatogram for the ^{99m}Tc labeling of nanogastrin-HYNIC **3a** with tricine/nicotinic acid as co-ligands; **c)** HPLC chromatogram for the ^{99m}Tc labeling of nanogastrin-HYNIC **3a** with tricine as co-ligand.

a)



b)



c)

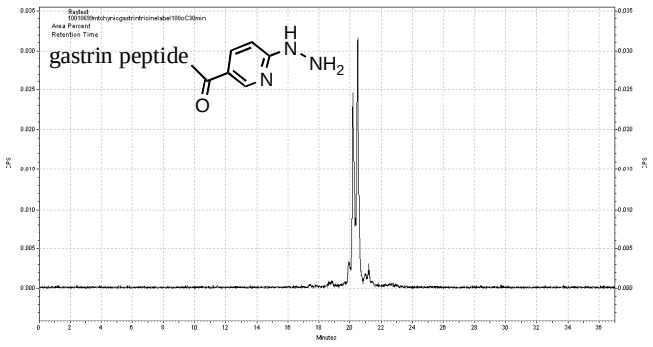
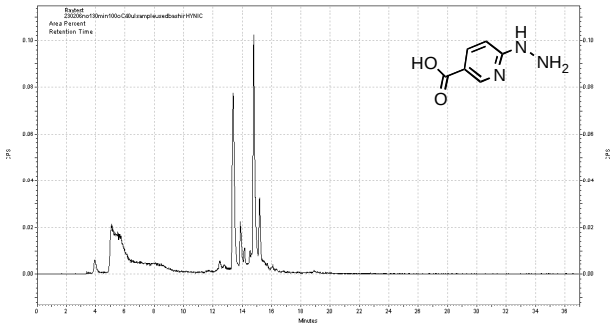


Figure C: a) Radiochromatogram for the ^{99m}Tc labeling of HYNIC **1b**; **b)** Radiochromatogram for the ^{99m}Tc labeling of trifluoroacetylHYNIC **1c**

a)



b)

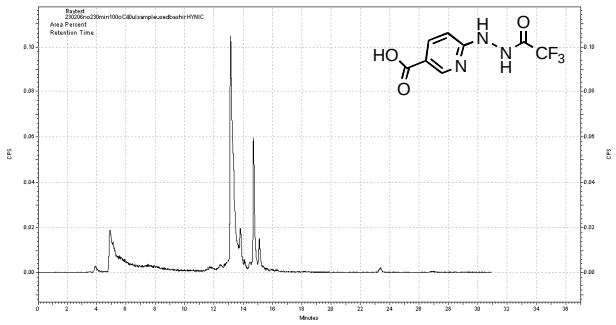
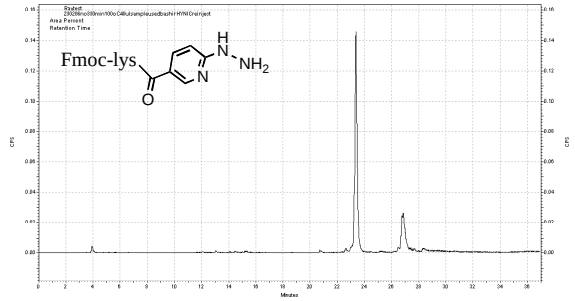
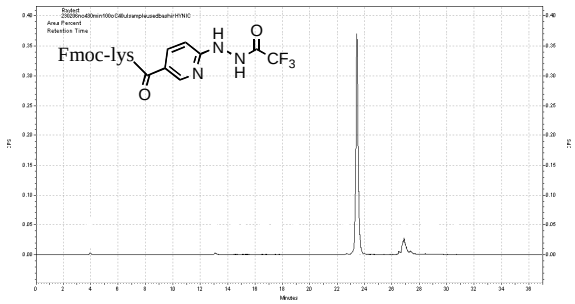


Figure D: a) HPLC radiochromatogram for the ^{99m}Tc labeling of Fmoc-lysine-HYNIC **2b**; **b)** HPLC radiochromatogram for the ^{99m}Tc labeling of Fmoc-lysine-HYNIC-TFA **2c**; **c)** HPLC radiochromatogram for the ^{99m}Tc labeling of nanogastrin-HYNIC-TFA **3c**, all with tricine as co-ligand.

a)



b)



c)

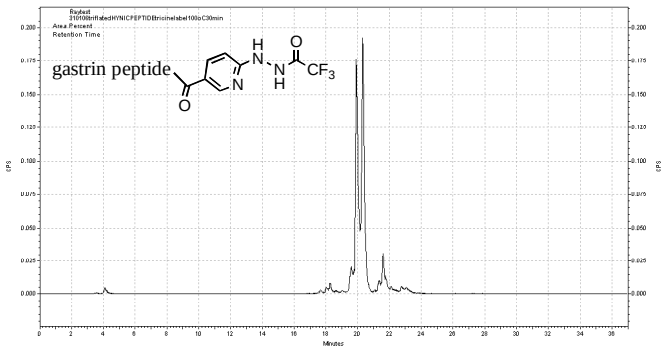


Figure E. HPLC radiochromatograms for the ^{99m}Tc labeling of nanogastrin-HYNIC **3b** (top) and nanogastrin-HYNIC-TFA **3c** (bottom), both with tricine as co-ligand, under identical labeling conditions.

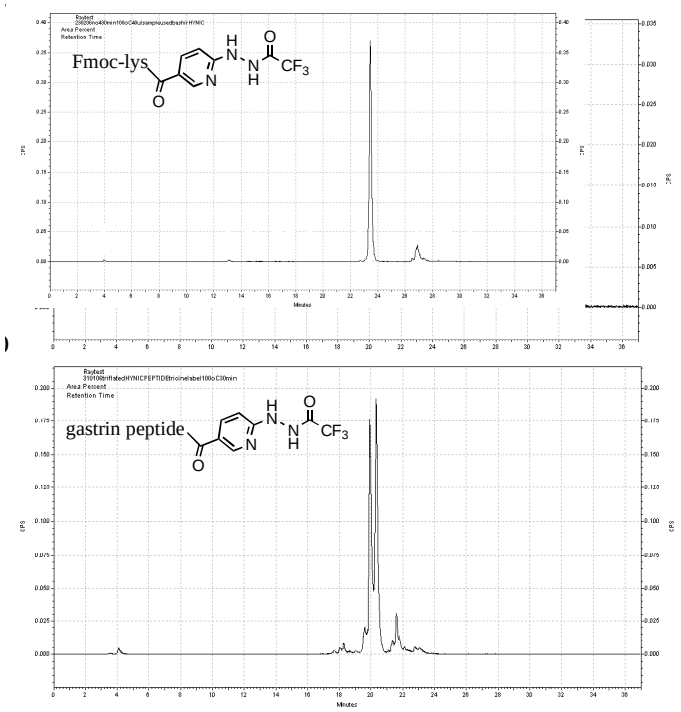


Table of analytical data for *Hydrazinonicotinic acid (**1b**)* A solution of Boc-hydrazinonicotinic acid **1a** [1] (350 mg, 1.383 mmol) in 5M HCl (5 ml) was stirred at room temperature for 3 h and the resulting solution was evaporated under reduced pressure at 40˚C. The crude white solid obtained was then stirred in diethylether (20 ml) and filtered. The resulting solid was washed further with ether and dried under high vacuum to afford the desired product as a white amorphous solid (174 mg, 82% yield). The desired product also was prepared in a similar manner from trifluoroacetylhydrazinonicotinic acid **1c** after incubation with 1 M HCl for 1 hour (186 mg, 88 % yield).

Characterization	Assignments
^{1}H-NMR δ_H[$(CD_3)_2SO$; 270 MHz]	7.05 (1 H, d, *J* 7.2 Hz, Ar-H), 8.31 (1 H, d, *J* 7.2 Hz, Ar-H) and 8.90 (1 H, s, Ar-H)
^{13}C-NMR δ_C[$(CD_3)_2SO$; 67.5 MHz]	110.58 (Ar-CH), 119.85 (C quaternary), 140.95 and 159.61 (2 x CH aromatic), 159.61 (C quaternary), and 167.18 (COOH)
Mass Spectrometry *m/z* (EI+)	153 (100%, $[M]^+$), 138 (7, $[M - NH_2 + H]^+$), 123 (5, $[M- N_2H_3 + H]^+$), 108 (5, $[M - CO_2H]^+$), 105 (8, $[M - N_2H_3 - OH]^+$), 78 (15, $[M - N_2H_3 - COOH + H]^+$), and 44 (25, $[M - C_4H_6N_3 - H]^+$)
HRMS	Calcd for $C_6H_7N_3O_2$ $[M]^+$: 153.0538; Found: 153.0531
Infra-Red Spectroscopy υ_{max}(KBr)/cm^{-1}	3210 br, 3058 s, 3039 s, 1696 s, 1636 s, 1613 s, 1536-1444 m, 1170 s, 757 s and 736 s

Table of analytical data for *Trifluoroacetylhydrazinonicotinic acid (**1c**):*

Characterization	Assignments
^{1}H-NMR δ_H[CD_3OD; 270 MHz]	7.02 (1 H, d, *J* 7.2 Hz, Ar-H), 8.34 (1 H, d, *J* 7.2 Hz, Ar-H) and 8.93 (1 H, s, Ar-H);
^{13}C-NMR δ_C[CD_3OD; 67.5 MHz]	106.48 (CH aromatic), 115.41 and 116.20 (q, *J* 201.0 Hz, *cis* and *trans*, *C*F_3), 117.79 (C quaternary), 138.81 and 149.61 (2 x CH
^{19}F-NMR (254 MHz; C^2HCl_3)	74.77 and -76.20 (*cis* and *trans*);
Mass Spectrometry (ES-)	248.0 (100%, $[M - H]^-$).
HRMS	Calcd. for $C_8H_5N_3O_3F_3$ $[M - H]^-$: 248.0283; Found: 248.0291;
Infra-Red Spectroscopy υ_{max}(KBr)/cm^{-1}	υ_{max}(KBr)/cm^{-1} 3311 br, 3056 s, 3037 s, 1697 s, 1635 s, 1610 s, 1529-1440 m, 1176 s, 756 s and 737 s;

Page S11

*Table of analytical data for N^{α}-(Fmoc)-N^{ϵ}-(t-butoxyhydrazinonicotinyl)-lysine (**2a**):*

Characterization	Assignments
^{1}H-NMR δ_H[$(CD_3)_2SO$; 270 MHz]	1.36-2.17 (15 H, m, 2 H_l, 9 H_t, 2 H_k and 2 H_j), 3.46 (2 H, m, H_m), 3.73 (1 H, m, H_h), 4.22 (2 H, m, H_e), 4.51 (2 H, m, 2 H_g), 6.82 (1 H, d, *J* 8.0, H_p), 7.77 (1 H, t, *J* 7.4, H_b), 7.82 (1 H, t, *J* 7.0, H_c), 7.90 (1 H, d, *J* 6.9, H_d), 8.07 (1 H, d, *J* 7.4, H_a), 8.16-8.30 (2 H, m, H_o and H_n), 8.49 (1 H, d, *J* 1.8 Hz, H_s), 8.83 (1 H, s, H_r), 8.92 (1 H, s, H_q) and 9.23 (1 H, s, H_g); no unassigned peaks
^{13}C-NMR δ_C[$(CD_3)_2SO$); 67.5 MHz]	21.1 (C_k), 28.5 (3 x C_t), 29.8 (C_l), 30.6 (C_j), 40.5 (C_m), 43.2 (C_e), 56.6 (C_i), 79.2 (C quaternary), 109.2 (CH aromatic), 111.3 (C_f), 121.6 (CH aromatic), 121.8 (C quaternary), 122.3 (CH aromatic), 127.3 and 127.5 (2 x C quaternary), 128.8 and 129.7 (2 x CH aromatic), 137.2 and 137.6 (C quaternary), 139.3 (CH aromatic), 148.9 (CH aromatic), 156.8 and 163.9 (2 x CO), 167.4 (C quaternary), 172.1 (CO) and 175.8 (COOH); no unassigned peaks
Mass Spectrometry *m/z* (ES+)	648.1 (25%, $[M - H + 2 Na]^+$) and 626.1 (100, $[M + Na]^+$).
HRMS	for $C_{32}H_{37}N_5O_4Na$ $[M + Na]^+$: 626.2591; found 626.2581;
Infra-Red Spectroscopy υ_{max}(KBr)/cm^{-1}	3323 br, 3066 s, 3036 s, 2977–2863 m, 1699 s, 1631 s, 1610 s, 1531-1446 m, 1277 s, 1252 s, 1163 s, 758 s and 737 s;
$[\alpha]_D$	$[\alpha]_D$4.0 [c 0.005 in $(CH_3)_2SO$]
% purity	100% by 2 HPLC methods (see p. S1)

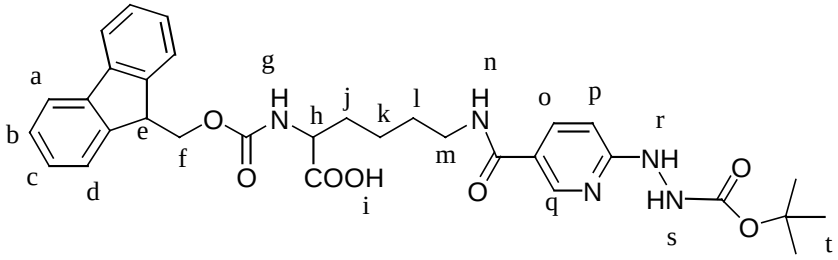


*Table of analytical data for N^{α}-(Fmoc)-N^{ε}-(hydrazinonicotinyl)-lysine (**2b**):* The desired product was prepared in a similar manner to the hydrazinonicotinic acid **1b** using Fmoc-lysine-HYNIC-Boc **2a** as starting material instead of HYNIC-Boc **1a** (145 mg, 75 % yield). The desired product was also prepared in a similar manner to the hydrazinonicotinic acid **1b** using Fmoc-lysine-HYNIC-TFA **2c** as starting material instead of HYNIC-TFA **1c** (164 mg, 85 % yield).

Characterization	Assignments
^{1}H-NMR δ_H[$(CD_3)_2SO$; 270 MHz]	1.32-2.10 (6 H, m, 2 H_l, 2 H_k and 2 H_j), 3.42 (2 H, m, H_m), 3.72 (1 H, m, H_h), 3.83 (5 H, br. s, H_g, H_r and 3 x H_s), 4.22 (2 H, m, H_e), 6.83 (1 H, d, *J* 7.7, H_p), 7.80 (1 H, t, *J* 7.4, H_b), 7.85 (1 H, t, *J* 7.0, H_c), 7.91 (1 H, d, *J* 6.9, H_d), 8.09 (1 H, d, *J* 7.4, H_a), 8.15-8.29 (1 H, d, *J* 7.7, H_o), 8.75 (1 H, br, H_n), 8.97 (1 H, s, H_q) and 9.27 (1 H, s, H_g);
^{13}C-NMR δ_C[$(CD_3)_2SO$); 67.5 MHz]	21.1 (C_k), 30.0 (C_l), 30.7 (C_j), 40.6 (C_m), 44.1 (C_e), 57.5 (C_i), 109.7 (CH aromatic), 113.3 (C_f), 122.7 (CH aromatic), 123.7 (C quaternary), 124.2 (CH aromatic), 128.6 and 128.9 (2 x C quaternary), 129.6 and 130.2 (2 x CH aromatic), 138.0 and 138.5 (C quaternary), 139.3 (CH aromatic), 149.7 (CH aromatic), 165.3 (CO), 167.6 (C quaternary), 172.2 (CO) and 171.5 (COOH);
Mass Spectrometry *m/z* (ES+)	548.2 (20%, $[M - H + 2 Na]^+$), 526.2 (100, $[M + Na]^+$) and 504.2 (25, $[M + H]^+$).
HRMS	calcd for $C_{27}H_{29}N_5O_5Na$ $[M + Na]^+$: 526.2066; Found: 526.2061;
Infra-Red Spectroscopy υ_{max}(KBr)/cm^{-1}	3215 br, 3060 s, 3031 s, 2988–2866 m, 1698 s, 1633 s, 1610 s, 1533-1442 m, 1275 s, 1251 s, 1165 s, 757 s and 736 s;

*Table of analytical data for N^{α}-(Fmoc)-N^{ε}-(trifluoroacetylhydrazinonicotinyl)-lysine **(2c)**:* The desired product was prepared in a similar manner to the hydrazinonicotinic acid derivative **1c** using Fmoc-lysine-HYNIC-Boc **2a** as starting material instead of HYNIC-Boc **1a** (122 mg, 80 % yield).

Characterization	Assignments
^{1}H-NMR δ_H[$(CD_3)_2SO$; 270 MHz]	1.31-2.15 (6 H, m, 2 H_l, 2 H_k and 2 H_j), 3.40 (2 H, m, H_m), 3.86 (13 H, br. s, H_g, H_r and H_s), 3.69 (1 H, m, H_h), 4.19 (2 H, m, H_e), 6.80 (1 H, d, *J* 7.7, H_p), 7.78 (1 H, t, *J* 7.4, H_b), 7.83 (1 H, t, *J* 7.0, H_c), 7.89 (1 H, d, *J* 6.9, H_d), 8.10 (1 H, d, *J* 7.4, H_a), 8.17-8.33 (1 H, d, *J* 7.7, H_o), 8.73 (1 H, br, H_n), 8.91 (1 H, s, H_q) and 9.25 (1 H, s, H_g);
^{13}C-NMR δ_C[$(CD_3)_2SO$); 67.5 MHz]	21.6 (C_k), 30.2 (C_l), 30.9 (C_j), 40.9 (C_m), 43.7 (C_e), 57.2 (C_i), 109.0 (CH aromatic), 112.7 (C_f), 117.33 and 118.71 (q, *J* 198.6 Hz, *cis* and *trans*, *C*F_3), 122.4 (CH aromatic), 123.2 (C quaternary), 123.9 (CH aromatic), 128.2 and 128.8 (2 x C quaternary), 129.8 and 130.5 (2 x CH aromatic), 138.2 and 138.7 (C quaternary), 139.7 (CH aromatic), 149.4 (CH aromatic), 156.8 and 158.4 (q, *J* 34.4 Hz, *cis* and *trans*, *C*OCF_3), 164.2 (CO), 167.4 (C quaternary), 172.7 (CO) and 171.4 (COOH);
^{19}F-NMR (254 MHz; C^2HCl_3)	-74.77 and -76.20;
Mass Spectrometry *m/z* (ES+)	644.1 (10%, $[M - H + 2 Na]^+$) and 622.1 (100, $[M + Na]^+$).
HRMS	calcd for $C_{29}H_{28}N_5O_6F_3Na$ $[M + Na]^+$: 622.1889; Found: 622.1898;
Infra-Red Spectroscopy υ_{max}(KBr)/cm^{-1}	3301 br, 3065 s, 3032 s, 2983–2869 m, 1697 s, 1631 s, 1611 s, 1536-1444 m, 1279 s, 1251 s, 1165 s, 758 s and 736 s;

*Experimental details for solid phase peptide synthesis:*Nanogastrin analog peptides were synthesized on a Shimadzu PSSM-8 Multiple Peptide Synthesizer using the Fmoc strategy. Standard side chain protecting groups were utilized and the peptide assembled on TGR amide resin (Novabiochem) using HBTU-mediated coupling. Briefly, the Fmoc group was removed by 2 x 5 min treatment with 30 % piperidine in DMF followed by 5 washes with DMF. Each amino acid (10 eq., except for Fmoc-Lys-HYNIC-Boc **2a** of which only 5 eq. were used) was dissolved in DMF, mixed with 0.5 M HOBt/0.5 M HBTU in DMF (10 eq.) and 1.0 M DIPEA in DMF (20 eq.) and added to the resin. The reaction was mixed by nitrogen bubbling for 30 min after which the resin was washed 5 times with DMF. This process was repeated until the required sequence was assembled. The final Fmoc group was removed, and the resin washed with DMF followed by methanol and then vacuum dried for 1 h.

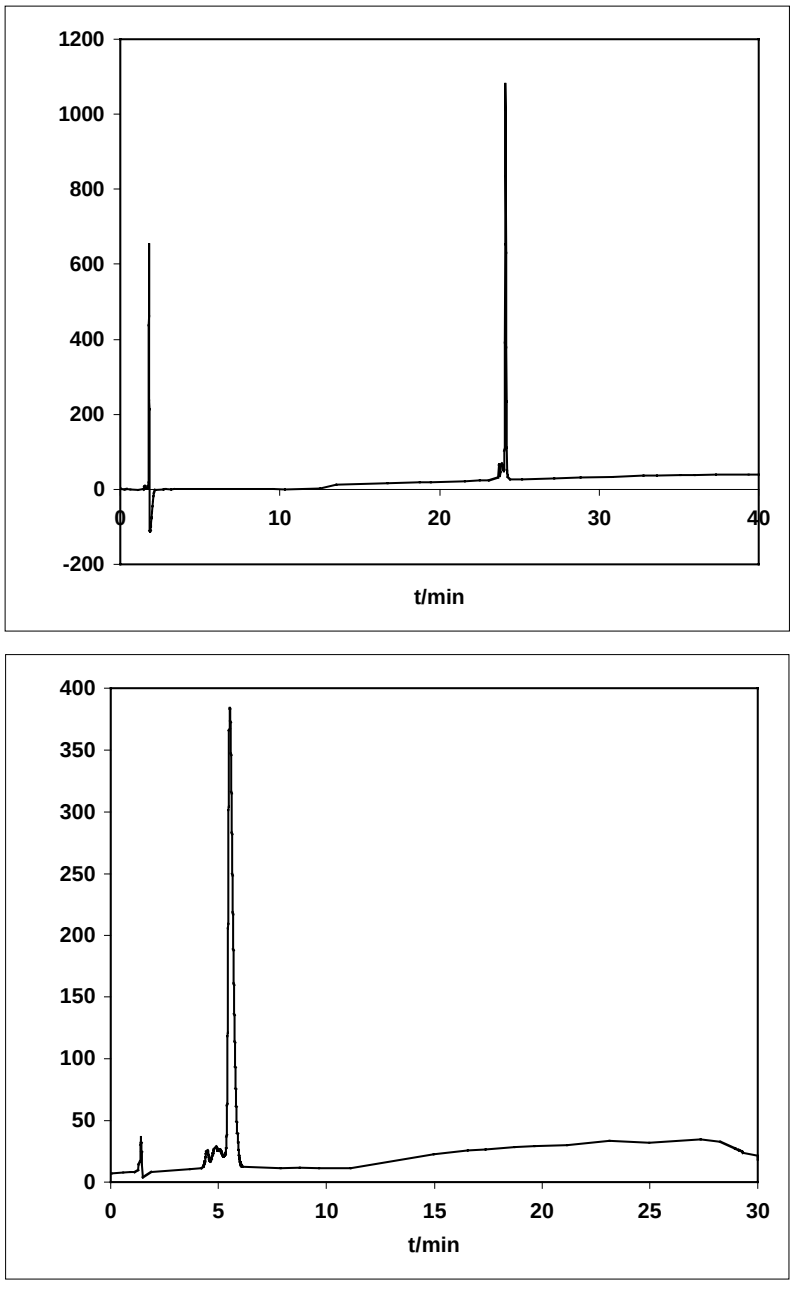
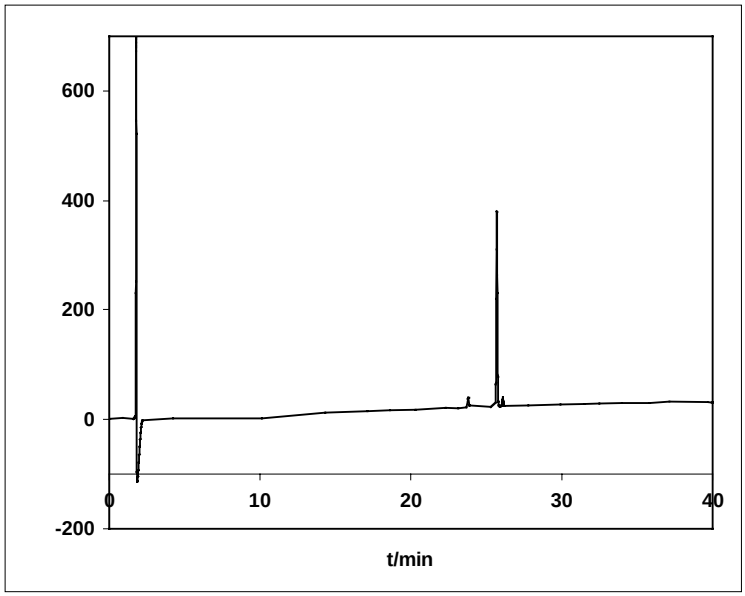


Figure F: top: RP HPLC of HYNIC-nanogastrin peptide **3b** using Method 4 (p. S17); wavelength 214 nm; **percentage purity: 93.5%;** retention time: 24.1 min; bottom: RP HPLC of HYNIC-nanogastrin peptide **3b** using Method 5 (p. S17); wavelength 214 nm;: **percentage purity 94.9%;** retention time: 5.5 min.



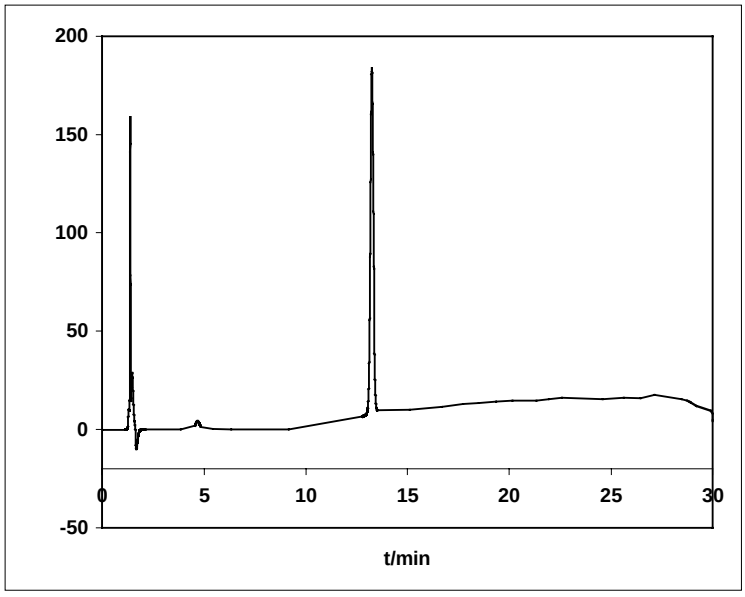


Figure G: top: RP HPLC of trifluroacetylHYNIC-nanogastrin peptide **3c** using Method 4 (p. S17); wavelength 214 nm; **percentage purity: 93.4%;** retention time: 25.7 min; bottom: RP HPLC of trifluoroacetylHYNIC-nanogastrin peptide **3c** using Method 5 (p. S17); wavelength 214 nm; **percentage purity: 97.6%;** retention time: 13.2 min.

HPLC methods

HPLC method 1

Phenomenex Polymer PRP-1 column (150 x 2 mm, 5 μm) mobile phase: linear gradient of increasing acetonitrile (ACN) in 0.05% aqueous TFA: 0-5 min 5% ACN, 5-35 min 5%-100% ACN, 35-40 min 100% ACN, 40-45 min 100%-5% ACN; flow rate: 0.2 ml/min; detection: UV absorbance at 214 nm and 254 nm).

HPLC method 2

{Vydac C18 column (250 x 10 mm, 5 μm); mobile phase: linear gradient of increasing acetonitrile (ACN) in 0.05% aqueous trifluoroacetic acid (TFA): 0-5 min 5% ACN, 5-35 min 5%-100% ACN, 35-40 min 100% ACN, 40-45 min 100%-5% ACN; flow rate: 4.5 ml/min; detection: UV absorbance at 214 nm}.

HPLC method 3

Phenomenex Jupiter C18 300A column, 250 x 4.60 mm 5 μm, flow rate 1 ml/min, UV detection at 220-350 nm were employed with the following gradient: ACN in 0.1% aqueous TFA: 0-5 min 0% ACN, 5-25 min 0%-60% ACN, 25-30 min 60% ACN, 30-35 min 60%-100% ACN, 35-37 min 100%-0% ACN.

HPLC method 4

Phenomenex Polymer RP-1 column 100 Å (150 x 2 mm, 5 μm) mobile phase: linear gradient of increasing acetonitrile (ACN) in 0.05% aqueous TFA: 0-10 min 5% ACN, 10-

35 min 5%-90% ACN, 35-40 min 100% ACN, 40-45 min 100%-5% ACN; flow rate: 1 ml/min; detection: UV absorbance at 214 nm and 254 nm).

HPLC method 5

Phenomenex Polymer RP-1 column 100 Å (150 x 2 mm, 5 μm) mobile phase: linear gradient of increasing acetonitrile (ACN) in 0.05% aqueous TFA: 0-10 min 40% ACN, 10-20 min 40%-90% ACN, 20-25 min 100% ACN, 25-30 min 40%-5% ACN; flow rate: 1 ml/min; detection: UV absorbance at 214 nm and 254 nm).