Supplementary Chemistry (SC), Methods, Tables and Figures

1. Materials

All reagents were purchased as reagent grade and used without further purification. *O*-(6-Chlorobenzotriazol-1-yl)-*N,N,N*',*N*'-tetramethyluronium hexafluorophosphate (HCTU), *O*-(7-azabenzotriazol-1-yl)-*N,N,N*',*N*'-tetramethyluronium hexafluorophosphate (HATU), *N*-(9-fluorenylmethoxycarbonyloxy) succinimide (FmocOSu), 4-[(*R,S*)-α-[1-(9*H*-fluoren-9-yl)]-methoxycarbonylamino]-2,4-dimethoxy]phenoxyacetic acid (Fmoc-Rink amide linker) and Fmoc-amino acids were purchased from GL Biochem (Shanghai, China). Fmoc-amino acids were supplied with the following side-chain protection: Fmoc-Tyr(*t*Bu)-OH, Fmoc-Thr(*t*Bu)-OH, Fmoc-Ser(*t*Bu)-OH, Fmoc-Asn(Trt)-OH (Trt = triphenylmethyl), Fmoc-His(Trt)-OH, Fmoc-Arg(Pbf)-OH (Pbf = 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)), Fmoc-Gln(Trt)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Lys(Boc)-OH (Boc = *tert*-butyloxycarbonyl).

Fmoc-Ser(*t*Bu)-Ser($\Psi^{Me,Me}$pro)-OH, Fmoc-Ala-Thr($\psi^{Me,Me}$pro)-OH and Fmoc-Leu-Ser($\psi^{Me,Me}$pro)-OH were purchased from Aapptec (Louisville, Kentucky). *N,N*-Diisopropylethylamine (*i*Pr_2NEt), 2,4,6-collidine, piperidine, *N,N'*-diisopropylcarbodiimide (DIC), 3,6-dioxa-1,8-octane-dithiol (DODT), triisopropylsilane (*i*Pr_3SiH), 1-methyl-2-pyrrolidinone (NMP), 6-chloro-1-hydroxybenzotriazole (6-Cl-HOBt), ninhydrin, phenol, potassium cyanide (KCN), methanol (MeOH), ethanol (EtOH), diethyl ether (Et_2O), *N*-methylmorpholine, 2,2'-dithiobis(5-nitropyridine) (DTNP), copper(II) sulphate pentahydrate ($CuSO_4 \cdot 5\ H_2O$), and triisopropylsilane (*i*Pr_3SiH) were purchased from Sigma-Aldrich (St. Louis, Missouri). Dichloromethane (CH_2Cl_2), magnesium sulphate ($MgSO_4$), sodium bicarbonate ($NaHCO_3$), sodium carbonate (Na_2CO_3), sodium ascorbate (Na ascorbate), ethyl acetate (EtOAc) and hexane were purchased from ECP limited (Auckland, New Zealand). Hydrochloric acid (HCl), sodium hydroxide (NaOH), *N,N*-dimethylformamide (DMF) (synthesis grade), and acetonitrile (MeCN), were purchased from Scharlau (Barcelona, Spain). Dimethyl sulfoxide (DMSO) was purchased from Romil Limited (Cambridge, United Kingdom). L-propargylglycine (L-Pra) was purchased from AK Scientific (Union City, California). Tetrahydrofuran (THF) was purchased from Avantor Performance Materials (Centre Valley, Pennsylvania). Trifluoroacetic acid (TFA) was purchased from Halocarbon (River Edge, New Jersey).

Aminomethyl Chemmatrix® resin (AM-CM) was purchased from Pcas BioMatrix Inc (Quebec, Canada). Aminomethyl polystyrene resin (AM-PS),[1] Fmoc-propargylglycine (Fmoc-L-Pra-OH),[2] and Fmoc-L-azidolysine[3] were synthesised following literature procedures.

2. General procedures for peptide synthesis

The general procedure for peptide synthesis is shown in Figure SC1.

2.1. General Method for attachment of linker to resin

The *C*-terminal amide of native human amylin was necessary to attain the final biologically active peptide, and this was installed by use of the Fmoc-Rink amide linker. Briefly, Fmoc-Rink amide, DIC (4 eq.) and 6-Cl-HOBt (4 eq.) were dissolved in in DMF (3 mL). The solution was added to either the pre-swollen (DMF, 3 mL, 20 min) aminomethyl polystyrene (AM-PS, loading 0.91 mmol/g) or aminomethyl Chemmatrix® (AM-CM, loading 0.69 mmol/g) resin and shaken for 2 h at room temperature (rt). The resin was then filtered and washed (DMF, 3 x 3 mL).

For synthesis of human amylin -COOH incorporating a *C*-terminal acid, a solution of Fmoc-Tyr(*t*Bu)-O-CH_2-phi-OCH_2-CH_2-COOH (Fmoc-Tyr-HMPP, 2 eq.) and DIC (2 eq.) in CH_2Cl_2/DMF (v/v; 2 : 1, 3 mL) was added to pre-swollen (CH_2Cl_2, 3 mL, 20 min) AM-CM resin, shaken for 2 h at rt, filtered and washed (CH_2Cl_2, 3 x 3 mL).

For synthesis of C2S-C7S and CAM human amylin analogs, the *C*-terminal amide was installed by use of a 5-[3,5-dimethoxy-4-(Fmoc-aminomethyl)phenoxy]pentanoic acid (Fmoc-PAL) linker.

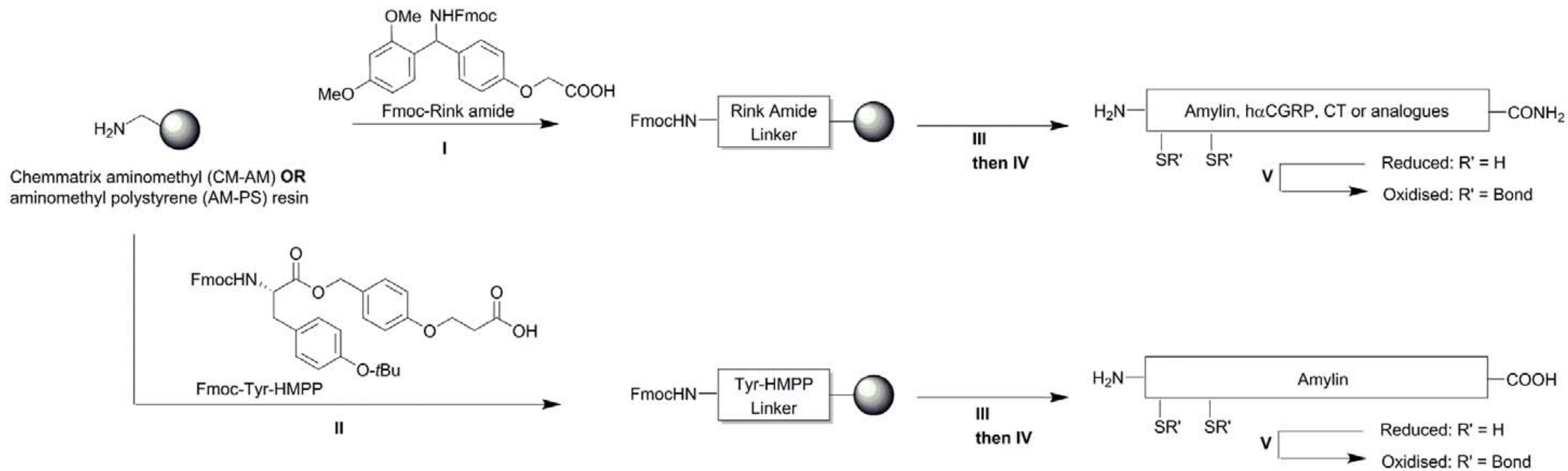

Figure SC1. Synthesis of human amylin, hαCGRP, calcitonin (CT) and analogues, excluding truncated peptides and disulfide modifications. Reagents and conditions: (I) DIC (4 eq.), 6-Cl HOBt (4 eq.), DMF, rt, 2 h; (II) Fmoc-Tyr(*t*Bu)-HMPP (2 eq.), DIC (2 eq.), CH_2Cl_2/DMF; (III) Fmoc SPPS: Fmoc-AA-OH coupling: Fmoc-AA-OH (5 eq.), activator, base; Fmoc-deprotection: 20% piperidine in DMF (see Supplementary Chemistry for details); (IV) TFA/$i$$Pr_3$SiH/DODT/$H_2O$ (94/1/2.5/2.5, v/v), rt, 2-3 h; (V) DTNP in DMSO, rt, 20 min.

2.2. General Method for Biotage® initiator + alstra and Liberty peptide synthesisers (microwave)

Stock solutions for Fmoc-protected amino acids (0.2 M in DMF), HATU (0.5 M in DMF), and *i*Pr_2NEt (2 M in NMP) were prepared prior to synthesis.

The Fmoc group was removed using 20% piperidine in DMF (2 x 3 min at maximum temperature of 70 °C and at 62 W). All amino acid couplings were performed as single coupling cycles with the exception of Fmoc-Arg(Pbf)-OH, Fmoc-His(Trt)-OH and Fmoc-Cys(Trt)-OH where a double coupling cycle was performed. Protected amino acids were incorporated using Fmoc-AA-OH (5.0 eq.), HCTU (4.5 eq.) and *i*Pr_2NEt (10 eq.) in NMP, for 5 min at a maximum temperature of 75 °C and at 25 W, except Fmoc-Arg(Pbf)-OH which was coupled for 25 min at room temperature followed by a second coupling for 5 min at a maximum temperature of 72 °C at 25 W, and Fmoc-His(Trt)-OH and Fmoc-Cys(Trt)-OH which were coupled for 10 min at room temperature followed by a second coupling for 5 min at a maximum temperature of 47 °C at 25 W. Coupling of Fmoc-Ser(*t*Bu)-Ser($\psi^{Me,Me}$pro)-OH (2 eq.) and Fmoc-Ala-Thr($\psi^{Me,Me}$pro)-OH (2 eq.) (Figure SC2) were undertaken via manual addition of reagents. The building blocks were dissolved in a solution of HATU (1.9 eq.) and 2,4,6-collidine (6 eq.) in DMF (2 mL) and coupled for 15 min at a maximum temperature of 75 °C at 25 W.

2.3. General Method for Tribute™ peptide synthesiser (room temperature)

Prior to synthesis, stock solutions were prepared for HCTU (0.23 M in DMF) and NMM (2 M in NMP). The Fmoc group was removed using 20% piperidine in DMF (2 x 5 min). All amino acids were double-coupled, with the exception of Fmoc-Arg(Pbf)-OH which was triple-coupled. Protected amino acids were incorporated using Fmoc-AA-OH (5.0 eq.), HCTU (4.6 eq.) and NMM (10 eq.) in DMF, for 10 min per coupling. Fmoc-Ser(*t*Bu)-Ser($\psi^{Me,Me}$pro)-OH and Fmoc-Ala-Thr($\psi^{Me,Me}$pro)-OH (2 eq.) were coupled via manual addition of reagents for 1.5 h at room temperature in the presence of HATU (1.9 eq.) and 2,4,6-collidine (6 eq.) in DMF.

2.4. General Method for PS3™ peptide synthesiser (room temperature)

A stock solution was prepared for NMM (0.4 M in DMF). The Fmoc group was removed using 20% piperidine in DMF (2 x 5 min). All amino acid couplings were performed as single coupling cycles. Protected amino acids were incorporated using Fmoc-AA-OH (5.0 eq.), HATU (4.5 eq.) and NMM (10 eq.) in DMF, for 20 min. Fmoc-Ser(*t*Bu)-Ser($\psi^{Me,Me}$pro)-OH, Fmoc-Ala-Thr($\psi^{Me,Me}$pro)-OH, and/or Fmoc-Leu-Ser($\psi^{Me,Me}$pro)-OH (5 eq.) (Figure SC2) were coupled using the same conditions.

2.5. General Method for cleavage from the resin

Cleavage from the resin with simultaneous side-chain deprotection was achieved by treatment with TFA/*i*Pr3SiH/H2O/DODT (94/1/2.5/2.5, v/v/v/v) for 2-3 hours. The resin was drained, washed with TFA (5 mL), precipitated with cold diethyl ether, isolated by centrifugation, dissolved in 50% aqueous acetonitrile containing 0.1% TFA and lyophilised.

3. General Method for disulfide bond formation

Crude peptides were dissolved in DMSO (10 mg/mL) and a solution of DTNP (0.5 eq.) in DMSO (20 mg/mL) was added and the mixture was shaken for 20 min. The mixture was diluted with H2O containing 0.1% TFA to a concentration of 1 mg/mL and immediately purified by semi-preparative reverse phase high-performance liquid chromatography (RP-HPLC).

4. Method for preparation of CAM-human amylin

CAM-human amylin was prepared by incubating purified human amylin in a solution of 13 mM DTT, 6 M GdnHCl, 0.19 M Tris HCl, and 10% DMSO at pH 8.0 for 4 hours at 4 °C under N_2 (g) to reduce the peptide. After reduction, iodoacetamide was added to the cocktail to a final concentration of 8 mM, for 4 hours at 4 °C in the dark. The reaction was quenched with 80 mM 2-mercaptoethanol. After purification by HPLC residual scavengers were removed via HFIP extraction.

5. General procedure for purification and analysis

Analytical RP-HPLC was performed on a Dionex Ultimate 3000 using the following columns: Vydac Diphenyl 300 Å, 3 µm, 4.6 mm x 250 mm; Agilent Zorbax 300SB-C3, 3.0 mm x 150 mm; Agilent TC-C18, 5 µm, 4.6 x 250 mm. Liquid-chromatography mass spectrometry (LCMS) was performed on an Agilent Technologies 1120 Compact LC connected to a HP Series 1100 MSD spectrometer using an Agilent Zorbax 300SB-C3, 3.5 µm, 3.0

mm x 150 mm column using linear gradient of 0.1% formic acid in water (A) and 0.1% formic acid MeCN (B). Semi-preparative RP-HPLC was performed using either a Waters 600E System with a Waters 2487 dual wavelength absorbance detector or a Dionex Ultimate 3000 using the following columns: Phenomenex Gemini C_{18} 110 Å, 5 µm, 10.0 mm x 250 mm (5 mL/min); Vydac Diphenyl 300 Å, 5 µm, 10.0 mm x 250 mm (5 mL/min); Vydac C4 300 Å, 5 µm, 10.0 mm x 250 mm (5 mL/min), Higgins Proto C18 300 Å, 5 µm, 10.0 x 250 mm column (5 mL/min). The columns and HPLC systems used for the semi-preparative RP-HPLC of individual peptides is outlined in Table SC1. A linear gradient of 0.1% TFA/water (A) and 0.1% TFA/MeCN (B) was used with detection at 210 nm. Gradient systems used for semi-preparative RP-HPLC were adjusted according to the elution and peak profiles obtained from the analytical RP-HPLC chromatograms.

Table SC1: Conditions used for the synthesis, and purity of individual peptides. Pseudoprolines (ψPro) refers to incorporation of pseudoproline in place of regular Fmoc amino acids (1: Fmoc-Ser(*t*Bu)-Ser(ψ^{Me,Me}pro)-OH at position 28 and 29, 2: Fmoc-Ser(*t*Bu)-Ser(ψ^{Me,Me}pro)-OH at position 19 and 20, 3: Fmoc-Ala-Thr(ψ^{Me,Me}pro)-OH at position 8 and 9, 4, Fmoc-Leu-Ser(ψ^{Me,Me}pro)-OH at position 27-28 5: Fmoc-Leu-Ser(ψ^{Me,Me}pro)-OH at position 16-17 (CGRP analogs only)). Purity was assessed by integration of the chromatogram at 210 nm. hAMY, human amylin; hCT, human calcitonin; hαCGRP, human alpha calcitonin gene-related peptide; pram, pramlintide.

	Scale (mM)	Synthesiser	Resin	Pseudoprolines	Purification Column	Purity (%)
hAMY	0.1	Liberty	AM-PS	1	Vydac Diphenyl	95
K1A	0.1	Liberty	AM-PS	1	Vydac Diphenyl	96
N3A	0.05	Liberty	AM-PS	1	Vydac Diphenyl	98
T4A	0.1	Liberty	AM-PS	1	Vydac Diphenyl	92
A5G	0.1	PS3	AM-CM	1,2,3	Vydac Diphenyl	96
T6A	0.1	Liberty	AM-PS	1,3	Vydac Diphenyl	95
A8G	0.1	PS3	AM-CM	1,2	Vydac Diphenyl	96
T9A	0.05	Liberty	AM-PS	1	Vydac Diphenyl	96
Q10A	0.1	Biotage	AM-CM	1,3	Phenomenex Gemini C$_{18}$	98
R11A	0.1	Tribute	AM-PS	1,2	Vydac Diphenyl	91
L12A	0.1	PS3	AM-CM	1,2,3	Vydac C4	98
A13G	0.1	PS3	AM-CM	1,2,3	Vydac C4	93
N14A	0.1	Tribute	AM-PS	1,2	Vydac Diphenyl	98
F15A	0.1	Tribute	AM-PS	1,2	Vydac Diphenyl	97
L16A	0.1	Tribute	AM-PS	1,2	Vydac Diphenyl	97
V17A	0.1	PS3	AM-CM	1,2,3	Vydac Diphenyl	96
I26A	0.1	PS3	AM-CM	2	Phenomenex Gemini C$_{18}$	99
L27A	0.1	PS3	AM-CM	2	Phenomenex Gemini C$_{18}$	98
S28A	0.1	PS3	AM-CM	2	Phenomenex Gemini C$_{18}$	94
S29A	0.1	PS3	AM-CM	2	Phenomenex Gemini C$_{18}$	95
T30A	0.1	PS3	AM-CM	2	Phenomenex Gemini C$_{18}$	94
N31A	0.1	PS3	AM-CM	2	Phenomenex Gemini C$_{18}$	97
V32A	0.1	PS3	AM-CM	2	Phenomenex Gemini C$_{18}$	96
G33A	0.1	PS3	AM-CM	2	Phenomenex Gemini C$_{18}$	98
S34A	0.1	PS3	AM-CM	2	Phenomenex Gemini C$_{18}$	94
N35A	0.1	PS3	AM-CM	2	Phenomenex Gemini C$_{18}$	94
T36A	0.1	PS3	AM-CM	2	Phenomenex Gemini C$_{18}$	93
Y37A	0.1	PS3	AM-CM	2	Phenomenex Gemini C$_{18}$	98
hAMY$_{1-17}$	0.1	PS3	AM-CM	3	Vydac C4	99
hAMY$_{8-37}$	0.1	PS3	AM-CM	1,2,3	Phenomenex Gemini C$_{18}$	85
hAMY$_{8-37(DR)}$	0.1	Liberty	AM-PS	2,4	Higgins Proto C$_{18}$	97
hAMY $_{Ac8-37(DR)}$	0.1	Liberty	AM-PS	2,4	Higgins Proto C$_{18}$	83
C2S-C7S	0.1	Liberty	AM-PS	2,3,4	Higgins Proto C$_{18}$	90
CAM-hAMY	0.1	Liberty	AM-PS	2,3,4	Higgins Proto C$_{18}$	93
A5S	0.1	PS3	AM-CM	1,2,3	Vydac Diphenyl	95
Y37F	0.1	PS3	AM-CM	1,2,3	Phenomenex Gemini C$_{18}$	99
Y37P	0.1	PS3	AM-CM	1,2,3	Phenomenex Gemini C$_{18}$	95
hαCGRP	0.1	PS3	AM-CM	5	Phenomenex Gemini C$_{18}$	97
hαCGRP F37Y	0.1	PS3	AM-CM	5	Phenomenex Gemini C$_{18}$	96
hCT	0.1	PS3	AM-CM	-	Phenomenex Gemini C$_{18}$	95
hCT P32Y	0.1	PS3	AM-CM	-	Phenomenex Gemini C$_{18}$	96
hAMY-COOH	0.1	Biotage Initiator + Alstra	AM-CM	2	Phenomenex Gemini C$_{18}$	99
Q10A pram	0.1	PS3	AM-CM	3	Vydac C4	99
DAGAR1	-	-	-	-	Phenomenex Gemini C$_{18}$	98

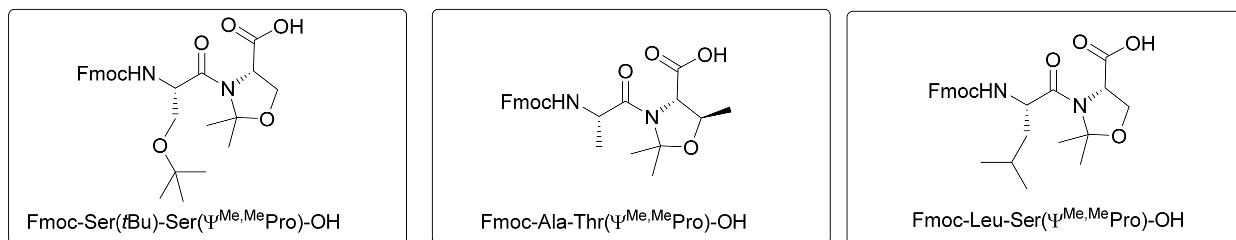

Figure SC2: Chemical structures of Fmoc-Ser(*t*Bu)-Ser(Ψ^{Me,Me}pro)-OH, Fmoc-Ala-Thr(ψ^{Me,Me}pro)-OH and Fmoc-Leu-Ser(ψ^{Me,Me}pro)-OH used for the synthesis of human amylin, hαCGRP, and analogs.

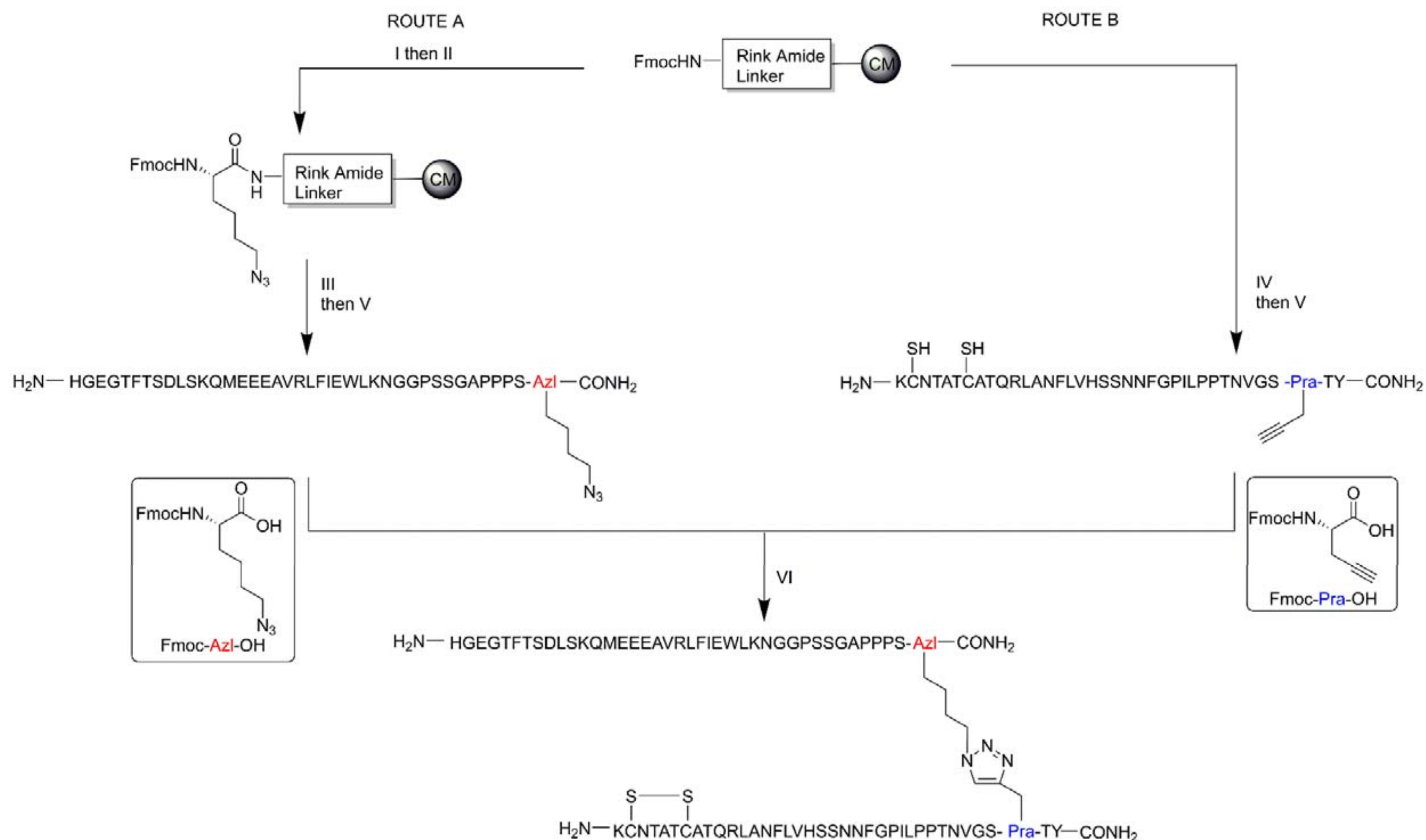

Figure SC3. Synthesis of a dual amylin and GLP-1 receptor agonist (DAGAR1). a) Synthesis of DAGAR1, and chemical structures of [Fmoc-Lys(N_3)-OH] and Fmoc-Pra-OH building blocks. ROUTE A: Synthesis of [Lys(N_3)]^{40}-exenatide and ROUTE B: Synthesis of [Pra]^{35}-pramlintide. Reagents and conditions: (I) 20% piperidine in DMF, rt, 2 x 5 min; (II) Fmoc-Lys(N_3)-OH (2 eq.), HATU (1.9 eq.), 2,4,6-collidine (6 eq.), rt, 1 h; (III) Fmoc SPPS using Biotage initiator + Alstra peptide synthesiser. Fmoc-AA-OH coupling: Fmoc-AA-OH (5 eq.), HATU (4.5 eq.), *i*Pr_2Net (10 eq.); Fmoc-deprotection: 20% piperidine in DMF; (IV) Fmoc SPPS using PS3™ peptide synthesiser. Fmoc-AA-OH coupling: Fmoc-AA-OH (5 eq), HATU (4.5 eq), NMM (10 eq.); Fmoc-deprotection: 20% piperidine in DMF; (V) TFA/*i*Pr_3SiH/DODT/H_2O (94/1/2.5/2.5, v/v); (VI) $CuSO_4 \cdot 5H_2O$ (0.0037 eq.), Na ascorbate (0.0037 eq.), DMSO, 80 °C, 5 min.

6. LCMS, RP-HPLC, and ESI-MS traces for peptides

Crude linear, and crude oxidised (Cys-2/Cys-7), and purified traces are provided for human amylin. For all other analogs, purified traces only are provided.

6.1. Traces for synthesis, oxidation and purification of human amylin

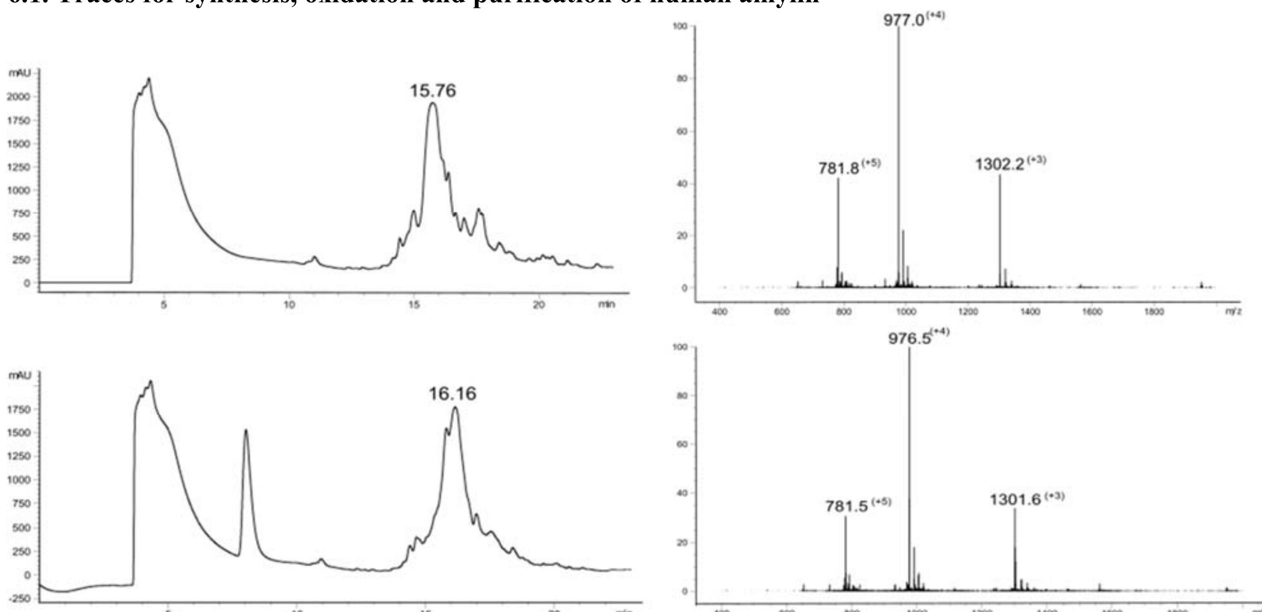

Figure SC4: LCMS traces for crude human amylin before oxidation (top) and after oxidation (bottom). Top *ca.* 42% as analysed by peak area of RP-HPLC at 214 nm; R_t 15.76 min; *m/z* (ESI-MS) 977.0 ($[M+4H]^{4+}$ requires 977.3); linear gradient of 5%B to 65%B over 20 min (*ca.* 3%B/min) at 40 °C, 0.3 mL/min. Bottom *ca.* 38% as analysed by peak area of RP-HPLC at 214 nm; R_t 16.16 min; *m/z* (ESI-MS) 976.5 ($[M+4H]^{4+}$ requires 976.8); linear gradient of 5%B to 65%B over 20 min (*ca.* 3%B/min) at 40 °C, 0.3 mL/min.

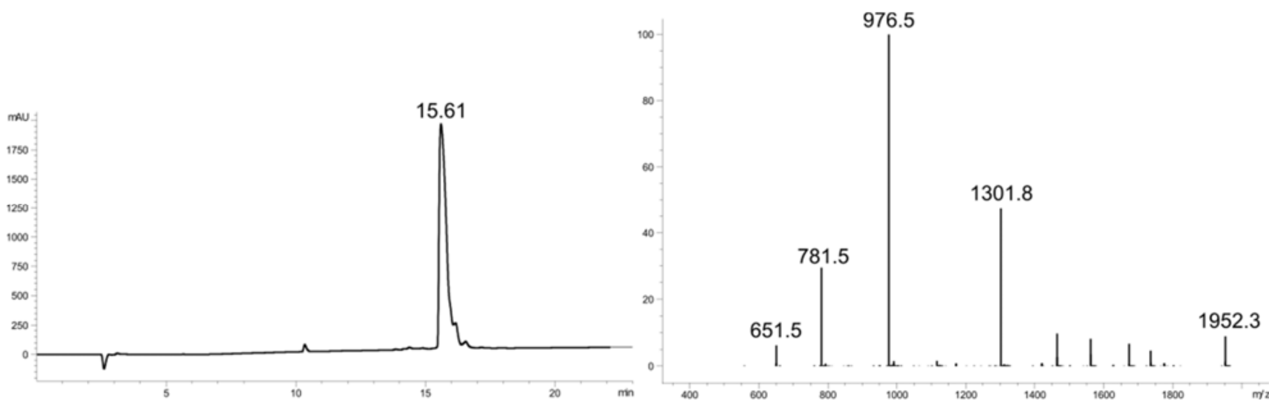

Figure SC5: LCMS trace of purified human amylin (95%); R_t 15.61 min; *m/z* (ESI-MS) 976.5 ($[M+4H]^{4+}$ requires 976.8); linear gradient of 5%B to 65%B over 60 min (*ca.* 1%B/min) at 40 °C, 0.3 mL/min.

Purified *N*-terminal Analogs

K1A

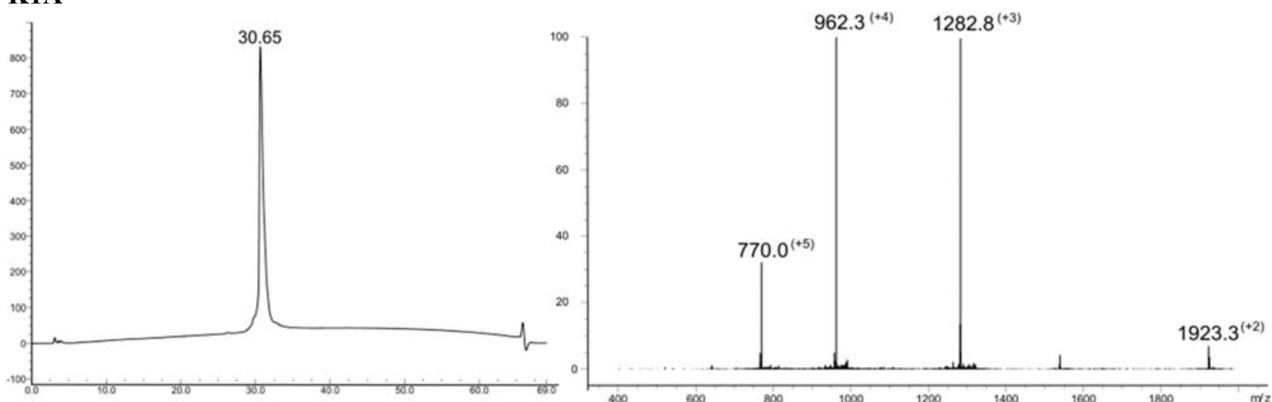

Figure SC6: LCMS trace of K1A analog of human amylin (96%); R_t 30.65 min; *m/z* (ESI-MS) 962.3 ($[M+4H]^{4+}$ requires 962.6); linear gradient of 5%B to 65%B over 60 min (*ca*. 1%B/min) at 40 °C, 0.3 mL/min.

N3A

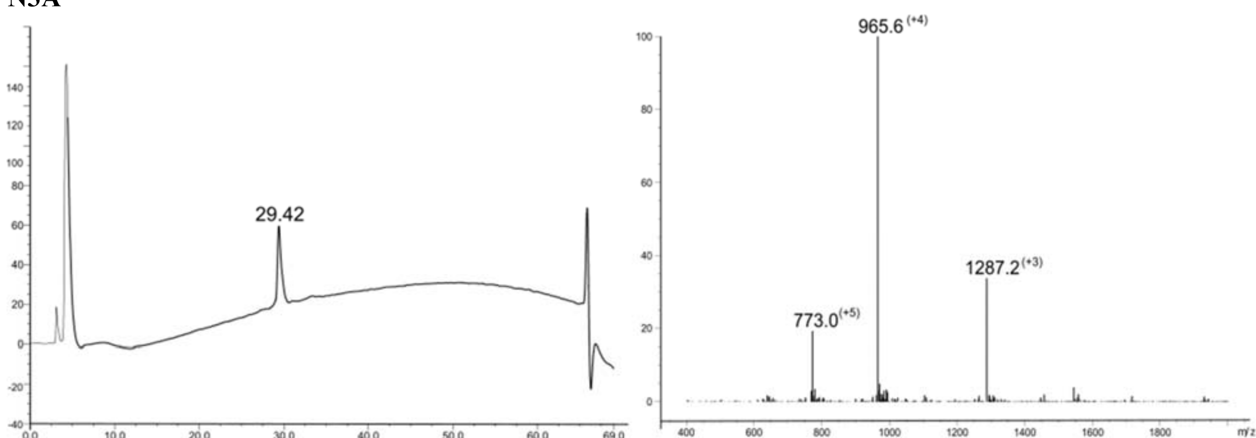

Figure SC7: LCMS trace of N3A analog of human amylin (98%); R_t 29.42 min; *m/z* (ESI-MS) 965.6 ($[M+4H]^{4+}$ requires 966.1); linear gradient of 5%B to 65%B over 60 min (*ca*. 1%B/min) at 40 °C, 0.3 mL/min. The peak at *ca.* 5 mins is attributed to the re-equilibration of the HPLC column.

T4A

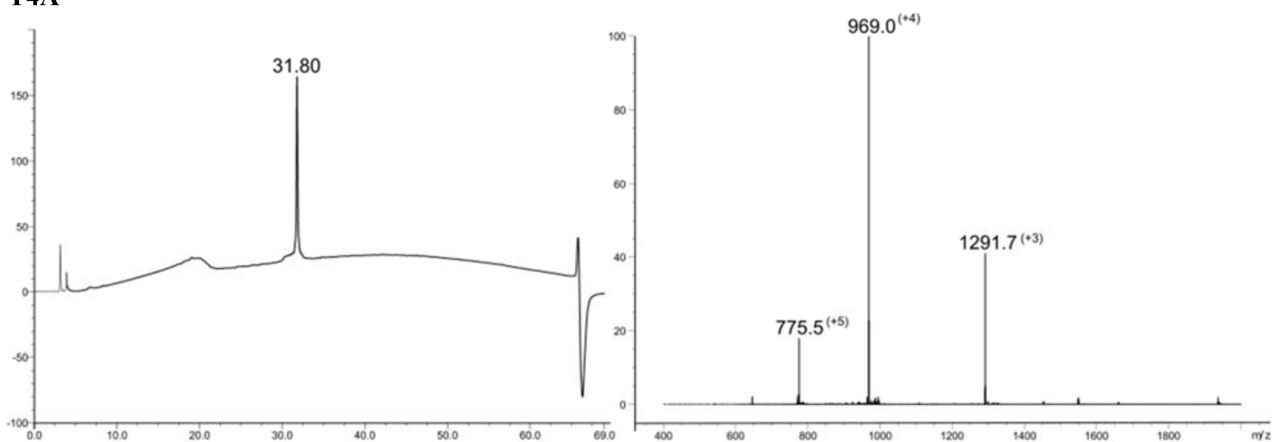

Figure SC8: LCMS trace of T4A analog of human amylin (92%); R_t 31.80 min; *m/z* (ESI-MS) 969.0 ($[M+4H]^{4+}$ requires 969.3); linear gradient of 5%B to 65%B over 60 min (*ca*. 1%B/min) at 40 °C, 0.3 mL/min.

A5G

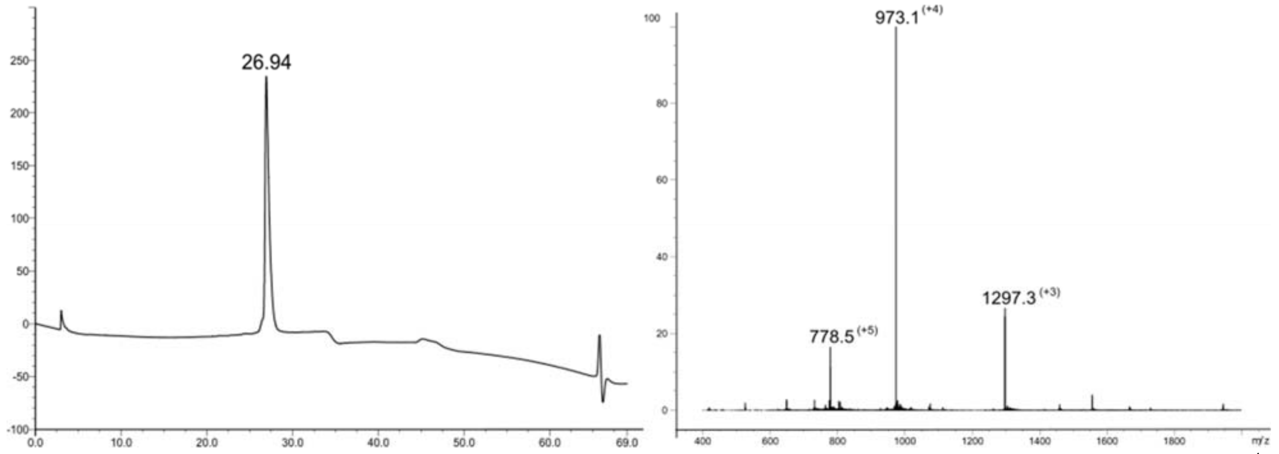

Figure SC9: LCMS trace of A5G analog of human amylin (96%); R_t 26.94 min; *m/z* (ESI-MS) 973.1 ($[M+4H]^{4+}$ requires 973.3); linear gradient of 5%B to 65%B over 60 min (*ca*. 1%B/min) at 40 °C, 0.3 mL/min.

T6A

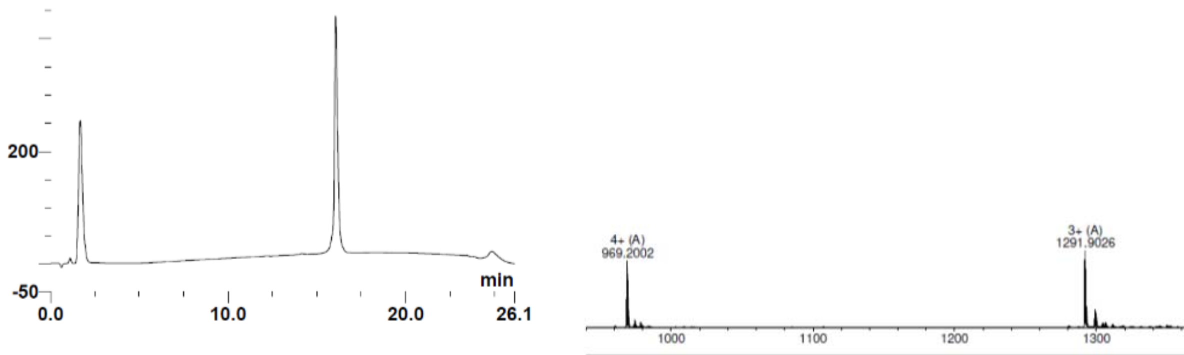

Figure SC10: RP-HPLC trace of T6A analog of human amylin (96%); R_t 15.50 min; *m/z* (ESI-MS) 969.2 ($[M+4H]^{4+}$ requires 969.3); linear gradient of 5%B to 65%B over 20 min (*ca*. 3%B/min) at 45 °C, 1 mL/min. The peak at *ca.* 2 mins is attributed to the re-equilibration of the HPLC column.

A8G

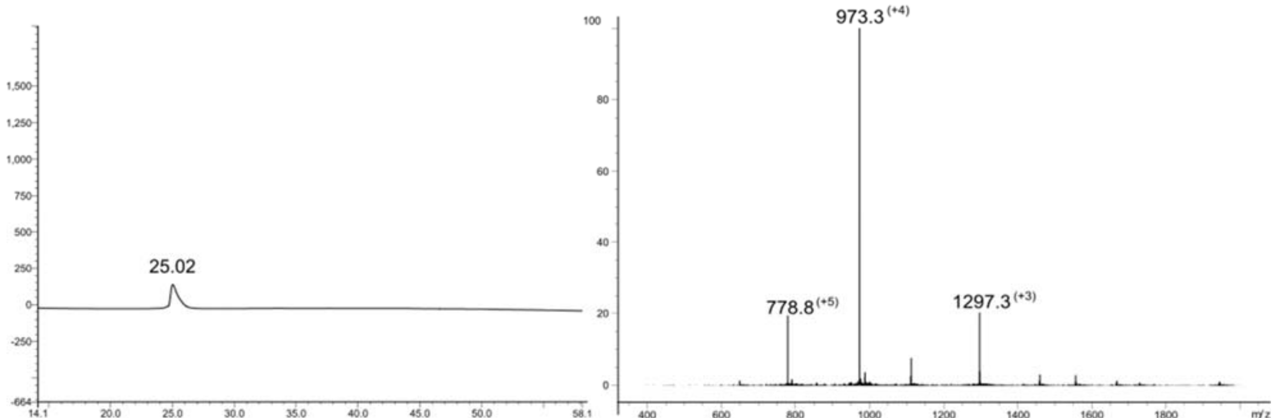

Figure SC11: LCMS trace of A8G analog of human amylin (96%); R_t 25.02 min; *m/z* (ESI-MS) 973.3 ($[M+4H]^{4+}$ requires 973.3); linear gradient of 5%B to 65%B over 60 min (*ca*. 1%B/min) at 40 °C, 0.3 mL/min.

T9A

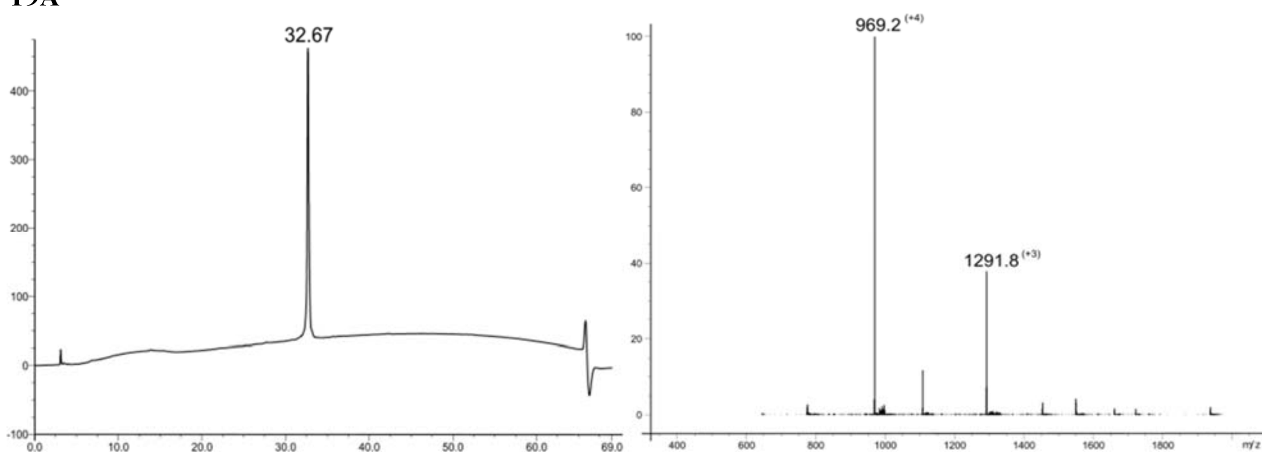

Figure SC12: LCMS trace of T9A analog of human amylin (96%); R_t 32.67 min; *m/z* (ESI-MS) 969.2 ($[M+4H]^{4+}$ requires 969.3); linear gradient of 5%B to 65%B over 60 min (*ca.* 1%B/min) at 40 °C, 0.3 mL/min.

Q10A

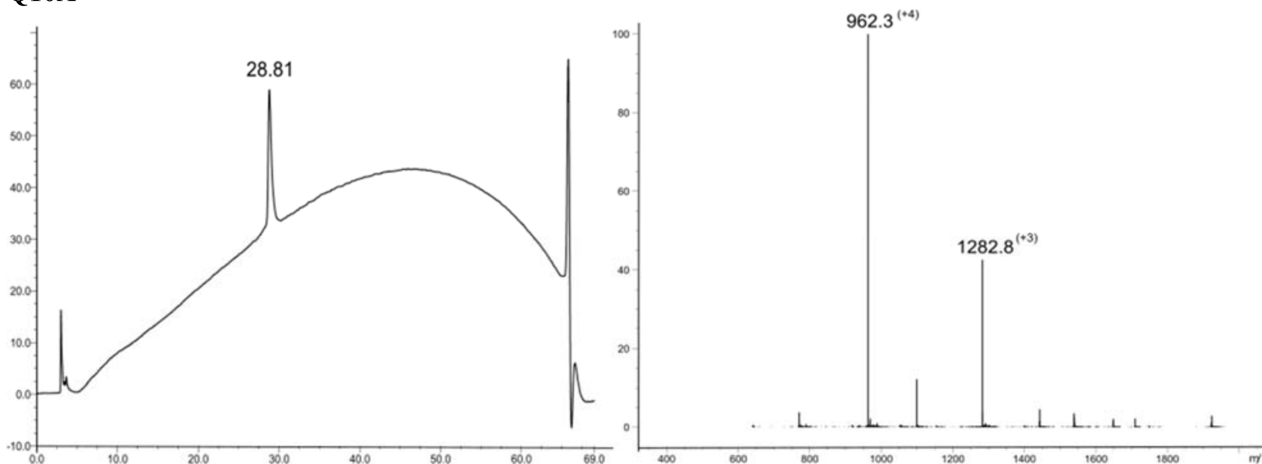

Figure SC13: LCMS trace of Q10A analog of human amylin (98%); R_t 28.81 min; *m/z* (ESI-MS) 962.3 ($[M+4H]^{4+}$ requires 962.6); linear gradient of 5%B to 65%B over 60 min (*ca.* 1%B/min) at 40 °C, 0.3 mL/min.

R11A

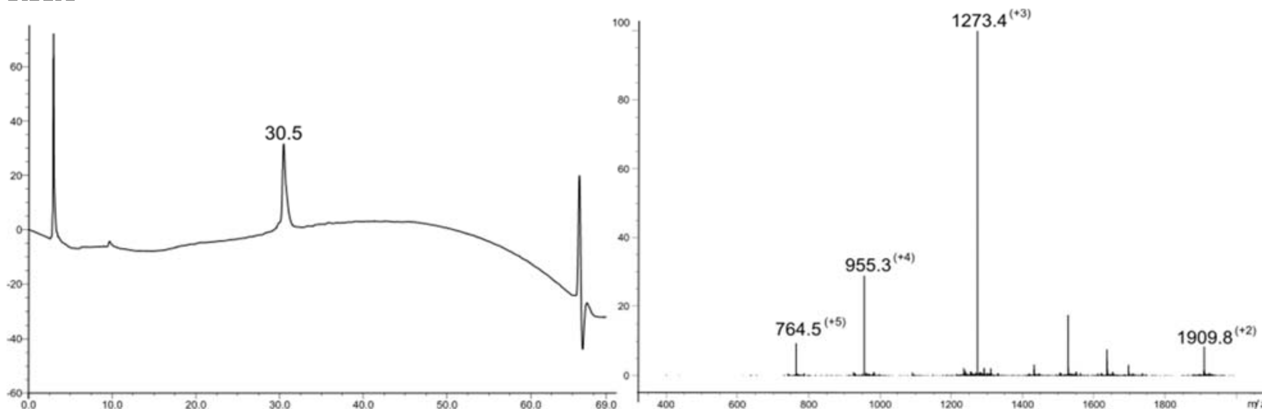

Figure SC14: LCMS trace of R11A analog of human amylin (91%); R_t 30.5 min; *m/z* (ESI-MS) 955.3 ($[M+4H]^{4+}$ requires 955.6); linear gradient of 5%B to 65%B over 60 min (*ca.* 1%B/min) at 40 °C, 0.3 mL/min. The peak at *ca.* 2 mins is attributed to the re-equilibration of the HPLC column.

L12A

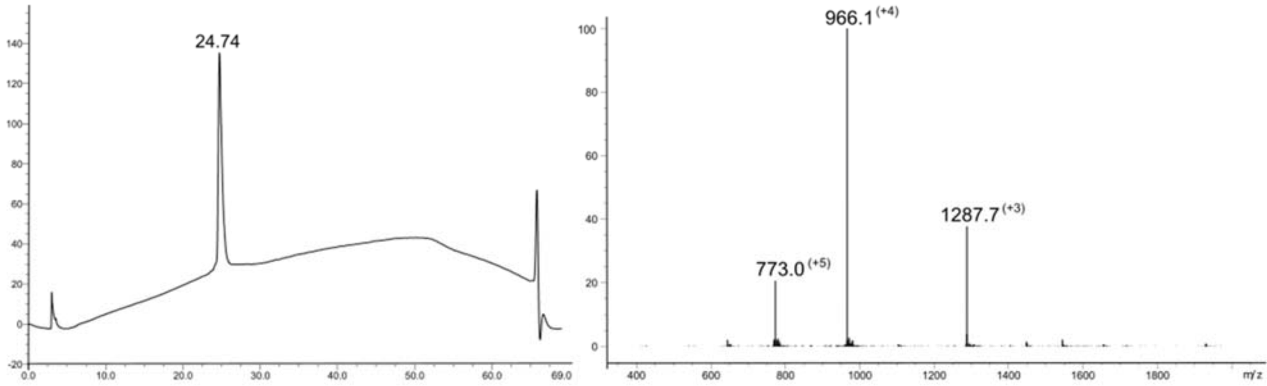

Figure SC15: LCMS trace of L12A analog of human amylin (98%); R_t 24.74 min; *m/*z (ESI-MS) 966.1 ($[M+4H]^{4+}$ requires 966.3); linear gradient of 5%B to 65%B over 60 min (*ca*. 1%B/min) at 40 °C, 0.3 mL/min.

A13G

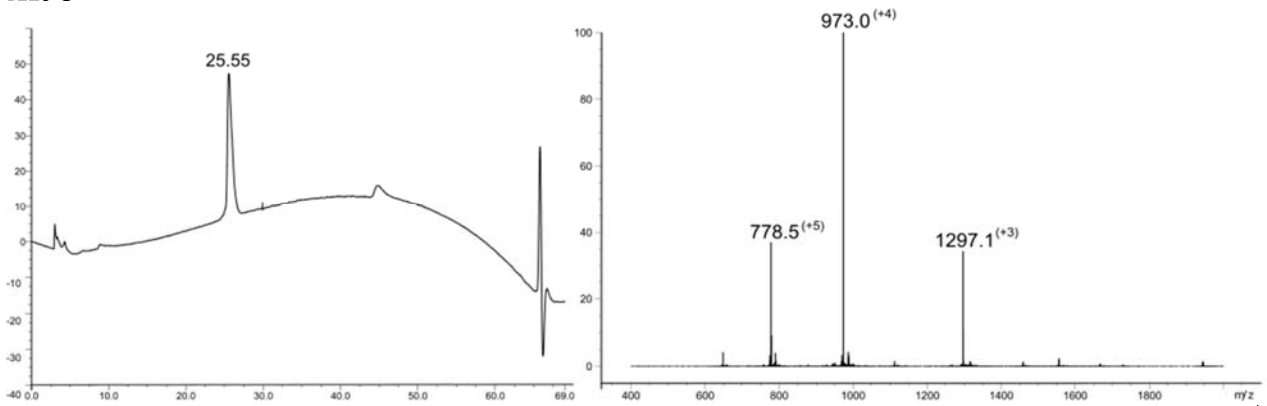

Figure SC16: LCMS trace of A13G analog of human amylin (93%); R_t 25.55 min; *m/*z (ESI-MS) 973.0 ($[M+4H]^{4+}$ requires 973.3); linear gradient of 5%B to 65%B over 60 min (*ca*. 1%B/min) at 40 °C, 0.3 mL/min.

N14A

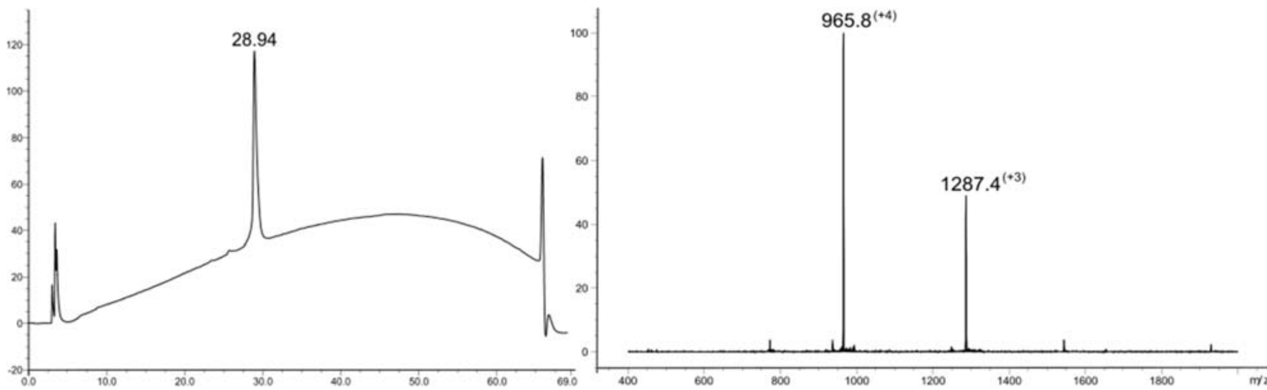

Figure SC17: LCMS trace of N14A analog of human amylin (98%); R_t 28.94 min; *m/*z (ESI-MS) 965.8 ($[M+4H]^{4+}$ requires 966.1); linear gradient of 5%B to 65%B over 60 min (*ca*. 1%B/min) at 40 °C, 0.3 mL/min.

F15A

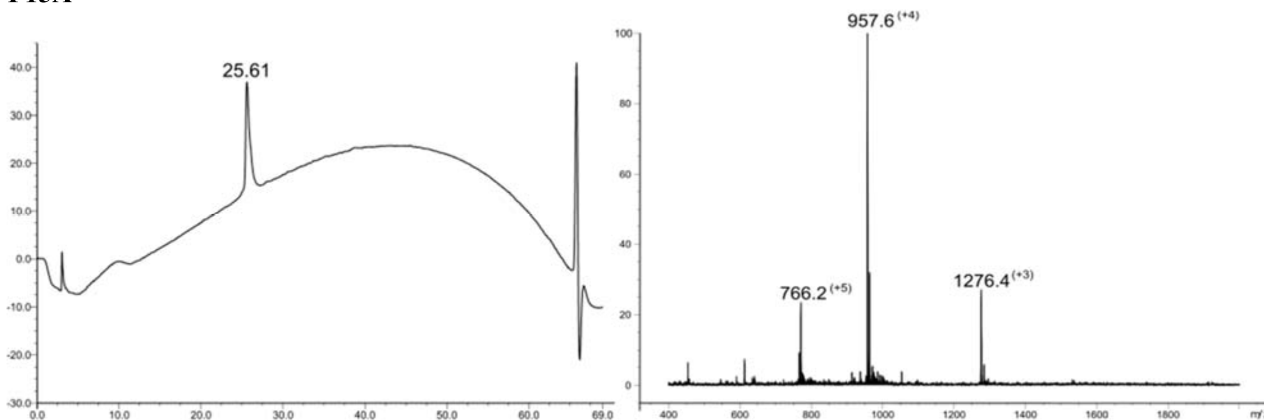

Figure SC18: LCMS trace of F15A analog of human amylin (97%); R_t 25.61 min; *m/*z (ESI-MS) 957.6 ($[M+4H]^{4+}$ requires 957.8); linear gradient of 5%B to 65%B over 60 min (*ca*. 1%B/min) at 40 °C, 0.3 mL/min.

L16A

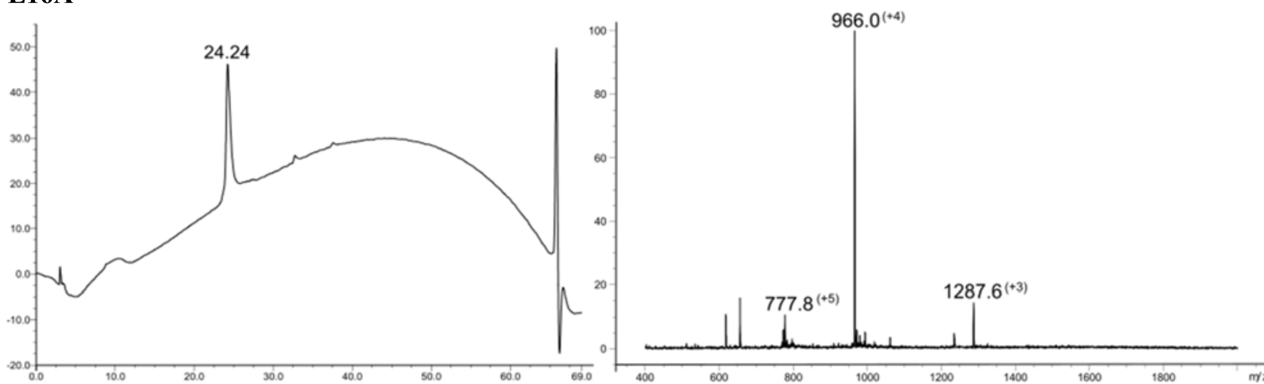

Figure SC19: LCMS trace of L16A analog of human amylin (97%); R_t 24.24 min; *m/*z (ESI-MS) 966.0 ($[M+4H]^{4+}$ requires 966.3); linear gradient of 5%B to 65%B over 60 min (*ca*. 1%B/min) at 40 °C, 0.3 mL/min.

V17A

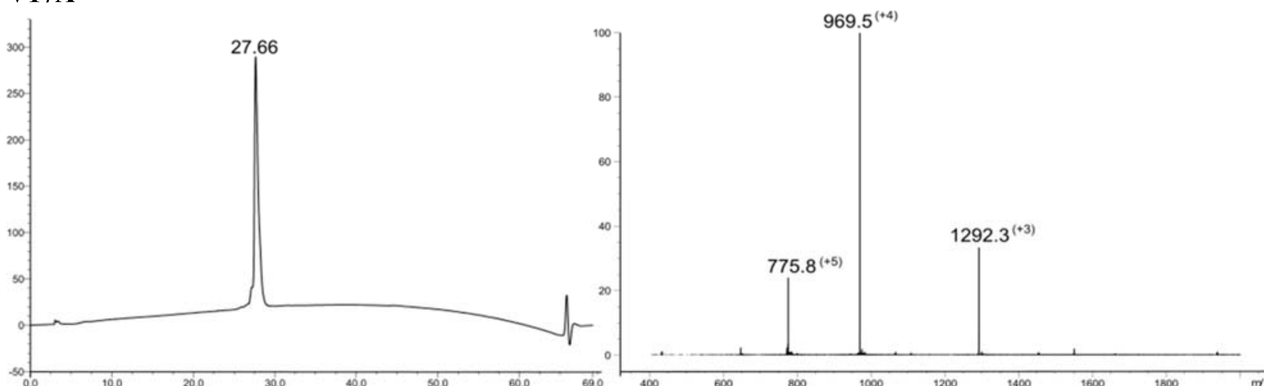

Figure SC20: LCMS trace of V17A analog of human amylin (96%); R_t 27.66 min; *m/*z (ESI-MS) 969.5 ($[M+4H]^{4+}$ requires 969.8); linear gradient of 5%B to 65%B over 60 min (*ca*. 1%B/min) at 40 °C, 0.3 mL/min.

Purified *N*-terminal Loop Analogs
C2S-C7S human amylin

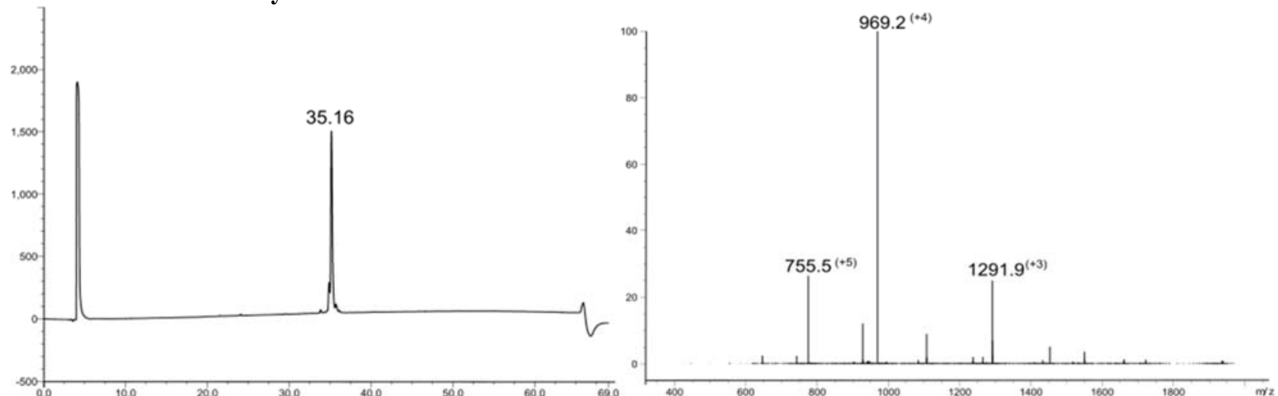

Figure SC21: LCMS trace of C2S-C7S analog of human amylin (90%); R_t 35.16 min; *m/*z (ESI-MS) 969.2 ($[M+4H]^{4+}$ requires 969.3); linear gradient of 5%B to 65%B over 60 min (*ca*. 1%B/min) at 40 °C, 0.3 mL/min. The peak at *ca.* 5 mins is attributed to the re-equilibration of the HPLC column.

CAM-human amylin

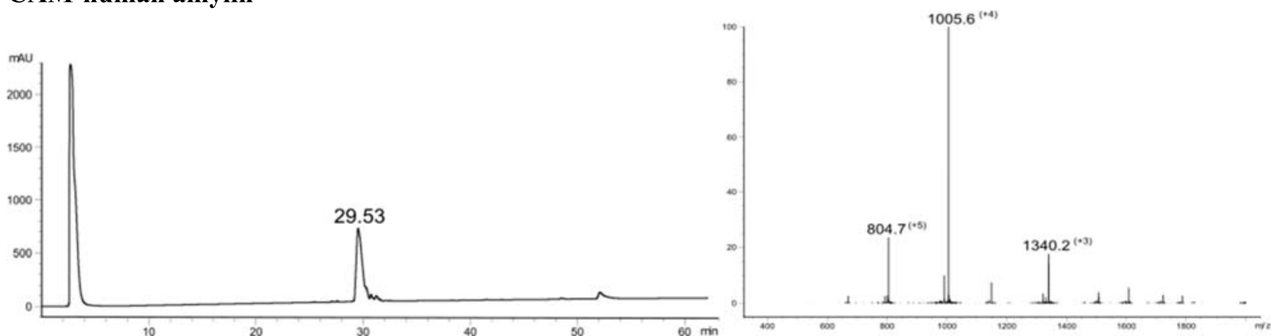

Figure SC22: LCMS trace of CAM-human amylin (93%); R_t 29.53 min; *m/*z (ESI-MS) 1005.6 ($[M+4H]^{4+}$ requires 1005.9); linear gradient of 5%B to 65%B over 60 min (*ca*. 1%B/min) at 40 °C, 0.3 mL/min. The peak at *ca.* 5 mins is attributed to the re-equilibration of the HPLC column.

Human amylin$_{8\text{-}37}$

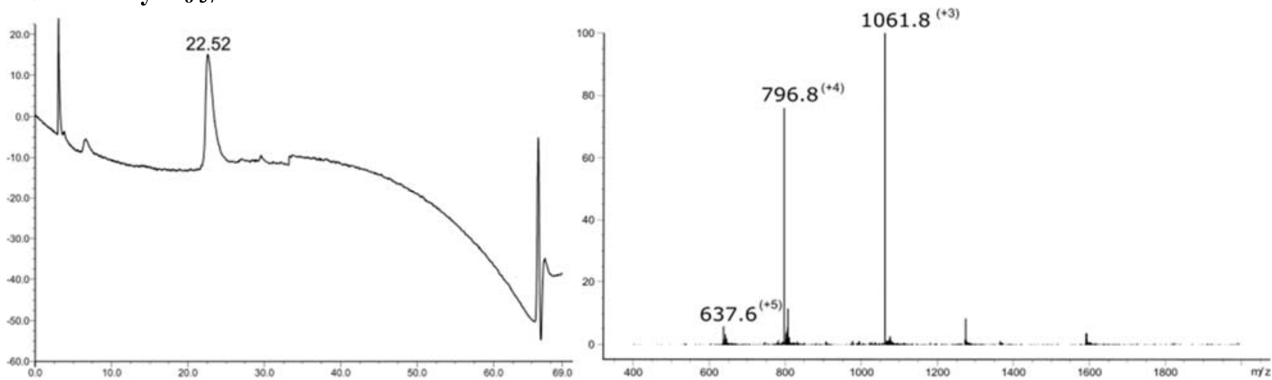

Figure SC23: LCMS trace of 8-37 fragment of human amylin (85%) R_t 22.52 min; *m/*z (ESI-MS) 796.8 ($[M+4H]^{4+}$ requires 796.9); linear gradient of 5%B to 65%B over 60 min (*ca*. 1%B/min) at 40 °C, 0.3 mL/min. The peak at *ca.* 2 mins is attributed to the re-equilibration of the HPLC column.

Human amylin$_{8\text{-}37\ (DR)}$

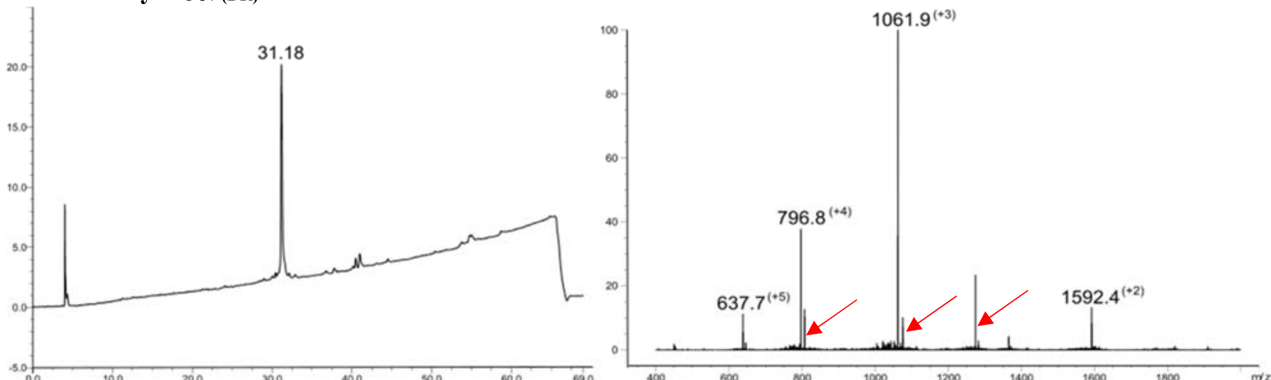

Figure SC24: LCMS trace of 8-37(DR) fragment of human amylin (83%) R_t 31.18 min; *m/z* (ESI-MS) 796.8 ($[M+4H]^{4+}$ requires 796.9); linear gradient of 5%B to 65%B over 60 min (*ca.* 1%B/min) at 40 °C, 0.3 mL/min. The peak at *ca.* 5 mins is attributed to the re-equilibration of the HPLC column.

There is a caveat regarding the purity of the two 8-37 peptides. Within the main peak an additional species eluted with the same retention time as 8-37, indicated by red arrows in the ESI-MS trace for 8-37(DR). This could not be removed by HPLC purification.

Human amylin$_{Ac8\text{-}37(DR)}$

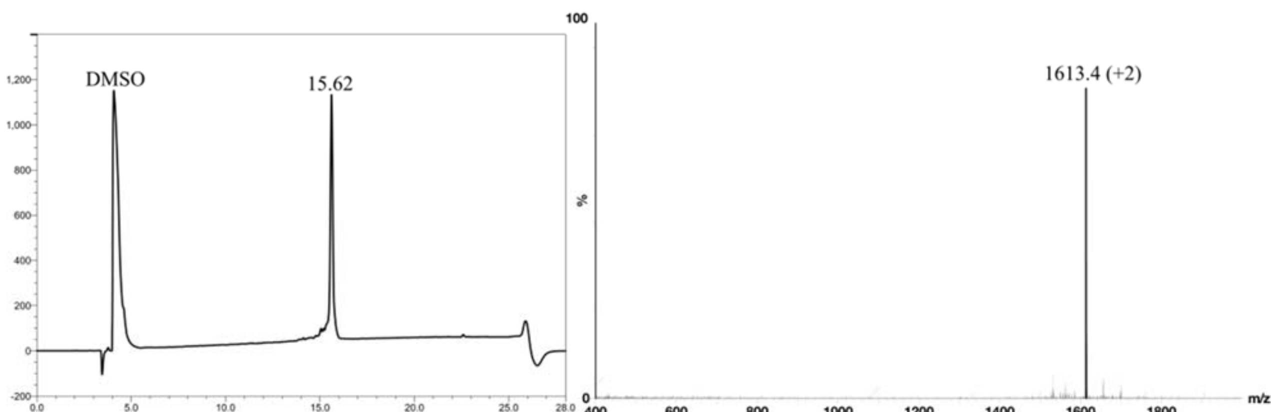

Figure SC25: LCMS trace of Ac8-37 (DR) human amylin (97%) R_t 15.62 min; *m/z* (ESI-MS) 1613.4 ($[M+2H]^{2+}$ requires 1614.8); linear gradient of 5%B to 65%B over 20 min (*ca.* 3%B/min) at 40 °C, 1 mL/min.

Human amylin$_{1\text{-}17}$

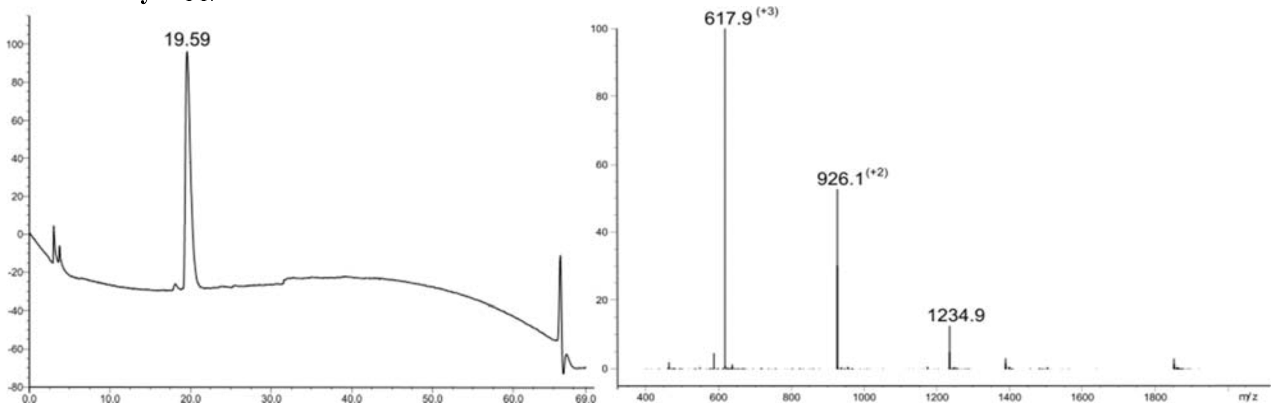

Figure SC26: LCMS trace of 1-17 fragment of human amylin (99%); R_t 19.59 min; *m/z* (ESI-MS) 926.1 ($[M+2H]^{2+}$ requires 926.6); linear gradient of 5%B to 65%B over 60 min (*ca.* 1%B/min) at 40 °C, 0.3 mL/min.

Purified Additional *N*-terminal Analogs
PramQ10A

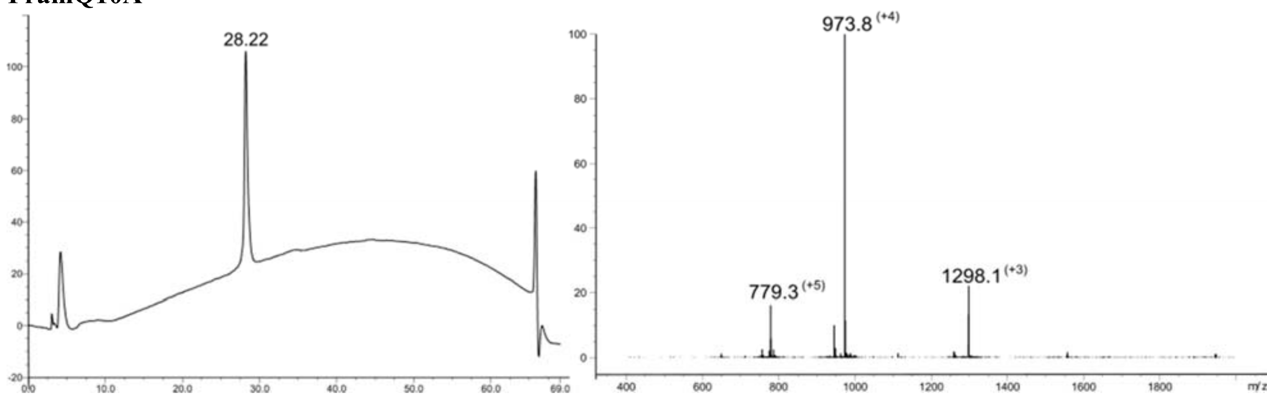

Figure SC27: LCMS trace of Q10A analog of pramlintide (99%); R_t 28.22 min; *m/*z (ESI-MS) 973.8 ($[M+4H]^{4+}$ requires 974.1); linear gradient of 5%B to 65%B over 60 min (*ca*. 1%B/min) at 40 °C, 0.3 mL/min. The peak at *ca.* 5 mins is attributed to the re-equilibration of the HPLC column.

A5S

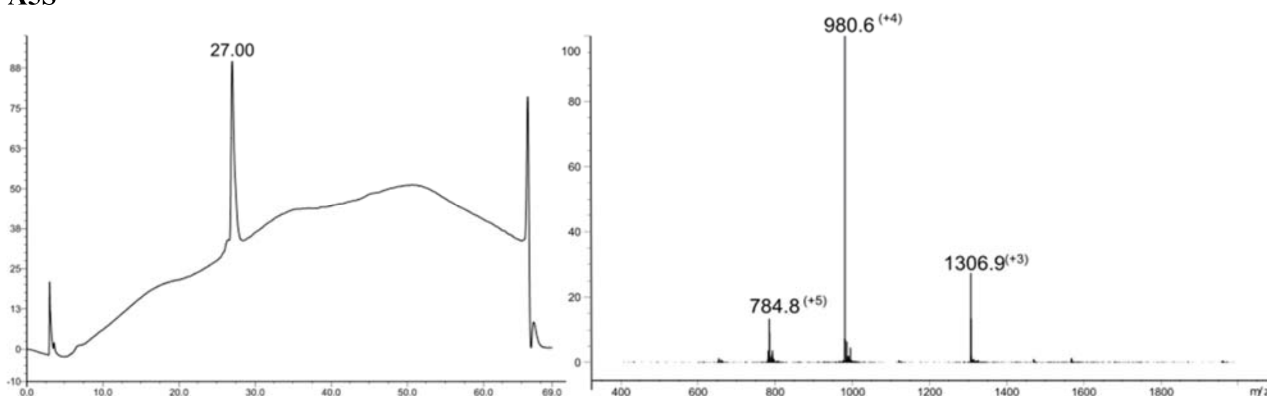

Figure SC28: LCMS trace of A5S analog of human amylin (95%); R_t 27.00 min; *m/*z (ESI-MS) 980.6 ($[M+4H]^{4+}$ requires 980.8); linear gradient of 5%B to 65%B over 60 min (*ca*. 1%B/min) at 40 °C, 0.3 mL/min.

Purified *C*-terminal Analogs

I26A

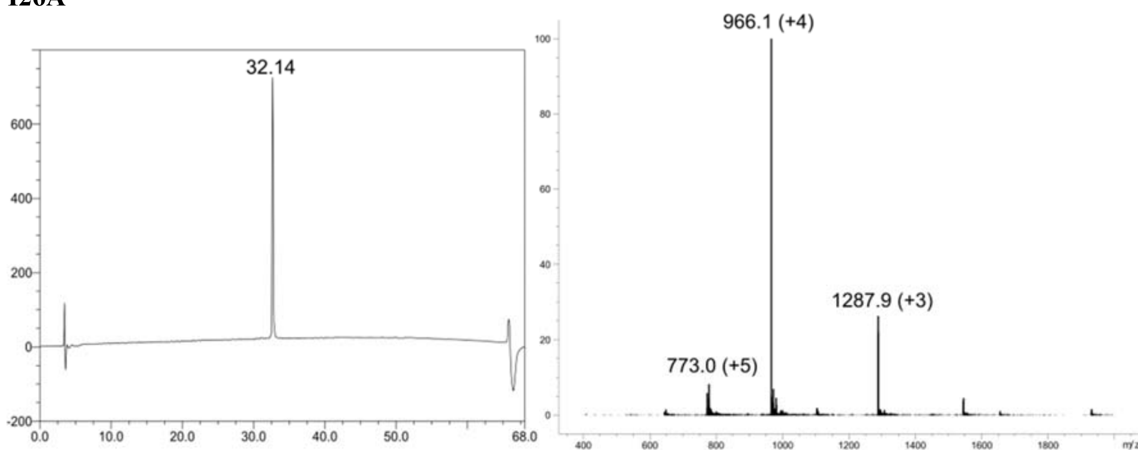

Figure SC29: RP-HPLC trace of I26A analog of human amylin (99%); R_t 32.14 min; *m/z* (ESI-MS) 966.1 ($[M+4H]^{4+}$ requires 966.3); linear gradient of 5%B to 65%B over 60 min (*ca.* 1%B/min) at 45 °C, 1 mL/min.

L27A

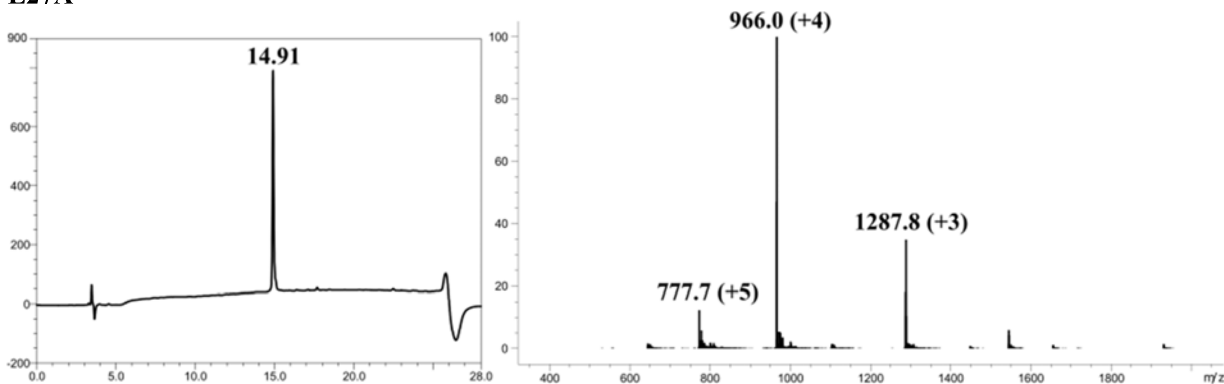

Figure SC30: RP-HPLC trace of L27A analog of human amylin (98%); R_t 32.14 min; *m/z* (ESI-MS) 966.0 ($[M+4H]^{4+}$ requires 966.3); linear gradient of 5%B to 65%B over 20 min (*ca.* 3%B/min) at 45 °C, 1 mL/min.

S28A

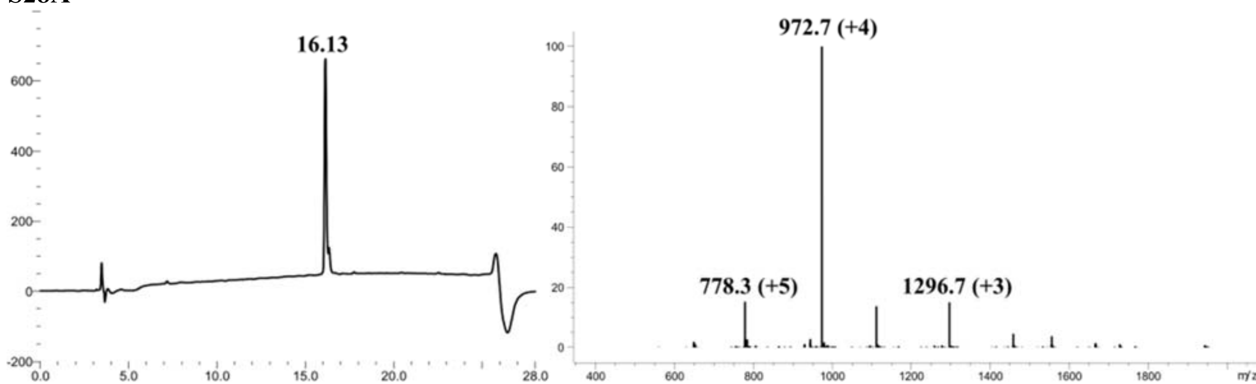

Figure SC31: RP-HPLC trace of S28A analog of human amylin (94%); R_t 16.13 min; *m/z* (ESI-MS) 972.7 ($[M+4H]^{4+}$ requires 972.8); linear gradient of 5%B to 65%B over 20 min (*ca.* 3%B/min) at 45 °C, 1 mL/min.

S29A

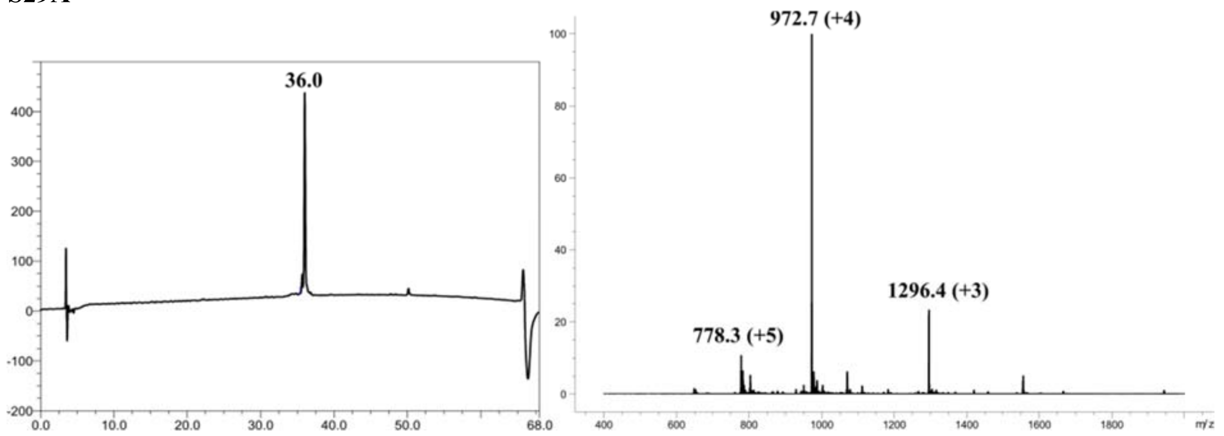

Figure SC32: RP-HPLC trace of S29A analog of human amylin (95%); R_t 36.0 min; *m/z* (ESI-MS) 972.7 ($[M+4H]^{4+}$ requires 972.8); linear gradient of 5%B to 65%B over 60 min (*ca.* 1%B/min) at 45 °C, 1 mL/min.

T30A

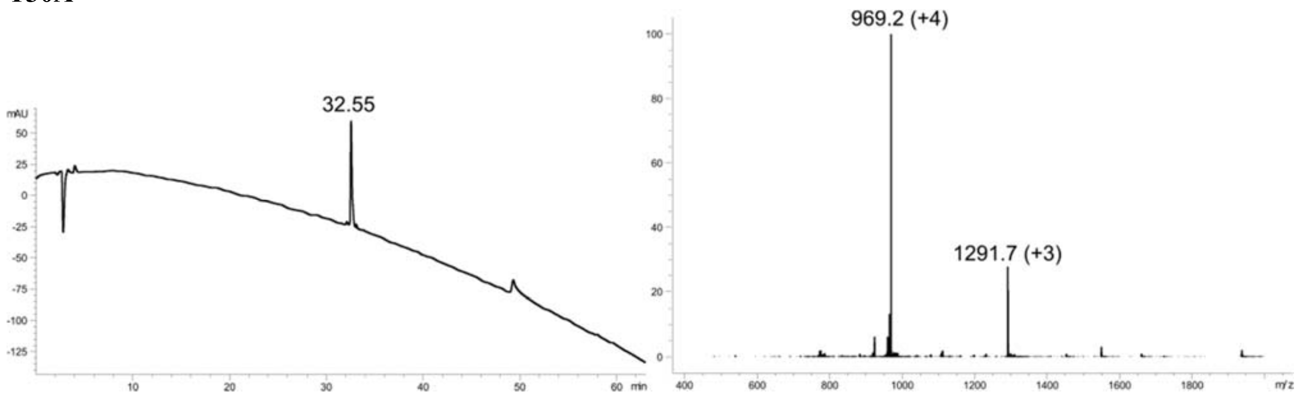

Figure SC33: LCMS trace of T30A analog of human amylin (94%); R_t 32.55 min; *m/z* (ESI-MS) 969.2 ($[M+4H]^{4+}$ requires 969.3); linear gradient of 5%B to 65%B over 60 min (*ca.* 1%B/min) at 40 °C, 0.3 mL/min.

N31A

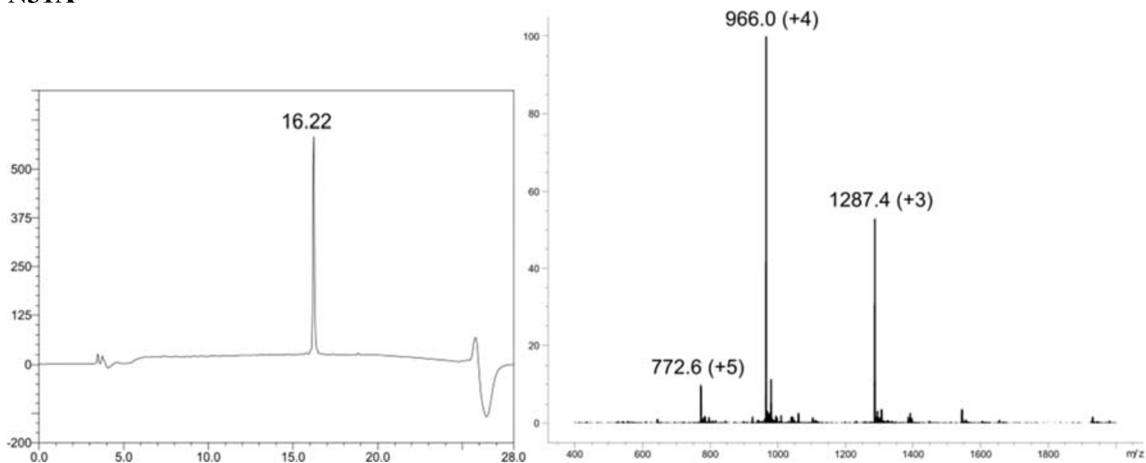

Figure SC34: RP-HPLC trace of N31A analog of human amylin (97%); R_t 16.22 min; *m/z* (ESI-MS) 966.0 ($[M+4H]^{4+}$ requires 966.1); linear gradient of 5%B to 65%B over 20 min (*ca.* 3%B/min) at 45 °C, 1 mL/min.

V32A

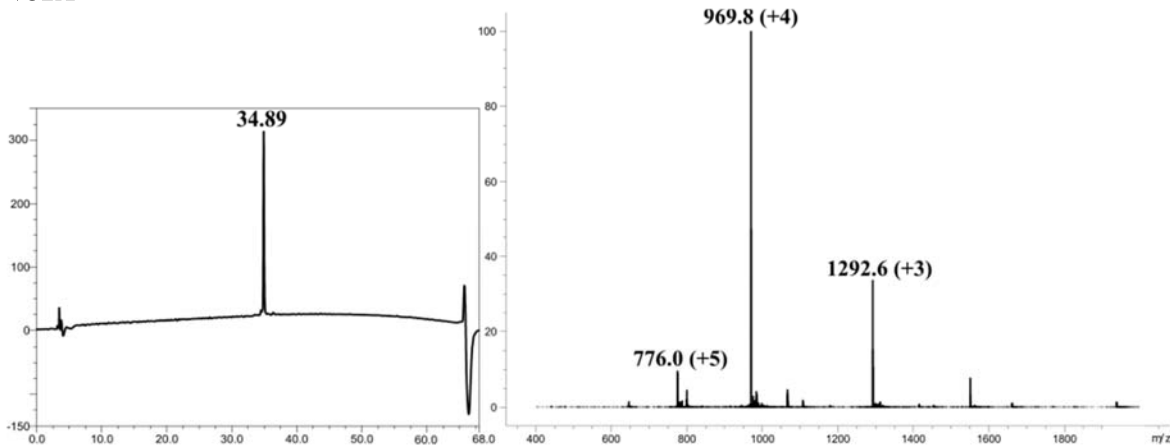

Figure SC35: RP-HPLC trace of V32A analog of human amylin (96%); R_t 34.89 min; *m/z* (ESI-MS) 969.8 ($[M+4H]^{4+}$ requires 969.8); linear gradient of 5%B to 65%B over 60 min (*ca*. 1%B/min) at 45 °C, 1 mL/min.

G33A

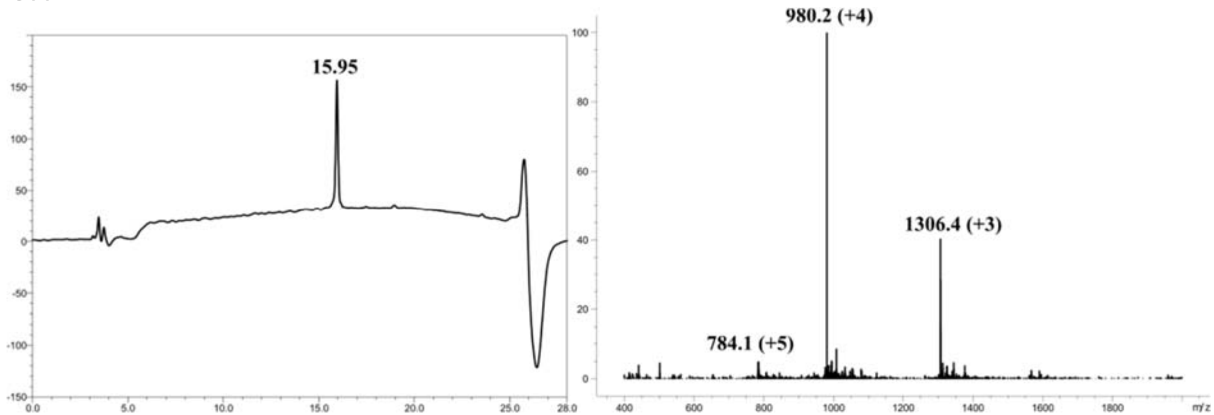

Figure SC36: RP-HPLC trace of G33A analog of human amylin (98%); R_t 15.95 min; *m/z* (ESI-MS) 980.2 ($[M+4H]^{4+}$ requires 980.3); linear gradient of 5%B to 65%B over 20 min (*ca*. 3%B/min) at 45 °C, 1 mL/min.

S34A

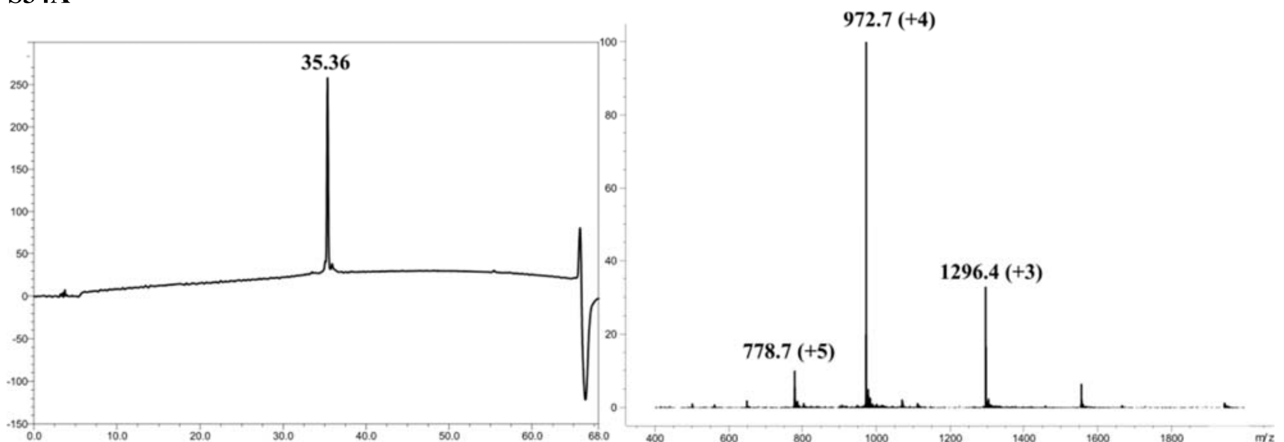

Figure SC37: RP-HPLC trace of S34A analog of human amylin (94%); R_t 35.36 min; *m/z* (ESI-MS) 972.7 ($[M+4H]^{4+}$ requires 972.8); linear gradient of 5%B to 65%B over 60 min (*ca*. 1%B/min) at 45 °C, 1 mL/min.

N35A

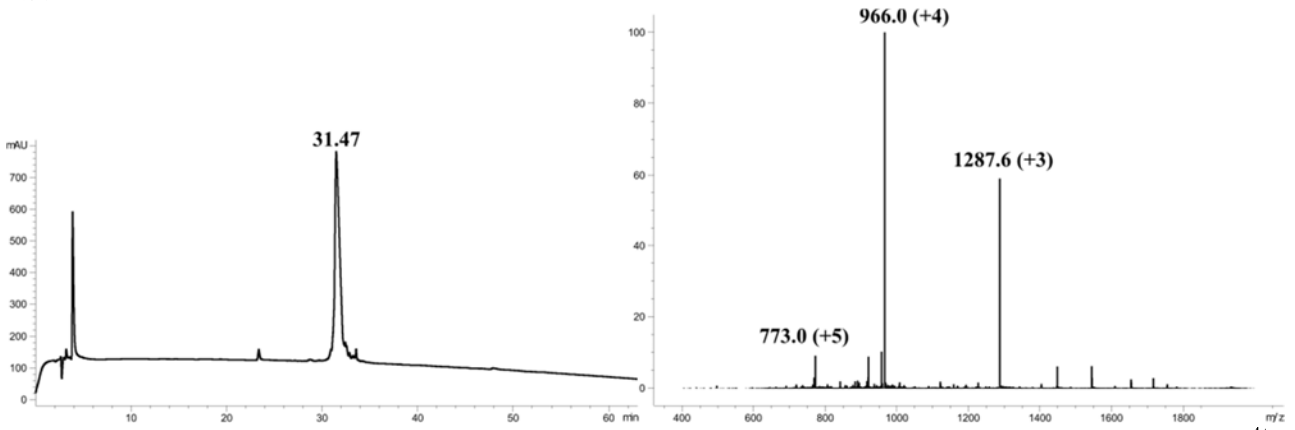

Figure SC38: LCMS trace of N35A analog of human amylin (94%); R_t 31.47 min; *m/z* (ESI-MS) 966.0 ($[M+4H]^{4+}$ requires 966.1); linear gradient of 5%B to 65%B over 60 min (*ca*. 1%B/min) at 40 °C, 0.3 mL/min.

T36A

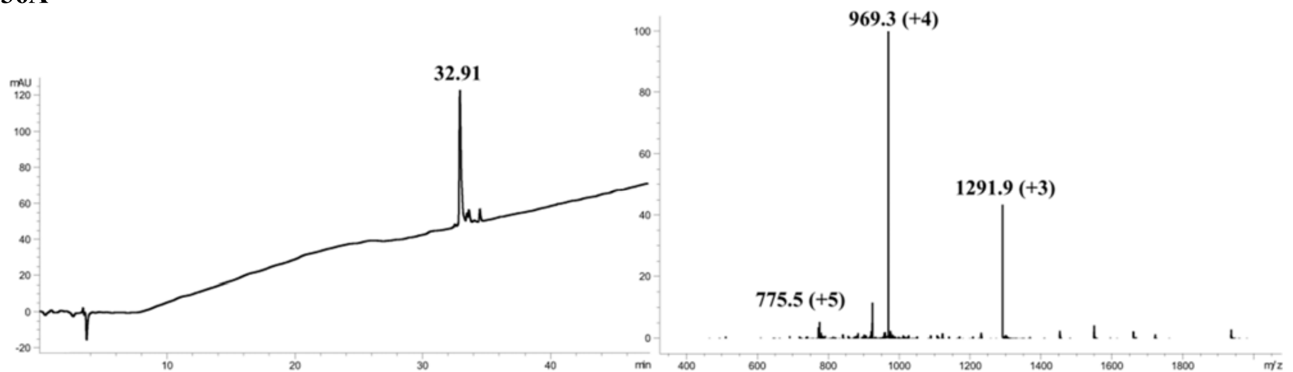

Figure SC39: LCMS trace of T36A analog of human amylin (93%); R_t 32.91 min; *m/z* (ESI-MS) 969.3 ($[M+4H]^{4+}$ requires 969.3); linear gradient of 5%B to 65%B over 60 min (*ca*. 1%B/min) at 40 °C, 0.3 mL/min.

Y37A

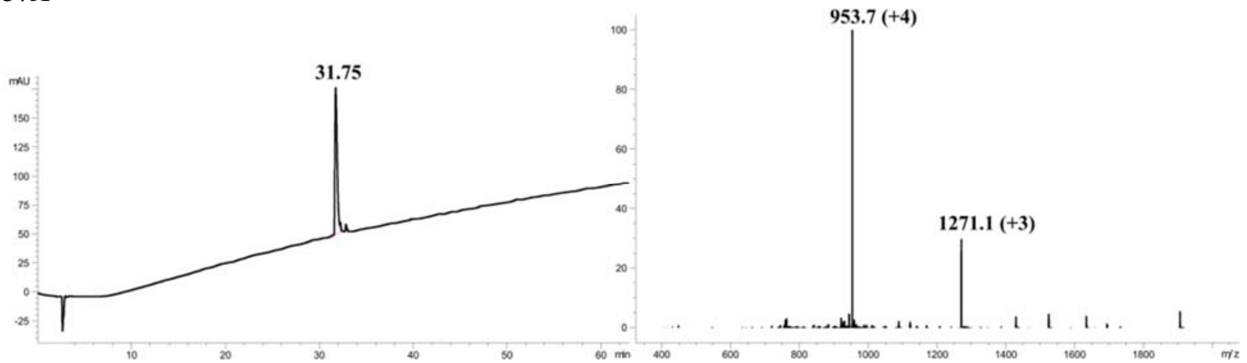

Figure SC40: LCMS trace of Y37A analog of human amylin (98%); R_t 31.75 min; *m/z* (ESI-MS) 953.7 ($[M+4H]^{4+}$ requires 953.8); linear gradient of 5%B to 65%B over 60 min (*ca*. 1%B/min) at 40 °C, 0.3 mL/min.

Human amylin-COOH

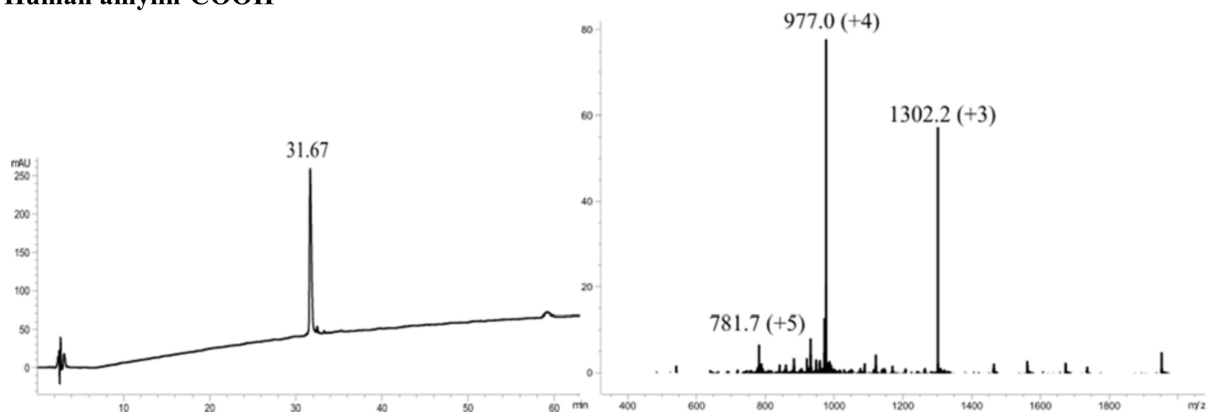

Figure SC41: LCMS trace of -COOH analog of human amylin (99%); R_t 31.67 min; *m/*z (ESI-MS) 977.0 ($[M+4H]^{4+}$ requires 977.1); linear gradient of 5%B to 65%B over 60 min (*ca*. 1%B/min) at 40 °C, 0.3 mL/min.

Purified human CGRP, calcitonin and amylin *C*-terminal exchanges/Analogs
CGRP

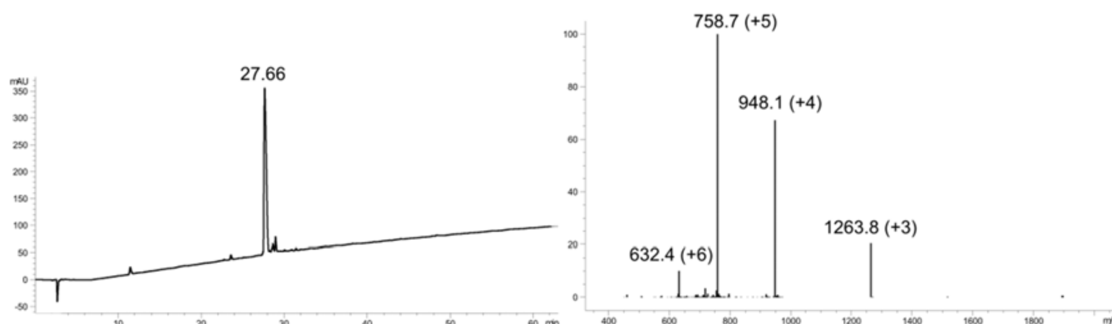

Figure SC42: LCMS trace of hαCGRP (97%); R_t 27.66 min; *m/z* (ESI-MS) 948.1 ($[M+4H]^{4+}$ requires 948.3); linear gradient of 5%B to 65%B over 60 min (*ca.* 1%B/min) at 40 °C, 0.3 mL/min.

CGRP F37Y

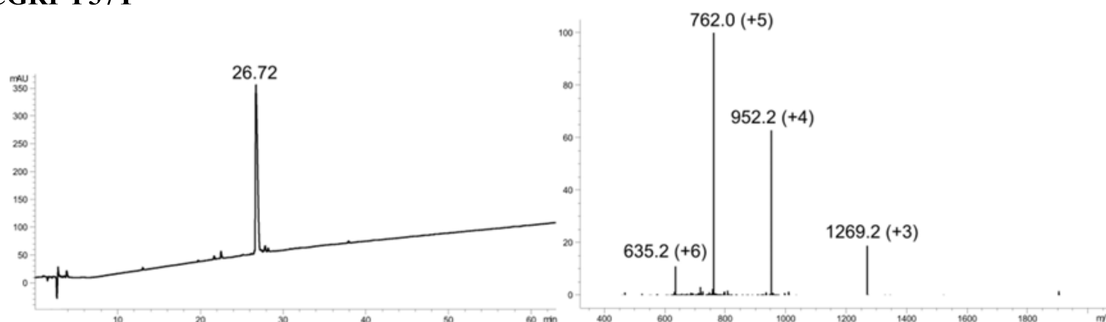

Figure SC43: LCMS trace of F37Y analog of hαCGRP (96%); R_t 26.72 min; *m/z* (ESI-MS) 952.2 ($[M+4H]^{4+}$ requires 952.3); linear gradient of 5%B to 65%B over 60 min (*ca.* 1%B/min) at 40 °C, 0.3 mL/min.

Human amylin Y37F

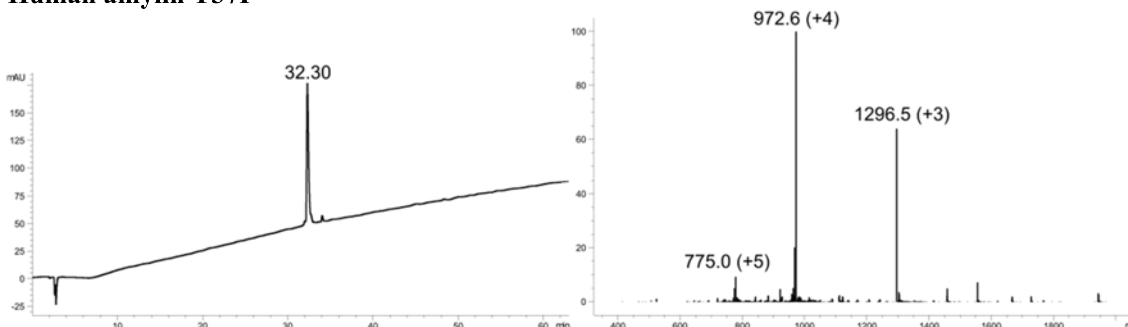

Figure SC44: LCMS trace of Y37F analog of human amylin (99%); R_t 32.30 min; *m/z* (ESI-MS) 972.6 ($[M+4H]^{4+}$ requires 972.8); linear gradient of 5%B to 65%B over 60 min (*ca.* 1%B/min) at 40 °C, 0.3 mL/min.

Human amylin Y37P

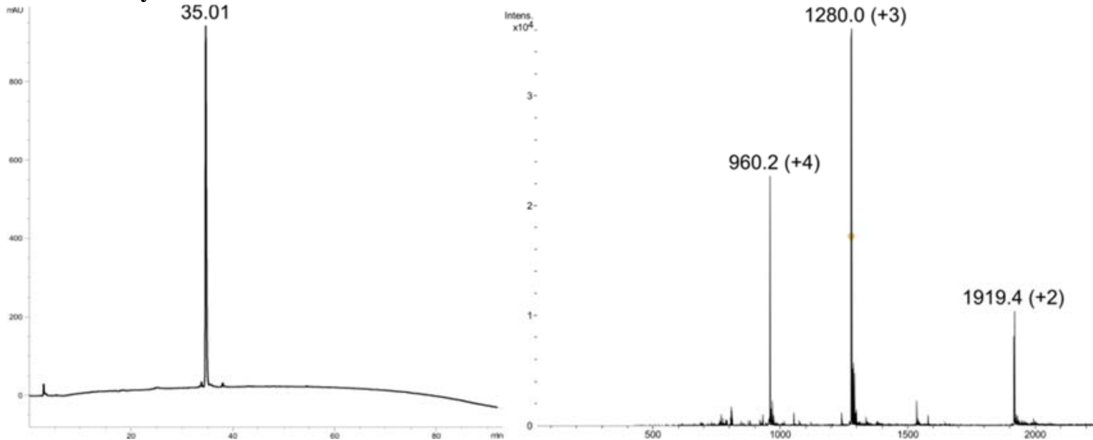

Figure SC45: RP-HPLC trace of Y37P analog of human amylin (95%); R_t 35.01 min; *m/z* (ESI-MS) 1280.0 ($[M+3H]^{3+}$ requires 1280.2); linear gradient of 5%B to 65%B over 60 min (*ca*. 1%B/min) at 45 °C, 1 mL/min.

Human calcitonin

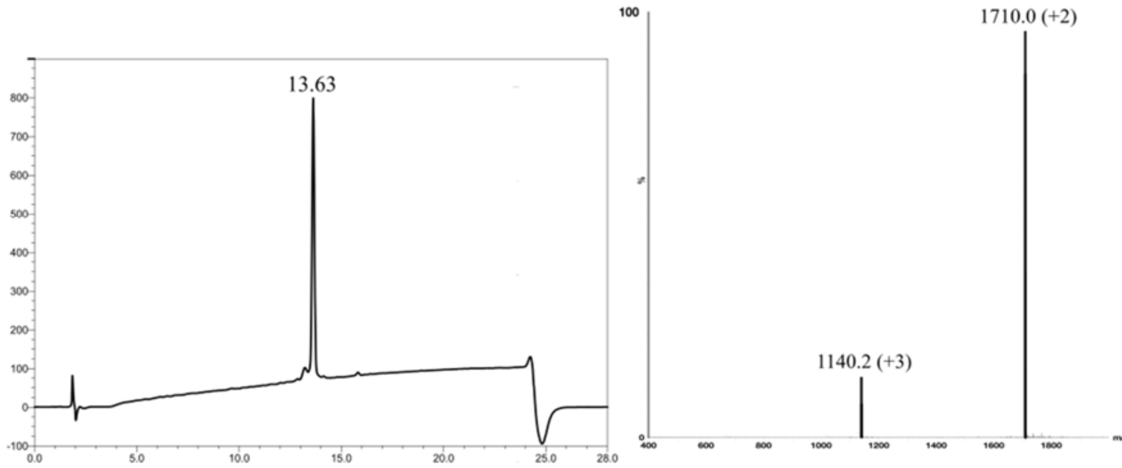

Figure SC46: RP-HPLC trace of human calcitonin (95%); R_t 13.63 min; *m/z* (ESI-MS) 1710.0 ($[M+2H]^{2+}$ requires 1709.9); linear gradient of 5%B to 65%B over 20 min (*ca*. 3%B/min) at 45 °C, 1 mL/min.

Human calcitonin P32Y

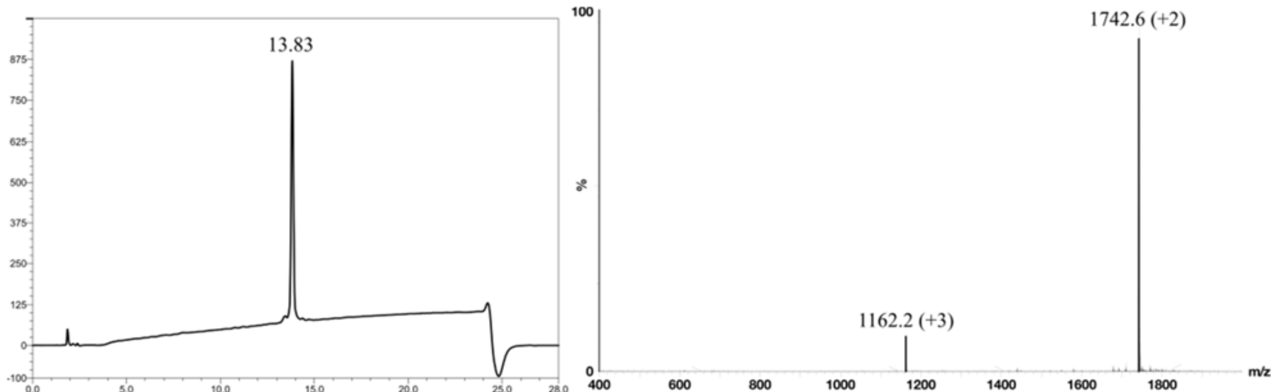

Figure SC47: RP-HPLC trace of P32Y analog of human calcitonin (96%); R_t 13.83 min; *m/z* (ESI-MS) 1742.6 ($[M+2H]^{2+}$ requires 1742.9); linear gradient of 5%B to 65%B over 20 min (*ca*. 3%B/min) at 45 °C, 1 mL/min.

Purified DAGAR1

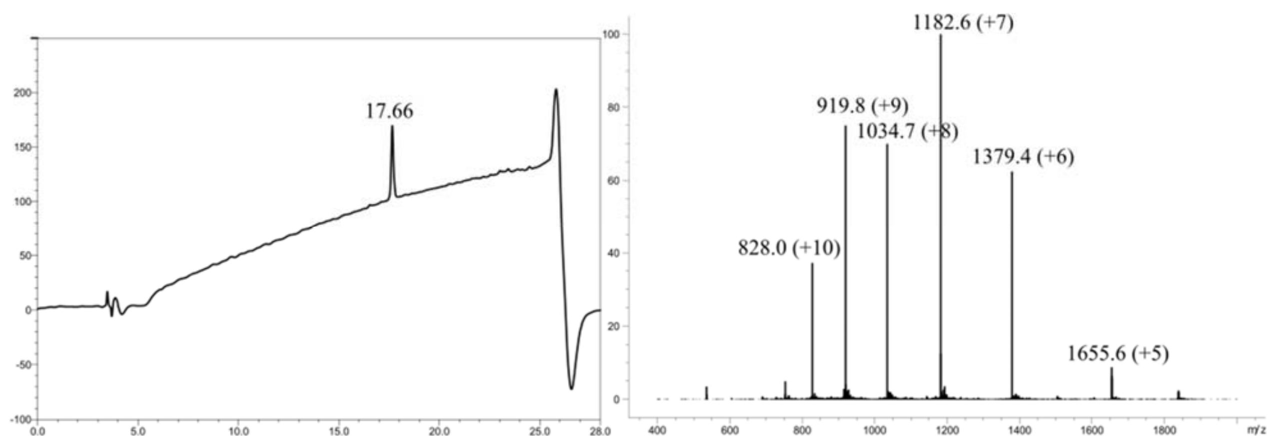

Figure SC48: RP-HPLC trace of purified DAGAR (98%); R_t 17.66 min; *m/z* (ESI-MS) 1182.6 ($[M+7H]^{7+}$ requires 1182.8); linear gradient of 5%B to 65%B over 20 min (*ca*. 3%B/min) at 45 °C, 1 mL/min.

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[1] Harris, P. W. R., Yang, S. H., and Brimble, M. A. (2011) An improved procedure for the preparation of aminomethyl polystyrene resin and its use in solid phase (peptide) synthesis, *Tetrahedron Letters 52*, 6024-6026. doi:10.1016/j.tetlet.2011.09.010

[2] Jensen, K. J., Meldal, M., and Bock, K. (1993) Glycosylation of phenols: preparation of 1, 2-cis and 1, 2-trans glycosylated tyrosine derivatives to be used in solid-phase glycopeptide synthesis, *Journal of the Chemical Society, Perkin Transactions 1*, 2119-2129.

[3] Pícha, J., Buděšínský, M., Macháčková, K., Collinsová, M., and Jiráček, J. (2017) Optimized syntheses of Fmoc azido amino acids for the preparation of azidopeptides, *Journal of Peptide Science 23*, 202-214.