

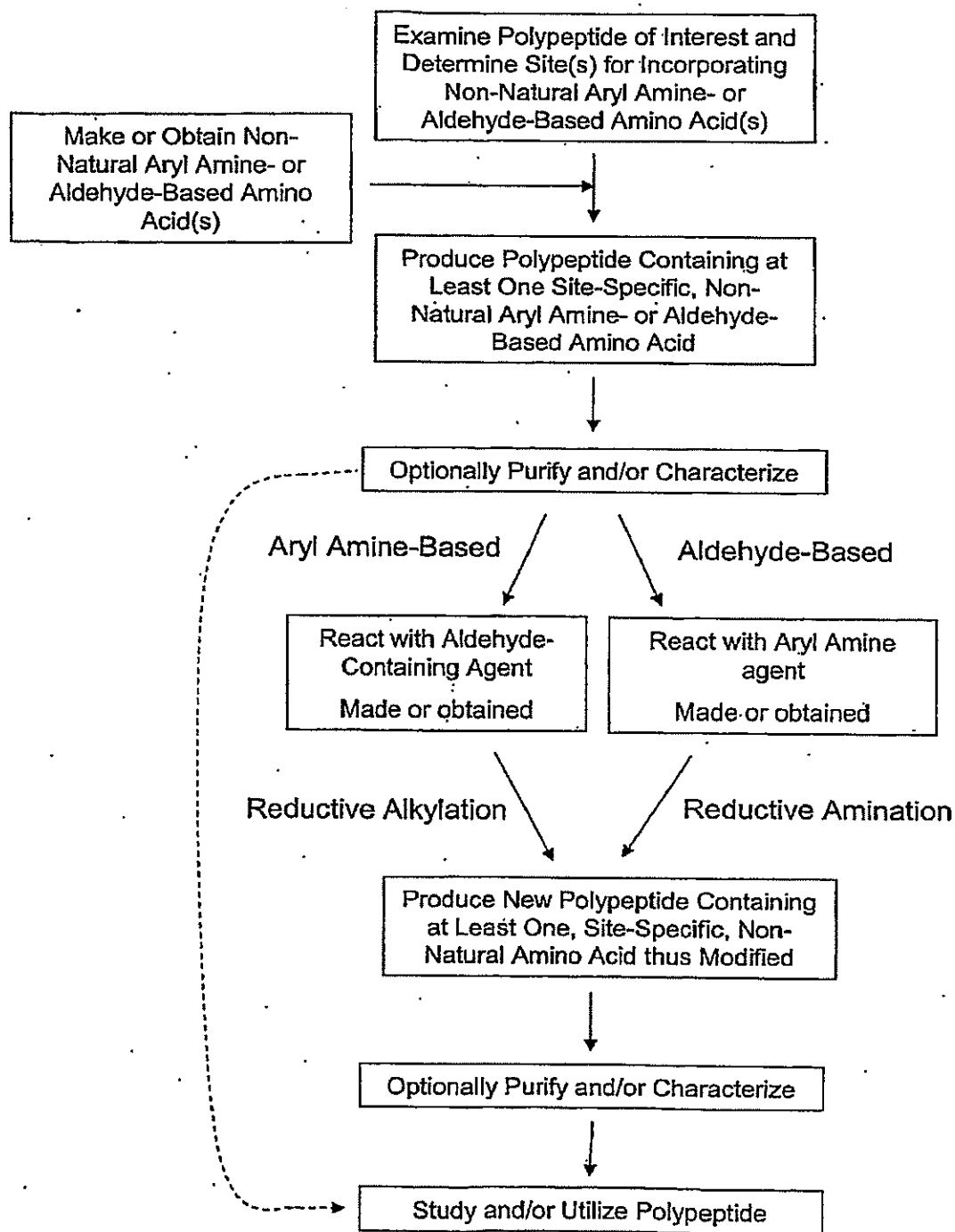
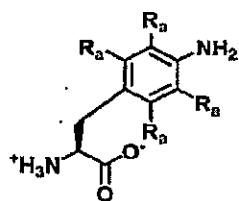


Figure 1

General Structure:



Examples:

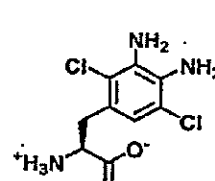
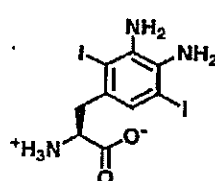
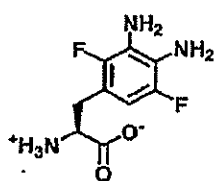
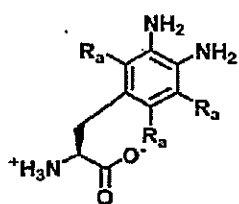
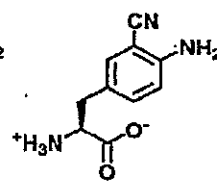
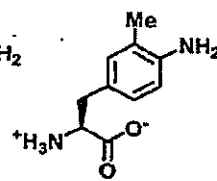
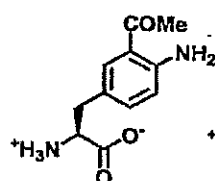
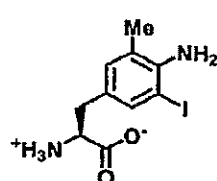
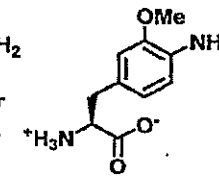
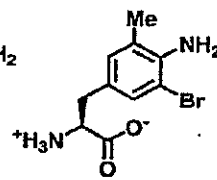
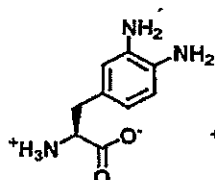
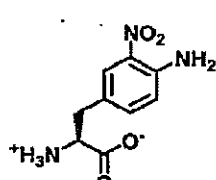
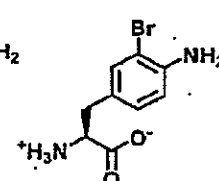
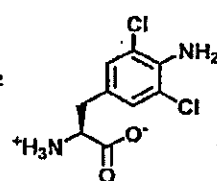
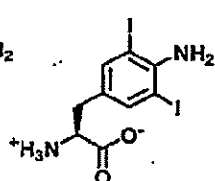
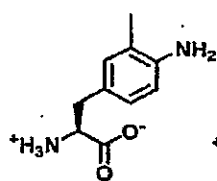
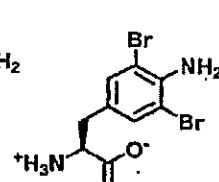
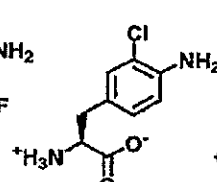
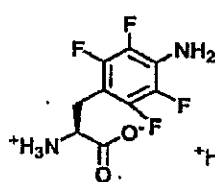
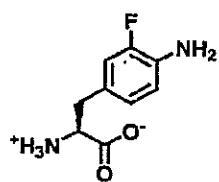
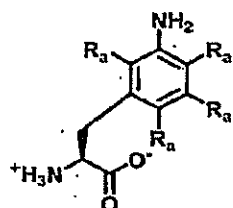


Figure 2

General Structure



Examples

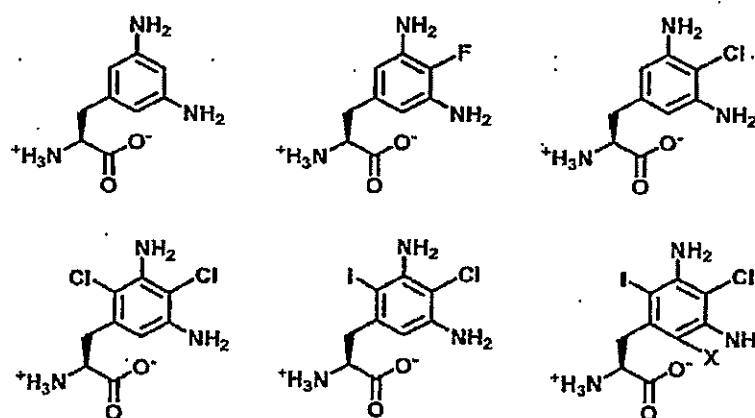
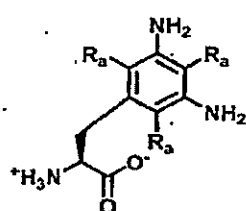
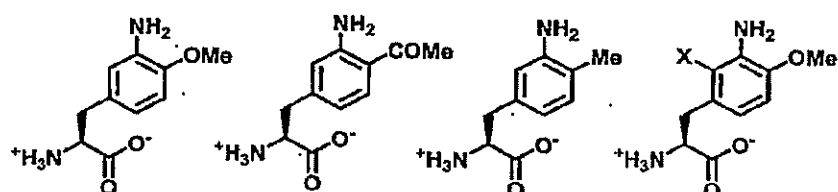
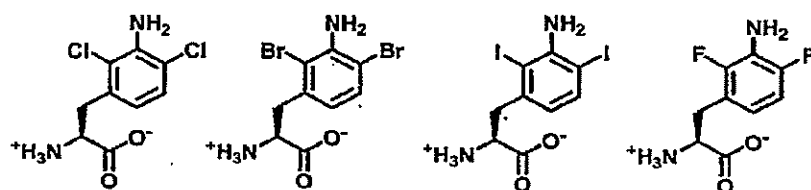
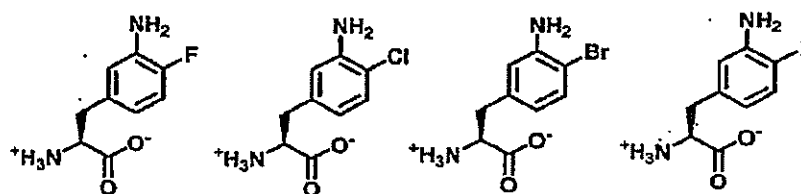


Figure 3

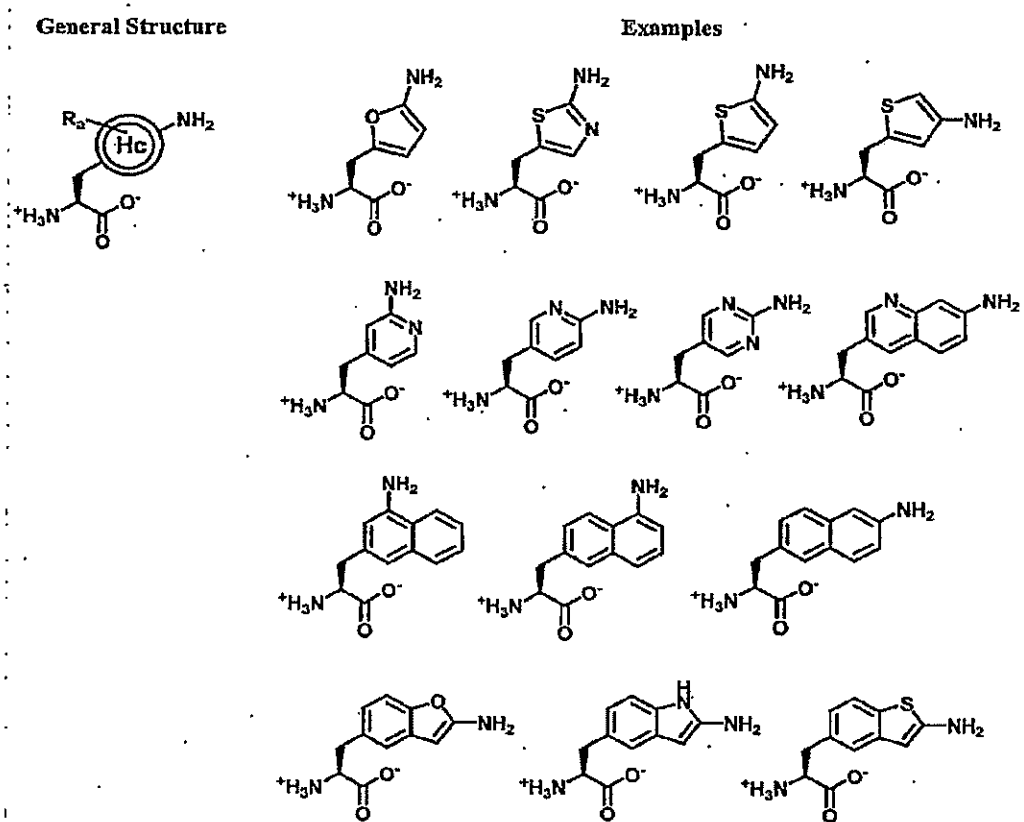
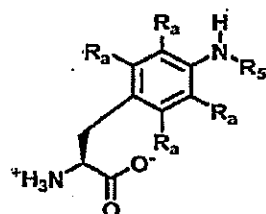


Figure 4

General Structure



Examples:

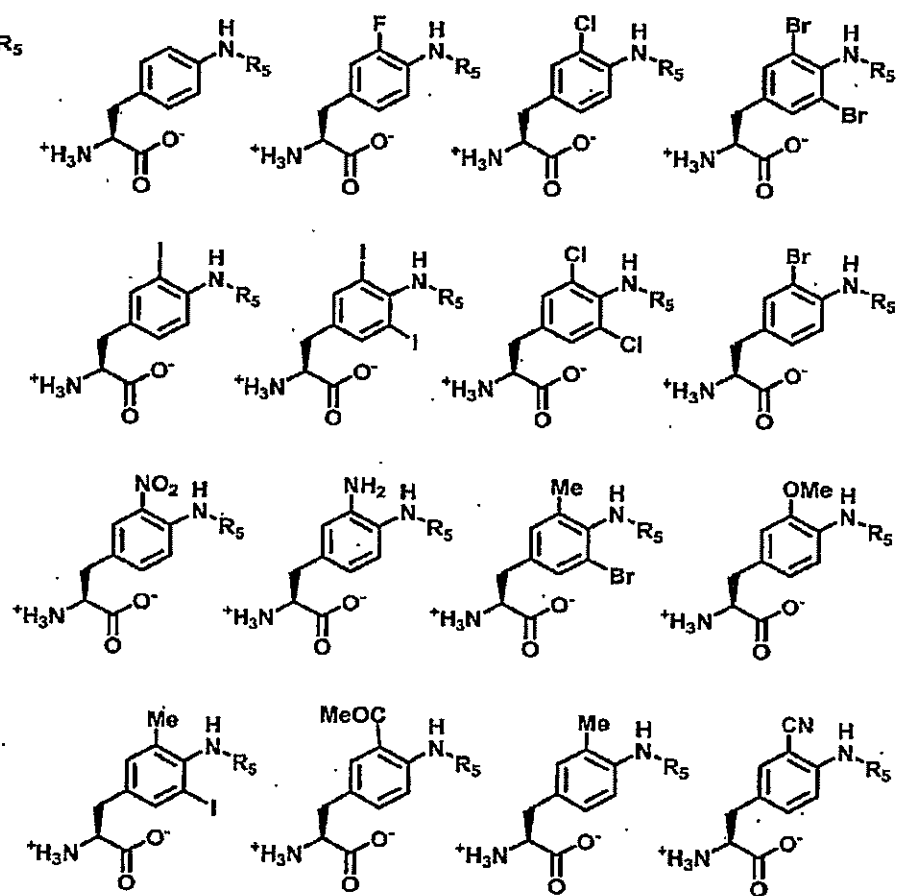
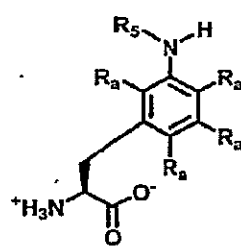


Figure 5

General Structure



Examples

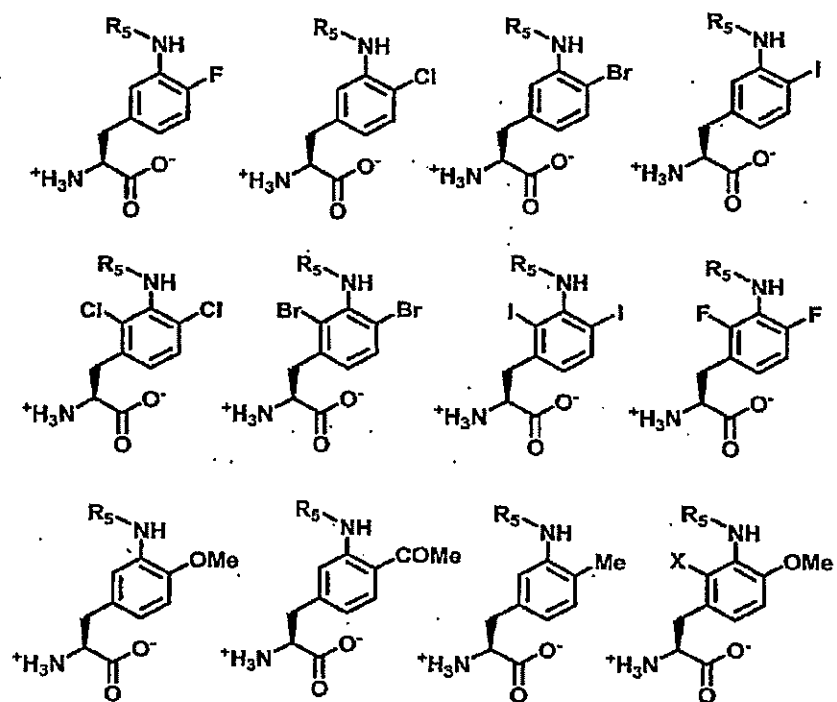


Figure 6

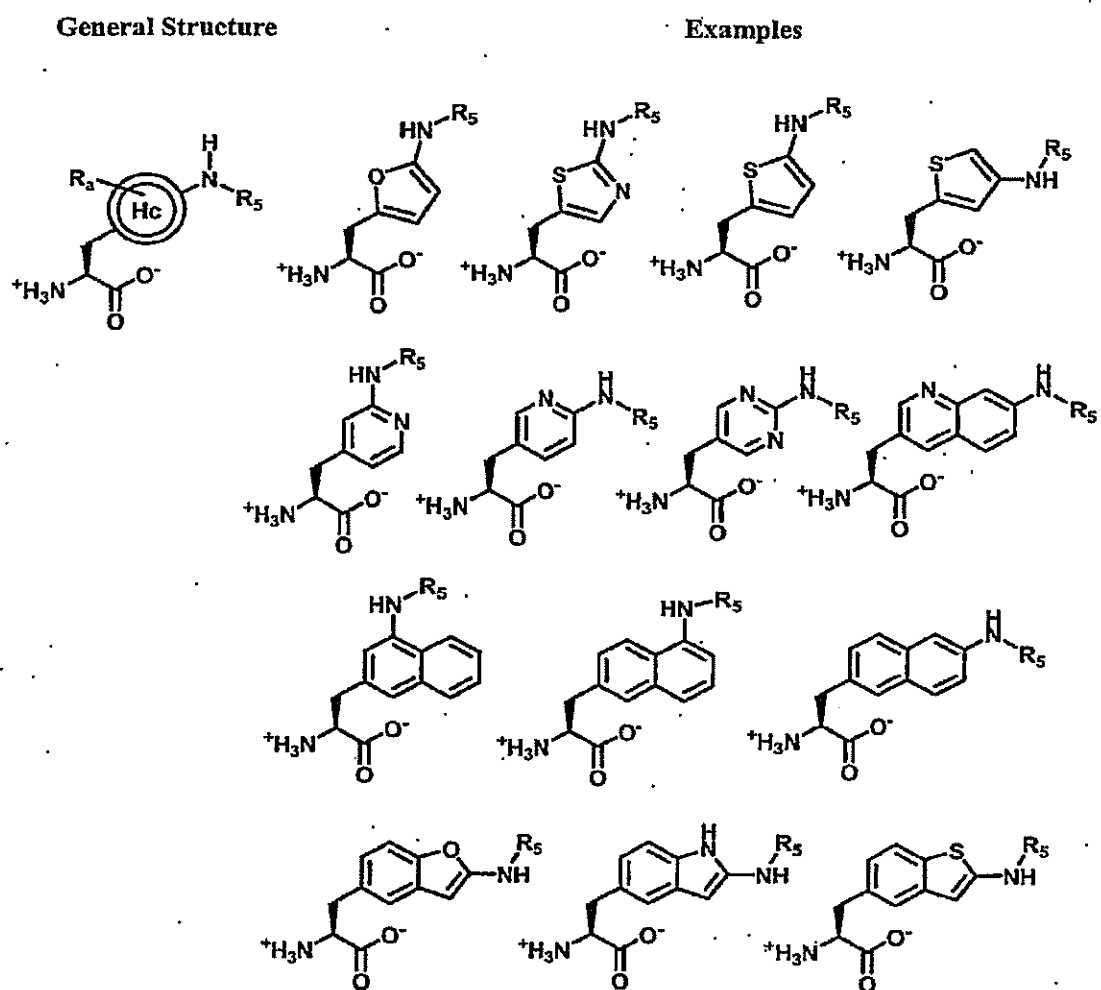
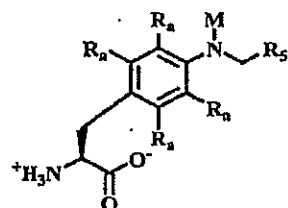


Figure 7

General Structure



Examples:

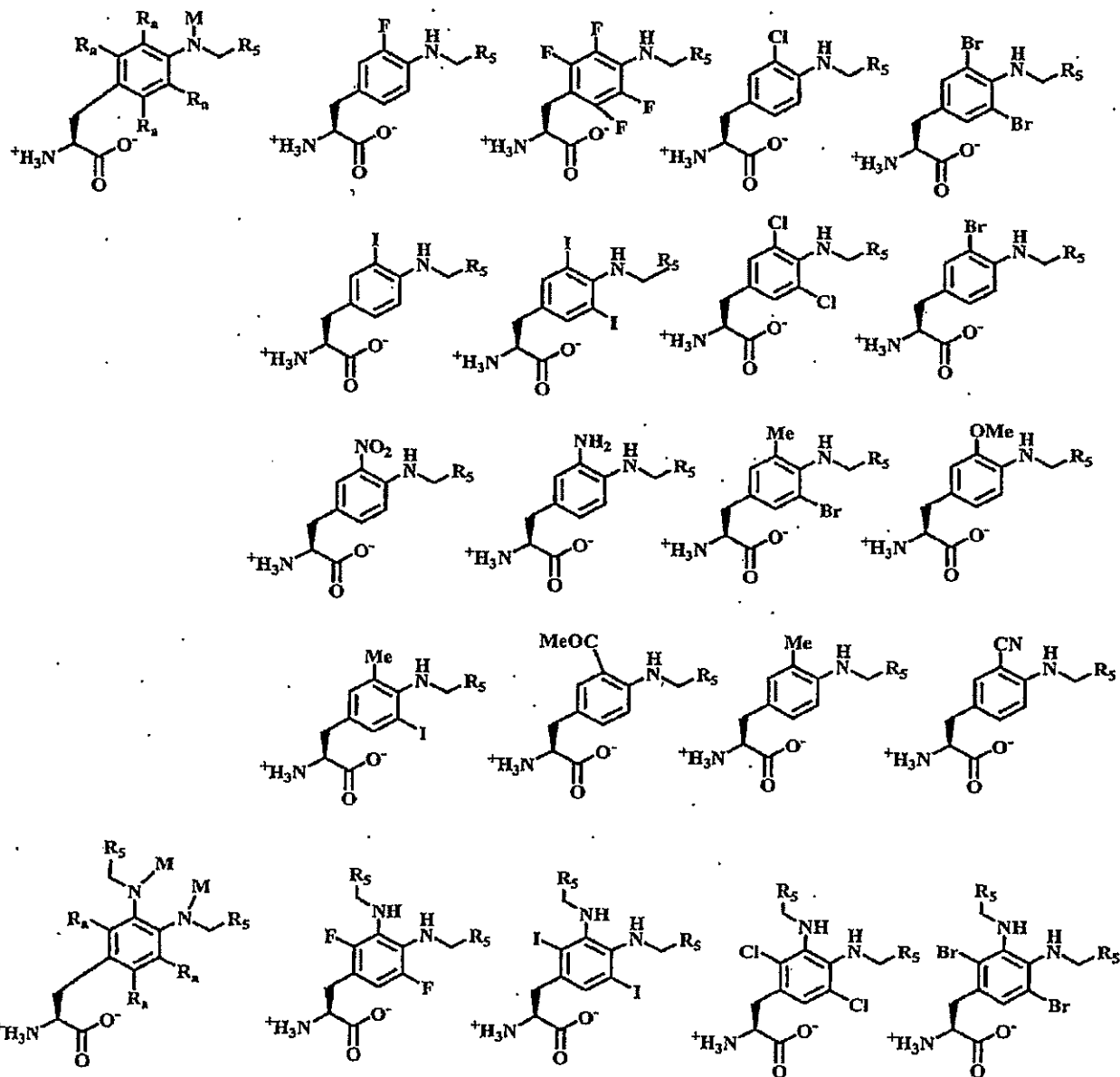
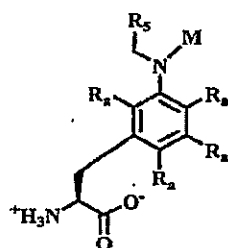


Figure 8

General Structure



Examples

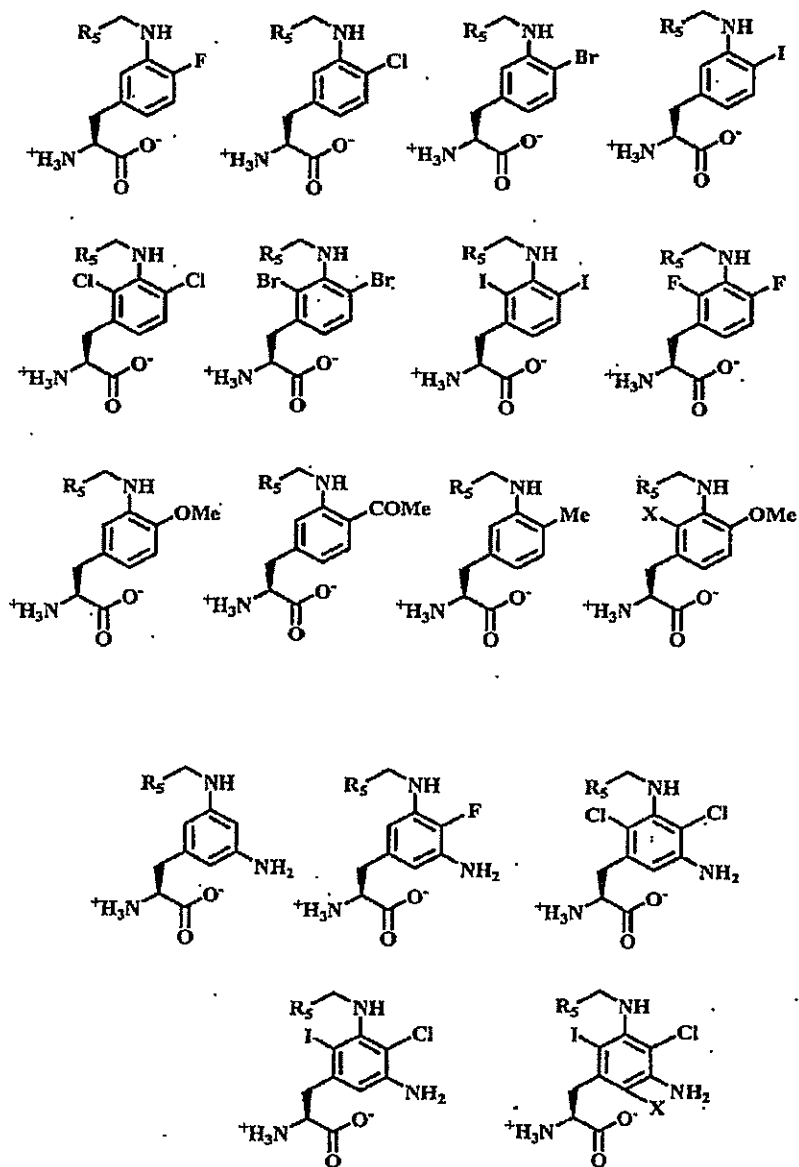
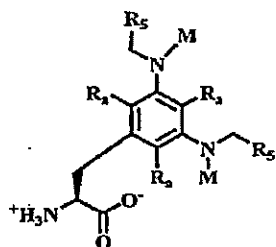


Figure 9

General Structure



Examples

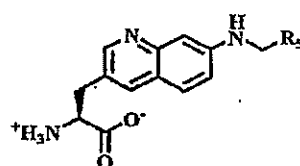
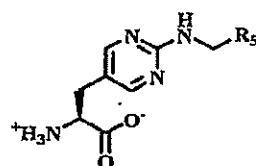
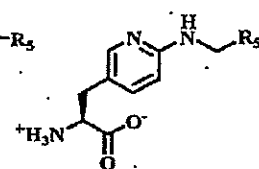
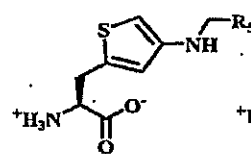
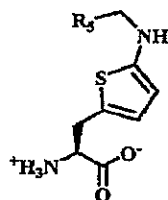
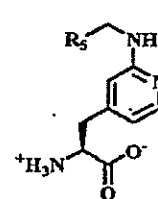
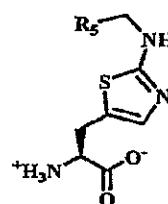
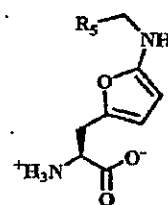
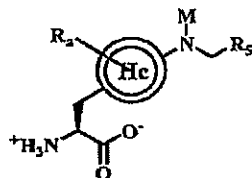
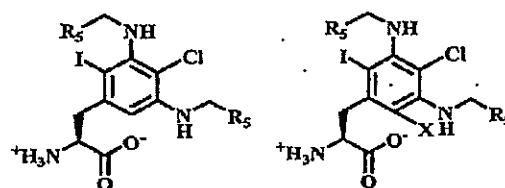
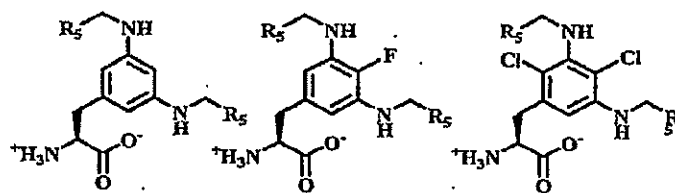
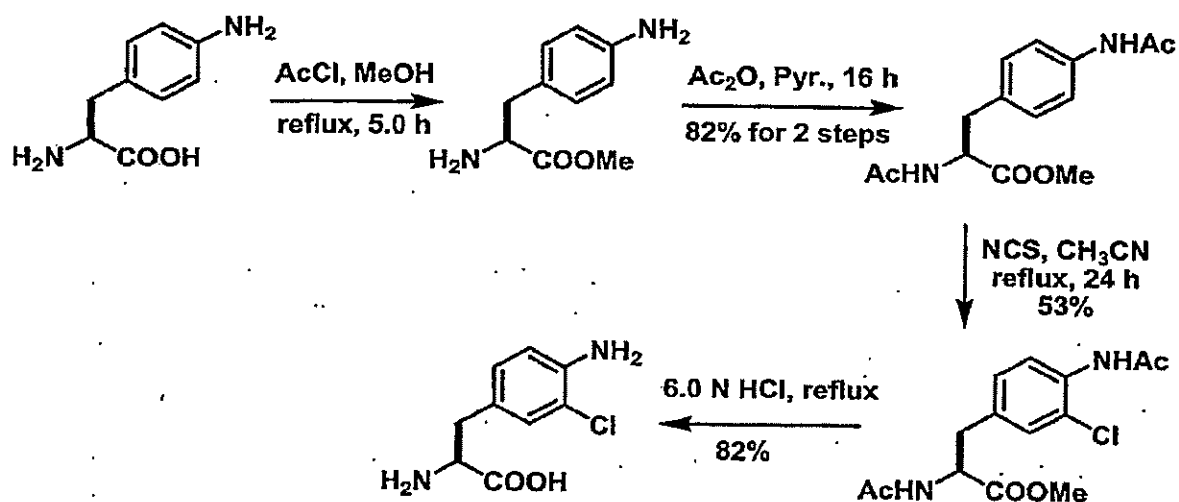


Figure 10

A. Synthesis of 3-chloro-4-amino-phenylalanine



B. Synthesis of 3-iodo-4-amino-phenylalanine

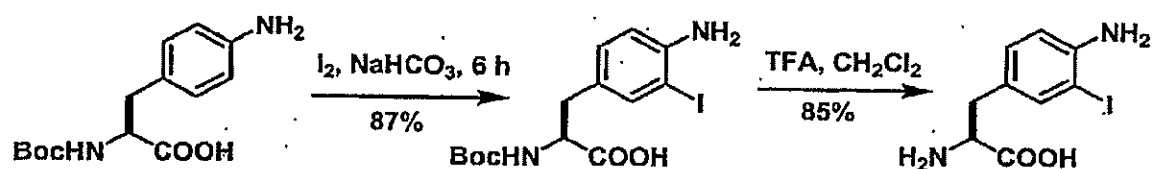
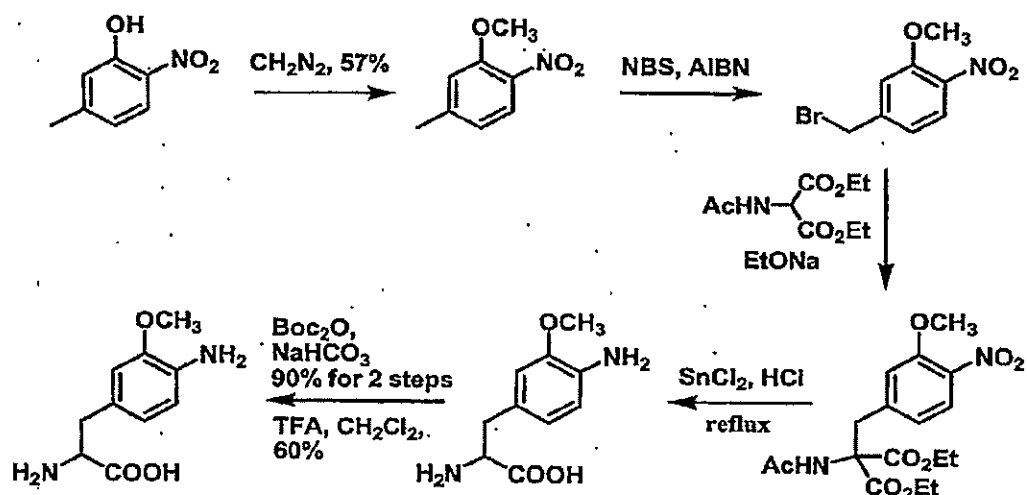


Figure 11

A. Synthesis of 3-methoxy-4-amino-phenylalanine



B. Synthesis of 3-fluoro-4-amino-phenylalanine

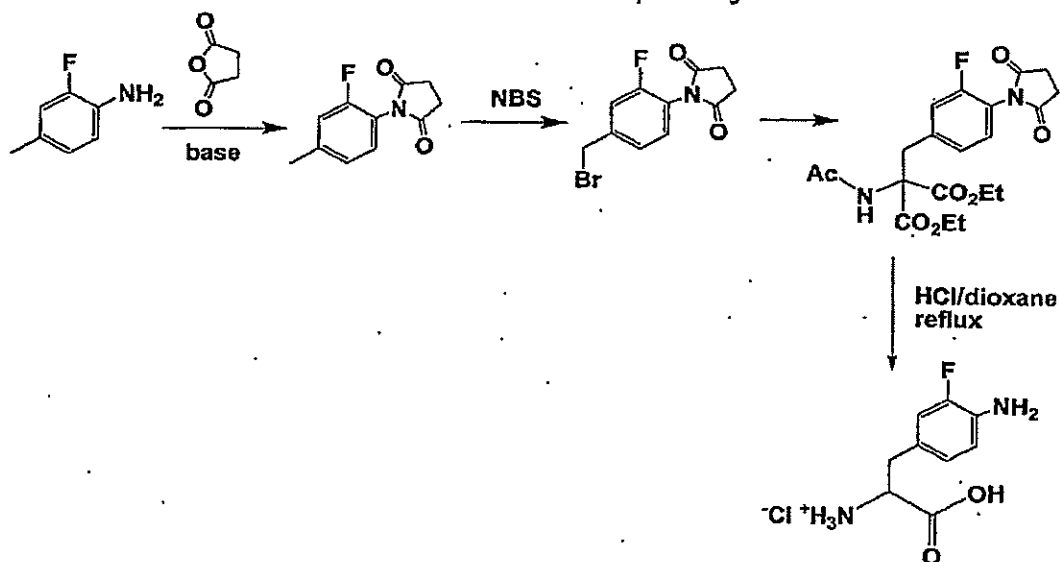
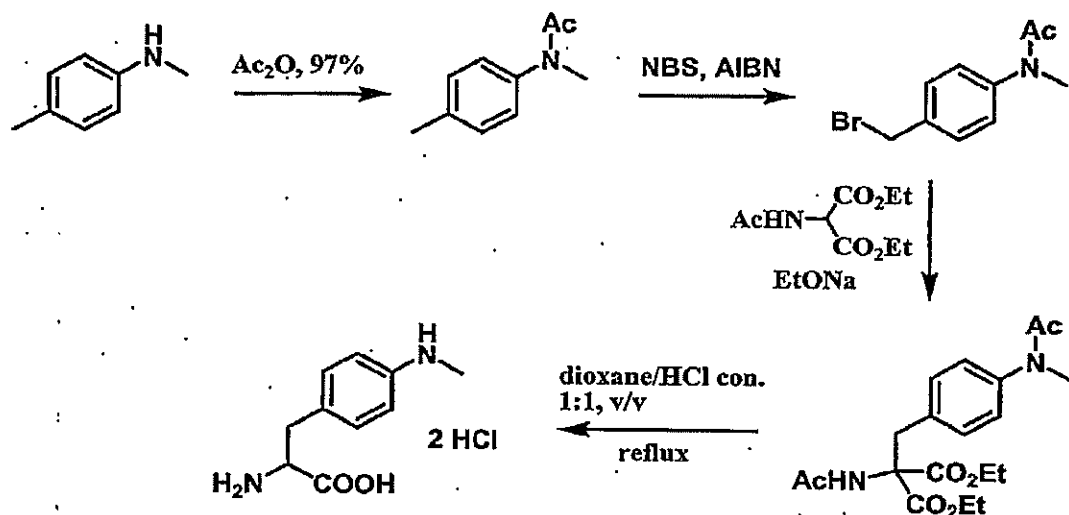


Figure 12

A. Synthesis of N-methyl-p-amino-phenylalanine



B. Synthesis of N-ethyl-p-amino-phenylalanine

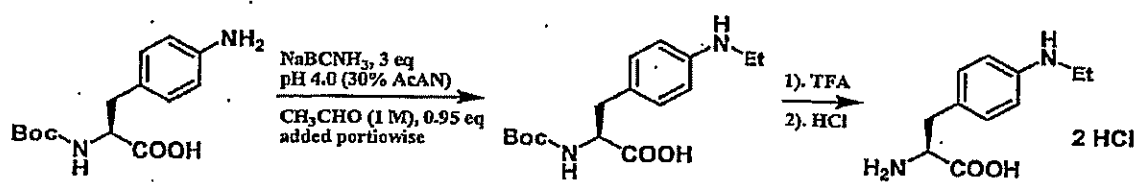


Figure 13

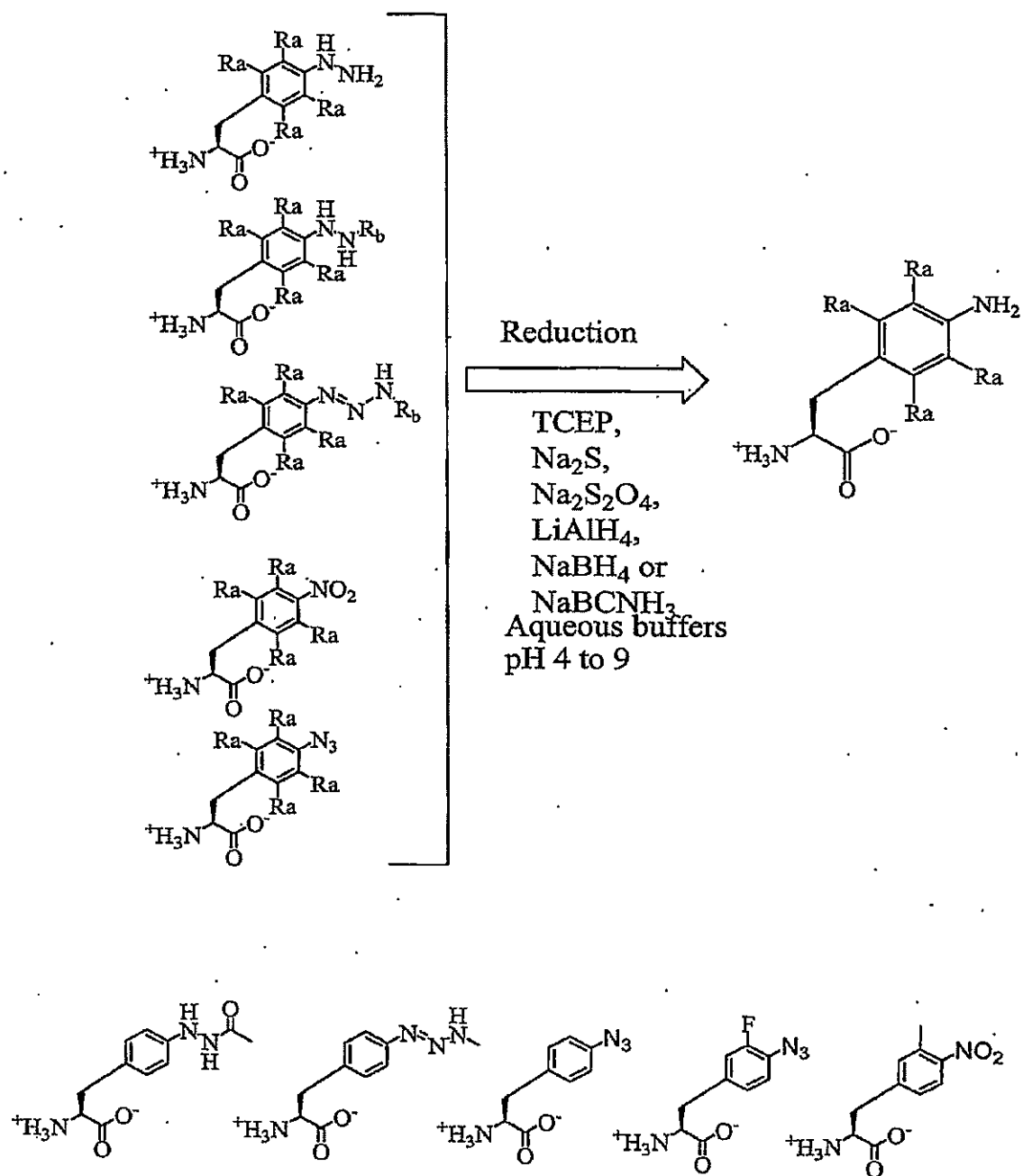


Figure 14

Formation of Polypeptides with Aromatic Amine-Containing Non-Natural Amino Acids by Post-Translational Reduction of the Masked Precursors, Followed by Reductive Alkylations with Aldehyde-Containing Reagents

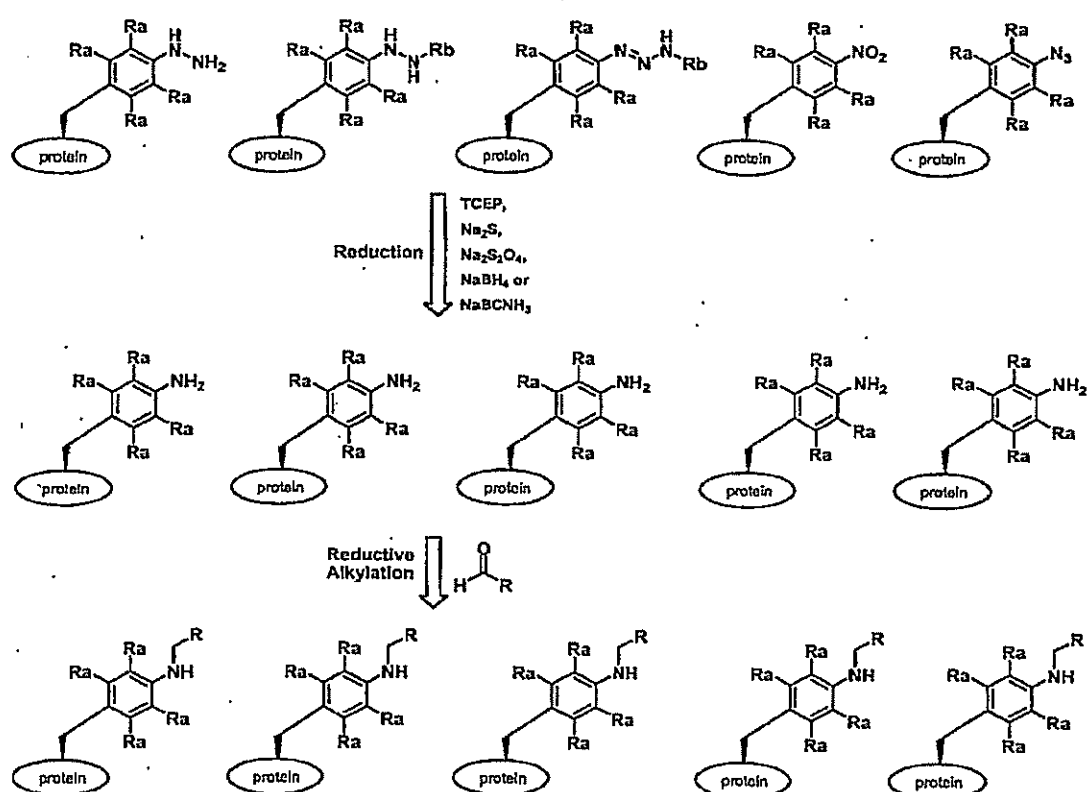


Figure 15

Reductive Alkylations of Mono-alkylated Aromatic Amine-Containing
Non-Natural Amino Acid Peptides

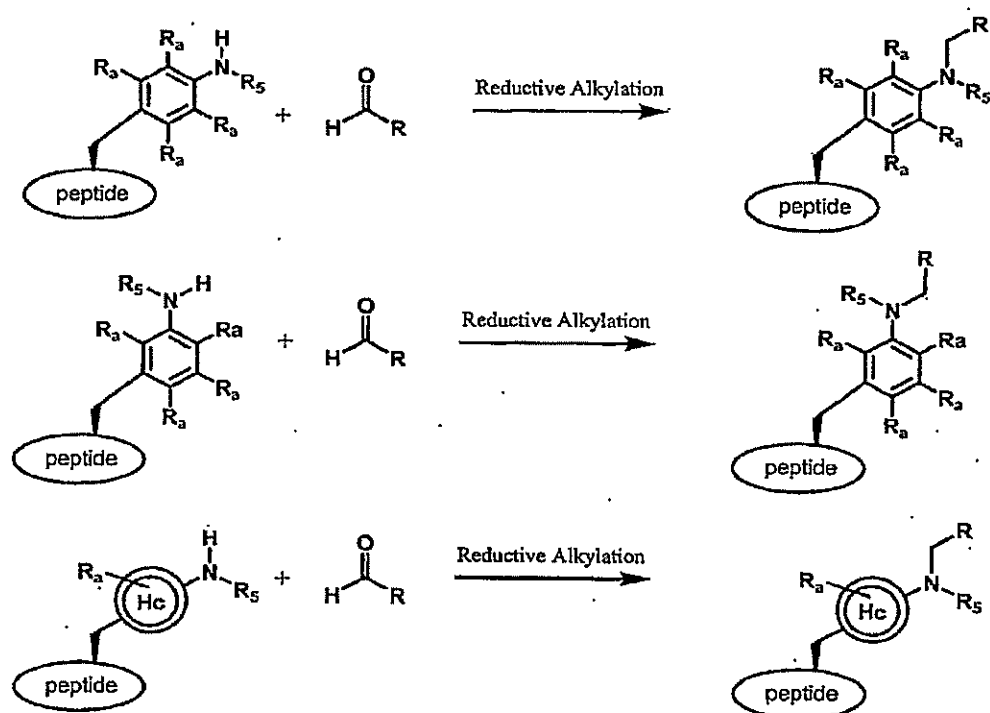
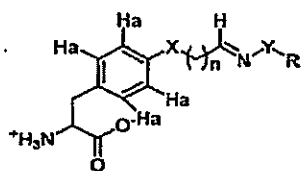
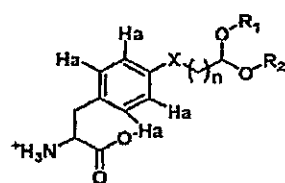


Figure 16

General Structure



Examples

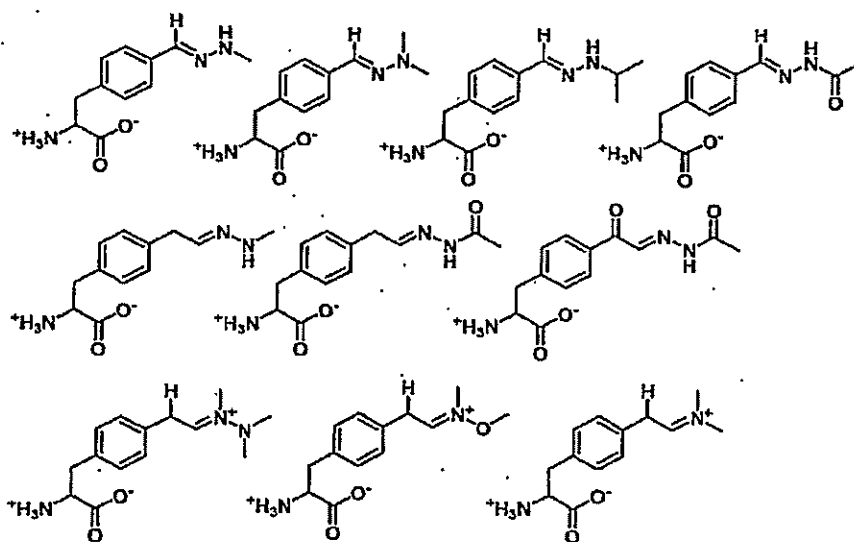
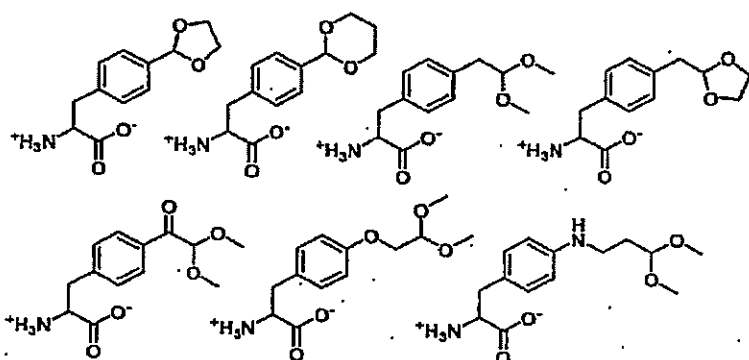


Figure 17

Formation of Polypeptides with Aldehyde-Containing Non-Natural Amino Acids by Post-Translational Deprotections of Masked Aldehyde Precursors, Followed by Reductive Aminations with Aromatic Amine-Containing Reagents

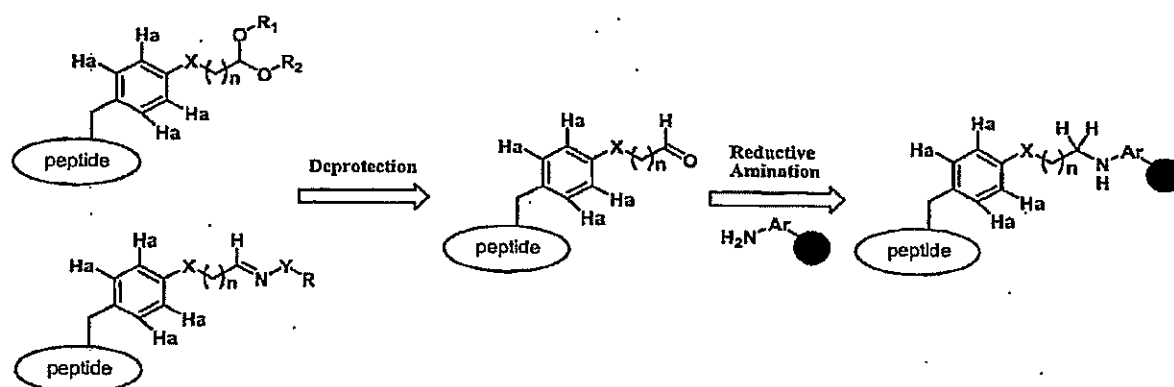
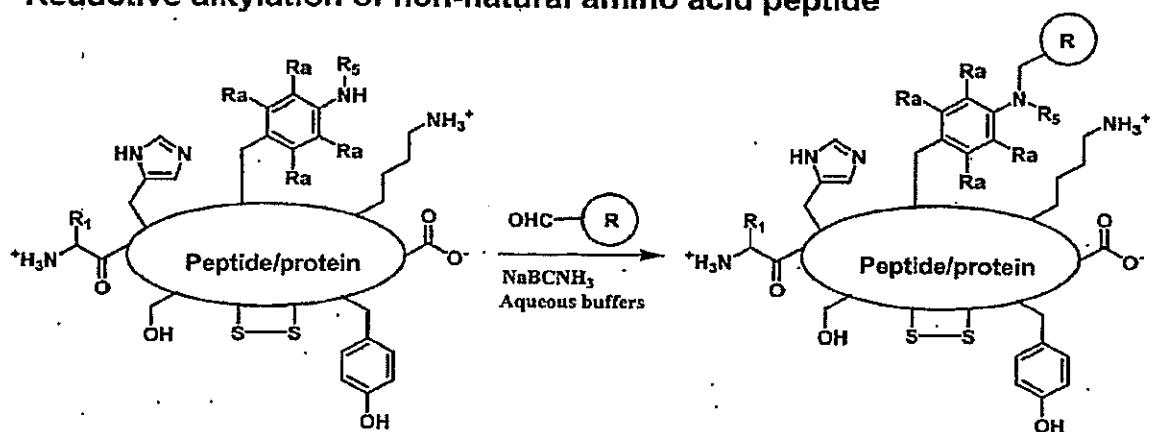


Figure 18

Reductive alkylation of non-natural amino acid peptide



Reductive amination of non-natural amino acid peptide

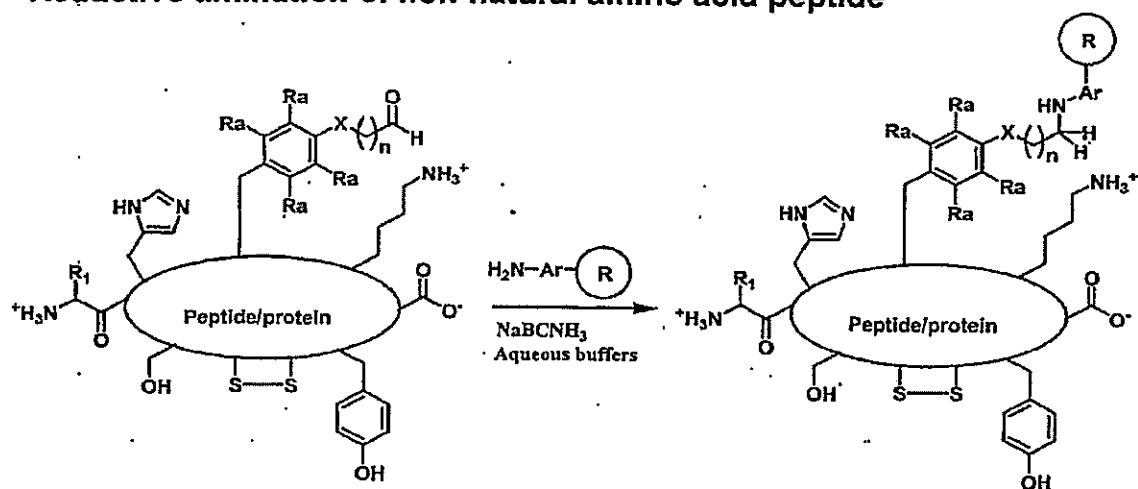
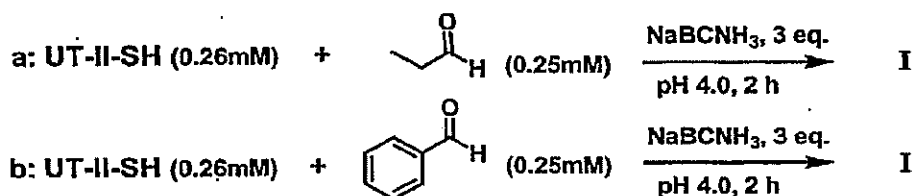
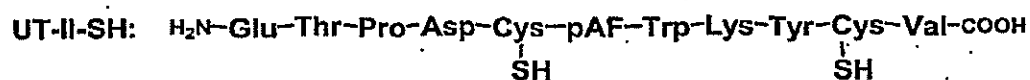


Figure 19

Reductive alkylations of reduced urotension-II (UT-II-SH) with propionaldehyde (a) and benzaldehyde (b)



pAF = L-para-Amino-phenylalanine

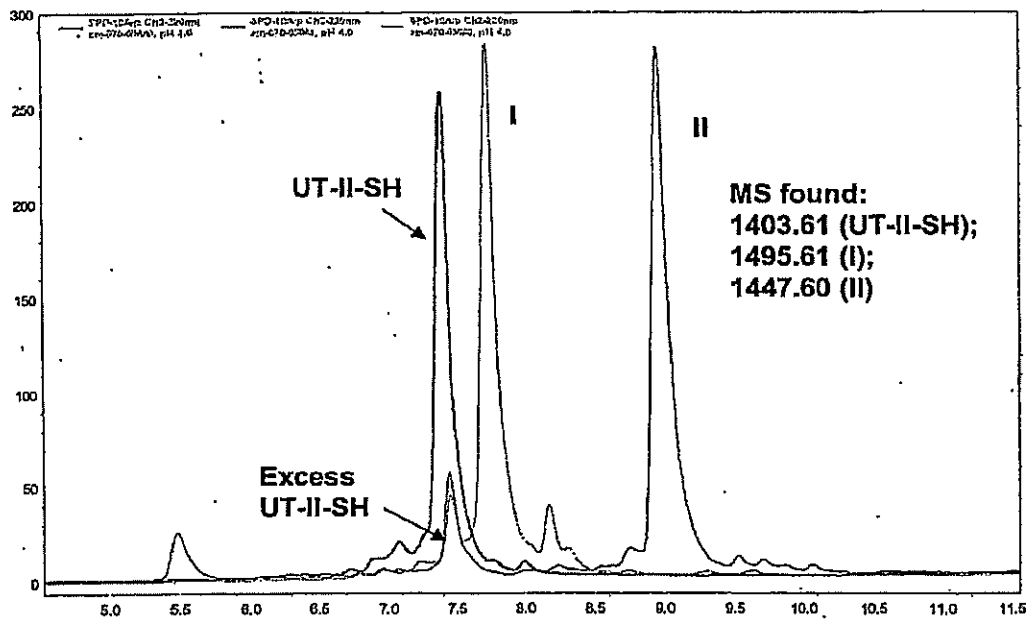
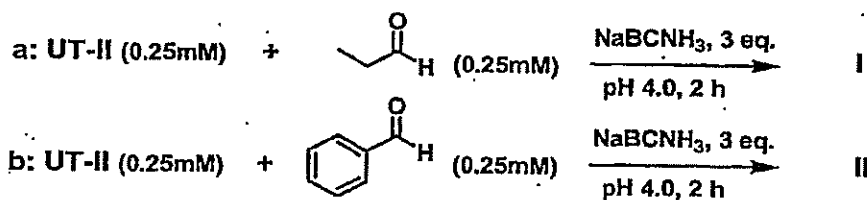
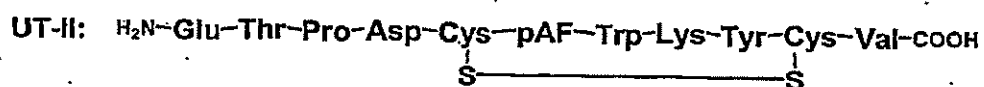


Figure 20

Reductive alkylations of urotension-II (UT-II) with propionaldehyde (a) and benzaldehyde (b), respectively.



pAF = L-para-Amino-phenylalanine

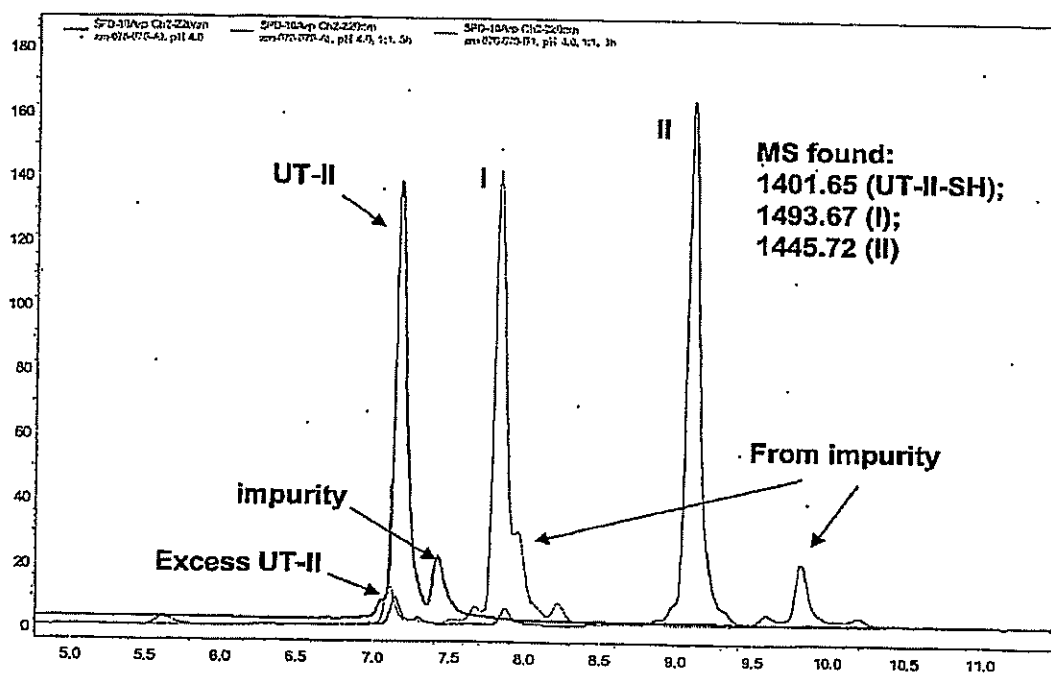
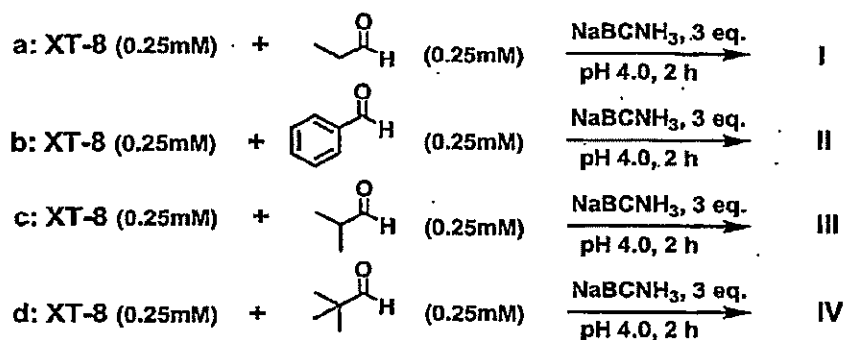
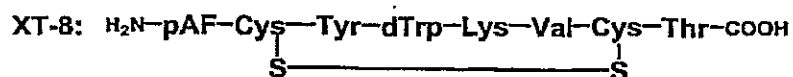


Figure 21

Reductive alkylations of peptide XT-8 with propionaldehyde (a) and benzaldehyde (b), isobutaldehyde (c) and pivalaldehyde (d)



pAF = L-Amino-phenylalanine

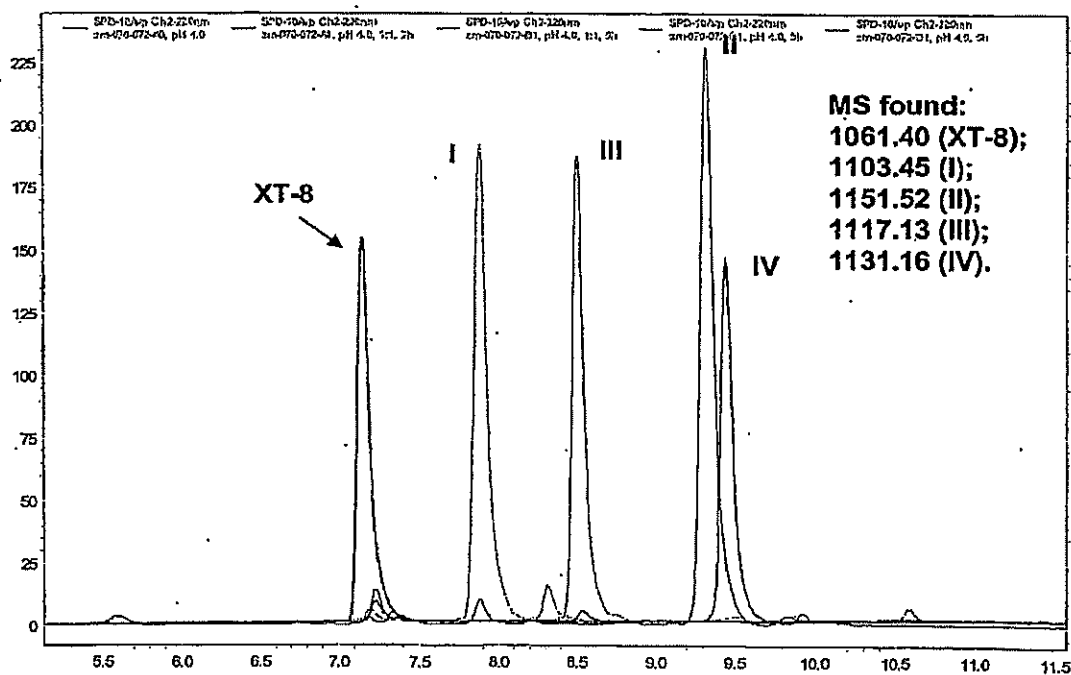
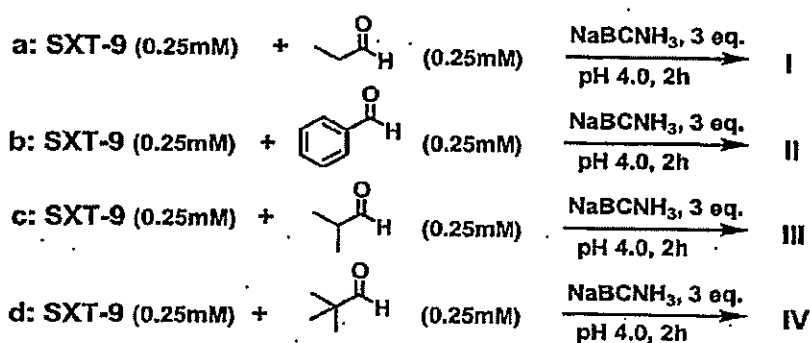
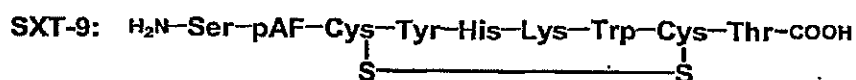


Figure 22

Reductive alkylations of peptide SXT-9 with propionaldehyde (a) and benzaldehyde (b), isobutaldehyde (c) and pivalaldehyde (d).



pAF = L-para-Amino-phenylalanine

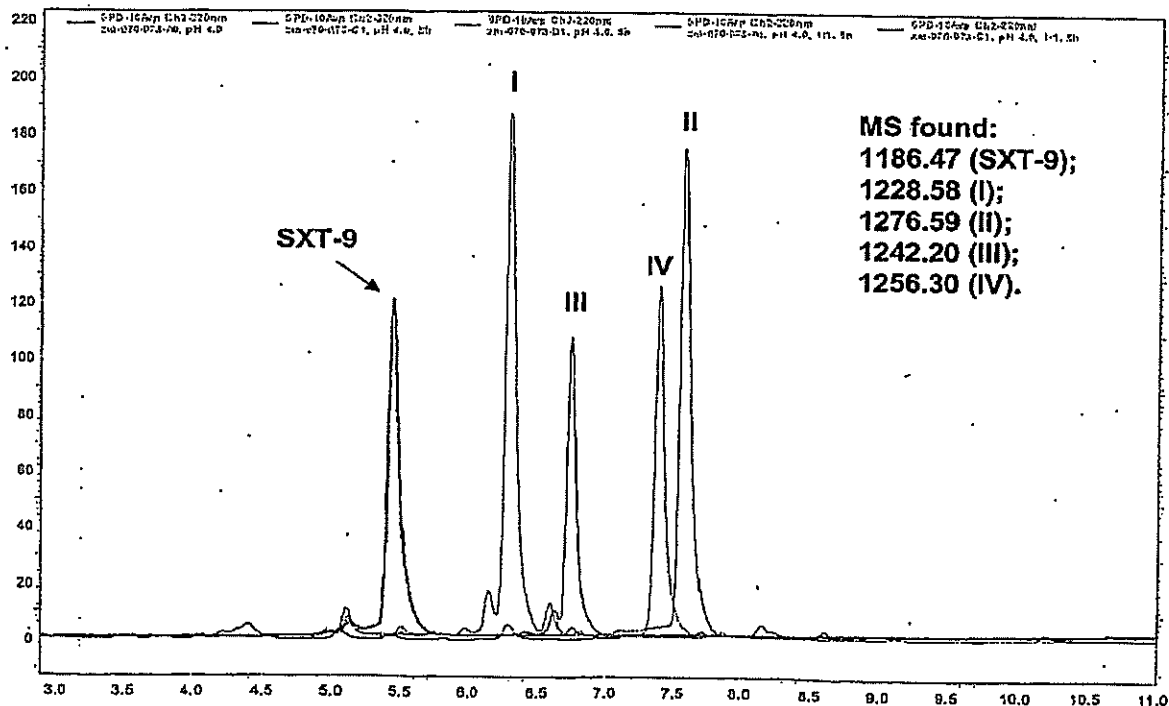
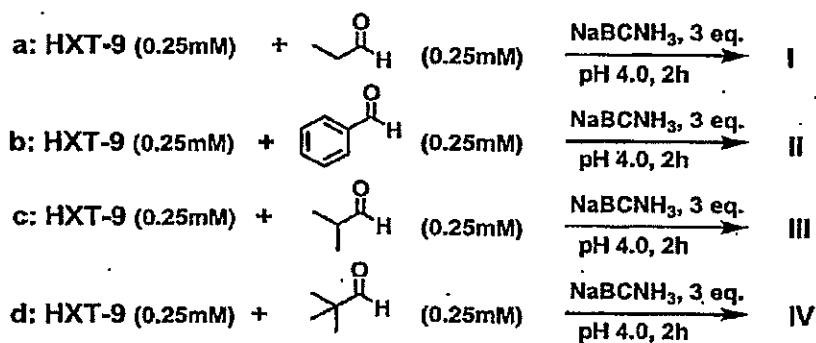
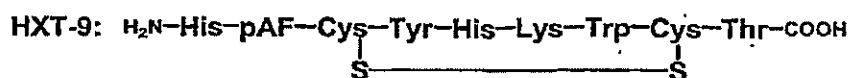


Figure 23

Reductive alkylations of peptide HXT-9 with propionaldehyde (a) and benzaldehyde (b), isobutaldehyde (c) and pivalaldehyde (d)



pAF = L-para-Amino-phenylalanine

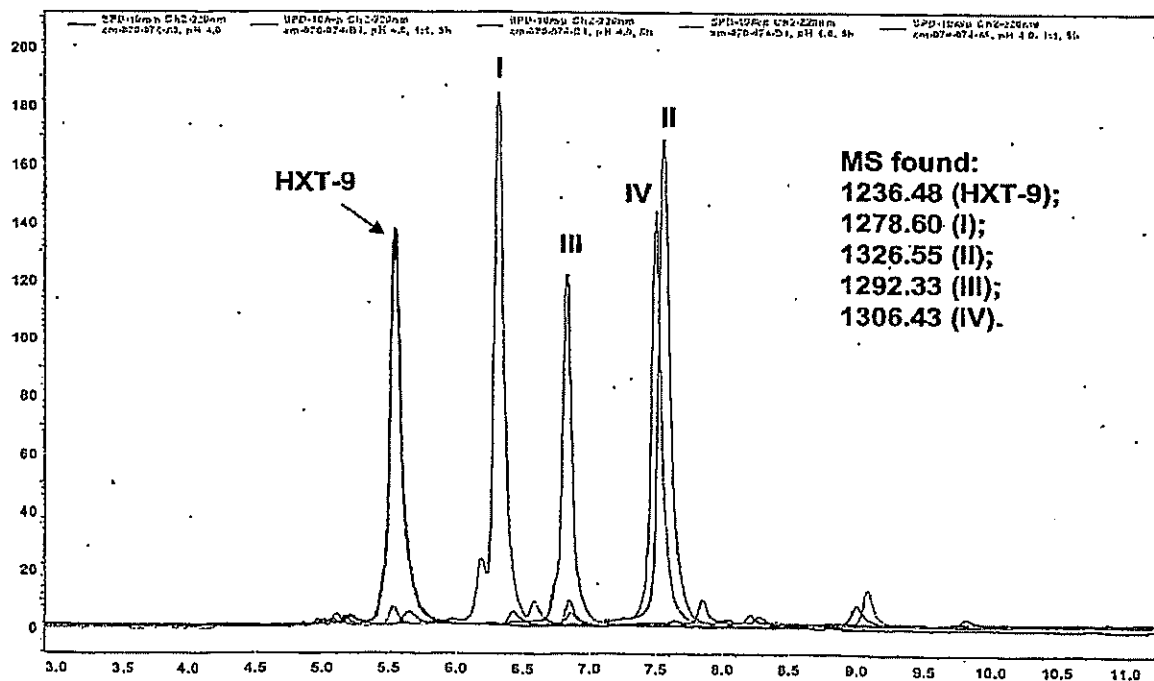
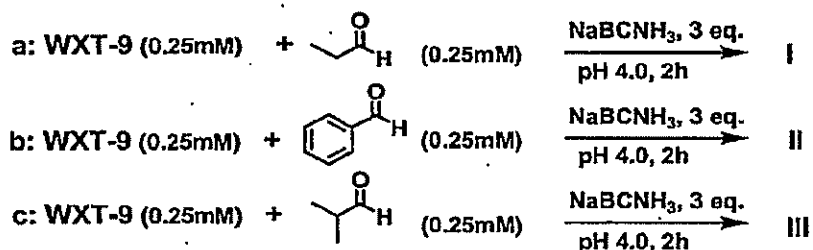
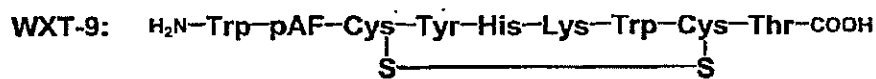


Figure 24

Reductive alkylations of peptide WXT-9 with propionaldehyde (a), benzaldehyde (b), and isobutaldehyde (c)



pAF = L-para-Amino-phenylalanine

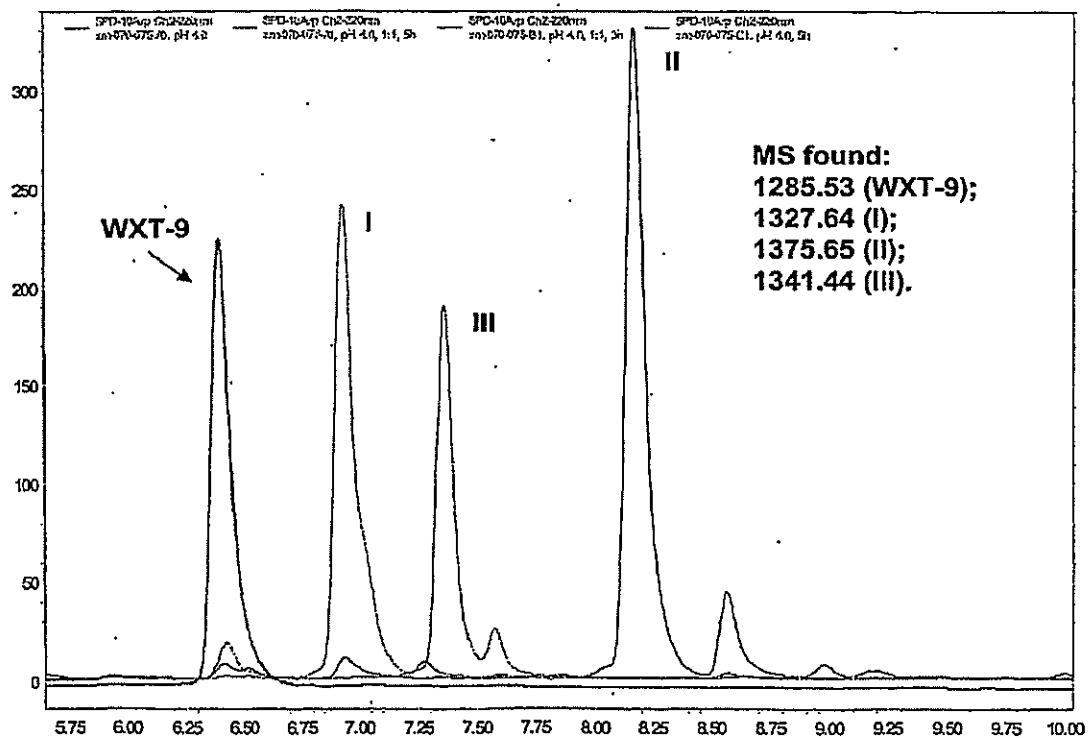
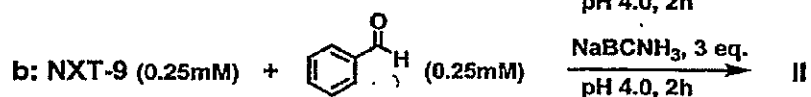
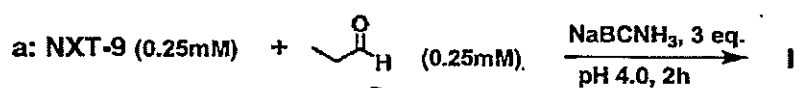
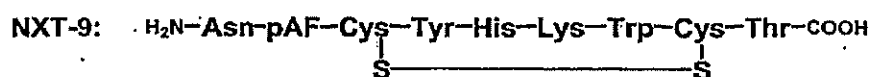


Figure 25

Reductive alkylations of peptide NXT-9 with propionaldehyde (a) and benzaldehyde (b)



pAF = L-para-Amino-phenylalanine

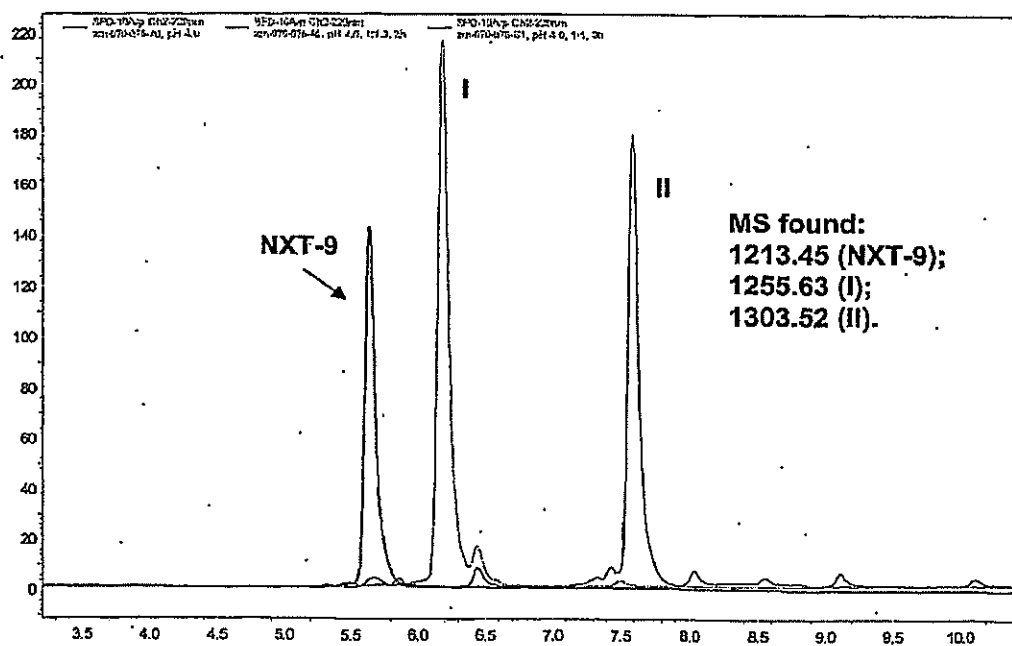
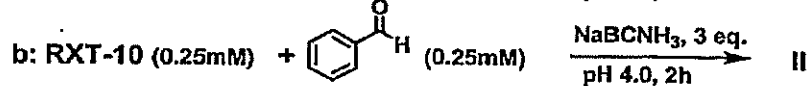
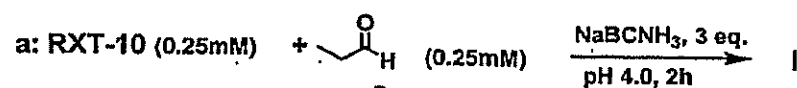
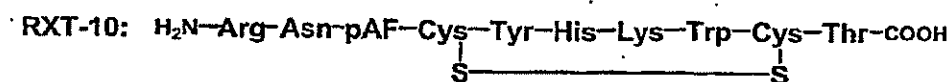


Figure 26

Reductive alkylations of peptide RXT-10 with propionaldehyde (a) and benzaldehyde (b)



pAF = L-para-Amino-phenylalanine

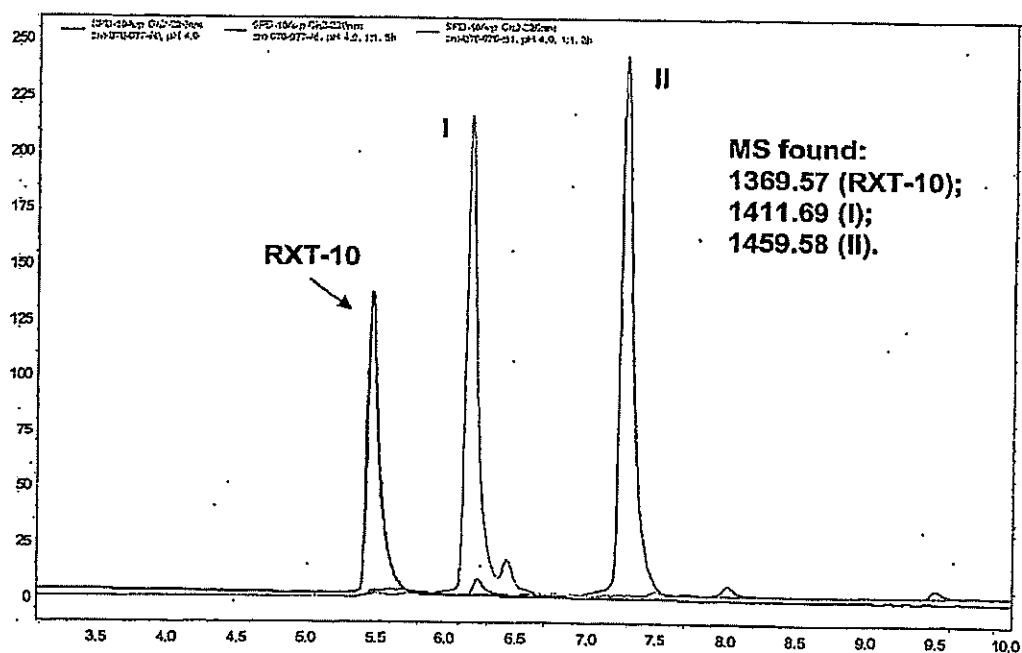
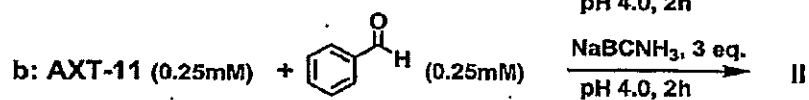
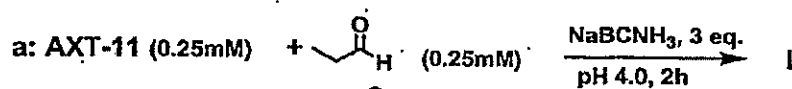
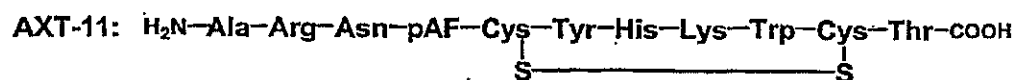


Figure 27

Reductive alkylations of peptide AXT-11 with propionaldehyde (a) and benzaldehyde (b)



pAF = L-para-Amino-phenylalanine

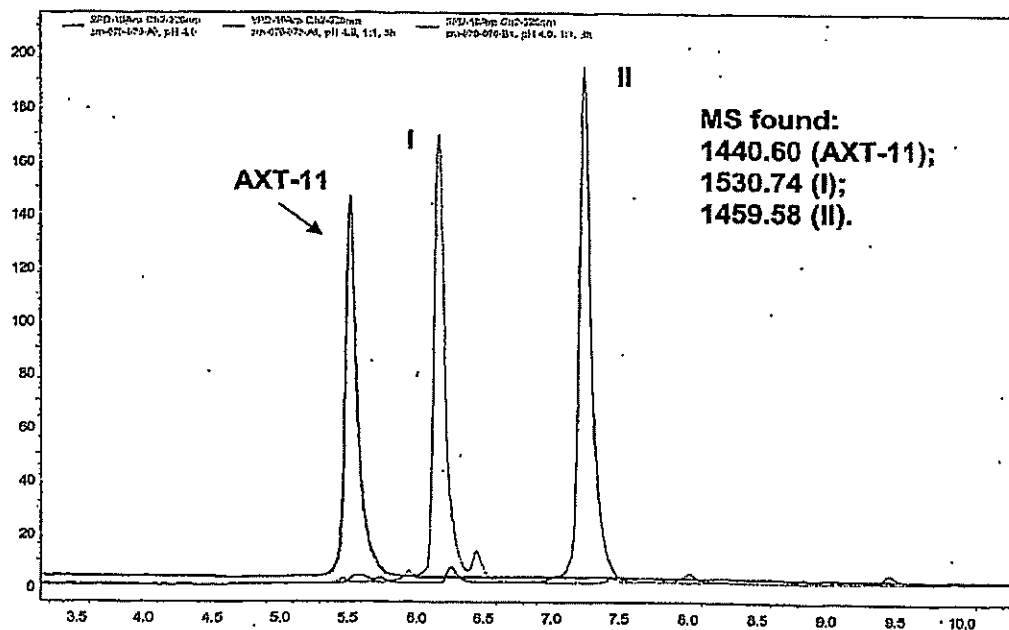
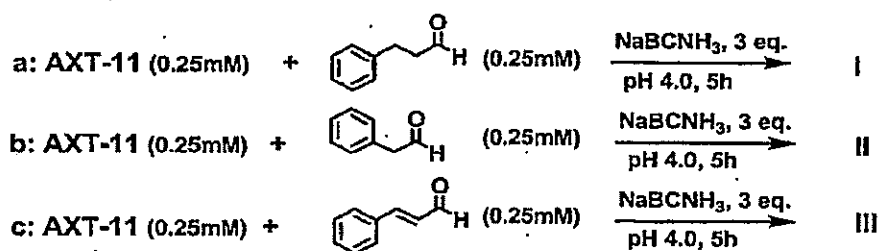
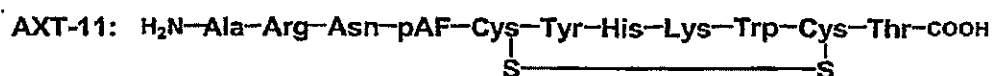


Figure 28

Reductive alkylations of peptide AXT-11 with various aldehydes (a-c).



pAF = L-para-Amino-phenylalanine

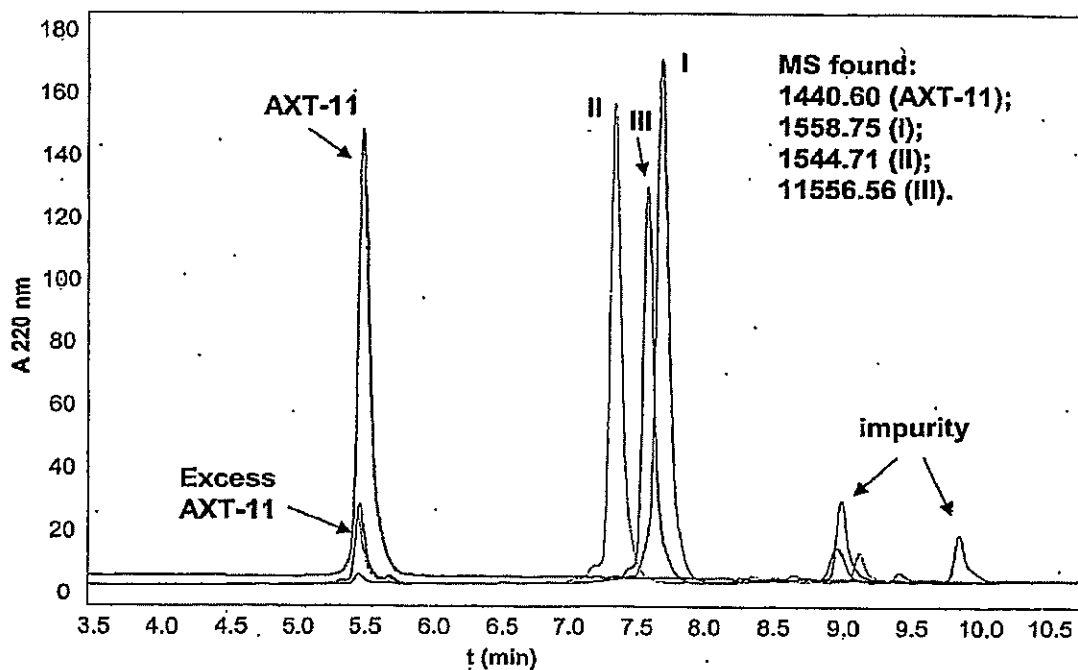
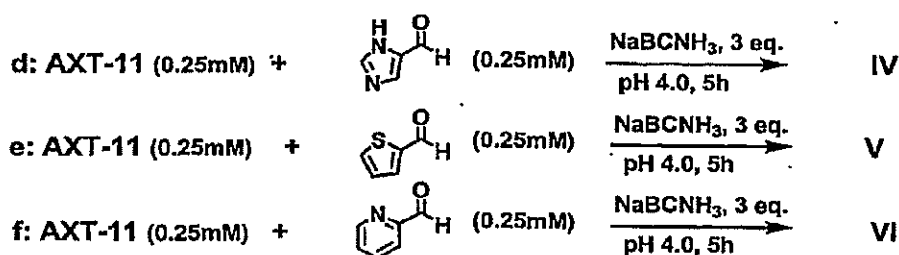
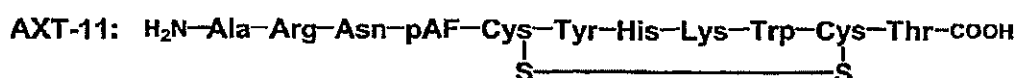


Figure 29

Reductive alkylations of peptide AXT-11 with various heteroaromatic aldehydes (d-g).



pAF = L-para-Amino-phenylalanine

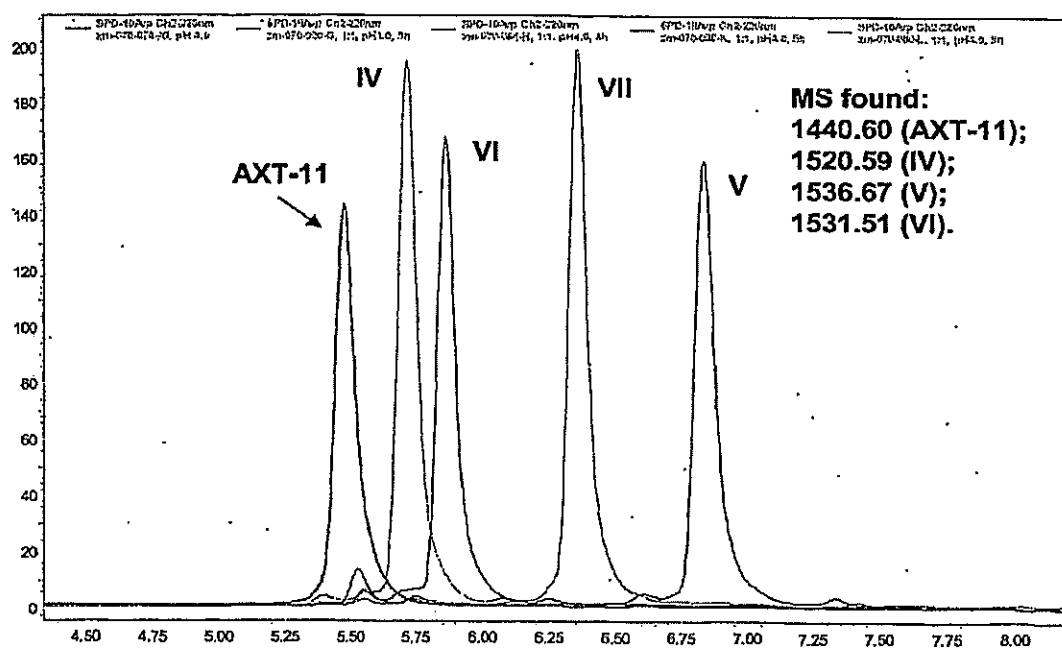


Figure 30

Competitive reactions of peptide AXT-11 with aldehyde and 1,3-diketone (I-III).

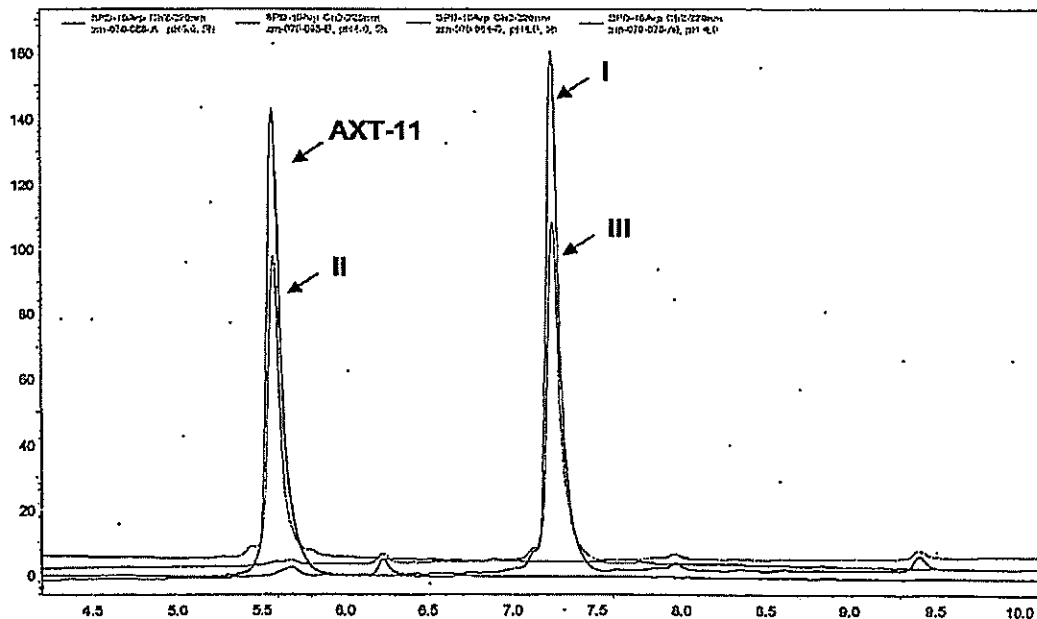
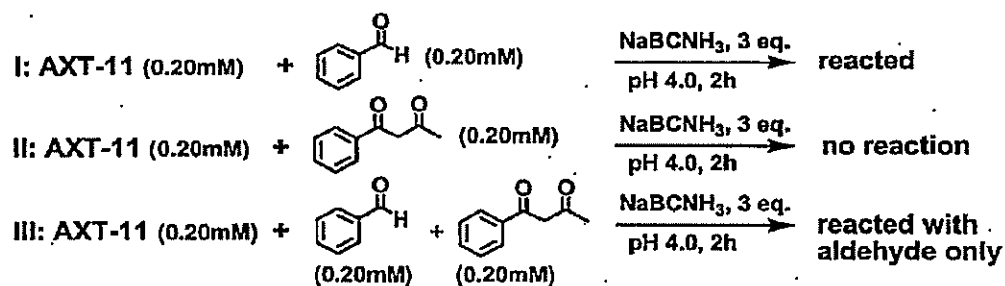
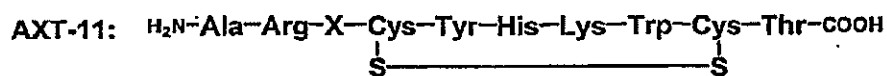


Figure 31

Competitive reactions of peptide NXT-9 with aldehyde and 1,2-diketone (I-III).

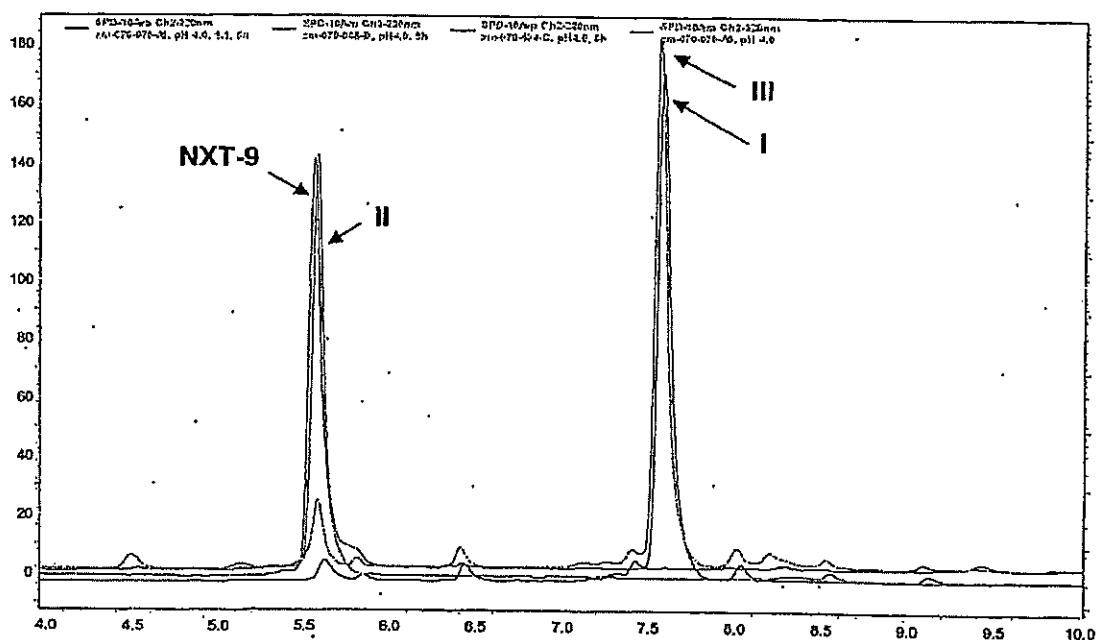
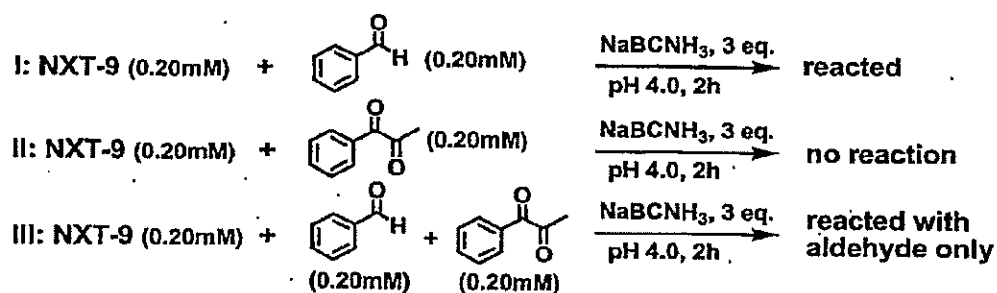
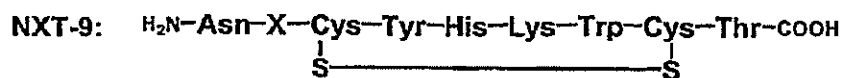
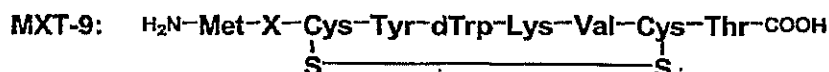


Figure 32

Competitive reactions of peptide MXT-9 with different aldehydes and ketones (I-V).



— I: MXT-9 (0.20mM)	+  (0.20mM)	$\xrightarrow[\text{pH 4.0}]{\text{NaBCNH}_3, 3 \text{ eq.}}$	reacted
— II: MXT-9 (0.20mM)	+  (0.20mM)	$\xrightarrow[\text{pH 4.0}]{\text{NaBCNH}_3, 3 \text{ eq.}}$	no reaction
— III: MXT-9 (0.20mM)	+  (0.20mM) +  (0.20mM)	$\xrightarrow[\text{pH 4.0}]{\text{NaBCNH}_3, 3 \text{ eq.}}$	only reacted with aldehyde
— IV: MXT-9 (0.20mM)	+  (0.20mM) +  (0.20mM)	$\xrightarrow[\text{pH 4.0}]{\text{NaBCNH}_3, 3 \text{ eq.}}$	dominantly reacted with alkyl aldehyde
— V: MXT-9 (0.20mM)	+  (0.20mM) +  (0.20mM)	$\xrightarrow[\text{pH 4.0}]{\text{NaBCNH}_3, 3 \text{ eq.}}$	only reacted with aldehyde

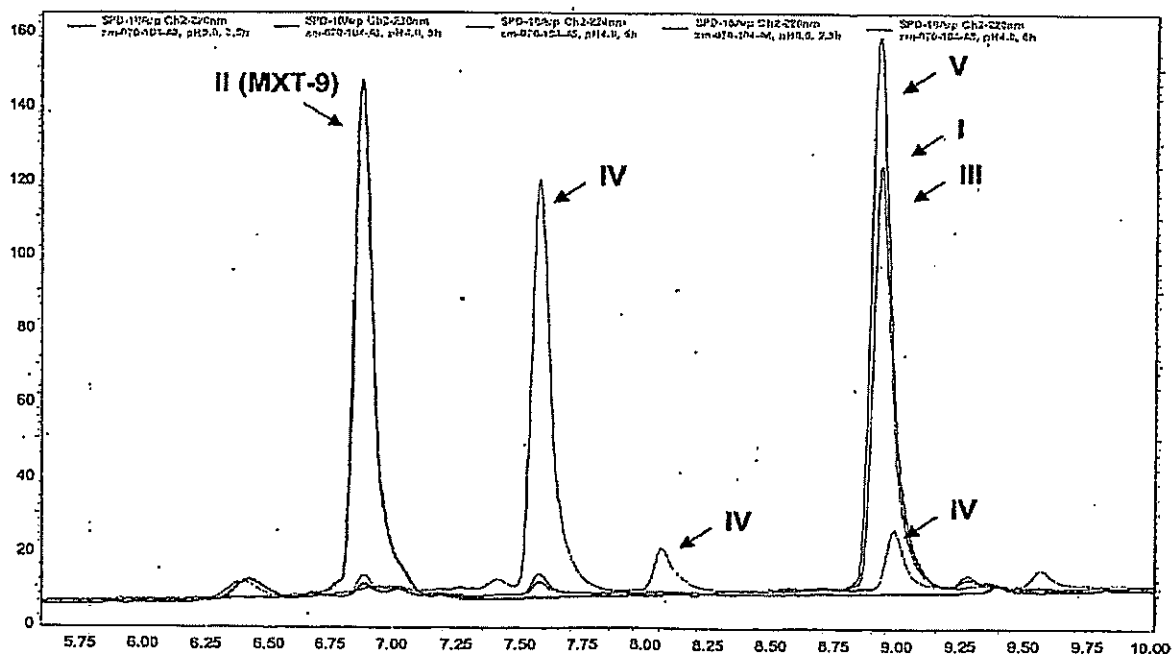


Figure 33

Reduction of peptide MXT-9-N3 to MXT-9NH2 (I), followed by reductive alkylations with propionaldehyde (II) and benzaldehyde (III)

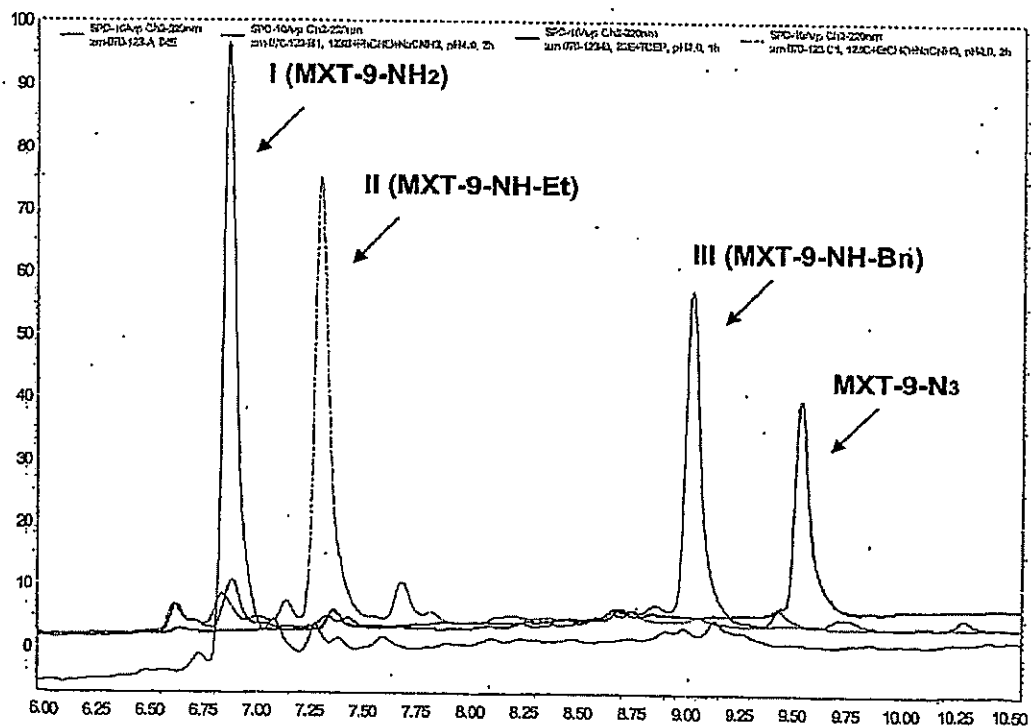
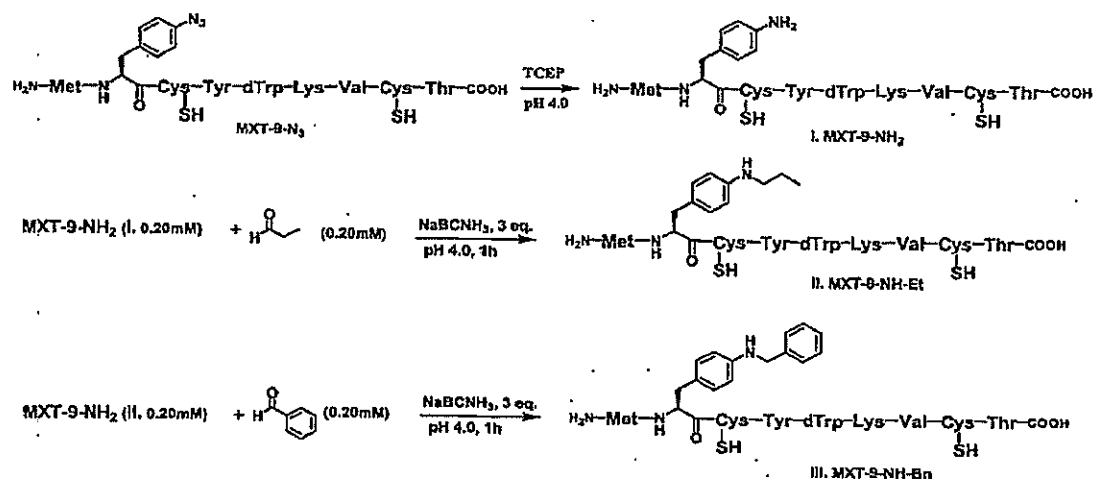
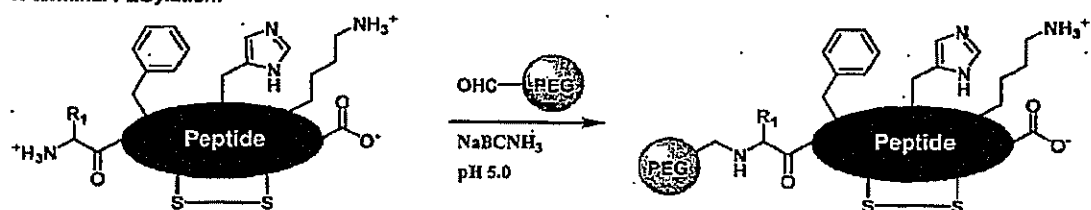


Figure 34

Peptide PEGylations.

N-terminal PEGylation:



pAF2-based PEGylation:

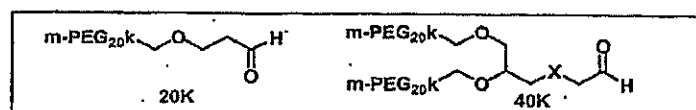
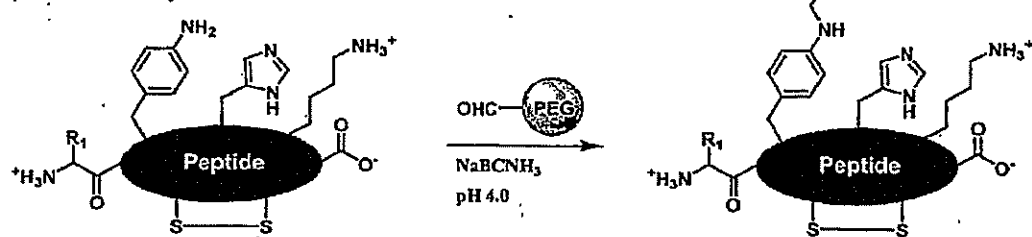
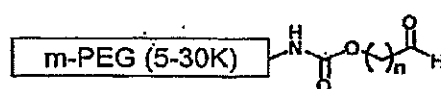
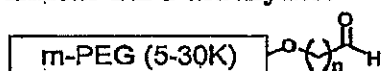


Figure 35

PEG Aldehydes used for PEGylations

Linear PEG aldehydes:



Branched PEG aldehydes derived from glycerol:

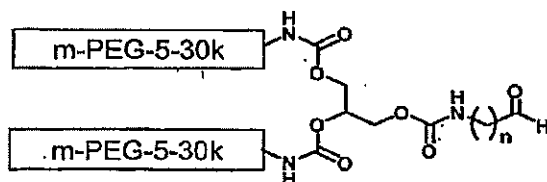
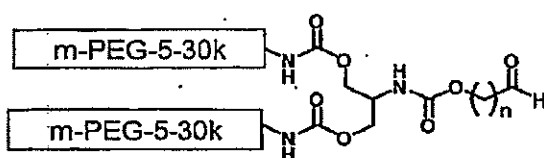
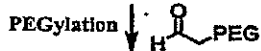
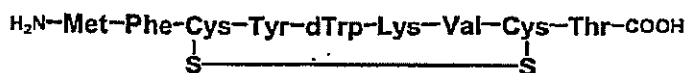


Figure 36

37/40

MT-9:



PEGylated
MT-9:

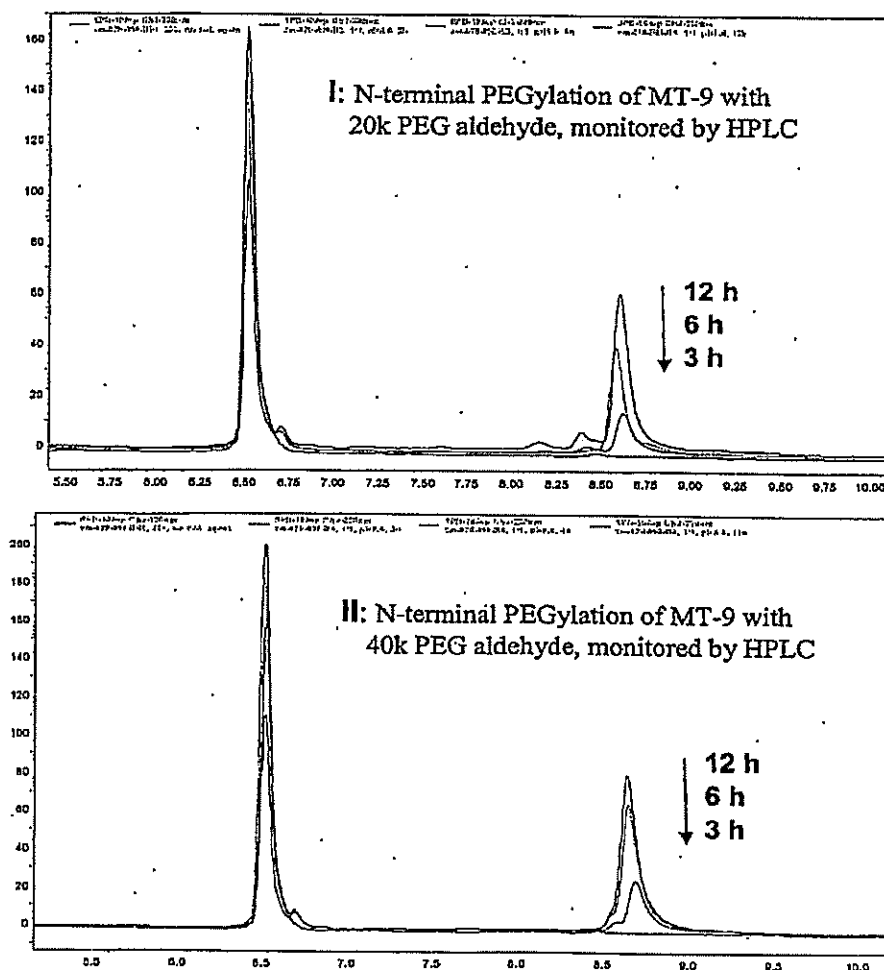
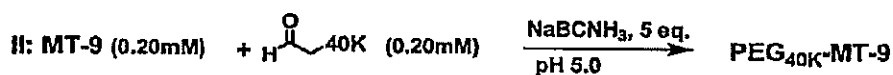
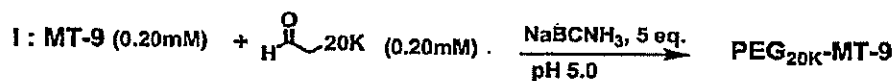
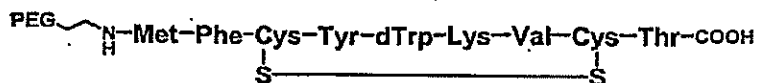


Figure 37

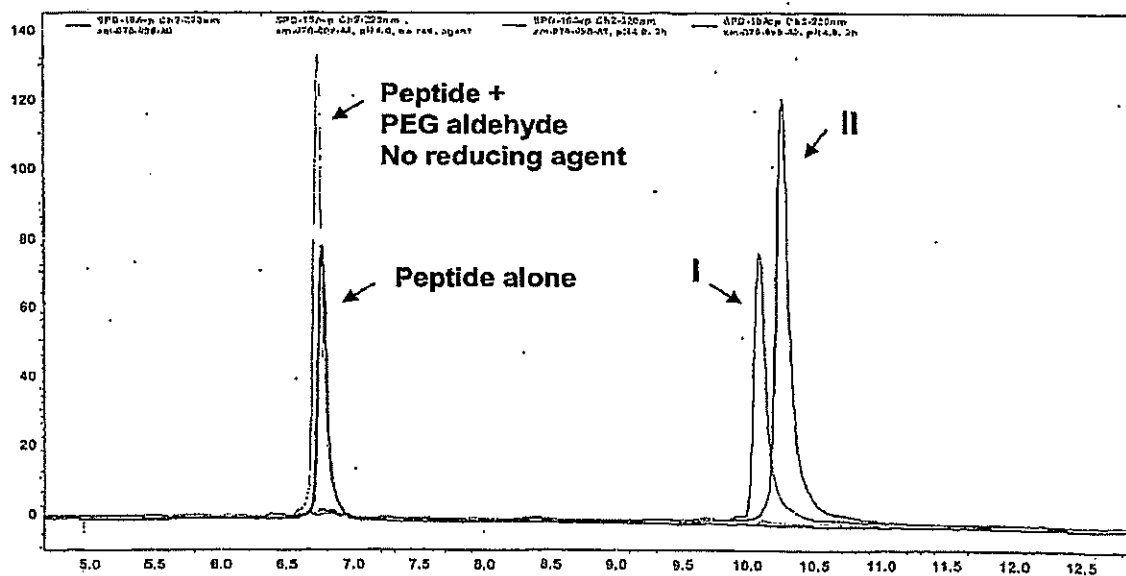
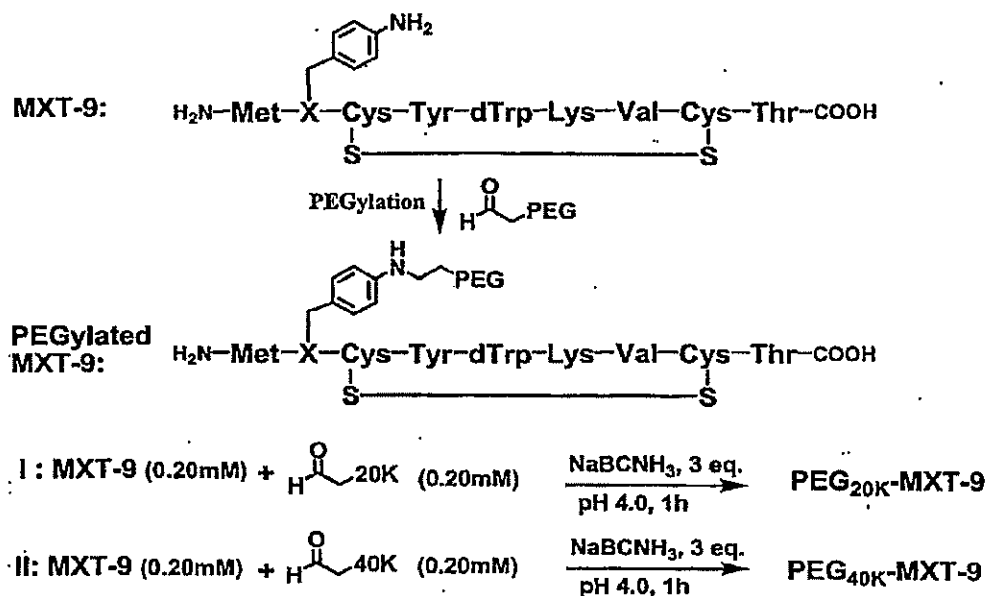
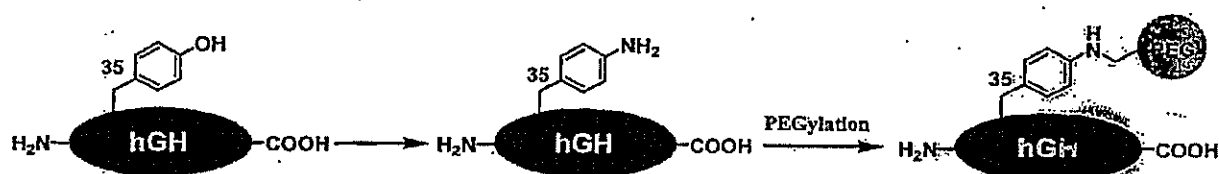


Figure 38

A)

Protein PEGylations — hGH



B)

Protein PEGylations — INFa

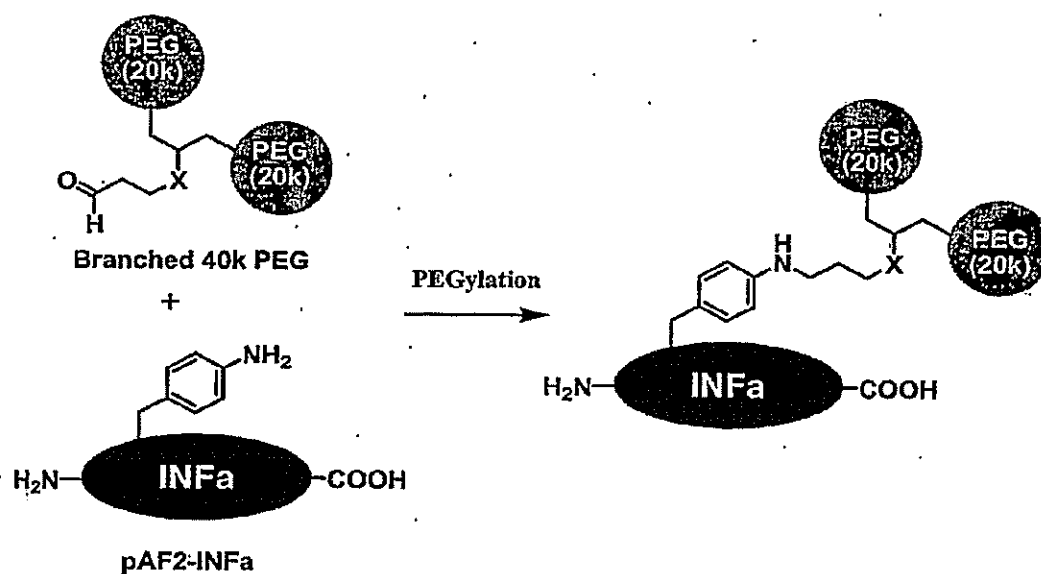


Figure 39

hGH Y35pAF2 PEGylation

PEG to protein ratio is 1:1.

NaCNBH₃ is 5:1.

pH = 4.0.

hGH is at 350uM.

1. H6-hGH Y35pAF2
2. H6-hGH Y35pAF2 + 20K PEG, 4°C
3. H6-hGH Y35pAF2 + 30K PEG, 4°C
4. H6-hGH Y35pAF2 + 40K, 4°C
5. H6-hGH Y35pAF2 + 20K PEG, RT
6. H6-hGH Y35pAF2 + 30K PEG, RT
7. H6-hGH Y35pAF2 + 40K, RT
8. Met-hGH Y35pAF1
9. Met-hGH Y35pAF1 + 40K, 4°C
10. Met-hGH Y35pAF1 + 40K, 4°C
11. H6-hGH Y35pAF2
12. H6-hGH Y35pAF2 + 40K, 4°C

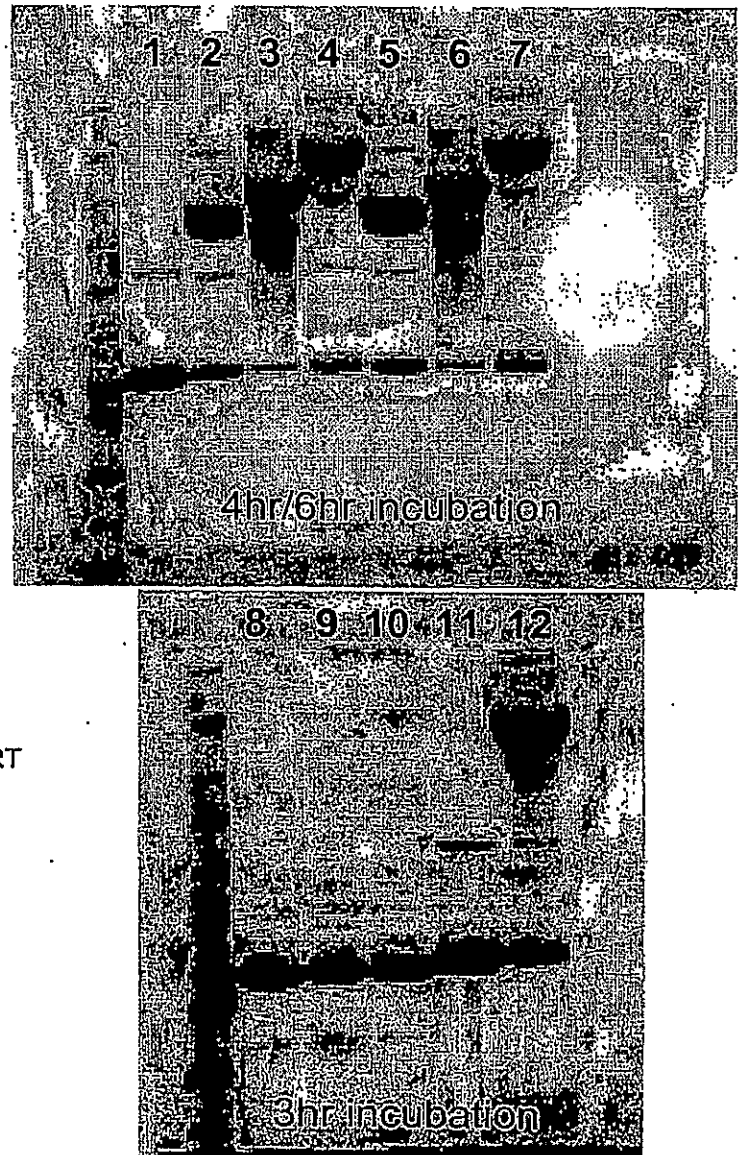


Figure 40