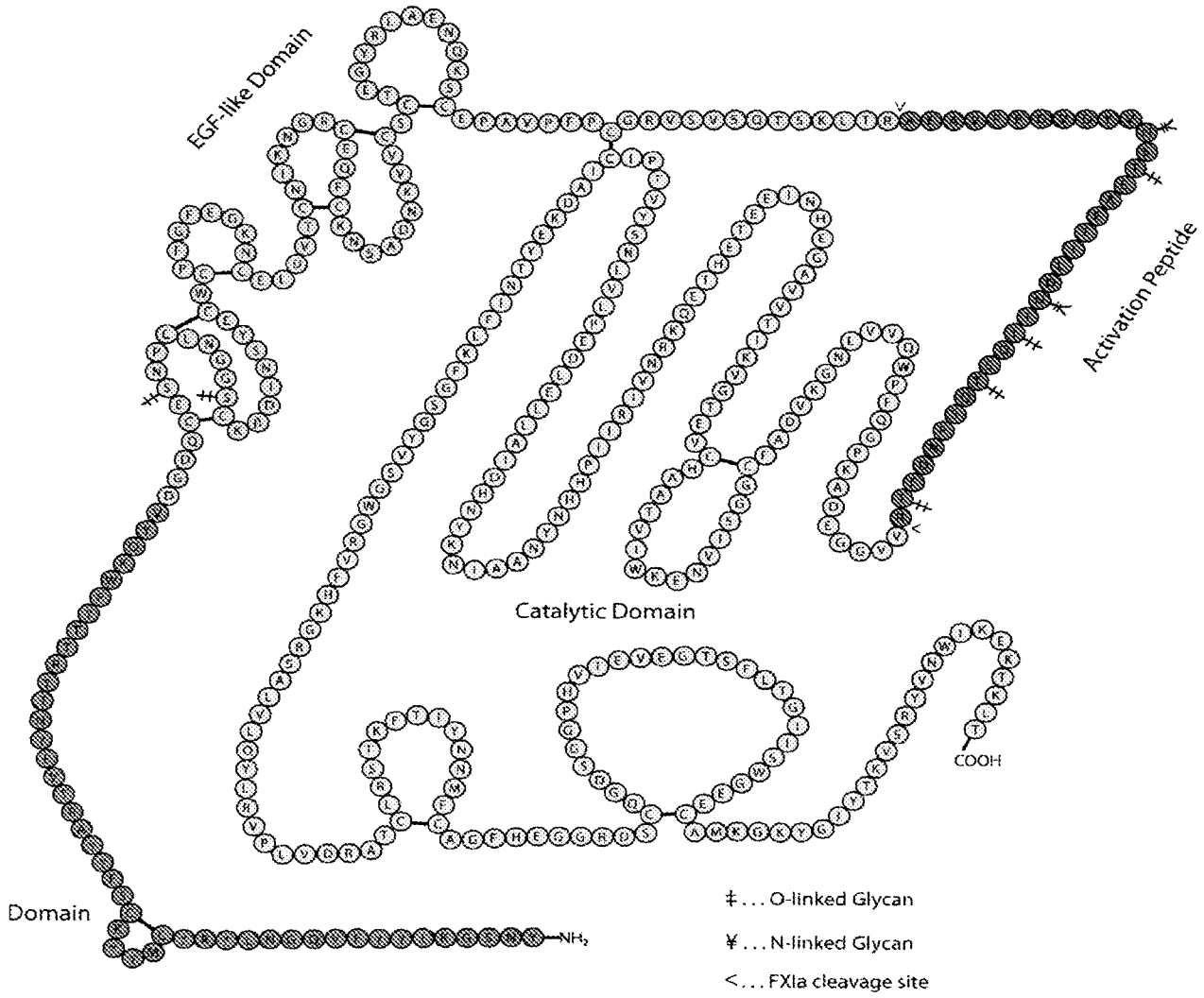

EGF-like Domain
Activation Peptide
Catalytic Domain
COOH
Domain
NH2
‡ . . . O-linked Glycan
¥ . . . N-linked Glycan
< . . . FXIa cleavage site

FIGURE 1

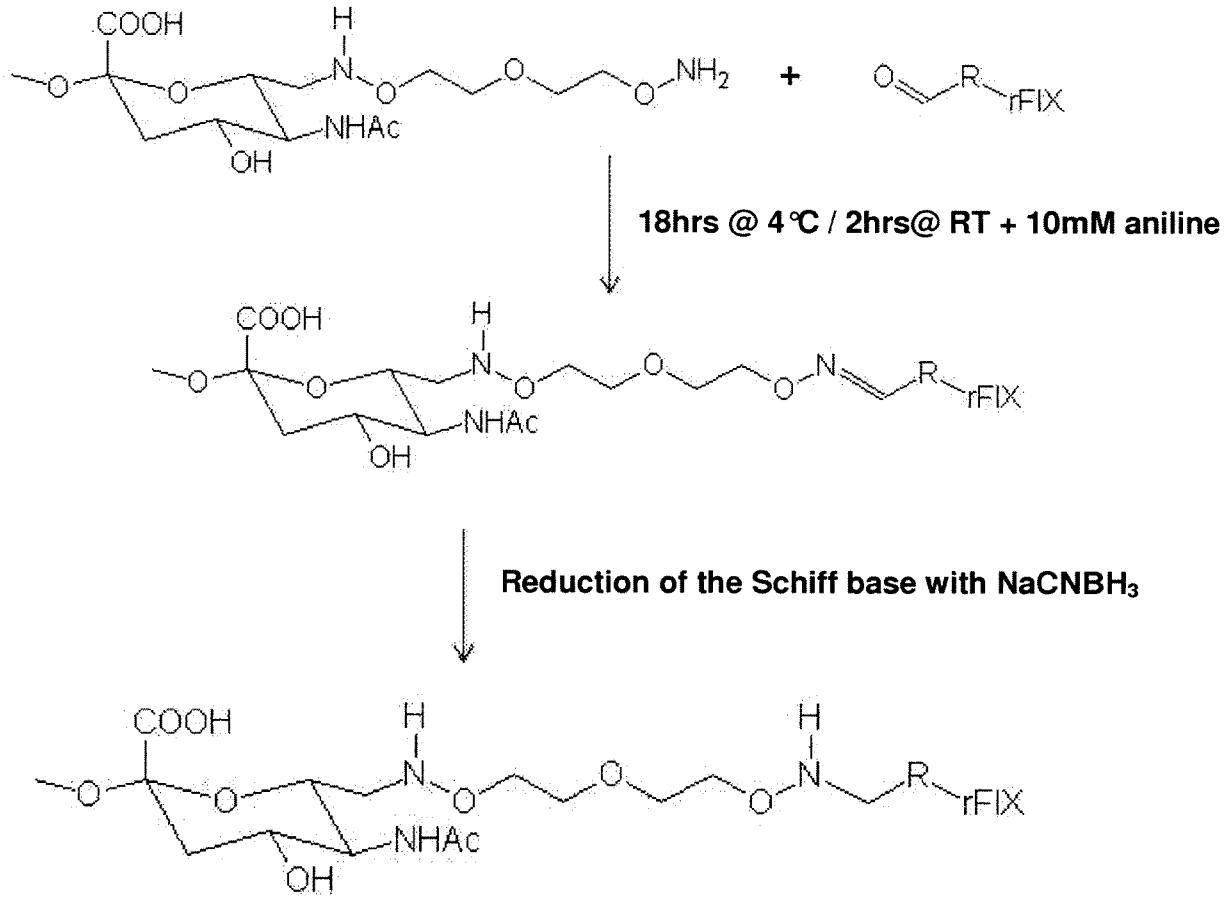

COOH
H
N
O
NHAc
OH
NH2
+
O
R
rFIX
18hrs @ 4°C / 2hrs@ RT + 10mM aniline
Reduction of the Schiff base with NaCNBH3

FIGURE 2

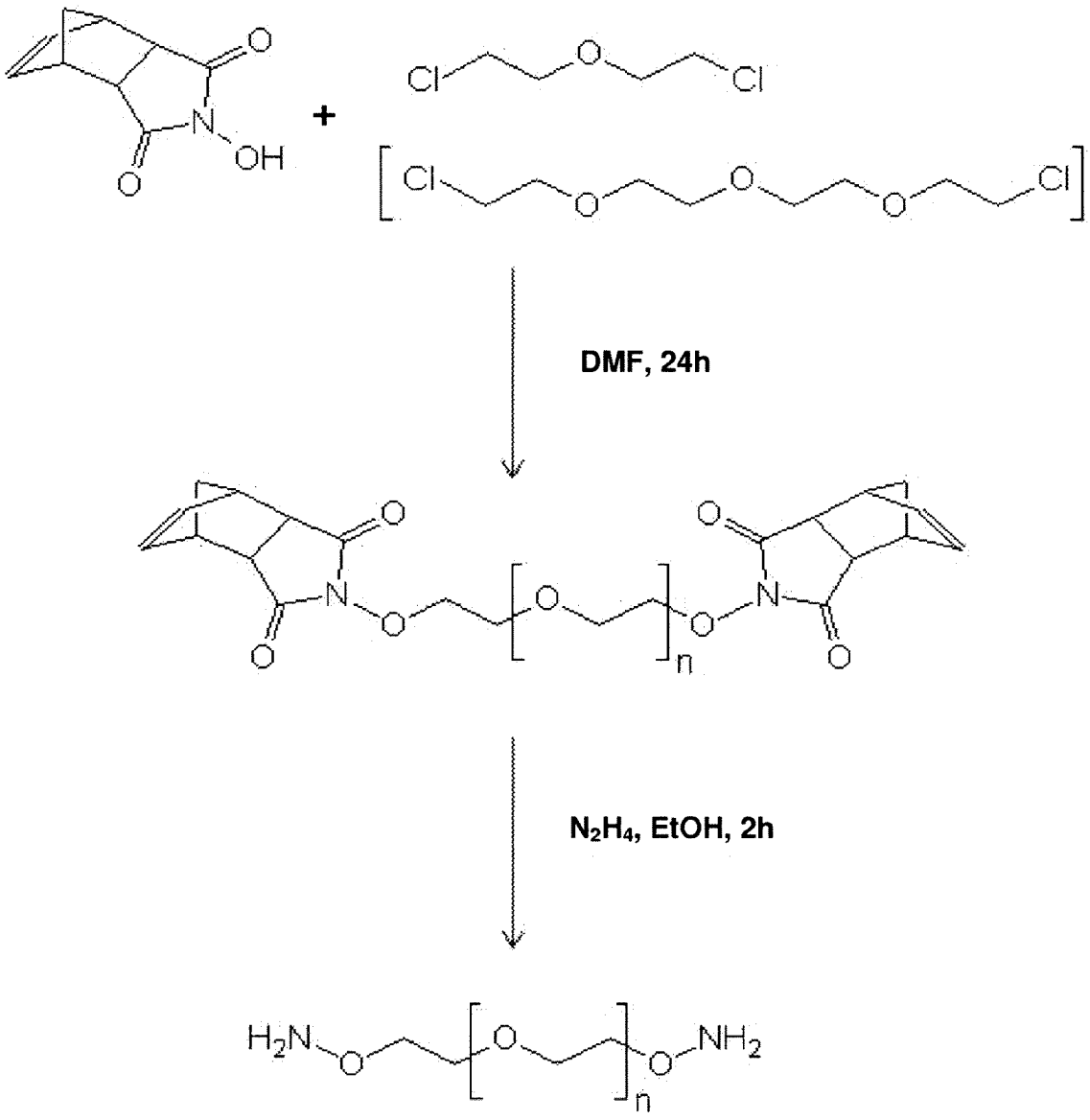
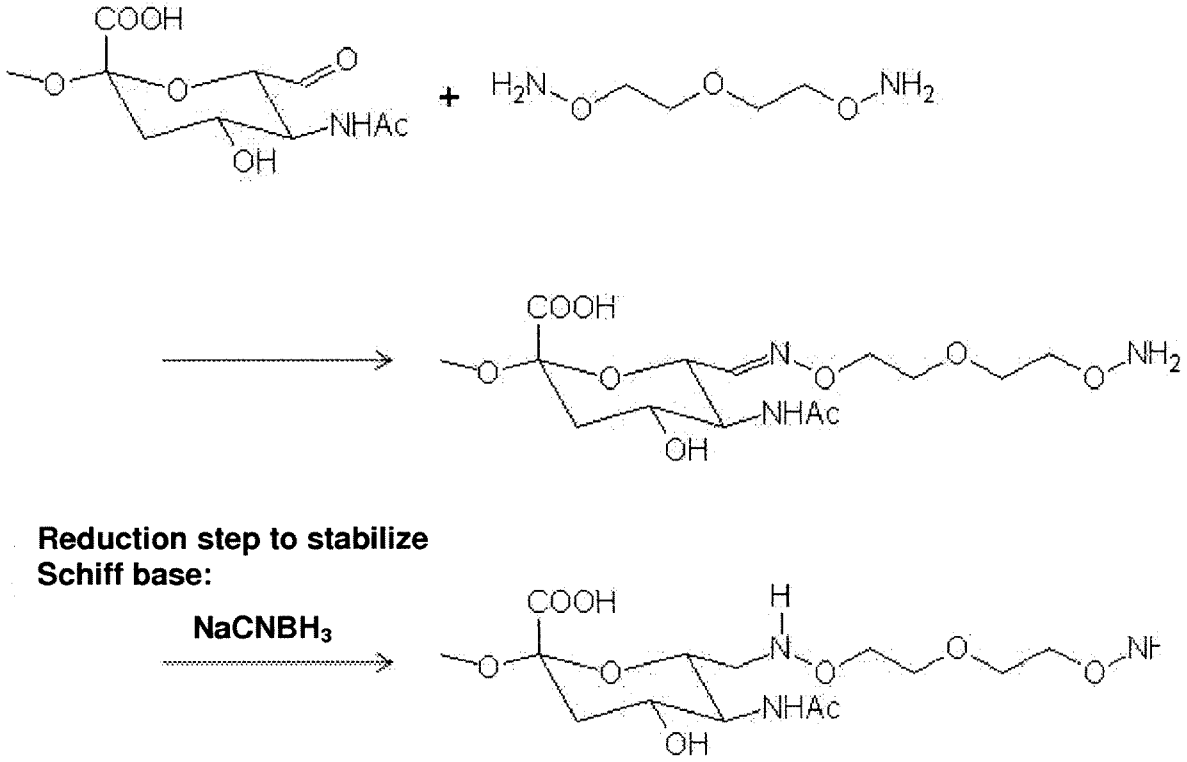

O
N
OH
O
+
Cl
O
Cl
Cl
O
O
O
Cl
DMF, 24h
O
N
O
O
O
n
O
N
O
O
N2H4, EtOH, 2h
H2N
O
O
n
O
NH2

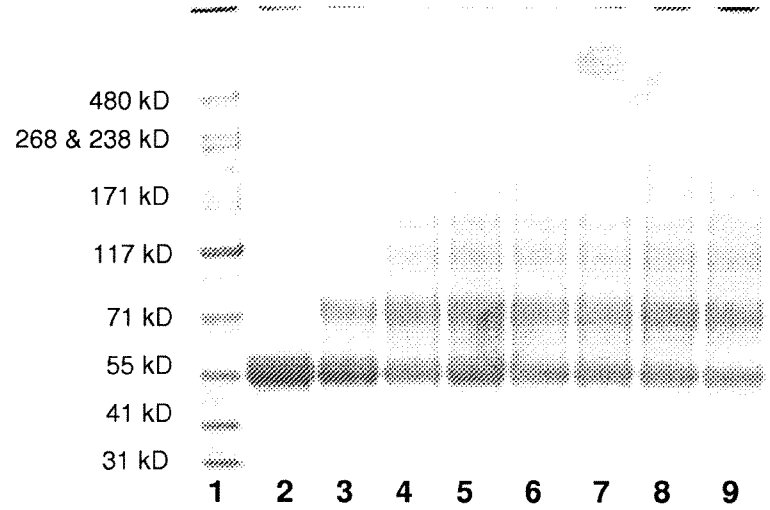
FIGURE 3



Reduction step to stabilize Schiff base:

FIGURE 4

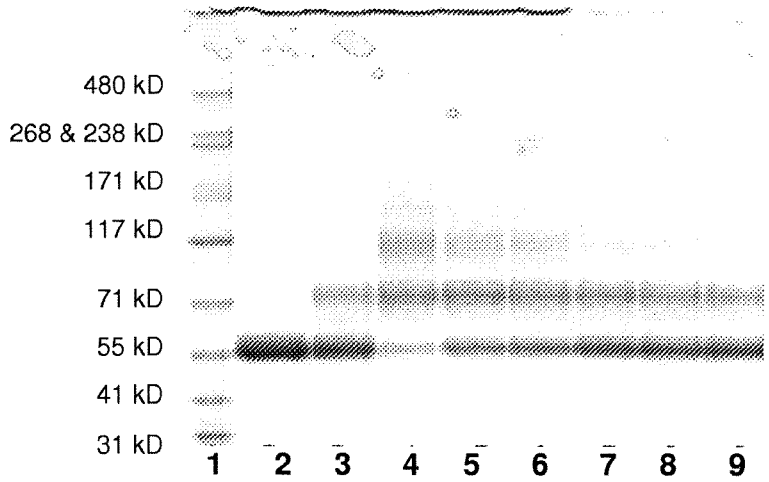
A



NuPAGE Novex 3-8% Tris-Acetate stained with coomassie

Lane 1: HiMark MW Standard
Lane 2: rFIX Standard
Lane 3: rFIX + 100µM NaIO4, 0mM catalyst, 5X PSA
Lane 4: rFIX + 100µM NaIO4, 2.5mM aniline, 5X PSA
Lane 5: rFIX + 100µM NaIO4, 5mM aniline, 5X PSA
Lane 6: rFIX + 100µM NaIO4, 10mM aniline, 5X PSA
Lane 7: rFIX + 100µM NaIO4, 2.5mM m-toluidine, 5X PSA
Lane 8: rFIX + 100µM NaIO4, 5mM m-toluidine, 5X PSA
Lane 9: rFIX + 100µM NaIO4, 10mM m-toluidine, 5X PSA

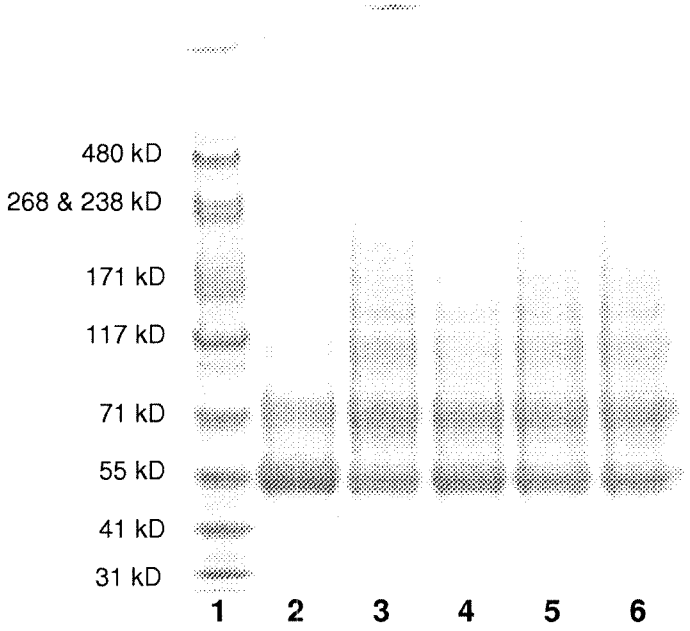
B



NuPAGE Novex 3-8% Tris-Acetate stained with coomassie

Lane 1: HiMark MW Standard
Lane 2: rFIX Standard
Lane 3: rFIX + 100µM NaIO4, 0mM catalyst, 5X PSA
Lane 4: rFIX + 100µM NaIO4, 10mM aniline, 5X PSA
Lane 5: rFIX + 100µM NaIO4, 10mM o-aminobenzoic acid, 5X PSA
Lane 6: rFIX + 100µM NaIO4, 10mM m-aminobenzoic acid, 5X PSA
Lane 7: rFIX + 100µM NaIO4, 10mM p-aminobenzoic acid, 5X PSA
Lane 8: rFIX + 100µM NaIO4, 10mM o-aminobenzamine acid, 5X PSA
Lane 9: rFIX + 100µM NaIO4, 10mM sulfanilic acid, 5X PSA

FIGURE 5



NuPAGE Novex 3-8% Tris-Acetate stained with coomassie

Lane 1: HiMark MW Standard

Lane 2: rFIX + 100µM NaIO4, 0mM catalyst, 5X PSA

Lane 3: rFIX + 100µM NaIO4, 10mM aniline, 5X PSA

Lane 4: rFIX + 100µM NaIO4, 10mM o-anisidine, 5X PSA

Lane 5: rFIX + 100µM NaIO4, 10mM m-anisidine, 5X PSA

Lane 6: rFIX + 100µM NaIO4, 10mM n-toluidine, 5X PSA

FIGURE 5

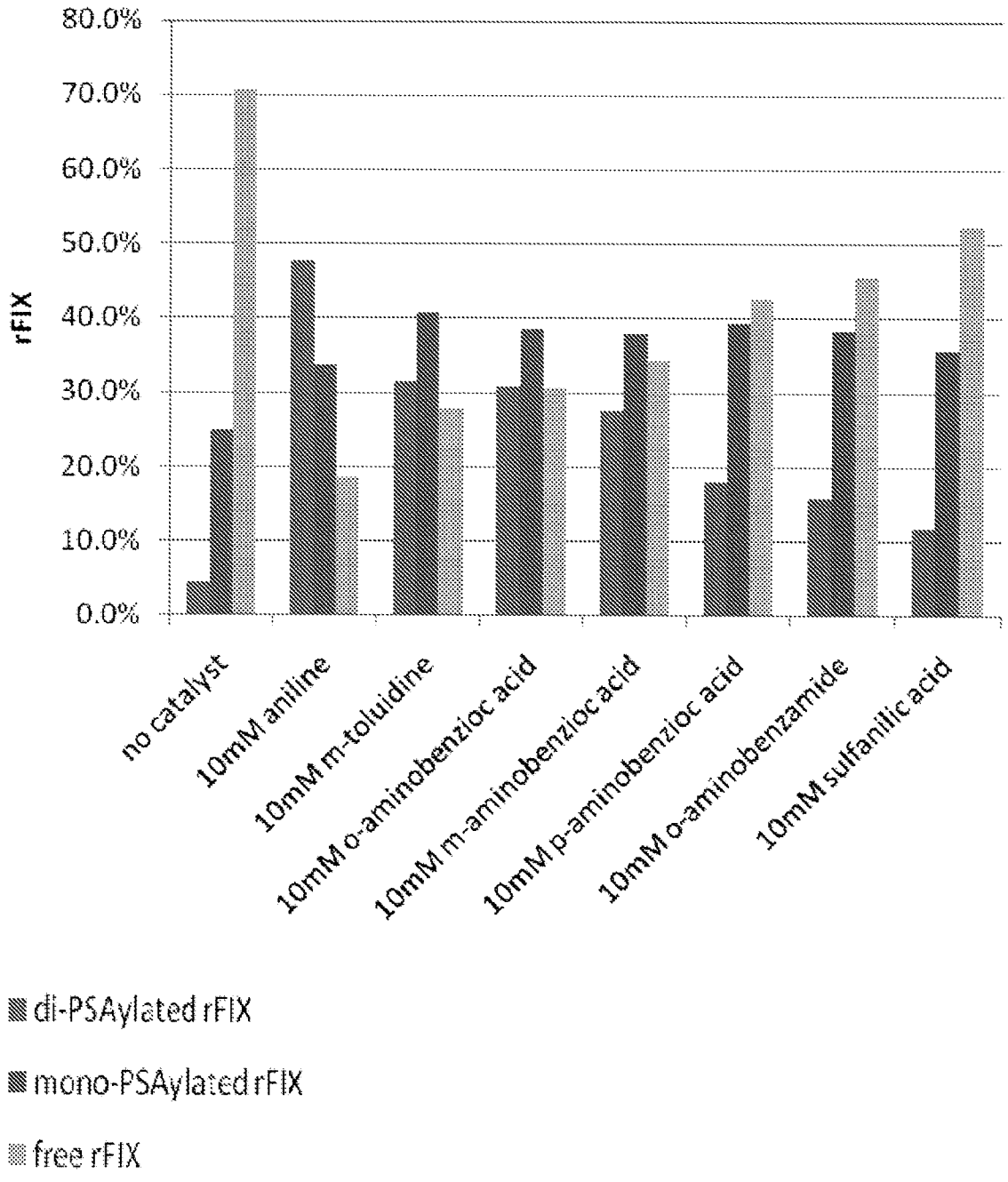

80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
rFIX
no catalyst
10mM aniline
10mM m-toluidine
10mM o-aminobenzioc acid
10mM m-aminobenzioc acid
10mM p-aminobenzioc acid
10mM o-aminobenzamide
10mM sulfanilic acid
di-PSAylated rFIX
mono-PSAylated rFIX
free rFIX

FIGURE 6