

eolf-seq1
SEQUENCE LISTING

<110> F. Hoffmann-La Roche AG

<120> Enzymatic one-pot reaction for double polypeptide conjugation in a single step

<130> P32477-WO

<150> EP14198534

<151> 2014-12-17

<160> 36

<170> PatentIn version 3.5

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Arg Arg Arg Arg Arg

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eolf-seql

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Lys Asp His Leu Ile His Asn Val His Lys Glu Phe His Ala His Ala
1 5 10 15

His Asn Lys

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Asp Tyr Lys Asp Asp Asp Asp Lys
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eolf-seql

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Asp Tyr Lys Asp His Asp Gly Asp Tyr Lys Asp His Asp Ile Asp Tyr
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Lys Asp Asp Asp Asp Lys
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Ala Trp Arg His Pro Gln Phe Gly Gly
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Trp Ser His Pro Gln Phe Glu Lys
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Met Asp Val Glu Ala Trp Leu Gly Ala Arg
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Met Asp Val Glu Ala Trp Leu Gly Ala Arg Val Pro Leu Val Glu Thr
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Met Asp Glu Lys Thr Thr Gly Trp Arg Gly Gly His Val Val Glu Gly
1 5 10 15

Leu Ala Gly Glu Leu Glu Gln Leu Arg Ala Arg Leu Glu His His Pro
20 25 30

Gln Gly Gln Arg Glu Pro
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Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu
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Lys Glu Thr Ala Ala Lys Phe Glu Arg Gln His Met Asp Ser
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Lys Arg Arg Trp Lys Lys Asn Phe Ile Ala Val Ser Ala Ala Asn Arg
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Phe Lys Lys Ile Ser Ser Ser Gly Ala Leu
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Pro Ala Thr Thr Thr Gly Ser Ser Pro Gly Pro Thr Gln Ser His Tyr
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Gly Gln Cys Gly Gly Ile Gly Tyr Ser Gly Pro Thr Val Cys Ala Ser
20 25 30

Gly Thr Thr Cys Gln Val Leu Asn Pro Tyr Tyr Ser Gln Cys Leu
35 40 45

<210> 16

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<213> Butyrivibrio fibrisolvens

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Met Asp Trp Asn Ala Asn Ile Ala Pro Gly Asn Ser Val Glu Phe Gly
1 5 10 15

Ile Gln Gly Ala Gly Ser Val Gly Asn Val Ile Asp Ile Thr Val Glu
20 25 30

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<223> chitin-binding-domain

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Thr Asn Pro Gly Val Ser Ala Trp Gln Val Asn Thr Ala Tyr Thr Ala
1 5 10 15

Gly Gln Leu Val Thr Tyr Asn Gly Lys Thr Tyr Lys Cys Leu Gln Pro
20 25 30

His Thr Ser Leu Ala Gly Trp Glu Pro Ser Asn Val Pro Ala Leu Trp

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35

40

45

Gln Leu Gln
50

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<211> 209

<212> PRT

<213> Chondrus crispus

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Met Pro Glu Ile Lys Leu Thr Tyr Phe Asp Met Arg Gly Arg Ala Glu
1 5 10 15

Ala Ser Arg Leu Ala Leu Val Val Gly Glu Ile Pro Phe Glu Asp Glu
20 25 30

Arg Val Val Phe Asp His Trp Lys Glu Ala Lys Pro Lys Thr Pro Tyr
35 40 45

Ala Ala Leu Pro Met Leu Thr Val Asp Gly Met Gln Val Ala Gln Ser
50 55 60

Asp Ala Ile Leu Arg Tyr Cys Gly Lys Leu Ala Gly Leu Tyr Pro Ser
65 70 75 80

Asp Pro Leu Glu Ala Ala Lys Val Asp Glu Val Gly Gly Val Ile Asp
85 90 95

Asp Val Thr His Ala Met Tyr Arg Tyr Arg Gly Asp Asp Lys Asp Lys
100 105 110

Leu Arg Glu Glu Arg Asp Lys Phe Ser Lys Val Asp Val Pro Arg Tyr
115 120 125

Val Gly Ala Leu Glu Lys Arg Leu Glu Ala Phe Gly Asp Gly Pro Trp
130 135 140

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Ala Val Gly Gly Asn Met Thr Ile Ala Asp Leu His Ile Cys His Leu
145 150 155 160

Val Thr Asn Ile Arg Cys Gly Met Leu Asp Phe Val Asp Lys Asp Leu
165 170 175

Leu Glu Gly Tyr Val Arg Ile Val Lys Ser Tyr Ser Ala Val Met Glu
180 185 190

His Pro Lys Val Thr Glu Trp Tyr Glu Lys Lys Pro Val Lys Met Phe
195 200 205

Ser

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<213> Escherichia coli

<400> 19

Met Lys Ile Lys Thr Gly Ala Arg Ile Leu Ala Leu Ser Ala Leu Thr
1 5 10 15

Thr Met Met Phe Ser Ala Ser Ala Leu Ala Lys Ile Glu Glu Gly Lys
20 25 30

Leu Val Ile Trp Ile Asn Gly Asp Lys Gly Tyr Asn Gly Leu Ala Glu
35 40 45

Val Gly Lys Lys Phe Glu Lys Asp Thr Gly Ile Lys Val Thr Val Glu
50 55 60

His Pro Asp Lys Leu Glu Glu Lys Phe Pro Gln Val Ala Ala Thr Gly
65 70 75 80

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Asp Gly Pro Asp Ile Ile Phe Trp Ala His Asp Arg Phe Gly Gly Tyr
85 90 95

Ala Gln Ser Gly Leu Leu Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln
100 105 110

Asp Lys Leu Tyr Pro Phe Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys
115 120 125

Leu Ile Ala Tyr Pro Ile Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn
130 135 140

Lys Asp Leu Leu Pro Asn Pro Pro Lys Thr Trp Glu Glu Ile Pro Ala
145 150 155 160

Leu Asp Lys Glu Leu Lys Ala Lys Gly Lys Ser Ala Leu Met Phe Asn
165 170 175

Leu Gln Glu Pro Tyr Phe Thr Trp Pro Leu Ile Ala Ala Asp Gly Gly
180 185 190

Tyr Ala Phe Lys Tyr Glu Asn Gly Lys Tyr Asp Ile Lys Asp Val Gly
195 200 205

Val Asp Asn Ala Gly Ala Lys Ala Gly Leu Thr Phe Leu Val Asp Leu
210 215 220

Ile Lys Asn Lys His Met Asn Ala Asp Thr Asp Tyr Ser Ile Ala Glu
225 230 235 240

Ala Ala Phe Asn Lys Gly Glu Thr Ala Met Thr Ile Asn Gly Pro Trp
245 250 255

Ala Trp Ser Asn Ile Asp Thr Ser Lys Val Asn Tyr Gly Val Thr Val
260 265 270

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Leu Pro Thr Phe Lys Gly Gln Pro Ser Lys Pro Phe Val Gly Val Leu
275 280 285

Ser Ala Gly Ile Asn Ala Ala Ser Pro Asn Lys Glu Leu Ala Lys Glu
290 295 300

Phe Leu Glu Asn Tyr Leu Leu Thr Asp Glu Gly Leu Glu Ala Val Asn
305 310 315 320

Lys Asp Lys Pro Leu Gly Ala Val Ala Leu Lys Ser Tyr Glu Glu Glu
325 330 335

Leu Ala Lys Asp Pro Arg Ile Ala Ala Thr Met Glu Asn Ala Gln Lys
340 345 350

Gly Glu Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe Trp Tyr Ala
355 360 365

Val Arg Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln Thr Val Asp
370 375 380

Glu Ala Leu Lys Asp Ala Gln Thr Arg Ile Thr Lys
385 390 395

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<223> sortase amino acid motif of Staphylococcus aureus

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<221> MISC_FEATURE

<222> (3)..(3)

<223> Xaa = can be any of the 20 proteinogenic amino acids

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eolf-seql

Leu Pro Xaa Thr Gly
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<223> mature soluble sortase

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Gln Ala Lys Pro Gln Ile Pro Lys Asp Lys Ser Lys Val Ala Gly Tyr
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Ile Glu Ile Pro Asp Ala Asp Ile Lys Glu Pro Val Tyr Pro Gly Pro
20 25 30

Ala Thr Pro Glu Gln Leu Asn Arg Gly Val Ser Phe Ala Glu Glu Asn
35 40 45

Glu Ser Leu Asp Asp Gln Asn Ile Ser Ile Ala Gly His Thr Phe Ile
50 55 60

Asp Arg Pro Asn Tyr Gln Phe Thr Asn Leu Lys Ala Ala Lys Lys Gly
65 70 75 80

Ser Met Val Tyr Phe Lys Val Gly Asn Glu Thr Arg Lys Tyr Lys Met
85 90 95

Thr Ser Ile Arg Asp Val Lys Pro Thr Asp Val Gly Val Leu Asp Glu
100 105 110

Gln Lys Gly Lys Asp Lys Gln Leu Thr Leu Ile Thr Cys Asp Asp Tyr
115 120 125

Asn Glu Lys Thr Gly Val Trp Glu Lys Arg Lys Ile Phe Val Ala Thr

eolf-seql
140

130

135

Glu Val Lys
145

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Gly Gly
1

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Gly Gly Gly
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<210> 24
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Gly Gly Gly Gly
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eolf-seql

<210> 25
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Gly Gly Gly Gly Gly
1 5

<210> 26
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Ala Ala
1

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Ala Ala Ala
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eolf-seq1

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<223> oligoalanine motif

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Ala Ala Ala Ala

1

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Ala Ala Ala Ala Ala

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5

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<223> sortase motif

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Leu Pro Glu Thr Gly

1

5

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eolf-seql

<222> (3)..(3)

<223> Xaa can be any one of the 20 protenaceous amino acid residues

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Leu Pro Xaa Thr Ala
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<211> 5

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<223> sortase motif LPETA

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Leu Pro Glu Thr Ala
1 5

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<223> bi-N-terminal polypeptide

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<222> (8)..(8)

<223> conjugated via the epsilon amino group - GSGSAAA

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Gly Gly Gly Gly Ser Gly Ser Lys
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eolf-seql

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<223> soluble Streptococcus pyogenes Sortase A

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Val Leu Gln Ala Gln Met Ala Ala Gln Gln Leu Pro Val Ile Gly Gly
1 5 10 15

Ile Ala Ile Pro Glu Leu Gly Ile Asn Leu Pro Ile Phe Lys Gly Leu
20 25 30

Gly Asn Thr Glu Leu Ile Tyr Gly Ala Gly Thr Met Lys Glu Glu Gln
35 40 45

Val Met Gly Gly Glu Asn Asn Tyr Ser Leu Ala Ser His His Ile Phe
50 55 60

Gly Ile Thr Gly Ser Ser Gln Met Leu Phe Ser Pro Leu Glu Arg Ala
65 70 75 80

Gln Asn Gly Met Ser Ile Tyr Leu Thr Asp Lys Glu Lys Ile Tyr Glu
85 90 95

Tyr Ile Ile Lys Asp Val Phe Thr Val Ala Pro Glu Arg Val Asp Val
100 105 110

Ile Asp Asp Thr Ala Gly Leu Lys Glu Val Thr Leu Val Thr Cys Thr
115 120 125

Asp Ile Glu Ala Thr Glu Arg Ile Ile Val Lys Gly Glu Leu Lys Thr
130 135 140

Glu Tyr Asp Phe Asp Lys Ala Pro Ala Asp Val Leu Lys Ala Phe Asn
145 150 155 160

His Ser Tyr Asn Gln Val Ser Thr
165

eolf-seql

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<400> 36

Met Arg Gly Ser His His His His His His Gly Ser
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