<110> HANMI PHARM. CO., LTD.

<120> Novel insulin analog and use thereof

<130> OPA14031-PCT

<150> KR 10-2013-0020703
<151> 2013-02-26

<150> KR 10-2013-0082511
<151> 2013-07-12

<150> KR 10-2014-0006937
<151> 2014-01-20

<160> 38

<170> KopatentIn 2.0

<210> 1
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 1
gggtccctgc agaagcgtgc gattgtggaa caatgctgt
39

<210> 2
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 2
acagcattgt tccacaatcg cacgcttctg cagggaccc
39

<210> 3
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 3

tccctgcaga agcgtggcgc ggtggaacaa tgctgtacc
39

<210> 4
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 4
ggtacagcat tgttccaccg cgccacgctt ctgcaggga
39

<210> 5
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 5
ctctaccagc tggaaaacgc gtgtaactga ggatcc
36

<210> 6
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 6
ggatcctcag ttacacgcgt tttccagctg gtagag
36

<210> 7
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 7
gttaaccaac acttgtgtgc gtcacacctg gtggaagct
39

<210> 8
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 8
agcttccacc aggtgtgacg cacacaagtg ttggttaac
39

<210> 9
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 9
ctagtgtgcg gggaacgagc gttcttctac acacccaag
39

<210> 10
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 10
cttgggtgtg tagaagaacg ctcgttcccc gcacactag
39

<210> 11
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 11
gtgtgcgggg aacgaggcgc gttctacaca cccaagacc
39

<210> 12
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 12
ggtcttgggt gtgtagaacg cgcctcgttc cccgcacac
39

<210> 13
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 13
tgcggggaac gaggcttcgc gtacacaccc aagacccgc
39

<210> 14
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 14
gcgggtcttg ggtgtgtacg cgaagcctcg ttccccgca
39

<210> 15
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 15
ccagcatctg ctcccctcgaa cagctggaga actactg
37

<210> 16
<211> 37

<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 16
cagtagttct ccagctgttc gagggagcag atgctgg
37

<210> 17
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 17
cagcatctgc tccctcaacc agctggagaa ctac
34

<210> 18
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 18
gtagttctcc agctggttga gggagcagat gctg
34

<210> 19
<211> 258
<212> DNA
<213> Artificial Sequence

<220>
<223> Analog 1

<400> 19
ttcgttaacc aacacttgtg tggctcacac ctggtggaag ctctctacct agtgtgcggg
60

gaacgaggct tcttctacac acccaagacc cgccgggagg cagaggacct gcaggtgggg
120

caggtggagc tgggcggggg ccctggtgca ggcagcctgc agcccttggc cctggagggg
180

tccctgcaga agcgtgcgat tgtggaacaa tgctgtacca gcatctgctc cctctaccag
240

ctggagaact actgcaac
258

<210> 20
<211> 86
<212> PRT
<213> Artificial Sequence

<220>
<223> Analog 1

<400> 20
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
1 5 10 15

Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Thr Arg Arg
20 25 30

Glu Ala Glu Asp Leu Gln Val Gly Gln Val Glu Leu Gly Gly Gly Pro
35 40 45

Gly Ala Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly Ser Leu Gln Lys
50 55 60

Arg Ala Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln
65 70 75 80

Leu Glu Asn Tyr Cys Asn
85

<210> 21
<211> 258
<212> DNA
<213> Artificial Sequence

<220>
<223> Analog 2

<400> 21
ttcgttaacc aacacttgtg tggctcacac ctggtggaag ctctctacct agtgtgcggg
60

gaacgaggct tcttctacac acccaagacc cgccgggagg cagaggacct gcaggtgggg
120

caggtggagc tgggcggggg ccctggtgca ggcagcctgc agcccttggc cctggagggg
180

tccctgcaga agcgtggcgc ggtggaacaa tgctgtacca gcatctgctc cctctaccag
240

ctggagaact actgcaac
258

<210> 22
<211> 86
<212> PRT
<213> Artificial Sequence

<220>
<223> Analog 2

<400> 22
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
1 5 10 15

Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Thr Arg Arg
20 25 30

Glu Ala Glu Asp Leu Gln Val Gly Gln Val Glu Leu Gly Gly Gly Pro
35 40 45

Gly Ala Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly Ser Leu Gln Lys
50 55 60

Arg Gly Ala Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln
65 70 75 80

Leu Glu Asn Tyr Cys Asn
85

<210> 23
<211> 258
<212> DNA
<213> Artificial Sequence

<220>
<223> Analog 3

<400> 23
ttcgttaacc aacacttgtg tggctcacac ctggtggaag ctctctacct agtgtgcggg
60

gaacgaggct tcttctacac acccaagacc cgccgggagg cagaggacct gcaggtgggg
120

caggtggagc tgggcggggg ccctggtgca ggcagcctgc agcccttggc cctggagggg
180

tccctgcaga agcgtggcat tgtggaacaa tgctgtacca gcatctgctc cctctaccag
240

ctggagaacg cgtgcaac
258

<210> 24
<211> 86
<212> PRT
<213> Artificial Sequence

<220>
<223> Analog 3

<400> 24
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
1 5 10 15

Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Thr Arg Arg
20 25 30

Glu Ala Glu Asp Leu Gln Val Gly Gln Val Glu Leu Gly Gly Gly Pro
35 40 45

Gly Ala Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly Ser Leu Gln Lys
50 55 60

Arg Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln
65 70 75 80

Leu Glu Asn Ala Cys Asn
85

<210> 25
<211> 258
<212> DNA
<213> Artificial Sequence

<220>
<223> Analog 4

<400> 25
ttcgttaacc aacacttgtg tgcgtcacac ctggtggaag ctctctacct agtgtgcggg
60

gaacgaggct tcttctacac acccaagacc cgccgggagg cagaggacct gcaggtgggg
120

caggtggagc tgggcggggg ccctggtgca ggcagcctgc agcccttggc cctggagggg
180

tccctgcaga agcgtggcat tgtggaacaa tgctgtacca gcatctgctc cctctaccag
240

ctggagaact actgcaac
258

<210> 26

<211> 86
<212> PRT
<213> Artificial Sequence

<220>
<223> Analog 4

<400> 26
Phe Val Asn Gln His Leu Cys Ala Ser His Leu Val Glu Ala Leu Tyr
1 5 10 15

Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Thr Arg Arg
20 25 30

Glu Ala Glu Asp Leu Gln Val Gly Gln Val Glu Leu Gly Gly Gly Pro
35 40 45

Gly Ala Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly Ser Leu Gln Lys
50 55 60

Arg Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln
65 70 75 80

Leu Glu Asn Tyr Cys Asn
85

<210> 27
<211> 258
<212> DNA
<213> Artificial Sequence

<220>
<223> Analog 5

<400> 27
ttcgttaacc aacacttgtg tggctcacac ctggtggaag ctctctacct agtgtgcggg
60

gaacgagcgt tcttctacac acccaagacc cgccgggagg cagaggacct gcaggtgggg
120

caggtggagc tgggcggggg ccctggtgca ggcagcctgc agcccttggc cctggagggg
180

tccctgcaga agcgtggcat tgtggaacaa tgctgtacca gcatctgctc cctctaccag
240

ctggagaact actgcaac
258

<210> 28
<211> 86
<212> PRT
<213> Artificial Sequence

<220>
<223> Analog 5

<400> 28
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
1 5 10 15

Leu Val Cys Gly Glu Arg Ala Phe Phe Tyr Thr Pro Lys Thr Arg Arg
20 25 30

Glu Ala Glu Asp Leu Gln Val Gly Gln Val Glu Leu Gly Gly Gly Pro
35 40 45

Gly Ala Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly Ser Leu Gln Lys
50 55 60

Arg Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln
65 70 75 80

Leu Glu Asn Tyr Cys Asn
85

<210> 29
<211> 258
<212> DNA
<213> Artificial Sequence

<220>
<223> Analog 6

<400> 29
ttcgttaacc aacacttgtg tggctcacac ctggtggaag ctctctacct agtgtgcggg
60

gaacgaggcg cgttctacac acccaagacc cgccgggagg cagaggacct gcaggtgggg
120

caggtggagc tgggcggggg ccctggtgca ggcagcctgc agcccttggc cctggagggg
180

tccctgcaga agcgtggcat tgtggaacaa tgctgtacca gcatctgctc cctctaccag
240

ctggagaact actgcaac
258

<210> 30
<211> 86
<212> PRT
<213> Artificial Sequence

<220>
<223> Analog 6

<400> 30

```
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
  1               5                  10                  15

Leu Val Cys Gly Glu Arg Gly Ala Phe Tyr Thr Pro Lys Thr Arg Arg
             20                  25                  30

Glu Ala Glu Asp Leu Gln Val Gly Gln Val Glu Leu Gly Gly Gly Pro
         35                  40                  45

Gly Ala Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly Ser Leu Gln Lys
     50                  55                  60

Arg Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln
 65                  70                  75                  80

Leu Glu Asn Tyr Cys Asn
                 85
```

<210> 31
<211> 258
<212> DNA
<213> Artificial Sequence

<220>
<223> Analog 7

<400> 31

```
ttcgttaacc aacacttgtg tggctcacac ctggtggaag ctctctacct agtgtgcggg
60

gaacgaggct tcgcgtacac acccaagacc cgccgggagg cagaggacct gcaggtgggg
120

caggtggagc tgggcggggg ccctggtgca ggcagcctgc agcccttggc cctggagggg
180

tccctgcaga agcgtggcat tgtggaacaa tgctgtacca gcatctgctc cctctaccag
240

ctggagaact actgcaac
258
```

<210> 32
<211> 86
<212> PRT
<213> Artificial Sequence

<220>
<223> Analog 7

<400> 32

Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
1 5 10 15

Leu Val Cys Gly Glu Arg Gly Phe Ala Tyr Thr Pro Lys Thr Arg Arg
20 25 30

Glu Ala Glu Asp Leu Gln Val Gly Gln Val Glu Leu Gly Gly Gly Pro
35 40 45

Gly Ala Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly Ser Leu Gln Lys
50 55 60

Arg Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln
65 70 75 80

Leu Glu Asn Tyr Cys Asn
85

<210> 33
<211> 261
<212> DNA
<213> Artificial Sequence

<220>
<223> Analog 8

<400> 33
ttcgttaacc aacacttgtg tggctcacac ctggtggaag ctctctacct agtgtgcggg
60

gaacgaggct tcttctacac acccaagacc cgccgggagg cagaggacct gcaggtgggg
120

caggtggagc tgggcggggg ccctggtgca ggcagcctgc agcccttggc cctggagggg
180

tccctgcaga agcgtggcat tgtggaacaa tgctgtacca gcatctgctc cctcgaacag
240

ctggagaact actgcaactg a
261

<210> 34
<211> 86
<212> PRT
<213> Artificial Sequence

<220>
<223> Analog 8

<400> 34
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
1 5 10 15

```
Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Thr Arg Arg
            20                  25                  30

Glu Ala Glu Asp Leu Gln Val Gly Gln Val Glu Leu Gly Gly Gly Pro
        35                  40                  45

Gly Ala Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly Ser Leu Gln Lys
    50                  55                  60

Arg Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Glu Gln
65                  70                  75                  80

Leu Glu Asn Tyr Cys Asn
                85
```

<210> 35
<211> 261
<212> DNA
<213> Artificial Sequence

<220>
<223> Analog 9

<400> 35
ttcgttaacc aacacttgtg tggctcacac ctggtggaag ctctctacct agtgtgcggg
60

gaacgaggct tcttctacac acccaagacc cgccgggagg cagaggacct gcaggtgggg
120

caggtggagc tgggcggggg ccctggtgca ggcagcctgc agcccttggc cctggagggg
180

tccctgcaga agcgtggcat tgtggaacaa tgctgtacca gcatctgctc cctcaaccag
240

ctggagaact actgcaactg a
261

<210> 36
<211> 86
<212> PRT
<213> Artificial Sequence

<220>
<223> Analog 9

<400> 36
```
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
  1               5                  10                  15

Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Thr Arg Arg
            20                  25                  30
```

```
Glu Ala Glu Asp Leu Gln Val Gly Gln Val Glu Leu Gly Gly Gly Pro
         35                  40                  45

Gly Ala Gly Ser Leu Gln Pro Leu Ala Leu Glu Gly Ser Leu Gln Lys
     50                  55                  60

Arg Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Asn Gln
 65                  70                  75                  80

Leu Glu Asn Tyr Cys Asn
                 85
```

<210> 37
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> A chain of insulin

<400> 37

```
Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu
  1               5                  10                  15

Glu Asn Tyr Cys Asn
             20
```

<210> 38
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> B chain of insulin

<400> 38

```
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
  1               5                  10                  15

Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Thr
             20                  25                  30
```