THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An engineered penicillin G acylase having a polypeptide sequence that is at least 90% identical to SEQ ID NO: 4, wherein said penicillin G acylase comprises a leucine residue at position ~~X2 as compared to the sequence~~ with reference to the position numbering of SEQ ID NO: 1220, wherein said penicillin G acylase is encoded by a polynucleotide sequence that is at least 85% identical to SEQ ID NO: 1219.

2. The engineered penicillin G acylase of Claim 1, wherein said penicillin G acylase comprises SEQ ID NO: 1194.

3. The engineered penicillin G acylase of Claim 1, wherein said penicillin G acylase comprises a polypeptide sequence that is at least 85% identical to at least one sequence ~~set forth in Table 5.1, 6.1, 7.1, 8.1, 8.2, 9.1, 10.1, 12.1, 12.2, 12.3, 12.4, 12.5, 12.6, and/or 12.7~~ having SEQ ID NO: (nt/aa) selected from the following: ~~:~~ 1193/1194, 1195/1196, 1197/1198, 1199/1200, 1201/1202, 1203/1204, 1205/1206, 1207/1208, 1209/1210, 1211/1212, 1213/1214, 1215/1216, 1217/1218, 1219/1220, 1221/1222, 1223/1224, 1225/1226, 1227/1228, 1229/1230, 1231/1232, 1233/1234, 1235/1236, 1237/1238, 1239/1240, 1241/1242, 1243/1244, 1245/1246, 1247/1248, 1249/1250, 1251/1252, 1253/1254..

4. The engineered penicillin G acylase of Claim 1, wherein said penicillin G acylase comprises a polypeptide sequence ~~set forth in Tables 9.1, and/or 10.1~~ having SEQ ID NO: (nt/aa) selected from the following: ~~:~~ 1193/1194, 1195/1196, 1197/1198, 1199/1200, 1201/1202, 1203/1204, 1205/1206, 1207/1208, 1209/1210, 1211/1212, 1213/1214, 1215/1216, 1217/1218, 1219/1220, 1221/1222, 1223/1224, 1225/1226, 1227/1228, 1229/1230, 1231/1232, 1233/1234, 1235/1236, 1237/1238, 1239/1240, 1241/1242, 1243/1244, 1245/1246, 1247/1248, 1249/1250, 1251/1252, 1253/1254.

5. The engineered penicillin G acylase of Claim 1, wherein said penicillin G acylase is capable of producing phenyl acetate mono-protected or di-protected insulin.

6. The engineered penicillin G acylase of Claim 1, wherein said penicillin G acylase comprises a polypeptide sequence that is at least 85% identical to at least one sequence provided in the even-numbered sequences of SEQ ID NOS: 6-1902.

~~7. The engineered penicillin G acylase of Claim 1, wherein said penicillin G acylase is encoded by a polynucleotide sequence that is at least 85% identical to SEQ ID NO: 1219.~~

7 ~~8~~. The engineered penicillin G acylase of Claim 1, wherein said penicillin G acylase comprises SEQ ID NO: 1220.

8 ~~9~~. An *in vitro* method for producing phenyl acetate mono-protected insulin, comprising: i) providing the engineered penicillin G acylase of Claim 1 and/or a composition comprising said engineered penicillin G acylase, and insulin comprising A1/B1/B29 tri-phenyl acetate protecting groups; and ii) exposing said engineered penicillin G acylase to said insulin comprising A1/B1/B29 tri-phenyl acetate protecting groups, under conditions such that said engineered penicillin G acylase removes the A1, B1 and/or B29 tri-phenyl acetate protecting groups from said insulin thereby producing phenyl acetate mono-protected insulin.

9 ~~10~~. The method of Claim 8 ~~9~~, wherein said penicillin G acylase removes the A1 tri-phenyl acetate protecting group of said insulin.

10 ~~11~~. The method of Claim 8 ~~9~~, wherein said penicillin G acylase removes the B1 tri-phenyl acetate protecting group of said insulin.

11 ~~12~~. The method of Claim 8 ~~9~~, wherein said penicillin G acylase removes the B29 tri-phenyl acetate protecting group of said insulin.

12 ~~13~~. The method of Claim 8 ~~9~~, wherein said penicillin G acylase removes the A1, B1, and B29 tri-phenyl acetate protecting group of said insulin.

13 ~~14~~. The method of Claim 8 ~~9~~, wherein said engineered penicillin G acylase produces more than 90% phenyl acetate mono-protected insulin, as compared to the production of phenyl acetate mono-protected insulin by the polypeptide of SEQ ID NO:2.