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 of SEQ ID NO: 1220.

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.

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.

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.

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

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.

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

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

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

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

14. The method of Claim 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.