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CLAIMS

- 1 A Cytochrome P450 BM3 monooxygenase (BM3) variant for catalyzing the C19 hydroxylation of a steroid or steroid derivative, wherein the BM3 variant comprises the mutation F87A and at least one and preferably two further mutations selected from
- 5 (i) a mutation at position V78, preferably V78F, V78Y, V78M, V78I or V78L, and
- (ii) a mutation at position A82, preferably A82E, A82Q or A82P.
- 2 The BM3 variant according to claim 1, characterized in comprising
- 10 (i) at least the mutations
- a) V78Y, A82E and F87A (BM3-254, SEQ ID No. 2),
- b) V78M, A82E and F87A (BM3-261, SEQ ID No. 3),
- c) V78I, A82E and F87A (BM3-263, SEQ ID No. 4),
- d) V78L, A82E and F87A (BM3-268, SEQ ID No. 5).
- 15 e) V78Y, A82P and F87A (SEQ ID No. 6),
- f) V78M, A82P and F87A (SEQ ID No. 7),
- g) V78I, A82P and F87A (SEQ ID No. 8),
- h) V78L, A82P and F87A (SEQ ID No. 9),
- i) V78Y, A82Q and F87A (SEQ ID No. 10),
- 20 j) V78M, A82Q and F87A (SEQ ID No. 11),
- k) V78I, A82Q and F87A (SEQ ID No. 12), or
- l) V78L, A82Q and F87A (SEQ ID No. 13),
- and at least one, two, three, four, five, six, seven, eight, nine or ten further mutation(s),
- 25 and/or
- (ii) at least the mutations
- a) V78F, A82E, F87A, M177Y and A184Y (SEQ ID No. 14),
- b) V78L, A82E, F87A, M177Y and A184Y (SEQ ID No. 15),
- c) V78I, A82E, F87A, M177Y and A184Y (SEQ ID No. 16),
- 30 d) V78M, A82E, F87A, M177Y and A184Y (SEQ ID No. 17),
- e) V78Y, A82E, F87A, M177Y and A184Y (SEQ ID No. 18),

- f) V78F, A82P, F87A, M177Y and A184Y (SEQ ID No. 19),
 g) V78L, A82P, F87A, M177Y and A184Y (SEQ ID No. 20),
 h) V78I, A82P, F87A, M177Y and A184Y (SEQ ID No. 21),
 i) V78M, A82P, F87A, M177Y and A184Y (SEQ ID No. 22),
 5 j) V78Y, A82P, F87A, M177Y and A184Y (SEQ ID No. 23),
 k) V78F, A82Q, F87A, M177Y and A184Y (SEQ ID No. 24),
 l) V78L, A82Q, F87A, M177Y and A184Y (SEQ ID No. 25),
 m) V78I, A82Q, F87A, M177Y and A184Y (SEQ ID No. 26),
 n) V78M, A82Q, F87A, M177Y and A184Y (SEQ ID No. 27),
 10 o) V78Y, A82Q, F87A, M177Y and A184Y (SEQ ID No. 28),
 p) V78L, A82E, F87A, S72G, M177Y, V178P, L181Y, A184Y and L188F (SEQ ID No. 117),
 q) V78L, A82E, F87A, S72G, T146F, M177Y, V178W, L181Y and A184Y (SEQ ID No. 118), or
 15 r) V78L, A82E, F87A, S72G, T146F, M177Y, V178W, L181Y, A184Y, G457S, I458G and A477N (SEQ ID No. 119).
- 3 A BM3 variant according to a previous claim, which is characterized by a product yield for at least one C19 hydroxylated steroid or derivative thereof or a secondary product thereof, which is > 5 %, preferably > 8 %, >10 %, or > 12 %, even more preferably > 15 %, >20 %, >25 %, > 30 %, >35 %, > 40 %, > 45 %, or > 50 %, most preferably > 55 %, > 60 %, > 65 %, > 70 % or > 75 %.
- 20 4 A BM3 variant according to a previous claim, which is characterized by a selectivity factor for the C19 hydroxylation of at least one steroid or steroid derivative, which is > 0.1, preferably > 0.2 or > 0.3, even more preferably > 0.4 or > 0.5, most preferably > 0.5, > 0.6, > 0.7, > 0.8 or > 0.9.
- 25 5 The BM3 variant according to any of claims 1 to 4, comprising mutations at at least one, two, three, four, five, six, seven, eight, nine, ten or more position(s) selected from (A184), (A191), (A221), (A264), (A321), (A328), (A33), (A330), (A399), (A74), (D168), (D208), (D222), (D363), (E13), (E143), (E267), (E352), (E64), (E82), (E93), (F173), (F205), (F261), (F331), (F393), (F77), (F81), (G240), (G271), (G402), (G415), (G570),
 30

(G677), (G85), (H266), (H659), (I153), (I174), (I258), (I259), (I263), (I401), (K210), (K224), (L150), (L181), (L188), (L20), (L262), (L272), (L29), (L324), (L333), (L356), (L437), (L75), (L78), (L86), (M118), (M177), (M185), (M212), (M354), (N70), (P243), (P326), (P329), (P392), (P9), (R147), (R161), (R190), (R203), (R255), (R323), (R47), (R50), (S164), (S176), (S54), (S72), (S89), (T146), (T149), (T260), (T268), (T269), (T327), (T365), (T436), (T438), (T49), (T88), (V178), (V26), (V299), (V314), (V371), (V48), (W325), (W367), (W90), (Y51), (A225), (A44), (D182), (D80), (E140), (E183), (E337), (E409), (G114), (G227), (G46), (H236), (H92), (K187), (K440), (K76), (M5), (N186), (P172), (Q189), (Q397), (Q403), (Q404), (Q73), (R179), (R79), (S270), (S53) and (T175).

6 A BM3 variant according to a previous claim, wherein said BM3 variant has an improved selectivity or product yield for C19 hydroxylation compared with at least one BM3 variant selected from the variants according to SEQ ID No. 2 to 5.

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7 A BM3 variant according to a previous claim, wherein said monooxygenase comprises the mutations M177Y and/or A184Y.

8 A BM3 variant according to a previous claim, wherein said variant comprises at least
20 the mutation(s) (A184D) or (A184D, L188K) or (A184E) or (A184F) or (A184G) or
(A184H) or (A184I) or (A184K) or (A184L) or (A184N) or (A184Q) or (A184R) or
(A184W) or (A184W, L188F) or (A184Y) or (A184Y, L188K) or (A184Y, R50S) or
(A221S) or (A264V) or (A321C) or (A321D) or (A321E) or (A321G) or (A321I) or
25 (A321N) or (A321T) or (A321V) or (A328P) or (A330C) or (A330D) or (A330E) or
(A330F) or (A330G) or (A330H) or (A330I) or (A330K) or (A330L) or (A330M) or
(A330N) or (A330Q) or (A330R) or (A330S) or (A330T) or (A330T, E352A) or (A330T,
F331V) or (A330V) or (A330W) or (A330Y) or (A330Y, W367C) or (A33V) or (A399C)
or (A399E) or (A399G) or (A399I) or (A399L) or (A399M) or (A399N) or (A399Q) or
(A399R) or (A399S) or (A399T) or (A399V) or (A74C) or (A74C, L75V) or (A74D) or
30 (A74D, F81V) or (A74E) or (A74F) or (A74G) or (A74G, L75C) or (A74H) or (A74I) or
(A74I, A184W, L188F) or (A74I, F77A, L181Y, A184W) or (A74I, L181Y, L188K) or
(A74I, L181Y, L188R) or (A74I, L188F) or (A74I, L188K) or (A74I, L188R) or (A74I,
M177Y, A184R, L188F) or (A74I, M177Y, V178P, A184G) or (A74I, T146F) or (A74I,
T146F, L181Y) or (A74I, T146F, L181Y, L188K) or (A74I, T146F, L181Y, L188R) or
35 (A74I, T146F, L188F) or (A74I, T146F, L188K) or (A74I, T146F, V178P) or (A74I,
T146F, V178P, L181Y, L188F) or (A74I, T146F, V178P, L181Y, L188K) or (A74I,
T146F, V178P, L188K) or (A74I, T146F, V178P, L188R) or (A74I, T146F, V178W) or

(A74I, T146F, V178W, L181Y, L188F) or (A74I, T146F, V178W, L181Y, L188R) or (A74I, T146F, V178W, L188F) or (A74I, T146F, V178W, L188K) or (A74I, T146F, V178W, L188R) or (A74I, V178P) or (A74I, V178P, L181Y) or (A74I, V178P, L181Y, L188R) or (A74I, V178P, L188F) or (A74I, V178P, L188K) or (A74I, V178P, L188R) or (A74I, V178W, L188F) or (A74I, V178W, L188K) or (A74I, V178W, L188R) or (A74K) or (A74L) or (A74M) or (A74N) or (A74P) or (A74Q) or (A74R) or (A74R, L75V) or (A74S) or (A74T) or (A74T, A184D) or (A74T, F77A, L181Y, A184D, L188K) or (A74T, F77A, V178W, A184Y) or (A74T, F77L, L181Y, A184D, L188K) or (A74T, L181Y) or (A74T, L181Y, A184D, L188K) or (A74T, L181Y, A184Y, L188K) or (A74T, L181Y, L188K) or (A74T, L181Y, L188R) or (A74T, L188K) or (A74T, M177Y, L181Y, A184D, L188K) or (A74T, T146F, L181Y, A184D, L188K) or (A74T, T146F, L181Y, L188K) or (A74T, T146F, L181Y, L188R) or (A74T, T146F, L188F) or (A74T, T146F, L188K) or (A74T, T146F, L188R) or (A74T, T146F, V178P) or (A74T, T146F, V178P, L181Y) or (A74T, T146F, V178P, L188K) or (A74T, T146F, V178P, L188R) or (A74T, T146F, V178W) or (A74T, T146F, V178W, L181Y) or (A74T, T146F, V178W, L181Y, L188K) or (A74T, T146F, V178W, L188R) or (A74T, V178P) or (A74T, V178P, L188F) or (A74T, V178P, L188K) or (A74T, V178P, L188R) or (A74T, V178W, L181Y, L188K) or (A74T, V178W, L188K) or (A74T, V178W, L188R) or (A74V) or (A74V, F77A, A184N, L188F) or (A74V, L181Y) or (A74V, L181Y, A184D, L188K) or (A74V, L181Y, A184W, L188F) or (A74V, L181Y, L188F) or (A74V, L188F) or (A74V, L188K) or (A74V, L75V) or (A74V, T146F, L181Y, L188K) or (A74V, T146F, L181Y, L188R) or (A74V, T146F, L188K) or (A74V, T146F, L188R) or (A74V, T146F, V178W) or (A74V, T146F, V178W, L181Y, L188F) or (A74V, V178P, A184W, L188K) or (A74V, V178P, L181Y, L188F) or (A74V, V178P, L181Y, L188K) or (A74V, V178P, L181Y, L188R) or (A74V, V178P, L188F) or (A74V, V178W, L188K) or (A74W) or (A74Y) or (D168Y, F173D) or (D363G, T438C) or (E13D, R47L) or (E143A) or (E143C) or (E143D) or (E143F) or (E143G) or (E143I) or (E143K) or (E143L) or (E143M) or (E143N) or (E143P) or (E143Q) or (E143R) or (E143S) or (E143V) or (E143W) or (E143Y) or (E267A) or (E267C) or (E267D) or (E267G) or (E267K) or (E267P) or (E267R) or (E267S) or (E267T) or (E267Y) or (E352A) or (E352D) or (E352F) or (E352G) or (E352I) or (E352L) or (E352M) or (E352N) or (E352P) or (E352R) or (E352S) or (E352T) or (E352V) or (E352W) or (E352Y) or (E64A) or (E64G) or (E64H) or (E64I) or (E64K) or (E64L) or (E64M) or (E64N) or (E64Q) or (E64V) or (E64W) or (E64Y) or (E82P) or (E93G) or (F173C) or (F173C, F205G) or (F173D) or (F173I) or (F173K) or (F173L) or (F173M) or (F173N) or (F173P) or (F173Q) or (F173R) or (F173S) or (F173S, F205P) or (F173V) or (F173W) or (F173Y) or (F173Y, I174F) or (F205A) or (F205C) or (F205D) or (F205D, D208N) or (F205E) or (F205G) or (F205H) or (F205I) or (F205K) or (F205L) or (F205M) or

(F205N) or (F205P) or (F205R) or (F205S) or (F205T) or (F205V) or (F205W) or (F205Y) or (F261A) or (F261C) or (F261D) or (F261G) or (F261I) or (F261L) or (F261M) or (F261N) or (F261Q) or (F261S) or (F261T) or (F261V) or (F261W) or (F261Y) or (F331C) or (F331H) or (F331I) or (F331L) or (F331M) or (F331N) or (F331P) or (F331T) or (F331V) or (F331W) or (F331Y) or (F393M) or (F393W) or (F77A) or (F77A, A184W) or (F77A, L181P, A184Y, L188K) or (F77A, M177Y) or (F77C) or (F77D) or (F77E) or (F77G) or (F77H) or (F77I) or (F77K) or (F77L) or (F77M) or (F77N) or (F77P) or (F77R) or (F77S) or (F77T) or (F77V) or (F77W, L86M) or (F77W) or (F77Y) or (F81A) or (F81C) or (F81D) or (F81I) or (F81L) or (F81P) or (F81R) or (F81S) or (F81T) or (F81V) or (F81W) or (F81Y) or (G271A) or (G271C) or (G271D) or (G271E) or (G271F) or (G271H) or (G271K) or (G271L) or (G271M) or (G271N) or (G271P) or (G271Q) or (G271R) or (G271S) or (G271T) or (G271V) or (G271W) or (G271Y) or (G402A) or (G415A) or (G415D) or (G415S) or (G415T) or (G415V) or (G85A) or (G85L) or (G85S) or (H266A) or (H266C) or (H266D) or (H266E) or (H266F) or (H266G) or (H266I) or (H266K) or (H266M) or (H266N) or (H266P) or (H266Q) or (H266R) or (H266S) or (H266T) or (H266V) or (H266W) or (H266Y) or (I153F, G271L) or (I153L) or (I153L, F173Y) or (I259A) or (I259C) or (I259D) or (I259F) or (I259G) or (I259H) or (I259K) or (I259L) or (I259M) or (I259N) or (I259Q) or (I259S) or (I259T) or (I259V) or (I259W) or (I259Y) or (I263A) or (I263C) or (I263E) or (I263F) or (I263G) or (I263H, A264G) or (I263K) or (I263L) or (I263M) or (I263N) or (I263Q) or (I263S) or (I263T) or (I263V) or (I263Y) or (I401A) or (I401L) or (I401M) or (I401T) or (I401V) or (K210E) or (K210T, G271V) or (K224C) or (K224E) or (K224F) or (K224H) or (K224I) or (K224L) or (K224M) or (K224P) or (K224Q) or (K224W) or (K224Y) or (L150A) or (L150C) or (L150D) or (L150E) or (L150F) or (L150G) or (L150H) or (L150I) or (L150K) or (L150M) or (L150N) or (L150Q) or (L150R) or (L150R, F205R) or (L150S) or (L150S, F173L) or (L150T) or (L150V) or (L150W) or (L150Y) or (L181H) or (L181I) or (L181M) or (L181P) or (L181V) or (L181Y) or (L181Y, A184D) or (L181Y, L188F) or (L181Y, L188K) or (L181Y, L188K, H659R) or (L188A) or (L188D) or (L188E) or (L188F) or (L188H) or (L188I) or (L188K) or (L188M) or (L188N) or (L188Q) or (L188R) or (L188S) or (L188W) or (L20C) or (L20D) or (L20E) or (L20F) or (L20G) or (L20G, R47L) or (L20I) or (L20M) or (L20N) or (L20P) or (L20R) or (L20S) or (L20T) or (L20V) or (L20W) or (L20Y) or (L262I) or (L262V) or (L262W) or (L262Y) or (L272A) or (L272C) or (L272E) or (L272F) or (L272G) or (L272I) or (L272K) or (L272M) or (L272N) or (L272Q) or (L272R) or (L272S) or (L272T) or (L272V) or (L272W) or (L272Y) or (L29A) or (L29C) or (L29D) or (L29F) or (L29H) or (L29I) or (L29M) or (L29M, R47G) or (L29P) or (L29Q) or (L29S) or (L29T) or (L29V) or (L29W) or (L29Y) or (L324F) or (L356C) or (L356F) or (L356H) or (L356I) or (L356M) or (L356N) or (L356Q) or (L356S)

or (L356T) or (L356V) or (L356W) or (L437I) or (L437M) or (L75H, F81S) or (L75I) or
 (L75I, F81C) or (L75I, F81G) or (L75I, F81H) or (L75I, F81I) or (L75I, F81L) or (L75I,
 F81S) or (L75I, F81V) or (L75I, F81Y) or (L75V) or (L75V, F81C) or (L75V, F81H) or
 (L75V, F81I) or (L75V, F81L) or (L75V, F81V) or (L75V, F81Y) or (L78F) or (L78I) or
 5 (L78M) or (L78V) or (L78Y) or (L86A, S89T) or (L86I) or (L86I, S89T) or (L86M) or
 (L86M, S89T) or (L86N, S89T) or (L86V) or (L86V, S89T) or (M118A) or (M118E) or
 (M118F) or (M118G) or (M118H) or (M118I) or (M118K) or (M118L) or (M118N) or
 (M118P) or (M118Q) or (M118S) or (M118T) or (M118V) or (M118W) or (M118Y) or
 (M177A) or (M177C) or (M177C, V178Y) or (M177D) or (M177E) or (M177F) or
 10 (M177G) or (M177H) or (M177I) or (M177K) or (M177L) or (M177N) or (M177P) or
 (M177Q) or (M177R) or (M177T) or (M177V) or (M177Y) or (M177Y, A184W) or
 (M177Y, A184Y) or (M177Y, M185V) or (M177Y, R50S) or (M177Y, V178P) or
 (M177Y, V178P, A184Y, L188F) or (M177Y, V178W) or (M177Y, V178W, A184Y) or
 (M177Y, V178W, A184Y, L188F) or (M185C) or (M185D) or (M185E) or (M185G) or
 15 (M185H) or (M185K) or (M185L) or (M185N) or (M185Q) or (M185R) or (M185V) or
 (M185Y) or (M212A) or (M212C) or (M212C, I259L) or (M212D) or (M212E) or (M212F)
 or (M212G) or (M212H) or (M212K) or (M212L) or (M212L, I259F) or (M212P) or
 (M212Q) or (M212R) or (M212S) or (M212T) or (M212V) or (M212W) or (M212Y) or
 (M354A) or (M354C) or (M354D) or (M354E) or (M354G) or (M354I) or (M354K) or
 20 (M354K, D363Y) or (M354L) or (M354N) or (M354Q) or (M354R) or (M354S) or
 (M354T) or (M354V) or (M354W) or (M354Y) or (N70A) or (N70C) or (N70F) or (N70G)
 or (N70H) or (N70K) or (N70R) or (N70W) or (N70Y) or (P243T, H266L) or (P326C) or
 (P326D) or (P326G) or (P326N) or (P326S) or (P326T) or (P329A) or (P329C) or
 (P329G) or (P329K) or (P329S) or (P329T) or (P392C) or (P392G) or (P392Q) or
 25 (P392R) or (P392V) or (P9S) or (R147C, H266G) or (R161C, G271D) or (R190L,
 F261V) or (R203C) or (R255A) or (R255F) or (R255G) or (R255I) or (R255K) or
 (R255L) or (R255M) or (R255N) or (R255P) or (R255T) or (R255W) or (R323C) or
 (R47A) or (R47C) or (R47D) or (R47E) or (R47F) or (R47G) or (R47H) or (R47H,
 A74W) or (R47I) or (R47K) or (R47L) or (R47M) or (R47N) or (R47P) or (R47Q) or
 30 (R47S) or (R47T) or (R47V) or (R47W) or (R50A) or (R50C) or (R50D) or (R50E) or
 (R50F) or (R50G) or (R50I) or (R50L) or (R50M) or (R50N) or (R50P) or (R50Q) or
 (R50S) or (R50S, A184W, L188F) or (R50S, A74I, A184W, L188F) or (R50S, A74M,
 A184Y, L188K) or (R50S, A74T, L181Y, A184D, L188K, G240R) or (R50S, A74V) or
 (R50S, M177Y, V178P, A184Y) or (R50S, M177Y, V178W, A184W) or (R50S, M177Y,
 35 V178W, A184Y) or (R50S, M177Y, V178W, A184Y, A330R) or (R50S, M177Y, V178W,
 A184Y, A74V, G677D) or (R50S, M177Y, V178W, A184Y, H266S) or (R50S, M177Y,
 V178W, A184Y, L181P) or (R50S, M177Y, V178W, A184Y, L181Y) or (R50S, M177Y,

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V178W, A184Y, L188K) or (R50S, M177Y, V178W, A184Y, L188R) or (R50S, M177Y, V178W, A184Y, T146F) or (R50S, M177Y, V178W, A184Y, V26N) or (R50S, M177Y, V178W, L181Y, A184Y) or (R50S, S72G, A74V) or (R50S, S72G, A74V, M177Y, A184Y) or (R50S, S72G, A74V, M177Y, V178P, A184Y) or (R50S, S72G, A74V, M177Y, V178W, A184Y) or (R50S, S72G, A74V, M177Y, V178W, A184Y, L188R) or (R50S, T146F, M177Y, V178W, A184Y) or (R50V) or (R50Y) or (S164N, F261L) or (S176A, A184Y, L188K) or (S176C) or (S176D) or (S176E) or (S176F) or (S176G) or (S176H) or (S176K) or (S176L) or (S176M) or (S176N) or (S176P) or (S176Q) or (S176R) or (S72C, A74C) or (S72C, A74I) or (S72C, A74L) or (S72C, A74V) or (S72C, A74Y) or (S72D, A74C) or (S72D, A74F) or (S72G) or (S72G, A74C) or (S72G, A74F) or (S72G, A74H) or (S72G, A74I) or (S72G, A74I, A184W, L188F) or (S72G, A74I, F77A, M177Y) or (S72G, A74I, F77L) or (S72G, A74I, F77L, M177Y) or (S72G, A74I, L181Y) or (S72G, A74I, L181Y, L188F) or (S72G, A74I, L181Y, L188K) or (S72G, A74I, L181Y, L188R) or (S72G, A74I, L188F) or (S72G, A74I, L188K) or (S72G, A74I, L188R) or (S72G, A74I, T146F) or (S72G, A74I, T146F, L181Y) or (S72G, A74I, T146F, L181Y, L188F) or (S72G, A74I, T146F, L181Y, L188K) or (S72G, A74I, T146F, L181Y, L188R) or (S72G, A74I, T146F, L188F) or (S72G, A74I, T146F, L188K) or (S72G, A74I, T146F, L188R) or (S72G, A74I, T146F, V178P) or (S72G, A74I, T146F, V178P, L181Y) or (S72G, A74I, T146F, V178P, L181Y, L188F) or (S72G, A74I, T146F, V178P, L181Y, L188K) or (S72G, A74I, T146F, V178P, L181Y, L188R) or (S72G, A74I, T146F, V178P, L188F) or (S72G, A74I, T146F, V178P, L188K) or (S72G, A74I, T146F, V178P, L188R) or (S72G, A74I, T146F, V178W) or (S72G, A74I, T146F, V178W, L181Y) or (S72G, A74I, T146F, V178W, L181Y, L188F) or (S72G, A74I, T146F, V178W, L181Y, L188K) or (S72G, A74I, T146F, V178W, L181Y, L188R) or (S72G, A74I, T146F, V178W, L188F) or (S72G, A74I, T146F, V178W, L188K) or (S72G, A74I, T146F, V178W, L188R) or (S72G, A74I, V178P) or (S72G, A74I, V178P, L181Y) or (S72G, A74I, V178P, L181Y, L188K) or (S72G, A74I, V178P, L181Y, L188R) or (S72G, A74I, V178P, L188F) or (S72G, A74I, V178P, L188K) or (S72G, A74I, V178P, L188R) or (S72G, A74I, V178W) or (S72G, A74I, V178W, L181Y, L188F) or (S72G, A74I, V178W, L181Y, L188K) or (S72G, A74I, V178W, L188R) or (S72G, A74L) or (S72G, A74M, A184Y, L188K) or (S72G, A74S) or (S72G, A74T) or (S72G, A74T, L181Y, A184D, L188K) or (S72G, A74T, L181Y, L188F) or (S72G, A74T, L181Y, L188K) or (S72G, A74T, L181Y, L188R) or (S72G, A74T, L188F) or (S72G, A74T, L188K) or (S72G, A74T, L188R) or (S72G, A74T, T146F) or (S72G, A74T, T146F, L181Y) or (S72G, A74T, T146F, L181Y, L188K) or (S72G, A74T, T146F, L181Y, L188R) or (S72G, A74T, T146F, L188F) or (S72G, A74T, T146F, L188K) or

(S72G, A74T, T146F, L188R) or (S72G, A74T, T146F, V178P) or (S72G, A74T, T146F, V178P, L181Y) or (S72G, A74T, T146F, V178P, L188F) or (S72G, A74T, T146F, V178P, L188K) or (S72G, A74T, T146F, V178P, L188R) or (S72G, A74T, T146F, V178W) or (S72G, A74T, T146F, V178W, L181Y) or (S72G, A74T, T146F, V178W, L181Y, L188F) or (S72G, A74T, T146F, V178W, L181Y, L188K) or (S72G, A74T, T146F, V178W, L181Y, L188R) or (S72G, A74T, T146F, V178W, L188F) or (S72G, A74T, T146F, V178W, L188K) or (S72G, A74T, T146F, V178W, L188R) or (S72G, A74T, V178P) or (S72G, A74T, V178P, L181Y) or (S72G, A74T, V178P, L181Y, L188F) or (S72G, A74T, V178P, L181Y, L188K) or (S72G, A74T, V178P, L188F) or (S72G, A74T, V178P, L188K) or (S72G, A74T, V178P, L188R) or (S72G, A74T, V178W) or (S72G, A74T, V178W, L181Y, L188F) or (S72G, A74T, V178W, L181Y, L188K) or (S72G, A74T, V178W, L188F) or (S72G, A74T, V178W, L188K) or (S72G, A74T, V178W, L188R) or (S72G, A74V) or (S72G, A74V, A184W) or (S72G, A74V, A221V) or (S72G, A74V, F77A) or (S72G, A74V, F77A, M177Y) or (S72G, A74V, F77L, M177Y) or (S72G, A74V, F77S) or (S72G, A74V, L181Y) or (S72G, A74V, L181Y, L188F) or (S72G, A74V, L181Y, L188K) or (S72G, A74V, L188F) or (S72G, A74V, L188K) or (S72G, A74V, L188R) or (S72G, A74V, T146F) or (S72G, A74V, T146F, L181Y) or (S72G, A74V, T146F, L181Y, L188F) or (S72G, A74V, T146F, L181Y, L188K) or (S72G, A74V, T146F, L181Y, L188R) or (S72G, A74V, T146F, L188F) or (S72G, A74V, T146F, L188K) or (S72G, A74V, T146F, L188R) or (S72G, A74V, T146F, V178P) or (S72G, A74V, T146F, V178P, L181Y) or (S72G, A74V, T146F, V178P, L181Y, L188F) or (S72G, A74V, T146F, V178P, L181Y, L188K) or (S72G, A74V, T146F, V178P, L181Y, L188R) or (S72G, A74V, T146F, V178P, L188F) or (S72G, A74V, T146F, V178P, L188R) or (S72G, A74V, T146F, V178W) or (S72G, A74V, T146F, V178W, L181Y) or (S72G, A74V, T146F, V178W, L181Y, L188K) or (S72G, A74V, T146F, V178W, L181Y, L188R) or (S72G, A74V, T146F, V178W, L188F) or (S72G, A74V, T146F, V178W, L188K) or (S72G, A74V, T146F, V178W, L188R) or (S72G, A74V, V178P) or (S72G, A74V, V178P, L181Y) or (S72G, A74V, V178P, L181Y, L188F) or (S72G, A74V, V178P, L181Y, L188K) or (S72G, A74V, V178P, L181Y, L188R) or (S72G, A74V, V178P, L188F) or (S72G, A74V, V178P, L188R) or (S72G, A74V, V178W) or (S72G, A74V, V178W, L181Y) or (S72G, A74V, V178W, L181Y, L188F) or (S72G, A74V, V178W, L181Y, L188K) or (S72G, A74V, V178W, L181Y, L188R) or (S72G, A74V, V178W, L188F) or (S72G, A74V, V178W, L188K) or (S72G, A74V, V178W, L188R) or (S72G, A74Y) or (S72G, L181Y) or (S72G, L181Y, L188F) or (S72G, L181Y, L188K) or (S72G, L181Y, L188R) or (S72G, L188F) or (S72G, L188K) or (S72G, L188R) or (S72G, M177Y, V178W) or (S72G, M177Y,

V178W, L188K) or (S72G, T146F) or (S72G, T146F, L181Y) or (S72G, T146F, L181Y, L188F) or (S72G, T146F, L181Y, L188K) or (S72G, T146F, L181Y, L188R) or (S72G, T146F, L188F) or (S72G, T146F, L188K) or (S72G, T146F, L188R) or (S72G, T146F, V178P) or (S72G, T146F, V178P, L181Y) or (S72G, T146F, V178P, L181Y, L188F) or
5 (S72G, T146F, V178P, L181Y, L188K) or (S72G, T146F, V178P, L181Y, L188R) or (S72G, T146F, V178P, L188F) or (S72G, T146F, V178P, L188K) or (S72G, T146F, V178P, L188R) or (S72G, T146F, V178W) or (S72G, T146F, V178W, L181Y) or (S72G, T146F, V178W, L181Y, L188F) or (S72G, T146F, V178W, L181Y, L188K) or
10 (S72G, T146F, V178W, L188F) or (S72G, T146F, V178W, L188K) or (S72G, T146F, V178W, L188R) or (S72G, V178P) or (S72G, V178P, L181Y) or (S72G, V178P, L181Y, L188F) or (S72G, V178P, L181Y, L188K) or (S72G, V178P, L181Y, L188R) or (S72G, V178P, L188F) or (S72G, V178P, L188K) or (S72G, V178P, L188R) or (S72G, V178W)
or (S72G, V178W, L181Y) or (S72G, V178W, L181Y, L188F) or (S72G, V178W, L181Y, L188K) or (S72G, V178W, L181Y, L188R) or (S72G, V178W, L188F) or (S72G, V178W, L188K) or (S72G, V178W, L188R) or (S72H, A74C) or (S72H, A74G) or
15 (S72H, A74S) or (S72H, A74Y) or (S72N, A74C) or (S72N, A74I) or (S72N, A74N) or (S72N, A74V) or (S72T) or (S72W) or (S72W, A74I, A184W, L188F) or (S72W, A74V) or (S72W, L333R) or (S72W, W90Y, V299G) or (S72Y, A74V) or (S89C) or (S89E) or (S89G) or (S89I) or (S89L) or (S89M) or (S89N) or (S89Q) or (S89R) or (S89T) or
20 (S89V) or (T146A) or (T146A, D222Y, I263M) or (T146A, F173D) or (T146A, F173V) or (T146A, F205G) or (T146A, F261C) or (T146A, F261S) or (T146A, F261V) or (T146A, G271R) or (T146A, G271S) or (T146A, G271T) or (T146A, H266T) or (T146A, H266V) or (T146A, I258T, I259S) or (T146A, I259H) or (T146A, I263F) or (T146A, I263L) or
(T146A, I263N) or (T146A, I263V) or (T146A, L150A) or (T146A, L150G) or (T146A, L150K) or (T146A, L150R, F173I) or (T146A, L272H) or (T146A, L272R) or (T146A, L272S) or (T146A, L272W) or (T146A, M212A) or (T146C) or (T146D) or (T146E) or
25 (T146F) or (T146F, L181Y) or (T146F, L181Y, L188F) or (T146F, L181Y, L188K) or (T146F, L188K) or (T146F, L188R) or (T146F, V178P) or (T146F, V178P, L181Y, L188R) or (T146F, V178P, L188K) or (T146F, V178P, L188R) or (T146F, V178W) or
(T146F, V178W, L181Y, L188R) or (T146F, V178W, L188F) or (T146F, V178W, L188K) or (T146F, V178W, L188R) or (T146G) or (T146H) or (T146L) or (T146M) or
30 (T146N) or (T146P) or (T146R) or (T146S) or (T146V) or (T146V, A191T) or (T146Y) or (T149P) or (T260A) or (T260S) or (T260W) or (T268A) or (T268S) or (T269K) or (T327A) or (T327A, A330W) or (T327C) or (T327D) or (T327E) or (T327G) or (T327I)
or (T327L) or (T327M) or (T327N) or (T327P) or (T327Q) or (T327S) or (T327V) or
35 (T365A) or (T365C) or (T365D) or (T365F) or (T365G) or (T365H) or (T365I) or (T365K) or (T365L) or (T365N) or (T365P) or (T365Q) or (T365V) or (T365W) or

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(T365Y) or (T436A) or (T436C) or (T436D) or (T436E) or (T436F) or (T436G) or
(T436H) or (T436I) or (T436K) or (T436L) or (T436M) or (T436N) or (T436P) or
(T436Q) or (T436R) or (T436S) or (T436V) or (T436W) or (T436Y) or (T438A) or
(T438C) or (T438G) or (T438I) or (T438L) or (T438S) or (T438V) or (T49A) or (T49C)
5 or (T49D) or (T49E) or (T49F) or (T49G) or (T49H) or (T49I) or (T49K) or (T49L) or
(T49L, S54N) or (T49M) or (T49N) or (T49P) or (T49Q) or (T49R) or (T49R, R50C) or
(T49R, R50S, M177Y, V178W, A184Y) or (T49S) or (T49V) or (T49W) or (T49Y) or
(T88A) or (T88C) or (T88S) or (T88V) or (V178A) or (V178D) or (V178F) or (V178H) or
(V178I) or (V178K) or (V178L) or (V178M) or (V178N) or (V178P) or (V178P, L181Y) or
10 (V178P, L181Y, L188R) or (V178P, L188F) or (V178P, L188K) or (V178P, L188R) or
(V178Q) or (V178R) or (V178S) or (V178T) or (V178W) or (V178W, A184Y) or
(V178W, L181Y, L188K) or (V178W, L181Y, L188R) or (V178W, L188R) or (V178W,
R50S) or (V178Y) or (V26A) or (V26C) or (V26E) or (V26F) or (V26G) or (V26G, R47S)
or (V26H) or (V26I) or (V26K) or (V26L) or (V26M) or (V26N) or (V26N, R50S, M177Y,
15 V178W, A184Y) or (V26Q) or (V26R) or (V26S) or (V26T) or (V26W) or (V26Y) or
(V314A) or (V314E) or (V314F) or (V314H) or (V314P) or (V314Q) or (V314S) or
(V314Y) or (V371P) or (V48A) or (V48D) or (V48E) or (V48F) or (V48G) or (V48H) or
(V48I) or (V48L) or (V48M) or (V48P) or (V48Q) or (V48R) or (V48S) or (V48T) or
(V48W) or (V48Y) or (W325A) or (W325C) or (W325D) or (W325E) or (W325F) or
20 (W325G) or (W325I) or (W325K) or (W325L) or (W325M) or (W325Q) or (W325R) or
(W325S) or (W325T) or (W325V) or (W325Y) or (W90D) or (W90Y) or (W90Y, V299G,
G570D) or (Y51A) or (Y51C) or (Y51E) or (Y51F) or (Y51G) or (Y51H) or (Y51I) or
(Y51L) or (Y51M) or (Y51N) or (Y51P) or (Y51Q) or (Y51S) or (Y51S, F77V) or (Y51T)
or (Y51V) or (Y51W) or (A225C) or (A225G) or (A225M) or (A225N) or (A225Q) or
25 (A225S) or (A225T) or (A225V) or (A225Y) or (A44G) or (A44Q) or (A44T) or (D182A)
or (D182C) or (D182E) or (D182H) or (D182I) or (D182M) or (D182N) or (D182V) or
(D182W) or (D80C) or (D80L) or (D80M) or (D80N) or (D80S) or (D80T) or (D80W) or
(E140A) or (E140D) or (E140K) or (E140L) or (E140M) or (E140T) or (E183D) or
(E183T) or (E337L) or (E337V) or (E409N) or (G114C) or (G114K) or (G114L) or
30 (G114N) or (G114W) or (G227N) or (G227R) or (G240A) or (G240C) or (G240H) or
(G240K) or (G240M) or (G240N) or (G240Q) or (G240R) or (G240S) or (G240V) or
(G46T) or (H236A) or (H236C) or (H236D) or (H236F) or (H236G) or (H236I) or
(H236K) or (H236M) or (H236P) or (H236R) or (H236S) or (H236Y) or (H92F) or
(H92Y) or (I174T) or (I174V) or (I258C) or (I258L) or (I258M) or (I258V) or (K187A) or
35 (K187G) or (K187N) or (K187R) or (K440C) or (K440M) or (K440N) or (K440T) or
(K76C) or (K76E) or (K76F) or (K76H) or (K76I) or (K76L) or (K76N) or (K76V) or
(M5C) or (M5E) or (M5P) or (M5Q) or (N186A) or (N186D) or (N186E) or (N186F) or

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(N186H) or (N186K) or (N186L) or (N186M) or (N186Q) or (N186T) or (N186V) or (N186Y) or (P172A) or (P172D) or (P172G) or (Q189E) or (Q189I) or (Q189K) or (Q189N) or (Q189V) or (Q397R) or (Q403K) or (Q403R) or (Q404F) or (Q404Y) or (Q73A) or (Q73E) or (Q73F) or (Q73L) or (Q73S) or (Q73T) or (Q73W) or (Q73Y) or (R179A) or (R179H) or (R179L) or (R179M) or (R179P) or (R179V) or (R190C) or (R190G) or (R190K) or (R190M) or (R190N) or (R190S) or (R190V) or (R190W) or (R79E) or (R79K) or (S270A) or (S270D) or (S270G) or (S53C) or (T175F) or (T175G) or (T175H) or (T175L) or (T175M) or (T175N) or (T175Q) or (T175R) or (T175Y) or (T269A) or (T269V) or (V299L) or (V299W) or (A225V, H236K, G240V, T269V) or (D80I, D182H, A225V, G240V, T269V) or (F173W, R179L, R190V, A225V) or (F173W, R179L, R190V, K440C) or (F173W, R179L, T269V, K440C) or (F173W, R190V, H236K, T269V) or (H236K, G240V, T269V, K440C) or (K76I, F173W, R179L, D182I, R190V, K440C) or (Q73W, D80I, N186V, G240V) or (R179L, A225S) or (R179L, A225V, G240V, K440C) or (R179L, R190V) or (R190V, A225V, T269V, K440C) or (T175M, H236D) or (T175M, R190V).

9 A BM3 variant according to a previous claim, wherein an amino acid sequence (insert) has been introduced between amino acid positions 458 and 477, and wherein the insert has at least 90 %, 95 %, 99 % or 100 % sequence identity with a sequence according to any of SEQ ID No. 123 to 173.

10 A Cytochrome P450 BM3 monooxygenase (BM3) variant characterized by an improved BM3 protein expression relative to BM3 wildtype (WT),

a) wherein the BM3 variant comprises mutations at at least one, two, three, four, five, six, seven, eight, nine, ten, or at all amino acid positions of (C62), (S106), (Q110), (A117), (Q128), (T152), (G157), (G227), (P243), (H285), (Q288), (A295), (N319), (G368), (V371), (K391), (N395) and (H408), and preferably

b) wherein said BM3 variant comprises at least one, two, three, four, five, six, seven, eight, nine, ten or all mutations of (C62L), (S106A), (Q110E), (A117P), (Q128E), (T152I), (G157L), (G227P), (P243E), (H285E), (Q288E), (A295D), (N319E), (G368E), (V371P), (K391L), (N395F) and (H408L).

11 The BM3 variant according to claim 10 wherein the BM3 variant comprises the mutations (S106A, Q128E) or (S106A, H285E, Q288E) or (S106A, Q128E, H285E) or (C62L, A117P, G157L, N319E) or (C62L, G227P, N319E, K391L) or (Q110E, Q128E, Q288E, N319E) or (Q128E, H285E, Q288E, N319E) or (C62L, S106A, A117P, A295D, V371P) or (C62L, S106A, A117P, G227P, P243E) or (C62L, S106A, Q128E, G227P, K391L) or

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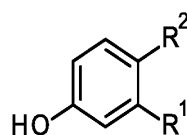
5 (S106A, Q110E, G227P, H285E, K391L) or (C62L, S106A, Q128E, G227P, Q288E, K391L) or (S106A, G227P, H285E, V371P, K391L, H408L) or (S106A, Q110E, G227P, N319E, K391L, N395F) or (C62L, S106A, Q128E, T152I, G227P, P243E, H408L) or (S106A, Q110E, Q128E, G227P, Q288E, N319E, K391L) or (C62L, S106A, Q128E, G157L, P243E, A295D, G368E, K391L) or (S106A, A117P, Q128E, T152I, G227P, N319E, V371P, N395F).

10 12 The BM3 variant according to any of claims 10 or 11 wherein the BM3 variant is a variant according to any of claims 1 to 9.

15 13 A nucleic acid encoding for a Cytochrome P450 BM3 monooxygenase variant according to a previous claim.

14 A host cell for the production of a Cytochrome P450 BM3 monooxygenase variant according to a previous claim.

20 15 Use of a Cytochrome P450 BM3 monooxygenase (BM3) variant for the production of a compound according to formula I wherein R¹ and R² form a six-membered ring as part of a steroid or steroid derivative.



(I).

25 16 Use of a BM3 variant according to claim 15, wherein said compound according to formula I is estrone or estradiol.

30 17 Use of a BM3 variant for the C19-hydroxylation of a steroid or steroid derivative, said steroid or steroid derivative comprising a 1,4-dien-3-one-A-ring or 4-en-3-one-A-ring.

18 Use of a BM3 variant for the C19-hydroxylation of a steroid according to claim 17, wherein said steroid derivative is androsta-1,4-dien-3,17-dione (ADD) or (17beta)-17-hydroxyandrosta-1,4-dien-3-one (delta1 testosterone).

- 19 Use of a BM3 variant according to any of claims 15 to 18, wherein the BM3 variant is a variant according to any of claims 1 to 8.
- 20 A process for C19-hydroxylation of a steroid derivative comprising (i)
- 5 a. culturing a recombinant Cytochrome P450 BM3 monooxygenase (BM3) variant producing microorganism in a culture medium, in the presence of an exogenous or intermediately formed substrate; or
- b. incubating a substrate-containing reaction medium with a BM3 variant;
- 10 and (ii) isolating from the medium the C19 hydroxylation product formed or a secondary product thereof;
- optionally further characterized in that said BM3 variant is a BM3 variant according to any of claims 1 to 12.
- 21 The process according to claim 20, wherein the steroid derivative comprises a 1,4-dien-3-one-A-ring or a 4-en-3-one-A-ring and a 19-methyl group.
- 15 22 A method for obtaining optimized BM3 variants for the C19 hydroxylation of steroids, said method comprising
- (i) (a) culturing a recombinant microorganism expressing a BM3 variant (test variant) in a culture medium, in the presence of an exogenous or intermediately formed steroid or steroid derivative; or
- 20 (b) incubating a steroid or steroid derivative-containing reaction medium with a BM3 variant (test variant); and
- (ii) Comparing the obtained product yield or titer and/or selectivity for the C19 hydroxylation product formed by the test variant or a secondary product thereof with the respective value obtained for a parent variant of the test variant capable of catalyzing the C19 hydroxylation of a steroid, and
- 25 (iii) Selecting the test variant as optimized for the C19 hydroxylation of steroids, if the test variant has an improved product yield or titer and/or selectivity compared to the parent variant.
- 30