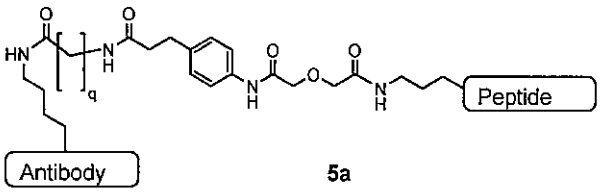
CLAIMS

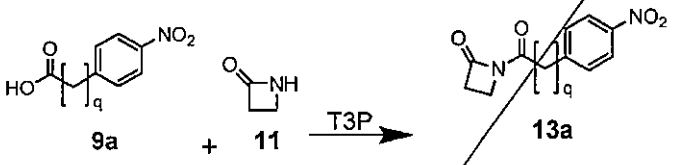
1. A process for preparing a compound according to formula **5a**



5a

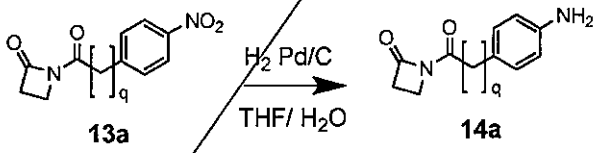
comprising

(i) reacting **9a** and **11** together in the presence of 1-propanephosphonic acid anhydride (T3P) to create compound **13a**



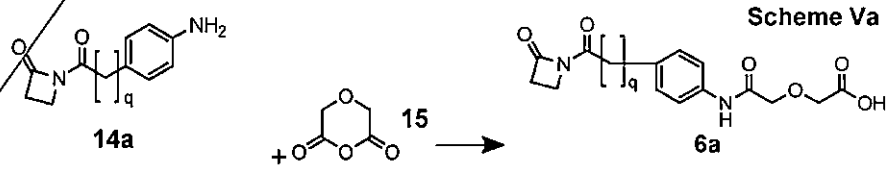
9a + **11** → **13a** **Scheme IIIa**

(ii) catalytic hydrogenation with Pd/C of compound **13a** in a THF:H_2O solution of at least about 50% THF to produce a compound of formula**14a**



13a → **14a** **Scheme IVa**

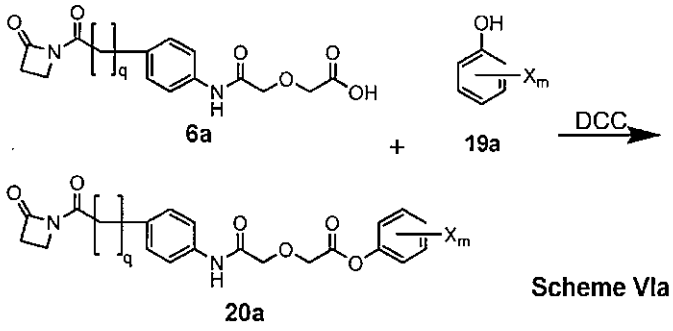
(iii) combining a solution of **14a** in THF with a compound according to formula **15** in a reaction substantially free of base to produce compound **6a**



Scheme Va

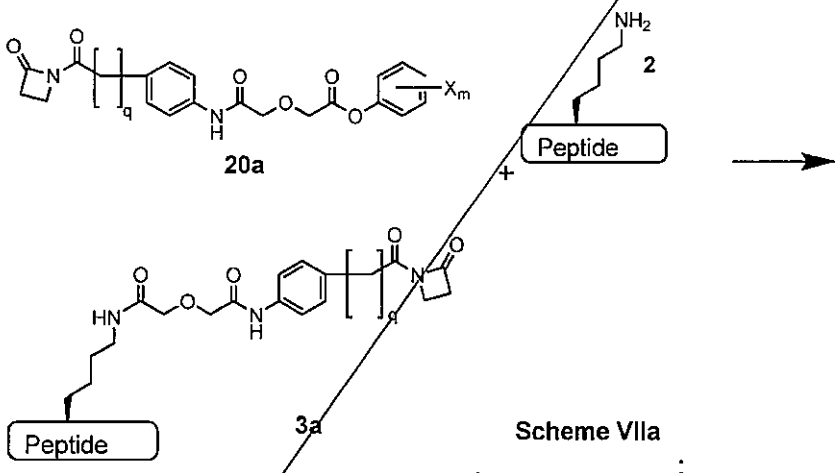
14a + **15** → **6a**

(iv) reacting a compound according to the formula **6a** with a compound according to formula **19a** in THF in the presence of DCC to produce compound **20a**



Scheme VIa

(v) combining **20a** with a ε-amino containing peptide **2** dissolved in an aprotic polar [15th] solvent to produce compound **3a**



Scheme VIIa

;

(vi) dissolving compound **3a** in DMSO;

(vii) adding histidine buffer at pH between about 5.5 to about 7.5 to the solution of DMSO and **3a** of step (vi);

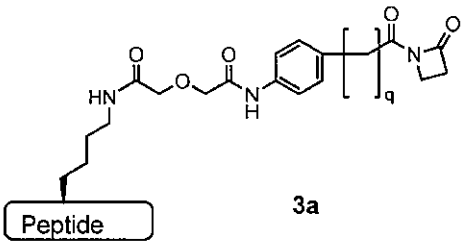
(viii) Adding an antibody comprising a variable light region comprising SEQ ID NO:5 and a variable heavy region comprising SEQ ID NO:6 to the solution of step (vii), so as to have a peptide:antibody molar ratio of between about 1.8:1 to about 3:1;

(ix) Agitating the mixture formed in step (viii) at a medium speed so as to avoid foaming the reaction mixture for at least about 1hr at between about pH 5.5 and about pH7.5 and at a temperature of between about 5°C and 35°C;

(x) Filtration of the solution from (ix) to extract the resultant peptide-linker antibody conjugate **5a**.

wherein q = 1, 2, 3, 4, or 5, X= F or Cl, and m=3, 4, or 5.

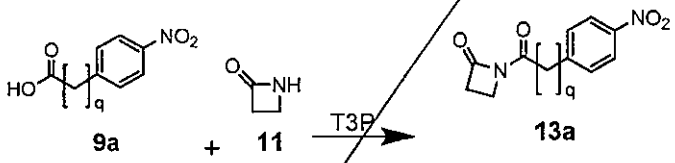
2. A process for preparing a compound according to Formula **3a**



3a

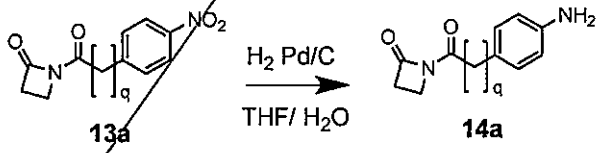
comprising

(i) reacting **9a** and **11** together in the presence of 1-propanephosphonic acid anhydride (T3P) to create compound **13a**



9a + **11** → **13a** **Scheme IIIa**

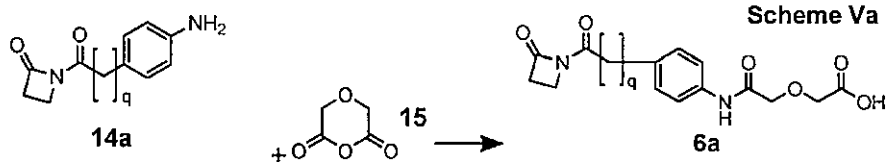
(ii) catalytic hydrogenation with Pd/C of compound **13a** in a THF:H_2O solution of at least about 50% THF to produce a compound of formula**14a**



13a **14a** **Scheme IVa**

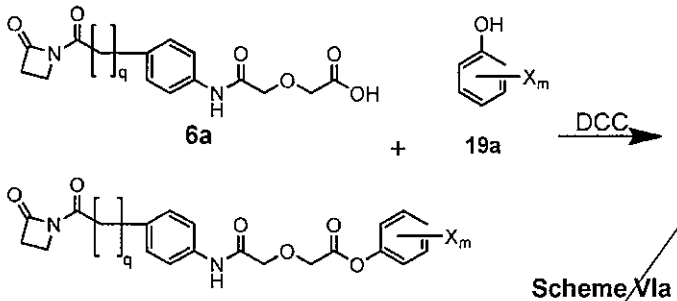
(iii) combining a solution of **14a** in THF with a compound according to formula **15** in a reaction substantially free of base to produce compound **6a**

Scheme Va



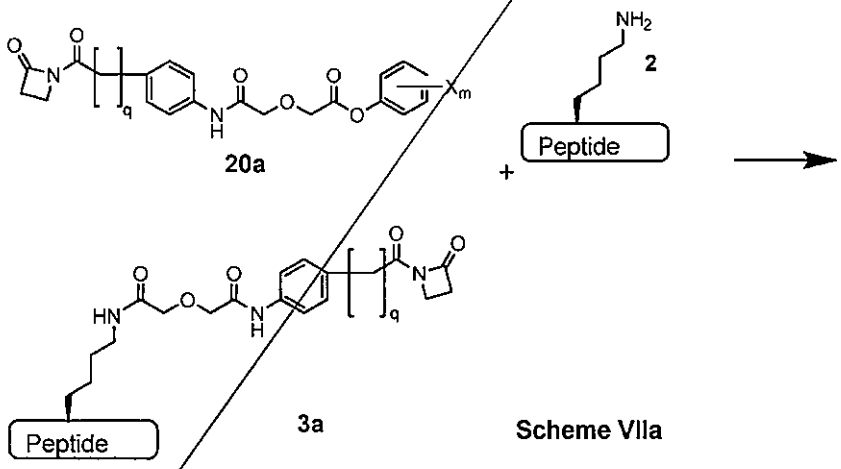
14a + **15** → **6a**

(iv) reacting a compound according to the formula **6a** with a compound according to formula **19a** in THF in the presence of DCC to produce compound **20a**



Scheme VIa

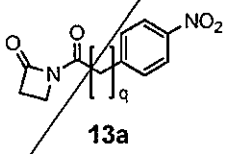
(v) combining **20a** with a ε-amino containing peptide **2** dissolved in an aprotic polar [15th] solvent to produce bioconjugate **3b**



Scheme VIIa

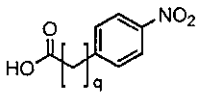
wherein q = 1, 2, 3, 4, or 5, X= F or Cl, and m=3, 4, or 5.

3. A process for preparing a compound of formula **13a**



13a

wherein q is 1, 2, 3, 4, or 5, characterized in that



9a



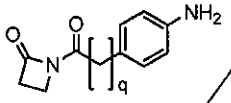
and **11**

are reacted together in the presence of 1-propanephosphonic acid anhydride (T3P).

4. The process as claimed in any one of claims 1-3, wherein the reaction between **9a** and **11** is carried out in the presence of a 1st solvent selected from the group consisting of of tetrahydrofuran (THF), 2-methyltetrahydrofuran, 1-methyl-2-pyrrolidinone (NMP), N,N-dimethylformamide (DMF), N,N-dimethylacetamide (DMAc), 1-methyl-2-pyrrolidinone, ethyl acetate (EtOAc), and acetonitrile (MeCN), and preferably MeCN.

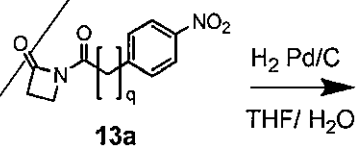
5. The process as claimed in claim 4, wherein the reaction is carried out in the presence of a 1st base selected from the group consisting of trimethylamine, triethylamine, tributylamine, DIPEA, pyridine, DBU, DABCO, 2,3-lutidine, 2,4-lutidine, 2,5-lutidine, 2,6-lutidine, 3,4-lutidine, 3,5-lutidine, and preferably DIPEA.

6. A process for preparing a compound of formula**14a**,



14a

wherein q is 1, 2, 3, 4, or 5, comprising catalytic hydrogenation with Pd/C of compound **13a**,



13a

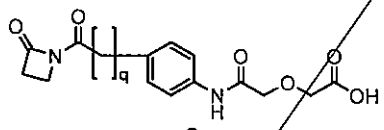
and characterized in that the reaction is conducted in a THF:H_2O solution of at least about 50% THF.

7. The process as claimed in any one of claims 1, 2 or 6, wherein the THF:H_2O solution comprises THF in an amount selected from the group consisting of at least about 60%, at least about 70%, at least about 80%, and at least about 90%.

8. The process as claimed in any one of claims 1, 2, 6 or 7, wherein prior to hydrogenation in THF/H_2O, compound **13a** is subjected to treatment with activated charcoal.

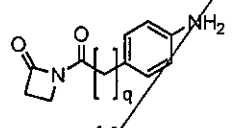
9. The process as claimed in claim 8, wherein the activated charcoal is selected from the group consisting of SX-Plus, Darco® S-51HF, E Supra USP, SX-Ultra, CASP, Darco® G-60 and CGSP.

10. A process for preparing a compound of formula **6a**



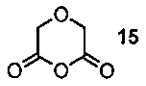
6a

wherein q =1, 2, 3, 4 or 5; comprising reacting a solution of compound **14a**



14a

in a 6th solvent comprising THF,

with compound **15** 

15

and characterized in that the reaction is carried out substantially free of a base.

11. The process as claimed in any one of claims 1, 2 or 10, wherein the compound **14a** is reacted with between about 1 and about 10 equivalants of compound **15**.

12. The process as claimed in any one of claims 1, 2, 10, or 11, wherein the solution of compound **6a** is distilled under vacuum, following which a 7th solvent comprising C_1-C_4 alkyl acetate is added to attain a solvent composition of between about 25%THF/75% C_1-C_4 alkyl acetate and about 100% C_1-C_4 alkyl acetate.

13. The process as claimed in claim 12, wherein the 7th solvent is selected from the group consisting of methyl acetate, ethyl acetate, *i*-propyl acetate, n-propyl acetate, n-butyl acetate, and preferably is *i*-propyl acetate.

14. The process as claimed in claim 12 or 13, wherein the ratio between 6th and 7th solvents is between 25:75 and 0:100.

15. A process for crystallization of a compound according to the formula **6a**, comprising

(i) dissolving acid **6a** in an 8th solvent comprising THF;

(ii) optionally treating with activated carbon and then filtering off said activated carbon;

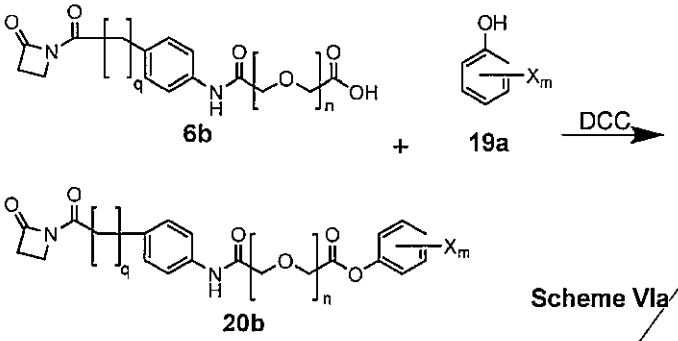
(iii) concentrating the acid **6a** in THF solution to between about 2 and about 20 vol;

(iv) adding between about 1 and about 50 vol of a 9th solvent consisting of 2-propanol;

(v) concentrating the solution of acid **6a** in THF and 2-propanol to between about 2 and about 50 vol;

(vi) cooling the concentrated solution of acid **6** to between about -25°C and about 10°C.

16. A process for preparing a compound according to the formula **20b** comprising reacting a compound according to the formula **6b** with a compound according to formula **19a** in a 10th solvent in the presence of DCC



Scheme VIa

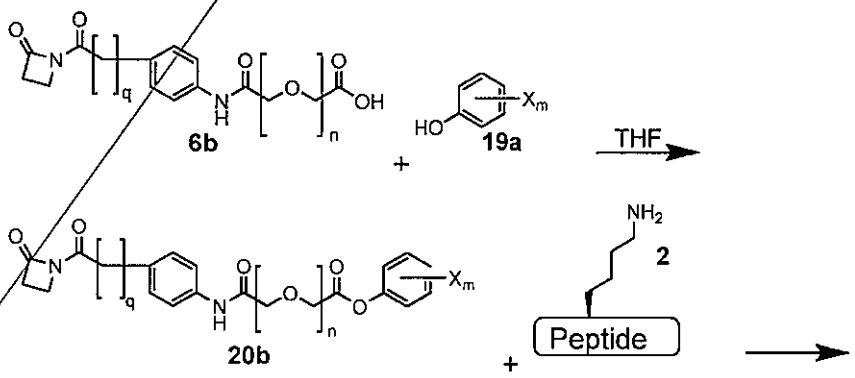
wherein q = 1, 2, 3, 4, or 5, n = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30, X = F or Cl, and m = 3, 4, or 5.

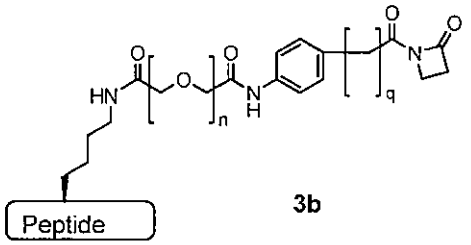
17. A process for conjugating an ε-amino containing peptide **2** to a linker of the formula **6b** comprising:

(i) dissolving peptide **2** in an aprotic, polar 15th solvent,

(ii) reacting **6b** with between about 1 and about 10 equivalents of **19a** in THF to produce compound **20b**;

(iii) combining **20b** with peptide **2** to produce bioconjugate **3b**





3b **Scheme XIIb**

wherein n = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30, q = 1, 2, 3, 4, or 5, X= F or Cl, and m=3, 4, or 5.

18. The process as claimed in any one of claims 1, 2, 16, or 17, wherein at least about 1 equivalant of compound **19a** is added to compound **6a** or **6b**.

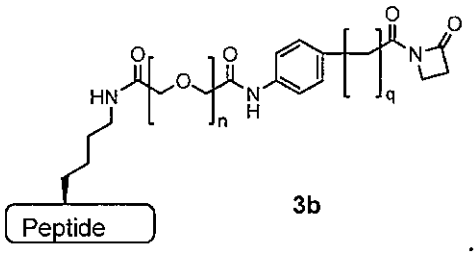
19. The process as claimed in any one of claims 1, 2, 16, 17, or 18, wherein the DCC is added to the reaction at a temperature of between -10°C and +10°C, and preferably between about +3°C and +5°C.

20. The process as claimed in any one of claims 1, 2, 16, 17, 18, or 19, wherein the reaction between compound **19a** and either **6a** or **6b** takes place in a 10th solvent selected from the group consisting of THF, dichloromethane, 2-methyltetrahydrofuran, N,N-dimethylformamide, and N,N-dimethylacetamide, and preferably THF.

21. The process as claimed in any one of claims 1, 2, and 16-20, wherein X is F, and m is 5.

22. A process for conjugating a peptide of formula **3b** to an antibody comprising the steps:

(i) dissolving compound **3b** in DMSO:



3b

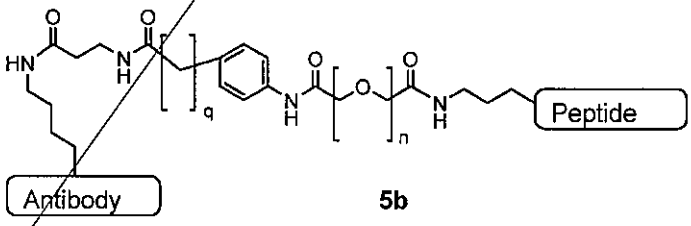
;

(ii) adding histidine buffer at pH between about 5.5 to about 7.5 to the solution of DMSO and **3b** of step (i);

(iii) Adding an antibody comprising comprises a variable light region comprising SEQ ID NO:5 and a variable heavy region comprising SEQ ID NO:6 to the solution of step (ii), wherein the antibody is in a solution of about 10 mM histidine and about 10 mM glycine buffer containing about 2 % (w/w) sucrose so as to have a peptide:antibody molar ratio of between about 1.8:1 to about 3:1;

(iv) Agitating the mixture formed in step (vi) at a medium speed so as to avoid foaming the reaction mixture for at least about 1hr at between about pH 5.5 and about pH7.5 and at a temperature of between about 5°C and 35°C;

(v) Filtration of the solution from (iv) to extract the resultant peptide-linker antibody conjugate **5b**,



5b

wherein n = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30, q = 1, 2, 3, 4, or 5.

23. The process as claimed in any one of claims claim 1or 22, wherein compound **3a** or compound **3b** is dissolved in DMSO at between about 5 and about 100 mg/ml, and preferably at between about 10 and about 15 mg/ml.

24. The process as claimed in any one of claims 1, 22, or 23, wherein the histidine buffer is added to the DMSO solution of compound **3a** or **3b** at an equal volume, plus or minus 10%.

25. The process as claimed in any one of claims 1, 22-24, wherein the concentration of compound **3a** or compound **3b** following addition of the histidine buffer is between about 2 and about 10mg/ml, and is preferably about 6mg/ml.

26. The process as claimed in any one of claims 1, or 22-25, whererin the antibody comprises a light chain constant region comprising a sequence selected from the group consisting of SEQ ID NOs:9, 10, 11, and 12, or variants thereof comprising between 1 and 5 amino acid substitutions, deletions or insertions, and a heavy chain constant region comprising a sequence selected from the group consisting of SEQ ID NOs:13, and 14, or variants thereof comprising between 1 and 5 amino acid substitutions, deletions or insertions.

27. The process as claimed in claim 26, whererin the antibody comprises a light chain comprising SEQ ID NO:3, or variants thereof comprising between 1 and 5 amino acid substitutions, deletions or insertions, and a heavy chain comprising SEQ ID NO:4, or variants thereof comprising between 1 and 5 amino acid substitutions, deletions or insertions.

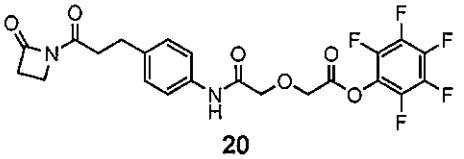
28. The process as claimed in any one of the preceding claims, wherein q=2, n when present =1, m when present = 5, and x when present =F.

29. A compound produced according to a process as claimed in any one of claims 1-28.

30. Use of a process as claimed in any one of claims 1-28, in the production of a compound selected from the group consisting of compound **13**, compound **13a**, compound **14**, compound **14a**, compound **6**, compound **6a**,

compound **6b**, compound **20**, compound **20a**, compound **20b**, compound **20c**, compound **20d**, compound **20e**, compound **3**, compound **3a**, compound **3b**, compound **5**, compound **5a**, and compound **5b**.

31. A compound comprising a structure selected from the group consisting of:



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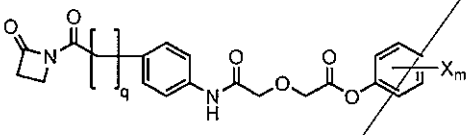

20a

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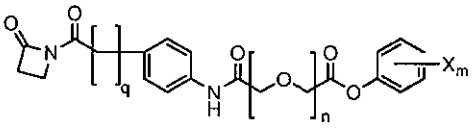

20b

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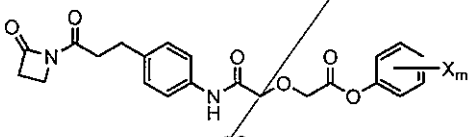

20c

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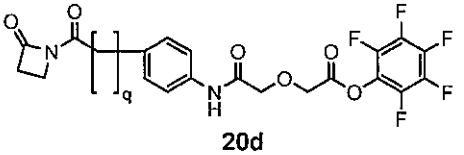

20d

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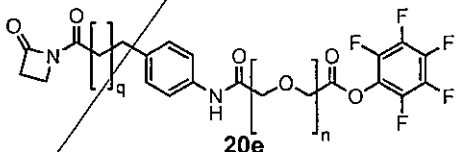


20e

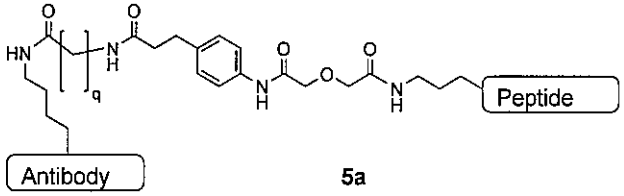
, and

,

wherein q is 1, 2, 3, 4, or 5, n = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, and X is any halogen, and m= 3, 4 or 5

CLAIMS

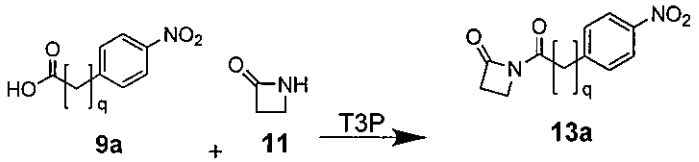
1. A process for preparing a compound according to formula **5a**



5a

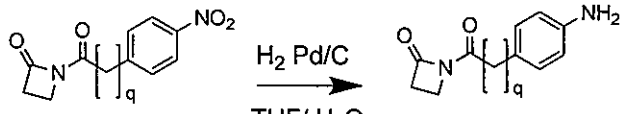
comprising

(i) reacting **9a** and **11** together in the presence of 1-propanephosphonic acid anhydride (T3P) to create compound **13a**



9a + **11** → **13a** **Scheme IIIa**

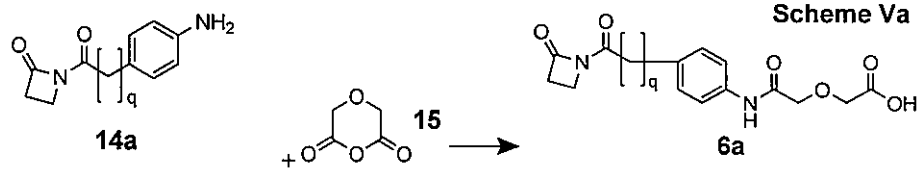
(ii) catalytic hydrogenation with Pd/C of compound **13a** in a THF:H_2O solution of at least about 50% THF to produce a compound of formula **14a**



13a **14a** **Scheme IVa**

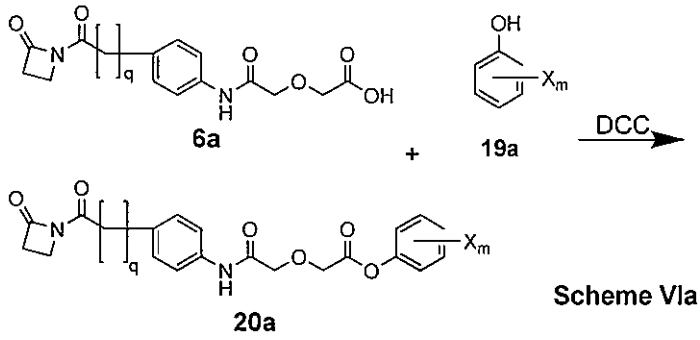
(iii) combining a solution of **14a** in THF with a compound according to formula **15** in a reaction substantially free of base to produce compound **6a**

Scheme Va



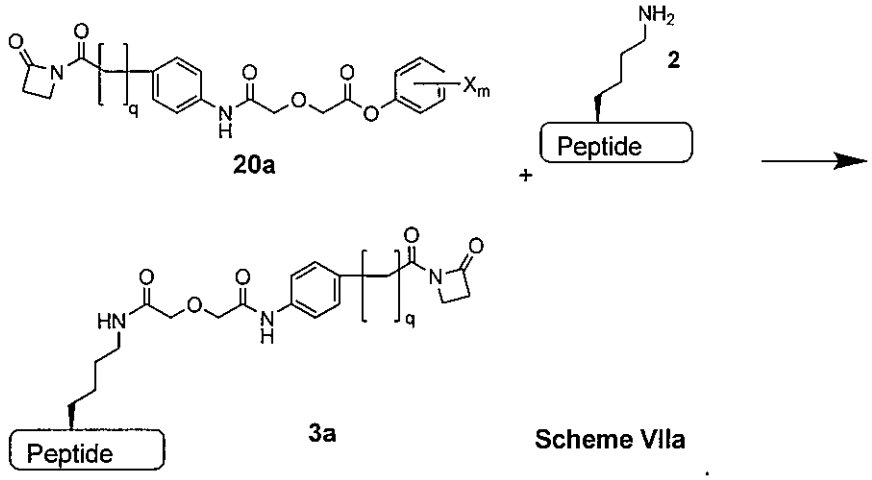
14a **15** → **6a**

(iv) reacting a compound according to the formula **6a** with a compound according to formula **19a** in THF in the presence of DCC to produce compound **20a**



Scheme VIa

(v) combining **20a** with a ϵ-amino containing peptide **2** dissolved in an aprotic polar [15th] solvent to produce compound **3a**



Scheme VIIa

,

;

(vi) dissolving compound **3a** in DMSO;

(vii) adding histidine buffer at pH between about 5.5 to about 7.5 to the solution of DMSO and **3a** of step (vi);

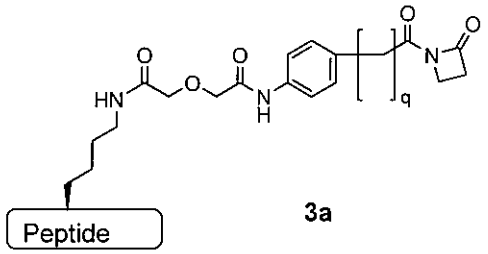
(viii) Adding an antibody comprising a variable light region comprising SEQ ID NO:5 and a variable heavy region comprising SEQ ID NO:6 to the solution of step (vii), so as to have a peptide:antibody molar ratio of between about 1.8:1 to about 3:1;

(ix) Agitating the mixture formed in step (viii) at a medium speed so as to avoid foaming the reaction mixture for at least about 1hr at between about pH 5.5 and about pH7.5 and at a temperature of between about 5°C and 35°C; and

(x) Filtration of the solution from (ix) to extract the resultant peptide-linker antibody conjugate **5a**

wherein q = 1, 2, 3, 4, or 5, X= F or Cl, and m=3, 4, or 5.

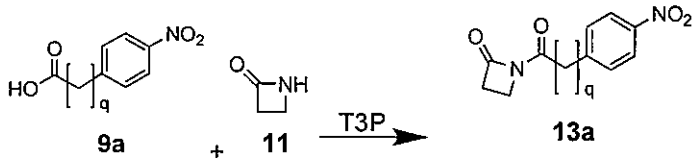
2. The process as claimed in claim 1, further comprising a process for preparing a compound according to Formula **3a**



3a

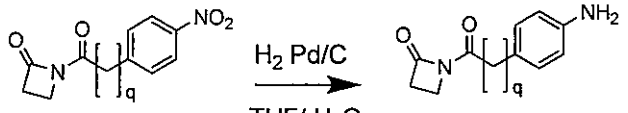
comprising

(i) reacting **9a** and **11** together in the presence of 1-propanephosphonic acid anhydride (T3P) to create compound **13a**



9a + **11** → **13a** **Scheme IIIa**

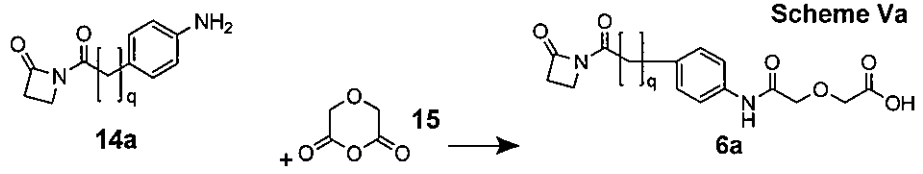
(ii) catalytic hydrogenation with Pd/C of compound **13a** in a THF:H_2O solution of at least about 50% THF to produce a compound of formula **14a**



13a **14a** **Scheme IVa**

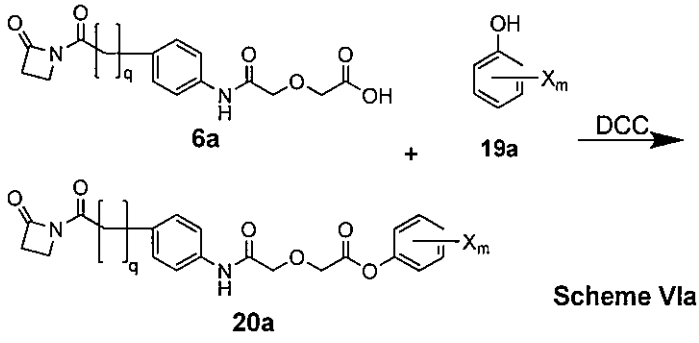
(iii) combining a solution of **14a** in THF with a compound according to formula **15** in a reaction substantially free of base to produce compound **6a**

Scheme Va



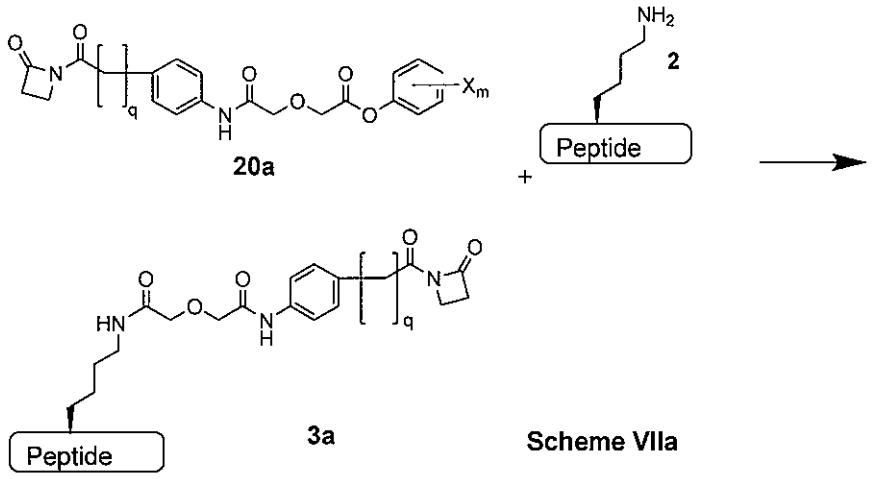
14a + **15** → **6a**

(iv) reacting a compound according to the formula **6a** with a compound according to formula **19a** in THF in the presence of DCC to produce compound **20a**



6a + **19a** → **20a** **Scheme VIa**

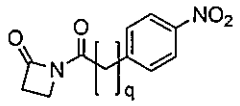
(v) combining **20a** with a ϵ-amino containing peptide **2** dissolved in an aprotic polar [15th] solvent to produce bioconjugate **3b**



Scheme VIIa

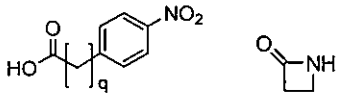
wherein q = 1, 2, 3, 4, or 5, X= F or Cl, and m=3, 4, or 5.

3. The process as claimed in claim 1, further comprising a process for preparing a compound of formula **13a**



13a

wherein q is 1, 2, 3, 4, or 5, characterized in that



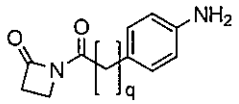
9a and **11**

are reacted together in the presence of 1-propanephosphonic acid anhydride (T3P).

4. The process as claimed claims 1, wherein the reaction between **9a** and **11** is carried out in the presence of a 1st solvent selected from the group consisting of of tetrahydrofuran (THF), 2-methyltetrahydrofuran, 1-methyl-2-pyrrolidinone (NMP), N,N-dimethylformamide (DMF), N,N-dimethylacetamide (DMAc), 1-methyl-2-pyrrolidinone, ethyl acetate (EtOAc), and acetonitrile (MeCN), and preferably MeCN.

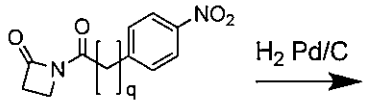
5. The process as claimed in claim 4, wherein the reaction is carried out in the presence of a 1st base selected from the group consisting of trimethylamine, triethylamine, tributylamine, DIPEA, pyridine, DBU, DABCO, 2,3-lutidine, 2,4-lutidine, 2,5-lutidine, 2,6-lutidine, 3,4-lutidine, 3,5-lutidine, and preferably DIPEA.

6. The process as claimed in claim 1, further comprising a_process for preparing a compound of formula **4a**,



14a

wherein q is 1, 2, 3, 4, or 5, comprising catalytic hydrogenation with Pd/C of compound **13a**,



13a

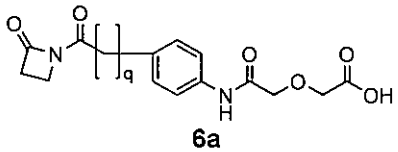
and characterized in that the reaction is conducted in a THF:H_2O solution of at least about 50% THF.

7. The process as claimed in claim 1, wherein the THF:H_2O solution comprises THF in an amount selected from the group consisting of at least about 60%, at least about 70%, at least about 80%, and at least about 90%.

8. The process as claimed in claim 1, wherein prior to hydrogenation in THF/H_2O, compound **13a** is subjected to treatment with activated charcoal.

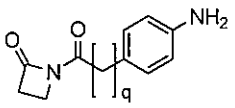
9. (Original) The process as claimed in claim 8, wherein the activated charcoal is selected from the group consisting of SX-Plus, Darco® S-51HF, E Supra USP, SX-Ultra, CASP, Darco® G-60 and CGSP.

10. The process as claimed in claim 1, further comprising a process for preparing a compound of formula **6a**



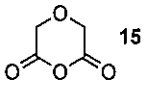
6a

wherein q =1, 2, 3, 4 or 5; comprising reacting a solution of compound **14a**



14a

in a 6th solvent comprising THF,

with compound **15** 

15

and characterized in that the reaction is carried out substantially free of a base.

11. The process as claimed in claim 1, wherein the compound **14a** is reacted with between about 1 and about 10 equivalents of compound **15**.

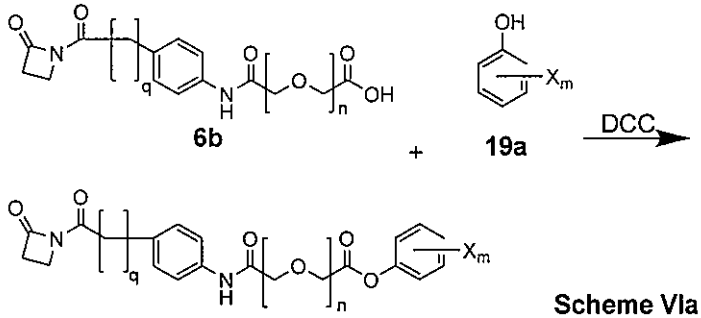
12. The process as claimed in claim 1, wherein the solution of compound **6a** is distilled under vacuum, following which a 7th solvent comprising C_1-C_4 alkyl acetate is added to attain a solvent composition of between about 25%THF/75% C_1-C_4 alkyl acetate and about 100% C_1-C_4 alkyl acetate.

13. The process as claimed in claim 12, wherein the 7th solvent is selected from the group consisting of methyl acetate, ethyl acetate, *i*-propyl acetate, n-propyl acetate, n-butyl acetate, and preferably is *i*-propyl acetate.

14. The process as claimed in claim 12, wherein the ratio between 6th and 7th solvents is between 25:75 and 0:100.

15. The process as claimed in claim 1, further comprising a process for crystallization of a compound according to the formula **6a**, comprising
    (i) dissolving acid **6a** in an 8th solvent comprising THF;
    (ii) optionally treating with activated carbon and then filtering off said activated carbon;
    (iii) concentrating the acid **6a** in THF solution to between about 2 and about 20 vol;
    (iv) adding between about 1 and about 50 vol of a 9th solvent consisting of 2-propanol;
    (v) concentrating the solution of acid **6a** in THF and 2-propanol to between about 2 and about 50 vol;
    (vi) cooling the concentrated solution of acid **6** to between about -25°C and about 10°C.

16. The process as claimed in claim 1, further comprising a process for preparing a compound according to the formula **20b** comprising reacting a compound according to the formula **6b** with a compound according to formula **19a** in a 10th solvent in the presence of DCC



Scheme VIa

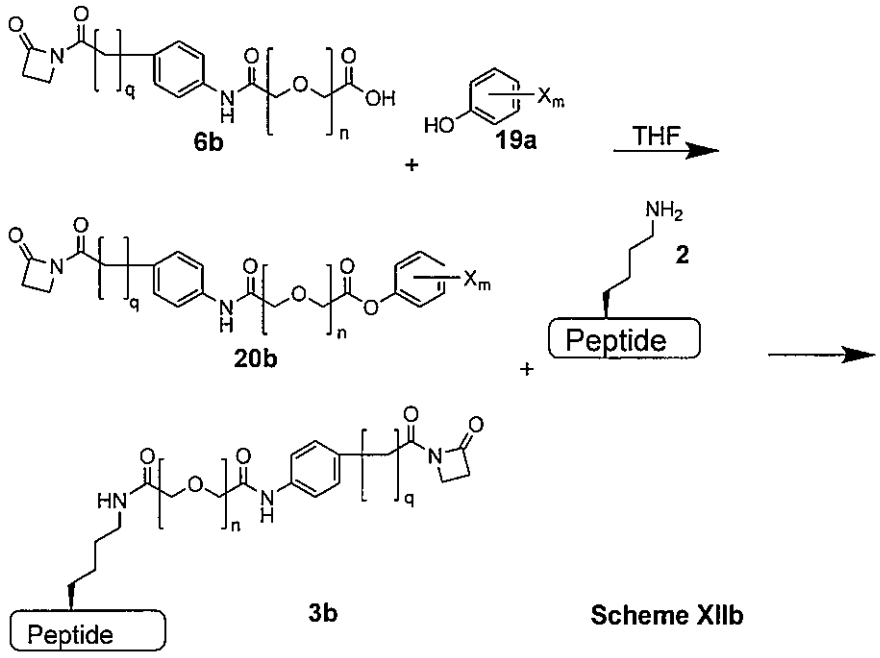
wherein q = 1, 2, 3, 4, or 5, n = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30, X = F or Cl, and m = 3, 4, or 5.

17. The process of claim 1, further comprising a process for conjugating an ϵ-amino containing peptide **2** to a linker of the formula **6b** comprising:

(i) dissolving peptide **2** in an aprotic, polar 15th solvent,

(ii) reacting **6b** with between about 1 and about 10 equivalents of **19a** in THF to produce compound **20b**;

(iii) combining **20b** with peptide **2** to produce bioconjugate **3b**



Scheme XIIb

wherein n = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30, q = 1, 2, 3, 4, or 5, X= F or Cl, and m=3, 4, or 5.

18. The process as claimed in claim 1, wherein at least about 1 equivalant of compound **19a** is added to compound **6a** or **6b**.

19. The process as claimed in claim 1, wherein the DCC is added to the reaction at a temperature of between -10°C and +10°C, and preferably between about +3°C and +5°C.

20. The process as claimed in claims 1, wherein the reaction between compound **19a** and either **6a** or **6b** takes place in a 10th solvent selected from the group consisting of THF, dichloromethane, 2-methyltetrahydrofuran, N,N-dimethylformamide, and N,N-dimethylacetamide, and preferably THF.

21. The process as claimed in claim 1, wherein X is F, and m is 5.

22. The process as claimed in claim 1 wherein compound **3a** or compound **3b** is dissolved in DMSO at between about 5 and about 100 mg/ml, and preferably at between about 10 and about 15 mg/ml.

23. The process as claimed in claim 1, wherein the histidine buffer is added to the DMSO solution of compound **3a** or **3b** at an equal volume, plus or minus 10%.

24. The process as claimed in claim 1, wherein the concentration of compound **3a** or compound **3b** following addition of the histidine buffer is between about 2 and about 10mg/ml, and is preferably about 6mg/ml.

25. The process as claimed in claim1, wherein the antibody comprises a light chain constant region comprising a sequence selected from the group consisting of SEQ ID NOs:9, 10, 11, and 12, or variants thereof comprising between 1 and 5 amino acid substitutions, deletions or insertions, and a heavy chain constant region comprising a sequence selected from the group consisting of SEQ ID NOs:13, and 14, or variants thereof comprising between 1 and 5 amino acid substitutions, deletions or insertions.

26. The process as claimed in claim 25, whererin the antibody comprises a light chain comprising SEQ ID NO:3, or variants thereof comprising between 1 and 5 amino acid substitutions, deletions or insertions, and a heavy chain comprising SEQ ID NO:4, or variants thereof comprising between 1 and 5 amino acid substitutions, deletions or insertions.

27. The process as claimed in claimed 1, wherein q=2, n when present =1, m when present = 5, and x when present =F.