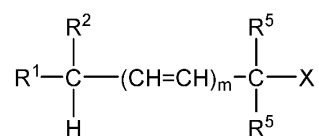


CLAIMS

1. A method to sterilize a hydrogel linked with a cross-linker that degrades by beta elimination, which method comprises adjusting the pH of a medium containing said hydrogel so as to minimize degradation of said cross-linker and heating to sufficient temperature and time to effect sterilization.
2. The method of claim 1 wherein the pH of the medium is determined by non-reactive buffering of said medium.
3. The method of claim 2 wherein said buffer is phosphate or acetate buffer.
4. The method of claim 1 wherein the adjusted pH is 2-5.
5. The method of claim 1 wherein the rate of degradation by beta elimination is controlled by one or more substituents contained in the cross-linker.
6. The method of claim 5 wherein hydrogel comprises a cross-linker of the formula:



wherein

m is 0 or 1;

X comprises a functional group connecting the crosslinker to a first polymer;

at least one of R<sup>1</sup>, R<sup>2</sup>, and R<sup>5</sup> comprises a functional group Z connecting the crosslinker to a second polymer;

wherein one and only one of R<sup>1</sup> and R<sup>2</sup> may be H or may be alkyl, arylalkyl or heteroarylalkyl, each optionally substituted;

at least one or both R<sup>1</sup> and R<sup>2</sup> is independently CN; NO<sub>2</sub>;

optionally substituted aryl;

optionally substituted heteroaryl;

optionally substituted alkenyl;

optionally substituted alkynyl;

COR<sup>3</sup> or SOR<sup>3</sup> or SO<sub>2</sub>R<sup>3</sup> wherein

R<sup>3</sup> is H or optionally substituted alkyl;

aryl or arylalkyl, each optionally substituted;

heteroaryl or heteroarylalkyl, each optionally substituted; or

OR<sup>9</sup> or NR<sup>9</sup><sub>2</sub> wherein each R is independently H or optionally substituted alkyl, or both R<sup>9</sup> groups taken together with the nitrogen to which they are attached form a heterocyclic ring;

SR<sup>4</sup> wherein

R<sup>4</sup> is optionally substituted alkyl;

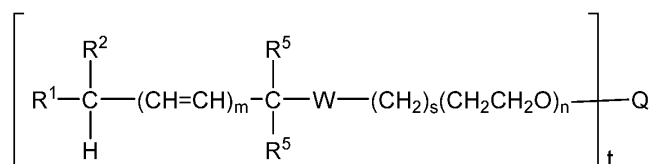
aryl or arylalkyl, each optionally substituted; or

heteroaryl or heteroarylalkyl, each optionally substituted;

wherein R<sup>1</sup> and R<sup>2</sup> may be joined to form a 3-8 membered ring; and

each R<sup>5</sup> is independently H or is alkyl, alkenylalkyl, alkynylalkyl, (OCH<sub>2</sub>CH<sub>2</sub>)<sub>p</sub> O-alkyl wherein p=1-1000, aryl, arylalkyl, heteroaryl or heteroarylalkyl, each optionally substituted;

or



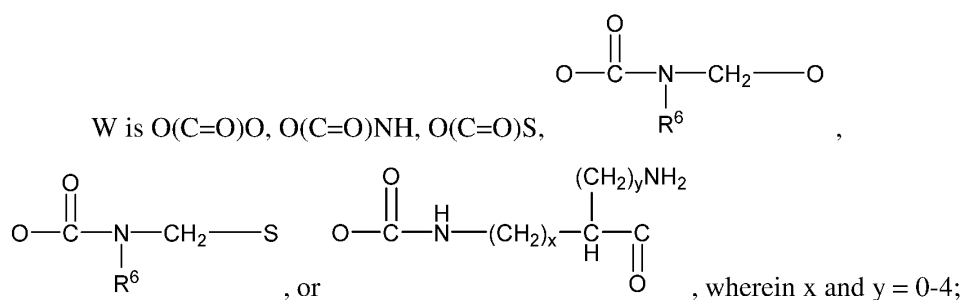
wherein

m is 0 or 1;

n is 1-1000;

s is 0-2;

t is 2,4, 8, 16 or 32;



Q is a core group having a valency=t;

wherein at least one of R<sup>1</sup>, R<sup>2</sup>, and R<sup>5</sup> comprises a functional group Z<sup>1</sup> capable of connecting to a polymer, and

at least one or both R<sup>1</sup> and R<sup>2</sup> is independently CN; NO<sub>2</sub>;

optionally substituted aryl;  
 optionally substituted heteroaryl;  
 optionally substituted alkenyl;  
 optionally substituted alkynyl;  
 $\text{COR}^3$  or  $\text{SOR}^3$  or  $\text{SO}_2\text{R}^3$  wherein

$\text{R}^3$  is H or optionally substituted alkyl;  
 aryl or arylalkyl, each optionally substituted;  
 heteroaryl or heteroarylalkyl, each optionally substituted; or  
 $\text{OR}^9$  or  $\text{NR}^9_2$  wherein each R is independently H or optionally

substituted alkyl, or both  $\text{R}^9$  groups taken together with the nitrogen to which they are attached form a heterocyclic ring;

$\text{SR}^4$  wherein

$\text{R}^4$  is optionally substituted alkyl;  
 aryl or arylalkyl, each optionally substituted; or  
 heteroaryl or heteroarylalkyl, each optionally substituted;

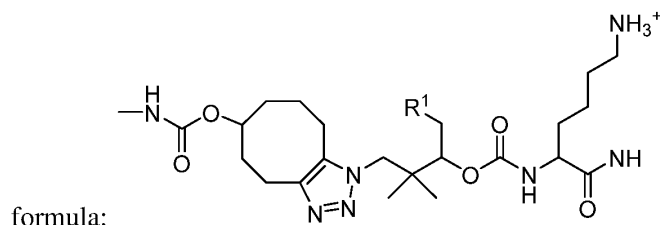
wherein  $\text{R}^1$  and  $\text{R}^2$  may be joined to form a 3-8 membered ring; and

wherein one and only one of  $\text{R}^1$  and  $\text{R}^2$  may be H or may be alkyl, arylalkyl or heteroarylalkyl, each optionally substituted; and

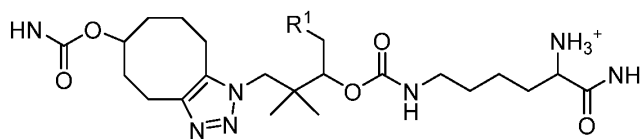
each  $\text{R}^5$  is independently H or is alkyl, alkenylalkyl, alkynylalkyl,  $(\text{OCH}_2\text{CH}_2)_p$  O-alkyl wherein  $p=1-1000$ , aryl, arylalkyl, heteroaryl or heteroarylalkyl, each optionally substituted.

7. The method of Claim 5 wherein X is a carbamate and Z is a triazole, carboxamide, or carbamate.

8. The method of Claim 5 wherein the hydrogel comprises a cross-linker of the



or



9. The method of Claim 8, wherein  $R^1$  is CN;  $NO_2$ ;  
 optionally substituted aryl;  
 optionally substituted heteroaryl;  
 optionally substituted alkenyl;  
 optionally substituted alkynyl;  
 $COR^3$  or  $SOR^3$  or  $SO_2R^3$  wherein  
 $R^3$  is H or optionally substituted alkyl;  
 aryl or arylalkyl, each optionally substituted;  
 heteroaryl or heteroarylalkyl, each optionally substituted; or  
 $OR^9$  or  $NR^9_2$  wherein each R is independently H or optionally  
 substituted alkyl, or both  $R^9$  groups taken together with the nitrogen to which they are  
 attached form a heterocyclic ring;  
 $SR^4$  wherein  
 $R^4$  is optionally substituted alkyl;  
 aryl or arylalkyl, each optionally substituted; or  
 heteroaryl or heteroarylalkyl, each optionally substituted.
10. The method of Claim 8 wherein  $R^1$  is CN or  $SO_2R^3$  wherein  
 $R^3$  is H or optionally substituted alkyl;  
 aryl or arylalkyl, each optionally substituted;  
 heteroaryl or heteroarylalkyl, each optionally substituted; or  
 $OR^9$  or  $NR^9_2$  wherein each R is independently H or optionally substituted alkyl, or  
 both  $R^9$  groups taken together with the nitrogen to which they are attached form a  
 heterocyclic ring.

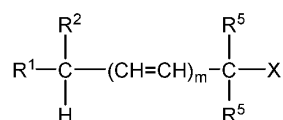
11. The method of Claim 8 wherein  $R^1$  is CN;  $SO_2Me$ ;  $SO_2NMe_2$ ;  $SO_2N(CH_2CH_2)_2X$  or  $SO_2(Ph-R^{10})$ , wherein X is absent, O, or  $CH-R^{10}$  and  $R^{10}$  is H, alkyl, alkoxy,  $NO_2$ , or halogen.

12. The method of Claim 1, wherein sterilization is achieved by heating to 121 °C with a hold time of 20 minutes.

13. A hydrogel comprising a cross-linker degradable by a beta-elimination mechanism that has been sterilized by heating to a sufficient temperature so as to effect sterilization.

14. The hydrogel of Claim 13 that is a suspension of microparticles.

15. The hydrogel of Claim 13 wherein the cross-linker degradable by a beta-elimination mechanism has the formula



wherein

m is 0 or 1;

X comprises a functional group connecting the crosslinker to a first polymer;

wherein at least one of  $R^1$ ,  $R^2$ , and  $R^5$  comprises a functional group Z connecting the crosslinker to a second polymer;

wherein one and only one of  $R^1$  and  $R^2$  may be H or may be alkyl, arylalkyl or heteroarylalkyl, each optionally substituted;

at least one or both  $R^1$  and  $R^2$  is independently CN;  $NO_2$ ;

optionally substituted aryl;

optionally substituted heteroaryl;

optionally substituted alkenyl;

optionally substituted alkynyl;

$COR^3$  or  $SOR^3$  or  $SO_2R^3$  wherein

$R^3$  is H or optionally substituted alkyl;

aryl or arylalkyl, each optionally substituted;

heteroaryl or heteroarylalkyl, each optionally substituted; or

OR<sup>9</sup> or NR<sup>9</sup><sub>2</sub> wherein each R is independently H or optionally substituted alkyl, or both R<sup>9</sup> groups taken together with the nitrogen to which they are attached form a heterocyclic ring;

SR<sup>4</sup> wherein

R<sup>4</sup> is optionally substituted alkyl;

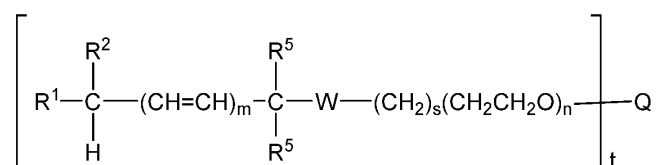
aryl or arylalkyl, each optionally substituted; or

heteroaryl or heteroarylalkyl, each optionally substituted;

wherein R<sup>1</sup> and R<sup>2</sup> may be joined to form a 3-8 membered ring; and

each R<sup>5</sup> is independently H or is alkyl, alkenylalkyl, alkynylalkyl, (OCH<sub>2</sub>CH<sub>2</sub>)<sub>p</sub> O-alkyl wherein p=1-1000, aryl, arylalkyl, heteroaryl or heteroarylalkyl, each optionally substituted;

or



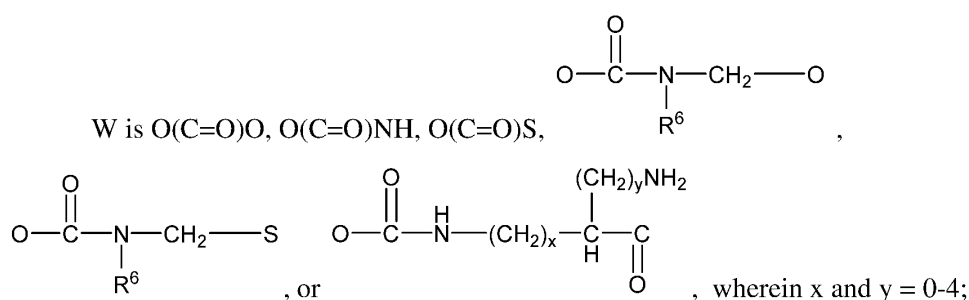
wherein

m is 0 or 1;

n is 1-1000;

s is 0-2;

t is 2,4, 8, 16 or 32;



Q is a core group having a valency=t;

wherein at least one of R<sup>1</sup>, R<sup>2</sup>, and R<sup>5</sup> comprises a functional group Z connecting the crosslinker to a second polymer, and

at least one or both R<sup>1</sup> and R<sup>2</sup> is independently CN; NO<sub>2</sub>;

optionally substituted aryl;

optionally substituted heteroaryl;

optionally substituted alkenyl;

optionally substituted alkynyl;

COR<sup>3</sup> or SOR<sup>3</sup> or SO<sub>2</sub>R<sup>3</sup> wherein

R<sup>3</sup> is H or optionally substituted alkyl;

aryl or arylalkyl, each optionally substituted;

heteroaryl or heteroarylalkyl, each optionally substituted; or

OR<sup>9</sup> or NR<sup>9</sup><sub>2</sub> wherein each R is independently H or optionally substituted alkyl, or both R<sup>9</sup> groups taken together with the nitrogen to which they are attached form a heterocyclic ring;

SR<sup>4</sup> wherein

R<sup>4</sup> is optionally substituted alkyl;

aryl or arylalkyl, each optionally substituted; or

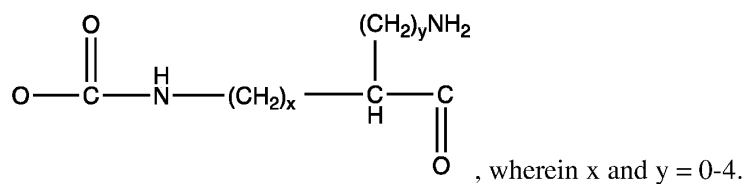
heteroaryl or heteroarylalkyl, each optionally substituted;

wherein R<sup>1</sup> and R<sup>2</sup> may be joined to form a 3-8 membered ring; and

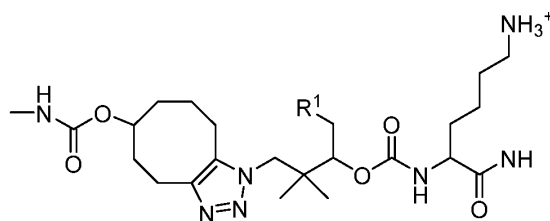
wherein one and only one of R<sup>1</sup> and R<sup>2</sup> may be H or may be alkyl, arylalkyl or heteroarylalkyl, each optionally substituted; and

each R<sup>5</sup> is independently H or is alkyl, alkenylalkyl, alkynylalkyl, (OCH<sub>2</sub>CH<sub>2</sub>)<sub>p</sub> O-alkyl wherein p=1-1000, aryl, arylalkyl, heteroaryl or heteroarylalkyl, each optionally substituted.

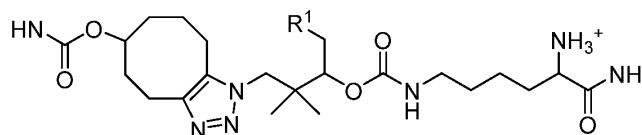
16. The hydrogel of Claim 15 wherein m = 0, R<sup>2</sup> is H, one R<sup>5</sup> is H and the other is C(Me)<sub>2</sub>(CH<sub>2</sub>)<sub>n</sub>Z wherein n = 0-6, and W is



17. The hydrogel of Claim 15 wherein the hydrogel comprises a cross-linker of the formula:



or



18. The hydrogel of Claim 13 wherein sterilization is achieved by heating a suspension of the hydrogel in buffer at pH 2-5 to a temperature of 121 °C for a hold time of 20 minutes.

19. A method of preparing a sterile hydrogel conjugate, which method comprises the steps of

(a) sterilizing a hydrogel by autoclaving at an appropriate pH, temperature, and time;

(b) optionally activating the sterile hydrogel for conjugation; and

(c) attaching a drug or linker-drug to the sterile hydrogel under conditions in which sterility of the conjugate is maintained.

20. The method of Claim 19 wherein the drug is a peptide, protein, or small molecule.