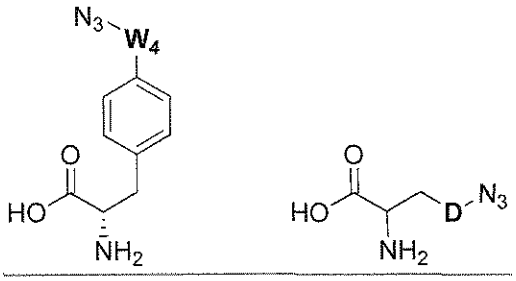
polypeptides are linked to the same conjugate at another location, and some polypeptides might not be linked at all.

[0007] There is a need, therefore, for polypeptides modified at site-specific positions optimized for uniformity, yield and/or activity to further the promising use of polypeptides in, for example, therapy and diagnostics.

SUMMARY

[0008] Provided herein are modified amino acids comprising an azido group, polypeptides, antibodies and conjugates comprising the modified amino acids, and methods of producing the polypeptides, antibodies and conjugates comprising the modified amino acids. The polypeptides, antibodies and conjugates are useful in methods of treatment and prevention, methods of detection and methods of diagnosis.

[0008a] In a first aspect of the present disclosure, there is provided a compound according to formula II or formula I:

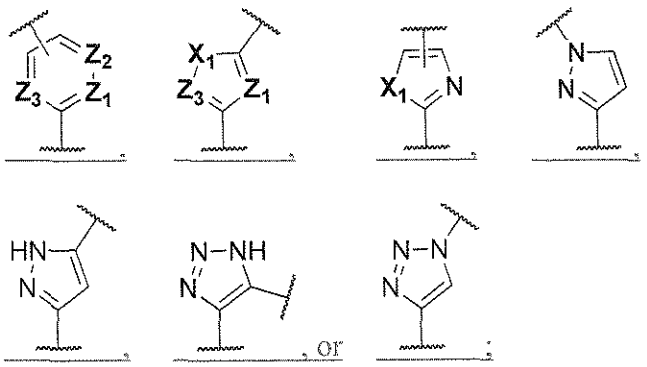


Formula II Formula I;

or a salt thereof, wherein:

D is –Ar-W_3– or –W_1-Y_1-C(O)-Y_2-W_2–;

Ar is



each of W_1, W_2, and W_3 is independently a single bond or lower alkylene;

W_4 is C_1-C_{10} alkylene;

each X_1 is independently –NH–, –O–, or –S–;

each Y_1 is independently a single bond, –NH–, or –O–;

each Y_2 is independently a single bond, –NH–, –O–, or an N-linked or C-linked pyrrolidinylene; and

one of Z_1, Z_2, and Z_3 is –N– and the others of Z_1, Z_2, and Z_3 are independently –CH–.

[0008b] In a second aspect of the present disclosure, there is provided a polypeptide comprising an amino acid residue corresponding to the compound of the first aspect.

[0008c] In a third aspect of the present disclosure, there is provided a conjugate comprising the polypeptide of the second aspect linked to a payload and optionally comprising a linking moiety between the polypeptide and the payload.

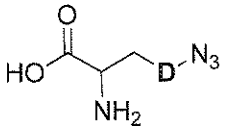
[0008d] In a fourth aspect of the present disclosure, there is provided an antibody comprising an amino acid residue corresponding to the compound of the first aspect.

[0008e] In a fifth aspect of the present disclosure, there is provided a conjugate comprising the antibody of the fourth aspect linked to a payload and optionally comprising a linking moiety between the antibody and the payload.

[0008f] In a sixth aspect of the present disclosure, there is provided an orthogonal tRNA aminoacylated with an amino acid residue corresponding to the compound of the first aspect.

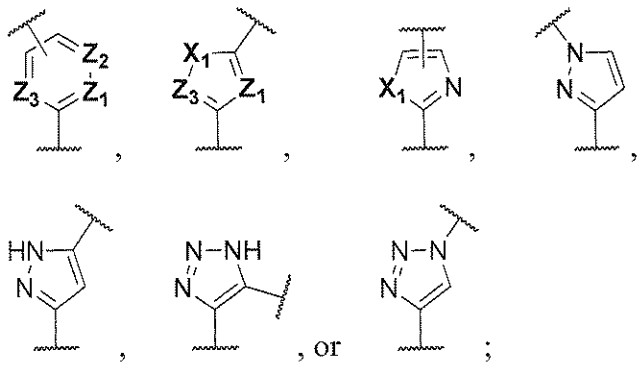
[0008g] In a seventh aspect of the present disclosure, there is provided a method of producing a polypeptide, comprising contacting a polypeptide with the orthogonal tRNA of the sixth aspect under conditions suitable for incorporating the amino acid residue into the polypeptide.

[0009] In one ~~aspect~~ embodiment a compound according to formula I is provided:



Formula I;

or a salt thereof, wherein: **D** is –**Ar**-W_3– or –W_1-Y_1-C(O)-Y_2-W_2–; **Ar** is



each of $\mathbf{W_1}$, $\mathbf{W_2}$, and $\mathbf{W_3}$ is independently a single bond or lower alkylene; each $\mathbf{X_1}$ is independently –NH–, –O–, or –S–; each $\mathbf{Y_1}$ is independently a single bond, –NH–, or –O–; each $\mathbf{Y_2}$ is independently a single bond, –NH–, –O–, or an N-linked or C-linked pyrrolidinylene; and one of $\mathbf{Z_1}$, $\mathbf{Z_2}$, and $\mathbf{Z_3}$ is –N– and the others of $\mathbf{Z_1}$, $\mathbf{Z_2}$, and $\mathbf{Z_3}$ are independently –CH–.

[0010] In a further ~~aspect~~embodiment, polypeptides and antibodies comprising an amino acid residue corresponding to a compound of formula I are provided. In particular embodiments, conjugates

of the polypeptides and payloads are provided. In further embodiments, conjugates of the antibodies and payloads are provided.

[0011] In another ~~aspect~~embodiment, an orthogonal tRNA is provided aminoacylated with an amino acid residue corresponding to a compound of formula I. In a related ~~aspect~~embodiment, a method of producing a polypeptide is provided, comprising contacting a polypeptide with an orthogonal tRNA aminoacylated with an amino acid residue corresponding to a compound of formula I.

[0012] The compounds of formula I described herein can be incorporated into any polypeptide known to those of skill in the art. Such polypeptides include, but are not limited to, proteins, antibodies, antibody fragments, enzymes, and nucleic acids.

BRIEF DESCRIPTION OF THE FIGURES

[0013] Figure 1 provides A schematic of the reaction of compounds (**30**), (**40**), and (**50**) with compound (**60**).

[0014] Figure 2 provides a plot of k_{obs} vs. [Azide] for reaction of compound (30), compound (40) and compound (50) with compound (**60**). In Figure 2, k_{obs} is plotted on the vertical axis in units of sec^{-1} from 0 to 0.006 and [Azide] is plotted on the horizontal axis in units of mM from 0 to 3. In Figure 2, results for compound (**30**) are provided as open circles, results for compound (**40**) are provided as filled triangles, and results for compound (**50**) are provided as filled circles. As provided in Figure 2, compounds (**30**) and (**40**) exhibited a first order rate constant of 1.4 M^{-1} sec^{-1}, approximately 7-fold higher than first order rate constant of 0.2 M^{-1} sec^{-1} for compound (**50**).

[0015] Figure 3 provides a plot of a time course for pAMF incorporation at the Y50TAG site in GFP. In Figure 3, RFU is plotted on the vertical axis from 0 to 400000 and time is plotted on the horizontal axis in units of minutes from 0 to 600. In Figure 3, results for turboGFP are provided as filled squares (top), results for pCNFRS D286R pAMF are provided as filled diamonds (middle), and results for Y50TAG are provided as filled triangles (bottom). As provided in Figure 3, turboGFP is approximately 250,000 RFU at 200 minutes, pCNFRS D286R pAMF is approximately 130,000 RFU at 200 minutes, and Y50TAG is approximately 0 RFU at 200 minutes. As provided in Figure 3, turboGFP is approximately 340,000 RFU at 400 minutes, pCNFRS D286R pAMF is approximately 160,000 RFU at 400 minutes, and Y50TAG is approximately 0 RFU at 400 minutes. As provided in Figure 3, turboGFP is approximately 370,000 RFU at 600 minutes, pCNFRS D286R pAMF is approximately 170,000 RFU at 600 minutes, and Y50TAG is approximately 0 RFU at 600 minutes.