

Table S1. Summary of trimers between intact globin chains for in vitro samples (RBCs incubated with DCVCS 0.9-450 μ M). See explanation in Table 2 (manuscript)

	DCVCS as cross-linker without additional monoadducts	DCVCS as cross-linker plus following monoadducts		
		DCVCS	DCVCS-GSH	DCVCS-Cys
DCVCS 0.9 μM				
Mass _{theo} (Da)	45865; 46631; 46645; 46675; 46733	47035; 47593; 47752	47563; 47729; 47884	-
Mass _{obs} (Da)	45869; 46629; 46650; 46675; 46735	47035; 47598; 47748	47564; 47727; 47884	
Cross-links	$\alpha 1\alpha 1\alpha 2+2cl$; $\alpha 2\alpha 2\beta 1+3cl$; $\alpha 2\alpha 2\beta 2+3cl$; $\alpha 1\alpha 2\beta 1+3cl$; $\alpha 1\alpha 1\beta 2+3cl$;	$\alpha 2\alpha 2\beta 2+3cl+2$; $\beta 2\alpha 1\beta 2+3cl+1$; $\alpha 2\beta 1\beta 1+2cl+2$	$\alpha 2\alpha 2\beta 1+3cl+2$; $\alpha 1\alpha 1\alpha 2+2cl+4$; $\alpha 2\beta 2\alpha 2+2cl+3$	
DCVCS 4.5 μM				
Mass _{theo} (Da)	46645	46667; 46681; 46725; 46950	46490	48079
Mass _{obs} (Da)	46650	46663; 46684; 46730; 46950	46492	48083
Cross-links	$\alpha 2\alpha 2\beta 2+3cl$	$\alpha 2\alpha 2\beta 1+2cl+1$; $\alpha 2\alpha 2\beta 2+2cl+1$; $\alpha 1\alpha 2\beta 2+2cl+1$; $\alpha 1\alpha 1\beta 1+2cl+2$	$\alpha 1\alpha 1\alpha 2+3cl+1$	$\alpha 1\beta 2\beta 2+2cl+3$
DCVCS 9 μM				
Mass _{theo} (Da)	45865; 46631; 46689	47752; 46681; 46725; 46755	46490; 47563; 47729	47519
Mass _{obs} (Da)	45860; 46634; 46688	47749; 46677; 46727; 46758	46492; 47558; 47731	47519
Cross-links	$\alpha 1\alpha 1\alpha 2+3cl$; $\alpha 2\alpha 2\beta 1+3cl$; $\alpha 1\alpha 2\beta 2+3cl$	$\alpha 2\beta 1\beta 1+2cl+3$; $\alpha 2\alpha 2\beta 2+2cl+1$; $\alpha 1\alpha 2\beta 2+2cl+1$; $\alpha 1\alpha 1\beta 1+2cl+1$	$\alpha 1\alpha 1\alpha 2+3cl+1$; $\alpha 2\alpha 2\beta 1+3cl+2$; $\alpha 1\alpha 1\alpha 2+2cl+4$	$\alpha 1\beta 2\beta 2+2cl+1$
DCVCS 90 μM				
Mass _{theo} (Da)	46645; 45821; 46689	46175; 46725; 46667	47729; 48356	47134
Mass _{obs} (Da)	46648; 45820; 46687	46177; 46730; 46665	47730; 48352	47129
Cross-links	$\alpha 2\alpha 2\beta 2+3cl$; $\alpha 1\alpha 2\alpha 2+2cl$; $\alpha 1\alpha 2\beta 2+3cl$	$\alpha 1\alpha 2\alpha 2+3cl+1$; $\alpha 1\alpha 2\beta 1+2cl+1$; $\alpha 2\alpha 2\beta 1+2cl+1$	$\alpha 1\alpha 1\alpha 2+2cl+4$; $\beta 1\beta 2\beta 2+2cl+1$	$\alpha 1\alpha 1\beta 2+2cl+2$
DCVCS 450 μM				
Mass _{theo} (Da)	46645	46667; 46725; 47593; 47752	47884; 47563; 47729	48079
Mass _{obs} (Da)	46643	46668; 46728; 47593; 47748	47882; 47562; 47732	48083
Cross-links	$\alpha 2\alpha 2\beta 2+3cl$	$\alpha 2\alpha 2\beta 1+2cl+1$; $\alpha 1\alpha 2\beta 2+2cl+1$; $\beta 2\alpha 1\beta 2+3cl+1$; $\alpha 2\beta 1\beta 1+2cl+3$	$\alpha 2\beta 2\alpha 2+2cl+3$; $\alpha 2\alpha 2\beta 1+3cl+2$; $\alpha 1\alpha 1\alpha 2+2cl+4$	$\alpha 1\beta 2\beta 2+2cl+3$

Table S2. Summary of dimers between intact globin chains for in vivo samples (SD rats dosed with DCVCS 230 $\mu\text{mol/kg}$). Cross-links containing DCVCS or NA-DCVCS as cross-linkers (between chains) with and without monoadducts are shown. See explanation for formation of DCVCS cross-link in Table 2 (manuscript). NA-DCVCS cross-link: 1cl (+201 Da) = 1 NA-DCVCS cross-linker (between chains), 2cl (+201*2 Da) = 2 NA-DCVCS cross-linkers, 3cl (+201*3 Da) = 3 NA-DCVCS cross-linkers. In addition to monoadducts listed in Table 2 (manuscript), other monoadducts included: NA-DCVCS (+237 Da, +237*2 Da, and +237*3 Da), NA-DCVCS-GSH (+508 Da with up to 3 adducts as described above for NA-DCVCS), NA-DCVCS-Cys (+322 Da with up to 3 adducts as described above for NA-DCVCS). Example: $\alpha\beta$ +1cl+2 defines a cross-link between the stated chains with 1 cross-linker (DCVCS or NA-DCVCS) and 2 monoadducts.

	Rat 1			Rat 2		
	Mass _{theo} (Da)	Mass _{obs} (Da)	Cross-links	Mass _{theo} (Da)	Mass _{obs} (Da)	Cross-links
DCVCS as cross-linker without additional monoadducts	30714	30716	$\alpha 1\alpha 1+2\text{cl}$	-		
DCVCS as cross-linker plus following monoadducts:						
DCVCS	31247	31246	$\alpha 2\alpha 2+1\text{cl}+4$	31245	31247	$\alpha 2\alpha 2+1\text{cl}+4$
DCVCS-GSH	30977; 31136	30974; 31137	$\alpha 1\alpha 2+1\text{cl}+1$; $\alpha 1\alpha 2+2\text{cl}+1$	30975; 31134	30973; 31138	$\alpha 1\alpha 2+1\text{cl}+1$; $\alpha 1\alpha 2+2\text{cl}+1$
DCVCS-Cys	-			-		
NA-DCVCS	-			-		
NA-DCVCS-GSH	32035	32039	$\alpha 1\alpha 2+1\text{cl}+3$	-		
NA-DCVCS-Cys	-			-		
NA-DCVCS as cross-linker without additional monoadducts	-			-		
NA-DCVCS as cross-linker plus following monoadducts:						
DCVCS	-			-		
DCVCS-GSH	31928	31932	$\alpha 1\beta 2+2\text{cl}+1$	-		
DCVCS-Cys	32056	32055	$\alpha 2\beta 2+1\text{cl}+3$	32057	32056	$\alpha 2\beta 2+1\text{cl}+3$
NA-DCVCS	-			-		
NA-DCVCS-GSH	32941	32945	$\beta 2\beta 2+1\text{cl}+2$	-		
NA-DCVCS-Cys	-			-		

Table S3. Summary of trimers between intact globin chains for in vivo samples (SD rats dosed with DCVCS 230 $\mu\text{mol/kg}$). See explanations in Table S2 *Experimental mass represents two different types of trimers.

	Rat 1			Rat 2		
	Mass _{theo} (Da)	Mass _{obs} (Da)	Cross-links	Mass _{theo} (Da)	Mass _{obs} (Da)	Cross-links
DCVCS as cross-linker without additional monoadducts	46576; 46722	46572; 46717	$\alpha 1\alpha 1\beta 2+2\text{cl}$; $\alpha 1\alpha 1\beta 1+3\text{cl}$	-		
DCVCS as cross-linker plus following monoadducts:						
DCVCS	-			-		
DCVCS-GSH	-			46938	46937*	$\alpha 2\alpha 2\beta 1+2\text{cl}+1$
DCVCS-Cys	47476	47472	$\alpha 2\beta 2\beta 2+2\text{cl}+1$	47475; 46864	47472; 46861	$\alpha 2\beta 2\beta 2+2\text{cl}+1$; $\alpha 1\alpha 1\alpha 2+3\text{cl}+3$
NA-DCVCS	-			-		
NA-DCVCS-GSH	-			-		
NA-DCVCS-Cys	-			-		
NA-DCVCS as cross-linker without additional monoadducts	-			46658	46655	$\alpha 1\alpha 1\beta 1+2\text{cl}$
NA-DCVCS as cross-linker plus following monoadducts:						
DCVCS	47896	47891	$\alpha 1\beta 2\beta 2+2\text{cl}+3$	-		
DCVCS-GSH	-			47532	47531	$\alpha 1\alpha 2\beta 2+2\text{cl}+2$
DCVCS-Cys	47591	47587	$\alpha 1\beta 2\beta 2+2\text{cl}+3$	46185; 46938	46184; 46937*	$\alpha 1\alpha 1\beta 2+2\text{cl}+1$
NA-DCVCS	-			-		
NA-DCVCS-GSH	-			-		
NA-DCVCS-Cys	-			-		

In vivo results for rats treated with 230 $\mu\text{mol/kg}$ DCVCS that refer to Tables S2 and S3 of the Supplemental Material

Globin chain cross-links. Analyses of intermolecular cross-links between various chains with DCVCS as a cross-linker (+159 Da) revealed a dimer between $\alpha 1$ chains (Table S2) and 2 trimers ($\alpha 1\alpha 1\beta 1$, $\alpha 1\alpha 1\beta 2$) in rat 1 (Table S3). The data was also analyzed for cross-links with NA-DCVCS (+201 Da) as cross-linker. Interestingly, 1 trimer ($\alpha 1\alpha 1\beta 1$) of this kind was detected with the other rat (rat 2; Table S3).

Globin chain cross-links with additional DCVCS monoadducts. Both rats had a dimer ($\alpha 2\alpha 2$) with 1 DCVCS moiety as a cross-linker (+159 Da) and four additional DCVCS moieties (+195 Da) as monoadducts (Table S2). Rat 1 also contained a trimer ($\alpha 1\beta 1\beta 2$) with 2 NA-DCVCS moieties as cross-linkers (+201 Da) and 3 DCVCS moieties as monoadducts (Table S3).

Globin chain cross-links with additional DCVCS-GSH monoadducts. There were 2 dimers with DCVCS moieties as cross-linkers (+159 Da) between $\alpha 1$ and $\alpha 2$ chains with a DCVCS-GSH adduct (+466 Da) in each rat (Table S2). It was not possible to determine if one of the rats (rat 2) also had an additional trimer ($\alpha 2\alpha 2\beta 1$) because the mass for that cross-link matches a different type of cross-link ($\alpha 1\alpha 1\beta 2$ with additional DCVCS-Cys monoadduct; Table S3). Each rat also had 1 cross-link ($\alpha 1\beta 2$ and $\alpha 1\alpha 2\beta 2$, respectively) containing 2 NA-DCVCS moieties as cross-linkers (+201 Da) that included 1 and 2 DCVCS-GSH adducts, respectively (Table S2 and S3).

Globin chain cross-links with additional DCVCS-Cys. The presence of cross-links containing DCVCS-Cys was also analyzed. Neither rat globin contained dimers with DCVCS as a cross-linker (+159 Da) and containing DCVCS-Cys monoadducts (+280 Da) (Table S2), however, one (rat 1) and two (rat 2) trimers were detected, with trimer ($\alpha 2\beta 2\beta 2$) being present in both rats

(Table S3). With NA-DCVCS as a cross-linker (+201 Da), one dimer ($\alpha_2\beta_2$) (Table S2) and one trimer (Table S3) were detected in each rat. Overall, 4 DCVCS-Cys moieties were detected on cross-links for each rat (Table S3).