

## CONFORMATIONAL PROPERTIES OF DISULPHIDE BRIDGES. 2. ROTATIONAL POTENTIALS OF DIETHYL DISULPHIDE

CARL HENRIK GÖRBITZ

*Department of Chemistry, University of Oslo, P.O. Box 1033 Blindern, N-0315 Oslo 3, Norway*

The conformational properties of diethyl disulphide, a model compound for the disulphide bridges in peptides and proteins, were studied with *ab initio* methods. Stationary point structures were optimized at the HF/6–31G\* level with consideration of electron correlation in subsequent single-point MP2 calculations. The six energy minima were also optimized at the MP2/6–31G\* level with calculation of zero-point vibrational frequencies and thermal corrections. Additional single-point MP2 energy calculations employed larger basis sets up to 6–311G(2d,p). With positive disulphide chirality, the global energy minimum is a 'spiral' conformation with *gauche* + C–C–S–S torsion angles. The further stability order for energy minima deviates from previous *ab initio* results. In particular, the extended *trans,trans* conformer is subject to a significant relative destabilization on inclusion of electron correlation in the calculations and is only the fifth most stable energy minimum with estimated *ab initio*  $\Delta H_{298} = 5.13 \text{ kJ mol}^{-1}$ . The results presented are relevant for the discussion of conformational properties for the structurally equivalent disulphide bridges in polypeptides and calculations of relative energies with molecular mechanics methods.

### INTRODUCTION

Detailed knowledge of the conformational properties of disulphide bridges is essential for studies of all molecules including this group, such as numerous extracellular proteins and biologically active oligopeptides.<sup>1</sup> The conformational problem can be approached without calculating relative energies, e.g. in the refinement of disulphides in protein structures by restrained least-squares programs<sup>2</sup> and in procedures developed for assessing the stereochemical suitability of potential sites for introduction of disulphide bridges by site-directed mutagenesis,<sup>3</sup> but frequently it is desirable to have some estimate of relative conformational energies. Molecular mechanics calculations<sup>4</sup> with specially designed force fields (AMBER,<sup>5</sup> CHARMM,<sup>6</sup> CVFF<sup>7</sup>) have provided such fundamental information for peptide and protein structures. While adequate for most purposes, it appears, however, that in the case of disulphide bridges many force fields suffer from poor parametrizations.<sup>8</sup> Accordingly, the results from calculations on molecules containing this cross-link may be of questionable quality.

In order to understand better and explain the conformational properties and preferences of disulphide bridges, a number of theoretical *ab initio* calculations have been carried out. The results are presented in a series of three papers. Part 1<sup>9</sup> described the sterically

unhindered C–S rotation in ethyl hydrodisulphide (ESSH), CH<sub>3</sub>–S–S–H. In this paper diethyl disulphide (ESSE), CH<sub>3</sub>–CH<sub>2</sub>–S–S–CH<sub>2</sub>–CH<sub>3</sub>, is used as a model molecule. The larger structure permits studies of several different C–S and S–S rotations which are unevenly affected by steric conflicts. Additionally, the relative energies of the minima, and hence their stability order, have been estimated at an unprecedented high level of theory. Part 3<sup>10</sup> will deal with the molecular flexibility of ESSE, with special regard to the incorporation of disulphides into protein structures.

### Terminology

Each C–S rotation has *gauche* + (*G*), *gauche* – (*G'*) and *trans* (*T*) minima in addition to two skew barriers (*S* and *S'*) and a *cis* barrier (*C*). An S–S rotation has *G* and *G'* '*gauche*' minima at *ca*  $\pm 90^\circ$  and *cis* (*C*) and *trans* (*T*) barriers. Any rotation is identified by the letter *x*, as in *GGx*. In the description of molecular geometry *r*(A–B) is used for the A–B bond distance and  $\alpha$ (A–B–C) is used for the A–B–C bond angle. The C–1–C–2–S–1–S–2, C–2–S–1–S–2–C–3 and S–1–S–2–C–3–C–4 torsion angles are denoted  $\chi_{CS}$ ,  $\chi_{SS}$  and  $\chi_{SC}$ , respectively.

## EXPERIMENTAL

All *ab initio* calculations were performed with the Gaussian 90<sup>11</sup> and Gaussian 92<sup>12</sup> molecular orbital program systems, and were run on Convex and Cray computers. The Z-matrix used for ESSE at the HF level employed a single methylene C—H distance and a set of five parameters for methyl group geometry: three C—C—H bond angles, one C—H bond length and one S—C—C—H torsion angle (assuming  $C_{3v}$  torsion symmetry). Although fully relaxed optimizations would have been feasible, the observed energy gain was so small ( $<0.01$  kJ mol<sup>-1</sup>) that, for simplicity, the constrained model was retained. For the time-consuming saddle point calculations the number of parameters was limited further by fixing the geometry of the two independent ethyl groups [except  $\alpha(C-C-S)$  and the H—C—C—S torsion angle], each as obtained in the corresponding symmetric structure. Accordingly, the geometries for ethyl groups in the *GGG* conformation were taken from the optimized *GGG* and *SGS* structures. Tests indicated that the error involved in this procedure is  $<0.03$  kJ mol<sup>-1</sup>.

Simultaneous rotations around both C—S bonds and the S—S bond in ESSE give a large number of stationary points, many of which are energy maxima of very limited interest. A study of the conformational properties can be simplified by regarding C—S and S—S rotations independently. Rotation around the two C—S bonds in ESSE (retaining positive disulphide chirality) yields nine energy minima, which are reduced to six owing to the symmetric nature of the molecule. Similarly, there are six regular energy maxima and nine saddle points. These stationary points (and their symmetry equivalents) can be interpreted in terms of three different C—S rotations *GGx*, *TGx* and *G'Gx*. All optimizations were straightforward, except for the *G'GS'* barrier. Further trials revealed that  $\chi_{SC}$  in this point is significantly distorted from  $-120^\circ$ , but led also to the discovery of an additional saddle point on the symmetry diagonal for a *G'GT*  $\rightarrow$  *TGG'* transition. The saddle point has been denoted *BGB*, and is accompanied by an energy maximum called *MGM*. The significance of these points is discussed in Figure 3.

There are four different potentials for S—S rotation in ESSE (mirror images disregarded), viz. *GxG*, *TxT*, *GxT* and *GxG'*. As S—S rotations are well documented in the literature,<sup>13,14</sup> only the first two were investigated in more detail. The calculation procedures were identical with those taken for C—S rotation maxima.

Electron correlation effects were recorded for all C—S and S—S rotation stationary points by performing single-point second-order Møller–Plesset (MP2) calculations<sup>15</sup> with the 6–31G\* basis set, using the previously refined HF geometries. In the text HF/6–31G\*//HF/6–31G\* and MP2/6–31G\*//HF/

6–31G\* levels of theory have been abbreviated HF//HF and MP2//HF, respectively.

In order to have more conclusive results with respect to the relative energies of the minima, fully relaxed geometries for the six structures were obtained at the MP2/6–31G\* level with calculation of zero-point vibrational frequencies. The frequencies were scaled by the empirical factor 0.9 and used to calculate zero-point vibrational energies (ZPVEs).<sup>16</sup> The corrected frequencies were also used to calculate thermal corrections for a temperature shift from 0 to 298.15 K. Additional single-point energy calculations employed three larger basis sets derived from the triple zeta valence 6–311G(d): (a) 6–311G(d,p) with p functions on H; (b) 6–311G(2d) with a second set of d functions on the heavy atoms; and (c) 6–311G(2d,p) with both of the above expansions. Full electron correlation effects were included at the MP2 level.

## RESULTS

The six energy minima with heavy atom bond lengths and bond angles are depicted in Figure 1 (the atomic numbering is omitted in the text and tables when redundant). Relative energies for the minima are listed in Table 1, which also includes *ab initio* values for  $\Delta H_0$  and  $\Delta H_{298}$ . Torsion angles for C—S rotation stationary points and relative energies for the rotational barriers and saddle points are given in Table 2. Geometry parameters and relative energies for S—S rotational barriers are given in Table 3.

## DISCUSSION

## Electron correlation and basis sets

The data in Tables 1 and 2 show that *gauche+* is generally the most favourable orientation for  $\chi_{CS}$  and  $\chi_{SC}$ , but with small energy differences relative to the *trans* rotamers at the HF level. For ESSH a substantial relative destabilization of the *trans* minimum on introduction of electron correlation in the calculations was discovered, and similar relative *trans* destabilizations are also evident for ESSE. On the other hand, electron correlation serves to stabilize the usually less favourable *gauche-* rotamers, as is evident for the *GGG*–*GGG'* pair with  $\Delta E_{HF//HF} = 1.97$  kJ mol<sup>-1</sup> and  $\Delta E_{MP2//HF} = 1.49$  kJ mol<sup>-1</sup>. With HF/6–31G\* optimized structures the average MP2 *trans* destabilization amounts to 1.66 kJ mol<sup>-1</sup>, while the average *gauche-* stabilization is 0.54 kJ mol<sup>-1</sup>. Several other basis sets were also tested with similar results. Examples include a 2.30 kJ mol<sup>-1</sup> *trans* destabilization with the 6–31G\* basis set and MP2/6–31G\* optimized structures, and a 2.02 kJ mol<sup>-1</sup> destabilization with the 3–21G\* basis set and HF/3–21G\* optimized structures.

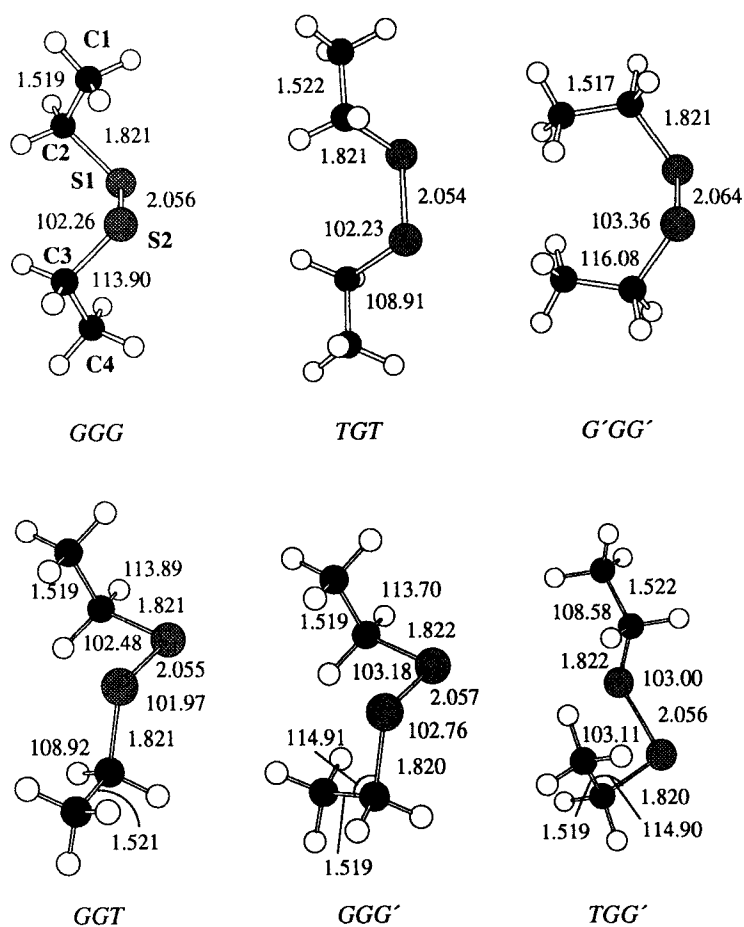


Figure 1. The six energy minimum conformations for diethyl disulphide with heavy atom numbering scheme, bond lengths and bond angles indicated

A *trans* destabilization, albeit smaller ( $0.84\text{--}1.63\text{ kJ mol}^{-1}$  depending on the basis set and optimization level), has also been observed for *n*-butane.<sup>17</sup> The inclusion of electron correlation in the calculations was furthermore required to reproduce the experimental *gauche* preferences for C—S rotations in methyl ethyl disulphide<sup>18</sup> and methyl ethyl sulphide.<sup>19</sup>

The selection of larger basis sets for further higher level calculations was guided by previous calculations for ESSH.<sup>9</sup> For ESSH MP2/6-311G\*\*//MP2/6-31G\* and MP2/6-311G(2d)//MP2/6-31G\* relative energies for minima were similar and close to the very high level MP4SDQ/6-311+G(2d,p)//MP2/6-31G\* values. As electron correlation beyond MP2 would have required excessive computer resources for ESSE, the single-point energies for the three symmetric ESSE minima were at first calculated at these two levels.

Given the ESSH results, it was surprising to find that MP2/6-311G\*\*//MP2/6-31G\* and MP2/6-311G(2d)//MP2/6-31G\* relative energies for ESSE were significantly different. An attempt to solve this controversy was made by employing the still larger basis set 6-311G(2d,p), which also yielded excellent results for ESSH at the MP2 level, for all six minima. The resulting relative energies for *TGT* and *G'GG'* lie between 6-311G\*\* and 6-311G(2d) values, but closer to the latter for *G'GG'*, and are in fact close to the MP2/6-31G\*\*//MP2/6-31G\* values. The contributions of vibrational energies and temperature are included in the estimates for  $\Delta H_0$  and  $\Delta H_{298}$  in Table 1. There are no dramatic changes to the relative energies, but with slightly higher values for  $\Delta H_{298}$  energies for structures with *trans* torsion angles.

Table 1. Relative energies (kJ mol<sup>-1</sup>) for diethyl disulphide minima

Conformation	Level of theory								$\Delta H_0^c$	$\Delta H_{298}^f$
	HF/3-21G <sup>a</sup>	HF/6-21G <sup>a,b</sup>	MP2/6-31G <sup>a,b</sup>	MP2/6-31G <sup>a,c</sup>	MP2/6-311G <sup>a,c</sup>	MP2/6-311(2d) <sup>c</sup>	MP2/6-311(2d, p) <sup>c,d</sup>	MP2/6-311(2d, p) <sup>c,d</sup>		
GGG	0.00	0.00 <sup>g</sup>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TGT	1.67	0.30	3.73	4.24	3.89	5.23	4.46	4.49	4.49	5.13
G'GG'	9.88	7.72	7.13	7.29	8.30	6.39	6.59	7.05	7.05	6.87
GGT	0.84	0.13	1.81	2.07			2.17	2.19	2.19	2.51
GGG'	2.76	1.97	1.49	1.39			1.11	1.39	1.39	1.26
TGG'	3.43	2.09	3.21	3.35			3.06	3.33	3.33	3.53

<sup>a</sup> From Ref. 22.

<sup>b</sup> Obtained with HF/6-31G<sup>\*</sup> optimized geometries.

<sup>c</sup> Obtained with MP2/6-31G<sup>\*</sup> optimized geometries.

<sup>d</sup> Best estimate for  $\Delta E$ , 214 basis functions.

<sup>e</sup>  $\Delta E$  + difference in ZPVE relative to GGG.

<sup>f</sup>  $\Delta H_0$  + difference in  $H_{298} - H_0$  relative to GGG.

<sup>g</sup> Total energies (hartree): HF/6-31G<sup>\*</sup>//HF/6-31G<sup>\*</sup>, -952.319067; MP2/6-31G<sup>\*</sup>//HF/6-31G<sup>\*</sup>, -953.130426; MP2/6-31G<sup>\*</sup>//MP2/6-31G<sup>\*</sup>, -953.131397; MP2/6-311G<sup>\*\*</sup>//MP2/6-31G<sup>\*</sup>, -953.601665; MP2/6-311G(2d)//MP2/6-31G<sup>\*</sup>, -953.622743; MP2/6-311G(2d, p)//MP2/6-31G<sup>\*</sup>, -953.698926.

Table 2. Torsion angles for HF/6-31G\* (roman type) and MP2/6-31G\* (italic type) diethyl disulphide energy minima and torsion angles and relative energies ( $GGG = 0.00$ ) for HF/6-31G\* C-S rotation saddle points and maxima

Conformation	$\chi_{cs}$ ( $^{\circ}$ )	$\chi_{ss}$ ( $^{\circ}$ )	$\chi_{sc}$ ( $^{\circ}$ )	Level of theory	
				HF/6-31G* (kJ mol $^{-1}$ )	MP2/6-31G* (kJ mol $^{-1}$ )
Minima					
<i>GGG</i>	70.53	88.86	70.53		
	<i>68.28</i>	<i>87.25</i>	<i>68.28</i>		
<i>TGT</i>	177.45	88.28	177.45		
	<i>178.34</i>	<i>85.79</i>	<i>178.34</i>		
<i>G'GG'</i>	-73.45	112.47	-73.45		
	<i>-70.89</i>	<i>111.41</i>	<i>-70.89</i>		
<i>GGT</i>	70.56	88.68	177.40		
	<i>68.07</i>	<i>86.63</i>	<i>178.25</i>		
<i>GGG'</i>	69.77	98.22	-70.46		
	<i>66.59</i>	<i>97.13</i>	<i>-65.89</i>		
<i>TGG'</i>	175.23	97.83	-70.06		
	<i>174.62</i>	<i>96.40</i>	<i>-65.00</i>		
Saddle points					
<i>GGC</i>	68.89	92.37	-4.21	17.04	15.85
<i>GGs</i>	69.32	88.46	120.38	6.90	7.82
<i>GGs'</i>	68.95	88.51	-123.23	7.69	8.24
<i>TGC</i>	174.31	92.20	-4.28	16.87	17.22
<i>TGS</i>	176.20	88.18	120.43	6.93	9.53
<i>TGS'</i>	175.72	88.11	-123.50	7.65	9.95
<i>G'GC</i>	-72.42	105.10	-11.35	20.23	18.44
<i>G'GS</i>	-70.93	94.28	118.17	7.52	7.73
<i>G'GS'</i>	-75.06	103.74	-109.27	11.01	10.80
<i>BGB</i>	-100.46	94.35	-100.46	11.49	11.45
Maxima					
<i>CGC</i>	-10.88	96.45	-10.88	32.95	30.03
<i>SGS</i>	119.10	90.04	119.10	14.23	16.01
<i>S'GS'</i>	-125.78	90.73	-125.78	15.84	17.14
<i>MGM</i>	-97.49	98.90	-97.49	11.53	11.51

 Table 3. Selected geometry parameters and relative energies ( $GGG = 0.00$ ) for diethyl disulphide HF/6-31G\* S-S rotational barriers

Conformation	C-C ( $\text{\AA}$ )	C-S ( $\text{\AA}$ )	S-S ( $\text{\AA}$ )	C-C-S ( $^{\circ}$ )	C-S-S ( $^{\circ}$ )	$\chi_{cs}/\chi_{sc}$ ( $^{\circ}$ )	$\chi_{ss}$ ( $^{\circ}$ )	Level of theory	
								HF/6-31G* (kJ mol $^{-1}$ )	MP2/6-31G* (kJ mol $^{-1}$ )
<i>GCG</i>	1.526	1.826	2.113	114.28	108.91	92.56	-8.79	57.97	59.44
<i>GTG</i>	1.525	1.822	2.095	114.84	99.36	73.61	180.44	25.30	29.56
<i>TCT</i>	1.528	1.823	2.110	107.90	107.04	180.00	0.00	48.20	53.50
<i>TTT</i>	1.526	1.822	2.089	109.58	98.47	180.00	180.00	24.05	31.59

### Relative stability of energy minima

Independent of the choice of basis set the *GGG* 'spiral' conformation is the global energy minimum for ESSE with positive disulphide chirality. This is in agreement with all recent experiments and calculations.<sup>20-22</sup> The

further stability order for the minima, derived from either the highest level *ab initio* energies, the  $\Delta H_0$  values or the  $\Delta H_{298}$  values, is  $GGG > GGG' > GGT > TGG' > TGT > G'GG'$ , with an even spacing of  $\Delta H_{298}$  energies from 0.00 to 6.87 kJ mol $^{-1}$ . Is it of particular interest that the extended *TGT* minimum

(5.13 kJ mol<sup>-1</sup>) is just number five in this sequence, succeeded only by the *G'GG'* minimum. The only other complete set of energies, calculated at the HF/3-21G\*/HF/3-21G\* level, yielded the sequence *GGG* > *GGT* > *TGT* > *GGG'* > *TGG'* > *G'GG'*.<sup>22</sup> When discussing the conformational preferences for disulphide bridges in peptides, the difference between these two stability orders is significant.

### C—S rotational potentials and steric conflict

The three different C—S rotational potentials are shown in Figure 2(a). The curve shapes obtained for *GGx* and *TGx* are almost identical and fairly symmetric at the centre, but the *G'Gx* potential displays significant asymmetry, indicating considerable steric conflict. In Figure 2(b) this effect was studied more comprehensively

by subtracting, from each of the curves in Figure 2(a), the MP//HF potential for the sterically unhindered C—S rotation in ESSH.<sup>9</sup> With the caveat that other factors than steric conflict could influence the potentials, Figure 2(b) gives an indication of the ethyl...ethyl steric interactions during C—S rotation. It is clear that the interaction level is low for all *GGx* and *TGx* stationary points, except for the *GGG'* and *TGG'* minima which are associated with the short H...H distances of 2.486 and 2.467 Å, respectively. In contrast, *G'Gx* stationary points are heavily affected by steric hindrance with *r*(H...H) values smaller than 2.55 Å. The *G'GG'* conformation is the most congested, explaining why it is the least stable energy minimum in Table 1. The *G'GS* saddle point makes an exception in this potential with a minimum *r*(H...H) = 2.864 Å. Consequently, *G'GS* has lower energy than both *GGG* and *TGS* at the MP2//HF level (Table 2), in agreement with the ESSH result that *gauche*- geometry is preferable to *gauche*+ in the absence of steric hindrance.

The skew torsional barriers average 6.5 kJ mol<sup>-1</sup> at the MP2//HF level, and range from 4.52 kJ mol<sup>-1</sup> (*TGG' → TGS*) to 9.30 kJ mol<sup>-1</sup> (*G'GT → G'GS'*). The eclipsed *cis* barriers average 15.5 kJ mol<sup>-1</sup> with range from 11.31 kJ mol<sup>-1</sup> (*G'GG' → G'GC*) to 16.95 kJ mol<sup>-1</sup> (*G'GG → G'GC*). These values are lower than the MP2//HF skew and *cis* barriers for ESSH (*S* = 8.99 kJ mol<sup>-1</sup>, *S'* = 9.68 kJ mol<sup>-1</sup>, *C* = 17.20 kJ mol<sup>-1</sup>),<sup>9</sup> as the comparatively most favourable ESSE stereochemistry occurs for the energy barrier structures (Figure 2). Inspection of H...H distances reveals that while minima always have at least one *r*(H...H) < 2.78 Å, the *GGx* and *TGx* skew barriers have no such contacts < 3.05 Å. Hence even H...H distances well above the sum of the van der Waals radii appear to be energetically unfavourable. The *cis* barriers remain enigmatic in this respect, however, with one *r*(H...H) < 2.56 Å for both *GGC* and *TGC*. This may be partly explained by the more 'head-on' geometry for H...H contacts in these structures, which thereby take advantage of the anisotropic van der Waals radius of hydrogen bonded to carbon ('polar flattening').<sup>23</sup>

With the data from Reference 9 and Tables 1 and 2, the stability order for the three C—S rotation minima at the MP2/6-31G\*/HF/6-31G\* level can be deduced. It is influenced by the amount of steric conflict experienced during rotation as follows:

Steric conflict (rotation)	Stability order
none (ESSH)	<i>G'</i> > <i>G</i> > <i>T</i>
small ( <i>GGx</i> , <i>TGx</i> )	<i>G</i> > <i>G'</i> > <i>T</i>
large ( <i>G'Gx</i> )	<i>G</i> > <i>T</i> > <i>G'</i>

It is essential that this differentiation is made when discussing the stability of C—S rotamers. The *G* > *G'* > *T* stability order should also apply to the C—S rotation in methyl ethyl disulphide, but deviates from the stability

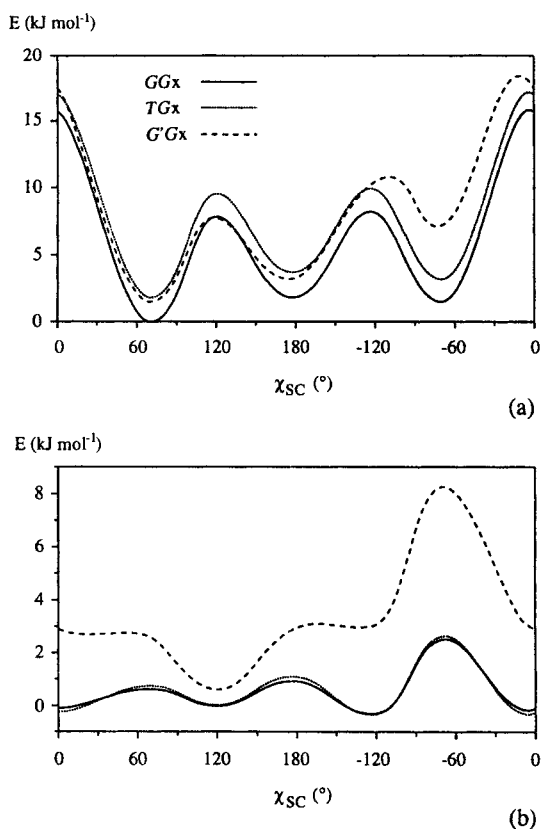


Figure 2. (a) MP2//HF energy potential curves for the three C—S rotations in diethyl disulphide. (b) Estimated magnitude for steric hindrance (same legend). The *TGx* and *G'Gx* curves have been adjusted to account for the permanent *trans* (1.66 kJ mol<sup>-1</sup>) and *gauche*- (-0.54 kJ mol<sup>-1</sup>) MP2//HF penalties, respectively. Note that the choice of 0-point on the energy axis is necessarily arbitrary. For other details, see text

order suggested by experimental data ( $G > T > G'$ ) for both compounds.<sup>20,21</sup> The reason for this discrepancy is unclear.

### C—S rotation and molecular geometry

The variations in bond lengths and bond angles are generally small and follow trends similar to those observed for C—S rotation in ESSH.<sup>9</sup> The only new feature is  $r(\text{S—S})$ , which varies between 2.047 and 2.063 Å. The smaller value is observed for the doubly eclipsed *CGC* maximum, which is surprising given the large value for  $\chi_{\text{SS}}$  (96.4°) and the known correlation between  $r(\text{S—S})$  and  $\chi_{\text{SS}}$ .<sup>14b</sup>

The  $\chi_{\text{SS}}$  torsion angle is mostly close to *ca* 90°, but Figure 3 shows that for  $C_2$  symmetric structures a dramatic change occurs when  $\chi_{\text{CS}}$  and  $\chi_{\text{SC}}$  are in the interval  $(-100^\circ, -90^\circ)$ . Thus, the 2.97° shift for  $\chi_{\text{CS}}/\chi_{\text{SC}}$  from  $-100.46^\circ$  to  $-97.49^\circ$  brings about a 4.55° increase for  $\chi_{\text{SS}}$  (Table 2). These major geometry modifications coincide with the curious *BGB* saddle point and *MGM* energy peak. The total range for  $\chi_{\text{SS}}$  in the HF refinements is substantial, from 88.11° for the *TGS* saddle point to 112.47° for the *G'GG'* minimum.

Geometry parameters from MP2/6-31G\* optimizations in general deviate only slightly from the corresponding HF/6-31G\* values. Changes to the torsion angles (Table 2) are the most evident, with the general trends being  $\chi_{\text{CS}}$  and  $\chi_{\text{SC}}$  values closer to 60° for

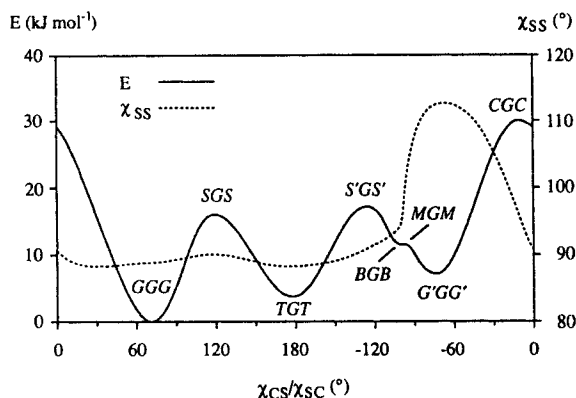


Figure 3. MP2//HF energy potential and  $\chi_{\text{SS}}$  values for simultaneous rotation of  $\chi_{\text{CS}}$  and  $\chi_{\text{SC}}$  (keeping  $C_2$  symmetry) in diethyl disulphide. The *MGM* and *BGB* stationary points are much more prominent features on the HF/3-21G\* energy surface (as shown by preliminary calculations) and may actually disappear all together with still larger basis sets than 6-31G\*. This would just leave a shoulder on the  $C_2$  symmetric energy curve. The geometry modifications taking place in this part of the  $\chi_{\text{CS}}/\chi_{\text{SC}}$  conformational space are, however, very similar at the HF/6-31G\* and HF/3-21G\* levels of theory

*gauche+* and  $-60^\circ$  for *gauche-* rotamers and overall smaller values for  $\chi_{\text{SS}}$ . The 2.5°  $\chi_{\text{SS}}$  reduction from 88.28° to 85.79° in the *TGT* minimum parallels the reduction from 87.4° (HF/6-31G\*) to 84.8° (MP2/6-31G\*\*) calculated for dimethyl disulphide.<sup>14a</sup> Experimental values for dimethyl disulphide are 84.7° (microwave spectroscopy)<sup>13a</sup> and 83.9° (electron diffraction).<sup>13b</sup> The close agreement between the theoretical MP2/6-31G\*\* results and experimental data for dimethyl disulphide gives confidence also to the calculated MP2/6-31G\* geometries for ESSE.

The modest inter-ethyl interactions means that the C-1—C-2—S-1 half of the molecule undergoes very small geometry modifications upon *TGx* and *GGx* rotations. Some effects are discernible, however, when  $\chi_{\text{CS}}$  or  $\chi_{\text{SC}}$  is *gauche-*, and in particular for the *G'GG'* minimum of the *G'Gx* rotation. It has  $\alpha(\text{S-2—C-3—C-4}) = 116.08^\circ$  compared with 114.91° and 114.90° observed in the *GGG'* and *TGG'* minima, respectively (MP2/6-31G\* values).

### S—S rotation

The *GxG* and *TxT* potentials are shown in Figure 4. The *TxT* MP2//HF *trans* and *cis* barriers are 27.86 and 49.77 kJ mol<sup>-1</sup>, respectively (Table 3, *TTT* minimum is 3.73 kJ mol<sup>-1</sup>). The corresponding HF//HF values are lower, 23.75 and 47.90 kJ mol<sup>-1</sup>, and close to the values of 23.02 and 47.18 kJ mol<sup>-1</sup> reported for an HF/6-31G\* refinement of dimethyl disulphide.<sup>14a</sup> Replacement of *trans* dimethyl disulphide H atoms with methyl groups therefore has very little effect on the S—S rotation. In a previous rigid rotor study of *TxT* potential for ESSE at the HF/STO-3G level, *trans* and *cis* barriers were 21.3 and 81.6 kJ mol<sup>-1</sup>, respectively.<sup>24</sup> These values are different from those obtained

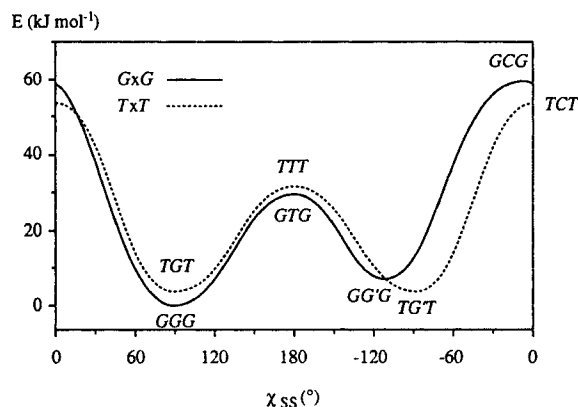


Figure 4. MP2//HF *GxG* and *TxT* potentials for S—S rotation in diethyl disulphide. Stationary points have been indicated on the curves

in the present study, demonstrating the need to use large basis sets and flexible models for estimation of rotational barrier heights.

For the  $GxG$  potential the *trans* barrier height is about the same as for the  $TxT$  potential, but inter-ethyl steric conflict becomes highly significant for the *cis* barrier. This is reflected by the  $59.44 \text{ kJ mol}^{-1}$   $GGG \rightarrow GCG$  energy compared with  $49.77 \text{ kJ mol}^{-1}$  for the  $TGT \rightarrow TCT$  rotation. Still, the observed increase is smaller than might have been expected from simple model considerations. This is because extremely short and energetically unfavourable inter-ethyl  $H \cdots H$  contacts are efficiently avoided by opening  $\chi_{CS}$  and  $\chi_{SC}$  from  $68.28^\circ$  in the  $GGG$  minimum to  $92.56^\circ$  for the  $GCG$  barrier. Supplementary STO-3G calculations indicate that the two torsion angles will even exceed  $100^\circ$  when  $\chi_{SS}$  is in the interval  $(-85^\circ, -25^\circ)$ . The  $GCG$  barrier has  $r(H-12 \cdots H-31)$  and  $r(H-21 \cdots H-42) = 2.252 \text{ \AA}$ , the shortest  $H \cdots H$  distances recorded in this study.

### Comparison with crystal structures

It is of interest to examine the correlation between the calculated stabilities for various ESSE minima and the frequency with which the corresponding disulphide bridge conformations are observed in crystal structures. Surveys of peptides<sup>25</sup> and proteins<sup>1,26</sup> have shown that the  $GGG$  conformation (P-chirality) or the mirror image  $G'G'G'$  conformation (N-chirality) are very common and recurs in numerous disulphide bridges. In particular, the most populated structural family in proteins is the  $G'G'G'G'G'$  ( $\chi_i^1 = \textit{gauche-}$ ,  $\chi_j^1 = \textit{gauche-}$ ) 'left-handed spiral' conformation.<sup>1,26</sup> Disulphides with central  $GGG'$  or  $G'GG$  combinations are also common. Accordingly, the experimental  $r(C^\alpha \cdots C^\alpha)$  distribution has clusters at  $5.8 \text{ \AA}$  ( $GGG/G'G'G$ ) and  $5.0 \text{ \AA}$  ( $GGG'/G'GG$ ),<sup>1</sup> which agree closely with the calculated values for  $r(C-1 \cdots C-4)$  in the  $GGG$  [ $5.84 \text{ \AA}$  (HF),  $5.70 \text{ \AA}$  (MP2)] and  $G'GG'$  [ $4.89 \text{ \AA}$  (HF),  $4.71 \text{ \AA}$  (MP2)] low-energy minima, respectively.

Structures with *trans*  $\chi_{CS}$  or  $\chi_{SC}$  torsions are rare and usually limited to immunoglobulins in association with large  $C^\alpha \cdots C^\alpha$  separations.<sup>1</sup> Only two closely related observations have been recorded for smaller peptides.<sup>27</sup> No example of an undistorted central  $TGT$  conformation has been found. The high-energy  $G'GG'$  conformation, characterized by short  $C^\alpha \cdots C^\alpha$  separations, is uncommon in proteins, but has been observed in  $G'G'GG'G'$  links between anti-parallel  $\beta$ -strands.<sup>1</sup> A similar role is played by this conformation in the model peptides Boc-Cys-Val-Aib-Ala-Leu-Cys-NHMe<sup>28</sup> and [Boc-Cys-Ala-Cys-NHMe]<sub>2</sub>.<sup>29</sup>

### CONCLUSION

The theoretical *ab initio* calculations for diethyl disulphide presented in this paper extend our knowledge of C—C—S—S and C—S—S—C rotations and the understanding of conformational properties of disulphide bridges in general. The estimated energy differences between the six minimum structures are small, but comparison with experimental results shows that nature nevertheless selectively chooses the low-energy conformations for the structurally equivalent disulphide bridges in peptides and proteins.

### SUPPLEMENTARY MATERIAL

Tables 4 and 5 giving complete MP2/6-31G\* molecular geometry for minima, Tables 6-9 giving complete HF/6-31G\* molecular geometry for C—S and S—S rotation stationary points, Table 10 giving unscaled harmonic vibrational frequencies and listings of the archive files from all correlated *ab initio* calculations are available from the author on request, and also by E-mail from c.h.gorbitz@kjemi.uio.no.

### ACKNOWLEDGEMENT

The author thanks the Norwegian Research Council (NFR) for a generous allocation of Cray computer time.

### REFERENCES

1. N. Srinivasan, R. Sowdhamini, C. Ramakrishnan and P. Balaram, *Int. J. Pept. Protein Res.* **36**, 147-155 (1990).
2. (a) J. H. Konnert and W. A. Hendrickson, *Acta Crystallogr., Sect. A* **36**, 344-350 (1980); (b) W. A. Hendrickson, *Methods Enzymol.* **115**, 252-270 (1985).
3. R. Sowdhamini, N. Srinivasan, B. Shoichet, D. V. Santi, C. Ramakrishnan and P. Balaram, *Protein Eng.* **3**, 95-103 (1989).
4. J. P. Bowen and N. L. Allinger, in *Reviews in Computational Chemistry II*, edited by K. B. Lipkowitz and D. B. Boyd, pp. 81-97, VCH, New York (1991).
5. S. J. Weiner, P. A. Kollman, D. T. Nguyen and D. A. Case, *J. Comput. Chem.* **7**, 230-253 (1986).
6. B. R. Brooks, R. E. Braccoleri, B. D. Olafson, D. J. States, S. Swaminathan and M. Karplus, *J. Comput. Chem.* **4**, 187-217 (1983).
7. P. Dauber-Osguthorpe, V. A. Roberts, D. J. Osguthorpe, J. Wolf, M. Genest and A. Hagler, *Proteins* **4**, 31-47 (1988).
8. C. H. Görbitz, unpublished results.
9. C. H. Görbitz, *J. Phys. Org. Chem.* **6**, 615-620 (1993).
10. C. H. Görbitz, *J. Chem. Soc., Perkin Trans. 2*, 259-263 (1994).
11. M. J. Frisch, M. Head-Gordon, G. W. Trucks, J. B. Foresman, H. B. Schlegel, K. Raghavachari, M. Robb, J. S. Binkley, C. Gonzalez, D. J. Defrees, D. J. Fox, R. A. Whiteside, R. Seeger, C. F. Melius, J. Baker, R. L.



- Martin, L. R. Kahn, J. J. P. Stewart, S. Topiol and J. A. Pople, *Gaussian 90*. Gaussian, Pittsburgh, PA (1990).
12. M. J. Frisch, G. W. Trucks, M. Head-Gordon, P. M. W. Gill, M. W. Wong, J. B. Foresman, B. G. Johnson, H. B. Schlegel, M. A. Robb, E. S. Replogle, R. Gomperts, J. L. Andres, K. Raghavachari, J. S. Binkley, C. Gonzalez, R. L. Martin, D. J. Fox, D. J. Defrees, J. Baker, J. J. P. Stewart and J. A. Pople, *Gaussian 92, Revision C*. Gaussian, Pittsburgh, PA (1992).
  13. (a) D. Sutter, H. Dreizler and H. D. Rudolph, *Z. Naturforsch., Teil A* **20**, 1676–1681 (1965); (b) B. Beagley and K. T. McAloon, *Trans. Faraday Soc.* **67**, 3216–3222 (1971).
  14. (a) D. Jiao, M. Barfield, J. E. Combariza and V. J. Hruby, *J. Am. Chem. Soc.* **114**, 3639–3643 (1992); (b) M. Aida and C. Nagata, *Theor. Chim. Acta* **70**, 73–80 (1986); (c) T.-K. Ha, *J. Mol. Struct.* **122**, 225–234 (1985); (d) A. Rauk, *J. Am. Chem. Soc.* **106**, 6517–6524 (1984); (e) V. Renugopalakrishnan and R. Walter, *Z. Naturforsch., Teil A* **39**, 495–498 (1984).
  15. (a) C. Møller and M. S. Plesset, *Phys. Rev.* **46**, 618–622 (1934); (b) J. S. Binkley and J. A. Pople, *Int. J. Quantum Chem.* **9**, 229–236 (1975).
  16. B. J. McClelland, *Statistical Thermodynamics*. Chapman and Hall, London (1973).
  17. K. B. Wiberg and M. A. Murcko, *J. Am. Chem. Soc.* **110**, 8029–8038 (1988).
  18. M. Ohsaku and N. L. Allinger, *J. Phys. Chem.* **92**, 4591–4594 (1988).
  19. M. Ohsaku and A. Imamura, *Mol. Phys.* **55**, 331–339 (1985).
  20. H. E. Van Wart, F. Cardinaux and H. A. Scheraga, *J. Phys. Chem.* **80**, 625–630 (1976).
  21. A. Yokozeki and S. H. Bauer, *J. Phys. Chem.* **80**, 618–625 (1976).
  22. W. Zhao and S. Krimm, *J. Mol. Struct.* **224**, 7–20 (1990).
  23. S. C. Nyburg, C. H. Faerman and L. Prasad, *Acta Crystallogr., Sect. B* **43**, 106–110 (1987).
  24. R. J. Boyd, J. S. Perkins and R. Ramani, *Can. J. Chem.* **61**, 1082–1085 (1983).
  25. C. H. Görbitz, *Acta Chem. Scand.* **44**, 584–590 (1990).
  26. (a) J. Richardson, *Adv. Protein Chem.* **34**, 167–339 (1981); (b) J. M. Thornton, *J. Mol. Biol.*, **151**, 261–287 (1981).
  27. C. M. Falcomer, Y. C. Meinwald, I. Choudhary, S. Talluri, P. J. Milburn, J. Clardy and H. A. Scheraga, *J. Am. Chem. Soc.* **114**, 4036–4042 (1992).
  28. I. L. Karle, R. Kishore, S. Raghobama and P. Balaram, *J. Am. Chem. Soc.* **110**, 1958–1963 (1988).
  29. I. L. Karle, J. L. Flippen-Anderson, R. Kishore and P. Balaram, *Int. J. Pept. Protein Res.* **34**, 37–41 (1989).