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The *trans* opening of ethylene diamine tetra acetic acid bis anhydride (EDTAA) with cystine-di-OMe: one-step synthesis of bihelical systems

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Respectfully dedicated to Professor M.V. George on the occasion of his 85th birthday

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ABSTRACT

The generation of a bihelical (figure of 8) motif has been illustrated by *trans* opening of EDTAA with L-cystine-di-OMe and D-penicillamine disulfide-di-OMe. In the former case the open cyclic system, arising by *cis* addition, was secured as a minor product.

Introduction

Bihelical (figure of 8) structures represent a versatile motif in several domains, with total synthesis of bihelical alanine t-RNA (Yeast), the role of such motifs in the initiation of transcription[1] and the key role it played in the first total synthesis of a gene[2] have made creation of such systems as an objective in several DNA–protein interaction studies. Figure of 8 motifs are increasingly found in toxic cyclic peptides.[3]

In continuation of our interest in figure of 8 motifs[4] we report here the one step formation to such systems by reaction of L-cyst-di-OMe(3) and EDTAA.[5,6] It was envisioned that compound **1** with a staggered NCH_2CH_2N bridge is likely to undergo a *trans* addition with cyst-di-OMe, harboring an orthogonally disposed –S–S– unit, leading to a bihelical system. In the event this proved largely correct (Scheme 1).

Synthesis

The reaction of L-cystine with trimethylsilyl chloride in dry MeOH solution stirring overnight and concentration, followed by crystallization from ether, afforded cyst-di-OMe di hydrochloride **2**, mp: 164 °C in quantitative yields.[7] The free base 3, generated in ~74% yields with aqueous sodium carbonate, extracted with methylene chloride and then evaporated, was used without delay. An ice cooled and stirred suspension of **1** in CH_2Cl_2, when mixed with, in drops, over 1 h, to an equivalent amount of freshly prepared **3** in CH_2Cl_2 gave a clear solution. The product precipitated slowly and was completed by leaving stirred for overnight and filtered to afford a powdery white solid, whose mass spectra confirmed the formation of a 1:1 adduct (73%, mp: 178–184 °C). The adduct was insoluble in most solvents. To a suspension of this in MeOH freshly prepared diazomethane was added and the resulting tetramethyl ester chromotographed on silica gel. Elution with chloroform/methanol = 98:2 afforded 0.150 g of solid that showed molecular weight expected for the 1:1 adduct ester (57%). However the ^1H NMR in $CDCl_3$ showed the presence of two amide protons at 8.45 and 8.1 ppm in the ratio of ~7:3 (in DMSO-d_6 both the amide protons were shifted to 8.36 and 8.22, respectively).

HPLC performed in a biomed C_4 column and elution with a linear gradient of A–B (A = H_2O, 0.1% TFA; B = CH_3CN, 0.1% TFA) showed largely a mixture of two peaks in the ratio of 75:25 with retention times, 9.016 and 12.039 min, respectively.

Careful chromatography enabled the separation of the mixture to their pure components. The mass spectra showed that both were

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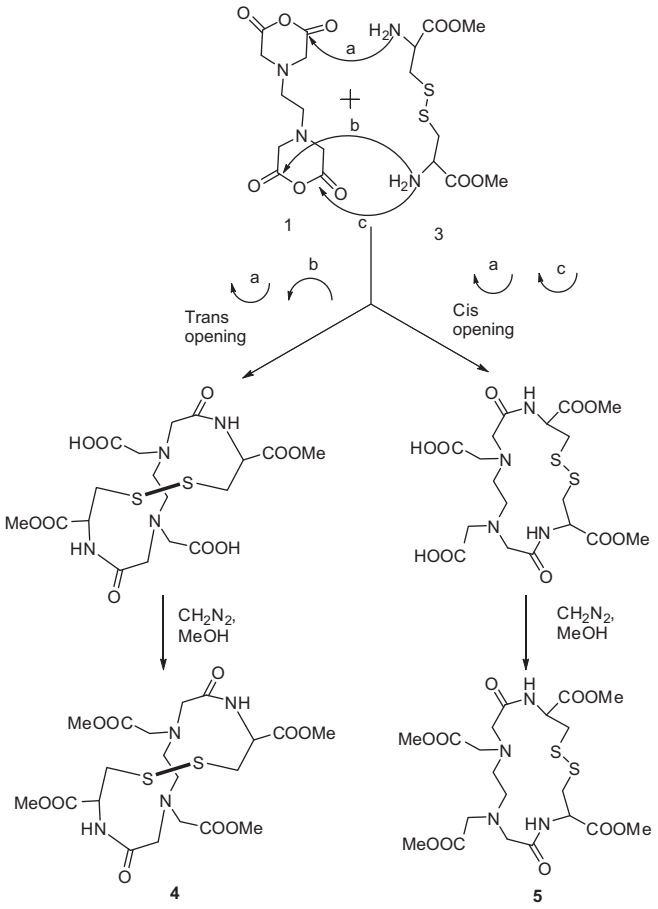
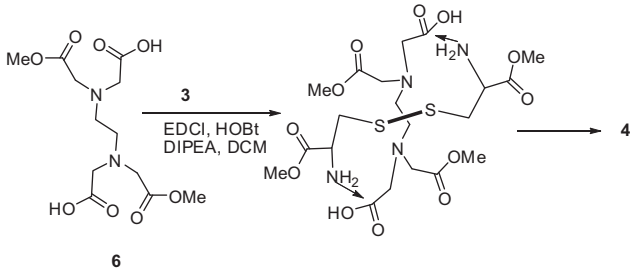


Scheme 1. Opening of EDTAA with L-cyst-di-OMe.

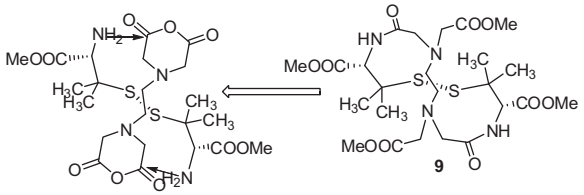
1:1 adducts. The major isomer is identified as **4** and the minor **5**. Their ^{1}H and ^{13}C NMR (**S1**–**S4**) had a similar profile excepting for the significant differences in the appearance of the amide and C$^{\alpha}$H Protons. Detailed studies (vide infra) have established the bihelical structure for **4,** arising from a *trans* opening of **1** and an open cyclic structure for **5** from the alternate *cis* mode (Scheme 1).

Further proof for the bihelical structure for **4** was secured from **6** obtained in quantitative yields from methanolic opening of **1** (Scheme 2) for which MO calculations showed an overwhelming preference for a configuration having transoriented CH_2COOMe groups and a staggered conformation for the $-NCH_2CH_2N-$ bridge, an arrangement that is expected to undergo cyclization, in a *trans* mode with cyst-di-OMe, leading to **4**. Indeed, the condensation of **6** with cyst-di-OMe (**3**) gave exclusively **4**.

To explore the effect of steric factors on the course of the adduct formation, **1** was condensed with D-penicillamine disulfide



Scheme 2. Condensation of **6** with cyst-di-OMe.



Scheme 3. Opening of EDTAA with D-penicillamine disulfide-di-OMe.

di-OMe, where the $-SCH_2-$ pairs of **3** are replaced by $-S(CH_3)_2-$, precisely under conditions described for **3**. The reaction exclusively afforded in 62% yields **9**, the methyl analog of the bihelical **4** (Scheme 3), whose spectral properties were completely in agreement with the assigned structure.

^{1}H NMR studies

The primary focus of NMR studies was on **4** and **9**, which have been assigned bihelical structures and **5**, a cyclic profile. Compounds **4** and **5** arise respectively, by the *trans* opening of **1** and the alternate *cis* mode with L-cystine di-OMe (Scheme 1). The sterically crowded D-pencillaminedisulfide-di-OMe offered only bihelical **9** by *trans* opening of **1** (Scheme 3). Extensive studies clearly show that **4** and **9** have a compact profile in contrast to a flexible one for **5**. Temperature dependent NMR studies in DMSO-d_6 in the range of 30–60 °C showed for the NH protons of pure **4** and **5**, dδ/dT values −3 ppb/K and −2.5 ppb/K and linear decay of their chemical shifts, suggesting strongly that the amide NH is involved in intra molecular hydrogen bonding in both cases.

The ^{1}H NMR of bihelical **4** as well as **9** and cyclic **5** is in support of the structural assignment and clearly distinguishes the structural profile. In **4**, **5**, and **9** each proton of CH_2COOMe and NCH_2CO is clearly resolved as doublet suggestive of distal positioning of these groups.

An expanded version of ^{1}H NMR of bihelical **4** and cyclic **5** (Fig. 1) in the region δ 2.7–3.6 ppm presented below suggests features that are in agreement with the proposed structures.

In **4** the $-NCH_2$ CH_2 N− protons appear as clean doublets at δ 2.7 and 2.92 ppm and in **5** as a clustered multiplet at 2.87. We suggest that in the bihelical structure **4** the orthogonal placement of S–S bridge makes such divergence in chemical shifts. The β CH_2 (doublets) protons in **4** and **5** are seen as a pair of doublet of doublets. The eight NCH_2CO protons (doublets) are seen in **4** (δ: 3.3, 3.49, 3.53, 3.6) and in **5** (δ: 3.34, 3.44, 3.50, 3.56). We feel that in the bihelical **4** the ring NCH_2CO protons appear as cluster with the external NCH_2CO as widely separated doublets. A similar profile like **4** was seen in the bihelical **9. I**n the cyclic **5** they are closely spaced.

The ROESY spectra of **4**, **5**, and **9** (500 MHz, $CDCl_3$) clearly provided support for the structural assignments. At the outset a ROESY spectrum of the mixture enabled a direct comparison of the spatial connectivities of the amide NH at δ 8.45 ppm of **4** and that of **5** at δ 8.2 ppm. The ROESY spectrum of **4** (Fig. S7, Supplementary data) showed that the NH peak at 8.45 ppm exhibited spatial relationship with $-NCH_2$ CH_2 N-(weak), β $CH_2$and NCH_2CO, and C$^{\alpha}$H protons.

The ROESY spectrum of **5** (Fig. S8, Supplementary data) showed that the NH peak at 8.2 ppm is spatially connected to $-NCH_2$ CH_2 N-(strong), β CH_2-, NCH_2CO, and C$^{\alpha}$H protons. The ROESY spectrum of **9** (Fig. S9, Supplementary data) exhibited the spatial relationship between the amide protons with that of the methyl protons, the well separated $-NCH_2CH_2N-$ protons as well as doublets formed by protons of NCH_2CO and $-CH_2COOMe$ with clarity.

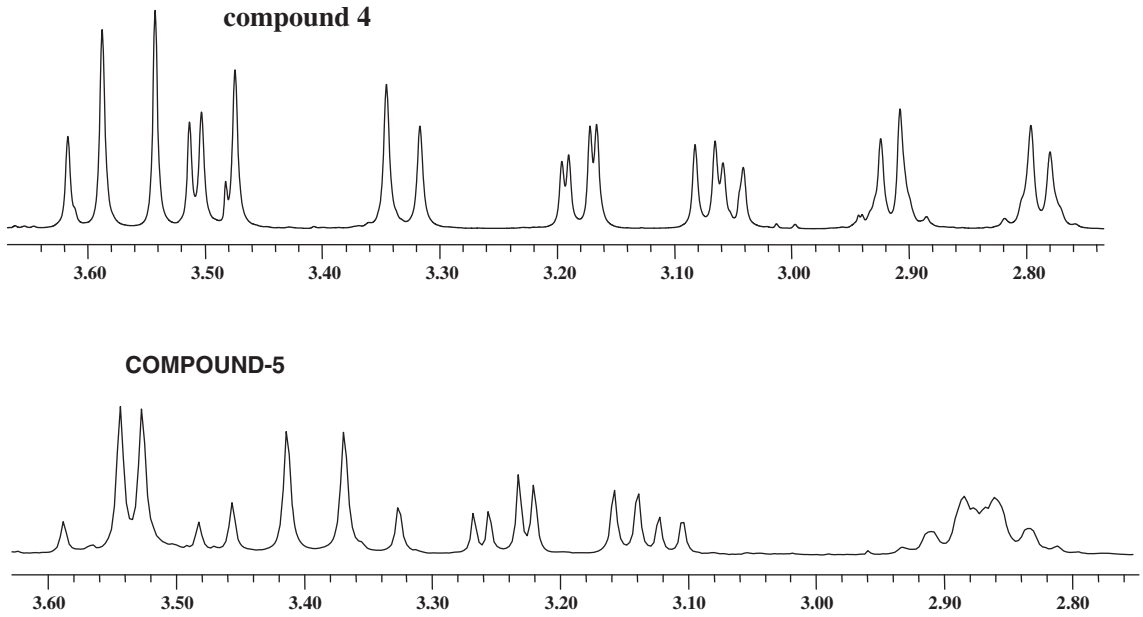


Figure 1. Expanded spectrum of compound **4** and **5**.

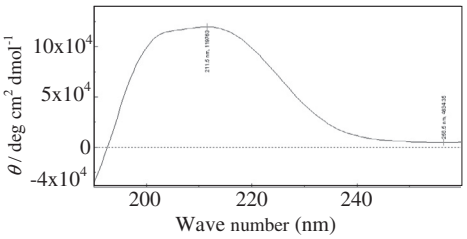


Figure 2. CD spectrum of **4**.

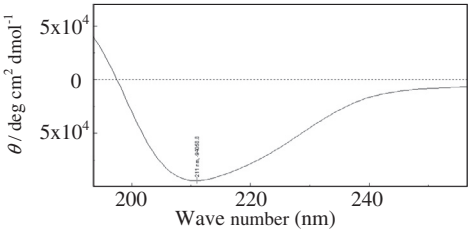


Figure 4. CD spectrum of **9**.

In conclusion the 2D NMR studies clearly support the assigned structures for **4**, **5**, and **9**.

Optical Rotation

The optical rotation of **4**, secured from L-cystine exhibited a value of $[\alpha]_D^{23}$ +112.4° (c = 0.5 $CHCl_3$) and for **9** from D-penicillamine disulfide $[\alpha]_D^{23}$ −106.8°(c = 0.25 $CHCl_3$). These suggest, as reported in our earlier work,[4] that the chirality of the bihelical topology is largely controlled by that of the linker. In sharp contrast, the optical rotation of the open module **5** was found to be $[\alpha]_D^{23}$ −30.0° (c = 0.4 $CHCl_3$).

Circular Dichroism Studies

The results from CD studies in trifluoroethanol(TFE) for compounds **4**, **5**, and **9**, provided strong support for the bihelical nature of **4** and **9**. The CD of **4**, secured from L-cystine, showed a band at 211 nm with positive ellipticity (Fig. 2) similar to the spectra of peptides adopting a β-turn configuration. The CD of **9** (Fig. 4), from D-penicillamine disulfide showed a mirror profile to that of **4**, with a negative band at 211 nm, again suggesting a β-turn configuration. The CD of **5** in TFE (Fig. 3) showed a shallow positive band at 219 nm, suggesting a flexible conformation.

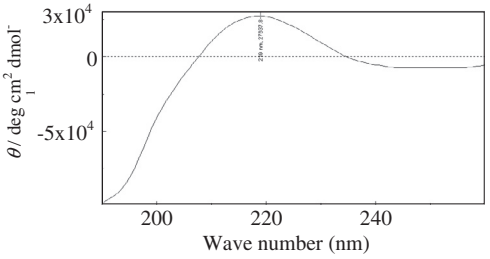
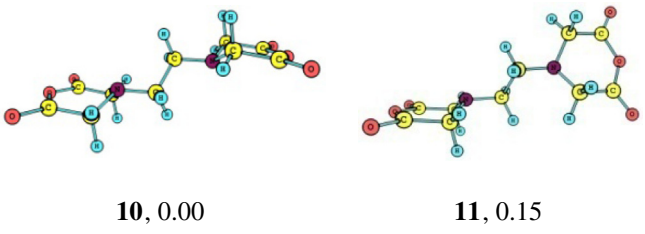


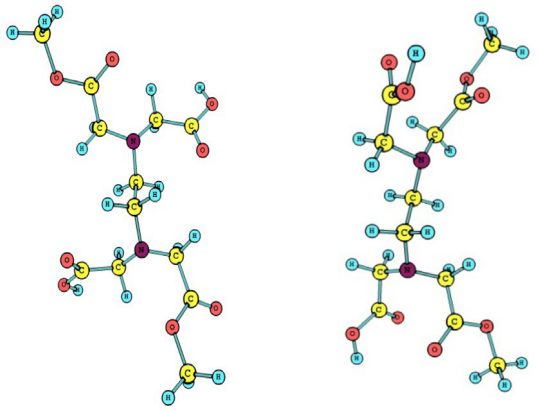
Figure 3. CD spectrum of **5**.

Molecular orbital calculations

Optimized energy calculations were done using *ab initio* and high level density functional theory (M06-2X/6-31G*) using GAUSSIAN 09 program.[8] As described in the present work, the bihelical compound **4** has been secured by transition state that overwhelmingly prefers a trans mode of amidation of EDTAA **(1)** and the



Scheme 4. The M06-2X/6-31G* energy minimized structures of EDTAA in two conformations. M06-2X/cc-pVTZ//M06-2X/6-31G*, relative energies are given in kcal/mol.[8]



Scheme 5. Enery minimized conformations of possible methanol opened product of **1,** optimized at M06-2X/6-31G* level of theory. The M06-2X/cc-pVTZ//M06-2X/6-31G* relative energies are given in kcal/mol.

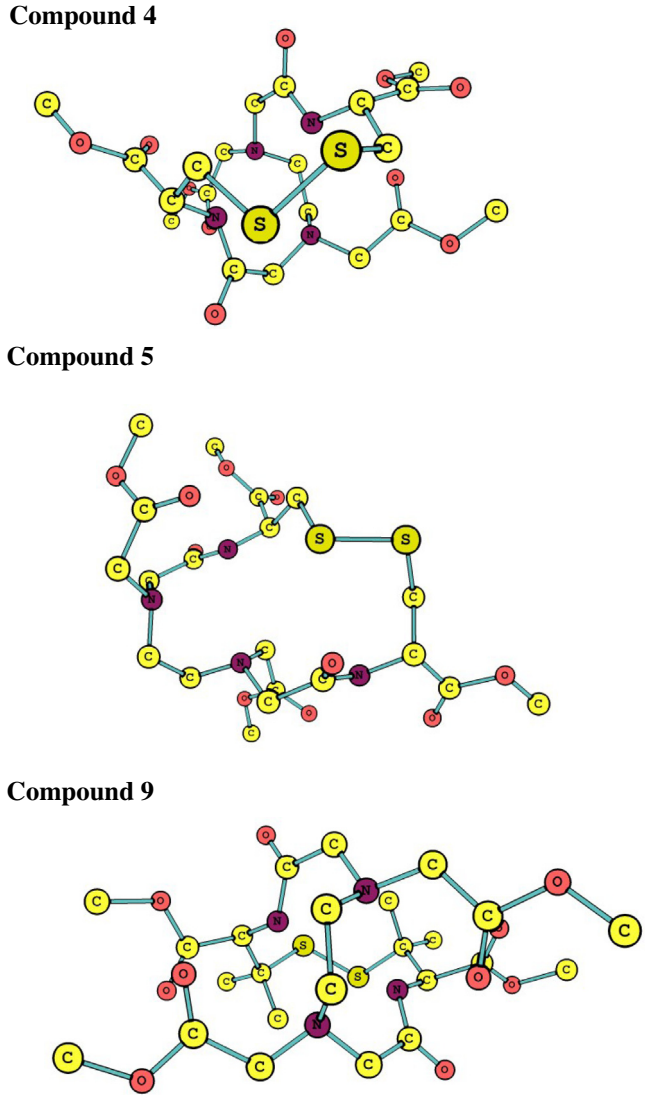


Figure 5. The M06-2X/6-31G* energy minimized structures of **4**, **5**, and **9**.

methanol opened di ester **6** with cyst-di-OMe harboring an orthogonally disposed –S–S– profile. Molecular orbital calculations show that **1** has two closely related minimum energy representations **10** and **11**, where both the representations have staggered $–NCH_2$ $CH_2N–$ conformation (Scheme 4).

Calculation shows that the methanolic opening overwhelmingly prefers a trans mode suggesting a conformation of **12** over **13** thus supporting the bihelical structure for the condensation product with cyst-di-OMe (Scheme 5). These findings clearly support the bihelical structure for the condensation product with cyst-di-OMe (Scheme 2).

The energy minimized conformations for the bihelical **4** and **9** and the cyclic **5** are presented in Figure 5.

Apart from mechanistic and spectral considerations the M.O calculations provide a clean picture of the double helical nature of **4**, **9** and a cyclic profile for **5**.

In compound **4** from L-cystine the –S–S– bridge forms part of a right handed helix while the $–NCH_2CH_2N–$ containing link is left handed. The situation is precisely reversed in **9** derived from D-D-penicillamine disulfide. As expected in the open **5** these two elements are nearly parallel.

Conclusion

The present work suggests a simple strategy for securing bihelical modules with diverse capabilities. Indeed **4** and **9** endowed with pair of CH_2COOMe and COOMe can be precursors for protein clusters, DNA recognition systems, and potential drug targets.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.11.078.

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