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Feasibility study on the reaction of 1,4-diazabicyclo[2.2.2]octane (DABCO) with (L-Serine-L-Serine) and (L-Phenylalanine-L-Serine) diketopiperazines

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This paper summarizes the reaction of DABCO with the enol tosylate derivatives made from (L-Ser-L-Ser) and (L-Phe-L-Ser) diketopiperazines (DKP's). The reaction between DABCO and *EE*-di-tosylate (L-Ser-L-Ser) DKP (**2**), results in the isomerization of the serine di-tosylate from *EE*-**2** to *ZZ*-**2.** This is the first direct example of the utility of DABCO as a reagent demonstrating the successful isomerization in a DKP derivative. The *E*-enol tosylate of (L-Phe-L-Ser) DKP (4) upon reaction with DABCO provided a unique bis-ylidiene product (**5**).

1. Introduction

2,5-diketopiperazines (DKP's), such as compounds (**1**) and (**3**), have potential to serve as building blocks for important class of compounds.[1-9] The presence of these 2,5-DKP's in bioactive natural products, their synthetic utility and applications in medicinal chemistry is excellently reviewed by Borthwick.[10] A detailed description of the application of these 2,5-DKP's in peptide and combinatorial chemistry is thoroughly reviewed by P.M. Fischer.[11] 2,5-DKP's have continued to establish impact on the frontiers of organic, structural, and medicinal chemistry.[12] DKP derivatives, such as enol di-tosylate (**2**) and enol tosylate (**4**), can serve as building blocks for a class of important compounds called endiamino peptides [compounds bearing a 1,3-diaminoethenyl functional group].[13] Endiamino bonds can induce a conformational rigidity in peptides and hydrogen bonding assisted enzyme sulfatases catalysis. It is worth to mention, that Callynormine A, is a recently isolated marine metabolite from Kenyan sponges *Callyspongia abnormis,* appears as a rare N-atom containing heterodetic peptide that

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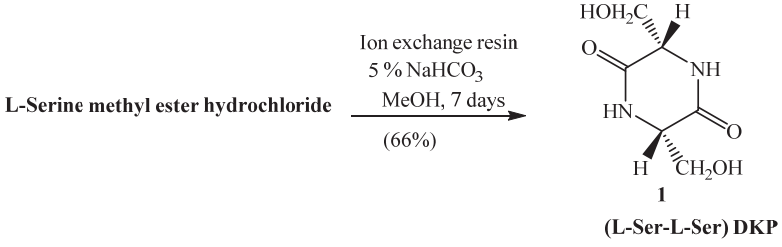
has the *Z*-endiamino group as the key structural element that is proposed to induce conformational rigidity.[14] The role of 1,3-diaminoethenyl structural unit in enzyme sulfatases activity via a serine modification (prokaryotes) or a cysteine modification (eukaryotes and prokaryotes) is studied also.[15] An example of a serine type sulfate is the well characterized bacterial arylsulfatase of *Klebsiella pneumoniae*.[16,17]

This paper details the preparation of the (L-Ser-L-Ser) DKP (Scheme 1), followed by oxidation to a stable *EE*- (L-Ser-L-Ser) DKP enol di-tosylate also referred here as *EE*-serine di-tosylate, using the dimethyl sulfoxide solution in dimethylformamide as oxidants in the presence of activator para-toluenesulfonyl chloride, i.e. the modified Moffat conditions (Scheme 2). Furthermore, the successful conversion of *EE* serine di-tosylate by reaction with DABCO to its *ZZ* isomer (Schemes 3 & 4) is probed by NMR studies and we now present the details of this isomerization reaction. The presented work serves as the first direct example where DABCO facilitates isomerization of the (L-Ser-L-Ser) DKP enol di-tosylate.

We also studied the reaction of DABCO with **4**, the enol tosylate prepared from (L-Phe-L-Ser) DKP. Scheme 5, shows the preparation of compound **3**, (L-Phe-L-Ser) DKP. Modified Moffat oxidation of **3** produced the *E*-enol tosylate **4** as shown in Scheme 6. We have previously reported, that compound **4** undergo reaction with 1˚and 2˚ amines to yield products bearing 1,3-diaminoethenyl moiety.[13,18] However, when 3 equivalents of DABCO were used as a tertiary amine to react with compound **4** (Scheme 7), it resulted in a unique elimination bis-ylidiene product **5**.

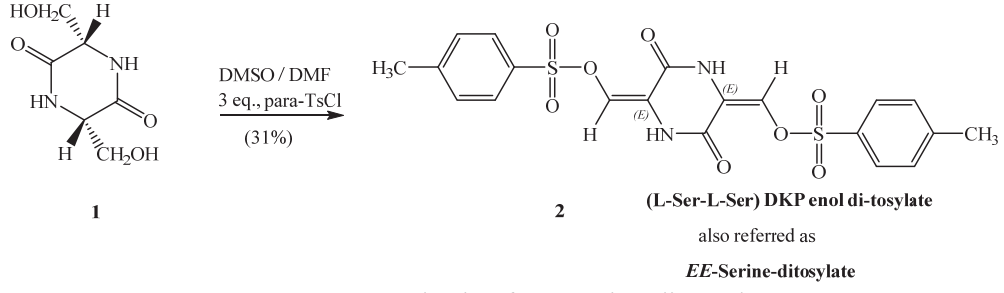
2. Results and Discussion

Scheme 1, shows the preparation of compound **1**, (L-Ser-L-Ser) DKP, obtained by passing of a solution of L-Serine methyl ester hydrochloride in methanol through an ion exchange resin column, that was pre-treated with 5% sodium bicarbonate. The desired dimer was obtained in 66% yield.



Scheme 1. Synthesis of (L-Ser-L-Ser) diketopiperazine

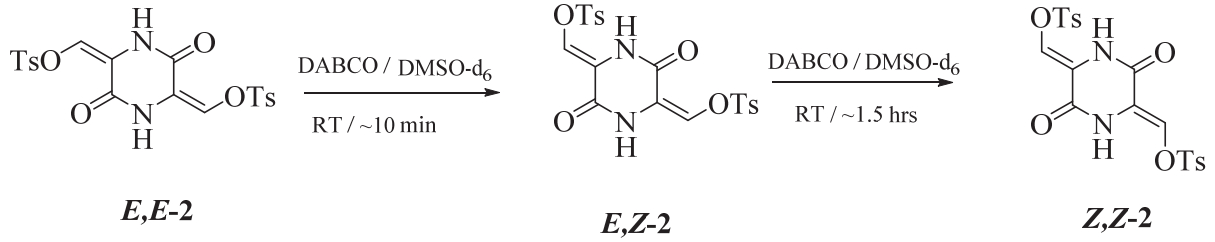
The oxidation of the L-Serine dimer (**1**) was carried out by employing modified Moffat oxidation conditions (Scheme 2) to yield **2** in 31% yield. The dimethyl sulfoxide solution in dimethylformamide was applied as oxidants in the presence of the para-toluenesulfonyl chloride and triethylamine at -5 ºC.



Scheme 2. Synthesis of *EE*-Serine di-tosylate

The ^{1}H and ^{13}C NMR spectra for compound **2** are consistent with the structure of di-tosylate. The ^{1}H-NMR spectrum showed the characteristic vinyl proton at δ 6.80 ppm, and the amide proton located downfield at δ 10.87 ppm. The stereochemistry at the double bonds was determined through 1D differential NOE experiments. Upon irradiating the amide proton at 10.87 ppm, we observed an NOE signal build up at the vinyl positioned at δ 6.80 ppm. The reciprocal experiment of irradiating the vinyl proton and the corresponding NOE signal build up at amide proton site at δ 10.87 ppm, confirmed and attested to the observation that only the *E,E* geometry assignment for the compound di-tosylate could give rise to such NOE signal effects. Based on these two correlating NOE signal results, the geometry around the double bond center is confirmed to be *E,E*. The ESI/MS mass spectrum of **2** contained a molecular ion $[M+NH_4]^+$ peak at 496 Da.

The isomerization reaction of serine di-tosylate (Scheme 3) was carried out in an NMR tube on a micro scale level. Experimentally, the ***E,E*-2** serine di-tosylate was dissolved in 0.6 mL of DMSO-d_6, and 3 equivalents of DABCO was added. The reaction, monitored by means of ^{1}H-NMR spectroscopy, was complete in less than 2 hours furnishing the isomeric product ***Z,Z*-2**.



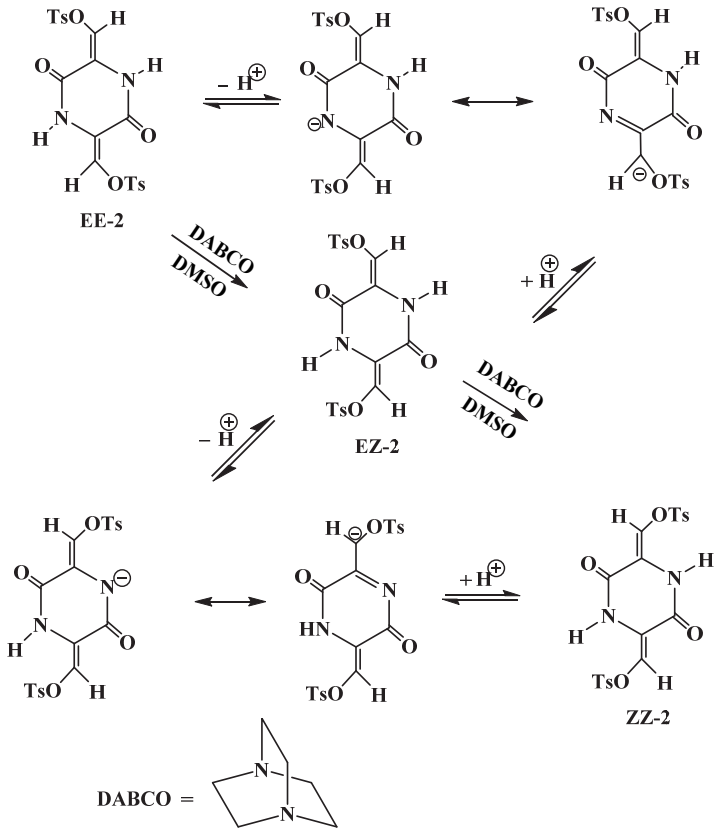
***E,E*-2** ***E,Z*-2** ***Z,Z*-2**

Scheme 3. Conversion of *E,E*- to *Z,Z*- isomer of L-Serine di-tosylate.

The ^{1}H-NMR spectra recorded at times ranging from 5 minutes to 2 hours of reaction run, led to a series of events that helps us to understand the molecular process behind the mechanism of this reaction. At first, upon addition of DABCO, we noticed that within 5 minutes the amide proton at 10.87 ppm for the *EE* isomer disappeared completely from the ^{1}H-NMR spectra either by chemical exchange or by deprotonation. Also, we noticed no change in chemical shift of vinyl group observed at δ 6.80 ppm. After the 10 minutes of reaction run, two new signals evolved at 6.59 ppm and 5.58 ppm. The intensity of the two signals appeared similar. Because of the proximity of the 6.59 ppm signal to *EE* isomer at 6.80 ppm, we assigned this to the *E* of *EZ* isomer in the test sample. The other signal which corresponds to the *Z* of *EZ* in the test sample was observed at 5.58 ppm. During this period, we observed the evolution of a less intense neighbor signal at 5.85 ppm, which we assign as the *ZZ* isomer.

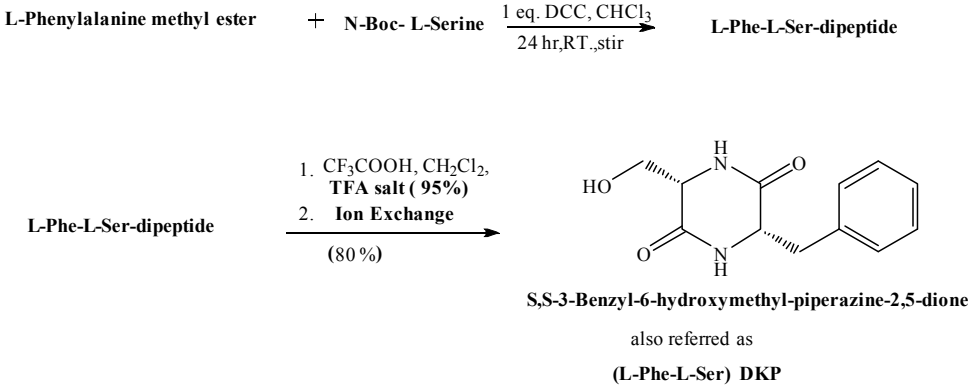
With the progress of time (47 min), the intensity of the *Z,Z* signal at 5.85 ppm continued to grow and the intensity of the signal for the Z of EZ part at 5.58 ppm diminished. Finally, after 110 minutes, we observed complete loss of the EZ component in the test sample. The reaction was complete with *ZZ* isomer as the sole product with a chemical shift of 5.85 ppm. Thus, we were able to monitor the molecular evolution for this isomeric conversion of *EE* di-tosylate to *ZZ* product including the formation of intermediate components and identify the signals for the *E* and *Z* features of *EZ* isomer, using simple ^{1}H NMR techniques.

Based on the above details of the sequence of ^{1}H NMR events, the following mechanism of conversion of *EE* di-tosylate to its *ZZ* isomer is depicted on Scheme 4. The further NOE experiments concentrated on observation of interaction on NH and CH protons in the *ZZ* form, as expected, have not shown any NOE correlations.



Scheme 4. Mechanism of DABCO reaction with enol di-tosylate of (L-Ser-L-Ser) DKP

Scheme 5, details the synthesis of (**3**). The free amino acid methyl ester of L-Phenylalanine was extracted by diethyl ether from a solution of L-phenylalanine methyl ester hydrochloride in 50% potassium carbonate. This freshly prepared L-Phenylalanine methyl ester was immediately dissolved in chloroform, and N-Boc-Serine as well as dicyclohexyl carbodiimide were added. The mixture was stirred at room temperature for 24 hours. Formed dicyclohexyl urea was filtered off, and after the solvent removal protected dipeptide was obtained.

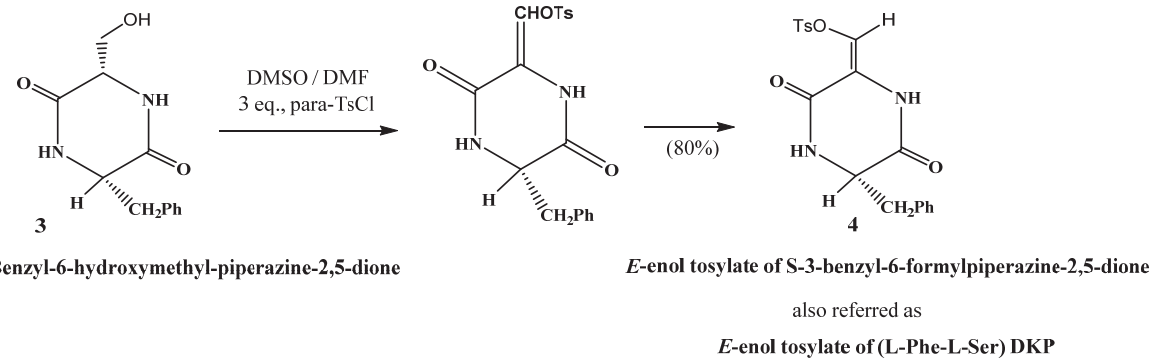


Scheme 5. Syntheses of (L-Phe-L-Ser) DKP

Treatment of the dipeptide with an excess of trifluoroacetic acid in dichloromethane resulted in the deprotection of Boc-group from the dipeptide. The triflouroacetate salt, obtained as a white powder, was next refluxed in methanol, and chloroform; however, these attempts to obtain the diketopiperazine product were unsuccessful. Removal of the triflouroacetate counter ion seems to be critical for the cyclization step. The triflouroacetate salt was dissolved in methanol and passed through an ion-exchange column, that was pre-treated with 5% $NaHCO_3$. Subsequent solvent evaporation resulted in

a white precipitate. The precipitate was refluxed in chloroform for 24 hours to yield the DKP product - *S,S*-3-benzyl-6-hydroxymethyl-piperazine-2,5-dione, in 80% yield. This methodology offers simple synthetic procedure with improved yield in comparison to existing DKP literature methods.[19,20]

The synthesis of the enol tosylate **4** was completed in 80% yield (Scheme 6) using the modified Moffatt conditions as it was mentioned above.[18] According to the NOE correlations we have state that enol tosylate **4** adopts an *E*-stereochemistry of the double bond.[13]



S,S-3-Benzyl-6-hydroxymethyl-piperazine-2,5-dione

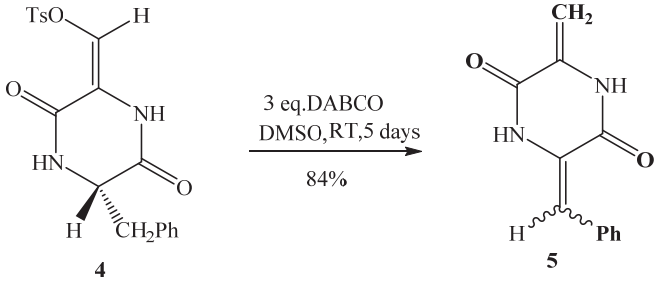
***E*-enol tosylate of S-3-benzyl-6-formylpiperazine-2,5-dione**

also referred as

***E*-enol tosylate of (L-Phe-L-Ser) DKP**

Scheme 6. Synthesis of *E*-enol tosylate of (L-Phe-L-Ser) DKP

The *E*-enol tosylate **4,** dissolved it in a minimum amount of dimethyl sulfoxide and in the presence of 3 equivalents of DABCO at room temperature during 5 days, undergo the transformation which after the quenching with cold water furnish bis-ylidiene **5** as an yellow tinted solid precepitate in 84% yield (Scheme 7).



Scheme 7. Formation of bis-ylidiene product **5**

The ^{1}H, ^{13}C, and COSY NMR spectra of **5** acquired in DMSO-d_6 were consistent with the structure of ylidiene product. In the ^{1}H NMR spectrum, the signals at 4.95 and 5.30, and 6.75 ppm are assigned to the methylene and the vinyl groups, respectively. The signals of two amide protons are observed at 10.11 and 10.95 ppm. In the ^{13}C NMR spectrum the characteristic vinyl group carbon appears at 100.97 ppm. The (^{1}H-^{1}H) COSY showed correlation between proton signals at 4.95 and 5.30 ppm, which confirmed the coupling between the two methylene protons, associated with this ylidiene product. The structure was further confirmed by mass spectrum, which contained a molecular ion peak of 214 Da.

3. Conclusions

2,5-diketopiperazines (DKP's) such as compounds **1** and **3** as well as corresponding enol tosylates **2** and **4** continue to generate interest in the chemical community through their presence in diverse chemical compounds having potential applications in organic and medicinal chemistry. We have demonstrated herein that, in the presence of DABCO, symmetric *EE* enol di-tosylate, derived from DKP of (L-Ser-L-Ser), undergo the isomerization reaction to form the *ZZ* enol di-tosylate. In the same time, the enol tosylate, derived from DKP of (L-Phe-L-Ser), in the presence of DABCO and DMSO

undergo an unique transformation to yield bis-ylidiene product **5**. Such DKP modifications have potential utility as synthons and can induce a conformational rigidity in peptides.

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4. Experimental

4.1. Materials and Methods

^{1}H and ^{13}C NMR spectra were recorded on a JEOL 400 NMR instrument operating at 400 and 100 MHz, respectively. Decoupling ^{1}H experiments were acquired on BRUKER AM 300 MHz. All spectra were recorded using DMSO-d_6 as the NMR solvent. Proton and carbon spectra were referenced to δ 2.50 and 39.50 ppm. Nuclear Overhauser (NOE) experiments were performed after measuring the necessary T1 relaxation for protons under study. Finnigan TSQ instrument with a direct probe insertion module, Mariner ESI/MS, and GC/MS from HP, were used to determine mass measurements. Optical rotations were measured by using either Rudolph Autopol III or Polyscience SR-6 polarimeter. Melting points are obtained using MEL-TEMP capillary melting point apparatus and are uncorrected. All solvents and amines were distilled prior to use and stored under nitrogen.

4.2. General procedure; and 4.3 Physical and Spectral data

Preparation of (1), (L-Ser-L-Ser) DKP

A 32 g ion-exchange column bed was made in a regular column chromatography set up. This was pretreated with 200 mL of 5% aqueous sodium bicarbonate, 240 mL of distilled water and 150 mL of methanol. 5 g of L-Serine methyl ester hydrochloride was dissolved in 50 mL methanol. This methanolic solution was passed through the treated ion exchange column and additional 200 mL of methanol was used to run the column to collect the free ester. The solvent was evaporated and the resulting oily substance was stored at room temperature for 7 days. A faint yellow colored solid was obtained and this was co washed with 2.5 mL each of ether and methanol and filtered, air dried to get (3.68 g) 66% yield of product cyclo (L-Ser-L-Ser) DKP.
^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.6 (s, 6H), 5.0 (s, 2H), 8.0 (s, 2 NH's) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 56.8, 63.4, 166.0 ppm.

Synthesis of (2), di-tosylate of (L-Ser-L-Ser) DKP

In a 10 mł round bottom flask cyclo L-Ser-L-Ser 0.0872 g (1 eq.) was dissolved in 1 mL of dry and distilled dimethyl sulfoxide in hot condition and 0.5 gm of 4 Aº of molecular sieves was added and stored overnight. In a separate 35 mL oven dried round bottom flask that was maintained under nitrogen at -5 ºC, para-toluensulfonyl chloride 0.958 g (2.5 eq.) was added and to this 1:1 (1.25 mL each) of dry dimethyl formamide and dry dimethyl sulfoxide was added to dissolve the solids. To this solution was added the dried DKP alcohol and continuously stirred under nitrogen at -5 ºC. After 18 minutes triethylamine 1130 µL was added and the reaction flask was brought to room temperature and was stirred for additional 1 hour. The reaction mixture was quenched with 25 mL pre cooled ice cold water to allow for the precipitation of the product di-tosylate 0.0568 g, 31% yield.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.4 (s, 6H), 7.5 (4H, d, *J* = 8.04 Hz), 7.9 (4H, d, *J* = 8.04 Hz), 10.8 (s, 2 NH's) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 21.75, 119.21, 125.08, 128.61, 130.91, 146.9, 155.8 ppm. Mass spectrum: ESI/MS; molecular ion peak $[M+NH_4]^+$ at 496 m/z.

Isomerization reaction of (2), *E,E* Serine di-tosylate by DABCO

In a typical micro-scale setup the reaction was done in a NMR tube. The *EE* di-tosylate (2) was dissolved in 0.6ml of DMSO-d_6 in an NMR tube and to this 3 eq. of DABCO was added and monitored using ^{1}H NMR at room temperature. The reaction of di-tosylate of dimer of L-Serine with DABCO is relatively much faster than in comparison to the enol tosylate made from Phenylalanine. The reaction was completed in less than 2 hours to form the *ZZ* Serine di-tosylate product isomer as judged by ^{1}H, and further confirmed by ^{13}C NMR.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.3 (s, 6H), 7.5 (4H, d, *J* = 7.72 Hz), 7.9 (4H, d, *J* = 7.68 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 21.34, 115.32, 126.06, 128.56, 128.72, 145.92, 158.60 ppm.

Procedure for the preparation of the L-Phe-L-Ser dipeptide

Preparation of the methyl ester of L-Phenylalanine

L-Phenylalanine methyl ester hydrochloride 5.0 g. (25 mmol), was dissolved in 20 mL water. This was treated with a solution of potassium carbonate K_2CO_3 (5.0 g, in 10 mL water). The mixture was extracted with ether (4 x 25 ml). The ether extracts are pooled and dried over magnesium sulfate and the solvent was evaporated to obtain 3.67 g, (20.11 mmol, 80.4% yield) of methyl ester. **Note:** The free ester is freshly prepared and used immediately. The free ester can potentially dimerize if allowed to stand for a long time even with storing in refrigerator.

Dipeptide formation

In a 250 mL round bottom flask, 3.6 g (20.11 mmol) of freshly prepared L-phenylalanine methyl ester was added to 50 mL chloroform. This was charged with 4.127 g (20.11 mmol) of N-Boc-L-Serine and (4.12 g, 20.11 mmol) of DCC (dicyclohexyl carbodiimide) and additional 100 mL of chloroform was added. The contents of the flask were stirred for 24 hours during which pronounced formation of white solid is seen. This obtained dicyclohexyl urea (DCU) was carefully filtered and the solvent was evaporated to get the protected dipeptide 10.9 gm by weight as a semi-solid and was used as such for further deprotection.

Deprotection of the Boc- group from the dipeptide.

The above dipeptide was dissolved in 50 mL dichloromethane and to this 25 mL of Trifluoroacetic acid was added in one portion. An additional 70 mL dichloromethane was added and stirred for 24 hours. The progress of the reaction can be monitored by TLC, chloroform: methanol (9:1) for the deprotection of the Boc-group as evidenced by the single spot for the product. Subsequent removal of the solvent yielded a yellow oily substance and this upon triturating with cold ether gave 7.35 g, (94.47%) of the triflouroacetate salt as a white solid. M.pt. = 145 ºC.

Synthesis of (3), S,S-3-benzyl-6-hydroxy-methyl-piperazine-2,5-dione (DKP)

A column chromatography type set up was made using a 32 g bed of weak base type ion exchange [Amberlyst 26 hydroxide ion (OH)] resin. The bed was previously washed with 200 mL of 5% aqueous sodium bicarbonate $NaHCO_3$, 240 mL of water, and 150 mL of methanol. The triflouroacetate salt (7

g) was dissolved in 50 mL of methanol. This methanolic solution was allowed to pass on the column. The flow rate of the column was adjusted so that the free dipeptide was obtained slowly upon washing the column by adding 300 mL of methanol. Evaporation of the solvent and subsequent reflux of the resulting white solid in 200 mL of chloroform for 24 hours afforded the title DKP compound in 80% yield (3.17g). M. Pt. 242 ºC. $[\alpha]_D^{25}$ = -71.6 (*c*0.1, DMSO). NMR data match the literature set of resonances reported in the case of structurally similar DKP found as a co metabolite in the natural product isolated from *Verticillium hemipterigenum* BCC 1449.[21]
^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.83 (ddd, 1H), 2.98 (dd, 1H), 3.09 (dd, 1H), 3.28 (ddd, 1H), 3.65 (m, 1H), 4.05 (m, 1H), 4.88 (t, 1H), 7.17 (dd, 2H), 7.22 (t, 1H), 7.28 (dd, 2H), 7.91 (s, 1NH), 8.02 (s, 1NH) ppm . ^{13}C NMR (100 MHz, DMSO-d_6): δ = 39.76, 55.39, 57.03, 63.03, 126.39, 128.0, 129.85, 136.55, 165.65, 166.44 ppm.

Preparation of (4), Enol tosylate of (L-Phe-L-Ser) DKP

In a 50 mL round bottom flask, 0.468 g (2 mmol) of the DKP S-3-benzyl-6-hydroxy-methyl-piperazine-2,5-dione was dissolved in 26 mL of hot tertiary butanol and 1.8 g of 4 Å molecular sieves was added to this and stored overnight. After removal of the molecular sieves the solvent was evaporated and a solid was obtained. This solid was taken into a 50 mL round bottom flask and was dissolved in 10 mL distilled dimethylformamide and the flask was maintained on an ice bath at -5 ºC under nitrogen. To this was added 1.98 g (5 mmol) of the activator para-toluene sulfonyl chloride, which was previously dissolved in (1:1) mixture of 5 mL each of dimethyl formamide solvent and dimethyl sulfoxide which acts as the oxidant. After 25 minutes of stirring time, triethylamine 4 mL (15 mmol) was added and upon this a dark coloration prevailed. The reaction flask was further maintained still at -5 ºC under nitrogen for an additional 5 minutes. The contents in the flask were continuously stirred under nitrogen atmosphere while it warmed to room temperature for additional 1 hour. The reaction mixture was quenched by pouring to a pre cooled ice water 100 mL and allowed for the solids within 1 hour to precipitate. The obtained solid product was filtered and washed with additional 25 mL cold water and air dried to yield the enol tosylate (4) in 80 % yield (0.663 g). M. Pt. 246-248 ºC. $[\alpha]_D^{21}$ = - 80.7 (*c* = 0.1, DMSO).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.42 (s,3H), 2.88 (1H, dd, ^{3}J = 13.74, 5.12Hz), 3.06 (1H, dd, ^{3}J = 13.56, 4.04Hz), 4.32 (1H), 6.43 (s, 1H), 7.04-7.06 (2H, d, *J* = 7.32 Hz), 7.15-7.16 (3H, t, *J* = 7.32 Hz), 7.51 (2H, d, *J* = 8.08 Hz), 7.82-7.84 (2H, d, *J* = 8.08Hz), 8.55 (s, 1NH), 10.29 (s, 1NH) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 21.74, 39.74, 56.26, 119.33, 122.14, 123.38, 127.29, 128.49, 128.56, 130.46, 131.80, 135.59, 135.68, 146.68, 158.09, 165.54 ppm. Mass spectrum: direct insertion/MS; 386 molecular ion, $M^{+\cdot}$

Synthesis of (5), bis-ylidiene compound

To a 25 mL oven dried round-bottom flask was added tosylate **4** (96 mg, 0.25 mmol), 2.1 mL of distilled dimethyl sulfoxide to dissolve the tosylate. To this solution DABCO (84 mg, 3 eq.) was added and stirred at room temperature for 5 days. The reaction mixture was quenched by adding 10 mL pre cooled ice water and was set aside for 30 min. The bis-ylidiene product precipitated out as a yellow tinted amorphous solid was filtered and washed with additional 10 mL cold water air dried to get 45 mg (84% yield). An analytical sample of product may be obtained by recrystallization from chloroform. Mp. 295-300 ºC (decomposes from yellow to brown color).
^{1}H NMR (400 MHz, DMSO-d_6): δ = 4.95 (s), 5.30 (s), 6.75 (s), 7.39 (d, 2H), 7.51 (t, 3H), 10.11 (s, 1 NH), 10.95 (s, 1 NH) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 100.97, 115.39, 127.05, 128.72, 129.27, 133.60, 135.06, 157.36, 157.73 ppm. Mass spectrum: direct insertion/MS; 214 molecular ion M^+.

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