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Nucleophilic reactivity of amines with an α-formylglycyl enol-tosylate fragment

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Abstract—The *E*-enol-tosylate of *S*-3-benzyl-6-formylpiperazine-2,5-dione reacts with 1°- and 2°-amines to yield its respective 3*S*-benzyl-6*E*-endiamine products while a 3°-amine, DABCO, exclusively yields a bis-3,6-ylidenepiperazine-2,5-dione product. These competitive reaction pathways with amine electron donors are shown to arise mechanistically via the same intermediate, or its tautomers, with an H-bond assisted nucleophilic substitution process being operative in the former case and an elimination reaction pathway in the latter instance.

The α-formylglycine (FGly) fragment is an important group that appears to be at the centre of enzymic reactivity in prokaryotic and eukaryotic sulfatases.[1] Deficiency in sulfatase reactivity is a rare human lysosomal storage disorder that has been attributed to insufficient formation of the FGly fragment at the sulfatase active site.[2] It has been shown that the FGly fragment of the sulfatase enzyme is stabilized in its hydrate form by extensive hydrogen bonding[1,2] to neighboring amino-acid fragments in the protein. Recently it has been shown that the normally unstable FGly fragment can be isolated in a small marine cyclic peptide, Callynormine A, as a *Z*-endiamino-fragment.[3]

The recent chemical synthesis of endiamines was achieved through the utilization of an enol-tosylate of FGly.[4] The enol-tosylate fragment of FGly has been shown to be reactive towards neutral-protic amine and thiol nucleophiles[4–6a] but the mechanism of the process is in doubt. Attempts to prepare formylglycine were initially unsuccessful until it was shown that its enol-tosylate could be isolated utilizing a Moffatt oxidation in which DMSO acts as the oxidant/solvent and where *p*-toluenesulfonyl chloride traps the unstable intermediate enol.[5] Subsequent substitution for the tosylate group by amines to provide the endiamine fragment, or by thiols to produce the enthioamine, has been suggested to be via an addition–elimination mechanism.[4]

The preparation of the enol-tosylate of *S*-3-benzyl-6-formylpiperazine-2,5-dione **1** has been reported but the configuration of the ylidene group was not indicated.[6a] We have established that **1** has an *E*-configuration by noting an NOE between the vinyl proton and the downfield or less shielded amide proton. We have thus utilized **1** as a surrogate in an attempt to understand the mechanism of the substitution reactions that occur when an enol-tosylate of the FGly fragment reacts with 1°- and 2°-amines.[6b] We have been able to follow the mechanistic process by NMR and have observed that a diastereoselective nucleophilic substitution reaction explains the results. Consequently we propose that the overall mechanistic process involves a nucleophilic substitution step during the sequence of reactions that lead to the *E*-endiamines **4a**–**g** via **2** when starting with the *E*-enol-tosylate **1**. When the 3°-amine DABCO, 1,4-diazabicyclo[2.2.2]-octane, is utilized bis-ylidene compound **3** is derived by elimination via this same key intermediate **2** (see Scheme 1).

Reaction of **1** with at least 3 equiv of various 1°- or 2°-amines in either DMF or DMSO solvent produced the optically active *E*-endiamine **4a**–**g** as the sole product in reasonable (40–70%) isolated yield.[7] The assignment of the *E*-geometry was evidenced by an NOE exhibited between the vinyl proton at ~6.1–6.4 ppm and the N–H proton at ~9 ppm in **4a**–**g**. The vinyl proton in an *E*-isomer is expected to exhibit such an NOE while the vinyl proton of the corresponding *Z*-isomer of a 3-ylidene-2,5-piperazinedione diastereomer is not expected to show an NOE. As is the case with

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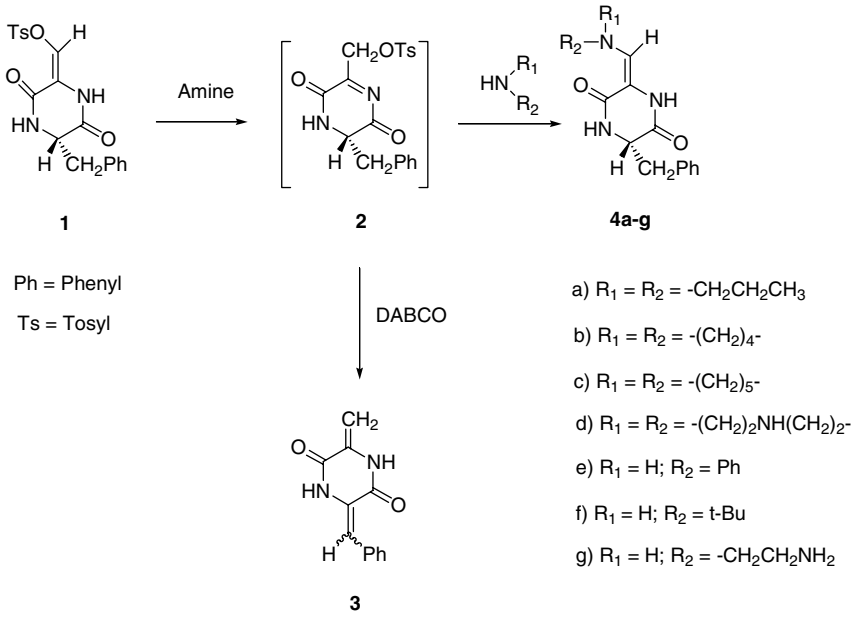


Scheme 1.

1 the product endiamine **4** exhibited such an NOE. Consequently the *Z*-endiamine diastereomer was not spectroscopically observable in **4a**–**g**.

Two mechanisms for this type of reaction have previously been proposed: (1) Reaction of an amine with the tosylate group to remove a *p*-toluene sulfonamide followed then by the trapping of the released formyl-function by excess amine to yield an endiamine;[5] or (2) an addition–elimination sequence whereby the tosylate-group is ultimately substituted for by an amino-group.[4] With either of these mechanisms one would expect that a diastereomeric mixture of the possible endiamine products would result unless there was a significant difference in energy between the isomers. It did not appear to us that there should be a significant energy difference between the two possible diastereomers of **1**. In apparent confirmation others have prepared one example of the enthioamine derived from **1**, utilizing a MeOH/DMSO solvent system with catalytic triethylamine (TEA), resulting in a 7:3 mixture of the *E*- and *Z*-diastereomers, respectively, supporting only a small energetic difference.[6b] In our hands, however, reaction of **1** with a large excess of 1-butanethiol, plus catalytic TEA in DMSO or DMF, produced the *E*-diastereomer only as judged by NMR. Thus we examined by ^{1}H NMR the molecular processes that occur when **1** reacts with either 1°-, 2°- or 3°-amines in DMSO-d_6 hoping to determine the mechanistic steps involved. Additionally we were also curious as to why neutral, protic nucleophiles, like 1°- or 2°-amines, led to an apparent diastereoselective substitution process in aprotic solvents while our attempts to introduce negatively charged nucleophiles, such as cyanide, thiocyanate, azide, etc., gave either decomposition at elevated temp or no reaction at room temperature.

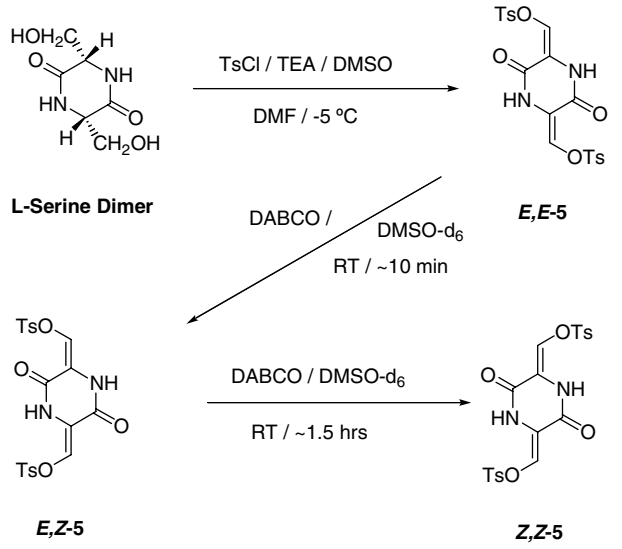
Observation of the kinetics for the transformation of **1** at 25 °C, in the presence of di-*n*-propylamine (DPA) in DMSO-d_6 by ^{1}H NMR, led to a second order rate constant $k = 0.289\ \mathrm{M^{-1}\ d^{-1}}$ for the production of **4a**. The rate of reaction at 25 °C for all the transformations of **1** by the linear 1°- and 2°-amines, as with DPA, were equally slow while temperature elevation led to considerable decomposition with lower yields albeit faster kinetics. The cyclic and more basic 2°-amines, pyrrolidine and piperidine, exhibited much faster bimolecular kinetics where the second order rate constant that produced **4b**, $k = 1.37\ \mathrm{M^{-1}\ h^{-1}}$, is two orders of magnitude greater[7] than for the linear amine DPA.

Spectroscopically the reaction of DPA with **1** to give **4a** was quantitative and diastereoselective at 25 °C. After noting the immediate disappearance of the N–H resonances in **1** upon addition of DPA, either by chemical exchange or deprotonation, the diastereoselective nature of the reaction was evidenced by the gradual and clean upfield shift of the resonances for the *E*-vinylic proton at 6.44 ppm and the chiral proton at 4.32 ppm in **1** to provide **4a** having these resonances at 6.42 and 3.96 ppm, respectively. Isolation of the pure *E*-endiamine product (**4a**–**g**) was accomplished in ~40–70% yield by quenching with H_2O followed by recrystallization. Characterization by ^{1}H and ^{13}C NMR as well as mass spectral analysis was sufficient in all cases.

Examination of the reaction of **1** with the 3°-amine DABCO in DMSO-d_6 at 25 °C produced a different result.[8] While the rate of reaction was similar to that exhibited by DPA the isolated product **3** was very different and optically inactive. Proof of the structure of **3** as the sole, isolable product (86%) was established by 1D- and 2D-NMR as well as GC/MS. Clearly the mechanism of this reaction involved an elimination of *p*-toluenesulfonic acid by the 3°-amine but whether this mechanism was competitive with the substitution process exhibited when a 1°- or 2°-amine was utilized as the electron pair donor, as above, was not obvious.

In an attempt to understand the mechanistic results obtained when **1** reacted with amines we explored, by ^{1}H NMR, the reactivity of **5** with DABCO in DMSO-d_6 at 25 °C. The preparation of **5** was easily accomplished utilizing a Moffatt oxidation of the cyclic dimer of L-serine with DMSO in DMF at −5 °C and trapping the formed bis-enol with *p*-toluenesulfonyl chloride/TEA. The bis-ylidene ditosylate **5** product was shown to be the *E*,*E*-diastereomer by the presence of an NOE between the vinylic (6.8 ppm) and amide (10.9 ppm) protons. The reaction of *E*,*E*-**5** with the 3°-amine DABCO was much faster than the reaction of **1** with linear 1°- or 2°-amines and was completed within 2 h at 25 °C. The reaction products were shown to result from isomerization with the ultimate product being the *Z*,*Z*-bis-ylidene ditosylate diastereomer of **5** which did not exhibit an NOE between its vinylic (5.85 ppm) and amide (8.95 ppm) protons. Thus it was immediately noted upon initiation of the reaction by the addition of DABCO to a solution of **5** in DMSO-d_6 that the single and exchangeable 10.9 ppm N–H resonance had disappeared, by chemical exchange or deprotonation, from the ^{1}H NMR spectrum. Further observation of the vinylic region clearly attested to the isomerization of the starting *E*,*E*-diastereomer of **5** to rapidly give the *E*,*Z*-diastereomer of **5**, which in turn was converted more slowly to the ultimate product the *Z*,*Z*-diastereomer of **5** (see Scheme 2).

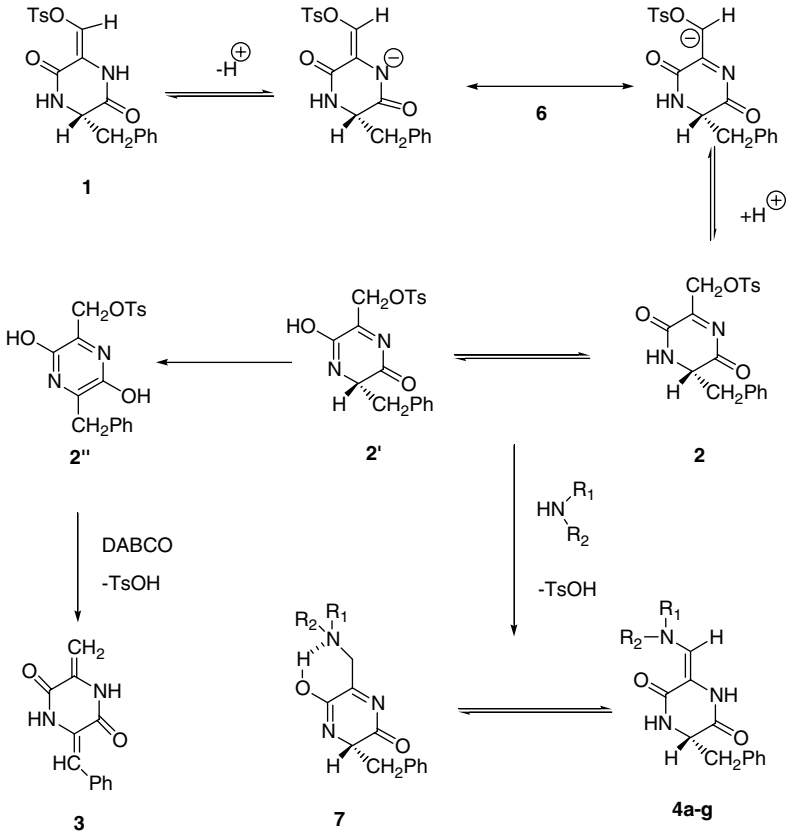
For this isomerization reaction we noted that within 10 min the vinylic singlet of the *E*,*E*-**5** isomer at 6.8 ppm disappeared completely to be replaced by two singlets of equal intensity at 6.6 ppm and 5.6 ppm. These two singlet resonances were, respectively, assigned to the *E*- and *Z*-vinylic protons of the *E*,*Z*-isomer of **5**. After another 1.5 h of reaction time the vinylic protons of the *E*,*Z*-**5** isomer also disappeared and were replaced by a singlet at 5.85 ppm, which corresponded to the *Z*,*Z*-**5** isomer. These diastereomeric assignments were based upon the observation that the specific *E*-vinylic proton of these 3,6-di-ylidenepiperazine-2,5-diones appeared downfield or at a less shielded chemical shift from the corresponding *Z*-vinylic proton of its isomer as well as whether an NOE was observable in the *E*-configuration. The proton resonances of the tosylate groups did not shift to any great extent during the course of the isomerization reaction although there were very minor changes in resonance sharpness and chemical shift position. ^{13}C NMR spectral analyses of these diastereomers were equally confirmatory. Since *E*,*E*-**5** was completely isomerized to *Z*,*Z*-**5** via *E*,*Z*-**5** in DMSO-d_6 at 25 °C within 2 h by DABCO we concluded that the *Z*,*Z*-diastereomeric configuration in **5** was thermodynamically more stable than the *E*,*E*-diastereomeric configuration in **5** when an appropriate 3°-amine base and an aprotic polar solvent are applied at room temperature.



Scheme 2.

Since we never observed the production of either the *Z*-isomer of **1** by isomerization or the *Z*-isomers of **4a**–**g** by substitution, at 25 °C in aprotic polar solvents, any mechanistic proposal will consequently have to take these observations into account. Our mechanistic proposal for the intermediacy of **2**, and/or its tautomers **2′** and **2″**, during the reaction leading from **1** to either **3** or **4a**–**g** can be outlined at the molecular level as shown (see Scheme 3).

The mechanistic processes of these reactions are initiated by deprotonation or rapid chemical exchange of *E*-**1** by an amine noting the absence of the *Z*-**1** diastereomer. The key intermediate **2** forms by the reprotonation of the resonance stabilized anion **6**. Intermediate **2**, or its tautomer **2′**, then undergoes nucleophilic substitution reactivity with a 1°- or 2°-amine that is present to exclusively yield the *E*-diastereomer of **4a**–**g** either directly or via the H-bonded intermediate **7**. Or in the presence of DABCO tautomer **2′**, or its irreversibly formed tautomer **2″**, can undergo a competitive elimination process to yield **3**. Only the *E*-isomers of **4a**–**g** are observed and thus it is implied that the active intermediates during the course of either the nucleophilic substitution or elimination reactions most probably have intramolecular H-bonded structures similar to **2′** or **2″** or even **7**. Intermediates **2′** and **2″** are tautomers derived from **2** and both can exhibit an intramolecular hydrogen bond between the tosylate group and a tautomeric amide group. Intermediate **7** is formed from **2′** by nucleophilic substitution where the N–H bond of the attacking nucleophile (the neutral 1° or 2° amine) guides the intermolecular reaction to yield *E*-product (**4a**–**g**). Hydrogen bonded intermediates like **2′**, **2″** or **7** help explain the diastereoselectivity observed in the substitution reaction mechanism in particular and to some extent the competitive elimination reaction mechanism. It is to be noted that the key intermediate **2**, or its tautomer **2′**, can easily accommodate and hydrogen bond to a charge neutral and protic nucleophile, such as a 1°- or 2°-amine, to yield the observed *E*-product. Negatively charged bases, like azide anion, would rapidly produce the resonance stabilized anionic **6** where reprotonation to intermediate **2** is unlikely. Additionally we note that the isomerization of *E*-**1** to *Z*-**1**, via a rotation and reprotonation of the resonance stabilized anion **6**, is apparently



Scheme 3.

precluded since we saw no evidence of this type of process in the NMR in the presence of protic or aprotic amines.

The *Z*-isomer of **4** is not observed as a product, via a nucleophilic substitution process, thus indicating that the less stable *E*-diastereomers of **4** must be the preferred product when a protic electron pair donor is the nucleophile. This is further confirmed with **5** where rotation with subsequent reprotonation occurs rapidly during the course of the isomerization reaction in the presence of an aprotic electron pair donor like DABCO to exclusively deliver the *Z*,*Z*-isomer. Additionally it is to be noted that the production of **3** from **1** implies that elimination is the preferred pathway when an aprotic electron pair donor is the base. We thus conclude that hydrogen bonds play an important role in these competitive reaction pathways.

The synthesis and general reactivity[9] of the 3-ylidenepiperazine-2,5-dione ring systems have been reviewed while the bioactivity of several different types of diketopiperazines have very recently been reviewed.[10] According to the literature the stability and reactivity of the exocyclic double bond in these types of compounds appear to be controlled in most examples by steric factors. Thus it has been shown that the *Z*-configuration is generally, but not exclusively, more stable while conjugate addition type reactivity, followed in some cases by elimination, delivers the product. We have shown that compound **1** does not adhere to these general considerations. In our system the nucleophilic substitution process exclusively delivers the thermodynamically less stable *E*-isomer and this mechanistic pathway is dependent upon the protic nature of the electron pair donor. Consequently, when a 1°- or 2°-amine is the nucleophilic and protic electron pair donor in an aprotic polar solvent *E*-product (**4a**–**g**) results. However when a 3°-amine like DABCO is present as the aprotic and basic electron pair donor in an aprotic solvent the product (**3**) results from the competitive mechanistic elimination pathway. Thus hydrogen bonds must play a dominant role in the control of the diastereoselective nucleophilic substitution that occurs when **1** reacts with a charge neutral and protic electron pair donor in aprotic solvent via either tautomeric intermediates **2** or **2′**. In contrast the competitive elimination pathway only occurs when **1** reacts with a charge neutral and sufficiently basic aprotic electron pair donor[11] via either intermediate **2′** or its tautomer **2″**.

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Supplementary data

Selected ^{1}H NMR, ^{13}C NMR and MS spectra for compounds **3**, **4a**,**b** and **5** are available, as are the kinetic results associated with the reaction of **1** to give **4a**,**b** and the time course ^{1}H NMR study of the isomerization reaction of *E*,*E*-**5** to produce *Z*,*Z*-**5**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.09.018.

References and notes

1. Dierks, T.; Miech, C.; Hummerjohann, J.; Schmidt, B.; Kertesz, M. A.; von Figura, K. *J. Biol. Chem.* **1998**, *273*, 25560–25564.
2. Chruszcz, M.; Laidler, P.; Monkiewicz, M.; Ortlund, E.; Lebioda, L.; Lewinski, K. *Inorg. Biochem.* **2003**, *96*, 386–392.
3. Berer, N.; Rudi, A.; Goldberg, I.; Benayahu, Y.; Kashman, Y. *Org. Lett.* **2004**, *6*, 2543–2545.
4. Pappo, D.; Varanian, M.; Lang, S.; Kashman, Y. *J. Am. Chem. Soc.* **2005**, *127*, 7682–7683.
5. Nakazawa, T.; Suzuki, T.; Ishii, M. *Tetrahedron Lett.* **1997**, *38*, 8951–8954.
6. (a) Pappo, D.; Kashman, Y. *Org. Lett.* **2006**, *8*, 1177–1179; (b) Note the following contrary quote taken from Ref. 6a: 'Whereas enol-tosylates of the esters of FGly indeed react with amines, amidation of the FGly carboxylic group reduces the reactivity of the double bond of the enol-tosylate, for example, in diketopiperazine **8** [our **1**] which no longer reacts with amines even under more severe conditions. On the contrary, compound **8** [our **1**] reacts with the thiol group...to give...a 7 to 3 mixture of the geometric isomers'.
7. *General experimental procedure; preparation of* **4b**: To a 25 mL oven dried flask was added **1**, (384 mg, 1 mmol) $[\alpha]_D^{21}$ −80.7 (*c* 1.0, DMSO). Dry and distilled DMF (8.5 mL) was used to dissolve **1** with stirring. Pyrrolidine (414 μL, 5 equiv) was added to the flask and the contents were stirred for 5 h at 25 °C. The resultant homogeneous solution was quenched with pre-cooled ice water (40 mL) and the mixture was set aside. A yellow, amorphous solid slowly formed and it was removed by filtration, washed with cold water and air dried. Endiamine **4b** was recrystallized (acetonitrile) to yield 114 mg of **4b** (40%); mp = 220 °C dec, $[\alpha]_D^{22}$ −250 (*c* 0.1, DMSO).
8. *Preparation of* **3**: To a 25 mL oven dried flask was added **1** (96 mg, 0.25 mmol) and 2.0 mL of dry and distilled DMSO. To this solution was added DABCO (84 mg, 3 equiv) and the mixture was stirred for 72 h at 25 °C. The reaction mixture was quenched by adding 10 mL of pre-cooled ice water and set aside to allow a yellow amorphous solid to slowly form. Product **3** was washed with an additional 10 mL of cold water, air dried and recrystallized from chloroform to yield 45 mg of **3** (83%); mp = 295 °C dec.
9. Liebscher, J.; Jin, S. *Chem. Soc. Rev.* **1999**, *28*, 251–259.
10. Martins, M. B.; Carvalho, I. *Tetrahedron* **2007**, *63*, 9923–9932.
11. The less basic TEA does not initiate elimination at 25 °C in aprotic solvent.