# σ-Complexes as biochemical and biophysical probes. Part 6‡. The interaction of *N*-2,4,6-trinitrophenyl-L-lysine with methoxide ion in dimethyl sulfoxide-methanol (95%-5% v/v) mixture: proton transfer and σ -complex formation

**Ikenna Onyido[a*], Masashi Hamaguchi,[b] and Erwin Buncel[b*]**

[a] *Department of Chemistry and Center for Agrochemical Technology, University of Agriculture, Makurdi, Nigeria*
*E-mail: ikennaonyido@yahoo.com*

[b] *Department of Chemistry, Queen's University, Kingston, Canada K7L 3N6*
*E-mail*: *buncele@chem.queensu.ca*

**This paper is dedicated to Professor Henry Shine in recognition of his distinguished contributions to chemistry**

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**Abstract**

The interaction of *N*-2,4,6-trinitrophenyl-L-lysine as the hydrochloride salt, **1**, with methoxide ion in dimethyl sulfoxide-methanol (DMSO-MeOH, 95/5, v/v) mixture has been investigated using UV-visible spectrophotometry at 25°C. An initial 1:1 interaction between $MeO^-$ and **1** results in the rapid and reversible deprotonation of N(1)-H while a 2:1 interaction involves the rapid transfer of a proton from $NH_3^+$ to the base. Deprotonation of N(1)-H in **1** is thus the kinetically and thermodynamically preferred process. At mole ratios $MeO^-$:**1** being > 3, addition of the $MeO^-$ nucleophile to the C-3 position of the doubly deprotonated substrate generates a σ-adduct. The order of acidity: N(1)-H > $NH_3^+$ > COOH which emerges in **1** is attributed to characteristic solute-solvent interactions involving delocalized anions in the DMSO-MeOH solvent mixture. The significance of the inversion of acid strengths observed in this study, in relation to general acid-base catalysis by protein residues in the non-aqueous environment of enzyme active sites in biological systems, is highlighted.

**Keywords:** Nitroaromatic-base interactions, σ -complex formation, acidity, salvation, solute-solvent interactions in DMSO-MeOH

‡ Part 5: ref. 6

## Introduction

Our continuing interest in σ -complexes of the Meisenheimer type, formed in the interactions of nitroaromatic compounds with bases, derives in part from the biological relevance and mechanistic significance ascribed to them.[1-3] For example, the anti-leukemic activity of certain nitrobenzofurazan and nitrobenzofuroxan derivatives has been linked to the σ -complexes derived in the interaction of these compounds with intracellular thiol groups.[4] Our studies of σ -complexes as biological, biophysical and environmental probes have been multi-pronged[5-8]. The synthesis of 3,5-dinitro-2-hydroxymethylpyridine 2-phosphate and related compounds and the reversible formation of anionic σ -adducts[5], demonstrated their utility as novel reporter groups for pyridoxal phosphate modifiable enzymes with lysyl residues[9]. The chemistry of dopamine and its 3,4-di-O-methyl derivative in their reaction with electrophilic centers in 1,3,5-trinitrobenzene, was explored in relation to the biological roles of these physiologically active catecholamines[6]. The herbicidal action of some substituted dinitroanilines has also been linked to their ability to form stable σ -complexes with nucleophilic protein residues in plant cells[10]. Our study[8] of the interaction of the dinitroaniline herbicides, trifluralin and benefin, with hydroxide and sulfite ions has firmly established the formation of anionic σ -adducts by these compounds; structures of the derived species and the pathways for their formation were elucidated.

σ-Complexes are recognized as models of the reaction intermediates postulated for nucleophilic aromatic substitution ($S_NAr$) processes[1-3,11,12]. A number of structure-reactivity relationships involving these species have been elucidated; the kinetic and thermodynamic data for the formation and decomposition of the σ-complexes, which have aided the understanding of the mechanism of $S_NAr$ reactions, have been the subject of authoritative reviews[1-3].

We have previously reported on the interactions of methoxide ion with *N*-methylpicramide (NMP)[13], *N*,*N*-dimethylpicramide (DMP)[14], *N*-picrylethylenediamine (PED) as its HCl salt[15], and *N*,*N*-dimethyl-picrylethylenediamine (DPED)[16] in dimethyl sulfoxide (DMSO)-methanol (MeOH) mixtures. The interactions yielded, variously, products of preferential picryl N-H deprotonation, $MeO^-$ addition at C-3, $MeO^-$ addition at C-3 and C-5, and intramolecular ring $NO_2$ displacement, depending on substrate structure and relative base concentration. In continuation of these studies, in this paper, we present the results obtained with the substrate N-2,4,6-trinitrophenyl-L-lysine as its HCl salt, **1**, reacting with increasing proportions of $MeO^-$ in DMSO-MeOH (95/5, v/v) solvent mixture, using UV-visible spectrophotometry. As an N-substituted L-lysine, **1** is a model system for the derivatization of amino acids in protein chemistry. The presence of three acidic sites in **1**, in addition to a number of potential electrophilic sites, raises interesting reactivity possibilities in this system. The species obtained in this system and the sequence of their evolution with increasing base concentration are identified and evaluated; these illustrate the importance of solute-solvent interactions inherent in DMSO-rich solvents.

## Results

In a medium of DMSO-MeOH (95/5, v/v), **1** exhibits an absorption band with an absorption maximum at 348 nm ($\varepsilon = 1.62 \times 10^4$ $M^{-1}cm^{-1}$) and shoulder with mid-point at 418 nm ($\varepsilon = 0.63 \times 10^4$ $M^{-1}$ $cm^{-1}$). The addition of progressively larger amounts of sodium methoxide to solutions of fixed concentration of **1** ($3.68 \times 10^{-4}$ M) results in the simultaneous disappearance of the absorption maximum due to **1** and the appearance of a new absorption band which has a maximum at 442 nm and shoulder with mid-point at 490 nm (Figure 1). Formation of this second absorbing species, **X**, was rapid, within the time required to mix the stock solutions of **1** and NaOMe and record the spectrum of the resulting solution. Associated with the conversion of **1** to **X** is an isosbestic point at 384 nm. The extent of conversion of **1** to **X** was dependent on NaOMe concentration. Conversion was essentially complete with the addition of 1 molar equivalent of base, thus we calculate the molar absorptivity ($\varepsilon$) values of $1.76 \times 10^4$ and $0.80 \times 10^4$ $M^{-1}$ $cm^{-1}$ at 442 and 490 nm, respectively, for the species **X**. This is termed a 1:1 $MeO^-$-**1** interaction. Addition of excess trifluoroacetic acid (TFA) to solutions containing **X** rapidly and quantitatively converted the species to the starting material **1**. The equilibrium constant ($K_1$) associated with the formation of **X** according to eqs. (1) and (2) is estimated to be $\geq 5.4 \times 10^4$ $M^{-1}$ at room temperature.

$$\mathbf{1} + MeO^- \rightleftarrows \mathbf{X} \quad (1)$$

$$K_1 = [\mathbf{X}] / [\mathbf{1}] [MeO \quad (2)$$

In pure, dry DMSO, the reaction of **1** with 1,4-diazabicyclo[2.2.2]octane (DABCO) also resulted in the conversion of **1** to the species **X**; the final spectrum generated is identical to that obtained with NaOMe, showing an absorption maximum at 443 nm and shoulder with mid-point at 492 nm. The conversion of **1** to **X** by DABCO is characterized by an isosbestic point at 385 nm; it is a rapid process which is easily reversed by the addition of excess TFA.

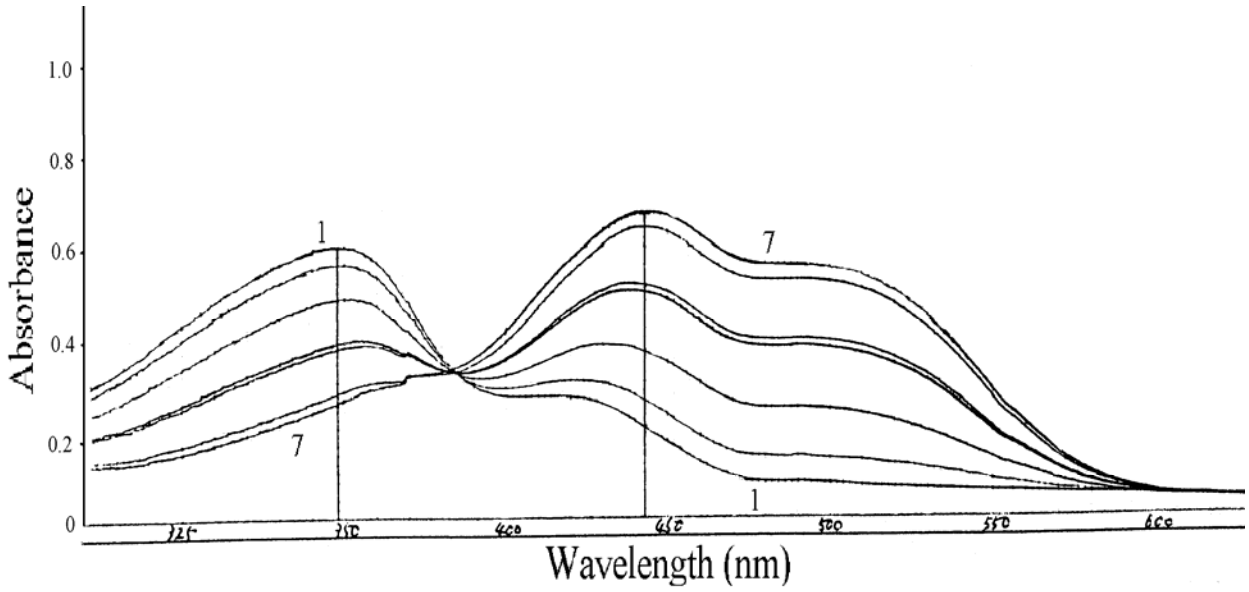



**Figure 1.** Absorption spectra of **1** (3.68 x $10^{-4}$ M) in DMSO-MeOH (95/5, v/v) containing various concentrations of NaOMe at 25°C: (1) 0; (2) 4.08 x $10^{-5}$ M; (3) 1.23 x $10^{-4}$ M; (4) 2.17 x $10^{-4}$ M; (5) 2.50 x $10^{-4}$ M; (6) 3.77 x $10^{-4}$ M; (7) 7.08 x $10^{-4}$ M.

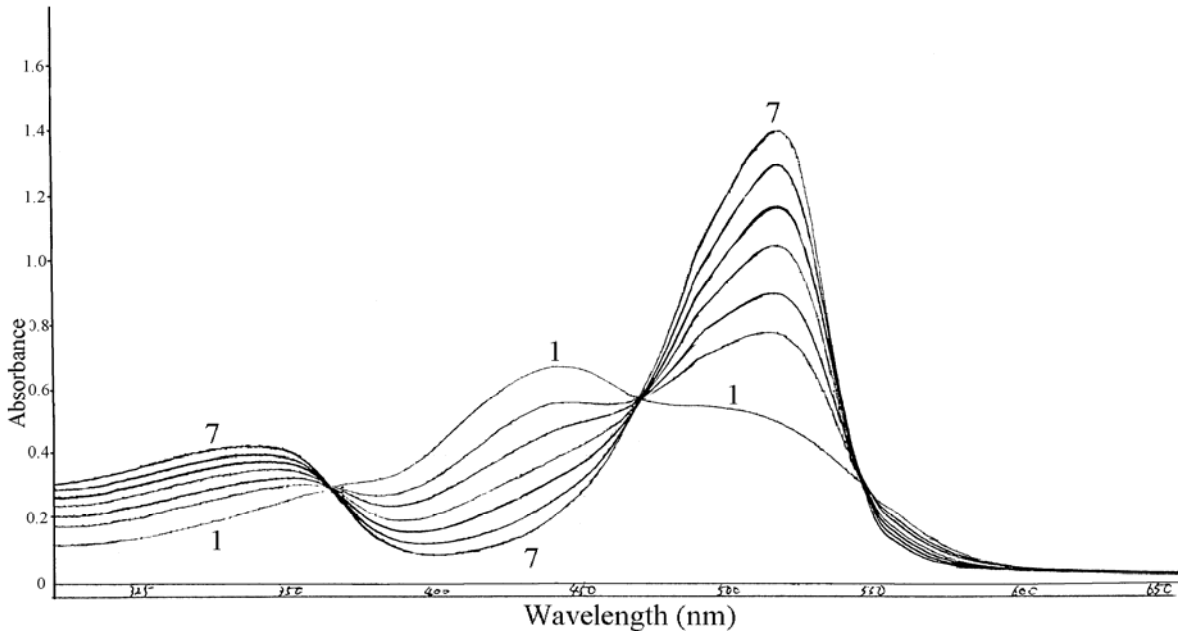



**Figure 2.** Final absorption spectra of **1** (3.50 x $10^{-4}$ M) in DMSO-MeOH (95/5, v/v) containing various concentrations of NaOMe at 25°C: (1) 8.89 x $10^{-4}$ M; (2) 1.33 x $10^{-3}$ M; (3) 1.68 x $10^{-3}$ M; (4) 2.24 x $10^{-3}$ M; (5) 2.91 x $10^{-3}$ M; (6) 4.35 x $10^{-3}$ M; (7) 8.25 x $10^{-3}$ M.

Beyond 1 molar equivalent of NaOMe, solutions of **1** of fixed concentration did not undergo any further spectral changes until another molar equivalent of the base had been consumed. This is termed a 2:1 base-substrate interaction. Although no spectral change accompanied the addition of a second molar equivalent of base, it is logical to infer the occurrence of another acid-base equilibrium between **1** and the base, in this case involving a proton that is remote from the chromophoric system. Thus the 2:1 interaction gives rise to another species **Y** which has the same spectral characteristics as **X**.

Addition of progressively increasing amounts of NaOMe (> 2 molar equivalents) leads to the replacement of the absorption due to **Y** by a new absorption band with maxima at 344 and 510 nm. The new maxima are attributed to the formation of a new absorbing species, **Z**, in solution, characterized by isosbestic points at 366, 472, and 546 nm. Formation of **Z** was rapid. It was followed by a slower process, $t_{½} \sim 30$ min, as a result of which the absorption maximum for **Z** at 510 nm shifts to 517 nm, with a slight change (< 3%) in intensity; the absorption at 344 remains essentially unchanged both in position and intensity. This new absorption with maxima at 344 and 517 nm (Figure 2) is tentatively ascribed to a species **Z'** . The similarity in the spectra of **Z** and **Z** ' is worthy of note. Conversion of **1** to **Z'** was essentially complete upon addition of >20 molar equivalents of NaOMe to the system, thus we calculate for the species **Z'** the molar absorptivity (ε) values of $1.10 \times 10^4$ and $3.60 \times 10^4$ $M^{-1}$ $cm^{-1}$ at 344 and 510 nm, respectively. Even with a very large excess of NaOMe, e.g. 1,600 molar equivalents, no further changes in the uv-visible spectrum were observed beyond the formation of **Z'.** Addition of TFA, in excess of total base, to a solution containing **Z'** causes the quantitative regeneration of the original reactant **1**.

On addition of DABCO, in pure, dry DMSO, there was no evidence for the formation of **Z** or **Z'** even at 480 molar equivalents of DABCO relative to **1**, since the spectrum resulting from the formation of **X** from **1** + DABCO remained unaffected thereby.

To aid subsequent discussion, we now summarize the results presented above as follows. **1** reacts reversibly with 1 molar equivalent of NaOMe (i.e. a 1:1 interaction) under the conditions of the experiment to give **X**, which has well defined spectral characteristics. **X**, in turn, consumes one molar equivalent of NaOMe (a 2:1 interaction) without any spectral change to produce **Y**. Further addition of NaOMe (> 2 molar equivalents) to the solution containing **Y**, produces **Z** in a 3:1 interaction. **Z** gives way to **Z'** in a slow process; **Z** and **Z'** exhibit essentially the same spectral features except for a small shift (ca. 7 nm) in the longer wavelength peak in favor of the latter.

## Discussion

### Reaction pathways

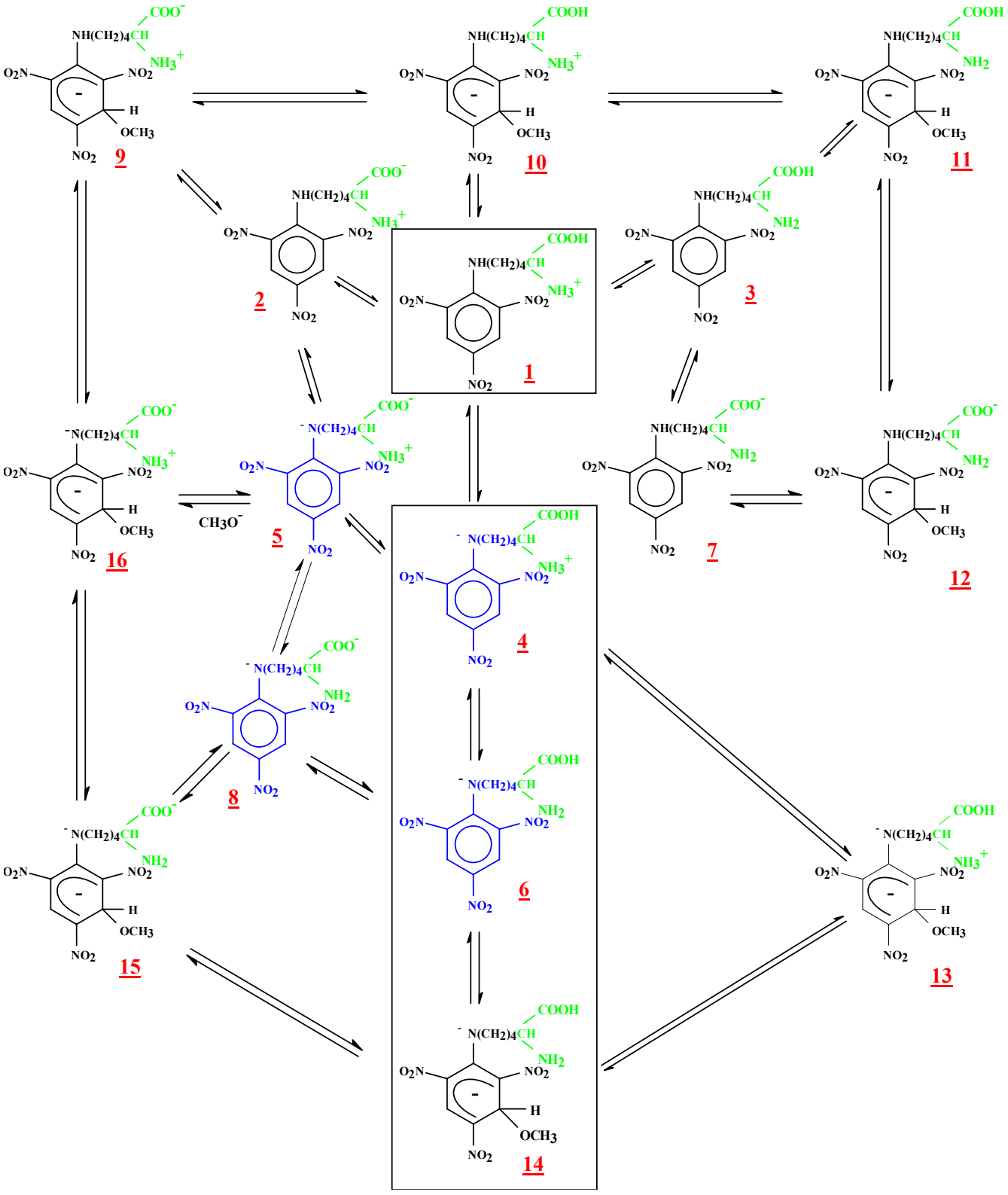
This study has revealed a number of spectral species that are formed consecutively in the DMSO-MeOH system. The overall reaction sequence is denoted by eq. (3).

$$\mathbf{1} \rightleftarrows \mathbf{X} \rightleftarrows \mathbf{Y} \rightleftarrows \mathbf{Z} \rightleftarrows \mathbf{Z'} \quad (3)$$

The presence of three acidic protons which can participate in acid-base equilibria and three potential electrophilic sites in **1** presents interesting possibilities for its interaction with a nucleophilic base like $MeO^-$ which are summarized in Schemes 1 and 2. The following discussion focuses on the identities of the species shown in eq. (3) and the solvation factors in DMSO-MeOH responsible for the observed reaction pathways. It is to be noted that the species obtained on proton abstraction and σ-adducts obtained from such studies exhibit characteristic uv-visible features, which can be employed diagnostically. In our previous studies involving the interaction of $MeO^-$ with NMP[13], DMP[14] and PED[15], substrates which are structurally related to **1**, we relied on the UV-visible spectral features of the derived species to satisfactorily establish the reaction pathways and the identities of the observed and inferred species.

**N(1)-H Deprotonation**

The structure of X is readily deduced by comparing the results obtained with $MeO^-$ in DMSO-MeOH (95/5, v/v) and DABCO in dry DMSO. Since DABCO, which cannot form σ-adducts[14,17] but can deprotonate N- and O-centers, generates the same species as $MeO^-$, it follows that X is the product of an acid-base equilibrium; hence the contending structures are 2, 3, and 4 in Scheme 1. That X is not a σ -adduct of the type represented by 9, 10, 11, 12 (or 13, 14, 15, 16) is evident from the dissimilarity of its spectrum from the spectra ascribed to such complexes, which show twin absorption maxima in the wavelength range of 400-600 nm, with the intensity of the shorter wavelength absorption being *ca*. twice that of the longer wavelength peak[1,18]. Structures 2 and 3 can also be readily ruled out in that their uv-visible characteristics cannot be radically different from those of 1; the former species differ from 1 by one $H^+$, removed from the side chain O or N. This leaves the anion 4 as the species formed under the given reaction conditions.




**Scheme 1**

This conclusion is supported by the similarity in the uv-visible features reported[14,15,19,20] for similar species (**19**) and those given above for **X** (see Table 1). In particular, the fact that the spectrum for **X** can be superimposed on the spectra for **19a** and **19b** obtained by the action of $MeO^-$ on NMP[14] and PED[15], respectively, in the same solvent system lends further credence to the assignment of structure **4** to species **X**. This 1:1 interaction, whereby **4** results from the reaction of 1 with $MeO^-$, leads to an estimate of the $pK_a$ of N(1)-H in **1**. In this regard, it is noted that *N*-*n*-butyl-2,4,6-trinitroaniline, the parent acid for anion **19d**, has a $pK_a$ of 10.61 in DMSO[19]. Since *N*-*n*-butyl-2,4,6-trinitroaniline is a good model for the N(1)-H acidity of **1**, we estimate the $pK_a$ of N(1)-H in **1** to be ~10.6 in DMSO-rich solvents. The relative acidity of N(1)-H in water and DMSO-rich solvents is discussed below (see also Table 2).

**Table 1.** Values of the absorption maxima ($\lambda_{max}$) and molar absorptivities ($\varepsilon$) of the species in the interaction of **1** with $MeO^-$ and of selected anions and σ-adducts from the literature

| Species | $\lambda_{max}$/nm($10^{-4}$ ε /$M^{-1}cm^{-1}$) | Solvent | Reference |
|---|---|---|---|
| **1** | 348 (1.62); 418 (0.63) | DMSO-MeOH (95/5, v/v) | This work |
| **X** (**4**) | 442 (1.76); 490 (1.35) | DMSO-MeOH (95/5, v/v) | This work |
| **Y** (**6**) | Same as **X** | DMSO-MeOH (95/5, v/v) | This work |
| **Z** (**14**) | 344; 510 | DMSO-MeOH (95/5, v/v) | This work |
| **Z'** [a] | 344 (1.10); 517 (3.60) | DMSO-MeOH (95/5, v/v) | This work |
| **19a** | 442 (1.71); 494 (1.29) | DMSO-MeOH (95/5, v/v) | 14 |
| **19b** | 430 (1.38); 490 (0.82) | DMSO-MeOH (95/5, v/v) | 15 |
| **19c** | 443 (1.76); 490 (1.29) | DMSO-MeOH (95/5, v/v) | 15 |
| **19d** | 444, 500 | DMSO | 19a |
| | 430 (2.0); 500 (1.4) | DMSO-MeOH (95/5, v/v) | 20 |
| | 410 | MeOH | 20 |
| **19e** | 435 | MeOH | 20 |
| **20a** | 335 (1.23); 508 (4.00) | DMSO-MeOH (95/5, v/v) | 14 |
| **20b** | 334 (1.16); 506 (3.37) | DMSO-MeOH (95/5, v/v) | 14 |

[a] See text.

**$NH_3^+$ Deprotonation**

Having formulated **X** as **4**, the product of its reaction with one further equivalent of $MeO^-$ (a 2:1 interaction), **Y** could be **5**, **6**, or **13** in Scheme 1. It is noted that for reasons given earlier, the spectral characteristics of **4** and **13** should be dissimilar. The fact that **Y** is formed from **4** without any spectral change rules out the formulation of **Y** as **13,** leaving **5** and **6** for further consideration. Since the processes **4** → **5** and **4** → **6** in Scheme 1 are proton transfer processes, the preferred route will be determined by the relative acidities of COOH and $NH_3^+$ in **4**. The $pK_a$ of the COOH and $NH_3^+$ groups in *l*-lysine in water are given[21] as 2.16 and 9.18, respectively. The possibility of formation of hydrogen bonds between the protons in these acidic functions and the

*ortho*-nitro groups in **1** and in **4** may attenuate their acidities. We therefore estimate their $pK_a$ to be close to the values for acetic acid[22] (4.8 in water; 12.6 in DMSO) and *n*-butylamine (10.6 in water; 11.1 in DMSO) in DMSO-rich solvents. From the foregoing estimates, the $NH_3^+$ function is a stronger acid than COOH in **1** and **4** in DMSO-MeOH (95/5, v/v) mixture. Hence deprotonation of **4** should yield **6**.

Table 2 presents a summary of the above discussion concerning the $pK_a$ estmates of the three acidic moieties in **1** in the DMSO-MeOH (95/5, v/v) and $H_2O$ media.

**Table 2.** $pK_a$ value estimates in DMSO-MeOH (95/5, v/v) and in $H_2O$ of the acidic moieties in *N*-2,4,6-trinitrophenyl-L-lysine[a], **1** (see text)

| | $pK_a$ | |
|---|---|---|
| | DMSO-MeOH (95/5, v/v) | $H_2O$ |
| TNP-**NH**$(CH_2)_4CH(NH_3^+)COOH$ | 10.6[b] | 13.5[c] |
| TNP-NH$(CH_2)_4CH($**$NH_3^+$**$)COOH$ | 11.1[d] | 10.6[d] |
| TNP-NH$(CH_2)_4CH(NH_3^+)$**COOH** | 12.6[e] | 4.8[e] |

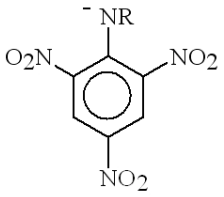
[a] TNP = 2,4,6-trinitrophenyl substituent in **1**.
[b] Based on the $pK_a$ (10.61) of *N*-*n*-butyl-2,4,6-trinitroaniline in DMSO (see ref. 19).
[c] Based on the $pK_a$ of picramide (see ref. 32).
[d] Value for *n*-$BuNH_3^+$ (see ref. 22).
[e] Value for $CH_3COOH$ (see ref. 22).

Support for the above assignment of order of acidity comes from the observed difference in the behavior of positively charged (e.g. ammonium cations) and uncharged (e.g. carboxylic acids and phenols) acids in water and DMSO. Generally speaking[22-29], the change from water to DMSO for a given acid usually results in a decrease in the acid strength of both acid types, an effect that is often greater for uncharged acids than for positively charged ones[22]. In some cases, mild increases in the acid strength of positively charged acids have been noted[22]. Hence increasing the DMSO content of an aqueous solution often leads to an inversion in the acid strength of charged and uncharged acids in DMSO-rich solvents. For example[22], phenol ($pK_a$ = 10.0) and ethylammonium ion ($pK_a$ = 10.6) have comparable acid strengths in water. However, in DMSO, ethylammonium ion ($pK_a$ = 11.0) is *ca*. 2.5 x $10^5$ times a stronger acid than phenol ($pK_a$ = 16.4). A study of the equilibrium protonation behavior of nicotinic[23,24], isonicotinic[24], and urocanic[25] acids, and of histidine hydrochloride[26] and trinitro-2,4,6 diphenylamine[27] in water and DMSO-water mixtures provided unambiguous proof of a different protonation sequence in DMSO than in water due to inversion of acidities. The acidity of COOH groups in various amino acids[30] has also been shown to be lowered by the addition of DMSO to water. We therefore conclude that **6** is the species formed through the deprotonation of **4**.

**Nucleophilic addition at C-3**

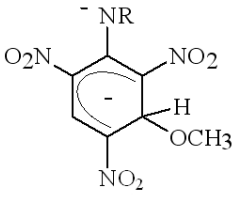
Further reaction of **6** (**Y**) with $MeO^-$ results in the formation of **Z** in a rapid reaction, which slowly gives way to **Z'**. The similarity in the spectral properties of the species **Z**, **Z'**, **20a** (derived from N-methylpicramide), and **20b** (derived from N-picrylethylenediamine) [see Table 1] argues for the formulation of species **Z** and **Z'** as 1,3 σ-adducts resulting from $MeO^-$ addition at C-3, consistent with the reversal of both species on acidification to give **1** (see above). In Scheme 1, there are two possible routes for the reaction of **6** with the base, either via nucleophilic addition to generate **14** or deprotonation to yield **8**. However, the latter process is not expected to give rise to a species with a distinctly different spectrum from that of **6**, since the proton being transferred is situated on the side chain and distant from the chromophoric moiety. We therefore conclude that the sequence is **6** (**Y**) → **14**(**Z**).

The species Z' could have the structure 15, so that the observed reaction sequence would be 14 → 15. However, this sequence is flawed on two grounds. Firstly, one of the characteristics of 1,3 σ -adducts is that their formation is rapid in the time scale of UV-visible spectrophotometry. Secondly, the sequence 14 → 15 is a proton transfer process between electronegative atoms which is diffusion controlled and therefore cannot be slow and observable under the conditions of the experiment. Hence we are of the view that the slow process cannot be 14 (Z) → 15 (Z'). Another possibility, not included in Schemes 1 and 2, is that 14 (Z) reacts with excess $MeO^-$ in the system to yield a σ -adduct of the type depicted in 21. This possibility is also discounted on the grounds that: (i) addition of a negatively charged base to the 5-position of an already doubly charged species (14) is highly unlikely on electrostatic grounds; (ii) species of the general structure 21, in which N-1 bears no negative charge, have markedly different spectra[1,14,15,18,20]; and (iii) to our knowledge, no precedent for 21 exists in the literature.



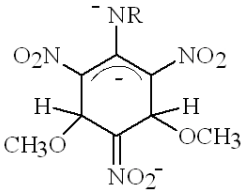
**19**

(a) R = $CH_3$
(b) R = $CH_2CH_2NH_3^+$
(c) R = $CH_2CH_2NH_2$
(d) R = n-Bu
(e) R = Ph

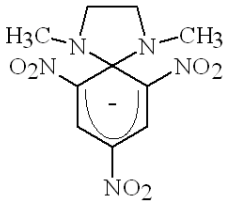


**20**

(a) R = $CH_3$
(b) R = $CH_2CH_2NH_2$



**21**



**22**

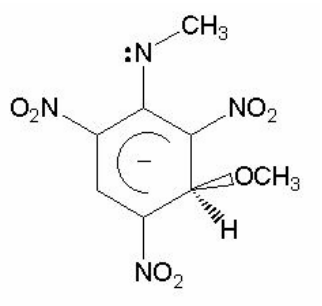
**Figure 3**

What then is the identity of Z'? The fact that 14 (Z) and Z' exhibit similar spectral properties suggests that they differ only slightly in their structures. The negative charge on N(1) is delocalized into the aromatic ring and this confers double bond character on the C(1)-N(1) bond. It is therefore conceivable that 14 exists in two geometrical isomeric forms in which the $(CH_2)_4CH(COOH)NH_2$ (R) group at N-1 is "*syn*", i.e. R is proximal with respect to the C(3)-$OCH_3$ group, or "*anti*", i.e. R is distal with respect to C(3)-$OCH_3$. Such isomers are not expected to give radically different UV-visible spectra. If one of these isomers is kinetically preferred but is less stable that the second, more slowly formed counterpart, then the slow sequence 14 (Z) → Z' reflects the conversion of the kinetically formed species to the more stable form. This kind of conversion was inferred previously in the interaction of PED with MeO[15] where a similar sequence of spectral changes was observed.

It is noted that in the presence of excess base one further deprotonation will occur, namely the COOH group in **14**, giving rise to **15**. This, however, will not result in any further spectral changes**.**

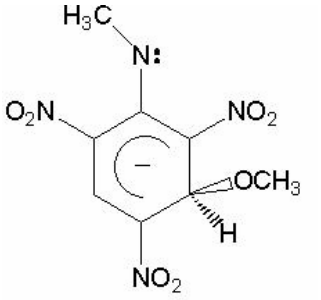
**PM3 modelling of the N-deprotonated methoxide adduct 14**

In order to test the above hypothesis concerning **Z** and **Z '** we have modelled the two isomers of **14** by the PM3 method. Modelling the N-alkyl (R) substituent in **14** as N-$CH_3$, one obtains structures **20a'** and **20a''** for the "*syn*" and "*anti*" forms with heat of formation for the




**20a'**

**-84.92 kcal**




**20a''**

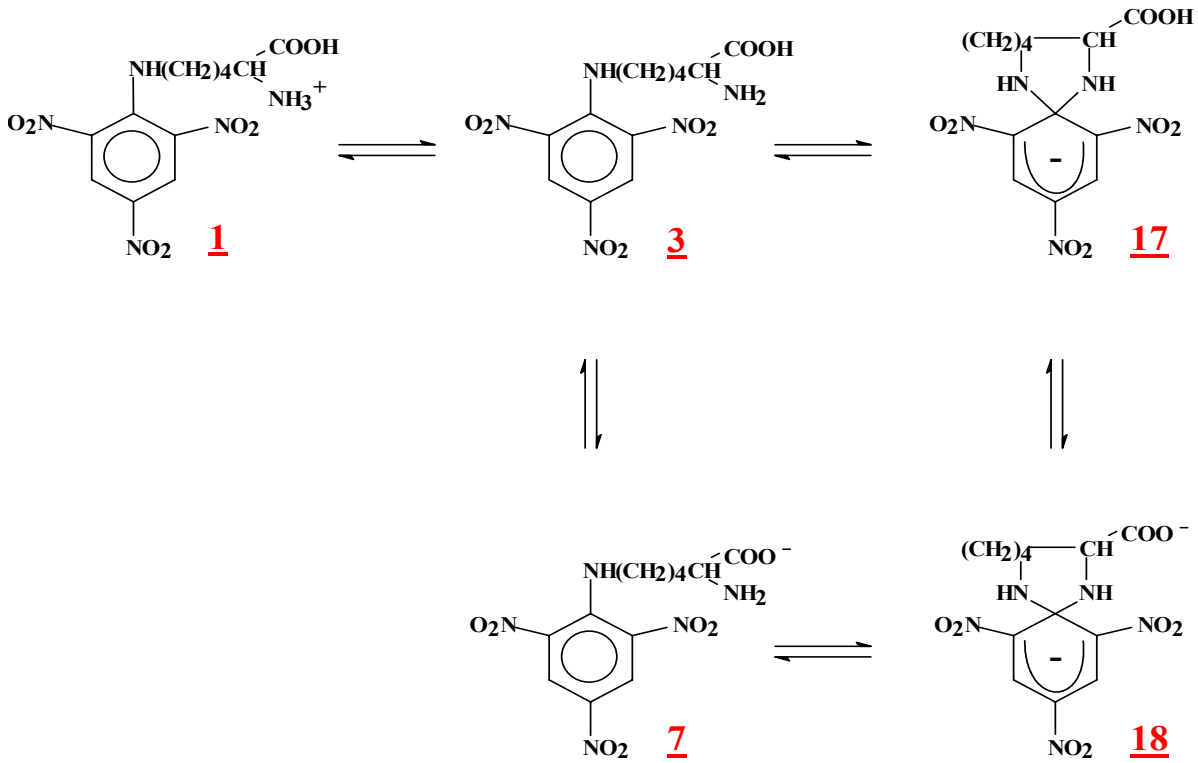
**-87.80 kcal**

**Figure 4**

"*anti*" form being almost 3 kcal greater than of the "*syn*". This enables one to identify **20a'** (**Z**) as the "*syn*" and **20a''** (**Z '**) as "*anti*". 3D structures of the modelled stereoisomers (see **Supplementary material**) reveal a further point of interest, namely that the N-$CH_3$ moiety is significantly out of plane with respect to the benzenoid ring; this is in accord with partial $sp^3$ character of the exocyclic nitrogen.

**Absence of spiro complexes**

We now turn to the structures in Scheme 2 and note that in this study no evidence was found for the formation of spiro complexes of the type **17** and **18**. Such σ-complexes have been reported in the literature [1,2,16,31-36] ; the kinetics and thermodynamics of their formation and decomposition have been carefully characterized in certain cases[1,2,31-33]. Significantly, formation of the spiro complex **22** was observed[16] in the reaction of DPED with NaOMe in DMSO-water and with $Et_3N$ in DMSO and DMF; the uv-visible spectrum of **22** is characteristically distinct from the spectra of the species observed in the present study. It is likely that spiro complexes analogous to **17** and **18** are not formed primarily because deprotonation of N(1)-H in **1** to give **4** is both kinetically and thermodynamically more favorable than the transformation **1** → **3**. The latter process should precede the formation of spiro adducts. Once **4** and similar species bearing a negative charge on N-1 are formed along the reaction route, intramolecular attack at C-1 by a free nucleophilic $NH_2$ to give **17** and/or **18** is disfavored on electrostatic grounds. Thus, the absence of N(1)-H in PED, the precursor of **22**, and the relief of steric strain in **22**[16] are important factors determining its stability[16].




**Scheme 2**

**Solvation factors and inversion of acidities in DMSO-MeOH**

Having accounted for the identities of the diverse species generated by the interaction of **1** with $MeO^-$ according to eq. (iii), which shows that sequential deprotonation of the N centers in **1** occurs in preference to σ-adduct formation, it is pertinent to consider the factors responsible for the observed reaction sequence.

The value of $K_1 \geq 5.4 \times 10^4$ $M^{-1}$ obtained in this study for the formation of **4** is of the same order of magnitude as that reported for **19a** ($2 \pm 1 \times 10^5$ $M^{-1}$)[13] and **19b** ($\geq 5 \times 10^5$ $M^{-1}$)[15] in DMSO-MeOH (95/5, v/v), consistent with similar acidity of N(1)-H in the precursors of these species in this solvent. This value can be compared with $K_1 = 20$ $M^{-1}$ for the formation of **19d** in MeOH[20]; the large change in the value of $K_1$ in going from MeOH to the DMSO-MeOH mixture reflects the very large (~$10^5$-fold) difference in N(1)-H acidity in the picramide substrates in the two solvent systems. The $pK_a$ of *N*-*n*-butyl-2,4,6-trinitroaniline, a good model for **1**, is 16.0 in MeOH[20]. Although *N*-*n*-butyl-2,4,6-trinitroaniline $pK_a$ in water is not known, a good estimate for it is that of picramide ($pK_a$ = 13.5)[31]. Hence it is estimated that in going from water to DMSO, there is a change of *ca*. 3 pK units in the acidity of **1** and similar substrates.

Preferential solvation by DMSO of delocalized anions of the type represented by **4**, **19a**, and **19b** is thought[13, 18, 37] to be mainly responsible for this very significant solvent effect on $K_1$. The formation of **4** in DMSO-MeOH, in preference to **2** and **3**, points to more favorable solvation energy for **4** than for **2** and **3** in this solvent system.

The results obtained in this study are fully analogous to those reported[13] for NMP in the same solvent, whereby NMP interacts sequentially with two molecules of $MeO^-$ to give **19a** and **20a**, the only difference being the intervention of an additional deprotonation process, i.e. **6** (**Y**) →**14** (**Z**). The results are also similar to those obtained in the study of the interaction of PED with $MeO^-$ in which two successive deprotonation reactions precede nucleophilic addition at the C-3 position[15].

Factors such as H-bonding and ion-dipole interactions, among others, are known to be important in determining the solvation energies and stabilization of different species in DMSO and DMSO-rich solvents[29,37]. As stated earlier, the negative charge on N(1) in **4** is delocalized into the aromatic ring. Consequently, polarizability effects should also be an important solvation factor in DMSO-rich solvents[29,37]. The complete inversion of acidities noted in this study, whereby the relative order of $pK_a$ changes from COOH (~ 4.8) > $NH_3^+$ (~ 10.6) > N(1)-H (13.5) in water to N(1)-H (10.6) > $NH_3^+$ (~ 11.1) > COOH (~ 12.6) in DMSO-MeOH is worthy of note. It is also noted that the greatest change occurs for the COOH function, consistent with poor solvation of negatively charged species in DMSO.

Clearly, the present results emphasize the importance of solvation effects in determining reaction pathways in these systems. DMSO-rich solvents have, on occasion, been used in probing the mechanisms of some important biological reactions[38,39]. The present results could be relevant in evaluating general acid-base catalysis by amino acid residues of proteins in biological processes that occur in the non-aqueous environment of enzyme active sites. The inversion in acidities recorded in this study underlines the need for caution on relying solely on the $pK_a$ values of protein residues measured in water in interpreting rate data in such systems.

## Experimental Section

**General Procedures.** *N*-2,4,6-Trinitrophenyl-L-lysine hydrochloride, **1**, was prepared by the procedure given by Okuyama and Satake[40] and recrystallized from hot HCl solution. DMSO was dried over calcium hydride and fractionated under reduced pressure, the middle fraction being collected. MeOH was distilled from magnesium turnings and the middle fraction was collected. NaOMe solutions were prepared by dissolving clean sodium in Analar methanol and were standardized with HCl, using phenolphthalein as indicator. DABCO was recrystallized twice from benzene and dried *in vacuo*. The resulting material was mixed with an equal amount of barium oxide and sublimed twice to yield white crystals, mp 157°C. Reagent grade trifluoroacetic acid was used as received.

Stock solutions of **1** in DMSO, DABCO in DMSO and NaOMe in MeOH were made up under nitrogen in a drybox and were protected from light. Solutions used for the uv-visible spectrophotometric experiments were made up in the drybox by introducing appropriate volumes of stock solutions and MeOH to known volumes of DMSO. After thorough mixing, a portion of

the solution was transferred to a clean dry spectrophotometric cell and its contents then placed in the spectrophotometer.

Spectral scans were performed on a Beckman Acta IV spectrophotometer using matched silica cells of 1.0 mm and 5.0 mm path length. Constant temperature in the reaction cells was maintained at 25.0 ± 0.1 $^{o}$C by water circulated from a constant temperature bath passing through a specially designed cell holder in the spectrophotometer.

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## Supplementary material

Modelling of 3D structures of the "*syn*" and "*anti*" forms of **14**. See Page 213

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