# DIHYDROISOXAZOLO[2,3-d][1,4]BENZODIAZEPINE RING SYSTEM: STEREOCHEMISTRY AND CONFORMATION

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Abstract - The configurational properties of dihydroisoxazolo[2,3-d][1,4]-benzodiazepine derivatives, obtained by 1,3-dipolar cycloaddition between chlordiazepoxide and various substituted alkynes, have been determined by $^1$H nmr spectroscopy, largely by computer-simulation of lanthanide induced shifts (LIS). Cycloadducts exist in solution as a 3:2 mixture of two interconvertible diastereoisomers, which are the result of the heptaatomic ring mobility. The stereochemistry of examined derivatives has been found appropriate to determine the further rearrangement to pyrrole-fused quinoxalines, observed in this new tricyclic system.

The chemistry of the benzodiazepine system draws an ever-growing interest with reference to the synthesis of variously functionalized derivatives with potential biological activity. 1,4-Benzodiazepines containing an additional heterocyclic ring annelated to "a"[1], "c"[2] or "d"[3] edge of the heptaatomic system have recently shown interesting pharmacological activity as minor tranquillizers[4]. Our interest in the configurational and conformational properties of the seven-membered benzodiazepine ring[5], also in consideration of the possibility of a structure-activity relationship, led us to investigate the cyclofunctionalization of the 1,4-benzodiazepine system by 1,3-dipolar cycloaddition[6,7].

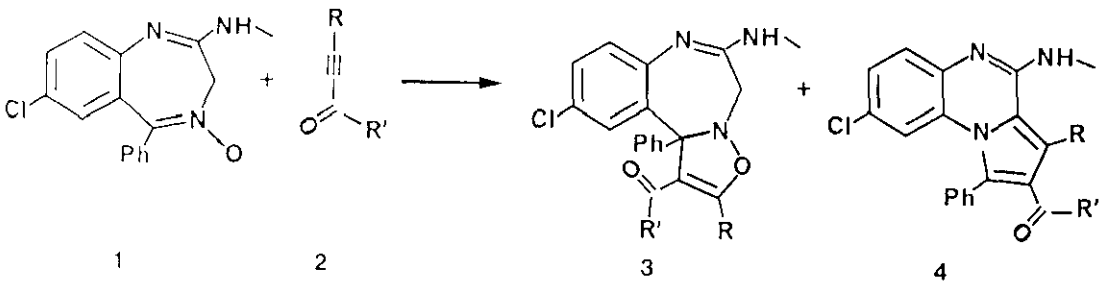
The reaction between chlordiazepoxide (7-chloro-2-methylamino-5-phenyl-3H [1,4]-benzodiazepine 4-oxide) and various substituted alkynes gave the dihydroisoxazolo [2,3-d][1,4]benzodiazepine derivatives, in which a 4-isoxazoline ring has been fused at the "d" edge of the heptaatomic ring. Synthesis and chemical behaviour of these compounds have been described elsewhere[8,9]: we report here a detailed analysis of the stereochemical and conformational properties of this novel system. The nature and substitution of the additional heterocyclic nucleus are related to the

conformational characteristics of the new tricyclic system.

## RESULTS AND DISCUSSION

Chlordiazepoxide 1 was submitted to 1,3-dipolar cycloaddition with the alkynes 2, in dry tetrahydrofuran (THF) at reflux[9]; by appropriate choice of temperature and reaction time, the cycloadducts 3 were obtained together with further-rearranged derivatives 4 (Scheme 1). The condensed 4-isoxazolines 3 showed analytical and physicochemical data consistent with the assigned structures. Table 1 summarizes the obtained adducts and yields. In all the investigated cases, the cycloaddition reaction has been found to be regiospecific, affording a single regioisomer with good yields. The dipolarophiles were assigned the orientations showed in Scheme 1 on the basis of $^1$H nmr data; in particular, for compounds 3a and 3b the 4-regioisomeric structure was assigned on the basis of the resonance of the vinyl hydrogen which is deshielded to 6.30 and 6.40 ppm respectively[10]. The assignments were confirmed by the LIS analysis (vide infra), which only allowed the characterization of the structure for compounds 3(c-e), since the simple $^1$H nmr spectra were not informative.

Scheme 1




1 2 3 4

Table 1

| Compound | R | R' | mp | yield |
|---|---|---|---|---|
| 3a | H | OMe | 173-76° | 45% |
| 3b | H | OEt | 172-75° | 55% |
| 3c | Ph | OMe | 162-65° | 38% |
| 3d | Ph | OEt | 171-74° | 41% |
| 3e | Ph | Me | 180-82° | 38% |

Although the cycloadducts 3(c-e) seemed to be a single material on tlc, their $^1$H nmr spectra were complex, indicating that they exist in $CDCl_3$ solution, at room temperature, as a 3:2 mixture of two diastereoisomers (A) and (B) in each case. As summarized in Table 2, all the resonances were doubled in number; moreover, the $^1$H nmr spectra in DMSO-$d_6$ allowed to evidence that the two resonances for the N-methyl groups are really two doublets with J=4.8 Hz, due to the coupling with the hydrogen atom. The assignments of the individual resonances to (A) and (B) have been performed by decoupling experiments and by analysis of the shifts induced by the addition of Eu(fod)$_3$.

A substantial difference was observed in the case of compounds 3a and 3b. At room temperature their $^1$H nmr spectra exhibited broadened resonances which, on cooling to -30° C, resulted in couples of absorptions assignable to the different protons of diastereoisomers (A) and (B) (Table 2). As the temperature was increased, each pair of resonances first broadened and then coalesced at 60° C into a single one, positionated at the centre of the initial resonance patterns of (A) and (B).

These spectral features and chemical data (two diastereoisomers rearrange in a single compound by heating[9]) suggested that two components present in solution at room temperature for 3(a-e) are conformational diastereoisomers, whose existence is attributable to the reduced mobility of the heptaatomic benzodiazepine ring. Similar conformational isomers as a consequence of a slow reversal of the seven-membered nucleus have been found also in the case of the cycloadducts of diazepam N-oxide with ethyl propiolate or dimethyl acetylenedicarboxilate[11-13]

The possibility of existence of rotamers about the C-N bond of the amidinic moiety can be ruled out by considering that chlordiazepoxide itself at room temperature appears as a mixture of interconvertible stereoisomers[14].

The attempt to accelerate the reversal of the benzodiazepine ring and to reach the coalescence of the resonances also for two diastereoisomers of 3(c-e), by recording the spectra at temperatures between 60° and 100° C, failed. The adduct with a 4-isoxazoline structure undergoes, with the increasing of the temperature, a thermal rearrangement in times comparable with those of the ring flipping. This chemical behaviour, assimilable to the behaviour of the N-O vinylic functionality, has been examined elsewhere[9,11,13].

The detailed analysis of the conformational equilibrium established in solution between two diastereoisomers (A) and (B) is quite complex: the assignment of the relative molecular geometry has been performed with the aid of the LIS analysis. Chlordiazepoxide exists in a boat (cycloheptatriene-like) conformation at room temperature; the interconversion gives rise to enantiomers[14]. The stereochemical consequences of a boat-boat interconversion in the obtained cycloadducts are quite complex. The presence of two additional chiral centres, the nitrogen atom and the

Table 2[a] – $^1$H nmr spectral data[b]

| Assignment | 3a | | 3b | | 3c | | 3d | | 3e | |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | A | B | A | B | A | B | A | B |
| | ppm J(Hz) | ppm J(Hz) | ppm J(Hz) | ppm J(Hz) | ppm J(Hz) | ppm J(Hz) | ppm J(Hz) | ppm J(Hz) | ppm J(Hz) | ppm J(Hz) |
| $COOCH_3$ | 4.10s | 4.15s | | | 3.50s | 3.61s | | | | |
| | 0.22 | 0.26 | | | 0.25 | 0.28 | | | | |
| | 0.27 | 0.31 | | | 0.28 | 0.30 | | | | |
| α-H of ester | | | 0.72t (7.0) | 1.25t (7.0) | | | 0.75t (7.15) | 0.89t (7.15) | | |
| | | | 0.17 | 0.20 | | | 0.25 | 0.28 | | |
| | | | 0.20 | 0.24 | | | 0.22 | 0.25 | | |
| $COCH_3$ | | | | | | | | | 1.77s | 2.12s |
| | | | | | | | | | 0.30 | 0.32 |
| | | | | | | | | | 0.26 | 0.29 |
| $N-CH_3$ | 3.04d (4.1) | 3.10d (4.1) | 3.14 (4.2) | 3.21 (4.2) | 2.95[d] | 3.04[d] | 2.98[d] | 3.08[d] | 2.76[d] | 2.94[d] |
| | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| $5-CH_2$ | 3.90br | 4.10br | 4.28br | 4.15br | 3.56br | 3.72br | 3.64br | 3.91br | 3.58br | 3.98br |
| | 1.04 | 1.06 | 0.97 | 0.96 | 0.98 | 0.98 | 0.99 | 0.97 | 0.98 | 0.96 |
| | 0.99 | 1.02 | 0.99 | 1.01 | 1.00 | 1.03 | 1.01 | 1.00 | 1.00 | 1.01 |
| β-H of ester | | | 3.90q (7.0) | 4.15q (7.0) | | | 3.88q (7.15) | 3.98q (7.15) | | |
| | | | 0.28 | 0.30 | | | 0.29 | 0.31 | | |
| | | | 0.25 | 0.28 | | | 0.26 | 0.27 | | |
| 1-H | 6.30s | 6.40s | 6.30s | 6.38s | | | | | | |
| | 0.32 | 0.63 | 0.42 | 0.65 | | | | | | |
| | 0.38 | 0.67 | 0.49 | 0.68 | | | | | | |
| 11-H | | | | | 6.40d (2.2) | 6.52d (2.2) | 6.41d (2.4) | 6.50d (2.4) | 6.42d (2.4) | 6.62d (2.4) |
| | | | | | 0.49 | 0.52 | 0.50 | 0.52 | 0.48 | 0.48 |
| | | | | | 0.52 | 0.55 | 0.53 | 0.55 | 0.52 | 0.54 |
| N-H | 8.95br | 9.78br | 8.94br | 9.81br | | | | | | |
| | 1.21 | 1.19 | 1.12 | 1.12 | | | | | | |
| | 1.16 | 1.16 | 1.15 | 1.16 | | | | | | |
| TQRF[18] | 0.1108 | 0.1092 | 0.0850 | 0.0901 | 0.1087 | 0.1105 | 0.0987 | 0.0950 | 0.1125 | 0.1098 |

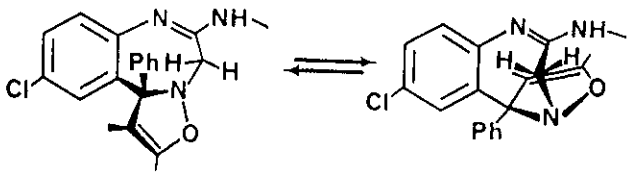
[a] Figures in the first row indicate chemical shifts in $CDCl_3$ of undoped spectra; figures in the second row indicate the observed molar induced shifts; figures in the third row indicate the calculated molar induced shifts.

[b] s=singlet; d=doublet; t=triplet; q=quadruplet; m=multiplet.

[c] Spectra recorded at 243° K

[d] Doublet in DMSO-$d_6$; J=4.8 Hz

[e] B=major component




(A) (B)

Scheme 2 - Conformational isomers for the dihydroisoxazolo[2,3-d][1,4]benzodiazepine ring system

C-3 of the 4-isoxazoline ring, introduces the possibility of four pairs of diastereoisomers: (A), (B), (C) and (D).

In Scheme 2, which is based on molecular models, (A) and (B) represent the conformational changes possible with the dihydroisoxazolo[2,3-d][1,4]benzodiazepine derivatives 3(a-e). The primary difference between conformations (A) and (C) or (B) and (D) may be attributed to the orientation of the unshared pair of electrons of the isoxazolinic nitrogen atom. (A) and (C) are nitrogen invertomers differentiated by having either a cis or trans ring junction; (B) and (D) may be similarly distinguished. (A) and (B) or (C) and (D) are interconverted by the reversal of the seven-membered ring. Moreover (A) and (C) are characterized by conformations in which the phenyl substituent is quasi-axial to the heptaatomic ring, while in the other two diastereoisomers, (B) and (D), the phenyl is in a quasi-equatorial situation.

From an examination of the molecular models, it appears that two of these diastereoisomers, (C) and (D), have higher strain energies than the others, i.e. two isomers which have a trans ring junction; the trans fusion of five- and seven-membered rings distorts the heptaatomic ring and imposes significant angle strain and non-bonded repulsions. Inspection of the model of (C) indicates that the axial phenyl ring is brought into close proximity to the amidinic nitrogen and severe repulsive interactions would be expected; moreover the conformation eclipsed of the N-4 versus the methylene group is energetically unfavourable. Isomer (D) exhibits a significant peri-like interaction between the phenyl substituent and the

H-10 of the chlorophenyl ring, besides a destabilizing interaction between the phenyl and the methylene groups. Furthermore, the following situation has been considered as far the nitrogen atom of the 4-isoxazoline ring is concerned: the N-4 is blocked in a preferred conformation by a high energy barrier[15].

On this basis, the two diastereoisomers (A) and (B), observed in solution, should correspond to two isomers originated from the cis-fusion of the 4-isoxazoline ring on two pseudo-equatorial bonds in 4 and 11-b. Furthermore, the cis ring fusion is consistent with a concerted cycloaddition process. (A) and (B) are interconvertible by the reversal of the seven-membered ring (Scheme 2).

The stereochemistry and the preferred conformations of the reaction products have been confirmed by $^1H$ nmr lanthanide probe analysis, which also supported the results concerning the regiospecificity of the cycloaddition reaction under investigation. The relative experiments have been carried out with $Eu(fod)_3$ as lanthanide shift reagent[16]. The choice represents a good compromise of two important factors, the shift towards low fields and the small broadening of the resonance signals. Moreover, the contact contribution to the observed LIS may be considered as negligible in the problem at hand[6]; consequently, a consistent average geometry for compounds 3(a-e) can result from the application of the equation of the pseudocontact interaction[17], which correlates the isotropic shifts with the geometric parameters of the complex and thus of the substrate. On the other hand, gross changes in substrate conformations are not to be expected by the lanthanide when the coordination site is easily accessible on steric grounds, as in examined compounds.

The 4-isoxazolinebenzodiazepines 3(a-e) coordinate $Eu(fod)_3$ preferentially to the amidinic nitrogen atom: this assumption is based on the fact that a linear dependence of LIS on LSR/substrate ratio is observed, together with a comparable high LIS for N-methyl and 5-methylene groups. Also the computer-simulation of LIS accounts well for the coordination of lanthanide to the amidinic nitrogen atom, taking into consideration that Eu is located in the direction of the $sp^2$ lone-pair orbital. Limiting shifts were obtained by mean-squares fitting of experimental values, obtained from ten solutions of different molar ratios, assuming at the equilibrium a 1:1 complex for the substrate and the paramagnetic reagent.

All four possible diastereoisomers (per regioisomer) for the obtained cycloadducts were tested by least-squares analysis to fit experimental and calculated shift; the geometrical interpretation of spectral parameters and correct assignment of resonances have been performed with the LISCA program[18]. The molecules at hand have been considered as systems of three rigid units, connected to one other by a bond rotatable without any restriction, so giving to the system under investigation the largest number of freedom degrees allowed by the LISCA approach. Calculations have

been performed for all different models, optimizing the rotatable bonds, the Eu-N distance and the bond angle Eu-N-C(6). The geometric parameters of the examined structures have been deduced by suitable Dreiding models. In the reported analysis, the magnetic axis of the complex was always aligned along the Eu-N bond: the fit optimized in all cases to $< 5°$. Deviations from planarity of the coordination site of the lanthanide ion with the main axis of the amidinic nitrogen lone-pair were not relevant, because preliminary trial calculations showed that calculated LIA did not vary significantly by exploring stepwise all the possible spatial locations of the lanthanide.

The obtained results allowed to assign confidently the relative molecular geometry to the diastereoisomers observed in solution. Only two structures (A) and (B), derived from the cis fusion of the five- and seven-membered rings, interconvertible by the reversal of the seven-membered nucleus, gave a good agreement between experimental and calculated shifts (Table 2). The calculated LIS for the other possible regioisomers and conformers are too far away to be reconciled with the experimental values.

The stereochemical characteristics of (A) and (B) are showed in Scheme 2: the folded conformer (B), more sterically hindered, is the minor isomer in solution. In particular, the torsion angle between the fused aromatic ring and the N-O bond of the 4-isoxazoline ring is 142°; thus the stereochemistry of the migrating bond at C-3 of the isoxazoline nucleus[12] is close to the ideal (180°) for the anti 1,2-migration, in accord with the mechanism proposed for the observed thermal rearrangement of these compounds to pyrrole-fused quinoxaline derivatives[9].

In conclusion, the stereochemistry of the dihydroisoxazolo[2,3-d][1,4]benzodiazepine compounds examined has been assigned. The nature of the added heterocyclic ring has been found to exert a certain influence on the conformational properties of the heptaatomic benzodiazepine ring. As reported[14], chlordiazepoxide exist in $CDCl_3$ solution as two boat conformers which are rapidly interconverting at room temperature; the ring inversion barrier is 15.2 kcal/mol. Compounds 3(a-e) show in solution a mixture of diastereoisomers slowly interconvertible at room temperature; in comparison with the precursor chlordiazepoxide, the annelation of a 4-isoxazoline ring to the "d" edge of the benzodiazepine nucleus results in an increase of the ring reversal barrier. The coalescence of two N-methyl doublets provides the free energy of activation for the conversion of the minor to major isomer: preliminary results employing temperature dependent nmr analysis indicated that the ring reversal barriers for 3a and 3b are 16.8 and 17.2 kcal/mol respectively. The reduced conformational mobility of the seven-membered ring is consistent with some of the results obtained for benzodiazepine derivatives which show a pentaatomic nucleus annelated to the "d" edge of the molecule with the

position of the phenyl substituent maintained[6,7].

## EXPERIMENTAL

Nmr spectra have been recorded at 200 MHz (Bruker WP 200 SY). Lanthanide induced shifts measurements were performed for solutions ~ 0.3 M in $CDCl_3$, containing increasing amounts of $Eu(fod)_3$ up to a value of 0.35 L/S ratio. $Eu(fod)_3$ was added stepwise from a standard solution (~300 mg/ml). Each signal monitored in the spectra and LIS were found to be directly proportional to the L/S ratio present. Computer-simulation of LIS has been carried out on a IBM 370/115 computer by the LISCA program. The interatomic distances, bond angles and dihedral angles were taken from pertinent Dreiding models. Methyl groups were treated as twelve equivalent points over which calculated LIS values are averaged.

Dihydroisoxazolo [2,3-d][1,4] benzodiazepines investigated have been synthesized as described[8,9]. Their analytical parameters were in accord with those reported.

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Received, 28th April, 1986