Configurational assignment of long-chain alkylated pyridinium aldoxime bromides by Noesy experiments

Mamta Sharma*, A.K. Gupta and S.K. Raza

Synthetic Chemistry Division, Defence Research and Development Establishment Gwalior-474002, M.P. India

A configurational study of long-chain *N*-alkylated pyridinium aldoxime derivatives and their positional isomers has been carried out by two-dimensional ^{1}H–^{1}H NOESY (nuclear Overhauser effect spectroscopy). Cross peak intensities were observed to be enhanced with increasing mixing time. Mixing times longer than 250 ms result in increasing contribution of spin diffusion that produces unrealistic hydrogen-hydrogen distances. The results of NOE measurements showed significant enhancement in the intensity of iminyl proton resonances on irradiation of hydroxyl proton resonances and vice versa. The chemical shift difference between hydroxyl proton resonances and iminyl proton resonances was found to be ~4 ppm for *syn* and ~5 ppm for *anti* configurations. The study reveals that these compounds exist in the *E* configuration *i.e.* the *syn* form in solution. The *syn* isomer predominates; the *anti* isomer amounts are 3% (2-oxime), 4% (3-oxime) and 6% (4-oxime).

Keywords: 1-D DIFNOE, 2-D NOESY, *N*-alkylated pyridinium aldoximes; 2-hydroxyiminomethylpyridinium methyl chloride

Nerve agents Tabun (GA), Sarin (GB), Soman (GD), Cyclosarin (GF) are considered as the most toxic chemical warfare (CW) agents.[1] Their use in World War II, Iran–Iraq conflicts and in terrorist activities has posed a threat to mankind and created an urgent necessity for effective antidotes for them.[2] At present, available antidotes against organophosphorous compound poisoning are heterocyclic mono and bispyridinium aldoximes, such as 2 PAM Cl^-, (2-hydroxyiminomethylpyridinium methyl chloride), Toxogonin [bis 1,1'-(4-hydroxyiminomethylpyridinium) dimethyl ether], HI-6 [1-(2-hydroxyiminomethylpyridinium)-1'-(4-carbamoyl-pyridinium) dimethyl ether] dichloride etc. whose functionality is responsible for reactivating the inhibited acetylcolinesterase enzyme (AchE).[3,4] We have selected the present class of compounds because it was found that due to the long-chain at nitrogen of pyridinium aldoximes these compounds may survive for longer periods and can cross the blood–brain barrier more effectively due to enhancement in lipid solubility. They may give a faster and more effective antidotal action. Some of these compounds were found more effective as adjuvants with PAM chloride and atropine, than atropine alone.[5,6]

In the search for suitable antidotes, the presently available compounds should be studied further with respect to their spatial arrangements to understand their antidotal efficacy. The effectiveness of an antidote depends on its molecular interactions (intra and inter) and the spatial arrangements of substrituent groups. Oximes can possess two isomeric configurations, *syn* and *anti*. Pozimek *et al.*[7] and Spohrer and Eyer[8] succeeded in isolating the two geometrical isomers of 4-PAM Cl^- [4-(hydroxyiminomethyl)pyridinium methyl chloride] and three isomers (*syn–syn*, *syn–anti*, *anti–anti*) of toxogonin respectively, which were characterised by ^{1}H NMR spectroscopy. The *syn* form is found to be more fitted to releasing the CW agents from inhibited acetylcolinesterase enzyme(AchE).[7-9] In this regard, a 2D NOE NMR study is reported as a potential tool for assigning the spatial orientation of proteins in the solution.[10] A large number of reports is available in the literature in which multiple NMR methods like ^{13}C, ^{15}N, ^{1}H NMR chemical shifts, coupling constant values and 1D DIFNOE (difference of nuclear Overhauser effect) were used to assign the geometry of various oximes.[11-15] We have initiated a two-dimensional NMR study of long-chain alkylated pyridinium aldoxime bromides, in order to determine the configuration and proton connectivities in space. NMR experiments such as $^{13}C\{^{1}H\}$NMR, and 1D DIFNOE have also been used to support our results. To assign the structure of both *syn* and *anti* forms we adopted a 2D HSQC NMR method. The use of HMQC 2D-NMR experiments was previously applied for determination of the *syn* and *anti* forms of selenium compounds in mixtures.[16] Proton NMR and 2D-HSQC NMR analysis revealed the existence of two geometrical isomers in all three-positional isomers. The ^{1}H and ^{13}C chemical shifts of *syn* and *anti* isomers are completely assigned. Comparison of NMR data may help to decide the geometry of newly synthesised compounds.

* Correspondent. E-mail: reekana@rediffmail.com

Experimental

A series of long-chain alkyl isomeric pyridinium oximes (Fig. 1), varying in the chain lengths of the alkyl groups from two to 16 carbon atoms, was prepared by a known method,[17] in which the mixture of pyridinium aldoxime and alkyl halide was refluxed in DMF (dimethyl formamide) at 100°C for 10 hours. These synthesised compounds were purified by flash chromatography on cellulose powder and purity was checked by thin layer chromatography (TLC cellulose DS-0-5 Fluka) by getting a single spot with 1-butanol : acetic acid : water (3 : 1 : 1) as solvent system. High performance liquid chromatography (HPLC) was also used to ensure the purity of these compounds. HPLC conditions were as follows: (i) column – C-18 micro bonda pack (Waters); (ii) mobile phase – acetonitrile: water (20:80) were used; (iii) detector – UV at 254 nm wavelength. The pH was adjusted in the range of 3–4 by adding 0.01 M heptane sulfonic acid solution. The flow rate was kept constant at 1.5 ml^{-1} min and UV absorbance was detected at 254 nm using a Perkin-Elmer spectrophotometer.

NMR experiments

The NMR spectroscopic data for ^{1}H and ^{13}C nuclei were recorded in DMSO-d_6 (dimethyl sulfoxide-d_6) using a Bruker DPX 400 MHz NMR instrument at observation frequencies of 400.1324 and 100.6228 MHz respectively. The chemical shift scale was adjusted to deuterated DMSO-d_6 at 2.5 ppm and 49.5 ppm for proton and carbon nuclei respectively. All ^{13}C spectra were recorded using proton-decoupled mode. All proton homonuclear NOESY NMR spectra were recorded using degassed (0.2–0.5 M) oxime solutions in DMSO-d_6. DIFNOE NMR spectra were recorded using non-degassed solution conditions. The spectroscopic parameters for ^{1}H NMR were adjusted in the frequency range of 1–14 ppm (5995.204 Hz), with pulse width 5 μs (flip angle 90^0), pulse delay 1 s., acquisition time 2.73 s and for ^{13}C NMR the frequency range was 220 ppm with a pulse width 6.75 μs (flip angle 90^0), pulse delay 2 s, acquisition time 1.37 s. Interpretation of ^{1}H and $^{13}C\{^{1}H\}$ δ was made with the help of two-dimensional ^{1}H–^{1}H COSY (homo nuclear correlation spectroscopy) and ^{1}H–^{13}C HSQC (heteronuclear single quantum correlation), and DEPT NMR experiments.

Result and discussion

The long-chain alkylated pyridinium aldoxime bromides with alkyl chains varying from C_2H_5 to $C_{16}H_{33}$, having an hydroxyliminomethyl group (–CH=NOH), at different positions of the pyridine ring shown in Fig. 1 were synthesised. Here ^{1}H and ^{13}C chemical shifts of the *syn* and *anti* forms of *N*-dodecyl-2,3-and 4-(hydroxyiminomethyl) pyridinium bromide are presented as representative for this class of compounds (shown in Tables 1 and 2). It is notable that a significant change in δ of alkyl groups was not observed in any of the positional isomers. The spectroscopic assignment for ^{1}H and ^{13}C nuclei was made on the basis of shielding and deshielding effects of attached groups and the splitting pattern of proton signals for all the aldoximes. Proton δ assignment was done on the basis of COSY experiments. δ Assignment of carbons was done with the help of proton–carbon correlation contours of HSQC. It was observed that during the synthesis of long-chain alkylated pyridinium aldoximes the *anti* form is also formed along with the *syn* form in small quantity. As a result small intensity signals were also observed along with high intensity signals in proton and carbon NMR spectra of all the positional isomers (Fig. 2). The observed proton chemical shift of the *anti* form was compared with previously reported chemical shifts for other oximes 4-PAM[7] and obidoxime.[8] The chemical shifts of the CH protons of the *anti* forms showed lower frequencies than the *syn* form of the pyridinium aldoximes[7] and obidoximes.[8] Furthermore, we have confirmed these values with the help of COSY and HSQC experiments. The proton NMR spectra of all the isomers showed complete separation in their chemical shift values for both the forms. However, carbon NMR spectrum of the *N*-alkylated 2-pyridinium aldoxime showed the distinct δ for the *anti* form while other 3 and 4 isomers showed a distinction in δ of C-3 and C-7 only. It was also observed that δ of other carbon signals of the *syn* and *anti* forms merged together. To check the observed facts, the chemical shifts of the 2, 3 and 4 pyridinium oximes were recorded and compared. Furthermore, the conclusions were also confirmed by standard addition of corresponding aldoximes. The result of standard addition showed that signals have different chemical shift values than the peaks of the *syn* and the *anti* forms.

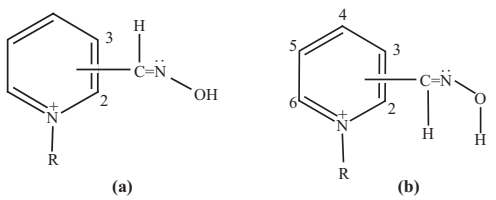

Fig. 1 (a) *N*-Alkyl pyridinium aldoxime bromides, showing the structure of *anti* forms, R is an alkyl group having general formula C_nH_{2n+1} where *n* varies from 2 to 16, –CH=NOH group present at 2, 3 or 4 positions of the pyridinium ring; (b) structure of *syn* forms.

Single bond connectivity of protons with carbons in the *syn* and *anti* forms of all the three isomers was observed in 2D-HSQC NMR. (Fig. 3) Fourteen intense peaks were observed in the ^{13}C NMR spectrum of *N*-dodecylpyridinium 2-aldoxime. C-2 was assigned easily because it showed a signal at high frequency at 146.7 ppm and the absence of a correlation contour with any proton. Secondly the deshielded C-6 (145.9 ppm) showed correlation with the H-6 proton (9.05 ppm). Thirdly the deshielded C-4 (145.1) showed attachment with H-4 (8.6 ppm). C-5 and C-3 showed attachment with assigned H-5 and H-3 at 127.4 ppm and 125.6 ppm in the f2 dimension of the HSQC

Table 1 The ^{1}H chemical shifts of 2, 3, 4 substituted *N*-dodecylpyridinium aldoximes bromides

Position	PA-I				PA-II				PA-III			
	Syn	*J*/Hz	*Anti*	*J*/Hz	*Syn*	*J*/Hz	*Anti*	*J*/Hz	*Syn*	*J*/Hz	*Anti*	*J*/Hz
2	–	–	–	–	9.25	–	9.60	–	9.02	6.6	9.30	5.6
3	8.39	8.0	8.32	7.5	–	–	–	–	8.22	6.6	8.51	5.5
4	8.55	7.8	8.65	7.9	8.71	8.2	8.81	7.6	–		–	
5	8.10	6.9	8.16	7.0	8.14	6.1	8.22	5.9	8.22	6.6	8.51	5.5
6	9.05	6	9.2	5.9	9.04	6.0	9.08	6.2	9.02	6.6	9.30	5.6
7	8.78	–	8.18	–	8.33	–	7.76	–	8.42	–	7.91	
8												
9	13.13	–	12.84	–	12.25	–	12.79	–	12.82	–	13.5	–
10	4.73	7.6	4.6	7.5	4.61	7.3		–	4.55	7.2	–	–
11	1.79	7.6	–	–	1.92	7.3			1.90	7.2	–	–
19*	0.85	6.6			0.85	6.6			0.85	6.6		

PA-I = *N*-Dodecyl-2-(hydroxyiminomethyl)pyridinium bromide.
PA-II = *N*-Dodecyl-3-(hydroxyiminomethyl)pyridinium bromide.
PA-III = *N*-Dodecyl-4-(hydroxyiminomethyl)pyridinium bromide.
*H-12 to H-18 appeared in the range of 1.25–1.09 ppm.

Table 2 The ^{13}C{^{1}H} chemical shifts of 2, 3, 4 substituted *N*-dodecylpyridinium aldoximes bromides

Position	PA-I		PA-II		PA-III	
	Syn	*Anti*	*Syn*	*Anti*	*Syn*	*Anti*
2	146.7	146.9	142.5	142.5	144.9	144.9
3	125.6	128.0	133.6	133.5	124	124
4	145.1	145.4	141.4	141.4	148.3	148.3
5	127.4	129.6	128.1	128.1	124	124
6	145.9	146.1	144.3	144.3	144.9	144.9
7	141.4	137.4	143.3	143.3	145.1	145.1
8	–	–	–	–	–	–
9	–	–	–	–	–	–
10	57.9	58.6	61.2	61.2	60.3	60.3
11	30.3	30.2	30.6	30.6	30.5	30.5
12	31.2	31.2	31.3	31.3	30.9	30.9
13	28.7	28.7	28.8	28.8	28.0	28.0
14	28.7	28.6	28.7	28.7	28.6	28.6
15	28.5	28.5	28.6	28.6	28.5	28.5
16	28.3	28.3	28.4	28.4	28.3	28.3
17	25.3	25.3	25.4	25.4	25.3	25.3
18	22.0	22.0	22.1	22.1	21.9	21.9
19	13.8	13.8	13.9	13.9	13.8	13.8

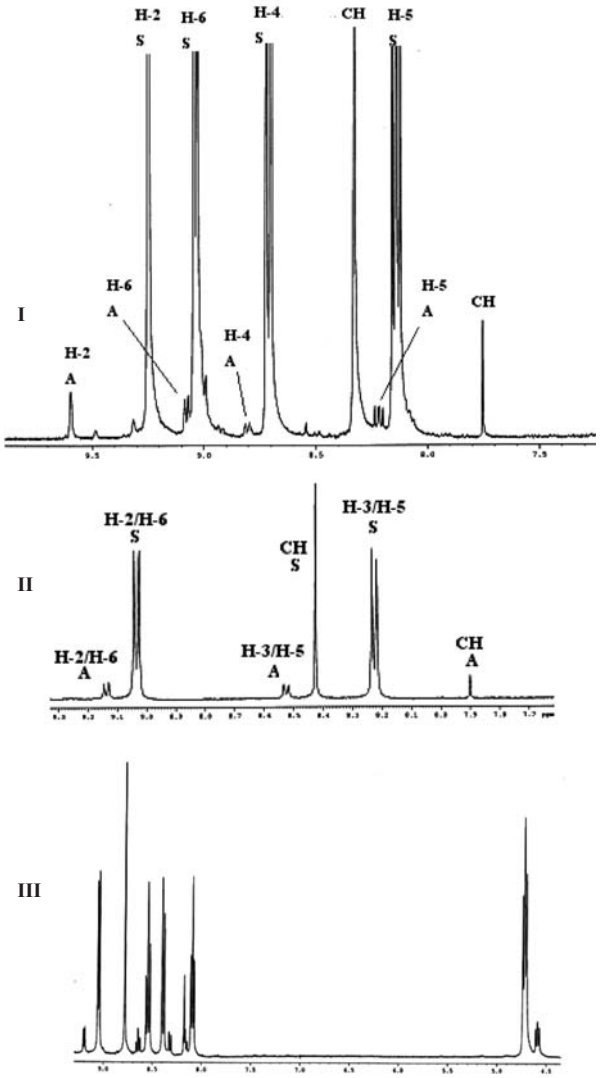

Fig. 2 ^{1}H NMR spectrum of *N*-dodecyl-3-(hydroxyiminomethyl) pyridinium bromide (I), *N*-dodecyl-4-(hydroxyiminomethyl) pyridinium bromide (II), *N*-dodecyl-2-(hydroxyiminomethyl) pyridinium bromide (III) (high intensity signals are for *syn* form and low intensity signals are for the *anti* form) showing proton signals for *syn* (S) and *anti* (A) form of compounds.

spectrum respectively. C-7 at 141.4 ppm showed correlation with H-7 (8.78 ppm). C-7, H-7 of the *anti* form were shielded more than in the *syn* form due to the shielding effect of the lone pair of electrons on nitrogen, appeared at 137.4 ppm and 8.18 ppm respectively. The same shielding effect was observed in the other isomers, the 2 and 3 substituted oximes being shown in Table 1. C-7 and H-7 of 4-substituted oxime showed correlation at 140 ppm of the carbon chemical shift scale and 7.91 ppm of the proton chemical shift scale.

DIFNOE NMR experiments

All the compounds were subjected to the DIFNOE experiment. *N*-octyl-3-(hydroxyiminomethyl)pyridinium bromide was chosen as model compound for presentation of results. In these measurements, the irradiation of the oxime hydroxyl proton resulted in the significant enhancement of the imino methine proton resonance. Similarly in reverse experiments irradiation of the imino methine proton caused enhancement of the hydroxyl proton resonance as shown in Fig. 4. Similar results were obtained for all of the isomeric compounds of ethyl to cetyl pyridinium aldoxime bromides even with the variation of the position of the hydroxyimino methyl moiety. The result of this study indicates that the hydroxyl and CH groups are in close proximity, which provides strong evidence that these compounds exist in the *E*/*Syn* configuration in DMSO-d_6 solution.

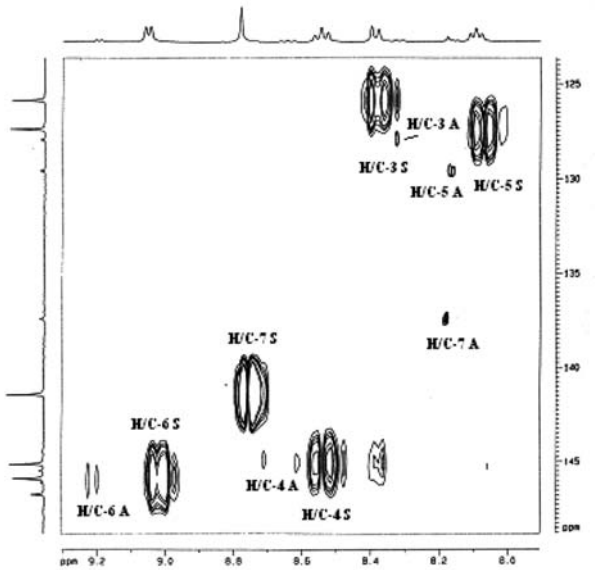

Fig. 3 ^{1}H–^{13}C HSQC NMR spectrum (partial) of *N*-dodecyl-2-(hydroxyiminomethyl)pyridinium bromide.

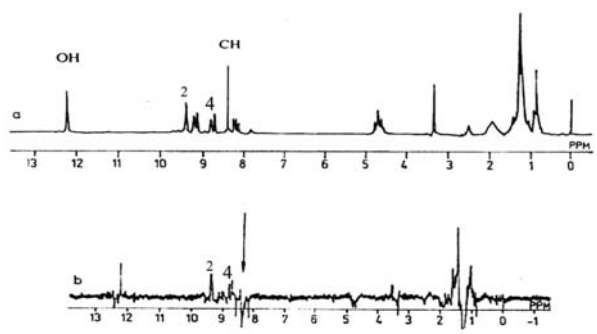

Fig. 4 (a) ^{1}H NMR spectrum of *N*-octyl-3-(hydroxyiminomethyl) pyridinium bromide (b) NOE Difference Spectrum of *N*-octyl-3-(hydroxyiminomethyl)pyridinium bromide resulting from irradiation of the CH proton.

2D-NOESY experiments (intramolecular interactions)

The spatial connectivity of OH and H protons and other closely placed protons of pyridinium ring and alkyl chain has been studied by 2D-NOESY NMR experiment.[18] This also depicts the orientation of alkyl chain and oxime group with respect to the pyridinium ring. In *N*-dodecyl-2-(hydroxyiminomethyl)pyridinium bromide CH is close to OH, H-10, H-11, H-12 and H-6 is present near to the H-10, H-11 and H-12 protons. In *N*-dodecyl-3-(hydroxyiminomethyl) pyridinium bromide CH is close to OH, H-2 and H-2/H-6 are close to H-10, H-11 and H-12 in space. In *N*-dodecyl-4-(hydroxyiminomethyl) pyridinium bromide CH is close to OH and H-2/H-6 are close to H-10, H-11 and H-12 in space. Seven of *N*-dodecyl-2(hydroxyiminomethyl) pyridinium bromide and four cross peaks of *N*-dodecyl-4(hydroxy-iminomethyl)pyridinium bromide are shown in Figs 5a, and 5b respectively. Seven high intensity cross peaks showing correlation between protons of *N*-dodecyl-2-(hydroxyiminomethyl) pyridinium bromide were taken to plot the graph. All the NOESY NMR experiments were performed with water suppression at 3.3 ppm. The NOESY experiments were done at different mixing times (50, 100, 150, 200, 250, 300, 350, 400 and 450 ms). Decrease in the cross peak intensity was observed in the order of CH-H-10>H-6-H-10>CH-CH-11>H-6-H-11>CH-H-12>H-6-H-12>CH-OH in *N*-dodecyl-2-(hydroxyiminomethyl) pyridinium bromide. Cross peaks obtained for CH and OH protons of the oximino group showed low intensity due to the large distance between them. These two groups are at the same side of the double bond. The build-up curves (Fig. 6) of the intensities of all the NOE peaks showed the characteristic parabolic shape. The maximum NOE intensities are obtained in the 100–150 ms range of mixing time. Above mixing

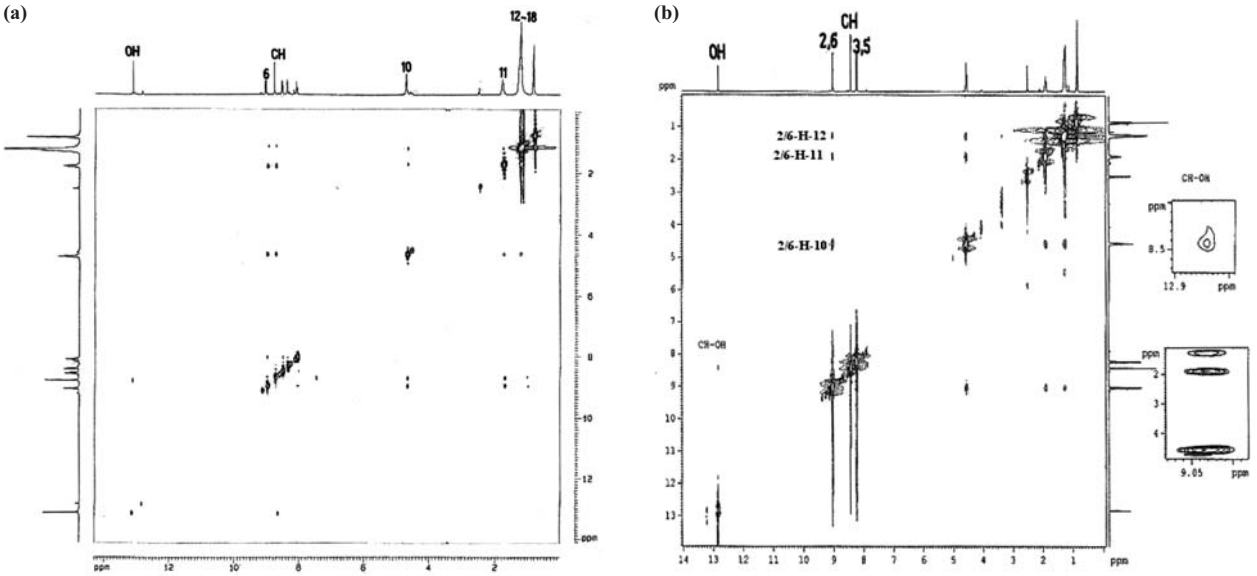

Fig. 5a NOESY Spectrum of *N*-dodecyl-2-(hydroxyiminomethyl)pyridinium bromide in DMSO solvent at a concentration of 20 mM. The numbering of the atom correspond to that shown in the structure. **5b** NOESY spectrum of *N*-dodecyl-4-(hydroxyiminomethyl) pyridinium bromide in DMSO solvent at a concentration of 20 mM. The numbering of the atoms corresponds to that shown in the structure. Expansion of contours is also shown.

time of 250 ms spins started getting diffused. Only five mixing times of 50, 100, 150, 200 and 250 ms are taken to plot the graph.

Conclusion

All the N-alkylated pyridinium aldoximes were found to be in the *syn* form. There is no effect of the long-chain and the placement of the oxime group at pyridinium ring, whether it is at 2,3 or 4 position, on the configuration of the hydroxyl imino group. This always remains in the *syn* form, that is the OH and H protons are at same side of the double bond. Decrease in cross peak intensity in NOESY NMR spectra is the indication of increase of inter protonic distances in the molecule. The CH proton of the *syn* form appeared at a high frequency value (~8 ppm) than the CH proton of the *anti* form (~7 ppm). This is due to the shielding effect of the lone pair electrons of nitrogen of the oxime group. The difference in chemical shifts of OH and CH protons for long-chain alkylated pyridinium aldoximes is found to be 4.35, 3.92 and 4.40 ppm for *syn* and 4.66, 5.03 and 5.59 ppm for the *anti* form of 2, 3, 4 substituted oxime derivatives respectively. This CH and OH δ difference is more for the *anti* than the *syn* structures. Similar observations are reported for the oximes of aliphatic and alicyclic aldehydes[19] also.

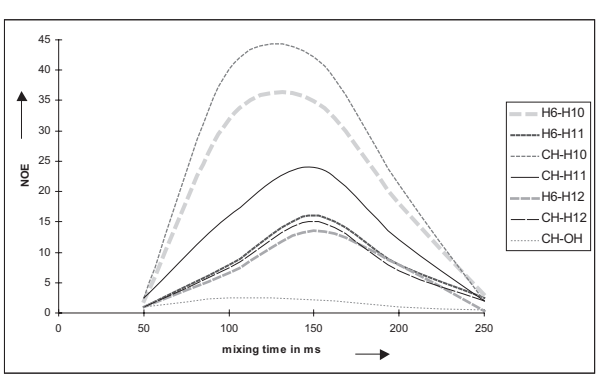

Fig. 6 Build-up curves of the seven most intense intraproton tr-NOE cross-peaks. (a) CH-OH; (b) H6-H12; (c)CH–H12; (d) H6–H11; (e) CH–H11; (f) H6–H10; (g) CH–H10. The parabolic behaviour is observed for all the peaks for *N*-dodecyl-2-(hydroxy-iminomethyl)pyridinium bromide.

We thank Dr. R. Vijayaraghavan, Director DRDE, Gwalior, for his keen interest, encouragement and fruitful discussion.

Received 18 February 2008; accepted 15 April 2008
Paper 08/5102 *doi: 10.3184/030823408X318424*

References

1 H.P. Benschop and L.P.A. De Jong, *Acct Chem. Res.*, 1988, **27**, 368-374.
2 Y. Miyata and H. Ando, *J. Hlth Sci.*, 2001, **47**, 75-77.
3 D. Waysbort, D. Balderman and G. Amitai, *Org Mag. Reson.*,1981, **16**, 7-10.
4 K. Peter, *Curr. Med. Chem.*, 2003, **10**, 2705-2709.
5 K. Rossmann, *Proc. Inter. Symp., Protection Against Chemical Warfare Agents, 6-9 June 1983,* Stockholm, Sweden, National 13 Defence Research Establishment Department of NBC Defence, S-90 182 UMEA°, Sweden, pp.233–237.
6 D.A. Ligtenstein, *Proc. Inter. Symp., Protection Against Chemical Warfare Agents, 6-9 June 1983,* Stockholm, Sweden, National Defence Research Establishment Department of NBC Defence, S-90182 UMEA°, Sweden, pp.129–140.
7 E.J. Poziomek, D.W. Karamer, W.A. Mosker and H.O. Michel, *J. Am. Chem.* Soc., **83**, 1961, 3916-3917.
8 U. Spohrer, P. Eyer, *J. Chromatogr. A,* 1995, **693**, 55-61.
9 K.D. Berlin and S. Rengaraju, *J. Org. Chem.*, 1971, **36**, 2912-2915.
10 J. Cavanagh, W.J. Fairbrother, AG. Palmer III and N.J. Skelton, 1996 *Protein NMR spectroscopy. principles and practic*e, Academic Press, San Diego, CA.
11 M. Idrissi and S. Faraj, *Phys. Chem. News*, 2003, **14**, 124-126.
12 G.E. Hawkes, K. Herwig and J.D. Roberts, *J. Org. Chem.*, 1974, **39**,1017-1027.
13 G. Heinisch and W. Holzer, *Collect. Czech. Chem. Commun.,* 1991, **56**, 2251-2257.
14 D. Crepaux and J.M. Lehn, *Org. Magn. Reson.*, 1975, **7**, 524-526.
15 G.E. Bachers and T. Schaefer *Chem. Rev.*, 1971, **71**, 617-626.
16 Valentin Ananikov, Zelinsky Institute of Organic Chemistry, Russian Academy of Science, Moscow, *Bruker Biospin report* 2005, pp.41-43.
17 J. Epstein, J.J. Kaminski, N. Bodor, R. Enever, J. Sowa and T. Higuchi, *J. Org. Chem.*, 1978, **43**(14), 2816-2821.
18 N. Heimer, S. Del, E. Rico and W.R. Carper, *Magn. Reson. Chem.*, 2004, **42**, 71-75.
19 G.G. Kleinspehn, J.A. Jung and S.A. Studnlarz, *J. Org. Chem*., 1967, **32**, 460.