On the regio- and stereoselectivity of pyridinyl radical dimerization

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The present work was carried out in order to elucidate the combined effects of the electron spin density on the ring carbons and the steric hindrance of the ring substituents upon the regio- and stereoselectivity of the dimerization of 3-carbamoyl- and 3-cyanopyridinyl radicals. To this purpose the composition of mixtures of diastereoisomeric dimers arising from the one-electron electrochemical reduction of several 3-carbamoyl and 3-cyano substituted pyridinium salts has been studied by ^{1}H NMR spectroscopy. In some cases, single diastereoisomers have been isolated from mixtures by preparative HPLC.

The results show that: (*a*) hindering steric effect of substituents at coupling sites prevails over electron spin density on coupling carbons in governing regioselectivity of 3-carbamoylpyridinyl radical dimerization; (*b*) large bulky N-ring substituents produce a significant shielding effect on the adjacent dimerization site; (*c*) the relative amounts of diastereoisomers in the mixtures of 4,4′- and 6,6′-linked dimers indicate that the dimerization process is largely stereoselective; (*d*) otherwise, nearly equal amounts of 4,4′- and 4,6′-linked dimers, and relative diastereoisomers as well, arise from the reduction of 3-cyano substituted pyridinium salts. This finding indicates that the presence of the carbamoyl substituent at the 3 position is a primary factor in inducing the regio- and stereoselectivity of pyridinyl radical dimerization.

In a recent paper[1] we reported the influence of steric factors on the regioselectivity of the dimerization of pyridinyl radicals arising from one-electron electrochemical reduction of 4- and 6-methyl substituted 3-carbamoylpyridinium salts. For example, 1-benzyl-3-carbamoyl-4-methylpyridinium bromide was found to afford, as the main product, a diastereoisomeric pair of 6,6′-linked dimers: this finding represents the first example of a symmetrical dimerization at the 6 positions of 3-carbamoyl substituted pyridinyl radicals. It is worth underlining that 4,4′-linked dimers were not obtained and that substantial amounts of 4,6′-linked dimers were, however, formed. These results indicate that, in this case, the symmetrical 4,4′-dimerization is sterically hindered by the 4-methyl substituents, but also show that the higher electron spin density on the 4 carbons[2] still favours these positions as coupling sites.

In the present work we further investigated the combined effects of the electron spin density on the ring carbons and of the steric hindrance by the ring nitrogen substituents upon the regioselectivity of the dimerization of 3-carbamoylpyridinyl radicals. To this purpose, an accurate ^{1}H NMR analysis was carried out on the dimer mixtures arising from a one-electron electrochemical reduction of the following salts: 1-(2,6-dichlorobenzyl)-3-carbamoyl-4-methylpyridinium bromide (**1a**), 1-(2,6-dichlorobenzyl)-3-carbamoyl-6-methylpyridinium bromide (**1b**), 1-(2,6-dichlorobenzyl)-3-carbamoylpyridinium bromide (**1e**) and 1-benzyl-3-carbamoylpyridinium chloride (**1f**). Furthermore, the composition of the dimer mixture obtained by reduction of the coenzyme NAD^+ was also studied.

Lastly, since steric interference between the 3-carbamoyl group and the 4 position of 1,4-dihydropyridine systems has been reported[3] and could affect the dimerization course of the 3-carbamoylpyridinyl radicals, we studied the composition of the dimer mixtures obtained by reduction of some 3-cyano substituted pyridinium salts such as 1-methyl-3-cyanopyridinium iodide (**1i**), 1-benzyl-3-cyanopyridinium bromide (**1l**) and 1-(2,6-dichlorobenzyl)-3-cyanopyridinium bromide (**1m**), in which any steric effect on the regioselectivity of the dimerization by the sterically undemanding cyano substituent should be absent.

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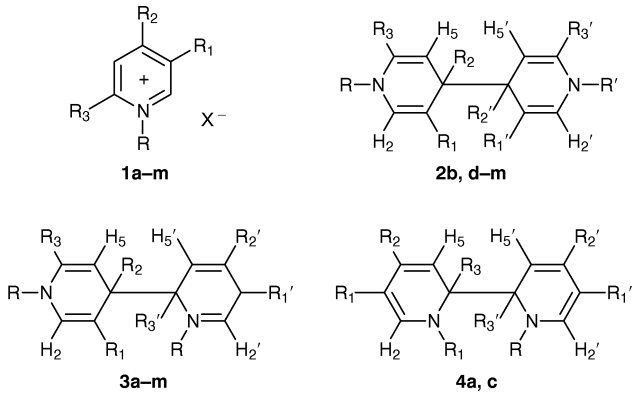
Results

Cyclic voltammetry of all the studied salts showed cathodic peaks in the range between −1.40 and −1.10 V *vs* a saturated calomel electrode (SCE). When the scan was reversed no corresponding anodic peaks were observed, but, in all voltammograms, a peak at about −0.02 V appeared instead. This behaviour closely agrees with the acquisition of one electron by the pyridinium cations, followed by irreversible dimerization of the resulting radicals to give dimers whose oxidation occurs at more positive potentials.[4]

The electrolysis of salts **1b**, **e**, **f**, **l**, **m** afforded mainly 4,4′-linked dimers (structures **2**) together with variable amounts of 4,6′-linked dimers (structures **3**), while 4,6′- and 6,6′-linked dimers (structures **4**) arose from the reduction of salt **1a**. These structures (shown in Scheme 1) are supported by molecular mass peaks and ^{1}H NMR and UV spectral data: in particular UV and NMR spectra indicate the presence in the dimers of only 1,4- and 1,6-dihydropyridine moieties. As the pyridinyl radical dimerization gives rise to two centers of asymmetry at the junction carbons, equivalent in 4,4′- and 6,6′-linked dimers and nonequivalent in 4,6′-linked dimers, two diastereoisomers (a *meso* form and a racemate) are possible in the first case and two again (two racemates) in the second. In the symmetric dimers (structures **2** and **4**) the corresponding protons of the two dihydropyridine moieties are stereochemically equivalent and, consequently, their chemical shifts are pairwise equal whereas, because of the molecular asymmetry, the signals of the protons of the whole molecule are present in ^{1}H NMR spectra of the 4,6′-linked dimers (structures **3**).

In some instances, as in the case of the dimer mixture arising from the reduction of salt **1a**, HPLC chromatography



Scheme 1 **a**: R = R′ = 2,6-dichlorobenzyl; $R_1 = R'_1 = CONH_2$; $R_2 = R'_2 = CH_3$; $R_3 = R'_3 = H$; $X^- = Br^-$. **b**: R = R′ = 2,6-dichlorobenzyl; $R_1 = R'_1 = CONH_2$; $R_2 = R'_2 = H$; $R_3 = R'_3 = CH_3$; $X^- = Br^-$. **c**: R = R′ = benzyl; $R_1 = R'_1 = CONH_2$; $R_2 = R'_2 = CH_3$; $R_3 = R'_3 = H$; $X^- = Br^-$. **d**: R = R′ = benzyl; $R_1 = R'_1 = CONH_2$; $R_2 = R'_2 = H$; $R_3 = R'_3 = CH_3$; $X^- = Br^-$. **e**: R = R′ = 2,6-dichlorobenzyl; $R_1 = R'_1 = CONH_2$; $R_2 = R'_2 = R_3 = R'_3 = H$; $X^- = Br^-$. **f**: R = R′ = benzyl; $R_1 = R'_1 = CONH_2$; $R_2 = R'_2 = R_3 = R'_3 = H$; $X^- = Cl^-$. **g**: R = R′ = ADPR;[a] $R_1 = R'_1 = CONH_2$; $R_2 = R'_2 = R_3 = R'_3 = H$. **h**: R = R″ = APDPR;[a] $R_1 = R'_1 = CONH_2$; $R_2 = R'_2 = R_3 = R'_3 = H$. **i**: R = R′ = methyl; $R_1 = R'_1 = CN$; $R_2 = R'_2 = R_3 = R'_3 = H$; $X^- = I^-$. **l**: R = R′ = 2,6-dichlorobenzyl; $R_1 = R'_1 = CN$; $R_2 = R'_2 = R_3 = R'_3 = H$; $X^- = Br^-$. **m**: R = R′ = benzyl; $R_1 = R'_1 = CN$; $R_2 = R'_2 = R_3 = R'_3 = H$; $X^- = Br^-$. [a] ADPR = adenosindiphosphoribosyl, APDPR = adenosin(2′-phosphate)diphosphoribosyl

allowed the separation of all the expected diastereoisomers. This made a careful ^{1}H NMR analysis of each single diastereoisomer (**3a$_1$**, **3a$_2$**, **4a$_1$** and **4a$_2$**) possible, thus facilitating both the identification of the diastereoisomers present in other dimer mixtures and the determination of their relative abundances, without separating them.

Moreover, a thorough study was carried out on the dimer mixture obtained from the reduction of NAD^+ (**1g**).[5] The ^{1}H NMR spectrum of the crude dimer mixture showed a complex pattern of signals, resulting from the several stereochemical arrangements possible from the NAD radical dimerization. Nevertheless, the known proton signal sequences of the symmetrical and asymmetrical structures **2** and **3** are clearly detectable. It is important to point out that, as already reported for NADP radicals,[6] in NAD dimers, besides the chiral centres already present in ribosyl-adenosine moieties, whose configuration (*A*) is retained, two additional chiral centres (*R* or *S*) are formed at the dimerization positions. Consequently, in the case of the 4,4′-linked dimers, a maximum of three diastereoisomers can exist, whose possible configurations are *ARRA*, *ASSA* or *ARSA*. The corresponding protons in the two molecule halves are stereochemically equivalent in the *ARRA* and *ASSA* configurations and thus must show chemical shift equivalence, whereas in the *ARSA* configuration they are nonequivalent and can give two different signals for each proton. Therefore, the ^{1}H NMR spectrum of a mixture of the three diastereoisomers can thus exhibit a maximum of four signals for each nucleus. In the case of the 4,6′-linked dimers, the formation of a mixture of four diastereoisomers may occur. As a matter of fact, the ^{1}H NMR spectrum of the crude NAD dimer mixture shows the presence of three diastereoisomeric 4,4′-linked dimers and, moreover, indicates that one of them is prevalent. Preparative HPLC allowed the separation of the three diastereoisomers, which have then been separately studied by ^{1}H NMR spectroscopy. Thirty-six proton signals can be detected in the spectrum of the prevalent dimer (**2g$_1$**), which, therefore, has an *ARSA* configuration, while only eighteen proton signals are present in the spectra of the other two diastereoisomers, **2g$_2$** and **2g$_3$**, to which the configurations *ARRA* or *ASSA* can be alternatively attributed. Furthermore, four diastereoisomeric 4,6′-linked dimers, present in low abundance, have been detected in the crude dimer mixture, but have not been isolated.

The crude mixtures arising from the reduction of salts **1i**, **1l** and **1m** were analysed by ^{1}H NMR without isolating every single dimer. Careful determination of the relative dimer abundance indicates the formation of nearly equal amounts of 4,4′- and 4,6′-linked dimers, and of the relative diastereoisomers as well.

Tables 1–3 summarise the ^{1}H NMR data of the dimers **2b**,**d–m**, **3a–m** and **4a**,**c** while Table 4 shows their relative abundances. The Tables also include the accurately revised ^{1}H

Table 1 ^{1}H NMR chemical shifts (ppm) and coupling constants (Hz) of the 4,4′-linked dimers **2b,d–m**[a]

					$N-CH_2 + N'-CH_2$						
	$H_2 + H_{2'}$	$H_4 + H_{4'}$[i]	$H_5 + H_{5'}$	$H_6 + H_{6'}$[j]	H_a[k]	H_b[k]	6 + 6′ CH_3	$J_{2,6}$	$J_{4,5}$	$J_{5,6}$	$J_{Ha,Hb}$
2b$_1$	6.84 s	3.02 d	4.35 d	—	4.82 d	4.48 d	1.80 s	—	4.90	—	14.30
2b$_2$	6.69 s	3.08 d	4.43 d	—	4.59 s		1.86 s	—	4.20	—	—
2d$_1$[b]	7.25 s	3.19 d	4.29 d	—	4.71 d	4.27 d	1.59 s	—	4.50	—	16.80
2d$_2$[b]	7.12 s	3.32 d	4.20 d	—	4.55 d	4.39 d	1.68 s	—	4.40	—	16.90
2e$_1$	7.22 d	3.02 d	4.26 dd	5.60 dd	4.51 d	4.47 d	—	1.30	4.75	7.85	14.50
2e$_2$	7.02 d	3.19 d	4.42 dd	5.82 dd	4.48 s		—	1.25	4.75	7.60	[d]
2f$_1$	7.13 d	3.35 d	4.47 dd	5.89 dd	4.31 s		—	1.40	4.60	7.80	[d]
2f$_2$	7.24 d	3.24 d	4.36 dd	6.02 dd	4.34 s		—	1.20	4.70	7.90	[d]
2g$_1$	7.06 br s, 7.01 s	3.20 dd, 2.82 dd	4.55 dd,[c]	6.07 d, 6.12 d	—		—	[d]	5.10, 4.30	7.30, 7.45	—
2g$_2$	7.16 d	2.91 d	4.42 dd	5.97 dd	—		—	1.10	4.05	7.80	—
2g$_3$	7.04 d	2.27 d	4.35 dd	6.10 dd	—		—	1.25	5.10	7.90	—
2h$_1$[e]	7.08 d	2.92 d	4.40 dd	6.01 dd	—		—	1.40	4.80	7.90	—
2h$_2$[e]	6.95 d, 6.93 d	3.19 dd, 2.91 dd	4.67 dd, 4.53 dd	6.11 dd, 6.03 dd	—		—	2.00, 1.50	5.50, 5.60	8.20, 7.90	—
2h$_3$[e]	6.96 d	2.28 d	4.41 dd	6.12 dd	—		—	1.40	5.10	8.20	—
2i$_1$	6.62 d	3.32 d	4.68 dd	5.87 dd	—		—	1.60	3.65	8.15	—
2i$_2$	6.60 d	3.18 d	4.60 dd	5.84 dd	—		—	1.60	4.10	8.05	—
2l$_1$	6.75 d	3.27 d	[f]	5.90 dd	[g]		—	1.70	4.00	8.00	[d]
2l$_2$	6.78 d	3.13 d	[f]	5.98 dd	[g]		—	1.70	4.20	8.00	[d]
2m$_1$	6.71 d	3.23 d	4.55 dd	5.86 dd	[h]		—	1.50	3.90	8.05	[d]
2m$_2$	6.70 d	3.38 d	4.69 dd	5.85 dd	[h]		—	1.50	3.85	8.10	[d]

[a] In the spectra of compounds **2b,d–f,l,m** the signals of the aromatic protons are in the region 7.10–7.60 ppm; in the spectra of compounds **2g** and **2h** the signals of the adenosine protons are in the regions 8.40–8.10, 6.10–6.00 and 4.70–4.00 ppm; in the spectra of compounds **2i** the methylic proton signals are in the region 2.90–3.10 ppm. [b] **2d$_1$** and **2d$_2$** correspond to dimers **5a** and **5b** of ref. 1. [c] Obscured by ribofuranosyl proton signals. [d] Not detected. [e] **2h$_1$**, **2h$_2$** and **2h$_3$** correspond to dimers XI, V and III, respectively, of ref. 6. [f] Obscured by the 2,6-dichlorobenzylic proton signals. [g] Overlapped with the **3l** 2,6-dichlorobenzylic proton signals. [h] Overlapped with the **3m** benzylic proton signals. [i] H_4 and $H_{4'}$ protons correspond to R_2 and $R_{2'}$ substituents of the structures **2**; [j] H_6 and $H_{6'}$ protons correspond to R_3 and $R_{3'}$ substituents of the structures **2**; [k] H_a and H_b are the benzylic or the dichlorobenzylic geminal protons.

Table 2 ^{1}H NMR chemical shifts (ppm) and coupling constants (Hz) of the 4,6′-linked dimers **3a–m**[a]

	H_2	H_4[l]	H_5	H_6[m]	4-CH_3	6-CH_3	$H_{2'}$	$H_{4'}$[l]	$H_{5'}$	$H_{6'}$[m]	4′-CH_3	6′-CH_3	$J_{2,6}$	$J_{4,5}$	$J_{5,6}$	$J_{4,6'}$	$J_{2',4'}$	$J_{4',5'}$	$J_{5',6'}$
3a$_1$	6.98 s	—	4.71 d	5.68 d	1.38 s	—	6.77 s	—	4.71 d	4.49 d	1.63 s	—	—	—	8.90	—	—	—	7.75
3a$_2$	7.12 d	—	4.67 d	5.88 dd	1.24 s	—	6.80 s	—	5.24 d	4.74 d	1.93 s	—	1.10	—	8.15	—	—	—	6.20
3b$_1$	6.78 s	3.95 d	4.73 d	—	—	1.98 s	6.48 s	6.16 d	4.64 d	—	—	1.24 s	—	5.40	—	—	—	10.00	—
3b$_2$	[b]	3.68 d	4.64 d	—	—	2.08 s	[b]	6.17 d	4.63 d	—	—	1.29 s	—	5.30	—	—	—	9.90	—
3c$_1$[c]	7.06 d	—	4.55 d	5.87 dd	1.14 s	—	7.18 s	—	4.70 d	4.64 d	1.91 s	—	1.40	—	8.10	—	—	—	5.90
3c$_2$[c]	6.95 d	—	4.53 d	5.77 dd	1.42 s	—	7.19 s	—	4.76 d	4.63 d	1.94 s	—	1.40	—	8.00	—	—	—	5.80
3d$_1$[c]	7.17 s	3.54 d	4.45 d	—	—	1.79 s	7.14 d	6.28 dd	4.63 d	—	—	1.09 s	—	5.50	—	—	1.30	9.20	—
3d$_2$[c]	7.18 s	3.68 d	4.49 d	—	—	1.74 s	7.12 d	6.20 dd	4.70 d	—	—	1.07 s	—	5.60	—	—	1.30	9.90	—
3e$_1$	[b]	[f]	[g]	6.03 dd	—	—	[b]	[h]	[g]	3.97 dd	—	—	1.05	[e]	7.95	[e]	[e]	[e]	6.70
3e$_2$	7.14 d	[f]	[g]	6.11 dd	—	—	6.77 s	6.46 d	[g]	3.73 dd	—	—	1.45	[e]	8.90	[e]	[e]	9.45	6.50
3f$_1$	[b]	3.70 dd	4.70 dd	6.10 dd	—	—	[b]	6.37 dd	4.88 dd	3.90 dd	—	—	1.40	5.25	7.75	2.50	1.65	9.70	5.25
3f$_2$	[b]	3.83 dd	4.80 dd	6.08 dd	—	—	[b]	6.58 dd	5.00 dd	4.12 dd	—	—	1.40	5.55	7.75	4.70	1.10	9.45	3.60
3g$_1$	7.13 s	2.98 m	[i]	[j]	—	—	7.23 s	[j]	[i]	3.78 m	—	—	[e]	[e]	[e]	1.80	[e]	[e]	5.70
3g$_2$	7.12 s (7.07)	3.34 d	[i]	[j]	—	—	7.37 s (7.29)	[j]	[i]	3.88 m	—	—	[e]	4.20	[e]	4.20	[e]	[e]	[e]
3g$_3$	7.07 s (7.12)	3.32 d	[i]	[j]	—	—	7.29 s (7.37)	[j]	[i]	3.95 m	—	—	[e]	5.70	[e]	2.50	[e]	[e]	[e]
3g$_4$	7.06 s	2.98 m	[i]	[j]	—	—	7.17 s	[j]	[i]	3.95 m	—	—	[e]	[e]	[e]	[e]	[e]	[e]	[e]
3h$_1$[k]	7.09 d	3.00 dd	[e]	[e]	—	—	7.16 d	[e]	5.02 m	3.73 dd	—	—	1.50	5.50	[e]	2.30	2.00	9.90	5.60
3h$_2$[k]	7.01 d	2.97 dd	[e]	[e]	—	—	7.23 d	6.18 dd	[e]	3.89 m	—	—	1.50	5.70	[e]	3.80	1.80	9.90	[e]
3h$_3$	7.02 d	3.28 dd	4.66 dd	6.09 dd	—	—	7.11 d	6.14 dd	5.03 dd	3.89 dd	—	—	1.50	5.50	8.00	2.80	1.30	9.90	5.60
3h$_4$[k]	7.10 d	3.33 dd	4.66 dd	5.93 dd	—	—	7.30 d	6.10 dd	4.97 dd	3.87 dd	—	—	1.50	5.00	8.00	3.80	1.50	9.90	5.40
3i$_1$	6.62 s	3.51 dd	4.65 dd	5.86 d	—	—	6.79 bs	5.99 dd	5.07 dd	4.08 dd	—	—	[e]	4.10	8.05	4.10	[e]	9.85	4.90
3i$_2$	6.61 d	3.54 dd	4.93 dd	5.90 dd	—	—	6.84 bs	6.03 dd	4.93 dd	4.05 dd	—	—	1.60	4.05	9.00	2.60	[e]	9.90	4.95
3l$_1$	6.86 d	3.43 dd	4.65 dd	5.74 m	—	—	6.82 d	6.12 dd	4.91 dd	4.12 m	—	—	1.50	4.75	8.20	[e]	1.35	9.90	5.50
3l$_2$	6.80 d	3.46 dd	4.72 dd	5.80 m	—	—	6.76 d	6.07 dd	5.03 dd	4.15 m	—	—	1.54	4.60	8.20	[e]	1.50	9.90	5.30
3m$_1$	6.80 d	3.37 dd	4.66 dd	5.96 m	—	—	6.87 d	5.96 dd	4.89 dd	4.04 m	—	—	1.50	4.70	8.00	[e]	1.55	9.75	5.30
3m$_2$	6.73 d	3.48 dd	4.74 dd	5.96 m	—	—	6.91 d	6.02 dd	5.05 dd	4.09 m	—	—	1.50	4.55	8.15	[e]	1.05	9.60	5.20

[a] In the spectra of compounds **3a–f,l,m** the signals of aromatic and benzylic protons are in the regions 7.10–7.60 and 4.00–5.00 ppm, respectively; in the spectra of compounds **3g** and **3h** the signals of adenosine protons are in the regions 8.40–8.10, 6.10–6.00 and 4.70–4.00 ppm; in the spectra of compounds **3i** the signals of methyl protons are in the region 2.90–3.10 ppm. [b] Overlapped by aromatic proton signals. [c] **3c$_1$**, **3c$_2$**, **3d$_1$** and **3d$_2$** correspond to dimers **3a**, **3b**, **6a** and **6b** of ref. 1. [e] Not detected. [f] Overlapped by the **2e** H_4 proton signals. [g] Overlapped by benzylic proton signals. [h] Overlapped by the **2e** H_6 proton signals. [i] Obscured by D_2O and ribosyl proton signals. [j] Covered by the ribosyl H_1 and **2g** H_6 proton signals. [k] **3h$_1$**, **3h$_2$**, **3h$_3$** and **3h$_4$** correspond to dimers VI, VII, VIII and IX, respectively, of ref. 6. [l] H_4 and $H_{4'}$ protons correspond to R_2 and $R_{2'}$ substituents of the structures **3**; [m] H_6 and $H_{6'}$ protons correspond to R_3 and $R_{3'}$ substituents of the structures **3**.

Table 3 ^{1}H NMR chemical shifts (ppm) and coupling constants (Hz) of the 6,6′-linked dimers **4a,c**[a]

					$N-CH_2+N'-CH_2$				
	$H_2 + H_{2'}$	$H_6 + H_{6'}$[c]	$H_5 + H_{5'}$	4 + 4 CH_3	H_a[d]	H_b[d]	$NH_2 + NH_{2'}$	$J_{5,6}$	J_{H_a, H_b}
4a$_1$	7.25 s	4.08 d	4.80 d	1.99 s	5.19 d	4.41 d	6.52 br s	4.20	14.7
4a$_2$	7.16 s	4.04 d	4.79 d	1.96 s	5.03 d	4.47 d	6.42 br s	4.70	14.7
4c$_1$[b]	7.38 s	3.82 d	4.64 d	1.93 s	4.44 d	4.34 d	6.45 br s	4.60	15.5
4c$_2$[b]	7.36 s	3.85 d	4.64 d	1.92 s	4.41 s		6.34 br s	4.600	—

[a] In the spectra of all compounds the signals of aromatic protons are in the region 7.10–7.60 ppm. [b] **4c$_1$** and **4c$_2$** correspond to dimers **2a** and **2b** of ref. 1. [c] H_6 and $H_{6'}$ protons correspond to R_3 and $R_{3'}$ substituents of the structures **4**; [d] H_a and H_b are the benzylic or the dichlorobenzylic geminal protons.

NMR spectral data and the composition of diastereoisomeric mixtures arising from the electrochemical reduction of salts **1c**,[1] **1d**[1] and **1h**,[6] already reported in previous papers.

Discussion

It is noteworthy to point out that 4,4′-linked dimers are missing from the reduction products of the 4-methyl substituted salts **1a**,**c**. This finding indicates that, in this case, the steric hindrance effect by substituents outweighs the highest electron spin density on 4 carbons as a governing factor of the regioselectivity of pyridinyl radical dimerization. Furthermore, a comparison between the compositions of the dimer mixtures obtained from the reduction of salts **1a** and **1c** indicates that a significant difference occurs between the 6,6′-linked dimers **4a** and **4c**, because the amount of dimer **4a** is considerably smaller (see Table 4). The larger steric hindrance effect on the adjacent coupling site by the *N*-dichlorobenzyl substituent, compared with the benzyl substituent, might account for this result. A similar effect, due to the bulky substituent on the ring nitrogen, can be observed for the reduction products of NAD^+ and $NADP^+$ coenzymes. The 4,4′-linked dimers largely prevail over the 4,6′-linked ones, as occurs for dimers **2b** and **2d** in comparison with dimers **3b** and **3d**, where a concurrent steric hindrance effect by both of the substituents on the ring nitrogen and on the 6-carbon is present. On account of these findings, the regioselectivity of the dimerization thus appears to be largely controlled by steric factors. Furthermore, the relative amounts of diastereoisomers in the pairs **2b$_1$**–**2b$_2$**, **2d$_1$**–**2d$_2$**, **2e$_1$**–**2e$_2$**, **2f$_1$**–**2f$_2$**, **4a$_1$**–**4a$_2$**, **4c$_1$**–**4c$_2$**, as well as in the triplets **2g$_1$**–**2g$_2$**–**2g$_3$** and **2h$_1$**–**2h$_2$**–**2h$_3$** (see Table 4), indicate that the dimerization is also stereoselective. Actually, the two faces of the nicotinamide ring are diastereotopic and their reciprocal approach in the coupling process can give rise to preferential configurations of the two additional chiral centres. Therefore, also in this nonenzymatic reaction a diastereofacial differentiation can occur.

Finally, the compositions of the dimeric mixtures obtained from the reduction of the 3-cyanopyridinium salts, **1i**, **1l** and **1m**, clearly indicate that both the regio- and stereoselectivity were lost in the dimerization process. Accordingly, these findings seem to indicate that the carbamoyl substituent at the 3 position is a primary factor in inducing both the regio- and stereoselectivity of pyridinyl radical dimerization.

Experimental

Materials and methods

2,6-Dichlorobenzyl bromide, benzyl bromide and methyl iodide were purchased from Fluka Chemie A.G.; 6-methylnicotinamide and β-NAD^+ were purchased from Aldrich Chemical Co. and Boehringer Mannheim GmbH, respectively. 4-Methylnicotinamide,[1] 1-(2,6-dichlorobenzyl)-3-carbamoyl-4-methylpyridinium bromide (**1a**),[8] 1-(2,6-dichlorobenzyl)-3-carbamoyl-6-methylpyridinium bromide (**1b**),[9] 1-(2,6-dichlorobenzyl)-3-carbamoylpyridinium bromide (**1e**),[10] 1-benzyl-3-carbamoylpyridinium chloride (**1f**),[11] 1-methyl-3-cyanopyridinium iodide (**1i**),[12] 1-(2,6-dichlorobenzyl)-3-cyanopyridinium bromide (**1l**)[10] and 1-benzyl-3-cyanopyridinium bromide (**1m**)[7] were prepared according to the literature procedures.

Melting points were taken on a Tottoli apparatus and are uncorrected. UV spectra were recorded on a Perkin Elmer 555 UV/VIS spectrophotometer. ε values are given in M^{-1} cm^{-1}. ^{1}H NMR measurements were performed at 500 MHz with a Bruker AMX − 500 spectrometer; chemical shifts are given in ppm *versus* TMS or DSS as internal standards, coupling constants are in Hz. The FAB-MS spectra, recorded on a ZAB 2SE spectrometer in positive ion mode, were obtained by adding the methanolic solution of the sample to a glycerol–thioglycerol matrix and placing them on a copper probe tip prior to bombardment with Cs^+ atoms having an energy of 25 kV and an emission current of 3 μA.

Cyclic voltammetry of the salts **1a**–**m** was carried out as previously reported[1] using a Amel 471 multipolarograph equipped with a water-jacketed cell, a glassy carbon electrode as the working electrode and a saturated calomel electrode (SCE) as the reference electrode. Controlled potential electro-

Table 4 Diastereoisomer relative abundances in the dimer mixtures arising from electrochemical reduction of salts **1a–m**.

4,4′-Linked dimers %	Total %	4,6′-Linked dimers %	Total %	4.6′-Linked dimers %	Total %
		3a$_1$ 21.3; **3a$_2$** 17.6	38.9	**4a$_1$** 47.2; **4a$_2$** 13.9	61.1
2b$_1$ 62.0; **2b$_2$** 31.0	93.0	**3b$_1$** 3.5; **3b$_2$** 3.5	7.0		
		3c$_1$ 10.7; **3c$_2$** 6.4	17.1	**4c$_1$** 69.7; **4c$_2$** 13.2	82.9
2d$_1$ 61.0; **2d$_2$** 30.0	91.0	**3d$_1$** 4.0; **3d$_2$** 5.0	9.0		
2e$_1$ 37.9; **2e$_2$** 25.7	63.6	**3e$_1$** 24.6; **3e$_2$** 11.8	36.4		
2f$_1$ 50.5; **2f$_2$** 20.7	71.2	**3f$_1$** 20.1; **3f$_2$** 8.7	28.8		
2g$_1$ 36.9; **2g$_2$** 30.8; **2g$_3$** 12.3	80.0	**3g$_1$** 7.5; **3g$_2$** 4.5; **3g$_3$** 4.5; **3g$_4$** 3.5	20.0		
2h$_1$ 42.9; **2h$_2$** 26.7; **2h$_3$** 12.2	81.8	**3h$_1$** 5.8; **3h$_2$** 5.2; **3h$_3$** 4.1; **3h$_4$** 3.1	18.2		
2i$_1$ 30.4; **2i$_2$** 26.1	56.5	**3i$_1$** 26.1; **3i$_2$** 17.4	43.5		
2l$_1$ 29.0; **2l$_2$** 27.0	56.0	**3l$_1$** 23.0; **3l$_2$** 21.0	44.0		
2m$_1$ 31.0; **2m$_2$** 27.0	58.0	**3m$_1$** 25.0; **3m$_2$** 17.0	42.0		

lyses were performed with an Amel 555/A potentiostat equipped with an Amel 721 analogic integrator and were carried out according to the reported procedure[1]. The lyophilisation of aqueous solutions was carried out with an Edwards Modulyo lyophilisator equipped with a perspex bell.

Analytical HPLC was carried out with an LC Perkin-Elmer Series 200 apparatus (equipped with a Perkin-Elmer 235C diode array detector and Merck RP-18 LiChroCart 250-4 7 μm and Supelco Hypersil 250-4 7 μm columns). A Waters GPCI system (equipped with a Hypersil APS 250-10 7 μm column, a U6K injector and with Lirec 804 UV variable wavelength and Waters 401 refractive index detectors) as well as a Perkin-Elmer Series 3 LC apparatus (equipped with a Merck RP-18 LiChroCART 250-25 7 μm column and a Perkin-Elmer LC55 UV detector) were used for preparative HPLC.

Electrochemical reductions

Salt 1a. 1a (180 mg) was dissolved in 70 ml of 0.1 M NH_4HCO_3 pH 8 buffer, benzene (25 ml) was then added and the mixture was electrolysed at −1.10 V *vs.* SCE. After separation of the benzene layer, the aqueous phase was extracted with dichloromethane. The combined dichloromethane and benzene solutions were dried and evaporated yielding 120 mg of a crude residue which, after washing with 5 × 1 ml portions of cold MeOH and crystallisation from MeOH, yielded pure 1,1′-di(2,6-dichlorobenzyl)-3,3′-dicarbamoyl-4,4′dimethyl-1,1′,4,6′-tetrahydro-4,6′-bipyridine (**3a$_1$**); mp 275–80 °C (dec.); UV (EtOH): λ_{max} 263 (ε 9,025) and 346 nm (ε 5,080); IR (cm^{-1}): 3500m, 3460, 3320, 3180b, 1685m, 1650s, 1605s, 1585s, 1540s, 1280, 1270, 1210, 965, 780; ^1H NMR (see Table 2). Anal. calcd (%) for $C_{28}H_{26}N_4O_2Cl_4$: C 56.77; H 4.42; N 9.46. Found: C 55.74; H 4.63; N 9.06. FAB-MS: $M^+ = 591$.

The crude residue (90 mg) from the evaporation of the methanolic leachate underwent a preparative HPLC separation (LiChroCART column; eluent: solvent A–solvent B 76:24; solvent A: 95% MeOH, 5% 0.01 M NH_4HCO_3; solvent B: 0.01 M NH_4HCO_3 in redistilled water; flow rate 7 ml min^{-1}). The effluent was monitored at 390 nm and two fractions of 30 ml and 40 ml were collected. The first fraction, after removal of the organic solvent, was extracted with CH_2Cl_2. The dichloromethane solution was washed with water, dried and evaporated under vacuum to yield a crude residue which, after recrystallisation from EtOH–H_2O, gave 15 mg of the dimer **3a$_2$**, diastereomeric with **3a$_1$**. **3a$_2$**: mp 175–76 °C (dec.); UV (EtOH), λ_{max} 273 (ε 13,100) and 359 nm (ε 6,240); IR (cm^{-1}) 3500s, 3140s, 1660s, 1640s, 1585s, 1280m, 1220m, 1205s, 880; ^1H NMR (see Table 2). Anal. calcd (%) for $C_{28}H_{26}N_4O_2Cl_4$, C 56.77; H 4.42; N 9.46. Found: C 56.97; H 4.49; N 9.03. FAB-MS: $M^+ = 591$.

The second fraction, after elimination of the solvent, was lyophilised to yield 40 mg of a crude solid. The solid was subjected to a preparative HPLC (Supelco Hypersil column; eluent: CH_2Cl_2–*n*-hexane–MeOH 50:50:2; flow rate 2 ml min^{-1}). In a typical run 10 mg of solid were dissolved in 300 μl of CH_2Cl_2 and processed using the peak sharing-recycle technique. Enriched material was collected from the first pass and from two consecutive recycles obtaining two fractions, which were further purified in a single pass. From three runs were obtained 18 mg of pure 1,1′-di(2,6-dichlorobenzyl)-3,3′-dicarbamoyl-4,4′-dimethyl-1,1′,6,6′-tetrahydro-6,6′-bipyridine **4a$_1$** and 9 mg of its pure diastereoisomer **4a$_2$**. **4a$_1$**: mp 168–72 °C (dec.); UV (MeOH), λ_{max} 266 (ε 14,980) and 338 nm (ε 8,875); IR (cm^{-1}) 3400w, 3320w, 3160b, 1640s, 1585s, 1565s, 1280m. ^1H NMR (see Table 3). Anal. calcd (%) for $C_{28}H_{26}N_4O_2Cl_4$: C 56.77; H 4.42; N 9.46. Found: C 56.97; H 4.49; N 9.03. FAB-MS: $M^+ = 591$. **4a$_2$** UV (MeOH), λ_{max} 273 (ε 13,550) and 356 nm (ε 5,480); ^1H NMR (see Table 3). Anal. calcd (%) for $C_{28}H_{26}N_4O_2Cl_4$, C 56.77; H 4.42; N 9.46. Found: C 56.90; H 4.39; N 9.20. FAB-MS: $M^+ = 591$.

^1H NMR analysis of the crude mixture arising from the electrolysis showed the following dimer relative abundances: **4a$_1$**, 47.2%, **4a$_2$**, 13.9%, **3a$_1$**, 21.3%; **3a$_2$**, 17.6%.

Salt 1b. 1b (180 mg) was dissolved in 70 ml of 0.1 M NH_4HCO_3 pH 8 buffer, benzene (25 ml) was then added and the mixture was electrolysed at −1.10 V *vs.* SCE. Thereafter, the benzene and the aqueous layers were separated and the aqueous layer was extracted with three 30 ml portions of CH_2Cl_2. The organic extract was washed with water, dried and then evaporated under vacuum to yield 120 mg of a crude residue, which after leaching with MeOH, left 50 mg of pure 1,1′-di(2,6-dichlorobenzyl)-3,3′-dicarbamoyl-6,6′-dimethyl-1,1′,4,4′-tetrahydro-4,4′-bipyridine (**2b$_1$**); mp 190–2 °C; UV (MeOH) λ_{max} 273 (ε 4,840) and 346 nm (ε 7,350) IR (cm^{-1}) 3440–3420w, 3320br, 1680–1640m, 1580s, 1320s, 1270s; ^1H NMR: (see Table 1). FAB-MS: $M^+ = 591$.

^1H NMR analysis of the residue from the evaporation of the methanolic leachate showed, in addition to **2b$_1$**, the presence of its diastereoisomer **2b$_2$** (see Table 1). The benzene solution was washed with water, dried and the solvent removed under reduced pressure to give 14 mg of a crude residue, whose ^1H NMR spectrum showed the presence, beside **2b$_1$** and **2b$_2$**, of the diastereomeric pair of 1,1′-di(2,6-dichlorobenzyl)-3,3′-dicarbamoyl-6,6′-dimethyl-1,1′,4,6′-tetrahydro-4,6′-bipyridines (**3b$_1$** and **3b$_2$**) (see Table 2).

^1H NMR analysis of the dimer mixture obtained from the electrolysis showed the following dimer relative abundances: **2b$_1$**, 62%; **2b$_2$**, 31%; **3b$_1$**, 3.5%; **3b$_2$**, 3.5%.

Salt 1e. 1e (150 mg) was dissolved in 60 ml of 0.1 M NH_4HCO_3 pH 8 buffer, benzene (30 ml) was then added and the mixture was electrolysed at −1.10 V *vs.* SCE. The aqueous phase was separated from the benzene layer and extracted with CH_2Cl_2. Removal of the solvent from the combined CH_2Cl_2 and benzene solutions yielded 90 mg of a crude residue, whose ^1H NMR analysis evidenced the following dimer relative abundances: 1,1′-di(2,6-dichlorobenzyl)-3,3′-dicarbamoyl-1,1′,4,4′-tetrahydro-4,4′-bipyridine (**2e$_1$**), 37.2%; its diastereoisomer **2e$_2$**, 25.7%; 1,1′-di(2,6-dichlorobenzyl)-3,3′-dicarbamoyl-1,1′,4,6′-tetrahydro-4,6′-bipyridine (**3e$_1$**) 24.6% and its diastereoisomer **3e$_2$**, 11.8% (see Tables 1 and 2).

Salt 1f. 1f (90 mg) was dissolved in 60 ml of 0.1 M NH_4HCO_3 pH 8 buffer, benzene (30 ml) was then added and the mixture was electrolysed at −1.10 V *vs.* SCE. The crude residue (90 mg), obtained according to the same procedure carried out for **1e**, was analysed by ^1H NMR and showed the following composition: 1,1′-dibenzyl-3,3′-dicarbamoyl-1,1′,4,4′-tetrahydro-4,4′-bipyridine (**2f$_1$**) 50.5%; its diastereoisomer **2f$_2$**, 20.7%; 1,1′-dibenzyl-3,3′-dicarbamoyl-1,1′,4,4′-tetrahydro-4,6′-bipyridine (**3f$_1$**), 20.1% and its diastereoisomer **3f$_2$**, 8.7% (see Tables 1 and 2).

Salt 1g. In a typical run, β-NAD^+ (0.5 g) was dissolved in 50 ml of a previously electrolysed 0.05 M NH_4HCO_3 pH 7.8 buffer. Then the electrolysis was carried out at −1.20 V *vs.* SCE. Thereafter, the solution was concentrated by ultrafiltration through a YC05 500 Da cutoff 76 mm membrane until the inorganic solute was quite eliminated. Freeze-drying of the solution yielded 0.45 g of a pale yellow solid. A sample of this dimer mixture was HPLC analysed using a Hibar Merck RP-18 LiChroCART 250-4 7 μm column with the following solvent system: solvent A = 12% EtOH: 30% H_2O: 58% aqueous 0.1 M NH_4HCO_3; solvent B = aqueous 0.07 M NH_4HCO_3; flow rate 1 ml min^{-1}. The eluate was monitored at 260 nm. The elution profile is shown in Fig. 1 and evidences

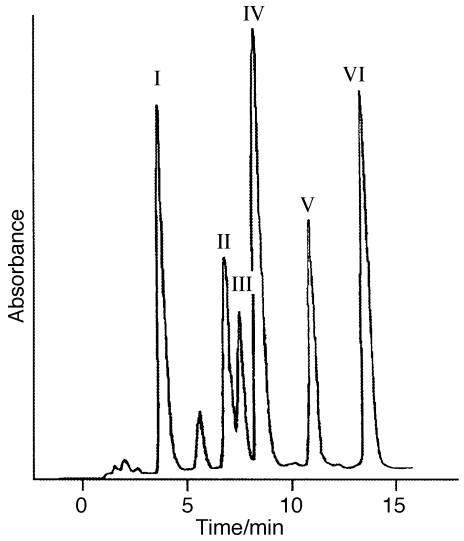


Fig. 1 HPLC elution profile of the NAD dimers mixture monitored at 260 nm. Retention times (t_R) are given neglecting the dead volume of the column and start from the injection point

three main peaks: I (t_R : 4.5 min, 12%), IV (t_R : 8.7 min, 37%) and VI (t_R : 13.4 min, 31%) and three secondary peaks: II (t_R : 7.3 min), III (t_R : 7.9 min) and V (t_R : 11.0 min). The UV spectra of all corresponding fractions show maxima at 260 and 340 nm. A sample of the dimer mixture (40 mg) was subjected to a two-step isocratic preparative HPLC separation [Merck RP-18 Hibar LiChroCART 250-25 7 μm column; eluent: 3.24% (v:v) absolute EtOH–aqueous 0.07 M NH_4HCO_3 ; flow rate 7.0 ml min^{-1}]. Two fractions, A (t_R : 18–36 min) and B (t_R : 81–96 min), were collected and analysed. Fraction A (18 mg) contained an enriched mixture of the components corresponding to peaks I and IV, while fraction B contains 9 mg of the almost pure component corresponding to peak VI, which was identified by ^{1}H NMR analysis as a 4*R*–4′*R* or 4*S*–4′*S* NAD dimer (**2g$_2$**) (see Table 1). The combined fractions A from three separations underwent a further preparative HPLC separation [Merck RP-18 LiChroCART 250-25 7 μm column; eluent: 1.80% (v:v) absolute EtOH–aqueous 0.05 M NH_4HCO_3 ; flow rate 7.0 ml min^{-1}]. Two fractions, C (t_R : 18–24 min) and D (t_R : 40–52 min) were collected. Fraction C contained 10 mg of the component corresponding to peak I, whose ^{1}H NMR analysis proved to be as well a 4*S*–4′*S* or 4*R*–4′*R* NAD dimer (**2g$_3$**) (see Table 1), while the component (22 mg) corresponding to peak IV was obtained from fraction D and was identified as the 4*R*–4′*S* NAD dimer **2g$_1$** (see Table 1).

^{1}H NMR analysis of the dimer mixture arising from the electrolysis showed the presence of four other NAD dimers, namely the 4,6′-linked diastereoisomer dimers **3g$_1$**, **3g$_2$**, **3g$_3$** and **3g$_4$** corresponding to peaks II, III and V of the HPLC elution profile (see Table 2). The relative abundance of the seven NAD dimers was: **2g$_1$**, 36.9%; **2g$_2$**, 30.8%; **2g$_3$**, 12.3%; **3g$_1$**, 7.5%; **3g$_2$**, 4.5%; **3g$_3$**, 4.5% and **3g$_4$**, 3.5%.

Salt 1i. The electrochemical reduction of the salt **1i** has been carried out according to literature procedure[13]. ^{1}H NMR analysis was performed on the crude reduction mixture whose composition was: 1,1′-dimethyl-3,3′-dicyano-1,1′,4,4′-tetrahydro-4,4′-bipyridine (**2i$_1$**), 30.4%; its diastereoisomer **2i$_2$**, 26.1%; 1,1′-dimethyl-3,3′-dicyano-1,1′,4,6′-tetrahydro-4,6′-bipyridine (**3i$_1$**), 26.1%, and its diastereoisomer **3i$_2$**, 17.4% (see Tables 1 and 2).

Salt 1l. The electrolysis was carried out on 150 mg of salt **1l** as described for **1i**. ^{1}H NMR analysis performed on the crude residue (100 mg), obtained according to the same procedure carried out for **1i**, showed the following dimer relative abundances: 1,1′-di(2,6-dichlorobenzyl)-3,3′-dicyano-1,1′,4,4′-tetrahydro-4,4′-bipyridine (**2l$_1$**), 29.0%; its diastereoisomer **2l$_2$**, 27.0%; 1,1′-di(2,6-dichlorobenzyl)-3,3′-dicyano-1,1′,4,4′-tetrahydro-4,6′-bipyridine (**3l$_1$**), 23.0% and its diastereoisomer **3l$_2$**, 21.0% (see Tables 1 and 2).

Salt 1m. The electrolysis was carried out on 150 mg of salt **1m** as described for **1i**. The crude residue (75 mg), obtained according to the same procedure carried out for **1i**, was analysed by ^{1}H NMR and showed the following composition: 1,1′-dibenzyl-3,3′-dicyano-1,1′,4,4′-tetrahydro-4,4′-bipyridine (**2m$_1$**), 31.0%; its diastereoisomer **2m$_2$**, 27.0%; 1,1′-dibenzyl-3,3′-dicyano-1,1′,4,6′-tetrahydro-4,6-bipyridine (**3m$_1$**), 25.0% and its diastereoisomer **3m$_2$**, 17.0% (see Tables 1 and 2).

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