Synthesis of Spin-Labelled 1,4-Dihydropyridines and Pyridines

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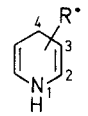
1,4-Dihydropyridines spin-labelled with 5- and 6-membered nitroxyl radicals in positions 1–4 of the pyridine ring were synthesized. The oxidation of these dihydropyridines to pyridines with active manganese dioxide was investigated.

The 1,4-dihydropyridines are biologically important compounds. Variations of the original Hantzsch synthesis[1–4] have been utilized to prepare a large number of derivatives, and numerous studies have been performed on their biochemical properties and pharmacological activities.[5,6] Some of them are used therapeutically as calcium antagonists or vasodilators (e.g. nifedipine and nitrendipine).

Dihydropyridines reduce unsaturated functionalities[3] (e.g. azomethines, conjugated olefins, keto esters and quinones) and are at the same time oxidized to the corresponding pyridines. This ability relates to their potential behavior as NADH mimics.[7]

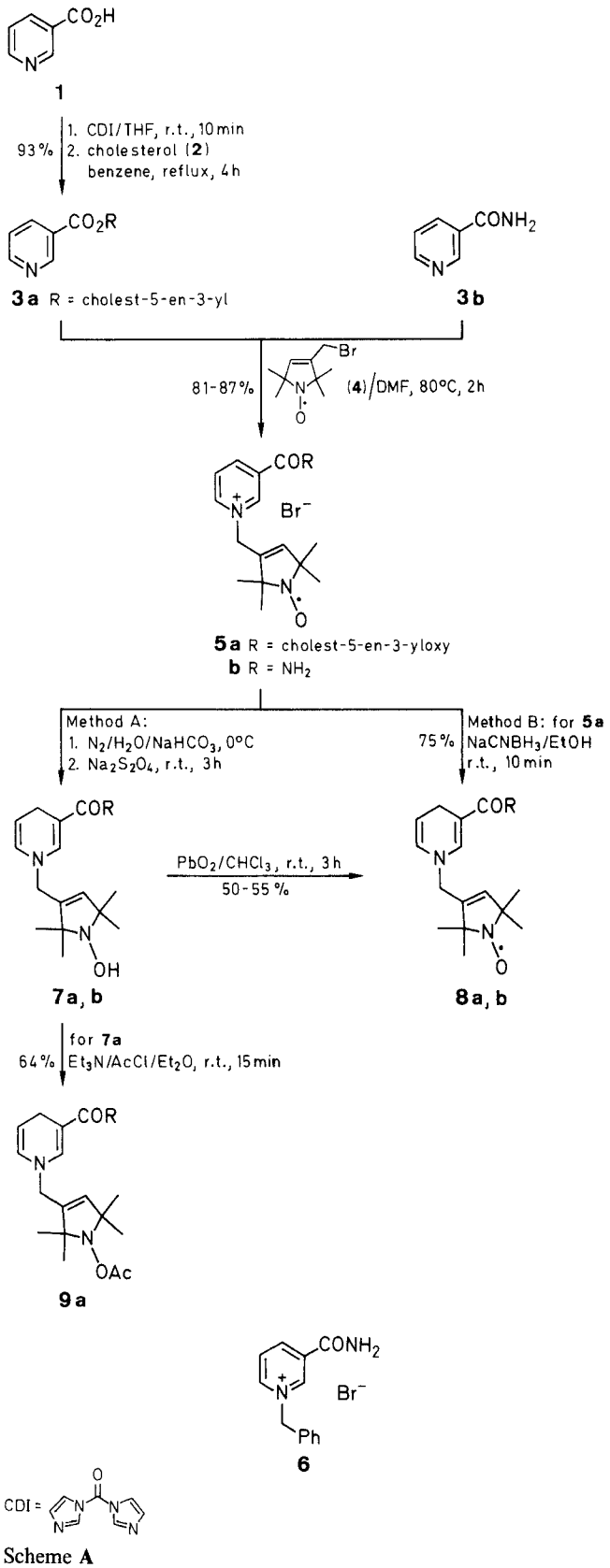
The oxidation mechanism may involve a one- or two-electron oxidation process. The mechanism of oxidation of several substituted 3,5-bis(ethoxycarbonyl)-1,4-dihydro-2,6-dimethylpyridines has been investigated intensively in the cytochrome P-450 system. These systematic investigations showed that a 4-alkyl group, and especially the 4-ethyl group, is transferred from the pyridine ring to a nitrogen of the prosthetic heme, resulting in an *N*-ethylprotoporphyrin derivative. The reaction takes place via a radical mechanism, as shown by spin-trapping studies.[8–10]

We report here on the synthesis of 1,4-dihydropyridines spin-labelled (SL) with groups containing 5- and 6-membered radicals offering series of paramagnetic dihydropyridines **(I)** for systematic biological studies.



I

For the preparation of 1-SL compounds, two pyridine derivatives were quaternized with a highly reactive halogenated spin label compound: cholesterol **(2)** was acylated with 3-(1*H*-imidazolylcarbonyl)pyridine, formed from the reaction of 3-pyridinecarboxylic acid **(1)** with 1,1′-carbonyldiimidazole (CDI) to give cholest-5-en-3-yl 3-pyridinecarboxylate **(3a)**. This ester or 3-pyridinecarboxamide **(3b)** was then quaternized with 3-bromomethyl-2,5-dihydro-2,2,5,5-tetramethyl-1*H*-pyrrol-1-yloxy radical[11–12] **(4)** to give **5**. These are close analogues of the frequently studied 3-carbamoyl-1-benzylpyridinio derivatives **(6)**. There are well-established routes for the reduction of pyridinium salts to 1,4-dihydropyridines:[13–16] reduction with sodium bisulfite or with sodium cyanoborohydride, which have been reported to lead mainly to the formation of the 1,4-dihydro isomer, with only a trace of the 1,2-isomer.[17]



Scheme A

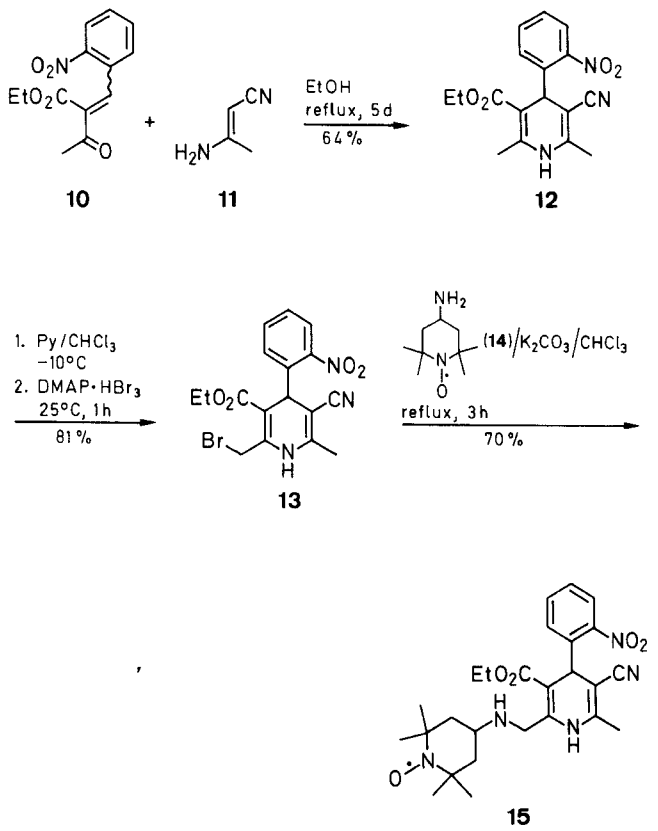
We found that SL-quaternized compounds **5** were reduced with sodium bisulfite to diamagnetic dihydropyridines **7**, which can be selectively oxidized to dihydropyridine radicals **8**. The reduction of a piperidin-1-yloxy radical, 4-amino-2,2,6,6-tetramethylpiperidin-1-yloxy radical **(14)** with sodium bisulfite was reported earlier to give a diamagnetic *N*-hydroxy derivative.[18] When the reductions of **5a** and **5b** were carried out with the milder reagent sodium cyanoborohydride, the radical function remained intact and the reaction products were **8a** and **8b**. The labile *N*-hydroxycholesteryl derivative **7a** could be protected to prevent the oxidation of *N*-hydroxy compound by *O*-acetylation to **9a** (Scheme **A**).

For the preparation of 2-SL-dihydropyridines, ethyl 2-(2-nitrobenzylidene)acetoacetate **(10)** and aminocrotonitrile **(11)** were first reacted according to the Hantzsch synthesis to give **12**. Following the method described earlier for the bromination of other 1,4-dihydropyridines with pyridinium bromide perbromide,[19,20] **12** was brominated with 4-dimethylaminopyridinium bromide perbromide (DMAP.HBr_3),[21] to yield the 2-bromomethyl derivative **13**. The bromine was then replaced with 4-amino-2,2,6,6-tetramethylpiperidin-1-yloxy radical **(14)**[22] to give **15** (Scheme **B**).

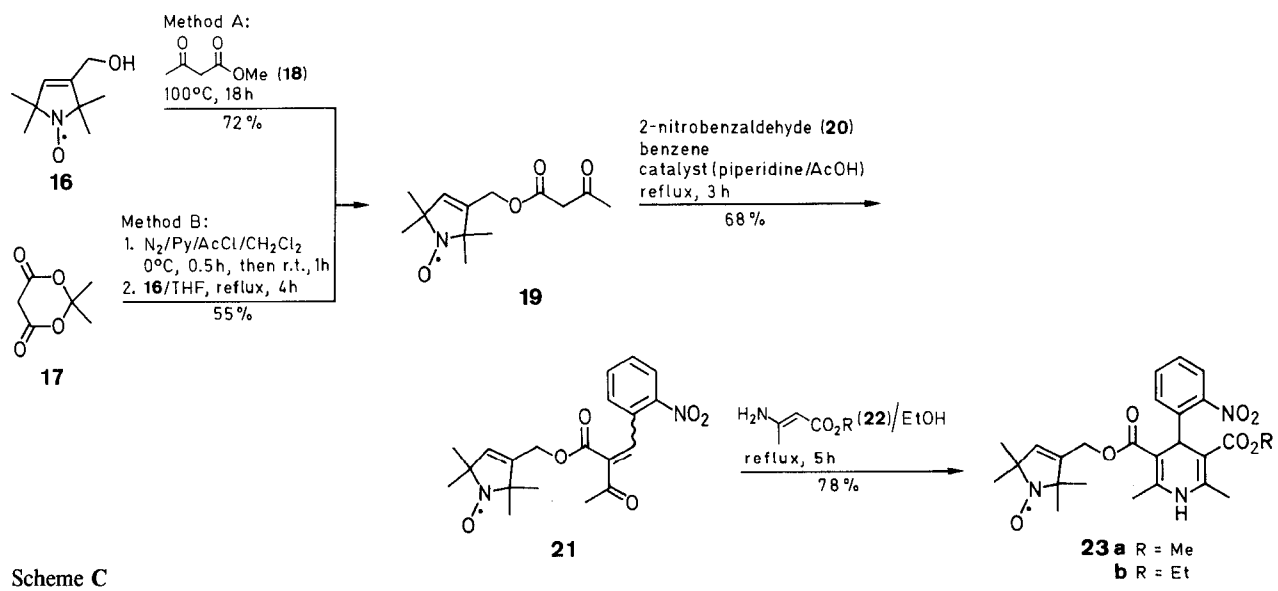
The 3-SL-dihydropyridines were obtained by the transesterification of methyl acetoacetate **(18)** or from Meldrum's acid **(17)**[23] with 2,5-dihydro-3-hydroxymethyl-2,2,5,5-tetramethyl-1*H*-pyrrol-1-yloxy radical **(16)** first to **19**, which was next reacted in a Knoevenagel condensation with 2-nitrobenzaldehyde **(20)** to **21**. Compound **21** was finally converted with aminocrotonate **(22)** to **23** (Scheme **C**).

The 4-SL-dihydropyridines **26a–i** were synthesized from aldehyde radical compounds **24a–f**[24–27] (differing in the size and degree of saturation of the hetero ring, and in the spacer between the aldehyde and the hetero ring) and alkyl aminocrotonate **22**, or from an aminocrotonate **22** and an α,β-unsaturated β-alkoxycarbonyl ketone **25** which is preformed from the nitroxide aldehyde **24** and alkyl acetoacetate **18** (Scheme **D**).

It was already known that dihydropyridines can be aromatized with several oxidizing agents. The oxidation takes place with or without cleavage of substituent R^1; when the oxidation was carried out with sodium nitrite in acetic acid, R^1 remained on the pyridine ring when R^1 = Me, Et or aryl, but it was cleaved off when R^1 = cyclohexyl or cyclohex-3-en-1-yl.[28] This partially correlates with results obtained on the cytochrome P-450 system, when R^1 = Et was cleaved off and *N*-ethylated



Scheme **B**



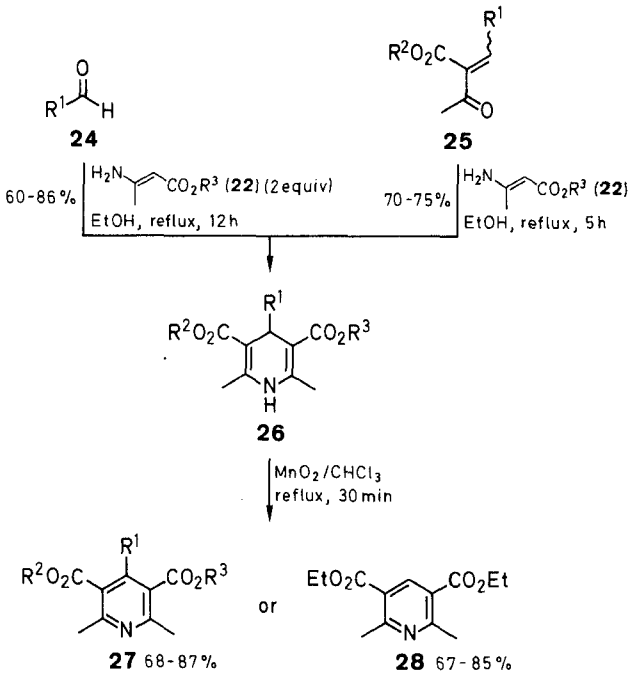
Scheme **C**

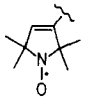
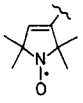
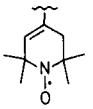
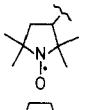
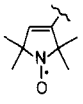
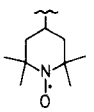
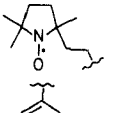
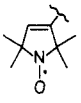
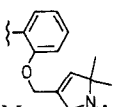
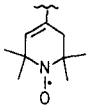
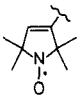
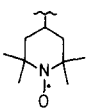
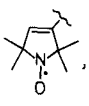
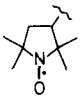
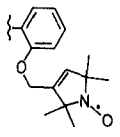
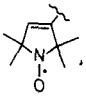
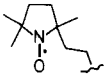
the protoporphyrin ring, whereas R^1 = 2-nitrophenyl remained on the pyridine.[8-10] We have found that active manganese dioxide (MnO_2) is a convenient reagent for the aromatization of 1,4-dihydropyridines to pyridines. We first investigated the oxidation of SL-dihydropyridines **26a–i** and found that when the connecting carbon has an sp^2 character and is part of a ring (**26a–d, 26g** and **26i**) R^1 remained on the pyridine ring, and that when the connecting carbon has an sp^3 character and is part of a ring (**26e, 26h**) it was cleaved off and gave **28**, but not when the radical was separated from the pyridine ring by an ethyl group (**26f**), because the product was then **27f**.

For comparison, the oxidation of a few diamagnetic dihydropyridines **26j–o** was also investigated. It was found that when R^1 = Me, $n = C_5H_{11}$, PhCH=CH, $PhCH_2CH_2$ and $2\text{-}NO_2C_6H_4$ (**26j–m** and **26o**), it was not cleaved off, and gave compounds **27j–m** and **27o**). The reaction of **26n** led to **28**.

In conclusion, the above SL dihydropyridines have a number of potentially practical applications, e.g.

(a) in studies of the metabolism of calcium channel drugs by ESR spectroscopy;
(b) in investigations of the mechanism of the hydride shift reaction in the cytochrome P-450 system;



24, 25	R^1			26, 27	R^1	R^2	R^3				
24a		**24g**	$n\text{-}C_5H_{11}$	**26a, 27a**		Me	Me	**26g, 27g**		Et	Et
24b		**24h**	(*E*)-CH=CHPh	**26b, 27b**		Me	Et	**26h**		Et	Et
24c		**24i**	CH_2CH_2Ph	**26c, 27c**		Et	Et	**26i, 27i**		Et	Et
		24j	3-cyclohexenyl								
24d		**24k**	$2\text{-}NO_2C_6H_4$	**26d, 27d**		*t*-Bu	Et	**26j, 27j**	Me 	Et	Et
								26k, 27k	$n\text{-}C_5H_{11}$	Et	Et
24e		**25a**	 , R^2 = Me	**26e**		Et	Et	**26l, 27l**	(*E*)-CH=CHPh	Me	Me
								26m, 27m	CH_2CH_2Ph	Et	Et
24f		**25b**	 , R^2 = Bu-*t*	**26f, 27f**		Et	Et	**26n**	3-cyclohexenyl	Et	Et
								26o, 27o	$2\text{-}NO_2C_6H_4$	Me	Me

Scheme **D**

Table. Compounds **5, 8, 9, 12, 13, 15, 23, 26, 27, 28** Prepared

Product	Yield (%)	mp (°C)	Molecular Formula[q]	IR (neat or Nujol) ν (cm^{-1})	MS (70 eV) *m/z* (%, rel. int.)
5a	81	193–195	$C_{42}H_{64}BrN_2O_3$ (724.9)	3650–3220, 1720, 1660, 1630, 1590	[k]
5b	87	208–209	$C_{15}H_{21}BrN_3O_2$ (355.3)	3375–3190, 1680, 1640, 1580	
8a[o]		140–141	$C_{42}H_{65}N_2O_3$ (646.0)	1690, 1662, 1590	645 (M^+, 53.6), 369 ($C_{27}H_{47}^{+}$[l], 9.2), 122 ($C_8H_{12}N$[d,j], 100)
8b	50	146–147	$C_{15}H_{22}N_3O_2$ (276.4)	3420–3200, 1680, 1640	276 (M^+, 64.3), 154 ($C_9H_{16}NO$[d,g], 44.3), 123 ($C_6H_7N_2O$[d], 100)
9a	64	138–140	$C_{44}H_{68}N_2O_4$ (689.1)	1768, 1690, 1660, 1590	(FAB) 687 ($[M-H]^+$[m], 74), 369 (cholestadienyl$^+$, 21), 319 ($[M-H]^+$-cholestadiene, 100)
12	65	191–192	$C_{17}H_{17}N_3O_4$ (327.4)	3360–3130, 2190, 1700, 1650, 1620, 1515	(FAB) 328 ($[M+H]^+$, 100), 310 ($[M+H]^+ - H_2O$, 33.5)
13	81	133	$C_{17}H_{16}BrN_3O_4$ (406.3)	3300–3120, 2200, 1690, 1640, 1520, 1500	(FAB) 406/408 ($[M+H]^+$)
15	70	161–162	$C_{26}H_{34}N_5O_5$ (496.6)	3290, 2200, 1690, 1655, 1525	496 (M^+, 38.5), 479 ($M^+ - {}^{\cdot}OH$, 49), 423 ($M^+ - C_3H_7N{}^{\cdot}O$[d], 100), 393 ($M^+ - {}^{\cdot}OH - C_4H_8NO$[d], 67.2)
23	78	153–154	$C_{26}H_{32}N_3O_7$ (498.6)	3260, 1690, 1640, 1620, 1530	498 (M^+, 62), 481 ($M^+ - {}^{\cdot}OH$, 76.6), 376 ([a,d], 12.2), 329 (M^+ – Pyr-$CH_2O{}^{\cdot}$[d], 100), 270 (*m/z* 329 $- {}^{\cdot}NO - {}^{\cdot}C_2H_5$[d], 29), 224 ([a] – $C_9H_{12}NO$[f], 28), 196 (*m/z* 224 – C_2H_4, 20.6), 138 (Pyr-$CH_2^+ - {}^{\cdot}CH$, 31.3)
26a	85	187–188	$C_{19}H_{27}N_2O_5$ (363.5)	3345, 1680, 1650, 1640, 1620	363 (M^+, 5.7), 224 ([a], 100)
26b	70	150–152	$C_{20}H_{29}N_2O_5$ (377.5)	3290, 1690, 1645, 1620	377 (M^+, 9.5), 238 ([a], 100), 210 ([a] $-C_2H_4$, 14.2)
26c	80	160–161	$C_{21}H_{31}N_2O_5$ (391.5)	3280, 1690, 1640, 1625	391 (M^+, 7.4), 252 ([a], 100), 224 ([a] $-C_2H_4$, 10.8), 196 ([a] – 2 × C_2H_4, 12.2)
26d	75	157–158	$C_{23}H_{35}N_2O_5$ (419.5)	3305, 1695, 1670, 1640, 1625	419 (M^+, 11), 280 ([a], 26.6), 224 ([a] $-C_4H_8$, 100), 196 ([a] $-C_4H_8-C_2H_4$, 12.9)
26e	72	162–163	$C_{21}H_{33}N_2O_5$ (393.5)	3260, 1690, 1635, 1615	393 (M^+, 2.8), 348 ($M^+ - {}^{\cdot}OEt$, 1.8), 252 ([a], 100), 224 ([a] $-C_2H_4$, 10.2), 196 ([a] – 2 × C_2H_4, 11.4)
26f	61	119–120	$C_{22}H_{35}N_2O_5$ (407.5)	3320, 1690, 1650, 1620	407 (M^+, 8), 252 ([a], 100), 224 ([a] $-C_2H_4$, 10), 196 ([a] – 2 × C_2H_4, 13), 128 (i + [b], 13.3)
26g	68	135–136	$C_{22}H_{33}N_2O_5$ (405.5)	3275, 1695, 1645, 1620	405 (M^+, 5), 252 ([a], 100), 224 ([a] $-C_2H_4$, 10.5), 196 ([a] – 2 × C_2H_4, 16.8), 154 ($C_9H_{16}NO^+$[d,e], 19)
26h	60	122–124	$C_{22}H_{35}N_2O_5$ (407.6)	3355, 1690, 1645	407 (M^+, 3.4), 252 ([a], 100), 224 ([a] $-C_2H_4$, 14), 196 ([a] – 2 × C_2H_4, 19)
26i	68	192–193	$C_{28}H_{37}N_2O_6$ (497.6)	3305, 1740, 1685, 1640, 1615	497 (M^+, 35.3), 344 (M^+ – Pyr-$CH_2{}^{\cdot}$[c,d], 100), 316 (*m/z* 344 $-C_2H_4$[d], 13.2), 252 ([a], 66), 138 (Pyr-$CH_2^+ - {}^{\cdot}CH_3$[d], 18)
26k	86	73–74	$C_{18}H_{29}N_1O_4$ (323.4)	3380–3100, 1688, 1638	(FAB) 324 ($[M+H]^+$, 26), 278 ($[M+H]^+$ – EtOH, 95), 252 ([a], 100)
26l	81	175–176	$C_{19}H_{21}N_1O_4$ (327.4)	3360–3150, 1725, 1690, 1640	(FAB) 328 ($[M+H]^+$, 9), 327 (10), 326 (14), 224 ([a], 100)
26m	72	116	$C_{21}H_{27}N_1O_4$ (357.5)	3370–3120, 1685, 1630	(FAB) 358 ($[M+H]^+$, 17), 312 ($[M+H]^+$ – EtOH, 25), 252 ([a], 88), 91 ($PhCH_2^+$, 100)
26n	79	146–148	$C_{19}H_{27}N_1O_4$ (333.5)	3380–3150, 1690, 1640	288 ($M^+ - {}^{\cdot}OEt$, 7.5), 252 ([a], 100), 224 ([a] $-C_2H_4$, 29.5), 196 ([a] – 2 × C_2H_4, 33)
27a	87	132–133	$C_{19}H_{25}N_2O_5$ (361.4)	1720, 1548	361 (M^+, 24.5), 331 ([g,d,l], 100), 240 ([g] $-CH_3OH - {}^{\cdot}COOCH_3$[d], 54.7)
27b	81	107–108	$C_{20}H_{27}N_2O_5$ (375.5)	1745, 1720, 1555	(FAB) 377 ($[M+2H]^+$[n], 72), 361 ($[M+H]^+ - {}^{\cdot}CH_3$, 58), 345 ($[M+2H]^+ - CH_3OH$, 100)
27c	77	100–101	$C_{21}H_{29}N_2O_5$ (389.5)	1735	389 (M^+, 24.7), 359 ($M^+ - {}^{\cdot}NO$[d], 100), 240 ($M^+ - {}^{\cdot}NO - EtOH - {}^{\cdot}COOEt$[d], 70.8)
27d	79	115–116	$C_{23}H_{33}N_2O_5$ (417.6)	1720, 1546	(FAB) 419 ($[M+2H]^+$[n], 8), 363 ($[M+2H]^+ - C_4H_8$, 50), 331 ($[M+2H]^+ - C_4H_8 - CH_3OH$, 100)
27f	68	oil	$C_{22}H_{33}N_2O_5$ (405.5)	1740, 1690, 1630	(FAB) 407 ($[M+2H]^+$[n], 73), 128 ([b], 100)
27g	70	79–82	$C_{22}H_{31}N_2O_5$ (403.5)	1730	403 (M^+, 59), 373 ($M^+ - CH_2O$[d], 98.8), 317 ($M^+ - C_4H_8NO$[d], 100), 288 (*m/z* 317–$^{\cdot}C_2H_5$[d], 99.4), 242 (*m/z* 288 – EtOH[d], 90.4)
27i	86	oil	$C_{28}H_{35}N_2O_6$ (495.6)	1770, 1720	(FAB) 496 ($[M+H]^+{}^{\cdot}$, 33), 344 ($[M+2H]^+$ – PyrCH$_2^{\cdot}$[c], 39), 297 ($[M+H]^+$ – EtOH – PyrCH$_2^{\cdot}$, 38), 138 (PyrCH$_2^{\cdot}$ – $^{\cdot}CH_3$, 100)

Table. (continued)

Product	Yield (%)	mp (°C)	Molecular Formula[q]	IR (neat or Nujol) ν(cm^{-1})	MS (70 eV) *m/z* (%, rel.int.)
27j	71	oil	$C_{14}H_{19}N_1O_4$ (265.3)	1720, 1560	(FAB) 266 ($[M+H]^+$, 100), 220 ($[M+H]^+ - EtOH$, 76)
27k	80	oil	$C_{18}H_{27}N_1O_4$ (321.4)	1714, 1565	(FAB) 322 ($[M+H]^+$, 100), 294 ($[M+H]^+ - C_2H_4$, 58)
27l	84	75–76	$C_{19}H_{19}N_1O_4$ (325.4)	1710, 1620, 1540	(FAB) 326 ($[M+H]^+$, 100), 294 ($[M+H]^+ - CH_3OH$, 12)
27m	75	oil	$C_{21}H_{25}N_1O_4$ (355.4)	1710, 1600, 1555	(FAB) 356 ($[M+H]^+$, 100), 310 ($[M+H]^+ - EtOH$, 30), 91 ($PhCH_2^+$, 44)
27o	82	102–103	$C_{17}H_{16}N_2O_6$ (344.3)	1720, 1610, 1555, 1515	(FAB) 345 ($[M+H]^+$, 100), 313 ($[M+H]^+ - CH_3OH$, 10), 330 ($[M+H]^+ - {}^{\cdot}CH_3$, 10), 277 ($[M+H]^+ - CH_3OH - {}^{\cdot}NO_2$, 11)
28[p]		69–70	$C_{13}H_{17}N_1O_4$ (251.3)	1720, 1586, 1540	251 (M^+, 38.5), 206 ($M^+ - {}^{\cdot}OEt$, 100), 178 (*m/z* 206 $-C_2H_4$, 46.8)

[a] The "dihydropyridinium ion", i.e. loss of the 4-substituent.
[b] The ion contains the pyrrolidine ring and is produced by a H-rearrangement, followed by loss of the dihydropyridine-ethyl side-chain, see ion i+ in [×].
[c] Pyr = the pyrrolinyl group.
[d] Corroborated by high-resolution mass measurement.
[e] Product of a H-rearrangement from the dihydropyridine to the tetrahydropyridine moiety; an intramolecular reduction process on EI.
[f] H-Rearrangement from the Pyr-CH_2 moiety to the COO group.
[g] H-Rearrangement: an intramolecular reduction of the pyrroline moiety by the dihydropyridine group upon EI.
[h] H-Rearrangement: an intramolecular reduction of the pyridine ring by the pyrrolinyl CH_2O moiety upon EI.
[i] The cholesteryl backbone.
[j] A fragment of the Pyr-CH_2 moiety.
[k] Upon evaporation, the salt undergoes a thermal decomposition to give 2,2,5,5-tetramethyl-3-(bromomethyl)pyrroline (M = 232/234), and nicotinic acid cholesteryl ester (M = 492) as products.
[l] Loss of $^{\cdot}NO$ from M^+, see ion g in ×. [×] Lit. [31]
[m] An oxidation of the 1-substituted 1,4-dihydropyridine takes place on the matrix' surface to give a pyridinium ion.[30]
[n] The radical is first reduced by the matrix to N–OH, then protonated upon evaporation.
[o] From Method A 55%; from Method B 75%.
[p] From **26e** 67%; from **26h** 70%; from **26n** 85%.
[q] Satisfactory microanalyses: C ±0.22, H ±0.27, N ±0.28; **5a, b**: Br ±0.20.

(c) as spin-labelled NADH mimic reagents;
(d) in the spin-labelling of brain-targeting drug delivery systems containing dihydropyridine.

Melting points were determined on a Boetius micro-mp apparatus and are not corrected. Elemental analyses (C, H, N) were performed on a Heraeus Micro U/E apparatus or (Hal) were carried out titrimetrically by Schöniger's method. The IR (Specord 75) and ^{1}H-NMR spectra (Perkin-Elmer R-12 and Bruker AC-250) of the compounds were in each case consistent with the assigned structures.

Fast atom bombardment (FAB) spectra were taken with an IonTech FAB gun at 8 kV, in glycerol/3-nitrobenzyl alcohol (4:1) matrix.

The mass spectra were recorded with a Finnigan MAT 8430 mass spectrometer – SS300 data acquisition system (EI: electron energy 70 eV, trap current 0.5 mA, resolution 1250/10%, sample introduction via the direct insertion probe with ±1 °C regulation of the evaporation temperature. HRMS measurements were made at R = 10000 by the peak matching technique, with PFK as the reference standard). Flash column chromatography on silica gel was performed with Merck Kieselgel 60 (0.040–0.063 mm). Qualitative TLC was carried out on commercially prepared plates (20 × 20 × 0.2 cm) coated with Merck Kieselgel GF_{254}.

The starting materials **1, 2, 3b, 11, 17, 18, 20, 22a,b, 24g–k** and **26j** were commercially available products and were used without purification. Compounds **4**,[11] **10**,[32] **14**,[22] **16**,[24] **24a,b**,[24] **24d–f**,[25,27,33] and **25a,b**[29,32] were prepared according to published procedures.

Cholest-5-en-3-yl 3-Pyridinecarboxylate (3a):
Nicotinic acid (**1**; 1.23 g, 10 mmol) and 1,1'-carbonylbis-(1*H*-imidazole) (1.62 g, 10 mmol) are dissolved in dry THF (15 mL) and stirred for 10 min. The THF is then evaporated, and benzene (20 mL) and cholesterol (**2**; 3.86 g, 10 mmol) are added. The mixture is refluxed for 4 h, then cooled, washed with brine, dried ($MgSO_4$) and evaporated. The ester **3a** is purified on silica gel with CCl_4/Et_2O (2:1); yield 4.55 g (93%); R_f = 0.62 (TLC); mp 149–150 °C (Lit.[34] mp 150–152 °C).

3-[3-(Cholest-5-en-3-yloxycarbonyl)-1-pyridiniomethyl]-2,5-dihydro-2,2,5,5-tetramethyl-1*H*-pyrrol-1-yloxy Bromide (5a) or 3-(3-Carbamoyl-1-pyridiniomethyl)-2,5-dihydro-2,2,5,5-tetramethyl-1*H*-pyrrol-1-yloxy Bromide (5b):
To a solution of cholest-5-en-3-yl 3-pyridinecarboxylate (**3a**; 491 mg, 1 mmol) or 3-pyridinecarboxamide (**3b**; 122 mg, 1 mmol) in dry DMF (10 mL) 3-bromomethyl-2,5-dihydro-2,2,5,5-tetramethyl-1*H*-pyrrol-1-yloxy radical (**4**; 233 mg, 1 mmol) is added and the mixture is heated at 80 °C for 2 h. The solvent is removed *in vacuo* and the residue is crystallized from acetone/Et_2O to give the quaternary salt **5a** or **5b**.

3-[3-(Cholest-5-en-3-yloxycarbonyl)-1,4-dihydro-1-pyridylmethyl]-2,5-dihydro-2,2,5,5-tetramethyl-1*H*-pyrrol-1-yloxy Radical (8a):
Method A: *Cholest-5-en-3-yl 1,4-Dihydro-1-(2,5-dihydro-1-hydroxy-2,2,5,5-tetramethyl-3-pyrrolylmethyl)-3-pyridinecarboxylate* (**7a**): To a solution of **5a** (3.62 g, 5 mmol) in deaerated water (20 mL), $NaHCO_3$ (2.50 g, 30 mmol) and EtOAc (20 mL) are added. The mixture is stirred in an ice-bath and $Na_2S_2O_4$ (3.48 g,

20 mmol) is added gradually over a period of 5 min. The mixture is stirred for 3 h unter N_2 at r.t. The organic phase is separated, dried ($MgSO_4$) and evaporated to give the *N*-hydroxy compound **7a**.

1H-Pyrrol-1-yloxy Radical **8a**: The residual *N*-hydroxy compound **7a** is dissolved in $CHCl_3$ (15 mL) and stirred for 3 h with PbO_2 (100 mg) to effect oxidation to **8a**.

The $CHCl_3$ solution is filtered and the filtrate is evaporated. Compound **8a** is crystallized from Et_2O/hexane.

Method B: The pyridinium salt **5a** (724 mg, 1 mmol) is dissolved in EtOH (10 mL), and $NaCNBH_3$ (126 mg, 2 mmol) is added. The mixture is stirred for 10 min, and the resulting precipitate **8a** is filtered off, chromatographed on silica gel with hexane/EtOAc (2:1) and crystallized from Et_2O/hexane; $R_f = 0.57$ (TLC).

3-(3-Carbamoyl-1,4-dihydro-1-pyridylmethyl)-2,5-dihydro-2,2,5,5-tetramethyl-1*H*-pyrrol-1-yloxy Radical (8b):

Following Method A, to a solution of **5b** (1.77 g, 5 mmol) in deaerated water (20 mL) $NaHCO_3$ (2.50 g, 30 mmol) is added. The mixture is stirred in an ice-bath and $Na_2S_2O_4$ (3.48 g, 20 mmol) is added gradually over a period of 5 min. The mixture is stirred for 3 h under N_2 at r.t. The bright-yellow crystalline solid that separates out is filtered off, washed with water and dried. The residual *N*-hydroxy compound **7b** is suspended in $CHCl_3$ (15 mL) and stirred for 2 h with PbO_2 (100 mg) to effect oxidation to **8b**. The $CHCl_3$ solution is dried ($MgSO_4$) and evaporated to dryness, and the residue is suspended in Et_2O (15 mL). The yellow crystals are filtered off, washed with Et_2O and dried.

Cholest-5-en-3-yl 1-(1-Acetoxy-2,5-dihydro-2,2,5,5-tetramethyl-1*H*-pyrrol-3-ylmethyl)-1,4-dihydro-3-pyridinecarboxylate (9):

To a dry ethereal solution (10 mL) of *N*-hydroxy compound **7a** (689 mg, 1 mmol), Et_3N (202 mg, 2 mmol) and AcCl (118 mg, 1.5 mmol) are added. After stirring for 15 min, the mixture is extracted with 1 N HCl solution (10 mL) and brine (10 mL). The organic phase is dried ($MgSO_4$), evaporated and chromatographed on silica gel with hexane/EtOAc (2:1) as eluent; $R_f = 0.75$ (TLC).

^{1}H-NMR ($CDCl_3$/TMS): $\delta = 0.40-2.50$ (m, 43 H, 5 CH_3, 11 CH_2, 6 CH), 1.22 (s, 12 H, 4 CH_3), 3.07 (br s, 2 H, CH_2), 3.62 (br s, 2 H, CH_2), 4.50–4.85 (m, 1 H, CH), 5.20–5.70 (m, 3 H, 3 CH), 6.92 (br s, 1 H, CH).

Ethyl 5-Cyano-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3-pyridinecarboxylate (12):

A solution of **10** (5.26 g, 20 mmol) and 3-aminocrotonitrile (**11**; 1.64 g, 20 mmol) in EtOH (50 mL) is refluxed for 5 d. The solution is then cooled to r.t. and the residual crystals are filtered off and washed with ice-cold EtOH; yield: 4.19 g (64%); mp 191–192°C.

^{1}H-NMR ($CDCl_3$/TMS): $\delta = 1.01$ (t, 3 H, $J = 6.6$ Hz, CH_3), 2.10 (s, 3 H, CH_3), 2.30 (s, 3 H, CH_3), 3.92 (q, 2 H, $J = 7.2$ Hz, CH_2), 5.42 (s, 1 H, CH), 6.88 (br s, 1 H, NH), 7.15–7.90 (m, 4 H_{arom}).

Ethyl 2-Bromomethyl-5-cyano-1,4-dihydro-6-methyl-4-(2-nitrophenyl)-3-pyridinecarboxylate (13):

To a solution of **12** (327 mg, 1 mmol) in $CHCl_3$ (10 mL) pyridine (0.1 mL) is added. The mixture is cooled to −10°C and DMAP.HBr (400 mg, 1.1 mmol) is added. The mixture is stirred at r.t. for 1 h, the organic phase is then washed with water, dried ($MgSO_4$), and evaporated *in vacuo*, and the residue is crystallized from Et_2O/hexane; yield: 329 mg (81%); mp 125–127°C.

^{1}H-NMR ($CDCl_3$/TMS): $\delta = 1.04$ (t, 3 H, $J = 7.2$ Hz, CH_3), 2.17 (s, 3 H, CH_3), 3.95 (q, 2 H, $J = 7.2$ Hz, CH_2), 4.65 (dd, 2 H, $J = 25.8$, 5.4 Hz, CH_2), 5.45 (s, 1 H, CH), 7.15–7.90 (m, 5 H, 4 H_{arom}, NH).

4-[5-Cyano-3-ethoxycarbonyl-1,4-dihydro-6-methyl-4-(2-nitrophenyl)-2-pyridylmethylamino]-2,2,6,6-tetramethylpiperidin-1-yloxy Radical (15):

To a solution of **13** (406 mg, 1 mmol) and 4-amino-2,2,6,6-tetramethylpiperidin-1-yloxy radical (**14**; 171 mg, 1 mmol) in dry $CHCl_3$ (10 mL), K_2CO_3 (138 mg, 1 mmol) is added. This mixture is stirred and refluxed for 3 h. The organic phase is then washed with water (3 × 20 mL), dried ($MgSO_4$) and evaporated. The residue is chromatographed on silica gel with $CHCl_3$/MeOH (9:1); $R_f = 0.57$ (TLC).

3-Acetoacetyloxymethyl-2,5-dihydro-2,2,5,5-tetramethyl-1*H*-pyrrol-1-yloxy Radical (19):

Method A: To alcohol **16** (1.70 g, 10 mmol), methyl acetoacetate (**18**; 5.80 g, 50 mmol) is added and the mixture is stirred at 100°C for 18 h. The mixture is then cooled to r.t. and the excess of **18** is removed by vacuum distillation (55°C/10 Torr). The remaining thick yellow oil is flash chromatographed with $CHCl_3/Et_2O$ (1:1) as eluent to give pure **19**; yield: 1.86 g (72%); $R_f = 0.78$ (TLC).

Method B: To a stirred solution of Meldrum's acid (**17**; 2.88 g, 20 mmol) in dry CH_2Cl_2 (50 mL) and dry pyridine (4 mL), AcCl (1.73 g, 22 mmol) in dry CH_2Cl_2 (15 mL) is added dropwise under N_2 at 0°C. After stirring for 0.5 h at 0°C and for 1 h at r.t., the reaction mixture is washed with 2 N HCl (30 mL) and water (2 × 30 mL), then dried ($MgSO_4$) and evaporated. The residue is dissolved in dry THF (50 mL), **16** (3.40 g, 20 mmol) is added, and the mixture is heated under reflux for 4 h. Removal of the THF and chromatography on silica gel with $CHCl_3/Et_2O$ (1:1) results in ester **19**; yield: 2.80 g (55%).

3-[2-(2-Nitrobenzylidene)-3-oxobutyryloxymethyl]-2,5-dihydro-2,2,5,5-tetramethyl-1*H*-pyrrol-1-yloxy Radical (21):

To a solution of 2-nitrobenzaldehyde (**20**; 4.53 g, 30 mmol) and **19** (7.63 g, 30 mmol) in benzene (40 mL), piperidine (0.2 mL) and AcOH (0.1 mL) are added. This mixture is refluxed for 3 h with a Dean-Stark trap. Et_2O (100 mL) is then added, and the organic layer is washed with 5% HCl solution (100 mL), 5% Na_2CO_3 solution (100 mL) and brine (100 mL). It is dried ($MgSO_4$) and evaporation of the solvents gives **21**. Pure **21** is obtained by chromatography on silica gel with Et_2O/hexane (2:1) as eluent; yield: 7.90 g (68%); $R_f = 0.29$ (TLC); mp 105°C.

4-Substituted 1,4-Dihydropyridines (23, 26b,d) from α,β-Unsaturated β-Alkoxycarbonyl Ketone 21, 25a,b and Aminocrotonate 22b; General Procedure:

Ethyl 3-aminocrotonate **22b** (1.29 g, 10 mmol) and the corresponding α,β-unsaturated β-alkoxycarbonyl ketone **21**, **25a,b** (10 mmol) are dissolved in EtOH (30 mL) and the mixture is refluxed for 5 h. The EtOH is then evaporated off and the residue is purified by column chromatography on silica gel with hexane/EtOAc (2:1) and crystallized from Et_2O/hexane.

1,4-Dihydropyridines (26a,c,e–i,k–o) from Aldehyde (24a–k) and Aminocrotonate (22a,b); General Procedure:

A mixture of methyl or ethyl 3-aminocrotonate **22a,b** (2.30 g or 2.58 g, 20 mmol) and the aldehyde **24a–k** (10 mmol) is heated under reflux in EtOH (50 mL) for 12 h. The reaction is monitored by TLC on silica gel ($CHCl_3/Et_2O$, 1:1). The solvent is evaporated off under reduced pressure and the residue is crystallized from Et_2O/hexane.

26k:

^{1}H-NMR ($CDCl_3$/TMS): $\delta = 0.82$ (t, 3 H, $J = 7.5$ Hz, CH_3), 1.10–1.35 (m, 8 H, 4 CH_2), 1.27 (t, 6 H, $J = 8.0$ Hz, 2 CH_3), 2.26 (s, 6 H, 2 CH_3), 3.9 (t, 1 H, $J = 6.6$ Hz, CH), 4.15 (m, 4 H, 2 CH_2), 5.85 (br s, 1 H, NH).

26l:

^{1}H-NMR ($CDCl_3$/TMS): $\delta = 2.33$ (s, 6 H, 2 CH_3), 3.72 (s, 6 H, 2 CH_3), 4.61 (d, 1 H, $J = 4.4$ Hz, CH), 5.75 (br s, 1 H, NH), 6.13 (d, 1 H, $J = 15.7$ Hz, CH), 6.21 (d, 1 H, $J = 15.7$ Hz, CH), 7.10–7.35 (m, 5 H_{arom}).

26m:

^{1}H-NMR ($CDCl_3$/TMS): $\delta = 1.29$ (t, 6 H, $J = 8.0$ Hz, 2 CH_3), 1.67 (m, 2 H, CH_2), 2.30 (s, 6 H, 2 CH_3), 2.55 (m, 2 H, CH_2), 4.06 (t, 1 H, $J = 5.8$ Hz, CH), 4.18 (m, 4 H, 2 CH_2), 5.61 (br s, 1 H, NH), 7.06–7.27 (m, 5 H_{arom}).

26n:

^{1}H-NMR ($CDCl_3$/TMS): $\delta = 1.30$ (t, 6H, $J = 7.2$ Hz, $2CH_3$), 1.50–2.14 (m, 7H, $3CH_2$, CH), 2.30 (s, 6H, $2CH_3$), 3.95–4.38 (m, 5H, $2CH_2$, CH); 5.50–5.65 (m, 2H, 2CH), 6.04 (br s, 1H, NH).

Oxidation of 4-Substituted 1,4-Dihydropyridines (26a–o) to Pyridines (27a–d,f,g,i–m,o and 28); General Procedure:

To a solution of the 1,4-dihydropyridine **26a–o** (1 mmol) in $CHCl_3$ (5 mL), MnO_2 (5 mmol) is added and the mixture is heated under reflux for 30 min. It is then filtered and the solid is washed with $CHCl_3$. The solvent is evaporated off and the residue is crystallized from Et_2O/hexane.

27i:

^{1}H-NMR ($CDCl_3$/TMS): $\delta = 1.40$ (t, 6H, $J = 7.2$ Hz, $2CH_3$), 2.30 (s, 3H, CH_3), 2.50 (s, 6H, $2CH_3$), 4.36 (q, 4H, $J = 7.2$ Hz, $2CH_2$).

27k:

^{1}H-NMR ($CDCl_3$/TMS): $\delta = 0.68–1.60$ (m, 9H, $3CH_2$, CH_3), 1.38 (t, 6H, $J = 6.6$ Hz, $2CH_3$), 2.49 (s, 6H, $2CH_3$), 2.40–2.74 (m, 2H, CH_2), 4.36 (q, 4H, $J = 6.6$ Hz, $2CH_2$).

27l:

^{1}H-NMR ($CDCl_3$/TMS): $\delta = 2.52$ (s, 6H, $2CH_3$), 3.80 (s, 6H, $2CH_3$), 6.88 (d, 2H, $J = 8.4$ Hz, 2CH), 7.15–7.50 (m, $5H_{arom}$).

27m:

^{1}H-NMR ($CDCl_3$/TMS): $\delta = 1.27$ (t, 6H, $J = 7.2$ Hz, $2CH_3$), 2.38 (s, 6H, $2CH_3$), 2.72 (s, 4H, $2CH_2$), 4.26 (q, 4H, $J = 7.2$ Hz, $2CH_2$), 7.05 (s, $5H_{arom}$).

28:

^{1}H-NMR ($CDCl_3$/TMS): $\delta = 1.40$ (t, 6H, $J = 7.2$ Hz, $2CH_3$), 2.82 (s, 6H, $2CH_3$), 4.35 (q, 4H, $2CH_2$), 8.61 (s, 1H, CH).

2-(Formylethyl)-2,5,5-trimethylpyrrolidin-1-yloxy Radical (24c):
(*analogously to Lit.*[35])

To a cooled (−60 °C) and stirred solution of oxalyl chloride (1.39 g, 11 mmol) in dry CH_2Cl_2 (25 mL), DMSO (1.89 g, 24 mmol) in dry CH_2Cl_2 (20 mL) is added. After 20 min, a solution of 2-(3-hydroxypropyl)-2,5,5-trimethylpyrrolidin-1-yloxy radical (1.86 g, 10 mmol) in dry CH_2Cl_2 (20 mL) is added. After 1 h Et_3N (5.05 g, 50 mmol) is added. The mixture is stirred at −60 °C for 30 min and allowed to warm to 0 °C, and water (10 mL) is added dropwise. The organic phase is washed in turn with 5% H_2SO_4 (20 mL), 5% aq $NaHCO_3$ (20 mL) and brine, dried ($MgSO_4$) and evaporated to dryness. The yellow residue is purified on silica gel with hexane/EtOAc (2:1); yield: 1.38 g (75%); $R_f = 0.82$ (TLC).

The work was supported by grants from the Hungarian Academy of Sciences (301/A/82 and 3/104/86). The authors wish to express their thanks to Mrs. M. Ott for microanalyses.

Received: 7 June 1990: revised: 13 August 1990

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