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New compounds

Spin labeled hydroxyproline analogues

Jean M.J. Tronchet [a,*], Chantal Jorand [a], Guido Zosimo-Landolfo [a,1], Michel Geoffroy [b]

[a] *Department of Pharmaceutical Chemistry, University of Geneva, Sciences 2, CH-1211 Geneva 4, Switzerland*
[b] *Department of Physical Chemistry, University of Geneva, Sciences 2, CH-1211 Geneva 4, Switzerland*

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Abstract

The synthesis and properties of both epimers of 4-(*N*-hydroxyamino)-*N*-tosyl-L-proline methyl ester and derivatives thereof are reported. In the presence of air, the compounds bearing a free *N*-hydroxyamino group oxidized in solution to give the corresponding aminoxyl free radicals, EPR spectra of which were recorded. None of these compounds showed any interesting biological activity as such, but they constitute potential synthetic building blocks towards spin labeled glycopeptide or oligopeptide analogues. © 1999 Elsevier Science S.A. All rights reserved.

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1. Introduction

Even if the alleged activity of thioproline against certain lung cancers [1] was not confirmed, [2] the interest of preparing other analogues of hydroxyproline still remains. The conformational stability of collagen is partially insured by hydrogen bonds, two molecules of water acting as a bridge between the hydroxy groups of two hydroxyproline moieties [3]. This entropically unfavorable situation could be improved by replacing the hydroxy group of hydroxyproline with a hydroxyamino group. Such an elongation of the hydrogen bonding groups could render unnecessary the second molecule of water. Other interests in the 4-hydroxyaminoproline derivatives reside in their spontaneous oxidation into aminoxyl radicals allowing the monitoring of these modified aminoacids by EPR spectroscopy [4] and their possible use as building blocks for the synthesis of oligopeptide or glycopeptide [5] analogues. We describe here the synthesis of derivatives of both diastereoisomers of 4-hydroxyamino-L-proline. A preliminary account of this work has been published [6].

2. Experimental procedures

General techniques have been described [7]. Reduction of oxime **1** has been performed using $NaBH_3CN$ in standard conditions [8] and nitrone **7** reduced by $NaBH_3CN$ in acidic medium following a described procedure [9]. Biological tests have been conducted as previously described [10]. Complementary information can be obtained from the authors (J.M.J. Tronchet).

3. Results and discussion

Upon cyanoborohydride reduction, oxime **1** [11] led to a 2:1 mixture (83%) of **2** and **3** from which only **2** could be obtained in pure state by crystallization (Scheme 1). The diastereoisomeric mixture of **2** and **3** was submitted to *O*-silylation and a chromatographic separation of the *O*-silyl derivatives afforded pure **4** (39%) and **5** (15%). Pure **2** and **3** were obtained almost quantitatively by de-*O*-silylation of **4** and **5** respec-

* Corresponding author. Fax: +41-22-702-6585.
E-mail address: jean.tronchet@pharm.unige.ch (J.M.J. Tronchet)
[1] Present address: Elsevier Science S.A., 50 avenue de la Gare, 1001 Lausanne, Switzerland.

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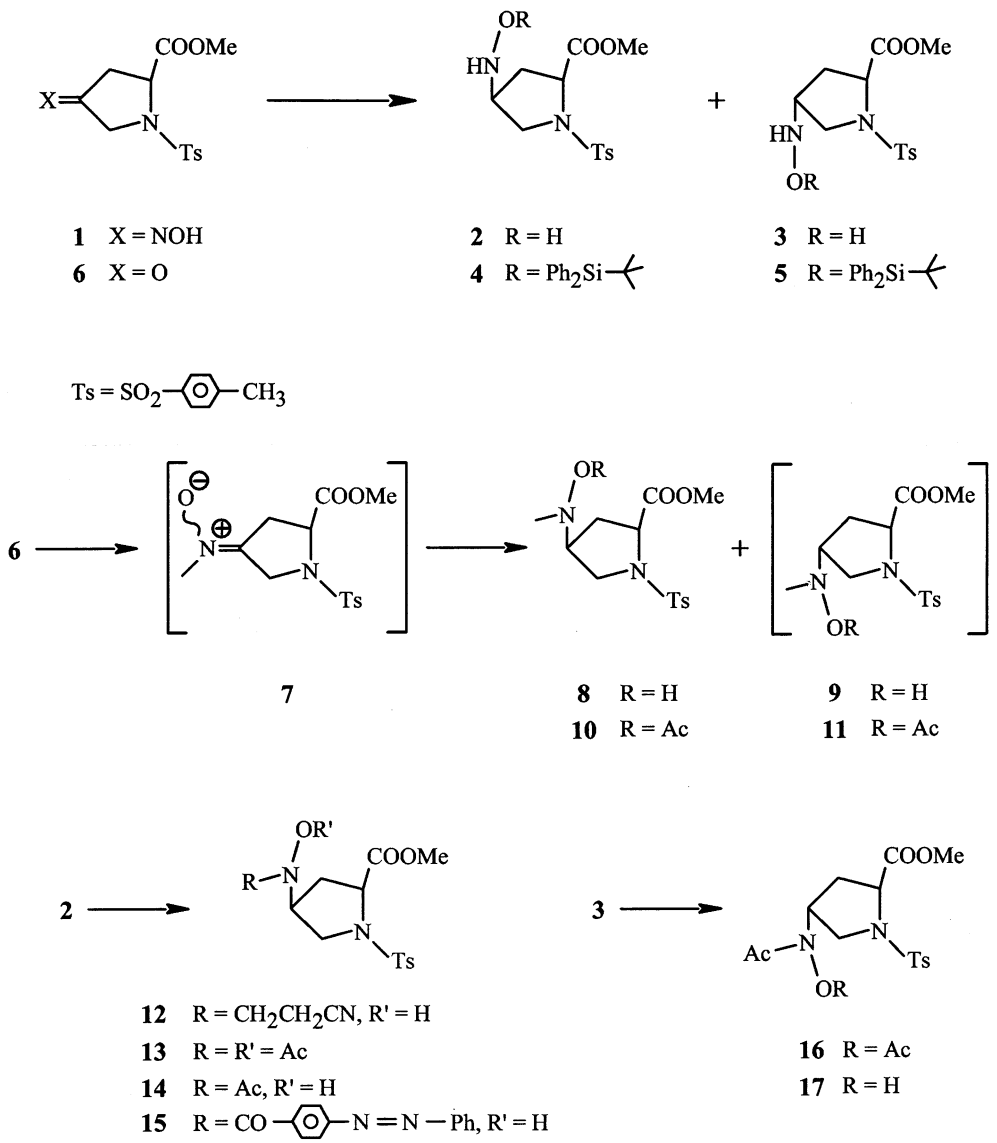
tively. The (2*S*,4*R*) configuration of **3** was established by X-ray diffraction [12]. Upon treatment with *N*-methylhydroxylamine hydrochloride, the ketone **6** [11] afforded an *E*/*Z* mixture of the unstable nitrone **7** which was directly reduced ($NaBH_3CN$) to a 2:1 mixture (94% from **6**) of **8** and **9** from which only **8** could be isolated in a pure state. From the acetylated mixture of **8** and **9**, the major isomer **10** (49%) was obtained by recrystallization. The (2*S*,4*S*) configuration of the major isomer **8** was assigned from the better accessibility of the *re* face of the electrophilic carbon atom of **7** as apparent from the issue of the reduction of **1**. From pure **2**, some derivatives of the hydroxyamino function were prepared. Thus, conjugate addition of **2** upon acrylonitrile led to **12** (92.5%) whereas, upon acetylation (Ac_2O), **2** gave the *N*,*O*-diacetylated derivative **13** (62%), selective deacetylation of which (one equivalent of NaOEt in ether/MeOH) afforded the hydroxamic acid **14** (75%). Treated with 4-phenylazobenzoyl chloride, **2** led to the hydroxamic acid **15** (67%). From **3**, **16** (69%) and **17** (70%) were prepared using the same procedures. Some properties of the novel compounds are collected in Tables 1 and 2.

Either spontaneously in the air or upon addition of traces of lead dioxide, compounds bearing a *N*-hydroxy group oxidized to the corresponding aminoxyl free radical. Except for the primary hydroxylamines **2** and **3** giving rise to unstable aminoxyls which decomposed into at least two paramagnetic species of unknown structure, other *N*-hydroxy derivatives afforded



Scheme 1.

Table 1
Some properties of modified proline derivatives

Compound	M.p. (°C)	$[\alpha]_D$ (t°C, c)[a]	R_F (solvent)	*Anal.* (C, H, N, S)[b]	EI MS
2	93.6–98.5	−71.4 [21, 0.3]	0.6 (5:1 AcOEt/MeOH)	$C_{13}H_{18}N_2O_5S$	314 (8, $M^{\bullet+}$)
3	112.0–113.5	−40.1 [23, 1.0]	0.7 (5:1 AcOEt/MeOH)	$C_{13}H_{18}N_2O_5S$	314 (9, $M^{\bullet+}$)
4	Syrup	−30.4 [23, 1.3]	0.53 (10:1:11 Et_2O/MeOH/hexane)	$C_{29}H_{36}N_2O_5SSi$	553 (1.2, $M^{\bullet+}+1$), 495 (69, $M^{\bullet+}-Me_3C$)
5	Syrup	−40.8 [23, 0.5]	0.45 (10:1:11 Et_2O/MeOH/hexane)	$C_{29}H_{36}N_2O_5SSi$	552 (2, $M^{\bullet+}$), 495 (89, $M^{\bullet+}-Me_3C$)
8	111.0–112.5	−69.0 [26, 1.0]	0.5 (AcOEt)	$C_{14}H_{20}N_2O_5S$	328 (0.6, $M^{\bullet+}$)
10	79.0–88.0	−50.8 [25, 1.2]	0.4 (2:1 AcOEt/$(Me_2CH)_2O$	$C_{16}H_{22}N_2O_6S$	370 (0.1, $M^{\bullet+}$), 328 (11, $M^{\bullet+}-CH_2CO$)
12	Syrup	−50.0 [22, 0.6]	0.45 (2:1 AcOEt/hexane)	$C_{16}H_{21}N_3O_5S$	367 (8, $M^{\bullet+}$), 307 ($M^{\bullet+}-$AcOH)
13	119.8–123.2	−37.4 [22, 0.8]	0.70 (AcOEt)	$C_{17}H_{22}N_2O_7S$	398 (0.5, $M^{\bullet+}$), 356 (36, $M^{\bullet+}-CH_2CO$)
14	148.0–150.0	−30.5 [25, 1.0]	0.5 (AcOEt)	$C_{15}H_{20}N_2O_6S$	356 (0.9, $M^{\bullet+}$), 281 (1.2, $M^{\bullet+}-$AcNOH)
15	195.2−198.0	−20.8 [23, 1.1]	0.5 (2:1 AcOEt/hexane)	$C_{26}H_{26}N_4O_6S$	351 (3, $M^{\bullet+}-$Ts−O)
16	Syrup	−45.3 [29, 1.2]	0.68 (AcOEt)	$C_{17}H_{22}N_2O_7S$	356 (2, $M^{\bullet+}-CH_2CO$)
17	148.5−149.3	−26.6 [21, 0.8]	0.45 (AcOEt)	$C_{15}H_{20}N_2O_6S$	356 (1.1, $M^{\bullet+}$)

[a] In $CHCl_3$.
[b] Within ± 0.3% of the calculated values.

Table 2
Selected spectroscopic data (UV, IR, ^{1}H NMR, ^{13}C NMR) of modified L-proline derivatives

Compound	UV (EtOH) λ_{max} (nm) (ε)	IR ν_{max} (cm^{-1})[a]	^{1}H NMR ($CDCl_3$) δ_H J (Hz)	^{13}C NMR ($CDCl_3$) δ
2	209 (3780) 230 (8970)	3460 (OH), 3150 (NH), 1725 (CO), 1590–1450 (C=C, Ar), 1340, 1160 (SO) [A]	Ha-3 2.07, $J_{3a,4}$ 5.5 Hb-3 2.17, $J_{3b,4}$ 3.0, $J_{3a,3b}$ 14.0 H-4 3.60, Ha–5 3.30 $J_{4,5a}$ 5.5 Hb-5 3.46 $J_{4,5b}$ 2.0, $J_{5a,5b}$ 10.5	C-3 33.02 C-4 60.77 C-5 51.12
3	202 (15900) 230 (11700)	3480, 3280 (OH, NH), 1750 (CO), 1600, 1495, 1455 (C=C, Ar), 1350, 1165 (SO) [B]	Ha-3 2.05, $J_{3a,4}$ 6.0 Hb-3 2.14, $J_{3b,4}$ 4.5, $J_{3a,3b}$ 14.0 H-4 3.73, $J_{4,5}$ 4.5, 2 H-5 3.48	C-3 33.72 C-4 60.28 C-5 50.81
4	202 (43800) 220 (24800) 265 (1840)	3275 (NH), 1747 (CO), 1601, 1500, 1474 (C=C, Ar), 1355, 1164 (SO) [A]	2 H-3 2.11, $J_{3,4}$ 4.0 H-4 3.43, Ha-5 3.20, $J_{4,5a}$ 5.5 Hb-5 3.75, $J_{4,5b}$ 2.0, $J_{5a,5b}$ 10.5	C-3 32.60 C-4 61.14 C-5 50.85
5	202 (46800) 220 (23400) 270 (11300)	3270 (NH), 1750 (CO), 1602, 1500, 1468 (C=C, Ar), 1355, 1168 (SO) [D]	Ha-3 1.93, $J_{3a,4}$ 7.0 Hb-3 2.05, $J_{3b,4}$ 4.0, $J_{3a,3b}$ 13.5 H-4 3.64, Ha-5 3.41, Hb-5 3.60	C-3 33.39 C-4 61.02 C-5 50.73
8	204 (13300) 230 (9900)	3140 (OH), 1746 (CO), 1598, 1499, 1449 (C=C, Ar), 1344, 1157 (SO) [A]	2 H-3 2.16, $J_{3,4}$ 7.5 H-4 3.00, Ha-5 3.40, $J_{4,5a}$ 6.0 Hb-5 3.57, $J_{4,5b}$ 6.8, $J_{5a,5b}$ 11.0	C-3 33.14 C-4 66.86 C-5 50.77 N–Me 46.47
10	202 (13500) 230 (9200)	1765 (CO), 1605, 1443 (C=C, Ar), 1360, 1166-1100 (SO), 1166-1100 (C-O-C) [C]	Ha-3 2.11, $J_{3a,4}$ 8.0 Hb-3 2.35, $J_{3b,4}$ 6.0, $J_{3a,3b}$ 13.2 H-4 3.24, Ha-5 3.30, $J_{4,5a}$ 8.0 Hb-5 3.67, $J_{5a,5b}$ 8.0	C-3 33.07 C-4 65.90 C-5 50.18 N-Me 45.05 N–C=O 168.84 **Me**–CON 19.26
12	202 (14700) 230 (11200)	3455 (OH), 2250 (CN), 1751 (CO), 1591, 1495, 1438 (C=C, Ar), 1346, 1159 (SO) [D]	Ha-3 2.12, $J_{3a,4}$ 7.0 Hb-3 2.22, $J_{3b,4}$ 6.0, $J_{3a,3b}$ 13.0 H-4 3.10, 2 H-5 3.50, $J_{4,5}$ 6.5	C-3 32.91 C-4 65.66 C-5 50.70 **CH_2**CN 16.10 N–**CH_2**CH_2 53.02 CN 118.57

Table 2 (continued)

Compound	UV (EtOH) λ_{max} (nm) (ε)	IR ν_{max} (cm^{-1})[a]	^1H NMR ($CDCl_3$) δ_H J (Hz)	^{13}C NMR ($CDCl_3$) δ
13	202 (18000) 230 (11900)	1810, 1760, 1685 (CO), 1605, 1500, 1450 (C=C, Ar), 1365, 1167 (SO) [C]	Ha-3 2.10, $J_{3a,4}$ ca 7 Hb-3 2.43, $J_{3b,4}$ ca 7 H-4 4.87, Ha-5 3.36, $J_{4,5a}$ 7.5 Hb-5 360, $J_{4,5b}$ 8.0, $J_{5a,5b}$ 13.0	C-3 32.15 C-4 53.99 C-5 48.73 N-CO 170.58 **Me**–CON 20.14
14	203 (21700) 229 (12700)	3280 (OH), 1727, 1640 (CO), 1595, 1440 (C=C, Ar), 1355, 1163 (SO) [C]	2 H-3 2.26, H-4 4.88 Ha-5 3.28, $J_{4,5a}$ 6.0 Hb-5 3.91, $J_{4,5b}$ 0.5, $J_{5a,5b}$ 10.5	C-3 35.09 C-4 54.80 C-5 49.16
15	202 (38700) 230 (24400) 324 (22100)	3130 (OH), 1750 (CO), 1600, 1585, 1500, 1460 (C=C, Ar), 1350, 1162 (SO) [A]	2 H-3 2.43, H-4 4.88 Ha-5 3.48, $J_{4,5a}$ 6.5 Hb-5 3.97, $J_{4,5b}$ 3.0, $J_{5a,5b}$ 11.0	C-3 34.76 C-4 56.98 C-5 59.04
16	203 (17700) 230 (12200)	1800, 1750, 1685 (CO), 1600, 1495, 1440 (C=C, Ar), 1370, 1175 (SO) [B]	2 H-3 2.26, H-4 5.21 Ha-5 3.49, $J_{4,5a}$ ca. 7 Hb-5 3.60, $J_{4,5b}$ ca. 7, $J_{5a,5b}$ 10.0	
17	202 (17700) 230 (12000)	3300 (OH), 1755, 1655 (CO), 1608, 1525, 1445 (C=C, Ar), 1350, 1170 (SO) [C]	2 H-3 2.26, H-4 4.88 Ha-5 3.28, $J_{4,5a}$ 6.0 Hb-5 3.91, $J_{4,5b}$ 0.5, $J_{5a,5b}$ 10.5	

[a] Solvent [A] = KBr, [B] = CCl_4, [C] = $CHCl_3$, [D] = film (no solvent).

Table 3
EPR spectra in diglyme of aminoxyl radicals generated from hydroxylamines or hydroxamic acids[a]

Diamagnetic species	t (°C)	Oxidizing agent	g	a_N	$a_{H\text{-}4}$	a_{CH_2}	a_{CH_3}	⌈[b]
8	45	None	2.0061	14.95	7.7		12.25	1.2
12	11.5	None	2.0060	14.15	7.1	10.6		1.5
14	25	None	2.0064	7.55	4.35			2.0
15	50	PbO_2	2.0069	7.45	4.7			1.2
17	25	None	2.0065	7.5	4.8			1.3

[a] Hyperfine coupling constants in Gauss.
[b] Linewidth in Gauss.

free radicals giving rise to well resolved EPR spectra (Table 3), exhibiting hyperfine coupling constants in the expected range of values [4].

None of these compounds showed any significant cytotoxic anticancer, or antiviral (HSV-1, RV-31, RV-1B, influenza A) activity.

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