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Note

Spacer arms bearing two *N*-hydroxyimino groups and nitroxyl free radicals thereof

Jean M. J. Tronchet*, Maria Koufaki, and Guido Zosimo-Landolfo
Institute of Pharmaceutical Chemistry, University of Geneva, Sciences II, CH-1211 Geneva 4 (Switzerland)

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The binding of a small bioactive molecule (*i.e.*, a drug) onto a large biopolymer (*i.e.*, a receptor) generally involves co-operativity between substructures, the relative separation distances of which are maintained by parts of the molecule, "spacer arms", not concerned directly in the fixation process[1]. Numerous diamagnetic spacer arms have been developed[2], but the introduction of a paramagnetic probe in these groups[3] could improve their usefulness.

We now report the synthesis and e.s.r. study of model compounds in which two blocked sugar units are connected by a spacer arm that includes two *N*-hydroxyimino groups, with the aim of testing the feasibility of generating diradical species from such precursors. Owing to the chemical lability of the *N*-hydroxyimino groups, a totally different blocking strategy will be required in order to prepare the corresponding unsubstituted analogs.

The blocked deoxy-*N*-hydroxyamino sugar **1** (ref. 4), on treatment with a deficiency of terephthalaldehyde, gave the dinitrone **2** (74.2%), which was reduced into the bis(*N*-hydroxyimino) sugar derivative **3** in excellent yield (82%). When treated with a small excess of terephthalaldehyde, **1** gave the mononitrone **4** (87%). The mononitrone **6**, obtained (61%) from **5** (ref. 4), reacted with **1** to give the unsymmetrical dinitrone **7** (89%). From **8**, prepared by reduction of the corresponding oxime[5] by a classical procedure[4], the symmetrical dinitrone **9** (70%) was prepared, whereas the mononitrone **11** was obtained (70%) from **10** (ref. 6).

When **10** reacted with an aliphatic dialdehyde (glutaraldehyde), the yields were smaller and the resulting nitrone (**12**) was not stable enough to be characterised fully. However, the structure was established by spectroscopic data and reduction to **13** (45% from **10**). The ^{1}H-n.m.r. data of the compounds prepared are collected in Tables I and II.

Spin-labelling is a useful technique for the monitoring of biochemical events[7], but, in the classical mode of application, there is a serious drawback. In order to deal with very stable free radicals that have long half-lives, heavily substituted spin-labelled

* Author for correspondence.

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TABLE I

^{1}H-N.m.r. chemical shifts ($CDCl_3$, 200 MHz) of some sugar nitrones and hydroxylamines

Compound	*N=CH*	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*Other*
2	7.51	5.65	4.31	4.68	4.28	4.65	H-6,6 (4.12)
3		5.52	4.25	4.55	4.21	4.18	H-6,6 (2.95), Ph-CH_2 (3.72 and 3.99)
4	7.51	5.51	4.34	4.70	4.29	4.67	H-6,6 (4.09 and 4.20), CHO (10.03)
6	7.55	4.08 4.39	4.60	3.81	4.00	4.22	CHO (10.0)
7 *Gal*	7.48 or 7.50	5.52	4.33	4.67	4.30	4.64	H-6,6 (3.98–4.20)
7 *Ara*	7.48 or 7.50	3.98–4.20 4.36	4.61	3.83	3.98–4.20	3.98–4.20	
8		5.97	4.67	3.93	4.53	3.22 3.30	Ph-CH_2 (4.52 and 4.71)
9	7.41	5.98	4.68	4.08	4.97	4.11 4.25	Ph-CH_2 (4.50 and 4.74)
11	7.57	5.90	5.03	4.41	4.75	4.32	H-6,6 (3.97 and 4.13), CHO (10.02)
12	6.90	5.83	4.98	4.22	4.54	4.26	H-6,6 (3.91 and 4.11), =CHCH_2 (2.62), $CH_2CH_2CH_2$ (2.83)
13		5.79	4.80	3.14	4.44	4.31	CH_2N (2.89 and 3.10) $CH_2(CH_2)_3CH_2$ (1.46–1.73) NOH (6.27)

TABLE II

^{1}H-N.m.r. coupling constants (Hz) for some sugar nitrones and hydroxylamines

Compound	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	*Others*
2	5.0	2.5	8.0	2.0	6.0	
3	5.0	2.0	12.5 ?	1.8	5.2[a] 7.0	$J_{5a,6b}$ 13.5, J_{N,CH_2} 13.0
4	5.0	2.5	7.5	2.0	4.0 8.0	$J_{6a,6b}$ 12.8
6	7.5 3.0	7.5	8.0	?	—	$J_{1a,1b}$ 12.5
7 *Gal*	5.0	2.7	8.0	2.0	?	
7 *Ara*	? 2.7	8.0	8.0	?	—	$J_{1a,1b}$ 13.5
8	3.8	0	3.5	4.8 7.5	—	$J_{5a,5b}$ 13.5
9	4.0	0	3.8	4.5 7.8	—	$J_{5a,5b}$ 13.0, $J_{CH_2,Ph}$ 12.0
11	4.0	5.2	9.0	6.0	6.0 7.0	$J_{6a,6b}$ 9.0
12	3.8	5.0	9.5	5.7 7.0	6.0	$J_{6a,6b}$ 8.5, J_{NCH,CH_2} 7.5, J_{CH_2,CH_2} 7.5
13	4.0	5.0	9.0	5.2	7.0	J_{NCH_2,CH_2} 12.5

[a] For two non-equivalent methylene protons, the values concerning the more shielded (a) are given first.

molecules are used that are different structurally from their natural congeners. We are exploring[8] the use of sugar hydroxylamines as precursors of free radicals that are less stable but much closer structurally to the natural sugars. *N*-Hydroxyimino and hydroxymethine groups have similar van der Waals volumes and electrostatic potentials. Stable diradicals could provide extra information on structure[9], derived from distance-sensitive through-space exchange coupling[10].

E.s.r. signals from a degassed solution of **3** in diglyme, previously left in contact with air, were observable at $>75°$. The spectral data recorded at 90° are collected in Table III. The four large hyperfine coupling constants were assigned[4] to two methylene groups adjacent to the nitroxyl group of a monoradical species, the small constant corresponding to the coupling[11] with H-5 of the Gal moiety. One methylene group (10.6 + 9.25 = 19.85 G) should be eclipsed, and the other (8.35 and 8.35 G) should exist in a more complicated conformational equilibrium also implying staggered forms[11]. The e.s.r. spectrum of **13** showed three large a_H hyperfine couplings, two of which corresponded to an eclipsed methylene group and the third to the often large hyperfine coupling with the *exo*-H-3 of the 1,2-*O*-isopropylidene furanose ring. Two small long-range couplings were also noted. In order to increase the stationary concentration of radical species, a chloroform solution of **13** was oxidised with lead dioxide and then filtered. The e.s.r. spectrum of the filtrate corresponded to the same monoradical as before, but the amplitude of the signals was increased. The same operation on a solution of **3** in diglyme also gave the spectrum of the monoradical, a small broadening of the

TABLE III

E.s.r. data of nitroxyl free radicals obtained by oxidation of **3** and **13**

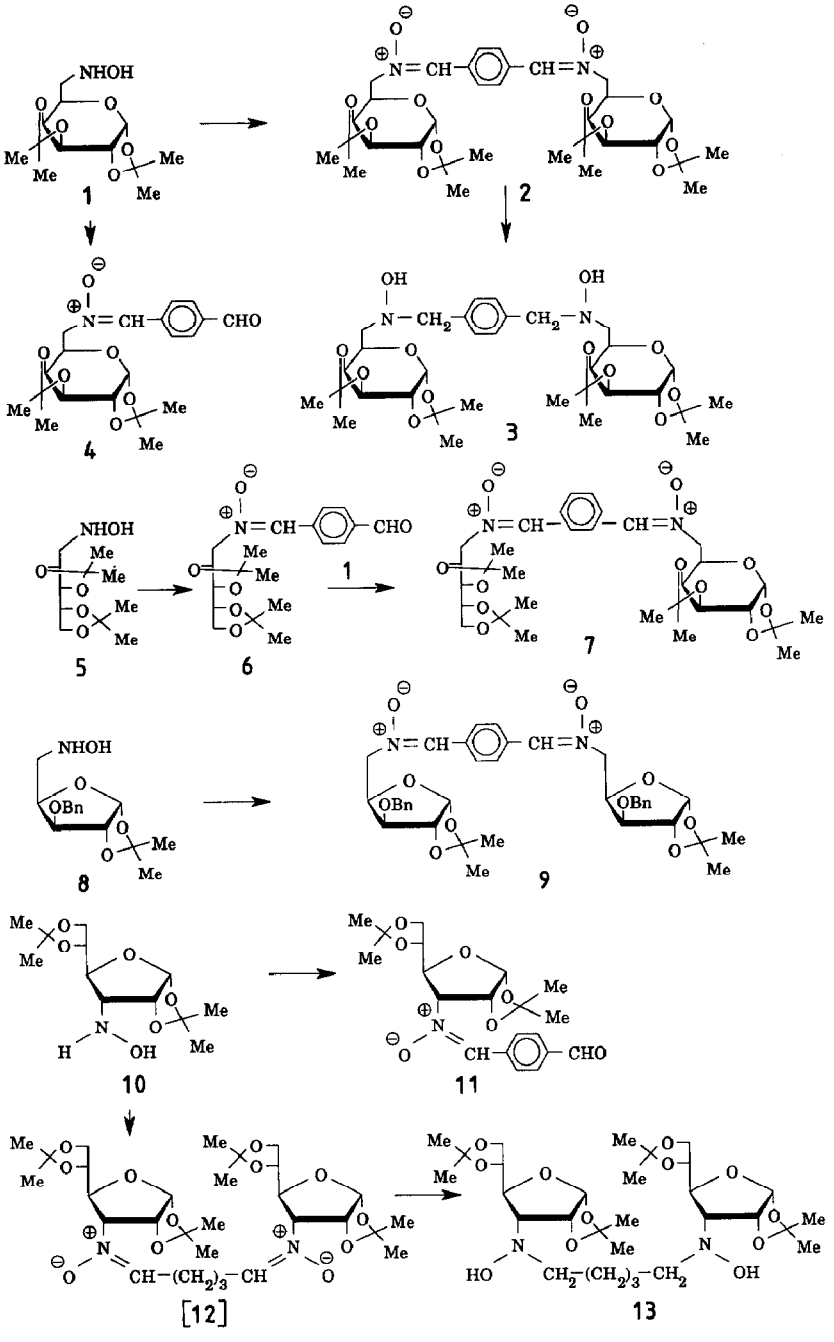
Compound	*Solvent*	*Temp.*	*g*	a_N	a_H	a_H	a_H	a_H	a_H
3	Diglyme	90°	2.0059	14.5	10.6	9.25	8.35	8.35	0.85
13	$CHCl_3$	50°		14.4	11.6	9.1	9.1	0.5	0.5
13	Diglyme	90°	2.0058	14.3	11.0	9.3	9.3	0.5	0.5
13	Diglyme	115°		14.4	11.0	9.0	9.8	0.7	0.7

signals being the only possible indication of weak through-space exchange coupling of a small amount of a diradical species.

These results indicated that such bis(*N*-hydroxyimino) derivatives could be useful as precursors of monoradical species, but are unable to yield diradicals in stationary concentrations sufficient to allow study of the intramolecular through-space spin–spin interaction.

EXPERIMENTAL

General methods. — See ref. 12. Optical rotations were obtained for solutions in chloroform. Column chromatography was conducted with silica gel (Merck 70–230 mesh). The n.m.r. data are collected in Tables I and II.



*1,4-Bis(6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-*D*-galactopyranos-6-ylimino-methyl)benzene bis-*N*-oxide* (**2**). — To a solution of **1** (800 mg, 2.9 mmol) in 2:1 pyridine–ethanol (30 mL) was added terephthalaldehyde (170 mg, 1.27 mmol), and the mixture was stirred at room temperature for 12 h. Removal of the solvents and

recrystallisation (ether–hexane) of the resulting solid gave **2** (610 mg, 74%), m.p. 185.0–187.0°, $[\alpha]_D^{23}$ −63.5° (*c* 1), R_F 0.2 (1:1 ethyl acetate–hexane); ν_{max}^{KBr} 1580 (C=N) and 1200 (N^+–O^-) cm^{-1}; λ_{max}^{EtOH} 204 (ε 10900), 233 (8100), 302 (19600), 340 (13700), and 359 nm (14350). Mass spectrum: *m/z* 59 (100%), 85 (82), 169 (66), 69 (55), 97 (52), 127 (29), 185 (26), 155 (14), 227 (5), 407 (3), 200 (2), 390 (2), 375 (0.3), and 648 (0.1, M^+).

Anal. Calc. for $C_{32}H_{44}N_2O_{12}$ (648.71): C, 59.25; H, 6.84; N, 4.32. Found: C, 59.15; H, 6.89; N, 4.36.

*1,4-Bis[*N*-(6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-*D*-galactopyranos-6-yl)-*N*-hydroxyaminomethyl]benzene* (**3**). — To a solution of **2** (500 mg, 0.77 mmol) in tetrahydrofuran (20 mL) was added sodium cyanoborohydride (100 mg, 1.6 mmol). The mixture was stirred at room temperature for 30 min and the pH was kept at 3.5 by the dropwise addition of 1:1 methanol–6M HCl. The mixture was then concentrated to dryness, the residue was extracted with chloroform (2 × 10 mL), and the combined extracts were washed with NaOH (10 mL) and then water (10 mL), dried ($MgSO_4$), and concentrated. Column chromatography (2:1 ethyl acetate–hexane) of the residue gave **3** (410 mg, 82%), m.p. 64.2–65.8°, $[\alpha]_D^{29}$ −68.75° (*c* 0.8), R_F 0.35; ν_{max}^{KBr} 3200 (OH), 1380, 1210, 1160, 1070, and 850 cm^{-1}; λ_{max}^{EtOH} 203 (ε 9670) and 212 nm (9410). Mass spectrum: *m/z* 57 (100%), 100 (95), 85 (83), 71 (77), 113 (59), 132 (26), 169 (17), 185 (7), 302 (5), 375 (5), 391 (4), and 328 (4).

Anal. Calc. for $C_{32}H_{48}N_2O_{12}$ (652.75): C, 58.88; H, 7.41; N, 4.29. Found: C, 58.60; H, 7.42; N, 4.36.

*6-Deoxy-6-(4-formylbenzylidenamino)-1,2:3,4-di-*O*-isopropylidene-α-*D*-galacto-pyranose* N*-oxide* (**4**). — To a solution of **1** (550 mg, 2 mmol) in 2:1 pyridine–ethanol (30 mL) was added terephthalaldehyde (350 mg, 2.6 mmol). The mixture was stirred for 12 h at room temperature and then concentrated. Column chromatography (1:1 ethyl acetate–hexane) of the residue gave **4** (650 mg, 87%), m.p. 64.2–65.8°, $[\alpha]_D^{26}$ −42° (*c* 0.8), R_F 0.4; ν_{max}^{KBr} 1690 (C=O), 1600 (C=N), and 1200 (N^+–O^-) cm^{-1}; λ_{max}^{EtOH} 202 (ε 9400), 232 (8640), and 324 nm (15300). Mass spectrum: *m/z* 59 (100%), 71 (77), 169 (46), 97 (37), 91 (33), 109 (19), 185 (15), 146 (15), 127 (11), 155 (7), 376 (0.8, M^+ − Me), and 391 M^+.

Anal. Calc. for $C_{20}H_{25}NO_7$ (391.42): C, 61.37; H, 6.53; N, 3.58. Found: C, 61.08; H, 6.53; N, 3.53.

*1-Deoxy-1-(4-formylbenzylidenamino)-2,3:4,5-di-*O*-isopropylidene-*D*-arabinitol* N*-oxide* (**6**). — Compound **5** (800 mg, 3.2 mmol) and terephthalaldehyde (550 mg, 4.1 mmol) were reacted as described for **4**, to give **6** (720 mg, 61%), m.p. 119.0–121.8°, $[\alpha]_D^{26}$ +36° (*c* 1.1), R_F 0.2 (2:3 ethyl acetate–hexane); ν_{max}^{KBr} 1680 (C=O), 1600 (C=N), and 1210 (N^+–O^-) cm^{-1}; λ_{max}^{EtOH} 202 (ε 10340), 232 (9150), and 324 nm (18700). Mass spectrum: *m/z* 59 (100%), 101 (42), 139 (10), 64 (9), 91 (9), 156 (6), 71 (6), 288 (5), 176 (5), 204 (5), 247 (4), and 348 (4, M^+ − Me).

Anal. Calc. for $C_{19}H_{25}NO_6$ (363.41): C, 62.80; H, 6.93; N, 3.85. Found: C, 62.52; H, 7.02; N, 3.93.

*1-(6-Deoxy-1,2:3,4-di-*O*-isopropylidene-α-*D*-galactopyranos-6-yliminomethyl)-4-(1-deoxy-2,3:4,5-di-*O*-isopropylidene-*D*-arabinitol-1-yliminomethyl)benzene bis-*N*-oxide* (**7**). — Compounds **6** (400 mg, 1.1 mmol) and **1** (300 mg, 1.1 mmol) were reacted,

as described for **4**. Column chromatography (2:1 ethyl acetate–hexane) of the product gave **7** (610 mg, 89%), isolated as a syrup, R_F 0.2, $[\alpha]_D^{27}$ −4° (*c* 1); ν_{max}^{film} 1580 (C=N) and 1200 (N^+–O^-) cm^{-1}; λ_{max}^{EtOH} 206 (ε 10600), 240 (10300), 341 (36150), and 360 nm (38100). Mass spectrum: *m/z* 58 (100%), 81 (73), 97 (63), 143 (56), 169 (44), 113 (37), 127 (33), 185 (22), 157 (20), 404 (8), 545 (7), 529 (4), 589 (4), and 620 (3, M^+).

Anal. Calc. for $C_{31}H_{44}N_2O_{11}$ (620.70): C, 59.99; H, 7.15; N, 4.51. Found: C, 59.79; H, 7.33; N, 4.38.

3-O-Benzyl-5-deoxy-5-hydroxyamino-1,2-O-isopropylidene-α-D-xylofuranose (**8**). — A solution of 3-*O*-benzyl-5-deoxy-5-hydroxyimino-1,2-*O*-isopropylidene-α-D-xylofuranose[4] (1.5 g, 5 mmol) and sodium cyanoborohydride (1.4 g, 22 mmol) in methanol (100 mL) was stirred for 2 h at room temperature and the pH was kept at 2–3 by the addition of M HCl. The mixture was then neutralised (saturated aqueous $NaHCO_3$), diluted with water (150 mL), and extracted with chloroform (3 × 50 mL). The combined extracts were dried (Na_2SO_4) and concentrated. Column chromatography on silica gel (6:1 ether–hexane) of the residue gave **8** (930 mg, 63%), isolated as a syrup, $[\alpha]_D^{26}$ −45° (*c* 1.3), R_F 0.2; ν_{max}^{KBr} 3450, 3270, 1380, 1220, 1170, and 1080 cm^{-1}; λ_{max}^{EtOH} 208 nm (ε 7540). Mass spectrum: *m/z* 91 (100%), 46 (10.04), 59 (9.04), 92 (8.28), 65 (7.49), 113 (5.86), 55 (5.23), 136 (3.55), 71 (2.40), 295 (0.1, M^+).

Anal. Calc. for $C_{15}H_{21}NO_5$ (295.34): C, 61.00; H, 7.17; N, 4.74. Found: C, 61.22; H, 7.25; N, 4.72.

1,4-Bis(3-O-benyl-5-deoxy-1,2-O-isopropylidene-α-D-xylofuranos-5-ylimino-methyl)benzene bis-N-oxide (**9**). — Compound **8** (600 mg, 2.16 mmol) and terephthalaldehyde (65 mg, 0.5 mmol) were reacted, as described for **4**. Column chromatography (8:4:1 ether–hexane–methanol) of the product gave **9** (240 mg, 69%), m.p. 88.3–90.5°, $[\alpha]_D^{29}$ −21.2° (*c* 0.85), R_F 0.2; ν_{max}^{KBr} 1580 (C=N) and 1170 (N^+–O^-) cm^{-1}; λ_{max}^{EtOH} 206 (ε 27800), 240 (9560), 341 (31600), and 360 nm (33500). Mass spectrum: *m/z* 91 (100%), 77 (9), 65 (5), 147 (5), 408 (3), 302 (3), 230 (3), 262 (1), and 655 (1).

Anal. Calc. for $C_{38}H_{44}N_2O_{10}$ (688.78): C, 66.27; H, 6.44; N, 4.07. Found: C, 66.12; H, 6.48; N, 4.08.

3-Deoxy-3-(4-formylbenzylidenamino)-1,2:5,6-di-O-isopropylidene-α-D-allofuranose N-oxide (**11**). — A solution of **10** (250 mg, 0.8 mmol) and terephthalaldehyde (120 mg, 1.1 mmol) in toluene (20 mL) was heated for 5 h under reflux, then concentrated. Column chromatography (ether) of the residue gave **11**(220 mg, 69.6%), m.p. 53.5–55.2°, $[\alpha]_D^{27}$ +232° (*c* 0.9), R_F 0.2; ν_{max}^{KBr} 1690 (C=O), 1600 (C=N), and 1200 (N^+–O^-) cm^{-1}; λ_{max}^{EtOH} 202 (ε 13300), 238 (9300), and 329 nm (15650). Mass spectrum: *m/z* 58 (100%), 101 (65), 77 (34), 85 (25), 133 (18), 91 (14), 149 (11), 119 (10), 158 (3), 201 (2), 230 (1), and 376 (0.3, M^+ −Me).

Anal. Calc. for $C_{20}H_{25}NO_7$ (391.42): C, 61.37; H, 6.44; N, 3.58. Found: C, 61.31; H, 6.45; N, 3.58.

1,5-Bis[N-(3-deoxy-1,2:5,6-di-O-isopropylidene-α-D-allofuranos-3-yl)-N-hydroxyamino]pentane (**13**). — A solution of glutaraldehyde (100 mg, 1 mmol; prepared by extraction of a commercial 50% aqueous solution with chloroform) and **10** (550 mg, 2 mmol) in 3:1 pyridine–ethanol (20 mL) was stirred at room temperature for 12 h, then

concentrated. Column chromatography (9:1 ethyl acetate–hexane) of the residue gave **12** (300 mg, 49%), m.p. 97.1–99.2°, $[\alpha]_D^{29}$ +148° (*c* 1), R_F 0.25; ν_{max}^{KBr} 1600 (C=N) and 1200 (N^+–O^-) cm^{-1}; λ_{max}^{EtOH} 231 nm (ε 15850). Mass spectrum: *m/z* 614 (0.2%, $M^{+\cdot}$).

To a solution of **12** (200 mg, 0.3 mmol) in methanol (10 mL) was added sodium borohydride (60 mg, 1.5 mmol), and the mixture kept at 0° for 15 min, then concentrated. The residue was partitioned between ethyl acetate (10 mL) and water (10 mL), and the organic layer was dried ($MgSO_4$) and concentrated. Column chromatography (ethyl acetate) of the residue gave **13** (180 mg, 90%; 45% from **10**), isolated as a syrup, R_F 0.4, $[\alpha]_D^{23}$ +60° (*c* 0.7); ν_{max}^{KBr} 3500 (OH) cm^{-1}. Mass spectrum: *m/z* 101 (100%), 84 (85), 59 (55), 72 (30), 138 (30), 126 (24), 240 (18), 325 (15), 166 (15), 154 (15), 603 (14, $M^+ - Me$), 214 (12), 112 (12), and 618 (10, $M^{+\cdot}$).

Anal. Calc. for $C_{29}H_{30}N_2O_{12}$ (618.73): C, 56.30; H, 8.15; N, 4.53. Found: C, 56.52; H, 8.09; N, 4.32.

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