Tetrahedron 72 (2016) 6368–6374

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Water soluble, chiral, verdazyl radicals derived from aldoses

Thanh-Ngoc Le, Harjot Grewal, Victor Changoco, Vinhly Truong, David J.R. Brook *

Department of Chemistry, San José State University, One Washington Square, San José, CA 95192, USA

ARTICLE INFO

Article history:
Received 12 May 2016
Received in revised form 10 August 2016
Accepted 11 August 2016
Available online 18 August 2016

Keywords:
Stable free radical
Fluorescence quencher
Spin-probe
Verdazyl
Water soluble

ABSTRACT

Condensation of 2,4-diisopropylcarbonobis(hydrazide) bis-hydrochloride with a series of aldoses gives rise to tetrazanes that can be oxidized with potassium ferricyanide to give stable verdazyl radicals in good yield. The radicals are stable under ambient conditions, and are soluble in water and polar organic solvents. Aqueous solutions are stable over a range of both acidic and basic pH and do not react significantly with ascorbic acid or hydrogen peroxide. The radicals quench fluorescence from long lived fluorophores such as pyrene, or when there is an association between the radicals and the fluorophore. These radicals thus provide the foundations of a new series of radical probes and fluorescence quenchers.

1. Introduction

Stable free radicals are important tools for probing structure and function of biological systems. In addition to the identification of radicals in a diamagnetic background by EPR, radicals can be spatially located through ESR imaging,[1,2] and can be indirectly detected through their ability as efficient fluorescence quenchers[3–11] and through NMR spin relaxation techniques[12–16] In particular, the return of fluorescence combined with loss of an ESR signal when a free radical is destroyed is a powerful method for the detection of various species; recent examples include urushiols,[17] ascorbic acid[18–20] and nicotine.[21] Attachment of radicals to a dendrimer core has been recently used to provide a novel, gadolinium free MRI contrast agent.[22] As the most widely known series of stable organic radicals, nitroxides have played a dominant role in these studies; however, they are not without problems. A particular challenge is stability. Nitroxides are reduced to hydroxylamines in vivo with a half life of a few minutes.[23,24] Bulky substituents can reduce the rate of reduction, but limit the interaction with the system of interest. Consequently development of other stable radicals with different structure and reactivity may broaden the range of application of these methods. Verdazyls (Fig. 1) are a series of paramagnetic, heterocyclic free radicals that are stable under ambient conditions and may provide an alternate series of radical probes with complementary properties to existing systems.

6-Oxoverdazyls (X=O) are generally more resistant to reduction than nitroxides, and variable substituents in the 1, 3 and 5 positions may be used to control interactions with other molecules. Of such interactions water solubility is desirable for biological applications, but most stable organic radicals are relatively non-polar as a result of the alkyl or aryl substituents required for stability. For verdazyls, several approaches have been used to improve water solubility. Early on in the study of verdazyls, Kuhn and Fischer-Schwarz reported water soluble verdazyls derived from sugar formazans.[25] The water solubility was limited to about 10^{-5} mol L^{-1} and the radical underwent disproportionation below pH 7. Two groups reported water solubility derived from anionic substituents: Bezvershenko and Premyslov synthesized a sulfonated verdazyl radical with solubility in water of about 0.1 mol L^{-1}.[26] More recently, Hicks and co-workers synthesized a verdazyl carboxylic acid,[27] though this was only soluble in aqueous base. With our development of the more robust diisopropyl-6-oxoverdazyls,[28] we considered that Kuhn's approach of synthesizing verdazyls from aldoses was worth revisiting. We have reported initial studies in this area at recent conferences;[29,30] we now report full details of the synthesis of a series of verdazyl radicals synthesized from aldoses, along with initial studies of their reactivity and properties as fluorescence quenchers.

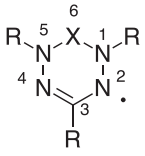


Fig. 1. Structure of verdazyl free radicals.

* Corresponding author. E-mail address: david.brook@sjsu.edu (D.J.R. Brook).

http://dx.doi.org/10.1016/j.tet.2016.08.035
0040-4020/

2. Results

Combination of a series of aldoses (Table 1) with 2,4-diisopropyl carbono-bis-hydrazide (**1**) and sodium acetate in water gave tetrazanes **2a**–**i**. Tetrazanes derived from the disaccharides maltose and lactose had complex ^{1}H NMR that may be indicative of more than one species in solution (as indicated by the number of peaks corresponding to isopropyl methyl groups); nevertheless, mass spectra were consistent with the tetrazane structure. Initial attempts at oxidation of the tetrazanes with benzoquinone[28,31] or sodium periodate[32] failed; the former because of the limited solubility of the tetrazanes in non-aqueous systems, the latter because periodate oxidation also resulted in cleavage of the carbohydrate side chain. Oxidation with potassium ferricyanide,[33] however, gave the verdazyls **3a**–**i** as bright yellow solids, purified by extraction with butanol (Scheme 1). While the disaccharide tetrazanes **2h**–**i** clearly gave verdazyl radicals **3h**–**i** (as indicated by ESR, UV–vis and HRMS), HPLC indicated other components to the samples that could not be easily separated.

Table 1
Starting aldoses, structure designations and absolute configurations of tetrazanes **2a**–**i** and verdazyls **3a**–**i**

Aldose	Designation	R	R′	Absolute configuration
D-Lyxose	a	–H	–H	1′R, 2′R, 3′R
D-Xylose	b	–H	–H	1′S, 2′R, 3′R
D-Ribose	c	–H	–H	1′R, 2′S, 3′R
D-Arabinose	d	–H	–H	1′S, 2′S, 3′R
D-Glucose	e	–CH_2OH	–H	1′S, 2′R, 3′R, 4′R
D-Mannose	f	–CH_2OH	–H	1′R, 2′R, 3′R, 4′R
D-Galactose	g	–CH_2OH	–H	1′S, 2′R, 3′S, 4′R
D-Maltose	h	–CH_2OH	–α-D-glucopyranosyl	1′S, 2′R, 3′R, 4′R
D-Lactose	i	–CH_2OH	–β-D-galactopyranosyl	1′S, 2′R, 3′R, 4′R

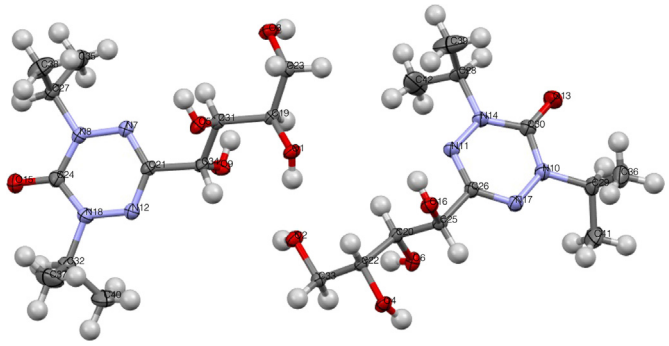
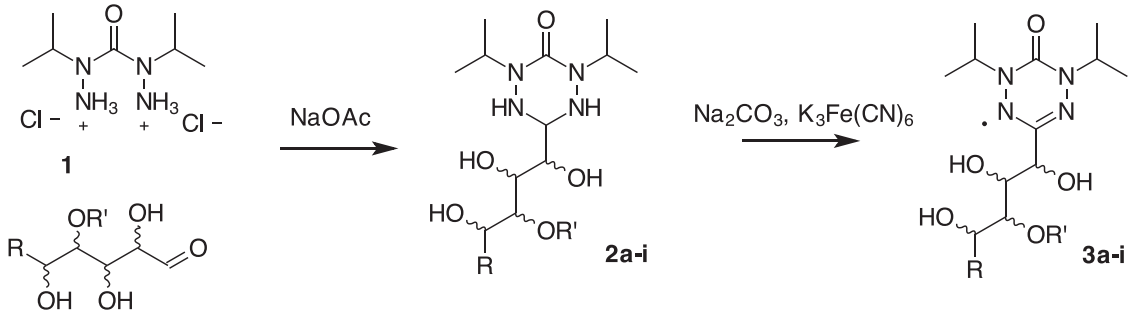


Fig. 2. Thermal ellipsoid plot of the two independent molecules of **3a** in the unit cell. Ellipsoids are drawn at the 50% probability level.

simulation for **3d**. Spectra and simulations for the remaining verdazyls are provided in Supplementary data while spectral parameters are reported in Table 2. There is very little variation in most of hyperfine parameters (+/−0.1G) between radicals; this is typical for such systems since the spin density is largely localized on the verdazyl nitrogens. The biggest variations are seen in coupling to the side chain hydrogen. These variations are probably due to small differences in preferred conformation.

Electronic spectra were similar to the spectra we reported for 3-methyl-1,5,-diisopropyl-6-oxoverdazyl[34] though there are differences in intensity and width of the contributing bands as a result of the change of solvent from hexane to methanol (Fig. 4).

All of the new verdazyls are quite soluble in water, but less soluble in less polar solvents. We estimated water solubility for the mannose derived verdazyl **3f** of at least 0.7 mol L^{-1}. Solubility of the other verdazyls was comparably large. Electronic spectra were unaffected by variation in pH from 4 to 10 though slow decomposition occurred at pH 0 (the UV–vis spectrum lost two thirds



Scheme 1. Synthesis of tetrazanes **2a**–**i** and verdazyls **3a**–**i**. Absolute configurations of the stereocenters on the side chain are given in Table 1.

To provide unambiguous confirmation of the verdazyl structure, radical **3a** was characterized by X-ray crystallography. Crystals were grown by slow evaporation of a methanol solution. Full details, including data collection and refinement have been deposited in the Cambridge Crystallographic Data Centre (CCDC), deposition number 1475223. Radical **3a** crystallizes in the monoclinic space group $P112_1$ (No. 4) with two independent molecules in the asymmetric unit. The two molecules differ only in slightly different conformations of the polyol side chain. A thermal ellipsoid plot is shown in Fig. 2. The polyol groups form a two dimensional hydrogen bonded network that separates layers containing verdazyl rings. Unlike other examples, the verdazyl ring does not participate in hydrogen bonding. The geometry of the verdazyls themselves is very similar to that observed for other 1,5-diisopropyl verdazyls.

All of the radicals gave ESR spectra characteristic of 1,5-diisopropyloxoverdazyls.[28,34] Fig. 3 shows the spectrum and

of its intensity over a 24 h period). Electronic spectra were also unaffected by addition of hydrogen peroxide or ascorbic acid at neutral pH.

To gain an initial idea of the potential of these molecules as probes we examined their ability to quench the fluorescence of organic fluorophores. In methanol solution, only very weak quenching of the fluorescence of riboflavin was observed. Significantly greater quenching was observed when the solvent was changed to chloroform (Fig. 5). Quenching was also observed with pyrene in acetonitrile. Stern-Volmer plots are shown in Fig. 6.

3. Discussion

Most stable organic free radicals are essentially lipophilic species. Nitroxides, nitronyl nitroxides, verdazyls and others typically sport alkyl or aryl substituents that contribute to stability through