

Synthesis of Alkylseleno-Carbohydrates and Evaluation of their Antioxidant Properties

Jaqueline P. Vargas,^a Lucimar M. Pinto,^b Lucielli Savegnago^{*,b} and Diogo S. Lüdtko^{*,a}

^aInstituto de Química, Universidade Federal do Rio Grande do Sul (UFRGS),
Av. Bento Gonçalves 9500, 91501-970 Porto Alegre-RS, Brazil

^bGrupo de Pesquisa em Neurobiotecnologia (GPN), CDTec, Universidade Federal de Pelotas
(UFPel), 96160-000 Pelotas-RS, Brazil

In the present study, we describe the synthesis of alkylseleno carbohydrates with different sugar scaffolds and the evaluation of their antioxidant activity profile. A compound possessing a C8 alkyl chain presented the best results in the free radical scavenging activity.

Keywords: organoselenium, organochalcogens, antioxidant, selenocarbohydrates

Introduction

The chemistry and biology of simple small molecules containing selenium in their structures has received increased attention over the past decades. The first of such compounds to be developed was ebselen and after the discovery of its properties,^{1,2} a number of different small molecules containing selenium have been synthesized.³⁻⁸ The great deal of attention that has been paid to these compounds stems from the discovery of a selenium atom at the catalytic site of glutathione peroxidase (GPx).^{9,10} GPx is a key enzyme present in mammals with antioxidant activity and its mechanism of action involves the reduction of H₂O₂ at the expense of the oxidation of glutathione.^{11,12} A number of studies have been reported in the literature describing that small organic selenium compounds can effectively scavenge and eliminate reactive oxygen species (ROS) thus mimicking the activity of GPx enzyme.¹³⁻¹⁶ ROS are commonly generated in normal cellular oxygen metabolism playing important biological roles.¹⁷ However, an increase in the ROS production over cellular endogenous antioxidant system capacity of response could result in a condition characterized as oxidative stress (OS), which causes damage to cells leading to age related degenerative diseases, cancer and a wide range of different human diseases.^{18,19} A simple and effective way of preventing these conditions is by inhibition of the oxidative damage and, therefore, there is a great interest in the search for new organoselenides which could represent good pharmacological alternatives

to counteract oxidative stress. In this context, our groups have been interested in the synthesis and biological properties of selenium-containing carbohydrates and as a result, we have developed a straightforward approach for the introduction of an organoselenium moiety at non-anomeric positions of sugars.²⁰⁻²² Most of our efforts have been focused on the synthesis of arylselenium derivatives of carbohydrates and of diselenides, which were used as building blocks for the synthesis of more complex non-glycosidically linked selenium-linked pseudodisaccharides and neoglycopeptides.²³ In addition, previous studies of our group demonstrated that arylseleno-furanosides and a carbohydrate-derived diselenide did not inhibited δ -aminolevulinatase (δ -ALA-D), demonstrating low toxicity and antioxidant effects.²¹ More recently, we demonstrated that arylseleno-furanoside therapy was effective in restoring δ -ALA-D activity from ovary that was inhibited by cadmium²⁴ and arylseleno- and aryltelluroxylofuranosides attenuate manganese-induced toxicity in *Caenorhabditis elegans*.²⁵

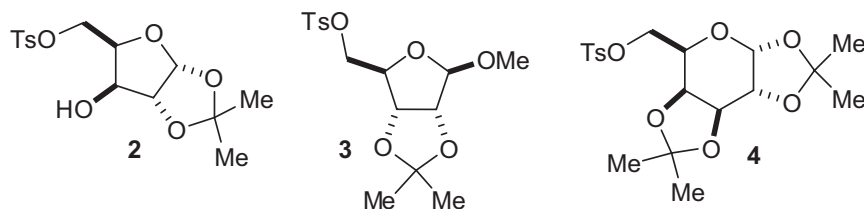
In view of the above, herein we report additional studies on this area, more specifically on the synthesis of selenocarbohydrates possessing an alkylselenium moiety and the evaluation of their *in vitro* antioxidant profile.

Results and Discussion

Chemistry

The synthesis of the selenocarbohydrates was straightforward, and we chose as sugar substrates two

*e-mail: luciellisavegnago@yahoo.com.br; dsludtke@iq.ufrgs.br

**Figure 1.** Sugar tosylates 2-4.

furanoside derivatives **2** and **3**, readily available from the parent carbohydrates D-xylose and D-ribose, respectively, and a pyranoside substrate, derived from D-galactose (Figure 1).

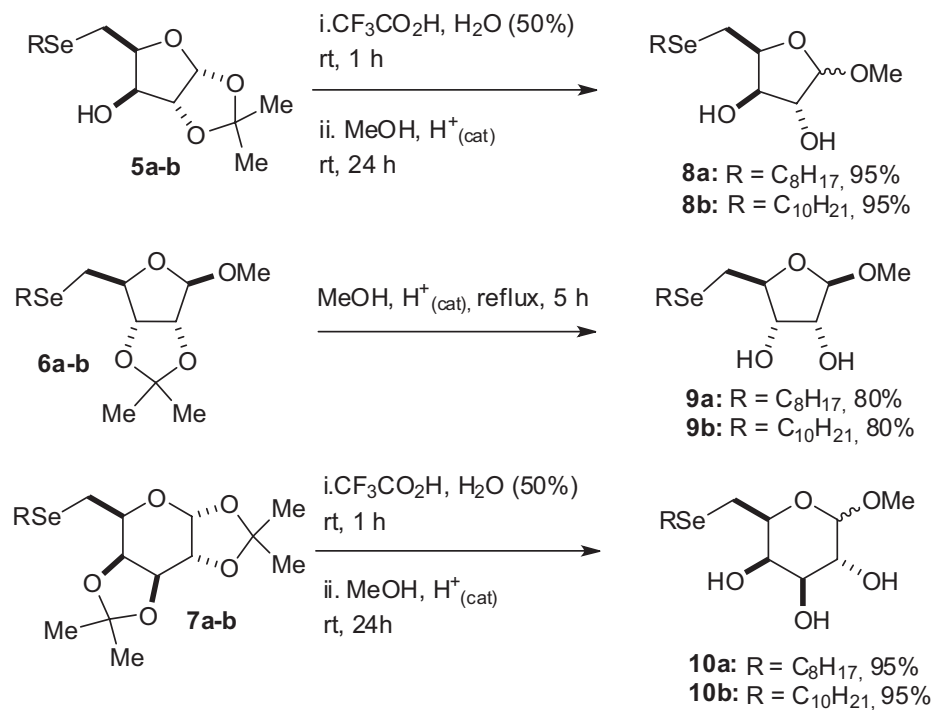
The tosylates **2-4** reacted with a selenium nucleophile, generated by reductive cleavage of the corresponding

dialkyldiselenide with NaBH₄, to yield seleno-carbohydrates **5-7** (Table 1). The reaction was performed using a 3:1 mixture of tetrahydrofuran (THF) and ethanol as solvents. The tosylate displacement reaction with furanoside derivatives **2** and **3** was easier than with the pyranoside derivative **4**, which took longer reaction times to afford

Table 1. Synthesis of alkyl-Se-carbohydrates

entry	Tosylate	Product	time / h	Yield / % ^a
1			24	65
2			24	53
3			24	66
4			24	60
5			72	51
6			72	50

^aIsolated yields.



Scheme 1. Synthesis of deprotected seleno-carbohydrates.

reasonable yields of the corresponding products. The reason for this behavior is the opposing influence of the fixed dipole caused by the presence of the axial C–O bond at the C-4, combined with the steric hindrance imposed by the acetonide protecting group that hinders the backside approach of the selenium nucleophile at C-6.^{26,27}

Deprotection of the acetonide, followed by acetal formation at the anomeric position with MeOH , under acidic conditions delivered the desired seleno-furanosides **8a-b**, **9a-b**, and **10a-b** (Scheme 1).

Antioxidant activity

Previous data have demonstrated that the biological activity of organoselenium compounds is significantly dependent of the substitution pattern of the molecule and subtle structural variations can lead to significant differences in the biological profile.²⁸⁻³⁰ With this in mind, we screened

selected selenocarbohydrates for their *in vitro* antioxidant activity. We first screened the new alkylseleno carbohydrate in the 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), ferric ion reducing antioxidant power (FRAP) assays. As can be seen in Table 2, xylo-pyranoside **8a** presented ABTS and DPPH radical scavenging at concentration of 50 and 500 μM , respectively. Compound **8a** possessed potent inhibitory activity against ABTS radical with IC_{50} value of 210 μM . In addition, compound **8a** exhibited ferric-reducing ability, and the reducing power was improved by increasing its concentration. Comparing the values of the DPPH, ABTS and FRAP assays, we can suggest that the mechanism of antioxidant action of compound **8a** is most likely based on single electron transfer.

On the other hand, compound **8b**, possessing the longer C-10 alkyl chain and displaying a ribo-furanoside backbone, exhibited ferric-reducing ability (Table 3) but

Table 2. Antioxidant activity of compound **8a** on ABTS, DPPH and FRAP assays^a

μM	Scavenging ABTS / %	μM	Scavenging DPPH / %	μM	FRAP (absorbance)
10	2.27 \pm 1.79	10	0.03 \pm 0.05	10	0.19 \pm 0.04
50	13.60 \pm 1.57 ^c	50	0.26 \pm 0.45	50	0.22 \pm 0.03
100	25.99 \pm 1.61 ^c	100	0.13 \pm 0.22	100	0.26 \pm 0.02
500	81.69 \pm 0.90 ^c	500	5.49 \pm 3.40 ^b	500	0.57 \pm 0.08 ^c
IC_{50}	210	–	–	–	–

^aDenote $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$ as compared to the respective control sample (one way ANOVA/Newman-Keuls). IC_{50} : concentration (μM) providing 50% inhibition in the assays. I_{max} : maximal inhibition (%).

didn't show significant results in both DPPH and ABTS assays (data not shown).

Table 3. Antioxidant activity of compound **8b** on FRAP assay^a

μM	FRAP (absorbance)
10	0.19 \pm 0.06
50	0.19 \pm 0.04
100	0.20 \pm 0.03
500	0.65 \pm 0.07 ^b

^aDenote $p < 0.05$; ^b $p < 0.01$ as compared to the respective control sample (one way ANOVA/Newman-Keuls).

The galactose-derived compounds **10a-b** were also evaluated. Galacto-pyranoside **10a** presented ABTS scavenging at concentration of 500 μM (Table 4) but didn't show significant result in both assays DPPH and FRAP (data not shown). On the other hand, results presented in Table 5 indicated that galacto-pyranoside derivative **10b** presented ABTS scavenging at concentrations 50-500 μM and exhibited ferric-reducing ability (at 500 μM). However, it didn't exhibit any protection against DPPH radical at any concentration tested (data not shown).

Table 4. Antioxidant activity of compound **10a** on ABTS assay^a

μM	Scavenging ABTS / %
10	3.47 \pm 0.47
50	4.24 \pm 3.93
100	7.64 \pm 4.90
500	31.77 \pm 19.26 ^b

^aDenote $p < 0.05$; ^b $p < 0.01$ as compared to the respective control sample (one way ANOVA/Newman-Keuls).

Table 5. Antioxidant activity of compound **10b** on ABTS and FRAP assays^a

μM	Scavenging ABTS / %	μM	FRAP (absorbance)
10	3.04 \pm 4.10	10	0.20 \pm 0.04
50	12.47 \pm 3.74	50	0.20 \pm 0.03
100	24.90 \pm 5.81 ^c	100	0.23 \pm 0.06
500	21.01 \pm 7.87 ^b	500	0.54 \pm 0.07 ^c

^aDenote $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$ as compared to the respective control sample (one way ANOVA/Newman-Keuls).

Compounds **5b** and **9b** were also evaluated against ABTS, DPPH and FRAP assays but didn't show any significant antioxidant effects. In addition to the ABTS, DPPH and FRAP studies, compounds **5b**, **8a**, **8b**, **9b**, **10a** and **10b** had their superoxide dismutase (SOD)-like, nitric oxide³¹ and hydroxyl radical³² scavenging activities evaluated (data not shown) and none of them displayed

significant activity, therefore ruling out these mechanisms as being responsible for their antioxidant profile.

Inspection of all the results gathered revealed that seleno-xylofuranoside **8a**, containing an alkyl group with eight carbon atoms, showed the highest antioxidant activity when compared with the closely related compound **8b** (with an alkyl group with ten carbon atoms) and seleno-galactopyranoside **10b**, which possesses a C-8 alkyl chain. Direct comparison between **8a** and the acetonide-protected compounds **5a-b** revealed that the presence of the acetonide moiety has a deleterious effect for the antioxidant activity, since **5** did not exhibit antioxidant activity, suggesting that the increased water-solubility of **8a** when compared to **5a** might play a role on its antioxidant profile.

Conclusions

Herein we have reported a straightforward synthesis of simple alkylseleno carbohydrates with different sugar scaffolds. Screening of selected compounds for antioxidant activity revealed that the presence of an alkyl group with eight carbon atoms has an important influence in the free radical scavenging activity of these compounds and therefore they can potentially prevent damage of protein and lipids.

Experimental

General procedure for the synthesis of **5-7**

Under an argon atmosphere, sodium borohydride (2.5 equiv) was added to a solution of the dialkyldiselenide (1.0 mmol) in THF (7.5 mL). Ethanol (2.5 mL) was then added dropwise and the clear solution formed was stirred at room temperature for 10 min. After this time, a solution of the appropriate tosylate (1.5 mmol in 1 mL THF) was added dropwise. After stirring under reflux for the time indicated in Table 1, the reaction mixture was quenched with aqueous saturated NH_4Cl (10 mL) and extracted with CH_2Cl_2 (3 \times 25 mL). The combined organic layers were dried with MgSO_4 , filtered and concentrated. The crude product was purified by flash chromatography, first eluting with hexanes and then with a mixture of hexanes/ethyl acetate.

Analytical data for compound **5a**: yield 65%; white solid; purified using hexane:EtOAc 80:20; $[\alpha]_D^{20} = -54$ (c 1.0, AcOEt); m.p. 62 °C; IR (film) $\nu_{\text{max}}/\text{cm}^{-1}$ 3398, 2922, 1372, 1217, 1089, 1014, 817; ¹H NMR (300 MHz, CDCl_3) δ 5.93 (d, 1H, J 3.7 Hz, CH), 4.53 (d, 1H, J 3.7 Hz, CH), 4.34 (ddd, 1H, J 9.0, 5.3, 2.5 Hz, CH), 4.27 (dd, 1H, J 5.3, 2.5 Hz, CH), 2.82 (dq, 2H, J 12.0, 7.3 Hz, CH_2), 2.69-2.60 (m, 2H, CH_2), 1.72-1.62 (m, 2H, CH_2), 1.50 (s, 3H, CH_3),

1.43-1.27 (m, 10H, 5 × CH₂), 1.31 (s, 3H, CH₃), 0.88 (t, 3H, *J* 6.7 Hz, CH₃); ¹³C NMR (75 MHz, CDCl₃) δ 111.5, 104.7, 85.0, 80.1, 75.0, 31.7, 30.5, 29.7, 29.0, 28.9, 26.6, 26.0, 24.9, 22.5, 19.9, 13.9; HRMS-ESI *m/z* calcd. for C₁₆H₃₀O₄Se + Na⁺: 389.1202; found: 389.1206.

General procedure for the synthesis of **8** and **10**

In a round bottomed flask, the appropriate selenocarbohydrate (0.5 mmol) was stirred in an aqueous solution of trifluoroacetic acid (50% v/v, 10 mL) for 1 h at room temperature. After this time, the reaction mixture was concentrated in vacuum, co-evaporated with toluene (3 × 10 mL) and the residue dissolved in MeOH (10 mL), in the presence of a catalytic amount of sulfuric acid, and stirred for additional 24 h, at room temperature. Following this time, the mixture was neutralized by the addition of solid sodium bicarbonate. The mixture was filtered and the solvents evaporated to afford the product.

Analytical data for compound **8a**: yield 95%; yellow oil; mixture of anomers (1.0:0.66); IR (film) ν_{\max} /cm⁻¹ 3421, 2925, 1456, 1208, 1112, 1024; ¹H NMR (300 MHz, CDCl₃) δ 4.99 (d, 0.46H, *J* 4.5 Hz, CH_β), 4.85 (s, 0.7H, CH_α), 4.51 (ddd, 0.7H, *J* 7.9, 6.9, 4.1 Hz, CH), 4.36 (ddd, 0.46H, *J* 7.4, 6.5, 5.1 Hz, CH), 4.24 (dd, 0.7H, *J* 4.5, 3.7 Hz, CH), 4.20 (s, 0.7H, CH), 4.13 (dd, 0.46H, *J* 4.1, 3.5 Hz, CH), 4.07 (d, 0.46H, *J* 4.6 Hz, CH), 3.49 (s, 1.38H, CH_{3α}), 3.38 (s, 2.1H, CH_{3β}), 2.82 (m, 4H, 2 × CH₂), 2.64 (m, 4H, 2 × CH₂), 1.67 (m, 4H, 2 × CH₂), 1.30 (m, 20H, 2 × 5 × CH₂), 0.88 (t, 6H, *J* 6.8 Hz, 2 × CH₃); ¹³C NMR (75 MHz, CDCl₃) δ 108.5, 101.7, 83.5, 79.6, 78.6, 78.5, 77.0, 76.2, 55.8, 55.1, 31.7, 30.6, 30.5, 29.87, 29.82, 29.12, 29.10, 29.06, 29.03, 24.91, 24.87, 22.65, 22.56, 21.9, 14.0.

Acknowledgements

This work was supported by CNPq, CAPES and FAPERGS. L. S. and D. S. L. are recipients of CNPq research fellowships. J. P. V. acknowledges CAPES for a PhD fellowship.

Supplementary Information

Supplementary information (full experimental procedures, analytical data and copies of NMR spectra) is available free of charge at <http://jbcs.sq.org.br> as PDF file.

References

- Müller, A.; Cadenas, E.; Graf, P.; Sies, H.; *Biochem. Pharmacol.* **1984**, *33*, 3235.

- Wendel, A.; Fausel, M.; Safayhi, H.; Tiegs, G.; Otter, R.; *Biochem. Pharmacol.* **1984**, *33*, 3241.
- Nogueira, C. W.; Rocha, J. B. T.; *Organoselenium and Organotellurium Compounds: Toxicology and Pharmacology. In Patai's Chemistry of Functional Groups*; John Wiley & Sons, 2011.
- Mugesh, G.; du Mont, W. W.; Sies, H.; *Chem. Rev.* **2001**, *101*, 2125.
- Nogueira, C. W.; Zeni, G.; Rocha, J. B. T.; *Chem. Rev.* **2004**, *104*, 6255.
- Alberto, E. E.; Nascimento, V.; Braga, A. L.; *J. Braz. Chem. Soc.* **2010**, *21*, 2032.
- Nogueira, C. W.; Rocha, J. B. T.; *J. Braz. Chem. Soc.* **2010**, *21*, 2055.
- Rocha, J. B. T.; Saraiva, R. A.; Garcia, S. C.; Gravina, F. S.; Nogueira, C. W.; *Toxicol. Res.* **2012**, *1*, 85.
- Rotruck, J. T.; Pope, A. L.; Ganther, H. E.; Swanson, A. B.; Hafeman, D. G.; Hoekstra, W. G.; *Science* **1973**, *179*, 588.
- Flohé, L.; Günzler, W. A.; Schock, H. H.; *FEBS Lett.* **1973**, *32*, 132.
- Alwi, S. S.; Cavell, B. E.; Donlevy, A.; Packham, G.; *Cell Stress Chaperones* **2012**, *17*, 529.
- Rayman, M. P.; *Lancet* **2000**, *356*, 233.
- Meotti, F. C.; Stangherlin, E. C.; Zeni, G.; Nogueira, C. W.; Rocha, J. B. T.; *Environ. Res.* **2004**, *94*, 276.
- Nogueira, C. W.; Rocha, J. B. T.; *Arch. Toxicol.* **2011**, *85*, 1313.
- Gerzson, M. F.; Victoria, F. N.; Radatz, C. S.; de Gomes, M. G.; Boeira, S. P.; Jacob, R. G.; Alves, D.; Jesse, C. R.; Savegnago, L.; *Pharmacol., Biochem. Behav.* **2012**, *102*, 21.
- Victoria, F. N.; Martinez, D. M.; Castro, M.; Casaril, A. M.; Alves, D.; Lenardão, E. J.; Salles, H. D.; Schneider, P. H.; Savegnago, L.; *Chem.-Biol. Interact.* **2013**, *205*, 100.
- Halliwell, B.; Gutteridge, J. M. C.; *Free Radicals in Biology and Medicine*, 4th ed.; Oxford University Press, Oxford, 2007.
- Rahal, A.; Kumar, A.; Singh, V.; Yadav, B.; Tiwari, R.; Chakraborty, S.; Dhama, K.; *BioMed Res. Int.* **2014**, *in press*, DOI: 10.1155/2014/761264.
- Brawek, B.; Loffer, M.; Wagner, K.; Huppertz, H. J.; Wendling, A. S.; Weyebrock, A.; Jackish, R.; Feuerstein, T. J.; *Brain Res. Bull.* **2010**, *81*, 484.
- Braga, A. L.; Severo-Filho, W. A.; Schwab, R. S.; Rodrigues, O. E. D.; Dornelles, L.; Braga, H. C.; Lüdtkke, D. S.; *Tetrahedron Lett.* **2009**, *50*, 3005.
- Braga, H. C.; Stefani, H. A.; Paixão, M. W.; Santos, F. W.; Lüdtkke, D. S.; *Tetrahedron* **2010**, *66*, 3441.
- Braga, H. C.; Wouters, A. D.; Zerillo, F. B.; Lüdtkke, D. S.; *Carbohydr. Res.* **2010**, *345*, 2328.
- Affeldt, R. F.; Braga, H. C.; Baldassari, L. L.; Lüdtkke, D. S.; *Tetrahedron* **2012**, *68*, 10470.
- Vargas, L. M.; Soares, M. B.; Izaguirry, A. P.; Lüdtkke, D. S.; Braga, H. C.; Savegnago, L.; Wollenhaupt, S.; Brum, D. S.;

- Leivas, F. G.; Santos, F. W.; *J. Appl. Toxicol.* **2013**, *33*, 679.
25. Wollenhaupt, S.; Soares, A. T.; Salgueiro, W. G.; Noremborg, S.; Bohrer, D.; Gubert, P.; Soares, F. A.; Affeldt, R. F.; Lüdtke, D. S.; Santos, F. W.; Denardin, C. C.; Aschner, M.; Avila, D. S.; *Food Chem. Toxicol.* **2014**, *64*, 192.
26. Richardson, A. C.; *Carbohydr. Res.* **1969**, *10*, 395.
27. Hough, L.; Richardson, A. C. In *Biological Compounds*; Barton, D.; Ollis, W. D.; Haslam, E., eds.; Pergamon Press: Oxford, 1979, vol. 5, ch. 26.1.
28. Prigol, M.; Wilhelm, E. A.; Schneider, C. C.; Nogueira, C. W.; *Chem.-Biol. Interact.* **2008**, *176*, 129.
29. Sabir, S. M.; Salman, S. M.; Rocha, J. B. T.; *Environ. Toxicol. Pharmacol.* **2012**, *34*, 446.
30. Acker, C. I.; Luchese, C.; Prigol, M.; Nogueira, C. W.; *Neurosci. Lett.* **2009**, *455*, 168.
31. Feelisch, M.; Schmidt, H. H.; Hofmann, H.; Schindler, U.; Shutenko, Z. S.; Cunningham, D. D.; *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 14492.
32. Yoshikawa, T.; Naito, Y.; Masui, K.; Fujii, T.; Boku, Y.; Nakagawa, S.; Yoshida, N.; Kondo, M.; *Biomed. Pharmacother.* **1997**, *51*, 328.

Submitted: October 23, 2014

Published online: February 6, 2015

FAPERGS has sponsored the publication of this article.