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Direct biocatalysed synthesis of first sulfur-, selenium- and tellurium- containing L-ascorbyl hybrid derivatives with radical trapping and GPx-like properties†

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6-O-L-Ascorbyl selenoesters, thioesters and telluroesters can be efficiently and directly prepared from L-ascorbic acid and suitable functionalised chalcogenoesters through lipase-catalysed transesterification reactions. Novel synthesised L-ascorbyl derivatives exhibited remarkable chain breaking and glutathione peroxidase-like activities.

L-Ascorbic acid (vitamin C) is a powerful water soluble antioxidant essential for the appropriate functioning of the body, and is involved in a number of biological processes ranging, amongst others, from the biosynthesis of collagen¹ and catecholamine to the modulation of neurotransmission.²

The enhanced concentration of harmful reactive oxygen species (ROS) has long been related to the onset of several human diseases such as cancer, immune disorders, cystic fibrosis, and neurodegenerative diseases.³ Liposoluble vitamin E and hydrosoluble vitamin C, together with phenolic compounds, carotenoids, and trace elements such as zinc and selenium, are the main exogenous defences against oxidative stress. In this context, the design and development of novel antioxidants have been attracting growing interest over the past few decades.⁴ Particularly, owing to their capability to mimic the glutathione peroxidase activity, the synthesis of chalcogen-containing antioxidants has recently attracted growing attention. Furthermore, organochalcogenides can possess anti-cancer, antibacterial, and enzyme inhibition activities.⁵ The functionalization of bioactive natural products with chalcogens represents an effective strategy to modulate or improve their biological properties. For example, the introduction of chalcogen-containing moieties into natural compounds such as tocopherols,^{6,7} tocotrienols,⁷ retinol,⁸ hydroxytyrosol,⁹ chrysin,¹⁰ quercetin,^{10a} and resveratrol^{10c} has been extensively studied. However, whilst sulfur-, selenium-, or tellurium-functionalised vitamin E and vitamin A derivatives have been reported, to the best of our knowledge, the synthesis of chalcogen-containing vitamin C derivatives has never

been described. The paucity of such results is reasonably due to the instability of the L-ascorbic acid core, which easily undergoes oxidation of the enediol moiety and ring opening of the lactone. Furthermore, several alcohol protecting group strategies cannot be applied since the conditions of the endgame protecting group cleavage are often not compatible with chalcogens or with the L-ascorbic acid core. For example, because the presence of chalcogens poisons the Pd-catalyst,¹¹ the most commonly employed benzyl protecting group cannot be cleaved under mild Pd/C catalysed hydrogenation conditions. Additionally, harsh bases- or acid-mediated deprotection procedures cause severe decomposition of products.

We sought to approach this problem from a different perspective and evaluated the possibility of applying lipase biocatalysed transesterifications¹² of chalcogen-containing esters and L-ascorbic acid. This approach would ideally allow a straightforward access to chalcogen-containing L-ascorbic acid derivatives, without requiring tedious and detrimental protection/deprotection steps. Lipase B catalysed transesterification is indeed a versatile tool to synthesise L-ascorbyl esters under green and mild conditions.¹³ However, to the best of our knowledge, its application to chalcogen-containing acyl donors has never been described and its feasibility and functional group tolerance were not obvious.

We commenced our studies by establishing the optimal conditions required to promote the biocatalysed transesterification reaction of β -selenoester **2a**, prepared through seleno-Michael addition from benzeneselenol **1a** and methyl acrylate (see ESI†), with L-ascorbic acid **3**. According to a literature survey, the optimal temperature for the enzyme activity was established to be 45 °C. Polar solvents, such as acetone and tertiary alcohols, are the media of choice for lipase-catalysed L-ascorbic acid esterifications.^{12,13} In our work, acetone proved to be the most effective, plausibly owing to its high capability to dissolve both **2a** and **3**. In addition non protic solvents such as acetone do not promote the dissociation of L-ascorbic acid to the more oxidisable L-ascorbate. Evaluation of different solvents commonly used for lipase B catalysed reactions, such as *tert*-butanol and 2-methyl-1-butanol, gave lower yields. Thus, we investigated the effect of the reaction stoichiometry and the

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Table 1 Optimization of the biocatalysed synthesis of selenium-containing 6-O-ascorbyl ester **4a**

| Entry | 2a (eq.) | Lipase B (U mmol ⁻¹) | Time (h) | Yield ^a (%) | Entry | 2a (eq.) | Lipase B (U mmol ⁻¹) | Time (h) | Yield ^a (%) |
|-------|----------|----------------------------------|----------|------------------------|-------|----------|----------------------------------|----------|------------------------|
| 1 | 1.0 | 500 | 48 | 12 | 6 | 0.3 | 1000 | 24 | <10 |
| 2 | 0.5 | 500 | 48 | <10 | 7 | 1.0 | 1000 | 48 | 26 |
| 3 | 0.3 | 500 | 48 | <10 | 8 | 3.0 | 1000 | 48 | 45 |
| 4 | 0.3 | 1000 | 48 | 22 | 9 | 3.0 | 1000 | 48 | 74 ^c |
| 5 | 0.3 | 1000 | 72 | 14 ^b | 10 | 0.3 | 1000 | 48 | 32 ^c |

^a Isolated yield is reported. ^b Partial decomposition of **4a** was observed. ^c 4 Å molecular sieves (300 mg) were added. See ESI† for details. The use of molecular sieves with a lower enzyme loading (500 U mmol⁻¹) resulted in a slight improvement in yield.

amount of lipase. We found that, whilst poor yields were obtained upon using 500 U mmol⁻¹ of enzyme (Table 1, entries 1–3), doubling of the enzyme amount brought about a significant improvement in the yield (Table 1, entry 4).

The optimal reaction time was found to be 48 h, as partial decomposition of the reaction product was observed after 72 h (Table 1, entry 5). On the other hand, shorter times resulted in much lower yields (Table 1, entry 6). Pleasingly, we found that by using an excess amount of β-selenoester **2a** the desired 6-O-L-ascorbyl ester **4a** was formed in a rather good yield for this type of biocatalysed transformation (45%, Table 1, entry 4).

Remarkably, a significant improvement in yield was achieved upon performing the reaction in the presence of 4 Å molecular sieves (Table 1, entries 9 and 10). Particularly, by using an excess amount of **2a** under these conditions, the ascorbyl derivative **4a** was formed in 74% yield, which represents a very good result for lipase-catalysed transesterification reactions. The striking effect of zeolites can be reasonably ascribed to the trapping of both MeOH and H₂O. Indeed, the removal of methanol produced by the transesterification displaces the equilibrium of the reaction toward the formation of **4a**. Furthermore, by trapping water, molecular sieves hamper the competitive lipase-catalysed hydrolysis of **4a**.

Having identified the optimal reaction conditions, we proceeded to investigate the scope of the transformation with respect to different chalcogen-containing esters. Thus, a large variety of differently substituted selenium-, sulfur-, and tellurium-containing esters (Scheme 1 and Scheme S1, ESI†) were synthesised as shown in Scheme 1. β-Arylseleno- and β-alkylseleno-esters **2a–g** were smoothly prepared through a novel seleno-Michael addition¹⁴ involving suitable aryl- or alkyl-selenols¹⁵ **1** and methyl acrylate. The reaction occurred under very mild conditions in the presence of Al₂O₃ (Scheme 1 and Scheme S1 (ESI†), *via* A). Furthermore, α-selenoesters **2h** and **2i** and γ-selenoester **2j** were easily obtained by exploiting the reactivity of selenols **1** with methyl bromoacetate

and ethyl 4-bromobutyrate, respectively (Scheme 1 and Scheme S1 (ESI†), *via* B). Similarly, variously substituted and functionalised α-, β-, and γ-thioesters **6a–i** were prepared from the corresponding aryl or alkyl thiols^{16a} and suitable electrophiles (Scheme S1 (ESI†), *via* A and *via* B). Disulfide **6j**, bearing two ester functions and four sulfur atoms, was synthesised from 1,9-nonanethiol through a two-step procedure involving thiol-Michael addition and a DCF (dicyano-fumarate) mediated oxidation sequence (see ESI† for details).^{16b} β-Aryltelluroesters **8a,b** were prepared from the corresponding ditellurides and methyl 3-bromopropionate (Scheme 1 and Scheme S1, ESI†).

With a range of differently substituted and functionalised chalcogen-containing esters in hand, we then explored the scope of the lipase-catalysed transesterification reaction with L-ascorbic acid (Scheme 2). Under the optimised reaction conditions, *o*-, *m*-, and *p*-methoxy phenylselenoesters **2b–d** gave selectively the corresponding 6-O-ascorbyl arylselenoalkanoates **4b–d** in good yields. β-Selenoester **2e**, bearing a *p*-F-C₆H₄ ring, also reacted efficiently with **3** to afford compound **4e**. The reaction was also successfully applied to the synthesis of L-ascorbyl derivatives **4f** and **4g** bearing different alkylseleno moieties, including the functionalised glycidol derivative (**4g**). Furthermore, methyl and ethyl α- and γ-selenoesters **2h–j** were efficiently transesterified with **3** yielding the corresponding 6-O-L-ascorbyl esters **4h–j** in rather good yields, therefore demonstrating the versatility of the biocatalysed approach towards the synthesis of variously functionalised homologous L-ascorbyl selenoesters. Next, we turned our attention to evaluating the generality of such a reaction with respect to sulfur-containing esters. β-Arylthioesters **6a–d** were smoothly converted into the corresponding sulfurated 6-O-ascorbyl derivatives bearing a phenyl ring (**9a**) or *o*- (**9b**), *m*- (**9c**), and *p*- (**9d**) bromo-substituted benzenes bonded to the S atom. This methodology could also be applied to more interesting highly functionalised alkyl sulfides. The hydroxy-substituted *S*-alkyl β-thioester **6e** and the enantio-enriched epichlorohydrin derivatives (*S*)-**6f** and (*R*)-**6f** were successfully transferred onto the L-ascorbic acid core, affording the corresponding functionalised ester **9e** and the enantio-enriched derivatives **9f** and **9g**, containing three controlled stereogenic centers and a further functionalisable chlorinated chain. Additionally, a chiral enantio-enriched *N*-tosyl amino-substituted β-thioester **6g**, synthesised from L-valine,^{14,15a} could be efficiently employed to access the enantio-enriched *S,N*-containing 6-O-L-ascorbyl ester **9h**.



Scheme 1 Synthesis of selenoesters **2**, thioesters **6**, and telluroesters **8**. See ESI† for reagents and conditions, and experimental details.



Scheme 2 Biocatalysed direct synthesis of selenium-, sulfur-, and tellurium-containing 6-*O*-ascorbyl esters **4**, **9**, and **10** from L-ascorbic acid. Isolated yields are reported.

Furthermore, α - and γ -thioesters **9i** and **9j** were also conveniently obtained by treating **3** with **6h** and **6i**, respectively, under the above described conditions. Remarkably, the disulfide **6j** was successfully used in a double biocatalysed transesterification to afford the potentially valuable bola type bis-ascorbyl ester **9k**, bearing two L-ascorbyl moieties and four sulfur atoms. Indeed, owing to the unique physicochemical properties of bola type structures, novel bifunctional chalcogen-containing amphiphilic ascorbic acid derivatives could find wide applications both in polymers and materials science and in medicinal chemistry for the drug delivery of lipophilic molecules.^{12a,17}

Finally, in order to extend the scope of this methodology to organotellurium derivatives, we evaluated the reactivity of β -aryltelluroesters **8a** and **8b** with **3**. 6-*O*-L-Ascorbyl aryltelluroalkanoates **10a** and **10b** were directly and selectively formed in good yield, thus highlighting the remarkably broad scope of the procedure and the possibility to use the lipase-catalysed approach with all chalcogens.

Owing to the presence of both the vitamin C free enediol moiety and the selenium or tellurium atom, ascorbyl derivatives **4** and **10** can exhibit both chain breaking and catalytic antioxidant activities, therefore representing excellent antioxidant candidates. Therefore, having developed a convenient procedure to access novel chalcogen-containing L-ascorbic acid derivatives, we wished to investigate their antioxidant properties. Pleasingly, according to the DPPH assay, all the synthesised 6-*O*-ascorbyl esters exhibited remarkable chain breaking activity,¹⁸ leading to a rapid free radical

quenching (complete decolouration of ethanolic DPPH[•] solution occurred within 3 seconds) and, thus, showing radical scavenger properties comparable to those of L-ascorbic acid, which represents the most powerful hydrophilic antioxidant. The stoichiometry of the reaction (n , number of radical trapped within 10 minutes) was found to be $n = 2$ for all the tested L-ascorbyl esters **4**, **9**, and **10**, thus indicating that under the experimental conditions the oxidation products of these compounds are not capable of reacting with DPPH. Furthermore, the catalytic thiol peroxidase like activity of selected L-ascorbyl derivatives was also pursued according to methods reported in the literature using dithiothreitol (DTT)¹⁹ or glutathione (GSH)¹⁹ as substrates (see Scheme S2, and Fig. S2 and S3, ESI[†]). We were delighted to discover that all tested selenium- and tellurium-containing vitamin C derivatives behave as catalysts in promoting the reduction of hydrogen peroxide in the presence of a thiol cofactor (Table 2). Intriguingly, when the DTT oxidation assay is accomplished using 1.0 eq. of hydrogen peroxide *ca.* 10–20% of DTT remained unreacted. Control experiments suggested that reaction of the L-ascorbate moiety with H₂O₂ proceeds slowly in the absence of catalysts. The observed results might be reasonably ascribed to the formation of tricarbonyl derivatives, proceeding through a redox reaction involving the enediol moiety and a selenoxide or telluroxide intermediate.²⁰ On the other hand, complete DTT oxidation was achieved upon using an excess amount (1.2 eq.) of H₂O₂. As can be noticed (Table 2 and Fig. 1), under these conditions all the tested compounds exhibited catalytic thiol-peroxidase like properties, being able to promote the oxidation of both DTT and GSH. Intriguingly, the β -alkylseleno-ester **4g** showed higher catalytic activity with respect to the arylseleno-substituted analogues **4a–c** and **4e**. Furthermore, according to both assays, L-ascorbyl β -aryltelluroalkanoates **10a** and **10b** behaved as more effective catalysts with respect to similar selenium-containing derivatives **4a–e**.

Furthermore, particularly interesting are the results obtained through the GSH/GR/NADPH coupled test, which better reproduces the cellular environment (Fig. 1). Indeed, under these conditions the novel synthesised L-ascorbyl derivatives exhibited GPx-like activity, thus demonstrating the effective enhanced antioxidant properties of these novel amphiphilic systems, thus offering new opportunities

Table 2 Thiol-peroxidase like activity of selenium- and tellurium-containing 6-*O*-L-ascorbyl esters **4** and **10** according to DTT and GSH/GR methods

| Entry | Compound | DTT (T_{50}) ^{ab} | GSH/GR (T_{50}) ^{ac} |
|-------|------------|--------------------------------|-----------------------------------|
| 1 | 4a | 3405 (\pm 268) | 43 (\pm 5) |
| 2 | 4b | 4270 (\pm 325) | 48 (\pm 7) |
| 3 | 4c | 4046 (\pm 314) | 45 (\pm 5) |
| 4 | 4e | 3862 (\pm 362) | 53 (\pm 4) |
| 5 | 4g | 1346 (\pm 151) | 37 (\pm 3) |
| 6 | 4h | 2846 (\pm 116) | 42 (\pm 6) |
| 7 | 10a | 654 (\pm 104) | 18 (\pm 3) |
| 8 | 10b | 386 (\pm 93) | 14 (\pm 3) |

^a T_{50} is the time required, in seconds, to halve the initial thiol concentration after the addition of H₂O₂; data in parentheses are the experimental error. ^b DTT oxidation was monitored by ¹H NMR spectroscopy; 10 mol% of **4** and 1 mol% of **10** were used. ^c NADPH consumption was monitored by UV spectroscopy (340 nm).



Fig. 1 NADPH-coupled GPx assay. Reaction conditions: $[\text{NADPH}]_0 = 0.3 \text{ mM}$, $[\text{GSH}]_0 = 1.0 \text{ mM}$, $[\text{H}_2\text{O}_2]_0 = 2.5 \text{ mM}$, $[\text{GR}] = 4 \text{ units per mL}$, $[\text{catalyst}] = 0.1 \text{ mM}$ in pH 7.4 phosphate buffer at ambient temperature. The mean \pm SD values of three separate experiments are reported.

for their potentially wide application in chemistry, biology, and materials science.

Preliminary physicochemical characterisation of these unreported amphiphilic antioxidants showed that most of them behave as semicrystalline solids. The evolution of the crystal and of the amorphous fraction depends on the thermal history of the sample, a common behaviour of polymers. As an example, the DSC curves obtained for **9c** are shown in Fig. S1 (see ESI†). The crystalline fraction in **9c** melted at about $75 \text{ }^\circ\text{C}$ producing an endothermic peak with $\Delta H = 21.6 \text{ J g}^{-1}$. When the melt was slowly cooled to $-20 \text{ }^\circ\text{C}$ (at $5 \text{ }^\circ\text{C min}^{-1}$), crystallisation occurred producing an exothermic peak at about $35.5 \text{ }^\circ\text{C}$ with $\Delta H = 28.2 \text{ J g}^{-1}$. The crystalline fraction was found to melt between $40 \text{ }^\circ\text{C}$ and $100 \text{ }^\circ\text{C}$ upon heating. Instead, when the sample was cooled quickly down to $-20 \text{ }^\circ\text{C}$ (at $50 \text{ }^\circ\text{C min}^{-1}$), no crystallisation was seen, and a cold crystallisation exothermic peak was detected in the following heating cycle followed by a broad endothermic melting peak. Remarkably, while this behaviour is typical for polymers, it is rather unusual for such small molecules and may represent an opportunity for their application in materials science.

In summary, we have found a convenient, green, mild, and direct route to synthesise novel chalcogen-containing L-ascorbyl derivatives, which would not have been accessible through classic methodologies. Owing to their enhanced chain breaking and catalytic antioxidant properties, these conjugate molecules represent potential valuable systems in biology, medicinal chemistry, and materials science.

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Conflicts of interest

There are no conflicts to declare.

Notes and references

- 1 R. S. Pinnell, *Yale J. Biol. Med.*, 1985, **58**, 553.
- 2 R. Figueroa-Méndez and S. Rivas-Arancibia, *Front. Physiol.*, 2015, **6**, 397.

- 3 (a) J. K. Andersen, *Nat. Rev. Neurosci.*, 2004, **5**, S18; (b) R. C. S. Seet, C.-Y. J. Lee, E. C. H. Lima, J. J. H. Tan, A. M. L. Quek, W.-L. Chong, W.-F. Looi, S.-H. Huang, H. Wang, Y.-H. Chand and B. Halliwell, *Free Radical Biol. Med.*, 2010, **48**, 560.
- 4 (a) S. Yoshida, F. Kumakura, I. Komatsu, K. Arai, Y. Onuma, H. Hojo, B. G. Singh, K. I. Priyadarsini and M. Iwaoka, *Angew. Chem., Int. Ed.*, 2011, **50**, 2125; (b) C. W. Nogueira, G. Zeni and J. B. T. Rocha, *Chem. Rev.*, 2004, **104**, 6255.
- 5 (a) A. R. Patra, S. S. Roy, A. Basu, A. Bhuniya, A. Bhattacharjee, S. Hajra, U. H. Sk, R. Baral and S. Bhattacharya, *Sci. Rep.*, 2018, **8**, 2194; (b) A. Angeli, D. Tanini, A. Capperucci and C. T. Supuran, *Bioorg. Chem.*, 2018, **76**, 268; (c) A. Angeli, D. Tanini, A. Capperucci, G. Malevolti, F. Turco, M. Ferraroni and C. T. Supuran, *Bioorg. Chem.*, 2018, **81**, 642; (d) A. Angeli, D. Tanini, A. Capperucci and C. T. Supuran, *ACS Med. Chem. Lett.*, 2017, **8**, 1213.
- 6 V. P. Singh, J. F. Poon and L. Engman, *Org. Lett.*, 2013, **15**, 6274.
- 7 P. S. Vraha, C. Drouza, M. P. Rikkou, A. D. Odysseos and A. D. Keramidias, *Bioorg. Med. Chem.*, 2006, **14**, 2684.
- 8 S. C. Welch and M. Gruber, *J. Med. Chem.*, 1979, **22**, 1532.
- 9 G. Rodríguez-Gutiérrez, F. Rubio-Senent, A. Gómez-Carretero, I. Maya, J. Fernández-Bolaños, G. G. Duthie and B. de Roos, *Eur. J. Nutr.*, 2018, DOI: 10.1007/s00394-018-1733-y.
- 10 (a) S. F. Fonseca, D. B. Lima, D. Alves, R. G. Jacob, G. Perin, E. J. Lenardão and L. Savegnago, *New J. Chem.*, 2015, **39**, 3043; (b) I. L. Martins, C. Charneira, V. Gandin, J. L. Ferreira da Silva, G. C. Justino, J. P. Telo, A. J. S. C. Vieira, C. Marzano and A. M. M. Antunes, *J. Med. Chem.*, 2015, **58**, 4250; (c) D. Tanini, L. Panzella, R. Amorati, A. Capperucci, E. Pizzo, A. Napolitano, S. Menichetti and M. d’Ischia, *Org. Biomol. Chem.*, 2015, **13**, 5757.
- 11 P. Albers, J. Pietsch and S. F. Parker, *J. Mol. Catal. A: Chem.*, 2001, **173**, 275.
- 12 (a) C. Dolle, P. Magrone, S. Riva, M. Ambrosi, E. Fratini, N. Peruzzi and P. Lo Nostro, *J. Phys. Chem. B*, 2011, **115**, 11638; (b) G. Carrea and S. Riva, *Angew. Chem., Int. Ed.*, 2000, **39**, 222.
- 13 (a) K. Nott, A. Brognaux, G. Richard, P. Laurent, A. Favrelle, C. Jérôme, C. Blecker, J. P. Wathet, M. Paquet and M. Deleu, *Prep. Biochem. Biotechnol.*, 2012, **42**, 348; (b) S. Park, F. Viklund, K. Hulta and R. J. Kazlauskas, *Green Chem.*, 2003, **5**, 715.
- 14 D. Tanini, S. Scarpelli, E. Ermini and A. Capperucci, *Adv. Synth. Catal.*, 2019, DOI: 10.1002/adsc.201900168.
- 15 (a) D. Tanini, C. Tiberi, C. Gellini, P. R. Salvi and A. Capperucci, *Adv. Synth. Catal.*, 2018, **360**, 3367; (b) A. Angeli, D. Tanini, A. Nocentini, A. Capperucci, M. Ferraroni, P. Gratteri and C. T. Supuran, *Chem. Commun.*, 2019, **55**, 648.
- 16 (a) D. Tanini, C. Borgogni and A. Capperucci, *New J. Chem.*, 2019, **43**, 6388; (b) G. Mlostoń, A. Capperucci, D. Tanini, R. Hamera-Fałdga and H. Heimgartner, *Eur. J. Org. Chem.*, 2017, 6831.
- 17 S. Palma, R. H. Manzo, P. Lo Nostro and D. Allemandi, *Int. J. Pharm.*, 2007, **345**, 26.
- 18 (a) E. Tempestini, M. Bucci, V. Mastromartino, M. Gori, D. Tanini, M. Ambrosi, E. Fratini, A. Capperucci and P. Lo Nostro, *Chem-PhysChem*, 2017, **18**, 1400; (b) P. Lo Nostro, G. Capuzzi, A. Romani and N. Mulinacci, *Langmuir*, 2000, **16**, 1744.
- 19 (a) D. Tanini, A. Grechi, L. Ricci, S. Dei, E. Teodori and A. Capperucci, *New J. Chem.*, 2018, **42**, 6077; (b) F. Kumakura, B. Mishra, K. I. Priyadarsini and M. Iwaoka, *Eur. J. Org. Chem.*, 2010, 440.
- 20 A reasonable catalytic cycle is the following. Ring opening of the oxidised lactone ring cannot be ruled out

