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Synthesis and Evaluation of Oligomeric Thioether-Linked Carbacyclic β-(1→3)-Glucan Mimetics

Peng Wen,[a] Václav Větvička,[b] and David Crich[a,*]

a) Department of Chemistry, Wayne State University, 5101 Cass Avenue, Detroit, MI 48202, USA

b) Department of Pathology, University of Louisville, 323 East Chestnut Street, Louisville, KY 40202, USA

Dcrich@chem.wayne.edu

Abstract

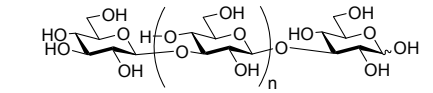
Extrapolating from lessons learnt with previous low molecular weight β-(1→3)-mimetics we designed a series of minimal 2,4-dideoxy-thioether-linked carbacyclic β-(1→3)-mimetics and synthesized the di-, tri- and tetramers in enantiomerically pure form by an iterative sequence based on a simple building block readily available from commercial (*S*)-(-)-3-cyclohexenecarboxylic acid. These substances were screened for their ability to inhibit anti-CR3-FITC staining of human neutrophils and anti-Dectin-1-FITC staining of mouse macrophages, as well as for their ability to stimulate phagocytosis and pinocytosis. In each assay the synthetic compounds displayed comparable activity to the corresponding native β-(1→3)-glucans, laminaritriose and tetraose suggesting that the exploitation of hydrophobic patches in the lectin-binding domains of CR3 and Dectin-1 is a promising strategy for the development of small molecule analogues of the β-(1→3)-glucans.

Introduction

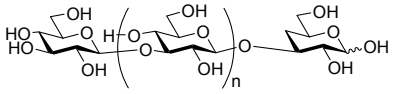
The β-(1→3)-glucans **1** (Fig 1) are a group of widely occurring immunostimulatory oligosaccharides with the potential for use as therapeutic agents and/or vaccines in a number of disease areas including cancer and inflammatory bowel disease.[1-8] Systematic investigation of these many beneficial properties is however hindered by the wide structural heterogeneity of β-(1→3)-glucans obtained from natural sources, which stems from variations in purity, of the degree of polymerization, and of β-(1→6)-branching. Nevertheless, it has been established that, following ingestion, the β-(1→3)-glucans are taken up in the small intestine by macrophages through interaction with the lectin domain of Dectin-1.[9] Degradation of the large β-(1→3)-glucans by the macrophages then provides smaller fragments which are taken up by circulating granulocytes, monocytes and macrophages, via binding to the lectin domain of complement receptor 3 (CR3), where they can induce the relevant immune responses.[10,11] The relative inaccessibility of structurally homogeneous β-(1→3)-glucans from nature has been palliated in recent years by the many advances in carbohydrate chemistry[12-19] culminating in numerous impressive syntheses of both linear and branched oligomeric glucans permitting further biological evaluation,[20-27] and conformational analysis of the glucan chains.[24,28] Work with pure oligomers obtained by degradation and extensive HPLC purification of natural isolates led to the conclusion that the shortest β-(1→3)-glucans capable of binding to Dectin-1 in a microarray format were the deca- and undecamers.[29] Subsequent work with synthetic material and an SPR-based assay demonstrated that even the heptasaccharide is capable of binding to recombinant murine Dectin-1.[22] The length of the natural β-(1→3)-glucans is such that multiple Dectin-1 units can bind to a single polymeric glucan chain through a multivalent interaction with affinity increasing in an additive manner.[22] STD NMR studies showed little or no binding between either recombinant

Dectin-1 or CR3 and a synthetic hexamer, whereas other STD-NMR studies revealed that a hexadecamer,[30] but not a hexamer, binds to the lectin domain of Dectin-1.[28] Work with glucans isolated from yeast cell walls showed the tetrasaccharide to be the smallest unit able to block the ingestion of zymosan (a natural β-(1→3)-glucan) by monocytes via CR3,[6] while study of a series of homogeneous synthetic oligomers revealed the tetra- and especially the pentasaccharides possess immunostimulatory effects including the potentiation of phagocytosis similar to those of phycarine (another natural β-(1→3)-glucan).[20] Chemical synthesis also revealed that penta- and hexameric glucans could be modified by replacement of the terminal reducing end glucopyranose residue by a gluco and/or manno-configured glucitol unit, by a mannopyranose ring, or by a 4-deoxyglucopyranose ring **2** (Fig 1) without loss of the ability to promote phagocytosis.[31,32] Likewise, it was demonstrated that the glycosidic oxygen could be replaced by a thioglycoside moiety **3-5** without loss of activity.[33]

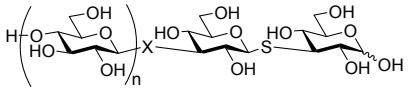
The lectin domain of Dectin-1 was revealed by X-ray crystallographic studies to consist of a shallow carbohydrate-binding groove in which the side chains of Trp 221 and His 223 line the walls of a hydrophobic pocket.[34] Further, laminarin, a natural β-(1→3)-glucan, was shown by STD-NMR studies to bind to this hydrophobic patch in recombinant Dectin-1 by the interaction with the α-faces of the terminal residues at either its reducing or non-reducing ends (Fig 2).[28,30] Overall, the picture is one of a relatively weak interaction of a short carbohydrate epitope with the lectin domain, as is commonly found in carbohydrate-protein interactions, that is bolstered by the multivalent effect due to the repetitive presentation of the epitope along the length of the polymeric oligosaccharide.[35-39]



β-(1→3)-Glucan **1** (n is highy variable and source dependent)



Pentasaccharide with terminal 4-deoxy modification **2**, n = 3



Thioglucans **3**: n = 1, X =O, **4**: n = 2, X = O, **5**: n = 2, X =S

Figure 1. Structures of β-(1→3)-glucan and synthetic analogs

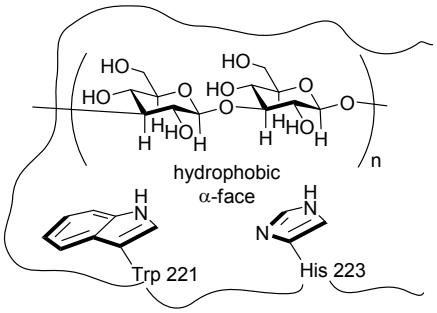
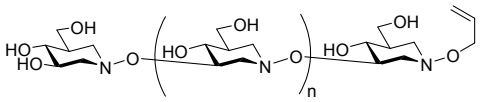


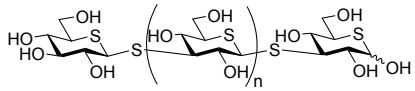
Figure 2. Schematic representation of the hydrophobic α-face of a disaccharide unit of a β-(1→3)-glucan in complex with the hydrophobic binding pocket of the Dectin-1 lectin domain

In our laboratory, pursuing a glycomimetic approach as opposed to a multivalent glycoconjugate approach to the synthesis of improved β-(1→3)-glucan analogues,[40,41] we prepared and evaluated the properties of the di- and trimeric hydroxylamine linked constructs **6** and **7** (Fig 3).[42] We found both **6** and **7** to display significant affinity for Dectin-1 and CR3, and hypothesized that this arises from the enhanced hydrophobicity of the α-face arising from removal of the C2-hydroxyl group, and replacement of the ring oxygen by a methylene group. Building on this hypothesis, and

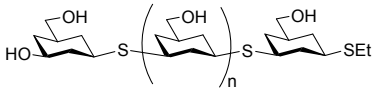
informed by the previous development of the monothioglucoside analogs **3-5** by the Ferrières laboratory,[33] and by the frequently observed enhancement of protein-small molecule and protein-carbohydrate interactions on replacement of ether units by thioethers,[43-52] we designed and prepared the di-, tri- and tetrameric 1,5-dithia analogs **8-10** (Fig 3), and were again rewarded by the observation of significant affinity for Dectin-1 and CR3, particularly for the trimer **9**.[53] Pursuing this line of investigation further, and noting the extensive history of carbacyclic motifs as carbohydrate analogs,[54-59] we now report the synthesis and evaluation of the thioether-linked carbasugar glucan mimetics **11-13** that incorporate features of the hydroxylamines **6** and **7**, the dithiaglucans **8-10**, and the 4-deoxyglucopyranose moiety in **2**.



Synthetic oligohydroxylamines **6**: n = 0, **7**: n = 1



Synthetic dithiaglucans **8**: n = 0, **9**: n = 1, **10**: n = 2

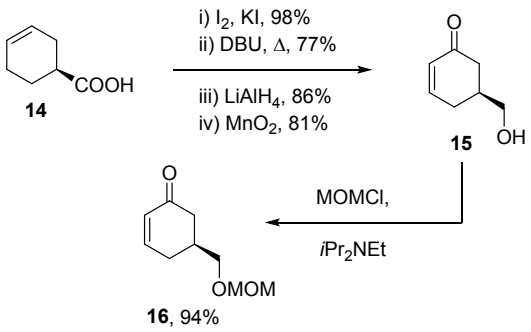


Thioether-linked oligomeric carbasugars
11: n = 0, **12**: n = 1, **13**: n = 2

Figure 3. Previous (**6-10**) and Targeted (**11-13**) β-(1→3)-Glucan Mimetics.

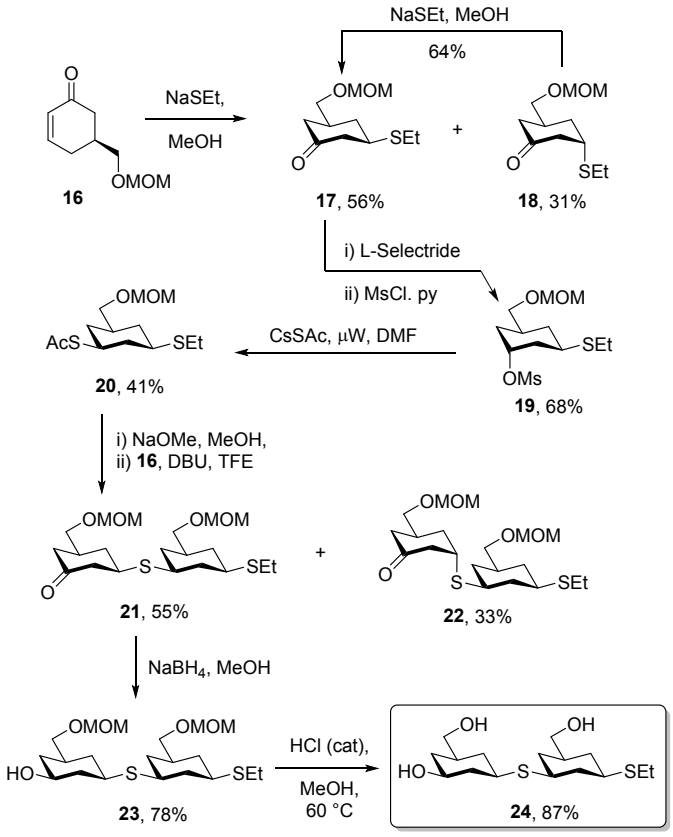
Results and Discussion

Synthesis. Following the protocol reported by Mori,[60] commercially available (*S*)-(-)-3-cyclohexenecarboxylic acid **14** was converted to the enone **15** in four known steps and 53% overall yield (Scheme 1). Installation of a methoxymethyl ether under standard conditions then gave the central building block **16** in 94% yield (Scheme 1).



Scheme 1. Synthesis of the Monomeric Building Block **16**

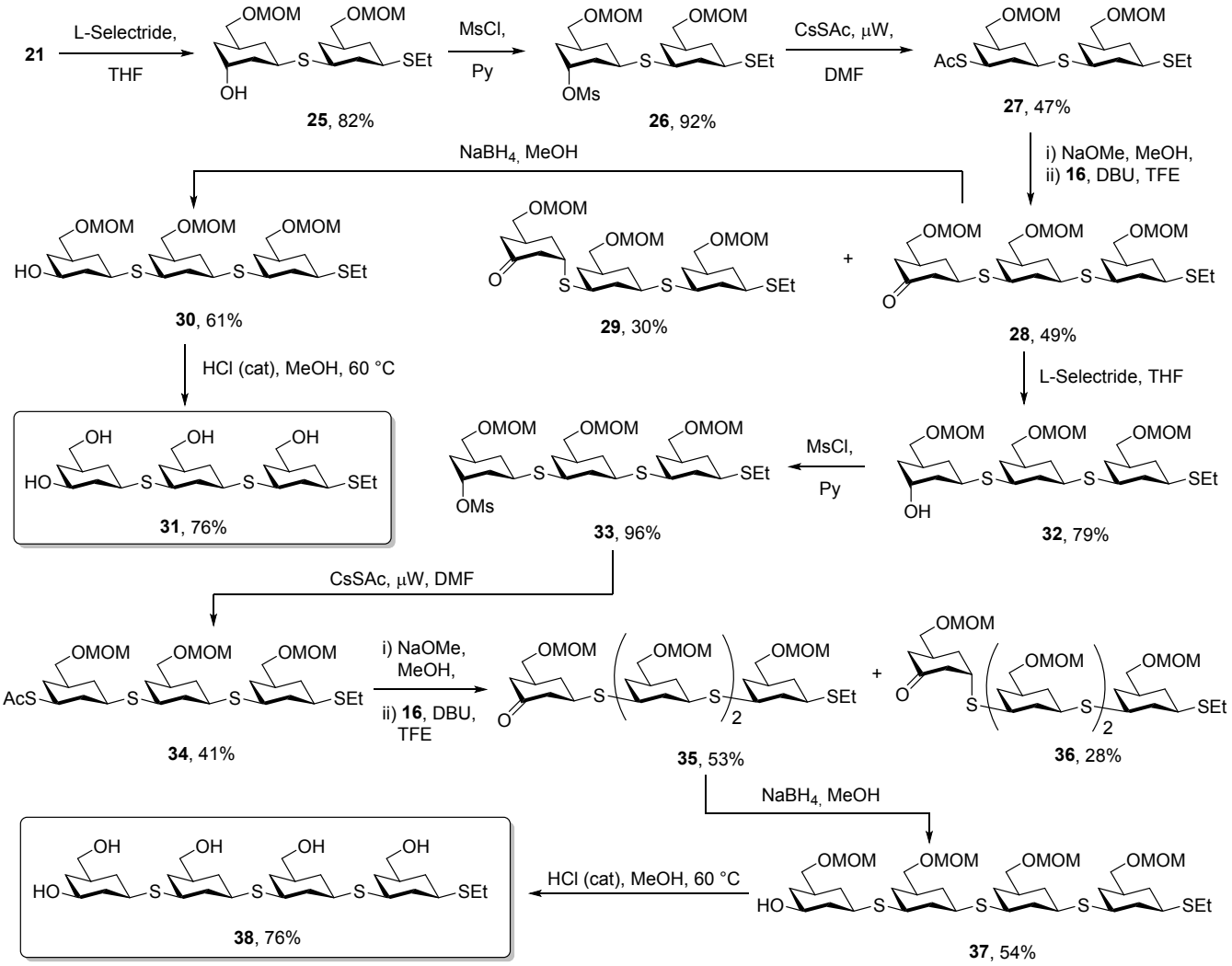
Treatment of **16** with sodium ethanethiolate in methanol at room temperature afforded the desired equatorial Michael adduct **17** and its axial isomer **18** in 56% and 31% yields, respectively. Resubjecting the axial isomer **18** to the reaction conditions resulted in its conversion to the equatorial adduct **17** in 64% yield. Reduction with L-Selectride followed by mesylation then afforded the mesylate **19** in 68% yield. Finally, mesylate displacement by cesium thioacetate in DMF under microwave irradiation gave the thioester **20** in 41% yield (Scheme 2). Cleavage of the thioacetyl moiety from **20** with sodium methoxide in methanol followed by exposure of the resultant thiol to enone **16** in the presence of diazabicycloundecene (DBU) in trifluoroethanol gave the desired equatorial adduct **21** in 55% yield, together with 33% of its axial isomer **22**. Reduction of **21** with sodium borohydride in methanol afforded 78% of the equatorial alcohol **23** from which the methoxymethyl ethers were removed with catalytic hydrogen chloride in methanol to give the mimetic **24** of laminaribiose (Scheme 2).



Scheme 2. Synthesis of the Laminaribiose Mimetic **24**

Reduction of the ketone **21** with L-Selectride in THF afforded the axial alcohol **25** in 82% yield, and was followed by treatment with mesyl chloride in pyridine to give the mesylate **26** in 92% yield. Mesylate displacement with cesium thioacetate in DMF under microwave irradiation next provided the thioacetate **27** in 47% yield which, on deactylation followed by stirring with enone **16** in the trifluoroethanol in the presence of DBU gave the all equatorial trimer **28** in 49% yield along with the axial isomer **29** in 30% yield. Sodium borohydride reduction of **28** to give the equatorial alcohol **30** in 61% yield was followed by removal of the MOM groups with HCl in methanol to afford the laminaritriose mimetic **31** in 76% yield. On the other hand reduction of ketone **28** with L-Selectride in THF gave 79% of the axial alcohol **32**, which was converted to the mesylate **33** and then to the thioacetate **34** in 96 and 41% yields, respectively. Iteration of the

protocol for the removal of the acetyl moiety followed by conjugate to enone **16** then gave the isomeric tetramers **35** and **36** in 53% and 28% yields, respectively. Sodium borohydride reduction of **35** afforded the equatorial alcohol **37** in 54% yield, and was followed by removal of the MOM groups to give the laminaritetraose mimetic **38** in 76% yield (Scheme 3).



Scheme 3. Synthesis of the Laminaritriose and Tetraose Mimetics **31** and **38**

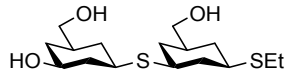
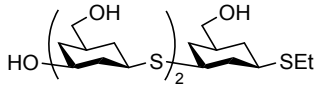
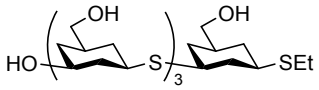
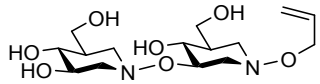
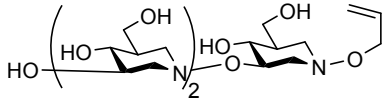
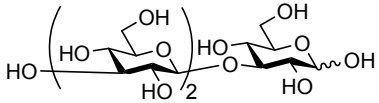
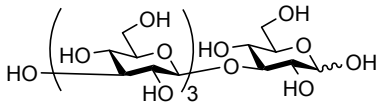
Binding to CR3 and Dectin-1 Receptors.

We screened the di-, tri-, and tetrasaccharide mimetics **24**, **31**, and **38**, respectively, for their ability to inhibit staining of human neutrophils and mouse macrophages by anti-CR3 or anti-Dectin-1

fluorescent antibody conjugates (FITC), as a measure of their affinity for CR3 and Dectin-1,[61] as reported in Table 1. For comparison purposes commercial laminaritriose **39** and tetraose **40** were screened in parallel as reported in Table 1 together with the values previously obtained for the hydroxylamine-based mimetics **6** and **7**.

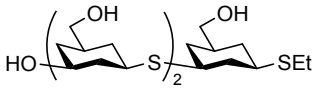
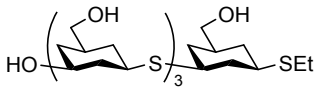
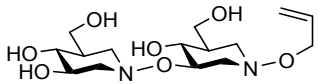
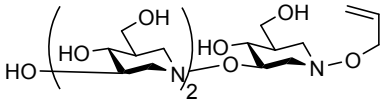
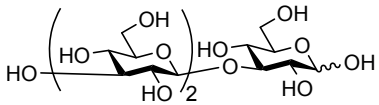
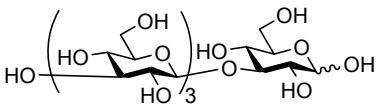
Stimulation of phagocytosis and pinocytosis. The ability of glycan mimetics **24**, **31**, and **38** to stimulate phagocytosis of synthetic polymeric 2-hydroxyethyl methacrylate particles[62] by human macrophage-like RAW 264 cells was examined. In addition the ability of the mimetics to stimulate pinocytosis, another important mechanism of cellular internalization, was examined by spectrophotometric measurement of neutral red dye accumulation by mouse macrophages (Table 2).[63] Laminaritriose **39** and tetraose **40** were again screened for comparison purposes as was the commercial highly purified yeast-derived insoluble Glucan #300.[64] Also for comparison purposes the previously recorded phagocytic activity of the di- and trimeric hydroxylamines **6** and **7** is reproduced in Table.

Table 1. Percentage Inhibition of anti-CR3 and anti-Dectin-1-FITC Antibody Staining of Neutrophils and Macrophages by 0.1 μg.mL[-1] Compound.

Cmpd	Oligomer No	% Inhibition of anti-CR3-FITC Staining of Human Neutrophils[a]	% Inhibition of anti-Dectin-1-FITC Staining of Mouse Macrophages[a]
 **24**	dimer	40.5 ± 3.6	48.9 ± 4.1
 **31**	trimer	33.2 ± 2.8	41.6 ± 3.6
 **38**	tetramer	45.3 ± 3.9	54.7 ± 3.8
 **6**	dimer	26.4 ± 2.7	28.2 ± 2.9
 **7**	trimer	34.2 ± 3.3	43.1 ± 3.5
 **39**	trimer	16.1 ± 1.7	19.9 ± 0.9
 **40**	tetramer	31.2 ± 2.1	44.8 ± 3.8
PBS[b]	-	1.3 ± 0.2	0.8 ± 0.3

[a] Mean ± SD. [b] phosphate buffered saline

Table 2. Percentage Stimulation of Phagocytosis and Pinocytosis.

Cmpd	Oligomer No	% Stimulation of Phagocytosis (RAW 264 macrophages, 10 μg/mL, 24 h) [a]	Stimulation of Pinocytosis (Uptake of neutral red dye by mouse macrophages after 2 h (ng/L x 10^5 cells) [a]
 **24**	dimer	17.7 ± 1.6	20.8 ± 2.9
 **31**	trimer	12.3 ± 1.5	9.1 ± 2.0
 **38**	tetramer	18.3 ± 0.9	16.2 ± 3.0
 **6**	dimer	7.8 ± 1.1	nd
 **7**	trimer	16.6 ± 2.0	nd
 **39**	trimer	7.9 ± 1.0	14.1 ± 1.3
 **40**	tetramer	20.5 ± 1.9	22.4 ± 2.1
Glucan #300		34.2 ± 2.6	36.8 ± 3.0
PBS[b]	-	2.2 ± 0.3	1.6 ± 0.2

[a] Mean ± SD at $P < 0.05$ level. [b] phosphate buffered saline

Inspection of Table 1 suggests that the simple thioether-linked carbocyclic di-, tri- and tetrasaccharide mimetics **24**, **31**, and **38**, respectively, display significant affinity for CR3 and Dectin-1. This activity is of a comparable magnitude to that of the earlier hydroxylamine based mimetics **6** and **7**,[42] of the more recent thiapyranoside mimetics **8-10** (data not shown)[53] and importantly of laminaribiose **39** and tetraose **40** themselves. These affinities are borne out by the ability of **24**, **31**, and **38** to stimulate both phagocytosis and pinocytosis (Table 2), again at levels comparable and even superior to the simple β-(1→3)-glucans of the same length. Considered as a whole with the earlier analogues prepared in the Ferrières laboratory (Figure 1),[31-33] these results strongly suggest that there is considerable scope for the development of potent small molecule analogs of the β-(1→3)-glucans.

Conclusions

Extrapolating from earlier small molecule β-(1→3)-glucans mimetics reported in the literature or prepared in our laboratories, we designed and synthesized a series of thioether linked 4-deoxycarbocyclic analogues of laminaribiose, triose, and tetraose to probe the minimal requirements for binding to the lectin domains of CR3 and Dectin-1. In the event the di-, tri- and tetrasaccharide mimetics, **24**, **31**, and **38** all displayed the ability to inhibit fluorescent antibody binding to CR3 and Dectin-1 and stimulated phagocytosis and pinocytosis at levels at least comparable to that of the minimal β-(1→3)-glucans laminaritriose and tetraose.

Experimental Section

General. All reactions were performed using oven-dried glassware under an atmosphere of argon. All reagents and solvents were purchased from commercial suppliers and were used without further purification unless otherwise specified. Chromatographic purifications were performed on silica gel (230-400 mesh) columns (20-50 g of silica gel per gram of crude compound). Reactions were monitored by analytical thin-layer chromatography on pre-coated glass backed plates (w/UV 254) and visualized by UV irradiation (254 nm) or by staining with 25% H_2SO_4 in EtOH or ceric ammonium molybdate (CAM) solution. Specific rotations were measured on an automatic polarimeter with a path length of 100 mm in the solvent specified. Concentrations are given in g/100 mL. High resolution mass spectra (HRMS) were recorded with an electrospray ionization (ESI) source coupled to a time-of-flight (TOF) mass analyzer or with an electron impact (EI) source coupled to a TOF mass analyzer. ^{1}H, ^{13}C, ^{19}F, spectra were recorded on a 400, 500 or 600 MHz spectrometer. NMR solvents were used without purification. Chemical shifts are given in ppm (δ) and coupling constants (*J*) are given in Hz. Multiplicities are given as singlet (s), broad singlet (br s), doublet (d), triplet (t), doublet of doublets (dd), triplet of doublets (td), multiplet (m), apparent quartet (app q), apparent pentet (app p), etc. Reactions requiring microwave irradiation were performed on a commercial microwave synthesizer with internal temperature control and magnetic stirring.

(5*S*)-5-((Methoxymethoxy)methyl)cyclohex-2-en-1-one (16). A stirred solution of enone **15**[60] (1.0 g, 7.93 mmol) in *N,N*-diisopropylethylamine (2 mL) and DCM (2 mL) at 0 °C was treated with chloromethyl methyl ether (1.2 mL, 15.85 mmol), and stirred at room temperature for 6 h before it was diluted with ethyl acetate, washed with 1 N HCl and brine, dried over Na_2SO_4, and concentrated under reduced pressure. Chromatographic purification (33% ethyl acetate/hexanes) afforded the title compound as a colorless oil (**16**, 1.27 g, 94%) with spectral data consistent with

the literature values:[65] $[\alpha]_D^{21}$ 73.0 (*c* 1.15, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 6.96 (ddd, *J* = 10.1, 5.4, 2.7 Hz, 1H), 6.06 – 5.95 (m, 1H), 4.60 (s, 2H), 3.52 – 3.41 (m, 2H), 3.33 (s, 3H), 2.55 – 2.18 (m, 5H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 199.1, 149.3, 129.7, 96.5, 70.6, 55.2, 41.0, 35.5, 28.9. HRMS (ESI-TOF) m/z: Calcd. for $C_9H_{14}O_3Na$ ($[M + Na]^+$): 193.0841; found: 193.0837.

(3*R*,5*R*)-3-(Ethylthio)-5-((methoxymethoxy)methyl)cyclohexan-1-one (17) and (3*S*,5*R*)-3-(Ethylthio)-5-((methoxymethoxy)methyl)cyclohexan-1-one (18). To a stirred solution of **16** (300 mg, 1.76 mmol) in MeOH (3.5 mL) at room temperature was added sodium ethanethiolate (444.8 mg, 5.29 mmol). The reaction mixture was stirred at the same temperature for 25 min before it was diluted with ethyl acetate, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. Chromatographic purification (18% ethyl acetate/hexanes) afforded the title compound as a colorless oil (**17**, 265.2 mg, 56%): $[\alpha]_D^{21}$ 58.6 (*c* 0.63, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 4.59 (s, 2H), 3.49 – 3.39 (m, 2H), 3.33 (s, 3H), 2.93 (tt, *J* = 12.7, 4.0 Hz, 1H), 2.68 (ddt, *J* = 14.1, 4.2, 2.0 Hz, 1H), 2.58 (q, *J* = 7.4 Hz, 2H), 2.42 (ddt, *J* = 13.9, 3.9, 2.0 Hz, 1H), 2.33 – 2.21 (m, 2H), 2.16 (t, *J* = 13.4 Hz, 1H), 2.10 – 1.98 (m, 1H), 1.51 (app q, *J* = 12.3 Hz, 1H), 1.24 (t, *J* = 7.4 Hz, 3H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 208.2, 96.5, 71.3, 55.3, 48.2, 44.0, 40.9, 37.8, 35.8, 24.4, 14.9. HRMS (ESI-TOF) m/z: Calcd. for $C_{11}H_{20}O_3SNa$ ($[M + Na]^+$): 255.1031; found: 255.1036.

Further elution (25% ethyl acetate/hexanes) gave **18** as a colorless oil (147.8 mg, 31%): $[\alpha]_D^{21}$ -22.9 (*c* 0.89, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 4.59 (s, 2H), 3.52 – 3.41 (m, 3H), 3.34 (s, 3H), 2.68 (ddd, *J* = 14.7, 4.7, 0.9 Hz, 1H), 2.59 – 2.42 (m, 5H), 2.23 (ddd, *J* = 14.3, 9.7, 1.2 Hz, 1H), 2.03 – 1.98 (m, 2H), 1.24 (t, *J* = 7.4 Hz, 3H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 208.3, 96.5, 70.9, 55.3, 46.5, 43.9, 40.1, 34.3, 33.1, 24.7, 14.5. HRMS (ESI-TOF) m/z: Calcd. for $C_{11}H_{20}O_3SNa$ ($[M + Na]^+$): 255.1031; found: 255.1038.

(1*R*,3*R*,5*R*)-3-(Ethylthio)-5-((methoxymethoxy)methyl)cyclohexan-1-ol. To a stirred solution of **17** (252.6 mg, 1.09 mmol) in THF (3 mL) at -78 °C was added a solution of L-Selectride in THF (1.0 M, 3.26 mL, 3.26 mmol). The reaction mixture was stirred at the same temperature for 5 h before it was warmed to room temperature. The reaction was quenched by adding MeOH and the mixture was concentrated under reduced pressure. Chromatographic purification (33% ethyl acetate/hexanes) afforded the title compound as a colorless oil (188.6 mg, 74%): $[\alpha]_D^{21}$ -12.5 (*c* 1.06, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 4.59 (s, 2H), 4.23 (app p, *J* = 3.0 Hz, 1H), 3.40 – 3.34 (m, 2H), 3.34 (s, 3H), 3.07 (tt, *J* = 12.5, 3.6 Hz, 1H), 2.58 (q, *J* = 7.4 Hz, 2H), 2.18 – 2.05 (m, 3H), 1.82 (ddt, *J* = 13.8, 3.4, 1.3 Hz, 1H), 1.44 (td, *J* = 13.2, 2.7 Hz, 1H), 1.29 – 1.19 (m, 4H), 1.05 (q, *J* = 12.8 Hz, 1H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 96.5, 72.6, 66.7, 55.1, 39.9, 37.1, 36.8, 35.6, 32.2, 24.0, 15.1. HRMS (ESI-TOF) m/z: Calcd. for $C_{11}H_{22}O_3SNa$ ($[M + Na]^+$): 257.1187; found: 257.1189.

(1*R*,3R,5*R*)-3-(Ethylthio)-5-((methoxymethoxy)methyl)cyclohexyl Methanesulfonate (19). To a stirred solution of (1*R*,3*R*,5*R*)-3-(ethylthio)-5-((methoxymethoxy)methyl)cyclohexan-1-ol (167.4 mg, 0.71 mmol) in pyridine (2 mL) at room temperature was added methanesulfonyl chloride (0.28 mL, 3.57 mmol). The reaction mixture was stirred at the same temperature for 11 h before it was diluted with ethyl acetate, washed with 1 N HCl and brine, dried over Na_2SO_4, and concentrated under reduced pressure. Chromatographic purification (25% ethyl acetate/hexanes) afforded the title compound as a colorless oil (**19**, 204.4 mg, 92%): $[\alpha]_D^{21}$ -1.3 (*c* 1.19, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 5.11 (app p, *J* = 2.9 Hz, 1H), 4.58 (s, 2H), 3.37 (d, *J* = 5.4 Hz, 2H), 3.33 (s, 3H), 3.05 – 2.95 (m, 4H), 2.58 (q, *J* = 7.4 Hz, 2H), 2.38 (dtt, *J* = 14.4, 3.7, 2.0 Hz, 1H), 2.17 – 2.03 (m, 3H), 1.52 (ddd, *J* = 14.8, 12.6, 2.5 Hz, 1H), 1.35 (t, *J* = 13.7 Hz, 1H), 1.24 (t, *J* = 7.4 Hz, 3H), 1.12 (app q, *J* = 13.3, 12.9 Hz, 1H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 96.5, 78.7,

71.8, 55.2, 38.6, 38.2, 36.6, 36.1, 33.7, 32.5, 24.1, 15.0. HRMS (ESI-TOF) m/z: Calcd. for $C_{12}H_{24}O_5S_2Na$ ([M + Na]$^+$): 335.0963; found: 335.0969.

***S*-((1*S*,3*R*,5*S*)-3-(Ethylthio)-5-((methoxymethoxy)methyl)cyclohexyl) Thioacetate (20).** A mixture of **19** (60.0 mg, 0.19 mmol) and cesium thioacetate (798.9 mg, 3.84 mmol) in DMF (0.6 mL) was heated to 100 ^{o}C by irridation with microwave for 1 h. After cooling to room temperature, the reaction mixture was diluted with ethyl acetate, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. Chromatographic purification (6% ethyl acetate/hexanes) afforded the title compound as a light yellow oil (**20**, 23.0 mg, 41%): $[\alpha]_D^{21}$ -24.3 (*c* 0.68, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.58 (s, 2H), 3.44 (tt, *J* = 12.7, 3.9 Hz, 1H), 3.36 (d, *J* = 6.1 Hz, 2H), 3.34 (s, 3H), 2.75 (tt, *J* = 12.2, 3.7 Hz, 1H), 2.58 (q, *J* = 7.4 Hz, 2H), 2.32 – 2.24 (m, 4H), 2.09 (ddq, *J* = 11.1, 3.5, 1.7 Hz, 1H), 2.02 (ddq, *J* = 12.7, 3.7, 1.9 Hz, 1H), 1.90 – 1.77 (m, 1H), 1.32 (q, *J* = 12.4 Hz, 1H), 1.24 (t, *J* = 7.4 Hz, 3H), 1.10 (q, *J* = 12.5 Hz, 1H), 1.03 (q, *J* = 12.4 Hz, 1H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 195.4, 96.5, 72.0, 55.2, 41.5, 40.9, 39.9, 38.5, 36.0, 35.4, 30.7, 24.2, 15.0. HRMS (ESI-TOF) m/z: Calcd. for $C_{13}H_{24}O_3S_2Na$ ([M + Na]$^+$): 315.1065; found: 315.1070.

(3*R*,5*R*)-3-(((1*S*,3*R*,5*S*)-3-(Ethylthio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexan-1-one (21) and (3*S*,5*R*)-3-(((1*S*,3*R*,5*S*)-3-(Ethylthio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexan-1-one (22). To a stirred solution of **20** (378.6 mg, 1.29 mmol) in MeOH (2 mL) at room temperature was added sodium methoxide (139.9 mg, 2.59 mmol). After stirring at room temperature for 30 min, the reaction mixture was diluted with ethyl acetate, washed with 1 N HCl and brine, dried over Na_2SO_4, and concentrated under reduced pressure to give the free thiol (328.2 mg). To a solution of the free thiol and enone **16** (330.5 mg, 1.94 mmol) in 2,2,2-trifluoroethanol (3 mL) at

room temperature was added a solution of DBU (197.1 mg, 1.29 mmol) in 2,2,2-trifluoroethanol (0.5 mL). After stirring at room temperature for 30 min, the reaction mixture was concentrated under reduced pressure at 40 °C. Chromatographic purification (33% ethyl acetate/hexanes) afforded the title compound as a colorless oil (**21**, 300.0 mg, 55%): $[\alpha]_D^{21}$ 19.6 (*c* 0.25, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ 4.59 (s, 2H), 4.58 (s, 2H), 3.47 – 3.41 (m, 2H), 3.37 – 3.33 (m, 5H), 3.33 (s, 3H), 3.01 (tt, *J* = 12.6, 4.0 Hz, 1H), 2.74 (tt, *J* = 12.2, 3.7 Hz, 1H), 2.70 – 2.61 (m, 2H), 2.57 (q, *J* = 7.4 Hz, 2H), 2.42 (ddt, *J* = 14.0, 4.0, 1.9 Hz, 1H), 2.29 (t, *J* = 13.6 Hz, 1H), 2.26 – 2.18 (m, 2H), 2.16 (t, *J* = 13.6 Hz, 1H), 2.10 – 2.01 (m, 3H), 1.76 – 1.68 (m, 1H), 1.51 (app q, *J* = 12.5 Hz, 1H), 1.29 (app q, *J* = 12.4 Hz, 1H), 1.23 (t, *J* = 7.4 Hz, 3H), 1.07 – 0.98 (m, 2H). $^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$) δ 208.0, 96.5 (2 Cs), 72.1, 71.2, 55.3, 55.2, 48.9, 44.0, 41.6, 41.1, 40.9, 39.8, 38.5, 37.8, 36.8, 36.3 (2 Cs), 24.0, 15.0. HRMS (ESI-TOF) m/z: Calcd. for $C_{20}H_{36}O_5S_2Na$ ($[M + Na]^+$): 443.1902; found: 443.1908.

Further elution (40% ethyl acetate/hexanes) gave **22** as a colorless oil (177.2 mg, 33%): $[\alpha]_D^{21}$ -28.6 (*c* 0.21, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ 4.59 (s, 2H), 4.58 (s, 2H), 3.57 (app p, *J* = 5.1 Hz, 1H), 3.48 – 3.42 (m, 2H), 3.36 – 3.33 (m, 5H), 3.33 (s, 3H), 2.71 – 2.60 (m, 3H), 2.57 (q, *J* = 7.4 Hz, 2H), 2.55 – 2.49 (m, 1H), 2.45 (m, 2H), 2.28 – 2.19 (m, 2H), 2.09 (ddt, *J* = 12.8, 3.6, 1.9 Hz, 1H), 2.06 – 1.94 (m, 3H), 1.78 – 1.68 (m, 1H), 1.29 (app q, *J* = 12.3 Hz, 1H), 1.23 (t, *J* = 7.4 Hz, 3H), 1.08 – 0.98 (m, 2H). $^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$) δ 208.2, 96.51, 96.49, 72.1, 70.9, 55.3, 55.2, 47.1, 43.8, 41.6, 41.1, 40.8, 38.9, 38.5, 36.4, 36.3, 34.4, 33.7, 24.0, 15.0. HRMS (ESI-TOF) m/z: Calcd. for $C_{20}H_{36}O_5S_2Na$ ($[M + Na]^+$): 443.1902; found: 443.1913.

(1*S*,3*R*,5*R*)-3-(((1*S*,3*R*,5*S*)-3-(Ethylthio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexan-1-ol (23). To a stirred solution of **21** (20.0 mg, 0.05 mmol) in MeOH (0.3 mL) at room temperature was added sodium borohydride (5.4 mg, 0.14

mmol). The reaction mixture was stirred at room temperature for 20 min before it was diluted with ethyl acetate, washed with 1 N HCl and brine, dried over Na_2SO_4, and concentrated under reduced pressure. Chromatographic purification (50% ethyl acetate/hexanes) afforded the title compound as a colorless oil (**23**, 15.7 mg, 78%): $[\alpha]_D^{21}$ -17.8 (*c* 0.32, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ 4.60 (s, 2H), 4.59 (s, 2H), 3.65 (tt, *J* = 10.9, 4.3 Hz, 1H), 3.41 – 3.37 (m, 2H), 3.37 – 3.35 (m, 2H), 3.34 (s, 3H), 3.33 (s, 3H), 2.79 – 2.71 (m, 2H), 2.66 (tt, *J* = 12.2, 3.7 Hz, 1H), 2.58 (q, *J* = 7.4 Hz, 2H), 2.30 – 2.23 (m, 2H), 2.12 – 1.99 (m, 4H), 1.77 – 1.68 (m, 2H), 1.52 (d, *J* = 4.8 Hz, 1H), 1.30 (app q, *J* = 12.3 Hz, 1H), 1.26 – 1.21 (m, 4H), 1.07 – 0.95 (m, 4H). $^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$) δ 96.53, 96.51, 72.2, 72.1, 69.6, 55.2 (2 Cs), 43.1, 41.7, 41.1, 40.7, 38.6, 38.5, 38.4, 36.9, 36.7, 36.4 (2 Cs), 24.0, 15.0. HRMS (ESI-TOF) m/z: Calcd. for $C_{20}H_{38}O_5S_2Na$ ($[M + Na]^+$): 445.2058; found: 445.2070.

(1*S*,3*R*,5*R*)-3-(((1*S*,3*R*,5*S*)-3-(Ethylthio)-5-(hydroxymethyl)cyclohexyl)thio)-5-(hydroxymethyl)cyclohexan-1-ol (24). A mixture of alcohol **23** (22.6 mg, 0.05 mmol), MeOH (1 mL) and concentrated HCl (10 μL) was heated to 60 °C for 6 h. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure. Chromatographic purification (9% methanol/dichloromethane) afforded the title compound as a colorless oil (**24**, 15.5 mg, 87%): $[\alpha]_D^{21}$ -16.4 (*c* 0.14, CH_3OH). ^1H NMR (600 MHz, CD_3OD) δ 3.58 (tt, *J* = 11.1, 4.2 Hz, 1H), 3.44 – 3.35 (m, 4H), 2.84 – 2.74 (m, 2H), 2.70 (tt, *J* = 12.1, 3.6 Hz, 1H), 2.57 (q, *J* = 7.4 Hz, 2H), 2.26 (dtd, *J* = 14.4, 3.6, 1.7 Hz, 1H), 2.23 – 2.18 (m, 1H), 2.08 – 1.99 (m, 3H), 1.99 – 1.93 (m, 1H), 1.66 – 1.54 (m, 2H), 1.22 (t, *J* = 7.4 Hz, 3H), 1.20 – 1.10 (m, 2H), 0.96 – 0.83 (m, 4H). $^{13}C\{^1H\}$ NMR (151 MHz, CD_3OD) δ 68.9, 66.33, 66.31, 42.9, 41.4 (2 Cs), 40.6, 40.5, 38.6, 38.5, 37.5, 36.6, 36.6, 36.0, 23.4, 14.0. HRMS (ESI-TOF) m/z: Calcd. for $C_{16}H_{30}O_3S_2Na$ ($[M + Na]^+$): 357.1534; found: 357.1543.

(1*R*,3*R*,5*R*)-3-(((1*S*,3*R*,5*S*)-3-(Ethylthio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexan-1-ol (25). To a stirred solution of **21** (277.7 mg, 0.66 mmol) in THF (3 mL) at -78 °C was added a solution of L-Selectride in THF (1.0 M, 1.98 mL, 1.98 mmol). The reaction mixture was stirred at the same temperature for 4 h before it was warmed to room temperature. The reaction was quenched by adding MeOH and the mixture was concentrated under reduced pressure. Chromatographic purification (15% acetone/hexanes) afforded the title compound as a colorless oil (**25**, 228.1 mg, 82%): $[\alpha]_D^{21}$ -22.2 (*c* 0.36, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.58 (s, 2H), 4.57 (s, 2H), 4.22 (app p, *J* = 2.9 Hz, 1H), 3.38 – 3.30 (m, 10H), 3.15 (tt, *J* = 12.4, 3.7 Hz, 1H), 2.74 (tt, *J* = 12.1, 3.7 Hz, 1H), 2.65 (tt, *J* = 12.1, 3.6 Hz, 1H), 2.57 (q, *J* = 7.5 Hz, 2H), 2.30 (dtd, *J* = 14.6, 3.6, 1.7 Hz, 1H), 2.17 – 2.01 (m, 5H), 1.80 (dtt, *J* = 13.9, 3.7, 2.1 Hz, 1H), 1.77 – 1.68 (m, 1H), 1.42 (td, *J* = 13.3, 2.7 Hz, 1H), 1.28 (app q, *J* = 12.5 Hz, 1H), 1.25 – 1.20 (m, 4H), 1.09 – 0.98 (m, 3H). ^{13}C{^{1}H} NMR (151 MHz, $CDCl_3$) δ 96.50, 96.47, 72.5, 72.2, 66.7, 55.2, 55.1, 41.7, 41.3, 40.7, 40.5, 38.6, 37.7, 36.9, 36.4, 36.0, 35.6, 32.2, 24.0, 15.0. HRMS (ESI-TOF) m/z: Calcd. for $C_{20}H_{38}O_5S_2Na$ ($[M + Na]^+$): 445.2058; found: 445.2066.

(1*R*,3*R*,5*R*)-3-(((1*S*,3*R*,5*S*)-3-(Ethylthio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexyl Methanesulfonate (26). To a stirred solution of **25** (88.4 mg, 0.21 mmol) in pyridine (2 mL) at room temperature was added methanesulfonyl chloride (80.9 µL, 1.05 mmol). The reaction mixture was stirred at the same temperature for 20 h before it was diluted with ethyl acetate, washed with 1 N HCl and brine, dried over Na_2SO_4, and concentrated under reduced pressure. Chromatographic purification (40% ethyl acetate/hexanes) afforded the title compound as a colorless oil (**26**, 95.9 mg, 92%): $[\alpha]_D^{21}$ -11.6 (*c* 0.56, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.08 (app p, *J* = 3.0 Hz, 1H), 4.57 (s, 2H), 4.56 (s, 2H), 3.37 – 3.33 (m, 4H), 3.32

(s, 3H), 3.31 (s, 3H), 3.07 (tt, *J* = 12.4, 3.7 Hz, 1H), 3.01 (s, 3H), 2.74 (tt, *J* = 12.2, 3.7 Hz, 1H), 2.65 (tt, *J* = 12.2, 3.6 Hz, 1H), 2.57 (q, *J* = 7.4 Hz, 2H), 2.36 (dtt, *J* = 14.4, 3.9, 2.0 Hz, 1H), 2.26 (ddt, *J* = 12.6, 3.8, 1.9 Hz, 1H), 2.14 – 2.02 (m, 5H), 1.77 – 1.68 (m, 1H), 1.51 (ddd, *J* = 14.8, 12.6, 2.5 Hz, 1H), 1.37 – 1.25 (m, 2H), 1.23 (t, *J* = 7.4 Hz, 3H), 1.12 (app q, *J* = 12.9 Hz, 1H), 1.06 – 0.98 (m, 2H). $^{13}C\{^{1}H\}$ NMR (151 MHz, $CDCl_3$) δ 96.5 (2 Cs), 78.6, 72.1, 71.8, 55.20, 55.17, 41.6, 41.1, 41.0, 38.8, 38.7, 38.5, 36.9, 36.8, 36.3, 35.7, 33.6, 32.5, 24.0, 15.0. HRMS (ESI-TOF) m/z: Calcd. for $C_{21}H_{40}O_7S_3Na$ ($[M + Na]^+$): 523.1834; found: 523.1841.

***S*-((1*S*,3*R*,5*S*)-3-(((1*S*,3*R*,5*S*)-3-(Ethylthio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexyl) Thioacetate (27).** A mixture of **26** (10.0 mg, 0.02 mmol) and cesium thioacetate (12.5 mg, 0.06 mmol) in DMF (0.05 mL) was heated to 100 °C by irridation with microwave for 50 min. After cooling to room temperature, the reaction mixture was diluted with ethyl acetate, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. Chromatographic purification (14% ethyl acetate/hexanes) afforded the title compound as a light yellow oil (**27**, 4.5 mg, 47%): $[\alpha]_D^{21}$ -16.7 (*c* 0.18, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.59 (s, 2H), 4.57 (s, 2H), 3.42 (tt, *J* = 12.6, 3.9 Hz, 1H), 3.37 – 3.33 (m, 7H), 3.32 (s, 3H), 2.81 (tt, *J* = 12.1, 3.8 Hz, 1H), 2.72 (tt, *J* = 12.2, 3.7 Hz, 1H), 2.66 (tt, *J* = 12.2, 3.6 Hz, 1H), 2.57 (q, *J* = 7.4 Hz, 2H), 2.29 (s, 3H), 2.27 – 2.20 (m, 2H), 2.11 – 1.97 (m, 4H), 1.88 – 1.78 (m, 1H), 1.77 – 1.69 (m, 1H), 1.36 – 1.26 (m, 2H), 1.24 (t, *J* = 7.4 Hz, 3H), 1.12 – 0.97 (m, 4H). $^{13}C\{^{1}H\}$ NMR (151 MHz, $CDCl_3$) δ 195.3, 96.52 (2 Cs), 96.50, 72.2, 72.0, 55.2, 41.7, 41.1, 40.9, 40.8, 40.6, 40.5, 38.5 (2 Cs), 37.0, 36.6, 36.3, 35.4, 30.7, 24.0, 15.0. HRMS (ESI-TOF) m/z: Calcd. for $C_{22}H_{40}O_5S_3Na$ ($[M + Na]^+$): 503.1936; found: 503.1946.

(3*R*,5*R*)-3-(((1*S*,3*R*,5*R*)-3-(((1*S*,3*R*,5*S*)-3-(Ethylthio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-

5-((methoxymethoxy)methyl)cyclohexan-1-one (28) and (3*S*,5*R*)-3-(((1*S*,3*R*,5*R*)-3-(((1*S*,3*R*,5*S*)-3-(Ethylthio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexan-1-one (29). To a stirred solution of **27** (24.6 mg, 0.05 mmol) in MeOH (0.6 mL) at room temperature was added sodium methoxide (5.5 mg, 0.10 mmol). After stirring at room temperature for 25 min, the reaction mixture was diluted with ethyl acetate, washed with 1 N HCl and brine, dried over Na_2SO_4, and concentrated under reduced pressure to give the free thiol (21.8 mg). To a solution of the free thiol and enone **16** (10.5 mg, 0.06 mmol) in 2,2,2-trifluoroethanol (0.6 mL) was added a solution of DBU (7.8 mg, 0.05 mmol) in 2,2,2-trifluoroethanol (0.1 mL). After stirring at room temperature for 30 min, the reaction mixture was concentrated under reduced pressure at 40 °C. Chromatographic purification (40% ethyl acetate/hexanes) afforded the title compound as a colorless oil (**28**, 15.4 mg, 49%): $[\alpha]_D^{21}$ 13.6 (*c* 0.39, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.59 (s, 2H), 4.58 (s, 2H), 4.57 (s, 2H), 3.48 – 3.41 (m, 2H), 3.38 – 3.29 (m, 13H), 3.01 (tt, *J* = 12.6, 4.1 Hz, 1H), 2.78 – 2.70 (m, 3H), 2.70 – 2.62 (m, 2H), 2.57 (q, *J* = 7.4 Hz, 2H), 2.42 (ddt, *J* = 14.0, 3.9, 1.9 Hz, 1H), 2.33 – 2.12 (m, 5H), 2.10 – 2.01 (m, 5H), 1.77 – 1.67 (m, 2H), 1.52 (app q, *J* = 12.5 Hz, 1H), 1.33 – 1.20 (m, 5H), 1.07 – 0.97 (m, 4H). ^{13}C{^{1}H} NMR (151 MHz, $CDCl_3$) δ 208.0, 96.5 (3 Cs), 72.2, 72.0, 71.2, 55.3, 55.22, 55.20, 48.9, 44.0, 41.7, 41.3, 41.11, 41.07, 40.6, 40.5, 39.8, 38.51, 38.48, 37.8, 36.94, 36.86, 36.7, 36.32, 36.28, 24.0, 15.0. HRMS (ESI-TOF) m/z: Calcd. for $C_{29}H_{52}O_7S_3Na$ ($[M + Na]^+$): 631.2773; found: 631.2780.

Further elution (50% ethyl acetate/hexanes) gave **29** as a colorless oil (9.4 mg, 30%): $[\alpha]_D^{21}$ -18.3 (*c* 0.30, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.60 – 4.56 (m, 6H), 3.57 (app p, *J* = 5.1 Hz, 1H), 3.50 – 3.42 (m, 2H), 3.40 – 3.28 (m, 13H), 2.78 – 2.62 (m, 5H), 2.57 (q, *J* = 7.4 Hz, 2H), 2.55 – 2.48 (m, 1H), 2.48 – 2.41 (m, 2H), 2.29 – 2.17 (m, 3H), 2.11 – 1.92 (m, 6H), 1.78 – 1.67 (m, 2H),

1.32 – 1.20 (m, 5H), 1.07 – 0.97 (m, 4H). $^{13}C\{^{1}H\}$ NMR (151 MHz, $CDCl_3$) δ 208.2, 96.53, 96.52, 96.49, 72.2, 72.0, 70.9, 55.3, 55.2 (2 Cs), 47.1, 43.8, 41.6, 41.2, 41.13, 41.09, 40.6, 40.5, 39.0, 38.53, 38.49, 37.0, 36.9, 36.33, 36.26, 34.4, 33.7, 24.0, 15.0. HRMS (ESI-TOF) m/z: Calcd. for $C_{29}H_{52}O_7S_3Na$ ($[M + Na]^+$): 631.2773; found: 631.2766.

(1*S*,3*R*,5*R*)-3-(((1*S*,3*R*,5*R*)-3-(((1*S*,3*R*,5*S*)-3-(Ethylthio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexan-1-ol (30). To a stirred solution of **28** (5.7 mg, 0.009 mmol) in MeOH (0.1 mL) at room temperature was added sodium borohydride (1.1 mg, 0.03 mmol). The reaction mixture was stirred at room temperature for 20 min before it was diluted with ethyl acetate, washed with 1 N HCl and brine, dried over Na_2SO_4, and concentrated under reduced pressure. Chromatographic purification (20% acetone/hexanes) afforded the title compound as a colorless oil (**30**, 3.5 mg, 61%): $[\alpha]_D^{21}$ -11.4 (*c* 0.14, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.62 – 4.55 (m, 6H), 3.65 (tt, *J* = 11.1, 4.2 Hz, 1H), 3.43 – 3.29 (m, 15H), 2.80 – 2.70 (m, 4H), 2.66 (tt, *J* = 12.2, 3.6 Hz, 1H), 2.58 (q, *J* = 7.4 Hz, 2H), 2.31 – 2.20 (m, 3H), 2.11 – 1.97 (m, 6H), 1.78 – 1.68 (m, 3H), 1.34 – 1.20 (m, 6H), 1.08 – 0.94 (m, 6H). $^{13}C\{^{1}H\}$ NMR (151 MHz, $CDCl_3$) δ 96.54 (2 Cs), 96.51, 72.22, 72.16, 72.1, 69.6, 55.22 (2 Cs), 55.19, 43.3, 41.70, 41.65, 41.1, 40.8, 40.6, 40.5, 38.7, 38.6, 38.5, 38.3, 36.92, 36.89, 36.8, 36.7, 36.4, 36.3, 24.0, 15.0. HRMS (ESI-TOF) m/z: Calcd. for $C_{29}H_{54}O_7S_3Na$ ($[M + Na]^+$): 633.2929; found: 633.2931.

(1*S*,3*R*,5*R*)-3-(((1*S*,3*R*,5*R*)-3-(((1*S*,3*R*,5*S*)-3-(Ethylthio)-5-(hydroxymethyl)cyclohexyl)thio)-5-(hydroxymethyl)cyclohexyl)thio)-5-(hydroxymethyl)cyclohexan-1-ol (31). A mixture of alcohol **30** (5.2 mg, 0.009 mmol), MeOH (0.5 mL) and concentrated HCl (5 μL) was heated to 60 °C for 5 h. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure. Chromatographic purification (9% methanol/dichloromethane) afforded the title

compound as a colorless oil (**31**, 3.1 mg, 76%): $[\alpha]_D^{21}$ -11.4 (*c* 0.07, CH_3OH). ^1H NMR (600 MHz, CD_3OD) δ 3.58 (tt, *J* = 10.9, 4.1 Hz, 1H), 3.45 – 3.35 (m, 6H), 2.88 – 2.75 (m, 4H), 2.72 (tt, *J* = 12.1, 3.6 Hz, 1H), 2.58 (q, *J* = 7.4 Hz, 2H), 2.31 – 2.23 (m, 2H), 2.20 (dtt, *J* = 12.1, 4.0, 2.1 Hz, 1H), 2.11 – 1.99 (m, 5H), 1.96 (d, *J* = 12.1 Hz, 1H), 1.73 – 1.52 (m, 3H), 1.22 (t, *J* = 7.4 Hz, 3H), 1.20 – 1.11 (m, 3H), 1.01 – 0.78 (m, 6H). $^{13}C\{^1H\}$ NMR (151 MHz, CD_3OD) δ 68.9, 66.3 (3 Cs), 42.8, 42.0, 41.44, 41.42, 40.6, 40.52, 40.51, 40.47 (2 Cs), 38.6, 38.5, 37.4, 36.64, 36.56 (2 Cs), 36.5, 35.9, 23.4, 14.1. HRMS (ESI-TOF) m/z: Calcd. for $C_{23}H_{42}O_4S_3Na$ ($[M + Na]^+$): 501.2143; found: 501.2155.

(1*R*,3*R*,5*R*)-3-(((1*S*,3*R*,5*R*)-3-(((1*S*,3*R*,5*S*)-3-(Ethylthio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexan-1-ol (32). To a stirred solution of **28** (14.9 mg, 0.02 mmol) in THF (0.3 mL) at -78 °C was added a solution of L-Selectride in THF (1.0 M, 73.4 µL, 0.07 mmol). The reaction mixture was stirred at the same temperature for 12 h before it was warmed to room temperature. The reaction was quenched by adding MeOH and the mixture was concentrated under reduced pressure. Chromatographic purification (14% acetone/hexanes) afforded the title compound as a colorless oil (**32**, 11.8 mg, 79%): $[\alpha]_D^{21}$ -18.7 (*c* 0.47, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ 4.59 (s, 2H), 4.58 (s, 2H), 4.57 (s, 2H), 4.22 (br s, 1H), 3.42 – 3.28 (m, 15H), 3.14 (tt, *J* = 12.4, 3.7 Hz, 1H), 2.78 – 2.69 (m, 3H), 2.66 (tt, *J* = 12.2, 3.6 Hz, 1H), 2.57 (q, *J* = 7.4 Hz, 2H), 2.31 – 2.22 (m, 2H), 2.17 – 1.99 (m, 7H), 1.80 (dtd, *J* = 14.1, 3.6, 1.8 Hz, 1H), 1.77 – 1.69 (m, 2H), 1.42 (td, *J* = 13.5, 2.6 Hz, 1H), 1.33 – 1.18 (m, 6H), 1.10 – 0.94 (m, 5H). $^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$) δ 96.51 (2 Cs), 96.47, 72.5, 72.21, 72.19, 66.6, 55.2 (2 Cs), 55.1, 41.9, 41.7, 41.2, 40.9, 40.7, 40.6 (2 Cs), 38.6, 38.5, 37.8, 37.0, 36.92, 36.85, 36.3, 36.2, 35.5,

32.2, 24.0, 15.0. HRMS (ESI-TOF) m/z: Calcd. for $C_{29}H_{54}O_7S_3Na$ ([M + Na]$^+$): 633.2929; found: 633.2932.

(1*R*,3*R*,5*R*)-3-(((1*S*,3*R*,5*R*)-3-(((1*S*,3*R*,5*S*)-3-(Ethylthio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexyl Methanesulfonate (33). To a stirred solution of **32** (10.5 mg, 0.02 mmol) in pyridine (0.25 mL) at room temperature was added methanesulfonyl chloride (6.7 μL, 0.09 mmol). The reaction mixture was stirred at the same temperature for 11 h before it was diluted with ethyl acetate, washed with 1 N HCl and brine, dried over Na_2SO_4, and concentrated under reduced pressure. Chromatographic purification (45% ethyl acetate/hexanes) afforded the title compound as a colorless oil (**33**, 11.4 mg, 96%): $[\alpha]_D^{21}$ -9.0 (*c* 0.41, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.09 (br s, 1H), 4.60 – 4.56 (m, 6H), 3.43 – 3.28 (m, 15H), 3.07 (tt, *J* = 12.4, 3.6 Hz, 1H), 3.01 (s, 3H), 2.80 – 2.70 (m, 3H), 2.67 (tt, *J* = 12.2, 3.6 Hz, 1H), 2.57 (q, *J* = 7.4 Hz, 2H), 2.38 (dd, *J* = 14.2, 3.7 Hz, 1H), 2.30 – 2.20 (m, 2H), 2.16 – 2.00 (m, 7H), 1.79 – 1.69 (m, 2H), 1.51 (ddd, *J* = 14.8, 12.7, 2.5 Hz, 1H), 1.34 (t, *J* = 13.5 Hz, 1H), 1.31 – 1.25 (m, 2H), 1.23 (t, *J* = 7.4 Hz, 3H), 1.12 (app q, *J* = 12.7 Hz, 1H), 1.07 – 0.97 (m, 4H). ^{13}C{^{1}H} NMR (151 MHz, $CDCl_3$) δ 96.52, 96.51 (2 Cs), 78.6, 72.2, 72.1, 71.8, 55.20 (2 Cs), 55.19, 41.63, 41.59, 41.1, 41.0, 40.6, 40.5, 38.9, 38.7, 38.49, 38.47, 37.0, 36.9 (2 Cs), 36.8, 36.4, 35.7, 33.6, 32.6, 24.0, 15.0. HRMS (ESI-TOF) m/z: Calcd. for $C_{30}H_{56}O_9S_4Na$ ([M + Na]$^+$): 711.2705; found: 711.2727.

***S*-((1*S*,3*R*,5*S*)-3-(((1*S*,3*R*,5*S*)-3-(((1*S*,3*R*,5*S*)-3-(Ethylthio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexyl) Thioacetate (34).**

A mixture of **33** (11.4 mg, 0.02 mmol) and cesium thioacetate (10.3 mg, 0.05 mmol) in DMF (0.1 mL) was heated to 100 °C by irridation with microwave for 35 min. After cooling to room

temperature, the reaction mixture was diluted with ethyl acetate, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. Chromatographic purification (22% ethyl acetate/hexanes) afforded the title compound as a light yellow oil (**34**, 4.5 mg, 41%): $[\alpha]_D^{21}$ -16.1 (*c* 0.18, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ 4.59 (m, 4H), 4.57 (s, 2H), 3.43 (tt, *J* = 12.6, 3.9 Hz, 1H), 3.39 – 3.29 (m, 15H), 2.81 (tt, *J* = 12.9, 4.1 Hz, 1H), 2.77 – 2.70 (m, 3H), 2.67 (tt, *J* = 12.2, 4.0 Hz, 1H), 2.58 (q, *J* = 7.4 Hz, 2H), 2.29 (s, 3H), 2.28 – 2.19 (m, 3H), 2.12 – 1.96 (m, 6H), 1.89 – 1.79 (m, 1H), 1.79 – 1.69 (m, 2H), 1.36 – 1.25 (m, 3H), 1.24 (t, *J* = 7.4 Hz, 3H), 1.09 (app q, 1H), 1.06 – 0.97 (m, 5H). $^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$) δ 195.3, 96.54 (2 Cs), 96.51, 72.22, 72.16, 72.0, 55.21, 55.19 (2 Cs), 41.7, 41.5, 41.1, 40.83, 40.80, 40.5 (4 Cs), 38.5 (3 Cs), 36.98, 36.96, 36.9, 36.6, 36.4, 35.3, 30.7, 24.0, 15.0. HRMS (ESI-TOF) m/z: Calcd. for $C_{31}H_{56}O_7S_4Na$ ($[M + Na]^+$): 691.2807; found: 691.2817.

(3*R*,5*R*)-3-(((1*S*,3*R*,5*R*)-3-(((1*S*,3*R*,5*S*)-3-(((1*S*,3*R*,5*S*)-3-(Ethylthio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexan-1-one (35) and (3*S*,5*R*)-3-(((1*S*,3*R*,5*R*)-3-(((1*S*,3*R*,5*S*)-3-(((1*S*,3*R*,5*S*)-3-(Ethylthio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexan-1-one (36). To a stirred solution of **34** (3.3 mg, 0.005 mmol) in MeOH (0.2 mL) at room temperature was added sodium methoxide (0.5 mg, 0. 01 mmol). After stirring at room temperature for 10 min, the reaction mixture was diluted with ethyl acetate, washed with 1 N HCl and brine, dried over Na_2SO_4, and concentrated under reduced pressure to give the free thiol (4.5 mg). To a solution of the free thiol and enone **16** (1.0 mg, 0.006 mmol) in 2,2,2-trifluoroethanol (0.1 mL) was added a solution of DBU (750 μg, 0.005 mmol) in 2,2,2-trifluoroethanol (10 μL). After stirring at room

temperature for 30 min, the reaction mixture was concentrated under reduced pressure at 40 °C. Chromatographic purification (45% ethyl acetate/hexanes) afforded the title compound as a colorless oil (**35**, 2.1 mg, 53%): $[\alpha]_D^{21}$ 2.0 (*c* 0.15, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ 4.61 – 4.58 (m, 8H), 3.49 – 3.41 (m, 2H), 3.41 – 3.30 (m, 18H), 3.02 (tt, *J* = 12.6, 4.1 Hz, 1H), 2.81 – 2.71 (m, 5H), 2.71 – 2.63 (m, 2H), 2.58 (q, *J* = 7.4 Hz, 2H), 2.43 (ddt, *J* = 13.9, 4.0, 1.9 Hz, 1H), 2.30 (t, *J* = 13.6 Hz, 1H), 2.29 – 2.20 (m, 4H), 2.17 (t, *J* = 13.7 Hz, 1H), 2.13 – 2.00 (m, 7H), 1.79 – 1.69 (m, 3H), 1.52 (t, *J* = 12.5 Hz, 1H), 1.32 – 1.22 (m, 6H), 1.09 – 0.97 (m, 6H). $^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$) δ 208.0, 96.6, 96.5 (3 Cs), 72.20, 72.17, 72.0, 71.2, 55.3, 55.23 (2 Cs), 55.20, 48.9, 44.0, 41.7, 41.5, 41.3, 41.2, 41.1, 40.6 (2 Cs), 40.54, 40.46, 39.8, 38.53, 38.49, 38.47, 37.8, 36.94 (3 Cs), 36.88, 36.8, 36.4, 36.3, 24.0, 15.0. HRMS (ESI-TOF) m/z: Calcd. for $C_{38}H_{68}O_9S_4Na$ ($[M + Na]^+$): 819.3644; found: 819.3643.

Further elution (50% ethyl acetate/hexanes) gave **36** as a colorless oil (1.1 mg, 28%): $[\alpha]_D^{21}$ -10.9 (*c* 0.11, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ 4.62 – 4.56 (m, 8H), 3.58 (app p, *J* = 4.9 Hz, 1H), 3.50 – 3.42 (m, 2H), 3.42 – 3.30 (m, 18H), 2.81 – 2.62 (m, 7H), 2.58 (q, *J* = 7.4 Hz, 2H), 2.56 – 2.49 (m, 1H), 2.48 – 2.41 (m, 2H), 2.32 – 2.17 (m, 4H), 2.13 – 1.93 (m, 8H), 1.80 – 1.69 (m, 3H), 1.34 – 1.21 (m, 6H), 1.09 – 0.96 (m, 6H). $^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$) δ 208.2, 96.6, 96.54 (2 Cs), 96.50, 72.20, 72.17, 72.1, 70.9, 55.3, 55.23, 55.22, 55.21, 47.1, 43.8, 41.7, 41.5, 41.2, 41.14, 41.08, 40.6 (2 Cs), 40.53, 40.46, 39.0, 38.53 (2 Cs), 38.48, 37.1, 37.0, 36.9 (2 Cs), 36.4, 36.3, 34.4, 33.7, 24.0, 15.0. HRMS (ESI-TOF) m/z: Calcd. for $C_{38}H_{68}O_9S_4Na$ ($[M + Na]^+$): 819.3644; found: 819.3656.

(1*S*,3*R*,5*R*)-3-(((1*S*,3*R*,5*R*)-3-(((1*S*,3*R*,5*S*)-3-(((1*S*,3*R*,5*S*)-3-(Ethylthio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexyl)thio)-5-((methoxymethoxy)methyl)cyclohexan-1-ol

(37). To a stirred solution of **35** (4.9 mg, 0.006 mmol) in MeOH (0.1 mL) at room temperature was added sodium borohydride (0.7 mg, 0.018 mmol). The reaction mixture was stirred at room temperature for 20 min before it was diluted with ethyl acetate, washed with 1 N HCl and brine, dried over Na_2SO_4, and concentrated under reduced pressure. Chromatographic purification (25% acetone/hexanes) afforded the title compound as a colorless oil (**37**, 2.7 mg, 54%): $[\alpha]_D^{21}$ -10.0 (*c* 0.11, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.61 – 4.57 (m, 8H), 3.65 (tt, *J* = 10.9, 3.8 Hz, 1H), 3.44 – 3.31 (m, 20H), 2.80 – 2.70 (m, 6H), 2.66 (tt, *J* = 12.2, 3.6 Hz, 1H), 2.58 (q, *J* = 7.4 Hz, 2H), 2.32 – 2.19 (m, 4H), 2.13 – 1.98 (m, 8H), 1.79 – 1.69 (m, 4H), 1.34 – 1.20 (m, 7H), 1.09 – 0.94 (m, 8H). ^{13}C{^{1}H} NMR (151 MHz, $CDCl_3$) δ 96.6, 96.54 (2 Cs), 96.51, 72.20 (2 Cs), 72.16, 72.1, 69.6, 55.3, 55.22, 55.20, 55.19, 43.3, 41.8, 41.7, 41.5, 41.2, 40.8, 40.60, 40.57 (3 Cs), 40.5, 38.8, 38.6, 38.54, 38.50, 38.3, 37.0, 36.92 (2 Cs), 36.88, 36.7, 36.37, 36.36, 24.0, 15.0. HRMS (ESI-TOF) m/z: Calcd. for $C_{38}H_{70}O_9S_4Na$ ($[M + Na]^+$): 821.3800; found: 821.3766.

(1*S*,3*R*,5*R*)-3-(((1*S*,3*R*,5*R*)-3-(((1*S*,3*R*,5*S*)-3-(((1*S*,3*R*,5*S*)-3-(Ethylthio)-5-(hydroxymethyl)cyclohexyl)thio)-5-(hydroxymethyl)cyclohexyl)thio)-5-(hydroxymethyl)cyclohexyl)thio)-5-(hydroxymethyl)cyclohexan-1-ol (38). A mixture of alcohol **37** (2.2 mg, 0.003 mmol), MeOH (0.3 mL) and concentrated HCl (3 µL) was heated to 60 °C for 6 h. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure. Chromatographic purification (11% methanol/dichloromethane) afforded the title compound as a colorless oil (**38**, 1.3 mg, 76%): $[\alpha]_D^{21}$ -22.5 (*c* 0.04, CH_3OH). ^{1}H NMR (600 MHz, CD_3OD) δ 3.62 – 3.55 (m, 1H), 3.44 – 3.35 (m, 8H), 2.88 – 2.75 (m, 6H), 2.72 (tt, *J* = 12.2, 3.6 Hz, 1H), 2.58 (q, *J* = 7.4 Hz, 2H), 2.30 – 2.23 (m, 3H), 2.21 (d, *J* = 11.9 Hz, 1H), 2.10 – 1.99 (m, 7H), 1.96 (d, *J* = 12.3 Hz, 1H), 1.68 – 1.54 (m, 4H), 1.22 (t, *J* = 7.4 Hz, 3H), 1.20 – 1.10 (m, 4H), 0.98 – 0.83 (m, 8H). ^{13}C{^{1}H} NMR (151 MHz, CD_3OD) δ 68.9, 66.3 (4 Cs), 42.9, 42.09, 42.05,

42.0, 41.5, 41.4, 40.54 (4 Cs), 40.48 (4 Cs), 38.7, 38.5, 37.4, 36.7, 36.6 (4 Cs), 36.0, 23.4, 14.1. HRMS (ESI-TOF) m/z: Calcd. for $C_{30}H_{54}O_5S_4Na$ ($[M + Na]^+$): 645.2752; found: 645.2753.

Laminaritriose 39 and tetraose 40 were purchased from commercial sources. They were found to be pure by ^1H and ^{13}C NMR spectroscopy and ESI mass spectrometry, and had spectral data consistent with the literature:[20]

39: ^1H NMR (600 MHz, D_2O) δ 5.09 (d, J = 3.7 Hz, 0.42H, H-1α), 4.65-4.60 (m, 2H), 4.53 (d, J = 8.1 Hz, 0.58H, H-1β), 3.81 – 3.55 (m, 9H), 3.44 – 3.22 (m, 9H).$^{13}C\{^1H\}$ NMR (151 MHz, D_2O) δ 102.7, 102.55, 102.46, 95.6, 92.0, 84.4, 84.21, 84.17, 82.2, 75.9, 75.6, 75.55, 75.52, 73.8, 73.4, 73.20, 73.17, 71.2, 71.0, 69.5, 68.05, 68.01, 60.6, 60.5. ESIHRMS calculated for $C_{18}H_{32}O_{16}$ $[M+Na]^+$, 527.1588; found, 527.1578.

40: ^1H NMR (600 MHz, D_2O) δ 5.09 (d, J = 3.7 Hz, 0.37H, H-1α), 4.66 – 4.61 (m, 3H), 4.53 (d, J = 8.0 Hz, 0.63H, H-1β), 3.81 – 3.54 (m, 12H), 3.45 – 3.22 (m, 12H). $^{13}C\{^1H\}$ NMR (151 MHz, D_2O) δ 102.7, 102.6, 102.5, 102.4, 95.6, 91.9, 84.4, 84.2, 84.03, 83.99, 82.2, 75.9, 75.6, 75.56, 75.54, 75.51, 73.8, 73.4, 73.3, 73.25, 73.22, 71.2, 71.0, 69.5, 68.05, 68.01, 60.62, 60.59, 60.5. ESIHRMS calculated for $C_{24}H_{42}O_{21}$ $[M+Na]^+$, 689.2116; found, 689.2111.

Inhibition of anti-CR3-FITC antibody staining of human neutrophils and of anti-Dectin 1-FITC antibody staining of mouse macrophages. For fluorescent staining, anti-CR3-FITC antibodies (MN-41 donated by Drs. Allison Eddy and Alfred Michael of the University of Minnesota, Minneapolis, MN, and rat anti Mouse Dectin-1 antibody labeled with FITC (purchased from AbD Serotec, Raleigh, NC) were employed. Either human neutrophils or mouse peritoneal macrophages were incubated with 0.1 μg.mL^{-1} of tested samples for 0.5 h on ice and washed. Subsequently, the

cells were stained with antibodies on ice using standard techniques. After centrifugation of cells through a 3 mL cushion of 12% BSA in PBS, the cells were re-suspended in PBS containing 1% BSA and 10 mM sodium azide. Cell cytometry was performed with a Becton Dickinson-LSRII instrument. The inhibition of CR3 receptor and Dectin-1 receptor staining was calculated as described.[61]

Stimulation of phagocytosis. The technique employing phagocytosis of synthetic polymeric microspheres was described earlier.[66] Human cells (cell line RAW 264) were incubated *in vitro* with 10 $\mu g.mL^{-1}$ of tested samples for 24 h at 37 ^{o}C. After washing, 0.05 mL of 2-hydroxyethyl methacrylate particles (HEMA; $5x10^{8}$/mL) was added. The test tubes were incubated at 37 ^{o}C for 1 h, with intermittent shaking. Smears were stained with Wright stain. Cells with three or more HEMA particles were considered positive. The insoluble glucan Glucan #300 used as comparison standard was obtained from Yeast-derived insoluble Glucan #300 (>85% dry w/w basis) was purchased from Transfer Point (Columbia, SC, USA). This glucan contains 96% carbohydrates and 2.1% proteins. Neutral sugar analysis confirmed 91.3% glucose and 8% mannose.

Stimulation of pinocytosis. Stimulation of pinocytosis was determined spectrophotometrically as described.[63]

Supporting Information. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.8b01645.

Copies of the ^{1}H and ^{13}C NMR spectra of all new compounds (PDF)

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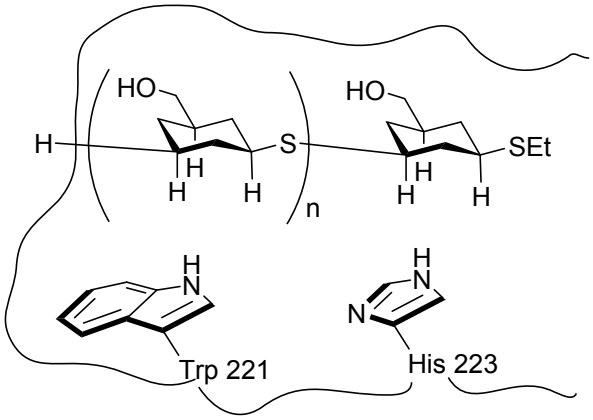
Table of Contents Graphic



- Synthesis of carbocyclic S-linked 2,4-dideoxy glucan mimetics
- Retention of glucan-like properties