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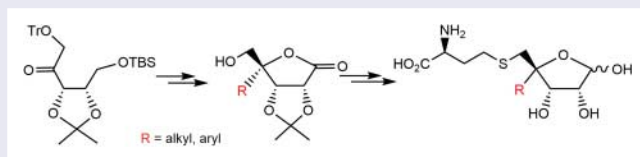
S-Ribosylhomocysteine analogues modified at the ribosyl C-4 position

Christiane Chbib, Adam J. Sobczak, Mukesh Mudgal, Cesar Gonzalez , Daniel Lumpuy, Justyna Nagaj, Kamila Stokowa-Soltys and Stanislaw F. Wnuk

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ABSTRACT

4-C-Alkyl/aryl-S-ribosylhomocysteine (SRH) analogues were prepared by coupling of homocysteine with 4-substituted ribofuranose derivatives. The diastereoselective incorporation of the methyl substituent into the 4 position of the ribose ring was accomplished by the addition of methylmagnesium bromide to the protected ribitol-4-ulose yielding the 4-C-methylribitol in 85% yield as single 4R diastereomer. The 4-C hexyl, octyl, vinyl, and aryl ribitols were prepared analogously. Chelation controlled addition of a carbanion to ketones from the Si-face was responsible for the observed stereochemical outcome. Oxidation of the primary alcohol of the 4-C ribitols with catalytic amounts of tetrapropylammonium per-ruthenate in the presence of N-methylmorpholine N-oxide produced 4-C-alkylribo-1,4-lactones in high yields. Mesylation of the latter compounds at the 5-hydroxyl position and treatment with a protected homocysteine thiolate afforded protected 4-C-alkyl/aryl-SRH analogues as the lactones. Reduction with lithium triethylborohydride and successive global deprotections with TFA afforded 4-C-alkyl/aryl SRH analogues. These analogues might impede the S-ribosylhomocysteine(LuxS)-catalyzed reaction by preventing β -elimination of a homocysteine molecule, and thus depleting the production of quorum sensing signaling molecule AI-2.



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1. Introduction

Quorum sensing (QS) is a process in which exchange of chemical signals enables bacterial population to take control of crucial functions in united communities for enhancement of symbiosis, virulence, and biofilm formation.[1–4] The interference in this chemical communication among bacteria could result in improving our control of bacterial infection.

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Numerous small and macromolecules that modulate QS pathways have been designed and synthesized.[5–8] The acetylhomoserine-based isothiocyanate and haloacetamide probes, which covalently inhibit bacterial QS [9] and probes for signaling molecules which utilize ‘click chemistry’, [10] have been developed.

The S-ribosylhomocysteinase (LuxS; EC 4.4.1.21) is a key enzyme in the biosynthetic pathway for conversion of S-ribosyl-L-homocysteine (SRH, **1**; Figure 1) to homocysteine (Hcy) and 4,5-dihydroxy-2,3-pentadione (DPD), the precursor for the type II autoinducer (AI-2) [11] which mediates the interspecies QS among bacteria (see Figure 2).[12–14] Various SRH analogues have been designed as mechanistic probes and/or inhibitors of the LuxS enzyme.[6] Among them, one of the most important are SRH analogues that target mechanistic steps of the LuxS catalytic cycle by effecting the initial ring opening step (e.g. 1-deoxy-SRH analog **2** [15] and [4-aza]-**3a** [16] or 4-[thio]-SRH analogs **3b** [17]; i.e. **1** → intermediate **A**, Figure 2) or one of the tautomerization/isomerization steps (**A** → **B** or **B** → **C**). These included substrates lacking the enolizable hydroxyl group at C3 (e.g. **4**; X = H or OMe),[18] including mechanistically significant C3 halogenated [3-Br or F]-SRH analogues **4**,[19] Zhou and co-workers synthesized substrate analogue S-homoribosyl-L-cysteine **5**, which was designed to prevent the final mechanistic step of the LuxS catalytic cycle.[15] Moreover, brominated furanone derivatives were found to modify LuxS selectively leading to the covalent inhibition.[20]

The substitution of hydrogen at C4 by an alkyl or aryl group in SRH (e.g. **18**) should impede the LuxS-catalyzed reaction by preventing β -elimination of a homocysteine molecule (i.e. **C** → **D**) since abstraction of the C4-proton by a general base (e.g. Glu158) from the intermediate **C**, when R = alkyl/aryl, will be disallowed (Figure 2). Consequently, the formation of DPD necessary for the production of AI-2 would be depleted with 4-C-alkyl-SRH analogues.

Since LuxS forms a dimer it is possible that the size and chemical nature of the group incorporated at C4 of the ribose ring might also play an additional role in inhibiting dimerization. Recently, the SRH analogues having the sterically demanding alkyl or aryl group at the Hcy fragment of the SRH have been designed and were attempted to be synthesized.[21] These analogues were thought to be able to bind to one monomer of the LuxS protein while blocking the correct association of the second monomer, possibly interfering with dimerization interfaces.[21–23] In theory, for example, the longer the alkyl chain incorporated at the C4 position in analogs **18**, the more potent the inhibition of dimerization of LuxS might be observed since the inhibitor can reach both homodimer parts of the protein. The inhibitor might also block one monomer leading to the alteration of the activity and as a consequence conformational changes of the second monomer. Herein, we report synthesis

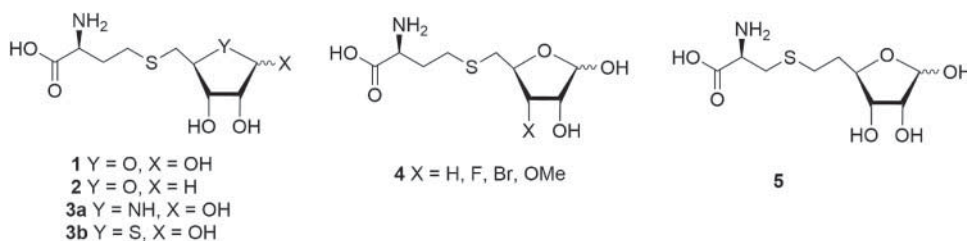


Figure 1. Selected LuxS inhibitors.

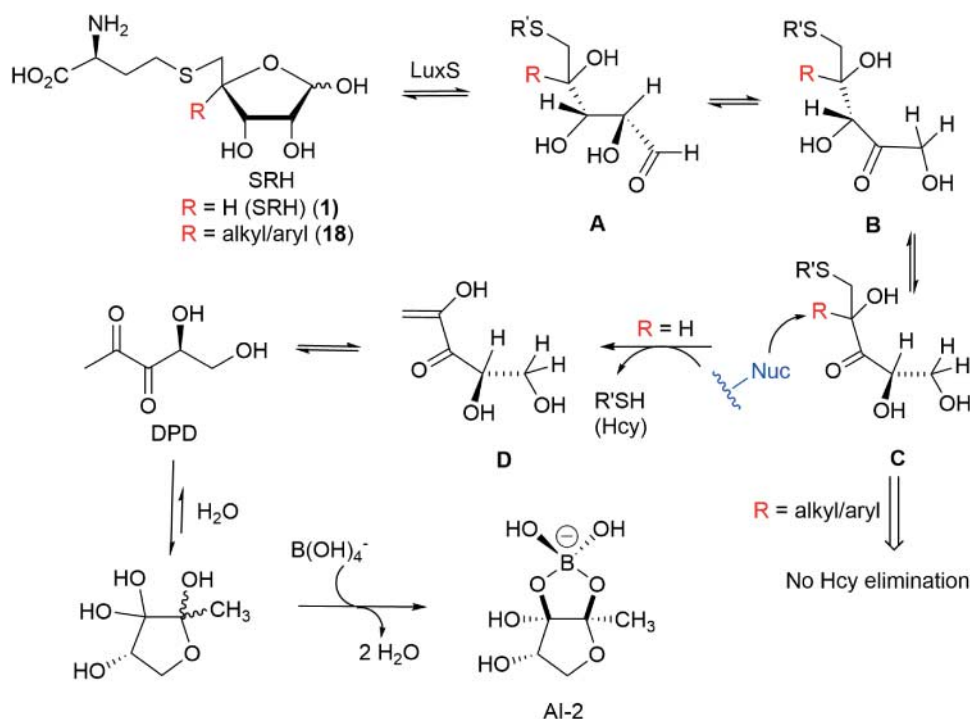


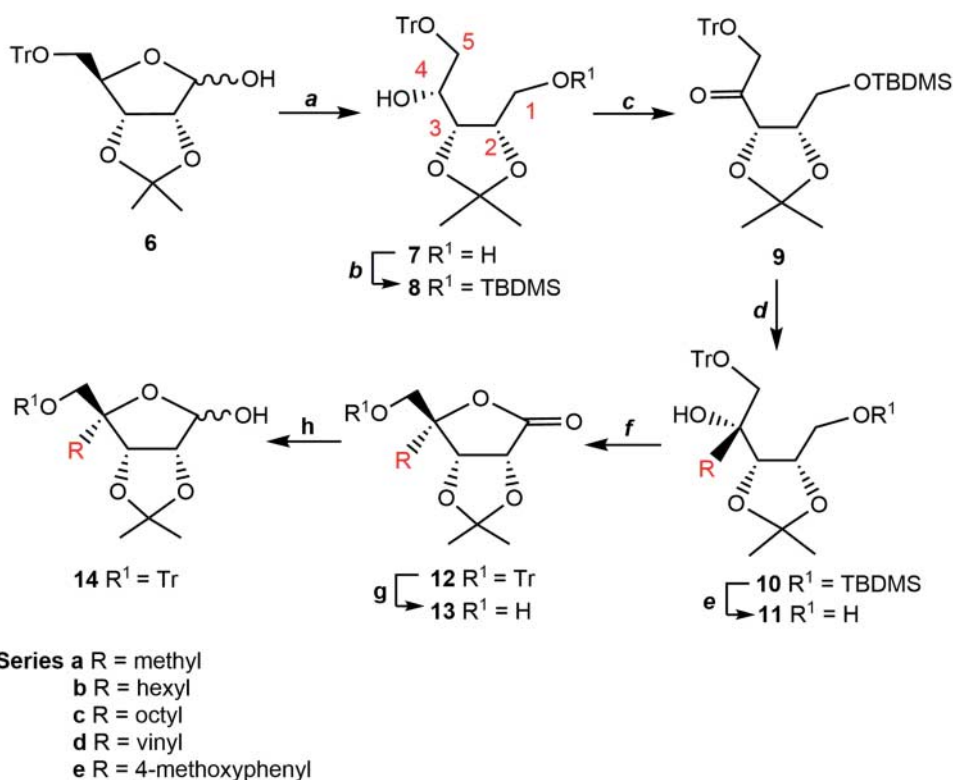
Figure 2. A plausible inhibition of LuxS enzyme by 4-C substituted SRH analogues.

of [4-alkyl/aryl]-SRH analogues which would deplete the production of AI-2 by preventing elimination of Hcy and could also act as dimerization inhibitors.

2. Results and discussion

The 4-C-alkyl/aryl-*S*-ribofuranose derivatives were prepared by coupling of the homocysteine with the 4-C-substituted ribofuranose derivatives. The 4-C-substituted riboses can be prepared either by manipulation of natural carbohydrates [24] or the chemoenzymatic strategy from non-sugar precursors.[25,26] From the method available, we chose Maddaford's method for diastereoselective incorporation of the alkyl substituent into the 4 position of the D-ribose ring by the addition of Grignard reagents to 4-ulose.[24] Reduction of the protected ribose **6** with NaBH₄ provided the acyclic ribitol **7** (Scheme 1). Regioselective silylation of the primary hydroxyl with TBDMSCl and subsequent Dess-Martin or Collins oxidation of the secondary hydroxyl in **8** yielded ketone **9**. The overall yield for the conversion of the ribose to the ribitol-4-ulose **9** [27] was 75% (5 steps).

Treatment of ketone **9** with methylmagnesium bromide at -78°C produced the 4-C-methylribitol 4*R*-**10a** in 85% yield as a single isomer after purification by silica gel chromatography. The addition of the hexylmagnesium bromide or octylmagnesium bromide to ketone **9** gave the corresponding 4-C-hexyl and 4-C-octyl ribitols **10b** and **10c** in 74% and 69% isolated yields, respectively. The 4-C-vinyl ribitol **10d** (61%) and 4-C-aryl ribitol **10e** (96%) were prepared analogously. The Grignard reagent addition to the ribitol-4-ulose **9**, which is an α -alkoxy ketone, is proposed to proceed via a 5-membered ring



Scheme 1. Reagents and conditions: (a) NaBH₄/EtOH/H₂O; (b) TBDMSCl/imidazole/CH₂Cl₂/16 h; (c) Dess-Martin (3 h) or Collins (1 h) reagents; (d) RMgX/Et₂O/−78°C; (e) TBAF/THF; (f) TPAP/NMO/CH₂Cl₂/6 h; (g) TFA/CH₂Cl₂/r.t.; and (h) LiEt₃BH/CH₂Cl₂/0°C/0.5 h.

chelate [28] with the complexation of the metal with the carbonyl group and the α -alkoxy group at C3 allowing transfer of the alkanide carbanion in the *anti*-fashion (*Si*-face) to the α -alkoxy group at C3.[24,29]

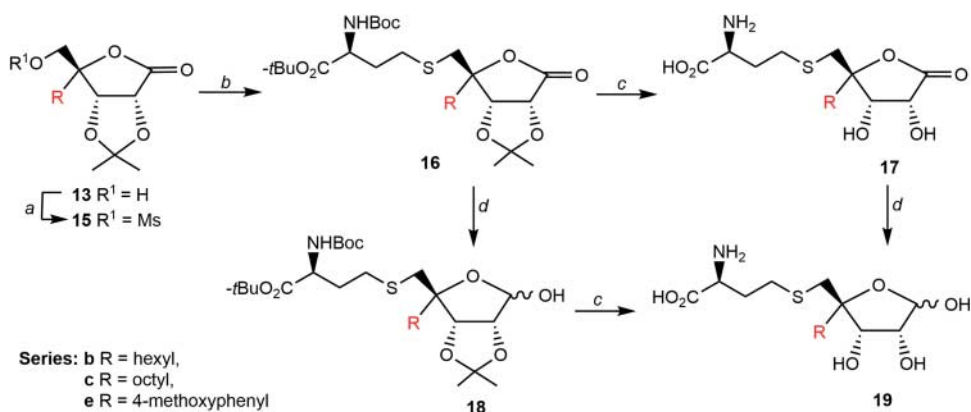
Treatment of the 4-*C*-methylribitol **10a** with TBAF (0°C/30 min) effected desilylation to give ribitol **11a** (78%). Analogous deprotection of **10b–e** produced the 4-*C*-substituted ribitols **11b–e** (75–87%) with a primary hydroxyl group at C1 and a tertiary hydroxyl group at C4. For the ring closure, we elected the oxidation of the primary alcohol at C1 to the carboxylic acid with the concomitant ring closure to the corresponding ribono-1,4-lactones,[30,31] since such an approach would provide convenient precursors for the synthesis of both 4-*C*-substituted SRH lactones [32] and, after reduction, 4-*C*-alkyl/aryl SRH analogues. Thus, oxidation (6 h) of the **11a** with a catalytic amount of tetrapropylammonium perruthenate (TPAP) in the presence of a stoichiometric amount of *N*-methylmorpholine *N*-oxide (NMO) [33,34] gave the corresponding 4-*C*-methylribono-1,4-lactone **12a** (80%). The formation of lactone was supported by the disappearance of the signals for H1 and H1' protons (¹H NMR) and the appearance of a peak at 172.1 ppm for the carbonyl carbon at C1 (¹³C NMR). Oxidation of 4-*C*-hexyl and 4-*C*-octyl ribitols **11b** or **11c** with TPAP/NMO also proceeded smoothly to give the 4-*C*-hexyl- and 4-*C*-octylribonolactones **12b** and **12c** (95%). The longer reaction time (e.g. 14 h), especially with

substrates with long alkyl chain (e.g. **11c**), led to the formation of by-product(s) ($\sim 30\%$). The 4-C-vinyl **12d** and 4-C-aryl **12e** lactones were obtained analogously by the oxidation of **11d** and **11e**, respectively.

Detritylation of **12a** (6 h, rt) with TFA/CH₂Cl₂ gave **13a** (66%). Analogous treatment of the hexyl **12b** and octyl **12c** with TFA (5 h, rt) gave ribono-1,4-lactones **13b** and **13c** in 80% and 75% yields, respectively. The longer reaction time should be avoided since the formation of by-product(s) was observed during detritylation of **12b** to **13b** when the reaction was carried out for 16 h.[35] Treatment of the vinyl analogue **12d** with TFA (5 h, rt) also gave detritylated ribonolactone **13d** but only in 35% yield after purification on silica gel column chromatography. Similarly, 4-C-aryl analogues **12e** were converted to **13e** (70%).

Careful reduction of the protected 4-C-hexyl ribonolactone **12b** with LiEtBH₃ (-20°C , 30 min) gave lactol **14b** as an anomeric mixture (α/β , 1:3) in 54% yield after column chromatography. The chemical shifts for the anomeric protons and the magnitude of vicinal $^3J_{\text{H1-H2}}$ coupling constants were diagnostic [36,37] for the determination of the composition of α/β anomers. The anomeric proton for α -isomer [5.61 ppm (dd, $J = 4.2, 11.5$ Hz)] appeared as doublet of doublets with splitting to H2 and OH group, while H1 proton for β -anomer appears upfield as a doublet [5.15 (d, $J = 8.4$ Hz)].

Next, the coupling of the selected ribono-1,4-lactones **13** with homocysteine was examined. Thus, treatment of **13b**, **13c**, or **13e** with methylsulfonyl chloride gave the primary 5-O-mesyl derivatives **15b**, **15c**, and **15e** (60–83%). From different approaches [13,15,38–40] tested for the nucleophilic displacement of the mesylate in **15** with homocysteine thiolates, we found that reactions with the (*N*-Boc, COOtBu) protected homocysteine, generated *in situ* by the reduction of the corresponding homocysteine [13] with water-extractable tris(2-carboxyethyl)phosphine [18] gave the best results. Thus, treatment of the **15b** with homocysteine thiolate (3 equiv) generated from BocNHCH(CH₂CH₂SH)CO₂t-Bu/LDA afforded protected 4-C-hexyl-SRH lactone **16b** (65%, Scheme 2). Analogous coupling of **15c** with Hcy gave **16c**, contaminated with the protected Hcy substrate ($\sim 1:1$), which was directly used in the subsequent deprotection step. 4-C-Aryl mesylate **15e** was coupled with homocysteine thiolate to give **16e** (48%). It is noteworthy to add that displacement of the



Scheme 2. MsCl/TEA/CH₂Cl₂; (b) BocNHCH(CH₂CH₂SH)CO₂t-Bu/LDA/DMF; (c) TFA/H₂O; and (d) LiEt₃BH/THF.

primary mesylate (or tosylate) from the highly branching ribonolactones **15** having trisubstituted carbon (C4) at the adjacent position needs to be carried out with great caution to avoid the formation of by-products. The structure of one such by-product isolated from the reaction of **15c** with Hcy was tentatively assigned as the 2,3-*O*-isopropylidene-4-*C*-octyl-D-ribo-1,5-lactone (see note under the experimental procedure for **17c**) based on the spectroscopic analysis (^1H and ^{13}C NMR and HRMS) and by comparison with the similar ribono-1,5-lactones.[41]

Treatment of **16b** with TFA effected global removal of the *N*-Boc, acetonide and *t*-butyl ester protecting groups to give 4-*C*-hexyl-SRH lactone **17b** in 55% yield after HPLC purification. Analogous treatment of the crude **16c** with TFA and purification of the resulting mixture on the Sep-Pak column gave 4-*C*-octyl-SRH lactone **17c** in 21% overall yield from **15c**. Deprotection of **16e** gave 4-*C*-(4-methoxyphenyl)-SRH lactone **17e** (75%).

Synthesis of the somehow unstable 4-*C*-alkyl/aryl-SRH derivatives **19** was accomplished by the reduction of either protected **16** or deprotected **17** lactones with lithium triethylborohydride. Thus, treatment of **17c** with LiEt_3BH /THF (2 equiv.) in CH_2Cl_2 at -20°C effected reduction of the lactone yielding 4-*C*-octyl SRH **19c** (α/β , $\sim 1:3$; 60%). Alternatively, reduction of the protected 4-*C*-hexyl-SRH lactone **16b** with LiEt_3BH followed by deprotection of the resulting **18b** with TFA and TFA/ H_2O afforded 4-*C*-hexyl-SRH **19b** (α/β , 1:9; 75%). Similarly, subjection of **16e** to the reduction and deprotection sequence afforded 4-*C*-(4-methoxyphenyl)-SRH **19e** (α/β , 1:9; 77%).

3. Conclusion

We have developed synthesis of *S*-ribosylhomocysteine analogues substituted at the ribosyl C-4 position with the alkyl or aryl group. The critical steps in this multistep synthesis starting from ribose were (i) diastereoselective addition of the alkyl/aryl-magnesium bromides to protected ribitol-4-ulose to produce the 4-*C*-alkyl/aryl-ribitols in high yields as single 4*R* diastereomers, (ii) oxidation of the primary alcohol at C1 of the 4-*C* substituted ribitols with the catalytic amounts of tetrapropylammonium perruthenate in the presence of a stoichiometric amount of *N*-methylmorpholine *N*-oxide to give 4-*C*-alkyl/aryl-ribo-1,4-lactones in good yields, (iii) displacement of 5-mesylate with the protected homocysteine thiolate to afford protected 4-*C*-alkyl/aryl-SRH analogues with a lactone carbonyl at the C1 position, and (iv) reduction with lithium triethylborohydride and successive global deprotections with TFA to give 4-*C*-alkyl/aryl-SRH analogues. Enzymatic and biological properties of these novel analogues of SRH will be published elsewhere.

4. Experimental section

4.1. General procedures

The ^1H (400 or 600 MHz) and ^{13}C (100 MHz) NMR spectra were determined with solutions in CDCl_3 unless otherwise noted. Mass spectra (MS) and HRMS were obtained in the AP-ESI or TOF-ESI mode. TLC was performed with Merck kieselgel 60-F₂₅₄ sheets, products were detected with 254 nm light or by visualization with the $\text{Ce}(\text{SO}_4)_2/(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}/\text{H}_2\text{SO}_4/\text{H}_2\text{O}$ reagent. Merck kieselgel 60 (230–400 mesh) was used for column chromatography. Final products were purified using the HPLC

[XTerra preparative RP₁₈ OBD column (5 μ m 19 \times 150 mm) with a gradient program using CH₃CN/H₂O as a mobile phase] or Sep-Pak cartridge (C18 classic column) using water and ethanol as eluting system. Reagent grade chemicals were used, and solvents were dried by reflux over and distillation from CaH₂ (except for THF/potassium) under argon. The 4-C-substituted SRH analogues need to be handled with care and stored in a refrigerator (\sim 4°C) in solid or dried oil state.

4.2. 2,3-O-Isopropylidene-5-O-tritylribitol (7)

NaBH₄ (91 mg, 2.4 mmol) was added to a stirred solution of **6** [42] (865 mg, 2.0 mmol) in EtOH (20 mL) at 0°C (ice bath) under N₂ atmosphere. After 1 h, the reaction mixture was partitioned between NaHCO₃/H₂O and EtOAc. The organic layer was dried over anhydrous MgSO₄ and evaporated. The residue was column chromatographed (30% hexane/EtOAc) to give **7** [24] (807 mg, 93%): ¹H NMR δ 1.35 (s, 3H, CH₃), 1.37 (s, 3H, CH₃), 2.96 (d, J = 3.6 Hz, 1H, OH), 3.08 (dd, J = 5.0, 8.4 Hz, 1H, H1), 3.34 (dd, J = 6.9, 9.8 Hz, 1H, H5), 3.50 (dd, J = 2.9, 9.8 Hz, 1H, H5'), 3.75–3.81 (m, 1H, H1'), 3.83–3.91 (m, 1H, H4), 4.10–4.17 (m, 1H, H2), 4.33–4.40 (m, 1H, H3), 7.25–7.38 (m, 15H, Ar); MS (ESI⁺) m/z 457 (M+Na⁺).

4.3. 1-O-tert-Butyldimethylsilyl-2,3-O-isopropylidene-5-O-tritylribitol (8)

TBDMSCl (302 mg, 2.0 mmol) and imidazole (204 mg, 3.0 mmol) were added to a solution of **7** (652 mg, 1.5 mmol) in DMF (10 mL) at room temperature and stirring was continued for 72 h. The volatiles were evaporated and the residue was partitioned between saturated NH₄Cl/H₂O and EtOAc. The separated organic layer was then washed with NaHCO₃/H₂O, dried over Mg₂SO₄, evaporated and the resulting residue was column chromatographed (50% hexane/EtOAc) to give **8** [24] (666 mg, 81%) as an amorphous white powder: ¹H NMR δ 0.08 (s, 6H, SiMe₂), 0.81 (s, 9H, *t*-Bu), 1.35 (s, 3H, CH₃), 1.37 (s, 3H, CH₃), 3.20 (dd, J = 5.3, 9.7 Hz, 1H, H5), 3.25 (dd, J = 2.8, 9.7 Hz, 1H, H5'), 3.49 (dd, J = 4.1, 10.6 Hz, 1H, H1), 3.68 (dd, J = 8.7, 10.5 Hz, 1H, H1'), 3.79–3.81 (m, 1H, H4), 4.13–4.15 (m, 1H, H2), 4.22 (dd, J = 5.5, 9.2 Hz, 1H, H3), 7.25–7.38 (m, 15H, Ar); MS (ESI⁺) m/z 571 (M+Na⁺).

4.4. 1-O-tert-Butyldimethylsilyl-2,3-O-isopropylidene-5-O-tritylribitol-4-ulose (9)

Method A. The Dess-Martin reagent (2.35 mL of 15 wt% solution/CH₂Cl₂; 381 mg, 0.9 mmol) was added to a solution of **8** (330 mg, 0.6 mmol) in CH₂Cl₂ (8 mL) at room temperature and stirred for 3 h. The reaction mixture was partitioned between saturated NaHCO₃ (10 mL)/dilute Na₂S₂O₃ (5 mL) and CH₂Cl₂ (15 mL). The organic layer was dried over anhydrous MgSO₄ and evaporated. The residue was column chromatographed (85% hexane/EtOAc) to give **9** [24] (300 mg, 91%) as an oil: ¹H NMR δ 0.08 (s, 6H, SiMe₂), 0.81 (s, 9H, *t*-Bu), 1.35 (s, 3H, CH₃), 1.37 (s, 3H, CH₃), 3.68 (dd, J = 4.2, 11.2 Hz, 1H, H1), 3.76 (dd, J = 4.0, 11.2 Hz, 1H, H1'), 4.04 (d, J = 17.7 Hz, 1H, H5), 4.20 (d, J = 17.8 Hz, 1H, H5'), 4.51–4.53 (m, 1H, H2), 4.71 (d, J = 7.8 Hz, 1H, H3), 7.25–7.38 (m, 15H, Ar); MS (ESI⁺) m/z 569 (M+Na⁺).

Method B. A freshly prepared solution of the Collins reagent [CrO_3 (144 mg, 1.44 mmol), pyridine (0.116 mL, 114 mg, 1.44 mmol), and Ac_2O (0.272 mL, 294 mg, 2.88 mmol) in CH_2Cl_2 (2 mL)] was added to a stirred solution of **8** (200 mg, 0.36 mmol) in CH_2Cl_2 (8 mL) at ambient temperature. The resulting mixture was stirred for 1 h and was immediately column chromatographed (EtOAc) to give **9** (185 mg, 93%) with spectra properties as above.

4.5. General procedure for the synthesis of 4-C-substituted ribitols 10

RMgBr reagent (0.75 mmol) was added to a stirred solution of **9** (205 mg, 0.375 mmol) in anhydrous THF (5 mL) at -78°C under N_2 atmosphere. After 15 min, the reaction mixture was allowed to warm up to ambient temperature and was kept stirring for 2 h. The reaction was then quenched by the addition of MeOH (1 mL) and diluted with EtOAc (15 mL). The resulting mixture was washed with 0.1 N HCl and the organic layer dried over anhydrous MgSO_4 . Volatiles were evaporated and the crude residue was then purified by flash column chromatography (90% hexane/EtOAc).

4.5.1. 1-O-tert-Butyldimethylsilyl-2,3-O-isopropylidene-4-C-methyl-5-O-tritylribitol (**10a**)

Treatment of **9** (205 mg, 0.375 mmol) with MeMgBr (1 M/THF; 0.75 mL, 0.75 mmol), using the procedure reported in Section 4.5, gave **10a** [24] (180 mg, 85%) as a clear oil: ^1H NMR δ 0.08 (s, 6H, SiMe_2), 0.81 (s, 9H, *t*-Bu), 1.35 (s, 3H, CH_3), 1.40 (s, 6H, $2 \times \text{CH}_3$), 3.01 (d, $J = 8.7$ Hz, 1H, H5), 3.12 (d, $J = 8.7$ Hz, 1H, H5'), 3.25 (dd, $J = 3.8, 10.9$ Hz, 1H, H1), 3.70 (dd, $J = 3.8, 10.9$ Hz, 1H, H1'), 3.90–3.95 (m, 1H, H2), 4.40 (d, $J = 5.5$ Hz, 1H, H3), 7.25–7.38 (m, 15H, Ar); HRMS calcd for $\text{C}_{34}\text{H}_{46}\text{O}_5\text{SiNa}^+ [\text{M}+\text{Na}]^+$ 585.3007, found 585.3009.

4.5.2. 1-O-tert-Butyldimethylsilyl-4-C-hexyl-2,3-O-isopropylidene-5-O-tritylribitol (**10b**)

Treatment of **9** (165 mg, 0.30 mmol) with *n*- $\text{C}_6\text{H}_{13}\text{MgBr}$ (0.8 M/THF; 0.75 mL, 0.6 mmol), using the procedure reported in Section 4.5 (flash column chromatography; 80% hexane/EtOAc), gave **10b** (140 mg, 74%) as a clear oil: ^1H NMR δ 0.08 (s, 6H, SiMe_2), 0.81 (s, 9H, *t*-Bu), 0.89 (t, $J = 6.6$ Hz, 3H, H6a), 1.30–1.40 (m, 8H, H2a–H5a), 1.40 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 1.50–1.60 (m, 2H, H1a), 3.06 (d, $J = 8.9$ Hz, 1H, H5), 3.22 (d, $J = 8.9$ Hz, 1H, H5'), 3.28 (dd, $J = 3.8, 11.1$ Hz, 1H, H1), 3.72 (dd, $J = 7.6, 11.0$ Hz, 1H, H1'), 3.80–3.85 (m, 1H, H2), 4.40 (d, $J = 5.2$ Hz, 1H, H3), 7.25–7.38 (m, 15H, Ar); HRMS calcd for $\text{C}_{39}\text{H}_{56}\text{O}_5\text{SiNa}^+ [\text{M}+\text{Na}]^+$ 655.3789, found 655.3799.

4.5.3. 1-O-tert-Butyldimethylsilyl-2,3-O-isopropylidene-4-C-octyl-5-O-tritylribitol (**10c**)

Treatment of **9** (480 mg, 0.87 mmol) with *n*- $\text{C}_8\text{H}_{17}\text{MgBr}$ (2 M/THF; 0.87 mL, 1.74 mmol), using the procedure reported in Section 4.5, gave **10c** (402 mg, 69%) as a clear oil: ^1H NMR δ 0.08 (s, 6H, SiMe_2), 0.81 (s, 9H, *t*-Bu), 0.89 (t, $J = 6.6$ Hz, 3H, H8a), 1.28–1.30 (m, 12H, H2a–H7a), 1.38 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 1.50–1.60 (m, 2H, H1a), 3.05 (d, $J = 8.9$ Hz, 1H, H5), 3.21 (d, $J = 8.9$ Hz, 1H, H5'), 3.26 (dd, $J = 3.8, 11.0$ Hz, 1H, H1), 3.70 (dd, $J = 3.9, 11.0$ Hz, 1H, H1'), 3.80–3.85 (m, 1H, H2), 4.40 (d, $J = 5.2$ Hz, 1H, H3), 7.25–7.38 (m, 15H, Ar); HRMS calcd for $\text{C}_{43}\text{H}_{68}\text{O}_5\text{SiNa}^+ [\text{M}+\text{Na}]^+$ 737.5007, found 737.5009.

H3), 7.25–7.38 (m, 15H, Ar); HRMS calcd for $C_{41}H_{60}O_5SiNa^+$ $[M+Na]^+$ 683.4102, found 683.4128.

4.5.4. 1-O-tert-Butyldimethysilyl-2,3-O-isopropylidene-5-O-trityl-4-C-vinylribitol (10d)

Treatment of **9** (120 mg, 0.21 mmol) with vinyl-MgBr (1 M/THF; 0.42 mL, 0.42 mmol), using the procedure reported in Section 4.5, gave **10d** (78 mg, 61%) as a clear oil: 1H NMR δ 0.08 (s, 6H, SiMe₂), 0.81 (s, 9H, *t*-Bu), 1.35 (s, 3H, CH₃), 1.37 (s, 3H, CH₃), 2.92 (d, J = 9.0 Hz, 1H, H5), 3.12 (d, J = 9.0 Hz, 1H, H5'), 3.42 (dd, J = 4.5, 10.7 Hz, 1H, H1), 3.80 (dd, J = 8.5, 10.7 Hz, 1H, H1'), 4.00–4.13 (m, 1H, H2), 4.69 (d, J = 6.1 Hz, 1H, H3), 5.16 (dd, J = 1.7, 10.9 Hz, 1H, CH=CHH), 5.40 (dd, J = 1.8, 17.4 Hz, 1H, CH=CHH), 6.20 (dd, J = 10.9, 17.4 Hz, 1H, CH=CHH), 7.25–7.38 (m, 15H, Ar); ^{13}C NMR δ –5.40 (SiMe₂), 21.02 and 25.29 (CMe₂), 25.77 (SiCMe₃), 31.58 (SiCMe₃), 60.47 (CPh₃), 61.98 (C1), 69.13 (C5), 74.87 (C4), 78.15 (C2), 78.45 (C3), 107.87 (CH=CH₂), 114.84 (CMe₂), 126.91 and 127.71 and 128.87 and 143.40 (Ar), 146.89 (CH=CH₂); HRMS calcd for $C_{35}H_{46}O_5SiNa^+$ $[M+Na]^+$ 597.3007; found 597.3006.

4.5.5. 1-O-tert-Butyldimethysilyl-2,3-O-isopropylidene-4-C-4-methoxyphenyl-5-O-tritylribitol (10e)

Treatment of **9** (165 mg, 0.30 mmol) with 4-MeOC₆H₄MgBr (1 M/THF; 0.60 mL, 0.60 mmol), using the procedure reported in Section 4.5, gave **10e** (190 mg, 96%) as a clear oil: 1H NMR δ 0.08 (s, 6H, SiMe₂), 0.81 (s, 9H, *t*-Bu), 1.35 (s, 3H, CH₃), 1.37 (s, 3H, CH₃), 3.01 (d, J = 9.1 Hz, 1H, H5), 3.15 (d, J = 9.1 Hz, 1H, H5'), 3.41 (dd, J = 4.5, 10.8 Hz, 1H, H1), 3.80 (s, 3H, CH₃O), 3.85 (dd, J = 4.5, 10.8 Hz, 1H, H1'), 4.19–4.22 (m, 1H, H2), 5.05 (d, J = 6.4 Hz, 1H, H3), 6.85 (d, J = 6.9 Hz, 2H, Ar), 7.25–7.38 (m, 15H, Ar), 7.61 (d, J = 8.9 Hz, 2H, Ar); HRMS calcd for $C_{40}H_{50}O_6SiNa^+$ $[M+Na]^+$ 677.3269, found 677.32567.

4.6. General procedure for desilylation of 4-C-substituted ribitols

TBAF (1 M/THF; 0.4 mL, 0.4 mmol) was added to a stirred solution of **10** (0.33 mmol) in THF (6 mL) at 0°C (ice bath). After 1 h, the volatiles were evaporated and the resulting residue was washed with NaHCO₃/H₂O and extracted with EtOAc. The organic layer was then dried over Mg₂SO₄ and evaporated to give the crude residue which was column chromatographed (80 → 70% hexane/EtOAc).

4.6.1. 2,3-O-Isopropylidene-4-C-methyl-5-O-tritylribitol (11a)

Treatment of **10a** (189 mg, 0.33 mmol) with TBAF, using the procedure reported in Section 4.6, gave **11a** (118 mg, 78%): 1H NMR δ 1.35 (s, 3H, CH₃), 1.40 (s, 3H, CH₃), 1.49 (s, 3H, CH₃), 3.05 (d, J = 9.0 Hz, 1H, H5), 3.32 (d, J = 9.0 Hz, 1H, H5'), 3.58 (dd, J = 5.2, 12.0 Hz, 1H, H1), 3.75 (dd, J = 5.5, 12 Hz, 1H, H1'), 4.10–4.20 (m, 1H, H2), 4.30 (d, J = 6.2 Hz, 1H, H3), 7.25–7.38 (m, 15H, Ar); ^{13}C NMR δ 14.21 (C1a), 25.17 and 27.28 (CMe₂), 60.42 (C5), 61.43 (C1), 67.95 (CPh₃), 68.38 (C4), 77.61 (C2), 79.10 (C3), 107.67 (CMe₂), 127.27 and 127.97 and 128.62 and 143.43 (Ar); HRMS calcd for $C_{28}H_{32}O_5Na^+$ $[M+Na]^+$ 471.2142; found 471.2158.

4.6.2. 2,3-O-Isopropylidene-4-C-hexyl-5-O-tritylribitol (11b)

Treatment of **10b** (280 mg, 0.44 mmol) with TBAF, using the procedure reported in Section 4.6, gave **11b** (145 mg, 87%) as a viscous oil: ^1H NMR δ 0.85 (t, $J = 6.6$ Hz, 3H, H6a), 1.30–1.40 (m, 8H, H2a–H5a), 1.25 (s, 3H, CH₃), 1.40 (s, 3H, CH₃), 1.50–1.60 (m, 2H, H1a), 2.91 (d, $J = 9.3$ Hz, 1H, H5), 3.20 (d, $J = 9.3$ Hz, 1H, H5'), 3.30 (dd, $J = 5.0, 12.2$ Hz, 1H, H1), 3.40 (dd, $J = 5.0, 12.0$ Hz, 1H, H1'), 3.80 (q, $J = 5.8$ Hz, 1H, H2), 4.15 (d, $J = 5.8$ Hz, 1H, H3), 7.25–7.38 (m, 15H, Ar); ^{13}C NMR δ 14.14 (C6a), 22.61, 23.06, 29.74, 31.83 (C2a–C5a), 25.45 and 27.57 (CMe₂), 36.05 (C1a), 61.88 (C1), 64.11 (C5), 74.35 (CPh₃), 77.55 (C4), 79.14 (C2), 86.92 (C3), 107.21 (CMe₂), 127.31 and 127.96 and 128.65 and 143.37 (Ar); HRMS calcd for C₃₃H₄₂O₅Na⁺ [M+Na]⁺ 541.2924; found 541.2924.

4.6.3. 2,3-O-Isopropylidene-4-C-octyl-5-O-tritylribitol (11c)

Treatment of **10c** (350 mg, 0.52 mmol) with TBAF, using the procedure reported in Section 4.6, gave **11c** (200 mg, 84%) as a viscous oil. ^1H NMR δ 0.80 (t, $J = 6.6$ Hz, 3H, H8a), 1.20–1.30 (m, 12H, H2a–H7a), 1.29 (s, 3H, CH₃), 1.40 (s, CH₃), 1.50–1.60 (m, 2H, H1a), 2.95 (d, $J = 9.4$ Hz, 1H, H5), 3.25 (d, $J = 9.3$ Hz, 1H, H5'), 3.35 (dd, $J = 4.92, 12.2$ Hz, 1H, H1), 3.45 (dd, $J = 5.4, 12.2$ Hz, 1H, H1'), 3.87–3.94 (m, 1H, H2), 4.10 (d, $J = 5.8$ Hz, 1H, H3), 7.25–7.38 (m, 15H, Ar); ^{13}C NMR δ 14.24 (C8a), 22.79, 23.17, 29.35, 29.68, 30.18, 32.00 (C2a–C7a), 25.54 and 27.65 (CMe₂), 36.13 (C1a), 61.94 (C1), 64.27 (C5), 74.41 (CPh₃), 77.65 (C4), 79.28 (C2), 87.00 (C3), 107.27 (CMe₂), 127.37, 128.03, 128.75 and 143.49 (Ar); HRMS calcd for C₃₅H₄₆O₅Na⁺ [M+Na]⁺ 569.3237; found 569.3237.

4.6.4. 2,3-O-Isopropylidene-4-C-vinyl-5-O-tritylribitol (11d)

Treatment of **10d** (200 mg, 0.34 mmol) with TBAF, using the procedure reported in Section 4.6, gave **11d** (124 mg, 77%) as a viscous oil: ^1H NMR δ 1.35 (s, 3H, CH₃), 1.37 (s, 3H, CH₃), 3.38 (d, $J = 11.2$ Hz, 1H, H5), 3.54 (d, $J = 11.2$ Hz, 1H, H5'), 3.58 (dd, $J = 5.1, 11.3$ Hz, 1H, H1), 3.90 (d, $J = 8.6, 11.3$ Hz, 1H, H1'), 4.25–4.30 (m, 1H, H2), 4.32 (d, $J = 6.5$ Hz, 1H, H3), 5.20 (dd, $J = 1.2, 11.0$ Hz, 1H, CH=CHH), 5.40 (dd, $J = 1.5, 17.6$ Hz, 1H, CH=CHH), 6.20 (dd, $J = 11.0, 17.5$ Hz, 1H, CH=CHH), 7.25–7.38 (m, 15H, Ar); ^{13}C NMR δ 24.65 and 27.07 (CMe₂), 60.29 (CPh₃), 60.62 (C1), 68.07 (C5), 77.90 (C2), 78.10 (C4), 82.01 (C3), 115.48 (CMe₂), 116.03 (CH=CH₂), 126.82 and 127.65 and 128.75 and 143.28 (Ar), 146.88 (CH=CH₂); HRMS calcd for C₂₉H₃₂O₅Na⁺ [M+Na]⁺ 483.2142, found 483.2131.

4.6.5. 2,3-O-Isopropylidene-4-C-4-methoxyphenyl-5-O-tritylribitol (11e)

Treatment of **10e** (190 mg, 0.29 mmol) with TBAF, using the procedure reported in Section 4.6, gave **11e** (117 mg, 75%) as a viscous oil: ^1H NMR δ 1.35 (s, 3H, CH₃), 1.37 (s, 3H, CH₃), 3.01 (dd, $J = 6.0, 11.7$ Hz, 1H, H1), 3.03 (d, $J = 9.0$ Hz, 1H, H5), 3.23 (dd, $J = 6.0, 11.7$ Hz, 1H, H1'), 3.45 (d, $J = 9.0$ Hz, 1H, H5'), 3.72 (s, 3H, CH₃O), 4.19–4.22 (m, 1H, H2), 4.75 (d, $J = 6.6$ Hz, 1H, H3), 6.85 (d, $J = 6.9$ Hz, 2H, Ar), 7.61 (d, $J = 8.9$ Hz, 2H, Ar), 7.25–7.38 (m, 15H, Ar); ^{13}C NMR δ 24.58 and 27.02 (CMe₂), 55.19 (CH₃O), 60.04 (CPh₃), 61.28 (C1), 69.73 (C5), 78.08 (C2), 79.05 (C3), 82.02 (C4), 113.48 (CMe₂), 127.25, 127.37, 127.92, 159.10 (Ar), 128.57 and 129.68 and 132.91 and 146.88 (Ar); HRMS calcd for C₃₄H₃₆O₆Na⁺ [M+Na]⁺ 563.2404; found 563.2386.

4.7. General procedure for the synthesis of 4-C-substituted ribono-1,4-lactones **12**

N-Methylmorpholine *N*-oxide (NMO; 50 mg, 0.42 mmol) and tetrapropylammonium per-ruthenate (TPAP; 1 mg, 0.002 mmol) were added to a stirred solution of **11** (0.11 mmol) in CH₂Cl₂ (3.5 mL) at ambient temperature under N₂ atmosphere. After 6 h, the reaction mixture was filtered off and the filtrate was dried over MgSO₄ and evaporated. The residue was purified by flash column chromatography (75 → 50% hexane/EtOAc) to give **12**.

Note: Treatment of **11** with NMO and TPAP, as described above, in the presence of 4 Å molecular sieves (100 mg) also gave lactones **12** in similar yields.

4.7.1. 2,3-O-Isopropylidene-4-C-methyl-5-O-trityl-D-ribono-1,4-lactone (**12a**)

Treatment of **11a** (51 mg, 0.11 mmol) with NMO/TPAP, using the procedure reported in Section 4.7, gave **12a** (40 mg, 80%): ¹H NMR δ 1.30 (s, 3H, CH₃), 1.35 (s, 3H, CH₃), 1.40 (s, 3H, CH₃), 2.91 (d, *J* = 10.2 Hz, 1H, H5), 3.50 (d, *J* = 10.2 Hz, 1H, H5'), 4.20 (d, *J* = 5.6 Hz, 1H, H2), 5.01 (d, *J* = 5.6 Hz, 1H, H3), 7.25–7.38 (m, 15H, Ar); ¹³C NMR δ 16.40 (C1a) 25.90 and 26.78 (CMe₂), 66.65 (CPh₃), 67.63 (C5), 77.70 (C2), 79.77 (C3), 88.56 (C4), 126.07 (CMe₂), 127.46 and 128.25 and 128.67 and 146.87 (Ar), 172.07 (C1); HRMS calcd for C₂₈H₂₈O₅Na⁺ [M+Na]⁺ 467.1829, found 467.1847.

4.7.2. 2,3-O-Isopropylidene-4-C-hexyl-5-O-trityl-D-ribono-1,4-lactone (**12b**)

Treatment of **11b** (37 mg, 0.09 mmol) with NMO/TPAP, using the procedure reported in Section 4.7, gave **12b** (35 mg, 94%): ¹H NMR δ 0.80 (t, *J* = 6.6 Hz, 3H, H6a), 1.19–1.21 (m, 8H, H2a–H5a), 1.24 (s, 3H, CH₃), 1.40 (s, 3H, CH₃), 1.50–1.60 (m, 2H, H1a), 2.85 (d, *J* = 10.2 Hz, 1H, H5), 3.51 (d, *J* = 10.2 Hz, 1H, H5'), 4.10 (d, *J* = 5.6 Hz, 1H, H2), 5.01 (d, *J* = 5.6 Hz, 1H, H3), 7.25–7.38 (m, 15H, Ar); ¹³C NMR δ 13.98 (C6a), 22.45, 23.33, 29.42, 31.30 (C2a–C5a), 25.91 and 26.76 (CMe₂), 31.60 (C1a), 66.29 (C5), 77.57 (C3), 80.11 (C2), 87.75 (C4), 88.26 (CPh₃), 112.92 (CMe₂), 127.41, 128.16, 128.52 and 142.95 (Ar), 174.41 (C1); MS (ESI⁺) *m/z* 532 (M+NH₄)⁺.

4.7.3. 2,3-O-Isopropylidene-4-C-octyl-5-O-trityl-D-ribono-1,4-lactone (**12c**)

Treatment of **11c** (48 mg, 0.08 mmol) with NMO/TPAP, using the procedure reported in Section 4.7, gave **12c** (32 mg, 95%): ¹H NMR δ 0.80–0.84 (m, 3H, H8a), 1.10–1.20 (s, 12H, H2a–H7a), 1.25 (s, CH₃), 1.40 (s, CH₃), 1.50–1.60 (m, 2H, H1a), 2.90 (d, *J* = 10.1 Hz, 1H, H5), 3.55 (d, *J* = 10.2 Hz, 1H, H5'), 4.10 (d, *J* = 5.6 Hz, 1H, H2), 5.01 (d, *J* = 5.6 Hz, 1H, H3), 7.25–7.38 (m, 15H, Ar); ¹³C NMR δ 14.23 (C8a), 22.77, 23.50, 29.24, 29.29, 30.06, 31.72 (C2a–C7a), 26.06 and 26.92 (CMe₂), 31.93 (C1a), 66.39 (C5), 77.57 (C3), 80.11 (C2), 87.94 (C4), 88.38 (CPh₃), 113.07 (CMe₂), 127.56, 128.31, 128.65, 143.07 (Ar), 174.63 (C1); MS (ESI⁺) *m/z* 560 (M+NH₄)⁺.

Note: When oxidation of **11c** was carried out overnight instead of 6 h, a second minor product (~30%) was formed in addition to **12c**. The minor product had the following ribosyl peaks: ¹H NMR δ 3.75 (d, *J* = 11.6 Hz, 1H), 3.91 (d, *J* = 11.6 Hz, 1H), 4.60 (d, *J* = 5.6 Hz, 1H), 4.90 (d, *J* = 5.6 Hz, 1H).

4.7.4. 2,3-O-Isopropylidene-5-O-trityl-4-C-vinyl-D-ribono-1,4-lactone (**12d**)

Treatment of **11d** (100 mg, 0.21 mmol) with NMO/TPAP, using the procedure reported in Section 4.7, gave **12d** (90 mg, 95%): ¹H NMR δ 1.22 (s, 3H, CH₃), 1.26 (s, 3H, CH₃), 2.91

(d, $J = 10.2$ Hz, 1H, H5), 3.50 (d, $J = 10.3$ Hz, H1, H5'), 4.20 (d, $J = 5.6$ Hz, 1H, H2), 5.00 (d, $J = 5.6$ Hz, 1H, H3), 5.21 (d, $J = 11.2$ Hz, 1H, CH=CHH), 5.34 (d, $J = 17.4$ Hz, 1H, CHH), 5.61 (dd, $J = 11.2, 17.4$ Hz, 1H, CH=CHH), 7.25–7.38 (m, 15 H); ^{13}C NMR δ 25.92 and 26.64 (CMe₂), 53.50 (CPh₃), 65.50 (C5), 80.01 (C2), 82.03 (C3), 88.87 (C4), 112.03 (CH=CH₂), 118.50 (CMe₂), 127.29, 127.95, 129.69 and 145.01 (Ar), 146.85 (CH=CH₂), 174.14 (C1); HRMS (TOF) m/z calcd for C₂₉H₂₈O₅Na⁺ [M+Na]⁺ 479.1829; found 479.1829.

4.7.5. 2,3-O-Isopropylidene-4-C-4-methoxyphenyl-5-O-trityl-D-ribo-1,4-lactone (12e)

Treatment of **11e** (90 mg, 0.16 mmol) with NMO/TPAP, using the procedure reported in Section 4.7, gave **12e** (73 mg, 82%): ^1H NMR δ 1.22 (s, 3H, CH₃), 1.25 (s, 3H, CH₃), 3.25 (d, $J = 10.5$ Hz, 1H, H5'), 3.35 (d, $J = 10.5$ Hz, 1H, H5), 3.80 (s, 3H, CH₃O), 4.48 (d, $J = 5.5$ Hz, 1H, H2), 5.20 (d, $J = 5.5$ Hz, 1H, H3), 6.82 (d, $J = 8.8$ Hz, 2H, Ar), 7.12 (d, $J = 9.7$ Hz, 2H, Ar), 7.25–7.38 (m, 15H, Ar); ^{13}C NMR δ 25.92 and 26.64 (CMe₂), 55.18 (CH₃O), 62.94 (CPh₃), 68.79 (C5), 77.71 (C2), 80.77 (C3), 88.87 (C4), 113.60 (CMe₂), 126.9, 127.25, 128.75 and 159.10, (Ar), 128.57, 129.68, 132.91 and 146.40 (Ar), 174.14 (C1); MS (ESI) m/z 554 (M+NH₄)⁺.

4.8. General procedure for detritylation of 4-C-substituted ribono-1,4-lactones

TFA (0.5 mL) and CH₂Cl₂ (5 mL) were added to a stirred solution of **12** (0.1 mmol) at ambient temperature for 6 h. The volatiles were evaporated and the residue co-evaporated with toluene. The oily residue was partitioned between aqueous NaHCO₃ and CH₂Cl₂. The separated organic layer was washed with brine, dried (MgSO₄), evaporated and was purified on a silica gel column (hexane/EtOAc, 8:2).

4.8.1. 2,3-O-Isopropylidene-4-C-methyl-D-ribo-1,4-lactone (13a)

Treatment of **12a** (44 mg, 0.1 mmol) with TFA, using the procedure reported in Section 4.8, gave **13a** (13 mg, 66%): ^1H NMR δ 1.32 (s, 3H, CH₃), 1.35 (s, 3H, CH₃), 1.42 (s, 3H, CH₃), 3.60 (d, $J = 11.6$ Hz, 1H, H5), 3.71 (d, $J = 11.6$ Hz, H1, H5'), 4.55 (d, $J = 5.6$ Hz, 1H, H2), 4.90 (d, $J = 5.6$ Hz, 1H, H3); ^{13}C NMR δ 16.42 (C1a), 25.81 and 26.76 (CMe₂), 67.61 (C5), 77.82 (C2), 80.01 (C3), 86.35 (C4), 113.02 (CMe₂), 174.46 (C1); HRMS (TOF) m/z calcd for C₉H₁₄O₅Na⁺ [M+Na]⁺ 225.0733; found 225.0734.

4.8.2. 2,3-O-Isopropylidene-4-C-hexyl-D-ribo-1,4-lactone (13b)

Treatment of **12b** (31 mg, 0.06 mmol) with TFA, using the procedure reported in Section 4.8 (flash column chromatography; 80% hexane/EtOAc), gave **13b** (12.5 mg, 80%): ^1H NMR δ 0.80 (t, $J = 6.6$ Hz, 3H, H6a), 1.19–1.21 (m, 8H, H2a–H5a), 1.41 (s, 3H, CH₃), 1.50 (s, 3H, CH₃), 1.50–1.60 (m, 2H, H1a), 3.75 (d, $J = 11.8$ Hz, 1H, H5), 3.85 (d, $J = 11.4$ Hz, 1H, H5'), 4.65 (d, $J = 5.6$ Hz, 1H, H2), 4.96 (d, $J = 5.6$ Hz, 1H, H3); ^{13}C NMR δ 14.06 (C6a), 22.47, 23.54, 29.42, 31.48 (C2a–C5a), 25.79 and 26.78 (CMe₂), 31.91 (C1a), 65.83 (C5), 77.20 (C3), 80.63 (C2), 89.73 (C4), 112.85 (CMe₂), 172.67 (C1); HRMS (TOF) m/z calcd for C₁₄H₂₄O₅Na⁺ [M+Na]⁺ 295.1516, found 295.1516.

4.8.3. 2,3-O-Isopropylidene-4-C-octyl-D-ribo-1,4-lactone (**13c**)

Treatment of **12c** (35 mg, 0.065 mmol) with TFA, using the procedure reported in Section 4.8, gave **13c** (16 mg, 75%): ^1H NMR δ 0.80 (t, $J = 6.6$ Hz, 3H, H8a), 1.30–1.32 (m, 12H, H2a–H7a), 1.40 (s, 3H, CH₃), 1.50 (s, 3H, CH₃), 1.50–1.60 (m, 2H, H1a), 3.75 (d, $J = 11.6$ Hz, 1H, H5), 3.85 (d, $J = 11.6$ Hz, 1H, H5'), 4.65 (d, $J = 5.6$ Hz, 1H, H2), 4.95 (d, $J = 5.6$ Hz, 1H, H3); ^{13}C NMR δ 14.25 (CH₃, C8a), 22.74, 23.64, 29.30, 29.41, 30.21, 31.27 (C2a–C7a), 25.93 and 26.9 (CMe₂), 31.98 (C1a), 65.92 (C5), 77.46 (C3), 79.91 (C2), 88.94 (C4), 113.0 (CMe₂), 174.98 (C1); HRMS (TOF) m/z calcd for C₁₆H₂₈O₅Na⁺ [M+Na]⁺ 323.1829; found 323.1915.

4.8.4. 2,3-O-Isopropylidene-4-C-vinyl-D-ribo-1,4-lactone (**13d**)

Treatment of **12d** (30 mg, 0.065 mmol) with TFA, using the procedure reported in Section 4.8 (flash column chromatography; 80% hexane/EtOAc), gave somehow an unstable product **13d** (5 mg, 35%): ^1H NMR δ 1.22 (s, 3H, CH₃), 1.26 (s, 3H, CH₃), 3.61 (d, $J = 11.7$ Hz, 1H, H5), 3.65 (d, $J = 11.8$ Hz, 1H, H5'), 4.60 (d, $J = 5.6$ Hz, 1H, H2), 4.82 (d, $J = 5.6$ Hz, 1H, H3), 5.24 (d, $J = 11.3$ Hz, 1H, CH=CHH), 5.34 (d, $J = 17.5$ Hz, 1H, C=CHH), 5.71 (dd, $J = 11.2, 17.5$ Hz, 1H, CH=CHH); ^{13}C NMR δ 25.87, 26.70 (CMe₂), 66.37 (C5), 76.70 (C2), 80.01 (C3), 87.95 (C4), 113.29 (CMe₂), 118.21 (CH=CH₂), 130.53 (CH=CH₂), 174.06 (C1); HRMS (TOF) m/z calcd for C₁₀H₁₄O₅Na⁺ [M+Na]⁺ 237.0733; found 237.0733.

4.8.5. 2,3-O-Isopropylidene-4-C-4-methoxyphenyl-D-ribo-1,4-lactone (**13e**)

Treatment of **12e** (70 mg, 0.13 mmol) with TFA, using the procedure reported in Section 4.8 (flash column chromatography; 80% hexane/EtOAc), gave **13e** (23 mg, 70%): ^1H NMR δ 1.22 (s, 3H, CH₃), 1.25 (s, 3H, CH₃), 3.80 (s, 3H, CH₃O), 3.85 (d, $J = 12.3$ Hz, 1H, H5), 3.95 (d, $J = 12.5$ Hz, 1H, H5'), 4.95 (d, $J = 5.4$ Hz, 1H, H2), 5.15 (d, $J = 5.3$ Hz, 1H, H3), 6.82 (d, $J = 8.8$ Hz, 2H, Ar), 7.12 (d, $J = 9.0$ Hz, 2H, Ar); ^{13}C NMR δ 25.83 and 26.65 (CMe₂), 55.25 (CH₃O), 68.50 (C5), 77.41 (C2), 80.57 (C3), 90.12 (C4), 112.93 (CMe₂), 113.76, 126.17, 126.89, 159.33 (Ar); 174.86 (C1); HRMS (TOF) m/z calcd for C₁₅H₁₈O₆Na⁺ [M+Na]⁺ 317.0996; found 317.0982.

4.9. 2,3-O-Isopropylidene-4-C-hexyl-5-O-trityl-D-ribofuranose (**14b**)

LiEt₃BH (1M/THF, 0.18 mL, 0.18 mmol) was added dropwise to a solution of **12b** (36 mg, 0.07 mmol) in CH₂Cl₂ (0.5 mL) and the resulting mixture was stirred for 30 min at –20°C under N₂ atmosphere. The reaction was quenched with MeOH and the volatiles were evaporated. The residue was partitioned between CH₂Cl₂ and NaHCO₃, washed with brine and dried with anhydrous MgSO₄. The resulting oil was column chromatographed (75:15, hexane/EtOAc) to give **14b** (α/β ; 1:3; 20 mg, 54%). The major β -anomer had: ^1H NMR δ 0.80 (t, $J = 6.6$ Hz, 3H, H6a), 1.20–1.28 (m, 8H, H2a–H5a), 1.33 (s, 3H, CH₃), 1.41 (s, 3H, CH₃), 1.50–1.60 (m, 2H, H1a), 3.16 (d, $J = 10.1$ Hz, 1H, H5), 3.32 (d, $J = 10.1$ Hz, 1H, H5'), 3.74 (d, $J = 8.8$ Hz, OH), 4.50 (d, $J = 6.0$ Hz, 1H, H3), 4.75 (d, $J = 6.0$ Hz, 1H, H2), 5.15 (d, $J = 8.4$ Hz, 1H, H1). 7.25–7.38 (m, 15H, Ar). The minor α -anomer had: ^1H NMR δ 0.80 (t, $J = 6.6$ Hz, 3H, H6a), 1.20–1.28 (m, 8H, H2a–H5a), 1.33 (s, 3H, CH₃), 1.41 (s, 3H, CH₃), 1.50–1.60 (m, 2H, H1a), 2.94 (d, $J = 9.9$ Hz, 1H, H5), 3.25 (d, $J = 9.9$ Hz, 1H, H5'), 3.86 (d, $J = 11.5$ Hz, OH), 4.20 (d, $J = 6.1$ Hz, 1H, H3), 4.62 (dd, $J = 4.2, 6.1$ Hz, 1H, H2),

5.61 (dd, $J = 4.2, 11.5$ Hz, 1H, H1), 7.25–7.38 (m, 15H, Ar). ^{13}C NMR for the mixture of α/β -anomers: δ 14.01 (C6a), 23.61 and 23.94 (CMe₂), 24.57 and 24.98 (CMe₂), 25.99, 26.22, 29.65, 31.51 (C2a–C5a), 31.83 (C1a), 67.33 and 68.18 (C5), 80.01 and 82.78 (C2), 83.09 and 83.82 (C3), 88.05 and 88.12 (C4), 96.55 and 102.85 (C1), 112.06 and 112.32 (CMe₂), 127.22, 127.45, 127.97, 128.05, 128.65, 128.76, 142.90, 143.49 (Ar); HRMS (TOF-ESI) m/z calcd for C₃₃H₄₀O₅Na⁺ [M+Na]⁺ 539.2768; found 539.2789.

4.10. General procedure for the synthesis of 5-O-mesyl-4-C-substituted ribono-1,4-lactones 15

TEA (48 μL , 34 mg, 0.33 mmol) and MsCl (11.4 μL , 19.5 mg, 0.15 mmol) were added to a stirred solution of **13** (0.1 mmol) in dry CH₂Cl₂ (2 mL) at 0°C (ice bath) under N₂ atmosphere. After 1 h, the reaction mixture was partitioned between CH₂Cl₂ and diluted HCl. The separated organic layer was washed with aqueous solution of NaHCO₃, brine and dried over anhydrous MgSO₄. Volatiles were evaporated and the residue was purified on silica column chromatography (hexane/EtOAc, 6:4).

4.10.1. 2,3-O-Isopropylidene-4-C-hexyl-5-O-mesyl-D-ribo-1,4-lactone (**15b**)

Treatment of **13b** (27 mg, 0.1 mmol) with MsCl, using the procedure reported in Section 4.10, gave **15b** (21 mg, 60%): ^1H NMR δ 0.80 (t, $J = 6.6$ Hz, 3H, H6a), 1.20–1.28 (m, 8H, H2a–H5a), 1.33 (s, 3H, CH₃), 1.41 (s, 3H, CH₃), 1.50–1.60 (m, 2H, H1a), 2.99 (s, 3H, Ms), 4.20 (d, $J = 11.0$ Hz, 1H, H5), 4.32 (d, $J = 11.0$ Hz, 1H, H5'), 4.60 (d, $J = 5.7$ Hz, 1H, H2), 4.88 (d, $J = 5.7$ Hz, 1H, H3); ^{13}C NMR δ 14.03 (CH₃, C6a), 22.46, 23.36, 29.55, 31.38 (C2a–C5a), 25.78 and 26.69 (CMe₂), 31.45 (C1a), 37.62 (Ms), 71.80 (C5), 76.70 (C3), 78.91 (C2), 85.77 (C4), 113.79 (CMe₂), 173.21 (C1); HRMS (TOF-ESI) m/z calcd for C₁₅H₂₆O₇SN⁺ [M+Na]⁺ 373.1291; found 373.1307.

4.10.2. 2,3-O-Isopropylidene-5-O-mesyl-4-C-octyl-D-ribo-1,4-lactone (**15c**)

Treatment of **13c** (32 mg, 0.1 mmol) with MsCl, using the procedure reported in Section 4.10, gave **15c** (19 mg, 68%): ^1H NMR δ 0.80 (t, $J = 6.6$ Hz, 3H, H8a), 1.20–1.28 (m, 12H, H2a–H7a), 1.35 (s, 3H, CH₃), 1.41 (s, 3H, CH₃), 1.50–1.60 (m, 2H, H1a), 3.05 (s, 3H, Ms), 4.20 (d, $J = 10.9$ Hz, 1H, H5), 4.32 (d, $J = 10.9$ Hz, 1H, H5'), 4.60 (d, $J = 5.6$ Hz, 1H, H2), 4.84 (d, $J = 5.6$ Hz, 1H, H3); ^{13}C NMR δ 14.24 (CH₃, C8a), 22.78, 23.52, 29.22, 29.30, 30.02, 31.58 (C2a–C7a), 25.92 and 26.82 (CMe₂), 31.94 (C1a), 37.76 (Ms), 71.93 (C5), 76.88 (C3), 79.05 (C2), 85.91 (C4), 113.92 (CMe₂), 173.34 (C1); MS (ESI) m/z 401 (M+Na)⁺.

4.10.3. 2,3-O-Isopropylidene-5-O-mesyl-4-C-4-methoxyphenyl-D-ribo-1,4-lactone (**15e**)

Treatment of **13e** (24 mg, 0.08 mmol) with MsCl, using the procedure reported in Section 4.10, gave **15e** (25 mg, 83%): ^1H NMR δ 1.35 (s, 3H, CH₃), 1.41 (s, 3H, CH₃), 3.0 (s, 3H, Ms), 3.80 (s, 3H, CH₃O), 4.20 (d, $J = 11.2$ Hz, 1H, H5), 4.55 (d, $J = 11.2$ Hz, 1H, H5'), 5.01 (d, $J = 5.5$ Hz, 1H, H2), 5.15 (d, $J = 5.5$ Hz, 1H, H3), 6.82 (d, $J = 8.8$ Hz, 2H, Ar), 7.12 (d, $J = 9.0$ Hz, 2H, Ar); ^{13}C NMR δ 25.90 and 26.58 (CMe₂), 44.10 (Ms), 55.33 (CH₃O), 73.26 (C5), 77.35 (C2), 79.71 (C3), 87.04 (C4), 113.79 (CMe₂), 114.10, 124.91, 127.00, 159.95

(Ar); 173.01 (C1); HRMS (TOF-ESI) m/z calcd for $C_{16}H_{20}O_8SNa^+$ $[M+Na]^+$ 395.0771; found 395.0793.

4.11. General procedure for the synthesis of 4-C-substituted S-ribosylhomocysteine lactones 16

Step a. H_2O (0.24 mL) and tris(2-carboxyethyl)phosphine hydrochloride (88 mg, 0.31 mmol) were added to a stirred solution of *N,N'*-di(*tert*-butoxycarbonyl)-l-homocysteine di(*tert*-butyl) ester[13] (160 mg, 0.28 mmol) in anhydrous DMF (2.4 mL) at ambient temperature under Ar atmosphere. After 20 h, the reaction mixture was partitioned between EtOAc, and saturated $NaHCO_3/H_2O$. The aqueous layer was extracted with EtOAc, and the combined organic layer was washed with brine, dried (Na_2SO_4), and concentrated to give *N-tert*-butoxycarbonyl-l-homocysteine *tert*-butyl ester (159 mg, 99%) as a colorless oil of sufficient purity to be directly used in the next step.

Step b. LDA (2M/THF and heptanes, 48 μ L, 0.96 mmol) was added slowly to a stirred solution of the freshly prepared protected l-homocysteine (29 mg, 0.1 mmol; from step (a)) in anhydrous DMF 1.5 mL under Ar atmosphere at 0°C (ice bath). After 30 min, a solution of **15** (0.035 mmol) in DMF (1 mL) was added by a syringe and the mixture was left stirring for 15 min at 0°C then at room temperature for 24 h. The reaction was quenched with NH_4Cl and the volatiles were evaporated under high vacuum. The residue was partitioned between EtOAc and $NaHCO_3$, washed with brine and dried over anhydrous $MgSO_4$. Volatiles were evaporated and the resulting oil was column chromatographed (hexane/EtOAc, 8:2).

4.11.1. S-(2,3-O-Isopropylidene-4-C-hexyl-D-ribono-1,4-lactone-5-yl)-N-tert-butoxycarbonyl-l-homocysteine tert-butyl ester (16b)

Treatment of **15b** (11 mg, 0.031 mmol) with homocysteinate salt, using the procedure reported in Section 4.11, gave **16b** (18 mg, 65%): 1H NMR δ 0.80 (t, J = 6.6 Hz, 3H, H6a), 1.20–1.28 (m, 8H, H2a–H5a), 1.31 (s, 3H, CH_3), 1.39 and 1.41 (2 \times s, 2 \times 9H, 2 \times *t*-Bu), 1.42 (s, 3H, CH_3), 1.50–1.60 (m, 2H, H1a), 1.75–1.85 (m, 1H, H8), 1.95–2.05 (m, 1H, H8'), 2.46–2.54 (m, 2H, H7,7'), 2.72 (d, J = 14.7 Hz, 1H, H5), 2.80 (d, J = 14.7 Hz, 1H, H5'), 4.20–4.25 (m, 1H, H9), 4.40 (d, J = 5.9 Hz, 1H, H3), 5.00 (br. d, J = 7.8 Hz, 1H, NH), 5.10 (d, J = 5.9 Hz, 1H, H2); ^{13}C NMR δ 14.01 (C6a), 22.5 and 22.7 (CMe_2), 23.45, 25.66, 26.56, 30.67 (C2a–C5a), 27.9 (C7), 28.08 (*t*-Bu), 28.32 (*t*-Bu), 34.34 (C1a), 35.8 (C8), 39.86 (C5), 53.6 (C9), 77.0 (C2), 77.20 (C3), 80.59 (*t*-Bu), 82.41 (*t*-Bu), 88.98 (C4), 113.30 (CMe_2), 156.1 (Boc), 171.3 and 174.0 (C1 and C10); HRMS (TOF-ESI) calcd for $C_{27}H_{48}NO_8S^+$ $[M+H]^+$ 546.3095; found 546.3104.

4.11.2. S-(2,3-O-Isopropylidene-4-C-4-methoxyphenyl-D-ribono-1,4-lactone-5-yl)-N-tert-butoxycarbonyl-l-homocysteine tert-butyl ester (16e)

Treatment of **15e** (22 mg, 0.07 mmol) with homocysteinate salt, using the procedure reported in Section 4.11, gave **16e** (20 mg, 48%): 1H NMR δ 1.35 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.39 and 1.41 (2 \times s, 2 \times 9H, 2 \times *t*-Bu), 1.75–1.89 (m, 1H, H8), 1.95–2.05 (m, 1H, H8'), 2.52–2.68 (m, 2H, H7,7'), 2.85 (d, J = 14.8 Hz, 1H, H5), 3.20 (d, J = 15.0 Hz, 1H, H5'), 3.80 (s, 3H, OCH_3), 4.20–4.25 (m, 1H, H9), 4.80 (d, J = 5.8 Hz, 1H, H3), 5.01 (br. d, J = 8.1 Hz, 1H, NH), 5.30 (d, J = 5.8 Hz, 1H, H2), 6.82 (d, J = 8.8 Hz, 2H, Ar), 7.12 (d,

$J = 9.0$ Hz, 2H, Ar); ^{13}C NMR δ 25.9 and 26.5 (CMe_2), 28.1 (*t*-Bu), 28.4 (*t*-Bu), 28.9 (C7), 31.0 (C8), 43.9 (C5), 53.1 (C9), 55.4 (OCH_3), 77.16 (C2), 77.37 (C3), 81.6 (*t*-Bu), 82.6 (*t*-Bu), 90.2 (C4), 113.5 (CMe_2), 113.7, 126.7, 130.1, 156.2 (Ar), 155.8 (Boc), 171.2 and 172.0 (C1 and C10); HRMS (TOF-ESI) calcd for $\text{C}_{28}\text{H}_{41}\text{NO}_9\text{SNa}^+$ $[\text{M}+\text{Na}]^+$ 590.2394; found 590.2378.

4.12. General procedure for deprotection of 4-C-substituted S-ribosylhomocysteine lactones

Compound **16** (0.03 mmol) was stirred in TFA (2 mL) at 0°C for 1 h and then at ambient temperature for 3 h. H_2O (0.1 mL) was then added and stirring was continued for an additional 1 h. Volatiles were evaporated *in vacuum* below 30°C and the residue was coevaporated with MeCN (2×0.5 mL). The crude product was redissolved in deionized water (2.5 mL) and washed with CHCl_3 (2×1 mL). The aqueous layer was evaporated *in vacuum* below 30°C .

4.12.1. S-(4-C-Hexyl-D-ribo-1,4-lactone-5-yl)-l-homocysteine (**17b**)

Treatment of **16b** (17 mg, 0.03 mmol) with TFA, using the procedure reported in Section 4.12, gave **17b** (7 mg, 60%). This product was additionally purified by HPLC ($\text{CH}_3\text{CN}/\text{H}_2\text{O}$, 15:85; $t_{\text{R}} = 21.0$ min) to give 6.5 mg (55%) of **17b**. ^1H NMR (D_2O) δ 0.82 (t, $J = 6.6$ Hz, 3H, H6a), 1.20–1.28 (m, 8H, H2a–H5a), 1.45–1.50 (m, 2H, H1a), 1.87–2.00 (m, 1H, H8), 2.05–2.12 (m, 1H, H8'), 2.45–2.55 (m, 2H, H7,7'), 2.82 (d, $J = 13.6$ Hz, 1H, H5), 2.87 (d, $J = 13.6$ Hz, 1H, H5'), 4.20 (d, $J = 5.4$ Hz, 1H, H3), 4.21–4.23 (m, 1H, H9), 4.72 (d, $J = 5.4$ Hz, 1H, H2); ^{13}C NMR (D_2O) δ 15.01 (C6a), 23.20, 23.56, 23.90, 29.20 (C2a–C5a), 27.3 (C7), 29.73 (C8), 32.10 (C1a), 41.99 (C5), 52.4 (C9), 72.55 (C2), 78.50 (C3), 88.50 (C4), 172.3 and 173.5 (C1 and C10); HRMS calcd for $\text{C}_{15}\text{H}_{27}\text{NO}_6\text{S}^+$ $[\text{M}+\text{Na}]^+$ 372.1451; found 372.1469.

4.12.2. S-(4-C-Octyl-D-ribo-1,4-lactone-5-yl)-l-homocysteine (**17c**)

Step a. Treatment of **15c** (24 mg, 0.063 mmol) with homocysteinate lithium salt, using the procedure reported in Section 4.11, gave **16c** contaminated with protected homocysteine ($\sim 1:1$, 40 mg). Compound **16c** had: ^1H NMR δ 0.80 (t, $J = 6.6$ Hz, 3H, H8a), 1.20–1.25 (m, 12H, H2a–H7a), 1.26 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.39 and 1.41 ($2 \times$ s, $2 \times 9\text{H}$, $2 \times t\text{-Bu}$), 1.50–1.60 (m, 2H, H1a), 1.75–1.89 (m, 1H, H8), 1.9–2.0 (m, 1H, H8'), 2.48–2.54 (m, 2H, H7,7'), 2.80–2.83 (m, 2H, H5,5'), 4.20–4.26 (m, 1H, H9), 4.40 (d, $J = 5.9$ Hz, 1H, H3), 5.05 (br. d, $J = 7.6$ Hz, 1H, NH), 5.15 (d, $J = 5.9$ Hz, 1H, H2). *Step b.* Treatment of the crude **16c** ($\sim 1:1$, 40 mg; *Step a*) with TFA (2 mL), using the procedure reported in Section 4.12, gave an oily residue that was partitioned between water and CHCl_3 . The aqueous layer was evaporated *in vacuum* below 30°C and the residue (20 mg) was divided into two portions. Each portion of crude **17c** was dissolved in deionized water/MeCN (2.5 mL, 19:1, v/v) and was injected into the Sep-Pak cartridge (C18 classic column). The columns were eluted with deionized water (5 mL), a second portion of deionized water (5 mL) and ethanol (5 mL). The combined water eluents contained mainly Hcy (TLC and ^1H NMR), while the combined ethanol eluents were evaporated *in vacuum* to give **17c** (5 mg, 21% from **15c**): ^1H NMR ($\text{MeOH}-d_4$) δ 0.82 (t, $J = 6.6$ Hz, 3H, H8a), 1.20–1.28 (m, 12H, H2a–H7a), 1.50–1.60 (m, 2H, H1a), 1.90–2.00 (m, 1H, H8), 2.05–2.12 (m, 1H, H8'),

2.55–2.65 (m, 2H, H7,7'), 2.80 (d, $J = 13.8$ Hz, 1H, H5), 2.87 (d, $J = 13.9$ Hz, 1H, H5'), 4.20 (d, $J = 5.4$ Hz, 1H, H3), 4.19–4.21 (m, 1H, H9), 4.75 (d, $J = 5.4$ Hz, 1H, H2); ^{13}C NMR (MeOH- d_4) δ 15.01 (C8a), 23.00, 23.50, 23.85, 29.00, 30.67, 30.51 (C2a–C7a), 27.40 (C7), 29.7 (C8), 32.07 (C1a), 39.86 (C5), 52.21 (C9), 71.54 (C2), 77.20 (C3), 84.59 (C4), 172.21 and 173.52 (C1 and C10); HRMS calcd for $\text{C}_{17}\text{H}_{31}\text{NNaO}_6\text{S}^+$ $[\text{M}+\text{Na}]^+$ 400.1764; found 400.1783.

Note. By varying the reaction conditions different quantities of 2,3-*O*-isopropylidene-4-*C*-octyl-*D*-ribo-1,5-lactone were isolated during the column chromatography of the crude reaction mixture from step *a*: ^1H NMR δ 0.88 (t, $J = 6.6$ Hz, 3H, H8a), 1.25–1.32 (m, 12H, H2a–H7a), 1.40 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 1.62–1.70 (m, 2H, H1a), 3.86 (m, 2H, H5,5'), 4.60 (d, $J = 5.7$ Hz, 1H, H2), 4.85 (d, $J = 5.7$ Hz, 1H, H3); ^{13}C NMR δ 14.23 (CH_3 , C8a), 22.76, 22.87, 29.29, 29.48, 29.91, 31.92 (C2a–C7a), 25.93 and 26.9 (CMe_2), 35.47 (C1a), 63.45 (C5), 76.55 (C3), 80.07 (C2), 87.05 (C4), 114.57 (CMe_2), 173.25 (C1); HRMS (TOF) m/z calcd for $\text{C}_{16}\text{H}_{28}\text{O}_5\text{Na}^+$ $[\text{M}+\text{Na}]^+$ 323.1798; found 323.1805.

4.12.3. *S*-(4-*C*-4-Methoxyphenyl-*D*-ribo-1,4-lactone-5-yl)-*L*-homocysteine (**17e**)

Treatment of **16e** (11.4 mg, 0.02 mmol) with TFA (1 mL), using the procedure reported in Section 4.12, gave **17e** (5.6 mg, 75%): ^1H NMR (MeOH- d_4) δ 1.80–1.83 (m, 1H, H8), 1.90–1.92 (m, 1H, H8'), 2.50–2.65 (m, 2H, H7,7'), 2.85 (d, $J = 14.8$ Hz, 1H, H5), 3.20 (d, $J = 15.1$ Hz, 1H, H5'), 3.80 (s, 3H, CH_3O), 4.22–4.27 (m, 1H, H9), 4.60 (d, $J = 5.8$ Hz, 1H, H3), 4.90 (d, $J = 5.8$ Hz, 1H, H2), 6.82 (d, $J = 8.8$ Hz, 2H, Ar), 7.12 (d, $J = 9.0$ Hz, 2H, Ar); ^{13}C NMR (MeOH- d_4) δ 27.33 (C7), 29.73 (C8), 41.37 (C5), 52.50 (C9), 55.33 (CH_3O), 74.40 (C2), 78.20 (C3), 85.40 (C4), 117.20, 125.81, 127.00, 162.28 (Ar), 172.31, 173.49 (C1 and C10); HRMS calcd for $\text{C}_{16}\text{H}_{21}\text{NO}_7\text{SNa}^+$ $[\text{M}+\text{Na}]^+$ 394.0931; found 394.0908.

4.13. General procedure for the reduction of lactones. Synthesis of 4-*C*-substituted *S*-ribosylhomocysteines **19**

LiEt_3BH (1M/THF, 0.045 mL, 0.045 mmol) was added dropwise to a solution of 0.02 mmol of **16** in CH_2Cl_2 (1 mL) or **17** in THF/ CH_2Cl_2 (1:1; 1 mL) and the resulting mixture was stirred at -20°C for 30 min under N_2 atmosphere. MeOH (0.5 mL) was then slowly added to quench the reaction and volatiles were evaporated *in vacuum* below 25°C . The residue for the protected products **18** was partitioned between $\text{CH}_2\text{Cl}_2/\text{NaHCO}_3$, washed with brine, dried (MgSO_4) and was column chromatographed (75:25, hexane/EtOAc); whereas the residue for the deprotected product **19** was redissolved in deionized $\text{H}_2\text{O}/\text{MeOH}$ (4:1, 2.5 mL) and washed with CHCl_3 (2×1 mL) and then the aqueous layer was evaporated *in vacuum* below 30°C . The 4-*C*-substituted SRH analogues **19** are somehow unstable and need to be manipulated with care but are stable when stored as powder or well-dried syrup in a refrigerator at 4°C for a month.

4.13.1. *S*-(5-Deoxy-4-*C*-hexyl-*D*-ribofuranos-5-yl)-*L*-homocysteine (**19b**)

Step a. Treatment of **16b** (10.9 mg, 0.02 mmol) with LiEt_3BH , using the procedure reported in Section 4.13, gave 2,3-*O*-isopropylidene-5-[(*tert*-butoxycarbonyl)-*L*-homocysteine *tert*-butyl ester]-4-*C*-hexyl-*D*-ribofuranose **18b** (α/β , $\sim 1:9$, 9.5 mg, 90%). The major β anomer had: ^1H NMR δ 0.80 (t, $J = 6.6$ Hz, 3H, H6a), 1.20–1.28 (m, 8H, H2a–H5a),

1.35 and 1.41 ($2 \times s$, $2 \times 9H$, $2 \times t\text{-Bu}$), 1.35 (s , $6H$, $2 \times CH_3$), 1.50–1.60 (m , $2H$, $H1a$), 1.91–2.02 (m , $2H$, $H8,8'$), 2.48–2.58 (m , $2H$, $H7,7'$), 2.60 (d , $J = 12.8$ Hz, $1H$, $H5$), 2.94 (d , $J = 12.6$ Hz, $1H$, $H5'$), 4.19–4.21 (m , $1H$, $H9$), 4.30 (d , $J = 5.9$ Hz, $1H$, $H3$), 4.92 (d , $J = 5.9$ Hz, $1H$, $H2$), 5.19–5.21 (m , $1H$, NH), 5.35 (s , $1H$, $H1$). The minor α anomer had a peak for $H1$ at δ 5.47 (d , $J = 3.9$ Hz). *Step b.* Treatment of **18b** (α/β , $\sim 1:9$; 9.5 mg, 0.02 mmol) with in TFA (1 mL), using the procedure reported in Section 4.12, gave **19b** (α/β , $\sim 1:9$; 4 mg, 75%). The major β anomer had: 1H NMR (D_2O) δ 0.80 (t , $J = 6.6$ Hz, $3H$, $H6a$), 1.20–1.28 (m , $8H$, $H2a\text{--}H5a$), 1.50–1.60 (m , $2H$, $H1a$), 1.91–2.01 (m , $2H$, $H8,8'$), 2.48–2.58 (m , $2H$, $H7,7'$), 2.63 (d , $J = 12.8$ Hz, $1H$, $H5$), 2.94 (d , $J = 12.6$ Hz, $1H$, $H5'$), 4.12 (t , $J = 5.9$ Hz, $1H$, $H2$), 4.19–4.21 (m , $1H$, $H9$), 4.20 (d , $J = 5.9$ Hz, $1H$, $H3$), 5.33 (s , $1H$, $H1$); MS (ESI^-) m/z 350 (MH^-). HRMS calcd for $C_{15}H_{29}NO_6SNa^+$ [$M+Na$] $^+$ 374.1608, found 374.1617.

4.13.2. S-(5-Deoxy-4-C-octyl-D-ribofuranos-5-yl)-l-homocysteine (**19c**)

Treatment of **17c** (6 mg, 0.01 mmol) with $LiEt_3BH$ (0.03 mL), using the procedure reported in Section 4.13, gave **19c** (α/β , $\sim 1:3$; 4 mg, 60%). The major β anomer had: 1H NMR ($MeOH\text{-}d_4$) δ 0.80 (t , $J = 6.6$ Hz, $3H$, $H8a$), 1.21–1.32 (m , $12H$, $H2a\text{--}H7a$), 1.50–1.60 (m , $2H$, $H1a$), 1.9–2.0 (m , $2H$, $H8,8'$), 2.48–2.58 (m , $2H$, $H7,7'$), 2.79 (d , $J = 12.8$ Hz, $1H$, $H5$), 2.90 (d , $J = 12.6$ Hz, $1H$, $H5'$), 4.15 (t , $J = 5.9$ Hz, $1H$, $H2$), 4.19–4.21 (m , $1H$, $H9$), 4.20 (d , $J = 5.9$ Hz, $1H$, $H3$), 5.19–5.21 (m , $1H$, NH), 5.39 (s , $1H$, $H1$), [the minor α anomer had a peak for $H1$ at δ 5.44 (d , $J = 3.5$ Hz)]; ^{13}C NMR ($MeOH\text{-}d_4$) δ 15.01 ($C8a$), 23.00, 23.50, 23.85, 29.00, 30.67, 30.51 ($C2a\text{--}C7a$), 27.3 ($C7$), 29.6 ($C8$), 32.07 ($C1a$), 41.99 ($C5$), 50.51 ($C9$), 69.77 ($C3$), 72.09 ($C2$), 87.16 ($C4$), 99.90 ($C1$), 172.62 ($C10$); HRMS calcd for $C_{17}H_{33}NO_6SNa^+$ [$M+Na$] $^+$ 402.1921, found 402.1933.

4.13.3. S-(5-Deoxy-4-C-4-methoxyphenyl-D-ribofuranos-5-yl)-l-homocysteine (**19e**)

Step a. Treatment of **16e** (11.3 mg, 0.02 mmol) with $LiEt_3BH$, using the procedure reported in Section 4.13, gave 2,3-O-isopropylidene-5-[(*tert*-butoxycarbonyl)-l-homocysteine *tert*-butyl ester]-4-C-4-methoxyphenyl-D-ribofuranose **18e** (α/β , $\sim 1:9$; 7.8 mg, 68%). The major β -anomer had: 1H NMR δ 1.35 (s , $3H$, CH_3), 1.41 (s , $3H$, CH_3), 1.39 and 1.41 ($2 \times s$, $2 \times 9H$, $t\text{-Bu}$), 1.75–1.89 (m , $1H$, $H8$), 1.95–2.05 (m , $1H$, $H8'$), 2.48–2.54 (m , $2H$, $H7,7'$), 3.01 (d , $J = 14.8$ Hz, $1H$, $H5$), 3.20 (d , $J = 15.1$ Hz, $1H$, $H5'$), 3.80 (s , $3H$, CH_3O), 4.20–4.25 (m , $1H$, $H9$), 4.65 (d , $J = 5.8$ Hz, $1H$, $H3$), 4.85 (d , $J = 5.8$ Hz, $1H$, $H2$), 5.01 (d , $J = 8.1$ Hz, $1H$, NH), 5.40 (s , $1H$, $H1$), 6.82 (d , $J = 8.8$ Hz, $2H$, Ar), 7.12 (d , $J = 9.0$ Hz, $2H$, Ar), [the minor α -anomer had a peak for $H1$ at δ 5.60 (d , $J = 3.8$ Hz)]; HRMS calcd for $C_{28}H_{43}NO_9SNa^+$ [$M+Na$] $^+$ 592.2551, found 592.2522. *Step b.* Treatment of **18e** (α/β , $\sim 1:9$; 7.6 mg, 0.013 mmol) with in TFA (1 mL), using the procedure reported in Section 4.12, gave **19e** (α/β , $\sim 1:9$; 3.7 mg, 77%). The major isomer had: 1H NMR ($MeOH\text{-}d_4$) δ 1.90–2.00 (m , $2H$, $H8,8'$), 2.48–2.58 (m , $2H$, $H7,7'$), 3.01 (d , $J = 14.8$ Hz, $1H$, $H5$), 3.20 (d , $J = 15.1$ Hz, $1H$, $H5'$), 3.80 (s , $3H$, CH_3O), 4.15 (d , $J = 5.8$ Hz, $1H$, $H2$), 4.20–4.25 (m , $1H$, $H9$), 4.40 (d , $J = 5.8$ Hz, $1H$, $H3$), 5.45 (s , $H1$, $1H$), 6.82 (d , $J = 8.8$ Hz, $2H$, Ar), 7.12 (d , $J = 9.0$ Hz, $2H$, Ar); ^{13}C NMR ($MeOH\text{-}d_4$) δ 27.51 ($C7$), 29.70 ($C8$), 52.52 ($C9$), 55.33 (CH_3O), 42.20 ($C5$), 72.61 ($C3$), 74.40 ($C2$), 85.40 ($C4$), 101.10 ($C1$), 117.20, 125.81, 127.00, 162.28 (Ar), 172.3 ($C10$). HRMS calcd for $C_{16}H_{23}NO_7SNa^+$ [$M+Na$] $^+$ 396.1087, found 396.1062.

Disclosure statement

No potential conflict of interest was reported by the authors.

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