# Synthesis of a Nitrogen Analogue of Salacinol and Its α-Glucosidase Inhibitory Activity

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**A nitrogen analogue 4 of the naturally occurring sulfonium ion salacinol (1), a potent α-glucosidase inhibitor isolated from the Ayruvedic medicine *Salacia reticulata*, was synthesized and its inhibitory activity against α-glucosidase tested. Substitution of the sulfur atom in 1 with a nitrogen reduced the activity considerably. The solid-state stereostructure of the related compound (5) was determined on the basis of single crystal X-ray measurement.**

**Key words** cyclic sulfate; erythritol; salacinol; glycosidase inhibitor; glucosidase inhibitor; azasugar

Salacinol (**1**) is a potent glycosidase inhibitor isolated from the aqueous extracts of the roots and stems of *Salacia reticulata* WIGHT (known as *Kotala himbutu* in Singhalese), which is traditionally used in Sri Lanka and India for the treatment of diabetes. The α-glucosidase inhibitory activity of **1** was confirmed to be as strong as that of acarbose, which is used clinically.[1)] The structure of **1** is unique in that the ring sulfonium ion is stabilized by a sulfate counteranion by forming a spirobicyclic-like configuration comprised of 1-deoxy-4-thioarabinofranosyl cation and 1-deoxyerythrosyl-3-sulfate anion, as shown in Chart 1. Due to both the extraordinary high glycosidase inhibitory activity and the intriguing structure, much attention has been focused on **1** and related compounds.[2)]

Total synthesis of **1** was accomplished recently by two groups[3)] independently on the basis of the same strategy. In both cases, the key step in the synthesis was the ring opening of the cyclic sulfate (**2**) by nucleophilic attack of 1,4-dideoxy-1,4-epitio-D-arabinitol (**3a**). Structure–activity relationships between some related sulfonium compounds and the glycosidase inhibitory activity have also been reported.[3b,4)]

On the other hand, polyhydroxylated pyrrolidines [azasugars, *e.g.* 1,4-dideoxy-1,4-imino-D-arabinitol (**3b**)] have been known to function as potent inhibitors of glycosidase because of their ability to mimic carbohydrates.[5)] Structural modification of **1** represents a promising approach in the search for new antidiabetic agents. One strategy is to replace the thiosugar sulfur in **1** with a nitrogen, which gives the azacyclic version (**4**) of **1**. The recent report by Ghavami *et al.*[6)] on the synthesis of nitrogen analogues of **1** involving **4** and their evaluation as glycosidase inhibitors prompts us to report our own findings. This communication describes the synthesis of **4** and comparison of its α-glucosidase inhibitory activity with that of **1**. The solid-state stereostructure of the related compound, 1′-(1-pyrrolidiniumyl)-2′,4′-*O*-isopropylidene-1′-deoxy-L-erythritol-3′-sulfate (**5**) is also presented.

Synthesis of **4** was carried out by applying the ring-opening method developed by Yuasa[3a)] and Ghavami[3b)] for the synthesis of **1**. The cyclic sulfate **2a**,[3a)] however, was synthesized starting from D-glucose (D-**6**), which is much less expensive than the L-isomer (L-**6**). Thus compound D-**6** was first converted into 4,6-*O*-benzylidene-D-glucose (**7**) according to the protocol developed by Barili *et al.*[7)] Practically pure **7** could be obtained only by the extraction of the ethereal solution of the crude reaction mixture with water. Work-up and flash-column chromatography were found unnecessary, since the yield was raised to 82% from the 72% previously reported.

Effective transformation of **7** into 1,3-*O*-bezylidene-L-erythritol (**8**) was accomplished by modification of the standard protocol.[8)] Thus compound **7** was first oxidized with sodium metaperiodate in the presence of excess sodium bicarbonate, and following sodium borohydride reduction of the resulting D-erythrose intermediate gave **8** in 94% yield. Protection of the diol group of **8** with 2-methoxypropene followed by hydrogenolysis of the benzylidene moiety of 1,3-*O*-benzylidene-2,4-*O*-isopropylidene-L-erythritol (**9**) provided 2,4-*O*-isopropylidene-L-erythritol (**10**) in quantitative yield. Compound **10** was then treated with thionyl chloride to afford a 1 : 2 diastereomeric mixture of 2,4-*O*-isopropylidene-L-erythritol-1,3-cyclic sulfite (**11**). The oxidation of **11** to the desired 2,4-*O*-isopropylidene-L-erythritol-1,3-cyclic sulfate (**2a**) was carried out efficiently by the addition of sodium bicarbonate to the reaction mixture to prevent decomposition of the product **2a**, and substantial improvement of the oxidation yield up to 95% was achieved. Thus the desired cyclic sulfate **2a** was synthesized in 73% overall yields from D-**6** *via* seven steps.

The coupling reaction of the cyclic sulfate **2a** with pyrrolidine (**12**) as a model compound proceeded selectively to give **5**[9)] in 80% yield. The FAB mass spectrometry of **5** run in the positive mode showed a molecular protonated ion $[M+H]^+$ peak at *m/z* 296. The IR spectrum of **5** showed absorptions

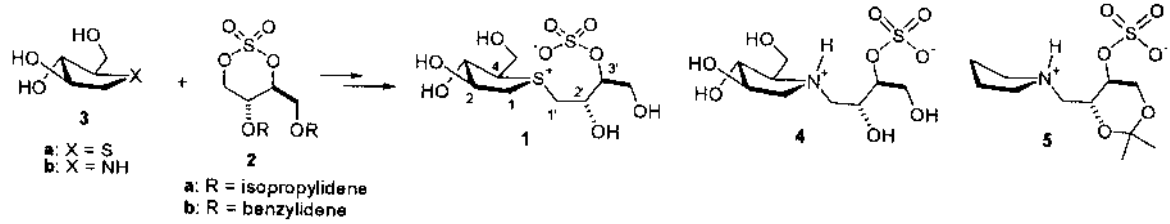


Chart 1

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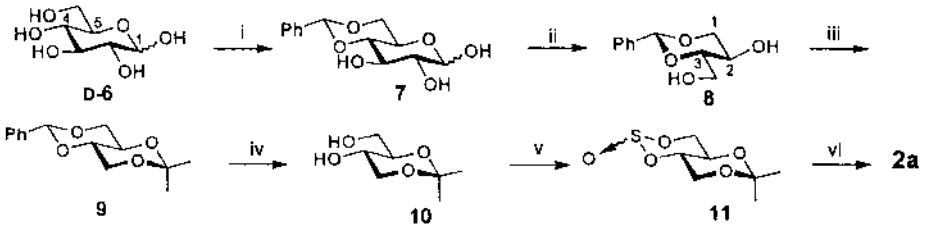


Reagents and conditions: (i) $PhCH(OCH_3)_2$, *p*-TsOH, DMF, 50 °C, 82%; (ii) $NaIO_4$, $NaHCO_3$, $H_2O$, THF, r.t. and then $NaBH_4$, r.t., 94%; (iii) $CH_3(CH_3O)C{=}CH_2$, DMF, 0—5 °C, quant.; (iv) $H_2$, Pd–C, EtOH, r.t., quant; (v) $SOCl_2$, $Et_3N$, $CH_2Cl_2$, 0 °C; (vi) $NaIO_4$, $RuCl_3$, $NaHCO_3$, $CCl_4$, $CH_3CN$, $H_2O$, r.t., 95%

Chart 2

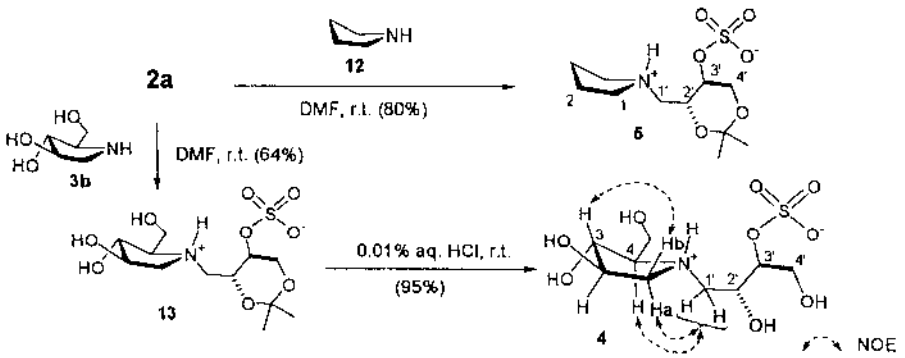


Chart 3

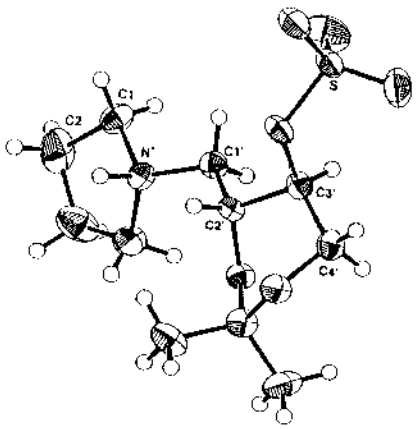

Fig. 1. Perspective View of **5**

due to two functionalities at 2770 $cm^{-1}$ ($N^+H$), and 1261 and 1234 $cm^{-1}$ ($OSO_3^-$). Observed downfield shift signals in its $^1$H-NMR spectrum appeared at $\delta$ 3.18—3.32 (H-1), 3.40 (H-1′a), and 3.61 (H-1′b), corresponding to $\alpha$-protons of the nitrogen, also supported the ammonium ion structure. The structural confirmation was performed on the basis of single-crystal X-ray measurement[10] as shown in Fig. 1, which detected the hydrogen atom on the ammonium nitrogen. In this molecule, the two ionic centers were far apart from each other, while **1** constructed the inner salt between these two moieties.[1]

Reaction of **2a** with azasugar **3b**[11] gave the corresponding *N*-alkylated product, 1′-[(1,4-dideoxy-1,4-imino-D-arabinitol)-4-*N*-ammonium]-2′,4′-*O*-isopropylidene-1′-deoxy-L-erythritol-3′-sulfate (**13**) in 64% yield. Deprotection of **13** using 0.01% HCl at room temperature provided the desired nitrogen analogue **4**[12] of salacinol in 95% yield. Compound

Table 1. $IC_{50}$ Values ($\times 10^{-5}$ M) of Compounds **1**, **4**, and **3b** against Disaccharidase

| Compound | Maltase | Sucrase | Isomaltase | Trehalase |
|---|---|---|---|---|
| **1**[a] | 0.96 | 0.25 | 0.18 | — |
| **4**[a] | 30.6 | 4.4 | 13.6 | >31.5 |
| **3b**[b] | 3.5 | 2.3 | 0.4 | 2.2 |

*a*) Rat small inteatinal brush border membrane vesicles were used. *b*) Mouse small intestinal mucosal homogenates were used.[5a]

**4** displayed similar $^1$H-NMR spectroscopic properties to those of the model compound **5**, with deshielded signals of five $\alpha$-protons due to ammonium ion formation detected. The relative stereochemistry of the side chain on the nitrogen was determined on the basis of nuclear Overhauser effect spectroscopy (NOESY) experiments, as shown in Chart 3. The observed NOE correlations between two ring protons $\alpha$ to the nitrogen (H-4 and one of the methylene protons H-1a), and the methylene protons on C-1′ suggested the depicted configuration of the side chain. The NOE correlation between the other methylene proton H-1b and H-3 was also detected.

The glycosidase inhibitory activity of **4** was tested for the intestinal $\alpha$-glucosidase *in vitro*[13] and compared with those of **1** and **3b**, as shown in Table 1. Upon substitution of the sulfur atom with the nitrogen, the inhibitory activities were reduced considerably. However, the magnitude of the decrease in the inhibitory activity against sucrase was not as great as those against maltase or isomaltase, and **4** sustained nearly equal inhibitory activity to **3b** against sucrase. No inhibition was detected against commercially available $\beta$-glucosidase originating from almonds. Further investigations on the origin of both the strong $\alpha$-glucosidase inhibitory activity of **1** and selectivity for certain glycosidase enzymes of **1** and

related compounds are in progress.

**References and Notes**


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9) Characterization of compound **5**. Colorless prisms. mp 175—181°C (decomp.). $[\alpha]_D^{20}$ +41.3 (*c*=0.5, $CH_3OH$). IR (KBr) $cm^{-1}$: 2770, 1261, 1234. $^1$H-NMR (500 MHz, $CD_3OD$) $\delta$: 1.39 (3H, s, $CH_3$), 1.52 (3H, s, $CH_3$), 2.01—2.15 (4H, br m, H-2), 3.18—3.32 (4H, m, H-1), 3.40 (1H, dd, *J*=13.0, 8.0 Hz, H-1′a), 3.61 (1H, dd, *J*=13.0, 2.0 Hz, H-1′b), 3.84 (1H, dd, *J*=11.0, 7.5 Hz, H-4′a), 4.09 (1H, dd, *J*=11.0, 5.0 Hz, H-4′b), 4.12—4.16 (2H, m, H-2′, H-3′). $^{13}$C-NMR (125 MHz, $CD_3OD$) $\delta$: 20.0 (q, $CH_3$), 23.9 (t, C-2), 28.1 (q, $CH_3$), 57.5 (t, C-1, C-1′), 63.4 (t, C-4′), 69.6 (d, C-2′), 71.2 (d, C-3′), 101.1 (s, $(CH_3)_2\underline{C}$). FAB-MS *m*/*z*: 296 $[M+H]^+$. HR-FAB-MS *m*/*z*: 296.1142 ($C_{11}H_{22}O_6NS$, calcd for 296.1168).
10) Crystal data for **5**: $C_{11}H_{21}O_6NS$, MW=295.35, orthorhombic, space group $P2_12_12_1$, *a*=11.222(4), *b*=13.390(2), *c*=9.529(2) Å, *V*=1431.9(6) $Å^3$, *Z*=4, $\mu$(Mo-K$\alpha$)=2.47 $cm^{-1}$, *F*(000)=632, $D_c$=1.370 $g/cm^3$, crystal dimensions: 0.12×0.20×0.24 mm. A total of 1906 reflections were collected using the $\omega$–2$\theta$ scan technique to a maximum 2$\theta$ value of 55°, and 1008 reflections with $I>3\sigma(I)$ were used in the structure determination. Final *R* and $R_w$ values were 0.039 and 0.039, respectively. The maximum and minimum peaks in the difference map were 0.19 $e^-$ $Å^{-3}$ and −0.19 $e^-$ $Å^{-3}$, respectively.
11) Fleet G. W. J., Witty D. R., *Tetrahedron*: *Asymmetry*, **1**, 119—136 (1990).
12) Characterization of compound **4**. A pale yellow oil. $[\alpha]_D^{20}$ +9.4 (*c*=2.0, $CH_3OH$). IR (neat) $cm^{-1}$: 2764, 1257, 1223. $^1$H-NMR (500 MHz, $CD_3OD$) $\delta$: 3.29 (1H, dd, *J*=13.5, 9.0 Hz, H-1′a), 3.57 (1H, td, *J*=6.5, 2.5 Hz, H-4), 3.59 (1H, dd, *J*=12.0, 4.0 Hz, H-1a), 3.77 (1H, br d, *J*=12.0 Hz, H-1b), 3.81 (1H, dd, *J*=12.0, 3.7 Hz, H-4′a), 3.83 (1H, dd, *J*=13.5, 2.5 Hz, H-1′b), 3.92 (1H, dd, *J*=12.0, 4.0 Hz, H-4′b), 3.94 (2H, d-like, *J*=6.5 Hz, H-5a, H-5b), 4.00 (1H, br s-like, H-3), 4.20 (ddd, *J*=7.0, 4.0, 3.7 Hz, H-3′), 4.18—4.22 (m, H-2), 4.28 (1H, ddd, *J*=9.0, 7.0, 2.5 Hz, H-2′). $^{13}$C-NMR (125 MHz, $CD_3OD$) $\delta$: 60.4 (t, C-5), 61.6 (t, C-4′), 61.7 (t, C-1′), 62.9 (t, C-1), 67.4 (d, C-2′), 75.9 (d, C-2), 77.7 (d, C-3), 78.7 (d, C-4), 81.0 (d, C-3′). FAB-MS *m*/*z*: 318 $[M+H]^+$. HR-FAB-MS *m*/*z*: 318.0855 ($C_9H_{20}O_9NS$, calcd for 318.0852).
13) $\alpha$-Glucosidase inhibitory activity of **4** was measured according to the method described in the literature.[1,2]