Pergamon

Tetrahedron Letters, Vol. 38, No. 2, pp. 273-276, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(96)02290-3

Synthesis of the Unique Trisaccharide: 6-d-L-Talpα(1→2)-L-Rhafβ(1→5)-DHA

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Abstract: Preparation of the title trisaccharide resulted from two remarkable facts: 1) during acetolysis of the anomeric *t*-butyl group in the fully protected disaccharide **4**, the rhamnopyranoside ring underwent an unusual conversion into the furanose form to furnish **5a**; 2) stereoselective coupling of 5-*O*-acetyl-3-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-acetyl-6-deoxy-α-L-talopyranosyl)-α-L-rhamnofuranosyl bromide (**5b**) with the glycosyl acceptor **7b** formed surprisingly the β-linkage, to give the corresponding trisaccharide **8a**. Copyright © 1996 Published by Elsevier Science Ltd

Recently has been shown[1] that lipopolysaccharides (LPSs) of two serotypes of Gram-negative bacteria *Rhizobium leguminosarum* bv. *trifolii* 24 and its exo⁻ mutant R20 contain an unique *O*-antigenic trisaccharide, composed of the solely rare 3-deoxy-D-*lyxo*-heptulosaric acid (DHA),[2] 6-deoxy-L-talose and L-rhamnose. This trisaccharide, possessing a structure 6-d-L-Talpα(1→2)-L-Rhapα(1→5)-DHA seems to represent a repeating unit of the carbohydrate *O*-chain.[1]

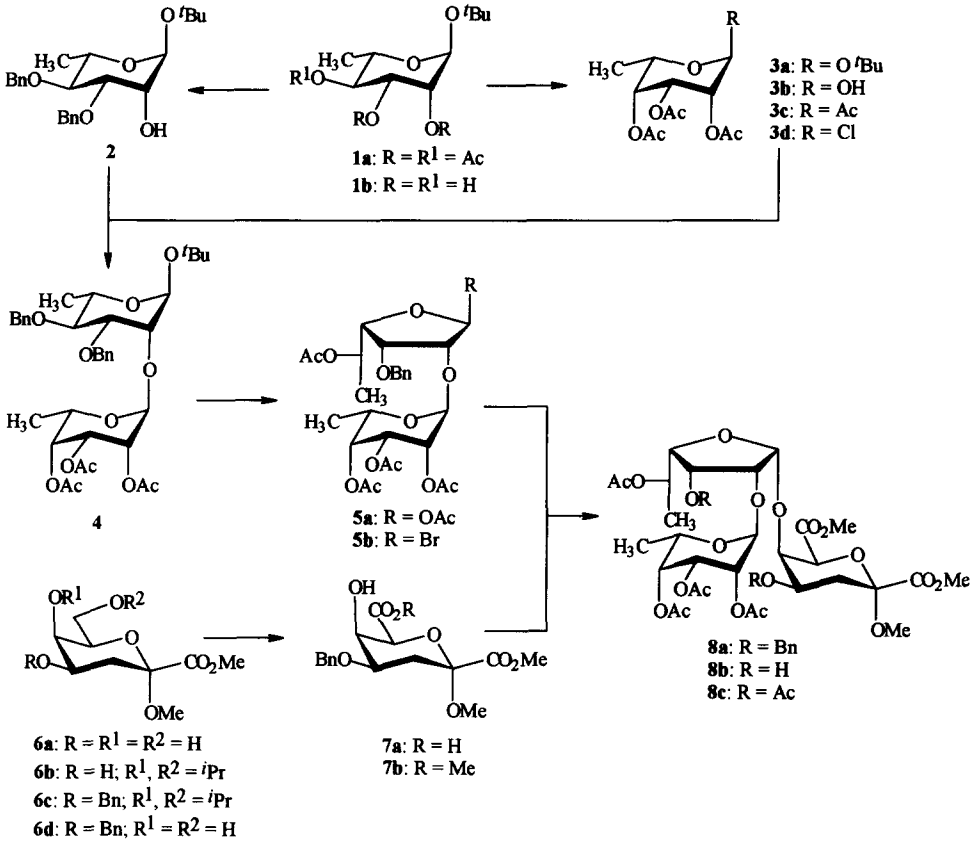
The preparation of this unusual trisaccharide, desirable for cloning, is an important synthetic target.

For the synthesis of the trisaccharide we needed to prepare the corresponding monosaccharide units. Although we recently described syntheses of DHA[3] as well as 6-deoxy-L-talose,[4] some modifications of those preparations, enabling the selective introduction of different protecting groups, were necessary. Thus, we decided to use *t*-butyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside (**1**) as a common substrate for the preparation of both the required 6-deoxy-hexoses **2** and **3** (**Scheme 1**). Applying the standard procedure[5] we obtained from **1** the glycosylic acceptor **2**. Transformation of **1** into the required *talo* isomer **3a** was achieved by the known reaction sequence.[6] After removal of the *t*-butyl group in **3a** with trifluoroacetic acid,[7] then acetylation, and subsequent treatment of **3c** with dichloromethyl methyl ether[8] the desired glycosyl donor **3d** was achieved.

Coupling of **2** and **3** was performed with classical reagents, i.e. silver triflate - sym. collidine under the base deficient conditions, usually used for construction of the trans-linked disaccharides.[8b] Disaccharide **4** thus obtained was isolated in 72% yield.[9a]

For the conversion of **4** to the glycosyl donor, needed for condensation with DHA acceptor, acetolysis of the *O*-*t*-Bu residue was applied. To our surprise, treatment of **4** with trifluoroacetic acid - acetic anhydride (1:15)

at room temperature gave rise not to the expected 1-*O*-acetyl derivative of **4**, but to the disaccharide **5a** (73%). The rhamnopyranose ring scission to furanose one was demonstrated by ^{1}H NMR data.[9b]



Sheme 1. Synthesis of trisaccharide **8c**: 6-d-L-Talpα(1→2)-L-Rhafβ(1→5)-DHA

Disaccharide **5** thus obtained can be considered as a kinetic product of dealkylation of 1-OH and 4-OH groups in the rhamnopyranoside unit, with spontaneous ring scission, followed by acetylation. Although selective acetolysis of the benzyl group in sugars is a known process, usually it proceeds under more drastic conditions.[10] On the other hand, easy cleavage of the anomeric *O*-*t*-butyl group by treatment with acids was well documented.[11] Therefore, an expectation that dealkylation at the anomeric center should precede debenzylation at C-4 of rhamnose, seemed to be reasonable. Moreover, a great preference of rhamnose to adopt the thermodynamically more favourable pyranose ring is well known.[12]

The disaccharide **5a** although initially unwanted, we found to be a very attractive glycosyl donor for coupling with sugar acceptors. As far as we can tell, there are no examples of condensation of rhamnofuranosyl halides with saccharide units. For this reason we decided to assemble disaccharide **5** with the DHA unit.

For the formation of a glycosyl donor compound **5a** was converted to the bromide **5b** by treatment with titanium tetrabromide.[13] The desired acceptor **7b** was prepared from heptulosonic acid **6a**,[3] in which 5,7-OH groups were selectively blocked[13] as *O*-isopropylidene derivative **6b**. This, in turn, was benzylated at 4-*O*-position with benzyl bromide/Ag_2O. After removal of *O*-isopropylidene protection the primary 7-OH group in **6c** was selectively oxidized using NaOCl/TEMPO[14] (TEMPO = 2,2,6,6-tetramethylpiperidine 1-oxyl radical) to afford **7a**. Esterification of 7-CO_2H group with methanol, promoted by Me_3SiCl furnished the acceptor **7b**.[15]

For the final coupling of **7b** with **5b**, $Hg(CN)_2$ mediated Koenigs-Knorr α-glycosylation reaction used for the formation of interglycosidic linkages between rhamnopyranoses was employed.[16]

The reaction proceeded smoothly to form exclusively one product **8a** in a high yield. Removal of the benzyl groups by catalytic hydrogenation, followed by acetylation provided the crystalline trisaccharide **8c**. Contrary to expectation, NMR data[17] of the rhamnoside unit in **8c**, comparable to those reported for methyl 2,3,5-tri-*O*-acetyl-β-L-rhamnofuranoside[12b] indicated β-configuration of the "new" glycosidic linkage. The final proof of this configuration was obtained by X-ray crystal structure determinations.[18]

Fig. 1 shows trisaccharide **8c** along with its atomic numbering. It demonstrates that all substituents in the rhamnofuranoside ring are *cis*-oriented. Its β-glycosidic configuration is supported by the torsion angles ϕ

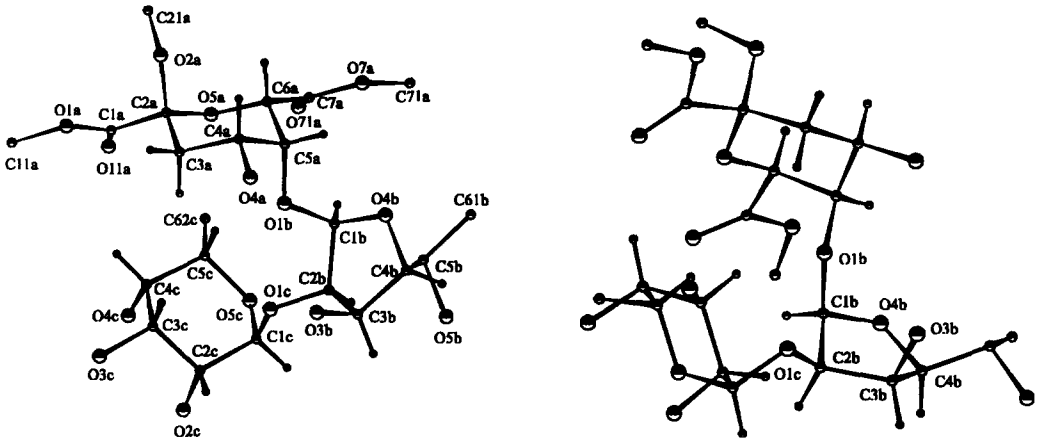


Fig. 1 X-ray structure of **8c**

which are defined as [O1b-C1b-C2b-O1c 30.7(5)°], [O1b-C1b-C2b-C3b -90.0(4)°] and [C4b-O4b-C1b-O1b 105.9(5)°], slightly deviating from those calculated by the MM2 program for β-L-rhamnofuranoside (19.03°, -106.36°, 131.49° respectively).

The unexpected formation of the interglycosidic β-linkage is difficult to explain. It has been known from many years that in furanosides having all substituents on the ring *cis* to each other (*manno*, *rhamno* and *lyxo*), the *cis* 1,2-anomer is particularly disfavoured.[19] As a result 1,2-*trans* glycosides are selectively formed, despite the use of a glycosyl donor, possessing a non participating group in the 2-position.[19] Evidently, further

investigations of this problem are desirable, particularly when the isomeric hexofuranoses have been identified as components of the bacterial polysaccharides.[20]

Acknowledgements: The authors would like to thank Prof. A. Zamojski and Dr. J. Krajewski for the helpful discussions. Financial support by the Grant 2. P303 from KBN is gratefully acknowledged.

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9. a) ^{1}HNMR selected data: for **4** (C_6D_6, 500 MHz) δ = 3.77 (t, 1H, $J_{4a,5a}$= 9.3 Hz, H-4a), 3.94 (dd, 1H, $J_{2a,3a}$= 3.0 Hz, H-2a), 4.08 (dd, 1H, $J_{3a,4a}$= 9.3 Hz, H-3a), 4.11 (pq, 1H, $J_{5a,6a}$= 6.2 Hz, H-5a), 4.23 (pq, 1H, $J_{5b,4b}$= 1.2 Hz, $J_{5b,6b}$= 6.6 Hz, H-5b), 5.23 (d, 1H, $J_{1a,2a}$= 2.0 Hz, H-1a), 5.28 (d, 1H, $J_{1b,2b}$= 1.3 Hz, H-1b); $[\alpha]_D$ -61.2° (c 1.27, $CHCl_3$).
   b) for **5a** (C_6D_6, 500 MHz) δ = 3.82 (pq, 1H, $J_{5b,4b}$= 1.4 Hz, $J_{5b,6b}$= 6.6 Hz, H-5b), 3.99 (dd, 1H, $J_{3b,4b}$= 4.5 Hz, H-3b), 4.17(dd, 1H, H-4b), 5.02 (d, 1H, $J_{1b,2b}$= 1.0 Hz, H-1b), 5.14 (t, 1H, $J_{3a,2a}$= 3.4 Hz, H-3a), 5.40 (dt, 1H, H-2a), 5.44 (t, 1H, $J_{4a,3a}$= 3.6 Hz, H-4a), 5.55 (q, 1H, $J_{5a,6a}$= 6.2 Hz, H-5a), 6.50 (d, 1H,$J_{1a,2a}$= 3.4Hz, H-1a).
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15. ^{1}HNMR selected data: for **7b** ($CDCl_3$, 500 MHz) δ = 2.08 (dd, 1H, $J_{3a,3e}$= 12.9 Hz, $J_{3a,4}$= 11.6 Hz, H-3ax) 2.24 (ddd, 1H, $J_{3eq,4}$= 5.1 Hz, H-4eq), 3.26 (s, 3H, OCH_3), 3.84, 3.86 (2s×3H, $2CO_2CH_3$), 3.97 (ddd, 1H, $J_{4,5}$= 3.0 Hz, H-4), 4.27 (d, 1H, $J_{5,6}$= 1.6 Hz, H-6), 4.36 (t, 1H, H-5); $[\alpha]_D$ 56.6° (c 0.61, $CHCl_3$).
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17. ^{1}HNMR selected data: (C_6D_6, 500 MHz) δ = 2.40 (dd, 1H, $J_{3eq,4a}$= 4.3 Hz, $J_{3eq,3ax}$= 12.6 Hz, H-3eq), 2.58 (t, 1H, $J_{3ax,4a}$= 12.3 Hz, H-3ax), 2.98 (s, 3H, OCH_3), 3.36, 3.55 (2×3H, 2 $COOCH_3$), 3.59 (q, 1H, $J_{2c,3c}$= 5.0 Hz, H-2c), 3.79 (t, 1H, $J_{4c,3c}$= 5.1 Hz, H-4), 4.03 (s, 1H, H-6a), 4.72 (pq, 1H, $J_{5c,6c}$= 6.4 Hz, $J_{5c,4c}$= 1.4 Hz, H-5c), 4.78 (d, 1H, $J_{5a,4a}$= 2.5 Hz, H-5a), 5.09 (d, 1H, $J_{1c,2c}$= 1.3 Hz, H-1c), 5.12 (d, 1H, $J_{1b,2b}$= 5.0 Hz, H-1b), 5.29 (t, 1H, H-3c), 5.30-535 (m, 2H, $J_{5b,6b}$= 6.2 Hz, H-5b, H-4a), 5.42 (dt, 1H, $J_{2b,3b}$= 1.8 Hz, H-2b), 5.63 (t, 1H, $J_{4b,3b}$= 3.5 Hz, H-4b), 5.80 (t, 1H, H-3b).
18. Crystal data for **8c**: $C_{34}H_{48}O_{22}$, M = 808.72, colourless blocks, orthorhombic, space group $P2_12_12_1$; $\omega/2\theta$ data collection on Kuma KM4 four-circe diffractometer with κ-geomerty and graphite-monochromated $Cu_{K\alpha}$ radiation (λ = 1.54069Å), a = 10.833(2), b = 13.531(2), c = 28.526(4)Å, V = 4181(1)Å^3, Z = 4, ρ_{calcd} = 1.285g·cm^{-3}; μ = 0.935mm^{-1}; F(000) = 1712; crystal dimensions 0.20×0.20×0.25 mm. The structure was solved by direct methods using SHELXS-86. The full-matrix least-squares refinement on F^2 was based on the unique total reflestions and carried out using SHELXL-93. For 4628 unique total data collected in room temperature ($2 < \theta < 82°$, $0 \le h \le 13$, $0 \le k \le 17$, $0 \le l \le 36$), corrected for Lorentz and polarisation factors but not absorption, the final *wR2* was 0.1402 [R = 0.0363, wR = 0.0971 for 1909 unique reflections with $F > 4\sigma(F)$]. All non-hydrogen atoms were refined anisotropically; all hydrogen atoms were geometry positioned and allowed to ride on their parent atoms with U_{iso} = 1.5 × binding atom. Maximum and minimum residual electron density: +0.27(5)/-0.20(5) eÅ^{-3}. Further details of the crystal structure investigation may be obtained from the Director of the Cambidge Crystallographic Data Centre, 12 Union Road, GB-Cambridge CB21EZ (UK), on quoting the full journal citation.
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(*Received in UK* 26 *July* 1996; *revised* 18 *November* 1996; *accepted* 22 *November* 1996)