Tetrahedron Letters, Vol. 33, No. 34, pp. 4971-4974, 1992
Printed in Great Britain

0040-4039/92 $5.00 + .0
Pergamon Press Ltd

STEREOCONTROLLED SYNTHESIS OF HYALURONAN TETRASACCHARIDE[1]

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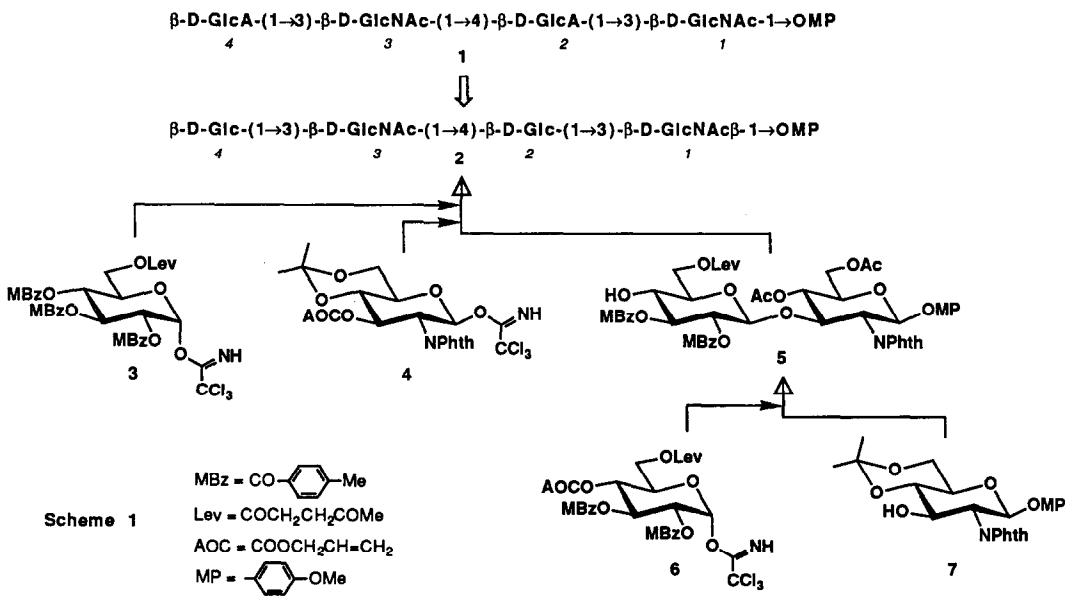
Abstract: A stereocontrolled synthesis of hyaluronan tetrasaccharide is described for the first time.

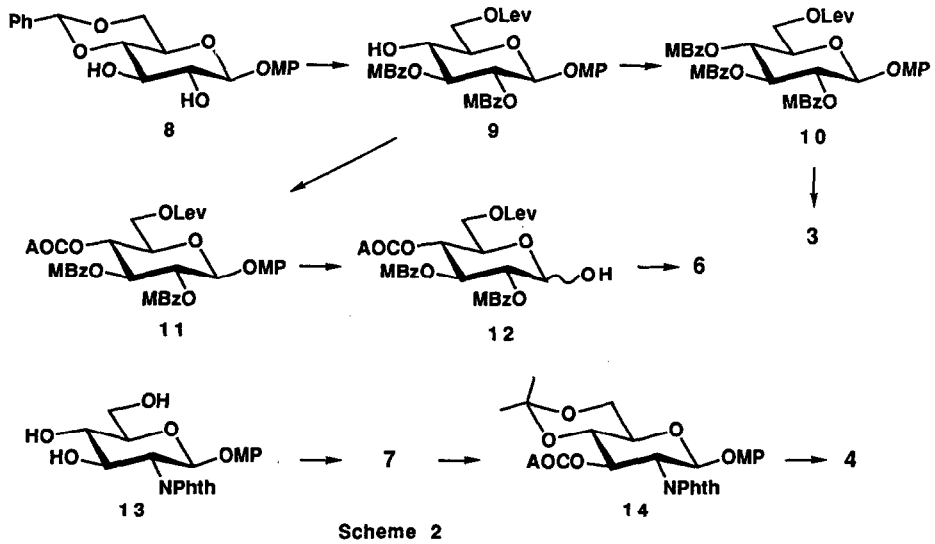
Hyaluronan is one of the major components of extracellular matrixes in which embryonic development and other significant cellular events are carried out[2]. Interaction of hyaluronan with cell surface is mediated by hyaluronan receptor glycoproteins and manifests crucial roles[3] of hyaluronan in various biological processes such as modulation of tissue morphogenesis and cellular proliferation. It is interesting to note that in cartilage differentiation exogenous addition of hyaluronan hexasaccharide disturbs the interaction between hyaluronan and cell surface and eventually normal differentiation of mesodermal cells into chondrocytes is blocked[4]. In order to provide tailor-made hyaluronan oligosaccharides as molecular probes for cell biological studies, we started a project on their synthesis. We describe here an efficient approach to the synthesis of hyaluronan tetrasaccharide derivative **1**. The target tetrasaccharide **1** was designed to carry 4-methoxyphenyl group at O-1[1] (O-1 of a sugar residue *1*) as the handle for further manipulation of the glycan chain at the reducing end. Because of the low reactivity of hydroxyl group at C-4 of uronic acid[5], we chose to oxidize two primary hydroxyl groups near to the last step of synthesis and designed a key intermediate **2** which may in turn be



Scheme 1

bond-disconnected into two glycosyl donors **3** and **4**, and a glycosyl acceptor **5**. The compound **5** may in turn be prepared by coupling of a glycosyl donor **6** with a glycosyl acceptor **7**.

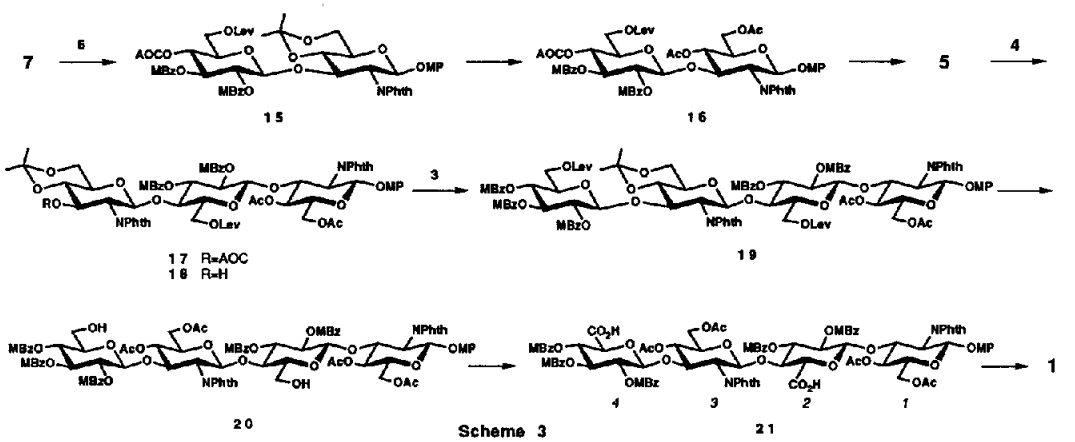
The necessary monosaccharide building units **3**, **4**, **6**, and **7** were synthesized in a straightforward manner. Compound **8**[6] was readily obtainable from penta-*O*-acetyl-β-D-glucopyranose in 3 steps (*1* MeOPhOH, TMSOTf in $(ClCH_2)_2$ 4.5h at 0°, *2* NaOMe in MeOH, *3* $PhCH(OMe)_2$, TsOH in DMF 16h at 25°, 84% overall). Conversion of **8** into a glucosyl donor **3** was carried out in 6 steps via **9**[6] and **10**[6] (*1* MBzCl, DMAP in Py, *2* 4:1 AcOH-H_2O, 80°, *3* LevOH, 2-chloro 1-methylpyridinium iodide (CMPI)[7], DABCO in $(ClCH_2)_2$, *4* MBzCl, DMAP in Py, *5* CAN[8] in 1:1:1 toluene-MeCN-H_2O, *6* Cl_3CCN[9], DBU in CH_2Cl_2; 59% overall). Another glucosyl donor **6** could be prepared from **9** via **11**[6] and **12**[6] in 3 steps (*1* AllOCOCl[10] in 1:1 CH_2Cl_2-Py, -35°, *2* CAN in 3:4:3 toluene-MeCN-H_2O, *3* Cl_3CCN, DBU in CH_2Cl_2, 51% overall). Next compound **13**[11] was transformed into a GlcNAc donor **4** via **7**[6] and **14**[6] in 4 steps (*1* $Me_2C(OMe)_2$, TsOH in DMF, *2* AllOCOCl in 1:1 CH_2Cl_2-Py, -35°, *3* CAN in 1:1:1.4 toluene-H_2O-MeCN, *4* Cl_3CCN, DBU in CH_2Cl_2, 58% overall).



Scheme 2

Having prepared monosaccharide building units, carbohydrate chain elongation was now examined as follows. Stereocontrolled glycosylation of **7** with 1.3 equivalents of **6** in CH_2Cl_2 in the presence of TMSOTf and powdered molecular sieves AW-300 (MSAW-300) at 0° afforded an 87% of **15**[6] which was converted into a glycosyl acceptor **5**[6] via **16**[6] in 3 steps (*1* TFA in CH_2Cl_2, *2* Ac_2O, DMAP in Py, *3* $(Ph_3P)_4Pd$[12], morpholine in THF reflux, 82% overall). Glycosylation of **5** with a GlcNAc donor **4** (2.5 equivalents) in the presence of $BF_3{\cdot}OEt_2$ and MSAW-300 in CH_2Cl_2 at 20° gave exclusively an 88% of **17**[6], which was again treated with $(Ph_3P)_4Pd$ and morpholine in THF to afford a 95% of glycotriosyl acceptor **18**[6]. TMSOTf-MSAW-300 promoted glycosylation of **18** with a Glc donor **3** (5.0 equivalents) in CH_2Cl_2 at 0° gave an 87% of tetrasaccharide **19**[6] which was further converted into **20**[6] equivalent to a designed key intermediate **2** in 3 steps (*1* 6:1 TFA-H_2O in CH_2Cl_2, *2* Ac_2O-DMAP in Py, *3* $NH_2NH_2{\cdot}AcOH$ in 2:1 EtOH-toluene[13], 62% overall). Crucial oxidation of **20** into **21**[6] was successfully achieved in 2 steps (*1* DMSO, $(COCl)_2$, iPr_2NEt, -78°, *2*

$NaClO_2$, NaH_2PO_4 in 3:2:1 *t*BuOH-H_2O-2-methylbutene[14], 86% overall). Finally deprotection of **21** into **1**[6] was carried out in 2 steps (*1* $MeNH_2$[15] in MeOH, *2* Ac_2O in MeOH, 82% overall).



In summary, a stereocontrolled approach to the synthesis of hyaluronan tetrasaccharide **1** was achieved in a highly efficient manner by employing four monosaccharide building units **3**, **4**, **6**, and **7**.

Acknowledgment. We thank Science and Technology Agency for providing T. Slaghek with STA fellowship (1989-1990). We thank Dr. J. Uzawa and Mrs. T. Chijimatsu for recording and measuring the n.m.r. spectra, Mr. Y. Esumi for FAB MS, Ms. M. Yoshida and her staff for the elemental analyses, and Ms. A. Takahashi, and Ms. K. Moriwaki for technical assistance.

References and Notes

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6 Physical data for new compounds are given below, values of $[\alpha]_D$ and $\delta_{H,C}$ were measured at 25°±3° for solutions in CH_2Cl_2 and $CDCl_3$, respectively, unless noted otherwise. Signal assignment such as 1^3 stands for a proton at C-1 of sugar residue *3*. **1**: $[\alpha]_D$ -19° (c 0.3, H_2O); R_F 0.18 in 4:2:2:1 nBuOH-EtOH-H_2O-AcOH; δ_H (1:1 D_2O-CD_3OD) 2.004 and 2.015 (2s, 2Ac), 3.789 (s, OMe), 4.444, 4.492, and 4.556 (3d, 7.3-8.3Hz, 1^2, 1^3, and 1^4), 5.025 (d, 8.6Hz, 1^1); FAB MS m/z 905 (M^++Na). **3**: $[\alpha]_D$ +38° (c 1.0); δ_H 2.179 (s, Lev), 2.292, 2.341 and 2.356 (3s, 3MeBz), 6.795 (d, 3.7Hz, H-1), 8.631 (s, NH). **4**: $[\alpha]_D$ +12° (c 1.0); δ_H 1.426 and 1.527 (2s, CMe_2), 5.661 (m, CH=CH_2), 6.635 (d, 8.8Hz, H-1), 8.632 (s, NH). **5**: $[\alpha]_D$ +86° (c 1.0); δ_H 2.159 and 2.181 (2s, 2Ac), 2.294, 2.393, and

2.792 (3s, 2MeBz and Lev), 3.665 (s, OMe), 4.852 (d, 8.1Hz, 1^2), 5.586 (d, 8.4Hz, 1^1). **6**: $[\alpha]_D$ +105° (c 1.0); δ_H 2.170 (s, Lev), 2.345 and 2.360 (2s, 2 MeBz), 5.695 (m, -C*H*=CH_2), 6.732 (d, 3.7Hz, H-1), 8.607 (s, NH). **7**: $[\alpha]_D$ +3° (c 1.0); δ_H 1.38 and 1.51 (2s, Me_2C), 3.67 (s, OMe), 5.73 (d, 7.9Hz, H-1). **8**: $[\alpha]_D$ -48° (c 1.0, MeOH); δ_H 3.791 (s, OMe), 4.903 (d, 7.7Hz, H-1), 5.587 (s, C*H*Ph). **9**: $[\alpha]_D$ +76° (c 1.0); δ_H 2.187 (s, Lev), 2.353 and 2.364 (2s, 2MeBz), 3.744 (s, OMe), 5.121 (d, 8.1Hz, H-1). **10**: $[\alpha]_D$ +22° (c 1.0); δ_H 2.160 (s, Lev), 2.289, 2.348 x 2 (2s, 3MeBz), 3.744 (s, OMe), 5.231 (d, 7.7Hz, H-1), 5.578 and 5.894 (2t, 9.5Hz, *3* and *4*). **11**: $[\alpha]_D$ +65° (c 1.0); δ_H 2.192 (s, Lev), 2.354 and 2.358 (2s, 2MeBz), 3.739 (s, OMe), 5.164 (d, 7.7Hz, H-1). **12**: $[\alpha]_D$ +101° (c 1.0); δ_C 90.4 (1α), 95.8 (1β). **14**: $[\alpha]_D$ +22° (c 1.0); δ_H 1.417 and 1.521 (2s, CMe_2), 3.708 (s, OMe), 5.869 (d, 8.4Hz, H-1). **15**: $[\alpha]_D$ +68° (c 1.0); δ_H(CD_3COCD_3), 1.471 and 1.677 (2s, CMe_2), 2.164 (s, Lev), 2.279 and 2.342 (2s, 2MeBz), 3.627 (s, OMe), 5.264 (d, 8.1Hz, 1^2), 5.652 (d, 8.4Hz, 1^1). **16**: $[\alpha]_D$ +79° (c 1.0); δ_H 2.101 and 2.122 (2s, 2Ac), 2.206, 2.299 and 2.389 (3s, 2MeBz and Lev), 3.676 (s, OMe), 4.603 (d, 8.1Hz, 1^2), 5.427 (d, 8.4Hz, 1^1). **17**: $[\alpha]_D$ +28° (c 1.0); δ_H 1.129 and 1.234 (2s, CMe_2), 1.981 and 2.070 (2s, 2Ac), 2.239 (s, Lev), 2.326 and 2.375 (2s, 2MeBz), 3.662 (s, OMe), 4.393 (d, 7.7Hz, 1^2), 5.220 and 5.354 (2d, ~8.2Hz, 1^1 and 1^3). **18**: $[\alpha]_D$ +35° (c 1.0); δ_H 1.160 and 1.261 (2s, CMe_2), 1.978 and 2.069 (2s, 2Ac), 2.231 (s, Lev), 2.322 and 2.374 (2s, 2MeBz), 3.659 (s, OMe), 4.399 (d, 8.1Hz, 1^2), 5.124 and 5.359 (2d, 8.4Hz, 1^1 and 1^3). **19**: $[\alpha]_D$ +5.5° (c 1.0); δ_H 1.216 and 1.302 (2s, CMe_2), 1.918 and 2.058 (2s, 2Ac), 2.160 and 2.224 (2s, 2Lev), 2.238, 2.315, 2.326, and 2.370 x 2 (4s, 5MeBz), 3.657 (s, OMe), 4.324 and 4.886 (2d, 7.7Hz, 1^2 and 1^4), 4.967 and 5.337 (2d, ~8.5Hz, 1^1 and 1^3). **20**: $[\alpha]_D$ +30° (c 1.0); δ_H 1.917, 1.937, 2.018, and 2.060 (4s, 4Ac), 2.228, 2.335 x 2, 2.355 and 2.385 (4s, 5MeBz), 3.668 (s, OMe), 4.559 and 4.603 (2d, ~7.5Hz, 1^2 and 1^4), 5.084 and 5.371 (2d, 8.4Hz, 1^1 and 1^3). **21**: $[\alpha]_D$ +9.5° (c 1.0); R_F 0.10 in 10:9:1 CH_2Cl_2-EtOAc-AcOH; δ_H (1:1 $CDCl_3$-CD_3OD) 1.849, 1.959, 2.077 x 2 (3s, 4Ac), 2.252, 2.304, 2.337, 2.396, and 2.413 (5s, 5MeBz), 3.672 (s, OMe), 4.115 and 4.184 (2d, 9.2 and 9.5Hz, respectively, 5^2 and 5^4), 4.442 and 4.651 (2d, 7.7 and 8.1Hz, respectively, 1^2 and 1^4), 4.981 and 5.373 (2d, 8.1 and 8.8Hz, respectively, 1^1 and 1^3). Corresponding dimethylester was prepared by treatment with CH_2N_2; δ_H 1.852, 1.892, 2.064, and 2.091 (4s, 4Ac), 2.241, 2.304, 2.332, 2.373, and 2.399 (5s, 5MeBz), 3.476, 3.603, and 3.663 (3s, 3OMe), 4.393 and 4.537 (2d, 7.3 and 7.7Hz, respectively, 1^2 and 1^4), 4.876 and 5.348 (2d, 8.1 and 8.4Hz, respectively, 1^1 and 1^3).

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(Received in Japan 16 April 1992)