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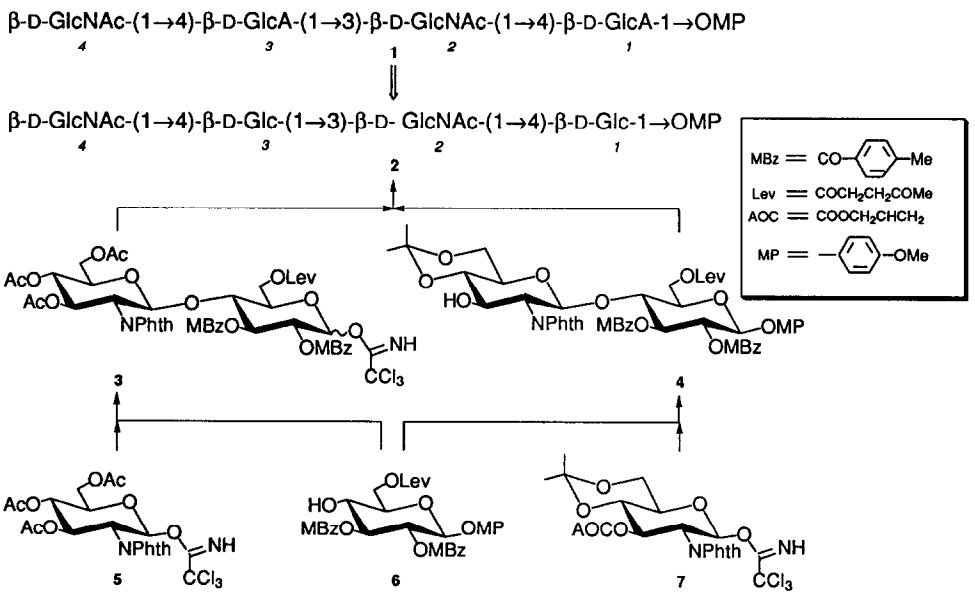
SYNTHESIS OF A TETRASACCHARIDE FRAGMENT OF HYALURONIC ACID HAVING A GLUCURONIC ACID AT THE REDUCING END[1]

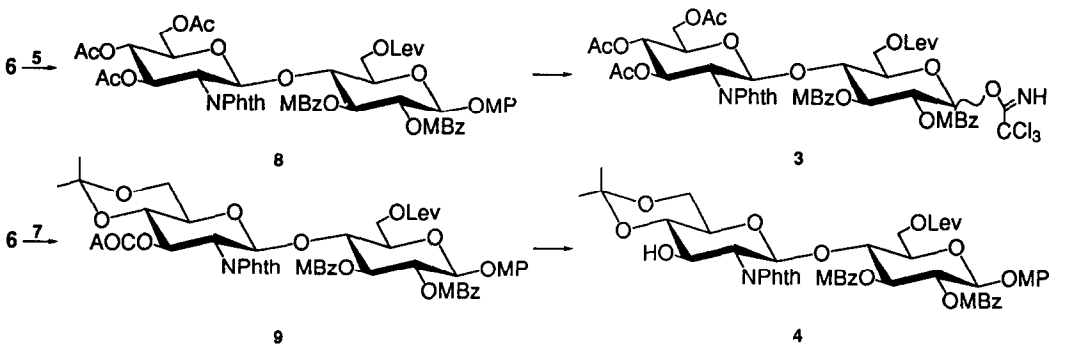
Ted M. Slaghek*[a], Teija K. Hyppönen[a], Tomoya Ogawa[b,c],
Johannis P. Kamerling[a], and Johannes F.G. Vliegenthart[a]
a) Bijvoet Center, Department of Bio-Organic Chemistry, Utrecht University, P.O. Box 80.075, NL-3508 TB Utrecht, The Netherlands
b) The Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama, 351-01 Japan
c) Faculty of Agriculture, University of Tokyo, Yayoi, Bunkyo-ku, Tokyo, 113 Japan

Abstract: A stereocontrolled synthesis of a tetrasaccharide fragment of hyaluronic acid, β-*p*-methoxyphenyl glycoside of β-D-GlcNAc-(1→4)-β-D-GlcA-(1→3)-β-D-GlcNAc-(1→4)-D-GlcA, is presented.

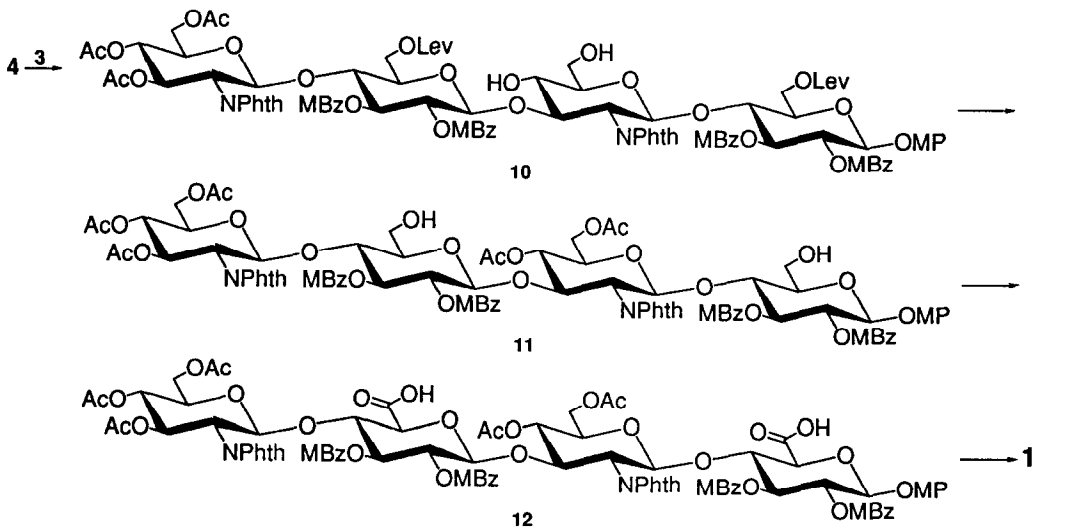
Hyaluronic acid (HA) is a linear, extracellular carbohydrate polymer consisting of disaccharide repeating units[2], namely [→4)-β-D-Glc*p*A-(1→3)-β-D-Glc*p*NAc-(1→]$_n$. It is a major component in extracellular matrices[3] and regulates various biological processes such as cellular proliferation[4,5], cell-cell recognition[3], cell migration[6], and cell adhesion. Interestingly, high concentrations of HA suppress vascularisation[7], while enzymically (hyaluronidase) generated medium-sized fragments of HA oligosaccharides at low concentrations stimulate the formation of new capillary bloodvessels[8,9]. Recently, it was suggested that higher concentrations of oligosaccharides of HA suppress the initial growth, and therefore it was postulated that they might act as a potential antitumor drug[10].



Isolation of the various oligosaccharides is laborious and so far mainly mixtures of even numbered oligosaccharides were used in biological testing[8,9]. The finding that these oligosaccharides can modulate angiogenesis initiated a synthetic program for the preparation of a wider range of medium-sized oligosaccharide fragments built up of even or odd number of monosaccharides with GlcA or GlcNAc at the reducing end. This report describes the stereoselective synthesis of HA fragment **1**. Oxidation of the primary hydroxyl groups (**2**) was carried out in the final stage of the synthesis since the C-4 hydroxyl group of D-glucuronic acid has a low reactivity towards glycosidation[11]. Therefore, key tetrasaccharide **2** was designed, and synthesised from glycosyl donor **3** and glycosyl acceptor **4**. These synthons can be prepared by coupling of glycosyl donor **5** with glycosyl acceptor **6** and glycosyl donor **7** with **6**, respectively.



The necessary monosaccharide building units **5**, **6**, and **7** were synthesised as described earlier[1,12]. Stereocontrolled glycosylation of **6**[13] with 1.5 equivalents of **5** in CH_2Cl_2 in the presence of $BF_3{\cdot}OEt_2$ and molecular sieves 4A at 25°C afforded in 81% **8**[13], which was converted into glycosyl donor **3**[13] in 2 steps (*1* CAN[14] in 1:1:1.4 toluene-H_2O-MeCN, *2* CCl_3CN[15], DBU in CH_2Cl_2, 88% overall).



Also stereocontrolled condensation of **6** with 1.5 equivalents of **7** in CH_2Cl_2 in the presence of $BF_3{\cdot}OEt_2$ and molecular sieves 4A gave **9**[13] in 81% yield, which was subsequently treated with $(Ph_3P)_4Pd$[16,17] and morpholine in THF to afford in 88% glycosyl acceptor **4**[13]. TMSOTf-MS4A promoted glycosylation of **4** with disaccharide donor **3** (2.5 equivalents) in CH_2Cl_2 at 0°C gave after acid hydrolysis with TFA in CH_2Cl_2 and water tetrasaccharide **10**[13] in 77% yield. Compound **10** was further converted into **11** in 2 steps (*1* Ac_2O-DMAP in py, *2* $NH_2NH_2{\cdot}HOAc$ in 2:1 EtOH-toluene[18,19], 85% overall). The oxidation of the two primary hydroxyl groups to obtain **12**[13] was successfully achieved in 2 steps (*1* DMSO, $(COCl)_2$, iPr_2NEt[20], -78°C, *2* $NaClO_2$, NaH_2PO_4 in 3:2:1 *t*BuOH-H_2O-2-methylbutene[21,22], 76% overall). The final deprotection of **12** into **1** was carried out in 2 steps (*1* $MeNH_2$[23] in MeOH, *2* Ac_2O in MeOH, 59% overall).

In summary, a stereocontrolled synthesis of HA tetrasaccharide **1** was carried out in a highly efficient manner by using one monosaccharide acceptor **6** and two monosaccharide donors **5** and **7**.

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References and Notes

1 Part 3. For part 2, see T.M. Slaghek, Y. Nakahara, T. Ogawa, J.P. Kamerling, and J.F.G. Vliegenthart, *Carbohydr. Res.*, submitted. For part 1, see: T.M. Slaghek, Y. Nakahara, and T. Ogawa, *Tetrahedron Lett.*, **33**, 4971 (1992).

2 K. Meyer, *Fed Am. Chem. Soc. Exp. Biol.*, **17**, 1075 (1958).

3 T.C. Laurent and J.R.E. Fraser, *FASEB J.*, **6**, 2397 (1992).

4 B.P. Toole, in E.D. Hay ed. *Cell Biology of Extracellular Matrix*, Plenum, New York, 259 (1981).

5 M. Yoneda, S. Shimizu, Y. Nishi, M. Yamagata, S. Suzuki, and K. Kimata, *J. Cell. Sci.*, **90**, 275 (1988).

6 I. Ellis, A.M. Grey, A.M. Schor, and S.L. Schor, *J. Cell. Sci* , **102**, 447 (1992).

7 R.N. Feinberg and D.C. Beebe, *Science*, **220**, 1177 (1983).

8 D.C. West, I.N. Hampson, F. Arnold, and S. Kumar, *Science*, **228**, 1324 (1985).

9 D.C. West and S. Kumar, in *The Biology of Hyaluronan*, Ciba Foundation symposium 143, Wiley, Chichester, England, 187 (1989).

10 S.D. Banerjee and B.P. Toole, *J. Cell. Biol.*, **119**, 643 (1992).

11 Y. Nakahara and T. Ogawa, *Tetrahedron Lett.*, **28**, 2731 (1987); *Carbohydr. Res.*, **173**, 306 (1988).

12 G. Grundler and R.R. Schmidt, *Carbohydr. Res.*, **135**, 203 (1985).

13 Physical data for new compounds are given below, values for $[\alpha]_D$ and δ_H were measured at 25°C 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 monosaccharide residue 3. **1**: $[\alpha]_D$ -45°

(c 1.0, H_2O); R_F 0.55 in 4:2:2:1 nBuOH-EtOH-H_2O-AcOH; δ_H (D_2O) 2.026 and 2.046 (2s, 2Ac), 3.810 (s, OMe), 4.491, 4.541, and 4.591 (3d, 7.8-8.4 Hz, 1^2, 1^3, and 1^4), 5.015 (d, 8.6 Hz, 1^1); FAB MS m/z 883 (M+H). **3**: $[\alpha]_D$ +80° (c 1.0) (α:β 3:2); δ_H 1.789, 1.912, and 1.978 (3s, 3Ac), 2.209 (s, Lev), 2.327 and 2.379 (2s, 2MeBz), 5.585 (d, 8.1 Hz, 1^2), 6.600 (d, 3.7 Hz, $1^1\alpha$), 6.631 (d, 8.8 Hz, $1^1\beta$). **4** : $[\alpha]_D$ +31° (c 1.0); δ_H 1.239 and 1.250 (2s, CMe_2), 2.160 (s, Lev), 2.316 and 2.370 (2s, 2MeBz), 3.678 (s, OMe), 5.048 (d, 7.5 Hz, 1^1), 5.287 (d, 8.2 Hz, 1^2). **8**: $[\alpha]_D$ +57° (c 1.0); δ_H 1.793, 1.918, and 1.965 (3s, 3Ac), 2.185 (s, Lev), 2.355 and 2.389 (2s, 2MeBz), 3.706 (s, OMe), 5.027 (d, 7.7 Hz, 1^1), 5.514 (d, 8.4 Hz, 1^2). **9**: $[\alpha]_D$ +25° (c 1.0); δ_H 1.224 and 1.254 (2s, CMe_2), 2.188 (s, Lev), 2.338 and 2.390 (2s, 2MeBz), 3.695 (s, OMe), 5.026 (d, 7.5 Hz, 1^1), 5.408 (d, 8.0 Hz, 1^2). **10**: $[\alpha]_D$ +70° (c 1.0); δ_H 1.768, 1.889, and 1.923 (3s, 3Ac), 2.162 and 2.166 (2s, 2Lev), 2.305, 2.341 x 2, and 2.380 (3s, 4MeBz), 3.683 (s, OMe), 4.443 and 4.957 (2d, 7.2 and 7.9 Hz, respectively, 1^1 and 1^3), 5.025 and 5.414 (2d, 8.3 Hz, 1^2 and 1^4). **11**: $[\alpha]_D$ +59° (c 1.0); δ_H 1.779, 1.836, 1.882, 1.913, and 1.946 (5s, 5Ac), 2.337 x 2, and 2.362 x 2 (2s, 4MeBz), 3.690 (s, OMe), 4.504 and 4.990 (2d, 7.2 and 7.7 Hz, respectively, 1^1 and 1^3), 5.149 and 5.458 (2d, 8.4 Hz, 1^2 and 1^4). **12**: $[\alpha]_D$ +6° (c 1.0); R_F 0.48 in 10:9:1 CH_2Cl_2-acetone-HOAc; δ_H 1.757, 1.824, 1.842, and 1.881 x 2 (4s, 5Ac), 2.298, 2.333, 2.344, and 2.382 (4s, 4MeBz), 3.689 (s, OMe), 3.783 and 3.859 (2d, 9.5 and 9.0 Hz, respectively, 5^1 and 5^3), 4.438 and 5.057 (2d, 7.5 and 7.7 Hz, respectively, 1^1 and 1^3), 5.370 and 5.567 (2d, 8.3 and 8.4 Hz, respectively, 1^2 and 1^4). The corresponding dimethylester was prepared by treatment with CH_2N_2: δ_H 1.767 x 2, 1.884, 1.894, and 1.908 (4s, 5Ac), 2.308, 2.340 x 2, and 2.385 (3s, 4MeBz), 3.386, 3.577, and 3.686 (3s, 3OMe), 3.722 and 3.794 (2d, 9.7 and 8.9 Hz, respectively, 5^1 and 5^3), 4.355 and 5.002 (2d, 7.6 and 7.0 Hz, respectively, 1^1 and 1^3), 4.971 and 5.285 (2d, 8.4 and 8.3 Hz, respectively, 1^2 and 1^4).

14 T. Fukuyama, A.A. Laird, and L.M. Hotchkiss, *Tetrahedron Lett.*, **26**, 6291 (1985).

15 R.R. Schmidt, J. Michel, and M. Roos, *Liebigs Ann Chem.*, 1343 (1984).

16 H. Kunz and H. Waldman, *Angew. Chem.*, **96**, 49 (1984).

17 Y. Hayakawa, H. Kato, M. Uchihyama, H. Kajino, and R. Noyori, *J Org. Chem.*, **51**, 2400 (1986).

18 J.H. van Boom and P.M.J. Burgers, *Tetrahedron Lett.*, 4875 (1976).

19 N. Jeker and C. Tamm, *Helv. Chim. Acta*, **71**, 1895, 1904 (1988).

20 K. Omura and D. Swern, *Tetrahedron*, **43**, 1651 (1978).

21 B.O. Lindgren and T. Nilsson, *Acta Chem. Scand.*, **27**, 888 (1973).

22 B. Kraus and B. Roth, *J. Org. Chem.*, **45**, 4825 (1980).

23 M.S. Motawai, J. Wengel, A.E.S. Abdel-Megid, and E.B. Pedersen, *Synthesis*, 384 (1989).

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