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SYNTHESIS AND BIOLOGICAL ACTIVITY OF A NEW ANTI-FACTOR Xa PENTASACCHARIDE WITH A *C*-INTERGLYCOSIDIC BOND

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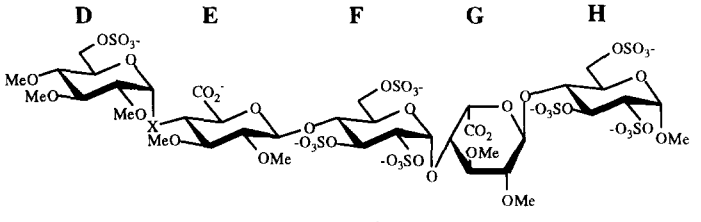
Abstract : A mixed synthetic C, O-pentasaccharide **17** has been synthesized and shown to display an anti-factor Xa activity similar to that of the corresponding O-pentasaccharide **18**. **17** represents the first example of a synthetic C-oligosaccharidic mimetic eliciting a significant biological response. © 1997 Elsevier Science Ltd.

Oligosaccharides are involved in an increasing number of biological processes[1]. This has stimulated the development of selective methods for the synthesis of complex oligosaccharides[2] of biological significance. The stereoselective chemical synthesis of pentasaccharides playing key roles in the antithrombotic activity of heparin[3], together with intense current efforts paid to the chemical synthesis of Lewis^x derivatives, within the context of inflammation[4], clearly demonstrate that synthetic oligosaccharides are more and more designated as active substances for potential drug development[5]. Among various analogues of di - or oligosaccharides, those arising from the replacement of the interglycosidic oxygen atom by a methylene group are of particular interest. The underlying question is indeed to evaluate to what extent such a replacement - which eradicates the *exo*-anomeric effect - affects the conformational properties of the molecule[6], resulting in enhanced or impaired biological activity. For this reason, the construction of so-called *C*-disaccharides[7] warrants significant attention[8]. Kishi has already demonstrated[9] that the *C*-trisaccharide analog of the human blood group antigenic determinant is still recognized by a lectin (UEA-I).

The present study was undertaken to assess the influence of introducing a stable carbon interglycosidic bond in an antithrombin III (AT III) binding synthetic pentasaccharide, this choice being dictated by the potential pharmaceutical importance of this class of molecules. We selected **17** as the target pentasaccharide, because the biological properties of the corresponding synthetic "*O*-pentasaccharide"**18** have been thoroughly investigated[10] and also because its specific methylated structure was well adapted to our synthetic strategy.



17 X= CH_2, **18** X=O

The substituted *C*-disaccharide trichloroacetimidate **10**[11] was first prepared (scheme 2) through a methodology that we recently developed for the expeditious synthesis of *C*-disaccharides[12]. This method is based on an 9 *endo*-trig radical cyclization between two tethered monosaccharides : the selenophenyl glucoside **3** and the *exo* methylene sugar **6**, which were uneventfully prepared, as shown in scheme 1, from the two known precursors **1**[13] and **4**[14]. The structure of the *C*-disaccharide **8** has been secured by X-ray analysis.

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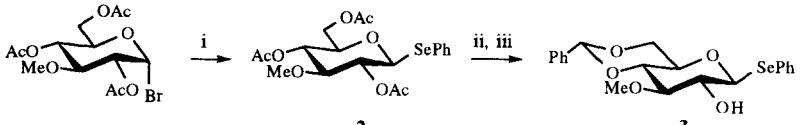
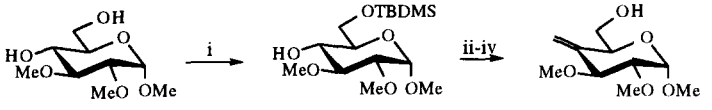
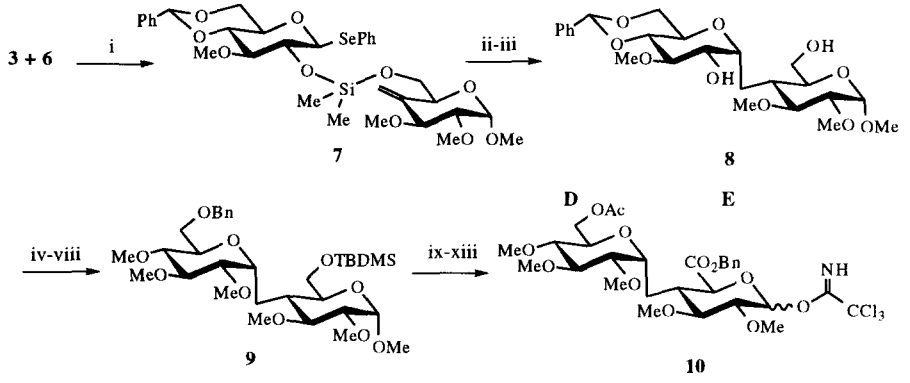


Reagents : i) PhSeSePh, $NaBH_4$, EtOH, CH_2Cl_2, reflux (86%); ii) NaOMe, MeOH, rt (94%); iii) $PhCH(OMe)_2$, APTS, CH_3CN, rt (88%).



Reagents : i) TBDMSCl, Et_3N, DMAP, CH_2Cl_2, rt (95%); ii) $(COCl)_2$, DMSO, Et_3N (93%); iii) $[Ph_3PCH_3]Br$, n-BuLi, -70°C → rt (60%); iv) CSA, CH_2Cl_2, MeOH 5:1, rt (95%).

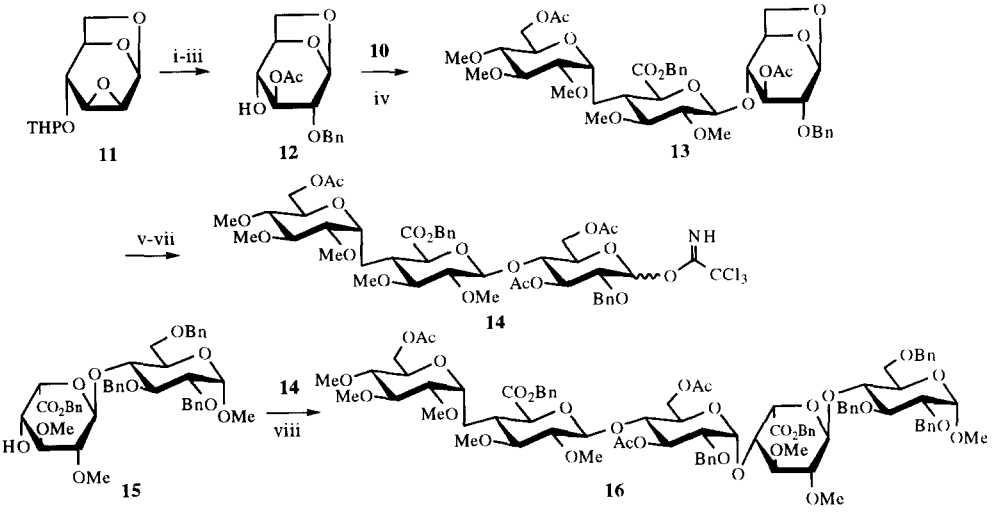
Scheme 1 : synthesis of the radical glycosyl donor **3** and of the radical acceptor **6**



Reagents :i) **3**, BuLi, Me_2SiCl_2, THF then **6** imidazole, THF (90% overall yield); ii) Bu_3SnH, AIBN, toluene; iii) aq. HF 40%, THF (36% overall yield). iv) Ac_2O, Pyr, DMAP (96%); v) HCl, Et_2O, $NaBH_3CN$, THF (87%); vi) NaOMe, MeOH (98%); vii) TBDMSCl, Et_3N, DMAP, CH_2Cl_2 (86%); viii) CH_3I, NaH, DMF (93%); ix) CrO_3, H_2SO_4, acetone; x) BnBr, Bu_4NI, $KHCO_3$, DMF (87% overall yield); xi) H_2SO_4, Ac_2O (76%); xii) NH_2NH_3AcO, DMF (80%); xiii) CCl_3CN, DBU, CH_2Cl_2 (87%).

Scheme 2 : synthesis of the **DE** glycosyl donor **10**

The various steps leading to the synthesis of the pentasaccharide **16** are now depicted in the self-explanatory scheme 3. The imidate **10** was condensed with the alcohol **12** [prepared from 1,6:2,3-di-anhydro-4-*O*-(terahydropyran-2-yl)-β-D-mannopyranose **11**[15]], the use as a solvent of acetonitrile at low temperature (-37°C) classically securing[16] - in the absence of a participating group - the selective formation of the trisaccharide **13** (β:α ratio 5.5:1, overall yield 88%). Classical conversion of **13** into the trichloroacetimidate donor **14** set up the stage for the final condensation step with the disaccharidic alcohol **15**[10]. The precious donor **14** was opposed to an excess (1.4 eq.) of the acceptor **15**, whereby the protected pentasaccharide **16** was obtained in 80% from **14**. The expected pentasaccharide **17** was obtained through a three-step sequence[17] (hydrogenation, saponification, and sulfation).



Reagents : i)1M BnONa, BnOH, 80 °C (95%); ii) Ac_2O, DMAP, Et_3N (97 %); iii) pTsOH, MeOH, reflux (95 %); iv)TMSOTf, CH_3CN, -37°C β:α 5.5:1 (88%); v) H_2SO_4, Ac_2O (100%); vi) NH_2NH_3AcO, DMF (90%); vii) CCl_3CN, DBU, CH_2Cl_2 (94%); viii) 0.2eq TMSOTf, CH_2Cl_2, -20°C (92%).

Scheme 3: synthesis of the protected mixed C, O-pentasaccharide **16**

Binding of the pentasaccharides to AT III induces a conformational change that results in an accelerated inhibition of blood coagulation factor Xa[3]. A comparison of the affinities of **17** and **18** for AT III, and the corresponding anti-factor Xa activities, are shown on table 1.

Table 1. Biological properties of the pentasaccharides **17** and **18** *in vitro*

Compounds	Affinity for AT III (Kd)	Anti-Factor Xa activity (units/mg)
17	2.8 ±0.1 nM	880±40
18	1.9 ±0.1 nM	1180±30

The binding constants of the pentasaccharides to AT III were determined by fluorescence spectroscopy using a method[17] based on the increase in intrinsic fluorescence of AT III when bound to heparin or active fragments. The anti-factor Xa activity was determined by an amidolytic method adapted from Teien and Lie[17]. The values reported on table 1 interestingly reveal that the substitution of an *O*-glycosidic bond by a *C*-glycosidic bond hardly affects the affinity for AT III, as well as the anti-factor Xa activity.

Conclusion

We thus can conclude that **17**, like **18**, is able to induce the conformational change in AT III resulting in active site loop exposure and trapping of the target serine protease factor Xa. Moreover, since it has recently been shown[18] that the DEF trisaccharidic part of the pentasaccharides is responsible for the initial recognition by AT III, this means that the active conformation is preserved in the DEF part of **17**. This work also demonstrates that the interglycosidic oxygen atom between D and E units of **18** is not directly and critically involved in any interaction with AT III. Compound **17** represents the first example of a new class of anti-factor Xa pentasaccharides containing *C*-interglycosidic bonds.

References and Notes

1. a) For selected references, see a) Perez, M.; Sindal, A. K.; Hakomori, S.; Paulson, J. C. *Science* **1990**, *250*, 1130; b) Lasky, L. A. *Science* **1992**, *258*, 964; c) Varki, A. *Glycobiology* **1993**, *3*, 97; d) Feizi, T. *Curr. Opin Struct. Biol.* **1993**, *3*, 701; e) Boren, T.; Falk, P.; Roth, K. A.; Larson, G.; Normak, S, *Science* **1993**, *262*, 1892; f), Varki, A. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 7390.
2. For a recent review, see Boons, G.-J. *Tetrahedron* **1996**, *52*, 1095.
3. Van Boeckel, C. A. A.; Petitou, M. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1671.
4. Uchiyama, T.; Vassilev, V. P.; Kajimoto, T.; Wong, W.; Huang, H.; Lin, C.-C.; Wong, C.-H. *J. Am. Chem. Soc.* **1995**, *117*, 5395 and references therein.
5. a) Petitou, M. *ACS, Symp. Series* **1994**, *560*, 19; b) Musser, J. F.; Fugedi, P.; Anderson, M. B.; Rao, N.; Peto, C.; Tyrrell, D.; Holme, K.; Tressler, R. *Drugs News & Perspect.* **1996**, *9*, 133; c) Zopf, D.; Roth, S. *Lancet* **1996**, *347*, 1017.
6. a) Wang, Y.; Goekjian, P. G.; Ryckmann, D. M.; Miller, W. H.; Babirad, S. A.; Kishi, Y. *J. Org. Chem.* **1992**, *57*, 482; b) Espinosa, J.-F.; Martín-Pastor, M.; Asensio, J. L.; Dietrich, H.; Martín-Lomas, M.; Schmidt, R. R.; Jiménez-Barbero, J. *Tetrahedron Lett.* **1995**, *36*, 6329; c) Berthault, P.; Birliradis, N.; Rubinstenn, G.; Sinaÿ, P.; Desvaux, H. *J. Biomol. NMR* **1996**, *8*, 23; see also 8f.
7. Rouzaud, D., Sinaÿ, P. *J. Chem. Soc., Chem. Commun.* **1983**, 1353.
8. Representative selection of stereoselective syntheses of C-disaccharides: a) Martin, O. R.; Lai, W. *J. Org. Chem.* **1993**, *58*, 176 and references therein; b) Mazéas, D.; Skrydstrup, T.; Doumeix, O.; Beau, J.-M. *Angew. Chem. In.t Ed. Engl.* **1994**, *33*, 1383; c) Ferritto R., Vogel P. *Tetrahedron: Asymmetry* **1994**, *5*, 2077; d) Dietrich, H.; Schmidt, R. R. *Liebigs Ann. Chem.* **1994**, 975; e) Armstrong, R. W.; Sutherlin, D. P.; *Tetrahedron Lett.* **1994**, 35, 7743; f) Wei, A.; Haudrechy, A.; Audin, C.; Jun, H.-S.; Haudrechy-Bretel, N.; Kishi, Y. *J. Org. Chem.* **1995**, *60*, 2160; g) Sutherlin, D. P.; Armstrong, R. W. *J. Am. Chem. Soc.* **1996**, *118*, 9802.
9. Wei, A.; Boy, K. M.; Kishi, Y. *J. Am. Chem. Soc.* **1995**, *117*, 9432.
10. Westerduin, P.; van Boeckel, C. A. A.; Basten, J. E. M.; Broekhoven, M. A.; Lucas, H.; Rood, A.; van der Heijden, H.; van Amsterdam, R. G. M.; van Dinther, T. G.; Meuleman, D. G. ; Visser, A.; Vogel, G. M. T.; Damm, J. B. L. ; Overklift, G. T. *Bioorg. Med. Chem.* **1994**, *2*, 1267.
11. All new compounds gave satisfactory elemental analysis and NMR data were in agreement with the proposed structures.
12. a) Xin, Y. C.; Mallet, J.-M.; Sinaÿ, P. *J. Chem. Soc., Chem. Commun.* **1993**, 864; b) Vauzeilles, B.; Cravo, D.; Mallet, J.-M.; Sinaÿ, P. *Synlett* **1993**, 522; c) Chénedé, A.; Perrin, E.; Rekaï, E. D.; Sinaÿ, P. *Synlett* **1994**, 420; d) Mallet, A.; Mallet, J.-M.; Sinaÿ, P. *Tetrahedron: Asymmetry* **1994**, 2593.
13. Sen, A. K.; Sarkar, K. K.; Banerji, N. *J. Carbohydr. Chem.* **1988**, *7*, 645.
14. Bredereck, H. *Ber.* **1935**, *68*, 781.
15. Paulsen, H.; Stenzel, W. *Chem. Ber.* **1978**, *111*, 2348.
16. a) Schmidt, R. R.; Behrendt, M.; Toepfer, A. *Synlett* **1990**, 694; b) Sinaÿ, P. *Pure Appl. Chem.* **1991**, *63*, 519; c) Marra, M.; Esnault, J.; Veyrières, A.; Sinaÿ, P. *J. Am. Chem. Soc.* **1992**, *114*, 6354.
17. Petitou, M.; Duchaussoy, P.; Jaurand, G.; Gourvenec, F.; Lederman, I.; Strassel, J.-M.; Barzû, T.; Crépon, B.; Hérault, J.-P.; Lormeau, J.-C.; Bernat, A.; Herbert, J.-M. *J. Med. Chem.*, in press.
18. Petitou, M.; Barzû, T.; Hérault J.-P.; Herbert J.-M. *Glycobiology*, in press.

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