



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

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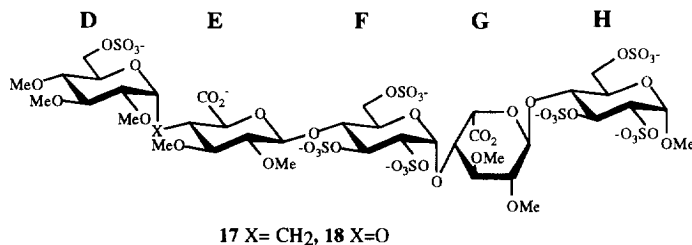
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Abstract : A mixed synthetic C, O-pentasaccharide **17** has been synthesized and shown to display an anti-factor Xa activity similar to that of the corresponding O-pentasaccharide **18**. **17** represents the first example of a synthetic C-oligosaccharidic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

Oligosaccharides are involved in an increasing number of biological processes¹. This has stimulated the development of selective methods for the synthesis of complex oligosaccharides² of biological significance. The stereoselective chemical synthesis of pentasaccharides playing key roles in the antithrombotic activity of heparin³, together with intense current efforts paid to the chemical synthesis of Lewis^x derivatives, within the context of inflammation⁴, clearly demonstrate that synthetic oligosaccharides are more and more designated as active substances for potential drug development⁵. 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 molecule⁶, resulting in enhanced or impaired biological activity. For this reason, the construction of so-called C-disaccharides⁷ warrants significant attention⁸. Kishi has already demonstrated⁹ that the C-trisaccharide analog of the human blood group antigenic determinant is still recognized by a lectin (UEA-I).

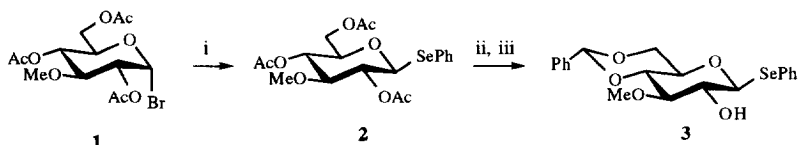
The present study was undertaken to assess the influence of introducing a stable carbon interglycosidic bond in an antithrombin III (AT III) binding synthetic pentasaccharide, this choice being dictated by the potential pharmaceutical importance of this class of molecules. We selected **17** as the target pentasaccharide, because the biological properties of the corresponding synthetic "O-pentasaccharide" **18** have been thoroughly investigated¹⁰ and also because its specific methylated structure was well adapted to our synthetic strategy.



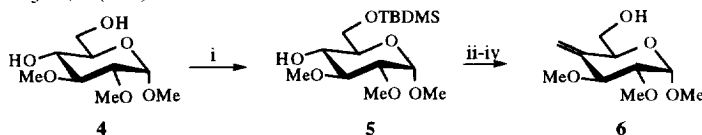
The substituted C-disaccharide trichloroacetimidate **10**¹¹ was first prepared (scheme 2) through a methodology that we recently developed for the expeditious synthesis of C-disaccharides¹². This method is based on an *endo*-trig radical cyclization between two tethered monosaccharides : the selenophenyl 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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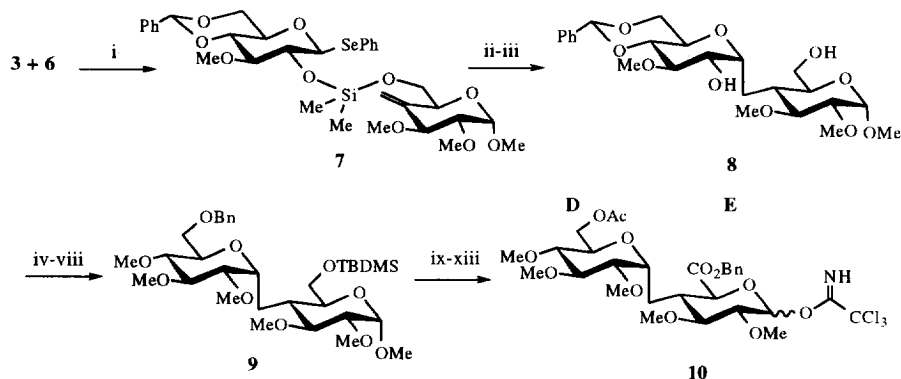


Reagents : i) PhSeSePh, NaBH₄, EtOH, CH₂Cl₂, reflux (86%); ii) NaOMe, MeOH, rt (94%); iii) PhCH(OMe)₂, APTS, CH₃CN, rt (88%).



Reagents : i) TBDMSCl, Et₃N, DMAP, CH₂Cl₂, rt (95%); ii) (COCl)₂, DMSO, Et₃N (93%); iii) [Ph₃PCH₃]⁺Br⁻, n-BuLi, -70°C → rt (60%); iv) CSA, CH₂Cl₂, MeOH 5:1, rt (95%).

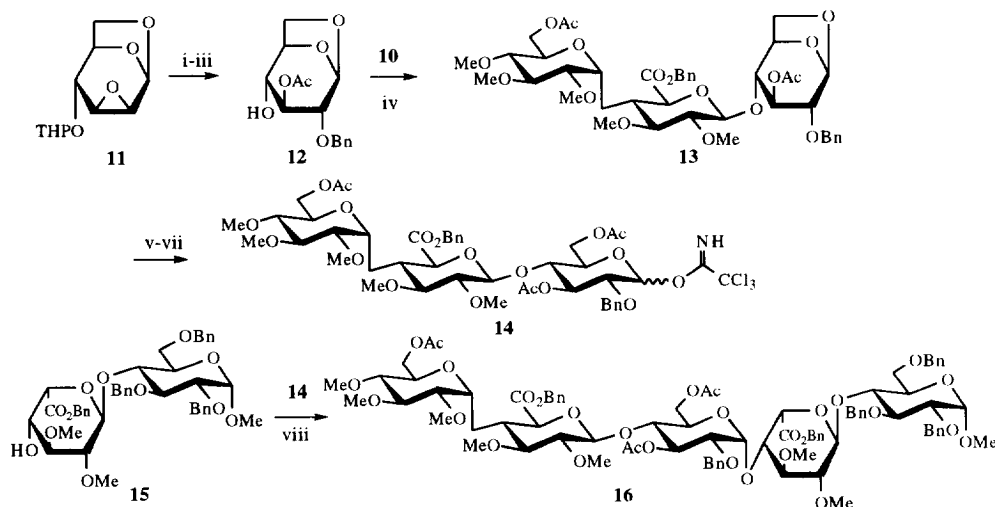
Scheme 1 : synthesis of the radical glycosyl donor **3** and of the radical acceptor **6**



Reagents : i) **3**, BuLi, Me₂SiCl₂, THF then **6** imidazole, THF (90% overall yield); ii) Bu₃SnH, AIBN, toluene; iii) aq. HF 40%, THF (36% overall yield). iv) Ac₂O, Pyr, DMAP (96%); v) HCl, Et₂O, NaBH₃CN, THF (87%); vi) NaOMe, MeOH (98%); vii) TBDMSCl, Et₃N, DMAP, CH₂Cl₂ (86%); viii) CH₃I, NaH, DMF (93%); ix) CrO₃, H₂SO₄, acetone; x) BnBr, Bu₄NI, KHCO₃, DMF (87% overall yield); xi) H₂SO₄, Ac₂O (76%); xii) NH₂NH₃AcO, DMF (80%); xiii) CCl₃CN, DBU, CH₂Cl₂ (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 securing¹⁶ - 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 sequence¹⁷ (hydrogenation, saponification, and sulfation).



Reagents : i) 1M BnONa, BnOH, 80 °C (95%); ii) Ac₂O, DMAP, Et₃N (97 %); iii) pTsOH, MeOH, reflux (95 %); iv) TMSOTf, CH₃CN, -37°C β:α 5.5:1 (88%); v) H₂SO₄, Ac₂O (100%); vi) NH₂NH₃AcO, DMF (90%); vii) CCl₃CN, DBU, CH₂Cl₂ (94%); viii) 0.2eq TMSOTf, CH₂Cl₂, -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 Xa³. 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 method¹⁷ 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 Lie¹⁷. 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 shown¹⁸ 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.

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