CHEMICAL SYNTHESIS OF CHITOOLIGOSACCHARIDES WITH CONTROLLED SIZES AND ARCHITECTURES [1]

Nadine Barroca, Astrid Pernet-Poil-Chevrier, Agnès Crepet, Stéphane Trombotto and Alain Domard*

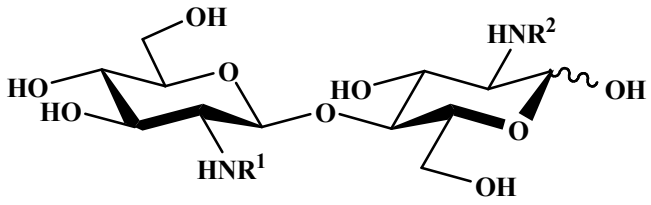
Laboratoire des Matériaux Polymères et des Biomatériaux, UMR 5627 CNRS/UCBL, Université Claude Bernard Lyon 1, Domaine Scientifique de La Doua, Bât. ISTIL, 15 Bd Latarjet, 69622 Villeurbanne Cedex, France.

Abstract

The chemical synthesis of perfect well-defined chitodisaccharides is reported for the first time. The use of 4-*O*-acetyl-3,6-di-*O*-benzyl-2-(trichloroacetamido or benzyloxycarbonylamino)-2-deoxy-α-D-glucopyranosyl trichloroacetimidate, readily prepared from the commercial D-glucosamine, allows the control of the distribution of the *N*-acetyl groups along the oligomer chain, the high yield and stereocontrolled coupling with the low reactive 4-*O*-hydroxyl group of D-glucosamine derivatives. Classical transformations of the protected dimers into the targeted disaccharides were achieved in high yield.

Introduction

Chitooligosaccharides are β-(1→4) linked homo- or hetero-oligomers of 2-acetamido-2-deoxy-D-glucopyranose (GlcNAc) and/or 2-amino-2-deoxy-D-glucopyranose (GlcN). Fairly recently, they have attracted considerable interest as functional materials for many applications in the field of medicine, food industry, cosmetics and pharmaceutics, taking advantage of their interesting biological properties, including antitumoral, antifungal, antimicrobial and eliciting activities.[2]
The biological activities of chitooligosaccharides are likely to depend on both the degrees of N-acetylation (DA) and polymerisation (DP), but also on the sequences of the GlcN and GlcNAc units along the oligomer chain.[3] However, at present time, any methods of preparing chitooligosaccharides do not allow a perfect control of the composition and the distribution of both acetylated and deacetylated units. Indeed, chitooligosaccharides are usually obtained by chemical or enzymatic partial hydrolysis of chitin or chitosan, generating mixtures of oligomers with different DPs and random distributions of *N*-acetyl groups.[2,4]
In order to propose an alternative route, we focused on the total chemical synthesis of chitin and chitosan oligomers. Previously, some chemical syntheses of homooligomers from chitin[5] or totally de-*N*-acetylated chitosan[6] have been reported. For the first time, we describe herein an efficient method leading to a perfect control of the size and the architecture of both homo- and hetero-oligomer structures, through the syntheses of convergent disaccharides **13**, **19**, **25** and **31** (Scheme 3)**,** which allow us to obtain fully deprotected chitodisaccharides **17**, **23**, **29** and **34** (Scheme 1)**,** and to open the way for the construction of fragments with higher molecular size.



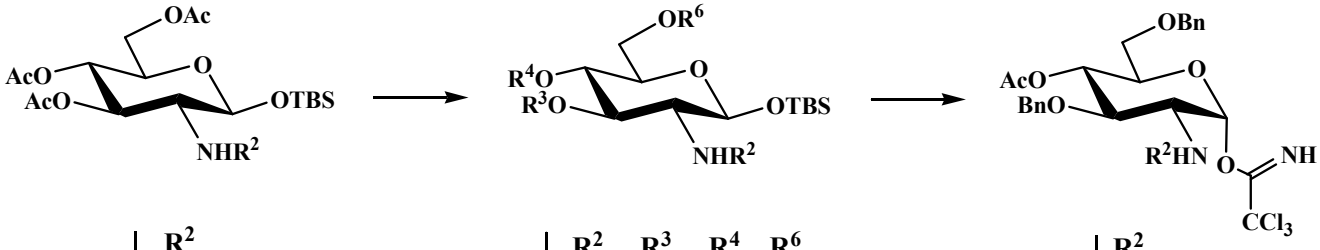
	R1	R2
17	Ac	Ac
23	Ac	H
29	H	Ac
34	H	H

SCHEME 1

Results and Discussion

Synthetic strategy. The availability of *D-gluco* convergent synthons which could build up convergent disaccharides synthons or upper size convergent synthons is a key point for the synthesis of our chitooligosaccharides. Each synthon would be able to be used as acceptor (**4** and **10**) or donor systems (**6** and **12**) in a minimum transformation steps. Therefore, we report herein the application of the *tert*-butyldimethylsilyl glycoside protection of the reducing end and the acetyl protecting group of the non-reducing end of our synthons. These two orthogonal protecting groups allow the growth of the saccharidic chain in each direction without affecting the other protecting groups present on the skeleton. The differentiation between the further *N*-acetyl and *N*-amino groups of the chitosan oligomers was obtained by the use of *N*-trichloroacetamido (TCA) and *N*-benzyloxycarbonylamino (Z) protections, respectively. The ease of attachment of these two protecting groups, their subsequent transformation or cleavage, as well as their enhanced capability to form a β-linkage[7] and their compatibility with different protecting groups manipulations made them versatile amino protecting groups in glucosamine-containing oligosaccharide synthesis.[8]

Monomers preparation. The preparation of the acceptor **4** was achieved in a straightforward manner as follows (Scheme 2). The treatment of the known 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-trichloroacetamido-D-glucopyranose[7] by hydrazine acetate in *N,N*-dimethylformamide, followed by the silylation with *tert*-butyldimethylsilyl chloride, afforded the crystalline **1** in 60% overall yield. The transesterification of **1** in Zemplèn conditions,[9] followed by the acetalation with 2,2-dimethoxybenzaldehyde and camphorsulfonic acid gave **2** in 83% yield. The protection at O-3 was then achieved by treatment with benzyl bromide and sodium hydride in *N,N*-dimethylformamide to give the crystalline **3** in 73% yield. No *N*-benzylation reaction was observed under these conditions.



	R2
1	TCA
7	Z

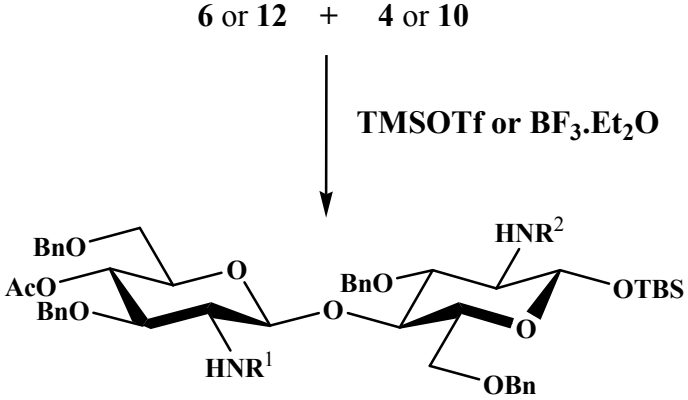
	R2	R3	R4	R6
2	TCA	H	PhCH	
3	TCA	Bn	PhCH	
4	TCA	Bn	H	Bn
5	TCA	Bn	Ac	Bn
8	Z	H	PhCH	
9	Z	Bn	PhCH	
10	Z	Bn	H	Bn
11	Z	Bn	Ac	Bn

	R2
6	TCA
12	Z

SCHEME 2

The regioselective reductive cleavage of the benzylidene acetal of **3** with triethylsilane and trifluoroacetic acid[10,11] gave the crystalline targeted 3,6-di-*O*-benzyl acceptor **4** in 81% yield. The acetylation of the acceptor **4** in conventional conditions (acetic anhydride/pyridine, 1:1 (v/v)) afforded **5**. The 6-regioselective reductive opening of the benzylidene acetal was confirmed from **5**. Indeed, compared to **3**, the ^{1}H NMR spectrum of **5** showed a chemical shift of the H-4 proton from δ 3.70 to 5.06 ppm, while its ^{13}C NMR spectrum highlighted a chemical shift of the C-4 carbon from δ 82.84 to 71.77 ppm.
The second targeted synthon **6** was obtained by removal of the *tert*-butyldimethylsilyl group of **5** by treatment with tetrabutylammonium fluoride and acetic acid in tetrahydrofuran. A subsequent reaction with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene as base afforded the α-imidate **6** with 90% overall yield. The α–anomeric configuration was deduced from the ^{1}H NMR spectrum, thanks to the signal of H-1α (doublet at δ 6.47 ppm with $J_{1,2}$ 3.4 Hz). The two other targeted synthons **10** and **12** were obtained in a similar synthetic route (Scheme 2) from the known 1,3,4,6-tetra-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-D-glucopyranose.[12] However, because of the relative instability of the *N*-benzyloxycarbonylamino group under very basic conditions, the O-3 protection could not be achieved by treatment with benzyl bromide and sodium hydride in *N,N*-dimethylformamide. Thus, **9** was obtained from **8** in 64% yield, by reaction with benzyl bromide in a milder basic system, composed of barium oxide and barium hydroxide octahydrate.[13] In these conditions, no *N*-benzylation reaction was observed. Then, the regioselective reductive cleavage of the benzylidene acetal of **9**, performed in similar conditions as **3**, allowed to get the expected acceptor **10** in 75% yield. The acetylation of **10** and the silyl removal followed by the trichloroacetimidoylation afforded the targeted donor **12** in 86% overall yield.

Coupling reactions. All combinations of the donors (**6** or **12**) and the acceptors (**4** or **10**) led to the preparation of the four different disaccharides **13**, **19**, **25** and **31** in excellent yields, respectively 90, 73, 70 and 70% (Scheme 3). Glycosylations were conducted in dry dichloromethane using as well trimethylsilyl trifluoromethanesulfonate (TMSOTf) or the complex boron trifluoride ethyl etherate ($BF_3.Et_2O$) as catalyst. The β-linkage was confirmed for both **13** (δ 4.96 ppm, $J_{1,2}$ 8.3 Hz, H-1a) and **19** (δ 4.87 ppm, $J_{1,2}$ 8.4 Hz, H-1a) from their ^{1}H NMR spectrum. In the case of **25** and **31**, the signal of the β-anomeric proton was positioned under the CH_2 singlet of the benzyloxycarbonyl peak resonance. In addition, it could not be possible to determine the β–coupling constant from neither the H-2a signal nor the 2D NMR COSY spectrum. Therefore, it was difficult to certify the β–configuration at this stage of the synthesis. However, because of the well known anchimeric assistance of carbamates, there was not so much doubt for the β–configuration of the new glycosidic linkage, as it will be shown below.

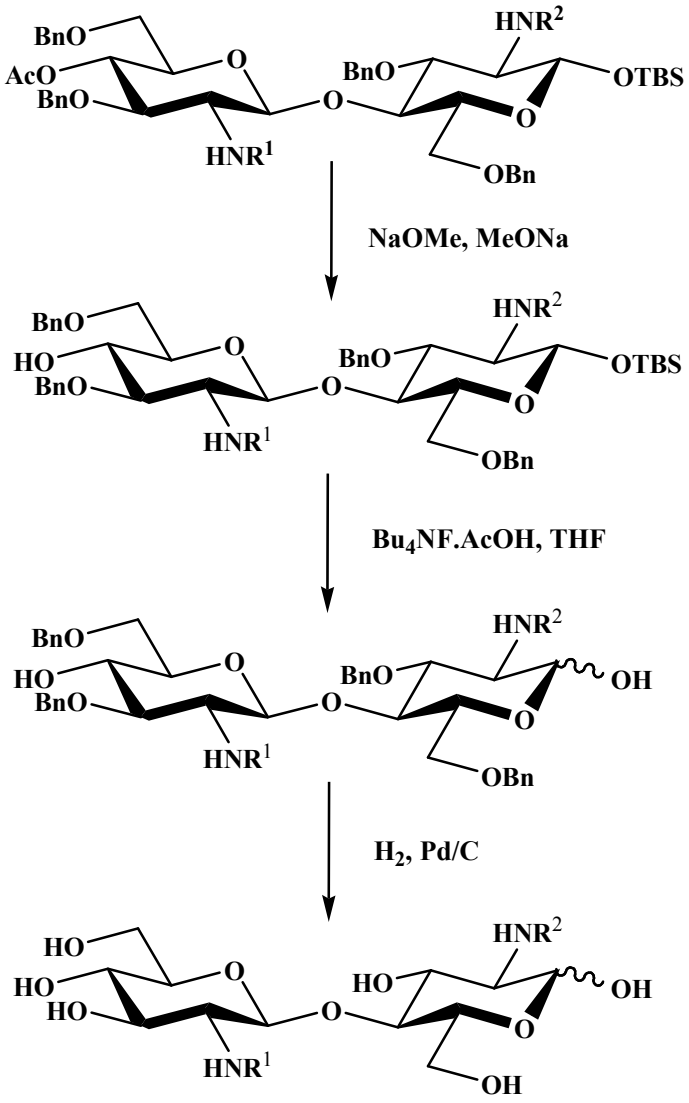


	R^1	R^2
13	TCA	TCA
19	TCA	Z
25	Z	TCA
31	Z	Z

SCHEME 3

Final oligomers deprotections. According to the initial strategy, after forming the perfect controlled skeleton, the next stage consisted in deprotecting completely the oligomers in order to lead to the expected chitodisaccharides (Scheme 4).

First, the *N*-trichloroacetyl groups in compounds **13**, **19** and **25** were readily transformed into the *N*-acetyl groups by the radical reduction[7] with tributyltin hydride and azobisisobutyronitrile (AIBN) leading to the acetamides **14**, **20** and **26** in excellent yields, respectively 97, 82 and 75%. At this stage of the synthesis, we got the confirmation of the β-linked configuration of the disaccharide **26** which could not be clearly identified previously. Thus, the ^{1}H NMR spectrum for **26** showed a signal for H-1 of the non-reducing sugar at δ 4.59 ppm with a large coupling constant $J_{1,2}$ 8.8 Hz, in agreement with a newly established 1,2-trans interglycosidic linkage. Then, the orthogonal protection at O-4 was removed by treatment with methanolic sodium methoxide to afford disaccharides **15**, **21**, **27** and **32** in good to excellent yields, respectively 83, 87, 95 and 79%. The treatment with tetrabutylammonium fluoride and acetic acid in tetrahydrofuran led to the reducing disaccharides **16**, **22**, **28** and **33** in excellent yields, respectively 89, 87, 86 and 89 %. Fortunately, after a 2 steps synthesis, the ^{1}H NMR spectrum showed for the disaccharide **33** a signal for H-1 of the non-reducing sugar at δ 4.72 ppm with a large coupling constant $J_{1,2}$ 7.9 Hz, highlighting the β–configuration of the glycosidic linkage. The final deprotection consisted in removing all benzyl groups by hydrogenolysis in the presence of Pd-C in aqueous methanol to afford the targeted chitodisaccharides **17**, **23**, **29** and **34** in good yields (70-80%).



	R^1	R^2
14	Ac	Ac
20	Ac	Z
26	Z	Ac
31	Z	Z

	R^1	R^2
15	Ac	Ac
21	Ac	Z
27	Z	Ac
32	Z	Z

	R^1	R^2
16	Ac	Ac
22	Ac	Z
28	Z	Ac
33	Z	Z

	R^1	R^2
17	Ac	Ac
23	Ac	H
29	H	Ac
34	H	H

SCHEME 4

In conclusion, the use of a combination of the *N*-trichloroacetamido (TCA) and *N*-benzyloxycarbonylamino (Z) protections for the differentiation between the further *N*-acetyl and the *N*-amino groups in glucosamine derived glycosyl donors and acceptors led to the efficient generation of four perfect well-defined chitodisaccharides. Moreover, we have developed an original synthesis strategy which will allow us to readily provide various chitooligosaccharidic structures. Thus, the way is now open for the construction of higher molecular size well-defined fragments, which is currently being investigated in our group.

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