Carbohydrates

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From Chitin to Bioactive Chitooligosaccharides and Conjugates: Access to Lipochitooligosaccharides and the TMG-chitotriomycin**

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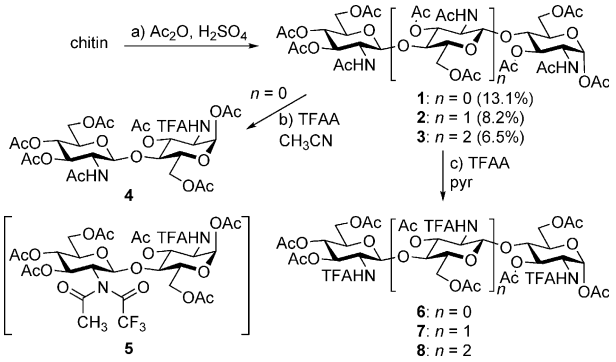
Abstract: *The direct and chemoselective N-transacylation of peracetylated chitooligosaccharides (COSs), readily obtained from chitin, to give per-N-trifluoroacetyl derivatives offers an attractive route to size-defined COSs and derived glycoconjugates. It involves the use of various acceptor building blocks and trifluoromethyl oxazoline dimer donors prepared with efficiency and highly reactive in 1,2-trans glycosylation reactions. This method was applied to the preparation of the important symbiotic glycolipids which are highly active on plants and to the TMG-chitotriomycin, a potent and specific inhibitor of insect, fungal, and bacterial N-acetylglucosaminidases.*

Chitin, a polysaccharide composed of β-1,4-linked 2-acetamido-2-deoxy-D-glucopyranose (GlcNAc) repeating units, is the second most abundant naturally occurring polysaccharide and is easily available as a major byproduct of the shrimp and crab industry.[1] Currently its hydrolysis is widely used for the industrial production of the glucosamine monomer, and to a much lessser extent, the production of chitooligosaccharides (COSs), because of low production yields of often heterogeneous mixtures. Furthermore, the degradation of chitin by acetolysis is well documented,[2,3] and leads only to or mainly to the dimer[2] as well as the tri- and tetramer being isolated in poor yield.[3] Higher oligomers exhibit numerous biological activities[4,5] and their elaboration to size-defined molecules with distinct substitution patterns is greatly desirable.

Using an improved procedure for chitin acetolysis to give short oligomeric products, our goal was to quickly re-build structurally modified chitooligomers, of defined sizes, en route to biologically important glycoconjugates. The N-acetyl moiety presents, however, three major limitations in any reconstruction procedure: the solubility of acetamido-containing building blocks is poor, 2-acetamido-2-deoxy glycosyl donors are usually impractical donors because of the formation of the relatively stable 1,2-*O*,*N*-oxazoline intermediates,[6] and 2-acetamido-2-deoxy glycosyl acceptors are notoriously poor nucleophiles, especially the 4-hydroxy group of GlcNAc derivatives.[7,8] Given the importance of COSs, a wide range of glycosylation procedures integrated in stepwise or blockwise assembly strategies have been developed for the effective construction of these oligomeric motifs from monomeric glucosamine precursors.[5c,7e,9,10]

A recently disclosed direct and chemoselective N-transacylation procedure by perfluoroacyl anhydrides of secondary acyl amides to their perfluorinated analogues was easily used for carbohydrate substrates.[11] We report herein a modified version of this procedure, which is particularly useful in the one-pot conversion of peracetylated COSs into per-N-trifluoroacetyl derivatives (per-NHTFA).[12] This N-protecting group is easily removable[13] and 2-deoxy-2-trifluoroacetamido sugar derivatives are well known as efficient donors in glycosylations.[7,14,15] We further show that these building blocks are soluble in common organic solvents and can be transformed into reactive oligomeric acceptor alcohols, in contrast to their N-acetylated precursors.

To obtain larger proportions of dimer and higher fragments, a controlled depolymerization of chitin by acetolysis (Ac_2O, H_2SO_4) was first established by tuning both the temperature and reaction time (55 °C for 3 h, room temperature for 16 h then heated at 55 °C for 1 h; see the Supporting Information). This procedure provided multigram quantities of pure peracetylated chitooligomers (**1**–**3**), which were easily purified by a standard work-up procedure (Scheme 1).



Scheme 1. Controlled depolymerization of chitin and one-pot *trans*-N-trifluoroacylation of the peracetylated chitooligomers. Reaction conditions: a) Ac_2O (300 mL), H_2SO_4 (30 mL). Run on 60 g of chitin. The suspension was stirred at 55 °C for 3 h then for 16 h at RT, and finally heated at 55 °C for 1 h; b) TFAA (7 equiv), CH_3CN, 135 °C, 10 min; c) TFAA (4 equiv per NHAc), pyridine, 135 °C, 15–30 min. TFAA = trifluoroacetic anhydride.

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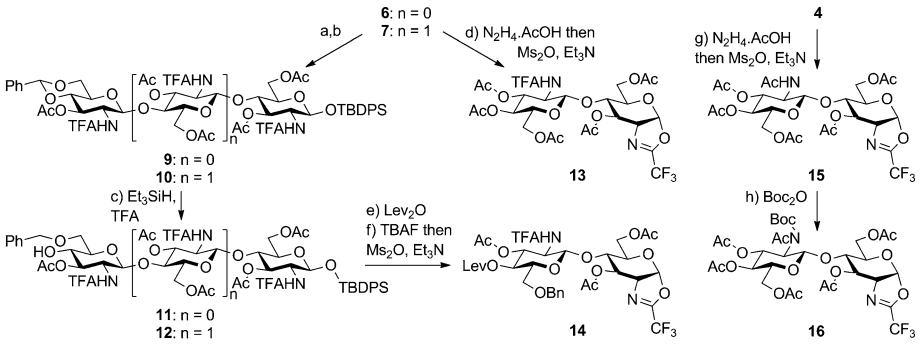
Interestingly, the initially reported conditions were tested on the dimer **1**, and after 10 minutes (excess of trifluoroacetic anhydride in acetonitrile at 135 °C)[11a] provided *trans*-N-acylation, only at the reducing unit, to give the N-differentiated disaccharide **4**[16] as the major product (69 % yield). It resulted from the mixed imide on the nonreducing unit **5**, which could be isolated before work up. Under the selected reaction conditions, the intermediary imide on the nonreducing unit was stable and degraded to the starting acetamide only after the solvolytic workup. This remarkable difference of reactivity between the α- and β-glycosidic units in **4** was confirmed by the stability of analogous imides derived from monomeric peracetylated 2-acetamido-2-deoxy derivatives with a β-anomeric substituent (OBn or SPh).[17] Under these reaction conditions, the starting acetamides were recovered after methanolysis of the imide intermediates.[18]

After optimization,[18] replacing acetonitrile by pyridine,[17] and increasing the excess of trifluoroacetic anhydride (4 equiv per NHAc) furnished the di-*N*-TFA chitobioside **6** in high yield (89 %; Scheme 1). Under these new reaction conditions, the trimer **2** and tetramer **3** gave the per-NHTFA products **7** and **8**, respectively, in good yields for multigram-scale experiments.

For the initial use of these NHTFA building blocks, we prepared glycosyl acceptors, which are useful in the synthesis of the lipochitooligosaccharides[5] or analogues,[5c,10c,19] with a free hydroxy group at C4 of the reducing unit. Standard anomeric acetate cleavage,[20] silylation,[21] deacetylation, benzylidene formation, acetylation, and reductive opening of the acetal in CH_2Cl_2 with triethylsilane and trifluoroacetic acid[22] gave acceptors **11** and **12** in respective overall yields of 57 and 53 % from **6** and **7** (Scheme 2).

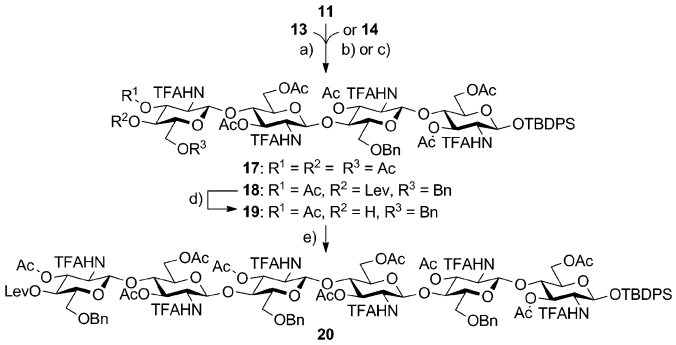
As glycosyl donors, and based on the excellent glycosylation results provided by the *N*-phenyl-trifluoroacetimidate glycosyl donors,[23–25] including an *N*-trichloromethyl acetamide building block at C2,[26] we first prepared *N*-trifluoromethyl acetamide units at C2 with a *N*-phenyl-trifluoroacetimidate at the anomeric position. These gave, however, not easily reproducible results with the formation of the trifluoromethyl oxazolines at both steps (donor formation and glycosylation).[27] We used this experimental observation to select instead the stable trifluoromethyl oxazolines as glycosyl donors,[16,28] thus expecting good results in glycosylation reactions, similar to that of the trichloromethyl oxazolines.[7c,29] The oxazoline **13** was readily prepared in 87 % yield from **6** by selective anomeric acetate cleavage[20] and cyclization to the oxazoline by the methanesulfonic anhydride/triethylamine reagent system (Scheme 2).[30] In addition, the oxazoline dimer **14**, orthogonally protected on the nonreducing unit, was derived from the 4-OH acceptor **11** in 79 % yield by levulinic ester formation,[31] anomeric desilylation, and oxazoline formation. Finally, the disaccharide **4** was used to prepare a useful N-differenciated building block donor, **16**, by oxazoline formation and installation of a *tert*-butyloxycarbonyl (Boc) group, in an overall yield of 70 %. This *N*,*N*-Boc,Ac motif has already been used at the C5 position of sialyl donors,[32] and proved to be stable under glycosylation conditions (NIS/cat·TfOH).

With all appropriate building blocks in hand, we first set up the preparation of a few oligomers to illustrate the versatility of the trifluoromethyl oxazolines (TFM-oxazolines) in glycosylation reactions. Although the application of 2-deoxy-2-trifluoroacetamido donors in glycosylations is well represented, the use of the corresponding oxazolines in oligosaccharide synthesis has not yet been reported. The ability of **13** to act as a donor in glycosylation was readily assessed by reacting it with the dimer acceptor **11**, in the presence of catalytic amounts of TMSOTf (Scheme 3). The reaction proceeded smoothly (−40 °C to RT), thus providing the tetrasaccharide **17** in good yield (82 %). This reaction revealed that this TFM-oxazoline is a reactive donor, just as the trichloromethyl counterparts are.[29,30] This reactivity was also demonstrated in the iterative synthesis of the chitohexaoside **20**, a synthesis carried out in two coupling steps, each using **14**. The same glycosylation conditions could be used (78 % of **18**). Better results were obtained by using a 9:1 mixture of dichloromethane and 1-butyl-3-methylimidazolium triflate as a cosolvent[33] at low temperature (−30 to −10 °C). Under these reaction conditions, **18** was isolated in 93 % yield. Applying the same reaction conditions with the acceptor tetramer **19** afforded the hexamer **20** in 77 % yield, with a protection pattern allowing site-selective modifications of the oligomer.[34]



Scheme 2. Preparation of oligomeric acceptors **11** and **12** and dimeric donors **13**–**16**. Reaction conditions: a) NH_2-NH_2·AcOH (1.1 equiv), DMF, 55 °C; TBDPSCl (2 equiv), imidazole (3 equiv), DMF, RT, $n=0$, 79 %, $n=1$, 80 %; b) NaOMe (1–2 equiv), MeOH, RT; PhCHO, $ZnCl_2$ (2.5 equiv), RT; Ac_2O (3 equiv per OH), pyridine, RT; $n=0$, 81 %, $n=1$, 78 %; c) Et_3SiH (7–10 equiv), TFA (15 equiv), AW300 M.S., 0 °C to RT, $n=0$, 88 %, $n=1$, 85 %; d) NH_2-NH_2·AcOH (1.1 equiv), DMF, 55 °C then Ms_2O (3 equiv), Et_3N (20 equiv), CH_3CN, RT, 86 %; e) Lev_2O (2 equiv), DMAP (0.2 equiv), pyr, RT, 98 %; f) TBAF (3 equiv), AcOH (6 equiv), THF, 0 °C to RT; Ms_2O (3 equiv), Et_3N (20 equiv), CH_3CN, RT, 82 %; g) NH_2-NH_2.AcOH (1.1 equiv), DMF, 55 °C, 35 min then Ms_2O (3 equiv), Et_3N (20 equiv), CH_3CN, RT, 78 %; h) Boc_2O (3 equiv), DMAP (0.2 equiv), THF, reflux, 30 min, 90 %. DMAP = 4-(*N*,*N*-dimethylamino)pyridine, DMF = *N*,*N*-dimethylformamide, Ms = methanesulfonyl, M.S. = molecular sieves, TBDPS = *tert*-butydiphenylsilyl, TFA = trifluoroacetic acid, THF = tetrahydrofuran.

The synthesis of these COS oligomers with an even number of units could also be completed by preparing a series of odd COS using the trimer acceptor **12** (not shown).



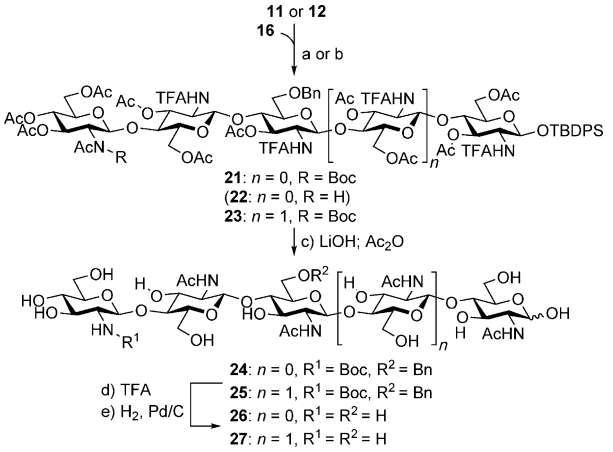
Scheme 3. Glycosylation with the trifluoromethyl oxazoline donors **13** and **14**. Reaction conditions: a) **13** (1.3 equiv), TMSOTf (0.3 equiv), 3 Å M.S., CH_2Cl_2, −40 °C to RT, 18 h, 82%; b) **14** (1.3 equiv), TMSOTf (0.2 equiv), 3 Å M.S., CH_2Cl_2, 0 °C to RT, 2 h, 78%; or c) **14** (1.3 equiv), TMSOTf (0.2 equiv), CH_2Cl_2:[bmim][OTf], 9:1, −30 to −10 °C, 93%; d) NH_2-NH_2·AcOH (2 equiv), CH_2Cl_2:MeOH, 6:1, RT, 20 h, 76%; e) **14** (1.3 equiv), TMSOTf (0.2 equiv), CH_2Cl_2:[bmim][OTf], 9:1, −30 to 0 °C, 3 h, 77%. [bmim][OTf] = 1-butyl-3-methylimidazolium triflate, Tf = trifluoromethanesulfonyl, TMS = trimethylsilyl.

The utility of this approach, using a variety of reactive oligomeric building blocks derived from chitin, was highlighted in the construction of important glycolipids derived from COSs. These are the Nod factors[5,35] (e.g., **30**–**31**; see Scheme 5) and the Myc factors[36] (e.g., **32**–**33**), decisive signal molecules which regulate two ecologically important *endo*-symbioses, the nitrogen-fixing nodulation of legumes, and the arbuscular mycorrhizal symbiosis of most plants. They have stimulated strong synthetic interest, especially because these molecules are produced by rhizobia or AM fungi as mixtures in very small quantities.[7d,e,19a,37] They are very similar glycolipids and structurally close to TMG-chitotriomycin (e.g., **34**) which is a potent and selective inhibitor of β-*N*-acetyl-hexosaminidases (HexNAcase, EC 3.2.1.52) from fungi, bacteria, and insects.[38] Their synthesis requires a selective differentiation of the amino group of the nonreducing unit present in the chitin fragment. The unexpectedly easy access to the N-differenciated **4**, the precursor to dimer donor **16**, provided a simple entry to these bioactive compounds.

Under our optimized glycosylation conditions, coupling of **11** with **16** afforded the tetramer **21** in 85% yield (Scheme 4). The Boc-cleavage product **22** was also isolated in 13% yield, thus indicating a quantitative conversion of the starting acceptor. The trimer **12** was also glycosylated in good yield (75%) to furnish the N-differentiated pentamer **23**.[39] After experimentation, the acyl (Ac and TFA) and the silyl groups in **21** were cleaved by treatment with an excess of aqueous LiOH in methanol at room temperature and, following neutralization with a proton exchange resin, the free amines were acetylated in the same pot to give the acetamides **24** or **25**. The N-Boc group was removed by aqueous TFA and the resulting crude reaction mixture was subjected to hydrogenolysis to remove the benzyl ether, thus providing the amines **26** and **27** in a good overall yield (85% from **21** and 67% from **23**, respectively).

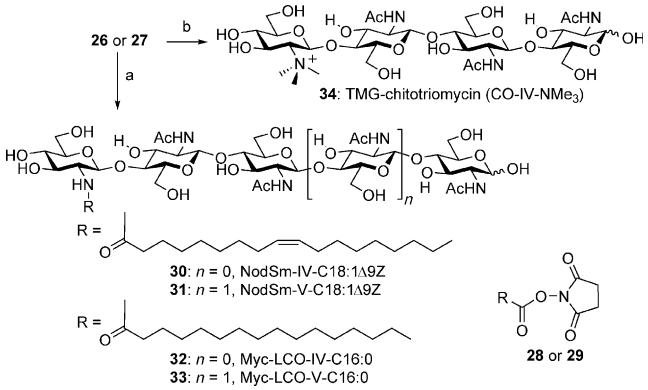
The final step of the synthesis involves a selective coupling reaction between the free amine and fatty acids. This coupling was done by selective acylation with the *N*-hydroxysuccinimide ester[37c,e,40] of oleic acid (C18:1Δ9Z; **28**)[41] or of palmitic acid (C16:0; **29**)[42] in DMSO at 60 °C in the presence of an excess of triethylamine (Scheme 5). This reaction afforded the targeted Nod factors **30** and **31** and the Myc factors **32** and **33** in yields ranging from 78% to quantitative and in good purities. Treating the tetrasaccharide **26** with a large excess of methyl iodide in 1-methyl-2-pyrrolidone at room temperature[43] gave the TMG-chitotriomycin **34** in 66% yield.

In conclusion, we have developed an efficient synthetic sequence for the conversion of chitin fragments into high-value COSs and their derived glycoconjugates. It involves a direct and chemoselective N-transacylation of peracetylated COSs, obtained from chitin, to give per-*N*-trifluoroacetyl derivatives. Various oligomeric acceptors and trifluoromethyl oxazoline dimer donors were provided with efficiency. Their high reactivity in 1,2-*trans* glycosylation is remarkable and makes these building blocks very attractive for other synthetic projects including the β-D-GlcNAc



Scheme 4. Preparation of protected COS N-differentiated at the non-reducing end. Reaction conditions. a) **16** (1.3 equiv) with acceptor dimer **11**: 25 mol% TMSOTf, 3 Å M.S., CH_2Cl_2:[bmim][OTf], 9:1, −30 to −5 °C, 45 min; 85%; b) **16** (1.3 equiv) with acceptor trimer **12**: 40 mol% TMSOTf, CH_2Cl_2:[bmim][OTf], 6:1, −35 to 4 °C, 5 h; 75%; c) 2 M aq. LiOH (50 equiv), MeOH, RT, then Ac_2O (30–50 equiv), K_2CO_3 (pH 11–12), methanol/water (1:1), RT; d) TFA/CH_3CN/water (1:1:1), sonication; e) H_2, Pd/C, methanol/water (1:1), RT; yield over 3 steps: 85% of **26** from **21** and 67% of **27** from **23**.

glycoside or the chitobiose unit, an essential motif in many biologically important oligosaccharides and glycoconjugates. This route also provides an easy access to the important plant symbiotic glycolipids or to the potent and specific inhibitor of *N*-acetylhexosaminidases, TMG-chitotriomycin, which will facilitate biochemical and physiological studies on these signaling molecules. Noteworthy, this work has revealed a positive influence of the trifluoroacetamide group on the reactivity of the 4-hydroxy group in chitobio- or trioside acceptors, and is in sharp contrast to their acetamide counterparts. This influence has not yet been rationalized and future



Scheme 5. Synthesis of the nodulation/mycorrhization factors (LCO **30**–**33**) and TMG-chitotriomycin **34** from the common amines **26** and **27**. Reaction conditions: a) **28** or **29** (1.1–1.2 equiv), Et_3N (1.3 equiv), DMSO, 60 °C, 3–5 days; b) MeI (50 equiv), 5 M aq. NaOH (4 equiv), 1-methyl-2-pyrrolidone, RT, 20 h. DMSO = dimethylsulfoxide.

experiments will aim at defining the intramolecular and/or solvation effects responsible for this appealing property.

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[1] a) R. Muzzarelli, C. Jeuniaux, G. W. Gooday, *Chitin in Nature and Technology*, Plenum, New York, NY, **1986**; b) M. Rinaudo, *Prog. Polym. Sci.* **2006**, *31*, 603 – 632; c) F. Khoushab, M. Yamabhai, *Mar. Drugs* **2010**, *8*, 1988 – 2012.

[2] a) S. A. Barker, A. B. Foster, M. Stacey, J. M. Webber, *J. Chem. Soc.* **1958**, 2218 – 2227; b) T. Osawa, *Carbohydr. Res.* **1966**, *2-3*, 435 – 443; c) R. Chang, A. R. Yeager, N. S. Finney, *Org. Biomol. Chem.* **2003**, *1*, 39 – 41; d) M. Mathiselvam, A. Srivastava, B. Varghese, S. Pérez, D. Loganathan, *Carbohydr. Res.* **2013**, *380*, 37 – 44.

[3] a) F. M. Delmotte, M. L. P. Monsigny, *Carbohydr. Res.* **1974**, *36*, 219 – 226; b) T. Inaba, T. Ohgushi, Y. Iga, E. Hasegawa, *Chem. Pharm. Bull.* **1984**, *32*, 1597 – 1603; c) S. I. Nishimura, H. Kuzuhara, Y. Takiguchi, K. Shimahara, *Carbohydr. Res.* **1989**, *194*, 223 – 231.

[4] a) B. B. Aam, E. B. Heggset, A. L. Norberg, M. Sørlie, K. M. Vårum, V. G. H. Eijsink, *Mar. Drugs* **2010**, *8*, 1482 – 1517; b) V. K. Mourya, M. N. Inamdar, Y. M. Choudhari, *Polym. Sci. Ser. A* **2011**, *53*, 583 – 612; c) W. Xia, P. Liu, J. Zhang, J. Chen, *Food Hydrocolloids* **2011**, *25*, 170 – 179.

[5] a) W. D'Haeze, M. Holsters, *Glycobiology* **2002**, *12*, 79R – 105R; b) C. Gough, J. Cullimore, *Mol. Plant-Microbe Interact.* **2011**, *24*, 867 – 878; c) J.-M. Beau, *Chimia* **2011**, *65*, 45 – 48; d) G. E. D. Oldroyd, J. D. Murray, P. S. Poole, J. A. Downie, *Annu. Rev. Genet.* **2011**, *45*, 119 – 144.

[6] For exceptions, see: a) R. Arihara, S. Nakamura, S. Hashimoto, *Angew. Chem. Int. Ed.* **2005**, *44*, 2245 – 2249; *Angew. Chem.* **2005**, *117*, 2285 – 2289; b) A. Stévenin, F.-D. Boyer, J.-M. Beau, *Eur. J. Org. Chem.* **2012**, 1699 – 1702.

[7] Reviews: a) H. Paulsen, *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 155 – 173; *Angew. Chem.* **1982**, *94*, 184 – 201; b) J. Banoub, P. Boullanger, D. Lafont, *Chem. Rev.* **1992**, *92*, 1167 – 1195; c) A. F. G. Bongat, A. V. Demchenko, *Carbohydr. Res.* **2007**, *342*, 374 – 406; d) R. Enugala, L. C. R. Carvalho, M. J. Dias Pires, M. M. B. Marques, *Chem. Asian J.* **2012**, *7*, 2482 – 2501; e) Y. Yang, B. Yu, *Tetrahedron* **2014**, *70*, 1023 – 1046.

[8] D. Crich, V. Dudkin, *J. Am. Chem. Soc.* **2001**, *123*, 6819 – 6825.

[9] For the chemical synthesis of chitriose derivatives and higher oligomers (up to a 16-mer) from monomeric glucosamine, see: a) H. Hashimoto, Y. Abe, S. Horito, J. Yoshimura, *J. Carbohydr. Chem.* **1989**, *8*, 307 – 311; b) H. Kuyama, Y. Nakahara, T. Nukada, Y. Ito, Y. Nakahara, T. Ogawa, *Carbohydr. Res.* **1993**, *243*, C1 – C7; c) C. Lamberth, J. O. Nagy, C. Kasper, M. D. Bednarski, *J. Carbohydr. Chem.* **1994**, *13*, 819 – 824; d) M. R. E. Aly, E. S. I. Ibrahim, E. S. H. El Ashry, R. R. Schmidt, *Carbohydr. Res.* **2001**, *331*, 129 – 142; e) S.-T. Liew, A. Wei, *Carbohydr. Res.* **2002**, *337*, 1319 – 1324; f) S. Yamago, T. Yamada, T. Maruyama, J.-I. Yoshida, *Angew. Chem. Int. Ed.* **2004**, *43*, 2145 – 2148; *Angew. Chem.* **2004**, *116*, 2197 – 2200; g) L. Huang, Z. Wang, X. Huang, *Chem. Commun.* **2004**, 1960 – 1961; h) D. Hesek, M. Lee, K.-I. Morio, S. Mobashery, *J. Org. Chem.* **2004**, *69*, 2137 – 2146; i) T. Yamada, S. Kinjyo, J.-I. Yoshida, S. Yamago, *Chem. Lett.* **2005**, *34*, 1556 – 1557; j) L. Huang, Z. Wang, X. Li, X.-S. Ye, X. Huang, *Carbohydr. Res.* **2006**, *341*, 1669 – 1679; k) S. Inamura, Y. Fujimoto, A. Kawasaki, Z. Shiokawa, E. Woelk, H. Heine, B. Lindner, N. Inohara, S. Kusumoto, K. Fukase, *Org. Biomol. Chem.* **2006**, *4*, 232 – 242; l) Z.-Y. Zhu, Y.-M. Zhang, C. Sun, Y.-P. Yao, *Chin. J. Chem.* **2008**, *26*, 1519 – 1522.

[10] For an interesting alternative method for the production of COS using metabolically engineered bacterial cells, see: a) E. Samain, S. Drouillard, A. Heyraud, H. Driguez, R. A. Geremia, *Carbohydr. Res.* **1997**, *302*, 35 – 42; b) E. Samain, V. Chazalet, R. A. Geremia, *J. Biotechnol.* **1999**, *72*, 33 – 47; c) N. Grenouillat, B. Vauzeilles, J. J. Bono, E. Samain, J.-M. Beau, *Angew. Chem. Int. Ed.* **2004**, *43*, 4644 – 4646; *Angew. Chem.* **2004**, *113*, 4744 – 4746.

[11] a) P. Rota, P. Allevi, R. Colombo, M. L. Costa, M. Anastasia, *Angew. Chem. Int. Ed.* **2010**, *49*, 1850 – 1853; *Angew. Chem.* **2010**, *122*, 1894 – 1897; b) P. Rota, P. Allevi, M. L. Costa, M. Anastasia, *Tetrahedron: Asymmetry* **2010**, *21*, 2681 – 2686.

[12] *N*-trifluoroacetylated chitooligomers (mono- to tetramer) have been prepared by a conventional way and used for binding studies: a) F. Millett, M. A. Raftery, *Biochemistry* **1972**, *11*, 1639 – 1643; b) P. Midoux, J.-P. Grivet, M. Monsigny, *FEBS Lett.* **1980**, *120*, 29 – 32; c) P. Gatellier, J.-P. Grivet, F. Delmotte, *Biochim. Biophys. Acta Protein Struct. Mol. Enzymol.* **1987**, *916*, 428 – 436.

[13] a) S. A. Gerber, F. Tureček, M. H. Gelb, *Bioconjugate Chem.* **2001**, *12*, 603 – 615; b) K. Sasaki, Y. Nishida, M. Kambara, H. Uzawa, T. Takahashi, T. Suzuki, Y. Suzuki, K. Kobayashi, *Bioorg. Med. Chem.* **2004**, *12*, 1367 – 1375; c) Y. Nagai, N. Ito, I. Sultana, T. Sugai, *Tetrahedron* **2008**, *64*, 9599 – 9606; d) Y. Chen, V. Thon, Y. Li, H. Yu, L. Ding, K. Lau, J. Qu, L. Hie, X. Chen, *Chem. Commun.* **2011**, *47*, 10815 – 10817.

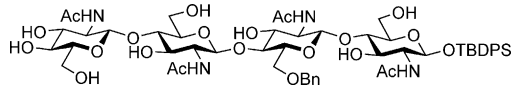
[14] M. L. Wolfrom, H. B. Bhat, *J. Org. Chem.* **1967**, *32*, 1821 – 1823.

[15] a) D. J. Silva, H. Wang, N. M. Allanson, R. K. Jain, M. J. Sofia, *J. Org. Chem.* **1999**, *64*, 5926 – 5929; b) H. Weiss, C. Unverzagt, *Angew. Chem. Int. Ed.* **2003**, *42*, 4261 – 4263; *Angew. Chem.* **2003**, *115*, 4389 – 4392; c) H. Myszka, D. Bednarczyk, M. Najder, W. Kaca, *Carbohydr. Res.* **2003**, *338*, 133 – 141; d) A. F. G. Bongat, M. N. Kamat, A. V. Demchenko, *J. Org. Chem.* **2007**, *72*, 1480 – 1483; e) A. Makino, K. Kurosaki, M. Ohmae, S. Kobayashi, *Biomacromolecules* **2006**, *7*, 950 – 957; f) S. Pajk, M. Garvas, J. Štrancar, S. Pečar, *Org. Biomol. Chem.* **2011**, *9*, 4150 – 4159; g) L. G. Weaver, Y. Singh, J. T. Blanchfield, P. L. Burn, *Carbohydr. Res.* **2013**, *371*, 68 – 76.

[16] V. W.-F. Tai, B. Imperiali, *J. Org. Chem.* **2001**, *66*, 6217 – 6228.

[17] For similar observations, see: Y. Li, C. Li, P. Wang, S. Chu, H. Guan, B. Yu, *Tetrahedron Lett.* **2004**, *45*, 611 – 613.

[18] Details (NMR) on the imides derivatives, optimization conditions on the peracetylated chitobiose **1**, including in CH_3CN containing Et_3N,[8b] are given in the Supporting Information.
[19] a) N. Demont-Caulet, F. Maillet, D. Tailler, J.-C. Jacquinet, J.-C. Promé, K. C. Nicolaou, G. Truchet, J.-M. Beau, J. Dénarié, *Plant Physiol.* **1999**, *120*, 83 – 92; b) J. Fliegmann, S. Canova, C. Lachaud, S. Uhlenbroich, V. Gasciolli, C. Pichereaux, M. Rossignol, C. Rosenberg, M. Cumener, D. Pitorre, B. Lefebvre, C. Gough, E. Samain, S. Fort, H. Driguez, B. Vauzeilles, J.-M. Beau, A. Nurisso, A. Imberty, J. Cullimore, J.-J. Bono, *ACS Chem. Biol.* **2013**, *8*, 1900 – 1906.
[20] G. Excoffier, D. Gagnaire, J.-P. Utile, *Carbohydr. Res.* **1975**, *39*, 368 – 373.
[21] a) T. Müller, R. R. Schmidt, *Tetrahedron Lett.* **1997**, *38*, 5473 – 5476; b) K. Routenberg Love, P. H. Seeberger, *J. Org. Chem.* **2005**, *70*, 3168 – 3177.
[22] M. P. DeNinno, J. B. Etienne, K. C. Duplantier, *Tetrahedron Lett.* **1995**, *36*, 669 – 672.
[23] a) B. Yu, H. Tao, *Tetrahedron Lett.* **2001**, *42*, 2405 – 2407; b) B. Yu, J. Sun, *Chem. Commun.* **2010**, *46*, 4668 – 4678.
[24] For the cyclic analogue 3,3-difluoro-3*H*-indol-2-yl, see: S. S. Nigudkar, K. J. Stine, A. V. Demchenko, *J. Am. Chem. Soc.* **2014**, *136*, 921 – 923.
[25] These imidates were developed from the Schmidt's trichloroacetimidate donors: a) R. R. Schmidt, *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 212 – 235; *Angew. Chem.* **1986**, *98*, 213 – 236; b) "Trichloroacetimidates": R. R. Schmidt, K.-H. Jung in *Carbohydrates in Chemistry and Biology*, *Part I: Chemistry of Saccharides*, *Vol. 1* (Eds.: B. Ernst, G. W. Hart, P. Sinaÿ), Wiley-VCH, Weinheim, **2000**, pp. 5 – 59.
[26] S. Hanashima, P. H. Seeberger, *Chem. Asian J.* **2007**, *2*, 1447 – 1459.
[27] As an example, the glycosyl donor derived from the peracetylated di-*N*-TFA chitobioside **6** [NH_2-NH_2·AcOH (1.1 equiv), DMF, 55 °C then $CF_3C(NPh)Cl$, (2 equiv), K_2CO_3, acetone, RT; 66% yield of the *N*-phenyl-trifluoroacetimidate donor + 25% yield of TFM-oxazoline **13**] was reacted with the dimer acceptor **11** [TMSOTf (0.3 equiv), 3 Å M.S., CH_2Cl_2, −30 °C], thus providing the tetrasaccharide **17** (see Scheme 3) in 69% yield and the TFM-oxazoline **13**.
[28] P. Busca, O. R. Martin, *Tetrahedron Lett.* **1998**, *39*, 8101 – 8104.
[29] G. Blatter, J.-M. Beau, J.-C. Jacquinet, *Carbohydr. Res.* **1994**, *260*, 189 – 202.
[30] T. J. Donohoe, J. G. Logan, D. D. P. Laffan, *Org. Lett.* **2003**, *5*, 4995 – 4998.
[31] a) A. Hassner, G. Strand, M. Rubinstein, A. Patchornik, *J. Am. Chem. Soc.* **1975**, *97*, 1614 – 1615; b) H. J. Koeners, J. Verhoeven, J. H. van Boom, *Tetrahedron Lett.* **1980**, *21*, 381 – 382.
[32] a) A. A. Sherman, O. N. Yudina, A. S. Shashkov, V. M. Menshov, N. E. Nifantiev, *Carbohydr. Res.* **2001**, *330*, 445 – 458; b) K. Ikeda, K. Miyamoto, M. Sato, *Tetrahedron Lett.* **2007**, *48*, 7431 – 7435.
[33] a) M. C. Galan, C. Brunet, M. Fuensanta, *Tetrahedron Lett.* **2009**, *50*, 442 – 445; b) M. C. Galan, K. Jouvin, D. Alvarez-Dorta, *Carbohydr. Res.* **2010**, *345*, 45 – 49.
[34] As a deprotection test, the per-NHTFA tetramer **17** was transformed [a) NaOMe (3 equiv), MeOH, RT, 39 h; b) 2 M aq. LiOH (8 equiv), THF, 0 °C, 4 h; c) Ac_2O (40 equiv), K_2CO_3 (pH 11–12), MeOH/water (1:1), RT, 19 h] into the following per-NHAc silyl glycoside in an overall yield of 55%.



[35] a) P. Lerouge, P. Roche, C. Faucher, F. Maillet, G. Truchet, J.-C. Promé, J. Dénarié, *Nature* **1990**, *344*, 781 – 784; b) G. Truchet, P. Roche, P. Lerouge, J. Vasse, S. Camut, F. de Billy, J.-C. Promé, J. Dénarié, *Nature* **1991**, *351*, 670 – 673.
[36] F. Maillet, V. Poinsot, O. André, V. Puech-Pagès, A. Haouy, M. Gueunier, L. Cromer, D. Giraudet, D. Formey, A. Niebel, E. A. Martinez, H. Driguez, G. Bécard, J. Dénarié, *Nature* **2011**, *469*, 58 – 63.
[37] Previous Nod/Myc factor syntheses: a) K. C. Nicolaou, N. J. Bockovich, D. R. Carcanague, C. W. Hummel, L. F. Even, *J. Am. Chem. Soc.* **1992**, *114*, 8701 – 8702; b) D. Tailler, J.-C. Jacquinet, J.-M. Beau, *J. Chem. Soc. Chem. Commun.* **1994**, 1827 – 1828; c) S. Ikeshita, A. Sakamoto, Y. Nakahara, Y. Nakahara, T. Ogawa, *Tetrahedron Lett.* **1994**, *35*, 3123 – 3126; d) L.-X. Wang, C. Li, Q.-W. Wang, Y.-Z. Hui, *J. Chem. Soc. Perkin Trans. 1* **1994**, 621 – 628; e) S. L. Sedinkin, A. I. Zinin, N. N. Malysheva, A. S. Shashkov, V. I. Torgov, V. N. Shibaev, *Mendeleev Commun.* **2007**, *17*, 10 – 13; f) J. S. Debenham, R. Rodebaugh, B. Fraser-Reid, *J. Org. Chem.* **1997**, *62*, 4591 – 4600; g) M. Ohsten Rasmussen, B. Hogg, J.-J. Bono, E. Samain, H. Driguez, *Org. Biomol. Chem.* **2004**, *2*, 1908 – 1910; h) L. Gillard, A. Stévenin, I. Schmitz-Afonso, B. Vauzeilles, F.-D. Boyer, J.-M. Beau, *Eur. J. Org. Chem.* **2013**, 7382 – 7390.
[38] a) H. Usuki, T. Nitoda, M. Ichikawa, N. Yamaji, T. Iwashita, H. Komura, H. Kanzaki, *J. Am. Chem. Soc.* **2008**, *130*, 4146 – 4152; b) Y. Yang, Y. Li, B. Yu, *J. Am. Chem. Soc.* **2009**, *131*, 12076 – 12077.
[39] Because of the rotameric mixture in **21** and **23**, their ^{1}H NMR spectra were correctly resolved only in [D_6]DMSO at 355 K. These compounds were further treated under Zemplen conditions (MeONa, MeOH) to provide the deacetylated **21′** and **23′**, which were characterized by ^{1}H and ^{13}C NMR spectroscopy in [D_6]DMSO at 355 K (see the Supporting Information).
[40] a) Y. Lapidot, S. Rappoport, Y. Wolman, *J. Lipid Res.* **1967**, *8*, 142 – 145; b) S. Ikeshita, Y. Nakahara, T. Ogawa, *Glycoconjugate J.* **1994**, *11*, 257 – 261.
[41] K. Vávrová, A. Hrabálek, P. Doležal, L. Šámalová, K. Palát, J. Zbytovská, T. Holas, J. Klimentová, *Bioorg. Med. Chem.* **2003**, *11*, 5381 – 5390.
[42] M. J. Clemente, J. Fitremann, M. Mauzac, J. L. Serrano, L. Oriol, *Langmuir* **2011**, *27*, 15236 – 15247.
[43] S. Halila, E. Samain, C. E. Vorgias, S. Armand, *Carbohydr. Res.* **2013**, *368*, 52 – 56.