Synthesis Design

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From Polymer to Size-Defined Oligomers: An Expeditious Route for the Preparation of Chondroitin Oligosaccharides**

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Chondroitin sulfates are structurally complex, polyanionic, microheterogeneous, linear polysaccharides belonging to the glycosaminoglycan family. They are ubiquitous components of the extracellular matrix of all connective tissues, but are also found on mammalian cell surfaces and in neural tissues.[1] They are copolymers made of dimeric units composed of D-glucuronic acid (GlcA) and 2-acetamido-2-deoxy-D-galactose (GalNAc) arranged in the sequence $[\rightarrow 4)$-β-D-GlcA-$(1\rightarrow 3)$-β-D-GalNAc-$(1\rightarrow]_n$, and contain on average one sulfate group per disaccharide unit, but other types with sulfate(s) at various positions are also known. These sulfation patterns give rise to numerous biologically important functions, such as cell–cell recognition,[2] brain development and regeneration,[3] or binding to selectins,[4] and many other that are not completely deciphered at the molecular level. Unfortunately, structurally defined oligosaccharide fragments of chondroitin and its sulfo forms are not easily prepared, even though they are in great demand. Thus, rapid and efficient methods for the preparation of chondroitin sulfate oligomers are critical to advance our understanding of this important class of biomolecules. Synthetic approaches should ideally provide ready access and involve a small number of steps. Several syntheses of chondroitin sulfate oligosaccharides have recently been reported,[5] all starting from monomeric units. Since D-GalNAc is a rare and somewhat expensive sugar, its derivatives have been generally prepared by long routes. In a continued effort to try to reduce the number of transformations in a multistep procedure, we turned our attention towards the possible chemical hydrolysis of chondroitin sulfate polymers. These compounds are abundant and readily available, and were formerly mainly obtained from bovine cartilage. However, the discovery of bovine spongiform encephalopathy has meant that they cannot be used anymore, at least for medical purposes. Commercial products are now obtained from cartilage of marine origin (shark, skate), and

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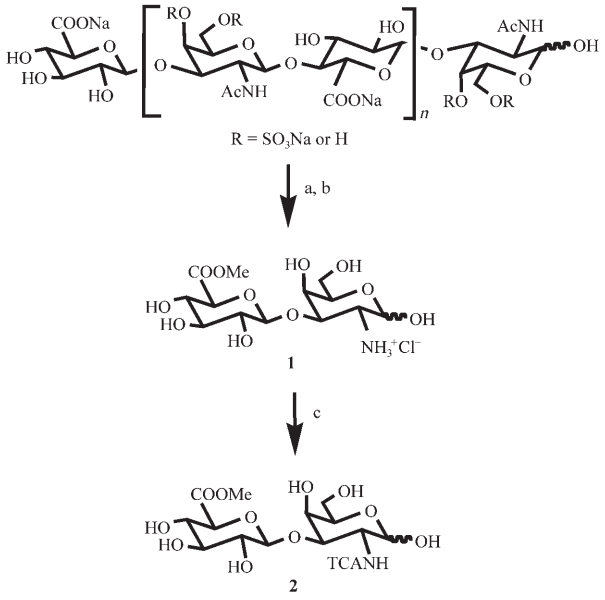
they should constitute an attractive starting material for organic chemists.

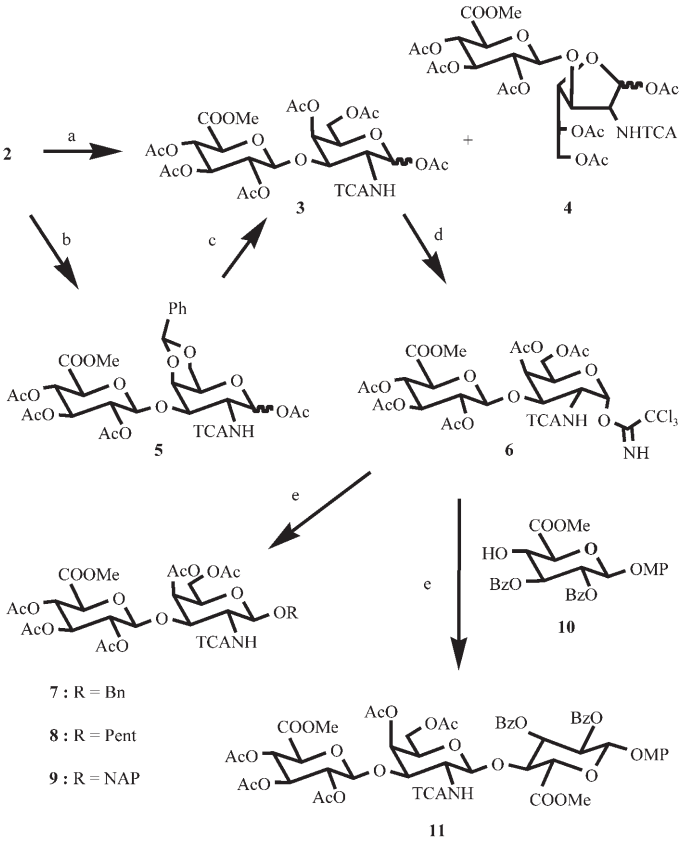
Since the pioneering works of Levene[6] and of Davidson and Meyer[7] on the structure of chondroitin sulfates, chondrosine, the basic repeating unit of chondroitin sulfates, can be obtained by acid hydrolysis of the polymer, a process that also results in desulfation and N-deacetylation of the resulting disaccharide. This product contains a number of attractive features. Besides its backbone, which still possesses D-GlcA and D-GalNH$_2$ arranged in the correct manner, there is an interesting possibility to substitute the free amine group with a powerful stereocontrolling auxiliary such as a trichloroacetyl group,[8] a process that was used successfully for the synthesis of chondroitin sulfate oligosaccharides.[9] We thus reinvestigated and improved the protocol devised by Davidson and Meyer, and the known[7] methyl ester **1** (Scheme 1)



Scheme 1. Preparation of building block **2**. Reagents and conditions: a) IR-120 [H$^+$] resin, H_2O; then 0.5 M H_2SO_4, 100 °C, 6 h; b) AcCl, MeOH, 0 °C, 72 h; c) Cl_3CCOCl, pyridine, 0 °C, 4 h; then CH_2Cl_2/MeOH/pyridine, room temperature, 3 h. TCA = trichloroacetyl.

was obtained in good yield. Attempted chemoselective N-trichloroacetylation of **1** failed, but trichloroacetamide **2** was readily obtained through O,N-pertrichloroacetylation followed by selective solvolysis of the trichloroacetate esters.

Unfortunately, acetylation of **2** was troublesome (Scheme 2), and variation of the conditions (acetylating reagent, solvent, temperature) led systematically to mixtures of pyranose **3** and furanose **4** in an approximately 3/1 ratio. This result is not a surprising[10] outcome in the D-galacto series as a consequence of the unfavorable interaction of the 4-axial hydroxy group in the pyranoid derivative. To avoid this ring equilibrium, **2** was first treated with benzaldehyde and trifluoroacetic acid under thermodynamic control to lock the galactosamine unit in the pyranose conformation through formation of a 4,6-benzylidene acetal, and then was peracetylated in situ to give **5**. Mild acid hydrolysis of **5** followed by



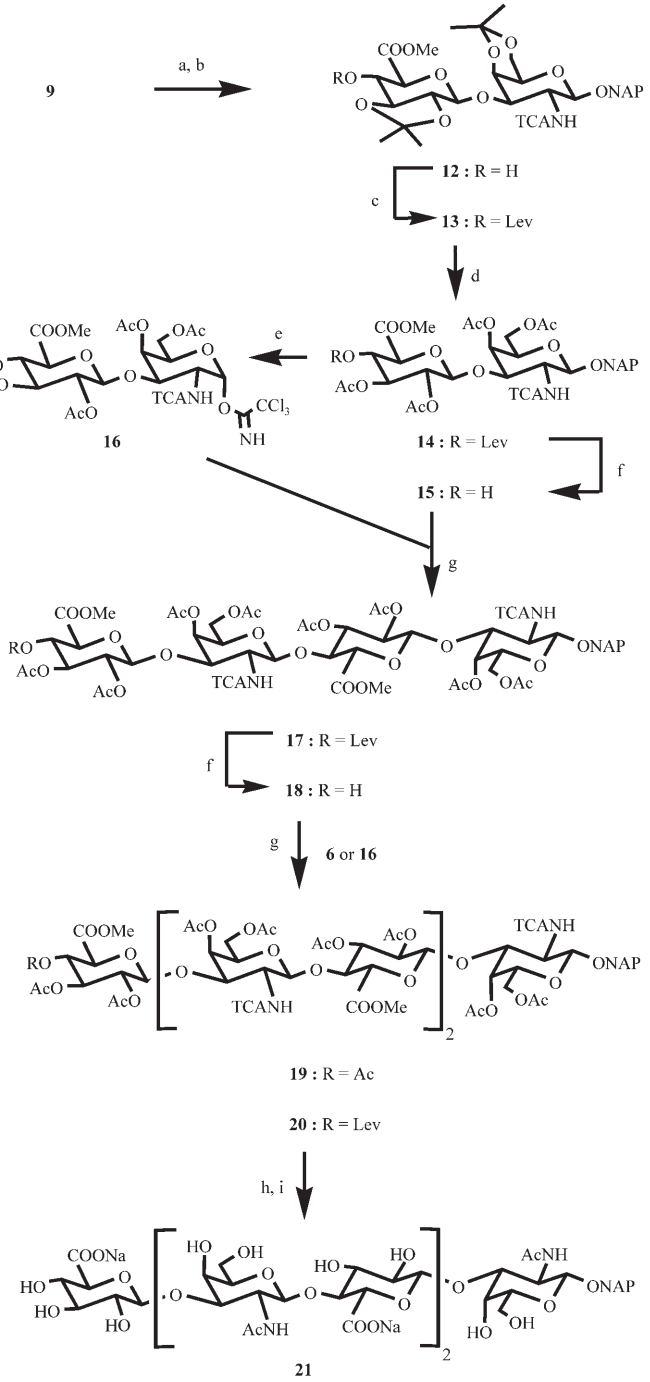
Scheme 2. Synthesis of glycosides **7**–**9** and **11**. Reagents and conditions: a) Ac_2O, pyridine, 0 °C, 8 h; b) PhCHO, TFA, 24 h; then Ac_2O, pyridine, room temperature, 16 h, 72%; c) 75% TFA, CH_2Cl_2, 0 °C, 4 h; then Ac_2O, pyridine, room temperature, 6 h, 78%; d) hydrazine acetate, DMF, 30 min; then Cl_3CCN, DBU, CH_2Cl_2, 30 min, 70%; e) alcohol, TMSOTf, CH_2Cl_2, 30 min, 80–90%. Bn = benzyl, Pent = pentenyl, NAP = 2-naphthylmethyl, MP = 4-methoxyphenyl, TFA = trifluoroacetic acid, DMF = *N*,*N*-dimethylformamide, DBU = 1,8-diazabicyclo-[5,4,0]undec-7-ene, TMSOTf = trimethylsilyl trifluoromethanesulfonate.

acetylation gave exclusively **3** in good yield. Removal of the 4,6-benzylidene acetal on **5** is a prerequisite since it has been demonstrated[11] that derivatives in the D-galacto series locked in an activated 4,6-acetal arrangement, despite the presence of a participating group at C-2, led to mixtures of glycosides in which the 1,2-*cis* species were predominant. The peracetate **3** was then transformed in two steps into the crystalline α-trichloroacetimidate **6**. The glycosylating power of **6** was then tested towards a set of alcohols. The coupling of imidate **6** with benzyl alcohol, 4-pentenol, 2-naphthylmethanol, and the poorly reactive **10**[12] in the presence of a trimethylsilyl triflate catalyst gave exclusively the corresponding 1,2-*trans*-glycosides **7**–**9** and **11** in excellent yields.

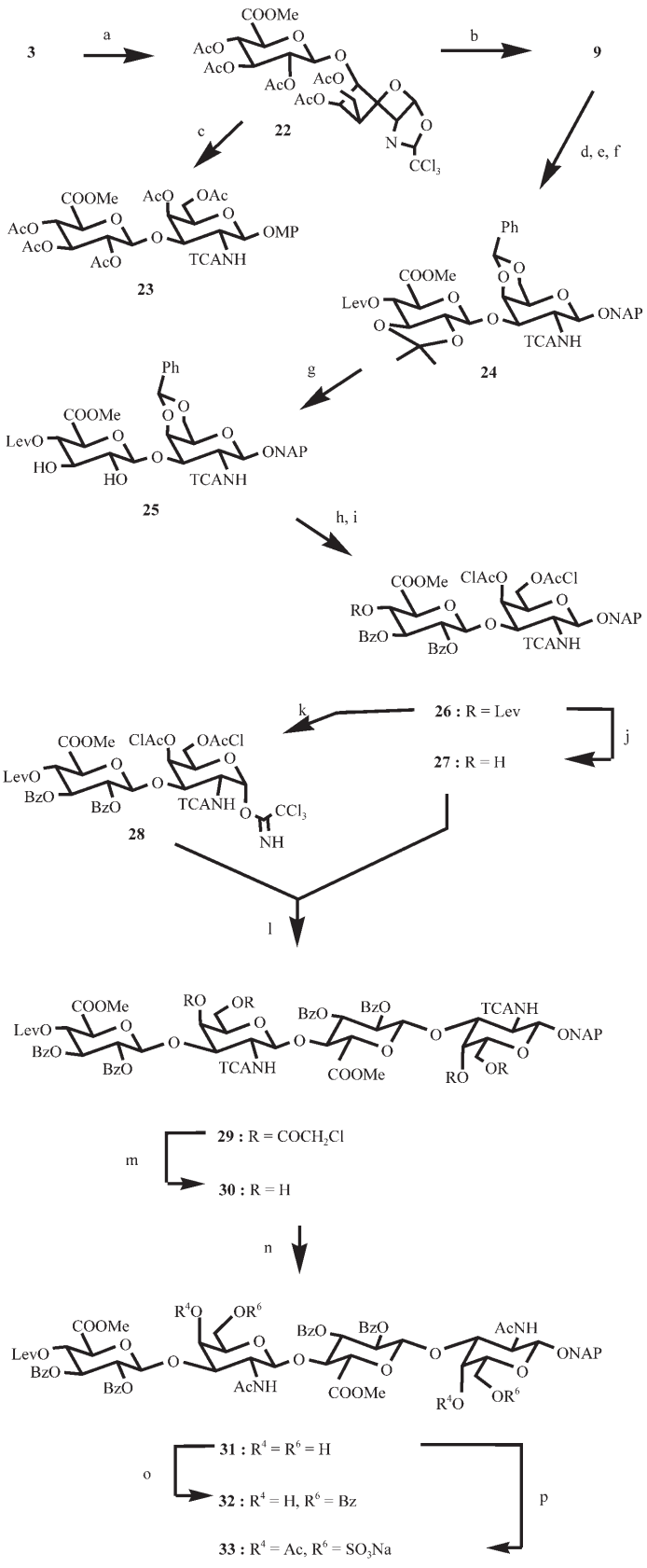
A block approach was designed for the construction of oligomers in which crucial coupling reactions were achieved by using Schmidt's trichloroacetimidate glycosylation procedure.[13] The 2-naphthylmethyl (NAP)-substituted glycoside **9** was selected as a starting material since the NAP group[14] should be retained after deprotection of the other hydroxy groups and serve as a fluorophore in biological assays or easily removed to afford reducing species—both useful tools

for the preparation of microarrays. Transesterification of **9** (Scheme 3) followed by acetalation with 2-methoxypropene and CSA under kinetic control gave the crystalline bisacetal **12** together with a small amount of its 3′,4′ isomer, which was easily recycled by acid hydrolysis. Levulinoylation of **12** gave **13**, which was submitted to mild acid hydrolysis and acetylation to afford the crystalline key intermediate **14**. This was then transformed into either acceptor **15** through selective cleavage of the levulinoyl group or crystalline donor **16** by treatment with DDQ[15] followed by imidoylation. Coupling of alcohol **15** with donor **16** in the presence of a TMSOTf catalyst gave the crystalline tetrasaccharide derivative **17**, which was readily transformed into acceptor **18** in good yield. Further coupling of **18** with imidate **6** or **16** under the same conditions gave hexasaccharide derivatives **19** and **20**, respectively, in 65% yield. Deprotection of **19** was achieved through transformation of the *N*-trichloroacetyl groups into their *N*-acetyl congeners by radical reduction with tributylstannane[8] followed by a two-step saponification with lithium hydroperoxide[16] and sodium hydroxide, to give the target hexasaccharide glycoside **21** in good yield. Thus, an efficient route is now open for the preparation of size-defined chondroitin oligomers containing a fluorescent glycoside as useful probes for the study of the biosynthesis as well as the polymerization of chondroitin sulfate chains.

The possibility was next examined of differentiating each sugar unit so as to prepare the way for the synthesis of chondroitin sulfo forms with important biological functions. Another route was explored in which the more economical 4-methoxyphenyl group was used as a temporary protection at the anomeric reducing center. Surprisingly, the attempted coupling of **3** or **6** with 4-methoxyphenol under various conditions led to anomeric mixtures of glycosides ($\beta \approx \alpha \approx 2:1$). Since anomerization of the β-glycoside was not observed in the reactions, this lack of stereoselectivity was attributed to the 2-deoxy-2-trichloroacetamido group participating too slowly, possibly as a result of the presence of the bulky group at O-3 which prevents a rapid change in the conformation of the D-galacto unit. To overcome this problem, the trichloromethyl oxazoline **22** (Scheme 4) was prepared directly from **3** by using a protocol reported for the D-gluco series.[8] Indeed, reaction of **22** with 4-methoxyphenol in the presence of TMSOTf at 0°C gave exclusively the β-glycoside **23** in excellent yield. It is to be noted that the NAP-substituted glycoside **9** could be obtained from **3** in 80% overall yield via **22**. Glycoside **9** was finally selected as a starting material on the basis of its accessability and because of the greater solubility of its derivatives compared to those of its 4-methoxyphenyl analogues. Transesterification of **9** followed by 4,6-benzylidene formation, 2′,3′-isopropylidenation, and 4′-levulinoylation, as reported previously, gave the intermediate **24** in 56% overall yield. Interestingly, the 2′,3′-isopropylidene acetal on **24** could be selectively removed in the presence of the 4,6-benzylidene group through mild acid hydrolysis to give the crystalline diol **25** in 79% yield. Variants in which the D-GalNAc moiety was sulfated were planned to be synthesized later on from the common precursor **31**, in which benzoate esters were selected as permanent protection for hydroxy groups that were not to be



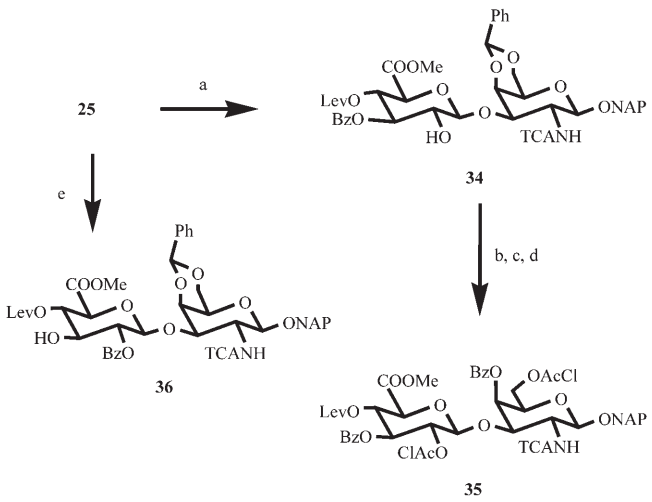
Scheme 3. Synthesis of chondrosine hexasaccharide glycoside **21**. Reagents and conditions: a) NaOMe, MeOH; b) 2-methoxypropene, CSA, DMF, 90 min, 67% from **9**; c) LevOH, DCC, DMAP, CH_2Cl_2, 3 h, 90%; d) 60% AcOH, 100°C, 1 h; then Ac_2O, pyridine, 16 h, 71%; e) DDQ, CH_2Cl_2/MeOH, 24 h; then Cl_3CCN, DBU, CH_2Cl_2, 30 min, 66%; f) hydrazine acetate, pyridine, 8 min, 89%; g) TMSOTf, CH_2Cl_2, 30 min, 57% for **17**, 65% for **19** and **20**; h) Bu_3SnH, AIBN, DMAC, 80°C, 2 h, 78%; then $LiOH/H_2O_2$, THF, −10°C to room temperature, 16 h; then 4 M NaOH, MeOH, room temperature, 4 h, 75%. CSA = camphorsulfonic acid, LevOH = 4-oxopentanoic acid, DCC = *N*,*N*-dicyclohexylcarbodiimide, DMAP = 4-dimethylaminopyridine, DDQ = 2,3-dichloro-5,6-dicyanobenzoquinone, AIBN = 2,2′-azobis(2-methylpropionitrile), DMAC = *N*,*N*-dimethylacetamide.



O-sulfonated. To achieve this goal, the intermediate **25** was submitted to 2′,3′-dibenzoylation, mild acid hydrolysis of the 4,6-benzylidene acetal, and chloroacetylation of the resulting 4,6-diol to give **26** in 76% overall yield. This latter compound was either transformed into acceptor **27**, through selective cleavage of the levulinoyl group, or donor **28**, through oxidative removal of the NAP glycoside with DDQ followed by imidoylation. Coupling of alcohol **27** with imidate **28** in the presence of a TMSOTf catalyst gave the tetrasaccharide derivative **29** in 71% yield. This intermediate should be ready for further elongation at the nonreducing end after selective cleavage of the *O*-lev group. Removal of the chloroacetate esters with thiourea gave the tetrol **30**, the *N*-trichloroacetyl groups of which were reduced by a radical method to give the key intermediate **31**, a precursor of the 4,6-disulfated species (chondroitin sulfate E). Selective benzoylation at O-6 of **31** with benzoyl cyanide gave **32**, a precursor of the 4-sulfated species (chondroitin sulfate A), whereas controlled regioselective sulfation at C-6[17] with the sulfur trioxide/trimethylamine complex followed by O-acetylation at C-4 (to assess the positions of sulfation) afforded **33**, a direct precursor of the 6-sulfated species (chondroitin sulfate C).

Differentiation between O-2′ and O-3′ was next studied (Scheme 5), and was achieved starting from diol **25**. Treatment of **25** with benzoyl cyanide gave exclusively the crystalline 3′-benzoate **34** in 80% yield. This was then transformed in good yield into the key intermediate **35** through chloroacetylation at O-2′, hydrolysis of the 4,6-benzylidene acetal, selective chloroacetylation at O-6 at −20 °C, and benzoylation at O-4, thus opening the way for the preparation of the 6,2′-sulfo form (chondroitin sulfate D). Tin-mediated[18] benzoylation of **25** gave the 2′-benzoate **36** in 68% yield, along with its 3′ isomer **34** (16% yield). It should now be possible to transform the 2′-benzoate **36** by the same strategy into precursors of the less common sulfo forms such as 4,3′-disulfated (chondroitin sulfate K), 6,3′-disulfated (chondroitin sulfate L), and 4,6,3′-trisulfated species (chondroitin sulfate M).

Scheme 4. Synthesis of precursors of chondroitin sulfates E (**31**), A (**32**), and C (**33**). Reagents and conditions: a) $BF_3{\cdot}OEt_2$, TMSBr, 2,4,6-collidine, Bu_4NBr, CH_2Cl_2, 48 h, 89%; b) 2-naphthylmethanol, TMSOTf, CH_2Cl_2, 30 min, 90%; c) 4-methoxyphenol, TMSOTf, CH_2Cl_2, 0 °C, 30 min, 90%; d) MeONa, MeOH; then PhCHO, TFA, 4 h, 75%; e) 2-methoxypropene, CSA, DMF, 90 min, 83%; f) LevOH, DCC, DMAP, CH_2Cl_2, 1 h, 92%; g) 80% AcOH, CH_2Cl_2, 20 h, 79%; h) PhCOCl, pyridine, 0 °C, 1 h, 95%; i) 75% AcOH, 100 °C, 30 min; then $(ClAc)_2O$, pyridine, CH_2Cl_2, 0 °C, 1 h, 80%; j) hydrazine acetate, pyridine, room temperature, 8 min, 89%; k) DDQ, CH_2Cl_2/MeOH, room temperature, 24 h; then Cl_3CCN, DBU, CH_2Cl_2, 20 min, 68%; l) TMSOTf, CH_2Cl_2, room temperature, 1 h, 71%; m) thiourea, pyridine/EtOH, 80 °C, 2 h, 90%; n) Bu_3SnH, AIBN, DMAC, 80 °C, 2 h, 89%; o) PhCOCN, pyridine, room temperature, 6 h, 88%; p) $Me_3N{\cdot}SO_3$, DMF, 40 °C, 2 h 30; then Ac_2O, pyridine, room temperature, 16 h; then SP-C25 [Na^+] resin, 81%. Bz = benzoyl.



Scheme 5. Access to precursors of CS-D (**35**) and chondroitin sulfates K, L, and M (**36**). Reagents and conditions: a) PhCOCN, pyridine, room temperature, 6 h, 80%; b) $(ClAc)_2O$, pyridine, 0°C, 1 h, 95%; c) 75% AcOH, 100°C, 20 min; then $(ClAc)_2O$, pyridine, CH_2Cl_2, −20°C, 30 min, 72%; d) PhCOCl, pyridine, 0°C, 1 h, 91%; e) Bu_2SnO, dioxane/benzene, reflux, 7 h; then PhCOCl, room temperature, 8 h, 68%.

In conclusion, **1** can be readily prepared on a multigram scale and its versatility has been demonstrated. This shortened sequence renders this approach very attractive and competitive for the preparation of size-defined chondroitin oligomers. Also relevant is the rapid access to precursors for the preparation of various sulfo forms starting from the common intermediate **25**. This route can also be applied to other biologically important members of the glycosaminoglycan family such as dermatan sulfate and hyaluronic acid. The synthesis of a collection of chondroitin oligomers and their various sulfo forms based on this approach is underway, and will be reported elsewhere in due course.

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