Exploring Cyclic Sulfamidate Building Blocks for the Synthesis of Sequence-Defined Macromolecules

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The preparation of sequence-defined macromolecules using cyclic sulfamidates on solid-phase is outlined. The challenges surrounding an AB+CD approach are described with focus on understanding the formation of ring-opened side products when using amide coupling reagents. To avoid undesired side product formation, a strategy of iterative ring-openings of cyclic sulfamidates on solid-phase is explored. Ring-opening on primary and secondary amines is successfully reported, generating both linear and branched chain growth. However, attempts to selectively cleave N-sulfate bearing sp^2-hybridized groups cannot be demonstrated, limiting the overall building block scope for this methodology. Consequently, the active ring-opening of cyclic sulfamidates on amine-functionalized oligo(amidoamine) backbones is successfully applied to produce sequence-defined, N-sulfated macromolecules.

1. Introduction

The relationship between any molecule’s shape and function is fundamental, with shape being governed by molecular composition and the molecule’s atomic arrangement. Similarly, a macromolecule’s sequence is known to influence its shape with natural examples, like DNA/RNA, proteins and polysaccharides, only functioning properly with the correct atomic or building block (BB) arrangement. The importance of sequence definition is well-understood in biopolymers and attention within the polymer community has turned toward artificial systems over the past decade. The focus on sequence-defined oligomers and polymers has led to many advances in both synthesis and application which have been recently reviewed.

In our own work, we have focused on the development of novel peptido- and glycomimetic macromolecules based on sequence-defined oligo(amidoamines) with pendant glycan side chains. We have achieved sequence-definition via the step-wise assembly of tailor-made BBs on solid phase (SP), which offers the chemist many advantages when assembling monodisperse, sequence-defined macromolecules (SDMs). Ever since Merrifield et al. reported the first solid-phase synthesis (SPS) of a tetrapeptide,^3 the versatility and appeal of this synthetic strategy has seen the purification and yield-maximizing benefits being applied to natural polymers, such as polysaccharides,^4 polypeptides,^5 and polynucleotides,^6 as well as non-natural or biomimetic polymers, such as polyurethanes,^7 triazine,^8 and thioether-based polymers.^

Our own work centers around standard peptide coupling strategies for BBs carrying a free carboxylic acid and a Fmoc-protected amine group. Our^2 have developed a toolbox of non-natural building blocks that allows us to vary main and side chain motifs in the final SDM and to site-selectively introduce different motifs, such as hydrophobic units or different glycans. In the recent past, we have looked to expand this toolbox and have become particularly interested in synthesizing glycomacromolecules containing sulfonated or sulfated structures. They are simplified mimetics of glycosaminoglycans (GAGs), densely-sulfated polysaccharides, which often bear other charged functionalities, e.g., amines and carboxylates, and perhaps heparin is the most widely-known example. Heparin/heparan sulfate’s O- and N-sulfation patterns can be highly heterogeneous, nevertheless it is known that specific patterns determine a given GAG’s function at the cell, tissue, and organism level influencing processes, such as signal transduction, and development of the nervous and skeletal system. Therefore, it is of high interest to create macromolecules with sequence-defined sulfation patterns and learn more about the correlation between the sequence and resulting biological activity. However, creating such natural or biomimetic sulfated macromolecules with sequence definition is highly challenging. Known synthetic methods include enzymatic modification of natural oligo- and polysaccharides and the use of noncarbohydrate containing polymers and complex protecting group strategies in SPS. In our recently reported work, we have used SPS in two distinct ways to generate sulfonated and

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sulfated SDMs. First, incorporating sulfation patterns onto the desired backbone or architecture on SP prior to cleavage. Alternatively, we reported that a resin-bound SDM may be produced and then cleaved from its support, before the desired sulfation is installed.

In this study, we explore an alternative way to produce N-sulfated SDMs that should be compatible with our previously-established SP strategies. Therefore, we identified cyclic sulfamidates (CS) as potential BBs to be added to our toolbox. CSs are a class of heterocycle identified by a N–SO₂–O functionality housed in either a 5 or 6-membered ring. CS reactivity is characterized by a site-selective, ring-opening reaction at the endocyclic C–O bond with a wide variety of nucleophiles including amines, alkoxides, halides, azide, sulfur-, and carbon-centered nucleophiles (Figure 1). The use of heterocyclic BBs that can be selectively ring-opened in the stepwise assembly of SDMs has been well-established by Du Prez et al. for thiolactones and Johnson et al. for epoxides. We envision two strategies for SDM chain elongation using CS BBs which can be categorized as either “latent” or “active” (Figure 1). A latent or active heterocyclic BB strategy describes the outcome of a BB’s coupling to a macro-molecule. If a strategy is latent then an unreacted heterocyclic functionality remains after SDM incorporation, retaining its reactivity to be used in a subsequent transformation. In contrast, an active strategy utilizes a heterocycle’s inherent chemistry in the coupling step. For a latent strategy an “AB+CD” monomer approach, akin to thiolactone examples, would be realized via creating a carboxylic acid-pended CS, e.g., 1 (“AB” monomer). In an initial step 1 would be coupled to an amine-functionalized resin via amide formation. After successful incorporation of the latent CS ring on the resin, treatment with an appropriate bifunctional nucleophile, e.g., diamine, N-protected aminol, or aminothiol...
("CD" monomer), would afford ring-opening/chain elongation and provide a reactive terminus for further iterative couplings of 1 (Figure 1B).

Alternatively, an active approach would use iterative ring-openings of CS BBs on nucleophile-presenting resins to grow the polymer backbone. Plus, in this approach the revealed N-sulfate group could be regarded as a masked protecting group, which after successful deprotection would yield a new amine end-group, primed for further chain elongation (Figure 1C). Secondary amine alkylation would result in linear growth, whereas primary amine termini would afford branching, allowing precise control over architecture. In both examples the role of the N-bound residual group could be: 1) to act as a side-chain (encoding information or additional functionality), or 2) provide an additional reactive group, potentially crucial for future chain elongation. Literature reports of CSs on SP have demonstrated both latent and active approaches but never in an iterated fashion. [18]

2. Results and Discussion

2.1. Latent Coupling Strategy

To begin with, a latent AB+CD monomer approach was investigated and to this end the gram-scale synthesis of novel cyclic sulfamidate 1 was proposed. 1 was synthesized via the Michael addition of ethanolamine (2) to tert-butyl acrylate (3) which afforded key, N-alkylated ethanolamine derivative (4) in multigram-scale quantities (Figure 2A, Supporting Information). [19] Treatment of 4 with thionyl chloride, followed by Ru-catalyzed oxidation afforded CS 5 in 64% over two steps. Deprotection of the tert-butyl ester protecting group by exposure to low pH afforded 1 quantitatively. Importantly, this protocol affords the desired building block in high purity and yield, vital for SPS which requires BBs to be used in high excess.

To investigate the stability of 1 tests were conducted. After 14 d bench storage 1H NMR showed degradation due to the presence of a complex set of peaks (Figure S1, Supporting Information). Similarly, 5 was not bench stable after 8 weeks’ storage, but it was found that long-term storage at −19 °C was sufficient for 5 (Figure S2, Supporting Information), hence all SP couplings reported herein were conducted with freshly synthesized 1.

For initial tests an oligomer precursor (6) was synthesized on acid-labile resin, forming an oligo(amicidioamine) backbone comprising three repeat units of a BB previously developed within our group (Figure 2). The EDS BB (short for Ethylene glycol—Diamine- Succinic acid) is applied in our group to precisely introduce ethylene glycol spacing units into oligo(amicidioamine) main chains (Figure 2). [20] 6 allowed reaction progress to be rapidly monitored by reverse phase high-performance liquid chromatography-mass spectrometry (RP-HPLC-MS) following resin cleavage. First coupling experiments were conducted akin to that reported by Cohen et al. using benzotriazol-1-yl oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), N-methylmorpholine and dimethylformamide (DMF) (Entry 1, Table S1 and Figure S3, Supporting Information). [18c] Analysis indicated that: 1) 100% conversion of 6 was not achieved, 2) no
evidence of latent electrophile 7 was noted, and 3) unexpected masses were noted, which after extensive consideration were assigned to desulfated-8 (Figure 2C; and Figures S4–S7, Supporting Information). Side product 8 was likely generated from the ring-opening of resin-bound 7, as successful PyBOP-mediated amide couplings release deprotonated hydroxybenzotriazole (HOBt), and owing to proximity, likely attacks the electrophilic amide couplings release deprotonated hydroxybenzotriazole 8.

Exploratory CS ring-opening experiments using 11 established a range of solvents, e.g., tetrahydrofuran, dichloromethane and DMF, plus, a range of bases, e.g., pyridine, 1,8-diazabiclo(5.4.0)undec-7-ene, triethylamine, and DIPEA facilitated ring-opening. Due to their broad SP and automated synthesizer compatibility DMF and DIPEA were taken forward for further investigation (Figure 3).

The active ring-opening with benzyl-functionalized CS 15 (25 eq.) proceeded with 100% conversion of 11 and characteristic desulfated masses for 12b were identified by ESI-MS (Entry 1, Table S2 (Supporting Information), see Figures S13–S16 (Supporting Information) for desulfated oligomer characterization). Indeed, sulfated 12b was observed by negative mode ESI-MS (Figure S17, Supporting Information). When ring-opening was attempted with ethyl-bearing 14 unreacted starting material 11 was noted (60 min, RT; Entry 2, Table S2 and Figure S13, Supporting Information). Increasing the coupling time fourfold achieved full conversion of 11 (Entry 3, Table S2 and Figure S14, Supporting Information). The reactivity differences between CS BBs were assigned to R group identity, e.g., donating effects via hyperconjugation (ethyl-functionalized 14) could lower the relative electrophilicity of the CS ring compared to 15. Further linear growth investigations with 5 and 16 probed reaction time, temperature, and required BB equivalents. It was found that, regardless of reaction time or temperature, a minimum of 10 eq. of BB was necessary to facilitate 100% conversion of 11 (Entries 4–14, Table S2 and see Figures S18–S21, Supporting Information).

Investigations were then undertaken to establish if branched architectures could be synthesized by reacting a primary amine end-group, e.g., EDS, precursor 6 with two equivalents of BB (Figure 3). Primary investigations reacted 6 with Boc-protected 16 in 10, 20, 50-fold excess for either 0.5, 2, or 24 h (Entries 15–23, Table S2, Supporting Information). In all cases, 100% conversion of starting materials was observed, but neither the desired bis-alkylated structure (13c) nor mono- or bis-desulfated equivalents were directly observed (Figure 3; and Figures S22 and S23, Supporting Information). The same profile was observed with 5 (Entries 24–26, Table S2, Supporting Information). After careful consideration, the masses were assigned to a “diethylamine” end-group akin to 18 (Figure 3). The assignment suggested successful bis-alkylation by 5 and 16 had occurred, but that branched architectures (with poly(ethyleneimine) character) were susceptible to degradation under ESI-MS conditions. Removing formic acid from the eluent still afforded the identical ESI-MS profile (Figures S22 and S23, Supporting Information). MALDI-MS analysis also showed similar degradation patterns (Figure S24, Supporting Information). As similar degradation patterns were not observed from the mono-alkylation of secondary amine-capped 11, we suggest the observed degradation is indeed architecture dependent. This hypothesis was further bolstered via 1H NMR and ESI-MS analysis of a cleaved 13c probe dissolved in D,O. ESI-MS analysis showed 13c masses for mono- and bis-desulfated 13c, and mono- and bis-deaminated end-groups, except with deuterium replacing exchangeable protons (Figure S25, Supporting Information). 1H NMR analysis of 13c did not indicate the presence of expected aminoethyl groups (18) and the global.
introduction of all protons matched the expected proton total (excluding exchangeable and amide protons – Figure S26, Supporting Information). Fourier-transformed infra-red spectroscopy (FTIR) analysis of 13c indicated the presence of sulfate groups (Figure S27, Supporting Information).[23] These results established that successful chain elongations with a range of CS BBs could be achieved to yield linear and branched architectures, therefore our attentions moved toward regenerating a nucleophilic chain terminus to allow iterative active couplings (Figure 1C).

N-sulfate cleavage following CS ring-opening is typically achieved via acid treatment.[15a,24] The acidic cleavage of carbamate-functionalized N-sulfates on SP at elevated temperatures has been demonstrated too.[18a,18b] We envisioned developing an effective deprotection strategy (mild acidic pH/elevated temperatures) that could achieve N-sulfate cleavage while not generating resin cleavage. It was evident that low pH conditions required for resin cleavage at room temperature, e.g., 95% trifluoroacetic acid solution did not affect N-sulfate cleavage (Figures S12, S17–S19, S21, and S24, Supporting Information). Negative mode ESI-MS after resin cleavage was used to confirm the presence of N-sulfate groups and it was discovered that Boc-functionalized N-sulfate groups (13c generated from 16) could be deprotected, but alkyl-bearing 13a/b could not be deprotected regardless of the conditions tested (Table S3, Supporting Information).

With increasing temperature or reducing pH the window between selective N-sulfate and resin cleavage shrinks, reducing the favorability of the deprotection conditions. For an iterative SPS of SDMs, conditions should ideally: 1) avoid unwanted resin cleavage to maximize yields and 2) be applicable to a broad range of functionality. As acidic cleavage was not effective for sp3-hybridized N-substituents with reasonable, mild conditions this limits the range of CS BBs possible for iterative chain elongations following this strategy. Moreover, we found that exposing deprotected 13c to a secondary CS BB did not yield the expected chain elongation when the previously optimized conditions were employed. Despite exploring a range of washing protocols at different pH values, with various organic and aqueous solvents, no effective method was discovered allowing a second BB to be employed iteratively. These limitations prevent the effective iterative coupling of CS BBs on SP, so this second strategy was too abandoned, but the successful ring-opening of CS BBs was reapplied to produce N-sulfated oligo(amiodeamine).

2.3. N-Sulfated, Sequence-Defined Oligomer Synthesis

Having established that a range of N-functionalized CSs could be actively ring-opened on amine-bearing SP, our attentions...
turned toward generating monodisperse, N-sulfated SDMs. We proposed constructing different architectures with varying levels of N-sulfation levels to examine the synthetic limitations available on SP, while generating structures of interest for potential GAG mimic structures. A single backbone (19) consisting of three EDS units, phenylalanine and two lysine residues bearing Boc-protected side-chains was assembled on SP (Figure 4). From this central scaffold the Fmoc- and Boc-protected amines could be orthogonally manipulated to precisely afford primary or secondary amines. Subsequent functionalization of the primary and secondary amines would target: branched or linear architectures, and variable N-sulfation levels. 5 or 16 were employed, as after acidic resin cleavage a carboxylic acid and unsubstituted N-sulfate groups would be, respectively, generated. Seven targets, as outlined in Figure 4, were chosen to understand potential synthetic limitations from the interplay of architecture, steric hindrance, and sulfation density. The structures chosen were functionalized either at the backbone terminus or at both backbone and side-chain termini. Terminus functionalization via primary amine bis-alkylation would afford exclusively branching generating bis-sulfated 20a/b or secondary amine alkylation would afford linear growth and mono-sulfated 21a/b. Removing both Fmoc and Boc groups would reveal three primary amines which upon global alkylation would afford branched, hexa-sulfated 22 and conversion to secondary amines would generate tri-sulfated 23a/b.

Having constructed 19 on solid-phase (Figures S28 and S29, Supporting Information) the terminal Fmoc group was removed and the subsequent primary amine was directly reacted with CS BBs 16 or 5 (25 eq. for 2 h) to generate the branching required for 20a/b. End-group transformation with Fmoc-protected 17 afforded a secondary amine to generate the scaffold for mono-sulfated 21a/b. For 20a, no starting material was observed after CS coupling and the ESI-MS mass peak profile corresponded to bis- and mono-desulfated 20a and the various adducts of a diethyleneglycol end-group, as previously discussed (Figure S30, Supporting Information). A similar profile was observed for 20b (Figure S30, Supporting Information). 1H NMR, FTIR, and elemental analysis of 20a/b showed the expected proton counts, presence of sulfate groups and sulfur content (Figures S31–S34 and Table S4, Supporting Information). Having synthesized and cleaved the end-functionalized, mono-sulfated 21a/b from solid-phase, analysis by 1H NMR, FTIR, ESI-MS and elemental analysis again indicated the desired products (Table S4 and Figure S35–S39, Supporting Information).

To access 22 sequential deprotection of Fmoc and Boc groups afforded a backbone bearing three primary amines, which was then reacted with 16. ESI-MS analysis identified characteristic peaks corresponding to hexa-desulfated oligomer and the expected branching architecture degradation (Figure S40, Supporting Information). The presence of the desired proton total by 1H NMR and FTIR and elemental analysis to indicate sulfur content suggested 22 was successfully generated on SP (Figure S42 and Table S4, Supporting Information). The global functionalization of main- and side chains to generate 23a/b was similarly achieved using CSs 5 and 16 and analyzed as previously described to show the desired synthesis of these peralkylated, N-sulfated
macromolecules (Figure S43–S47 and Table S4, Supporting Information). All targeted structures were produced in good yields (Table S5, Supporting Information) signifying that the ring-opening of CSs for SDM synthesis is an option for future studies when creating functionally diverse N-sulfated SDMs, e.g., GAG mimetics.

Given the evidence herein reported for the facile ring-opening of CS BBs, we speculate that the application of Cs to larger oligo- or polymer constructs on solid-phase or in-solution is a viable possibility. Limiting factors likely arising during syntheses would probably be steric hindrance or electronic of either nucleophile or electrophile, which would lower overall reactivity.

3. Conclusion

To the best of our knowledge, this is the first comprehensive study of the use of cyclic sulfamidates for the generation of N-sulfated SDMs using latent and active strategies. We have demonstrated the limitations of a latent, AB+CD strategy when an amide bond is used to couple CS BBs to the resin. Alternatively, actively ring-opening cyclic sulfamidates on amine-functionalized resins has been broadly demonstrated with a range of CS BBs on primary- and secondary amine-functionalized oligo(amidoamine) backbones. However, the iterative ring-opening of CS BBs is limited, as acidic N-sulfate cleavage was not shown for N-sulfate groups bearing sp²-hybridized residual groups. Therefore, a number of N-sulfated SDMs were produced on solid-phase to demonstrate the wide applicability of this method for producing SDMs with variable yet sequence-defined N-sulfation levels and architectures. Future work will look to investigate: 1) alternative latent coupling strategies, e.g., click chemistry, 2) alternative N-sulfate cleavage methodologies, 3) potential application of CS-based oligo(amidoamine)s as GAG mimetics, and 4) to the best of our knowledge, given no reports exist on either the polymerization of cyclic sulfamidate-based monomers or the postpolymerization functionalization of larger polymers, we will look to expand this methodology.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Research data are not shared.

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