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Total synthesis of trifluorobutyryl-modified, protected sialyl Lewis X by a convergent [2+2] approach

Gizem Akçay [a], John Y. Ramphal [b,†], Marc d'Alarcao [b,*], Krishna Kumar [a,*]

[a] Department of Chemistry, Tufts University, Medford, MA 02155, United States
[b] Department of Chemistry, San José State University, San José, CA 95192, United States

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ABSTRACT

Structural and quantitative changes in the expression of sialic acid residues on the surface of eukaryotic cells profoundly influence a broad range of biological processes including inflammation, antigen recognition, microbial attachment and tumour metastasis. Uptake and incorporation of sialic acid analogues in mammalian cells enable structure–function studies and perturbation of specific recognition events. Our group has recently shown that a trifluorobutyryl-modified sialic acid metabolite diminishes the adhesion of mammalian cells to E and P-Selectin, presumably by leading to the expression of fluorinated sLe^x epitopes on cell surfaces, and interfering with the sLe^x–selectin interactions that are well known in mediating tumour cell migration (*J. Med. Chem.* **2010**, *53*, 4277). For studies directed towards understanding the molecular basis of this reduced adhesion, chemical synthesis of trifluorobutyrylated sialyl Lewis X (C_4F_3-sLe^x) was crucial. We have developed a highly efficient [2+2] approach for the assembly of C_4F_3-sLe^x on a preparative scale that contains versatile protective groups allowing the glycan to be surface immobilized or solubilized as needed for biophysical studies to investigate selectin interactions. This strategy can, in principle, be used for preparation of other *N*-modified sLe^x analogues.

Introduction

Sialic acids (*N*-acylneuraminic acids) are the most prevalent monosaccharides found at the termini of glycoconjugates on cell surfaces and are involved in many biologically critical ligand–receptor interactions.[2] Sialylation patterns of cell surfaces are dynamic in order to accommodate specific carbohydrate–protein interactions. Most of the diversity is generated by substitution patterns at the C4, C5, C7, C8 and C9 positions associated with linkage variation.[3] In humans, sialic acids appear principally in the form of α(2–3)-linked galactosides or α(2–6)-linked 2-acetamino-2-deoxygalactosides.[4,5]

Modification of sialoglycoconjugates in living cells by metabolic incorporation of non-natural sialic acids expands this structural diversity, and proffers the ability to interfere with binding events that are implicated in disease development.[6–8] To investigate the therapeutic potential of sialic acid analogues in cancer progression by targeting selectin-mediated cell adhesion, we designed fluorinated sialic acid precursors. Selectins are membrane-bound glycoproteins expressed on a variety of cells including activated vascular endothelium and leucocytes, and they interact with sLe^x displayed on the surface of their partner cells. This facilitates the recruitment of leucocytes into inflamed tissues. Cancer cells utilize the same mechanism of selectin adhesion in order to exit the bloodstream and form metastatic tumours at different sites. Notably, high levels of sialosides, particularly sLe^x on cell surfaces have been shown to correlate with malignant transformation of gastrointestinal, pancreatic and breast cancer cells.[9–12]

In studies directed towards inhibition of sLe^x–selectin interactions, modified sLe^x structures that have higher binding affinities for selectins have been generated.[13] However, inhibition of selectin-mediated cell–cell interactions via monovalent sLe^x analogues appears to be limited since efficient binding to selectins requires multivalent interactions in the biological context. Our approach alters cellular adhesion through glycoengineering of surface sialoconjugates using synthetic fluorinated sialic acids. The observed decrease in the adhesion of these engineered cells was most pronounced with the trifluorobutyryl modified sialic acid precursor.[1] We hypothesized that fluorination of the endogenous sLe^x ligand on cell surfaces may reduce selectin-mediated cellular adhesion by lowering the affinity of the glycan towards selectins. Thus, we

* Corresponding authors at present addresses: Department of Chemistry, Tufts University, Medford, MA 02155, United States (K.K.), Department of Chemistry, San José State University, San José, CA 95192, United States (M.d'A.). Fax: +1 617 627 3443 (K.K.), +1 408 924 4945 (M.d'A.).
E-mail addresses: marc.dalarcao@sjsu.edu (M. d'Alarcao), krishna.kumar@tufts.edu (K. Kumar).
† InterMune, Brisbane, CA 94005, United States.

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are particularly interested in characterizing trifluorobutyrylated sLe^x–selectin binding in vitro. Accordingly, we focused our efforts on the synthesis of C_4F_3-sLe^x.

The assembly of the tetrasaccharide sLe^x has been a nontrivial task for synthetic chemists, as it requires selective formation of glycosidic bonds with highly functionalized substrates. Persistent challenges in synthesizing sLe^x include the spatial proximity of the galactose and fucose at positions C-4 and C-3 of *N*-acetylglucosamine,[14] resulting in low reactivity of C4-OH or C3-OH in glycosylation reactions, pronounced acid lability of the α-L-fucose linkage[15] and difficulties associated with chemical sialylation.[2,16] Choosing a suitable set of orthogonal protecting groups to enable anomeric control and high yielding glycosylations has been the key to several successful sLe^x syntheses reported to date. Although a variety of chemical and chemo-enzymatic methods are available for the synthesis of naturally occurring sLe^x and sLe^x-containing complex structures,[17–21] only few methods have been reported to make *N*-modified sialic acid containing oligosaccharides[22–24] and no efficient protocols exist for the preparation of *N*-modified sLe^x analogues. We devised a versatile solution phase convergent chemical strategy for the construction of *N*-substituted unnatural sLe^x structures. Key features of our synthesis include simple and efficient protecting group manipulation and orchestrated use of glycosyl halide, phosphite and trifluoroimidate donors to ensure sufficient reactivity and stereoselectivity. Furthermore, our synthetic route offers the opportunity to install a wide range of C-5 modifications on the sialic acid that would allow construction of *N*-modified sLe^x or more complex oligosaccharide libraries.

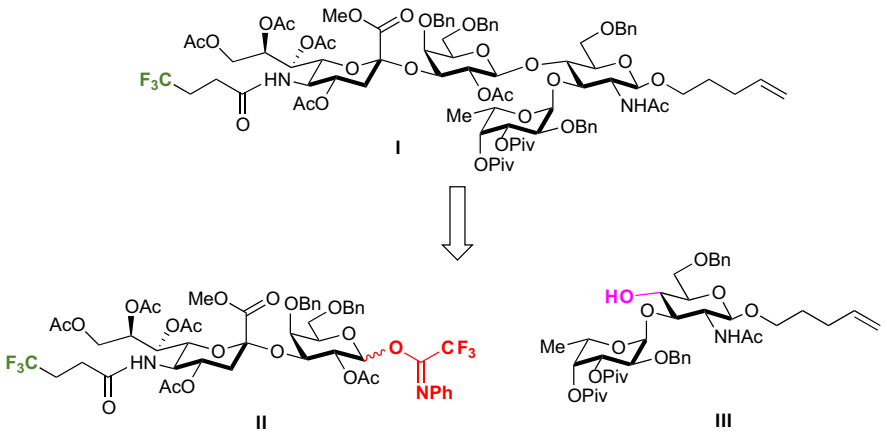
Results and discussion

For the synthesis of desired tetrasaccharide **I**, not only must the strategy afford suitable quantities but it also must accommodate structural variation to allow preparation of analogue structures. We envisioned that sufficient quantities of such a complex target could be obtained by employing a convergent approach that uses orthogonally protected building blocks that can be assembled into the target by using a minimal number of synthetic steps. In planning the synthetic route, target tetrasaccharide **I** was disconnected into two blocks at the Galβ(1–4)GlcNAc linkage, generating two disaccharide precursors. Accordingly, the final tetrasaccharide structure can be assembled through a [2+2] glycosylation of novel building blocks, disaccharide donor **II** and disaccharide acceptor **III** (Scheme 1).

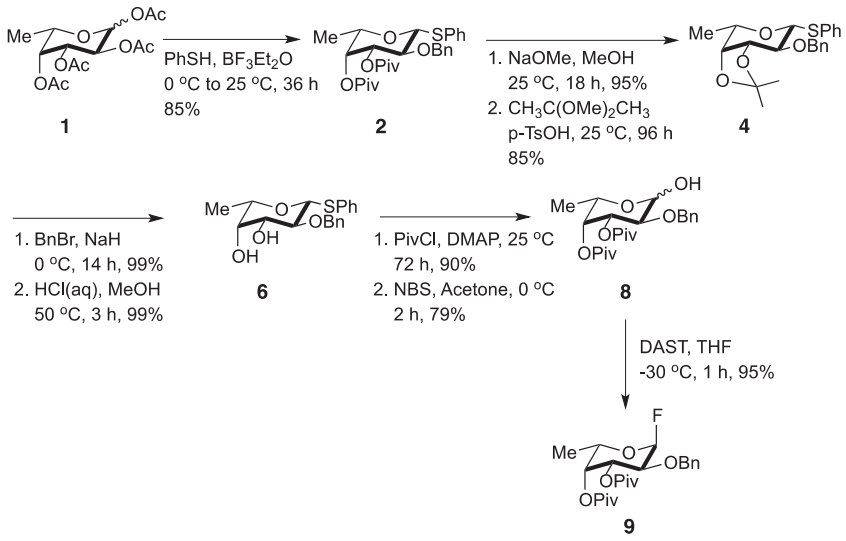
Important features of the disaccharide acceptor **III** include, (1) the protecting group pattern on the fucose building block, that improves stability of the α fucoside linkage while preserving a non-participating group at 2-OH to enable a 1,2-*cis*-L-fucopyranosyl glycosidic bond, (2) a 4,6-*O*-benzylidine acetal protection on the glucosamine building block, that provides a 6-*O*-benzyl substituted disaccharide acceptor that is sterically less demanding at the site of the [2+2] glycosylation and (3) the presence of a flexible *O*-pentenyl functionality at the anomeric oxygen that allows access to soluble sLe^x constructs by simple removal of the *O*-pentenyl moiety through aqueous *N*-bromosuccinimide (NBS) treatment or enables surface conjugation of the compounds via thiol linkers. A thiol-terminated linker can be introduced by addition of thiolacetic acid to the olefin in the presence of azobisisobutyronitrile (AIBN).[25] Other types of linkers can also be presented via olefin metathesis reaction.[26,27]

For the construction of disaccharide acceptor **III**, we proposed new building blocks **9** and **17** that can be prepared in a facile manner, using both reported and new intermediate monosaccharides. The preparation of fucosyl donor **9** started with the conversion of L-fucose tetraol to the peracetylated derivative **1**. The reaction of tetraacetate **1** with thiophenol (PhSH) in the presence of $BF_3{\cdot}Et_2O$ afforded thioglycoside **2**[28] in 85% yield. Next, global deprotection of *O*-acetyl groups followed by 3,4-*ortho* ester formation using 2,2-dimethoxypropane with *p*-toluenesulfonic acid monohydrate ($pTsOH{\cdot}H_2O$) generated derivative **4**.[28] Benzylation of the 2-OH and removal of the isopropylidene protection gave diol **6**[19] in high yields. Pivaloate esters were installed on the free hydroxyls by treating **6** with pivaloyl chloride and DMAP. A final deprotection step with NBS in aqueous medium furnished the reducing sugar **8**[29] in 79% yield. Glycosyl fluoride donors in combination with promoters, stannous chloride ($SnCl_2$) and silver perchlorate ($AgCl_4$) are often used to achieve good α-stereoselectivity.[30,31] Thus, we synthesized fucosyl fluoride **9** from the reaction of **8** with diethylaminosulfur trifluoride (DAST) at low temperatures (Scheme 2).

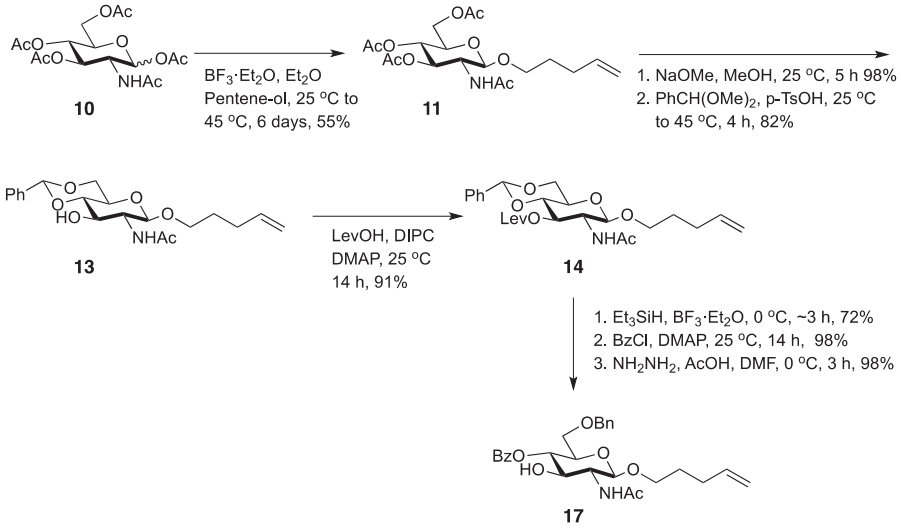
A carefully designed protection strategy enabled access to the *N*-acetyl glucosamine acceptor **17** efficiently. Glycosylation of acetate **10** with pentene-1-ol mediated by TMSOTf gave the known compound **11**.[32] Zemplén deacetylation followed by addition of the 4,6-*O*-benzylidine protection using benzaldehyde dimethyl acetal and *p*-toluenesulfonic acid resulted in previously reported intermediate **13**.[33] Next, the free alcohol was masked by treatment with levulinic acid in the presence of an activating system composed of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) and DMAP to yield **14**. Regioselective opening of the benzylidene acetal at 4-OH with triethylsilane and $BF_3{\cdot}Et_2O$ followed by benzoyl protection of the free alcohol and finally the cleavage of the levulinate ester via aqueous hydrazine afforded glycosyl acceptor **17** in 69% yield starting from **14** (Scheme 3).



Scheme 1. Target molecule and building blocks.



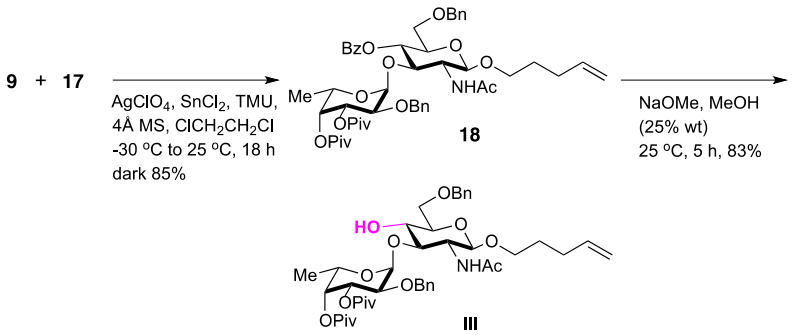
Scheme 2. The fucosyl donor was selectively protected to support α glycosidic bond formation and to generate an acid stable α-fucoside linkage.



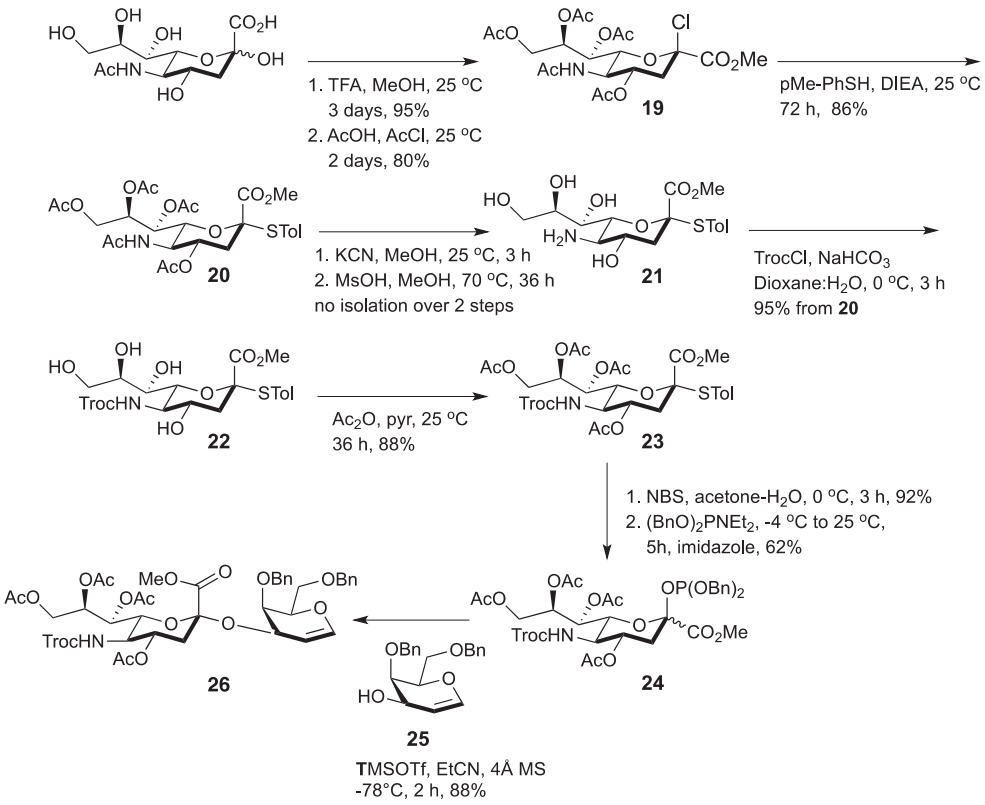
Scheme 3. Synthesis of the new glucosamine building block **17**.

The coupling of fucosyl donor **9** with acceptor **17** provided **18** in 85% yield. Disaccharide **18** was then subjected to optimized reaction conditions with NaOMe for selective removal of the benzoyl ester in the presence of pivaloyl esters, generating disaccharide acceptor **III** in 83% yield (Scheme 4).

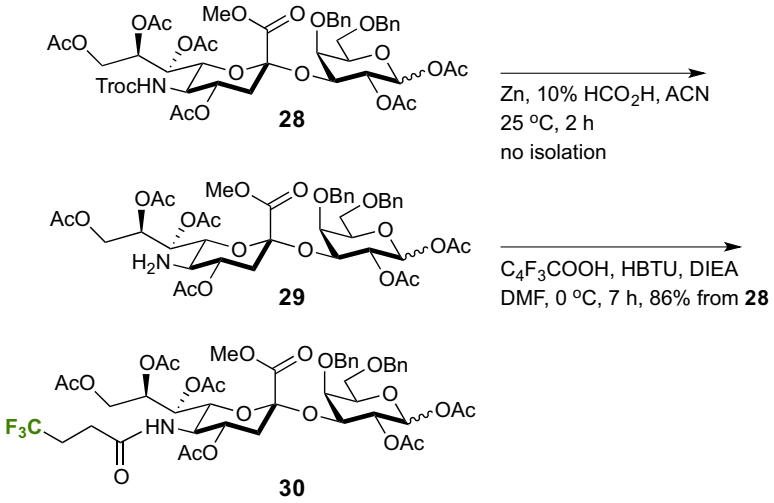
The next stage of the synthesis entailed the preparation of disaccharide donor **II**. We envisaged that *N*-Troc protection of the sialic acid amine would be suitable because selective Troc deprotection can be accomplished under mild acidic or neutral conditions to produce a sialic acid α(2–3)-galactose intermediate



Scheme 4. Formation of disaccharide acceptor **III**.



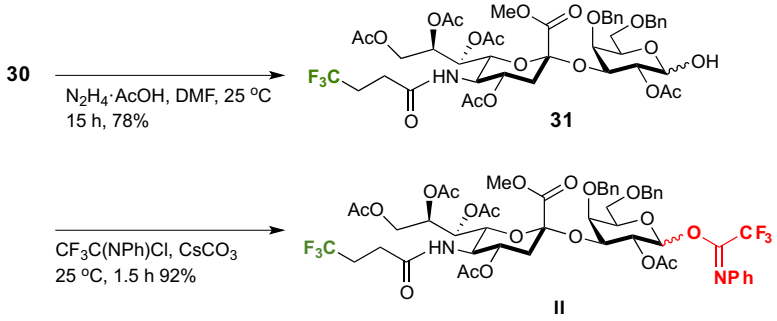
Scheme 5. Synthesis of donors **23** and **24** based on slight modification of the literature procedures ((i) separate steps for de-N-acetylation and de-O-acetylation during preparation of **21**. (ii) Switching from 1*H*-tetrazole to imidazole in the formation of phosphite donor **24**).[30,31]



Scheme 6. Synthesis of *N*-trifluorobutyrylated sialic acid α(2–3)galactose.

bearing a free amino group for derivatization. The additional advantage of this modification would be improved sialylation yields as reported by Hanashima et al.[34] For the construction of *N*-Troc sialic acid α(2–3)-galactose precursor, we explored the utility of both aryl sulfide and phosphite-based sialic acid donors. Aryl sulfide-based donors that are associated with the NIS/TfOH glycosylation system offer the advantage of being highly stable and non-hygroscopic. While phosphite-based donors are relatively less stable, they exhibit high activity when a catalytic amount of promoter is used and result in predominant formation of the α product.[35,36]

Synthesis of sialic acid donors **23**[35] and **24**[34] are outlined in Scheme 5. Glycosyl chloride **19** was treated with thiocresol in the presence of *N*,*N*-diisopropylethylamine (DIEA), affording the corresponding thioglycoside **20** in 71% yield. Conversion of thioglycoside **20** to **21** via a single de-acetylation step followed by Troc protection of the free amine resulted in poor yields, likely due to the formation of a bicyclic lactam side product. This side reaction was prevented by a two-step de-acetylation procedure. Fully de-acetylated amino intermediate **21** was obtained after treatment of **20** with potassium cyanide (KCN) in methanol at ambient temperature for selective de-O-acetylation followed by warming at reflux with methanolic



Scheme 7. Transformation of N-trifluorobutyrylated **30** to activated disaccharide donor **II**.

Table 1
Summary of [2+2] glycosylation reactions performed under various conditions

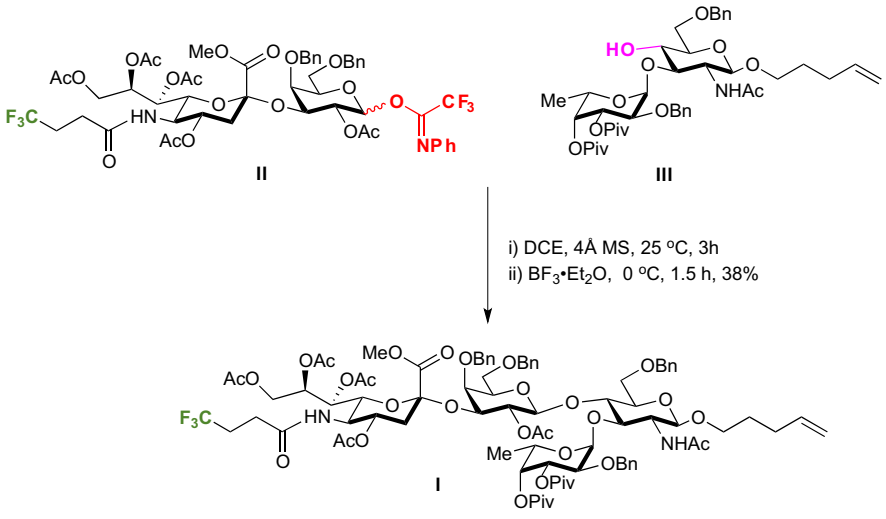
Entry	Promoter	Solvent	Temperature (°C)	Yield (%)
1	TMSOTf[a,b]	DCM	0	0[c]
2	TMSOTf[a,b]	DCE	rt	0[c]
3	TfOH[a,b]	DCM	0	0[c]
4	TfOH[a,b]	DCE	rt	0[c]
5	$Yb(OTf)_3$[a,b]	DCE	0	10
6	$Yb(OTf)_3$[a,b]	DCE	rt	10
7	4 Å AW MS*	Toluene	rt	15
8	4 Å AW MS*	Toluene	MW 40	15
9	$BF_3{\cdot}Et_2O$[b]	DCE	0	12
10	$BF_3{\cdot}Et_2O$[b]	DCE	rt	38
11	$BF_3{\cdot}Et_2O$[b]	DCE	MW 40	0[d]
12	$BF_3{\cdot}Et_2O$[b]	DCE	MW 60	0[d]

[a] 0.01 equiv of promoter.
[b] 0.02 equiv promoter.
[c] Both the donor and acceptor recovered unaltered.
[d] Hydrolysis of the donor is observed, rt ~25 °C.
* AW MS: acid-washed molecular sieves.

methanesulfonic acid to remove the *N*-acetyl function at the C-5 position. The resulting crude amine salt was directly used in the next reaction to generate tetra-ol **22** in 95% yield over three steps. Global acetylation of **22** afforded sialic acid donor **23** in 88% yield. Sialic acid phosphite donor **24** was prepared by removal of the thiocresol group followed by treatment with dibenzyl diisopropylphosphoramidite and imidazole in 60% yield.

Next, glycosylation reactions were performed to test the performance of sialic acid donors using 4,6-di-*O*-benzyl-D-galactal **25**[34] as the acceptor. NIS/TfOH-mediated coupling of thioglycoside **23** with **25** was attempted in acetonitrile at low temperatures, but no desired product could be isolated. The glycosylation reaction between phosphite donor **24** and alcohol **25** however, in the presence of TMSOTf at −78 °C, produced known α-sialoside **26** in 86% yield. The transformation of disaccharide **26** to corresponding sialic acid α(2–3)-galactose **28** was accomplished in two steps as described previously.[34] *N*-Troc removal was performed by treatment of **28** with activated zinc powder and 10% formic acid in acetonitrile to furnish the free amine **29** in quantitative yield. With the key intermediate **29** in hand, we sought an efficient procedure for appending the desired fluoroalkyl function at C-5. Initially we attempted to prepare the 5-trifluorobutyryl modified sialic acid α(2–3)-galactose by reaction of **29** with *N*-hydroxysuccinimidyl 4,4,4-trifluorobutyrate in the presence of DIEA. The results were unsatisfactory due to sluggish reactivity. However, treatment of **29** with the HOBt ester of 4,4,4-trifluorobutyric acid in the presence of DIEA at 0 °C, provided the desired fluoroalkyl product **30** in 86% yield (Scheme 6).

Having established an expedient synthesis of trifluorobutyryl modified sialic acid α(2–3)-galactose, the conversion of this product to an activated disaccharide donor was investigated. For this purpose, disaccharide **30** was treated with hydrazine acetate to selectively remove the anomeric acetate and generate hemiacetal **31**. Considering the good balance of stability and reactivity found with *N*-phenyl trifluoroacetimidate donors in complex oligosaccharide synthesis, we chose this methodology.[37–39] Treatment of **31** with *N*-phenyl trifluoroacetimidoyl chloride ($CF_3C(NPh)Cl$) in the presence of $CsCO_3$ produced the corresponding trifluorobutyryl-modified sialic acid α(2–3)-galactose donor **II** in 92% yield (Scheme 7).



Scheme 8. A final [2+2] glycosylation successfully assembles **I**.

Finally with both disaccharide building blocks in hand, we explored the viability of [2+2] glycosylation reaction to access trifluorobutyryl modified sLe^x **I**. We screened a variety of glycosylation conditions using promoter systems including TMSOTf, $Yb(OTf)_3$, acid washed molecular sieves and $BF_3{\cdot}Et_2O$ The best result was with $BF_3{\cdot}Et_2O$ mediated [2+2] coupling affording the desired tetrasaccharide **I** in 38% yield (Table 1, entry 10) (Scheme 8).

Conclusion

We have demonstrated the utility of a convergent [2+2] strategy in the synthesis of a versatile trifluorobutyryl-modified sLe^x analogue, **I**. This material can be used to generate the soluble fluorinated tetrasaccharide C_4F_3-sLe^x suitable for ITC studies to determine the binding constant with P- and E-Selectin. In parallel, **I** can be immobilized on a maleimide functionalized surface after selective modification of the *O*-pentenyl moiety into a thiol function, followed by removal of all the protecting groups for SPR binding studies.[40–43] Moreover, **I** could be elaborated further to obtain the high affinity P-Selectin glycoprotein ligand 1 (PSGL1). The results of these biophysical studies will be reported in due course.

The chemical strategy and the key building blocks described in this work should be particularly useful for preparative scale synthesis of biologically relevant oligosaccharides with unnatural sialic acid residues and offer a way to study interactions of *N*-5 (sialic acid) modified sLe^x epitopes for a variety of interrogations using biophysical and cell biological techniques.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.11.029.

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