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Chemoenzymatic Synthesis of GM_3, Lewis x and Sialyl Lewis x Oligosaccharides in ^{13}C-Enriched Form

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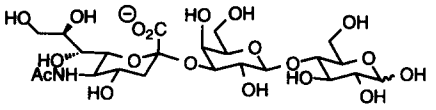
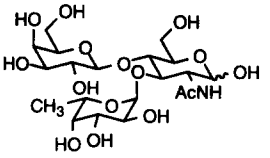
Abstract: In connection with studies on the solution and protein-bound conformation of oligosaccharides, we had reason to consider the application of ^{13}C-enriched sugars. Herein we describe the synthesis of ganglioside GM_3 trisaccharide (**1**), Lewis x trisaccharide (**2**) and the sialyl Lewis x tetrasaccharide (**3**) from [U-^{13}C]-D-Glc, [U-^{13}C]-L-Gal and [U-^{13}C]-pyruvate. © 1997 Elsevier Science Ltd.

The 3-D structure and dynamics of oligosaccharides are thought to play a crucial role in their biological function.[1] A description of the solution and bound conformation of oligosaccharides should therefore provide key information about complex biological recognition events, and aid the design of glycomimetics. To date, conformational analysis of such molecules by NMR spectroscopy has largely relied on analysis of ^1H-^1H nuclear Overhauser effects (nOe's), ^1H-^1H and ^1H-^{13}C coupling constants.[2,3] However, the few constraints provided by such techniques can limit their usefulness. Isotopic enrichment provides higher definition solution structures since heteronuclear and ^{13}C-edited NOESY, and ^{13}C-^{13}C COSY experiments become possible; we have recently reported the resonance assignment of exchangeable protons in ^{13}C-enriched *N*-acetyllactosamine, and ^1H-^1H nOe's involving these protons.[4] In addition, isotopically-enriched sugars offer scope for description of a more accurate molecular picture of carbohydrates in the bound state. Whilst transferred nOe techniques have been used to analyse relatively weak protein-carbohydrate complexes ($K_D > 50\mu M$),[5] this approach is unsuitable for studying tightly bound carbohydrate ligands. However, with the aid of ^{13}C-enriched ligand the conformation of estrone 3-glucuronide bound to a recombinant antibody Fv fragment (K_D = 1nM) can be defined.[6] Other studies employing partially-enriched sugars have reported the definition of the ring conformation of 2-deoxyribonucleosides by exploitation of ^{13}C-^1H and ^{13}C-^{13}C spin coupling constants,[7] and methods for the analysis of sialyl lactone formation based on isotope-edited inversed detection techniques.[8] The synthesis of ^{13}C-ribonucleosides uniformly isotopically enriched in the sugar ring has also recently be described.[9]

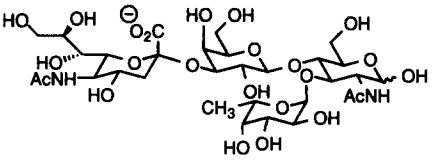
Peters and Pinto[3] note that "Unfortunately, isotopically-labelled oligosaccharides are not readily available and therefore, many heteronuclear NMR experiments are not applicable to the study of oligosacharides". Herein we describe the synthesis of ^{13}C-enriched ganglioside GM_3 trisaccharide [NeuNAc-α-2,3-Gal-β-1,4-Glc (**1**)], Lewis x trisaccharide [Gal-β-1,4-(Fuc-α-1,3)-GlcNAc (**2**)] and sialyl Lewis x tetrasaccharide [NeuNAc-α-2,3-Gal-β-1,4-(Fuc-α-1,3)-GlcNAc (**3**)] from [U-^{13}C]-D-Glc, [U-^{13}C]-L-Gal and [U-^{13}C]-pyruvate.



GM_3 trisaccharide (**1**)



Lewis x trisaccharide (**2**)

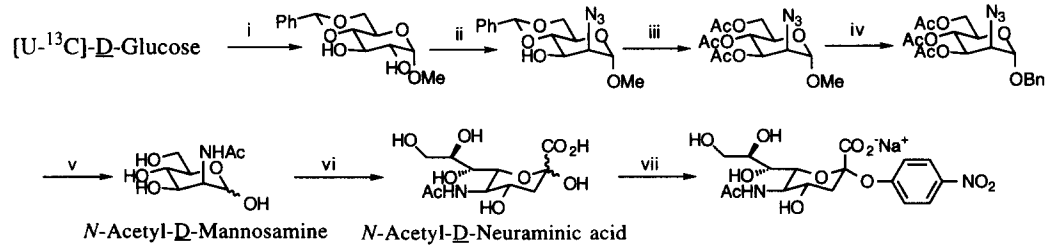


Sialyl Lewis x tetrasaccharide (**3**)

The principal limitation in the preparation of isotopically-enriched saccharides has been the lack of suitably labelled monosaccharide building blocks. However, recent developments in the culture of microalgae under photosynthetic conditions have enabled production of [U-^{13}C]-D-glucose up to kilogram scale.[10] The conversion of this material into other commonly occurring sugars serves as a starting point for the preparation of ^{13}C-labelled oligosaccharides.[11] Below we describe the conversion of [U-^{13}C]-D-glucose into isotopically-enriched *N*-acetyl-D-mannosamine and its conversion to *N*-acetyl-D-neuraminic acid. Recent work has identified an algal species capable of producing [U-^{13}C]-L-galactose when grown in the presence of [^{13}C]-carbon dioxide (P.W.Behrens, unpublished data). We have used the latter material as a precursor to ^{13}C-labelled L-fucose.

Synthesis of the p-Nitrophenyl Glycoside of N-Acetyl-D-[U-^{13}C]-Neuraminic acid

The synthesis of *N*-acetyl-D-mannosamine from [U-^{13}C]-D-glucose and its conversion to *N*-acetylneuraminic acid p-nitrophenylglycoside is outlined in Scheme 1. The synthetic procedures employed[13] are standard with a couple of exceptions. Firstly, to cut down the number of steps required overall generation of the 2-azido-2-deoxy-mannose derivative from the protected glucoside was effected without protection of the 3-OH group.[14] With careful control of the reaction conditions, and immediate use of the unstable triflate intermediate,



N-Acetyl-D-Mannosamine *N*-Acetyl-D-Neuraminic acid

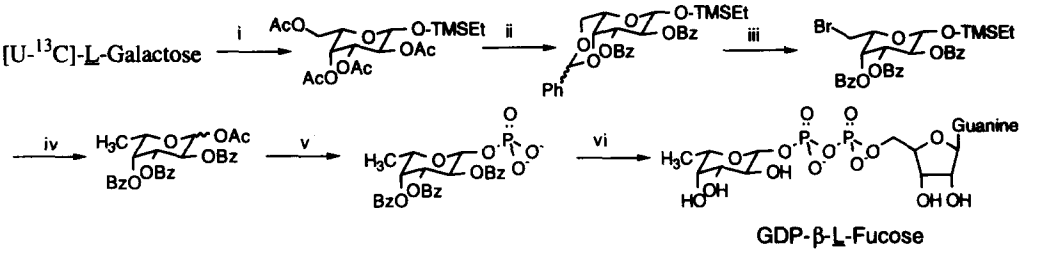
i. (a) MeOH, Dowex AG-50-X8(H^+), reflux; (b) PhCHO, $ZnCl_2$ (50%); ii. (a) Tf_2O, pyridine, CH_2Cl_2; (b) NaN_3, 15-crown-5, DMF, 35°C (71%); iii. (a) AcOH, H_2O, 80°C; (b) Ac_2O, pyridine (62%); iv. (a) Cl_2CHOCH_3, $ZnCl_2$; (b) BnOH, AgOTf, collidine, CH_2Cl_2 (70%); v. (a) NaOMe, MeOH; (b) H_2, $Pd(OH)_2$-C, Ac_2O, MeOH (98%); vi. [U-^{13}C]pyruvic acid , NeuNAc aldolase; vii. (a) MeOH, Dowex AG-50-X8(H^+), room temp.; (b) Ac_2O, pyr; (c) p-nitrophenol, CH_2Cl_2, H_2O, NaOH, $Bu_4N^+HSO_4^-$; (d) NaOMe, MeOH; (e) NaOH, H_2O (70% from ManNAc).

Scheme 1 : Synthesis of *N*-Acetyl-[U-^{13}C]-D-Neuraminic Acid p-Nitrophenyl Glycoside

competing 2,3-*manno*-epoxide formation could be avoided. Secondly, in our hands attempts to hydrolyse the methyl glycoside of ManNAc under acidic conditions gave rise to appreciable C-2 epimerisation.[15] For practical reasons we therefore chose to convert methyl α-$ManN_3$ to the α-benzyl glycoside, followed by reductive cleavage of both the glycoside and azide in one pot with *in situ N*-acetylation of the resulting aminosugar.

Synthesis of GDP-[U-^{13}C]-L-Fucose

The synthesis of GDP-β-L-fucose from [U-^{13}C]-L-galactose, largely using literature procedures,[16,17] is outlined in Scheme 2.

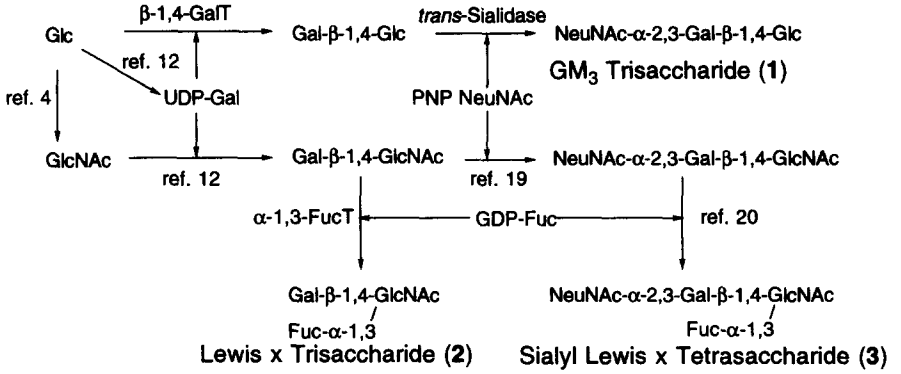


i. (a) Ac_2O, HBr, AcOH; (b) TMSEtOH, Ag_2CO_3, CH_2Cl_2, 4Å mol. sieves (53%); ii. (a) NaOMe, MeOH; (b) $PhCH(OMe)_2$, TsOH, CH_3CN; (c) BzCl, pyr (77%); iii. NBS, $BaCO_3$, CCl_4, reflux; iv. (a) H_2, Pd-C, Et_3N, MeOH; (b) Ac_2O, BF_3:OEt_2, toluene (91% over steps iii and iv); v. (a) HBr, AcOH; (b) $(BnO)_2PO.O^-Et_3NH^+$, Ag_2CO_3, CH_2Cl_2, 3Å mol. sieves; (c) H_2, Pd-C, toluene, pyridine, Et_3N; cyclohexylamine, MeOH (96%); (c) vi. GMP-morpholidate, pyridine (41%).

Scheme 2 : Synthesis of GDP-[U-^{13}C]-L-Fucose

Preparation of the GM_3 Trisaccharide (**1**), *Lewis x Trisaccharide* (**2**) *and Sialyl Lewis x Tetrasaccharide* (**3**)

With the building blocks described above (and those described previously[4]) in hand, saccharides (**1**), (**2**) and (**3**) can be prepared by enzymatic coupling methods, as outlined in Scheme 3.



Scheme 3 : Enzymatic Synthesis of Saccharides (1), (2) and (3)

The labelled GM_3 trisaccharide (**1**) was prepared by enzymatic sialylation of [U-^{13}C]-lactose, which was prepared enzymatically from [U-^{13}C]-glucose using methods described by Wong and co-workers.[12] In conjunction with recombinant *Trypanosoma cruzi trans*-sialidase,[18] *N*-acetylneuraminic acid PNP glycoside is an effective substrate for carrying out this α-2,3-sialylation reaction,[19] and typically gives rise to >80% yield for this step (when 2 x 0.75mol equivalents of PNP NeuNAc were employed). Isotopically enriched trisaccharide

(**1**) could therefore be obtained on a 10mg scale without complication. We have already reported the enzymatic synthesis of ^{13}C-enriched *N*-acetyllactosamine.[4] Fucosylation on this compound witth GDP-[U-^{13}C]-L-fucose catalysed by a milk fucosyltransferase preparation[22] proceeded in straightforward manner giving trisaccharide (**2**) in milligram quantities. Combining the enzymatic sialylation and fucosylation steps described above,[21] the sialyl Lewis x tetrasaccharide (**3**) was prepared on a milligram scale.

In conclusion, we have completed the chemoenzymatic synthesis of three biologically important oligosaccharides containing complete ^{13}C-enrichment in the sugar backbone.[23] Conformational analysis of these molecules in solution and bound to appropriate receptors will be reported in due course.

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23. ^{1}H and ^{13}C NMR data for compounds (**1**) - (**3**) were in accord with those of the corresponding unlabelled compounds. ES-MS data:-
    GM_3 (**1**) - Calcd. for $^{12}C_2{}^{13}C_{21}H_{39}O_{19}N$ = 654, found $[M+Na]^+$ = 676.7, $[M+K]^+$ = 692.7.
    Lewis x (**2**) - Calcd. for $^{12}C_2{}^{13}C_{18}H_{35}O_{15}N$ = 547, found $[M+Na]^+$ = 570.0, $[M+K]^+$ = 585.9.
    Sialyl Lewis x (**3**) - Calcd. for $^{12}C_4{}^{13}C_{27}H_{52}O_{23}N_2$ = 847,
    found $[M+Na]^+$ = 869.6 (+ ve ion mode), $[M-H]^-$ = 845.8 (- ve ion mode).

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