



Synthesis of Thio-Linked Ganglioside Analogues for Use as Immunogens



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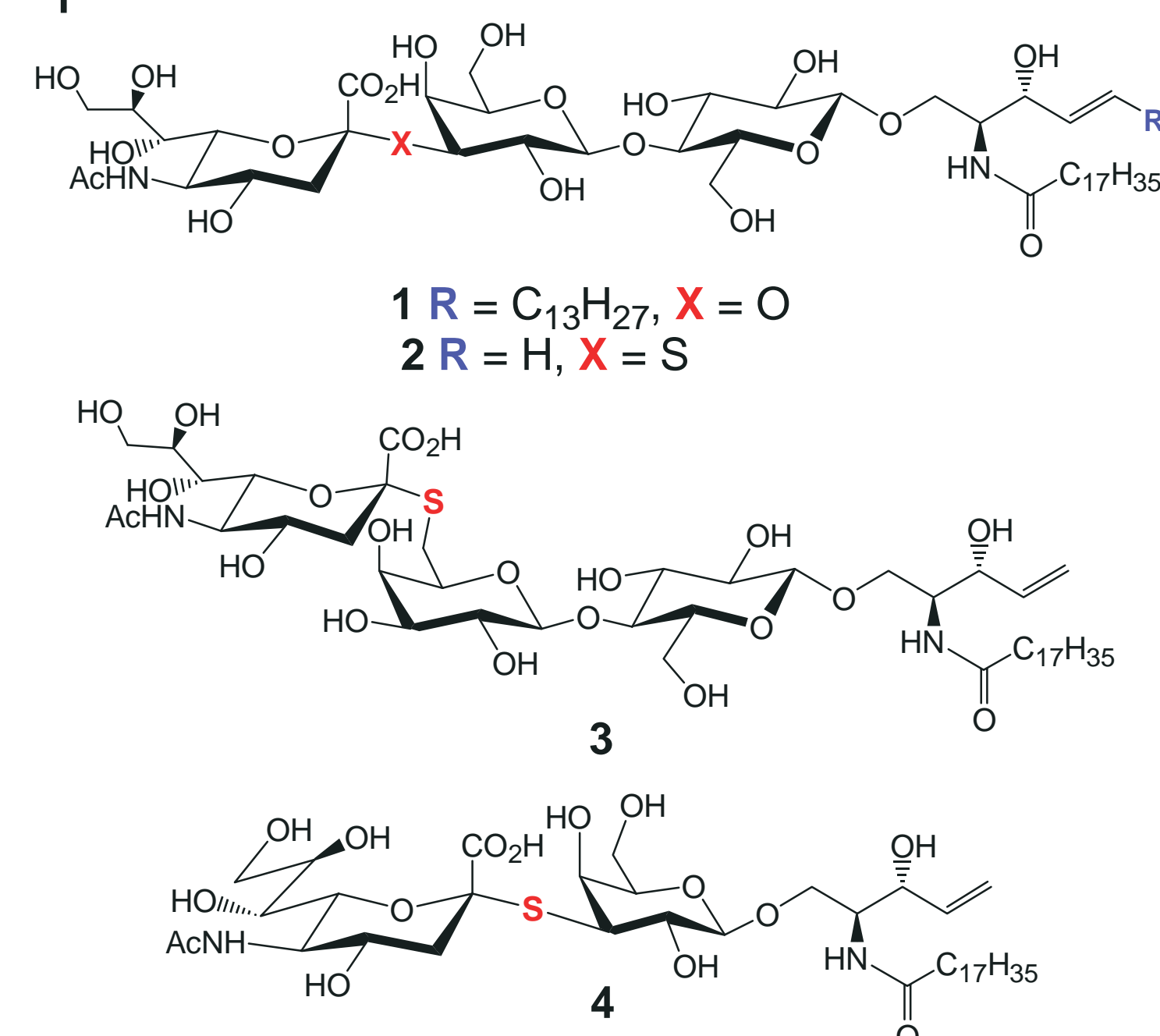
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INTRODUCTION

The over-expression of glycolipid and glycoprotein antigens on the surface of cancerous cells make these structures attractive targets for use in the active immunotherapy of tumors.¹ Gangliosides are associated with a variety of cancers, including melanomas, and have been employed in clinical trials as constituents of conjugate vaccines. Unfortunately, ganglioside vaccine preparations have proven to be poorly immunogenic. This may be due, in part, to the lability of terminal N-acetyl neuraminic acid (Neu5Ac) residues *in vivo*. We hypothesized that glycolipid analogues incorporating hydrolytically resistant Neu5Ac residues may prove effective in enhancing the immunogenicity of ganglioside containing conjugate vaccine preparations.

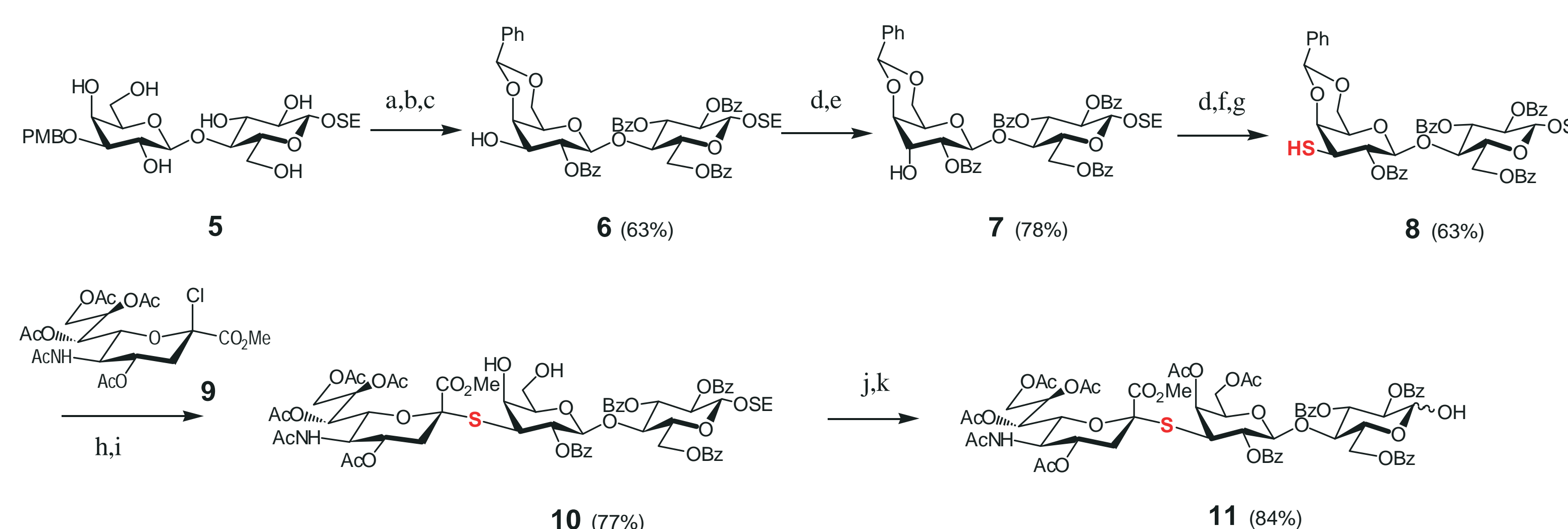
Glycolipid analogues in which the non-reducing terminal glycosidic oxygen is replaced by sulfur have been shown to resist enzymatic degradation by exoglycosidases. Although S-linked oligosaccharides are more flexible than their O-linked counterparts, the geometry about the glycosidic linkage remains similar. Earlier work on S-linked oligomers has shown that an LPS specific response can be induced by a sulfur containing glycoconjugate.² It is thus expected that the incorporation of thio-linked Neu5Ac containing ganglioside antigens into anti-cancer vaccines may promote a more robust immune response towards tumor associated glycolipid antigens than do vaccines containing natural ganglioside epitopes.

Herein we describe the synthesis of sulfur containing analogues (**2,3**) of the tumor associated glycolipid antigen GM₃ (**1**), and a thio-linked analogue (**4**) of the ganglioside GM₄. These structures contain a truncated ceramide aglycon, functionalized to allow for conjugation to a carrier protein. As an initial proof of concept, antibodies will be raised against **1-4** and tested for cross reactivity with the O-linked compounds.



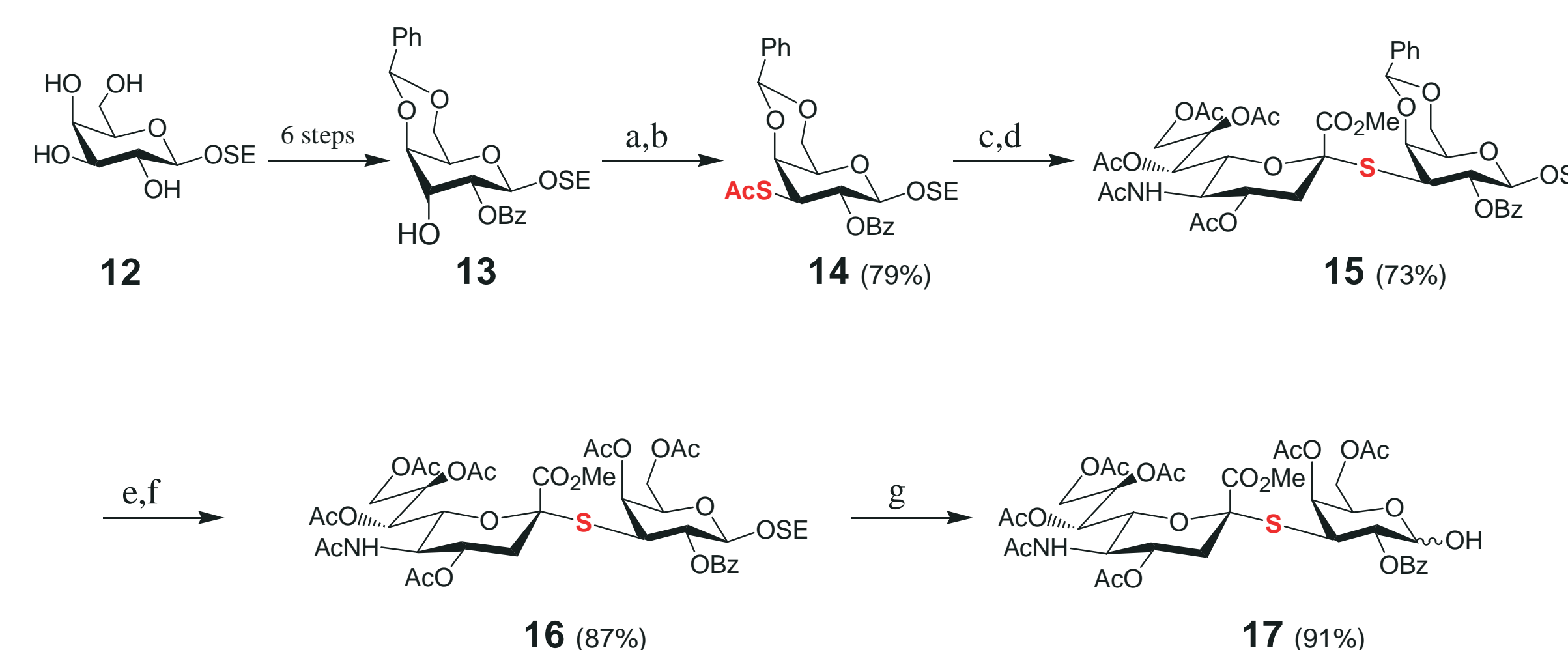
SYNTHESIS OF THIOOLIGOSACCHARIDES

A. Thiosialo-GM₃ Oligosaccharide



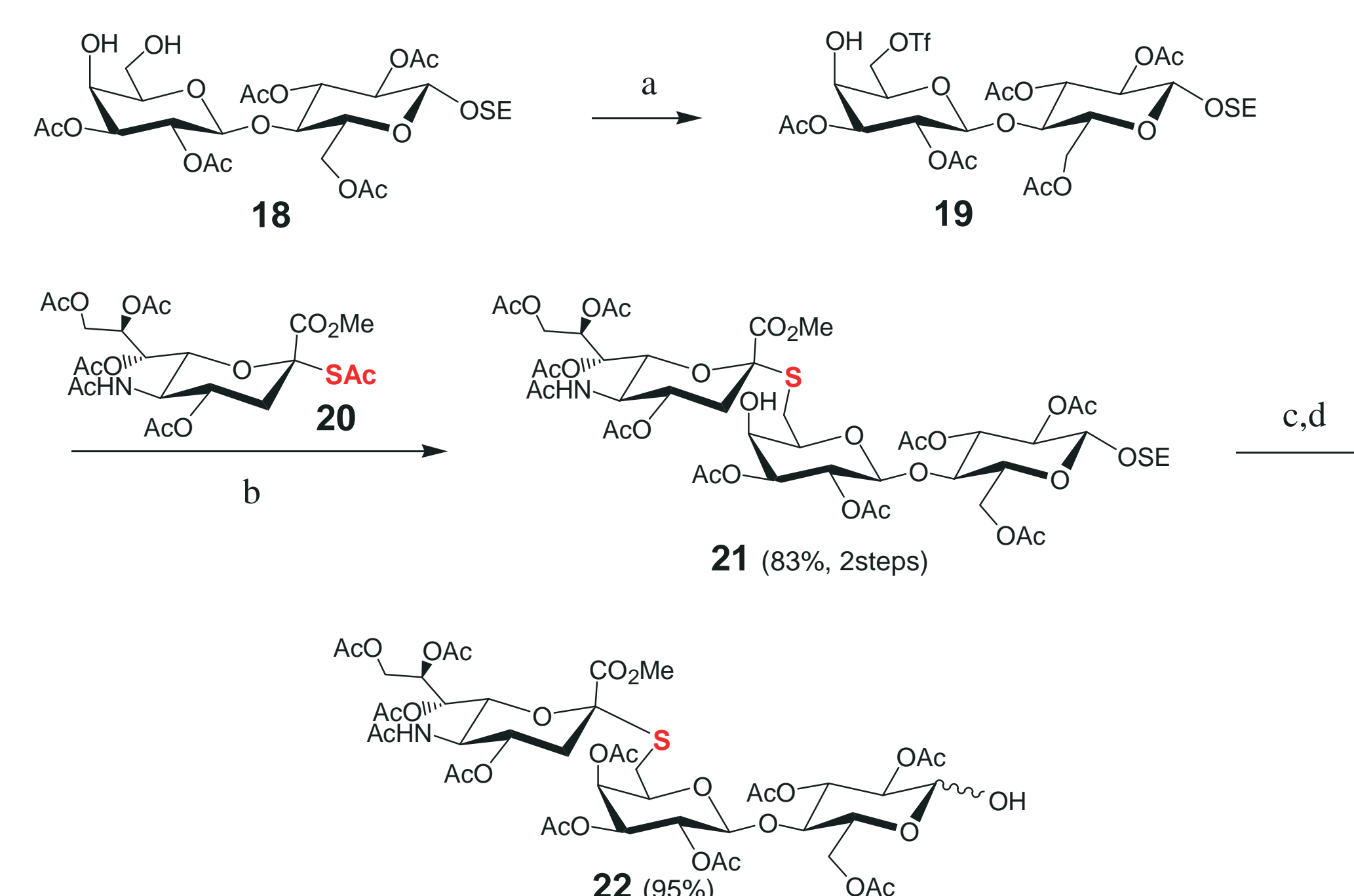
Reagents and Conditions: a) (MeO)₂CHPh, CSA, DMF b) BzCl, pyridine c) CAN, MeCN/H₂O d) Tf₂O, pyridine, -20°C e) Bu₄NNO₂, DMF f) KSac, DMF, -50°C g) hydrazinium acetate, DMF h) **9**, Kryptofix-21, DMF, 50°C i) 80% AcOH, 50°C j) Ac₂O, pyridine k) TFA, toluene

B. Thiosialo-GM₄ Oligosaccharide



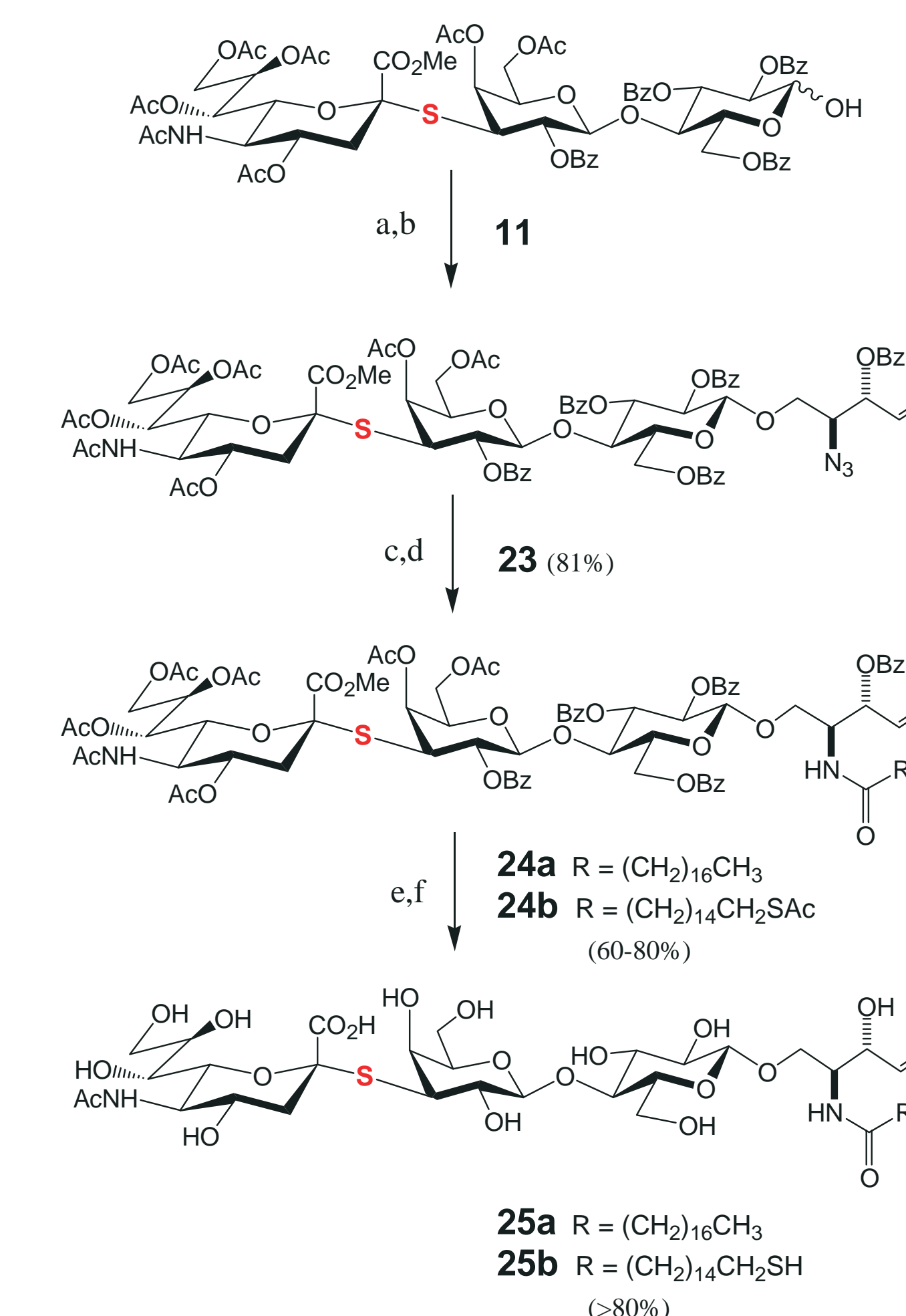
Reagents and Conditions: a) Tf₂O, pyridine, 0°C b) KSac, DMF, 60°C c) Hydrazine Acetate, DMF d) NaH, **9**, DMF e) 80% HOAc, 60°C f) Ac₂O, pyridine g) TFA, toluene

C. α(2-6) Thiosialyllactose



Reagents and Conditions: a) Tf₂O (1.1 eq.), pyridine, CH₂Cl₂, -20°C b) Me₂NH (10 eq.), **20** (1.2 eq.), DMF c) Ac₂O, pyridine d) TFA/toluene (2:1)

REPRESENTATIVE SYNTHESIS OF GLYCOLIPIDS



Reagents and Conditions: a) Cl₃CCN, DBU, CH₂Cl₂ b) (2S,3R)-2-Azido-3-O-benzoyl-4-penten-ol, BF₃Et₂O, DCM, Drierite c) PPh₃, pyridine/H₂O d) NHS-CO(CH₂)₁₆CH₃ or NHS-CO(CH₂)₁₄CH₂SAc, pyridine/H₂O e) NaOMe, MeOH f) NaOH, MeOH

- Trichloroacetimidate glycosyl donors are coupled with an azidosphingosine analogue
- Azide reduction followed by *N*-acylation with various esters yields ceramide analogues

SUMMARY

- a variety of sulfur containing ganglioside analogues have been prepared
- compounds such as GM₃ analogue **25b** are suitable for coupling to protein to generate conjugate vaccines
- Antibodies will be raised against thio glycolipid structures and tested for cross reactivity with O-linked antigens
- Glycosyl ceramide analogues bearing a terminal thiol are suitable for immobilization on a gold surface or for coupling to a fluorophore
- Protected sulfur containing GM₃ and GM₄ oligosaccharides **10** and **17** will serve as intermediates in our syntheses of analogues of higher gangliosides including GM₂, GM₁, and GD_{1a}

REFERENCES:

1. Allen, J.R., Danishefsky, S.J. *Angew. Chem. Int. Ed.* **2000**, *39*, 836.
2. Yu, H.N., Bundle, D.R. *unpublished results.*