Front. Chem. China 2009, 4(1): 10–31
DOI 10.1007/s11458-009-0016-9

REVIEW ARTICLE

Fanzuo KONG

Synthesis of plant arabinogalactans

Abstract Plant arabinogalactans consisting of a β-(1→6)-linked D-galactopyranosyl oligosaccharide backbone with α-(1→2)-L-arabinofuranosyl branches are synthesized based on the 1,2-anhydro galactopyranose technique, orthogonal (methoxydimethyl)methyl (MIP) and (2-naphthyl)methyl (NAP) protection strategy, and selective acylation or glycosylation method. The third method is the most simple and effective and it is also used for the synthesis of arabinogalactans composed of a β-(1→6)-linked D-galactopyranosyl oligosaccharide backbone with α-(1→3)-L-arabinofuranosyl branches.

Keywords arabinogalactan, oligosaccharide synthesis

1 Introduction

One of the main problems in studying the biological functions of oligosaccharides is their limited availability in pure form with enough quantity from natural sources. Therefore, efficient chemical synthesis would make complex carbohydrates more accessible to general chemists and biochemists who want to keep pace with the exploding area of glycobiology [1]. Great effort has been devoted to the development of new strategies for glycosidic coupling, and methods for construction of glycosidic linkages have progressed considerably [2–5]. This review will only focus on the synthesis of plant arabinogalactans.

Arabinogalactans are often classified into three groups: arabino-4-galactans (Type I), arabino-3,6-galactans (Type II), and polysaccharides with arabinogalactan side chains (Type III) [6]. The latter types are also called the real pectins. Some arabinogalactans from certain sources show biological activity. One of the arabinogalactans which shows an activity on the complement system is an arabinogalactan from a hot water extract of the roots of the Chinese herb *Angelica acutiloba* [7]; such activity is not found in the arabinogalactan from larch wood [8]. An arabinogalactan isolated from the roots of *Saposhnikova divaricata* or *Panex* notoginseng has reticuloendothelial system activating properties [9]. The arabinogalactans with a β-(1→6)-linked galactopyranose backbone and α-(1→2)-linked arabinofuranose side chains may exist in *Echinacea purpurea*, and have immunomodulating activity [10]. Thus, studies on identification of the arabinogalactan structures with bioactivity are very important in glycoscience. Also, it is known [11] that an expanding field in phytobiology is the use of monoclonal antibodies for the study of complex carbohydrates present at plant cell surfaces. Antibodies generated against specific plant cell wall carbohydrates may serve as probes in identifying cognate structural elements in outer membrane saccharides of other plant species. The usefulness of this concept relies upon characterization of the epitopes recognized by the antibodies. Albersheim *et al*. reported [12] that a β-(1→6)-linked galactan containing at least three galactopyranosyl residues functionalized at 3-OH with an α-linked L-arabinofuranose unit is supposed to be the epitope recognized by the CCRC-M7 antibody. The production or the specificity elucidation of the monoclonal antibodies requires well-defined oligosaccharides which are the epitopes of the antigens. Besides, although the presence of 2,6- and 3,6-branched residues in arabinogalactans is well known, the exact structure of these saccharides remains to be established. Thus far, there has been no definite conclusion regarding the core structure or fragment of arabinogalactans with immunomodulating activity. Is the arabinogalactan with a β-(1→6)-linked galactopyranose backbone and α-(1→2)-linked arabinofuranose side chains active, or the arabinogalactan with the same backbone but with α-(1→3)-linked arabinofuranose branches active, or the arabinogalactan with the same backbone but with mixed α-(1→2)- and α-(1→3)-linked arabinofuranose branches active? To solve the above problems, the synthesis of a variety of model arabinogalactan structures, and consequent study of the biological activity of the synthetic samples are necessary.

Received August 26, 2008

Fanzuo KONG (✉)
Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
E-mail: fzkong@rcees.ac.cn

2 Synthesis of arabinogalactans

There are some reports dealing with the synthesis of arabinogalactans which have been contributed mainly from three research groups led by van Boom [13], Lipták [14–17], and Kong [18–26]. They have developed quite different methods, i.e., the 1,2-anhydrogalactose-based method [13], the (methoxydimethyl)methyl (MIP) and (2-naphthyl)methyl (NAP) protection technique [14–17], and the selective acylation or glycosylation strategy [18–26].

2.1 Synthesis of arabinogalactans with 1,2-anhydrogalactose derivatives as the key intermediates

With 1,2-anhydrogalactose derivatives as the key intermediates, van Boom's group have successfully accomplished the synthesis of three tetrasaccharides which are composed of the same β-(1→6)-linked D-galactopyranosyl trisaccharide backbone and one α-linked L-arabinofuranosyl branch attached at O-2, O-2', and O-2" of the backbone, respectively. The synthetic routes for the tetramers with O-2 and O-2'-arabinofuranose branches are depicted in Scheme 1.

In the synthesized tetramers, the reducing end of the downstream galactose unit is functionalized with a dodecanoate spacer to enable conjugation to a solid support via a peptide linkage. In the synthesis, oxidative coupling of galactal is used for construction of the trisaccharide backbone. Thus, benzylation of 6-O-tert-butyldiphenylsilyl (TBDPS)-D-galactal **1** followed by 3,3-dimethyldioxirane (DMD)-mediated epoxidation gives the key intermediate, the fully protected 1,2-anhydrogalactose **2**. Condensation of **2** with methoxycarbonylundecanol gives the acceptor β-galactoside **3** with a 2-free hydroxyl group, and the consequent NIS/TfOH catalyzed condensation with the donor thioethyl 2,3,4-tri-O-benzoyl-α-L-arabinofuranoside **4** affords the α-(1→2)-linked disaccharide **5**. Desilylation of **5** (to **6**) followed by $ZnCl_2$-catalyzed coupling with **2** yields the trisaccharide **7**. Finally, desilylation of **7** and subsequent condensation with **2**, followed by routine deprotection affords the target tetramer **8** with an α-linked arabinofuranose branch at O-2. Similarly, benzoylation of **3** (to **9**) and consequent desilylation and condensation with **2** give the disaccharide acceptor **10**, coupling of which, with **4**, followed by desilylation, produces the trisaccharide acceptor **11**. Condensation of **11** with **2** followed by deprotection affords the other tetramer **12** with an α-linked arabinofuranose branch at O-2'. Meanwhile, the third tetramer **13** with an α-linked arabinofuranose branch at O-2" is also similarly synthesized.

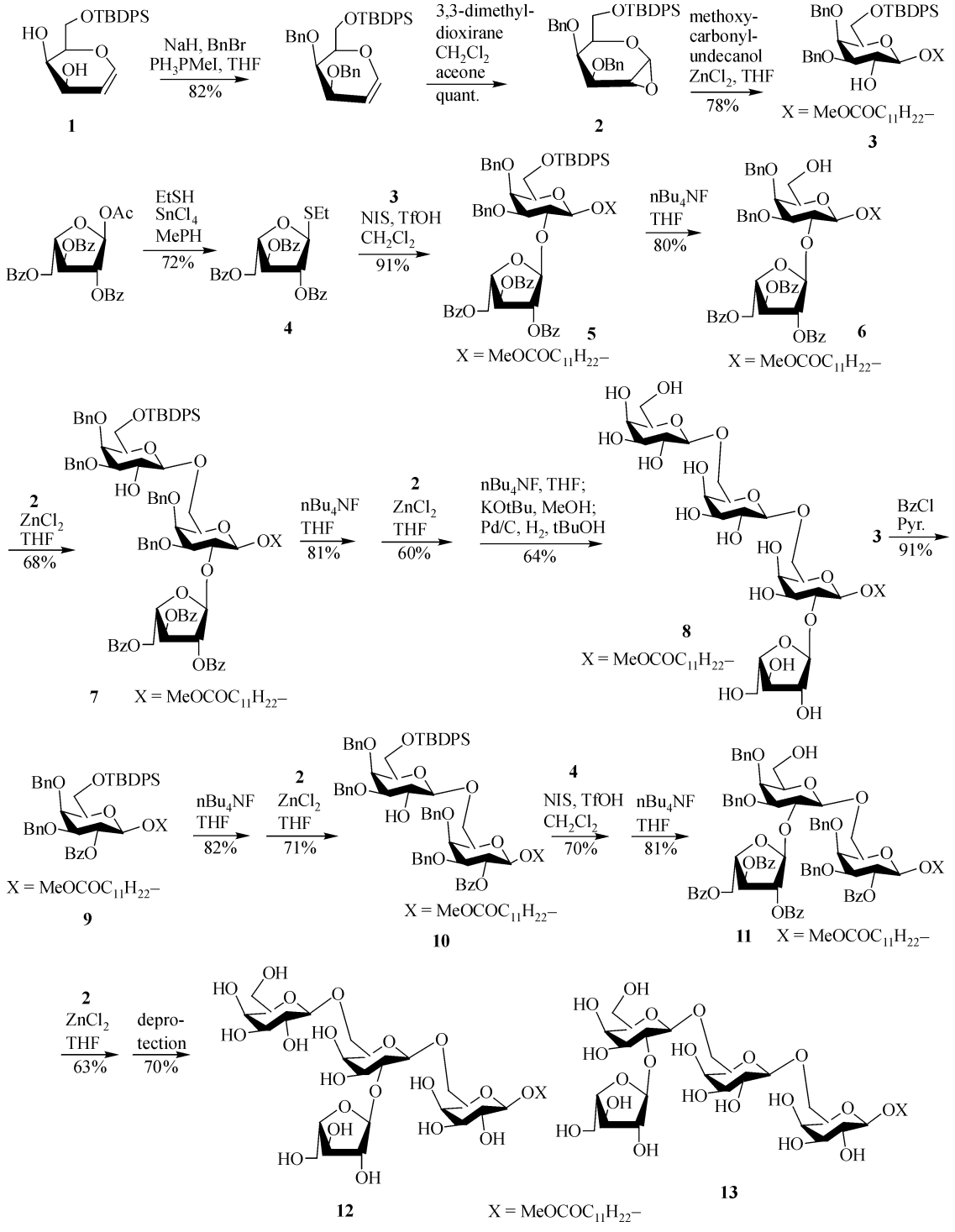
A propitious feature of this approach is the concomitant formation of an unprotected 2-hydroxyl function during the couplings with 1,2-anhydrogalactose **2** as the donor, and hence direct arabinofuranosylation is possible. Theoretically, any arabinogalactan composed of a β-(1→6)-linked galactopyranose oligosaccharide backbone and α-(1→2)-linked arabinofuranose branches can be synthesized by this method. However, the use of an unnatural starting galactal derivative prepared from galactose through several steps limits its application in synthesizing higher arabinogalactans.

2.2 Synthesis of arabinogalactans based on the (methoxydimethyl)methyl (MIP) and (2-naphthyl)methyl (NAP) protection technique

Lipták's group is the first to report [27] that the reaction of alkyl β-D-galactopyranosides with 2,2-dimeth-oxypropane in the presence of a catalytic amount of tolue-nesulfonic acid gives alkyl 3,4-O-isopropylidene-6-O-(methoxydimethyl)methyl(MIP)-β-D-galactopyranosides at high yields. Since the products contain a free 2-hydroxyl group and a very easily removed 6-O-MIP group, they are successfully applied in the synthesis of 2-O-arabinofuranosylated β-(1→6)-galacopyranosyl oligosaccharides.

2.2.1 Synthesis of simple arabinogalactans based on (methoxydimethyl)methyl (MIP) technique

Some 2'-O-, 6'-O- and 2',6'-di-O-(α-L-arabinofuranosyl)-β-D-galactopyranosyl-(1→6)-galactoses are synthesized using the MIP technique by Lipták's group, as shown in Scheme 2 [14]. In the synthesis, (1→6)-linked galactose disaccharide **14**, prepared by coupling of acetobromogalactose with galactose 1,2:3,4-diacetonide, is used as the starting material. Thus, the disaccharide **14** is deacetylated and then treated with excess 2,2-dimethoxypropane in the presence of toluenesulfonic acid to give disaccharides **15** and **16** at a ratio of 4:1. An attempt to obtain a 2'-O-linked trisaccharide is not successful as arabinosylation of **15** with perbenzoylated α-L-arabinofuranosyl bromide using $Hg(CN)_2$ as the promoter produces a mixture of 2',6'-di-O-linked tetrasaccharide **17** (25%), 6'-O-linked trisaccharide **18** (25%), and 2'-O-linked trisaccharide **19** (5%), indicating that the MIP group is very labile even at weakly acidic coupling conditions. To avoid the cleavage of the MIP group, a rather basic condensation with AgOTf as the promoter in the presence of 2,4,6-trimethylpyridine is executed. It is found, however, that the condensation proceeds to a moderate yield giving two products, **20** and **21**, at a ratio of 5:4. Compound **20** is the request trisaccharide and **21** is a trisaccharide dimer linked at 6'-O- through an isopropylidene acetal linkage, being able to transform to **19** *via* mild acidic hydrolysis. Compounds **17**, **18**, and **19** are easily deprotected by routine methods to give the corresponding 2',6'-branched tetrasaccharide,

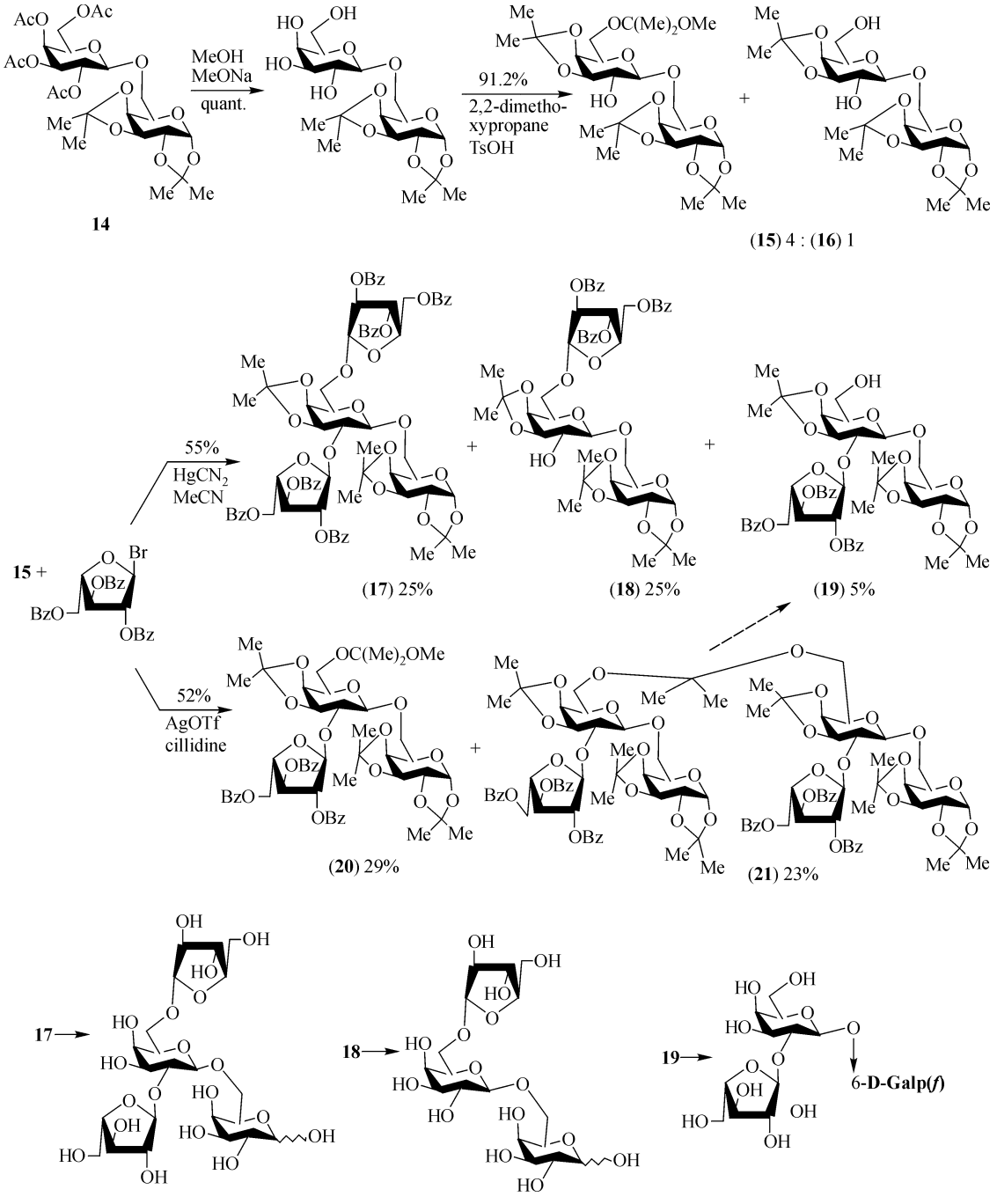


Scheme 1 Synthesis of arabinogalactans based on 1,2-anhydrogalactose derivatives

6'-branched trisaccharide, and 2'-branched trisaccharide, respectively. It seems that the MIP-containing acceptor **15** can be used in the synthesis of small arabinogalactans but its instability complicates the coupling reactions and decreases the coupling yields, and thus **15** is not a good glycosyl acceptor for the synthesis of higher arabinogalactans.

2.2.2 Synthesis of arabinogalactan hexasaccharide based on combination of MIP and benzyl protection

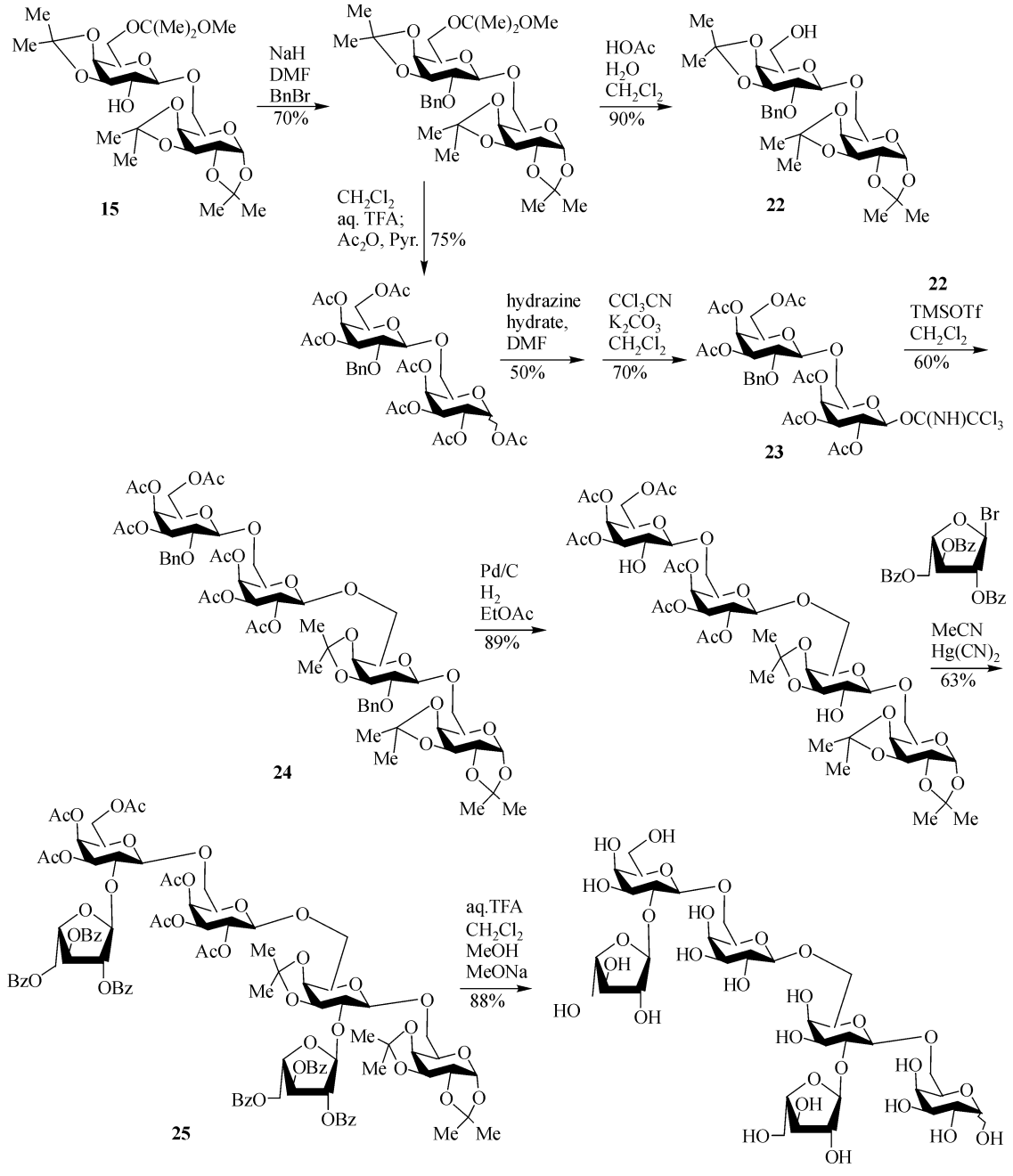
To overcome the instability of the MIP group during the coupling of **15** with the arabinose donor, Lipták *et al.* improved [15] the synthetic method to synthesize a



Scheme 2 Synthesis of simple arabinogalactans based on the MIP technique

hexasaccharide, α-L-Ara*f*-(1→2)-β-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→6)-[α-L-Ara*f*-(1→2)-]β-D-Gal*p*-(1→6)-D-Gal, the anticipated repeating unit of arabinogalactan from the cell culture of E. purpurea, as shown in Scheme 3.

In this synthesis the disaccharide **15** is not directly arabinosylated at O-2', instead, it is benzylated to block O-2' to obtain a fully protected disaccharide, and following with mild hydrolysis selectively removes the MIP group in the presence of isopropylidene groups to give the disaccharide acceptor **22** at a high yield. Meanwhile, hydrolysis of the fully protected disaccharide for removal of the acidic labile groups and then acetylation, selective 1-O-deacetylation and trichloroacetimidation afford the disaccharide donor **23**. Condensation of the donor **23** with the acceptor **22** is carried out smoothly, giving the tetrasaccharide **24** with benzylated O-2' and O-2'''. Consequently, under neutral conditions hydrogenolysis to remove benzyl groups is executed smoothly, giving a



Scheme 3 Synthesis of the arabinogalactan hexasaccharide

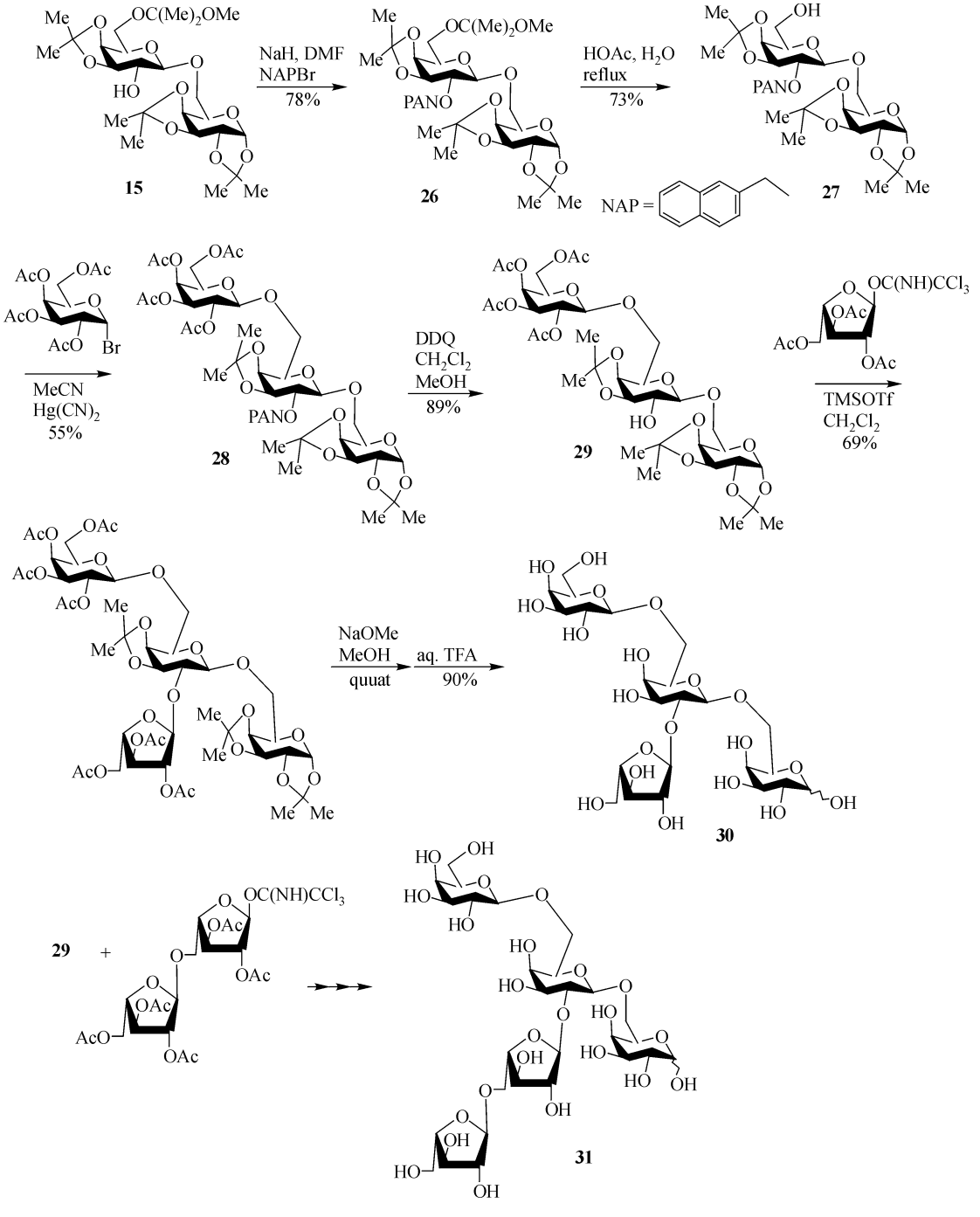
tetrasaccharide acceptor with O-2' and O-2''' free hydroxyl groups. Subsequent coupling with the benzoylated arabinofuranosyl bromide donor in the presence of $Hg(CN)_2$ as the promoter affords the hexasaccharide **25** at a fair yield, and routine deprotection gives the target hexasaccharide.

This method prevents the difficulties caused by the MIP group due to its hydrolysis or participation in condensation reactions, and successfully makes use of the difference between MIP and the isopropylidene group for preparation of the disaccharide acceptor, thus it is an effective method for preparation of 2-branched arabinogalactans.

2.2.3 Synthesis of arabinogalactans based on combination of MIP and NAP protection

Similarly, Lipták *et al*. also successfully use (2-naphthyl) methyl (NAP) as the protective group of O-2' in combination [16] with the use of MIP at O-6'. The NAP group, like the benzyl group, can be removed by catalytic hydrogenolysis, but NAP is more sensitive and can be selectively removed in the presence of the latter [28]. Besides, the NAP group is very similar to 4-methoxybenzyl, but NAP is more resistant to acids, being stable under the acidic

conditions used for removal of the isopropylidene groups. As shown in Scheme 4, the disaccharide **15** with a 2'-free hydroxyl group is readily (2-naphthyl)methylated with (2-naphthyl)methyl bromide and sodium hydride in DMF to give **26** at good yield. Then very mild hydrolysis of **26** cleaves the MIP group, giving the disaccharide acceptor **27** with a 6'-free hydroxyl group. Condensation of **27** with the acetylated galactosyl bromide in the presence of $Hg(CN)_2$ as the promoter affords the β-(1→6)-linked trisaccharide **28** in a moderate yield, and subsequent oxidative cleavage of the NAP group is achieved readily with DDQ in excellent yield, giving the trisaccharide acceptor **29** with a 2'-free hydroxyl group. Arabinosylation of **29** with 2,3,4-tri-O-acetyl-L-arabinofuranosyl trichloroacetimidate in the presence of TMSOTf as the catalyst and consequent deprotection give the target tetrasaccharide **30**. Meanwhile, condensation of **29** with fully acetylated α-L-(1→5)-linked disaccharide trichloroacetimidate followed



Scheme 4 Synthesis of arabinogalactans based on a combination of MIP and NAP protection

by deprotection affords the pentasaccharide **31** in high yields. Thus, the combination of MIP and the NAP protection method is effective for the synthesis of 2-branched arabinogalactans.

2.2.4 Synthesis of higher arabinogalactans based on a combination of MIP, NAP, and Benzyl protection

Lipták's group further report [17] on the synthesis of higher arabinogalactans by the use of orthogonal MIP, NAP, and benzyl groups. In the synthesis, as shown in Scheme 5, the trisaccharide **28** is deacetonated, acetylated, 1-O-deacetylated, and trichloroacetimidated to give the trisaccharide donor **32** with NAP at O-2'. Meanwhile, the disaccharide acceptor **22** is galactosylated with acetobromogalactose in the presence of $Hg(CN)_2$ as the promoter to afford the trisaccharide **33**. Zemplén deacetylation of **33** followed by reaction with 2,2-dimethoxypropane in the presence of catalytic toluenesulfonic acid produces the trisaccharide **34** with MIP at O-6" and a free hydroxyl function at O-2". Acetylation of **34** and consequent removal of the 6"-MIP afford the trisaccharide acceptor **35** with benzyl at O-2'. TMSOTf-catalyzed condensation of the acceptor **35** with the donor **32** yields the β-(1→6)-linked galactopyranose hexasaccharide framework **36** with NAP and benzyl at O-2"" and O-2', respectively. Since the cleavage conditions for NAP and benzyl can be orthogonal, assembly of the arabinofuranose branch can be carried out separately, thus making either the same or different branches at O-2' and O-2"". For example, oxidative removal of the NAP of **36** with DDQ produces the hexasaccharide acceptor **37** with a O-2"" free hydroxyl group, and its condensation with 2,3.4-tri-O-acetyl-α-L-arabinofuranosyl trichloroacetimidate gives the heptasaccharide **38**. Cleavage of the benzyl group of O-2' of **38** is smoothly carried out by catalytic hydrogenolysis, giving the heptasaccharide acceptor **39**. Then, coupling of **39** with the fully acetylated α-L-(1→5)-linked disaccharide trichloroacetimidate affords the nonasaccharide **40**, and consequent deprotection gives the target nonasaccharide **41**. Similarly, the octasaccharide **42** with α-L-arabinofuranose branches at O-2' and O-2"", and the nonasaccharide **43** with α-L-arabinofuranose and α-L-(1→5)-linked arabinofuranose disaccharide branches at O-2' and O-2"", respectively, are successfully synthesized by the same method.

Thus, the combined use of orthogonal groups such as MIP, NAP, and benzyl is an effective technique for the synthesis of complex 2-branched arabinogalactans.

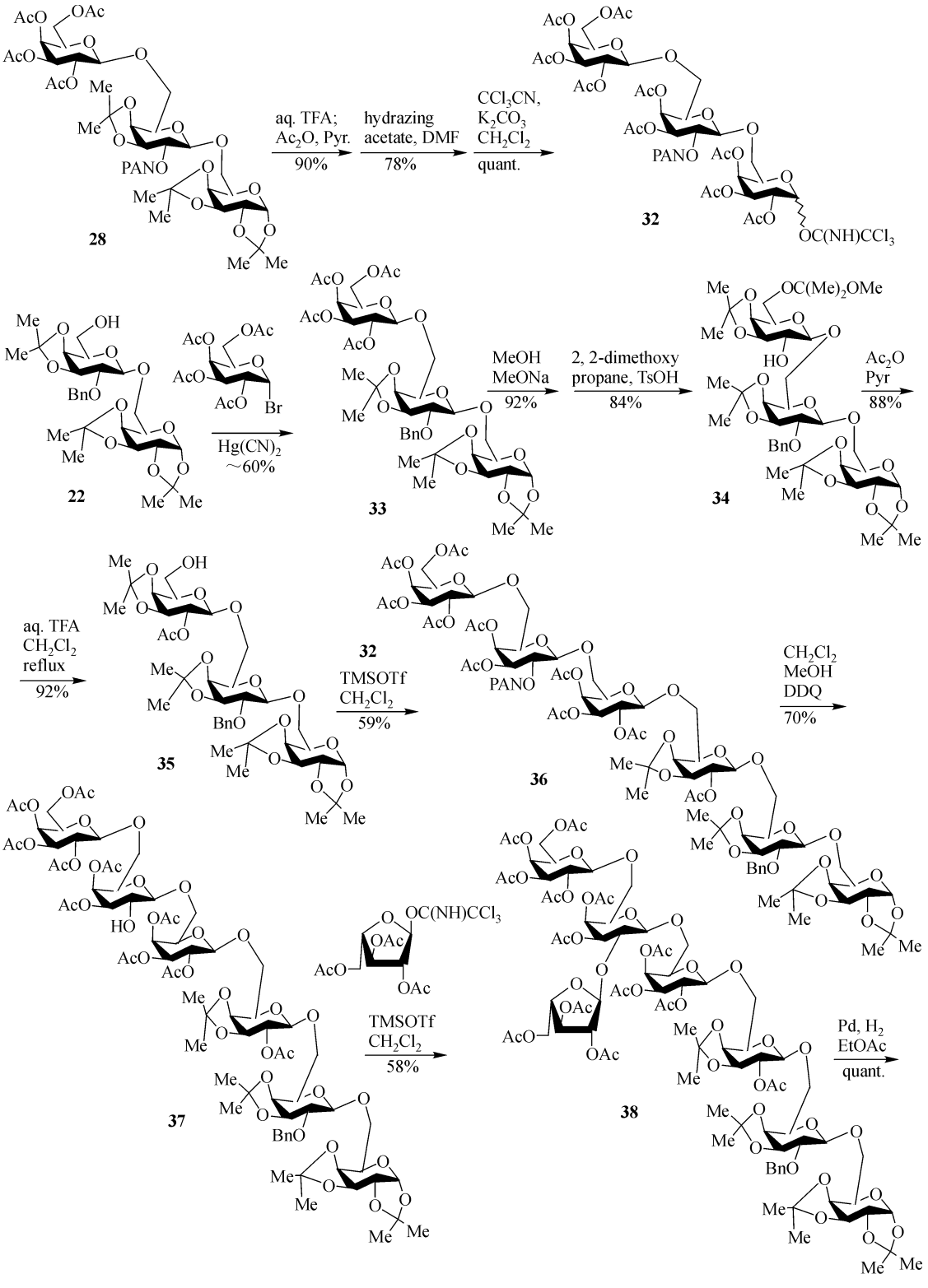
2.3 Synthesis of arabinogalactans based on selective deacetylation or galactosylation strategy

Kong's group reports [29–31] that formation of sugar-sugar orthoesters consisting of a fully acylated mono- or disaccharide donor and a partially protected mono- or disaccharide acceptor is regioselective, and Lewis acid-catalyzed rearrangement of the orthoesters *via* RO—(orthoester)C bond cleavage gives a dioxolenium ion intermediate leading to 1,2-*trans*-glycosidic linkage. They also reveal [32] that the coupling reactions with acylated glycosyl trichloroacetimidates as the donors usually give orthoesters as the intermediates especially when the coupling is carried out at slowed rates, and this is successfully used in regio- and stereoselective syntheses of oligosaccharides. With a partially protected mono- or oligosaccharide as the acceptor, and 2-branched arabinogalactans are also readily prepared. Further, most of the glycosyl acceptors used in the synthesis are prepared by methanolysis to selectively remove the acetyl groups in the presence of benzoyl groups with methanolic hydrogen chloride obtained by mixing 0.2%–2% AcCl and MeOH-CH_2Cl_2 (a modification of a procedure proposed by Kochetkov *et al*.) [33].

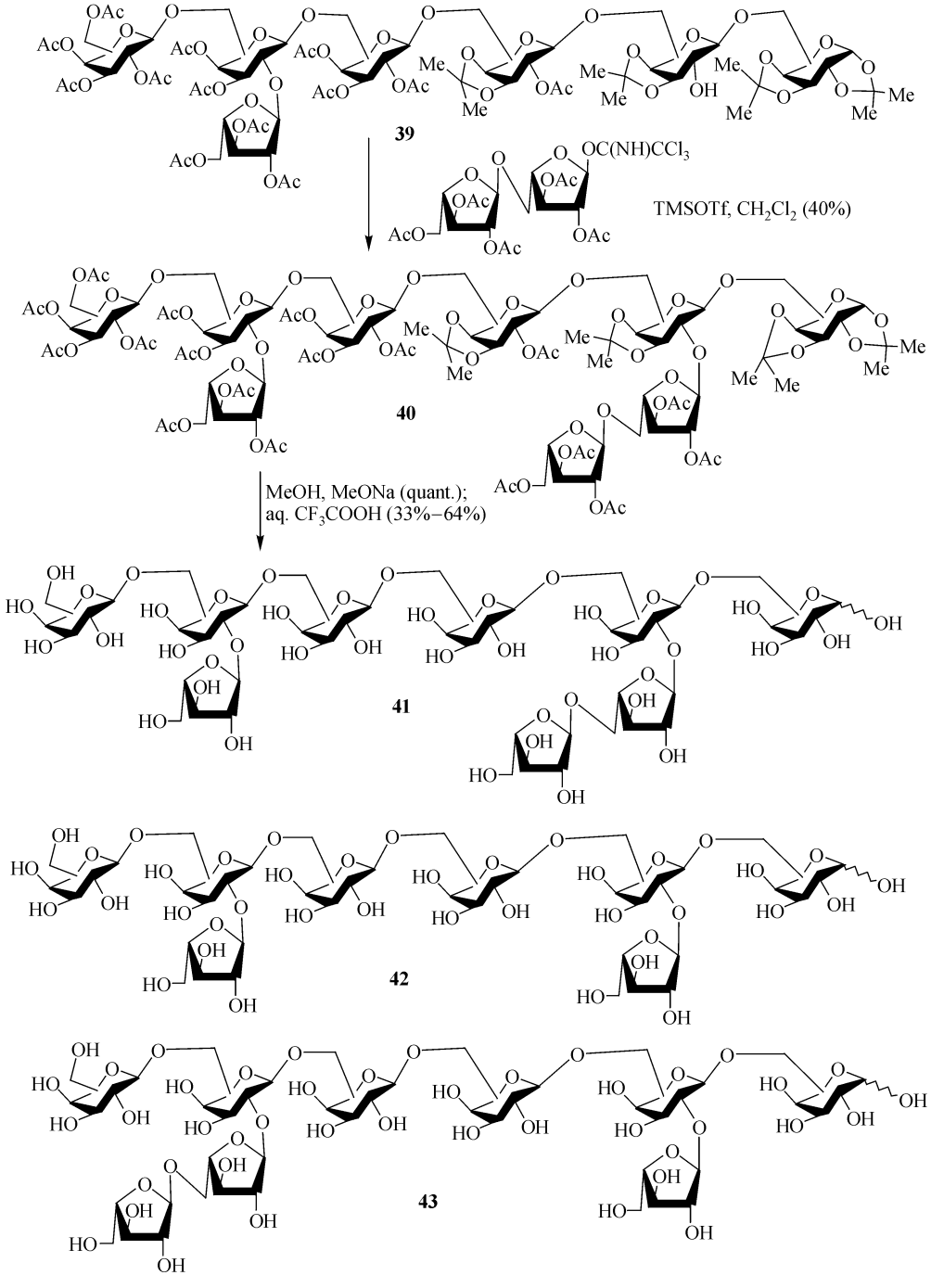
2.3.1 Synthesis of 2- and 3-branched arabinogalactans based on selective glycosylation

The synthesis [18] of O-2 branched arabinogalactan tetrasaccharide by selective glycosylation is shown in Scheme 6. In the synthesis, the disaccharide **14** is hydrolyzed to remove isopropylidene groups, and consequent acetylation, selective 1-O-deacetylation, and trichloroacetimidate formation give the fully acetylated disaccharide trichloroacetimidate donor **44**. Meanwhile, deacetylation of dodecyl tetra-O-acetyl-β-D-galactopyranoside **45** followed by isopropylidenation yields the monosaccharide acceptor **46** with 2,6-free hydroxyl groups. The coupling of the disaccharide donor **44** with the monosaccharide acceptor **46** is a regioselective reaction, giving the β(1→6)-linked trisaccharide **47** with a 2-free hydroxyl group at an acceptable yield. Then, direct condensation of **47** with benzoylated α-L-arabinofuranosyl trichloroacetimidate **48** [34,35] produces the tetrasaccharide **49**, and consequent deprotection affords the target O-2 branched tetrasaccharide glycoside **50**.

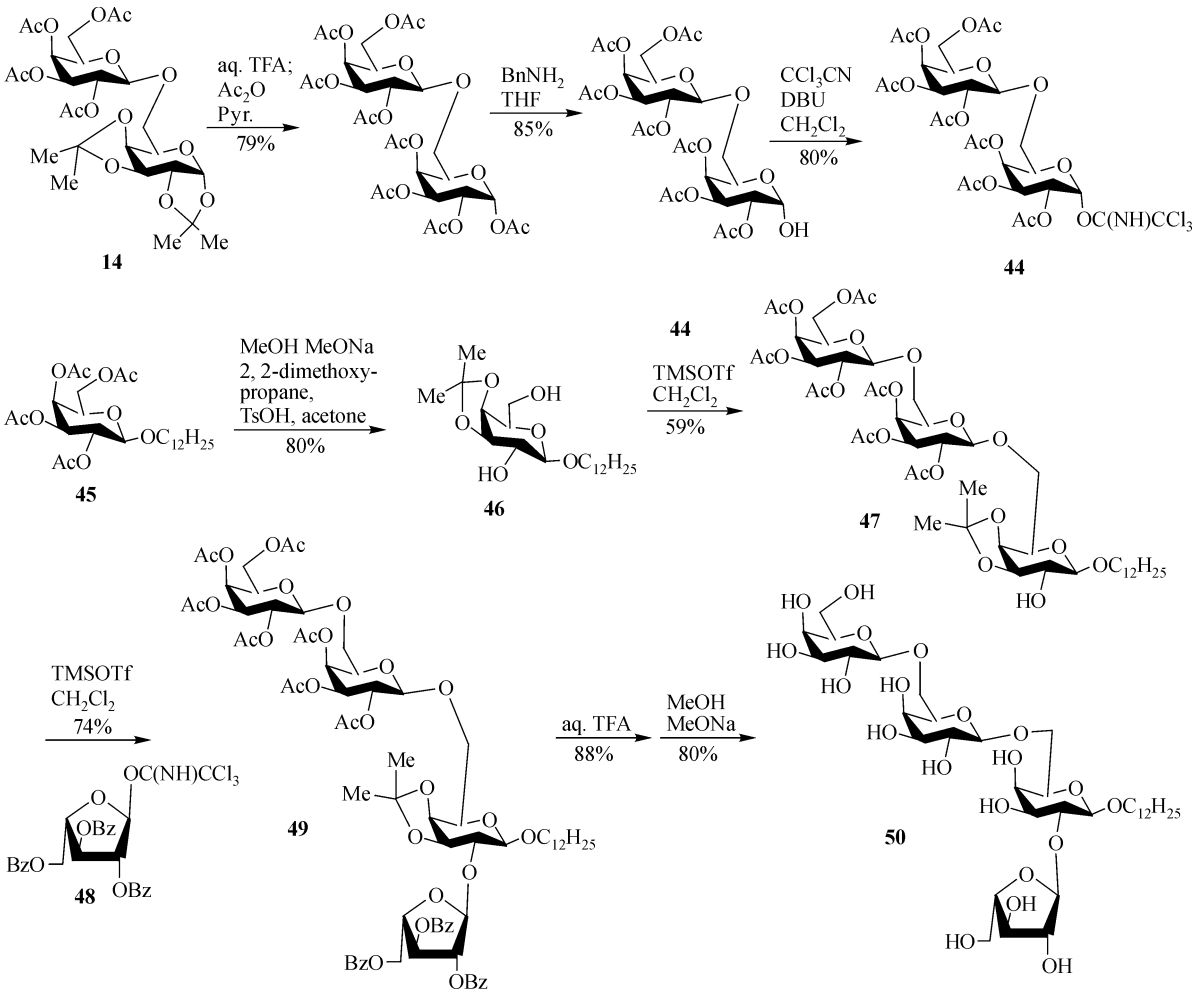
Their study [19] also reveals that with 6-O-trityl-1,2-O-isopropylidene-α-D-galactopyranose **51**, obtained readily from 1,2-O-isopropylidene-α-D-galactopyranose by tritylation, as the starting material, a 3-branched arabinogalactan tetrasaccharide is easily prepared, as shown in Scheme 7. Thus, selective coupling of the galactose acceptor **51** with the arabinose donor **48** gives the α-(1→3)-linked disaccharide **52**. Removal of the trityl group with ferric trichloride hexahydrate [36] affords the disaccharide acceptor **53** with O-4 and O-6 free hydroxyl groups. Again, selective glycosylation of **53** with the disaccharide donor **44** is carried out smoothly, producing the tetrasaccharide **54**, and subsequent deprotection gives the target O-3 branched tetramer.


aq. TFA; Ac2O, Pyr. 90%
hydrazing acetate, DMF 78%
CCl3CN, K2CO3 CH2Cl2 quant.
28
32
22
Hg(CN)2 ~60%
MeOH MeONa 92%
2, 2-dimethoxy propane, TsOH 84%
Ac2O Pyr 88%
33
34
aq. TFA CH2Cl2 reflux 92%
35
TMSOTf CH2Cl2 59%
CH2Cl2 MeOH DDQ 70%
36
TMSOTf CH2Cl2 58%
37
38
Pd, H2 EtOAc quant.

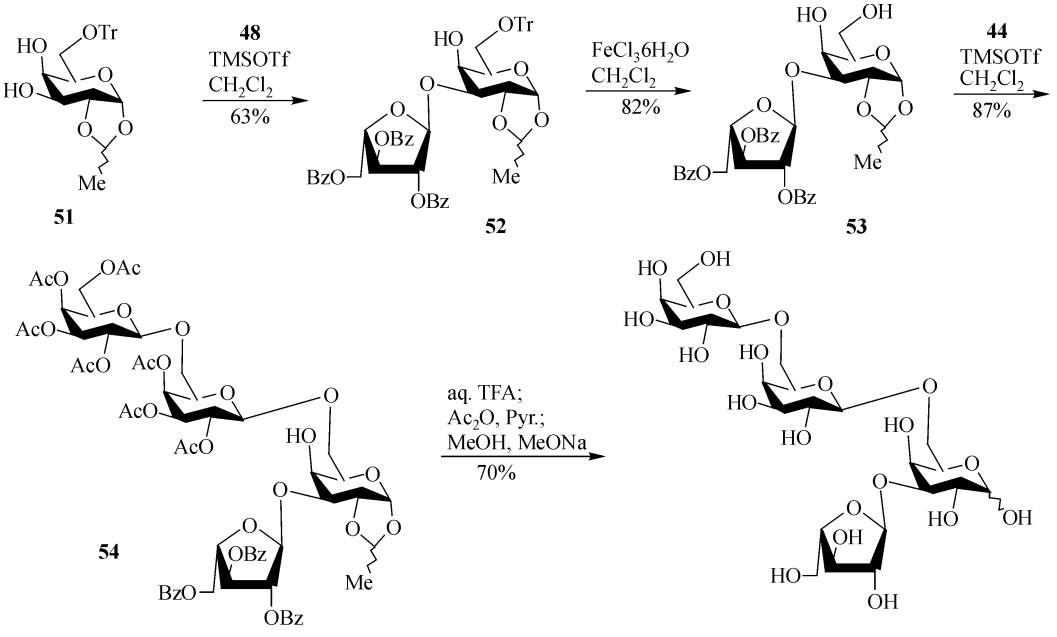
(*continued*)



Scheme 5 Synthesis of higher arabinogalactans



Scheme 6 Synthesis of O-2 branched arabinogalactan tetrasaccharide by selective galactosylation



Scheme 7 Synthesis of O-3 branched arabinogalactan tetrasaccharide from 1,2-O-isopropylidenated galactose

2.3.2 Synthesis of higher 2-branched arabinogalactans by combination of selective glycosylation and deacetylation

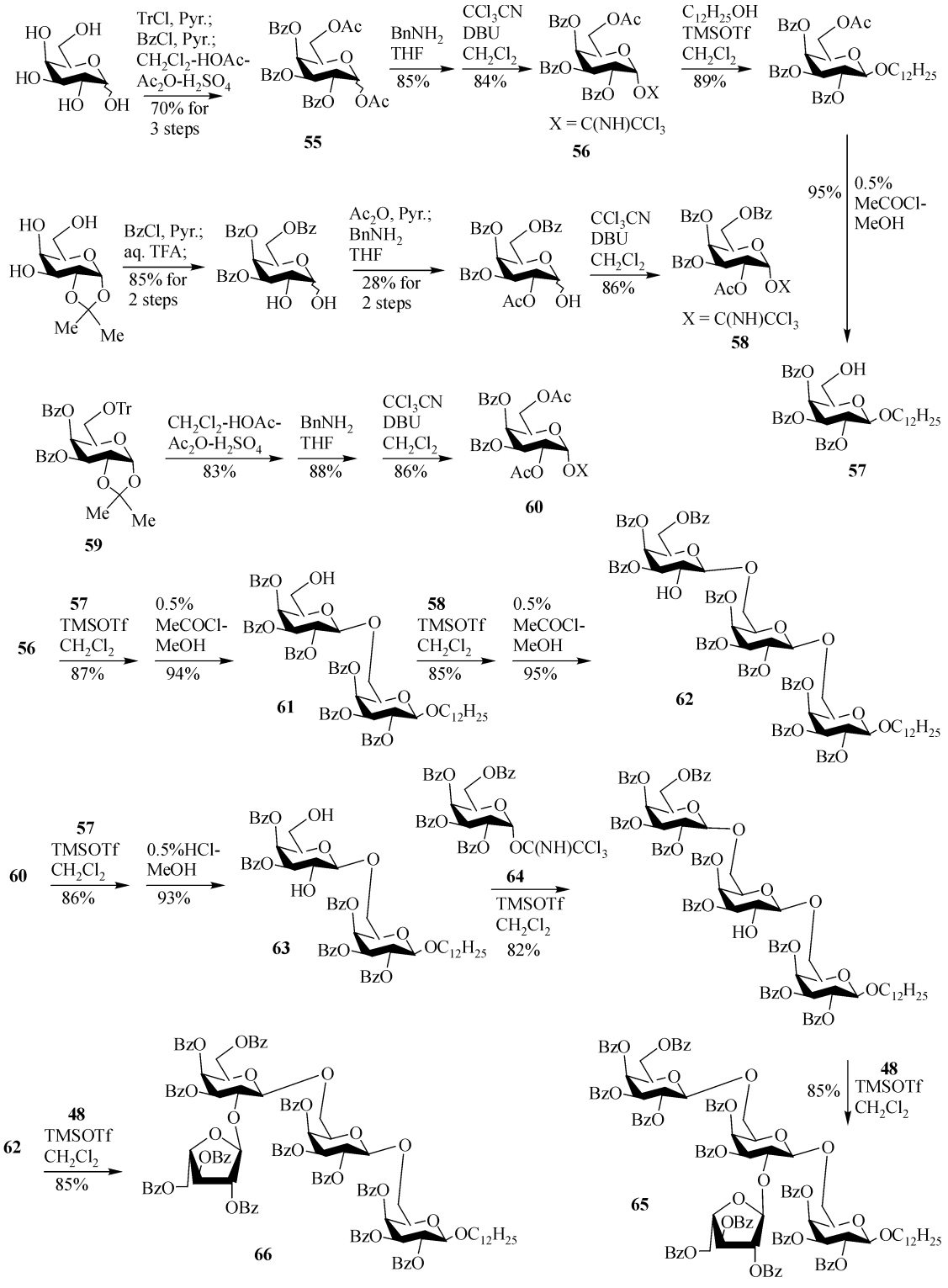
Higher arabinogalactan oligosaccharides are also synthesized by a combination of selective glycosylation and deacetylation, as shown in Scheme 8. In the synthesis [20], several key building blocks, i.e. the glycosyl donors **56**, **58**, and **60**, and the glycosyl acceptors **57** and **67,** are prepared first. The three glycosyl donors have the same leaving group but with acetyl groups at O-6, O-2, and O-2,6, respectively, and the two glycosyl acceptors have free hydroxyl groups at O-6 and O-2,6 respectively.

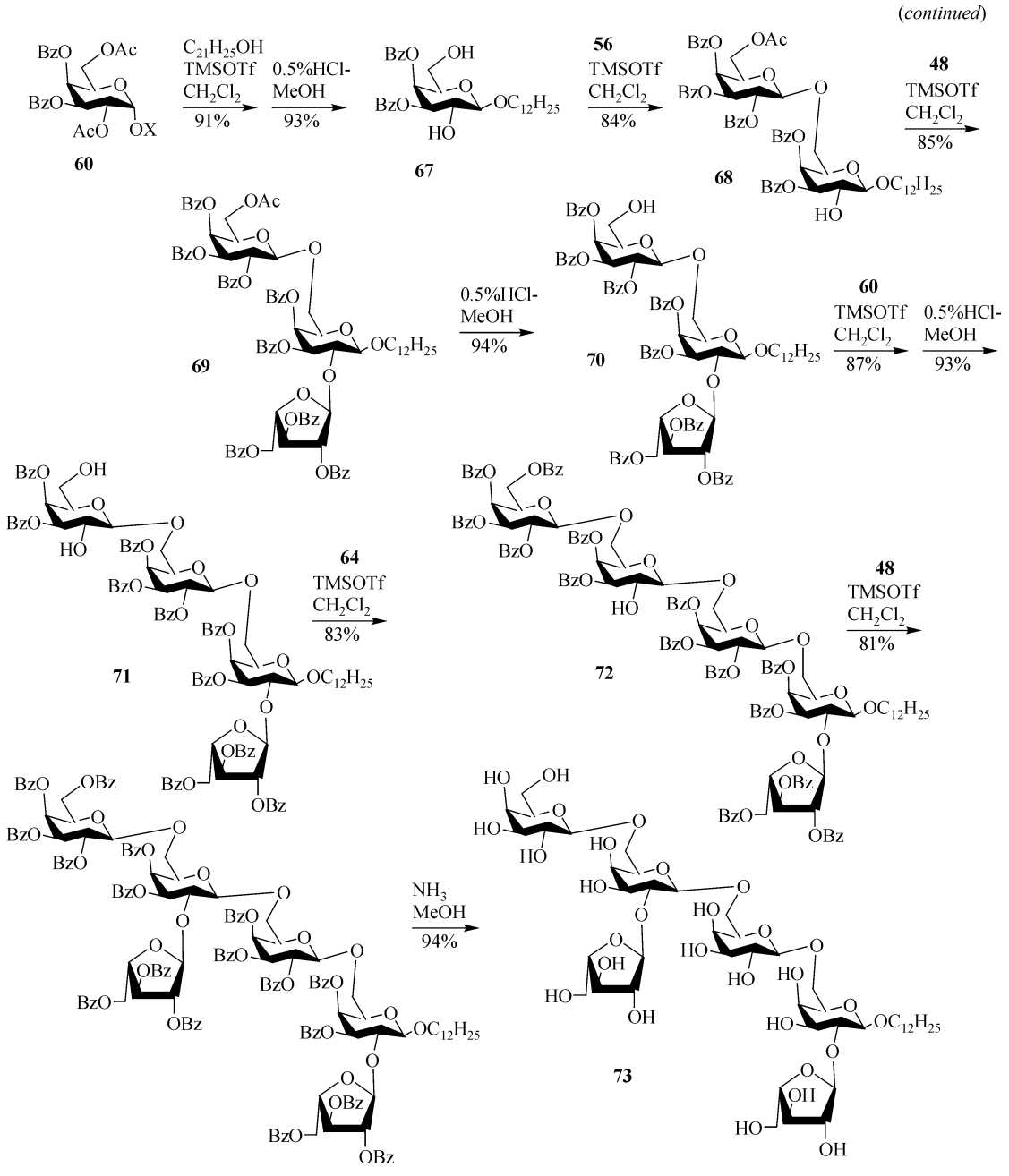
The donor **56** is prepared from 3,4,6-tri-O-benzoyl-1,2-O-acetyl-D-galctopyranose **55** by O-1 selective deacetylation, and trichloroacetimidate formation, while **55** is obtained from galactose in a one-pot manner through 6-O-tritylation, benzoylation, and acetolysis. The donor **58** is readily produced from 1,2-O-isopropylidene-α-D-galactopyranose *via* benzoylation, hydrolysis, O-1,2 acetylation, O-1 selective deacetylation and trichloroacetimidate formation, and the donor **60** is obtained from 3,4-di-O-bezoyl-1,2-O-isopropylidene-6-O-trityl-α-D-galactopyranose **59** by acetolysis, O-1 selective deacetylation and trichloroacetimidate formation. The glycosyl acceptor **57** or **67** is easily obtained from coupling of the donor **56** or **60** with dodecyl alcohol followed by O-6 or O-2,6 selective deacetylation with 0.5% MeCOCl/MeOH. With these glycosyl donors and acceptors at hand, the 2-branched arabinogalactans are readily prepared. For example, coupling of the donor **56** with the acceptor **57** followed by O-6 selective deacetylation gives the disaccharide acceptor **61**, and subsequent condensation of **61** with the donor **58** followed by O-2 selective deacetylation affords the trisaccharide acceptor **62** with an O-2" free hydroxyl group. Similarly, coupling of the donor **60** with the acceptor **57** followed by O-2,6 deacetylation yields the disaccharide acceptor **63**, while selective glycosylation of **63** with tetra-O-benzoyl-α-D-galactopyranosyl trichloroacetimidate **64** gives the (1→6)-linked trisaccharide acceptor, and consequent reaction with the arabinofuranose donor **48** affords the 2'-branched tetrasaccharide **65**. Also, direct condensation of the trisaccharide acceptor **62** with **48** gives the 2"-branched **66**. Meanwhile, selective glycosylation of the acceptor **67** with the donor **56** affords the (1→6)-linked disaccharide acceptor **68** with an O-2 free hydroxyl group. Condensation of the acceptor **68** with the arabinofuranose donor **48** gives the 2-branched trisaccharide **69**, and the following O-6' deacetylation affords the trisaccharide acceptor **70**. Then, coupling of **70** with the donor **60** followed by O-2",6" deacetylation yields the tetrasaccharide acceptor **71**. Again, selective glycosylation of **71** with **64** produces the (1→6)-linked pentasaccharide acceptor **72** with an O-2" free hydroxyl group. Direct condensation of **72** with the arabinofuranose donor **48** followed by deprotection affords the 2,2"-branched arabinogalactan hexasaccharide glycoside **73**. It is seen from the description above that selective glycosylation in combination with selective deacetylation is certainly an efficient method for the synthesis of 2-branched arbinogalactan oligosaccharides.

2.3.3 Synthesis of 2-branched arabinogalactans by sequential selective O-6 and O-2 deacetylation

Further study [21,22] reveals that not only can the benzoate and acetate groups be differentiated under the acidic condition of $MeCOCl/CH_3OH/CH_2Cl_2$ (1∶50∶50), but the O-6 acetyl and the O-2 acetyl groups can also be differentiated under the much milder condition of $MeCOCl/CH_3OH/CH_2Cl_2$ (1∶500∶500), where the former is removed under this condition without affecting the latter and the benzoate groups. This finding greatly simplifies the synthesis of 2-branched arabinogalactans since the use of orthogonal protective groups is avoided.

As shown in Scheme 9, TMSOTf-promoted condensation of **60** with 4-methoxyphenol followed by selective O-6 selective deacetylation gives the glycosyl acceptor **74**. Then coupling of **74** with **64** gives the β-(1→6)-linked disaccharide glycoside, and the following oxidative cleavage of the O-1 MP group and trichloroacetimidate formation afford the disaccharide donor **75**. Reaction of **75** with 4-methoxyphenol 3,4,6-tri-O-benzoyl-β-D-galactopyranoside **76**, prepared from **56** through its condensation with 4-methoxyphenol followed by selective O-6 selective deacetylation, affords a trisaccharide, and consequent O-2 selective deacetylation with $MeCOCl/MeOH/CH_2Cl_2$ (1∶50∶50) gives the trisaccharide acceptor **77** with an O-2' free hydroxyl group. Condensation of **77** with the α-(1→5)-linked arabinofuranose disaccharide trichloroacetimidate **78** [34,35] followed by routine protection yields the free pentasaccharide glycoside **79**. Meanwhile, coupling of the glycosyl acceptor **74** with the donor **56** gives the disaccharide **80** and the following O-6' selective deacetylation affords the disaccharide acceptor **81** with an O-6' free hydroxyl group. Then reaction of **81** with the disaccharide donor **75** yields the β-(1→6)-linked tetrasaccharide **82**, and consequent selective O-2,2" deacetylation, condensation with the arabinofuranose disaccharide donor **78**, and deprotection produce the target 2,2"-branched arabinogalactan octasaccharide **83**. More complex arabinogalactans can also be prepared by this technique. For example, coupling of the trisaccharide acceptor **77** with the arabinofuranose donor **48** gives the tetrasaccharide **84**, and the following oxidative cleavage of O-1 MP and trichloroacetimidate formation afford the tetrasaccharide donor **85**. Meanwhile, coupling of the disaccharide acceptor **86**, prepared from coupling **60** with **76** followed by O-6'


TrCl, Pyr.; BzCl, Pyr.; CH_2Cl_2-HOAc-Ac_2O-H_2SO_4 70% for 3 steps
55
$BnNH_2$ THF 85%
CCl_3CN DBU CH_2Cl_2 84%
X = $C(NH)CCl_3$
56
$C_{12}H_{25}OH$ TMSOTf CH_2Cl_2 89%
BzCl, Pyr.; aq. TFA; 85% for 2 steps
Ac_2O, Pyr.; $BnNH_2$ THF 28% for 2 steps
58
95% 0.5% MeCOCl-MeOH
57
59
CH_2Cl_2-HOAc-Ac_2O-H_2SO_4 83%
$BnNH_2$ THF 88%
60
57 TMSOTf CH_2Cl_2 87%
0.5% MeCOCl-MeOH 94%
61
58 TMSOTf CH_2Cl_2 85%
62
57 TMSOTf CH_2Cl_2 86%
0.5%HCl-MeOH 93%
63
64 TMSOTf CH_2Cl_2 82%
48 TMSOTf CH_2Cl_2 85%
66
65



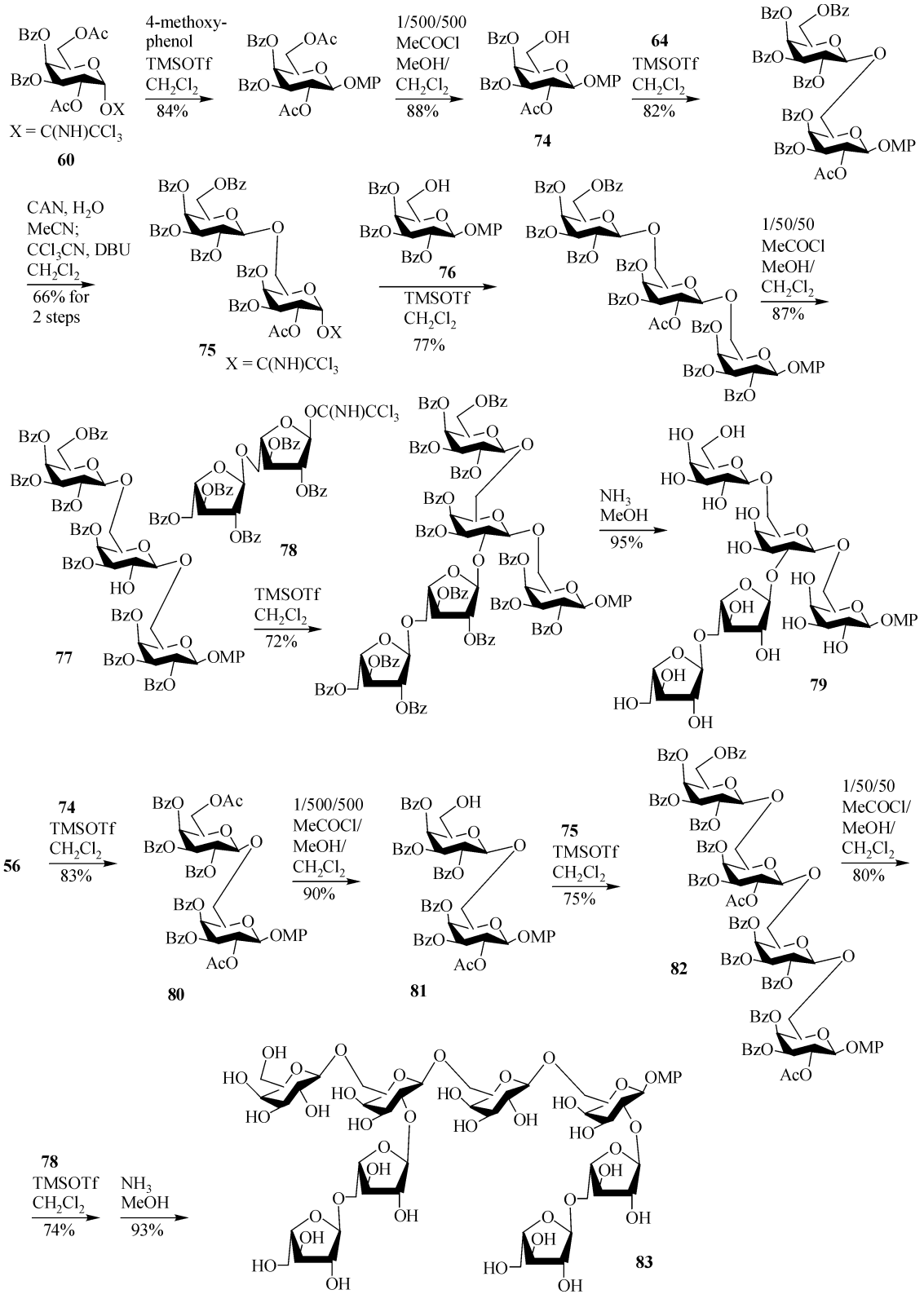
Scheme 8 Synthesis of arabinogalactans by combination of selective glycosylation and deacetylation.

selective deacetylation, with the tetrasaccharide donor **85** and consequent O-2' selective deacetylation produces the hexasaccharide acceptor **87**. Finally, condensation of **87** with the arabinose disaccharide donor **78** followed by deprotection produces the octasaccharide glycoside **88** with structurally different branches at O-2' and O-2'''.

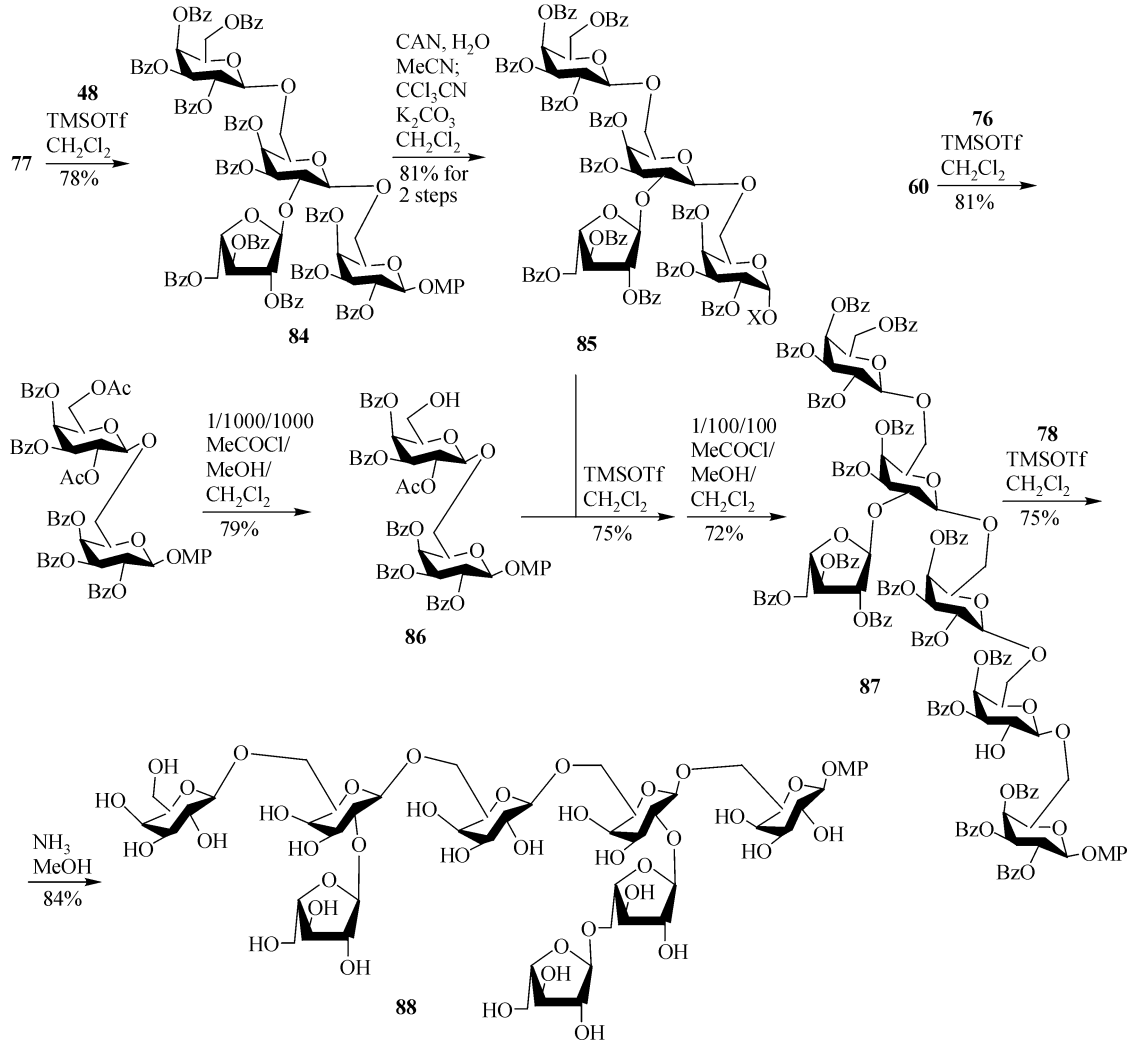
It is seen from the description above that the technique based on sequential O-6 and O-2 selective deacetylation gives the same outcome that selective glycosylation makes, however, the former method is more readily handled in large quantities, for general chemists.

2.3.4 Synthesis of higher 3-branched arabinogalactans by 3,6-selective protection of galactoside in combination with selective glycosylation

3-Arabinofufanose branched arabinogalactans are synthesized [23] based on a 3,6-selective protection technique [37,38] in combination with selective glycosylation, as shown in Scheme 10. In the synthesis, detritylation of isopropyl 2,4-di-O-benzoyl-3-O-*tert*-butyldimethylsilyl-6-O-trityl-1-thio-β-D-galactopyranoside **89** [37,38] with $FeCl_3$ hexahydrate [36], followed by condensation with


4-methoxy-phenol TMSOTf CH2Cl2 84%
1/500/500 MeCOCl MeOH/ CH2Cl2 88%
64 TMSOTf CH2Cl2 82%
X = C(NH)CCl3
60
74
CAN, H2O MeCN; CCl3CN, DBU CH2Cl2 66% for 2 steps
75
X = C(NH)CCl3
76 TMSOTf CH2Cl2 77%
1/50/50 MeCOCl MeOH/ CH2Cl2 87%
78
77
TMSOTf CH2Cl2 72%
NH3 MeOH 95%
79
56
74 TMSOTf CH2Cl2 83%
1/500/500 MeCOCl/ MeOH/ CH2Cl2 90%
80
81
75 TMSOTf CH2Cl2 75%
82
1/50/50 MeCOCl/ MeOH/ CH2Cl2 80%
78 TMSOTf CH2Cl2 74%
NH3 MeOH 93%
83

(*continued*)

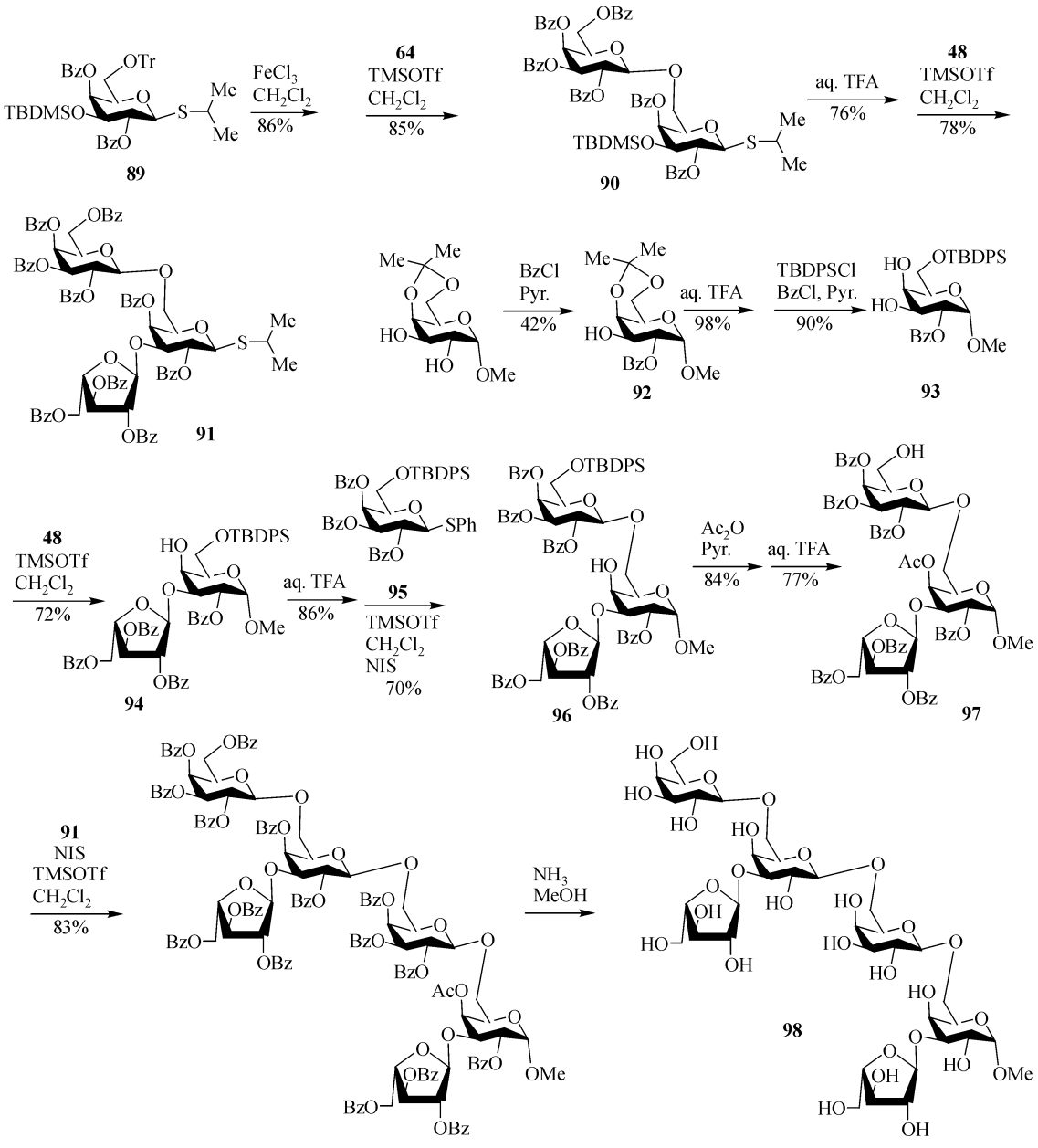


Scheme 9 Synthesis of 2-branched arabinogalactans by selective deacetylation

tetra-O-benzoyl-α-D-galactopyranosyl trichloroacetimidate **64** gives the β-(1→6)-linked disaccharide **90**. Desilylation of **90** and consequent coupling with the arabinose donor **48** afford the trisaccharide donor **91**. Meanwhile, the glycoside acceptor **93** with 3,4-free hydroxyl groups is prepared *via* selective O-2 benzoylation of methyl 4,6-O-isopropylidene-α-D-galactopyranoside (to **92**) and the following deisopropylidenation and selective 6-O-*tert*-butyldiphenylsilylation. Then, selective coupling of **93** with the arabinose donor **48** affords the (1→3)-linked disaccharide **94**, and the following desilylation and selective coupling with phenyl 2,3,4-tri-O-benzoyl-6-O-*tert*-butyldiphenyl-1-thio-β-D-galactopyranoside **95** give the β-(1→6)-linked trisaccharide **96**. Acetylation of **96** followed by desilylation affords the trisaccharide acceptor **97**. Finally, coupling of **97** with the trisaccharide donor **91** followed by deprotection yields the target 3- and 3''-branched arabinogalactan hexasaccharide **98**. It is seen from the above descriptions that this method successfully uses selective glycosylation, however, it needs some special silyl reagents and a rather complex procedure.

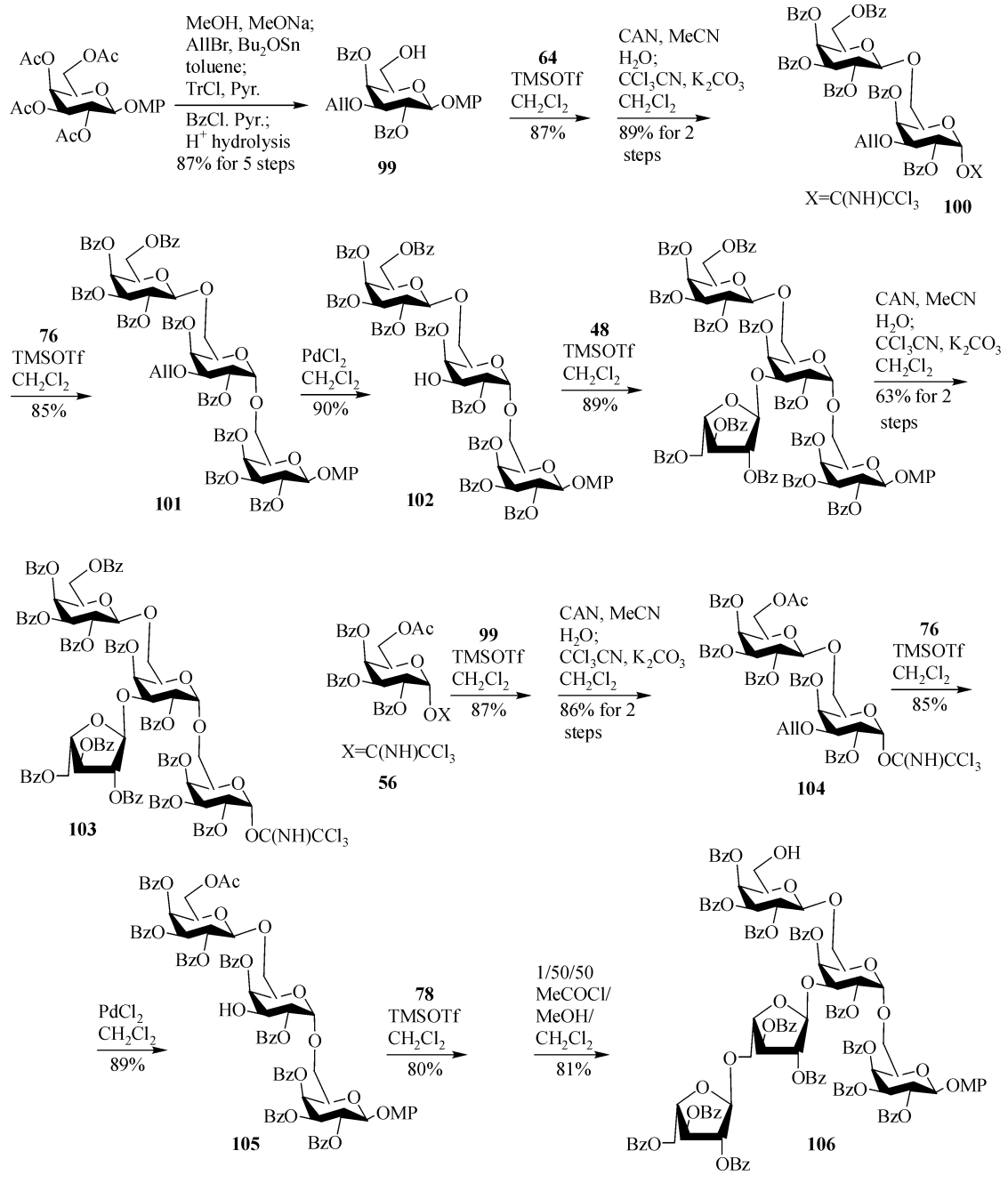
2.3.5 Synthesis of higher 3-branched arabinogalactans by O-3 selective allylation of galactoside in combination with selective O-6 deacetylation

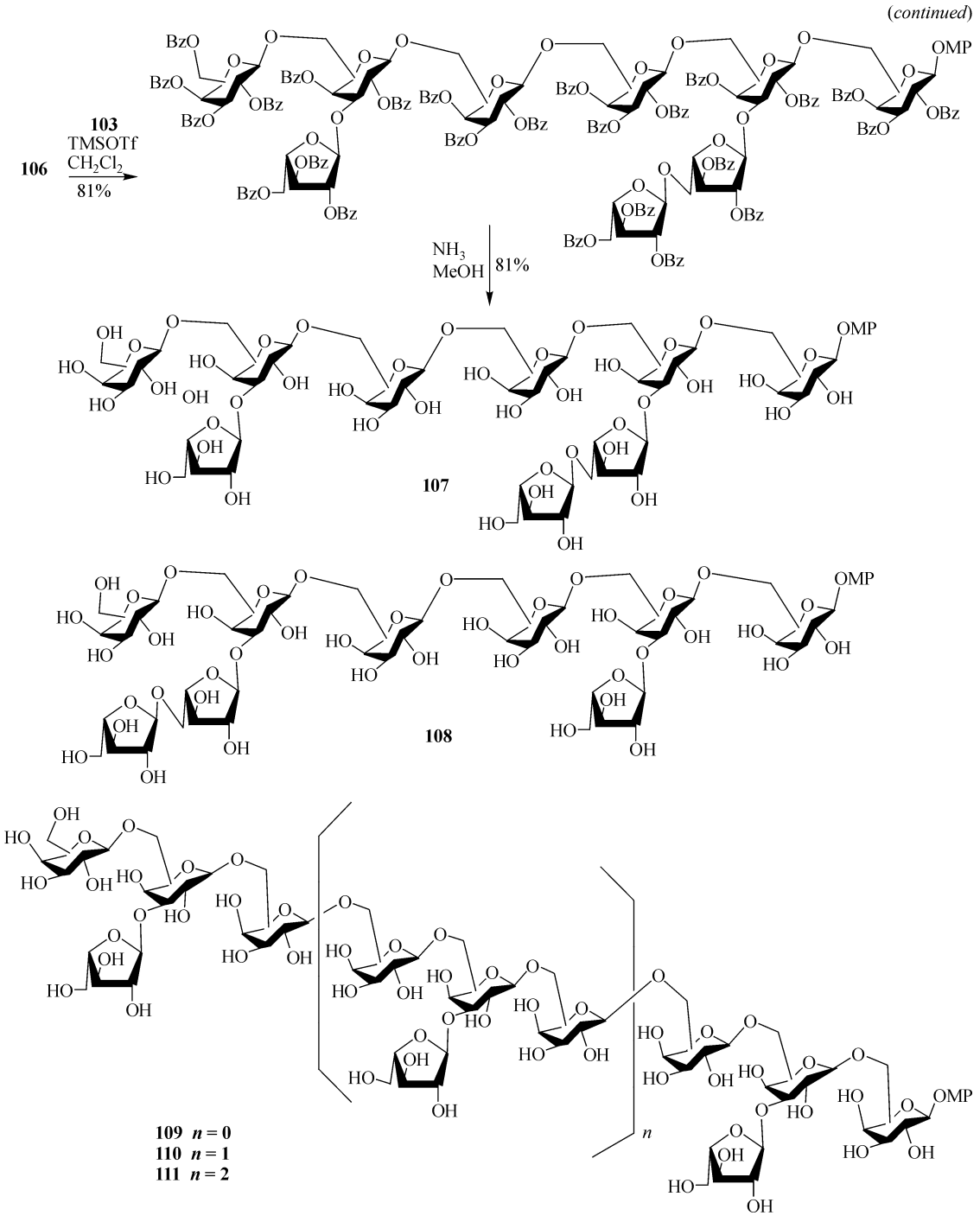
A more accessible method [24,25] based on 3-selective allylation of galactoside in combination with O-6 deacetylation is developed, as shown in Scheme 11. The key building block 4-methoxybenzyl 3-O-allyl-2,4-di-O-benzoyl-β-D-galactopyranoside **99** is obtained in high yield from 4-methoxybenzyl tetra-O-acetyl-β-D-galactopyranoside *via* Zémplen deacetylation, dibutylstannylene-mediated 3-O-selective allylation [39], 6-O-tritylation, benzoylation, and detritylation. Coupling of **99** with the fully benzoylated galactopyranosyl trichloroacetimidate **64** and consequent



Scheme 10 Synthesis 3-branched arabinogalactans based on 3,6-selective protection of galactoside in combination with selective glycosylation

oxidative cleavage of O-1 MP and trichloroacetimidate formation produce the disaccharide donor **100**. Then, condensation of the glycosyl acceptor **76** with **100** gives the trisaccharide **101** and the following deallylation affords the trisaccharide acceptor **102**. Direct coupling of **102** with the arabinose donor **48** and consequent transformation of the 1-O-MP to 1-O-trichloroacetimidate produce the tetrasaccharide donor **103**. Meanwhile, coupling of **99** with **56** followed by activation of the anomeric center yields the disaccharide donor **104**, and consequent condensation with **76** followed by deallylation gives the trisaccharide **105**. Reaction of **105** with the arabinobiose trichloroacetimidate **78** followed by selective O-6" deacetylation yields the pentasaccharide acceptor **106**. Finally, condensation of **106** with **103** followed by deprotection yields the 3'-arabinobiose- and 3""-arabinose-branched target nonasaccharide **107**. By the same strategy, 3'-arabinose- and 3""-arabinobiose-branched nonasaccharide **108**, 3'-, 3""-arabinose-branched octamer **109**, 3'-, 3""-, $3^{8\prime}$-branched dodecamer **110**, and 3'-, 3""-, $3^{8\prime}$-, $3^{12\prime}$-branched sixteenmer **111** are also concisely synthesized. It can be seen from the descriptions above that this method is very effective and concise for the synthesis of 3-branched arabinogalactans in large quantities.

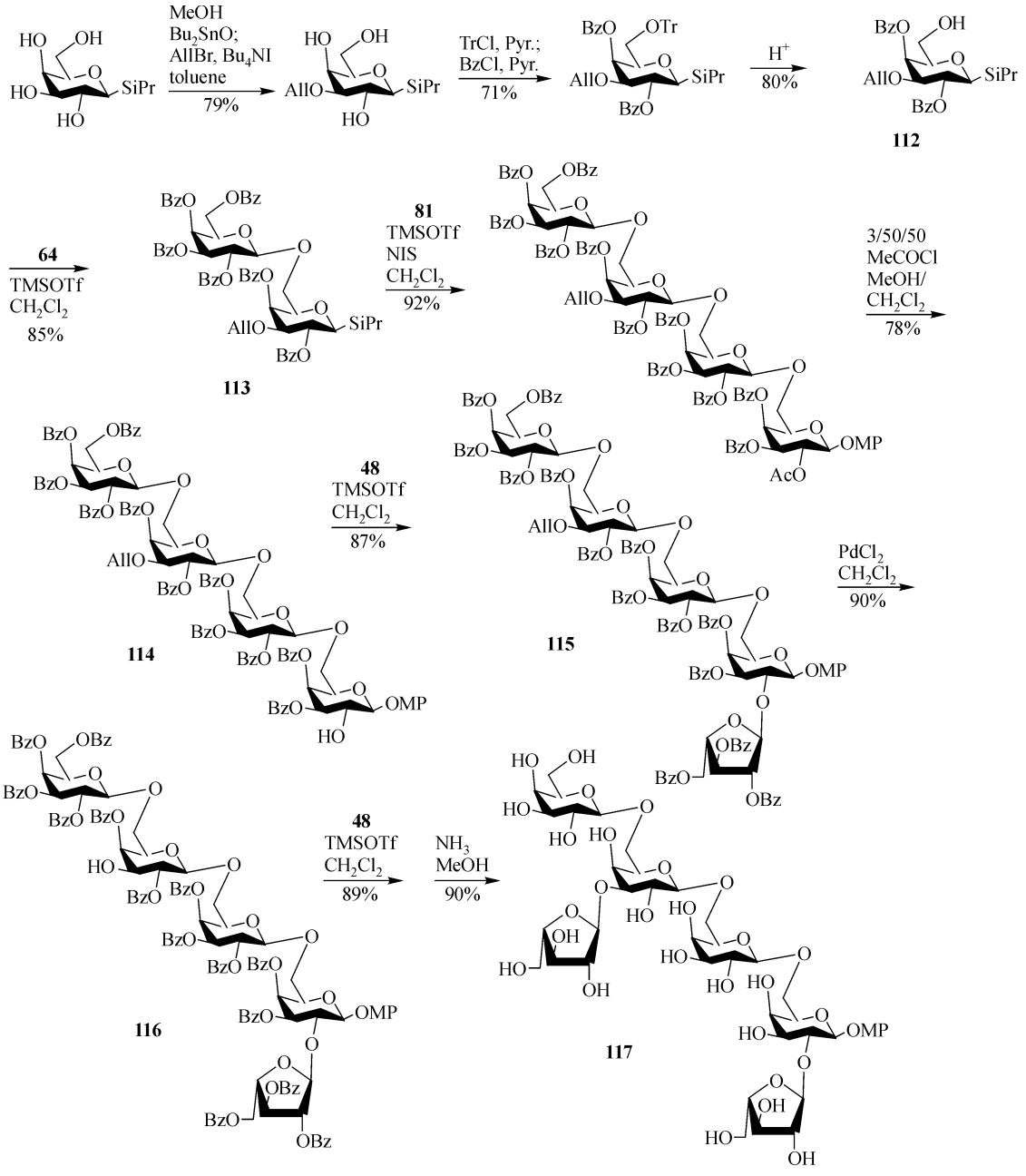

MeOH, MeONa;
AllBr, Bu2OSn
toluene;
TrCl, Pyr.
BzCl. Pyr.;
H+ hydrolysis
87% for 5 steps
99
64
TMSOTf
CH2Cl2
87%
CAN, MeCN
H2O;
CCl3CN, K2CO3
CH2Cl2
89% for 2 steps
X=C(NH)CCl3
100
76
TMSOTf
CH2Cl2
85%
101
PdCl2
CH2Cl2
90%
102
48
TMSOTf
CH2Cl2
89%
CAN, MeCN
H2O;
CCl3CN, K2CO3
CH2Cl2
63% for 2 steps
103
99
TMSOTf
CH2Cl2
87%
X=C(NH)CCl3
56
CAN, MeCN
H2O;
CCl3CN, K2CO3
CH2Cl2
86% for 2 steps
104
76
TMSOTf
CH2Cl2
85%
PdCl2
CH2Cl2
89%
105
78
TMSOTf
CH2Cl2
80%
1/50/50
MeCOCl/
MeOH/
CH2Cl2
81%
106



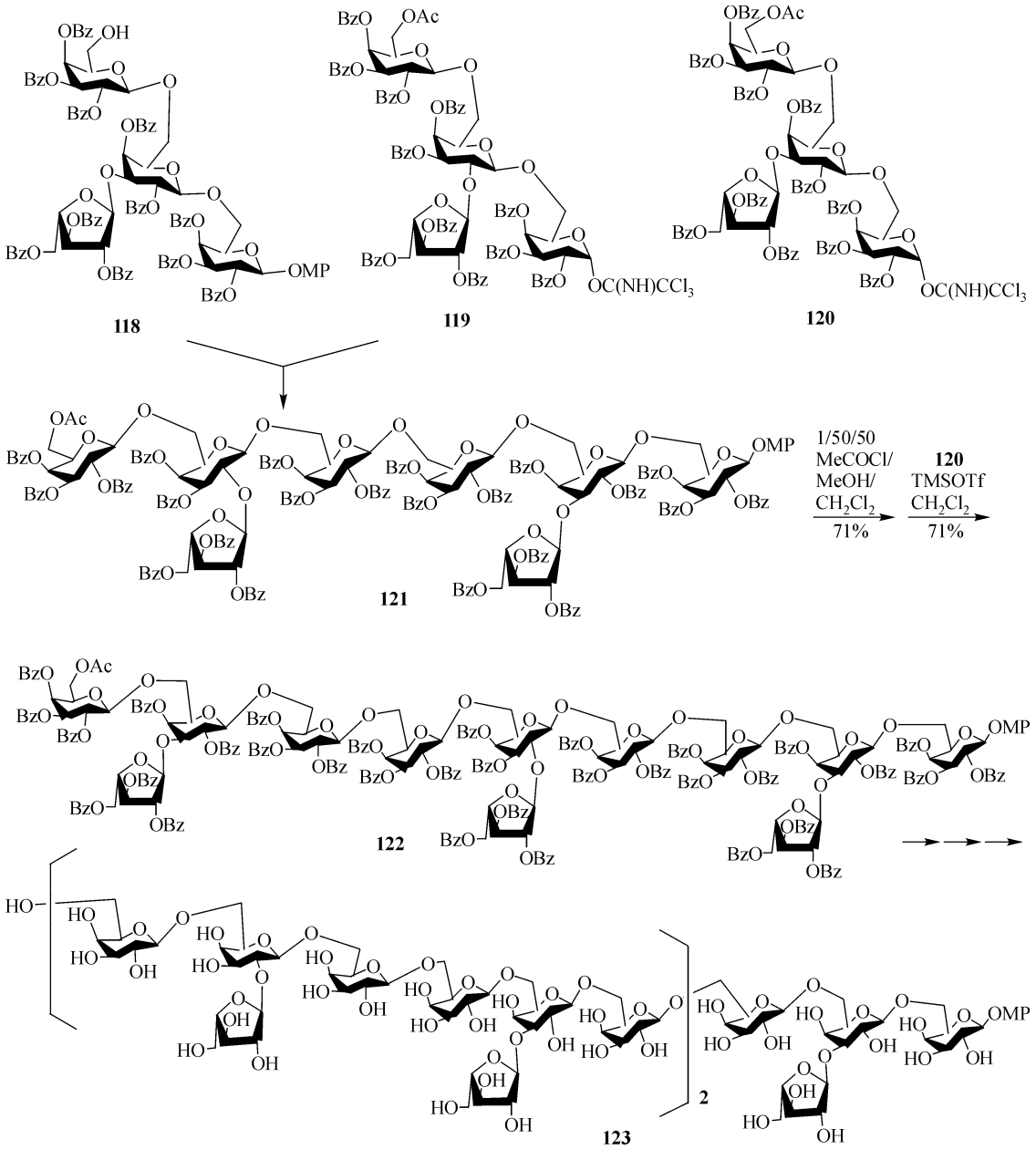
Scheme 11 Synthesis of 3-branched arabinogalactans based on selective O-3 allylation in combination with selective O-6 deacetylation

2.3.6 Synthesis of arabinogalactans with both 2- and 3-arabinose branches

As shown in Scheme 12, concise synthesis of the arabinogalactans with both α-(1→3)- and α-(1→2)-linked arabinose branches is achieved [26]. Isopropyl 1-thio-β-D-galactopyranoside, prepared readily by coupling of penta-O-acetyl-β-D-galactopyranose with isopropyl thiol and subsequent deacetylation, is chosen as the starting material. Its selective 3-O-allylation *via* dibutyltin complex and the following 6-O-tritylation, 2,4-di-O-benzoylation and 6-O-detritylation give the glycosyl acceptor **112** at a satisfactory yield. Condensation of **112** with perbenzoylated galactopyranosyl trichloroacetimidate **64** affords the disaccharide donor **113**. Then, condensation of **113** with the disaccharide acceptor **81** followed by selective 2-O-deacetylation gives the tetrasaccharide acceptor **114**. Condensation of **114** with the arabinose donor **48** produces


MeOH
Bu2SnO;
AllBr, Bu4NI
toluene
79%
TrCl, Pyr.;
BzCl, Pyr.
71%
H+
80%
112
64
TMSOTf
CH2Cl2
85%
113
81
TMSOTf
NIS
CH2Cl2
92%
3/50/50
MeCOCl
MeOH/
CH2Cl2
78%
48
TMSOTf
CH2Cl2
87%
114
115
PdCl2
CH2Cl2
90%
48
TMSOTf
CH2Cl2
89%
NH3
MeOH
90%
116
117

(*continued*)



Scheme 12 Synthesis of arabinogalactans with alternative 2- and 3-arabinose branches

the pentasaccharide **115**, and subsequent deallylation affords the acceptor **116**. Coupling of **116** with the arabinose donor **48** followed by deacylation give the target hexasaccharide **117** consisting of a β-(1→6)-linked galactopyranosyl tetrasaccharide backbone and α-L-arabinofuranose side chains at O-2 and O-3", respectively.

The octamer **121**, dodecamer **122**, and twentymer **123** are also effectively synthesized with three tetrasaccharide building blocks, i.e., the 3-branched acceptor **118**, the 2-branched donor **119**, and the 3-branched donor **120**; they are prepared readily by the developed method. In the synthesis, the oligosaccharide chain is elongated from the non-reducing end. Thus, coupling of **118** with **119** affords the octamer **121**, and consequent deprotection gives the free 4-methoxyphenyl glycoside of octasaccharide. Selective 6''''-O-deacetylation of **121** is carried out under stronger acidic conditions, i.e., 1∶50∶50 MeCOCl/MeOH/CH_2Cl_2, because of the weaker reactivity of the long chain, and the following coupling with the 3-branched donor **120** yields **122**, deprotection of which gives the free glycoside of dodecasaccharide. Starting from **122**, reiteration of selective deacetylation, coupling (with **119**), and deprotection produces the target twentymer glycoside **123**.

It can be seen from the above statements that this method is simple, highly regio- and chemoselective, and can

be used in the preparation of structurally different arabinogalactans required in glycobiology research. With the synthesized arabinogalactans at hand, the study of structure-bioactivity is readily performed. Preliminary mice testing results (unpublished) indicate that some synthesized arabinogalactans show strong immunomodulating activity. It is expected that a conclusion on structure-bioactivity of arabinogalactans will be drawn in the near future.

References

1. Bertozzi C R, Kiessling L L. Chemical glycobiology. Science, 2001, 291: 2357–2364
2. Kong F. Regio- and stereoselective synthesis of oligosaccharides with unprotected or lightly protected sugars as glycosyl acceptors. Curr Org Chem, 2003, 7(9): 841–865
3. Davis B G. Recent development in oligosaccharide synthesis, J Chem Soc Perkin Trans 1, 2000, (14): 2137–2160
4. Schmidt R R, Kinzy W. Adv Carbohydr Chem Biochem, 1994, 50: 21–125
5. Toshima K, Tatsuta K. Recent progress in O-glycosylation methods and its application to natural products synthesis. Chem Rev, 1993, 93: 1503–1531
6. Clarke A E, Anderson R L, Stone B A. Form and function of arabinogalactans and arabinogalactan-protein. Phytochemistry, 1979, 18: 521–528
7. Yamada H, Kiyohara H, Cyong J C, Otsuka Y. Studies on polysaccharides from *Angelica acutiloba*-IV, characterization of anti-complementary arabinogalactan from the roots of *Angelica acutiloba* Kitagawa. Mol Immunol, 1985, 22: 295–304
8. Kiyohara H, Yamada H, Cyong J C, Otsuka Y. Characterization and function of arabinogalactans. J Pharmacobio-Dyn, 1986, 9: 339–346
9. Shimizu N, Tomoda M, Gonda R. Studies on the constituents of edible and medicinal plants. Chem Pharm Bull (Tokyo), 1989, 37: 1329–1332
10. Egert D, Beuscher N. Studies on antigen specificity of immunoreactive arabinogalactan proteins extracted from Baptisa tinctoria and *Echinacea purpurea*. Planta Med, 1992, 58: 163–165
11. Hoson T. Structure and function of plant cell walls: immunological approaches. Int Rev Cytol, 1991, 130: 233–268
12. Steffan W, Kovac P, Albersheim P, Darvill A G, Hahn M G. Characterization of a monoclonal antibody that recognizes an arabinosylated β-1-6-galactan epitope in plant complex carbohydrates. Carbohydr Res, 1995, 275: 295–307
13. Timmers C M, Wigchert S C M, Leeuwenburgh M A, van der Marel G A, van Boom J H. Synthesis of tetrameric arabinogalactans based on 1,2-anhydrosugars. Eur J Org Chem, 1998, 1: 91–97
14. Borbás A, Jánossy L, Lipták A. Scope and limitation of the application of the (methoxydimethyl)methyl group in the synthesis of 2'-O-, 6'-O- and 2',6'-di-O-(α-L-arabinofuranosyl)-β-D-galactopyranosyl-(1→6)-D-galactoses. Carbohydr Res, 1999, 318: 98–109
15. Csávás M, Borbás A, Jánossy L, Batta G, Lipták A. Synthesis of the α-L-Araf-(1→2)-β-D-Galp-(1→6)-β-D-Galp-(1→6)-[α-L-Araf-(1→2)]-β-D-Galp-(1→6)-D-Gal hexasaccharide as a possible repeating unit of the cell-cultured exudates of *Echinacea purpurea* arabinogalactan. Carbohydr Res, 2001, 336: 107–115
16. Csávás M, Borbás A, Szilagyi L, Lipták A. Successful combination of (methoxydimethyl)methyl (MIP) and (2-naphthyl)methyl (NAP) ethers for the synthesis of arabinogalactan-type oligosaccharides. Synlett, 2002, 6: 887–890
17. Csávás M, Borbás A, Jánossy L, Lipták A. Synthesis of an arabinogalactan-type octa- abd two isomeric nonasaccharides. Suitable tuning of protecting groups. Tetrahedron Lett, 2003, 44: 631–635
18. Du Y, Pan Q, Kong F. Synthesis of a tetrasaccharide representing minimal epitope of arabinogalactan. Carbohydr Res, 2000, 323: 28–35
19. Du Y, Pan Q, Kong F. Synthesis of 3-O-arabinosylated (1-6)-galactan. J Carbohydr Chem, 2001, 20: 297–306
20. Ning J, Yi Y, Yao Z. An efficient method for the synthesis of 2,6-branched galacto-oligosaccharides and its application to the synthesis of three tetrasaccharides and a hexasaccharide related to the arabinogalactans (AGs). Synlett, 2003, 14: 2208–2212
21. Zeng Y, Li A, Kong F. A concise synthesis of arabinogalactan with β-(1→6) galactopyranose backbone and α-(1→2) arabinofuranose side chains. Tetrahedron Lett, 2003, 44: 8325–8329
22. Li A, Zeng Y, Kong, F. An effective synthesis of arabinogalactan with β-(1→6) galactopyranose backbone and α-(1→2) arabinofuranose side chains. Carbohydr Res, 2004, 339: 673–681
23. Gu G, Du Y, Kong, F. Synthesis of a hexasaccharide that relates to the arabinogalactan epitope. Carbohydr Res, 2001, 336: 99–106
24. Li A, Kong F. Concise syntheses of arabinogalactans consisting of β-(1→6)-linked galactopyranosyl backbone and α-(1→3)-linked arabinofuranosyl side chains. Chin J Chem, 2005, 23: 7–10
25. Li A, Kong, F. Syntheses of arabinogalactans consisting of β-(1→6)-linked D-galactopyranosyl backbone and α-(1→3)-linked L-arabinofuranosyl side chains. Carbohydr Res, 2004, 339: 1847–1856
26. Li A, Kong, F. Concise syntheses of arabinogalactans with β-(1→6)-linked galactopyranose backbones and α-(1→3)- and α-(1→2)-linked arabinofuranose side chains. Biorg Med Chem, 2005, 13: 839–853
27. Lipták A, Fugedi P, Kerekgyarto J, Nanasi P. Preparation of mixed-acetal derivatives of carbohydrates by acetal-exchange reaction. Carbohydr Res, 1983, 113: 225–231
28. Gaunt M J, Yu J, Spencer J B. Rational design of benzyl-type protecting groups allows sequential deprotection of hydroxyl groups by catalytic hydrogenolysis. J Org Chem, 1998, 63: 4172–4173
29. Wang W, Kong F. New method for regio- and stereoselective synthesis of oligosaccharides. J Org Chem, 1998, 63: 5744–5745
30. Wang W, Kong F. A highly convergent synthesis of the elicitor hexasaccharide. Tetrahedron Lett, 1998, 39: 1937–1940
31. Wang W, Kong F. Regio- and steroselective synthesis of mannose-containing oligosaccharides. Angew Chem Int Ed, 1999, 38: 1247–1250
32. Kong F. Recent studies on reaction pathways and applications of sugar orthoresters in synthesis of oligosaccharides. Carbohydr Res, 2007, 342: 345–373

33. Byramova N E, Ovchinnikov M V, Backinowsky L V, Kochetkov N K. Selective removal of O-acetyl groups in the presence of O-benzoyl groups by acid-catalyzed methanolysis. Carbohydr Res, 1983, 124: c8–c11
34. Du Y, Pan Q, Kong F. Regio- and steroselective synthesis of 1-5-linked arabinofuranosyl oligosaccharides using unprotected furanoside donors. Synlett, 1999: 1648–1652
35. Du Y, Pan Q, Kong, F. A facile synthesis of 1-5-linked arabinofuranosyl oligosaccharides. Carbohydr Res, 2000, 329: 17–24
36. Ding X, Wang W, Kong F. Selective O-detritylation of sugar derivatives. Carbohydr Res, 1997, 303: 445–448
37. Du Y, Zhang M, Kong F. Concise synthesis of 3,6-branched oligosaccharides. Org Lett, 2000, 2: 3797–3800
38. Du Y, Zhang M, Kong F. Facile synthesis biologically important pentasaccharides. Tetrahedron, 2001, 57: 1757–1763
39. Kong F, Lu D, Zhou S. Synthesis and conformation of 1,3-anhydro-2,4,6-tri-O-benzyl-D-galactose. Carbohydr Res, 1990, 198: 141–147