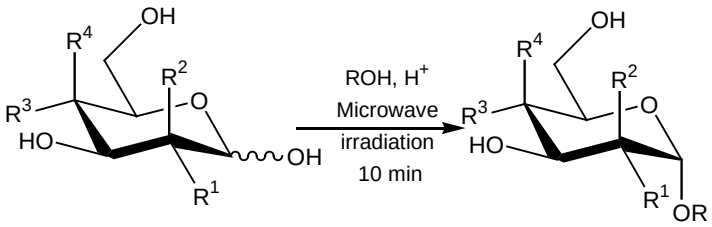
Graphical Abstract

Microwave-Accelerated Fischer Glycosylation

Laurent F. Bornaghi* and Sally-Ann Poulsen



N-Acetyl-D-glucosamine, R^1 = NHAc, R^2 = H, R^3 = OH, R^4 = H,
N-Acetyl-D-galactosamine, R^1 = NHAc, R^2 = H, R^3 = H, R^4 = OH,
D-Glucose, R^1 = OH, R^2 = H, R^3 = OH, R^4 = H,
D-Galactose, R^1 = OH, R^2 = H, R^3 = H, R^4 = OH,
D-Mannose, R^1 = H, R^2 = OH, R^3 = OH, R^4 = H.

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Abstract
Fischer glycosylation has been used for decades for the synthesis of simple alkyl and aryl glycosides from free sugars. The reaction proceeds under reflux in the presence of catalytic acid with the alcohol as solvent. The main deficiency of this reaction is the long reaction time required. In this study microwave heating has been utilized for the Fischer glycosylation reaction of *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, D-glucose, D-galactose and D-mannose with a variety of alcohols (methanol, ethanol, benzyl alcohol and allyl alcohol). Remarkable acceleration of the glycosylation reactions (minutes compared to hours) over conventional reflux heating was observed with good yields and production of the α-glycoside as the dominant product.

Keywords
Microwave; Fischer glycosylation; Glycosides.

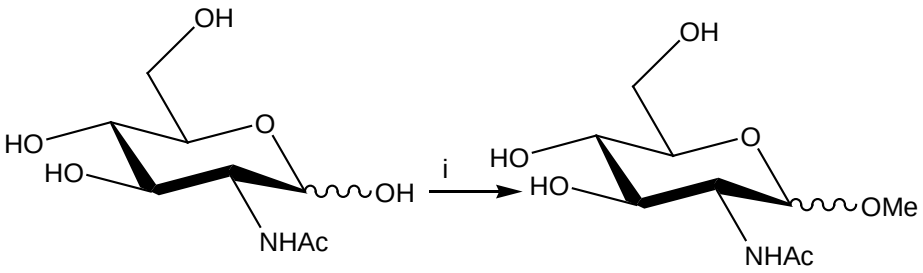
Since the inception of Fischer glycosylation in 1896 many methods have been developed for the preparation of glycosides. However, for the synthesis of simple glycosides, Fischer glycosylation remains the method of choice despite the long reaction times required.[1,2] Microwave irradiation, using either a domestic microwave oven or mono-mode reactors, has become a powerful tool for the preparation of various organic compounds.[3,4] Thermally driven organic transformations can take place by conventional heating or microwave-accelerated heating. In the microwave system of this study, a circular single-mode cavity directs the microwave energy into a defined area, resulting in a homogenous field pattern surrounding the sample and an instantaneous coupling to all polar and ionic components in the sample, leading to a rapid rise in temperature.[§] The microwave approach is simple and usually decreases reaction times whilst increasing product yield and purity. Fischer glycosylation appeared an ideal candidate for the application of microwave irradiation and herein we report a systematic analysis of the effect of microwave heating on the Fischer glycosylation reaction.

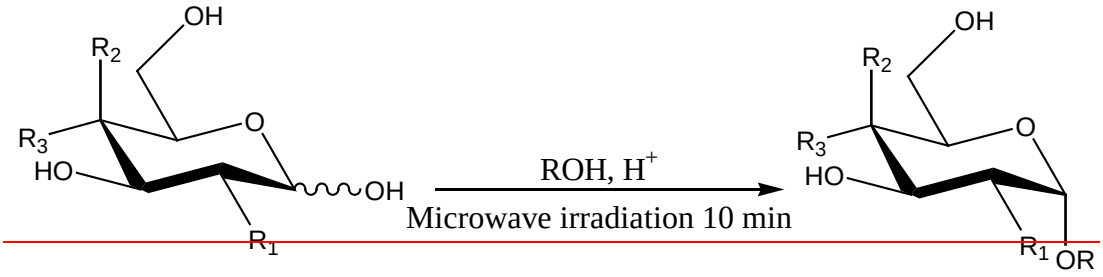
Fischer glycosylation of the anomeric hydroxyl of a sugar occurs upon reflux with an alcohol in the presence of acid catalyst.[5] An equilibrium mixture of α–

and β–glycosidic products is formed in a ratio that is dependent upon the relative thermodynamic stabilities of the isomers. When Fischer glycosylation reactions are stopped prior to reaching equilibrium the product ratios are different. The reaction mixtures contain not only α– and β–pyranosides but also furanosides and a small amount of acyclic dimethyl acetal.

As part of our work on the development of new carbohydrate building blocks for dynamic combinatorial chemistry,[6] we needed to prepare some simple glycosides as starting materials. The methyl glycoside of *N*-acetyl-D-glucosamine was the first of these materials. Conventional Fischer glycosylation reaction conditions using anhydrous methanol as solvent and Amberlite resin IRN 120 H^+ as acid catalyst were adopted for this preparation (Scheme 1).[7] The use of an acidic resin as catalyst offers work-up advantages as the catalyst can be removed by simple filtration, avoiding the need for neutralisation and resulting salt formation at the end of the reaction.

Insert scheme 1





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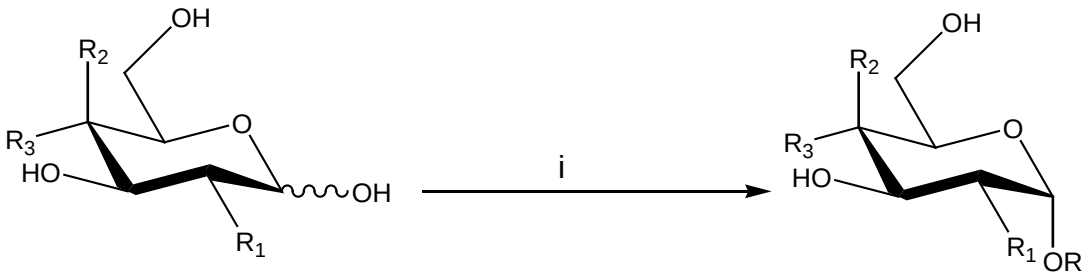

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Scheme 1. (i) MeOH, Amberlite resin IRN 120 H^+, reflux.

Optimisation of reaction time to give both high yield and a high proportion of the desired α-glycoside was carried out in methanol at 70 ^oC. After 4 hours, total consumption of the starting material was verified by thin layer chromatography and ^1H NMR spectroscopy. The reaction proceeded cleanly to give an improving α/β glycoside ratio up to 8 hours. Continuing beyond 8 hours (18 hours and 24 hours) gave only a minor improvement of the α/β glycoside ratio with an increase in decomposition products (Table 1).

Insert Table 1

[a]Anomeric composition of reaction mixture was monitored by ^{1}H NMR spectroscopy (200 MHz, D_2O, ppm): δ 4.80 (d, 1H, $J_{1,2}$ = 8.50 Hz, H-1β), 4.71 (d, 1H, $J_{1,2}$ = 4.00 Hz, H-1α).
[b]Reaction performed on 5.00 g (0.023 mol) scale to provide 3.81 g of purified product after recrystallisation (70%).[8]

Table 1. Fischer glycosylations under conventional reflux conditions.

We then investigated the effect of microwave heating on the synthesis of the methyl glycoside of *N*-acetyl-D-glucosamine again using Amberlite resin IRN 120 H^+ as catalyst.[§] Our procedure used microwave irradiation at both 90 °C and 120 °C for 10 minutes.[9] After 2 minutes there was almost complete consumption of starting material at both temperatures studied, compared with 4 hours at reflux. A similar α/β ratio was obtained after 10 minutes of microwave irradiation at 120 °C in comparison with 18 hours at reflux (Table 2).

Insert table 2

[a]Anomeric composition of reaction mixture was monitored by ^{1}H NMR spectroscopy (200 MHz, D_2O, ppm): δ 4.80 (d, 1H, $J_{1,2}$ = 8.50 Hz, H-1β), 4.71 (d, 1H, $J_{1,2}$ = 4.00 Hz, H-1α).
[b]Reactions performed on 0.5 g (0.0023 mol) scale to provide at 90 °C, 0.33 g of purified product after recrystallisation (61%) and 0.44 g (80%) at 120 °C. [9]

Table 2. Microwave assisted Fischer glycosylations of *N*-acetyl-D-glucosamine.

Carbohydrates that are selectively protected at the anomeric position are useful building blocks, provided that the protecting groups are easily removable as is the case with benzyl and allyl glycosides. Encouraged by the microwave irradiation results in methanol we conducted a study using a small range of starting materials (*N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, D-glucose, D-galactose and D-mannose) in methanol, ethanol, benzyl alcohol and allyl alcohol, to prepare the corresponding glycosides (Scheme 2).

Reactions were performed at a range of temperatures. Good α/β glycoside ratios were observed in all cases (Table 3) with less than 5% of starting material remaining after just 10 minutes of irradiation.

Insert scheme 2

Scheme 2. (i) Alcohol (ROH), Amberlite resin IRN 120 H^+, microwave irradiation, 10 min, various temperatures.

Insert table 3

[a]Anomeric composition of reaction mixture was monitored by ^{1}H NMR spectroscopy, following microwave irradiation for 10 minutes.
[b]Reaction performed on 0.5 g (0.0027 mol) scale to provide 0.39 g of purified product after recrystallisation (74%).[10]

Table 3. Microwave-accelerated Fischer glycosylations of *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, D-glucose, D-galactose and D-mannose in various alcohols.

We have applied microwave irradiation to conventional Fischer glycosylation resulting in an impressive acceleration of reaction time (minutes compared to hours) with good α-glycoside product selectivity. The synthesis is both practical and efficient for each substrate (*N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, D-glucose, D-galactose and D-mannose). This study demonstrates that microwave irradiation has potential as one of the easiest and quickest routes to prepare simple glycosides using Fischer glycosylation. Microwave equipment with scale-up capabilities (automated reaction handling modules or continuous flow through systems) is commercially available to support the transfer from feasibility studies to large-scale synthesis with either minimal or no need for further optimisation. This equipment will allow synthesis on a multigram or even kilogram scale. A larger investigation of various glycosylation reactions and sugar protecting group manipulations using microwave irradiation is underway.

Acknowledgements
We thank Professor I. Jenkins and Dr J. Ripper for their helpful discussions and for the opportunity to use their microwave oven. We thank the Australian Research Council for financial assistance.

References and notes
§ A CEM Discover microwave system was used in this study. This microwave is a circular single-mode cavity and can accommodate a single 10 mL 25 bar pressure rated sealed reaction vial. The system incorporates both temperature and pressure feedback systems for control of the reaction conditions. The temperature feedback system uses an infrared temperature sensor positioned below the reaction vessel to permit reproducible temperature control. Reactions were quenched following heating by forced gas cooling with nitrogen gas.

1. Igarashi, K. *Adv. Carbohydr. Chem.* **1977**, *34*, 243-244; Capon, B. *Chem. Rev.* **1969**, *69*, 407-498; Ferrier, R. J.; Hatton, L. R. *Carbohydr. Res.* **1968**, *6*, 75-86; Heard, D. D.; Barker, R. *J. Org. Chem.* **1968**, *33*, 740-746; Freudenberg, K. *Adv. Carbohydr. Chem.* **1966**, *21*, 2-38.
2. Kappe, C. O. *Angew. Chem. Int. Ed.* **2004**, *43*, 6250-6284; Pietruszka, J. *Carbohydr.* **2003**, 219-238; Magnusson, G.; Nilsson, U. J. *Glycoscience* **2001**, *2*, 1543-1587; Panza, L.; Lay, L. *Carbohydr. Chem. Biol.* **2000**, *1*, 195-237; Tamura, J.-I. *Carbohydr. Chem. Biol.* **2000**, *1*, 177-193; Fraser-Reid, B.;

Anilkumar, G.; Gilbert, M. R.; Joshi, S.; Kraehmer, R. *Carbohydr. Chem. Biol.* **2000**, *1*, 135-154; Zhang, Z.; Wong, C.-H. *Carbohydr. Chem. Biol.* **2000**, *1*, 117-134; Schmidt, R. R.; Jung, K.-H. *Carbohydr. Chem. Biol.* **2000**, *1*, 5-59.

3. Das, S. K. *Synlett* **2004**, *6*, 915-932; Adam, D. *Nature* **2003**, *421*, 571-572; Mohan, H.; Gemma, E.; Ruda K.; Oscarson, S. *Synlett* **2003**, *6*, 1255-1256; Nuchter, M.; Ondruschka, B.; Lautenschlager, W. *Synth. Commun.* **2001**, *31*, 1277-1283; Cleophax, J.; Liagre, M.; Loupy, A.; Petit, A. *Org. Process Res. Dev.* **2000**, *4*, 498-504; Varma, R. S. *Green Chem.* **1999**, 43-55.
4. Perreux, L.; Loupy, A. *Tetrahedron* **2001**, *57*, 2199-2223; Limousin, C.; Cleophax, J.; Petit, A.; Loupy, A.; Lukacs, G. *J. Carbohydr. Chem.* **1997**, *16*, 327-342.
5. Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21-73; Schmidt, R. R. *Angew. Chem. Int. Ed.* **1986**, *25*, 212-235; Capon, B. *Chem. Rev.* **1969**, *69*, 407-498.
6. Bornaghi, L. F.; Wilkinson, B. L.; Kiefel M. J.; Poulsen, S.-A. *Tetrahedron Lett.* **2004**, *45*, 9281-9284.
7. Lein, M. W. *J. Am. Chem. Soc.* **1955**, *77*, 1663-1967.
8. To a mixture of *N*-acetyl-D-glucosamine (5.00 g, 0.023 mol) in anhydrous methanol (50 mL) was added Amberlite IRN 120 H^+ resin (5.00 g). The reaction mixture was stirred at 70 ^oC for 8 h and then filtered. The filtrate was evaporated under reduced pressure. The residue was purified by recrystallisation from isopropanol. The desired methyl glycoside was isolated as a white solid (3.69 g, 68%). The product obtained has analytical data identical to the reference material described in the Spectral Database for Organic Compounds (SDBS) on http://www.aist.go.jp/RIODB/SDBS/cgi-bin/cre_index.cgi.
9. To a mixture of *N*-acetyl-D-glucosamine (0.5 g, 0.0023 mol) in anhydrous methanol (5 mL) was added Amberlite IRN 120 H^+ resin (0.5 g). The reaction was carried out in a pressure tube, sealed with a Teflon septum. The pressure tube was introduced in the microwave oven for 10 minutes at 90 ^oC and then the reaction mixture filtered. The filtrate was evaporated under reduced pressure. The residue was purified by recrystallisation from isopropanol. The desired methyl glycoside was isolated as a white solid (0.33 g, 61%). When the reaction was carried out at 120 ^oC, 0.43 g of the glycoside was isolated (80%).
10. To a mixture of D-glucose (0.5 g, 0.0027 mol) in anhydrous methanol (5 mL) was added Amberlite IRN 120 H^+ resin (0.5 g). The reaction was carried out in a pressure tube, sealed with a Teflon septum. The pressure tube was introduced in the microwave oven for 10 minutes at 120 ^oC and then the reaction mixture filtered. The filtrate was evaporated under reduced pressure. The residue was purified by recrystallisation from methanol. The desired methyl glycoside was isolated as a white solid (0.39 g, 74%). The product obtained has analytical data identical to the reference material described in the Spectral Database for Organic Compounds (SDBS) on http://www.aist.go.jp/RIODB/SDBS/cgi-bin/cre_index.cgi.
11. *General experimental procedure for microwave accelerated Fischer glycosylation:*
All reactions were carried out in a pressure tube, sealed with a Teflon septum. Reactions contained the sugar substrate (0.1 g, 0.45–0.56 mmol), Amberlite

IRN 120 H^+ resin (0.1 g) and alcohol (1 mL). The pressure tube was introduced to the centre of a CEM Discover microwave oven and then heated to the desired temperature for the appropriate time. On completion of the heating cycle an aliquot was removed from the reaction mixture, concentrated and analysed by ^{1}H NMR. All products obtained are described in the literature and were confirmed by ^{1}H NMR spectroscopy.

Tables

Table 1:

Entry	Reaction Time	α/β Ratio[a]	% of starting material
1	15 min	0.1 / 1	85
2	30 min	0.5 / 1	76
3	45 min	0.8 / 1	51
4	60 min	1.0 / 1	22
5	90 min	1.3 / 1	9
6	120 min	1.8 / 1	< 5
7	4 h	7.2 / 1	0
8	6 h	9.8 / 1	0
9	8 h	11.6 / 1[b]	0
10	18 h	12.4 / 1	0
11	24 h	12.8 / 1	0

Table 2:

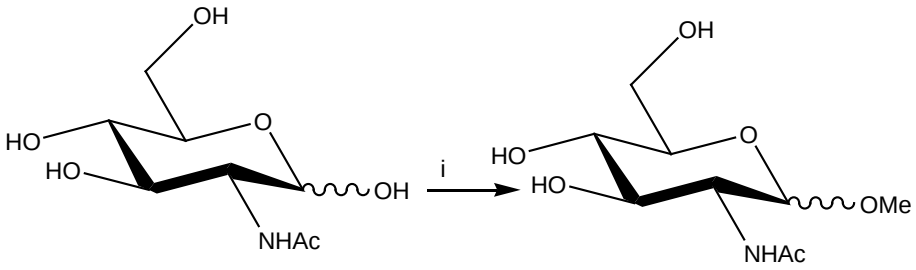
Entry	Reaction Time (min)	Temperature (^{o}C)	α/β Ratio[a]
1	2		0.8 / 1
2	4		1.2 / 1
3	6	90	2.1 / 1
4	8		3.4 / 1
5	10		5.0 / 1[b]
6	2		6.1 / 1
7	4		8.9 / 1
8	6	120	9.6 / 1
9	8		10.5 / 1
10	10		13.2 / 1[b]

Table 3:

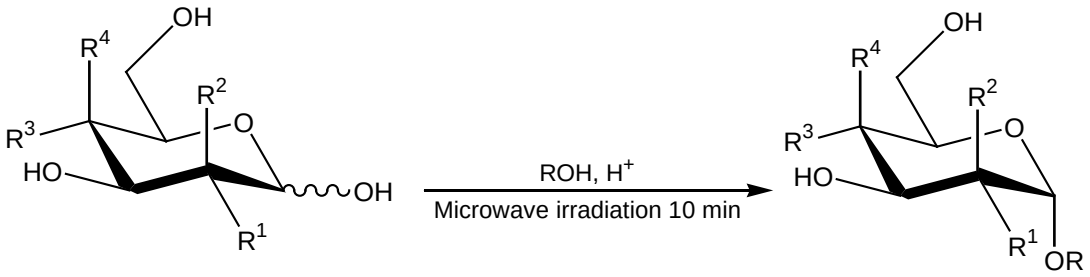
Entry	Solvent	Starting material	Temperature (^{o}C)	α/β Ratio[a]
1	Methanol	*N*-Acetyl-D-glucosamine	90	5.0 / 1
2	Methanol		120	13.0 / 1
3	Ethanol		120	10.6 / 1
4	Benzyl alcohol		120	8.9 / 1
5	Benzyl alcohol		150	10.3 / 1
6	Benzyl alcohol		200	Decomposition
7	Allyl alcohol		120	12.7 / 1
8	Allyl alcohol		150	Decomposition
9	Methanol	*N*-Acetyl-D-galactosamine	90	Only α
10	Methanol		120	Only α
11	Benzyl alcohol		120	Only α
12	Benzyl alcohol		150	Only α
13	Allyl alcohol		120	Only α
14	Methanol	D-Glucose	90	10.0 / 1
15	Methanol		120	15.1 / 1[b]
16	Benzyl alcohol		120	7.6 / 1
17	Benzyl alcohol		150	9.0 / 1
18	Allyl alcohol		120	8.3 / 1
19	Methanol	D-Galactose	90	9.3 / 1
20	Methanol		120	12.0 / 1
21	Benzyl alcohol		120	7.0 / 1
22	Benzyl alcohol		150	10.6 / 1
23	Allyl alcohol		120	9.7 / 1
24	Methanol	D-Mannose	90	6.1 / 1
25	Methanol		120	9.8 / 1
26	Benzyl alcohol		120	6.9 / 1
27	Benzyl alcohol		150	10.2 / 1
28	Allyl alcohol		120	7.7 / 1

Schemes

Scheme 1:



Scheme 2:



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