NEW SYNTHESIS OF O- AND S-GLYCOSYL DERIVATIVES OF 2-CHLORO-3-CYANO-5-NITROPYRIDINE

G. Pastuch[a], I. Wandzik[a], W. Szeja[a], G. Grynkiewicz[b], J. Ramza[b], W. Priebe[c], W. Pucko[b]

[a] *Silesian University of Technology, Dept. of Chemistry, ul. Krzywoustego 8, 44-100 Gliwice, Poland*
[b] *Pharmaceutical Research Institute, ul. Rydygiera 8, 01-793 Warszawa, Poland*
[c] *The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, Box 60, TX 77030, USA*

Abstract

Titled glycosides were formed in mild conditions in reaction between 2-chloro-3-cyano-5-nitropyridine as alkylating reagent and reducing (or 1-thiolo respectively) monosaccharides.

Introduction

Simple aryl and heteroaryl O- and S-glycosides are widely used as glycosyl donors and acceptors in chemical synthesis of oligosaccharides and complex glycosides[1,2] alike. Moreover, these compounds also demonstrate good compatibility with technologies employed for biocatalytic transglycosylation reactions, which constantly gain significance because their high stereoselectivities and ability to adopt unprotected saccharides as substrates. Until recently, 2- and 4-nitrophenyl glycosides were the most commonly used enzyme substrates[3] for analytical as well as preparative purposes.

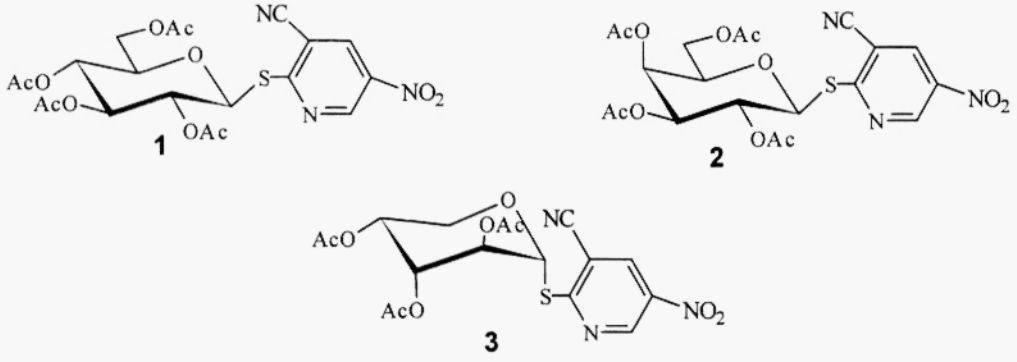
Heterocycles are by far the most numerous group of organic compounds and their role in medicinal chemistry and pharmacology as selective ligands of functional biopolymers is paramount[4]. However, many prospective drug candidates from this class suffer from poor bioavailibility and unfavourable biodistribution. It is well known that glycoconjugation constitutes one of the principal modes of metabolic conversion. Glycosides often constitute a biologically active form of complex, water insoluble natural aglycones. Likewise, there are numerous examples of synthetic drugs for which favourable tune up of efficacy have been achieved by glycosidation[5].

For the reasons stated above, extending methodology of chemical synthesis of glycosides to heteroaromatic and heterocyclic compounds is timely goal. As the first step of a project devoted to the prodrug strategy we have elaborated facile method for synthesis of heteroaromatic O- and S-glycosides. Obtained model compounds will be used for studies of enzymatic reactions, as well, as for studies of bioavailibility.

Result and Discussion

While traditional methods of synthesis leading to O- and S- aryl[6,7] and heterocyclic glycosides[8,9,10] employ anomeric substituent exchange reactions, the reverse approach utilising nucleophilic substitution of an activated aromatic halogen atom is becoming increasingly popular. Efficiency of such aromatic substitution is determined by a substrate ability to form an intermediate Meisenheimer complex[11], hence deprotonation of incoming nucleophile is advocated. Naturally, in case of sugar substrates, protected 1-OH and 1-SH derivatives, which can be easily deprotonated by action of sodium hydride, seem the first choice. However, considering this method of a nucleophile activation too harsh for many complex prospective aglycons, we have decided to look for the mildest conditions under which arylation of sugars with free hemiacetal position is still reasonably effective. Although polar aprotic solvents favouring cation solvation, like dimethylformamid (DMF) and dimethylsulfoxide (DMSO), in combination with medium-strength bases (e.g. K_2CO_3) are immediate candidates for reaction medium which enhances a "naked nucleophile" reactivity, we have tested many other possibilities. It is worth mentioning, that very cheap and convenient in work up solvent such as acetone offers excellent performance as the reaction medium.

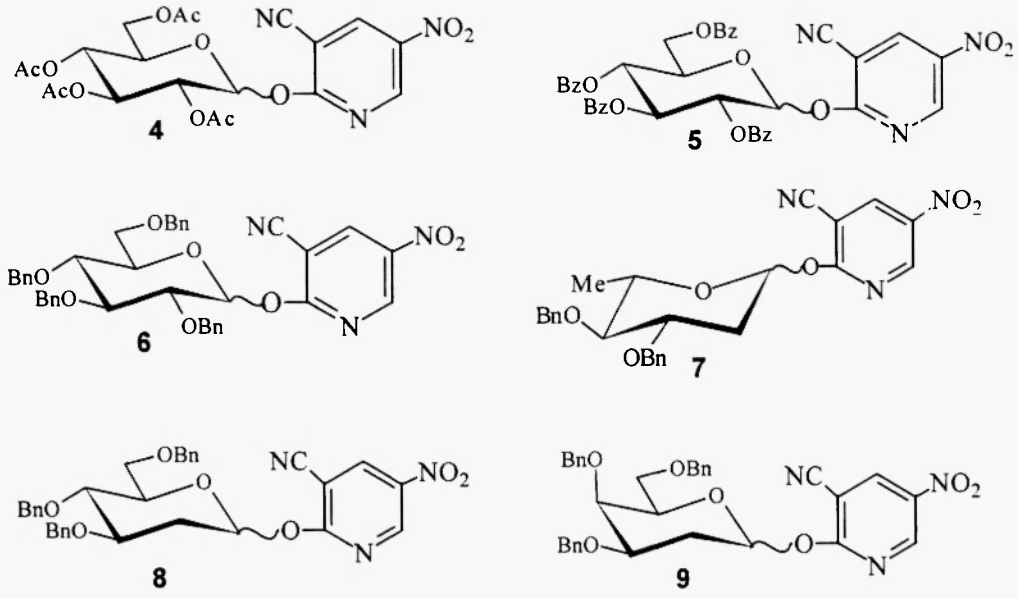
At first, formation of S-glycosyl compounds from 1-thiolo derivatives of D-*glucose*, D-*galactose* and D-*arabinose* were tested. In our previous report 2-chloro-5-nitropyridine[9] and 2-bromopyridine N-oxide[10] were chosen as alkylating reagents, now we report application of 2-chloro-3-cyano-5-nitropyridine (2-chloro-5-nitronicotinonitrile). We considered it important to limit not only the strength of a basic component of the reaction mixture, but also its amount in order to check compatibility of acyl protecting group with designed experimental protocol. It turned out that arylation of acetylated 1-thiopyranoses proceeded smoothly in acetone solution at room temperature, in the presence of only two equivalents of potassium carbonate. This simple procedure afforded heteroaryl thioglycosides **1-3** (Scheme 1) in very good yields. The reaction is highly stereoselective, affording only 1,2-trans glycosides, as evidenced by examination of the products ^{1}H NMR spectra. Results of our experiments are collected in Table 1.



Scheme 1. Heteroaryl S-glycosides.

In the next step, the scope of the new procedure was tested with an array of less nucleophilic substrates, such as differently protected reducing monosaccharides. We have found that in case of reaction between D-*glucose* derivatives and 2-chloro-5-nicotinonitrile, potassium carbonate is effective enough to secure formation of a product, as an anomeric mixture, in a good yield. In case of 2,3,4,6-tetra-O-acetyl-D-glucopyranose β-D-aryl glucosides **4**, (Scheme 2) prevailed, indicating higher reactivity of the equatorial anomeric alcoxylate, in agreement with earlier observations of Schmidt[6]. However, glucopyranosyl substrates protected with benzoyl or benzyl groups underwent arylation affording mainly

products with α-anomeric configuration (compounds **5**, **6**). An attempt to extend the procedure for 2-deoxypyranosyl series was unsuccessful, due to the low rate of the anomeric deprotonation. In this case both: the solvent and the base had to be replaced in order to achieve good yields. Under typical alcoxide generation conditions, comprising employment of sodium hydride – tetrahydrofuran mixture, only benzylated 2-deoxypyranoses could be efficiently glycosylated. As could be predicted, anomeric mixtures of compounds **7-9** were formed in such cases.



Scheme 2. Heteroaryl O-glycosides.

Table 1. Reactions of sugars with 2-chloro-5-nitronicotinonitrile.

Entry	Product	Reaction time [min]	Yield [%]	α:β*
Reactions of 1-thiosugars with 2-chloro-5-nitronicotinonitrile[a]				
1	**1**	15	70	0:1
2	**2**	20	87	0:1
3	**3**	20	64	0:1
Reactions of reducing sugars with 2-chloro-5-nitronicotinonitrile[a]				
4	**4**	48	45	1:6
5	**5**	24	65	4:1
6	**6**	48	71	5:1
Reactions of 2-deoxysugars with 2-chloro-5-nitronicotinonitrile[b]				
7	**7**	24	46	1:5
8	**8**	22	78	1:3
9	**9**	20	79	6:5

Reaction conditions, A: sugar (0.1 mol), 2-chloro-5-nitronicotinonitrile (0.1 mol), K_2CO_3, acetone, r.t., **B:** sugar (0.1 mol), 2-chloro-5-nitronicotinonitrile (0.1 mol), NaH, THF, molecular sieves 4Å, r.t

* determined by 300 MHz ^{1}H NMR analysis

Described method of aryl and heterocyclic glycoside synthesis is exceedingly simple and quite effective. Thioglycosyl compounds are obtained stereoselectively, while anomeric mixtures of O-glycosides are separable chromatographically, either in protected or deprotected form, providing an interesting entry into new biologically active glycoconjugates.

Experimental

General methods. ^{1}H NMR spectra were recorded for solutions in $CDCl_3$ (internal Me_4Si) on Varian 300 MHz spectrometer. Reactions were monitored by TLC on precoated plates of silica gel 60 (Riedel-de Haen, 37360), components were detected by spraying the plates with 10% sulphuric acid in ethanol followed by heating. Chromatographic purification was done with silica gel 60 (Merck) 0.063-0.2 mm. All glycosylation reactions were carried out in anhydrous solvents. All organic solutions were concentrated under reduced pressure at 40°C. Solvents and molecular sieves 4Å were commercially available (Aldrich, Merck, POCh) and were used without purification. Benzyl protected 2-deoxy sugars[12] were prepared as described in the literature.

General methods for the preparation of 1-thioglycosides derivatives of 5-nitronicotinonitrile (1-3):
Thiosugar (0.1 mmol) was dissolved in acetone (10 ml) and than the 2-chloro-5-nitronicotinonitrile (0.1 mmol) and potassium carbonate (0.1 mmol) were added. Resulting mixture was stirred at room temperature for about 20 minutes. When the reaction was completed off (TLC), reaction mixture was filtrated. The filtrate was evaporated. The residue was purified by column chromatography on silica gel using hexane-ethyl acetate 4:1 as a solvent.

General methods for the preparation of O-glycosides derivatives of 5-nitronicotinonitrile (4-9):
Procedure A: Per-O-acetylated sugar with non-protected 1-OH group (0.1 mmol) was dissolved in acetone (10 ml) and than the 2-chloro-5-nitronicotinonitrile (0.1 mmol) and potassium carbonate (0.1 mmol) were added. Resulting mixture was stirred at room temperature. When the reaction was completed off (TLC), reaction mixture was diluted with toluene, neutralised with 5% solution of acetic acid and washed with water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel using hexane-ethyl acetate 3:1 as a solvent.

Procedure B: To a solution of benzyl protected 2-deoxy sugar with non-protected 1-OH group (0.1 mmol) in dry THF (10 ml) 2chloro-5-nitronicotinonitrile (0.1 mmol) was added and the solution was stirred for 1 hour with molecular sieves 4Å. Sodium hydride (1 mmol) was added and resulting mixture was stirred at room temperature for about 24 hours. The reaction mixture was diluted with toluene, neutralised with 5% solution of acetic acid and washed with water. The organic layer was dried (anhydr. $MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using hexane-ethyl acetate 4:1 as a solvent.

2-(2',3',4',6'-tetra-O-acetyl-1'-thio-β-D-glucopyranosyl)-5-nitronicotinonitrile, (1). Yield 70%; $[\alpha]_D$ +12.6° (c 0.2, $CHCl_3$), only β. ^{1}H NMR δ: 2.04, 2.05, 2.06, 2.07 (4s, 12H, CH_3CO), 3.91 (ddd, 1H, J=2.2, 4.4, 10.0 Hz, H-5'), 4.10 (dd, 1H, J=2.2, 12.5 Hz, H-6'b), 4.26 (dd, 1H, J=4.4, 12.5 Hz, H-6'a), 5.19 (dd, 1H, J=10.0, 10.0 Hz, H-4'), 5.32 (dd, 1H, J=9.3, 9.3 Hz, H-3'), 5.38 (dd, 1H, J=9.0, 9.0 Hz, H-2'), 5.96 (d, 1H, J=10.3 Hz, H-1'), 8.64 (d, 1H, J=2.4 Hz, H-4), 9.40 (d, 1H, J=2.4 Hz, H-6).

2-(2',3',4',6'-tetra-O-acetyl-1'-thio-β-D-galactopyranosyl)-5-nitronicotinonitrile, (2). Yield 87%; $[\alpha]_D$ +48.6° (c 0.4, $CHCl_3$), only β. ^{1}H NMR δ: 2.01, 2.02, 2.06, 2.19 (4s, 12H, CH_3CO), 4.07-4.17 (m, 3H, H-5', H-6'a, H-6'b), 5.21 (dd, 1H, J=3.4, 10.0 Hz, H-3'), 5.48-5.55 (m, 2H, H-2', H-4'), 5.97 (d, 1H, J=10.5 Hz, H-1'), 8.65 (d, 1H, J=2.4 Hz, H-4), 9.41 (d, 1H, J=2.4 Hz, H-6).

2-(2',3',4'-tri-O-acetyl-1'-thio-α-D-arabinopyranosyl)-5-nitronicotinonitrile, (3). Yield 64%; $[\alpha]_D$ +13.2° (c 0.3, $CHCl_3$), only α. ^{1}H NMR δ: 2.11, 2.16, 2.20 (3s, 9H, CH_3CO), 3.83 (dd, 1H, J=3.6, 11.7 Hz, H-5'b), 4.11 (dd, 1H, J=7.3, 11.7 Hz, H-5'a), 5.30-5.39 (m, 3H, H-2', H-3', H-4'), 6.40 (d, 1H, J=2.4 Hz, H-1'), 8.63 (d, 1H, J=2.4 Hz, H-4), 9.42 (d, 1H, J=2.4 Hz, H-6).

2-(2',3',4',6'-tetra-O-acetyl-1'-O-D-glucopyranosyl)-5-nitronicotinonitrile, (4). Yield 45%; α:β=1:6. ^{1}H NMR δ: 2.05, 2.06, 2.07, 2.08 (4s, 12H, CH_3CO), 3.98 (ddd, J=2.4, 4.2, 9.3 Hz, H-5'α,β), 4.14 (dd, J=2.4, 12.2 Hz, H-6'b α,β), 4.30 (dd, J=4.2, 12.2 Hz, H-6'a α,β), 5.22-5.29 (m, H-2'α), 5.25 (dd, J=9.3, 9.3 Hz, H-4'β), 5.27 (dd, J=9.3, 9.3 Hz, H-4'α), 5.35 (dd, J=8.8, 8.8 Hz, H-2'β), 5.41 (dd, J=8.1, 8.1 Hz, H-3'β), 5.65 (dd, J=9.8, 9.8 Hz, H-3'α), 6.24 (d, J=7.6 Hz, H-1'β), 6.92 (d, J=3.9 Hz, H-1'α), 8.75 (d, J=2.7 Hz, H-4), 9.23 (d, J=2.7 Hz, H-6).

2-(2',3',4',6'-tetra-O-benzoyl-1'-O-D-glucopyranosyl)-5-nitronicotinonitrile, (5). Yield 65%; α:β=4:1. ^{1}H NMR δ: 4.52 (dd, J=4.6, 12.2 Hz, H-6'a α,β), 4.65 (dd, J=2.7, 12.2 Hz, H-6'b α,β), 4.71 (ddd, J=2.7, 4.6, 9.8 Hz, H-5'α,β), 5.66 (dd, J=3.7, 3.7 Hz, H-2'α), 5.67 (dd, J=6.2, 6.2 Hz, H-2'β), 5.89 (dd, J=9.8, 9.8 Hz, H-4'α,β), 6.03 (dd, J=8.7, 8.7 Hz, H-3'β), 6.36 (dd, J=10.0, 10.0 Hz, H-3'α), 6.64 (d, J=6.6 Hz, H-1'β), 7.15 (d, J=3.6 Hz, H-1'α), 7.26-8.01 (m, Ph), 8.52 (d, J=2.7 Hz, H-4β), 8.68 (d, J=2.7 Hz, H-4α), 8.86 (d, J=2.7 Hz, H-6α), 8.98 (d, J=2.7 Hz, H-6β).

2-(2',3',4',6'-tetra-O-benzyl-1'-O-D-glucopyranosyl)-5-nitronicotinonitrile, (6). Yield 71%; α:β=5:1. ^{1}H NMR δ: 3.61 (dd, J=1.9, 10.8 Hz, H-6'a α,β), 3.74 (dd, J=3.4, 10.8 Hz, H-6'b α,β), 3.79-3.83 (m, H-2', H-4'), 4.04 (ddd, J=1.9, 3.4, 10.2 Hz, H-5'α,β), 4.16 (dd, J=9.4, 9.4 Hz, H-3'α,β), 4.45 and 4.55 (AB, J=12.1, 12.1 Hz, CH_2Ph), 4.54 and 4.87 (AB, J=11.3, 11.3 Hz, CH_2Ph), 4.60 and 4.76 (AB, J=12.0, 12.0 Hz, CH_2Ph), 4.89 and 4.97 (AB, J=11.0, 11.0 Hz, CH_2Ph), 6.08 (d, J=6.9 Hz, H-1'β), 6.83 (d, J=3.3 Hz, H-1'α), 7.15-7.35 (m, Ph), 8.63 (d, J=2.7 Hz, H-4α,β), 9.07 (d, J=2.7 Hz, H-6α), 9.16 (d, J=2.7 Hz, H-6β).

2-(2'-deoxy-3',4'-di-O-benzyl-1'-O-L-rhamnopyranosyl)-5-nitronicotinonitrile, (7). Yield 46%; α:β=1:5. ^{1}H NMR δ: 1.41 (d, J=6.3 Hz, CH_3(H-6')β), 1.47 (ddd, J=10.5, 11.0, 12.5 Hz, H-2'$_{ax}$β), 1.48 (d, J=6.3 Hz, CH_3(H-6')α), 1.84 (ddd, J=2.9, 10.5, 13.0 Hz, H-2'$_{ax}$α), 2.56-2.60 (m, H-2'$_{eq}$α), 2.78 (ddd, J=2.2, 4.9, 12.5 Hz, H-2'$_{eq}$β), 3.27 (dd, J=8.5, 9.0 Hz, H-4'β), 3.38-3.40 (m, H-4'α), 3.66 (dq, J=6.3, 9.0 Hz, H-5'β), 3.85 (ddd, J=4.9, 8.5, 11.0 Hz, H-3'β), 3.91-3.94 (m, H-3'α), 4.43-4.47 (m, H-5'α), 4.61 and 4.67 (AB, J=11.5, 11.5 Hz, CH_2Ph), 4.69 and 4.96 (AB, J=11.0, 11.0 Hz, CH_2Ph), 5.81 (dd, J=2.2, 10.5 Hz, H-1'β), 6.83 (dd, J=2.9, 10.5 Hz, H-1'α), 7.26-7.35 (m, Ph), 8.59 (d, J=3.0 Hz, H-4α,β), 9.02 (d, J=3.0 Hz, H-6β), 9.13 (d, J=3.0 Hz, H-6α).

2-(2'-deoxy-3',4',6'-tri-O-benzyl-1'-O-D-glucopyranosyl)-5-nitronicotinonitrile, (8). Yield 78%; α:β=1:3. ^{1}H NMR δ: 1.67-1.74 (m, H-2'$_{ax}$α), 1.83 (ddd, J=2.5, 10.3, 13.0 Hz, H-2'$_{ax}$β), 2.66-2.70 (m, H-2'$_{eq}$α), 2.76 (ddd, J=2.2, 4.6, 12.9 Hz, H-2'$_{eq}$β), 3.48-3.52 (m, H-5'α), 3.60-3.92 (m, H-6'aα,β, H-6'bα,β, H-5'β, H-4'α,β), 4.34-4.41 (m, H-3'α), 4.44-4.46 (m, H-3'β), 4.55 (s, CH_2Ph) 4.59 and 4.91 (AB, J=10.8, 10.8 Hz, CH_2Ph), 4.60 and 4.67 (AB, J=11.6, 11.6 Hz, CH_2Ph), 5.83 (dd, J=2.2, 10.2 Hz, H-1'β), 6.16 (dd, J=3.2, 10.2 Hz, H-1'α), 7.19-7.37 (m, Ph), 8.59 (d, J=3.3 Hz, H-4α,β), 9.10 (d, J=3.3 Hz, H-6β), 9.31 (d, J=3.3 Hz, H-6α).

2-(2'-deoxy-3',4',6'-tri-O-benzyl-1'-O-D-galactopyranosyl)-5-nitronicotinonitrile, (9). Yield 79%; α:β=5:6. ^{1}H NMR δ: 1.22-1.33 (m, H-2'$_{ax}$α), 1.44 (ddd, J=2.7, 10.0, 13.6 Hz, H-2'$_{ax}$β), 2.42-2.50 (m, H-2'$_{eq}$α), 2.63 (ddd, J=2.9, 4.4, 13.6 Hz, H-2'$_{eq}$β), 3.58 (dd, J-6.1, 9.5 Hz, H-6'aβ), 3.67 (dd, J=6.1, 9.5 Hz, H-6'aα), 3.75-3.94 (m, H-5'α, H-5'β, H-

4'α,β), 4.42-4.98 (m, H-3'α, β, CH_2Ph), 5.85 (dd, J=2.7, 10.7 Hz, H-1'β), 6.19 (dd, J=2.4, 9.8 Hz, H-1'α), 7.25-7.37 (m, Ph), 8.57 (d, J=2.9, H-4α,β), 9.04 (d, J=2.9 Hz, H-6β), 9.08 (d, J=2.9 Hz, H-6α).

References:

1. Garegg, P. J. *Adv. Carbohydr. Chem. Biochem.* 1997, **52**, 179 and references cited therein.
2. Huchel, U.; Schmidt, C.; Schmidt, R. R. *Eur. J. Org. Chem.* 1998, 1353.
3. For recent rewievs, see: Palcic, M. M.; Hisdsgaul, O. *Trends Glycosci. Glucotechnol.* 1996, **8**, 37; Kren, V.; Thiem, J. *J. Chem. Soc. Rev.* 1997, **26**, 463.
4. Kleemann, A.; Engel, J.; Kutscher, B.; Reichert, D. "Pharmaceutical Substances"; Thieme Stuttgart: New York, 1999.
5. Hasegawa, O. H.; Suami, T. "Carbohydrates-Synthetic Methods and Application in Medicinal Chemistry"; Kadanska, Tokyo&VCH: Weinheim, 1992; Witczak, Z. J.; Nieforth, K. A."Carbohydrates in Drug Design"; Marcel Dekker, Inc.: New York, 1997.
6. Huchel, U.; Schmidt, C.; Schmidt, R. R. *Tetrahedron Lett.* 1995, **36**, 9457.
7. Palme, M.; Vasella, A. *Helv. Chim. Acta* 1995, **78**, 959.
8. Driguez, H.; Szeja, W. *Synthesis* 1994, 1413.
9. Pastuch, G.; Szeja, W. *Carbohydr. Lett.* 1997, **2**, 281.
10. Pastuch, G.; Szeja, W. *Polish J. Chem.* 2000, **74**, 227.
11. March, J. Advanced Organic Chemistry; A Wiley-Interscience Publication, Fourth Edition, 1992; 13; pp 641.
12. Wandzik, I., Szeja, W., *Polish. J. Chem.*, 1998, **72**, 703.

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