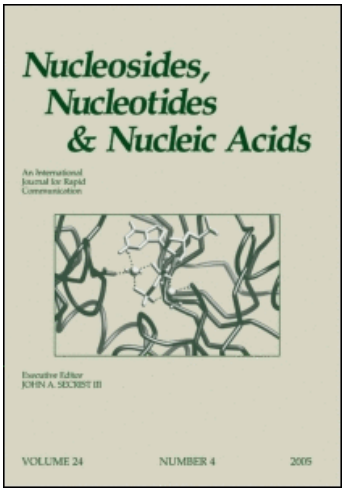


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Microwave Induced Synthesis of Ribonucleosides on Solid Support

Mariola Andrzejewska[a]; Jarosław Kamiński[b]; Zygmunt Kazimierczuk[a]
[a] Institute of Chemistry, Agricultural University, Warsaw, Poland [b] Institute of Industrial Chemistry, Warsaw, Poland

Online publication date: 06 March 2002

To cite this Article Andrzejewska, Mariola , Kamiński, Jarosław and Kazimierczuk, Zygmunt(2002) 'Microwave Induced Synthesis of Ribonucleosides on Solid Support', Nucleosides, Nucleotides and Nucleic Acids, 21: 1, 73 — 78
To link to this Article: DOI: 10.1081/NCN-120006532
URL: http://dx.doi.org/10.1081/NCN-120006532

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 21(1), 73–78 (2002)

MICROWAVE INDUCED SYNTHESIS OF RIBONUCLEOSIDES ON SOLID SUPPORT

Mariola Andrzejewska,[1] Jarosław Kamiński,[2] and Zygmunt Kazimierczuk[1,*]

[1]Institute of Chemistry, Agricultural University, 159C Nowoursynowska St., 02-787 Warsaw, Poland
[2]Institute of Industrial Chemistry, 8 Rydygiera St., 01-793 Warsaw, Poland

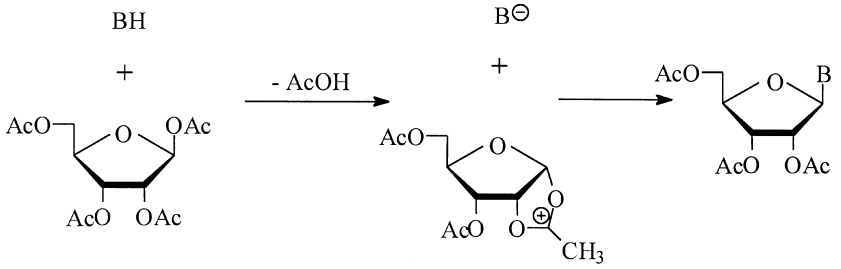
In recent years, the interest is growing in employing microwave irradiation (MWI) for the synthesis of organic compounds. So far this approach did not, except for few cases, result in obtaining different spectrum of products as compared with traditional methods. However, due to the relatively short duration of irradiation-generated heat pulses and shortening of reaction times, MWI gives usually better yields and lower amounts of by-products (for reviews see[1,2]). The data on the use of MWI in the chemistry of nucleosides, nucleotides and nucleic acids are extremely scarce. The only three cases known to us are: (i) MWI deprotection of oligodeoxyribonucleotides[3], (ii) synthesis and deprotection of 5′-trityl(acyl)-2′5′-dideoxynucleosides[4], and (iii) synthesis of imidazole *C*-nucleosides from appropriate unprotected hexoses or hexuloses and formamidine acetate[5]. Recently, we have obtained by MWI a number of adamantylated pyrimidines[6]. The aim of this preliminary study was to test the utility of the microwave-assisted procedure for nucleoside synthesis and to attract attention to the possible use of microwave induced reactions in nucleic acid chemistry. We have chosen the so-called 'fusion' approach. In this approach, the heterocyclic base reacts with the appropriate peracylated sugar at an elevated temperature (that is, above the melting points of the substrates), and vacuum is applied to remove the acetic acid generated. Catalytic amounts of Lewis acids promote the formation of the electrophilic sugar cation (Scheme 1). This approach has been previously

*Corresponding author. Fax +48-22-8221848; E-mail: kazimierczuk@delta.sggw.waw.pl

used for the synthesis of many purine and pyrimidine nucleosides and other heterocyclic systems [7, and references cited herein]. In some cases, substituted deoxyriboses, dideoxyriboses and glucals were used as sugar components instead of acylated ribose [e.g., [8–10]]. For Lewis acids, sulfonic acids, chlorine-substituted acetic acids, or phosphoric acid derivatives were commonly used. Occasionally, e.g., when using 1-trichloroacetyl-substituted ribose or 1,5-diacetyl-2,3-dideoxyribose, no acid catalyst appeared necessary[9,11].

In the present study, we have chosen 2,6-dichloropurine, 6-chloropurine, benzotriazole and benzimidazole and its disubstituted derivatives as the model bases. 1,2,3,5-Tetra-*O*-acetyl-β-*D*-ribofuranose was used as a sugar derivative in most experiments. The analysis of reaction products was performed by HPLC and UV detection at 254 nm. Tetraacetylribose shows no absorption at this wavelength, and the extinction coefficients of the bases used do not differ considerably from those of their respective acylated nucleosides. Therefore, the corresponding reaction yields can be directly estimated from the HPLC-profile with an approximately 5% accuracy. In experiments employing 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-*D*-ribofuranose, an appropriate correction was made for the UV absorption by the substituted ribose.

In a typical experiment, 1 mmol of a heterocyclic base and 1 mmol of acylated ribose were dissolved in ethyl acetate, then 1 g of silica gel 60 (200–400 mesh, Merck) was added and the solvent was removed by evaporation. The dry residue was transferred into a glass beaker placed in an alumina bath (250 g) and irradiated for 3 min at 350 W in a Whirlpool domestic microwave oven (2450 MHz). The reaction mixture was treated with 100 mL methanol, and a sample of the methanolic solution was analyzed by HPLC. The volume of the remaining methanolic solution was next reduced to about 20 mL and treated with sodium methanolate or saturated methanolic ammonia for deacylation and subsequent amination (in case of chloropurines), respectively. The resulting nucleosides were then isolated by flash chromatography and compared, using both chromatographic and



Scheme 1.

spectroscopic methods, with those prepared according to the previously described 'classical' procedures.

In our experience, the best results were obtained employing silica gel as the solid support phase. Our synthetic attempts using no solid support or using other solid carriers, such as aluminum oxide and montmorillonites KSF and K10, brought disappointing results. Using Lewis acids for catalysts did not improve the respective chromatographic patterns. As expected, the nucleosides formed were the thermodynamically controlled anomers 1β (for benzimidazoles) and 9β (for purines). In the case of benzotriazole, a considerable contribution from 2β isomer was observed. Our attempts to employ MWI for transglycosylation using nitrogen bases and N^2,2′,3′,5′,-tetracetylguanosine as the sugar donor (adsorbed on silica gel) were discouraging and resulted in negligible yields. Under the conditions used in this study, there was no need for the use of vacuum, as the acetic acid formed was immediately leaving the molten reaction mixture.

Table 1. Selected Results Showing the Effect of Reaction Conditions on Nucleoside Yields

Base	Sugar Derivative and Reaction Condition	Base Recovery %	(Nucleoside Isomer and % Yield)
2,6-dischloropurine	TAcR MWI	36	(9β) 47
	TAcR F(−TSA)	17	(9β) 69[a]
	AcTBzR MWI	18	(9β) 37
6-chloropurine	TacR MWI	35	(9β) 34
benzimidazole	TacR MWI	71	(1β) 17
	TAcR F(−TSA)	90	(1β) 3
	TAcR F(+Sg)	66	(1β) 17
5,6-dimetylobenzimidazole	TAcR MWI	52	(1β) 21
	TAcR F(−TSA)	88	(1β) 4
5,6-dichlorobenzimidazole	TAcR MWI	40	(1β) 26
	TAcR(H+Sg)	63	(1β) 27
	TAcR F(−TSA)	75	(1β) 14
	TAcR F(+TSA)	37	(1β) 51
	TAcR F(+Sg)	36	(1β) 59
benzotriazole	TAcR MWI	45	(1β)44 (2β) 5
	TAcR F(−TSA)	85	(1β)8 (2β) 2
	TAcR F(+TSA)[b]	28	(1β)27 (2β) 2
	TAcR F(+Sg)	27	(1β)63 (2β) 3

TacR = 1,2,3,5-*O*-tetraacetyl-β-*D*-ribofuranose; AcTBzR = 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-*D*-ribofuranose. [a]Ref. [7] 55–90%; [b]Degradation products were observed; MWI - microwave irradiation; F(−TSA) – fusion reaction using base : TAcR = 1 : 1, no p-toluenesulfonic acid, 150°C, 10mm Hg; F(+TSA) – fusion reaction as above, using 2% of p-toluenesulfonic acid; H(+Sg) – hot dryer, 150°C, 15min, reaction mixture as for MWI, that is, using silica gel (Sg) as adsorbent.

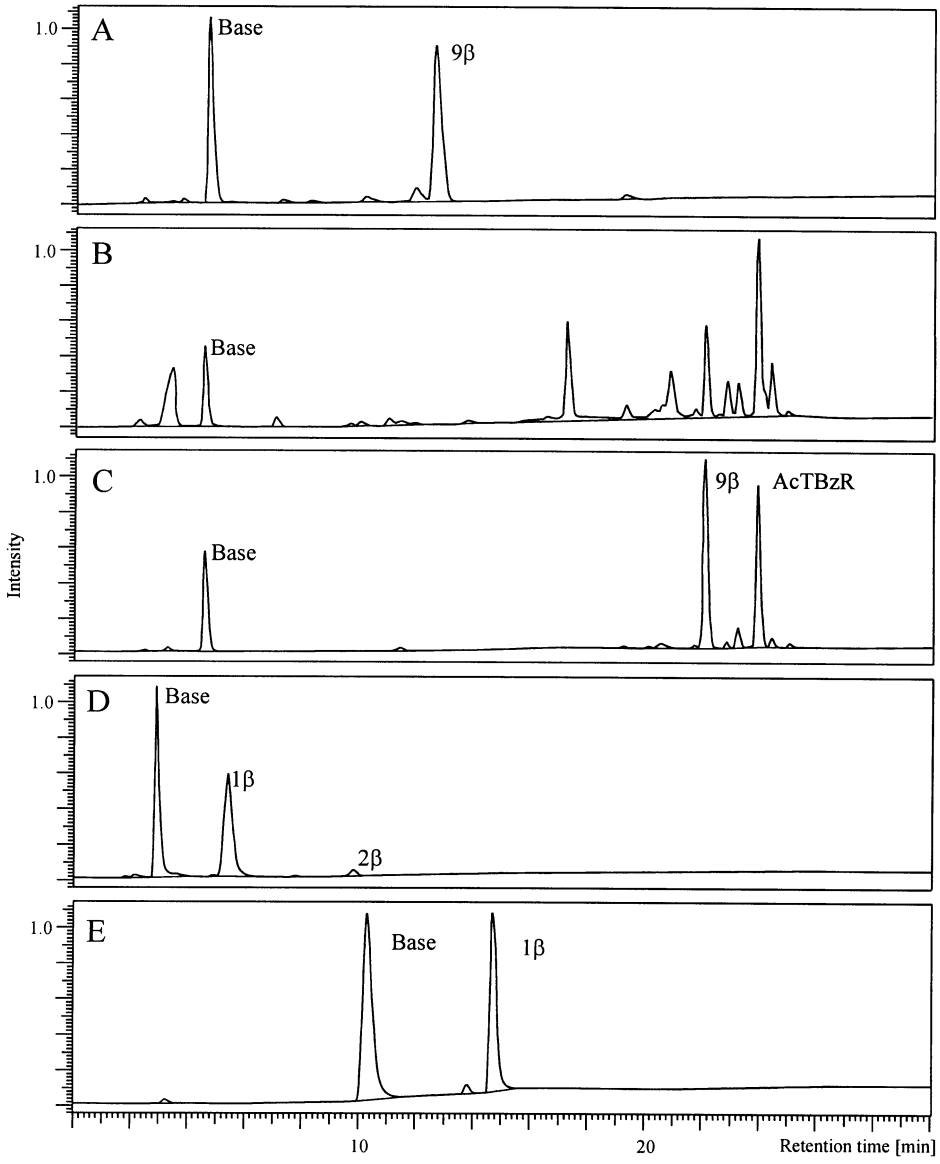


Figure 1. HPLC profiles of MWI fusion synthesis of ribonucleosides. Lichrospher 100 RP-18 (5μ) column (250 × 4.6 mm) was used. The solvent used for elution: water/methanol gradient (50% → 90%).A) 2,6-dichloropurine and TAcR onsilica gel; B) 2,6-dichloropurine and TAcR on alumina (an example of unsuccessful synthetic attempt); C) 2,6-dichloropurine and AcTBzR on silica gel; D) benzotriazole and TAcR on silica gel; E) 5,6-dichlorobenzimidazole and TAcR on silica gel.

There were two questions we tried to answer with these experiments. The first one was whether microwave heating will result in better yields than the 'conventional' method. The second one was the significance of the use of silica gel for catalyst instead of, e.g., *p*-toluenesulfonic acid that is commonly used in the fusion reaction. An exemplary experiment with 5,6-dichlorbenzimidazole and 1,2,3,5-tetraacetyl-β-*D*-ribofuranose adsorbed onto silica gel showed no difference in nucleoside yield between microwave and hot dryer heating. Thus, the role of microwaves was obviously that of a heating factor with no catalytic role.

Additionally, we tested the effect of *p*-toluenesulfonic acid and silica gel on ribonucleoside synthesis in a number of experiments. Whereas the use of an acid catalyst brought no extra benefit in the case of bases carrying an acidic hydrogen, e.g., for 2,6-dichloropurine, it played a significant role for benzimidazole and benzotriazole derivatives. Interestingly, the silica gel-aided fusion procedure resulted in markedly better yields of benzimidazole nucleosides than its *p*-toluenesulfonic acid-catalyzed counterpart. That was despite the possibility that reagents adsorbed onto silicagel surface may contact each other less effectively than in a molten reaction mixture. The improvement in reaction yields was particularly well seen for benzotriazole, as the formation of degradation products using silica gel was inconsiderable compared with that using *p*-toluenesulfonic acid.

The results of this study are summarized in Table 1. For typical HPLC profiles see Fig. 1.

ACKNOWLEDGMENTS

The study was supported in part by Warsaw Agricultural University (grant No. 50409270012) and by the Foundation for Development Diagnostic and Therapy. The authors are grateful to Dr S. J. Chrapusta for helpful discussions.

REFERENCES

1. Loupy, A.; Petit, A.; Hamelin, J.; Texier-Boullet, F; Jacquault, P.; Mather, D. Synthesis **1998**, 1213–1234.
2. Deshaves, S.; Liagre, M.; Loupy, A.; Luche, J-L.; Petit, A. Tetrahedron **1999**, *55*, 10,851–10,870.
3. Kumar, P.; Gupta, K.C. Nucleic Acids Res. **1997**, *25*, 5127–5129.
4. Kumar, P.; Gupta, K.C.; Chem. Lett. **1996**, *8*, 635–636.
5. Tschamber, T.; Rudyk, H.; Le Nouen, D. Helv. Chim. Acta. **1999**, *82*, 2015–2019.
6. Maurin, J.K.; Lasek, W.; Górska, A.; Świtaj, T.; Wamil, M.; Mlynarczuk, J.; Kazimierczuk, Z. Anticancer Drug Des. 2000 in press.

7. Organic Reactions, Vol. **55**(Willey & Sons Inc. New York, **2000**, Eds. Ruh-Polenz, C. and Vorbruggen, H.).
8. Robins, M.J.; Khwaja, T.A.; Robins, R.K.J. Org. Chem. **1970**, *35*, 636–639.
9. Diekmann, E.; Friedrich, K.; Fritz, K-G. J. prakt. Chem. **1993**, *335*, 425–429.
10. Leutzinger, E.E.; Robins, T.K.; Townsend L.B. Tetrahedron Lett. **1968**, 4475–4478.
11. Onodera, K; Fukumi, H. Agric. Biol. Chem. **1963**, *27*, 526; through Chem. Abstr., **1964**, *57*, 15216b.

Received June 15, 2001
Accepted December 5, 2001