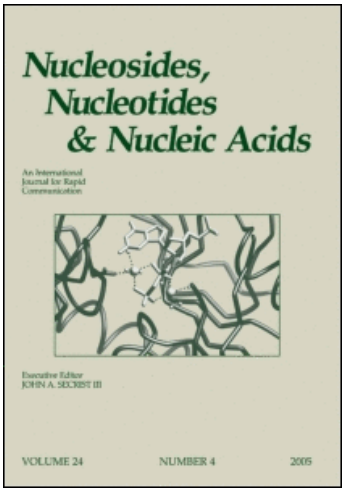

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Microwave-Assisted Synthesis of O′-Adamantylated Uracil-Derived Nucleosides

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ABSTRACT

Microwave-induced synthesis of O′-adamantyl derivatives of AZT, thymidine, 2′-deoxyuridine and uridine was investigated. Contrary to heterocyclus adamantylation of uracil and uridine in trifluoroacetic acid, the microwave-induced reaction provided sugar-substituted compounds.

Key Words: Microwave irradiation; O′-adamantylation; Solvent-free reactions; AZT; Thymidine; 2′-Deoxyuridine.

Our interest in adamantylated heterocyclic compounds was caused by our earlier findings that showed tumor necrosis factor alpha (TNF-α) production-enhancing activity of a series of pyrimidines and pyridines in a murine melanoma cell line transfected with human TNF-α gene.[1,2] We have previously reported the synthesis of a

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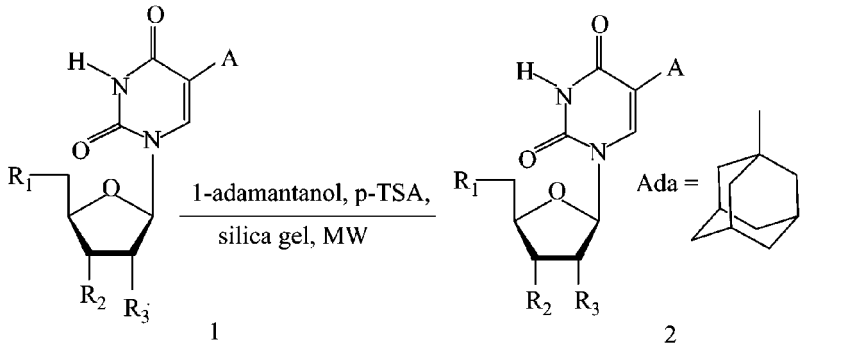
series of N- and C-adamantylated heterocycles obtained by adamantyl cation attack in refluxed trifluoroacetic acid.[3,4] These reaction conditions appeared unacceptably harsh for most biologically derived compounds, such as nucleosides and peptides. Yet the N-adamantylation of some pyridines and pyrimidines induced by microwave irradiation in a solventless system was found to yield much better synthetic effects.[3] In the last years, microwave-induced synthesis has become of growing interest because of increased reaction rates and yields. So far, this approach did not, except for few cases, result in obtaining different spectrum of products as compared with traditional methods (reviewed in Ref.[5,6]).

The data showing the use of microwave-aided synthesis in nucleic acids chemistry are yet extremely scarce. The only three previously published relevant studies known to us include: (i) microwave-induced deprotection of oligodeoxyribonucleotides,[7] (ii) synthesis and deprotection of 5′-S-trityl (acyl)-2′,5′-dideoxynucleosides,[8] and (iii) synthesis of imidazole C-nucleosides from the appropriate unprotected hexoses and hexuloses and formamidine acetate,[9] Recently, we have investigated the influence of microwave irradiation on the time course of the 'fusion' method used in the synthesis of ribonucleosides.[10] Below, we report the results of microwave-induced adamantylation of nucleosides adsorbed on silica gel with catalytic amounts of p-toluenesulfonic acid. The addition of the acid catalyst was necessary to induce the formation of reactive adamantyl cation from 1-adamantanol. The absence of a strong acid, or the presence of a weaker acid, for instance, the trichloroacetic acid, did not result in the formation of detectable amounts of any adamantylation product. Of all ribo- and deoxyribonucleosides tested, only uracil and thymine derivatives gave the O′-adamantylated daughter compounds (Sch. 1). Ribo- and deoxyribonucleosides derived from cytosine, adenine or guanine formed only decomposition products. The rational explanation for these findings is the formation of



1

1a A = CH, R_1 = OH, R_2 = N, R_3 = H
1b A = CH, R_1 = OH, R_2 = OH, R_3 = H
1c A = H, R_1 = OH, R_2 = OH, R_3 = H
1d A = H, R_1 = OH, R_2 = OH, R_3 = OH

2

2a A = CH, R_1 = OAda, R_2 = N, R_3 = H
2b A = CH, R_1 = OAda, R_2 = OH, R_3 = H
2c A = CH, R_1 = OAda, R_2 = OAda, R_3 = H
2d A = H, R_1 = OAda, R_2 = OH, R_3 = H
2e A = H, R_1 = OAda, R_2 = OAda, R_3 = H

Scheme 1.

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protonated form of the nucleosides by consuming p-toluenesulfonic acid followed by accelerated cleavage of the glycosylic bond.

It is well known that, of all nucleosides, the most resistant to acidic hydrolysis are those of uracil and thymine that can hardly be protonated. Adamantylation of uridine in refluxed trifluoroacetic acid resulted in the formation of 5-adamantyluridine, whereas thymidine was completely resistant under the same conditions.[4] Any O′-adamantylated nucleoside derivatives that could theoretically form in this reaction mixture will be immediately destroyed because of the liability of the ether bond in strong acid solution. In our experiments, the solventless acidic medium was constructed by adsorbing the respective nucleoside, 1-adamantanol and catalytic amounts of p-toluenesulfonic acid onto silica gel. Of the other support materials tested, only the use of montmorillonite K-10 has resulted in an acceptable yield, comparable to that employing silica gel, whereas the use of alumina and montmorillonite KSF ended in total failure – HPLC analysis showed no formation of adamantylated products on these supports. Following microwave irradiation of the mixture, the only products formed were O′-adamantylated nucleosides. The HPLC profiles of the reactions employing AZT (3′-azidothymidine), thymidine (using microwave irradiation and thermal heating), 2′-deoxyuridine and uridine are shown in Fig. 1.

The H-NMR studies of the isolated products revealed substitution of the nucleoside sugar moiety hydroxyl groups, whereas the heterocyclus remained intact. In the case of AZT that bears only one free 5′-hydroxyl group, a single reaction product was detected and isolated. The use of thymidine and 2′-deoxyuridine resulted, as expected, in the formation of three daughter compounds. We have isolated 3′,5′-diadamantyl- and 5′-O′-adamantyl derivatives of thymidine and 2′-deoxyuridine, whereas the attempts to obtain pure 3′-O-adamantylated compounds by flash chromatography were unsuccessful because we could not get rid of the contamination with the related 5′-monoadamantylated product. In the case of 'trihydroxylic' uridine, our attempts to isolate individual adamantylated products failed. We were unable to obtain any pure O′-adamantylated uridine with satisfactory analytical data. Even the major peak in the HPLC profile was found to be a mixture of mono-O-substituted derivatives. The yields of adamantylation reaction were generally low, most likely because of the elimination of water molecule from 1-adamantanol taking place concurrently with reactive adamantyl cation formation. The investigated reaction had, similarly to the majority of previously described microwave-assisted procedures, evidently thermal character. However, as we have found using thymidine for substrate, the yields of O′-adamantylated compounds using this approach were about twice higher than those obtained by optimized ''classical'' heating (250°C, 6 min) (see panels B and C in Fig. 1). The newly obtained adamantylated nucleosides were characterized by UV, H-NMR, elemental analysis and electrospray MS spectra.

General Procedure for Microwave Irradiation

A solution of nucleoside (1 mmol), 1-adamantanol (1 mmol, 152 mg) and p-toluenesulfonic acid (20 mg) in EtOH/water (1 : 1, 25 mL) was adsorbed onto silica gel.

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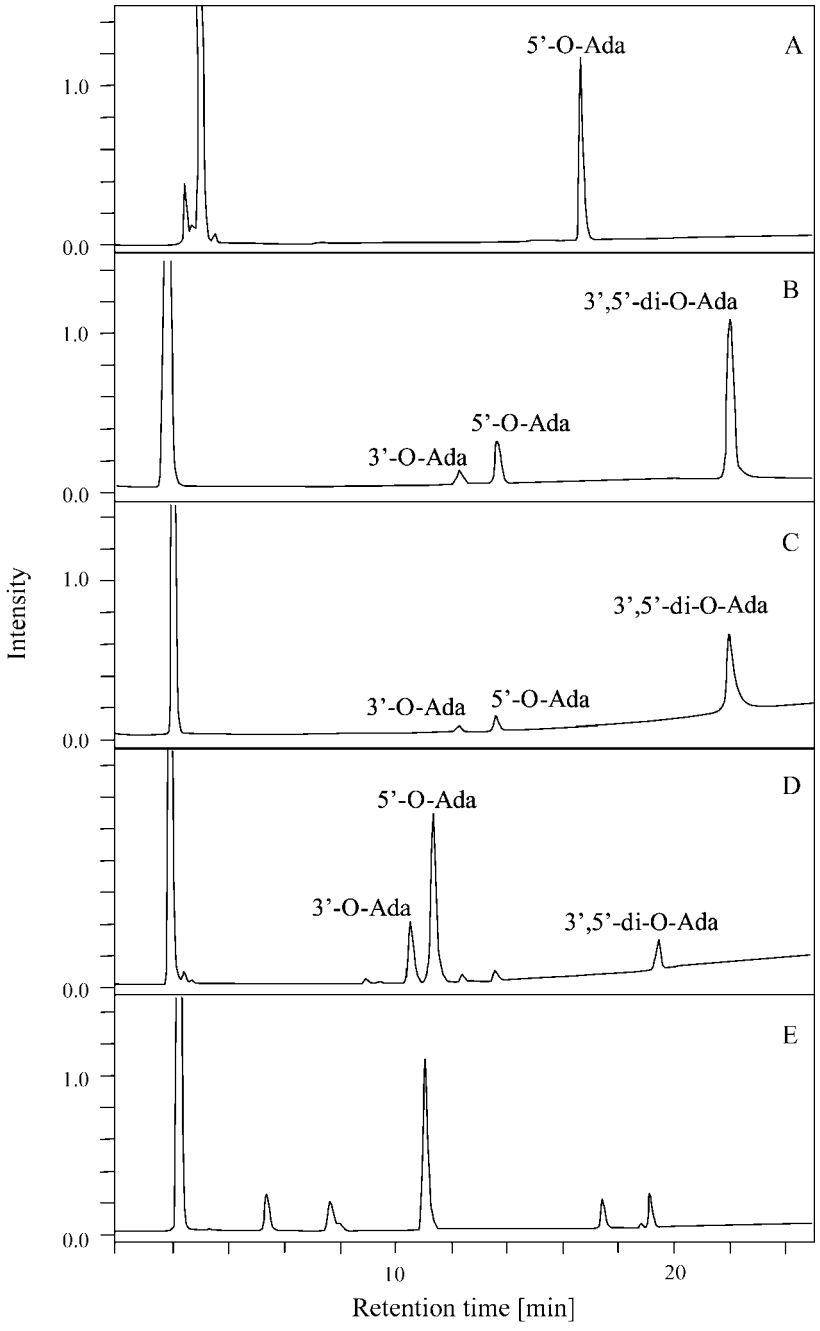

Figure 1. HPLC-profile of the reaction mixture after microwave irradiation and thermal heating. A) 3′-azido-3′-deoxythymidine, B) thymidine, C) thymidine (250°C, 6 min, metal bath), D) 2′-deoxyuridine, E) uridine.

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The solvent was removed by evaporation. The dry residue was transferred into a glass beaker placed in an alumina bath (200 g) and irradiated for 3 min at 350 W in a Whirlpool domestic microwave oven (2450 MHz). The reaction mixture was placed on the top of silica gel column and chromatographed with A) hexane (150 mL), then hexane-EtOAc (95 : 5, v/v) or B) $CHCl_3$ (150 mL) , then $CHCl_3$-MeOH (95 : 5, v/v). The corresponding fractions were evaporated and crystallized.

EXPERIMENTAL

All chemicals and solvents were purchased from Sigma-Aldrich. Melting points (uncorr.) were measured in open capillary tubes on a Gallenkamp melting point apparatus. Ultraviolet absorption spectra were recorded in a Techcomp UV 8500 spectrophotometer. ^{1}H-NMR spectra (in ppm) were measured with Varian Gemini 200 MHz and a Varian UNITYplus spectrometer at 298°K in D_6 (DMSO) using tetramethylsilane as internal standard. Flash chromatography was performed with Merck silica gel 60 (200–400 mesh). Analytical TLC was carried out on precoated silica gel F_{254} (Merck0 plates (0.25 mm thickness). Profiles HPLC were performed using Lichrospher 100RP-18 (5 μ) column (250 × 4.6 mm). The solvent used for elution; water/MeOH gradient (0 → 90%). Mass spectrum (ESI) was obtained with a model Mariner (Applied Biosystems) Intectra spectrometer.

5′-O-(1-adamantyl)-3′-azido-3′-deoxythymidine (2a). Yield: 20%. M.p. 168–170°C (from MeOH-H_2O). TLC: ($CHCl_3$/MeOH, 9:1) Rf 0.8.^{1}H-NMR (D_6 (DMSO)): 1.47–2.20 (20H, m, H-adamantyl, CH_3 2′-H), 3.61 (2H, d, 5′- and 5″-H), 3.93 (1H, q, 4′-H), 4.38 (1H, m, 3′-H), 6.09 (1H, t, 1′-H), 7.65 (1H, s, 6-H), 11.35 (1H, bs, H-N). UV: (MeOH/H_2O, 1 : 1) 267 (9 900); (MeOH/0.1N NaOH, 1 : 1) 266 (7 500). MS-ESI: (MNa^+) 424.5. Elemental analysis calcd. For $C_{20}H_{27}N_5O_4$: C, 59.84; H, 6.78; N, 17.44; found: C, 60.01; H, 6.80; N, 17.33.

5′-O-(1-adamantyl)-thymidine (2b). Yield: 11% (the first 2/3 of the fractions comprising the second zone of the silica gel column eluate). M.p. 201–202°C (decomp.) (from EtOH-H_2O). TLC: ($CHCl_3$/MeOH, 9:1) Rf 0.59.^{1}H-NMR (D_6 (DMSO)): 1.59–2.40 (20 H, m, H-adamantyl, CH_3 2′-H), 3.49–3.62 (2H, m, 5′- and 5″-H), 3.90 (1H, q, 4′-H), 4.19 (1H, m, 3′-H), 5.24 (1H, d, 3′-OH), 6.19 (1H, t, 1′-H), 7.69 (1H, s, 6-H), 11.31 (1H, bs, H-N). UV: (MeOH/H_2O, 1 : 1) 267 (9 800); (MeOH/0.1N NaOH, 1:1) 267 (7 800). MS-ESI: (MNa^+) 399.4. Elemental analysis calcd. for $C_{20}H_{28}N_2O_5$: C 63.81; H, 7.49; N, 7.44; found: C, 63.94; H, 7.60; N, 7.35.

3′-5′-di-O-(1-adamantyl)-thymidine (2c). Yield: 5%. 225–228°C (from MeOH-H_2O). TLC: ($CHCl_3$/MeOH, 9:1) Rf 0.82.^{1}H-NMR (D_6 (DMSO)): 1.59–2.40 (20H, m, H-adamantyl, CH_3 2′-H), 3.49–3.62 (2H, m, 5′- and 5″-H), 3.85 (1H, q, 4′-H), 4.32 (1H, m, 3′-H), 6.09 (1H, t, 1′-H), 7.63 (1H, s, 6-H), 11.28 (1H, bs, H-N). UV: (MeOH/H_2O, 1 : 1) 268 (9 600); (MeOH/0.1N NaOH, 1 : 1) 269 (7 500). MS-ESI: (MNa^+) 533.7. Elemental analysis calcd. for $C_{30}H_{42}N_2O_5$: C, 70.56; H, 8.29; N, 5.48; found: C, 70.68; H, 8.39; N, 5.33.

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5′-O-(1-adamantyl)-2′-deoxyuridine (2d). Yield: 9% (the first 2/3 of the fractions comprising the second zone of the silica gel column eluate). M.p. 241–244°C (decomp.) (from MeOH-H_2O). TLC: ($CHCl_3$/MeOH, 9:1) Rf 0.64.^{1}H-NMR (D_6 (DMSO)): 1.59–2.40 (17H, m, H-adamantyl, 2′-H), 3.40–3.59 (2H, m, 5′- and 5″-H), 3.86 (1H, q, 4′-H), 4.40 (1H, m, 3′-H), 5.26 (1H, d, 3′-OH), 5.61 (1H, d, 5-H), 6.07 (t, 1′-H), 7.99 (1H, d, 6-H), 11.28 (1H, bs, H-N). UV: (MeOH/H_2O, 1 : 1) 262 (10 000); (MeOH/0.1N NaOH, 1 : 1) 264 (8 100). MS-ESI: (MNa^+) 385.4 Elemental analysis calcd. for $C_{19}H_{26}N_2O_5$: C 62.97; H, 7.23; N, 7.73; found: C, 63.09; H, 7.36; N, 7.20.

3′,5′-di-O-(1-adamantyl)-2′-deoxyuridine (2e). Yield: 5%. M.p. 158–161°C (from EtOH-H_2O). TLC: ($CHCl_3$/MeOH, 9:1) Rf 0.83. ^{1}H-NMR (D_6 (DMSO)): 1.47–2.40 (32H, m, H-adamantyl, 2′-H), 3.40–3.61 (2H, m, 5′- and 5″-H), 3.82 (1H, q, 4′-H), 4.34 (1H, m, 3′-H), 5.61 (1H, d, 5-H), 5.61 (1H, d, 5-H), 6.09 (1H, t, 1′-H), 7.97 (1H, d, 6-H), 11.28 (1H, bs, H-N). UV: (MeOH/H_2O, 1 : 1) 264 (9 700); (MeOH/0.1N NaOH, 1 : 1) 266 (6 900). MS-ESI: (MNa^+) 519.6. Elemental analysis calcd. for $C_{29}H_{40}N_2O_5$: C, 70.13; H, 8.12; N, 5.64; found: C, 70.28; H, 8.18; N, 5.51.

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