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EFFICIENT SOLID-PHASE SYNTHESIS OF ISOCYTOSINE DERIVATIVES

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We have investigated the potential use of a solid-phase synthesis of novel 6-(arylmethyl)-5-methyl-isocytosine derivatives based on the immobilization of the corresponding 2-thiothymine on a Merrifield resin, oxidation of the immobilized form to the sulfone, and aminolysis of the latter under mild conditions.

Keywords: isocytosines, "addition-elimination" mechanism, antiviral activity, solid-phase synthesis.

Isocytosine derivatives are characterized by a number of practically useful properties amongst which a prominent place is occupied by the antiviral activity of specific members of this series which contain specific hydrocarbon radicals at atoms C-5 and C-6 of the pyrimidine ring and also at the exocyclic nitrogen atom [1]. The corresponding 2-N-unsubstituted and disubstituted derivatives [1, 2] can be readily prepared *via* condensation of the corresponding guanidine derivatives with 3-oxoesters in a basic medium. At the same time the synthesis of 2-N-monosubstituted members of this series unavoidably leads to the formation of a difficult to separate mixture of condensation products [3]. In this connection the preparation of the corresponding derivatives broadly involves the use of indirect methods based on the aminolysis of the corresponding S-alkylated 2-thiouracil derivatives [1, 2, 4-6]. This process generally occurs under rigid conditions and demands the use of a cosolvent [2, 4-6]. The absence of the latter usually leads to a lower yield of the target isocytosine derivatives and to difficulties in their separation and purification [1].

In this connection we have studied the use of a solid-phase synthetic strategy which is well established in the chemistry of peptides [7] in order to prepare the corresponding isocytosine derivatives. It should be noted that a solid-phase synthesis has already been successfully used in the chemistry of pyrimidine derivatives [3, 8].

The choice of starting materials and target products in the synthesis was carried out on the basis of a previously established structure – antiviral activity in this series of compounds [1].

We have used a Merrifield resin as solid carrier which contained 1-1.3 milliequivalents of chloromethyl groups per gram and 1% of divinylbenzene cross-links. Reaction of this resin with the sodium salts of the corresponding 2-thiothymines **1a-c** in anhydrous DMF medium caused the heterocyclic compound to be attached to the resin through the sulfur atom. The sulfides obtained **2a-c** were oxidized by *m*-chloro-perbenzoic acid [8, 9] to the corresponding sulfones **3a-c** and then treated with excess amine in anhydrous THF

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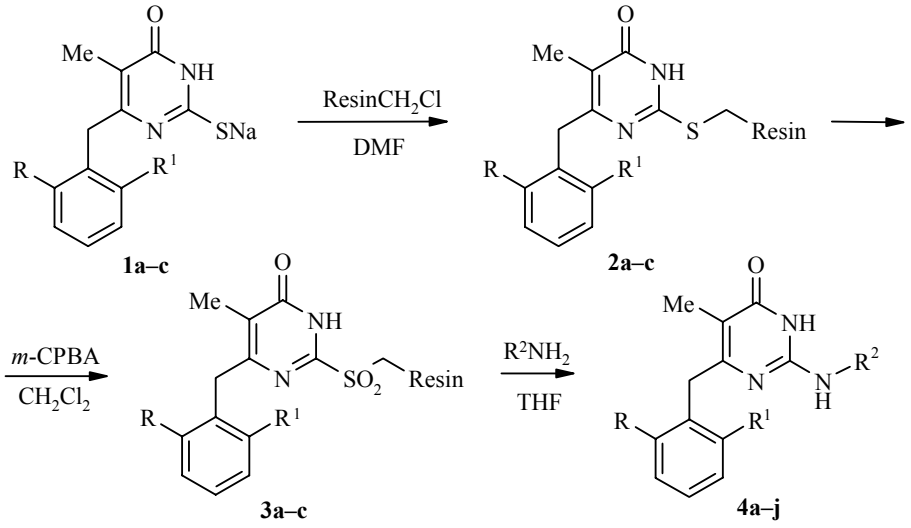
medium to give high yields of the target isocytosine derivatives **4a-j** (Scheme 1). The yields of the target products are given in the Experimental section. The starting 2-thiothymines **1a-c** were obtained using methods reported in the literature [10-12].

The synthetic scheme developed is characterized by a series of advantages over known analogs. In the first place its realization does not demand rigid conditions. In the second, S-methylation of the 2-thiouracil derivatives is not employed thus avoiding working with iodomethane which is both a toxic and a volatile compound.

Scheme 1



4 a, d–j R = R^1 = H; **b** R = R^1 = F, **c** R = F, R^1 = Cl; **a–c** R^2 = $PhCH_2CH_2$,
d R^2 = 4-$FC_6H_4CH_2CH_2$, **e** R^2 = 4-$MeOC_6H_4CH_2CH_2$, **f** R^2 = $PhCH_2CH_2CH_2$,
g R^2 = 1-$AdCH_2$, **h** R^2 = 1-$AdCH_2CH_2$, **i** R^2 = 1-$AdCH_2CH(Me)$,
j R^2 = 3-(1-naphthyl)bicyclo[2.2.1]hept-2-yl; *m*-CPBA = *m*-chloroperbenzoic acid

The main feature of the proposed method is the use of an immobilized form of 2-thiouracil which permits a series of operations connected with the removal of reaction side products to be achieved without loss of the starting compound. In addition, activation of the immobilized form of the 2-thiouracil is accomplished via oxidation of the sulfide group to a sulfone. The high lability of the sulfone towards nucleophilic attack (when compared with the starting sulfide) is achieved through the presence the significantly greater positive charge on the C-2 atom of the pyrimidine ring and also the improved properties of the leaving group. The key solvolysis reaction is likely realized through an "addition-elimination" reaction.

Hence we can conclude that a solid-phase synthesis is a convenient route for the preparation of differently structured isocytosine derivatives. The target products are formed in good yield and purity under mild conditions.

EXPERIMENTAL

^{1}H NMR spectra were recorded on a Varian Mercury 300B (300 MHz) instrument in $CDCl_3$ (compounds **4a-j**) with HMDS as internal standard. The melting points of the synthesized compounds were measured on a Cole-Palmer instrument.

Reagents from Alfa Aesar (2-phenylethylamine, 99%; 2-(4-methoxyphenyl)ethylamine, 98%; 3-phenylpropylamine, 98%; *m*-chloroperbenzoic acid, ~ 70%; and Merrifield resin) and from Acros Organics (2-(4-fluorophenyl)ethylamine, 99%) were used in the synthesis. Adamant-1-ylmethylamine [13], 2-adamant-1-ylethylamine [13], 1-adamant-1-ylpropyl-2-amine [14], and *trans*-[3-(1-naphthyl)bicyclo[2.2.1]hept-2-yl]amine [15] were prepared by methods reported before. Solvent purification and drying was carried out according to standard methods [16].

6-Benzyl-5-methyl-2-[(2-phenylethyl)amino]pyrimidin-4(3H)-one (4a). The Merrifield resin (9 g) was stirred with anhydrous DMF (45 ml) until swollen, excess solvent was decanted off, and there was added the sodium salt of 6-benzyl-2-thiothymine (**1a)**, (prepared by treating 6-benzyl-2-thiothymine (2 g, 8.6 mmol) with an alcohol solution of sodium hydroxide (380 mg, 9.5 mmol) and subsequent evaporation of the reaction product). An additional volume of DMF (120 ml) was added. The reaction mixture was stirred at room temperature to full conversion of the starting compound (TLC monitoring of an acidified sample in the system 3% MeOH in CH_2Cl_2). Solvent was decanted off and the residue was washed with DMF (3×100 ml), a mixture of CH_2Cl_2–ethanol (19:1 by volume, 3×100 ml), CH_2Cl_2 (3×100 ml), and petroleum ether (40-70ºC, 3×100 ml). The 6-benzyl-2-thiothymine immobilized on the polymer matrix was evacuated and suspended in anhydrous CH_2Cl_2 (25 ml) and a solution of *m*-chloroperbenzoic acid (~ 70%, 6.4 g, 25.8 mmol) in dry CH_2Cl_2 (125 ml) was added. The reaction product was stirred at room temperature for 24 h, solvent was decanted, and the residue was washed with 2-propanol (3×90 ml), petroleum ether (40-70ºC, 3×90 ml), ether (3×90 ml), and anhydrous THF (3×90 ml). The washed residue was evacuated and suspended in anhydrous THF (75 ml) and the mixture obtained was treated with 2-phenylethylamine (3.2 ml, 3.07 g, 25.3 mmol) and stirred for 24 h at room temperature. The solution was decanted and evaporated, the residue was washed with a mixture of CH_2Cl_2 and ethanol (19:1 by volume, 3×150 ml), and the solution obtained was mixed with the residue after evaporation of THF. The obtained solution was treated with a 10% aqueous solution of citric acid monohydrate (2×75 ml) and water (3×50 ml), and dried over $MgSO_4$. After filtration and evaporation of solvent the residue crystallized. Yield 2.33 g (85%); mp 146-148ºC (MeCN). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.77 (3H, s, CH_3); 2.78 (2H, t, *J* = 7.3, $NHCH_2C\underline{H}_2$); 3.49-3.56 (2H, m, $NHC\underline{H}_2CH_2$); 3.77 (2H, s, CH_2); 6.33 (1H, br. s, NH); 7.06-7.24 (10H, m, $2C_6H_5$); 11.55 (1H, br. s, NH pyrimidine). Found, %: C 74.90; H 6.70; N 12.81. $C_{20}H_{12}N_3O$. Calculated, %: C 75.21; H 6.63; N 13.16.

6-(2,6-Difluorobenzyl)-5-methyl-2-[(2-phenylethyl)amino]pyridimin-4(3H)-one (4b) was prepared similarly to compound **4a** apart from the use of 6-(2,6-difluorobenzyl)-2-thiothymine in place of the 6-benzyl-2-thiothymine. Yield 81%; mp 175-176ºC (MeCN). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.83 (3H, s, CH_3); 2.59 (2H, t, *J* = 7.3, $NHCH_2C\underline{H}_2$); 3.26-3.32 (2H, m, $NHC\underline{H}_2CH_2$); 3.82 (2H, s, CH_2); 6.17 (1H, t, *J* = 6.2, NH); 6.79 (2H, t, *J* = 7.7, H-3,5 2,6-$F_2C_6H_3$); 6.98 (2H, d, *J* = 7.3, H-2,6 Ph); 7.07-7.22 (4H, m, H-4 2,6-$F_2C_6H_3$, H-3,4,5 Ph); 11.47 (1H, br. s, NH pyrimidine). Found, %: C 67.39; H 5.20; F 10.69; N 12.20. $C_{20}H_{19}F_2N_3O$. Calculated, %: C 67.59; H 5.39; F 10.69; N 11.82.

6-(6-Chloro-2-fluorobenzyl)-5-methyl-2-[(2-phenylethyl)amino]pyrimidin-4(3H)-one (4c) was prepared similarly to compound **4a** apart from the use of 6-(2-fluoro-6-chlorobenzyl)-2-thiothymine in place of the 6-benzyl-2-thiothymine. Yield 84%; mp 160.5-161.5ºC (MeCN). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.83 (3H, s, CH_3); 2.54 (2H, t, *J* = 7.3, $C\underline{H}_2Ph$); 3.20-3.26 (2H, m, $C\underline{H}_2NH$); 3.93 (2H, s, 2-F-6-$ClC_6H_3C\underline{H}_2$); 6.27 (1H, br. s, NH); 6.88-6.96 (3H, m, H-3,4 2-F-6-ClC_6H_3, H-4 Ph); 7.03-7.20 (5H, m, H-5 2-F-6-ClC_6H_3, H-2,3,5,6 Ph); 11.41 (1H, br. s, NH pyrimidine). Found, %: C 65.00; H 4.99; Cl 9.53; F 5.11; N 10.92. $C_{20}H_{19}ClFN_3O$. Calculated, %: C 64.60; H 5.15; Cl 9.53; F 5.11; N 11.30.

6-Benzyl-2-{[(4-fluorophenyl)ethyl]amino}-5-methylpyrimidin-4(3H)-one (4d) was prepared similarly to compound **4a** apart from the use of 2-(4-fluorophenyl)ethylamine in place of 2-phenylethylamine. Yield 78%; mp 190-191ºC (MeCN). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.77 (3H, s, CH_3); 2.74 (2H, t, *J* = 7.3, 4-$FC_6H_4CH_2$); 3.49 (2H, m, $NHC\underline{H}_2$); 3.77 (2H, s, CH_2Ph); 6.24 (1H, br. s, NH); 6.86 (2H, t, *J* = 8.8, H-3,5 4-FC_6H_4); 6.96-7.06 (2H, m, H-2,6 Ph); 7.08-7.24 (5H, m, H-2,6 4-FC_6H_4, H-3,4,5 Ph); 11.54 (1H, br. s, NH pyrimidine). Found, %: C 71.01; H 6.00; F 5.64; N 12.12. $C_{20}H_{20}FN_3O$. Calculated, %: C 71.20; H 5.97; F 5.63; N 12.45.

6-Benzyl-2-{[2-(methoxyphenyl)ethyl]amino}5-methylpyrimidin-4(3H)-one (4e) was prepared similarly to compound **4a** apart from the use of 2-(4-methoxyphenyl)ethylamine in place of 2-phenylethylamine. Yield 79%; mp 156-157ºC (MeCN). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.79 (3H, s, CH_3); 2.71 (2H, t, *J* = 7.7, 4-$MeOC_6H_4C\underline{H}_2$); 3.48 (2H, m, $NHC\underline{H}_2$); 3.69 (3H, s, OCH_3); 3.77 (1H, s, $C\underline{H}_2$Ph pyrimidine); 6.20-6.31 (1H, m, NH); 6.73 (2H, d, *J* = 8.1, H-3,5 4-$MeOC_6\underline{H}_4$); 7.01 (2H, d, *J* = 8.1, H-2,6 4-$MeOC_6\underline{H}_4$); 7.10-7.27 (5H, m, C_6H_5); 11.56 (1H, br. s, NH pyrimidine). Found, %: C 72.20; H 6.64; N 12.10. $C_{21}H_{23}N_3O_2$. Calculated, %: C 72.18; H 6.63; N 12.03.

6-Benzyl-5-methyl-2-[(3-phenylpropyl)amino]pyrimidin-4(3H)-one (4f) was prepared similarly to compound **4a** using 3-phenylpropylamine in place of the 2-phenylethylamine. Yield 75%; mp 124-125ºC (MeCN). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.72-1.80 (2H, m, $CH_2C\underline{H}_2CH_2Ph$); 1.84 (3H, s, CH_3); 2.45-2.62 (2H, m, $CH_2C\underline{H}_2Ph$); 3.21-3.37 (2H, m, $C\underline{H}_2NH$); 3.78 (2H, s, $C\underline{H}_2$Ph pyrimidine); 7.03-7.19 (11H, m, $2C_6H_5$, NH). Found, %: C 75.61; H 6.89; N 12.74. $C_{21}H_{23}N_3O$. Calculated, %: C 75.65; H 6.95; N 12.60.

2-[(Adamant-1-ylmethyl)amino]-6-benzyl-5-methylpyrimidin-4(3H)-one (4g) was prepared similarly to compound **4a** apart from the use of adamant-1-ylmethylamine in place of the 2-phenylethylamine. Yield 97%; mp 228-229ºC (MeCN). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.27-1.43 (6H, m, $C\underline{H}_2$ Ad); 1.46-1.62 (6H, m, $C\underline{H}_2$ Ad); 1.76-1.93 (6H, m, $C\underline{H}_3$, H-3,5,7 Ad); 2.92-3.02 (2H, m, $C\underline{H}_2NH$); 3.75 (2H, s, CH_2Ph); 6.07-6.23 (1H, m, NH); 7.09-7.26 (5H, m, C_6H_5); 11.48 (1H, br. s, NH pyrimidine). Found, %: C 76.16; H 8.04; N 11.96. $C_{23}H_{29}N_3O$. Calculated, %: C 76.00; H 8.04; N 11.56.

2-[(2-Adamant-1-ylethyl)amino]-6-benzyl-5-methylpyrimidin-4(3H)-one (4h) was prepared similarly to compound **4a** apart from the use of 2-adamant-1-ylethylamine in place of 2-phenylethylamine. Yield 92%; mp 182-184ºC (MeCN). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.23 (2H, dd, J_1 = 8.4, J_2 = 16.5, $C\underline{H}_2$ Ad); 1.43 (6H, br. s, $C\underline{H}_2$ Ad); 1.51-1.67 (6H, m, CH_2 Ad); 1.79-1.95 (6H, m, CH_3, H-3,5,7 Ad); 3.21-3.37 (2H, m, $C\underline{H}_2NH$); 3.77 (2H, s, $C\underline{H}_2Ph$); 6.00 (1H, t, *J* = 5.5, NH); 7.09-7.26 (5H, m, C_6H_5); 11.49 (1H, br. s, NH pyrimidine). Found, %: C 76.70; H 8.28; N 10.75. $C_{24}H_{31}N_3O$. Calculated, %: C 76.35; H 8.28; N 11.13.

2-[(1-Adamant-1-ylprop-2-yl)amino]-6-benzyl-5-methylpyrimidin-4(3H)-one (4i) was prepared similarly to compound **4a** except for the use of 1-adamant-1-ylprop-2-ylamine in place of 2-phenylethylamine. Yield 97%; mp 228-229ºC (MeCN). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.06 (4H, d, *J* = 6.8, CH_2 Ad); 1.12 (1H, d, *J* = 3.4, CH_2 Ad); 1.22 (1H, d, *J* = 8.5, CH_2 Ad); 1.27 (1H, d, *J* = 8.5, CH_2 Ad); 1.35-1.51 (8H, m, $C\underline{H}_3CH$, CH_2 Ad); 1.53-1.60 (2H, m, $CHC\underline{H}_2$ Ad); 1.80-1.86 (3H, m, CH_3 pyrimidine); 1.93 (3H, s, H-3,5,7 Ad); 3.70-3.87 (2H, m, CH_2Ph); 4.16-4.32 (1H, m, $C\underline{H}NH$); 5.87 (1H, d, *J* = 8.5, NH); 7.12 (1H, d, *J* = 6.8, H-4 Ph); 7.15-7.31 (4H, m, H-2,3,5,6 Ph); 11.23 (1H, br. s, NH pyrimidine). Found, %: C 77.00; H 8.40; N 11.01. $C_{25}H_{33}N_3O$. Calculated, %: C 76.69; H 8.49; N 10.73.

6-Benzyl-5-methyl-2-{[*trans*-3-(1-naphthyl)bicyclo[2.2.1]hept-2-yl]amino}pyrimidin-4(3H)-one (4j) was prepared similarly to compound **4a** apart from the use of *trans*-3-(1-naphthyl)bicyclo[2.2.1]hept-2-ylamine in place of the 2-phenylethylamine. Yield 70%; mp 232.5-233.5ºC (MeCN). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.11-1.19 (1H, m, H-7 bicycle); 1.27-1.31 (1H, m, H-6 bicycle); 1.34-1.49 (6H, m, CH_3, H-5,6,7 bicycle); 1.56 (1H, br. s, H-5 bicycle); 1.98-2.14 (1H, m, H-4 bicycle); 2.43 (1H, br. s, H-1 bicycle); 3.34-3.48 (2H, m, $C\underline{H}_2Ph$); 3.88-4.04 (1H, m, H-2 bicycle); 4.19-4.35 (1H, m, H-3 bicycle); 6.80 (1H, br. s, NH); 6.88-6.96 (3H, m, H-4 Ph, H-2,7 $C_{10}H_7$); 6.98-7.10 (7H, m, H-2,3,5,6 Ph, H-3,4,6 $C_{10}H_7$); 7.14-7.18 (1H, m, H-5 $C_{10}H_7$); 7.58 (1H, d, *J* = 8.5, H-8 $C_{10}H_7$); 10.61 (1H, br. s, NH pyrimidine). Found, %: C 80.00; H 6.72; N 10.02. $C_{29}H_{29}N_3O$. Calculated, %: C 79.97; H 6.71; N 9.65.

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