Microwave assisted synthesis of N-(ethoxycarbonylmethyl)-nucleobases: building blocks for PNAs

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The synthesis of N1/N9- (Ethoxycarbonylmethyl)pyrimidine/purine using as synthons for peptide nucleic acids has been described. Microwave irradiation provided the desired products by alkylation of the appropriately protected natural and substituted nucleobases with ethyl bromoacetate within 4–7 min in 48–85% yields.

Keywords: **N**-(ethoxycarbonylmethyl)-nucleobases, PNAs, alkylation

Peptide nucleic acids (PNAs) are oligonucleotide analogues, in which the entire phosphodiester pentose backbone of DNA or RNA is replaced by a polyamide or peptide backbone.[1] The most widely known PNAs are based on a N-(2-aminoethyl)glycine backbone which recognise and bind strongly to specific DNA or RNA sequences.[2,3] These characteristics make them potentially useful as antisense and antigene drugs or molecular probes, which have numerous applications in the field of molecular and experimental medicine.[4-6] N1/N9-alkylation of nucleobases with different alkyl bromoacetates afforded the important building blocks for PNAs, which have been widely described in the literature.[7-10] These synthetic methods, however, required the long reaction time (overnight), harsh reaction conditions (using NaH) and gave N-alkylation products in moderate yields.

Microwave irradiation (MWI) as a non-conventional energy source has become a very popular and useful technology in organic chemistry. Its efficiency in dramatically accelerating reaction rates has been proven in several different organic synthesis fields,[11-15] but in nucleic acid chemistry only a few of examples of the use of microwave assisted synthesis have been reported.[16] Here we report the microwave assisted efficient synthesis of N1/N9 (ethoxycarbonylmethyl) pyrimidine/purines through the reaction between different nucleobases and ethyl bromoacetate in the presence of K_2CO_3. This shortened the reaction times under mild reaction conditions and gave the same or higher yield compared to the literature.

Uracil can be directly alkylated with ethyl bromoacetate under microwave irradiation in the presence of K_2CO_3 in CH_3CN (Scheme 1). The reaction time was reduced to 5 min and the yield was 52%. When the N^1, N^3-bisalkylated products appeared, the reaction was quenched. This procedure appears to be regioselective and gives only N^1-isomer **2a**. After completing the N^1-alkylation of uracil, we undertook the extension of this protocol to other nucleobases under similar conditions (Schemes 1 and 2). Thymine also can be directly alkylated at the N^1 position and provided **2b** in the similar yield as uracil. No N^1, N^3-bisalkylated products was found.

For the synthesis of **2c**, **2d**, **2e** and **2f**, however, we selected to protect the exocyclic amine group with appropriate group. Protection at these sites not only blocks undesired side reaction, but also increases the solubility of nucleobases.[7] Under 300W microwave irradiation and within 4–7 min, these protected nucleobases were alkylated to provide the desired N^1-alkylated pyrimidine and N^9-alkylated purines derivatives alone or with other regioisomers in 48–85% yield (Table 1). It is interesting that N^6-benzyladenine and 2,6-dichloropurine when reacted with ethyl bromoacetate under microwave irradiation afforded only the expected N^9-alkylated product in 85% and 70% yield respectively. However, the alkylation of N^2-acetylguanine resulted in a 73/27 ratio of N^9/N^7 isomers and the total yield was only 48%.

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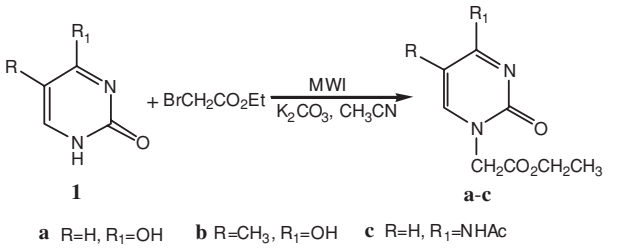
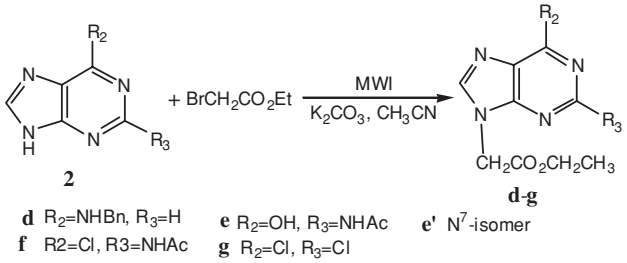


Scheme 1



Scheme 2

In our experiments, the best results were obtained using acetonitrile as solvent. We found that the good solubility of nucleobases afforded the corresponding products with higher yield (Table 1 entry 4, 6). Attempts using different solvents such as N, N-dimethylformamide and dimethylsulfoxide to increase the solubility of heterocycle bases did not give positive results and only afforded a dark reaction mixture. We also tried the solventless and solid support synthesis, such as silica gel or alumina. However the results were disappointing and resulted in negligible yields.

The compounds of **2a**, **2b**, **2e** and **2e'** have been synthesised previously[10] but the melting points were not reported. The compounds of **2c**, **2d**, **2f** and **2g** were reported for the first time. All products were characterised fully by ^{1}H NMR, ^{13}C NMR and elemental analysis, which we found to be in accordance with the proposed structures. In conclusion, we reported our microwave assisted efficient synthesis of acetate-

Table 1 Microwave assisted alkylation of different nucleobases

Entry	Nucleobase	product	Time/min	Yield/%[a]
1	Uracil	**2a**	5	52
2	Thymine	**2b**	7	55
3	N^4-acetylcytosine	**2c**	4	65
4	N^6-benzyladenine	**2d**	5	85
5	N^2-acetylguanine	**2e**	5	48[b]
6	N^2-acetyl-6-chloroguanine	**2f**	4	70
7	2,6-dichloropurine	**2g**	4	64

[a]Purification by silica gel chromatograph.
[b]N^9/N^7=73/27 yield ratio.

nucleobases within greatly reduced reaction time and in same or higher yields comparable to that reported in the literature.

Experimental

All melting points were uncorrected and measured on a Kofler micromelting point apparatus. ^{1}H NMR and ^{13}C NMR were recorded on a Bruker AV 400 spectrometer (400MHz) in DMSO-d_6 with TMS as internal reference. Elemental analysis was performed with an EA-1110 instrument. All the reactions were carried out in a modified domestic microwave oven at 300W (SANYO EM-202MS1, 2450MHz). A hole is designed in the microwave oven through which a round-bottomed flask is fixed and can be refluxed directly.

A general experimental procedure for the synthesis of **a**–**g** is described for uracil. To a mixture of uracil (0.50g, 4.50mmol) and ethyl bromoacetate (1.00ml, 2 equiv.) in dry acetonitrile (20ml) was added 0.2g KI and 1.00g of K_2CO_3.Then the mixture was irradiated in microwave oven at 300W for 5 min. When the mixture was still hot, it was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography and gave 0.46g of **2a** as white crystals.

1-(Ethoxycarbonylmethyl)uracil (**2a**): M.p. 137–139°C; ^{1}H NMR (DMSO-d_6)δ: 1.22(t, 3H, *J*=7.2Hz, CH_3); 4.17(q, 2H, *J*=7.2Hz, OCH_2); 4.51(s, 1H, NCH_2); 5.61(d, 1H, *J*=8.0Hz, 5-H); 7.61(d, 1H, *J*=8.0Hz, 6-H); 11.32 (s, 1H, NH); ^{13}C NMR: 15.0, 49.6, 62.2, 102.1, 146.8, 152.0, 164.67, 169.1; Anal. Calcd for $C_8H_{10}N_2O_4$: C, 48.48; H, 5.10; N, 14.14; Found: C, 48.52; H, 5.15; N, 14.33.

1-(Ethoxycarbonylmethyl)thymine (**2b**): M.p. 169–171°C; ^{1}H NMR δ:1.22(t, 3H, *J*=7.2Hz, CH_3CH_2); 1.77(s, 3H, 5-CH_3); 4.16(q, 2H, *J*=7.2Hz, OCH_2); 4.47(s, 2H, NCH_2); 7.49(s, 1H, 6-H); 11.31(s, 1H, NH); ^{13}C NMR: 12.8, 15.0, 49.5, 62.1, 109.6, 142.6, 152.0, 165.3, 169.2; Anal. Calcd for $C_9H_{12}N_2O_4$: C, 50.93; H, 5.71; N, 13.20 Found: C, 50.83; H, 5.71; N, 13.15.

N^4-Acetyl-1-(ethoxycarbonylmethyl)cytosine (**2c**): M.p. 189–191°C; ^{1}H NMRδ: 1.22(t, 3H, *J*=7.2Hz, CH_3CH_2); 2.12(s, 3H, CH_3CO); 4.16(q, 2H, *J*=7.2Hz, OCH_2); 4.62(s, 2H, NCH_2); 7.19(d, 1H, *J*=7.2Hz, 5-H); 8.04(d, 1H, *J*=7.2Hz, 6-H); 10.82(s, 1H, NH); ^{13}C NMR: 15.0, 25.4, 51.7, 62.1, 96.2, 151.6, 156.2, 164.0, 168.9, 171.9; Anal. Calcd for $C_{10}H_{14}N_3O_4$: C, 49.99; H, 5.89; N, 17.49; Found: C, 49.84; H, 5.45; N, 17.51.

N^6-Benzyl-9-(ethoxycarbonylmethyl)adenine (**2d**): M.p. 182–183°C; ^{1}H NMRδ: 1.22(t, 3H, *J*=7.2Hz, CH_3CH_2); 4.18(q, 2H, *J*=7.2Hz, OCH_2); 4.77(s, 2H, $NHCH_2$); 5.08(s, 2H, NCH_2); 7.22 (t, 1H, *J*=7.2Hz, p-H, Benzyl); 7.30(t, 2H, *J*=7.2Hz, m-H, Benzyl); 7.36(d, 2H, *J*=7.2Hz, o-H, Benzyl); 8.14(s, 1H, 8-H); 8.20(s, 1H, 2-H); 8.25(s, 1H, NH); ^{13}C NMR: 15.0, 44.2, 45.0, 62.4, 119.6, 127.8, 128.2, 129.2, 141.2, 142.2, 150.4, 153.6, 155.6, 168.9; Anal. Calcd for $C_{16}H_{17}N_5O_2$: C, 61.71; H, 5.51; N, 22.50; Found: C, 61.61; H, 5.47; N, 22.74.

N^2-Acetyl-9-(ethoxycarbonylmethyl)guanine (**2e**): M.p. 234–236°C(Dec.); ^{1}H NMRδ: 1.23(t, 3H, *J*=7.2Hz, CH_3CH_2); 2.17(s, 3H, CH_3CO); 4.18(q, 2H, *J*=7.2Hz, OCH_2); 5.20(s, 2H, NCH_2); 8.13 (s, 1H, 8-H); 11.53(s, 1H, NH); 12.09(NHAc); ^{13}C NMR: 15.0, 24.7, 48.3, 62.3, 112.8, 145.9, 148.1, 153.7, 157.9, 168.8, 174.3; Anal. Calcd for $C_{11}H_{13}N_5O_4$: C, 47.30; H, 4.70; N, 25.08. Found: C, 47.25; H, 4.73; N, 25.16.

N^2-Acetyl-7-(ethoxycarbonylmethyl)guanine (**2e'**): M.p. 247–249°C(Dec.); ^{1}H NMRδ: 1.22(t, 3H, *J*=7.2Hz, CH_3CH_2); 2.17 (s, 3H, CH_3CO); 4.17(q, 2H, *J*=7.2Hz, OCH_2); 5.00(s, 2H, NCH_2); 7.95 (s, 1H, 8-H); 11.63(s, 1H, NH); 12.03(NHAc); ^{13}C NMR: 15.0, 24.7, 45.3, 62.5, 120.8, 141.2, 149.0, 150.0, 155.8, 168.6, 174.5; Anal. Calcd for $C_{11}H_{13}N_5O_4$: C, 47.30; H, 4.70; N, 25.08. Found: C, 47.35; H, 4.75; N, 24.95.

2,6-Dichloro-9-ethoxycarbonylmethyl)purine (**2f**): M.p. 112–114°C; ^{1}H NMRδ: 1.23(t, 3H, *J*=7.2Hz, CH_3CH_2); 4.21 (q, 2H, *J*=7.2Hz, OCH_2); 5.25(s, 2H, NCH_2); 8.71(s, 1H, 8-H); ^{13}C NMR: 15.0, 45.9, 62.8, 131.2, 149.9, 151.0, 152.4, 154.6, 168.0; Anal. Calcd for $C_9H_8Cl_2N_4O_2$: C, 39.29; H, 2.93; N, 20.37; Found: C, 39.35; H, 2.85; N, 20.28.

N^2-Scetyl-6-chloro-9-(ethoxycarbonylmethyl)guanine (**2g**): M.p. 159–161°C; ^{1}H NMRδ: 1.23(t, 3H, *J*=7.2Hz, CH_3CH_2); 2.20 (s, 3H, CH_3CO); 4.20(q, 2H, *J*=7.2Hz, OCH_2); 5.14(s, 2H, NCH_2); 8.49(s, 1H, 8-H); 10.74(NHAc); ^{13}C NMR: 15.0, 25.5, 45.5, 62.7, 127.8, 147.7, 150.2, 153.3, 154.0, 168.3, 169.8; Anal. Calcd for $C_{11}H_{12}ClN_5O_3$: C, 44.38; H, 4.06; N, 23.53. Found: C, 44.25; H, 4.12; N, 23.23.

We are grateful to the National Natural Science Foundation of China (No: 20372018) for financial support.

Received 23 September 2004; accepted 4 January 2005
Paper 04/2787

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