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Synthesis of pyrrole carboxamide nucleotide triphosphates—putative labelled nucleotide analogues

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Abstract—The syntheses of pyrrole carboxamide nucleotide triphosphates and the initial results of their incorporation by Klenow fragment are reported.

Nucleoside analogues in which the natural pyrimidine or purine base moiety has been substituted by a pyrrole nucleus have been shown to be good universal bases. The 3-nitropyrrole nucleoside **1** (Fig. 1) has been incorporated into oligonucleotides using phosphoramidite chemistry and has been shown to be effective in the preparation of universal sequencing primers.[1] However, enzymatic incorporation of this class of analogue from the triphosphate level has been found to be less efficient, possibly due to the poor hydrogen bonding ability of the nitro group. With this limitation in mind, interest in pyrrole nucleosides that have more effective hydrogen bonding donor–acceptor groups has been growing. Pyrroles with one **2** or two carboxamide **3** substituents have recently been prepared.[2] The triphosphates of these analogues are also better substrates for polymerase enzymes and oligonucleotides that contain these base analogues have been synthesised via phosphoramidites.

In connection with our ongoing programme of research into the properties of nucleic acids and their analogues, we wished to prepare analogues of the pyrrole dicarboxamides which are capable of being labelled, either as the triphosphate or after incorporation. In this communication, we report the synthesis of two related carboxamide pyrrole nucleotide analogues. We have prepared a 3,4-dicarboxamide pyrrole nucleotide, in which both amide groups are directly attached to the pyrrole nucleus, and a 3-carboxamide pyrrole nucleotide in which only one amide is attached to the pyrrole nucleus. Both analogues have additional functionalisation to allow the attachment of a reporter group (such as a fluorophore) to the molecule. Labelled nucleoside analogues of this type have a possible role in nucleic acid analysis.

The pyrrole monocarboxamide (Scheme 1) was prepared starting from methyl 4-chloroacetoacetate **4**.[3] The

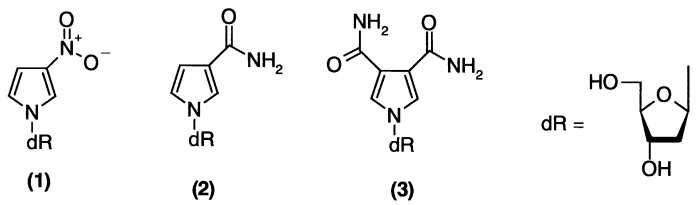

Figure 1.

Keywords: amides; nucleic acid analogues; nucleotides; pyrroles.

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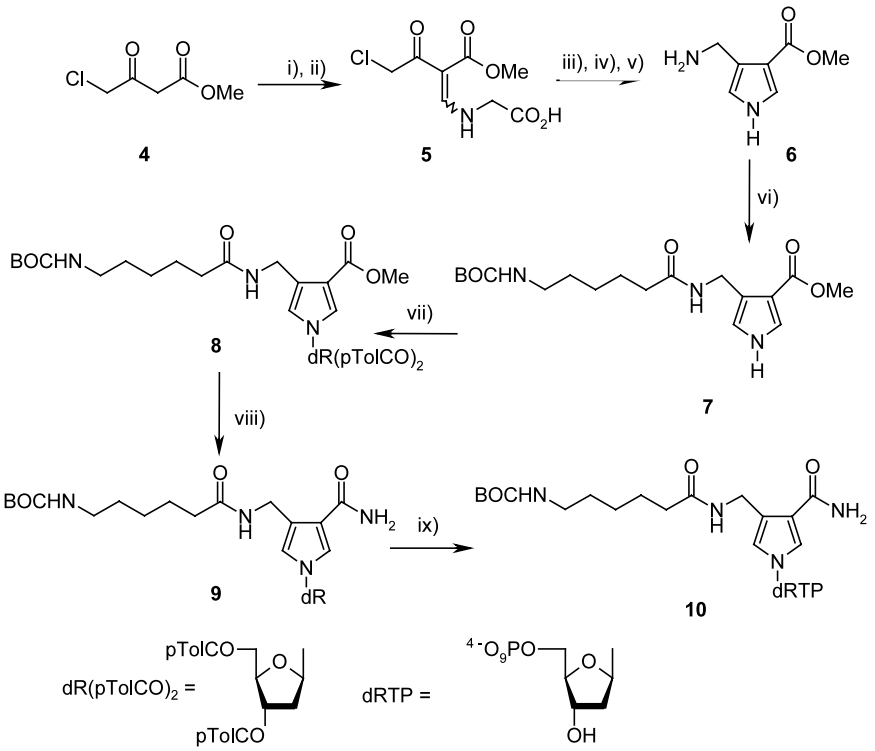
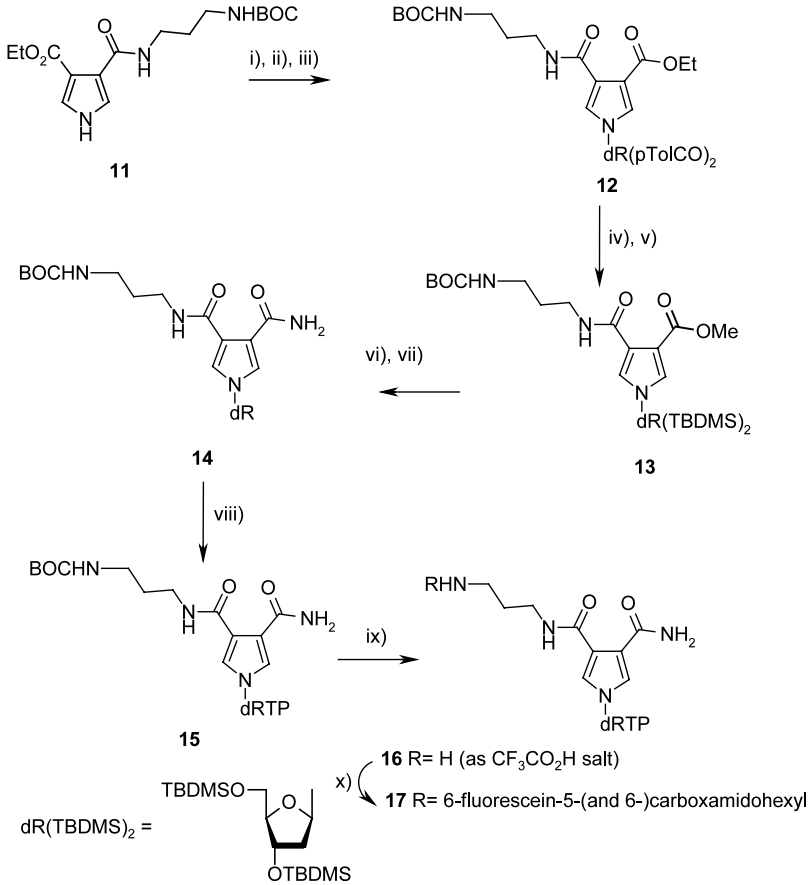


Scheme 1. *Reagents and conditions*: (i) $(EtO)_3CH$, Ac_2O, reflux, 100%; (ii) glycine, KOH, MeOH, reflux, 58%; (iii) Ac_2O, reflux, 36%; (iv) NaN_3, DMF then $NaHCO_3$, H_2O, 56%; (v) PPh_3, pyridine, H_2O, not isolated; (vi) *O*-succinimidyl-*N*-(*t*BOC) aminocaproate, MeCN, 68%; (vii) α-chloro-(3,5-di-*O*-*p*-toluoyl)-2-deoxy-D-ribose, NaH, MeCN, 68%; (viii) $HCONH_2$, MeONa, MeOH, THF, reflux, 54%; (ix) $POCl_3$, $(MeO)_3PO$, $(EtO)_3PO$ then $Bu_3N{\cdot}H_3P_2O_7$, DMF.

β-keto ester was reacted with triethyl orthoformate, and then glycine to give the vinyl amine **5**. Conversion to the aminomethyl pyrrole **6** was effected in three steps; cyclisation to form the pyrrole ring with concomitant decarboxylation was accomplished using acetic anhydride in 36% yield. Displacement of the chloride with sodium azide and subsequent reduction using triphenylphosphine in pyridine gave the amine **6**. The amine was reacted directly with the NHS ester of BOC-protected 6-aminocaproic acid to give the functionalised pyrrole **7**. Glycosylation was effected by alkylation of the sodium salt of the pyrrole with α-chloro-(3,5-di-*O*-*p*-toluoyl)-2-deoxy-D-ribose in acetonitrile. A single product **8** was obtained. ^{1}H NMR spectra of the product showed features that were entirely consistent with the product having the β configuration.[4] This was followed by amidation of the methyl ester function using formamide and sodium methoxide in refluxing methanol[5] with concomitant removal of the toluoyl protecting groups to give the nucleoside **9**. The nucleoside analogue was converted to the nucleotide analogue **10** in a solvent mixture of trimethyl and triethyl phosphate using phosphorous oxychloride, followed by addition of tributylammonium pyrophosphate in DMF.

Preparation of the functionalised pyrrole-3,4-dicarboxamide **17** was initiated from the commercially available 3,4-bis(ethoxycarbonyl) pyrrole. This was converted in two steps to the mono(amide) **11** by monosaponification according to the procedure of Nicolaus and Mangoni[6] followed by EDC coupling with mono-BOC-1,3-propanediamine. This pyrrole was glycosylated under identical conditions as **7** (Scheme 2). Once again, ^{1}H NMR showed that only one isomer, **12**, was formed and that this gave spectra consistent with the formation of the β-anomer.[7] The toluoyl groups were removed using sodium methoxide in methanol resulting in transesterification of the 4-ethoxycarbonyl moiety, and the sugar was then silylated **13** with TBDMS-Cl. This latter step proved necessary for purification of the nucleoside analogue **14**. Amidation was carried out under identical conditions to the formation of **9**[5] and the synthesis of the triphosphate was completed by desilylation using ammonium fluoride and phosphosphorylation. The triphosphate **15** was treated with TFA to remove the BOC protecting group, and the resultant amine **16** was reacted with the NHS ester of 5(6)-fluoresceinyl-6-aminocaproic acid in DMSO to give the fluorescently labelled triphosphate **17**.

The triphosphates were screened for incorporation using Klenow fragment in a primer extension assay. The pyrrole dicarboxamides **15**, **16** and **17** were incorporated as dA and dC, the monocarboxamide **10** was incorporated as dT.



Scheme 2. *Reagents and conditions*: (i) 10% $K_2CO_{3(aq.)}$, 52%; (ii) $BOCNH(CH_2)_3NH_2$, EDC, HOBt, THF, 77%; (iii) NaH, MeCN, then α-chloro-(3,5-di-*O*-*p*-toluoyl)-2-deoxy-D-ribose, 90%; (iv) MeONa, MeOH, 77%; (v) TBDMS-Cl, imidazole, DMF, 90%; (vi) $HCONH_2$, MeONa, MeOH, THF, reflux; (vii) NH_4F, MeOH, 72% from **13**; (viii) $POCl_3$, $P(O)(OMe)_3$, $P(O)(OEt)_3$, then $Bu_3NH{\cdot}P_2O_7$, DMF; (ix) TFA; (x) 6-fluorescein-5-(and 6-)carboxamidohexanoic acid *N*-hydroxysuccinimidyl ester, DIPEA, DMSO.

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3. Japanese Patent JP 7-157466.
4. ^{1}H NMR data for the nucleoside analogue **8** (300 MHz, $CDCl_3$): δ 1.20–1.26 (2H, m, C$\underline{H}_2$), 1.33 (9H, s, C(C$\underline{H}_3$)$_3$), 1.45–1.55 (2H, m, C$\underline{H}_2$), 1.91–1.94 (2H, m, C$\underline{H}_2$), 2.07 (2H, app t, C$\underline{H}_2$NHBOC, J=7 Hz), 2.22 (3H, s, ArC$\underline{H}_3$), 2.39 (3H, s, ArC$\underline{H}_3$), 2.64 (2H, m, 2′-C$\underline{H}_2$), 2.95 (2H, dd, J=6 and 13 Hz, C$\underline{H}_2$CONH), 3.68 (3H, s, $CO_2C\underline{H}_3$), 4.29 (2H, d, J=6 Hz, NHC$\underline{H}_2$-pyrrole), 4.47–4.59 (3H, m, 4′-C$\underline{H}_2$, 5′-C$\underline{H}_2$), 5.28 (1H, s, br, amide N$\underline{H}$), 5.60 (1H, m, 3′-C$\underline{H}$), 6.03 (1H, dd, J=7.7 and 6.2 Hz, 1′-C$\underline{H}$), 6.67 (1H, br, amide N$\underline{H}$), 6.81 (1H, d, J=2.5 Hz, pyrrole-$\underline{H}$), 7.30 (2H, d, J=8 Hz, 3, 5-Ar-$\underline{H}$), 7.31 (2H, d, J=8.4 Hz, 3, 5-Ar-$\underline{H}$), 7.46 (1H, J=2.5 Hz, pyrrole-$\underline{H}$), 7.89 (2H, d, J=8 Hz, 2, 6-Ar-$\underline{H}$), 7.94 (2H, d, J=8.4 Hz, 3, 5-Ar-$\underline{H}$).
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7. ^{1}H NMR data for the nucleoside **12** (300 MHz, $CDCl_3$): δ 1.14 (3H, t, J=7 Hz, $CH_2C\underline{H}_3$), 1.36 (9H, s, C(C$\underline{H}_3$)$_3$), 1.68 (2H, quintet, J=6.6 Hz, $NCH_2C\underline{H}_2CH_2N$), 2.33 (3H, s, ArC$\underline{H}_3$), 2.36 (3H, s, ArC$\underline{H}_3$), 2.62 (2H, br m, 2′-C$\underline{H}_2$), 3.11 (2H, app q, J=6.2 Hz, C$\underline{H}_2$CO), 3.40 (2H, app q, J=7 Hz, C$\underline{H}_2$NHCO$_2$′Bu), 4.10 (2H, m, $CO_2C\underline{H}_2CH_3$), 4.52 (1H, m, 4′-C$\underline{H}$), 4.54 (2H, m, 5′-C$\underline{H}_2$), 5.27 (1H, br s, $CH_2N\underline{H}CO$), 5.55 (1H, m, 3′-C$\underline{H}$), 5.95 (1H, dd, J=7.5 and 6.3 Hz, 1′-C$\underline{H}$), 7.15 (2H, d, J=8.1 Hz, 3,5-Ar$\underline{H}$), 7.20 (2H, d, J=8.1 Hz, 3,5-Ar$\underline{H}$), 7.52 (1H, d, J=2.6 Hz, pyrrole-$\underline{H}$), 7.61 (1H, d, J=2.6 Hz, pyrrole-$\underline{H}$), 7.82 (2H, d, J=8.1 Hz, 2,6-Ar-$\underline{H}$), 7.85 (2H, d, J=8.1 Hz, 2,6-Ar-$\underline{H}$), and 9.71 (1H, br t, N$\underline{H}$CO$_2$′Bu).