Fmoc/Acyl protecting groups in the synthesis of polyamide (peptide) nucleic acid monomers

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The chemical synthesis of polyamide (peptide) nucleic acid (PNA) monomers 22–25 has been accomplished using Fmoc [N-(2-aminoethyl)glycine backbone], anisoyl (adenine), 4-tert-butylbenzoyl (cytosine) and isobutyryl/diphenylcarbamoyl (guanine) protecting-group combinations, thus allowing oligomer synthesis on both peptide and oligonucleotide syntheses. An alternative method for the preparation of (N'-anisoyladenin-9-y)acetic acid 7 is described using partial hydrolysis of a dianisoylated derivative. Different methods were studied for guanine alkylation including (a) Mitsunobu reaction; (b) low-temperature, sodium hydride- and (c) N,N-diisopropylethylamine-mediated alkylation reactions to give preferentially N'-substituted derivatives. Empirical rules are proposed for differentiating N'/N'-substituted guanines based on their 13C NMR chemical-shift differences.

Introduction

Polyamide (or as originally referred: peptide) nucleic acids (PNA) are one of the most powerful analogues of oligonucleotides in terms of chemical and enzymic stability, double- and triple-helix formation, with potential applications in antisense diagnosis and therapeutics.25–27 In these compounds the entire sugar–phosphate backbone is replaced with an N-(2-aminoethyl)glycine moiety and the nucleobases are attached through an N-acetyl linkage.

The chemical synthesis of PNA mostly relies on the assembly of the protected N-(2-aminoethyl)glycine backbone and protected nucleobase-substituted acetic acid structural units followed by standard oligomerization protocols.4,4 The pioneering efforts of a Danish group resulted in the application of Boc (backbone) and Z (cytosine, adenine) or O-benzyl (guanine) protection.5 Later the Uhmann group used a monomethoxytrityl (MMTr)/acyl (anisoyl, 4-tert-butylbenzoyl, isobutyryl ethyl) strategy.5,8 All these methods require the use of (strong) acidic conditions (e.g., TFA, HF) in the oligomer construction and final cleavage from the support. The need for milder methods led to the employment of the Fmoc group for backbone protection and Z3 or MMTr groups9 for the nucleobases. The Fmoc group is a convenient alternative to acid-sensitive backbone-protecting groups (Boc, MMTr) and allows easy monitoring of the coupling process.10 The combination Fmoc/acyl should also be feasible since the former group can be cleaved without affecting the more stable base-protecting acyl groups.12–14 Herein we report on our results concerning the use of Fmoc (backbone)/acyl (4-tert-butylbenzoyl for cytosine; anisoyl for adenine; isobutyryl/N,N-diphenylcarbamoyl for guanine) protecting groups in the synthesis of PNA monomers. The prior protecting-group combinations (with the exception of Fmoc/MMTr) were used either for peptide or oligonucleotide synthesis protocols. With biologically important PNA–DNA and PNA–peptide conjugates in mind our approach offers a substantial advantage over the existing ones since both oligomerization methodologies are possible with the same monomers. Beside this, use of the frequently applied urethane protecting groups (e.g., Z) for nucleobases is not practical as in our experience the yields are often very low. This paper complements and details our preliminary account.15

Results and discussion

The choice of nucleobase-protecting groups was motivated by different considerations since uniform protection, though attractive, is not possible. Thymine does not require protection and our synthesis of the thymine monomer, starting from acid 1, was based on the procedure of Thomson et al.9 The anisoylated cytosine derivative 2 was alkylated to give acid 3 (Scheme 1) but the solubilities of these substances were so low in common solvents that we had to abandon this group. The 4-tert-butylbenzoyl group proved to be more rewarding; acid 5 was easily obtained via intermediate 4 and used later.

![Scheme 1](Image)

Scheme 1 Reagents and conditions: a, (1) BrCH2COOMe, K2CO3, DMF; (2) NaOH, then HCl; b, AnCl, py, 80°C; c, 4-Bu2C5H3COCl, Et3N, DMF; d, NaH, DMF, BrCH2CH2COEt; e, NaOH,aq, 1,4-dioxane, then HCl. An = 4-MeOC2H5CO

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instead of methyl bromoacetate followed by acidolysis. In an alternative approach ethyl (adenin-9-yl)acetate \(^8\) was anisoylated with excess of anisoyl chloride and the resulting \(N^6,N^\alpha\)-dianisoylated derivative (not isolated) was subjected to partial hydrolysis to give acid 7 in an improved yield (70%; overall 45% from adenine).

The substitution of guanine is notorious for giving \(N^9/N^\beta\)-regioisomers.\(^6\) Although the 2-amino group is not really nucleophilic enough to interfere with many transformations, the poor solubility of unprotected guanine excludes its use in most reactions. The application of \(N^2\)-acyl (acetyl, propionyl, isobutryl, \textit{etc.})-protected derivatives increases the solubility but \(N^2\)-acylation alone cannot solve the fundamental problem of the selectivity of alkylation.\(^16\) Constraining guanine from its dominant 6-lactam structure to lactim (enolate) form by different groups has a beneficial effect on the ratio of \(N^9/N^\beta\)-regioisomers. The most successful in this respect is the \(N,N\)-diphenylcarbamoyl protecting group\(^17\) which reportedly gives in some cases a 100:1 ratio in favour of the \(N^9\)-regioisomer.\(^18,19\) To see how the introduction of this group alters the selectivity of alkylation, first 2-\(N\)-isobutylyguanine \(^9\) was alkylated with tert-butyl bromoacetate in the presence of sodium hydride to afford a nearly 1:1 ratio of \(N^9/N^\beta\)-isomers (11 and 12, respectively) in 74% yield (Scheme 3). The selection of the isobutyl group was motivated by the fact that although its removal under basic conditions is more sluggish than that of other simple acyl groups (acetyl, propionyl)\(^17,20\) it confers steric hindrance on the 2-amino group and thus prevents unwanted alkylation/glycosylation on \(N^\beta\).

Next the \(N,N\)-diphenylcarbamoyl derivative \(^10\) was chosen for alkylation studies under different conditions. Its transformation with tert-butyl glycolate \(^21\) in the Mitsunobu reaction\(^22,23\) provided the product (13, Scheme 4) with good regioselectivity; however, its purification was very difficult and it was contaminated with significant amounts of triphenylphosphine oxide (\(4\)-Dimethylaminophenyl)diarylphosphine,\(^24,25\) claimed to give a phosphine oxide which can be removed by acidic extraction,\(^26\) proved to be unsatisfactory since the product was still contaminated with the corresponding phosphine oxide. Tributylphosphine, the oxide of which is water-soluble, gave a cleaner product but the yield was low (36%). In the next experiments sodium hydride-mediated alkylation with tert-butyl bromoacetate was used to obtain the desired compound. We noticed that at ambient temperature the relative proportion of \(N^9\)-regioisomer was relatively high, while lowering the temperature favoured the formation of the desired \(N^9\)-regioisomer. At \(-20^\circ\text{C}\) a clean reaction gave negligible amounts of the \(N^\beta\)-isomer but the yield of \(N^9\)-isomer was still low (40%). Acidolysis of ester 13 in dilute TFA–\(\text{CH}_2\text{Cl}_2\) (\(0^\circ\text{C}\); 18 h) removed the \(N,N\)-diphenylcarbamoyl (Dpc) group without affecting the tert-butyl ester functionality \(\rightarrow\) 11. Albeit there is some evidence for the lability of this group in 50% (\(v/v\)) TFA–\(\text{CH}_2\text{Cl}_2\)\(^1\) or in the presence of Lewis acids\(^2\) it was surprising that the Dpc group was more sensitive towards acid than was the tert-butyl group. The latter was expected to cleave under similar conditions.\(^14,19\)

The application of \(N,N\)-diisopropylpropyleneamine as a hindered base and methyl bromoacetate\(^*\) to circumvent premature cleavage of the Dpc group in the subsequent hydrolysis gave a 71% yield of the product 14, of which 58% was available without chromatography, along with 15% of the \(N^\beta\)-isomer 15. Basic hydrolysis of ester 14 led to acid 16 in a clean transformation. The surprisingly high yield of the unwanted isomer 15 in the first reaction underlines the fact that even the sterically hindered Dpc protecting group is not sufficient to steer the reaction to complete regioselectivity. Thus the claim that the use of the Dpc group has solved the historic problem of regioselective \(N^9\)-substitution of guanine\(^19,19\) seems to be restricted to the realm of glycosylation reactions, while alkylation transformations require further experimentation.

The coupling of nucleobase-substituted acetic acids 1, 5, 7, 16 with the backbone unit 17 under standard peptide-coupling conditions afforded the PNA esters 18–21 which were subsequently acylolysed (TFA in dichloromethane) to give the PNA monomers 22–25 (Scheme 5). As expected from our previous experience (13 \(\rightarrow\) 11, Scheme 4) in the latter reaction the Dpc protecting group was removed along with the tert-butyl group. It is clear that in this final deprotection step the protect-
Scrutinizing the $^{13}$C NMR chemical-shift parameters of compounds 11–16, 21, 25, 26 (Table 1) and a further 45 $N^9/N^2$-substituted guanines$^{19,35–39}$ (altogether 54 compounds) show that $\delta_{C_{4}}$ is the most sensitive to the $N^9/N^2$-substitution pattern (Fig. 1, $N^9$: 113.75–123.70 ppm; $N^2$: 104.56–115.09 ppm; for regioisomers the difference $[\Delta C = \delta(C(N^9)) – \delta(C(N^2))]$ is 7.86–9.82 ppm), insensitive to the lactam/lactim tautomerism (data not shown) and this signal alone can be of diagnostic value, especially if data for both regioisomers are available. However, due to the overlapping of chemical-shift ranges for regioisomers (Fig. 1), has been assessed and the following conclusions could be drawn:

1. The parameter $a$ distinctly differs for the regioisomers ($N^9$: 28.20–35.41 ppm; $N^2$: 41.35–54.25 ppm; for regioisomers the difference $[\Delta a = \delta(a(N^9)) – \delta(a(N^2))]$ is $-9.93$ to $-20.53$ ppm), shows little variation for lactam/lactim tautomerism (data not shown) and presents no overlapping ranges. The diagnostic value of this observation is slightly diminished since $\delta_{C_{4}}$ usually cannot be simply identified without having recourse to more sophisticated assignment techniques (selective INEPT, HMOC, HMBC, etc.).

2. The parameter $b$ shows similar characteristics ($N^9$: 16.10–26.91 ppm; $N^2$: 29.70–41.17 ppm; for regioisomers the difference $[\Delta c = \delta(c(N^9)) – \delta(c(N^2))]$ is $-11.61$ to $-16.15$ ppm) and its utility is further enhanced by the fact that $\delta_{C_{4}}$ is unmistakable among the skeletal carbons and $\delta_{C_{9}}$ can simply be located in a J-modulated spin-echo experiment.

3. The parameter $c$ can be clustered according to the nature of attached substituent rather than lactam/lactim tautomerism and it gives useful values for glycosylated derivatives ($N^9$: 33.38–35.81 ppm; $N^2$: 16.30–23.83 ppm; for regioisomers the difference $[\Delta c = \delta(c(N^9)) – \delta(c(N^2))]$ is $10.61–12.79$ ppm) while for (cyclo)alkylated compounds it is of less use ($N^9$: 53.40–83.45 ppm; $N^2$: 54.57–71.15 ppm; for regioisomers the difference $[\Delta c = \delta(c(N^9)) – \delta(c(N^2))]$ is $10.68–13.33$ ppm). The identification of $\delta_{C_{9}}, \delta_{C_{4}}$, involved in this parameter, often requires more sophisticated techniques.

As a conclusion it can be seen that the values $a$, $b$ [both for (cyclo)alkyl and glycosylated derivatives] and $c$ [for glycosylated derivatives] are useful for characterizing the $N^9/N^2$-substitution pattern of guanines. From a practical point of view the parameter $b$ is the most convenient one for the reasons explained above. It is noteworthy that $\delta_{C_{4}}$ (118.81 ppm) and the parameters $a$ (35.38 ppm) and $b$ (26.06 ppm) for compound 10$^{3}$ are in good agreement with those for $N^2$-substituted derivatives, suggesting that its dominant tautomer is H in DMSO-$d_6$ solution.

Further studies relating to the application of the above monomers in the preparation of PNA oligomers and our quest for novel combinations of protecting groups are in progress and will be reported in due time.

**Experimental**

**General**

The following abbreviations are employed: diisopropyl azodicarboxylate (DIAD); $N,N$-diisopropylethylamine (DIPEA); 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU); 1-hydroxybenzotriazole (HOBt); trifluoroacetic acid (TFA). Chemicals were purchased from Aldrich or Fluka. Sodium hydride refers to a 55% suspension in mineral oil. Anhydrous solvents were prepared as described.$^{40}$ Light petroleum refers to the fraction with distillation range 40–60 °C. Thymine derivatives 1, 18 and 22 were prepared using the procedure of Thomson$^{4,g}$ $N^9$-Benzylguanine hydrochloride$^{26,27}$ was prepared for comparison of its $^{13}$C NMR parameters with those of other guanine derivatives (see Table 1). Organic solutions were dried using magnesium sulfate and evaporated in Büchi rotary evaporators, TLC: Kieselgel 60 F$_{254}$ (Merck), visualization: UV light. Column chromatography: Kieselgel 60 (0.063–0.200 mm, Merck). Mp: Electrothermal IA 8103 apparatus. Elemental analysis: Perkin-Elmer CHN

analyser model 2400. UV: PE Lambda 10 spectrometer, λmax nm (lg ε), sh: shoulder. IR: Bio-Rad FTS-60A (KBr pellets, cm⁻¹; s: strong, m: medium, w: weak). NMR: Bruker Avance DRX 400 and 500 spectrometers (H: 400.13 MHz and 500.13 MHz; C: 100.62 MHz and 125.76 MHz, respectively). DMSO-d₆, solutions, δ (ppm), J (Hz). Spectral patterns: s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet; br, broad; deuter, deuterable. The superscripts *, # denote interchangeable assignments. For the 2D experiments (HMQC, HMBCC) the standard Bruker software packages (INV4GSSW, INV4GSLRNDSW) were applied. For the 2D experiments (HMBC) the standard Bruker software packages (INV4GSSW, INV4GSLRNDSW) were applied. For the 2D experiments (HMBC) the standard Bruker software packages (INV4GSSW, INV4GSLRNDSW) were applied. For the 2D experiments (HMBC) the standard Bruker software packages (INV4GSSW, INV4GSLRNDSW) were applied. For the 2D experiments (HMBC) the standard Bruker software packages (INV4GSSW, INV4GSLRNDSW) were applied.

[4-(4-Methoxybenzoylamino)-2-oxo-1,2-dihydropyrimidin-1-yl)acetic acid 3](#)

Cytosine (1.11 g, 10.0 mmol) suspended in pyridine (50 mL) was stirred at room temperature while 4-methoxybenzoyl chloride (2.56 g, 15.0 mmol) was added and the reaction mixture was stirred in an oil-bath at 80 °C. The cytosine rapidly dissolved, and then the product precipitated from the solution. After 2 h the mixture was evaporated in vacuo and coevaporated with methanol (2 × 400 mL). The product was insoluble in water (200 mL) and dichloromethane (200 mL). The resulting precipitate was filtered off and dried (5.66 g, 61%). The majority (2.20 g, 8.97 mmol) of this substance was suspended in pyridine (50 mL), heated to 80 °C, and then the product precipitated from the solution. After 2 h the mixture was evaporated in vacuo and the residue was dissolved in dichloromethane (70 mL), and the solution was washed with 10% (w/v) citric acid (2 × 30 mL), dried and evaporated in vacuo. The crude product (14.96 g) was dissolved in warm ethanol (100 mL), cooled to room temperature, 2 M aq. NaOH (30 mL) was added, and the solution was left at room temperature and checked from time to time by TLC. After 175 min more aq. NaOH (5 mL) was added. The reaction was stopped after 4 h by addition of 1 M HCl (35 mL), pH 5+, and the solution was evaporated in vacuo. The crude product was recrystallized from methanol (1 L) to afford a white powder (4.58 g, 70%), mp 215 °C (darkens), 254 °C (decomp.) [lit. [7] 222–223 °C (decomp.)]. The ‘H NMR and mass spectra of this compound were in good agreement with the published values [7] and with those of the substance prepared in procedure B.

B. Controlled hydrolysis of a diansolated derivative. Ethyl (adenin-9-yl)acetate 8° (4.42 g, 20 mmol) was suspended in anhydro pyridine (50 mL), heated to 80 °C for 30 min, then cooled to room temperature. 4-Methoxybenzoyl chloride (8.53 g, 50.0 mmol) was added in portions and the mixture was stirred for 18 h, then evaporated in vacuo and the residue was coevaporated with tolulene (3×). The residue was dissolved in dichloromethane (70 mL), and the solution was washed with 10% (w/v) citric acid (2 × 30 mL), dried and evaporated in vacuo. The crude product (14.96 g) was dissolved in warm ethanol (100 mL), cooled to room temperature, 2 M aq. NaOH (30 mL) was added, and the solution was left at room temperature and checked from time to time by TLC. After 175 min more aq. NaOH (5 mL) was added. The reaction was stopped after 4 h by addition of 1 M HCl (35 mL), pH 5+, and the solution was evaporated in vacuo. The crude product was recrystallized from methanol (1 L) to afford a white powder (4.58 g, 70%), mp 215 °C (darkens), 254 °C (decomp.) [lit. [7] 222–223 °C (decomp.)]. The ‘H NMR and mass spectra of this compound were in good agreement with the published values [7].

[2-(2-Isobutyrylamino-6-oxo-1,6-dihydropurin-9-yl)acetic acid tert-butyl ester 11 and (2-isobutyrylamino-6-oxo-1,6-dihydropurin-7-yl)acetic acid tert-butyl ester 12](#)

N-(6-Oxo-6,9-dihydro-1H-purin-2-yl)isobutryramide 9°(2) (1.11 g, 5.0 mmol) was suspended in anhydro DMF and the mixture was chilled to 0 °C. Sodium hydride (0.36 g, 8.25 mmol) was added and the mixture was stirred at 0 °C for 30 min. tert-Butyl bromoacetate (0.81 mL, 5.5 mmol) was added and the reaction was stopped after 2 h by addition of a small amount of solid CO₂ and methanol (2 mL). The reaction mixture was evaporated in vacuo and the residue was chromatographed using 3–5% (v/v) methanol in dichloromethane. Eluted first was the less polar N°-isomer 12 (0.43 g, 26%), second a mixture (in ≤1:1 ratio as judged by TLC and ‘H NMR) of N°- and N°′-isomer (0.24 g, 14%), and third the pure N°′-isomer (0.56 g, 34%).

Ester 11: white powder, mp 204 °C (decomp., from EtOH); R₁ 0.13 (CH₃Cl₂–MeOH 95:5). Found: C, 53.9; H, 6.4; N, 21.1. Calcd. for C₁₅H₁₅N₄O₂: C, 53.7; H, 6.3; N, 20.9%. λmax 50% (v/v) 1 M HCl in EtOH, pH 0±0.1: 206 (lg ε 4.26), 265 (4.23); λmax 50% (v/v) phosphate buffer in EtOH, pH 6±0.1: 260 (lg ε 4.17), 282 (4.08). For 2°-isomer λmax 50% (v/v) 1 M NaOH in EtOH, pH 13±0.1: 263 (4.06), 263 (4.06); vmax/cm⁻¹ 3151, 2980, 2932, 1735, 1693, 1673, 1614, 1549, 1483, 1411, 1233, 1154, 1143, 795; δH (500 MHz) 1.11 [6 H, d, J 6.8, (CH₃)₂CH₂]; 1.40 (9 H, s, Bu⁴); 2.78 [1 H, pseudoquintet, (CH₂)₂CH]; 4.88

### References

Table 1 ¹³C NMR chemical shifts of guanine derivatives (δ, ppm) *

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* In DMSO-d₆; 125.76 MHz; J-modulated spin-echo experiments; for guanine numbering see ester XII, Scheme 3. Ref. 19. * The C-4, C-5, C-6, C-8 signals were observed only after adding trifluoroacetic acid. The assignment of signals corresponding to C-2 and C-6 carbons is tentative. * Assignment based on HMOC and HMBC experiments. * Some signals were doubled due to the presence of rotamers. * N-Benzylguanine hydrochloride, prepared according to Bridson et al. ³²

(2, H, s, CH₂COO), 7.95 (1 H, s, H-8), 11.65 (1 H, br s, deut, NH), 12.10 (1 H, br s, deut, NH); m/z (ESI) 693 (20%, [2M + Na⁺]), 671 (55, [2M + H⁺]), 336 (100, [M + H⁺]).

Ester XII: white powder, mp 202.5 °C (decomp., from EtOH); Rᵣ 0.19 (CHCl₃–MeOH 95:5). Found: C, 53.65; H, 6.15; N, 21.1%; ¹³Cmax (δ/v) 1 M HCl in EtOH, pH 0/nm 206 (lg ε 4.24), 265 (4.20); ¹³Cmax (δ/v) phosphate buffer in EtOH, pH 6/nm 221 (lg ε 4.24), 265 (4.11), 2828 (3.98); ¹³Cmax (δ/v) 0.1 M NaOH in EtOH, pH 13/nm 224 (lg ε 4.31), 269 (4.01); ηmax/cm⁻¹ 3240w, 2981w, 2937s, 1741m, 1695s, 1675s, 1644s, 1535w, 1441w, 1390m, 1307m, 1283m, 1160m, 747w, δmax (500 MHz) 1.11

[6 H, d, J 6.6, (CH₂)₃CH], 1.39 (9 H, s, Bu), 2.73 [1 H, pseudo-quiet, (CH₂)₂CH], 5.07 (2 H, s, CH₂COO), 8.11 (1 H, s, H-8), 11.55 (1 H, br s, deut, NH), 12.14 (1 H, br s, deut, NH); m/z (ESI) 693 (40%, [2M + Na⁺]), 671 (25, [2M + H⁺]), 358 (27, [M + Na⁺]), 336 (100, [M + H⁺]).

[6-Diphenylcarbamoyloxy-2-(isobutyrylaminio)purin-9-yl]acetate tert-buty ester XIII

A. Mitsuboh reaction, general procedure. Compound 10¹⁻² (1.00 g, 2.40 mmol) was suspended in anhydrous THF (50 mL) and the mixture was refluxed for 20 min to achieve partial dissolution of the starting material. The suspension was cooled to room temperature, tert-buty glycolate²¹ (0.40 g, 3.0 mmol), the appropriate phosphate (3.19 mmol) and DIAD (0.62 mL, 3.19 mmol) were added dropwise, and the mixture was stirred at room temperature. The reaction mixture completely dissolved and became yellow coloured. After completion of the reaction (TLC) the solution was evaporated in vacuo and the residue was subjected to chromatographic purification.

AI. With triphenylphosphine.—Reaction time: 4 h at room temperature. Chromatography: 50–70% (v/v) ethyl acetate in light petroleum. Eluted first was the product XIII (0.31 g), slightly contaminated with triphenylphosphine oxide. Further fractions were also obtained containing varying proportions of the product and triphenylphosphine oxide. The different, partly crystalline fractions were triturated with methanol upon which the product crystallized. This was filtered off and washed with light petroleum. The clearest product (0.40 g, 31%), a white powder, melted at 183.2–185.5 °C. A further crystalline crop (0.39 g) containing the product and triphenylphosphine oxide (TLC) was also obtained; Rᵣ 0.50 (CHCl₃–MeOH 95:5) (Found: C, 63.5; H, 5.5; N, 15.7. Calc. for C₂₈H₂₈N₂O₂: C, 63.4; H, 5.7; N, 15.8%); ¹³Cmax (EtOH)/nm 205 (lg ε 4.60), 229 (4.53), 2588 (4.15), 279 (4.08); ¹³Cmax/cm⁻¹ 3462w, 3346w, 2979w, 294w, 1738s, 1715m, 1624m, 1587m, 1524m, 1449m, 1411m, 1305m, 1240m, 1184s, 1164s, 1056m, 758w, 700m; δmax (500 MHz) 1.09

[6 H, d, J 6.8, (CH₂)₃CH], 1.43 (9 H, s, Bu), 2.87 [1 H, pseudo-quiet, J 6.8, (CH₂)₂CH], 5.03 (2 H, s, CH₂), 7.29–7.53 (10 H, m, ArH), 8.45 (1 H, s, H-8), 10.69 (1 H, br s, deut, NH); m/z (ESI) 557% (8%, [2Ph₂PO + H⁺]), 531 (100, [M + H⁺]).

A₂. With 4-(dimethylamino)phenyl(diphenyl)phosphine ²₆⁻²₇.

Reaction time: 2.5 h at 0 °C. Work-up: the crude product was dissolved in dichloromethane (50 mL) and extracted successively with 4 M HCl (3 × 25 mL) and with 5% (v/v)aq. NaHCO₃ (50 mL). TLC revealed that most of the 4-(dimethylamino)phenyl(diphenyl)phosphine oxide remained in the organic phase. The organic phase was dried and purified by column chromatography using 0–1% (v/v) methanol in dichloromethane. Methanolic trituration and filtration (light petroleum) afforded the product (0.42 g, 33%), mp 182.5–185.0 °C. The IR, ¹H NMR and mass spectra of this compound were in good agreement with those of the substance obtained in AI.

A₃. With tributylphosphine.—Reaction time: 1.5 h at 0 °C. Work-up: the crude product was dissolved in dichloromethane (50 mL) and extracted with water (3 × 25 mL) to remove
the tributylphosphine oxide. Chromatography: 0.1–1.5% (v/v) methanol in dichloromethane. Methanolic titration and filtration (light petroleum) afforded the product (0.46 g, 36%), mp 182.2–184.8 °C. TLC: IR, 1H NMR and mass spectra of this compound were in good agreement with those of the substance obtained in procedure A.

B. Low-temperature, sodium hydride-mediated alkylation. To compound 10 (1.40 g, 3.36 mmol) suspended in anhydrous DMF (20 mL) was added sodium hydride (0.16 g, 7.50 mmol) at room temperature. After 30 min the reaction mixture was chilled to −20 °C and maintained at this temperature. tert-Butyl bromoacetate (0.60 mL, 4.0 mmol) was added dropwise. After 2 h the reaction was stopped by addition of a small amount of solid CO₂ and methanol, and the mixture was evaporated in vacuo and coevaporated with toluene (2×). The residue was dissolved in a mixture of water and dichloromethane (20 mL each). The aqueous phase was extracted with dichloromethane (3 × 15 mL), and the combined organic phases were dried, and evaporated in vacuo. Chromatography: 0–1% (v/v) methanol in dichloromethane. Methanolic titration and subsequent crystallization from methanol (15 mL) afforded the product (0.70 g, 40%) as a white powder, mp 183.1–184.5 °C. The IR, 1H NMR and mass spectra of this compound were in good agreement with those of the substance obtained in procedure A.

Hydrolysis of [6-diphenylcarbamoyloxy-2-(isobutylamino)-purin-9-yl]acetic acid tert-butyl ester (13 → 11)

To ester 13 (0.210 g, 0.38 mmol) dissolved in anhydrous dichloromethane (6 mL) were added 1,3-dimethoxybenzene (0.070 mL, 0.53 mmol) and TFA (0.50 mL, 6.5 mmol) at 0 °C and the mixture was stirred for 18 h. The reaction mixture was diluted with dichloromethane (20 mL), and extracted with satd. aq. NaHCO₃ solution (3 × 10 mL) to remove the excess of acid. Chromatography: 0–10% (v/v) methanol in dichloromethane to give the lactam 11 (0.069 g, 55%) as an amorphous foam. The IR, 1H NMR and mass spectra of this product were in good agreement with those of the substance obtained in a previous experiment (vide supra).

[6-Diphenylcarbamoyloxy-2-(isobutylamino)purin-9-yl]acetic acid methyl ester 14 and [6-diphenylcarbamoyloxy-2-(isobutylamino)purin-7-yl]acetic acid methyl ester 15 (cf. ref. 8)

To compound 10 (3.53 g, 8.47 mmol) suspended in anhydrous DMF (40 mL) was added DIPEA (2.87 mL, 16.74 mmol) and the mixture was briefly heated to 80 °C until a clear solution was obtained (10 min). The mixture was cooled to room temperature, methyl bromoacetate (0.87 mL, 9.32 mmol) was added, and the mixture was stirred for 20 h. The reaction mixture was evaporated in vacuo and the residue was coevaporated with methanol (3×). The partly crystalline material was suspended in methanol (40 mL) and added dropwise to water (120 mL) with vigorous stirring. The precipitate was filtered off (3.99 g, 96%) and recrystallized from EtOAc (190 mL) to afford the title products (2.01 g, 49%), mp 167.0–168.4 °C; from the mother liquor was obtained a further crop (0.37 g, 9%), mp 168.0–170.4 °C. The mother liquor was evaporated and chromatographed by using 1–4% (v/v) methanol in dichloromethane. Eluted first was ester 14 (0.54 g, 13%) then its resiosomer 15 (0.060 g, 15%). Overall yield of 14: 2.92 g, 71%.

Ester 14: white powder, mp 170.0–171.4 °C (from EtOAc); Rₚ 0.37 (CHCl₃-MeOH 95:5); Found: C, 61.35; H, 5.1; N, 17.4. Calc. for C₃₄H₃₈N₆O₃: C, 61.5; H, 4.95; N, 17.2%. 1H NMR (50%, 1 H, 1 H, 2H, 1H-CH₂CH₂CO₂); (CH₃CO₂)CH₂CH₂CH₂CO₂; 3.73 (3 H, s, CH₃), 5.15 (2 H, s, CH₂), 7.31–7.49 (10 H, m, ArH), 8.43 (1 H, s, H-8), 10.64 (1 H, br s, deuter, NH); m/z (ESI) 511 (25%, [M + Na⁺]), 489 (100, [M + H⁺]).

[6-Diphenylcarbamoyloxy-2-(isobutylamino)purin-9-yl]acetic acid 16 (cf. ref. 8)

Ester 14 (2.24 g, 4.59 mmol) was suspended under sonication in a mixture of methanol (6 mL), 1,4-dioxane (24 mL) and water (12 mL). 1 Maq NaOH (5 mL) was added and the mixture was stirred for 30 min. The pH of the mixture was brought to pH 6 by addition of 1 M HCl and the organics were evaporated off in vacuo. The solution was diluted with water (120 mL) and acidified to pH 3 by addition of 1 M HCl. The precipitate was filtered off, washed with ice water to give acid 16 (1.49 g, 91%), white powder, mp 156 °C (decomp.). Attempted recrystallization from EtOAc resulted in gel formation; Rₚ 0.40 (MeCN–MeOH–AcOH 8:1:1) (Found: C, 60.5; H, 4.4; N, 17.4. Calc. for C₂₂H₁₆N₂O₃: C, 60.75; H, 4.7; N, 17.7%). 1H NMR (45.1), 260(4.11), 279(4.06); νmax/cm⁻¹ 3397, 1723s, 1672m, 1591, 1522m, 1493m, 1414m, 1411m, 1307m, 1200s, 1187s, 760w, 701m; δOH (500 MHz) 1.10 [6 H, d, J 6.8, (CH₃)₂CH], 2.85 [1 H, pseudoquintet, J 6.8, (CH₃)₂CH], 3.56 (3 H, s, CH₃), 5.20 (2 H, s, CH₂), 7.28–7.48 (10 H, m, ArH), 8.53 (1 H, s, H-8), 10.57 (1 H, br s, NH); m/z (ESI) 511 (40%, [M + Na⁺]), 489 (100, [M + H⁺])

Esters 19–21

General procedure. To acid 5, 7 or 16 (2.00 mmol) dissolved in anhydrous DMF (20 mL) were added HOBt hydrate (0.61 g, 4.0 mmol) and HBTU (1.52, 4.0 mmol). Meanwhile ester 17* (1.95 g, 3.0 mmol for acids 5, 7 and 1.30 g, 2.0 mmol for acid 16) was suspended in dichloromethane (20/30 mL), extracted with satd. aq. NaHCO₃ (10 mL) and dried. The above dichloromethane solution of free acid 17 base and dipeptide (0.70 mL, 4.0 mmol) were added after 5 min and the reaction mixture was stirred at room temperature. Work-up: after evaporation of the solution in vacuo, the residue was dissolved in dichloromethane (30 mL), extracted with 1 M HCl (19.3 × 10 mL) or with satd. aq. NaHCO₃ (10 mL) and washed successively with satd. aq. NaHCO₃ (5 mL) and brine (25 mL). In the case of 21, after evaporation of the reaction mixture the residue was triturated with EtOAc (5 mL) and filtered, followed by crystallization. The resulting crude products were purified chromatographically (19, 20) or by crystallization (21).

(2-[4-(4-tert-Butylbenzoyl)amino]-2-oxo-1,2-dihydropyrimidin-1-yl)acetyl]-[2-(9H-fluoren-9-ylmethoxy carbamoyl)-ethyl]amino]acetic acid tert-butyl ester 19. — Reaction time: 2 h at room temperature. Chromatography: 1–3% (v/v) methanol in dichloromethane. Yield: 699 (77%); colorless oil. A repeated experiment the extractive work-up was omitted and the crude product was triturated with methanol (2 mL) to yield a cleaner product (0.82 g, 58%), amorphous foam; Rₚ 0.49 (CHCl₃-MeOH 95:5); Found: C, 67.6; H, 6.4; N, 9.7. Calc. for
[2-(9H-Fluoren-9-ylmethoxy carbonyl amino)ethyl]-[2-(4-iso- 
butylaminol-6-oxo-1,6-dihydropurin-9-yl]acetyl]aminoacetic acid 25

To ester 21 (0.08 g, 0.79 mmol) suspended in dichloromethane (20 mL) was added 1,3-dimethylbenzene (0.125 mL, 0.95 mmol) followed by TFA (0.74 mL, 95.3 mmol) and the mixture was stirred for 6 h at room temperature. The solution was evaporated in vacuo, and the residue was coevaporated with EtOAc (4×). The solid residue was triturated with EtOAc, and filtered (0.48 g, quant.), mp 202.0–206.0°C (decomp.) from the mother liquor a further crop was obtained (0.026 g, 5%), mp 206.0–208.1°C. Overall yield of the recrystallized product: 0.236 g, 50%. This reaction was repeated on a 3.0 mmol scale and afforded a quantitative yield of the crude acid 25 (1.80 g). mp 202.0–206.0°C; Rᵣ 0.16 (MeCN–MeOH–AcOH 8:1:1) (Found: C, 59.7; H, 5.3; N, 16.1. Calc. for C₂₃H₂₃NO₅ C: 59.9; H: 5.2; N: 16.3%).

[2-(9H-Fluoren-9-ylmethoxy carbonylamino)ethyl]-[2-(4-tert-
Butylzoinolyl-2-oxo-1,2-dihydropirimidin-
1-yl]acetyl]-[2-(9H-fluoren-9-ylmethoxy carbonylamino)ethyl]aminoacetic acid 23

To ester 19 (0.40 g, 0.56 mmol) dissolved in dichloromethane (20 mL) was added 1,3-dimethylbenzene (0.19 mL, 1.45 mmol) followed by TFA (4.0 mL, 52.3 mmol) and the mixture was stirred for 6 h at room temperature. The solution was evaporated in vacuo, and the residue was coevaporated with acetonitrile (5×). The residue was triturated under diethyl ether, filtered and recrystallized from methanol (20 mL) to give a white powder (0.27 g, 73%), mp 197.4–199.0°C (decomp.). Rᵣ 0.16 (MeCN–MeOH–AcOH 8:1:1) (Found: C, 59.7; H, 5.3; N, 16.1. Calc. for C₂₃H₂₃NO₅ C: 59.9; H: 5.2; N: 16.3%).

[2-(9H-Fluoren-9-ylmethoxy carbonylamino)ethyl]-[2-(6-
((Diphenylcarbamoyloxy)-2-((sobutylamino)purin-9-
yl]acetyl]aminoacetic acid tert-butylic ester 21 — Reaction time: 1.5 h at room temperature. Chromatography: 0/ν (v/v) EtOAc in methanol, yield 1.20 g (85%), amorphous foam; Rᵣ 0.42 (CH₃Cl–MeOH 95:5) (Found: C, 64.8; H, 5.4; N, 13.7. Calc. for C₂₃H₂₃NO₅ C: 64.7; H, 5.6; N, 13.9%; Rᵣmax(EtOH)/nm 205 (lg ε 4.78), 266 (4.41), 278 (4.41), 289 (6.32), 300 (4.32); νmax/cm⁻¹ 3065, 2980, 2943w, 1705m, 1706m, 1699m, 1568m, 1513w, 1458m, 1411w, 1252s, 1157m, 845s, 763m, 743m; δmax (500 MHz, rotamers) 1.32 (9 H, s, Bu'), 2.81 (2 H, S, CH₂), 3.35 (2 H, m, CH₂), 3.56 (2 H, m, CH₂), 3.90 (3 H, S, CH₃), 5.00 (2 H, m, CH₂), 5.25/5.44 (2 H, 2 s, CH₂), 7.16 (2 H, d, J = 8.7, anisoyl CH), 7.39 (2 H, d, J = 7.4 and 7.2, fluoroceryl CH), 7.49 (2 H, d, J = 7.4 and 7.2, fluoroceryl CH), 7.76 (2 H, d, J = 7.4, fluoroceryl CH), 7.96 (2 H, d, J = 7.4, fluoroceryl CH), 8.13 (2 H, d, J = 8.6, anisoyl CH), 8.40 (1 H, s, H-8*), 8.70 (1 H, s, H-2*), 11.10 (1 H, br s, NH); m/z (ESI) 706 (100%, [M + H]⁺).

[2-(9H-Fluoren-9-ylmethoxy carbonylamino)ethyl]-[2-iso-
butylaminol-6-oxo-1,6-di hydropurin-9-yl]acetyl]aminoacetic acid 24

To ester 20 (0.92 g, 1.30 mmol) dissolved in dichloromethane (20 mL) were added 1.3-dimethylbenzene (0.23 mL, 1.82 mmol) and TFA (15.0 mL, 196.0 mmol) and the mixture was stirred for 6 h at room temperature. The solution was evaporated in vacuo, and the residue was coevaporated with acetonitrile (5×). The residue was dissolved in methanol (1 mL), diethyl ether (4.5 mL) was added, and the mixture was stored at 4°C overnight. The resulting gum was triturated with diethyl ether, filtered and recrystallized from methanol (80 mL) to afford a white powder (0.59 g, 70%), mp 160.8–163.9°C; Rᵣ 0.76 (CH₃Cl–MeOH 6:4) (Found: C, 62.8; H, 4.65; N, 14.9. Calc. for C₂₃H₂₃NO₅ C: 62.9; H, 4.8; N, 15.1%; δmax(EtOH)/nm 206 (lg ε 4.74), 266 (4.43), 278 (4.43), 289 (6.35); νmax/cm⁻¹ 3440, 3222w, 3102w, 3069w, 2978w, 2946w, 1713m, 1695s, 1647m, 1582w, 1525m, 1401w, 1215w, 1275m, 1762m; δmax (500 MHz, rotamers) 3.15/5.9 (2 H, m, CH₂), 3.18 (2 H, s, CH₂), 3.85 (3 H, s, CH₃), 4.03/4.10 (2 H, m, CH₂), 4.12 (2 H, s, CH₂), 4.30/4.39 (2 H, S, 2 amidesyl CH), 4.78 (2 H, s, 4 adenyl CH), 7.08 (2 H, d, J = 8.7, anisoyl CH), 7.29/7.42 (1 H, 2 br t, NH), 7.32 (2 H, dd, J = 7.4 and 7.3, fluoroceryl CH), 7.41 (2 H, dd, J = 7.4 and 7.3, fluoroceryl CH), 7.70 (2 H, d, J = 7.4, fluoroceryl CH), 7.88 (2 H, d, J = 7.4, fluoroceryl CH), 8.06 (2 H, d, J = 7.8, anisoyl CH), 8.33 (1 H, s, H-8*), 8.62/8.67 (1 H, s, H-2*); m/z (ESI) 650 (100%, [M + H]⁺).
CH), 11.59/11.65 (1 H, 2 s, NH*), 12.07 (1 H, s, NH*), 12.50 (1 H, br s, OH*); m/z (APCI) 602 (100%, [M + H]+).

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