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**Full Paper**

# Peptide Nucleic Acid Monomers: A Convenient and Efficient Synthetic Approach to Fmoc/Boc Monomers

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A convenient and cost-effective method for the synthesis of Fmoc/Boc-protected peptide nucleic acid monomers is described. The Fmoc/Boc strategy was developed in order to eliminate the solubility issues during peptide nucleic acid solid-phase synthesis, in particular that of the cytosine monomer, that occurred when using the commercialized Bhoc chemistry approach.

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## Introduction

Peptide nucleic acid (PNA) is an unnatural oligonucleotide polymer analogous to the biopolymers deoxyribonucleic acid and ribonucleic acid, which carry and encode genetic information. PNA consists of a backbone of repeating *N*-(2-aminoethyl) glycine units connected by peptide bonds with methylene carbonyl linkers attaching the traditional nucleotide bases: adenine (A), guanine (G), cytosine (C), thymine (T) or uridine (U).[1] Advantages of PNA chemistry include the ability to form an achiral polymer with unnatural amino acid monomers that are less susceptible to proteases, have a greater pH stability and are amenable to peptide–base coupling techniques.[2–3]

A considerable amount of interest has developed around PNA and similar oligonucleotides owing to their potential applications in diagnostics, biosensors and antisense therapy.[1,3,4] Our work has focussed on the development of PNA sequences that have shown efficacy in a transgenic mouse model for amyotrophic lateral sclerosis, a form of motor neurone disease.[5–6] Synthesis of the PNA of interest has required significant amounts of the corresponding monomers. These building blocks are commercially available, with fluorenylmethyloxycarbonyl (Fmoc) protection of the *N*-(2-aminoethyl) glycine backbone and, for the adenine, guanine and cytosine monomers, benzhydryloxycarbonyl (Bhoc)-protection of the exocyclic amines of the nucleobase. The desired PNA molecules are synthesized on resin by automated techniques, isolated manually, purified by reverse-phase (RP)-HPLC and typically characterized by matrix-assisted laser desorption–ionization time-of-flight (MALDI-TOF) mass spectrometry. Although this approach follows standard protocols for PNA synthesis, it is not without problems. The monomers are available from few commercial sources, are very expensive (AU$270–308 per gram of monomer) and can be obtained only in the Bhoc-protected form. As a consequence of their expense, automated synthesis is usually undertaken on a very small scale (2 μmol), which results in correspondingly poor yields and difficulties in handling the purified material. The PNA monomers also require great care in storage and use to avoid degradation or the absorption of moisture, again resulting in a significant decrease in reaction yield. Particular difficulties are also encountered when solubilizing the monomers in the carrier solvent of *N*-methyl-2-pyrrolidone (NMP) for automated synthesis, particularly for the cytosine monomer, which appears to be the most problematic. This results in large amounts of time spent preparing the monomer solutions using agitation, with sonication also often required. Clearing the lengthy delivery lines of the automated PNA synthesizer, if later precipitation occurs, is also very time-consuming and inconvenient.

As a result, we were interested in finding an improved means of obtaining PNA monomers by developing a convenient synthetic route using an *N*-protecting group that would be more soluble in NMP than the Bhoc group. Although there are some synthetic methodologies in the literature outlining the synthesis of monomers with different protecting groups for both the backbone and base,[7–10] none of the procedures met our needs very well. In particular, we were interested in the work by Porcheddu and coworkers in producing a series of *N*-*tert*-butoxycarbonyl (Boc)-protected nucleobases;[11] however, their synthesis of the *N*-(-2-Fmoc-aminoethyl)glycine backbone was temperamental at best. Fmoc protection of ethanolamine was effective and straightforward; however, significant difficulties were encountered when attempting to oxidize the alcohol into the corresponding aldehyde under the conditions described. Oxidation using stabilized 2-iodoxybenzoic acid (SIBX, 45 wt-% IBX with benzoic acid and isophthalic acid) rather than the neat IBX as described, as a result of the potential explosive hazard, appeared to work in moderate yield. However, we were unable to isolate the corresponding aldehyde with sufficient purity. These difficulties prompted us to find a better, more efficient and cleaner solution.

Herein, we describe a convenient synthesis of the four Fmoc-protected PNA monomers of C, T, G and A with Boc nucleobase protection as required (Fig. 1).

## Results and Discussion

Our approach uses an efficient route centred on the production of an ethyl ester monomer **1**. It was originally envisaged that a *tert*-butyl ester would be critical for efficiency of preparation, as it was suggested in the literature that this functionality would be selectively cleaved by zinc bromide while retaining *N*-Boc protection.[12] Unfortunately, this desired orthogonal cleavage was not observed. Indeed, HPLC monitoring of the reaction showed that *N*-Boc deprotection was occurring preferentially. Therefore, the ethyl ester was determined to be a more suitable candidate for the protection of the carboxylic acid group. The ethyl ester was desired as cleavage of benzyl or allyl esters, such as those used by Hudson et al.,[9,13] require expensive and toxic reagents such as Pd/C and $Pd(PPh_3)_4$ whereas LiOH could be used for the ethyl ester hydrolysis. This is particularly beneficial when undertaking synthesis on a larger scale.

The Fmoc protected *N*-(2-aminoethyl)glycine backbone was prepared as shown in Scheme 1. Although this compound has been claimed in the patent literature,[14] no synthetic methodology or characterization data could be found. In our method, ethylenediamine was alkylated with one equivalent of *tert*-butyl bromoacetate in approximately 92 % yield, followed by Fmoc protection of the primary amine in 95 % yield to give the *tert*-butyl *N*-(2-(*N*-9-fluorenylmethoxycarbonyl)aminoethyl) glycinate hydrochloride salt **4**, according to the procedure by Thomson et al.[8] The bulky *tert*-butyl ester was employed to prevent self-cyclization, which was observed when alkylating with methyl or ethyl bromoacetate. Hydrolysis of the *tert*-butyl ester **4** was easily achieved by acidolysis using trifluoroacetic acid in dichloromethane in near-quantitative yield. The ethyl ester was installed by refluxing **5** in ethanol under acidic conditions to give the ethyl ester backbone **1** in 98 % yield. This series of reactions is able to be performed in excellent yields, with compounds obtained easily in very good purity. With an overall yield of 85 % from an inexpensive and readily available starting material, we consider this route preferable to those describing the synthesis of the equivalent methyl ester backbone.[10,11,15,16]

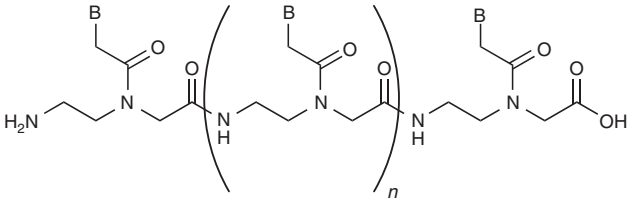
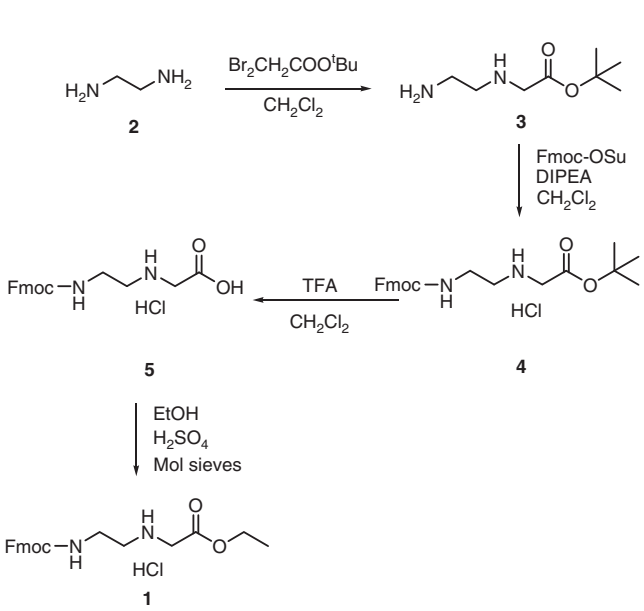


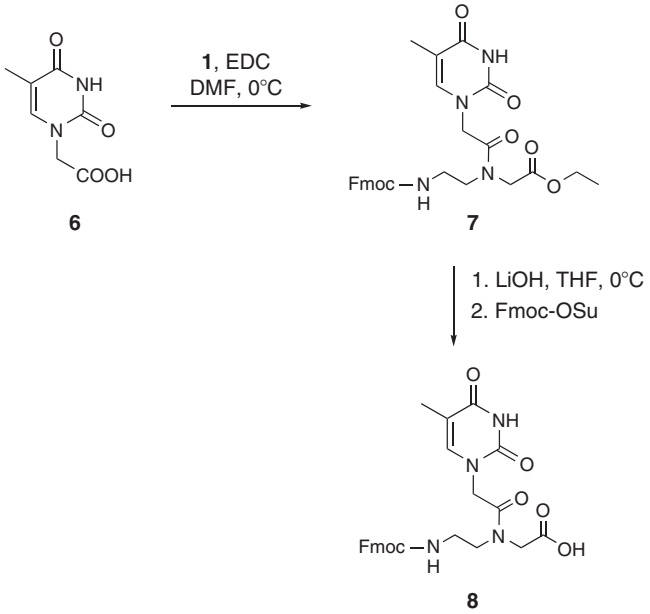
**Fig. 1.** Peptide nucleic acid where B = A, G, C, T or U.




**Scheme 1.** Synthesis of the Fmoc-protected ethyl ester backbone.

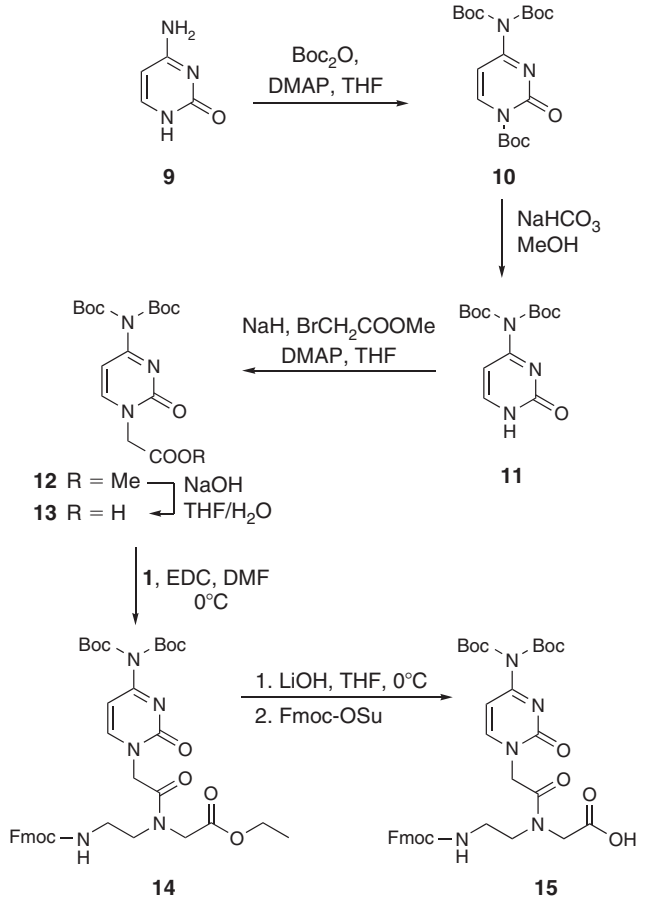
Synthesis of the four nucleobases was then undertaken in three to seven steps depending on the protection required owing to potential side reaction. The synthesis of the thymine PNA monomer **8** (Scheme 2) was straightforward following the synthesis of thymine acetic acid **6**.[16] Coupling of **6** to the Fmoc-protected backbone **1** to obtain **7** was achieved in 74 % yield using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC). EDC coupling was desired over other coupling methods such as HBTU/HOBt,[10] *N*,*N*′-dicyclohexylcarbodiimide,[16,18] isobutylchloroformate (IBC)[11] and ByBop[19] as nucleophilic attack of the secondary amine easily displaces the *O*-acylisourea intermediate and the resulting urea by-product is water-soluble. In addition to this, EDC is safer to handle and the coupling conditions used herein did not require the addition of HOBt as described in other coupling procedures.[9,13] Base hydrolysis of the ethyl ester was best achieved using 1 M LiOH in THF at 0°C. The use of 2.5 M NaOH[11] led to Fmoc cleavage at a faster rate than that of the ethyl ester. Attempted optimization of NaOH concentration, reaction time and temperature were unsuccessful. Using LiOH also led to cleavage of the Fmoc group, but to a lesser extent. To overcome this issue, Fmoc *N*-hydroxysuccinimide ester was added to the reaction mixture to reprotect the cleaved amine to give the completed thymine PNA monomer **8**.[20]

Synthesis of the remaining three nucleobase monomers required protection of the exocyclic amines in order to improve




**Scheme 2.** Synthesis of the thymine peptide nucleic acid monomer.

coupling efficiency and hence prevent side reactions. The acid-labile protecting group Boc was installed over the classical Bhoc group in order to improve the solubility of the PNA monomers in NMP, particularly the cytosine derivative. Synthesis of the Boc-protected cytosine monomer is shown in Scheme 3. Preparation of the cytosine acetic acid **13** was achieved by Boc protection of **9**, followed by selective deprotection, alkylation and saponification.[11] The *N*-(2-aminoethyl)glycinate ethyl ester backbone **1** was coupled to the protected cytosine acetic acid derivative **13** using EDC in 71 % yield. This was preferred over the IBC,[11] as the IBC reaction was not high-yielding and led to several undesired side products. Saponification of **14** with LiOH and subsequent reprotection with Fmoc *N*-hydroxysuccinimide ester afforded the completed cytosine PNA monomer **15**. The solubility of the Boc-protected cytosine monomer **15** was determined to be more than six times that of the corresponding Bhoc-protected monomer in NMP. Furthermore, **15** did not precipitate out of solution after several days.




**Scheme 3.** Synthesis of the cytosine peptide nucleic acid monomer.

The synthesis of the final two Boc-protected purine monomers is shown in Scheme 4. Preparation of the adenine nucleobase **16** and the more complex guanine analogue **19** followed the methodology of Porcheddu.[11] **16** and **19** were coupled with the Fmoc-protected *N*-(2-aminoethyl)glycine backbone **1,** following activation with EDC, in 73 and 69 % yield respectively. Deprotection of **17** and **20** was undertaken using LiOH and subsequent reprotection with Fmoc *N*-hydroxysuccinimide ester resulted in the completed nucleobases **18** and **21** in high yields (81 and 89 % respectively).

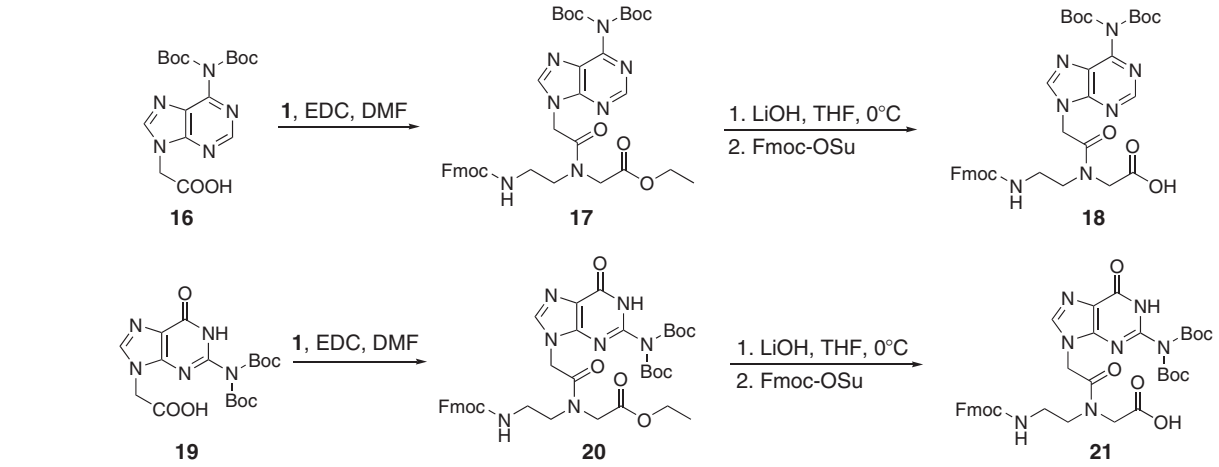
## Conclusion

A practical and effective synthetic route to the preparation of Fmoc/Boc protected PNA monomers from inexpensive starting materials has been reported. This is a much easier, more convenient method compared with those currently available. The synthesis and characterization of *N*-(2-(Fmoc)aminoethyl) glycinate ethyl ester and the subsequent four novel monomer ethyl esters have not been reported in the literature to date.

The coupling of the nucleobases to the backbone is simple and efficient using EDC. The novel ethyl esters are hydrolysed in mild basic conditions although reprotection of the Fmoc backbone is required as a fraction of the protecting group is cleaved, contrary to other reports in the literature. Most importantly, the introduction of Boc protection has increased the solubility of the cytosine building block over six-fold without crystallization over time.

## Experimental

NMR spectra were recorded on a Bruker Avance-300 spectrometer ($^1$H at 300.13 MHz and $^{13}$C at 75.47 MHz). TLC was performed using Merck Kieselgel 60 $F_{254}$ plates. Drying and purification methods for solvents and reagents were followed from procedures described by Armarego and Chai.[21] Melting




**Scheme 4.** Synthesis of the adenine and guanine peptide nucleic acid monomers.

points were measured on a hot-stage Reichert Thermopan apparatus and are uncorrected. Low-resolution electrospray ionization (ESI) mass spectrometry was carried out using an Esquire 6000 ion-trap mass spectrometer (Bruker Daltonics, Germany). The samples were introduced at a flow rate of 4 μL $min^{-1}$ and a mass range of 50–3000 *m*/*z* was recorded. A scan rate of 5500 *m*/*z* $s^{-1}$ was used with the temperature set at 300°C. High-resolution electrospray ionization mass spectrometry (HR-ESI) was performed on an Agilent Technologies 6220 accurate-mass TOF (time of flight) LC/MS as the solutions specified. $[M]^+$ denotes the molecular ion. Analyses were performed in positive ion mode ($ESI^+$) unless otherwise stated.

### *Ethyl 2-((2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino) ethyl)amino)acetate Hydrochloride Salt* **1**

A solution of **5** (3 g, 7.96 mmol) in dry ethanol (30 mL) and $H_2SO_4$ (500 μL) was left to reflux over 3-Å molecular sieves overnight. The hot solution was filtered and concentrated, and the addition of cold 1 M HCl precipitated **1** as a white solid (3.15 g, 98 %), mp 150–151°C. $\delta_H$ (300 MHz, [D6]DMSO) 9.52 (s, 2H, NH), 7.85 (d, *J* 7.5, 2H, 2 × CH), 7.66 (d, *J* 7.5, 2H, 2 × CH), 7.53 (t, *J* 5.4, 1H), 7.38 (t, *J* 7.5, 2H, 2 × CH), 7.30 (t, *J* 7.5, 2H, 2 × CH), 4.29 (d, *J* 6.3, 2H, $CHCH_2$), 4.20–4.131 (m, 4H, $CH_2CH_3$ and $CHCH_2$), 3.93 (s, 2H, $CH_2NH$), 3.30 (m, 2H, $CH_2CH_2$), 2.99 (t, *J* 6.0), 1.20 (t, *J* 7.2, 3H, $CH_2CH_3$). $\delta_C$ (75 MHz, [D6]DMSO) 167.0, 156.7, 144.3, 144.3, 141.2, 128.1, 127.5, 125.6, 120.6, 66.1, 62.1, 47.1, 14.9, 37.0, 14.4. *m*/*z* (HR-ESI) 369.1816 (M + H), 391.1635 (M + Na).

### *2-((2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)ethyl) amino)acetic Acid Hydrochloride Salt* **5**

A solution of **4** (3.0 g, 6.93 mmol) in a 1 : 1 solution of TFA/$CH_2Cl_2$ (20 mL) was stirred for 1 h. Toluene (20 mL) was added to the solution and the solvents evaporated under vacuum. This process was repeated twice. $NaHCO_3$ was added to the residue, followed by acidification using 6 M HCl until precipitation ceased. The product was filtered by vacuum filtration, washed with water and further dried in a desiccator overnight to give **5** as a white solid (2.48 g, 98 %), mp 156–159°C (lit.[22] 154–156°C). $\delta_H$ (300 MHz, [D6]DMSO) 9.32 (bs, 1H, OH), 7.86 (d, *J* 7.5, 2H, 2 × CH), 7.65 (d, *J* 7.2, 2H, 2 × CH), 7.48 (bt, 1H), 7.38 (t, *J* 7.2, 2H, 2 × CH), 7.30 (t, *J* 7.2, 2H, 2 × CH), 4.30 (d, *J* 6.9, H, $CHCH_2$), 4.19 (t, *J* 6.6, 1H, $CHCH_2$), 3.29 (m, 2H, $CH_2CH_2$), 2.98 (t, *J* 6.0, 2H, $CH_2CH_2$). $\delta_C$ (75 MHz, [D6]DMSO) 183.8, 168.4, 144.3, 141.2, 128.1, 127.5, 125.6, 120.6, 66.07, 47.4, 47.1, 46.9, 37.1.

### *Ethyl 2-(*N*-(2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino) ethyl)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido)acetate* **7**

To a solution of **6** (275 mg, 1.49 mmol) in anhydrous DMF (5 mL) at 0°C under argon, EDC (514 mg, 2.68 mmol) was added and stirred for 30 min. To this, **1** (635 mg, 1.57 mmol) was added portionwise over a period of 30 min and the reaction stirred overnight. The reaction mixture was poured over ice and the resultant solid was filtered off and purified by flash chromatography (EtOAc) to give **7** as a white solid (583 mg, 74 %), mp 160–162°C. $\delta_H$ (300 MHz, [D6]DMSO, two rotamers 3 : 2) 11.22 (s, NH), 7.85 (d, *J* 7.5, 2H), 7.65 (d, *J* 7.5, 2H), 7.40–7.22 (m, 6H, 4 × Ar–CH, NH, vinylic H), 4.63 (s, 1.2H, N–$CH_2$–C=O), 4.45 (s, 0.8H, N–$CH_2$–C=O), 4.32–3.98 (m, 7H, $CH_2$–$CH_3$, CH–$CH_2$, N–$CH_2$–C=O), 3.42–3.04 (m, 4H, $CH_2$–$CH_2$), 1.70 (s, 18H, 3 × $CH_3$), 1.22–1.12 (m, 3H, $CH_2$–$CH_3$). $\delta_C$ (75 MHz, [D6]DMSO) 169.4, 167.8, 164.8, 156.8, 151.4, 144.3, 142.5, 141.2, 128.1, 127.5, 125.6, 120.6, 65.9, 61.0, 48.4, 48.6, 47.3, 47.2, 14.5, 12.4. *m*/*z* (HR-ESI) 535.2196 (M + H), 557.2014 (M + Na).

### *2-(*N*-(2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino) ethyl)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido)acetic Acid* **8**

Compound **7** (173 mg, 0.32 mmol) was dissolved in THF (2 mL) and chilled to 0°C. To this, 2 equiv. of LiOH (1 M) was added dropwise and the reaction was stirred for 30 min, followed by the addition of Fmoc-succinimide (109 mg, 0.32 mmol), and the reaction stirred for 12 h. The resultant solid was filtered and the residue dissolved in hot EtOAc and precipitated with hexane to give **8** as a white solid (163 mg, 85 %), mp 220–222°C (lit.[4] 216–219°C). $\delta_H$ (300 MHz, [D6]DMSO) 11.23 (s, 1H, OH), 7.84 (d, *J* 7.2, 2H, 2 × CH), 7.64 (d, *J* 7.2, 2H, 2 × CH), 7.48 (bt, 1H), 7.38 (t, *J* 7.2, 2H, 2 × CH), 7.30 (t, *J* 7.2, 2H, 2 × CH). $\delta_C$ (75 MHz, [D6]DMSO) 171.4, 167.8, 164.5, 156.2, 151.1, 144.0, 142.28, 140.8, 127.7, 127.2, 125.4, 120.2, 108.0, 65.6, 51.7, 47.8, 47.5, 46.9, 46.8, 12.0. *m*/*z* (LR-ESI) 507.4 (M + H), 529.4 (M + Na).

### *Ethyl 2-(*N*-(2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino) ethyl)-2-(4-(bis(*tert*-butoxycarbonyl)amino)-2-oxopyrimidin-1(2H)-yl)acetamido)acetate* **14**

A solution of **13** (400 mg, 1.08 mmol) in anhydrous DMF was cooled to 0°C under a blanket of argon. To this, EDC (372 mg, 7.94 mmol) was added and the reaction stirred for 30 min, followed by the addition of **1** (460 mg, 1.13 mmol), and the reaction left to stir overnight. The reaction was quenched on ice, the resulting solid filtered, purified by column chromatography (7 : 3 EtOAc : hexane) and trituated with diethyl ether to give **14** as white solid (550 mg, 71 %), mp 81–83°C. $\delta_H$ (300 MHz, [D6] DMSO, two rotamers 3 : 2) 7.98–7.92 (m, 1H, rotamer vinylic H), 7.85 (d, *J* 7.5, 2H), 7.65 (d, *J* 7.2, 2H), 7.34–7.23 (m, 5H, 4 × Ar–CH, NH), 6.80–6.76 (m, 1H, rotamer vinylic H), 4.83 (s, 12.H, N–$CH_2$–C=O), 4.65 (s, 0.8H, N–$CH_2$–C=O), 4.32–4.01 (m, 7H, $CH_2$–$CH_3$, CH–$CH_2$, N–$CH_2$–C=O), 3.43–3.06 (m, 4H, $CH_2$–$CH_2$), 1.70 (s, 18H, 3 × $CH_3$), 1.23–1.12 (m, 3H, $CH_2$–$CH_3$). $\delta_C$ (75 MHz, [D6]DMSO) 169.4, 167.5, 162.4, 156.8, 154.5, 152.0, 149.7, 144.3, 141.2, 128.0, 127.5, 125.6, 120.6, 95.7, 85.0, 66.0, 61.0, 50.2, 48.4, 47.5, 47.1, 27.7, 14.5. *m*/*z* (HR-ESI) 720.3245 (M + H), 742.3061 (M + Na).

### *2-(*N*-(2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino) ethyl)-2-(4-(bis(*tert*-butoxycarbonyl)amino)-2-oxopyrimidin-1(2H)-yl)acetamido)acetic Acid* **15**

Compound **14** (150 mg, 0.20 mmol) was dissolved in THF (2 mL) and chilled to 0°C. To this, 2 equiv. of LiOH (1 M) was added dropwise and the reaction was stirred for 30 min, followed by the addition of Fmoc-succinimide (70 mg, 0.20 mmol), and the reaction stirred for 12 h. The resultant solid was filtered and recrystallized by ethyl acetate and hexane to give **15** as a white solid (120 mg, 83 %), mp 115–116°C (lit.[5] 110–112°C). $\delta_H$ (300 MHz, [D6]DMSO, two rotamers 3 : 2) 7.97–7.92 (m, 1H, rotamer vinylic H), 7.86 (d, *J* 7.2, 2H), 7.65 (d, *J* 7.2, 2H), 7.40–7.22 (m, 5H, 4 × Ar–CH, NH), 6.80–6.74 (m, 1H, rotamer vinylic H), 4.83 (s, 12.H, N–$CH_2$–C=O), 4.65 (s, 0.8H, N–$CH_2$–C=O), 4.32–4.20 (m, 3.8H, CH–$CH_2$, N–$CH_2$–C=O, 0.8H), 3.97 (s, 1.2H, $CH_2$), 3.42–3.09 (m, 4H, $CH_2$–$CH_2$), 1.45 (s, 18H,

3 × $CH_3$). $\delta_C$ (75 MHz, [D6]DMSO) 170.8, 167.3, 162.4, 156.8, 154.6, 152.0, 149.7, 144.4, 141.2, 128.1, 127.5, 125.6, 120.6, 95.7, 85.0, 66.0, 50.2, 48.2, 47.4, 47.2, 27.7. *m/z* (LR-ESI) 692.3 (M + H), 714.3 (M + Na).

*Ethyl 2-(*N*-(2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino) ethyl)-2-(6-(bis(*tert*-butoxycarbonyl)amino)-9*H*-purin-9-yl) acetamido)acetate* ***17***

A solution of **16** (450 mg, 1.14 mmol) in anhydrous DMF (5 mL) was cooled to 0°C under a blanket of argon. To this, EDC (395 mg, 2.06 mmol) was added and the reaction stirred for 30 min, followed by the addition of **1** (508 mg, 1.25 mmol), and the reaction left to stir overnight. The reaction was quenched on ice, the resulting pink solid filtered, purified by column chromatography (4 : 1 EtOAc : hexane) and coevaporated with diethyl ether to give **17** as white solid (617 mg, 73 %), mp 85–88°C. $\delta_H$ (300 MHz, [D6]DMSO, two rotamers 3 : 2) 8.76 (s, 0.3H, minor vinylic rotamer), 8.44 (s, 0.7H, major vinylic rotamer), 8.44 (d, 1H, vinylic rotamer), 7.86–7.82 (m, 2H, 2 × Ar–H), 7.66–7.62 (m, 2H, 2 × Ar–H), 7.46–7.24 (m, 5H, 2 × Ar–H, NH), 5.37 (s, 1.2H, N–$CH_2$–C=O), 5.20 (s, 0.8H, N–$CH_2$–C=O), 4.41–4.00 (m, 7H, C$H_2$–$CH_3$, CH–$CH_2$, N–$CH_2$–C=O), 3.54–3.09 (m, 4H, $CH_2$–$CH_2$), 1.34 (s, 18H, 3 × $CH_3$), 1.24–1.05 (m, 3H, $CH_2$–C$H_3$). $\delta_C$ (75 MHz, [D6]DMSO) 169.3, 167.0, 156.9, 153.9, 151.9, 150.5, 149.4, 148.1, 144.3, 141.2, 128.1, 127.8, 127.7, 127.5, 125.5, 120.6, 83.7, 66.0, 61.0, 48.4, 47.5, 47.2, 44.9, 44.6, 39.3, 27.7, 14.4. *m/z* (HR-ESI) 744.3353 (M + H), 766.3159 (M + Na).

*2-(*N*-(2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino) ethyl)-2-(6-(bis(*tert*-butoxycarbonyl)amino)-9*H*-purin-9-yl) acetamido)acetic Acid* ***18***

Compound **17** (200 mg, 0.27 mmol) was dissolved in THF (2 mL) and chilled to 0°C. To this, 2 equiv. of LiOH (1 M) was added dropwise and the reaction was stirred for 30 min, followed by the addition of Fmoc-succinimide (90 mg, 0.27 mmol), and the reaction stirred for 12 h. The resultant solid was filtered and recrystallized by ethyl acetate and hexane to give **18** as a white solid (156 mg, 81 %), mp 105–106°C (lit.[5] 103–104°C). $\delta_H$ ([D6]DMSO, two rotamers 3 : 2) 9.98 (s, 1H, OH), 8.75 (s, 0.4H, CH), 8.70 (s, 0.6H, CH), 8.44 (s, 1H, CH), 7.90–7.81 (m, 2H, 2 × CH), 7.67–7.58 (m, 2H, 2 × CH), 7.50–7.35 (m, 5H, 4 × CH, NH), 5.37 (s, 1.2H, $NCH_2C$=O), 5.19 (s, 0.8H, $NCH_2C$=O), 4.34–4.20 (complex m, 5H), 3.98 (s, 2H, $CH_2$), 3.53–3.12 (m, 4H), 1.34 (s, 18H, 2 × $C(CH_3)_3$). $\delta_C$ (75 MHz, [D6]DMSO) 173.2, 170.8, 166.8, 156.9, 153.9, 151.5, 150.2, 149.4, 148.3, 144.3, 141.2, 141.1, 128.1, 127.8, 127.5, 125.6, 125.5, 120.6, 83.7, 66.0, 49.8, 48.1, 47.4, 47.2, 44.8, 38.4, 27.7. *m/z* (LR-ESI) 716.6 (M + H), 738.5 (M + Na).

*Ethyl 2-(*N*-(2(((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino) ethyl)-2-(2-(bis(*tert*-butoxy carbonyl)amino)-6-oxo-1*H*-purin-9(6*H*)-yl-acetamido)acetate* ***20***

To a solution of **19** (912 mg, 2.23 mmol) and **1** (890 mg, 2.23 mmol) in anhydrous DMF (10 mL), EDC (855 mg, 4.45 mmol) was added portionwise over a period of 1 h and stirred under argon overnight. The reaction mixture was poured over ice and the resultant solid was filtered off. The resulting solid was purified by column chromatography (4 : 1 EtOAc : hexane) and trituated with diethyl ether to give **20** as a white crystalline solid (1.16 g, 69 %), mp 87–89°C. $\delta_H$ ([D6]DMSO, two rotamers 3 : 2) 7.98 (s, 1H, NCH), 7.84 (d, *J* 7.5, 2H, 2 × CH), 7.65 (d, *J* 7.2, 2H, 2 × CH), 7.37 (t, *J* 7.5, 2H, 2 × CH), 7.28 (t, *J* 7.5, 2H, 2 × CH), 5.18 (s, 1.2H, $NCH_2C$=O), 5.18 (s, 0.8H, $NCH_2C$=O), 4.37–3.96 (complex m, 7H), 3.50 (br t, 2H), 3.10 (br t, 2H), 1.31 (s, 18H, 2 × $C(CH_3)_3$), 1.13 (t, *J* 7.2, 3H, $CH_2CH_3$). $\delta_C$ (75 MHz, [D6]DMSO) 169.3, 167.2, 156.8, 156.6, 154.9, 152.1, 151.4, 144.3, 141.8, 141.2, 128.1, 127.5, 125.5, 120.6, 117.6, 82.5, 65.9, 61.0, 49.6, 48.4, 47.4, 44.0, 27.8, 14.4. *m/z* (HR-ESI) 760.3303 (M + H), 782.3120 (M + Na).

*2-(*N*-(2((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)ethyl)-2-(2-(bis(*tert*-butoxy carbonyl)amino)-6-oxo-1*H*-purin-9(6*H*)-yl-acetamido)acetic Acid* ***21***

Compound **20** (200 mg, 0.23 mmol) was dissolved in THF (2 mL) and chilled to 0°C. To this, 2 equiv. of LiOH (1 M) was added dropwise and the reaction was stirred for 30 min, followed by the addition of Fmoc-succinimide (13 mg, 0.23 mmol), and the reaction stirred for 12 h. The reaction was quenched with 1 M HCl, extracted with EtOAc (3 × 15 mL) and washed with water. The residue was purified by column chromatography ($CH_2Cl_2$ : MeOH 19 : 1) yielding **21** as a white solid (171 mg, 89 %), mp 253°C (decomp.) (lit.[5] 255°C decomp.). $\delta_H$ (300 MHz, [D6] DMSO, two rotamers 3 : 2) 7.98 (d, 1H, rotamer vinylic H), 7.84–7.82 (m, 2H, 2 × Ar–H), 7.49 (bs, 1H, NH), 7.36–7.27 (m, 4H, 4 × Ar–H), 5.15 (s, 0.8H, N–$CH_2$–C=O), 4.96 (s, 1.2H, N–$CH_2$–C=O), 4.30–3.92 (m, 5H, CH–$CH_2$, N–$CH_2$–C=O), 3.47–3.12 (m, 4H, $CH_2$–$CH_2$), 1.32 (s, 18H, 3 × $CH_3$). $\delta_C$ (75 MHz, [D6] DMSO) 171.9, 167.6, 156.77, 156.54, 154.8, 152.1, 151.4, 144.3, 142.0, 141.2, 128.0, 127.6, 125.7, 120.5, 117.5, 82.5, 66.0, 51.8, 48.1, 48.0, 47.1, 44.2, 27.8. *m/z* (LR-ESI) 732.2 (M + H).

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