# Chiral peptide nucleic acid monomers (PNAM) with modified backbones†

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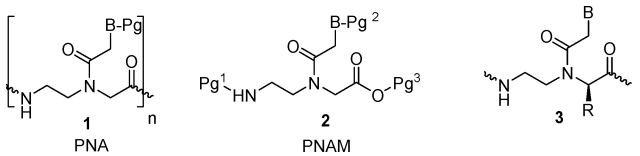
Convenient high yielding syntheses of optically pure PNAMs comprising L- or D-serine, L-lysine and L-arginine units linked to thymine or Cbz-cytosine are described. Simple workup and inexpensive reagents are employed and free amino acids are used as coupling components.

## Introduction

Peptide nucleic acids are synthetic analogs of nucleic acids in which the phosphate–sugar polynucleotide backbone is replaced by a flexible pseudo-peptide polymer to which the nucleobases are linked. Nielsen and co-workers have reported "classical" PNA **1** derived from PNA monomer (PNAM) units **2** comprising *N*-protected (2-aminoethyl)glycine with an attached protected nucleobase. The PNA **1** has the capacity to bind with high affinity and specificity to a complementary sequence of DNA[1] or RNA[2] and **1** is also resistant to DNAses and proteinases.[3]

The unique physico-chemical characteristics of PNAs **1** have led to their use in a wide range of biological assays,[4,5] *in vitro* antigen and antisense studies,[6–9] and *in situ* studies of cancer and aging.[10–12] However, **1** lacks the formal charges found in DNA and classical transfection agents are often unable to deliver a PNA into cells. Other limitations include low aqueous solubility, ambiguity in DNA binding orientation and poor membrane permeability.[13] During the last decades modified PNAs aiming to improve the characteristics of Nielsen's PNA have been reported,[13–15] however, the search for new ligands with alternative modes of binding to DNA duplexes remains of paramount importance. Ideal ligands would: (i) bind DNA (and RNA) with both high affinity and high selectivity, (ii) possess sufficient biostability and (iii) lack sequence restrictions.

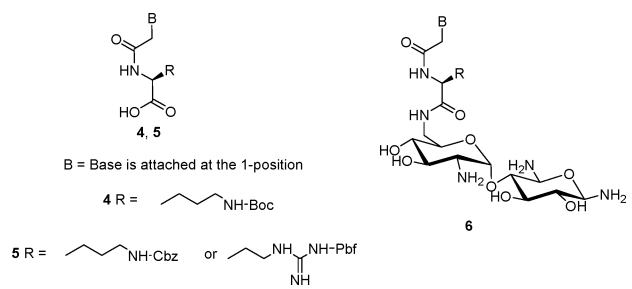
One important strategy for improving the properties of PNA **1** is the utilization of monomeric PNA building blocks with modified peptide backbones.[13,15–18] The PNA backbone has been modified in several ways: importantly, the replacement of glycine by α-amino acids having hydrophobic, hydrophilic or charged α-substituents leads to chiral PNA **3** with different orientational selectivity in complementary DNA binding, as well as better solubility and cell uptake rate.[13–15,19]




Lysine, arginine and serine are of considerable interest among the α-amino acids utilized for backbone modifications of PNA. Positively charged lysine-based monomers are considered to be efficient in creating stable PNA–DNA duplexes.[20] Replacements with D-lysine exhibited DNA hybridization properties equal to that of the original PNA.[20–22] The lysine-containing oligomers were also readily soluble in aqueous systems.[22] The lysine moiety allows modification of backbones and the introduction of a broad range of functional groups without interfering with the binding to DNA or RNA.[23] In many cases, PNAs were constructed with a lysine residue at the C-terminus[24,25] in order to improve the solubility of the whole molecule or to avoid self-aggregation. Zhang *et al.* designed novel chiral PNA molecules with lysine as the main chain unit; however, monomer **4** was obtained utilizing a multistep synthetic methodology.[23c,d]

Incorporation of the arginine side chain (guanidinium functional group) into the PNA backbone facilitates PNA uptake into mammalian cells.[16] However, application of the recent methodology for the preparation of nucleobase–lysine and arginine monomers **5** suffers from the use of DMF as the solvent, long reaction times (23–25h), the need to deprotect esters, and moderate yields of products.[26]

In addition to their utilization in the construction of PNA oligomers, nucleobase–amino acid (lysine, arginine) building blocks are appropriate for the design of new drugs with a neamine scaffold such as **6**. Neamine derivatives, bearing a nucleobase with lysine and arginine as linkers, are active inhibitors of the HIV (human immunodeficiency virus) TAR-Tat (trans-activator responsive region-trans-activator protein) interaction.[26]




Utilization of serine-containing PNA monomers for the construction of PNA oligomers leads to SNE **7** (serine-based nucleobase-linked polyester), polyester analogs of nucleic acids.[27] The replacement of the amide linkages by ester linkages should improve the water solubility and backbone flexibility of a PNA; however, the increased liability of esters to strong acids or bases

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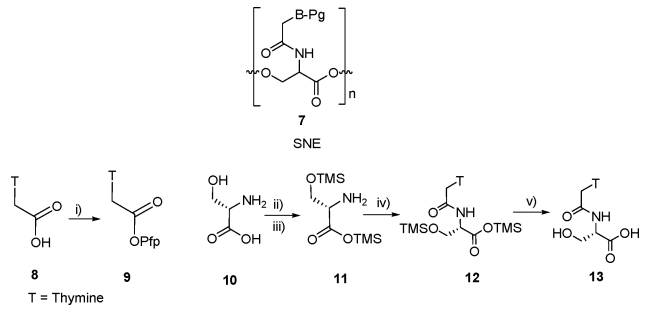
as compared with amide linkages requires the development of new synthetic strategies for SNE oligomers. Lacking an efficient synthetic procedure, SNEs have received little attention. Recently Murata and Wada first reported[27] the multistep synthesis of the optically active unprotected thymine–serine monomer **13** (Scheme 1). (Thymin-1-yl)acetic acid **8** was converted to the pentafluorophenyl ester 9 (37%), and coupled with TMS-protected L-serine **11** to obtain protected SNE monomer **12**, which undergoes subsequent deprotection of the TMS groups to give **13**.[27]




**Scheme 1** *Reagents and conditions*: (i) pentafluorophenol, DCC, DMF, rt, 17 h; (ii) TMS-Cl, $CH_2Cl_2$, 60 °C, 1.5 h; (iii) MeOH, $CH_2Cl_2$, rt; (iv) **9**, $CH_2Cl_2$, rt, 12 h; (v) TFA, $CH_2Cl_2$, MeOH, rt, 1 h.

We consider the published synthetic protocols[23c,d,26,27] challenging for the preparation of nucleobase–lysine, arginine and serine monomers as they include complex procedures, low yields and difficulties with product purification.

We have previously reported the extensive use of *N*-acylbenzotriazoles for *N*-,[28] *C*-,[29] and *O*-acylation[30] reactions. We now report a simple and efficient synthesis, utilizing *N*-acylbenzotriazoles, of backbone modified chiral PNA and SNE monomers comprising L- and D-serine, L-lysine and L-arginine amino acids.

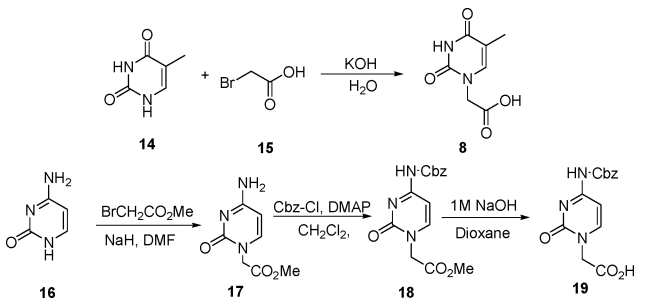
## Results and Discussion

Our strategy was to utilize benzotriazole mediated solution phase methodology, which enables the incorporation of nucleobases and important amino acids in two simple steps, affording backbone modified, chiral PNA and SNE monomers and building blocks appropriate for new drug design strategies.

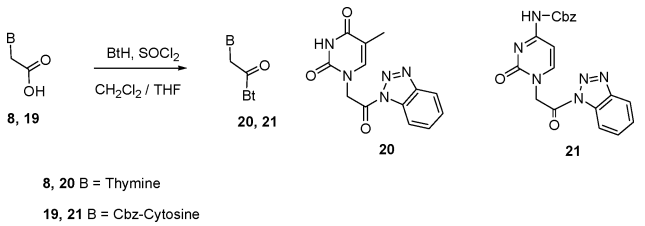
The synthesis of our desired monomers starts with the preparation of the nucleobase (thymine, cytosine) acetic acid derivatives **8**, **19**. (Thymin-1-yl)acetic acid[31] **8** was prepared in 80% yield from thymine **14** (Scheme 2). The Cbz-cytosine derivative[32] **19** was prepared (43% overall) from cytosine **16** by (i) alkylation with methyl bromoacetate, (ii) Cbz-protection of the exocyclic amine **17** using Cbz-Cl–DMAP reagents and (iii) saponification of the corresponding methyl ester **18** (Scheme 2).

### 1. Preparation of *N*-(nucleobase-1-yl-acetyl)-1*H*-benzotriazoles 20, 21

Conversion of **8** and **19** into the corresponding *N*-acylbenzotriazoles **20**, **21** was performed by modified literature procedures.[28–30,33] Reaction of nucleobase-1-yl-acetic acids with 1*H*-benzotriazole and thionyl chloride in $CH_2Cl_2$ or THF at




**Scheme 2** Synthesis of nucleobase (thymine and Cbz-protected cytosine)-1-acetic acid derivatives.

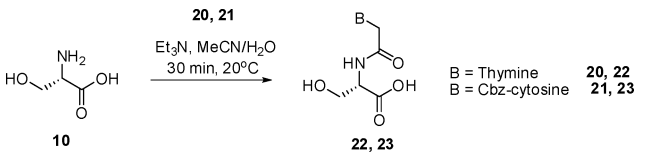



**Scheme 3**

room temperature (Scheme 3) took 12 h to complete, but pure **20** (82%) and **21** (75%) were isolated directly from the reaction media. Compounds **20** and **21** are stable indefinitely at 20 °C.

### 2. Preparation of nucleobase–serine SNE monomers 22, 22′, 23, 23′

*N*-Acylbenzotriazoles **20**, **21** were treated with L- and D-serine **10**, **10′** in aqueous acetonitrile at 20 °C for 30 min (Scheme 4, Table 1). After acidifying, and evaporation of the solvent, the residue was washed with water to achieve complete removal of side product BtH and afford optically pure nucleobase (thymine and Cbz-cytosine)–serine monomers **22**, **22′** and **23**, **23′** in 78–85% yields. Advantageously, no protection of the side chain functionality is required to selectively form an amide bond to serine.




**Scheme 4**

**Table 1** Yields of nucleobase–serine monomers **22**, **22′**, **23**, **23′**

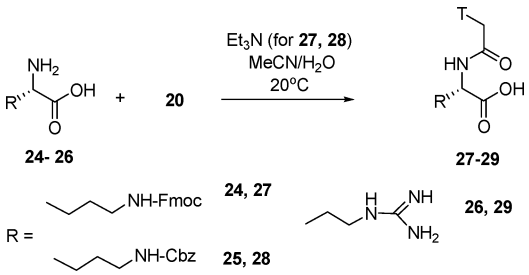
| Thymine–serine monomers (Yield[a]) | Amino acid | Cbz-cytosine–serine monomers (Yield[a]) |
|---|---|---|
| Thymine–L-serine **22** (85%) | L-Serine **10** | Cbz-cytosine–L-serine **23** (82%) |
| Thymine–D-serine **22′** (83%) | D-Serine **10′** | Cbz-cytosine–D-serine **23′** (78%) |

[a] Isolated yield.

In comparison with the recent literature procedure[27] for the preparation of SNE monomers, our methodology offers simple preparative and workup procedures, short times for completion, uses inexpensive reagents, gives high yields, and allows the use of free amino acids as coupling components.

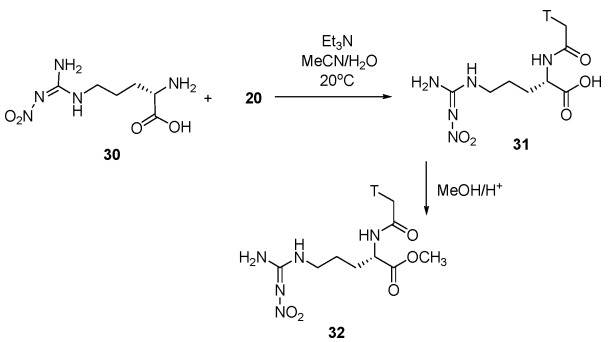
### 3. Preparation of nucleobase–lysine/–arginine PNE monomers 27–29, 32, 33

Thymine was chosen for initial further study because it presented the fewest synthetic challenges; however, the methodology should be extendable to other natural and unnatural nucleobases. Optically active monomers **27**, **28** containing thymine linked to L-lysine were obtained similarly, by coupling (in aqueous acetonitrile) benzotriazole activated (thymin-1-yl)acetic acid **20** with $N^{\varepsilon}$-Fmoc and -Cbz protected L-lysines **24**, **25**, respectively, in the presence of $Et_3N$. Simple work up procedures afforded pure monomers **27**, **28** (80–92%) without chromatography. The preparation of the free L-arginine PNA building block **29** (92%) did not require the utilization of $Et_3N$ or any protecting group (Scheme 5).



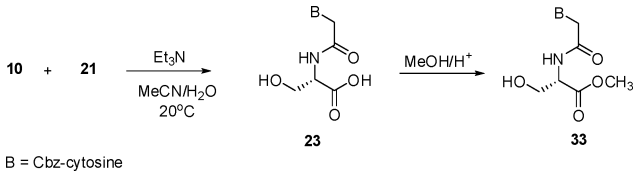

**Scheme 5**

The synthesis of optically active monomer **31**, derived from thymine and $N^{\omega}$-$NO_2$-L-arginine **30**, was accompanied by unexpected esterification, when crude **31** was crystallized from MeOH–DCM–ether–HCl (Scheme 6) to give **32** (89%).




**Scheme 6**

Esterification was also observed under the isolation conditions used (MeOH–$H^+$) in the synthesis of the Cbz-cytosine–L-serine monomer **23** (Scheme 7) to give **33** (75%). Such esterification could be useful for the synthesis of methyl esters of thymine and Cbz-cytosine monomers derived from $N^{\omega}$-$NO_2$-arginine and serine.




**Scheme 7**

## Conclusion

In conclusion we have developed novel approaches to the convenient and efficient synthesis of backbone modified serine-, lysine- and arginine-containing chiral PNA monomers, utilizing a simple two-step synthetic route involving: (i) activation of nucleobase-1-yl acetic acids as stable benzotriazole derivatives and (ii) coupling with amino acids in aqueous media requiring neither anhydrous reaction conditions nor the use of expensive coupling reagents.

## Experimental

### General Methods

Melting points were determined on Fisher melting apparatus. $^1$H NMR (300 MHz) and $^{13}$C NMR (75 MHz) spectra were recorded on a 300 MHz NMR spectrometer in $CDCl_3$ or DMSO-$d_6$. *N*-Cbz-, *N*-Fmoc-, and free amino acids were purchased from Fluka and Acros and used without further purification. Elemental analyses were performed on a Carlo Erba-1106 instrument. MALDI analyses were performed on a Bruker Reflex II TOF mass spectrometer retrofilled with delayed extraction. Optical rotation values were measured with the use of the sodium D line.

**(Thymine-1-yl)acetic acid (8).** Thymine (3.78 g, 30 mmol) was dissolved in a solution of KOH (6.458, 115 mmol) in 20 ml of water. While this solution was warmed in a 40 °C water bath, a solution of bromoacetic acid (6.25 g, 45 mmol) in 10 ml of water was added over 30 minutes. The reaction was stirred for another 30 minutes at this temperature. It was allowed to cool to room temperature and the pH was adjusted to 5.5 with conc. HCl. The solution was then cooled in a refrigerator for 2 h. Any precipitate formed was removed by filtration. The solution was then adjusted to pH 2 with conc. HCl and put in a freezer for 2 h. The resultant white precipitate was isolated by filtration, washed with water and dried, affording **8** (4.4 g, 80%). White solid; mp 252–253 °C, (lit.[31]); $^1$H NMR (300 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$ 1.76 (s, 3H), 4.37 (s, 2H), 7.50 (s, 1H), 11.35 (s, 1H), 13.20 (s, 1H). $^{13}$C NMR (DMSO-$d_6$, $Me_4Si$): $\delta$ 11.9, 48.4, 108.3, 141.8, 151.0, 164.4, 169.6. Anal. calcd for $C_7H_8N_2O_4$: C, 45.66; H, 4.38; N, 15.21. Found: C, 45.30; H, 4.24; N, 15.19.

**Methyl (cytosine-1-yl)acetate (17).** To a suspension of cytosine (5.0 g, 45.0 mmol) in DMF (100mL) was added NaH (1.08 g, 45.0 mmol) under $N_2$ at 0 °C. The mixture was stirred for 2 h at rt, then methyl bromoacetate (4.3 mL, 45.0 mmol) was added. The mixture was stirred for 48 h at rt. The solvent was evaporated *in vacuo*. The crude residue was triturated with water (100mL) and the precipitate was filtered off. Recrystallization from MeOH–$H_2O$ gave **17** (5.27 g, 64%) as white microcrystals. Mp 225–226 °C, (lit.[32] mp 225–227 °C); $^1$H NMR (300 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$ 3.68 (s, 3H), 4.54 (s, 2H), 5.87 (d, $J$ = 7.3 Hz, 1H), 7.60–7.75 (m, 2H),

8.14 (br s, 1H). $^{13}$C NMR (75 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$ 49.7, 52.2, 93.7, 147.6, 153.0, 164.0, 168.8.

**Methyl ($N^4$-benzyloxycarbonyl)cytosin-1-ylacetate (18).** Cbz-Cl (3.12 mL, 21.8 mmol) and DMAP (2.67 g, 21.8 mmol) were dissolved at −15 °C in $CH_2Cl_2$ (20 mL). The mixture was stirred for 15 min then **17** (2.0 g, 10.9 mmol) was gradually added. After stirring for 15 min at −15 °C then 5 h at rt, the mixture was evaporated *in vacuo* and the crude residue was taken up in $CHCl_3$. The organic layer was washed with 1M HCl and with water, then dried over $Na_2SO_4$ and lastly evaporated under reduced pressure. Trituration of the crude residue in ether gave a white precipitate which was then filtered off, affording **18** (2.65 g, 77%) as white microcrystals. Mp 161–162 °C, (lit.[32]); $^1$H NMR (300 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$ 3.68 (s, 3H), 4.63 (s, 2H), 5.20 (s, 2H), 7.05 (d, $J = 7.1$ Hz, 1H), 7.30–7.450 (m, 5H), 8.06 (d, $J = 7.1$ Hz, 1H), 10.89 (bs, 1H). $^{13}$C NMR (75 Mz, DMSO-$d_6$, $Me_4Si$): $\delta$ 50.5, 52.3, 66.5, 94.2, 128.0, 128.2, 128.5, 135.9, 150.3, 151.5, 154.9, 163.5, 168.5. Anal. calcd for $C_{15}H_{15}N_3O_5$: C, 56.78; H, 4.76; N, 13.24. Found: C, 56.92; H, 4.67; N, 12.95.

**($N^4$-Benzyloxycarbonyl)cytosin-1-ylacetic acid (19).** **18** (0.2 g, 6.3 mmol) was dissolved in dioxane (50 mL) and aqueous 1M NaOH (8.82 mL) was added. The mixture was stirred for 5 h at rt and then concentrated *in vacuo*. The residue was taken up in aqueous 1M $KHSO_4$. The resultant white precipitate was isolated by filtration, washed with water and dried, affording **19** (1.66 g, 87%) as white microcrystals. Mp 264–266 °C, (lit.[32]); $^1$H NMR (300 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$ 4.53 (s, 2H), 5.19 (s, 2H), 7.03 (d, $J = 7.2$ Hz, 1H), 7.33–7.43 (m, 5H), 8.04 (d, $J = 7.3$ Hz, 1H), 10.81 (br s, 1H). $^{13}$C NMR (75 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$ 50.5, 66.5, 94.0, 128.0, 128.2, 128.5, 136.0, 150.5, 153.2, 155.0, 163.3, 169.4. Anal. calcd for $C_{14}H_{13}N_3O_5$: C, 55.45; H, 4.32; N, 13.86. Found: C, 55.50; H, 4.35; N, 13.72.

**General procedure for the preparation of nucleobase–*N*-acylbenzotriazoles: thymine–Bt (20), Cbz-cytosine–Bt (21).** To a solution of 1*H*-benzotriazole (0.476 g, 4 mmol) in 20 mL of dry $CH_2Cl_2$ [for **20**] [THF for **21**], was added thionyl chloride (0.1 mL, 1.2 mmol) and the reaction mixture was stirred for 20 min at 20 °C. Nucleobase acetic acid **8** or **13** (1 mmol) was added to the reaction mixture and stirred overnight at room temperature. The precipitate obtained was filtered off and dried. Water (7 ml) was added to the dry solid and stirred for 2 min to dissolve BtH·HCl. The suspension was filtered off then the solid was washed with water until the pH was neutral and dried to obtain pure product.

**1-(2-Benzotriazol-1-yl-2-oxo-ethyl)-5-methyl-1*H*-pyrimidine-2,4-dione (20).** (234 mg, 82%). White microcrystals; mp 242–243 °C; $^1$H NMR (300 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$ 1.81 (s, 3H), 5.60 (s, 2H), 7.60–7.72 (m, 2H), 7.84 (t, $J = 7.7$ Hz, 1H), 8.22 (d, $J = 8.3$ Hz, 1H), 8.33 (d, $J = 8.3$ Hz, 1H), 11.56 (s, 1H). $^{13}$C NMR (75 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$ 12.1, 50.3, 108.9, 113.6, 120.4, 127.0, 130.5, 131.4, 141.6, 145.3, 151.2, 164.4, 167.1. Anal. calcd for $C_{13}H_{11}N_5O_3$: C, 54.74; H, 3.89; N, 24.55. Found: C, 54.45; H, 3.94; N, 24.31.

**[1-(2-Benzotriazol-1-yl-2-oxo-ethyl)-2-oxo-1,2-dihydro-pyrimidin-4-yl]-benzyl carbamate (21).** (303 mg, 75%). Yellowish microcrystals; mp 128–130 °C; $^1$H NMR (300 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$ 5.22 (s, 2H), 5.73 (s, 2H), 7.17 (d, $J = 7.1$ Hz, 1H), 7.33–7.45 (m, 5H), 7.68 (t, $J = 7.8$ Hz, 1H), 7.84 (t, $J = 7.8$ Hz, 1H), 8.20 (t, $J = 8.0$ Hz, 2H), 8.33 (d, $J = 8.2$ Hz, 1H), 10.96 (s, 1H). $^{13}$C NMR (75 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$ 52.4, 66.6, 94.6, 113.7, 120.4, 127.0, 128.0, 128.2, 128.5, 130.5, 131.4, 135.9, 145.4, 150.6, 153.1, 155.1, 163.8, 166.8. *m/z* (TOF MS) 405.1306 [$M^+$ + H] 405.1298 (100%).

**General procedure for the preparation of nucleobase–serine monomers: thymine–L-serine (22), thymine–D-serine (22′), Cbz-cytosine–L-serine (23), Cbz-cytosine–D-serine (23′).** Nucleobase–*N*-acylbenzotriazole **20** [for **22**, **22′**] or **21** [for **23**, **23′**] (1 mmol) was added at 20 °C to a solution of L-serine (or D-serine) (1 mmol) in MeCN–$H_2O$ (10 : 5 mL), in the presence of $Et_3N$ (1.2 mmol). The reaction mixture was stirred at room temperature for 30 min. After adding 4 N HCl (1 mL) the solvent was removed under reduced pressure until fully dry. Water (3 mL) was added to the dry residue and the mixture was stirred for 2 min to dissolve BtH·HCl. The suspension was filtered off and the solid washed with water until the pH was neutral, giving the pure product.

**(2*S*)-3-Hydroxy-2-({2-[5-methyl-2,4-dioxo-3,4-dihydro-1(2*H*)-pyrimidinyl]acetyl}amino)propanoic acid (22).** (230 mg, 85%); white microcrystals; mp 218–219 °C, (lit.[27]); $[\alpha]_D^{23}$ −16.4 (*c* 1.68 in DMF); $^1$H NMR (300 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$ 1.74 (s, 3H), 3.61 (dd, $J = 10.7$, 4.1 Hz, 1H), 3.70 (dd, $J = 10.7$, 4.9 Hz, 1H), 4.25–4.32 (m, 1H), 4.37 (s, 2H), 5.06 (br s, 1H), 7.42 (s, 1H), 8.46 (d, $J = 8.0$ Hz, 1H), 11.27 (s, 1H), 12.69 (br s, 1H). $^{13}$C NMR (75 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$ 12.0, 49.0, 54.7, 61.4, 107.9, 142.5, 151.0, 164.5, 167.1, 171.8. Anal. calcd for $C_{10}H_{13}N_3O_6$: C, 44.28; H, 4.83; N, 15.49. Found: C, 43.91; H, 4.69; N, 15.22

**(2*R*)-3-Hydroxy-2-({2-[5-methyl-2,4-dioxo-3,4-dihydro-1(2*H*)-pyrimidinyl]acetyl}amino)propanoic acid (22′).** (225 mg, 83%); white microcrystals; mp 234–235 °C, (lit.[27]); $[\alpha]_D^{23}$ +19.2 (*c* 1.78 in DMF); $^1$H NMR (300 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$ 1.74 (s, 3H), 3.61 (dd, $J = 10.7$, 3.9, Hz, 1H), 3.70 (dd, $J = 10.7$, 4.1, Hz, 1H), 4.25–4.33 (m, 1H), 4.37 (s, 2H), 7.42 (s, 1H), 8.46 (d, $J = 7.7$ Hz, 1H), 11.28 (s, 1H). $^{13}$C NMR (75 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$ 12.0, 49.0, 54.7, 61.4, 107.9, 142.5, 151.0, 164.5, 167.1, 171.8. Anal. calcd for $C_{10}H_{13}N_3O_6$: C, 44.28; H, 4.83; N, 15.49. Found: C, 44.42; H, 4.85; N, 15.21.

**(*S*)-2-[2-(4-Benzyloxycarbonylamino-2-oxo-2*H*-pyrimidin-1-yl)-acetylamino]-3-hydroxypropanoic acid (23).** (320 mg, 82%). White microcrystals; mp 192–194 °C; $[\alpha]_D^{23}$ −11.5 (*c* 1.63 in DMF); $^1$H NMR (300 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$ 3.62 (dd, $J = 10.7$, 4.0 Hz, 1H), 3.72 (dd, $J = 10.7$, 4.8, Hz, 1H), 4.28–4.34 (m, 1H), 4.52–5.63 (m, 3H), 5.20 (s, 2H), 6.97–7.06 (m, 1H), 7.30–7.49 (m, 5H), 8.00 (d, $J = 7.3$, 0.8H), 8.06 (d, $J = 7.3$ Hz, 0.2H), 8.56 (d, $J = 7.4$ Hz, 1H). $^{13}$C NMR (75 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$ 51.1, 54.8, 61.5, 66.6, 93.7, 128.0, 128.2, 128.5, 136.0, 151.5, 153.2, 154.7, 162.9, 166.7, 171.8. *m/z* (TOF MS) 413.1068 [$M^+$ + Na] 413.1053 (100%).

**(*R*)-2-[2-(4-Benzyloxycarbonylamino-2-oxo-2*H*-pyrimidin-1-yl)-acetylamino]-3-hydroxypropanoic acid (23′).** (304 mg, 78%); white microcrystals; mp 183–185 °C; $[\alpha]_D^{23}$ +7.4 (*c* 0.68 in DMF); $^1$H NMR (300 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$ 3.62 (dd, $J = 10.3$, 4.0 Hz, 1H), 3.72 (dd, $J = 10.7$, 4.7, Hz, 1H), 4.26–4.34 (m, 1H), 4.52–5.62 (m, 3H), 5.19 (s, 2H), 6.95–7.04 (m, 1H), 7.30–7.52 (m, 5H), 8.00 (d, $J = 7.4$, 0.8H), 8.05 (d, $J = 7.4$ Hz, 0.2H), 8.55 (d, $J = 7.7$ Hz, 1H). $^{13}$C NMR (75 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$

51.1, 54.8, 61.4, 66.7, 93.7, 128.0, 128.3, 128.6, 135.9, 151.6, 153.1, 154.5, 162.8, 166.7, 171.8. *m/z* (TOF MS) 413.1068 [M$^+$ + Na] 413.1063 (100%).

**(*S*)-6-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-2-[2-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-acetylamino]hexanoic acid (27).** **20** (0.285 g, 1 mmol) was added at 20 °C to a solution of $N^{\varepsilon}$-Fmoc–L-Lys-OH (0.384 g, 1 mmol) in MeCN–$H_2O$ (10 : 5 mL) in the presence of $Et_3N$ (2 mmol). The reaction mixture was stirred for 1 h. After adding 4 N HCl (1 mL) the acetonitrile was removed under reduced pressure. The water layer was extracted with EtOAc (50 mL) and washed with 4 N HCl (10 mL). White crystals precipitated from the organic solution and were filtered to give **27** (0.427 g, 80%) which was reprecipitated from $CH_2Cl_2$–hexanes. White microcrystals, mp 223–224 °C; $[a]_D^{23}$ −2.46 (*c* 1.83 in DMF); $^1$H NMR (300 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$ 1.22–1.70 (m, 6H), 1.77 (s, 3H), 2.88–3.02 (m, 2H), 4.12–4.26 (m, 2H), 4.27–4.32 (m, 2H), 4.37 (s, 2H), 7.27–7.48 (m, 6H), 7.67 (d, $J$ = 7.1 Hz, 2H), 7.88 (d, $J$ = 7.4 Hz, 2H), 8.48 (d, $J$ = 7.8 Hz, 1H), 11.29 (s, 1H). $^{13}$C NMR (75 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$ 12.1, 22.7, 29.1, 31.0, 46.9, 49.2, 52.1, 65.4, 108.1, 120.3, 125.3, 127.3, 127.8, 140.9, 142.6, 144.1, 151.2, 156.3, 164.7, 167.2, 173.6. *m/z* (TOF MS) 557.2007 [M$^+$ + Na] 557.2028 (100%).

**(*S*)-6-Benzyloxycarbonylamino-2-[2-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-acetylamino]hexanoic acid (28).** **20** (0.285 g, 1 mmol) was added at 20 °C to a solution of $N^{\varepsilon}$-Cbz–L-Lys-OH (0.280 g, 1 mmol) in MeCN–$H_2O$ (10 : 5 mL), in the presence of $Et_3N$ (2 mmol). The reaction mixture was stirred for 1 h. After adding 4 N HCl (1 mL) the acetonitrile was removed under reduced pressure. The water layer was extracted with EtOAc (50 mL), washed with 4 N HCl soln (3 × 30 mL) and sat. NaCl soln (20 mL), and dried over $MgSO_4$. Evaporation of the solvent gave **28** (392 mg, 92%) in pure form, which was reprecipitated from $CH_2Cl_2$–hexanes. White microcrystals, mp 204–206 °C, (lit.[26]); $[a]_D^{23}$ −5.9 (*c* 1.70 in DMF); $^1$H NMR (300 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$ 1.22–1.48 (m, 4H), 1.50–1.74 (m, 2H), 1.74 (s, 3H), 2.91–3.04 (m, 2H), 4.12–4.24 (m, 1H), 4.33 (s, 2H), 5.00 (s, 2H), 7.23–7.29 (m, 1H), 7.29–7.39 (m, 5H), 7.41 (s, 1H), 8.47 (d, $J$ = 7.6 Hz, 1H), 11.3 (s, 1H), 12.66 (br s, 1H). $^{13}$C NMR (75 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$ 12.0, 22.6, 29.0, 30.9, 49.0, 52.0, 65.2, 107.9, 127.8, 128.4, 137.3, 142.5, 151.0, 156.2, 164.5, 167.0, 173.4. Anal. calcd for $C_{21}H_{26}N_4O_7$ : C, 56.50; H, 5.87; N, 12.55. Found: C, 56.30; H, 5.94; N, 12.45.

**(*S*)-5-Guanidino-2-[2-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-acetylamino]pentanoic acid, (29).** **20** (0.1 g, 0.35 mmol) was added at 20 °C to a solution of L-arginine (0.061 g, 0.35 mmol) in MeCN–$H_2O$ (8 : 8 mL). After several minutes a white solid precipitated and the reaction mixture was stirred for a further 1 h. The acetonitrile was removed under reduced pressure and most of the water was evaporated by a current of air. The obtained suspension was filtered off to give pure **29** (312 mg, 92%), which was then recrystallized from water. White microcrystals, mp 290–292 °C; $^1$H NMR (300 MHz, TFA, $Me_4Si$): $\delta$ 1.76–1.97 (m, 3H), 1.99 (s, 3H), 2.08–2.24 (m, 1H), 3.28–3.42 (m, 2H), 4.55–4.88 (m, 3H), 7.40 (m, 1H). $^{13}$C NMR (75 MHz, TFA, $Me_4Si$): $\delta$ 13.5, 27.3, 31.6, 43.8, 54.5, 55.6, 116.2, 146.8, 155.6, 159.9, 170.6, 172.5, 179.2. *m/z* (TOF MS) 341.1568 [M$^+$ + H] 341.1575 (100%).

**(*S*)-5-Nitroguanidino-2-[2-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-acetylamino]pentanoic acid methyl ester (32).** **20** (0.15 g, 0.5 mmol) was added at 20 °C to a solution of $N^{\omega}$-$NO_2$-L-arginine (0.115 g, 0.5 mmol) in MeCN–$H_2O$ (10 : 5 mL), in the presence of $Et_3N$ (2 mmol). The reaction mixture was stirred for 1 h. 4 N HCl (1 mL) was then added, the acetonitrile removed under reduced pressure and the water evaporated by a current of air. The residue was dissolved with a minimum amount of methanol (2–3 mL), then dichloromethane–ether ($CH_3OH$ : $CH_2Cl_2$ : ether; 1 : 1 : 0.5) were added and the clear solution was allowed to stand to give pure **32** (355 mg, 89%). White microcrystals, mp 215–217 °C; $[a]_D^{23}$ −5.03 (*c* 1.45 in DMF); $^1$H NMR (300 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$ 1.43–1.72 (m, 4H), 1.75 (s, 3H), 3.05–3.23 (m, 2H), 3.64 (s, 2H), 4.20–4.35 (m, 1H), 4.35 (s, 2H), 7.43 (s, 1H), 7.52–8.60 (m, 3H), 8.65 (d, $J$ = 7.3 Hz, 1H), 11.29 (s, 1H). $^{13}$C NMR (75 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$ 11.9, 24.7, 28.3, 49.1, 51.8, 52.0, 108.0, 142.4, 151.0, 159.3, 164.5, 167.3, 172.2. Anal. calcd for $C_{14}H_{21}N_7O_7$ : C, 42.10; H, 5.30; N, 24.55. Found: C, 42.33; H, 5.40; N, 24.20.

**(*S*)-2-[2-(4-Benzyloxycarbonylamino-2-oxo-2*H*-pyrimidin-1-yl)-acetylamino]-3-hydroxypropionic acid methyl ester (33).** **21** (0.2 g, 0.5 mmol) was added at 20 °C to a solution of L-serine (0.05 g, 0.5 mmol) in MeCN–$H_2O$ (10 : 5 mL), in the presence of $Et_3N$ (2 mmol). The reaction mixture was stirred for 1 h. 4 N HCl (1 mL) was then added, the acetonitrile removed under reduced pressure and the water evaporated by a current of air. The residue was dissolved with a minimum amount of methanol (2–3 mL) and dichloromethane–ether ($CH_3OH$ : $CH_2Cl_2$ : ether; 1 : 1 : 0.5) were added. The clear solution was allowed to stand to give pure **33** (303 mg, 75%). White microcrystals, mp 204–205 °C; $^1$H NMR (300 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$ 3.01–3.09 (m, 1H), 3.64 (s, 3H), 3.59–3.76 (m, 1H), 4.32–4.42 (m, 1H), 4.57 (s, 2H), 5.20 (s, 2H), 7.00 (d, $J$ = 7.3 Hz, 1H), 7.30–7.46 (m, 5H), 8.00 (d, $J$ = 7.1 Hz, 1H), 8.73 (d, $J$ = 7.3 Hz, 1H). $^{13}$C NMR (75 MHz, DMSO-$d_6$, $Me_4Si$): $\delta$ 51.1, 52.0, 54.9, 61.3, 66.8, 93.7, 128.1, 128.3, 128.6, 135.8, 152.0, 153.0, 153.9, 162.5, 166.8, 170.9. *m/z* (TOF MS) 427.1224 [M$^+$ + Na] 427.1278 (100%).

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