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Synthesis of new unnatural N^{α}-Fmoc pyrimidin-4-one amino acids: use of the *p*-benzyloxybenzyloxy group as a pyrimidinone masking group†

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The *p*-benzyloxybenzyloxy group is used to mask the oxo function of the 4(3*H*)-pyrimidinone ring in the synthesis of new unnatural amino acids. The synthetic approach is based on an aromatic nucleophilic substitution reaction between 4-[4-(benzyloxy)benzyloxy]-2-(benzylsulfonyl)pyrimidine and the nucleophilic side chain of several N^{α}-Boc amino esters, as the key step, followed by a series of standard protecting group transformations. *p*-Benzyloxybenzyloxy is efficiently removed under mild acid conditions to recover the 4(3*H*)-pyrimidinone system.

Introduction

Unnatural amino acids, the non-genetically-coded amino acids, including α-, β- and γ-amino acids,[1] represent a nearly infinite array of compounds useful in different fields such as drug discovery, medicinal chemistry and protein engineering.[2] They can be either naturally[3] occurring or chemically synthesized. Amongst many other applications, synthetic α-amino acids[4] are particularly suitable for the replacement of proteinogenic α-amino acids into bioactive peptide sequences.[5] It is well known that the introduction of non-coded amino acids, which generates modifications in the secondary and tertiary structures of a peptide, is a helpful approach to increase its proteolytic stability, and enhance its biological selectivity and activity.[6] In addition, the recent advances in genetic encoding of unnatural amino acids into proteins and peptides has provided a potent tool to design and synthesize new customized peptides.[7] Therefore, the development of efficient synthetic routes towards new unnatural α-amino acids is of a great importance to date.[4] Among them, heterocyclic α-amino acids are particularly interesting, due to their diverse range of chemical and biomedicinal applications, not only as components of peptides, but also as a result of their intrinsic biological properties.[3] Several examples include L-azatyrosine,[8] a pyridine-containing α-amino acid, which displays anticancer and antibacterial activities, synthetic tryptophan derivatives,[9] as key precursors of biologically active compounds, or willardiine[10] and L-lathyrine,[11] two naturally occurring α-amino acids carrying a pyrimidine ring on their side chain, which exhibit a diverse range of bioactivities. In peptides incorporating heterocycle-containing α-amino acids, the nature of the heterocycle plays an essential role in improving their biological properties and preventing their enzymatic degradation. The pyrimidine ring and its various oxo derivatives, pyrimidinones, are valuable scaffolds present in many natural and synthetic therapeutics, including anticancer, antiviral and antibacterial agents.[12] Thus, we focused our attention on the development of synthetic methods[14] to access unusual pyrimidine α-amino acids[13] with the objective of preparing new antimicrobial peptides. In particular, we recently reported the synthesis of a set of N^{α}-Fmoc-pyrimidinyl α-amino acids, types **1** and **2**, containing a pyrimidine ring in their side chain.[15] These compounds are useful building blocks for solid-phase peptide synthesis following the Fmoc/*tert*-butyl strategy. As an extension of this work the preparation of N^{α}-Fmoc-pyrimidin-4-one α-amino acids type **3** is described in this manuscript (Fig. 1).

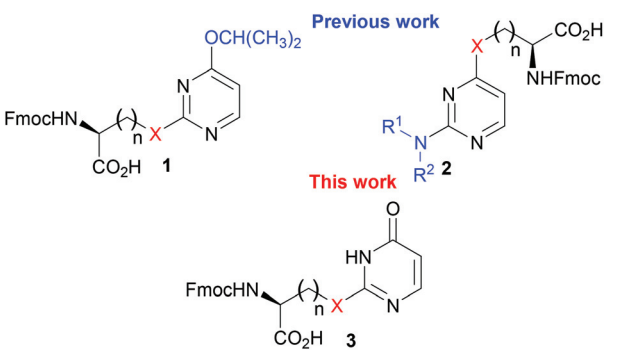

Fig. 1 New unnatural pyrimidinyl α-amino acids.

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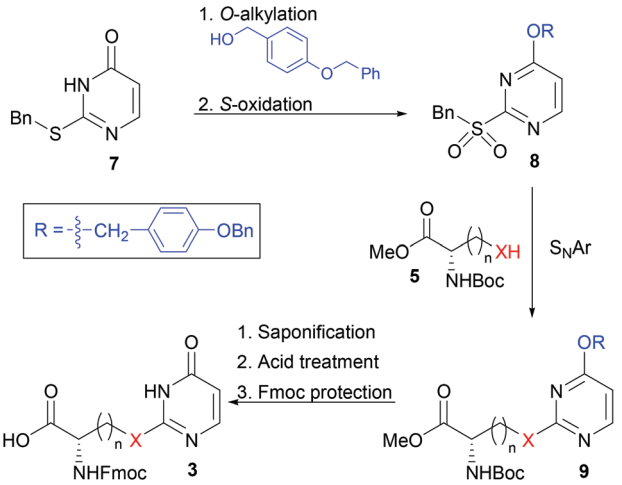
† Electronic supplementary information (ESI) available: Experimental details and copies of NMR spectra (^{1}H, ^{13}C, dept) for all new compounds. See DOI: 10.1039/c4ob02235a

Results and discussion

The synthesis of N^{α}-Fmoc-pyrimidin-2-yl amino acids **1** was achieved *via* an aromatic nucleophilic substitution reaction (S_NAr) between sulfone **4** and the nucleophilic side chain of several natural α-amino acids **5**, properly protected, followed by a series of standard protecting group transformations: (i) ester saponification, (ii) acid treatment to remove the Boc group, and (iii) Fmoc protection of the free amino acid (Scheme 1).[15b] The preparation of pyrimidin-4-one amino acids **3** was initially envisioned by a simple cleavage of the isopropoxy group of the pyrimidin-2-yl amino esters **6** under acidic conditions (H_2SO_4) as previously described.[14c] Under these conditions, simultaneous cleavage of both the isopropoxy and Boc groups should take place. Disappointingly, the required strong acidic media to remove the isopropoxy moiety caused the decomposition of the starting substrates (Scheme 1). These results revealed the need for a more labile group to mask the pyrimidinone function during the nucleophilic substitution step. It is mandatory to mask the pyrimidinone function because the nucleophilic substitution of a leaving group at position 2 of the 4(3*H*)-pyrimidinone ring is too labile and easily decomposes to the corresponding uracil.[14c]

Our first alternative was the use of the more labile *tert*-butoxy group to mask the pyrimidinone function instead of isopropoxy one.[14c] However, the synthesis of 4-*tert*-butoxy-pyrimidine derivatives afforded poor yields rendering this approach inefficient.[14c] Instead the *p*-benzyloxybenzyloxy group was chosen due to its structural similarity to Wang resin,[16] which can be cleaved under mild acidic conditions (TFA at 25 °C) in the solid-phase.[17] For example, Lam *et al.* reported the synthesis of a library of purine analogues using solid-phase synthesis where the final cleavage from Wang resin released the pyrimidinone ring employing 30% TFA in CH_2Cl_2 at 25 °C.[18]

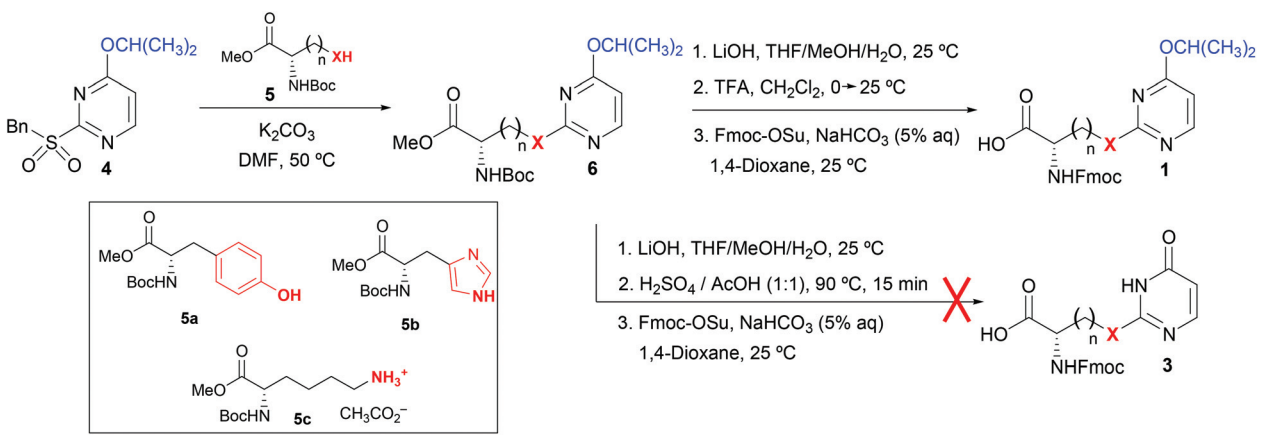
Hence, we planned the synthesis of target amino acids **3** *via* a S_NAr between the 2-benzylsulfonylpyrimidine **8** (R = *p*-benzyloxybenzyloxy group) and the nucleophilic side chain of the protected amino esters **5** followed by a series of protecting group transformations (Scheme 2).



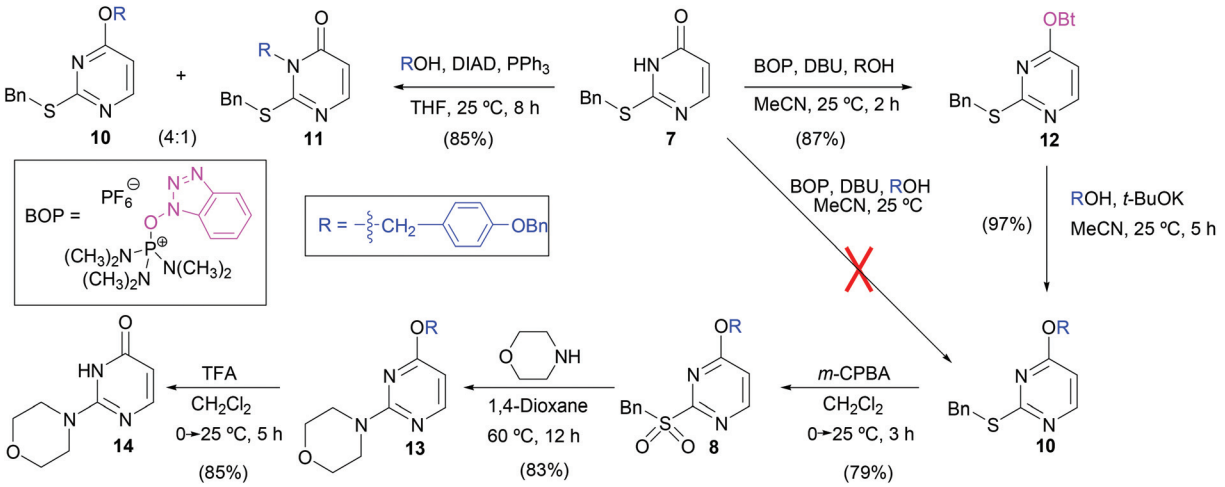
Scheme 2 Synthetic approach to N^{α}-Fmoc-pyrimidin-4-one amino acids **3**.

First, the introduction of the *p*-benzyloxybenzyloxy group at position 4 of the pyrimidine ring was attempted through a selective *O*-alkylation of 2-benzylsulfanyl-4(3*H*)-pyrimidinone **7** with *p*-benzyloxybenzyl alcohol under Mitsunobu conditions as was performed in the synthesis of sulfone **4**.[14c] In this case, the Mitsunobu reaction was not completely selective and along with the desired *O*-alkylated product **10**, a significant amount of *N*-alkylated product **11** was observed in a 4 : 1 ratio, respectively.

Chromatography separation of these isomers was problematic rendering this reaction impractical. This byproduct could be avoided using the phosphonium coupling reaction previously employed in the synthesis of pyrimidin-4yl α-amino acids **2**.[15a] While direct coupling of pyrimidinone **7** with *p*-benzyloxybenzyl alcohol mediated by (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) failed, the use of a two-step sequence through the isolation of the benzotriazolyloxy (OBt) intermediate **12** successfully afforded compound **10**. Thus, pyrimidinone **7** was reacted with BOP and DBU in CH_3CN to form the OBt-intermediate **12**, fol-



Scheme 1 Synthesis of N^{α}-Fmoc-pyrimidinyl amino acids **1**.



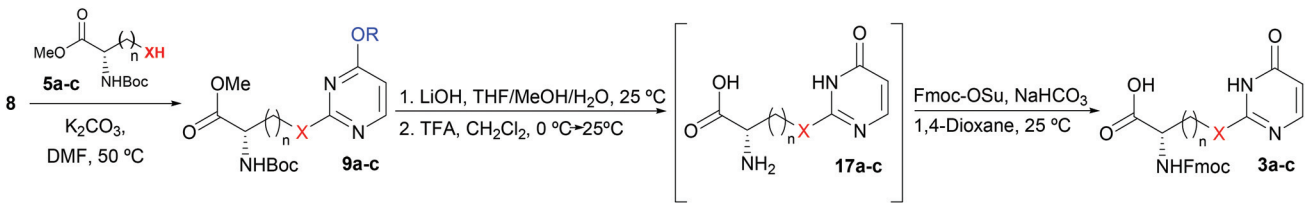
Scheme 3 Synthesis of pyrimidinylsulfone **8**.

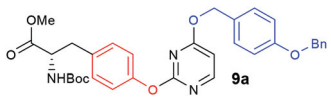
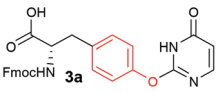
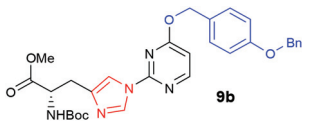
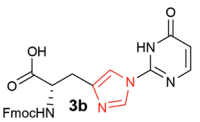
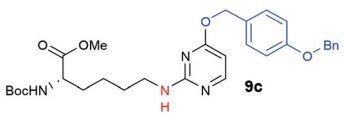
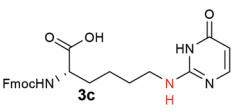
lowed by treatment with *p*-benzyloxybenzyl alcohol in the presence of *t*-BuOK to provide the *O*-alkylated product **10** in 84% yield over two steps. The latter was then oxidized with *m*-CPBA to afford the desired sulfone **8** in 79% yield (Scheme 3).

In our previous studies,[15a] we observed an unusual nucleophilic substitution of several alkoxy groups at position 4 of the pyrimidine ring. The stability of the *p*-benzyloxybenzyloxy group under S_NAr conditions was tested by subjecting sulfone **8** to an excess of morpholine at 60 °C. Compound **13** was obtained in 83% yield as the sole product with no traces of any side product corresponding to the displacement of the alkoxy group. In addition, we also tested the deprotection conditions of this group using 30% TFA in CH_2Cl_2 at room temperature. After 5 hours, the 4-alkoxy pyrimidine was fully converted to 2-morphonlino-4(3*H*)-pyrimidinone **14** in 85% yield (Scheme 3).

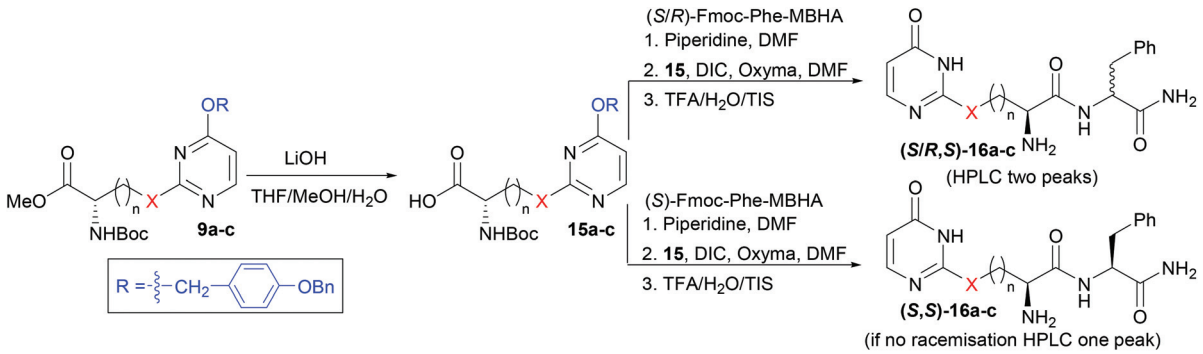
The base and reaction temperature are crucial parameters to avoid racemization during the S_NAr using N^{α}-Boc-amino esters **5** as nucleophiles.[15] The S_NAr between pyrimidinyl sulfone **8** and the suitably protected amino esters **5a–c** – tyrosine, histidine and lysine – was carried out using potassium carbonate as a base in DMF under a controlled temperature ranging from 25 °C to 50 °C. Under these conditions, the corresponding N^{α}-Boc pyrimidin-2-yl amino esters **9** were isolated in excellent yields and without appreciable racemization (Table 1). In the case of amino ester **5b**, the nucleophilic attack of the imidazole ring took place through $N(\tau)$nitrogen

Table 1 Synthesis of N^{α}-Boc-pyrimidinyl amino esters **9** and N^{α}-Fmoc-pyrimidin-4-one amino acids **3**



Entry	Amino ester **5**	Compound **9**	Yield of **9** [a] (%)	Compound **3**	Yield of **3** [b] (%)
1	**5a**		89		68
2	**5b**		94		65
3	**5c**		94		63

[a] Isolated yields. [b] Isolated yields over three steps.



Scheme 4 Solid-phase synthesis of dipeptides **16a–c**.

affording the compound **9b** as a single regioisomer. Interestingly, the ^{1}H-NMR spectrum of the lysine derivative **9c** revealed a dynamic process confirmed by variable temperature NMR experiments. While at room temperature, the pyrimidine ring protons and benzylic protons appeared as broad signals, these signals became sharper with increasing temperature most probably due to the fast exchange of the protons on the guanidine function.[19]

The optical integrity of compounds **9a–c** was determined using a chromatography method established in our laboratory. Pyrimidinyl amino acids **9a–c** were saponificated with LiOH and the resulting Boc-amino acids **15a–c** were coupled with both a resin-supported racemic phenylalanine and a resin supported *S*-phenylalanine using standard solid-phase peptide synthesis. Final cleavage from the resin with TFA concomitantly removed both the Boc and *p*-benzyloxybenzyloxy groups leading to dipeptides **16a–c**. In all cases, the HPLC analysis of compounds **(*S*,*S*)-16a–c** resulted in only one peak corresponding to a single diastereoisomer, while the analysis of **(*S*/*R*,*S*)-16a–c** showed two peaks (Scheme 4).

The N^{α}-Boc-pyrimidin-2-yl amino esters **9a–c** were then converted to the target N^{α}-Fmoc-pyrimidin-2-one amino acids **3a–c**. Hydrolysis of the methyl ester followed by TFA treatment that removed both the Boc and the *p*-benzyloxybenzyloxy groups as expected. The resulting amino acids **17a–c** were immediately protected as Fmoc carbamate to achieve the target N^{α}-Fmoc-pyrimidin-2-one amino acids **3a–c** in good yields over three steps (Table 1). Determination of the optical integrity of products **3a–c** was also realized through the chromatography method described above, including the Fmoc removal step before cleavage from the resin.[19] These tests showed that no detectable racemization occurred, in addition, proved the stability of these substances by solid-phase peptide synthesis following the Fmoc strategy.

Conclusions

In summary, we have demonstrated the use of the *p*-benzyloxybenzyloxy group as a masking group of the pyrimidinone moiety during the synthesis of new unnatural α-amino acids **3**. The *p*-benzyloxybenzyloxy group at position 4 of the pyrimidine ring proved to be stable under the basic conditions in the presence of nucleophiles and could be easily removed under mild acid conditions. The features of this group allow the successful synthesis of N^{α}-Fmoc-pyrimidin-4-one amino acids **3** through an aromatic nucleophilic substitution reaction between sulfone **8** and the nucleophilic side chain of several natural α-amino acids properly protected, followed by a series of standard protecting group transformations. These Fmoc derivatives **3a–c** are useful building-blocks for the solid-phase peptide synthesis following a Fmoc/*tert*-butyl strategy. The biological effect of the incorporation of these unusual amino acids into antimicrobial peptides is currently underway.

Experimental

General remarks

All commercially available chemicals were used as purchased without further purification. Melting points (capillary tube) were measured with an Electrothermal digital melting point apparatus IA 91000 and are uncorrected. IR spectra were recorded on a Mattson-Galaxy Satellite FT-IR using a single reflection ATR system as a sampling accessory. NMR spectra were recorded on a Bruker DPX200 Advance spectrometer. ^{1}H NMR spectra were recorded at 300 or 400 MHz.^{13}C NMR spectra and DEPT experiments were performed at 75 or 100 MHz. Spectra recorded in $CDCl_3$ were referenced to residual $CHCl_3$ at 7.26 ppm for ^{1}H or 77.0 ppm for ^{13}C. Spectra recorded in DMSO-d_6 were referenced to residual DMSO at 2.50 ppm for ^{1}H or 39.5 ppm for ^{13}C. Coupling constants (J) are given in hertz (Hz). The following abbreviations were used for spin multiplicity: s = singlet, d = doblet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, br = broad. ESI mass spectra were recorded using a Navigator quadrupole instrument. High resolution mass spectra (HRMS) were determined under conditions of ESI on a Bruker Micro Q-TOF instrument using a hybrid quadrupole time-of-flight mass spectrometer. Optical rotations were measured on a Perkin Elmer polarimeter 343 Plus, using the sodium D line. Specific rotation $[\alpha]_D$ is given in 10^{-1} cm^2 g^{-1}, and the concentration (c) are expressed in g per 100 mL. Analytical thin layer chromatography (TLC) was performed on precoated TLC plates, silica

gel 60 F_{254} (Merck). The spots on the TLC plates were visualized under a UV lamp (254 nm) and/or with an aqueous solution of potassium permanganate (1.5% w/w). Flash chromatography purifications were performed on silica gel 60 (230–400 mesh, Merck).

Synthesis of 1-[2-(benzylsulfanyl)pyrimidin-4-yloxy]-1*H*-[*b*]-[1,2,3]-benzotriazole (12). To a stirred solution of pyrimidinone **7** (2 g, 9.17 mmol) in CH_3CN (27 mL), DBU (2 mL, 13.75 mmol) and BOP (6 g, 13.75 mmol) were added successively. The mixture was stirred at room temperature for 3 hours and the reaction completion was confirmed by TLC analysis. The solvent was evaporated under reduced pressure, and the resulting residue was purified by flash chromatography (*n*-hexane–EtOAc, 7 : 3) to afford OBt-adduct **12** (2.66 g, 87%) as a white solid. mp 131–132 °C; TLC: R_f (*n*-hexane–EtOAc, 1 : 1): 0.59; IR (neat): 3062, 3028, 1581, 1542, 1424, 1336, 1258, 1236, 1213, 1081 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.53 (d, J = 5.6 Hz, 1H, $H(6)_{pyrim}$), 8.09 (dd, J = 8.1, 1.5 Hz, 1H, H_{OBt}), 7.58–7.52 (m, 1H, H_{OBt}), 7.47–7.41 (m, 2H, H_{OBt}), 7.15–7.12 (m, 3H, H_{aryl}), 6.94–6.91 (m, 2H, H_{aryl}), 6.79 (d, J = 5.6 Hz, 1H, $H(5)_{pyrim}$), 3.79 (s, 2H, CH_2S); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.1 (s), 168.8 (s), 160.1 (d), 143.4 (s), 136.4 (s), 128.9 (d), 128.8 (s), 128.6 (d, 2C), 128.4 (d, 2C), 127.2 (d), 125.0 (d), 120.6 (d), 108.7 (d), 100.4 (d), 35.1 (t); HRMS (ESI) *m*/*z*: calculated for $C_{17}H_{14}N_5OS$ $[M + H]^+$ 336.0914, found 336.0900; calculated for $C_{17}H_{13}N_5NaOS$ $[M + Na]^+$ 358.0733, found 358.0717.

Synthesis of 4-[4-(benzyloxy)benzyloxy]-2-(benzylsulfanyl)pyrimidine (10). To a stirred solution of OBt-adduct **12** (3.0 g, 9.0 mmol) in CH_3CN (30 mL), 4-benzyloxybenzyl alcohol (2.9 g, 13.5 mmol) and *t*-BuOK (2.0 g, 18 mmol) were added successively. The mixture was stirred at 25 °C for 90 minutes. The solvent was evaporated under reduced pressure, and the resulting residue was purified by flash chromatography (*n*-hexane–EtOAc, 9 : 1) to afford compound **10** (3.62 g, 97%) as a white solid. mp 65–66 °C; TLC: R_f (*n*-hexane–EtOAc, 1 : 1): 0.72; IR (neat): 3060, 3030, 1610, 1553, 1511, 1437, 1307, 1225, 1173 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.24 (d, J = 5.7 Hz, 1H, $H(6)_{pyrim}$), 7.45–7.26 (m, 12H, H_{aryl}), 6.98 (d, J = 8.7 Hz, 2H, H_{aryl}), 6.42 (d, J = 5.7 Hz, 1H, $H(5)_{pyrim}$), 5.32 (s, 2H, CH_2O), 5.08 (s, 2H, CH_2O), 4.44 (s, 2H, CH_2S); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2 (s,), 168.5 (s), 158.9 (s), 157.4 (d), 137.6 (s), 136.9 (s), 130.1 (d, 2C), 128.9 (d, 2C), 128.7 (d, 2C), 128.6 (d, 2C), 128.4 (s), 128.0 (d), 127.5 (d, 2C), 127.2 (d), 115.0 (d, 2C), 104.2 (d), 70.1 (t), 68.1 (t), 35.4 (t); HRMS (ESI) *m*/*z*: calculated for $C_{25}H_{23}N_2O_2S$ $[M + H]^+$ 415.1475, found 415.1478; calculated for $C_{25}H_{22}N_2NaO_2S$ $[M + Na]^+$ 437.1294, found 437.1301.

Synthesis of 4-[4-(benzyloxy)benzyloxy]-2-(benzylsulfonyl)pyrimidine (8). To an ice-cooled solution of benzylsulfanylpyrimidine **10** (3.5 g, 8.4 mmol) in CH_2Cl_2 (42 mL), *m*-CPBA (3.6 g, 21.1 mmol) was added in small portions. The resulting mixture was allowed to warm up to room temperature and stirred for 1 h. The reaction completion was confirmed by TLC analysis. Next, the solvent was removed under reduced pressure and the residue was dissolved in EtOAc (100 mL), washed with saturated aqueous $NaHCO_3$ solution (2 × 20 mL) and brine (1 × 20 mL). The organic layer was dried over $MgSO_4$, filtered, concentrated under reduced pressure and the resulting crude purified by flash chromatography (*n*-hexane–EtOAc, 6 : 4) to afford sulfone **8** (2.89 g, 79%) as a colourless oil. TLC: R_f (*n*-hexane–EtOAc, 1 : 1): 0.41; IR (neat): 3062, 3032, 1578, 1535, 1511, 1467, 1449, 1318, 1239, 1174, 1123 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.52 (d, J = 5.7 Hz, 1H, $H(6)_{pyrim}$), 7.42–7.3 (m, 12H, H_{aryl}), 6.99 (d, J = 8.7 Hz, 2H, H_{aryl}), 6.85 (d, J = 5.7 Hz, 1H, $H(5)_{pyrim}$), 5.42 (s, 2H, CH_2O), 5.06 (s, 2H, CH_2O), 4.71 (s, 2H, CH_2S); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.9 (s), 164.3 (s), 159.2 (s), 157.7 (d), 136.7 (s), 131.2 (d, 2C), 130.6 (d, 2C), 128.8 (d), 128.7 (d, 2C), 128.6 (d, 2C), 128.0 (d), 127.4 (d, 2C), 127.3 (s), 126.8 (s), 115.0 (d, 2C), 111.6 (d), 70.0 (t), 69.6 (t), 57.6 (t); HRMS (ESI) *m*/*z*: calculated for $C_{25}H_2N_2NaO_4S$ $[M + Na]^+$ 469.1192, found 469.1177.

Synthesis of 4-[4-(benzyloxy)benzyloxy]-2-morpholinopyrimidine (13). To a stirred solution of pyrimidinyl sulfone **8** (132 mg, 0.29 mmol) in 1,4-dioxane (1 mL), morpholine (75 mL, 0.87 mmol) was added. The mixture was stirred at 60 °C for 12 hours. The solvent was evaporated under reduced pressure, and the resulting residue was purified by flash chromatography (*n*-hexane–EtOAc, 7 : 3) to afford compound **13** (91 mg, 83%) as a white solid. Mp 101–104 °C; TLC: R_f (*n*-hexane–EtOAc, 1 : 1): 0.65; IR (neat): 3060, 3030, 1610, 1553, 1511, 1437, 1307, 1225, 1173, 983, 819, 696 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 5.6 Hz, 1H, $H(6)_{pyrim}$), 7.45–7.33 (m, 7H, H_{aryl}), 6.80 (d, J = 8.5 Hz, 2H, H_{aryl}), 6.04 (d, J = 5.6 Hz, 1H, $H(5)_{pyrim}$), 5.28 (s, 2H, CH_2O), 5.08 (s, CH_2O), 3.80–3.76 (m, 8H, CH_{2morph}); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.3 (s), 161.6 (s), 158.6 (s), 157.9 (d), 136.8 (s), 129.7 (d, 2C), 129.0 (s), 128.5 (d, 2C), 127.9 (d), 127.4 (d, 2C), 114.8 (d, 2C), 97.3 (d), 70.0 (t), 67.1 (t), 66.7 (t, 2C), 44.3 (t, 2C); MS (ESI) *m*/*z*: 378.1 $[M + H]^+$.

General procedure for the synthesis of N^{α}-Boc pyrimidin-2-yl amino esters (9)

To a stirred solution of the corresponding N^{α}-Boc-amino ester **5** (1.1 equiv.) in dry DMF (0.4 M), K_2CO_3 (1.2–2.4 equiv.) was added and the resulting mixture was stirred at room temperature for 15 min. Then, pyrimidinyl sulfone **8** (1.0 equiv.) was added dissolved in DMF (1 M). The resulting mixture was stirred at the temperature specified for each compound. The solvent was removed under reduced pressure, and the resulting residue was purified by flash chromatography (*n*-hexane–EtOAc, from 15 : 1 to 1 : 1) to afford N^{α}-Boc-pyrimidin-2-yl amino esters **9**.

Methyl (2*S*)-*N*-Boc-2-amino-3-{4-[4-(4-(benzyloxy)-benzyloxy)-pyrimidin-2-yloxy]phenyl}propanoate (9a). Synthesized according to the general procedure from pyrimidinyl sulfone **8** (250 mg, 0.56 mmol), N^{α}-Boc-(*S*)-tyrosine methyl ester **5a** (182 mg, 0.61 mmol) and K_2CO_3 (93 mg, 0.67 mmol) at 50 °C for 24 h, compound **9a** (291 mg, 89%) was obtained as a colourless oil. TLC: R_f (*n*-hexane–EtOAc, 3 : 2): 0.25; $[\alpha]_D^{20}$ +22.9 (*c* 1.36, $CHCl_3$); IR (neat): 3433, 1741, 1709, 1568, 1508, 1380, 1334, 1271, 1240, 1213, 1166 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, J = 5.7 Hz, 1H, $H(6)_{pyrim}$), 7.44–7.25 (m, 7H, H_{aryl}),

7.19 (d, J = 8.5 Hz, 2H, H_{aryl}), 7.13 (d, J = 8.5 Hz, 2H, H_{aryl}), 6.95 (d, J = 8.6 Hz, 2H, H_{aryl}), 6.45 (d, J = 5.7 Hz, 1H, $H(5)_{pyrim}$), 5.26 (s, 2H, CH_2O), 5.08 (s, 2H, CH_2O), 5.05 (br s, 1H, NH), 4.61 (m, 1H, CH_α), 3.71 (s, 3H, OCH_3), 3.15 (dd, J = 13.9, 5.8 Hz, 1H, C$H_{2\beta}$), 3.07 (dd, J = 13.9, 6.1 Hz, 1H, C$H_{2\beta}$), 1.42 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.3 (s), 171.2 (s), 164.9 (s), 159.0 (s), 158.9 (d), 152.0 (s), 136.8 (s), 133.1 (s, 2C), 130.4 (d, 2C), 130.3 (d, 2C), 128.6 (d, 2C), 128.1 (s), 128.0 (d), 127.5 (d, 2C), 122.0 (d, 2C), 114.9 (d, 2C), 103.6 (d), 80.2 (s), 70.1 (t), 68.2 (t), 54.5 (d), 52.3 (q), 37.8 (t), 28.4 (q, 3C); HRMS (ESI) m/z: calculated for $C_{33}H_{36}N_3O_7$ $[M + H]^+$ 586.2548, found 586.2555; calculated for $C_{33}H_{35}N_3NaO_7$ $[M + Na]^+$ 608.2367, found 608.2384.

Methyl (2*S*)-*N*-Boc-2-amino-3-{1-[4-(4-(benzyloxy)benzyloxy)-pyrimidin-2-yl]-1*H*-imidazol-4-yl}propanoate (9b). Synthesized according to the general procedure from pyrimidinyl sulfone **8** (250 mg, 0.56 mmol), N^α-Boc-(*S*)-histidine methyl ester **5b** (164 mg, 0.61 mmol) and K_2CO_3 (93 mg, 0.67 mmol) at 50 °C for 24 h, compound **9b** (293 mg, 94%) was obtained as a colourless oil. TLC: R_f (*n*-hexane–EtOAc, 3 : 2): 0.25; $[\alpha]_D^{20}$ +17.2 (c 1.5, $CHCl_3$); IR (neat): 2961, 2919, 1745, 1710, 1590, 1565, 1512, 1486, 1446, 1361, 1298, 1247, 1170, 1027 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (s, 1H, $H(2)_{imid}$), 8.33 (d, J = 5.7 Hz, 1H, $H(6)_{pyrim}$), 7.64 (s, 1H, $H(5)_{imid}$), 7.44–7.31 (m, 7H, H_{aryl}), 7.00 (d, J = 8.7 Hz, 2H, H_{aryl}), 6.61 (d, J = 5.7 Hz, 1H, $H(5)_{pyrim}$), 5.83 (d, J = 8.2 Hz, 1H, NH), 5.41 (s, 2H, CH_2O), 5.08 (s, 2H, CH_2O), 4.62 (m, 1H, CH_α), 3.73 (s, 3H, H_{17}), 3.17 (dd, J = 14.7, 5.5 Hz, 1H, C$H_{2\beta}$), 3.07 (dd, J = 14.7, 4.8 Hz, 1H, C$H_{2\beta}$), 1.44 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.5 (s), 170.3 (s), 159.2 (s), 158.5 (d), 155.6 (s), 136.9 (s), 135.9 (s), 132.2 (d), 130.2 (d, 2C), 128.7 (d, 2C), 128.1 (d), 127.9 (s, 2C), 127.5 (d, 2C), 115.2 (d, 2C), 114.3 (d), 106.4 (d), 79.8 (s), 70.2 (t), 68.7 (t), 53.5 (d), 52.3 (q), 29.8 (t), 28.4 (q, 3C); HRMS (ESI) m/z: calculated for $C_{30}H_{34}N_5O_6$ $[M + H]^+$ 560.2504, found 560.2511; calculated for $C_{30}H_{33}N_5NaO_6$ $[M + Na]^+$ 582.2323, found 582.2294.

Methyl (2*S*)-*N*-Boc-2-amino-6-{*N*-[4-(4-(benzyloxy)benzyloxy)-pyrimidin-2-yl]amino}hexanoate (9c). Synthesized according to the general procedure from pyrimidinyl sulfone **8** (250 mg, 0.56 mmol), N^α-Boc-(*S*)-lysine methyl ester **5c** (200 mg, 0.61 mmol) and K_2CO_3 (155 mg, 1.22 mmol) at 45 °C for 24 h, compound **9c** (280 mg, 84%) was obtained as a colourless oil. TLC: R_f (*n*-hexane–EtOAc, 3 : 7): 0.51; $[\alpha]_D^{20}$ +7.8 (c 1.0, $CHCl_3$); IR (neat): 3365, 3255, 2935, 1739, 1685, 1584, 1528, 1511, 1453, 1427, 1282, 1243, 1225, 1171, 1159 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, J = 5.7 Hz, 1H, $H(6)_{pyrim}$), 7.44–7.31 (m, 7H, H_{aryl}), 6.98 (d, J = 8.7 Hz, 2H, H_{aryl}), 6.01 (d, J = 5.7 Hz, 1H, $H(5)_{pyrim}$), 5.27 (s, 2H, CH_2O), 5.13 (br s, 1H, NH), 5.07 (s, 2H, CH_2O), 4.30 (br s, 1H, CH_α), 3.71 (s, 3H, OCH_3), 3.40 (q, J = 6.7 Hz, 2H, NCH_2), 1.83 (m, 1H, C$H_{2\beta}$), 1.73–1.56 (m, 3H, CH_2, C$H_{2\beta}$), 1.43 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.5 (s), 170.1 (s), 161.9 (s), 159.0 (s), 156.8 (d), 155.6 (s), 137.1 (s), 130.1 (d, 2C), 129.0 (s), 128.8 (d, 2C), 128.2 (d), 127.6 (d, 2C), 115.1 (d, 2C), 97.7 (d), 80.1 (s), 70.3 (t), 67.6 (t), 53.6 (d), 52.4 (q), 41.3 (t), 32.7 (t), 29.4 (t), 28.5 (q), 23.0 (t); HRMS (ESI) m/z: calculated for $C_{30}H_{39}N_4O_6$ $[M + H]^+$ 551.2864, found 551.2884.

General procedure for the synthesis of N^α-Fmoc pyrimidin-2-one amino acids 3

To a stirred solution of appropriate N^α-Boc-amino methyl esters **9** (1 equiv.) in THF–MeOH–H_2O (1 : 2 : 2; 0.12 M), LiOH monohydrate (2.5 equiv.) was added. The mixture was stirred at room temperature for 3–4 h. The organic solvents were removed under reduced pressure and the pH of the resulting aqueous solution was then adjusted to 4 with glacial acetic acid. The solution was extracted with CH_2Cl_2 (3 × 5 mL) and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting residue was used without further purification in the next step. The free N^α-Boc-amino acid was dissolved in CH_2Cl_2 (0.7 M) and the solution was cooled in an ice bath. TFA (0.3 M) was added dropwise and the resulting mixture was stirred at 0 °C for 5 h. The solvent was removed under reduced pressure and the crude material was dissolved in 1,4-dioxane (0.3 M). The resulting solution was adjusted with 5% aqueous $NaHCO_3$ solution to pH 7. Fmoc-Osu (1.02–1.05 equiv.) was then added slowly. During this addition, pH was readjusted with 5% aqueous $NaHCO_3$ solution to pH 7. The resulting mixture was stirred at rt for 8–12 h and the reaction completion was confirmed by TLC analysis. After this time, the solvent was removed under reduced pressure and the resulting residue was diluted in water (10 mL) and extracted with EtOAc (3 × 5 mL). The combined organic layers were back extracted with saturated $NaHCO_3$ solution (3 × 5 mL). The combined basic aqueous layers were then acidified to pH 1–2 with 1% aqueous HCl solution, and extracted with EtOAc (3 × 5 mL). These organic layers were combined and dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting residue was triturated with diethyl ether, filtered, washed with diethyl ether and *n*-pentane, and dried to afford N^α-Fmoc pyrimidin-2-one amino acids **3**.

(2*S*)-*N*-Fmoc-2-amino-3-[4-(6-oxo-1,6-dihydropyrimidin-2-yloxy)phenyl]propanoic acid (3a). Synthesized according to the general procedure from N^α-Boc-pyrimidin-2-yl amino ester **9a** (140 mg, 0.25 mmol), compound **3a** (84 mg, 68%) was obtained as a colourless solid. Mp: 117–118 °C; TLC: R_f (EtOAc–MeOH–AcOH, 10 : 2 : 0.1): 0.45; $[\alpha]_D^{20}$ −1.3 (c 0.4, DMF); IR (neat): 3318, 1663, 1592, 1551, 1502, 1306, 1193, 1137 cm^{-1}; ^{1}H NMR (400 MHz, d_6-DMSO) δ 12.81 (br, 1H, OH), 7.88 (d, J = 7.4 Hz, 2H, H_{Fmoc}), 7.68–7.64 (m, 3H, $H(6)_{pyrim}$, H_{Fmoc}), 7.42–7.38 (m, 2H, H_{Fmoc}), 7.34–7.28 (m, 4H, H_{aryl}), 7.12 (d, J = 8.4 Hz, 2H, H_{Fmoc}), 6.07 (d, J = 6.3 Hz, 1H, $H(5)_{pyrim}$), 4.24–4.12 (m, 4H, CHCH_2O, CH_α), 3.10 (dd, J = 13.9, 4.1 Hz, 1H, C$H_{2\beta}$), 2.89 (dd, J = 13.9, 10.4 Hz, 1H, C$H_{2\beta}$); ^{13}C NMR (75 MHz, d_6-DMSO) δ 173.5 (s), 165.2 (s), 159.3 (s), 155.9 (s), 153.9 (d), 150.1 (s), 143.8 (s, 2C), 140.6 (s, 2C), 135.7 (s), 130.2 (d, 2C), 127.6 (d, 2C), 127.0 (d, 2C), 125.2 (d), 125.1 (d), 121.3 (d, 2C), 120.0 (d, 2C), 108.4 (d), 65.5 (t), 55.7 (d), 46.6 (d), 35.9 (t); HRMS (ESI) m/z: calculated for $C_{28}H_{24}N_3O_6$ $[M + H]^+$ 498.1660, found 498.1683; calculated for $C_{28}H_{23}N_3NaO_6$ $[M + Na]^+$ 520.1479, found 520.1493.

(2*S*)-*N*-Fmoc-2-amino-3-[1-(6-oxo-1,6-dihydropyrimidin-2-yl)-1*H*-imidazol-4-yl]propanoic acid (3b). Synthesized according

to the general procedure from N^{α}-Boc-pyrimidin-2-yl amino ester **9b** (150 mg, 0.25 mmol), compound **3b** (76 mg, 65%) was obtained as a colourless solid. Mp: 163–164 °C; TLC: R_f (EtOAc–MeOH–AcOH, 10 : 2 : 0.1): 0.25; $[\alpha]_D^{20}$ +17.4 (*c* 0.3, DMF); IR (neat): 3135, 1685, 1598, 1526, 1494, 1445, 1389, 1206, 1142 cm^{-1}; ^1H NMR (400 MHz, d_6-DMSO) δ 12.81 (br, 1H, O*H*), 8.37 (s, 1H, $H(2)_{imid}$), 8.24 (d, J = 5.6 Hz, 1H, $H(6)_{pyrim}$), 7.86 (d, J = 7.2 Hz, 2H, H_{Fmoc}), 7.66–7.63 (m, 4H, $H(5)_{imid}$, N*H*, H_{Fmoc}), 7.37 (t, J = 7.4 Hz, 2H, H_{Fmoc}), 7.29–7.24 (m, 2H, H_{Fmoc}), 6.47 (d, J = 5.6 Hz, 1H, $H(5)_{pyrim}$), 4.31–4.17 (m, 4H, C*H*CH_2O, CH_{α}), 3.00 (dd, J = 14.7, 4.4 Hz, 1H, C$H_{2\beta}$), 2.90 (dd, J = 14.7, 9.4 Hz, 1H, C$H_{2\beta}$); ^{13}C NMR (75 MHz, d_6-DMSO) δ 173.4 (s), 171.2 (s), 157.8 (d), 155.9 (s), 153.8 (s), 143.7 (s, 2C), 140.6 (s, 2C), 139.0 (s), 134.9 (d), 127.5 (d, 2C), 127.0 (d, 2C), 125.2 (d), 125.1 (d), 120.0 (d, 2C), 113.8 (d), 106.4 (d), 65.6 (t), 53.7 (d), 46.5 (d), 29.8 (t); HRMS (ESI) *m*/*z*: calculated for $C_{25}H_{22}N_5O_5$ $[M + H]^+$ 472.1615, found 472.1615.

(2*S*)-*N*-Fmoc-2-amino-6-[*N*-(4-oxo-3*H*-pyrimidin-2-yl) amino]-hexanoic acid (3c). Synthesized according to the general procedure from N^{α}-Boc-pyrimidin-2-yl amino ester **9c** (150 mg, 0.27 mmol), compound **3c** (78 mg, 63%) was obtained as a colourless solid. Mp: 121–122 °C; R_f (EtOAc–MeOH–AcOH, 10 : 2 : 0.1): 0.69; $[\alpha]_D^{20}$ +6.6 (*c* 1.0, DMF); IR (neat): 2944, 1686, 1645, 1534, 1446, 1193, 1132 cm^{-1}; ^1H NMR (400 MHz, d_6-DMSO) δ 12.59 (br s, 1H, O*H*), 7.89 (d, J = 7.5 Hz, 2H, H_{Fmoc}), 7.72 (d, J = 7.1 Hz, 2H, H_{Fmoc}), 7.65–7.61 (m, 2H, $H(6)_{pyrim}$, N*H*), 7.42 (t, J = 7.4 Hz, 2H, H_{Fmoc}), 7.33 (t, J = 7.4 Hz, 2H, H_{Fmoc}), 5.67 (d, J = 6.6 Hz, 1H, $H(5)_{pyrim}$,), 4.30–4.20 (m, 3H, C*H*CH_2O), 3.93 (m, 1H, CH_{α}), 3.25 (q, 2H, CH_2N), 1.75–1.68 (m, 2H, CH_2CH_2N), 1.66–1.54 (m, 2H, C$H_2CH_2CH_2CH_2$N); ^{13}C NMR (100 MHz, d_6-DMSO) δ 173.9 (s), 162.5 (s), 162.3 (s), 156.2 (s), 153.8 (d), 143.8 (s, 2C), 140.7 (s, 2C), 127.6 (d, 2C), 127.1 (d, 2C), 125.3 (d, 2C), 120.1 (d, 2C), 102.9 (d), 65.6 (t), 53.8 (d), 46.7 (d,), 40.4 (t), 30.4 (t), 28.1 (t), 22.9 (t); HRMS (ESI) *m*/*z*: calculated for $C_{25}H_{27}N_4O_5$ $[M + H]^+$ 463.1976, found 463.1984.

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