Synthesis of Nucleo Aminooxy Acid Derivatives

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Abstract: Nucleobase-functionalized peptides have attracted increasing interest because of their well-ordered secondary structures and stability toward enzymatic degradations. We have designed and synthesized nucleo aminooxy acids as novel building blocks for nucleopeptides. Four nucleo aminooxy acid derivatives with cytosine or thymine in the side chain linked by an amide or a triazole moiety have been synthesized from L-serine.

Key words: nucleo aminooxy acids, nucleopeptides, *N*-oxy nucleopeptides, cytosine, thymine, amides, triazoles, L-serine, eliminations

Nucleo amino acids are synthetic amino acids bearing nucleobases covalently linked to their side chains. Various peptides containing nucleo α- and β-amino acids have been reported as being able to form rigid and helical structures as well as well-defined double strands with complementary sequences.[1–7] Moreover, nucleopeptides have recently emerged as a promising alternative to peptide nucleic acids,[8,9] able to penetrate into a cell nucleus without cytotoxic effects.[10]

Aminooxy acids are analogues of amino acids bearing an oxyamine function (O–NH_2) in the place of amine. Peptides of aminooxy acids have an ease of forming well-defined structures like α-, β- and γ-turns or helices thanks to intramolecular hydrogen-bond formation.[11,12] It would therefore be interesting to synthesize nucleo aminooxy acids containing nucleobases on the side chain of aminooxy acids in order to study the secondary structure and DNA/RNA binding properties of the corresponding *N*-oxy nucleopeptides, since both the *N*-oxy peptide and the nucleobases could contribute to structure organization. As part of a continuing program on the synthesis of sugar- and nucleoside-derived aminooxy acids,[13–17] we report herein the synthesis of nucleo aminooxy acid derivatives with thymine or cytosine connected to the side chain of a β-aminooxy acid through either an amide or a triazole linkage (Figure 1). To the best of our knowledge, nucleo aminooxy acids have not been previously reported in the literature.

The target nucleo aminooxy acid derivatives are accessible from the β-phthalimidooxy ester **8** (Scheme 1). This compound has been previously prepared from L-serine by Burke and co-workers.[18] We have synthesized the phthalimidooxy ester **7** from L-Ser–OMe (**5**) by N-tritylation and Mitsunobu reactions, and purified compounds **6** and **7** by simple precipitation, without column chromatography. Treatment of **7** with 36% hydrochloric acid in dichloromethane as reported led, however, to a mixture of compounds. Removal of the trityl group was then achieved with acetyl chloride in methanol, leading to the amine salt **8** in 65% yield. Coupling of **8** with N^4-Cbz-protected cytosin-1-ylacetic acid **9**[19] using BOP reagent furnished the cytosin-1-yl-substituted aminooxy ester **2a** in 53% yield; however, reaction of **8** with thymin-1-ylacetic acid (**14**)[20] using EDC/HOAt led to the corresponding polar thymin-1-yl-substituted aminooxy ester which proved to be difficult to purify.

We then decided to replace the phthaloyl protecting group of the oxyamine in **8** by Boc, through Cbz protection of the amine function, hydrazinolysis and treatment with Boc_2O;[18] however, saponification of **10** led to a mixture of the desired carboxylic acid **11**[18] and the elimination product **12**[21] in a 1:1 ratio (Scheme 1), showing that the *N*-Boc-protected aminooxy ester **10** is sensitive to basic conditions. Deprotection of the Cbz group under hydrogenolysis conditions was also troublesome. In fact, prolonged reaction induced homolytic cleavage of the N–O bond.[15]

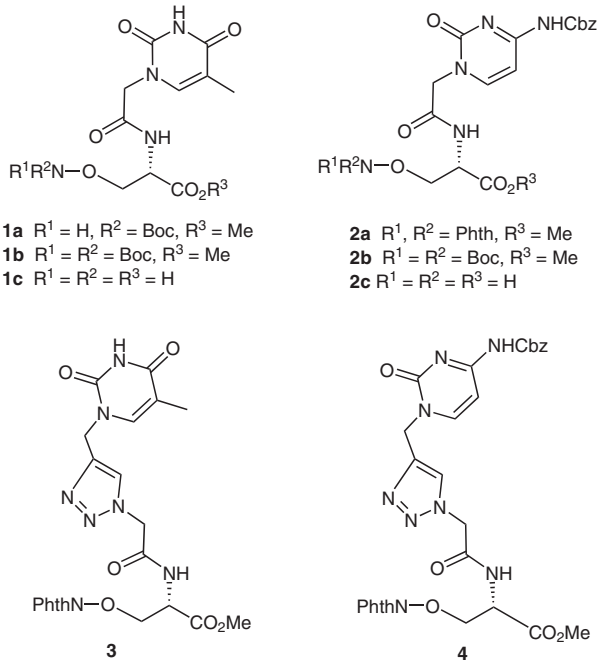

Figure 1 Structures of the target nucleo aminooxy acid derivatives

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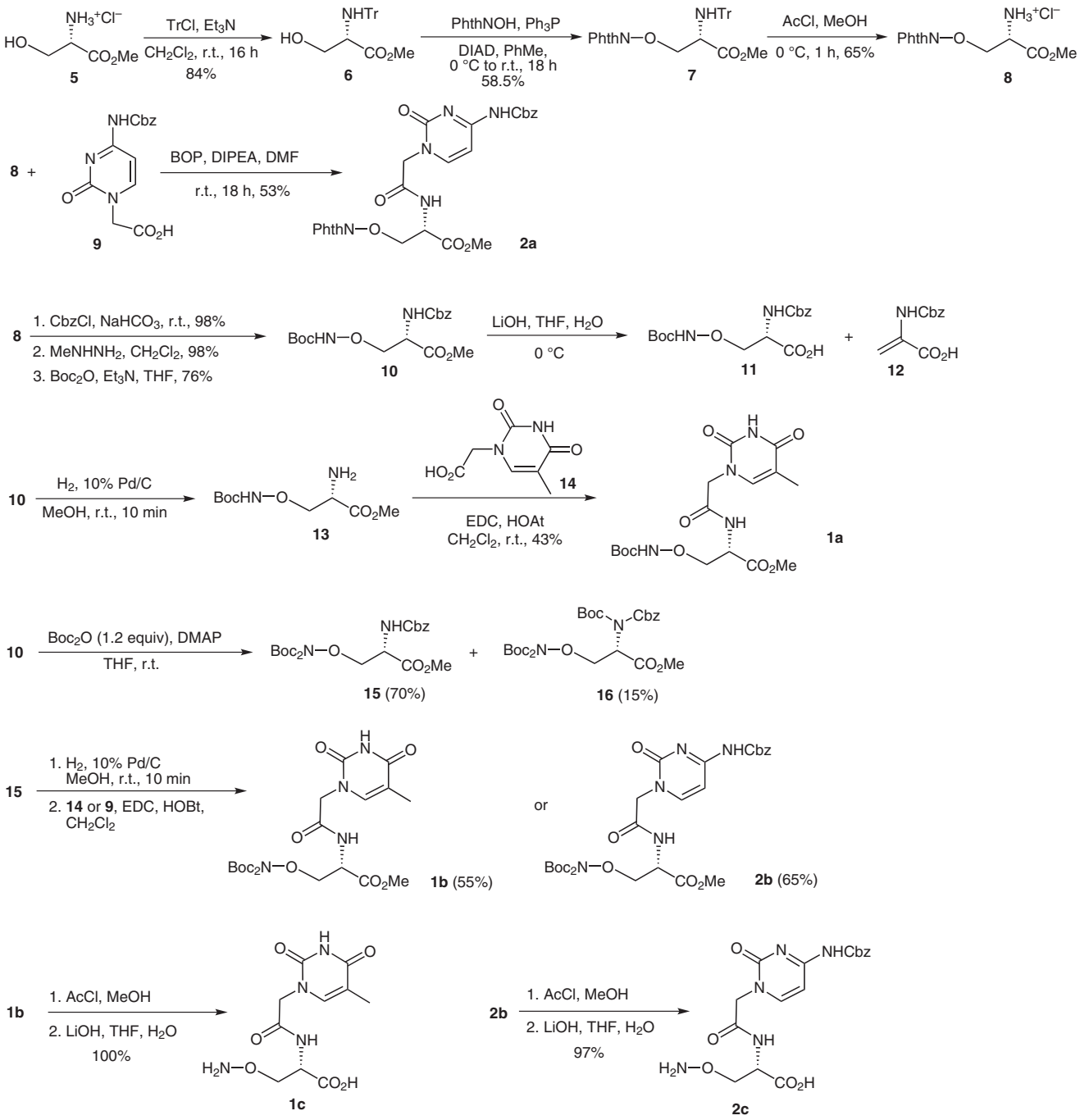
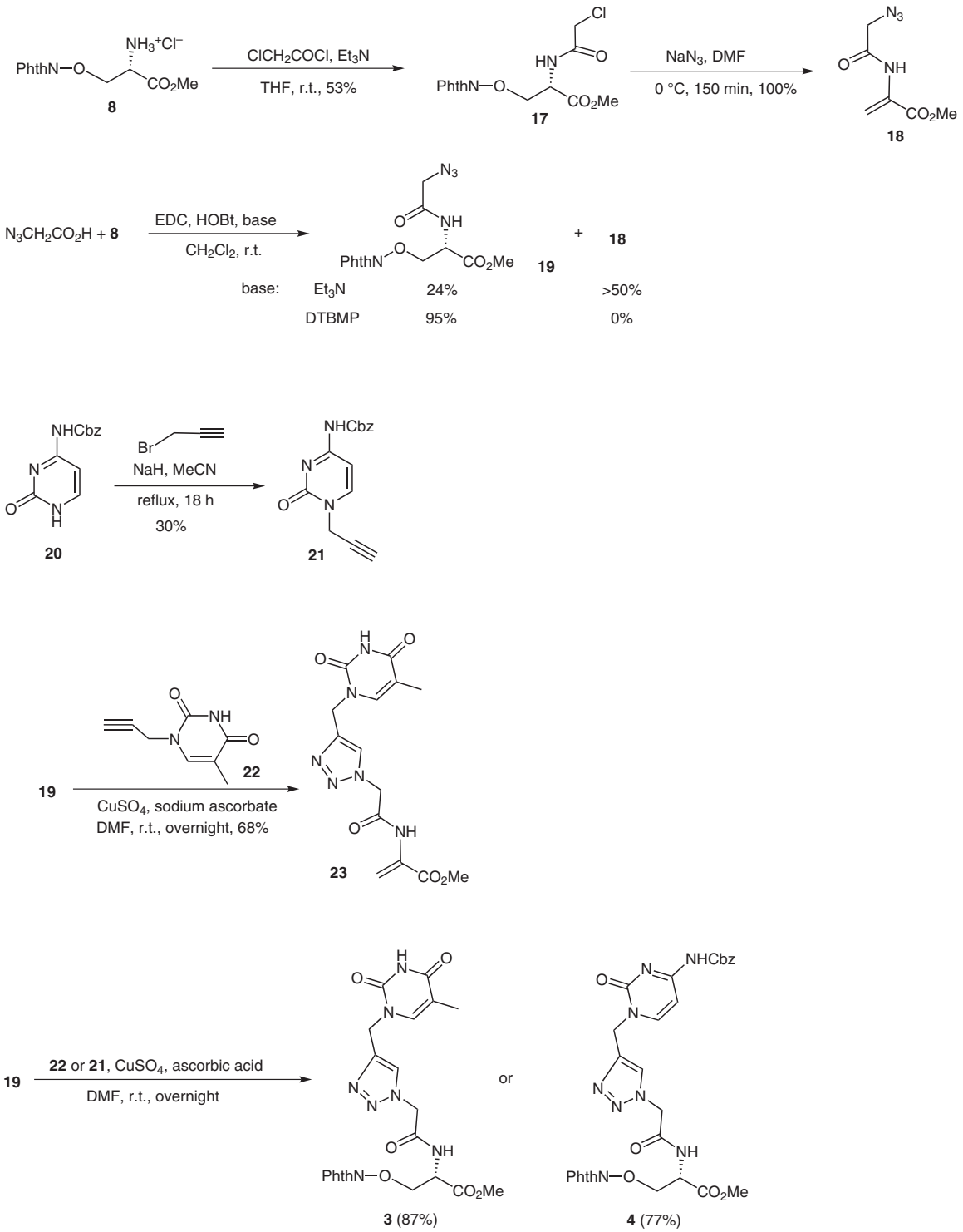


Scheme 1 Synthesis of nucleo aminooxy acid derivatives **1** and **2**

Nevertheless, it was possible to obtain the amine **13** in acceptable yield when the reaction time did not exceed 10 minutes. Coupling of amine **13** with thymin-1-ylacetic acid (**14**) promoted by EDC/HOAt furnished the thymin-1-yl-substituted aminooxy ester **1a** in 43% yield (two steps), along with a small quantity of over-acylation product of the HN–O nitrogen.[22] Boc protection of the HN–O nitrogen in **10** was then realized with Boc_2O in the presence of 4-(dimethylamino)pyridine to afford a 70% yield of **15** and a 15% yield of the tris-Boc derivative **16**. Fast hydrogenolysis of **15** followed by coupling with **14** or **9** gave the corresponding nucleo aminooxy esters **1b** and **2b** in 55% and 65% yield, respectively. To prepare the fully deprotected nucleo aminooxy acids **1c** and **2c**, it is preferable to remove the Boc groups before saponification in order to avoid the elimination reaction of **1b** and **2b** under basic conditions. Compounds **1c** and **2c** were obtained in quantitative yield (Scheme 1).

Triazole-linked nucleo aminooxy acid derivatives **3** and **4** could be prepared by click reaction between the azido intermediate **19** and the alkyne derivatives **22** and **21**[23] (Scheme 2). Compound **8** was firstly acylated with chloroacetyl chloride to give compound **17**; however, subse-



Scheme 2 Synthesis of nucleo aminooxy esters **3** and **4**; DTBMP = 2,6-di(*tert*-butyl)-4-methylpyridine

quent substitution with sodium azide at 0 °C quantitatively afforded the elimination product **18**. We then decided to prepare **19** by condensation of amine salt **8** with azidoacetic acid[24] promoted by EDC/HOBt in the presence of 1 equivalent of triethylamine. Once again, the elimination reaction mainly occurred: the desired compound **19** was isolated in only 24% yield. To avoid this side reaction, hindered 2,6-di-*tert*-butyl-4-methylpyridine was chosen to neutralize the amine salt **8**, successfully leading to compound **19** in 95% yield. Click reaction of **19** with 1-propargylthymine (**22**) catalyzed by copper(II) sulfate and sodium ascorbate accomplished the cycloaddition reaction, followed, however, by elimination of the phthalimidooxy moiety to give compound **23** in 68% yield. Fortunately, the use of ascorbic acid[25] avoided the elimination reaction and afforded the desired nucleo aminooxy acid derivatives **3** and **4** in 87% and 77% yield, respectively.

In summary, two series of nucleo aminooxy acid derivatives have been synthesized by linking thymine or cytosine to the side chain of an α-amino-β-aminooxy acid prepared from L-serine methyl ester. A more convenient procedure for the synthesis of phthaloyl-protected β-aminooxy ester **8** has been developed. The high polarity of phthaloyl-protected amide-linked nucleo β-aminooxy esters led us to prepare Boc-protected derivatives **1a**, **1b** and **2b** which were fully deprotected to the free nucleo β-aminooxy acids **1c** and **2c**. Triazole-linked nucleo aminooxy esters **3** and **4** have also been successfully synthesized via click chemistry. During our synthesis, we also observed the instability of phthaloyl- or Boc-protected β-aminooxy esters under basic conditions, leading to the corresponding acrylate elimination products. This side reaction could be avoided by the use of a hindered base or nonbasic conditions. These newly synthesized nucleo aminooxy acid derivatives might constitute useful building blocks for the synthesis of *N*-oxy nucleopeptides for investigation of their secondary structure and DNA/RNA binding properties.

Commercially available solvents and reagents were used without further purification, except DMF which was distilled over CaH_2. Melting points were measured on a Kofler bench. Optical rotations were measured using a JASCO P-2000 polarimeter. Column chromatography was performed on Carlo Erba silica gel 60A (40–63 μm). Analytical thin-layer chromatography was performed on E. Merck aluminum precoated plates of silica gel 60F-254 with detection by UV light and by spraying with 10% H_2SO_4 in EtOH or ninhydrin soln (3 g·L^{-1}) and heating for about 20 seconds at 400 °C. ^{1}H and ^{13}C NMR spectra were recorded on a Jeol ECS-400 spectrometer. ESI-HRMS data were recorded on a Bruker micrOTOF-Q II or a Bruker maXis spectrometer using standard conditions.

_N_-Trityl-L-serine Methyl Ester (6)[18]

To a soln of L-Ser–OMe·HCl (**5**; 9.29 g, 59.9 mmol) in CH_2Cl_2 (250 mL) were added Et_3N (23 mL, 163.7 mmol) and TrCl (19.98 g, 71.9 mmol). The resulting mixture was stirred for 16 h at r.t. and then concentrated under reduced pressure. The crude material was triturated in EtOAc and compound **6** was isolated by precipitation as a white solid; yield: 18.64 g (84%); mp 138 °C.

R_f = 0.5 (EtOAc–PE, 1:1).

_O_-Phthalimido-_N_-trityl-L-serine Methyl Ester (7)[18]

To a soln of **6** (10.02 g, 27.76 mmol) in toluene (150 mL) were added *N*-hydroxyphthalimide (6.33 g, 38.86 mmol) and Ph_3P (10.18 g, 38.86 mmol). At 0 °C, DIAD (7.65 mL, 41.64 mmol) was then added dropwise. The resulting mixture was stirred for 18 h at r.t. and then washed with 1 N NaOH (2 × 50 mL) and brine (50 mL), dried over $MgSO_4$ and concentrated under reduced pressure. To the orange crude material dissolved in Et_2O (100 mL), ice (100 g) was added. After 2 h of vigorous stirring, the precipitate was collected by filtration to obtain **7** as a white solid; yield: 8.23 g (58.5%); mp 113 °C.

R_f = 0.74 (EtOAc–PE, 1:1).

_O_-Phthalimido-L-serine Methyl Ester Hydrochloride (8)

To a soln of **7** (11.12 g, 21.98 mmol) in MeOH (300 mL) was added AcCl (1.73 mL, 24.18 mmol) at 0 °C. After 1 h of stirring, MeOH was evaporated under reduced pressure to give a white crude material which precipitated in CH_2Cl_2 to give **8** as a white solid; yield: 4.28 g (65%); mp 154 °C.

$[\alpha]_D^{27}$ +2.6 (*c* 0.5, MeOH).

^{1}H NMR (CD_3OD): δ = 7.89–7.86 (m, 4 H, H-Phth), 4.65–4.63 (m, 2 H, CH_2), 4.61–4.58 (m, 1 H, CH), 3.88 (s, 3 H, OCH_3).

^{13}C NMR (CD_3OD): δ = 170.6, 167.2 (CO), 138.9 (CH-Phth), 132.7 (Cq), 127.3 (CH-Phth), 78.3 (CH_2), 56.5 (OCH_3), 55.3 (CH).

HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{12}H_{13}N_2O_5$: 265.0824; found: 265.0820.

Methyl (*S*)-2-[2-(4-{[(Benzyloxy)carbonyl]amino}-2-oxopyrimidin-1(2*H*)-yl)acetamido]-3-[(1,3-dioxoisoindolin-2-yl)oxy]propanoate (2a)

To a soln of {*N*^{4}-[(benzyloxy)carbonyl]cytosin-1-yl}acetic acid (**9**; 1.31 g, 4.33 mmol) in DMF (20 mL) were added DIPEA (1.1 mL, 6.66 mmol) and BOP reagent (1.91 g, 4.33 mmol) at r.t. After 10 min, compound **8** (1.00 g, 3.33 mmol) was added. The reaction mixture was stirred for 18 h, then concentrated and dissolved in EtOAc (100 mL). The organic layer was washed with sat. aq NH_4Cl (1 × 30 mL), sat. aq $NaHCO_3$ (2 × 30 mL) and brine (1 × 30 mL), then dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography (CH_2Cl_2 to CH_2Cl_2–MeOH, 96:4) to give **2a** as white crystals; yield: 863 mg (53%); mp 118 °C.

$[\alpha]_D^{27}$ +13.3 (*c* 0.5, $CHCl_3$); R_f = 0.40 (CH_2Cl_2–MeOH, 95:5).

^{1}H NMR ($CDCl_3$): δ = 8.01 (d, *J* = 7.8 Hz, 1 H, NH), 7.85–7.79 (m, 2 H, H-Phth), 7.75–7.72 (m, 2 H, H-Phth), 7.69 (d, *J* = 7.3 Hz, 1 H, H-Ar), 7.37 (m, 5 H, CH), 7.26 (d, *J* = 7.3 Hz, 1 H, CH), 5.20 (s, 2 H, OCH_2), 4.88–4.86 (m, 1 H, CH), 4.85 (dd, *J* = 3.2, 7.3 Hz, 1 H, OCH_2), 4.73 (s, 2 H, NCH_2), 4.38 (dd, *J* = 3.2, 7.3 Hz, 1 H, OCH_2), 3.68 (s, 3 H, OCH_3).

^{13}C NMR ($CDCl_3$): δ = 169.1, 166.8, 163.5, 163.0, 156.0, 149.3 (Cq), 149.5, 135.2 (CH), 134.9 (Cq), 128.8, 128.7, 128.6 (CH), 128.4 (Cq), 123.9, 95.8 (CH), 77.7, 68.0 (OCH_2), 53.6 (NCH_2), 53.1 (OCH_3), 52.2 (CH).

HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{26}H_{23}N_5NaO_9$: 572.1393; found: 572.1387.

Methyl (*S*)-3-{[(*tert*-Butoxycarbonyl)amino]oxy}-2-{2-[5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]acetamido}propanoate (1a)

To a soln of methyl (*S*)-2-{[(benzyloxy)carbonyl]amino}-3-{[(*tert*-butoxycarbonyl)amino]oxy}propanoate[18] (**10**; 204 mg, 0.55 mmol) in MeOH (10 mL) was added 10% Pd/C (30 mg). H_2 was bubbled into the mixture for 10 min. The mixture was filtered and the filtrate was concentrated under reduced pressure. The resultant oily residue in CH_2Cl_2 (4 mL) was added to a mixture of 2-[5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]acetic acid (**14**; 131 mg, 0.72 mmol), EDC (136 mg, 0.72 mmol) and HOAt (97 mg, 071 mmol) in CH_2Cl_2 (26 mL) at r.t. After 18 h of stirring, the mixture was washed with sat. aq $NaHCO_3$ (2 × 10 mL) and brine (1 × 10 mL). The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography (CH_2Cl_2–MeOH, 96:4) to obtain **1a** as a white foam; yield: 94 mg (43%); mp 110 °C.

$[\alpha]_D^{27}$ +2.0 (*c* 0.5, $CHCl_3$); R_f = 0.40 (CH_2Cl_2–MeOH, 9:1).

^{1}H NMR ($CDCl_3$): δ = 9.57 (s, 1 H, NH), 8.07 (d, *J* = 8.2 Hz, 1 H, NH), 7.85 (s, 1 H, NH), 7.11 (s, 1 H, CH), 4.81–4.78 (m, 1 H, CH), 4.50 (s, 2 H, NCH_2), 4.30 (dd, *J* = 3.7, 11.0 Hz, 1 H, OCH_2), 4.02 (dd, *J* = 3.7, 11.0 Hz, 1 H, OCH_2), 3.71 (s, 3 H, OCH_3), 1.91 (s, 3 H, CH_3), 1.45 (s, 9 H, *t*-Bu).

^{13}C NMR ($CDCl_3$): δ = 170.3, 167.2, 164.5, 157.5, 151.5 (CO), 141.0 (CH-Ar), 111.2 (Cq), 82.6 (Cq-*t*-Bu), 75.6 (OCH_2), 53.0 (OCH_3), 51.7 (CH), 50.2 (NCH_2), 28.2 (*t*-Bu), 12.5 (CH_3).

HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{16}H_{24}N_4NaO_8$: 423.1492; found: 423.1488.

Methyl (*S*)-2-{[(Benzyloxy)carbonyl]amino}-3-{[bis(*tert*-butoxycarbonyl)amino]oxy}propanoate (15)

To a soln of **10** (106 mg, 0.29 mmol) in THF (3 mL) were added a soln of Boc_2O (75 mg, 0.35 mmol) in THF (3 mL) and DMAP (70 mg, 0.58 mmol) at r.t. After 17 h of stirring, the reaction mixture was concentrated. The crude product was purified by column chromatography (EtOAc–PE, 1:9 to 2:8) to give compound **15** as a colorless oil [yield: 94 mg (70%)] and methyl (*S*)-2-{[(benzyloxy)carbonyl](*tert*-butoxycarbonyl)amino}-3-{[bis(*tert*-butoxycarbonyl)amino]oxy}propanoate (**16**) as a colorless oil [yield: 25 mg (15%)].

Compound **15**

$[\alpha]_D^{27}$ –2.0 (*c* 0.5, $CHCl_3$); R_f = 0.61 (EtOAc–PE, 3:7).

^{1}H NMR ($CDCl_3$): δ = 7.36–7.33 (m, 5 H, H-Ph), 6.16 (d, *J* = 8.2 Hz, 1 H, NH), 5.14 (s, 2 H, OCH_2), 4.57–4.54 (m, 1 H, OCH_2), 4.57–4.54 (m, 1 H, CH), 4.02 (dd, *J* = 3.2, 9.2 Hz, 1 H, OCH_2), 3.78 (s, 3 H, OCH_3), 1.52 (s, 18 H, 2 × *t*-Bu).

^{13}C NMR ($CDCl_3$): δ = 169.9, 156.2, 149.8 (CO), 136.4 (Cq-Ph), 128.5, 128.2, 128.1 (CH-Ph), 84.6 (Cq-*t*-Bu), 75.6, 67.1 (CH_2), 53.3 (CH), 52.8 (OCH_3), 28.1 (*t*-Bu).

HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{22}H_{32}N_2NaO_9$: 491.2006; found: 491.2015.

Compound **16**

$[\alpha]_D^{27}$ –16.4 (*c* 0.5, $CHCl_3$); R_f = 0.68 (EtOAc–PE, 3:7).

^{1}H NMR ($CDCl_3$): δ = 7.35–7.30 (m, 5 H, H-Ph), 5.37–5.34 (m, 1 H, CH), 5.21 (s, 2 H, OCH_2), 4.62–4.59 (m, 1 H, OCH_2), 4.25–4.23 (m, 1 H, OCH_2), 3.64 (s, 3 H, OCH_3), 1.46 (s, 18 H, 2 × *t*-Bu), 1.41 (s, 9 H, *t*-Bu).

^{13}C NMR ($CDCl_3$): δ = 168.5 (CO), 153.5, 151.1, 149.9, 135.2 (Cq), 128.6, 128.5, 128.4 (CH-Ph), 84.1, 83.8 (Cq-*t*-Bu), 75.1, 69.0 (OCH_2), 57.5 (CH), 52.6 (OCH_3), 28.2, 28.1 (*t*-Bu).

HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{27}H_{40}N_2NaO_{11}$: 591.2530; found: 591.2520.

Methyl (*S*)-3-{[Bis(*tert*-butoxycarbonyl)amino]oxy}-2-{2-[5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]acetamido}propanoate (1b)

To a soln of **15** (100 mg, 0.21 mmol) in MeOH (2 mL) was added 10% Pd/C (40 mg). H_2 was bubbled into the mixture for 10 min. The mixture was filtered and the filtrate was concentrated under reduced pressure. The resultant oily residue in CH_2Cl_2 (2 mL) was added to a mixture of **14** (55 mg, 0.29 mmol), EDC (57 mg, 0.29 mmol) and HOBt (41 mg, 0.29 mmol) in CH_2Cl_2 (8 mL) at r.t. After 18 h of stirring, the mixture was washed with sat. aq $NaHCO_3$ (2 × 10 mL) and brine (1 × 10 mL). The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography (EtOAc–PE, 4:6 to 6:4) to obtain **1b** as a white foam; yield: 59 mg (55%); mp 100 °C.

$[\alpha]_D^{27}$ +11.0 (*c* 0.5, $CHCl_3$); R_f = 0.43 (CH_2Cl_2–MeOH, 9:1).

^{1}H NMR ($CDCl_3$): δ = 9.00 (s, 1 H, NH), 7.86 (d, *J* = 7.8 Hz, 1 H, NH), 7.05 (s, 1 H, CH), 4.70–4.67 (m, 1 H, CH), 4.61 (dd, *J* = 2.8, 9.6 Hz, 1 H, OCH_2), 4.48 (s, 2 H, NCH_2), 3.97 (dd, *J* = 3.7, 10.1 Hz, 1 H, OCH_2), 3.73 (s, 3 H, OCH_3), 1.88 (d, *J* = 0.9 Hz, 3 H, CH_3), 1.49 (s, 18 H, 2 × *t*-Bu).

^{13}C NMR ($CDCl_3$): δ = 169.2, 166.8, 164.2, 151.0, 150.4 (Cq), 140.6 (CH-Ar), 111.2, 85.1 (Cq), 75.6 (OCH_2), 53.0 (OCH_3), 51.9 (CH), 49.8 (NCH_2), 28.1 (*t*-Bu), 12.4 (CH_3).

HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{21}H_{32}N_4NaO_{10}$: 523.2016; found: 523.2007.

Methyl (*S*)-2-{2-[4-{[(Benzyloxy)carbonyl]amino}-2-oxopyrimidin-1(2*H*)-yl]acetamido}-3-{[bis(*tert*-butoxycarbonyl)amino]oxy}propanoate (2b)

Compound **15** (676 mg, 1.44 mmol) was hydrogenolyzed in the presence of 10% Pd/C (270 mg). The resultant oily residue in CH_2Cl_2 (10 mL) was added to a mixture of **9** (623 mg, 2.06 mmol), EDC (600 mg, 2.02 mmol) and HOBt (278 mg, 2.06 mmol) in CH_2Cl_2 (70 mL) at r.t. After 18 h of stirring, the mixture was washed with sat. aq $NaHCO_3$ (2 × 50 mL) and brine (1 × 50 mL). The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography (EtOAc–PE, 4:6 to 6:4) to obtain **2b** as a white solid; yield: 587 mg (65%); mp 97 °C.

$[\alpha]_D^{27}$ +4.1 (*c* 0.5, $CHCl_3$); R_f = 0.33 (EtOAc).

^{1}H NMR ($CDCl_3$): δ = 8.16 (s, 1 H, NH), 7.94 (d, *J* = 7.3 Hz, 1 H, NH), 7.67 (d, *J* = 7.4 Hz, 1 H, CH), 7.32–7.28 (m, 5 H, H-Ph), 7.20 (d, *J* = 7.4 Hz, 1 H, CH), 5.14 (s, 2 H, OCH_2), 4.68–4.65 (m, 1 H, CH), 4.63 (s, 2 H, NCH_2), 4.52 (dd, *J* = 3.2, 10.1 Hz, 1 H, OCH_2), 3.98 (dd, *J* = 3.7, 10.1 Hz, 1 H, OCH_2), 3.62 (s, 3 H, OCH_3), 1.40 (s, 18 H, 2 × *t*-Bu).

^{13}C NMR ($CDCl_3$): δ = 169.3, 168.0, 166.8, 162.9, 155.9, 152.4, 150.2 (Cq), 149.6 (CH), 135.2 (Cq), 128.7, 128.6, 128.3, 95.3 (CH), 84.8 (Cq-*t*-Bu), 75.4, 67.9 (OCH_2), 52.9 (OCH_3), 52.0 (CH), 50.8 (NCH_2), 28.0 (*t*-Bu).

HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{28}H_{38}N_5O_{11}$: 620.2568; found: 620.2561.

(*S*)-3-(Aminooxy)-2-{2-[5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]acetamido}propanoic Acid (1c)

To a soln of **1b** (46 mg, 0.092 mmol) in MeOH (2 mL) was added AcCl (200 μL, 2.88 mmol) at 0 °C. After 4 h of stirring, the mixture was concentrated under reduced pressure. The residue was dissolved in THF (1.5 mL) and H_2O (1.5 mL). LiOH (6.6 mg, 0.28 mmol) was added to the solution at r.t. After an overnight stirring, the mixture was neutralized with H^+ resin (Dowex) and concentrated under reduced pressure to give **1c** as a white solid; yield: 26 mg (100%); mp 124 °C.

$[\alpha]_D^{27}$ +3.4 (*c* 0.5, MeOH).

IR (KBr): 3481.6, 3329.1, 2976.3, 1724.2, 1670.0, 1625.5, 1618.3, 1557.3 cm^{-1}.

^{1}H NMR (DMSO-d_6): δ = 11.29 (s, 1 H, OH), 8.80 (d, *J* = 7.3 Hz, 1 H, NH), 7.43 (s, 1 H, CH), 4.77–4.70 (m, 1 H, CH), 4.51 (m, 1 H, OCH_2), 4.38–4.28 (m, 2 H, NCH_2), 3.91–3.86 (m, 1 H, OCH_2), 1.73 (s, 3 H, CH_3).

^{13}C NMR (DMSO-d_6): δ = 168.0, 165.0, 151.5 (Cq), 142.8 (CH), 108.5 (Cq), 73.0 (OCH_2), 51.8 (CH), 49.6 (NCH_2), 12.5 (CH_3).

HRMS (ESI): *m*/*z* $[M - H_2O + Na]^+$ calcd for $C_{10}H_{12}N_4NaO_5$: 291.0705; found: 291.0701.

(*S*)-3-(Aminooxy)-2-{2-[4-{[(benzyloxy)carbonyl]amino}-2-oxopyrimidin-1(2*H*)-yl]acetamido}propanoic Acid (2c)

To a soln of **2b** (49 mg, 0.079 mmol) in MeOH (2 mL) was added AcCl (200 μL, 2.88 mmol) at 0 °C. After 4 h of stirring, the mixture was concentrated under reduced pressure. The residue was dissolved in THF (1.5 mL) and H_2O (1.5 mL). LiOH (6.6 mg, 0.28 mmol) was added to the solution at r.t. After an overnight stirring, the mixture was neutralized with H^+ resin (Dowex) and concentrated under reduced pressure to give **2c** as a white solid; yield: 31 mg (97%); mp 113 °C.

$[\alpha]_D^{27}$ –6.8 (*c* 0.5, DMSO).

IR (KBr): 3403.3, 3319.1, 1734.4, 1709.2, 1666.0, 1644.7, 1606.9 cm^{-1}.

^{1}H NMR (DMSO-d_6): δ = 11.28 (s, 1 H, OH), 9.82 (s, 1 H, NH), 8.88 (d, *J* = 7.8 Hz, 1 H, NH), 7.96 (d, *J* = 7.8 Hz, 1 H, CH), 7.38–7.30 (m, 5 H, H-Ph), 6.95 (d, *J* = 7.8 Hz, 1 H, CH), 5.14 (s, 2 H, OCH_2), 4.74–4.68 (m, 1 H, CH), 4.55–4.44 (m, 3 H, NCH_2 + OCH_2), 3.90–3.86 (m, 1 H, OCH_2).

^{13}C NMR (DMSO-d_6): δ = 167.8, 163.7, 160.5, 155.5, 153.7 (Cq), 151.5 (CH), 136.5 (Cq), 129.0, 128.7, 128.5, 92.3 (CH), 73.0, 67.0 (OCH_2), 51.9 (CH), 51.6 (NCH_2).

HRMS (ESI): *m/z* $[M - H_2O + H]^+$ calcd for $C_{17}H_{18}N_5O_6$: 388.1257; found: 388.1250.

Methyl (*S*)-2-(2-Chloroacetamido)-3-[(1,3-dioxoisoindolin-2-yl)oxy]propanoate (17)

To a soln of **8** (209 mg, 0.70 mmol) in THF (10 mL) were added Et_3N (196 μL, 1.40 mmol) and chloroacetyl chloride (83 μL, 1.05 mmol) at 0 °C. After 17 h of stirring at r.t., the reaction mixture was washed with brine (2 × 10 mL). The aqueous layer was extracted with EtOAc (2 × 10 mL). The combined organic layers were dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography (EtOAc–PE, 2:8 to 6:4) to give **17** as a white solid; yield: 126 mg (53%); mp 138 °C.

$[\alpha]_D^{27}$ +62.5 (*c* 0.5, $CHCl_3$); R_f = 0.37 (EtOAc–PE, 1:1).

^{1}H NMR ($CDCl_3$): δ = 7.98 (d, *J* = 5.0 Hz, 1 H, NH), 7.88–7.83 (m, 2 H, H-Phth), 7.80–7.77 (m, 2 H, H-Phth), 4.89–4.87 (m, 1 H, CH), 4.89–4.87 (m, 1 H, OCH_2), 4.42 (dd, *J* = 4.6, 11.9 Hz, 1 H, OCH_2), 4.18 (s, 2 H, CH_2Cl), 3.74 (s, 3 H, OCH_3).

^{13}C NMR ($CDCl_3$): δ = 168.9, 166.6, 163.4 (CO), 135.0 (CH-Phth), 128.7 (Cq), 123.9 (CH-Phth), 77.1 (OCH_2), 53.2 (OCH_3), 52.0 (CH), 42.5 (CH_2Cl).

HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{14}H_{14}ClN_2O_6$: 341.0540; found: 341.0535.

Methyl 2-(2-Azidoacetamido)acrylate (18)

To a soln of **17** (211 mg, 0.62 mmol) in DMF (3 mL) was added NaN_3 (61 mg, 0.93 mmol) at 0 °C. After 150 min at 0 °C, EtOAc (20 mL) was added to the mixture and the resultant solution was washed with brine (2 × 15 mL). The aqueous layer was extracted with EtOAc (1 × 20 mL). The combined organic layers were dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography (EtOAc–PE, 2:8 to 3:7) to give **18** as a colorless oil; yield: 155 mg (100%).

R_f = 0.47 (EtOAc–PE, 3:7).

^{1}H NMR ($CDCl_3$): δ = 8.47 (s, 1 H, NH), 6.54 (s, 1 H, =CH), 5.87 (s, 1 H, =CH), 4.04 (s, 2 H, CH_2N_3), 3.78 (s, 3 H, OCH_3).

^{13}C NMR ($CDCl_3$): δ = 165.5, 164.1 (CO), 130.5 (Cq), 109.8 (=CH_2), 53.1 (OCH_3), 52.9 (CH_2N_3).

Methyl (*S*)-2-(2-Azidoacetamido)-3-[(1,3-dioxoisoindolin-2-yl)oxy]propanoate (19)

To a soln of 2-azidoacetic acid (111 mg, 1.0 mmol) in CH_2Cl_2 (5 mL) were added EDC (83 mg, 0.43 mmol), HOBt (58 mg, 0.43 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (68 mg, 0.33 mmol) at r.t. After 10 min, compound **8** (100 mg, 0.33 mmol) was added. After 17 h of stirring, the reaction mixture was washed with sat. aq $NaHCO_3$ (1 × 10 mL) and brine (1 × 10 mL). The organic layer was dried over $MgSO_4$ and concentrated. The resulting residue was purified by column chromatography (EtOAc–PE, 5:5 to 10:0) to give **19** as a white solid; yield: 110 mg (95%); mp 158 °C.

$[\alpha]_D^{27}$ +45.9 (*c* 0.5, $CHCl_3$); R_f = 0.38 (EtOAc–PE, 1:1).

^{1}H NMR ($CDCl_3$): δ = 7.85–7.83 (m, 2 H, H-Phth), 7.79–7.75 (m, 2 H, H-Phth), 4.90–4.88 (m, 2 H, OCH_2), 4.39–4.36 (m, 1 H, CH), 4.10 (s, 2 H, CH_2N_3), 3.73 (s, 3 H, OCH_3).

^{13}C NMR ($CDCl_3$): δ = 169.0, 167.3, 163.4 (CO), 135.0 (CH-Phth), 128.6 (Cq), 123.9 (CH-Phth), 77.3 (OCH_2), 53.1 (OCH_3), 52.7 (CH_2N_3), 51.6 (CH).

HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{14}H_{14}N_5O_6$: 348.0944; found: 348.0939.

Benzyl [2-Oxo-1-(prop-2-yn-1-yl)-1,2-dihydropyrimidin-4-yl]carbamate (21)

To a soln of benzyl (2-oxo-1,2-dihydropyrimidin-4-yl)carbamate[26] (**20**; 556 mg, 2.27 mmol) in MeCN (25 mL) was added 60% NaH (108 mg, 4.53 mmol) at 0 °C. Propargyl bromide (80% in toluene; 401 μL, 2.72 mmol) was then added. After 18 h of stirring at reflux, the reaction mixture was washed with brine (2 × 15 mL). The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography (EtOAc–PE, 5:5 to 8:2) to give **21** as a pale yellow solid; yield: 192 mg (30%); mp 154 °C.

R_f = 0.60 (EtOAc).

^{1}H NMR (CD_3OD): δ = 8.10 (d, *J* = 7.3 Hz, 1 H, =CH), 7.38–7.30 (m, 6 H, H-Ar), 5.19 (s, 2 H, OCH_2), 4.67 (s, 2 H, NCH_2), 2.96 (s, 1 H, ≡CH).

^{13}C NMR (CD_3OD): δ = 163.9, 156.4, 153.2 (Cq), 147.9 (CH), 135.8 (Cq), 128.3, 128.1, 128.0, 95.9 (CH), 75.1 (≡CH), 70.3 (≡Cq), 67.2 (OCH_2), 38.6 (NCH_2).

Methyl 2-[2-(4-{[5-Methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]methyl}-1*H*-1,2,3-triazol-1-yl)acetamido]acrylate (23)

To a soln of **19** (230 mg, 0.66 mmol) in DMF (3 mL) were added 5-methyl-1-(prop-2-yn-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione (**22**; 109 mg, 0.66 mmol), sodium ascorbate (66 mg, 0.33 mmol) and $CuSO_4 \cdot 5H_2O$ (41 mg, 0.17 mmol) at r.t. After an overnight stirring, EtOAc (50 mL) was added to the mixture. The solution was washed with sat. aq $NaHCO_3$ (1 × 30 mL) and brine (2 × 30 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to give **23** as a white solid; yield: 157 mg (68%); mp 150 °C.

R_f = 0.66 (CH_2Cl_2–MeOH, 9:1).

^{1}H NMR (DMSO-d_6): δ = 11.29 (s, 1 H, NH), 9.88 (s, 1 H, NH), 8.01 (s, 1 H, H-triazole), 7.60 (s, 1 H, =CH), 6.22 (s, 1 H, =CH), 5.75 (s, 1 H, =CH), 5.31 (s, 2 H, CH_2N), 4.87 (s, 2 H, CH_2N), 3.73 (s, 3 H, OCH_3), 1.71 (s, 3 H, CH_3).

^{13}C NMR (DMSO-d_6): δ = 165.9, 164.8, 164.0, 151.2, 142.9 (Cq), 141.7 (CH-Ar), 132.6 (Cq), 125.8, 110.9 (CH), 109.4 (Cq), 53.3 (OCH_3), 52.5, 42.7 (NCH_2), 12.5 (CH_3).

HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{14}H_{17}N_6O_5$: 349.1260; found: 349.1255.

Methyl (*S*)-3-[(1,3-Dioxoisoindolin-2-yl)oxy]-2-[2-(4-{[5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]methyl}-1*H*-1,2,3-triazol-1-yl)acetamido]propanoate (3)

To a soln of **19** (200 mg, 0.58 mmol) in DMF (4 mL) were added **22** (95 mg, 0.58 mmol), ascorbic acid (51 mg, 0.29 mmol) and $CuSO_4 \cdot 5H_2O$ (36 mg, 0.14 mmol) at r.t. After 18 h of stirring, EtOAc (40 mL) was added to the solution. The precipitate formed was filtered and the filtrate was washed with brine (2 × 20 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to give **3** as a white solid; yield: 255 mg (87%); mp 156 °C.

$[\alpha]_D^{27}$ +3.9 (*c* 0.5, DMSO); R_f = 0.14 (EtOAc).

^{1}H NMR (DMSO-d_6): δ = 11.31 (s, 1 H, NH), 9.08 (d, *J* = 7.8 Hz, 1 H, NH), 8.03 (s, 1 H, H-triazole), 7.85–7.79 (m, 4 H, H-Phth), 7.60 (d, *J* = 1.4 Hz, 1 H, CH), 5.23 (s, 2 H, CH_2N), 4.91 (s, 2 H, CH_2N), 4.79–4.77 (m, 1 H, CH), 4.51–4.42 (m, 2 H, OCH_2), 3.68 (s, 3 H, OCH_3), 1.74 (s, 3 H, CH_3).

^{13}C NMR (DMSO-d_6): δ = 169.6, 166.4, 164.8, 163.4, 162.8, 151.3, 142.8 (Cq), 141.7, 135.4 (CH), 129.1 (Cq), 125.6, 123.9 (CH), 109.4 (Cq), 76.8 (OCH_2), 53.0 (OCH_3), 52.2 (CH), 51.9, 42.6 (NCH_2), 12.5 (CH_3).

HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{22}H_{22}N_7O_8$: 512.1530; found: 512.1522.

Methyl (*S*)-2-(2-{4-[(4-{[(Benzyloxy)carbonyl]amino}-2-oxopyrimidin-1(2*H*)-yl)methyl]-1*H*-1,2,3-triazol-1-yl}acetamido)-3-[(1,3-dioxoisoindolin-2-yl)oxy]propanoate (4)

To a soln of **19** (50 mg, 0.14 mmol) in DMF (1 mL) were added **21** (41 mg, 0.14 mmol), ascorbic acid (13 mg, 0.07 mmol) and $CuSO_4 \cdot 5H_2O$ (9 mg, 0.035 mmol) at r.t. After 18 h of stirring, EtOAc (15 mL) was added to the solution. The precipitate formed was filtered and the filtrate was washed with brine (2 × 10 mL). The

organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to give **4** as a white solid; yield: 70 mg (77%); mp 126 °C.

$[\alpha]_D^{27}$ +0.9 (*c* 0.5, $CHCl_3$); R_f = 0.15 (EtOAc).

^{1}H NMR ($CDCl_3$): δ = 8.18 (s, 1 H, H-triazole), 7.99 (d, *J* = 7.8 Hz, 1 H, NH), 7.89 (d, *J* = 8.0 Hz, 1 H, CH), 7.73–7.70 (m, 2 H, H-Phth), 7.68–7.65 (m, 2 H, H-Phth), 7.31–7.28 (m, 5 H, H-Ph), 7.13 (d, *J* = 8.0 Hz, 1 H, CH), 5.32 (s, 2 H, CH_2N), 5.12 (s, 2 H, CH_2N), 5.10 (s, 2 H, OCH_2), 4.86–4.84 (m, 1 H, CH), 4.73–4.71 (m, 1 H, $NOCH_2$), 4.39–4.36 (m, 1 H, $NOCH_2$), 3.62 (s, 3 H, OCH_3).

^{13}C NMR ($CDCl_3$): δ = 169.1, 165.9, 163.3, 155.9, 152.5 (Cq), 148.8 (CH), 142.0 (Cq), 134.9, 128.7 (CH), 128.6 (Cq), 128.5, 128.3, 126.3, 123.9, 95.5 (CH), 77.3, 67.8 (OCH_2), 53.1 (CH), 52.5 (CH_2N), 51.9 (OCH_3), 45.3 (NCH_2).

HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{29}H_{27}N_8O_9$: 631.1901; found: 631.1891.

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