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AN IMPROVED SYNTHESIS OF 5-ACYLAMINO-6-OXO-2-PHENYL-1(6*H*)-PYRIMIDINEACETIC ACID FROM GLYCINE WITH READILY REMOVABLE PROTECTING GROUPS

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Abstract – Concise synthesis of *N*-acyl-5-amino-6-oxo-2-phenyl-1(6*H*)-yrimidineacetic acid was achieved by cyclization reaction of 2-alkyl-4-lkoxymethylene-5(4*H*)-oxazolone with *N*-(carboxymethyl)benzamidine, while a similar reaction with sodium salt of 2-alkyl-4-hydroxymethylene-5(4*H*)-xazolones gave a mixture of regioisomers of the pyrimidinone. *N*-Acyl groups (acetyl or phenylacetyl) of the pyrimidinone derivatives were readily cleaved under very mild conditions with weak base or enzyme. Thus, the process enabled us to synthesize the drug candidate without exchanging *N*-protecting group. Since the starting oxazolones were easily prepared from *N*-acylglycine, the synthetic route can be used for the large scale synthesis of the key intermediate for several enzyme inhibitors.

INTRODUCTION

5-Amino-6-oxo-2-phenyl-1(6*H*)-pyrimidineacetic acid **1** was designed by mimicking the Ala-Pro moiety of a known serine protease inhibitor along with 3-amino-2-oxo-1(2*H*)-pyridineacetic acid, and was used as a core structure for the development of orally active non-peptidic inhibitors of human leukocyte elastase and chymase. Typical drug candidates (**2**-**5**) that contain **1** as a core molecule are shown in Figure 1.[1-4]

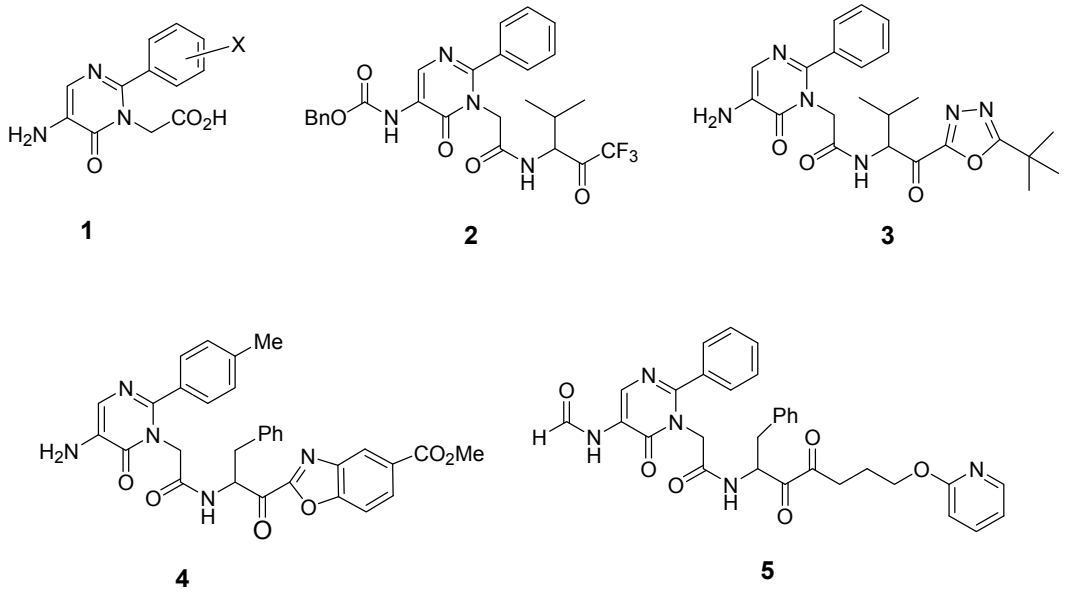
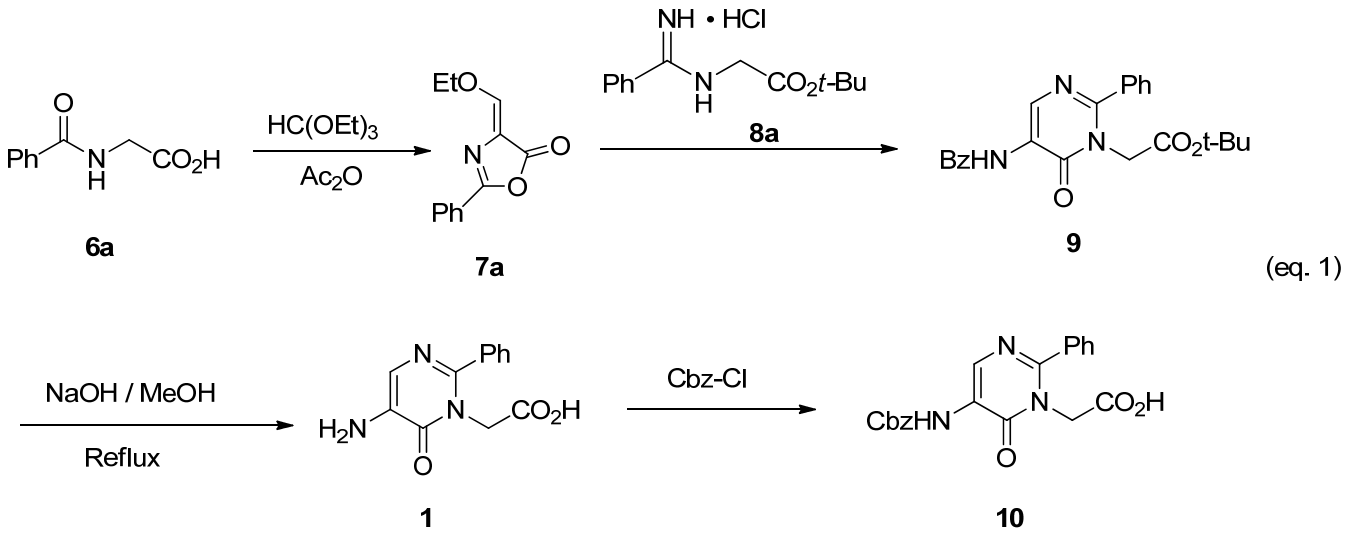


Figure 1. Serine protease inhibitors containing **1**

Several methods are available for the synthesis of pyrimidinone **1**.[5] To avoid the hazardous conditions and reagents used in the previous syntheses and to improve the economy and safety of the process, we have recently developed a new and efficient method (eq. 1) for synthesizing pyrimidinone **1** by using 2-phenyl-4-alkoxymethylene-5(4*H*)-oxazolone **7a**, which is readily prepared from *N*-benzoylglycine **6a**.[6]



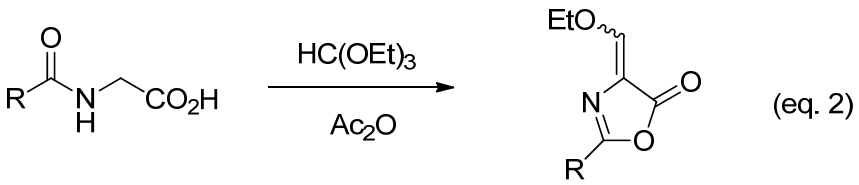
This new process eliminated hazardous reactions. However, there were several drawbacks in the synthesis of the enzyme inhibitors shown in Figure 1. For example, **1** is easily dimerized under the conditions for peptide condensation if the amino group is not protected. In addition, we could selectively cleave the *t*-butyl ester of **9** to make the peptide bond, but it is difficult to remove the *N*-Bz (benzoyl) group selectively under mild conditions. To avoid these drawbacks, it was necessary to re-protect the 5-amino

group with a Cbz (carbobenzyloxy) group for the following steps. Meanwhile, we found that an *N*-acetyl group was easily deprotected under remarkably mild reaction conditions. Based on these results, we decided to develop a new process to synthesize the pyrimidone intermediates efficiently.

RESULTS AND DISCUSSION

Synthetic approach to 1a *via N*-(benzyloxycarbonyl)-2-(hydromethylene)glycinate

To prepare the *N*-acyl (acetyl or phenacetyl) derivative of 5-amino-2-phenyl-4-pyrimidinone, it is necessary to obtain 2-alkyl(methyl or benzyl)-4-ethoxymethylene-5-oxazolone **7b** or **7c** in good yield. However, there are few reports on the synthesis of not only **7b** but also 2-alkyl-4(*H*)-5-oxazolone itself.[7] It has also been reported that the conventional Erlenmeyer method using *N*-acylglycine and acetic anhydride gave the desired products in very poor yield.[8a] To the best of our knowledge, there have been no reports on the direct synthesis of **7b** and **7c** from *N*-acylglycine, although there have been reports on the synthesis of **7a** from hippuric acid (*N*-benzoylglycine).[8]



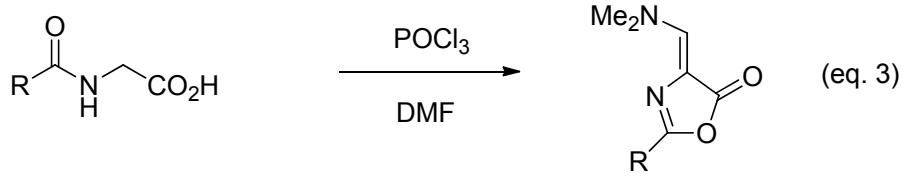
(eq. 2)

6a; R=Ph
6b; R=Me
6c; R=CH_2Ph

7a; R=Ph
7b; R=Me
7c; R=CH_2Ph

Thus, we re-examined the synthesis of **7b** and **7c** by the Erlenmeyer method and confirmed that the HPLC yields for **7b** (25%, R=methyl) and **7c** (33%, R=benzyl) were much less than that (>70%) for **7a**. Inspired by suggestions in the literature,[9] we tested the effect of additives on the reaction yield. As a result, we found that the HPLC yield was improved to 52% for **7b** and 56% for **7c** when we used a catalytic amount (0.1 equiv.) of $ZnCl_2$/NaOAc as an additive. Though the reaction yield was slightly improved, there were still problems such as the formation of byproducts, difficult purification, and silica gel column chromatography was required.

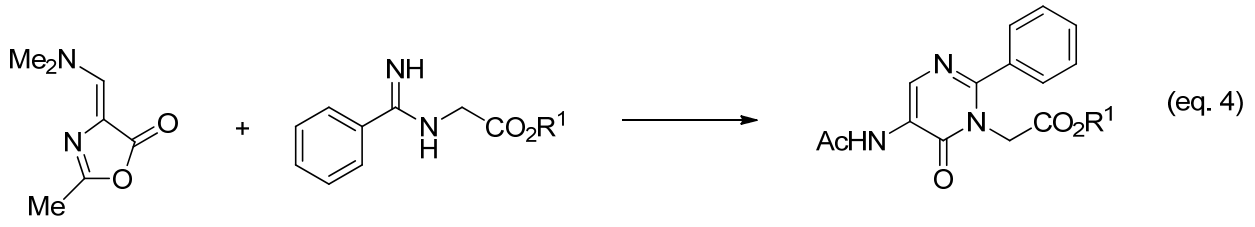
Next, we tried to find an alternative way to obtain the 2-alkyl 4-oxymethylene-5-oxazolone in good yield. Stanovnik and co-workers reported that 4-[(*N,N*-dimethylamino)methylene]oxazolone **11b** was prepared from *N*-acetylglycine **6b** in yields of 51% and 55% by treatment with $POCl_3$/DMF and DCC/DMF-DMA, respectively.[10] According to this method, we could obtain **11b** and **11c** as crystalline solids in isolated yields of 76% and 73%, respectively.



6b; R=Me
6c; R=CH_2Ph

11b; R=Me (76%)
11c; R=CH_2Ph (73%)

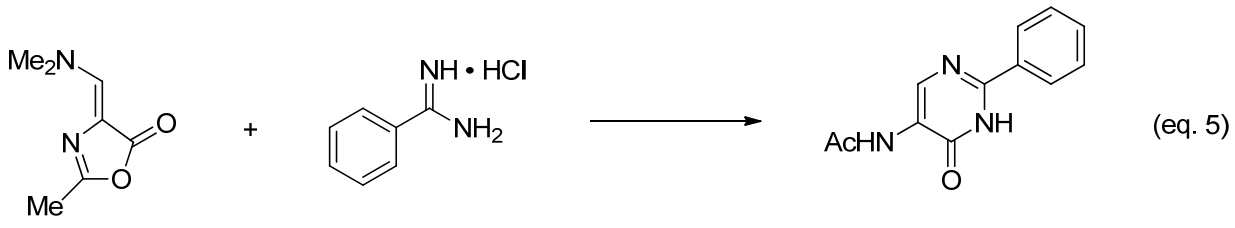
Singh and Singh reported the addition-elimination reaction of **11b** to replace the *N,N*-dimethylamino group at the β-position of the *exo*-methylene bond with a monoalkylamine.[11] With this in mind, we examined the reaction of **11b** with *N*-*tert*-butoxycarbonylmethylbenzamidine **8a**. However, the reaction did not take place in toluene at reflux temperature to give the pyrimidinone **12b**, and instead **11b** was recovered almost quantitatively. When we used *N*-carboxymethylbenzamidine (**8b**) in DMF as a solvent, the desired cyclized product **13b** was obtained in 32% yield at 130 °C after 12 h. We also changed the β-substituent of the *exo*-olefin from a *N,N*-dimethylamino- to a morpholine- or piperidino-group, but the yield was not improved. When we used unsubstituted benzamidine **14,** we obtained the cyclization product **15** in modest yield (38%).



11b

8a; (R^1=*t*-Bu) • HCl
8b; (R^1=H)

12b; no reaction (R^1=*t*-Bu)
13b; 32% (R^1=H)



11b

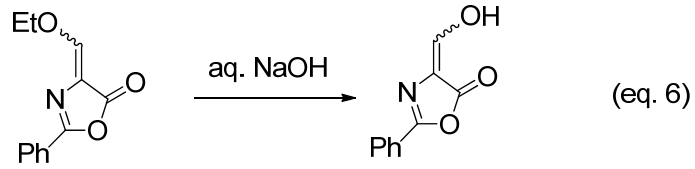
14

15

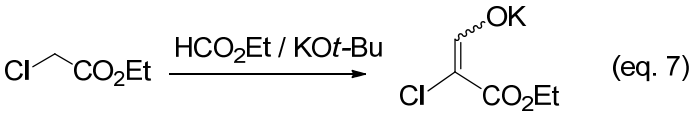
Based on these results, it seemed that the major reason for the low yield of the desired reaction might be the lower reactivity of not only *N*-substituted benzamidines **8a** and **8b** but also the dimethylamino group of the *exo*-methylene bond of the oxazolone **11b**.

In a previous paper,[6] we reported that the reactivity of *tert*-butoxycarbonylmethylbenzamidine hydrochloride (**8a**) toward methyl 2-oxymethyleneglycinate was strongly influenced by the β-substituent.

Thus, we became interested in the conversion of **11b** to its hydroxyl- or methoxy-derivative. Benoiton *et al.* obtained 2-phenyl-4-hydroxymethylene-5-oxazolone (**16**) in 88% yield from the ethoxy compound **7a** by treatment with 1 equivalent of NaOH in water without decomposition of the oxazolone ring.[12] Furthermore, Ikemoto *et al.* synthesized (α-hydroxymethylene)chloroacetate from ethyl chloroacetate (**17**) and isolated **18** as a stable potassium salt.[13]

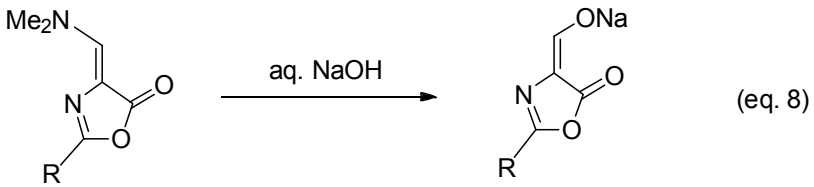


7a **16**



17 **18**

Encouraged by these results, we carried out the hydrolysis of **11c** under basic conditions, and found that the 2-benzyl-4-hydroxymethylene-5-oxazolene was obtained as a sodium salt (**19c**) as a mixture of *E/Z* isomer (80:20 from ^{1}H-NMR) in 92% yield after precipitation from ethyl acetate. We also obtained the 2-methyl derivative **19b** in a similar manner (94%) after precipitation from acetonitrile. **19b** was also a mixture of *E/Z* isomer (80:20 from 1H-NMR). The ring-opening reaction of the oxazolone was observed when ethanol was used as a solvent for hydrolysis.[14]



11a; R=Ph
11b; R=Me
11c; R=Bn

19b; R=Me (94%)
19c; R=Bn (92%)

To clarify the molecular structure of **19c**, we obtained single crystals from methanol as a methanol solvate, and performed X-ray crystallographic analysis. Interestingly, **19c** was found to exist as a dicarbonyl form coordinated with sodium, as shown in Figure 2. It is well known that **11a** exists as the *Z*-isomer.[15] However, the stable form of the corresponding monoalkylamine analog of **11a** was revealed to be the *E*-isomer based on an NMR study (inverse gated decoupling technique of *exo*-olefinic H and carbonyl C)

by Norton Matos *et al*.[8b] It was assumed that an intermolecular hydrogen-bonding interaction may contribute to this stabilization. Coordination of oxygen atom to the sodium ion is reasonably presumed to stabilize the *E*-isomer (**19c**) in our case.

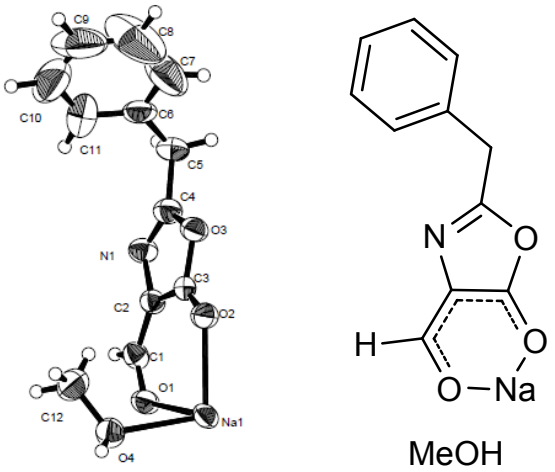
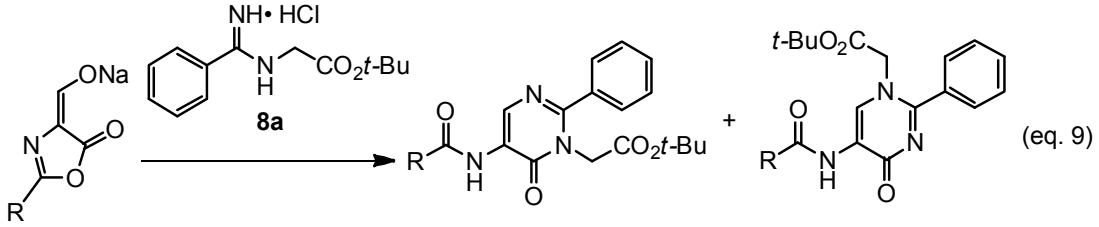


Figure 2

The ORTEP drawing of crystal structure of **19c**. One methanol molecule is included in the crystal, and carbon and oxygen atoms of it are represented C12 and O4, respectively. The compound crystallized in the space group *C*2/*c* with unit cell dimensions of a = 25.67(3) Å, b = 6.276(8) Å, c = 15.52(3) Å, and b = 99.78(8)°. The diffraction data was collected to a maximum $sin\theta / \lambda$ value of 0.694, using the fully automatic data-collection system[16] installed on the beam line 6C at the Photon Factory of the National Laboratory for High Energy Physics, Tsukuba, Japan. The crystal structure was determined by direct methods and refined by full-matrix least-squares to *R1* = 6.4% and *GOF* = 1.06.

We further transformed sodium salts **19b** and **19c** into their corresponding β-methoxy, β-TMSO, β-acetoxy as well as β-benzyloxy derivatives in order to compare the relative reactivity in this series. First, we examined the cyclization reaction of 2-alkyl-4-hydroxymethylene-5-oxazolone with benzamidine derivatives. Thus, the sodium salts (**19a-c**) were reacted with *tert*-butoxycarbonylmethyl-benzamidine hydrochloride (**8a**) in acetonitrile at 80 °C for 18 h. Surprisingly, the desired reaction took place to give the cyclization products **12a-c** along with the formation of a substantial amount of the isomers **20a-c** in each case, though the conversions from **19a-c** were relatively high (eq. 9). Attempts to improve the selectivity by protecting the OH group by acetylation or silylation were not very effective, though the cyclization yield including the isomer was increased to 95% in the case of TMS protection of **19c**.

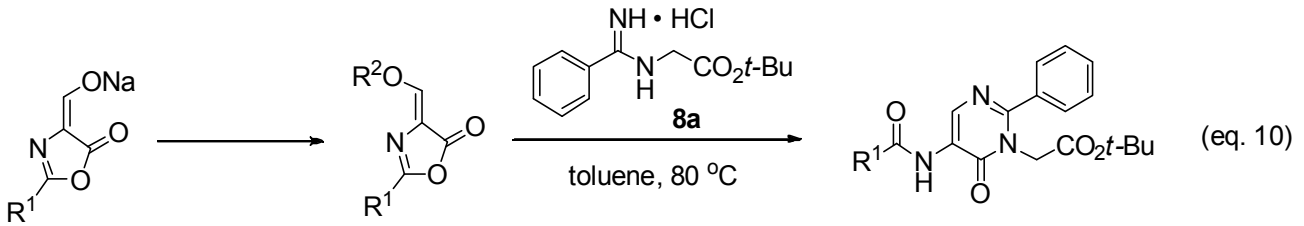


19a; R=Ph
19b; R=Me
19c; R=Bn

12a; R=Ph (47%)
12b; R=Me (28%)
12c; R=Bn (44%)

20a; R=Ph (29%)
20b; R=Me (40%)
20c; R=Bn (41%)

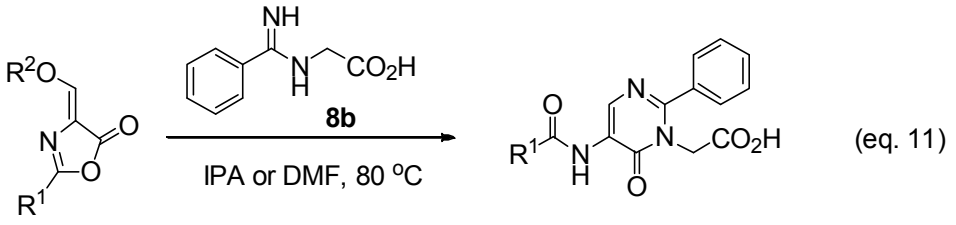
Next, we tried to prepare the alkoxymethyleneoxazolone derivatives **7d-f** by methylation, and benzylation. Interestingly, the products mainly exist in the *Z*-form probably due to very facile isomerization. We then examined the cyclization reactions of **7d-f** with **8a** after the free form was created by treatment with a base. The reaction proceeded very smoothly in toluene at 80 °C to give **12b** and **12c** in very high yields with less than 3% of **20b-c**. When we used **8b** as an amidine derivative, the desired products were also obtained, though the yields were slightly lower than in the case of **8a** and the reactions required a polar solvent such as isopropyl alcohol or DMF to dissolve **8b**.



19b; R^1=Me
19c; R^1=Bn

7d; R^1=Me, R^2=Me
7e; R^1=Me, R^2=Bn
7f; R^1=Bn, R^2=Me

12b; R^1=Me (85%)
12b; R^1=Me (87%)
12c; R^1=Bn (69%)

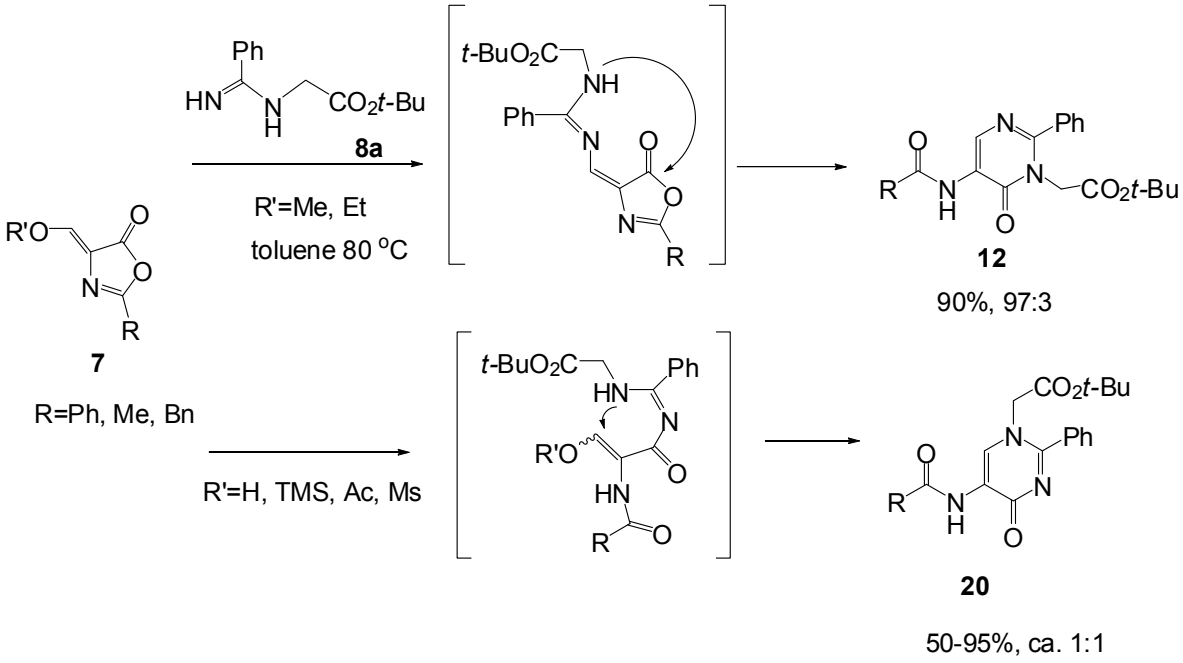


7d; R^1=Me, R^2=Me
7e; R^1=Me, R^2=Bn
7f; R^1=Bn, R^2=Me

13b; R^1=Me (78%)
13b; R^1=Me (76%)
13c; R^1=Bn (48%)

A possible reaction mechanism is shown in Scheme 1. It is well known that the alkoxy group in **7a** can be easily replaced by an amine via addition-elimination at the β-position. In a similar fashion, an imino

group of the amidine will add to the β-position of the *exo*-olefin bond of **7** to give an adduct, which is followed by a cyclization reaction to selectively give the pyrimidinone **12**. In contrast, the *N*-1 isomer should be obtained if the imino nitrogen attacks the carbonyl group of oxazolone. Although the reason is not clear at this moment, the proportion of the undesired isomer with β-OH and β-OAc is greater than that with β-OMe.



Scheme 1. Possible reaction pathways

This can likely be best understood in the context of why other compounds (R' = OH, mesylate, acetate, TMS) give some of the undesired compound **20**. The hydroxymethylene compound may provide the other product because of a possible intramolecular H-bonded enol structure as shown below. This should activate the lactone carbonyl group, which results in an initial competing attack at the oxazolone carbonyl residue to give the undesired product (**20**).

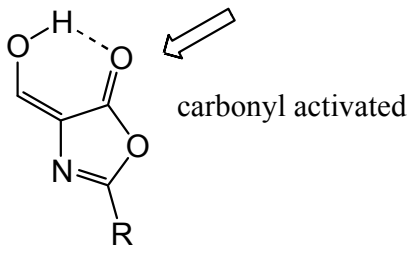


Figure 3. Activation of carbonyl group via hydrogen bonding

The other compounds (mesylate, acetate and silyl) that also give undesired isomers are likely to have an electron-withdrawing effect through the sigma bond framework of the enol ether system and also activate the carbonyl for attack. This electron-withdrawing effect is at odds with the resonance effect as shown for the alkoxy substrates shown below. The alkoxy substrates (R = Me, Et) likely exist primarily as the *Z*-isomer and do not activate the carbonyl for nucleophilic attack; rather, these electron-donating groups reduce the electrophilicity of the lactone carbonyl through the aromatic resonance form shown below. This is very likely a delicate balance of electron-donating effects as shown below and electron-withdrawing effects and H-bonded activation of the carbonyl group that bifurcates the two reaction pathways.

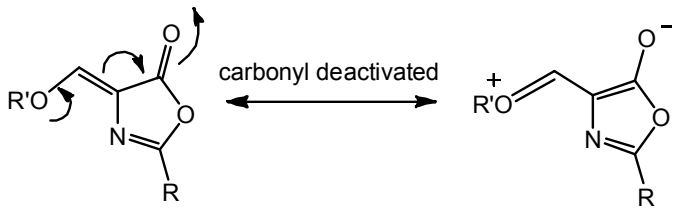


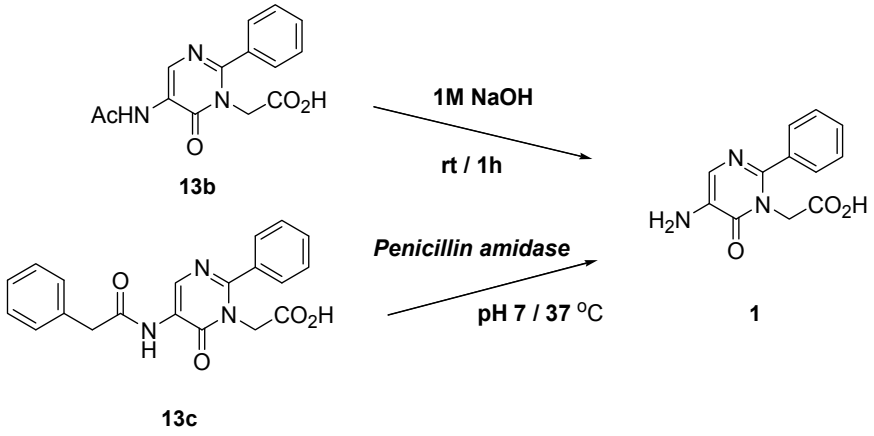
Figure 4. Carbonyl deactivation by resonance

Deprotection

Deprotection of the *N*-benzoyl group of **9** required severe conditions such as NaOH in methanol at reflux-temperature for 24 h. Thus, we examined the effect of the substituent on the benzene ring on hydrolysis. However, no dramatic changes were observed with *p*-MeO-, *p*-Me, and *p*-Cl substitution, though the pyrimidine derivatives were readily prepared via their corresponding oxazolones.

Next, we tried to deprotect the *N*-acetyl group of **13b**, which was found to be easily hydrolyzed to give **1** under extremely mild conditions, such as by exposure to 1M aqueous NaOH solution at room temperature for 1 h. We then further investigated hydrolysis under milder conditions using an enzyme. Although we examined several commercially available enzymes to hydrolyze the *N*-acyl group, only one, penicillin amidase, was effective for deprotecting the *N*-phenylacetyl group of the pyrimidinone **13c** under very mild conditions.

In summary, *t*-butyl ester of the pyrimidinone **9** is easily deprotected under acidic conditions while deprotection of the *N*-benzoyl group required more severe conditions such as refluxing in MeOH with NaOH. However, it was found that the *N*-acetyl group of (**13b**) was readily cleaved under very mild conditions, such as at ambient temperature for 1 h in 1M aqueous NaOH solution. In the case of **13c**, the *N*-phenylacetyl group was deprotected enzymatically (penicillin amidase) under neutral conditions at pH 7 in almost quantitative yield.



Scheme 2. Hydrolysis of the *N*-acyl group

This methodology might be applicable to the late-stage deprotection of drug candidates, since they contain an amino acid moiety and labile acyl residues and therefore it should be possible to perform deprotection on other substrates under very mild conditions. In the present methodology, it is not necessary to exchange the initial protecting group, rendering the overall process highly efficient.

CONCLUSION

We have successfully synthesized 2-alkyl-4-hydroxymethylene-oxazolone sodium salts that were difficult to prepare by the conventional Erlenmeyer method. Alkylation of the hydroxyl group of the *exo*-olefin bond leads to the desired product by minimizing formation of the undesired regioisomers. The pyrimidine thus obtained was confirmed to be readily deprotected under very mild hydrolytic conditions and therefore this process could be further applied to the synthesis of several drug candidates and enzyme inhibitors.

EXPERIMENTAL

All reagents were purchased and used without further purification. Thin-layer chromatography (TLC) was conducted on precoated TLC plates (Merck 60F250). High-performance liquid chromatography (HPLC) was performed with a Hitachi L-6000 pump and L-4000 UV detector system using an Inertsil ODS-2 column. Melting points were measured with a Büchi B-545 or a Yanaco melting point apparatus MP model and are uncorrected. NMR spectra were obtained on a Varian XL-300 spectrometer. All proton NMR spectra were measured in $CDCl_3$ or DMSO-d_6 solvent, and chemical shifts are reported as δ values in parts per million relative to tetramethylsilane (δ 0.00) or $CDCl_3$ (δ 7.26) as an internal standard. Data are reported as follows: chemical shift (integrated intensity or assignment, multiplicity, coupling constants in hertz, assignment). All carbon NMR spectra were measured in $CDCl_3$ or DMSO-d_6 solvent, and chemical shifts are reported as δ values in parts per million relative $CDCl_3$ (δ 77.0) or DMSO-d_6 (δ 39.5)

as an internal standard. Infrared (IR) spectra were recorded on a SHIMAZU IR Prestige-21 Fourier transform infrared spectrophotometer Smith Dura Sample IR II and are reported in wave number (cm^{-1}). Mass spectra (MS) were obtained with a ThermoQuest TSQ700 a JEOL JMS-HX110 instrument with ESI (electrospray) or FAB (fast atom bombardment) ionization. High-resolution mass spectra (HRMS) were obtained with a JEOL MS700V by JEOL datum Ltd.

2-Alkyl(methyl or benzyl)-4-ethoxymethylene-5-oxazole-1-one (7b, 7c)

Compounds **7b** and **7c** were prepared according to the method reported by Singh[11] except for the use of additives. Analytical data of **7b** and **7c** were fully consistent with those reported.

4-Dimethylaminomethylene-2-alkyl-5-oxazole-1-one (11b, 11c)

Compounds **11b** and **11c** were prepared according to the method reported by Stanovnik[10] after some modifications. Analytical data of **11b** and **11c** were fully consistent with those reported.

5-Acetylamino-6-oxo-2-phenyl-1(6H)-pyrimidineacetic acid (13b)

To a solution of *N*-(carboxymethyl)benzamidine **8b** (1.26 g, 7.09 mmol) and 21% of NaOEt/EtOH (2.23 g, 7.09 mmol) in DMF (15 mL) was added oxazolone **11b** (1.09 g, 7.09 mmol) and the mixture was stirred at 130 °C for 12 h. The reaction mixture was concentrated and EtOAc (15 mL) and water (10 mL) were added. The aqueous layer was acidified with hydrochloric acid and extracted with EtOAc. To the organic layer was added hexane and the precipitate was collected and dried under reduced pressure to give **13b** (651 mg, 31%) as pale yellow solid.

Mp 216-218 °C, IR (neat): 1732, 1680, 1523, 1365, 1246, 1102, 772, 714, 601 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 2.15 (3H, s), 4.52 (2H, s), 7.48-7.56 (5H, m), 8.83 (1H, s), 9.58 (1H, s). ^{13}C-NMR (DMSO-d_6) δ 24.0, 48.4, 125.4, 128.4, 129.0, 130.5, 134.4, 137.6, 153.9, 157.5, 169.1, 170.0. MS (ESI) m/z $[M+H]^+$ 288, $[M-H]^+$ 286. Anal. Calcd for $C_{14}H_{13}N_3O_4$: C, 58.53; H, 4.56; N, 14.63. Found: C, 58.26; H, 4.48; N, 14.50.

5-Acetylamino-2-phenyl-4(3H)-pyrimidinone (15)

To a solution of oxazolone **11b** (500 mg, 3.24 mmol) in EtOH (7 mL) were added benzamidine hydrochloride **14** (560 mg, 3.58 mmol) and Et_3N (0.5 mL) and stirred at 70 °C for 16 h. The reaction mixture was concentrated and purified by silica gel chromatography (hexane/EtOAc) to give **15** in 38% yield (282 mg, 1.23 mmol).

Mp 281-281.5 °C. IR (neat): 1636, 1603, 1533, 1505, 1372, 1310, 1258, 1071, 957, 882, 764, 696, 671, 658, 617, 596, 576, 530 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 2.15 (3H, s), 7.50-7.58 (3H, m), 8.06-8.09 (2H, m),

8.85 (1H, s), 9.50 (1H, s), 13.0 (1H, brs). ^{13}C-NMR (DMSO-d_6) δ 23.6, 125.2, 127.2, 128.6, 131.1, 132.0, 169.5, 150.5, 150.6, 169.5. MS (ESI) m/z $[M+H]^-$ 228. HRMS: Calcd for $C_{12}H_{11}N_3O_2$; m/z $[M+H]^+$ 230.0929. Found: 230.0918.

Sodium salt of 4-hydroxymethylene-2-methyl-5-oxazol-1-one (19b)

To a solution of **11b** (4.0 g, 26.0 mmol) in MeCN (50 mL) was added 2M NaOH (15 mL) in an ice bath and the mixture was stirred at room temperature for 14 h. The mixture was evaporated to dryness. To the residue was added acetonitrile (5 mL), and the mixture was stirred at 60 °C for 1 h. The precipitates were collected by filtration, washed with acetonitrile, and dried under reduced pressure at 80 °C to give **19b** (3.65 g, 94%) as a crystalline solid.

Mp 251 °C, IR (neat): 1714, 1641, 1067, 904, 763, 630, 505, 470 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 2.00 (3H, s), 8.67 (0.8H, s), 8.90 (0.2H, s). ^{13}C-NMR (D_2O) δ 13.8, 111.6, 154.3, 173.8, 174.5. MS (APCI) m/z $[M+H]^+$ 126. Anal. Calcd for $C_5H_4NO_3Na$: C, 40.28; H, 2.70; N, 9.40. Found: C, 40.31; H, 2.42; N; 9.15.

Sodium salt of 4-hydroxymethylene-2-benzyl-5-oxazol-1-one (19c)

92% (crystallized from EtOAc)

Mp 235 °C, IR (neat): 1710, 1620, 1085, 916, 781, 696, 597, 491 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 3.66 (2H, s), 7.22-7.32 (5H, m), 8.71 (0.8H, s), 8.96 (0.2H, s). ^{13}C-NMR (D_2O) δ : 34.8, 111.5, 127.7, 129.2, 129.3, 135.4, 155.1, 173.6, 175.2. MS (ESI) m/z $[M-H]^+$ 202. Anal. Calcd for $C_{11}H_8NO_3Na$: C, 58.67; H, 3.58; N, 6.22. Found: C, 58.55; H. 3.34; N, 6.09.

General procedure for the reaction of oxazolone sodium salt 19 with amidine 8a.

To a suspension of *N*-(*tert*-butoxycarbonylmethyl)benzamidine hydrochloride **8a** (120 mg, 0.44 mmol) in MeCN (3 mL) was added oxazolone sodium salt **19** (1.0 eq.) and vigorously stirred at 80 °C for 17 h. After the reaction, the mixture was filtered and concentrated. The residue was purified by silica gel chromatography to give **12** and **20**.

5-Benzoylamino-6-oxo-2-phenyl-1(6H)-pyrimidineacetic acid tert-butyl ester (12a)

Mp 189-190 °C, IR: 1734, 1649, 1512, 1485, 1365, 1238, 1149, 771, 698, 597 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 1.31 (9H, s), 4.57 (2H, s), 7.51-7.65 (8H, m), 797-8.00 (2H, m), 8.79 (1H, s), 9.58 (1H, s). ^{13}C-NMR ($CDCl_3$) δ 27.9, 49.0, 83.3, 125.2, 125.3, 127.3, 128.2, 128.9, 128.9, 129.0, 130.5, 132.3, 133.7, 153.4, 158.4, 165.6, 166.2. MS (ESI) m/z $[M+H]^+$ 406, Anal. Calcd for $C_{23}H_{23}N_3O_4$: C, 68.13; H, 5.72; N, 10.36. Found: C, 68.20; H, 5.65; N, 10.30.

5-Benzoylamino-4-oxo-2-phenyl-1(4H)-pyrimidineacetic acid tert-butyl ester (20a)

IR: 1731, 1633, 1516, 1610, 1458, 1240, 1149, 700, 686 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.45 (9H, s), 4.44 (2H, s), 7.46-7.55 (5H, m), 7.82-7.90 (5H, m), 8.83 (1H, s), 9.07 (1H, s). ^{13}C-NMR ($CDCl_3$) δ 28.3, 56.2, 84.6, 123.4, 126.5, 128.5, 129.0, 129.3, 129.4, 129.7, 130.8, 133.6, 133.8, 159.0, 164.6, 165.0, 171.1. HRMS: Calcd for $C_{23}H_{23}N_3O_4$; m/z $[M+H]^+$ 406.1767. Found: 406.1762.

5-Acetylamino–6-oxo-2-phenyl-1(6H)-pyrimidineacetic acid tert-butyl ester (12b)

Mp 150-152 °C, IR: 3329, 1735, 1645, 1508, 1354, 1226, 1147, 771, 667, 590 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.45 (9H, s), 2.23 (3H, s), 4.54 (2H, s), 7.48 (5H, m), 8.03 (1H, s), 9.09 (1H, s). ^{13}C-NMR ($CDCl_3$) δ 24.4, 27.9, 49.0, 94.1, 125.1, 128.1, 128.9 130.0, 134.0, 136.6, 153.3, 158.0, 166.1, 168.8. MS(ESI) m/z $[M+H]^+$ 344. HRMS: Calcd for $C_{18}H_{21}N_3O_4$; $[M+H]^+$ 344.1610. Found: 344.1584.

5-Acetylamino-4-oxo-2-phenyl-1(4H)-pyrimidineacetic acid tert-butyl ester (20b)

Mp 197-199 °C. IR (neat): 1748, 1505, 1516, 1465, 1433, 1234, 1201, 1166, 754, 705, 540 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.43 (9H, s), 2.22 (3H, s), 4.41 (2H, s), 7.45-7.51 (5H, m), 8.32 (1H, s), 8.62 (1H, s). ^{13}C-NMR ($CDCl_3$) δ 24.3, 27.9, 55.8, 84.3, 123.5, 128.1, 128.8, 130.7, 133.2, 158.6, 164.4, 165.8, 169.8. HRMS: Calcd for $C_{15}H_{15}N_3O_4$ $[M+H]^+$ 344.1610. Found: 344.1587.

5-Phenylacetylamino-6-oxo-2-phenyl-1(6H)-pyrimidineacetic acid tert-butyl ester (12c)

Mp 111.0-111.5 °C. IR (neat): 1692, 1635, 1510, 1482, 1363, 1151, 760, 704, 524 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.40 (9H, s), 3.81 (2H, s), 4.58 (2H, s), 7.33-7.44 (5H, m), 7.53-7.67 (5H, s), 8.02 (1H, s), 9.10 (1H, s). ^{13}C-NMR ($CDCl_3$) δ 27.9, 44.2, 50.0, 84.2, 125.2, 125.8, 127.9, 128.5, 129.5, 130.0, 133.5, 133.5, 155.5, 156.4, 165.5, 170.4. HRMS: Calcd for $C_{24}H_{25}N_3O_4$ $[M+H]^+$ 420.1923. Found: 420.1898.

5-Phenylacetylamino-4-oxo-2-phenyl-1(4H)-pyrimidineacetic acid tert-butyl ester (20c)

Mp 162-163 °C. IR (neat): 1741, 1610, 1593, 1510, 1470, 1230, 1215, 1160, 746, 696, 540 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.44 (9H, s), 3.75 (3H, s), 4.35 (2H, s), 7.30-7.37 (5H, m), 7.42-7.50 (5H, m), 8.38 (1H, s), 8.63 (1H, s). ^{13}C-NMR ($CDCl_3$) δ 28.3, 44.7, 56.2, 84.7, 123.8, 127.9, 128.5, 129.2, 129.3, 129.4, 129.7, 131.1, 133.6, 134.2, 159.1, 164.7, 166.1, 171.1. HRMS: Calcd for $C_{24}H_{25}N_3O_4$ $[M+H]^+$ 420.1923. Found: 420.1903.

2-Methyl-4-methoxymethylene-5-oxazol-1-one (7d)

To a suspension of **19b** (3.0 g, 20.1 mmol) in DMF (30 mL) was added Me_2SO_4 (2.20 mL, 23.2 mmol), and the mixture was stirred at 60 ^{o}C for 5 h. The solvent was removed by evaporation, EtOAc was added, and mineral salt was filtered off. The filtrate was washed with aq. $NaHCO_3$ solution and brine. The

organic layer was evaporated and purified by silica gel column chromatography (eluted with hexane/EtOAc) to give **7d** as crystalline solid (1.73 g, 61%).

Mp 109-111 °C. IR (neat): 1689, 1636, 1531, 1433, 1248, 1142, 961, 916, 598, 552, 448 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 2.28 (3H, s), 4.11 (3H, s), 7.13 (1H, s). ^{13}C NMR ($CDCl_3$) δ 15.5, 63.5, 117.5, 152.9, 161.8, 168.9. MS (APCI) m/z $[M+H]^+$ 142.1. HRMS: Calcd for $C_6H_7NO_3$ $[M+H]^+$ 142.0504. Found: 142.0488.

2-Methyl-4-benzyloxymethylene-5-oxazol-1-one (7e)

To a suspension of **19b** (420 mg, 2.81 mmol) in DMF (5 mL) was added benzyl bromide (530 mg, 3.10 mmol) , and the mixture was stirred at 80 ^{o}C for 5 h. The solvent was removed by evaporation, toluene was added, and mineral salt was filtered off. The filtrate was washed with aq. $NaHCO_3$ solution and brine. The organic layer was evaporated and purified by silica gel column chromatography (eluted with hexane/EtOAc) to give **7e** as crystalline solid (370 mg, 60%).

Yield: 60%

Mp 76-77 °C. IR (neat): 1700, 1651, 1628, 1522, 1265, 1179, 1142, 976, 743, 698, 597, 476 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 2.27 (3H, s), 5.35 (2H, s), 7.27 (1H, s), 7.35-7.40 (5H, m). ^{13}C NMR ($CDCl_3$) δ 15.2, 78.0, 117.5, 128.2, 128.4, 128.8, 129.0, 151.0, 161.6, 168.5. MS (ESI) m/z $[MH]^+$ 218.3. HRMS: Calcd for $C_{12}H_{11}NO_3$ $[M+H]^+$ 218.0817. Found: 218.0802.

2-Benzyl-4-methoxymethylene-5-oxazol-1-one (7f)

To a suspension of oxazolone **19c** (3.0 g, 13.3 mmol) in MeCN (30 mL) was added Me_2SO_4 (1.64 mL, 17.3 mmol), and the mixture was stirred at 60 °C for 4 h. The reaction mixture was concentrated and EtOAc was added. The insoluble salt was filtered off. The filtrate was washed with aq. $NaHCO_3$ solution and brine. The organic layer was evaporated and purified by silica gel column chromatography (eluted with hexane/EtOAc) to give **7f** (1.99 g, 69%).

Mp 92-94 °C.[17] IR (neat): 1771, 1755, 1668, 1254, 1148, 1036, 895, 878, 756, 698, 638 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 3.86 (2H, s), 4.10 (3H, s), 7.15 (1H, s), 7.25-7.37 (5H, m). ^{13}C NMR ($CDCl_3$) δ 35.9, 63.3, 117.2, 127.5, 128.8, 129.2, 133.3, 153.2, 162.5, 168.4. MS (ESI) m/z $[M+H]^+$ 218.2. HRMS: Calcd for $C_{12}H_{11}NO_3$ $[M+H]^+$ 218.0817. Found: 218.0789.

General procedure for the preparation of 5-acylamino-6-oxo-2-phenyl-1(6H)-pyrimidineacetic acid tert-butyl ester 12 from oxazolone derivatives 7d-f and amidine 8a.

To a suspension of *N*-(*tert*-butoxycarbonylmethyl)benzamidine hydrochloride **8a** (2.00 g, 7.39 mmol) in toluene (25 mL) was added Na_2CO_3 (2.3 g) and water (10 mL) with vigorous stirring until the substrate dissolved. After separation, the aqueous layer was extracted with toluene. The combined organic layer

was washed with brine and dried over Na_2SO_4. The organic layer was added dropwise to a solution of the oxazolone **7d-f** (5.97 mmol) in MeCN (5 mL) and stirred at 80 °C for 17 h. The mixture was washed with 1M aqueous HCl, saturated aqueous $NaHCO_3$ solution and brine. After concentration, the resulting slurry was added dropwise to hexane and stirred. The crystals were filtered and dried under reduced pressure to give **12** as crystals. Analytical data of **12a-c** were fully consistent with those obtained from **19a-c** (vide infra).

General procedure for the preparation of 5-acylamino-6-oxo-2-phenyl-1(6H)-pyrimidineacetic acid 13 from oxazolone derivatives 7d-f and amidine 8b.

To a solution of *N*-(carboxymethyl)benzamidine **8b** (1.26 g, 7.09 mmol) in ^{i}PrOH (15 mL) was added 21% of NaOEt/EtOH (2.23 g, 7.09 mmol). The mixture was stirred for 0.5 h. To the mixture was added oxazolone derivative **7d-f** (7.09 mmol) in 4 portions for 1 h at 50 °C, and the mixture was stirred at 80 °C for 18 h. The reaction mixture was concentrated and EtOAc (15mL) and water (10mL) were added. The aqueous layer was acidified with aq. HCl and extracted with EtOAc. To the organic layer was added hexane and the precipitate was collected and dried under reduced pressure to give **13** as pale yellow solid.

5-Acetylamino-6-oxo-2-phenyl-1(6H)-pyrimidineacetic acid (13b) from 7d

Yield: 78%

Analytical data of **13b** were fully consistent with those obtained from **11b** (vide infra).

5-Phenylacetylamino-6-oxo-2-phenyl-1(6H)-pyrimidineacetic acid (13c)

Yield: 48%

Mp 154-155 °C. IR (neat): 1722, 1654, 1508, 1488, 1217, 1199, 774, 721, 705 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 3.84 (2H, s), 4.52 (2H, s), 7.25-7.27 (1H, m), 7.31-7.34 (4H, m), 7.47-7.55 (5H, m), 8.83 (1H, s), 9.72 (1H, s). ^{13}C-NMR (DMSO-d_6) δ 42.9, 48.5, 125.3, 127.0, 128.4, 128.8, 129.4, 129.6, 130.5, 134.4, 136.1, 137.6, 154.0, 157.5, 169.1, 170.8. MS (ESI) m/z $[M+H]^+$ 364. $[M-H]^+$ 362. HRMS: Calcd for $C_{20}H_{17}N_3O_4$ $[M+H]^+$ 364.1297. Found: 364.1267.

Deprotection of N-acetyl group of 4-aminopyrimidinones under weak basic conditions

5-Acetylamino-6-oxo-2-phenyl-1(6*H*)-pyrimidineacetic acid **13b** (200 mg, 0.697 mmol) was stirred in 1 M aqueous NaOH (2 mL) for 1 h at room temperature. The mixture was neutralized to pH 3 with 6M hydrochloric acid. Resulting precipitates were collected by filtration and washed with water and vacuum dried to give **1** as crystals in 97% yield (165 mg, 0.676 mmol).

Analytical data of **1** were fully consistent with those reported previously by us.[6]

Deprotection of N-phenylacetyl group with enzyme

5-Phenylacetylamino-6-oxo-2-phenyl-1(6*H*)-pyrimidineacetic acid **13c** (150 mg, 0.413 mmol) was dissolved in 0.01 M potassium phosphate buffer (pH 7.4, 3 mL) and aqueous *penicillin amidase* solution (Sigma) were added. The mixture was stirred overnight at 37 °C. The reaction mixture was neutralized to pH 3 with 6M hydrochloric acid and added MTBE. The precipitates were collected by filtration, washed with water and vacuum dried to give **1** as crystals in 95% yield (96 mg, 0.392 mmol).

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REFERENCES

1. C. A. Veale, P. R. Bernstein, C. Bryant, C. Ceccarelli, J. R. Damewood, Jr., R. Earley, S. W. Feeney, B. Gomes, B. J. Kosmider, G. B. Steelman, R. M. Thomas, E. P. Vacek, J. C. Williams, D. J. Wolanin, and S. Woolson, *J. Med. Chem.,* 1995, **38**, 98.
2. Y. Odagaki, K. Ohmoto, S. Matsuoka, N. Hamanaka, H. Nakai, M. Toda, and Y. Katsuya, *Bioorg. Med. Chem*., 2001, **9**, 647.
3. F. Akahoshi, A. Ashimori, H. Sakashita, T. Yoshimura, M. Eda, T. Imada, M. Nakajima, N. Mitsutomi, S. Kuwahara, T. Ohtsuka, C. Fukaya, M. Miyazaki, and N. Nakamura, *J. Med. Chem*., 2001, **44**, 1297.
4. S. Takai, D. Jin, M. Nishimoto, A. Yuda, M. Sakaguchi, K. Kamoshita, K. Ishida, Y. Sukenaga, S. Sasaki, and M. Miyazaki, *Life Sciences,* 2001, **69**, 1725.
5. (a) K. Ohmoto, T. Yamamoto, T. Horiuchi, T. Kojima, K. Hachiya, S. Hashimoto, M. Kawamura, H. Nakai, and M. Toda, *Synlett,* 2001, 299; (b) T. Kojima, T. Okada, K. Hachiya, T. Motoi, and S. Hashimoto, PCT Int. Appl., 2001, WO 2001023361; (c) T. Sugiura, T. Horiuchi, T. Miyazaki, T. Kojima, and S. Hashimoto, PCT Int. Appl., 2002, WO 2002051815.
6. D. Takahashi, Y. Honda, and K. Izawa, *Heterocycles,* 2012*,* **85**, 1089.
7. (a) P. Kumar, H. D. Mishra, and A. K. Mukerjee, *Synthesis*, 1980, 836; (b) P. K. Tripathi and A. K. Mukerjee, *Synthesis*, 1985, 285; (c) R. Cotton, P. M. Hardy, and A. E. Langran-Goldsmith, *Int. J. Peptide Protein Res*., 1986, **28**, 230.
8. (a) I. Ojima, K. Kato, and K. Nakahashi, *J. Org. Chem.,* 1989, **54**, 4511; (b) M. R. P. Norton Matos, P. M. P. Gois, M. L. E. N. Mata, E. J. Cabrita, and C. A. M. Afonso, *Synth. Commun.*, 2003, **33**, 1285; (c) D. Takahashi, Y. Honda, and K. Izawa, PCT Int. Appl., 2003 WO 2003106434.

9. (a) J. Kashyap, A. B. Chetry, and J. Das Pranab, *Synth. Comm*., 1998, **28**, 4187; (b) R. V. Vnkataratnam and P. S. Rao, *Indian J. Chem*., 1994, **33B**, 984.
10. L. Kralj, A. Hvala, J. Svete, L. Golic, and B. Stanovnik, *J. Heterocycl. Chem.,* 1997, **34**, 247.
11. R. S. Singh and R. M. Singh, *Indian J. Chem*., 2000, **39B**, 688.
12. N. L. Benoiton, F. Hudecz, and M. F. Chen, *Int. J. Peptide Protein Res*., 1995, **45**, 266.
13. T. Ikemoto, T. Kawamoto, K. Tomimatsu, M. Takatani, and M. Wakimasu, *Tetrahedron*, 2000, **56**, 7915.
14. K. K. Singh, R. S. Radhey, and R. M. Singh, *Indian J. Chem*., 1998, **37B**, 120.
15. M. K. Singh, K. K. Singh, R. S. Singh, and R. M. Singh, *Indian J. Chem*., 1997, **36B**, 137.
16. K. Sakabe, K. Sasaki, N. Watanabe, M. Suzuki, Z. G. Wang, J. Miyahara, and N. Sakabe, *J. Synchrotron Rad.,* 1997, **4**, 136.
17. S. T. Rolfson, J. H. Hunter, and J. W. Hinman, US 2555006 (1951).