Discovery and Synthesis of HIV Integrase Inhibitors: Development of Potent and Orally Bioavailable N-Methyl Pyrimidones

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The human immunodeficiency virus type-1 (HIV-1) encodes three enzymes essential for viral replication: a reverse transcriptase, a protease, and an integrase. The latter is responsible for the integration of the viral genome into the human genome and, therefore, represents an attractive target for chemotherapeutic intervention against AIDS. A drug based on this mechanism has not yet been approved. Benzyl-dihydroxypyrimidine-carboxamides were discovered in our laboratories as a novel and metabolically stable class of agents that exhibits potent inhibition of the HIV integrase strand transfer step. Further efforts led to very potent compounds based on the structurally related N−Me pyrimidone scaffold. One of the more interesting compounds in this series is the 2-N-Me-morpholino derivative **27a**, which shows a CIC_{95} of 65 nM in the cell in the presence of serum. The compound has favorable pharmacokinetic properties in three preclinical species and shows no liabilities in several counterscreening assays.

Introduction

The HIV genome exists within the virus as a positive sense RNA strand and encodes three constitutive viral enzymes that are required for viral replication: a protease, a reverse transcriptase, and an integrase. Inhibitors of the viral reverse transcriptase,[1−4] along with protease inhibitors,[5−7] have been combined to form the basis of the so-called triple combination therapy or "HAART". Attacking the virus on as many fronts as possible has proven to be the most effective way of suppressing viral replication in patients. While triple therapy has extended the lives of many, there are others for whom drug treatment has failed, either because of the emergence of viral strains resistant to the current drugs or simply because of the intolerable side effects of the drugs themselves. For these reasons, many laboratories have been involved in unraveling the structure and function of HIV integrase, with the ultimate goal of finding small molecule HIV integrase inhibitors with clinical utility.[8−11] Integration is believed to be mediated by integrase[12] in three steps: assembly of a stable nucleoprotein complex with viral DNA sequences, cleavage of the two nucleotides from the 3′ termini of the linear proviral DNA, and covalent joining of the recessed 3′OH termini of the proviral DNA at a staggered cut made at the host target site. The fourth step in the process, repair synthesis of the resultant gap, may be accomplished by cellular enzymes. The viral integrase is expressed as a 32 kDa, 288 amino acid residue protein containing three distinct regions: the N-terminus of the enzyme contains a zinc binding domain, including amino acid residues 1−50, with a highly conserved "HH−CC" motif, followed by the catalytic core from residues 51 to 212 and the C-terminal DNA binding domain from residues 213 to 288, whose function is binding and orientation of viral DNA during the integration process. The catalytic core domain contains two aspartate (Asp64, Asp116) and one glutamate (Glu152) residues that are essential for the catalytic activity of the integrase and are believed to bind Mg^{2+} or Mn^{2+} ions.[13] The presence of either of these divalent ions is required for HIV integrase catalytic activity and also the activity of integrase inhibitors such as diketoacids **1**, which were recently disclosed by us and others,[14] was shown to be metal-dependent. The diketoacid structure **1** was believed to be essential for the activity of many integrase inhibitors. The structures of diketotriazole (S1360),[15] diketotetrazoles (5CITEP),[16] diketopyridine,[17] and 7-carbonyl-8-hydroxy-(1,6)-naphthyridine[17] are examples of bioisosteres of the diketoacid pharmacophore. Naphthyridine **2** was shown to be efficacious against replication of simian-human immunodeficiency virus (SHIV) 89.6P in infected rhesus macaques, demonstrating that integrase inhibitors can be engineered with all the appropriate properties required for an effective therapy to treat HIV infections.[18] In our laboratories, studies on inhibitors of the hepatitis C virus (HCV) RNA-dependent RNA polymerase, such as diketoacids **1**[19] and 3-hydroxypyran-4-one carboxylic acid derivative **3**,[20] led to the preparation of a series of 2-aryl-5,6-dihydroxypyrimidine-4-carboxylic acids **4** (Figure 1).[21−26] HIV integrase and HCV RNA polymerase are mechanistically related enzymes in which divalent magnesium cations (Mg^{2+}) play a pivotal role in catalysis. Thus, metal-chelating inhibitors such as diketoacids or mimics thereof are effective against both enzymes. Due to their highly charged nature, compounds **4** showed only moderate inhibition in the subgenomic cell-based assay of HCV replication, despite good in vitro potency.[22] Conversion to carboxamides such as **5** abolished the activity on the HCV polymerase, but promising inhibition of

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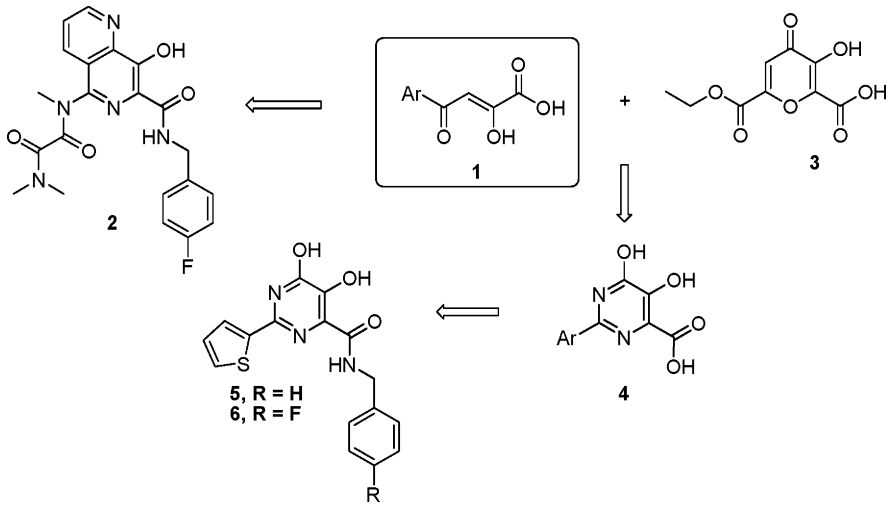


Figure 1. From diketoacid inhibitors **1** to naphthyridine **2** and dihydroxypyrimidine carboxamide **6**.

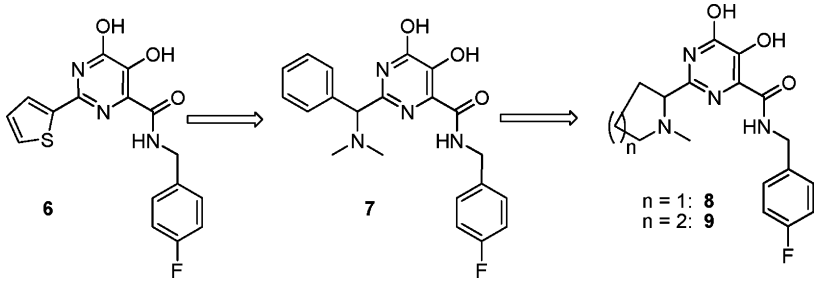


Figure 2. From aromatic derivatives to aliphatic heterocyclic compounds.[29]

Table 1. Enzymatic and Antiviral Activity, Rat and Human Plasma Protein Binding, Oral Bioavailability, and Plasma Clearance in Rat of Compounds **8**−**11**

cmpd	IC_{50}[a] (μM)	CIC_{95} (μM) (10% FBS)[b]/(50% NHS)[c]	protein binding rat[d]/human[e]	rat F[f]/Clp[g]
8	0.12	0.15/0.62	89/97	nd/nd
9	0.22	0.14/0.40	95/95	27/75
10	0.10	5.00/5.00	67/52	nd/nd
11	0.44	0.83/1.00	48/55	100/31

[a] HIV strand transfer assay results are the mean of at least three independent experiments; IC_{50} is the concentration of inhibitor that reduces the HIV integrase activity by 50%.[13,30,31] [b] Spread assay results are the mean of at least three independent experiments; CIC_{95} is the concentration of compound that inhibits HIV replication in the cell-based assay by 95% in the presence of 10% of fetal bovine serum (FBS).[32] [c] Spread assay results are the mean of at least three independent experiments; CIC_{95} is the concentration of compound that inhibits HIV replication in the cell-based assay by 95% in the presence of 50% normal human serum (NHS).[32] [d] Percentage of compound bound to rat plasma proteins.[33] [e] Percentage of compound bound to human plasma proteins.[33] [f] Oral bioavailability (%). [g] Plasma clearance (mL/min/kg).

the HIV integrase in the strand transfer assay was observed, where **5**[26] displayed an IC_{50} value of 85 nM.

A focused library of 200 carboxamides was assembled,[27] and the 4-fluorobenzylamide **6** emerged as the optimal substituent, displaying an 8-fold improvement in potency (**6**, IC_{50} = 10 nM) although with low cellular activity (CIC_{95} >10 μM in the presence of 10% FBS). Improvement of potency in the cell-based assay was obtained by the introduction of a basic residue in position 2 of the pyrimidine, leading to **7** and ultimately to equipotent 2-pyrrolidinyl or 2-piperidinyl-substituted dihydroxypyrimidines **8** or **9**, which inhibited the strand transfer process of integration, with an IC_{50} of 120 and 220 nM, respectively (Figure 2 and Table 1). Both compounds were potent inhibitors of HIV-1 replication in cell culture, with CIC_{95} of 150 nM in the presence of 10% fetal bovine serum, which shifted 3−4-fold when the assay was conducted in the presence of 50% normal human serum as a consequence of extensive binding of these molecules to human plasma proteins.[28] Compound **9** was profiled further and showed modest oral bioavailability (F = 27%) and high plasma clearance (Cl = 75 mL/min/kg) in rats.

In parallel with the further development of the dihydroxypirimidines, we studied the effect of methylation on the N-1 pyrimidine nitrogen, with the goal to improve the in vivo potency and the pharmacological properties of the molecules.

In this paper we describe an extensive and consistent structure−activity relationship that led to the identification of compounds that inhibit HIV integrase in vitro at nanomolar concentration, block effectively HIV replication in cell culture in the presence of high serum concentration, and show excellent physicochemical and pharmacokinetic (PK[a]) properties.

Results and Discussion

The structure−activity relationship around **8** and **9** had previously established that the amine contained in the ring had to occupy the benzylic position with respect to the pyrimidine and that small alkyl groups, such as methyl or ethyl on the nitrogen of the saturated heterocycle, are preferred.[29] We hoped that conversion of the dihydroxypyrimidine to the more polar *N*-methylpyrimidone would be beneficial for cell-based potency by reducing binding to plasma proteins and targeted first the direct analogs of **8** and **9** (Figure 3).

Despite the reduction in intrinsic and in cell-based potency, compound **11** showed only a small shift between 10% and 50%

[a] Abbreviations: TFA, trifluoroacetic acid; TMS, tetramethylsilane; DMSO, dimethyl sulfoxide; DCM, dichloromethane; THF, tetrahydrofuran; TEA, triethylamine; *m*-CPBA, *meta*-chloroperbenzoic acid; TFAA, trifluoroacetic anhydride; MTBE, *tert*-butyl methyl ether; DMAP, 4-dimethylaminopyridine; DAST, (diethylamino)sulfur trifluoride; PK, pharmacokinetic.

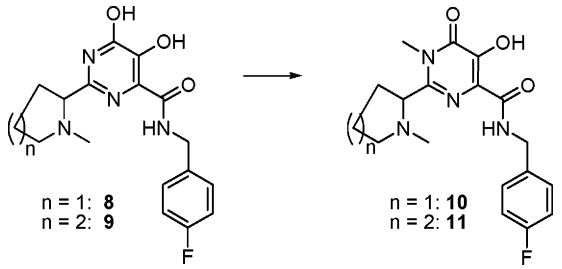


Figure 3. From dihydroxypyrimidines **8** and **9** to N−Me pyrimidones **10** and **11**.

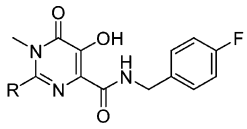
serum, because the polar pyrimidone has a lower affinity for plasma proteins. The pyrrolidine derivative **10** is 5-fold less potent than compound **11**, but also here no shift in the cell-based assay was observed. The more potent compound **11** was characterized further and its PK profile in rat was encouraging. The compound showed moderate clearance (Cl = 31 mL/min/kg) and high oral bioavailability (F = 100%). Encouraged by these preliminary data, we continued the SAR studies on five- and six-membered heterocycles, with the aim to further probe both the cell-based activity and the pharmacological properties of these compounds.

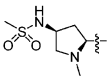
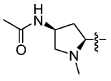
The structure−activity relationship surrounding the pyrrolidine ring revealed a certain degree of tolerance for diverse chemical functionalities that could be incorporated, and this confirmed the previous observation that the moiety at the 2-position is not involved in a specific interaction with the enzyme (Table 2). For better comparison of the data, compound **10** is reported in Table 2 in its enantiomerically pure form, having a 2*S*-configuration, **10a**. Initially, a methyl group was scanned on the pyrrolidine ring by preparation of compounds **12**−**15**, and the substitution on the position 4 of the ring, as in compounds **13** and **14**, gave the best enzymatic activity. The (*S*)-configuration was preferred, leading to compound **13**, with an IC_{50} = 10 nM. Based on these data, further SAR was conducted. As position 4 was the most appropriate for substitution, to explore the possibility to vary the physicochemical properties of this class of molecules to achieve activity also in cells, the *trans*-4-hydroxy pyrrolidine was initially selected based on its synthetic accessibility and ease of decoration; this strategy proved to be both informative and productive.

Starting from this building block, a series of compounds were prepared. The free hydroxyl group present in compound **16** gave a moderate activity in the cell, whereas the methoxy, ethoxy, and benzyloxy compounds **17**−**19** were potent both in vitro and in cell-based assays. Interestingly, no shift was observed for the small alkyloxy groups, while the benzyloxy derivative **19** showed at least a 4-fold drop in activity in high serum. The sulfonamide **20** and the acetamide **21** were potent in vitro, but had low activity in the cell, probably as a result of an increased polar surface area. The replacement of the hydrogen with a fluorine atom gave potent compounds on the enzyme: a *cis*- or *trans*-fluorine atom in the 4-position was well accepted. The stereochemistry of the fluorine proved to be not crucial in vitro, indeed the two diastereoisomers **22** and **23** and also the 4,4-difluorinated compound **24** showed similar potency. In the cell-based assay, compound **22** had CIC_{95} = 0.13 μM, with a 2-fold shift of the low serum value; the diastereoisomeric *trans* compound **23** was 2-fold less potent. The difluoroderivative **24** exhibited good potency in cells in 10% serum, but lost almost 6-fold in high serum.

We then analyzed a six-membered-based substitution in position 2 of the pyrimidine, exploiting the possibility in this

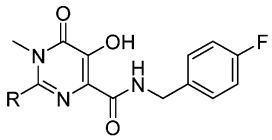
Table 2. Enzymatic and Antiviral Activity of 2-Pyrrolidinyl N-Me-pyrimidones

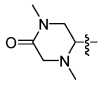


Compd	R	IC_{50} (nM)[a]	CIC_{95} (μM) (10% FBS)[b] / (50% NHS)[c]
10a		62	>1.00 / >1.00
12[d]		710	2.50 / 5.00
13		10	>1.00 / >1.00
14		190	>1.00 / >1.00
15[d]		690	>1.00 / >1.00
16		100	0.63 / 0.63
17		180	0.15 / 0.17
18		200	0.17 / 0.21
19		130	<0.08 / 0.31
20		30	0.50 / 0.50
21		61	>1.00 / >1.00
22		20	0.06 / 0.13
23		20	0.13 / 0.25
24		30	0.03 / 0.17

[a] HIV strand transfer assay results are the mean of at least three independent experiments; IC_{50} is the concentration of inhibitor that reduces the HIV integrase activity by 50%.[13,30,31] [b] Spread assay results are the mean of at least three independent experiments; CIC_{95} is the concentration of compound that inhibits HIV replication in the cell-based assay by 95% in the presence of 10% of fetal bovine serum (FBS).[32] [c] Spread assay results are the mean of at least three independent experiments; CIC_{95} is the concentration of compound that inhibits HIV replication in the cell-based assay by 95% in the presence of 50% normal human serum (NHS).[32] [d] Mixture of diastereoisomers (2:1), undetermined stereochemistry.

Table 3. Six-Membered Heterocycles in the 2-Position of the Pyrimidone



Compd	R	IC_{50} (nM)[a]	CIC_{95} (μM) (10% FBS)[b] / (50% NHS)[c]
11		440	0.83 / 1.04
25		21	0.50 / 0.50
26		62	0.25 / 1.00
27		60	0.06 / 0.10
28		70	0.05 / 0.13
29[d]		37	>1.00 / >1.00
30		140	>1.00 / >1.00
31		100	0.25 / 0.19
32		50	1.25 / 1.25

[a] HIV strand transfer assay results are the mean of at least three independent experiments; IC_{50} is the concentration of inhibitor that reduces the HIV integrase activity by 50%.[13,30,31] [b] Spread assay results are the mean of at least three independent experiments; CIC_{95} is the concentration of compound that inhibits HIV replication in the cell-based assay by 95% in the presence of 10% of fetal bovine serum (FBS).[32] [c] Spread assay results are the mean of at least three independent experiments; CIC_{95} is the concentration of compound that inhibits HIV replication in the cell-based assay by 95% in the presence of 50% normal human serum (NHS).[32] [d] A 9:1 mixture, undetermined stereochemistry.

case to introduce heteroatoms both inside and outside of the ring (Table 3).

Also in the case of six-membered derivatives, the introduction of a heteroatom was beneficial; the 4-OH-N-Me piperidine derivative **25** was 20-fold more potent in the enzyme assay and it did not show any shift in cells between low and high serum conditions. This shift was more pronounced with the benzyloxy derivative **26**, as we observed in the pyrrolidine series. The introduction of a heteroatom inside the ring was beneficial: the morpholine derivative **27** showed a high affinity for the enzyme and displayed equal activity in the spread assay, with less than a 2-fold shift between low and high serum. Also, the thiomorpholine **28** was potent, whereas the oxidation of the sulfur to the sulfoxide **29** and sulfone **30** caused reduced activity in cells. The piperazine **31** was potent in cells, while the lactam **32**, lowering the basicity of the N1 nitrogen, was less active. The interesting data relative to the piperazine compound **31** led us to further explore this versatile scaffold, and several derivatives were prepared (Table 4).

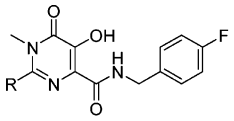
As for N1, in the case of the nitrogen in the 4 position, the methyl and ethyl residues gave potent compounds such as **31** and **34**; a free NH or a bigger substituent gave compounds (**33** and **35)** with comparable enzymatic activity but with lower potency in the spread assay. A sterically hindered carbamate **36** was well tolerated by the enzyme but exhibited an almost 7-fold shift from 10% to 50% serum in cells. The acetyl group was used as a substituent on the N4-nitrogen, as in compound **37** where it was well-tolerated, and also to reassess the need of an amino group in the position 1 of the piperazine: compound **38**, having the acetyl group on the N1 nitrogen was as expected of low activity. Other substitutions on the nitrogen in the 4 position, such as benzoate, amino acetyl, and ethylurea (**39**−**41**), were accepted by the enzyme but did not translate into high potency in the cell-based assay. The activity on the enzyme was further increased with sulfonamide and sulfamide moieties, and in the case of a simple mesyl derivative **42**, the CIC_{95} was 125 nM without any shift in high serum. The phenyl sulfonamide **43** was more potent in the presence of 10% of fetal bovine serum, but the shift was more than 16-fold between the two serum conditions. The sulfamide **44** had CIC_{95} = 125 nM.

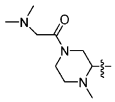
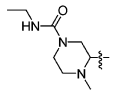
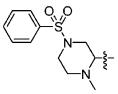
With all these compounds having good potency on the enzyme and in the cell-based assay available, we decided to further develop some of them to see if the PK profiles might be able to differentiate among these molecules. The PKs of the most potent and structurally different compounds were studied in rats, and the results are summarized in Table 5 together with some measured log D and the plasma protein binding data.

Apart from compound **16**, which displayed a low oral bioavailability (F = 2%), and the piperazine derivatives **37** and **42** that in rat showed high clearance (42 and 78 mL/min/kg, respectively) and moderate oral bioavailability (17 and 38, respectively), all the other compounds had moderate-low clearance, from 10 to 28 mL/min/kg and oral bioavailability from 60 to 100%. Two structurally different compounds, such as **24** and **27**, were further profiled in dog, where they displayed excellent PKs: both had 100% oral bioavailability and low plasma clearance (5 and 3 mL/min/kg, respectively, Table 6).

Due to the better potency in the presence of 50% normal human serum, the lower serum shift, and the lower clearance in dog, compound **27** was one of the more interesting compounds made so far and it was the focus of further SAR studies. The dihydroxypyrimidine analog **45** was tested as well, and although it showed better in vitro potency than the analog piperidine compound **9** and the corresponding N-Me pyrimidone **27**, it still exhibited an 8-fold shift between low and high serum and its oral bioavailability in rat was relatively low, 16%. The role of the amino group was revisited, and the most indicative analogs, such as compounds **46**−**49**, were prepared (Table 7). The need of an amino group on the nitrogen of the ring linked to the pyrimidine was reassessed, and the tertiary amino group was shown to be optimal for cell activity. Whereas the free NH compound **46** exhibited low activity in the spread assay, probably as a consequence of a high number of hydrogen bond donors, the ethyl derivative **47** was equipotent with the methyl

Table 4. 2-Piperazinyl N-Me-pyrimidones



Compd	R	IC_{50} (nM)[a]	CIC_{95} (μM) (10% FBS)[b] / (50% NHS)[c]
31		100	0.25 / 0.19
33		200	0.75 / 0.50
34		250	0.25 / 0.25
35		130	0.50 / 0.50
36		41	0.13 / 0.81
37		32	0.23 / 0.30
38		4000	>1.00 / >1.00
39		12	nd[d] / 0.25
40		4	0.50 / 1.00
41		52	0.25 / >1.00
42		11	0.13 / 0.13
43		7	0.03 / 0.50
44		8	0.06 / 0.13

[a] HIV strand transfer assay results are the mean of at least three independent experiments; IC_{50} is the concentration of inhibitor that reduces the HIV integrase activity by 50%.[13,30,31] [b] Spread assay results are the mean of at least three independent experiments; CIC_{95} is the concentration of compound that inhibits HIV replication in the cell-based assay by 95% in the presence of 10% of fetal bovine serum (FBS).[32] [c] Spread assay results are the mean of at least three independent experiments; CIC_{95} is the concentration of compound that inhibits HIV replication in the cell-based assay by 95% in the presence of 50% normal human serum (NHS).[32] [d] No data.

Table 5. Rat Pharmacokinetic Parameters, Log D, Rat, and Human Plasma Protein Binding

cmpd	F^a	Clp[b]	$t_{1/2}$[c]	AUC[d] after $PO_{(mg/kg)}$	LogD	protein binding rat[e]/human[f]
16	2	21	1.8	$0.1_{(2.3)}$	[g]	78/72
17	79	19	1.5	$5.2_{(3)}$	[g]	89/75
19	75	28	2.3	$2.3_{(2.4)}$	2.2	93/95
22	94	21	1.7	$4.5_{(3)}$	[g]	92/94
23	100	12	2.0	$12.5_{(3)}$	0.66	97/86
24	93	11	0.6	$11.8_{(3)}$	[g]	94/88
27	92	22	1.6	$4.3_{(2.3)}$	0.59	78/69
28	76	10	0.5	$9.7_{(3)}$	[g]	[g]/83
31	62	21	0.7	$5.1_{(3)}$	[g]	[g]/72
37	17	42	0.9	$0.8_{(3)}$	0.09	[g]/70
42	38	78	0.8	$0.6_{(3)}$	[g]	[g]/69

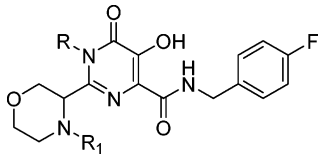
[a] Oral bioavailability (%). [b] Plasma clearance (mL/min/kg). [c] Plasma half-life following iv administration (h). [d] Area under the curve following oral administration at the dose indicated in brackets (μM × h). [e] Percentage of compound bound to rat plasma proteins.[33] [f] Percentage of compound bound to human plasma proteins.[33] [g] No data.

Table 6. Dog Pharmacokinetic Parameters and Dog Plasma Protein Binding of Compounds **24** and **27**

cmpd	F^a	Clp[b]	$t_{1/2}$[c]	AUC[d] after PO (mg/kg)	dog protein binding[e]
24	100	5	6	$9.9_{(1)}$	85
27	100	3	10	$11_{(0.8)}$	47

[a] Oral bioavailability (%). [b] Plasma clearance (mL/min/kg). [c] Plasma half-life following iv administration (h). [d] Area under the curve following oral administration at the dose indicated in brackets (μM × h). [e] Percentage of compound bound to dog plasma proteins.

Table 7. Morpholino-N-Me-pyrimidones



cmpd	R	R_1	IC_{50}[a] (nM)	CIC_{95} (nM) (10% FBS)[b]/(50% NHS)[c]
27	Me	Me	60	60/100
45[d]	H	Me	30	30/230
46	Me	H	50	>1000/>1000
47	Me	Et	33	50/90
48	Me	Ac	15	>1000/>1000
49	Me	Boc	20	>1000/>1000

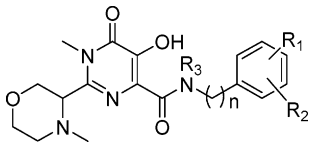
[a] HIV strand transfer assay results are the mean of at least three independent experiments; IC_{50} is the concentration of inhibitor that reduces the HIV integrase activity by 50%.[13,30,31] [b] Spread assay results are the mean of at least three independent experiments; CIC_{95} is the concentration of compound that inhibits HIV replication in the cell-based assay by 95% in the presence of 10% of fetal bovine serum (FBS).[32] [c] Spread assay results are the mean of at least three independent experiments; CIC_{95} is the concentration of compound that inhibits HIV replication in the cell-based assay by 95% in the presence of 50% normal human serum (NHS).[32] [d] See ref 29.

derivative **27**. Acetyl compound **48** and the carbamate **49**, although potent on the enzyme, showed low activity in the cell-based assay.

The structure−activity relationship around the benzylamide portion was assessed by the preparation of different amides (Table 8).

The unsubstituted benzylamide **50** was shown to have the ideal length, while the homologous phenethylamide **51** and the N-Me benzylamide **52** had CIC_{95} higher than 8 μM. Different halogens were tested in the three different positions of the phenyl ring, and considering the activity in the cells in the presence of 50% normal human serum, the 4-position was the favorite for

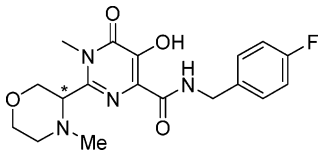
Table 8. 2-N-Me-morpholinyl-N-Me-pyrimidone Carboxamides



cmpd	R_1	R_2	R_3	*n*	IC_{50}[a] (nM)	CIC_{95} (nM) (10% FBS)[b]/(50% NHS)[c]
50	H	H	H	1	66	156/156
51	H	H	H	2	71	>8000/>8000
52	H	H	Me	1	>5000	>8000/>8000
53	2-F	H	H	1	175	156/625
54	3-F	H	H	1	62	125/250
55	3-Cl	H	H	1	62	16/63
56	3-Br	H	H	1	19	16/63
57	3-OMe	H	H	1	140	125/125
58	4-F	3-F	H	1	21	31/63
59	4-F	3-Me	H	1	86	16/42
60	4-F	3-Cl	H	1	28	<8/31
61	4-F	3-Br	H	1	44	31/125

[a] HIV strand transfer assay results are the mean of at least three independent experiments; IC_{50} is the concentration of inhibitor that reduces the HIV integrase activity by 50%.[13,30,31] [b] Spread assay results are the mean of at least three independent experiments; CIC_{95} is the concentration of compound that inhibits HIV replication in the cell-based assay by 95% in the presence of 10% of fetal bovine serum (FBS).[32] [c] Spread assay results are the mean of at least three independent experiments; CIC_{95} is the concentration of compound that inhibits HIV replication in the cell-based assay by 95% in the presence of 50% normal human serum (NHS).[32]

Table 9. N-Me-morpholine Derivatives **27**, **27a**, and **27b**



cmpd	IC_{50}[a] (nM)	CIC_{95} (μM) (10% FBS)[b]/(50% NHS)[c]	human protein binding[d]
27(±)[e]	60	65/100	70
27a(+)[f]	20	40/65	81
27b(−)[f]	25	90/190	74

[a] HIV strand transfer assay results are the mean of at least three independent experiments; IC_{50} is the concentration of inhibitor that reduces the HIV integrase activity by 50%.[13,30,31] [b] Spread assay results are the mean of at least three independent experiments; CIC_{95} is the concentration of compound that inhibits HIV replication in the cell-based assay by 95% in the presence of 10% of fetal bovine serum (FBS).[32] [c] Spread assay results are the mean of at least three independent experiments; CIC_{95} is the concentration of compound that inhibits HIV replication in the cell-based assay by 95% in the presence of 50% normal human serum (NHS).[32] [d] Percentage of compound bound to human plasma proteins.[33] [e] Trifluoroacetate salt. [f] Hydrochloride salt.

substitution; although, also in the *meta*-position, substitution was well-accepted. The combination of the two different substitutions was beneficial, and compounds **59**−**61** were potent, although with a higher serum shift passing from **59** to **61** probably as consequence of an increased log D.

Separating the two single enantiomers of compound **27** was possible by chiral HPLC resolution, and compounds **27a** and **27b** were submitted for testing (Table 9). The intrinsic activity on the enzyme was the same for the two enantiomers: IC_{50} = 20 and 25 nM for **27a** and **27b**. Compound **27a** was 3-fold more potent than its enantiomer **27b** in the spread assay in the presence of 50% normal human serum, so it was further characterized with respect to its PK properties. The hydrochloride salt of compound **27a** was a white crystalline solid, with a solubility of 5.8 mg/mL at physiological pH and a measured log D = 0.47.

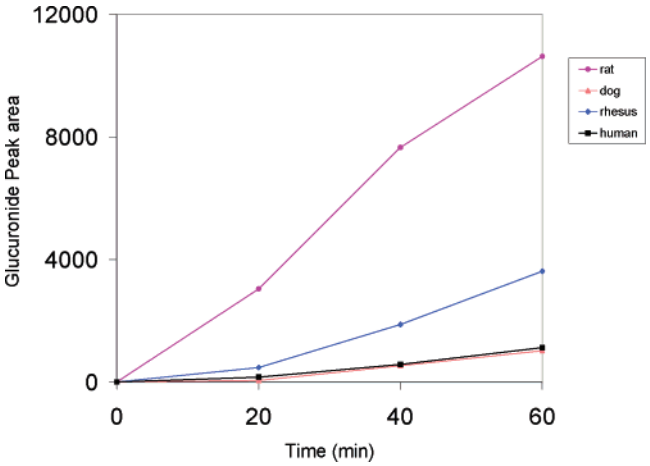


Figure 4. Glucuronide formation rate of compound **27a** in rat, dog, rhesus, and human liver microsomes in the presence of UDPGA.

An important feature was that compound **27a** maintained low affinity toward human plasma protein (hPPB = 81), which translated into a less than 2-fold shift in potency against HIV-1 replication in the presence of 50% NHS. The metabolic stability of compound **27a** was assessed in rat, dog and human liver microsomes in the presence of UDPGA and NADPH.[34] In the presence of NADPH, it was stable in microsomes from all species and no turnover was observed. Similarly, the glucuronidation rate was minimal; to rank the rate of glucuronidation among the species, we analyzed the rate of appearance of the glucuronide, and this was higher in rat and lower in dog and human microsomes (Figure 4). It did not show significant degradation both in human and in rat hepatocytes up to 4 h of incubation (data not shown).

Compound **27a** was dosed in rats intravenously and orally at 3 mg/kg: it exhibited low clearance (9 mL/min/kg) and it was found to be well-absorbed, with an oral bioavailability of 56%, a good exposure (AUC = 8.0 μM × h), and high C_{max} (5.6 μM).

When dosed in dogs intravenously and orally (4 mg/kg iv and 10 mg/kg p.o.), it exhibited low clearance (2.2 mL/min/kg) and good half-life ($t_{1/2}$ = 7.2 h) and it was found to be well-absorbed, with an oral bioavailability of 69%, excellent exposure (AUC = 136 μM × h), and high C_{max} (35 μM). After 48 h, the plasma concentration was still higher than the CIC_{95} of the compound (Figure 5). Compound **27a** was dosed intravenously and orally also in rhesus monkeys (1 mg/kg): it exhibited moderate plasma clearance (14 mL/min/kg) and it was found to be well-absorbed, with an oral bioavailability of 73% (Table 10).

Compound **27a** presented low to moderate clearance in three preclinical species and good oral bioavailability, thus, was an ideal candidate for further investigation. In counterscreening, it was a selective HIV integrase inhibitor, displaying IC_{50} higher than 50 μM with respect to HCV polymerase, HIV reverse transcriptase, and human DNA polymerases α, β, and γ. It was tested on a panel of 170 assays for potential ancillary activities and no significant responses were noted (MDS Pharma Services-Panlabs).

Compound **27a** was tested for hERG-channel activity, and it showed IC_{50} = 24 μM.[35] It did not inhibit the major CyP450 enzymes (3A4, 2D6, 1A2, and 2C19) up to 100 μM. It did not react with glutathione after incubation at 37 °C for 24 h, and the [^{3}H]-**27a** after administration in rat (30 mpk, po) did not

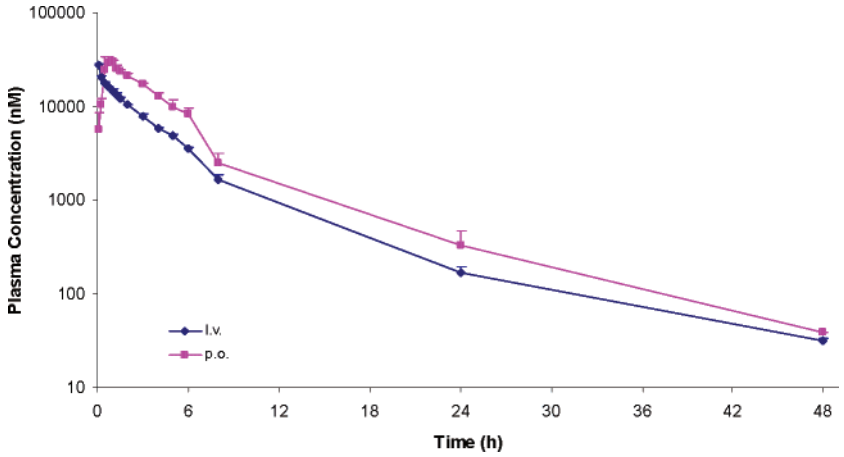


Figure 5. Pharmacokinetic profile for compound **27a** in dogs dosed intravenously (4 mg/kg) and orally (10 mg/kg) as a solution in 35%DMSO/65% (0.9% NaCl) and in 1% methylcellulose, respectively.

Table 10. Rat, Dog and Rhesus Monkey Pharmacokinetic Parameters for the Racemic Compound **27** and Its Single Enantiomers **27a** and **27b**

cmpd	F[a] rat/dog/ rhesus	Clp[b] rat/dog/ rhesus	$t_{1/2}$[c] rat/dog/ rhesus	AUC[d] after $PO_{(mg/kg)}$ rat/dog/rhesus
27(±)	92/100/53	22/3/14	1.5/10/1.4	$4.3_{(2.3)}/22_{(1.5)}/1.7_{(1)}$
27a(+)	56/69/73	9/2.2/14	1.1/7.2/2.0	$8_{(3)}/136_{(10)}/2.2_{(1)}$
27b(−)	84/87/[e]	18/2.6/[e]	1.0/1.9/[e]	$6_{(3)}/29_{(2)}$/[e]

[a] Oral bioavailability (%). [b] Plasma clearance (mL/min/kg). [c] Plasma half-life following iv administration (h). [d] Area under the curve following oral administration at the dose indicated in brackets (μM × h). [e] No data.

show measurable covalent binding to liver, kidney, and plasma proteins. Glucuronidation was the major metabolic pathway of compound **27a** in rats and dogs. The major metabolite in rat urine accounting for about 30% of the dose on the basis of ^{19}F analysis, was the glucuronide conjugate in position 5 of the pyrimidine.[36] LC-MS and ^{19}F-NMR experiments demonstrated that no racemization occurs in vivo. A second minor metabolite was the *N*-demethylated morpholine.

Biology

Compounds were routinely assessed for activity against the purified HIV-1 integrase enzyme.[13] Integrase-mediated strand transfer activity was determined as published.[37] Compounds were tested in HIV-1 replication assays: antiviral activity was assessed by measuring the decrease in HIV-1 p24 core antigen in MT-4 human T-lymphoid cells/HIV-1IIIb cultured in the presence of an increasing concentration of inhibitor, as published.[32] Cells were infected en masse at low multiplicity (0.01) using HIV-1 strain IIIb and were incubated for 24 h. At this time, cells were washed and distributed into 96-well microtiter dishes. Serial 2-fold dilutions of inhibitor were added to the wells, and the cultures were maintained for three additional days. Control cultures in the absence of inhibitor were fully infected at 4 days.

Synthesis

The chemistry used to prepare analogs reported in Tables 3 and 7 is presented in Scheme 1 for final compounds **11**, **25**−**30**, and **46**−**49**. A procedure reported by Culbertson[38] was followed starting from the appropriate nitrile **62**. Formation of the aldoxime **63** followed by Michael reaction with dimethylacetylene dicarboxylate (DMAD) gave the mixture of the *cis*/*trans* adducts **64**.

This mixture was heated in refluxing *o*-xylene to effect a thermal rearrangement that afforded the dihydroxypyrimidine **65**,[39,40] which was selectively benzoylated to compound **66**. Methylation with LiH and dimethyl sulfate gave the desired *N*-alkylated heterocycle; in the case of compound **66a**, a 10:1 ratio between the *N*- and *O*-regioisomers **67a** and **68a** was obtained.[41] Amidation and debenzoylation were accomplished by refluxing with 4F-benzylamine in MeOH, and the corresponding benzylamide **49** was obtained. Removal of the Boc group under acidic conditions or of the Cbz group by hydrogenation afforded compounds **46**, which were submitted to reductive alkylation to give compounds **11**, **26**−**28**, and **47** or to acetylation to obtain compound **48**. Compound **25** was obtained from **26** by catalytic hydrogenation. The oxidized thiomorpholine compounds **29** and **30** were obtained from thiomorpholine **28** through $NaIO_4$ and *m*-CPBA oxidations, respectively.

As far as the preparation of the starting material, **62a**, this was initially prepared through a five-step synthesis, arriving at the methyl-morpholine-3-carboxylate. According to a procedure reported in the literature,[42] the carboxylate was then manipulated to the required morpholine-3-nitrile. To overcome the low yield and the length of this pathway, a novel and efficient synthesis to morpholine-3-nitrile **71**, reported in Scheme 2, was developed based on a literature procedure for piperidine 2-carbonitrile.[43]

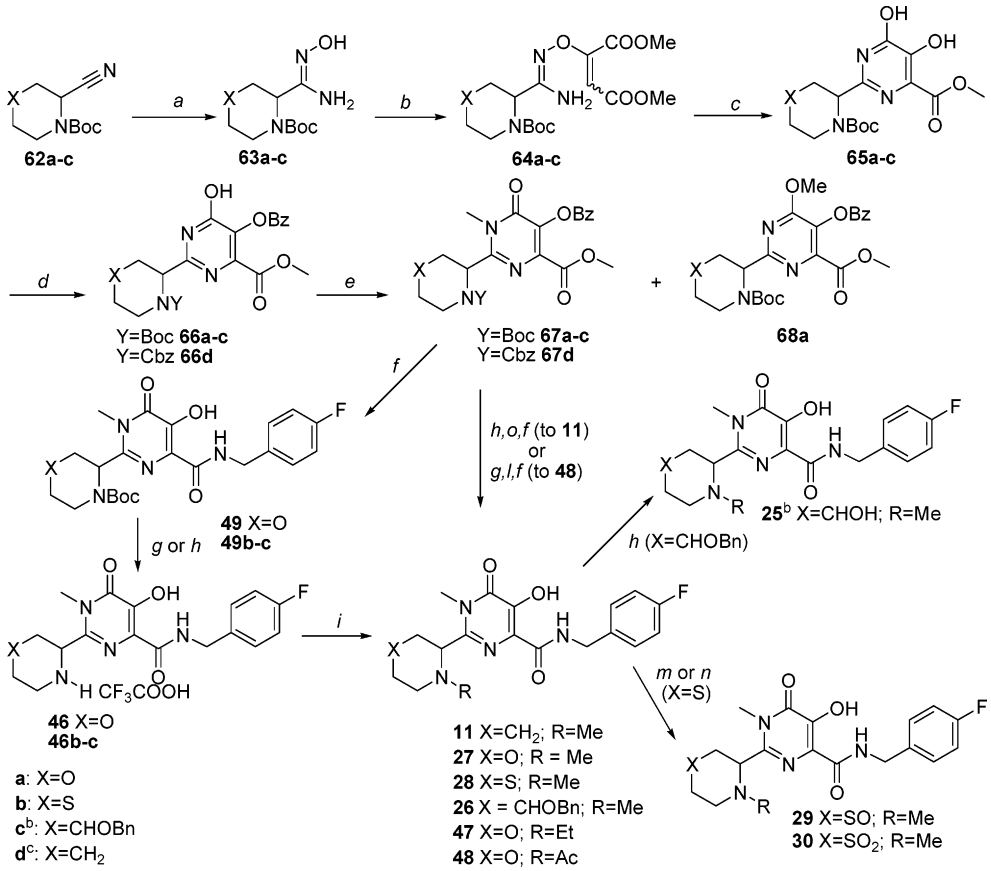
Morpholine nitrile **71** was prepared from morpholine via oxidation to *N*-chloromorpholine, followed by elimination/trimerization to **70**, which was subsequently cyanated. Treatment of **71** with BOC anhydride gave the nitrile **62a**. For the formation of *N*-chloromorpholine, different solvents were evaluated, among these were ethyl ether, THF, and MeOH, but switching to MTBE, the exotherms were milder and the possible formation of an explosive compound such as MeOCl was eliminated.

Nitrile **62b** was prepared from the ethyl thiomorpholine carboxylate **72** obtained according literature procedure.[44] Nitrile **62c** was synthesized from the oxygenated pipecolic acid derivative **75** that was obtained according to a literature procedure[45] (Scheme 3).

A different synthetic pathway was followed for the synthesis of compound **32**, and it is illustrated in Scheme 4; in this case, the lactam was formed after having built the N−Me pyrimidone carboxamide scaffold **79**.

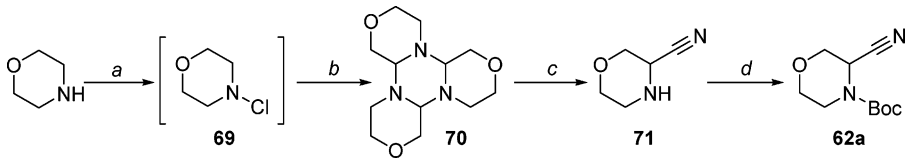
The five-membered derivatives were synthesized in an analogous manner to compounds reported in Scheme 1, and the synthesis is summarized in Scheme 5. Compound **10** was also prepared in the enantiomerically pure form **10a**. This led us to a careful monitoring of the enantiomeric excess at each step of

Scheme 1. Synthesis of Final Compounds **11**, **25**−**30**, and **46**-**49**[a]



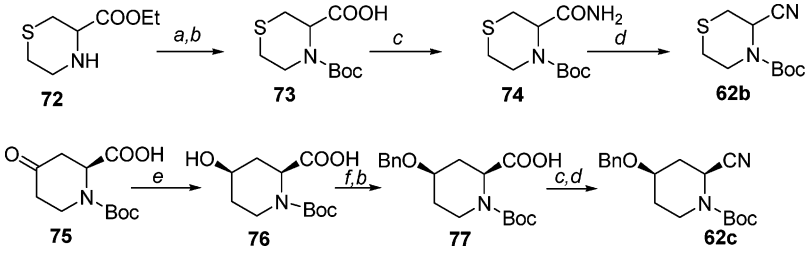
[a] Reagents and conditions (yields are reported for compound **27**): (a) $NH_2OH{\cdot}HCl$, Et_3N, EtOH, reflux, 5 h, 89%; (b) DMAD, $CHCl_3$, reflux, 1 h, 78%; (c) *o*-xylenes, reflux, 12 h, 54%; (d) Bz_2O, Py, 3 h, 71%; (e) LiH, dioxane, Me_2SO_4, 64%; (f) 4F-$BnNH_2$, MeOH, reflux in a sealed tube, 2 h, 68%; (g) TFA-DCM, rt, quant.; (h) H_2, Pd/C, MeOH, rt, 5 h; (i) HCOH or CH_3COH, $NaCNBH_3$, MeOH; (l) Ac_2O, Py; (m) $NaIO_4$, H_2O, EtOH; (n) *m*-CPBA, DCM; (o) NEt_3, MeI, THF. [b]*cis*-Configuration. [c]Reference 29.

Scheme 2. Novel Synthesis of Boc-Protected Morpholine-3-nitrile **62a**[a]



[a] Reagents and conditions: (a) *t*-BuOCl, MTBE; (b) NaOMe, MeOH; (c) HCN, H_2O; (d) Boc_2O, DMAP, DCM.

Scheme 3. Synthesis of Boc-Protected Nitriles **62b** and **62c**[a]



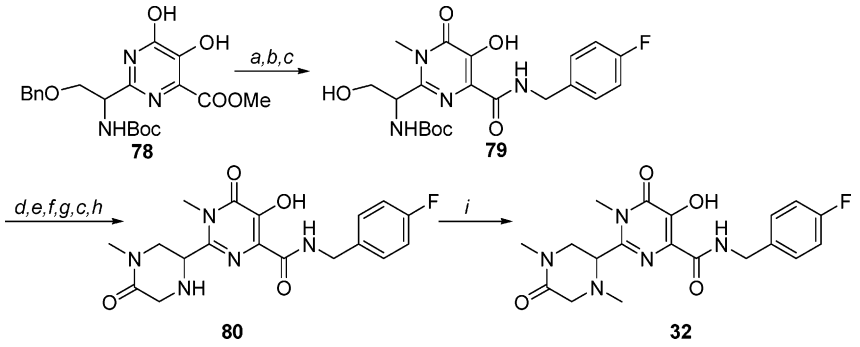
[a] Reagents and conditions: (a) Boc_2O, $NaHCO_3$, H_2O, $CHCl_3$; (b) NaOH, MeOH; (c) Boc_2O, NH_4HCO_3, Py, dioxane, rt;[46] (d) TFAA, Et_3N, DCM, rt; (e) L-selectride, THF, 0 °C, 2.5 h, 95%; (f) NaH, THF, BnBr.

the synthesis, starting from optically active *N*-Boc-L-proline. After the cyclization step, followed by benzoylation of the 5-OH group, the enantiomeric excess of compound **85a** was 84%,[47] and this e.e. was retained after the methylation step for compound **86a**. Recrystallization from ethyl acetate and hexanes gave **86a** as a white solid with 99.8% e.e., and this high enantiomeric purity was maintained until the end of the synthesis.

The necessary nitriles **81b**−**e** were synthesized through manipulation of the appropriate pyrrolidine building blocks according to Scheme 6. Nitriles **81b** and **81e** were prepared from the corresponding pyrrolidinones **91b** and **91e** that were Cbz-protected and then reduced with super hydride to the corresponding lactam alcohols **93b** and **93e**. These were then submitted to the reaction with TMSCN in the presence of ZnI_2 to give the desired nitriles **81b** and **81e**.

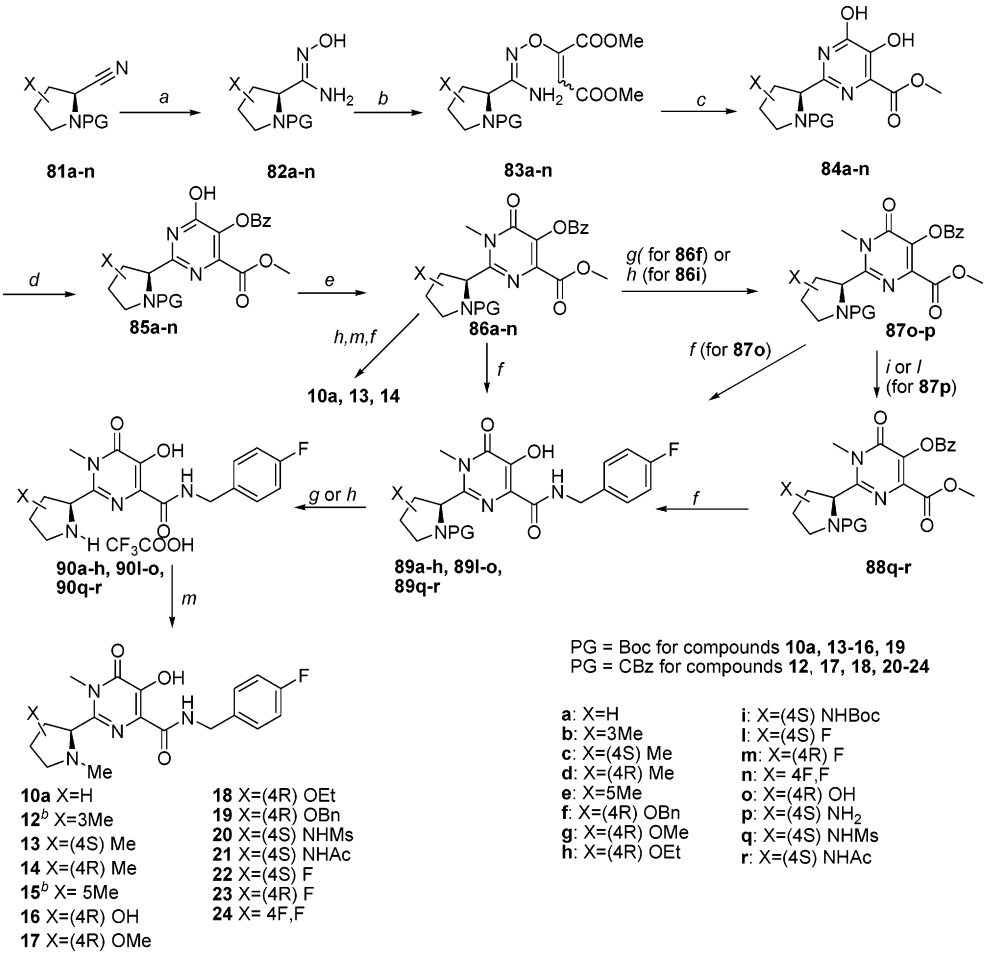
The nitriles **81c** and **81d**, as a mixture, were prepared through the usual steps from the 4-methyl proline carboxylate **95**. Compound **95** was obtained by catalytic hydrogenation of

Scheme 4. Synthesis of Compound **32**[a]



[a] Reagents and conditions: (a) LiH, dioxane, Me_2SO_4; (b) 4F-$BnNH_2$, MeOH, reflux, 2 d; (c) H_2, Pd/C, MeOH, 1 N HCl; (d) Et_3N, DCM, MsCl, rt, 2 h; (e) BnMeNH, CH_3CN, reflux, 3 h; (f) TFA-DCM, rt, 3 h; (g) EtOCOCOH, $NaCNBH_3$, MeOH, Et_3N; (h) Et_3N, MeOH, reflux, 5 h; (i) HCOH, $NaCNBH_3$, MeOH, Et_3N.

Scheme 5. Synthesis of Final Compounds **10a**, and **12**−**24**[a]



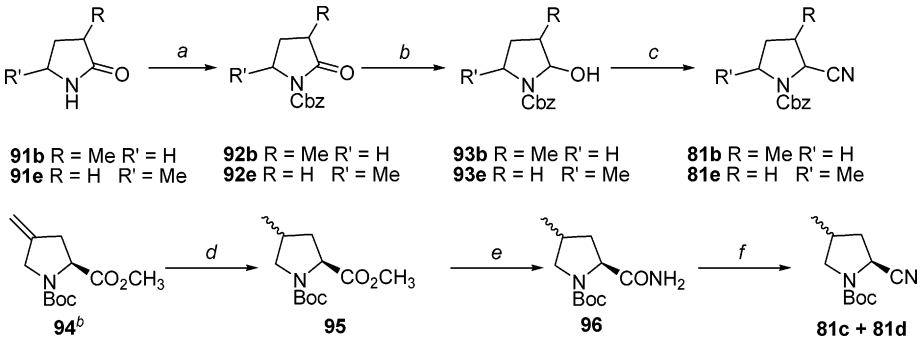
[a] Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, Et_3N, EtOH, reflux, 5 h; (b) DMAD, $CHCl_3$, reflux, 1 h; (c) *o*-xylenes, reflux, 12 h; (d) Bz_2O, Py, 3 h; (e) LiH, dioxane, Me_2SO_4 or Cs_2CO_3, THF, Me_2SO_4; (f) 4F-$BnNH_2$, MeOH, reflux in a sealed tube, 2 h; (g) H_2, Pd/C, MeOH; (h) TFA-DCM, rt; (i) MsCl, Et_3N, DCM, rt, 1 h; (l) Ac_2O, Py, rt, 1 h; (m) HCOH, $NaCNBH_3$, MeOH. [b]Mixture of diastereoisomers, undetermined stereochemistry.

methylene compound **94** that was prepared in three steps, starting from the commercially available (2*S*,4*R*)-4-hydroxy-2-(methoxycarbonyl)pyrrolidinium chloride, using a procedure reported in the literature.[48]

The nitriles **81f**−**h**, having an alkoxy substituent in the 4(*R*)-position, were synthesized from the corresponding carboxylic acids **98**, prepared according to the literature.[49] The nitrile **81i** was obtained through transformation of the (4*R*)-hydroxyl group into a (4*S*)-NHBoc moiety, followed by the usual formation of the primary amide and its dehydration. Fluorinated nitriles **81l**−**n** were obtained by DAST reaction from the appropriate 4-OH-proline (from 4*R*-OH for **81l** and 4*S*-OH for **81m**) or 4-oxo-proline (Scheme 7).

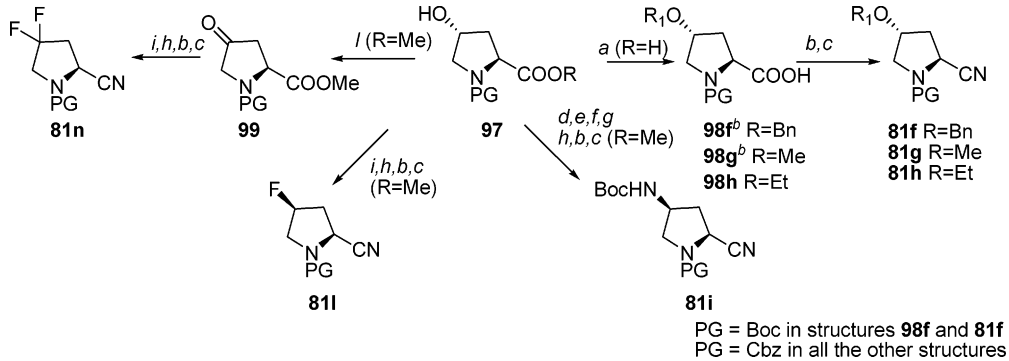
The elaboration steps toward the piperazine compounds of Table 4 are reported in Scheme 8. The orthogonal protection of the piperazine ring with the Boc and the Cbz groups allowed the distinct derivatization of the N1 and N4 nitrogens. The Cbz deprotection of the N−Me pyrimidone **106**, followed by reductive alkylation and formation of the 4F-benzylamide gave the advanced intermediate **36**. This compound was submitted to Boc-deprotection to obtain **33**, which was reductively alkylated to the corresponding compounds **31**, **34**, and **35**.

Scheme 6. Protected Methylpyrrolidine Nitriles **81b**−**e**[a]



[a] Reagents and conditions: (a) NaH, CbzCl, DMF; (b) $LiBEt_3H$, THF; (c) TMSCN, ZnI_2; (d) H_2, PtO_2, MeOH; (e) NH_3 32%, THF; (f) TFAA, Et_3N, DCM. [b]Reference 48.

Scheme 7. Synthesis of Cbz 4-Substituted Pyrrolidine Nitriles **81f**−**n**[a]



[a] Reagents and conditions: (a) NaH, THF, R_1X; (b) Boc_2O, NH_4HCO_3, Py, dioxane, rt, 72%;[46] (c) TFAA, Et_3N, DCM, rt; (d) TsCl, Py; (e) NaN_3, DMF; (f) PPh_3; H_2O, THF; (g) Boc_2O, Et_3N, DCM; (h) NaOH, MeOH; (i) DAST, dry DCM, −78 °C; (l) DMSO, $(COCl)_2$, Et_3N, DCM. [b] Reference 49.

Compound **33** was acylated to derivatives **37**, **39**, and **40** or sulfonylated to compounds **43** and **44**. The ethylurea **41** was obtained by treatment of compound **33** with ethyl isocyanate in pyridine. Compound **38** was obtained by acetylation of the N1 nitrogen followed by Boc-removal and reductive methylation of the N4. The mesylate compound **42** was obtained by leaving the formation of the 4F-benzylamide and the concomitant deprotection of the benzoyl group as the last step.

The SAR on the benzylamide portion of the morpholino series reported in Table 8 was performed following a more convergent approach based on the formation of the carboxamide at the last step (Scheme 9).

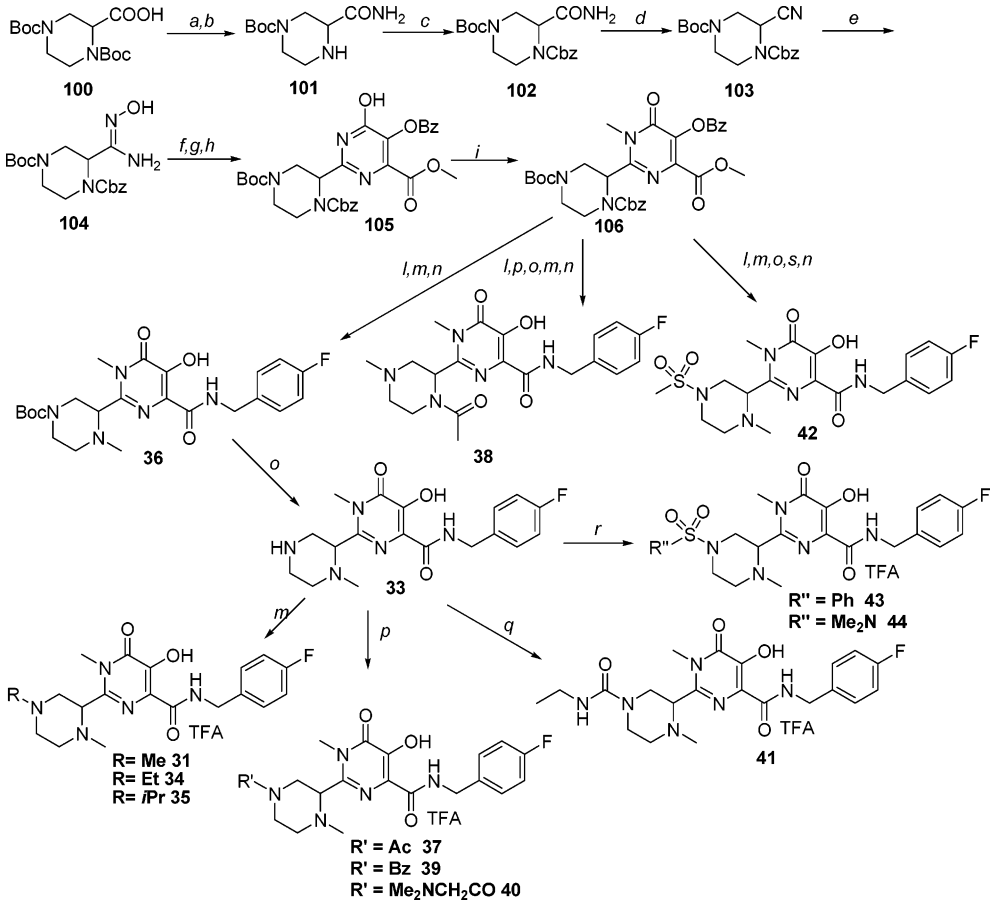
Conclusions

The development of a potent series of *N*-methyl pyrimidone derivatives exhibiting nanomolar potency against replication of HIV-1 in cell-based assay was described. The N-Me morpholine analog **27a** inhibited viral growth with a CIC_{95} of 65 nM in the presence of 50% normal human serum. This level of antiviral potency is comparable to that of the clinically effective protease inhibitors indinavir and nelfinavir (CIC_{95} values of 50 nM and 400 nM, respectively). Compound **27a** displayed excellent biological and physical properties that reflect excellent PK profiles in rats, dogs, and monkeys. It did not show any liabilities in a great number of counterscreening assays and no CyP450 inhibition. Based on this data, it represents a promising antiviral agent against HIV-1.

Experimental Section

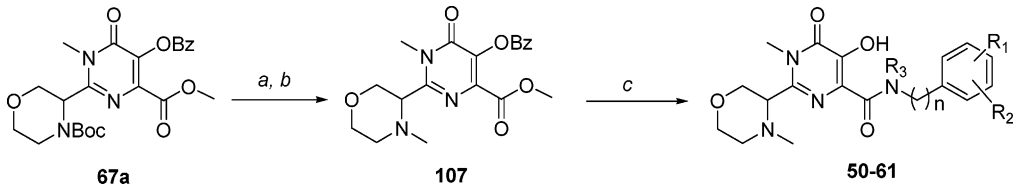
Solvents and reagents were obtained from commercial suppliers and were used without further purification. Flash chromatography purifications were performed on Merck silica gel (200−400 mesh) as the stationary phase or were conducted using prepacked cartridges on a Biotage system, eluting with petroleum ether/ethyl acetate mixtures. HPLC-MS analyses were performed on a Waters Alliance 2795 apparatus, equipped with a diode array and a ZQ mass spectrometer, using an X-Terra C_{18} column (5 μm, 4.6 × 50 mm). The solvent system was acetonitrile−0.1% HCOOH/water−0.1% HCOOH, gradient 10−90% over 6 min, with a flow rate of 1 mL/min. Preparative reversed-phase high-performance liquid chromatography (RP-HPLC) was performed using a Waters Delta Prep 4000 operating with a flow rate of 20 mL/min and incorporating a dual λ absorbance detector 2487 or a Shimadzu HPLC 10AV-VP operating with a flow rate of 15 mL/min and incorporating a diode array detector SPD10AV-VP. The stationary phases used were for compound **27**, PrepNovaPack HR C_{18}, 6 μm-60A (40 × 100 mm); for the other final compounds, Waters Symmetry C_{18} 5 μm, 19 × 100 mm, C_{18} 7 μm, 19 × 150 mm, and C_{18} 7 μm, 19 × 300 mm. The mobile phase comprised a linear gradient of binary mixtures of acetonitrile (containing 0.1% TFA) and H_2O (containing 0.1% TFA). The purity of final compounds was more than 99% by area. As criterion of purity, chromatographic systems with NMR and MS data were employed. Nuclear magnetic resonance spectra (^1H NMR recorded at 600, 500, 400, or 300 MHz, ^{13}C NMR recorded at 100 or 75 MHz) were obtained on Bruker AMX spectrometers and are referenced in ppm relative to TMS. Unless indicated, spectra were acquired at 300 K. Low-resolution mass spectra (*m*/*z*) were recorded on a Perkin-Elmer API 100 (electrospray ionization) mass spectrometer. All the accurate mass measurements were carried out by electrospray ionization (ESI) on TSQ Quantum Ultra AM triple quadrupole, operating in enhanced mass-resolution mode (peak width of 0.2 Th FWMH). Accurate mass determinations by ESI-MS were performed using positive negative ionization mode. HPLC analysis of the final compounds was carried out on two systems: Agilent 1100 HPLC, with chromatography performed on a Waters Sunfire C_{18} column (30 × 2.1 mm, 5 μm) at room temperature, and detection was carried out using a ThermoFinnigan TSQ Quantum Ultra mass-spectrometer equipped with an electrospray (ESI) ion source; Waters Acquity UPLC equipped with a PDA

Scheme 8. Synthesis of Piperazines **31** and **33**−**44**[a]



[a] Reagents and conditions: (a) $SOCl_2$, Py, DMF, THF, 4 h, 40 °C; (b) NH_3 0.5 M in dioxane, DCM, 2 h, 40 °C; (c) CbzCl, Et_3N, DCM, rt, o/n; (d) TFAA, Et_3N, DCM, 0 °C; (e) NH_2OH, Et_3N, MeOH, rt, o/n; (f) DMAD, $CHCl_3$, reflux, 12 h; (g) *o*-xylenes, 160 °C for 8 h and then 120 °C for 48 h; (h) Bz_2O, Py, rt, 3 h; (i) Cs_2CO_3, THF, Me_2SO_4, 40 °C, 1 h; (l) H_2, Pd/C, MeOH; (m) HCOH or CH_3COH or acetone, $NaCNBH_3$, MeOH; (n) 4F-$BnNH_2$, MeOH, reflux in sealed tube, 12 h; (o) TFA−DCM, rt; (p) Ac_2O or Bz_2O in pyridine or *N*,*N*-dimethylglycine hydrochloride, WSCDI, HOBt, DIPEA, DCM; (q) EtNCO, Py, rt, 0.5 h; (r) $PhSO_2Cl$ or Me_2NSO_2Cl, NaOH (1 M), DCM; (s) MsCl, NEt_3, DCM.

Scheme 9. Synthesis of Carboxamides **50**−**61**[a]



[a] Reagents and conditions: (a) TFA−DCM, rt; (b) HCOH, $NaCNBH_3$, MeOH; (c) $R_3R'NH$, MeOH, reflux.

Detector Acquity, and chromatography was performed on an Acquity UPLC BEH C_{18} column (50 × 2.1 mm, 1.7 μM).

***tert*-Butyl 3-[Amino(hydroxyimino)methyl]morpholine-4-carboxylate (63a).** Compound **62a** (40.0 g, 188 mmol) was dissolved in ethanol (530 mL), then NH_2OH·HCl (18.57 g, 266 mmol) and Et_3N (45.0 mL, 320.4 mmol) were added. The reaction mixture was stirred at reflux for 5 h. Ethanol was concentrated and the residue was taken up in EtOAc and water; the aqueous layer was extracted with EtOAc three times, dried (Na_2SO_4), filtered, and concentrated under vacuum to give the desired compound **63a**, 40 g (89% yield), as a yellow solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.16 (bs, 1H), 5.32 (bs, 2H), 4.30 (bs, 1H), 4.08 (d, J = 11.6 Hz, 1H), 3.75 (d, J = 6.8 Hz, 1H), 3.50−3.33 (m, 4H), 1.38 (s, 9H). MS m/z 246 $(M + H)^+$.

Dimethyl 2-[({-Amino[4-(*tert*-butoxycarbonyl)morpholin-3-yl]methylene}amino)oxy]but-2-enedioate (64a). A solution of dimethylacetylene dicarboxylate (228 mL, 226.2 mmol) and compound **63a** (45 g, 188.5 mmol) was refluxed in chloroform (230 mL) for 1 h. The $CHCl_3$ was evaporated under vacuum and the residue was purified by column chromatography (SiO_2, petroleum ether/EtOAc = from 7:3 to 1:1), yielding the desired adducts **64a** as a mixture of configurational isomers *E* and *Z*, 57 g (78% yield). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 6.60 and 6.20 (2bs, 2H), 5.58 and 5.41 (2s, 1H), 4.36 (bs, 1H), 4.04 (bs, 1H), 3.8 (bs, 1H), 3.76 and 3.72 (2s, 3H), 3.63 and 3.58 (2s, 3H), 3.53 (td, J = 13.6, 3.7 Hz, 1H), 3.44 (t, J = 10.4 Hz, 1H), 3.31 (m, 2H), 1.35 (s, 9H). MS m/z 388 $(M + H)^+$.

***tert*-Butyl 3-[4,5-Dihydroxy-6-(methoxycarbonyl)pyrimidin-2-yl]morpholine-4-carboxylate (65a).** The mixture of adducts **64a** (30 g, 77.4 mmol) was refluxed in *o*-xylene (110 mL) for 12 h. The reaction mixture was cooled down; petroleum ether was added until formation of a precipitate, which was filtered to give the desired compound **65a** as a light brown solid, 15.3 g (54% yield). ^{1}H NMR (DMSO-d_6, 400 MHz, 340 K) δ 4.62 (s, 1H), 4.15 (d, J = 12 Hz, 1H), 3.84 (bs, 1H), 3.82 (s, 3H), 3.70 (dd, J = 12.3, 4.0 Hz, 1H), 3.61 (dd, J = 12.2, 3.8 Hz, 1H), 3.56 (t, J = 13 Hz, 1H), 3.43 (td, J = 11.5, 3.4 Hz, 1H), 1.35 (s, 9H). MS m/z 356 $(M + H)^+$.

***tert*-Butyl 3-[5-(Benzoyloxy)-4-hydroxy-6-(methoxycarbonyl)-pyrimidin-2-yl]morpholine-4-carboxylate (66a).** The pyrimidine

65a (23 g, 64.8 mmol) in dry pyridine (260 mL) was treated with benzoic anhydride (29.32 g, 129.6 mmol) and stirred overnight at rt. The mixture was evaporated, taken in ethyl acetate, and washed with HCl (1 N), $NaHCO_3$ satd soln, and brine. Organics were dried (Na_2SO_4), filtered, evaporated, and purified by flash chromatography (SiO_2, petroleum ether/EtOAc = 3:7) to obtain **66a** as a brown solid (21 g, 71% yield). ^{1}H NMR (DMSO-d_6, 400 MHz, 340 K) δ 13.3 (bs, 1H), 8.07 (d, J = 7.5 Hz, 2H), 7.76 (t, J = 7.5 Hz, 1H), 7.61 (t, J = 7.5 Hz, 2H), 4.73 (s, 1H), 4.22 (d, J = 12.4 Hz, 1H), 3.86 (d, J = 11.0 Hz, 1H), 3.78 (dd, J = 12.4, 3.9 Hz, 1H), 3.73 (s, 3H), 3.58 (t, J = 13.9 Hz, 2H), 3.47 (td, J = 10.7, 3.6 Hz, 1H), 1.36 (s, 9H). MS m/z 600 $(M + H)^+$.

Methyl 5-(Benzoyloxy)-2-[4-(*tert*-butoxycarbonyl)morpholin-3-yl]-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxylate (67a). The benzoyl-protected pyrimidine **66a** (82 g, 87 wt %, 156 mmol) was added to a suspension of LiH (1.36 g, 1.1 equiv) in dioxane (714 mL) at rt. The mixture was aged 45 min at 38 °C and was then cooled to rt. Dimethylsulfate (19 mL, 1.3 equiv) was added, and the mixture was warmed to 38 °C (4 h) and 56 °C (4 h). The reaction mixture was cooled to 16 °C and glacial acetic acid (0.89 mL, 0.1 equiv) was added, followed by water (714 mL) and EtOAc (714 mL). The aqueous layer was separated and extracted with EtOAc (400 mL). The combined organic layer was dried (Na_2SO_4) and concentrated to an oil containing the two regioisomeric products N−Me (**67a**) and O−Me (**68a**) in the ratio 9.4:1.[41] The two regioisomers were separated by column chromatography (SiO_2, EtOAc/hexanes = 1:1). The fractions containing the desired compound **67a** were evaporated to a foamy solid. This solid was dissolved in ethyl ether and re-evaporated to a foamy solid that could be scraped out easily. This solid was dried in a vacuum oven overnight at 40 °C to afford *N*-methylpyrimidone **67a** as a pale yellow solid, 48 g (64% yield). Crystalline *N*-methylpyrimidone **67a** was obtained from ether. (**67a**) ^{1}H NMR (DMSO-d_6 + TFA, 400 MHz, 330 K) δ 8.09 (d, J = 7.3 Hz, 2H), 7.77 (t, J = 7.5 Hz, 1H), 7.62 (t, J = 7.8 Hz, 2H), 5.08 (d, J = 3.4 Hz, 1H), 4.21 (d, J = 12.3 HZ, 1H), 3.95−3.85 (m, 3H), 3.76 (s, 3H), 3.58 (s, 3H), 3.55−3.50 (m, 2H), 1.34 (s, 9H). MS m/z 474 $(M + H)^+$. (**68a**) ^{1}H NMR (DMSO-d_6, 400 MHz, 330 K) δ 8.09 (d, J = 7.3 Hz, 2H), 7.77 (t, J = 7.3 Hz, 1H), 7.61 (t, J = 7.4 Hz, 1H), 4.94 (bs, 1H), 4.50 (d, J = 11.6 Hz, 1H), 4.0 (s, 3H), 3.85−3.81 (m, 2H), 3.76 (s, 3H), 3.66 (d, J = 10.4 Hz, 1H), 3.49−3.45 (m, 2H), 1.35 (bs, 9H). MS m/z 474 $(M + H)^+$.

***tert*-Butyl 3-(4-{[(4-Fluorobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)morpholine-4-carboxylate (49).** The methyl ester **67a** (9.2 g, 19.43 mmol) in dry MeOH (190 mL) was treated with 4-fluorobenzylamine (5.55 mL, 48.6 mL) at reflux in a sealed tube for 2 h. Solvent was removed in vacuum and the residue was triturated with Et_2O to obtain the title compound **49** as a white solid (6.14 g, 68% yield). ^{1}H NMR (DMSO-d_6, 400 MHz, 320 K) δ 11.95 (bs, 1H), 8.32 (t, J = 6.0 Hz, 1H), 7.39−7.35 (m, 2H), 7.19−7.13 (m, 2H), 4.96 (dd, J = 4.2, 2.4 Hz, 1H), 4.62 (dd, J = 6.9, 4.2 Hz, 1H), 4.49 (dd, J = 14.9, 5.8 Hz, 1H), 4.16 (dd, J = 12.2, 2.0 Hz, 1H), 3.87−3.79 (m, 2H), 3.70−3.64 (m, 1H), 3.55−3.45 (m, 5H), 1.23 (s, 9H). MS m/z 463 $(M + H)^+$.

3-(4-{[(4-Fluorobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)morpholin-4-ium Trifluoroacetate (46). Compound **49** (6.13 g, 13.25 mmol) was treated with a mixture of DCM/TFA (2:1, 420 mL) for 1 h at rt. Volatiles were removed under vacuum to give a residue that upon addition of Et_2O formed a precipitate that was filtered as a pale pink solid **46** (quantitative yield). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.45 (bs, 1H), 7.39−7.36 (m, 2H), 7.19−7.15 (m, 2H), 4.93 (d, J = 9.2 Hz, 1H), 4.64 (dd, J = 15.4, 6.7 Hz, 1H), 4.55 (dd, J = 15.4, 6.2 Hz, 1H), 4.35 (d, J = 12.8 Hz, 1H), 4.08 (d, J = 12.6 Hz, 1H), 3.77 (t, J = 12.4 Hz, 1H), 3.55 (s, 3H), 3.54−3.46 (m, 2H), 3.40−3.34 (m,1H). MS m/z 363 $(M + H)^+$.

3-(4-{[(4-Fluorobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-4-methylmorpholin-4-ium Trifluoroacetate (27). Compound **46** (4.3 g, 9 mmol) was dissolved in MeOH (9 mL) and treated with TEA (1.37 mL, 9.9 mmol), sodium acetate (1.18 g, 14.4 mmol), formaldehyde 37% w/w aq soln (2.2 mL, 27 mmol), and sodium cyanoborohydride (0.798 g, 12.87 mmol). The mixture was left stirring at rt for 1 h. Volatiles were removed under vacuum and the crude was purified by preparative HPLC giving compound **27** as trifluoroacetate salt. ^{1}H NMR (DMSO-d_6 + TFA, 600 MHz) δ 12.33 (bs, 1H), 10.05 (bs, 1H), 9.48 (t, J = 6.4 Hz, 1H), 7.35−7.33 (m, 2H), 7.15−7.12 (m, 2H), 4.98 (d, J = 8.8 Hz, 1H), 4.57 (d, J = 6.4 Hz, 2H), 4.36 (d, J = 12.7 Hz, 1H), 4.13 (d, J = 12.4 Hz, 1H), 3.77 (t, J = 12.5 Hz, 1H), 3.69 (d, J = 12.8 Hz, 1H), 3.54 (s, 3H), 3.48−3.41 (m, 2H), 2.83 (s, 3H). ^{13}C NMR (DMSO-d_6 + TFA, 100 MHz) δ 168.4, 161.7, 157.8, 148.0, 142.7, 134.6, 129.2, 124.8, 115.5, 68.1, 64.2, 62.7, 53.4, 41.7, 41.4, 30.7. MS m/z 377 $(M + H)^+$. HRMS calcd for $C_{18}H_{22}FN_4O_4$ $(M + H)^+$, 377.16196; found, 377.16156. Compound **27** was resolved into its enantiomers by semipreparative chiral HPLC on column Chiralpak AS, 250 × 46 mm at 1.0 mL/min, collected by absorption at 260 nM, mobile phase: hexane:ethanol=1:1 both containing 0.2% TFA. The first eluted was the enantiomer **27a** $[\alpha]_D$ = +55.42 (MeOH, *c* 0.24, 25 °C). The second eluted was the enantiomer **27b** $[\alpha]_D$ = −51.63 (MeOH, *c* 0.215, 25 °C). Compound **27a** as trifluoroacetate was converted in the hydrochloride salt by dissolving it in dry MeOH and adding to that solution HCl (2 N) in Et_2O in a ratio of 5:1. The methanolic solution was evaporated under vacuum, and the procedure was repeated five times; the dry material was recrystallized by MeOH to give **27a** hydrochloride salt as a white crystalline solid. Anal. Calcd for $C_{18}H_{22}FN_4O_4Cl$: C, 52.32: H, 5.35; N, 13.56. Found: C, 52.61; H, 5.64; N, 13.62.

4-Ethyl-3-(4-{[(4-fluorobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)morpholin-4-ium Trifluoroacetate (47). Following the same procedure used to synthesize compound **27**, compound **47** was prepared from compound **46** using acetaldehyde instead of formaldehyde. ^{1}H NMR (DMSO-d_6 + TFA, 300 MHz) δ 10.03 (bs, 1H), 9.65 (t, J = 6.2 Hz, 1H), 7.36 (dd, J = 8.3, 5.8 Hz, 2H), 7.17 (t, J = 8.7 Hz, 2H), 5.05 (d, J = 8.3 Hz, 1H), 4.65 (dd, J = 15.4, 6.9 Hz, 1H), 4.51 (dd, J = 15.4, 5.8 Hz, 1H), 4.40 (d, J = 12.9 Hz, 1H), 4.19 (d, J = 12.2 Hz, 1H), 3.85 (t, J = 13.4 Hz, 2H), 3.55 (s, 3H), 3.55−3.48 (m, 1H), 3.40−3.25 (m, 2H), 3.20−3.07 (m, 1H), 1.19 (t, J = 7.2 Hz, 3H). MS m/z 391 $(M + H)^+$.

2-(4-Acetylmorpholin-3-yl)-*N*-(4-fluorobenzyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide (48). Compound **67a** (0.184 g, 0.39 mmol) was treated with a TFA/DCM/H_2O solution (65:35:10, 10 mL) at rt for 30 min and then evaporated under vacuum to give a compound that was taken up in Et_2O and evaporated several times before being dissolved in pyridine (2 mL) and treated with acetic anhydride (0.073 mL, 0.78 mmol). The reaction mixture was stirred at rt overnight and then concentrated to dryness. The resulting crude (0.39 mmol) was then dissolved in MeOH (3.0 mL), and 4-fluorobenzylamine (0.133 mL, 1.17 mmol) was added; the mixture was refluxed overnight. The reaction mixture was then evaporated under vacuum to give a residue that was purified by RP-HPLC to provide compound **48**. ^{1}H NMR (DMSO-d_6, 300 MHz, 330 K) δ 12.00 (bs, 1H), 8.45 (bs, 1H), 7.37 (t, J = 7.8 Hz, 2H), 7.16 (t, J = 8.6 Hz, 2H), 5.25 (s, 1H), 4.61−4.48 (m, 2H), 4.22 (d, J = 12.4 Hz, 1H), 3.88−3.76 (m, 3H), 3.58−3.48 (m, 2H), 3.48 (s, 3H), 2.03 (s, 3H). MS m/z 405 $(M + H)^+$.

Methyl 5-(Benzoyloxy)-2-{1-[(benzoyloxy)carbonyl]piperidin-2-yl}-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxylate (67d). Pyrimidine **66d** was dissolved in THF and treated with Cs_2CO_3 (2.0 equiv) and methyl iodide (5.0 equiv). The reaction mixture was stirred at 40 °C for 2 h, cooled to rt, and concentrated. The residue was taken up in ethyl acetate and washed with 1 N HCl and brine. The organic layer was separated, dried (Na_2SO_4), filtered, and concentrated, and the residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = 6:4) to give compound **67d** in 52% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.14 (d, J = 7.9 Hz, 2H), 7.66 (t, J = 7.4 Hz, 1H), 7.61 (t, J = 7.5 Hz, 2H), 7.37−7.34 (m, 5H), 5.30 (bs, 1H), 5.22 (d, J = 12.2 Hz, 1H), 5.17 (d, J = 12.2 Hz, 1H), 4.14−4.11 (m, 1H), 3.83 (s, 3H), 2.99

(s, 3H), 2.91−2.88 (m, 1H), 2.55−2.52 (m, 1H), 1.78−1.52 (m, 5H). MS *m*/*z* 506 $(M + H)^+$.

***N*-(4-Fluorobenzyl)-5-hydroxy-1-methyl-2-(1-methylpiperidin-2-yl)-6-oxo-1,6-dihydropyrimidine-4-carboxamide (11).** Compound **67d** was taken up in methanol and hydrogenated at atmospheric pressure in the presence of 10% Pd/C. The mixture was stirred 5 h at rt, filtered through celite, and concentrated. The residue was suspended in THF and treated with TEA (3 equiv) and methyl iodide (3 equiv). The reaction mixture was stirred at 40 °C for 0.5 h, then concentrated and partitioned between EtOAc and 1 N HCl. The organic layer was washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure to an oily residue that was dissolved in *N*-methylpyrrolidinone and treated with 4-fluorobenzylamine (3 equiv) at 90 °C for 15 min. The reaction mixture was cooled to rt, and the title product **11** was isolated as its trifluoroacetate salt by RP-HPLC. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.28 (bs, 1H), 9.50 (bt, 1H), 9.31 (bs, 1H), 7.37 (dd, J = 8.4, 5.6 Hz, 2H), 7.18 (t, J = 8.8 Hz, 2H), 4.8−4.6 (m, 1H), 4.57 (d, J = 6.4 Hz, 2H), 3.70−3.60 (m, 1H), 3.50 (s, 3H), 3.40−3.30 (m, 1H), 2.78 (bs, 3H), 2.4−2.3 (m, 1H), 1.92−1.46 (m, 5H). MS *m*/*z* 375 $(M + H)^+$.

Morpholine-3-carbonitrile (71). In a flask fitted with a condenser and a thermocouple, a solution of morpholine (43.6 g, 0.5 mol) in THF (81 mL) was prepared and cooled to −15 °C. To this, *t*-BuOCl (56.5 mL, 0.5 mol) was added over 15 min, keeping the internal temperature <0 °C to generate **69**. After 30 min at this temperature, 25% NaOMe/MeOH (114 mL, 0.5 mol) was added over 15 min, keeping the internal temperature <3 °C. The mixture was allowed to warm to 45 °C over 1 h then cooled to rt to generate **70**. (***Caution:*** Warm up gradually to prevent a violent reflux.) The resulting slurry was filtered over celite and rotary evaporated. The residue was diluted with water (100 mL) and solid KCN (32.6 g, 0.5 mol) was added, stirred until most of the solid dissolved, and cooled to 0 °C. Concd HCl (60 mL) was added over 20 min, keeping the internal temperature <20 °C then stirring for 2 h at rt. (***Caution:*** Acidification generates HCN, therefore, use of fumehood is required and use of caustic scrubber advised to catch any vented gas.) This mixture was cooled and basified to pH 10 with 50% NaOH (12 mL). This was extracted five times with DCM (50 mL), dried (Na_2SO_4), rotary evaporated to a residue that was chromatographed (SiO_2, EtOAc containing 0.2% TEA), and concentrated to an oil, which crystallized on standing. The solids were stirred in EtOAc/hexanes = 1:1 and filtered to afford **71** in two crops, 23 g (41% yield). Mp: 81.4 − 87.3 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.9−3.7 (m, 4H), 3.60 (m, 1H), 3.22 (m, 1H), 2.79 (td, J = 12.3, 2.9 Hz, 1H), 2.16 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 118.7, 68.1, 67.5, 46.4, 42.8. LRMS *m*/*z* 113 (M + H). HRMS *m*/*z* 113.0713 (M + H), 113.0715 (expected).

***tert*-Butyl 3-Cyanomorpholine-4-carboxylate (62a).** Morpholine nitrile **71** (51.6 g) was dissolved in DCM (350 mL), and DMAP (2.2 g, 0.04 equiv) was added. This was cooled over ice and BOC-anhydride (110 g, 1.1 equiv) was added. The mixture was stirred 3 h at rt and then was quenched with the addition of water (200 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated to a solid, which was crystallized from EtOAc/hexanes in two crops to afford **62a**, 52.5 g. The mother liquor was purified by flash chromatography (ethyl acetate/hexanes = 3:7) to afford 28 g additional **62a** (82% yield). Mp 99.7−101.3 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.88 (brs, 1H), 4.04 (d, J = 11.9 Hz, 1H), 3.94 (brd, J = 10.5 Hz, 1H), 3.80 (brs, J = 12.7 Hz, 1H), 3.61 (dd, J = 11.8, 3.1 Hz, 1H), 3.47 (dt, J = 11.9, 2.8 Hz,1H), 3.22 (brs, 1H), 1.48 (9H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 153.7, 116.6, 82.3, 67.6, 66.6, 44.0, 41.3, 28.2. LRMS *m*/*z* 157 (M + H − *t*-Bu). HRMS *m*/*z* 235.1055 (M + Na), 235.1059 (expected).

4-(*tert*-Butoxycarbonyl)thiomorpholine-3-carboxylic Acid (73). To a solution of ethyl thiomorpholine-3-carboxylate **72**[44] (15.5 g, 0.11 mol) in $CHCl_3$, $NaHCO_3$ (9.2 g, 0.11 mol), and NaCl (22 g) in H_2O (168 mL), followed by $(Boc)_2O$ (26.4 g, 0.12 mol), were added. The reaction mixture was stirred at reflux for 4 h. The aqueous phase was washed with $CHCl_3$, and the organic phase was washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure. The yellow oil obtained was dissolved in MeOH (200 mL) and NaOH (2 M) was added (160 mL). The reaction mixture was stirred at rt for 3 h. MeOH was removed under reduced pressure and the aqueous phase was washed with Et_2O. To the aqueous phase AcOEt was added, and the mixture was cooled on ice bath and acidified with HCl (6 N). The organic phase was washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure to afford compound **73** as a yellow solid, which was used in the next step as such (67% yield). ^{1}H NMR (DMSO-d_6, 400 MHz, 340 K) δ 12.52 (bs, 1H), 4.92 (bs, 1H), 4.13 (d, J = 13.7 Hz, 1H), 3.20−2.82 (m, 3H, (1H hidden by water signal), 2.66−2.52 (m, 2H), 1.41 (s, 9H). MS *m*/*z* 248 $(M + H)^+$.

***tert*-Butyl 3-Cyanothiomorpholine-4-carboxylate (62b)**. To a solution of carboxylic acid **73** (18 g, 73 mmol) in dioxane (110 mL) and pyridine (3.65 mL), $(Boc)_2O$ (20.7 g, 95 mmol) and NH_4HCO_3 (7.3 g, 92 mmol) were added. The reaction mixture was stirred at rt overnight. The solvent was removed under reduced pressure, and the crude was taken into EtOAc, washed with water, 1 N HCl, and brine, dried (Na_2SO_4), and evaporated under reduced pressure to give a crude compound **74** as a yellow oil (18 g, 73 mmol), which was suspended in DCM (500 mL) and TEA (21.3 mL, 153 mmol) and treated with TFAA (11.2 mL, 80 mmol) added dropwise. After 20 min, $NaHCO_3$ satd soln was added, DCM was washed with water and brine, dried (Na_2SO_4), and evaporated under reduced pressure to give compound **62b** as a yellow solid (85% yield). ^{1}H NMR (DMSO-d_6, 400 MHz, 340 K) δ 5.58 (t, J = 3.4 Hz, 1H), 4.23 (d, J = 14.0 Hz, 1H), 3.10−2.81 (m, 3H, 1H hidden by water signal), 2.71−2.46 (m, 2H), 1.45 (s, 9H).

***tert*-Butyl 3-[5-(Benzoyloxy)-4-(methoxycarbonyl)-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl]thiomorpholine-4-carboxylate (67b).** The product was obtained in five steps from *tert*-butyl 3-cyanothiomorpholine-4-carboxylate **62b**, following the same steps described for the synthesis of compound **67a** (4.8% yield over five steps). ^{1}H NMR (DMSO-d_6 + TFA, 340 K) δ 8.08 (d, J = 7.6 Hz, 2H), 7.75 (t, J = 6.8 Hz, 1H), 7.64.7.57 (m, 2H), 5.54−5.23 (m, 1H), 3.98−3.82 (m, 2H), 3.74 (s, 3H), 3.58 (s, 3H), 3.36−3.10 (m, 2H), 2.87−2.65 (m, 2H), 1.31 (s, 9H). MS *m*/*z* 490 $(M + H)^+$.

***tert*-Butyl 3-(4-{[(4-Fluorobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)thiomorpholine-4-carboxylate (49b).** From compound **67b**, following the same step described for compound **49**, compound **49b** was obtained in 51% yield. ^{1}H NMR (DMSO-d_6 + TFA, 340 K) δ 8.77 (bs, 1H), 7.40−7.33 (m, 2H), 7.15−7.08 (m, 2H), 5.12 (bs, 1H), 4.56−4.49 (m, 2H), 3.85−3.18 (m, 2H), 3.48 (s, 3H), 3.25 (dd, J = 14.0, 7.5 Hz, 1H), 3.01 (dd, J = 14.0, 4.0 Hz, 1H), 2.70−2.58 (m, 2H), 1.28 (s, 9H). MS *m*/*z* 479 (M + H).

***N*-(4-Fluorobenzyl)-5-hydroxy-1-methyl-2-(4-methylthiomorpholin-3-yl)-6-oxo-1,6-dihydropyrimidine-4-carboxamide (28).** Compound **49b** (330 mg, 0.67 mmol) was deprotected and submitted to the next reductive alkylation, following the same step described for compound **27**, to obtain compound **28** in 47% yield. ^{1}H NMR (DMSO-d_6 + TFA, 400 MHz) δ 10.80 (bs, 1H), 10.09 (bs, 1H), 7.41 (t, J = 7.8 Hz, 2H), 7.13 (t, J = 8.7 Hz, 2H), 5.05−4.90 (m, 1H), 4.56−4.40 (m, 2H), 3.88 (d, J = 12.2 Hz 1H), 3.55 (s, 3H), 3.50−3.26 (m, 3H), 3.05−2.95 (m, 2H), 2.76 (s, 3H). MS *m*/*z* 393 (M + H).

***N*-(4-Fluorobenzyl)-5-hydroxy-1-methyl-2-(4-methyl-1-oxidothiomorpholin-3-yl)-6-oxo-1,6-dihydropyrimidine-4-carboxamide (29).** To a solution of compound **28** (20 mg, 0.04 mmol) in EtOH (0.5 mL), a solution of 0.34 M of $NaIO_4$ (117 μL) was added dropwise at rt. After 18 h, the solvent was removed under reduced pressure. Purification by prep HPLC gave compound **29** as a 9:1 mixture of diastereoisomers in 45% yield. ^{1}H NMR (DMSO-d_6 + TFA, 400 MHz) δ 10.15 (bs, 1H), 7.41 (t, J = 8.0 Hz, 2H), 7.11 (t, J = 8.4 Hz, 2H), 5.41 (d, J = 11.2 Hz, 0.9H), 5.26 (d, J = 11.2 Hz, 0.1H), 4.56−4.34 (m, 2H), 4.08−3.82 (m, 1H), 3.80−3.57 (m, 2H), 3.56−3.37 (m, 4H), 3.35−3.04 (m, 2H), 2.85 (s, 2.7H), 2.73 (s, 0.3H). MS *m*/*z* 409 (M + H).

***N*-(4-Fluorobenzyl)-5-hydroxy-1-methyl-2-(4-methyl-1,1-dioxidothiomorpholin-3-yl)-6-oxo-1,6-dihydropyrimidine-4-carboxamide (30).** To a solution of compound **28** (20 mg, 0.04 mmol)

in DCM (0.5 mL), *m*-CPBA (19.7 mg, 0.08 mmol) was added portionwise at 0 °C. After 18 h at rt, solvent was removed under reduced pressure. Purification by prep HPLC gave compound **30**. ^{1}H NMR (DMSO-d_6 + TFA, 300 MHz) δ 9.18 (bs, 1H), 7.39−7.33 (m, 2H), 7.15−7.05 (m, 2H), 5.67 (d, J = 10.4 Hz, 1H), 4.85−4.7 (m, 1H), 4.60−4.40 (m, 2H), 4.15−3.95 (m, 2H), 3.70−3.25 (m, 3H), 3.60 (s, 6H). MS *m*/*z* 425 (M + H).

***tert*-Butyl-4-(benzyloxy)-2-cyanopiperidine-1-carboxylate (62c).** Compound **75** (prepared according to a literature procedure;[45] 7.18 g, 30 mmol) was dissolved in dry THF (200 mL), then L-Selectride (1 M in THF, 70 mL, 70 mmol) was added at 0 °C, and the mixture was stirred at 0 °C for 2.5 h. After quenching with water, boranes were removed by extraction, the aqueous phase was acidified with 1 N HCl and extracted with EtOAc, and the organic layer was washed with water and brine, dried (Na_2SO_4), filtered, and evaporated under vacuum. Crude **76** (6.85 g, 95%) was used as such. Part of crude **76** (2.96 g, 12.5 mmol) was dissolved in THF (150 mL) and 60% NaH in mineral oil (2.0 g, 50 mmol) was added at 0 °C; after stirring at rt for 40 min, BnBr (6.0 mL, 50 mmol) was added, again at 0 °C, and the mixture was stirred at rt overnight. Quenching with water and evaporation of solvent gave a bis-benzylated product that was taken in MeOH (50 mL) and hydrolyzed with 2 N aq NaOH (25 mL) at 50 °C for 4 h. After acidification with $KHSO_4$, the product was extracted with EtOAc, and the organic layer was washed with water and brine, dried (Na_2SO_4), filtered, and evaporated under vacuum. The crude was purified by chromatography to provide compound **77** (2.86 g, 68%). Compound **77** (2.85 g, 8.5 mmol) was dissolved in pyridine (25 mL), then Boc_2O (2.14 g, 9.8 mmol) and NH_4HCO_3 (0.77 g, 9.8 mmol) were sequentially added, and the mixture was stirred at rt overnight. After removing volatiles, the product was taken in EtOAc, the organic layer was washed with 1 N HCl, $NaHCO_3$ satd soln, and brine, dried (Na_2SO_4), filtered, and evaporated under vacuum. The crude was dissolved in DCM (30 mL), and TEA (3.5 mL, 25.5 mmol) was added. The resulting mixture was cooled at 0 °C, TFAA (1.8 mL, 12.7 mmol) was added, and the mixture was stirred at rt for 2 h. Solvent was evaporated under vacuum, the residue was taken up in EtOAc, the organic layer was washed with 1 N HCl, satd $NaHCO_3$, and brine, dried (Na_2SO_4), filtered, and evaporated under vacuum. The crude was purified by chromatography to provide compound **62c** (1.87 g, 70%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.43 (bd, J = 7.2 Hz, 2H), 7.36 (bt, J = 7.4 Hz, 2H), 7.27−7.31 (m, 1H), 5.19 (bs, 1H), 4.73 (d, J = 12.0 Hz, 1H), 4.54 (d, J = 12.0 Hz, 1H), 3.90 (bs, 1H), 3.85 (t, J = 2.7 Hz, 1H), 3.41 (bs, 1H), 2.32 (d, J = 14.5 Hz, 1H), 1.99 (bd, J = 13.5 Hz, 1H), 1.83 (ddd, J = 14.6, 6.3, 2.5 Hz, 1H), 1.55−1.64 (m, 1H), 1.50 (s, 9H).

Methyl 5-(Benzoyloxy)-2-[4-(benzyloxy)-1-(*tert*-butoxycarbonyl)piperidin-2-yl]-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxylate (67c). A solution of hydroxylamine hydrochloride (688 mg, 9.9 mmol) in MeOH (20 mL) was added at 0 °C to a solution of KOH (557 mg, 9.9 mmol) in MeOH (20 mL). After stirring for 10 min, KCl was filtered off, and the filtrate was added to a solution of **62c** (1.87 g, 5.9 mmol) in MeOH (25 mL); the mixture was stirred at 55 °C overnight. Additional hydroxylamine (0.8 equiv, prepared in the same way) was added and stirring was continued for 5 h. Evaporation to dryness gave a crude **63c** (2.3 g, 100%) that was used as such. A solution of amidoxime **63c** (5.9 mmol) and dimethyl acetylene dicarboxylate (1.1 g, 7.7 mmol) in $CHCl_3$ (50 mL) was stirred at rt overnight. The crude product **64c**, obtained after evaporation of the solvent under vacuum, was used as such for the following cyclization reaction. A solution of **64c** (theoretical, 5.9 mmol from previous step) in toluene (120 mL) was refluxed (Dean−Stark trap) for 48 h. The mixture was evaporated under vacuum, the resulting crude **65c** was dissolved in pyridine (30 mL), and benzoic anhydride (3.0 g, 13 mmol) was added. After 5 h of stirring at rt, the mixture was evaporated under vacuum and the residue was dissolved in EtOAc, washed with 1 N HCl, $NaHCO_3$ satd soln, and brine, dried over Na_2SO_4, filtered, and evaporated under vacuum to provide crude benzoate, which was purified by flash chromatography to provide **66c** (632 mg, 19% yield from amidoxime **63c**) and about 10% of the corresponding *trans*-isomer. A solution of compound **66c** (547 mg, 1.0 mmol) in dry 1,4-dioxane (5 mL) was added at rt to a suspension of LiH (9.3 mg, 1.2 mmol) in dry dioxane (3 mL). After stirring 45 min at 40 °C, Me_2SO_4 (0.14 mL, 1.5 mmol) was slowly added at rt, and the mixture was stirred at 55 °C for 4 h (additional Me_2SO_4 was added). After pouring into brine, the reaction mixture was extracted with AcOEt, washed with brine, and dried over Na_2SO_4, filtered, and evaporated under vacuum. Crude was purified by column chromatography (SiO_2, AcOEt/petroleum ether = 1:1) to afford **67c** (345 mg, yield 62%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.18 (d, J = 7.3 Hz, 2H), 7.63 (t, J = 7.3 Hz, 1H), 7.50 (t, J = 7.6 Hz, 2H), 7.19−7.33 (m, 5H), 4.90 (bs, 1H), 4.50 (d, J = 11.9 Hz, 1H), 4.39 (d, J = 11.9 Hz, 1H), 4.02−4.16 (m, 1H), 3.85−3.90 (m, 1H), 3.77 (s, 3H), 3.60−3.67 (m, 1H), 3.58 (s, 3H), 2.11−2.28 (m, 2H), 1.85−1.95 (m, 2H), 1.38 (s, 9H). MS *m*/*z* 578 $(M + H)^+$.

2-[4-(Benzyloxy)-1-methylpiperidin-2-yl]-*N*-(4-fluorobenzyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide (26). Compound **67c** (340 mg, 0.59 mmol) was dissolved in MeOH (20.0 mL) and 4-fluorobenzylamine (0.30 mL, 2.7 mmol) was added, and the mixture was refluxed for 4 h. Evaporation to dryness gave crude **49c**, which was used as such. Crude **49c** was then treated for 2 h at rt with a TFA/DCM (2.5:1) solution, then evaporated under vacuum to give a crude (1.38 g) that was purified by RP-HPLC to provide **46c** (61% overall yield from **67c**). ^{1}H NMR (CD_3OD, 400 MHz) δ 7.30−7.44 (m, 7H), 7.10 (t, J = 8.7 Hz, 2H), 4.76 (bd, J = 12.1 Hz, 1H), 4.58−4.67 (m, 4H), 3.91−4.00 (m, 1H), 3.62−3.70 (m, 1H), 3.62 (s, 3H), 3.25 (t, J = 12.2 Hz, 1H), 2.71 (bd, J = 13.7 Hz, 1H), 2.43 (bd, J = 13.1 Hz, 1H), 1.69−1.80 (m, 1H), 1.57−1.66 (m, 1H). MS *m*/*z* 467 $(M + H)^+$. Crude **46c** (0.11 mmol) was dissolved in MeOH (2.0 mL), TEA (0.04 mL, 0.3 mmol), sodium acetate (33 mg, 0.4 mmol), acetic acid (0.023 mL, 0.4 mmol), and sodium cyanoborohydride (25 mg, 0.4 mmol) were added to the solution, followed by a 37% formaldehyde solution in water (0.15 mL, 2.0 mmol). The reaction mixture was stirred at rt for 5 h, then evaporated to get the *N*-methylated product **26** that was purified by RP-HPLC (35 mg, 56%). ^{1}H NMR (CD_3OD, 400 MHz) δ 7.39−7.43 (m, 2H), 7.27−7.35 (m, 5H), 7.09 (bt, J = 8.8 Hz, 2H), 4.76−4.84 (m, 1H), 4.63 (s, 2H), 4.61 (bs, 2H), 3.85−3.93 (m, 1H), 3.78 (bd, J = 13.1 Hz, 1H), 3.62 (s, 3H), 3.33−3.39 (m, 1H), 2.86 (s, 3H), 2.67 (bd, J = 14.1 Hz, 1H), 2.42 (bd, J = 14.1 Hz, 1H), 1.78−2.00 (m, 1H), 1.67−1.77 (m, 1H). MS *m*/*z* 481 $(M + H)^+$.

***N*-(4-Fluorobenzyl)-5-hydroxy-2-[4-hydroxy-1-methylpiperidin-2-yl]-1-methyl-6-oxo-1,6-dihydro-pyrimidine-4-carboxamide (25).** Compound **26** (25 mg of TFA salt, 0.04 mmol) was dissolved in MeOH (5.0 mL), some drops of 1 N HCl and cat. Pd/C 10% was added, and the reaction mixture was stirred under H_2 atmosphere at rt for 3.5 h. Filtration on celite gave a solution from which solvent was evaporated under vacuum. The residue was purified by RP-HPLC to provide **25** as trifluoroacetate salt (10.2 mg, yield 50%). ^{1}H NMR (CD_3OD, 400 MHz) δ 7.40 (dd, J = 8.2, 5.5 Hz, 2H), 7.40 (t, J = 8.7 Hz, 2H), 4.85 (under H_2O peak, 1H), 4.57−4.65 (m, 2H), 3.97−4.05 (m, 1H), 3.76 (bd, J = 12.5 Hz, 1H), 3.62 (s, 3H), 3.39 (t, J = 13.3 Hz, 1H), 2.87 (s, 3H), 2.49 (bd, J = 13.7 Hz, 1H), 2.24 (bd, J = 13.7 Hz, 1H), 1.76−1.86 (m, 1H), 1.63−1.72 (1H, m). MS *m*/*z* 391 $(M + H)^+$.

***N*-(4-Fluorobenzyl)-5-hydroxy-1-methyl-2-(4-methyl-5-oxopiperazin-2-yl)-6-oxo-1,6-dihydropyrimidine-4-carboxamide (80).** To a solution of compound **78** (9.8 g, 18.7 mmol) in dry 1,4-dioxane (150 mL) was added LiH (200 mg, 25 mmol). After stirring 90 min at 100 °C, Me_2SO_4 (2.7 mL, 28 mmol) was slowly added at rt, and the mixture was stirred at 60 °C for 1.5 h. After pouring into brine, the reaction mixture was extracted with AcOEt, washed with brine, and dried (Na_2SO_4), filtered, and evaporated under vacuum to give a crude that was purified by column chromatography (SiO_2, AcOEt/petroleum ether = 1:1) to afford the corresponding N−Me pyrimidone (7.0 g, yield 69%). The N−Me pyrimidone (4.86 g, 9.0 mmol) was dissolved in MeOH (70.0 mL), 4-fluorobenzylamine (2.38 mL, 20.8 mmol) was added, and the mixture was then refluxed for 2 days. After cooling to rt, cat. Pd/C

10% and 1 N HCl (5 mL) were added, and the reaction mixture was stirred under H_2 atmosphere, at rt overnight. Filtration on celite gave a solution from which solvent was evaporated under vacuum to give a crude compound **79** (3.9 g) that was used as such. The primary alcohol **79** (8.9 mmol) was dissolved in dry DCM (100 mL), TEA (6.3 mL, 45 mmol) and MsCl (1.7 mL, 22 mmol) were added, and the mixture was stirred at rt for 2 h. Evaporation to dryness gave a residue (mono- and bismesylate) that was (0.84 mmol) taken up in CH_3CN (12 mL), and excess BnMeNH (1 mL) was added. The mixture was stirred at reflux for 3 h and then overnight at rt; the residue obtained by evaporation was purified by RP-HPLC to provide a tertiary amine compound that (235 mg, 0.38 mmol) was dissolved in DCM (5 mL); TFA (2 mL) was added, and the mixture was stirred at rt for 3 h. Evaporation to dryness gave a residue that was taken in MeOH (2.0 mL), TEA (0. 4 mL, 3 mmol), and acetic acid (0.25 mL, 4.5 mmol) were added to the solution, followed by ethyl oxoacetate (2.0 mmol) and sodium cyanoborohydride (100 mg, 1.6 mmol). The reaction mixture was stirred at rt for 3 h, then Pd/C 10% and 1 N HCl (1.5 mL) were added, and the mixture was stirred under a H_2 atmosphere overnight; filtration on celite, followed by evaporation, gave a residue that was used as such. This crude (0.38 mmol) was taken up in MeOH, excess TEA was added, and the mixture was refluxed for 5 h. The residue obtained after removing volatiles was purified by chromatography to give the desired **80** (28 mg of TFA salt, 32% yield over four steps). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.20 (bs, 1H), 9.39 (t, J = 6.5 Hz, 1H), 7.43 (dd, J = 8.6, 5.5 Hz, 2H), 7.24 (t, J = 8.6 Hz, 2H), 5.32 (bd, J = 6.6 Hz, 1H), 4.62 (bd, J = 4.9 Hz, 2H), 3.90−3.99 (m, 3H), 3.61 (s, 3H), 3.55−3.65 (m, 1H), 2.97 (s, 3H). MS m/z 390 $(M + H)^+$.

2-(1,4-Dimethyl-5-oxopiperazin-2-yl)-*N*-(4-fluorobenzyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydro-pyrimidine-4-carboxamide (32). Et_3N (0.025 mL, 0.18 mmol), acetic acid (0.03 mL), and 37% aq HCHO (0.1 mL) were added to a solution of compound **80** (30 mg of TFA salt, 0.06 mmol) in MeOH (2 mL); excess $NaCNBH_3$ (10 mg) was added and the mixture was stirred at rt for 2.5 h. The residue obtained by evaporation was purified by RP-HPLC to provide **32** as trifluoroacetate salt (15 mg, yield 49%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.28 (t, J = 6.3 Hz, 1H), 7.38 (dd, J = 8.2, 5.8 Hz, 2H), 7.18 (t, J = 8.8 Hz, 2H), 5.23 (dd, J = 3.9, 10.4 Hz, 1H), 4.55 (d, J = 6.3 Hz, 2H), 4.23 (d, J = 16.5 Hz, 1H), 4.04 (d, J = 16.5 Hz, 1H), 3.92 (dd, J = 3.9, 13.7 Hz, 1H), 3.66−3.75 (m, 1H), 3.59 (s, 3H), 2.91 (s, 3H), 2.82 (s, 3H). MS m/z 404 $(M + H)^+$.

Methyl-5-(benzoyloxy)-2-[(2*S*)-1-(*tert*-butoxycarbonyl)-pyrrolidin-2-yl]-1-methyl-6-oxo-1,6-dihydro-pyrimidine-4-carboxylate (86a). Methyl 5-(benzoyloxy)-2-[(2*S*)-1-(*tert*-butoxycarbonyl)-pyrrolidin-2-yl]-6-hydroxy-pyrimidine-4-carboxylate **85a** (0.5 g, 1.1 mmol) was added at rt to a suspension of LiH (10 mg, 1.2 mmol) in dioxane (5 mL). The mixture was stirred for 45 min at 38 °C and was then cooled to rt. Dimethylsulfate (0.14 mL, 1.4 mmol) was added dropwise, and the mixture was warmed to 58 °C. After 3 h, the crude was cooled to rt, and brine (2 mL) was added, followed by EtOAc (10 mL). The aqueous phase was separated and extracted two times with EtOAc (2 × 10 mL). The combined extracts were dried (Na_2SO_4) to afford a residue that was purified by column chromatography (SiO_2, EtOAc/petroleum ether = 1:1), 80% yield; N−Me/O−Me = 13:1; e.e. 85.5%.[47] Recrystallization from EtOAc/hexanes gave compound **86a** as a white solid; e.e. 99.8%; $[\alpha]_D = -19.13$ (CH_3CN, c 0.05, 20 °C). ^{1}H NMR ($CDCl_3$, 300 MHz, mixture of rotamers) δ 8.18 (d, J = 7.5 Hz, 2H), 7.63 (t, J = 7.2 Hz, 1H), 7.50 (t, J = 7.6 Hz, 2H), 5.10−5.00 (m, 0.5H), 5.00−4.90 (m, 0.5H), 3.80 (s, 1.5H), 3.78 (s, 1.5H), 3.69 (s, 1.5H), 3.65 (s, 1.5H), 3.65−3.45 (m, 2H), 2.50−2.30 (m, 1H), 2.30−2.10 (m, 1H), 2.10−1.90 (m, 2H), 1.56 (s, 5H), 1.45 (s, 4H). MS m/z 458 $(M + H)^+$.

2(*S*)-(4-{[(4-Fluorobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-1-methylpyrrolidinium Trifluoroacetate (10a). Compound **86a** (75 mg, 0.16 mmol) was treated with DCM/TFA = 7:3 (7 mL) at 0 °C. The solution was allowed to warm to rt and stirred for 1 h. Volatiles were removed under reduced pressure, the residue was triturated with Et_2O, and the corresponding free pyrrolidinium trifluoroacetate was collected by filtration in 97% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.18 (d, J = 7.5 Hz, 2H), 7.67 (t, J = 7.6 Hz, 1H), 7.50 (t, J = 7.6 Hz, 2H), 5.46 (t, J = 6.6 Hz, 1H), 3.82 (s, 3H), 3.63 (s, 3H), 3.70−3.50 (m, 2H), 2.80−2.70 (m, 1H), 2.40−2.00 (m, 3H). MS m/z 358 $(M + H)^+$. To a stirred solution of the pyrrolidinium trifluoroacetate (0.1 g, 0.21 mmol) in 1,2-dichloroethane (10 mL), Et_3N (0.04 mL, 0.25 mmol), AcOH glacial (0.02 mL, 0.34 mmol), 37% HCHO (0.03 mL, 0.42 mmol), and $NaCNBH_3$ (0.02 g, 0.30 mmol) were added. The reaction mixture was stirred at rt overnight, then poured in aqueous $NaHCO_3$ satd soln, and extracted in EtOAc. The organic layer was collected, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was used in the next step without further purification. A solution of the *N*-methylpyrrolidine compound (0.073 g, 0.20 mmol) in *N*-methyl-2-pyrrolidinone (0.5 mL) was treated with 4-fluorobenzylamine (0.067 mL, 0.60 mmol), and the solution was heated at 90 °C for 30 min. After cooling to rt, the reaction mixture was purified by preparative RP-HPLC to give compound **10a** in 40% yield. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.47 (bs, 1H), 9.63 (t, J = 6.6 Hz, 1H), 9.42 (bs, 1H), 7.35 (dd, J = 8.6, 5.6 Hz, 2H), 7.18 (t, J = 8.8 Hz, 2H), 4.95−4.80 (m, 1H), 4.59 (d, J = 6.4 Hz, 2H), 3.80−3.70 (m, 1H), 3.42 (s, 3H), 2.94 (bs, 3H), 2.70−2.10 (m, 1H), 2.30−2.10 (m, 1H), 2.10−1.80 (m, 2H). $[\alpha]_D = -62$ (CH_3CN, c 0.01, 20 °C). MS m/z 361 $(M + H)^+$.

Methyl 5-(Benzoyloxy)-2-{1-[(benzyloxy)carbonyl]-3-methylpyrrolidin-2-yl}-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxylate (86b). Compound **85b** (1.1 g, 2.2 mmol) was added at rt to a suspension of LiH (20 mg, 2.4 mmol) in dioxane (10 mL). The mixture was stirred for 45 min at 38 °C and was then cooled to rt. Dimethylsulfate (0.28 mL, 2.9 mmol) was added dropwise, and the mixture was warmed to 58 °C. After 2 h, the crude was cooled to rt and added to NaCl satd soln, followed by the addition of EtOAc (10 mL). The aqueous phase was separated and extracted with EtOAc. The combined extracts were dried (Na_2SO_4) to afford a residue that was purified by flash column chromatography (EtOAc/petroleum ether = 1:1) to give compound **86b** in 66% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.25−8.12 (m, 2H), 7.70−7.60 (m, 1H), 7.55−7.45 (m, 2H), 7.40−7.30 (m, 5H), 5.20−4.80 (m, 3H), 3.80, 3.79 (2s, 1.5H), 3.78, 3.76 (2s, 3H), 3.60−3.30 (m, 1H), 3.37, 3.34 (2s, 1.5H), 2.80−2.60 (m, 1H), 2.40−2.20 (m, 1H), 2.00−1.90 (m, 1H), 1.70−1.60 (m, 1H), 1.30−0.80 (m, 3H).

Methyl 5-(Benzoyloxy)-2-{1-[(benzyloxy)carbonyl]-5-methylpyrrolidin-2-yl}-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxylate (86e). Following the same reaction described to obtained compound **86b**, compound **86e** was obtained from **85e**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.25−8.15 (m, 2H), 7.70−7.60 (m, 1H), 7.60−7.40 (m, 2H), 7.40−7.30 (m, 5H), 5.20−4.80 (m, 3H), 4.50−4.30 (m, 1H), 3.78 (s, 2H), 3.75 (s, 1H), 3.69 (s, 1.5H), 3.41 (s, 1.5H), 2.60−2.30 (m, 2H), 1.95−1.80 (m, 1H), 1.60−1.50 (m, 1H), 1.32 (d, J = 6.4 Hz, 1.5H), 1.22 (d, J = 6.4 Hz, 1.5H).

2-(4-{[(4-Fluorobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-1,3-dimethylpyrrolidinium Trifluoroacetate (12). A solution of compound **86b** (0.6 g, 1.2 mmol) in MeOH (20 mL) was treated with 4-fluorobenzylamine (0.34 mL, 3.0 mmol), and the solution was stirred at reflux overnight. The solvent was removed in vacuo, and the residue was taken up in EtOAc, washed with 1 N HCl and brine, and dried (Na_2SO_4). Evaporation of the solvent gave a residue **89b**, which was used in the next step without further purification. To a solution of compound **89b** (0.5 g, 1.0 mmol) in MeOH (20 mL), Pd/C (10 mg, 0.1 mmol) and HCl (1 N, 1 mL, 1.0 mmol) was added and the reaction mixture was stirred under a H_2 atmosphere (1 atm) for 2 h. Then the mixture was filtered through celite, and the solvent was removed under vacuum affording **90b**, which was used in the next step without further purification. To a stirred solution of compound **90b** (0.07 g, 0.17 mmol) in $CHCl_3$ (10 mL), Et_3N (0.029 mL, 0.20 mmol), AcOH glacial (0.016 mL, 0.27 mmol), 37% HCHO (0.032 mL, 0.23 mmol), and $NaCNBH_3$ (0.015 g, 0.24 mmol) were added. The reaction mixture was stirred at rt overnight, then poured in aqueous

$NaHCO_3$ satd soln, and extracted in EtOAc. The organic layer was collected, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by preparative RP-HPLC to give compound **12** as a 2:1 mixture in 26% yield. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.50 (bs, 1H), 9.64 (bs, 1H), 7.38 (t, J = 8.8 Hz, 2H), 7.18 (t, J = 8.8 Hz, 2H), 5.00−4.90 (m, 1H), 4.60−4.50 (m, 2H), 3.90−3.80 (m, 1H), 3.48 (s, 1H), 3.45 (s, 2H), 3.10−3.00 (m, 1H), 2.88 (s, 3H), 2.40−2.20 (m, 1H), 2.00−1.60 (m, 2H), 1.21 (d, J = 6.5 Hz, 1H), 0.70 (d, J = 6.5 Hz, 2H). MS m/z 375 $(M + H)^+$.

Methyl 5-(Benzoyloxy)-2-[1-(*tert*-butoxycarbonyl)-4-methylpyrrolidin-2-yl]-6-hydroxypyrimidine-4-carboxylate (85c,d). Following the usual chemistry toward 5-*O*-benzoylated pyrimidine **85**, compounds **85c** and **85d** were obtained from nitrile **81c**,**d** as a mixture. The two diastereoisomeric products **85c** and **85d** were separated by column chromatography (SiO_2, petroleum ether/EtOAc = from 8:2 to 1:1) as yellow solids: (**85c**) **Methyl 5-(Benzoyloxy)-2-[(2*S*,4*S*)-1-(*tert*-butoxycarbonyl)-4-methylpyrrolidin-2-yl]-6-hydroxypyrimidine-4-carboxylate** (34%); R_f = 0.3 (EtOAc/petroleum ether = 1:1). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.10 (d, J = 7.5 Hz, 2H), 7.80 (t, J = 7.2 Hz, 1H), 7.60 (t, J = 7.6 Hz, 2H), 4.60−4.50 (m, 1H), 3.75 (s, 3H), 3.70−3.60 (m, 1H), 3.10−3.00 (m, 1H), 2.50−2.40 (m, 1H), 2.40−2.20 (m, 1H), 1.70−1.50 (m, 1H), 1.40−1.20 (2s, 9H), 1.05 (d, J = 6.6 Hz, 3H). MS m/z 458 $(M + H)^+$; (**85d**) **Methyl 5-(Benzoyloxy)-2-[(2*S*,4*R*)-1-(*tert*-butoxycarbonyl)-4-methylpyrrolidin-2-yl]-6-hydroxypyrimidine-4-carboxylate** (16%); R_f = 0.4 (EtOAc/petroleum ether = 1:1). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.10 (d, J = 7.5 Hz, 2H), 7.80 (t, J = 7.2 Hz, 1H), 7.60 (t, J = 7.6 Hz, 2H), 4.70−4.50 (m, 1H), 3.75 (s, 3H), 3.70−3.60 (m, 1H), 3.00−2.80 (m, 1H), 2.50−2.40 (m, 1H), 2.10−1.80 (m, 2H), 1.40,1.20 (2s, 9H), 1.00 (d, J = 6.6 Hz, 3H). MS m/z 458 $(M + H)^+$.

M**ethyl 5-(Benzoyloxy)-2-[(2*S*,4*S*)-1-(*tert*-butoxycarbonyl)-4-methylpyrrolidin-2-yl]-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxylate (86c).** Compound **85c** (0.4 g, 0.9 mmol) was added at rt to a suspension of LiH (10 mg, 1.0 mmol) in dioxane (5 mL). The mixture was stirred for 45 min at 38 °C and was then cooled to rt. Dimethylsulfate (1.3 equiv, 0.11 mL, 1.1 mmol) was added dropwise, and the mixture was warmed to 58 °C. After 3 h, the crude was cooled to rt and brine (2 mL) was added, followed by EtOAc (10 mL). The aqueous phase was separated and extracted two times with EtOAc (2 × 10 mL). The combined extracts were dried (Na_2SO_4) to afford a residue, which was purified by column chromatography (SiO_2, EtOAc/petroleum ether = 1:1) to give compound **86c** in 68% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.17 (d, J = 7.5 Hz, 2H), 7.62 (t, J = 7.6 Hz, 1H), 7.50 (t, J = 7.6 Hz, 2H), 5.00−4.80 (m, 1H), 3.80, 3.78 (2s, 3H), 3.69 (s, 1.5H), 3.90−3.80 (m, 1H), 3.65 (s, 1.5H), 3.20−2.90 (m, 1H), 2.60−2.50 (m, 1H), 2.40−2.20 (m, 1H), 1.80−1.60 (m, 1H), 1.44, 1.28 (2s, 9H), 1.15 (d, J = 6.6 Hz, 1.5H), 1.12 (d, J = 6.6 Hz, 1.5H). MS m/z 472 $(M + H)^+$.

(2*S*,4*S*)-2-(4-{[(4-Fluorobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-1,4-dimethylpyrrolidinium trifluoroacetate (13). Compound **86c** (0.35 g, 0.74 mmol) was treated with DCM/TFA = 7:3 (7 mL) at 0 °C. The solution was allowed to warm to rt and stirred for 1 h. Volatiles were removed under vacuum. The residue was triturated with Et_2O, and the corresponding free pyrrolidinium trifluoroacetate was collected by filtration in 97% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.16 (d, J = 7.5 Hz, 2H), 7.67 (t, J = 7.6 Hz, 1H), 7.50 (t, J = 7.6 Hz, 2H), 5.50−5.40 (m, 1H), 3.90−3.80 (m, 1H), 3.80 (s, 3H), 3.63 (s, 3H), 3.60−3.50 (m, 1H), 3.10−3.00 (m, 1H), 2.90−2.70 (m, 2H), 1.60−1.50 (m, 1H), 1.20 (d, J = 6.5 Hz, 3H). MS m/z 372 $(M + H)^+$. The pyrrolidinium trifluoroacetate (0.1 g, 0.21 mmol) was dissolved in 1,2-dichloroethane (10 mL), Et_3N (0.035 mL, 0.25 mmol), AcOH glacial (0.02 mL, 0.34 mmol), 37% HCHO (0.03 mL, 0.42 mmol), and $NaCNBH_3$ (0.02 g, 0.30 mmol) were added. The reaction mixture was stirred at rt overnight, then poured in aqueous $NaHCO_3$ satd soln, and extracted in EtOAc. The organic layer was collected, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was used in the next step without further purification. It was (0.063 g, 0.16 mmol) dissolved in *N*-methyl-2-pyrrolidinone (0.5 mL) and treated with 4-fluorobenzylamine (0.047 mL, 0.40 mmol), and the solution was heated at 90 °C for 30 min. After cooling to rt, the reaction mixture was purified by preparative RP-HPLC to give the desired compound **13** as trifluoroacetate salt in 10% yield. ^{1}H NMR (DMSO-d_6, 400 MHz, 330 K) δ 12.50 (bs, 1H), 9.62 (bs, 2H), 7.36 (t, J = 8.8 Hz, 2H), 7.16 (t, J = 8.8 Hz, 2H), 5.00−4.90 (m, 1H), 4.60−4.50 (m 2H), 3.90−3.80 (m, 1H), 3.43 (s, 3H), 2.95 (bs, 3H), 2.90−2.80 (m, 1H), 2.80−2.70 (m, 1H), 1.60−1.50 (m, 1H), 1.07 (d, J = 6.5 Hz, 3H). MS m/z 375 $(M + H)^+$.

Methyl 5-(Benzoyloxy)-2-[(2*S*,4*R*)-1-(*tert*-butoxycarbonyl)-4-methylpyrrolidin-2-yl]-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxylate (86d). Compound **86d** was obtained from compound **85d** using the same reaction described to obtain compound **86c**. ^{1}H NMR ($CDCl_3$, 300 MHz, 330 K) δ 8.17 (d, J = 7.5 Hz, 2H), 7.62 (t, J = 7.6 Hz, 1H), 7.49 (t, J = 7.6 Hz, 2H), 5.00−4.80 (m, 1H), 3.79, 3.77 (two s, 3H), 3.66 (s, 1.5H), 3.90−3.80 (m, 1H), 3.63 (s, 1.5H), 3.20−3.00 (m, 1H), 2.70−2.60 (m, 1H), 2.10−2.00 (m, 1H), 1.80−1.70 (m, 1H), 1.45, 1.29 (2s, 9H), 1.09 (7, J = 6.6 Hz, 3H). MS m/z 472 $(M + H)^+$.

(2*S*,4*R*)-2-(4-{[(4-Fluorobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-1,4-dimethylpyrrolidinium Trifluoroacetate (14). Compound **14** was obtained from the intermediate **86d** following the same three steps used to prepare compound **13**. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.47 (bs, 1H), 9.61 (bs, 2H), 7.35 (m, 2H), 7.18 (t, J = 8.8 Hz, 2H), 5.00−4.90 (m, 1H), 4.60−4.50 (m 2H), 3.90−3.80 (m, 1H), 3.42 (s, 3H), 2.94 (bs, 3H), 2.30−2.20 (m, 3H), 1.12 (d, J = 6.5 Hz, 3H). MS m/z 375 $(M + H)^+$.

2-(4-{[(4-Fluorobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-1,5-dimethylpyrrolidinium Trifluoroacetate (15). Following the same steps described for the preparation of compound **12**, compound **15** from nitrile **81e** was obtained as a 1:2 mixture. ^{1}H NMR (DMSO-d_6 + TFA, 400 MHz) δ 9.55, 9.37 (2bs, 1H), 7.38 (t, J = 7.5 Hz, 2H), 7.17 (t, J = 8.7 Hz, 2H), 5.00−4.90 (m, 1H), 4.70−4.60 (m 2H), 4.20−4.00 (m, 0.5H), 3.70−3.60 (m, 0.5H), 3.46 (s, 1.5H), 3.45 (s, 1.5H), 2.96 (s, 1H), 2.82 (s, 2H), 2.80−2.60 (m, 1H), 2.40−2.30 (m, 0.5H), 2.30−2.10 (m, 0.5H), 2.10−1.90 (m, 1.5H), 1.80−1.70 (m, 0.5H), 1.47 (d, J = 6.6 Hz, 1H), 1.39 (d, J = 6.6 Hz, 2H). MS m/z 375 $(M + H)^+$.

Methyl 5-(Benzoyloxy)-2-[(2*S*,4*R*)-4-(benzyloxy)-1-(*tert*-butoxycarbonyl)-pyrrolidin-2-yl]-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxylate (86f). To compound **85f** (1.91, 3.58 mmol), dissolved in THF (30 mL), Cs_2CO_3 (1.75 g, 1.5 equiv) and dimethyl sulfate (678 mg, 1.5 equiv) were added, and the reaction mixture was stirred at 50 °C for 1 h. The reaction mixture was cooled down to 0 °C and HCl (1 N) was added. The reaction mixture was extracted with ethyl acetate, and the organic phase was washed with HCl (1 N) and brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. The title product **86f** was isolated by column chromatography (SiO_2, petroleum ether/ethyl acetate = 7:3) as a 2:3 mixture of rotamers by NMR (0.816 g, 40% yield). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.18 (d, J = 8.1 Hz, 2H), 7.63 (t, J = 7.5 Hz ,1H), 7.49 (t, J = 7.5 Hz, 2H), 7.41−726 (m, 5H), 5.13 (t, J = 6 Hz, 0.4H), 5.05 (t, J = 6 Hz ,0.6H),), 4.65−4.45 (m, 2H), 4.41 (bs, 0.4H), 4.32 (bs, 0.6H), 3.95−3.63 (m, 8H), 2.57−2.40 (m, 1H), 2.30−2.20 (m, 1H), 1.44 (s, 3.6H), 1.30 (s, 5.4 H). MS m/z 564 $(M + H)^+$.

(2*S*,4*R*)-4-(Benzyloxy)-2-(4-{[(4-fluorobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-1-methylpyrrolidinium Trifluoroacetate (19). To compound **86f** (0.20 g, 0.36 mmol) dissolved in methanol (16 mL), 4-F-benzylamine (0.155 g, 3.5 equiv) was added. The reaction mixture was stirred at reflux overnight. Methanol was removed in vacuo, and the residue was dissolved in ethyl acetate and washed with HCl (1 N) and brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was triturated with diethyl ether to give the compound **89f** that was submitted to the next step without further purification. ^{1}H NMR (DMSO-d_6, 300 MHz, 330 K) 3:7 mixture of rotamers δ

11.98 (bs, 1H), 8.68 (bs, 0.7H), 8.52 (bs, 0.3H), 7.49−7.22 (m, 7H), 7.19 (t, J = 8.8 Hz, 2H), 5.2−4.90 (m, 1H), 4.63−4.42 (m, 4H), 4.25 (bs, 1H), 3.85−3.72 (m, 1H), 3.58 (s, 0.9H), 3.51 (s, 2.1H), 2.15−2.29 (m, 1H), 1.26 (bs, 2.7H), 1.11 (bs, 6.3H). MS m/z 553 $(M + H)^+$. Compound **89f** was treated with DCM−TFA (6 mL, 1:1), and the mixture was stirred at rt. After 1 h, volatiles were evaporated and the residue was triturated with diethyl ether obtaining compound **90f**, which was submitted to the next step without further purification. Formaldehyde (37 wt. % solution in water; 53 μL, 2 equiv) and sodium acetate (26 mg, 1.6 equiv) were added to a mixture of the compound **90f** and Et_3N (49 μL, 1 equiv) in methanol (3 mL), followed after stirring for 10 min by $NaCNBH_3$ (31 mg, 1.4 equiv). After stirring overnight, volatiles were evaporated under reduced pressure, affording a residue, which was redissolved in DMSO and purified by RP-HPLC. Fractions containing the pure compound were combined and freeze-dried yielding compound **19** (82 mg, 40% yield over three steps). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 12.54 (s, 1H), 9.78 (s, 1H), 9.68 (t, J = 6.2 Hz, 1H), 7.43−7.30 (m, 7H), 7.19 (t, J = 8.8 Hz, 2H), 5.11 (bs, 1H), 5.68−4.53 (m, 4H), 4.48−4.30 (m, 1H), 4.22−3.98 (m, 1H), 3.58−3.50 (m, 1H, partially obscured by water), 3.46 (s, 3H), 2.87 (dd, J = 13.5, 7.7 Hz, 1H), 3.00 (d, J = 3.7 Hz, 3H), 2.19−2.06 (m, 1H), 2.24−2.16 (m, 1H). MS m/z 467 $(M + H)^+$.

(2*S*,4*R*)-2-(4-{[(4-Fluorobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydro-pyrimidin-2-yl)-4-hydroxy-1-methyl-pyrrolidinium Trifluoroacetate (16). Compound **86f** (0.30 g, 0.53 mmol) was dissolved in methanol (15 mL) and Pd/C (10%, 150 mg, 50% w/w) was added. The mixture was stirred under a H_2 atmosphere at rt overnight. The reaction mixture was filtered and methanol was removed in vacuo to give compound **87o** (86% yield). Compound **87o** (0.215 g, 0.45 mmol) was dissolved in methanol (15 mL) and 4-F-benzylamine (0.176 g, 3.1 equiv) was added. The reaction mixture was stirred at reflux overnight. Methanol was removed in vacuo, and the residue was dissolved in ethyl acetate and washed with HCl (1 N) and brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was triturated with diethyl ether to give the desired compound **89o** that was submitted to the next step without further purification. ^{1}H NMR (DMSO-d_6, 400 MHz) 3:7 mixture of rotamers δ 12.15 (bs, 0.7H), 12.08 (s, 0.3H), 8.90 (t, J = 6.6 Hz, 0.7H), 8.71 (t, J = 6.6 Hz, 0.3H), 7.34−7.25 (m, 2H), 7.20−7.05 (m, 2H), 5.05 (bs, 1H), 4.95 (t, J = 7.7 Hz, 1H), 4.98−4.40 (m, 2H), 4.34 (bs, 1H), 3.76 (dd, J = 10.9, 4.0 Hz, 1H), 3.53 (s, 0.9H), 3.50 (s, 2.1H), 2.35−2.05 (m, 2H), 1.26 (bs, 2.7H), 1.10 (bs, 6.3H). MS m/z 463 $(M + H)^+$. Compound **89o** was treated with DCM−TFA (6 mL, 1:1), and the mixture was stirred at rt. After 1 h, volatiles were evaporated, and the residue was triturated with diethyl ether obtaining compound **90o**, which was submitted to the next step without further purification. Formaldehyde (37 wt. % solution in water; 136 μL, 4 equiv) and sodium acetate (119 mg, 3.2 equiv) were added to the amine **90o**, dissolved in methanol (10 mL), followed after stirring for 10 min by $NaCNBH_3$ (80 mg, 2.8 equiv). After stirring overnight, volatiles were evaporated under reduced pressure, affording a residue, which was redissolved in DMSO and purified by RP-HPLC. Fractions containing the pure compounds were combined and freeze-dried, yielding compound **16** (89 mg, 60% yield over three steps). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 12.52 (s, 1H), 9.64 (t, J = 5.5 Hz, 1H), 9.54 (s, 1H), 7.35 (dd, J = 8.6 Hz, 5.7 Hz, 2H), 7.18 (t, J = 8.8 Hz, 2H), 5.80 (bs, 1H), 5.09 (q, J = 8,2 Hz, 1H), 4.57 (d, J = 6.3 Hz, 2H), 4.47 (bs, 1H), 4.01−3.90 (m, 1H), 3.44 (s, 3H), 3.26−3.18 (m, 1H), 3.00 (d, J = 3.7 Hz, 3H), 2.65−2.55 (m, 1H, partially obscured by DMSO), 2.22−2.09 (m, 1H). MS m/z 377 $(M + H)^+$.

***N*-(4-Fluorobenzyl)-5-hydroxy-2-[(2*S*,4*R*)-4-methoxypyrrolidin-2-yl]-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide (90g).** Compound **89g** (0.51 g, 1.02 mmol) was dissolved in methanol (50 mL) and Pd/C (10%, 70 mg, 14% w/w) was added. The mixture was stirred under a H_2 atmosphere at rt. After 2 h, the reaction mixture was filtered and methanol was removed in vacuo to give the title product **90g**, which was used in the following step without further purification. ^{1}H NMR (DMSO-d_6 + TFA, 400 MHz) δ 12.58 (bs, 1H), 10.16 (bs, 1H), 9.74 (t, J = 6.3 Hz, 1H), 8.90 (bs, 1H), 7.36 (dd, J = 8.5, 5.7 Hz, 2H), 7,19 (t, J = 8.8 Hz, 2H), 5.01 (bs, 1H), 4.50−4.60 (m, 2H), 4.19 (bs, 1H), 3.55−3.45 (m, 1H), 3.47 (s, 3H), 3.45−3.35 (m, 1H), 3.32 (s, 3H), 2.74 (dd, J = 13.9, 7.5 Hz, 1H), 2.17−2.10 (m, 1H). MS m/z 377 $(M + H)^+$.

(2*S*,4*R*)-2-(4-{[(4-Fluorobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-4-methoxy-1-methylpyrrolidinium Trifluoroacetate (17). Formaldehyde (37 wt. % solution in water; 32 μL, 3 equiv) and sodium acetate (20 mg, 1.6 equiv) were added to the amine **90g** (50 mg, 0.133 mmol) in methanol (3 mL) followed by $NaCNBH_3$ (12 mg, 1.4 equiv) after 10 min. After stirring overnight, volatiles were evaporated under reduced pressure, affording a residue, which was redissolved in DMSO and purified by RP-HPLC. Fractions containing the pure compound were combined and freeze-dried, yielding compound **17** (20 mg, 36% yield over three step). ^{1}H NMR (DMSO-d_6 + TFA, 300 MHz) δ 10.65 (bs, 1H), 10.42 (bs, 1H), 7.44 (m, 2H), 7.16 (t, J = 8.7, 2H), 5.05 (t, J = 10.2 Hz, 1H), 4.50−4.42 (m, 2H), 4.21−4.07 (m, 2H), 3.47 (s, 3H), 3.33 (s, 3H), 3.39−3.21 (m, 1H), 2.94 (d, J = 4.5, 3H), 2.76 (dd, J = 7.5, 7.2 Hz 1H), 2.19−2.06 (m, 1H). MS m/z 391 $(M + H)^+$.

(2*S*,4*R*)-4-Ethoxy-2-(4-{[(4-fluorobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-1-methylpyrrolidinium Trifluoroacetate (18). Following the same reactions performed to obtain compound **17**, compound **18** was prepared in 34% yield. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.50 (s, 1H), 9.60 (s, 1H), 9.49 (bs, 1H), 7.37 (m, 2H), 7.20 (t, J = 8.8 Hz ,2H), 5.08 (m, 1H), 4.60 (m, 2H), 4.28 (m, 1H), 3.98 (m, 1H), 3.59−3.40 (m, 6H, partialy obscured by water), 3.00 (d, J = 5.6 Hz, 3H), 2.78 (m, 1H), 2.16 (m, 1H), 1.17 (t, J = 7.2 Hz, 3H). MS m/z 405 $(M + H)^+$.

Methyl 5-(Benzoyloxy)-2-{(2*S*,4*S*)-1-[(benzyloxy)carbonyl]-4-[(*tert*-butoxycarbonyl)-amino]-pyrrolidin-2-yl}-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxylate (86i). The pyrimidine **85i** (1.9 g, 3.1 mmol) in THF (70 mL) was treated with dimethylsulfate (360 μL, 1.2 equiv) and cesium carbonate (1.2 g, 1.2 equiv). The reaction mixture was stirred at 50 °C for 1.5 h. Volatiles were removed under vacuum, the residue was dissolved in EtOAc, washed with HCl (1 N) and brine, dried (Na_2SO_4), and filtered to give after concentration a residue, which was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate = from 4:6 to 3:7), yielding compound **86i** (1.2 g, 64% yield). ^{1}H NMR (DMSO-d_6, 300 MHz, 340 K) δ 8.09 (d, J = 7.5 Hz, 2H), 7.45 (t, J = 7.5 Hz, 1H), 7.63 (t, J = 7.6 Hz, 2H), 7.00−6.91 (bm, 5H), 6.92 (bd, J = 5.7 Hz, 1H), 5.18 (dd, J = 8.3, 5.9 Hz, 1H), 5.13−4.80 (bm, 2H), 4.22 (m, 1H), 3.96−3.82 (m, 1H), 3.76 (s, 3H), 3.72−3.31 (br m, 4H), 2.75 (m, 1H), 1.99 (m, 1H), 1.39 (s, 9H). MS m/z 607 $(M + H)^+$.

(3*S*,5*S*)-5-[5-(Benzoyloxy)-4-(methoxycarbonyl)-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl]-1-[(benzyloxy)carbonyl]pyrrolidin-3-aminium Trifluoroacetate (87p). The Boc-protected pyrimidine **86i** (496 mg, 0.82 mmol) was stirred for 40 min in TFA/DCM/H_2O (65:35:10, 18 mL). Volatiles were removed iv, and the residue was taken up several times with ethyl ether until a pale brown solid appeared upon removal of volatiles iv, yielding compound **87p** (556 mg, quantitative yield), which was submitted to the next step without purification.^{1}H NMR (DMSO-d_6, 300 MHz, 340 K) δ 8.37 (bs, 3H), 8.12 (d, J = 7.7 Hz, 2H), 7.81 (t, J = 7.4 Hz, 1H), 7.66 (t, J = 7.6 Hz, 2H), 7.44−7.03 (bm, 5H), 5.32 (bd, J = 7.9 Hz, 1H), 5.18−4.92 (bm, 2H), 4.07 (bm, 1H), 3.93−3.33 (bm partially obscured by H_2O, 8H), 2.89−2.73 (m, 1H), 2.40−2.26 (m, 1H). MS m/z 507 $(M + H)^+$.

(2*S*,4*S*)-2-(4-{[(4-Fluorobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-1-methyl-4-[(methylsulfonyl)amino]pyrrolidinium Trifluoroacetate (20). Methyl sulfonyl chloride (25 μL, 1.1 equiv) was added dropwise to a solution of compound **87p** (182 mg, 0.29 mmol) and Et_3N (102 μL, 2.5 equiv) in DCM (2 mL), cooled at 0 °C, and stirring was continued at rt. After 1 h, volatiles were evaporated under vacuum iv, the residue was dissolved in EtOAc, washed with water, HCl (1 N) and brine, dried (Na_2SO_4), and filtered to give after concentration a residue, which was purified by column chroma-

tography (SiO_2, DCM/MeOH = 80:1), yielding compound **88q** (122 mg, 71% yield). Compound **88q** (118 mg, 0.20 mmol) and 4-F-benzylamine (92 μL, 4 equiv) were heated at 90 °C in methanol (2 mL) in a closed vessel. After 4 d, volatiles were evaporated iv to give a residue, which was washed with ethyl ether leaving the desired compound **89q**, which was submitted to the next step without purification. Pd/C (10%, 12 mg, 10% w/w) and the pyrimidine **89q** in methanol (30 mL) were stirred under a hydrogen atmosphere. After 3 h, solids were filtered away and volatiles were evaporated iv to give a residue **90q**, which was submitted to the next step without purification. Formaldehyde (37 wt. % solution in water; 50 μL, 3 equiv) and sodium acetate (29 mg, 1.6 equiv) were added to the amine **90q** in methanol (4 mL) followed by $NaCNBH_3$ (20 mg, 1.4 equiv) after 5 min. After stirring for 4 h, volatiles were evaporated under reduced pressure affording a residue, which was redissolved in DMSO and purified by RP-HPLC. Fractions containing the pure compounds were combined and freeze-dried yielding compound **20** (82 mg, 72% yield over three steps). ^{1}H NMR (DMSO-d_6, 300 MHz, 340 K) δ 12.80−12.23 (bs, 1H), 9.78−9.47 (bm, 2H), 7.44−7.36 (m, 2H), 7.28 (d, J = 4.3 Hz, 1H), 7.22−7.12 (m, 2H), 4.96 (bm, 1H), 4.63 (m, 2H), 4.38 (bm, 1H), 3.88−3.75 (bm, 1H), 3.67−3.43 (bm partially obscured by H_2O, 4H), 3.14−2.94 (m, 7H), 2.04 (m, 1H). MS m/z 454 $(M + H)^+$.

Methyl 2-{(2*S*,4*S*)-4-(Acetylamino)-1-[(benzyloxy)carbonyl]-pyrrolidin-2-yl}-5-(benzoyloxy)-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxylate (88r). Acetic anhydride (58 μL, 2 equiv) was added dropwise to a solution of compound **87p** (190 mg, 0.31 mmol) in pyridine (2.5 mL). After 2 h, volatiles were evaporated under vacuum iv, the residue was dissolved in EtOAc, washed with water, HCl (1 N), and brine, dried over Na_2SO_4, and filtered to give after concentration compound **88r**, which was submitted to the next step without further purification. ^{1}H NMR (DMSO-d_6, 300 MHz, 340 K) δ 8.11 (d, J = 7.6 Hz, 2H), 7.92 (bd, J = 6.9 Hz, 1H), 7.80 (t, J = 7.4 Hz, 1H), 7.65 (t, J = 7.6 Hz, 2H), 7.43−6.99 (bm, 5H), 5.21 (dd, J = 8.4, 5.6 Hz, 1H), 5.13−4.82 (bm, 2H), 4.47 (m, 1H), 3.96−3.84 (m, 1H), 3.77 (s, 3H), 3.70−3.33 (m partially obscured by H_2O, 4H), 2.76 (dt, J = 13.2, 7.8 Hz, 1H), 2.05−1.94 (m, 1H), 1.83 (s, 3H). MS m/z 549 $(M + H)^+$.

(2*S*,4*S*)-4-(Acetylamino)-2-(4-{[(4-fluorobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-1-methylpyrrolidinium Trifluoroacetate (21). Following the same three steps used for the synthesis of compound **20**, final compound **21** was obtained from **88r** in 48% yield. ^{1}H NMR (DMSO-d_6, 300 MHz, 340 K) δ 12.97−11.87 (bs, 1H), 10.43−9.09 (bs, 1H), 9.60 (bm, 1H), 7.98 (d, J = 4.4 Hz, 1H), 7.46−7.32 (m, 2H), 7.23−7.09 (m, 2H), 4.95 (bm, 1H), 4.60 (m, 2H), 4.42 (bm, 1H), 3.83 (bd, J = 11.3 Hz, 1H), 3.58−3.22 (m partially obscured by H_2O, 2H), 3.46 (s, 3H), 3.07−2.92 (m, 1H), 2.96 (s, 3H), 2.01 (m, 1H), 1.76 (s, 3H). MS m/z 418 $(M + H)^+$.

(2*S*,4*S*)-4-Fluoro-2-(4-{[(4-fluorobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-1-methylpyrrolidinium Trifluoroacetate (22). Compound **86l** (1.53 g, 3.00 mmol) and 4-F-benzylamine (1.0 mL, 3 equiv) were heated at 90 °C in methanol (23 mL) in a closed vessel. After 24 h, the reaction mixture was diluted with ethyl ether and the precipitated solid was collected by filtration, affording the desired compound **89l**, which was submitted to the next step without purification. Pd/C (10%, 100 mg, 10% w/w) and the pyrimidine **89l** in methanol (110 mL) were stirred under a hydrogen atmosphere. After 17 h, TFA (308 μL, 2 equiv) was added to dissolve the desired product. Solids were filtered away and volatiles were evaporated iv to give a residue containing compound **90l**, which was submitted to the next step without purification. Formaldehyde (37 wt. % solution in water; 34 μL, 3 equiv), sodium acetate (20 mg, 1.6 equiv), and TEA (42 μL, 2 equiv) were added to compound **90l** (1/15 of the amount obtained from the previous step) in methanol (3 mL), followed by $NaCNBH_3$ (14 mg, 1.4 equiv) after 10 min. After stirring for 4 h, volatiles were evaporated under reduced pressure affording a residue, which was redissolved in DMSO and purified by RP-HPLC. Fractions containing the pure compounds were combined and freeze-dried, yielding compound **22** (57 mg, 57% yield over three steps). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.59 (bs, 1H), 10.05−9.87 (bs, 1H), 9.77 (bm, 1H), 7.41−7.32 (m, 2H), 7.25−7.16 (m, 2H), 5.61 (bd, J = 49.9 Hz, 1H), 5.04 (bm, 1H), 4.69−4.53 (m, 2H), 4.17−4.02 (bm, 1H), 3.79−3.62 (m partially obscured by H_2O, 1H), 3.39 (s, 3H), 3.32−3.01 (m, 1H), 2.93 (bs, 3H), 2.47−2.34 (m, 1H). MS m/z 379 $(M + H)^+$.

(2*S*,4*R*)-4-Fluoro-2-(4-{[(4-fluorobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-1-methylpyrrolidinium Trifluoroacetate (23). Following the same steps followed to obtain compound **22**, compound **23** was obtained from **86m** in 27% yield. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.57 (bs, 1H), 9.76 (bs, 1H), 9.63 (bs, 1H), 7.41−7.32 (m, 2H), 7.25 (t, J = 9 Hz, 2H), 5. (bd, J = 52.5 Hz, 1H), 5.33 (bt, J = 8.2 Hz, 1H), 4.69−4.53 (m, 2H), 4.25 (dd, J = 31.5, 15.0 Hz, 1H), 3.81 (dd, J = 23.3, 15.0 Hz, 1H), 3.52 (s, 3H), 3.12−2.91 (m, 4H), 2.47−2.34 (m, 1H). MS m/z 379 $(M + H)^+$.

2-[(2*S*)-4,4-Difluoro-1-methylpyrrolidin-2-yl]-*N*-(4-fluorobenzyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide (24). Following the same steps followed to obtain compound **22**, compound **24** was obtained from **86n** in 47% yield. ^{1}H NMR (DMSO-d_6 + TFA, 300 MHz, 340 K) δ 9.43 (t, J = 6.0 Hz, 1H), 7.43 (t, J = 8.2 Hz, 2H), 7.19 (t, J = 8.9 Hz, 2H), 5.32 (t, J = 9.0 Hz, 1H), 4.60 (dd, J = 15.7, 6.6 Hz, 1H), 4.56 (dd, J = 15.7, 6.0 Hz, 1H), 4.34 (td, J = 12.3, 4.1 Hz, 1H), 3.96 (dt, J = 18.5, 12.2 Hz, 1H), 3.44 (s, 3H), 3.39−3.30 (m, 1H), 3.00 (s, 3H), 2.88−2.74 (m, 1H). MS m/z 397 $(M + H)^+$.

Benzyl 3-Methyl-2-oxopyrrolidine-1-carboxylate (92b). To a suspension of NaH (4.8 g, 121.5 mmol, 60% dispersion in mineral oil) in dry DMF (100 mL) was added 3-methylpyrrolidin-2-one **91b** (10.0 g, 100.8 mmol) at 0 °C, and after 15 min, benzyl chloroformate (17.3 mL, 121.0 mmol) was added and the solution was stirred at rt for 2 h. Then NH_4Cl satd soln was added, the aqueous phase was extracted with EtOAc, and the organic phase was dried (Na_2SO_4). Evaporation of solvent gave a residue, which was purified by column chromatography (SiO_2, petroleum ether/EtOAc = from 8:2 to 1:1) to obtain compound **92b** (60% yield). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.50−7.30 (m, 5H), 5.28 (s, 2H), 3.90−3.80 (m, 1H), 3.70−3.60 (m, 1H), 2.65−2.50 (m, 1H), 2.30−2.20 (m, 1H), 1.75−1.55 (m, 1H), 1.24 (d, J = 7.0 Hz, 3H).

Benzyl 2-Cyano-3-methylpyrrolidine-1-carboxylate (81b). To a solution of compound **92b** (4.0 g, 17.2 mmol) in dry THF (20 mL) was added lithium triethylboron hydride (superhydride, 20.6 mL, 1 M solution in THF, 20.6 mmol) at −78 °C under nitrogen. After the mixture was stirred for 30 min at this temperature, the reaction was quenched with $NaHCO_3$ satd soln, and the mixture was allowed to reach 0 °C. Then H_2O_2 (33%) was added, and the mixture was stirred for 40 min. The aqueous layer was extracted with EtOAc, and the combined organic layers were dried (Na_2SO_4). Evaporation of solvent gave a residue of the lactam alcohol **93b**, which was used in the next step without further purification. To a solution of compound **93b** in dry DCM (20 mL), TMSCN (6.9 mL, 51.0 mmol) and ZnI_2 (0.54 g, 1.7 mmol) were added, and the reaction mixture was stirred overnight at rt. The solvent was removed in vacuo, and the residue was partitioned between water and EtOAc. The organic layer was washed with brine and dried (Na_2SO_4). Evaporation of solvent gave a residue that was purified by column chromatography (SiO_2, petroleum ether/EtOAc = 8:2) to obtain compound **81b** (50% yield). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40−7.30 (m, 5H), 5.20−5.10 (m, 2H), 4.70−4.50 (m, 1H), 3.70−3.60 (m, 1H), 3.50−3.30 (m, 1H), 2.50−2.30 (m, 1H), 2.20−2.10 (m, 1H), 1.90−1.80 (m, 1H), 1.34−1.24 (m, 3H).

Benzyl 2-Cyano-5-methylpyrrolidine-1-carboxylate (81e). Following the same three steps for the preparation of nitrile **81b**, compound **81e** was prepared from **92e**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.50−7.30 (m, 5H), 5.30−5.10 (m, 2H), 4.60−4.50 (m, 1H), 4.20−4.10 (m, 1H), 2.40−2.20 (m, 3H), 1.80−1.70 (m, 1H), 1.30−1.10 (m, 3H).

1-*tert*-Butyl 2-Methyl (2*S*)-4-Methylpyrrolidine-1,2-dicarboxylate (95). To a solution of 1-*tert*-butyl 2-methyl (2*S*)-4-methylenepyrrolidine-1,2-dicarboxylate[48] **94** (0.2 g, 0.8 mmol) in MeOH

(10 mL), PtO_2 (20 mg, 0.08 mmol) was added and the reaction mixture was stirred under a H_2 atmosphere (1 atm) for 12 h. Then the mixture was filtered through celite and solvent was removed under vacuum affording compound **95** as an inseparable mixture of *cis*/*trans*-isomers (5:1, based on NMR data), which was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.20−4.00 (m, 1H), 3.64 (s, 3H), 3.60−3.50 (m, 1H), 3.00−2.80 (m, 1H), 2.40−2.20 (m, 1H), 2.20−2.10 (m, 1H), 1.50−1.40 (m, 1H), 1.37 and 1.31 (2s, 9H), 0.98 (d, J = 6.5 Hz, 3H). MS m/z 244 $(M + H)^+$.

1-*tert*-Butyl (2*S*)-2-(Aminocarbonyl)-4-methylpyrrolidine-1-carboxylate (96). Compound **95** (0.3 g, 1.23 mmol) was heated in a mixture of THF/32% aq NH_3 solution (1:9, 20 mL) at 60 °C overnight in a sealed tube. The solvents were reduced in vacuo affording the primary amide **96** as a white solid, which was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.30−5.90 (m, 2H), 4.30−4.10 (m, 1H), 3.80−3.60 (m, 1H), 3.00−2.80 (m, 1H), 2.40−2.20 (m, 1H), 2.20−2.10 (m, 1H), 1.80−1.50 (m, 1H), 1.43 and 1.39 (2s, 9H), 1.04 (d, J = 6.6 Hz, 3H). MS m/z 229 $(M + H)^+$.

1-*tert*-Butyl (2*S*)-2-Cyano-4-methylpyrrolidine-1-carboxylate (81c + 81d). A solution of compound **96** (0.25 g, 1.1 mmol) and Et_3N (0.5 mL, 3.7 mmol) in DCM(10 mL) was cooled to 0 °C and TFAA (0.18 mL, 1.3 mmol) was added dropwise under nitrogen. Stirring was continued at rt for 1 h, and volatiles were removed in vacuo. The residue was taken up in EtOAc, washed with water and brine, and dried (Na_2SO_4). Evaporation of solvent gave a residue that was purified by column chromatography (SiO_2, petroleum ether/EtOAc = 8:2) to obtain a mixture of *cis*/*trans*-nitriles **81c** and **81d** in 75% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.50−4.30 (m, 1H), 3.70−3.50 (m, 1H), 3.00−2.80 (m, 1H), 2.50−2.30 (m, 1H), 2.30−2.10 (m, 1H), 1.90−1.80 (m, 1H), 1.48, 1.45 (2s, 9H), 1.12 (d, J = 6.6 Hz, 3H). MS m/z 211 $(M + H)^+$.

Benzyl-(2*S*,4*R*)-2-cyano-4-methoxypyrrolidine-1-carboxylate (81g). To compound **98g** (10.94 g, 39.18 mmol) prepared according literature[49] and dissolved in dioxane (60 mL), Boc-anhydride (11.11 g 1.3 equiv), NH_4HCO_3 (3.46 g, 1.26 equiv), and pyridine (2 mL) were added. The mixture was stirred overnight at rt. Dioxane was removed in vacuo and the residue, dissolved in ethyl acetate, was washed with HCl (1 N), saturated aqueous $NaHCO_3$, and brine, dried (Na_2SO_4), filtered, and concentrated in vacuo to get the primary amide, which was submitted to the next step without further purification, 7.86 g (72% yield). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.50−7.22 (m, 6H), 7.02−7.11 (m, 1H), 5.14−5.01 (m, 1H), 4.28−4.12 (m, 1H), 3.95 (bs, 1H), 3.61−3.41 (m, 2H), 3.21 (s, 3H), 2.48−2.18 (m, 1H), 1.99−1.82 (m, 1H). The primary amide (7.86 g, 28.3 mmol) was dissolved in DCM (250 mL) and treated with TEA (8.28 mL, 2.1 equiv). The mixture was cooled down to 0 °C and TFAA (4.39 mL, 1.1 equiv) was added. After 1 h, the organic solution was diluted, washed with HCl (1 N), $NaHCO_3$ satd soln, and brine, dried (Na_2SO_4), filtered, and evaporated in vacuo. The title product was obtained after purification by column chromatography (SiO_2, petroleum ether/ethyl acetate = 8:2), yielding compound **81g** as a 4:6 mixture of rotamers by NMR (4.62 g, 63% yield). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.45−7.3 (m, 5H), 5.14 (s, 1.2H), 5.20 (d, J = 12.0 Hz, 0.4H), 5.12 (d, J = 13.0 Hz, 0.4H), 4.75 (t, J = 7.0 Hz, 0.4H), 4.64 (t, J = 7.8 Hz, 0.6H), 4.02 (bs, 1H), 3.6−3.45 (m, 2H), 3.21 (s, 3H), 2.45−2.40 (partially under DMSO, m, 1H), 2.40−2.25 (m, 1H).

***tert*-Butyl (2*S*,4*R*)-4-(Benzyloxy)-2-cyanopyrrolidine-1-carboxylate (81f) and Benzyl-(2*S*,4*R*)-2-cyano-4-ethoxypyrrolidine-1-carboxylate (81h)** were prepared by using the procedure described above for the synthesis of compound **81g**. (**81f**) ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.34−7.26 (m, 5H), 4.62 (t, J = 7.9 Hz, 1H), 4.53−4.45 (m, 2H), 4.19 (bs, 1H), 3.57−3.41 (m, 2H), 2.45−2.40 (partially under DMSO, m, 1H), 2.40−2.22 (m, 1H), 1.44 (s, 9H). MS m/z 303 $(M + H)^+$. (**81h**) ^{1}H NMR (DMSO-d_6, 300 MHz, 330 K) δ 7.53−7.24 (m, 5H), 5.25−5.11 (m, 2H), 4.72 (m, 1H), 4.21−4.10 (m, 1H), 3.63−3.37 (m, 4H), 2.47−2.25 (partially under DMSO, m, 2H), 1.09 (t, J = 7.0 Hz, 3H).

Benzyl (2*S*, 4*S*)-4-[(*tert*-Butoxycarbonyl)amino]-2-cyanopyrrolidine-1-carboxylate (81i). ^{1}H NMR (DMSO-d_6, 300 MHz, 340 K) δ 7.41 (m, 5H), 7.03 (m, 1H), 5.16 (s, 2H), 4.73 (bm, 1H), 4.07−3.99 (m, 1H), 3.63 (dd, J = 107, 6.3 Hz, 1H), 3.35 (dd, J = 10.7, 4.9 Hz, 1H), 2.47 (m, 1H), 2.30−2.24 (m, 1H), 1.41 (s, 9H). MS m/z 346 $(M + H)^+$.

Benzyl-(2*S*,4*S*)-2-cyano-4-fluoropyrrolidine-1-carboxylate (81l) and **Benzyl-(2*S*)-2-cyano-4,4-difluoropyrrolidine-1-carboxylate (81n)** were obtained from the corresponding carboxylic acids prepared according literature.[50] **Benzyl-(2*S*,4*R*)-2-cyano-4-fluoropyrrolidine-1-carboxylate (81m)** was used as such in the next step of the synthesis and it was not isolated. (**81l**) ^{1}H NMR (DMSO-d_6, 400 MHz, 340 K) δ 7.42−7.30 (m, 5H), 5.40 (dbt, J_{H-F} = 52.3 Hz, 1H), 5.20 (d, J = 12.7 Hz, 1H), 5.16 (d, J = 12.7 Hz, 1H), 4.94 (d, J = 8.4 Hz, 1H), 3.68−3.56 (m, 2H), 2.63−2.41 (m, 2H). MS m/z 249 $(M + H)^+$. (**81n**) ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.40−7.34 (m, 5H), 5.20−5.03 (m, 3H), 3.99−3.72 (m, 2H), 3.06−2.69 (m, 2H).

***tert*-Butyl 3-(Aminocarbonyl)piperazine-1-carboxylate (101).** Compound **100** (25.0 g, 75.7 mmol) was suspended in THF (125 mL), then pyridine (9.19 mL, 113.6 mmol), DMF (1.925 mL, 25.0 mmol), and thionyl chloride (7.25 mL, 100.8 mmol) were sequentially added, and the mixture was stirred at 40 °C for 4 h. After dilution with brine, the product was extracted with EtOAc, and the organic layer was dried (Na_2SO_4), filtered, and evaporated under vacuum. The crude was dissolved in DCM (100 mL) and added to a 0.5 M solution of ammonia in dioxane (350 mL, 175 mmol), and the resulting mixture was stirred at 40 °C for 2 h, then at rt overnight. Solvent was evaporated under vacuum, the residue was dissolved in 1 N HCl, washed with diethyl ether, basified with sodium hydroxide solution, extracted with DCM, dried (Na_2SO_4), filtered, and evaporated under vacuum to provide **101** (15.4 g, 89%). ^{1}H NMR (DMSO-d_6, 400 MHz, 340 K) δ 6.92 (bs, 2H), 3.84 (dd, J = 13.0, 3.0 Hz, 1H), 3.60 (d, J = 13.0 Hz, 1H), 3.09 (dd, J = 9.0, 3.0 Hz, 1H), 2.90−2.78 (m, 3H), 2.60−2.50 (m, 2H), 1.41 (s, 9H).

1-Benzyl 4-*tert*-Butyl 2-(Aminocarbonyl)piperazine-1,4-dicarboxylate (102). To a stirred mixture of compound **101** (3.85 g, 16.8 mmol) and Et_3N (5.6 mL, 40.4 mmol) in DCM (150 mL), CbzCl (5.8 mL, 38.7 mmol) was added, and the mixture was stirred at rt overnight. The mixture was washed with 1 N HCl, $NaHCO_3$ satd soln, then brine, dried (Na_2SO_4), filtered, and evaporated under vacuum to provide the product as an oil, which solidified upon treatment with petroleum ether to give **102** (5.38 g, 88%). ^{1}H NMR (DMSO-d_6, 400 MHz, 340 K) δ 7.39−7.27 (m, 5H), 7.10 (bs, 2H), 5.09 (s, 2H), 4.46−4.42 (m, 1H), 4.26 (d, J = 13.5 Hz, 1H), 3.82−3.72 (m, 2H), 3.46−3.35 (m, 1H), 3.20 (dd, J = 13.5, 4.8 Hz, 1H), 2.97−2.87 (m, 1H), 1.40 (s, 9H).

1-Benzyl 4-*tert*-Butyl 2-Cyanopiperazine-1,4-dicarboxylate (103). To a 0 °C solution of compound **102** (15.0 g, 41.32 mmol) and TEA (12.1 mL, 87.0 mmol) in DCM (400 mL), TFAA (6.4 mL, 45.5 mmol) was added dropwise. After 2 h, the mixture was washed with brine, dried (Na_2SO_4), filtered, and evaporated under vacuum to provide the desired product **103** in quantitative yield. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.45−7.28 (m, 5H), 5.32 (bs, (1H), 5.15 (s, 2H), 4.22−4.08 (m, 1H), 4.00−3.45 (m, 2H), 3.20−3.04 (m, 1H), 3.02−2.80 (m, 2H), 1.42 (s, 9H).

1-Benzyl 4-*tert*-Butyl 2-[(Amino(hydroxyimino)methyl]piperazine-1,4-dicarboxylate (104). A solution of the nitrile **103** (10.0 g, 29.0 mmol) in MeOH (40 mL) was added to a stirred mixture of hydroxylamine hydrochloride (2.60 g, 37.7 mmol) and TEA (6.0 mL, 43.5 mmol) in MeOH (30.0 mL), and the mixture was stirred at rt overnight. After dilution with water and extraction with EtOAc, the organic layer was dried (Na_2SO_4), filtered, and evaporated under vacuum to provide the product **104** in quantitative yield. ^{1}H NMR (DMSO-d_6, 400 MHz, 340 K) δ 9.15 (bs, 1H), 7.40−7.30 (m, 5H), 5.42 (bs, 2H), 5.07 (s, 2H), 4.53 (bs, 1H), 4.25 (d, J = 13.0 Hz, 1H), 3.85 (bs, 1H), 3.71 (d, J = 13.0 Hz, 1H), 3.42 (bs, 1H), 3.08 (bs, 1H), 2.81 (bs, 1H), 1.37 (s, 9H). MS m/z 379 $(M + H)^+$.

1-Benzyl 4-*tert*-Butyl-2-[5-(benzoyloxy)-4-hydroxy-6-(methoxycarbonyl)pyrimidin-2-yl]-piperazine-1,4-dicarboxylate (105).

A solution of amidoxime **104** (10.96 g, 29.0 mmol) and dimethyl acetylene dicarboxylate (4.95 g, 34.8 mmol) in $CHCl_3$ (85 mL) was refluxed for 12 h. The crude product, obtained after evaporation of the solvent under vacuum, (theoretical 29.0 mmol from previous step) was used as such for the following cyclization reaction, and it was heated at 160 °C in xylene (85 mL) for 8 h and at 120 °C for 48 h. The mixture was evaporated under vacuum, the resulting crude was dissolved in pyridine (103 mL), and benzoic anhydride (13.12 g, 58.0 mmol) was added. After 3 h stirring at rt, the mixture was evaporated under vacuum, and the residue was dissolved in EtOAc, washed with water, dried over anhydrous sodium sulfate, filtered, and evaporated under vacuum to provide crude benzoate, which was purified by column chromatography (SiO_2, petroleum ether/EtOAc) to provide **105** (4.01 g, 23% yield over three steps). ^{1}H NMR (DMSO-d_6, 400 MHz, 340 K) δ 13.35 (bs, 1H), 8.07 (d, J = 7.2 Hz, 2H), 7.76 (t, J = 7.2 Hz, 1H), 7.62 (t, J = 7.8 Hz, 2H), 7.40−7.25 (m, 5H), 5.20−5.10 (m, 2H), 4.97−4.94 (m, 1H), 4.29 (d, J = 14.2 Hz, 1H), 3.93 (d, J = 13.8 Hz, 1H), 3.85−3.75 (m, 2H), 3.73 (s, 3H), 3.46 (dd, J = 14.3, 4.5 Hz, 1H), a signal hidden under H_2O, 1.30 (s, 9H). MS m/z 593 $(M + H)^+$.

1-Benzyl 4-*tert*-Butyl 2-[5-(Benzoyloxy)-4-(methoxycarbonyl)-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl]piperazine-1,4-dicarboxylate (106). Compound **105** (1.78 g, 3.0 mmol) was dissolved in dry THF (70 mL), then Cs_2CO_3 (1.07 g, 3.3 mmol) and dimethysulfate (0.404 mL, 3.3 mmol) were added. After 1 h at 60 °C, the reaction mixture was concentrated, diluted with AcOEt, washed with HCl (1 N), water, and brine, dried (Na_2SO_4), filtered, and evaporated under vacuum to give a crude that was purified by column chromatography (SiO_2, AcOEt/petroleum ether = 2:3; 0.74 g, yield 41%). The regioisomeric OMe compound was not isolated. ^{1}H NMR (DMSO-d_6, 400 MHz, 340 K) δ 8.08 (d, J = 7.3 Hz, 2H), 7.77 (t, J = 7.4 Hz, 1H), 7.63 (t, J = 7.7 Hz, 2H), 7.35−7.20 (m, 5H), 5.35−5.30 (m, 1H), 5.15−5.05 (m, 2H), 4.15 (bd, J = 14.7 Hz, 1H), 4.00−3.90 (m, 2H), 3.80−3.70 (m, 1H), 3.73 (s, 3H), 3.65−3.50 (m, 4H), 1.27 (s, 9H). MS m/z 607 $(M + H)^+$.

***tert*-Butyl 3-(4-{[(4-Fluorobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydro-pyrimidin-2-yl)-4-methyl-piperazine-1-carboxylate (36).** Compound **106** (0.68 g, 1.12 mmol) was dissolved in MeOH (45.0 mL), Pd/C (10%, 0.068 g, 10% w/w) was added, and the suspension was hydrogenated at atm pressure. After 30 min, the catalyst was filtered and the filtrate was evaporated under vacuum to provide the crude compound, which was used as such for the following reaction. It was dissolved (0.60 g, 1.26 mmol) in MeOH (10.0 mL), $NaCNBH_3$ (0.11 g, 1.76 mmol) and sodium acetate (0.17 g, 2.02 mmol) were added to the solution, followed by a 37% formaldehyde solution in water (0.204 mL, 2.52 mmol). The reaction mixture was stirred at rt for 2 h, then evaporated to give the *N*-methylated product that, without further purification, was suspended in MeOH (8.0 mL), where 4-F-benzylamine (1.15 mL, 10.09 mmol) was added, and the mixture was stirred at 80 °C for 12 h. From the crude mixture solvent was evaporated under vacuum, and the crude was purified by RP-HPLC to provide pure **36** as trifluoroacetate salt. ^{1}H NMR (DMSO-d_6 + TFA, 400 MHz, 340 K) δ 7.40−7.35 (m, 2H), 7.18−7.10 (m, 2H), 4.83 (d, J = 7.3 Hz, 1H), 4.59 (d, J = 6.3 Hz, 2H), 4.41 (d, J = 14.9 Hz, 1H), 4.20−4.10 (m, 1H), 3.75−3.60 (m, 1H), 3.54 (s, 3H), 3.38−3.25 (m, 2H), 3.15−3.05 (m, 1H), 2.85 (s, 3H), 1.45 (s, 9H). MS m/z 476 $(M + H)^+$.

***N*-(4-Fluorobenzyl)-5-hydroxy-1-methyl-2-(1-methylpiperazin-2-yl)-6-oxo-1,6-dihydropyrimidine-4-carboxamide (33).** Compound **36** was treated with a TFA/DCM solution, then evaporated under vacuum to give a residue that was purified by RP-HPLC to provide **33** (0.065 g, 11% over 4 steps). ^{1}H NMR (DMSO-d_6, 400 MHz, 340 K) δ 12.25 (bs, 1H), 9.03 (bs, 1H), 7.42−7.35 (m, 2H), 7.20−7.10 (m, 2H), 4.62−4.45 (m, 2H), 4.14−4.09 (m, 1H), 3.62 (s, 3H), 3.62−3.52 (m, 1H), 3.48−3.32 (m, 1H), 3.25−3.15 (m, 1H), 3.15−3.05 (m, 2H), 2.44−2.32 (m, 1H), 2.34 (s, 3H). MS m/z 376 $(M + H)^+$.

2-(1,4-Dimethylpiperazin-2-yl)-*N*-(4-fluorobenzyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide (31). Compound **33** (0.05 g, 0.13 mmol) was dissolved in MeOH (2.0 mL). TEA (0.02 mL, 0.13 mmol), $NaCNBH_3$ (0.02 g, 0.37 mmol), and sodium acetate (0.04 g, 0.43 mmol) were added to the solution, followed by a 37% formaldehyde solution in water (0.04 mL, 0.67 mmol). The reaction mixture was stirred at rt for 1 h. Solvent was evaporated under vacuum, to give a residue that was purified by RP-HPLC to provide **31** as trifluoroacetate salt (0.02 g, yield 75%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.19 (bs, 1H), 7.45−7.35 (m, 2H), 7.20−7.08 (m, 2H), 4.75−4.65 (m, 1H), 4.54 (d, J = 6.3 Hz, 2H), 3.75−3.45 (m, 6H), 3.48−3.25 (m, 2H), 3.22−3.09 (m, 1H), 2.81 (s, 3H), 2.66 (s, 3H). MS m/z 390 $(M + H)^+$.

2-(4-Ethyl-1-methylpiperazin-2-yl)-*N*-(4-fluorobenzyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide (34). Compound **33** (0.1 g, 0.2 mmol) was dissolved in MeOH (2 mL), Et_3N (0.056 mL, 0.4 mmol), $NaCNBH_3$ (17.4 mg, 0.28 mmol), AcONa (26.2 mg, 0.32 mmol), and CH_3CHO (1 mL) were added, and the reaction mixture was stirred at rt for 1 h, and solvent was removed under reduced pressure. The crude was purified by preparative HPLC purification to give compound **34** (yield 8%). ^{1}H NMR (DMSO-d_6 + TFA, 300 MHz) δ 9.38 (t, J = 5.9 Hz, 1H), 7.40−7.30 (m, 2H), 7.20−7.10 (m, 2H), 5.10−4.98 (m, 1H), 4.60−4.50 (m, 2H), 4.04−3.75 (m, 3H), 3.60−3.32 (m, 4H), 3.33−3.12 (m, 4H), 2.87 (s, 3H), 1.21 (t, J = 7.14 Hz, 3H). MS m/z 404 $(M + H)^+$.

***N*-(4-Fluorobenzyl)-5-hydroxy-2-(4-isopropyl-1-methylpiperazin-2-yl)-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide (35).** Compound **33** (0.10 g, 0.2 mmol) was dissolved in MeOH (2 mL), and Et_3N (0.166 mL, 1.2 mmol), $NaCNBH_3$ (17.4 mg, 0.28 mmol), AcONa (26.2 mg, 0.32 mmol), and acetone (1 mL) were added. The reaction mixture was stirred at rt for 3 h, solvent was removed under reduced pressure, and the crude was purified by preparative HPLC purification. The product obtained was dissolved in CH_3CN (0.5 mL), HCl (1 N, 1.5 mL), and water (5 mL) and lyophilized to give **35** as hydrochloride salt (14% yield). ^{1}H NMR (DMSO-d_6 + TFA, 400 MHz) δ 10.08 (bs, 1H), 7.48−7.38 (m, 2H), 7.18−7.08 (m, 2H), 5.21−5.12 (m, 1H), 4.57−4.43 (m, 2H), 4.08−3.80 (m, 3H), 3.70−3.50 (m, 6H), 3.32−3.20 (m, 1H), 2.87 (s, 3H), 1.28 (d, J = 6.6 Hz, 6H). MS m/z 418 $(M + H)^+$.

2-(4-Acetyl-1-methylpiperazin-2-yl)-*N*-(4-fluorobenzyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide (37). Compound **33** (0.10 g, 0.2 mmol) was dissolved in pyridine (1 mL) and acetic anhydride (1 equiv) was added. The reaction mixture was stirred at rt for 30 min. Solvent was evaporated under vacuum, to give a residue that was purified by RP-HPLC to provide **37** as trifluoroacetate salt (0.015 g, 14% yield). ^{1}H NMR (DMSO-d_6 + TFA, 400 MHz; two conformers are present with a 1:1 ratio) δ 10.11 (bs, 1 H), 9.45 (bs, 1 H), 7.42−7.31 (m, 2 H), 7.21−7.12 (m, 2 H), 5.03−5.59 (m, 0.5 H), 4.87−4.75 (m, 1 H), 4.64−4.52 (m, 2.5 H), 4.48−4.39 (m, 0.5 H), 4.22−4.12 (m, 0.5 H), 3.66−3.77 (m, 1 H), 3.59−3.46 (m, 3.5 H), 3.42−3.16 (m, 1.5 H), 3.15−2.95 (m, 0.5 H), 2.85−2.71 (m, 3.5 H), 2.14−2.05 (m, 3 H). MS m/z 418 $(M + H)^+$.

2-(4-Benzoyl-1-methylpiperazin-2-yl)-*N*-(4-fluorobenzyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide (39). To a solution of compound **33** (30 mg, 0.06 mmol) in pyridine (1 mL), benzoic anhydride (16 mg, 0.07 mmol) was added. The reaction mixture was stirred at rt for 20 min, solvent was removed under reduced pressure, and the crude was purified by preparative HPLC purification to obtain a compound that was dissolved in CH_3CN (0.5 mL), HCl (1 N, 0.5 mL), and water (1 mL) and characterized as hydrochloride salt **39** (53% yield). ^{1}H NMR (DMSO-d_6 + TFA, 300 MHz, 340 K) δ 9.75 (bs, 1H), 7.60−7.38 (m, 7H), 7.20−7.10 (m, 2H), 5.02−4.92 (m, 1H), 4.68−4.20 (m, 4H), 3.75−3.20 (m, 7H), 2.83 (s, 3H). MS m/z 480 $(M + H)^+$.

2-[4-(*N*,*N*-Dimethylglycyl)-1-methylpiperazin-2-yl]-*N*-(4-fluorobenzyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide (40). To a solution of compound **33** (30 mg, 0.06 mmol) in DCM (2 mL), HOBt (10.5 mg, 0.08 mmol), WSCDI (15 mg, 0.08 mmol), DIPEA (0.06 mL, 0.32 mmol), and *N*,*N*-dimethylglycine hydrochloride (17.3 mg, 0.12 mmol) were added. The reaction mixture was stirred at rt for 1 h, solvent was removed under vacuo, and the crude was purified by preparative HPLC to

give compound **40** as trifluoroacetate salt (40% yield). ^{1}H NMR (DMSO-d_6 + TFA, 300 MHz) δ 9.80 (bs, 1H), 9.50 (bs, 1H) 7.43−7.32 (m, 2H), 7.23−7.13 (m, 2H), 5.08−4.80 (m, 1.5H), 4.68−3.70 (m, 6.5H), 3.65−3.20 (m, 5H), 318−2.70 (m, 10H). MS *m*/*z* 461 (M + H)$^+$.

2-{4-[(Ethylamino)carbonyl]-1-methylpiperazin-2-yl}-*N*-(4-fluorobenzyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine carboxamide (41). To a solution of compound **33** (0.12 g, 0.2 mmol) in Py (2.0 mL), ethyl isocyanate (0.016 mL, 0.2 mmol) was added. The reaction mixture was stirred at rt for 30 min. Solvent was evaporated under vacuum, to give a residue that was purified by RP-HPLC to provide **41** as trifluoroacetate salt. ^{1}H NMR (DMSO-d_6 + TFA, 400 MHz, 340 K) δ 9.30 (bs, partially hidden by water signal, 1 H), 7.44−7.32 (m, 2 H), 7.20−7.08 (m, 2 H), 4.82−4.70 (m, 1 H), 4.65−4.52 (m, 2 H), 4.50−4.38 (m, 1H), 4.28−4.15 (m, 1H), 3.72−3.64 (m, 1H), 3.58 (s, 3H), 3.35−3.21 (m, 2 H), 3.20−3.16 (m, 2 H), 3.04−2.90 (m, 1 H), 2.84 (s, 3 H), 1.12−1.00 (m, 3 H). MS *m*/*z* 447 (M + H)$^+$.

***N*-(4-Fluorobenzyl)-5-hydroxy-1-methyl-2-[1-methyl-4-(phenylsulfonyl)piperazin-2-yl]-6-oxo-1,6-dihydropyrimidine-4-carboxamide (43).** To a solution of compound **33** (39 mg, 0.08 mmol) in DCM (0.5 mL), benzenesulphonyl chloride (0.07 mL, 0.56 mmol), and NaOH (1 M, 0.56 mL) were added. The mixture was stirred at rt for 18 h and then warmed to 60 °C. After 2 h, the reaction mixture was cooled and the solvent was removed under reduced pressure. Purification by RP-HPLC gave compound **43** (35% yield). ^{1}H NMR (DMSO-d_6 + TFA, 300 MHz, 340 K) δ 9.20−9.08 (m, 1H), 7.87−7.62 (m, 5H), 7.38−7.25 (m, 2H), 7.20−7.10 (m, 2H), 5.03−4.91 (m, 1H), 4.60−4.48 (m, 2H), 4.30−4.18 (m, 1H), 4.07−3.92 (m, 1H), 3.79−3.69 (m, 1H), 3.56 (s, 3H), 3.50−3.38 (m, 1H), 2.92−2.60 (m, 5H). MS *m*/*z* 516 (M + H).

2-{4-[(Dimethylamino)sulfonyl]-1-methylpiperazin-2-yl}-*N*-(4-fluorobenzyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide (44). To a solution of compound **33** (30 mg, 0.06 mmol) in DCM (0.8 mL), TEA (0.03 mL, 0.21 mmol) followed by dimethylsulfamoyl chloride (0.084 mL, 0.08 mmol) was added. After stirring for 18 h at rt, solvent was removed under reduced pressure and the crude was purified by RP-HPLC to give compound **44** (28% yield). ^{1}H NMR (DMSO-d_6 + TFA, 600 MHz) δ 9.48−9.40 (m, 1H), 7.39−7.33 (m, 2H), 7.20−7.15 (m, 2H), 5.02−4.94 (m, 1H), 4.59−4.54 (m, 2H), 4.17−4.09 (m, 1H), 3.93−3.84 (m, 1H), 3.78−3.70 (m, 1H), 3.52 (s, 3H), 3.46−3.08 (m, 2H), 3.14−3.04 (m, 1H), 2.86 (s, 3H), 2.78 (s, 6H). MS *m*/*z* 483 (M + H).

***N*-(4-Fluorobenzyl)-5-hydroxy-1-methyl-2-[1-methyl-4-(methylsulfonyl)piperazin-2-yl]-6-oxo-1,6-dihydropyrimidine-4-carboxamide (42).** Compound **106** was deprotected and methylated on the N1 nitrogen, the Boc was removed as previously described, and the resulting compound (0.05 g, 0.1 mmol) was dissolved in DCM (2 mL) and TEA (0.03 mL, 0.21 mmol), and methanesulfonyl chloride (0.01 mL, 0.11 mmol) was added. After 1 h of stirring at rt, the solvent was evaporated and the residue was dissolved in MeOH (1.5 mL), 4-F-benzylamine (0.034 mL, 0.3 mmol) was added, and the mixture was stirred at 80 °C overnight. The residue was purified by RP-HPLC and the product was then treated with a mixture of 3 N HCl/acetonitrile and lyophilized to obtain product **42** as a hydrochloride salt (0.013 g). ^{1}H NMR (DMSO-d_6 + TFA, 400 MHz, 340 K) δ 9.95−9.88 (m, 1H), 7.45−7.39 (m, 2H), 7.15−7.05 (m, 2H), 5.00−4.93 (m, 1H), 4.52 (d, J = 6.4 Hz, 2H), 4.20−4.14 (m, 1H), 3.95−3.89 (m, 1H), 3.76−3.70 (m, 1H), 3.50−3.37 (m, 2H), 3.21−3.12 (m, 1H), 3.04 (s, 3H), 2.83 (s, 3H). MS *m*/*z* 454 (M + H)$^+$.

2-(1-Acetyl-4-methylpiperazin-2-yl)-*N*-(4-fluorobenzyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidine-4-carboxamide (38). Compound **106** was deprotected on the N1 nitrogen of the piperazine, as previously reported, and a solution of the obtained compound (0.10 g, 0.2 mmol) was dissolved in THF (2 mL) and treated with pyridine (0.05 mL, 0.6 mmol), followed by acetyl chloride (0.02 mL, 0.3 mmol). The reaction mixture was stirred at rt for 1 h and then concentrated to dryness. The resulting crude was diluted with AcOEt, washed with HCl (1 N), dried (Na_2SO_4), filtered, and evaporated under vacuum. The resulting crude was treated with a TFA/DCM solution (2 mL) at rt for 30 min and then evaporated under vacuum to give a compound deprotected on the N4 nitrogen of the piperazine, which was used as such in the next step. To a solution of this crude (theoretical 0.12 mmol) in MeOH (3 mL) and TEA (0.017 mL, 0.12 mmol), $NaCNBH_3$ (0.01 g, 0.17 mmol), sodium acetate (0.016 g, 0.19 mmol), followed by a 37% formaldehyde solution in water (0.01 mL, 0.12 mmol), was added. The reaction mixture was stirred at rt overnight and then evaporated. The resulting crude was diluted with AcOEt, washed with water, dried (Na_2SO_4), filtered, and evaporated under vacuum. The resulting crude was then dissolved in MeOH (2.0 mL) and 4-F-benzylamine (0.036 mL, 0.32 mmol) was added; the mixture was stirred at 60 °C overnight. The reaction mixture was then evaporated under vacuum to give a residue that was purified by RP-HPLC to provide **38** (0.012 g, 11% yield over five steps).^{1}H NMR (DMSO-d_6 + TFA, 400 MHz, 340 K) δ 8.88 (bs, 1H), 7.44−7.35 (m, 2H), 7.14−7.05 (m, 2H), 6.07 (m, 1H), 4.61−4.46 (m, 2H), 4.38−4.25 (m, 1H), 4.06−3.92 (m, 1H), 3.48−3.12 (m, 7H), 2.90 (s, 3H), 2.15 (s, 3H). MS *m*/*z* 418 (M + H)$^+$.

Methyl 5-(Benzoyloxy)-1-methyl-2-(4-methylmorpholin-3-yl)-6-oxo-1,6-dihydropyrimidine-4-carboxylate (107). A solution of compound **67a** (1.6 g, 3.4 mmol) in DCM (30 mL) was treated with TFA (25 mL) at 0 °C. The mixture was stirred and allowed to warm to rt over 2 h. Volatiles were removed under reduced pressure to obtain 3-[5-(benzoyloxy)-4-(methoxycarbonyl)-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl]morpholin-4-ium trifluoroacetate as a pale yellow solid (1.65 g, quantitative yield). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.63 (bs, 1H), 9.44 (bs, 1H), 8.09 (d, J = 7.7 Hz, 2H), 7.81 (t, J = 7.1 Hz, 1H), 7.64 (t, J = 7.6 Hz, 2H), 5.05 (d, J = 8.1 Hz, 1H), 4.40 (d, J = 12.1 Hz, 1H), 4.01 (d, J = 11.0 Hz, 1H), 3.87 (t, J = 10.4 Hz, 1H), 3.79 (s, 3H), 3.66−3.57 (m, 4H), 3.41−3.30 (m, 2H). MS *m*/*z* 374 (M + H)$^+$. This intermediate (1.65 g, 3.4 mmol) was solubilized in 1,2-dichloroethane (20 mL), and Et_3N (0.57 mL, 4.08 mmol), acetic acid glacial (0.20 mL, 3.57 mmol), and formaldehyde 37% (0.50 mL, 6.8 mmol) were added at rt, followed by $NaCNBH_3$ (0.256 mg, 4.08 mmol). The mixture was stirred at rt overnight, and then it was diluted with 1,2-dichloroethane (150 mL) and washed with $NaHCO_3$ satd soln (2 × 50 mL). Organics were dried (Na_2SO_4), filtered, and concentrated under reduced pressure to obtain compound **107** as a white solid (1.3 g, 98% yield) that was used as crude in the next step. ^{1}H NMR (DMSO-d_6, 400 MHz) δ. 8.07 (d, J = 7.5 Hz, 2H), 7.79 (t, J = 7.3 Hz, 1H), 7.63 (t, J = 7.8 Hz, 2H), 3.89 (d, J = 9.4 Hz, 1H), 3.77 (s, 3H), 3.76 (s, 3H), 3.72−3.57 (m, 2H), 3.32 (s, 3H), 3.10 (q, J = 7.1 Hz, 2H), 2.90 (d, J = 11.8 Hz, 1H), 2.38 (t, J = 9.8 Hz, 1H). MS *m*/*z* 388 (M + H)$^+$.

General Procedure for the Preparation of Compounds 50−61. Compound **107** (45 mg, 0.18 mmol) in MeOH (2 mL) was treated with the appropriate amine (2 equiv) under microwave irradiation (1500 s, 100 °C). Volatiles were removed under reduced pressure and the final compounds were purified by preparative RP-HPLC. The final compounds were isolated as trifluoracetic salts (yields 20−45%).

3-{4-[(Benzylamino)carbonyl]-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl}-4-methylmorpholin-4-ium Trifluoroacetate (50). ^{1}H NMR (DMSO-d_6 + TFA, 300 MHz) δ 10.12 (bs, 1 H), 9.69 (t, J = 6.5 Hz, 1H), 7.60−7.49 (m, 5H), 5.19 (bs, 1H), 4.83 (d, J = 5.6 Hz, 2 H), 4.60 (d, J = 4.6 Hz, 1H), 4.37 (d, J = 11.3 Hz, 1 H), 3.99 (t, J = 12.5 Hz, 1 H), 3.90 (d, J = 12.4 Hz, 1H), 3.76 (s, 3H), 3.71−3.63 (m, 2H), 3.06 (s, 3H). MS *m*/*z* 359 (M + H)$^+$.

3-(5-Hydroxy-1-methyl-6-oxo-4-{[(2-phenylethyl)amino]carbonyl}-1,6-dihydropyrimidin-2-yl)-4-methylmorpholin-4-ium Trifluoroacetate (51). ^{1}H NMR (DMSO-d_6 + TFA, 400 MHz) δ 10.08 (bs, 1 H), 9.11 (t, J = 5.8 Hz, 1H), 7.32−7.28 (m, 2H), 7.25−7.20 (m, 3H), 4.96 (bs, 1H), 4.39 (dd, J = 13.2, 2.9 Hz, 1 H), 4.17 (dd, J = 13.1, 2.2 Hz, 1 H), 3.78 (t, J = 12.2 Hz, 1H), 3.70 (d, J = 13.2 Hz, 1H), 3.57 (q, J = 7.0 Hz, 2H), 3.53 (s, 3H), 3.43 (dd, J = 12.7, 10.5 Hz, 2H), 2.87 (t, J = 7.0 Hz, 2H), 2.81 (s, 3H). MS *m*/*z* 373 (M + H)$^+$.

3-(4-{[Benzyl(methyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-4-methylmorpholin-4-ium Trifluoroacetate (52). Two sets of signals, two rotamers (ratio 1:1) were present. ^{1}H NMR (CD_3CN + TFA, 400 MHz) δ 7.50−7.34 (m, 5H), 4.86 (bs, 1H), 4.86−4.59 (m, 2H), 4.47−4.12 (m, 2H), 3.98−3.77 (m, 1H), 3.54 (s, 3H), 3.53−3.49 (m, 1H), 3.45−3.34 (m, 2H), 3.10 (s, 3H), 2.92 (s, 1.5 H), 2.86 (s, 1.5 H). MS *m*/*z* 373 $(M + H)^+$.

3-(4-{[(2-Fluorobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-4-methylmorpholin-4-ium Trifluoroacetate (53). ^{1}H NMR (DMSO-d_6 + TFA, 400 MHz) δ 10.08 (bs, 1 H), 9.49 (t, J = 6.1 Hz, 1H), 7.36−7.31 (m, 2H), 7.23−7.16 (m, 2H), 4.98 (bs, 1H), 4.63 (d, J = 6.1 Hz, 2 H), 4.38 (dd, J = 13.0, 2.7 Hz, 1H), 4.16 (dd, J = 13.1, 2.5 Hz, 1 H), 3.80 (t, J = 12.0 Hz, 1 H), 3.70 (d, J = 12.9 Hz, 1H), 3.54 (s, 3H), 3.48−3.35 (m, 2H), 2.84 (s, 3H). MS *m*/*z* 377 $(M + H)^+$.

3-(4-{[(3-Fluorobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-4-methylmorpholin-4-ium Trifluoroacetate (54). ^{1}H NMR (DMSO-d_6 + TFA, 400 MHz) δ 10.04 (bs, 1 H), 9.50 (bt, 1H), 7.43−7.34 (m, 1H), 7.17−7.06 (m, 2H), 5.00 (bs, 1H), 4.63 (d, J = 5.9 Hz, 2 H), 4.39 (d, J = 13.4 Hz, 1 H), 4.15 (d, J = 13.8 Hz, 1 H), 3.79 (t, J = 12.6 Hz, 1H), 3.71 (d, J = 13.2 Hz, 1H), 3.59 (s, 3H), 3.51−3.45 (m 2H), 2.86 (s, 3H). MS *m*/*z* 377 $(M + H)^+$.

3-(4-{[(3-Chlorobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-4-methylmorpholin-4-ium Trifluoroacetate (55). ^{1}H NMR (DMSO-d_6 + TFA, 300 MHz) δ 10.18 (bs, 1 H), 9.71 (t, J = 6.3 Hz, 1H), 7.64−7.49 (m, 4H), 5.18 (bs, 1H), 4.83 (d, J = 6.4 Hz, 2 H), 4.61 (d, J = 13.3 Hz, 1H), 4.38 (d, J = 12.6 Hz, 1 H), 3.99 (t, J = 13.7 Hz, 1 H), 3.91 (d, J = 13.3 Hz, 1H), 3.76 (s, 3H), 3.72−3.64 (m, 2H), 3.07 (s, 3H). MS *m*/*z* 393 $(M + H)^+$.

3-(4-{[(3-Bromobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-4-methylmorpholin-4-ium Trifluoroacetate (56). ^{1}H NMR (DMSO-d_6 + TFA, 300 MHz) δ 10.15 (bs, 1 H), 9.70 (t, J = 6.1 Hz, 1H), 7.73−7.69 (m, 2H), 7.55−7.54 (m, 2H), 5.19 (bs, 1H), 4.83 (d, J = 6.6 Hz, 2 H), 4.61 (d, J = 12.0 Hz, 1H), 4.38 (d, J = 12.2 Hz, 1 H), 4.00 (t, J = 13.3 Hz, 1 H), 3.90 (d, J = 13.1 Hz, 1H), 3.76 (s, 3H), 3.72−3.61 (m, 2H), 3.07 (s, 3H). MS *m*/*z* 437/439 $(M + H)^+$.

3-(5-Hydroxy-4-{[(3-methoxybenzyl)amino]carbonyl}-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-4-methylmorpholin-4-ium Trifluoroacetate (57). ^{1}H NMR (DMSO-d_6 + TFA, 300 MHz) δ 9.97 (bs, 1 H), 9.46 (t, J = 6.5 Hz, 1H), 7.27 (t, J = 7.9 Hz, 1 H), 6.90−6.83 (m, 3H), 4.97 (bs, 1H), 4.59 (d, J = 6.4 Hz, 2 H), 4.39 (dd, J = 13.4, 2.0 Hz, 1H), 4.17 (dd, J = 12.8, 2.0 Hz, 1 H), 3.82−3.67 (m, 5H), 3.55 (s, 3H), 3.49−3.41 (m, 2H), 2.45 (s, 3H). MS *m*/*z* 389 $(M + H)^+$.

3-(4-{[(3,4-Difluorobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-4-methylmorpholin-4-ium Trifluoroacetate (58). ^{1}H NMR (DMSO-d_6 + TFA, 400 MHz) δ 10.08 (bs, 1 H), 9.60 (t, J = 6.3 Hz, 1H), 7.43−7.32 (m, 2H), 7.17−7.14 (m, 1H), 4.97 (bs, 1H), 4.60 (d, J = 6.1 Hz, 2 H), 4.38 (dd, J = 12.9, 2.3 Hz, 1H), 4.15 (dd, J = 12.5, 1.9 Hz, 1 H), 3.80 (t, J = 12.5 Hz, 1 H), 3.68 (d, J = 12.6 Hz, 1H), 3.54 (s, 3H), 3.50−3.36 (m, 2H), 2.84 (s, 3H). MS *m*/*z* 395 $(M + H)^+$.

3-(4-{[(4-Fluoro-3-methylbenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-4-methylmorpholin-4-ium Trifluoroacetate (59). ^{1}H NMR (DMSO-d_6 + TFA, 400 MHz) δ 10.04 (bs, 1 H), 9.46 (t, J = 6.4 Hz, 1H), 7.23 (d, J = 7.5 Hz, 1H), 7.19−7.08 (m, 2H), 4.98 (bs, 1H), 4.55 (d, J = 6.4 Hz, 2H), 4.39 (dd, J = 12.9, 2.9 Hz, 1H), 4.20 (dd, J = 12.7, 2.9 Hz, 1H), 3.80 (t, J = 11.8 Hz, 1H), 3.70 (d, J = 12.9 Hz, 1H), 3. 56 (s, 3H), 3.49−3.36 (m, 2H), 2.85 (s, 3H), 2.22 (d, J = 1.3 Hz, 3H). MS *m*/*z* 391 $(M + H)^+$.

3-(4-{[(3-Chloro-4-fluorobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-4-methylmorpholin-4-ium Trifluoroacetate (60). ^{1}H NMR (DMSO-d_6 + TFA, 400 MHz) δ 10.06 (bs, 1 H), 9.50 (t, J = 6.3 Hz, 1H), 7.50 (d, J = 7.2 Hz, 1 H), 7.37−7.32 (m, 2H), 5.00 (d, J = 8.8 Hz, 1 H), 4.59 (d, J = 6.1 Hz, 2 H), 4.38 (d, J = 12.7 Hz, 1 H), 4.15 (d, J = 12.5 Hz, 1 H), 3.79 (t, J = 13.0 Hz, 1H), 3.71 (d, J = 13.4 Hz, 1H), 3.56 (s, 3H), 3.51−3.41 (m 2H), 2.86 (s, 3H). MS *m*/*z* 411 $(M + H)^+$.

3-(4-{[(3-Bromo-4-fluorobenzyl)amino]carbonyl}-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-4-methylmorpholin-4-ium Trifluoroacetate (61). ^{1}H NMR (DMSO-d_6 + TFA, 400 MHz) δ 12.21 (bs, 1H), 9.88 (bs, 1H), 9.52 (t, J = 6.0 Hz, 1H), 7.66 (d, J = 6.8 Hz, 1H), 7.38 (d, J = 7.0 Hz, 2H), 5.00−4.90 (m, 1H), 4.70−4.50 (m, 2H), 4.39 (d, J = 12.6 Hz, 1H), 4.16 (d, J = 12.6 Hz, 1H), 3.79 (t, J = 12.4 Hz, 1H), 3.68 (d, J = 12.2 Hz, 1H), 3.55 (s, 3H), 3.50−3.30 (m, 2H), 2.85 (bs, 3H). MS *m*/*z* 455/457 $(M + H^+)$.

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Note Added after ASAP Publication. This paper was published on September 8, 2007 with incorrect data reported in Scheme 6. The correct version was published ASAP on September 10, 2007.

Supporting Information Available: Experimental section relative to the synthesis of compounds **66d**, **78**, **89g**, **89h**, **85i**, **90n**, **86m**, **85b**, and **85e**, analytical data such as chromatographic and high-resolution mass data used as criterion of purity, and PK procedures are reported in details. This material is available free of charge via the Internet at http://pubs.acs.org.

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