Design and Synthesis of Bicyclic Pyrimidinones as Potent and Orally Bioavailable HIV-1 Integrase Inhibitors

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HIV integrase is one of the three enzymes encoded by HIV genome and is essential for viral replication, but integrase inhibitors as marketed drugs have just very recently started to emerge. In this study, we show the evolution from the *N*-methylpyrimidinone structure to bicyclic pyrimidinones. Introduction of a suitably substituted amino moiety modulated the physical−chemical properties of the molecules and conferred nanomolar activity in the inhibition of spread of HIV-1 infection in cell culture. An extensive SAR study led to sulfamide (*R*)-**22b**, which inhibited the strand transfer with an IC_{50} of 7 nM and HIV infection in MT4 cells with a CIC_{95} of 44 nM, and ketoamide (*S*)-**28c** that inhibited strand transfer with an IC_{50} of 12 nM and the HIV infection in MT4 cells with a CIC_{95} of 13 nM and exhibited a good pharmacokinetic profile when dosed orally to preclinical species.

Introduction

Human immunodeficiency virus type 1 (HIV-1) is responsible for human acquired immunodeficiency syndrome (AIDS), one of the most urgent world health threats. Currently approved therapies[1,2] are based on inhibitors of HIV protease (PIs),[3] reverse transcriptase (NRTIs and NNRTIs, nucleoside and non-nucleoside reverse transcriptase inhibitors),[4] and more recently, fusion inhibitors (enfuvirtide).[5] During the past years, the combination of protease and reverse transcriptase inhibitors (HAART, highly active antiretroviral therapy)[6,7] has demonstrated efficacy in strongly reducing morbidity and mortality. Nevertheless, the rapid emergence of drug resistant viral strains and the toxicity derived from chronic treatment remain serious issues that highlight the need for the development of new therapeutic strategies.

The HIV-1 genome, together with protease and reverse transcriptase, encodes a third enzyme, integrase, which is necessary for the viral life cycle and is an appealing target for drug discovery for its essential function in viral replication and the absence of a direct counterpart in the host cells.[8,9] HIV-1 integrase catalyzes the incorporation of the viral DNA into the host cell's genome through a multistep process. In the initial assembly step, integrase binds to specific sequences of the double-stranded viral DNA, newly formed by reverse transcription. An endonucleolytic cleavage of the last two nucleotides from the 3′ terminus of both strands of the viral DNA generates a processed DNA/integrase preintegration complex (PIC), which crosses the nuclear membrane to access the host genome. The cell DNA is then cleaved by integrase in a nonspecific way, and the shortened strands of viral DNA are integrated into the host DNA sequence.

Selective inhibition of the HIV-1 integrase activity causes an interruption of the HIV-1 replication cycle and could represent an answer to the unmet medical need for new and improved treatments.[10] Just very recently the first integrase inhibitor (raltegravir) was approved in the U.S. for treatment of HIV infection.[11]

We recently reported[12–14] that dihydroxypyrimidine carboxamide **1** (Figure 1) is a potent HIV integrase strand transfer[15,16] inhibitor (IC_{50} = 0.06 μM), but despite the high intrinsic potency, it showed a much lower activity in the cell based assays (CIC_{95} > 10 μM in inhibiting the spread of HIV-1 infection in MT4 cell culture in the presence of 10% fetal bovine serum (FBS) or 50% normal human serum (NHS) (spread assay).[15,17] An extensive SAR study on the substitution at the 2-position of the dihydroxypyrimidine scaffold[13] demonstrated that various substituents are tolerated, providing compounds equipotent to **1** in the enzymatic assay. This observation was interpreted as an indication that the 2-substituent is not directly interacting with the HIV integrase enzyme and that this position could be exploited to modify the physical−chemical properties of the molecule (lipophilicity, solubility, plasma protein binding) in order to modulate the pharmacological properties.

Introduction of a basic amine proved to be beneficial for cellular activity, as in **2**,[14] which proved to be a potent inhibitor both in the strand transfer and in the spread assays.

Cyclization of the tertiary amine of **2** as in **3** provided a further enhancement in the spread activity, and the introduction of a methyl on the 1-nitrogen of the pyrimidine led to 1-Me-pyrimidinone structure **4**,[18] which was more than 2 orders of magnitude more potent than **1** in the spread assay in the presence of 50% normal human serum (NHS).

Capitalizing on the above observations of the importance of installing a β-amino substituent in the 2-position of the

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[a] Abbreviations: TFA, trifluoroacetic acid; TMS, tetramethylsilane; DMSO, dimethyl sulfoxide; DMF, dimethylformamide; DCM, dichloromethane; THF, tetrahydrofuran; TEA, triethylamine; HOBt, 1-hydroxybenzotriazole; EDC, *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide; DIPEA, *N*,*N*-diisopropylethylamine; PEG, poly(ethylene glycol); NBS, *N*-bromosuccinimide; PK, pharmacokinetic; NADPH, nicotinamide adenine dinucleotide phosphate, reduced form; UDPGA, uridine 5′-diphosphoglucuronic acid; PPB, plasma protein binding; HPLC, high-performance liquid chromatography; RP-HPLC, reverse-phase high-performance liquid chromatography; UPLC, ultraperformance liquid chromatography; HRMS, high-resolution mass spectrometry.

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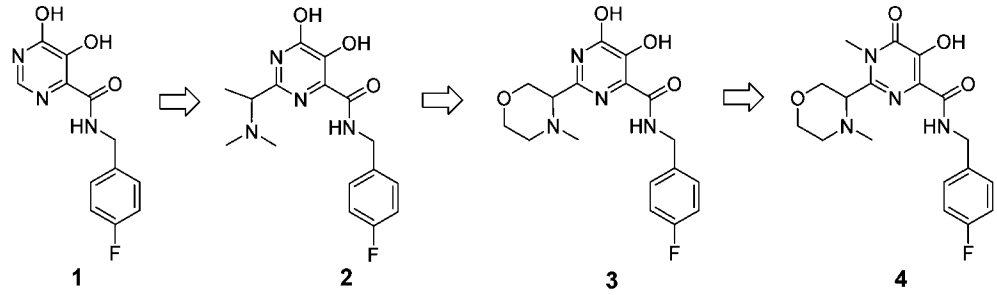


Cmpd.	Strand transfer IC_{50}[a] (μM)	Spread CIC_{95} (μM)[b] 10% FBS	50% NHS
1	0.06	>10	>10
2	0.10	0.13	0.50
3	0.01	0.03	0.23
4	0.06	0.06	0.10

[a] Strand transfer inhibition assay. For details see ref. in Table 1 [b] HIV-1 infection spread inhibition in cell culture. For details see ref. in Table 2.

Figure 1. Evolution from dihydroxypyrimidine to *N*-methylpyrimidinones.

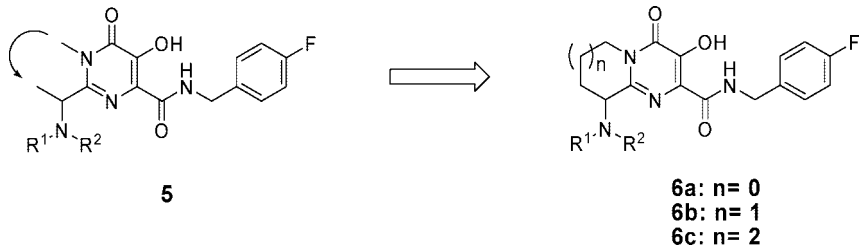


Figure 2. Strategic approach from *N*-methylpyrimidinones to bicyclic pyrimidinones.

pyrimidine scaffold and the beneficial effect of the 1N-methylation, we envisaged the possibility of constraining the 1*N*-Me of the 1*N*-methylpyrimidinone scaffold **5** into a saturated cycle as in **6** ($n = 0, 1, 2$) (Figure 2).

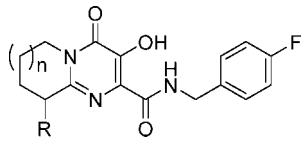
Results and Discussion

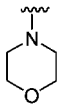
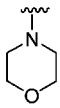
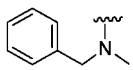
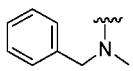
In order to probe the hypothesis summarized in Figure 2, our initial investigations focused on the evaluation of structure **6** where R^1 and R^2 are alkyls, which should provide the closest cyclized analogues of **3**–**4**. We were pleased to see that dimethylamino derivatives **7a**−**c**, the closest analogues of **2**, indeed displayed intrinsic activity ($IC_{50} = 0.007$, 0.069, and 0.55 μM, respectively) comparable to that for **1**–**4** for $n = 0$–1 and lower activity than **1**−**4** for $n = 2$. Substitution of the dimethylamino moiety with the cyclic amine pyrrolidine (**8b**) provided a compound that displayed the same activity as **7b**, but the introduction of more bulky cyclic amines (piperidine as in **9b**, morpholine as in **10b**,**c**) and the more lypophilic benzylmethylamine as in **11b**,**c** gave a 1 order of magnitude increase of the potency in the enzymatic assay. The positive influence of the benzyl moiety on integrase inhibition has also been suggested by a pharmacophore model previously reported by the Chimirri research group.[19]

Interestingly, the unsubstituted derivatives **12a**−**c** also showed very high enzymatic activity ($IC_{50} = 0.008$, 0.005, 0.019 μM, respectively), comparable to that of **9** and **10**. The activities displayed in Table 1 seem to follow a trend according to the size of the saturated ring in the bicyclic system, the seven-membered cycle ($n = 2$) being less active than the six-membered ($n = 1$), which is equally (or less as in **7b**) potent as the corresponding five-membered ($n = 0$) one. Unfortunately, all the above compounds show lower inhibitory effect on the spread of HIV infection in cell culture ($CIC_{95} \geq 1$ μM in the presence of 50% NHS). Nevertheless, these observations confirmed the potential of the bicyclic pyrimidinone series as HIV-1 integrase inhibitors and prompted us to undertake a further investigation on the 2-β-nitrogen in **6**, aimed at modifying the physical−chemical properties of the cyclized structures, in order to gain activity in the cell based assays.

The initial efforts concentrated on the exploration of the amide SAR, as shown in Table 2. Changing one of the two nitrogen alkyls into an acetyl group as in **13a**−**c** proved to be well tolerated for $n = 0$–1 and very favorable for $n = 2$, providing equipotent low nanomolar inhibitors in the strand transfer assay. When $n = 2$ (**13c**), activity was also observed in the cell based assay. A large variety of acyl groups was accepted: small acyl groups as in **14b** and more elaborate residues as in **15b** and **16b**. We were very pleased to discover that the introduction of a sulfonamide (as in **17b**) or a sulfone (as in **18b**,**c**) moiety in the β position with respect to the amide carbonyl resulted in very active inhibitors of the viral spread. The low shift in activity displayed in the low serum conditions (10% fetal bovine serum) with respect to the intrinsic enzymatic activity (less than 2-fold for **17b** and 3- to 4-fold for **18b**,**c**) confirmed the ability of the compounds to cross the cellular membrane. Moreover, the very low shift between the low and high serum (50% normal human serum) assay conditions can be rationalized as the presence of a significant unbound fraction of compound in human plasma,[20]

Table 1. Effect of Amino Substituents on Activity of Bicyclic Pyrimidinones[a]



Cmpd.	R	n	Strand transfer IC_{50}[b] (μM)
7a		0	0.007
7b		1	0.069
7c		2	0.55
8b		1	0.063
9b		1	0.004
10b		1	0.005
10c		2	0.062
11b		1	0.004
11c		2	0.062
12a	H	0	0.008
12b	H	1	0.005
12c	H	2	0.019

[a] Results are the mean of at least three independent experiments. [b] Assays were performed with recombinant HIV-1 integrase (0.1 μM) preassembled on immobilized oligonucleotides. Inhibitors were added after assembly and washings. For details, see ref 15 and references therein.

which was confirmed by a low value of human plasma protein binding (81% and 70% bound to human plasma proteins, respectively, for **17b** and **18b**).[21] Removal of one of the R^2 alkyl, as in **19b**, produced no change in the intrinsic potency but produced a marked drop of activity in the cell based assays.

The two enantiomers of **17b**, (*R*)-**17b** and (*S*)-**17b**, were prepared and further profiled in vitro and in vivo. Although showing the same in vitro enzymatic activity, enantiomer (*S*)-**17b** was 2-fold more potent in the spread assays (CIC_{95}: 10% FBS, 0.016 μM; 50% NHS, 0.031 μM) than (*R*)-**17b**. The pharmacokinetic profile in rat of (*S*)-**17b**, as reported in Table 4, was characterized by high plasma clearance (Clp = 55 (mL/min)/kg) and modest oral bioavailability (F = 17%). The metabolic stability in liver microsomes was studied in the presence of NADPH and UDPGA to evaluate the metabolism rate by oxidation and glucuronidation. (*S*)-**17b** displayed moderate stability in the presence of NADPH (turnover; rat 51, dog 15, human 36 %/h) and UDPGA (turnover; rat 47, dog 24, human 46 %/h).[22] The same pharmacokinetic profile was observed also for the enantiomer (*R*)-**17b** (data not shown). This finding, together with the low values of plasma protein binding measured in all the species investigated ((*S*)-**17b** plasma protein binding; rat 74, dog 71, human 78% bound), were consistent with the high plasma clearance observed in vivo (rat). The same profile observed for (*R*)-**17b** and (*S*)-**17b** was also observed for the enantiomers of derivative **18b** (data not shown).

We then turned our attention to sulfonamide derivatives (Table 3). Sulfonamides **20b**,**c** proved to be potent compounds both on the enzyme and in the cellular assays. Pharmacokinetic evaluation of **20b** in rat (Table 4) showed that, though characterized by a good oral bioavailability (F = 47%), the compound presents similar relatively high plasma clearance (Clp 48 (mL/min)/kg) already observed in the amide series. Metabolic stability studies in liver microsomes in the presence of UDPGA, in agreement with the observed in vivo data, showed high turnover in all species and in particular in rat and human (turnover; rat 88, dog 29, human 74 %/h).[22] Structurally more complex sulfonamides, such as **21b**, displayed similar high potency both on the enzyme and in the cell based assays and even lower metabolic stability in liver microsomes (turnover; rat 94, dog 78, human 90 %/h, in the presence of UDPGA).[22]

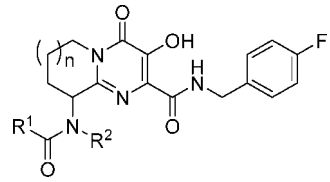
A significant improvement was obtained by modification of the sulfonamide moiety into a tetrasubstituted sulfamide as in **22a**−**c**. Compound **22b** was a low nanomolar inhibitor of HIV integrase strand transfer (IC_{50} = 0.005 μM), displaying only a 4-fold shift in activity in the spread assay in the presence of 50% human plasma serum (CIC_{95} = 0.021 μM). The corresponding analogue with n = 2 (**22c**), though only 2-fold less potent than **22b** in the enzymatic assay, showed a lower activity in the high serum cell based assay (CIC_{95} = 0.125 vs 0.021 μM). Surprisingly, the corresponding analogue for n = 0 (**22a**) was 9-fold less potent than **22b** in the strand transfer assay and not active up to 1 μM in both the cell based assays.

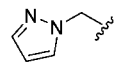
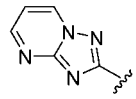
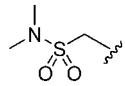
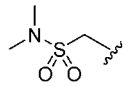
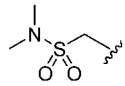
Further studies on the effects of different alkylation patterns on the sulfamide nitrogen were performed. Internal cyclization of the sulfamide as in **23b**,**c** provided compounds equipotent with the corresponding open derivatives **22b**,**c**. Replacement of the two methyls of **22b** with cycloalkyls as in **24b** and **25b** lowered activity in the cell based assay (50% NHS). Activity could be restored to previous levels by introduction of the more polar morpholine (**26b**), obtaining a compound that is essentially equipotent with **22b** in the cell based assays. This observation prompted us to further increase polarity by introducing a protonable nitrogen in the sulfamide moiety; the change of morpholine into *N*-methylpiperazine gave an extremely potent compound (**27b**), and interestingly, it was characterized by a higher stability toward glucuronidation with respect to **22b** (data not shown).

The two enantiomers (*S*)- and (*R*)-**27b** were profiled in vitro and in vivo showing that the (*S*)-enantiomer was 3-fold more potent than the (*R*)-enantiomer in the high serum spread assay (CIC_{95} = 0.006 μM for (*S*)-**27b** vs CIC_{95} = 0.020 μM for (*R*)-**27b**). (*S*)-**27b** thus proved to be the most active compound in the series.

Metabolic stability of (*S*)-**27b** in liver microsomes in the presence of both NADPH and UDPGA showed a moderately low turnover (turnover; rat 10, dog 8 (mL/min)/mg P),[23] but plasma protein binding was also low, consistent with a positively charged molecule[24] (PPB; rat 76, dog 82% bound), and the

Table 2. Effect of Amide Substitution[a]



Cmpd.	R^1	R^2	n	Strand transfer IC_{50} (μM)[b]	Spread CIC_{95} (μM)[c] 10% FBS	50% NHS
13a	CH_3	CH_3	0	0.009	>1	>1
13b	CH_3	CH_3	1	0.006	1	1
13c	CH_3	CH_3	2	0.008	0.250	0.500
14b		CH_3	1	0.018	0.500	1
15b		CH_3	1	0.003	>1	>1
16b		CH_3	1	0.010	0.250	0.250
17b		CH_3	1	0.009	0.016	0.047
(R)-17b		CH_3	1	0.007	0.031	0.062
(S)-17b		CH_3	1	0.007	0.016	0.031
18b		CH_3	1	0.006	0.016	0.022
18c		CH_3	2	0.017	0.063	0.125
19b		H	1	0.007	1	1

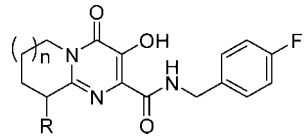
[a] Results are the mean of at least three independent experiments. [b] Assays were performed with recombinant HIV-1 integrase (0.1 μM) preassembled on immobilized oligonucleotides. Inhibitors were added after assembly and washings. For details, see ref 15 and references therein. [c] Cell culture inhibitory concentrations (CIC_{95}) are defined as those that inhibited by ≥95% the spread of HIV-1 infection in susceptible cell culture MT-4 human T-lymphoid cells. For details see ref 15 and references therein.

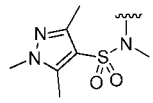
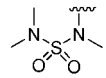
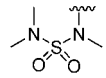
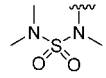
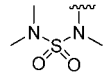
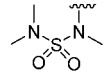
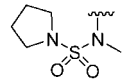
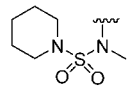
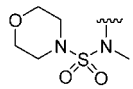
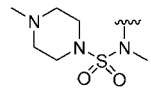
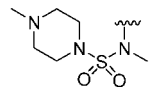
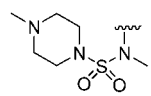
compound presented a rat and dog PK[a] profile (Table 4) characterized by high plasma clearance (98 and 23 (mL/min)/kg, respectively, in rat and dog).

22b and its two enantiomers ((*S*)-**22b** and (*R*)-**22b**) were also evaluated in vitro and in vivo in preclinical species; they displayed almost the same potency in the enzymatic and in the cell based assays and similar moderately low human PPB ((*S*)-**22b**, 87% bound, (*R*)-**22b**, 81% bound). Pharmacokinetic profiles of the two enantiomers (Table 4) showed significant differences; in rat, (*R*)-**22b** showed a 1.8 (mL/min)/kg plasma clearance value, while the corresponding value for (*S*)-**22b** was almost 8-fold higher (Clp = 14 (mL/min)/kg). Furthermore, oral bioavailability was higher for (*R*)-**22b** (*F* = 63%) than for (*S*)-**22b** (*F* = 49%). Comparison of the dog PK parameters revealed that although the plasma clearance was identical for the two enantiomers, the oral bioavailability was again higher for (*R*)-**22b** (*F* = 42%) than for (*S*)-**22b** (*F* = 16%). Rhesus monkey PK profile of (*R*)-**22b** displayed a plasma clearance of 27 (mL/min)/kg and 9% oral bioavailability.

The stability of (*R*)-**22b** was also investigated in liver microsomes in the presence of NADPH and UDPGA, revealing that the compound was not subject to significant oxidative metabolism and was metabolized mainly by glucuronidation. Despite the low (rat and dog) and moderate (rhesus) plasma clearance in vivo (Table 4), (*R*)-**22b** displayed unexpectedly high turnover in liver microsomes in the presence of UDPGA (turnover; rat 81, dog 46, rhesus 84 %/h).[25] The high plasma protein binding values observed (PPB; rat 99, dog 89, rhesus 86% bound) may partially

Table 3. SAR of Sulfonamides and Sulfamides[a]



Cmpd.	R	n	Strand transfer IC_{50} (μM)[b]	Spread CIC_{95} (μM)[c] 10% FBS	50% NHS
20b		1	0.012	0.026	0.086
20c		2	0.016	0.031	0.125
21b		1	0.007	0.021	0.063
22a		0	0.045	>1	>1
22b		1	0.005	0.016	0.021
(R)-22b		1	0.007	0.016	0.044
(S)-22b		1	0.005	0.012	0.031
22c		2	0.010	0.031	0.125
23b		1	0.014	0.016	0.047
23c		2	0.006	0.078	0.156
24b		1	0.007	0.016	0.047
25b		1	0.009	0.063	0.125
26b		1	0.011	0.015	0.031
27b		1	0.007	0.006	0.020
(R)-27b		1	0.010	0.013	0.020
(S)-27b		1	0.007	0.006	0.006

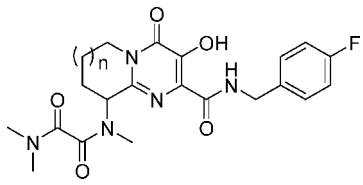
[a] Results are the mean of at least three independent experiments. [b] Assays were performed with recombinant HIV-1 integrase (0.1 μM) preassembled on immobilized oligonucleotides. Inhibitors were added after assembly and washings. For details, see ref 15 and references therein. [c] Cell culture inhibitory concentrations (CIC_{95}) are defined as those that inhibited by ≥ 95% the spread of HIV-1 infection in susceptible cell culture MT-4 human T-lymphoid cells. For details, see ref 15 and references therein.

Table 4. Pharmacokinetics

compd	species	F (%)[a]	Clp[b] ((mL/min)/kg)
(*S*)-**17b**	rat[c]	17	55
20b	rat[d]	47	48
(*S*)-**22b**	rat[d]	49	14
	dog[e]	16	3.8
(*R*)-**22b**	rat[d]	63	1.8
	dog[e]	42	3.8
	rhesus[f]	9	27
(*S*)-**27b**	rat[g]	15	98
	dog		23

[a] Oral bioavailability. [b] Plasma clearance. [c] Dose: iv = po, 3 mg/kg. Formulation: iv, DMSO/PEG400/H_2O (60%−20%−20%); po, 1% methylcellulose. [d] Dose: iv = po, 3 mg/kg. Formulation: iv, DMSO/PEG400/H_2O (20%−40%−40%); po, 1% methylcellulose. [e] Dose: iv, 1 mg/kg; po, 2 mg/kg. Formulation: iv, DMSO/PEG400/H_2O (20%−40%−40%); po, 1% methylcellulose. [f] Dose: iv, 1 mg/kg; po, 6.5 mg/kg. Formulation: iv, DMSO; po, 0.5% methylcellulose. [g] Dose: iv = po, 3 mg/kg. Formulation: iv, DMSO/PEG400/H_2O (20%−60%−20%); po, 1% methylcellulose.

Table 5. Ketoamides: Effect of Ring Size on Activity[a]



compd	n	strand transfer IC_{50} (μM)[b]	spread CIC_{95} (μM)[c] 10% FBS	spread CIC_{95} (μM)[c] 50% NHS
28a	0	0.020	>1	>1
28b	1	0.007	0.038	0.040
(*R*)-**28b**	1	0.019	0.067	0.098
(*S*)-**28b**	1	0.012	0.033	0.043
28c	2	0.013	0.094	0.063
(*R*)-**28c**	2	0.035	0.021	0.042
(*S*)-**28c**	2	0.012	0.010	0.013

[a] Results are the mean of at least three independent experiments. [b] Assays were performed with recombinant HIV-1 integrase (0.1 μM) preassembled on immobilized oligonucleotides. Inhibitors were added after assembly and washings. For details, see ref 15 and references therein. [c] Cell culture inhibitory concentrations (CIC_{95}) are defined as those that inhibited by ≥ 95% the spread of HIV-1 infection in susceptible cell culture MT-4 human T-lymphoid cells. For details, see ref 15 and references therein.

account for this apparent discrepancy by reducing the in vivo turnover, thus resulting in a low plasma clearance.

Further profiling of (*R*)-**22b** demonstrated that it does not inhibit human DNA polymerases α, β, and γ (IC_{50} > 200 μM), HIV-RNase-H (IC_{50} > 25 μM), or HCV polymerase (IC_{50} > 50 μM), and when tested for hERG-channel activity, it showed IC_{50} = 20 μM. It was not an inhibitor of CyP450 (1A2, 2C19, 2C9, 2D6, and 3A4, IC_{50} > 100 μM)).

In order to further optimize the pharmacological properties of our inhibitors, we turned our attention to other possible nitrogen functionalizations to improve metabolic stability and maintain the high potency.

In other series of HIV-1 integrase inhibitors[26,27] that had been developed in our company, it had been found that the introduction of a ketoamide functionality had positive effects on activity and pharmacological profile. We thus investigated the ketoamide derivatization in the bicyclic series (Table 5).

As already observed in the amide and sulfonamide series, when n = 0, compound **28a**, though active in the enzymatic assay (IC_{50} = 0.020 μM), displayed no significant activity up to 1 μM in the cell based assays. The enlargement of the saturated ring as in **28b** (n = 1) and **28c** (n = 2) resulted in potent inhibitors both of the strand transfer and of the viral spread in cells (spread CIC_{95}: **28b** 0.040 μM and **28c** 0.063 μM in the presence of 50% NHS). In order to further evaluate the series, the enantiomers of both compounds were tested and, in both cases, they shared the common feature of the (*S*)-enantiomers (*S*)-**28b** and (*S*)-**28c**, being about 2-fold more active than the corresponding (*R*)-enantiomers (*R*)-**28b** and (*R*)-**28c** in the enzyme assay and in the cell based assay in high serum conditions. Plasma protein binding values for (*S*)-**28c** (PPB; rat 94, dog 76, rhesus 88, human 81% bound) were higher in all species tested with respect to (*S*)-**28b** (PPB; rat 84, dog 66, human 63% bound).

Table 6. Pharmacokinetics Data for Ketoamides Derivatives

compd	species	F (%)[a]	Clp[b] ((mL/min)/kg)	$T_{1/2}$ (h)[c]	AUC[d] (μM·h)	C_{max}[e] (μM)	V_{dss}[f] (L/kg)
(*S*)-**28b**	rat[g]	47	11	14	2.5	2.0	3.0
	dog[h]	39	15	1.7	3.8	2.5	1.2
(*S*)-**28c**[i]	rat[j]	64	7	7.4	37	27	0.7
	dog[k]	61	7.5	2.1	31	25	0.9
	rhesus[l]	31	8	2.6	14	10	0.7

[a] Oral bioavailability. [b] Plasma clearance. [c] Plasma half-life after iv administration. [d] Area under the curve after po administration. [e] After PO administration. [f] Volume of distribution. [g] Dose: iv = po, 3 mg/kg. Formulation: iv, DMSO/PEG400/H_2O (20%−40%−40%); po, 1% methylcellulose suspension. [h] Dose: iv, 1 mg/kg; po, 4 mg/kg. Formulation: iv, DMSO/PEG400/H_2O (20%−40%−40%); po, 1% methylcellulose suspension. [i] Dosed as potassium salt. [j] Dose: iv, 3 mg/kg; po, 10 mg/kg. Formulation: iv, DMSO/PEG400/H_2O (20%−60%−20%); po, 1% methylcellulose suspension. [k] Dose: iv, 1 mg/kg; po, 2 mg/kg. Formulation: iv, DMSO/PEG400/H_2O (20%−60%−20%); po, 1% methylcellulose suspension. [l] Dose: iv, 2 mg/kg; po, 10 mg/kg. Formulation: iv, DMSO; po, 0.5% methylcellulose suspension.

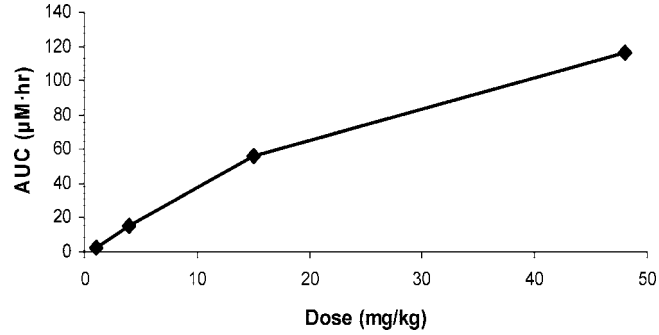


Figure 3. Rat oral administration of (*S*)-**28c**, exposure dose proportionality.

Rat PK profiles of both (*S*)-**28b** and (*S*)-**28c** were then evaluated (Table 6). Both compounds proved to be orally bioavailable, and (*S*)-**28c** showed a lower plasma clearance with respect to (*S*)-**28b** (7 vs 11 (mL/min)/kg). The C_{max} of (*S*)-**28c** was 13-fold higher than that for (*S*)-**28b** (25 vs 2 μM). The same behavior was also observed after administration to dog, when (*S*)-**28c** showed a 2-fold lower plasma clearance and 10-fold higher C_{max} than (*S*)-**28b**.

Because of the above results, (*S*)-**28c** was further profiled. A PK study in rhesus monkey showed a moderately low plasma clearance (8 (mL/min)/kg), acceptable oral bioavailability (F = 31%), and also in this species a high C_{max} (10 μM).

In rat the compound displayed dose proportionality of plasma exposure after oral administration of the compound (dose: 1, 4, 16, 48 mg/kg) as reported in Figure 3.

Plasma concentration in the three preclinical species studied (rat, dog, rhesus) was monitored after a 10 mg/kg oral administration, revealing that up to 24–30 h after administration, all species tested maintained concentration values that are higher than the CIC_{95} (Figure 4).

Metabolic stability of (*S*)-**28c** in liver microsomes in the presence of NADPH showed no appreciable turnover in dog

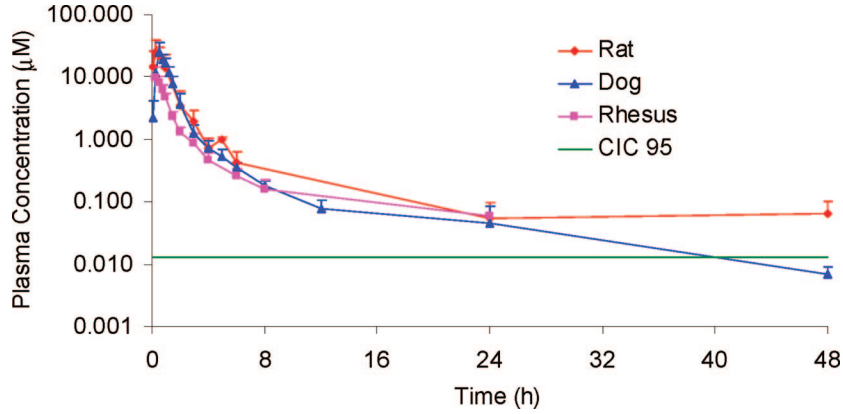
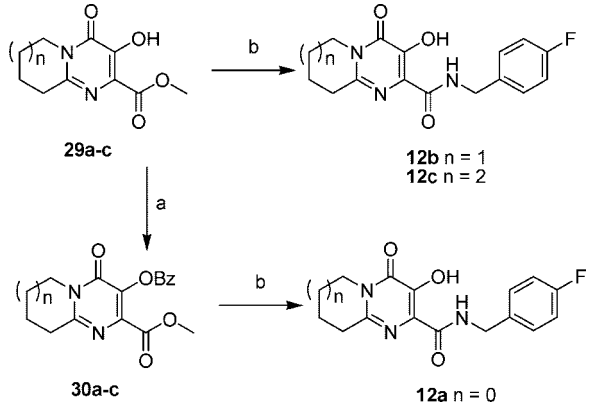


Figure 4. The po dosing of (*S*)-**28c** (potassium salt) at 10 mg/kg in three preclinical species.

Scheme 1. Synthesis of Unsubstituted Bicyclic Pyrimidinones[a]



[a] Reagents and conditions: (a) benzoic anhydride, pyridine or THF; (b) 4-fluorobenzylamine, MeOH, 60 °C.

and human and only minor oxidative metabolism in rat and rhesus. In the presence of both NADPH and UDPGA the compound exhibited low turnover (turnover; rat 11, dog 4, human 3 (mL/min)/mg P) and was cleared mainly by 5-O-glucuronidation.[28] Likewise, glucuronide formation was observed in human hepatocytes, while no significant disappearance of (*S*)-**28c** was detected from three different donors during a 4 h incubation with 10^6 cells/mL.

Selectivity of (*S*)-**28c** was demonstrated by testing it for inhibition of human DNA polymerase α, *β*, and *γ* (IC_{50} > 10 *μ*M), HIV RNaseH (IC_{50} > 50 *μ*M), and it was also counter-screened on a panel of 170 enzymes (MDS Pharma Services-Panlabs) to evaluate potential off-target activities, resulting in no significant notable responses. It did not significantly inhibit the main isoforms of CyP450 (3A4, 2D6, 2C19; IC_{50} > 100 *μ*M; 2C9, IC_{50} = 75 *μ*M), and when tested for hERG-channel activity, it showed IC_{50} > 10 *μ*M.

Chemistry

The synthesis of the bicyclic scaffolds was performed as shown in Scheme 1. Treatment of pyrimidinones **29a**−**c**[29,30] or the corresponding *O*-benzoyl protected **30a**−**c** (more easily isolated and purified) with 4-fluorobenzylamine provided benzylamides **12a**−**c**.

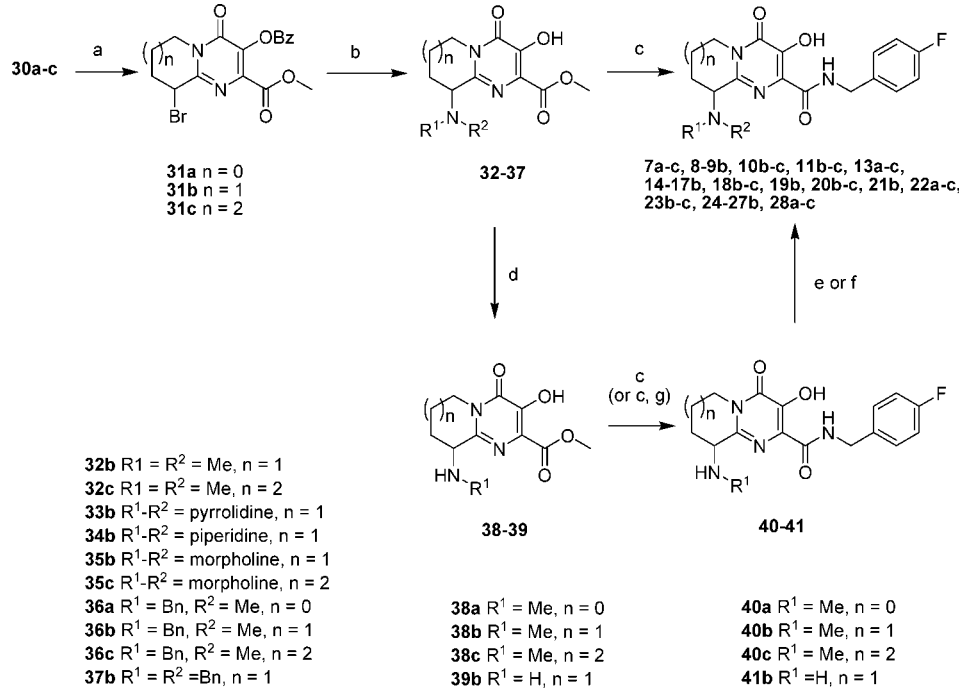
To obtain the functionalized derivatives shown in Tables 1−3 and 5, the synthetic pathway shown in Scheme 2 was followed. Most of the steps could also be performed in one pot or sequentially without isolating the intermediate steps (as described in Experimental Section). The *O*-benzoyl protected pyrimidinones **30a**−**c** were brominated with NBS,[29] and the resulting bromides (**31a**−**c**) were displaced with the appropriate secondary amine, which provided also O-deprotection (**32**–**37**). Treatment with 4-fluorobenzylamine, upon heating in MeOH, afforded amino substituted compounds **7b**,**c, 8**–**9b, 10b**,**c, 11b**,**c**. Compounds **36a**−**c** and **37b**, where R^1 (or both R^1 and R^2 as in **37b**) was a benzyl, gave access to further functionalized compounds by removal of the benzyl group(s) by Pd-catalyzed hydrogenation to **38a**−**c** and **39b** that, when treated with 4-fluorobenzylamine as before, furnished the versatile advanced intermediates **40a**−**c** and **41b**, which allowed the synthesis of the racemic compounds by reductive amination (**7a**) or functionalization with acyl chlorides or carboxylic acids to give the amide derivatives shown in Table 2, or with sulfonyl and sulfamoyl chlorides to give the sulfonamide and sulfamide derivatives shown in Table 3 and with methyl chlorooxoacetate, followed by in situ amidation with dimethylamine, to provide ketoamides shown in Table 5. To facilitate the purification of the advanced intermediates **40**, *N*-Boc derivatization can be performed, followed by flash chromatography and Boc deprotection in acidic conditions, as described in the Experimental Section for **40b**.

The syntheses of the chiral derivatives present in Tables 2, 3, and 5 were performed, as described for the corresponding racemic compounds in Scheme 2 (step e), by functionalizing the chiral benzylamide intermediates (R)- or (S)-**43b**,**c** (Scheme 3). Chiral advanced intermediates **43b** (*n* = 1) were obtained by treatment with 4-fluorobenzylamine of the corresponding chiral esters **42b**,[29] while **43c** (*n* = 2) had been prepared as recently described.[30] The amide derivatives (*R*)/(*S*)-**17b** were obtained by conventional amide coupling with [(dimethylamino)sulfonyl]acetic acid,[31] and the sulfamides (*R*)/(*S*)-**22b** and (*R*)/(*S*)-**27b** were obtained by reaction with the appropriate sulfamoyl chlorides in the presence of TEA. (*R*)/(*S*)-**28b**,**c** could be obtained by a two-step one-pot procedure by acylating the pyrimidone *N*-methylamine derivatives (*R*)/(*S*)-**43b**,**c** with methyl chlorooxoacetate, followed by addition of a THF solution of dimethylamine ((*R*)/(*S*)-**28b**) or alternatively by conventional amide coupling with potassium (dimethylamino)(oxo)acetate ((*R*)/(*S*)-**28c**).

Conclusions

Design and synthesis of bicyclic analogues of *N*-methylpyrimidinones led to a new class of HIV-1 integrase inhibitors. Prompted by the results of the unsubstituted bicyclic compounds **12a**−**c**, which were nanomolar inhibitors of the strand transfer of the integration process, we undertook a wide study on the substitutions on the saturated ring of the bicyclic structures. Amine, amide, sulfonamide, sulfamide, and ketoamide deriva-

Scheme 2. Synthesis of Substituted Bicyclic Pyrimidinones[a]



[a] Reagents and conditions: (a) NBS, benzoyl peroxide, CCl_4, reflux; (b) R^1R^2NH, DMF; (c) 4-fluorobenzylamine, MeOH, 60 °C; (d) H_2 atmospheric pressure, 10% Pd/C, MeOH; (e) appropriate acyl chloride (or sulfonyl chloride or sulfamoyl chloride), base or carboxylic acid, coupling reagent; (f) for **40a**, to obtain **7a**, 37% HCHO, $NaCNBH_3$, AcONa, MeOH; (g) (1) Boc_2O, dioxane; (2) 4 M HCl/dioxane.

tives were evaluated as well as the size of the saturated ring (Tables 1−3 and 5). A number of low nanomolar inhibitors of the HIV-1 infection spread in cell culture could be identified and further profiled in vitro and in vivo, leading to the ketoamide (*S*)-**28c**, which proved to be a very potent and selective HIV integrase inhibitor and orally biovailable and with good pharmacological profile in preclinical species (rat, dog, rhesus monkey) and was taken into further development.

Experimental Section

Reagents and solvents were obtained from commercial suppliers and were used as obtained without further purification. Flash chromatography purifications were performed on a Biotage system using prepacked silica gel (200–400 mesh) cartridges. Analytical HPLC-MS (X-Terra C_{18} column, 4.6 mm × 50 mm, 5 μm) and UPLC−MS (Acquity UPLC BEH C_{18} column, 2.1 mm × 50 mm, 1.7 μm) were performed on a Waters Alliance 2795 apparatus equipped with a diode array and a ZQ mass spectrometer (solvent system acetonitrile/water + 0.1% HCOOH). Purity of final compounds was more than 98% by area. Preparative RP-HPLC was performed on a Waters Delta Prep 4000 connected to a dual-wavelength absorbance detector 2487 (gradient of CH_3CN/H_2O + 0.1% TFA[a], flow rate of 20 mL/min), typically using a Waters Symmetry preparative column C_{18}, 5 μm, 19 mm × 300 mm. After RP-HPLC, the final compounds were isolated by lyophilization. Enantiomeric excesses were determined by chiral HPLC using a Chiralpak AD column, mobile phase *n*-hexane/ethanol + 0.2% TFA + 3% methanol, or or *n*-hexane + isopropanol + 0.2% TFA. Nuclear magnetic resonance spectra (^{1}H NMR recorded at 300, 400, or 500 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 otherwise indicated, spectra were acquired at 300 K. Microwave irradiation was performed in a Personal Chemistry (now Biotage) Optimizer.

General Procedure for the Synthesis of Amines 7b−11c. To a solution of bromide **31**[29,30] (0.245 mmol) in DMF (0.5 mL) (or MeOH), the appropriate amine (0.75 mmol) was added. The mixture was stirred at room temperature for 1 h. The solvent was removed under reduced pressure, and the crude material was dissolved in methanol (0.5 mL). 4-Fluorobenzylamine (0.084 mL, 0.75 mmol) was added, and the mixture was stirred for 1.5 h at 65 °C. The solvent was removed under reduced pressure, and the product was purified by preparative RP-HPLC and isolated as the trifluoroacetate salt unless otherwise indicated.

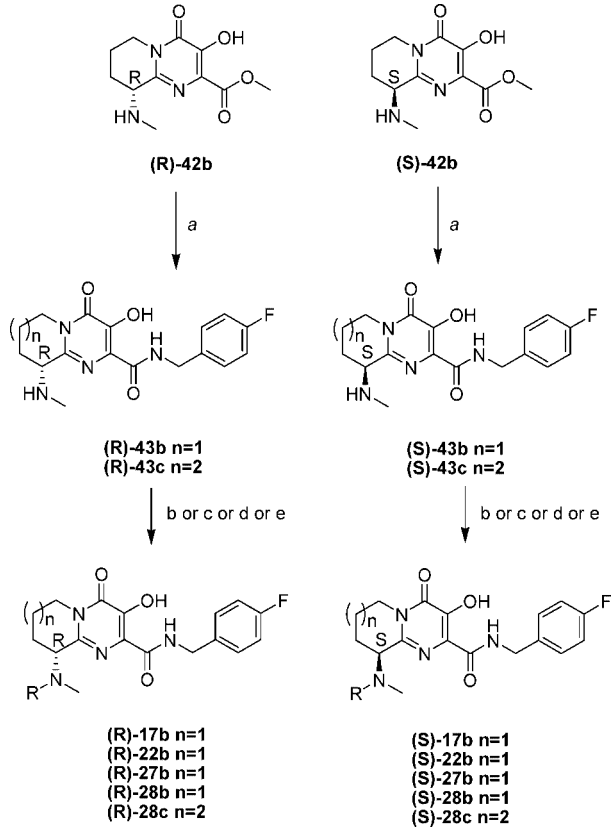
(±)-9-(Dimethylamino)-*N*-(4-fluorobenzyl)-3-hydroxy-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide (7b). A solution 2 N in MeOH of dimethylamine was used as amine. Yield: 26%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.51 (s, 1H), 10.69 (s, 1H), 10.59 (t, J = 3.6 Hz, 1H), 7.50–7.40 (m, 2H), 7.22–7.10 (m, 2H), 4.79–4.68 (m, 1H), 4.60–4.38 (m, 2H), 4.13–4.03 (m, 1H), 3.61–3.48 (m, 1H), 2.97 (d, J = 5.1 Hz, 3H), 2.66 (d, J = 5.1 Hz, 3H), 2.34–2.17 (m, 2H), 2.00–1.79 (m, 2H). MS *m*/*z*: 361 (M + H)$^+$.

(±)-10-(Dimethylamino)-*N*-(4-fluorobenzyl)-3-hydroxy-4-oxo-4,6,7,8,9,10-hexahydropyrimido[1,2-*a*]azepine-2-carboxamide (7c). A solution 2 N in MeOH of dimethylamine was used as amine. Yield: 18%. ^{1}H NMR (300 MHz, pyridine-d_5) δ 10.01 (bs, 1H), 7.52 (dd, J = 8.6, 5.7 Hz, 2H), 7.13 (t, J = 8.6 Hz, 2H), 5.12–4.91 (m, 1H), 4.79 (dd, J = 14.4, 6.2 Hz, 1H), 4.68 (dd, J = 14.7, 5.9 Hz, 1H), 4.42 (t, J = 11.8 Hz, 1H), 2.95 (bs, 1H), 2.00 (m, 6H), 1.92–1.61 (m, 3H), 1.60–1.10 (m, 3H). MS *m*/*z*: 375 (M + H)$^+$.

(±)-*N*-(4-Fluorobenzyl)-3-hydroxy-4-oxo-9-pyrrolidin-1-yl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide (8b). Yield: 22%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.44 (s, 1H), 11.14 (s, 1H), 10.66 (t, J = 6.4 Hz, 1H), 7.47–7.40 (m, 2H), 7.18–7.10 (m, 2H), 4.85–4.74 (m, 1H), 4.55–4.37 (m, 2H), 4.07–4.00 (m, 1H), 3.76–3.65 (m, 1H), 3.65–3.52 (m, 1H), 3.42–3.25 (m, 2H), 3.22–3.15 (m, 1H), 2.37–2.29 (m, 1H), 2.25–2.15 (m, 1H), 2.10–1.76 (m, 6H). MS *m*/*z*: 387 (M + H)$^+$.

(±)-*N*-(4-Fluorobenzyl)-3-hydroxy-4-oxo-9-piperidin-1-yl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide (9b). Yield: 17%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.32 (s, 1H), 10.45 (t, J = 5.4 Hz, 1H), 10.07 (s, br, 1H), 7.52–7.40 (m, 2H), 7.22–7.11 (m, 2H), 4.83–4.70 (m, 1H), 4.62–4.40 (m, 2H), 4.13–4.02 (m, 1H), 3.60–3.15 (m, 4H), 2.98–2.82 (m, 1H), 2.65–2.50 (m, 1H), 2.44–2.22 (m, 2H), 2.00–1.67 (m, 6H), 1.56–1.37 (m, 1H). MS *m*/*z*: 401 (M + H)$^+$.

Scheme 3. Synthesis of Chiral Pyrimidinone Benzylamides[a]



[a] Reagents and conditions: (a) 4-fluorobenzylamine, MeOH, 65 °C, 3 h. (b) For (*R*)/(*S*)-**17b**: HOBt, EDC, DIPEA, [(dimethylamino)sulfonyl]acetic acid, DMF, room temperature, 24 h. (c) For (*R*)/(*S*)-**22b** and (*R*)/(*S*)-**27b**: appropriate sulfamoyl chloride, DCM, TEA, room temperature, 90 min. (d) For (*R*)/(*S*)-**28b**: (1) methyl chlorooxoacetate, TEA, DCM, room temperature, 50 min; (2) 2 M *N*,*N*-dimethylamine/THF, 55 °C, 16 h. (e) For (*R*)/(*S*)-**28c**: potassium (dimethylamino)(oxo)acetate, HOBt, EDC, TEA, DCM, room temperature overnight.

(±)-*N*-(4-Fluorobenzyl)-3-hydroxy-9-morpholin-4-yl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-a]pyrimidine-2-carboxamide (10b). Yield: 9%. The trifluoroacetate salt obtained after RP-HPLC was dissolved in 1 N HCl, the solvent was removed under reduced pressure, and the residue was lyophilized from water/acetonitrile to afford *N*-(4-fluorobenzyl)-3-hydroxy-9-morpholin-4-yl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide **10b** as the hydrochloride salt. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.34 (s, 1H), 10.99 (s, 1H), 10.47 (s, 1H), 7.44 (m, 2H), 7.16 (m, 2H), 4.85 (m, 1H), 4.60–4.40 (m, 3H), 4.10–3.85 (m, 4H), 3.60–3.05 (m, 5H), 2.35–2.15 (m, 2H), 2.03–1.80 (m, 2H). MS *m*/*z*: 403 (M + H)$^+$.

(±)-*N*-(4-Fluorobenzyl)-3-hydroxy-10-morpholin-4-yl-4-oxo-4,6,7,8,9,10-hexahydropyrimido[1,2-*a*]azepine-2-carboxamide (10c). Yield: 31%.^{1}H NMR (300 MHz, pyridine-d_5) δ 10.04 (bs, 1H), 7.52 (dd, J = 8.3, 5.6 Hz, 2H), 7.14 (t, J = 8.7 Hz, 2H), 5.13 (dd, J = 13.2, 6.1 Hz, 1H), 4.81 (dd, J = 14.4, 6.6 Hz, 1H), 4.69 (dd, J = 14.5, 6.5 Hz, 1H), 4.41 (t, J = 12.1 Hz, 1H), 3.77–3.57 (m, 4H), 2.96 (d, J = 5.3 Hz, 1H), 2.42–2.19 (m, 2H), 2.00–1.64 (m, 5H), 1.56–1.23 (m, 3H). MS *m*/*z*: 417 (M + H)$^+$.

(±)-9-[Benzyl(methyl)amino]-*N*-(4-fluorobenzyl)-3-hydroxy-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide (11b). Yield: 26%.^{1}H NMR (300 MHz, DMSO-d_6) δ 12.64 (s, 0.4H), 12.34 (s, 0.6H), 10.92 (t, J = 6.2 Hz, 0.4H), 10.77 (s, 0.4H), 10.32 (s, 0.6H), 10.17 (t, J = 6.3 Hz, 0.6H), 8.00 (m, 1H), 7.53–7.11 (m, 9H), 4.93–4.82 (m, 0.6H), 4.82–4.73 (m, 0.4H), 4.65–4.49 (m, 2H), 4.45–4.32 (m, 1H), 4.23–4.05 (m, 1.6H), 3.98–3.85 (m, 0.4H), 3.52–3.45 (m, 1H), 2.96 (d, J = 4.8 Hz, 1.8H), 2.89 (d, J = 4.8 Hz, 1.2H), 2.49–1.60 (m, 4H). MS *m*/*z*: 438 (M + H)$^+$.

(±)-10-[Benzyl(methyl)amino]-*N*-(4-fluorobenzyl)-3-hydroxy-4-oxo-4,6,7,8,9,10-hexahydropyrimido[1,2-*a*]azepine-2-carboxamide (11c). Yield: 36%. ^{1}H NMR (400 MHz, pyridine-d_5) δ 9.85 (bs, 1H), 7.51 (dd, J = 8.3, 5.6 Hz, 2H), 7.43–7.25 (m, 5H), 7.10 (t, J = 8.7 Hz, 2H), 5.13 (dd, J = 13.2, 6.1 Hz, 1H), 4.61–4.82 (m, 3H), 3.42–3.11 (m, 3H), 1.98–1.75 (m, 6H), 1.62–1.22 (m, 3H). MS *m*/*z*: 451 (M + H)$^+$.

(±)-8-(Dimethylamino)-*N*-(4-fluorobenzyl)-3-hydroxy-4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]pyrimidine-2-carboxamide (7a). The title compound was prepared using the following five-step procedure. Step a: A mixture of methyl 3-(benzoyloxy)-4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]pyrimidine-2-carboxylate **30a**[31] (0.095 g, 88% w/w, 0.26 mmol), NBS (0.061 g, 0.35 mmol), and catalytic dibenzoylperoxide in CCl_4 (1.6 mL) was stirred under reflux for 6 h. The mixture was cooled to room temperature and filtered over silica gel (petroleum ether/ethyl acetate, 65:35) to afford methyl 3-(benzoyloxy)-8-bromo-4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]pyrimidine-2-carboxylate **31a** (MS *m*/*z*, 393/395 (M + H)$^+$), which was used as such in the next step. Step b: To a stirred solution of the above bromide in anhydrous DMF (0.3 mL) was added *N*-benzyl-*N*-methylamine (0.064 mL, 0.5 mmol). The mixture was stirred for 2 h at room temperature and then concentrated to dryness under reduced pressure to provide crude methyl 8-[benzyl(methyl)amino]-3-hydroxy-4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]pyrimidine-2-carboxylate **36a** (MS *m*/*z*, 330 (M + H)$^+$), which was used as such in the next step. Step c: A solution of crude as above in methanol (0.9 mL), containing palladium on charcoal (6 mg, 10% w/w), was stirred under hydrogen at atmospheric pressure for 1.5 h. The catalyst was filtered off, and the solution was concentrated to dryness under reduced pressure. Crude methyl 3-hydroxy-8-(methylamino)-4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]pyrimidine-2-carboxylate **38a** (MS *m*/*z*, 240 (M + H)$^+$) was used as such in the next step. Step d: To a solution of the above crude and TEA (0.023 mL, 0.16 mmol) in dry methanol (0.7 mL) was added 4-fluorobenzylamine (0.028 mL, 0.25 mmol). The mixture was stirred and heated to 65 °C overnight. After the mixture was cooled to room temperature, the solvent was removed under reduced pressure and the crude *N*-(4-fluorobenzyl)-3-hydroxy-8-(methylamino)-4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]pyrimidine-2-carboxamide **40a** (MS *m*/*z*, 333 (M + H)$^+$) obtained was used as such. Step e: To a solution of the above crude in methanol (2 mL) were added formaldehyde (37% aqueous solution, 0.03 mL, 0.33 mmol), sodium acetate (0.022 g, 0.27 mmol), and sodium cyanoborohydride (0.014 g, 0.23 mmol). The mixture was stirred for 16 h at room temperature. The mixture was concentrated under reduced pressure, and 8-(dimethylamino)-*N*-(4-fluorobenzyl)-3-hydroxy-4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]pyrimidine-2-carboxamide **7a** was isolated by preparative RP-HPLC as the trifluoroacetate salt. Yield: 5%. ^{1}H NMR (400 MHz, DMSO-d_6 + 2% TFA, 340 K) δ 9.03 (bs, 1H), 7.42–7.33 (m, 2H), 7.20–7.09 (m, 2H), 4.96 (t, J = 8.3 Hz, 1H), 4.55 (ddd, J = 6.3, 15.0, 35.9 Hz, 2H), 4.21–4.09 (m, 1H), 4.00–3.89 (m, 1H), 2.96 (s, 6H), 2.72–2.59 (m, 1H), 2.47–2.39 (m, 1H, partially under solvent signal). MS *m*/*z*: 347 (M + H)$^+$.

***N*-(4-Fluorobenzyl)-3-hydroxy-4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]pyrimidine-2-carboxamide (12a).** To a solution of **30a** (0.100 g, 88% w/w, 0.28 mmol) in dry methanol (1.0 mL) was added 4-fluorobenzylamine (0.11 g, 0.84 mmol). The mixture was irradiated in a sealed vessel in a microwave apparatus (140 °C, 500 s). After the mixture was cooled, the solvent was removed under reduced pressure and the product was isolated by preparative RP-HPLC (0.070 g, 82%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.74 (bs, 1H), 8.96 (bs, 1H), 7.94–7.89 (m, 2H), 7.66–7.60 (m, 2H), 5.06 (d, J = 6.6 Hz, 2H), 4.56 (t, J = 7.2 Hz, 2H), 3.47 (t, J = 7.9 Hz, 2H), 2.77 (m, partially overlapped by H_2O). ^{1}H NMR (300 MHz, DMSO-d_6 + 2% TFA) δ 9.41 (t, J = 6.2 Hz, 1H), 7.38–7.31 (m, 2H), 7.19–7.07 (m, 2H), 4.42 (d, J = 6.2 Hz, 2H), 3.98 (t, J = 7.4 Hz, 2H), 2.97 (t, J = 7.8 Hz, 2H), 2.14 (q, J = 7.4 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.6, 161.2 (d, J = 242 Hz),

156.3, 153.7, 147.0, 134.8 (d, $J = 3$ Hz), 129.5 (d, $J = 8$ Hz), 126.6, 115.0 (d, $J = 21$ Hz), 46.8, 41.4, 31.1, 19.3. MS *m/z*: 304 $(M + H)^+$.

***N*-(4-Fluorobenzyl)-3-hydroxy-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide (12b).** A solution of **29b** (0.050 g, 0.22 mmol) and 4-fluorobenzylamine (0.055 g, 0.44 mmol) in methanol (0.5 mL) was stirred and heated to 65 °C for 22 h. After the mixture was cooled, the solvent was removed under reduced pressure and the product was isolated by preparative RP-HPLC. Yield: 45%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.12 (s, 1H), 9.35 (m, 1H), 7.36 (m, 2H), 7.15 (m, 2H), 4.44 (m, 2H), 3.84 (t, $J = 6.4$ Hz, 2H), 2.80 (t, $J = 6.8$ Hz, 2H), 1.90–1.73 (m, 4H). MS *m/z*: 318 $(M + H)^+$.

***N*-(4-Fluorobenzyl)-3-hydroxy-4-oxo-4,6,7,8,9,10-hexahydropyrimido[1,2-*a*]azepine-2-carboxamide (12c). 12c** was prepared as described for **12b**. The product was isolated by preparative RP-HPLC (yield 54%). ^{1}H NMR (500 MHz, DMSO-d_6 + 2% TFA) δ 9.35 (bs, 1H), 7.36 (dd, $J = 8.4$, 5.6 Hz, 2H), 7.14 (t, $J = 8.9$ Hz, 2H), 4.44 (d, $J = 6.4$ Hz, 2H), 4.33–4.20 (m, 2H), 3.05–2.90 (m, 2H), 1.78–1.62 (m, 6H). MS: *m/z*: 332 $(M + H)^+$.

(±)-2-{[(4-Fluorobenzyl)amino]carbonyl}-3-hydroxy-*N*-methyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-9-aminium chloride (40b). The title compound was prepared using the following five-step procedure. Step a: To a stirred solution of methyl 3-(benzoyloxy)-9-bromo-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxylate **31b** (5.9 g, 14.43 mmol) in DMF (25 mL) at room temperature were added *N*-methyl-*N*-benzylamine (4.03 mL, 30.3 mmol) and TEA (2.21 mL, 16.0 mmol). The mixture was stirred for 75 min, and Et_2O (900 mL) and 2 M HCl/Et_2O (23.6 mL) were added. The formed yellow precipitate was filtered off, washed with Et_2O, and dried under high vacuum to afford a yellow solid (9.3 g). Crude methyl 9-[benzyl(methyl)amino]-3-hydroxy-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxylate hydrochloride **36b** was used as such without further purification (MS *m/z*: 344 $(M + H)^+$). Step b: The above crude material was dissolved in MeOH (160 mL), palladium on charcoal (0.980 g, 10% w/w) was added, and the mixture was stirred under a hydrogen atmosphere for 3.5 h. The catalyst was filtered off, and the solution was concentrated to dryness under reduced pressure to afford crude methyl 3-hydroxy-9-(methylamino)-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxylate **38b** as the hydrochloride salt (6.9 g), which was used as such in the next step without further purification (MS *m/z*: 254 $(M + H)^+$). Step c: An amount of 4.72 g of this crude material was dissolved in MeOH (60 mL). 4-Fluorobenzylamine (1.15 mL, 10 mmol) and TEA (2.78 mL, 20 mmol) were added, and the mixture was stirred at 60 °C for 14 h. The mixture was concentrated to dryness. The residue was triturated with Et_2O, and the residue was dried under vacuum to afford 6.4 g of crude *N*-(4-fluorobenzyl)-3-hydroxy-9-(methylamino)-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide **40b** as a yellow solid, which was used without further purification (MS *m/z*: 347 $(M + H)^+$). Step d: An amount of 5.9 g of this crude material was suspended in a mixture of water (52 mL) and 1,4-dioxane (67 mL). Di-*tert*-butyl dicarbonate (11.0 g, 50.7 mmol), and $NaHCO_3$ (4 g, 22.5 mmol) were added, and the mixture was stirred at 62 °C for 3.5 h. After cooling to room temperature, the mixture was partitioned between EtOAc (400 mL) and 0.3 M aqueous HCl (200 mL). The organic phase was separated, and the aqueous phase was extracted with EtOAc (200 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The crude product was purified by flash chromatography on silica gel, using EtOAc/petroleum ether (4:1) as eluent. The pooled product fractions were concentrated to dryness under reduced pressure to afford *tert*-butyl (2-{[(4-fluorobenzyl)amino]carbonyl}-3-hydroxy-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-9-yl)methylcarbamate as a light-pink oil (1.67 g, 3.74 mmol, 41% from **31b**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.02 (s, 0.6H), 11.86 (s, 0.4H), 8.60 (s, 0.6H), 8.24 (s, 0.4H), 7.40–7.08 (m, 4H), 4.80–4.75 (m, 0.4H), 4.60–4.40 (m, 2.6H), 4.16–7.00 (m, 1H), 3.67–3.45 (m, 1H), 2.93 (s, 1.8H), 2.78 (s, 1.2H), 2.18–1.75 (m, 4H), 1.25 (s, 3.6H), 1.14 (s, 5.4H). MS *m/z*: 447 $(M + H)^+$. Step e: A solution of the above *tert*-butyl (2-{[(4-fluorobenzyl)amino]carbonyl}-3-hydroxy-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-9-yl)methylcarbamate (1.62 g, 3.62 mmol) in 4 M HCl/1,4-dioxane (31 mL) was stirred at room temperature for 3 h. The solvents were removed under reduced pressure and the residue was triturated with Et_2O, filtered, and left under high vacuum to afford **40b** as the hydrochloride salt as a yellow solid (1.37 g, 99%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.38 (s, 1H), 10.39 (t, $J = 6.2$ Hz, 1H), 9.90 (s, br, 1H), 9.43 (s, br, 1H), 7.49–7.05 (m, 4H), 4.60–4.43 (m, 3H), 4.06–3.99 (m, 1H), 3.70–3.60 (m, 1H), 2.64 (s, 3H), 2.35–2.25 (m, 1H), 2.20–2.11 (m, 1H), 1.96–1.73 (m, 2H). MS *m/z*: 347 $(M + H)^+$.

(±)-*N*-(4-Fluorobenzyl)-3-hydroxy-10-(methylamino)-4-oxo-4,6,7,8,9,10-hexahydropyrimido[1,2-*a*]azepine-2-carboxamide (40c). 40c was prepared as described for **40b**, steps a−c, starting from methyl 10-bromo-3-hydroxy-4-oxo-4,6,7,8,9,10-hexahydropyrimido[1,2-*a*]azepine-2-carboxylate **31c**. The compound was used as a crude for the next reactions or isolated by RP-HPLC as the trifluoroacetate salt (yield 49%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.33 (s, 1H), 9.52 (s, 1H), 9.22 (bs, 1H), 8.82 (bs, 1H), 7.39 (t, $J = 7.12$ Hz, 2H), 7.21 (t, $J = 8.6$ Hz, 2H), 4.94 (dd, $J = 14.3$, 4.6 Hz, 1H), 4.71 (m, 1H), 4.63–4.51 (m, 2H), 3.50 (dd, partially overlapped by water, 1H), 2.69 (s, 3H), 2.23 (d, $J = 12.7$ Hz, 1H), 1.97 (d, $J = 11.5$ Hz, 1H), 1.91–1.80 (m, 2H), 1.70–1.60 (m, 1H), 1.45–1.30 (m, 1H). MS *m/z*: 361 $(M + 1)^+$.

General Procedure for the Synthesis of Amides 13c, 14–17b, 18b,c. Compound **40b** or crude **40c** (0.052 mmol) was dissolved in DMF (0.5 mL) or DCM (1 mL). DIPEA (0.030 mL, 0.15 mmol), HOBt (0.009 g, 0.068 mmol), EDC (0.038 g, 0.16 mmol), and the appropriate carboxylic acid (0.16 mmol) were added, and the mixture was stirred for 24 h at room temperature. The mixture was diluted with 0.5 mL of DMF and filtered, and the product was isolated by preparative RP-HPLC.

(±)-10-[Acetyl(methyl)amino]-*N*-(4-fluorobenzyl)-3-hydroxy-4-oxo-4,6,7,8,9,10-hexahydropyrimido[1,2-*a*]azepine-2-carboxamide (13c). Yield: 17% from **31c**. ^{1}H NMR (400 MHz, DMSO-d_6 + 2% TFA) δ 8.13 (bs, 1H), 7.41–7.36 (m, 2H), 7.16 (t, $J = 8.8$ Hz, 2H), 5.61 (bs, 1H), 5.00 (dd, $J = 14.4$, 5.4 Hz, 1H), 4.61–4.49 (m, 2H), 3.63–3.50 (m, 1H), 3.05 (bs, 2H), 2.09–1.77 (m, 9H), 1.44–1.29 (m, 1H). MS *m/z*: 403 $(M + H)^+$.

(±)-9-[(Difluoroacetyl)(methyl)amino]-*N*-(4-fluorobenzyl)-3-hydroxy-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide (14b). Yield: 19%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.20–11.90 (m, 1H), 8.67 (t, $J = 6.0$ Hz, 0.7H), 8.57 (t, $J = 6.0$ Hz, 0.3H), 7.39–7.25 (m, 2H), 7.21–7.10 (m, 2H), 6.92–6.55 (m, 1H), 5.26–5.02 (m, 1H), 4.50 (d, $J = 6.0$ Hz, 2H), 4.18–4.02 (m, 1H), 3.80–3.59 (m, 1H), 3.02 (s, 2.1H), 2.73 (s, 0.9H), 2.18–1.82 (m, 4H). MS *m/z*: 425 $(M + H)^+$.

(±)-*N*-(4-Fluorobenzyl)-3-hydroxy-9-[methyl(1*H*-pyrazol-1-ylacetyl)amino]-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide (15b). Yield: 44%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.16 (bs, 1H), 9.07 (t, $J = 6.7$ Hz, 0.44H), 8.81 (t, $J = 6.7$ Hz, 0.56H), 7.68 (d, $J = 2.0$ Hz, 0.44H), 7.56 (d, $J = 2.2$ Hz, 0.56H), 7.41–7.28 (m, 3H), 7.22–7.06 (m, 2H), 6.28–6.21 (m, 1H), 5.48 (d, $J = 16.1$ Hz, 0.44H), 5.29–5.10 (m, 2.56H), 4.65–4.51 (m, 2H), 4.20–4.05 (m, 1H), 3.72–3.55 (m, 1H), 3.00 (s, 1.68H), 2.66 (s, 1.32 H), 2.18–1.85 (m, 4H). MS *m/z*: 455 $(M + H)^+$.

(±)-*N*-(4-Fluorobenzyl)-3-hydroxy-9-[methyl([1,2,4]triazolo[1,5-*a*]pyrimidin-2-ylcarbonyl)amino]-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide (16b). Yield: 68%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.20–11.5 (m, 1H), 9.60–9.50 (m, 0.6H), 9.45–9.40 (m, 0.6H), 9.35–9.23 (m, 0.8H), 7.56–7.38 (m, 3H), 7.21–7.03 (m, 2H), 5.57–5.50 (m, 0.6H), 5.25–5.15 (m, 0.4H), 4.80–4.60 (m, 2H), 4.15–3.90 (m, 1H), 3.76–3.55 (m, 1H), 2.89 (s, 1.2H), 2.85 (s, 1.8H), 2.23–1.71 (m, 4H). MS *m/z*: 493 $(M + H)^+$.

(±)9-[{[(Dimethylamino)sulfonyl]acetyl}(methyl)amino]-*N*-(4-fluorobenzyl)-3-hydroxy-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide (17b). Yield: 53%. ^{1}H NMR (300 MHz, DMSO-d_6) mixture of two rotamers in ratio 4/1: δ 12.07 (s, 1H), 8.90 (t, $J = 6.3$ Hz, 0.8H), 8.53 (t, $J = 6.3$ Hz, 0.2H),

7.46–7.32 (m, 2H), 7.26–7.13 (m, 2H), 5.56–5.44 (m, 0.8H), 5.24–5.12 (m, 0.2H), 4.64–4.04 (m, 5H), 3.80–3.64 (m, 1H), 3.10 (s, 2.4H), 2.83 (s, 6H), 2.74 (s, 0.6H), 2.19–1.90 (m, 4H). MS *m*/*z*: 496 $(M + H)^+$.

(*R*)-9-[{[(Dimethylamino)sulfonyl]acetyl}(methyl)amino]-*N*-(4-fluorobenzyl)-3-hydroxy-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide ((*R*)-17b). (*R*)-**17b** was prepared from (*R*)-**43b**. $[\alpha]^{20}_D$ +89 ± 3 (EtOH), 99.5% ee. Spectral properties are identical to those of the corresponding racemic compound **17b**.

(*S*)-9-[{[(Dimethylamino)sulfonyl]acetyl}(methyl)amino]-*N*-(4-fluorobenzyl)-3-hydroxy-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide ((*S*)-17b). (*S*)-**17b** was prepared from (*S*)-**43b**. $[\alpha]^{20}_D$ −93 ± 3 (EtOH), 96.7% ee. Spectral properties are identical to those of the corresponding racemic compound **17b**.

(±)-*N*-(4-Fluorobenzyl)-3-hydroxy-9-{methyl[(methylsulfonyl)acetyl]amino}-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide (18b). Yield: 70%. ^{1}H NMR (300 MHz, DMSO-d_6) mixture of two rotamers in ratio 4/1: δ 12.11 (s, 1H), 8.89 (t, J = 6.3 Hz, 0.8H), 8.68 (t, J = 6.3 Hz, 0.2H), 7.45–7.33 (m, 2H), 7.25–7.13 (m, 2H), 5.55–5.43 (m, 0.8H), 5.25–5.15 (m, 0.2H), 4.74–4.44 (m, 4H), 4.22–4.10 (m, 1H), 3.80–3.60 (m, 1H), 3.25 (s, 0.6H), 3.10 (s, 2.4H), 3.08 (s, 2.4H), 2.73 (s, 0.6H), 2.20–1.92 (m, 4H). MS *m*/*z*: 467 $(M + H)^+$.

(±)-*N*-(4-Fluorobenzyl)-3-hydroxy-10-{methyl[(methylsulfonyl)acetyl]amino}-4-oxo-4,6,7,8,9,10-hexahydropyrimido[1,2-*a*]azepine-2-carboxamide (18c). To crude *N*-(4-fluorobenzyl)-3-hydroxy-10-(methylamino)-4-oxo-4,6,7,8,9,10-hexahydropyrimido[1,2-*a*]azepine-2-carboxamide **40c** (0.33 mmol) in DCM (6 mL) were added DIPEA (0.043 g, 0.33 mmol), HATU (0.152 g, 0.4 mmol), methylsulfonylacetic acid (0.055 g, 0.4 mmol), and TEA (0.121 g, 1.2 mmol), and the mixture was stirred at room temperature. The reaction mixture, diluted with DCM, was washed with 1 M HCl in water, dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by RP-HPLC. Yield: 14% from **31c**. ^{1}H NMR (400 MHz, DMSO-d_6) mixture of two rotamers in ratio 4/1: δ 12.78 (bs, 1H), 8.60 (bs, 0.8H), 8.09 (bs, 0.2H), 7.34–7.30 (m, 2H), 7.14 (t, J = 8.8 Hz, 2H), 5.58 (d, J = 8.8 Hz, 0.8H), 5.26 (bs, 0.2H), 4.98 (dd, J = 14.1, 5.2 Hz, 1H), 4.69 (d, J = 14.3 Hz, 1H), 4.56 (dd, J = 14.7, 6.7 Hz, 1H), 4.45 (dd, J = 15.0, 6.2 Hz, 1H), 4.32 (d, J = 14.5 Hz, 1H), 3.53 (t, J = 12.8 Hz, 1H), 3.18 (s, 2.4H), 3.02 (s, 3H), 2.95 (s, 0.6H), 2.15–1.71 (m, 5H), 1.45–1.31 (m, 1H). MS *m*/*z*: 481 $(M + H)^+$.

(±)-8-[Acetyl(methyl)amino]-*N*-(4-fluorobenzyl)-3-hydroxy-4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]pyrimidine-2-carboxamide (13a). The compound was prepared by reacting a solution of crude *N*-(4-fluorobenzyl)-3-hydroxy-8-(methylamino)-4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]pyrimidine-2-carboxamide **40a** (prepared as described for **7a**, step d) in dry DCM (2 mL) with DIPEA (0.062 g, 0.48 mmol), HOBt (0.045 g, 0.33 mmol), EDC (0.064 mg, 0.33 mmol), and acetic acid (0.034 g, 0.56 mmol). The mixture was stirred at room temperature for 16 h and then concentrated. The product was isolated by preparative RP-HPLC. Yield: 2% from **30a**. ^{1}H NMR (400 MHz, DMSO-d_6 + 2% TFA) mixture of two rotamers in ratio 3/1: δ 9.02 (bs, 1H), 7.40–7.32 (m, 2H), 7.18–7.08 (m, 2H), 5.75–5.65 (m, 0.75H), 5.59–5.48 (m, 0.25H), 4.50 (ddd, J = 6.6, 15.0, 24.8 Hz, 2H), 4.19–4.05 (m, 1H), 3.91–3.78 (m, 1H), 2.93 (s, 2.1H), 2.71–2.64 (bs, 0.9H), 2.46–2.35 (m, 1H, partially under solvent signal), 2.20–2.00 (m, 4H). MS *m*/*z*: 375 $(M + H)^+$.

(±)-9-[Acetyl(methyl)amino]-*N*-(4-fluorobenzyl)-3-hydroxy-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide (13b). The compound was prepared starting from **31b** (0.055 g, 0.138 mmol), according to the procedure described for the synthesis of **40b** (step c) and then the following step d: Crude **40b** was dissolved in DCM (2 mL). DIPEA (0.105 g, 0.82 mmol), acetic acid (0.029 g, 0.48 mmol), HOBt (0.081 g, 0.60 mmol), and EDC (0.115 g, 0.60 mmol) were added, and the mixture was stirred for 4 h at room temperature. The solvent was removed under reduced pressure, and the product was isolated by preparative RP-HPLC to afford the title compound (0.020 g, 26%). ^{1}H NMR (400 MHz, DMSO-d_6) mixture of two rotamers in ratio 4/1: δ 12.08 (s, br, 1H), 8.72 (t, J = 6.4 Hz, 0.8H), 8.49 (t, J = 6.4 Hz, 0.2H), 7.38–7.30 (m, 2H), 7.21–7.11 (m, 2H), 5.23–5.18 (m, 0.8H), 5.02–4.954 (m, 0.2H), 4.58 (d, J = 6.4 Hz, 2H), 4.10–4.01 (m, 1H), 3.74–3.49 (m, 1H), 2.92 (s, 2.4H), 2.60 (s, 0.6H), 2.09 (s, 0.6H), 2.02 (s, 2.4H), 2.01–1.84 (m, 4H). MS *m*/*z*: 389 $(M + H)^+$.

(±)-9-({[(Dimethylamino)sulfonyl]acetyl}amino)-*N*-(4-fluorobenzyl)-3-hydroxy-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide (19b). 19b was prepared from **31b** as described as for **13b**, using dibenzylamine in the first step. Yield: 8% from **31b**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.16 (s, 1H), 9.08 (t, J = 6.2 Hz, 1H), 8.84 (d, J = 7.8 Hz, 1H), 7.42–7.33 (m, 2H), 7.22–7.13 (m, 2H), 4.92–4.81 (m, 1H), 4.60–4.40 (m, 2H), 4.31 (d, J = 13.8 Hz, 1H), 4.13 (d, J = 13.8 Hz, 1H), 4.12–4.02 (m, 1H), 3.90–3.80 (m, 1H), 3.11 (s, 3H), 2.18–1.87 (m, 3H), 1.75–1.55 (m, 1H). MS *m*/*z*: 453 $(M + H)^+$.

General Procedure for the Synthesis of Sulfonamides 20, 21, 22b,c, 24–27. 40b (or **40c**) (0.14 mmol) was suspended in DCM (4 mL). TEA (0.3 mL, 2.15 mmol) and the appropriate sulfonyl (or sulfamoyl) chloride (1.86 mmol) were added, and the mixture was stirred at room temperature for 90 min. The solvent was removed under reduced pressure, and the product was isolated by preparative RP-HPLC.

(±)-*N*-(4-Fluorobenzyl)-3-hydroxy-9-[methyl(methylsulfonyl)amino]-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide (20b). Yield: 39%. Prior to RP-HPLC, the residue was suspended in a mixture of 1 M NaOH and MeOH (1:1, 20 mL), stirred at room temperature for 16 h, and neutralized with 1 N aqueous HCl. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.85 (s, 1H), 8.87 (t, J = 6.4 Hz, 1H), 7.36–7.28 (m, 2H), 7.20–7.12 (m, 2H), 4.97–4.88 (m, 1H), 4.57–4.42 (m, 2H), 4.01–3.94 (m, 1H), 3.74–3.65 (m, 1H), 3.09 (s, 3H), 2.71 (s, 3H), 2.10–1.92 (m, 4H). MS *m*/*z*: 425 $(M + H)^+$.

(±)-*N*-(4-Fluorobenzyl)-3-hydroxy-10-[methyl(methylsulfonyl)amino]-4-oxo-4,6,7,8,9,10-hexahydropyrimido[1,2-*a*]azepine-2-carboxamide (20c). 20c was treated with NaOH prior to RP-HPLC, as described for **20b**. Yield: 8% from **31c**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.13 (bs, 1H), 8.68 (t, J = 6.0 Hz, 1H), 7.35 (dd, J = 8.6, 5.7 Hz, 2H), 7.16 (t, J = 8.9 Hz, 2H), 4.99 (d, J = 8.8 Hz, 2H), 4.88 (dd, J = 14.0, 5.5 Hz, 1H), 4.59–4.47 (m, 2H), 3.58–3.48 (m, partially hidden under water), 3.03 (s, 1H), 2.94 (s, 3H), 2.11–1.78 (m, 5H), 1.38–1.22 (m, 1H). MS *m*/*z*: 439 $(M + H)^+$.

(±)-*N*-(4-Fluorobenzyl)-3-hydroxy-9-{methyl[(1,3,5-trimethyl-1*H*-pyrazol-4-yl)sulfonyl]amino}-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide (21b). Yield: 29%. ^{1}H NMR (400 MHz, DMSO-d_6 + 2% TFA) δ 9.04 (t, J = 6.0 Hz, 1H), 7.42–7.35 (m, 2H), 7.20–7.11 (m, 2H), 5.17 (t, J = 8.8 Hz, 1H), 4.60 (dd, J = 7.2, 15.0 Hz, 1H), 4.44 (dd, J = 5.6, 15.0 Hz, 1H), 4.10–4.02 (m, 1H), 3.67 (s, 3H), 3.60–3.49 (m, 1H), 2.37 (s, 3H), 2.31 (s, 3H), 2.16 (s, 3H), 2.12–1.92 (m, 4H). MS *m*/*z*: 519 $(M + H)^+$.

(±)-9-[[(Dimethylamino)sulfonyl](methyl)amino]-*N*-(4-fluorobenzyl)-3-hydroxy-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide (22b). Yield: 32%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.95 (s, 1H), 9.13 (m, 1H), 7.38 (m, 2H), 7.04 (m, 2H), 4.98 (m, 1H), 4.56 (m, 2H), 4.36 (m, 1H), 3.62 (m, 1H), 2.84 (s, 6H), 2.57 (s, 3H), 2.38–1.85 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.53, 162.55, 160.11, 157.74, 145.76 144.11, 132.70, 128.89, 128.81, 124.82, 114.60, 114.39, 58.06, 42.93, 41.53, 37.00, 29.03, 23.89, 20.09. MS *m*/*z*: 454 $(M + H)^+$.

(*S*)-9-[[(Dimethylamino)sulfonyl](methyl)amino]-*N*-(4-fluorobenzyl)-3-hydroxy-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide ((*S*)-22b). (*S*)-**22b** was prepared from (*S*)-**43b** as already described for **22b**. Yield: 37%. $[\alpha]^{20}_D$ +33 ± 3 (EtOH), 97% ee. Spectral properties are identical to those of the corresponding racemic compound **22b**.

(*R*)-9-[[(Dimethylamino)sulfonyl](methyl)amino]-*N*-(4-fluorobenzyl)-3-hydroxy-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide ((*R*)-22b). (*R*)-**22b** was prepared from (*R*)-**43b** as already described for **22b**. The pooled product

fractions, lyophilized after RP-HPLC purification, afforded the title compound as a fluffy white material (90.7% ee). The compound was dissolved in ethanol and left standing at room temperature for 3 days. The supernatant was taken off and concentrated to dryness under reduced pressure. The residue was redissolved in ethanol, and the solution was left standing at room temperature for 1 day. The supernatant was taken off and concentrated under reduced pressure and the residue lyophilized from water/acetonitrile to afford the title product with an enantiomeric excess of 99.4% (ee determined by chiral HPLC Chiralpak AD, mobile phase *n*-hexane/isopropanol + 0.2% TFA). Yield: 61%. $[\alpha]^{20}_{D}$ −33 ± 2 (EtOH). Spectral properties are identical to those of the corresponding racemic compound **22b**.

(±)-10-[[(Dimethylamino)sulfonyl](methyl)amino]-*N*-(4-fluorobenzyl)-3-hydroxy-4-oxo-4,6,7,8,9,10-hexahydropyrimido[1,2-*a*]azepine-2-carboxamide (22c). Yield: 10% from **31c**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.78 (bs, 1H), 8.85 (t, J = 5.8 Hz, 1H), 7.38 (dd, J = 8.4, 5.6 Hz, 2H), 7.18 (t, J = 8.9 Hz, 2H), 4.96–4.85 (m, 2H), 4.61–4.48 (m, 2H), 3.49 (dd, J = 13.8, 11.6 Hz, 1H), 2.81 (s, 3H), 2.71(s, 6H), 2.18–2.08 (m, 1H), 2.03–1.79 (m, 4H), 1.41–1.28 (m, 1H). MS *m*/*z*: 468 (M + H)$^+$.

(±)-*N*-(4-Fluorobenzyl)-3-hydroxy-9-[methyl(pyrrolidin-1-ylsulfonyl)amino]-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide (24b). The appropriate sulfamoyl chloride was prepared according to the literature.[32] Yield: 27%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.64 (s, 1H), 9.15 (t, J = 6.3, 1H), 7.40–7.27 (m, 2H), 7.20–7.10 (m, 2H), 4.95–4.82 (m, 1H), 4.50–4.40 (m, 2H), 4.10–4.05 (m, 1H), 3.62–3.48 (m, 1H), 3.30–3.11 (m, 4H), 2.53 (s, 3H), 2.15–1.91 (m, 4H), 1.90–1.79 (m, 4H). MS *m*/*z*: 480 (M + H)$^+$.

(±)-*N*-(4-Fluorobenzyl)-3-hydroxy-9-[methyl(piperidin-1-ylsulfonyl)amino]-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide (25b). The appropriate sulfamoyl chloride was prepared according to the literature.[32] Yield: 26%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.64 (bs, 1H), 9.09 (t, J = 6.4 Hz, 1H), 7.41–7.30 (m, 2H), 7.21–7.11 (m, 2H), 4.90–4.78 (m, 1H), 4.61–4.42 (m, 2H), 4.12–3.98 (m, 1H), 3.65–3.48 (m, 1H), 3.15–3.04 (m, 4H), 2.53 (s, 3H), 2.19–1.95 (m, 4H), 1.62–1.41 (m, 6H). MS *m*/*z*: 494 (M + H)$^+$.

(±)-*N*-(4-Fluorobenzyl)-3-hydroxy-9-[methyl(morpholin-4-ylsulfonyl)amino]-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide (26b). The appropriate sulfamoyl chloride was prepared according to the literature.[32] Yield: 43%. ^{1}H NMR (300 MHz, CD_3CN) δ 11.90 (bs, 1H), 9.13 (bs, 1H), 7.51–7.35 (m, 2H), 7.21–7.10 (m, 2H), 5.00–4.88 (m, 1H), 4.65–4.50 (m, 2H), 4.28–4.12 (m, 1H), 3.81–3.69 (m, 4H), 3.69–3.55 (m, 1H), 3.26–3.13 (m, 4H), 2.65 (s, 3H), 2.32–1.92 (m, 4H). MS *m*/*z*: 496 (M + H)$^+$.

(±)-*N*-(4-Fluorobenzyl)-3-hydroxy-9-{methyl[(4-methylpiperazin-1-yl)sulfonyl]amino}-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide (27b). The appropriate sulfamoyl chloride was prepared according to the literature.[32] Yield: 33%. Isolated as the trifluoroacetate salt after RP-HPLC. ^{1}H NMR (300 MHz, CD_3CN) δ 11.67 (bs, 1H), 9.73 (bs, 1H), 8.96 (t, J = 6.3 Hz, 1H), 7.41–7.29 (m, 2H), 7.21–7.10 (m, 2H), 4.93–4.80 (m, 1H), 4.61–4.40 (m, 2H), 4.12–4.00 (m, 1H), 3.81–3.36 (m, 5H), 3.21–2.97 (m, 4H), 2.84 (s, 3H), 2.59 (s, 3H), 2.19–1.91 (m, 4H). MS *m*/*z*: 510 (M + H)$^+$.

(*S*)-*N*-(4-Fluorobenzyl)-3-hydroxy-9-{methyl[(4-methylpiperazin-1-yl)sulfonyl]amino}-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide ((*S*)-27b). (*S*)-**27b** was prepared from (*S*)-**42b** as described for **27b**. Yield: 11%. $[\alpha]^{20}_{D}$ +22 ± 3 (EtOH), 93% ee. Spectral properties are identical to those of the corresponding racemic compound **27b**.

(*R*)-*N*-(4-Fluorobenzyl)-3-hydroxy-9-{methyl[(4-methylpiperazin-1-yl)sulfonyl]amino}-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide ((*R*)-27b). (*R*)-**27b** was prepared from (*R*)-**42b** as described for **27b**. Yield: 33%. $[\alpha]^{20}_{D}$ −23 ± 3 (EtOH), 98% ee. Spectral properties are identical to those of the corresponding racemic compound **27b**.

(±)-8-[[(Dimethylamino)sulfonyl](methyl)amino]-*N*-(4-fluorobenzyl)-3-hydroxy-4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]pyrimidine-2-carboxamide (22a). The compound was prepared by reacting a solution of crude *N*-(4-fluorobenzyl)-3-hydroxy-8-(methylamino)-4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]pyrimidine-2-carboxamide **40a** (prepared as described for **7a** step d) in dry DCM (0.5 mL) and TEA (0.04 mL, 0.29 mmol). *N,N*-Dimethylsulfamoyl chloride (0.031 mL, 0.29 mmol) was added. The mixture was stirred at room temperature for 16 h, then diluted with DCM and washed with 1 N HCl and brine. The organic phase was dried over Na_2SO_4 and concentrated. The product was isolated by preparative RP-HPLC. Yield: 2% from **30a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.42 (bs, 1H), 8.78 (t, J = 6.3 Hz, 1H), 7.42–7.35 (m, 2H), 7.23–7.15 (m, 2H), 5.27 (t, J = 9.1 Hz, 1H), 4.51 (ddd, J = 6.3, 4.8, 6.8 Hz, 2H), 4.13–4.05 (m, 1H), 3.88–3.72 (m, 1H, partially hidden under the signal of water), 2.78 (s, 6H), 2.73 (s, 3H), 2.48–2.38 (m, 1H), 2.35–2.23 (m, 1H). MS *m*/*z*: 440 (M + H)$^+$.

(±)-*N*-(4-Fluorobenzyl)-3-hydroxy-9-(6-methyl-1,1-dioxido-1,2,6-thiadiazinan-2-yl)-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-2-carboxamide (23b). 23b was prepared from **31b** as described below for **23c**. Yield: 30%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.59 (bs, 1H), 9.14 (t, J = 6.2 Hz, 1H), 7.45–7.32 (m, 2H), 7.25–7.12 (m, 2H), 4.87–4.78 (m, 1H), 4.61–4.42 (m, 2H), 4.12–3.96 (m, 1H), 3.70–3.47 (m, 2H), 3.31–3.22 (m, 1H), 3.18–3.12 (m, 2H), 2.68 (s, 3H), 2.12–1.86 (m, 5H), 1.48–1.35 (m, 1H). MS *m*/*z*: 466 (M + H)$^+$.

(±)-*N*-(4-Fluorobenzyl)-3-hydroxy-10-(6-methyl-1,1-dioxido-1,2,6-thiadiazinan-2-yl)-4-oxo-4,6,7,8,9,10-hexahydropyrimido[1,2-*a*]azepine-2-carboxamide (23c). To a solution of methyl 3-(benzoyloxy)-10-bromo-4-oxo-4,6,7,8,9,10-hexahydropyrimido[1,2-*a*]azepine-2-carboxylate **31c** (0.100 g, 0.238 mmol) in DMF (5 mL) were added 2-methyl-1,2,6-thiadiazinane 1,1-dioxide (0.178 g, 1.19 mmol) and Cs_2CO_3 (0.387 g, 1.19 mmol), and the mixture was stirred at 0 °C for 20 min. The solvent was removed under reduced pressure and to crude methyl 3-hydroxy-10-(6-methyl-1,1-dioxido-1,2,6-thiadiazinan-2-yl)-4-oxo-4,6,7,8,9,10-hexahydropyrimido[1,2-*a*]azepine-2-carboxylate dissolved in methanol (4 mL) was added 4-fluorobenzylamine (0.127 g, 0.952 mmol). The mixture was stirred at reflux overnight. The solvent was removed under reduced pressure, and the product was purified by preparative RP-HPLC. Yield: 6%. ^{1}H NMR (500 MHz, DMSO-d_6 + 2% TFA) δ 9.02 (bs, 1H), 7.39 (dd, J = 8.4, 5.7 Hz, 2H), 7.18 (t, J = 8.9 Hz, 2H), 4.80 (d, J = 8.6 Hz, 1H), 4.68 (dd, J = 14.0, 6.1 Hz, 1H), 4.58–4.44 (m, 2H), 3.77 (t, J = 12.2 Hz, 1H), 3.55–3.47 (m, 2H), 3.43–3.29 (m, 2H), 2.70 (s, 3H), 2.07–1.90 (m, 2H), 1.91–1.62 (m, 5H), 1.39–1.49 (m, 1H). MS *m*/*z*: 480 (M + H)$^+$.

General Procedure for the Synthesis of Ketoamides 28. Method A. To a stirred 0.1 M solution of crude **40a** or **40b**,**c** in DCM were added TEA (or DIPEA) (6 equiv) and methyl chlorooxoacetate (6 equiv). The mixture was stirred at room temperature for 2 h, the solvent was removed under reduced pressure, and the residue was dissolved in a solution of 2 M dimethylamine in THF (6 equiv). The mixture was stirred at 57 °C overnight. After the mixture was cooled to room temperature, the solvent was removed under reduced pressure and the product was isolated by preparative RP-HPLC.

Method B. To a stirred 0.1 M solution of **40a**−**c** in DCM were added TEA (3 equiv), potassium (dimethylamino)(oxo)acetate (2 equiv), EDC (2.2 equiv), and HOBt (2.2 equiv). The mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure, and the residue was partitioned between ethyl acetate and 1 M aqueous HCl. The aqueous phase was extracted with ethyl acetate, and the combined organic phases were dried over sodium sulfate, filtered, and concentrated to dryness under vacuum. The product was isolated by preparative RP-HPLC.

(±)-*N*-(2-{[(4-Fluorobenzyl)amino]carbonyl}-3-hydroxy-4-oxo-4,6,7,8-tetrahydropyrrolo[1,2-*a*]pyrimidin-8-yl)-*N*,*N*′,*N*′-trimethylethanediamide (28a). 28a was prepared from crude **40a** (method A) (yield 2% from **30a**). ^{1}H NMR (400 MHz, DMSO-d_6) mixture of rotamers 1:1: δ 12.57 (s, 0.5H), 12.48 (s, 0.5H), 9.17

(m, 0.5H), 9.01 (m, 0.5H), 7.37–7.29 (m, 2H), 7.21–7.13 (m, 2H), 5.53 (m, 0.5H), 5.18 (m, 0.5H), 4.61–4.34 (m, 2H), 4.18–4.04 (m, 1H), 3.96–3.86 (m, 0.5H), 3.81–3.71 (m, 0.5H), 2.97 (s, 1.5H), 2.91 (s, 1.5H), 2.87–2.78 (m, 6H), 2.57–2.54 (m, partially overlapped by solvent signal), 2.37–2.16 (m, 1.5H). MS *m*/*z*: 432 $(M + H)^+$.

(±)-N^1-(2-{[(4-Fluorobenzyl)amino]carbonyl}-3-hydroxy-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-9-yl)-N^1,N^2,N^2-trimethylethanediamide (28b). 28b was prepared from **40b** (method A). Yield: 39%. ^{1}H NMR (400 MHz, DMSO-d_6) mixture of rotamers 4:1, δ 12.05 (s, 0.2H), 11.89 (s, 0.8H), 9.21 (m, 0.8H), 8.74 (m, 0.2H), 7.40–7.28 (m, 2H), 7.20–7.10 (m, 2H), 5.17 (m, 0.8H), 4.63–4.35 (m, 2.2H), 4.13–4.00 (m, 1H), 3.65–3.53 (m, partially overlapped by water signal), 2.95–2.75 (m, 9H), 2.15–1.80 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.87, 167.73, 165.92, 165.46, 164.51, 164.30, 162.42, 160.01, 157.50, 157.41, 146.27, 146.18, 145.76, 145.49, 134.44, 129.43, 129.35, 129.08, 129.00, 125.17, 125.05, 115.07, 114.85, 57.47, 53.60, 43.14, 41.37, 36.49, 35.95, 32.92, 32.64, 32.36, 28.19, 23.88, 22.12, 19.67, 19.35. MS *m*/*z*: 446 $(M + H)^+$.

(*R*)-N^1-((9*R*)2-{[(4-Fluorobenzyl)amino]carbonyl}-3-hydroxy-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-9-yl)-N^1,N^2,N^2-trimethylethanediamide ((*R*)-28b). (*R*)-**28b** was prepared from (*R*)-**43b**. To a solution of (*R*)-**43b** (0.115 g, 0.313 mmol) in MeOH (0.5 mL) were added 4-fluorobenzylamine (0.079 g, 0.63 mmol) and TEA (0.041 g, 0.41 mmol). The mixture was stirred and heated to 65 °C for 3 h. After the mixture was cooled to room temperature, the solvent was removed under reduced pressure and the residue was dissolved in DCM (4 mL). TEA (0.126 g, 1.25 mmol) and methyl chlorooxoacetate (0.133 g, 1.25 mmol) were added, and the mixture was left stirring at room temperature for 50 min. The solvent was removed under reduced pressure, and the residue was dissolved in a 2 M solution of *N*,*N*-dimethylamine in THF (6.0 mL, 12.0 mmol). The mixture was stirred and heated at 55 °C for 16 h. After the mixture was cooled to room temperature, the solvent was removed under reduced pressure and the product was purified by preparative RP-HPLC. After lyophilization of the pooled product fractions, the title compound was obtained as a fluffy white powder (0.079 g, yield 52%, 94% ee). The compound was dissolved in ethyl acetate/heptane (3:2.5 mixture) and left standing at room temperature for 4 days. The supernatant was taken off from the formed precipitate and concentrated under reduced pressure, and the residue was lyophilized from water/acetonitrile to afford enantiopure title product (100% ee, determined by chiral HPLC Chiralpak AS, mobile phase *n*-hexane/isopropanol + 0.2% TFA). $[\alpha]^{20}_D$ +39 ± 2 (EtOH). Spectral properties are identical to those of the corresponding racemic compound **28b.**

(*S*)-N^1-((9*S*)2-{[(4-Fluorobenzyl)amino]carbonyl}-3-hydroxy-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-9-yl)-N^1,N^2,N^2-trimethylethanediamide ((*S*)-28b). (*S*)-**28b** was prepared from (*S*)-**43b**, as described above for (*R*)-**28b**. The compound obtained after RP-HPLC (93% ee) was dissolved in ethanol, and the precipitate formed was filtered off. The filtrate was concentrated to dryness under vacuum and the residue was redissolved in ethanol. The solution was left standing at room temperature for 1 day. The supernatant was taken off, concentrated under reduced pressure, and lyophilized from water/acetonitrile to afford the title compound(99.6% ee, determined by chiral HPLC Chiralpak AS, mobile phase *n*-hexane/isopropanol + 0.2% TFA). $[\alpha]^{20}_D$ −40 ± 2 (EtOH). Spectral properties are identical to those of the corresponding racemic compound **28b.**

(±)-*N*-(2-{[(4-Fluorobenzyl)amino]carbonyl}-3-hydroxy-4-oxo-4,6,7,8,9,10-hexahydropyrimido[1,2-*a*]azepin-10-yl)-*N*,*N*′,*N*′-trimethylethanediamide (28c). 28c was prepared from **40c** (method B). Yield: 10%. Spectral properties are identical to those of the corresponding enantiomer (*S*)-**28c**.[30] MS *m*/*z*: 460 $(M + H)^+$.

(*R*)-*N*-(2-{[(4-Fluorobenzyl)amino]carbonyl}-3-hydroxy-4-oxo-4,6,7,8,9,10-hexahydropyrimido[1,2-*a*]azepin-10-yl)-*N*,*N*′,*N*′-trimethylethanediamide ((*R*)-28c). Spectral properties are identical to those of the corresponding enantiomer (*S*)-**28c**.[30] $[\alpha]^{20}_D$ +72 ± 2 ($CHCl_3$).

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Supporting Information Available: Synthesis and analytical data for the intermediates **30a**, **30c**, **31c**, and [(dimethylamino)sulfonyl]acetic acid; HPLC, UPLC, and high-resolution mass measurements (HRMS) data for final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(22) Single-point measurement after 1 h. Experimental conditions: 2 μM compound, 1 mg/mL microsomes. The degradation of the compound in the presence of UDPGA (or NADPH) was measured by LC/MS/MS after 1 h of incubation.

(23) Multiple-points measurement during 1 h. Experimental conditions: 1 μM compound, 1 mg/mL microsomes. The degradation of the compound in the presence of UDPGA and NADPH was measured by LC/MS/MS during 1 h of incubation.

(24) A positive charge on a molecule is reported to often disrupt the high-affinity interaction with HSA (human serum albumin). See ref 20.

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