# Synthesis and Structure−Activity Relationships of 1-Arylmethyl-5-aryl-6-methyluracils as Potent Gonadotropin-Releasing Hormone Receptor Antagonists

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Based on the SAR from bicyclic gonadotropin-releasing hormone (GnRH) antagonists such as 6-aminomethyl-7-aryl-pyrrolo[1,2-*a*]pyrimid-4-ones (**5**) and 2-aryl-3-aminomethyl-imidazolo-[1,2-*a*]pyrimid-5-ones (**6a**,**b**), a series of novel uracil compounds (**8**) were derived as GnRH antagonists. The synthesis and SAR studies of 6-methyluracils as human GnRH receptor antagonists are discussed herein. Introduction of a small methyl substituent at the $\beta$-position of the N3 side-chain improved the GnRH binding potency by 5−10-fold. Introduction of a methyl group of (*R*)-configuration at the α-carbon of the N-3 side-chain gave a modest improvement in binding affinity over the unsubstituted ethylene analogues. This modification enabled us to make uracil compounds without the labile 2-pyridylethyl motif on the basic nitrogen while still maintained excellent potency against the *h*GnRH receptor.

## Introduction

Gonadotropin-releasing hormone (GnRH), also known as luteinizing hormone-releasing hormone (LHRH), is the decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-$NH_2$), hormone produced in and secreted by the hypothalamus in a pulsatile manner.[1,2] It acts on the pituitary gland to stimulate the secretion of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These gonadotropins, in turn, act on the reproductive organs, where they participate in the regulation of gonadal steroid production, spermatogenesis in males, and follicular development in females. Several reproductive disease conditions such as endometriosis, uterine fibroids, and prostate cancer are caused by overstimulation of the reproductive organs by the gonadal steroids and thus can be treated by suppression of the pituitary−gonadal axis. Continuous administration of high dose of GnRH or its analogues (agonists) desensitizes the receptor with consequent decline in gonadal gametogenesis and steroid production. This phenomenon has successfully led to an extensive application of GnRH peptide agonists for treatment of a variety of reproductive diseases. Currently, depot forms of peptidic GnRH agonists, represented by leuprorelin are used to treat such conditions through a receptor down-regulation mechanism to suppress gonadal steroid production.[3] On the another hand, recent clinical evidence has shown that peptidic GnRH antagonists can act immediately at the receptor through competition with endogenous GnRH to inhibit the reproductive system and therefore alleviate disease symptoms without the concomitant "flare effect", which is exhibited by the peptide agonists due to their initial overstimulation of the receptor.[4] Nevertheless, both peptide agonists and antagonists require parenteral administration, typically in depot form due to their poor oral bioavailability. By contrast, small molecule GnRH antagonists offer the potential for oral administration and therefore could gain wider acceptance from patients. In response to that need, intensive efforts have been initiated for the development of orally active small molecule GnRH antagonists by many laboratories.[5−10]

Compound **1** (T-98475, Figure 1) and its analogues are the first small molecules reported[5] to have high affinity on the human GnRH receptor ($IC_{50}$: 0.2 nM) albeit with reduced binding affinity for the rat receptor ($IC_{50}$: 60 nM).[5] In addition, a series of papers on quinolones and tryptamines as potent GnRH antagonists were recently published. For example, quinolone **2** possesses high binding affinity ($IC_{50}$: 0.4 nM) for the human GnRH receptor as well as the rat GnRH receptor ($IC_{50}$: 4 nM).[6] Indole derivatives such as **3** were reported to be potent and orally bioavailable GnRH antagonists.[7] Most recently, TAK-013 ($IC_{50}$: 0.2 nM) and its analogues were reported as potent and orally active hGnRH antagonists.[8] Most of the above compounds have high binding affinity to the hGnRH receptor, but they all have high molecular weight (**1** and **4** above 650; **2** and **3** above 500).

In the previous papers,[9,10] we disclosed the SAR study of 6-aminomethyl-7-aryl-pyrrolo[1,2-*a*]pyrimid-4-ones (**5**, Figure 2**)** and 2-aryl-3-aminomethyl-imidazolo[1,2-*a*]-pyrimid-5-ones (**6a**,**b**) as potent hGnRH receptor antagonists. The SAR of **5** revealed that the binding affinity was enhanced by a 2-pyridyl group on the side chain of the 6-aminomethyl group and a para substituent ($R^3$) on the 7-phenyl group (e.g. **5**, $R^1 = CH_3$, $R^2 =$

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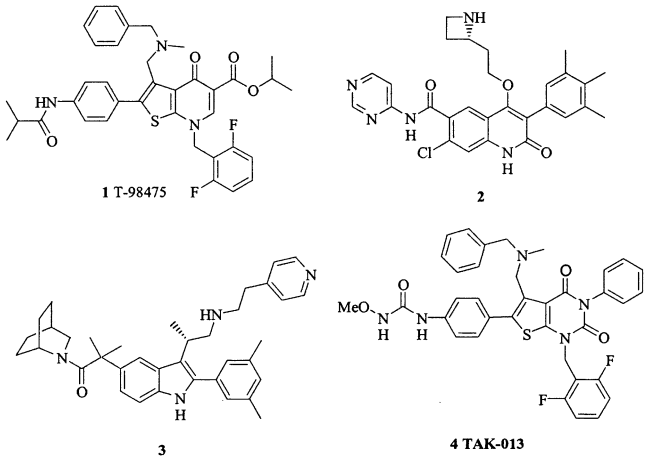



**Figure 1.** Small molecule GnRH antagonists.

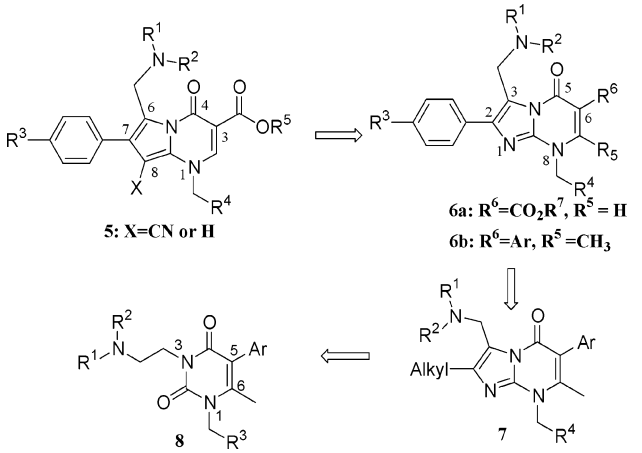



**Figure 2.** From bicyclic to monocyclic GnRH antagonists.

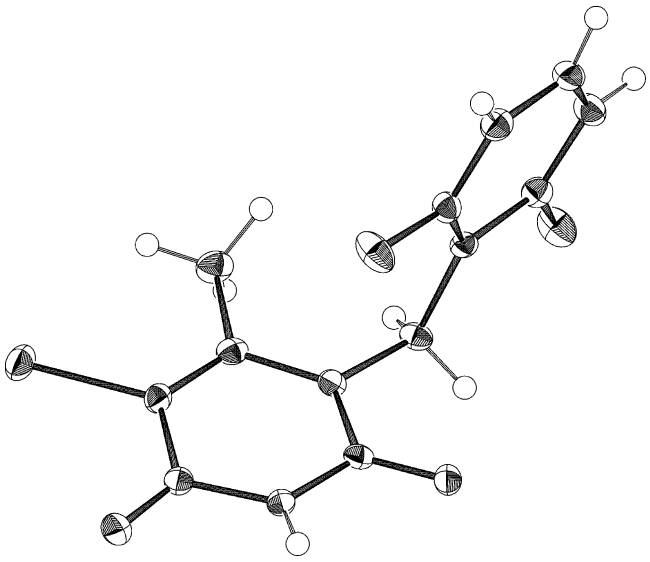
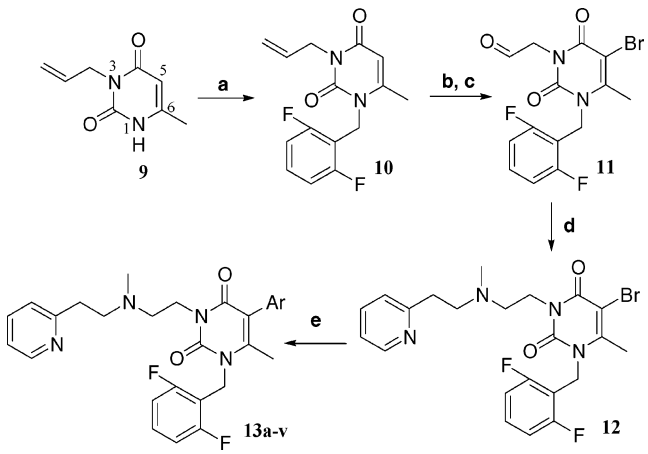


**Figure 3.** X-ray structure for **16**.

2-Py$CH_2CH_2$; $R^3$ = *n*-PrCONH). Furthermore, SAR studies of **6b** indicated that an aryl group, preferably the 3-methoxyphenyl group, could replace the 6-carboxylate of **6a**. Introduction of the 6-aryl group led to a different SAR profile in the two series represented by **6a** and **6b**. Thus, while the para substituent ($R^3$) on the phenyl ring of the molecule **6a** was crucial for binding, it had minimal effect on the activity of **6b**. This observation led us to successfully design the 2-alkyl analogues **7**, which possess lower molecular weights but maintain the potent GnRH antagonistic activity of **6b**.[11] On the basis of these SAR results, we further postulated that the whole left side of **7** including the alkyl group and the five-membered ring might not be necessary for binding, which would lead to much smaller monocyclic compounds. To test this hypothesis, the uracil was chosen as one of several possible monocyclic core structures due to its synthetic accessibility. The initial SAR studies of this novel monocyclic series (**8**) as human GnRH receptor antagonists were discussed recently.[12] The best compound (**13b**) from the initial SAR study has a $K_i$ value of 34 nM, which is less potent than some of the bicyclic analogues. We rationalized that reducing the flexibility of the alkylamino side chain at the 3-position should be beneficial for binding energy, as long as one of the low-energy conformers fits into the binding pocket. A small alkyl group such as a methyl substituent at the ethylene linker could serve such a purpose. Here we report the synthesis and SAR studies of the 1-arylmethyl-3-(2-aminoethyl)-5-aryl-6-methyluracil series.

**Scheme 1**[a]



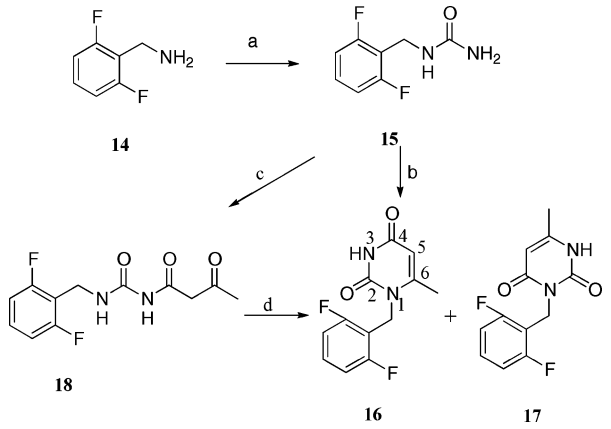

[a] Reagents and conditions: (a) 2,6-difluorobenzyl bromide, TBAF, THF/DMF, 59%; (b) $NaIO_4$, OsO4 (cat.), THF/$H_2O$, quant.; (c) $Br_2$, HOAc, 58%; (d) 2-(*N*-methyaminoethyl)-pyridine, $NaBH(OAc)_3$, DCM, 73%; (e) boronic acid or boronic acid pinacol ester, $Pd(PPh_3)_4$, $K_2CO_3$, toluene/$H_2O$, reflux, 30−80%.

## Chemistry

The initial approach to synthesize the fully substituted uracils is outlined in Scheme 1. 3-Allyl-6-methyluracil (**9**) was obtained by condensation of *N*-allylurea with ethyl acetoacetate using a known procedure.[13] It was then treated with 2,6-difluorobenzyl bromide in the presence of n-tetrabutylammonium fluoride in THF to afford **10** in 59% yield, which underwent oxidative cleavage of the olefin with $NaIO_4$, followed by bromination at the 5-position to yield **11** in 58% yield (two steps). Reductive amination of the aldehyde **11** with 2-(*N*-methyl-2-aminoethyl)pyridine, one of the favored side-chains from the bicyclic series, in the presence of $NaBH(OAc)_3$, gave **12** in 73% yield. Finally, **12** was subjected to Suzuki coupling reactions with a variety of arylboronic acids or boronic acid pinacol esters to afford the desired products **13(a−v)** in 30−80% yields.

While the above synthesis of the 6-methyluracil core offers quick access to the desired products **13**, its

**Scheme 2**[a]




[a] Reagents and conditions: (a) urea, water, HCl, reflux, 79%; (b) diketene, HOAc, reflux, 40 min, 69%; (c) diketene, pyridine, rt, 24 h, 60%; (d) HOAc, reflux, 1 h, 95%.

application is limited in several respects. First, the condensation of allylurea with ethyl acetoacetate gave low yield (∼7%) and a long reaction time (12 days). Second, the N3 substituent was introduced into the molecule at the beginning of synthesis, thus limiting the SAR study at this site. Accordingly, a new and improved synthetic route was then developed, as shown in Scheme 2 and 3. Thus, 2,6-difluorobenzylamine and urea were refluxed in HCl/water solution to form the benzyl urea **15** (Scheme 2), which was crystallized from EtOAc in 79% yield. Urea **15** and diketene were then refluxed in acetic acid for 40 min to yield a mixture of N1- and N3-substituted 6-methyluracils (**16** and **17**) in 3:1 ratio. 1-(2,6-Difluorobenzyl)-6-methyluracil (**16**) was separated from its N3-isomer (**17**) by recrystallization in acetic acid. This two-step sequence afforded **16** in about 25% yield from **14**.[12b] A more efficient procedure was subsequently developed to afford the N1-substituted 6-methyluracil **16** exclusively, wherein compound **15** was first treated with diketene in pyridine at ambient temperature for 24 h, and the intermediate **18** was separated in 60% yield. **18** was then refluxed in acetic acid for 1 h and the desired product **16** crystallized out from the reaction mixture in 95% yield (Scheme 2). The structure assignments of **16** and **17** were confirmed by NOE NMR experiments. (In compound **16**, for instance, there was a clear NOE of the 6-methyl signal upon irradiation of the benzylic protons at N1; this effect was absent in **17**.)[14]

Uracil **16** was brominated in acetic acid to yield **19** in 88% yield. Compound **19** was treated with either *N*-*t*-Boc-*R*-alaninol or its corresponding *S*-isomer under Mitsunobu conditions in THF using di-*tert*-butyl-azodicarboxylate as the coupling reagent to give *R*-**20** or *S*-**20** in high yield (94% and 96%, respectively) with complete retention of their chirality (Scheme 3). Coupling of **20** with 3-methoxyphenylboronic acid or 2-fluoro-3-methoxyphenylboronic acid under the Suzuki coupling conditions catalyzed by Pd(0) yielded **21** or **22** in about 45% yield. Deprotection of the amino-Boc group of **21** and **22** with TFA gave the amino compounds **23** and **24** in quantitative yields. These amines (**23** and **24**) were treated, under reductive alkylation conditions, with a variety of aldehydes and ketones ($R_1R_2CO$) in dichloroethane, followed by reaction with $NaBH(OAc)_3$ to afford the desired chiral amines **25** and **26**.

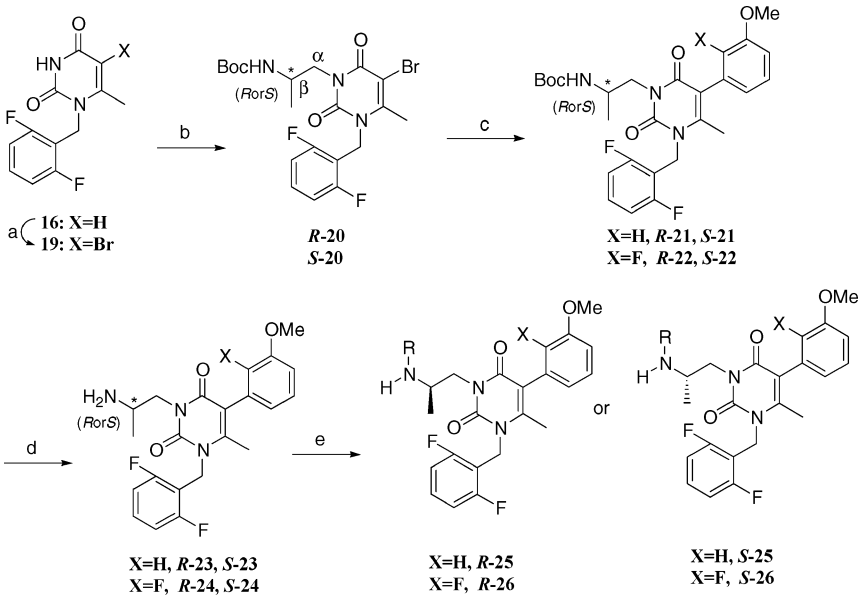
To examine the substitution effect at the α-position of the N3 side chain, we also prepared compounds **34** and **35**, and the synthesis is shown in Scheme 4. Commercial (*R*)- or (*S*)-1-amino-2-propanol (*R*-**27** or *S*-**27,** Aldrich, 97% ee) was protected as its *tert*-butyl carbamate derivate and then used in the Mitsunobu alkylation of uracil **19** (Scheme 4). The Mitsunobu reaction on the secondary alcohol *R*-**28**, or *S*-**28**, occurred with complete inversion of the chiral center, leading to the corresponding alkylated uracil *S*-**29**, or *R*-**29**, with (*S*)-, or (*R*)-configurations, in ∼70% yield. The stereochemical integrity of *S*-**29** and *R*-**29** was confirmed by chiral HPLC analysis,[15] and the absolute (*R*)-stereochemistry of *R*-**29** was determined by X-ray crystallography,[12c] which was possible due to the anomalous contributions of the heavy atom (bromine).[16] The 5-(3-methoxyphenyl)- or 5-(2-fluoro-3-methoxyphenyl) group was introduced via a Suzuki coupling reaction of the appropriate boronic acids with bromides *S*-**29** or *R*-**29**. For example, compound **31** was obtained in 53% yield, which was treated with trifluoroacetic acid in $CH_2Cl_2$ to furnish the corresponding amine **33** in 95% yield. **32** and **33** were then reacted with a variety of aldehydes and ketones, under reductive conditions, to give the corresponding secondary amines **34** and **35**.

## Results and Discussions

**In Vitro Receptor Binding Studies.** All synthesized compounds were evaluated for their ability to inhibit des-Gly$^{10}$[$^{125}$I-Tyr$^5$,D-Leu$^6$,NMeLeu$^7$,Pro$^9$-NEt]-GnRH radioligand binding to the cloned hGnRH receptor stably expressed in HEK293 cells using a 96-well filtration assay format.[17]

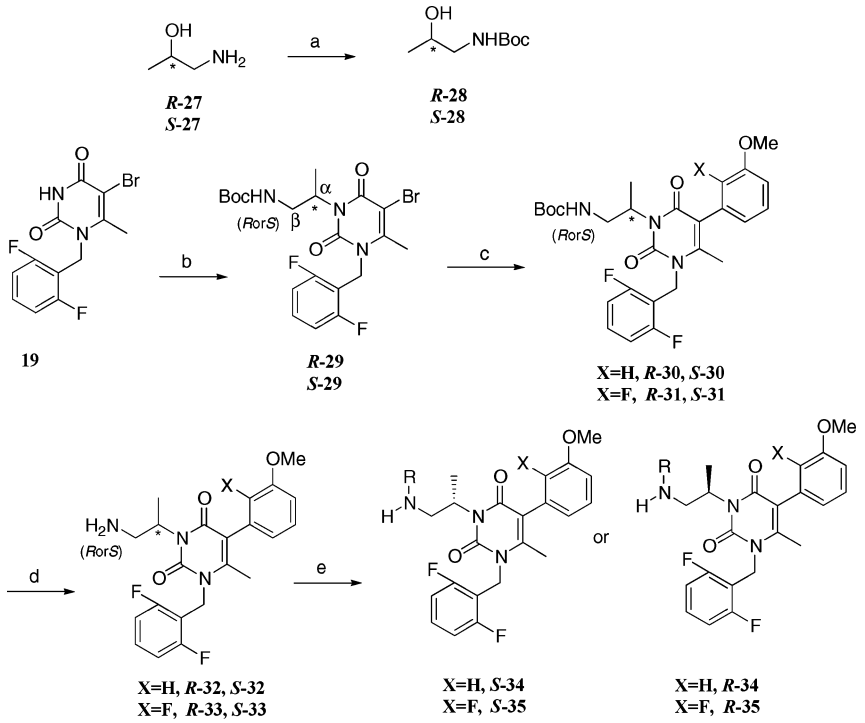
Our initial SAR studies of the uracil series indicated that at N3 position, [*N*-methyl-*N*-(2-pyridylethyl)]-aminoethyl group was the key feature for obtaining highly potent hGnRH antagonists.[12a] Thus, this preferred side-chain was fixed at N3 position, while the impact of the substitution on the 5-phenyl group to binding affinities was examined and the data are summarized in Table 1.[18] The unsubstituted phenyl compound **13a** was more than 10-fold less potent than the corresponding 3-methoxyphenyl analogue **13b**, indicating that the 3-methoxy group may function as a possible hydrogen-bond acceptor. Replacement of the methoxyl group by a 3,4-methylenedioxy (**13c**) or 3,4-ethylenedioxy (**13d**) generated equally potent molecules. However, replacement with the difluoro-3,4-methylenedioxy group (**13e**, 470 nM) reduced the binding affinity significantly, which might be caused by reduced hydrogen bonding ability of the substituent. Shifting the methoxy group from the 3- to the 4-position also resulted in a substantial reduction in potency (**13f**, 230 nM), while extending the 4-methoxy to 4-ethoxy led to further decrease in potency (**13g**, 680 nM). Combining 3- and 4-methoxy groups into the phenyl ring resulted in compound (**13h**) with very poor binding, and incorporation of the 3,4,5-trimethoxyphenyl group yielded an inactive compound (**13i**). The importance of hydrogen bonding ability of the substituents to the binding affinity was further demonstrated by **13m**−**o**, in which simple alkyl substituents without any oxygen atom led to lower potency. Although a strong

**Scheme 3**[a]




[a] Reagents and conditions: (a) $Br_2$, HOAc, 88%; (b) *N*-*t*-Boc-*R*-alaninol or *N*-*t*-Boc-*S*-alaninol, $PPh_3$, di-*tert*-butyl-azodicarboxylate, THF, 94% or 96%; (c) 3-methoxyphenyl boronic acid or 2-fluoro-3-methoxyphenyl boronic acid, $Pd(Ph_3P)_4$, $Na_2CO_3$, toluene/$H_2O$, reflux, ~45%; (d) TFA/$CH_2Cl_2$ (1:1), quant.; (e) aldehyde or ketone, $NaBH(OAc)_3$, dichloroethane.

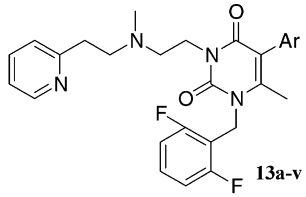
**Scheme 4**[a]



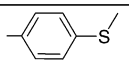
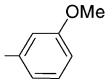
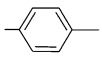
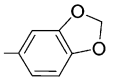
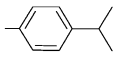
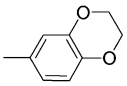
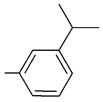
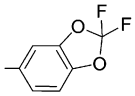
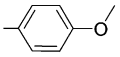
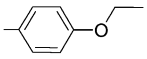
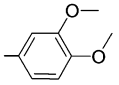
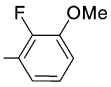
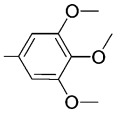
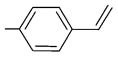
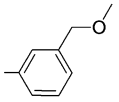
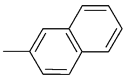
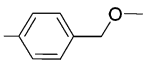
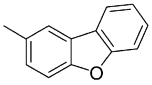

[a] Reagents and conditions: (a) $(Boc)_2O$, $Et_3N$, $CH_2Cl_2$, 0 °C to rt, 2 h, quant.; (b) *R*-**28** or *S*-**28**, DEAD, $Ph_3P$, THF, rt, 17 h, ~70%; (c) 3-methoxyphenyl boronic acid or 2-fluoro-3-methoxyphenyl boronic acid, $Pd(Ph_3P)_4$, $K_2CO_3$, toluene/EtOH/$H_2O$, 110 °C, 18 h, 90% or 53%; (d) TFA/$CH_2Cl_2$ 1:1 v/v, rt, 1 h, 95%; (e) aldehyde or ketone, MeOH, $NaBH_4$, rt, 1 h.

electron-withdrawing fluorine atom at 3-position resulted in weakly active analogue (**13q**, 1200 nM), switching the fluorine to the 2-position gave a 5-fold increase in binding affinity in comparison to unsubstituted phenyl analogue (**13a**). When the 2-fluorine and the 3-methoxy substituents were combined into the 5-phenyl group of the uracil, a dramatic increase in binding affinity was achieved and **13s** became the most potent compound in this series (3 nM). This phenomenon may be explained by a combination of effects caused by the stereo-effect of the 2-fluoro and hydrogen bond acceptor by the 3-methoxy group. We also speculate that the 2-fluoro group, being slightly larger than a proton, could impede the free rotation of the 5-phenyl ring,[19] thus force the 5-phenyl ring into a perpendicular conformation with respect to the uracil core, which in turn, may be preferred for a $\pi-\pi$ interaction with the GnRH receptor. Extending the 5-phenyl ring to a larger aromatic ring system (**13u** and **13v**) did not give noticeable change in binding affinity.

**Table 1.** Binding Affinities of Compounds **13a**−**v** to the Human GnRH Receptor[18]



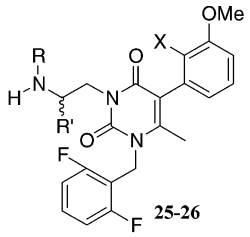

**13a-v**

| compd | Ar | $K_i$(nM) | compd | Ar | $K_i$(nM) |
|---|---|---|---|---|---|
| **13a** |  | 400 | **13l** |  | 120 |
| **13b** |  | 34 | **13m** |  | 220 |
| **13c** |  | 38 | **13n** |  | 110 |
| **13d** |  | 24 | **13o** |  | 240 |
| **13e** |  | 470 | **13p** |  | 370 |
| **13f** |  | 230 | **13q** |  | 1200 |
| **13g** |  | 680 | **13r** |  | 81 |
| **13h** |  | 4800 | **13s** |  | 3 |
| **13i** |  | 10800 | **13t** |  | 340 |
| **13j** |  | 1500 | **13u** |  | 460 |
| **13k** |  | 1400 | **13v** |  | 170 |

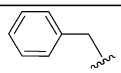
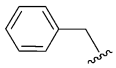
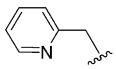
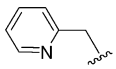
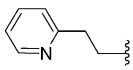
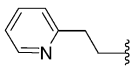
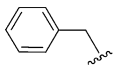
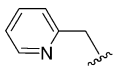
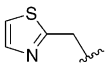
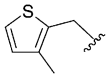
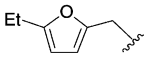
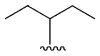
With this success, we also rationalized that restriction of the flexible ethylene side chain on the N3 of uracil may also be beneficial for the binding, and introducing a small alkyl group such as a methyl at the α- or *β*-position could serve such purpose. In addition, the readily available chiral 2-amino-1-propanol (alaninol) and 1-amino-2-propanol enabled us to examine the stereo-effect. The results from compounds **25** and **26**, prepared from *R*- and *S*-2-amino-1-propanols, are summarized in Table 2. The previous SAR studies from both bicyclic **5**−**7** and uracil **8** demonstrated the 2-(2-pyridyl)-ethylamino side-chain was the most preferred, and this preference continued in compound **25** and **26** with a *β*-methylated ethylene side-chain, albeit not significantly. For example, *R*-**25c**, which has the 2-(2-pyridyl)-ethylamino group, was merely 2-fold better in binding affinity than the corresponding benzyl analogue *R*-**25a**, while the 2-pyridylmethyl derivative *R*-**25b** was 5-fold more active than *R*-**25c**. Interestingly, for *N*-benzyl derivatives, *R*- and *S*-isomers were almost equally active. As expected, introducing the 2-fluoro-3-methoxyphenyl group at 5-position greatly improved the binding affinity. *S*-**26a** was 6 times more potent than *S*-**25a**, while *R*-**26b** was more than 10 times better than *R*-**25b**. Compound *R*-**26d** with a thiazolylmethyl side-chain also showed good activity**.** For (*S*)-derivatives, both benzyl **26a** and benzylic-like side-chains, such as thiophene-methyl and furan-methyl **26e**, **26f** demonstrated good activities as well as small alkyl groups such as **26g**, **26h**, and **26i** ($K_i$ = 4.1, 4.5, and 5.0 nM, respectively) also generated highly potent compounds.

The SAR of compounds **34** and **35** with a methyl group on the α-position of the N-3 side-chain was also examined and the results are shown in Table 3. Unlike the compounds **25** and **26**, the (*R*)-enantiomeric preference for the receptor was consistently confirmed by alkylation of amines *S*-**32** and *R*-**32** with various aldehydes and ketones.[12c] For instance, the N-benzylated compound *S*-**34a** ($K_i$ = 370 nM) was about 5 times less potent than its counterpart *R*-**34a** ($K_i$ = 75 nM). It is clear that benzylic side-chains were preferred over aliphatic ones for this subseries. The substitution pattern of the benzylic side-chain was also investigated. Introduction of an *o*-methyl group *R*-**34b** caused little change in binding affinity compared to *R*-**34a** whereas *o*-methoxy compound (*R*-**34c**) was about 4-fold more potent. Interestingly, there was no obvious difference in the binding affinity between these compounds in the

**Table 2.** Binding Affinities of Compounds **25** and **26** to the Human GnRH Receptor[18]



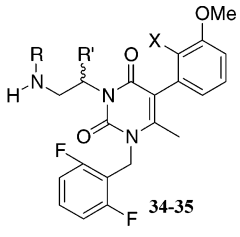

**25-26**

| compd | X | R | R' Chirality | $K_i$(nM) |
|---|---|---|---|---|
| *R*-**25a** | H |  | *R*-Me | 130 |
| *S*-**25a** | H |  | *S*-Me | 140 |
| *R*-**25b** | H |  | *R*-Me | 15 |
| *S*-**25b** | H |  | *S*-Me | 460 |
| *R*-**25c** | H |  | *R*-Me | 79 |
| *S*-**25c** | H |  | *S*-Me | 780 |
| *S*-**26a** | F |  | *S*-Me | 25 |
| *R*-**26b** | F |  | *R*-Me | 1.1 |
| *R*-**26d** | F |  | *R*-Me | 13 |
| *S*-**26e** | F |  | *S*-Me | 4.0 |
| *S*-**26f** | F |  | *S*-Me | 15 |
| *S*-**26g** | F |  | *S*-Me | 4.1 |
| *S*-**26h** | F |  | *S*-Me | 4.5 |

*S*-isomers, **34a**−**c** all had $K_i$ values around 350 nM. As expected, introducing a 5-(2-fluoro-3-methoxy)-phenyl group generated highly potent compounds. *R*-**35b** ($K_i$ = 7 nM) was about 10 times more potent than its counterpart *R*-**34b**, while *R*-**35c** ($K_i$ = 3.9 nM) was about 5 times better than *R*-**34c**.

**Further in Vitro and in Vivo Studies.** To ensure that these high binding affinity molecules were functional antagonists, selected compounds were tested for their ability to inhibit $Ca^{2+}$ influx induced by GnRH in RBL cells transfected with the GnRH receptor. The results shown in Table 4 indicate that all these compounds are potent functional antagonists with comparable $IC_{50}$ values versus their $K_i$ values. For example, compound *S*-**26h** exhibited a $K_i$ value of 4.5 nM on human GnRH receptor binding, and it's also a functional antagonist with $IC_{50}$ value of 5.5 nM in the $Ca^{2+}$ influx assay. Similar to our previous results,[9a] these compounds displayed species differences in their binding to the GnRH receptors.[20] They exhibited reduced binding affinity on the monkey GnRH receptor and much lower affinity on the rat GnRH receptor. For example, compound *S*-**26h** exhibited 10 times weaker binding affinity on the monkey GnRH receptor ($K_i$ = 50 nM) and 1000 times lower affinity on the rat GnRH receptor ($K_i$ = 7.5 $\mu$M) than the high affinity on the human GnRH receptor ($K_i$ = 4.5 nM).

**Table 3.** Binding Affinities of Compounds **34** and **35** to the Human GnRH Receptor[18]




**34-35**

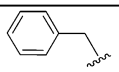
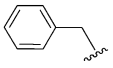
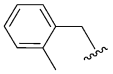
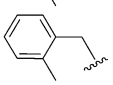
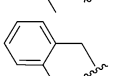
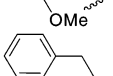
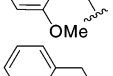
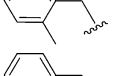
| compd | X | R | R' Chirality | $K_i$(nM) |
|---|---|---|---|---|
| *R*-**34a** | H |  | *R*-Me | 75 |
| *S*-**34a** | H |  | *S*-Me | 370 |
| *R*-**34b** | H |  | *R*-Me | 70 |
| *S*-**34b** | H |  | *S*-Me | 400 |
| *R*-**34c** | H |  | *R*-Me | 20 |
| *S*-**34c** | H |  | *S*-Me | 320 |
| *R*-**35b** | F |  | *R*-Me | 7.0 |
| *R*-**35c** | F |  | *R*-Me | 3.9 |

**Table 4.** Functional Assay Data to the Human GnRH Receptor, and Binding Affinities to the Rats and Monkey GnRH Receptors for Selected Compounds[18]

| compd | human $K_i$ (nM) | $IC_{50}$ (nM) | rats $K_i$ (nM) | monkey $K_i$ (nM) |
|---|---|---|---|---|
| **13b** | 34 | 16 | 2200 | 610 |
| *S*-**26a** | 25 | 38 | 25000 | 550 |
| *R*-**26b** | 1.1 | 0.5 | 6400 | 43 |
| *R*-**26d** | 13 | 6.1 | 10000 | 820 |
| *S*-**26e** | 4.0 | 4.0 | 3200 | 87 |
| *S*-**26f** | 15 | 27 | 4900 | 260 |
| *S*-**26g** | 4.1 | 14 | 7600 | 200 |
| *S*-**26h** | 4.5 | 5.5 | 7500 | 50 |
| *S*-**26i** | 5.0 | 10 | 22000 | 87 |
| *R*-**35c** | 3.9 | 5.7 | 7900 | 30 |

Selected compounds were also studied for their pharmacokinetic properties in mice, and the results are summarized in Table 5. Compound **13b** from the initial series with a 2-pyridylethylamino side-chain was metabolically labile.[12a] The high clearance rate data in mice (CL = 120 mL/min·kg) suggested other degradation routes in addition to hepatic removal (hepatic blood flood in mice is about 90 mL/min·kg). Sample analysis from hepatic portal vein indicated that this compound was also poorly absorbed (4.7% by comparing oral hepatic concentration versus iv plasma concentration). Compounds from the alaninol series had improved pharma-

**Table 5.** In Vivo Pharmacokinetics of Selected Compounds in Mice

| compd | *F* (%) | $t_{1/2}$ (h) | clearance (mL/min·kg) | AUC (oral) (ng/mL·h) |
|---|---|---|---|---|
| **13b**[b] | 1.6 | 0.4 | 120 | 23 |
| *R*-**26d**[a] | 6.0 | 1.3 | 97.3 | 104 |
| *S*-**26g**[a] | 8.2 | 0.9 | 129 | 110 |
| *S*-**26h**[c] | 2.2 | 0.6 | 70.7 | 57 |

[a] Dosed at 10 mg/kg iv and 10 mg/kg po. [b] Dosed at 5 mg/kg iv and 10 mg/kg po. [c] Dosed at 2.5 mg/kg iv and 10 mg/kg po.

cokinetic profiles. For example, *R*-**26d** and *S*-**26g** had reasonable oral exposure in mice with absolute oral bioavailability (*F*) of 6.0 and 8.2%, respectively, despite high clearance in this species. Compound *S*-**26h** also had high clearance in mice, which may account for its low oral bioavailability, but this compound exhibited reasonable absorption (42%) by comparing the blood sample concentration from hepatic portal vein of oral dose versus iv plasma concentration. This compound had moderate volume distribution (4 L/kg) and short half-life in mice (0.6 h). However, in an in vitro human liver microsome assay, this compound was projected to have a systemic clearance of 8.1 mL/min·kg, a much lower value than **13b**, which had a clearance value of 18.1 mL/min·kg. On the basis of in vitro human liver microsomes stability and in vivo mouse absorption data, one can predict this compound should have oral bioavailability of 27% in humans.

## Conclusion

In conclusion, we have successfully designed and synthesized a series of novel and potent monocyclic uracil GnRH antagonists. A novel, convergent cyclization procedure for assembly of the 6-methyl uracil core was developed. Introduction of a small methyl substituent at the $\beta$-position of the N3 side-chain improved the GnRH binding potency by 5−10-fold. We have also demonstrated that introduction of a methyl group of (*R*)-configuration at the α-carbon of the N-3 side-chain gave a modest improvement in binding affinity over the unsubstituted ethylene analogues. This modification enabled us to make uracil compounds without the labile 2-pyridylethyl motif on the basic nitrogen while still maintaining excellent potency against the *h*GnRH receptor. More importantly, these new uracil compounds have lower molecular weights (∼500), which should be beneficial in the discovery of bioavailabile compounds. Further modification in terms of metabolic stability and oral bioavailability will be published in due course.

## Experimental Section

**Competitive Radioligand Binding Assay.** The affinity of the compounds for the human GnRH receptor was determined by competitive displacement of the GnRH receptor radioligand, [$^{125}$I-Tyr$^5$,D-Leu$^6$,NMeLeu$^7$,Pro$^9$-NEt]GnRH. HEK293 cells stably expressing the full-length human GnRH receptor were harvested, resuspended in 5% sucrose, and homogenized using a polytron homogenizer (2 × 15 s). Nuclei debri were removed by centrifugation (3000*g* for 5 min), and the supernatant centrifuged (20 000*g* for 30 min, 4 °C) to collect the membrane fraction. The final membrane preparation was resuspended in binding buffer (10 mM HEPES (pH 7.5), 150 mM NaCl, and 0.1% BSA) and stored at −70 °C. Binding reactions were performed in a Millipore MultiScreen 96-well filtration plate assembly with polyethylenimine-coated GF/C membranes. The reaction was initiated by adding membranes (25 $\mu$g protein in 130 $\mu$L binding buffer) to 50 $\mu$L of [$^{125}$I]-labeled GnRH peptide (∼100 000 cpm), and 20 $\mu$L of competitor at increasing concentrations. The reaction was terminated after 90 min by filtration and washing (2×) with phosphate-buffered saline. Bound radioactivity was measured by removing the filters from the plate, and the filters were counted directly on a gamma counter. $K_i$ values were calculated from competition binding data using nonlinear least squares regression analysis by the Prism software package (GraphPad Software) with the Cheng−Prusoff equation.

**$Ca^{2+}$ Influx Assay. Inhibition of GnRH-Stimulated $Ca^{2+}$ Flux.** Functional activity of the compounds for the human GnRH receptor was determined by inhibition of GnRH-stimulated $Ca^{2+}$ flux. RBL cells stably expressing the full-length human GnRH receptor were seeded into 96-well, black-wall clear bottom plates (Corning) at a density of 50 000 cells/well and the cells allowed to attach overnight. The attached cells were then loaded with the $Ca^{2+}$ sensitive dye, Fluo-4 (Molecular Probes), by incubation in loading medium [(DMEM with 20 mM Hepes, 10% FBS, 2 $\mu$M Fluo-4, 0.02% pluronic acid (Molecular Probes), and 2.5 mM probenecid (Sigma)] for 1 h at 37 °C. The cells were then washed three times with assay buffer (Hanks balanced salt, 20 mM Hepes, 2.5 mM probenecid). Compounds at increasing concentrations in assay buffer were preincubated with the cells for 1 min prior to stimulation with GnRH (5 nM). Measurement of fluorescence due to GnRH-stimulated $Ca^{2+}$ flux was performed according to manufacturer's instructions on a FLIPR system (Molecular Devices, FLIPR$^{384}$ system). $IC_{50}$ values for the inhibition of GnRH-stimulated $Ca^{2+}$ flux were calculated using the Prism software package (GraphPad Software) with a "sigmoidal dose−response (variable slope)" option for curve fitting.

**Chemistry.** Elemental analysis results are indicated by atom symbols and are within 0.4% of theoretical values except where indicated. $^1$H NMR spectra were recorded on a Varian Spectrometer (Mercury 300 Hz) using TMS as the internal standard and $CDCl_3$ as solvent except where indicated. Final products were purified by Gilson preparatory HPLC system, which was connected to a mass spectrometer and fraction collector. The fraction collector was triggered by the desired mass. All final compounds after purification were reanalyzed by reverse phase HPLC-MS system (HP-4500 series with APCI mode for mass detection) and determined to be at least 98% pure based on two UV absorbance wavelengths (220 nM, 254 nM) and total ion current (TIC) monitoring from the mass spectrometer.

**3-Allyl-6-methyluracil (9).** To the allylic urea (25 g, 0.25 mol) in anhydrous ethanol (10 mL) were added ethyl acetoacetate (31.86 mL, 0.25 mol) and 10 drops of concentrated HCl solution. The mixture was stored at rt for 12 days in a desiccator and then concentrated and redissolved in MeOH (200 mL). KOH (22.5 g, 0.40 mol) was added and the solution refluxed for 1 h, acidified slowly by 6 N HCl. The resulting precipitates were collected by filtration. The solid was washed with water and dried under vacuum at 50 °C for 1 day to give 2.7 g of white solid (**9**, 6.5%). $^1$H NMR ($CDCl_3$): $\delta$ 2.16 (s, 3H), 4.52 (d, $J$ = 5.3 Hz, 2H), 5.18 (d, $J$ = 10.2 Hz, 1H), 5.23 (d, $J$ = 18.2 Hz, 1H), 5.60 (s, 1H), 5.82−5.93 (m, 1H), 10.30 (s, 1H). Anal. ($C_8H_{10}N_2O_2$) C, H, N.

**1-(2,6-Difluorobenzyl)-3-allyl-6-methyluracil (10).** To **9** (2.6 g, 15.7 mmol) in DMF (20 mL) was added tetrabutylammonium fluoride (1M in THF, 25 mmol, 25 mL), followed by addition of 2,6-difluorobenzyl bromide (4.14 g, 20 mmol). The mixture was stirred at r.t for 2 days and then concentrated. The crude product was partitioned between ethyl acetate (200 mL) and water (100 mL). The organic layer was separated, dried over $Na_2SO_4$, and purified by column chromatography with silica gel (ethyl acetate/hexane) to recover the starting material (0.39 g) and the desired product (**10**, 2.7 g, 59%). $^1$H NMR ($CDCl_3$): $\delta$ 2.26 (s, 3H), 4.52−4.55 (m, 2H), 5.18(s, 2H), 5.13−5.23 (m, 2H), 5.63 (s, 1H), 5.81−5.92 (m, 1H), 6.86−6.95 (m, 2H), 7.23−7.33 (m, 1H). MS (CI) *m*/*z* 293.1 ($MH^+$). Anal. ($C_{15}H_{14}N_2O_2$) C, H, N.

**1-(2,6-Difluorobenzyl)-3-(2-oxoethyl)-5-bromo-6-methyluracil (11).** To **10** (1.46 g, 5 mmol) in THF/$H_2O$ (20 mL/10 mL) were added $OsO_4$ (200 mg, 0.79 mmol) and $NaIO_4$ (3.2 g, 15 mmol). After the solution was stirred at rt for 2 h, additional $NaIO_4$ (1 g, 4.6 mmol) was added and stirred for another 1 h, followed by the addition of ethyl acetate (200 mL) and water (100 mL). The organic layer was separated and dried to yield the crude aldehyde (1.5 g). MS: 372.9/374.9 ($MH^+$).

To the crude aldehyde (1.5 g, 5.1 mmol) in acetic acid (20 mL) was added bromine (0.28 mL, 5.5 mmol) in acetic acid (5 mL) dropwise over 10 min. The mixture was stirred at rt for 1 h and then concentrated and resuspended in ethyl acetate (200 mL). The organic layer was washed with water (2 × 100 mL), dried, concentrated, and purified by column chromatography with silica gel to give the compound **11** as a white solid (1.1 g, 58%). $^1$H NMR ($CDCl_3$): $\delta$ 2.55 (s, 3H), 4.87 (s, 2H), 5.33 (s, 2H), 6.90−6.96 (m, 2H), 7.26−7.33 (m, 1H), 9.59 (s, 1H). MS (CI) *m*/*z* 373.0/375.0 ($MH^+$).

**1-(2,6-Difluorobenzyl)-3-{2-[*N*-methyl-*N*-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5-bromo-6-methyluracil (12).** To a mixture of the aldehyde **11** (6.71 g, 18.0 mmol) and *N*-methyl-2-(2-pyridyl)ethylamine (2.5 g, 18.3 mmol) in 1,2-dichloroethane (100 mL) was added $NaBH(OAc)_3$ (7.6 g, 36 mmol) in 4 portions within 10 min. The mixture was stirred at rt for 2 h and then diluted with water (100 mL) and chloroform (200 mL) with stirring for 5 min. The organic layer was separated, dried over $Na_2SO_4$, and concentrated to yield a brown oil, which was purified by silica gel chromatography using the $CHCl_3$/MeOH system to yield the desired product **12** (6.5 g, 73%). $^1$H NMR ($CDCl_3$): $\delta$ 2.39 (s, 3H), 2.50 (s, 3H), 2.70 (t, $J$ = 7.5 Hz, 2H), 2.78−2.95 (m, 4H), 4.14 (t, $J$ = 7.5 Hz, 2H), 5.32 (s, 2H), 6.69−6.87 (m, 2H), 7.06−7.13 (m, 1H), 7.20 (d, $J$ = 7.5 Hz, 1H), 7.25−7.31 (m, 1H), 7.58 (dt, $J$ = 7.5, 1.8 Hz, 1H), 8.50 (d, $J$ = 4.5 Hz, 1H). MS (CI) *m*/*z* 493.1/495.1 ($MH^+$).

**1-(2,6-Difluorobenzyl)-3-{2-[*N*-methyl-*N*-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5-phenyl-6-methyl-uracil (13a). Procedure A**: To a sealable tube containing **12** (49.3 mg, 0.1 mmol) were added phenylboronic acid (18 mg, 0.15 mmol) and $K_2CO_3$ (47.4 mg, 0.3 mmol), followed by addition of toluene (5 mL) and water (2 mL). The slurry was degassed by $N_2$ for 10 min, and then $Pd(Ph_3P)_4$ (12 mg, 0.01 mmol) was added. The tube was sealed and heated at 90 °C overnight. After being cooled to rt, the organic layer was separated and concentrated. The crude product was purified by prep TLC plate ($CHCl_3$/MeOH/$NH_4OH$ = 850/150/2) to give **13a** (24 mg, 49%). $^1$H NMR ($CDCl_3$): $\delta$ 2.14 (s, 3H), 2.41 (s, 3H), 2.72−2.78 (m, 2H), 2.84−2.93 (m, 4H), 4.15 (t, $J$ = 7.2 Hz, 2H), 5.29 (s, 2H), 6.90 (t, $J$ = 8.1 Hz, 2H), 7.06−7.41 (m, 8H), 7.56 (dt, $J$ = 1.8 and 7.9 Hz, 1H), 8.48 (d, $J$ = 4.4 Hz, 1H). MS (CI) *m*/*z* 491.1 ($MH^+$). Anal. ($C_{28}H_{28}F_2N_4O_2$·0.4MeOH) C, H, N.

**1-(2,6-Difluorobenzyl)-3-{2-[*N*-methyl-*N*-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5-(3-methoxyphenyl)-6-methyluracil di-trifluoroacetate (13b).** To **12** (49.3 mg, 0.1 mmol) in toluene/$H_2O$ (3 mL/2 mL) in a sealable tube were added 3-methoxyphenylboronic acid (30.2 mg, 0.2 mmol) and $K_2CO_3$ (47.4 mg, 0.3 mmol). The mixture was degassed with $N_2$ for 10 min, and then Pd $(PPh_3)_4$ (11 mg, 0.01 mmol) was added. The mixture was sealed and stirred vigorously at 90 °C for 14 h. After being cooled to rt, the aqueous layer was removed and the organic layer was concentrated and purified by Prep LC-MS to obtain the desired product **13b** (45 mg, 60%) as TFA salt. $^1$H NMR ($CDCl_3$): $\delta$ 2.10 (s, 3H), 3.00 (s, 3H), 3.45−3.78 (m, 6H), 3.80 (s, 3H), 4.30 (br s, 2H), 5.25 (s, 2H), 6.81−6.83 (m, 2H), 6.88−6.95 (m, 3H), 7.24−7.35 (m, 2H), 7.60 (m, 1H), 7.89 (d, $J$ = 7.0 Hz, 1H), 8.13 (t, $J$ = 8.0 Hz, 1H), 8.62 (s, 1H). HRMS (FAB) *m*/*z* calcd for $C_{29}H_{30}F_2N_4O_3$ ($MH^+$) 521.2364, found 521.2369. Anal. ($C_{29}H_{30}F_2N_4O_3$·2TFA·$H_2O$) C, H, N.

**1-(2,6-Difluorobenzyl)-3-{2-[*N*-methyl-*N*-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5-(3,4-methylenedioxy-phenyl)-6-methyl-uracil (13c).** Prepared according to Procedure A, 37 mg, 70%. $^1$H NMR ($CDCl_3$): $\delta$ 2.15 (s, 3H), 2.40 (s, 3H), 2.71−2.77 (m, 2H), 2.84−2.92 (m, 4H), 4.13 (t, $J$ = 7.0 Hz, 2H), 5.28 (s, 2H), 5.96 (s, 2H), 6.63 (t, $J$ = 7.9 Hz, 2H), 6.81−7.30 (m, 6H), 7.58 (dt, $J$ = 1.8 and 7.9 Hz, 1H), 8.48 (d, $J$ = 4.2 Hz, 1H). HRMS (FAB) *m*/*z* calcd for $C_{29}H_{28}F_2N_4O_4$ ($MH^+$) 535.2157, found 535.2134.

**1-(2,6-Difluorobenzyl)-3-{2-[*N*-methyl-*N*-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5-(3,4-ethylenedioxy-phenyl)-6-methyl-uracil (13d). Procedure B**: To a sealable tube containing **12** (49.3 mg, 0.1 mmol) was added 3,4-ethylenedioxyphenylboronic acid pinacol ester (45 mg, 0.16 mmol), followed by addition of toluene (2 mL), EtOH (0.8 mL), $K_2CO_3$ (0.12 mL of a 2 M solution in water), and $Ba(OH)_2$ (40 $\mu$L of a saturated aqueous solution). The slurry was bubbled with $N_2$ for 10 min, and then $Pd(Ph_3P)_4$ (12 mg, 0.01 mmol) was added. The tube was sealed and heated at 90 °C for 12 h. After being cooled to ambient temperature, the organic layer was separated and concentrated. The crude product was purified by prep TLC plate ($CHCl_3$/MeOH/$NH_4OH$ = 850/150/2) to give **13d** (38 mg, 69%). $^1$H NMR ($CDCl_3$): $\delta$ 2.14 (s, 3H), 2.39 (s, 3H), 2.70−2.75 (m, 2H), 2.78−2.88 (m, 2H), 2.90−2.98 (m, 2H), 4.12 (m, 2H), 4.25 (s, 4H), 5.27 (s, 2H), 6.65−6.70 (m, 2H), 6.85−6.92 (m, 3H), 7.06−7.10 (m, 1H), 7.18−7.29 (m, 2H), 7.56 (dt, $J$ = 2.0 and 7.6 Hz, 1H), 8.48 (d, $J$ = 4.4 Hz, 1H). MS (CI) *m*/*z* 549.2 ($MH^+$). Anal. ($C_{30}H_{30}F_2N_4O_4$) C, H; N: calcd, 10.21; found, 9.29.

**1-(2,6-Difluorobenzyl)-3-{2-[*N*-methyl-*N*-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5-[3,4-(2,2-difluoro-methylenedioxy)-phenyl]-6-methyl-uracil (13e).** Prepared according to Procedure B, 26 mg, 46%. $^1$H NMR ($CDCl_3$): $\delta$ 2.15 (s, 3H), 2.39 (s, 3H), 2.70−2.75 (m, 2H), 2.82−2.91 (m, 4H), 4.13 (t, $J$ = 7.0 Hz, 2H), 5.28 (s, 2H), 6.87−6.93 (m, 3H), 7.05−7.30 (m, 5H), 7.57 (dt, $J$ = 1.8 and 7.9 Hz, 1H), 8.48−8.50 (m, 1H). MS (CI) *m*/*z* 571.0 ($MH^+$). Anal. ($C_{29}H_{26}F_4N_4O_4$) C, H, N.

**1-(2,6-Difluorobenzyl)-3-{2-[*N*-methyl-*N*-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5-(4-methoxy-phenyl)-6-methyl-uracil (13f).** Prepared according to Procedure A, 14 mg, 27%. $^1$H NMR ($CDCl_3$): $\delta$ 2.15 (s, 3H), 2.40 (s, 3H), 2.72−2.77 (m, 2H), 2.83−2.93 (m, 4H), 3.81 (s, 3H), 4.13 (dd, $J$ = 7.0 and 14.1 Hz, 2H), 5.28 (s, 2H), 6.88−6.93 (m, 4H), 7.10−7.26 (m, 5H), 7.56 (dt, $J$ = 1.8 and 7.9 Hz, 1H), 8.48 (d, $J$ = 4.1 Hz, 1H). HRMS (FAB) *m*/*z* calcd for $C_{29}H_{30}F_2N_4O_3$ ($MH^+$) 521.2364, found 521.2339.

**1-(2,6-Difluorobenzyl)-3-{2-[*N*-methyl-*N*-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5-(4-ethoxy-phenyl)- 6-methyl-uracil (13g).** Prepared according to Procedure A, 22 mg, 42%. $^1$H NMR ($CDCl_3$): $\delta$ 1.25 (t, $J$ = 7.0 Hz, 3H), 2.14 (s, 3H), 2.40 (s, 3H), 2.71−2.77 (m, 2H), 2.83−2.92 (m, 4H), 4.03 (q, $J$ = 7.0 Hz, 2H), 4.13 (t, $J$ = 7.0 Hz, 2H), 5.28 (s, 2H), 6.88−6.93 (m, 4H), 7.08−7.29 (m, 5H), 7.56 (dt, $J$ = 1.8 and 7.9 Hz, 1H), 8.48 (d, $J$ = 4.5 Hz, 1H). HRMS (FAB) *m*/*z* calcd for $C_{30}H_{32}F_2N_4O_3$ ($MH^+$) 535.2521, found 535.2494.

**1-(2,6-Difluorobenzyl)-3-{2-[*N*-methyl-*N*-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5-(3,4-dimethoxy-phenyl)-6-methyluracil (13h).** Prepared according to Procedure A, 19 mg, 34%. $^1$H NMR ($CDCl_3$): $\delta$ 2.16 (s, 3H), 2.50 (s, 3H), 2.77−2.83 (m, 2H), 2.93−3.02 (m, 4H), 3.84 (s, 3H), 3.85 (s, 3H), 4.18 (t, $J$ = 7.0 Hz, 2H), 5.28 (s, 2H), 6.71−6.93 (m, 3H), 7.07−7.36 (m, 5H), 7.56 (dt, $J$ = 1.8 and 7.5 Hz, 1H), 8.48 (d, $J$ = 4.0 Hz, 1H). HRMS (FAB) *m*/*z* calcd for $C_{30}H_{32}F_2N_4O_4$ ($MH^+$) 551.2469, found 551.2459.

**1-(2,6-Difluorobenzyl)-3-{2-[*N*-methyl-*N*-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5-(3,4,5-trimethoxy-phenyl)-6-methyluracil (13i).** Prepared according to Procedure A, 45 mg, 77%. $^1$H NMR ($CDCl_3$): $\delta$ 2.15 (s, 3H), 2.40 (s, 3H), 2.72−2.77 (m, 2H), 2.83−2.93 (m, 4H), 3.83 (s, 6H), 3.85 (s, 3H), 4.15 (t, $J$ = 7.0 Hz, 2H), 5.28 (s, 2H), 6.39 (s, 2H), 6.91 (t, $J$ = 8.3 Hz, 2H), 7.07−7.30 (m, 3H), 7.57 (dt, $J$ = 1.8 and 7.9 Hz, 1H), 8.48 (d, $J$ = 4.1 Hz, 1H). Anal. ($C_{31}H_{34}F_2N_4O_5$) C, H, N.

**1-(2,6-Difluorobenzyl)-3-{2-[*N*-methyl-*N*-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5-(3-methoxymethyl-phenyl)- 6-methyluracil (13j).** Prepared according to Procedure B, 24 mg, 45%. $^1$H NMR ($CDCl_3$): $\delta$ 2.13 (s, 3H), 2.43 (s, 3H), 2.75−2.82 (m, 2H), 2.88−3.01 (m, 4H), 3.39 (s, 3H), 4.14−4.18 (m, 2H), 4.42 (d, $J$ = 13.6 Hz, 2H), 5.28 (s, 2H), 6.73−6.93 (m, 3H), 7.08−7.47 (m, 5H), 7.56 (dt, $J$ = 1.8 and 7.9 Hz, 1H), 7.76−7.81 (m, 1H), 8.48 (d, $J$ = 4.1 Hz, 1H). HRMS (FAB) *m*/*z* calcd for $C_{30}H_{32}F_2N_4O_3$ ($MH^+$) 535.2520, found 535.2499.

**1-(2,6-Difluorobenzyl)-3-{2-[*N*-methyl-*N*-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5-(4-methoxymethyl-phenyl)-6-methyluracil (13k).** Prepared according to Procedure B, 13 mg, 25%. $^1$H NMR ($CDCl_3$): $\delta$ 2.14 (s, 3H), 2.40 (s, 3H), 2.72−2.77 (m, 2H), 2.82−2.93 (m, 4H), 3.40 (s, 3H), 4.13 (t, $J$ = 7.0 Hz, 2H), 4.46 (s, 2H), 5.28 (s, 2H), 6.88−6.93 (m, 2H), 7.06−7.37 (m, 7H), 7.56 (dt, $J$ = 1.8 and 7.9 Hz, 1H), 8.48 (d, $J$ = 4.3 Hz, 1H). HRMS (FAB) $m/z$ calcd for $C_{30}H_{32}F_2N_4O_3$ ($MH^+$) 535.2520, found 535.2510.

**1-(2,6-Difluorobenzyl)-3-{2-[*N*-methyl-*N*-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5-(4-methylsulfanyl-phenyl)-6-methyluracil (13l).** Prepared according to Procedure A, 37 mg, 69%. $^1$H NMR ($CDCl_3$): $\delta$ 2.14 (s, 3H), 2.40 (s, 3H), 2.48 (s, 3H), 2.72−2.77 (m, 2H), 2.83−2.93 (m, 4H), 4.13 (t, $J$ = 7.0 Hz, 2H), 5.26 (s, 2H), 6.75−6.93 (m, 3H), 7.07−7.47 (m, 5H), 7.58 (dt, $J$ = 1.8 and 7.8 Hz, 1H), 7.78 (dd, $J$ = 3.5 and 8.3 Hz, 1H), 8.49 (d, $J$ = 4.2 Hz, 1H). HRMS (FAB) $m/z$ calcd for $C_{29}H_{30}F_2N_4O_2S$ ($MH^+$) 537.2136, found 537.2137.

**1-(2,6-Difluorobenzyl)-3-{2-[*N*-methyl-*N*-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5-(*p*-tolyl)-6-methyluracil (13m).** Prepared according to Procedure A, 24 mg, 47%. $^1$H NMR ($CDCl_3$): $\delta$ 2.14 (s, 3H), 2.35 (s, 3H), 2.40 (s, 3H), 2.71−2.77 (m, 2H), 2.83−2.93 (m, 4H), 4.14 (t, $J$ = 7.0 Hz, 2H), 5.28 (s, 2H), 6.90 (t, $J$ = 8.3 Hz, 2H), 7.06−7.30 (m, 7H), 7.56 (dt, $J$ = 1.8 and 7.8 Hz, 1H), 8.48 (d, $J$ = 4.2 Hz, 1H). MS (CI) $m/z$ 505.0 ($MH^+$). Anal. ($C_{29}H_{30}F_2N_4O_2$·0.4MeOH) C, H, N.

**1-(2,6-Difluorobenzyl)-3-{2-[*N*-methyl-*N*-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5-(4-isopropyl-phenyl)-6-methyluracil (13n).** Prepared according to Procedure A, 28 mg, 52%. $^1$H NMR ($CDCl_3$): $\delta$ 1.24 (d, $J$ = 7.0 Hz, 6H), 2.15 (s, 3H), 2.39 (s, 3H), 2.71−2.76 (m, 2H), 2.83−2.93 (m, 5H), 4.13 (t, $J$ = 7.3 Hz, 2H), 5.29 (s, 2H), 6.90 (t, $J$ = 8.3 Hz, 2H), 7.06−7.30 (m, 7H), 7.55 (dt, $J$ = 1.8 and 7.5 Hz, 1H), 8.49 (d, $J$ = 4.8 Hz, 1H). MS (CI) $m/z$ 533.1 ($MH^+$). Anal. ($C_{31}H_{34}F_2N_4O_2$·$H_2O$) C, H, N.

**1-(2,6-Difluorobenzyl)-3-{2-[*N*-methyl-*N*-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5-(3-isopropyl-phenyl)-6-methyluracil (13o).** Prepared according to Procedure A, 22 mg, 41%. $^1$H NMR ($CDCl_3$): $\delta$ 1.25 (d, $J$ = 6.6 Hz, 6H), 2.14 (s, 3H), 2.40 (s, 3H), 2.72−2.78 (m, 2H), 2.84−2.93 (m, 5H), 4.13 (t, $J$ = 7.5 Hz, 2H), 5.29 (s, 2H), 6.91 (t, $J$ = 8.3 Hz, 2H), 6.99−7.33 (m, 7H), 7.56 (dt, $J$ = 1.8 and 7.5 Hz, 1H), 8.49 (d, $J$ = 4.2 Hz, 1H). HRMS (FAB) $m/z$ calcd for $C_{31}H_{34}F_2N_4O_2$ ($MH^+$) 533.2728, found 533.2726.

**1-(2,6-Difluorobenzyl)-3-{2-[*N*-methyl-*N*-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5-(3-chloro-phenyl)-6-methyluracil (13p).** Prepared according to Procedure A, 28 mg, 53%. $^1$H NMR ($CDCl_3$): $\delta$ 2.14 (s, 3H), 2.39 (s, 3H), 2.70−2.75 (m, 2H), 2.83−2.92 (m, 4H), 4.12 (t, $J$ = 7.0 Hz, 2H), 5.29 (s, 2H), 6.91 (t, $J$ = 7.9 Hz, 2H), 7.06−7.33 (m, 7H), 7.56 (dt, $J$ = 1.8 and 7.9 Hz, 1H), 8.49 (d, $J$ = 4.6 Hz, 1H). MS (CI) $m/z$ 524.8 ($MH^+$). Anal. ($C_{28}H_{27}ClF_2N_4O_2$) C, H, N.

**1-(2,6-Difluorobenzyl)-3-{2-[*N*-methyl-*N*-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5-(3-fluoro-phenyl)-6-methyluracil (13q).** Prepared according to Procedure A, 15 mg, 29%. $^1$H NMR ($CDCl_3$): $\delta$ 2.14 (s, 3H), 2.40 (s, 3H), 2.71−2.76 (m, 2H), 2.84−2.93 (m, 4H), 4.13 (t, $J$ = 7.3 Hz, 2H), 5.29 (s, 2H), 6.88−7.39 (m, 9H), 7.58 (dt, $J$ = 1.8 and 7.5 Hz, 1H), 8.48 (d, $J$ = 4.2 Hz, 1H). HRMS (FAB) $m/z$ calcd for $C_{28}H_{27}F_3N_4O_2$ ($MH^+$) 509.2164, found 509.2187.

**1-(2,6-Difluorobenzyl)-3-{2-[*N*-methyl-*N*-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5-(2-fluoro-phenyl)-6-methyluracil Trifluoroacetate (13r).** Prepared according to Procedure A and then purified on Prep LC-MS to give **13r** (35 mg, 56%) as TFA salt. $^1$H NMR ($CDCl_3$): $\delta$ 2.17 (s, 3H), 3.03 (s, 3H), 3.47−3.55 (m, 2H), 3.65−3.76 (m, 4H), 4.27−4.46 (m, 2H), 5.15 (d, $J$ = 15.9 Hz, 1H), 5.37 (d, $J$ = 15.9 Hz, 1H), 6.92 (t, $J$ = 8.3 Hz, 2H), 7.08−7.36 (m, 5H), 7.69 (t, $J$ = 6.6 Hz, 1H), 7.98 (d, $J$ = 7.9 Hz, 1H), 8.23 (t, $J$ = 7.7 Hz, 1H), 8.65 (d, $J$ = 5.2 Hz, 1H). HRMS (FAB) $m/z$ calcd for $C_{28}H_{27}F_3N_4O_2$ ($MH^+$) 509.2164, found 509.2169.

**1-(2,6-Difluorobenzyl)-3-{2-[*N*-methyl-*N*-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5-(2-fluoro-3-methoxy-phenyl)-6-methyluracil (13s).** Prepared according to Procedure A, 33 mg, 62%. $^1$H NMR ($CDCl_3$): $\delta$ 2.15 (s, 3H), 2.46 (s, 3H), 2.61−2.69 (m, 2H), 3.10−3.17 (m, 4H), 3.89 (s, 3H), 4.23−4.28 (m, 2H), 5.29 (s, 2H), 6.84−7.32 (m, 8H), 7.59 (td, $J$ = 6.0 and 1.8 Hz, 1H), 8.48 (d, $J$ = 4.8 Hz, 1H). HRMS (FAB) $m/z$ calcd for $C_{29}H_{29}F_3N_4O_3$ ($MH^+$) 539.2272, found 539.2268.

**1-(2,6-Difluorobenzyl)-3-{2-[*N*-methyl-*N*-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5-(4-vinyl-phenyl)-6-methyluracil (13t).** Prepared according to Procedure A, 24 mg, 46%. $^1$H NMR ($CDCl_3$): $\delta$ 2.15 (s, 3H), 2.40 (s, 3H), 2.71−2.77 (m, 2H), 2.83−2.93 (m, 4H), 4.14 (t, $J$ = 7.2 Hz, 2H), 5.23 (d, $J$ = 11.0 Hz, 1H), 5.29 (s, 2H), 5.76 (d, $J$ = 17.5 Hz, 1H), 6.70 (dd, $J$ = 11.0 and 17.5 Hz, 1H), 6.91 (t, $J$ = 8.3 Hz, 2H), 7.06−7.43 (m, 7H), 7.57 (dt, $J$ = 1.8 and 7.5 Hz, 1H), 8.49 (d, $J$ = 4.7 Hz, 1H). HRMS (FAB) $m/z$ calcd for $C_{30}H_{30}F_2N_4O_2$ ($MH^+$) 517.2415, found 517.2391.

**1-(2,6-Difluorobenzyl)-3-{2-[*N*-methyl-*N*-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5-(naphthalen-2-yl)-6-methyluracil (13u).** Prepared according to Procedure A, 19 mg, 35%. $^1$H NMR ($CDCl_3$): $\delta$ 2.18 (s, 3H), 2.41 (s, 3H), 2.72−2.78 (m, 2H), 2.84−2.95 (m, 4H), 4.17 (t, $J$ = 7.5 Hz, 2H), 5.32 (s, 2H), 6.93 (t, $J$ = 8.1 Hz, 2H), 7.06−7.88 (m, 11H), 8.49 (d, $J$ = 3.9 Hz, 1H). MS (CI) $m/z$ 541.1 ($MH^+$). Anal. ($C_{32}H_{30}F_2N_4O_2$·0.4MeOH) C, H, N.

**1-(2,6-Difluorobenzyl)-3-{2-[*N*-methyl-*N*-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5-(dibenzofuran-3-yl)-6-methyluracil (13v).** Prepared according to Procedure A, 30 mg, 52%. $^1$H NMR ($CDCl_3$): $\delta$ 2.14 (s, 3H), 2.40 (s, 3H), 2.76−2.94 (m, 6H), 4.18 (t, $J$ = 7.0 Hz, 2H), 5.25 (d, $J$ = 16.2 Hz, 1H), 5.46 (d, $J$ = 16.2 Hz, 1H), 6.93 (t, $J$ = 8.3 Hz, 2H), 7.05−7.56 (m, 9H), 7.93 (dd, $J$ = 2.2 and 6.6 Hz, 2H), 8.48 (d, $J$ = 4.1 Hz, 1H). MS (CI) $m/z$ 581.1 ($MH^+$). Anal. ($C_{34}H_{30}F_2N_4O_3$·0.5MeOH) C, H, N.

**1-(2,6-Difluoro-benzyl)-3-(3-oxo-butyryl)-urea (18).** Diketene (12.9 mL, 166.7 mmol, reagent stabilized with $CuSO_4$) was added dropwise over 10 min to a stirred solution of 2,6-difluorobenzyl urea (**15**, 28.18 g, 151.5 mmol) in pyridine (300 mL), cooled at 0 °C (ice/water bath) under $N_2$. The resulting gold-yellow solution was allowed to reach room temperature and stirred for 24 h. The resulting suspension was filtered, the solids were rinsed with diethyl ether and dried under vacuum to give **18** (24.38 g, 60%). $^1$H NMR ($CDCl_3$): $\delta$ 2.12 (s, 3H), 3.55 (s, 2H), 4.45 (d, $J$ = 5.7 Hz, 2H), 7.06−7.15 (m, 2H), 7.35−7.45 (m, 1H), 8.60 (br, 1H), 10.42 (br, 1H). MS (CI) $m/z$ 271.1 ($MH^+$).

**1-(2,6-Difluoro-benzyl)-6-methyl-uracil (16).** The acyl urea obtained above (**18**, 24.3 g, 90.0 mmol) was suspended in glacial acetic acid (180 mL) and the mixture refluxed for 1 h. The resulting solution was then cooled and poured onto ice/water (~1.5 L). The white solids were collected by vacuum filtration and dried in a vacuum oven at 50 °C for 24 h to give **16** (21.3 g, 94%). $^1$H NMR (DMSO-$d_6$): $\delta$ 2.22 (s, 3H), 5.07 (s, 2H), 5.53 (s, 1H), 7.06−7.13 (m, 2H), 7.36−7.42 (m, 1H), 11.24 (s, 1H). MS (CI) $m/z$ 253.0 ($MH^+$).

**5-Bromo-1-(2,6-difluoro-benzyl)-6-methyl-uracil (19).** To a solution of **16** (1.26 g, 5.0 mmol) in acetic acid (20 mL) was added bromine (0.28 mL, 5.5 mmol) in acetic acid (5 mL) dropwise over 10 min. The mixture was stirred at rt for 1 h and then purged with $N_2$ to remove most of the bromine. The resulting solid was collected by filtration and washed with ether (three times) to give compound **19** as a white solid (1.46 g, 88%). $^1$H NMR (DMSO-$d_6$): $\delta$ 2.46 (s, 3H), 5.20 (s, 2H), 7.06−7.12 (m, 2H), 7.36−7.46 (m, 1H), 11.86 (s, 1H). MS (CI) $m/z$ 330.0/332.9 ($MH^+$).

**5-Bromo-1-(2,6-difluorobenzyl)-3-[2-(*S*)-*N*-Boc-amino-propyl]-6-methyluracil (*S*-20).** A solution of *N*-(*tert*-butyloxycarbonyl)-L-alaninol (1.75 g, 10 mmol) in anhydrous THF (15 mL) was treated with 5-bromo-1-(2,6-difluorobenzyl)-6-methyluracil (**19**, 3.31 g, 10 mmol) and triphenylphosphine (3.15 g, 12 mmol) at ambient temperature, and then di-*tert*-butylazo dicarboxylate (2.76 g, 12 mmol) was introduced. The reaction mixture was stirred at ambient temperature for 16

h, and volatiles were evaporated. The residue was partitioned between saturated $NaHCO_3/H_2O$ and EtOAc. The organic layer was dried (sodium sulfate), evaporated, and purified by flash chromatography (silica, 1:2 EtOAc/hexanes) to give compound *S*-**20** (4.69 g, 96.1%). $^1$H NMR ($CDCl_3$): $\delta$ 1.15 (d, 3H, $J$ = 6.3 Hz), 1.36 (s, 9H), 2.51 (s, 3H), 3.86 (d, $J$ = 9.3 Hz, 1H), 4.09−4.15 (m, 2H), 4.75−4.80 (m, 1H), 5.15 (d, $J$ = 15.6 Hz, 1H), 5.46 (d, $J$ = 15.6 Hz, 1H), 6.90 (t, $J$ = 8.2 Hz, 2H), 7.22−7.32 (m, 1H). MS (CI) *m*/*z* 388.0, 390.0 ($MH^+$ − Boc).

**5-Bromo-1-(2,6-difluorobenzyl)-3-[2-(*R*)-*N*-Boc-aminopropyl]-6-methyluracil (*R*-20).** The procedure used for preparation of *S*-**20** was repeated here with *N*-(*tert*-butyloxycarbonyl)-D-alaninol (1.75 g, 10 mmol) and 5-bromo-1-(2,6-difluorobenzyl)-6-methyluracil (**19**, 3.31 g, 10 mmol) as the starting materials to give compound *R*-**20** (4.61 g, 94.2%). $^1$H NMR ($CDCl_3$): $\delta$ 1.15 (d, 3H, $J$ = 6.0 Hz), 1.46 (s, 9H), 2.51 (s, 3H), 3.83 (d, $J$ = 9.3 Hz, 1H), 4.09−4.15 (m, 2H), 4.76−4.81 (m, 1H), 5.15 (d, $J$ = 15.8 Hz, 1H), 5.46 (d, $J$ = 15.8 Hz, 1H), 6.90 (t, $J$ = 8.1 Hz, 2H), 7.22−7.32 (m, 1H). MS (CI) *m*/*z* 388.0, 390.0 ($MH^+$−Boc).

**3-[2-(*R*)-Amino-propyl]-1-(2,6-difluoro-benzyl)-5-(2-fluoro-3-methoxy-phenyl)-6-methyluracil Trifluoroacetate (*R*-24).** To a solution of compound *R*-**20** (1.0 g, 2.05 mmol) in bezene/EtOH/ethylene glycol dimethyl ether (20/2/22 mL) were added 2-fluoro-3-methoxyphenylboronic acid (435 mg, 2.56 mmol) and saturated $Ba(OH)_2$/water (∼0.5 M, 15 mL). The reaction mixture was deoxygenated with $N_2$ for 10 min, tetrakis(triphenylphosphine) palladium (0) (242 mg, 0.21 mmol) was added, and the reaction mixture was heated at 80 °C overnight under the protection of $N_2$. The reaction mixture was partitioned between brine and EtOAc. The organic layer was dried (sodium sulfate), evaporated, and purified by flash chromatography (silica, 40% EtOAc/hexanes) to give compound *R*-**22** (450 mg, 41.2%): MS (CI) *m*/*z* 434.2 ($MH^+$ − Boc).

A solution of *R*-**22** (267 mg, 0.5 mmol) in dichloromethane (2 mL) was added TFA (2 mL), and the reaction mixture was stirred at ambient temperature for 1 h. Volatiles were evaporated, and the residue was partitioned between saturated $NaHCO_3$/water and EtOAc. The organic layer was dried (sodium sulfate) and evaporated to give compound *R*-**24** (241 mg, 97%); a sample of the product was purified by reverse phase HPLC (C-18 column, 15−75% ACN/water) to give a TFA salt: $^1$H NMR ($CDCl_3$): $\delta$ 1.07 and 1.17 (d, $J$ = 6.6 Hz, 3H), 2.13 and 2.14 (s, 3H), 3.86 (s, 3H), 3.81−3.98 (m, 2H), 4.23 and 4.35 (dd, $J$ = 14.0, 9.6 Hz, 1H), 5.12−5.36 (m, 2H), 6.73−7.33 (m, 6H), 7.66 (br s, 2H). MS (CI) *m*/*z* 434.10 ($MH^+$).

**3-(2-(*S*)-Amino-propyl)-1-(2,6-difluoro-benzyl)-5-(2-fluoro-3-methoxy-phenyl)-6-methyl-uracil Trifluoroacetate (*S*)-24.** The procedure used for the preparation of *R*-**24** was repeated here with *S*-**20** (978 mg, 2 mmol) as the starting materials to give compound *S*-**24** in two steps (390 mg, 45%); a sample was purified on HPLC as the TFA salt: $^1$H NMR ($CDCl_3$): $\delta$ 1.09 and 1.18 (d, $J$ = 6.6 Hz, 3H), 2.13 and 2.14 (s, 3H), 3.86 (s, 3H), 3.80−3.97 (m, 2H), 4.23 and 4.35 (dd, $J$ = 14.0, 9.6 Hz, 1H), 5.11−5.38 (m, 2H), 6.75−7.31 (m, 6H), 7.73 (br s, 2H). MS (CI) *m*/*z* 434.10 ($MH^+$).

**General Procedure C for the Preparation of Compounds 26.** To compound **24** (216 mg, 0.5 mmol) in dichloroethane (10 mL) was added a carbonyl compound (0.6 mmol), and the mixture was stirred at room temperature for 10 min. Then $NaBH(OAc)_3$ (255 mg, 1.2 mmol) was added, and the mixture was further stirred for 10 h and then diluted with DCM, washed with water, dried (sodium sulfate), and evaporated. The crude product was purified by flash chromatography (silica, 20 to 50% EtOAc/hexanes). Alternately, the crude product was purified by Prep LC-MS to give the desired compound as a TFA salt.

**Procedure D.** The free base was converted to the hydrochloride salt by dissolution in dichloromethane and addition of 1 N HCl solution in ether, followed by removal of the solvent under vacuum to provide the HCl salt as amorphous solid.

**1-(2,6-Difluorobenzyl)-3-[2-(*S*)-benzylaminopropyl]-5-(2-fluoro-3-methoxyphenyl)-6-methyluracil Trifluoroacetate (*S*-26a).** Prepared according to Procedure C as the TFA salt, 203 mg, 56%. $^1$H NMR ($CDCl_3$): $\delta$ 1.25 and 1.27 (d, $J$ = 6.4 Hz, 3H), 2.13 and 2.14 (s, 3H), 3.39 and 3.48 (m, 1H), 3.87 and 3.88 (s, 3H), 3.96 (d, $J$ = 9.6 Hz, 1H), 4.02 (m, 1H), 4.15 and 4.16 (d, $J$ = 14.0 Hz, 1H), 4.31 (m, 1H), 5.10 and 5.22 (d, $J$ = 16.0 Hz, 1H), 5.25 and 5.28 (d, $J$ = 16.0 Hz, 1H), 6.78 (m, 1H), 6.88 (t, $J$ = 8.4 Hz, 2H), 6.96 (m, 1H), 7.09 (m, 1H), 7.22−7.36 (m, 6H). MS (CI) *m*/*z* 524.1 ($MH^+$). Anal. ($C_{29}H_{28}F_3N_3O_3$·TFA) C, H, N.

**1-(2,6-Difluorobenzyl)-3-[2-(*R*)-2-pyridylmethylaminopropyl]-5-(2-fluoro-3-methoxyphenyl)-6-methyluracil Dihydrochloride (*R*-26b). Procedure E**: A solution of *R*-**24** (216 mg, 0.5 mmol) in MeOH (5 mL) was added 2-pyridinecarboxyaldehyde (80 mg, 0.75 mmol), and the reaction mixture was stirred at ambient temperature for 10 h. $NaBH_4$ (56 mg, 1.5 mmol) was added, and the reaction mixture was kept at ambient temperature for 10 min. Volatiles were evaporated, and the residue was partitioned between saturated $NaHCO_3$/water and dichloromethane. The organic layer was dried (sodium sulfate), evaporated, and purified by flash chromatography to give *R*-**26b**, which was converted to the HCl salt as described in procedure D (160 mg, 46%). $^1$H NMR (DMSO-$d_6$): $\delta$ 1.25 and 1.26 (d, $J$ = 6.8 Hz, 3H), 2.19 (s, 3H), 3.58 (m, 1H), 3.95 (s, 3H), 4.07 (m, 1H), 4.23 (dd, $J$ = 6.4, 14.0 Hz, 1H), 4.35 (d, $J$ = 14.0 Hz, 1H), 4.42 (d, $J$ = 14.0 Hz, 1H), 5.25 (s, 2H), 6.76 (m, 1H), 7.12 (t, $J$ = 8.4 Hz, 2H), 7.17 (m, 1H), 7.19 (m, 1H), 7.42 (m, 1H), 7.44 (dd, $J$ = 4.4, 8.2 Hz, 1H), 7.48 (d, $J$ = 7.6 Hz, 1H), 7.89 (dt, $J$ = 2.0, 8.0 Hz, 1H), 8.60 (d, $J$ = 4.4 Hz, 1H), 9.17 (br s, 2H), 9.98 (br s, 1H). MS (CI) *m*/*z* 525.2 ($MH^+$). Anal. ($C_{28}H_{27}F_3N_4O_3$·2HCl) C, H, N.

**1-(2,6-Difluorobenzyl)-3-{2-(*R*)-[(2-thiazolylmethyl)-amino]propyl}-5-(2-fluoro-3-methoxyphenyl)-6-methyluracil Hydrochloride (*R*-26d).** Prepared according to Procedure E and D to give *R*-**26d** as the HCl salt (201 mg, 71%). $^1$H NMR (DMSO-$d_6$): $\delta$ 1.25 and 1.27 (d, $J$ = 5.1 Hz, 3H), 2.19 (s, 3H), 3.60−3.65 (m, 1H), 3.86 (s, 3H), 4.02−4.09 (m, 1H), 4.23 (dd, $J$ = 10.5, 5.1 Hz, 1H), 4.62−4.67 (m, 2H), 5.25 (s, 2H), 6.74−6.78 (m, 1H), 7.11 (t, $J$ = 6.3 Hz, 2H), 7.18−7.42 (m, 3H), 7.87 (dd, $J$ = 0.6, 2.4 Hz, 1H), 7.91 (d, $J$ = 2.4 Hz, 1H), 9.39 (br s, 1H), 9.54 (br s, 1H). MS (CI) *m*/*z* 531.2 ($MH^+$). Anal. ($C_{26}H_{25}F_3N_4O_3S$·HCl·$H_2O$) C, H, N.

**1-(2,6-Difluorobenzyl)-3-{2-(*S*)-[(3-methyl-2-thiophenylmethyl)amino]propyl}-5-(2-fluoro-3-methoxyphenyl)-6-methyluracil Hydrochloride (*S*-26e).** Prepared according to Procedure E and D to give *S*-**26e** as the HCl salt (151 mg, 52%). $^1$H NMR (DMSO-$d_6$): $\delta$ 1.23−1.28 (m, 3H), 2.20 (s, 3H), 2.23 (s, 3H), 3.60−3.65 (m, 1H), 3.86 (s, 3H), 4.01−4.07 (m, 1H), 4.15−4.21 (m, 1H), 4.33−4.38 (m, 2H), 5.26 (s, 2H), 6.77−6.81 (m, 1H), 6.93 (d, $J$ = 3.6 Hz, 1H), 7.11 (t, $J$ = 6.3 Hz, 2H), 7.19−7.54 (m, 4H), 8.98 (brs, 2H). MS (CI) *m*/*z* 502.2 ($MH^+$). Anal. ($C_{28}H_{28}F_3N_3O_3S$·HCl) C, H, N.

**1-(2,6-Difluorobenzyl)-3-{2-(*S*)-[(5-ethyl-2-furanylmethyl)amino]propyl}-5-(2-fluoro-3-methoxyphenyl)-6-methyluracil Hydrochloride (*S*-26f).** Prepared according to Procedure E and D to give *S*-**26f** as the HCl salt (237 mg, 82%). $^1$H NMR (DMSO-$d_6$): $\delta$ 1.17 (t, $J$ = 6.7 Hz, 3H), 1.20 and 1.21 (d, $J$ = 6.7 Hz, 3H), 2.19 (s, 3H), 2.61 (q, $J$ = 6.7 Hz, 2H), 3.50−3.55 (m, 1H), 3.86 (s, 3H), 4.03−4.08 (m, 1H), 4.14−4.23 (m, 3H), 5.25 (s, 2H), 6.12 (d, $J$ = 2.4 Hz, 1H), 6.45 (d, $J$ = 2.4 Hz, 1H), 6.73−6.79 (m, 1H), 7.11 (t, $J$ = 6.3 Hz, 2H), 7.18−7.42 (m, 3H), 9.18 (br s, 2H). MS (CI) *m*/*z* 542.3 ($MH^+$). Anal. ($C_{29}H_{30}F_3N_3O_4$·HCl) C, H, N.

**1-(2,6-Difluorobenzyl)-3-[2-(*S*)−(1-ethylpropyl)aminopropyl]-5-(2-fluoro-3-methoxyphenyl)-6-methyluracil Hydrochloride (*S*-26g).** Prepared according to Procedure C and D to give *S*-**26g** as the HCl salt (214 mg, 79%). $^1$H NMR (DMSO-$d_6$): $\delta$ 0.89 (t, $J$ = 7.6 Hz, 6H), 1.15 and 1.16 (t, $J$ = 6.0 Hz, 3H), 1.24−1.28 (m, 1H), 1.35−1.39 (m, 1H), 1.43−1.46 (m, 1H), 1.62−1.66 (m, 1H), 2.17 and 2.18 (s, 3H), 3.33 (m, 1H), 3.53−3.58 (m, 1H), 3.86 (s, 3H), 4.03−4.08 (m, 1H), 4.16 (dd, $J$ = 9.9, 6.0 Hz, 1H), 5.28 (s, 2H), 6.74−6.79 (m, 1H), 7.12 (t, $J$ = 8.0 Hz, 2H), 7.18−7.42 (m, 3H), 8.60 (br s, 1H), 8.83 (br s, 1H). MS (CI) *m*/*z* 504.2 ($MH^+$). Anal. ($C_{27}H_{32}F_3N_3O_3$·HCl·0.5$Et_2O$) C, H, N.

**1-(2,6-Difluorobenzyl)-3-[2-(*S*)-cyclopentylaminopropyl]-5-(2-fluoro-3-methoxyphenyl)-6-methyluracil Hydrochloride (*S*-26h).** Prepared according to Procedure C and D to give *S*-**26h** as the HCl salt (226 mg, 84%). $^1$H NMR (DMSO-$d_6$): $\delta$ 1.17 (d, $J$ = 6.8 Hz, 3H), 1.51 (brs, 2H), 1.62 (brs, 2H), 1.71 (br s, 2H), 1.92 (br s, 2H), 2.17 (s, 3H), 3.40−3.45 (m, 1H), 3.57−3.64 (m, 1H), 3.85 (s, 3H), 4.11 (br s, 1H), 4.13 (br s, 1H), 5.27 (s, 2H), 6.74−6.79 (m, 1H), 7.12 (t, $J$ = 8.4 Hz, 2H), 7.17 (d, $J$ = 5.2 Hz, 1H), 7.19−7.42 (m, 2H), 9.01 (br s, 1H), 9.10 (br s, 1H). MS (CI) $m/z$ 502.2 ($MH^+$). Anal. ($C_{27}H_{30}F_3N_3O_3$·HCl) C, H, N.

**1-(2,6-Difluorobenzyl)-3-[2-(*S*)-cyclobutylaminopropyl]-5-(2-fluoro-3-methoxyphenyl)-6-methyluracil Hydrochloride (*S*-26i).** Prepared according to Procedure C and D to give *S*-**26i** as the HCl salt (202 mg, 77%). $^1$H NMR (DMSO-$d_6$): $\delta$ 1.12 (d, $J$ = 5.1 Hz, 3H), 1.72−1.79 (m, 2H), 2.18 (s, 3H), 2.20−2.26 (m, 4H), 3.37−3.42 (m, 1H), 3.77−3.83 (m, 1H), 3.86 (s, 3H), 4.03−4.08 (m, 2H), 5.27 (s, 2H), 6.75−6.80 (m, 1H), 7.12 (t, $J$ = 6.3 Hz, 2H), 7.18−7.42 (m, 3H), 9.13 (br s, 2H). MS (CI) $m/z$ 488.3 ($MH^+$). Anal. ($C_{26}H_{28}F_3N_3O_3$·HCl·0.5$H_2O$) C, H, N.

**1-*N*-Boc-amino-propan-2-ol (*S*-28).** To a solution of (*S*)-(+)-1-amino-2-propanol (5.18 g, 67 mmol) and TEA (4.14 g, 70 mmol) in DCM (80 mL) was added di-*tert*-butyl dicarbonate (15.5 g, 69 mmol), and the reaction mixture was stirred at 0 °C under $N_2$ for 1 h. The reaction mixture was partitioned between saturated $NaHCO_3$/$H_2O$ and DCM and washed with brine. The organic layer was dried (sodium sulfate) and evaporated to give *R*-**28** (11.6 g, 99%). $^1$H NMR ($CDCl_3$): $\delta$ 1.18 (d, 3H, $J$ = 6.3 Hz), 1.45 (s, 9H), 2.95−3.05 (m, 1H), 3.24−3.29 (m, 1H), 3.87−3.93 (m, 1H), 5.30 (s, 1H).

**3-[2-*N*-Boc-amino-1-(*R*)-methyl-ethyl]-5-bromo-1-(2,6-difluoro-benzyl)-6-methyluracil (*R*-29).** A solution of 1-*N*-Boc-amino-propan-2-ol (*S*-**28**, 1.92 g, 11 mmol) in anhydrous THF (30 mL) was treated with 5-bromo-1-(2,6-difluorobenzyl)-6-methyluracil (**19**, 3.31 g, 10.5 mmol) and triphenylphosphine (4.11 g, 15.7 mmol) at ambient temperature, and then diethyl azodicarboxylate (2.73 g, 15.7 mmol) was added dropwise. The reaction mixture was stirred at ambient temperature for 12 h, and volatiles were evaporated. The residue was partitioned between saturated $NaHCO_3$/$H_2O$ and EtOAc. The organic layer was dried (sodium sulfate), evaporated, and purified by flash chromatography (silica, 1:9 EtOAc/hexanes) to give compound *R*-**29** (3.58 g, 70%). $^1$H NMR (DMSO-$d_6$): $\delta$ 1.21 (d, 3H, $J$ = 6.9 Hz), 1.29 (s, 9H), 2.46 (s, 3H), 3.14−3.19 (m, 1H), 3.36−3.41 (m, 1H), 4.87−4.93 (m, 1H), 5.14 (d, $J$ = 16.5 Hz, 1H), 5.28 (d, $J$ = 16.5 Hz, 1H), 7.04−7.09 (m, 2H), 7.33−7.43 (m, 1H), 8.97 (br s, 1H). MS (CI) $m/z$ 388.0, 390.0 ($MH^+$−Boc).

**3-[2-*N*-Boc-amino-1-(*R*)-methyl-ethyl]-1-(2,6-difluoro-benzyl)-5-(2-fluoro-3-methoxy-phenyl)-6-methyluracil (*R*-31).** To a solution of compound *R*-**29** (2.83 g, 5.8 mmol) in toluene/water (20/10 mL) were added 2-fluoro-3-methoxyphenylboronic acid (1.87 g, 11 mmol) and $K_2CO_3$ (1.60 g, 11.6 mmol). The reaction mixture was deoxygenated with $N_2$ for 10 min, tetrakis(triphenylphosine)palladium(0) (693 mg, 0.6 mmol) was added, and the reaction mixture was heated at 90 °C for 12 h in a sealed flask under the protection of $N_2$. The reaction mixture was partitioned between brine and EtOAc. The organic layer was dried (sodium sulfate), evaporated, and purified by flash chromatography (silica, 10 to 40% EtOAc/hexanes) to give compound *R*-**31** (1.64 g, 53%). $^1$H NMR ($CDCl_3$): $\delta$ 1.41 (s, 9H), 1.59 (s, 3H), 2.11 (s, 3H), 3.28−3.37 (m, 1H), 3.88 (s, 3H), 4.96−5.01 (m, 1H), 5.20−5.31 (m, 3H), 6.79−7.28 (m, 6H), 7.68 (br s, 1H). MS (CI) $m/z$ 434.1 ($MH^+$ − Boc).

**3-[2-Amino-1-(*R*)-methyl-ethyl]-1-(2,6-difluoro-benzyl)-5-(2-fluoro-3-methoxy-phenyl)-6-methyluracil (*R*-33).** To a solution of *R*-**31** (1.12 g, 2.1 mmol) in dichloromethane (20 mL) was added TFA (20 mL), and the reaction mixture was stirred at ambient temperature for 2 h. Volatiles were evaporated, and the residue was partitioned between saturated $NaHCO_3$/water and EtOAc. The organic layer was dried (sodium sulfate) and evaporated, to give compound *R*-**33** (863 mg, 95%). $^1$H NMR ($CDCl_3$): $\delta$ 1.43 (d, $J$ = 6.9 Hz, 3H), 2.11 (s, 3H), 2.97 (dd, $J$ = 12.9, 6.0 Hz, 1H), 3.36 (dt, $J$ = 13.5, 5.4 Hz, 1H), 3.88 (s, 3H), 5.00−5.08 (m, 1H), 5.23−5.39 (m, 2H), 6.78−7.30 (m, 6H). MS (CI) $m/z$ 434.10 ($MH^+$).

**1-(2,6-Difluorobenzyl)-3-[1-(*R*)-methyl-2-(2-methyl-benzylamino)-ethyl]-5-(2-fluoro-3-methoxyphenyl)-6-methyluracil Hydrochloride (*R*-35b).** To a solution of *R*-**33** (650 mg, 1.5 mmol) in MeOH (15 mL) was added *o*-tolualdehyde (1.80 g, 15 mmol), and the mixture stirred for 1 h at rt. $NaBH_4$ (283 mg, 7.5 mmol) was added and reaction mixture stirred at ambient temperature for 15 min, concentrated, and purified by flash chromatography (20% EtOAc/hexanes). The crude material was treated according to procedure D to yield a white solid *R*-**35b** as the HCl salt (232 mg, 27%). $^1$H NMR ($CDCl_3$): $\delta$ 1.41 (d, $J$ = 6.2 Hz, 3H), 2.07 (s, 3H), 2.28 and 2.29 (s, 3H), 2.94 (t, $J$ = 10.4 Hz, 1H), 3.84 (s, 3H), 3.93 (m, 1H), 4.06 (d, $J$ = 13.0 Hz, 1H), 4.23 (d, $J$ = 13.0 Hz, 1H), 5.14 and 5.15 (d, $J$ = 16.4 Hz, 1H), 5.25 and 5.32 (d, $J$ = 16.4 Hz, 1H), 5.45 (m, 1H), 6.73 (m, 1H), 6.87 (m, 2H), 6.92−7.30 (m, 7H). MS (CI) $m/z$ 538.2 ($MH^+$). Anal. ($C_{30}H_{30}F_3N_3O_3$·HCl) C, H, N.

**1-(2,6-Difluoro-benzyl)-5-(2-fluoro-3-methoxy-phenyl)-3-[1-(*R*)-methyl-2-(2-methoxy-benzylamino)-ethyl]-6-methyluracil Hydrochloride (*R*-35c).** The procedure used for the preparation of *R*-**35b** was repeated here with *R*-**33** (650 mg, 1.5 mmol) and *o*-anisaldehyde (2.04 g, 15 mmol) to give *R*-**35c** as a white solid, which was treated under procedure D to yield the product as the HCl salt (274 mg, 31%). $^1$H NMR ($CDCl_3$): $\delta$ 1.60 and 1.61 (d, $J$ = 6.4 Hz, 3H), 2.08 (s, 3H), 2.84 and 2.94 (m, 1H), 3.58 and 3.67 (s, 3H), 3.62 (m, 1H), 3.87 and 3.90 (s, 3H), 3.87 (m, 1H), 4.41 and 4.43 (m, 1H), 5.16 and 5.17 (d, $J$ = 16.0 Hz, 1H), 5.26 and 5.27 (d, $J$ = 16.0 Hz, 1H), 5.43−5.47 (m, 1H), 6.65−7.37 (m, 10H), 8.62 and 8.76 (br s, 1H), 10.7 and 10.8 (br s, 1H). MS (CI) $m/z$ 554.2 ($MH^+$). Anal. ($C_{30}H_{30}F_3N_3O_4$·HCl) C, H, N.

**Human Liver Microsomes Stability Studies.** Microsomal incubations were performed in the presence of an NADPH-generating system composed of 10 mM $MgCl_2$, 1 mM NADP+, 46 mM glucose-6-phosphate, and 3 units/mL glucose-6-phosphate dehydrogenase in 70 mM potassium phosphate buffer (pH 7.4); all concentrations are relative to the final incubation volume. The GnRH compounds were added in DMSO to a final concentration of 50 $\mu$M (1% final DMSO concentration). Incubations were conducted at 37 °C in duplicate and were terminated by the addition of 0.1 mL of acetic acid in acetonitrile (6:94; v/v) to each 0.25 mL of incubation volume. Precipitated proteins were removed by centrifugation, and supernatants were analyzed by HPLC with UV detection. Separation of parent compound from putative metabolites was obtained on a Zorbax C8 HPLC column (5 $\mu$m, 150 × 4.6 mm) and a mobile phase gradient from 100% trifluoroacetic acid in water (1:1000; v/v) to 100% trifluoroacetic acid in acetonitrile (1:1000; v/v) in 15 min; injection volumes were 50 $\mu$L. The GnRH compounds were detected at UV absorbance wavelengths of 240 and 255 nm. Testosterone (Sigma Chemical Co., St Louis, MO) was used as a positive control for viability of the microsomal preparation; testosterone assays were performed as described above, and with UV detection at 240 nm.

**Oral Absorption in Mice.** Oral bioavailability studies were conducted in CD-1 mice, and test articles were administered to the mice by oral gavage and intravenous injection (10 mg/kg; $N$ = 3/time point) in water solution. Terminal blood samples were taken at predetermined time points for composite sampling. For in vivo first-pass effect studies, the mice were anesthetized with isoflurane by using either a calibrated vaporizer and induction chamber, anesthetic chamber, or open drop method at the time of sampling. A retro-orbital eye bleed was performed on each mouse so that 300−600 $\mu$L of whole blood was drawn. The mouse was then checked to ensure a deep anesthesia. The mouse was laid on its back and the midline of the stomach opened up. The hepatic portal vein was occluded as near as possible to the liver. A catheter was placed into the hepatic portal vein such that the catheter was pointed toward the intestines. A 300−600 $\mu$L sample was taken from the hepatic portal vein using a 600 $\mu$L tube that contained 1.2−2 mg EDTA/mL of blood. Blood samples from each dosing

route were taken at predetermined time points for pharmacokinetic analysis. All plasma samples were flash frozen in liquid nitrogen within 10 min of sampling and stored in −76 °C or below until analysis. The bioanalytical method applied for the measurement of test articles in plasma along with added internal standard consisted of precipitation with 200 $\mu$L of acetonitrile from 50 $\mu$L of plasma, centrifugation, and recovery the supernatant, which was evaporated in a vacuum and then reconstituted in acetonitrile−water solution before introduction into an LC-MS/MS system for analysis. The lower limit of quantification (LLOQ) for the analytical methods was 5 ng/mL of test article in plasma. All pharmacokinetic parameters were calculated from a noncompartmental model using WinNonlin program version 3.2.

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(14) The crystal structure of **16** was also determined (Figure 3).

(15) The enantiomeric excesses (% ee) of compounds *S*-**29** and *R*-**29** were determined by HPLC, using a long Pirkle Covalent (R,R) WHELK column (25 cm × 4.6 mm), manufactured by REGIS. The samples were eluted with an isocratic 80:20 (v/v) mixture of hexanes and ethanol, respectively. For *S*-**29**, $t_R$ = 11.10 min, ee = >99%. For *R*-**29**, $t_R$ = 9.93 min, ee = >99%.

(16) *R*-**29** was crystallized from slow diffusion of diethyl ether into a dichloromethane solution. Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 204509. Copies of the data can be obtained, free of charge, on application to CCDC,

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(18) On each assay plate a standard antagonist of comparable affinity to those being tested was included as a control for plate-to-plate variability. Overall, $K_i$ values were highly reproducible with an average standard deviation of 45% for replicate $K_i$ determinations. Key compounds were assayed in three to eight independent experiments.
(19) Atropical diastereoisomers were observed by NMR and HPLC for compounds with 2-fluoro or 2-fluoro-3-methoxy analogues.
(20) Two compounds (*R*-**26d** and *S*-**26g**) were selected and tested for their binding activity against over 10 different G-protein-coupled receptors in-house. Neither of them showed more than 30% inhibition against any of these GPCR receptors at 10 $\mu$M concentration.

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