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5-Aryluracils as potent GnRH antagonists—Characterization of atropisomers

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Abstract—Optimization of a series of uracils bearing a 2-fluoro- or 2-chloro-3-methoxyphenyl group at the 5-position resulted in compounds such as **3d** and **3f** with subnanomolar binding affinity at the human GnRH receptor. While the 2-fluoro-3-methoxyphenyl compound **3a** was characterized as a mixture of interchangeable atropisomers, the diastereoisomers of 2-chloro-3-methoxyphenyl analogs were separated. It was found that the a*R*-atropisomer was much more potent than the a*S*-isomer based on the X-ray crystal structure of **3h-II**.

Gonadotropin-releasing hormone (GnRH), also known as luteinizing hormone-releasing hormone (LH-RH), is a linear decapeptide amide originally isolated and characterized from porcine and ovine hypothalami.[1] GnRH exerts its biological activity via the activation of its cell surface G-protein-coupled receptor (GnRH-R)[2] in the pituitary gland to stimulate the secretion of the gonadotropins—luteinizing hormone (LH) and follicle-stimulating hormone (FSH).[3] Several disease conditions, such as endometriosis and prostate cancer, can be treated by the suppression of the pituitary-gonadal axis. Clinical evidence demonstrates that peptidic GnRH-R antagonists directly lower gonadal sex hormone levels alleviating disease symptoms without the concomitant flare effect caused by agonists.[4] An orally bioavailable small molecule not only avoids injection site reactions and doctor's office visits, but also may provide improved control over the degree of pituitary suppression (including rapid withdrawal of therapy), which in turn may provide improved clinical management options in reproductive hormone related diseases.

We have previously described a series of uracils exemplified by **1** (Fig. 1) as potent antagonists of the human gonadotropin-releasing hormone receptor (*h*GnRH-R).[5] Optimization of this series led to the identification of NBI-42902 (**2a**, $K_i = 0.56$ nM),[6] which is potent and selective in vitro,[7] and suppresses serum LH in postmenopausal women after oral administration.[8] While the 2-fluoro substituent at the 5-phenyl group in **2a** is important for high binding affinity, it causes the compound to exist as a pair of atropisomers,[9] due to a slow rotation of the 5-aryl group around the carbon–carbon bond connecting the uracil core.[10] The existence of two atropisomers of **2a** at room temperature in solution is evidenced by HPLC and NMR spectroscopic studies.[11]

Atropisomerism of biaryl and other compounds have been previously encountered during drug discovery programs,[12] and this characteristic may present some challenges due to analytical and manufacturing concerns.[13] Atropisomerism can be eliminated by making interconversion more rapid, in the case of uracil derived GnRH antagonists, it disappears when the 2-fluorine at the 5-phenyl group (**2b**, $K_i = 3.2$ nM)[6] or the 5-methyl group at the uracil core (**2c**, $K_i = 5.3$ nM) is deleted.[14] These changes, however, result in a 6- or 10-fold reduction in binding affinity, respectively. Alternatively, increasing

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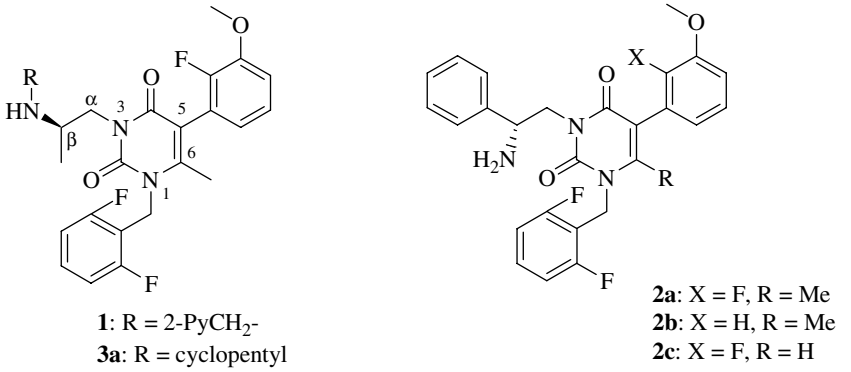
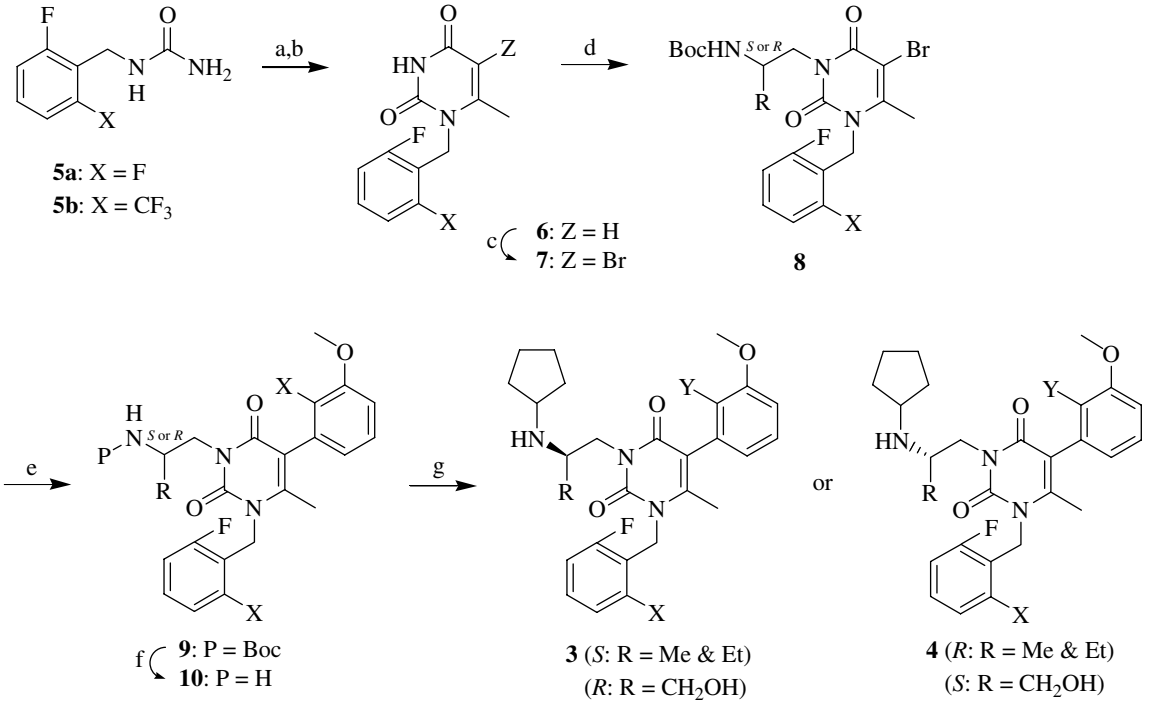


Figure 1. Uracil GnRH-R antagonists.

the rotation barrier can allow the separation of thermally stable atropisomers. In our previous studies, we have found that aryluracils bearing an *N*-alkyl aminoalkyl side chain at the 3-position are potent *h*GnRH-R antagonists.[15] Among them, the 2*S*-(cyclopentylamino)propyl derivative **3a** (Fig. 1) possesses high binding affinity (K_i = 4.5 nM).[16] To investigate the most suitable approach to optimize **3a**, we prepared several structurally-close analogs including thermally stable atropisomers that could be separated.

Compounds **3** and **4** were synthesized from 1-(2,6-difluorobenzyl)-5-bromo-6-methyluracil **7**, which was obtained in three steps from readily available chemicals using a procedure similar to that reported previously (Scheme 1).[17] Thus, coupling reactions of **7** with either (*R*)- or (*S*)-*N*-Boc-aminoalaninol, or its variants, under Mitsunobu conditions (DEAD/Ph_3P in THF) gave the corresponding uracils **8** in about 70% yields, which were then subjected to Suzuki coupling reactions with 2-fluoro- or 2-chloro-3-methoxyphenylboronic acids, catalyzed by palladium to afford the 5-aryluracils **9** in 20–90% yields. Deprotection of **9** with TFA afforded the corresponding free amines **10** in almost quantitative yields, which were alkylated with cyclopentanone in the presence of sodium triacetoxyborohydride to give the desired products **3** or **4** in about 50% yield (Scheme 1). For compound **3b**, an additional hydrogenation step was applied before the final purification.

Compound **3a** was initially synthesized as a potent *h*GnRH-R antagonist, and it possessed a K_i value of 4.5 nM and an IC_{50} of 8.2 nM in inhibition of calcium flux.[15] Compound **3a** existed in solution as a 1:1 mixture. The rotameric property of this compound was identified based on NMR spectra.[18] Fluorine NMR spectrum of **3a** clearly showed two sets of signals with an equal intensity for the 2-fluorine of the 5-phenyl



Scheme 1. Reagents and conditions: (a) urea/aq HCl/heat; (b) diketene/TMSCl/NaI/ACN; (c) Br_2/AcOH; (d) (*S*)- or (*R*)-RCH(NHBoc)CH_2OH/DEAD/Ph_3P/THF (for **3b**, R=$BnOCH_2$); (e) 2-Y-3-$MeOC_6H_3B(OH)_2$/$Pd(PPh_3)_4$/Na_2CO_3/H_2O/dioxane/reflux; (f) TFA; (g) cyclopentanone/$NaBH(OAc)_3$/CH_2Cl_2.

group, and carbon-13 displayed several double peaks, especially for the uracil ring (Table 1). Since there is only one conventional chiral center at the 3-side-chain-derived from the chiral alaninol, the possible existence of atropisomers due to a slow rotation of the carbon–carbon bond between the 5-phenyl group and 6-methyluracil core (Fig. 2) were suspected.[19] Although a pair of peaks for **3a** was observed by HPLC, attempt to separate them was unsuccessful. Rapid interconversion of the two isomers resulted in failure of separation. In order to prove these signals corresponded to the interchangeable rotamers, VT NMR experiments were performed in an attempt to observe coalescence of two sets of signals at an elevated temperature. Fluorine NMR, however, indicated that the temperature for coalescence would be higher than 125 °C in DMSO solution, since two sets of fluorine signals corresponding to the 5-(2-fluoro-3-methoxylphenyl) were still observed at this temperature.

To confirm that the two sets of NMR signals are caused by atropisomerism, we performed HMQC and HMBC NMR experiments. Thus, all the carbons connected with a proton were assigned by a ^{1}H-detected heteronuclear one bond ^{1}H–^{13}C correlation experiment (HMQC). Further information regarding the skeletal structure was sought from multiple bond proton–carbon couplings, which were identified by ^{1}H-detected heteronuclear multiple bond ^{1}H–^{13}C correlation experiment (HMBC). These results are summarized in Tables 1 and 2.

Replacement of one fluorine of the 1-(2,6-difluorobenzyl) moiety with a larger and stronger electron-withdrawing trifluoromethyl group resulted in an about 7-fold increase in binding affinity (**3d**, K_i = 0.61 nM). Switching the

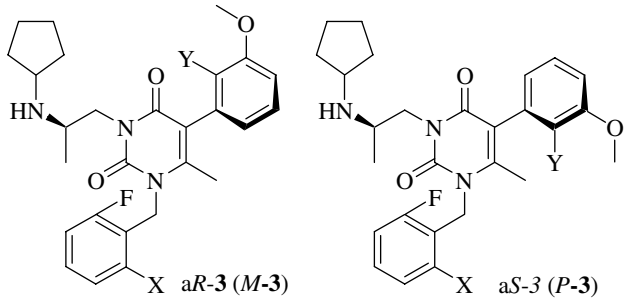
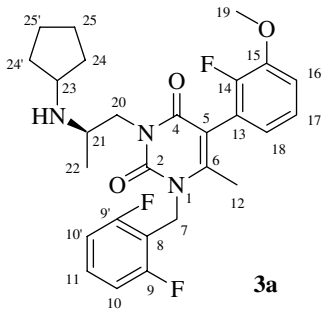


Figure 2. Atropisomers of **3**.

Table 1. ^{1}H, ^{19}F, and ^{13}C NMR assignments of **3a** (in DMSO-d_6)[a]



No.	Proton or [Fluorine]	Carbon
2	—	151.0 and 151.1
4	—	160.5 and 160.6
5	—	106.8 and 106.9
6	—	151.2 and 151.3
7	5.26 (s, 2H)	38.6
8	—	112.1 (t, J = 17.0 Hz)
9 and 9′	[−115.0 (m, 2F)][b]	160.5 (dd, J = 7.5, 246.5 Hz)
10 and 10′	7.12 (m, 2H)	111.9 (m, AA′XX′)
11	7.42 (m, 1H)	130.1 (t, J = 10.7 Hz)
12	2.17 (s, 3H)	17.5
13	—	122.2 (d, J = 13.6 Hz)
14	[−135.3 & 135.5 (m, 1F)][b]	149.5 (d, J = 242.7 Hz)
15	—	147.4 (d, J = 10.7 Hz)
16	7.18 (d, J = 5.2 Hz, 1H)	124.0 (d, J = 3.8 Hz)
17	7.19 (m, 1H)	113.5
18	6.76 (m, 1H)	123.8
19	3.85 (s, 3H)	55.9
20	4.14 (m, 2H)	42.4
21	3.43 (m, 1H)	50.3 and 50.5
22	1.17 (d, J = 5.7 Hz, 3H)	14.5 and 14.6
23	3.62 (m, 1H)	55.5
24 and 24′	H_a: 1.62 (br s, 2H); H_b: 1.92 (br s, 2H)	28.85 and 28.96
25 and 25′	H_a: 1.51 (br s, 2H); H_b: 1.71 (br s, 2H)	23.5

[a] On a Varian Mercury 300.
[b] $CFCl_3$ as an internal standard.

Table 2. $^{2\text{-}4}J_{C-H}$ Correlation from HMBC experiment of **3a** (in DMSO-d_6)

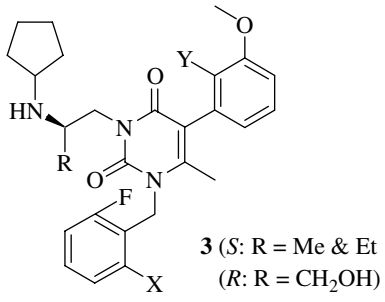
Proton number	HMBC	HMQC
	Long-range correlations to carbon number	$^{1}J_{C-H}$ correlation
H-7a, H-7b	C-2, C-6, C-8, C-9, C-9′	C-7
H-10, H-10′	C-8, C-9	C-10
H-11	C-9, C-9′, C-10, C-10′	C-11
H-12 (3H)	C-5, C-6	C-12
H-16	C-14, C-15	C-16
H-17	C-13	C-17
H-18	C-5, C-14, C-16, C-17	C-18
H-19 (3H)	C-15	C-19
H-20a, H-20b	C-2, C-4, C-21, C-22	C-20
H-21	—	C-21
H-22 (3H)	C-20, C-21	C-22
H-23	—	C-23
H-24a, H-24a′	C-23, C-25, C-25′	C-24
H-24b, H-24b′	C-25, C-25′	C-24
H-25a, H-25a′	C-24, C-24′	C-25
H-25b, H-25b′	C-24, C-24′	C-25

β-methyl of **3d** with a larger ethyl group decreased its binding affinity about 7-fold (**3e**, K_i = 4.2 nM, Table 3). The removal of the 2-fluorine group of the 5-phenyl ring in **3d** resulted in a significant reduction in binding affinity (**3c**, K_i = 4.5 nM). Compound **3c** existed in solution as a single isomer based on its NMR spectra,[20] suggesting that the atropisomeric characteristic of **3d** may be important for receptor binding. This is further supported by the 2-chlorine analog **3f** (K_i = 0.30 nM), which improved the potency about 2-fold compared to **3d**.

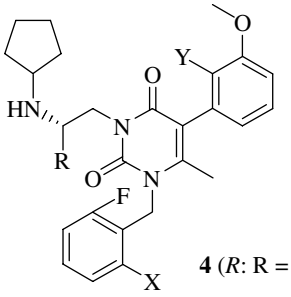
Based on the previous reports,[21] we suspected that the rotational barrier in the chlorinated compound **3f** would be sufficient to allow the separation of the distinct atropisomers. The SAR of **3c**, **3d**, and **3f** strongly suggested that only one of the atropisomers had the appropriate topological profile to fit into a putative binding site of *h*GnRH-R. Therefore, we investigated whether a single atropisomer would have sufficient thermal stability for isolation and characterization.

The atropisomers of **3f** were readily separated by chiral HPLC[22] into the individual isomers **3f-I** and **3f-II**.[23] The individual single isomers were very stable at room temperature. No interconversion was observed for either isomer after one week storage either dry or as a DMSO solution. As expected, one isomer (**3f-II**, K_i = 0.30 nM) exhibited much higher binding affinity than the other (**3f-I**, K_i = 61 nM) at *h*GnRH-R. Therefore, it could be concluded that the 15-fold increase in binding affinity of the 2-chloro-3-methoxyphenyl **3f-II** from the 3-methoxyphenyl analog **3c** results from the exclusive orthogonal orientation of the 2-chlorophenyl ring. For isomer **3f-II**, the 3-methoxy moiety of the 5-phenyl ring is at the right location for a possible hydrogen-bond

Table 3. SAR of uracils at *h*GnRH-R



3 (*S*: R = Me & Et
(*R*: R = CH_2OH)



4 (*R*: R = Me & Et)

Compound	R	X	Y	K_i [a] (nM)	IC_{50} [c,d] (nM)
3a	Me	F	F	4.5	110
3b[b]	CH_2OH	F	F	4.9	n.d.
3c	Me	CF_3	H	4.5	n.d.
3d	Me	CF_3	F	0.61	3.3
3e	Et	CF_3	F	4.2	n.d.
3f	Me	CF_3	Cl	0.30	1.5
3f-I				61	385
3f-II				0.30	1.5
3g	Et	CF_3	Cl	3.7	n/a
3h[b]	CH_2OH	CF_3	Cl	0.70	5.4
3h-I [b]				190	2500
3h-II [b]				0.30	2.6
4a	Me	F	F	68	n.d.
4d	Me	CF_3	F	33	n.d.
4e	Et	CF_3	F	20	n.d.
4g	Et	CF_3	Cl	7.4	n.d.

[a] Data are average of two or more independent measurements.
[b] *R*-configuration due to the higher priority of $HOCH_2$ group.
[c] Functional activity was determined using an IP_3 assay.
[d] n.d., not determined.

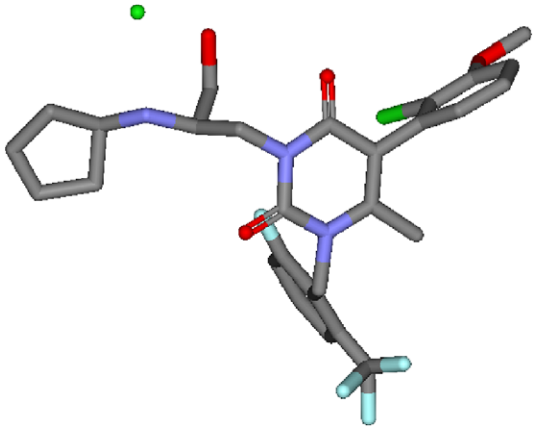


Figure 3. The X-ray crystal structure of **3h-II** hydrochloride indicating an a*R*-configuration of the higher affinity atropisomer.

interaction with the receptor. The X-ray crystal structure of **3h-II** as a hydrochloride salt was obtained (Fig. 3) and its absolute stereochemistry was determined to be a*R*-configured.[24]

Compared to **3f**, the β-ethyl analog **3g** (K_i = 3.7 nM) was about 12-fold less potent, while the β-hydroxymethyl **3h** (K_i = 0.7 nM) displayed only slightly reduced binding affinity. The *R*-configured **4a** and **4d** were significantly less potent than their *S*-isomers **3a** and **3d**. In contrast, the discrepancy between the two stereoisomers for the β-ethyl compounds was small. Thus, **4g** (K_i = 7.4 nM) was only 2-fold less potent than **3g** in binding affinity. The functional antagonist activity of these compounds was demonstrated in an IP_3 assay (Table 3).[25] Thus, **3f-II** exhibited an IC_{50} of 1.5 nM. **3f-I** also shown dose-dependent inhibition in this assay, but with much lower potency (IC_{50} = 385 nM).

In summary, several uracils were characterized as a novel class of GnRH-R antagonists. These 5-aryl-6-methyluracils existed as atropisomers at room temperature. While the 2-fluoro-5-methoxyphenyluracils such as **3a** presented a pair of interchangeable atropisomers a*R*-**3a** and a*S*-**3a**, replacement of the 2-fluorine with a chlorine produced thermally stable atropisomers, which were separable by HPLC. It was found that the atropisomer II was much more potent as a GnRH-R antagonist than isomer I, demonstrating a stereo-preference for receptor interactions. The X-ray crystal structure of **3h-II** showed an a*R*-configuration of this active isomer. Reports have shown that stable biphenyl atropisomers can be synthesized using a chiral Suzuki coupling reaction.[26] In addition to the classical separation of atropisomers by chromatography, crystallization of atropisomers with a chiral salt has been described recently.[27] Thus, a thermally stable atropisomer may be a viable alternative for further development of nonpeptide GnRH antagonists.

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18. *3-[(2S)-Cyclopentylaminopropyl]-1-(2,6-difluorobenzyl)-6-methyl-5-(2-fluoro-3-methoxyphenyl)pyrimidin-2,4-dione hydrochloride* (***3a***)*:* ^{1}H NMR ($CDCl_3$): 1.17 (s, 3H), 1.51 (br s, 2H), 1.62 (br s, 2H), 1.71 (br s, 2H), 1.92 (br s, 2H), 2.17 (s, 3H), 3.43 (m, 1H), 3.62 (m, 1H), 3.85 (s, 3H), 4.14 (m, 2H), 5.26 (s, 2H), 6.76 (m, 1H), 7.12 (m, 2H), 7.18 (d, J = 5.2 Hz, 1H), 7.19 (m, 1H), 7.42 (m, 1H); 19F NMR: −115.0 (m, 2F), −135.4 (m, 1F); ^{13}C NMR: 14.5 and 14.6, 17.5, 23.5 (2C), 28.8 and 28.9, 28.9, 38.6, 42.6, 59.1 and 50.3, 55.5, 55.9, 106.8 and 106.9, 111.9 (m, 2C), 112.1 (t, J = 17 Hz), 113.5, 115.2, 118.5, 122.2 (d, J = 13.6 Hz), 123.6, 124.0 (d, J = 3.8 Hz), 130.1 (t, J = 10.7 Hz), 147.4 (d, J = 10.7 Hz),

149.5 (d, J = 242.7 Hz), 151.0 and 151.1, 151.2 and 152.3,160.5 (dd, J = 7.5, 246.5 Hz, 2C), 160.5 and 160.6.

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20. ^{1}H *NMR (*$CDCl_3$*) for* ***3c****:* 1.14 (d, J = 6.0 Hz, 3H), 1.19–1.95 (m, 8H), 2.06 (s, 3H), 3.23 (m, 2H), 3.80 (s, 3H), 3.98 (dd, J = 5.7 Hz, 1H) <4.11 (dd, J = 8.8, 12.6 Hz, 1H), 6.73 (t, J = 2.1 Hz, 1H), 6.77 (d, J = 7.8 Hz, 1H), 6.87 (dd, J = 2.1, 8.1 Hz, 1H), 7.25 (m, 1H), 7.30 (dd, J = 7.8, 8.1 Hz, 1H), 7.41 (m, 1H), 7.54 (d, J = 7.5 Hz, 1H); ^{13}C NMR: 18.2, 18.9, 24.0, 29.9, 32.9, 33.4, 42.9, 47.1, 51.1, 55.5, 57.4, 113.5, 113.6 (d, J = 5.0 Hz), 115.0, 116.8, 121.0, 121.3, 122.5 (m), 122.9, 123.3, 123.8 (q, J = 300 Hz), 129.6 (d, J = 9.8 Hz), 129.8, 135.5, 150.3 (d, J = 295 Hz), 159.9 (d, J = 6.6 Hz), 162.7, 163.2.
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22. Separation was performed on a Beckman 322 semipreparative HPLC equipped with a 10×250 nm Chirex 3020AL column at a solvent flow-rate of 0.3 ml/min. Mobile phase A consisted of hexane: 1,2-dichloroethane (65:35) while mobile phase B was composed of hexane:1,2-dichloroethane: EtOH (55:35:10) with 0.5% TFA. Retention time was 15.93 min for peak 1 (**3f-I**) and 17.08 for peak 2 (**3f-II**).
23. ^{1}H *NMR (*$CDCl_3$*) for* ***3f-I*** *:* 1.48 (d, J = 6.3 Hz, 3H), 1.55–1.90 (m, 8H), 2.00 (s, 3H), 2.09 (br s, 1H), 3.47 (br s, 1H), 3.60 (br s, 1H), 3.92 (s, 3H), 4.29 (d, J = 15.3 Hz, 1H), 4.54 (m, 1H), 5.46 (d, J = 17.1 Hz, 1H), 5.53 (d, J = 17.1 Hz, 1H), 6.87 (d, J = 6.9 Hz, 1H), 6.98 (d, J = 7.4 Hz, 1H), 7.29 (m, 2H), 7.45 (m, 1H), 7.57 (d, J = 7.5 Hz, 1H); for **3f-II** : 1.39 (d, J = 6.9 Hz, 3H), 1.55–1.70 (m, 7H), 2.00 (s, 3H), 2.15 (br s, 1H), 3.40–3.70 (br s, 3H), 3.92 (s, 3H), 4.20 (d, J = 13.8 Hz, 1H), 4.54 (dd, J = 6.3, 14.4 Hz, 1H), 5.35 (d, J = 17.1 Hz, 1H), 5.52 (d, J = 16.5 Hz, 1H), 6.84 (d, J = 7.5 Hz, 1H), 6.97 (d, J = 7.4 Hz, 1H), 7.30 (m, 2H), 7.44 (m, 1H), 7.57 (d, J = 8.1 Hz, 1H).
24. CCDC 684002 contains the supplementary crystallographic data for this paper. These data can be obtained via the CDCC website (www.ccdc.cam.ac.uk), or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK.
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