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A convenient one-pot synthesis of asymmetric 1,3,5-triazine-2,4,6-triones and its application towards a novel class of gonadotropin-releasing hormone receptor antagonists

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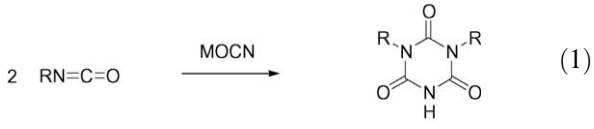
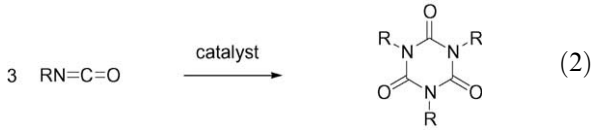
Abstract—A convenient one-pot synthetic route was developed for the preparation of asymmetric 1,3-dialkyl-1,3,5-triazine-2,4,6-triones from readily available alkyl- or aryl-isocyanates, primary amines and *N*-chlorocarbonyl isocyanate in excellent yields. Subsequent alkylation with N-protected amino alcohols afforded the desired 1,3,5-triazine-2,4,6-triones in good yields. This methodology was applied to the synthesis of a chemical library acting as antagonists of the *h*GnRH receptor.

1,3,5-Triazine-2,4,6-trione, also known as isocyanurate, is a class of simple heteroaromatic molecules.[1] Symmetrical 1,3-disubstituted triazinetriones can be prepared by condensation of two molecules of isocyanates with a metal isocyanate (Na, K, Li, as in MNCO) in a dipolar aprotic solvent (Eq. 1),[2] and symmetrical trisubstituted triazinetriones are synthesized by trimerization of isocyanates using a variety of catalysts such as Lewis bases,[3] anions,[4] and metallic compounds (Eq. 2).[5] In addition to the severe conditions required for these reactions, these approaches are limited to the 1,3,5-triazine-2,4,6-triones substituted with two or three identical groups. However, there are very few literature reports on the synthesis of asymmetric triazinetriones with two or three different substituents. Kappe and co-workers synthesized 1-(1*H*-benzimidazol-2-ylmethyl)-3-phenyl-*s*-triazine-2,4,6(1*H*,3*H*,5*H*)-trione as an antimicrobial agent by cyclization of the corresponding urea and *N*-ethoxycarbonyl isocyanate in refluxing bromobenzene in 60% yield (Eq. 3).[6] A more efficient cyclization of *N*,*N*′-dialkylureas with *N*-chlorocarbonyl isocyanate to yield asymmetric disubstituted triazinetriones is described in an early patent literature (Eq. 4).[7] Recently, this cyclization has been applied to the solid-support synthesis of asymmetric 1,3,5-triazine-2,4,6-triones using resin-bound amino acids for a chemical library (Eq. 5).[8]



(1)



(2)

We have reported the discovery of a new series of 6-methyluracils such as **1** (Fig. 1) as potent antagonists of the human gonadotropin-releasing hormone (*h*GnRH) receptor.[9] The early lead compound **1a** (K_i = 34 nM) has been optimized to much more potent analogues such as **1b** (K_i = 1.1 nM); interestingly, the regio-isomer of **1a**, **2**, did not display measurable binding affinity (K_i > 20 μM). This data suggest the important role of the 4-carbonyl functionality of uracil **1a** in

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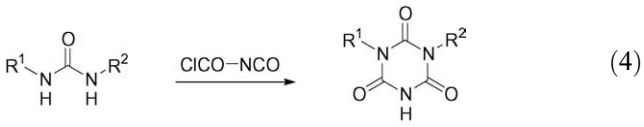
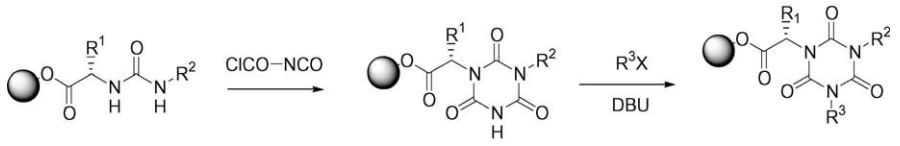
(3)



(4)

than **1a**. While this data did not provide a conclusive answer to the question, it prompted us to further investigate this class of compounds as potential small molecule antagonists of the *h*GnRH receptor.

In order to explore the structure–activity relationship of this class of compounds, a general synthetic method to yield the 1,3,5-triazine-2,4,6-triones with three different substitution groups that would be amenable to the efficient preparation of large numbers of analogues is required. If we could achieve rapid access to the asymmetric 1,3-disubstituted 1,3,5-triazine-2,4,6-trione, this key intermediate could be suitable for Mitsunobu alkylation with readily available amino alcohols. This would incorporate a side-chain bearing a basic nitrogen, an important pharmacophore feature for this class of GnRH antagonists. In this paper, we report an efficient one-pot synthesis of 1,3-disubstituted 1,3,5-triazine-2,4,6-triones bearing two different substituents from



(5)

receptor binding possibly through hydrogen bonding. Alternatively, the change from the 6-methyl group of **1a** to the carbonyl moiety of **2** between the 3-methoxyphenyl and the 2,6-difluorobenzyl groups could also cause a conformational change of molecule **2**, which may account for its loss of potency. To explore the impact of this carbonyl group on GnRH receptor binding we designed and synthesized the 1,3,5-triazine-2,4,6-trione analogue **3**, using the method described in Eq. 3. Interestingly, compound **3** exhibited a K_i value of 4.2 μM, which was superior to **2**, but much less potent readily available material, and subsequent Mitsunobu reaction with N-protected amino alcohols to prepare the monocyclic compounds in a screening library for the human GnRH receptor.

The initial approach to synthesize the targeted 1,3,5-triazine-2,4,6-triones **3** is summarized in Scheme 1. 1-Allyl-3-(3-methoxy-phenyl)-1,3,5-triazine-2,4,6-trione **5a** was obtained by a cyclization of *N*-allyl-*N*′-(3-methoxyphenyl)urea **4** with *N*-ethoxycarbonyl isocyanate in refluxing bromobenzene in ~30% yield. Compound **5a** was then

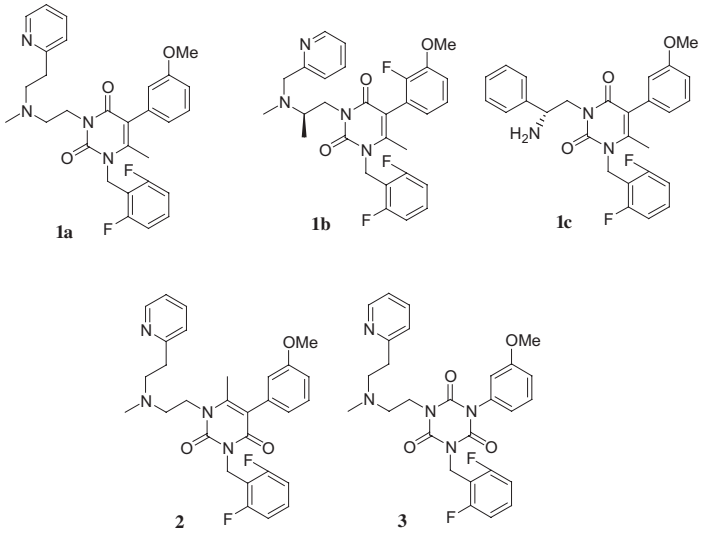
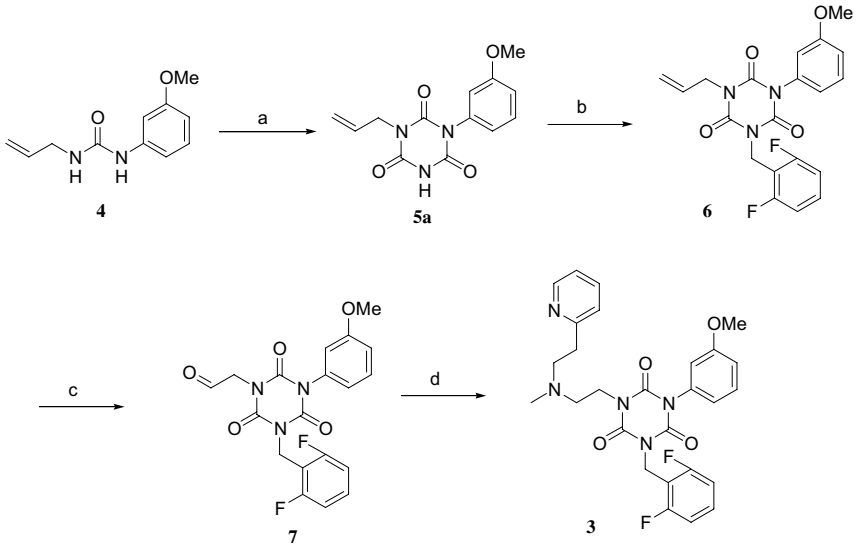
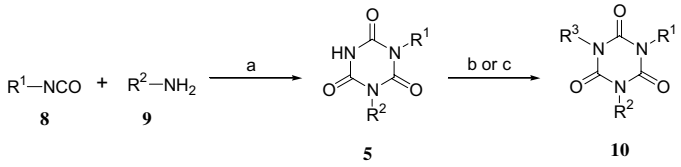


Figure 1. Small molecule GnRH antagonists.



Scheme 1. Reagents and conditions: (a) EtOCONCO, bromobenzene, 150 °C, 30%; (b) 2,6-difluorobenzyl bromide, TBAF, THF, 62%; (c) $NaIO_4$, OsO_4 (cat), THF/H_2O, 92%; (d) 2-(*N*-methylaminoethyl)pyridine, $NaBH(OAc)_3$, dichloroethane, 85%.



Scheme 2. Reagents and conditions: (a) 1. dichloromethane, ambient temperature, 2. ClCONCO, dichloromethane; (b) R^3OH, triphenylphosphine, di-*t*-butyl-azodicarboxylate, THF; (c) R^3Br, K_2CO_3, DMF.

treated with 2,6-difluorobenzyl bromide and *n*-tetrabutylammonium fluoride in THF to afford **6**, which was subjected to an oxidative cleavage of the olefin by $NaIO_4/OsO_4$ to yield **7**. Finally, the aldehyde **7** was reacted with 2-(*N*-methyl-2-aminoethyl)pyridine under reductive conditions to give the desired product **3**.

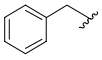
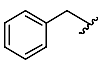
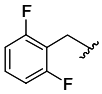
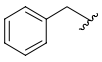
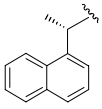
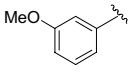
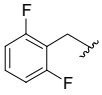
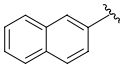
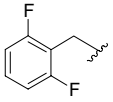
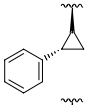
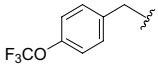
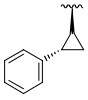
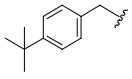
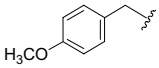
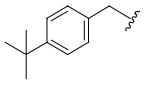
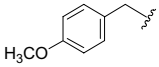
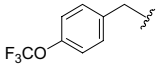
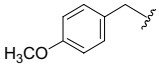
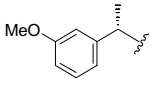
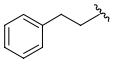
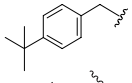
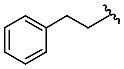
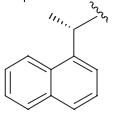
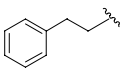
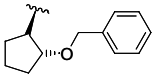
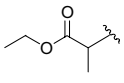
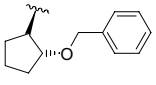
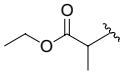
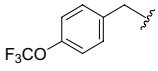
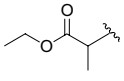
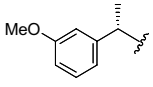
Since this route was inefficient, we focused on the development of a new synthetic method for 1,3,5-triazine-2,4,6-triones with two different groups at the 1- and 3-positions. Typically, an *N*,*N*′-dialkylurea is prepared by the treatment of a primary amine with an isocyanate, then the isolated urea is cyclized into the triazinetrione. We found that by combining these two steps in dichloromethane, first by mixing an equimolar amount of amine and isocyanate, followed by addition of *N*-chlorocarbonyl isocyanate (1.5–2 equiv, excess amount was required for the reaction to completion), the desired triazinetrione usually precipitated out from the reaction mixture to afford the pure product. Thus, a convenient method to synthesize the asymmetric disubstituted 1,3,5-triazine-2,4,6-trione **5** in an one-pot procedure was developed (Scheme 2).

A general procedure for this synthesis of 1,3-dialkyl-1,3,5-triazine-2,4,6-triones is described as follows.[10] To a solution of a primary amine (7.5 mmol) in CH_2Cl_2 (10 mL), an isocyanate (7.5 mmol) was added slowly. The resulting mixture was stirred at ambient temperature for 1 h. *N*-Chlorocarbonyl isocyanate (1 equiv, 0.6 mL, 7.5 mmol) was then added slowly (caution: the reaction was usually exothermic), and the reaction mixture was stirred for one additional hour. An additional equivalent of *N*-chlorocarbonyl isocyanate (0.6 mL) was slowly introduced and the resulting mixture was stirred overnight. If precipitate formed, it was filtered, and washed with cold dichloromethane to yield the desired triazinetrione product. Otherwise, the reaction was diluted with dichloromethane, washed with saturated aqueous $NaHCO_3$ and then brine, and the organic layer dried over Na_2SO_4, and evaporated to give the final product as white solid.[11]

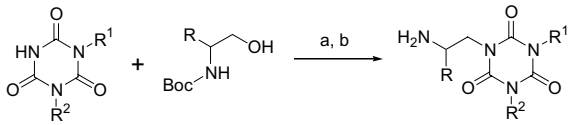
In general, this efficient, two-step, one-pot reaction sequence afforded 1,3-dialkyl-1,3,5-triazine-2,4,6-triones in very good to excellent yields, while minimal workup and purification were required. Over 100 compounds with a wide variety of substitutions were prepared using this method. A selection of compounds and their yields are listed in Table 1.

These disubstituted 1,3,5-triazine-2,4,6-triones **5** were further modified by introduction of the third substituent, by either a conventional alkylation with an alkyl halide in the presence of a base such as potassium carbonate (Scheme 2) or by Mitsunobu reaction with

Table 1. Synthesis of disubstituted 1,3,5-triazine-2,4,6-diones **5b–t** by one-pot procedure

Compound	R^1 (isocyanates)	R^2 (amines)	Yield[a] (%)
5b			87
5c			91
5d			76
5e			82
5f			83
5g			95
5h			81
5i			87
5j			98
5k			100
5l			90
5m			100
5n			94
5o			95
5p			94
5q			99
5r			96
5s			93
5t			92

[a] Isolated yield.



Scheme 3. Reagents and conditions: (a) PPh_3, di-*t*-butyl-azodicarboxylate, THF; (b) TFA/CH_2Cl_2 (1:1).

alcohols to afford the asymmetric triazinetriones **12** (Scheme 3). The successful Mitsunobu reaction of the disubstituted triazinetriones allowed us to introduce, by utilizing readily available N-protected amino alcohols, an aminoalkyl side-chain, which is important for high affinity GnRH receptor binding. Examples of this Mitsunobu alkylation followed by deprotection of Boc group are listed in Table 2.[12]

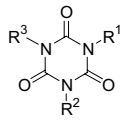
We have also successfully adopted solid-supported triphenylphosphine (Aldrich, 1 mmol/g loading) in the synthesis of library compounds, which overcame the difficulty of separating the triphosphine oxide by-product from the reaction mixture. Despite the large excess of solid-supported triphenylphosphine (~10 equiv) that was required (instead of 1.5 equiv of triphenylphosphine in solution) and the longer reaction times (10–16 vs 1–4 h), the solid-supported reaction offered an easy purification of the desired product by filtration. This method was especially useful when the product had similar polarity to triphenylphosphine oxide.

All final compounds were evaluated for their ability to inhibit des-Gly^{10}[^{125}I-Tyr^5, DLeu^6, NMeLeu^7, Pro^9-NEt]-GnRH radioligand binding to the cloned *h*GnRH receptor stably expressed in HEK293 cells using a 96-well filtration assay format.[13] The binding affinities of a few selected compounds **12** are summarized in Table 3. Several compounds from this collection were found to exhibit moderate potency with the 3-methoxyphenyl compound derived from (*R*)-phenylglycinol (**12c**) having the highest binding affinity (K_i = 37 nM). The 2-fluorophenyl analogue **12g** displayed a K_i value of 89 nM. Interestingly, the 2,6-difluorophenyl analogue (**12h**, 220 nM) was slightly less potent than **12g**.

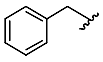
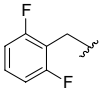
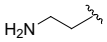
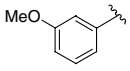
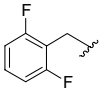
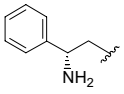
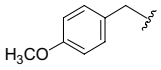
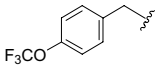
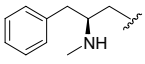
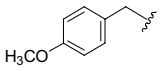
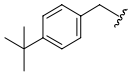
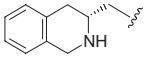
In comparison with the corresponding uracil derivative **1c** (K_i = 2.3 nM), **12c** displayed lower affinity, but only by about 16-fold. This data indicates that the change from a methyl group to a carbonyl moiety between the 3-methoxyphenyl and 2,6-difluorobenzyl groups in **1c** has some impact, but is not a key factor in *h*GnRH receptor binding. Thus the loss of potency in **2** is most likely caused by the loss of the carbonyl group between the 3-methoxyphenyl and the aminoethyl groups for possible hydrogen bonding.

In summary, we have developed a convenient two-step, one-pot cyclization procedure for assembly of the 1,3,5-triazine-2,4,6-triones with disubstitutions in excellent yields. Mitsunobu reaction of these disubstituted triazinetriones was also developed and applied to introduce, from readily available amino alcohols, a third

Table 2. Synthesis of 1,3,5-triazine-2,4,6-diones **12a–e** by Mitusnobu reaction[12]

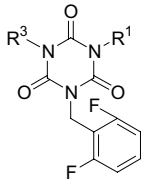


12a-e

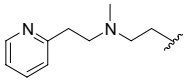
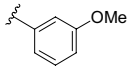
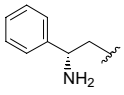
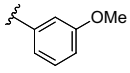
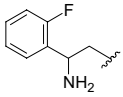
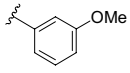
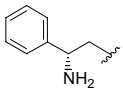
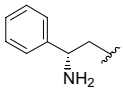
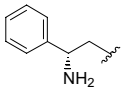
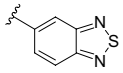
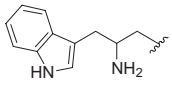
Compound	R^1 (isocyanates)	R^2 (amines)	R^3	Yield[a] (%)
12a				78
12b				92
12c				89
12d				90
12e				86

[a] Isolated yield.

Table 3. Binding affinities of compounds **3**, **12** on the *h*GnRH receptor[14]



3, 12c, 12f-j

Compound	R^3	Chirality	R^1	K_i (nM)
3				4200
12c		*R*		37
12f		*RS*		120
12g		*R*		89
12h		*R*		220
12i		*R*		410
12j		*RS*		380

substitution group bearing a basic nitrogen. This convenient synthetic route was utilized to synthesize a screening library for the human gonadotropin-releasing hormone receptor. The initial work on this series provided us with compounds of moderate affinity. The best compound (**12c**) from the initial SAR study had a K_i value of 37 nM. Further structure–activity relationships of this series of compounds as *h*GnRH antagonists will be presented elsewhere.

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11. (*S*)-1-((*S*)-2-Phenyl-cyclopropyl)-3-(4-trifluoromethoxybenzyl)-[1,3,5]triazinane-2,4,6-trione (**5j**) was obtained as a white solid (98% yield): mp 192.8–193.0 °C; ^{1}H NMR (TMS/$CDCl_3$): δ 1.43–1.48 (m, 1H), 1.62–1.67 (m, 2H), 2.36–2.37 (m, 1H), 2.74–2.78 (m, 1H), 5.023 (s, 1H), 7.17–7.56 (m, 9H), 8.48 (br s, 1H). HRMS (ESI) *m*/*z* calcd for $C_{20}H_{16}F_3N_3O_4$ (MNa^+) 442.0985, found 442.0988. Anal. Calcd for $C_{20}H_{16}F_3N_3O_4$: C, 57.28; H, 3.85; N, 10.02. Found: C, 57.26; H, 3.69; N, 10.19. 1-(4-*t*-Butyl-benzyl)-3-(4-methoxybenzyl)-[1,3,5]triazinane-2,4,6-trione (**5l**) was obtained as a white solid (90% yield): mp 131.1–131.8 °C; ^{1}H NMR (TMS/$CDCl_3$): δ 1.29 (s, 9H), 3.78 (s, 3H), 4.94 (s, 2H), 4.97 (s, 2H), 6.83–7.44 (m, 8H), 8.69 (br s, 1H). HRMS (ESI) *m*/*z* calcd for $C_{22}H_{25}N_3O_4$ ($M-H^-$) 394.1772, found 394.1765. Anal. Calcd for $C_{22}H_{25}N_3O_4$: C, 66.82; H, 6.37; N, 10.63. Found: C, 67.05; H, 6.16; N, 10.68.
12. Typical procedure: a solution of **5l** (32 mg, 0.08 mmol) in anhydrous THF (1.0 mL) was treated with *N*-Boc-(*R*)-1-(1,2,3,4-tetrahydro-isoquinolin-3-yl)-methanol (22 mg, 0.08 mmol), Ph_3P (42 mg, 0.12 mmol) and di-*t*-butyl-azodicarboxylate (28 mg, 0.12 mmol) at ambient temperature. The reaction mixture was stirred at ambient temperature for 3 h. Volatiles were evaporated, and the residue was treated with 1:1 TFA/DCM (2 mL) for 1 h. The reaction mixture was then evaporated and purified by reverse phase HPLC (C-18 column, 15–75% ACN/water) to give 1-(4-*t*-butyl-benzyl)-3-(4-methoxybenzyl)-5-[(*R*)-1-(1,2,3,4-tetrahydro-isoquinolin-3-yl)methyl]-[1,3,5]triazinane-2,4,6-trione (**12e**, 45 mg, 86% yield) was obtained as TFA salt: ^{1}H NMR (TMS/$CDCl_3$): δ 1.28 (s, 9H), 3.10 (d, J = 7.5 Hz, 2H), 3.77 (s, 3H), 3.91–4.13 (m, 2H), 4.48–4.93 (m, 7H), 6.82 (d, J = 9.0 Hz, 2H), 7.09–7.37 (m, 10H); MS (CI) *m*/*z* 541.2. Anal. Calcd for $C_{32}H_{36}N_4O_4$·TFA: C, 62.38; H, 5.70; N, 8.56. Found: C, 62.19; H, 5.62; N, 8.45.
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14. 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 3–8 independent experiments.