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Multicomponent Approach to Libraries of Substituted Dihydroorotic Acid Amides

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Abstract

A process featuring a sequential multicomponent reaction followed by a regioselective post-cyclization strategy was implemented for the facile synthesis of *N*,*N*'-disubstituted dihydroorotic acid amides under mild conditions. We obtained, for the first time, a library of 29 derivatives, encompassing 19 N^{α}-substituted-N^{4}-dihydroorotyl-4-aminophenylalanine derivatives, a key residue of gonadotropin-releasing hormone antagonist Degarelix. The corresponding products were prepared from easily accessible starting materials in good to excellent yields with broad substrate scope.

KEYWORDS Combinatorial Chemistry; Multicomponent Reactions; Domino Process; Carbodiimide; Dihydroorotic acid.

INTRODUCTION

Dihydroorotic acid (2,6-dioxo-4-hexahydro-pyrimidinecarboxylic acid, DHO acid **1**, Figure 1) derivatives are chemically and biologically very interesting compounds since DHO acid is a key component of pyrimidine nucleotide biosynthesis.[1] DHO acid is formed by cyclization of *N*-carbamoyl-aspartic acid precursor triggered by zinc metalloenzyme dihydroorotase (DHOase) and converted to orotic acid by an oxidation catalyzed by the dihydroorotate dehydrogenase (DHODH) enzyme.[2] DHO acid derivatives able to target and inhibit DHOase or DHODH are potential anticancer and antimalarial drugs.[3] Since the biological activity of heterocycles in general often do not arise only from the heterocycle itself, but from the substituents attached to it, the development of a practical and straightforward way to synthesize libraries of substituted DHO acid derivatives is of great interest.

Moreover, DHO acid is found in marketed drugs, such as Degarelix **2**, a decapeptide where N^4-(dihydroorotyl)-4-aminophenylalanine (4-Aph(Hor)) amino acid is incorporated in position 5. (Figure 1).

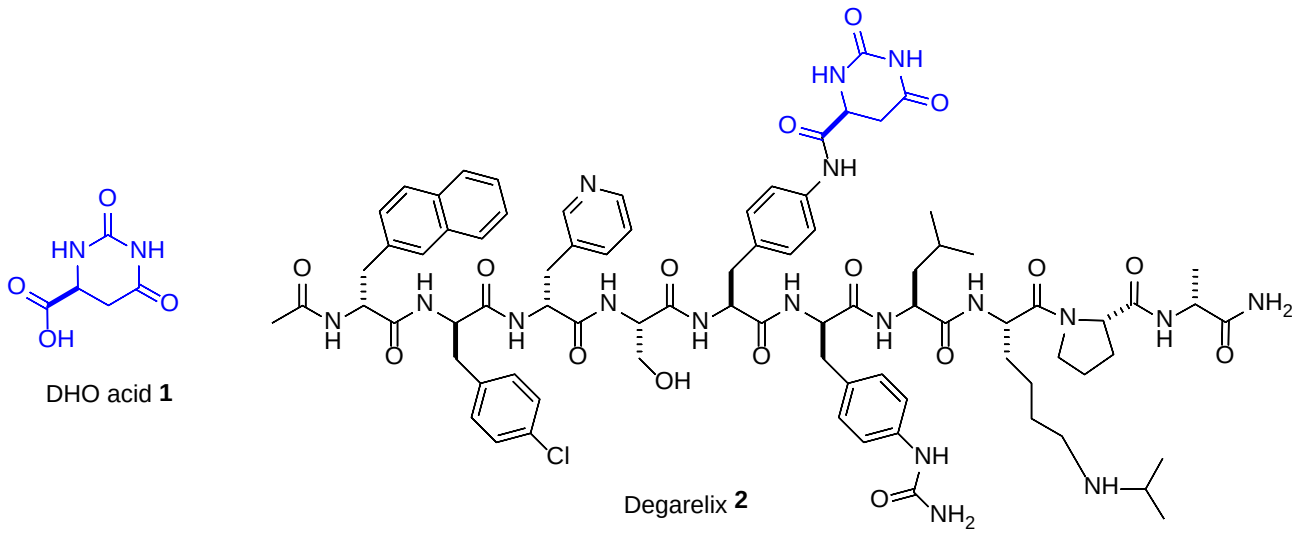


Figure 1. Structures of DHO acid **1** and Degarelix **2**.

The introduction of a DHO acid residue, along with a urea moiety in position 5 and 6, respectively, of known peptides acting as gonadotropin-releasing hormone (GnRH) antagonists, led to the

discovery of analogues with improved hydrophilicity, lesser propensity to form gels, and a dramatic increase in duration of action when submitted subcutaneously.[4] However, so far there is not a mechanistic insight to explain these unique features of Degarelix and new studies are still ongoing.[5] Since the introduction of hydrophobic side chains, as well as new hydrogen bonding sites, may modulate the activity/solubility/bioavailability, the incorporation of *N*-substituted DHO acid in Degarelix, or more general in GnRH antagonist of similar structure, could be an important goal in order to shed light on the relationship between the peptide's structure and the morphology of the self-assembled nanostructures and/or to find biologically and pharmaceutically more active peptides. Thus, also for this purpose, the development of a process that would make accessible libraries of substituted 4-Aph(Hor) derivatives is of great interest.

However, an extensive literature survey revealed that there are few reports describing the synthesis of *N*-substituted DHO acid. To date, the most utilized strategies rely in a step-wise ethoxycarbonylation of *N*-substituted asparagine followed by cyclization under strongly basic conditions[6] or by alkylation of DHO esters using strong bases such as NaH or potassium *tert*-butoxide in dry solvents and highly reactive methyl or benzyl halides (or more reactive trifluoromethansulphonates), followed by ester saponification.[7]

The most explored tool in drug discovery programs, both in industry and in academia, for the combinatorial synthesis of new entities in a programmed and efficient manner, and in particular for heterocycles, is the application of multicomponent reactions (MCRs), which allow the simultaneous formation of multiple bonds starting from three or more reactants in one-step.[8] However, the use of MCR strategy suffers from a key issue, *i.e.* a limited scaffold diversity due to the difficulty to discover a new MC process.[9] In particular, to the best of our knowledge, there are no reports in the literature describing the combinatorial synthesis of DHO acid derivatives either by MCRs or by multi-step synthetic pathways.

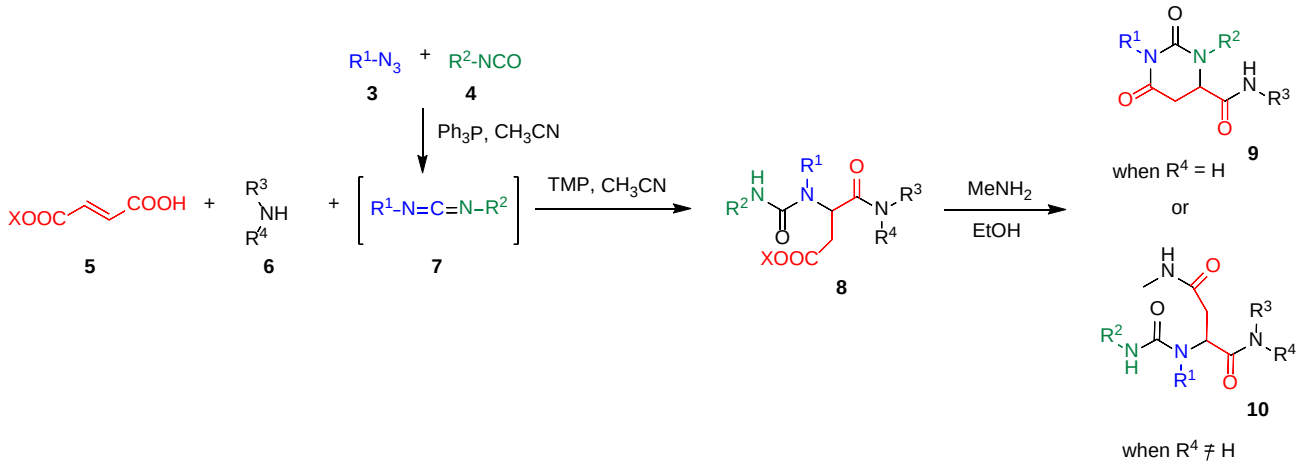
During the last years, we have developed a new sequential MCR that allowed us to prepare libraries of hydantoin derivatives and urea-peptide conjugates incorporating a hexafluorovaline or aspartic (Asp) ester moieties starting from easily accessible reagents under very mild conditions.[10] In order to exploit known MCRs to increase scaffold diversity and complexity, along with the single

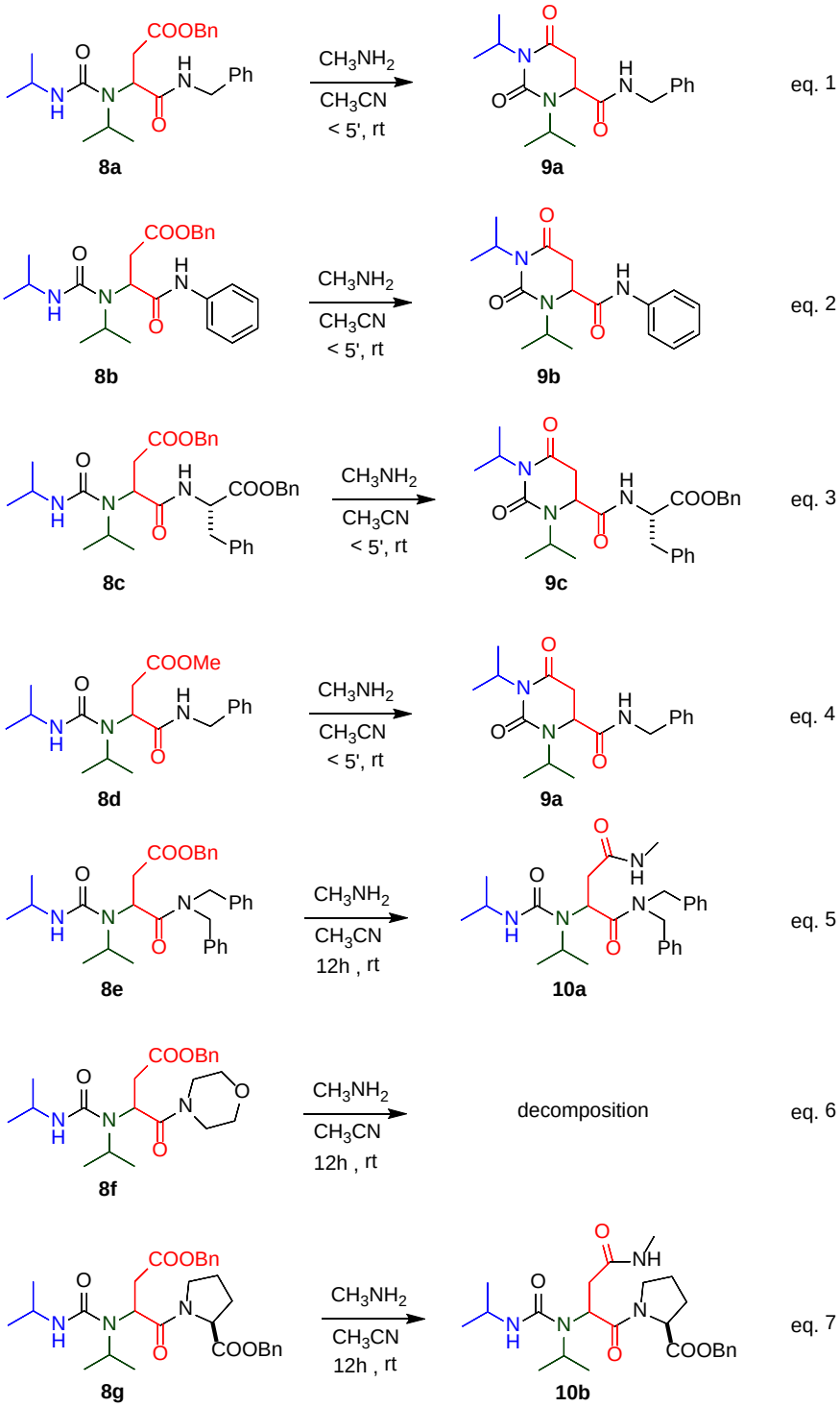
reactant replacement (SRR)[11] and condition-based divergence (CBD)[12] strategies, a particularly attractive and synthetically productive manipulation of MCRs is the post-cyclization strategy which triggers the formation of (poly)cyclic compounds, mainly (poly)heterocycles.[13] In this Research Article, we present for the first time the combinatorial synthesis of amides and peptides incorporating substituted DHO or 4Aph(Hor) moieties, which arises by fast and regioselective, one-pot, post-cyclization of *N*-urea-aspartate derivatives obtained by our MCR.

RESULTS AND DISCUSSION

We recently demonstrated that carbodiimides **7**, generated *in situ* from azides **3** and iso(thio)cyanates **4** through Staudinger/aza-Wittig process, react straightforwardly in the presence of fumaric acid monoesters **5** and amines or aminoacids **6** leading to the formation of, respectively, urea-amide or -peptide conjugates incorporating an aspartic unit **8** (Scheme 1).[10] In a previous work concerning the application of our MC process to the synthesis of neomycin-sugar conjugates, when the conjugates were treated with a solution of methylamine, along with sugar de-acetylation, we obtained the immediate formation of a DHO heterocycle probably through the nucleophilic attack of the urea NH to the ester function of the Asp unit.[14] This result was quite unexpected since cyclization of ureido-esters to pyrimidine-2,4-diones was known to occur with strong bases (such as *t*-BuOK or NaH) or weaker inorganic bases ($CsCO_3$) in very polar solvent, DMF, and long reaction time (typically over-night). We decided to study in depth this process by treating urea-Asp amide conjugates **8a-g** in the same reaction conditions used for the MC process, *i.e.* by dissolving the adducts **8** in CH_3CN and adding a 10% in volume of a commercially available 8.03 M solution of methylamine in ethanol (Scheme 1). Starting from urea-Asp secondary amides, whatever alkyl **8a**, aryl **8b** or α-amido ester **8c**, cyclization occurred in less than five minutes at room temperature (rt) giving rise the formation of DHO amides **9a-c**, respectively, in almost quantitative yields (equations 1-3, Scheme 1). Moreover, starting from urea-Asp amide **8d** bearing a methyl ester instead of benzyl ester, after cyclization and work-up with 1N aqueous solution of HCl, product **9a** was recovered very clean without the need of a chromatographic purification (equation 4, Scheme 1). Quite surprisingly, starting from urea-Asp tertiary amides **8e-g** the cyclization reaction did not occur. Indeed, after

stirring at rt for 12 hours, we obtained the formation of the corresponding *N*-methyl amides **10a,b**, arising from nucleophilic attack of methylamine to the Asp benzyl ester moiety, in the case of *N*,*N*-dibenzyl and prolyl amides **8e,g**, respectively, while decomposition of the starting material starting from morpholine amide **8f** (equations 5-7, Scheme 1).





Scheme 1. Reaction of urea-Asp amides **8** with a solution of methylamine.

After the observation that cyclization could be obtained in the same condition used for the MC process, namely using CH_3CN as solvent, we explored the scope of this strategy for the sequential MC synthesis of *N,N'*-disubstituted DHO amides. More in detail, azide **3** and isocyanate **4** were mixed in CH_3CN in the presence of Ph_3P at room temperature (rt). Once carbodiimide **7** was formed (TLC monitoring), the temperature was lowered to 0°C and amine **6**, 2,4,6-trimethylpyridine (TMP) followed by acid **5** were added and the solution stirred at rt for 3 hours. Once urea-Asp amide **8** is formed, a commercial available solution of $MeNH_2$ (8.03 M in ethanol) was added (10% in volume), the solution stirred for 5 minutes and quenched with a 1N aqueous solution of HCl (Figure 3). We started from a variety of azides **3**{*1-7*} (chemset 1, Figure 2) and isocyanates **4**{*1-4*} (chemset 2, Figure 2), or available carbodiimides **7**{*a-b*} (chemset 4, Figure 2), fumaric acid methyl and benzyl monoesters **5**{*1-2*} (chemset 3, Figure 2), and amines **6**{*1-8*} (chemset 5, Figure 2). All the corresponding final DHO amides were recovered in very good yields (Figure 3). Moreover, as expected, the reaction was completely regioselective when asymmetric carbodiimides were used (see mechanism in Scheme 3). Starting from chiral aminoacids as nucleophiles, a 1:1 distereoisomeric mixture of DHO-containing dipeptides **9**{*a,1,5*}, **9**{*a,1,6*}, **9**{*1,1,2,5*}, and **9**{*a,1,7*} was obtained. It is worth noting that compounds **9**{*a,1,7*} could be considered a precursor for the synthesis of *N,N'*-disubstituted analogues of Taltirelin, a thyrotropin-releasing hormone (TRH) analog containing DHO acid moiety.[16] The same 1:1 mixture of diastereoisomers **9**{*a,2,8*} were obtained when lysine amide **6**{*8*} was used as nucleophile. These results were expected since the chiral nucleophiles step in the reaction when the new chiral stereocenter at the Asp moiety is already formed (see mechanism in Scheme 3).

The scope of this strategy was further extended to the synthesis of 4-Aph(Hor) conjugates **9** by using 4-Aph derivatives **6**{*9-13*} as nucleophiles (chemset 5, Figure 2). The first attempt using commercially available DCC **7**{*b*} lead to the formation of a 2:1 mixture of two inseparable products (Scheme 2). Along with the desired DHO amide **9**{*b,2,9*}, we obtained the formation of the side product **11**{*b,2,9*} arising from intramolecular attack of the aromatic amidic nitrogen instead of the nitrogen belonging the urea moiety (stated from ^{1}H NMR spectrum, Scheme S2). Thus, we turned our attention in the optimization of the cyclization process starting from 4Aph-Asp-urea **8**{*b,2,9*}

isolated after the MC process. Among different conditions suitable with the use CH_3CN as organic solvent, we found that the addition of a 10% in volume of a 1N aqueous solution of NaOH produced the regiospecific formation of DHO amide **9**{*b*,*2*,*9*} in almost quantitative yields (Scheme 2). Similarly, a 1:3 mixture in favor of the undesired derivatives **11**{*1*,*1*,*2*,*11*} was obtained by cyclization triggered by methylamine solution of intermediate **8**{*1*,*1*,*2*,*11*} (stated from ^{1}H NMR spectrum, Scheme S3) that was obtained from the MC process with asymmetric carbodiimide **7**{*1*,*1*}, whereas regiospecific cyclization leading to the formation of target compound **9**{*1*,*1*,*2*,*11*} was triggered by using 10% in volume of a 1N aqueous solution of NaOH (Scheme 2).

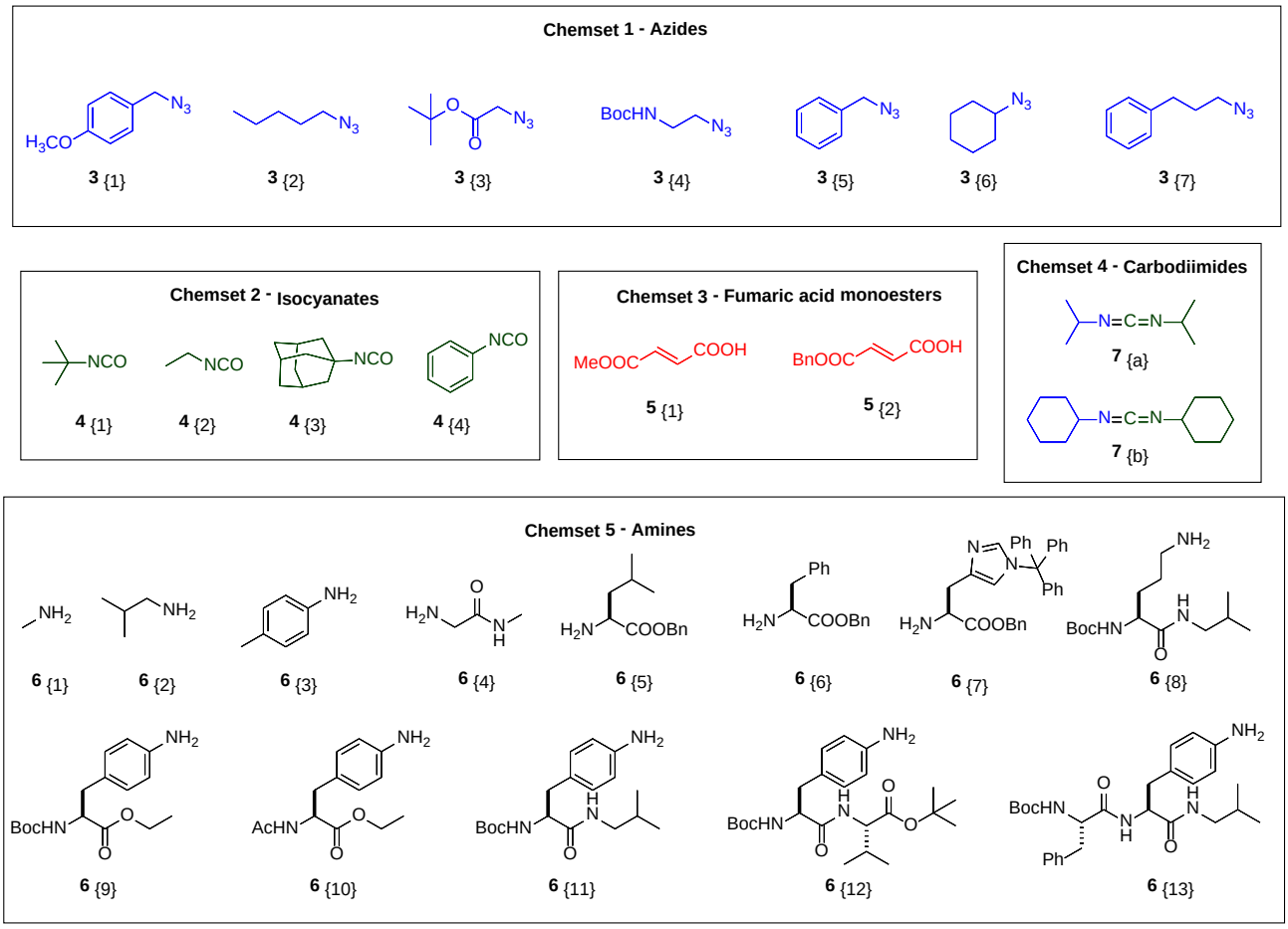
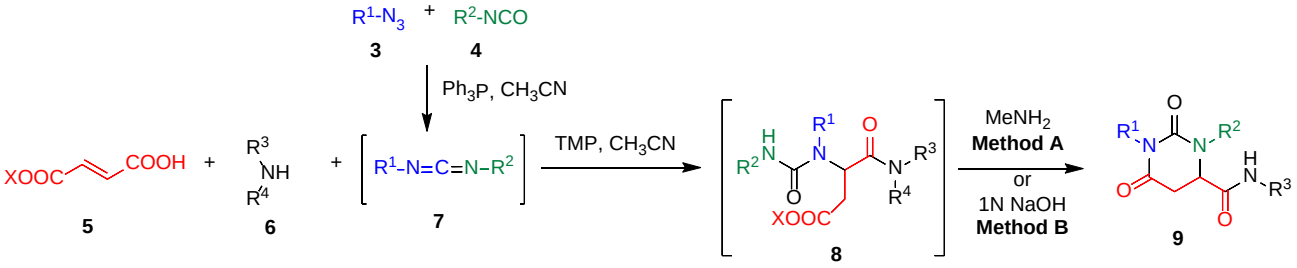
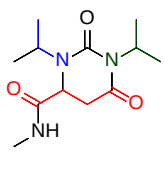
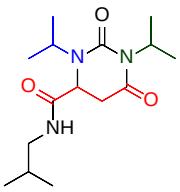


Figure 2. Selected chemsets for the synthesis of the library of *N*,*N*'-disubstituted DHO amides: azides (**chemset 1**), isocyanates (**chemset 2**), fumaric acid monoesters (**chemset 3**), commercial available carbodiimides (**chemset 4**), and amines (**chemset 5**).





9 {a,1,1} (93%)
Method A



9 {a,1,2} (88%)
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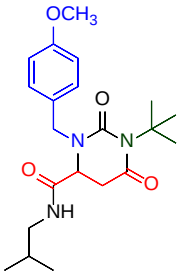
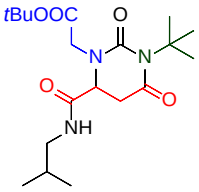


9 {1,1,2,2} (68%)
Method A



9 {3,1,2,2} (62%)
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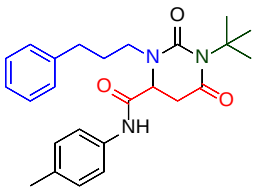

9 {7,1,2,3} (63%)
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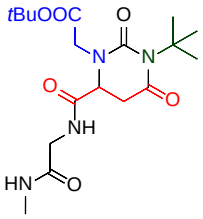

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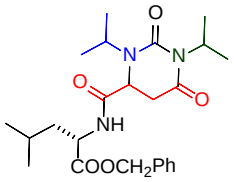

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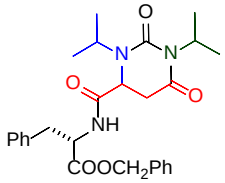
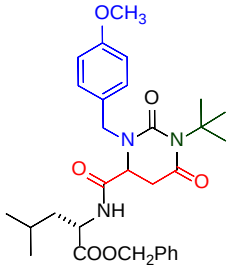


9 {a,1,6} (84%)
Method A



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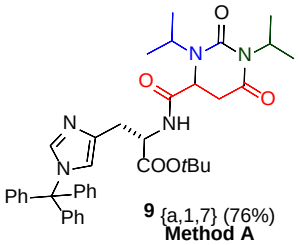

9 {a,1,7} (76%)
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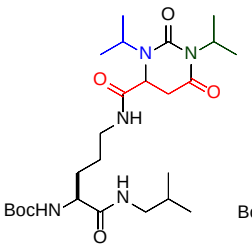

9 {a,2,8} (87%)
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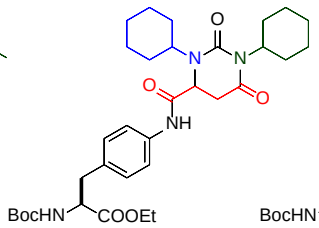
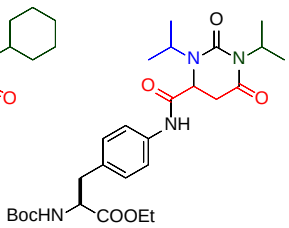


9 {b,2,9} (82%)
Method B



9 {a,2,9} (88%)
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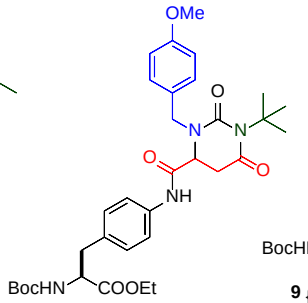
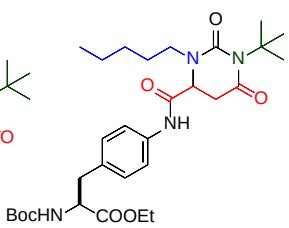


9 {1,1,1,9} (61%)
Method B



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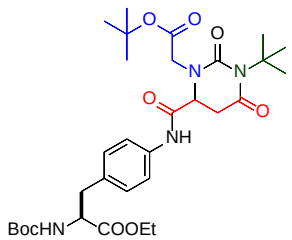

9 {3,1,1,9} (72%)
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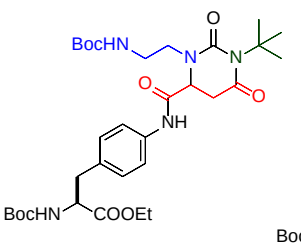

9 {4,1,1,9} (61%)
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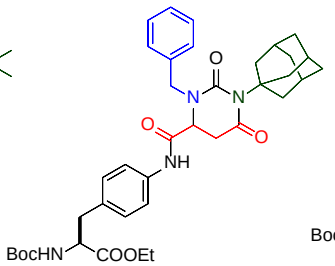
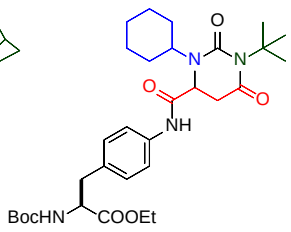


9 {5,3,1,9} (66%)
Method B



9 {6,1,2,9} (57%)
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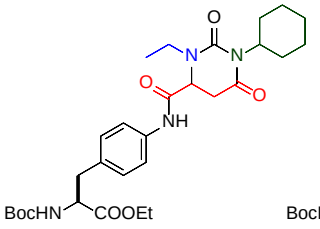

9 {6,2,2,9} (62%)
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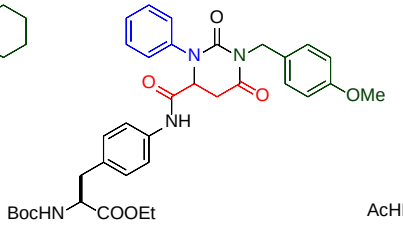

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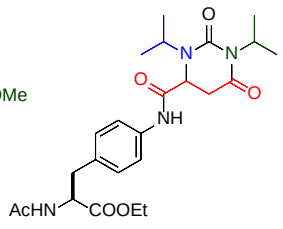
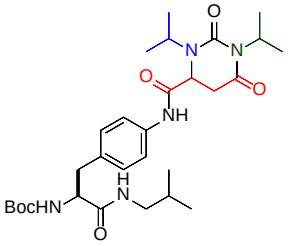


9 {a,1,10} (71%)
Method B



9 {a,1,11} (82%)
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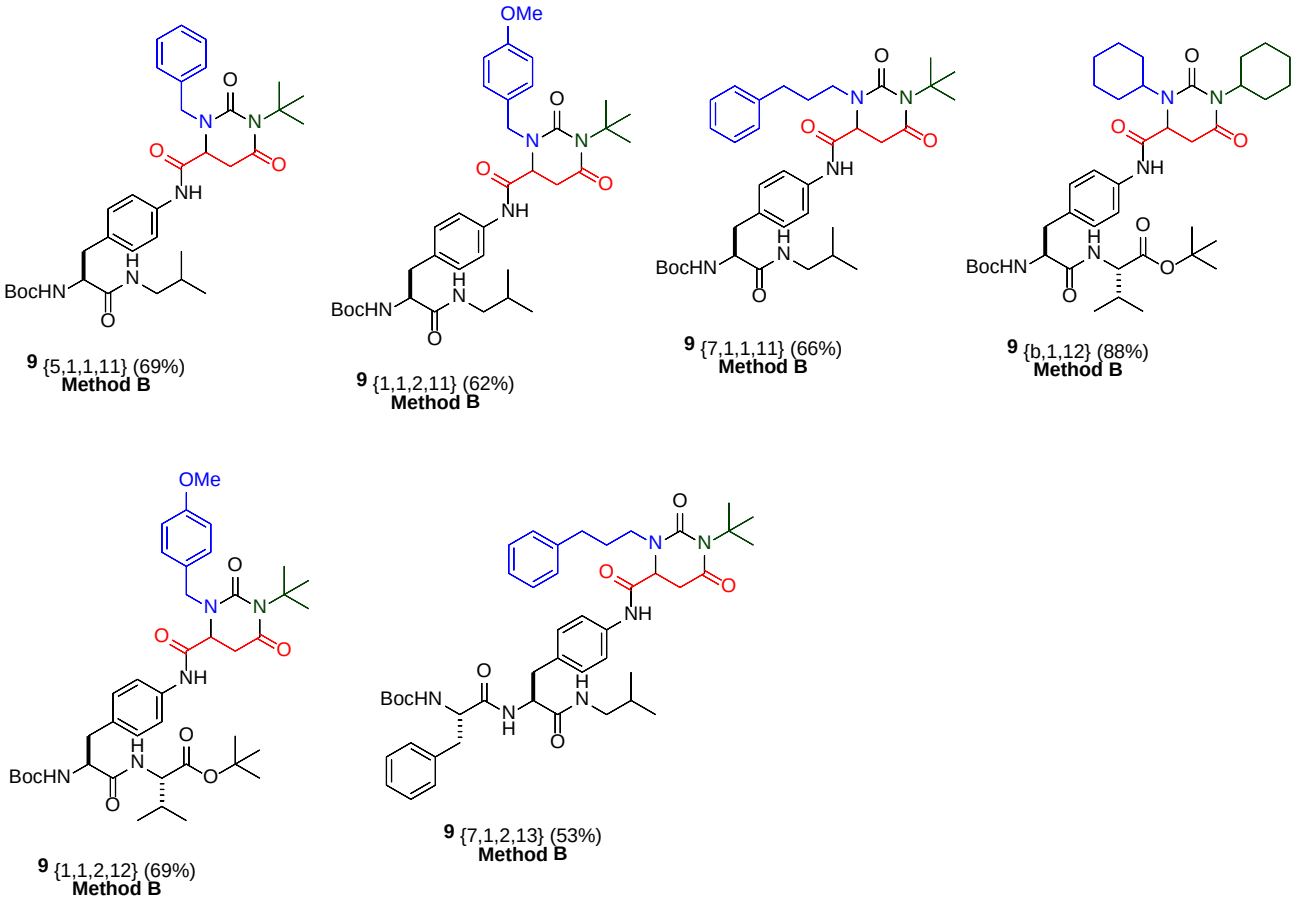
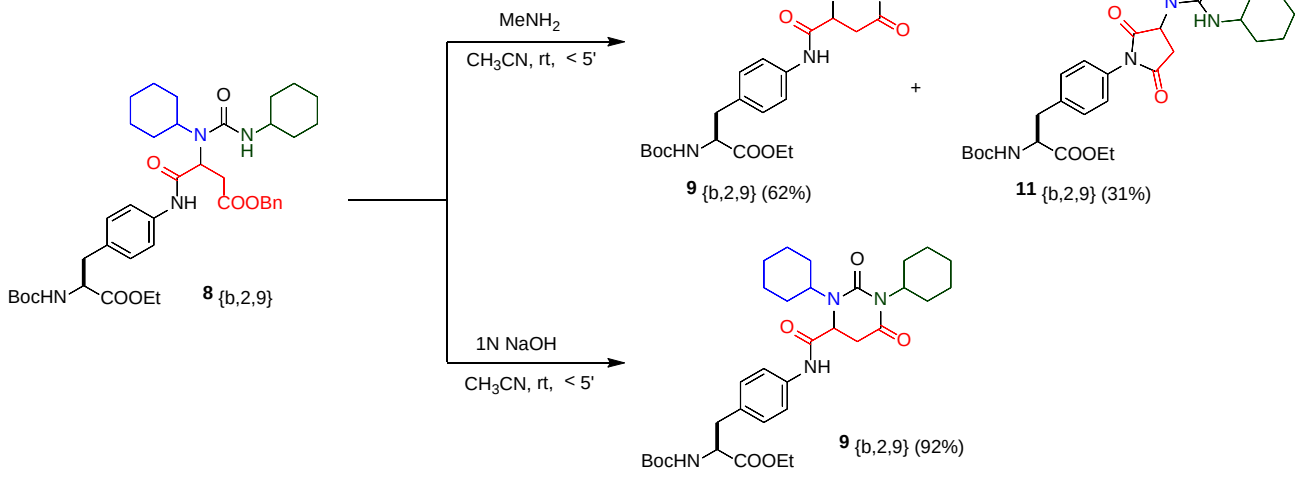
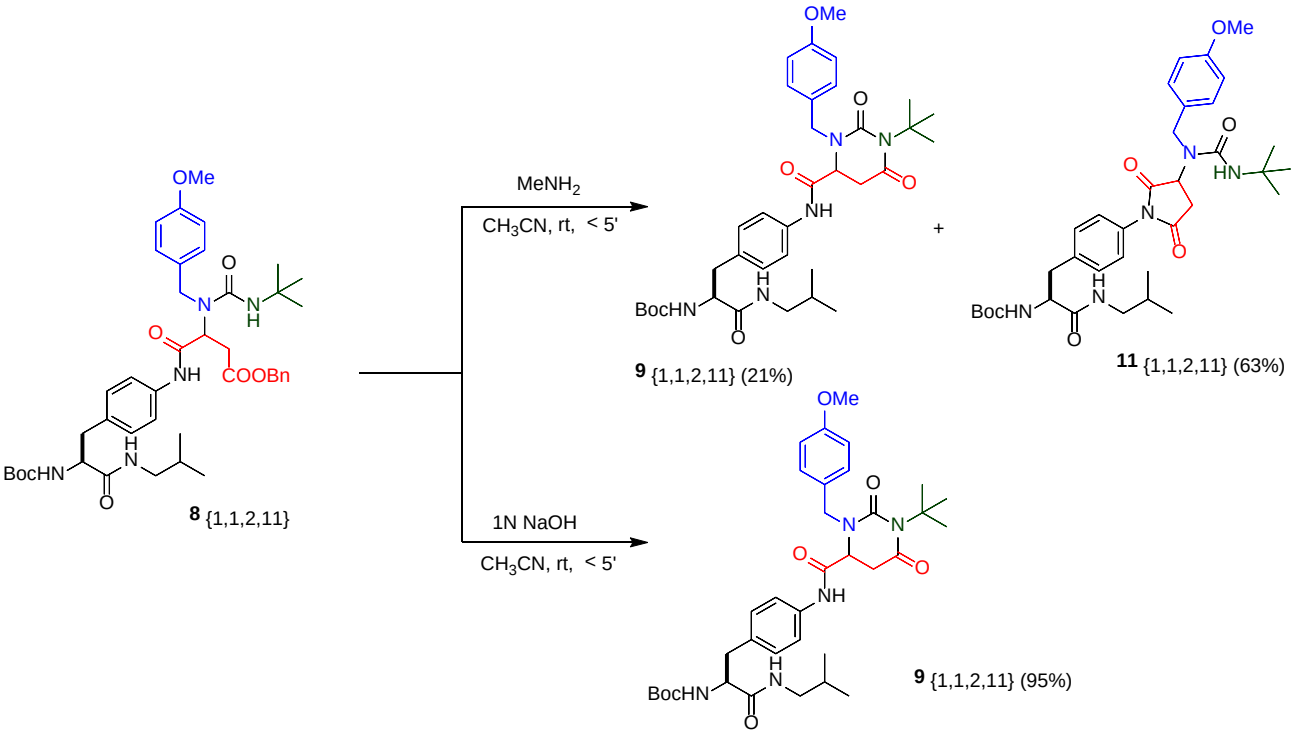


Figure 3. Library of *N*,*N*'-disubstituted DHO amides encompassing 4-Aph(Hor) derivatives.



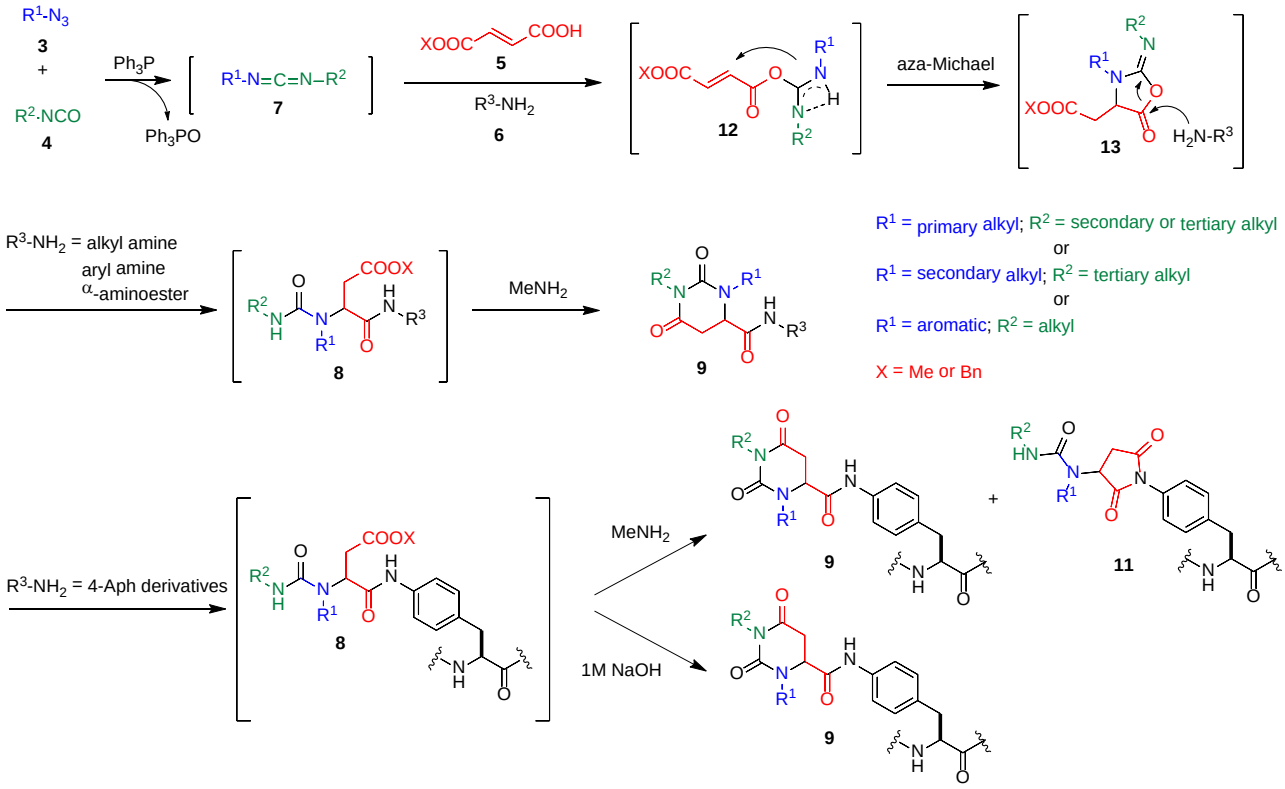


Scheme 2. Attempts to regioselective cyclization of 4-Aph-Asp-urea **8**.

With this result in hand, we checked the possibility to apply these conditions for the MC sequential synthesis of 4-Aph(Hor) conjugates **9**. We were delighted to observe that in all cases the formation of desired conjugates **9** arose with good yields through a one pot, completely regiospecific cyclization process, *i.e.* we did not detect the formation of byproducts **11** in any reaction performed. Indeed, the reaction worked efficiently starting either from 4-Aph esters with different protecting groups at the α-nitrogen, such as N^{α}-Boc-4-Aph ethyl ester **6**{*9*}, and N^{α}-Ac-4-Aph ethyl ester **6**{*10*}, from 4-Aph amide **6**{*11*}, and from 4-Aph dipeptides **6**{*12-13*} (chemset 5, Figure 2). Again, it was not surprising that starting from azides and isocyanates with different substituents, the reaction was totally regiospecific leading to the formation of DHO derivatives having the less sterically hindered substituent (or aromatic substituent as for compound **9**{*1,5,1,9*}) at the N-3 position. Quite surprisingly, the diastereosisomers formed during the reactions with 4-Aph derivatives **6**{*9-13*} are isochrones in all cases. Indeed, although a 1:1 mixture of two diastereoisomers is formed, the ^{1}H and ^{13}C NMR spectra of the inseparable mixtures are very clean showing a single set of signals (see copies of the NMR spectra in SI). In order to prove the presence of the two diastereoisomers, we recovered intermediate **8**{*5,1,2,10*} and submitted to hydrogenolysis of the benzyl ester (which

cleaved also the benzyl moiety at N-3) followed by coupling with (S)-alanine methyl ester (Scheme S4). NMR spectra of the final adduct **14** showed two sets of signals belonging to the two diasterosisomers (see ^{1}H and ^{13}C NMR in SI).[17]

The currently accepted mechanism of the sequential MC process[18] starts with reaction between azide **3** and isocyanate **4** in the presence of Ph_3P that produces carbodiimide **7** through Staudinger/aza-Wittig reactions (Scheme 3).



Scheme 3. Proposed mechanism of the sequential MC process

It is worth noting that this is the yield-limiting step since when we run the sequential MC/post-cyclization process with commercially available carbodiimides **7**{*a*,*b*}, the final adducts are obtained with excellent yields in all cases. Once carbodiimide **7** is formed (TLC monitoring), the addition in the reaction solution of amine **6** followed by acid **5** leads to the formation of *O*-acylisourea intermediate **12** which readily cyclizes to **13** before the amine can acts as nucleophile as it happens in usual carbodiimide-promoted coupling reactions. The cyclization process is totally regiospecific and arises from nucleophilic attack of the less hindered nitrogen. Once **13** has formed, the amine steps-in the reaction mechanism leading to the formation of urea-Asp amide **8**. The addition of a base triggers

the immediate cyclization of urea-Asp secondary amides to afford DHO amides **9**, while starting from urea-Asp tertiary amides the cyclization process did not occur. In general, $MeNH_2$ works efficiently. However, when the amidic proton of **8** is more acidic, such as in DHO amide arising from the nucleophilic attack of 4-Aph derivatives **6**{*9-13*}, cyclization leading to the formation of succinimide ring **11** becomes competitive. In these cases, the use of a stronger base such as a 1N aqueous solution of NaOH is required to obtain a regiospecific cyclization.

To explain these results, we first reasoned that some conformational effects, due to hydrogen bond networking, could be responsible for different reactivity of the urea-NH nitrogen in affording product **9** or **10**.[15] Firstly, we computationally investigated the conformational behavior of compounds **8a,b,d** and **8e** to gather information about the presence of hydrogen bonds involving the urea-NH hydrogen, thus inhibiting the reactivity toward the intramolecular cyclization. In compound **8a,b,d** two NH (a urea-NH and an amide-NH) are present which are capable of forming four distinct hydrogen bonds with carbonyls: *A* and *B* H-bonds involve the urea moiety whereas *C* and *D* H-bonds belong to the amide bond (*A*-*D*, Figure 4). For compound **8e** only the urea-NH can be involved in two distinct H-bond (*A*, *B*, Figure 4)

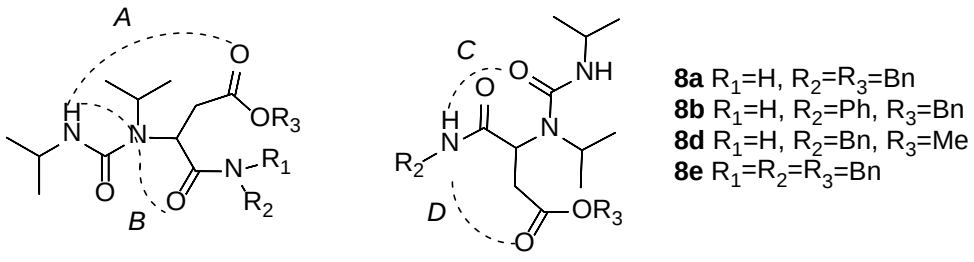


Figure 4. Intramolecular hydrogen bond patterns for compounds **8a,b,d,e**.

A conformational analysis was performed with a combined Monte Carlo search and a Molecular Mechanics energy minimization (MM/MC). Results are reported in Table 1 as percentage of conformers, which have a specific intramolecular hydrogen bond.

Table 1. Results from conformational analysis.

	Urea NH H-Bond		Amide NH H-Bond	
Compound	*A*	*B*	*C*	*D*
8a	4%	17%	27%	12%
8b	3%	5%	61%	5%
8d	8%	25%	22%	27%
8e	36%	5%	--	--

From this analysis, it appears that for compounds **8a,b,d** there is a preference to form an intramolecular H-bond *C* and *D* with the amide-NH (39% of conformers for **8a**, 66% for **8b** and 49% for **8d**) over the urea-NH hydrogen. As obvious, for **8e** only the urea-NH can be considered to form an H-bond (41% of conformers). For compound **8a** we also compared the lowest energy conformers having the urea-NH or the amide-NH hydrogen bonds by computing their energy by DFT calculations at the 6-31G(d,p) level and results confirmed that there is a preference for the formation of a type *C* intramolecular hydrogen bond (Figure 5).

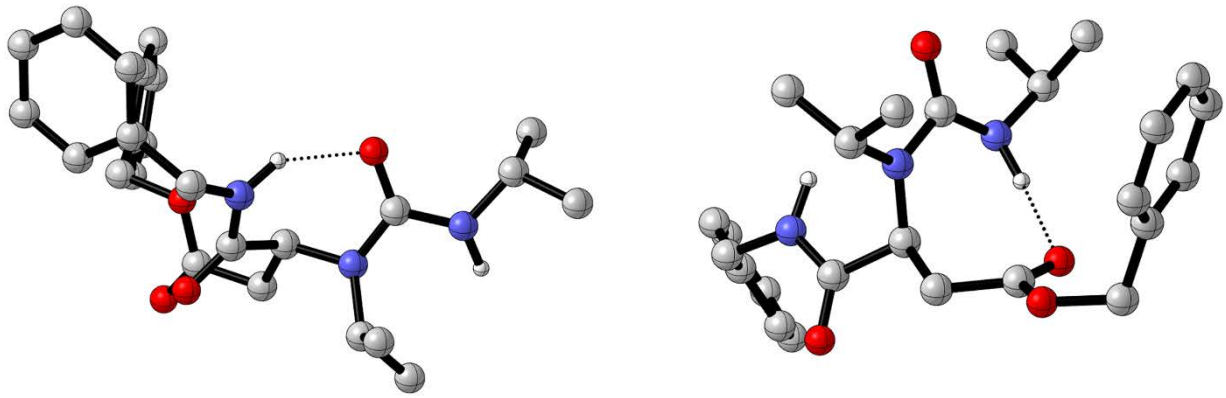


Figure 5. Lowest energy conformer for **8a** (left, rel. E = 0.00 Kcal/mol) and the first conformer having an urea-NH hydrogen bond (right, rel. E = 0.42 Kcal/mol).

These observations prompted us to suppose that the presence of an intramolecular hydrogen bond with the urea-NH in compound **8e** locked the nitrogen atom in a less reactive conformation and this could be one of the reasons why the intramolecular cyclization cannot occur. Moreover, this H bond can activate the ester function toward nucleophilic attack, thus favoring the formation of amide

product **10**. For compounds **8a,b,d**, the urea-NH nitrogen is free to react because of a preference to form the intramolecular H-bond with the amide moiety and the cyclization to form **9a,b,d** can take place. Then, we turned our attention to the possible importance of the second substituent on the urea nitrogen not involved in the cyclization process.[19] Indeed, a preliminary study on the acidity of the NH groups revealed that the urea-NH becomes much more acidic (four order of magnitude) when the other nitrogen belonging the urea moiety is disubstituted, becoming almost two order of magnitude more acidic than the amide-NH (Figure 6).

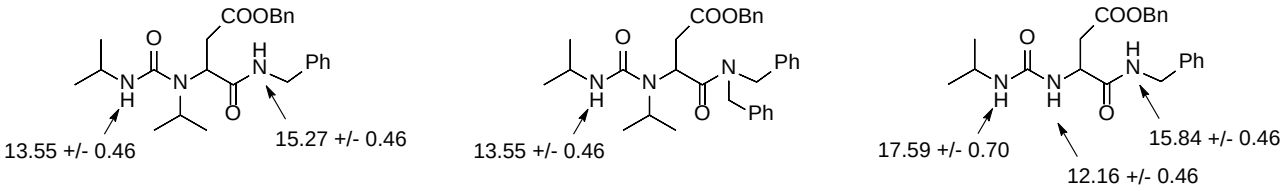
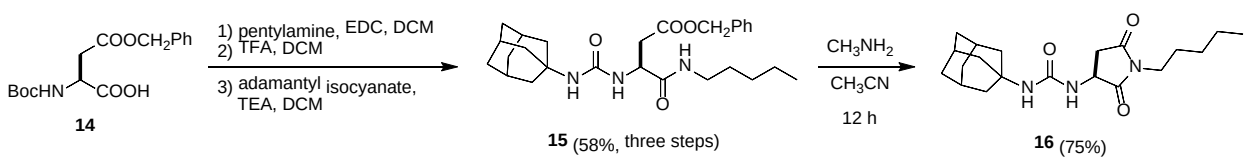


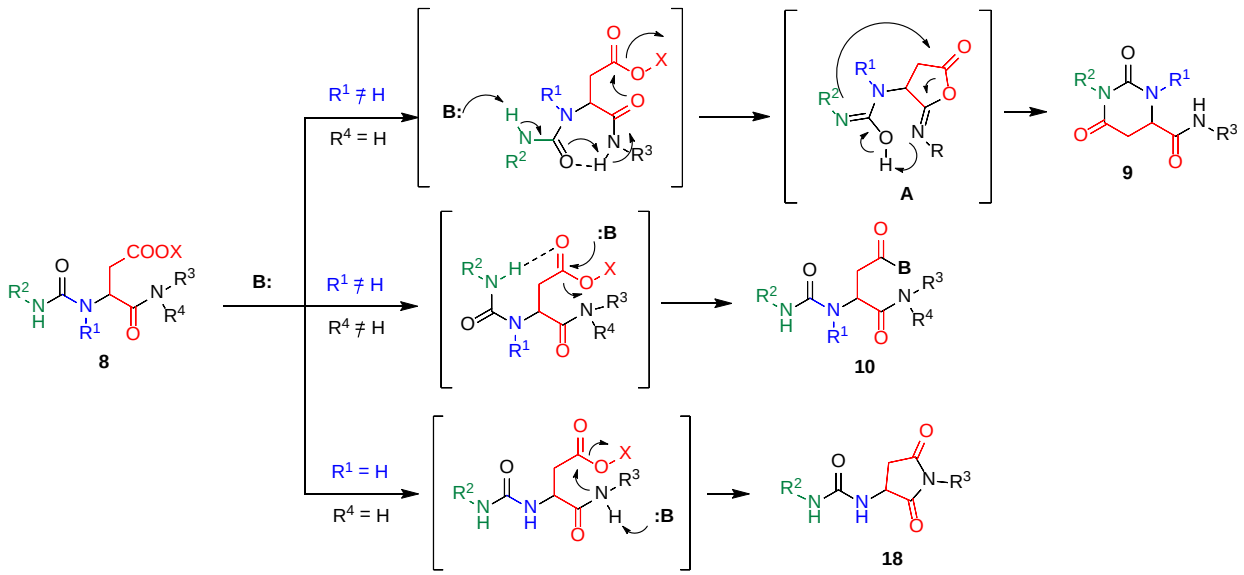
Figure 6. pKa values of the NH moieties. pKa values were calculated with the software ACD Labs 6.0

Thus, we synthetized urea-Asp conjugate **15** through a stepwise strategy, namely coupling Boc-L-aspartic acid 4-benzyl ester **14** with pentyl amine followed by *N*-deprotection and reaction with adamantly isocyanate (lipophilic admantyl and pentyl substituents were chosen to make compound **15** soluble in acetonitrile). Compound **15** was dissolved in CH_3CN and treated with a 10% in volume of a 8.03 M solution of methylamine in ethanol. Under these conditions we slowly obtained (12 hours) the five-member imide ring **16** which proved to be very stable (Scheme 4).[20] This result clearly demonstrates that in order to obtain the fast cyclization to DHO amides **9**, it is mandatory that the urea nitrogen not involved in the process is disubstituted.[21]



Scheme 4. Cyclization of disubstituted urea-Asp **15**.

Based on the experimental and computational results, and on literature reports, a possible mechanism for the cyclization process is shown in Scheme 5. We speculate that, when $R^1 \neq H$ and $R^4 = H$, deprotonation of the more acidic urea-NH triggers the formation of a formal negative charge on the amide oxygen which attacks the ester moiety generating a highly reactive 5-membered lactone-like intermediate **A**.[22] In the next step, **A** can undergo opening by the attack of nucleophilic urea nitrogen producing final DHO amide **9**. It is worth noting that these two processes are very fast, occurring in less than five minutes. Starting from tertiary amides (R^1 and $R^4 \neq H$) this mechanism cannot occur. In this case, the ester moiety, activated by the intramolecular hydrogen bond, slowly reacts with the nucleophilic base giving rise the formation of the methyl amide. Finally, starting from *N,N'*-monosubstituted urea-Asp conjugates (R^1 and $R^4 = H$) deprotonation occurs at the more acidic NH-amide producing the slow formation of stable five-membered imide ring **18** when the base is methylamine, while hydrolysis of the ester moiety occurs when the base is aqueous NaOH.[19]



Scheme 5. Proposed mechanism for the cyclization process.

CONCLUSION

In conclusion, we have developed for the first time a sequential MC domino process for the synthesis of intriguing DHO amides by a one-pot, post-cyclization of *N*-urea-aspartate which arises

under mild conditions. The process is high yielding and, by selecting the suitable base to trigger the last cyclization step, totally regioselective. The process is also modular since it works efficiently with different alkyl and aromatic azides and isocyanates, fumaric acid monoesters and primary amines. In particular, starting from α-aminoacid or peptides as nucleophiles it is possible to prepare libraries of peptidomimetics having at the *N*-terminus a *N,N'*-disubstituted DHO heterocycle. Moreover, by running the process in the presence of 4-Aph derivatives, we were able to prepare a large array of structurally diverse conjugates bearing *N*-substituted 4-Aph(Hor) moiety which is a key component of GnRH antagonists such as Degarelix. The application of the described methodology, which looks also suitable for solid-phase/combinatorial synthesis, to incorporate *N*-substituted DHO amides into known DHO-containing biologically relevant compounds or to find new molecular hits, as well as the development of a stereoselective version of the process, is currently in progress in our laboratories.

Experimental Section

General Methods: Commercially available reagent-grade solvents were employed without purification. TLC were run on silica gel 60 F_{254} Merck. Flash chromatography (FC) was performed with silica gel 60 (60-200 μm, Merck). ^{1}H NMR spectra were recorded on 400 MHz spectrometers. Chemical shifts are expressed in ppm (δ), using tetramethylsilane (TMS) as internal standard for ^{1}H and ^{13}C nuclei (δ_H and δ_C = 0.00). 4-Aph derivatives **6**{*9-13*} were prepared starting from commercial available Boc-4-nitro-L-phenylalanine with usual coupling reaction followed by hydrogenation at ambient pressure catalyzed by Pd/C of the nitro group. Compound **8a,g** were obtained as described in reference 10 b.

General procedure for the MC process followed by post-cyclization with a solution of $MeNH_2$ in ethanol. To a solution of *para*-methoxybenzyl azide **3**{*1*} (0.31 mmol, 1 equiv) and *tert*-butyl isocyanate **4**{*1*} (0.32 mmol, 1.05 equiv) in CH_3CN (3 mL), solid Ph_3P (0.32 mmol, 1.05 equiv) was added at rt. The solution is left to stir overnight. The temperature is lowered to 0 °C and TMP (0.32 mmol, 1.05 equiv), neat *iso*-butyl amine **6**{*2*} (0.32 mmol, 1.05 equiv) followed by a

solution of acid **5**{*2*} (0.26 mmol, 1.2 equiv) in a minimum amount of CH_3CN were added. The reaction was left to stir at rt until complete disappearance of the carbodiimide was detected (TLC monitoring, typically 3 hours). A commercial available 8.03 M ethanolic solution of $MeNH_2$ (0.3 mL) was added at rt. After 5 minutes the reaction was acidified with a 1N aqueous solution of HCl and extracted with AcOEt. The collected organic phases were dried over Na_2SO_4, filtered, and the solvent evaporated. The crude was purified by flash chromatography affording compound **9**{*1,1,2,2*} in 68% isolated yield.

Representative example of DHO acid derivative. *1-(tert-butyl)-N-isobutyl-3-(4-methoxybenzyl)-2,6-dioxohexahydropyrimidine-4-carboxamide* ***9****{1,1,2,2}: Rf* = 0.37 (hexane:AcOEt = 50:50); ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.21 (d, *J* = 8.8 Hz, 2H, aromatics), 6.84 (d, *J* = 8.8 Hz, 2H, aromatics), 5.83 (br s, 1H, NH), 4.72 (d, *J* = 14.8 Hz, 1H), 4.21 (d, *J* = 14.8 Hz, 1H), 3.98 (t, *J* = 5.2 Hz, 1H), 3.78 (s, 3H), 3.02-2.98 (m, 2H), 2.62 (dd, *J* = 15.2 and 5.2 Hz, 1H), 2.51 (dd, *J* = 15.2 and 5.2 Hz, 1H), 1.70 (septet, *J* = 6.4 Hz, 1H), 1.61 (s, 9H), 0.87 (d, *J* = 6.4 Hz, 6H); ^{13}C-NMR (100.6 MHz, $CDCl_3$); δ = 173.7, 168.1, 159.2, 157.3, 129.6, 128.4, 114.1, 58.1, 55.6, 55.2, 47.1, 44.5, 36.8, 28.7, 28.3, 20.09, 20.08; ESI (m/z) 412.2, $[M+Na, (100)]^+$; Anal. calcd. for $C_{21}H_{31}N_3O_4$: C 64.76, H 8.02, N 10.79; found: C 64.77, H 8.02, N 10.80.

General procedure for the MC process followed by post-cyclization with 1M aqueous solution of NaOH. To a solution of *para*-methoxybenzyl azide **3**{*1*} (0.31 mmol, 1 equiv) and *tert*-butyl isocyanate **4**{*1*} (0.32 mmol, 1.05 equiv) in CH_3CN (3 mL), solid Ph_3P (0.32 mmol, 1.05 equiv) was added at rt. The solution is left to stir overnight. The temperature is lowered to 0 °C and TMP (0.32 mmol, 1.05 equiv), a solution of amine **6**{*9*} (0.32 mmol, 1.05 equiv) in a minimum amount of CH_3CN followed by a solution of acid **5**{*1*} (0.26 mmol, 1.2 equiv) in a minimum amount of CH_3CN were added. The reaction was left to stir at rt until complete disappearance of the carbodiimide was detected (TLC monitoring, typically 3 hours). A 1N aqueous solution of NaOH (0.3 mL) was added at rt. After 5 minutes the reaction was acidified with a 1N aqueous solution of HCl and extracted with AcOEt. The collected organic phases were dried over Na_2SO_4, filtered, and

the solvent evaporated. The crude was purified by flash chromatography affording compound **9** {1,1,1,9} in 61% isolated yield.

Representative example of 4-Aph(Hor) derivative. *ethyl (2S)-2-((tert-butoxycarbonyl)amino)-3-(4-(1-(tert-butyl)-3-(4-methoxybenzyl)-2,6-dioxohexahydropyrimidine-4-carboxamido)phenyl)propanoate* ***9****{1,1,1,9}*. *Rf* = 0.35 (hexane:AcOEt = 40:60); ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.74 (br s, 1H, NH-amide), 7.28 (d, *J* = 8.0 Hz, 2H, aromatics), 7.12 (d, *J* = 8.4 Hz, 2H, aromatics), 6.99 (d, *J* = 8.4 Hz, 2H, aromatics), 6.71 (d, *J* = 8.0 Hz, 2H, aromatics), 4.90 (br d, *J* = 7.6 Hz, 1H, NH-carbamate), 4.57 (d, *J* = 15.2 Hz, 1H), 4.45 (m, 1H), 4.30 (d, *J* = 15.2 Hz, 1H), 4.08 (q, *J* = 7.2 Hz, 2H), 3.96 (m, 1H), 3.65 (s, 3H), 3.00 (dd, *J* = 11.6 and 5.6 Hz, 1H), 2.94 (dd, *J* = 11.6 and 5.6 Hz, 1H), 2.63 (m, 2H), 1.55 (s, 9H), 1.35 (s, 9H), 1.17 (t, *J* = 7.2 Hz, 3H); ^{13}C-NMR (100.6 MHz, $CDCl_3$); δ = 173.9, 171.7, 166.1, 159.3, 157.2, 136.5, 132.2, 129.9, 129.7, 128.6, 128.3, 119.7, 114.2, 79.9, 61.4, 58.3, 55.8, 55.2, 54.5, 44.7, 37.9, 37.7, 28.7, 28.3, 14.2; ESI (m/z) 663.1 $[M+K, (5)]^+$, 647.2, $[M+Na, (100)]^+$; Anal. calcd. for $C_{33}H_{44}N_4O_8$: C 63.44, H 7.10, N 8.97; found: C 63.42, H 7.10, N 8.98.

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Abbreviations

DHO, dihydroorotic; DHODH, dihydroorotate dehydrogenase; GnRH, gonadotropin-releasing hormone; 4-Aph, 4-aminophenylalanine; 4-Aph(Hor), N^4-(dihydroorotyl)-4-aminophenylalanine; Asp, aspartic.

Supporting Information Available: Characterization of all the new compounds not present in the Experimental Section. Copies of ^{1}H NMR, ^{13}C NMR, and ESI-MS spectra of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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15. In literature we found only one example for the synthesis of DHO acid amides by cyclization of ureido-Asp cyclic amides: Herrero, S., Salgado, A., Garcia-Lopez, M. T., Herranz, R. Synthesis of chiral 1,6,8-trioxoperhydropyrazino[1,2-*c*]pyrimidines as novel highly functionalized scaffolds for peptidomimetics. *Terahedron Lett.* **2002**, *43*, 4899-4902. In this work, the cyclization process was influenced by the stereochemistry of tryptophan of urea-oxopiperazine derivatives. Indeed, cyclization of compounds bearing L-tryptophan arose with an equimolar amount of DBU at rt, while starting from the analogues bearing D-tryptophan it was necessary to heat at refluxing temperature.

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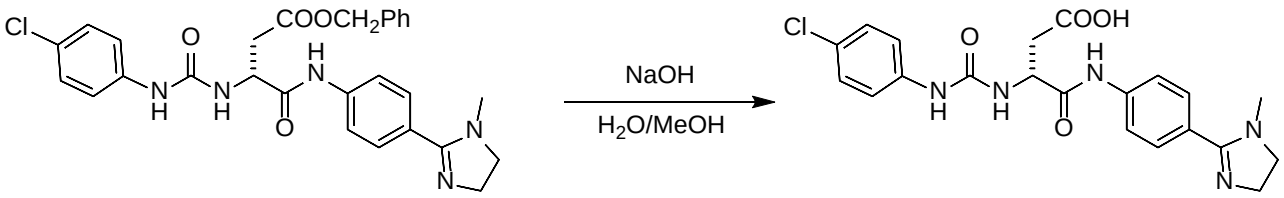
17. We tried to perform HPLC analysis on different compounds **9**. We were able to obtain good separation only with compound **9**{*a,2,9*} which chromatogram showed the presence of two peaks with an area ratio of 1:1 (see SI, page S67).

18. Marcelli, T., Olimpieri, F., Volonterio, A. Domino synthesis of 1,3,5-trisubstituted hydantoins: a DFT study. *Org. Biomol. Chem.* **2011**, *9*, 5156-5161.

19. Looking at the literature, we found only one example where treatment of *N*-aryl-*N'*-urea-Asp amide 4-benzyl ester with a 1N aqueous solution of NaOH lead to the hydrolysis of the ester function rather than cyclization to DHO amide:

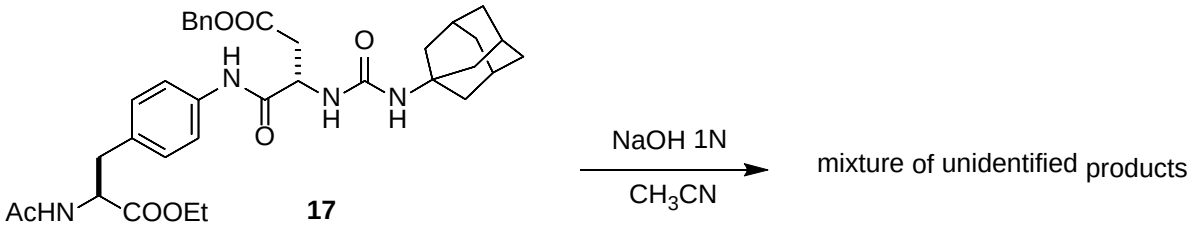


Song, Y.; Zhu, B.; Wang, S.; Bhakta, C.; Scarborough. Preparation of amino acid urea derivatives as factor Xa inhibitors. **2006**, WO 2006063113.

20. The structure of compound **16** was determined through ^{1}H and ^{1}H-^{1}H COSY NMR (see SI, Scheme S5). The spectra clearly show the presence of two signals belonging to NH protons: one

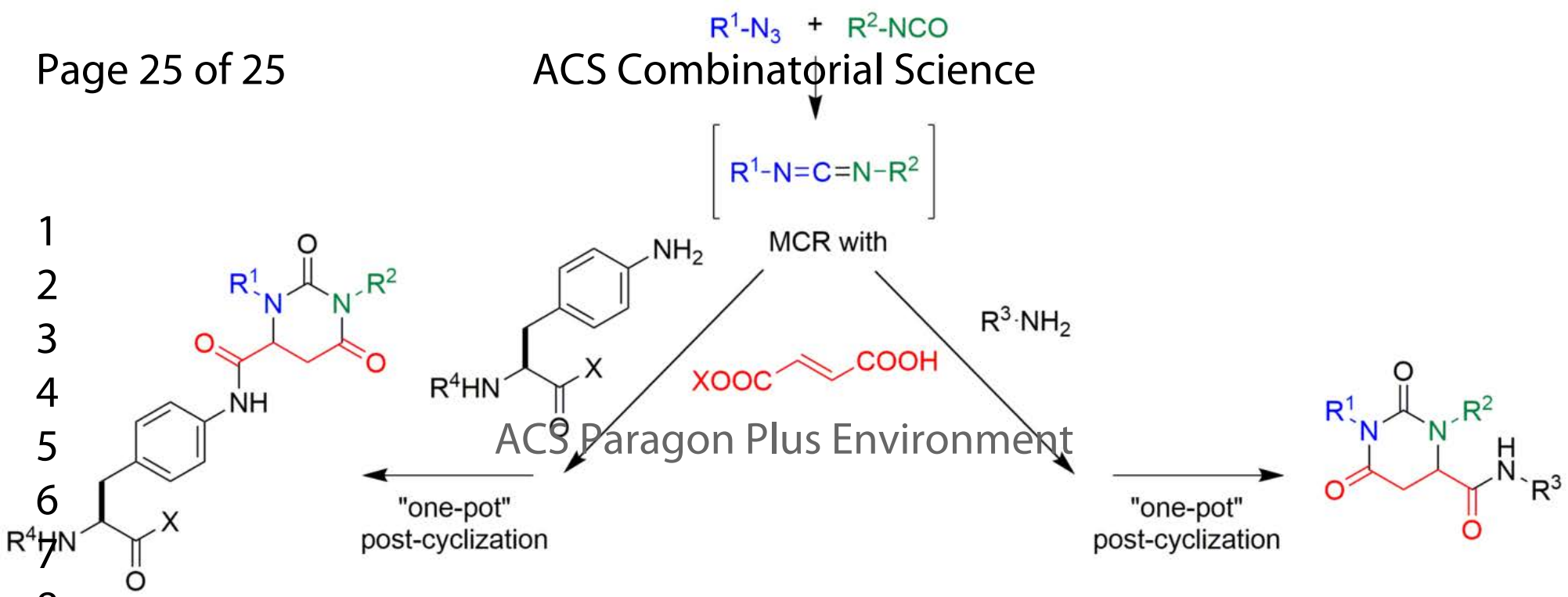
singlet resonating at 5.38 ppm (urea-NH linked to adamantane) and one doublet (other urea-NH) resonating around 6.24 ppm, which shows a cross peak with a multiplet resonating around 4.26 ppm belonging to the Cα of the Asp moiety.

21. A similar attempt was made by adding a 10% in volume of a 1N aqueous solution of NaOH to 4-Aph-Asp derivative **17** dissolved in CH_3CN:



However, in this case we obtained a mixture of unidentified products.

22. In hope of trapping a reactive intermediate, we performed the cyclization of 4-Aph-Asp **8**{*b,2,9*} in the presence of 1, 5, and 10 equivalents of highly electrophilic benzyl bromide. However, by quenching the reaction after 5 minutes, we always recovered 4-Aph(Hor) **9**{*b,2,9*} as the only product.


R1-N3 + R2-NCO
R1-N=C=N-R2
MCR with
NH2
R4HN
X
O
XOOC
COOH
R3-NH2
ACS Paragon Plus Environment
"one-pot"
post-cyclization
"one-pot"
post-cyclization
R1
N
R2
O
NH
R4HN
X
O
H
N
R3