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Combinatorial Aid for Underprivileged Scaffolds: Solution and Solid-phase Strategies for a Rapid and Efficient Access To Novel Aza-diketopiperazines (Aza-DKP)

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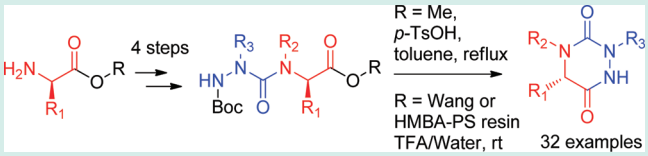
Supporting Information

ABSTRACT: An efficient solution-phase synthesis of aza-diketopiperazines (aza-DKP, triazinediones) is reported. A structurally diverse collection of *c*-[aza-alkylGly-Pro] derivatives and yet unreported 2,4,5-trisubstituted-1,2,4-triazine-3,6-diones has been synthesized starting from Fmoc-L-Pro-OH and various Fmoc-L-amino acids. To extend the practical value of this class of dipeptidomimetics, a general solid-phase synthesis approach amenable to library production was developed on both Wang-PS and HMBA-PS resins. The final acidic treatment of the resins in TFA/water mixture at room temperature enabled the rapid and quantitative cyclization/release highly pure triazinediones. The conformational preferences and the spatial organization of the three substituents of a representative 2,4,5-trisubstituted-1,2,4-triazine-3,6-dione were investigated by X-ray diffraction and ^{1}H NMR spectroscopy.



KEYWORDS: *aza-DKP, triazinedione, heterocycle, peptidomimetic, solid-phase synthesis, molecular diversity*

INTRODUCTION

The vast majority of biologically active organic compounds are heterocyclic in nature, a fact that has been heavily exploited by the pharmaceutical industry.[1,2] Among these compounds, 2,5-diketopiperazines (DKPs) occupy a special position as they link peptide and heterocycle chemical space. DKPs result from the cyclization of a linear dipeptide and are the smallest possible cyclopeptides. This motif is present in the core of many natural products and synthetic compounds that display interesting therapeutic properties.[3,4] DKPs have been shown to inhibit several enzymes including PDE-5 by the clinically approved drug Cialis, as well as to recognize, modulate, and control the activity of many receptors, such as calcium channels and opioid,[5] GABAergic,[6] serotoninergic $5HT_{1A}$,[7] and oxytocin receptors.[8] Thus, the DKPs are an excellent example of a privileged scaffold that confers biological activity across a variety of drug targets.[9−11] From the combinatorial point of view, DKPs have the further advantage of ease of synthesis from dipeptides that can be assembled with high diversity from commercially available amino acids. While the concept of privileged scaffolds is a useful strategy in drug discovery, it is nevertheless self-fulfilling as it is based on precedent. A scaffold that leads to biologically active compounds will attract interest by medicinal chemists who will then produce more examples of the same and discover new active compounds that further confirm the hypothesis. There should then exist examples of "underprivileged scaffolds" that are intrinsically suitable for drug discovery applications but in practice are underrepresented or absent. For example, scientists at Celltech have recently generated a list of heterocycles that have yet to be synthesized and would have the advantage of novelty compared to the patent literature.[12] In the same way, we believe the attractive features of DKPs as a privileged scaffold can be carried over to novel DKP-like scaffolds that are less well-known and provide interesting opportunities for lead discovery. One such example is the 1,2,4-triazine-3,6-dione (aza-DKP, Figure 1). This class of heterocycles can be viewed as constrained cyclic dipeptidomimetic DKP analogues whereby one C_α-stereogenic center has been replaced by a planar nitrogen. Compared to the extensive medicinal and combinatorial chemistry literature on DKPs, there are very few examples on the synthesis of aza-DKPs. Pinnen et al. described the pioneering synthesis of *c*-[aza-Phe-Pro] in their research toward pseudopeptidic ergopeptines.[13] More recently, two groups have devised synthetic routes to access *c*-[aza-alkylGly-Pro] derivatives.[14,15] A few examples of 1,4-di- and 1,4,5-trisubstituted-1,2,4-triazine-3,6-diones synthesis have also been described

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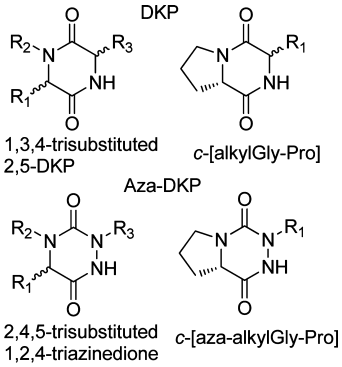


Figure 1. General structure of diketopiperazines (DKP) and aza-diketopiperazines (Aza-DKP).

either in solution or on solid-phase.[16,17] However, to date, no examples of 2,4,5-trisubstituted-1,2,4-triazine-3,6-diones have been reported in the literature.

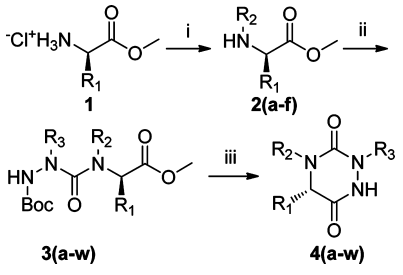
This novel heterocycle is particularly appealing in term of "drug-likeness" because it fulfills the following requirements: (1) it is a compact six-membered ring heterocycle of low molecular weight, which can then be decorated by a set of three variable substituents; (2) it contains five heteroatoms able to increase the overall hydrophilicity of compounds; (3) the total number of H-bond donors and acceptors in the core is one and five, respectively, meaning that decoration of the core can add several such groups without infringing Lipinski's "Rule of Five".

To exploit the potential of this "underprivileged scaffold", a combinatorial route that can generate new examples of the heterocycle are needed. In this article, we report a concise and rapid strategy toward *c*-[aza-alkylGly-Pro] derivatives and 2,4,5-trisubstituted-1,2,4-triazine-3,6-diones that has been developed in solution-phase and advantageously adapted to solid support to afford a set of novel triazinediones. Conformational and structural features of one representative example were investigated by X-ray diffraction and ^{1}H NMR spectroscopy.

RESULTS AND DISCUSSION

Solution-Phase Synthesis. On the basis of the results published by Obreza et al.[14] a synthetic approach was devised starting from commercially available α-amino acid esters (Scheme 1). This approach features mainly three steps: (1) the reductive alkylation of α-amino acid esters with various aldehydes; (2) the semicarbazide formation with presynthesized *N*′-alkyl *tert*-butyl carbazate; and (3) the final deprotection and cyclization of the semicarbazide precursor to afford triazinediones.

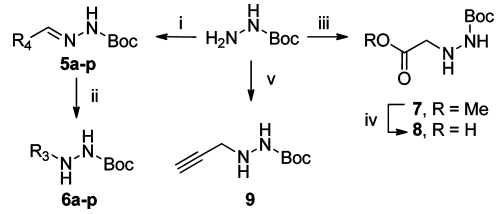
Scheme 1. Solution-Phase Approach Towards aza-DKP[a]



[a]Reagents and conditions: (i) (a) aldehyde, NEt_3, MeOH, overnight, rt; (b) $NaBH_4$, MeOH, 30 min, rt; (ii) $R_3NHNHBoc$, BTC, CH_2Cl_2, or THF, Hünig's base, 10 min, rt; (iii) *p*-TsOH, toluene, reflux, 2.5 h (method A) or Amberlyst 15 resin, MeOH, μwave 150 W, 120–140 °C, 15–20 min (method B).

N′*-Alkyl/Aryl tert-Butyl Carbazates Synthesis.* On the basis of literature,[18] commercially available *tert*-butyl carbazate was reacted with benzaldehyde in THF for 2 days. Without isolation, the corresponding hydrazone was subsequently reduced in the presence of sodium cyanoborohydride ($NaCNBH_3$), AcOH 1% to provide hydrazine **6a** in a moderate 56% yield. In an attempt to improve the yield of the reaction, hydrazone formation was performed in toluene at 50 °C overnight. Following isolation by simple precipitation and purification by recrystallization, hydrazone **5a** was reduced as described above to furnish hydrazine **6a** in an improved 85% yield (Scheme 2).

Scheme 2. Synthesis of *N*′-Alkyl/Aryl *tert*-Butyl Carbazates[a]



[a]Reagents and conditions: (i) R_4CHO, toluene, 50 °C; (ii) (a) $NaBH_3CN$, THF, *p*-TsOH, (b) NaOH, MeOH, rt; (iii) Ethyl bromoacetate, water, 1 h, rt; (iv) LiOH, dioxane/water, 1 h, 40 °C; (v) propargyl bromide, K_2CO_3, THF/DMF (9/1), overnight, rt.

With these optimized conditions, a set of structurally diverse hydrazines **6b–n** were thus obtained with yields ranging from 40% to 75% for the two steps (Table 1). However, for compounds **6o**, **p** the reduction of the precursor hydrazones was found more difficult to achieve. Prolonged heating at 40 °C overnight failed to provide the expected hydrazines. Therefore, an alternative protocol was developed on the basis of the use of *p*-toluenesulphonic acid (*p*-TsOH) instead of acetic acid.[19] Hydrazones **5o**, **p** were refluxed in THF for four hours in the presence of $NaBH_3CN$ and *p*-TsOH to successfully provide hydrazines **6o**, **p** in 73% and 69% yields, respectively, for the two steps. Hydrazines **7** and **9** (Scheme 2) were prepared by direct alkylation of *tert*-butyl carbazate with either ethyl bromoacetate or propargyl bromide.[20,21] The slow addition of bromo derivatives to an excess of *tert*-butyl carbazate (3–5 equiv) enabled the formation of dialkylated hydrazine by-products to be minimized. Propargyl **9** and ester **7** were isolated by column chromatography in 48% and 76% yield respectively. A subsequent hydrolysis of ester **7** under basic conditions gave access to hydrazine **8** in 79% yield. Finally, one example of aryl hydrazine (compound **10**) was also prepared by condensation of phenylhydrazine to di-*tert*-butyl dicarbonate (Boc_2O) in refluxing dichlororomethane for 2 h (72% yield).

Alkylated Amino Ester Synthesis. Their preparation was performed as previously described in the literature starting from α-amino acid esters **1** (Scheme 1).[22] Imine formation was accomplished in the presence of NEt_3 either in THF or MeOH. The addition of $MgSO_4$ was found to facilitate the imine formation. The subsequent reduction was carried out with sodium borohydride ($NaBH_4$) in MeOH for 1 h. According to this one-pot process, six alkylated amino esters **2a–f** were prepared in yields ranging from 65 to 79% (Table 2).

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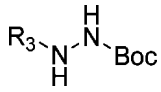
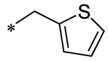
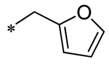
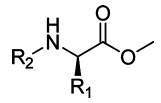
Table 1. Representative *N*′-Alkyl *tert*-Butyl Carbazate Derivatives



compound	R_3	yield (%)[a]
6a	Bn-	85
6b	4-MeOBn-	73
6c	4-Me_2NBn-	64
6d	$CH_3(CH_2)_4$-	70
6e	2-BrBn-	75
6f	3-BrBn-	71
6g	4-BrBn-	65
6h	2-MeOBn-	51
6i	3-MeOBn-	57
6j	3,5-$(MeO)_2$Bn-	61
6k	2,4,6-$(MeO)_3$Bn-	45
6l		68
6m		57
6n	Me_2CHCH_2-	52
6o	4-NO_2Bn-	73[b]
6p	3,4,5-$(MeO)_3$Bn-	69[b]
8	HOOC-CH_2-	60[c]
9	HC≡C−CH_2−	48
10	Ph-	72[d]

[a]Overall yields from *tert*-butyl carbazate. [b]*p*-TSOH was used instead of AcOH. [c]Two steps overall yields. [d]Boc_2O, CH_2Cl_2, phenylhydrazine, 2.5 h.

Table 2. Alkylated Amino Esters 2a−f



compound	R_1	R_2	yield (%)[a]
2a	$(CH_3)_2CHCH_2$-	Bn-	67
2b	$HOCH_2$-	Bn-	95
2c	2-indoleCH_2-	Bn-	65
2d	Bn-	Bn-	74
2e	$(CH_3)_2CHCH_2$-	4-NO_2Bn-	79
2f	$(CH_3)_2CHCH_2$-	4-MeOBn-	72

[a]Overall yields from L-amino esters **1**.

Semicarbazide Formation. Starting from alkylated amino esters **2a−f**, optimized conditions for the synthesis of the semicarbazide derivatives were investigated by the fine-tuning of various parameters such as: the nature of the carbonyl activation reagents (bis(trichloromethyl)carbonate (BTC), phosgene, carbonyldiimidazole), the nature of the solvent (CH_2Cl_2 vs THF), the sequence of addition of the reagents and the reaction time. Best results were obtained by activating *N*′-alkyl/aryl *tert*-butyl carbazates with BTC in the presence of Hünig's base before adding dropwise the resulting mixture to the alkylated amino esters. The nature of the solvent used to carry out this latter reaction was found to have a profound effect on the yield. Table 3 describes semicarbazides synthesized following the optimal experimental conditions. For compounds **3a**, **c**, **h**, the reaction performed in CH_2Cl_2 failed to provide the expected semicarbazides whereas in THF the yields were 81%, 48% and 55% respectively. These results could be ascribed to the general better solubility of *N*′-alkyl/aryl *tert*-butyl carbazates and alkylated amino esters in THF.

Semicarbazide Cyclization Studies. This final step was investigated by using model semicarbazide **3i**. Attempts to perform the cyclization applying Obreza conditions,[14] that is, HCl in acetic acid, failed to afford the expected triazinedione **4i**. Following optimization, the triazinedione was finally obtained (58% yield) in the presence of *p*-TsOH in refluxing toluene for 30 min (Method A). Microwave-assisted heating in the presence of Amberlyst 15 resin, MeOH at 140 °C for 20 min (Method B) gave similar results (55% yield). Nevertheless, the use of polymer-supported *p*-TSOH (Amberlyst 15 resin) made the final workup easier. Structure assignment for **4i** was done by ^1H, ^{13}C NMR and IR analysis and compared to the data previously described in the literature.[9] The optimized synthetic protocol was then employed with semicarbazides **3a−w** above to access both novel *c*-[Aza-alkylGly-Pro] derivatives and 2,4,5-trisubstituted-1,2,4-triazine-3,6-diones (Table 4). In all cases but five (compounds **4k**, **l**, **p**, **t**), the expected triazinediones were isolated in low to moderate yields. Extending the time or increasing the temperature did not improve yields but led in most cases to the decomposition of semicarbazides. For triazinediones **4s** and **4u**, the best conversion was obtained by replacing MeOH by acetonitrile. Noteworthy, when R_3 = aryl (semicarbazide **3c**), the cyclization did not proceed and only deprotected semicarbazide was recovered. Semicarbazides **3k**, **l**, **p**, **t**, decomposed under the reaction conditions without forming any corresponding triazinediones.

The solution-phase approach developed in the first part of this study allowed for the access to novel and structurally diverse collections of aza-DKP heterocycles. Given the promising results obtained above, we decided to undertake a solid-phase approach with the aim: (1) to facilitate the access to such compounds by avoiding intermediate purification; (2) to improve both the yield and purity of final compounds; (3) to develop a strategy amenable to libraries production in a multiparallel format.

Solid-Phase Approach. *Synthesis on Wang-PS Resin.* The optimization of the process was performed on the model 2,4,5-tribenzyl-1,2,4-triazine-3,6-dione **16a** starting from the commercially available Fmoc-L-phenylalanine-Wang-PS resin **11** (Scheme 3). All solid-phase reactions were monitored by using different colorimetric tests (chloranil, trinitrobenzene sulfonate (TNBS), Ninhydrin)[23] but also by treatment of an aliquot of resin in trifluoroacetic acid (TFA)/water (95:5) mixture followed by liquid chromatography−mass spectrometry (LC-MS) analysis of the crude sample.

Our approach to the solid-phase synthesis of model triazinedione **16a** involved a reductive amination step of the resin-bound primary amine **12** with benzaldehyde in trimethylorthoformate (TMOF), followed by the reduction of the imine with $NaBH_3CN$ in the presence of 1% AcOH.[24,25] To avoid bis-alkylation, the excess of benzaldehyde was removed by filtration prior to the imine reduction, leading to the clean secondary amine **13**. Next, the preparation of solid-supported semicarbazide **15** was attempted following procedures described in the literature for the solid-phase synthesis of azapeptides, that is, the solution-phase activation of carbazate **6a** with phosgene prior to the addition to the resin-bound secondary amine **13**.[26] However, in our hand, this approach failed to provide the expected semicarbazide, only the starting secondary amine being detected by LC-MS. This result suggests that the steric hindrance of the resin-bound secondary amine **13** prevents the reaction with the activated carbazate. Hence, the semicarbazide formation

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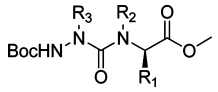
Table 3. Semicarbazides 3a−w



compound	R_1	R_2	R_3	yield (%)[a]	
				CH_2Cl_2	THF
3a	$HOCH_2$-	Bn-	Bn-	0	81
3b	$(CH_3)_2CHCH_2$-	Bn-	Bn-	66	
3c	$(CH_3)_2CHCH_2$-	Bn-	Ph-	0	48
3d	$(CH_3)_2CHCH_2$-	Bn-	4-MeOBn-	90	
3e	$(CH_3)_2CHCH_2$-	4-NO_2Bn-	4-MeOBn-		76
3f	Bn-	Bn-	4-NO_2Bn-		82
3g	Bn-	Bn-	$CH_3(CH_2)_4$-		88
3h	2-indoleCH_2-	Bn-	3,4,5-$(MeO)_3$Bn-	0	55
3i	-$(CH_2)_3$-		Bn-		
3j	-$(CH_2)_3$-		3,4,5-$(MeO)_3$Bn-		
3k	-$(CH_2)_3$-		2-furanCH_2-		62
3l	-$(CH_2)_3$-		2-thiopheneCH_2-		73
3m	-$(CH_2)_3$-		2-MeOBn-		68
3n	-$(CH_2)_3$-		3-MeOBn-		64
3o	-$(CH_2)_3$-		3,5-$(MeO)_2$Bn-		86
3p	-$(CH_2)_3$-		2,4,6-$(MeO)_3$Bn-		56
3q	-$(CH_2)_3$-		2-BrBn-		98
3r	-$(CH_2)_3$-		3-BrBn-		90
3s	-$(CH_2)_3$-		4-BrBn-		52
3t	-$(CH_2)_3$-		4-MeOBn-		98
3u	-$(CH_2)_3$-		4-NO_2Bn-		94
3v	-(*R*)-$CH_2CHOHCH_2$-		3,4,5-$(MeO)_3$Bn-		92
3w	-(*R*)-$CH_2CHOHCH_2$-		Bn-		93

[a]Overall yields from alkylated amino esters **2a−f**. Following secondary amines treatment with BTC, resulting activated compounds were treated with *N*′-alkyl/aryl *tert*-butyl carbazate either in CH_2Cl_2 or in THF.

was envisaged by solid-phase activation of secondary amine before addition of an excess of carbazate, as previously described for the solid-phase urea formation.[27] Resin **13** was then treated with BTC, 2,4,6-collidine in THF for 30 min before addition of a 10-fold excess of carbazate **6a**. After one night stirring at room temperature, the resin was washed, dried and treated with a 95% TFA/H_2O mixture for 1 h. The LC-MS analysis of the crude material indicated the presence of triazinedione **16a** together with unreacted secondary amine. Unexpectedly, no trace of uncyclized semicarbazide was detected suggesting that the conversion of the resin-bound semicarbazide **15** to triazinedione **16a** was quantitative during the final acidic treatment at room temperature. This promising result led us to optimize various parameters of the reaction such as the nature of the solvent (CH_2Cl_2, 1,2-dichloroethane (DCE), THF), the nature of the base (Hünig's base, Et_3N, Pyridine/4-dimethylaminopyridine (DMAP)) and the temperature (rt, 40 °C, 70 °C, microwave (μw)). Both the temperature of the reaction and the nature of the solvent were found crucial to drive the semicarbazide formation to completion. Indeed, following secondary amine activation with BTC, the resin treated with an excess of carbazate **6a** in CH_2Cl_2 at room temperature provided triazinedione **16a** in 15% yield, whereas in THF the yield reached 33% overall for the six-step solid-phase process. The reaction performed overnight at 70 °C in THF further increased the yield up to 61%. Finally, best results were obtained with Hünig's base over the other bases evaluated. Isolated triazinedione **16a** was fully characterized by ^1H, ^{13}C, and 2D NMR techniques (HSQC, HMBC and NOESY experiments) and high-resolution mass spectrometry (HRMS).

To the best of our knowledge, the solid-phase semicarbazide cyclization initiated in highly acidic conditions has not been described. Apart from the work of Obreza[10] and our solution-phase study herein described, the reaction between hydrazine derivatives and electrophilic ester bond proceeds in basic conditions.[28] Here, an acidic treatment at room temperature for one hour enabled a one-pot three-step process i.e. semicarbazide deprotection followed by the concomitant cyclization/release to provide trisubstituted triazinedione. It is noteworthy that the cyclization did not proceed when R_2 = H (Scheme 4). In this case, the disubstituted triazinedione **16b** was isolated in a very low 3% yield, whereas the corresponding linear semicarbazide **18** was obtained in 62% yield. Similarly, the influence of the *N*-alkylation of the primary amine upon the cyclization has been previously reported for the synthesis of thiohydantoins.[29]

We also investigated the possibility to prepare D-triazinediones starting from the Fmoc-D-amino acid-Wang-PS resins (Figure 2). According to the same protocol and starting from Fmoc-D-phenylanine-Wang-PS resin, D-triazinedione **19** was obtained in a comparable 54% yield with optical rotation of opposite value compared to L-triazinedione **16a** (−24.9 vs +28.0, respectively).

With optimized conditions in hand, the scope of our solid-phase methodology was demonstrated by the synthesis of a small library (Table 5) starting from various Fmoc-L-amino acids such as Phe, Leu, Ser(*t*Bu), Glu(*t*Bu), Lys(Boc). Resin **12** was reacted with benzaldehyde, isovaleraldehyde, Boc-aminoethanal, 3,5-dimethoxybenzaldehyde, *p*-nitrobenzaldehyde and

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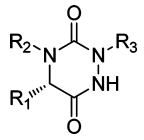
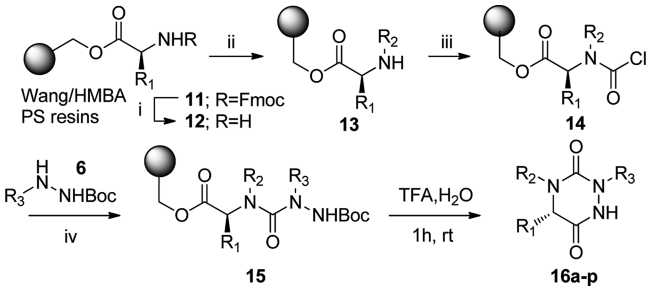
Table 4. Solution-Phase Cyclization Towards Aza-DKP 4a−w



compound	R_1	R_2	R_3	yield (%)[a] method A[b]	yield (%)[a] method B[c]
4a	$HOCH_2$-	Bn-	Bn-		72
4b	$(CH_3)_2CHCH_2$-	Bn-	Bn-	48	
4c	$(CH_3)_2CHCH_2$-	Bn-	Ph-	nd[d]	nd
4d	$(CH_3)_2CHCH_2$-	Bn-	4-MeOBn-	43	
4e	$(CH_3)_2CHCH_2$-	4-NO_2Bn-	4-MeOBn-		66
4f	Bn-	Bn-	4-NO_2Bn-		38
4g	Bn-	Bn-	$CH_3(CH_2)_4$-		59
4h	2-indoleCH_2-	Bn-	3,4,5-$(MeO)_3$Bn-	40	
4i	-$(CH_2)_3$-		Bn-	58	55
4j	-$(CH_2)_3$-		3,4,5-$(MeO)_3$Bn-	28	
4k	-$(CH_2)_3$-		2-furanCH_2-		nd
4l	-$(CH_2)_3$-		2-thiopheneCH_2-		nd
4m	-$(CH_2)_3$-		2-MeOBn-		20
4n	-$(CH_2)_3$-		3-MeOBn-		50
4o	-$(CH_2)_3$-		3,5-$(MeO)_2$Bn-		66
4p	-$(CH_2)_3$-		2,4,6-$(MeO)_3$Bn-		nd
4q	-$(CH_2)_3$-		2-BrBn-		65
4r	-$(CH_2)_3$-		3-BrBn-		50
4s	-$(CH_2)_3$-		4-BrBn-		19
4t	-$(CH_2)_3$-		4-MeOBn-		nd
4u	-$(CH_2)_3$-		4-NO_2Bn-		21
4v	-(*R*)-$CH_2CHOHCH_2$-		3,4,5-$(MeO)_3$Bn-		63
4w	-(*R*)-$CH_2CHOHCH_2$-		Bn-		44

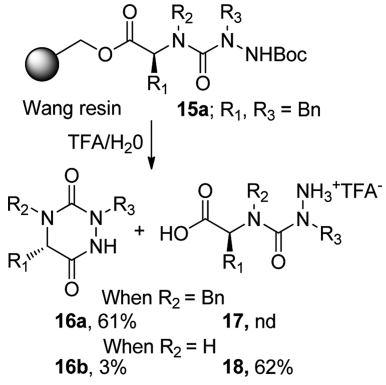
[a]Isolated yield starting from protected semicarbazides **3a−w**. [b]Method A: *p*-TsOH, toluene, reflux, 2.5 h. [c]Method B: Amberlyst 15 resin, MeOH, μwave 150 W, 120−140 °C, 15−20 min. [d]Not detected by LC-MS.

Scheme 3. Solid-Phase Strategy Towards Aza-DKP 16a−p[a]



[a]Reagents and conditions: (i) 20% piperidine/DMF; (ii) aldehyde or ketone, TMOF, $NaBH_3CN$, AcOH 1%; (iii) BTC, Et_3N, CH_2Cl_2; (iv) R_3-NHNHBoc, THF, 70 °C, overnight.

Scheme 4. Influence of the *N*-Alkylation upon the Cyclization



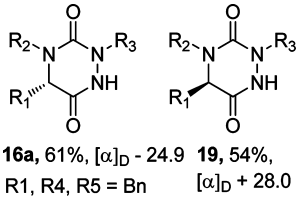


Figure 2. Synthesis of L- and D-triazinediones **16a** and **19**.

cyclohexanone to form the corresponding imines that were reduced in presence of $NaBH_3CN$ and 1% AcOH. Following activation of the secondary amines **13** with BTC, the semicarbazide formation was performed in presence of various carbazates *N*-alkylated with Bn-, isopentyl-, *p*-nitrobenzyl-, and propargyl moieties at 70 °C in THF, overnight. The final deprotection, cleavage and cyclization were initiated in presence of a TFA/water (95/5) mixture for 1 h at rt. In all cases, triazinediones were obtained in >60% purity (HPLC analysis, UV detection at 220 nm) and in yields ranging from 9 to 78% for the 6 steps. These results show that the process herein developed is compatible with the presence of hydrophobic, polar, cationic or anionic aminoacids (Table 5, compounds **16a−e**), neutral, electron-rich, and aliphatic aldehydes (Table

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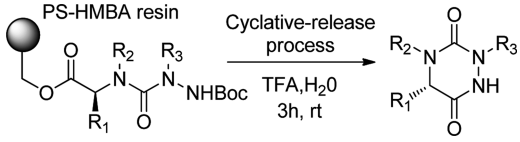
Table 5. Representative 2,4,5-Trisubstituted-1,2,4-triazine-3,6-diones 16a−o, 4b, and *c*-[Aza-Phe-Pro] 4i Synthesized from Fmoc-L-amino Acid Wang-PS Resins

compound	R_1	R_2	R_3	mass $[M + H]^+$	t_R,[a] (min)	HPLC[b] (%)	yield[c] (%)
16a	Bn-	Bn-	Bn-	386.47	11.4	94	61
16b	Bn-	H-	Bn-	296.34	8.1	<10	3
16c	$HOCH_2$-	Bn-	Bn-	326.37	7.7	91	37
16d	$HOOC(CH_2)_2$-	Bn-	Bn-	368.41	8.1	91	57
16e	$NH_2(CH_2)_4$-	Bn-	Bn-	366.47	6.8	90	35
16f	Bn-	$(CH_3)_2CH(CH_2)_2$-	Bn-	366.48	11.7	90	48
16g	Bn-	NH_2-$(CH_2)_2$-	Bn-	339.41	6.9	65	25
16h	Bn-	3,5-$(MeO)_2$Bn-	Bn-	446.52	11.5	62	9
16i	Bn-	*p*-NO_2Bn-	Bn-	431.47	11.3	75	34
16j	Bn-	*c*-C_6H_{11}-	Bn-	378.49	11.8	60	26
16k	Bn-	Bn-	$(CH_3)_2CH(CH_2)_2$-	366.48	11.7	90	67
16l	Bn-	Bn-	*p*-NO_2Bn-	431.47	11.4	77	37
16m	Bn-	Bn-		334.39	9.9	91	50
16n	Bn-	Bn-	HOOC−CH_2-	354.14	10.7	84	47
16o	Me-	*p*-NO_2Bn-	Bn-	355.36	7.5	88	54
4b	$(CH_3)_2CHCH_2$-	Bn-	Bn-	352.45	11.5	>98	63
4i	-$(CH_2)_3$-		Bn-	Bn-	6.4	>98	78

[a]Retention times from analytical RP-HPLC profile (UV detection at 220 nm, see Supporting Information for HPLC experimental conditions). [b]Determined by RP-HPLC analysis of the crude product. [c]Isolated yield based on manufacturer's loading of Fmoc-L-aminoacid Wang resin.

Table 6. Triazinediones Obtained Starting from HMBA-PS Resin Following a Final Cyclative-Release Step



compound	R_1	R_2	R_3	HPLC (%)	yield (%)
16a	Bn-	Bn-	Bn-	94[a]/99[b]	61[a]/93[b]
16f	Bn-	$(CH_3)_2CH(CH_2)_2$-	Bn-	90/96	48/57
16h	Bn-	3,5-$(MeO)_2C_6H_3CH_2$-	Bn-	62/96	9/53
16i	Bn-	*p*-$NO_2C_6H_5CH_2$-	Bn-	75/95	34/62

[a]Wang-PS resin. [b]HMBA-PS resin.

5, compounds **16f−i**) but also cyclohexanone (Table 5, compound **16j**). The propargyl derived triazinedione **16m** could be further functionalized by Cu^I-catalyzed-1,3-dipolar cycloaddition ("Click chemistry") to provide new chemical entities containing a 1,2,3-triazole ring.[30−33] Carboxylic acid derived triazinedione **16o** could be also readily functionalized by coupling reaction with commercially available amine building blocks to increase further the molecular and structural diversity of the compounds. The successful synthesis of compound **4i** shows that the strategy is also efficient to rapidly access *c*-[aza-Phe-Pro] analogues and represents a straightforward alternative to the solution-phase strategies already described in the literature.[10,15] Interestingly, the comparison of the yields obtained for triazinediones **4b** and **4i** by both strategies highlighted the efficacy of the solid-phase approach (23 and 33% in solution respectively versus 63% and 78% on solid-phase).

To improve further the purity of the crude triazinediones, we envisaged the use of an acid-stable linker. The potential side-products formed during the synthesis (bis-alkylated compounds or secondary amines) would then be expected to remain attached to the resin while the desired product is released by intramolecular cyclization. This cyclative-release process has already been advantageously considered for the synthesis of potential bioactive compounds such as DKP,[4] hydantoin,[14] or tetrahydro-*β*-carbolinehydantoin[34] scaffolds on various resins.

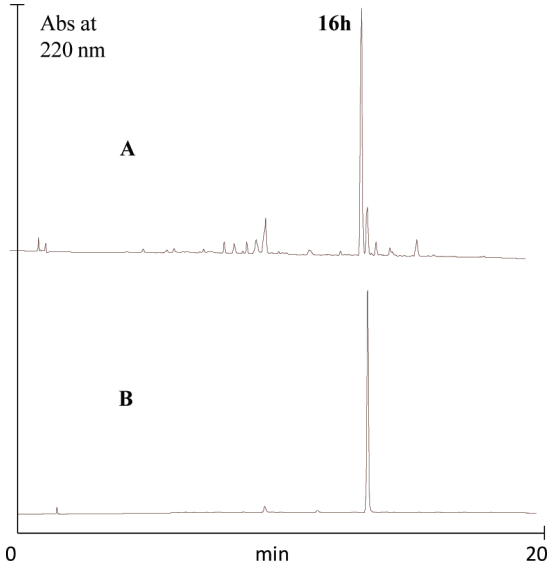


Figure 3. RP-HPLC profiles of triazinedione **16h** obtained either on (A) Wang or (B) HMBA resins. Chromatographic conditions: C18 Symmetry Shield column (4.6 × 150 mm). Flow rate 1 mL.min^{-1}. Buffer A: 0.1% aqueous TFA. Buffer B: 0.1% TFA in CH_3CN. Gradient: 0−100% B over 20 min. Detection at 220 nm.

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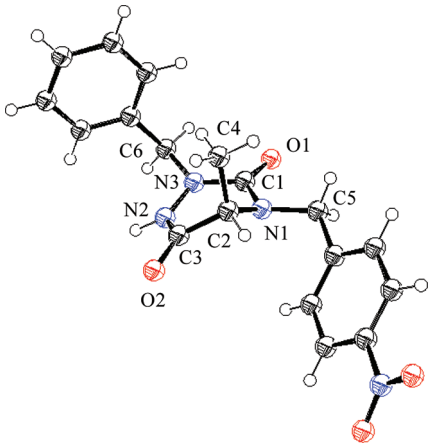

Figure 4. ORTEP view of compounds **16o** (displacement ellipsoids at the 50% probability level).

The final cyclization was generally initiated under basic conditions and not in highly acidic medium such as TFA.

Synthesis on HMBA-PS Resin. The potential of our approach was evaluated on 4-Hydromethyl benzoic acid-polystyrene (HMBA-PS) resin which was esterified with Fmoc-L-Phe-OH using the symmetrical anhydride method.[35] Following Fmoc resin loading determination,[36] the same protocol that was developed on Wang resin was applied for the synthesis of representative compounds **16a**, **f**, **h**, **i**. Resin-bound semicarbazides were treated in TFA/water (95:5) mixture for 3 h. As observed on Wang resin, in such acidic conditions, all the target triazinediones were released in solution. As expected, the cyclative-release process ensured better purities than those obtained on Wang resin (Table 6).

Figure 3 shows the RP-HPLC profiles of crude compound **16h** obtained either on Wang (A) or HMBA (B) resins enabling the improvement of the purity from 62% to 96%, respectively. Unexpectedly, yields were also increased significantly for all compounds (Table 6). Thus, the strategy developed on HMBA resin represents a convenient and efficient entry to highly pure triazinediones.

*Triazinedione **16o** Conformational Analysis by X-ray and ^1H NMR.* Single-crystal X-ray analysis of triazinedione **16o** enabled the determination of both the conformation of the scaffold and also the spatial arrangement of the side chains. The ORTEP diagram (Figure 4) shows that the six-membered heterocyclic ring adopts a pseudo boat conformation. The C2−C3−N2−N3 atoms are planar whereas N3−C1−N1−C2 display a torsion angle of −33 °C. The methyl group C4 (R_1 substituent) is oriented pseudoaxial in the same side of the plane as the benzyl group at N3 (R_3 substituent), but slightly displaced from each other. The pseudoquatorial hydrogen at C2 displays the same spatial orientation as the *p*-nitrobenzyl group at N1 (R_2 group).

Figure 5 shows the ^1H NMR spectrum of triazinedione **16o** in various deuterated solvents and temperature. In $CDCl_3$ at 298 K, benzyl protons featured an AB quartet for H6a,b ($\Delta\delta_{AB}$ = 0.11, J_{AB} = 15.2 Hz) and an AX pattern centered at 4.89 ppm and 4.17 ppm for H5a,b (Δppm =0.72, J_{AX} = 15.9 Hz). When recorded in a $CDCl_3$/MeOD (1:1) mixture, both H5a,b and H6a,b resonated as an AX system (Δppm =0.71/0.68, J_{AX} = 15.3/15.8 Hz respectively) with slight modification of their chemical shifts compared to those in $CDCl_3$. In DMSO-d_6, the peaks were line broadening at 298 K but were found sharper and better resolved with increasing temperature. At 390 K, diastereotopic benzyl protons H5a,b and H6a,b displayed also an AX system (Δppm =0.45/0.48, J_{AX} = 15.4/15.9 Hz respectively) and did not give rise to the coalescence of the signal. The pseudoequatorial H2 proton came up as a quadruplet (J = 6.9 Hz). Both the solvents and temperature had a very limited influence on its chemical shift indicating that no changing in the scaffold conformation occurred. Altogether, these results indicate that in protic and/or polar solvents, regardless of the temperature, triazinedione **16o** is a rather rigid and constrained structure with hindered rotation about the phenyl-CH_2 bonds.

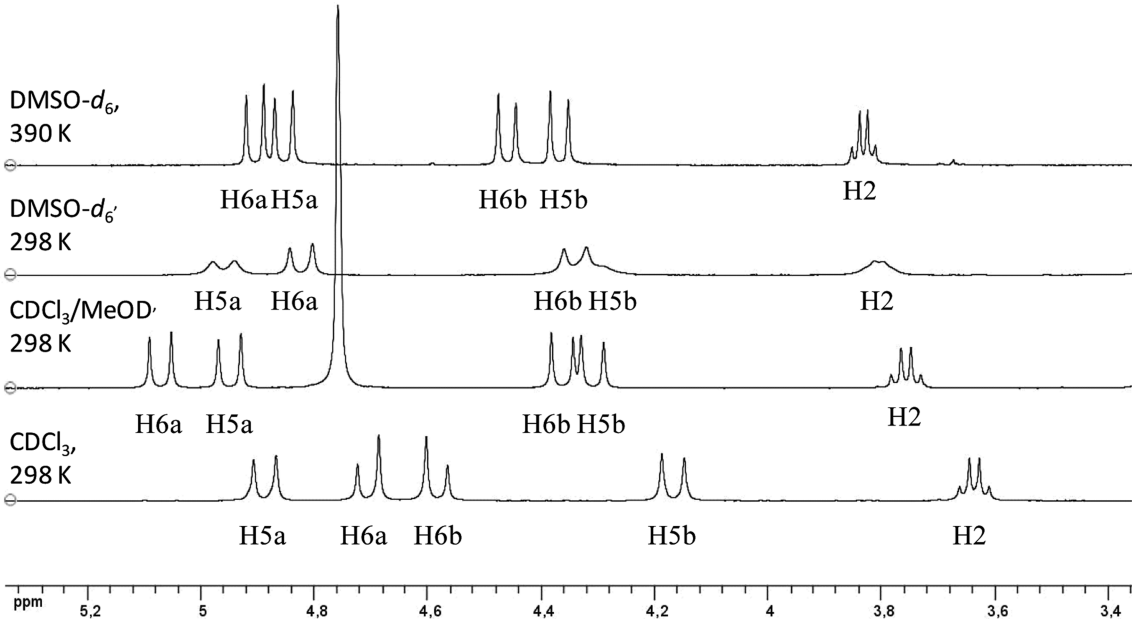

Figure 5. ^1H NMR spectrum of **16o** in various deuterated solvents.

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CONCLUSION

In conclusion, we have described in this paper a solution-phase approach toward a set of novel aza-DKP, both *c*-[aza-alkylGly-Pro] derivatives and 2,4,5-trisubstituted-1,2,4-triazine-3,6-diones. The solid-phase approach enabled a rapid access to triazinediones in good yields and high purities. The efficiency of the method makes this approach readily amenable to the parallel synthesis of structurally diverse collections of aza-DKP and will facilitate the evaluation of this underprivileged scaffold for potential interesting biological activity.

EXPERIMENTAL PROCEDURES

General Methods. Reagents were obtained from commercial sources and used without any further purification. Fmoc-protected amino acid loaded on Wang resin were purchased from Aldrich and the overall yields for the solid-phase syntheses of aza-DKPs were calculated based on the initial loadings provided by the supplier. Solid-phase reactions conducted at room temperature were performed in polypropylene tubes equipped with polyethylene frits and polypropylene caps using an orbital agitator shaking device. Solid-phase reactions at 70 °C were conducted in sealed glassware tubes using the Chemflex rotating oven from Robbins Scientific as the shaking device. Thin-layer chromatography was performed on silica gel $60F_{254}$ plates. Flash chromatography was performed on silica gel (40 μm, Grace) or RP18 (25−40 μm, Merck) prepacked columns on a SpotII ultima from Armen. ^{1}H NMR spectra were recorded at 200, 300, and 500 MHz on a Bruker Advance spectrometer. Chemical shifts are reported in parts per million (ppm), coupling constants (J) are reported in hertz (Hz). Signals are described as s (singlet), d (doublet), t (triplet), m (multiplet), and brs (broad singlet). LC-MS spectra were obtained on a ZQ (Z quadripole) Waters/Micromass spectrometer equipped with an X-Terra C18 column (4.6 × 50 mm, 3.5 μm) using electrospray ionization mode (ESI). Analytical HPLC analyses were performed on a Chromolith SpeedROD column (50 × 4.6 mm, C_{18}) from Merck using the following conditions: flow rate 7 mL.min^{-1}. Buffer A: 0.1% aqueous TFA. Buffer B: 0.1% TFA in CH_3CN. Gradient: 0% B in 1 min than 0−100% B over 5 min. Detection at 220/254 nm. Retention times (t_R) from analytical RP-HPLC are reported in minutes.

General Procedure for the Synthesis of *N*′-Alkylidene-*tert*-butyl Carbazates 5a−5p. A solution of *tert*-butylcarbazate (15.0 mmol, 1.0 equiv) and the aldehyde (16.5 mmol, 1.1 equiv) in toluene (10 mL) was stirred at 50 °C for 40 min. The reaction mixture was allowed to cool to room temperature overnight and the crude product crystallized out of solution. The product was then filtered, washed with excess cold toluene and dried in vacuo (where the product did not crystallize out of solution the solvent was removed in vacuo to give the crude product). The crude product was recrystallized.

General Procedure for the Synthesis of *N*′-Alkyl-*tert*-butyl Carbazates 6a−6p. *N*′-Hydrazone-carboxylic acid tert-butyl esters (10 mmol, 1.0 equiv) and sodium cyanoborohydride (25 mmol, 2.5 equiv) were suspended in tetrahydrofuran (10 mL). Acetic acid (2 mL) was added and the reaction mixtures stirred at room temperature overnight. In two specific cases, **5d** and **5e**, *p*-toluene-sulfonic acid was used instead of acetic acid and the reaction mixtures were refluxed for 4 h. All reactions were then concentrated in vacuo, ethyl acetate (20 mL), and water (20 mL) were added to the reaction mixture, followed by solid $NaHCO_3$ until basic. The layers were separated and the organics were washed with brine (20 mL), saturated $NaHCO_{3\ (aq)}$ (20 mL), and brine (20 mL). The organic layers were dried over $MgSO_4$, filtered and the solvent was removed in vacuo. The residues were redissolved in methanol (10 mL) and $NaOH_{\ (aq)}$ (2N, 10 mL) and stirred for 2 h. The solvent was removed in vacuo and the residues redissolved in ethyl acetate (50 mL). The organics were washed with brine (2 × 20 mL) and dried over $MgSO_4$. The solvent was removed in vacuo to give the crude products.

General Procedure for the Solution-Phase Synthesis of Semicarbazides 3a−3w. A solution of the alkyl hydrazine carboxylic acid *tert*-butyl ester (1.51 mmol, 2.25 equiv) and diisopropyl ethylamine (1.68 mmol, 2.50 equiv) in tetrahydrofuran or dichloromethane (5 mL) was added dropwise to a stirred solution of triphosgene (0.67 mmol, 1.00 equiv in dry tetrahydrofuran or dichloromethane (5 mL). After it was stirred for 5 min, a solution of amino acid methyl ester (1.51 mmol, 2.25 equiv) and diisopropyl ethylamine (1.68 mmol, 2.50 equiv) in tetrahydrofuran or dichloromethane (5 mL) was added in one-portion. The reaction mixture was stirred at room temperature for a further 10 min before the solvent was removed in vacuo. The crude product was rapidly purified by column chromatography (10−100% ethyl acetate/hexane) to give the title semicarbazide as a mixture of rotamers.

General Procedure for the Solution-Phase Synthesis of 1,2,4-Triazine-3,6-diones 4a−4w. Semicarbazide (0.43 mmol, 1.0 equiv) and *p*-toluene sulfonic acid hydrate (0.65 mmol, 1.5 equiv) were dissolved in toluene (10 mL). The reaction mixture was heated to reflux and after stirring for 30−40 min was allowed to cool to room temperature. The solvent was removed in vacuo and the crude product was purified by column chromatography (50−100% ethyl acetate/hexane) to give the title 1,2,4-triazine-3,6-dione.

(S)-2,4-Dibenzyl-5-(hydroxymethyl)-1,2,4-triazine-3,6-dione ***4a.*** Colorless oil. $[\alpha]_D$ +14.2 (*c* 0.3, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34−7.26 (m, 10H), 5.08 (d, 1H, J = 15.1 Hz), 4.91 (d, 1H, J = 15.1 Hz), 4.52 (d, 1H, J = 15.1 Hz), 4.20 (d, 1H, J = 15.1 Hz), 3.76−3.70 (m, 3H), 2.56 (br s 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.2, 154.8, 136.3, 134.8, 129.1, 128.8, 128.3, 128.2, 60.5, 60.1, 53.3, 49.2. HRMS: calcd for $C_{18}H_{20}N_3O_3$ 326.14284, found 326.15012.

(S)-2,4-Dibenzyl-5-isobutyl-[1,2,4]triazine-3,6-dione ***4b.*** White solid. mp 124−126 °C $[\alpha]_D$ +21° (*c* 1.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37−7.27 (m, 10H), 5.04 (d, 1H, J = 15.2 Hz), 4.72 (AB, 2H, $\Delta\delta$ = 0.44, J = 14.8 Hz), 4.05 (d, 1H, J = 15.2 Hz), 3.62 (dd, 1H, J = 6.4 Hz), 1.58−1.54 (m, 1H), 1.28−1.23 (m, 2H), 0.79 (dd, 6H, J = 6.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.1, 154.1, 136.4, 135.1, 128.9, 128.8, 128.3, 128.1, 127.9, 57.3, 51.2, 49.38.7, 24.4, 23.0, 21.8. MS (ESI) m/z 352.4 [M + H$^+$].

(S)-2-(4-Methoxy-benzyl)-4-benzyl-5-isobutyl-[1,2,4]-triazine-3,6-dione ***4d.*** Colorless oil. $[\alpha]_D$ +16.4 (*c* 0.8, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28 (br s, 1H), 7.34−7.27 (m, 7H), 6.87−6.85 (m, 2H), 5.02 (d, 1H, J = 15.0 Hz), 4.72 (d, 1H, J = 15.0 Hz), 4.59 (d, 1H, J = 15.0 Hz), 4.05 (d, 1H, J = 15.0 Hz), 3.80 (s, 3H), 3.62 (m, 1H), 1.61−1.24 (m, 3H, m) 0.82 (d, 3H, J = 6.6 Hz), 0.78 (d, 3H, J = 6.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.1, 159.9, 154.5, 136.6, 130.4, 129.0, 128.3, 128.1, 127.0, 114.5, 57.6, 55.4, 51.1, 49.5, 38.9, 24.6, 23.2, 21.9. MS (ESI) m/z 382.0 [M + H$^+$].

(S)-2-(4-Methoxybenzyl)-4-(4-nitrobenzyl)-5-isobutyl-1,2,4-triazine-3,6-dione ***4e.*** White crystalline solid. mp 128−130 °C (Ethyl acetate/hexane). $[\alpha]_D$ +10.4 (*c* 0.4, $CHCl_3$). ^{1}H

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NMR (400 MHz, $CDCl_3$) δ 8.22−8.20 (m, 2H), 7.47−7.45 (m, 2H), 7.28−7.26 (m, 2H), 6.89−6.87 (m, 2H), 5.09 (d, 1H, J = 15.6 Hz), 4.70 (d, 1H, J = 15.1 Hz), 4.64 (d, 1H, J = 15.1 Hz), 4.14 (d, 1H, J = 15.6 Hz), 3.81 (s, 3H), 3.63 (dd, 1H, J = 9.5, 5.5 Hz), 1.39−1.24 (m, 3H), 0.86 (d, 3H, J = 6.5 Hz), 0.81 (d, 3H, J = 6.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.6, 160.1, 154.3, 147.8, 144.4, 130.4, 128.7, 126.6, 124.3, 114.6, 58.9, 55.5, 51.1, 49.3, 39.1, 24.7, 23.1, 22.0. MS (ESI) m/z 449.0 [M + Na^+].

(S)-2-(4-Nitrobenzyl)-4,5-dibenzyl-1,2,4-triazine-3,6-dione ***4f.*** Colorless oil. $[\alpha]_D$ −105.6 (c 0.7, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.12 (br s, 1H), 8.13−8.10 (m, 2H), 7.36 − 7.07 (m, 12H), 5.11 (d, 1H, J = 15.4 Hz), 4.52 (d, 1H, J = 15.4 Hz), 3.97−3.86 (m, 3H), 2.96 (dd, 1H, J = 13.9, 5.1 Hz), 2.85 (dd, 1H, J = 13.9, 5.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.7, 153.4, 148.7, 142.8, 135.9, 135.1, 129.9, 129.3, 129.1, 129.0, 128.4, 127.7, 124.0, 60.0, 51.2, 49.4, 35.9. MS (ESI) m/z 431.0 [M + H^+].

(S)-4,5-Dibenzyl-2-pentyl-1,2,4-triazine-3,6-dione ***4g.*** Colorless oil. $[\alpha]_D$ +15.5 (c 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27−7.19 (m, 6H), 7.10−7.08 (m, 4H), 4.97 (d, 1H, J = 15.1 Hz), 3.90 (dd, 1H, J = 6.5, 5.5 Hz), 3.60 (d, 1H, J = 15.1 Hz), 3.15−3.10 (m, 2H), 2.96 (dd, 1H, J = 14.1, 5.5 Hz), 2.87 (dd, 1H, J = 14.1, 6.5 Hz), 1.46−1.31 (m, 2H), 1.25−1.15 (m, 4H), 0.82 (t, 3H, J = 7.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.0, 153.5, 136.4, 135.6, 129.8, 129.0, 128.9, 128.3, 128.1, 127.5, 60.2, 49.4, 47.9, 35.8, 28.8, 26.9, 22.4, 14.1. MS (ESI) m/z 366.0 [M + H^+].

(S)-5-(1H-Indol-2-ylmethyl)-2-(3,4,5-trimethoxy-benzyl)-4-benzyl-1,2,4-triazine-3,6-dione ***4h.*** Crystalline white solid. mp 82−84 °C. $[\alpha]_D$ +34.4 (c 0.9, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (br s, 1H), 7.54 (d, 1H, J = 6.0 Hz), 7.36−6.99 (m, 9H), 5.97 (s, 2H), 5.11 (d, 1H, J = 14.6 Hz), 4.45 (d, 1H, J = 14.1 Hz), 4.00−3.95 (m, 2H), 3.70 (s, 3H) and 3.67 (s, 6H), 3.23 (dd, 1H, J = 15.0, 4.0 Hz), 3.09 (dd, 1H, J = 15.0, 5.0 Hz), 2.18 (d, 1H, J = 14.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.1, 165.2, 153.7, 136.3, 136.1, 129.3, 129.1, 128.5, 128.2, 124.5, 122.5, 120.1, 119.4, 111.5, 109.0, 106.0, 60.9, 59.3, 56.3, 52.8, 49.2, 25.9. MS (ESI) m/z 515.0 [M + H^+].

c-[Aza-phenylalaninyl-prolyl] ***4i.*** White solid. mp 220−221 °C. $[\alpha]_D$ −45° (c 1.1, chloroform). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36−7.33 (m, 5H), 4.69 (s, 2H), 3.88 (t, 1H, J = 8.0 Hz), 3.57−3.53 (m, 2H), 2.22−2.14 (m, 2H), 2.01−1.93 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 154.3, 135.1, 128.9, 128.5, 128.3, 57.6, 50.6, 45.2, 26.7, 23.3. MS (ESI) m/z 246.5 [M + H^+].

c-[Aza-3,4,5-trimethoxyphenylalaninyl-prolyl] ***4j.*** Crystalline green solid. mp 270−271 °C. $[\alpha]_D$ −56.8 (c 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (br s, 1H), 6.60 (s, 2H), 4.77 (d, 1H, J = 14.8 Hz), 4.46 (d, 1H, J = 14.8 Hz), 3.92 (t, 1H, J = 7.9 Hz), 3.85 (s, 9H), 3.58−3.53 (m, 2H), 2.25−2.16 (m, 2H), 2.03 − 2.94 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.6, 154.5, 153.8, 130.6, 106.1, 60.8, 57.9, 56.4, 51.1, 45.4, 26.9, 23.5. MS (ESI) m/z 336.1 [M + H^+].

c-[Aza-2-methoxyphenylalaninyl-prolyl] ***4m.*** Colorless oil. $[\alpha]_D$ +14.0 (c 0.5, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (br s, 1H), 7.25 (dd, 1H, J = 7.5, 1.5 Hz), 7.25 (ddd, 1H, J = 8.0, 7.5, 2.0 Hz), 6.97−6.80 (m, 2H), 4.66 (d, 1H, J = 15.0 Hz), 4.59 (d, 1H, J = 15.0 Hz), 3.90−3.75 (m, 4H), 3.46−3.41 (m, 2H), 2.28−1.99 (m, 2H), 1.94−1.75 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.4, 156.2, 153.2, 131.4, 129.0, 122.7, 120.6, 109.7, 56.7, 54.8, 44.3, 44.2, 26.0, 22.3. MS (ESI) m/z 276.1 [M + H^+].

c-[Aza-3-methoxyphenylalaninyl-prolyl] ***4n.*** White crystalline solid. mp 170−171 °C. $[\alpha]_D$ −25.4 (c 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (br s, 1H), 7.28−7.23 (m, 1H), 6.93−6.83 (m, 3H), 4.66 (s, 2H), 3.89 (t, 1H, J = 8.0 Hz), 3.79 (s, 3H), 3.54 (t, 2H, J = 7.0 Hz), 2.27−2.10 (m, 2H), 2.05−1.85 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.4, 160.1, 154.3, 136.7, 129.9, 120.8, 114.2, 113.8, 57.7, 55.3, 50.6, 45.3, 26.8, 23.4.MS (ESI) 276.2 [M + H^+].

c-[Aza-3,5-dimethoxyphenylalaninyl-prolyl] ***4o.*** Colorless oil. $[\alpha]_D$ −9.2 (c 1.6, MeOH,). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (br s, 1H), 6.50 (d, 2H, J = 2.1 Hz), 6.39 (t, 1H, J = 2.1 Hz), 4.64 (d, 1H, J = 15.4 Hz), 4.59 (d, 1H, J = 15.4 Hz), 3.91 (t, 1H, J = 8.0 Hz), 3.77 (s, 6H), 3.54 (t, 2H, J = 7.0 Hz), 2.28−2.09 (m, 2H), 2.04−1.85 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.3, 161.2, 154.2, 136.6, 106.5, 100.0, 57.7, 55.3, 50.6, 45.2, 26.8, 23.3. MS (ESI) m/z 306.1 [M + H^+].

c-[Aza-2-bromophenylalaninyl-prolyl] ***4p.*** Yellow oil $[\alpha]_D$ −33.2 (c 0.5, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (br s, 1H), 7.59 (dd, 1H, J = 7.6, 1.0 Hz), 7.39 (dd, 1H, J = 7.6, 1.6 Hz), 7.20 (td, 1H, J = 7.6, 1.0), 7.10 (td, 1H, J = 7.6, 1.6 Hz), 5.01 (d, 1H, J = 15.7 Hz), 4.69 (d, 1H, J = 15.7 Hz), 3.97 (t, 1H, J = 7.7 Hz), 3.56 (t, 2H, J = 6.2 Hz), 2.17−2.10 (m, 2H), 2.10−1.86 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.4, 154.2, 134.8, 133.2, 130.3, 129.8, 127.9, 123.9, 57.7, 50.4, 45.3, 26.7, 23.4. MS (ESI) m/z 348.0, 346.0 ([M + Na^+], 1:1 ratio).

c-[Aza-3-bromophenylalaninyl-prolyl] ***4q.*** White crystalline solid. mp 236−237 °C. $[\alpha]_D$ −6.0 (c 0.5, DMSO). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.75 (br s, 1H), 7.80 (m, 2H), 7.64 (m, 2H), 5.19 (d, 1H, J = 16.6 Hz), 4.61 (d, 1H, J = 16.6 Hz), 4.38 (m, 2H), 4.28 (t, 1H, J = 7.8 Hz), 2.44−2.14 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 203.8, 181.2, 137.8, 134.2, 130.8, 130.6, 126.9, 121.6, 59.8, 48.8, 44.9, 28.9, 22.9. MS (ESI) m/z 348.0, 346.0 ([M + Na^+], 1:1 ratio).

c-[Aza-4-bromophenylalaninyl-prolyl] ***4r.*** Colorless oil. $[\alpha]_D$ +50.5 (c 0.5, DMSO). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.43 (br s, 1H), 7.53 (d, 2H, J = 8.4 Hz), 7.23 (d, 2H, J = 8.4 Hz), 4.86 (d, 1H, J = 15.3 Hz), 4.23 (d, 1H, J = 15.3 Hz), 3.93 (t, 1H, J = 7.9 Hz), 3.39−3.34 (m, 2H), 2.07−1.86 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.2, 154.7, 147.3, 145.8, 129.1, 123.9, 57.6, 49.0, 45.2, 27.2, 23.2. MS (ESI) m/z 324.0, 322.0 ([M + H^+], 1:1 ratio).

c-[Aza-4-nitrophenylalaninyl-prolyl] ***4u.*** White crystalline solid. mp 200−201 °C. $[\alpha]_D$ −6.0 (c 0.5, DMSO). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.51 (br s, 1H), 8.21 (d, 2H, J = 8.8 Hz), 7.57 (d, 2H, J = 8.8 Hz), 5.01 (d, 1H, J = 16.5 Hz), 4.44 (d, 1H, J = 16.5 Hz), 4.03 (t, 1H, J = 7.3 Hz), 3.40−3.36 (m, 2H), 2.11−1.85 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.6, 162.1, 157.9, 152.1, 134.0, 128.8, 54.3, 53.9, 50.1, 31.9, 28.0. MS (ESI) m/z 291.1 [M + H^+].

c-[Aza-3,4,5-trimethoxyphenylalaninyl-trans-4-hydroxy-prolyl] ***4v.*** Colorless oil. $[\alpha]_D$ −87.5 (c 0.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.59 (s, 2H), 4.81 (d, 1H, J = 15.1 Hz), 4.59 (t, 1H, J = 4.5 Hz), 4.43 (d, 1H, J = 15.1 Hz), 4.30 − 4.26 (m, 1H), 3.85 (s, 9H), 3.72−3.71 (m, 1H), 3.63−3.60 (m, 1H), 2.35−2.23 (m, 1H), 2.23−2.11 (m, 1H), 1.25 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.6, 153.7, 138.2, 130.4, 115.1, 105.9, 69.4, 60.8, 56.8 56.2, 54.0, 51.2, 36.5. MS (ESI) m/z 352.1 [M + H^+].

c-[Aza-phenylalaninyl-trans-4-hydroxyprolyl] ***4w.*** Colorless crystalline solid. mp 126−128 °C. $[\alpha]_D$ −48.8 (c 0.4, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48−7.27 (m, 5H), 4.83 (d, 1H, J = 15.0 Hz), 4.65−4.51 (m, 1H), 4.27 (dd, 1H, J = 10.6, 7.0 Hz), 3.72 (dd, 1H, J = 12.1, 4.4 Hz), 3.62 (dd, 1H, J =

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12.1, 2.9 Hz), 2.36−2.06 (m, 2H), 1.88 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.6, 154.2, 134.9, 129.0, 128.5, 69.4, 56.4, 54.1, 50.7, 36.5. MS (ESI) m/z 262.1 [M + H$^+$].

General Procedure for the Solid-Phase Synthesis of 1,2,4-Triazine-3,6-diones. The Fmoc-protected amino acid (∼ 70 μmol), loaded on Wang or HMBA resin, was swollen in DCM for 1 h. The solution was drained and a 20% solution of piperidine in DMF (1.5 mL) was added. The mixture was shaken at room temperature for 15 min. The solution was drained and the deprotection step repeated once. The solution was drained and the resin was successively washed with DMF, DCM, DMF, and anhydrous TMOF. The resin was swollen in anhydrous TMOF (1.5 mL) and the aldehyde (10 equiv) was added. The mixture was shaken at room temperature for 90 min. The solution was drained and the resin washed with anhydrous TMOF. The resin was swollen in anhydrous TMOF and sodium cyanoborohydride (10 equiv) was added. The mixture was shaken at room temperature for 15 min then acetic acid (10 equiv) was added and the mixture was further shaken at room temperature for 1 h. The solution was drained and the resin was successively washed with methanol (3×), DCM, and THF. The resin was swollen in anhydrous THF (1.5 mL), 2,4,6-collidine (10 equiv) and BTC (10 equiv) were added, and the mixture was shaken at room temperature for 30 min. The solution was drained and the resin was successively washed with anhydrous THF, anhydrous DCM, anhydrous THF and ether. The resin was placed in a microwave tube and a solution of the *N*′-alkylhydrazine-carboxylic acid *tert*-butyl ester (10 equiv) in anhydrous THF (1.5 mL) was added. The tube was sealed and the mixture shaken at 70 °C overnight. The mixture was filtrated and the resin successively washed with DCM, methanol, and ether. TFA/water: 95/5 (1.2 mL) was added to the resin and the mixture was shaken at room temperature for 1 h. The mixture was filtrated and the filtrate evaporated in vacuo. The crude product was purified by RP chromatography.

(S)-2,4,5-Tribenzyl-[1,2,4]triazine-3,6-dione ***16a.*** White solid. mp 65−66 °C. $[\alpha]_D$ −24.4° (*c* 1, chloroform). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39−7.13 (m, 9H), 7.24−7.2 (m, 4H), 7.12 (d, 2H, *J* = 4.8 Hz), 5.1 (d,1H, *J* = 14.8 Hz), 4.55 (d, 1H, *J* = 14.8 Hz), 3.97 (X of ABX, 1H, *J* = 5.6 Hz), 3.84 (d, 1H, *J* = 14.8 Hz), 3.79 (d, 1H, *J* = 14.8 Hz), 2.85 (AB of ABX, 2H, J_{AB} = 14.0 Hz, J_{AX} = 6.0 Hz, J_{BX} = 5.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.7, 153.5, 135.7, 135.1, 134.3, 12937, 129, 128.9, 128.8, 128.7, 128.6, 128.2, 128.1, 127.5, 59.8, 51.8, 19.4, 35.9. HRMS: calcd for $C_{24}H_{24}N_3O_2$ 386.17522, found 386.18655.

(S)-2,5-Dibenzyl-[1,2,4]triazine-3,6-dione ***16b.*** White solid. mp 216−218 °C. $[\alpha]_D$ −94.8° (*c* 1.1, chloroform). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28−7.16 (m, 8H), 7.09 (d, 2H, *J* = 6.8 Hz), 4.38 (AB, 2H, $\Delta\delta$ = 0.09, *J* = 15.2 Hz), 3.92 (X of ABX, 1H, *J* = 4 Hz), 2.76 (AB of ABX, 2H, J_{AB} = 14.0 Hz, J_{AX} = 8.4 Hz, J_{BX} = 4.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.8, 135.2, 134.9, 129.4, 128.7, 128.6, 128.1, 128.0, 127.1, 55.8, 50.5, 38.3. MS (ESI) m/z 296.3 [M + H$^+$].

(S)-2,4-Dibenzyl-5-(hydroxymethyl)-[1,2,4]triazine-3,6-dione ***16c.*** Clear oil. $[\alpha]_D$ −8.1° (*c* 0.9, chloroform). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36−7.26 (m, 10H), 5.10 (d, 2H, *J* = 14.8 Hz), 4.85 (d, 1H, *J* = 14.8 Hz), 4.55 (d, 1H, *J* = 14.8 Hz),), 4.16 (d, 1H, *J* = 15.2 Hz), 3.76−3.73 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.2, 154.5, 135.9, 134.6, 129.0, 128.9, 128.6, 128.4, 128.1, 60.3, 59.9, 52.9, 49.0. MS (ESI) m/z 326.4 [M + H$^+$].

(S)-3-(2,4-Dibenzyl-3,6-dioxo-1,2,4-triazinan-5-yl)-propanoic acid ***16d.*** White solid. mp 75 −77 °C. $[\alpha]_D$ +52.8° (*c* 1.6, chloroform). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40−7.28 (m, 10H), 6.97 (sl, 2H), 5.06 (d, 1H, *J* = 15.2 Hz), 4.73 (AB, 2H, $\Delta\delta$ = 0.20, *J* = 15.2 Hz), 4.11 (d, 1H, *J* = 14.8 Hz),), 3.75 (dd, 1H, *J* = 8.8,O 4.4 Hz), 2.35 (t, 2H, *J* = 6.8 Hz), 2.01−1.94 (m, 1H), 1.89−1.84 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.7, 164.7, 153.3, 136.1, 134.7, 128.9, 128.8, 128.7, 128.4, 128.1, 57.8, 51.3, 49.0, 29.2, 24.0. MS (ESI) m/z 368.4 [M + H$^+$].

(S)-5-(4-aminobutyl)-2,4-dibenzyl-1,2,4]triazine-3,6-dione ***16e.*** Clear oil. $[\alpha]_D$ +64.5° (*c* 1.1, chloroform). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (bs, 2H),7.32−7.22 (m, 10H), 7.13 (sl, 1H), 4.91 (d, 1H, *J* = 15.2 Hz), 4.70 (AB, 2H, $\Delta\delta$ = 0.20, *J* = 15.2 Hz), 4.05 (d, 1H, *J* = 15.2 Hz),), 3.59 (dd, 1H, *J* = 8.4, 4.8 Hz), 2.72 (bs, 2H), 1.45−1.14 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.5, 136.2, 135.1, 128.9, 128.7, 128.6, 128.3, 128.0, 58.5, 51.2, 49.2, 39.3, 29.0, 26.6, 21.3. MS (ESI) m/z 367.5 [M + H$^+$].

(S)-2,5-Dibenzyl-4-isopentyl-[1,2,4]triazine-3,6-dione ***16f.*** Clear oil. $[\alpha]_D$ −33.9° (*c* 1.1, chloroform). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37−7.28 (m, 6H), 7.23−7.20 (m, 2H), 7.13 (dd, 2H, *J* = 6.8, 1.6 Hz), 4.16 (AB, 2H, $\Delta\delta$ = 0.7, *J* = 14.8 Hz), 4.03 (X of ABX, 1H, *J* = 6.0 Hz), 3.88−3.81 (m, 1H), 2.89 (AB of ABX, 2H, J_{AB} = 13.6 Hz, J_{AX} = 6.4 Hz, J_{BX} = 5.2 Hz), 2.63−2.57 (m, 1H), 1.54−1.51 (m, 1H), 1.44−1.37 (m, 2H), 0.90−0.87 (m, 6H) . ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.8, 153.4, 135.1, 134.6, 129.7, 128.9, 128.8, 128.7, 128.4, 127.4, 61.2, 51.5, 44.7, 36.7, 36.4, 25.7, 22.5, 22.2. MS (ESI) m/z 366.5 [M + H$^+$].

(S)-4-(2-aminoethyl)-2,5-dibenzyl-[1,2,4]triazine-3,6-dione ***16g.*** White solid. mp 95−96 °C. $[\alpha]_D$ −76.9° (*c* 1, chloroform). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (sl, 2H), 7.37−7.19 (m, 8H), 6.93 (d, 2H, *J* = 6.7 Hz), 4.87 (d, 1H, *J* = 14.8 Hz), 4.11−4.07 (m, 2H), 3.85 (m, 1H), 3.78 (d, 1H, *J* = 14.8 Hz), 3.03−3.00 (m, 1H), 2.86−2.85 (m, 1H), 2.64−2.59 (m, 2H), 2.53−2.47 (m, 1H) . ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.9, 153.5, 135.2, 135.0, 129.4, 128.8, 128.4, 127.3, 63.1, 50.8, 45.0, 38.2, 36.4, 31.2. MS (ESI) m/z 339.4 [M + H$^+$].

(S)-2,5-Dibenzyl-4-(3,5-dimethoxybenzyl)-[1,2,4]triazine-3,6-dione ***16h.*** White solid. mp 56−57 °C. $[\alpha]_D$ −18.1° (*c* 1.8, chloroform). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37−7.31 (m, 7H), 7.24−7.21 (m, 2H), 7.14 (d, 2H, *J* = 6.8 Hz), 6.38 (t, 1H, *J* = 4.4 Hz), 6.35 (d, 2H, *J* = 2.4 Hz), 5.05 (d, 1H, *J* = 15.2 Hz), 4.62 (d, 1H, *J* = 14.4 Hz), 4.00 (X of ABX, 1H, *J* = 5.6 Hz), 3.76 (s, 6H), 3.73 (d, 1H, *J* = 11.6 Hz), 3.69 (d, 1H, *J* = 11.2 Hz), 2.89 (AB of ABX, 2H, J_{AB} = 14.0 Hz, J_{AX} = 6.4 Hz, J_{BX} = 5.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.4, 161.2, 153.6, 138.1, 135.2, 134.3, 129.8, 129.0, 128.8, 128.7, 128.6, 127.5, 106.2, 99.8, 59.8, 55.3, 51.8, 49.3, 35.9. MS (ESI) m/z 446.5 [M + H$^+$].

(S)-2,5-Dibenzyl-4-(4-nitrobenzyl)-[1,2,4]triazine-3,6-dione ***16i.*** White solid. mp 73−74 °C. $[\alpha]_D$ +32.7° (*c* 1, chloroform). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21−8.17 (m, 2H), 7.41−7.32 (m, 8H), 7.26−7.23 (m, 2H), 7.15 (dd, 2H, *J* = 6.8, 1.6 Hz), 5.13 (d, 1H, *J* = 15.6 Hz), 4.26 (AB, 2H, $\Delta\delta$ = 0.72, *J* = 14.8 Hz), 3.97 (X of ABX, 1H, *J* = 4.8 Hz), 3.78 (d, 1H, *J* = 16.0 Hz), 2.90 (AB of ABX, 2H, J_{AB} = 13.6 Hz, J_{AX} = 6.8 Hz, J_{BX} = 4.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.4, 153.3, 147.7, 143.6, 134.8, 133.9, 129.6, 129.1, 129.0, 128.8, 128.7, 128.6, 127.7, 124.1, 61.2, 51.7, 49.1, 36.2. MS (ESI) m/z 431.5 [M + H$^+$].

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(S)-4-Cyclohexyl-2,5-dibenzyl-[1,2,4]triazine-3,6-dione ***16j***. White solid. mp 78−79 °C. $[\alpha]_D$ +70.2° (*c* 1.1, chloroform). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37−7.23 (m, 8H), 7.11 (m, 2H), 4.20 (AB, 2H, $\Delta\delta$ = 0.74, *J* = 14.8 Hz), 4.07 (X of ABX, 1H, *J* = 12.8 Hz), 4.03 (m, 1H), 2.92 (AB of ABX, 2H, J_{AB} = 13.6 Hz, J_{AX} = 8.4 Hz, J_{BX} = 6.4 Hz), 1.97 (m, 1H), 1.83 (m, 2H), 1.67 (m, 2H), 1.41 (m, 4H), 1.11 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.3, 153.8, 135.2, 134.6, 129.6, 129.0, 128.8, 128.6, 128.5, 127.3, 57.4, 55.9, 50.9, 38.6, 32.2, 31.4, 26.0, 25.7, 25.3. MS (ESI) *m*/*z* 378.5 [M + H$^+$].

(S)-4,5-Dibenzyl-2-isopentyl-[1,2,4]triazine-3,6-dione ***16k***. White solid. mp 89−90 °C. $[\alpha]_D$ +14.8° (*c* 1.3, chloroform). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37−7.22 (m, 6H), 7.17 (d, 4H, *J* = 7.2 Hz), 5.07 (d, 1H, *J* = 14.8 Hz), 3.98 (X of ABX, 1H, *J* = 6.0 Hz), 3.69 (d, 1H, *J* = 15.2 Hz), 3.33−3.17 (m, 2H), 2.99 (AB of ABX, 2H, J_{AB} = 13.6 Hz, J_{AX} = 6.4 Hz, J_{BX} = 4.8 Hz), 1.56−1.50 (m, 1H), 1.39−1.32 (m, 1H), 1.30−1.22 (m, 1H), 0.89 (dd, 6H, *J* = 6.0, 1.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.0, 153.0, 136.1, 135.3, 129.6, 128.9, 128.8, 128.2, 127.9, 127.4, 59.8, 49.1, 46.0, 35.6, 35.5, 25.6, 22.3. MS (ESI) *m*/*z* 366.5 [M + H$^+$].

(S)-2-(4-nitrobenzyl)-4,5-dibenzyl-[1,2,4]triazine-3,6-dione ***16l***. White solid. mp 207−208 °C. $[\alpha]_D$ −25.5° (*c* 1.1, chloroform). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, 2H, *J* = 8.8 Hz), 7.38−7.29 (m, 8H), 7.23 (m, 2H), 7.11 (m, 2H), 5.11 (d, 1H, *J* = 15.2 Hz), 4.52 (d, 1H, *J* = 16.0 Hz), 3.98 (X of ABX, 1H, *J* = 5.2 Hz), 3.90−3.83 (m, 2H), 2.91 (AB of ABX, 2H, J_{AB} = 14.0 Hz, J_{AX} = 5.6 Hz, J_{BX} = 5.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.4, 151.4, 147.3, 139.6, 139.0, 133.6, 132.8, 132.7, 132.6, 132.1, 132.0, 131.4, 127.7, 63.6, 54.8, 39.7. MS (ESI) *m*/*z* 431.5 [M + H$^+$].

(S)-4,5-Dibenzyl-2-(prop-2-yn-1-yl)-[1,2,4]triazine-3,6-dione ***16m***. Clear oil $[\alpha]_D$ −12.9° (*c* 1, chloroform). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39−7.27 (m, 6H), 7.18 (d, 4H, *J* = 6.8 Hz), 6.61 (sl, 1H), 5.02 (d, 1H, *J* = 15.2 Hz), 4.26 (dd, 1H, *J* = 17.6, 2.4 Hz), 4.02 (X of ABX, 1H, *J* = 6.4 Hz), 3.75 (d, 1H, *J* = 14.8 Hz), 3.43 (dd, 1H, *J* = 17.6, 2.4 Hz), 3.04 (AB of ABX, 2H, J_{AB} = 13.6 Hz, J_{AX} = 6.4 Hz, J_{BX} = 5.2 Hz), 2.33 (dd, 1H, *J* = 4.8, 2.8 Hz) . ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.0, 153.1, 135.5, 135.0, 129.7, 129.0, 128.9, 128.2, 128.1, 127.5, 60.0, 49.4, 37.8, 36.2. MS (ESI) *m*/*z* 334.4 [M + H$^+$].

(S)-2-(4,5-Dibenzyl-3,6-dioxo-1,2,4-triazinan-2-yl)acetic acid ***16n***. White solid. mp 96 °C. $[\alpha]_D$ +11.9° (*c* 1, chloroform). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37−7.30 (m, 6H), 7.21 (d, 2H, *J* = 7.6 Hz), 7.15 (d, 2H, *J* = 8.4 Hz), 5.78 (bs, 3H), 5.01 (A of AX, d, 1H, J_{AX} = 14.8 Hz), 4.23 (A′ of A′X′, d, 1H, *J* = 18.0 Hz), 4.04 (t, 1H, *J* = 7.6 Hz), 3.85 (X of AX, d, 1H, J_{AX} = 14.8 Hz), 3.10 (X′ of A′X′, d, 1H, *J* = 18.0 Hz), 3.02−3.00 (bs, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 165.9, 153.6, 135.3, 134.8, 129.8, 129.0, 128.9, 128.3, 127.7, 59.8, 49.5, 48.4, 35.6. MS (ESI) *m*/*z* 400.5 [M + H$^+$].

(S)-2-Benzyl-5-methyl-4-(4-nitrobenzyl)-[1,2,4]triazine-3,6-dione ***16o***. White solid. mp 171−172 °C. $[\alpha]_D$ +72.1° (*c* 1, chloroform). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, 2H, *J* = 8.6 Hz), 7.37 (d, 2H, *J* = 8.6 Hz), 7.26−7.22 (m, 4H), 7.15 (s, 1H), 4.89 (A of AX, d, 1H, J_{AX} = 15.9 Hz), 4.65 (AB, 2H, $\Delta\delta_{AB}$ = 0.11, J_{AB} = 15.2 Hz), 4.17 (X of AX, d, 1H, J_{AX} = 15.9 Hz), 3.64 (q, 1H, *J* = 7.0 Hz), 1.07 (d, 3H, *J* = 7.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 154.1, 147.9, 144.5, 134.8, 129.2, 128.9, 128.8, 124.3, 55.9, 51.7, 48.7, 15.1. MS (ESI) *m*/*z* 355.0 [M + H$^+$].

■ ASSOCIATED CONTENT

Supporting Information

Experimental data for compounds **2a−2f**, **5a−5p**, **6a−6p**, **7**, **8**, **9**, **10**, general experimental procedure for the synthesis of *N*-alkyl-amino acid methyl esters, 2D NMR data (HSQC, HMBC and NOESY experiments) for compound **16a**, X-ray crystallographic data of compound **16o**. This material is available free of charge via Internet at http://pubs.acs.org.

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Notes

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