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Solution-phase submonomer diversification of aza-dipeptide building blocks and their application in aza-peptide and aza-DKP synthesis

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Aza-peptides have been used as tools for studying SARs in programs aimed at drug discovery and chemical biology. Protected aza-dipeptides were synthesized by a solution-phase submonomer approach featuring alkylation of *N*-terminal benzophenone semicarbazone aza-Gly-Xaa dipeptides using different alkyl halides in the presence of potassium *tert*-butoxide as base. Benzophenone protected aza-dipeptide *tert*-butyl ester 31c was selectively deprotected at the *C*-terminal ester or *N*-terminal hydrazone to afford, respectively, aza-dipeptide acid and amine building blocks 36c and 40c, which were introduced into longer aza-peptides. Alternatively, removal of the benzophenone semicarbazone protection from aza-dipeptide methyl esters 29a–c led to intramolecular cyclization to produce aza-DKPs 39a–c. In light of the importance of aza-peptides and DKPs as therapeutic agents and probes of biological processes, this diversity-oriented solution-phase approach may provide useful tools for studying peptide science. Copyright © 2010 European Peptide Society and John Wiley & Sons, Ltd.

Supporting information may be found in the online version of this article

Keywords: alkylation; aza-Gly-Xaa; aza-peptide; β-turn conformation; benzophenone semicarbazone protecting group; DKP; aza-DKP; triazine

Introduction

Peptide mimics have been used to enhance the bioavailability, potency, specificity and duration of action of natural parent peptides [1–3]. Investigations in peptide mimicry have also shown that certain structural changes, when introduced into a peptide, can prevent enzyme degradation and induce conformational preferences that mimic the biologically active secondary structure [4]. Among the modifications found in peptide mimicry, aza-peptides, in which one or more residue is replaced by an aza-amino acid [5], have found promising utility in enhancing the drug-like character of peptide candidates [6], by increasing the duration of action [7] and resistance to enzymatic degradation [8,9] (see Figure 1 for relevant examples). Furthermore, aza-amino acid residues have been shown to stabilize β-turn conformations in peptides as detected by computational [10–12], spectroscopic [13–15] and crystallographic analyses [13].

The introduction of aza-amino acids into peptides involves a combination of hydrazine and peptide chemistry (Figure 2) [5]. For example, hydrazine precursors were used in solution to synthesize *N*-Boc protected aza[1]-dipeptide **3** [20,21] and *N*-Fmoc protected aza-amino acid chloride **4**, which were used successfully in solid-phase syntheses to introduce aza-residues into biologically relevant peptides [22,23]. On the other hand, attempts to prepare aza-peptide by the reaction of protected *N*-alkyl hydrazine [24] **11** onto resin-bound active carbamate **9** or isocyanate **10** have suffered from low yields due to the formation of hydantoin **12** upon intramolecular acylation of the amide of the preceding *C*-terminal residue (Figure 2) [24–26].

The prerequisite to synthesize the *N*-alkyl hydrazine prior to incorporation into aza-peptide has limited the diversity of the aza-residue side chain, because of the inherent difficulty to differentiate selectively the two nitrogen of the hydrazine moiety [27]. Alternatively, alkylation of a suitably *N*-protected aza-glycine moiety surmounts the issues associated with hydrazine synthesis [28,29]. Although *N*-Fmoc-aza-glycine acid chloride **4** could not be introduced into aza-peptide in solution, likely because of competitive formation of oxadiazalone **6** (Figure 2) [23], aza-Gly peptides were synthesized using the activated methylidenecarbazate **20** from mixing benzophenone hydrazone **17** and *p*-nitrophenyl chloroformate **18** (Scheme 2) [28,29]. Subsequent alkylation of solid-phase bound benzophenone semicarbazone-protected aza-Gly peptide, semicarbazone deprotection, peptide elongation and cleavage has provided a submonomer approach for aza-peptide synthesis [29]. To compliment this solid-phase approach, solution-phase synthesis by a submonomer approach has been explored. At the time, we were also aware that alkylation of *N*-phthalimido aza-glycine *tert*-butyl ester (*N*-*tert*-butyloxycarbonyl-aminophthalimide **13**, Scheme 1) had been achieved by using different alcohols under Mitsunobu conditions [30]. Both phthalimide and benzophe-

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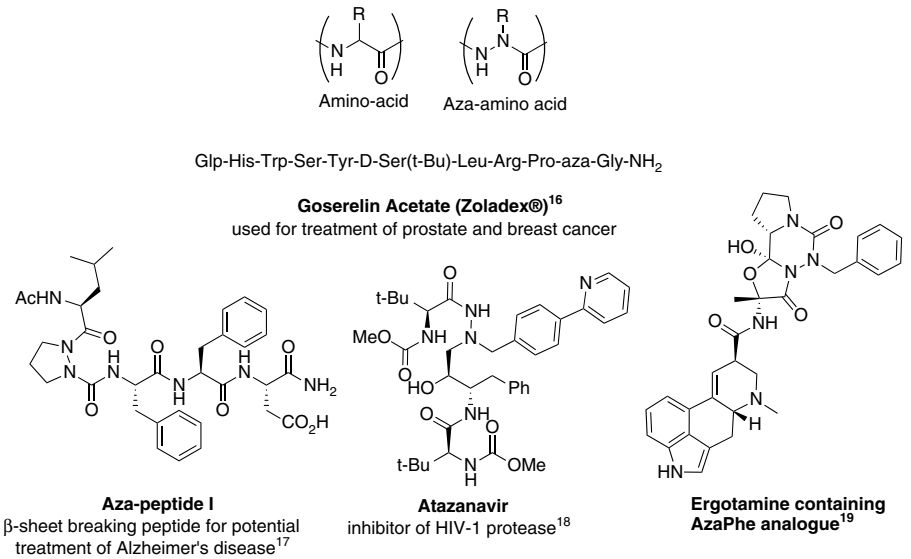


Figure 1. Representive biologically active aza-peptide and aza-DKP analogs [16–19].

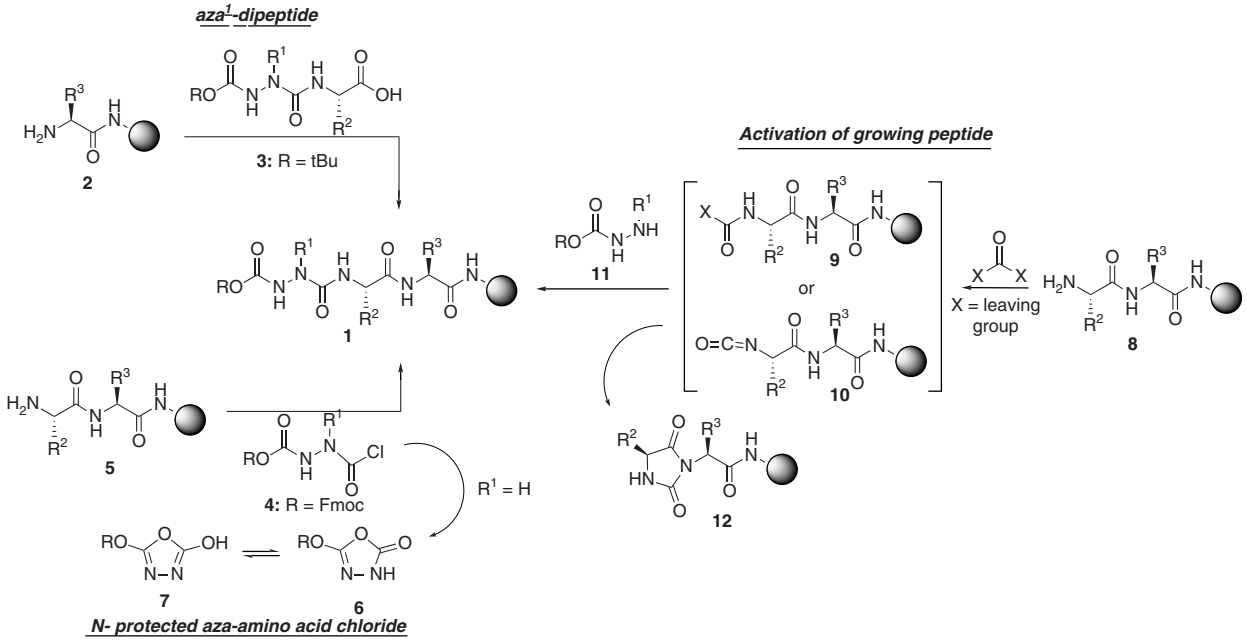


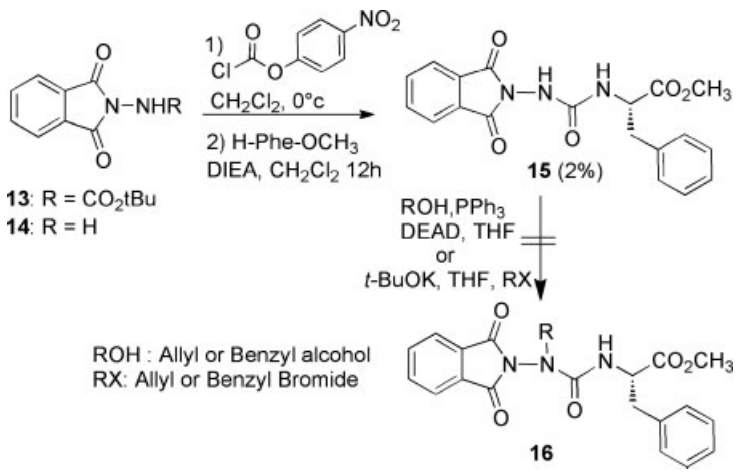
Figure 2. Methods and pitfalls to avoid in introducing aza-residues into peptides.

none semicarbazone protection strategies were studied to avoid oxadiazolone formation during incorporation of aza-Gly residues and to orient regioselective nitrogen alkylation for the synthesis of protected aza-dipeptide building blocks in solution. Although *N*-phthalimido aza-glycinamides could not be successfully diversified, a variety of *N*-benzophenone semicarbazone aza-glycine analogs were alkylated to furnish a diverse set of aza-dipeptides. Moreover, selective removal of the benzophenone or the ester protection gave suitable building blocks for aza-peptide synthesis, respectively, by *N*- or *C*-terminal elongation. Finally, examination of the removal of the *N*-protection from the aza-dipeptide methyl ester under acidic conditions has provided access to a relatively rare class of heterocycles, the so-called aza-diketopiperazines (aza-DKPs). This class of triazines merits study in light of the broad spectrum of biological activities associated with the parent DKP structure [31].

Results and Discussion

Synthesis and Attempted Alkylation of Phthalimido Gly-Xaa Dipeptides

As mentioned earlier, phthalimide protection has been used for the synthesis of *N*-alkyl, *N*-acyl hydrazines in solution- and on solid-phase [30,32–35]. Mitsunobu alkylation of *N*-acylaminophthalimides provided good yields of alkylated products using primary, secondary and benzyl alcohols [30]. Removal of the phthaloyl group with methylhydrazine afforded the 1,1-disubtituted hydrazines [30]. In light of the success with



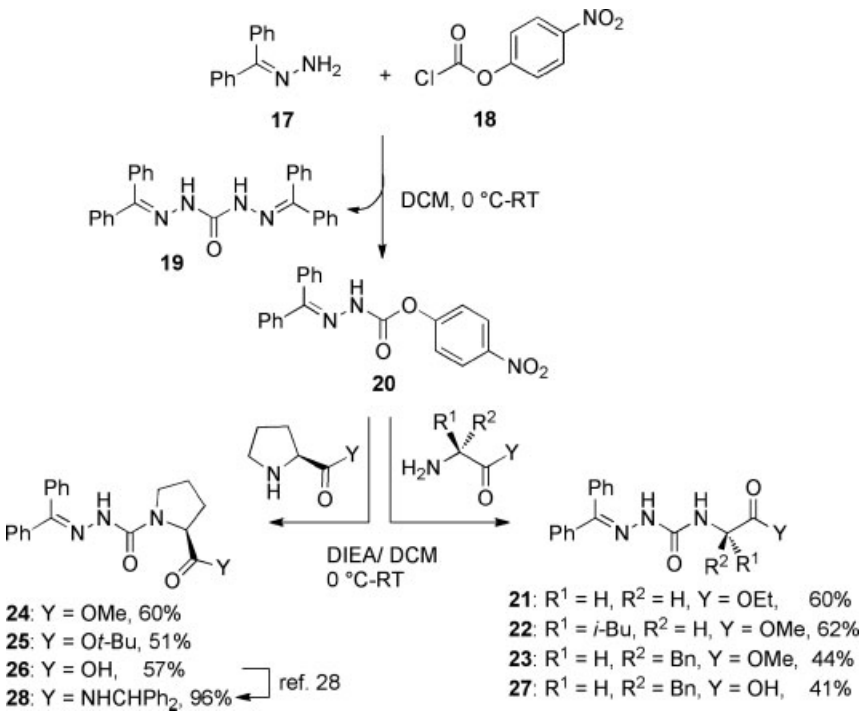
Scheme 1. Synthesis and attempted alkylation of phthalimido aza-Gly-Phe-OMe **15**.

N-acylaminophthalimides **13**, the related ureidophthalimide **15** was synthesized and examined as a protected aza-Gly residue to explore related alkylation chemistry to provide other aza-amino acids (Scheme 1).

N-aminophthalimide **14** was synthesized as reported [36], reacted with *p*-nitrophenyl chloroformate at 0 °C for 2 h and coupled with Phe-OMe in a sluggish reaction giving a mixture of multiple products, as detected by TLC analysis, from which silica gel column chromatography finally provided the desired *N*-phthalimido aza-Gly-Phe-OMe **15**, albeit in 2% yield. Attempts failed to add diversity onto the phthalimido aza-Gly residue of **15** using benzyl and allyl alcohols under Mitsunobu conditions, which were reported to be successful for alkylation of Pht-aza-Gly-O*t*Bu **13** [30], likely due to the weaker acidity of the *N*-ureidophthalimide versus the *N*-carbamatophthalamide. Furthermore, recovered starting material was obtained from attempts to alkylate *N*-ureidophthalimide **15** using allyl bromide or allyl iodide in the presence of potassium *tert*-butoxide in THF at 0 °C (Scheme 1). Both the low coupling yields and the lack of alkylation of ureidophthalimide **15** suggested that an alternative protecting group strategy was needed to build and modify the aza-Gly-Xaa dipeptide.

Benzophenone Semicarbazone Protection for aza-Gly-Xaa Synthesis

Hydrazone protection has recently been used to prepare activated aza-Gly analogs without oxadiazolone formation [28,29]. Moreover, the alkylation of supported aza-Gly peptide has been used to make aza-peptide on solid phase [29].



Scheme 2. Hydrazone activation and insertion of aza-glycine into dipeptides.

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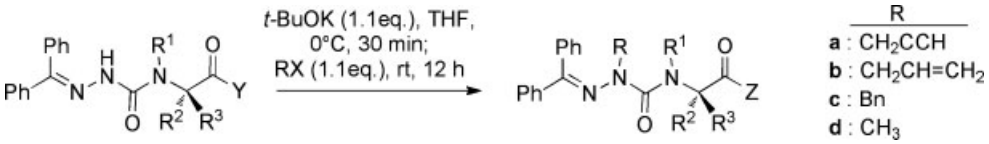

Scheme 3. General method for alkylation of benzophenone protected aza-Gly-Xaa-Y.

Table 1. Alkylated benzophenone protected aza-Gly-Xaa-Y

Entry	Starting material	Y	R^1, R^2	R^3	RX	Z	Product	% Yields
1	**24**	OCH_3	$(CH_2)_3$	H	$HC{\equiv}CCH_2Br$	OCH_3	**29a**	37
2	**24**	OCH_3	$(CH_2)_3$	H	$CH_2{=}CHCH_2Br$	OCH_3	**29b**	20
3	**24**	OCH_3	$(CH_2)_3$	H	BnBr	OCH_3	**29c**	15
4	**24**	OCH_3	$(CH_2)_3$	H	CH_3I	OCH_3	**29d**	80
5	**23**	OCH_3	H, H	Bn	BnBr	OCH_3	**30**	14
6	**25**	$OC(CH_3)_3$	$(CH_2)_3$	H	$HC{\equiv}CCH_2Br$	$OC(CH_3)_3$	**31a**	72
7	**25**	$OC(CH_3)_3$	$(CH_2)_3$	H	$CH_2{=}CHCH_2Br$	$OC(CH_3)_3$	**31b**	56
8	**25**	$OC(CH_3)_3$	$(CH_2)_3$	H	BnBr	$OC(CH_3)_3$	**31c**	84
9	**25**	$OC(CH_3)_3$	$(CH_2)_3$	H	CH_3I	$OC(CH_3)_3$	**31d**	73
10	**21**	OCH_2CH_3	H, H	H	$CH_2{=}CHCH_2Br$	OCH_2CH_3	**32**	60
11	**26**	OH	$(CH_2)_3$	H	$HC{\equiv}CCH_2Br$	$OCH_2C{\equiv}CH$	**33**	51
12	**27**	OH	H, H	Bn	$HC{\equiv}CCH_2Br$	$OCH_2C{\equiv}CH$	**34a**	22[a]
13	**27**	OH	H, H	Bn	BnBr	OBn	**34b**	63[b]
14	**28**	$NHCH(Ph)_2$	$(CH_2)_3$	H	$HC{\equiv}CCH_2Br$	$NHCH(Ph)_2$	**35**	48

[a] Benzophenone aza-Gly-D-Phe-$OCH_2CH{\equiv}CH$ was obtained as major side product determined by LCMS analyses.
[b] Benzophenone aza-Gly-D-Phe-OBn was obtained as major side product determined by LCMS analyses.

In solution, benzophenone semicarbazone-protected aza-Gly dipeptides **21–27** were synthesized in 41–62% yields by activation of diphenylhydrazone **17** with *p*-nitrophenyl chloroformate **18** in DCM at 0 °C for 1 h, followed by addition of a solution of the coupling partner (amino acid or ester, Xaa-Y as hydrochloride or free base) and DIEA in DCM or methanol (Scheme 2) [28]. The reagents CDI [37] and phosgene [22] in solution gave exclusively symmetric urea **19**.

Activated carbazate **20** was coupled to amino methyl ester hydrochlorides with best conditions involving a premixing of the salt with 2 eq. of DIEA in DCM prior to addition. Proline *tert*-butyl ester was used as the free amine with only 1 eq. of DIEA, which gave the desired aza-Gly dipeptide **25** in 51% yield. The amino acids Pro and D-Phe were also coupled to the *p*-nitrophenyl carbazate **20** with 1 eq. of DIEA, using methanol as solvent in the case of proline to furnish benzophenone protected aza-Gly-Pro **26** and aza-Gly-Phe **27** in 57% and 41% respective yields. Benzophenone protected aza-Gly-Pro diphenylmethylamide **28** was synthesized in 96% yield as previously described by coupling the corresponding aza-Gly-Pro **26** and diphenylmethylamine [28].

Alkylation of Benzophenone Semicarbazones

Alkylation of the benzophenone imines of glycine esters was first introduced by O'Donnell as a practical method for the synthesis of racemic and optically active α-amino acids [38]. In analogy to the Gly Schiff-base, in which imine double-bond resonance favors stabilization of the α-carbanion [39], semicarbazones **21–28** could be regioselectively deprotonated and alkylated. A general protocol was explored in which the aza-Gly dipeptide was treated with potassium *tert*-butoxide in THF at 0 °C under argon followed by treatment with alkyl halide. According to this procedure, the regioselective alkylation was achieved on methyl ester **24**, using propargyl bromide (37%), benzyl bromide (15%), allyl bromide (20%) and methyl iodide (80%; Table 1 entries 1–4 and Scheme 3).

Four products were initially obtained from alkylation with propargyl bromide on benzophenone semicarbazone-protected aza-Gly-Pro-OMe **24** using potassium *tert*-butoxide (Figure 3). The aza-propargylGly-Pro-OMe **29a** was isolated in 20% yield and shown to be contaminated with its corresponding acid and propargyl ester counterparts **36a** and **33**, as well as hydantoin **37** in, respectively, 10%, 7% and 20% yields. Intramolecular acylation of the semicarbazone anion by the methyl ester may likely lead to the formation of hydantoin **37** which was isolated in 20% yield and characterized by ^1H and ^{13}C NMR.

Residual hydroxide ion caused likely the solvolysis of the methyl ester, leading to alkylation of the resulting carboxylate to prepare propargyl ester **33**. Alkylation conditions were subsequently improved to yield the aza-propargylGly-Pro-OMe **29a** in 37% yield (Table 1, entry 1). Ions characteristic of similar side products were detected in the LCMS traces of semicarbazone-protected aza-allylGly-Pro-OMe **29b** and aza-Phe-Pro-OMe **29c** (Table 1, entries 2 and 3); however, only starting material (20%) and aza-Ala-Pro-OMe **29d** (80%) were recovered from alkylation with iodomethane (Table 1, entry 4). Attempts to effect alkylation under Mitsunobu conditions failed. Isomerization of the tertiary amide in aza-Gly-Pro analogs may be partially responsible for the low yields of methyl ester **24**. A similar attempt to alkylate benzophenone semicarbazone-protected aza-Gly-D-Phe-OMe **23** using benzyl bromide in the presence of potassium *tert*-butoxide (1.1 eq.) gave aza-Phe-D-Phe-OMe **30** in only 14% yield (Table 1, entry 5). Similarly, aza-allylGly-Gly-OEt **32** was synthesized in 60% yield by alkylation of benzophenone semicarbazone-protected aza-Gly-Gly-OEt **21** (Scheme 3 and Table 1, entry 10). Neither

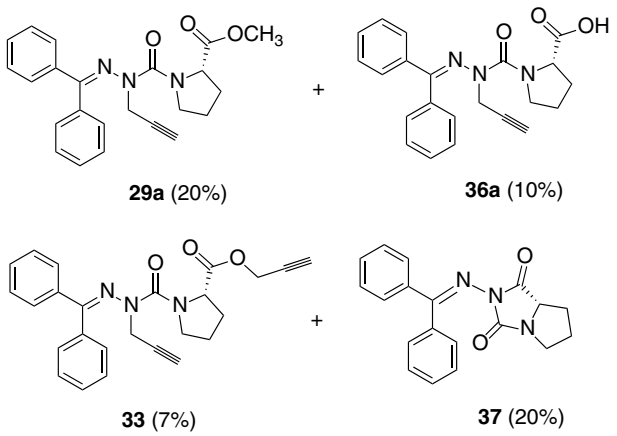

Figure 3. Aza-propargylGly-Pro-OMe and side products obtained from alkylation of methyl ester 24.

C- nor *N*-amide alkylation was observed by a ^{1}H–^{1}H COSY NMR experiment which indicated a three-bond coupling between the urea NH and the neighboring αCH$_2$ of the glycine residue.

tert-Butyl protection alleviated the issues of trans-esterification and low yields in the alkylation of aza-Gly-Pro dipeptide methyl esters. Under similar alkylation conditions using *tert*-BuOK and propargyl, benzyl and allyl bromides, semicarbazone-protected aza-propargylGly-Pro, aza-allylGly-Pro and aza-Phe-Pro *tert*-butyl esters **31a–c** were afforded in improved yields (56–84%); methyl iodide gave aza-Ala-Pro dipeptide *tert*-butyl ester **31d** in 73% yield.

Alkylation of aza-Gly-dipeptide acids was also achieved by double deprotonation with 2 eq. of base and treatment with excess alkyl halide to provide the *N*,*O*-dialkylated products. Benzophenone semicarbazone-protected aza-Gly-Pro **26** and aza-Gly-D-Phe **27** reacted with propargyl bromide under these conditions to give the aza-propargylGly-Pro and aza-propargylGly-D-Phe propargyl esters **33** and **34a** in 51 and 22% yields, respectively (Table 1, entries 11 and 12) [40]. Treatment of benzophenone semicarbazone-protected aza-Gly-D-Phe **27** with benzyl bromide gave aza-Phe-D-Phe benzyl ester **34b** in 63% yield (Table 1, entry 13).

Finally, alkylation of aza-Gly-Pro dipeptide amide **28** with propargyl bromide and potassium *tert*-butoxide provided aza-propargylGly-Pro amide **35** in 48% yield (Table 1, entry 14). Examination of the ^{1}H-^{1}H COSY spectra of **35** showed the coupling between the amide and the methyl protons of the diphenylmethyl amide indicating that no *N*-alkylation occurred at the *C*-terminal amide.

C-Terminal deprotection and coupling of N-benzophenone aza-dipeptides

N-Protected aza^1-dipeptides have been used as configurationally stable building blocks for assembling aza-peptides in solution and on solid phase for subsequent SAR studies [21,26]. With means in hand for alkylation of *N*-benzophenone semicarbazone aza-Gly-Xaa dipeptide esters to generate diverse aza-dipeptide analogs, ester removal was next examined to provide the building blocks for introduction into longer peptides. Methyl esters **29a–d** were hydrolyzed using LiOH (2 eq.) in 3 : 1 methanol : water to give acids **36a–d** in 38–89% yield. Acid **36a** was also prepared in 72% yield by the treatment of *tert*-butyl ester **31a** with 8 : 2 TFA in DCM for 12 h followed by chromatography. Alternatively, *tert*-butyl ester was removed by treatment with bubbling HCl in DCM for 2 h and acids **36a–d** were directly used without further purification in amide couplings. For the synthesis of amides **35a–d**, isobutyl chloroformate and NMM at −15 °C proved most effective giving 12–74% yields (Scheme 4). Other conditions for *tert*-butyl ester removal were less successful often causing benzophenone removal as observed by LCMS analyses [41–43].

N-Terminal deprotection and aza-diketopiperazine formation

Previously, *N*-benzophenone semicarbazone aza-Gly-Pro-OMe **24** was converted to hydrochloride salt **38** in 99% yield using 1 N HCl in THF at 40 °C for 12 h [28]. On the other hand, applying the same conditions to the alkylated methyl esters **29a–c** afforded aza-DKPs **39a–c** (Scheme 5).

The synthesis and activity of aza-DKPs has been rather unexplored [44], yet offers interesting potential for studying the influence of C_α-stereochemistry on activity. In this light, the pioneering synthesis of the aza-DKP, *c*-[aza-Phe-Pro], and its incorporation into an analog of the peptidic moiety of ergotamine (Figure 1), led to a notable rearrangement giving imidazolinone surrogates of the oxazolidin-4-one moiety characteristic of ergotamine and related ergopeptins [19,45]. Aza-DKPs have been previously synthesized by condensations of proline esters with preformed Boc-aza-amino acid chlorides [44] and nitrophenyl esters [46]. Our entry to aza-DKPs offers efficient means for diversifying the aza-residue, and may be useful for examining active DKPs [47,48], and related triazines [49–51].

N-terminal deprotection without aza-DKP formation and enantiomeric purity

To avoid aza-DKP formation and afford a free amine building block for aza-peptide synthesis, *N*-benzophenone semicarbazone aza-Phe-Pro *tert*-butyl ester **31c** was treated with hydroxylamine hydrochloride in pyridine at 60 °C for 12 h [29]. Purification by chromatography on silica gel gave the free amine **40c** in 88% yield (Scheme 6).

Amine **40c** was subsequently used for determining enantiomeric purity to ascertain whether α-carbon racemization had occurred during semicarbazone alkylation and removal. Aza-tripeptides (*R*,*S*)- and (*S*,*S*)-**41** were synthesized by coupling **40c,** respectively, to Fmoc-D- and L-alanine by the way of a mixed anhydride, formed using isobutylchloroformate and NMM. Without purification, aza-tripeptide (*S*,*S*)-**41** was then demonstrated to be of >98% diastereomeric excess using normal phase HPLC with subsequent incremental additions of the (*R*,*S*)-**41** diastereomer to establish the limits of detection (Scheme 6; for HPLC analyses see supporting information). Hence, no racemization was deemed to occur during the submonomer aza-peptide synthesis leading to pure (*S*,*S*)-**41** and (*R*,*S*)-**41** diastereomers. Moreover, the use of the *N*-benzophenone semicarbazone aza-dipeptide *tert*-butyl ester as building block was validated by the synthesis of these aza-tripeptides and their purification in 92 and 95% yields.

Conclusion

The construction and alkylation of aza-Gly dipeptides to prepare a variety of aza-dipeptides in solution has been achieved using benzophenone semicarbazone protection. Using a variety of alkyl halides, methyl, allyl and propargyl side chains, which

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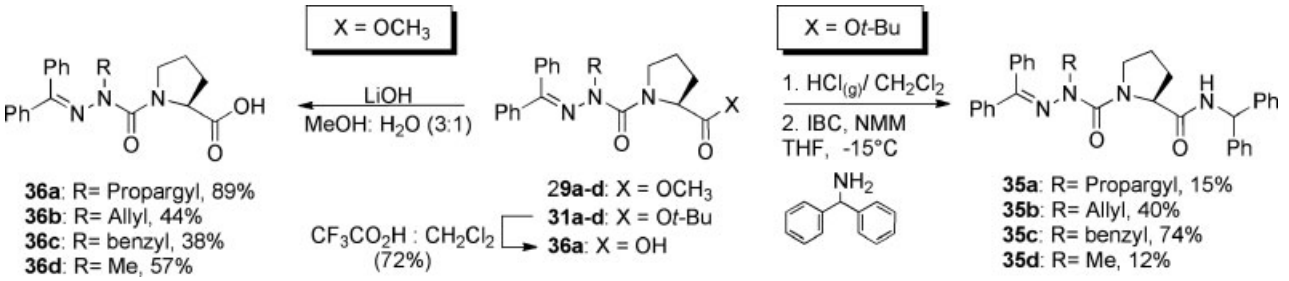
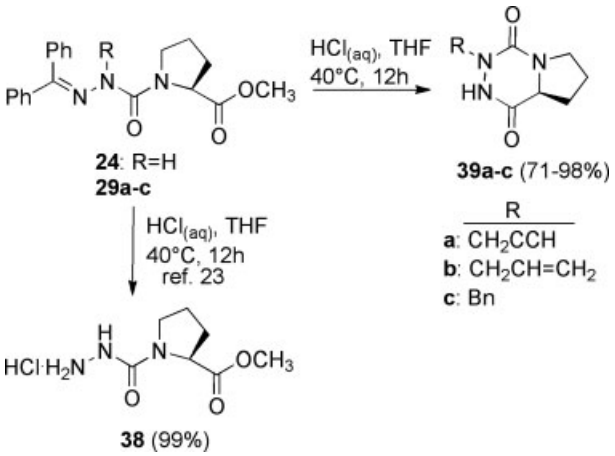
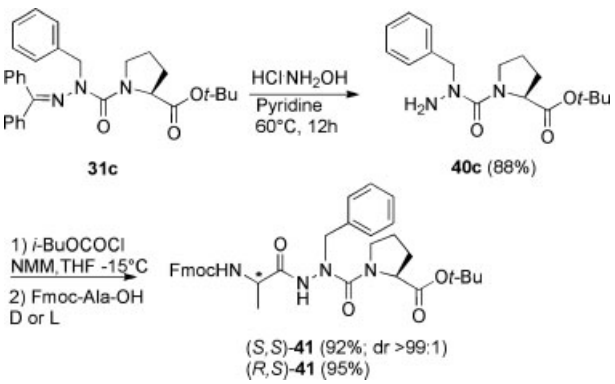


Scheme 4. *C*-terminal deprotection of benzophenone semicarbazone aza-dipeptide esters.



Scheme 5. Deprotection of benzophenone semicarbazone and aza-DKP synthesis.



Scheme 6. Synthesis and diastereomeric purity of aza-tripeptides (*R*,*S*)- and (*S*,*S*)-**41**.

are challenging to introduce by traditional hydrazine chemistry, were incorporated specifically onto the aza-Gly residue by this submonomer synthesis. *C*-terminal amino acid residues bearing aliphatic and aromatic side chains were tolerated such that removal of the ester and benzophenone semicarbazone protection could be accomplished selectively to afford dipeptide units for elongation on the *C*- and *N*-termini, respectively, to afford aza-peptides with diastereomeric purity >98%. Using acidic conditions to remove the benzophenone semicarbazone moiety from aza-Xaa-Pro methyl esters gave aza-DKPs **39a–c** possessing a diverse series of side chains at the aza-residue. By circumventing the issues of hydrazine synthesis, this method for aza-dipeptide and aza-DKP synthesis has opened the way for preparing diverse analogs of these interesting structures in solution.

Experimental

Unless otherwise noted, all reactions were performed under an argon atmosphere and distilled solvents were transferred by syringe. Benzophenone hydrazone and *p*-nitrophenyl chloroformate were purchased from Aldrich Chemicals. Anhydrous solvents (THF, CH_2Cl_2 and CH_3OH) were obtained by passage through solvent filtration systems (GlassContour, Irvine, CA, USA). DIEA was distilled over ninhydrin and CaH_2. Final reaction mixture solutions were dried over $MgSO_4$ or Na_2SO_4. Chromatography was on 230–400 mesh silica gel, and TLC was on glass-backed silica plates. Melting points were made on a Gallankamp apparatus and are uncorrected. Specific rotations $[\alpha]_D$ were measured at 20 °C at the specified concentrations (*c* in g/100 ml) using a 1-dm cell on a PerkinElmer Polarimeter 341 and the general formula: $[\alpha]_D^{20} = (100 \times \alpha)/(d \times c)$. Accurate mass measurements were performed on a LC-MSD instrument from Agilent technologies in positive electrospray (ES) mode at the Université de Montréal Mass Spectrometry facility. Analytical HPLC for enantiomeric purity determination was made by using an analytical Phenomenex Luna normal phase HPLC column (150 × 4.60 mm, 3 µm). Either protonated molecular ions $[M + H]^+$ or sodium adducts $[M + Na]^+$ were used for empirical formula confirmation. ^1H NMR spectra were measured in $CDCl_3$, CD_3OD or DMSO-d_6 at 400 MHz and referenced to $CDCl_3$ (7.26 ppm), CD_3OD (3.31 ppm) or DMSO-d_6 (2.50 ppm). ^{13}C NMR spectra were measured in $CDCl_3$, CD_3OD or DMSO-d_6 at 100 MHz and, respectively, referenced to $CDCl_3$ (77.0 ppm), CD_3OD (49.0 ppm) or DMSO (39.52 ppm). Coupling constants, *J* values were measured in hertz (Hz) and chemical shift values in parts per million (ppm).

Synthesis of Phtalimido Aza-Glycinyl-Phenylalanine-OMe (15)

N-Aminophthalimide **14** (2 g, 12.3 mmol, prepared according to the literature procedure [36]) was added to a solution of *p*-nitrophenylchloroformate (2.5 g, 12.3 mmol) in anhydrous DCM (31 ml) at 0 °C under argon. The reaction mixture was stirred at room temperature for 2 h, and when no more starting material appeared by TLC (R_f = 0.5, 1 : 1 v/v hexanes : EtOAc), the reaction was treated dropwise with DIEA (2.1 ml, 12.3 mmol), stirred for 15 min and treated with a premixed solution of Phe-OMe•HCl (2.7 g, 12.4 mmol) and DIEA (2.1 ml, 12.3 mmol) in CH_2Cl_2. After stirring at room temperature for 12 h, the mixture was diluted with CH_2Cl_2 (100 ml) and extracted with a saturated solution of aqueous $NaHCO_3$ (3 × 100 ml) and brine (1 × 100 ml). The combined organic

phases were dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel with a 1 : 1 mixture of hexanes : ethyl acetate. Evaporation of the collected fractions gave aza-dipeptide **15** as a white solid (92 mg, 2%): mp 184–185 °C; R_f 0.42 (hexanes : EtOAc 1 : 1); $[\alpha]^{20}_D$ 104 (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.09 (2H, m), 3.67 (3H, s), 4.79 (1H, dd, $J = 5.8$, 2 Hz), 6.06 (1H, d, $J = 7.8$ Hz), 7.11 (3H, t, $J = 7.2$ Hz), 7.19 (2H, t, $J = 7,2$ Hz), 7.51 (1H, s), 7.75 (2H, dd, $J = 5.4$, 3.1 Hz), 7.86 (2H, dd, $J = 5.4$, 3.1 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 37.6, 52.1, 53.8, 123.5, 126.6, 128.0, 129.1, 129.7, 134.2, 135.3, 154.8, 165.7, 172.3. HRMS (ESI) *m/z* calcd for $C_{19}H_{18}N_3O_5$ 368.1249, found $[M + H]^+$ 368.1241.

Benzhydrylidene Aza-Glycinyl-Glycine Ethyl Ester (21)

A stirred solution of *p*-nitrophenyl chloroformate (464 mg, 2.3 mmol) in 20 ml of DCM at 0 °C was treated dropwise with a solution of benzophenone hydrazone **17** (451.4 mg, 2.3 mmol) in 10 ml of DCM. The ice bath was removed and the reaction was allowed to warm to room temperature. After 1 h, complete disappearance of the hydrazone and the formation of the activated methylidine carbazate intermediate **20** were observed by TLC R_f 0.83 (EtOAc : hexane 1 : 1). The reaction mixture was treated with a premixed solution of ethyl glycinate hydrochloride (0.32 g, 2.3 mmol) and DIEA (0.8 ml, 4.6 mmol) in DCM. After stirring overnight, the crude reaction mixture was concentrated under vacuum and purified by column chromatography on silica gel using 2 : 1 hexanes : EtOAc as eluant. Evaporation of the collected fractions gave semicarbazone **21** as a white solid (0.5 g, 60%). R_f 0.25 (hexanes : EtOAc 2 : 1); mp 140–142 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.19 (3H, t, $J = 7$ Hz), 4.02 (2H, d, $J = 5.6$ Hz), 4.13 (2H, dd, $J = 7$, 14.4 Hz), 6.78 (1H, t, $J = 5.5$ Hz), 7.1–7.4 (10H, m), 7.61 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 13.8, 41.4, 61.0, 126.8, 127.8, 127.9, 128.1, 129.0, 129.1, 129.4, 129.4, 129.5, 131.4, 136.5, 148.2, 151.0, 154.9, 170. HRMS (ESI) *m/z* calcd for $C_{18}H_{20}N_3O_3$ $[M + H]^+$ 326.1499; found 326.1503.

Benzhydrylidene Aza-Glycinyl-Leucine Methyl Ester (22)

This was synthesized according to the procedure described above for the synthesis of aza-Gly dipeptide **21** from methyl leucinate hydrochloride (0.3 g, 1.65 mmol), and the product was purified by column chromatography using 2 : 1 hexanes : EtOAc as eluant. Evaporation of the collected fractions gave semicarbazone **22** as a white solid (0.4 g, 62%): R_f 0.47 (2 : 1 hexanes : EtOAc); mp 148–150 °C; $[\alpha]^{20}_D$ 26.7 (*c* 0.25, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.91 (6H, dd, $J = 4$, 8 Hz), 1.55–1.75 (3H, m), 3.67 (3H, s), 4.55 (1H, dt, $J = 3.5$, 9.7 Hz), 6.61 (1H, d, $J = 8.8$ Hz), 7.15–7.46 (10H, m), 7.56 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 13.8, 21.6, 22.6, 24.5, 41.6, 50.9, 51.8, 53.1, 126.8, 128.0, 128.1, 129.0, 129.3, 129.4, 129.5, 131.5, 136.6, 148.1, 154.9, 173.5. HRMS (ESI) *m/z* calcd for $C_{21}H_{26}N_3O_3$ $[M + H]^+$ 368.1969; found 368.1968.

Benzhydrylidene Aza-Glycinyl-D-Phenylalanine Methyl Ester (23)

This was synthesized from D-phenylalanine methyl ester hydrochloride (4 g, 22.3 mmol) according to the procedure described above and purified by column chromatography using 1 : 1 hexanes : EtOAc as eluant. Evaporation of the collected fractions gave semicarbazone **23** as a white solid (3.6 g, 44%). R_f 0.32 (ether : petroleum ether 4 : 1); mp 119.4–122.6, $[\alpha]^{20}_D$ −34.6 (*c* 0.68, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.20 (2H, m), 3.73 (3H, s), 4.85 (1H, m), 6.78 (1H, dd, $J = 12$, 24 Hz), 7.19–7.51 (15H, m) 7.58 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 38.2, 38.4, 52.1, 52.2, 53.3, 53.8, 126.8, 127.1, 128.1, 128.3, 128.4, 128.6, 129.2, 129.3, 129.6, 129.7, 131.5, 135.9, 136, 136.7, 148.2, 154.6, 172.2, 172.9. HRMS (ESI) *m/z* calcd for $C_{24}H_{24}N_3O_3$ 402.1812; found $[M + H]^+$ 402.1825.

Benzhydrylidene Aza-Glycinyl-Proline Methyl Ester (24)

This was synthesized according to the procedure described above from proline methyl ester hydrochloride (640 mg, 4.96 mmol) and purified by column chromatography using 1 : 1 hexanes : EtOAc as eluant. Evaporation of the collected fractions gave semicarbazone **24**, as a light yellow oil (1 g, 60%): R_f 0.36 (hexanes : EtOAc 1 : 1); $[\alpha]^{20}_D$ −41.0 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.88–2.06 (3H, m), 2.14–2.23 (1H, m), 3.55–3.65 (2H, m), 3.67 (3H, s), 4.64 (1H, d, $J = 8$ Hz), 7.25–7.34 (5H, m), 7.48–7.56 (5H, m), 7.76 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 24.4, 30.2, 48.2, 52.6, 60.8, 116.4, 126.5, 127.8, 128.6, 128.9, 129.6, 130.2, 132.5, 137.4, 150.5, 154.3, 164.4, 173.8. HRMS (ESI) *m/z* calcd for $C_{20}H_{22}O_3N_3$ $[M + H]^+$ 352.1656; found 352.1671.

Benzhydrylidene Aza-Glycinyl-Proline *tert*-Butyl Ester (25)

This was synthesized according to the general procedure described above from a premixed solution of free amino proline *tert*-butyl ester (2.3 g, 11.68 mmol) with 1 eq. of DIEA (2 ml, 11.68 mmol) in DCM and purified by column chromatography using 3 : 2 hexanes : EtOAc as eluant. Evaporation of the collected fractions gave semicarbazone **25**, as a light yellow foam (2.32 g, 51%): R_f 0.37 (hexanes : EtOAc 1 : 1); $[\alpha]^{20}_D$ −42.3 (*c* 0.208, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.26 (9H, s), 1.73–1.87 (3H, m), 2.06 (1H, s), 3.46 (2H, m), 4.51 (1H, s), 7.08–7.16 (5H, m), 7.38–7.43 (5H, m), 7.68 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 24.8, 28.2, 31.0, 48.1, 61.0, 81.4, 127.6, 128.5, 128.8, 129.3, 130.0, 130.1, 132.3, 137.6, 148.9, 154.0, 172.1. HRMS (ESI) *m/z* calcd for $C_{23}H_{28}O_3N_3$ $[M + H]^+$ 394.2125; found 394.2142.

Benzhydrylidene Aza-Glycinyl-Proline (26)

This was synthesized according to the procedure described above from proline (570 mg, 4.96 mmol) dissolved in methanol with 1 eq. of DIEA (0.86 ml, 4.96 mmol) and purified by column chromatography using 20 : 1 EtOAc : AcOH as eluant. Evaporation of the collected fractions gave semicarbazone **26** as a pale yellow foam (1 g, 57%): R_f 0.5 (EtOAc : AcOH 20 : 1); mp 86.2–88.2 °C; $[\alpha]_D^{20}$ −97.3 (*c* 1.09, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.94–2.04 (3H, m), 2.28 (1H, s), 3.44–3.51 (2H, m), 4.57 (1H, dd, $J = 2.8$, 4.8 Hz), 7.28–7.33 (5H, m), 7.50–7.58 (5H, m), 7.92 (1H, br s); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 24.3, 28.2, 47.3, 60.4, 127.3, 128.1, 128.2, 129.3, 129.7, 129.8, 131.6, 136.8, 150.9, 155.0. HRMS (ESI) *m/z* calcd for $C_{19}H_{20}O_3N_3$ $[M + H]^+$ 338.1499; found 338.1499.

Benzhydrylidene Aza-Glycinyl-D-Phenylalanine (27)

This was synthesized from D-phenylalanine (4 g, 20.4 mmol) in DCM according to the procedure described above and purified by column chromatography using 9 : 1 DCM : MeOH as eluant. Evaporation of the collected fractions gave semicarbazone **27** as a white solid (3.23 g, 41%): R_f 0.35 (MeOH : DCM 1 : 9); mp = 210.1–213.0; $[\alpha]^{20}_D$ 21.2 (*c* 0.84, $CHCl_3$). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.12–3.17 (2H, m), 4.48 (1H, dd, $J = 6.6$, 13.6 Hz), 7.09

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(1H, d, J = 8.2 Hz), 7.25–7.42 (12H, m), 7.52–7.60 (3H, m), 8.50 (1H, br s), 12.85 (1H, br s); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 38.2, 40.5, 40.8, 41.1, 41.3, 41.6, 54.8, 127.9, 128.0, 129.6, 129.7, 130.3, 130.5, 130.7, 130.8, 133.4, 138.6, 138.7, 148.5, 155.7, 174.6. HRMS (ESI) m/z calcd for $C_{23}H_{22}O_3N_3$ $[M + H]^+$ 388.1656; found 388.1662.

Benzhydrylidene Aza-Propargylglycinyl-Proline Methyl Ester (29a)

This was synthesized from benzophenone aza-Gly-Pro-OMe **24** (400 mg, 1.14 mmol) dissolved in 5 ml of anhydrous THF at 0 °C. This solution was treated with 1.1 eq. of *t*BuOK (141 mg, 1.25 mmol) and stirred for 15 min prior to the dropwise addition of 1.1 eq. of propargyl bromide (112 µl, 1.25 mmol). The reaction was allowed to warm to room temperature and left to react overnight. After evaporation under reduced pressure, the crude material was purified by chromatography on silica gel using 20% EtOAc in hexanes as solvent system. Evaporation of the collected fractions gave the propargyl semicarbazone **29a** as a pale yellow foam (165 mg, 37%): R_f 0.52 (hexanes : EtOAc 1 : 1); $[\alpha]^{20}_D$ −126 (c 0.21, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.76–1.83 (1H, m), 1.86–1.94 (2H, m), 2.09 (1H, t, J = 2.4 Hz), 2.10–2.12 (1H, m), 3.45 (3H, s), 3.69–3.76 (3H, m), 4.31 (1H, dd, J = 2.4, 17.6 Hz), 4.52 (1H, dd, J = 3.2, 8.4 Hz), 7.36–7.38 (2H, m), 7.41–7.43 (3H, m), 7.46–7.48 (3H, m), 7.52–7.56 (2H, m); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 38.1, 49.7, 52.1, 61.3, 72.0, 77.1, 79.6, 128.5, 129.1, 129.2, 129.3, 129.9, 130.5, 136.3, 136.7, 159.9, 161.0, 173.8. HRMS (ESI) m/z calcd for $C_{23}H_{24}O_3N_3$ $[M + H]^+$ 390.1739; found 390.1812.

Benzhydrylidene Aza-Allylglycinyl-Proline Methyl Ester (29b)

This was synthesized according to the procedure described for **29a** but using allyl bromide (0.11 ml, 1.25 mmol) as alkyl agent. After purification by chromatography on silica gel using 20% EtOAc in hexanes as solvent system, the evaporation of the collected fractions gave the allyl semicarbazone **29b** as a pale yellow foam (87 mg, 20%): R_f 0.50 (hexanes : EtOAc 1 : 1); $[\alpha]^{20}_D$ −179 (c 0.267, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.78–2.04 (3H, m), 2.12–2.19 (1H, m), 3.43 (3H, s), 3.63–3.69 (3H, m), 4.11 (1H, dd, J = 5.2 Hz, 16 Hz), 4.50 (1H, dd, J = 3.6 Hz, 8.4 Hz), 4.88 (1H, dd, J = 1.2 Hz, 17.2 Hz), 5.01 (1H, dd, J = 1.2 Hz, 9.2 Hz), 5.61–5.69 (1H, m), 7.34–7.41 (5H, m), 7.46–7.49 (5H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 23.8, 29.9, 49.1, 51.1, 51.4, 60.8, 116.9, 127.8, 128.5, 128.6, 128.7, 129.2, 129.7, 133.5, 136.4, 138.5, 159.3, 160.1, 173.3. HRMS (ESI) m/z calcd for $C_{23}H_{26}O_3N_3$ $[M + H]^+$ 390.1896; found 390.1969.

Benzhydrylidene Aza-Phenylalaninyl-Proline Methyl Ester (29c)

This was synthesized according to the procedure described for **29a** but using benzyl bromide (0.15 ml, 1.25 mmol) as alkylating agent. After purification by chromatography on silica gel using 20% EtOAc in hexanes as solvent system, the evaporation of the collected fractions gave the benzyl semicarbazone **29c** as a yellow foam (77 mg, 15%): R_f 0.53 (hexanes : EtOAc 1 : 1); $[\alpha]^{20}_D$ −101 (c 0.467, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.81–1.95 (3H, m), 2.14–2.19 (1H, m), 3.47 (3H, s), 3.68–3.79 (2H, m), 4.36 (1H, d, J = 16 Hz), 4.55 (1H, dd, J = 3.6 Hz, 8 Hz), 4.76 (1H, d, J = 16 Hz), 7.06–7.45 (15H, m); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 23.9, 29.5, 29.9, 49.3, 51.5, 51.9, 60.9, 126.5, 127.5, 127.7, 127.8, 128.3, 128.6, 128.8, 129.3, 129.6, 136.1, 137, 138.6, 159.3, 160.0, 173.4. HRMS (ESI) m/z calcd for $C_{27}H_{28}O_3N_3$ $[M + H]^+$ 442.2052; found 442.2124.

Benzhydrylidene Aza-Alaninyl-Proline Methyl Ester (29d)

This was synthesized according to the procedure described for **29a** but using methyl iodide (0.15 ml, 1.25 mmol) as alkylating agent. After purification by chromatography on silica gel using 20% EtOAc in hexanes as solvent system, the evaporation of the collected fractions gave the methyl semicarbazone **29d** as a light yellow foam (248 mg, 80%): R_f 0.39 (hexanes : EtOAc 1 : 1); $[\alpha]^{20}_D$ −173 (c 0.33, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.84–1.96 (3H, m), 2.13–2.16 (1H, m), 2.76 (3H, s), 3.39 (3H, s), 3.73 (2H, t, J = 6.8 Hz), 4.56 (1H, dd, J = 3.6 Hz, 8.4 Hz), 7.33–7.39 (5H, m), 7.44–7.48 (5H, m); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 23.7, 30.1, 37.6, 49.4, 51.4, 61.1, 127.8, 127.9, 128.1, 128.5, 128.9, 129.1, 129.5, 136.4, 138.7, 156.7, 159.9, 173.5. HRMS (ESI) m/z calcd for $C_{21}H_{24}O_3N_3$ $[M + H]^+$ 366.1739; found 366.1814.

Benzhydrylidene Aza-Phenylalanilyl-D-Phenylalanine Methyl Ester (30)

This was synthesized according to the procedure described for **29c** from**23** (0.46 mmol) using benzyl bromide (65.8 µl, 0.55 mmol) as alkylating agent. After purification by chromatography on silica gel using 15% EtOAc in hexanes as solvent system, the evaporation of the collected fractions gave the benzyl semicarbazone **30** as a yellow oil (33.3 mg, 14%): R_f 0.52 (1 : 1 hexane : EtOAc); $[\alpha]^{20}_D$ 27.6 (c 0.47, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.17–3.30 (2H, m), 3.78 (3H, s), 4.45 (1H, d, J = 16.4 Hz), 4.65 (1H, d, J = 16.4 Hz), 4.94 (1H, dd, J = 7.2 Hz, 13.2 Hz), 6.79 (2H, d, J = 7.2 Hz), 7.08–7.49 (19H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 38.1, 49.0, 52.1, 54.4, 126.6, 126.9, 127.0, 127.9, 128.3, 128.4, 128.5, 129.1, 129.2, 129.6, 129.7, 135.5, 136.2, 136.5, 138.7, 157.1, 158.3, 172.6. HRMS (ESI) m/z calcd for $C_{31}H_{29}O_3N_3$ $[M + Na]^+$ 514.2101; found 514.2119.

Benzhydrylidene Aza-Propargylglycinyl-Proline *tert*-Butyl Ester (31a)

This was synthesized from benzophenone aza-Gly-Pro-O*t*Bu **25** (200 mg, 0.51 mmol) dissolved in 2 ml of anhydrous THF at 0 °C. This solution was treated with 1.1 eq. of *t*BuOK (63 mg, 0.56 mmol) and the reaction mixture was stirred for 15 min followed by dropwise addition of 1.1 eq. of propargyl bromide (50 µl, 0.56 mmol). The reaction was allowed to warm to room temperature and left to react overnight. After evaporation under reduced pressure, the crude material was purified by chromatography on silica gel using 20% EtOAc in hexanes as solvent system. Evaporation of the collected fractions gave the propargyl semicarbazone **31a** as a white foam (158 mg, 72%): R_f 0.40 (hexanes : EtOAc 7 : 3); $[\alpha]^{20}_D$ −17.6 (c 0.193, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.44 (9H, s), 1.74–1.77 (1H, m), 1.86–1.91 (2H, m), 2.10–2.13 (2H, m), 3.60–3.64 (2H, m), 3.65 (1H, d, J = 2.4 Hz), 3.92 (1H, d, J = 17.6 Hz) 4.49 (1H, m), 7.33–7.36 (2H, m), 7.39–7.45 (6H, m), 7.57–7.59 (2H, m); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 24.6, 28.4, 30.1, 38.7, 49.7, 61.8, 71.9, 79.9, 81.3, 128.5 128.9, 129, 129.2, 129.7, 130.4, 136.4, 138.7, 159.7, 161,1, 172.6. HRMS (ESI) m/z calcd for $C_{26}H_{30}O_3N_3$ $[M + H]^+$ 432.2282; found 432.2301.

Benzhydrylidene Aza-Allylglycinyl-Proline *tert*-Butyl Ester (31b)

This was synthesized according to the procedure described for the synthesis of aza-propargylglycinyl dipeptide **31a** but using allyl bromide (47 µl, 0.56 mmol) as alkylating agent. After purification by chromatography on silica gel using 20% EtOAc in hexanes as

solvent system, the evaporation of the collected fractions gave the allyl semicarbazone **31b** as a yellow oil (124 mg, 56%): R_f 0.46 (hexanes : EtOAc 7 : 3); $[\alpha]^{20}_D$ −3.6 (*c* 0.165, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.45 (9H, s), 1.65–1.72 (1H, m), 1.84–1.90 (2H, m), 2.07–2.14 (1H, m), 3.55–3.62 (2H, m), 3.82 (1H, dd, J = 6 Hz, 15.9 Hz), 4.02 (1H, dd, J = 5.6 Hz, 15.9 Hz), 4.39 (1H, dd, J = 4.4 Hz, 8.4 Hz), 4.93 (1H, dd, J = 1.6 Hz, 17.2 Hz), 5.02 (1H, dd, J = 1.2 Hz, 10.4 Hz), 5.64–5.71 (1H, m), 7.32–7.45 (8H, m), 7.52–7.55 (2H, m); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 24.8, 28.4, 30.0, 49.7, 52.2, 61.8, 81.1, 117.3, 128.4, 128.9, 129.2, 129.5, 130.1, 134.4, 137.0, 139.0, 159.7, 160.4, 172.7. HRMS (ESI) *m/z* calcd for $C_{26}H_{32}O_3N_3$ $[M + H]^+$ 434.2607; found 434.2481.

Benzhydrylidene Aza-Phenylalaninyl-Proline *tert*-Butyl Ester (31c)

This was synthesized according to the procedure described for **31a** but using benzyl bromide (67 µl, 0.56 mmol) as alkylating agent. After purification by chromatography on silica gel using 20% EtOAc in hexanes as solvent system, the evaporation of the collected fractions gave the benzyl semicarbazone **31c** as a yellow oil (207 mg, 84%): R_f 0.52 (hexanes : EtOAc 7 : 3); $[\alpha]^{20}_D$ 10 (*c* 0.22, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.49 (9H, s), 1.71–1.78 (1H, m), 1.82–1.98 (2H, m), 2.14–2.19 (1H, m), 3.59–3.68 (2H, m), 4.42–4.46 (1H, m), 4.58 (1H, d, J = 16 Hz), 4.66 (1H, d, J = 16 Hz), 7.12–7.18 (3H, m), 7.22–7.29 (6H, m), 7.42–7.43 (6H, m); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 24.9, 28.4, 30.1, 49.9, 53.2, 62.0, 81.2, 127.0, 128.3, 128.4, 128.6, 128.8, 128.9, 129.3, 129.6, 130.1, 136.8, 137.9, 139.1, 159.6, 160.4, 172.7. HRMS (ESI) *m/z* calcd for $C_{30}H_{34}O_3N_3$ $[M + H]^+$ 484.2414; found 484.2641.

Benzhydrylidene Aza-Alaninyl-Proline *tert*-Butyl Ester (31d)

This was synthesized according to the procedure described for **31a** but using methyl iodide (35 µl, 0.56 mmol) as alkylating agent. After purification by chromatography on silica gel using 20% EtOAc in hexanes as solvent system, the evaporation of the collected fractions gave the methyl semicarbazone **31d** as a light yellow oil (152 mg, 73%): R_f 0.38 (hexanes : EtOAc 7 : 3); $[\alpha]^{20}_D$ −9.7 (*c* 0.27, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.43 (9H, s), 1.72–1.76 (1H, m), 1.77–1.95 (2H, m), 2.14–2.19 (1H, m), 2.77 (3H, s), 3.63–3.74 (2H, m), 4.51 (1H, dd, J = 4.4 Hz, 8 Hz), 7.29–7.35 (5H, m), 7.41–7.43 (3H, m), 7.52–7.54 (2H, m); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 24.7, 28.4, 30.3, 38.4, 50.1, 62.1, 81.1, 128.5, 128.8, 128.9, 129.4, 129.5, 129.9, 137.0, 139.3, 156.4, 160.2, 172.9. HRMS (ESI) *m/z* calcd for $C_{24}H_{30}O_3N_3$ $[M + H]^+$ 408.2282; found 408.2299.

Benzhydrylidene Aza-Allylglycinyl-Glycine Ethyl Ester (32)

This was synthesized according to the procedure described for aza-allylglycinyl dipeptide **29b** from the semicarbazone aza-Gly-Gly-OEt (50 mg, 0.15 mmol) using allyl bromide (20 µl, 0.18 mmol) as alkylating agent. After purification by flash chromatography on silica gel using EtOAc in hexanes (1 : 2) as solvent system, the evaporation of the collected fractions gave the allyl semicarbazone **32** as an oil (35 mg, 60%): R_f 0.45 (hexanes : EtOAc 2 : 1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.23 (3H, t, J = 7 Hz), 3.86 (2H, dd, J = 1.6 Hz, 3.8 Hz), 4.05 (2H, d, J = 5.7 Hz), 4.16 (2H, q, J = 7 Hz), 4.78 (1H, dd, J = 1 Hz, 10 Hz), 4.92 (1H, dd, J = 1 Hz, 10 Hz) 5.33–5.43 (1H, m), 6.89 (1H, t, J = 5.5 Hz), 7.18–7.42 (10H, m); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 13.8, 42.2, 47.9, 60.8, 116.3, 127.8, 128.2, 128.3, 128.7, 129.3, 129.6, 132.4, 135.6, 138.4, 157.7, 158.4, 170.3. HRMS (ESI) *m/z* calcd for $C_{21}H_{24}N_3O_3$ $[M + H]^+$ 366.1813; found 366.1812.

Benzhydrylidene Aza-Propargylglycinyl-Proline Propargyl Ester (33)

This was synthesized from benzophenone aza-Gly-Pro-OH **26** (200 mg, 0.59 mmol) dissolved in 5 ml of anhydrous THF at 0 °C. This solution was treated with 2 eq. of *t*BuOK (133 mg, 1.18 mmol) and the reaction mixture was stirred for 15 min followed by the dropwise addition of 5 eq. of propargyl bromide (0.26 ml, 2.97 mmol). The reaction was allowed to warm to room temperature and left to react overnight. After evaporation under reduced pressure, the crude material was purified by flash chromatography on silica gel using 20% EtOAc in hexanes as solvent system. Evaporation of the collected fractions gave the propargyl semicarbazone **33** as a light yellow foam (125 mg, 51%): R_f 0.42 (hexane : EtOAc 1 : 1); $[\alpha]^{20}_D$ −225 (*c* 0.09, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.83 (1H, m), 1.92–1.99 (2H, m), 2.03 (1H, s), 2.08–2.16 (1H, m), 2.41 (1H, s), 3.62–3.72 (3H, m), 4.32 (1H, d, J = 2 Hz), 4.36 (1H, d, J = 2 Hz), 4,52 (2H, m), 7.31–7.51 (10H, m); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 23.2, 29.3, 29.7, 37.1, 49.0, 51.5, 60.6, 71.4, 74.6, 76.5, 77.2, 78.7, 127.8, 127.9, 128.3, 128.4, 128.5, 129.3, 129.8, 135.5, 138.0, 159.3, 160.1, 171.5. HRMS (ESI) *m/z* calcd for $C_{25}H_{24}O_3N_3$ $[M + H]^+$ 414.1812; found 414.1826.

Benzhydrylidene Aza-Propargylglycinyl-D-Phenylalanine Propargyl Ester (34a)

This was synthesized from benzophenone aza-Gly-D-Phe-OH **27** (500 mg, 1.29 mmol) according to the procedure described for dialkylated aza-analog **33** using propargyl bromide. After purification by flash chromatography using a gradient solvent of 1 : 9 ethyl acetate in hexanes, the evaporation of the collected fractions afforded **34a** as a yellow oil in 22% yield (131 mg). R_f 0.61 (hexanes : EtOAc 1 : 1); $[\alpha]^{20}_D$ 50.5 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.06 (1H, s), 2.51 (1H, s), 3.21–3.24 (2H, m), 3.85 (1H, d, J = 20.4 Hz), 4.19 (1H, d, J = 18 Hz), 4.24 (1H, d, J = 2.4 Hz), 4.78 (1H, d, J = 2.4 Hz), 4.89 (1H, m), 7.01 (1H, d, J = 7.7 Hz), 7.21–7.28 (5H, m), 7.32–7.41 (4H, m), 7.44–7.46 (6H, m); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 35.0, 37.8, 52.4, 54.2, 71.8, 75.2, 78.3, 126.9, 128.0, 128.5, 129.0, 129.4, 129.6, 130.0, 135.2, 135.7, 138.2, 157.6, 158.2, 171.0. HRMS (ESI) *m/z* calcd for $C_{29}H_{26}O_3N_3$ $[M + H]^+$ 464.1968; found 464.1991.

Benzhydrylidene Aza-Phenylalanine-D-Phenylalanine Benzyl Ester (34b)

This was synthesized from benzophenone aza-Gly-D-Phe-OH **27** (500 mg, 1.29 mmol) according to the procedure described for dialkylated aza-analog **33** using benzyl bromide. After purification by flash chromatography using a gradient solvent from 1 : 9 to 1 : 1 ethyl acetate in hexanes, the evaporation of the collected fractions afforded **34b** as a yellow oil in 63% yield (457 mg). R_f 0.62 (hexanes : EtOAc 1 : 1); $[\alpha]^{20}_D$ 35.2 (*c* 0.97, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.20–3.25 (2H, m), 4.55 (1H, d, J = 22 Hz), 4.63 (1H, d, J = 22 Hz), 4.99 (1H, m), 5.19 (2H, m), 6.79 (2H, m), 7.08–7.46 (25H, m); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 39.1, 50.0, 55.3, 67.8, 127.5, 127.7, 128.0, 128.8, 129.1, 129.2, 129.3, 129.4, 130.0, 130.2, 130.5, 130.6, 136.3, 136.5, 137.1, 137.5, 139.6, 158.1, 159.2, 172.8. HRMS (ESI) *m/z* calcd for $C_{37}H_{35}O_3N_3$ $[M + H]^+$ 568.2595; found 568.2603.

Benzhydrylidene Aza-Propargylglycinyl-Prolinyl-Diphenylmethylamide (35)

This was synthesized according to the procedure described for **29a** using propargyl bromide (11.3 µl, 0.127 mmol) as alkylating

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agent. After purification by chromatography on silica gel using 1 : 1 EtOAc : hexanes as solvent system, the evaporation of the collected fractions gave the propargyl semicarbazone **35** as a pale yellow foam (30 mg, 48%): R_f 0.46 (hexanes : EtOAc 1 : 1); $[\alpha]^{20}_D$ 196 (*c* 0.17, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.75–1.88 (2H, m), 2.01 (1H, t, J = 2.4 Hz), 2.10–2.17 (2H, m), 3.53–33.62 (3H, m), 4.33 (1H, dd, J = 2.3 Hz, 17.6 Hz), 4.78 (1H, dd, J = 6 Hz, 7.75 Hz), 6.21 (1H, d, J = 8.3 Hz), 7.23–7.38 (13H, m), 7.38–7.40 (5H, m), 7.45–7.47 (3H, m), 7.48 (1H, d, J = 8.3 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 25.2, 28.3, 37.4, 50.1, 56.7, 61.5, 71.5, 78.9, 127.0, 127.1, 127.2, 128.0, 128.3, 128.4, 128.5, 128.7, 129.5, 130.1, 135.4, 137.8, 141.5, 141.9, 161.0, 161.6, 171.0. HRMS (ESI) *m/z* calcd for $C_{35}H_{34}O_4N_3$ $[M + H]^+$ 541.2598; found 541.2603.

Benzhydrilidene Aza-Propargylglycinyl-Proline Diphenylmethylamide (35a)

This was synthesized from *tert*-butyl ester **31a** (88 mg, 0.204 mmol) dissolved in 10 ml of DCM. This solution was treated with HCl gas bubbles via Teflon canula. After striring for 2 h, the reaction was evaporated under reduced pressure and placed under vacuum for 2 h. The crude salt was dissolved in THF, cooled to −15 °C and treated sequentially with isobutyl chloroformate (27 µl, 0.204 mmol) and *N*-methyl morpholine (22 µl, 0.204 mmol). After 5 min, dibenzylmethyl amine (53 µl, 0.306 mmol) was added to the reaction mixture, which was stirred at −15 °C for 2 h. After the removal of the volatiles by rotary evaporation under reduced pressure, the crude was amide was purified by chromatography on silica gel using 50% EtOAc in hexane as eluent. Evaporation of the collected fractions afforded 16.7 mg (15% yield) of **35a** which exhibited the same spectral and physical properties of material, as described earlier.

Benzhydrilidene Aza-Allylglycinyl-Proline Diphenylmethylamide (35b)

This was synthesized according to the procedure described above for the synthesis of amide **35a** using *tert*-butyl ester **31b** (80 mg, 0.18 mmol), which afforded 39.7 mg (40% yield) of **35b**: R_f 0.38 (hexanes : EtOAc 1 : 1); $[\alpha]^{20}_D$ 228 (*c* 0.17, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.79–1.86 (2H, m), 2.11–2.16 (2H, m), 3.39–3.44 (1H, dd, J = 7.6 Hz, 16 Hz), 3.54–3.62 (2H, m), 4.18–4.23 (1H, dd, J = 4.4 Hz, 20 Hz), 4.80–4.86 (2H, m), 4.96–4.98 (1H, d, J = 10.4 Hz), 5.51–5.62 (1H, m), 6.25 (1H, d, J = 8.4 Hz), 7.22–7.42 (17H, m), 7.48–7.50 (3H, m), 7.60 (1H, d, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 25.1, 28.3, 50.0, 50.6, 56.2, 61.2, 117.2, 126.9, 126.9, 127.0, 127.1, 127.8, 128.1, 128.2, 128.3, 128.4, 129.3, 129.7, 133.0, 135.7, 137.8, 141.4, 141.5, 159.9, 161.9, 171.0. HRMS (ESI) *m/z* calcd for $C_{35}H_{35}O_2N_4$ $[M + H]^+$ 543.2755; found 543.2771.

Benzhydrylidene Aza-Phenylalaninyl-Proline Diphenylmethylamide (35c)

This was synthesized according to the procedure described for **35a** from *tert*-butyl ester **31c** (50 mg, 0.1 mmol), which afforded 47 mg (74% yield) of amide **35c**: R_f 0.55 (hexanes : EtOAc 1 : 1); $[\alpha]^{20}_D$ 240 (*c* 0.197, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.78–1.90 (2H, m), 2.14–2.24 (2H, m), 3.57–3.70 (2H, m), 4.06 (1H, d, J = 16 Hz), 4.88–4.92 (1H, m), 4.98 (1H, d, J = 16 Hz), 6.23 (1H, d, J = 8 Hz), 7.02–7.36 (22H, m), 7.38–7.47 (3H, m), 7.57 (1H, d, J = 8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 25.0, 28.3, 50.2, 51.3, 56.7, 61.5, 126.5, 126.8, 126.9, 127.0, 127.6, 127.7, 128.1, 128.2, 128.3, 128.4, 128.5, 129.3, 129.6, 135.6, 136.4, 137.9, 141.3, 141.7, 159.3, 162.1, 171.1. HRMS (ESI) *m/z* calcd for $C_{39}H_{37}O_2N_4$ $[M + H]^+$ 593.2911; found 593.2928.

Benzhydrylidene Aza-Alaninyl-Proline Diphenylmethylamide (35d)

This was synthesized according to the procedure described for **35a** from *tert*-butyl ester **31d** (70 mg, 0.17 mmol), which gave 11 mg (12% yield) of **35d**: R_f 0.36 (hexanes : EtOAc 1 : 1); $[\alpha]^{20}_D$ 166 (*c* 0.22, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.78–1.89 (2H, m), 2.11–2.17 (2H, m), 2.74 (3H, s), 3.55–3.69 (2H, m), 4.84 (1H, t, J = 6 Hz), 6.24 (1H, d, J = 8 Hz), 7.22–7.33 (20H, m), 7.38 (1H, d, J = 8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 25.0, 28.4, 36.8, 50.1, 56.1, 61.5, 126.9, 126.9, 127.0, 127.1, 127.2, 127.8, 127.9, 128.2, 128.3, 128.4, 128.5, 128.6, 129.1, 129.5, 135.6, 137.9, 141.4, 141.6, 158.0, 161.8, 171.3. HRMS (ESI) *m/z* calcd for $C_{33}H_{33}O_2N_4$ $[M + H]^+$ 517.2598; found 517.2609.

Benzhydrylidene Aza-Propargylglycinyl-Proline (36a)

This was synthesized from benzophenone aza-propargylGly-Pro-OMe **29a** (27 mg, 0.069 mmol) dissolved in 5 ml of a mixture of 3 : 1 MeOH : H_2O at 5 °C. To this solution was added 2 eq. of LiOH (3.3 mg, 0.138 mmol) and the reaction was stirred overnight. After evaporation, the crude material was diluted with 5 ml of 1 N HCl and extracted with EtOAc (3 × 10 ml). The organic phase was dried over Na_2SO_4 and concentrated *in vacuo* to give the acid **36a** as a yellow foam (23 mg, 89%): R_f 0.64 (EtOAc : AcOH 20 : 1); $[\alpha]^{20}_D$ −168 (*c* 0.1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.81–1.84 (1H, m), 1.89–2.00 (3H, m), 2.10–2.14 (1H, s), 2.14–2.25 (1H, m), 3.64–3.79 (3H, m), 4.26–4.51 (1H, dd, J = 1.6 Hz, 18 Hz), 4.51 (1H, m), 7.34–7.56 (10H, m); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 24.1, 37.7, 49.2, 71.6, 78.4, 127.8, 127.9, 128.3, 128.4, 128.5, 129.4, 129.7, 130.0, 132.1, 135.1, 137.6, 160.2, 161.7. HRMS (ESI) *m/z* calcd for $C_{22}H_{22}O_3N_3$ $[M + H]^+$ 376.1656; found 376.1652.

Benzhydrylidene Aza-Allylglycinyl-Proline (36b)

This was synthesized according to the procedure described for **36a**. Evaporation of the collected fractions gave the allyl semicarbazone **36b** as a pale yellow oil (12.4 mg, 44%): R_f 0.58 (EtOAc : AcOH 20 : 1); $[\alpha]^{20}_D$ −219 (*c* 0.148, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.73–2.00 (3H, m), 2.32–2.35 (1H, m), 3.58–3.73 (3H, m), 4.09 (1H, dd, J = 5.2 Hz, 15.9 Hz), 4.50 (1H, m), 4.92 (1H, dd, J = 1.2 Hz, 15.6 Hz), 5.08 (1H, dd, J = 1.1 Hz, 10.3 Hz), 5.63–5.68 (1H, m), 7.34–7.37 (4H, m), 7.43–7.53 (6H, m); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 24.6, 27.8, 29.5, 49.6, 51.6, 61.5, 117.5, 128.1, 128.2, 128.5, 128.6, 128.7, 129.6, 129.9, 130.2, 132.9, 135.7, 137.8, 161.5, 173.6. HRMS (ESI) *m/z* calcd for $C_{22}H_{24}O_3N_3$ $[M + H]^+$ 378.1812; found 378.1805.

Benzhydrylidene Aza-Phenylalaninyl-Proline (36c)

This was synthesized according to the procedure described for **36a**. Evaporation of the collected fractions gave the benzyl semicarbazone **36c** as a pale yellow foam (10.9 mg, 38%): R_f 0.61 (EtOAc : AcOH 20 : 1); $[\alpha]^{20}_D$ −154 (*c* 0.122, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.81–2.06 (3H, m), 2.33–2.35 (1H, m), 3.58–3.73 (3H, m), 4.36 (1H, d, J = 15.7 Hz), 4.51 (1H, m), 4.76 (1H, d, J = 15.7 Hz), 7.00–7.10 (2H, m) 7.12–7.48 (13H, m); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 24.5, 26.4, 27.6, 29.4, 44.9, 49.5, 52.1, 61.5, 126.8, 127.6, 127.7, 127.8, 127.9, 128.4, 128.6, 129.6, 129.7, 130.0, 132.1, 135.2, 136.1, 137.2, 137.6, 161.0, 161.7, 173.6. HRMS (ESI) *m/z* calcd for $C_{26}H_{26}O_3N_3$ $[M + H]^+$ 428.1969; found 428.1963.

Benzhydrylidene Aza-Alaninyl-Proline (36d)

This was synthesized according to the procedure described for **36a**. Evaporation of the collected fractions gave the methyl semicarbazone **36d** as a light yellow foam (20.2 mg, 57%): R_f 0.51 (EtOAc : AcOH 20 : 1); $[\alpha]^{20}_D$ −193 (*c* 0.267, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.81–2.12 (3H, m), 2.18–2.31 (1H, m), 2.81 (3H, s), 3.68–3.78 (2H, m), 4.58 (1H, m), 7.28–7.53 (10H, m), 7.79–7.82 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 24.6, 28.0, 37.8, 49.9, 61.7, 128.0, 128.1, 128.4, 128.6, 128.7, 129.5, 129.9, 130.1, 132.3, 135.7, 138.0, 159.3, 161.1, 174.2. HRMS (ESI) *m/z* calcd for $C_{20}H_{22}O_3N_3$ $[M + H]^+$ 352.1656; found 352.1649.

Aza-Hydantoin 37

This was obtained as side product during the synthesis of aza-propargylglycinyl proline methyl ester **29a**, and purified by chromatography on silica gel using 20% EtOAc in hexanes as solvent system. Second to be eluted was aza-hydantoin **37**, which gave after evaporation of the collected fractions a white foam (30.5 mg, 20%): R_f 0.47 (hexanes : EtOAc 1 : 1); $[\alpha]^{20}_D$ −166 (*c* 0.12, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.89–1.94 (2H, m), 2.08–2.19 (1H, m), 3.12–3.23 (1H, m), 3.61 (1H, m), 3.94 (1H, t, J = 8 Hz), 7.33–7.44 (8H, m), 7.71–7.74 (2H, m); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 27.0, 27.8, 46.5, 62.5, 77.7, 128.4, 128.5, 128.7, 130.2, 130.4, 132.7, 134.9, 136.01, 157.9, 168.4, 180.1. HRMS (ESI) *m/z* calcd for $C_{19}H_{18}O_2N_3$ $[M + H]^+$ 320.1394; found 320.1398.

***c*-[Aza-Propargylglycinyl-Prolyl] (39a)**

This was synthesized by the treatment of benzophenone protected aza-propargylglycinyl proline methyl ester **29a** (30 mg, 0.077 mmol) with a mixture of 5 ml of HCl 1 N and 5 ml of THF at 40 °C overnight. After concentration under reduced pressure, the aqueous crude mixture was extracted with EtOAc (3 × 10 ml) and the organic phase was dried over Na_2SO_4 and concentrated under vacuum. The crude material was purified by flash chromatography on silica gel using 100% EtOAc to finally give the aza-DKP **39a** as a pale yellow foam (12.5 mg, 84%): R_f 0.25 (100% EtOAc); $[\alpha]^{20}_D$ −18 (*c* 0.103, $CHCl_3$). ^{1}H NMR (CD_3OD, 400 MHz): δ 1.92–2.15 (3H, m), 2.21–2.31 (1H, m), 2.76 (1H, t, J = 2.4 Hz), 3.52 (2H, t, J = 7.2 Hz), 4.11 (1H, dd, J = 7.6 Hz, 9.2 Hz), 4.17 (1H, dd, J = 2.4 Hz, 17.6 Hz), 4.36 (1H, dd, J = 2.4 Hz, 17.6 Hz); ^{13}C NMR (CD_3OD, 100 MHz): δ 22.9, 27.4, 37.2, 45.3, 57.7, 73.4, 77.9, 167.3, 175.8. HRMS (ESI) *m/z* calcd for $C_9H_{12}O_2N_3$ $[M + H]^+$ 194.0924; found 194.0931.

***c*-[Aza-Allylglycinyl-Prolyl] (39b)**

This was synthesized by the treatment of benzophenone semicarbazone-protected aza-allylglycinylproline methyl ester **29b** (44.2 mg, 0.113 mmol) as described for the synthesis of **39a.** Purification by flash chromatography on silica gel using 100% EtOAc as solvent provided the aza-DKP **39b** as a pale yellow foam (15.7 mg, 71%): R_f 0.20 (100% EtOAc); $[\alpha]^{20}_D$ −54 (*c* 0.121, $CHCl_3$). ^{1}H NMR (CD_3OD, 400 MHz): δ 1.91–1.99 (2H, m), 2.01–2.13 (1H, m), 2.15–2.27 (1H, m), 3.50 (2H, t, J = 6.8 Hz), 3.91 (1H, dd, J = 6 Hz, 16 Hz), 4.05 (1H, dd, J = 7.2 Hz, 8.4 Hz), 4.32 (1H, dd, J = 5.6 Hz, 16 Hz), 5.26 (1H, dd, J = 1.2 Hz, 16.4 Hz), 5.31 (1H, dd, J = 1.2 Hz, 23.6 Hz), 5.86 (1H, m); ^{13}C NMR (CD_3OD, 100 MHz): δ 23.1, 27.2, 45.2, 49.1, 57.8, 118.0, 132.4, 154.9, 166.7. HRMS (ESI) *m/z* calcd for $C_9H_{14}O_2N_3$ $[M + H]^+$ 196.1081; found 196.1090.

***c*-[Aza-Phenylalaninyl-Prolyl] (39c)**

This was synthesized by the treatment of benzophenone protected aza-phenylalaninylproline methyl ester **29c** (45 mg, 0.102 mmol) as described for **39a**. Purification by flash chromatography on silica gel using 100% EtOAc as solvent provided the aza-DKP **39c** as a pale yellow foam (21.3 mg, 85%): R_f 0.23 (100% EtOAc); $[\alpha]^{20}_D$ −38 (*c* 0.112, $CHCl_3$). ^{1}H NMR (CD_3OD, 400 MHz): δ 1.95–2.05 (2H, m), 2.12–2.26 (2H, m), 3.51–3.64 (2H, m), 3.92 (1H, t, J = 7.9 Hz), 4.66 (1H, d, J = 15.05 Hz), 4.78 (1H, d, J = 15 Hz), 7.37 (5H, s); ^{13}C NMR (CD_3OD, 100 MHz) δ 23.8, 27.2, 45.7, 51.1, 58.2, 128.9, 129.0, 129.4, 135.4. HRMS (ESI) *m/z* calcd for $C_{13}H_{15}O_2N_3$ $[M + Na]^+$ 268.1057; found 268.1069.

Aza-Phenylalaninyl-Proline *tert*-Butyl Ester (40c)

Benzhydrylidene aza-phenylalaninyl-glycinyl-proline *tert*-butyl ester **31c** (50 mg, 0.1 mmol) was stirred with hydroxylamine hydrochloride (29 mg, 0.42 mmol) in 20 ml of pyridine as solvent overnight at 60 °C. The volatiles were removed by rotary evaporation followed by co-evaporation with DCM and ethyl acetate until solidification. Purification by flash chromatography on silica gel using a 1 : 1 mixture of ethyl acetate in hexane afforded amine **40c** (28 mg, 88%) as a pale yellow foam: R_f 0.31 (hexanes : EtOAc 1 : 1); $[\alpha]^{20}_D$ −35 (*c* 0.1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.44 (9H, s), 1.83–1.95 (3H, m), 2.12–2.19 (1H, m), 3.64–3.67 (2H, m), 4.46 (1H, m), 4.59 (1H, d, J = 16 Hz), 4.61 (1H, d, J = 16 Hz), 7.28–7.35 (5H, m); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 23.4, 27.6, 30.1, 49.1, 56.1, 61.7, 80.1, 127.2, 127.3, 128.1, 128.3, 136.3, 172.7. HRMS (ESI) *m/z* calcd for $C_{17}H_{26}O_3N_3$ $[M + H]^+$ 320.1971; found 320.1969.

(*s*,*s*)- and (*R*, *s*)-Fmoc-Alaninyl-Aza-Phenylalaninyl-Proline *tert*-Butyl Ester (*s*,*s*)- and (*R*,*s*)-41

L- or D-Fmoc-Alanine (58 mg, 0.188 mmol) was dissolved in THF, cooled to −15 °C, treated sequentially with isobutyl chloroformate (25 µl, 0.188 mmol) and *N*-methyl morpholine (26 µl, 0.236 mmol), stirred for 5 min and treated with a solution of aza-phenylalaninyl-proline *tert*-butyl ester **40c** (50 mg, 0.157 mmol) in a minimum volume of ethyl acetate (5 ml). After stirring at −15 °C for 1 h, the volatiles were removed under reduced pressure. The crude material was dissolved in ethyl acetate and washed with brine. The organic phase was filtered through a pad of silica gel and evaporated to, respectively, afford crude (***s***, ***s***)**-41** and (***R***, ***s***)-**41** from which 2 mg of each was used for enantiomeric purity determination by analytical HPLC. The rest of the crudes was purified by chromatography on silica gel using a mixture of ethyl acetate in hexane (1 : 1) to afford 88 mg of (***s***, ***s***)**-41** (92%) and 92 mg of (***R***, ***s***)**-41** (95%) which were analyzed as below.

(*s*,*s*)-41

R_f 0.35 (hexane : EtOAc 1 : 1) and 0.32 (hexane : iPrOH 9 : 1); $[\alpha]^{20}_D$ 24 (*c* 0.133, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.26 (3H, d, J = 7.2 Hz), 1.47 (9H, s), 1.75–1.81 (2H, m), 1.85–1.97 (1H, m), 2.10–2.15 (1H, m), 3.40–3.49 (2H, m), 4.14–4.25 (3H, m), 4.28–4.30 (1H, m), 4.39 (1H, t, J = 7.2 Hz), 4.48 (1H, d, J = 14 Hz), 4.78 (1H, d, J = 14 Hz), 5.55 (1H, d, J = 7.6 Hz), 7.20–7.35 (7H, m), 7.41 (2H, t, J = 7.6 Hz), 7.57 (2H, t, J = 6 Hz), 7.77 (2H, d, J = 7.6 Hz), 8.45 (1H, br); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 18.9, 25.7, 28.4, 29.9, 47.4, 49.3, 53.6, 61.9, 67.6, 77.7, 81.8, 120.4, 125.4, 125.5, 127.5, 128.1, 128.2, 128.8, 129.7, 136.5, 141.5, 144.1, 156.2, 159.7, 171.4, 172.9. HRMS (ESI) *m/z* calcd for $C_{35}H_{40}O_6N_4$ $[M + H]^+$ 613.3025; found 613.3021.

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(*R,S*)-41

R_f 0.33 (hexane : EtOAc 1 : 1) and 0.26 (hexane : iPrOH 9 : 1); $[\alpha]^{20}_D$ 33 (*c* 0.1, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ 1.27 (3H, d, $J = 7.2$ Hz), 1.46 (9H, s), 1.77–1.82 (2H, m), 1.89–1.92 (1H, m), 2.12–2.17 (1H, m), 3.48–3.53 (2H, m), 4.13–4.24 (3H, m), 4.28–4.32 (1H, m), 4.39 (1H, t, $J = 6.4$ Hz), 4.53 (1H, d, $J = 14.4$ Hz), 4.72 (1H, d, $J = 14.4$ Hz), 5.61 (1H, d, $J = 7.2$ Hz), 7.15–7.24 (3H, m), 7.28–7.33 (5H, m), 7.40 (2H, t, $J = 7.6$ Hz), 7.56 (2H, d, $J = 6.8$ Hz), 7.76 (2H, d, $J = 7.2$ Hz), 8.45 (1H, br); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 18.6, 25.5, 28.4, 29.9, 47.4, 49.3, 53.9, 61.9, 67.6, 77.6, 81.7, 120.4, 125.4, 127.5, 128.1, 128.2, 128.8, 129.6, 136.6, 141.7, 144.1, 156.3, 159.8, 171.4, 172.7. HRMS (ESI) *m/z* calcd for $C_{35}H_{40}O_6N_4$ $[M + H]^+$ 613.3025; found 613.3021.

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Supporting information

Supporting information may be found in the online version of this article.

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