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# Synthesis of *N*′-substituted Ddz-protected hydrazines and their application in solid phase synthesis of aza-peptides

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ABSTRACT

Hydrazine derivatives are of considerable scientific and industrial value. Substituted hydrazines are precursors for many compounds of great interest and importance, among them aza-peptides. (Aza-peptides are peptide analogues in which one or more of the α-carbons, bearing the side chain residues, has been replaced by a nitrogen atom.) Aza-amino acid residues conserve the pharmacophores necessary for biological activity while inducing conformational changes and increased resistance to proteolytic degradation. These properties make aza-peptides attractive tools for structure–activity relationship studies and drug design. We describe the synthesis of *N*′-substituted 2-(3,5-dimethoxyphenyl)propan-2-yloxycarbonyl (Ddz) protected hydrazines. A general approach for solid phase synthesis of aza-peptides has been developed based on the in-situ activation of the *N*-Ddz,*N*′-substituted hydrazines with phosgene, followed by introduction to the N-terminus of a resin-bound peptide. The Ddz-aza-amino building units include aliphatic, aromatic and functionalized side chains, protected for synthesis by the Fmoc strategy. Solid phase aza-peptide synthesis is demonstrated including selective mild deprotection of Ddz with $Mg(ClO_4)_2$ and coupling of the next amino acid with triphosgene. Ddz deprotection is orthogonal with the Fmoc and Boc protecting groups, making the solid phase Ddz-aza-peptide synthesis compatible with both the Fmoc and the Boc strategies. The Ddz-protected hydrazines have wide applications in the synthesis of substituted hydrazines and in the synthesis of aza containing peptidomimetics.



## 1. Introduction

Substituted hydrazines have found many scientific and commercial applications.[1,2] The importance of hydrazine derivatives is reflected in the great number of such compounds synthesized to date and the synthesis of substituted hydrazines has been the subject of many studies.[3] Hydrazine derivatives are employed as drugs, insecticides, reagents in organic chemistry of diverse functions, precursors for synthesis of heterocycles,[4] and in peptidomimetic synthesis.[5] The vast interest in peptidomimetic structures is enthused by their value to understand structure–activity relationships and the ongoing search for in vivo active drug lead compounds.[6,7] Peptidomimetics are designed in an attempt to enhance biological activity while overcoming undesirable peptide properties such as rapid metabolism by proteolysis, poor bioavailability and nonselective receptor binding.[8–10] Substituted hydrazines are key components in the synthesis of aza-peptides,[11–13] a peptidomimetic in which one or more of the α-carbons, bearing the side chain residues, has been replaced by a nitrogen atom (Fig. 1). Aza-amino acid residues impart special conformational properties[14] to the parent peptide structure due to the loss of stereogenicity and reduction of flexibility.

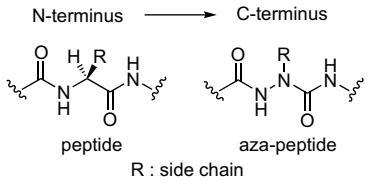


**Figure 1.** Peptide and aza-peptide.

*Abbreviations:* ACN, acetonitrile; AcOH, acetic acid; Alloc, allyloxycarbonyl; Boc, *tert*-butoxycarbonyl; BTC, bis(trichloromethyl)carbonate; Cbz, benzyloxycarbonyl; DBE, 1,2-dibromoethane; $CH_2Cl_2$, dichloromethane; Ddz, 2-(3,5-dimethoxyphenyl)propan-2-yloxycarbonyl; DIEA, *N*,*N*-diisopropylethylamine; DMAP, 4-(*N*,*N*-dimethylamino)pyridine; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; ES-MS, electron spray mass spectrometry; EtOAc, ethyl acetate; Fmoc, 9-fluorenylmethoxycarbonyl; HBTU, *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; HPLC, high performance liquid chromatography; HRMS, high resolution mass spectrometry; MBHA, methylbenzhydrylamine; MS, mass spectrometry; NMP, *N*-methyl-2-pyrrolidinone; NMR, nuclear magnetic resonance; PE, petroleum ether (40–60); PyBOP, benzotriazole-1-yl-oxy-trispyrrolidino-phosphonium hexafluorophosphate; RP, reverse phase; rt, room temperature; SPPS, solid phase peptide synthesis; *t*-Bu, *tert*-butyl; TDW, triple distilled water; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TIS, triisopropylsilane; TLC, thin layer chromatography; Ts, *para*-toluenesulfonyl; UV, ultraviolet.

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The incorporated aza-amino acid is evidently able to adopt the proper pharmacophore orientation required for activity and selectivity yet confers resistance towards proteolytic degradation. Replacement of a $C^{\alpha}$ by N in a peptide results in an additional possibility for the formation of hydrogen bonds and was also found to affect the acidity of the neighbouring amide N–H bonds.[13] The considerable reduction of the flexibility, brought about by the inclusion of hydrazide and urea structural elements, has been shown to induce β-turn conformations in aza-peptides.[15,16] Hence, the replacement of a certain $C^{\alpha}$ by N in a biologically active peptide will most probably affect its conformation and hence its absorption, transport, distribution, enzyme or receptor binding and metabolic stability. The presence of an aza-amino acid residue may increase the biological activity and/or improve the pharmacokinetic properties of the parent peptide. These properties suggest that aza-peptides could be useful tools for peptidomimetic drug design and for structure–activity relationship studies, and indeed, application of aza-peptides in the study of biologically active peptides has had significant success.[11,17] In addition, an aza-amino acid scan of biologically active peptides was recently introduced as a method for identifying biologically active conformations.[16]

The 2-(3,5-dimethoxyphenyl)propan-2-yloxycarbonyl (Ddz) group is an attractive protecting group, which can be removed under extremely mild conditions.[18] The Ddz protecting group is normally removed with dilute TFA solutions (0.2–3%) in a few minutes. However, Ddz can also be selectively removed by photolysis or by Lewis acids such as $Mg(ClO_4)_2$ or $ZnCl_2$. Ddz deprotection during solid phase peptide synthesis (SPPS) utilizing $Mg(ClO_4)_2$ in acetonitrile (ACN) at 50 °C was found to be fully orthogonal with side chain Boc protection. These extremely mild conditions offer an additional degree of orthogonality and enable Ddz to replace Fmoc in the SPPS Fmoc/*t*-Bu chemistry strategy.[19] To our surprise, our literature search came up with few substituted Ddz-protected hydrazines. For these reasons we have chosen to prepare Ddz-protected substituted hydrazines and examine their application for the SPPS of aza-peptides.

In this report we present two general methods for the synthesis of *N*′-substituted 2-(3,5-dimethoxyphenyl)propan-2-yl carbazates (*N*′-substituted Ddz protected hydrazines). We then evaluate the application of *N*-substituted Ddz hydrazines in solid phase aza-peptide synthesis using acid labile Rink amide methylbenzhydrylamine (MBHA) resin with mild Lewis acid mediated Ddz deprotection.

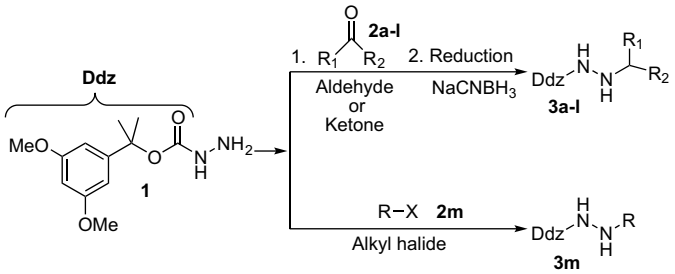
## 2. Results and discussion

### 2.1. Synthesis of *N*′-substituted 2-(3,5-dimethoxyphenyl)propan-2-yl carbazates

Our synthesis of *N*′-substituted Ddz hydrazines was designed according to the literature procedures for the synthesis of Boc, Fmoc and Cbz substituted hydrazines.[3,15,20–22] Two general synthetic pathways were used to prepare *N*′-substituted Ddz hydrazines **3a**–**m** (Scheme 1). (1) Reduction of the Ddz hydrazones, derived from the reaction of commercially available 2-(3,5-dimethoxyphenyl)propan-2-yl carbazate (Ddz hydrazide) **1** with either aldehyde or ketone, to yield the desired *N*′-substituted Ddz hydrazines **3a**–**l** in satisfying yields (methods A–C, Table 1). (2) Introduction of side chain corresponding to aspartic acid was achieved by nucleophilic substitution of alkyl halide, *tert*-butyl bromoacetate, with Ddz hydrazide to obtain compound **3m** (method D).

### 2.2. Reduction of Ddz hydrazones

Condensation of Ddz hydrazide **1** with the appropriate aldehyde or ketone **2a**–**l** in THF readily gave the corresponding Ddz hydrazone, which was reduced without further purification. Reduction was performed with sodium cyanoborohydride under mild acidic conditions achieved by acetic acid (methods B and C) or by gradual addition of *p*-toluenesulfonic acid monohydrate solution managed by means of indicator toning (method A).[20,21] The obtained $BH_2CN$ adduct was subsequently hydrolized with aq NaOH in methanol to give the desired *N*′-substituted Ddz hydrazine **3a**–**l**.




**Scheme 1.** Synthetic scheme of *N*′-alkyl,*N*-Ddz hydrazines **3a**–**m**.

Synthetic difficulties were encountered in the reduction of the aryl hydrazone derivatives. Reported reduction of Fmoc, Boc and Cbz aryl hydrazones suggests that catalytic hydrogenation is preferred over reduction with sodium cyanoborohydride.[15,21] However, other reports indicate that catalytic conditions and the duration of the reaction substantially affect the yield and that care must be taken to avoid over reduction.[20,22] In the case of benzaldehyde hydrazone, 30 min of catalytic hydrogenation over 10% palladium–carbon gave the desired aza-Phe precursor **3j** in 74% yield.[†] However, the same conditions did not suffice for reduction of the 4-*tert*-butoxy benzaldehyde hydrazone to obtain the aza-Tyr(*t*-Bu) precursor **3k**. Attempts to prepare **3j** and **3k** by method A (THF, 1.5 mol equiv $NaBH_3CN$, 1.1 mol equiv $TsOH\cdot H_2O$, indicator toning) or by method B (1.5 mol equiv $NaBH_3CN$, 2 mol equiv AcOH) resulted in considerably long reaction times even with further addition of reducing agent. Ultimately, reaction of the benzaldehyde hydrazone with excess reducing agent and excess acetic acid, method C (3 mol equiv $NaBH_3CN$, 5 mol equiv AcOH), gave complete reduction overnight. These conditions were also successful in preparation of the aryl derivatives **3k**–**l** without apparent deprotection of the Ddz protecting group.

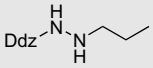
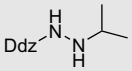
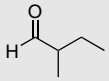
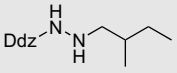
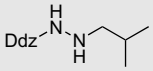
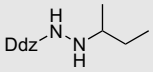
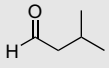
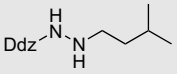
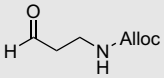
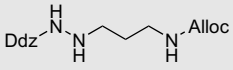
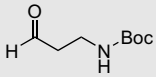
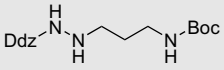
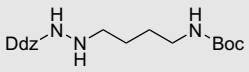
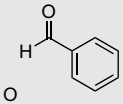
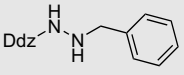
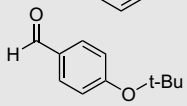
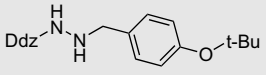
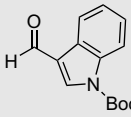
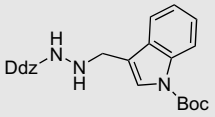
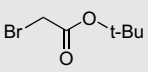
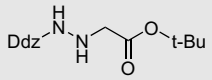
### 2.3. Nucleophilic substitution with alkyl halide

Reaction of Ddz hydrazide **1** with the appropriate alkyl halide, *tert*-butyl bromoacetate and potassium carbonate in dry DMF gave the desired Ddz substituted hydrazine **3m**. Although dialkylation was expected to be a major by-product, only mono alkylation was observed. This method is still under investigation in our lab with other alkyl halides. Recent reports demonstrated that nucleophilic substitution of *tert*-butyl bromoacetate with Fmoc carbazate, performed under different conditions, proceeded in considerably lower yield (20%) than the corresponding Boc or Cbz carbazates (80%).[23] The above conditions gave the Aza-Asp(*t*-Bu) precursor in 62% yield.

Thirteen *N*′-substituted Ddz hydrazines were prepared in satisfying yields (62–95%) using two synthetic methods: reductive alkylation and nucleophilic addition. The conveniently orthogonal removal of the Ddz protecting group provides these compounds with a wide variety of applications in the synthesis of substituted hydrazines.

[†] Amino acids abbreviations are according to the IUPAC-IUB Commission of Biochemical Nomenclature (http://www.chem.qmul.ac.uk/iupac/AminoAcid).

**Table 1**
Summary of the synthesis of **3a–m**

| Entry | Structure of **2a–m** | Structure of **3a–m** | Method | Yield % | Precursor for the incorporation of |
|---|---|---|---|---|---|
| a |  |  | A | 86 | Aza-norvaline |
| b |  |  | A | 78 | Aza-valine |
| c |  |  | A | 83 | Aza-homoisoleucine (racemic) |
| d |  |  | B | 81 | Aza-leucine |
| e |  |  | B | 86 | Aza-isoleucine (racemic) |
| f |  |  | B | 87 | Aza-homoleucine |
| g |  |  | B | 73 | Aza-ornithine(Alloc) |
| h |  |  | B | 70 | Aza-ornithine(Boc) |
| i |  |  | B | 76 | Aza-lysine(Boc) |
| j |  |  | C | 95 | Aza-phenylalanine |
| k |  |  | C | 78 | Aza-tyrosine(O*t*-Bu) |
| l |  |  | C | 68 | Aza-tryptophan(Boc) |
| m |  |  | D | 62 | Aza-aspartic acid(*t*-Bu) |

Method A: THF, aldehyde or ketone 1 mol equiv, $NaBH_3CN$ 1.5 mol equiv, $TsOH \cdot H_2O$ 1.1 mol equiv, indicator toning. Method B: THF, aldehyde or ketone 1 mol equiv, $NaBH_3CN$ 1.5 mol equiv, AcOH 2 mol equiv. Method C: THF, aldehyde or ketone 1 mol equiv, $NaBH_3CN$ 3 mol equiv, AcOH 5 mol equiv. Method D: DMF, alkyl halide 1 mol equiv, $K_2CO_3$ 1.1 mol equiv.

### 2.4. In-situ activation of *N*′-substituted Ddz hydrazines and solid phase synthesis of aza-peptides (Fig. 2)

We examined the use of *N*′-substituted Ddz hydrazines in the solid phase synthesis of aza-peptides as previously described for the *N*′-alkyl Fmoc carbazates.[15,24,25] Procedures for activation of *N*′-substituted Ddz hydrazines and their coupling as activated aza-amino acids to the free amine of peptidyl-resin are similar to those reported for *N*′-alkyl Fmoc carbazates with minor modifications. *N*′-Substituted Ddz hydrazines with excess diisopropyl ethylamine (DIEA) in dry dichloromethane ($CH_2Cl_2$) at 0 °C were slowly treated with excess phosgene solution in toluene. These conditions converted the *N*′-substituted Ddz hydrazines to their corresponding *N*′-substituted-*N*′-chlorocarbonyl Ddz hydrazines, which were subsequently reacted with the free N-terminal amine of the peptidyl-resin as activated aza-amino building units. After indication by TLC that the activation reaction was complete (10 min), excess phosgene and toluene were removed under reduced pressure and the resulted activated aza-amino acid was introduced to the solid supported deprotected peptide without further purification. This coupling of activated aza-amino acid was performed with excess of activated unit and DIEA in $CH_2Cl_2$ as previously described.[15] All couplings were monitored by Kaiser-ninhydrin[26] and Chloranil[27] tests and were repeated when tests showed positive free amine. The Ddz protecting group was removed conveniently and selectively with $Mg(ClO_4)_2$ in ACN at 50 °C as previously described.[19] Prior Fmoc

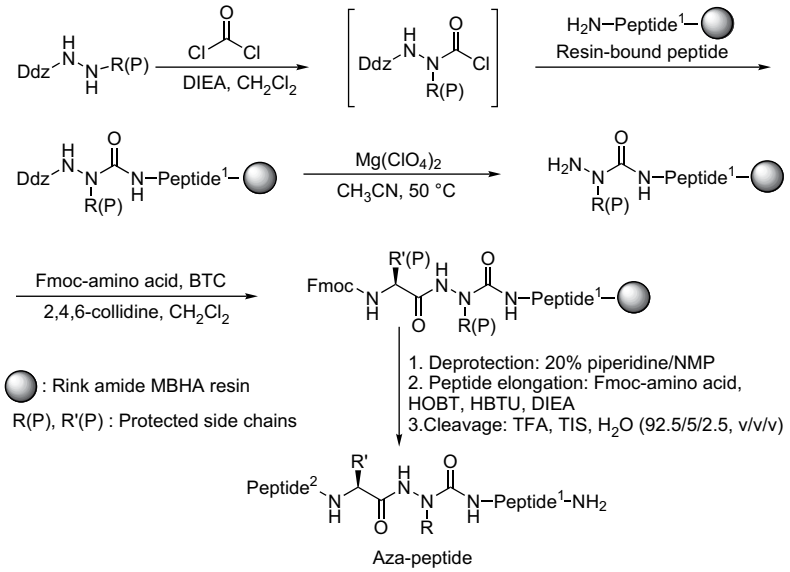



**Figure 2.** General protocol for solid phase synthesis of aza-peptides using *N*′-substituted Ddz hydrazines.

loading test[28,29] of resin-bound Fmoc-phenylalanine ensured that these conditions do not cause any apparent cleavage of substrate from the resin. Removal of Ddz from the aza-amino acid residue was monitored by UV absorbance of the deprotection solution[18] and was found to be slower than described for the removal of Ddz from N-terminus amino acid. After 3 h the deprotection solution was monitored and renewed every half an hour to ensure complete deprotection. Difficult coupling of the next Fmoc-amino acid to the aza-amino acid was achieved with bis(trichloromethyl)carbonate (BTC) and 2,4,6-colidine in $CH_2Cl_2$[30] and subsequently repeated. The Kaiser-ninhydrin test could not be used to monitor this coupling. The Chloranil test occasionally gave red coloured beads instead of the usual green colour. Aza-glycine seemed to give the fastest and strongest response to colour the beads red, other aza-residues reacted very slowly and only gave a faint red colour after several hours up to a day. Consequently, the coupling to the aza-amino acid was monitored by cleavage with TFA of a very small bead sample ('small cleavage') followed by MS analysis of the crude obtained. If N-terminal aza-peptide mass was observed, additional coupling under more forcing conditions of 60 °C in 1,2-dibromoethane (DBE) overnight was performed and the aza-peptide was subsequently acylated using acetic anhydride solution in DMF.

As a model, the aza-peptide sequence Fmoc-Lys-azaVal-Ala-Ala-Phe-$NH_2$ **4** was chosen. Two parallel aza-peptides were synthesized (1) using Ddz and (2) using Fmoc-protected isopropyl substituted hydrazine as aza-Val building unit precursors. Quantitative Fmoc substitution of the peptidyl-resin[28,29] prior to cleavage indicated that in both cases the aza-peptide was obtained in an overall yield of 50%. Crude HPLC of both aza-peptides were very similar (Supplementary data). The crude peptide was purified by preparative HPLC and characterized by mass spectrometry (Supplementary data).

We demonstrate here the utilization of *N*′-substituted Ddz hydrazines in the synthesis of aza-peptides. Similar to the Fmoc aza-peptide synthesis strategy,[15,24,25] acylation of *N*′-substituted Ddz hydrazines with phosgene may afford activated Ddz-protected aza-amino acids possessing aliphatic (Val **3a**, Nvl **3c**, Leu **3d**, Hol **3f**) aromatic (Phe **3j**, Tyr **3k**, Trp **3l**), basic (Orn **3h**, Lys **3i**) and acidic (Asp **3m**) side chains protected for the Fmoc/*t*-Bu SPPS strategy. Activated aza-amino acids applied to resin-bound peptides followed by selective Ddz deprotection and subsequent routine peptide elongation afforded the desired aza-peptides. Selective Alloc deprotection from aza-Orn(Alloc) precursor **3g** and subsequent guanidilation can afford the Aza-arginine residue as previously described.[15] This convenient procedure for solid phase synthesis of aza-peptides using Ddz orthogonal protection facilitates the generation of novel peptidomimetics such as branched aza-peptides and cyclic aza-peptides. In addition, Ddz deprotection is orthogonal with the Fmoc and Boc protecting groups, making the solid phase Ddz-aza-peptide synthesis compatible with both the Fmoc and the Boc strategies. Substituted Ddz hydrazines have a wide range of applications in the synthesis aza-peptide, aza-peptoide and other aza-peptidomimetics. Of note is the possible incorporation of these compounds in the synthesis of aza-$\beta^3$-amino acids, which utilizes Fmoc, Boc and Cbz substituted hydrazines.[23]

## 3. Conclusion

We present the synthesis of novel *N*′-substituted Ddz hydrazines in which the *N*′-substituents are mainly derived from the side chains of amino acids. We believe these compounds will find a wide use in peptidomimetic synthesis and in the general production of substituted hydrazines. We demonstrate the incorporation of these aza-amino acid precursors into peptides to afford aza-peptides using standard Fmoc chemistry SPPS. Considering the convenience of the presented procedure for aza-peptide synthesis and the diversity of Ddz protecting group, this method should be of general use in the synthesis of aza-peptides as well as other aza-peptidomimetic structures. We reason that the various possibilities for Ddz removal and applications brought about in this study will promote a wider use of this attractive protecting group.

## 4. Experimental section

### 4.1. Hazards

Phosgene solution and triphosgene, bis(trichloromethyl)-carbonate (BTC), are highly toxic and may cause death by inhalation. These substances should be handled in a well-ventilated hood with extreme caution.

### 4.2. Reagents

Ddz hydrazide **1** and Fmoc-protected amino acids were purchased from Nova Biochemicals (Laüfelfingen, Switzerland). Unless otherwise noted, aldehydes and ketones **2** and other starting materials and reagents were purchased from commercial suppliers and used without further purification. The 20% solution of phosgene in toluene was purchased from Fluka (Seelze, Germany). Rink amide MBHA resin (200–400 mesh) was purchased from CBL (Patras, Greece; catalog no. BR-1305, lot no. 2536) and loading was determined to be 0.6 mmol/g. Solvents for organic synthesis, trifluoroacetic acid (TFA) and solvents for high performance liquid chromatography (HPLC) were purchased from Bio-Lab (Jerusalem, Israel).

### 4.3. General methods

Thin layer chromatography (TLC) was performed on Merck $F_{245}$ 60 silica gel plates (Darmstadt, Germany). Visualization was achieved with UV light when applicable or developed by iodine staining. Flash chromatography was performed using silica gel 60 (230–400 mesh) (Merck Darmstadt, Germany). Nuclear magnetic resonance (NMR) spectra were recorded on Bruker AMX-300 MHz or Bruker DRX-400 MHz spectrometers. High resolution mass spectrometry (HRMS) was recorded on a Thermo Scientific LTQ Orbitrap mass spectrometer. Electron spray mass spectrometry (ES-MS) was recorded on a Finnigan ThermoQuest LCQ-DUO ion trap mass spectrometer. MALDI-TOF mass spectrometry was recorded on a PerSeptive Biosystems Voyager-DE PRO BioSpectrometry workstation. Analytical RP-HPLC analysis was performed on a Phenomenex Gemini RP-C18 column (5 μ, 250×4.6 mm) using a Merck-Hitachi LaChrom D6000 interface system equipped with L-4250 UV–vis detector set at 220 nm, with an L-6200 intelligent pump and AS-4000 autosampler. Eluants A (0.05% TFA in TDW) and B (0.05% TFA in ACN) were used in a 35 min linear gradient (5% B to 95% B) and a flow of 1 mL/min. Semi-preparative RP-HPLC was performed on a Phenomenex Gemini RP-C18 column (5 μ, 250×10.0 mm) using a Merck-Hitachi LaChrom D6000 interface system equipped with L-4250 UV–vis detector set at 220 nm and an L-6200 intelligent pump. Eluants A (0.05% TFA in TDW) and B (0.05% TFA in ACN) were used in a 50 min linear gradient (5% B to 95% B) and a flow of 5 mL/min. UV–vis spectrometry was recorded on a Shimadzu UV-1650PC spectrometer. IR spectra were recorded on a Bruker Vector 22 spectrometer. Melting points were recorded on a Fisher-Johns melting point apparatus.

### 4.4. Chemistry

Commercially available aldehydes and ketones **2a–f,j** were used without further purification. Aldehydes **2g–i,l** were prepared by known procedures: β-amino aldehydes **2g** and **2h** for the synthesis of the aza-Orn precursors were prepared by reduction of their corresponding β-alanine Weinreb amides[31] with lithium aluminium hydride,[32] 1-*tert*-butyloxycarbonyl-2-hydroxypyrrolidine **2i** for the synthesis of the aza-Lys(Boc) precursor was prepared by hydrolysis of Boc protected 4-amino butyraldehyde diethyl acetal, and Boc protection of indole-3-carboxaldehyde gave aldehyde **2l** for the synthesis of aza-Trp(Boc) precursor. Condensation of 1-*tert*-butyloxycarbonyl-2-hydroxypyrrolidine **2i** with Ddz hydrazide **1**, to form the corresponding hydrazone, was carried out in the presence of *p*-toluenesulfonic acid.

#### *4.4.1. 3-(Allyloxycarbonylamino)propanal **2g***

Reduction of the corresponding Alloc protected β-alanine Weinreb amide, [2-(*N*-methoxy-*N*-methylcarbamoyl)ethyl] carbamic acid allyl ester, with $LiAlH_4$ was performed as recently described.[33] The crude yellowish oil product was used without further purification. Yield: 75%. $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 2.73 (t, $J$=5.8 Hz, 2H), 3.47 (q, $J$=6.0 Hz, 2H), 4.53 (d, $J$=5.3 Hz, 2H), 5.16 (br s, 1H), 5.19 (dd, $J$=10.3, 0.7 Hz, 1H), 5.28 (dd, $J$=17.2, 1.3 Hz, 1H), 5.81–5.99 (m, 1H), 9.80 (s, 1H).

#### *4.4.2. 3-(tert-Butyloxycarbonylamino)propanal **2h***

Reduction of the corresponding Boc protected β-alanine Weinreb amide, [2-(*N*-methoxy-*N*-methylcarbamoyl)ethyl] carbamic acid *tert*-butyl ester, with $LiAlH_4$ was performed as previously described.[34,35] The crude colourless oil product was used without further purification. Yield: 55%. $^1$H NMR ($CDCl_3$, 300 MHz): $\delta$ 1.42 (s, 9H), 2.70 (t, $J$=5.9 Hz, 2H), 3.42 (q, $J$=5.9 Hz, 2H), 4.88 (br s, 1H), 9.80 (s, 1H).

#### *4.4.3. 1-tert-Butyloxycarbonyl-2-hydroxypyrrolidine **2i**[36,15]*

Overnight hydrolysis of Boc protected 4-amino butyraldehyde diethyl acetal in AcOH/water 2:1 v/v solution gave a colourless oil that was used without further purification. Yield: 85%. $^1$H NMR (DMSO-$d_6$, 400 MHz): $\delta$ 1.40 (s, 9H), 1.59–1.70 (m, 1H), 1.71–1.83 (m, 2H), 1.87–2.00 (m, 1H), 3.07–3.17 (m, 1H), 3.25–3.30 (m, 1H), 5.20–5.32 (m, 1H), 5.51 (br d, 1H).

#### *4.4.4. 1-tert-Butoxycarbonylindole-3-carboxaldehyde **2l**[37]*

Boc protection of indole-3-carboxaldehyde gave yellow solid that was used without further purification. Yield: 96%. $^1$H NMR ($CDCl_3$, 300 MHz): $\delta$ 1.71 (s, 9H), 7.34–7.46 (m, 2H), 8.15 (dd, $J$=7.3, 1.4 Hz, 1H), 8.23 (s, 1H), 8.29 (dd, $J$=7.0, 2.0 Hz, 1H), 10.10 (s, 1H).

### 4.5. General method A: synthesis of *N′*-alkyl 2-(3,5-dimethoxyphenyl)propan-2-yl carbazates (3a–c)

A solution of 2-(3,5-dimethoxyphenyl)propan-2-yl carbazate **1** and the appropriate aldehyde or ketone **2a–c** (1 mol equiv for substances with high bp or 1.5 mol equiv for substances easily removed by vacuum) in dry THF (0.65 M) was stirred overnight at rt and concentrated in vacuo to obtain the corresponding hydrazone, which was subsequently used without further purification. The hydrazone was dissolved in dry THF (0.15 M) and treated with $NaBH_3CN$ (1.5 mol equiv) with vigorous stirring. A few granules of bromocresol green were introduced and the resulting blue solution was treated dropwise with *p*-toluenesulfonic acid monohydrate (1.1 mol equiv) in THF (2 mL). Every addition of the acid solution was performed after indicator toning to maintain the reaction pH between 3.5 and 5.[16,20,21] After the yellowish colour persisted for 1 h, the solvent was removed under reduced pressure and the residue was partitioned between EtOAc (50 mL) and brine (50 mL). The aqueous phase was extracted with EtOAc (3×25 mL). The combined organic layers were washed with saturated aq $NaHCO_3$ (50 mL) and brine (50 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was dissolved in MeOH (10 mL), treated with 1 M NaOH (1.2 mol equiv) and stirred for 1 h at rt. The solvent was removed under reduced pressure and the residue was dissolved in EtOAc (50 mL), washed with brine (50 mL), dried over $MgSO_4$ and concentrated under reduced pressure to provide the desired compound.

#### *4.5.1. N′-Propyl-2-(3,5-dimethoxyphenyl)propan-2-yl carbazate (**3a**)*

Product of the reaction of Ddz hydrazide **1** (0.51 g, 2.0 mmol) and propionaldehyde **2a** (0.105 mL, 2.05 mmol). Purification by flash chromatography on silica using petroleum ether (PE)/EtOAc 6:4 as eluant gave a white solid. Yield 86% (0.51 g); mp=56–58 °C; $R_f$=0.22 (PE/EtOAc 7:3). IR (KBr) $\nu_{max}$ 3300, 2958, 1716, 1686, 1601 cm$^{-1}$. $^1$H NMR (DMSO-$d_6$, 400 MHz): $\delta$ 0.83 (t, $J$=7.3 Hz, 3H), 1.28–1.41 (m, 2H), 1.65 (s, 6H), 2.58 (br t, 2H), 3.73 (s, 6H), 4.31 (br s,

1H), 6.37 (t, $J$=2.2 Hz, 1H), 6.47 (d, $J$=1.6 Hz, 2H), 8.46 (br s, 1H). $^{13}$C NMR (DMSO-$d_6$, 100 MHz): $\delta$ 11.5, 20.5, 28.9, 52.7, 55.0, 79.7, 97.8, 102.6, 149.4, 155.6, 160.1. HRMS calcd for $C_{15}H_{25}N_2O_4$ 297.18088 ($MH^+$), found 297.18060.

#### 4.5.2. *N′-Isopropyl-2-(3,5-dimethoxyphenyl)propan-2-yl carbazate (**3b**)*

Product of the reaction of Ddz hydrazide **1** (0.51 g, 2.0 mmol) and acetone **2b** (0.151 mL, 2.05 mmol). Purification by flash chromatography on silica using PE/EtOAc 7:3 as eluant gave a white solid. Yield 78% (0.46 g); mp=88–89 °C; $R_f$=0.19 (PE/EtOAc 7:3). IR (KBr) $\nu_{max}$ 3283, 2979, 1725, 1600 cm$^{-1}$. $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 1.07 (d, $J$=6.2 Hz, 6H), 1.75 (s, 6H), 3.16–3.26 (m, 1H), 3.78 (s, 6H), 6.35 (t, $J$=2.2 Hz, 1H), 6.50 (d, $J$=2.1 Hz, 2H). $^{13}$C NMR ($CDCl_3$, 100 MHz): $\delta$ 19.8, 28.9, 51.7, 55.3, 82.3, 98.6, 102.9, 148.4, 155.7, 160.7. HRMS calcd for $C_{15}H_{25}N_2O_4$ 297.18088 ($MH^+$), found 297.18063.

#### 4.5.3. *(R,S)-N′-(2-Methyl-butyl)-2-(3,5-dimethoxyphenyl)propan-2-yl carbazate (**3c**)*

Product of the reaction of Ddz hydrazide **1** (0.25 g, 1.0 mmol) and (*R,S*)-2-methyl butyraldehyde **2c** (0.108 mL, 1.01 mmol). Purification by flash chromatography on silica using PE/EtOAc 8:2 as eluant gave a white solid. Yield 83% (0.27 g); mp=68 °C; $R_f$=0.50 (PE/EtOAc 7:3). IR (KBr) $\nu_{max}$ 3277, 2961, 2873, 1721, 1599 cm$^{-1}$. $^1$H NMR (DMSO-$d_6$, 400 MHz): $\delta$ 0.75–0.93 (m, 6H), 0.99–1.11 (m, 1H), 1.32–1.47 (m, 2H), 1.65 (s, 6H), 2.35–2.44 (m, 1H), 2.51–2.59 (m, 1H), 3.73 (s, 6H), 4.28 (br s, 1H), 6.37 (t, $J$=2.2 Hz, 1H), 6.47 (d, $J$=1.8 Hz, 2H), 8.46 (br s, 1H). $^{13}$C NMR (DMSO-$d_6$, 100 MHz): $\delta$ 11.0, 17.4, 26.7, 29.0, 32.5, 54.9, 56.9, 79.6, 97.7, 102.6, 149.3, 155.6, 160.1. HRMS calcd for $C_{17}H_{29}N_2O_4$ 325.21218 ($MH^+$), found 325.21198.

### 4.6. General method B: synthesis of *N′*-alkyl 2-(3,5-dimethoxyphenyl)propan-2-yl carbazates (3d–i)

A solution of 2-(3,5-dimethoxyphenyl)propan-2-yl carbazate **1** and the appropriate aldehyde or ketone **2d**–**i** (1 mol equiv for substances with high bp or 1.5 mol equiv for substances easily removed by evaporation under reduced pressure) in dry THF (0.65 M) was stirred overnight at rt and concentrated under reduced pressure to obtain the corresponding hydrazone, which was subsequently used without further purification. The hydrazone was dissolved in dry THF (0.15 M) and treated with $NaBH_3CN$ (1.5 mol equiv) with vigorous stirring. To this mixture, acetic acid (2 mol equiv) was added and the reaction was stirred overnight at rt. Additional $NaBH_3CN$ was added if necessary to ensure completion of the reaction verified by TLC (PE/EA 1:1). The solvent was removed by evaporation under reduced pressure and the residue was partitioned between EtOAc (50 mL) and brine (50 mL). The organic layer was washed with saturated aq $NaHCO_3$ (50 mL) and brine (50 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was dissolved in MeOH (10 mL), treated with 1 M NaOH (1.2 mol equiv) and stirred for 1 h at rt. The solvent was removed by reduced pressure and the residue was dissolved in EtOAc (50 mL), washed with brine (50 mL), dried over $MgSO_4$ and concentrated under reduced pressure to provide the desired compound.

#### 4.6.1. *N′-Isobutyl-2-(3,5-dimethoxyphenyl)propan-2-yl carbazate (**3d**)*

Product of the reaction of Ddz hydrazide **1** (0.51 g, 2.0 mmol) and isobutyraldehyde **2d** (0.275 mL, 3 mmol). Product was recrystallized from DMF/water to give a white solid. Yield 81% (0.50 g); mp=55 °C. IR (KBr) $\nu_{max}$ 3295, 2931, 2971, 1727, 1600 cm$^{-1}$. $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 0.90 (d, $J$=6.9 Hz, 6H), 1.66–1.78 (m, 1H), 1.75 (s, 6H), 2.63 (d, $J$=5.8 Hz, 2H), 3.78 (s, 6H), 3.91 (br s, 1H), 6.19 (br s, 1H), 6.35 (t, $J$=2.3 Hz, 1H), 6.50 (d, $J$=2.5 Hz, 2H). $^{13}$C NMR ($CDCl_3$, 100 MHz): $\delta$ 20.5, 26.8, 29.0, 55.2, 59.8, 81.5, 98.4, 102.9, 148.7, 156.0, 160.6. HRMS calcd for $C_{16}H_{27}N_2O_4$ 311.19653 ($MH^+$), found 311.19641.

#### 4.6.2. *(R,S)-N′-sec-Butyl-2-(3,5-dimethoxyphenyl)propan-2-yl carbazate (**3e**)*

Product of the reaction of Ddz hydrazide **1** (0.51 g, 2.0 mmol) and ethyl methyl ketone **2e** (0.270 mL, 3.0 mmol). Product was recrystallized from DMF/water to give a white solid. Yield 86% (0.53 g); mp=53 °C. IR (KBr) $\nu_{max}$ 3304, 3283, 2981, 2949, 1728, 1599 cm$^{-1}$. $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 0.87 (t, $J$=7.4 Hz, 3H), 0.97 (d, $J$=6.0 Hz, 3H), 1.18–132 (m, 1H), 1.41–153 (m, 1H), 1.74 (s, 6H), 2.83–2.93 (m, 1H), 3.77 (s, 6H), 3.95 (br s, 1H), 6.35 (t, $J$=2.1 Hz, 1H), 6.38 (br s, 1H), 6.50 (d, $J$=2.3 Hz, 2H). $^{13}$C NMR ($CDCl_3$, 100 MHz): $\delta$ 9.9, 17.6, 27.3, 28.91, 28.99, 55.1, 56.4, 81.4, 98.4, 102.8, 148.7, 156.0, 160.6. HRMS calcd for $C_{16}H_{27}N_2O_4$ 311.19653 ($MH^+$), found 311.19635.

#### 4.6.3. *N′-Isopentyl-2-(3,5-dimethoxyphenyl)propan-2-yl carbazate (**3f**)*

Product of the reaction of Ddz hydrazide **1** (0.76 g, 3.0 mmol) and isovaleraldehyde **2f** (0.483 mL, 4.5 mmol). Product was recrystallized from DMF/water to give a white solid. Yield 87% (0.85); mp=66 °C. IR (KBr) $\nu_{max}$ 3316, 2954, 1690, 1600 cm$^{-1}$. $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 0.87 (d, $J$=6.5 Hz, 6H), 1.27–1.35 (m, 2H), 1.55–166 (m, 1H), 1.74 (s, 6H), 2.81 (t, $J$=7.0 Hz, 2H), 3.78 (s, 6H), 6.29 (br s, 1H), 6.35 (t, $J$=2.3 Hz, 1H), 6.50 (d, $J$=2.3 Hz, 2H). $^{13}$C NMR ($CDCl_3$, 100 MHz): $\delta$ 22.6, 25.9, 28.9, 36.7, 50.3, 55.2, 81.5, 98.3, 102.9, 148.7, 156.0, 160.6. HRMS calcd for $C_{17}H_{29}N_2O_4$ 325.21218 ($MH^+$), found 325.21201.

#### 4.6.4. *N′-1-(3-(tert-Allyloxycarbonylamino)propyl)-2-(3,5-dimethoxyphenyl)propan-2-yl carbazate (**3g**)*

Product of the reaction of Ddz hydrazide **1** (0.64 g, 2.5 mmol) and 3-(*tert*-3-(allyloxycarbonylamino))propanal **2g** (0.39 g, 2.5 mmol). Purification by flash chromatography on silica using hexane/EtOAc 7:3 as eluant gave a yellowish oil. Yield 73% (0.72 g); $R_f$=0.15 (hexane/EtOAc 1:1). IR (KBr) $\nu_{max}$ 3318, 2939, 1705, 1599 cm$^{-1}$. $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 1.58–168 (m, 2H), 1.73 (s, 6H), 2.88 (br t, $J$=6.5 Hz, 2H), 3.16–3.28 (m, 2H), 3.77 (s, 6H), 4.53 (d, $J$=5.2 Hz, 2H), 5.19 (dd, $J$=10.4, 1.3 Hz, 1H), 5.26 (br s, 1H), 5.28 (dd, $J$=17.2, 1.5 Hz, 1H), 5.84–5.96 (m, 1H), 6.34 (t, $J$=2.2 Hz, 1H), 6.49 (d, $J$=2.3 Hz, 2H). $^{13}$C NMR ($CDCl_3$, 100 MHz): $\delta$ 27.2, 28.9, 38.9, 49.4, 55.2, 65.4, 81.9, 98.3, 102.9, 117.5, 132.9, 148.5, 155.8, 156.3, 160.6. HRMS calcd for $C_{19}H_{30}N_3O_6$ 396.21291 ($MH^+$), found 396.21289.

#### 4.6.5. *N′-1-(3-(tert-Butyloxycarbonylamino)propyl)-2-(3,5-dimethoxyphenyl)propan-2-yl carbazate (**3h**)*

Product of the reaction of Ddz hydrazide **1** (0.61 g, 2.4 mmol) and 3-(*tert*-Butyloxycarbonylamino)propanal **2h** (0.41 g 2.4 mmol). Purification by flash chromatography on silica using 1% MeOH in $CH_2Cl_2$ as eluant gave a white solid. Yield 70% (0.69 g); mp=86–89 °C; $R_f$=0.17 (hexane/EtOAc 1:1). IR (KBr) $\nu_{max}$ 3318, 2981, 2949, 1692, 1600 cm$^{-1}$. $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 1.42 (s, 9H), 1.55–165 (m, 2H), 1.73 (s, 6H), 2.85 (t, $J$=6.1 Hz, 2H), 3.15 (br s, 2H), 3.77 (s, 6H), 4.93 (br s, 1H), 6.34 (t, $J$=2.3 Hz, 1H), 6.48 (d, $J$=2.3 Hz, 2H). $^{13}$C NMR ($CDCl_3$, 100 MHz): $\delta$ 27.6, 28.3, 28.9, 38.5, 49.4, 55.2, 79.0, 81.7, 98.3, 102.9, 148.6, 155.9, 156.0, 160.6. HRMS calcd for $C_{20}H_{34}N_3O_6$ 412.24421 ($MH^+$), found 412.24408.

#### 4.6.6. *N′-1-(4-(tert-Butyloxycarbonylamino)butyl)-2-(3,5-dimethoxyphenyl)propan-2-yl carbazate (**3i**)*

Product of the reaction of Ddz hydrazide **1** (0.45 g, 1.76 mmol) and 1-*tert*-Butyloxycarbonyl-2-hydroxypyrrolidine **2i** (0.33 g, 1.76 mmol). Formation of the hydrazone was catalyzed by

*p*-toluenesulfonic acid monohydrate (33 mg, 0.17 mmol). Purification by flash chromatography on silica using hexane/EtOAc 7:3 as eluant gave a yellowish oil. Yield 76% (0.57 g); $R_f$=0.21 (hexane/EtOAc 1:1). IR (KBr) $\nu_{max}$ 3335, 2976, 2935, 1699, 1599 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 1.36–1.52 (m, 13H), 1.72 (s, 6H), 2.79 (br t, $J$=6.0 Hz, 2H), 3.09 (br q, $J$=6.0 Hz, 2H), 3.76 (s, 6H), 3.92 (br s, 1H), 4.71 (br s, 1H), 6.33 (t, $J$=2.2 Hz, 1H), 6.45 (br s, 1H), 6.48 (d, $J$=2.2 Hz, 2H). $^{13}C$ NMR ($CDCl_3$, 100 MHz): $\delta$ 24.8, 27.5, 28.3, 28.9, 40.3, 51.4, 55.1, 78.9, 81.5, 98.2, 102.9, 148.6, 155.9, 156.0, 160.6. HRMS calcd for $C_{21}H_{36}N_3O_6$ 425.25986 ($MH^+$), found 425.25937.

### 4.7. General method C: synthesis of *N′*-aryl 2-(3,5-dimethoxyphenyl)propan-2-yl carbazates (3j–l)

A solution of 2-(3,5-dimethoxyphenyl)propan-2-yl carbazate **1** and aldehyde or ketone **2j–l** (1 mol equiv) in dry THF (0.65 M) was stirred overnight at rt and concentrated in vacuo to obtain the corresponding hydrazone, which was subsequently used without further purification. The hydrazone was dissolved in dry THF (0.15 M) and treated with $NaBH_3CN$ (3 mol equiv) with vigorous stirring. To this mixture, acetic acid (5 mol equiv) was added and the reaction was stirred for 24 h. Additional $NaBH_3CN$ was added if necessary to ensure completion of the reaction as verified by TLC (hexane/EA 1:1 or $CH_2Cl_2$/MeOH/TEA 98.5:1:0.5). The solvent was removed by evaporation under reduced pressure and the residue was partitioned between EtOAc (50 mL) and brine (50 mL). The organic layer was washed with saturated aq $NaHCO_3$ (50 mL) and brine (50 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was dissolved in MeOH (10 mL), treated with 1 M NaOH (1.2 mol equiv) and stirred for 1 h at rt. The solvent was removed by reduced pressure and the residue was dissolved in EtOAc (50 mL), washed with brine (50 mL), dried over $MgSO_4$ and concentrated under reduced pressure to provide the desired compound.

#### 4.7.1. *N′-Benzyl-2-(3,5-dimethoxyphenyl)propan-2-yl carbazate (**3j**)*

Product of the reaction of Ddz hydrazide **1** (0.66 g, 2.6 mmol) and benzaldehyde **2j** (0.26 mL, 2.6 mmol). Purification by flash chromatography on silica using 1% MeOH in $CH_2Cl_2$ as eluant gave a colourless oil. Yield 95% (0.85 g); $R_f$=0.58 (hexane/EtOAc 1:1). IR (KBr) $\nu_{max}$ 3311, 2980, 2937, 1717, 1598 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 1.74 (s, 6H), 3.77 (s, 6H), 3.94 (s, 2H), 6.36 (t, $J$=2.2 Hz, 1H), 6.51 (d, $J$=2.2 Hz, 2H), 7.22–7.39 (m, 5H). $^{13}C$ NMR ($CDCl_3$, 100 MHz): $\delta$ 28.9, 55.2, 55.5, 81.8, 98.3, 102.9, 127.6, 128.4, 129.1, 137.0, 148.5, 155.8, 160.7. HRMS calcd for $C_{19}H_{25}N_2O_4$ 345.18088 ($MH^+$), found 345.18079.

#### 4.7.2. *N′-(4-(tert-Butoxy)-benzyl)-2-(3,5-dimethoxyphenyl)-propan-2-yl carbazate (**3k**)*

Product of the reaction of Ddz hydrazide **1** (0.76 g, 3.0 mmol) and 4-(*tert*-butoxy)-benzaldehyde **2k** (0.522 mL, 3.0 mmol). Purification by flash chromatography on silica using 1% MeOH in $CH_2Cl_2$ as eluant gave a colourless oil. Yield 78% (0.97 g); $R_f$=0.56 (hexane/EtOAc 1:1). IR (KBr) $\nu_{max}$ 3383, 3334, 2978, 2933, 1719, 1598 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 1.33 (s, 9H), 1.74 (s, 6H), 3.77 (s, 6H), 3.94 (s, 2H), 6.35 (t, $J$=2.3 Hz, 1H), 6.50 (d, $J$=2.1 Hz, 2H), 6.94 (d, $J$=8.4 Hz, 2H), 7.23 (d, $J$=8.4 Hz, 2H). $^{13}C$ NMR ($CDCl_3$, 100 MHz): $\delta$ 28.8, 28.9, 55.1, 55.2, 78.5, 82.2, 98.4, 103.0, 124.1, 129.9, 130.7, 148.4, 155.2, 155.6, 160.7. HRMS calcd for $C_{23}H_{33}N_2O_5$ 417.23840 ($MH^+$), found 417.23785.

#### 4.7.3. *N′-3-((N-Boc-Indolyl)methyl)-2-(3,5-dimethoxyphenyl)-propan-2-yl carbazate (**3l**)*

Product of the reaction of Ddz hydrazide **1** (0.51 g, 2.0 mmol) and 1-*tert*-butoxycarbonylindole-3-carboxaldehyde **2l** (0.49 g, 2.0 mmol). Purification by flash chromatography on silica using hexane/EtOAc 8:2 as eluant gave a colourless oil. Yield 68% (0.66 g); $R_f$=0.65 (hexane/EtOAc 1:1). IR (KBr) $\nu_{max}$ 3356, 2979, 2936, 1732, 1598 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 1.66 (s, 9H), 1.72 (s, 6H), 3.75 (s, 6H), 4.22 (s, 2H), 6.33 (t, $J$=2.2 Hz, 1H), 6.50 (d, $J$=2.1 Hz, 2H), 7.22–7.27 (m, 1H), 7.29–7.34 (m, 1H), 7.64 (s, 1H), 7.70 (d, $J$=7.8 Hz, 1H), 8.13 (d, $J$=7.8 Hz, 1H). $^{13}C$ NMR ($CDCl_3$, 100 MHz): $\delta$ 28.2, 28.8, 46.5, 55.2, 82.4, 83.8, 98.4, 102.9, 115.2, 119.4, 122.8, 124.6, 125.6, 129.8, 135.6, 148.3, 149.5, 155.6, 160.6, 160.7. HRMS calcd for $C_{26}H_{34}N_3O_6$ 484.24421 ($MH^+$), found 484.24350.

### 4.8. General method D: synthesis of *N′*-alkyl 2-(3,5-dimethoxyphenyl)propan-2-yl carbazates (3m)

A well stirred suspension of 2-(3,5-dimethoxyphenyl)propan-2-yl carbazate **1** and $K_2CO_3$ (1.1 mol equiv) in dry DMF (0.35 M) was cooled down to 0 °C in an ice bath. Then, alkyl halide (1 mol equiv) was added, the ice bath was removed after 10 min and the reaction mixture was stirred overnight at rt. The reaction mixture was diluted with water (50 mL) and extracted with EtOAc (3×30 mL). The combined organic extracts were washed with water (50 mL) and brine (50 mL), dried over $MgSO_4$ and concentrated under reduced pressure to provide the desired compound.

#### 4.8.1. *N′-(tert-Butyl acetate)-2-(3,5-dimethoxyphenyl)propan-2-yl carbazate (**3m**)*

Product of the reaction of Ddz hydrazide **1** (0.38 g, 1.5 mmol) and *tert*-butyl bromoacetate **2m** (0.22 mL, 1.5 mmol). Purification by flash chromatography on silica using PE/EtOAc 7:3 as eluant gave a white solid. Yield 62% (0.34 g); mp=97–104 °C; $R_f$=0.46 (hexane/EtOAc 1:1). IR (KBr) $\nu_{max}$ 3368, 3316, 2978, 2939, 1742, 1709, 1595 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 1.46 (s, 9H), 1.74 (s, 6H), 3.53 (s, 2H), 3.78 (s, 6H), 4.05 (br s, 1H), 6.34 (t, $J$=2.3 Hz, 1H), 6.50 (d, $J$=2.3 Hz, 2H), 6.73 (br s, 1H). $^{13}C$ NMR ($CDCl_3$, 100 MHz): $\delta$ 28.1, 28.8, 53.3, 55.2, 81.9, 98.4, 102.9, 148.5, 155.4, 160.6, 170.0. HRMS calcd for $C_{18}H_{29}N_2O_6$ 369.20201 ($MH^+$), found 369.20169.

### 4.9. General procedures for solid phase peptide synthesis

Solid phase reactions were performed in a manual glass reaction vessel with an outer glass blanket to enable heating and cooling and equipped with a sintered glass bottom and Teflon valve for draining. All solid phase reactions were performed using Rink amide MBHA resin and reactions were carried out in an amount of solvent that was enough to cover the resin (0.1–0.15 M).

Solid phase peptide synthesis was carried out using the standard Fmoc/*t*-Bu strategy. The Fmoc protecting group was removed by piperidine solution in NMP. Standard Fmoc-amino acid coupling reactions were performed with HBTU/HOBt and DIEA in NMP/$CH_2Cl_2$ mixture. Cleavage of aza-peptides and deprotection of side chain protecting groups was carried out in TFA/TIS/$H_2O$ solution. Cleavage of aza-peptides containing an aza-amino acid with an aromatic side chain was performed at 0 °C to avoid loss of aromatic side chain promoted by acidic cleavage conditions.[15] Crude aza-peptides were precipitated with $Et_2O$/hexane (1/1 mixture), dissolved in ACN/$H_2O$ solution and lyophilized to obtain isolated aza-peptides as white foams.

#### 4.9.1. *Fmoc deprotection*

The resin was treated twice with a 20% solution of piperidine in *N*-methyl-2-pyrrolidinone (NMP) for 20 min each. The resin was then washed with NMP (5×) to remove the Fmoc by-products (dibenzofulvene and its piperidine adduct) and residual piperidine.

#### 4.9.2. *HOBt/HBTU coupling*

Fmoc-protected amino acid (1.5 equiv), 1-hydroxybenzotriazole hydrate (HOBt) (1.5 equiv) and diisopropyl-ethylamine (DIEA)

(1.5 equiv) were dissolved in NMP (about 7.5 vol) at rt. The solution was chilled to 0–5 °C and *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) (1.5 equiv) was added followed by stirring for 10 min to dissolve. The solution of activated amino acid was applied to the resin in $CH_2Cl_2$ (about 2.5 vol). The reaction was agitated for 1 h, the resin was then drained and washed with NMP (3×). Coupling completion was monitored by Kaiser-ninhydrin and Chloranil tests as described below. If a positive ninhydrin or chloranil test was observed after 1 h, the coupling reaction was repeated using 1 equiv of activated amino acid.

*4.9.3. Capping*

The resin was treated twice for 30 min each with a mixture of acetic anhydride (0.5 M), DIEA (0.125 M) and HOBt (0.015 M) in DMF. The resin was subsequently washed with DMF (3×) and $CH_2Cl_2$ (3×).

*4.9.4. Kaiser-ninhydrin test*

A sample containing 1–3 mg of the resin was withdrawn, placed into a test tube and washed clean with ethanol, which was removed by decantation. To the sample were added 3 drops of 80% phenol in ethanol (w/v) solution, 4 drops of 1 mL of 1 mM aq KCN in 49 mL of pyridine solution and 3 drops of 5% ninhydrin in ethanol (w/v) solution. The solution was mixed well and placed in a preheated heating block at 110 °C for 5 min. A blue or violet colour of solution is a positive indication for the presence of free amines, indicating deprotection of the Fmoc protecting group or incomplete coupling reaction.[26]

*4.9.5. Chloranil test*

A sample containing 1–3 mg of the resin was withdrawn, placed into a test tube and washed clean with ethanol, which was removed by decantation. To the sample were added 3 drops of 2% acetaldehyde in DMF and 3 drops of 2% chloranil in DMF. The solution was agitated at rt for 5 min. A green or blue colour of the beads is a positive indication for the presence of free amines, indicating deprotection of the Fmoc protecting group or incomplete coupling reaction.[27]

*4.9.6. Small cleavage*

A sample of 5–10 mg peptidyl-resin was treated with a TFA/TDW/TIS (95:2.5:2.5, v/v/v) solution precooled to 0 °C. The solution was shaken for 0.5 h at rt. The resin was removed by filtration and the solvents were evaporated by a stream of nitrogen. The residue was dissolved in ACN/TDW 1:1 solution and subsequently analyzed by MS and HPLC.

*4.9.7. Loading test*

Quantitative Fmoc substitution of the resin was determined by Fmoc cleavage and optical density measurements at 304 nm according to Novabiochem's proposed procedure.[28,29]

### 4.10. General procedure for introduction of an aza-amino acid on solid support by the Ddz method

*N*′-Alkyl 2-(3,5-dimethoxyphenyl)propan-2-yl carbazate **3** (3 mol equiv relative to peptidyl-resin) was dissolved in dry $CH_2Cl_2$ (0.1 M) and DIEA (6 mol equiv) was added. The reaction mixture was cooled to 0 °C in an ice bath under argon and phosgene in toluene (20% solution, 6 mol equiv) was added dropwise. Slow addition of the phosgene solution, low temperature and basic conditions during activation are necessary to avoid deprotection of the acid labile Ddz protecting group. After completion (~10 min observed by TLC (hexane/EA 1:1)), the excess phosgene and solvents were evaporated under reduced pressure. The obtained Ddz-aza-amino acid chloride was dissolved in dry $CH_2Cl_2$, DIEA (6 mol equiv relative to peptidyl-resin) was added and the solution was applied to the vessel containing resin-bound N-terminal peptide. The reaction mixture was shaken overnight, the resin was drained and washed with $CH_2Cl_2$ (2×). The described Ddz-aza-amino acid coupling was repeated once more. Coupling was monitored by Kaiser-ninhydrin and Chloranil tests as described above.

### 4.11. General procedure for Ddz removal

The resin was washed with ACN (2×), $Mg(ClO_4)_2$ (10 mol equiv relative to resin) was dissolved in ACN and applied to the vessel, heated to 50 °C and shaken for 1.5 h. The resin was washed with ACN (2×), $CH_2Cl_2$ (2×), ACN (2×), and this procedure was repeated once more. The procedure was further repeated for 0.5 h till no UV absorption of the deprotection solution is visible on TLC plate. The resin is then washed with ACN (3×) and $CH_2Cl_2$ (2×).

### 4.12. General procedure for coupling of amino acid to the N-terminus aza-amino acid peptidyl-resin

Difficult coupling to aza-amino acid was achieved according to the procedure previously described using Fmoc-amino acid chlorides:[30] Fmoc-amino acid-OH (3 mol equiv relative to resin) and bis-(trichloromethyl) carbonate (BTC, triphosgene) (1 mol equiv) were suspended in $CH_2Cl_2$ and cooled down to 0 °C in an ice bath. 2,4,6-Collidine (14 mol equiv) was added and after all the solids were dissolved (about 1 min), the solution was poured onto the resin and shaken for 3 h at rt. The resin was drained and this coupling procedure was repeated once more. At the end of the second coupling cycle, the resin was drained and washed with $CH_2Cl_2$ (5×). The coupling was monitored by small cleavage followed by MS analysis of the crude obtained. If N-terminus aza-peptide (starting material) mass was observed, the above procedure was repeated using 5 mol equiv Fmoc-amino acid in DBE at 60 °C overnight and the aza-peptide was subsequently capped.

*4.12.1. Peptide cleavage from the resin and removal of side chain protecting groups*

Prior to cleavage, the peptidyl-resin was washed with $CH_2Cl_2$ (2×) and MeOH (2×) and thoroughly dried under reduced pressure. A freshly made solution of TFA/TDW/triisopropylsilane (TIS) (95:2.5:2.5, v/v/v) was cooled to 0 °C (14 mL/g peptidyl-resin). The resin was added, the mixture was maintained in the ice bath for 30 min and was subsequently shaken for 3 h at rt. The resin was removed by filtration, washed with a small amount of neat TFA and the combined TFA filtrates were concentrated (to about 2 mL) under a stream of nitrogen. The oily residue was treated with a previously cooled to 0 °C mixture of diethylether/hexane (1:1, v/v) and the ether-hexane was decanted. The peptide precipitate was dissolved in ACN/TDW 1:1 and lyophilized. This complete cleavage process as well as the small cleavage of aza-peptides containing an aza-amino acid with aromatic side chain was carried out at 0 °C as previously recommended.[15]

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### Supplementary data

$^{1}H$ and $^{13}C$ NMR spectral data for compounds **3a**–**m**, analytical HPLC and mass spectra for aza-peptide **4** are provided. This material is available free of charges via the internet. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.11.038.

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