Novel Solid-Phase Synthesis of Azapeptides and Azapeptoides via Fmoc-Strategy and Its Application in the Synthesis of RGD-Mimetics

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The cell adhesion motif Arg-Gly-Asp (RGD) has been used as a starting point for the development of several antagonists for the αIIbβ3 and αvβ3 integrins, which are implicated in various pathological processes. In this paper, an efficient method for the solid-phase synthesis and biological evaluation of linear RGD-mimetics containing an azaamino acid instead of glycine are described. Activation of the Fmoc-protected hydrazines **1**, **4**, and **6** with a solution of phosgene in toluene provided Fmoc-protected activated azaglycine (**2**), azasarcosine (**5**), and azaalanine (**7**) in high yields. Six aza-RGD-mimetics have been synthesized on solid support using Fmoc peptide synthesis and individually optimized reaction conditions for the incorporation of activated azabuilding blocks. Due to orthogonal anchoring and side-chain protection, our strategy yielded TentaGel-bound RGD-mimetics, which meet all requirements of the one-bead−one-compound concept. We observed differing activity and selectivity in bioassays for the αIIbβ3- and αvβ3-integrin receptor depending on the substitution pattern of the azabuilding blocks. Our biological data suggest that azapeptides and azapeptoides can be employed as selectivity- and activity-inducing templates in pseudobio-oligomers.

Introduction

Solid-phase synthesis (SPS) is one of the most promising methods in automated synthesis and combinatorial chemistry directed at high-throughput drug discovery.[1] Due to their enhanced metabolic stability, bioavailability, and biological absorption, nonpeptidic compounds have been the focus of research interests over the past several years.[2] However, in contrast to peptide and oligonucleotide synthesis, organic reactions often do not meet the requirements of SPS, due to formation of side products, low reaction rates, or unsuitable reaction conditions. A potential class of peptidomimetics for a broad application in SPS are azapeptides and azapeptoides.[3] In previous investigations, we have demonstrated that replacement of glycine by azaglycine in Arg-Gly-Asp (RGD)-containing antagonists can influence the biological activity and selectivity.[4] The amino acid sequence RGD is a cell-recognition motif that has been used for the development of different antagonists.[5] Originally, the attention of this medicinal research was directed toward the αIIbβ3 integrin as a target for the inhibition of platelet aggregation.[6] More recently, other integrins, including αvβ3, have received increasing attention, due to their potential application as therapeutic targets in pathologies as diverse as osteoporosis, restenosis, angiogenesis, and acute renal failure.[5,7]

Theoretical studies of diacylhydrazines indicated that, in contrast to azaglycine, N-substituted azapeptides should be more rigid than their conventional peptide counterparts.[8] As a result, we regard azapeptides and azapeptoides as conformationally restrained and there-

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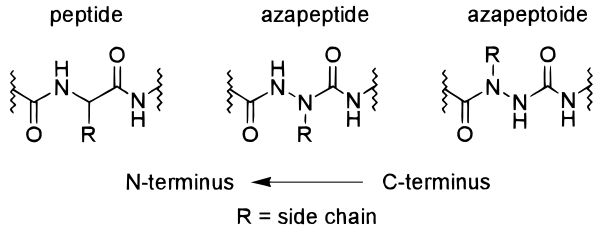
fore as templates that have the potential to induce biological selectivity in pseudo biooligomers.

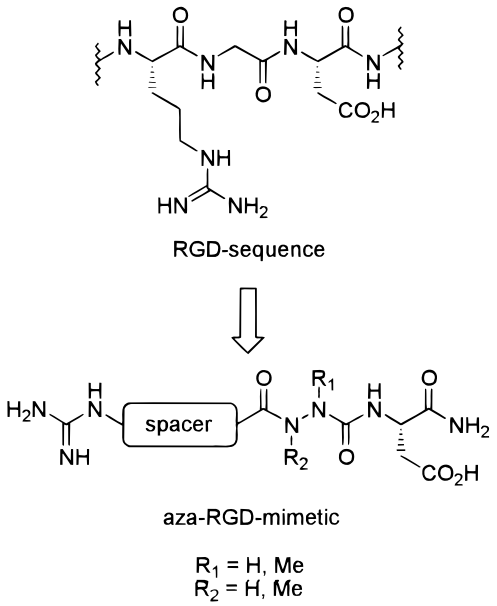


Apart from the various solution-phase syntheses of azapeptides[9] and the PEG-supported synthesis of azatides[10] only two approaches for the SPS of azapeptides have been reported. The first synthetic route starts with the conversion of the N-terminus of resin-bound peptide to an isocyanate or an activated ester, followed by coupling with the 9*H*-fluoren-9-ylmethoxycarbonyl (Fmoc)-protected hydrazine. However, this method is limited by the formation of hydantoines as side products and rather low reaction rates.[11] The transformation of the N-protected hydrazines into activated carbazic acids in solution seems much more promising, since resin-bound peptides can be treated with an excess of activated agent to enforce complete reaction. In the case of azapeptides, the fundamental feasibility of this method was recently shown by the use of triphosgene as carbonylating agent.[12] This method works well for azapeptides, although the activated species can only be prepared in situ. Furthermore, we observed that this method is inappropriate for the synthesis of azapeptoides and azaglycine, due to formation of considerable amounts of side products.

Here, we describe new approaches to SPS of azapeptides and azapeptoides using the Fmoc-strategy, commonly used in solid-phase peptide synthesis (SPPS).[13] We found suitable reaction conditions to prepare activated N-protected carbazic acids, depending on their substitution pattern, by using easy-to-handle and commercially available solutions of phosgene in toluene. To check the feasibility of preparing azapeptides and azapeptoides on a solid support, we carried out a systematic study of the coupling conditions and targeted the preparation of six RGD-mimetics, all of which contain aza building blocks instead of glycine. The biological activity of the aza-RGD-mimetics on the $\alpha v\beta 3$- and $\alpha IIb\beta 3$-integrin receptor was determined to test the potential use of azabuilding blocks as biological activity and selectivity inducing elements.

Results and Discussion

Synthesis and Activation of Aza Building Blocks in Solution. The synthetic route for the preparation of the activated azaglycine, azaalanine, and azasarcosine



is outlined in Scheme 1. The azaglycine precursor Fmoc-hydrazine (**1**) was prepared by monoprotection of aqueous hydrazine with FmocCl.[14] The transformation of Fmoc-protected hydrazines into azaamino acids or azapeptoides requires double-activated carbonic acid. For this reaction, we used a commercially available solution of phosgene as the reagent, because of its high reactivity and easy removal of excess reagents by evaporation under reduced pressure. Thus, utilizing conditions recently described for the preparation of isocyanates,[15] the treatment of Fmoc-hydrazine (**1**) with 2 equiv of phosgene in a biphasic 1:1 mixture of CH_2Cl_2 and saturated aqueous $NaHCO_3$ afforded the 1,3,4-oxadiazol-2(3*H*)-one **2** within 10 min in good yield as a highly activated azaglycine building block.

For the syntheses of the activated azasarcosine **5** and azaalanine **7** we utilized the enhanced nucleophilicity of the methylated N-atom of methylhydrazine (**3**), which could be regioselectively converted with FmocCl and Et_3N to 1-Fmoc-1-methylhydrazine (**4**) in high yield. To obtain the regioisomer 1-Fmoc-2-methylhydrazine (**6**) in a one-pot procedure, methylhydrazine (**3**) was treated first with an excess of di-*tert*-butyl dicarbonate (Boc_2O) followed by addition of FmocCl and *N*,*N*-diisopropylethylamine (DIEA). After 8 h, the *tert*-butyloxycarbonyl (Boc) protecting group was cleaved by acidification with trifluoroacetic acid (TFA). Our attempts to convert the Fmoc-protected methylhydrazines **4** and **6** into the corresponding 1,3,4-oxadiazol-2(3*H*)-ones with the procedure described above did not afford the activated heterocyclic compounds. Instead of the expected reaction, compound **4** was converted quantitatively into the bis-Fmoc-carbonic dihydrazide, whereas **6** afforded impure carbazic acid chloride **7**. The in situ activation of Boc-protected hydrazines with triphosgene in the presence of *N*-methylmorpholine was reported by Marraud et al.[12,16] Unfortunately, our attempts to activate **4** with phosgene in the

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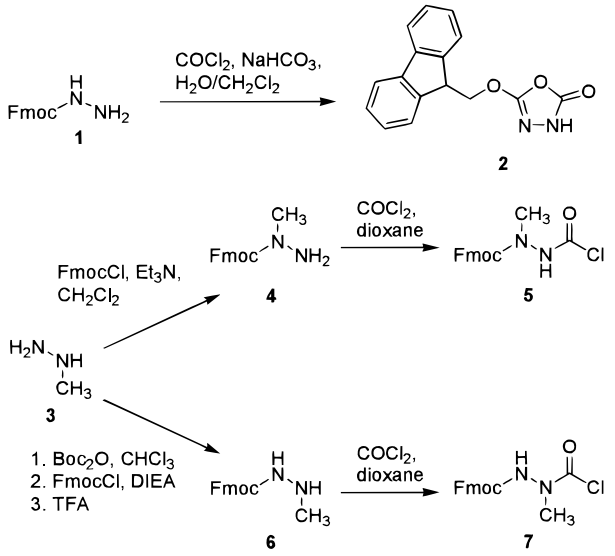
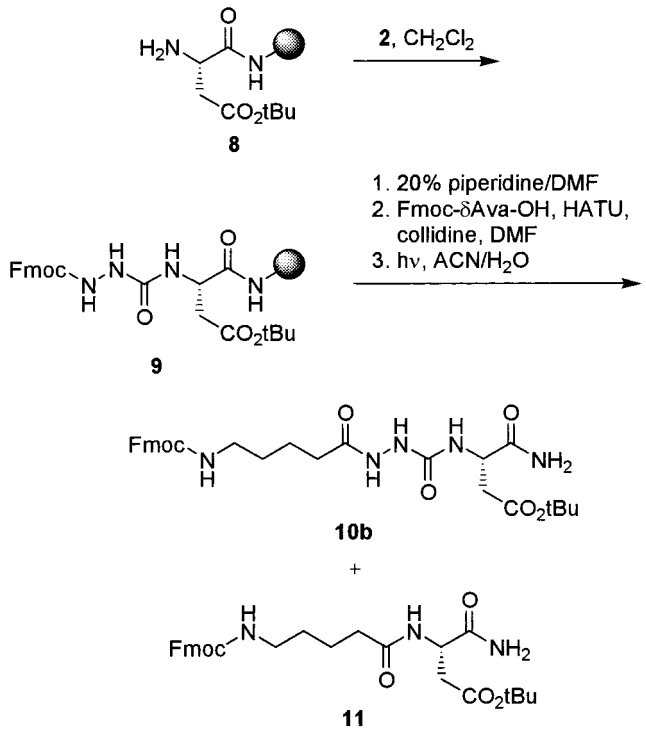
Scheme 1. Synthesis and Activation of the Azabuilding Blocks



Scheme 2. Synthesis of AzaGly Containing Peptide



presence of tertiary bases such as Et_3N, DIEA, pyridine, collidine, or *N*-methylmorpholine afforded a mixture of side products. In contrast to the activation of Boc-protected hydrazines, we did not need to neutralize the hydrogen chloride formed during the reaction, since the Fmoc-protected hydrazines are stable under acidic conditions. Therefore, we investigated the reaction of the Fmoc-methylhydrazines **4** and **6** with phosgene in the absence of a tertiary base. As expected, **4** and **6** precipitated as hydrochloride salt in CH_2Cl_2 during the treatment with a solution of phosgene in toluene. However, the precipitation could be avoided by using anhydrous dioxane as a solvent. Under these conditions, the Fmoc-methylhydrazines **4** and **6** could be converted quantitatively into the corresponding carbazic acid chlorides **5** and **7** with 2 equiv of phosgene at ambient temperature. All activated azabuilding blocks were isolated as pure solids and were stable in long-term storage at 4 °C.

The Use of Activated Aza Building Blocks in SPS. All reactions on solid support were performed on Fmoc-aminoethyl-photolinker[17] TentaGel resin to meet the requirements of the one-bead−one-compound concept, which can be used directly for biological on-bead screening.[18] Fmoc-Asp(Bu)-OH was attached to the resin using *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′,-tetramethyluronium hexafluorophosphate (HATU) and collidine. The Fmoc-protecting group was removed with a solution of 20% piperidine in *N*,*N*-dimethylformamide (DMF) to obtain the immobilized side-chain protected L-aspartic acid amide (Asp(Bu)-amide resin) **8**.

We studied the coupling reaction between **8** and 1,3,4-oxadiazol-2(3*H*)-one **2** to obtain resin-bound Fmoc-azaGly-Asp(Bu)-NH_2 (**9**) (Scheme 2). The progress of the conversion was monitored through quenching the reaction by washing the resin with CH_2Cl_2 or DMF. After Fmoc removal, Fmoc-protected δ-aminovaleric acid (Fmoc-δAva-OH) was coupled using standard conditions described above. Photolytic cleavage for 1.5 h in a 4:1 mixture of acetonitrile (ACN)/H_2O released the desired product **10b** along with **11** indicating incomplete conversion of **8** to **9**. The predominant UV-chromophoric Fmoc-δAva-group of both **10b** and **11** facilitates reliable quantification of their molar ratio by HPLC chromatogram detected at 220 nm and monitoring of the conversion from **8** to **9**. Indeed, we monitored a remarkable rapid consumption of **8** in anhydrous CH_2Cl_2 at room temperature, although a 3-fold excess of **2** was necessary to complete conversion within 50 min. The reaction courses of a 0.04 M solution of 2 and 3 equiv of **2** in CH_2Cl_2 are presented in Figure 1 (solid lines). Furthermore, we observed that this reaction is not limited to the use of CH_2Cl_2 but also works quite well in DMF, ACN, or tetrahydrofuran (THF). In the past, various 1,3,4-oxadiazol-2(3*H*)-ones have been described as stable heterocycles, which require temperatures higher than 100 °C for ring opening by semicarbazides or amines.[19] In contrast to these 1,3,4-oxadiazol-2(3*H*)-ones, compound **2** is substituted by a heteroatom at the 5-*C*-atom (resulting from the urethane group of **1**). This also appears to be the reason for the extraordinarily high reactivity of our 1,3,4-oxadiazol-2(3*H*)-one **2**.

Next, we investigated the suitability of the activated azaSar building block **5** in SPS. To monitor the progress of these reactions, we used the strategy described above (Scheme 3). As expected, the reaction of Asp(Bu)-amide resin (**8**) with an excess of carbazic acid chloride **5** stopped at approximately 50% conversion due to increased formation of hydrogen chloride. However, treatment of the resin with a solution of carbazic acid chloride **5** and DIEA in CH_2Cl_2 led to a substantial quantity of side products, probably caused by ultrahighly activated species resulting from β-elimination of HCl in **5**. Fortunately, the byproduct formation could be suppressed completely by changing the reaction protocol: treatment of Asp(Bu)-

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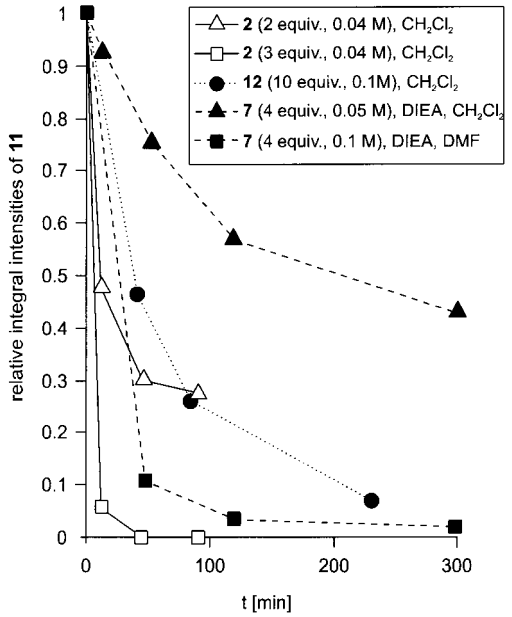
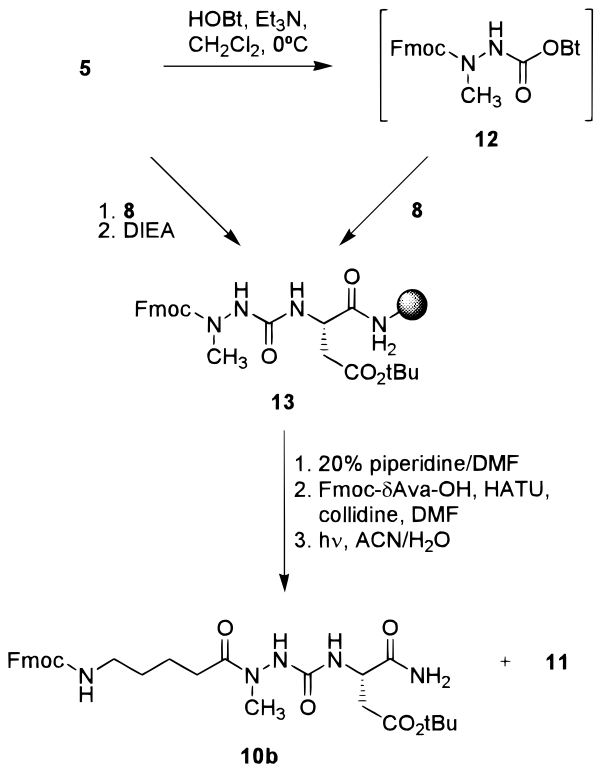


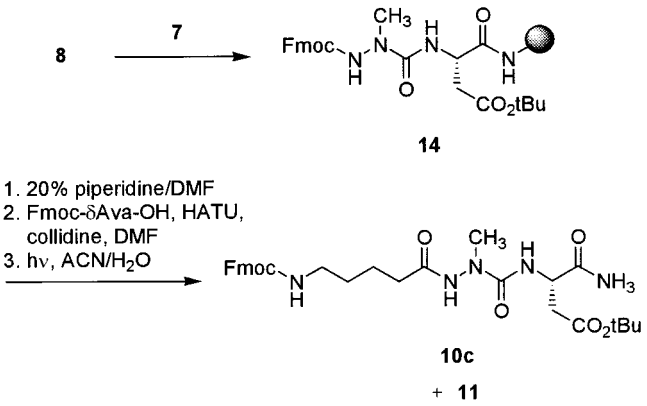
Figure 1. Reaction course of activated azabuilding blocks **2**, **7**, and **12** with Asp(Bu)-amide resin (**8**), visualized by the decrease of the deletion sequence Fmoc-δAva-Asp(Bu)-NH_2 (**11**); the relative integral intensities were obtained from the HPLC chromatogram at 220 nm. Although the 1,3,4-oxadiazol-2(3*H*)-one **2** exhibited a high reaction rate, 3 equiv of **2** was necessary to complete conversion within 50 min (solid lines). The unoptimized reaction of 10 equiv of carbazic acid active ester **12** revealed an acceptable reaction rate (dotted line). The conversion of the carbazic acid chloride **7** showed a significant enhancement in reaction rate by increasing the concentration and employing DMF instead of CH_2Cl_2 as the solvent (dashed lines).

Scheme 3. Synthesis of AzaSar Containing Peptide



amide resin (**8**) with an excess of carbazic acid chloride **5** in CH_2Cl_2 at room temperature followed by addition of DIEA afforded the desired product **13** in >95% purity (HPLC). Deprotecting, coupling with Fmoc-δAva-OH, and photolysis afforded **10b** without detectable amounts of **11**.

Scheme 4. Synthesis of AzaAla Containing Peptide



However, some techniques in SPS such as SPOT synthesis on cellulose paper[20] or light-directed synthesis on chips[21] complicate additional pH adjustment. To meet these requirements, we developed an alternative route. This approach involved in situ generation of the active ester **12** by treatment of **5** with a solution of 2.2 equiv of *N*-hydroxybenzotriazole (HOBt) and 2.0 equiv of Et_3N in CH_2Cl_2 at 0 °C to obtain a reaction solution for the conversion of Asp(Bu)-amide resin (**8**) (Scheme 3). The reaction of **8** with 10 equiv of a 0.1 M solution of **12** in CH_2Cl_2 proved to proceed with an acceptable reaction rate as shown in Figure 1 (dotted line), without significant side-product formation. Obviously, an excess of Et_3N/HOBt mixture helped to buffer the reaction medium and prevented HCl elimination. We did not optimize these reaction conditions, since the acid chloride route worked reliably when the resin-supported reactions were performed in syringes as reaction vessels.

Compared to the activated azabuilding blocks discussed, the carbazic acid chloride **7** turned out to be much less reactive. Thus, conversion of Asp(Bu)-amide resin (**8**) by treatment with 4 equiv of a 0.05 M solution of **7** and DIEA at room temperature for 5 h was only half-complete, as indicated by the ratio of the coupling products **10c** and **11** (Scheme 4 and Figure 1 (dashed lines)). The low reaction rate is in agreement with the observation recently made by the group of Janda,[10] who found that C-terminal activation of Boc-protected azaamino acids with bis(pentafluorphenyl) carbonate failed, due to the poor electrophilic nature of the resulting pentafluorophenylester.

Next, we attempted to catalyze the reaction of the acid chloride using 4-dimethylamino pyridine (DMAP), but in this case the acetylating catalyst completely inhibited the formation of the desired Fmoc-azaAla-Asp(Bu)-NH_2 (**14**). The use of tributylphosphine (Bu_3P) as an alternative acylation catalyst[22] did not influence the reaction at all. A significant enhancement of the reaction rate was observed by increasing the concentration and employing

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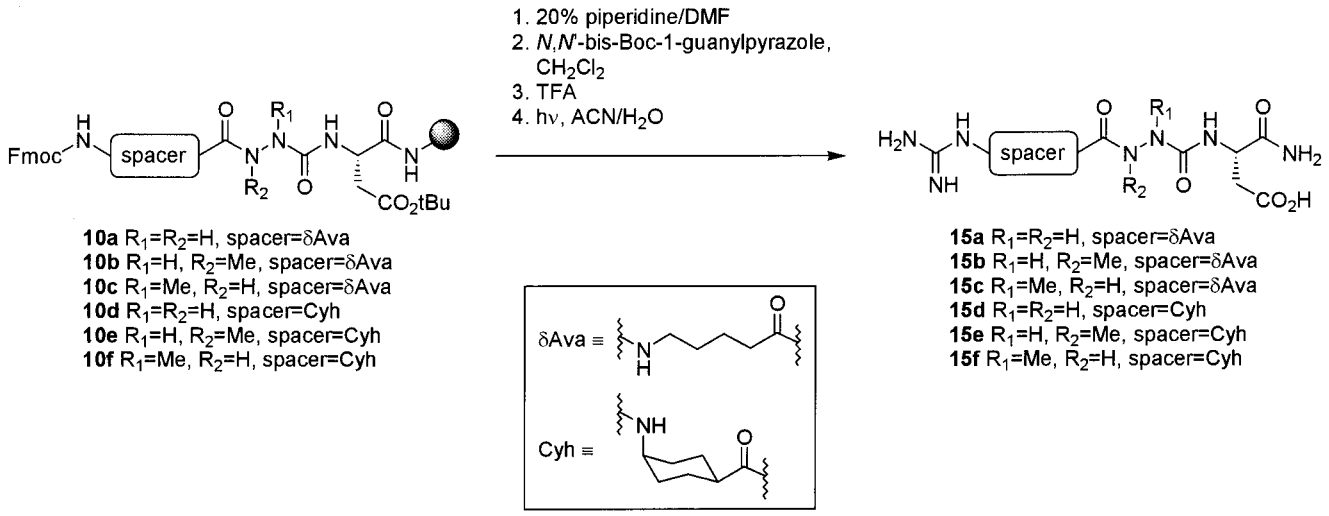
Scheme 5. Synthesis of Aza-RGD-mimetics Involving Solid-Phase Supported Guanylation



10a R_1=R_2=H, spacer=δAva
10b R_1=H, R_2=Me, spacer=δAva
10c R_1=Me, R_2=H, spacer=δAva
10d R_1=R_2=H, spacer=Cyh
10e R_1=H, R_2=Me, spacer=Cyh
10f R_1=Me, R_2=H, spacer=Cyh

15a R_1=R_2=H, spacer=δAva
15b R_1=H, R_2=Me, spacer=δAva
15c R_1=Me, R_2=H, spacer=δAva
15d R_1=R_2=H, spacer=Cyh
15e R_1=H, R_2=Me, spacer=Cyh
15f R_1=Me, R_2=H, spacer=Cyh

DMF as the solvent instead of CH_2Cl_2. The reaction of Asp(Bu)-amide resin (**8**) with 4 equiv of a 0.1 M solution of carbazic acid chloride **7** and DIEA in DMF is presented in Figure 1 (dashed lines). For the final synthesis of azaAla-containing compounds, we treated the resin with an excess of 5 equiv of activated azaAla **7** overnight to ensure complete reaction. It is noteworthy that our strategy consequently avoids the use of toxic phosgene or triphosgene in SPS.

Syntheses of the RGD-Mimetics. With a reproducible and reliable approach for the insertion of aza building blocks in hand, we synthesized the 4-*cis*-aminocyclohexanecarbonic acid (Cyh)-containing compounds **10d**−**f** in addition to the Fmoc-protected δAva containing compounds **10a**−**c** (Scheme 5). To introduce the guanidine group at the N-terminus, Fmoc deprotected **10a**−**f** were individually treated with an excess of *N*,*N*′-bis-Boc-1-guanylpyrazole,[23] followed by removal of the acid-labile protecting groups with TFA. Photolysis in a 1:1 mixture of ACN and H_2O afforded the crude products **15a**−**f** in 70−100% yield, whereas representative purification of **15a**, **15b**, and **15d** by HPLC decreased the yield to 51−59%.

Biological Evaluation. The azaAla-containing compounds **15c** and **15f** were inactive in both αvβ3- and αIIbβ3-integrin ligand-binding assays (IC_{50} > 100 μM) (Table 1). The azaGly-containing RGD-mimetics showed weak inhibitory activity on αvβ3: compound **15a** had an IC_{50} of 6.8 μM on αvβ3 but at 100 μM no activity on αIIbβ3. On the other hand, **15d** with an IC_{50} of 5.4 μM on αvβ3 and 4.0 μM on αIIbβ3 showed no selectivity. Remarkably, azaSar-containing compounds **15b** and **15e** exhibited a distinct selectivity on αIIbβ3, in contrast to azaGly. Thus, compound **15b** had an IC_{50} of 2.7 μM on αIIbβ3 and **15e** had an IC_{50} of 22 μM, whereas both were inactive (IC_{50} > 100 μM) on αvβ3. These results demonstrate that both activity and selectivity can be influenced through the substitution pattern of the azabuilding block.

Table 1. Effect of Compounds 15a−f on Ligand Binding to Integrins αvβ3 and αIIbβ3[a]

	αvβ3		αIIbβ3	
compd	IC_{50} (μM)	*Q*	IC_{50} (μM)	*Q*
cyclo(−RGDfV−)	0.0015	1.0	3.7	1.0
15a	6.8	4500	>100	
15b	>100		2.7	0.73
15c	>100		>100	
15d	5.4	3600	4.0	1.1
15e	>100		22	5.9
15f	>100		>100	

[a] The Peptide *cyclo*(−RGDfV−) was included for ref 24. The *Q* value designates the activity of the compound relative to the activity of *cyclo*(−RGDfV−).

Conclusion

New approaches to the solid-phase synthesis of azapeptides as well as azapeptoides using the Fmoc-strategy are described. We have developed syntheses of the activated azabuilding blocks **2**, **5**, and **7**. In a systematic investigation, we have found mild and therefore suitable reaction conditions for the solid-phase-supported incorporation of these azabuilding blocks. We have demonstrated the feasibility of our strategy by the solid-phase syntheses of the RGD-mimetics **15a**−**f**. The synthesized aza-RGD-mimetics exhibit varying activity and selectivity on the integrin receptors αvβ3 or αIIbβ3 depending on the substitution pattern of the azabuilding block. The results offer a potential application of azapeptides and azapeptoides as selectivity- and activity-inducing templates in pseudobiooligomers. We want to emphasize that our strategy afforded completely deprotected TentaGel-bound RGD-mimetics **15a**−**f**, which meet all requirements of the one-bead−one-compound concept[18] and allow biological on-bead screening and subsequent chemical characterization, due to orthogonal anchoring. For that purpose, we are currently developing an on-bead assay for biological evaluation of aza-RGD-libraries.

Experimental Section

General Methods. Fmoc-aminoethyl-photolinker TentaGel resin (0.18 mmol/g) was purchased from Calbiochem-Novabiochem. The manufacturer's reported loading of the resin was used in the calculation of the theoretical yields of the final products. Phosgene in toluene 20% was purchased from Fluka. All reagents and solvents were from Bachem, Aldrich, and Fluka and were used as received. Reactions on solid supports were performed in filter columns (2 and 10 mL) from Abimed. Melting points were uncorrected. ^1H and ^{13}C NMR spectra were obtained in $CDCl_3$ or DMSO-d_6 as the solvent and

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internal reference. Thin-layer chromatography was performed on silica gel 60 F_{254} plates from Merck. Flash chromatography was performed on silica gel 60 (230−400 mesh ASTM) from Merck. Analytical HPLC analyses were performed on a YMC column, 4.6 mm × 25 mm, 5 μm C_{18}, 1 mL/min, 30 min linear gradient from water (0.1% TFA) and CH_3CN (0.1% TFA), 220 nm.

General Procedure for Photolysis. Resin was suspended in a solution of ACN and H_2O in a filter column. Photolysis was performed by irradiation of the sample with a 150 W high-pressure Hg lamp (TQ 150 Z2 from Heraeus). The samples were irradiated from above for 1−6 h with gentle mixing by a magnetic stirrer. The supernatant was analyzed by HPLC and ESI-MS after photolysis. After quantitative cleavages were performed, the supernatant was concentrated under reduced pressure and lyophilized from H_2O.

General Procedure for Fmoc Deprotectings and HATU Couplings. Fmoc-aminoethyl-photolinker TentaGel resin (1.10 g, 0.18 mmol/g, 0.20 mmol) was washed with DMF (2 × 7 mL) and treated with a solution of 20% piperidine in DMF (2 × 7 mL) for 10 and 20 min. The resin was washed with DMF (6 × 7 mL), MeOH (3 × 7 mL), and Et_2O (2 × 7 mL). A solution of Fmoc-Asp(Bu)-OH (0.198 g, 0.482 mmol), HATU (0.183 g, 0.482 mmol), and collidine (1.28 mL, 1.17 g, 9.46 mmol) in DMF (6 mL) was added to the resin. The reaction mixture was shaken at room temperature for 1 h and washed with DMF (4 × 7 mL). The Fmoc protecting group was removed under the standard conditions described above. The resin was washed with DMF (6 × 7 mL), MeOH (3 × 7 mL), and Et_2O (2 × 7 mL) and dried under vacuo to give Asp(Bu)-amide resin **(8)**.

1-Fmoc-1-methylhydrazine (4). A solution of 9*H*-fluoren-9-ylmethyl chloroformate (3.802 g, 14.70 mmol) in CH_2Cl_2 (10 mL) was slowly added at −78 °C to a stirring solution of methylhydrazine (**3**) (1.0 mL, 0.88 g, 19 mmol) and Et_3N (2.9 mL, 2.1 g, 21 mmol) in CH_2Cl_2 (20 mL). The reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction mixture was extracted with H_2O, dried over Na_2SO_4, and concentrated under reduced pressure to give a yellow oil. Compound **4** (3.31 g, 84%) was isolated by flash chromatography as a white solid: mp 84 °C; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.26 (d, J = 7.3 Hz, 2H), 7.72 (br d, J = 7.2 Hz, 2H), 7.36 (tm, J = 7.2 Hz, 2H), 7.30 (tm, J = 7.4 Hz, 2H), 4.42 (d, J = 6.8 Hz, 2H), 4.24 (t, J = 6.9 Hz, 3H), 4.10 (br s, 3H); ^{13}C NMR ($CDCl_3$, 125.0 MHz) δ 157.6, 143.8, 141.3, 127.7, 127.0, 124.9, 120.0, 67.8, 47.2, 38.4; HRMS calcd for $C_{16}H_{16}N_2O_2$ 268,1212, found 268.1213.

1-Fmoc-2-methylhydrazine (6). Methylhydrazine (0.25 mL, 0.36 g, 7.8 mmol) was added in a single portion at −78 °C to a stirring solution of di-*tert*-butyl dicarbonate (1.76 g, 10.1 mmol) in anhydrous $CHCl_3$ (10 mL). The reaction mixture was allowed to warm to room temperature and stirred for 30 min. To this reaction mixture was added 9*H*-fluoren-9-ylmethyl chloroformate (2.02 g, 7.81 mmol) in a single portion, and DIEA (1.6 mL, 1.2 g, 9.3 mmol) was added dropwise over a period of 10 min. After 8 h, TFA (10 mL) was carefully added to the reaction mixture. The reaction was stirred until complete consumption of FmocHN-NMeBoc was observed by TLC (educt R_f = 0.8; product R_f = 0.5; 30% hexane in EtOAc), approximately 2 h. The mixture was concentrated under reduced pressure. The remaining, dark brown residue was redissolved in EtOAc (70 mL) and washed with aqueous $NaHCO_3$ (10%, 3 × 70 mL) and brine (1 × 50 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give a yellow solid. Compound **6** (1.33 g, 64%) was isolated by flash chromatography as a white solid: mp 155 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.75 (d, J = 7.5 Hz, 2H), 7.57 (d, J = 7.5 Hz, 2H), 7.39 (t, J = 7.4 Hz, 2H), 7.30 (t, J = 7.4 Hz, 2H), 6.27 (br s, 2H), 4.45 (br s, 2H), 4.22 (br s, 1H), 3.88 (br s, 1H), 2.62 (br s, 3H); ^{13}C NMR ($CDCl_3$, 125.0 MHz) δ 157.2, 143.8, 141.4, 127.8, 127.1, 125.0, 120.0, 67.0 (br s), 47.2 (br s), 39.3 (br s); HRMS calcd for $C_{16}H_{16}N_2O_2$ 268.1212, found 268.1212.

5-(9*H*-Fluoren-9-ylmethoxy)-1,3,4-oxadiazol-2(3*H*)-one (2). Fmoc-hydrazine (**1**) (0.995 g, 3.91 mmol) was suspended in CH_2Cl_2 (40 mL) and saturated aqueous $NaHCO_3$ (40 mL). The biphasic mixture was cooled to 0 °C in an ice bath with vigorous stirring for 5 min. Stirring was stopped, the layers were allowed to separate, and phosgene (4.2 mL, 1.89 M in toluene, 7.8 mmol) was added in a single portion via syringe to the lower (organic) phase. Stirring was resumed immediately, and the ice-cooled reaction mixture was vigorously stirred for 10 min. The layers were then separated, the aqueous phase was extracted with CH_2Cl_2 (3 × 40 mL), and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield the product **2** as a white solid (0.958 g, 87%): ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.19 (br s, 1H), 7.75 (dm, J = 7.4 Hz, 2H), 7.58 (dm, J = 7.9 Hz, 2H), 7.45 (tm, J = 7.4 Hz, 2H), 7.35 (td, J = 7.5, 1.3 Hz, 2H), 4.50 (d, J = 7.2 Hz, 2H), 4.36 (t, J = 7.3 Hz, 1H); ^{13}C NMR ($CDCl_3$, 62.50 MHz) δ 156.1, 152.5, 142.4, 141.3, 128.2, 127.3, 125.1, 120.2, 72.9, 46.2; IR (KBr, cm^{-1}) $\tilde{\nu}$ 3300s, 1780s, 1650s, 1451m, 1426m, 1347m, 1224m, 918m, 758w, 740m. Anal. Calcd for $C_{16}H_{12}N_2O_3$: C, 68.56; H, 4.32. Found: C, 68.17; H, 4.51.

2-(Chlorocarbonyl)-1-Fmoc-methylhydrazine (5). Phosgene (4.2 mL, 1.89 M in toluene, 7.8 mmol) was slowly added at 10 °C to a stirring solution of **4** (0.925 g, 3.30 mmol) in anhydrous dioxane (20 mL). The temperature was kept at 10 °C for 5 min, after which time the reaction mixture was allowed to warm to room temperature and stirred for 70 min. The reaction mixture was lyophilized to yield the product **5** as a white powder (1.092 g, 100%): mp 146 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.75 (d, J = 7.5 Hz, 2H), 7.54 (dm, J = 7.4 Hz, 2H), 7.39 (tm, J = 7.9 Hz, 2H), 7.31 (tm, J = 7.5 Hz, 2H), 4.48 (d, J = 6.6 Hz, 2H), 4.23 (t, J = 6.5 Hz, 1H), 3.19 (s, 3H); ^{13}C NMR ($CDCl_3$,125.0 MHz) δ = 155.9, 148.2, 143.8, 141.8, 128.4, 127.7, 125.4, 120.5, 69.3, 47.5, 37.8; IR (KBr, cm^{-1}) $\tilde{\nu}$ 3250m, 3048w, 1782s, 1694s, 1479m, 1452m, 1403w, 1355m, 1301w, 1200m, 1141m, 941w. Anal. Calcd for $C_{17}H_{15}ClN_2O_3$: C, 61.73; H, 4.57. Found: C, 62.27; H, 4.72.

2-(Chlorocarbonyl)-1-Fmoc-2-methylhydrazine (7). Phosgene (1.7 mL, 1.89 M in toluene, 3.2 mmol) was slowly added at 10 °C to a stirring solution of **6** (0.449 g, 1.60 mmol) in anhydrous dioxane (5 mL). The temperature was kept at 10 °C for 5 min, after which time the reaction mixture was allowed to warm to room temperature and stirred for 90 min. The solvent was removed under reduced pressure to yield the product **7** as a yellow solid (0.531 g, 100%): mp 62 °C; ^{1}H NMR (300 K, $CDCl_3$, 250 MHz) δ 7.75 (d, J = 7.4 Hz, 2H), 7.56 (br d, J = 7.3 Hz, 2H), 7.40 (tm, J = 7.1 Hz, 2H), 7.31 (tm, J = 7.4 Hz, 2H), 6.95 and 6.72 (2 × br s, 1H), 4.64−4.36 (m, 2H), 4.23 (t, J = 6.2 Hz, 1H), 3.37, 3.24 and 2.99 (3 × br s, 3H); (335 K, $CDCl_3$, 250 MHz) δ 7.75 (d, J = 7.3 Hz, 2H), 7.56 (dm, J = 7.3 Hz, 2H), 7.39 (tm, J = 7.4 Hz, 2H), 7.30 (tm, J = 7.4 Hz, 2H), 6.76 (br s, 1H), 4.59 (br d, J = 6.1 Hz, 2H), 4.23 (t, J = 6.2 Hz, 1H), 3.17 (br s, 3H); ^{13}C NMR (335 K, $CDCl_3$, 62.50 MHz) δ 154.4, 151.6, 143.3, 141.5, 127.9, 127.2, 124.8, 120.1, 68.1, 47.2, 39.2; IR (KBr, cm^{-1}) $\tilde{\nu}$ 3298m, 2954w, 1749s, 1257m, 1103m, 916w, 740m, 667m; HRMS calcd for $C_{17}H_{15}N_2O_3Cl$ 330.0771, found 330.0778.; Anal. Calcd for $C_{17}H_{15}ClN_2O_3$: C, 61.73; H, 4.57. Found: C, 61.99; H, 4.62.

Fmoc-azaGly-Asp(Bu)-amide Resin (9). Asp(Bu)-amide resin (**8**) (1.12 g, 0.18 mmol/g, 0.20 mmol) was washed with anhydrous CH_2Cl_2 (3 × 7 mL), and a solution of **2** (0.176 g, 0.629 mmol) in anhydrous CH_2Cl_2 (5 mL) was added. The reaction mixture was shaken at room temperature for 90 min, washed with CH_2Cl_2 (3 × 7 mL), NMP (5 × 7 mL), and Et_2O (4 × 7 mL), and dried under vacuo. A small sample of resin (3.1 mg) was photolyzed in a solution of 20% H_2O in ACN (1 mL) for 1.5 h to yield the product **9** (88% purity) in solution: HPLC (10 → 90) t_R 28.2 min.

Fmoc-azaSar-Asp(Bu)-amide Resin (13). Asp(Bu)-amide resin (**8**) (0.372 g, 0.18 mmol/g, 67 μmol) was washed with anhydrous CH_2Cl_2 (3 × 5 mL), and a solution of **5** (0.112 g, 0.339 mmol) in anhydrous CH_2Cl_2 (3 mL) was added. The reaction mixture was shaken at room temperature for 10 min. A solution of DIEA (69 μL, 52 mg, 0.40 mmol) in CH_2Cl_2 (0.9 mL) was then added to the reaction mixture in three equal portions over a period of 1 h. After being shaken for 1 h, the resin was washed with CH_2Cl_2 (3 × 7 mL), NMP (5 × 7 mL),

and Et_2O (4 × 7 mL) and dried under vacuo. A small sample of resin (10.4 mg) was photolyzed in a solution of 20% H_2O in ACN (1 mL) for 1 h to yield the product **13** (>95% purity) in solution: HPLC (10 → 90) t_R 23.5 min.

Fmoc-azaAla-Asp(Bu)-amide Resin (14). Asp(Bu)-amide resin (**8**) (0.577 g, 0.17 mmol/g, 98.1 μmol) was washed with anhydrous DMF (3 × 5 mL), and a solution of **7** (0.164 g, 0.496 mmol) and DIEA (92 μL, 69 mg, 0.54 mmol) in anhydrous DMF (5 mL) was added. The reaction mixture was shaken at room temperature for 15 h, washed with NMP (5 × 7 mL) and Et_2O (4 × 7 mL), and dried under vacuo. A small sample of resin (9.5 mg) was photolyzed in a solution of 20% H_2O in ACN (1 mL) for 1.5 h to yield the product **14** (89% purity) in solution: HPLC (10 → 90) t_R 20.8 min.

Gua-δAva-azaGly-Asp-NH_2 (15a). Fmoc-azaGly-Asp-amide resin (**9**) (0.336 g, 0.17 mmol/g, 57 μmol) was deprotected, and Fmoc-δAva-OH was coupled under the standard conditions. After removal of the Fmoc protecting group, the resin was washed with DMF (6 × 7 mL), MeOH (3 × 7 mL), Et_2O (2 × 7 mL), and CH_2Cl_2 (2 × 7 mL), and a solution of *N*,*N*′-bis-Boc-1-guanylpyrazole (35.6 mg, 0.115 mmol) in CH_2Cl_2 (1.6 mL) was added. The reaction mixture was shaken at room temperature for 15 h and washed with CH_2Cl_2 (6 × 7 mL), Et_2O (3 × 7 mL), and CH_2Cl_2 (2 × 7 mL). The resin was treated with a solution of 5% H_2O in TFA (5 mL) for 1.5 h, washed with CH_2Cl_2 (3 × 7 mL), and neutralized with a solution of 10% pyridine in CH_2Cl_2 (5 mL). The resin was washed with CH_2Cl_2 (5 × 7 mL), MeOH (3 × 7 mL), and Et_2O (3 × 7 mL) and dried under vacuo. Standard photolysis in a 1:1 solution of H_2O and ACN (7 mL) for 8 h yielded the product **15a** (13.2 mg, 70%) as a yellow solid. Purification by preparative chromatography (reversed-phase C_{18}, 0 → 20% ACN/H_2O) yielded **15a** (9.6 mg, 51%) as a white solid: ^{1}H NMR (320 K, $CDCl_3$, 500 MHz) δ 9.45 (s, 1H), 7.91 (s, 1H), 7.54 (br s, 1H), 7.25−6.85 (m, 6 H), 6.57 (d, J = 8.3 Hz, 1H), 4.35 (m_c, 1H), 2.60 (m_c, 2H), 2.13 (t, J = 7.0 Hz, 2H), 1.61−1.44 (m, 4H); ^{13}C NMR (320 K, $CDCl_3$, 125.0 MHz) δ 172.8, 171.8, 171.7, 157.3, 156.8, 49.7, 40.3, 36.4, 32.4, 27.8, 21.7; HPLC (0 → 30) t_R = 4.4 min; ESI-MS m/z 332.3 (M + H^+).

***cis*-Gua-4-aminocyclclohexylcarbonyl-azaGly-Asp-NH_2 (15d).** Product **15d** (20.8 mg, 100% crude yield) was obtained from **9** (0.310 g, 0.17 mmol/g, 53 μmol) and Fmoc-Cyh-OH as a yellow solid. Purification by preparative chromatography (reversed-phase C_{18}, 0 → 20% ACN/H_2O) yielded **15d** (11.0 mg, 59%) as a white solid: ^{1}H NMR (320 K, DMSO, 500 MHz) δ 9.44 (s, 1H), 7.87 (s, 1H), 7.61 (br s, 1H), 7.22−6.83 (m, 6H), 6.64 (br s, 1H), 4.33 (m_c, 1H), 3.63 (br s, 1H), 2.59 (m_c, 2H), 2.30 (m_c, 1H), 1.81−1.53 (m, 8H); ^{13}C NMR (300 K, DMSO, 128.0 MHz) δ 174.7, 173.1, 172.4, 157.5, 155.9, 49.7, 46.2, 39.0, 36.6, 28.6, 23.8 and 23.6 (a pair of s);[25] HPLC (0 → 30) t_R = 10.8 min; ESI-MS m/z 358.3 (M + H^+).

Gua-δAva-azaSar-Asp-NH_2 (15b). Product **15b** (22.3 mg, 96% crude yield) was obtained from **13** (0.372 g, 0.18 mmol/g, 67 μmol) and Fmoc-δAva-OH as a yellow solid. Purification by preparative chromatography (reversed-phase C_{18}, 0 → 20% ACN/H_2O) yielded **15b** (12.8 mg, 55%) as a white solid: ^{1}H NMR (320 K, DMSO, 500 MHz) δ 8.48 (s, 1H), 7.54 (br s, 1H), 7.29−6.83 (m, 6H), 6.68 (d, J = 7.7 Hz, 1H), 4.39 (q, J = 6.8 Hz, 1H), 3.09 (q, J = 6.1 Hz, 2H), 2.95 (s, 3H), 2.64 (dd, J = 16.1, 5.3 Hz, 1H), 2.56 (dd, 16.0, 7.4 Hz, 1H), 2.29 (br s, 2H), 1.48 (br s, 4H); ^{13}C NMR (300 K, DMSO, 128.0 MHz) δ 175.0, 172.9, 172.0, 156.7, 156.4, 50.1, 40.5, 36.8, 35.3, 30.8, 28.1, 21.3; HPLC (0 → 30) t_R = 14.0 min; ESI-MS m/z 346.3 (M + H^+).

***cis*-Gua-4-aminocyclclohexylcarbonyl-azaSar-Asp-NH_2 (15e).** Product **15e** (16.6 mg, 83% crude yield) was obtained from **13** (0.300 g, 0.18 mmol/g, 54 μmol) and Fmoc-Cyh-OH as a yellow solid; ^{1}H NMR (320 K, DMSO, 500 MHz) δ 8.49 (br s, 1H), 7.40 (d, J = 8.2 Hz, 1H), 7.23−6.82 (m, 4H), 6.70−6.58 (m, 2H), 6.02 (d, J = 8.0 Hz, 1H), 4.40 (m_c, 1H), 3.74−3.62 (m, 1H), 2.96 (s, 3H), 2.79−2.69 (m, 1H), 2.65 (dd, J = 15.9, 5.4 Hz, 1H), 2.57 (dd, J = 16.0, 7.3 Hz, 1H), 1.74−1.37 (m, 8H); ^{13}C NMR (300 K, DMSO, 128.0 MHz) δ 177.9 and 177.5 (a pair of s),[25] 172.8, 171.9, 156.5, 155.9, 50.0, 45.8 and 43.1 (a pair of s),[25] 36.8 (2C), 35.4, 28.6, 23.3 (br s); HPLC (0 → 20) t_R = 16.0 min; ESI-MS m/z 372.4 (M + H^+).

Gua-δAva-azaAla-Asp-NH_2 (15c). Product **15c** (18.9 mg, 100% crude yield) was obtained from **14** (0.306 g, 0.18 mmol/g, 55 μmol) and Fmoc-δAva-OH as a yellow solid: ^{1}H NMR (320 K, DMSO, 500 MHz) δ 9.94 (s, 1H), 8.11 (br s, 1H), 7.30−6.75 (m, 7H), 8.54 (m_c, 1H), 3.11 (m_c, 2 H), 2.93 (s, 3H), 2.59−2.55 (m, 2H), 2.23−2.11 (m, 2H) 1.66−1.43 (m, 4H); ^{13}C NMR (300 K, DMSO, 128.0 MHz) δ 173.6, 173.1, 172.1, 157.1, 156.9, 50.7, 40.3, 36.4, 35.7, 32.7, 27.8, 21.8; HPLC (0 → 20) t_R = 16.0 min; ESI-MS m/z 372.4 (M + H^+).

***cis*-Gua-4-aminocyclclohexylcarbonyl-azaAla-Asp-NH_2 (15f).** Product **15f** (17.1 mg, 98% crude yield) was obtained from **14** (0.261 g, 0.18 mmol/g, 47 μmol) and Fmoc-Cyh-OH as a yellow solid: ^{1}H NMR (320 K, DMSO, 500 MHz) δ 9.91 (s, 1H), 7.48 (br s, 1H), 7.19−6.78 (m, 6H), 6.66 (d, J = 8.0 Hz, 1H), 4.34 (m_c, 1H), 3.65 (br s, 1H), 2.92 (s, 3H), 2.62 (d, J = 5.9 Hz, 2H), 2.31 (m_c, 1H), 1.78−1.55 (m, 8H); ^{13}C NMR (300 K, DMSO, 128.0 MHz) δ 174.5, 173.2, 172.5, 157.2, 155.9, 50.4, 46.1, 38.9, 36.1, 35.6, 28.5, 23.6 and 23.3 (a pair of s);[25] HPLC (0 → 20) t_R = 19.2 min; ESI-MS m/z 372.2 (M + H^+).

Receptor Binding Assays. Vitronectin and fibrinogen from human plasma were prepared as previously described;[26] αIIbβ3 was purified from outdated thrombocytes;[27] αvβ3 was obtained from term human placenta.[28] The integrins were > 95% pure as judged by SDS-PAGE and by ELISA. Receptor inhibition assays were performed as previously described.[29]

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