Novel acylation catalysts in peptide synthesis: derivatives of *N*-hydroxytriazoles and *N*-hydroxytetrazoles

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Six new derivatives of 1-hydroxy-1,2,3-triazole (1-hydroxytriazole in the following) and *N*-hydroxytetrazole have been evaluated in a direct competition assay to investigate their efficiency as catalysts in the formation of peptide bonds. Furthermore, three well known catalysts, 1-hydroxy-7-azabenzotriazole (HOAt), 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (Dhbt-OH) and 1-hydroxybenzotriazole (HOBt) have been compared. All nine compounds have been used for activation in combination with *N*,*N*′-diisopropylcarbodiimide (DIPCDI) in solid-phase synthesis using the fluoren-9-ylmethoxycarbonyl (Fmoc) strategy. The capability of the catalysts to suppress racemization has also been analysed. The results demonstrate that three of the new compounds are competitive with HOAt and HOBt in the suppression of racemization. 5-Chloro-1-hydroxytriazole is found to be a highly efficient acylation catalyst; however, it does not show sufficient suppression of racemization. Its catalytic effect in the synthesis of Aib–Aib-containing peptides is superior to that of HOAt. Also, 2-hydroxytetrazole has a catalytic efficiency superior to that of HOAt and suppressed racemization as efficiently as HOBt. The hydroxytetrazoles are explosive in a hammer test whereas the triazoles are stable compounds.

Introduction

The addition of coupling additives to *in situ* coupling reagents such as carbodiimides[1,2] can favorably influence reactivity and decomposition of intermediates in the formation of amide bonds during the stepwise assembly of peptides on a solid phase.[3] Thus the rate of acylation can be enhanced between sterically hindered amino acids and the racemization can be suppressed. HOBt is a commonly used catalyst, which in carbodiimide-mediated coupling prevents racemization and other side-reactions such as dehydration of the side chains of Asn and Gln.[4] Recently, a new racemization-suppressing catalyst for solid-phase peptide synthesis, 1-hydroxy-7-azabenzotriazole (HOAt), has been described.[5] The very favorable properties of 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (Dhbt-OH) as a catalyst have also been reported.[6,7] Dhbt-OH furthermore indicates progress of the reaction visually by a color change when the acylation is complete. Unfortunately a ring-opening side-reaction leading to 2-azidobenzoic acid Dhbt ester is associated with the use of Dhbt-OH as an additive to DCC reactions. However, fully protected amino acid esters of Dhbt-OH are stable and crystalline and have increased activity compared with other active esters.[6]

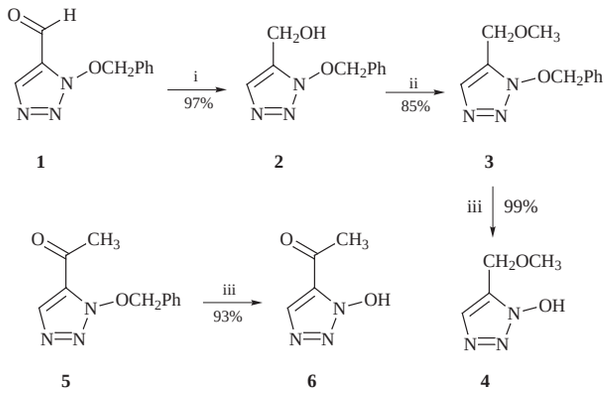
Coupling efficiency and rates have been examined for several coupling procedures.[8–10] The coupling-efficiency studies have been performed in either solution or on a solid phase using a one-to-one mixture of the two components containing free amino and carboxy groups.[8–10]

The present work compares nine different compounds, including modified derivatives of 1-hydroxytriazoles and *N*-hydroxytetrazoles and commercially available peptide-coupling additives. Their efficiency as catalysts in the formation of peptide bonds and the suppression of racemization was determined. The novel catalysts were hydroxy-triazole and -tetrazole derivatives: 1-hydroxy-5-(methoxymethyl)-1,2,3-triazole **4**, 5-acetyl-1-hydroxy-1,2,3-triazole **6**, 5-chloro-1-hydroxy-1,2,3-triazole **7** (either free or as the hydrochloride), 1-hydroxy-1,2,3-triazole **8**, 2-hydroxytetrazole **9** and 1-hydroxytetrazole **10**. The preparation of compounds **7**–**10** has been described elsewhere.[11–14] The core of these structures is a five-membered ring containing at least three vicinal nitrogens in the ring and the first nitrogen is hydroxylated. Additionally the neighboring carbon is substituted to provide a lone pair in the vicinity of the hydroxy group, thereby mimicking the structure and catalytic mechanism of HOAt and Dhbt-OH. These compounds have not previously been employed as acylation catalysts in peptide synthesis. HOBt[4] **11**, HOAt[5] **12** and Dhbt-OH[6,7] **13** are commercially available. The investigation of catalysis of peptide-bond formation was performed using poly(ethylene glycol)-cross-linked polyamide (PEGA) as a resin.[15] PEGA swells 10–20-fold in both organic solvents and aqueous buffer solution and has been successfully employed in synthesis and biochemical assaying of peptide libraries.[16,17]

The coupling additives were evaluated under conditions similar to those used in solid-phase Fmoc-based peptide synthesis.[18,19] Thus all reactions were performed with a three-molar excess of reagents. In the assay Fmoc-Ile-OH was coupled to Val-Gly-PEGA resin by adding the catalyst in the presence of diisopropylcarbodiimide (DIPCDI).[2] The coupling times were kept relatively short (exactly 5 min, followed by quenching of the reaction) in order to determine the reaction rates with essentially constant concentration of amino groups on the resin. The relative ratio of Ile to Val was determined with good accuracy by amino acid analysis. In studies of the racemization, the tripeptide benzyloxycarbonyl-Phe-Val-Pro-NH_2 (Z-Phe-Val-Pro-NH_2) was selected, since the two diasteroisomers Z-Phe-Val-Pro-NH_2 and Z-Phe-val-Pro-NH_2 (val indicates D-Val according to the recommendation by UIPAC) can easily be separated by analytical reversed-phased HPLC.[8]

Results and discussion

Nine compounds **4**, **6**–**13** have been evaluated for their ability to catalyze formation of peptide bonds by analysis of coupling efficiency and suppression of racemization in model reactions



Scheme 1 *Reagents and conditions:* i, $NaBH_4$; ii, CH_3I, NaH; iii, H_2, Pd/C

on a solid phase. The compounds analyzed are *N*-hydroxytriazole and *N*-hydroxytetrazole derivatives designed to mimic the catalytic mechanism of HOAt and Dhbt-OH.

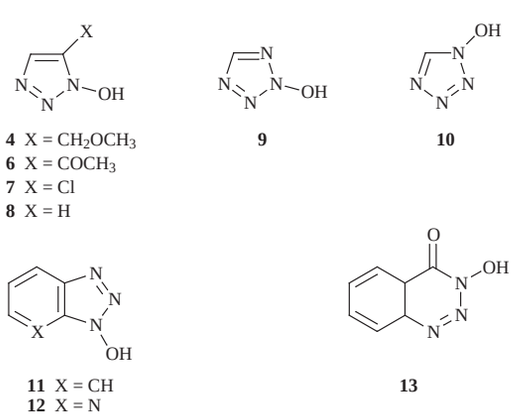
1-Hydroxy-5-(methoxymethyl)-1,2,3-triazole **4** and 5-acetyl-1-hydroxy-1,2,3-triazole **6** (see Scheme 1) were first synthesized. Thus 1-benzyloxy-5-formyl-1,2,3-triazole[14] **1** was reduced with $NaBH_4$ to afford 1-benzyloxy-5-(hydroxymethyl)-1,2,3-triazole **2** in 97% yield. The intermediate 1-benzyloxy-5-(methoxymethyl)-1,2,3-triazole **3** was formed in 85% yield by treatment of compound **2** with MeI in the presence of NaH, and compound **3** was further converted into 1-hydroxy-5-(methoxymethyl)-1,2,3-triazole **4** in 99% yield by hydrogenation using palladium/charcoal (10%). Similarly, 5-acetyl-1-hydroxy-1,2,3-triazole **6** was obtained in 93% yield by hydrogenolytic debenzylation of 5-acetyl-1-benzyloxy-1,2,3-triazole[13] **5**. The first series of experiments was designed to evaluate the relative efficiency in forming peptide bonds on a solid phase (Fig. 1). The couplings were mediated by the *in situ* reagent DIPCDI[2] and formation of the peptide bond was performed on the PEGA-resin.[15] In this assay Fmoc-Ile-OH was coupled to Val-Gly-PEGA resin. The resin was equally divided into 20 columns of a library generator.[20] Amino acid, catalyst and DIPCDI were dissolved in DMF and used in three-fold excess in order to perform the experiment under the usual solid-phase peptide synthesis conditions. First, the amino acid and catalyst were added to the resin. After 2 min pre-incubation, DIPCDI was added. A relatively short coupling time of 5 min was selected in order to obtain a low degree of conversion, while still allowing reagents to diffuse into the resin. The result ranked the compounds as potential acylation catalysts and peptide-coupling additives. The ratio of Ile to Val, which is a value for the relative efficiency of formation of the peptide bond was calculated from the relative Ile and Val content found by amino acid analysis. The results are presented in Table 1. The experiment showed that triazole derivative **7** was the best catalyst and considerably more efficient than HOAt. The 2-hydroxytetrazole derivative **9** was also slightly more efficient as compared with HOAt in forming the peptide bond. Compound **4** had a catalytic activity between those of HOBt and HOAt. The remaining compounds **6**, **8**, **10** and **13** were poor catalysts with relative coupling efficiencies comparable to that of *in situ* activation with no addition of catalyst.

In the second series of experiments, the ability to suppress racemization was studied for the nine compounds mentioned above. Previously, a peptide model reaction in which the mixture of Z-Phe-Val-Pro-NH-Resin and Z-Phe-val-Pro-NH-Resin is formed by coupling Z-Phe-Val to a Pro residue on the solid phase has been employed in racemization studies.[13] The two LLL and LDL diastereoisomers are easily separated by analytical HPLC. This model was selected to study the ability of the new catalysts to suppress racemization. The acid-labile *p*-[(α-Fmoc-amino)-2,4-dimethoxybenzyl]phenoxyacetic acid (Rink amide linker)[21] was employed. The two diastereoisomers

Table 1 Evaluation of analogs of *N*-hydroxytriazoles and *N*-hydroxytetrazoles, Dhbt-OH, HOBt and HOAt as catalysts in peptide synthesis using a comparison study

Coupling catalyst	Yield (% Ile)[a]	LDL (%)	Aib–Aib bond formation (%)
4	6.0	34.3[b]	
6	3.7	20.3[b]	
7	9.0	35.6[b], 47.2[c], 28.7[d]	43
8	4.1	18.3[b], 24.9[c]	
9	7.0	14.1[b], 18.1[d]	
10	3.7	4.5[b]	
11	5.4	10.9[b]	6
12	6.6	0.95[b]	38
13	4.0	26.0[b]	
no catalyst	4.1	50.0[b]	2

[a] The amino acid Fmoc-Ile-OH and catalyst were added to Val-Gly-PEGA resin. After 2 min, DIPCDI was added and the reaction was allowed to proceed for 5 min. The yield of Ile was calculated based on the ratio of Ile to Val found after hydrolysis of the peptide-resin. The couplings with the 3 best catalysts (**7**, **9** and **12**) were repeated and standard deviations were < ±0.5. The low conversions secure that concentrations of amine component are essentially constant for all catalysts investigated. [b] The dipeptide Z-Phe-Val-OH and catalyst were added to Pro-Rink linker-PEGA resin. After 1 min, DIPCDI was added. The reaction was carried out overnight and the peptide was cleaved with 95% aq. TFA. The degree of racemization was calculated based on the areas between the two diastereoisomers obtained from HPLC. The experiments with compounds **7**, **9**, **11** and **12** with a deviation < ±2. [c] Catalyst and DIPCDI were dissolved in DMF at 0 °C and the solution was left for 5 min. Then, Z-Phe-Val-OH was added to the above solution at 0 °C. After 2 min, the mixture was added to the Pro-Rink linker-PEGA resin. The coupling reaction was carried out overnight followed by cleavage of the peptide with 95% aq. TFA. The optical purity was analyzed as described above.[b] [d] Same procedure as described above[c] except that three mole equivalents of catalyst were used.



4 X = CH_2OCH_3
6 X = $COCH_3$
7 X = Cl
8 X = H

9 **10**

11 X = CH
12 X = N

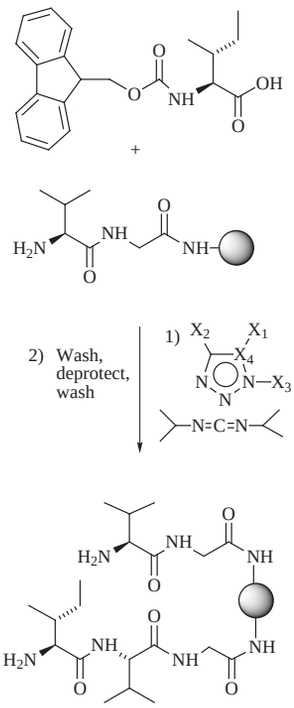
13

Fmoc-Aib-Lys(Boc)-Ser(*t*Bu)-Ser(*t*Bu)-Tyr(*t*Bu)-Lys(Boc)-Rink linker-PEGA

14

Structures of the derivatives of triazole (**4**, **6**–**8**) and tetrazole (**9**, **10**), HOBt **11**, HOAt **12**, Dhbt-OH **13** and **14**

LLL and LDL were first obtained in a 6:4 ratio by reaction of dipeptide with Pro-linker-PEGA resin and 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) in the presence of *N*-ethylmorpholine (NEM) and pre-activation for 8 min.[22] Cleavage of the peptide from the resin was carried out by treatment with 95% aq. (TFA) and HPLC separation of the two diasteroisomers is presented in Fig. 2. Throughout this assay, the coupling procedure was the same as that described above for the coupling efficiency assay except that four-fold excess of reagents was used, preactivation was 1 min and the acylation reaction mixture was left overnight. Subsequently, the peptides were cleaved from the linker by 95% aq. TFA and analyzed by HPLC (Fig. 1). This model reaction proved to be extremely sensitive in testing carbodiimide-mediated couplings since the racemization ranged between 0.95% and 50.0% for the



X_1	X_2	X_3	X_4
Cl	H	OH	C
CH_3OCH_2	H	OH	C
Ac	H	OH	C
OH	H	H	N
H	H	OH	N
H	H	OH	C

Dhbt-OH, HOBt or HOAt

Fig. 1 Evaluation of the relative efficiency in forming peptide bonds on a solid phase. Racemization in the solid-phase reaction was investigated in a similar system where Z-Phe-Val-OH was coupled to solid-phase-linked Pro using different catalysts (see Fig. 2).

evaluated compounds (Table 1). The excellent racemization-suppressing property of HOAt afforded the product with only 0.95% racemization, whereas for HOBt, and the two tetrazoles **9** and **10**, the amount of the LDL diastereoisomer was 10.9, 14.1 and 4.5%, respectively. The modified triazole **7**, which in the efficiency assay was found to be the most effective catalyst, gave the highest degree of racemization (35.6%) of the evaluated compounds. However, this is still a large improvement as compared with the reaction without catalyst (50%) and the catalyst could still be valuable in peptide synthesis. It may be speculated that a different mechanism of catalysis is in operation with this catalyst. For the coupling with DIPCDI and no addition of catalyst, a ratio of one to one of the two diastereoisomers was found. Racemization studies with the most efficient additives **7** and **9**–**12** were repeated without significant deviation of results.

In an effort to suppress the racemization and avoid the usual formation of *O*-acylisourea, a reversed-addition protocol was performed.[23] DIPCDI and a small excess of catalyst were mixed for 5 min at 0 °C to generate the intermediate adduct. The protected dipeptide was added to the formed intermediate RO-(R′O)C-$(NHR)_2$ which probably reacted to give the protected active dipeptide ester of the catalyst and *N*,*N*′-diisopropylurea. This coupling procedure was employed with the modified triazole **7** as well as for *N*-hydroxytriazole (Table 1), but unexpectedly it resulted in a small increase in the racemization. However, using the protocol with a 3 molar excess of catalyst decreased the amount of the LDL form from 47.2 to 28.7% for the modified triazole **7** (Table 1).

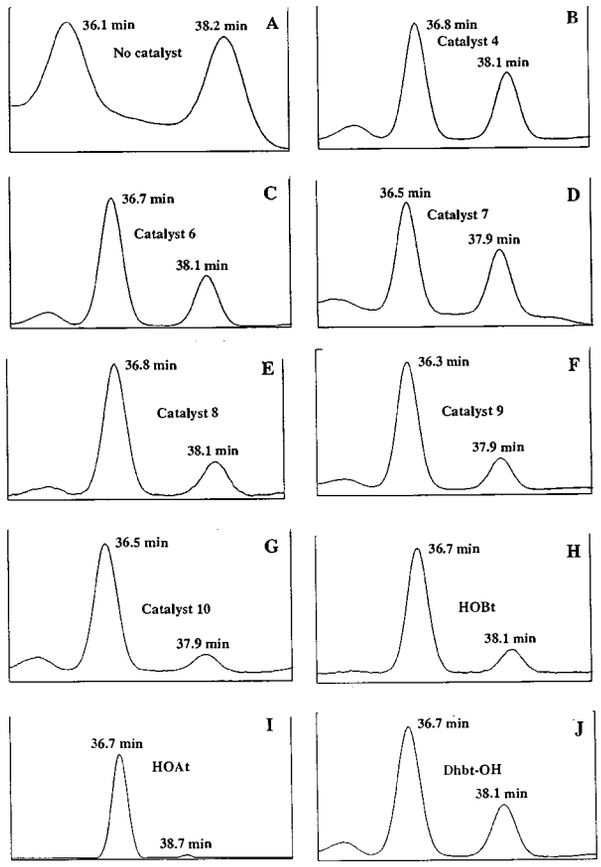


Fig. 2 Separation of the two diastereoisomers LLL (R_f 36 min) and LDL (R_f 38 min) by analytical HPLC and obtained from DIPCDI-mediated coupling of Z-Phe-Val-OH with Pro linked to PEGA-resin. DIPCDI in combination with the evaluated compounds derivatives of triazole, tetrazole and commercially available peptide coupling additives: A, no additive; B, **4**; C, **6**; D, **7**; E, **8**; F, **9**; G, **10**; H, **11** (HOBt); I, **12** (HOAt) and J, **13** (Dhbt-OH)

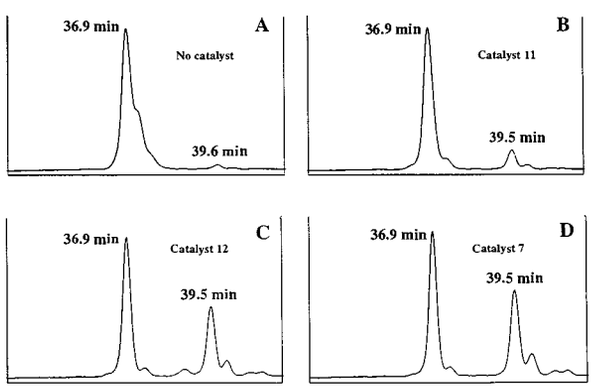


Fig. 3 Determination of the efficiency of catalyst **11** (B), **12** (C) and **7** (D) in Aib–Aib bond formation on a solid phase. In trace A no catalyst was added. After deprotection of the peptides and cleavage from the resin HPLC separation showed the relative formation of Aib-Aib-Lys-Ser-Ser-Tyr-Lys-NH_2 (R_f 39.5) from Aib-Lys-Ser-Ser-Tyr-Lys-NH_2 (R_f 36.9) (Table 1).

The efficiency of compounds **7**, **11** and **12** as catalysts in the synthesis of peptides containing sterically hindered bonds, such as Aib–Aib for which racemization is not problematic, was analyzed. The Fmoc-protected peptide Fmoc-Aib-Lys(Boc)-Ser(*t*Bu)-Ser(*t*Bu)-Tyr(*t*Bu)-Lys(Boc)- was synthesized on a Rink amide linker on a PEGA resin (compound **14**). The peptide resin was first deprotected with piperidine and then it was mixed with 3 equiv. of catalyst and amino acid and, after equilibrium had been established, DIPCDI was added. The reactions were terminated after 30 min by filtration and washing, and after cleavage with 95% aq. TFA the amount of

coupling was monitored by HPLC. The results are presented in Fig. 3. The reaction without addition of catalyst was a control reaction, affording only 3% formation of an Aib–Aib bond. Addition of HOBt or HOAt gave 6 and 38% conversion to product respectively, whereas compound **7** afforded 43% conversion to the peptide containing the Aib–Aib bond. Compound **7** is therefore superior to HOAt in the catalysis of αα-dialkylglycine-coupling reactions.

The synthesis of fluorine-substituted analogs of compound **7** was attempted by a range of different strategies with both electrophilic and nucleophilic substitution reactions; however, none were successful.

In conclusion, efficiency of the formation of a peptide bond and measurement of the racemization during peptide coupling have been determined using 6 new hydroxy-triazole and -tetrazole derivatives and 3 commonly used compounds as coupling catalysts in DIPCDI mediated couplings on a solid phase. The compounds were modified 1-hydroxytriazoles and *N*-hydroxytetrazoles.[11–14] The synthesis of compounds **4** and **6** was achieved and afforded between 85% and 99% yield in the four reaction steps reported. The results showed that three of the new triazole and tetrazole derivatives could compete with HOAt and HOBt. One of the catalysts was even found to be superior to HOAt with respect to coupling efficiency. These compounds contain only one five-membered ring and thus are less sterically crowded. In particular, compound **7** could have an advantage over HOAt and HOBt in coupling reactions, especially between sterically hindered amino acids such as Aib residues. Furthermore, 5-chloro-1-hydroxy-1,2,3-triazole **7** was a superior catalyst, giving faster coupling reactions than HOAt but with less suppression of racemization. The results showed that 2-hydroxytetrazole was as effective as HOAt in forming peptide bonds. 2-Hydroxytetrazole afforded optical purity comparable to that obtained with HOBt. 1-Hydroxytetrazole had no significant catalytic activity but suppressed racemization quite efficiently. **CAUTION:** The *N*-hydroxytetrazoles were both explosive in a hammer test.

Experimental

General

Analytical TLC was performed on Merck silica gel $60F_{254}$ alumina sheets with detection of the protected triazoles by charring with mostain $[Ce(SO_4)_2{\cdot}H_2O$ (0.4 g) and $(NH_4)_6Mo_7O_{24}$ (20 g) dissolved in 10% H_2SO_4 (400 ml)]. All organic solvents were purchased from Labscan Ltd. (Dublin, Ireland). Concentrations were performed under reduced pressure at temperatures <40 °C. Suitable protected N^{α}-Fmoc-amino acids, Z-Phe-Val and the Rink-amide-linker were purchased from Nova Biochem (Switzerland); TBTU, $NaBH_4$, DIPCDI, HOBt and Dhbt-OH from Fluka (Switzerland); NEM from Merck (Germany); NaCl and $MgSO_4$ from Aldrich; and HOAt from Millipore. The optical purity of Z-Phe-Val-Pro-NH_2 was analyzed by analytical HPLC. The catalysts 1-hydroxy-1,2,3-triazole **8**[12,14] 5-chloro-1-hydroxy-1,2,3-triazole **7**[11,14] 1-hydroxytetrazole **10**,[12] and 2-hydroxytetrazole **9**[12] were prepared as previously described. The relative molecular masses of the peptide compounds were determined, using matrix-assisted laser desorption time-of-flight mass spectroscopy (MALDITOF-MS), recorded on a Lasermat 2000 (Finnigan Mat) using a matrix of α-cyano-4-hydroxycinnamic acid. Quantitative amino acid analyses were performed on a Pharmacia LKB Alpha Plus amino acid analyser following hydrolysis with 6 M HCl at 110 °C for 36 h. Analytical HPLC was performed using a Waters RCM 8 × 10 module and with a Deltapak C-18 column (19 × 300 mm). The solvent system for the analytical HPLC was buffer A; 0.1% TFA in water and buffer B; 0.1% TFA in 90% acetonitrile–10% water and UV detection was at 215 and 280 nm. The gradient for analytical HPLC (1 cm^3 min^{-1}); a linear gradient of 0–100% buffer B over a period of 50 min. ^1H and ^{13}C NMR spectra were recorded on a 200 MHz or 300 MHz Bruker instrument. *J*-Values are given in Hz. Hammer tests were performed on compounds **7**–**10** by placing a 2–4 mg sample of a compound on a block of iron and dropping a hammer-head on the compound from height of 50 cm. Compounds **7** and **8** were not explosive *whereas compounds* **9** *and* **10** *both exploded.*

1-Hydroxy-5-(methoxymethyl)-1,2,3-triazole 4

A solution of 1-benzyloxy-5-formyl-1,2,3-triazole **1**[14] (163 mg, 0.80 mmol) and MeOH (10 cm^3) was cooled to 0 °C and was then treated with $NaBH_4$ (41 mg, 1.08 mmol). After 40 min, the solvent was removed *in vacuo*. The obtained solid was dissolved in CH_2Cl_2 (15 cm^3) and washed with saturated aq. NaCl (3 × 10 cm^3). The organic phase was dried ($MgSO_4$) and the solvent was removed *in vacuo* to obtain 160 mg (97%) of 1-*benzyloxy*-5-(*hydroxymethyl*)-1,2,3-*triazole* **2**, R_f (EtOAc–heptane, 1:1) 0.05; mp 71–72 °C; δ_H ($CDCl_3$) 7.49 (1 H, s), 7.42–7.31 (5 H, m), 5.47 (2 H, s), 4.33 (2 H, d, *J* 4.9) and 3.06 (1 H, br t, *J* ~5); δ_C ($CDCl_3$) 132.5 (s), 131.6 (s), 131.2 (d), 130.1 (d), 130.0 (d), 128.9 (d), 82.6 (t) and 52,1 (t) (Calc. for $C_{10}H_{11}N_3O_2$: C, 58.53; H, 5.40; N, 20.48%. Found: C, 58.60; H, 5.59; N, 20.24).

1-Benzyloxy-5-(hydroxymethyl)-1,2,3-triazole **2** (160 mg, 0.78 mmol) was dissolved in dry THF (5 cm^3) and MeI (166 mg, 1.17 mmol) was added. The reaction mixture was cooled to −78 °C and then NaH (24 mg, 1.0 mmol) was added. The cooling bath was removed and the solution was stirred at room temperature for 1 h. Water (2 cm^3) was added and the solution was extracted with CH_2Cl_2 (3 × 10 cm^3). The organic phase was dried ($MgSO_4$) and the solvent was removed *in vacuo*. Purification by column chromatography (EtOAc–heptane, 1:1) gave 145 mg (85%) of 1-benzyloxy-5-(methoxymethyl)-1,2,3-triazole **3**, R_f (EtOAc–heptane, 1:1) 0.43; δ_H ($CDCl_3$) 7.58 (1 H, s), 7.46–7.22 (5 H, m), 5.45 (2 H, s), 4.17 (2 H, s) and 3.25 (3 H, s); δ_C ($CDCl_3$) 132.2 (s), 131.7 (s), 129.7 (d), 129.5 (d), 128.5 (d), 128.3 (d), 82.4 (t), 60.8 (q) and 57.8 (t).

1-Benzyloxy-5-(methoxymethyl)-1,2,3-triazole **3** (315 mg, 1.43 mmol) was dissolved in EtOH (20 cm^3), the solution was cooled to 0 °C, 10% palladium on charcoal (50 mg) was added, and the reaction mixture was stirred under hydrogen for 30 min. After filtration through Celite and evaporation *in vacuo*, 182 mg (99%) of 1-hydroxy-5-(methoxymethyl)-1,2,3-triazole **4** was obtained, mp 108–110 °C; δ_H (D_2O) 8.04 (1 H, s), 4.53 (2 H, s) and 3.36 (3 H, s); δ_C (D_2O) 128.7 (s), 127.4 (d), 61.4 (q) and 58.6 (t) (Calc. for $C_4H_7N_3O_2$: C, 37.21; H, 5.46; N, 32.54%. Found: C, 37.52; H, 5.25; N, 32.30).

5-Acetyl-1-hydroxy-1,2,3-triazole 6

5-Acetyl-1-benzyloxy-1,2,3-triazole **5** (138 mg, 0.64 mmol)[13] was dissolved in MeOH (10 cm^3) and the solution was cooled to 0 °C. Then, palladium on charcoal (30 mg) was added and the reaction mixture was stirred under hydrogen for 30 min. After filtration through Celite and evaporation *in vacuo* 76 mg (93%) of 5-*acetyl*-1-*hydroxy*-1,2,3-*triazole* **6** was obtained, mp 143–145 °C (decomp.); δ_H (CD_3OD) 8.40 (1 H, s) and 2.65 (3 H, s); δ_C (CD_3OD) 186.2 (s), 132.3 (d), 128.7 (s) and 27.22 (q) (Calc. for $C_4H_5N_3O_2$: C, 37.80; H, 3.97; N, 33.06%. Found: C, 37.91; H, 3.81; N, 32.71).

Solid-phase synthesis and analysis, general procedure

The synthesis of the peptides using the PEGA resin[15] were performed by the plastic syringe technique[24] or a 20-column library generator.[25] The protected N^{α}-Fmoc-L-amino acid pentafluorophenyl (OPfp) esters (3 equiv.)[22] were coupled in DMF with the addition of Dhbt-OH (1 equiv.) as an acylation catalyst and an indicator of the end-point of the acylation reaction. The dipeptide Z-Phe-Val was coupled as its free acid using TBTU *in situ* activation[22] to prepare the two diastereoisomers Z-Phe-Val-Pro-NH_2 and Z-Phe-val-Pro-NH_2.[7] The N^{α}-Fmoc group was removed by 20% piperidine in DMF. The resin was

then washed with DMF (×5) and CH_2Cl_2 (×5) and dried. The peptides which were used for racemization studies were cleaved simultaneous from the Rink-linker[20] by treatment with 95% aq. TFA for 2 h. The resin was then rinsed with 95% aq. acetic acid (× 4). Both TFA and acetic acid were removed under reduced pressure and, after precipitation with diethyl ether, the crude product was analyzed by analytical HPLC. However, determination and comparison of the ratio Ile : Val was performed by quantitative amino acid analysis.

Anchoring of the Rink linker

Rink-amide-linker (539.6 mg, 1 mmol) was dissolved in DMF. The solution was cooled to 0 °C and TBTU (308.3 mg, 0.96 mmol) and NEM (0.239 cm^3, 1.6 mmol) were added. After 8 min, the solution was added to the PEGA resin (1 g, 0.4 mmol) which had been swelling in DMF (1 h). The flask was gently shaken for 2 h and the resin was then filtered off, and rinsed with DMF (× 6) and CH_2Cl_2 (× 5). The remaining amino groups were capped by addition of 10% acetic anhydride in DMF (20 min).

Procedure used for peptide formation comparison studies (see Table 1 [a])

Synthesis of the Fmoc-Val-Gly-PEGA-resin was carried out as described above in a plastic syringe using the PEGA-resin (0.3–0.4 mmol g^{-1}; 1 g). The resin was then dried under high vacuum. The dried Fmoc-peptide resin (200 mg) was treated with 20% piperidine in DMF for 20 min, washed with DMF and then transferred to a 20-column library generator (10 mg of Fmoc-peptide resin in each column), such that the resin was equally divided into the 20 columns. Then, DMF (0.1 cm^3), Fmoc-Leu-OH (4.7 mg, 13.5 μmol) in DMF (50 mm^3) and coupling additive (3 equiv.) in DMF (0.1 cm^3) were added in each column. After 2 min, a solution of DIPCDI (2.2 mm^3, 13.5 μmol) in DMF (50 mm^3) was added and the mixture was allowed to react for exactly 5 min. The evaluated catalysts were 1-hydroxy-5-(methoxymethyl)-1,2,3-triazole **4** (1.76 mg, 13.5 μmol), 5-acetyl-1-hydroxy-1,2,3-triazole **6** (1.70 mg, 13.5 μmol), 5-chloro-1-hydroxy-1,2,3-triazole hydrochloride **7**·HCl (2.10 mg, 13.5 μmol), 1-hydroxy-1,2,3-triazole **8** (1.15 mg, 13.5 μmol), 2-hydroxytetrazole **9** (1.91 mg, 13.5 μmol), 1-hydroxytetrazole **10** (1.91 mg, 13.5 μmol), HOBt **11** (1.84 mg, 13.5 μmol), HOAt **12** (1.84 mg, 13.5 μmol) and Dhbt-OH **13** (2.20 mg, 13.5 μmol). For 5-chloro-1-hydroxy-1,2,3-triazole hydrochloride **7**·HCl diisopropylethylamine (DIEA) (0.0023 cm^3, 13.5 μmol) was added for neutralization. One experiment was performed without catalyst and the protocol was the same as described above except that catalyst was not added. Then, resin was drained, washed and the Fmoc-group was removed by treatment with 20% piperidine in DMF followed by washing with DMF. The peptides were hydrolyzed by 6 M HCl at 110 °C for 36 h and the Val : Ile ratio was determined by amino acid analysis.

The racemization assay A (see Table 1 [b])

The two stereoisomers for racemization studies,[8] Z-Phe-Val-Pro-NH_2 and Z-Phe-val-Pro-NH_2, were synthesized as follows; Z-Phe-Val-OH (87.6 mg, 0.22 mmol) was dissolved in DMF, then NEM (0.044 cm^3, 0.35 mmol) and TBTU (67.1 mg, 0.20 mmol) were added. The mixture was stirred at 0 °C for 8 min and was then added to the Pro-Rink-linker PEGA resin (180 mg, 0.054 mmol). The mixture was allowed to react for 2 h. The resin was then washed thoroughly with DMF. The resin was dried and the two stereoisomers were cleaved from the resin by 95% aq. TFA. They were analyzed by analytical HPLC using a linear gradient of 0–100% buffer B over a period of 50 min [t_R 36.7 min (LLL) and t_R 38.1 min (LDL)] as well as by MALDITOF-MS; *m/z* 517.5 $(M + Na)^+$ (LLL) and 517.3 $(M + Na)^+$ (LDL), respectively ($C_{27}H_{34}N_4NaO_5$ requires *m/z*, 517.2). The dried Fmoc-Pro-Rink linker-PEGA resin (50 mg × 9, 0.135 mmol/g) was deprotected with 20% piperidine in DMF and, after washing with DMF, transferred to a 20 column Teflon multiple synthesizer. The dipeptide Z-Phe-Val-OH (23.9 mg, 0.06 mmol, 4 equiv.) was dissolved in DMF (0.1 cm^3) and catalyst (4 equiv.) in DMF (0.5 cm^3) were added to the resin. After 1 min, DIPCDI (8 mm^3, 0.06 mmol, 4 equiv.) was simultaneously added to all columns and the coupling reaction was left overnight. All the nine compounds **4**, **6**–**13** were examined. For the hydrochloride of 5-chloro-1-hydroxy-1,2,3-triazole **7**, DIEA (10 mm^3, 0.06 mmol) was added. In one experiment, the acylation was performed as described above but without catalyst. The peptide was cleaved from the resin by 95% aq. TFA and the degree of racemization was analyzed by HPLC using the conditions as described above. For those compounds showing catalytic activity the experiment was repeated.

The racemization assay B (see Table 1 [c])

In these experiments, DIPCDI (0.008 cm^3, 0.06 mmol, 4 equiv.) and catalyst (4.1 equiv.) were dissolved in DMF (0.5 cm^3) at 0 °C. For hydrochloride of 5-chloro-1-hydroxy-1,2,3-triazole **7**, DIEA (0.011 cm^3, 0.066 mmol) was added. The solution was left for 5 min. Then, Z-Phe-Val-OH (23.9 mg, 0.06 mmol, 4 equiv.) as a solution in DMF (0.2 cm^3) was added at 0 °C. After 2 min, the mixture was transferred to the Pro-linker(Rink)-resin (50 mg, 0.015 mmol). The coupling reaction was carried out overnight. The peptide was cleaved and analyzed as described above (see Table 1 [b]).

The racemization assay B (see Table 1 [d])

In these experiments, the racemization assay B protocol was used, but with an increase to 12 equiv. of catalyst as compared with resin-bound amino groups.

Preparation of Fmoc-Aib-Lys(Boc)-Ser(*t*Bu)-Ser(*t*Bu)-Tyr(*t*Bu)-Lys(Boc)-Rink linker-PEGA resin 14

The precursor Fmoc-Lys(Boc)-Ser(*t*Bu)-Ser(*t*Bu)-Tyr(*t*Bu)-Lys(Boc)-Rink linker-PEGA resin (0.12 mmol g^{-1}) was synthesized as described above using the PEGA resin. After Fmoc deprotection with 20% piperidine in DMF, Fmoc-Aib-OH (54 mg, 0.166 mmol) was coupled to the protected peptide PEGA resin (460 mg, 0.055 mmol) using TBTU (51.1 mg, 0.16 mmol) and NEM (0.0033 cm^3, 0.266 mmol). The coupling reaction was carried out for 24 h. A small amount of the peptide resin was then Fmoc-deprotected. The side-chain deprotection and cleavage from the resin was mediated by 95% aq. TFA. The crude product was analyzed by analytical HPLC (t_R 37.8 min) using a isotropic gradient of 100% buffer A during 20 min followed by a linear gradient of 0–100% buffer B during 70 min. Analysis by MALDITOF-MS [*m/z* 696.3 $(M + H)^+$ and 718.2 $(M + Na)^+$, ($C_{31}H_{53}N_9O_9$ requires *M*, 695.8)] confirmed the expected product.

Coupling of Fmoc-Aib-OH to Aib-Lys(Boc)-Ser(*t*Bu)-Ser(*t*Bu)-Tyr(*t*Bu)-Lys(Boc)-Rink linker-PEGA resin using catalyst-mediated couplings

The dried protected-peptide resin **14** (400 mg, 0.12 mmol g^{-1}) was Fmoc-deprotected with 20% piperidine in DMF for 20 min, washed with DMF and then divided into eight plastic syringes. The protected amino acid Fmoc-Aib-OH in DMF (5.86 mg, 18 μmol in 0.050 cm^3) and either HOBt (2.43 mg, 18 μmol), HOAt (2.44 mg, 18 μmol) or 5-chloro-1-hydroxy-1,2,3-triazole hydrochloride **7**·HCl (2.8 mg, 18 μmol) in DMF (0.05 cm^3) were added to the syringe (for 2 sets of reactions). After 2 min, a solution of DIPCDI in DMF (0.0028 cm^3, 18 μmol in 0.1 cm^3) was added and the coupling reactions were performed in 25 min and 4 h respectively. In one experiment, the reaction was carried out in the absence of catalyst. For 5-chloro-1-hydroxy-1,2,3-triazole hydrochloride **7**·HCl, one mole equiv. of DIEA (0.0031 cm^3, 18 μmol) was added for neutralization. The

Fmoc group was removed and the peptide was side-chain-deprotected and cleaved with 95% aq. TFA. The progress of the reaction was monitored by analytical HPLC (t_R 39.5 min). The yield of the desired peptide Aib-Aib-Lys-Ser-Ser-Tyr-Lys-NH_2 was calculated based on the areas of the two obtained HPLC peaks (see Fig. 3). The product was characterized by MALDITOF-MS; *m*/*z* 782.5 $(M + H)^+$, 806.5 $(M + Na)^+$ and 822.4 $(M + K)^+$ ($C_{35}H_{60}N_{10}O_{10}$ requires *M*, 780.9).

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