Stereochemistry as a Diversity Element: Solid-Phase Synthesis of Cyclic RGD Peptide Derivatives by Asymmetric Catalysis[**]

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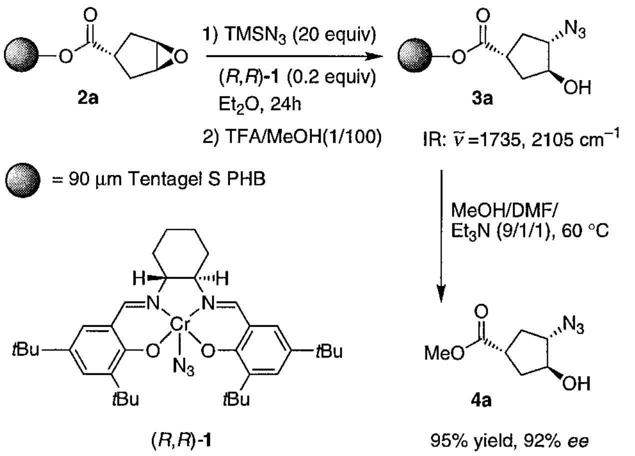
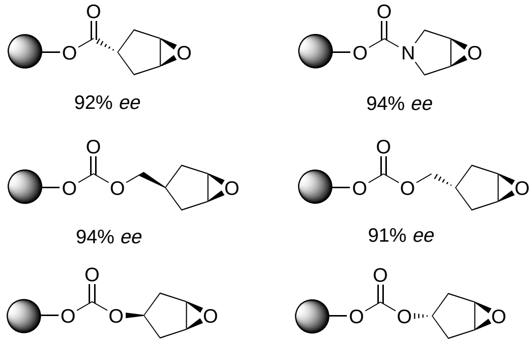
The emergence of combinatorial and parallel library synthesis as an important strategy for the generation and optimization of pharmaceutical lead structures has resulted in major and exciting new challenges in the field of synthetic methodology.[1] The preparation of libraries with significant structural and functional diversity requires synthetic reactions that are not only highly effective, but also generally applicable. Since solid-phase synthesis is the method of choice for the generation of libraries, the development of methods applicable to immobilized substrates adds yet another dimension to the challenge.

Despite the enormous recent progress in solid-phase synthesis,[2] stereoselective reactions have played a relatively minor part.[3, 4] A possible explanation is that absolute stereocontrol is not important in library synthesis. Indeed, if the goal is to generate as many compounds as possible and the screening process allows for the evaluation of mixtures and the deconvolution of individual components, then stereochemical control may not be necessary or even desirable. However, if both specificity and affinity for a target are sought, then evaluation of stereoisomerically pure compounds may be essential. For example, cyclic compounds that contain the amino acid sequence Arg-Gly-Asp (RGD) can switch specificities to different targets as a result of subtle structural and stereochemical variation of the peptide backbone.[5, 6] With the goal of developing enantioselective methods for solid-phase syntheses and highlighting their possible utility for lead discovery and optimization, we describe here the asymmetric catalytic ring opening of polymer-bound *meso*-epoxides with trimethylsilyl azide ($TMSN_3$),[7] and the transformation of the resulting products into stereochemically diverse templates for the synthesis of compounds with RGD pharmacophores.

The reaction of the TentaGel S PHB-immobilized[8] epoxy ester **2a** with an excess of $TMSN_3$ in the presence of the [(salen)CrN_3] complex[9] (*R*,*R*)-**1** proceeded to completion within 24 h, as monitored by IR spectroscopy (Scheme 1). The product was isolated, excess reagents and catalyst were removed by filtration, and the product was desilylated with a dilute methanolic solution of trifluoroacetic acid to afford resin-bound azido alcohol **3a**. Transesterification with MeOH/DMF/Et_3N (9/1/1) then provided soluble **4a** in greater than 95% yield with 92% *ee*, as established by ^1H NMR spectroscopy and gas chromatography. The scope of this solid-phase asymmetric reaction was evaluated on a series of other *meso*-epoxides with five-membered rings (Scheme 2). In all cases, epoxide ring opening occurred cleanly to provide the corresponding azido alcohol derivatives with good to excellent enantiomeric excesses.



Scheme 1. Asymmetric ring opening of polymer-bound *meso*-epoxides catalyzed by [(salen)CrN_3] (**1**).



Scheme 2. Enantioselectivities of the asymmetric ring opening of some polymer-bound *meso*-epoxides.

Having established polymer-bound epoxides as viable substrates for the asymmetric ring-opening reaction, we evaluated the optically active azido alcohol products for their utility as conformationally constrained templates for the synthesis of RGD-containing compounds. The synthesis of cyclic compounds (*S*,*S*)-**9**, (*R*,*R*)-**9**, (*R*,*S*)-**12**, and (*S*,*R*)-**12** is outlined in Schemes 3 and 4. Acylation of the resin-bound alcohol (*S*,*S*)-**3a** with FmocAsp(O*t*Bu) provided the *trans* azido ester (*S*,*S*)-**5** in quantitative yield. In a transformation that permitted the introduction of additional stereochemical diversity, esterification under Mitsunobu conditions[10] pro-

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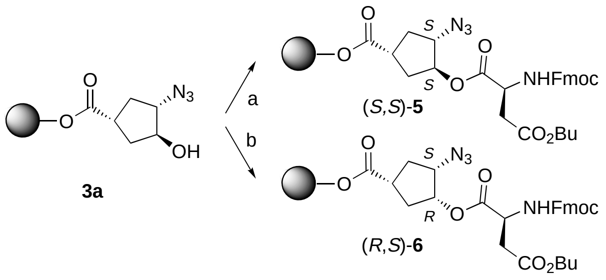
Supporting information for this article (structural coordinates for (*S*,*S*)-**9**, (*R*,*R*)-**9**, (*R*,*S*)-**12**, and (*S*,*R*)-**12** in PDB format) is available on the WWW under http://www.wiley-vch.de/home/angewandte/ or from the author.



Scheme 3. Stereochemical control by means of the acylation conditions. a) FmocAsp(O*t*Bu), DIC, DMAP, DMF, 100%; b) premixed FmocAsp(O*t*Bu) and PPh_3/DEAD, THF, 0°C, 90% (+5% *trans* product). Fmoc = 9-fluorenylmethoxycarbonyl, DIC = *N*,*N*′-diisopropylcarbodiimide, DMAP = 4-dimethylaminopyridine, DEAD = diethyl azodicarboxylate.

vided the corresponding *cis* azido ester (*R*,*S*)-**6** with greater than 15:1 diastereoselectivity, as determined by HPLC analysis. The synthesis of cyclic peptides containing the RGD sequence from **5** and **6** was completed by using well-established solid-phase methods.[11] Cleavage from the resin and deprotection of the side chain were accomplished in one step to provide the desired macrocycles (*S*,*S*)-**9** and (*R*,*R*)-**9** with the cyclopentyl ring in the *trans* configuration as colorless solids after preparative HPLC purification (64% and 58% overall yield, respectively). Macrocycles (*R*,*S*)-**12** and (*S*,*R*)-**12**, in which the cyclopentyl ring has the *cis* configuration, were obtained in a similar manner in 59% and 48% overall yield.

To assess the influence of backbone configuration on the conformation of the RGD pharmacophore, the structures of the macrocycles in solution were estimated from 2D NOESY spectra in [D_6]DMSO by distance geometry/energy minimization methods.[12] Centroids of the lowest energy conformational families of the cyclic peptides are shown in Figure 1.

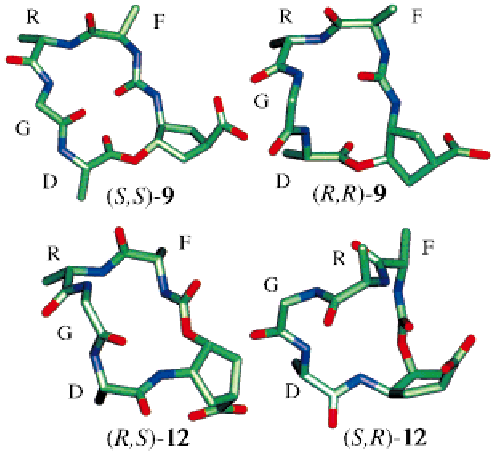
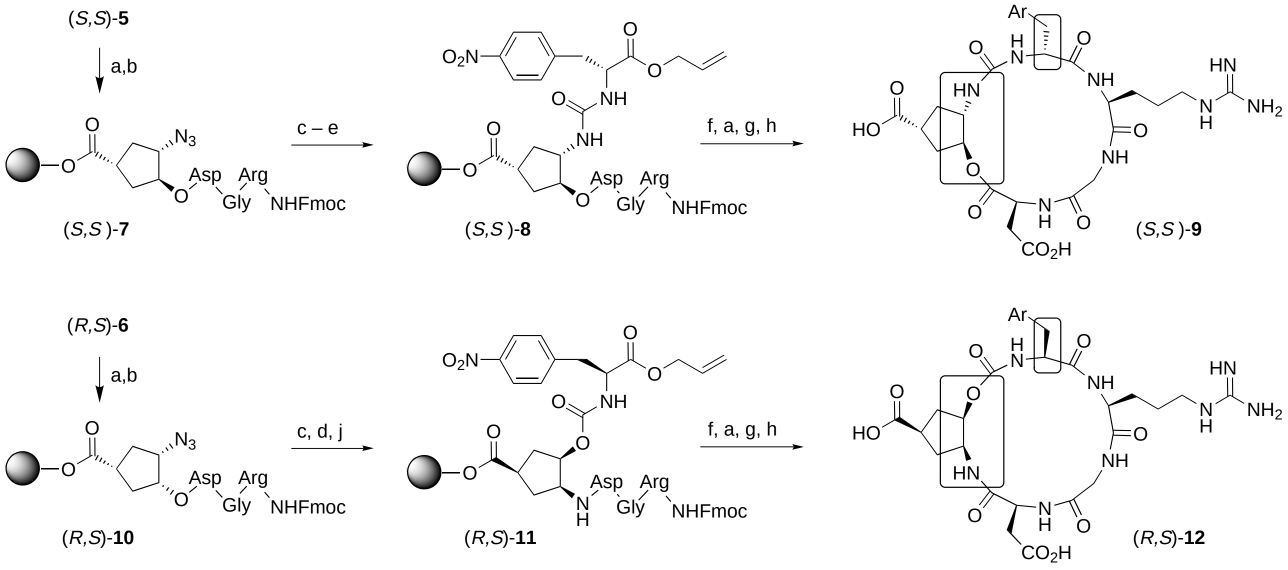


Figure 1. Solution structures of cyclic RGB peptides that were obtained by asymmetric ring opening of polymer-bound epoxides (side chains omitted for clarity).

Compound (*S*,*S*)-**9** adopts a flat structure with a type II′ *β* turn that results from a hydrogen bond between the carbonyl group of urea and the NH group of glycine, and the RGD sequence has an extended conformation. In contrast, (*R*,*R*)-**9**, which was constructed on the enantiomeric *trans*-amino alcohol template, adopts a type I *β* turn, and the RGD sequence is bent in the region of the glycine residue. Thus, enantiomeric templates induce different *β* turns, resulting in a dramatic change in the conformation of the RGD pharmacophore. Compounds (*R*,*S*)-**12** and (*S*,*R*)-**12**, constructed on enantiomeric *cis*-amino alcohol templates, both contain *γ* turns about the glycine residues; however, the orientations of the *γ* turns differ with respect to the Asp and Arg side chains because of rotation about the D–G and G–R amide planes. As a result, the RGD conformations in (*R*,*S*)-**12** and (*S*,*R*)-**12** are puckered in opposite directions.



Scheme 4. Synthesis of macrocycles containing the RGD sequence. a) Piperidine/DMF (1/4); b) FmocArg(Pmc)Gly, HBTU, DIPEA, DMF; c) $SnCl_2$, Et_3N, PhSH, THF; d) 2 M phosgene in PhMe, DIPEA, DMAP, THF, 0°C; e) D-4-NO_2PheOAll, DIPEA, DMAP, DMF; f) [$Pd(PPh_3)_4$], $CHCl_3$/HOAc/morpholine (37/2/1); g) HATU, HOAt, DIPEA, THF; h) TFA/H_2O/Me_2S (90/5/5); j) L-4-NO_2PheOAll, DIPEA, DMAP, DMF. Pmc = 2,2,5,7,8-pentamethylchroman-6-sulfonyl, HBTU = benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, DIPEA = diisopropylethylamine, All = allyl, HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HOAt = 1-hydroxy-7-azabenzotriazole, TFA = trifluoroacetic acid.

The significant conformational differences in the stereoisomeric cyclic peptides prompted us to compare their affinity toward the $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ integrins.[13] Selective inhibitors of $\alpha_v\beta_3$ hold great promise in cancer treatment as suppressors of tumor-induced angiogenesis, while $\alpha_{IIb}\beta_3$ inhibitors are of interest as potential antithrombotic agents.[6] All compounds displayed affinity for $\alpha_v\beta_3$, but substantial differences were observed in selectivity for the two integrin receptors (Table 1). In the most striking example, (*S*,*R*)-**12** binds three times more strongly to $\alpha_v\beta_3$ but over four orders of magnitude times less strongly to $\alpha_{IIb}\beta_3$ than its diastereomer (*R*,*S*)-**12**.[14] If **12** had been prepared as a mixture from racemic azido alcohol **3a**, the specificity for the critically important $\alpha_v\beta_3$ target would not have been recognized.

Table 1. Inhibition of integrin binding by cyclic compounds containing the RGD sequence.

Compound	$\alpha_v\beta_3$[a]	$\alpha_{IIb}\beta_3$[b]	$\alpha_v\beta_3$ selectivity[c]
(*S*,*S*)-**9**	0.6	280	470
(*R*,*R*)-**9**	24	$>10^6$	>40000
(*R*,*S*)-**12**	6.0	120	20
(*S*,*R*)-**12**	1.7	$>10^6$	>500000

[a] IC_{50} [μM], platelet adhesion to osteopontin. [b] IC_{50} [μM], platelet adhesion to fibrinogen. [c] $IC_{50}(\alpha_{IIb}\beta_3)/IC_{50}(\alpha_v\beta_3)$.

The differences in conformation, affinity, and selectivity of the cyclic peptides prepared in this study emanate from stereochemical alterations in their amino alcohol backbones, and thus illustrate the possible future importance of enantioselective reactions in library synthesis. Product isolation and purification are greatly simplified by performing the enantioselective reaction on polymer-immobilized substrates. More important, substrate immobilization opens the possibility of library synthesis in a split-pool manner, in which simultaneous ring opening of families of polymer-bound epoxides is performed.

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