20 mol %, with respect to alkene) in benzene over 3 h. Workup was performed according to usual methods.[12]

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Carbohydrate Derivatives for Use in Drug Design: Cyclic α_v-Selective RGD Peptides**

Elisabeth Lohof, Eckart Planker, Christian Mang, Fred Burkhart, Michael A. Dechantsreiter, Roland Haubner, Hans-Jürgen Wester, Markus Schwaiger, Günther Hölzemann, Simon L. Goodman, and Horst Kessler*

The improvement of pharmacokinetic and dynamic properties of pharmaceutically active compounds with retention of activity and selectivity is an important task in modern drug design. The potential of carbohydrates for the development of new drugs is not yet fully exploited. Here we will show that the modification of peptides with carbohydrate derivatives leads to an improvement of the properties of the peptides. Sugar derivatives can be incorporated in the peptide backbone and/or in the side chains. Both strategies and the different influences on structure and activity of the modified peptides are exemplified for biologically active, cyclic RGD peptides.

Earlier work in our lab demonstrated that the use of sugar amino acids (SAA) makes it possible to predict the conformation of cyclic peptides.[1] Now we show for the first time how this knowledge can be used to obtain high-affinity peptidic compounds. As a lead structure for the derivatization of the RGD motif the cyclic pentapeptide *cyclo*(-Arg-Gly-Asp-D-Phe-Val-) was chosen, which binds selectively $\alpha_v\beta_3$-integrins.[2] Integrins are located at the cell surface of a number of different cell types and play a major role in cell – matrix interactions and in tumorgenesis. This aroused pharmaceutical interest in $\alpha_v\beta_3$ antagonists, especially with regard to blocking tumor-induced angiogenesis.[2] The cyclic peptide *cyclo*(-Arg-Gly-Asp-D-Phe-*N*(Me)Val-),[3] which was the best hit in an extensive screening of peptidomimetics,[2e] is now being tested for its potency as an antitumor drug in phase II clinical trials as EMD121974 from Merck KGaA (Germany).

In earlier attempts the modification of cyclic RGD peptides with carbohydrates impaired the biological activity of the compounds.[4] In order to match the receptor's steric demands, a structurally modified sugar amino acid was incorporated into the sequence of the above-mentioned cyclic peptide. The sugar amino acid was intended to replace the two amino acids D-Phe-Val (Figure 1). From structure – activity investigations

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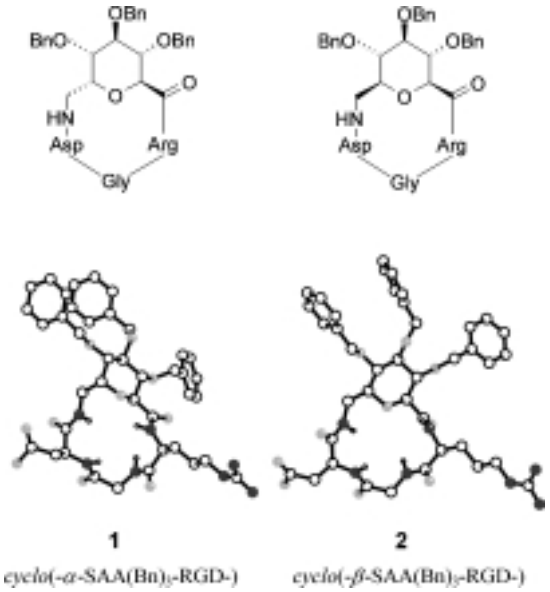

Figure 1. The benzyled sugar amino acid (SAA) built in as scaffold mimetic in the cyclic RGD peptides **1** and **2**. The conformations in DMSO were resolved by NMR spectroscopy and molecular dynamics calculations.[7]

of the lead structure it is known that the hydrophobic character of the phenylalanine improves activity,[2d] and that the backbone conformation of the residues D-Phe-Val should resemble a βII′-turn to force the RGD sequence, which acts as pharmacophore, into a kinked, $\alpha_v\beta_3$-selective conformation (Figure 2).[2, 3] Both demands are met by the new sugar amino acid,[5] whose structure is given in Figure 1: the SAA is a hydrophobic β-turn mimetic. Peptides with both SAA anomers[5] were synthesized (Figure 1, **1** and **2**), the respective structures were determined by NMR spectroscopy, and the biological activity was tested.[2d]

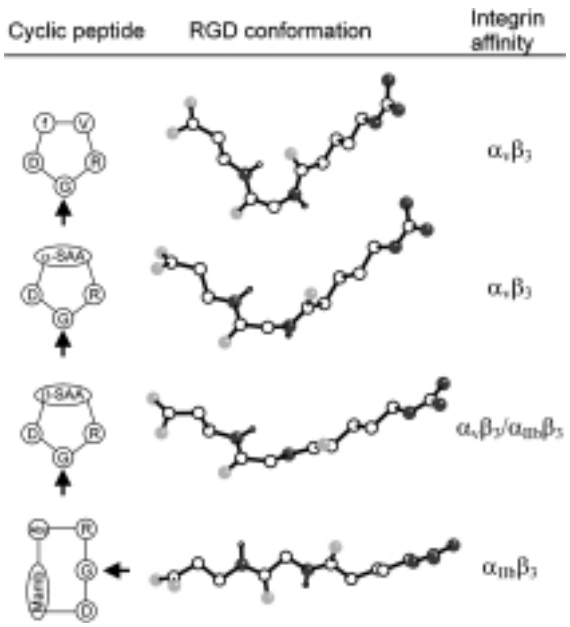

Figure 2. Structure–activity relationship of cyclic RGD peptides. The RGD conformations of the α (**1**) and β compounds (**2**) are compared with typical representatives of $\alpha_v\beta_3$- and $\alpha_{IIb}\beta_3$-antagonists, the lead peptide *cyclo*(-RGDfV-), and the compound *cyclo*(-D-Abu-*N*MeArg-Gly-Asp-Mamb-) (Abu = *A*-aminobutyric acid, Mamb = *m*-(anminomethyl)benzoic acid. [6] The view along the pharmacophoric RGD moiety (center; direction indicated by an arrow in the left-hand column) is oriented parallel to the ring plane of the cyclic peptide.

As hoped, the SAA-modified cyclic peptides exhibit a high $\alpha_v\beta_3$ activity ($IC_{50} = 150$ nM (**1**) and 25 nM (**2**); Table 1). In contrast, the high activity of **2** against the $\alpha_{IIb}\beta_3$ receptor ($IC_{50} = 13.4$ nM) was unexpected. $\alpha_{IIb}\beta_3$-selective antagonists typically adopt a stretched RGD conformation (Figure 2).[6] Structure analysis and molecular dynamics simulations[7] of both the SAA peptides provided an explanation for this unexpected behavior: whereas the conformation of the α-compound is similar to that of the lead structure (the peptidomimetic adopts the $\alpha_v\beta_3$-selective kinked conformation), the conformation of the β-SAA-peptide **2** is in between the conformations of the selective $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ antagonists. In addition, differences in the dynamic behavior of the two compounds are observable. For instance, the β-SAA peptide **2** is more flexible than the α-SAA peptide **1**. Therefore a kinked as well as a stretched conformation can be realized in solution. The compound is therefore able to re-adjust its conformation, matching the steric demands of both the receptor pockets: high activity with a loss of selectivity is the consequence.

Table 1. Inhibition behavior of the different RGD peptides with regard to binding of vitronectin to the isolated $\alpha_v\beta_3$ receptor as well as the binding of fibrinogen to the isolated $\alpha_{IIb}\beta_3$ receptor. The linear peptide GRGDSPK was chosen as standard.

Compound	IC_{50} [nm]		
	$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$	$\alpha_v\beta_5$
GRGDSPK	210	1700	> 10000
cyclo(-RGDfV-)	2.5	8000	320
1	150	720	935
2	25	13.4	> 10000
11	55	> 10000	2750
12	0.8	1910	24.7
13	15	450	> 10000
14	21	5000	970
15	30	> 10000	1000
16	5.9	> 10000	4000
17	6.9	6000	6000
18	2.7	6000	480
19	15	> 10000	6000
20	1.4	> 10000	> 10000
21	1.0	> 10000	610
22	1.8	7300	260

A further possibility to modify the lead peptide is by the glycosylation of the side chains of the peptide. The main focus here is the modification of the pharmacokinetic properties[8] with retention of activity and selectivity. In contrast to the above-introduced scaffold mimetica **1** and **2**, the structure-inducing effects of the side chain modifications on the RGD entity should be negligible. Nonconserving substitutions of the valine residue in the lead peptide diminish activity only slightly, as shown in earlier studies.[2d]

Systematic substitutions of the side chain of the valine residue with carbohydrates of differing length and functionality were intended to elucidate the influence of these substitutions on the biological properties of the peptide. Scheme 1 shows the novel compounds **11**–**14**,[9a] **15**, **16**–**17**,[9b]

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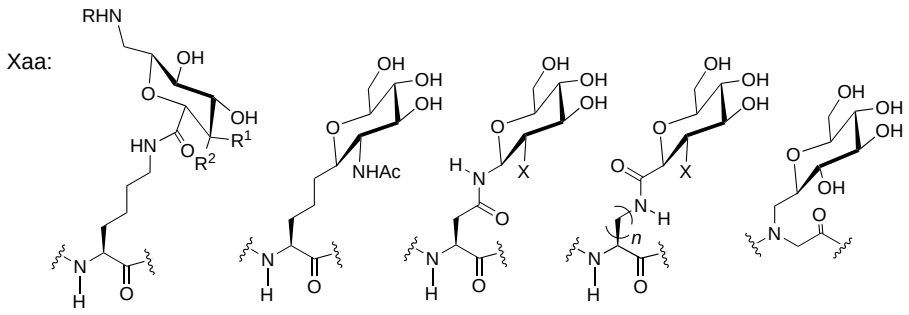

Scheme 1. *C*- and *N*-glycosylated RGD peptides *cyclo*(-Arg-Gly-Asp-D-Tyr-Xaa-) **11**–**14**, and *cyclo*(-Arg-Gly-Asp-D-Phe-Xaa-) **15**–**22**. Shown are the structural formulas of the side chain modified residue Xaa.

18–**21**,[9c, 9f, 10] and **22**[9e]. The syntheses of compounds **12**, **14**, and **16** are modeled on the natural *N*-glycosidic protein glycosylation with glucosamine. To study a possible influence of the *N*-acetyl moiety, the glucose derivatives **11**, **13**, and **15** were incorporated. Especially the C-glucosidic derivatives **11**–**15** and **18**–**22** should be pharmacologically interesting, as this link is expected to be enzymatically very stable.

All compounds exhibit a very high $\alpha_v\beta_3$ affinity ($IC_{50} = 0.8$ to $IC_{50} = 1.8$ nM) and selectivity; some even outperform the lead structure. Noteworthy is the very high selectivity of compound **20** and the high activity of compound **12** against the $\alpha_v\beta_5$ receptor. This might indicate an otherwise unnoticed interaction of the side chain with the appropriate subtype of the receptor. Meanwhile it has also been shown that the side chain glycosylation improves the pharmacokinetic properties of peptides which have been used as tracer substances in nuclear medicinal applications.[11] In **20** D-Phe was substituted by D-Tyr to enable radioactive labeling with iodine isotopes. Pharmacokinetic studies demonstrate that the uptake in the liver was reduced compared to that of the nonglycosylated compound, the initial concentration in the blood was doubled, and, the accumulation of the tracer in tumor tissues was dramatically enhanced. Furthermore the first γ-camera pictures of osteosarcom-carrying mice with the ^{123}I-marked peptide revealed images in which the tumor could be clearly delimited from the surrounding tissue.[11]

The presented studies demonstrate that new areas in drug design can be exploited by the incorporation of sugar amino acids in biologically active peptidic compounds. We have illustrated that sugar amino acids as a part of the peptidic backbone broaden the dynamic spectrum and widen the conformational space of this class of peptidomimetics. In the process of direct drug design they help to structurally adjust the bioactive moiety to the appropriate subtypes of the receptor. Additionally they assist in tracing bioactive conformations. Derivatization of side chains with sugars can also influence activity and selectivity, though the main purpose of this kind of modification remains the improvement of pharmacokinetic properties. The results shown here lead to the assumption that the application of sugar amino acids will be used in future as a standard tool in the optimization of peptidic drugs.

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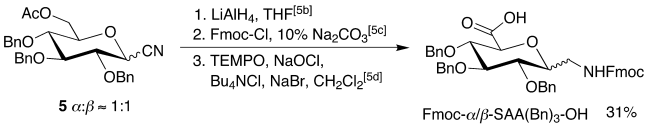

Scheme 2. Synthesis of Fmoc-α/β-SAA$(Bn)_3$-OH. Fmoc = 9-fluorenylmethoxycarbonyl, TEMPO = 2,2,6,6-tetramethylpiperidinoxyl radical.

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[9] a) **11**–**14:** In this case the cyclic peptide *cyclo*(-Arg-Gly-Asp-D-Tyr-Lys-) was synthesized first, followed by glycosylation of the amino moiety of the lysine. **11, 12**: ref. [1a]; **13**–**14**: ref. [9f] followed by an oxidation as described in ref. [5d]; b) F. Burkhart, M. Hoffmann, H. Kessler, *Angew. Chem.* **1997**, *109*, 1240–1241; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1191–1192; c) H. Paulsen, S. Peters, T. Bielefeldt, *New Compr. Biochem.* **1995**, *29a*, 87–121; d) M. Hoffmann, F. Burkhart, G. Hessler, H. Kessler, *Helv. Chim. Acta* **1996**, *79*, 1519–1532; e) glycopeptoid: by reductive amination of the nitro sugar[9f] with glyoxylic acid, followed by a Fmoc derivatization (Scheme 3); f) L. Petrus, S. Bystricky, T. Sticzay, V. Bilik, *Chem. Zvesti* **1982**, *36*, 103–110.

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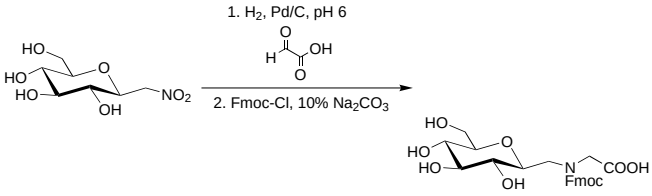

Scheme 3. Synthesis of a glycopeptoid building block.

[10] The building blocks **15**–**22** were made as Fmoc derivatives, and were as such used under standard solid-phase conditions with TBTU/HOBt [R. Knorr, A. Trzeciak, W. Bannwarth, D. Gillessen, *Tetrahedron Lett.* **1989**, *30*, 1927–1930].

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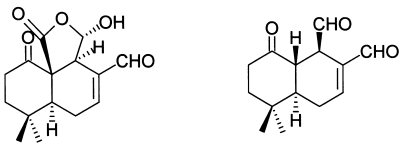
A Short Total Synthesis of Kuehneromycin A**

Johann Jauch*

Dedicated to Professor Volker Schurig on the occasion of his 60th birthday

The kuehneromycins were isolated from the fermentation broth of the basidiomycete *Kuehneromyces* sp. 8758 in 1995.[1] Kuehneromycin A (**1**) is a noncompetitive inhibitor of avian myeloblastosis virus reverse transcriptase[2] as well as moloney murine leukemia virus reverse transcriptase.[2] Kuehneromycin B (**2**) is a strong inhibitor of platelet aggregation and both compounds show cytotoxic and antimicrobial activities. Structurally, the kuehneromycins are related to the mniopetals,[3] which inhibit HIV reverse transcriptase.



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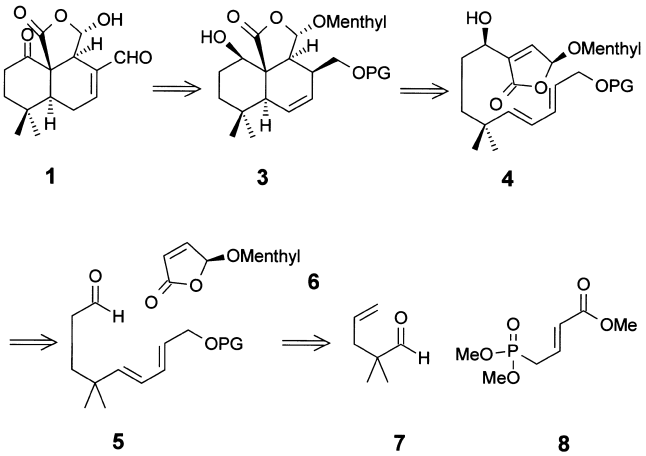
In the course of our project towards the synthesis of new sesquiterpenoids[4] with interesting biological activities we decided to synthesize kuehneromycin A and here we report the first synthesis of naturally occurring (−)-kuehneromycin A.

Retrosynthetic analysis of kuehneromycin A (**1**) leads to the protected alcohol **3** which should readily be obtainable from the trienolide **4** in an *endo*-selective intramolecular Diels–Alder reaction (IMDA reaction; Scheme 1). Trienolide **4** is the result of a Baylis–Hillman reaction of aldehyde **5** and Feringa's butenolide **6**.[5] Aldehyde **5** is disconnected into aldehyde **7** and phosphonate **8** through retrosynthetically applying a hydroboration/oxidation Horner–Wadsworth–Emmons sequence.



Scheme 1. Retrosynthetic analysis of kuehneromycin A (**1**). PG = protecting group.

Our synthesis (Scheme 2) started with a Horner–Wadsworth–Emmons reaction[6] of 2,2-dimethyl-4-pentenal (**7**)[7] and phosphonate **8**[8] from methyl *E*-4-bromobutenoate, which afforded a mixture of triene esters (*trans*:*cis* > 20:1) from which **9** was readily separated in 85% yield by flash chromatography. Reduction of the ester functionality with diisobutylaluminum hydride (DIBALH)[9] gave the alcohol **10** in 97% yield, which subsequently was protected as the *tert*-butyldiphenylsilyl (TBDPS) ether **11**[10] in quantitative yield. Hydroboration[11] with 9-borabicyclo[3.3.1]nonane (9-BBN), followed by oxidation with H_2O_2/NaOH under standard conditions led regioselectively to alcohol **12** in 91% yield. Oxidation of the primary alcohol with 2,2,6,6-tetramethylpiperidin-*N*-oxyl (TEMPO)/diacetoxyiodobenzene[12] gave exclusively the aldehyde **5**, which served as the starting material for the planned Baylis–Hillman reaction.[13]

The Baylis–Hillman reaction under standard conditions using 1,4-diazabicyclo[2.2.2]octane (DABCO) as a nucleophile was not applicable in our case since Feringa's butenolide[5] **6** is highly base sensitive and the DABCO-catalyzed reaction only works well for β-unsubstituted acrylic acid derivatives. Therefore we developed a new and highly diastereoselective variant[14] of the Baylis–Hillman reaction that used lithium phenylselenide as a strong but only weakly basic nucleophile. PhSeLi was readily prepared from diphenyl diselenide through reductive cleavage with either *n*BuLi[15] or

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