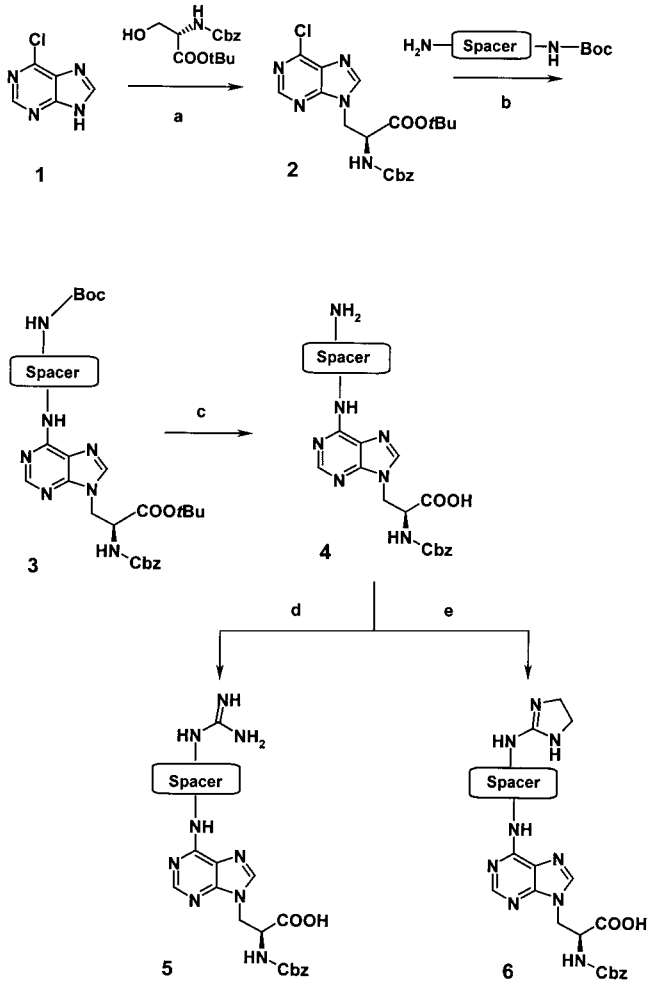
Vitronectin Receptor Antagonists: Purine-Based Peptidomimetics

Anusch Peyman,* Jean-François Gourvest, Thomas R. Gadek, and Jochen Knolle

Integrins are a widely expressed family of α/β heterodimeric cell surface receptors which bind to extracellular matrix adhesive proteins such as fibrinogen, fibronectin, vitronectin, laminin, and osteopontin.[1–3] They are composed of dimers of at least fifteen α subunits and eight β subunits. The β_3 class of the integrin family, $\alpha_{IIb}\beta_3$ (GPIIb/IIIa or fibrinogen receptor) and $\alpha_V\beta_3$ (vitronectin receptor), has received special attention in recent drug research.[4, 5] $\alpha_{IIb}\beta_3$ is prevalent on platelets and plays a role in thromboembolic disorders,[5] while $\alpha_V\beta_3$ is the dominant receptor for mediating the attachment of osteoclasts to bone during bone resorption[6, 7] and has been implicated in tumor progression, angiogenesis,[5, 8, 9] and restenosis.[10] Many integrins, including $\alpha_{IIb}\beta_3$ and $\alpha_V\beta_3$, interact with a common Arg-Gly-Asp (RGD) binding motif in their target proteins.[11–13] Kessler et al. were able to identify the structural properties required for the selective inhibition of either $\alpha_{IIb}\beta_3$ or $\alpha_V\beta3$ using stereoisomeric cyclic peptide libraries.[14–16] In drug development peptidomimetic compounds are often preferred over peptides[17], and several selective peptidomimetic antagonists of $\alpha_V\beta_3$ have been reported recently.[18–25] This has led to an increased interest in finding new building blocks for integrin antagonists that allow the correct spatial arrangement of pharmacophorically important groups. Although the purine scaffold has been used in the synthesis of a variety of chemical libraries, especially in the design of kinase inhibitors,[26, 27] there are to our knowledge no reports on its use as a central scaffold in peptidomimetics. Here we introduce the purine scaffold in the design and synthesis of selective antagonists of $\alpha_V\beta_3$ and present the first structure–activity relationship within this series.

The synthesis of the $\alpha_V\beta_3$ antagonists **5** and **6** is outlined in Scheme 1. Alkylation of 6-chloropurine **1** with *N*-Cbz-L-serine *tert*-butyl ester under Mitsunobu conditions afforded **2** in 50% yield. Nucleophilic substitution with building blocks containing one *tert*-butyloxycarbonyl (Boc) protected and one unprotected amino group gave aminopurines **3**. Only (4-piperidylmethyl)amine was used without Boc-protection, reacting selectively (>95%) with the secondary amine. (No trace of the corresponding product of the primary amine was found.) The Boc group and the *tert*-butyl ester were cleaved simultaneously by treatment with 95% TFA/5% water and



Scheme 1. Synthesis of the $\alpha_V\beta_3$ antagonists **5** and **6**. a) PPh_3, DEAD, THF, 0 °C; 50%; b) DMF, DIPEA, 6 h 50 °C; 60–80%; c) 95% TFA, 2 h, RT; 100%; d) 1*H*-pyrazole-1-carboxamidine, H_2O, DIPEA, 48 h, RT; 50–85%; e) 2-methylsulfanyl-2-imidazoline, DIPEA, 24 h, 50 °C; 50–70%. DEAD = diethyl azodicarboxylate, TFA = trifluoroacetic acid, DIPEA = diisopropylethylamine.

the resulting molecules **4** were converted into the guanidines **5** by treatment with 1*H*-pyrazole-1-carboxamidine[28] and DIPEA in water with yields ranging from 50 to 85%. Alternatively **4** was converted to the (1*H*-imidazolin-2-yl)amines **6** using 2-methylsulfanyl-2-imidazoline and DIPEA in DMF with yields of 50 to 70%. The synthesis of antagonist **7**, which contains a 2-aminobenzimidazole group as an arginine mimetic, is depicted in Scheme 2. *N*-(1*H*-benzimidazol-2-yl)butane-1,4-diamine, prepared by standard methods, was treated with **2** to give antagonist **7** after cleavage of the *tert*-butyl ester. A second series of $\alpha_V\beta_3$ antagonists (**10** and **11**) is characterized by a "reversed" orientation of the central purine scaffold (amino acid substituent not on the imidazole but on the pyrimidine ring), and its synthesis is shown in Scheme 3. Compound **1** was first alkylated with N-Boc-protected aminoalkyl tosylates to give the chloropurines **8** in high yields. These compounds reacted with the amino group of *tert*-butyl (*S*)-N^2-Cbz-2,3-diaminopropionate and its homologues, and after treatment with TFA, compounds **9** are obtained. Subsequent conversion to the guanidines **10** and the

[*] Dr. A. Peyman
Aventis Pharma, Medicinal Chemistry G838
65926 Frankfurt (Germany)
Fax: (+49) 69-305-80679
E-mail: anusch.peyman@aventis.com
Dr. J.-F. Gourvest
Aventis Pharma, 102 Route de Noisy, 93235 Romainville Cédex (France)
Dr. T. R. Gadek
Genentech, 1 DNA Way, South San Francisco, CA 94080 (USA)
Dr. J. Knolle
Axys Inc., 385 Oyster Point Blvd., South San Francisco, CA 94080 (USA)

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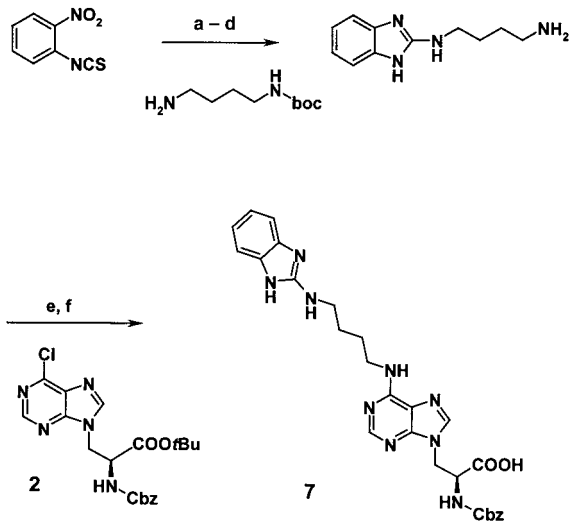
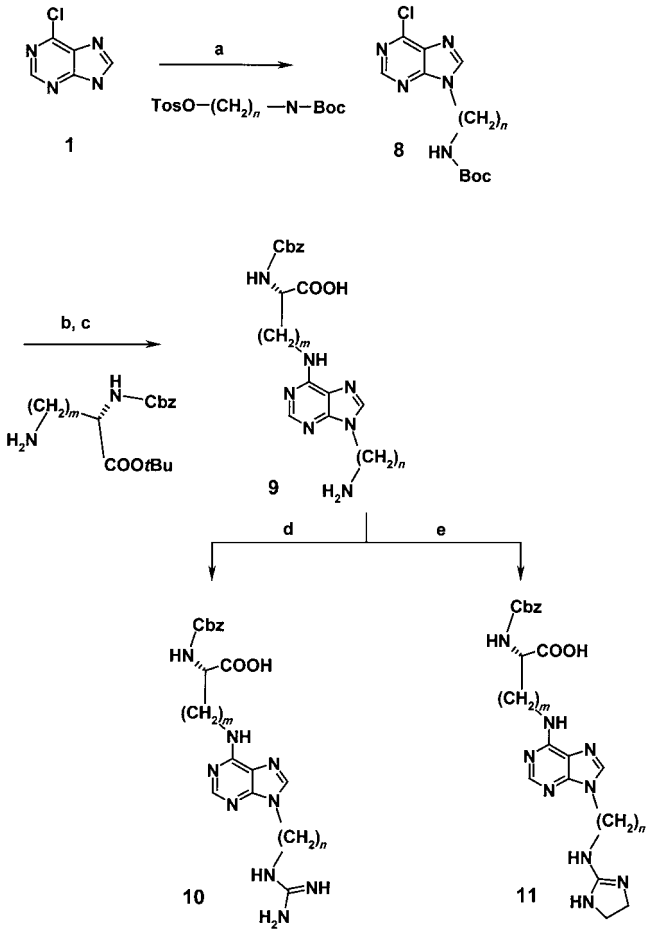


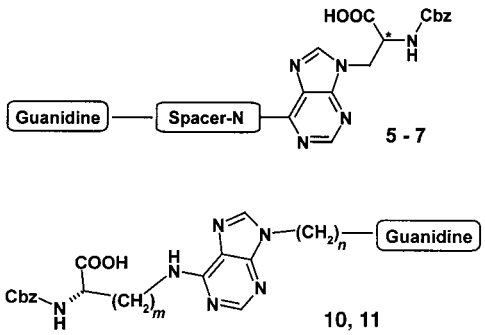
Scheme 2. Synthesis of the $\alpha_V\beta_3$ antagonist **7**. a) DMF, 0 °C, 1 h; 95 %; b) H_2/Pd-C; 100 %; c) HgO, sulfur, 50 °C; 43 %; d) 95 % TFA, 2 h, 0 °C; 100 %; e) DMF, DIPEA, 32 h, 50 °C; 32 %; f) 95 % TFA, 2 h, 0 °C; 100 %.



Scheme 3. Synthesis of the $\alpha_V\beta_3$ antagonists **10** and **11**. a) DMF, K_2CO_3, RT; 70–80 %; b) DMF/DIPEA (2/1), 70 °C, 7 d; 40–50 %; c) 95 % TFA, 2 h, 0 °C; 100 %; d) 1*H*-pyrazol-1-carboxamidine, H_2O, DIPEA, 48 h, RT; 50–60 %; e) 2-methylsulfanyl-2-imidazoline, DIPEA, 24 h, 50 °C; 50–60 %.

amino-2-imidazolines **11** was accomplished with the same methods as for the synthesis of **5** and **6**.

One of the most interesting challenges in this area is the design of selective antagonists since both $\alpha_V\beta_3$ and $\alpha_{IIb}\beta_3$ bind to the same recognition motif RGD and there seem to be only subtle differences in the ligand structure that determine selectivity. The IC_{50} values of compounds **5**–**7**, and **10**, **11** (Scheme 4) for the inhibition of binding of fibrinogen to $\alpha_{IIb}\beta_3$ and of Kistrin (a disintegrin with high affinity to $\alpha_V\beta_3$[7, 29]) to $\alpha_V\beta_3$ are summarized in Tables 1 ("normal" orientation) and 2 ("reversed" orientation).



Scheme 4. General formulas for the antagonists **5**–**7**, and **10**, **11**.

The most striking criterion for selectivity is the distance between the carboxy group and the N-terminal guanidino group, as can be seen from a comparison of **5a**, **5b**, and **5c** or in the "reversed" series by comparing **10a**, **10b**, and **10c**. The optimum distance for $\alpha_V\beta_3$ binding within these two series is 12 bonds (**5b**, **10b**), while $\alpha_{IIb}\beta_3$ prefers a substrate where the two groups are further apart, as in **5a** or **10a** with a distance of

Table 1. Activity and selectivity of $\alpha_V\beta_3$ antagonists **5**–**7** (Scheme 4). The IC_{50} values [μM] denote the concentration required to reduce binding of fibrinogen (Fg) to $\alpha_{IIb}\beta_3$ or of Kistrin (K) to $\alpha_V\beta_3$ by 50 %.[30]

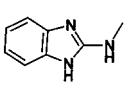
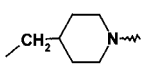
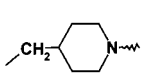
	Configuration (*)	Guanidine	Spacer	IC_{50} K/V_nR	IC_{50} Fg/IIbIIIa
5a	*S*		-$(CH_2)_5$-NH-	1.1	1.9
5b	*S*		-$(CH_2)_4$-NH-	0.7	> 10
5c	*S*		-$(CH_2)_3$-NH-	2.2	> 10
6a	*S*		-$(CH_2)_4$-NH-	0.15	> 10
7	*S*		-$(CH_2)_4$-NH-	0.16	> 10
6b	*S*			0.05	4.0
6c	*R*			0.34	> 10
6d	*R*		-$(CH_2)_4$-NH-	1.0	> 10

Table 2. Activity and selectivity of $\alpha_V\beta_3$ antagonists **10** and **11** (Scheme 4). The IC_{50} values [μM] denote the concentration required to reduce binding of fibrinogen (Fg) to $\alpha_{IIb}\beta_3$ or of Kistrin (K) to $\alpha_V\beta_3$ by 50%.[30]

	Guanidine	*m*	*n*	IC_{50} K/V_nR	IC_{50} Fg/IIbIIIa
10a		1	5	0.65	0.6
10b		1	4	0.17	5.0
10c		1	3	0.54	3.5
10d		2	3	0.58	> 10
11a		1	4	0.075	> 10
11b		1	5	0.21	> 10
11c		2	3	0.19	> 10

13 bonds. This observation is in good agreement with the results obtained for other inhibitors[18, 19] and with Kessler's observation that in cyclopeptides $\alpha_V\beta_3$-selective molecules are strongly bent, while $\alpha_{IIb}\beta_3$-selective molecules bind in a more extended conformation.[14, 16]

A comparison of **5b**, **6a**, and **7** illustrates that cyclic guanidines have a higher affinity for $\alpha_V\beta_3$ than noncyclic ones. The cyclic guanidino antagonist **6a** ($IC_{50} = 150$ nM) exhibits a fivefold affinity over the open guanidino compound **5b** ($IC_{50} = 700$ nM). The 2-aminobenzimidazole antagonist **7** shows approximately the same potency as **6a**. Interestingly this increase in affinity is not observed for $\alpha_{IIb}\beta_3$ binding. A similar trend can be observed in the "reversed" series; however, the increase in affinity from **10b** ($IC_{50} = 170$ nM) to the cyclic guanidino compound **11a** ($IC_{50} = 75$ nM) is only by a factor of 2, but at the same time there is a decrease in affinity to $\alpha_{IIb}\beta_3$. This decreased affinity to $\alpha_{IIb}\beta_3$ for cyclic guanidines can also be observed in a comparison of the extended antagonists **10a** and **11b**. One could speculate that the antagonists bind to $\alpha_{IIb}\beta_3$ through an "end-on" interaction, while $\alpha_V\beta_3$-binding is achieved through "side-on" binding on the guanidine.

Another enhancement in $\alpha_V\beta_3$ binding is obtained through the rigidification of the spacer from aminobutane in **6a** ($IC_{50} = 150$ nM) to methylpiperidine in **6b** ($IC_{50} = 50$ nM), keeping the length of the spacer constant. The piperidine spacer seems to bring the guanidino group into the correct spatial orientation and, in addition, it has the advantage that no additional stereocenter is introduced.

The *S* configuration is clearly preferred for the only stereocenter that is connected with the lipophilic side chain, the benzyloxycarbonylamino group, as can be seen from the IC_{50} values for **6c** (340 nM) and **6d** (1000 nM) which both have the R configuration and which are approximately sevenfold less active than the S enantiomers **6b** and **6a**, respectively.

Unexpectedly, the binding affinity is quite insensitive to the orientation of the central purine scaffold—a comparison of, for example, **6a** ($IC_{50} = 150$ nM) and **11a** ($IC_{50} = 75$ nM) reveals only a small preference for the "reversed" orientation. The difference is of the same order of magnitude as the one induced by changing the position of the purine scaffold within a given orientation ("slidomers"); compare, for example, **11a** ($IC_{50} = 75$ nM) and **11c** ($IC_{50} = 190$ nM).

In conclusion, we have been able to show that the purine scaffold is a highly versatile building block in the synthesis of peptidomimetics. To mimic the RGD peptide recognition sequence of the vitronection receptor, we could easily vary spacer length, rigidity, and pharmacophoric groups. This led to a very consistent structure – activity relationship and resulted in highly active and selective new antagonists of $\alpha_V\beta_3$. The ease of derivatization will certainly encourage many future uses of this scaffold in peptidomimetic chemistry.

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Highly Efficient and Versatile Acylation of Alcohols with $Bi(OTf)_3$ as Catalyst

Akihiro Orita, Chiaki Tanahashi, Atsushi Kakuda, and Junzo Otera*

The acylation of alcohols is an important transformation in organic synthesis.[1] Despite a number of precedents, new efficient methods are still in strong demand. Acid anhydrides are the most commonly used reagents in the presence of an acid or base catalyst[2] and the utility of this protocol was boosted by the discovery of the dimethylaminopyridine (DMAP) catalyst.[2a] More recently, metal triflates, such as scandium triflate,[2d] trimethylsilyl triflate,[2e] and indium triflate,[2f] were found to be effective as well. These catalysts are very useful, but also suffer from some drawbacks. Scandium triflate is rather expensive and must be used under anhydrous conditions. Trimethylsilyl triflate is labile towards moisture, and its acidity is too strong for acid-sensitive alcohols as reagents.[2e] In the development of organotin acylation catalysts,[2g,h, 3] we have established that they are so mild that various selective acylation reactions are feasible but their acidity is not strong enough to perform acylation of sterically hindered alcohols. In this context, we were intrigued to employ bismuth triflate $Bi(OTf)_3$[4] since it had proved to be easy to handle due to its stability in the air and to be acidic enough to catalyze Friedel – Crafts,[5] Diels – Alder,[6] and ene reactions.[7]

As shown in Table 1, $Bi(OTf)_3$ can promote the acetylation of primary, secondary, and tertiary alcohols in acetic anhydride at 25 °C [Eq. (1)]. For the acetylation of 2-phenylethanol a catalyst loading as low as 0.01 mol % was sufficient to afford the acetate quantitatively within 10 min (entry 1).

Table 1. $Bi(OTf)_3$-catalyzed acetylation of alcohols [Eq. (1)].[a]

$$R{-}OH + Ac_2O \xrightarrow{Bi(OTf)_3} R{-}OAc \qquad (1)$$

Entry	Alcohol	Mol % of the catalyst	Time [h]	Yield [%][b]
1	2-phenylethanol	0.01	0.167	98
2	2-phenylethanol	0.005	2	99
3[c]	2-phenylethanol	0.5	4	92
4	1-phenylethanol	0.005	2	95
5	2-octanol	0.005	17	98
6[d]	2-octanol	0.005	3	98
7	1-adamantanol	0.01	6	98

[a] Reaction conditions: alcohol (1.0 mmol), acetic anhydride (10 equiv), 25 °C. [b] Determined by GC. [c] Acetic anhydride (1.5 equiv), CH_2Cl_2 (wet, 1.0 mL). [d] At 40 °C.

Even with 0.005 mol % of $Bi(OTf)_3$, this acetylation proceeded quantitatively, but took longer to complete (entry 2). It is possible to employ a solvent different from the anhydride as well. For example, 2-phenylethanol in CH_2Cl_2 was acetylated by 1.5 equivalents of Ac_2O, where CH_2Cl_2 was used without any purification (vide infra and entry 3). Secondary alcohols such as 1-phenylethanol and 2-octanol were also smoothly converted into the corresponding acetates in the presence of 0.005 mol % of $Bi(OTf)_3$ (entries 4 and 5). Although 2-octanol is less reactive than 1-phenylethanol, quantitative acetylation was possible by using longer reaction times or higher reaction temperatures (entries 5 and 6). Surprisingly, even for the acetylation of 1-adamantanol a catalyst loading of 0.01 mol % was enough (entry 7).

After these preliminary examinations, acetylation of functionalized alcohols was scrutinized at 25 °C (Table 2). Although acid-sensitive geraniol and furfuryl alcohol were acetylated in 81 % and 80 % yields, respectively (entries 1 and 3), further improvements of the yields were attained by adding a donor solvent, such as acetonitrile or THF (entries 2 and 4). Not only primary but also secondary and tertiary alcohols with functional groups or phenol units underwent smooth acetylation (entries 5 – 15). Alcohols having an ester or amine function did not react more slowly or show decomposition of the functional groups. More importantly, neither racemization nor epimerization was detected (entries 7 – 9).

Notably, tertiary alcohols can be acetylated at ambient temperatures (entries 10 – 14). For example, the $Bi(OTf)_3$-catalyzed acetylation of an α,α-dimethyl-substituted tertiary

[*] Prof. Dr. J. Otera, Dr. A. Orita, C. Tanahashi, A. Kakuda
Department of Applied Chemistry, Okayama University of Science
Ridai-Cho, Okayama 700-0005 (Japan)
Fax: (+81) 86-256-4292
E-mail: otera@high.ous.ac.jp