Experimental Section

The antimony thin films were synthesized in ultra-high vacuum (base pressure: 4×10^{-11} mbar) by evaporation of Sb_4 molecules from a resistance-heated effusive oven (temperature: 330 °C; deposition rate: 0.1 nm s^{-1}; Sb: 99.9999 %, Johnson-Matthey). The layer thickness is given in monolayers of Sb atoms. The MoS_2 substrates were prepared by cleavage according to the procedure given in reference [13]. The $AuSb_2$ surface alloy was prepared starting from Au(111).[14] On this substrate the surface alloy $AuSb_2$ with the (100) orientation forms spontaneously, if less than one monolayer of antimony is deposited at room temperature.[15] The beetle-type STM[16] is located in an analysis chamber directly attached to the preparation chamber.[17] To increase image contrast, the STM data were electronically differentiated directly during acquisition. Therefore the STM images appear as if they are illuminated from the side.

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Conformationally Restricted Peptides

Analogues of Neuropeptide Y Containing β-Aminocyclopropane Carboxylic Acids are the Shortest Linear Peptides That Are Selective for the Y_1 Receptor**

Norman Koglin, Chiara Zorn, Raphael Beumer, Chiara Cabrele, Christian Bubert, Norbert Sewald, Oliver Reiser, and Annette G. Beck-Sickinger**

Dedicated to Professor Peter Welzel on the occasion of his 65th birthday.

Neuropeptide Y (NPY) is one of the most abundant neuropeptides in the mammalian central nervous system. It consists of 36 amino acids and has an amide group at the C terminus. To date NPY is the strongest known stimulator of food intake in rat and mice models. Other important biological functions are vasoconstriction in the periphery, regulation of behavior and modulation of pain and epileptic seizures.[1]

In mammals, the activities of NPY are mediated by at least three different G-protein-coupled receptors (Y_1, Y_2, and Y_5). Because NPY shows sub-nanomolar affinity towards all of them, it is still difficult to distinguish the physiological roles of each receptor in vivo. To address this problem, the knowledge of the particular bioactive conformation at each receptor is indispensable for the further development of subtype-selective ligands. Substitutions of single amino acids have revealed that especially the highly conserved C-terminal part of NPY, with its two positively charged arginine side chains in positions 33 and 35 and the tyrosine amide in position 36, plays a crucial role during the recognition process by the respective receptor. Because of its flexibility, no defined structure could be assigned to this important part of the molecule to date. It is assumed that different secondary structure motifs of the C terminus are responsible for receptor subtype selectivity.

[*] Prof. Dr. O. Reiser, Dr. C. Zorn, Dr. R. Beumer, Dr. C. Bubert
Institut für Organische Chemie
Universität Regensburg
Universitätsstrasse 31, 93040 Regensburg (Germany)
Fax: (+49) 941-9434-121
E-mail: oliver.reiser@chemie.uni-regensburg.de
Prof. Dr. A. G. Beck-Sickinger, Dipl.-Biochem. N. Koglin, Dr. C. Cabrele
Institut für Biochemie
Universität Leipzig
Talstrasse 33, 04103 Leipzig (Germany)
Fax: (+49) 341-9736-998
E-mail: beck-sickinger@uni-leipzig.de
Prof. Dr. N. Sewald
Institut für Organische und Bioorganische Chemie
Universität Bielefeld
Postfach 100131, 33501 Bielefeld (Germany)

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It has been shown that substitutions close to these major C-terminal positions allow a distinction to be made between some receptor subtypes. For example, the introduction of a proline residue in position 34 of the NPY sequence significantly reduced the Y_2 receptor affinity and maintained the affinity toward the Y_1 and Y_5 receptors.[2] The introduction of the turn-inducing Ala–Aib sequence in positions 31 and 32, respectively, led to a Y_5-selective analogue.[3] Whereas the influence of the Ala–Aib substitution on the peptidic structure could be further elucidated by NMR spectroscopic investigations, the proline substitution at position 34 did not sufficiently restrain the C terminus to enable the structural requirements of NPY binding at other receptor subtypes to be deduced.[4] Accordingly, novel approaches are required for the identification of the bioactive conformation at the Y_1 receptor.

To rigidify the peptide backbone and to induce or to stabilize distinct secondary structure motifs, analogues of the C-terminal NPY sequence with new conformationally restricted building blocks were investigated. We expected that the incorporation of such constraints would be especially effective at positions 32 and 34, which are in direct proximity to the most important amino acids Arg33 and Arg35. By using this strategy, we identified β-aminocyclopropane carboxylic acids (β-ACC) as novel constricted building blocks that are highly effective for the stabilization of secondary structures in peptides, and moreover, complement known constrained α-amino acids such as proline or α-aminoisobutyric acid (Aib) in their properties.

The incorporation of β-ACCs into peptides, however, turned out to be challenging. Because of the 1,2-donor-acceptor substituted cyclopropane structure, β-ACCs are unstable in the N-unprotected form, which necessitates special coupling techniques to incorporate them into peptides.[5] The β-amino acid **1** (Scheme 1) can be readily synthesized in diastereo- and enantiomerically pure form starting from *N*-Boc-pyrrole.[6] We synthesized the prerequisite dipeptides **2** and **3**, which then were used as dipeptide building blocks in solid-phase peptide synthesis. The β-ACC-containing dipeptides were introduced into the growing peptide chain by a manual coupling step after TBTU activation[7] and further elongation by automated Fmoc/*tert*-butyl coupling procedures.[3] The resulting peptides were analyzed by HPLC and MALDI-MS, which confirmed the intact cyclopropyl ring system. The conformational properties of the C-terminal NPY analogues containing these β-ACC dipeptides were investigated by circular dichroism (CD) spectroscopy, and their affinity to the Y receptors was probed by competition binding assays with living cells that selectively express one receptor subtype (compounds **4**–**15**, Table 1).

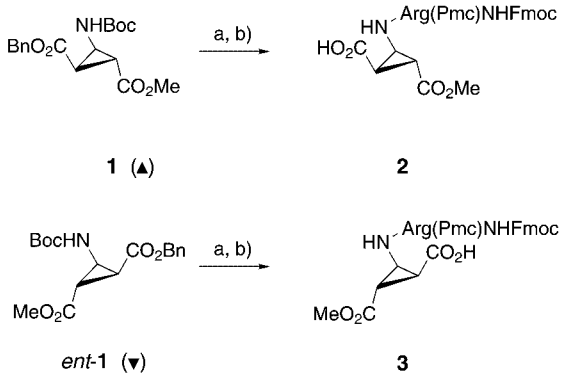
From previous studies it is known that the full-length molecule of NPY is necessary for binding to the Y_1 receptor. N-terminally truncated analogues, such as NPY(13–36) or NPY(18–36) bind only to the Y_2 receptor, whereas NPY(2–36) is recognized by Y_2 and Y_5.[2b] However, in contrast to the unsubstituted C-terminal fragment NPY(25–36)[8] we obtained β-ACC-containing NPY analogues, which bind to the Y_1 receptor (peptides **4**, **8**, and **10**, Table 1) with high affinity and selectivity.

The affinity of the β-ACC-containing NPY analogues is strongly dependent on the position of the substitution and the absolute configuration of the β-ACC derivative. The introduction of a β-ACC-moiety with the (▲)-configuration in position 34 was crucial for binding to the Y_1 receptor. Accordingly, only a single β-ACC substitution with this configuration in position 34 (peptide **4**) was sufficient to evoke nanomolar biological affinity at the Y_1 receptor and high selectivity, whereas a (▼)-configured β-ACC-residue in position 34 (peptide **5**) remained biologically inactive at all

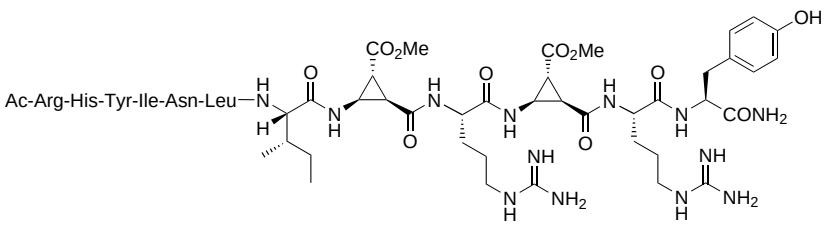
Table 1: Sequences and affinities of shortened β-ACC-containing NPY analogues at the Y receptors. The affinities are expressed as K_i values [nM].[a] For more details see text.

	Sequences[b]	Y_1	Y_2	Y_5	$\Delta[\Theta]_R$[c]
	RHYINLITRQRY-NH_2	>1000	21[d]	>1000	17.5
4	RHYINLITR▲RY-NH_2	37(±20)	>1000	724	5.6
5	RHYINLITR▼RY-NH_2	>1000	>1000	>1000	3.7
6	RHYINLI▲RQRY-NH_2	>1000	>1000	>1000	1.5
7	RHYINLI▼RQRY-NH_2	>1000	>1000	>1000	1.6
8	RHYINLI▲R▲RY-NH_2	50(±10)	>1000	617	2.9
9	RHYINLI▼R▲RY-NH_2	>150	>1000	>1000	3.5
10	RHYINL**R**▲R▲RY-NH_2	29(±13)	>1000	118	3.3
11	RHYINL**R**▼R▼RY-NH_2	1000	>1000	>1000	2.2
12	NL**R**▲R▲RY-NH_2	235	>1000	>1000	2.0
13	RHYINLITR**P**RY-NH_2	>1000	>1000	>1000	5.1
14	RHYINLI**P**R**P**RY-NH_2	>1000	>1000	>1000	1.4
15	RHYINLITR**β**RY-NH_2	>1000	>1000	>1000	9.9

For explanations of ▲ and ▼, see Scheme 1; **β** = β-homoglutamine; other substitutions are marked in bold. [a] Competitive binding assays were performed as described previously using ^{3}H-NPY (Amersham) as the radioligand.[3] The K_i values were determined by using the Cheng–Prusoff equation with K_d values for ^{3}H-NPY of 0.18 nM for the Y_1 receptor and 2.4 nM for the Y_5 receptor. [b] All peptides were N-terminally acetylated, except for peptides **6** and **7**. [c] $\Delta[\Theta]_R$ [10^{-3} deg cm^2 dmol^{-1}] is the difference of the mean-residue molar ellipticity values $[\Theta]_R$ at 222 nm, obtained from the CD spectra in buffer and in the presence of 30% TFE. Peptide concentrations were 30 μM. [d] K_i value previously published.[8]



Scheme 1. The synthesis of β-ACC-containing dipeptides for facile introduction into peptide sequences: a) 1) HCl(g)/EtOAc; 2) Fmoc-Arg(Pmc)-OH, EDC, pyridine, 88–97%; b) Pd/C, 1,4-cyclohexadiene (5 equiv), MeOH, 77–88%. Bn = benzyl, Boc = *tert*-butoxycarbonyl, EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, Fmoc = 9-fluorenylmethyloxycarbonyl, Pmc = 2,2,5,7,8-pentamethylchroman-6-sulfonyl.



Scheme 2. A C-terminal NPY analogue (peptide **8**) containing two (▲)-configured β-ACC residues.

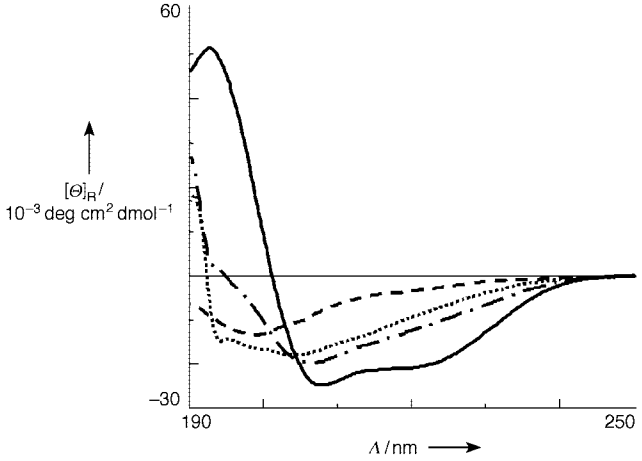


Figure 1. CD-spectra of the unsubstituted C-terminal NPY fragment NPY(25–36) in 20 mM phosphate buffer (pH 7.0; – – –) and in the presence of 30% TFE (——), in comparison with the β-ACC disubstituted analogue (peptide **8**) in buffer (••••) and in 30% TFE (•–•–), respectively. Peptide concentrations were 30 μM.

investigated receptors. Peptides **6** and **7**, which contain a β-ACC residue at position 32, were biologically inactive in both configurations. Combination of the two β-ACC-substitutions with the same (▲)-configuration of the cyclopropane ring in positions 32 and 34 did not improve affinity at the Y_1 receptor (peptides **8** and **10**, Scheme 2), but led to more rigid peptides such as can be seen in Figure 1. In peptide **10**, even the substitution of an Ile by an Arg residue in position 31 is tolerated at the Y_1 receptor, if it is combined with the bioactive (▲)-configuration of the β-ACC residues in positions 32 and 34. However, peptide **10** is slightly less selective, as its affinity at the Y_5 receptor increased by approximately five fold. This increase could arise from a new ionic interaction of the Arg31 side chain with the Y_5 receptor. If the opposite (▼)-configuration of the β-ACC residue is used in position 32 and 34, the disubstituted peptide **11** will remain biologically inactive. Peptide **9**, with the (▼)-configured β-ACC residue in position 32 but with a bioactive (▲)-β-ACC derivative in position 34, shows reduced affinity to the Y_1 receptor. Interestingly, the further shortened β-ACC-disubstituted octapeptide **12**, with two (▲)-configured β-ACCs, still shows moderate affinity. Accordingly, these four peptides (**4**, **8**, **10**, and **12**) are the first and only linear NPY segments with affinity and selectivity at the Y_1 receptor.

A recently reported disulfide-bridged, cyclic, C-terminal NPY analogue shows similar affinities at the Y_1 receptor to those of the linear β-ACC-substituted peptides **4**, **8**, and **10** reported herein.[9] Accordingly it can be assumed that β-ACC substitutions cause a similarly constrained arrangement of the critical side chains in linear peptides to that which can otherwise be obtained by simple cyclization. It is remarkable that similar NPY analogues containing the natural conformationally restricted amino acid proline are biologically inactive at all investigated receptors (peptides **13** and **14**). Similarly, the introduction of the β-amino acid β-homoglutamine[10] (**β**) into position 34 yielded also only an inactive peptide **15**. Accordingly, the observed affinities at the Y_1 receptor are exclusively evoked by a substitution with the conformationally constricted (▲)-β-ACC derivative in position 34 or in combination with a similarly configured β-ACC residue in position 32.

For the unsubstituted C-terminal fragment, conformational studies by circular dichroism (CD) showed the typical CD spectrum of a random peptide in buffer and also a high α-helical content in the presence of the α-helix-promoting additive 2,2,2-trifluoroethanol (TFE), whereas the β-ACC-containing analogues behaved completely differently (Figure 1). A higher content of structured peptide with a minimum around 206 nm was found and a significantly reduced tendency of α-helix formation was observed in the presence of TFE, as shown by the $\Delta[\Theta]_R$ values in Table 1. The $\Delta[\Theta]_R$ values are obtained by subtracting the $[\Theta]_R$ values at 222 nm in the presence of TFE from those obtained in buffer. From these studies it can be concluded that β-ACC substitutions stabilize distinct backbone conformations and thereby significantly reduce the tendency towards α-helix formation in helix-promoting environments such as TFE or membranes.

Thus the β-ACC disubstituted and truncated NPY analogues **8** and **10** are the most rigidified peptides but have still the structural requirements for biological activity at the Y_1 receptor; to date such reqirements can neither be induced with natural amino acids nor with analogous β-amino acids. These peptides are therefore most suitable for further structural investigations and for the development of new lead structures in pharmaceutical chemistry. Further studies are currently under investigation to gain more insights into the peptide structure and the influence of the configuration of the cyclopropane ring system on the secondary structure. Accordingly, we could show that β-ACC residues are extremely useful for the rigidification of peptides. The application of this concept to other systems could lead to new types of peptidomimetics.

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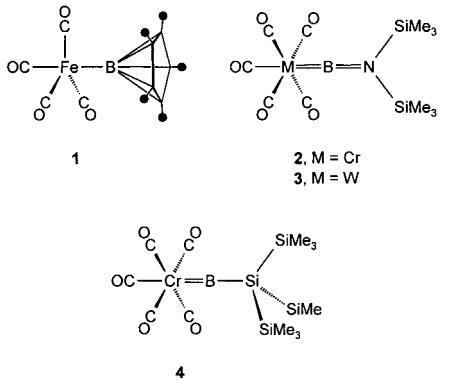
Borylene Half-Sandwich Complexes

$[(\eta^5\text{-}C_5H_5)(OC)_3V{=}B{=}N(SiMe_3)_2]$: A Half-Sandwich Complex with a Terminal Borylene Ligand**

Holger Braunschweig, Miriam Colling, Chunhua Hu, and Krzysztof Radacki*

Dedicated to Professor Thomas P. Fehlner on the occasion of his 65th birthday

In contrast to the rather well-developed chemistry of bridged borylene complexes $L_xM{-}B(R){-}ML_x$[1] their terminal counterparts $L_xM{=}B{-}R$ are still exceptionally rare and restricted to five structurally authentic examples[2]—two of which,



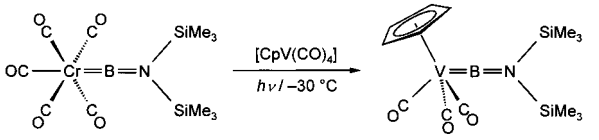
1 — 2, M = Cr; 3, M = W — 4

Figure 1. Structurally authentic terminal borylene complexes that have been obtained by salt elimination reactions.

however, comprise boron in higher coordination numbers than two.[3] Most of these borylene complexes (Figure 1) were obtained by salt elimination reactions from dianionic carbonylmetallates and suitable dihaloboranes. Although this method of preparation was initially very successful, it appears to be limited to the use of the homoleptic carbonylmetallates $Na_2[Fe(CO)_4]$ and $Na_2[M(CO)_5]$ (M = Cr, W), despite the rather broad availability of dianionic transition metal complexes and corresponding synthetic equivalents.[4] Thus, combination of terminal borylenes with any coligand other than carbonyl is precluded as yet, although such complexes with various ligands are of significant interest.[5]

Recently, we reported on the photochemically induced intermetallic borylene transfer as a novel and potentially useful way of synthesizing both bridged and terminal borylene complexes.[2b] However, as far as the latter were concerned at that time, this route provided only an alternative access to the chromium complex $[(OC)_5Cr{=}B{=}N(SiMe_3)_2]$, which we obtained somewhat earlier by conventional salt elimination.[2a] Further exploitation of this method has now led to the synthesis and full characterization of $[(\eta^5\text{-}C_5H_5)(OC)_3V{=}B{=}N(SiMe_3)_2]$ (**5**), the first half-sandwich complex with a terminal borylene ligand.

The title compound **5** was formed upon irradiation of $[(OC)_5Cr{=}B{=}N(SiMe_3)_2]$ (**2**) in the presence of $[(\eta^5\text{-}C_5H_5)V(CO)_4]$ for four days at −30 °C in toluene according to Equation (1). Compound **5** was separated by fractional crystallization and isolated in 42 % yield as a dark yellow



(1)

2 — 5

crystalline material, which is readily soluble in all common aliphatic and aromatic solvents and only modestly air- and moisture sensitive.

Single crystals of **5** suitable for X-ray structural analysis were grown from hexane solutions at −80 °C. The compound crystallizes in the space group $P2_1/c$ and the molecule adopts a

[*] Prof. Dr. H. Braunschweig,[+] Dr. K. Radacki
Department of Chemistry
Imperial College
London SW7 2AY (UK)
E-mail: h.braunschweig@mail.uni-wuerzburg.de
Dr. M. Colling, Dr. C. Hu
Institut für Anorganische Chemie
Technische Hochschule
52056 Aachen (Germany)
[+] New address:
Institut für Anorganische Chemie der Universität Würzburg
Am Hubland, 97074 Würzburg (Germany)
Fax: (+49) 0931-8884623

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