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Alicyclic β-amino acids in Medicinal Chemistry

Review Article

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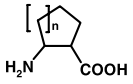
Summary. The structural element of alicyclic β-amino acids shows some remarkable biological effects: For some 5- and 6-membered β-amino acids a unique anti fungal activity has been observed, 7-membered β-amino acid derivatives have been investigated for neurological disorders. The application of 5-, 6- and 7-membered alicyclic β-amino acids in Medicinal Chemistry will be reported.



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Keywords: Beta-amino acid – Medicinal Chemistry – Antifungal – Cispentacin – Icofungipen – Asymmetric synthesis

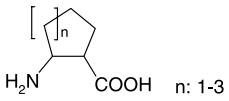
Introduction

Although an increasing number of powerful tools for the synthesis of aliphatic β-amino acids are available to the synthetic chemist (Juaristi, 1997; Cole, 1994) only a few attempts have been made to synthesize alicyclic derivatives thereof. Cyclic β-amino acids of the general formula **1**, in which both the β-amino and the acids functionality are vicinally attached to an aliphatic ring still represent a demanding challenge to the synthetic chemist. One reason for this lies in the intriguing difficulty associated with controlling the absolute and relative stereochemistry of two adjacent stereocenters. Herein we give a comprehensive review (see also v. Nussbaum and Spiteller, 2004) on the synthesis and pharmacological activity of five, six and seven membered alicyclic β-amino acids with relevance to medicinal chemistry.

Antifungal cyclic five and six membered β-amino acids

Alicyclic five and six membered β-amino acids of the general formula **1** have received only little interest in Medicinal Chemistry until the late eighties, when Hashimoto and Konishi et al. independently reported on the isolation of a potent antifungal from the cell broth of *Bacillus cereus* or *Strepomyces setonii*. The structure elucidation of the natural product named FR-109615 or cispentacin revealed an alicyclic β-amino acid with the structure **2** (Scheme 2). Cispentacin (**2**) exhibits a strong antifungal *in vitro* activity against various Candida strains, e.g. *Candida albicans*, *Candida krusei* and *Candida utilis*. It showed weak *in vitro* activity against *Trichophyton mentagrophytes*, while no *in vitro* activity was observed against *Cryptococcus* and *Aspergillus* species. In addition, cispentacin (**2**) displayed a potent therapeutic efficacy against a lethal lung *Candida albicans* infection in immuno-compromised mice. *I.v.* administration of 50 mg/kg cispentacin (**2**) led to a 100% survival rate (Tomatsu et al., 1989).

Life-threatening systemic fungal infections have been recognized as a major cause of mortality and morbidity during the last 20 years with a continuously rising incidence caused by the increasing number of immunocompromised patients (Pfaller, 1995; Dixon et al., 1996; Edmond et al., 1999). There are only a few therapeutic options that are limited due to either narrow therapeutic window, limited dosages forms or rapid emergence of resistance as in the case of azole-antimycotics such as fluconazole. Therefore,



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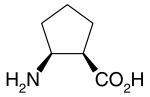
Scheme 1. General formula of alicyclic β-amino acids



Cispentacin 2

Scheme 2. Antifungal β-amino acid cispentacin (**2**)

new orally available antifungals with improved efficacy and tolerability are urgently needed (Hossain et al., 2000).

In this context, cispentacin (**2**) displayed an intriguing, new lead structure for an antifungal, for which an structure-activity-relationship (SAR) had to been exploited. For this purpose several synthetic approaches to cispentacin (**2**) and derivatives have been investigated. However, the first synthesis of racemic cispentacin (**4**) was accomplished years before its isolation by Nativ and Rona et al. in 1972 *via* [2 + 2] cycloaddition of chlorosulfonyl isocycante to cyclopentene followed by hydrolysis of the intermediate β-lactam **6** (Scheme 3).

Enantiomerically pure cispentacin (**2**) and its antipode **3** were obtained for the first time by Kawabata et al. (1990) through separation of diastereomeric dipeptides followed by Edman degradation (Scheme 4). He determined the absolute configurations of *cis*-(1*R*, 2*S*) cispentacin (**2**) and its (1*S*, 2*R*)-antipode **3** through X-ray crystallographic analyse of diastereomers **7a** and **7b**.

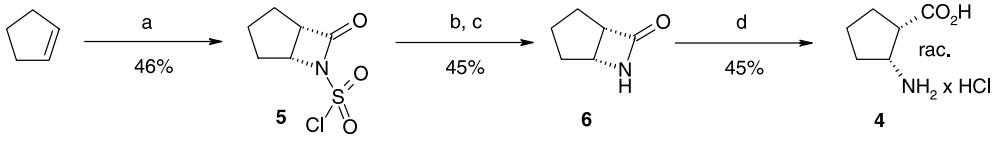
The first asymmetric synthesis of cispentacin (**2**) was accomplished by Davies et al. (1993) *via* diastereoselective Michael addition. The homochiral lithium (*S*)-(α-methylbenzyl)-benzylamide **8** was added to *tert*-butyl 1-cyclopentene-1-carboxylate **9** to afford the protected β-amino acid **10** in 65% yield and >98% diastereomeric excess. Cispentacin (**2**) was obtained through debenzylation, ester hydrolysis and ion exchange chromatography in 72% yield (Scheme 5).

Recently, Aggarwal et al. (2003) reported a new asymmetric approach to (−)-cispentacin (**2**) *via* a highly diastereoselective intramolecular nitrone cycloaddition. 1,3-Dithiane **11** was converted in two steps to the enantiomerically pure phosphonate **12**. Subsequent, Horner-Wadsworth-Emmons olefination with 5,5-dimethoxypentanal provided ketene thioacetal **13**. Hydrolysis of the dimethoxyacetal **13** followed by addition of *N*-benzylhydroxylamine led to the [3 + 2]-cycloaddition product **14**. (−)-Cispentacin (**2**) was obtained in 85% yield after reductive N–O bond cleavage and debenzylation (Scheme 6).

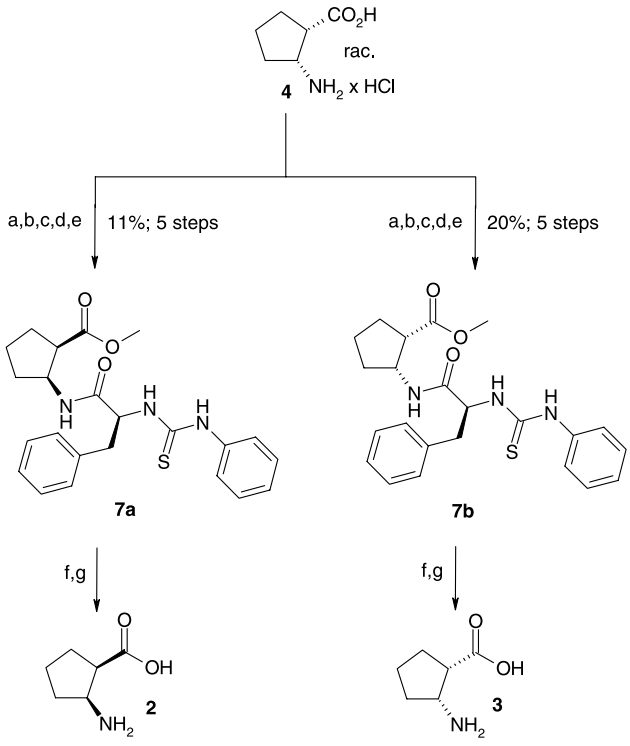
Further asymmetric syntheses of cispentacin (**2**) have been reported (Konosu et al., 1993; Theil et al., 1996; Mittendorf et al., 1995, 1997; Bolm et al., 2001; Fülöp et al., 2001; Zhang et al., 2003).

Cispentacin (**2**) and its stereoisomers **3** and **15** were tested for their antifungal activity against *C. albicans* (Kawabata et al., 1990). Only cispentacin (**2**) showed antifungal activity. Both, its antipode **3** and *trans*-diastereomer **15** were inactive *in vitro* against *Candida albicans* and *Candida tropicalis* (Table 1).

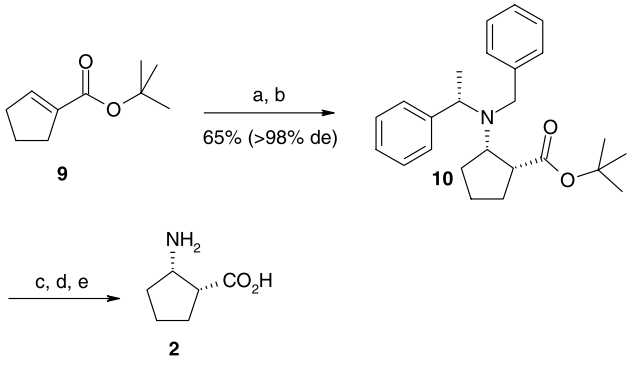
To explore the SAR of antifungal cispentacin β-amino acids Ohki et al. (1991) synthesized several derivatives of cispentacin (**2**) and described their antifungal *in vitro* activity against *Candida albicans* (Table 2). Among the derivatives modified at the carboxyl and amino group, the simple amides **16**, **17**, the primary alcohol **19** and the methyl ester **20** were inactive. The *N*-dimethylamino carboxylic acid **18** and ring expanded 2-aminohexane carboxylic acid **21** were also inactive. From these results, Ohki et al. concluded that both functional groups, the carboxylic acid and the primary amino group, were necessary for potent antifungal activity. On the other hand, Ohki et al. observed that several dipeptides of cispentacin exhibited potent *in vitro* activity against *Candida albicans*. The configuration of the α-amino acid in these dipeptides was critical to activity. Only the (*S*)-amino acid derivatives **22** and **24** showed a potent activity against *C. albicans*, whereas the (*R*)-amino acid derivatives **23** and **25** were almost inactive (Table 2). It seems reasonable to assume that the strong antifungal activity of the (*S*)-amino acid derivatives was caused by proteolytic cleavage under the



Scheme 3. Synthesis of racemic β-amino acid **4** *via* [2 + 2] cycloaddition. **a** $ClSO_2NCO$, −78°C to 8 h 0°C then rt overnight; **b** KI; $NaHSO_4$; **c** NaOH, pH 7; **d** conc. HCl, 0°C 3 h



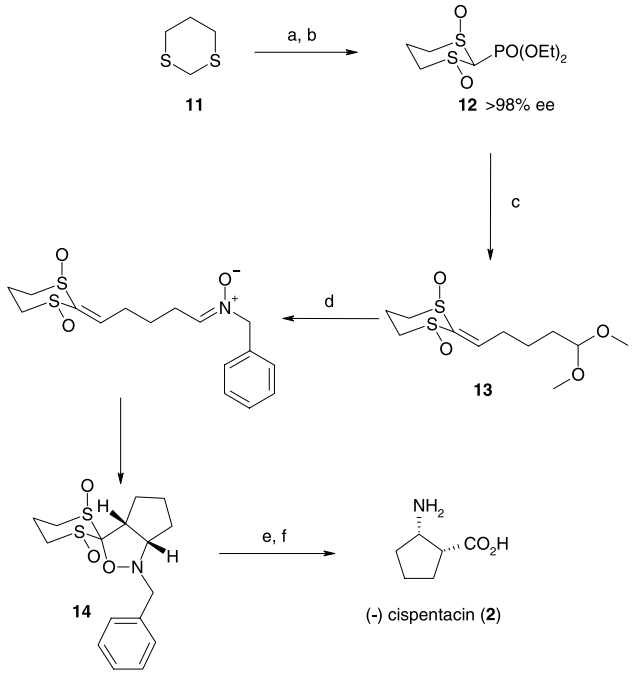
Scheme 4. Synthesis of (−)-cis-(1*R*, 2*S*)-cispentacin (**2**) and (+)-cis-(1*S*, 2*R*) **3**. **a** $SOCl_2$, MeOH; **b** *N*-Boc-L-Phenylalanin, WSCD, HOBT CH_2Cl_2; **c** fractional crystallization; **d** 4-*N*-HCl, EtOAc; **e** Phenyl isothiocyanate, EtOH, reflux; **f** 4-*N*-HCl, CH_2Cl_2; **g** water, pH 6.7, anion exchange resin. WSCD: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide



Scheme 5. Synthesis of (−)-cis-(1*R*, 2*S*)-cispentacin (**2**) by Davies et al. (1993). **a** Lithium (*S*)-(α-methylbenzyl)benzylamide **8**; THF, −95°; **b** 2,6-di-*tert*-butylphenol, −78°C, 65%, >98% de; **c** Acetic acid, H_2, Pd/C; **d** methanolic HCl; **e** ion exchange chromatography Dowex 50X8-200

assay conditions to release cispentacin (**2**). Overall, Ohki et al. observed a strict structural requirement for antifungal activity in their limited SAR study on cyclic β-amino acids.

Independently, scientists at BAYER AG discovered that the 2-aminohexene carboxylic acid **26**, which was originally designed as a pyridoxal phosphate suicide inhibitor

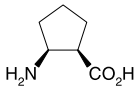
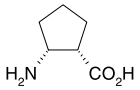
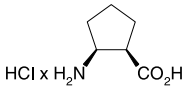
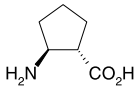


Scheme 6. Synthesis of (−)-cis-(1*R*, 2*S*)-cispentacin (**2**) by Aggarwal et al. (2003). **a** (i) NCS, benzene, rt, 24 h (ii) $P(OEt)_3$, 60°C, 4 h, 78%; **b** $PhC(CH_3)_2OOH$, $Ti(O^iPr)_4$, (+)-DET, CH_2Cl_2, 72 h, 43%, >98% ee; **c** 5,5-Dimethoxypentanal, LiOH, THF, 80°C, 4 h, 80%; **d** $PdCl_2(CH_3CN)_2$, acetone, 60°C, 1 h then BnNHOH × HCl and $NaHCO_3$, rt, 16 h, 70%; **e** Pd/C, AcOH, H_2, 48 h, 65%; **f** $Pd(OH)_2$-C, Et_3N, EtOH, 40°C, H_2, 4 h, 85%. (+)-DET = diethyl tartrate

(Kunisch et al., 1992), exhibited a strong antifungal activity against *Candida albicans* (Table 3). However, in toxicological studies cyclohexene β-amino acids **26** showed a less favorable profile. Along with the reported antifungal activity of the natural β-amino acid cispentacin (**2**) these findings prompted Mittendorf et al. (1993a, 2003a) to initiate a derivatization program in order to investigate the SAR of cyclohexene β-amino acid **26** and cispentacin (**2**) with the aim to identify cyclic β-amino acids with a superior efficacy/tolerability profile.

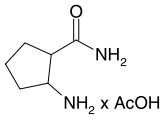
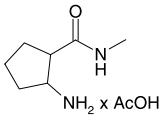
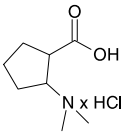
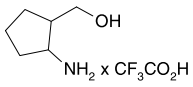
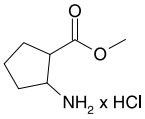
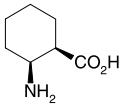
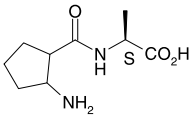
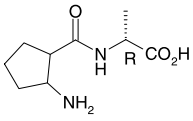
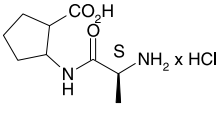
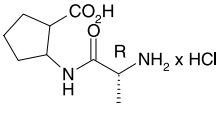
In this context, a variety of structurally interesting β-amino acids listed in Tables 3–5 was synthesized and evaluated for their antifungal *in vitro* activity against *C. albicans*. Many of these β-amino acids were prepared following known methods (see Fülöp et al., 2001; Juaristi et al., 1999). However, for several β-amino acids new synthetic routes were developed. Most notably, a short and efficient asymmetric synthesis *via* asymmetric desymmetrisation of *meso*-anhydrides was established, as exemplified for the *exo*-methylen β-amino acid **43** (Scheme 7). In the key step, a highly enantioselective, quinine-mediated alcoholysis of the *meso*-anhydride **62** provided cinnamyl ester **63** (84% yield) with ee ≥97%. Subsequent Curtius

Table 1. Antifungal activity of 2-aminocyclopentane-1-carboxylic acid derivatives (**2**, **3**, **4** and **15**) against *Candida albicans* and *Candida tropicals*

Compound	Structure	MIC [μg/ml] *C. albicans* FP 578[b]	MIC [μg/ml] *C. tropicalis* FP 583[b]
2		6.25	6.25
3		>100	>100
4[a]		12.5	12.5
15[a]		>100	>100

[a] Racemic
[b] MICs were determined by the agar dilution method using minimum essential medium (MEM) agar after incubation at 37°C for 18 h with inoculum size of about 10^6 cfu/ml

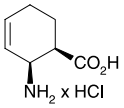
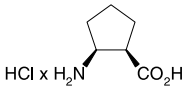
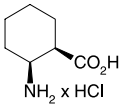
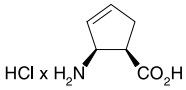
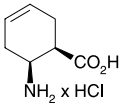
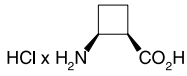
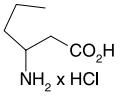
Table 2. SAR studies by Ohki et al. (1991)

Compound	Structure	MIC (μg/ml)[b] *C. albicans*	Compound	Structure	MIC (μg/ml)[b] *C. albicans*
16		>100	**17**		>100
18[a]		>100	**19**[a]		>100
20[a]		>100	**21**[a]		>100
22[a]		1.56	**23**[a]		100
24[a]		12.5	**25**[a]		>100

[a] Racemic
[b] MICs were determined by EAGLE's MEM agar (Nissui), 10^5 cfu/ml. Streak method, 30°C, 24 h

rearrangement and Pd-catalyzed removal of the cinnamyl protecting groups afforded **43** with ee $\geq$99.5%. This process was successfully used to produce 5 kg of β-amino acid **43**. The absolute (1*R*, 2*S*)-configuration of **43** was assigned by X-ray crystallography. Other β-amino acids, that were prepared following this new synthetic route include cispentacin (**2**) and the heterocyclic β-amino acid **33**. The corresponding antipodes with (1*S*, 2*R*)-configuration,

Table 3. Influence of double bond geometry and ring size on antifungal activity

Compound	Structure	IC_{50} (mg/l)[b] *C. albicans*	Compound	Structure	IC_{50} (mg/l)[b] *C. albicans*
26		0.03	**2**		0.13
27[a]		128	**28**[a]		0.5
29[a]		128	**30**[a]		128
31[a]		256			

[a] Racemic

[b] IC_{50} values were determined after incubation with *C. albicans* ATCC 36082 YNB medium (yeast nitrogen base powder). Read-out was performed photo-/nephelometrically on Spectra III Elisa reader at 360 nm

e.g. *exo*-methylene derivative **44** could be obtained following the same synthetic route except that quinidine instead of quinine was used as chiral auxiliary (Mittendorf et al., 1995, 1997). In addition, several structurally interesting new β-amino acids like the cyclopropyl β-amino acid **42** were prepared starting from protected *exo*-methylene derivative **43** (Mittendorf et al., 2003a).

The structurally unique β-amino acids **51** and **52** were prepared *via* Hofmann degradation. [2 + 2] Cycloaddition of 1-*H*-pyrrole-2,5-dione (**65**) and allene provided cycloadduct **66** in 43% yield. A subsequent Hofmann degradation using potassium hypochlorite afforded a mixture of the 2-amino-cyclobutane carboxylic acid derivatives **67** and **68**. These were separated and hydrolyzed to provide the *exo*-methylen-amino acids **51** and **52** as racemic mixtures in 68% and 23% yield, respectively (Scheme 8).

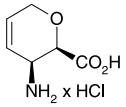
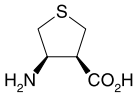
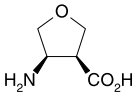
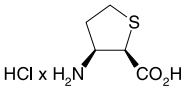
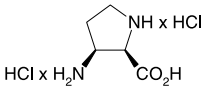
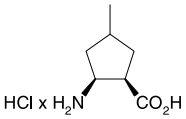
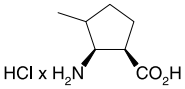
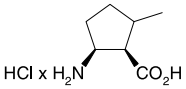
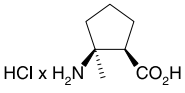
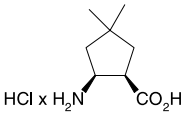
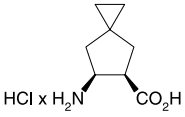
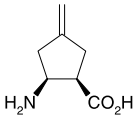
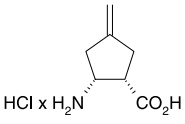
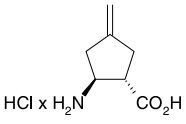
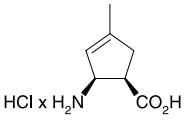
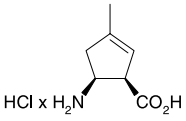
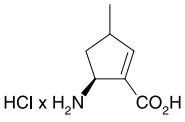
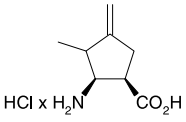
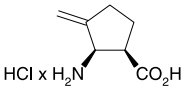
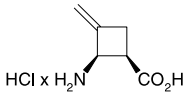
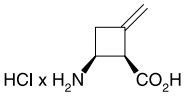
All compounds illustrated in Tables 3–5 were tested for their inhibitory activity against *C. albicans* (for assay condition see Mittendorf et al., 2003a). In this assay, the natural product cispentacin (**2**) and the β-amino cyclohexene carboxylic acid **26** demonstrated a strong antifungal *in vitro* activity against *C. albicans* (IC_{50} 0.13 and 0.03 mg/l, respectively) (Table 3). Ring size and double bond position have a strong impact on the antifungal activity (Table 3). Dehydro-cispentacin **28** showed only slightly lower potency ($IC_{50} = 0.5$ mg/l). Transposition or hydrogenation of the double bond in **26** resulted in a significant loss of activity (examples **27**, **29**). Additionally, a ring contraction and a ring opening led to a dramatic loss of *in vitro* potency (examples **30**, **31**).

Similarly, the introduction of heteroatoms on different positions in the five and six membered ring led to less active compounds (see Table 4, Examples **32**–**36**). Even a mono substitution (example **37**–**40**) on almost all positions of the cyclopentane ring or a dimethyl substitution (example **41**) had a negative impact on antifungal activity (Table 4).

The SAR at position 4 of the cyclopentane ring was investigated in more detail. Among these derivatives (**42**–**48**), only the introduction of an *exo*-methylene group resulted in strong antifungal activity. The *exo*-methylen derivative **43** ($IC_{50} = 0.13$ mg/l) was equipotent to cispentacin (**2**) and was selected for further derivatizations. Again the (1*R*, 2*S*)-configuration of **43** turned out to be essential since stereoisomeres **44** and **45** demonstrated only weak antifungal activity. Once more, a very narrow SAR was observed, resulting in significant loss of potency, when the double bond of **43** was shifted to different *endo* positions and small substituents were introduced (example **46**–**49**). Also a shift of the *exo*-methylene group from the 4- to the 3-position of the cyclopentane ring or a ring size contraction led to a significant loss of potency in comparison to **43** (see example **50**–**52**, Table 4).

In order to investigate the role of the carboxylic acid moiety on the SAR, Mittendorf et al. (2003a) introduced several acid isosteres. Similar to Ohki's studies (see above), they observed that the carboxylic acid functionality

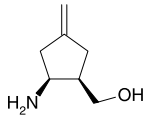
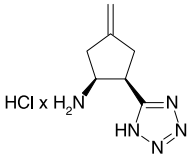
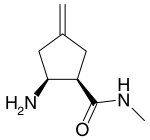
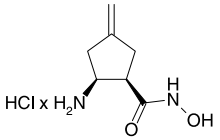
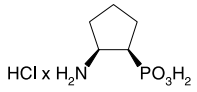
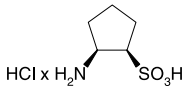
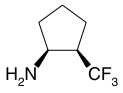
Table 4. *In vitro* activity of different cyclopentane derivatives against *Candida albicans*

Compound	Structure	IC_{50} (mg/l)[d] *C. albicans*	Compound	Structure	IC_{50} (mg/l)[d] *C. albicans*
32		64	**33**		64
34[a]		32	**35**[a]		>256
36[a]		>256	**37**[b,c] **(5:1 m.d.)**		4
38[b,c] **(4:1 m.d.)**		128	**39**[a]		diast. A[c] 8 diast. B[c] 16
40[a]		64	**41**[a]		256
42		32	**43**		0.13
44		128	**45**		128
46		8	**47**		32
48[b,c] **(3:1 m.d.)**		16	**49**[a,c] **(single diast)**		64
50[a]		32	**51**[a]		>256
52[a]		>256			

[a] Racemic
[b] m.d., mixture of diastereomers
[c] Configuration not known
[d] IC_{50} values were determined after incubation with *C. albicans* ATCC 36082 YNB medium (yeast nitrogen base powder). Read-out was performed photo-/nephelometrically on Spectra III Elisa reader at 360 nm

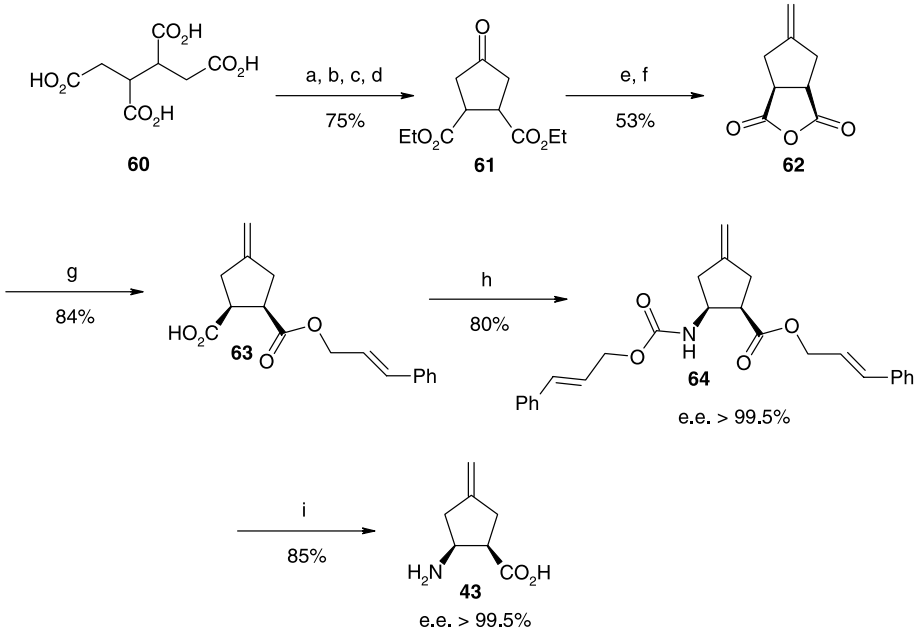
was essential for potency against *Candida albicans* (example **53**–**58**). Surprisingly, only a replacement of the carboxylic acid group by trifluoromethyl resulted in the moderately active derivative **59** ($IC_{50} = 1$ mg/l; racemic mixture) (Table 5). However, the trifluoromethyl substituted derivative **59** showed no antifungal efficacy *in vivo*,

Table 5. *In vitro* activity against of different acid isosteres *Candida albicans*

Compound	Structure	IC_{50} (mg/l)[b] *C. albicans*	Compound	Structure	IC_{50} (mg/l)[b] *C. albicans*
53		>256	**54**[a]		128
55[a]		32	**56**[a]		32
57[a]		>256	**58**[a]		>256
59[a]		1			

[a] Racemic

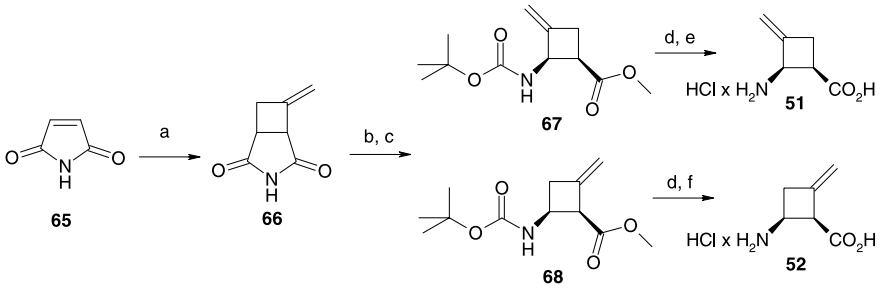
[b] IC_{50} values were determined after incubation with *C. albicans* ATCC 36082 YNB medium (yeast nitrogen base powder). Read-out was performed photo-/nephelometrically on Spectra III Elisa reader at 360 nm



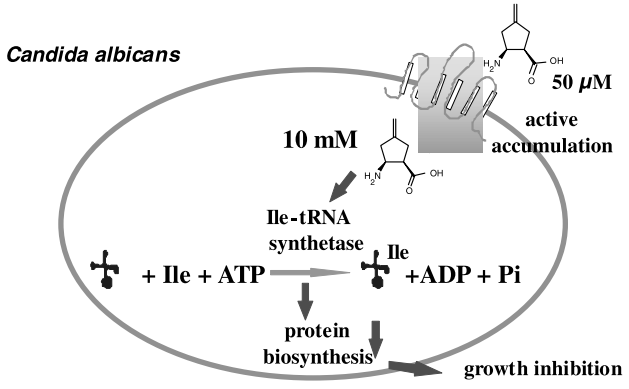
Scheme 7. Asymmetric synthesis of β-amino acid **43**. **a** EtOH, H_2SO_4; **b** NaOMe, MeOH; **c** HCl, H_2O; **d** EtOH, H_2SO_4, 75%; **e** $Ph_3PMe^+Br^-$, KotBU, THF, then KOH, THF, H_2O, 71%; **f** $(EtCO)_2O$, 135°C, 75%; **g** 1.0 equiv. Quinine, 1.5 equiv. (2E)-3-phenyl-2-propene-1-ol, toluene, −15°C, 4 h, 84%; **h** $(PhO)_2PON_3$, NEt_3, toluene, 90°C, then 3-phenyl-2-propane-1-ol, toluene, reflux, 80%; **i** 0.05 mol% $Pd(OAc)_2$, PPh_3, morpholine, EtOH, 85%

probably due to high plasma protein binding (Mittendorf et al., 1993b,c).

In summary, after an extensive chemical optimization program an unusually steep SAR for antifungal activity of β-amino acids was observed. Only the novel β-amino acids **43** was identified as an analogue displaying antifungal activity in the same range as the lead structures cispentacin (**2**) and the cyclohexene derivative **26**.



Scheme 8. Synthesis of 2-amino-*exo*-methylen-cyclobutane carboxylic acids **51** and **52** *via* Hofmann degradation. **a** Allene, CH_2Cl_2, hv, $-70°C$, 43%; **b** KOCl, KOH, H_2O, then $(Boc)_2O$, Na_2CO_3, dioxane; **c** DCC, DMAP, MeOH, CH_2Cl_2, 34% (**51**) and 8% (**52**); **d** LiOH, H_2O, THF; **e** 4-*N*-HCl, dioxane, 68%; **f** TBDMS-OTf, 2,6-lutidine, CH_2Cl_2, 23%



Scheme 9. Unique dual mode of action of β-amino acid **43**. Unique dual mode of action of β-amino acid **43**

Ziegelbauer et al. (1998) recently elucidated β-amino acids **43** displays its antifungal activity against *C. albicans* through a unique dual mode of actions. First, **43** is accumulated about 200-fold in yeast cells by active transport *via* permeases specific for branched-chain α-amino acids. Inside the cell, **43** inhibited specifically iso-leucyl-tRNA synthetase, resulting in inhibition of protein synthesis and cell growth (Scheme 9). In contrast, active transport and inhibition of protein synthesis of cispentacin (**2**) appears to be mediated by the corresponding enzymes specific for proline (see also Ziegelbauer et al., 1998). The dual mode of action makes the optimization on the actual target impossible and allows the generation of SAR only for combination of both targets. This and the fact that the β-amino acid have to act as mimetics of small α-amino acids may explain the observed narrow SAR of antifungal β-amino acids.

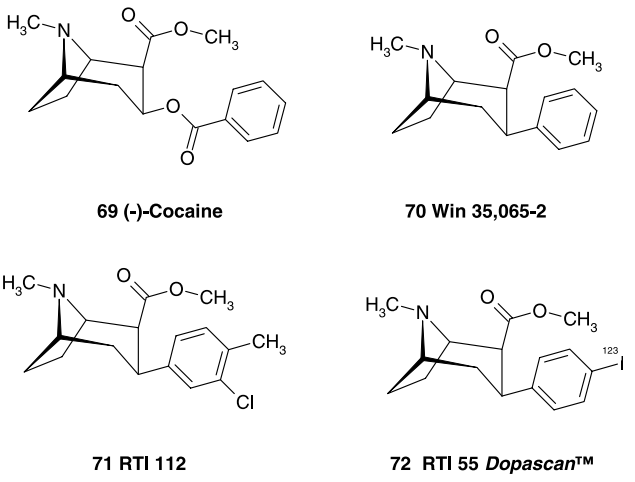
β-Amino acid **43** showed the most favorable activity-tolerability profile of all β-amino acids so far prepared and was therefore selected for further development. β-Amino acid **43** showed strong *in vivo* activity for clinical strains of the main pathogen *C. albicans* but also on all other non-*albicans* Candida strains, including, most importantly, azol resistant strains. It showed also activity against dermatophytes but no activity against *Aspergillus fumigatus* and *Pneumocystic carinii*. In *in vivo* animal studies β-amino acid **43** showed high efficacy in mouse, rat and rabbit model of systemic *C. albicans* infection, including fluconazole resistant strains. In a lethal challenge model the β-amino acid **43** achieved a 100% survival rate at various dosing regimes (10 mg/kg/day, rats; 10 mg/kg twice daily, mice). With *in-vivo* efficacy against fluconazole resistant strains **43** showed no cross-resistance, as expected by the previously described novel mode of action. Even if the treatment was started 24 h after infection a complete protection in rats was achieved (Schoenfeld et al., 2001). In addition, it showed significant activity in non-lethal *Candida glabrata* and *Candida krusei* infection models. Again, no activity was observed in systemic *Aspergillus fumigatus* and *Pneumocystic carinii* models. This seems to be the only drawback of β-amino acid **43**. However, Candidosis is the most frequent systemic fungal infections and accounts for more than 80% of reported cases. Recently, Walsh et al. (2004) reported a dosage dependent *in vivo* efficacy (immunocompromised rabbits) of **43** against experimental oropharynlgeal and esophageal candidiasis caused by fluconazole-resistant *C. albicans*.

The pharmacokinetic profile was also remarkable. After oral administration the β-amino acid **43** revealed a high bioavailability of 60% in mice and 100% in rats, rabbits and dogs. The plasma half life increased from 3 hours (mouse) to 10 hours (dog). Phase I studies showed that these pharmocokinetic profile is transferable to men. The β-amino acid **43** was rapidly absorbed after an oral administration with an oral bioavailability over 80%. The half life was constant over a various dose range with a mean value of approximately 7 hours. Moreover, β-amino acid **43** showed a favorable tolerability and safety profile after multiple oral dosing in healthy volunteers (see also Schoenfeld et al., 2001).

In 2000, β-amino acid **43** (BAY 10-8888) was licensed to the Croatian pharmaceutical company PLIVA, where it is now named icofungipen (PLD-118). Based on the very promising results in preclinical and phase I clinical studies, a phase II clinical study was initiated with this compound being the only new compound in development for oral treatment of yeast infection (see King et al., 2004).

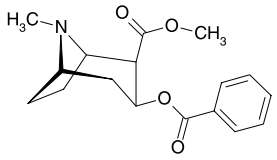
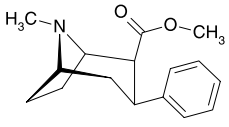
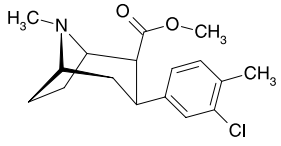
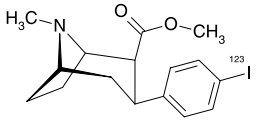
CNS-active cyclic seven membered β-amino acids

Alicyclic seven membered β-amino acids of the general formula **1** did not find wide application in medicinal chemistry: The seven membered homologues of β-amino acid **26** and **43** neither exhibit antifungal activity (Mittendorf et al., 1993b) nor do they hit targets related to the central nerve system due to the special obstacle the blood brain barrier presents. However, some ester derivatives easily penetrate into the brain, especially, if they are related to the tropane alkaloid framework in which the amino functionality is embedded in an additionally fused five membered ring (Scheme 10). The tropane alkaloide cocaine (**69**), formally a seven membered cyclic β-amino acids methyl ester displays high affinity to the dopamine transporter. This enzyme is located at the presynaptic nerve terminals and exerts a major role in the signal transduction between dopaminergic neurons. The inhibition of the re-uptake of previously released dopamine leads to an increased concentration of the neurotransmitter in the synaptic cleft and as consequence to a potentiation of the dopaminergic signal transduction. Therefore, the dopamine transporter presents a suitable target for addiction therapy (Caroll et al., 2003). The aim of potential therapeutics (Scheme 10) is hereby to block the binding of cocaine (**69**), which inhibits not only the re-uptake of dopamine and further monoamine neurotransmitters but also exerts effects on cholinergic muscarine receptors and Na^+-channels in the brain (Schoemaker et al., 1985;



Scheme 10. Seven membered cyclic β-amino acid esters. Seven membered cyclic β-amino acid esters

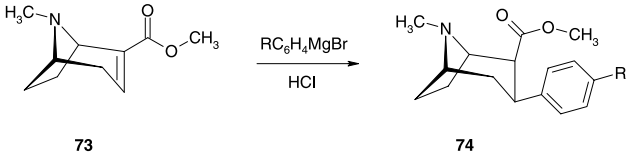
Table 6. Comparison of monoamine binding properties of different 3-aryltropanes to cocaine, (*DA*, dopamine; *5HT*, serotonin; *NE*, norepinephrine; *T*, transporter)

Compound	Structure	Name	IC_{50} DAT	IC_{50} 5HTT	IC_{50} NET
69		(−)-Cocaine	89 nM	1050 nM	3300 nM
70		Win 35,065-2	23 nM	1962 nM	920 nM
71		RTI 112	0.8 nM	10.5 nM	36.2 nM
72		RTI 55	1.3 nM	4.2 nM	36 nM

Madras et al., 1989; Reith et al., 1986; Caroll et al., 1992a, 2002; Kuhar et al., 1991).

A potential cocaine abuse treatment drug should therefore be selective for the dopamine transporter and have a favorable pharmacokinetic with flat dose response and long duration of action (Caroll et al., 1999). For this purpose, WIN 35,065-2 (**70**), RTI-112 (**71**) and related 3β-aryl tropane analogues are being investigated as dopamine re-uptake inhibitors with more than a 100-fold higher potency than cocaine (**69**) at the dopamine transporter (Table 6) (Caroll et al., 1992b, 1993, 1994; Deutsch et al., 1999; Kozikowski et al., 1993).

The synthesis of the homochiral analogues **70**–**72** usually commenced from naturally derived anhydroecgonine methyl ester (**73**) by Kharasch reaction (Scheme 11) (Carroll et al., 1991). Diastereoselective protonation yielded the 2β-orientated diastereomer. Many efforts were spent in order to achieve this step with the desired stereochemistry: Applying dry HCl at -78°C proved to give best results, yielding the 2β-orientated diastereomer as major isomer in a ratio of 9:1 (Davies et al., 1994).



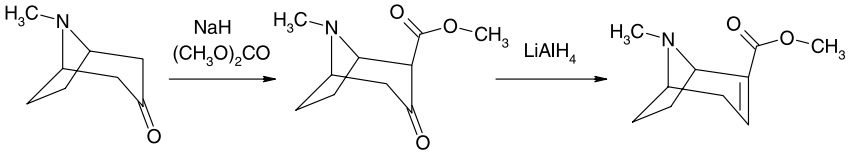
73 **74**

Scheme 11. Synthesis of enantiopure dopamine transporter inhibitors. Synthesis of enantiopure dopamine transporter inhibitors

An other suitable starting material is tropinone (**75**). Upon deprotonation and subsequent methoxycarbonylation the desymmetrized β-keto ester **76** is obtained. Further direct transformation to *rac*-anhydroecgonine methylester (*rac*-**73**) is accomplished upon treatment with $LiAlH_4$ (Findlay, 1957). It is also possible to prepare enantiopure (*R*)-analogues by this route, if the optical antipodes of **76** are separated *via* the corresponding *L*-tartaric acid salts (Scheme 12, Meltzer et al., 1994).

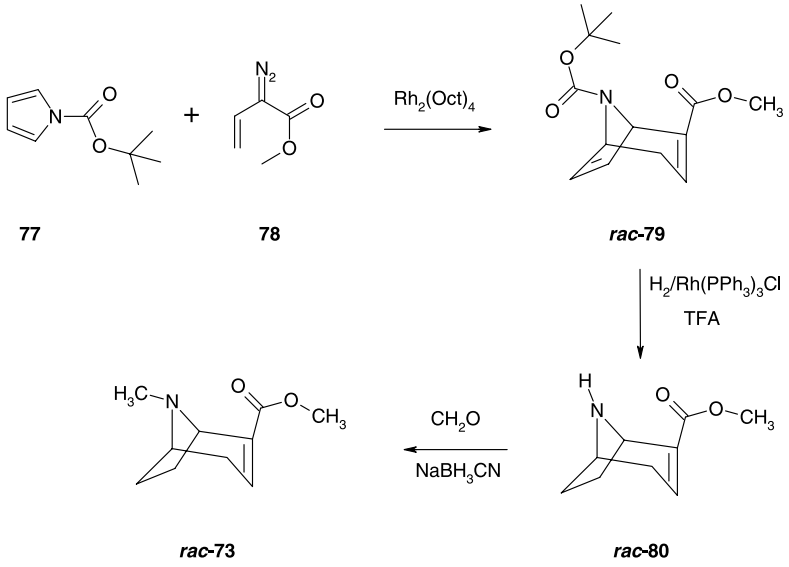
A straightforward diastereoselective total synthesis of racemic anhydroecgonine methyl ester (*rac*-**73**) applying a formal [3 + 4] cycloaddition (Scheme 13) was introduced by Davies et al. (1991). The reaction of protected pyrrole **77** with vinyl-diazomethane **78** led *via* cyclopropanation and subsequent Cope rearrangement to bicyclic diene **79**. Its partial hydrogenation followed by deprotection and reductive methylation of the bridging nitrogen afforded *rac*-**73**.

The SAR of the tropane alkaloid **69** and analogues thereof at the dopamine transporter was intensively exploited by



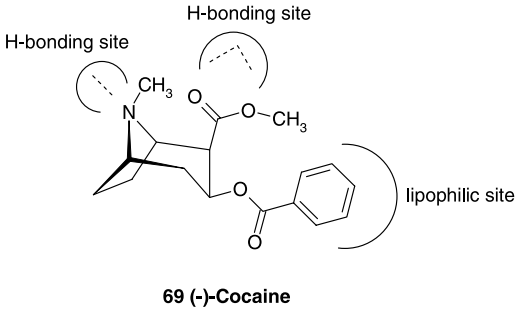
rac-75 **76** **rac-73**

Scheme 12. Synthesis of *rac*-dopamine transporter inhibitors *via* tropinone **75**



77 **78** ***rac*-79** ***rac*-73** ***rac*-80**

Scheme 13. Total synthesis of *rac*-dopamine transporter inhibitors *via* *rac*-**79**



Scheme 14. Structural requirements for binding at the dopamine transporter

Caroll (1991, 1992a), Meltzer (1994) and Davies et al. (1994). There are four structural requirements for binding at the dopamine transporter (Scheme 14).

Of major importance is the stereochemistry of the tropane ligand: only the (*R*)-enantiomer with 2β- and 3-β-configuration led to highly potent derivatives (Caroll and Melzer, 1994). In addition to the absolute stereochemistry, the tropane nitrogen is required for tide binding. An H-bond acceptor site displays the 2β-methoxy-carbonyl moiety, which can be replaced by bioisosteric groups (Fleckenstein et al., 1996). Next to these two polar sites a lipophilic 3β-aryl substituent is necessitated for binding. While a replacement of the 3β-benzoate group in cocaine (**69**) with a shorter linked phenyl group was only accompanied by a slight improvement of binding affinity, e.g. phenyl derivative **70**, a tremendous increase was observed, when the 3β-aryl group was substituted with lipophilic alkyl or halide groups in the *meta*- and *para*-position, e.g. analogue **71**. As a result of the described SAR, iodine ^{123}I labeled dopamine re-uptake inhibitors, e.g. RTI-55 (**72**), were prepared and are successfully used as dopamine transporter imaging agents. These radiopharmaceuticals allow the secure *in vivo* diagnosis of Parkinson's disease in an early, non-symptomatic stage due to the fact that the quantity of dopamine transporter corresponds to the density of dopaminergic neurons in the stratial region of the brain (Morgan et al., 1999; Booji et al., 2001).

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