Solid-Phase Synthesis and Biological Evaluation of a Teleocidin Library—Discovery of a Selective PKCδ Down Regulator

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Abstract: Protein kinase C (PKC) is linked to the signal-induced modulation of a wide variety of cellular processes, such as growth, differentiation, secretion, apoptosis, and tumor development. The design and synthesis of small molecules that regulate these different cellular signaling systems is at the forefront of modern drug design. Herein we report a) an efficient method for the synthesis of indolactam V (**6**), a PKC activator, and its N^{13}-des(methyl) analogues (**19**) using a regioselective organometallic transformation, a convenient aminomalonate derivative (**10**) to introduce the appropriate functionality and an enantiospecific enzymic hydrolysis as key steps; b) the use of this method in the first solid-phase synthesis of a teleocidin library modifying the N-13, C-12 and C-7 alkyl chains, and, therefore, producing a library of potential activators and/or inhibitors of PKC of the general structure (**32**); c) the activation of PKC by selected members of the library using a MARCKS translocation in vivo assay system; d) the observation that some of these analogues are nearly as effective as the natural PKC activators phorbol dibutyrate and (−)-indolactam V (**6**), and e) the observation that some of these analogues have different potential to induce down-regulation of members of the PKC gene family after chronic stimulation.

Keywords: alkaloids · combinatorial chemistry · enzyme catalysis · lactams · protein kinase C · signal transduction

Introduction

The combinatorial synthesis of molecular libraries on polymeric supports is a powerful approach for the rapid identification of new compounds that are efficient tools for the study of biological phenomena and new leads for the development of new drugs.[1] However, its efficiency depends critically on the choice of the molecular scaffolds onto which different functional groups are grafted in the process of combinatorial synthesis. The generation of large libraries alone is not sufficient, the underlying basic structure of the individual library members must be biologically relevant. Natural products with proven biological activity offer such relevant molecular frameworks and, therefore, the development of methods for the combinatorial synthesis of compound libraries embodying the molecular architecture of natural products[2] must be of great relevance to combinatorial, bioorganic, and medicinal chemistry. This calls for the development of synthetic techniques and multistep reaction sequences that proceed with high efficiency on a polymeric support.

Kinase-mediated protein phosphorylation is a crucial component of the signal transduction pathways by which extracellular signaling molecules influence their target cells.[3a] The identification of cellular signaling systems, and the design and synthesis of small molecules that control these systems is at the forefront of modern drug design.[3b, 4] However, these transduction pathways are significantly more complex than simple linear arrays of enzyme-catalyzed reactions that run from the cell surface to the nucleus. Protein kinase C (PKC), a family of at least eleven closely related serine/threonine kinase isoenzymes, plays key roles in signal transduction pathways that regulate numerous cellular responses including gene expression, proliferation, differentiation, apoptosis, and tumor development.[3] PKC is expressed in all cell types, however, the different isoforms often are distributed in a tissue-specific manner.[3c–e] Altered PKC activity has been implicated in many disease states, and the synthesis and study of modulators (selective activators or inhibitors) of PKC activity may be useful to establish the structural requirements

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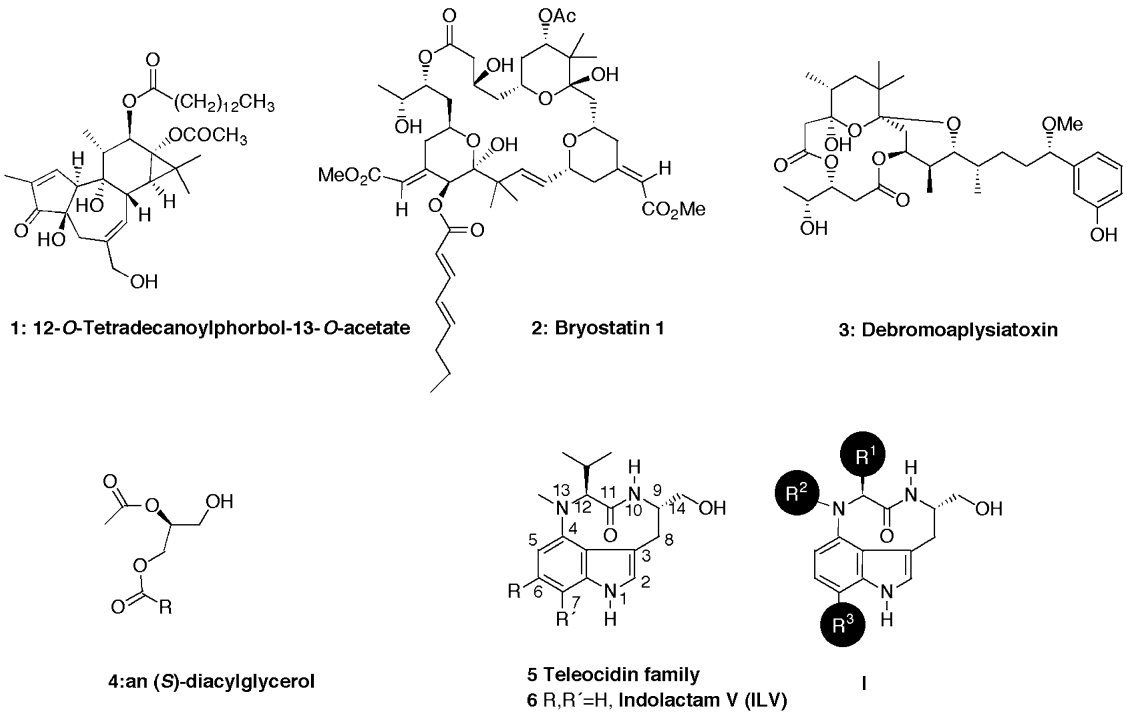

Figure 1. Molecular structures of 12-*O*-tetradecanoylphorbol-13-*O*-acetate (**1**), bryostatin 1 (**2**), debromoaplysiatoxin (**3**), an (*S*)-diacylglycerol (**4**), the teleocidin family (**5**), indolactam V (**6**) and general structure of indolactam V analogues (**I**) to be built up by combinatorial synthesis.

for the activation of PKC and ultimately lead to the development of new medicinal leads for the treatment of, for example, cancer, asthma, rheumatoid arthritis, diabetic complications, psoriasis, and central nervous system disorders.[3e, 4] PKC is believed to be activated by translocation to the membrane and a subsequent conformational change caused by the binding of phosphatidyl-L-serine (L-PS) or diacylglycerol (DAG, **4**), an endogenous PKC activator, to the cysteine rich domain (CDR) in the regulatory site.[3d] The function of DAG can be mimicked by (exogenously applied) agents like phorbol esters (**1**), bryostatin (**2**), aplysiatoxin (**3**), teleocidins (**5**), and their derivatives which bind to the regulatory domain of PKC, and thus serve as potent activators of this enzyme (Figure 1). Indeed, computer-assisted molecular-modeling studies of these tumor promoters, while somewhat controversial, have suggested a commonality of their hydrophobic regions and certain heteroatoms.[3]

In particular, (−)-indolactam V (**6**) the core structure of tumor-promoting teleocidins (**5**),[3, 5a] has attracted substantial interest as the key component for the investigation of the structural requirements for the activation of PKC, and synthesis, molecular modeling, and structure–activity relationships of indolactam V and its analogues have been described.[5]

Herein we describe an efficient method for the synthesis of indolactam V (**6**), and consequently the preparation of both enantiomeric series of N^{13}-des(methyl)indolactam V analogues. The use of this method in the development of solid-phase chemistry and the generation of the first combinatorial teleocidin library are also described.[6] The biological evaluation of some members of this library allowed the discovery of analogues with nearly equal potencies of PKC activation compared with the natural products. The knowledge gained within the indolactam V–teleocidin molecular framework sets the stage for further advances in the field.

Results and Discussion

Design of the solid-phase strategy: As shown in Figure 1 the indolactam core structure displays a pattern which allows the set up of a multidimensional library, based on a natural product derived template with unique spatial properties (see **I**). For the design of the library, it had to be taken into account that the structure of substituents at C-12 and at N-13 (see structure **IV**, Figure 2 for numbering) influence the conformation of the nine-membered lactam ring (twist vs. sofa) and determine the PKC binding ability of the heterocycles (i.e. the twist form represents the active conformation of the indolactams).[5c] Furthermore substituents at C-7 mediate membrane binding of the PKC activators, and a free OH group is required at C-14 for biological activity.[7] Therefore, indolactam analogues **I** (Figure 2) were chosen as promising targets. It was planned to vary substituents R^1–R^3 by means of appropriate reactions preferably on a solid support, and to link the indolactam core to the polymeric carrier by the primary OH group. Thus, compounds **I** should be obtained by cleavage from the resin **II**. Structure **II** could be tailored from **III** by reductive amination followed by regioselective functionalization of C-7 (e.g. iodination) and elongation in this position (e.g. Pd mediated C–C bond formation). Finally, disconnection of the conjugates **III** from the polymeric support unravels possible precursors, N^{13}-des(methyl)indolactam analogues **IV**, an appropriate linker **V**, and resin **VI**.

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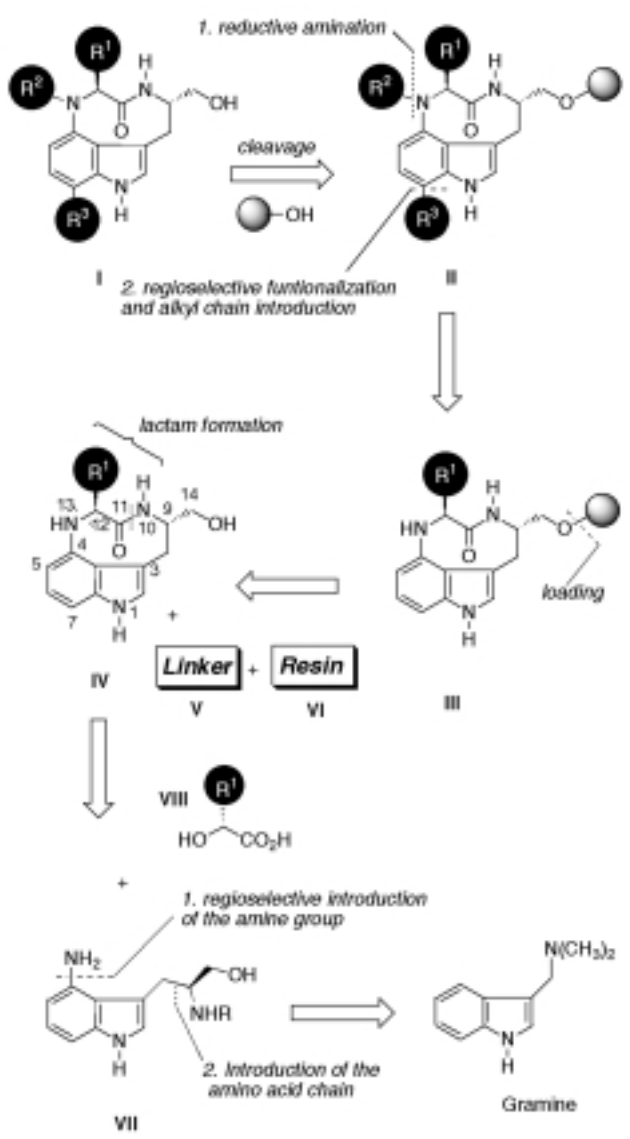
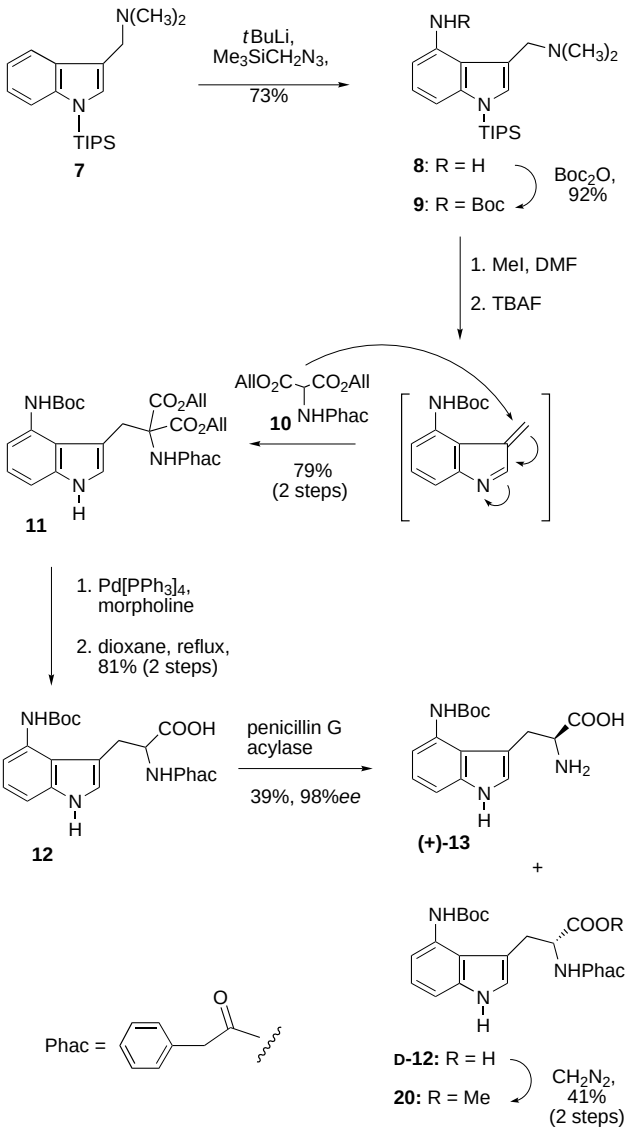


Figure 2. Retrosynthetic analysis of the teleocidin library (**I**).



Scheme 1. Synthesis of enantiomerically pure L-4-*N*-Boc-tryptophan (**13**) and D-4-*N*-Boc-*N*-phenylacetyltryptophan methyl ester (**20**).

Analogues **IV** could be built up in solution from the central intermediate **VII** and different α-hydroxy acids **VIII**, whereas the structure **VII** would be obtained from gramine by regioselective introduction of an amino group at C-4 and introduction of the appropriate chiral functionalized chain at C-3.

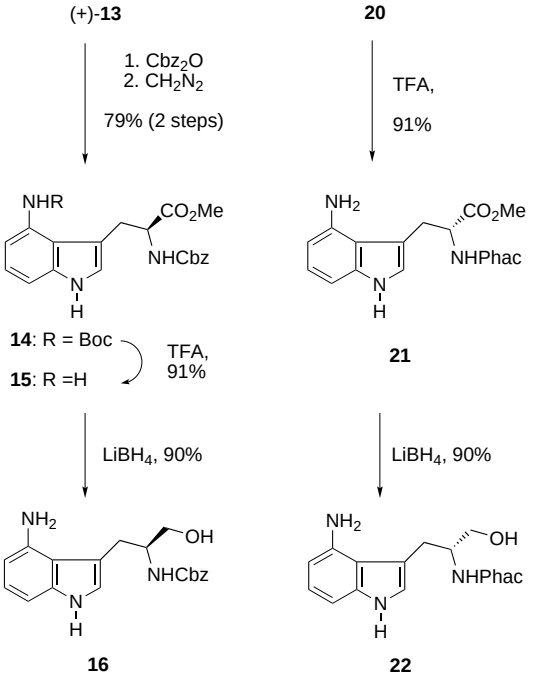
Synthesis of enantiomerically pure L- and D-4-*N*-Boc-tryptophan derivatives: In order to construct the central enantiomerically pure building block for the combinatorial introduction of residues R^1-R^3 on the solid phase, L-4-*N*-Boc-tryptophan ((+)-**13**) and D-4-*N*-Boc-*N*-phenylacetyltryptophan methyl ester **20**, were synthesized as shown in Scheme 1. *N*-Triisopropylsilyl (TIPS)-protected gramine **7** was regioselectively lithiated in the 4-position,[8] and an NH_2 group was introduced by treatment of the aryllithium intermediate with trimethylsilylmethyl azide.[9] The intermediary aryllithium compound is stabilized by the 3-dimethylaminomethyl substituent, and furthermore, the bulky N-protecting group prevents deprotonation at C-2 and C-7.[8] After protection of the amino group, gramine derivative **9** was elaborated to the corresponding substituted tryptophan. To this end, the tertiary amine was *N*-methylated.[10] Unexpectedly the quaternization did not proceed in the nonpolar solvent benzene and instead DMF had to be used. We assume that the NHBoc group forms an hydrogen bond with the dimethylamino group and prevents the methylation in benzene. TIPS-deprotection with tetrabutylammonium fluoride (TBAF) was accompanied by elimination of trimethylamine, and yielded an *exo*-methylene imine intermediate that was attacked by phenylacetamidomalonic acid bis(allyl ester) **10** in a Mannich-type reaction.[10] The nucleophile **10** carries a phenylacetyl group that allows enzymatic resolution of the racemate (vide infra), and furthermore it possesses a diallyl ester function that can easily be cleaved and decarboxylated under mild conditions. *N*-Phenylacetylation of diethylamino malonate hydrochloride in the presence of *N*,*N*-diisopropylethylamine (DIPEA),[11a,b] and transesterification with allyl alcohol, using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as a base and LiCl[11c] afforded **10** in good yield (see Experimental Section).

The resulting α-alkylated malonic acid bis ester **11** was saponified by Pd^0-mediated allyl transfer to morpholine,[12] and the malonic acid formed was decarboxylated to give 4-aminotryptophan derivative **12** in high yield. In order to construct enantiomerically pure indolactam analogues racemic *N*-acylated amino acid **12** was subjected to enantioselective hydrolysis of the phenylacetamide with penicillin G acylase.[13] The enzymatic hydrolysis virtually stopped at 50% conversion and from the reaction mixture the desired L-amino acid (+)-**13** was obtained in 39% yield (maximum yield 50%) and with an enantiomeric excess of >98%.

In addition, the remaining D-amino acid phenylacetamide (>98% *ee*) was isolated as its methyl ester **20** in 41% yield (two steps) (see Scheme 1). Thus, both enantiomers of 4-*N*-Boc tryptophan are accessible by this method in a straightforward and efficient manner.

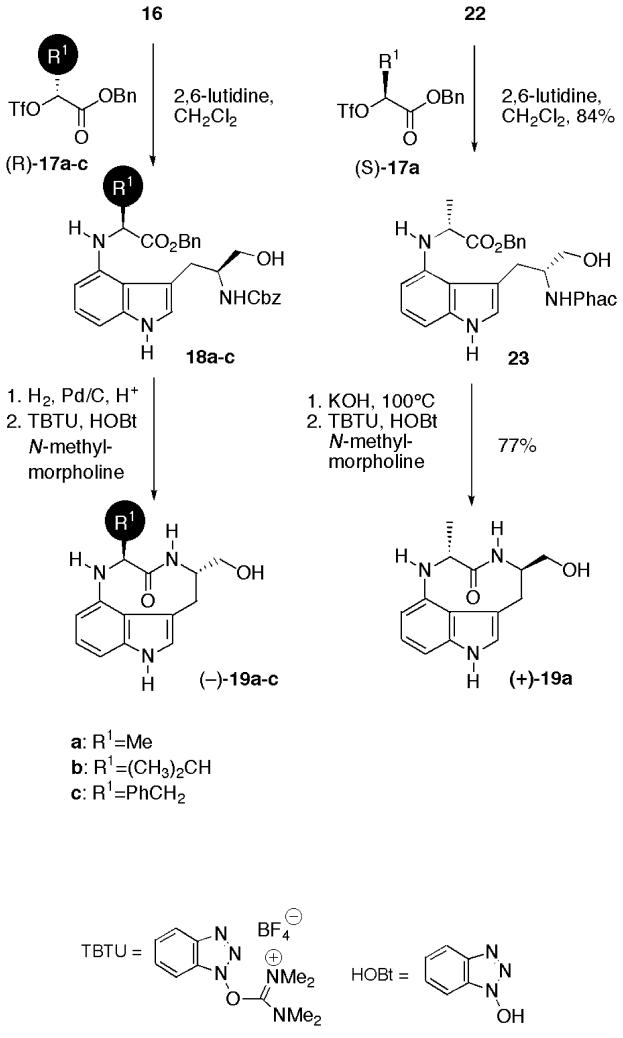
Formal synthesis of (−)-indolactam V (6): L-Amino acid (+)-**13** was converted into the selectively masked amino alcohol **16** in 65% overall yield by: a) protection of the amino group by a *Z* group by means of dibenzyl dicarbonate,[14] and methylation with diazomethane; b) Boc-deprotection with trifluoroacetic acid (TFA) in the presence of 1,3-dimethoxybenzene,[15] which acts as a trapping reagent for the carbocation liberated, and c) reduction of the methyl ester to the alcohol. 4-Aminoindole derivative **16** has previously been prepared as an advanced intermediate in a synthesis of (−)-indolactam V (**6**). The spectral and physical data of **16** were identical with those previously reported;[16] thus the reaction sequence depicted on the left in Scheme 2 represents a novel formal total synthesis of this PKC activator.

In addition, using a parallel strategy the D-amino acid methyl ester **20** was converted into the selectively masked amino alcohol **22** in 82% overall yield (see Scheme 2).[17]



Scheme 2. Synthesis of the 4-aminotryptophan derivatives (**16**) and (**22**)—a formal total synthesis of (−)-indolactam V (**6**).

Synthesis of N^{13}-des(methyl)indolactam-V analogues 19: In order to prepare a library of teleocidin analogues, amino alcohol **16** was employed as the central intermediate in a solid-phase synthesis of teleocidin analogues. The second stereocenter characteristic of the indolactam nucleus was introduced by alkylation of the aromatic amine with different α-hydroxy acid ester triflates **17** in an S_N2 process.[16] Displacement of these triflates with the aminoindole **16** was achieved in 1,2-dichloroethane at 70 °C for 18 h ((*R*)-**17b**) as described[16] or in CH_2Cl_2 at rt for 30 min ((*R*)-**17a**, **c**)[18a] to give pseudodipeptides **18a**,[18b]**b, c** in high yields (Scheme 3,



Scheme 3. Synthesis of N^{13}-des(methyl)indolactam V analogues (**19**).

Table 1). Subsequent hydrogenolytic removal of the Z protecting group and the benzyl ester followed by amide formation using TBTU as a coupling reagent yielded nine-membered lactams (−)-**19a–c** in high yield (Scheme 3, Table 1). The N^{13}-des(methyl)indolactam-V analogues, (−)-**19a,c** and (−)-**19b**, proved to be spectroscopically identical with the racemates[19] and the enantiomerically pure compound,[7] respectively.

Using the same strategy after triflate formation of the benzyl ester of L-lactic acid the coupling between the two chiral compounds, (*S*)-**17a** and **22**, proceeded with good yield

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Table 1. Results of the synthesis of resin-bound indole derivatives **26**.[a]

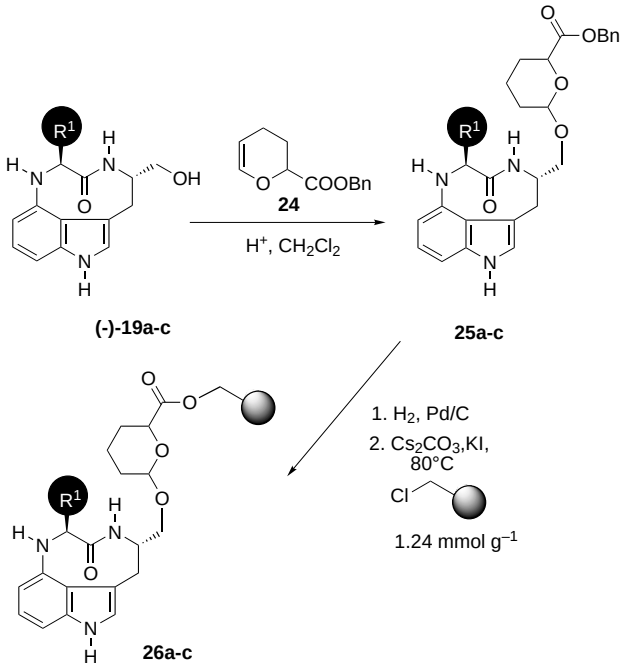
	16 → 18		**18 → 19**		**19 → 25**		**25 → 26**	
R^1	Compd	Yield [%]	Cmpd	Yield [%]	Compd	Yield [%]	Compd	Yield [%]
CH_3	**18a**	87	**19a**	87	**25a**	25	**26a**	81
*i*Pr	**18b**	80	**19b**	83	**25b**	50	**26b**	73
CH_2Ph	**18c**	84	**19c**	77	**25c**	68	**26c**	77

[a] All yields were determined by gravimetry after chromatography.

affording **23**. The hydrolysis of both protecting groups by means of a refluxing ethanolic solution of KOH (1M) and further lactam formation under the same conditions as above yielded the indolactam (+)-**19a** in high yield. After many attempts we found that acidifying the solution after the work-up of the basic hydrolysis was important to achieve the subsequent lactam formation in high yield. Compound (+)-**19a** proved to be spectroscopically identical with its enantiomer (−)-**19a** except for the sign of the optical rotation. This result opens up the possibility to prepare indolactams **19** with different substituents at C-12.

This is the first enantioselective synthesis of the N^{13}-des(methyl)indolactam-V analogues (−)-**19c**, (−)-**19a** and (+)-**19a** which had been prepared as racemates by Endo et al.[19] Thus the solution syntheses we have developed allows the preparation of both enantiomeric series of N^{13}-des(methyl)indolactam-V **19** with different hydrophobic substituents at C-12.

Loading of N^{13}-des(methyl)indolactams-V analogues 19 onto the solid support: Taking into account that a free OH group is required at C-14 for biological activity,[7] we chose to attach indolactams (−)-**19a–c** to the solid support through this alcohol group (Scheme 4). The use of the THP linker Merrifield resin developed by Ellman et al.[20a] should allow alcohol attachment and cleavage under mild acid catalysis and linker stability under the expected reactions. In order to avoid possible problems in the attachment of our substrates regioselectively through OH-14, we decided to protect N-13. Surprisingly the reaction of (−)-**19b** with FmocCl and pyridine at rt led to a single product identified as O^{14}-Fmoc-**19b**. This fact forced us to explore the direct attachment of unprotected indolactams **19**. Although the reaction in solution of the indolactam (−)-**19a** with 0.5 equivalents of 3,4-dihydropyrane (DHP) in the presence of a catalytic amount of pyridinium toluene sulfonate (PPTS) afforded only the expected monoprotected O^{14}-THP derivative, the direct attachment of indole derived alcohols **19** to a polystyrene support equipped with a THP linker[20a] unexpectedly was inefficient, showing a maximum loading level of approximately 0.1 mmol g^{-1}. This problem could, however, be circumvented by rapid acetal formation with the known prelinker **24**.[20b] After this transformation, two of the four possible diastereomers **25** (ratio ca. 2:1) were isolated. Both compounds could be easily separated by chromatography, but separation is not necessary owing to the fact that the cleavage of both conjugates after the solid-phase sequence would afford a single teleocidin analogue. Hydrogenolysis of the benzyl ester and coupling of the resulting linker/substrate conjugates to chloromethylated polystyrene beads by nucleophilic esterification using Cs_2CO_3 and catalytic amounts of KI at 80 °C in DMF afforded substrate/linker/resins **26** (Scheme 4). Resins **26** were obtained typically with loading levels of approximately 0.9–1 mmol g^{-1}[21] corresponding to coupling yields of 73–81 % (Table 1).



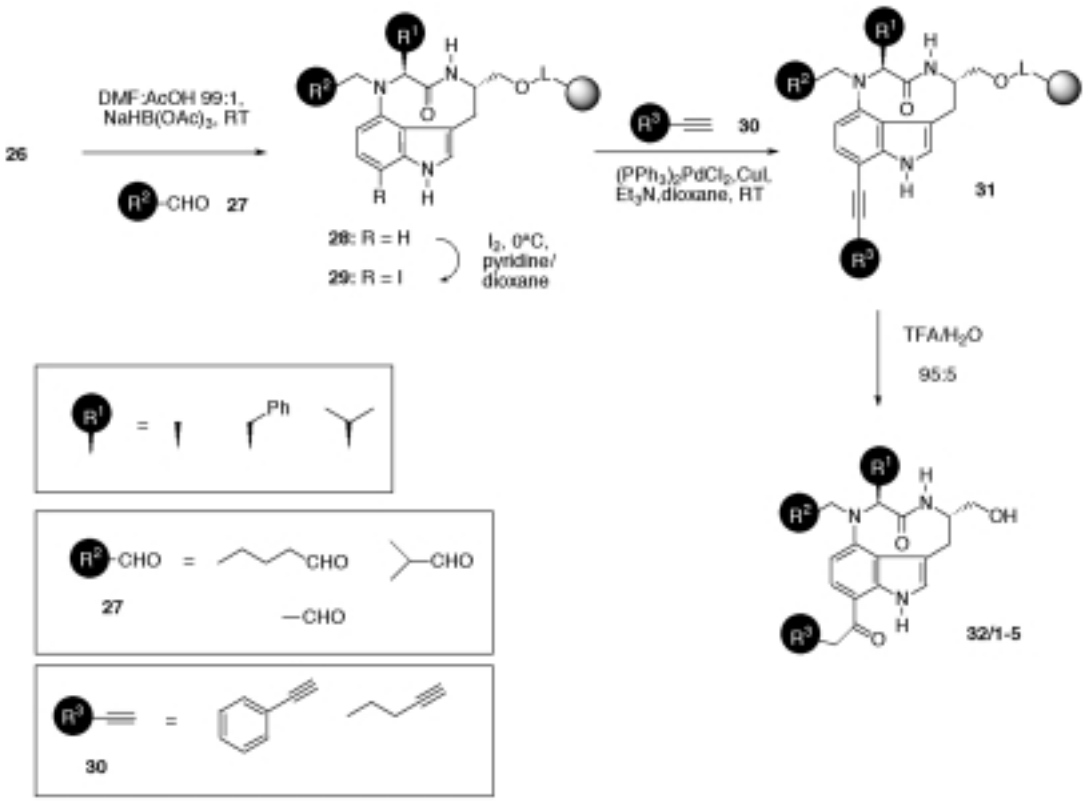
Scheme 4. Loading of N^{13}-des(methyl)-indolactam V analogues (**19**) to the solid support.

Solid-phase synthesis of teleocidin analogues 32: Resin-linked indole derivatives **26** were then subjected to a four step sequence to introduce substituents R^2 and R^3 and to cleave the desired teleocidin analogues from the solid support (Scheme 5). As a prelude to a library construction, and in order to develop the required chemistry, we first targeted compounds **32/1–5** (Table 2). To this end, N-13 of the indolactam nucleus was N-alkylated by reductive amination with aldehydes **27** and $NaHB(OAc)_3$ to yield the corresponding immobilized tertiary amines **28**.[22] Initially in order to

Table 2. Results of the solid-phase synthesis of indolactam V analogues **32/1–5**.[a]

	26 → 32/1–5			
Compd	R^1	R^2	R^3	Yield [%]
32/1	CH_2Ph	*n*Bu	*n*Pr	40
32/2	CH_2Ph	*n*Bu	Ph	50
32/3	CH_3	*i*Pr	*n*Pr	43
32/4	CH_3	*i*Pr	Ph	47
32/5	*i*Pr	CH_3	*n*Pr	20

[a] Yields were determined by gravimetry after chromatographic purification and are based on the loading level of the corresponding resin.



Scheme 5. Synthesis of teleocidin analogues **32/1 – 5** on the solid phase.

functionalize C-7, we decided to make use of a regioselective Vilsmaier formylation. Although this transformation was described in solution for indolactam V **6** to proceed regioselectively with high yield,[23] the formylation of **28/2** on solid phase afforded the desired 7-formyl derivative in only 10% yield. As an alternative we planned to introduce substituents at C-7 by Pd° catalyzed reaction. Gratifyingly, C-7 of the aromatic nucleus was regioselectively iodinated by treatment with iodine in pyridine/dioxane at 0 °C,[24a] and the resulting aryl iodides **29** were subjected to Sonogashira coupling with acetylenes **30** on the polymeric support[25] to give immobilized alkynes **31** (Scheme 5). Finally, the multiply substituted indole derivatives were released from the polymeric carrier by cleavage of the acetal linker with aqueous trifluoroacetic acid. Under these conditions the alkynyl groups attached to C-7 were hydrated to the corresponding ketones **32**. We speculate by analogy to a transformation previously described in the literature,[26a] that this unexpected hydration process is mediated by hydrogen-bond formation to the neighboring N – H group. By means of this four step sequence, teleocidin analogues **32/1 – 5** were obtained in yields ranging from 40% to 50%, that is, with high overall efficiency (average yield per step: 80 – 84%). In the case of **32/5** the iodination step was less efficient and resulted in a lower overall yield.

The structures of these teleocidin analogues were assigned to **32** by analysis of their spectroscopic data (^{1}H NMR, HRMS, $[\alpha]_D$, IR),[26b] and fully confirmed by comparison with the previously synthesized compound[24b] in the solution phase (**32a**, $R^1 = (CH_3)_2CH$, $R^2 = H$, $R^3 = Et$). A significant difference in the chemical shift of the indole NH-1 of these compounds is observed in their ^{1}H NMR spectra in comparison with N^{13}-des(methyl)indolactams; the proton resonates at $\delta = 2.87 - 2.17$ downfield in **32** compared with **19** ($\delta = 10.87 - 10.67$ and $8.00 - 8.50$ in **32** and **19**, respectively), due to the influence of the carbonyl group present at C-7 in **32**. Inspection of the ^{1}H NMR spectra reveals several characteristic signals and coupling constants (e.g. the twist conformers are characterized by peaks at $\delta = 6.18 - 6.56$ (assigned to H-5), and peaks at $\delta = 4.51 - 5.20$ (assigned to H-12)) which indicate that products **32** exist only as twist conformers, that is in the biologically active conformation. Probably the introduction of an electron-withdrawing group at C-7 increases the resonance among the lone-pair electrons on N-13, aromatic electrons and the substituent at C-7, fixing the molecule in the twist conformer, in which the lone-pair electrons on N-13 are more delocalized onto the indolic ring.[24b]

With an efficient solid-phase sequence in hand we then constructed a library of indolactam analogues (Table 3). Resin-bound indole derivative **26c** served as a starting material which was derivatized with six different aldehydes and eight alkynes in parallel syntheses according to the protocol described above in order to build up a library of teleocidin-analogues (Scheme 6). The results given in Table 3 demonstrate that differently substituted aldehydes and alkynes with additional functional groups can be applied successfully in the developed solid-phase sequence. In this way an additional 27 indolactam analogues were synthesized in overall yields ranging from 65% to 10% (average yield per step 90% to 57%). Unexpectedly, 22 analogues were not formed since the desired compounds could not be detected in

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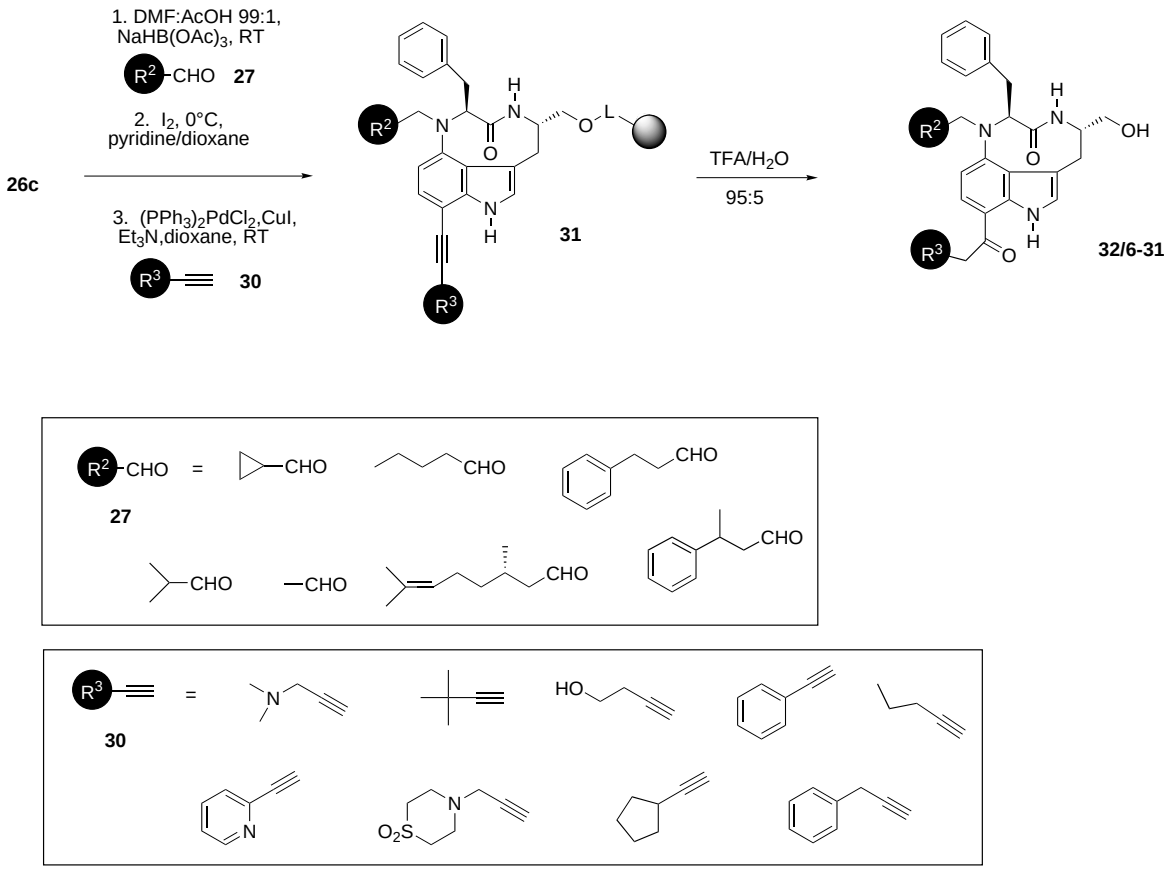


Scheme 6. Synthesis of a teleocidin library **32/6–31** on the solid phase.

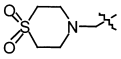
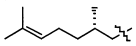
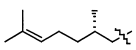
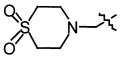
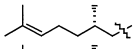
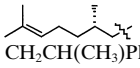
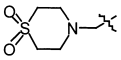
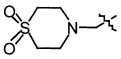
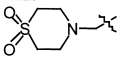
the reaction mixtures after release from the solid support by LC-MS (Table 4). Analysis of the mass spectra suggests that the reductive amination was not efficient for some individual members (e.g. for compounds **32/44–51** 2-ethylhexanal could present steric problems for the imine formation). The surprising fact that the Sonogashira coupling with pyridine-2-acetylene and propargyl alcohol was ineffective, may be attributed to the lower reactivity of these alkynes[25] and/or instability of the individual products under the acidic cleavage conditions.

However, numerous further aldehydes, alkynes and α-hydroxy acids are readily available. Furthermore, the aryl iodide intermediates may also be subjected to Pd^0-mediated Suzuki, Stille, and Heck reactions opening up the opportunity to introduce various aryl and alkenyl groups. Thus, the developed synthetic route should provide an efficient and flexible access to numerous indolactam and teleocidin analogues.

Biological evaluation of teleocidin analogues: In order to investigate if the teleocidin analogues built up by the route described above are indeed PKC activators, eleven selected members[27] of the compound library were subjected to a cell-based assay system. In this assay Swiss 3T3 fibroblasts are treated with PKC modulators. Activation of PKC causes the phosphorylation of the major PKC substrate, namely MARCKS (myristoylated alanine-rich C kinase substrate).[28] The unphosphorylated 80 kDa MARCKS protein is targeted to membranes by its N-terminal myristoylated domain and the unphosphorylated phosphorylation domain in the middle of the protein (Figure 3A). MARCKS is a specific PKC substrate.[29] After activation of PKC, MARCKS becomes phosphorylated in the phosphorylation domain resulting in its rapid and extensive translocation from the membrane to the cytosol. Thus analysis of subcellular MARCKS localization (membrane bound vs. cytosolic) allows determination of the activation of PKC. Confluent and quiescent cultures of Swiss 3T3 cells were exposed for 30 min to a saturated concentration of phorbol dibutyrate (PDB) (200 nM) for maximal activation of PKC. The cells were homogenized and the extracts separated into cytosolic and membrane fractions.

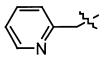
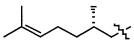
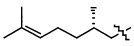
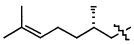
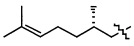
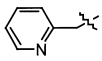
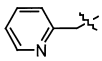
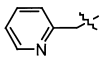
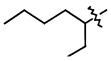
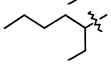
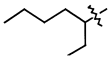
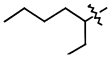
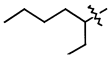
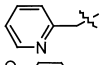
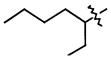
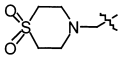
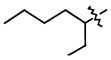
The levels of MARCKS protein in these fractions were determined by Western-blot analyses using an antiserum raised against recombinant GST-MARCKS protein. This antiserum detects a single band corresponding to MARCKS.[28] In quiescent cells, only small amounts of MARCKS were detectable in the cytoplasmic fraction (Figure 3B; control), while the majority of this protein was found in association with the membrane (data not shown). This system was then utilized to examine the potency of members of our library of teleocidin analogues (200 nM) to activate PKC in 3T3 fibroblasts and to promote MARCKS translocation. All substances tested caused a striking translocation of MARCKS to various degrees (Figure 3B). Scanning of autoradiographs revealed a MARCKS translocation induced by the indolactam V-derivatives by three- to fivefold, which

Table 3. Results of the solid-phase synthesis of a teleocidin library **32/6–31**.[a]

	26 → 32/6–31			
Compd	R^1	R^2	R^3	Yield [%]
32/6	CH_2Ph		CH_2Ph	13
32/7	CH_2Ph			17
32/8	CH_2Ph		*t*Bu	17
32/9	CH_2Ph			52
32/10	CH_2Ph		Ph	20
32/11	CH_2Ph		$CH_2N(CH_3)_2$	33
32/12	CH_2Ph		CH_2Ph	10
32/13	CH_2Ph			30
32/14	CH_2Ph		Ph	11
32/15	CH_2Ph		$CH_2N(CH_3)_2$	20
32/16	CH_2Ph	$CH_2CH(CH_3)Ph$	CH_2Ph	12
32/17	CH_2Ph	$CH_2CH(CH_3)Ph$		14
32/18	CH_2Ph	$CH_2CH(CH_3)Ph$	*t*Bu	15
32/19	CH_2Ph	$CH_2CH(CH_3)Ph$	CH_2CH_2OH	17
32/20	CH_2Ph	$CH_2CH(CH_3)Ph$		56
32/21	CH_2Ph	$CH_2CH(CH_3)Ph$	$CH_2N(CH_3)_2$	65
32/22	CH_2Ph	*N*Bu	CH_2Ph	20
32/23	CH_2Ph	*N*Bu		22
32/24	CH_2Ph	*N*Bu	*t*Bu	15
32/25	CH_2Ph	*N*Bu		49
32/2	CH_2Ph	*N*Bu	Ph	40
32/26	CH_2Ph	*N*Bu	$CH_2N(CH_3)_2$	53
32/27	CH_2Ph	CH_2CH_2Ph	CH_2Ph	18
32/28	CH_2Ph	CH_2CH_2Ph		16
32/29	CH_2Ph	CH_2CH_2Ph		53
32/30	CH_2Ph	CH_2CH_2Ph	Ph	17
32/31	CH_2Ph	CH_2CH_2Ph	$CH_2N(CH_3)_2$	51

[a] Yields were determined by analytical HPLC ($\lambda = 190$ nm) and gravimetry after chromatographic separation.

Table 4. Members of the teleocidin library that were not formed (**32/32–51**).

	32/32–51		
Compd	R^1	R^2	R^3
32/32	CH_2Ph		CH_2CH_2OH
32/33	CH_2Ph		
32/34	CH_2Ph		
32/35	CH_2Ph		*t*Bu
32/36	CH_2Ph		CH_2CH_2OH
32/37	CH_2Ph		
32/38	CH_2Ph	$CH_2CH(CH_3)Ph$	
32/39	CH_2Ph	$CH_2CH(CH_3)Ph$	Ph
32/40	CH_2Ph	*n*Bu	CH_2CH_2OH
32/41	CH_2Ph	*n*Bu	
32/42	CH_2Ph	CH_2CH_2Ph	*t*Bu
32/43	CH_2Ph	CH_2CH_2Ph	CH_2CH_2OH
32/44	CH_2Ph		CH_2Ph
32/45	CH_2Ph		
32/46	CH_2Ph		*t*Bu
32/47	CH_2Ph		CH_2CH_2OH
32/48	CH_2Ph		
32/49	CH_2Ph		
32/50	CH_2Ph		Ph
32/51	CH_2Ph		$CH_2N(CH_3)_2$

was slightly less efficient compared with indolactam V itself and with the phorbol ester PDB (both seven-fold) (Figure 3B). Preincubation of the Swiss 3T3 cells with 2.5 μM GF 109203X, a specific PKC inhibitor,[28] completely abrogated the MARCKS translocation (data not shown). MARCKS phosphorylation by PKC is a prerequisite for MARCKS translocation.[28a] Thus, these results demonstrate that the indolactam-V analogues are potent PKC activators.

MARCKS is a specific PKC substrate for all conventional and novel PKC isoforms in vitro and in fibroblasts.[29] Our data on the differently pronounced activation of PKC in Swiss 3T3 cells by different indolactam analogues may be explained by the varying potency of these indolactam analogues for binding to and activating PKC in general. This possibility might open new routes for the establishment of structure–function relationships. Alternatively, the individual indolactam analogues tested may bind the individual members of the PKC family with different efficiency. In this context it should be noted that the murine Swiss 3T3 fibroblasts used in this study express mainly the conventional PKC-isoform α, the novel isoforms δ and ε, and the atypical isoform ζ.[30]

To explore the effect on PKC isoforms in more detail, we investigated the potency of indolactam and of the different indolactam derivatives to induce PKC down-regulation. Chronic stimulation of PKC, for example, with phorbol esters leads to a marked decrease in the cellular content of most PKC isoforms. This phenomenon has been termed down-regulation and desensitizes the cell to a subsequent PKC-mediated signal. The role and mechanism of PKC down-regulation is not well characterized. To examine PKC down-regulation, quiescent Swiss 3T3 cultures were treated with compounds **32/9**, **32/20**, **32/25**, **32/15**, **32/26**, **32/31**, **32/4**, and for control with PDB and indolactam V (200 nM each) for 48 hours. Then, cellular proteins were extracted, separated by SDS-polyacrylamide gel electrophoresis and PKC levels determined by Western-blot analysis as described previously[31] (Figure 4). PKCα became efficiently down-regulated by the phorbol ester PDB and only slightly by indolactam V. Indolactam V derivatives had no significant effect on PKCα expression. Levels of PKCδ were drastically reduced in cells

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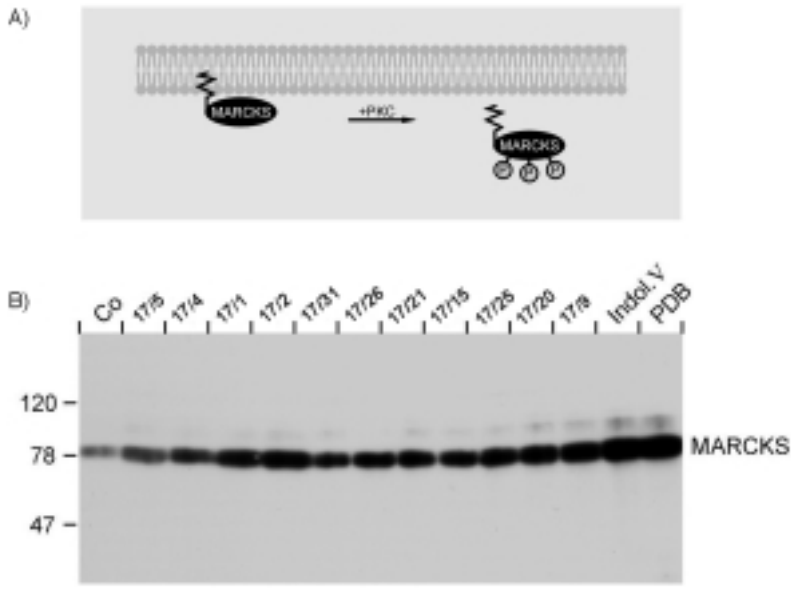

Figure 3. Activation of PKC induces translocation of MARCKS.

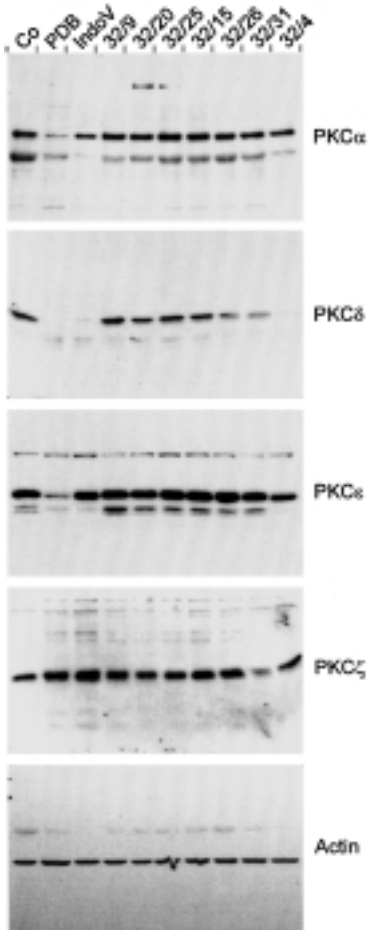

Figure 4. Down-regulation of PKC isoforms. Quiescent Swiss 3T3 cultures were treated with the indicated compounds (200 nM each) for 48 h or remained untreated (Co). Then, cells were extracted and 15 μg protein per lane loaded on 7.5% SDS polyacrylamide gels. The levels of PKCα, PKCδ, PKCε and PKCζ were determined by Western blotting as described.[31] While PDB caused down-regulation of PKCα, PKCδ and PKCε, indolactam V and its analogue **32/4** induced significant down-regulation only of PKCδ. The other indole derivatives caused activation of PKC (see Figure 3B), but no down-regulation.

treated with PDB and indolactam V. Additionally, the compound **32/4** also effectively induced PKCδ down-regulation while **32/26** and **32/31** had minor and all other indolactam analogues tested had no effect. PKCε was down-regulated by PDB, but hardly by indolactam V or derivatives. Interestingly, **32/4** caused a 50% reduction of PKCε level. Neither PDB nor indolactam V nor derivatives had any effect on PKCζ, which does not bind to phorbol esters.[32] Taken together, these data show, that PKC can be activated by low molecular weight compounds like indole alkaloids (Figure 3) without causing down-regulation (Figure 4). The result, that PKCα and PKCδ, but not PKCε were down-regulated by indolactam V can be explained by its different affinities for these PKCs. It was shown, that indolactam V binds with a 2.5-fold lower affinity to PKCε compared with PKCα and PKCδ.[32]

It is particularly noteworthy that out of the tested subset of indolactam V analogues one compound (**32/4**) selectively down-regulates only one of the four PKC isoenzymes (PKCδ). It suggests that further selective mediators of PKC activity may be discovered by means of the combinatorial approach described above.

Conclusion

Herein we have described an efficient synthesis of the PKC activator indolactam V (**6**) which allows the preparation of both enantiomeric series of N^{13}-des(methyl)indolactams-V analogues **19** and its application to the development of the first teleocidin combinatorial library. Some members of this library were biologically evaluated with regards to PKC activation and down regulation. The results were useful in terms of new synthetic methodology developments and open up the possibility to develop new PKC activators and/or inhibitors. The gained knowledge within the indolactam V-teleocidin molecular framework sets the stage for further advances in the development of new medicinal leads.

Experimental Section

General: All melting points were determined using a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer polarimeter 241 using a 10 cm path length cell. $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. IR spectra were measured as KBr pellets or liquid films. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC250, AM400, or DRX-500 spectrometers. The signal of the residual protonated solvent ($CDCl_3$ or CD_3OD) was taken as the reference (the singlet at $\delta = 7.24$ ($CHCl_3$) or 3.31 (CH_3OH) for ^{1}H and the triplet centered at $\delta = 77.0$ ($CHCl_3$) or the quadruplet at 49.0 (CH_3OH) for ^{13}C NMR data).

Mass spectra were obtained by electron impact (EI): at 70 eV on a Finnigan MAT MS70 spectrometer. Column chromatography refers to flash chromatography and was performed on Baker silica gel (230–400 mesh ASTM). Reaction progress was monitored by TLC using Merck silica gel $60F_{254}$ aluminum sheets. Penicillin G acylase was obtained in immobilized form from encapsulated Eupergit C from Boehringer Mannheim. Chloromethylated polystyrene beads (1.24 mmol g^{-1}, 1% DVB, 100–200 mesh) was purchased from Nova Biochem. All reactions were carried out under an inert atmosphere of dry argon using oven-dried glassware and freshly distilled and dried solvents. Unless stated otherwise, reaction mixtures were worked up by addition of water and extraction with the appropriate solvent (indicated). The organic extracts were washed with water and brine, and dried using anhydrous sodium sulfate. Evaporation was performed under reduced pressure. The ee % was determined by HPLC using a chriobiotic T column (250 × 4.6 mm; particle size 5 micrometer; ASTEC®) and a mixture of H_3CCN/MeOH/NEt_3/AcOH (545:455:4:2) as eluent at a flow rate of 1 ml min^{-1} or 0.4 mL min^{-1}, and gave the retention times at 8.56 and 9.09 min for the L and D enantiomer of **12**, respectively.

3-Dimethylaminomethyl-1-triisopropylsilylindole (7):[8] Powdered gramine (45.28 g, 259.82 mmol) was added over 20 min at 0 °C to a stirred suspension of NaH (80% in mineral oil, 8.57 g, 285.80 mmol) (prewashed with dry pentane) in anhydrous THF (520 mL). After 3 h at this temperature, triisopropylsilyl chloride (52.60 g, 272.80 mmol) was added dropwise. The reaction mixture was stirred overnight at 0 °C, and then carefully quenched with H_2O (100 mL). The product was extracted with Et_2O and worked up to afford a residue, which was purified by distillation under reduced pressure to give TIPS-gramine **7** (72.95 g, 84.9%) as a yellowish oil. B.p. 150–155 °C, 0.3 mbar; $R_f = 0.3$ (silica gel, EtOAc/MeOH/NH_3, 10:3:0.1); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 7.70$ (m, 1H), 7.50 (m, 1H), 7.20 (s, 1H), 7.10 (m, 2H), 3.65 (s, 2H), 2.30 (s, 6H), 1.65 (sept., $J = 6.5$ Hz, 3H), 1.15 (d, $J = 6.5$ Hz, 18H); ^{13}C NMR (63 MHz, $CDCl_3$): $\delta = 141.3$, 131.3, 130.5, 121.4, 119.6, 119.1, 114.9, 113.8, 54.6, 45.2, 18.1, 12.8; MS (EI): *m/z*: 330 [M^+] (25), 286 (100), 173 (9), 157 (17), 129 (15), 115 (47), 87 (25), 73 (33), 59 (73), 59 (73), 45 (25).

3-Dimethylaminomethyl-4-amino-1-triisopropylsilylindole (8): *t*BuLi (1.5 M, 19.6 mL, 29.4 mmol) was added dropwise to a stirred solution of TIPS-gramine **7** (8.08 g, 24.45 mmol) in dry Et_2O (125 mL) at −78 °C. The solution was stirred for 15 min at −78 °C and then allowed to warm to 0 °C, and the vessel was immersed in an ice/water bath for 1.5 h. The mixture was cooled to −78 °C, and a solution of trimethylsilylmethyl azide (4.74 g, 37.24 mmol) in dry Et_2O (14 mL) was added dropwise. After 1 h at this temperature the reaction mixture was allowed to warm to room temperature, and then the reaction was quenched with saturated aqueous NH_4Cl solution (75 mL). Extraction with Et_2O and work-up afforded an oily residue, which was purified by chromatography on neutral alumina. Elution with a mixture of hexane/EtOAc (10:1) as eluent furnished **8** as a colorless oil (6.16 g, 73%), which on standing solidified. $R_f = 0.3$ (neutral alumina, hexane/EtOAc, 10:1); m.p. 96–97 °C. FT-IR (neat): $\tilde{\nu}_{max} = 3415$, 3281, 3164, 2941, 2866, 2824, 2775, 1619, 1585, 1560, 1491, 1460, 1438, 1373, 1315, 1284, 1245, 1130, 1073, 1035, 1018, 1001, 884, 725, 694, 659 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.92$ (m, 2H), 6.75 (dd, $J = 8.5$, 0.5 Hz, 1H), 6.23 (dd, $J = 7.3$, 0.5 Hz, 1H), 5.37 (br s, 2H), 3.47 (s, 2H), 2.17 (s, 6H), 1.58 (sept, $J = 7.5$ Hz, 3H), 1.05 (d, $J = 7.5$ Hz, 18H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 143.5$, 142.6, 128.3, 122.8, 119.8, 116.1, 104.4, 104.2, 56.7, 44.6, 18.2, 12.8; MS (EI): *m/z*: 347.3 [M^++2] (6), 346 [M^++1] (25), 345 [M^+] (94), 330 (37), 302 (24), 301 (11), 300 (100), 257 (12), 115 (13), 59 (27); HRMS (EI): calcd for $C_{20}H_{35}N_3Si$ [M^+]: 345.2600, found: 345.2611.

3-Dimethylaminomethyl-4-(*N*-*tert*-butyloxycarbonyl)amino-1-triisopropylsilylindole (9): A solution of **8** (5.62 g, 16.26 mmol) and di-*tert*-butyl dicarbonate (3.73 g, 17.1 mmol) in THF (500 mL) was stirred overnight at room temperature. After addition of water (150 mL), the resulting mixture was extracted with Et_2O and work-up afforded a residue, which was purified by chromatography on neutral alumina using hexane/EtOAc (20:0.4) as eluent. Evaporation of the selected fractions gave pure Boc-aminoindole **9** (6.64 g, 91.7%) as a white solid. $R_f = 0.3$ (neutral alumina, hexane/EtOAc, 20:1); m.p. 103–104 °C (hexane); FT-IR (neat): $\tilde{\nu}_{max} =$ 2946, 2868, 2824, 2780, 1719, 1626, 1586, 1561, 1490, 1464, 1419, 1290, 1249, 1157, 1017, 1003, 883, 848, 775, 761, 740, 697, 685, 666 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.70$ (br s, 1H), 7.06–7.11 (m, 3H), 6.96 (s, 1H), 3.54 (s, 2H), 2.32 (s, 6H), 1.66 (sept., $J = 7.5$ Hz, 3H), 1.53 (s, 9H), 1.12 (d, $J =$ 7.5 Hz, 18H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 154.3$, 143.0, 133.4, 129.1, 122.6, 121.8, 115.0, 109.8, 108.4, 79.8, 56.1, 44.1, 28.6, 18.2, 12.8; MS (EI): *m/z*: 446 [M^++1] (30), 445 [M^+] (94), 388 (14), 346 (27), 345 (100), 344 (16), 330 (31), 302 (21), 301 (54), 300 (84), 115 (11), 87 (15), 73 (16), 59 (28), 57 (20); HRMS (EI): calcd for $C_{25}H_{43}SiO_2N_3$ [M^+]: 445.3124, found: 445.3135.

Bis(allyl) phenylacetamidomalonate (10): A mixture of bis(ethyl) phenylacetamidomalonate (35.0 g, 119.3 mmol), LiCl (25.0 g, 588.6 mmol), dry allyl alcohol (100 mL), and DBU (18 mL) in THF/CH_2Cl_2 (3:1) (420 mL) was refluxed through a compensated pressure funnel with 4 Å molecular sieves under Argon overnight. The mixture was warmed to room temperature and HCl (1 N, 250 mL) was added followed by ethyl acetate (500 mL). The organic layer was separated. and work-up afforded a solid, which was purified by recrystallization from propan-2-ol to afford **10** (29.92 g, 79.0% yield) as white needles. $R_f = 0.40$ (silica gel, EtOAc/hexane, 1:2); m.p. 59–60 °C (propan-2-ol); IR (KBr): $\tilde{\nu}_{max} = 3322$, 3067, 3031, 2940, 1751, 1741, 1533, 1468, 1374, 1343, 1272, 1235, 1177, 981, 943, 727 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.34–7.38$ (m, 2H), 7.28–7.32 (m, 3H), 6.49 (d, $J =$ 6.0 Hz, 1H), 5.81–5.90 (m, 2H), 5.24–5.34 (m, 4H), 5.22 (d, $J = 6.0$ Hz, 1H), 4.60–4.72 (m, 4H), 3.64 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta =$ 170.7, 165.8, 134.0, 130.8, 129.4, 129.0, 127.5, 119.2, 66.9, 56.4, 43.1; MS (EI): *m/z*: 318 [M^++1] (5), 317 [M^+] (31), 232 (18), 226 (15), 114 (50), 91 (100); HRMS (EI): calcd for $C_{17}H_{19}NO_5$ [M^+]: 317.1263, found: 317.1274.

Bis(allyl) [4-(*N*-*tert*-butyloxycarbonyl)amino-1*H*-indol-3-yl]methylphenylacetamidomalonate (11): A stirred solution of **9** (6.55 g, 14.7 mmol) in DMF (125 mL) at room temperature, was treated with MeI (5.5 mL, 58.7 mmol). The mixture was stirred overnight and then evaporated under reduced pressure. The resulting methiodide was further dried in vacuo. A stirred suspension of the methiodide and **10** (5.12 g, 16.15 mmol) in THF (500 mL), was treated with TBAF (1 M in THF, 22.5 mL, 74.2 mmol). After 1 h at room temperature, the solvent was evaporated to dryness under reduced pressure. The residue was taken up in Et_2O (800 mL) and worked up. The remaining residue was purified by chromatography using hexanes/EtOAc (4:3) as eluent, to afford diallyl ester **11** (6.51 g, 78.9%) as white solid. $R_f = 0.4$ (silica gel, hexane/EtOAc, 2:1). M.p. 66–67 °C (Et_2O); FT-IR (neat): $\tilde{\nu}_{max} = 3373$, 3290, 2979, 2943, 1761, 1701, 1650, 1621, 1518, 1496, 1454, 1419, 1367, 1282, 1248, 1201, 1163, 1088, 745 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.25$ (br s, 1H), 7.21–7.27 (m, 4H), 7.18 (s, 1H), 7.04–7.11 (m, 2H), 6.99 (m, 2H), 6.68 (s, 1H), 6.45 (d, $J = 2.0$ Hz, 1H), 5.78 (m, 2H), 5.23 (m, 4H), 4.60 (m, 4H), 3.93 (s, 2H), 3.48 (s, 2H), 1.56 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 28.4$, 29.2, 43.4, 67.0, 67.5, 79.9, 106.9, 108.4, 115.9, 119.2, 120.9, 122.2, 123.7, 127.3, 128.9, 129.2, 130.1, 130.9, 134.0, 137.2, 154.5, 167.2, 170.4; MS (EI): *m/z*: 562 [M^++1] (12), 561 [M^+] (36), 461 (31), 326 (16), 317 (13), 189 (62), 171 (52), 156 (12), 145 (91), 114 (36), 91 (100), 57 (38), 41.0 (42); HRMS (EI): calcd for $C_{31}H_{35}N_3O_7$ [M^+]: 561.2475, found: 561.2486

4-(*N*-*tert*-Butyloxycarbonyl)amino-*N*-phenylacetyltryptophan (12): A stirred solution of diallyl ester **11** (5.4 g, 9.62 mmol) and morpholine (15 mL, 172.2 mmol) in THF (125 mL) was degassed for 20 min with argon at room temperature. Then tetrakis(triphenylphosphane)palladium(**0**) (0.50 g, 0.46 mmol) was added and stirring was continued for a further 30 min. After addition of NaOH (150 mL, 0.15 M) and Et_2O (150 mL), the aqueous layer was extracted with Et_2O. The pH was adjusted to pH = 1.6 with HCl (1 M), and the mixture extracted with $CHCl_3$ and then with AcOEt. The organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was taken up in dioxane (150 mL) and refluxed for 30 min. Concentration under reduced pressure and crystallization from diethyl ether/pentane afforded *N*-phenylacetyltryptophan **12** (3.42 g, 80.8%) as a white solid. $R_f = 0.8$ (silica gel, *n*BuOH/AcOH/H_2O, 4:1:1); m.p. 94–100 °C (decomposition); FT-IR (KBr): $\tilde{\nu}_{max} = 3305$, 3064, 2978, 2931, 1705, 1665, 1515, 1496, 1454, 1414, 1368, 1351, 1246, 1163, 1080, 743 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.24$ (d, $J = 8.0$ Hz, 1H), 7.17–7.19 (m, 3H), 7.03–7.07 (m, 3H), 6.94 (s, 1H), 6.86 (d, $J = 7.5$ Hz, 1H), 4.71 (dd, $J = 10.5$, 4.5 Hz, 1H), 3.40–3.52 (m, 3H), 3.18 (dd, $J = 15.0$, 10.5 Hz, 1H), 1.51 (s, 9H); ^{13}C NMR (100.5 MHz, CD_3OD): $\delta = 175.3$, 173.9, 157.8, 139.7, 136.6, 130.6, 130.0, 129.4, 127.7, 125.3, 124.7, 122.4, 118.7, 111.0, 110.7, 80.9, 55.2, 43.5, 29.1, 28.8; MS (EI): *m/z*: 438 [M^++1] (35), 437 [M^+] (41), 383 (13), 382 (50), 364 (16), 338 (73), 246 (10), 202 (18), 189 (49), 171 (40), 157 (63), 145 (100), 91 (47); HRMS (FAB) calcd for $C_{24}H_{27}N_3O_5$ [M^+]: 437.1951, found: 437.2020.

(*S*)-4-(*N*-*tert*-Butyloxycarbonyl)aminotryptophan (13): NaOH (0.1 N) was added to a stirred suspension of *N*-phenylacetyltryptophan **12** (3.5 g, 8.0 mmol) in MeOH (72 mL) and water (468 mL) to adjust the pH to 7.6.

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The volume was adjusted to 900 mL with water, penicillin G acylase immobilized on Eupergit C (2.5 g) was added, and the solution was incubated at 37 °C. After 50% of conversion (see HPLC conditions in general) the reaction was stopped by filtration. Then HCl (4 N) was added to adjust the pH to 1.5, and the reaction mixture was extracted three times with AcOEt (300 mL). The aqueous solution was brought to pH 7.0 with NaOH (1 N) and evaporated to dryness in vacuo. Recrystallization from MeOH/water gave L-tryptophan derivative **13** (0.997 g, 39%) as white powder. $R_f = 0.5$ (silica gel, *n*BuOH/AcOH/H_2O, 4:1:1); m.p. 212–213 °C (MeOH/H_2O); $[\alpha]_D^{20} = +47.0$ ($c = 0.17$, CH_3OH); FT-IR (KBr): $\tilde{\nu}_{max}$ 3280, 2978, 1689, 1619, 1513, 1452, 1383, 1327, 1274, 1244, 1162, 1082, 993, 771, 744 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): $\delta = 7.24$ (dd, $J = 8.0$, 0.5 Hz, 1H), 7.18 (s, 1H), 7.07 (t, $J = 8.0$, 1H), 6.92 (d, $J = 7.0$ Hz, 1H), 3.83 (dd, $J = 8.5$, 4.5 Hz, 1H), 3.56 (dd, $J = 15.5$, 4.0 Hz, 1H), 3.30 (m, 1H), 1.53 (s, 9H); ^{13}C NMR (100.5 MHz, CD_3OD): $\delta = 174.0$, 157.8, 139.7, 130.7, 125.9, 123.9, 122.5, 118.6, 110.7, 108.8, 81.0, 56.7, 28.7, 28.3; MS (EI): *m/z* 320 [M^++1] (2), 319 [M^+] (18), 190 (10), 189 (73), 171 (30), 156 (14), 145 (60), 59 (26), 56 (28), 44 (100), 41 (47), 39 (19); HRMS (EI): calcd for $C_{16}H_{21}N_3O_4$ [M^+]: 319.1532, found: 319.1518.

(*S*)-*N*-Benzyloxycarbonyl-4-(*N*-*tert*-butyloxycarbonyl)aminotryptophan methyl ester (14): Dibenzyl dicarbonate (0.72 g, 2.43 mmol) in dioxane (2.44 mL) was added dropwise to a stirred solution of L-tryptophan derivative **13** (0.78 g, 2.44 mmol) in NaOH (1 N, 2.44 mL) and dioxane (2.44 mL). After 1 h at room temperature the solvent was evaporated under reduced pressure. The mixture was diluted with water (60 mL), the pH was adjusted to 2 with sulfuric acid (1 N), and the mixture extracted with EtOAc. After work-up, the crude residue (1.35 g) was dissolved in MeOH (45 mL), and CH_2Cl_2 (135 mL) at room temperature, and diazomethane (1 M in CH_2Cl_2, 20 mL) was added at −78 °C. The solution was stirred for 30 min at room temperature, and then degassed with argon to eliminate the excess of diazomethane. The solvent was evaporated under reduced pressure and the crude residue was purified by chromatography on silica gel using hexane/AcOEt (3:2) as eluent. Evaporation of the selected fractions gave methyl ester **14** (0.903 g, 79.1%) as a yellowish solid. $R_f = 0.3$ (silica gel, hexane/EtOAc, 3:2); m.p. 166–167 °C (acetone/hexane); $[\alpha]_D^{20}$ −29.0 ($c = 1.0$, MeOH); FT-IR (KBr) $\tilde{\nu}_{max} = 3330$, 3297, 3120, 2976, 2953, 2938, 1751, 1708, 1675, 1621, 1538, 1514, 1496, 1429, 1210, 1167, 1060, 1051 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 8.48$ (s, 1H), 7.28–7.35 (m, 5H), 7.19 (dd, $J = 6.0$, 2.0 Hz, 1H), 7.05–7.10 (m, 2H), 6.99 (s, 1H), 6.73 (s, 1H), 5.48 (d, $J = 7.5$ Hz, 1H), 5.10 (d, $J = 12.0$ Hz, 1H), 5.07 (d, $J = 12.0$ Hz, 1H), 4.66 (dd, $J = 6.0$, 13.5 Hz, 1H), 3.67 (s, 3H), 3.38 (dd, $J = 15.0$, 5.0 Hz, 1H), 3.28 (dd, $J = 15.0$, 6.5 Hz, 1H), 1.55 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.6, 156.0, 154.7, 137.7, 136.2, 129.9, 128.5, 128.2, 128.1, 123.7, 122.3, 121.3, 115.9, 109.1, 108.6, 80.3, 67.0, 55.2, 52.4, 29.0, 28.4; MS (EI): *m/z* 468 [M^++1] (8), 467 [M^+] (29), 367 (18), 190 (11), 189 (97), 172 (13), 171 (64), 156 (10), 146 (11), 145 (100), 91 (42), 57 (24); HRMS (EI): calcd for $C_{25}H_{29}N_3O_6$ [M^+]: 467.2056, found: 467.2070.

(*S*)-*N*-Benzyloxycarbonyl-4-aminotryptophan methyl ester (15): CF_3COOH (40 mL) was added to a stirred suspension of methyl ester **14** (0.80 g, 1.72 mmol) and 1,3-dimethoxybenzene (1.64 mL) at room temperature. The solution was stirred for 30 min and then concentrated under reduced pressure. The residue was taken up in $KHCO_3$ (1 N, 50 mL), extracted with AcOEt and worked up to give a residue, which was purified by column chromatography using hexanes/EtOAc (1:1) as eluent. Evaporation of the selected fractions gave amino compound **15** (0.58 g, 91.4%) as a white foam. $R_f = 0.25$ (silica gel, EtOAc/hexanes, 1:1); $[\alpha]_D^{20}$ +3.6 ($c = 0.65$, MeOH); FT-IR (KBr): $\tilde{\nu}_{max} = 3389$, 3032, 2951, 1708, 1625, 1585, 1507, 1444, 1354, 1271, 1218, 1057, 1027, 738 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 8.14$ (br s, 1H), 7.28–7.35 (m, 5H), 6.97 (t, $J = 8.0$ Hz, 1H), 6.82 (dd, $J = 8.0$, 0.5 Hz, 1H), 6.79 (d, $J = 2.0$ Hz, 1H), 6.43 (d, $J = 7.5$ Hz, 1H), 6.36 (d, $J = 7.5$ Hz, 1H), 5.07 (d, $J = 12.5$ Hz, 1H), 5.04 (d, $J = 12.5$ Hz, 1H), 4.61 (dd, $J = 13.5$, 6.5 Hz, 1H), 4.00 (br s, 2H), 3.69 (s, 3H), 3.35 (d, $J = 5.5$ Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.7, 156.3, 139.8, 137.9, 136.4, 128.5, 128.0, 128.0, 123.2, 122.1, 116.8, 109.7, 106.7, 103.3, 66.8, 56.3, 52.3, 29.6; MS (EI): *m/z* 368 [M^++1] (8), 367 [M^+] (30), 146 (14), 145 (100), 91 (14), 43.0 (16); HRMS (EI): calcd for $C_{20}H_{21}N_3O_4$ [M^+]: 367.1532, found: 367.1541.

Phenylmethyl (*S*)-[2-(4-amino-1*H*-indol-3-yl)-1-(hydroxymethyl)ethyl]-carbamate (16): $LiBH_4$ (2 M in THF, 1.05 mL, 2.11 mmol) was added dropwise to a stirred solution of **15** (0.55 g, 1.48 mmol) in dry THF (9 mL) at room temperature and under an argon atmosphere. The solution was stirred for 1 h at room temperature and for a further 1 h at reflux. Then the reaction mixture was cooled to room temperature and quenched with MeOH. The solvent was removed by evaporation under reduced pressure, and the residue was diluted with water (40 mL). Extraction with EtOAc and work-up afforded a residue, which was purified by column chromatography using hexanes/EtOAc (2:3) as eluent. Evaporation of the selected fractions furnished amino alcohol **16** (0.45 g, 89.9%) as a white foam. $R_f = 0.28$ (silica gel, EtOAc/hexanes, 3:2); $[\alpha]_D^{20}$ −16.5 ($c = 0.89$, ethanol) (ref. [16] $[\alpha]_D^{20}$ −16.3); FT-IR (KBr): $\tilde{\nu}_{max} = 3390$, 3034, 2928, 2873, 1700, 1624, 1584, 1507, 1454, 1348, 1264, 1058, 737, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 7.92$ (br s, 1H), 7.28 (br s, 5H), 6.90 (t, $J = 8.0$ Hz, 1H), 6.88–6.82 (m, 2H), 6.33 (d, $J = 7.5$ Hz, 1H), 5.58 (br d, $J = 7.5$ Hz, 1H), 5.03 (s, 2H), 3.99 (br s, 3H), 3.73 (m, 1H), 3.52 (dd, $J = 11.5$, 2.5 Hz, 1H), 3.42 (dd, $J = 11.5$, 3.5 Hz, 1H), 3.19 (dd, $J = 14.5$, 5.0 Hz, 1H), 2.95 (dd, $J = 14.5$, 9.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 156.5$, 139.2, 137.9, 136.6, 128.5, 128.1, 128.0, 122.9, 122.6, 117.6, 111.1, 107.6, 104.2, 66.7, 62.1, 55.3, 28.2; MS (EI): *m/z*: 339 [M^+] (40), 146 (33), 145 (100), 108 (30), 107 (20), 91 (39), 79 (29), 77 (18); HRMS (EI): calcd for $C_{19}H_{21}N_3O_3$ [M^+]: 339.1583, found: 339.1573.

Phenylmethyl (*S*,*S*)-2-{4-[1-(Phenylmethyloxycarbonyl)ethylamino]-1*H*-indol-3-yl}-1-(hydroxymethyl)ethylcarbamate (18a): A solution of (*R*)-2-phenylmethyl hydroxypropanoate (53 mg, 0.30 mmol) in dry CH_2Cl_2 (1 mL) was cooled to 0 °C and trifluoromethanesulfonic acid anhydride (0.054 mL, 0.33 mmol) was added in one portion under an argon atmosphere. After five minutes, 2,6-lutidine (0.087 mL, 0.82 mmol) was added in one portion, and the reaction mixture was stirred for a further five minutes. Then a solution of amino alcohol **16** (92 mg, 0.27 mmol) in CH_2Cl_2 (1 mL) was added dropwise to the solution of the formed triflate **(*R*)-17a**. The reaction mixture was stirred at room temperature for 2 h and the solution was concentrated and chromatographed using EtOAc/hexane (1/1) as eluent affording **18a** (118 mg, 87%) as an oil. $R_f = 0.60$ (silica gel, EtOAc/hexane, 3:2); $[\alpha]_D^{19}$ −8.9 ($c = 0.85$, EtOH); FT-IR (neat): $\tilde{\nu}_{max} =$ 3399, 2939, 1708, 1513, 1216, 1158, 735, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 8.24$ (br s, 1H), 7.40–7.25 (m, 10H), 6.98 (t, $J = 8.0$ Hz, ^{1}H), 6.88 (br s, 1H), 6.85 (d, $J = 8.0$ Hz, 1H), 6.23 (d, $J = 7.5$ Hz, 1H), 5.72 (br d, $J = 8.0$ Hz, ^{1}H), 5.18 (m, 2H), 5.12 (m, 2H), 4.29 (q, $J = 7.0$ Hz, 1H,), 3.80 (m, 1H), 3.62 (m, 1H), 3.51 (m, 1H), 3.18 (m, 2H), 1.57 (d, $J = 7.0$ Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 176.3, 156.3, 140.9, 137.7, 136.6, 135.4, 128.6, 128.6, 128.6, 128.5, 128.5, 128.3, 128.3, 128.2, 128.1, 128.1, 123.0, 122.5, 116.6, 111.3, 103.6, 102.2, 67.1, 66.7, 62.0, 55.6, 52.6, 28.4, 18.7; MS (EI): *m/z*: 502 [M^++1] (5), 501 [M^+] (25), 366 (4), 258 (4), 187 (5), 171 (15), 145 (7), 108 (100), 107 (65), 101 (50), 79 (80), 77 (55), 43 (100); HRMS (EI): calcd for $C_{29}H_{31}N_3O_5$ [M^+]: 501.2264, found: 501.2283.

Phenylmethyl (*S*,*S*)-2-{4-[1-(Phenylmethyloxycarbonyl)-2-methylpropyl-amino]-1*H*-indol-3-yl}-1-(hydroxymethyl)ethylcarbamate (18b):[16] A solution of **16** (68 mg, 0.20 mmol), **(*R*)-17b** (78 mg, 0.23 mmol), and 2,6-lutidine (0,026 mL, 0.24 mmol) in dry 1,2-dichloroethane (1 mL) was heated at 70 °C for 18 h. After cooling to room temperature the solution was purified by chromatography using EtOAc/hexane (2:3) as an eluent to afford **18b** (84 mg, 80%) as an oil. $R_f = 0.70$ (silica gel, EtOAc/hexane, 3:2); ^1H NMR (250 MHz, $CDCl_3$): δ 7.99 (br s, 1H, H-1), 7.33–7.20 (m, 10H, 2Ph), 6.91 (t, $J = 8.0$ Hz, ^{1}H, H-6), 6.90 (br s, 1H; H-2), 6.74 (d, $J = 8.0$ Hz, 1H; H-7), 6.15 (d, $J = 7.5$ Hz, 1H; H-5), 5.52 (br d, $J = 8.0$ Hz, 1H; H-10), 5.08 (m, 2H; CH_2OCO), 5.04 (m, 2H; CH_2OCO'), 3.90 (d, $J = 8.0$ Hz, 1H; H-12), 3.74 (m, 1H; H-9), 3.53 (m, 1H; H-14), 3.11 (m, 2H; H-8), 2.08 (m, 1H; H-15), 1.04 (d, $J = 6.5$ Hz, 3H; H-16), 0.92 (d, $J = 6.5$ Hz, 3H; H-17).

Phenylmethyl (*S*,*S*)-2-{4-[1-(Phenylmethyloxycarbonyl)-2-phenylethyl-amino]-1*H*-indol-3-yl}-1-(hydroxymethyl)ethylcarbamate (18c): According to the procedure described for the synthesis of **18a**, phenylmethyl (*R*)-2-hydroxy-3-phenylpropanoate (0.31 mg, 1.22 mmol) was allowed to react with trifluoromethanesulfonic acid anhydride (0.22 mL, 1.35 mmol) and 2,6-lutidine (0.39 mL, 3.67 mmol) in dry CH_2Cl_2 (6 mL) at −78 °C. Then a solution of amino alcohol **16** (0.41 g, 1.20 mmol) in CH_2Cl_2 (6 mL) was added dropwise to the solution of the formed triflate (*R*)-**17c**. The reaction mixture was stirred at room temperature for 22 h and the solution was concentrated and purified by chromatography using EtOAc/hexane (3:2) as eluent to afford **18c** (0.58 g, 84%) as an oil. $R_f = 0.40$ (silica gel, EtOAc/hexane, 1:1); $[\alpha]_D^{19}$ −24.3 ($c = 0.78$, EtOH); FT-IR (neat): $\tilde{\nu}_{max} =$ 3399, 2923, 1707, 1513, 1215, 1158, 735, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.04 (br s, 1H), 7.39–7.14 (m, 15H), 6.96 (t, $J = 8.0$ Hz, ^{1}H), 6.93 (br s, 1H), 6.83 (d, $J = 8.0$ Hz, 1H), 6.23 (d, $J = 7.5$ Hz, 1H), 5.50 (br d, $J =$ 8.0 Hz, ^{1}H), 5.39 (br s, 1H), 5.13 (m, 2H), 5.06 (m, 2H), 4.51 (t, $J = 6.5$ Hz,

1H), 3.75 (m, 1H), 3.59 (m, 1H), 3.49 (m, 1H), 3.20 (m, 2H), 3.09 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.1, 156.3, 140.9, 137.6, 136.6, 136.4, 135.1, 129.4, 129.4, 128.6, 128.6, 128.6, 128.6, 128.6, 128.5, 128.5, 128.5, 128.5, 128.5, 128.1, 128.1, 127.0, 123.1, 122.3, 116.5, 111.5, 103.4, 101.7, 67.2, 66.7, 61.8, 58.1, 55.6, 38.8, 28.4; MS (FAB): *m/z*: 578 [M^++1] (70), 577 [M^+] (40), 488 (10), 444 (50), 185 (55), 133 (30), 93 (50), 91 (100); HRMS (FAB) calcd for $C_{35}H_{36}N_3O_5$ [M^++1]: 578.2655, found: 578.2597.

(−)-*N*^{13}-Desmethyl-*C*^{12}-desisopropyl-*C*^{12}-methylindolactam V ((−)19a): 10% Palladium on carbon (18 mg) and (+)-camphorsulfonic acid ((+)-CSA) (18 mg) were added to a solution of **18a** (105 mg, 0.21 mmol) in MeOH (3.5 mL) and the mixture was stirred under a H_2 atmosphere for 5 h. The reaction mixture was filtered through Celite and the solvent removed by evaporation to give a solid which was dissolved in DMF (5 mL). To this stirred solution was added hydroxybenzotriazole (32 mg, 0.21 mmol), *N*-methylmorpholine (0.08 mL, 0.73 mmol), and (1-benzotriazoloxy-bis-dimethylamino) tetrafluoroborate (132 mg, 0.42 mmol), and the solution was stirred at room temperature for 16 h. Evaporation of the solvent gave a residue that was dissolved in EtOAc. The solution was washed with water, saturated sodium bicarbonate, brine, and dried over Na_2SO_4, and concentrated to give an oil. The desired compound was purified by chromatography with AcOEt/MeOH as an eluent (9:1) to afford **19a** (47 mg, 87%) as an amorphous solid R_f = 0.40 (silica gel, EtOAc/MeOH, 9:1); $[\alpha]_D^{20}$ −114 (*c* = 0.98, EtOH); FT-IR (neat): $\tilde{\nu}_{max}$ = 3305, 2931, 1645, 1502, 1084, 1059, 748 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD, only twist): δ = 7.03 (d, *J* = 8.0 Hz, ^{1}H), 6.95 (m, 2H, H-2), 6.65 (dd, *J* = 7.5, 0.5 Hz, 1H), 5.25 (br s, 1H), 4.16 (q, *J* = 7.0 Hz, 1H), 3.69 (dd, *J* = 11.5, 4.5 Hz, 1H), 3.59 (dd, *J* = 11.5, 7.0 Hz, 1H), 3.08 (dd, *J* = 15.5, 6.0 Hz, 1H), 2.98 (dd, *J* = 16.0, 9.0 Hz, 1H), 1.53 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 178.6, 142.7, 140.9, 124.5, 122.9, 122.8, 114.2, 112.3, 108.1, 65.9, 63.1, 55.0, 31.3, 18.9; MS (EI): *m/z*: 260 [M^++1] (37), 259 [M^+] (75), 173 (25), 172 (70), 171 (45), 158 (20), 157 (70), 156 (25), 130 (25), 116 (35), 91 (10), 72 (100), 44 (35); HRMS (EI): calcd for $C_{14}H_{17}N_3O_2$ [M^+]: 259.1321, found: 259.1302.

(−)-*N*^{13}-Desmethylindolactam-V (19b):[16] According to the procedure described for the synthesis of **19a**, 10% palladium on carbon (12 mg) and (+)-CSA (12 mg) were added to a solution of **18b** (83 mg, 0.156 mmol) in MeOH (2.6 mL), and the mixture stirred under H_2 atmosphere for 5 h. After filtration and evaporation, the residue was allowed to react with hydroxybenzotriazole (24 mg, 0.156 mmol), *N*-methylmorpholine (0.058 mL, 0.54 mmol), and (1-benzotriazoloxy-bis-dimethylamino) tetrafluoroborate (100 mg, 0.31 mmol) in DMF (4 mL) at room temperature for 24 h. Work-up and column chromatography (EtOAc/MeOH, 9:1) afforded **19b** (37 mg, 83%) as an amorphous solid. R_f = 0.60 (silica gel, EtOAc/MeOH, 9:1); $[\alpha]_D^{22}$ −70 (*c* = 0.91, EtOH) (ref.[7] $[\alpha]_D^{20}$ −76); FT-IR (Film): $\tilde{\nu}_{max}$ = 3305, 2958, 2928, 2872, 1650, 1501, 1350, 1258, 1049, 747 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD, twist:sofa, 3:1): twist conformer: δ = 7.03 (d, *J* = 8.0 Hz, 1H; H-7), 6.96 (m, 1H; H-2), 6.95 (t, *J* = 8.0 Hz, 1H; H-6), 6.67 (d, *J* = 7.5 Hz, 1H; H-5), 5.10 (br s, 1H; H-9), 3.71 (dd, *J* = 11.0, 5.0 Hz, 1H; H-14), 3.63 (dd, *J* = 11.0, 7.0 Hz, 1H; H′-14), 3.56 (d, *J* = 9.5 Hz, 1H; H-12), 3.14 (dd, *J* = 15.5, 6.5 Hz, 1H; H-8), 2.98 (dd, *J* = 16.5, 10.5 Hz, 1H; H′-8), 2.28 (m, 1H; H-15), 1.23 (d, *J* = 6.5 Hz, 3H; H-16), 1.03 (d, *J* = 6.5 Hz, 3H; H-17); ^{13}C NMR (125 MHz, CD_3OD): δ = 177.5, 142.2, 140.8, 124.6, 123.5, 122.9, 114.9, 111.9, 108.2, 66.2, 55.1, 38.9, 32.6, 30.8, 20.7, 20.6; MS (EI): *m/z*: 288 [M^++1] (15), 287 [M^+] (100), 256 (3), 213 (3), 201 (20), 200 (20), 198 (20), 171 (10), 157 (95), 149 (35), 130 (10), 73 (40), 43 (70); HRMS (EI): calcd for $C_{16}H_{21}N_3O_2$ [M^+]: 287.1634, found: 287.1645.

(−)-*N*^{13}-Desmethyl-*C*^{12}-desisopropyl-*C*^{12}-phenylmethylindolactam V (19c): According to the procedure described for the synthesis of **19a** above, 10% palladium on carbon (60 mg) and (+)-CSA (60 mg) were added to a solution of **18c** (0.394 g, 0.68 mmol) in MeOH (11 mL) and the mixture was stirred under a H_2 atmosphere for 5 h. After filtration and evaporation the residue was allowed to react with hydroxybenzotriazole (90 mg, 0.68 mmol), *N*-methylmorpholine (0.23 mL, 2.04 mmol), and (1-benzotriazoloxy-bis-dimethylamino) tetrafluoroborate (0.382 mg, 1.36 mmol) in DMF (17 mL) at room temperature for 15 h. Work-up and column chromatography (EtOAc/MeOH, 25:1) afforded **19c** (176 mg, 77%) as an amorphous solid. R_f = 0.40 (silica gel, EtOAc/MeOH, 25:1); $[\alpha]_D^{22}$ −100.3 (*c* = 0.90, $CHCl_3$); FT-IR (neat): $\tilde{\nu}_{max}$ = 3350, 3026, 2963, 1636, 1492, 1454, 1262, 1098, 1050, 802, 749 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD, twist:sofa, 7:1): twist conformer: δ = 7.39 – 7.23 (m, 5H), 7.02 (dd, *J* = 8.0, 1.0 Hz, ^{1}H), 6.96 (s, 1H), 6.89 (t, *J* = 8.0 Hz, 1H), 6.44 (dd, *J* = 7.5, 0.5 Hz, 1H), 5.30 (m, 1H), 4.16 (t, *J* = 7.5 Hz, 1H), 3.60 (dd, *J* = 11.3, 5.0 Hz, 1H), 3.50 (dd, *J* = 11.3, 7.0 Hz, 1H), 3.31 (dd, *J* = 13.5, 6.5 Hz, 1H), 3.18 (dd, *J* = 13.5, 8.0 Hz, 1H), 3.13 (dd, *J* = 15.5, 6.5 Hz, 1H), 2.97 (dd, *J* = 15.5, 11.5 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD): δ = 177.9, 141.8, 140.8, 139.0, 130.5, 130.5, 129.7, 129.7, 127.9, 124.7, 123.3, 122.8, 114.5, 111.9, 108.4, 69.8, 66.1, 55.1, 40.4, 30.9; MS (EI): *m/z*: 335 [M^+] (100), 247 (10), 201 (10), 157 (70), 116 (20), 91 (10), 72 (40), 44 (8); HRMS (EI): calcd for $C_{20}H_{21}N_3O_2$ [M^+]: 335.1634, found: 335.1646.

(*R*)-4-(*N*-*tert*-Butyloxycarbonyl)amino-*N*-phenylacetyltryptophan methyl ester (20): The combined organic layers resulting from the work-up of the enzymic resolution of **12** were washed with brine, and dried (Na_2SO_4), and concentrated to give a residue (2.49 g) which was dissolved in CH_2Cl_2 (45 mL) at room temperature. To this solution diazomethane (1M in CH_2Cl_2, 30 mL) at −78 °C was added and the solution was stirred for 30 min at room temperature, and then degassed with argon, the solvent was evaporated under reduced pressure and the crude residue was purified by chromatography on silica gel using hexane/AcOEt (1:1) as eluent affording methyl ester **20** (1.42 g, 41%, two steps) as an amorphous solid. R_f = 0.40 (silica gel, hexane/AcOEt, 1:1); $[\alpha]_D^{20}$ +19.7 (*c* = 0.45, EtOH); IR (KBr): $\tilde{\nu}_{max}$ = 3303, 3062, 2950, 1733, 1700, 1660, 1516, 1248, 1164, 745 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.18 (s, 1H), 7.25 (m, 3H), 7.18 (m, 3H), 7.10 (m, 2H), 6.78 (s, 1H), 6.63 (s, 1H), 6.28 (br d, *J* = 7.5 Hz, 1H), 4.83 (m, 1H), 3.70 (s, 3H), 3.52 (m, 2H), 3.35 (dd, *J* = 15.0, 5.0 Hz, 1H), 3.25 (dd, *J* = 15.0, 8.0 Hz, 1H), 1.53 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 172.4, 171.3, 155.0, 137.6, 134.6, 129.3, 129.3, 128.6, 128.6, 128.6, 127.1, 123.7, 122.1, 121.8, 116.5, 109.4, 108.8, 80.5, 53.8, 52.4, 43.3, 28.4, 28.4, 28.4, 28.0; MS (EI): *m/z* 452 [M^++1] (3), 451 [M^+] (10), 352 (7), 351 (30), 242 (8), 216 (12), 189 (20), 145 (100), 91 (19), 56 (20), 43 (33), 41 (39); HRMS (EI): calcd for $C_{25}H_{29}N_3O_5$ [M^+]: 451.2107, found 451.2095.

(*R*)-4-Amino-*N*-phenylacetyltryptophan methyl ester (21): According to the procedure described above for amino compound **15**, to a suspension of **20** (1.14 g, 2.61 mmol) and 1,3-dimethoxybenzene (0.55 mL), was added at room temperature CF_3COOH (25 mL) to provide after work-up and column chromatography (hexanes/EtOAc, 1:1) amino alcohol **21** (0.81 g, 91%) as a white foam. R_f = 0.25 (silica gel, EtOAc/hexanes, 1:1); $[\alpha]_D^{20}$ −4.8 (*c* = 0.81, EtOH); IR (KBr): $\tilde{\nu}_{max}$ = 3373, 3030, 2951, 1738, 1657, 1218, 737 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.98 (br s, 1H), 7.49 (br d, *J* = 7.0 Hz, ^{1}H), 7.30 (m, 3H), 7.15 (m, 2H), 7.01 (t, *J* = 7.0 Hz, 1H), 6.89 (d, *J* = 8.0 Hz, 1H), 6.77 (s, 1H), 6.37 (d, *J* = 7.5 Hz, 1H), 4.72 (q, *J* = 7.0 Hz, 1H), 3.88 (br s, 2H), 3.72 (s, 3H), 3.53 (s, 2H), 3.31 (d, *J* = 7.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.6, 171.6, 139.1, 137.8, 134.7, 129.5, 129.5, 128.7, 128.7, 127.0, 122.9, 122.3, 117.2, 109.7, 107.3, 103.9, 55.3, 52.2, 43.4, 28.9; MS (EI): *m/z*: 352 [M^++1] (8), 351 [M^+] (40), 216 (9), 157 (8), 146 (10), 145 (100), 91 (12); HRMS (EI): calcd for $C_{20}H_{21}N_3O_3$ [M^+]: 351.1583, found: 351.1593.

(*R*)-[2-(4-Amino-1*H*-indol-3-yl)-1-(hydroxymethyl)ethyl]phenylacetamide (22): According to the procedure described above for amino alcohol **16**, to a solution of methyl ester **20** (0.675 g, 1.92 mmol) in dry THF (2 mL) was added $LiBH_4$ (2M in THF, 1.1 mL, 2.21 mmol). The solution was stirred for 1 h at room temperature and for a further 1 h at reflux to provide after work-up and column chromatography with EtOAc as eluent amino alcohol **22** (0.558 g, 90%) as a white foam. R_f = 0.3 (silica gel, EtOAc); $[\alpha]_D^{19}$ −4.9 (*c* = 0.66, EtOH); IR (KBr): $\tilde{\nu}_{max}$ = 3372, 3061, 2935, 1645, 1507, 1038, 732 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.39 (br s, 1H), 7.29 – 7.23 (m, 3H), 7.15 – 7.13 (m, 2H), 6.95 (t, *J* = 8.0 Hz, 1H), 6.89 (br d, *J* = 9.5 Hz), 6.83 (d, *J* = 8.0 Hz, 1H), 6.66 (d, *J* = 2.5 Hz, 1H), 6.34 (d, *J* = 7.5 Hz, 1H), 4.23 (br s, 3H), 3.97 (m, 1H), 3.51 (dd, *J* = 11.5, 3.5 Hz, 1H), 3.47 (s, 2H), 3.40 (dd, *J* = 11.0, 4.0 Hz, 1H), 3.11 (dd, *J* = 14.5, 6.0 Hz, 1H), 2.88 (dd, *J* = 14.5, 9.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.8, 139.2, 137.9, 134.9, 129.3, 129.3, 128.8, 128.8, 127.1, 122.8, 122.6, 117.5, 110.8, 107.4, 104.1, 62.5, 54.1, 43.6, 27.6; MS (EI): *m/z*: 324 [M^++1] (12), 323 [M^+] (75), 188 (40), 157 (8), 146 (18), 145 (100), 91 (20), 58 (12), 43 (38); HRMS (EI): calcd for $C_{19}H_{21}N_3O_2$ [M^+]: 323.1634, found: 323.1623.

(*R*,*R*)-2-{4-[1-(Phenylmethyloxycarbonyl)ethylamino]-1*H*-indol-3-yl}-1-(hydroxymethyl)ethylphenylacetamide (23): According to the procedure described for the synthesis of **18a** above, phenylmethyl (*S*)-2-hydroxy-3-phenylpropanoate (0.233 g, 1.27 mmol) was allowed to react with trifluoromethanesulfonic acid anhydride (0.232 mL, 1.39 mmol) and 2,6-lutidine (0.41 mL, 3.81 mmol) in dry CH_2Cl_2 (5 mL) at 0 °C. Then a solution of amino alcohol **22** (0.4 g, 1.24 mmol) in CH_2Cl_2 (5 mL) was added dropwise to the solution of triflate **(−)-17a**. The reaction mixture was stirred at room temperature for 2 h, concentrated, and purified by chromatography using

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EtOAc as an eluent affording **23** (0.49 g, 83 %) as an amorphous solid. R_f = 0.50 (silica gel, EtOAc); $[\alpha]_D^{19}$ −10.4 (c = 1.00, EtOH); FT-IR (neat): $\tilde{\nu}_{max}$ = 3395, 3063, 3031, 2926, 1734, 1653, 1513, 1159, 730 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 8.21 (br s, 1H), 7.36–7.18 (m, 10H), 6.96 (t, J = 8.0 Hz, ^1H), 6.85 (d, J = 8.0 Hz, 1H), 6.76 (d, J = 2.5 Hz, 1H), 6.43 (br d, J = 8.0 Hz, ^1H), 6.22 (d, J = 7.5 Hz, 1H), 5.15 (m, 2H), 4.26 (q, J = 7.0 Hz, 1H), 4.03 (m, 1H), 3.60 (dd, J = 11.0, 3.0 Hz, 1H), 3.51 (s, 2H), 3.48 (dd, J = 11.0, 3.5 Hz, 1H), 3.08 (m, 2H), 1.52 (d, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 176.0, 171.2, 140.7, 137.7, 135.4, 135.0, 129.3, 129.3, 128.8, 128.8, 128.6, 128.6, 128.5, 128.3, 128.3, 127.1, 123.0, 122.5, 116.7, 111.0, 103.8, 102.0, 67.1, 62.5, 54.3, 52.6, 43.8, 28.0, 18.8; MS (EI): m/z 486 [M^++1] (25), 485 [M^+] (100), 378 (5), 365 (8), 350 (60), 197 (20), 171 (58), 157 (20), 91 (95), 43 (22); HRMS (EI): calcd for $C_{29}H_{31}N_3O_4$ [M^+]: 485.2314, found: 485.2299.

(+)-N^{13}-desmethyl-C^{12}-desisopropyl-C^{12}-methylindolactam V ((+)19a): A solution of phenylacetamide **23** (92 mg, 0.190 mmol) in EtOH/KOH (2 M in H_2O) (1:1) (12 mL) was refluxed for 17 h. After cooling to room temperature the EtOH was evaporated in vacuo, and the pH of the resulting aqueous mixture was adjusted to pH = 1.5 by slow addition at 0 °C of concentrated HCl. The mixture was diluted with H_2O and washed with AcOEt. The aqueous phase was concentrated to give a solid residue which according to the procedure described above for the synthesis of **19a**, was allowed to react with hydroxybenzotriazole (57 mg, 0.430 mmol), *N*-methylmorpholine (0.44 mL, 1.94 mmol), and (1-benzotriazoloxy-bis-dimethylamino) tetrafluoroborate (123 mg, 0.40 mmol) in DMF (5.5 mL) at room temperature for 22 h. Work-up and column chromatography (EtOAc/MeOH, 9:1) afforded **(+)-19b** (39 mg, 77 %) as an amorphous solid. $[\alpha]_D^{20}$ +123 (c = 0.75, ethanol).

Conjugate (25a): A solution of (−)-**19a** (210 mg, 0.81 mmol), **24** (195 mg, 0.89 mmol), and *para*-toluenesulfonic acid (PTSA) (recrystallized from AcOEt and dried) (170 mg, 0.89 mmol) in dry CH_2Cl_2 (10 mL) was heated at 40 °C for 1 h. After cooling to room temperature the reaction mixture was poured into AcOEt and washed with saturated aqueous $NaHCO_3$, and dried over Na_2SO_4, and concentrated. The crude mixture was purified by chromatography using EtOAc/hexane (2:3) as an eluent to afford **25a** (100 mg, 25 %) as a mixture of two diastereoisomers (ratio ca. 2:1) and unreacted **(−)-19a** (145 mg, 69 %). **25a**: FT-IR (neat): $\tilde{\nu}_{max}$ = 3344, 3033, 2949, 2871, 1747, 1654, 1499, 1456, 1197, 1172, 1126, 1084, 1035, 751, 698 cm^{-1}; MS (EI): m/z: 479 [M^++2] (20), 477 [M^+] (60), 401 (20), 343 (2), 259 (25), 258 (15), 218 (40), 172 (80), 171 (45), 157 (40), 127 (70), 91 (100), 83 (40), 55 (20); HRMS (EI): calcd for $C_{27}H_{31}N_3O_5$ [M^+]: 477.2264, found: 477.2275.

Conjugate (25b): According to the procedure described above for the synthesis of **25a**, a solution of **19b** (35 mg, 0.118 mmol), **24** (31 mg, 0.142 mmol), and PTSA (36 mg, 0.142 mmol) in dry CH_2Cl_2 (3.5 mL) was heated under an argon atmosphere at 40 °C for 1 h. After work-up and chromatographic purification using EtOAc/hexane (3:2) as an eluent unreacted **19b** (8.4 mg, 35 %) and **25b** (31 mg, 50 %) was obtained as a mixture of two diastereoisomers (ratio ca. 2:1). **25b**: FT-IR (neat): $\tilde{\nu}_{max}$ = 3350, 3034, 2955, 2870, 1747, 1656, 1499, 1456, 1197, 1170, 1129, 1036, 750, 698 cm^{-1}; MS (EI): m/z: 506 [M^++1] (20), 505 [M^+] (100), 287 (20), 286 (15), 200 (35), 199 (20), 157 (70), 156 (10), 130 (10), 91 (65), 43 (20); HRMS (EI): calcd for $C_{29}H_{35}N_3O_5$ [M^+]: 505.2577, found: 505.2597.

Conjugate (25c): According to the procedure described above for the synthesis of **25a**, a solution of **19c** (125 mg, 0.373 mmol), **24** (98 mg, 0.447 mmol), and PTSA (78 mg, 0.41 mmol) in dry CH_2Cl_2 (13 mL) was heated under an argon atmosphere at 40 °C for 1 h. After work-up and chromatographic purification using EtOAc/hexane (3:2) as an eluent unreacted **19c** (37 mg, 29 %) and **25c** (140 mg, 68 %) was obtained as a mixture of two diastereoisomers (ratio ca. 2:1). **25c**: FT-IR (neat): $\tilde{\nu}_{max}$ = 3354, 3031, 2951, 2870, 1746, 1658, 1497, 1455, 1262, 1198, 1034, 750, 700 cm^{-1}; MS (EI): m/z: 553 [M^+] (80), 477 (3), 417 (5), 335 (35), 248 (15), 247 (20), 183 (15), 157 (100), 155 (20), 91 (80), 43 (50); HRMS (EI): calcd for $C_{33}H_{35}N_3O_5$ [M^+]: 553.2577, found: 553.2560.

General procedure for solid-phase synthesis: Parallel solid-phase synthesis was performed with a FlexChem reaction block (Robbins Scientific). For compounds **32/1–5**, reactions carried out on solid phase were monitored by TLC and/or ^1H NMR analysis of the product obtained upon acidic cleavage from the resin and yields of the four-step solid-phase sequence refer to the mass balance after cleavage, work up and chromatographic purification. Compounds **32/6–31** were characterized using HPLC and flow injection ESI-MS. Yields refer to the contents by analytical HPLC performed on a HP1100 instrument using a ProntoSil 120–3-C18 AQ column (250 × 2.1 mm, 3 μm, Bischoff®) and a gradient from 90 % aqueous media (0.05 % HCOOH) to 100 % CH_3CN (0.05 % HCOOH) as eluent at a flow rate of 0.3 mL min^{-1} with UV detection at 190–400 nm range. All compounds biologically tested were purified by flash chromatography. All compounds were isolated in multi milligram amounts (typically 5–20 mg).

Attachment of conjugate 25a to the Merrifield resin: 10 % palladium on carbon (12 mg) was added to a solution of conjugate **25a** (84 mg, 0.176 mmol) in MeOH (6 mL), and the resulting mixture was stirred under a H_2 atmosphere for 1.5 h. Then the mixture was filtered through Celite, concentrated, and dried under high vacuum to afford a white solid. The solid was dissolved in DMF (4 mL) and chloromethylpolystyrene resin (1.24 mmol g^{-1}, 128 mg, 0.16 mmol), Cs_2CO_3 (156 mg, 0.48 mmol), and KI (13.2 mg, 0.08 mmol) were added under an argon atmosphere. The resulting mixture was stirred gently over 28 h at 80 °C. Then the suspension was poured into a fritted funnel, washed with DMF, DMF/H_2O (1:1), DMF, MeOH, and CH_2Cl_2 and dried under vacuum to constant weight to furnish resin **26a** (156 mg). The IR-spectroscopic analysis of the resin shows intensive signals at ν = 3431 (^{13}N–H), 1746 (C=O, ester), 1666 cm^{-1} (C=O, amide). Determination of the loading level based on recovered starting material: the combined filtrates were concentrated and allowed to react with benzyl bromide (excess) and CO_3Cs_2 (excess) in DMF (2 mL) at room temperature for 15 h to afford after work-up and column chromatography pure **25a** (8 mg, 0.017 mmol; 0.156 mmol **25a** attached to 156 mg of **26a**, 1 mmol g^{-1}, 81 % for coupling yield). Determination of the loading level by gravimetry: a sample of resin **26a** (12 mg) was treated with CF_3COOH/H_2O (95:5) (0.5 mL), and the resulting mixture was shaken at room temperature for 0.5 h. The mixture was filtered, washed with CH_2Cl_2, and concentrated. The residue was taken up in AcOEt, and the solution was washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated. The crude mixture was purified by chromatography using EtOAc/MeOH (9:1) as eluent to afford 3 mg of pure **19a** (loading level: 1 mmol g^{-1}, 81 % for coupling yield).

Attachment of conjugates 25b, c to the Merrifield resin: According to the procedure described above for the synthesis of **26a**, conjugates **25b** and **25c** afforded resins **26b** and **26c** with loadings levels of 0.90 and 0.95 mmol g^{-1} respectively.

General reductive amination procedure: A solution of aldehyde **27** (5.0 equiv) in DMF (1 % HOAc) (3 mL per 0.04 mmol of **27**) was added to dried resin **26** (1.0 equiv). The suspension was gently stirred for 1 h after which $NaBH(OAc)_3$ (5.0 equiv) was added. The suspension was gently stirred for 35 h, and CH_3OH was added to the resin to quench the excess of reducing reagent and dissolve the borate salts. The suspension was filtered, washed with DMF, DMF/H_2O (1:1), THF, and CH_2Cl_2, and dried to constant weight in vacuo to afford **28**.

General iodination procedure: To a suspension of resin **28** (1.0 equiv) in dry dioxane/pyridine (1:1) (1 mL per 0.025 mmol of **28**) was added doubly sublimated iodine (3 equiv) and the reaction was gently stirred at 0 °C for 1 h. The suspension was poured into a fritted funnel, and the resin was washed with dioxane, DMF/$Na_2S_2O_3$ (aq. 3 %) (1:1), DMF/H_2O (1:1), DMF, THF, and CH_2Cl_2 and dried to constant weight in vacuo to afford **29**.

General Sonogashira coupling procedure: To resin **29** (1.0 equiv) was added dioxane/Et_3N (2:1) (1 mL per 0.01 mmol of **29**), CuI (0.4 equiv) and alkyne **30** (8.0 equiv), and the resulting suspension was degassed for 10 min. Bis(triphenylphosphane)palladium(II) chloride (0.2 equiv) was added and the reaction mixture was shaken at room temperature for 15 h. The suspension was filtered and the resin was washed with dioxane, H_2O, DMF/H_2O (1:1), DMF, MeOH, and CH_2Cl_2, and dried to constant weight in vacuo to afford **31**.

Cleavage of polymer-bound teleocidin analogues: To resin **31** (1.0 equiv) was added CF_3COOH/H_2O (95:5) (1 mL/0.01 mmol of **31**) and the resulting mixture was shaken at room temperature for 1 h. The mixture was filtered, washed with CH_2Cl_2 and concentrated. The residue was diluted with AcOEt, washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated. For compounds **32/1,5** the crude mixture was purified by chromatography using EtOAc/hexane mixtures as an eluent to afford **32**.

(−)-N^{13}-Desmethyl-C^{12}-desisopropyl-N^{13}-pentyl-C^{12}-phenylmethyl-7-phenylethanoyl indolactam V (32/1): Overall yield for four steps: 40 %, based

on **26c** loading level: 0.95 mmol g^{-1}; $R_f = 0.45$ (silica gel, EtOAc/hexane, 1:1); $[\alpha]_D^{22}$ −148 ($c = 0.07$, $CHCl_3$); FT-IR (neat): $\tilde{\nu}_{max} = 3413, 3062, 3027, 2929, 2868, 1661, 1574, 1502, 1293, 1146, 1080, 799, 722$ cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$): δ 10.67 (s, 1H), 7.56 (d, $J = 9.5$ Hz, 1H), 7.30–7.20 (m, 5H), 6.88 (m, 6H, H-2), 6.58 (s, 1H), 6.18 (d, $J = 9.5$ Hz, 1H), 5.13 (t, $J =$ 7.5 Hz, 1H), 4.36 (m, 1H), 4.27 (d, $J = 14.5$ Hz, 1H), 4.15 (d, $J = 14.5$ Hz, 1H), 3.69 (m, 1H), 3.6–3.4 (m, 2H), 3.31 (dd, $J = 12.5$, 8.0 Hz, 1H), 3.12 (m, 1H), 2.99 (br s, 2H), 2.88 (dd, $J = 13.5$, 7.5 Hz, 1H), 1.81 (br s, 1H), 1.60–1.45 (m, 2H), 1.3–1.15 (m, 4H), 0.81 (m, 3H); HRMS (EI): calcd for $C_{33}H_{37}N_3O_3$ [M^+]: 523.2835, found: 523.2849.

(−)-*N*^{13}-Desmethyl-*C*^{12}-desisopropyl-*N*^{13}-pentyl-*C*^{12}-phenylmethyl-7-pentanoyl indolactam V (32/2): Overall yield for four steps: 50%, based on **26c** loading level: 0.95 mmol g^{-1}; $R_f = 0.50$ (silica gel, EtOAc/hexane 1:1); $[\alpha]_D^{22}$ −174 ($c = 0.05$, $CHCl_3$); FT-IR (neat): $\tilde{\nu}_{max} = 3408, 3087, 3063, 3026, 2956, 2857, 1665, 1575, 1540, 1454, 1345, 1221, 1154, 1078, 798, 733, 699$ cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 10.77$ (s, 1H), 7.54 (d, $J = 8.5$ Hz, 1H), 7.01 (m, 5H), 6.96 (s, 1H), 6.26 (s, 1H), 6.26 (d, $J = 8.5$ Hz, 1H), 5.20 (t, $J =$ 8.5 Hz, 1H), 4.46 (m, 1H), 3.76 (dd, $J = 10.5$, 4.0 Hz, 1H), 3.57 (m, 2H), 3.38 (dd, $J = 14.0$, 8.0 Hz, 1H), 3.19–2.90 (m, 6H), 2.35 (t, $J = 7.5$ Hz, 1H), 1.75 (quint., $J = 7.5$ Hz, 2H), 1.67 (m, 2H), 1.43 (sext., $J = 7.5$ Hz, 2H), 1.30 (m, 4H), 0.97 (t, $J = 7.5$ Hz, 3H), 0.86 (m, 3H); HRMS (EI): calcd for $C_{30}H_{39}N_3O_3$ [M^+]: 489.2950, found: 489.2991.

(−)-*N*^{13}-Desmethyl-*C*^{12}-desisopropyl-*N*^{13}-isobutyl-*C*^{12}-methyl-7-phenylmethylindolactam V (32/3): Overall yield for four steps: 43%, based on **26a** loading level: 1.0 mmol g^{-1}; $R_f = 0.45$ (silica gel, EtOAc/hexane, 3:1); $[\alpha]_D^{22}$ −223 ($c = 0.02$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 10.78$ (s, 1H), 7.84 (d, $J = 8.5$ Hz, 1H), 7.32 (m, 5H), 6.99 (s, 1H), 6.57 (s, 1H), 6.53 (d, $J =$ 8.5 Hz, 1H), 4,92 (q, $J = 8.0$ Hz, 1H), 4.65 (m, 1H), 4.32 (s, 1H), 3.79 (dd, $J = 11.5$, 4.5 Hz, 1H), 3.59 (t, $J = 11.5$ Hz, 1H), 3.42 (d, $J = 13.5$ Hz, 1H), 3.10 (m, 2H), 2.59 (t, $J = 13.5$ Hz, 1H), 1.98 (m, 1H), 1.24 (d, $J = 7.5$ Hz, 3H), 0.95 (d, $J = 8.0$ Hz, 3H), 0.85 (d, $J = 7.5$ Hz, 3H); MS (MALDI): $C_{32}H_{39}N_3O_3$ [M^+]: 433.

(−)-*N*^{13}-Desmethyl-*C*^{12}-desisopropyl-*N*^{13}-isobutyl-*C*^{12}-methyl-7-pentanoylindolactam V (32/4): Overall yield for four steps: 47%, based on **26a** loading level: 1.0 mmol g^{-1}; $R_f = 0.50$ (silica gel, EtOAc/hexane, 3:1); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 10.73$ (s, 1H), 7.68 (d, $J = 8.5$ Hz, 1H), 6.95 (s, 1H), 6.66 (s, 1H), 6.47 (d, $J = 8.0$ Hz, 1H), 4.88 (q, $J = 8.0$ Hz, 1H), 4.62 (m, 1H), 3.73 (m, 1H), 3.56 (m, 1H), 3.37 (m, 1H), 3.15–2.85 (m, 6H), 1.90 (m, 1H), 1.75 (m, 2H), 1.38 (sext., $J = 7.5$ Hz, 2H), 1.20 (d, $J = 7.5$ Hz, 3H), 0.91 (t, $J = 7.5$ Hz, 3H), 0.81 (m, 6H); MS (MALDI): $C_{29}H_{39}N_3O_3$ [M^+]: 399.

(−)-*N*^{13}-Desmethyl-*N*^{13}-ethyl-7-pentanoylindolactam V (32/5): Overall yield for four steps: 20%, based on **26b** loading level: 0.9 mmol g^{-1}; $R_f =$ 0.45 (silica gel, EtOAc/hexane, 4:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 10.87$ (s, 1H), 7.71 (d, $J = 8.5$ Hz, 1H), 7.00 (s, 1H), 6.83 (s, 1H), 6.56 (d, $J =$ 8.5 Hz, 1H), 4.51 (d, $J = 10.5$ Hz, 1H), 4.22 (m, 1H), 3.60 (m, 4H), 3.20–3.00 (m, 2H), 2.96 (m, 2H), 2.62 (m, 1H), 1.75 (m, 2H), 1.45 (m, 2H), 1.10 (m, 3H), 0.93 (t, $J = 7.0$ Hz, 3H), 0.93 (d, $J = 7.5$ Hz, 3H), 0.59 (d, $J = 7.5$ Hz, 3H); MS (MALDI): $C_{32}H_{39}N_3O_3$ [M^+]: 433.

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[17] Initial attemps in order to deprotect the amino group at *N*-10 before the manipulation of the functional groups, forced us to carry out this operation just before the lactam formation. 1) the hydrolysis of the remaining Phac group, present in D-**12** and **20**, in the same enzymatic reaction but with longer reaction time, gave only unreacted starting material. The same result was observed at pH 11. For related hydrolyses, see: G. Cardillo, A. Tolomilli, C. Tomasini, *J. Org. Chem*. **1996**, *61*, 8651–8654; 2) the hydrolysis of the two protecting groups present in **20** using HCl at reflux gave the respective diamino indole which we were not able to monoprotect.

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[18] a) In these cases once the triflate was formed, the amino indol **16** was added to the same flask with excess of base (2,6-lutidine) and the pseudopeptides **18a** and **18c** were isolated in very high yield, see: R. W. Feenstra, E. H. M. Stokkingreef, R. J. F. Nivard, H. C. J. Ottenheijm, *Tetrahedron* **1988**, *44*, 5583–5595. b) Although compound **18a** was chromatographically homogeneous, its ^{1}H NMR spectrum showed the presence of a second minor component (ca. 7%), which could not be separated by any conventional purification procedure. We speculate that the alkylating reagent (*R*)-**17a** contains a small portion of its enantiomer (*S*)-**17a**, which would react with **16** in the same way. A similar percentage was found in the ^{1}H NMR spectrum of (−)-**19a**, probably corresponding to the C-12 epimer of (−)-**19a**.

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