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Synthesis and Pharmacological Evaluation of 8- and 9-Substituted Benzolactam-V8 Derivatives as Potent Ligands for Protein Kinase C, a Therapeutic Target for Alzheimer's Disease

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*A central element in the pathophysiology of Alzheimer's disease (AD) is the formation of amyloid plaques, which result from abnormal processing of the amyloid precursor protein (APP). The processing of APP is largely provided by three key enzymes, namely the α-, β-, and γ-secretases. As the latter two contribute to the formation of neurotoxic Aβ fragments while α-secretase does not, a decrease in the amyloidogenic products can be brought about either by inhibition of the β- and γ-secretases or through the activation of α-secretase. It is now known that the activation of protein kinase C (PKC) enhances α-secretase activity and therefore represents a possible target for the development of agents urgently needed for the treatment of this devastating neurodegenerative disorder. In the present study, new benzolactam-V8-based PKC activators were synthesized and tested for their binding affinity toward PKCα. All compounds tested showed binding values in the nanomolar concentration range. In accordance with previous publications, 9-substitution dramatically increased PKC binding affinity in comparison with the corresponding 8-substituted analogues. In addition to the location of the side chain on the aromatic ring, the binding affinities of these benzolactams were found to depend on the orientation, length, and electronic properties of this appendage. An interesting decrease in binding affinity was found for the 9-thienyl analogue **13**, suggesting adverse electronic interactions of the sulfur atom with PKC or parts of the cellular membrane.*

Introduction

Protein kinase C (PKC) is a dynamic, multienzyme family of serine/threonine kinases that mediates intracellular signal transduction pathways which regulate a number of cellular events such as gene transcription, differentiation, cell cycle, apoptosis, cell migration, and drug resistance in response to hormonal, neuronal, and other stimuli.[1–4] Based on their divergent structures and responses to external factors, these enzymes have been classified into three groups of isoforms: the classical, novel, and atypical PKCs. Classical and novel PKCs are found in the cytosol. In the presence of diacylglycerol (DAG) and (for the classical PKCs) Ca^{2+} they translocate to the membranes and become active.[5] The binding of DAG and other ligands such as phorbol esters or indolactams occurs at the two cysteine-rich zinc fingers (C1 domains) of the N-terminal regulatory domain of the classical and novel PKCs.

PKC has been shown to play a major role in a variety of disease states including diabetes, heart disease, cancer, and Alzheimer's disease. In recent years, there has been a growing interest in studying the relationship between Alzheimer's disease and PKC. In addition to the detection of defective PKC in brains [6–11] and peripheral tissues of AD patients,[12–14] PKC is known to be involved in the processing of the amyloid precursor protein (APP) by modulating the activity of α-secretase.[15,16] Cleavage of APP by α-secretase results in the formation of pathologically harmless fragments such as sAPPα and C83, whereas the competitive, successive processing of APP by β- and γ-secretase generates neurotoxic fragments, namely Aβ40 or Aβ42, and ultimately leads to plaque formation.[17,18] Thus, it seems reasonable to assume that the activation of α-secretase results in a decrease of amyloidogenic products of β-secretase. In accordance with the finding that PKC activators of the phorbol family dramatically enhance α-secretase activity,[19–21] it has been shown that PKC activation results in a direct decrease in Aβ. Although other studies could not verify this result, they do

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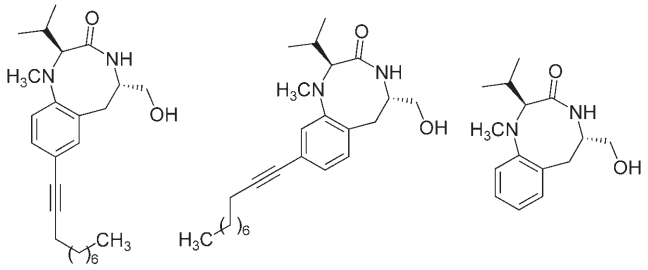
confirm effects of PKC activation that are consistent with an improvement of the pathological condition.[22–24]

Earlier publications have shown the ability of benzolactam-V8 derivatives to act as potent PKC activators.[25] The presence of alkynyl or other hydrophobic chains such as those in compounds **1a** and **1b**, however, was required for robust PKC activation, as compounds lacking such appendages (compound **1c**) showed a considerably decreased ability to activate PKC.[26]



1a **1b** **1c**

In addition, the position and nature of the hydrophobic side chain seems to have a significant effect on PKC activation, as steric hindrance was reported in positions 7 and 8, even though compound **1a** shows K_i values in the low nanomolar range.[27] Saturated and unsaturated chains as well as chains containing heteroatoms have been introduced that have diverging impact on binding affinity and PKC activation.[27–29] In this context, possible tumor-promoting activities, which seem to be closely connected to the nature of the side chain, must be addressed.[30–32]

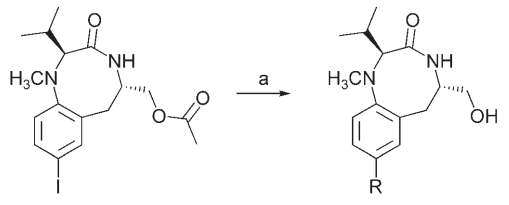
Design and synthesis

In continuation of our previous studies, we decided to more closely investigate modifications at position 9. We introduced new aryl substituents in this location and, for better comparison, prepared the corresponding novel 8-substituted analogues.

Apart from aryl substitution, we established a convenient way to introduce arylamide and closely related groups, which are proposed to be favorable for PKC activation due to their putative ability to interact with proximal amino acid residues that constitute the binding loops of PKCα and polar regions of the phospholipids in the cellular membrane.[28] Furthermore, those compounds containing an amide moiety display reasonable Clog *P* values with regard to therapeutic applications.

The preparation of 8- and 9-substituted derivatives commenced from hydroxy-protected, 8-iodinated or 9-triflated benzolactams, the syntheses of which have previously been published.[27,29] Phenyl and thienyl substitution of the benzolactams was carried out through reaction with substituted arylboronic acids under Suzuki conditions as shown in Scheme 1.[33]

The required decynylphenyl and decynylthienylboronic acids were prepared starting from diiodobenzene and diiodothiophene (Experimental Section). Monoalkynyl substitution was



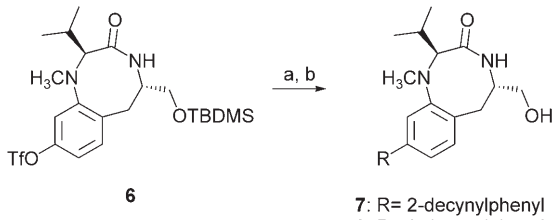
1

2: R= 2-decynylphenyl
3: R= 3-decynylphenyl
4: R= 4-decynylphenyl
5: R= 5-decynylthienyl

Scheme 1. Reagents and conditions for compounds **2**–**5**: a) arylboronic acid, $[Pd(PPh_3)_4]$, KOH, toluene, EtOH, H_2O, argon, reflux.

carried out with Sonogashira coupling,[34,35] and the remaining iodo substituent was exchanged by lithium metallation, subsequent reaction with trimethoxyborane, and acidic workup.[36]

In the case of 8-substituted benzolactams, an acetyl moiety was chosen as a hydroxy protective group, and was readily cleaved under the Suzuki coupling conditions to yield the desired compounds **2**–**5**. Formation of the ligands in a one-pot synthesis could not, however, be observed in the case of 9-substituted benzolactams (Scheme 2), hence the TBDMS group



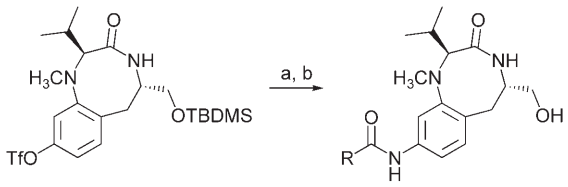
6

7: R= 2-decynylphenyl
8: R= 3-decynylphenyl
9: R= 4-decynylphenyl
10: R= 2-hexynylphenyl
11: R= 3-hexynylphenyl
12: R= 4-hexynylphenyl
13: R= 5-decynylthienyl
14: R= 4-(Boc-amino)phenyl

Scheme 2. Reagents and conditions for compounds **7**–**14**: a) arylboronic acid, $[Pd(PPh_3)_4]$, K_2CO_3, argon, reflux; b) TBAF, THF. Boc = *tert*-butoxycarbonyl, TBAF = tetra-*n*-butylammonium fluoride, TBDMS = *tert*-butyldimethylsilyl.

was introduced to protect the hydroxy group.[37] For compounds **7**–**14**, cleavage of the silyl ether after the coupling reaction with TBAF in THF succeeded in high yields.

The palladium-catalyzed introduction of nitrogen at position 9 was attempted with several nitrogen-containing species such as benzylamines[38] and diphenylmethanimine[39,40] and different palladium-binding ligands, but was not successful until carboxamides were allowed to react under the conditions given in 'Scheme 3. Cleavage of the TBDMS group for compounds **15**–**23** was carried out with hydrochloric acid in ethanol, as the amide moieties seemed to be unstable in the presence of TBAF. The synthesis of compound **24** was published previously.[37]



6

15: R= phenyl
16: R= 2-phenylvinyl
17: R= 2-phenylethyl
18: R= 4-phenylbutadienyl
19: R= 4-phenylbutyl
20: R= biphenyl
21: R= 4-butylphenyl
22: R= 3-hexynylphenyl
23: R= 3-hexylphenyl

Scheme 3. Reagents and conditions for compounds **15**–**23**: a) carboxamide, $[Pd_2(dba)_3]$, xantphos, Cs_2CO_3, 1,4-dioxane, argon, 100 °C; b) EtOH, HCl (conc.). dba = *trans*,*trans*-dibenzylideneacetone, xantphos = 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene.

Binding studies

The binding affinities of all new benzolactam compounds toward PKCα were evaluated in displacement studies against bound [20-^{3}H]phorbol-12,13-dibutyrate (PDBU) on recombinant PKCα in the presence of phosphatidylserine.[41] Partition coefficients (Clog *P*) were calculated according to the fragment-based program KOWWIN 1.63,[42,43] and the results are presented in Table 1. The Clog *P* values ranged between 4.6 and 7.8 in the case of the phenyl-substituted benzolactams and varied from 3 to 5.5 for the carbonylamino-substituted compounds. The K_i values were all in the nanomolar concentration range, but depended strongly on the position of the benzolactam substituent.

Results and Discussion

The position and nature of the side chain attached to the benzolactam had a marked impact on its affinity for PKC. As previously reported, substitution at position 8 generally results in decreased affinity, which may partially result from steric interaction with certain amino acid residues present in the PKC structure. In accordance with these findings, the direction of the alkynyl chain had a pronounced influence on the K_i values within the 8-substituted benzolactam series. The *o*-decynyl derivative showed 5- to 10-fold higher affinities than its *meta* and *para* analogues, respectively. Although it is not clear whether these differences are due to interactions with PKC or the cellular membrane, this effect strongly diminished in the corresponding 9-substituted benzolactams. Within the series of 9-aryl-substituted compounds, different chain lengths and positions of substitution showed only subtle differences in binding affinity. As the *para* derivatives displayed both the lowest (decynyl, **9**) and the highest (hexynyl, **12**) affinities, while the others showed values in between, we suggest that *ortho* and *meta* substitutions result in a binding mode similar to that of the *para* substitutions, but with weaker interactions between alkyl groups and the membrane. Given an existing "optimum chain length", as proposed in previous publications, that is exceeded by compound **9**, shorter effective chain lengths are predicted to lead to increased binding values. In the series of compounds **2**–**13**, lipophilicity does not seem to play an important role, as differing Clog *P* values did not have a significant effect on the K_i values.

Of all compounds, the highest affinity was displayed by compound **12**, which almost reached K_i values in the picomolar concentration range. Compared with other 8-substituted benzolactams with similar Clog *P* values between 5.5 and 6, it showed a binding affinity that was 5–10-fold better. This result may be a consequence of the previously mentioned steric hindrance caused by the substituent located at position 8. On the other hand, the bulkiness of the phenyl ring of **12** should also result in a diminished affinity.

Interestingly, the 9-thiophene analogue **13** displayed a notable ~5-fold decrease in affinity relative to its phenyl analogue. This unexpected effect could be the result of adverse electronic interactions of the benzolactam binding pocket with the sulfur atom. It will be of interest to probe the consequence of such structural changes more fully, and in particular, as it relates to the selectivity of such ligands for the full range of PKC isozymes.

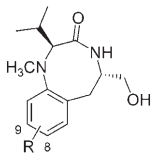
A wider range of binding values was shown by the ligands with more heteroatoms and functional groups (**14**–**24**). The binding data correlate well with the Clog *P* values. By increasing the distance between the phenyl ring and the amide bond by means of an alkyl (**16** and **18**) or alkenyl group (**17** and **19**), a dramatic increase in binding affinity was found. This effect was similar for both the unsaturated and saturated ligands, as was reported earlier for certain 8-substituted benzolactams. The position of the amide substituent did not seem to be of great importance, as the previously reported 8-substituted analogues of **18** and **19** displayed very similar binding values.[28]

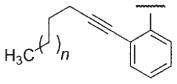
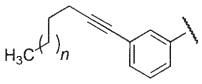
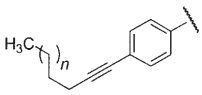
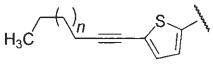
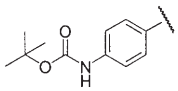
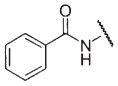
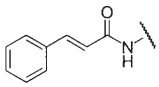
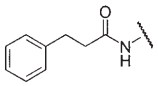
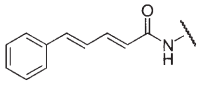
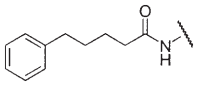
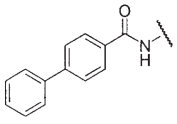
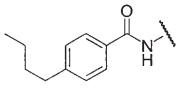
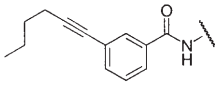
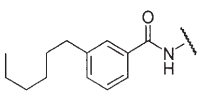
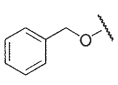
An improvement of the membrane interaction could also be achieved by the addition of alkyl or alkynyl groups to the phenyl ring of **15**, leading to **21**–**23**. These ligands showed K_i values in the low nanomolar concentration range.

Although Clog *P* is only a calculated value, it would seem that the nature of the residue borne by the amide group contributes to the affinity of the ligand primarily through the resulting increase of lipophilicity. Location of the substituent groups at position 9 brings about some improvement in affinity in relative to location of the substituent at position 8; however, the amide group does not show the kind of interaction that was proposed by earlier predictions.[28]

Interestingly, compound **24**, which represents a key intermediate in the preparation of the other compounds presented herein, can be considered to be a bioisostere of compound **15.** It was thus deemed worthy of testing, and much to our delight, was found to show a K_i value of 3.8 nM, despite its lower Clog *P* value. The aryl benzylether function thus appears to be quite favorable for the ligand–PKC–membrane interactions. Additionally, its Clog *P* value is in the range shown by many drug molecules, and thus we anticipate that the study of other aryl ethers of this type should be made. Efforts are also currently being made to explore the activity of selected ligands described herein in mouse models of Alzheimer's disease.

Table 1. K_i values of benzolactams **2–24.**



Compd	position	Appendage R	*n*	Clog *P*	K_i [nM] ± SEM
2	8		5	7.77	65.9 ± 3.1
7	9		5	7.77	3.6 ± 0.4
10	9		1	5.81	3.1 ± 0.5
3	8		5	7.77	309.4 ± 42.5
8	9		5	7.77	5.9 ± 0.7
11	9		1	5.81	3.0 ± 0.4
4	8		5	7.77	703.0 ± 135.8
9	9		5	7.77	6.0 ± 0.9
12	9		1	5.81	1.2 ± 0.1
5	8		5	7.59	396.2 ± 18.6
13	9		5	7.59	25.1 ± 3.5
14	9			4.64	31.5 ± 3.3
15	9			2.46	173.9 ± 10.5
16	9			2.85	114.4 ± 11.8
17	9			3.06	115.7 ± 19.3
18	9			3.6	49.8 ± 6.6
19	9			4.0	18.3 ± 1.1
20	9			4.23	25.9 ± 6.5
21	9			4.48	9.5 ± 1.1
22	9			4.75	4.1 ± 0.1
23	9			5.47	3.2 ± 0.0
24	9			3.54	3.8 ± 0.4

Experimental Section

Analysis of inhibition of [^{3}H]PDBU binding by non-radioactive ligands: Enzyme–ligand interactions were analyzed by competition with [^{3}H]PDBU binding to the single isozyme PKCα as described previously.[41]

Chemistry: Analytical and preparative thin-layer chromatography (TLC) was performed in a solvent-vapor-saturated chamber on EM Science silica gel 60 F-254 plates. Spots were visualized by UV light. NMR spectra were determined on a Bruker Avance 400 (^{1}H frequency of 400 MHz) using TMS as an internal standard (^{1}H and ^{13}C). High-resolution mass analysis was performed on a Waters QTOF-2 mass spectrometer with an electrospray source. All compounds were measured in positive ion mode; reserpine was used as an internal standard. Optical rotations were obtained on a Rudolph Research Analytic Autopole IV. Determinations of purity by HPLC were performed with a Shimadzu LC-10 AD pump and a Waters 484 tunable absorbance detector using the following conditions: A) Waters μBondapak C_{18} 300 mm × 3.9 mm; flow rate = 1.0 mL min^{-1}; detection at 254 nm; 0–30 min, 40–75% acetonitrile in water; 30–50 min, 75–100% acetonitrile in water. B) Waters μBondapak C_{18} 300 mm × 3.9 mm; flow rate = 1.0 mL min^{-1}; detection at 254 nm; 0–30 min, 30–75% acetonitrile in water, 30–50 min, 75–100% acetonitrile in water. C) Supelco Supelcosil LC-F 250 mm × 4.6 mm; flow rate = 1.0 mL min^{-1}; detection at 254 nm; 0–10 min, 40–50% acetonitrile in water, 10–30 min 50–100% acetonitrile in water.

General procedure A for the synthesis of 8-phenyl-substituted benzolactams-V8 2–5: A mixture of (2*S*,5*S*)-*O*-acetyl-8-iodobenzolactam **1** (50 mg, 0.12 mmol), KOH (100 mg), [Pd(PPh$_3$)$_4$] (26 mg), and the appropriately substituted phenylboronic acid (0.4 mmol) in toluene (2 mL), ethanol (1 mL), and water (1 mL) was degassed and heated at reflux for 24 h under an argon atmosphere. The desired product, easily detectable by its blue color under UV light

($\lambda = 254$ nm), was purified by TLC using ethyl acetate as the eluent.

General procedure B for the synthesis of 9-phenyl-substituted benzolactams-V8 7–14: A mixture of (2*S*,5*S*)-*O*-TBDMS-9-(trifluoromethylsulfonyloxy)benzolactam-V8 (70 mg, 0.13 mmol), K_2CO_3 (80 mg), $[Pd(PPh_3)_4]$ (20 mg), and the appropriately substituted phenylboronic acid (0.4 mmol) in toluene (4 mL) was degassed and heated at reflux for 16 h under an argon atmosphere. After purification by TLC, the compound was dissolved in THF (3 mL), and a few drops of TBAF solution (1 M in THF) were added. The solution was stirred for 1 h at room temperature, and the product was purified by TLC using ethyl acetate as the eluent.

General procedure C for the synthesis of (2*S*,5*S*)-9-(acylamino)-benzolactams-V8 15, 16, 18, 22, 23: A mixture of (2*S*,5*S*)-*O*-TBDMS-9-(trifluoromethylsulfonyloxy)benzolactam-V8 (50 mg, 0.1 mmol), Cs_2CO_3 (50 mg, 0.15 mmol), 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (xantphos, 10 mg), $[Pd_2(dba)_3]$ (5 mg) and the appropriate primary amide (0.2 mmol) in dry dioxane (5 mL) was degassed and stirred at 100 °C for 14–18 h under an argon atmosphere. The crude product was purified by TLC. To cleave the TBMDS group, the compound was dissolved in ethanol (8 mL) and a few drops of concentrated HCl were added. After starting material was no longer detectable by TLC (4–6 h), the product was partitioned between EtOAc and a saturated solution of $NaHCO_3$, the organic phase was dried ($MgSO_4$), and the product was then purified by HPLC.

(2*S*,5*S*)-8-(2-(1-Decynyl)phenyl)benzolactam-V8 (2): The title compound was obtained according to the general procedure A in 8% yield as a colorless oil: $[\alpha]_D^{20} = -316.8$ ($c = 0.27$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 0.88$–0.98 (m, 6H), 1.13 (d, 3H, $J = 6.8$ Hz), 1.25–1.45 (m, 10H), 1.48–1.58 (m, 2H), 2.35 (t, 2H, $J = 7.1$ Hz), 2.45–2.57 (m, 1H), 2.75 (br s, 1H), 2.80–2.93 (m, 4H), 3.13 (dd, 1H, $J = 16.6$, 7.9 Hz), 3.50–3.62 (m, 2H), 3.73–3.82 (m, 1H), 4.03 (m, 1H), 6.65 (s, 1H), 7.05 (d, 1H, $J = 8.4$ Hz), 7.22–7.40 (m, 3H), 7.50 ppm (d, 2H, $J = 7.0$ Hz); ^{13}C NMR ($CDCl_3$): $\delta = 14.1$, 19.5, 19.8, 20.5, 22.6, 28.3, 28.9, 29.1, 29.2, 31.8, 35.1, 37.5, 54.2, 66.1, 69.9, 80.4, 93.3, 118.9, 122.0, 126.5, 126.7, 128.7, 129.1, 129.9, 132.6, 133.2, 133.5, 142.9, 150.8, 173.7 ppm; HRMS: *m/z* $[M+Na^+]$ for $C_{31}H_{42}N_2O_2Na$: found 497.3129, calcd 497.3144; HPLC: $t_R = 31.9$ min (99.4% purity) under conditions A, $t_R = 32.8$ min (96.1% purity) under conditions C.

(2*S*,5*S*)-8-(3-(1-Decynyl)phenyl)benzolactam-V8 (3): 1,3-diiodobenzene (3 g, 9.1 mmol), CuI (0.27 g, 1.4 mmol), *n*-butanamine (1.73 g, 23.6 mmol), 1-decyne (1.57 g, 11.4 mmol) and $[Pd(PPh_3)_4]$ (0.32 g, 0.3 mmol) were dissolved in dry and degassed ethyl ether (40 mL), degassed, and stirred at room temperature for 6 h. After evaporation of all volatiles under high vacuum, the residue was purified by column chromatography using petroleum ether as eluent. The pure product (1.99 g, 5.9 mmol) was dissolved in dry THF (30 mL) and cooled to −78 °C before *n*BuLi (1.6 M in hexane, 4.5 mL, 7.2 mmol) was added dropwise. After 30 min stirring at the same temperature, $B(OMe)_3$ (0.75 mL) was added in one portion, and the mixture was allowed to warm to room temperature. After acidic workup, the boronic acid was used without further purification. The title compound was obtained according to the general procedure A in 18% yield as a colorless oil: $[\alpha]_D^{20} = -296.3$ ($c = 0.265$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 0.85$–0.96 (m, 6H), 1.13 (d, 3H, $J = 6.8$ Hz), 1.21–1.42 (m, 8H), 1.45–1.55 (m, 2H), 1.60–1.72, (m, 3H), 2.1 (br s, 1H), 2.41–2.56 (m, 3H), 2.82–2.92 (m, 4H), 3.23 (dd, 1H, $J = 16.6$, 7.9 Hz), 3.50–3.65 (m, 2H), 3.72–3.83 (m, 1H), 4.04 (br s, 1H), 6.38 (s, 1H), 7.07 (d, 1H, $J = 8.3$ Hz), 7.21 (s, 1H), 7.33–7.38 (m, 2H), 7.43–7.50 (m, 1H), 7.60 ppm (s, 1H); ^{13}C NMR ($CDCl_3$): $\delta = 14.1$, 19.4, 19.8, 20.5, 22.6, 28.2, 28.8, 28.9, 29.1, 29.2, 29.7, 31.8, 35.1, 37.6, 54.0, 66.1, 80.6, 90.5, 119.8, 124.4, 125.8, 126.3, 128.6, 129.8, 130.5, 130.8, 133.3, 140.6, 151.2, 173.2 ppm; HRMS: *m/z* $[M+Na^+]$ for $C_{31}H_{42}N_2O_2Na$: found 497.3144, calcd 497.3144; HPLC: $t_R = 36.2$ min (96.7% purity) under conditions A, $t_R = 32.2$ min (97.2% purity) under conditions C.

(2*S*,5*S*)-8-(4-(1-Decynyl)phenyl)benzolactam-V8 (4): The title compound was obtained according to the general procedure A in 11% yield as a colorless oil: $[\alpha]_D^{20} = -222.0$ ($c = 0.06$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 0.88$–0.98 (m, 6H), 1.13 (d, 3H, $J = 6.8$ Hz), 1.25–1.42 (m, 8H), 1.42–1.52, m, 2H), 1.52–1.70 (m, 2H), 2.1 (br s, 1H), 2.40–2.56 (m, 3H), 2.82–2.95 (m, 4H), 3.13 (dd, 1H, $J = 16.6$, 7.9 Hz), 3.50–3.63 (m, 2H), 3.73–3.82 (m, 1H), 4.0 (m, 1H), 6.33 (s, 1H), 7.07 (d, 1H, $J = 8.4$ Hz), 7.29 (s, 2H), 7.42–7.52 ppm (m, 4H); ^{13}C NMR ($CDCl_3$): $\delta = 14.1$, 19.5, 19.8, 20.5, 22.6, 28.2, 28.8, 28.9, 29.1, 29.2, 31.8, 35.1, 37.7, 54.0, 66.1, 70.0, 80.4, 91.0, 119.9, 122.4, 126.2, 126.3, 130.4, 130.9, 131.9, 133.0, 139.5, 151.2, 173.2 ppm; HRMS: *m/z* $[M+H^+]$ for $C_{31}H_{43}N_2O_2$: found 475.3326, calcd 475.3325; HPLC: $t_R = 35.7$ min (99.7% purity) under conditions A, $t_R = 32.8$ min (96.6% purity) under conditions C.

(2*S*,5*S*)-8-(5-(1-Decynyl)thienyl)benzolactam-V8 (5): 5-(1-decynyl)thienylboronic acid was prepared according to the method described for compound **3**. The title compound was obtained according to the general procedure A in 25% yield as a colorless oil: $[\alpha]_D^{20} = -254.9$ ($c = 0.94$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 0.8$–1.0 (m, 6H), 1.1 (d, 3H, $J = 6.8$ Hz), 1.25–1.40 (m, 8H), 1.40–1.51 (m, 2H), 1.57–1.70 (m, 3H), 2.41–2.52 (m, 3H), 2.76–2.89 (m, 3H), 3.07–3.25 (m, 2H), 3.47–3.62 (m, 2H), 3.72–3.80 (m, 1H), 3.95 (br s, 1H), 6.82 (s, 1H), 6.96–7.08 (m, 3H), 7.26 (s, 1H), 7.48–7.52 ppm (m, 1H); ^{13}C NMR ($CDCl_3$): $\delta = 14.1$, 19.7, 19.8, 20.5, 22.6, 28.2, 28.6, 28.9, 29.1, 29.2, 31.8, 35.0, 37.4, 54.1, 65.9, 69.9, 73.9, 95.2, 119.8, 121.7, 122.5, 125.2, 127.0, 129.3, 131.1, 132.0, 144.2, 151.4, 173.7 ppm; HRMS: *m/z* $[M+H^+]$ for $C_{29}H_{41}N_2O_2S$: found 481.2901, calcd 481.2889; HPLC: $t_R = 36.5$ min (99.2% purity) under conditions A, $t_R = 31.9$ min (95.1% purity) under conditions C.

(2*S*,5*S*)-9-(2-(1-Decynyl)phenyl)benzolactam-V8 (7): 2-(1-decynyl) phenylboronic acid was prepared according to the method described for compound **3**. The title compound was obtained according to the general procedure B in 22% yield as a colorless oil: $[\alpha]_D^{20} = -290.7$ ($c = 0.57$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 0.85$–0.97 (m, 6H), 1.13 (d, 3H, $J = 6.8$ Hz), 1.23–1.45 (m, 10H), 1.48–1.60 (m, 2H), 2.33 (t, 2H, $J = 7.1$ Hz), 2.41–2.55 (m, 1H), 2.82–2.95 (m, 4H), 3.08–3.20 (m, 2H), 3.50–3.65 (m, 2H), 3.72–3.83 (m, 1H), 4.18 (br s, 1H), 6.83 (s, 1H), 7.08–7.17 (m, 1H), 7.23–7.39 (m, 5H), 7.55 ppm (d, 1H, $J = 7.3$ Hz); ^{13}C NMR ($CDCl_3$): $\delta = 14.1$, 19.6, 20.0, 20.3, 22.6, 28.4, 28.7, 28.9, 29.1, 29.2, 31.8, 35.4, 37.1, 54.1, 65.8, 71.0, 80.4, 93.9, 118.5, 119.7, 120.2, 126.8, 127.6, 129.3, 130.3, 131.3, 135.5, 139.8, 140.3, 146.0, 152.1, 173.6 ppm; HRMS: *m/z* $[M+Na^+]$ for $C_{31}H_{42}N_2O_2Na$: found 497.3146, calcd 497.3144; HPLC: $t_R = 32.0$ min (99.9% purity) under conditions A, $t_R = 31.7$ min (96.8% purity) under conditions C.

(2*S*,5*S*)-9-(3-(1-Decynyl)phenyl)benzolactam-V8 (8): The title compound was obtained according to the general procedure B in 19% yield as a colorless oil: $[\alpha]_D^{20} = -319.6$ ($c = 0.345$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 0.85$–0.97 (m, 6H), 1.13 (d, 3H, $J = 6.8$ Hz), 1.23–1.45 (m, 8H), 1.45–1.55 (m, 2H), 1.60–1.72, (m, 3H), 2.41–2.56 (m, 3H), 2.80–2.96 (m, 5H), 3.13 (dd, 1H, $J = 16.6$, 7.9 Hz), 3.52–3.65 (m, 2H), 3.72–3.83 (m, 1H), 4.08 (br s, 1H), 6.83 (s, 1H), 7.08–7.17 (m, 1H), 7.21 (s, 1H), 7.33–7.43 (m, 2H), 7.46–7.52 (m, 1H), 7.62 ppm (s, 1H); ^{13}C NMR ($CDCl_3$): $\delta = 14.1$, 19.5, 19.9, 20.4, 22.7, 28.4, 28.8, 29.0, 29.1, 29.2, 29.7, 31.9, 35.4, 37.1, 54.1, 66.0, 70.4, 80.5, 90.8, 118.5,

120.2, 124.5, 126.2, 128.6, 130.1, 130.3, 130.4, 132.3, 140.2, 140.9, 152.1, 173.8 ppm; HRMS: *m/z* [*M*+Na$^+$] for $C_{31}H_{42}N_2O_2Na$: found 497.3145, calcd 497.3144; HPLC: t_R = 36.1 min (99.3% purity) under conditions A, t_R = 30.2 min (99.5% purity) under conditions C.

(2*S*,5*S*)-9-(4-(1-Decynyl)phenyl)benzolactam-V8 (9): 4-(1-decynyl)-phenylboronic acid was prepared according to the method described for compound **3**. The title compound was obtained according to the general procedure B in 23% yield as a colorless oil: $[\alpha]_D^{20} = -298.5$ (c = 0.95 in $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 0.88–0.98 (m, 6H), 1.13 (d, 3H, J = 6.8 Hz), 1.20–1.43 (m, 8H), 1.44–1.56 (m, 2H), 1.60–1.70 (m, 2H), 2.42–2.53 (m, 3H), 2.80–2.93 (m, 4H), 3.13 (dd, 1H, J = 16.6, 7.9 Hz), 3.40 (br s, 1H), 3.50–3.63 (m, 2H), 3.70–3.80 (m, 1H), 4.03 (br s, 1H), 6.88 (s, 1H), 7.10 (s, 1H), 7.21 (s, 1H), 7.43–7.56 ppm (m, 4H); ^{13}C NMR ($CDCl_3$): δ = 14.1, 19.5, 19.9, 20.4, 22.6, 28.4, 28.8, 28.9, 29.1, 29.2, 31.8, 35.5, 37.0, 54.1, 65.8, 70.6, 80.4, 91.2, 118.5, 120.2, 123.1, 126.7, 130.5, 131.8, 132.3, 139.8, 140.2, 152.1, 173.9 ppm; HRMS: *m/z* [*M*+H$^+$] for $C_{31}H_{43}N_2O_2$: found 475.3333, calcd 475.3325; HPLC: t_R = 35.7 min (95.0% purity) under conditions A, t_R = 33.6 min (98.9% purity) under conditions C.

(2*S*,5*S*)-9-(2-(1-Hexynyl)phenyl)benzolactam-V8 (10): 2-(1-hexynyl)phenylboronic acid was prepared according to the method as described for compound **3**. The title compound was obtained according to the general procedure B in 19% yield as a colorless oil: $[\alpha]_D^{20} = -280.8$ (c = 0.73 in $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 0.85–0.97 (m, 6H), 1.08 (d, 3H, J = 6.8 Hz), 1.32–1.45 (m, 2H), 1.46–56 (m, 2H), 2.35 (t, 2H, J = 7.0 Hz), 2.42–2.53 (m, 1H), 2.82–2.91 (m, 4H), 3.1–3.3 (m, 2H), 3.51–3.65 (m, 2H), 3.73–3.81 (m, 1H), 4.10–4.20 (m, 1H), 6.87 (br s, 1H), 7.11 (s, 2H), 7.25–7.40 (m, 4H), 7.56 ppm (d, 1H, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$): δ = 13.7, 19.3, 20.0, 20.3, 21.9, 28.4, 30.7, 35.7, 37.1, 54.1, 65.9, 70.8, 80.2, 93.4, 121.4, 122.2, 122.6, 127.6, 129.3, 130.2, 131.3, 133.4, 140.3, 143.0, 151.1, 174.0 ppm; HRMS: *m/z* [*M*+H$^+$] for $C_{27}H_{35}N_2O_2$: found 419.2711, calcd 419.2699; HPLC: t_R = 22.4 (99.9% purity) under conditions A, t_R = 18.4 (98.3% purity) under conditions C.

(2*S*,5*S*)-9-(3-(1-Hexynyl)phenyl)benzolactam-V8 (11): 3-(1-hexynyl)phenylboronic acid was prepared according to the method as described for compound **3**. The title compound was obtained according to the general procedure B in 5% yield as a colorless oil: $[\alpha]_D^{20} = -287.0$ (c = 0.02 in $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 0.93 (d, 3H, J = 6.8 Hz), 1.0 (t, 2H, J = 7.2 Hz), 1.08 (d, 3H, J = 6.8 Hz), 1.50–1.70 (m, 4H), 1.88 (br s 1H), 2.42–2.57 (m, 3H), 2.82–2.96 (m, 4H), 3.19 (dd, 1H, J = 16.6, 7.9 Hz), 3.53–3.67 (m, 2H), 3.73–3.81 (m, 1H), 4.03–4.10 (br s, 1H), 6.26 (s, 1H), 7.07–7.15 (m, 2H), 7.23 (s, 1H), 7.34–7.42 (m, 2H), 7.44–7.51 (m, 1H), 7.70 ppm (s, 1H); ^{13}C NMR ($CDCl_3$): δ = 13.6, 19.1, 19.9, 20.2, 22.0, 28.3, 30.8, 35.5, 36.9, 53.9, 65.8, 70.5, 80.5, 90.6, 118.3, 120.1, 126.2, 126.6, 128.6, 130.1, 130.3, 130.4, 132.5, 140.3, 140.9, 151.9, 173.6 ppm; HRMS: *m/z* [*M*+H$^+$] for $C_{27}H_{35}N_2O_2$: found 419.2707, calcd 419.2699; HPLC: t_R = 24.9 min (97.0% purity) under conditions A, t_R = 18.9 min (97.2% purity) under conditions C.

(2*S*,5*S*)-9-(4-(1-Hexynyl)phenyl)benzolactam-V8 (12): 4-(1-hexynyl)phenylboronic acid was prepared according to the method as described for compound **3**. The title compound was obtained according to the general procedure B in 14% yield as a colorless oil: $[\alpha]_D^{20} = -334.7$ (c = 0.75 in $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 0.91 (d, 3H, J = 6.8 Hz), 1.00 (t, 3H, J = 7.3 Hz), 1.1 (d, 3H, J = 6.8 Hz), 1.47–1.57 (m, 2H), 1.57–1.73 (m, 2H), 2.42–2.57 (m, 3H), 2.81–2.95 (m, 4H), 3.1–3.35 (m, 2H), 3.5–3.63 (m, 2H), 3.72–3.80 (m, 1H), 4.08 (br s, 1H), 6.85 (br s, 1H), 7.12 (s, 2H), 7.23 (s, 1H), 7.42–7.52 ppm (m, 4H); ^{13}C NMR ($CDCl_3$): δ = 13.6, 19.2, 19.9, 20.4, 22.0, 28.4, 30.8, 35.5, 37.0, 54.1, 65.9, 70.5, 80.4, 91.2, 118.4, 120.2, 123.1, 126.7, 130.4, 131.9, 132.3, 139.8, 140.2, 152.1, 173.8 ppm; HRMS: *m/z* [*M*+H$^+$] for $C_{27}H_{35}N_2O_2$: found 419.2703, calcd 419.2699; HPLC: t_R = 25.1 min (98.9% purity) under conditions A, t_R = 19.1 min (98.6% purity) under conditions C.

(2*S*,5*S*)-9-(5-(1-Decynyl)thienyl)benzolactam-V8 (13): The title compound was obtained according to the general procedure B in 15% yield as a colorless oil: $[\alpha]_D^{20} = -278.5$ (c = 0.11 in $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 0.8–1.0 (m, 6H), 1.1 (d, 3H, J = 6.8 Hz), 1.20–1.40 (m, 8H), 1.40–1.51, m, 2H), 1.57–1.70 (m, 3H), 2.05 (br s, 1H), 2.38–2.52 (m, 3H), 2.76–2.89 (m, 3H), 3.12 (dd, 1H, J = 16.6, 7.9 Hz), 3.47–3.62 (m, 2H), 3.70–3.80 (m, 1H), 3.97 (br s, 1H), 6.3 (s, 1H), 7.03–7.2 ppm (m, 5H); ^{13}C NMR ($CDCl_3$): δ = 14.1, 19.7, 19.8, 20.4, 22.7, 28.2, 28.6, 28.9, 29.1, 29.2, 31.8, 35.0, 37.2, 53.9, 66.1, 69.9, 73.8, 95.4, 116.7, 118.8, 122.6, 123.3, 125.5, 131.8, 132.4, 133.6, 144.1, 152.1, 173.1 ppm; HRMS: *m/z* [*M*+Na$^+$] for $C_{29}H_{40}N_2O_2SNa$: found 503.2718, calcd 503.2708; HPLC: t_R = 36.0 min (98.6% purity) under conditions A, t_R = 31.5 min (96.3% purity) under conditions C.

(2*S*,5*S*)-9-(4-(*tert*-Butyloxycarbonylamino)phenyl)benzolactam-V8 (14): The title compound was obtained according to the general procedure B in 5% yield as a colorless oil: $[\alpha]_D^{20} = -261.1$ (c = 0.12 in $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 0.92 (d, 3H, J = 6.8 Hz), 1.12 (d, 3H, J = 6.8 Hz), 1.25–1.45 (s, 9H), 2.03 (br s, 1H), 2.45–2.59 (m, 1H), 2.83–2.96 (m, 4H), 3.18 (dd, 1H, J = 16.6, 7.9 Hz), 3.52–3.64 (m, 2H), 3.75–3.83 (m, 1H), 4.07 (br s, 1H), 6.31 (s, 1H), 6.55 (s, 1H), 7.08–7.15 (m, 2H), 7.19 (s, 1H), 7.43–7.48 (m, 2H), 7.49–7.56 ppm (m, 2H); ^{13}C NMR ($CDCl_3$): δ = 19.9, 20.4, 28.2, 28.3, 29.7, 35.2, 37.2, 54.0, 66.0, 70.4, 118.0, 118.7, 119.8, 127.5, 129.4, 132.2, 135.6, 137.7, 140.4, 152.0, 152.8, 173.2 ppm; HRMS: *m/z* [*M*+Na$^+$] for $C_{26}H_{35}N_3O_4Na$: found 476.2538, calcd 476.2526; HPLC: t_R = 25.5 min (99.4% purity) under conditions A, t_R = 21.4 min (97.5% purity) under conditions C.

(2*S*,5*S*)-9-(Benzoylamino)benzolactam-V8 (15): The title compound was obtained according to the general procedure C in 9% yield as a yellow oil: $[\alpha]_D^{20} = -486.0$ (c = 0.15 in $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 0.90 (d, 3H, J = 6.8 Hz), 1.08 (d, 3H, J = 6.8 Hz), 2.40–2.53 (m, 1H), 2.75–2.87 (m, 4H), 2.90–3.00 (br s, 1H), 3.07 (dd, 1H, J = 16.6, 7.9 Hz), 3.54 (d, 2H, J = 8.8 Hz), 3.69–3.78 (m, 1H), 3.98 (br s, 1H), 6.55 (s, 1H), 7.03 (d, 1H, J = 8.1 Hz), 7.12 (d, 1H, 8.12 Hz), 7.41 (s, 1H), 7.50 (t, 1H, J = 7.7 Hz), 7.52–7.63 (m, 1H), 7.90 (d, 2H, J = 7.2 Hz), 7.95 ppm (m, 2H); ^{13}C NMR ($CDCl_3$): δ = 19.9, 20.3, 28.2, 35.1, 36.9, 54.1, 65.8, 70.7, 111.5, 113.5, 126.8, 127.0, 128.8, 131.8, 132.3, 134.9, 137.6, 152.1, 170.5, 173.5 ppm; HRMS: *m/z* [*M*+Na$^+$] for $C_{22}H_{27}N_3O_3Na$: found 404.1957, calcd 404.1950; HPLC: t_R = 12.3 min (98.8% purity) under conditions A, t_R = 7.1 min (97.9% purity) under conditions C.

(2*S*,5*S*)-(*E*)-9-(3-Phenyl-2-propenoylamino)benzolactam-V8 (16): The title compound was obtained according to the general procedure C in 17% yield as a yellow oil: $[\alpha]_D^{20} = -554.7$ (c = 0.42 in $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 0.88 (d, 3H, J = 6.8 Hz), 1.06 (d, 3H, J = 6.8 Hz), 2.36–2.48 (m, 1H), 2.71, (s, 3H), 2.73–2.87 (m, 1H), 3.07 (dd, 1H, J = 16.6, 7.9 Hz), 3.45–3.61 (m, 3H), 3.65–3.75 (m, 1H), 3.98 (br s, 1H), 6.57–6.71 (m, 2H), 6.93–6.98 (m, 1H), 7.02–7.08 (m, 1H), 7.40 (s, 4H), 7.50–7.58 (m, 2H), 7.72–7.81 (m, 1H), 7.95 ppm (s, 1H); ^{13}C NMR ($CDCl_3$): δ = 19.9, 20.1, 28.2, 35.2, 36.9, 54.1, 65.6, 70.7, 111.6, 113.4, 121.0, 126.9, 127.9, 128.3, 128.8, 129.1, 129.9, 132.2, 134.6, 137.7, 142.2, 152.0, 164.2, 173.8 ppm; HRMS: *m/z* [*M*+Na$^+$] for $C_{24}H_{29}N_3O_3Na$: found 430.2098, calcd 430.2107; HPLC: t_R = 21.7 min (96.9% purity) under conditions B, t_R = 8.3 min (95.4% purity) under conditions C.

(2*S*,5*S*)-9-(3-Phenyl-2,4-propanoylamino)benzolactam-V8 (17): A mixture of **16** (10 mg, 0.025 mmol), Pd/C (10%, 5 mg), and methanol (5 mL) was stirred under H_2 (1 atm) at room temperature for 1 h. Filtration from the catalyst, evaporation, and TLC (EtOAc as eluent) provided **17** (9.5 mg, 95%) as a colorless oil: $[\alpha]_D^{20} = -366.7$ ($c = 0.475$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 0.88$ (d, 3H, $J = 6.8$ Hz), 1.08 (d, 3H, $J = 6.8$ Hz), 2.37–2.48 (m, 1H), 2.66 (t, 2H, $J = 7.3$ Hz), 2.70–2.82 (m, 4H), 3.00–3.10 (m, 3H), 3.40–3.55 (m, 3H), 3.67–3.73 (m, 1H), 3.95 (br s, 1H), 6.69 (s, 1H), 6.85–6.97 (m, 2H), 7.15 (s, 1H), 7.20–7.37 ppm (m, 6H); ^{13}C NMR ($CDCl_3$): $\delta = 19.9$, 20.2, 28.2, 31.5, 35.1, 36.8, 39.4, 54.2, 65.7, 70.4, 111.4, 113.1, 126.3, 126.7, 128.2, 128.6, 132.1, 137.4, 140.6, 152.0, 170.4, 173.8 ppm; HRMS: *m/z* [*M*+Na$^+$] for $C_{24}H_{31}N_3O_3Na$: found 432.2257, calcd 432.2263; HPLC: $t_R = 15.8$ min (99.5% purity) under conditions B, $t_R = 7.2$ min (98.9% purity) under conditions C.

(2*S*,5*S*)-(*E*,*E*)-9-(5-Phenyl-2,4-pentadienoylamino)benzolactam-V8 (18): 5-phenylpentadienoic acid (2 g, 11.5 mmol) were dissolved in $SOCl_2$ (20 mL) and stirred at 60 °C for 3 h. After the volatiles were evaporated under vacuum, the residue was dissolved in $CHCl_3$ (40 mL) and the solution was added dropwise to a cooled and well-stirred solution of NH_4OH (50 mL). The organic phase was separated, dried, and evaporated under vacuum. The residue was recrystallized from EtOAc and used in the next reaction. The title compound was obtained according to the general procedure C in 37% yield as a yellow oil: $[\alpha]_D^{20} = -295.6$ ($c = 0.20$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 0.90$ (d, 3H, $J = 6.8$ Hz), 1.07 (d, 3H, $J = 6.8$ Hz), 2.39–2.50 (m, 1H), 2.70–2.83 (m, 4H), 3.05–3.18 (m, 2H), 3.50–3.59 (m, 2H), 3.69–3.76 (m, 1H), 3.98 (br s, 1H), 6.13 (d, 1H, $J = 14.8$ Hz), 6.58 (s, 1H), 6.90–7.07 (m, 4H), 7.30–7.42 (m, 3H), 7.43–7.62 ppm (m, 4H); ^{13}C NMR ($CDCl_3$): $\delta = 19.9$, 20.2, 28.1, 35.0, 36.9, 54.2, 65.7, 70.3, 111.2, 113.1, 124.1, 126.1, 127.1, 128.8, 128.9, 132.2, 136.1, 137.7, 140.0, 142.2, 152.0, 164.1, 173.6 ppm; HRMS: *m/z* [*M*+Na$^+$] for $C_{26}H_{31}N_3O_3Na$: found 456.2272, calcd 456.2263; HPLC: $t_R =$ 24.7 min (97.5% purity) under conditions B, $t_R = 18.4$ min (96.8% purity) under conditions C.

(2*S*,5*S*)-9-(5-Phenylpentanoylamino)benzolactam-V8 (19): A mixture of **18** (10 mg, 0.023 mmol), Pd/C (10%, 5 mg), and methanol (5 mL) was stirred under H_2 (1 atm) at room temperature for 1 h. Filtration from the catalyst, evaporation, and TLC (EtOAc as eluent) provided the title compound **19** (1 mg, 10%) as a colorless oil: $[\alpha]_D^{20} = -415.0$ ($c = 0.04$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 0.88$ (d, 3H, $J = 6.8$ Hz), 1.08 (d, 3H, $J = 6.8$ Hz), 1.70–1.88 (m, 4H), 2.15 (m, 1H), 2.38 (t, 2H, $J = 4.9$ Hz), 2.42–2.53 (m, 1H), 2.60–2.70 (m, 2H) 2.81–2.90 (m, 4H), 3.13 (dd, 1H, $J = 16.6$, 7.9 Hz), 3.47–3.61 (m, 2H), 3.71–3.80 (m, 1H), 4.07 (br s, 1H), 6.45 (s, 1H), 6.85–6.93 (m, 2H), 7.02–7.10 (m, 1H), 7.18–7.40 ppm (m, 6H); ^{13}C NMR ($CDCl_3$): $\delta =$ 19.9, 20.3, 25.1, 28.1, 31.0, 35.0, 35.7, 37.0, 37.6, 54.1, 66.0, 70.7, 111.0, 112.6, 125.8, 128.3, 128.4, 132.1, 137.5, 142.1, 152.1, 164.2, 170.9, 173.2 ppm; HRMS: *m/z* [*M*+Na$^+$] for $C_{26}H_{35}N_3O_3Na$: found 460.2568, calcd 460.2576; HPLC: $t_R = 22.5$ min (97.7% purity) under conditions B, $t_R = 20.4$ min (95.8% purity) under conditions C.

(2*S*,5*S*)-9-(Biphenyl-4-carbonylamino)benzolactam-V8 (20): The title compound was obtained according to the general procedure C in 41% yield as a colorless oil: $[\alpha]_D^{20} = -266.3$ ($c = 0.24$ in MeOH); ^{1}H NMR ([D$_6$]acetone): $\delta = 0.88$ (d, 3H, $J = 6.8$ Hz), 1.08 (d, 3H, $J = 6.8$ Hz), 2.35–2.47 (m, 1H), 2.88–2.95 (m, 4H), 3.11 (dd, 1H, $J = 16.6$, 7.9 Hz), 3.47–3.55 (m, 1H), 3.57–3.64 (m, 1H), 3.65–3.73 (m, 1H), 3.85 (br s, 1H), 4.35 (t, 1H, $J = 4.7$ Hz), 6.20 (s, 1H), 7.06 (d, 1H, 8.1 Hz), 7.39–7.48 (m, 2H), 7.58 (t, 1H, $J = 7.7$ Hz), 7.61 (s, 1H), 7.72–7.88 (m, 5H), 8.11 (d, 2H, $J = 8.2$ Hz), 9.51 ppm (s, 1H); ^{13}C NMR ([D$_6$]acetone): $\delta = 19.4$, 19.7, 34.3, 36.9, 54.3, 65.3, 69.9, 111.1, 112.8, 126.8, 127.0, 128.0, 128.9, 131.8, 134.3, 138.8, 139.8, 143.8, 152.0, 164.8, 171.7 ppm; HRMS: *m/z* [*M*+Na$^+$] for $C_{28}H_{31}N_3O_3Na$: found 480.2271, calcd 480.2263; HPLC: $t_R = 34.9$ min (97.0% purity) under conditions B, $t_R = 18.8$ min (98.3% purity) under conditions C.

(2*S*,5*S*)-9-(4-Butylbenzoylamino)benzolactam-V8 (21): The title compound was obtained according to the general procedure C in 24% yield as a colorless oil: $[\alpha]_D^{20} = -458.7$(c=0.315 in $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 0.85$–1.03 (m, 6H), 1.03–1.13 (m, 3H), 1.31–1.45 (m, 2H), 1.56–1.72 (m, 3H), 2.35–2.50 (br s, 1H), 2.62–2.85 (m, 5H), 3.03–3.16 (m, 1H), 3.23 (s, 1H), 3.47–3.61 (m, 2H), 3.68–3.73 (m, 1H), 3.95 (br s, 1H), 6.61 (s, 1H), 6.89–7.01 (m, 1H), 7.08 (s, 1H), 7.25–7.41 (m, 3H), 7.78–7.86 (m, 2H), 7.96 ppm (s, 1H); ^{13}C NMR ($CDCl_3$): $\delta = 14.3$, 20.3, 20.7, 22.7, 28.6, 33.7, 35.6, 36.0, 37.3, 54.6, 66.2, 70.8, 112.0, 113.7, 127.2, 127.5, 129.2, 132.6, 138.1, 147.7, 152.5, 166.2, 174.1 ppm; HRMS: *m/z* [*M*+Na$^+$] for $C_{26}H_{35}N_3O_3Na$: found 460.2586, calcd 460.2576; HPLC: $t_R = 32.1$ min (99.2% purity) under conditions B, $t_R = 20.4$ min (96.9% purity) under conditions C.

(2*S*,5*S*)-9-(3-(1-Hexynyl)benzoylamino)benzolactam-V8 (22): 4-iodophenylbenzoylamide (1 g, 4 mmol), CuI (12.6 mg, 0.06 mmol), $PdCl_2$ (6 mg, 0.03 mmol), PPh_3 (17.3 mg, 0.06 mmol), and 1-hexyne (0.54 g, 6.6 mmol) were dissolved in diethylamine (15 mL) and stirred for 6 h. After evaporation of all volatiles under vacuum, the compound was purified by column chromatography with EtOAc/PE (2:1) as the mobile phase. The title compound was obtained according to the general procedure C in 32% yield as a colorless oil: $[\alpha]_D^{20} = -337.7$ ($c = 0.475$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 0.88$ (d, 3H, $J = 6.8$ Hz), 0.93 (t, 3H, $J = 7.4$ Hz), 1.08 (d, 3H, $J = 6.8$ Hz), 1.44–1.74 (m, 4H), 2.38–2.52 (m, 3H), 2.72–2.85 (m, 4H), 3.01–3.17 (m, 2H), 3.47–3.60 (m, 2H), 3.68–3.73 (m, 1H), 3.95 (br s, 1H), 6.59 (s, 1H), 7.00 (d, 1H, $J = 8.2$ Hz), 7.11 (d, 1H, $J = 8.1$ Hz), 7.32–7.43 (m, 2H), 7.50–7.60 (m, 1H), 7.8 (d, 1H, $J = 7.8$ Hz), 7.88 ppm (s, 1H), 7.95 (s, 1H); ^{13}C NMR ($CDCl_3$): $\delta = 13.6$, 19.1, 19.9, 20.3, 22.0, 28.2, 30.7, 35.2, 36.9, 54.1, 65.8, 70.4, 79.6, 91.9, 111.7, 113.4, 124.8, 126.3, 127.0, 128.7, 129.8, 132.2, 134.6, 135.0, 137.5, 152.1, 165.1, 173.6 ppm; HRMS: *m/z* [*M*+Na$^+$] for $C_{28}H_{35}N_3O_3Na$: found 484.2580, calcd 484.2576; HPLC: $t_R = 37.1$ min (99.0% purity) under conditions B.

(2*S*,5*S*)-9-(3-Hexylbenzoylamino)benzolactam-V8 (23): A mixture of **22** (10 mg, 0.022 mmol), Pd/C (10%, 5 mg), and methanol (5 mL) was stirred under H_2 (1 atm) at room temperature for 1 h. Filtration from the catalyst, evaporation, and TLC (EtOAc as eluent) provided **23** (1 mg, 9.8%) as a colorless oil: $[\alpha]_D^{20} = -453.3$ ($c = 0.03$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 0.88$–0.96 (m, 6H), 1.08 (d, 3H, $J = 6.8$ Hz), 1.27–1.43 (m, 6H), 1.52–1.72 (m, 2H), 1.97 (br s, 1H), 2.43–2.54 (m, 1H), 2.70 (t, 2H, $J = 7.6$ Hz), 2.78–2.90 (m, 4H), 3.11 (dd, 1H, $J =$ 16.6, 7.9 Hz), 3.50–3.62 (m, 2H), 3.68–3.73 (m, 1H), 3.95 (br s, 1H), 6.26 (s, 1H), 7.00–7.13 (m, 2H), 7.35–7.43 (m, 3H), 7.63–7.70 (m, 1H), 7.70–7.73 (m, 1H), 7.74–7.78 ppm (m, 1H); ^{13}C NMR ($CDCl_3$): $\delta = 13.6$, 19.1, 19.9, 20.3, 22.0, 28.2, 30.7, 35.2, 36.9, 54.1, 65.8, 70.4, 79.6, 91.9, 111.7, 113.4, 124.8, 126.3, 127.0, 128.7, 129.8, 132.2, 134.6, 135.0, 137.5, 152.1, 165.1, 173.6 ppm; HRMS: *m/z* [*M*+Na$^+$] for $C_{28}H_{39}N_3O_3Na$: found 488.2889, calcd 488.2889; HPLC: $t_R =$ 42.6 min (98.2% purity) under conditions B, $t_R = 28.1$ min (95.3% purity) under conditions C.

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[1] H. Mellor, P. J. Parker, *Biochem. J.* **1998**, *332*, 281.
[2] A. C. Newton, *J. Biol. Chem.* **1995**, *270*, 28495.
[3] A. F. Quest, *Enzyme Protein* **1996**, *49*, 231.
[4] D. Ron, M. G. Kazanietz, *FASEB J.* **1999**, *13*, 1658.
[5] H. Hennings, P. M. Blumberg, G. R. Pettit, C. L. Herald, R. Shores, S. H. Yuspa, *Carcinogenesis* **1987**, *8*, 1343.
[6] G. Cole, K. R. Dobkins, L. A. Hansen, R. D. Terry, T. Saitoh, *Brain Res.* **1988**, *452*, 165.
[7] E. Masliah, G. Cole, S. Shimohama, L. Hansen, R. DeTeresa, R. D. Terry, T. Saitoh, *J. Neurosci.* **1990**, *10*, 2113.
[8] S. Shimohama, M. Narita, H. Matsushima, J. Kimura, M. Kameyama, M. Hagiwara, H. Hidaka, T. Taniguchi, *Neurology* **1993**, *43*, 1407.
[9] H. Y. Wang, M. R. Pisano, E. Friedman, *Neurobiol. Aging* **1994**, *15*, 293.
[10] E. Masliah, G. M. Cole, L. A. Hansen, M. Mallory, T. Albright, R. D. Terry, T. Saitoh, *J. Neurosci.* **1991**, *11*, 2759.
[11] M. Chachin, S. Shimohama, Y. U. Kunugi, T. Taniguchi, *Jpn. J. Pharmacol.* **1996**, *71*, 175.
[12] T. Van Huynh, G. Cole, R. Katzman, K. P. Huang, T. Saitoh, *Arch. Neurol.* **1989**, *46*, 1195.
[13] S. Govoni, S. Bergamaschi, M. Racchi, F. Battaini, G. Binetti, A. Bianchetti, M. Trabucchi, *Neurology* **1993**, *43*, 2581.
[14] S. Govoni, M. Racchi, S. Bergamaschi, M. Trabucchi, F. Battaini, A. Bianchetti, G. Binetti, *Ann. N. Y. Acad. Sci.* **1996**, *777*, 332.
[15] S. Gandy, P. Greengard, *Int. Rev. Neurobiol.* **1994**, *36*, 29.
[16] J. D. Buxbaum, K. N. Liu, Y. Luo, J. L. Slack, K. L. Stocking, J. J. Peschon, R. S. Johnson, B. J. Castner, D. P. Cerretti, R. A. Black, *J. Biol. Chem.* **1998**, *273*, 27765.
[17] H. Cai, Y. Wang, D. McCarthy, H. Wen, D. R. Borchelt, D. L. Price, P. C. Wong, *Nat. Neurosci.* **2001**, *4*, 233.
[18] R. Vassar, M. Citron, *Neuron* **2000**, *27*, 419.
[19] F. Desdouits, J. D. Buxbaum, J. Desdouits-Magnen, A. C. Nairn, P. Greengard, *J. Biol. Chem.* **1996**, *271*, 24670.
[20] S. Efthimiopoulos, S. Punj, V. Manopoulos, M. Pangalos, G. P. Wang, L. M. Refolo, N. K. Robakis, *J. Neurochem.* **1996**, *67*, 872.
[21] T. Kinouchi, H. Sorimachi, K. Maruyama, K. Mizuno, S. Ohno, S. Ishiura, K. Suzuki, *FEBS Lett.* **1995**, *364*, 203.
[22] S. J. Fuller, E. Storey, Q.-X. Li, A. I. Smith, K. Beyreuther, C. L. Masters, *Biochemistry* **1995**, *34*, 8091.
[23] A. C. LeBlanc, M. Koutroumanis, C. G. Goodyer, *J. Neurosci.* **1998**, *18*, 2909.
[24] M. J. Savage, S. P. Trusko, D. S. Howland, L. P. Pinsker, S. Mistretta, A. G. Reaume, B. D. Greenberg, R. Siman, R. W. Scott, *J. Neurosci.* **1998**, *18*, 1743.
[25] Y. Endo, M. Ohno, H. Masaaki, A. Itai, K. Shudo, *J. Am. Chem. Soc.* **1996**, *118*, 1841.
[26] A. P. Kozikowski, D. Ma, Y.-P. Pang, P. Shum, V. Likic, P. K. Mishra, A. Basu, J. S. Lazo, R. G. Ball, *J. Am. Chem. Soc.* **1993**, *115*.
[27] A. P. Kozikowski, S. Wang, D. Ma, J. Yao, S. Ahmad, R. I. Glazer, K. Bogi, P. Acs, S. Modarres, N. E. Lewin, P. M. Blumberg, *J. Med. Chem.* **1997**, *40*, 1316.
[28] A. P. Kozikowski, I. Nowak, P. A. Petukhov, R. Etcheberrigaray, A. Mohamed, M. Tan, N. Lewin, H. Hennings, L. L. Pearce, P. M. Blumberg, *J. Med. Chem.* **2003**, *46*, 364.
[29] D. Ma, T. Zhang, G. Wang, A. P. Kozikowski, N. E. Lewin, P. M. Blumberg, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 99.
[30] N. A. Sharkey, H. Hennings, S. H. Yuspa, P. M. Blumberg, *Carcinogenesis* **1989**, *10*, 1937.
[31] G. von Fürstenberger, E. Hecker, *Planta Med.* **1972**, *22*, 241.
[32] Q. J. Wang, T. W. Fang, D. Fenick, S. Garfield, B. Bienfait, V. E. Marquez, P. M. Blumberg, *J. Biol. Chem.* **2000**, *275*, 12136.
[33] N. Miyaura, A. Suzuki, *Chem. Rev.* **1995**, *95*, 2457.
[34] M.-J. Wu, L.-J. Chang, L.-M. Wei, C.-F. Lin, *Tetrahedron* **1999**, *55*, 13193.
[35] G. T. Crisp, T. A. Robertson, *Tetrahedron* **1992**, *48*, 3239.
[36] C.-W. Wan, A. Burghart, J. Chen, F. Bergström, L. Johansson, M. F. Wolford, T. G. Kim, M. R. Topp, R. M. Hochstrasser, K. Burgess, *Chem. Eur. J.* **2003**, *9*, 4430.
[37] D. Ma, W. Tang, A. P. Kozikowski, N. E. Lewin, P. M. Blumberg, *J. Org. Chem.* **1999**, *64*, 6366.
[38] J. P. Wolfe, H. Tomori, J. P. Sadighi, J. Yin, S. L. Buchwald, *J. Org. Chem.* **2000**, *65*, 1158.
[39] T. Matsui, T. Kondo, Y. Nishita, S. Itadani, S. Nakatani, N. Omawari, M. Sakai, S. Nakazawa, A. Ogata, H. Mori, K. Terai, W. Kamoshima, H. Ohno, T. Obata, H. Nakai, M. Toda, *Bioorg. Med. Chem.* **2002**, *10*, 3757.
[40] J. P. Wolfe, J. Ahman, J. P. Sadighi, R. A. Singer, S. L. Buchwald, *Tetrahedron Lett.* **1997**, *38*, 6367.
[41] N. E. Lewin, P. M. Blumberg, *Methods Mol. Biol.* **2003**, *233*, 129.
[42] W. M. Meylan, P. H. Howard, 1.63 ed., Syracuse Research Corp., North Syracuse, NY 13212-2510 (USA).
[43] W. M. Meylan, P. H. Howard, *J. Pharm. Sci.* **1995**, *84*, 83.

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