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Design, synthesis, and BK channel-opening activity of hexahydrodibenzazepinone derivatives

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Abstract—In order to explore new scaffolds for large-conductance Ca^{2+}-activated K^+ channel (BK channel) openers, we carried out molecular design and synthesis on the basis of the following two concepts: (1) introduction of a heteroatom into the dehydroabietic acid (BK channel opener) skeleton would allow easier introduction of substituents. (2) Because of the fourfold symmetrical structure of BK channels, dimeric compounds in which two pharmacophores are linked through a tether are expected to have a greater binding probability to the channels, resulting in increased channel-opening activity. Herein, we explore the usefulness of the hexahydrodibenzazepinone structure as a new scaffold for BK channel openers. The synthesized monomer compounds of hexahydrodibenzazepinone derivatives, which can be derived from dehydroabietic acid, were subjected to electrophysiological patch–clamp studies, followed by Magnus contraction–relaxation assay using rabbit urinary bladder smooth muscle strips to assess overall activities. Dimeric compounds were designed by linking the monomeric hexahydrodibenzazepinone derivatives through a diacetylenebenzene tether, and their channel-opening activities were evaluated by electrophysiological methods. Finally, we concluded that the critical structure for BK channel-opening activity is the hexahydrodibenzazepinone monomer substituted with a phenyl-bearing alkynyl substituent on the lactam amide.

1. Introduction

Ion-selective channels are membrane proteins, which generate electrical ionic signals and regulate signal transduction events in living systems. Among such channels, K^+ channels are widely expressed in various tissues, such as smooth muscles and neurons, and play an important role in modulating membrane potential. In particular, large-conductance Ca^{2+}-activated K^+ channels (BK channels) elicit such a large K^+ conductance that depolarized membrane potential can be effectively quenched by the opening of BK channels.[1] The BK channels are distributed in both excitable and non-excitable cells, which are involved in many cellular functions,[2] such as action potential repolarization, neuronal excitability, neurotransmitter release, hormone secretion, tuning of cochlear hair cells,[3] innate immunity,[4] and modulation of the tone of vascular, airway, uterine, gastrointestinal, and urinary bladder smooth muscle tissues. Since BK channels are activated by both elevation of intracellular Ca^{2+} concentration and membrane depolarization, the K^+ efflux via BK channels results in membrane repolarization and the suppression of Ca^{2+} influx through voltage-dependent Ca^{2+} channels. Accordingly, BK channels serve as a negative-feedback mechanism to relax excessive muscle contraction in smooth muscles[5] and to prevent aberrant cellular excitability of neurons.

Like other members of the voltage-dependent K^+ channel superfamily, BK channels are tetrameric proteins composed of pore-forming α-subunits and auxiliary β-subunits.[6] Thus, the K^+ channels are fourfold symmetrical. Each BK channel α-subunit contains seven transmembrane spanning segments S0–S6 at the N-terminus

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and the four hydrophobic segments S7–S10 at the large intracellular C-terminus. Unlike other classes of Ca^{2+}-activated K^+ channels, SK (small-conductance) and IK (intermediate conductance) channels, the α-subunit of the BK channels has an extra hydrophobic transmembrane segment (S0) that leads to the extracellular N-terminus. The N-terminus acts as a binding domain for β-subunits.[7] Each α-subunit has a S4 voltage sensor[8] and a pore-forming region formed by S5–S6 and the P-loop.

BK channel openers have potential therapeutic applications because of possible involvement of BK channels in various pathophysiological conditions such as hypertension,[9] coronary artery spasm, urinary incontinence,[10] progressive deafness,[11] and several neurological disorders.[12] The BK channel has an advantage as a therapeutic target compared to other K^+ channels, such as the ATP-sensitive potassium channel (K_{ATP}), because it is mostly absent in cardiac myocytes, except for mitochondria.[13] The BK channel openers comprise a large series of synthetic benzimidazolone derivatives (Chart 1), such as NS004[14] and NS1619,[15] the biaryl amines, such as mefenamic and flufenamic acids,[16] the biarylureas, such as NS1608,[17] the aryloxindoles (BMS-204352),[18] the arylpyrroles (NS-8),[19] indole-3-carboxylic acid esters (CGS-7184, CGS-7181)[20] and natural modulators, including dihydrosoyasaponin-1 (dehydrosoyasaponin-1, DHS-1),[21] and terpenes such as maxikdiol (**1**, Chart 2).[22] Most of these compounds activate BK channels as a subsidiary action in addition to their primary action.[23] In this context, the available scaffolds for BK channel openers are rather limited in structural diversity. Both of the pioneering drugs NS004 and NS1619 are α-subunit-selective BK openers. We have found that

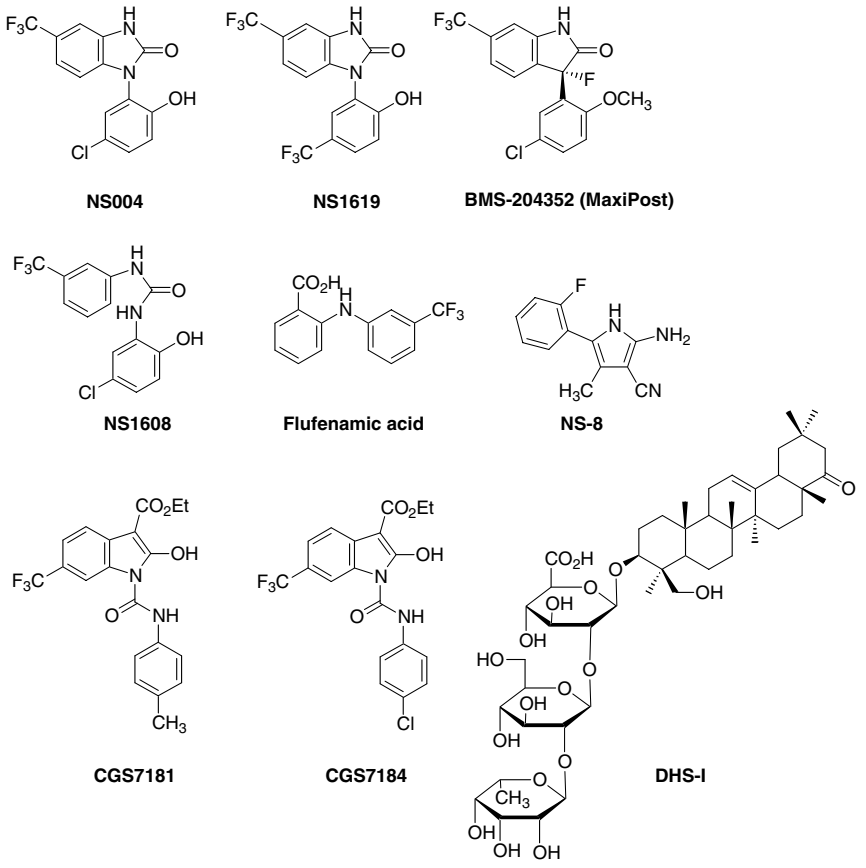


Chart 1.

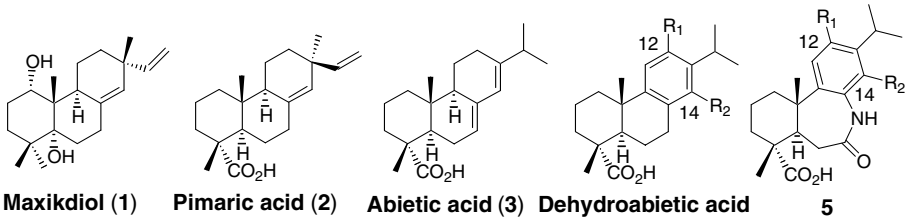


Chart 2.

pimaric acid and dehydroabietic acid act as BK channel openers via direct interaction with the α-subunit of BK channels.[24] Several possible mechanisms can explain the effect of the activators on BK channels: (i) modulation of the gating (=open/close) kinetics of the α subunit, (ii) modulation of the Ca^{2+} binding site at the C-terminal end of the α-subunit, (iii) strengthening of the interaction between the α- and β-subunits, and (iv) mimicking the interaction of β- with the α-binding site. Novel synthetic channel modulators would be useful tools to reveal the mechanism of the channel gating at the atomic level, in addition to having therapeutic potential.

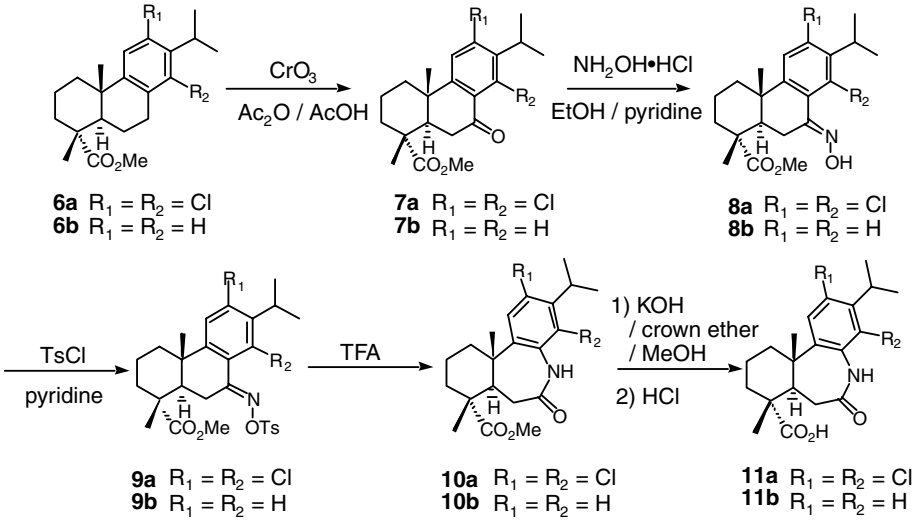
We have already found that pimaric acid (**2**) and dehydroabietic acid (**4**) exhibit BK channel opening activities, while the structurally related abietic acid (**3**) has weak activity (Chart 2).[24] In order to explore new structural scaffolds for BK channel openers, we carried out molecular design on the basis of two concepts: (1) introduction of a heteroatom into the dehydroabietic acid skeleton would allow easier introduction of substituents. (2) Because of the fourfold symmetrical structure of the BK channels, dimeric compounds in which two pharmacophores are linked through a tether may have a greater binding probability to the channels, resulting in increased channel opening activity. Herein we describe our exploration of the hexahydrodibenzazepinone derivatives (**5**), which can be generated from dehydroabietic acid (**4**), to examine the value of the hexahydrodibenzazepinone structure as a new scaffold for BK channel openers.

The synthesized monomer compounds were evaluated by means of electrophysiological patch–clamp studies in HEK293 cells expressing BK channels, followed by the Magnus contraction–relaxation assay with rabbit urinary bladder smooth muscle strips to evaluate overall actions on smooth muscle. Dimeric compounds were designed on the basis of the monomeric scaffolds, and their channel opening activities were evaluated by electrophysiological methods. Our results demonstrate that the hexahydrodibenzazepinone structure is a potential new scaffold for novel BK channel openers.

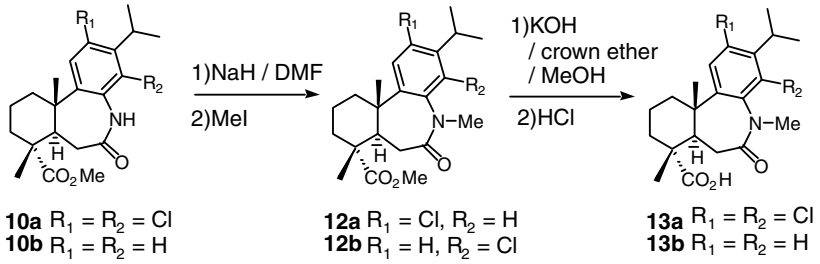
2. Chemistry

2.1. Hexahydrodibenzazepinone derivatives

The hexahydrodibenzazepinone derivatives (**11a** and **b**) were synthesized as shown in Scheme 1. The benzylic oxidation of the dehydroabietic acid derivatives (**6a** and **b**) was carried out with CrO_3 in Ac_2O/AcOH to give the corresponding ketone compounds (**7a** and **b**). The ketones were converted to the oximes (**8a** and **b**), and the hydroxyl group of the oximes was tosylated. These tosylated oximes (**9a** and **b**) were subjected to the Beck-

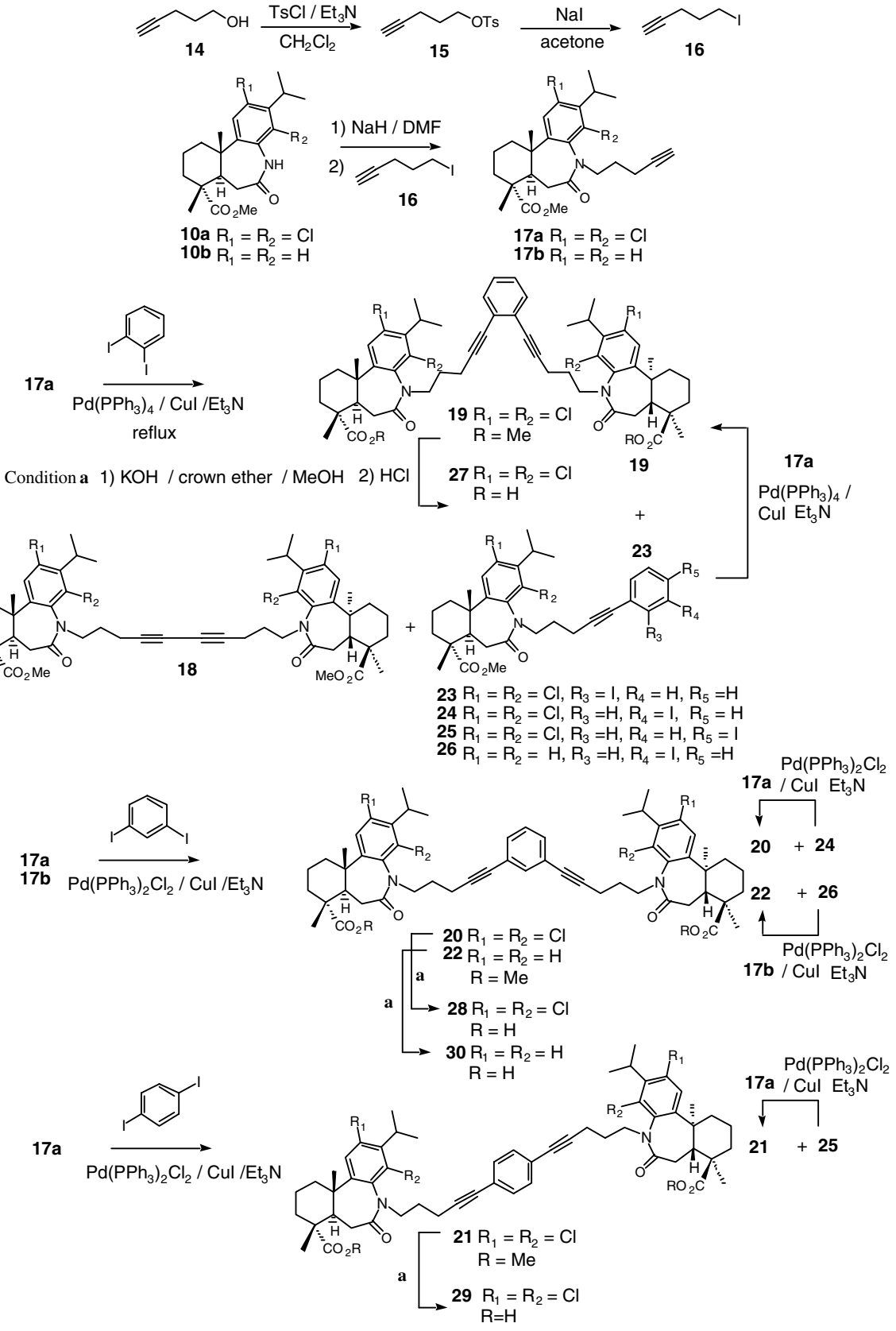


Scheme 1.

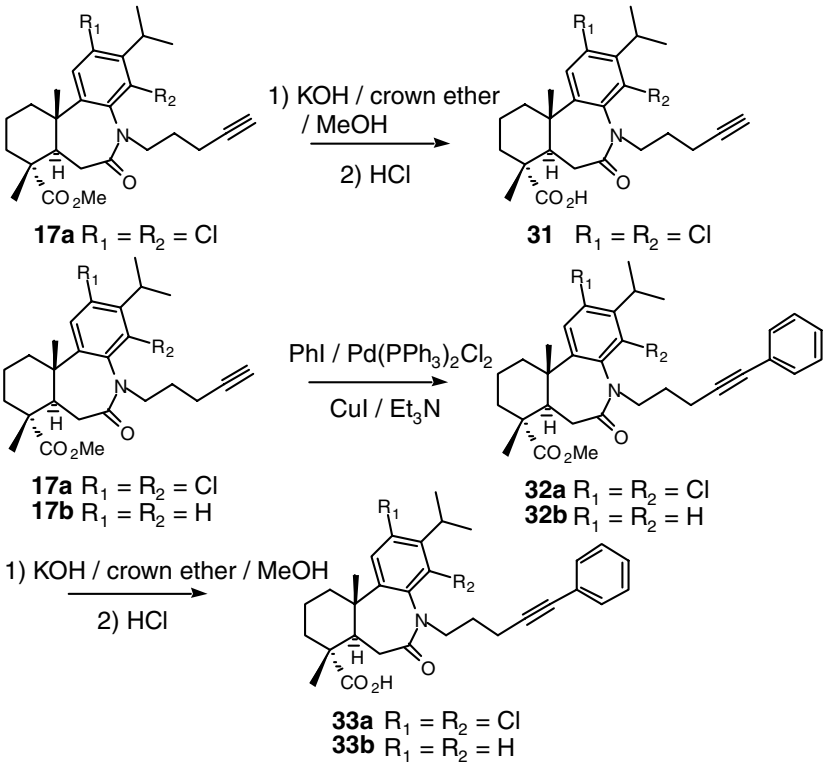


Scheme 2.

mann rearrangement in the presence of TFA at rt to give the hexahydrodibenzazepinone derivatives (**10a** and **b**). Finally, the methyl ester was hydrolyzed with KOH and crown ether in methanol to give the corresponding carboxylic acids (**11a** and **b**). The *N*-methyl group was introduced with methyl iodide after deprotonation of



Scheme 3.



Scheme 4.

the amide NH group with NaH (Scheme 2). Hydrolysis of the methyl ester was carried out in a similar manner to that described for **10**, to give the carboxylic acids (**13a** and **b**). The lactam structure of hexahydrodibenzazepinone derived from the Beckmann rearrangement was reported previously,[25] and our ^{1}H NOE analysis between the N–CH_3 group and the aromatic protons of the compound **12a** confirmed the structure.

The syntheses of the dimeric compounds (**27**–**30**) are illustrated in Scheme 3. Sonogashira coupling was the key reaction. With respect to the linker moiety, 4-pentyn-1-ol (**14**) was tosylated and converted to the iodide (**16**) with NaI. N-Alkylation was carried out with the iodide **16** to afford the alkyne derivatives (**17a**,**b**), which were coupled with *m*-diiodobenzene or *p*-diiodobenzene in the presence of $Pd(PPh_3)_2Cl_2$/CuI in Et_3N at rt (the Sonogashira coupling reaction). The acetylene disubstituted products (**20**–**22**) and/or the acetylene monosubstituted products (**24**–**26**) were obtained. In the case of the monosubstituted products, a second Sonogashira coupling reaction was carried out to obtain the acetylene disubstituted products (**20**–**22**). Finally, the ester group was hydrolyzed with KOH and crown ether in methanol to afford the corresponding dimeric compounds (**27**–**29**). The alkyne (**17**) and *o*-diiodobenzene did not readily undergo the Sonogashira coupling reaction with $Pd(PPh_3)_2Cl_2$/CuI in Et_3N at rt. Under these reaction conditions, in addition to the acetylene monosubstituted product (**23**), the acetylene homodimeric product (**18**) was obtained, probably because of steric hindrance. Thus, on coupling of the alkyne (**17a**) with *o*-diiodobenzene, $Pd(PPh_3)_4$ was used as a catalyst and purified CuI was used under reflux in Et_3N, to give the desired ortho dimeric compound (**19**). Hydrolysis of the ester provided the final carboxylic acid (**27**). The monomeric compounds (**31** and **33a**,**b**), which bear a alkynyl substituent on the lactam amide, were also synthesized as shown in Scheme 4.

2.2. Molecular design of dimers

The X-ray structures of several K^+ channels revealed that the diameters of the pores (the gate region) in the closed state are 6 Å (for the KcsA channel) and 8 Å (KirBac channel), and those in open state are 25 Å (for the MthK channel) and 19 Å (KvAP channel). In the cases of other ion channels, the binding sites of small organic molecules, such as channel blockers, are located on the inner side of the pore.[26] Moreover, the dichlorodehydroabietic acid (**4a**) was shown to activate the BK channel through interaction with the α subunit.[24c] It seems reasonable to assume that the hexahydrodibenzazepinone derivatives (**13**) also interact with the α subunits of the BK channels.

We thus designed several dimeric compounds, **27**–**29**, in which two units of the pharmacophore, dichlorohexahydrodibenzazepinone **13a**, are linked through the rigid diacetylenebenzene unit. Because we have little information about the arrangement and topology of the binding sites, we added adjustable molecular-dynamic flexibility by using an *n*-propyl chain as a tether between the nitrogen atom of the hexahydrodibenzazepinone core and the rigid diacetylenyl benzene core. Ortho, meta, and para substitution can change the direction of extension of

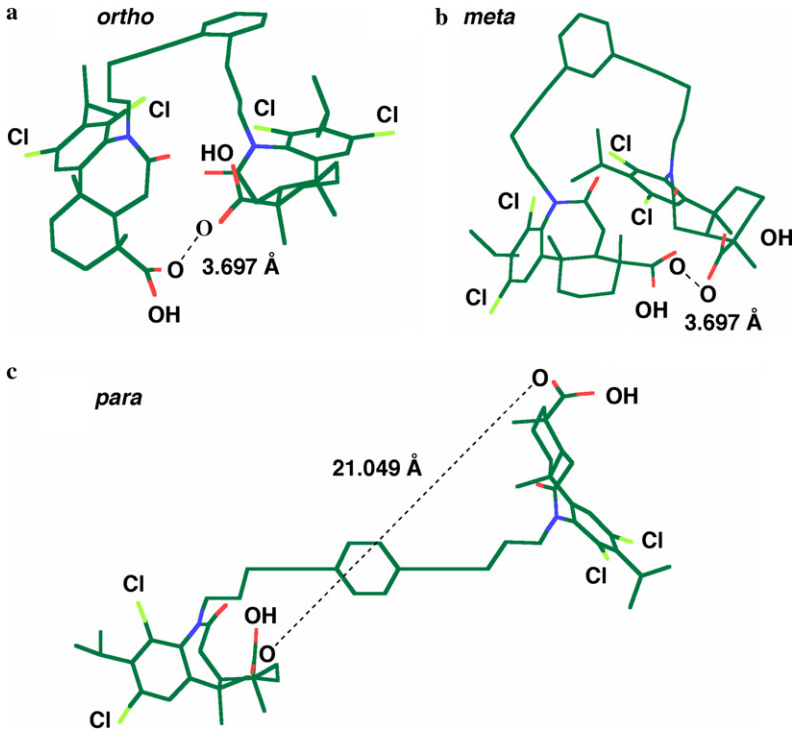


Figure 1. Conformational spaces of isomeric dimer compounds energy-minimized structures of the dimers in an *n*-octanol environment.

the two pharmacophores, and this molecular design allowed us to change the distance between the two pharmacophores in the dimer. In fact, molecular dynamic calculations on the basis of conformational search with OPLS2001 force field parameters in an *n*-octanol environment (Macromodel 8.5) generated a range of dimer structures.[27] We obtained various numbers of independent structures as follows: ortho (**27**): 2852 structures; meta (**28**): 2581 structures; para (**29**): 2838 structures, when the number of generated trial structures was set to 3000 in each case. The minimum-energy structures in each case are shown in Figure 1. The structural space was not significantly different in the gas-phase or in water as a solvent (data not shown). Because the carboxylic acid group of dehydroabietic acid **4** is known to be essential for BK channel opening activity,[24] this group presumably plays an important role in the interaction with the channel proteins. A similar role of the carboxylic acid group was expected for the hexahydrodibenzazepinone derivatives. The average (with standard deviation) minimum and maximum distances between the two carbonyl oxygen atoms of the individual carboxylic acids in the calculated dimer structures are as follows: ortho (**27**): 6.54 ± 4.02 Å (minimum: 3.01 Å; maximum: 15.37 Å); meta (**28**): 7.04 ± 5.54 Å (minimum: 2.99 Å; maximum: 18.42 Å); para (**29**): 22.23 ± 1.66 Å (minimum: 4.40 Å; maximum: 25.09 Å). The distances between the pharmacophores, particularly those of the carboxylic acids of **27**–**29**, accord well with the size of the pores of the K^+ channels, which are well conserved among the K^+ channel superfamily. The present designed dimeric compounds should be able to bind to the two putative binding sites of a single BK channel, if the binding sites are located close to the pore regions, and this should result in an increase of channel opening activity.

3. Results and discussion

3.1. Electrophysiological studies of hexahydrodibenzazepinones derivatives

The activity of the monomeric hexahydrodibenzazepinone derivatives as BK channel openers was evaluated by the patch–clamp technique with inside-out configuration. Human BK channel subunits, hSlo α and β_1, were transiently expressed in tsA201 cells, a derivative of the human embryonic kidney (HEK) cell line 293. In the patch–clamp measurement, when a compound has channel opening activity, the conductance–voltage curve shifts towards the negative voltage direction (leftward), that is, a larger BK channel current is activated by the same voltage step under the voltage–clamp condition. The BK channel opening activities of the tested compounds at 10 μM are represented as the shift of $V_{1/2}$ value (mV, $n = 3$–5), the voltage activating the half-maximum current, in comparison with the negative control in the presence of 100 μM Ca^{2+} on the intracellular side of the membrane patch (Fig. 2). In this work, we used dichlorodehydroabietic acid **4a** as a reference compound which has a related structure.[24]

The voltage shift of $V_{1/2}$ of **4a** was −5.3 ± 3.7 mV, and that of the *N*-methyl derivative of the hexahydrodibenzazepinone **13a** was −9.2 ± 3.7 mV. Compound **13a** can open the BK channel, while **13b**, its counterpart lacking the dichloro-substituent, did not show channel

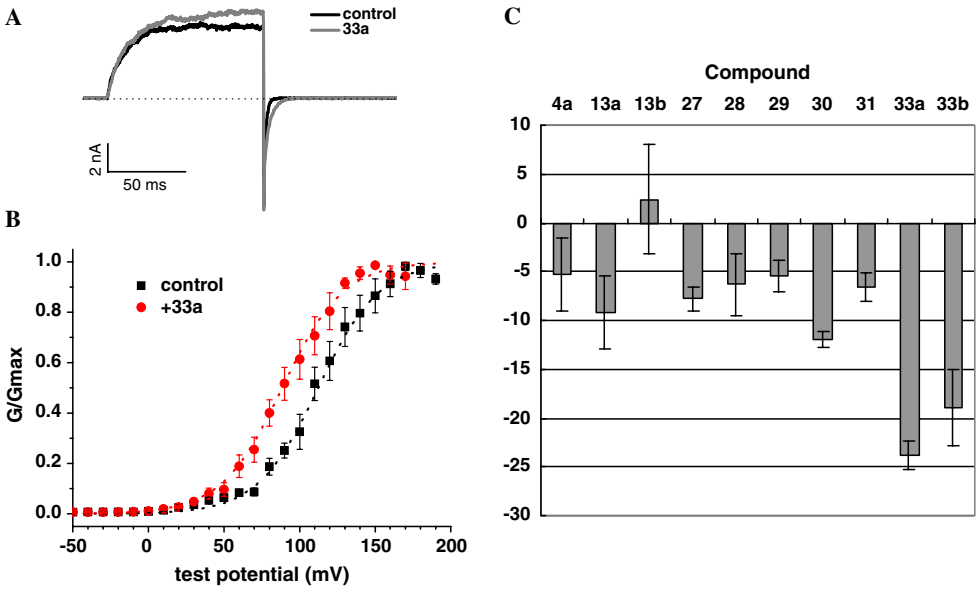


Figure 2. Effects of test compounds on the BK channel current. (A) Effect of **33a** on BK channel currents recorded in the inside-out macro patch-clamp configuration. BK channel currents were activated by test pulses to 100 mV from a holding potential at −100 mV. Current zero level is indicated by a dotted line. Outward BK channel currents are shown in upward direction. (B) Conductance–voltage relationship (*G–V* curve) of BK channel currents ($n = 4$). Compound **33a** shifted *G–V* curve toward hyperpolarized direction. (C) Shifts of $V_{1/2}$ value (mV) of *G–V* curve by test compounds ($n = 3$–5). A negative value indicates a shift to hyperpolarizing voltages.

opening activity. In this context, the results of the smooth muscle relaxation assay are consistent with those of the patch–clamp experiments.

Thus, the results of the electrophysiological study supported the idea that the dichloro-substituted *N*-methyl-hexahydrodibenzazepinone **13a** can serve as a pharmacophore for BK channel openers.

3.2. Electrophysiological study of the dimers and N-substituent effects

These dimeric compounds bearing dichloro substituents on the aromatic ring (**27**–**29**) were studied by means of an inside-out patch–clamp configuration. They showed BK channel opening activities, the left-shift of $V_{1/2}$ being -7.7 ± 1.2 mV, -6.3 ± 3.2 mV, and -5.4 ± 1.6 mV, respectively. While the channel opening activity of the ortho derivative **27** is likely to be stronger than those of the other isomers (**28** and **29**), the differences are not significant. In addition, the magnitude of the shift of **27** was as large as that of the monomer **13a**. These results imply that a part of the structures common to these dimeric isomers could be responsible for the activity, rather than the dual binding of the dimeric compounds to multiple binding sites of the BK channel. Thus, we synthesized monomeric fragments of the dimers, that is, **31** and **33a**, the former bearing a *N*-alkyl acetylene substituent and the latter bearing an additional terminal benzene moiety. We examined the BK channel opening activity of these compounds in patch clamp experiments (Fig. 2). Unexpectedly, we found that **33a** showed significant channel opening activity (-23.9 ± 1.4 mV left-shift of $V_{1/2}$), while **31** showed activity (-6.6 ± 1.5 mV) comparable in magnitude with that of the *N*-methyl derivative **13a**.

The prototype *N*-methyl hexahydrodibenzazepinone **13b**, lacking the aromatic dichloro-substituents, did not show BK channel opening activity in patch–clamp experiments (Fig. 2). However, the *m*-dimeric compound **30** showed channel opening activity with a hyperpolarizing shift of $V_{1/2}$ by -11.9 ± 0.9 mV. This activity is also probably derived from the fragment structure of the dimer. In fact, the monomeric compound **33b** also showed a strong opening activity, with a leftward shift of $V_{1/2}$ by -18.9 ± 3.8 mV, which is less than the shift caused by **33a**, but comparable in magnitude. The most potent compound **33a** expanded the width of the tail current in the patch-clamp measurements (data not shown), which suggests that this compound stabilizes the BK channel in the open state.

3.3. Isolated rabbit detrusor smooth muscle assay as overall activity

We evaluated the overall activities of hexahydrodibenzazepine derivatives on detrusor smooth muscle. Figure 3 shows the results of the rabbit detrusor smooth muscle assay. The K^+ channel-dependent muscle dilating effect was evaluated as follows.[28] Prior to the application of test compounds, detrusor smooth muscle strips were precontracted by depolarization caused by raising the extracellular K^+ concentration to either 30 mM or 120 mM. With the extracellular K^+ concentration at 30 mM, the small depolarization allows Ca^{2+} influx through Ca^{2+} channels to produce contraction. If a test compound activates the BK channel, the K^+ efflux will shift the membrane potential toward the equilibrium potential for K^+ (E_K) of approximately −40 mV where Ca^{2+} channels are closed, thus causing relaxation of the muscle strip. In contrast, with the extracellular K^+ concentration at 120 mM, the opening

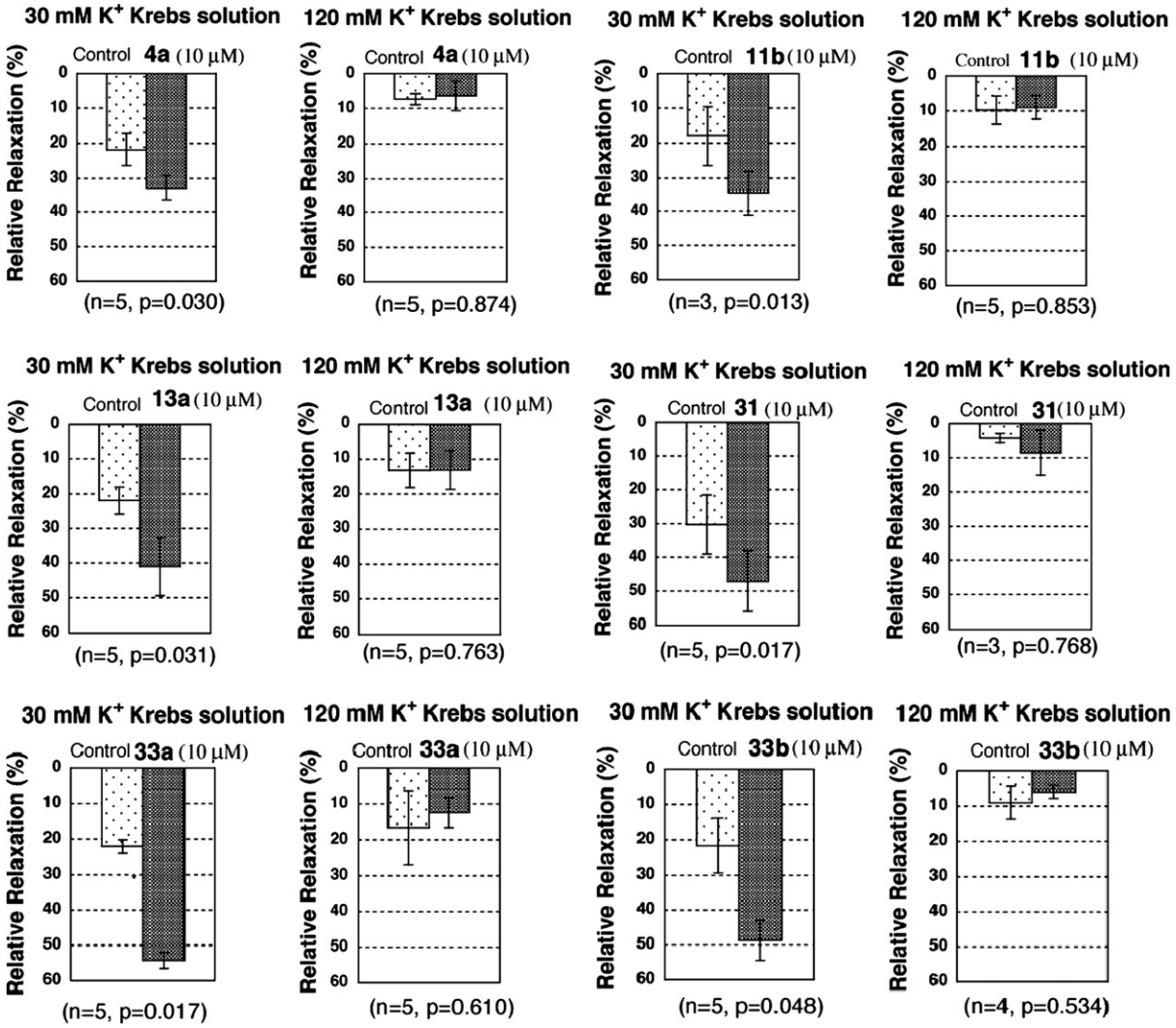


Figure 3. Detrusor smooth muscle relaxation assay The relative magnitude of relaxation was normalized to the precontraction caused by high K^+ (30 mM or 120 mM) as 100%. The relaxant activities of the test compounds were compared with that of 0.1% DMSO as the vehicle (control).

of the BK channel will shift the membrane potential toward E_K of around 0 mV, where Ca^{2+} channels are open, and the dilating activity of the BK channel opener should be abolished. The relative magnitude of relaxation was normalized to the contraction caused by high K^+ precontraction as 100%. The concentration of DMSO as a vehicle was set to be no more than 0.1% (v/v), because DMSO at high concentration caused relaxation by itself. Moreover, the concentration of the test compounds was fixed at 10 μM because of their relatively poor solubility in DMSO–water. The relaxant activities of the test compounds were compared with that of vehicle (0.1% DMSO) measured in the same muscle strip. All values are expressed as means ± SEM. Statistical significance of differences was determined by paired Student's *t*-test, and *P* values less than 0.05 were considered to be significant.

The results of the isolated rabbit detrusor smooth muscle relaxation study of the dehydroabietic acid derivatives were consistent with what we had found in the previous[24] and present electrophysiological studies. The dichloro-substituted **4a** showed a statistically significant relaxation activity, while the unsubstituted **4b** showed weak activity (data not shown). The smooth muscle relaxation study showed that the *N*-methyl derivative **13a**, bearing aromatic dichloro substituents, showed a statistically significant relaxation effect, while the prototype NH amide **11a** showed less potent activity (data not shown). Even in the absence of the aromatic dichloro substituents, the NH derivative **11b** showed distinct relaxation activity, while the *N*-methyl derivative **13b** did not.

The monomeric compounds **31** and **33a** showed distinct rabbit urinary bladder relaxation activities in detrusor smooth muscle strips (54.3 ± 2.3% and 48.68 ± 5.8%, respectively, $n = 5$), the activity of **33a** being statistically significant (Fig. 3). Reasonably, the N-alkylated monomeric compound **33b** also exhibited potent relaxation activities (48.68 ± 5.8%, $n = 5$).

4. Conclusion

In this work, we first demonstrated that the hexahydrodibenzazepinone structure represents a new scaffold for BK channel openers. Second, we carried out molecular design based on our hypothesis that dual binding of linked pharmacophores might provide strong activation even if the single pharmacophore is a rather weak opener. We found that the dimerization retained (in the case of **27**) or increased (in the case of **30**) the channel opening activity as compared with the corresponding monomeric pharmacophores (**13a** and **13b**, respectively). In the present design, ortho, meta, and para isomers (**27**–**29**) of the dimeric compounds showed similar, not increased, magnitudes of activity as compared with the minimal monomer **13a**, probably because the two pharmacophores cannot bind two putative binding sites at the same time. However, a portion of the molecules clearly can gain access to the binding site. Because of the highly hydrophobic nature of **33** and the dimers **27**–**30**, and retention of the opening activities of the dimers **27**–**30**, it is possible that there are hydrophobic binding sites in the transmembrane region of the channels which allow these hydrophobic molecules to gain access in a well-ordered conformation (at least partially) via a membrane bilayer pathway, rather than a direct aqueous pathway.[29] Finally, we found that the phenyl-bearing alkynyl substituent on the lactam amide nitrogen atom is crucial for BK channel opening activity. Our findings provide a new basis for development of novel BK channel openers.

5. Experimental

5.1. Chemistry

Commercially available reagents were purchased and were used without further purification unless otherwise stated. ^{1}H NMR spectra were recorded on a Bruker Advance (400 MHz) spectrometer. The chemical shifts (ppm) were shown by using tetramethylsilane (TMS) as an internal standard in deuterated chloroform ($CDCl_3$), and the peak of the solvent was used as an internal standard in the case of deuterated methanol (CD_3OD). Mass spectra were recorded on a JEOL MStation JMS-700 spectrometer. Elemental analyses were recorded on a Yanaco CHN CORDER spectrometer and were carried out at the Analysis Center of the Graduate School of Pharmaceutical Sciences, the University of Tokyo. Flash column chromatography was carried out with silica gel (Kanto Chemical 60 N, particle size 40–50 mm). Melting points were measured on a Yanaco hot-stage microscope and are uncorrected. Yields are shown in terms of those of isolated pure materials.

12,14-Dichlorodehydroabietic acid (4a). To a suspension of dehydroabietic acid **4b** (2.0 g, 6.66 mmol), 2% w/w $FeCl_3$ on SiO_2 (1 g), and 1% w/w DDQ on SiO_2 (0.1 g) in 25 mL CCl_4 was added a solution of 2.8 M Cl_2 in 35.7 mL CCl_4 (99.9 mmol, 15 equiv) at 0 °C. The reaction mixture was vigorously stirred at rt for one day in a sealed tube. Then to this mixture was added a solution of 2.8 M Cl_2 in 10 mL CCl_4 (28.0 mmol, 4 equiv) at rt, and the whole was stirred for 4 days. The insoluble materials were filtered off and the filtrate was then evaporated in vacuum. The residue was recrystallized from *n*-hexane to afford **4a** (574 mg, 1.554 mmol, yield 23%). Compound **4a**: colorless fine needles. Mp 218–222 °C (*n*-hexane/AcOEt). ^{1}H NMR ($CDCl_3$) 1.20 (3H, s, CH_3), 1.28 (3H, s, CH_3), 1.39 (6H, d, J = 7.3 Hz, 2CH_3CH), 1.61–1.66 (2H, m, 2CH), 1.72–1.86 (5H, m, 5CH), 2.13 (1H, dd, J = 12.6 Hz, 2.2 Hz, CH), 2.24 (1H, d, J = 11.9 Hz, CH), 2.70–2.79 (1H, m, CH), 2.94 (1H, dd, J = 18.4 Hz, 6.0 Hz, CH), 3.91 (1H, br s, CH_3CH), 7.15 (1H, s, ArH). MS (FAB$^+$) *m*/*z* 369 ([M($^{35}Cl_2$)+H]$^+$), 371 ([M($^{35}Cl^{37}Cl$)+H]$^+$), 373 ([M($^{37}Cl_2$)+H]$^+$). Anal. Calcd for $C_{20}H_{26}Cl_2O_2$: C, 65.04; H, 7.10. Found: C, 64.95; H, 7.16.

12,14-Dichlorodehydroabietic acid methyl ester (6a). To a solution of 12,14-dichlorodehydroabietic acid **4a** (442 mg, 1.197 mmol) in MeOH (2 mL) and PhMe (4 mL) was dropwise added 2.0 M $TMSCHN_2$ in Et_2O (0.78 mL, 1.557 mmol, 1.3 equiv) at rt over 5 min, and the whole was stirred at rt for 30 min. Excess $TMSCHN_2$ was quenched with Et_2O, then the reaction mixture was evaporated in vacuum, and the residue was purified by flash chromatography (*n*-hexane only to *n*-hexane/AcOEt = 10:1) to afford **6a** (453 mg, 1.181 mol, yield 99%) as a colorless solid. **6a**: colorless needles. Mp 126–129 °C. ^{1}H NMR ($CDCl_3$) 1.19 (3H, s, CH_3), 1.26 (3H, s, CH_3), 1.39 (6H, d, J = 7.1 Hz, 2CH_3CH), 1.47–1.53 (2H, m, 2CH), 1.65–1.75 (5H, m, 5CH), 2.12 (1H, d, J = 10.6 Hz, CH), 2.23 (1H, d, J = 11.7 Hz, CH), 2.71–2.73 (1H, m, CH), 2.92 (1H, did, J = 18.3 Hz, 6.2 Hz, CH), 3.67 (3H, s, CO_2CH_3), 3.91 (1H, br s, CH_3CH), 7.15 (1H, s). LRMS (FAB$^+$) *m*/*z* 383 ([M($^{35}Cl_2$)+H]$^+$), 385 ([M($^{35}Cl^{37}Cl$)+H]$^+$), 387 ([M($^{35}Cl_2$)+H]$^+$). Anal. Calcd for $C_{21}H_{28}Cl_2O_2$: C, 65.79; H, 7.36. Found; C, 65.86; H, 7.14.

Dehydroabietic acid methyl ester (6b). A mixture of dehydroabietic acid **4b** (5.1 g, 0.017 mol) and concd H_2SO_4 (6 mL) in MeOH (200 mL) was stirred at 85 °C for 19 h. Then concd H_2SO_4 (2 mL) was carefully added to the reaction mixture. Stirring was continued for 2 days, then the mixture was cooled, the solvent was evaporated in vacuum, and the residue was diluted with water (300 mL). The whole was extracted with $CHCl_3$ (1× 200 mL, 2× 100 mL). The combined organic layer was washed with brine (1× 100 mL), dried over Na_2SO_4, filtered, and then the solvent was evaporated in vacuum. The residue was purified by flash chromatography (*n*-hexane/AcOEt = 10:1) to afford **6b** (4.4 g, 0.014 mol, yield 83%) as a colorless solid. Compound **6b**: colorless needles. Mp 45–49 °C (*n*-hexane). ^{1}H NMR ($CDCl_3$) 1.21–1.23 (9H, m, 2CH_3CH and CH_3), 1.27 (3H, s, CH_3), 1.42 (1H, m, CH), 1.50 (1H, m, CH), 1.61–1.70 (5H, m, 5CH_2), 2.24 (1H, dd, J = 12.5 Hz, 2.1 Hz, CH), 2.30 (1H, d, J = 12.3 Hz, CH), 2.80–2.84 (1H, m, CH_3CH), 2.86–2.90 (1H, m, CH), 3.66 (3H, s, $COCH_3$), 6.88 (1H, s, ArH), 6.99 (1H, d, J = 6.4 Hz, ArH), 7.16 (1H, d, J = 8.1 Hz, ArH). LRMS (FAB$^+$) *m*/*z* 315

($[M+H]^+$). Anal. Calcd for $C_{21}H_{30}O_2$: C, 80.21; H, 9.62. Found: C, 80.07; H, 9.82.

13-Isopropyl-12,14-dichloro-7-oxopodocarpe-8,11,13-triene-15-carboxylic acid methyl ester (7a). To a solution of CrO_3 (287 mg, 2.867 mmol, 1.1 equiv) in Ac_2O (9 mL) and AcOH (4 mL) was dropwise added a suspension of the methyl ester **6a** (999 mg, 2.606 mmol) in AcOH (15 mL) at 0 °C over 10 min. The reaction mixture was stirred at 50 °C for 9 h, the whole was cooled, and poured into ice-water (40 mL) and then the whole was extracted with $CHCl_3$ (3× 30 mL). The combined organic layer was washed with water (1× 30 mL), satd $NaHCO_3$ (2× 30 mL), and brine (1× 30 mL), dried over Na_2SO_4, filtered, and then the solvent was evaporated in vacuum. The residue was purified by flash chromatography (*n*-hexane/AcOEt = 5:1) to afford **7a** (847 mg, 2.130 mmol, yield 82%) as a pale yellow amorphous solid. Compound **7c**: ^1H NMR ($CDCl_3$) 1.17 (3H, s, CH_3), 1.34 (3H, s, CH_3), 1.41 (6H, d, J = 7.3 Hz, 2CH_3CH), 1.75–1.79 (5H, m, 5CH), 2.20 (1H, d, J = 12.5 Hz, CH), 2.51 (1H, dd, J = 16.9 Hz, 5.2 Hz, CH), 2.64 (2H, m, 2CH), 3.66 (3H, s, CO_2CH_3), 3.94 (1H, br s, CH_3CH), 7.19 (1H, br s, ArH).

12,14-Dichloro-13-isopropyl-7-hydroxyiminepodocarpe-8,11,13-triene-15-carboxylic acid methyl ester (8a). A mixture of the methyl ester **7a** (198 mg, 0.498 mmol), pyridine (0.06 mL), and $NH_2OH{\cdot}HCl$ (54 mg, 0.772 mmol, 1.6 equiv) in EtOH (2 mL) was stirred at 100 °C for 3.5 h, the whole was cooled, and the solvent was evaporated in vacuum. The residue was purified by flash chromatography (*n*-hexane/AcOEt = 5:1) to afford **8a** (207 mg, quantitative yield) as a colorless amorphous solid. Compound **8a**: ^1H NMR ($CDCl_3$) 1.06 (3H, s, CH_3), 1.22 (6H, d, J = 8.4 Hz, 2CH_3CH), 1.26 (1H, dd, J = 18.7 Hz, 6.4 Hz, CH), 1.72–1.77 (5H, m, 5CH), 2.15 (2H, dd, J = 12.1 Hz, 6.1 Hz, CH), 2.38 (1H, dd, J = 18.7 Hz, 6.4 Hz, CH), 3.09 (1H, dd, J = 18.7 Hz, 13.0 Hz, CH), 3.66 (3H, s, CO_2CH_3), 3.69 (1H, br s, CH_3CH), 7.15 (1H, s, ArH).

12,14-Dichloro-13-isopropyl-7-tosyloxyiminepodocarpe-8,11,13-triene-15-carboxylic acid methyl ester (9a). To a solution of the methyl ester **8a** (207 mg, 0.503 mmol) in pyridine (1 mL) was added TsCl (144 mg, 0.754 mmol, 1.5 equiv) at rt. The reaction mixture was stirred for 17 h at rt, and then the solvent was evaporated in vacuum. The residue was purified by flash chromatography (*n*-hexane/AcOEt = 5:1) to afford **9a** (282 mg, 0.497 mmol, yield 99%) as a colorless oil. Compound **9a**: ^1H NMR ($CDCl_3$) 1.03 (3H, s, CH_3), 1.25 (3H, d, J = 7.1 Hz, CH_3CH), 1.27 (3H, d, J = 7.1 Hz, CH_3CH), 1.37 (3H, s, CH_3), 1.74–1.77 (5H, m, 5CH), 2.06–2.15 (2H, m, 2CH), 2.46 (3H, s, CH_3), 2.51 (1H, dd, J = 13.8 Hz, 5.0 Hz, CH), 3.02 (1H, dd, J = 19.0 Hz, 12.9 Hz, CH), 3.67 (3H, s, CO_2CH_3), 3.94 (1H, br s, CH_3CH), 7.12 (1H, br s, ArH), 7.35 (2H, d, J = 8.4 Hz, ArH), 7.94 (2H, d, J = 8.2 Hz, ArH).

12,14-Dichloro-13-isopropyl-8,11a-dimethyl-6-oxo-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepine-8-carboxylic acid methyl ester (10a). The mixture of the methyl ester **9a** (282 mg, 0.497 mmol) in TFA (2 mL) was stirred at rt for 1 h, and then the solvent was evaporated in vacuum. The residue was purified by flash chromatography (*n*-hexane/AcOEt = 1:1) to afford **10a** (166 mg, 0.403 mmol, yield 81%) as a colorless amorphous solid. Compound **10a**: ^1H NMR ($CDCl_3$): 1.42 (6H, d, J = 7.1 Hz, 2CH_3CH), 1.45 (3H, s, CH_3), 1.58 (3H, s, CH_3), 1.67–1.76 (2H, m, 2CH), 1.81–1.83 (2H, m, 2CH), 1.90–1.93 (2H, m, 2CH), 2.29 (1H, d, J = 8.6 Hz, CH), 2.92 (1H, dd, J = 15.9 Hz, 6.8 Hz, CH), 3.47–3.55 (1H, m, CH), 3.64 (3H, s, CO_2CH_3), 3.96 (1H, br s, CH_3CH), 7.26 (1H, br s, ArH), 7.85 (1H, br s, NH). LRMS (FAB^+) *m*/*z* 412 ($[M(^{35}Cl_2)+H]^+$), 414 ($[M(^{35}Cl^{37}Cl)+H]^+$), 416 ($[M(^{37}Cl_2)+H]^+$).

12,14-Dichloro-13-isopropyl-8,11a-dimethyl-6-oxo-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepine-8-carboxylic acid (11a). A mixture of the methyl ester **10a** (80 mg, 0.194 mmol), KOH (109 mg, 3.068 mmol, 10.0 equiv), and 18-crown ether-6 (128 mg, 0.485 mmol, 2.5 equiv) in MeOH (2 mL) was stirred at 80 °C for 7.5 h, the whole was cooled, and then the solvent was evaporated in vacuum. The residue was diluted with water (10 mL), acidified with 2 N HCl (2 mL), and the whole was extracted with $CHCl_3$ (3× 20 mL). The combined organic layer was washed with brine (1× 20 mL), dried over Na_2SO_4, filtered, and then the solvent was evaporated in vacuum. The residue was purified by flash chromatography (AcOEt only to AcOEt/MeOH = 10:1) to afford **11a** (35 mg, 0.087 mmol, yield 45%) as a colorless solid. Compound **11a**: colorless powder. Mp 192–197 °C (*n*-hexane/AcOEt/MeOH). ^1H NMR (CD_3OD): 1.32 (6H, d, J = 7.1 Hz, 2CH_3CH), 1.36 (3H, s, CH_3), 1.47 (3H, s, CH_3), 1.60 (2H, m, 2CH), 1.70 (2H, m, 2CH), 1.70 (2H, m, 2CH), 1.85 (2H, m, 2CH), 2.10 (1H, d, J = 8.4 Hz, CH), 2.86 (1H, dd, J = 15.9 Hz, CH), 3.27 (1H, m, CH), 3.90 (1H, br s, CH_3CH), 7.23 (1H, br s, ArH). LRMS (FAB^+) *m*/*z* 398 ($[M(^{35}Cl_2)+H]^+$), 400 ($[M(^{35}Cl^{37}Cl)+H]^+$), 402 ($[M(^{37}Cl_2)+H]^+$). Anal. Calcd for $C_{20}H_{25}Cl_2NO_3{\cdot}3/4H_2O$: C, 58.33; H, 6.30; N, 3.40. Found: C, 58.29; H, 6.39; N, 3.39.

13-Isopropyl-7-oxopodocarpe-8,11,13-triene-15-carboxylic acid methyl ester (7b). To a solution of CrO_3 (790 mg, 7.886 mmol, 1.1 equiv) in Ac_2O (28 mL) and AcOH (14 mL) was dropwise added a suspension of dehydroabietic acid methyl ester **6b** (2.35 g, 7.456 mmol) in AcOH (6 mL) at rt for 11 min. The reaction mixture was stirred for 15 min at rt, then stirred at 50 °C for 8.5 h. The mixture was cooled, and the whole was poured into icewater (100 mL) and then extracted with $CHCl_3$ (1× 200 mL, 2× 100 mL). The combined organic layer was washed with brine (1× 100 mL), dried over Na_2SO_4, filtered, and then the solvent was evaporated in vacuum. The residue was purified by flash chromatography (*n*-hexane/AcOEt = 5:1) to afford the desired compound **7b** (570 mg, 1.746 mmol, yield 23%) as a pale yellow oil and the undesired overreacted compound (550 mg, 1.431 mmol, yield 19%) as a yellow oil. Compound **7b**: ^1H NMR ($CDCl_3$) 1.24–1.26 (9H, m, 2CH_3CH and CH_3), 1.34 (3H, s, CH_3), 1.61–1.80 (5H, m, 5CH), 2.32–2.38 (2H, m, 2CH), 2.71–2.73 (2H, m, 2CH), 2.91–2.95 (1H, m, CH_3CH), 3.65 (3H, s, CO_2CH_3), 7.29 (1H, d, J = 8.1 Hz, ArH), 7.40 (1H, dd, J = 8.2 Hz, 2.1 Hz, ArH), 7.87 (1H, d, J = 2.0 Hz,

ArH). LRMS (FAB$^+$) *m/z* 329 ([M+H]$^+$). *By-product:* (13-(1-Acetoxy-1-methylethyl)-7-oxopodocarpe-8,11,13-triene-15-carboxylic acid methyl ester): ^{1}H NMR ($CDCl_3$) 1.26 (3H, s, CH_3), 1.34 (3H, s, CH_3), 1.72–1.79 (1H, m, 5CH and 2C*H*$_3$CPh), 2.10 (3H, s, CH_3CO), 2.32–2.36 (2H, m, 2CH), 2.70–2.74 (2H, m, 2× CH), 3.66 (3H, s, CO_2CH_3), 7.33 (1H, d, *J* = 8.2 Hz, ArH), 7.52 (1H, dd, *J* = 8.3 Hz, 2.3 Hz, ArH), 7.97 (1H, d, *J* = 2.4 Hz, ArH).

13-Isopropyl-7-hydroxyiminepodocarpe-8,11,13-triene-15-carboxylic acid methyl ester (8b). A mixture of the methyl ester **7b** (598 mg, 1.667 mmol), pyridine (0.2 mL), and NH_2OH·HCl (180 mg, 2.585 mmol, 1.6 equiv) in EtOH (4 mL) was stirred at 100 °C for 3 h, the mixture was cooled, and the solvent was evaporated in vacuum. The residue was purified by flash chromatography (*n*-hexane/AcOEt = 5:1) to afford **8b** (390 mg, 1.137 mmol, yield 68%) as a colorless amorphous solid. Compound **8b**: ^{1}H NMR ($CDCl_3$) 1.12 (3H, s, CH_3), 1.25 (6H, d, *J* = 7.0 Hz, 2C*H*$_3$CH), 1.38 (3H, s, CH_3), 1.42–1.76 (5H, m, 5CH), 2.28–2.35 (2H, m, 2CH), 2.64–2.67 (2H, m, 2CH), 2.88–2.91 (1H, m, CH_3C*H*), 3.65 (3H, s, CO_2CH_3), 7.21 (2H, m, ArH), 7.70 (1H, s, ArH). LRMS (FAB$^+$) *m/z* 344 ([M+H]$^+$).

13-Isopropyl-7-tosyloxyiminepodocarpe-8,11,13-triene-15-carboxylic acid methyl ester (9b). To a solution of the methyl ester **8b** (390 mg, 1.137 mmol) in pyridine (1 mL) was added TsCl (325 mg, 1.705 mmol, 1.5 equiv) at rt. The reaction mixture was stirred for 14 h at rt, and then the solvent was evaporated in vacuum. The residue was purified by flash chromatography (*n*-hexane/AcOEt = 5:1) to afford **9b** (545 mg, 1.095 mmol, yield 96%) as a colorless solid. Compound **9b**: colorless powder. Mp 158–160 °C (*n*-hexane/AcOEt). ^{1}H NMR ($CDCl_3$) 1.04 (3H, s, CH_3), 1.20–1.28 (6H, m, 2C*H*$_3$CH), 1.34 (3H, s, CH_3), 1.68–1.74 (5H, m, 5CH), 2.26 (2H, t, *J* = 9.3 Hz, CH_2), 2.45 (3H, s, PhC*H*$_3$), 2.66 (2H, d, *J* = 8.6 Hz, CH_2), 2.85–2.88 (1H, m, CH_3C*H*), 3.66 (3H, s, CO_2CH_3), 7.18 (1H, d, *J* = 8.2 Hz, ArH), 7.25 (1H, dd, *J* = 8.2 Hz, 2.0 Hz, ArH), 7.35 (2H, d, *J* = 8.6 Hz, ArH), 7.58 (1H, d, *J* = 1.8 Hz, ArH), 7.95 (2H, d, *J* = 8.4 Hz, ArH). LRMS (FAB$^+$): *m/z* 498 ([M+H]$^+$). Anal. Calcd for $C_{28}H_{35}NO_5S$: C, 67.58; H, 7.09; N, 2.81. Found: C, 69.39; H, 7.21; N, 2.91.

13-Isopropyl-8,11a-dimethyl-6-oxo-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepine-8-carboxylic acid methyl ester (10b). A mixture of the methyl ester **9b** (505 mg, 1.015 mmol) in TFA (3 mL) was stirred at rt for 40 min, and then the solvent was evaporated in vacuum. The residue was purified by flash chromatography (*n*-hexane/AcOEt = 1:1) to afford **10b** (392 mg, quantitative yield) as a colorless amorphous solid. Compound **10b**: colorless needles. Mp 148–149 °C (*n*-hexane/AcOEt). ^{1}H NMR ($CDCl_3$) 1.24 (6H, d, *J* = 7.0 Hz, 2C*H*$_3$CH), 1.42 (3H, s, CH_3), 1.45 (3H, s, CH_3), 1.69–1.93 (6H, m, 6CH), 2.18 (1H, d, *J* = 14.8 Hz, CH), 2.57 (1H, dd, *J* = 14.7 Hz, 8.2 Hz, CH), 2.78–2.81 (1H, dd, *J* = 8.2 Hz, 3.6 Hz, CH), 2.85–2.88 (1H, m, CH_3C*H*), 3.65 (3H, s, CO_2CH_3), 6.76 (1H, d, *J* = 2.0 Hz, ArH), 7.04 (1H, d, *J* = 8.2 Hz, ArH), 7.33 (1H, d, *J* = 8.4 Hz, ArH), 8.62 (1H, s, NH). LRMS (FAB$^+$) *m/z* 343 ([M+H]$^+$). Anal. Calcd for $C_{21}H_{29}NO_3$: C, 73.44; H, 8.51; N, 4.08. Found: C, 73.21; H, 8.29; N, 4.10.

13-Isopropyl-8,11a-dimethyl-6-oxo-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*] azepine-8-carboxylic acid (11b). A mixture of the methyl ester **10b** (100 mg, 0.291 mmol), KOH (163 mg, 2.911 mmol, 10.0 equiv) and 18-crown ether-6 (192 mg, 0.728 mmol, 2.5 eq.) in MeOH (2 mL) was stirred at 80 °C for 15 h, the whole was cooled, and the solvent was evaporated in vacuum. The residue was diluted with water (10 mL), acidified with 2 N HCl (2 mL), and then the mixture was extracted with $CHCl_3$ (3× 20 mL). The combined organic layer was washed with brine (1× 20 mL), dried over Na_2SO_4, filtered, and then the solvent was evaporated in vacuum. The residue was purified by flash chromatography (*n*-hexane/AcOEt = 1:2) to afford **11b** (75 mg, 0.228 mmol, yield 78%) as a colorless oil. Compound **11b**: colorless powder (*n*-hexane/AcOEt); Mp 135–140 °C; ^{1}H NMR ($CDCl_3$) 1.22 (6H, d, *J* = 6.8 Hz, 2C*H*$_3$CH), 1.42 (3H, s, CH_3), 1.45 (3H, s, CH_3), 1.74–1.88 (5H, m, 5CH), 1.95 (1H, d, *J* = 11.3 Hz, CH), 2.28 (1H, dd, *J* = 14.9 Hz, 3.4 Hz, CH), 2.67 (1H, dd, *J* = 14.9 Hz, 7.8 Hz, CH), 2.80 (1H, dd, *J* = 7.9 Hz, 3.7 Hz, CH), 2.83–2.86 (1H, m, CH_3C*H*), 6.70 (1H, d, *J* = 1.8 Hz, ArH), 7.00 (1H, dd, *J* = 8.0 Hz, 1.8 Hz, ArH), 7.31 (1H, d, *J* = 8.4 Hz, ArH), 7.99 (1H, s, ArH). LRMS (FAB$^+$) *m/z* 330 ([M+H]$^+$). Anal. Calcd for $C_{20}H_{27}NO_3$·1/6H_2O: C, 72.26; H, 8.28; N, 4.21. Found: C, 72.21; H, 8.15; N, 4.16.

12,14-Dichloro-3-isopropyl-5,8,11a-trimethyl-6-oxo-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepine-8-carboxylic acid methyl ester (12a). To a suspension of NaH (60% in mineral oil, washed twice with *n*-hexane) (10 mg, 0.244 mmol, 1.1 equiv) in DMF (1 mL) was dropwise added a solution of the methyl ester **10a** (91 mg, 0.222 mmol) in DMF (1 mL) at 0 °C for 2 min in a flask fitted with a $CaCl_2$ tube. After stirring at 0 °C for 15 min, MeI (0.07 mL, 1.108 mmol, 5.0 equiv) was added to the reaction mixture at 0 °C. Stirring was continued at rt for 7 h, the solvent was evaporated in vacuum, and the residue was diluted with 1NHCl (10 mL) and then extracted with $CHCl_3$ (3× 20 mL). The combined organic layer was washed with brine (1× 20 mL), dried over Na_2SO_4, filtered, and then the solvent was evaporated in vacuum. The residue was purified by flash chromatography (*n*-hexane/AcOEt = 2:1) to afford **12a** (73 mg, 0.171 mmol, yield 77%) as a colorless oil. Compound **12a**: ^{1}H NMR ($CDCl_3$) 1.36 (3H, s, CH_3), 1.42 (6H, d, *J* = 7.1 Hz, 2C*H*$_3$CH), 1.46 (3H, s, CH_3), 1.72–1.73 (2H, m, CH_2), 1.82–1.91 (4H, m, 4CH), 2.21 (1H, d, *J* = 8.6 Hz, CH), 2.74 (1H, d, *J* = 16.3 Hz, CH), 3.19 (3H, s, NCH_3), 3.64 (3H, s, CO_2CH_3), 3.81 (1H, dd, *J* = 16.2 Hz, 8.8 Hz, CH), 4.09 (1H, br s, CH_3C*H*), 7.20 (1H, br s, ArH).

12,14-Dichloro-13-isopropyl-5,8,11a-trimethyl-6-oxo-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]-azepine-8-carboxylic acid (13a). A mixture of the methyl ester **12a** (73 mg, 0.171 mmol), KOH (96 mg, 1.705 mmol, 10.0 equiv) and 18-crown ether-6 (113 mg, 0.426 mmol, 2.5 equiv) in MeOH (1 mL) was stirred at 80 °C for 13 h, the whole was cooled, and the solvent was evaporat-

ed in vacuum. The residue was diluted with water (10 mL), acidified with 2 N HCl (2 mL), and the whole was extracted with $CHCl_3$ (3× 20 mL). The combined organic layer was washed with brine (1× 20 mL), dried over Na_2SO_4, filtered, and the solvent was evaporated in vacuum. The residue was purified by flash chromatography (AcOEt only) to afford **13a** (65 mg, 0.156 mmol, yield 92%) as a colorless solid. Compound **13a**: colorless powder. Mp 155–159 °C (*n*-hexane). ^{1}H NMR ($CDCl_3$) 1.34 (3H, s, CH_3), 1.39 (6H, d, J = 7.1 Hz, 2CH_3CH), 1.43 (3H, s, CH_3), 1.54 (2H, m, 2CH), 1.73–1.88 (4H, m, 4CH), 2.18 (1H, d, J = 8.6 Hz, CH), 2.83 (1H, d, J = 16.3 Hz, CH), 3.19 (3H, s, NCH_3), 3.79–3.85 (1H, m, CH), 3.94 (1H, br s, CH_3CH), 7.19 (1H, br s, ArH), 10.03 (1H, br s, CO_2H). LRMS (FAB^+) *m*/*z* 412 ([M($^{35}Cl_2$)+H]$^+$), 414 ([M($^{35}Cl^{37}Cl$)+H]$^+$), 416 ([M($^{37}Cl_2$)+H]$^+$). Anal. Calcd for $C_{21}H_{27}Cl_2NO_2 \cdot 1/4H_2O$: C, 60.51; H, 6.64; N, 3.36. Found: C, 60.72; H, 6.82; N, 3.26.

13-Isopropyl-5,8,11a-trimethyl-6-oxo-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepine-8-carboxylic acid methyl ester (12b). To a suspension of NaH (60% in mineral oil, washed twice with *n*-hexane) (13 mg, 0.032 mmol, 1.1 equiv) in DMF (1 mL) was dropwise added a solution of the carboxylic acid **10b** (100 mg, 0.291 mmol) in DMF (1 mL) at 0 °C for 1 min, in a flask fitted with a $CaCl_2$ tube. After stirring at 0 °C for 15 min, MeI (0.091 mL, 1.456 mmol, 5.0 equiv) was added to the reaction mixture at 0 °C. Stirring was continued at rt for 14 h, the solvent was evaporated in vacuum, and the residue was diluted with 1NHCl and then extracted with $CHCl_3$ (3× 20 mL). The combined organic layer was washed with brine (1× 20 mL), dried over Na_2SO_4, filtered, and then the solvent was evaporated in vacuum. The residue was purified by flash chromatography (*n*-hexane/AcOEt = 2:1) to afford **12b** (90 mg, 0.252 mmol, yield 87%) as a pale orange oil. Compound **12b**: colorless plates. Mp 149–150 °C (*n*-hexane/AcOEt). ^{1}H NMR ($CDCl_3$) 1.26 (6H, d J = 7.0 Hz, 2CH_3CH), 1.31 (3H, s, CH_3), 1.48 (3H, s, CH_3), 1.65–1.68 (2H, m, 2CH), 1.74–1.77 (2H, m, 2CH), 1.90 (1H, d, J = 13.6 Hz, CH), 1.89–1.98 (3H, m, 3CH), 2.50–2.63 (2H, m, 2CH), 2.88–2.92 (1H, m, CH_3CH), 3.27 (3H, s, NCH_3), 3.60 (3H, s, CO_2CH_3), 7.01 (1H, d, J = 1.8 Hz, ArH), 7.07 (1H, dd, J = 8.2 Hz, 1.9 Hz, ArH), 7.28 (1H, d, J = 8.2 Hz, ArH). LRMS (FAB^+) *m*/*z* 358 ([M+H]$^+$). Anal. Calcd for $C_{22}H_{31}NO_3$: C, 73.91; H, 8.74; N, 3.92. Found: C, 73.78; H, 8.89; N, 3.97.

13-Isopropyl-5,8,11a-trimethyl-6-oxo-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepine-8-carboxylic acid (13b). A mixture of the methyl ester **12b** (50 mg, 0.141 mmol), KOH (79 mg, 1.410 mmol, 10.0 equiv) and 18-crown ether-6 (93 mg, 0.352 mmol, 2.5 equiv) in MeOH (1 mL) was stirred at 80 °C for 18 h, the mixture was cooled, and the solvent was evaporated in vacuum. The residue was diluted with water (10 mL), acidified with 2 N HCl (2 mL), and then extracted with $CHCl_3$ (3× 20 mL). The combined organic layer was washed with brine (1× 20 mL), dried over Na_2SO_4, filtered, and then the solvent was evaporated in vacuum. The residue was purified by flash chromatography (*n*-hexane/AcOEt = 1:2) to afford **13b** (42 mg, 0.121 mmol, yield 86%) as a colorless amorphous solid. Compound **13b**: colorless flakes. Mp 123–127 °C (*n*-hexane/AcOEt). ^{1}H NMR ($CDCl_3$) 1.24 (6H, d, J = 7.0 Hz, 2CH_3CH), 1.31 (3H, s, CH_3), 1.50 (3H, s, CH_3), 1.67–1.78 (4H, m, 2CH), 1.98–2.04 (3H, m, 3CH), 2.50–2.62 (2H, m, 2CH), 2.86–2.90 (1H, m, CH_3CH), 3.28 (3H, s, NCH_3), 6.97 (1H, d, J = 1.8 Hz, ArH), 7.06 (1H, dd, J = 8.2 Hz, 1.9 Hz, ArH), 7.26 (1H, d, J = 8.2 Hz, ArH). LRMS (FAB^+) *m*/*z* 344 ([M+H]$^+$). Anal. Calcd for $C_{21}H_{29}NO_3$: C, 73.44; H, 8.51; N, 4.08. Found: C, 73.28; H, 8.31; N, 4.03.

Toluene-4-sulfonic acid pent-4-ynyl ester (15). To a solution of 4-pentyn-1-ol **14** (1.10 mL, 0.012 mol) and Et_3N (1.81 mL, 0.013 mol, 1.1 equiv) in CH_2Cl_2 (8 mL) was dropwise added a solution of TsCl (2.49 g, 0.0113 mol, 1.1 equiv) in CH_2Cl_2 (20 mL) at 0 °C for 20 min, in a flask fitted with a $CaCl_2$ tube. Stirring was continued at rt for 20 h, and the whole was poured into icewater (50 mL). The separated aqueous layer was extracted with $CHCl_3$ (3× 30 mL). Then the combined organic layer was washed with water (1× 30 mL) and brine (1× 30 mL), dried over Na_2SO_4, filtered, and then the solvent was evaporated in vacuum. The residue was purified by flash chromatography (*n*-hexane/AcOEt = 4:1) to afford **15** (2.37 g, 0.010 mol, yield 84%) as a colorless liquid. Compound **15**: ^{1}H NMR ($CDCl_3$) 1.85–1.89 (2H, m, $CH_2C\mathit{H}_2CH_2$), 1.89 (1H, t, J = 2.7 Hz, HCC), 2.26 (2H, dt, J = 6.9 Hz, 2.7 Hz, CCH_2), 2.45 (3H, s, CH_3), 4.15 (2H, t, J = 6.1 Hz, CH_2O), 7.35 (2H, d, J = 8.2 Hz, ArH), 7.80 (2H, d, J = 8.4 Hz, ArH).

5-Iodopent-1-yne (16). A mixture of toluene-4-sulfonic acid pent-4-ynyl ester **15** (817 mg, 3.430 mmol) and NaI (1.234 g, 8.232 mmol, 2.4 equiv) in acetone (8 mL) was stirred at 70 °C for 1 h, the mixture was cooled, and poured into ice-water (20 mL). The whole was extracted with Et_2O (3× 20 mL). The combined organic layer was washed with satd $NaHCO_3$ (1× 20 mL) and brine (1× 20 mL), dried over Na_2SO_4, filtered, and then the solvent was evaporated in vacuum to afford **16** (600 mg, 3.093 mmol, yield 90%) as a yellow liquid. Compound **16**: ^{1}H NMR ($CDCl_3$) 2.00 (1H, t, J = 2.6 Hz, HCC), 2.01 (2H, tt, J = 6.7 Hz, 6.7 Hz, $C\mathit{H}_2CH_2I$), 2.35 (2H, dt, J = 6.7 Hz, 2.6 Hz, CCH_2), 3.32 (2H, t, J = 6.7 Hz, CH_2I).

12,14-Dichloro-13-isopropyl-8,11a-dimethyl-6-oxo-5-pent-4-ynyl-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepine-8-carboxylic acid methyl ester (17a). To a suspension of NaH (60% in mineral oil, washed twice with *n*-hexane) (6 mg, 0.138 mmol, 1.1 equiv) in DMF (1 mL) was dropwise added a solution of the methyl ester **10a** (52 mg, 0.125 mmol) in DMF (1.5 mL) at 0 °C for 2 min, in a flask fitted with a $CaCl_2$ tube. After stirring at 0 °C for 30 min, a solution of 5-iodopent-1-yne **16** (122 mg, 0.627 mmol, 5.0 equiv) in DMF (1.5 mL) was added to the reaction mixture at 0 °C. Stirring was continued at rt for 17 h, the solvent was evaporated in vacuum, and the residue was diluted with water (20 mL) and the whole was extracted with $CHCl_3$ (3× 20 mL). The combined organic layer was washed with brine (1× 20 mL), dried over Na_2SO_4, filtered, and then evaporated in vacuum. The residue was purified by flash chromatography (*n*-hexane/AcOEt = 4:1) to afford **17a** (44 mg, 0.093 mmol, yield 74%) as a colorless

oil. Compound **17a**: ^{1}H NMR ($CDCl_3$) 1.42 (3H, s, CH_3), 1.42 (6H, d, J = 7.0 Hz, 2CH_3CH), 1.47 (3H, s, CH_3), 1.72 (2H, m, 2CH), 1.83–1.91 (5H, m, 5CH), 2.01 (1H, t, J = 2.3 Hz, HCC), 2.21 (1H, d, J = 8.5 Hz, CH), 2.30 (2H, t, J = 7.0 Hz, CCH_2), 2.90 (1H, d, J = 16.2 Hz, CH), 3.05–3.12 (1H, m, CH), 3.66 (3H, s, CO_2CH_3), 3.70 (1H, dd, J = 16.4 Hz, 8.9 Hz, CH), 3.96 (1H, br s, CH_3CH), 4.11–4.28 (1H, m, CH), 7.21 (1H, br s, ArH). LRMS (FAB^+) m/z 478 ([M($^{35}Cl_2$)+H]$^+$), 480 ([M($^{35}Cl^{37}Cl$)+H]$^+$), 482 ([M($^{37}Cl_2$)+H]$^+$).

12,14-Dichloro-13-isopropyl-8,11a-dimethyl-6-oxo-5-[5-(4-iodophenyl)pent-4-ynyl]-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepine-8-carboxylic acid methyl ester (23) and 1,4-bis[[5-(2,4-dichloro-3-isopropyl-8,11a-dimethyl-8-methoxycarbonyl-6-oxo-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepin-5-yl)]pent-1-ynyl]benzene (21). A mixture of the methyl ester **17a** (44 mg, 0.093 mmol), *p*-diiodobenzene (34 mg, 0.102 mmol, 1.1 equiv), $Pd(PPh_3)_2Cl_2$ (1 mg, 0.001 mmol, 0.02 equiv), and CuI (1 mg, 0.005 mmol, 0.06 equiv) in Et_3N (2 mL) was stirred at rt for 39 h under Ar atmosphere. The reaction mixture was filtered, the solvent was evaporated in vacuum, and the resultant residue was purified by flash chromatography (*n*-hexane/AcOEt = 2:1) to afford the mixture of the monosubstituted compound **25** and *p*-diiodobenzene (55 mg) as pale yellow oil and the disubstituted compound **21** (14 mg, 0.013 mmol, yield 28%) as pale yellow amorphous solid. Then the former mixture was purified by flash chromatography (*n*-hexane/AcOEt = 4:1) again to afford the monosubstituted compound **25** (32 mg, 0.046 mmol, yield 50%) as a pale orange amorphous solid. Compound **25**: ^{1}H NMR ($CDCl_3$) 1.41 (6H, d, J = 5.3 Hz, 2CH_3CH), 1.42 (3H, s, CH_3), 1.46 (3H, s, CH_3), 1.70 (2H, m, 2CH), 1.81–1.97 (5H, m, 5CH), 2.02–2.09 (1H, m, CH), 2.21 (1H, d, J = 8.5 Hz, CH), 2.50 (2H, t, J = 6.9 Hz, CH_2), 2.86 (1H, d, J = 16.2 Hz, CH), 3.06–3.50 (1H, m, CH), 3.51 (3H, s, CO_2CH_3), 3.72 (1H, dd, J = 16.1 Hz, 8.6 Hz, CH), 3.95 (1H, br s, CH_3CH), 4.33–4.39 (1H, m, CH), 7.14 (2H, d, J = 8.1 Hz, ArH), 7.20 (1H, br s, ArH), 7.62 (2H, d, J = 8.1 Hz, ArH). LRMS (FAB^+) m/z 680 ([M($^{35}Cl_2$)+H]$^+$), 682 ([M($^{35}Cl^{37}Cl$)+H]$^+$), 684 ([M($^{37}Cl_2$)+H]$^+$). Compound **21**: ^{1}H NMR ($CDCl_3$) 1.43 (12H, d, J = 6.0 Hz, 4CH_3CH), 1.44 (6H, s, $2CH_3$), 1.48 (6H, s, $2CH_3$), 1.71 (4H, m, 4CH), 1.83–1.99 (10H, m, 10CH), 2.04–2.09 (2H, m, 2CH), 2.22 (2H, d, J = 8.5 Hz, 2CH), 2.53 (4H, d, J = 6.9 Hz, 4CH), 2.89 (2H, d, J = 16.3 Hz, 2CH), 3.09–3.16 (2H, m, 2CH), 3.53 (6H, s, $2CO_2CH_3$), 3.74 (2H, dd, J = 16.1 Hz, 8.6 Hz, 2CH), 3.97 (2H, br s, $2CH_3CH$), 4.35–4.41 (2H, m, 2CH), 7.22 (2H, br s, ArH), 7.35 (4H, s, ArH). LRMS (FAB^+) m/z 1029 ([M($^{35}Cl_4$)+H]$^+$), 1031 ([M($^{35}Cl_3{}^{37}Cl$)+H]$^+$), 1033 ([M($^{35}Cl_2{}^{37}Cl_2$)+H]$^+$), 1035 ([M($^{35}Cl^{37}Cl_3$)+H]$^+$).

1,4-Bis[[5-(12,14-dichloro-13-isopropyl-8,11a-dimethyl-8-methoxycarbonyl-6-oxo-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepin-5-yl)]pent-1-ynyl]benzene (21). The mixture of the alkyne **17a** (17 mg, 0.036 mmol), the iodide **25** (32 mg, 0.047 mmol, 1.3 equiv), $Pd(PPh_3)_2Cl_2$ (1 mg, 0.001 mmol, 0.04 equiv), and CuI (1 mg, 0.005 mmol, 0.14 equiv) in Et_3N (2 mL) was stirred at rt for 72 h under Ar atmosphere. The reaction mixture was filtered, the solvent was evaporated in vacuum, and the resultant residue was purified by flash chromatography (*n*-hexane/AcOEt = 2:1) to afford **21** (32 mg, 0.031 mmol, yield 84%) as a colorless oil.

1,4-Bis[[5-(12,14-dichloro-13-isopropyl-8,11a-dimethyl-8-hydroxycarbonyl-6-oxo-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepin-5-yl)]pent-1-ynyl]benzene (29). A mixture of the methyl ester **21** (46 mg, 0.045 mmol), KOH (25 mg, 0.445 mmol, 10.0 equiv) and 18-crown ether-6 (59 mg, 0.223 mmol, 5.0 equiv) in MeOH (2 mL) was stirred at 80 °C for 19 h, the mixture was cooled, and the solvent was evaporated in vacuum. The residue was diluted with water (20 mL), acidified with 2 N HCl (2 mL) and then extracted with $CHCl_3$ (3× 20 mL). The combined organic layer was washed with brine (1× 20 mL), dried over Na_2SO_4, filtered, and then the solvent was evaporated in vacuum. The residue was purified by flash chromatography (AcOEt/MeOH = 10:1) to afford **29** (29 mg, 0.029 mmol, yield 65%) as a colorless solid. Compound **29**: colorless powder. Mp 211–214 °C (*n*-hexane/AcOEt). ^{1}H NMR (CD_3OD) 1.31 (6H, s, $2CH_3$), 1.43–1.49 (12H, m, 2CH_3CH and $2CH_3$), 1.76–2.18 (14H, m, 14CH), 2.27 (2H, d, J = 8.6 Hz, 2CH), 2.54–2.55 (4H, m, 4CH), 3.15 (2H, d, J = 16.5 Hz, 2CH), 3.65–3.72 (2H, m, 2CH), 4.02 (2H, br s, $2CH_3CH$), 4.30 (2H, m, 2CH), 7.32 (4H, s, ArH), 7.35 (2H, br s, ArH). LRMS (FAB^+) m/z 1001 ([M($^{35}Cl_4$)+H]$^+$), 1003 ([M($^{35}Cl_3{}^{37}Cl$)+H]$^+$), 1005 ([M($^{35}Cl_2{}^{37}Cl_2$)+H]$^+$), 1007 ([M($^{35}Cl^{37}Cl_3$)+H]$^+$), 1009 ([M($^{37}Cl_4$)+H]$^+$). HRMS (FAB^+) found: 1001.3432. Calcd for $C_{56}H_{64}{}^{35}Cl_4N_2O_6$: 1001.3449. Anal. Calcd for $C_{56}H_{64}Cl_4N_2O_6\cdot 3/2H_2O$: C, 65.30; H, 6.56; N, 2.72. Found: C, 65.34; H, 6.59; N, 2.60.

13-Isopropyl-8,11a-dimethyl-6-oxo-5-pent-4-ynyl-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]-azepine-8-carboxylic acid methyl ester (17d). To a suspension of NaH (60% in mineral oil, washed twice with *n*-hexane) (12 mg, 0.292 mmol, 1.1 equiv) in DMF (1 mL) was dropwise added a solution of the methyl ester **10b** (91 mg, 0.265 mmol) in DMF (1.5 mL) at 0 °C over 1 min, in a flask fitted with a $CaCl_2$ tube. After stirring at 0 °C for 30 min, a solution of 5-iodopent-1-yne **16** (257 mg, 1.326 mmol, 5.0 equiv) in DMF (1.5 mL) was added to the reaction mixture at 0 °C. Stirring was continued at rt for 18 h, and the solvent was evaporated in vacuum, and the residue was diluted with water (20 mL) and then extracted with $CHCl_3$ (3× 20 mL). The combined organic layer was washed with brine (1× 20 mL), dried over Na_2SO_4, filtered and the solvent was evaporated in vacuum. The residue was purified by flash chromatography (*n*-hexane/AcOEt = 4:1) to afford **17b** (76 mg, 0.85 mmol, yield 70%) as a colorless oil. Compound **17b**: ^{1}H NMR ($CDCl_3$) 1.27 (6H, d, J = 6.9 Hz, 2CH_3CH), 1.32 (3H, s, CH_3), 1.48 (3H, s, CH_3), 1.66–1.68 (2H, m, 2CH), 1.75–1.78 (2H, m, 2CH), 1.90 (1H, d, J = 13.3 Hz, CH), 1.95–2.04 (2H, m, 2CH), 1.96 (1H, t, J = 2.6 Hz, HCC), 2.10–2.17 (1H, m, CH), 2.19–2.37 (2H, m, 2CH), 2.50–2.59 (2H, m, 2CH), 2.88–2.96 (1H, m, CH_3CH), 3.48–3.56 (1H, m, CH), 3.60 (3H, s, CO_2CH_3), 3.90–3.97 (1H, m, CH), 7.08 (1H, dd, J = 8.2 Hz, 1.9 Hz, ArH), 7.11 (1H, d,

J = 1.9 Hz, ArH), 7.29 (1H, d, J = 8.2 Hz, ArH). LRMS (FAB^+) m/z 410 ($[M+H]^+$).

12,14-Dichloro-13-isopropyl-8,11a-dimethyl-6-oxo-5-[5-(3-iodophenyl)pent-4-ylnyl]-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepine-8-carboxylic acid methyl ester (24) and 1,3-bis[[5-(12,14-dichloro-13-isopropyl-8,11a-dimethyl-8-methoxycarbonyl-6-oxo-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepin-5-yl)]pent-1-ynyl]benzene (20). A mixture of the alkyne **17b** (62 mg, 0.129 mmol), *m*-diiodobenzene (21 mg, 0064 mmol, 0.5 equiv), $Pd(PPh_3)_2Cl_2$ (1 mg, 0.001 mmol, 0.01 equiv), and CuI (1 mg, 0.005 mmol, 0.04 equiv) in Et_3N (3 mL) was stirred at rt for 49 h under Ar atmosphere. The reaction mixture was filtered, the solvent was evaporated in vacuum. The resultant residue was purified by flash chromatography (*n*-hexane/AcOEt = 4:1) to afford the monosubstituted compound **24** (32 mg, 0.046 mmol, yield 36%) as an orange oil and the disubstituted compound **20** (31 mg, 0.030 mmol, yield 47%) as an orange oil. Compound **24**: ^1H NMR ($CDCl_3$) 1.41 (6H, d, J = 5.9 Hz, $2CH_3CH$), 1.42 (3H, s, CH_3), 1.47 (3H, s, CH_3), 1.70–1.71 (2H, m, 2CH), 1.82–1.93 (5H, m, 5CH), 2.03–2.10 (1H, m, CH), 2.20 (1H, d, J = 8.5 Hz, CH), 2.51 (2H, t, J = 7.0 Hz, CH_2), 2.87 (1H, d, J = 16.2 Hz, CH), 3.07–3.14 (1H, m, CH), 3.52 (3H, s, CO_2CH_3), 3.72 (1H, dd, J = 16.1 Hz, 8.6 Hz, CH), 3.96 (1H, br s, CH_3CH), 4.32–4.39 (1H, m, CH), 7.02 (1H, t, J = 7.9 Hz, ArH), 7.20 (1H, br s, ArH), 7.37 (1H, td, J = 7.8 Hz, 1.2 Hz, ArH), 7.61 (1H, td, J = 8.5 Hz, 1.4 Hz, ArH), 7.77 (1H, t, J = 1.6 Hz, ArH). LRMS (FAB^+) m/z 680 ($[M(^{35}Cl_2)+H]^+$), 682 ($[M(^{35}Cl^{37}Cl)+H]^+$), 684 ($[M(^{37}Cl_2)+H]^+$). Compound **20**: ^1H NMR ($CDCl_3$) 1.41 (6H, s, $2CH_3$), 1.41 (12H, d, J = 6.3 Hz, $4CH_3CH$), 1.47 (6H, s, $2CH_3$), 1.69–1.70 (4H, m, 4CH), 1.81–1.93 (10H, m, 10CH), 2.03–2.08 (2H, m, 2CH), 2.20 (2H, d, J = 8.5 Hz, 2CH), 2.50 (4H, d, J = 7.0 Hz, 4CH), 2.88 (2H, d, J = 16.2 Hz, 2CH), 3.07–3.14 (2H, m, 2CH), 3.51 (6H, s, $2CO_2CH_3$), 3.73 (2H, dd, J = 16.2 Hz, 8.7 Hz, 2CH), 3.96 (2H, br s, $2CH_3CH$), 4.32–4.39 (2H, m, 2CH), 7.20 (2H, br s, ArH), 7.21 (2H, t, J = 7.7 Hz, ArH), 7.32 (2H, d, J = 7.8 Hz, ArH), 7.44 (1H, s, ArH). LRMS (FAB^+) m/z 1029 ($[M(^{35}Cl_4)+H]^+$), 1031 ($[M(^{35}Cl_3{}^{37}Cl)+H]^+$), 1033 ($[M(^{35}Cl_2{}^{37}Cl_2)+H]^+$), 1035 ($M(^{35}Cl^{37}Cl_3)+H)^+$).

1,3-Bis[[5-(12,14-dichloro-13-isopropyl-8,11a-dimethyl-8-methoxycarbonyl-6-oxo-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepin-5-yl)]pent-1-ynyl]benzene (20). A mixture of the alkyne **17a** (22 mg, 0.046 mmol), the iodide **24** (32 mg, 0.046 mmol), $Pd(PPh_3)_2Cl_2$ (1 mg, 0.001 mmol, 0.03 equiv), and CuI (1 mg, 0.005 mmol, 0.1 equiv) in Et_3N (2 mL) was stirred at rt for 48 h under Ar atmosphere. The reaction mixture was filtered, and the solvent was evaporated in vacuum, and the residue was purified by flash chromatography (*n*-hexane/AcOEt = 2:1) to afford **20** (33 mg, 0.032 mmol, yield 70%) as an orange oil.

1,3-Bis[[5-(12,14-dichloro-13-isopropyl-8,11a-dimethyl-8-hydroxycarbonyl-6-oxo-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepin-5-yl)]pent-1-ynyl]benzene (28). A mixture of the methyl ester **20** (69 mg, 0.067 mmol), KOH (38 mg, 0.672 mmol, 10.0 equiv), and 18-crown ether-6 (89 mg, 0.336 mmol, 5.0 equiv) in MeOH (2 mL) was stirred at 80 °C for 18 h, the whole was cooled, and the solvent was evaporated in vacuum. The resultant residue was diluted with water (20 mL), acidified with 2 N HCl (2 mL), and then extracted with $CHCl_3$ (3× 20 mL). The combined organic layer was washed with brine (1× 20 mL), dried over Na_2SO_4, filtered, and then the solvent was evaporated in vacuum. The residue was purified by flash chromatography (AcOEt/MeOH = 10:1) to afford the desired compound **28** (50 mg, 0.050 mmol, yield 75%) as a colorless solid. Compound **28**: colorless powder. Mp 207–213 °C (*n*-hexane/$CHCl_3$). ^1H NMR ($CDCl_3$) 1.29 (6H, s, $2CH_3$), 1.32 (12H, d, J = 7.1 Hz, $4CH_3CH$), 1.33 (6H, s, $2CH_3$), 1.61 (4H, m, 4CH), 1.75–1.89 (10H, m, 10CH), 2.13 (2H, d, J = 8.6 Hz, 2CH), 2.21 (4H, m, 4CH), 2.92–3.00 (2H,, m, 2CH), 2.98 (2H, d, J = 16.7 Hz, 2CH), 3.50–3.57 (2H, m, 2CH), 3.91 (2H, br s, $2CH_3CH$), 4.13–4.20 (2H, m, 2CH), 7.09 (3H, m, ArH), 7.24 (2H, br s, ArH), 7.30 (1H, s, ArH). LRMS (FAB^+) m/z 1001 ($[M(^{35}Cl_4)+H]^+$), 1003 ($[M(^{35}Cl_3{}^{37}Cl)+H]^+$), 1005 ($[M(^{35}Cl_2{}^{37}Cl_2)+H]^+$), 1007 ($[M(^{35}Cl^{37}Cl_3)+H]^+$), 1009 ($[M(^{37}Cl_4)+H]^+$). HRMS ($FAB^+$) found: 1001.4380. Calcd for $C_{56}H_{65}{}^{35}Cl_4N_2O_6$: 1001.4302. Anal. Calcd for $C_{56}H_{64}Cl_4N_2O_6{\cdot}3/2H_2O$: C, 65.30; H, 6.56; N, 2.72. Found: C, 65.03; H, 6.78; N, 2.62.

13-Isopropyl-8,11a-dimethyl-6-oxo-5-[5-(3-iodophenyl)pent-4-ylnyl]-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepine-8-carboxylic acid methyl ester (26) and 1,3-bis[[5-(13-isopropyl-8,11a-dimethyl-8-methoxycarbonyl-6-oxo-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepin-5-yl)]pent-1-ynyl]benzene (22). A mixture of the alkyne **17b** (66 mg, 0.161 mmol), *m*-diiodobenzene (53 mg, 0.161 mmol), $Pd(PPh_3)_2Cl_2$ (1 mg, 0.001 mmol, 0.01 equiv), and CuI (1 mg, 0.005 mmol, 0.03 equiv) in Et_3N (2 mL) was stirred at rt for 19 h under Ar atmosphere. The reaction mixture was filtered, the solvent was evaporated in vacuum, and the resultant residue was purified by flash chromatography (*n*-hexane/AcOEt = 4:1–2:1) to afford the monosubstituted compound **26** (63 mg, 0.103 mmol, yield 64%) as yellow oil and the disubstituted compound **22** (26 mg, 0.029 mmol, yield 36%) as a yellow oil. Compound **26**: ^1H NMR ($CDCl_3$) 1.23 (6H, d, J = 6.9 Hz, $2CH_3CH$), 1.34 (3H, s, CH_3), 1.50 (3H, s, CH_3), 1.68–1.69 (2H, m, 2CH), 1.77–1.79 (2H, m, 2CH), 1.92 (1H, d, J = 8.3 Hz, CH), 1.97–2.00 (2H, m, 2CH), 2.09 (1H, m, CH), 2.22–2.23 (1H, m, CH), 2.46–2.64 (4H, m, 4CH), 2.83–2.90 (1H, m, CH_3CH), 3.55–3.61 (1H, m, CH), 3.62 (3H, s, CO_2CH_3), 3.95–4.02 (1H, m, CH), 7.00 (1H, t, J = 7.9 Hz, ArH), 7.08 (1H, dd, J = 8.2 Hz, 1.8 Hz, ArH), 7.13 (1H, d, J = 1.8 Hz, ArH), 7.30 (1H, t, J = 8.3 Hz, ArH), 7.32 (1H, dt, J = 8.0 Hz, 1.4 Hz, ArH), 7.60 (1H, td, J = 7.9 Hz, 1.2 Hz, ArH), 7.73 (1H, t, J = 1.6 Hz, ArH). LRMS (FAB^+) m/z 612 ($[M+H]^+$). Compound **22**: ^1H NMR ($CDCl_3$) 1.19 (12H, d, J = 6.9 Hz, $4CH_3CH$), 1.33 (6H, s, $2CH_3$), 1.48 (6H, s, $2CH_3$), 1.67–1.68 (4H, m, 4CH), 1.75–1.77 (4H, m, 4CH), 1.90 (2H, d, J = 7.3 Hz, 2CH), 2.01–2.11 (2H, m, 2CH), 2.17–2.24 (2H, m, 2CH), 2.40–2.62 (8H, m, 8CH), 2.80–2.90 (2H, m, $2CH_3CH$), 3.42–3.60 (2H, m, 2CH), 3.61 (6H, s, $2CO_2CH_3$), 3.92–4.00 (2H, m, 2CH), 7.05 (2H, dd, J = 8.2 Hz, 1.9 Hz, ArH), 7.11 (2H, d, J = 1.9 Hz, ArH), 7.16 (1H, d,

J = 8.4 Hz, ArH), 7.24 (2H, dd, J = 8.6 Hz, 1.5 Hz, ArH), 7.28 (2H, d, J = 8.3 Hz, ArH), 7.36 (1H, m, ArH). LRMS (FAB^+) m/z 893 ($[M+H]^+$).

1,3-Bis[[5-(13-isopropyl-8,11a-dimethyl-8-hydroxycarbonyl-6-oxo-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepin-5-yl)]pent-1-ynyl]benzene (30). A mixture of the methyl ester **22** (26 mg, 0.029 mmol), KOH (16 mg, 0.289 mmol, 10.0 equiv), and 18-crown ether-6 (38 mg, 0.145 mmol, 5.0 equiv) in MeOH (2 mL) was stirred at 80 °C for 17 h, the whole was cooled, and the solvent was evaporated in vacuum. The residue was diluted with water (20 mL), acidified with 2 N HCl (2 mL), and then extracted with $CHCl_3$ (3× 20 mL). The combined organic layer was washed with brine (1× 20 mL), dried over Na_2SO_4, filtered, and then the solvent was evaporated in vacuum. The residue was purified by flash chromatography (AcOEt/MeOH = 10:1) to afford **30** (9 mg, 0.010 mmol, yield 36%) as a colorless amorphous solid. Compound **30**: colorless powder. Mp 159–163 °C (*n*-hexane/$CHCl_3$). ^1H NMR (CD_3OD) 1.04 (12H, d, J = 6.9 Hz, 4CH_3CH), 1.22 (6H, s, 2CH_3), 1.33 (6H, s, 2CH_3), 1.57–1.68 (8H, m, 8CH), 1.84–1.88 (8H, m, 8CH), 2.21–2.27 (2H, m, 2CH), 2.38–2.48 (8H, m, 8CH), 2.73–2.78 (2H, m, 2CH_3CH), 3.30–3.38 (2H, m, 2CH), 3.80 (2H, m, 2CH), 7.01–7.08 (6H, m, ArH), 7.23–7.25 (4H, m, ArH). LRMS (FAB^+) m/z 866 ($[M+H]^+$). Anal. Calcd for $C_{56}H_{68}N_2O_6{\cdot}2H_2O$: C, 74.64; H, 8.05; N, 3.11. Found: C, 74.45; H, 8.31; N, 2.83.

12,14-Dichloro-13-isopropyl-8,11a-dimethyl-6-oxo-5-[5-(2-iodophenyl)pent-4-ynyl]-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepine-8-carboxylic acid methyl ester (23) and 1,10-bis[5-(12,14-dichloro-13-isopropyl-8,11a-dimethyl-8-methoxycarbonyl-6-oxo-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepin-5-yl)]decane-4,6-diyne (18). A mixture of the alkyne **17a** (77 mg, 0.161 mmol), *o*-diiodobenzene (0.010 mL, 0.080 mmol, 0.5 equiv), $Pd(PPh_3)_2Cl_2$ (1 mg, 0.001 mmol, 0.01 equiv), and CuI (1 mg, 0.005 mmol, 0.03 equiv) in Et_3N (2 mL) was stirred at rt for 48 h under Ar atmosphere. The reaction mixture was filtered, the solvent was evaporated in vacuum and the resultant residue was purified by flash chromatography (*n*-hexane/AcOEt = 2:1) to afford the monosubstituted compound **23** (17 mg, 0.025 mmol, yield 15%) as orange oil and the homodimeric compound **18** (52 mg, 0.054 mmol, yield 67%) as an orange oil. Compound **23**: ^1H NMR ($CDCl_3$) 1.43 (6H, d, J = 7.5 Hz, 2CH_3CH), 1.44 (3H, s, CH_3), 1.49 (3H, s, CH_3), 1.70 (2H, m, 2CH), 1.85–1.92 (4H, m, 2CH), 2.02–2.03 (1H, m, CH), 2.12–2.17 (1H, m, CH), 2.22 (1H, d, J = 8.5 Hz, CH), 2.61 (2H, t, J = 6.9 Hz, CH_2), 2.91 (1H, d, J = 16.3 Hz, CH), 3.20–3.27 (1H, m, CH), 3.52 (3H, s, CO_2CH_3), 3.77 (1H, dd, J = 16.1 Hz, 8.6 Hz, CH), 3.98 (1H, br s, CH_3CH), 4.34–4.38 (1H, m, CH), 6.98 (1H, dt, J = 7.7 Hz, 1.7 Hz, ArH), 7.22 (1H, br s, ArH), 7.29 (1H, dt, J = 7.6 Hz, 1.2 Hz, ArH), 7.44 (1H, dd, J = 7.7 Hz, 1.6 Hz, ArH), 7.84 (1H, dd, J = 8.0 Hz, 1.6 Hz, ArH). LRMS (FAB^+) m/z 680 ($[M(^{35}Cl_2)+H]^+$), 682 ($[M(^{35}Cl^{37}Cl)+H]^+$), 684 ($[M(^{37}Cl_2)+H]^+$). Compound **18**: ^1H NMR ($CDCl_3$) 1.40 (6H, s, 2CH_3), 1.41 (12H, d, J = 5.8 Hz, 4CH_3CH), 1.46 (6H, s, 2CH_3), 1.71 (4H, m, 4CH), 1.79–1.92 (10H, m, 10CH), 1.95–2.04 (2H, m, 2CH), 2.20 (2H, d, J = 8.5 Hz, 2CH), 2.36 (4H, d, J = 7.1 Hz, 4CH), 2.83 (2H, d, J = 16.2 Hz, 2CH), 2.98–3.05 (2H, m, 2CH), 3.67 (6H, s, 2CO_2CH_3), 3.69 (2H, dd, J = 16.0 Hz, 8.6 Hz, 2CH), 3.94 (2H, br s, 2CH_3CH), 4.26–4.32 (2H, m, 2CH), 7.19–7.23 (2H, br s, ArH). LRMS (FAB^+) m/z 1029 ($[M(^{35}Cl_4)+H]^+$), 1031 ($[M(^{35}Cl_3{}^{37}Cl)+H]^+$), 1033 ($[M(^{35}Cl_2{}^{37}Cl_2)+H]^+$), 1035 ($[M(^{35}Cl^{37}Cl_3)+H]^+$), 1037 ($[M(^{37}Cl_4)+H]^+$).

1,2-Bis[[5-(12,14-dichloro-13-isopropyl-8,11a-dimethyl-8-methoxycarbonyl-6-oxo-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepin-5-yl)]pent-1-ynyl]benzene (19). A mixture of the alkyne **17a** (15 mg, 0.032 mmol), the iodide **23** (22 mg, 0.032 mmol), $Pd(PPh_3)_4$ (2 mg, 0.002 mmol, 0.05 equiv), and CuI (1 mg, 0.010 mmol, 0.3 equiv) in Et_3N (1 mL) was stirred at rt for 19 h under Ar atmosphere. The reaction mixture was filtered, the solvent was evaporated in vacuum, and the resultant residue was purified by flash chromatography (*n*-hexane/AcOEt = 2:1) to afford **19** (11 mg, 0.011 mmol, yield 33%) as an orange oil. Compound **19**: ^1H NMR ($CDCl_3$) 1.41 (6H, s, 2CH_3), 1.42 (12H, d, J = 6.6 Hz, 4CH_3CH), 1.46 (6H, s, 2CH_3), 1.68 (4H, m, 4CH), 1.81–1.92 (8H, m, 8CH), 1.95–2.00 (2H, m, 2CH), 2.07–2.14 (2H, m, 2CH), 2.20 (2H, d, J = 8.0 Hz, 2CH), 2.57 (4H, t, J = 6.9 Hz, 2CH_2), 2.89 (2H, d, J = 16.2 Hz, 2CH), 3.13–3.20 (2H, m, 2CH), 3.47 (6H, s, 2CO_2CH_3), 3.74 (2H, dd, J = 16.2 Hz, 8.6 Hz, 2CH), 3.96 (2H, br s, 2CH_3CH), 4.33–4.38 (2H, m, 2CH), 7.20 (2H, dd, J = 5.7 Hz, 3.3 Hz, ArH), 7.19–7.20 (2H, m, ArH), 7.41 (2H, dd, J = 5.7 Hz, 3.4 Hz, ArH). LRMS (FAB^+) m/z 1029 ($[M(^{35}Cl_4)+H]^+$), 1031 ($[M(^{35}Cl_3{}^{37}Cl_3)+H]^+$), 1033 ($[M(^{35}Cl_2{}^{37}Cl_2)+H]^+$), 1035 ($[M(^{35}Cl^{37}Cl_3)+H]^+$).

1,2-Bis[[5-(12,14-dichloro-13-isopropyl-8,11a-dimethyl-8-hydroxycarbonyl-6-oxo-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepin-5-yl)]pent-1-ynyl]benzene (27). A mixture of the methyl ester **19** (11 mg, 0.011 mmol), KOH (60 mg, 0.108 mmol, 10.0 equiv), and 18-crown ether-6 (14 mg, 0.054 mmol, 5.0 equiv) in MeOH (2 mL) was stirred at 80 °C for 16 h, the whole was cooled, and the solvent was evaporated in vacuum, and the resultant residue was diluted with water (20 mL), acidified with 2 N HCl (2 mL) and then extracted with $CHCl_3$ (3× 20 mL). The combined organic layer was washed with brine (1× 20 mL), dried over Na_2SO_4, filtered, and then the solvent was evaporated in vacuum. The residue was purified by flash chromatography (AcOEt/MeOH = 20:1) to afford **27** (4 mg, 0.004 mmol, yield 41%) as a colorless solid. Compound **27**: pale yellow powder. Mp 181–184 °C (*n*-hexane/AcOEt). ^1H NMR (CD_3OD) 1.28 (6H, s, 2CH_3), 1.34 (12H, m, 2CH_3CH and 2CH_3), 1.73 (14H, m, 14CH), 2.21 (6H, m, 6CH), 2.98 (4H, m, 4CH), 3.54 (2H, m, 2CH), 3.91 (2H, br s, 2CH_3CH), 4.15 (2H, m, 2CH), 7.06 (2H, s, ArH), 7.27 (4H, m, ArH). LRMS (FAB^+) m/z 1001 ($[M(^{35}Cl_4)+H]^+$), 1003 ($[M(^{35}Cl_3{}^{37}Cl)+H]^+$), 1005 ($[M(^{35}Cl_2{}^{37}Cl_2)+H]^+$), 1007 ($[M(^{35}Cl^{37}Cl_3)+H]^+$), 1009 ($[M(^{37}Cl_4)+H]^+$). Anal. Calcd for $C_{56}H_{64}Cl_4N_2O_6{\cdot}3/2H_2O$: C, 65.30; H, 6.56; N, 2.72. Found: C, 65.17; H, 6.79; N, 2.69.

12,14-Dichloro-13-isopropyl-8,11a-dimethyl-6-oxo-5-pent-4-ynyl-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepine-8-carboxylic acid (31). A mixture of the methyl ester **17a** (41 mg, 0.086 mmol), KOH (48 mg, 0.863 mmol, 10.0 equiv) and 18-crown ether-6 (57 mg, 0.216 mmol, 2.5 equiv) in MeOH (1 mL) was stirred at 80 °C for 17 h, the whole was cooled, and the solvent was evaporated in vacuum. The residue was diluted with water (10 mL), acidified with 2 N HCl (2 mL), and then extracted with $CHCl_3$ (3× 20 mL). The combined organic layer was washed with brine (1× 20 mL), dried over Na_2SO_4, filtered, and then the solvent was evaporated in vacuum. The residue was purified by flash chromatography (AcOEt/MeOH = 10:1) to afford **31** (29 mg, 0.0624 mmol, yield 72%) as a colorless solid. Compound **31**: colorless flakes. Mp 122–126 °C (*n*-hexane/AcOEt). ^{1}H NMR ($CDCl_3$) 1.39 (3H, s, CH_3), 1.40 (6H, d, J = 5.5 Hz, 2CH_3CH), 1.44 (3H, s, CH_3), 1.69–1.74 (2H, m, 2CH), 1.73 (1H, t, J = 2.4 Hz, HCC), 1.81–1.89 (5H, m, 5CH), 2.16 (1H, d, J = 8.5 Hz, CH), 2.50–2.53 (2H, m, 2CH), 2.94–3.01 (1H, s, CH), 3.04 (1H, d, J = 16.4 Hz, CH), 3.74 (1H, dd, J = 15.9 Hz, 9.0 Hz, CH), 3.95 (1H, br s, CH_3CH), 4.36–4.43 (1H, m, CH), 7.19 (1H, br s, ArH). LRMS (FAB$^+$) *m*/*z* 464 ([M(^{35}Cl$_2$)+H]$^+$), 466 ([M(^{35}Cl^{37}Cl)+H]$^+$), 468 ([M(^{37}Cl$_2$)+H]$^+$). Anal. Calcd for $C_{25}H_{31}Cl_2NO_3$: C, 64.65; H, 6.73; N, 3.02. Found: C, 64.63; H, 6.87; N, 2.94.

12,14-Dichloro-13-isopropyl-8,11a-dimethyl-6-oxo-5-(5-phenylpent-4-ynyl)-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepine-8-carboxylic acid methyl ester (32c). A mixture of the alkyne **17a** (20 mg, 0.063 mmol), iodobenzene (0.008 mL, 0.075 mmol, 1.2 equiv), $Pd(PPh_3)_2Cl_2$ (1 mg, 0.001 mmol, 0.02 equiv), and CuI (1 mg, 0.005 mmol, 0.08 equiv) in Et_3N (1 mL) was stirred at rt for 14 h under Ar atmosphere. The reaction mixture was filtered, the solvent was evaporated in vacuum, and the resultant residue was purified by flash chromatography (*n*-hexane/AcOEt = 5:1) to afford **32a** (37 mg, quantitative yield) as a pale yellow amorphous solid. Compound **32a**: ^{1}H NMR ($CDCl_3$) 1.42 (6H, d, J = 6.7 Hz, 2CH_3CH), 1.42 (3H, s, CH_3), 1.47 (3H, s, CH_3), 1.69 (2H, m, 2CH), 1.81–1.98 (5H, m, 5CH), 2.06–2.11 (1H, m, CH), 2.20 (1H, d, J = 8.5 Hz, CH), 2.52 (2H, t, J = 7.0 Hz, CH_2), 2.89 (1H, d, J = 16.3 Hz, CH), 3.10–3.17 (1H, m, CH), 3.48 (3H, s, CO_2CH_3), 3.73 (1H, dd, J = 16.2 Hz, 8.5 Hz, CH), 3.96 (1H, br s, CH_3CH), 4.32–4.39 (1H, m, CH), 7.20 (1H, br s, ArH), 7.27–7.29 (3H, m, ArH), 7.41 (2H, dd, J = 7.9 Hz, 1.9 Hz, ArH). LRMS (FAB$^+$), *m*/*z* 554 ([M(^{35}Cl$_2$) +H]$^+$), 556 ([M(^{35}Cl^{37}Cl)+H]$^+$), 558 ([M(^{37}Cl$_2$)+H]$^+$).

12,14-Dichloro-13-isopropyl-8,11a-dimethyl-6-oxo-5-(5-phenylpent-4-ynyl)-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepine-8-carboxylic acid (33a). A mixture of the methyl ester **32a** (37 mg, 0.067 mmol), KOH (38 mg, 0.669 mmol, 10.0 equiv), and 18-crown ether-6 (44 mg, 0.167 mmol, 2.5 equiv) in MeOH (1 mL) was stirred at 80 °C for 15 h, the whole was cooled, and the solvent was evaporated in vacuum. The residue was diluted with water (10 mL), acidified with 2 N HCl (2 mL), and then extracted with $CHCl_3$ (3× 20 mL). The combined organic layer was washed with brine (1× 20 mL), dried over Na_2SO_4, filtered, and then evaporated in vacuum. The residue was purified by flash chromatography (*n*-hexane/AcOEt = 10:1) to afford **33a** (31 mg, 0.058 mmol, yield 87%) as a colorless oil. Compound **33a**: colorless powder. Mp 127–130 °C (*n*-hexane/AcOEt). ^{1}H NMR ($CDCl_3$) 1.37–1.44 (12H, m, 4CH_3), 1.70 (2H, m, 2CH), 1.79–1.88 (5H, m, 5CH), 1.90–2.04 (1H, m, CH), 2.11 (1H, d, J = 8.5 Hz, CH), 2.47 (2H, t, J = 6.9 Hz, CH_2), 2.89 (1H, d, J = 16.3 Hz, CH), 3.07–3.14 (1H, m, CH), 3.48 (1H, dd, J = 16.1 Hz, 8.6 Hz, CH), 3.94 (1H, br s, CH_3CH), 4.27–4.36 (1H, m, CH), 7.13–7.36 (6H, m, ArH). LRMS (FAB$^+$) *m*/*z* 540 ([M(^{35}Cl$_2$)+H]$^+$), 542 ([M(^{35}Cl^{37}Cl)+H]$^+$), 544 ([M(^{37}Cl$_2$)+H]$^+$). Anal. Calcd for $C_{22}H_{29}Cl_2NO_3$: C, 68.88; H, 6.53; N, 2.59. Found: C, 68.62; H, 6.65; N, 2.58.

13-Isopropyl-8,11a-dimethyl-6-oxo-5-(5-phenylpent-4-ynyl)-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepine-8-carboxylic acid methyl ester (32b). A mixture of the alkyne **17b** (88 mg, 0.215 mmol), iodobenzene (0.029 mL, 0.258 mmol, 1.2 equiv), $Pd(PPh_3)_2Cl_2$ (2 mg, 0.002 mmol, 0.01 equiv), and CuI (1 mg, 0.005 mmol, 0.02 equiv) in Et_3N (1 mL) was stirred at rt for 21 h under Ar atmosphere. The reaction mixture was filtered, the solvent was evaporated in vacuum, and the resultant residue was purified by flash chromatography (*n*-hexane/AcOEt = 2:1) to afford **32b** (81 mg, 0.167 mmol. yield 78%) as a pale orange amorphous solid. Compound **32b**: ^{1}H NMR ($CDCl_3$) 1.20 (6H, d, J = 6.9 Hz, 2CH_3CH), 1.33 (3H, s, CH_3), 1.49 (3H, s, CH_3), 1.66–1.68 (2H, m, 2CH), 1.74–1.77 (2H, m, 2CH), 1.91 (1H, d, J = 13.3 Hz, CH), 1.92–1.98 (2H, m, 2CH), 2.05–2.12 (1H, m, CH), 2.16–2.27 (1H, m, CH), 2.41–2.62 (4H, m, 4CH), 2.79–2.90 (1H, m, CH_3CH), 3.56–3.63 (1H, m, CH), 3.60 (3H, s, CO_2CH_3), 3.95–4.02 (1H, m, CH), 7.06 (1H, dd, J = 8.2 Hz, 1.7 Hz, ArH), 7.13 (1H, d, J = 1.9 Hz, ArH), 7.24–7.26 (3H, m, ArH), 7.28 (1H, d, J = 8.3 Hz, ArH), 7.34–7.37 (2H, m, ArH). LRMS (FAB$^+$) *m*/*z* 486 ([M+H]$^+$).

13-Isopropyl-8,11a-dimethyl-6-oxo-5-(5-phenylpent-4-ynyl)-6,7,7a,8,9,10,11,11a-octahydro-5*H*-dibenzo[*b*,*d*]azepine-8-carboxylic acid (33b). A mixture of the methyl ester **32b** (67 mg, 0.139 mmol), KOH (93 mg, 0.167 mmol, 1.2 equiv), and 18-crown ether-6 (92 mg, 0.347 mmol, 2.5 equiv) in MeOH (1 mL) was stirred at 80 °C for 15 h, the whole was cooled, and the solvent was evaporated in vacuum. The residue was diluted with water (20 mL), acidified with 2 N HCl (2 mL), and then extracted with $CHCl_3$ (3× 20 mL). The combined organic layer was washed with brine (1× 20 mL), dried over Na_2SO_4, filtered, and then evaporated in vacuum. The residue was purified by flash chromatography (*n*-hexane/AcOEt = 1:1) to afford the unreacted compound **32b** (24 mg, 0.049 mmol, yield 35% after recrystallization) as a colorless amorphous solid and the desired compound **33b** (32 mg, 0.068 mmol, yield 49%) as a colorless amorphous solid. Compound **33b**: pale yellow powder. Mp 76–79 °C (*n*-hexane). ^{1}H NMR ($CDCl_3$) 1.24 (6H, d, J = 7.0 Hz, 2CH_3CH), 1.26 (3H, s, CH_3), 1.46 (3H, s, CH_3), 1.65–1.77 (4H, m, 4CH), 1.88–2.03 (2H, m, 2CH), 1.97 (1H, d, J = 13.3 Hz, CH), 2.05–2.12 (1H, m, CH), 2.46–2.57 (4H, m, 4CH), 2.84–2.91 (1H, m, CH_3CH), 3.23–3.31 (1H, m, CH), 3.74–3.80

(1H, m, CH), 7.05 (1H, dd, $J = 8.2$ Hz, 1.8 Hz, ArH), 7.08 (1H, d, $J = 1.8$ Hz, ArH), 7.18 (2H, d, $J = 8.2$ Hz, ArH), 7.21–7.25 (2H, m, ArH), 7.29 (1H, d, $J = 7.4$ Hz, ArH). LRMS (FAB^+) *m/z* 472 ($[M+H]^+$). Anal. Calcd for $C_{31}H_{37}Cl_2NO_3$: C, 78.95; H, 7.91; N, 2.97. Found: C, 78.65; H, 8.07; N, 2.80.

5.2. Biological assays

5.2.1. Electrophysiological measurements. Human BK channel subunits (hslo α and hslo β1) were subcloned into pcDNA3 (Invitrogen, CA, USA) and pTracer-CMV2 (Invitrogen), respectively, and transiently co-expressed in TSA201 cells with FuGENE6 transfection reagent (Roche Diagnostics Corporation, IN, USA). BK channel currents were measured with the inside-out patch clamp technique (Patch/Whole Cell Clamp Amplifier CEZ-2400, Nihon Koden). Experiments was performed within 24–48 h after the transfection. All the experiments were carried out at room temperature (20–25 °C). Test compounds were diluted in the intracellular solution (in mM; 140 K-Mes (Alfa Aesar, MA, USA), 10 HEPES (Sigma–Aldrich, Inc., MO, USA), 5 HEDTA (Nacalai Tesque, Inc., Kyoto, Japan), and 100 nM free-Ca^{2+}, pH 7.4) at the final concentration of 10 μM and applied to the intracellular side of the patch membrane. The resistance of the patch pipette was 2–5 MΩ when filled with the pipette solution. Test voltages from −100 mV to 190 mV were applied at 10 mV steps. The BK channel conductance was determined by measuring the tail current at each test voltage, and plotted as conductance–voltage curves. $V_{1/2}$ (half-maximal activation voltage) values were calculated based on Boltzmann's fit of the conductance–voltage curves. The BK channel opening activities of tested compounds were evaluated as the hyperpolarizing shift of $V_{1/2}$.

5.2.2. Measurement of isolated detrusor smooth muscle relaxation. The experimental protocol complied with the Guidelines for Animal Experiments approved by Toho University. Urinary bladders were isolated from male Japanese white rabbits (3–4 kg, 3–4 months old) that had been euthanized with an overdose of pentobarbital. The isolated tissue was immediately immersed in Krebs-bicarbonate solution (in mM; 118 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 2.6 $CaCl_2$, 1.2 KH_2PO_4, 25 $NaHCO_3$, and 11 glucose). The longitudinal strips of detrusor smooth muscle (5–7 mm length, 2–3 mm width), after removal of mucosa by dissection, were suspended in an organ bath chamber containing 10 mL of Krebs solution, maintained at 37 °C and bubbled with 95% O_2/5% CO_2 gas. Muscle tension was increased isotonically using a force–displacement transducer (TB-612T, Nihon Koden, Tokyo, Japan) connected to a carrier amplifier (AP-601G, Nihon Koden) with a basal preload of ca. 2 gW as initial tension. The tissues were allowed to equilibrate for 1 h. The strips were precontracted with high-K^+ (30 mM or 120 mM) Krebs solution by replacement of the whole bath solution and allowed to equilibrate for 5–30 min (typically) before the addition of test compounds. When high-K^+ solutions were used to contract preparations, equimolar concentrations of Na^+ in Krebs solution were replaced with K^+ to maintain isotonicity. The relaxant activities of the test compounds were compared with that of vehicle (0.1% DMSO) measured in the same muscle strip. All values are expressed as means ± SEM. Statistical significance of differences was determined by paired Student's *t*-test, and *P* values less than 0.05 were considered to be significant.

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References and notes

1. Marty, A. *Nature* **1981**, *291*, 497–500.
2. Srinivas Ghatta, S.; Nimmagadda, D.; Xu, X.; Stephen, T.; O'Rourke, S. T. *Pharmacol. Ther.* **2006**, *110*, 103–116.
3. Jones, E. M. C.; Gray-Keller, M.; Fettiplace, R. *J. Physiol.* **1999**, *518*, 653–665.
4. Ahluwalia, J.; Tinker, A.; Clapp, L. H.; Duchen, M. R.; Abramov, A. Y.; Pope, S.; Nobles, M.; Segal, A. W. *Nature* **2004**, *427*, 853–858.
5. Kotlikoff, M.; Hall, I. *J. Clin. Invest.* **2003**, *112*, 654–656.
6. (a) Shen, K. Z.; Lagrutta, A.; Davies, N. W.; Standen, N. B.; Adelman, J. P.; North, R. A. *Pflugers Arch.* **1994**, *426*, 440–445; (b) Garcia-Calvo, M.; Knaus, H. G.; McManus, O. B.; Giangiacomo, K. M.; Kaczorowski, G. J.; Garcia, M. L. *J. Biol. Chem.* **1994**, *269*, 676–682; (c) McManus, O. B.; Helms, L. M.; Pallanck, L.; Ganetzky, B.; Swanson, R.; Leonard, R. J. *Neuron* **1995**, *14*, 645–650.
7. Wallner, M.; Meera, P.; Toro, L. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14922–14927.
8. Papazian, D. M.; Timpe, L. C.; Jan, Y. N.; Jan, L. Y. *Nature* **1991**, *349*, 305–310.
9. Brenner, R.; Jegla, T. J.; Wickenden, A.; Liu, Y.; Aldrich, R. W. *J. Biol. Chem.* **2000**, *275*, 6453–6461.
10. (a) Meredith, A. L.; Thorneloe, K. S.; Werner, M. E.; Nelson, M. T.; Aldrich, R. W. *J. Biol. Chem.* **2004**, *279*, 36746–36752; (b) Petkov, G. V.; Bonev, A. D.; Heppner, T. J.; Brenner, R.; Aldrich, R. W.; Nelson, M. T. *J. Physiol.* **2001**, *537*, 443–452; (c) Thorneloe, K. S.; Meredith, A. L.; Knorn, A. M.; Aldrich, R. W.; Nelson, M. T. *Am. J. Physiol. Renal Physiol.* **2003**, *289*, F604–F610.
11. Ruttiger, L.; Sausbier, M.; Zimmermann, U.; Winter, H.; Braig, C.; Engel, J.; Knirsch, M.; Arntz, C.; Langer, P.; Hirt, B.; Müller, M.; Köpschall, I.; Pfister, M.; Münkner, S.; Rohbock, K.; Pfaff, I.; Rüsch, A.; Ruth, P.; Knipper, M. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 12922–12927.
12. Sausbier, M.; Hu, H.; Arntz, C.; Feil, S.; Kamm, S.; Adelsberger, H.; Sausbier, U.; Sailer, C. A.; Feil, R.; Hofmann, F.; Korth, M.; Shipston, M. J.; Knaus, H.-G.; Wolfer, D. P.; Pedroarena, C. M.; Storm, J. F.; Ruth, P. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 9474–9478.
13. Sato, T.; Saito, T.; Saegusa, N.; Nakaya, H. *Circulation* **2005**, *111*, 198–203.
14. Olesen, S.-P.; Munchm, E.; Watjen, F.; Drejer, J. *Neuroreport* **1994**, *5*, 1001–1004.

15. Olesen, S. P.; Munch, E.; Moldt, P.; Drejer, J. *Eur. J. Pharmacol.* **1994**, *251*, 53–59.
16. Ottolia, M.; Toro, L. *Biophys. J.* **1994**, *67*, 2272–2279.
17. StrØbæk, D.; Christopherso, P.; Holm, N. R.; Moldt, P.; Ahring, P. K.; Johansen, T. E.; Olesen, S.-P. *Neuropharmacology* **1996**, *35*, 903–914.
18. (a) Gribkoff, V. K.; Starrrett, J. E., Jr.; Dworetzky, S. I.; Hewawasam, P.; Boissard, C. G.; Cook, D. A.; Frantz, S. W.; Heman, K.; Hibbard, J. R.; Huston, K.; Johnson, G.; Krishnan, B. S.; Kinney, G. G.; Lombardo, L. A.; Meanwell, N. A.; Molinoff, P. B.; Myers, R. A.; Moon, S. L.; Oritiz, A.; Pajor, L.; Pieschl, R. L.; Post-Munson, D. J.; Signor, L. J.; Srinivas, N.; Taber, M. T.; Thalody, G.; Trojnacki, J. T.; Wiener, H.; Yeleswaram, K.; Yeola, S. W. *Nat. Med.* **2001**, *7*, 471–477; (b) Hewawasam, P.; Erway, M.; Moon, S. L.; Knipe, J.; Weiner, H.; Boissard, C. G.; Post-Munson, D. J.; Gao, Q.; Huang, S.; Gribkoff, V. K.; Nicholas, A.; Meanwell, N. A. *J. Med. Chem.* **2002**, *45*, 1487–1499.
19. Tanaka, M.; Sasaki, Y.; Hukui, T.; Kyotani, J.; Hayashi, S.; Hamada, K.; Kimura, Y.; Ukai, Y.; Kitano, M.; Kimura, K. *BJU Int.* **2003**, *92*, 1031–1036.
20. Hu, S.; Fink, C. A.; Lappe, R. W. *Drug Dev. Res.* **1997**, *41*, 10–21, and *Drug Dev.Res.* **1997**, *41*, 109..
21. McManus, O. B.; Harris, G. H.; Giangiacomo, K. M.; Feigenbaum, P.; Reuben, J. P.; Addy, M. E.; Burka, J. F.; Kaczorowski, G. J.; Garcia, M. L. *Biochemistry* **1993**, *32*, 6128–6133.
22. (a) Singh, S. B.; Goetz, M. A.; Zink, D. L.; Dombrowski, A. W.; Polishook, J. D.; Garcia, M. L.; Schemalhofer, W.; McManus, O. B.; Kaczorrowski, G. J. *J. Chem. Soc., Perkin Trans. 1* **1994**, 3349–3352; (b) Kaczorowski, G. J.; Knaus, H. G.; Leonard, R. J.; McManus, O. B.; Garcia, M. L. *J. Bioenerg. Biomembr.* **1996**, *28*, 255–267.
23. Coghlan, M. J.; Carroll, W. A.; Gopalakrishnan, M. *J. Med. Chem.* **2001**, *44*, 1628–1653.
24. (a) Ohwada, T.; Nonomura, T.; Maki, K.; Sakamoto, K.; Ohya, S.; Muraki, K.; Imaizum, Y. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3971–3974; (b) Imaizumi, Y.; Sakamoto, K.; Yamada, A.; Hotta, A.; Ohya, S.; Muraki, K.; Uchiyama, M.; Ohwada, T. *Mol. Pharmacol.* **2002**, *62*, 836–846; (c) Sakamoto, K.; Nonomura, T.; Ohya, S.; Muraki, K.; Ohwada, T.; Imaizumi, Y. J. *Pharmacol. Exp. Ther.* **2006**, *316*, 144–153.
25. Nelson, W. L.; Miller, D. D.; Wilson, R. S. *J. Heterocycl. Chem.* **1969**, *6*, 131–133.
26. (a) Bruhova, I.; Zhorov, B. Z. *Biophys. J.* **2005**, *89*, 1020–1029; (b) Lipkind, G. M.; Fozzard, H. A. *Mol. Pharmacol.* **2005**, *68*, 1611–1622.
27. Macromolecule 8.5, 2003, Shrödinger, L.L.C., Portland, Oregon, USA.
28. (a) Seth, A.; Capello, E.; Chou, C.-L.; Longhurst, P. A. *BJU Int.* **2005**, *95*, 157–162; (b) Uchida, W.; Masuda, M.; Shirai, Y.; Shibasaki, K.; Satoh, N.; Takenaka, T. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1994**, *350*, 398–402; (c) Kobayashi, H.; Satomi Adachi-Akahane, S.; Nagao, T. *Eur. J. Pharmacol.* **2000**, *404*, 231–238.
29. (a) Mason, R. P.; Rhodes, D. G.; Herbette, L. G. *J. Med. Chem.* **1991**, *34*, 869–877; (b) Yamaguchi, S.; Zhorov, B. S.; Yoshioka, K.; Nagao, T.; Ichijo, H.; Adachi-Akahane, S. *Mol. Pharmacol.* **2003**, *64*, 235–248.