A novel series of indole alkaloid derivatives inhibit dengue and Zika virus infection by interference with the viral replication complex

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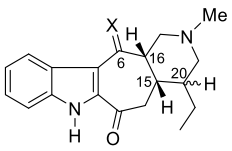
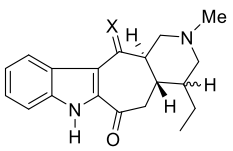
Supporting Information Available

Synthesis of compounds

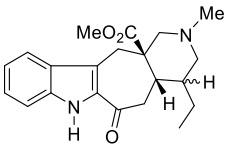
Compounds subjected to antiviral activity assays were prepared in the context of our studies in the total synthesis of Corynantean-type indole alkaloids of the ervatamine-silicine group (1), (2), (3), which are characterized by a rearranged skeleton lacking the characteristic tryptamine moiety present in most monoterpenoid indole alkaloids. Ervatamine-silicine-type alkaloids (4), (5) share a tetracyclic structure with an indole and a piperidine fused to a seven-membered carbocyclic ring, but they differ in the relative stereochemistry of C-16 and C-20 stereocenters (there are also C-20 *E*-ethylidene derivatives), the oxidation level at C-6, and the presence or absence of a C-16 methoxycarbonyl group. However, because of their common biogenetic origin from secologanin, the configuration of the C-15 stereocenter is usually *S*.



Silicine X = H, H; 20-β-H
20-Episilicine X = H, H; 20-α-H
6-Oxosilicine X = O; 20-β-H



16-Episilicine X = H, H; 20-β-H
16,20-Episilicine X = H, H; 20-α-H
6-Oxo-16-Episilicine X = O; 20-β-H
6-Oxo-16,20-Episilicine X = O; 20-α-H

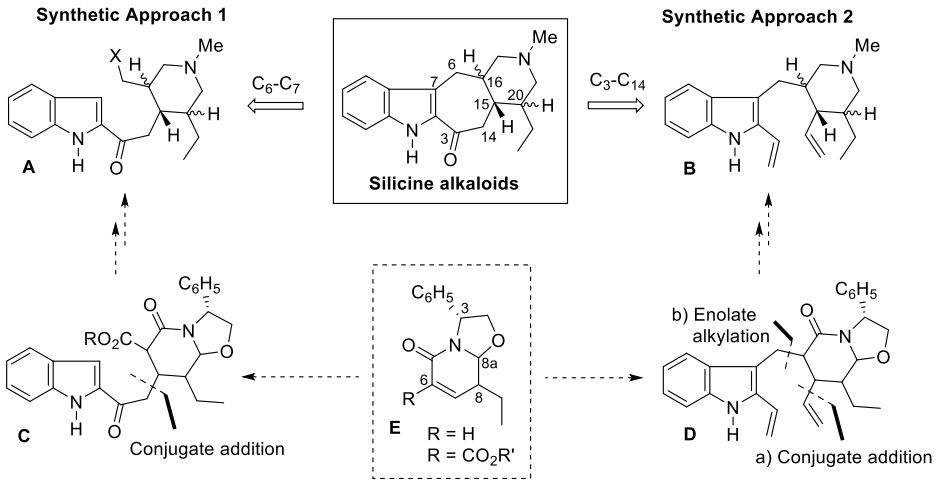


20-Epiervatamine X = H, H; 20-β-H
Ervatamine X = H, H; 20-α-H

Representative alkaloids of the ervatamine-silicine group.

Chiral oxazolopiperidone lactams, easily available in a single step by cyclocondensation of δ-oxo-acid derivatives with (*R*)- or (*S*)-phenylglycinol, bear a strategically versatile functionalized piperidine ring embedded in a conformationally rigid bicyclic system allowing the stereoselective introduction of substituents in most positions of the aza-heterocycle. In previous work, we have established a versatile

methodology for the preparation of a variety of enantiopure nitrogen heterocycles with a high degree of stereoselectivity and a predictable absolute configuration as a result of extensive studies on the scope and limitations of this procedure. The potential of this approach has been demonstrated in our research group with the synthesis of a variety of natural products, among them indole alkaloids of the ervatamine-silicine group.

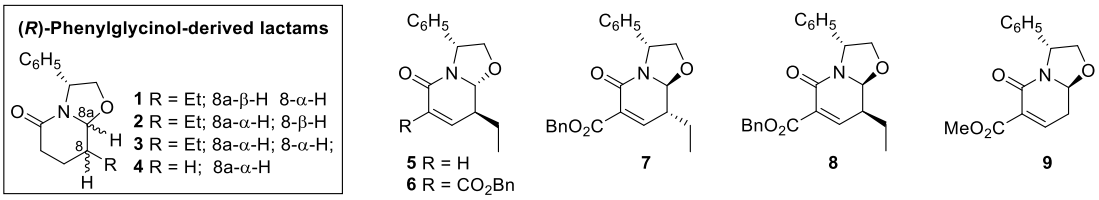


Scheme 1. Synthetic strategies.

Two strategies were devised for the construction of the tetracyclic system of silicine alkaloids from a piperidine precursor bearing substituents at the C-3, C-4, and C-5 positions (Scheme 1, **A** and **B**). In both cases the seven-membered carbocyclic ring was constructed at the last stages of the synthesis, either by intramolecular alkylation (or acylation) of the indole 3-position (Synthetic Approach 1, C_6-C_7 bond formed) from type-**A** precursors or by ring-closing metathesis (Synthetic Approach 2, C_3-C_{14} bond formed) from intermediates **B**. As can be observed in Scheme 1, the three stereogenic centers of silicine alkaloids, at C-15, C-16, and C-20, were embedded in the piperidine moiety. Unsaturated ethyl-substituted (*R*)-phenylglycinol-derived lactams of type **E**

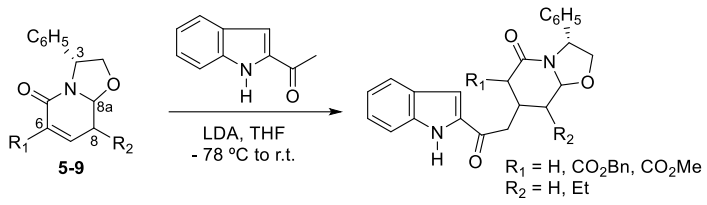
enabled the stereoselective introduction of substituents at the 4-position of the piperidine ring by conjugate addition reactions, and at the 3-position in a good degree of stereoselectivity by enolate alkylation, giving access to a broad variety of enantiopure 3,4,5-trisubstituted piperidines. Therefore, synthetic intermediates of type **C** may be accessible by conjugate addition reactions of the enolate of 2-acylindole to unsaturated lactams **E** whereas intermediates of type **D** can be prepared from **E** by conjugate addition of a vinyl residue and a subsequent alkylation to introduce a 2-vinyl-3-indolylmethyl fragment.

The required unsaturated lactams **6-8**, which differ in the configuration of the stereocenters at the C-8 and/or C-8a positions, were prepared from the corresponding (*R*)-phenylglycinol-derived bicyclic lactams (**1-3**), as previously reported. Unsaturated lactams **5** and **9**, prepared from **1** and **4**, respectively, were also included in our studies to analyze the influence of the benzyloxycarbonyl and the ethyl substituents in the bioactivity of the resulting conjugate addition products (Scheme 2).



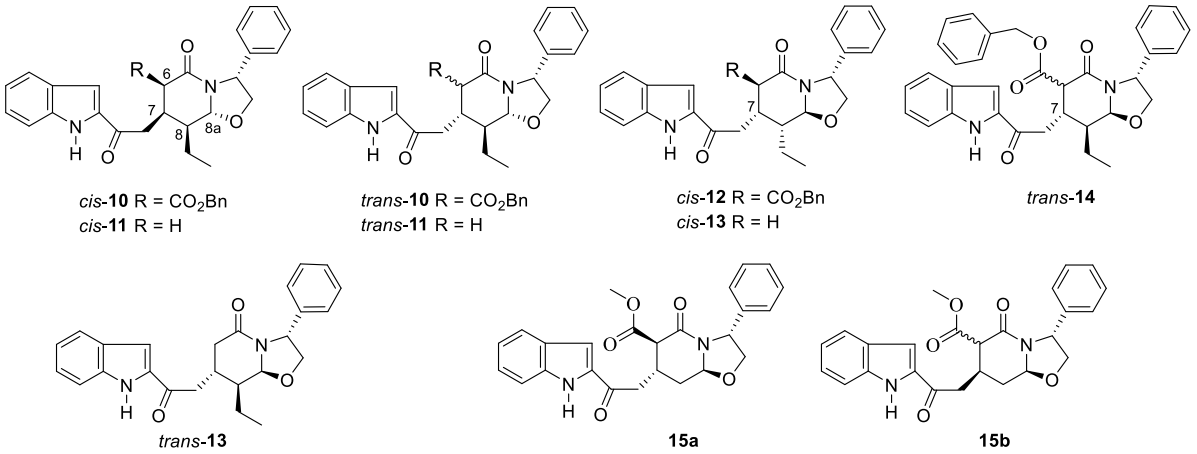
Scheme 2. Unsaturated (*R*)-phenylglycinol bicyclic lactams

Conjugate addition reactions of 2-acetylindole enolates to unsaturated lactams was carried out using an excess of 2-acetylindole (5 equivalents) using LDA as a base in THF (Scheme 3).



Scheme 3. Conjugate addition reactions of 2-acetylindole enolate to unsaturated lactams.

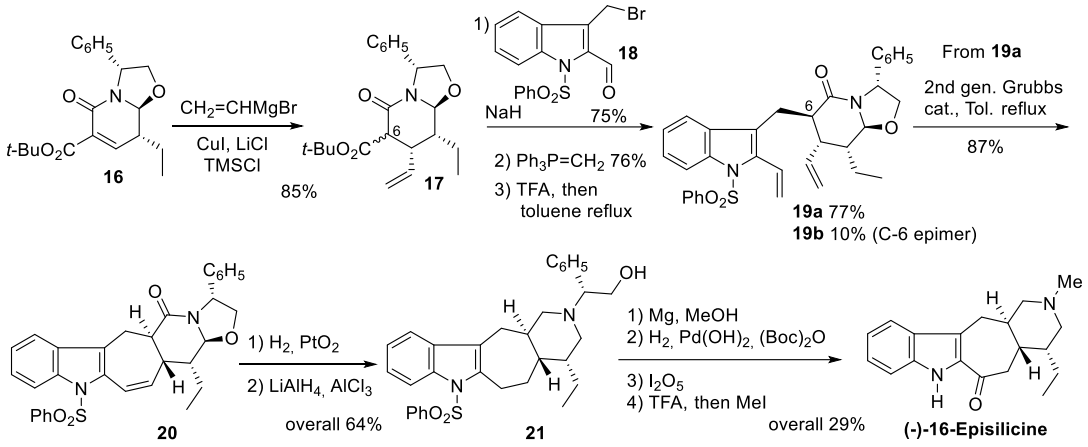
Following the above general procedure, compounds *cis*-**10** and *trans*-**10** were isolated in 90% yield from unsaturated lactam **6** in a 77:23 ratio, respectively (Scheme 4). A similar result was observed when lactam **7** was subjected to the above conditions, affording *cis*-**12** and its C-7 epimer (*trans*-**12**) in a 77:23 ratio and 62% overall yield. Compound *trans*-**14** was obtained from the H-8/H-8a *cis* unsaturated lactam **8**, along with its C-7 isomer (*cis*-**14**), in 86% overall yield and a 84:16 ratio. Oxazolopiperidones *cis*-**10**, *trans*-**10**, *cis*-**12**, and *trans*-**14** were selected to analyze the influence of the relative configuration of the stereocenters at the oxazolopiperidone C-6, C-7, C-8, and C-8a positions in the viral activity. Conjugate addition of 2-acetylindole enolate to the non-activated unsaturated lactam **5** resulted in a mixture of products *cis*-**11** and *trans*-**11** in good chemical yield (73%) but lower stereoselectivity (42:58), whereas lactam **9**, lacking the ethyl substituent, afforded a mixture of **15a** and **15b** (70:30) in 50% yield. Conjugate addition adducts *trans*-**10**, *trans*-**14**, and **15b**, with a H-7/H-8a *cis* relative configuration, were isolated as epimeric mixtures at the isomerizable stereocenter of the C-6 position. Finally, compounds *cis*-**13** and *trans*-**13** were obtained by isomerization of the C-8 and/or the C-8a stereocenters of *trans*-**11** under acidic conditions. Derivatives *cis*-**11**, *cis*-**13**, and *trans*-**13**, lacking the benzyloxycarbonyl substituent at C-6, were prepared to analyze the influence of this substituent in the bioactivity, whereas **15a** and **15b** can be considered deethyl analogues of *cis*-**12** and *trans*-**14** bearing a methoxycarbonyl instead of a benzyloxycarbonyl group.



Scheme 4. Synthetic adducts resulting from conjugate addition reactions.

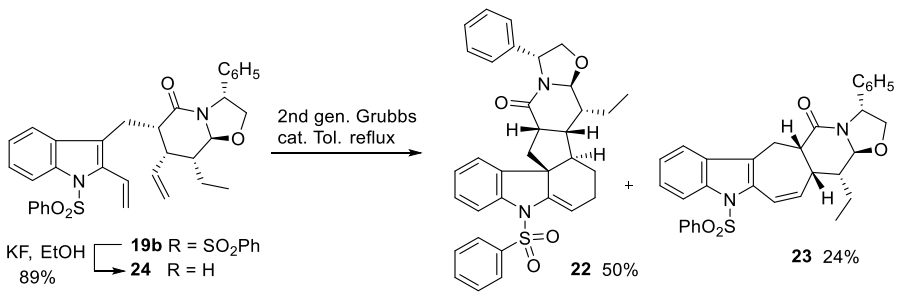
The moderate stereoselectivity observed in the above conjugate addition reactions prompted us to explore an alternative approach for the synthesis of silicine alkaloids in which the closure of the seven-membered carbocyclic ring is performed by ring-closing metathesis from an appropriate diene (Scheme 1). The overall sequence for the synthesis of (-)-16-episilicine is outlined in Scheme 5. Conjugate addition of the non-stabilized nucleophile vinylmagnesium bromide to the unsaturated lactam **16** took place in very high facial selectivity, leading to compound **17** (mixture of C-6 epimers). Preparation of the required diene **19a** was carried out by alkylation of the sodium enolate of **17** with indolylmethyl bromide **18**, followed by Wittig methylenation and removal of the *tert*-butoxycarbonyl group under acidic conditions. Compound **19a** was isolated in good overall yield (43%, three steps from **17**) along with minor amounts of the C-6 epimer **19b**. The crucial ring-closing metathesis of **19a** was performed using the second-generation Grubbs catalyst under refluxing toluene to give the expected compound **20** in 87% yield. Catalytic hydrogenation of the double bond of **20** followed by treatment with $LiAlH_4$ - $AlCl_3$, which provoked the reduction of the lactam carbonyl and the reductive opening of the oxazolidine ring, gave tetracycle **21**. Conventional synthetic transformations from **21**, including the removal of the benzenesulfonyl

protecting group, debenzylation of the nitrogen in the presence of $(Boc)_2O$, chemoselective oxidation of the methylene next to the indole 2-position, and nitrogen methylation, furnished the alkaloid (-)-16-episilicine.



Scheme 5. Enantioselective total synthesis of (-)-16-episilicine.

Surprisingly, when compound **19b**, the C-6 epimer of **19a**, was subjected to the above ring-closing metathesis conditions, the main product observed was the Diels-Alder adduct **22** (50% yield), whereas the expected *cis*-fused pentacyclic compound **23** was formed in only 24% yield (Scheme 6). The different behavior of **19a** and **19b** under the thermal conditions used in the ring-closing metathesis reaction could have a conformational origin due to their opposite configuration at the 6-position of the rigid oxazolopiperidone system. Finally, removal of the benzenesulfonyl group of **19b** with potassium fluoride afforded compound **24**.

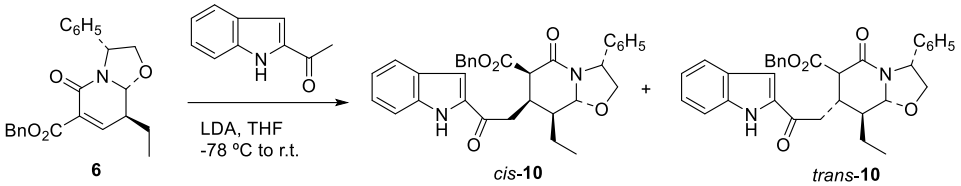


Scheme 6. RCM reaction from isomer **19b**.

Compounds **20** and **21** shared the tetracyclic system present in (-)-16-episilicine. The pentacyclic derivative **20** constituted a conformationally restricted analogue of **21** due to the lactam function and the presence of the oxazolidine ring. Product **22** displayed a unique complex structure and was also selected for biological assays.

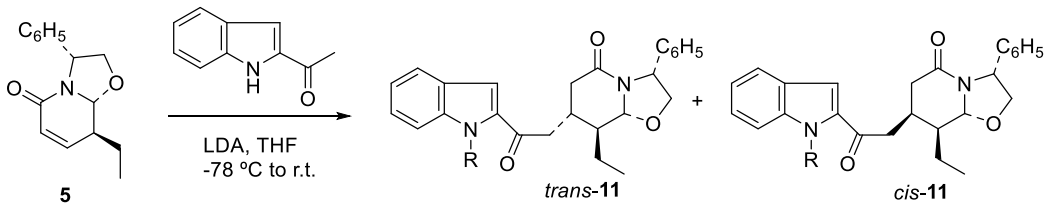
Experimental procedures and spectroscopic data

General Procedures. All air sensitive reactions were carried out under a dry argon or nitrogen atmosphere, with dry, freshly distilled solvents using standard procedures. Drying of organic extracts during the work-up of reactions was performed over anhydrous $MgSO_4$ or Na_2SO_4. Evaporation of solvent was accomplished with a rotatory evaporator. Thin-layer chromatography was done on SiO_2 (silica gel 60 F_{254}), and the spots were located by UV and either a 1% $KMnO_4$ solution or 3% ethanolic *p*-anysaldehyde. Chromatography refers to flash column chromatography and was carried out on SiO_2 (silica gel 60, 230-400 mesh). NMR spectra were recorded at 400 MHz (^1H) and 100.6 MHz (^{13}C), and chemical shifts were reported in δ values, in parts per million (ppm) relative to Me_4Si (0 ppm) as an internal standard. Data were reported in the following manner: chemical shift, multiplicity, coupling constant (*J*) in Hertz (Hz), integrated intensity, and assignment (when possible). Assignments and stereochemical determinations were deduced from definitive two-dimensional NMR experiments (*g*-HSQC-COSY). IR spectra were performed in a spectrophotometer Nicolet Avatar 320 FT-IR and only noteworthy IR absorptions (cm^{-1}) were listed. Optical rotations were measured in a Perkin-Elmer 241 polarimeter, using a Na lamp. $(\alpha)_D$ values were given in 10^{-1} deg cm^2 g^{-1}. High-resolution mass spectra (HRMS) were performed by the *Centres Científics i Tecnològics de la Universitat de Barcelona*.



(3*R*,6*R*,7*R*,8*S*,8a*R*)-6-(Benzyloxycarbonyl)-8-ethyl-7-[2-(2-indolyl)-2-oxoethyl]-5-oxo-3-phenyl-2,3,6,7,8,8a-hexahydro-5*H*-oxazolo[3,2-*a*]pyridine (*cis*-10) and its 6*S*,7*S* diastereoisomer (*trans*-10): LDA (27.4 mL of a solution 1.5 M in THF, 41.1 mmol) was slowly added to a cooled (– 78ºC) solution of 2-acetylindole (3.27 g, 20.5 mmol) in 100 mL in THF (100 mL), and the mixture was stirred at this temperature for 1 h. Then, a solution of the crude unsaturated lactam **6** (6) (1.55 g, 4.11 mmol) in THF (50 mL) was added and the resulting mixture was stirred at room temperature for 5 h. The reaction was quenched by the addition of saturated aqueous NH_4Cl, and the aqueous layer was extracted with EtOAc. The combined organic extracts were dried, filtered, and concentrated. Flash chromatography (hexane to 1:1 hexane–EtOAc) of the residue afforded *cis*-**10** (1.53 g, 69%) and *trans*-**10** (457 mg, 21%). *cis*-**10** as a yellow foam. *cis*-**10**: $[\alpha]^{22}_D$ − 87.0 (*c* 0.2, $CHCl_3$), IR (film): *v* 1655, 1735, 3320 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, COSY, HETCOR) δ 1.02 (t, *J* = 7.6 Hz, 3H, CH_3 ethyl), 1.40-1.50 (m, 1H, CH_2 ethyl), 1.83-1.94 (m, 1H, CH_2 ethyl), 2.42-2.51 (m, 1H, H-8), 2.92 (dd, *J* = 16.2, 11.1 Hz, 1H, CH_2CO), 3.10 (dd, *J* = 16.2, 2.7 Hz, 1H, CH_2CO), 3.16 (m, 1H, H-7), 3.47 (d, *J* = 1.2 Hz, 1H, H-6), 4.03 (dd, *J* = 9.0, 1.5 Hz, 1H, H-2), 4.19 (dd, *J* = 9.0, 7.2 Hz, 1H, H-2), 4.67 (d, *J* = 9.6 Hz, 1H, H-8a), 4.97 (dd, *J* = 7.2, 1.5 Hz, 1H, H-3), 5.03 (d, *J* = 16.8 Hz, 1H, C*H*$_2C_6H_5$), 5.08 (d, *J* = 16.8 Hz, 1H, C*H*$_2C_6H_5$), 7.12-7.41 (m, 14H, ArH), 7.69 (dd, *J* = 8.4, 1.2 Hz, 1H, H-4 ind), 9.22 (s, 1H, NH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 11.1 (CH_3 ethyl), 20.7 (CH_2 ethyl), 33.4 (C-7), 35.8 (*C*H_2CO), 40.1 (C-8), 53.1 (C-6), 59.5 (C-3), 66.7 (*C*$H_2C_6H_5$), 73.8 (C-2), 90.0 (C-8a), 109.3 (C-3 ind),

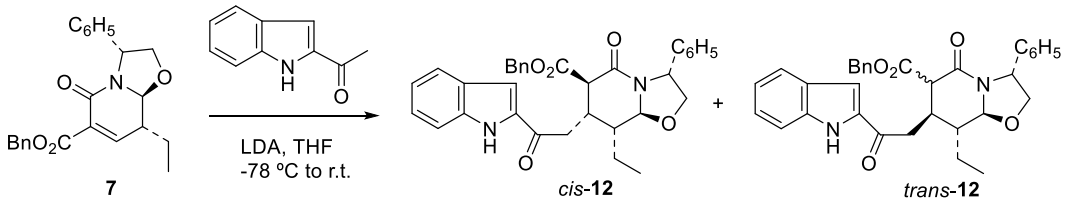
112.6 (C-7 ind), 120.6 (C-2 ind), 122.5 (C-6 ind), 126.1 (C-5 ind), 126.2 (C-4 ind), 126.9, 127.2, 127.5, 127.7, 127.9, 128.1 (C-*o*, *m*, *p*), 134.6 (C-3a ind), 135.2 (C-7a ind), 140.5 (C-*i*), 137.7 (C-*i*), 162.2 (COO), 169.2 (NCO), 189.9 (CO). Anal. Calcd. For $C_{33}H_{32}N_2O_5 \cdot 1/4$ EtOAc: C, 73.10; H, 6.13; N, 5.01. Found C, 73.08; H, 6.00; N, 4.99. *trans*-**10**: ^{1}H NMR (400 MHz, $CDCl_3$, COSY, HETCOR) δ 0.99 (t, *J* = 7.6 Hz, 3H, CH_3 ethyl), 1.71-2.04 (m, 3H, CH_2 ethyl, H-8), 2.87-3.18 (m, 3H, $2CH_2CO$, H-7), 3.56 (d, *J* = 5.6 Hz, 1H, H-6), 4.01-4.20 (m, 2H, H-2), 4.71 (d, *J* = 8.4 Hz, 1H, H-8a), 4.97 (d, *J* = 6.0 Hz, 1H, H-3), 5.09 (d, *J* = 11.6 Hz, 1H, $CH_2C_6H_5$), 5.14 (d, *J* = 11.6 Hz, 1H, $CH_2C_6H_5$), 6.95 (d, *J* = 1.6 Hz, 1H, H-3 ind), 7.12-7.41 (m, 13H, ArH), 7.65 (d, *J* = 7.6 Hz, 1H, H-4 ind), 9.09 (bs, 1H, NH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 10.4 (CH_3 ethyl), 23.1 (CH_2 ethyl), 34.9 (C-7), 41.1 (CH_2CO), 44.7 (C-8), 55.0 (C-6), 58.9 (C-3), 67.3 ($CH_2C_6H_5$), 73.8 (C-2), 90.4 (C-8a), 109.7 (C-3 ind), 112.3 (C-7 ind), 120.9 (C-2 ind), 123.0 (C-6 ind), 126.4 (C-5 ind), 126.5 (C-4 ind), 126.9-128.6 (C-*o*, *m*, *p*), 134.8 (C-3a ind), 137.5 (C-7a ind), 140.6 (C-*i*), 140.8 (C-*i*), 162.7 (COO), 169.9 (NCO), 190.0 (CO).



(3*R*,7*R*,8*S*,8a*R*)-8-Ethyl-7-[2-(2-indolyl)-2-oxoethyl]-5-oxo-3-phenyl-2,3,6,7,8,8a-hexahydro-5*H*-oxazolo[3,2-*a*]pyridine (*trans*-11) and its 7*S* epimer (*cis*-11): LDA (11 mL of a solution 1.5 M in cyclohexane, 16.5 mmol) was slowly added at – 78 ºC to a solution of 2-acetylindole (1.3 g, 8.2 mmol) in THF (50 mL), and the mixture was stirred at – 78 ºC for 1 h. Then, a solution of the unsaturated lactam **5** (7) (400 mg, 1.64 mmol) in THF (30 mL) was added and the resulting mixture was stirred at room

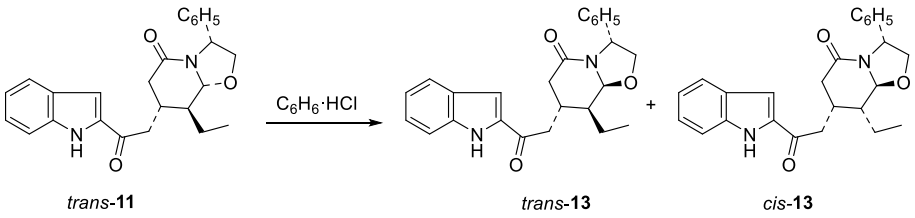
temperature for 3 h. The reaction was quenched by the addition of saturated aqueous NH_4Cl, and the aqueous layer was extracted with EtOAc. The combined organic extracts were dried, filtered, and concentrated. Flash chromatography (from 1:1 to 3:7 hexane–EtOAc) afforded *trans*-**11** (279 mg, 42 %) and *cis*-**11** (202 mg, 31 %) as a yellow foam. *trans*-**11**: $[\alpha]^{22}_D$ − 7.0 (*c* 0.5, $CHCl_3$); IR (film): *v* 1657, 1735, 3312 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, COSY, HETCOR) δ 1.04 (t, *J* = 7.2 Hz, 3H, CH_3 ethyl), 1.64-1.88 (m, 3H, CH_2 ethyl, H-8), 2.16 (dd, *J* = 16.5, 7.6 Hz, 1H, CH_2CO), 2.57 (m, 1H, H-7), 2.68 (dd, *J* = 16.5, 6.0 Hz, 1H, H-6), 2.79 (dd, *J* = 16.5, 9.2 Hz, 1H, CH_2CO), 3.00 (dd, *J* = 16.5, 4.5 Hz, 1H, H-6), 4.10 (dd, *J* = 9.0, 1.2 Hz, 1H, H-2), 4.18 (dd, *J* = 9.0, 6.6 Hz, 1H, H-2), 4.71 (d, *J* = 8.1 Hz, 1H, H-3), 4.98 (d, *J* = 5.7 Hz, 1H, H-8a), 6.87 (d, *J* = 1.5 Hz, 1H, H-3 ind), 7.04-7.08 (m, 1H, H-6 ind), 7.18-7.25 (m, 1H, H-5 ind), 7.28-7.41 (m, 7H, ArH, H-4 ind), 10.05 (s, 1H, NH); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 10.1 (CH_3 ethyl), 22.4 (CH_2 ethyl), 30.8 (C-7), 37.2 (*C*H_2CO), 42.0 (C-6), 44.6 (C-8), 58.3 (C-3), 73.8 (C-2), 90.7 (C-8a), 109.3 (C-3 ind), 112.5 (C-7 ind), 120.3 (C-4 ind), 122.6 (C-6 ind), 125.8 (C-5 ind), 126.7 (C-*o*), 127.0 (C-3a ind), 127.5 (C-*m*), 128.5 (C-*p*), 135.0 (C-7a ind), 137.7 (C-*i*), 141.3 (C-2 ind), 167.2 (NCO), 190.4 (CO); $C_{25}H_{26}N_2O_3 \cdot 1/4$ EtOAc (424.52): calcd. C 73.56, H 6.65, N 6.60; found C 73.77, H 6.63, N 6.47. *cis*-**11**: $[\alpha]^{22}_D$ − 70.4 (*c* 0.5, $CHCl_3$); IR (film) *v* 1657, 1735, 3325 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, COSY, HETCOR) δ 1.10 (t, *J* = 7.3 Hz, 3H, CH_3 ethyl), 1.45-1.54 (m, 1H, CH_2 ethyl), 1.89-2.03 (m, 2H, CH_2 ethyl, H-8), 2.45 (m, 2H, CH_2CO), 3.00 (m, 3H, 2H-6, H-7), 4.04 (dd, *J* = 9.3, 4.3 Hz, 1H, H-2), 4.18 (dd, *J* = 9.3, 6.9 Hz, 1H, H-2), 4.68 (d, *J* = 9.3 Hz, 1H, H-3), 4.94 (d, *J* = 5.7 Hz, 1H, H-8a), 6.87 (d, *J* = 1.5 Hz, 1H, H-3 ind), 7.11-7.36 (m, 9H, ArH, H ind), 7.68 (dd, *J* = 8.1, 0.9 Hz, 1H, H-4 ind), 9.50 (s, 1H, NH); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 11.3 (CH_3 ethyl), 21.2 (CH_2 ethyl), 29.0 (C-7), 36.5 (*C*H_2CO), 37.9 (C-6), 43.6 (C-8), 59.4 (C-3), 73.8 (C-2), 90.2 (C-8a), 109.4 (C-3

ind), 112.4 (C-7 ind), 120.8 (C-4 ind), 122.8 (C-6 ind), 126.2 (C-5 ind), 126.3 (C-*o*), 127.2 (C-3a ind), 127.4 (C-*m*), 128.4 (C-*p*), 135.0 (C-7a ind), 137.6 (C-*i*), 141.3 (C-2 ind), 166.8 (NCO), 190.7 (CO). Anal. Calcd. For $C_{25}H_{26}N_2O_3 \cdot 1/2\ H_2O$: C, 70.78; H, 6.56; N, 6.88. Found C, 73.40; H, 6.27; N, 6.98.



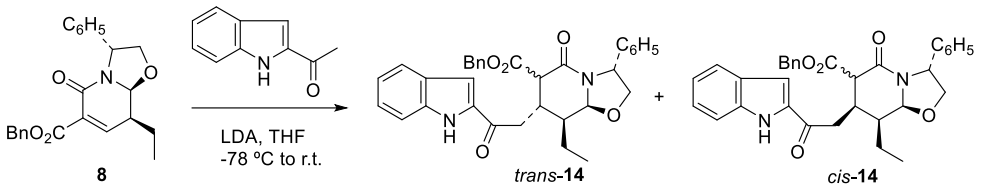
(3*R*,6*R*,7*S*,8*R*,8a*S*)-6-(Benzyloxycarbonyl)-8-ethyl-7-[2-(2-indolyl)-2-oxoethyl]-5-oxo-3-phenyl-2,3,6,7,8,8a-hexahydro-5*H*-oxazolo[3,2-*a*]pyridine (*cis*-12): LDA (2.86 mL of a solution 1.5 M in THF, 4.3 mmol) was slowly added to a cooled (-78 ºC) solution of 2-acetylindole (342 mg, 2.15 mmol) in THF (15 mL), and the mixture was stirred at this temperature for 1 h. Then, a solution of unsaturated lactam **7** (161 mg, 0.43 mmol) in THF (10 mL) was added and the resulting mixture was stirred at room temperature for 20 h. The reaction was quenched by the addition of saturated aqueous NH_4Cl, and the aqueous layer was extracted with EtOAc. The combined organic extracts were dried, filtered, and concentrated. Flash chromatography (9:1 hexane–EtOAc to EtOAc) afforded *cis*-**12** (110 mg, 48%) and *trans*-**12** (1:1 mixture of C-6 epimers; 32 mg, 14%). *cis*-**12** as a yellow foam: $[\alpha]^{22}_D$ − 101.1 (*c* 1.2, $CHCl_3$); IR (KBr): *v* 1656, 1735 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, COSY, HETCOR) δ 0.98 (t, *J* = 7.5 Hz, 3H, CH_3 ethyl), 1.61 (m, 1H, CH_2 ethyl), 1.76 (m, 1H, CH_2 ethyl), 1.91 (m, 1H, H-8), 2.91 (m, 1H, H-7), 3.12 (dd, *J* = 5.4, 2.4 Hz, 2H, CH_2CO), 3.67 (d, *J* = 9.3 Hz, 1H, H-6), 3.73 (dd, *J* = 9.0, 7.8 Hz, 1H, H-2), 4.49 (dd, *J* = 9.0, 7.8 Hz, 1H, H-2), 4.93 (d, *J* =

8.4 Hz, 1H, H-8a), 5.04 (d, *J* = 12.3 Hz, 1H, C*H*$_2$C$_6$H$_5$), 5.13 (d, *J* = 12.3 Hz, 1H, C*H*$_2$C$_6$H$_5$), 5.29 (t, *J* = 7.8 Hz, 1H, H-3), 7.12-7.37 (m, 14H, ArH, H-ind), 7.67 (d, *J* = 8.1 Hz, 1H, H-4 ind), 9.21 (bs, 1H, NH); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 9.9 (CH_3 ethyl), 21.5 (CH_2 ethyl), 33.4 (C-7), 38.6 (*C*H$_2$CO), 40.3 (C-8), 55.4 (C-6), 58.7 (C-3), 67.4 (*C*H$_2$C$_6$H$_5$), 72.6 (C-2), 90.6 (C-8a), 109.4 (C-3 ind), 112.3 (CHind), 121.0 (CHind), 123.1 (CHind), 125.7 (CHAr), 126.5 (CHAr), 127.4 (C-*i*), 127.6 (CHAr), 128.0 (CHAr), 128.2 (CHAr), 128.4 (CHAr), 128.8 (CHAr), 134.8 (C-*i*), 135.2 (C-2 ind), 137.4 (C-3a ind), 138.6 (C-7a), 164.1 (NCO), 170.1 (COO), 190.0 (CO). Anal. Calcd. For $C_{33}H_{32}N_2O_5$: C, 73.86; H, 6.01; N, 5.22. Found C, 73.46; H, 6.08; N, 5.34.



(3*R*,7*R*,8*S*,8a*S*)-8-Ethyl-7-[2-(2-indolyl)-2-oxoethyl)]-5-oxo-3-phenyl-2,3,6,7,8,8a-hexahydro-5*H*-oxazolo[3,2-*a*]pyridine (*trans*-13) and (3*R*,7*R*,8*R*,8a*S*)-8-Ethyl-7-[2-(2-indolyl)-2-oxoethyl)]-5-oxo-3-phenyl-2,3,6,7,8,8a-hexahydro-5*H*-oxazolo[3,2-*a*]pyridine (*cis*-13): A solution of pure *trans*-**11** (70 mg, 0,17 mmol) in benzen-·HCl 1 M (7 ml) was stirred at reflux temperature for 44 h. The resulting acidic solution was neutralized with saturated aqueous solution of $NaHCO_3$ (15 mL). The organic phase was separated, and the aqueous layer was extracted with EtOAc. The combined organic solutions were dried and concentrated, and the residue was chromatographed (hexane to 9:1 hexane:EtOAc) to give *cis*-**13** (29 mg, 41%) and *trans*-**13** (19 mg, 27%) as a yellow foam. *cis*-**13**: $[\alpha]^{22}_D$ − 74.7 (*c* 0.67, $CHCl_3$); IR (KBr): v 1648 cm^{-1}; ^{1}H NMR

(400 MHz, $CDCl_3$, COSY, HETCOR) δ 1.08 (t, *J* = 7.6 Hz, 3H, CH_3 ethyl), 1.45-1.54 (m, 1H, CH_2 ethyl), 1.75-1.90 (m, 2H, CH_2 ethyl, H-8), 2.54-2.66 (m, 2H, H-6, H-7), 2.90 (m, 1H, H-6), 2.97 (d, *J* = 11.8 Hz, 1H, CH_2CO), 3.08 (d, *J* = 11.8 Hz, 1H, CH_2CO), 3.80 (t, *J* = 8.4 Hz, 1H, H-2), 4.56 (t, *J* = 8.4 Hz, 1H, H-2), 4.78 (d, *J* = 9.2 Hz, 1H, H-8a), 5.35 (t, *J* = 8.0 Hz, 1H, H-3), 7.12-7.41 (m, 9H, ArH, H ind), 7.66 (d, *J* = 8.4 Hz, 1H, H-ind), 9.26 (bs, 1H, NH); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 11.5 (CH_3 ethyl), 22.1 (CH_2 ethyl), 29.0 (C-7), 36.1 (*C*H_2CO), 37.4 (C-6), 44.0 (C-8), 58.3 (C-3), 72.4 (C-2), 90.9 (C-8a), 109.4 (C-3 ind), 112.3 (C-7 ind), 121.1 (C-4 ind), 123.0 (C-5 ind), 125.9 (C-*o*), 126.7 (C-6 ind), 127.4 (C-2 ind), 127.8 (C-*m*), 129.0 (C-*p*), 135.0 (C-*i*), 137.5 (C-3a), 139.7 (C-7a), 168.3 (NCO), 190.9 (CO); HRMS calcd. for $C_{25}H_{27}N_2O_3$ $[M + H]^+$: 403.2016; found: 403.2018. Anal. Calcd. For $C_{25}H_{26}N_2O_3 \cdot 1/2\ H_2O$: C, 72.97; H, 6.61; N, 6.81. Found C, 72.94; H, 6.61; N, 6.45. *trans*-**13**: $[\alpha]^{22}_D$ − 21.6 (*c* 0.5, $CHCl_3$); IR (KBr): 1649, 3312 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, COSY, HETCOR) δ 1.01 (t, *J* = 7.4 Hz, 3H, CH_3 ethyl), 1.21-1.28 (m, 1H, CH_2 ethyl), 1.71-1.79 (m, 1H, CH_2 ethyl), 2.18 (ddd, *J* = 14.0, 6.0, 4.8 Hz, 1H, H-8), 2.36 (dd, *J* = 18.4, 3.2 Hz, 1H, CH_2CO), 2.63 (dd, *J* = 18.4, 6.4 Hz, 1H, CH_2CO), 2.84 (m, 1H, H-7), 3.09-3.11 (m, 2H, H-6), 3.79 (dd, *J* = 8.8, 7.6 Hz, 1H, H-2), 4.48 (t, *J* = 8.8 Hz, 1H, H-2), 5.23 (d, *J* = 4.4 Hz, 1H, H-8a), 5.30 (t, *J* = 7.6 Hz, 1H, H-3), 7.14-7.41 (m, 9H, ArH), 7.69 (d, *J* = 8.0 Hz, 1H, H-4 ind), 9.35 (bs, 1H, NH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 11.6 (CH_3 ethyl), 18.7 (CH_2 ethyl), 29.6 (C-7), 33.7 (*C*H_2CO), 40.7 (C-8), 41.9 (C-6), 58.3 (C-3), 72.2 (C-2), 88.0 (C-8a), 109.5 (C-3 ind), 112.3 (C-7 ind), 121.1 (C-5 ind), 123.1 (C-6 ind), 126.0 (C-*o*), 126.7 (C-2 ind), 127.7 (C-*m*), 128.9 (C-*p*), 134.9 (C-3a ind), 137.5 (C-7a ind), 139.6 (C-*i*), 168.1 (NCO), 190.7 (CO). HRMS cald. for $C_{25}H_{27}N_2O_3$ $[M + H]^+$: 403.2016; found: 403.2014.



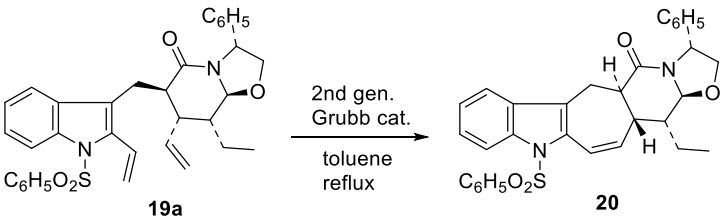
[3*R*,6*R*(and 6*S*),7*S*,8*S*,8a*S*]-6-(Benzyloxycarbonyl)-8-ethyl-7-[2-(2-indolyl)-2-oxoethyl]-5-oxo-3-phenyl-2,3,6,7,8,8a-hexahydro-5*H*-oxazolo[3,2-*a*]pyridine (*trans*-14): LDA (26.5 mL of a solution 1 M in THF, 26.5 mmol) was slowly added to a cooled (-78 ºC) solution of 2-acetylindole (2.11 g, 13.25 mmol) in THF (100 mL), and the mixture was stirred at this temperature for 1 h. Then, a solution of unsaturated lactam **8** (1 g, 2.65 mmol) in THF (50 mL) was added and the resulting mixture was stirred at room temperature for 5 h. The reaction was quenched by the addition of saturated aqueous NH_4Cl, and the aqueous layer was extracted with EtOAc. The combined organic extracts were dried, filtered, and concentrated. Flash chromatography (9:1 hexane–EtOAc to EtOAc) afforded *trans*-**14** as a yellow foam (9:1 mixture of C-6 epimers; 1.02 g, 72%) and *cis*-**14** (mixture of C-6 epimers; 211 mg, 14%). *trans*-**14** (major 6*S*-epimer): IR (film) ν 1655, 1736, 3324 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, COSY, HETCOR) δ 0.95 (t, *J* = 7.2 Hz, 3H, CH_3 ethyl), 1.26-1.41 (m, 1H, CH_2 ethyl), 1.64-1.71 (m, 1H, CH_2 ethyl), 2.23-2.31 (m, 1H, H-8), 2.98-3.04 (m, 1H, H-7), 3.09 (dd, *J* = 12.4, 6.0 Hz, 1H, CH_2CO), 3.13 (dd, *J* = 12.4, 6.4 Hz, 1H, CH_2CO), 3.66 (d, *J* = 8.8 Hz, 1H, H-6), 3.86 (dd, *J* = 8.8, 6.8 Hz, 1H, H-2), 4.45 (dd, *J* = 8.8, 8.0 Hz, 1H, H-2), 5.09 (d, *J* = 9.2 Hz, 1H, H-8a), 5.16 (d, *J* = 12.5 Hz, 1H, $CH_2C_6H_5$), 5.19 (d, *J* = 12.5 Hz, 1H, $CH_2C_6H_5$), 5.33 (t, *J* = 7.6 Hz, 1H, H-3), 7.10 (d, *J* = 1.4 Hz, 1H, H-3 ind), 7.15-7.42 (m, 13H, ArH, H-ind), 7.69 (t, *J* = 8.0 Hz, 1H, H-ind), 9.09 (bs, 1H, NH); ^{13}C NMR (100.6 MHz, $CDCl_3$,) δ 11.3 (CH_3 ethyl), 19.9 (CH_2 ethyl), 33.1 (C-7), 40.5 (CH_2CO), 42.1 (C-8), 53.8 (C-6), 58.8 (C-3), 67.2 ($CH_2C_6H_5$), 72.3 (C-2), 88.0 (C-

8a), 109.5 (C-3 ind), 112.2 (C-7 ind), 121.1 (C-4 ind), 123.2 (C-5 ind), 125.6 (C-6 ind), 126.3, 126.7, 127.8, 128.1, 128.3, 128.4, 128.8, (C-*o*, C-*m*, C-*p*, C-3a ind), 134.7 (C-2 ind), 135.5 (C-7a ind), 139.1 (C-*i*), 166.0 (NCO), 169.6 (COO), 190.3 (CO); HRMS calcd. for $C_{33}H_{32}N_2NaO_5$ $[M + Na]^+$: 559.2203; found 559.2207. *trans*-**14** (minor 6*R*-epimer): ^{1}H NMR (400 MHz, $CDCl_3$, COSY, HETCOR) δ 1.00 (t, *J* = 7.4 Hz, 3H, CH_3 ethyl), 1.30-1.42 (m, 2H, CH_2 ethyl), 3.15-3.20 (m, 2H, H-7, H-8), 3.25 (dd, *J* = 16.0, 7.2 Hz, 1H, CH_2CO), 3.30 (dd, *J* = 16.0, 5.6 Hz, 1H, CH_2CO), 3.74 (dd, *J* = 8.7, 7.6 Hz, 1H, H-2), 3.78 (d, *J* = 5.6 Hz, 1H, H-6), 4.52 (t, *J* = 8.4 Hz, 1H, H-2), 5.07 (d, *J* = 12.4 Hz, 1H, $CH_2C_6H_5$), 5.13 (d, *J* = 12.4 Hz, 1H, $CH_2C_6H_5$), 5.16 (masked, 1H, H-8a), 5.38 (t, *J* = 7.6 Hz, 1H, H-3), 7.00 (d, *J* = 1.4 Hz, 1H, H-3 ind), 7.10-7.42 (m, 13H, ArH, H-ind), 7.68 (t, *J* = 8.6 Hz, 1H, H-ind), 9.07 (bs, 1H, NH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 11.6 (CH_3 ethyl), 19.5 (CH_2 ethyl), 32.0 (C-7), 38.4 (CH_2CO), 39.6 (C-8), 50.5 (C-6), 58.1 (C-3), 67.3 ($CH_2C_6H_5$), 71.9 (C-2), 87.6 (C-8a), 109.5 (C-3 ind), 112.2 (C-7 ind), 121.1 (C-4 ind), 123.2 (C-5 ind), 125.6 (C-6 ind), 126.6, 127.5, 128.4, 128.5, 128.6, 128.8, 128.9 (C-*o*, C-*m*, C-*p*, C-3a ind), 135.1 (C-2 ind), 134.8 (C-7a ind), 137.3 (C-*i*), 164.8, (NCO), 169.2 (COO), 190.1.

3*R*,6*R*,7*R*,8a*S*)- and (3*R*,7*S*,8a*S*)-7-(2-(2-Indolyl)-2-oxoethyl)-6-(methoxycarbonyl)-5-oxo-3-phenyl-2,3,6,7,8,8a-hexahydro-5*H*-oxazolo(3,2-*a*)pyridine (15a and 15b): LDA (4.05 mL of a 2 M solution THF, 8.1 mmol) was slowly added at –78ºC to a solution of 2-acetylindole (1.29 g, 8.1 mmol) in THF (20 mL), and the mixture was stirred at –78ºC for 1 h. Then, a solution of unsaturated lactam **9** (444 mg, 1.62 mmol) in THF (8 mL) was added and the resulting mixture was stirred at –

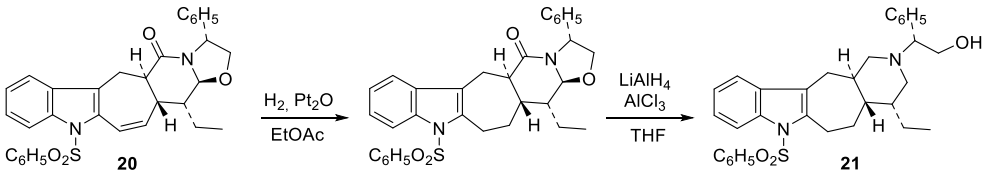
78ºC for 5 h and at room temperature for 12 h. The reaction was quenched by the addition of saturated aqueous NH_4Cl, and the aqueous layer was extracted with EtOAc. The combined organic extracts were dried, filtered, and concentrated. Flash chromatography (9:1 to 1:1 hexane-EtOAc) of the residue afforded **15a** as a mixture of C-6 epimers (105 mg, 15%) and **15b** (246 mg, 35%). **15a**: IR (film): ν = 1658 (NCO), 1739 (CO and COO), 3323 (NH) cm^{-1}; ^1H NMR ($CDCl_3$, COSY, *g*-HSQC) δ 2.39-2.46 (m, 2H, H-8), 2.83-2.91 (m, 2H, CH_2CO), 3.30 (m, 1H, H-7), 3.47 (s, 3H, OCH_3), 3.72 (d, *J* = 12.8 Hz, 1H, H-6), 3.88 (dd, *J* = 8.8, 6.8 Hz, 1H, H-2), 4.50 (m, 1H, H-2), 5.17 (dd, *J* = 5.2, 2.8 Hz, 1H, H-8a), 5.51 (t, *J* = 7.6 Hz, H-3); 7.20-7.37 (m, 9H, ArH, H-ind), 7.74 (d, *J* = 8.4 Hz, 1H, H-ind), 9.13 (bs, 1H, NH); ^{13}C RMN ($CDCl_3$, 100,6 MHz): δ 28.8 (C-7), 30.1 (C-8), 48.9 (*C*H_2CO), 52.1 (C-6), 53.2 (OCH_3), 58.8 (C-3), 71.5 (C-2), 86.3 (C-8a), 112.5 (C-3 ind), 120.9 (C-7 ind), 121.7 (C-4 ind), 125.7 (C-5 ind), 126.3 (C-*o*), 126.4 (C-6 ind), 127.6 (C-2 ind), 127.8 (C-*m*), 128.8 (C-*p*), 134.7 (C-*i*), 136.0 (C-3a), 139.3 (C-7a), 169.4 (NCO), 190.6 (CO). HRMS calcd. for $C_{25}H_{25}N_2O_5$ $(M + H)^+$: 433.1758; found: 433.1759. **15b**: **IR** (film): ν = 1655 (NCO), 1736 (CO and COO), 3323 (NH) cm^{-1}; ^1H NMR (from a mixture of C-6 epimers, $CDCl_3$, COSY, *g*-HSQC) δ 2.19-2.26 (m, 2H, H-8), 2.30-2.36 (m, 2H, H-8), 2.99 (dd, *J* = 14.8, 8.4 Hz, 2H, CH_2CO), 3.01 (m, 1H, H-7), 3.20 (dd, *J* = 14.8, 5.6 Hz, 2H, CH_2CO), 3.30 (m, 1H, H-7), 3.46 (d, *J* = 6.8 Hz, 1H, H-6), 3.73-3.75 (m, 2H, H-2, H-6), 3.74 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.83 (m, 1H, H-2), 4.50-4.54 (m, 2H, H-2), 5.10 (m, 2H, H-8a), 5.39 (m, 2H, H-3), 7.16-7.44 (m, 18H, ArH, H-ind), 7.72 (d, *J* = 8.4 Hz, 2H, H-ind), 9.13 (bs, 2H, NH); ^{13}C RMN ($CDCl_3$): δ 28.6 and 29.6 (C-7), 29.9 and 30.1 (C-8), 39.8 and 41.4 (*C*H_2CO), 52.5 and 52.7 (C-6), 53.6 (OCH_3), 58.0 and 58.6 (C-3), 71.7 and 71.8 (C-2), 85.9 (C-8a), 109.6 and 109.9 (C-3 ind), 112.2 (C-7 ind), 121.2 and 121.3 (C-4 ind), 123.1 and 123.2 (C-5 ind), 125.6 and 126.8 (C-*o*), 126.3 and 126.7 (C-6 ind), 127.4 and 127.6

(C-2 ind), 127.8 and 128.9 (C-*m*), 129.0 (C-*p*), 134.6 (C-*i*), 137.5 (C-3a), 139.2 and 139.4 (C-7a), 169.2 and 169.9 (NCO), 190.9 and 191.0 (CO). HRMS calcd. for $C_{25}H_{25}N_2O_5$ $(M + H)^+$: 433.1758; found: 433.1755.



(1*R*,3a*S*,4*R*,4a*S*,12a*R*)-7-(Benzenesulfonyl)-4-ethyl-13-oxo-1-phenyl-1,2,3a,4,4a,12,12a,13-octahydrooxazolo[2'',3'':6',1']pyrido[3',4':4,5]cyclohepta[1,2-*b*]indole (20). Second-generation Grubbs catalyst (263 mg) was added to a solution of vinylindole **19[a]** (8) (1.31 g, 2.32 mmol) in anhydrous toluene (300 mL) under argon atmosphere. The mixture was stirred at reflux for 5 days and concentrated. The resulting residue was purified by flash column chormatography (4:1 to 1:1 hexane-EtOAc) to yield pentacyclic lactam **20** (1.09 g, 87%): $[\square]^{22}_D$ –244.0 (*c* 0.3, $CHCl_3$); IR (KBr) 1656 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, COSY, HETCOR) □ 0.99 (t, *J* = 7.4 Hz, 3H, CH_3 ethyl), 1.53-1.64 (m, 2H, CH_2 ethyl), 1.89-1.95 (m, 1H, H-4), 2.62 (dt, *J* = 10.0, 3.6 Hz, 1H, H-12a), 2.77 (ddd, *J* = 10.0, 5.4, 2.1 Hz, 1H, H-4a), 2.87 (dd, *J* = 16.8, 10.0 Hz, 1H, H-12), 3.46 (dd, *J* = 16.8, 3.6 Hz, 1H, H-12), 3.84 (dd, *J* = 9.0, 6.6 Hz, 1H, H-2), 4.40 (dd, *J* = 9.0, 7.8 Hz, 1H, H-2), 4.92 (d, *J* = 4.8 Hz, 1H, H-3a), 5.31 (t, *J* = 6.9 Hz, 1H, H-1), 6.10 (dd, *J* = 11.7, 5.1 Hz, 1H, H-5), 7.23-7.50 (m, 13H, ArH, H-6, H-9, H-10), 7.66 (dd, *J* = 8.4, 1.2 Hz, 1H, H-8), 8.24 (d, *J* = 8.1 Hz, 1H, H-11); ^{13}C NMR ($CDCl_3$, 100.6 MHz) □□12.1 (CH_3 ethyl), 19.9 (CH_2 ethyl), 24.3 (C-12), 39.8 (C-4a), 43.8 (C-4), 44.3 (C-12a), 58.6 (C-1), 71.8 (C-2), 90.2 (C-3a), 115.5 (C-8), 118.9 (C-9), 121.1 (C-6a), 123.8 (C-11), 124.1 (C-10), 125.5 (C-11a), 126.1 (C-11b), 126.4 (C-*o*), 127.6 (C-*m*), 128.7

(C-*p*), 128.8 (C-*o*), 130.8 (C-6), 132.6 (C-5), 132.8 (C-*m*), 133.5 (C-*p*), 136.7 (C-*i*), 137.9 (C-7a), 139.4 (C-*i*), 170.8 (NCO); mp 161–163ºC (MeOH). Anal. Calcd. For $C_{32}H_{30}N_2O_4S{\cdot}1/2H_2O$: C, 70.57; H, 5.67; N, 5.14; S, 5.89. Found C, 70.53; H, 5.74; N, 5.09; S, 5.89.

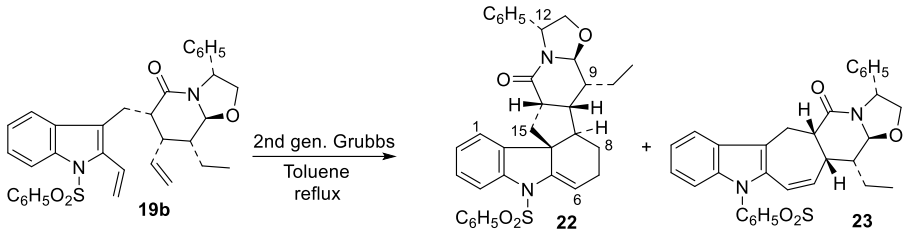


(4*R*,4a*S*,12a*R*)-7-Benzenesulfonyl-4-ethyl-2-[(1*R*)-2-hydroxy-1-phenylethyl]-1,3,4,4a,5,6,12,12a-octahydropyrido[3',4':4,5]cyclohepta[1,2-*b*]indole (21). A suspension of compound **20** (500 mg, 0.93 mmol) in EtOAc (50 mL) and 20% PtO_2 (100 mg) was hydrogenated at room temperature and atmospheric pressure for 24 h. The catalyst was removed by filtration, the solvent was evaporated, and the resulting residue was chromatographed (9:1 hexane-EtOAc) to afford the saturated pentacycle (360 mg, 72%): $[\square]^{22}_D$ – 22.5 (*c* 1.0, $CHCl_3$); IR (KBr) 1662 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, COSY, HETCOR) □ 1.00 (3H, t, *J* 7.6 Hz, CH_3 ethyl), 1.43-1.62 (2H, m, CH_2 ethyl), 1.75 (1H, ddd, *J* 18.0, 9.2 and 3.6 Hz, H-5), 1.87-1.95 (2H, m, H-4, H-5), 2.08-2.16 (1H, m, H-4a), 2.57 (1H, ddd, *J* 11.2, 8.4 and 4.0 Hz, H-12a), 2.87 (1H, dd, *J* 16.0 and 8.4 Hz, H-12), 3.21 (1H, ddd, *J* 17.2, 9.2 and 4.4 Hz, H-6), 3.41-3.50 (1H, m, H-6), 3.43 (1H, dd, *J* 16.0 and 4.0 Hz, H-12), 3.78 (1H, dd, *J* 9.2 and 7.4 Hz, H-2), 4.38 (1H, t, *J* 8.4 Hz, H-2), 4.74 (1H, d, *J* 2.8 Hz, H-3a), 5.36 (1H, t, *J* 7.4 Hz, H-3), 7.23-7.55 (11H, m, H-8, H-9, H-10 and ArH), 7.70 (2H, dd, *J* 8.8 and 1.2 Hz, ArH), 8.19 (1H, dd, *J* 6.4 and 1.6 Hz, H-11); ^{13}C NMR ($CDCl_3$, 100.6 MHz) □□12.6 (CH_3 ethyl), 19.6 (CH_2 ethyl), 22.2 (C-12), 25.3 (C-6), 28.1 (C-5), 37.6 (C-4a), 41.2 (C-12a), 44.6 (C-4),

58.5 (C-1), 71.4 (C-2), 89.8 (C-3a), 114.8 (C-8), 118.5 (C-9), 119.5 (C-6a), 123.6 (C-10), 124.2 (C-11), 126.0 (C-*o*), 126.2 (C-*m*), 127.6 (C-*p*), 128.8 (C-*o*), 129.2 (C-*m*), 130.7 (C-11b), 133.5 (C-*p*), 136.4 (C-11a), 136.6 (C-7a), 139.2 (C-*ipso*), 139.9 (C-*ipso*), 172.1 (NCO); HMRS calcd for [$C_{32}H_{32}N_2O_4S$ + H]: 541.2155, found: 541.2160. Anal. Calcd. For $C_{32}H_{32}N_2O_4S \cdot 1/3CHCl_3$: C, 66.94; H, 5.62; N, 4.83; S, 5.53. Found C, 66.57; H, 5.81; N, 4.58; S, 5.13.

$LiAlH_4$ (436 mg, 11.5 mmol) was slowly added to a suspension of $AlCl_3$ (498 mg, 3.73 mmol) in THF (50 mL) at 0 ºC. After the mixture was stirred at 25 ºC for 30 min and cooled to –78 ºC, a solution of dihydro compound prepared above (940 mg, 1.74 mmol) in anhydrous THF (10 mL) was slowly added. The stirring was continued at –78 ºC for 10 min and at 0 ºC for 1 h 30 min. The reaction was quenched with water. The aqueous layer was extracted with EtOAc, and the combined organic extracts were dried and concentrated to give a foam, which was chromatographed (9:1 hexane–EtOAc to 4:1 hexane–EtOAc) to afford compound **21** (805 mg, 88% yield): $[\square]^{22}_D$ +73.2 (*c* 0.37, $CHCl_3$); IR (KBr) 3441 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, COSY, HETCOR) □□0.90 (t, *J* = 7.2 Hz, 3H, CH_3 ethyl), 1.17-1.54 (m, 7H, CH_2 ethyl, H-4, H-4a, H-5, H-12a), 2.10 (dd, *J* = 15.2, 10.8 Hz, 1H, H-3), 2.28 (d, *J* = 11.6 Hz, H-12), 2.57 (dd, *J* = 15.2, 1.6 Hz, 1H, H-3), 2.66 (dd, *J* = 15.2, 10.8 Hz, 1H, H-6), 2.85 (d, *J* = 8.8 Hz, 1H, H-1), 2.95 (d, *J* = 11.6 Hz, 1H, H-12), 3.62-3.77 (m, 3H, H-6, NCH, CH_2O), 4.01-4.09 (m, 1H, CH_2O), 7.19-7.51 (m, 11H, ArH, H-8, H-9, H-10), 7.66 (dd, *J* = 8.4, 0.8 Hz, 2H, ArH), 8.20-8.23 (m, 1H, H-11); ^{13}C NMR ($CDCl_3$, 100.6 MHz) □□12.7 (CH_3 ethyl), 18.8 (CH_2 ethyl), 25.4 (C-12), 28.8 (C-6), 31.5 (C-5), 35.3 (C-4a), 43.7 (C-12a), 49.7 (C-4), 53.4 (C-3), 55.6 (C-1), 60.2 (CH_2O), 70.0 (NCH), 115.3 (C-8), 117.7 (C-9), 121.1 (C-6a), 123.4 (C-10), 123.9 (C-11), 126.2 (C-*o*), 127.9 (C-*p*), 128.2 (C-*m*), 128.9 (C-*o*), 129.1 (C-*m*), 130.4 (C-11b), 133.5 (C-*p*), 135.2 (C-11a), 136.3 (C-7a), 139.3 (C-*i*), 139.6 (C-*i*);

HRMS calcd for [$C_{32}H_{36}N_2O_3S$ + H]: 529.2519, found: 529.2526. Anal. Calcd. For $C_{32}H_{36}N_2O_3S$: C, 72.70; H, 6.86; N, 5.30. Found C, 72.62; H, 7.12; N, 5.01.



RCM reaction from 19b.

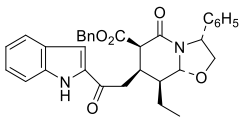
Second–generation Grubbs catalyst (480 mg) was added to a solution of diene **19b**[4b] (1.08 g, 1.91 mmol) in toluene (500 mL) and the resulting mixture was heated at reflux for 16 days, with an additional 30 mg of catalyst being added each 24h. The solvent was evaporated and the resulting residue was chromatographed (9:1 hexane–EtOAc to 4:1 hexane–EtOAc) to yield pentacycle **23** (240 mg, 24% yield) and hexacycle **22** (540 mg, 50% yield). **23:** [□]$^{22}_{D}$ –252.0 (*c* 0.3, $CHCl_3$); IR (film) 1655 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, COSY, HETCOR) □ 0.97 (t, *J* = 7.4 Hz, 3H, CH_3 ethyl), 1.46-1.73 (m, 2H, CH_2 ethyl), 1.89 (ddd, *J* = 14.0, 7.6, 5.2 Hz, 1H, H-4), 2.89-2.95 (m, 1H, H-4a), 2.92 (dd, *J* = 14.4, 10.4 Hz, 1H, H-12), 3.18 (dd, *J* = 14.4, 5.2 Hz, 1H, H-12), 3.31 (dt, *J* = 7.2, 5.2 Hz, 1H, H-12a), 3.63 (dd, *J* = 8.8, 8.4 Hz, 1H, H-2), 4.50 (dd, *J* = 8.8, 8.4 Hz, 1H, H-2), 4.78 (d, *J* = 9.2 Hz, 1H, H-3a), 5.20 (t, *J* = 8.0 Hz, 1H, H-1), 6.21 (dd, *J* = 11.6, 5.2 Hz, 1H, H-5), 6.88-7.79 (m, 14H, ArH, H-6, H-8, H-9, H-10), 8.20 (d, *J* = 8.4 Hz, 1H, H-11); ^{13}C NMR ($CDCl_3$, 100.6 MHz) □□11.4 (CH_3 ethyl), 22.1 (CH_2 ethyl), 25.4 (C-12), 38.6 (C-4a), 43.6 (C-4), 54.2 (C-12a), 58.3 (C-1), 72.6 (C-2), 90.7 (C-3a), 114.6 (C-8), 119.1 (C-9), 123.0 (C-6a), 124.0 (C-11), 124.2 (C-10), 125.2 (C-*o*), 125.3 (C-*p*), 126.5 (C-*m*), 127.0 (C-11a), 127.3 (C-11b), 128.7 (C-*o*), 129.2 (C-*m*), 130.1 (C-

p), 130.6 (C-6), 133.6 (C-5), 136.2 (C-7a ind), 138.7 (C-*i*), 138.8 (C-*i*), 169.9 (NCO); HRMS calcd for [$C_{32}H_{30}N_2O_4S$ + H]: 539.1999, found: 539.1997. **22**: [□]$^{22}_D$ –85.5 (*c* 0.6, $CHCl_3$); IR (film) 1655 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, COSY, HETCOR) □ 1.02 (dd, *J* = 13.2, 8.4 Hz, 1H, H-15), 1.10 (t, *J* = 7.4 Hz, 3H, CH_3 ethyl), 1.25-1.33 (m, 1H, H-15), 1.30 (d, *J* = 13.2 Hz, 1H, H-8), 1.56-1.69 (m, 1H, CH_2 ethyl), 1.71 (d, *J* = 13.2 Hz, 1H, H-8), 1.84 (ddd, *J* = 13.2, 9.2, 4.0 Hz, 1H, H-9), 1.99 (ddd, *J* = 13.6, 8.4, 2.4 Hz, 1H, CH_2 ethyl), 2.04 (dt, *J* = 13.2, 2.8 Hz, 1H, H-7), 2.17 (dt, *J* = 12.4, 2.8 Hz, 1H, H-7), 2.39 (t, *J* = 8.4 Hz, 1H, H-14a), 2.50 (ddd, *J* = 10.4, 6.0, 3.6 Hz, 1H, H-8b), 2.57 (dd, *J* = 10.4, 6.0 Hz, 1H, H-8a), 4.14 (dd, *J* = 9.2, 6.8 Hz, 1H, H-11), 4.44 (dd, *J* = 9.2, 8.0 Hz, 1H, H-11), 5.18 (d, *J* = 8.4 Hz, 1H, H-9a), 5.27 (t, *J* = 7.4 Hz, 1H, H-12), 6.03 (dt, *J* = 7.2, 0.8 Hz, 1H, H-1), 6.30 (dd, *J* = 8.0, 0.8 Hz, 1H, H-2), 6.35 (dd, *J* = 8.4, 2.8 Hz, 1H, H-6), 6.95 (dt, *J* = 8.0, 1.2 Hz, 1H, H-3), 7.35-7.70 (m, 11H, ArH, H-4); ^{13}C NMR ($CDCl_3$, 100.6 MHz) □□11.7 (CH_3 ethyl), 19.0 (C-7), 22.9 (CH_2 ethyl), 28.3 (C-8), 41.0 (C-8b), 41.5 (C-8a), 42.7 (C-9), 45.7 (C-14a), 47.1 (C-15), 59.5 (C-12), 71.8 (C-11), 90.3 (C-9a), 114.6 (C-6), 115.3 (C-4), 121.8 (C-2), 124.5 (C-1), 127.0 (C-*o*), 127.4 (C-*p*), 128.2 (C-*p*), 128.6 (C-*m*), 128.8 (C-15b), 128.9 (C-*o*), 129.1 (C-*m*), 133.4 (C-3), 138.1 (C-4a), 138.4 (C-5a), 139.0 (C-15a), 139.3 (C-*i*), 146.7 (C-*i*), 169.9 (NCO); mp 193–195 ºC (MeOH): HRMS calcd for [$C_{34}H_{34}N_2O_4S$ + H]: 567.2312, found: 567.2310.

(*3R,6S,7S,8R,8aS*)-8-Ethyl-5-oxo-3-phenyl-7-vinyl-6-(1-(2-vinyl-3-indolyl)methyl)-2,3,6,7,8,8a-hexahydro-5*H*-oxazolo(3,2-*a*) pyridine (24): KF (61 mg, 1.05 mmol) was added to a solution of **19b** (120 mg**,** 0.21 mmol) in EtOH (50 mL), and the mixture was stirred at reflux temperature for 24 h. The solution was concentrated under reduced pressure and the residue was dissolved with CH_2Cl_2 and quenched with H_2O. The organic layer was separated, and the aqueous phase was extracted with CH_2Cl_2.

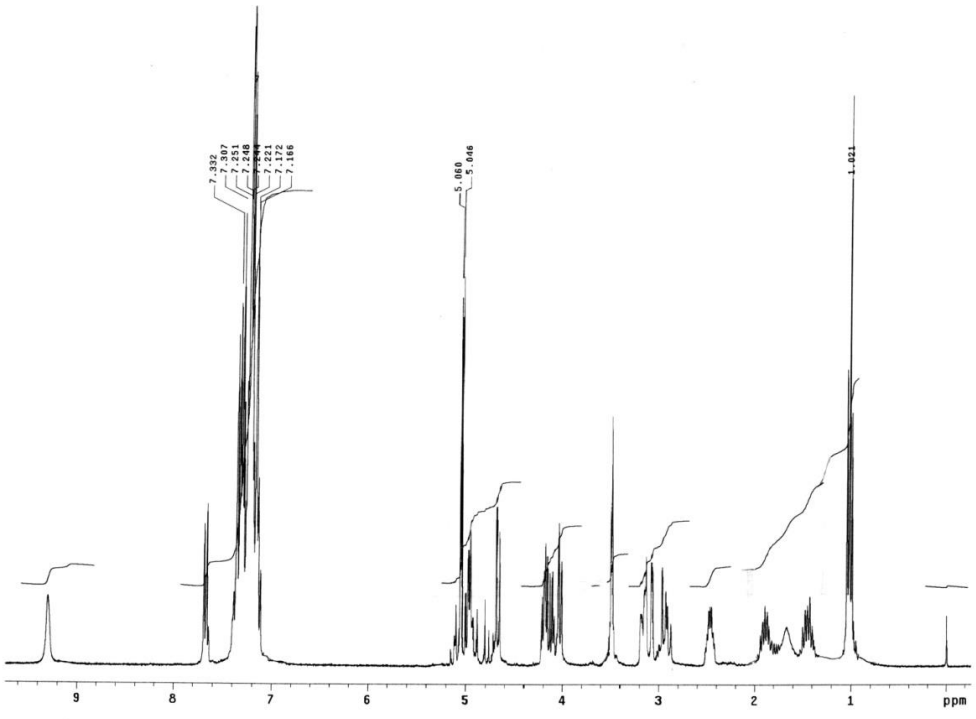
The organic extracts were dried, filtered, and concentrated. Flash chromatography (99.1 CH_2Cl_2-EtOAc) of the residue afforded **24** (80 mg, 89%) as a yellow foam: $(\alpha)^{22}_D$ –55.0 (*c* 0.2, $CHCl_3$); IR (KBr) ν =1635 (NCO), 3311 (NH) cm^{-1}; ^1H NMR ($CDCl_3$, COSY, *g*-HSQC) δ 0.90 (t, *J* = 7.3 Hz, 3H, CH_3), 1.33-1.41 (m, 1H, CH_3CH_2), 1.70-1.80 (m, 2H, CH_3CH_2, H-8), 2.54 (dd, *J* = 8.0, 2.8 Hz, 1H, H-7), 2.92 (dd, *J* = 11.0, 3.2 Hz, 1H, H-6), 3.00 (dd, *J* = 14.0, 11.0 Hz, 1H, CH_2ind), 3.50 (dd, *J* = 14.0, 3.2 Hz, 1H, CH_2ind), 3.70 (dd, *J* = 8.8, 8.0 Hz, 1H, H-2), 4.51 (dd, *J* = 8.8, 8.0 Hz, 1H, H-2), 4.64 (d, *J* = 8.8 Hz, 1H, H-8a), 4.85 (dt, *J* = 17.2, 1.2 Hz, 1H, $HC=CH_2$), 5.02 (d, *J* = 10.4 Hz, 1H, $HC=CH_2$), 5.27 (d, *J* = 11.2 Hz, 1H, ind$CH=CH_2$), 5.29 (t, *J* = 8.0 Hz, 1H, H-3), 5.50 (d, *J* = 17.6 Hz, 1H, ind$CH=CH_2$), 5.76 (ddd, *J* = 17.2, 10.4, 8.4 Hz, 1H, $HC=CH_2$), 6.86 (dd, *J* = 17.6, 11.2 Hz, 1H, ind$C=CH_2$), 7.06 (dt, *J* = 8.4, 1.2 Hz, 1H, H-ind), 7.18 (dt, *J* = 7.2, 1.2 Hz, 1H, H-ind), 7.24-7.37 (m, 5H, ArH, H-ind), 7.63 (d, *J* = 8.4 Hz, 2H, ArH), 8.15 (sa, 1H, NH); ^{13}C RMN ($CDCl_3$): δ 11.3 (CH_3), 21.6 (CH_3CH_2), 26.0 (CH_2ind), 39.1 (C-7), 39.8 (C-8), 46.8 (C-6), 58.8 (C-3), 72.7 (C-2), 90.9 (C-8a), 110.6 (C-7 ind), 111.7 (ind-$CH=CH_2$), 113.2 (C-3 ind), 117.3 ($CH=CH_2$), 119.2 (C-4 ind), 119.5 (C-5 ind), 123.0 (C-6 ind), 125.4 (C-3a ind), 126.0 (C-*o*), 128.6 (C-*p*), 128.9 (C-*m*), 133.2 (C-2 ind), 135.9 ($CH=CH_2$), 136.2 (C-7a ind), 139.7 (C-*i*), 170.7 (NCO). Anal. Calcd. for $C_{28}H_{30}N_2O_2 \cdot 3/4H_2O$**:** C, 76.42; H, 7.21; N, 6.37. Found: C, 76.21; H, 6.89; N, 6.10.

Copies of ^{1}H and ^{13}C NMR spectra

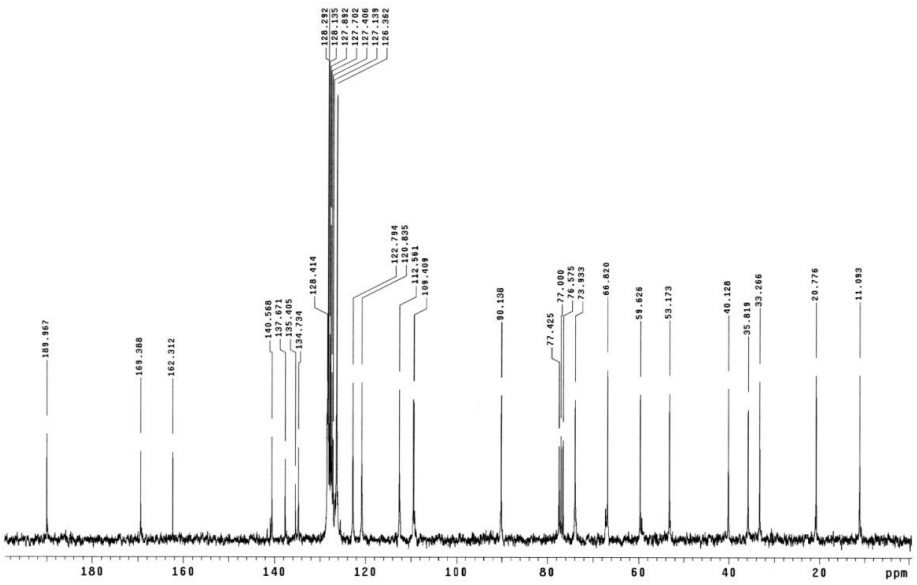


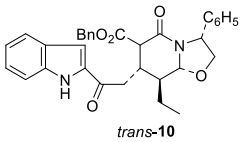
cis-**10**

^{1}H NMR (300 MHz, $CDCl_3$)



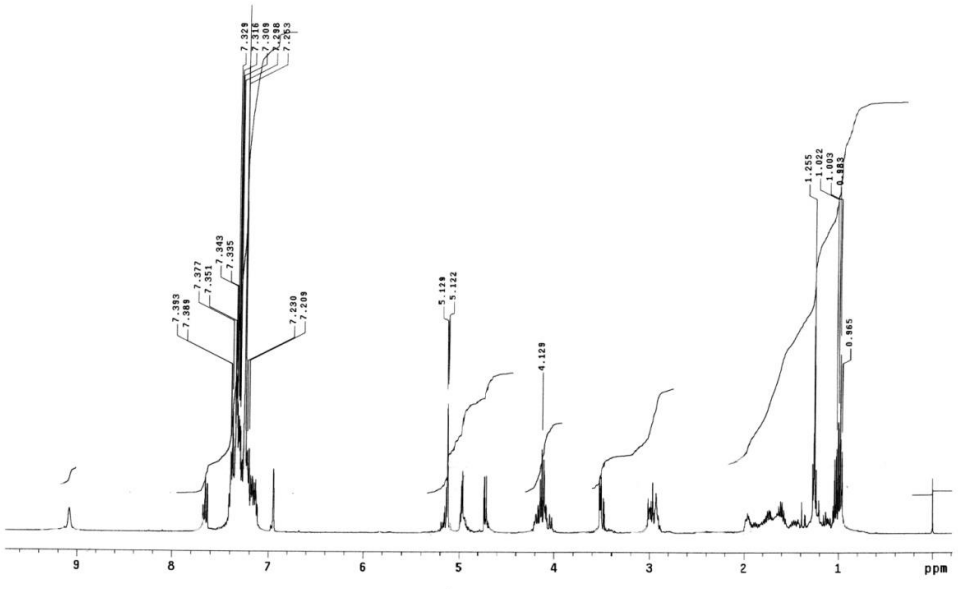
^{13}C NMR (75.4 MHz, $CDCl_3$)



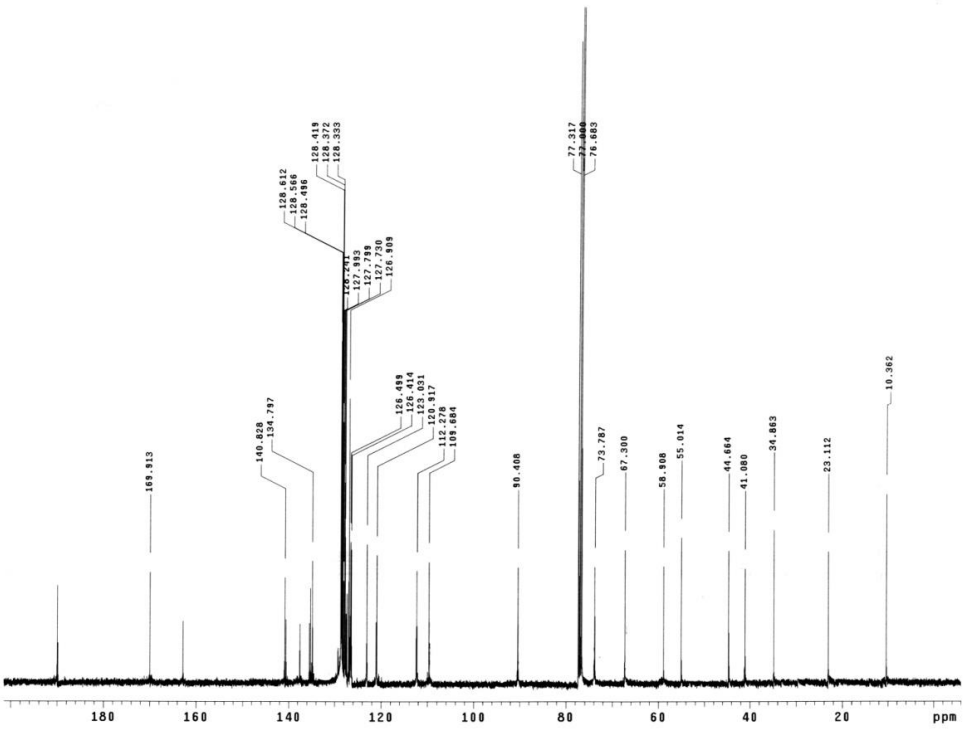


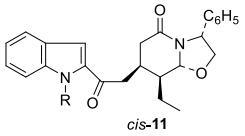
trans-**10**

^{1}H NMR (400 MHz, $CDCl_3$)



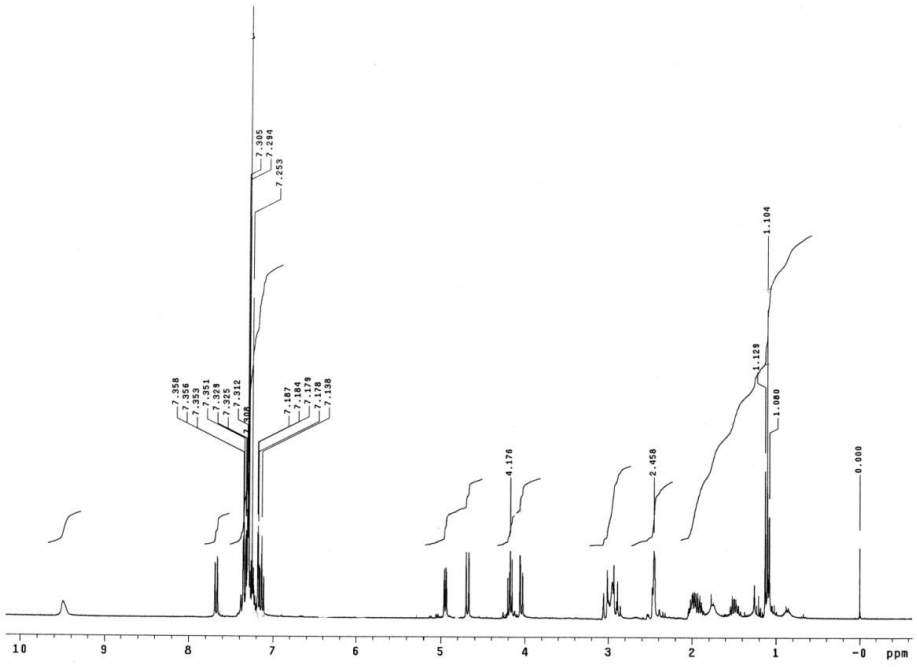
^{13}C NMR (100.6 MHz, $CDCl_3$)



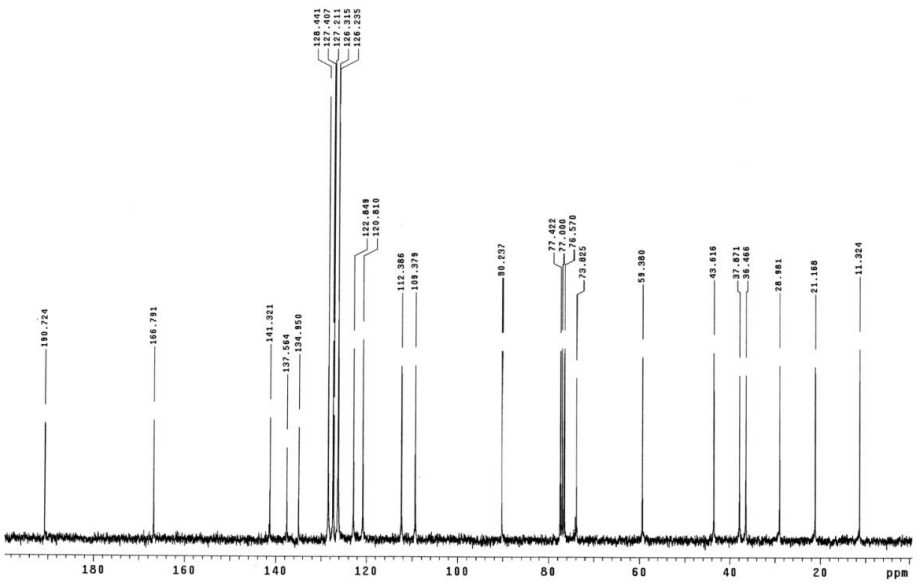


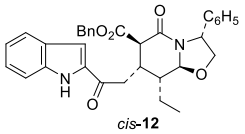
cis-**11**

^{1}H NMR (300 MHz, $CDCl_3$)



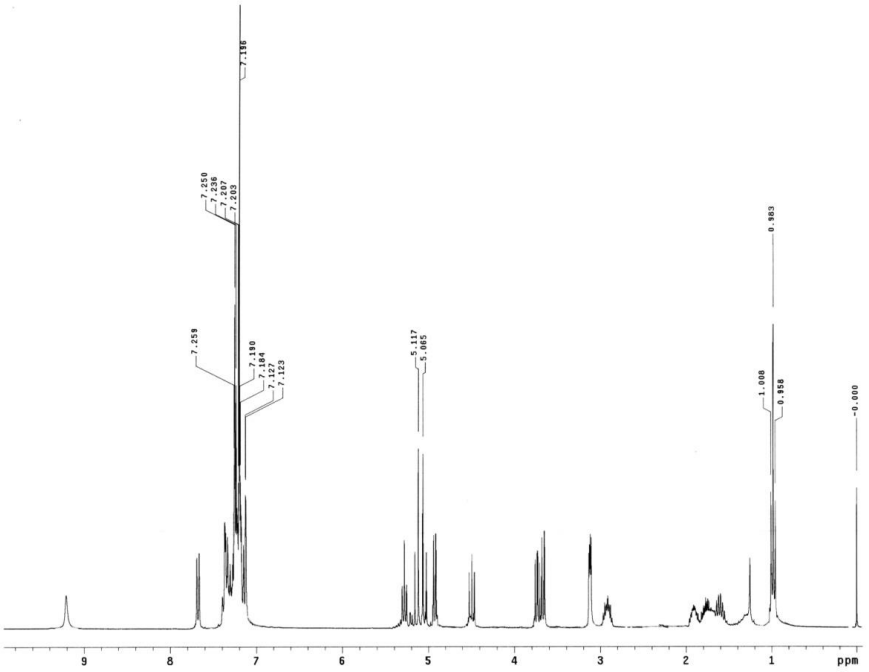
^{13}C NMR (75.4 MHz, $CDCl_3$)



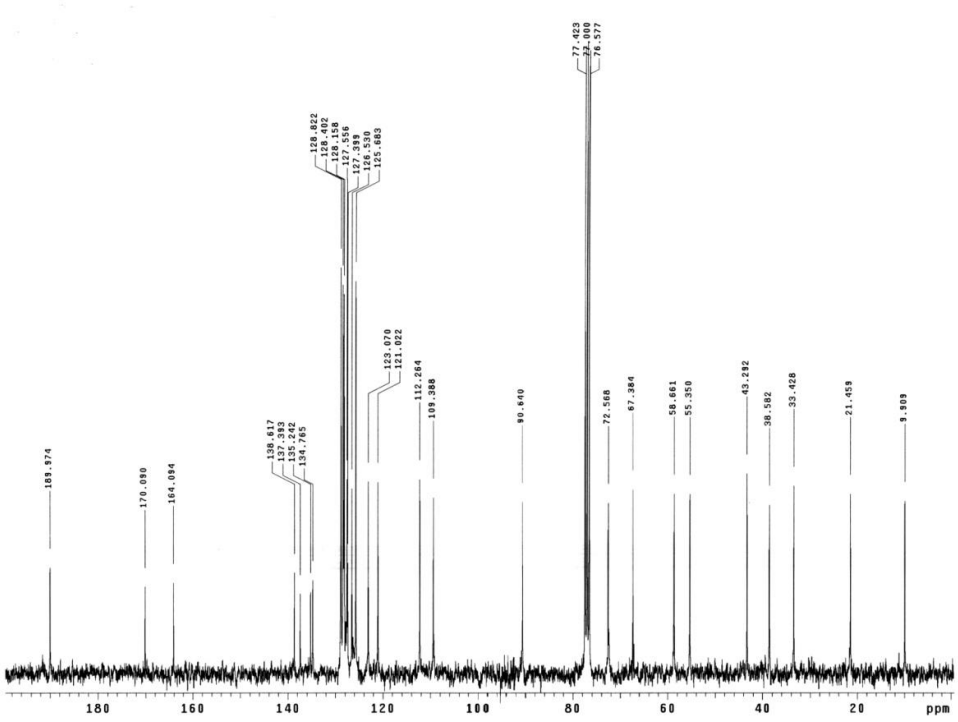


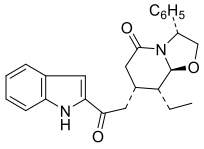
cis-**12**

^{1}H NMR (300 MHz, $CDCl_3$)



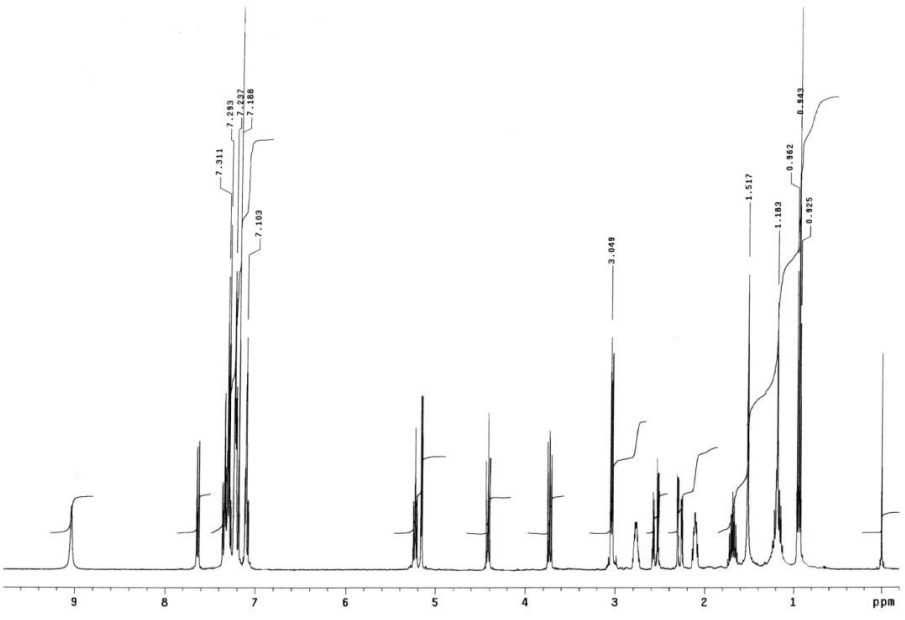
^{13}C NMR (75.4 MHz, $CDCl_3$)



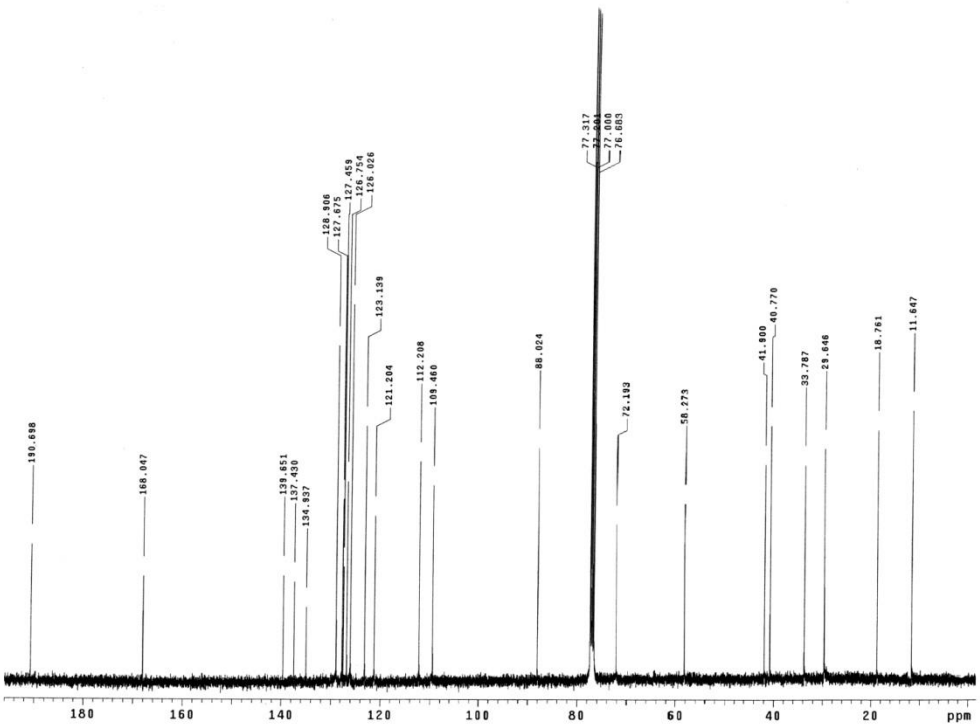


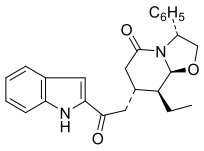
cis-**13**

^{1}H NMR (400 MHz, $CDCl_3$)



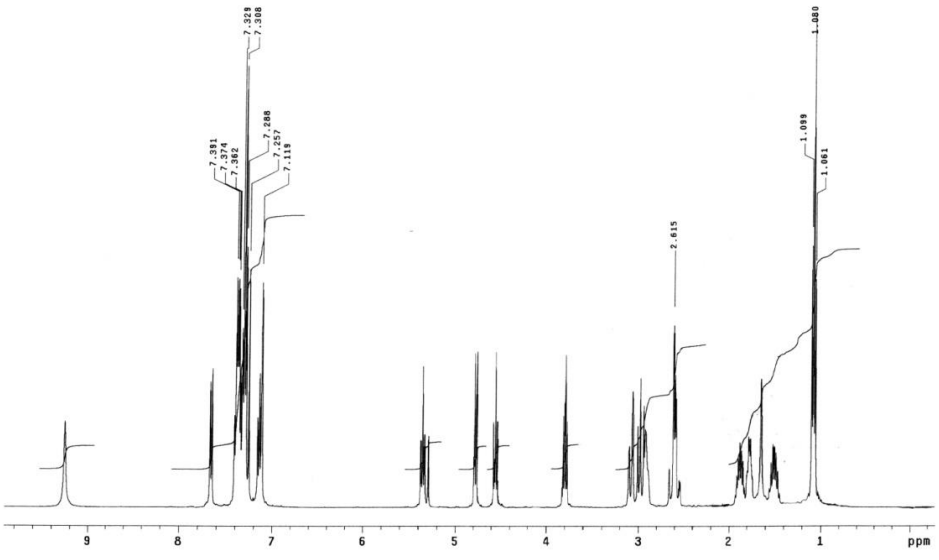
^{13}C NMR (100.6 MHz, $CDCl_3$)



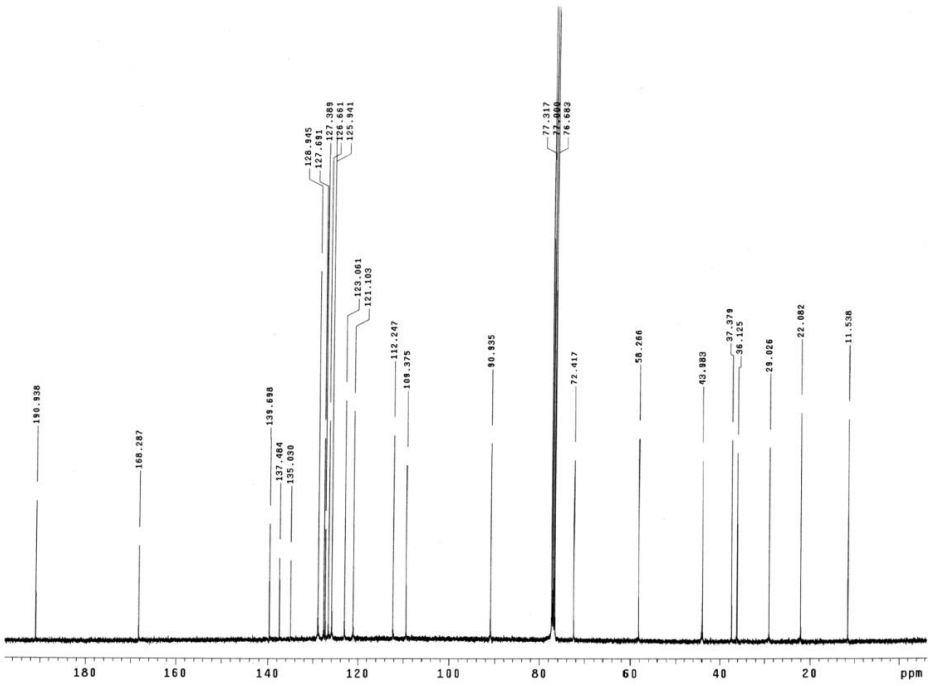


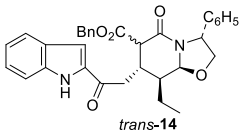
trans-**13**

^{1}H NMR (400 MHz, $CDCl_3$)



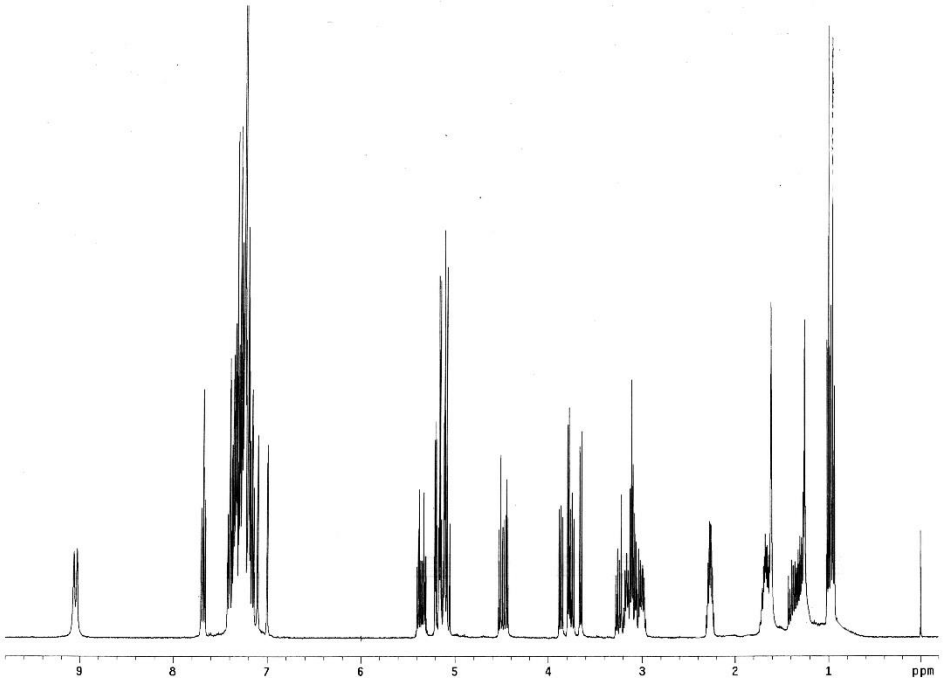
^{13}C NMR (100.6 MHz, $CDCl_3$)



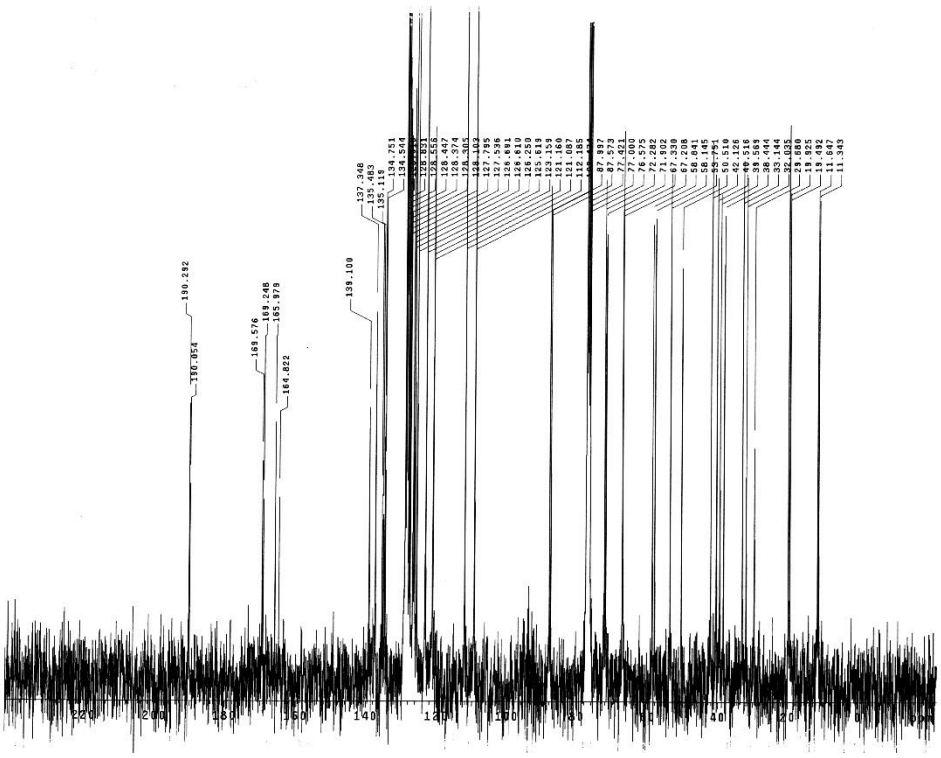


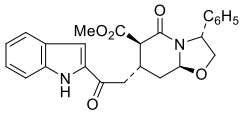
trans-**14**

^{1}H NMR (400 MHz, $CDCl_3$)



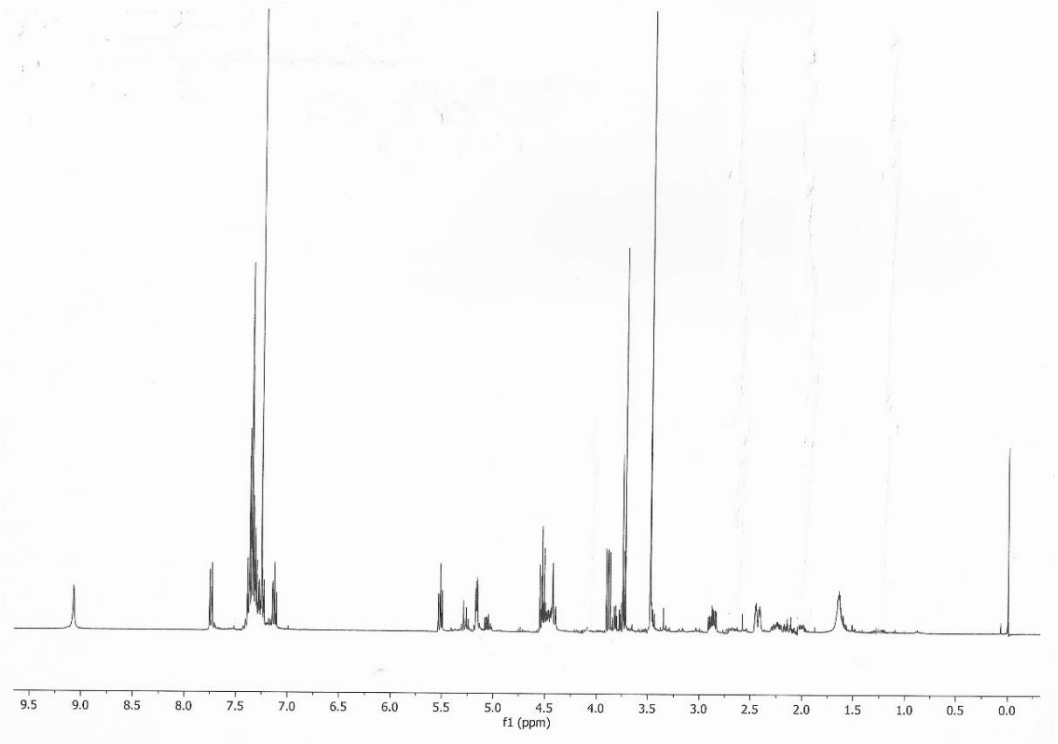
^{13}C NMR (100.6 MHz, $CDCl_3$)



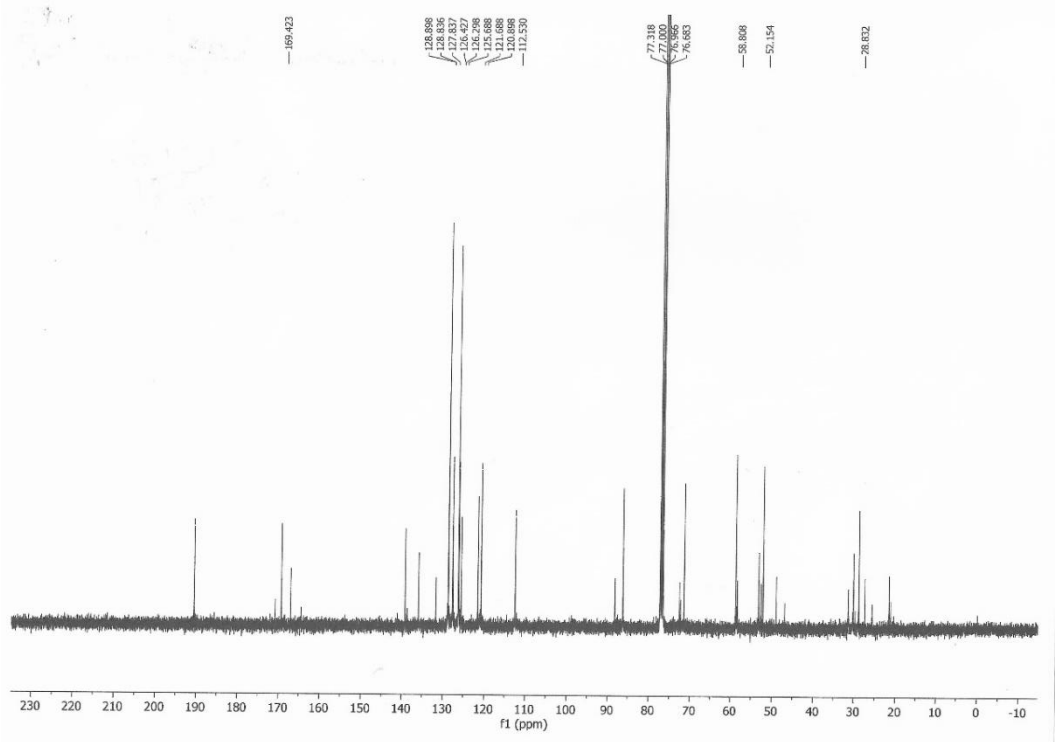


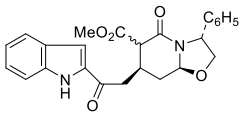
15a

^{1}H NMR (400 MHz, $CDCl_3$)



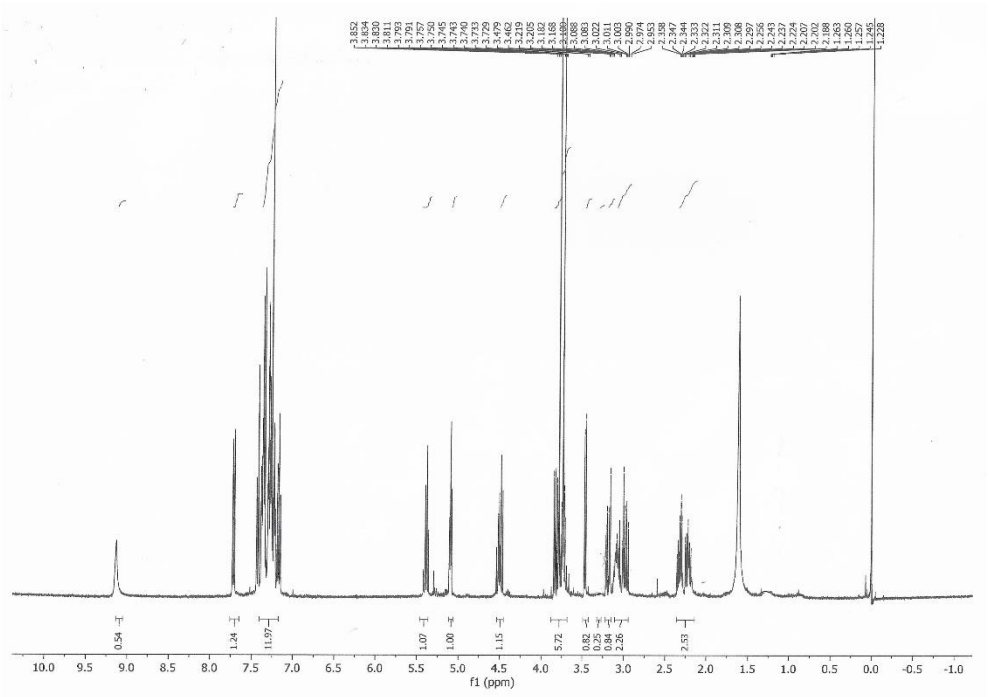
^{13}C NMR (100.6 MHz, $CDCl_3$)





15b

^{1}H NMR (400 MHz, $CDCl_3$)



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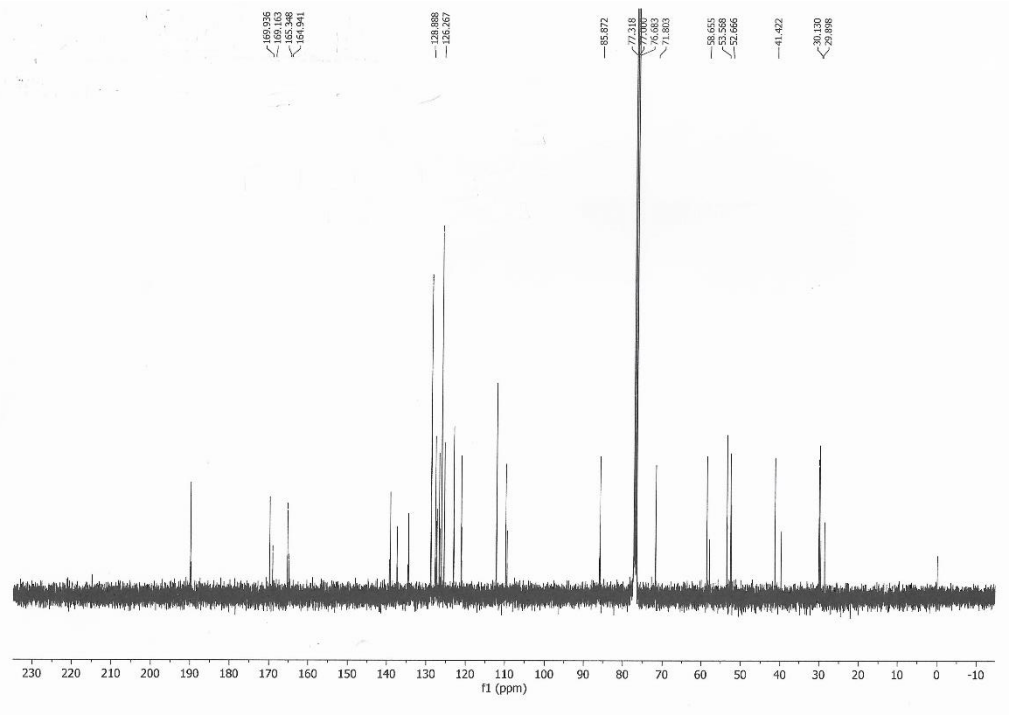
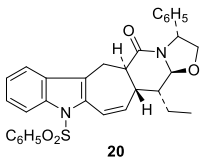
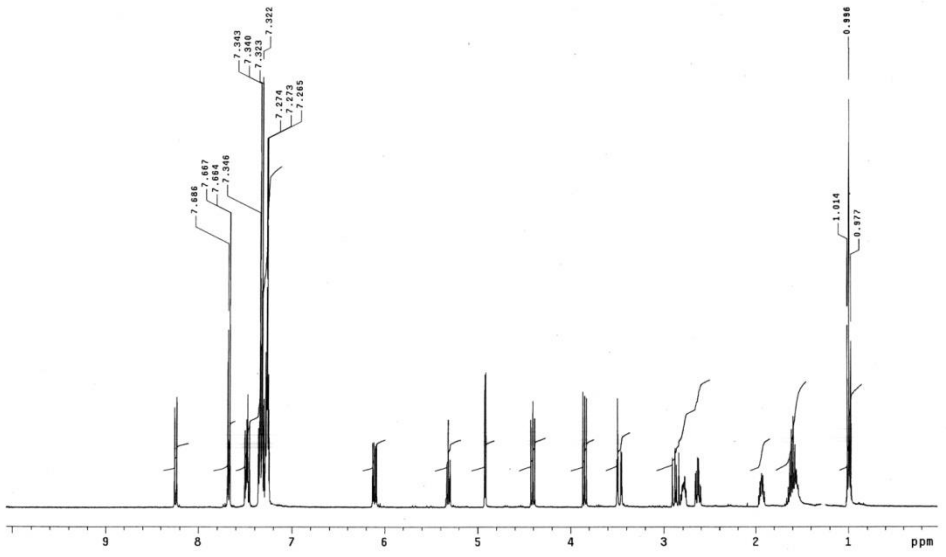


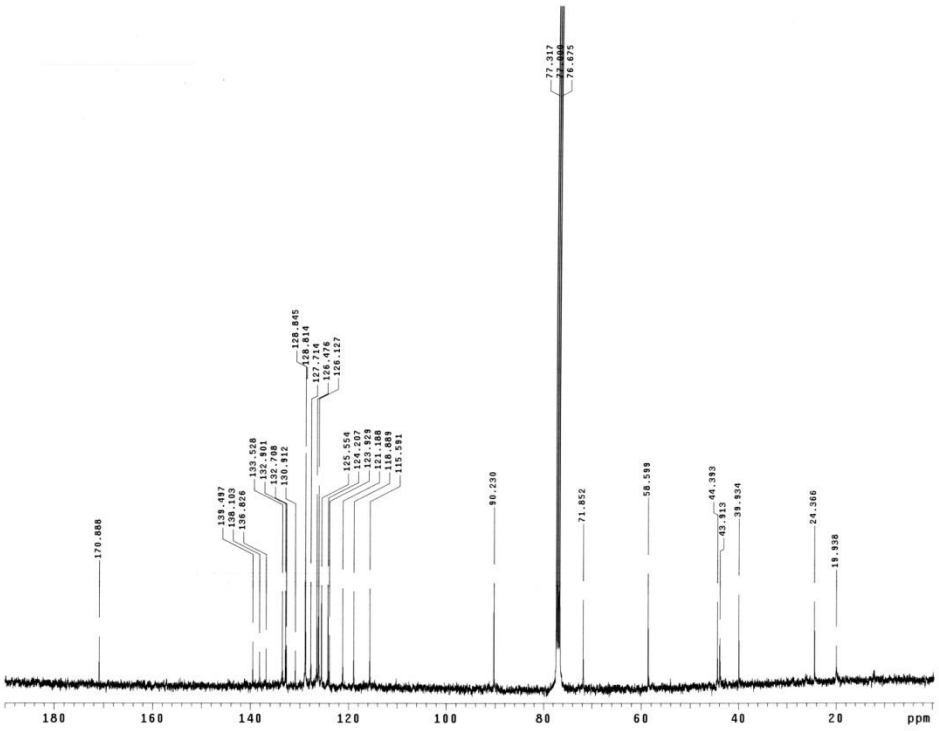


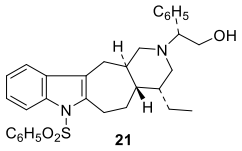
20

^{1}H NMR (400 MHz, $CDCl_3$)



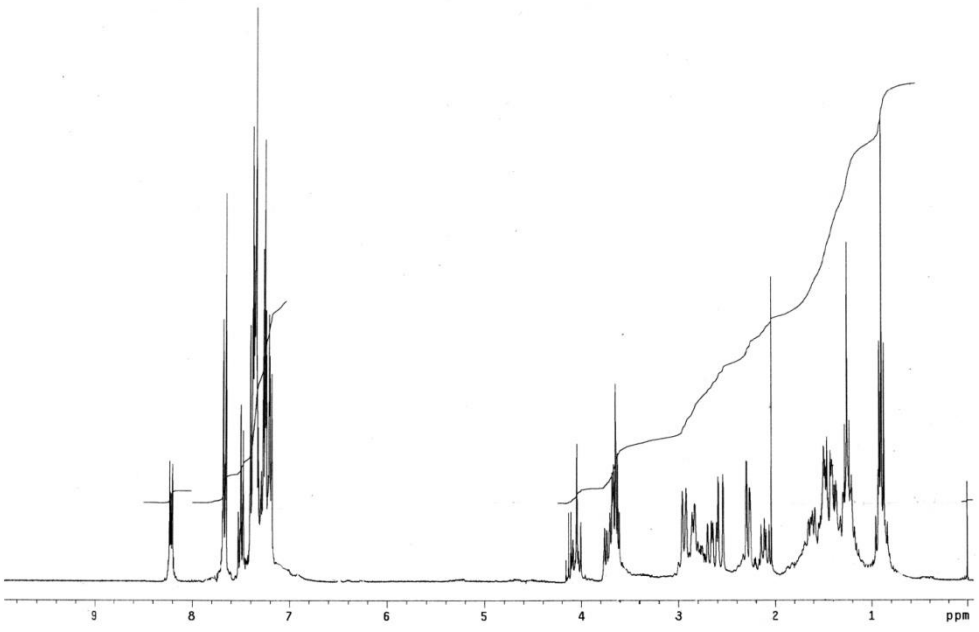
^{13}C NMR (100.6 MHz, $CDCl_3$)



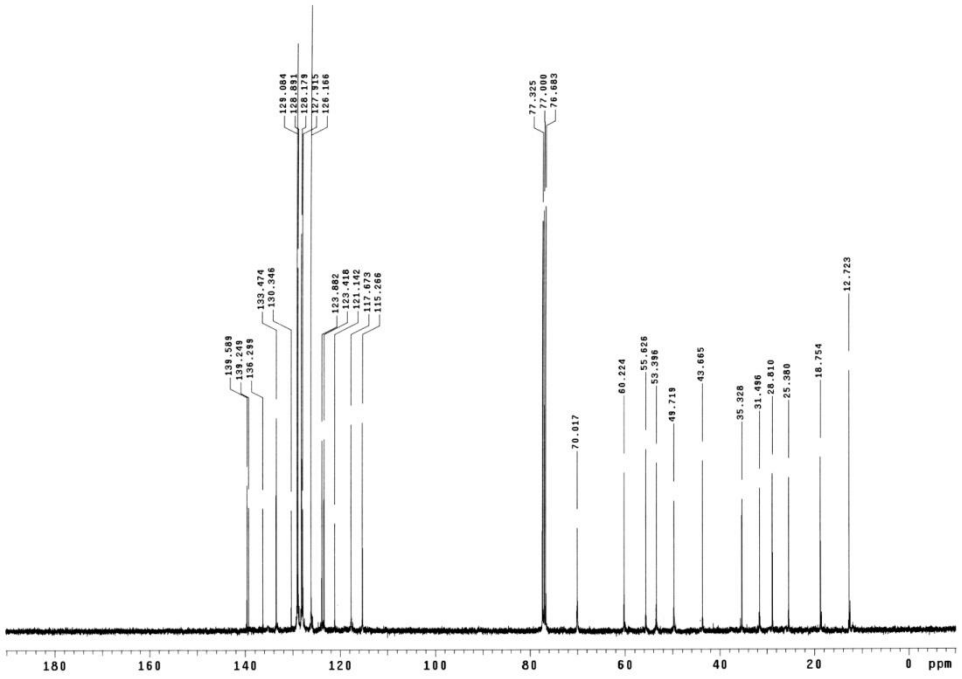


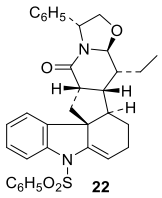
21

^{1}H NMR (400 MHz, $CDCl_3$)



^{13}C NMR (100.6 MHz, $CDCl_3$)





22

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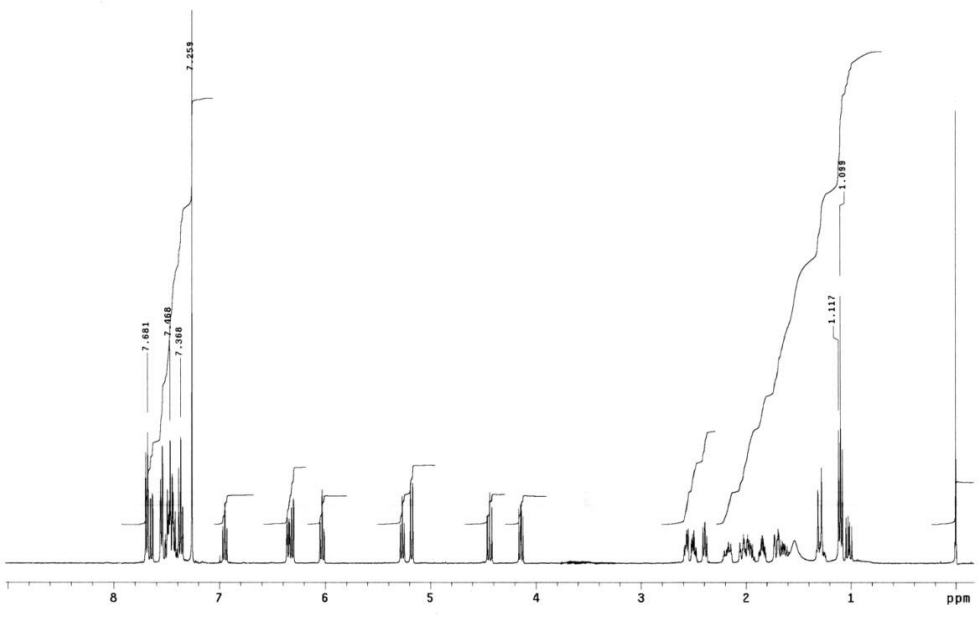
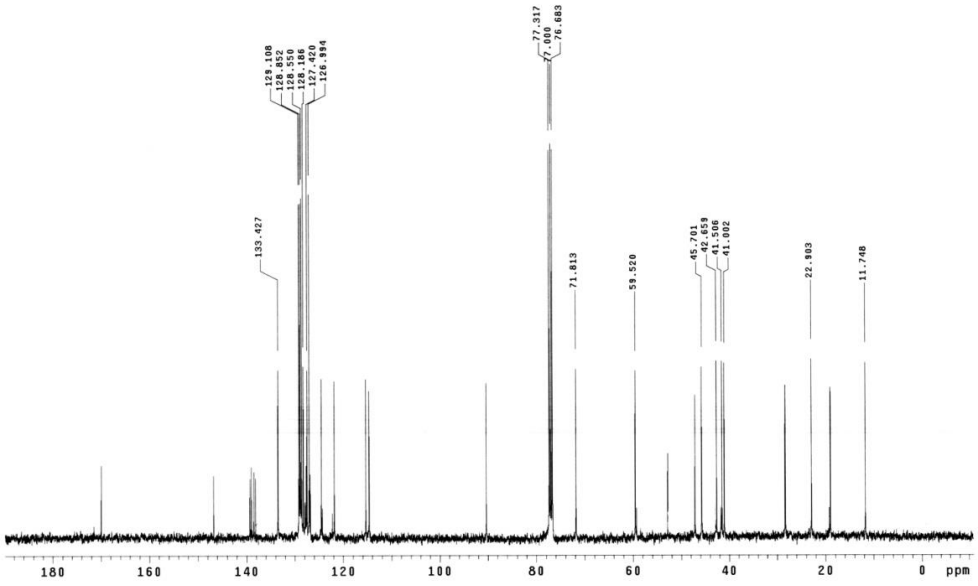
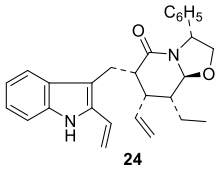


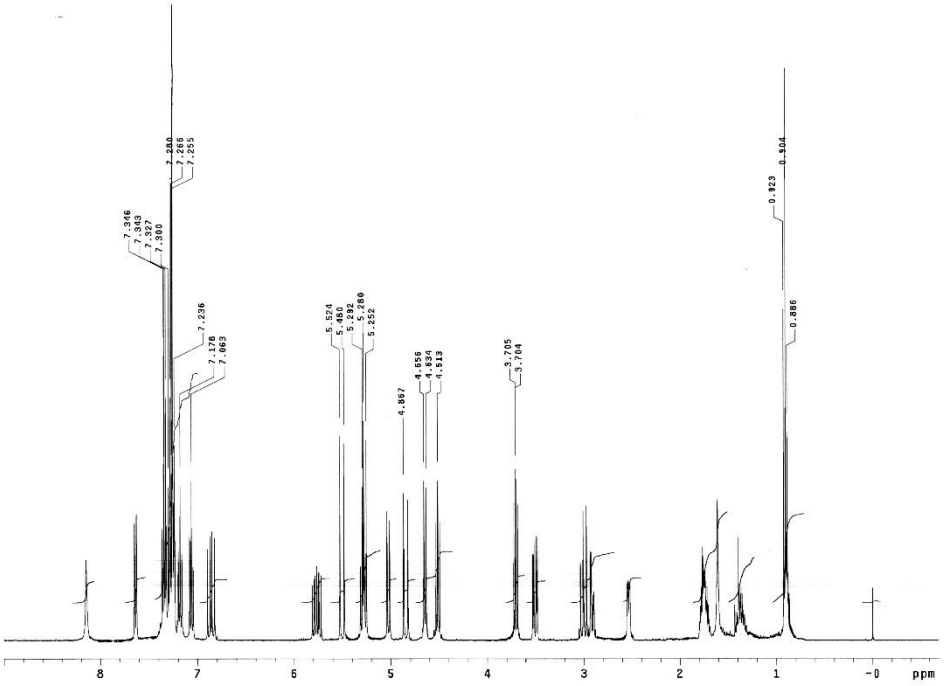
^{13}C NMR (100.6 MHz, $CDCl_3$)


133.427
128.108
128.852
128.550
128.186
127.680
126.894
77.317
77.000
76.683
71.813
59.520
45.701
43.615
41.506
41.002
22.903
11.748
180
160
140
120
100
80
60
40
20
0
ppm

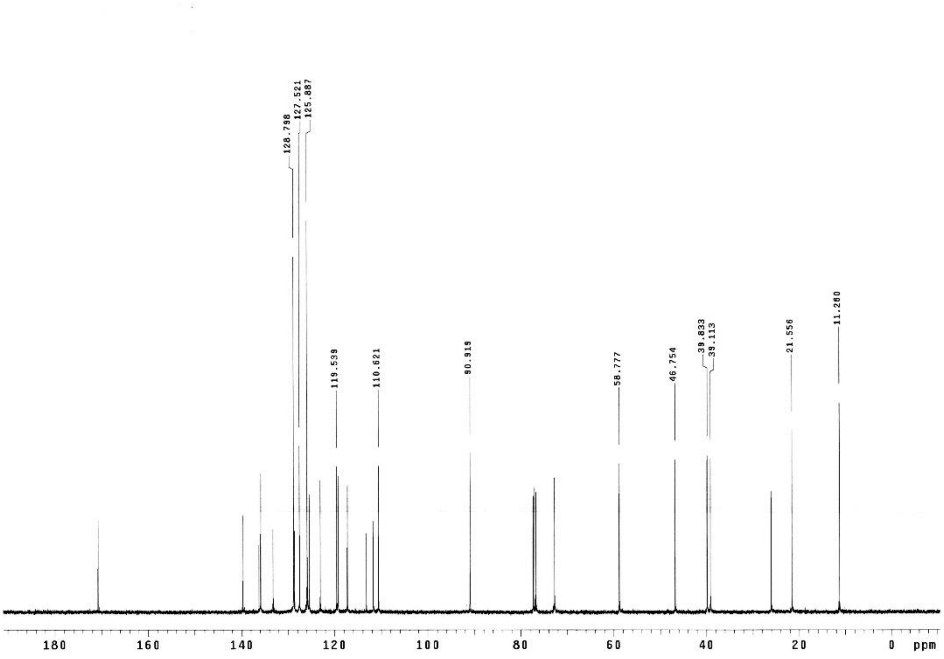


24

^{1}H NMR (400 MHz, $CDCl_3$)



^{13}C NMR (100.6 MHz, $CDCl_3$)



Variant calling analysis

In an attempt to extract more information on nucleotide differences between 22-res and trans-14-res in passage 16, **LoFreq*** (9) was utilized against the indexed virus control consensus sequence, which was used as a reference. Upon **variant calling**, only consensus level variants (frequency > 50%) were considered for our comparative mutation analysis (Figures A and B), according to the methodology previously described (10).

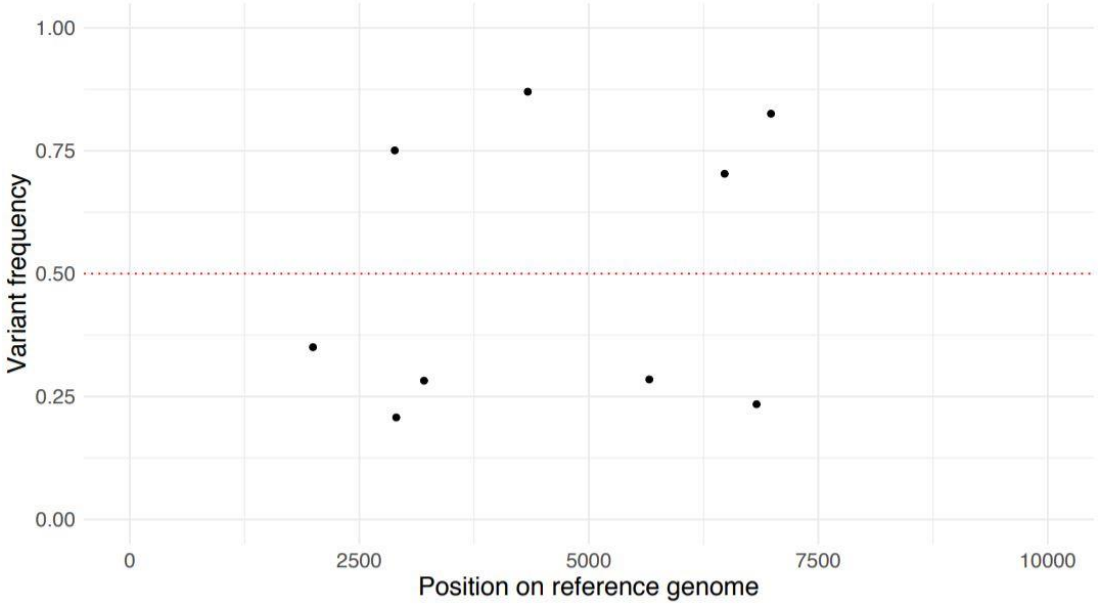


Figure A: Variant frequencies of **22**-res compared to virus control (passage 16)

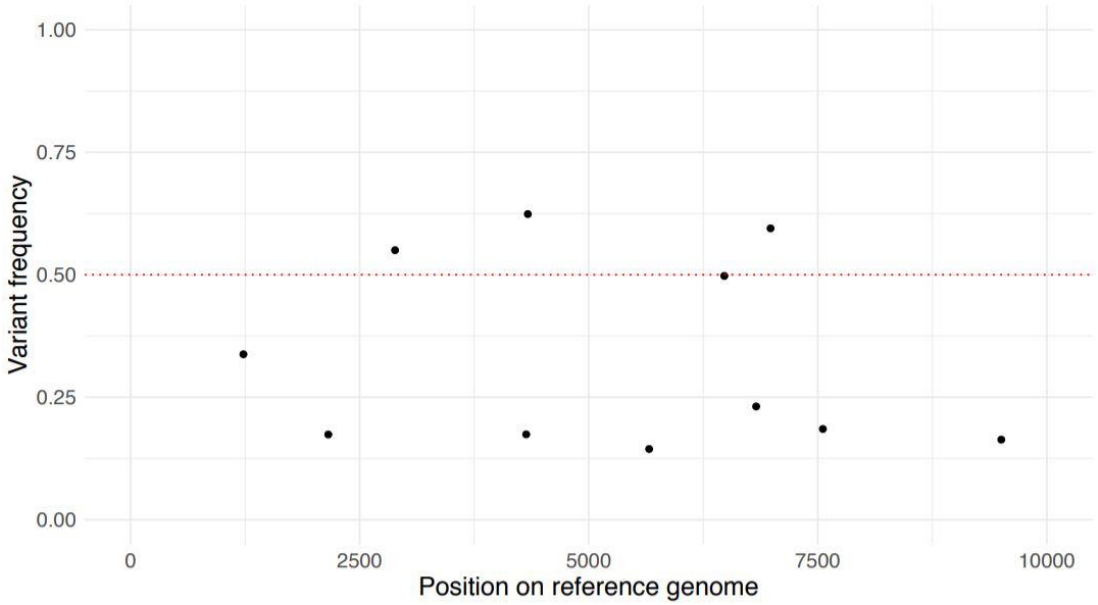


Figure B: Variant frequencies of *trans*-**14**-res compared to virus control (passage 16)

In the figure, X-axis represents the position of the mutation across the virus control genome (passage 16), while Y-axis depicts the frequency at which each mutation is present in the sequencing data. A dashed red line indicates our threshold of 50% frequency.

Based on our findings, we showed that there are no qualitative differences on the amino acid level between the two resistant mutants. However, after implementation of the variant calling workflow, we observe that the frequency of the variants (above 50%) at the indicated positions between both strains **slightly differ**. For example, at nucleotide position 6984, the mutation for **22**-resistant seems to be present in approximately 80% of the sequence reads, while the same mutation for *trans*-**14**-resistant is found in less than 60% of the reads. Differences on the frequencies at which each variant occurs, highly reflect differences on the population of the variants present within each sampled mutant strain. We hypothesize that the varying rate of occurrence of these mutations could possibly explain the deviations on the phenotypic inhibitory activity of trans-10 and cis-10 against these two mutants.

Statistical analysis

One-way ANOVA, followed by Bonferroni correction test were performed to evaluate significant differences in Figure panels 3A, 3B and 4A. Unpaired t-test was performed in Figure panel 4B.

Significance was calculated based on p values:

Non-significant, $p > 0.05$

*, $p < 0.05$

**, $p < 0.005$

***, $p < 0.0005$

****, $p < 0.0001$

Particularly, for Fig 3A, we compared the percentage of detected viral load after the treatment of cells with the tested compounds (**22**, *Trans*-**14**, DS, 7DMA) with this observed for virus control (no treatment with compounds, red dashed line in the panel) at each time point, separately. As clearly shown, our molecules suppress viral replication 8h-12h post infection (as we proposed in the manuscript).

In addition, we provide the statistical differences on the replicon levels among the three compounds (luciferase values from different concentrations among the molecules), in order to compare their inhibitory activity profile (Fig 3B). As shown from the figure panel 3B, significant differences on the replicon levels were noticed, especially at highest concentrations (20 μM and 100 μM).

Fig 3A:

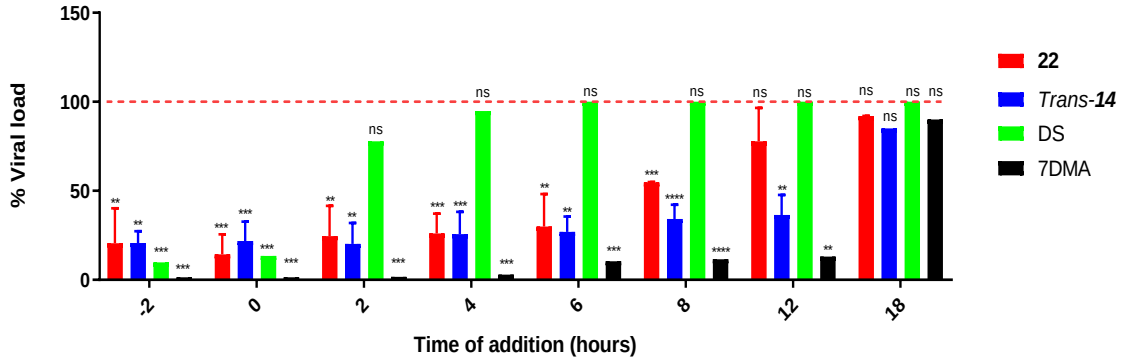
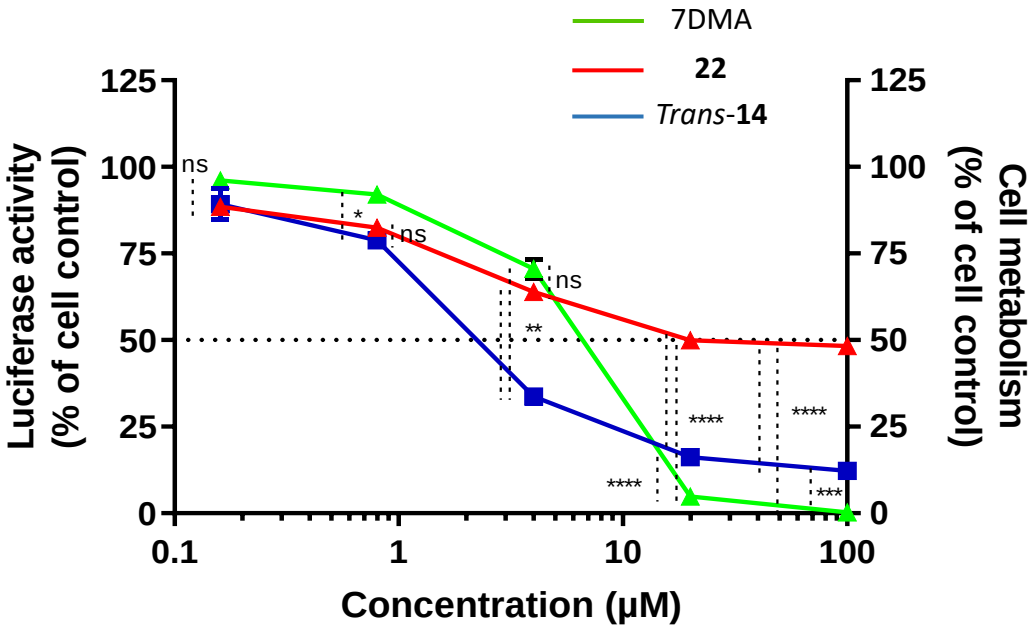


Fig 3B:



For Figure 4, one-way ANOVA (4A) and unpaired t-test (4B) were performed. In both panels, each condition was compared to the corresponding virus control for the statistical analysis.

Fig 4A:

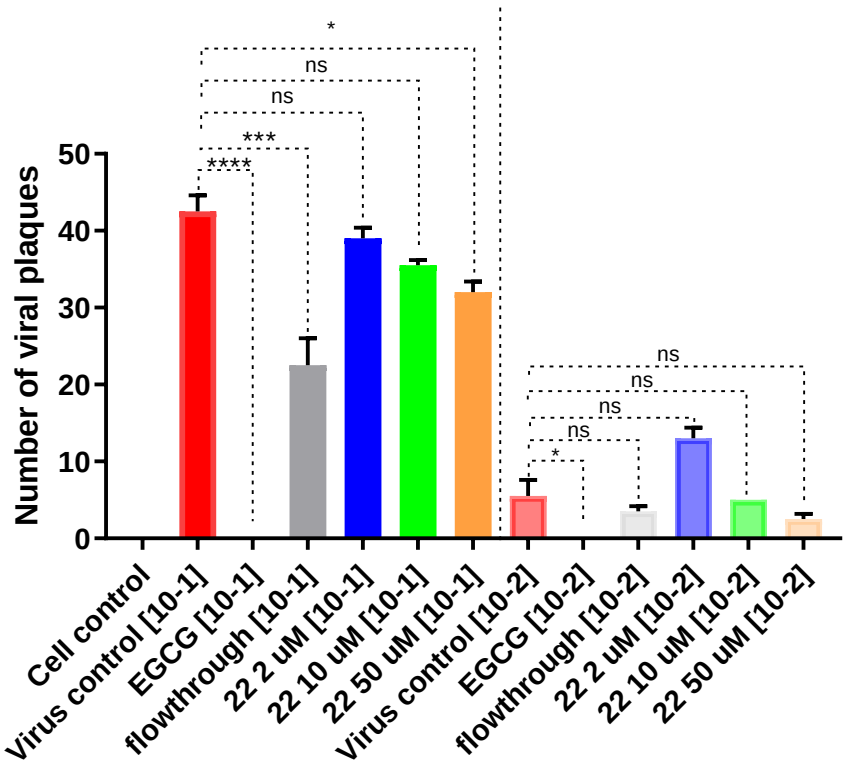
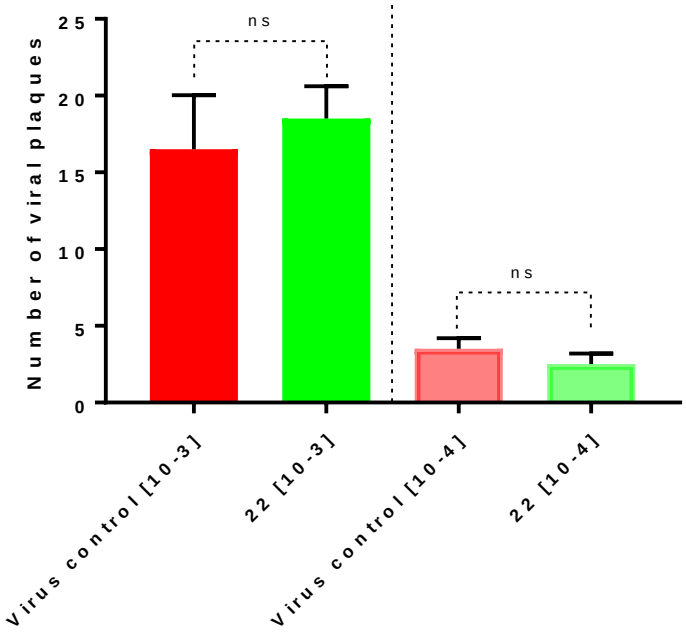

Number of viral plaques
50
40
30
20
10
0
*
ns
ns
***
****
ns
ns
ns
ns
*
Cell control
Virus control [10-1]
EGCG [10-1]
flowthrough [10-1]
22 2 uM [10-1]
22 10 uM [10-1]
22 50 uM [10-1]
Virus control [10-2]
EGCG [10-2]
flowthrough [10-2]
22 2 uM [10-2]
22 10 uM [10-2]
22 50 uM [10-2]

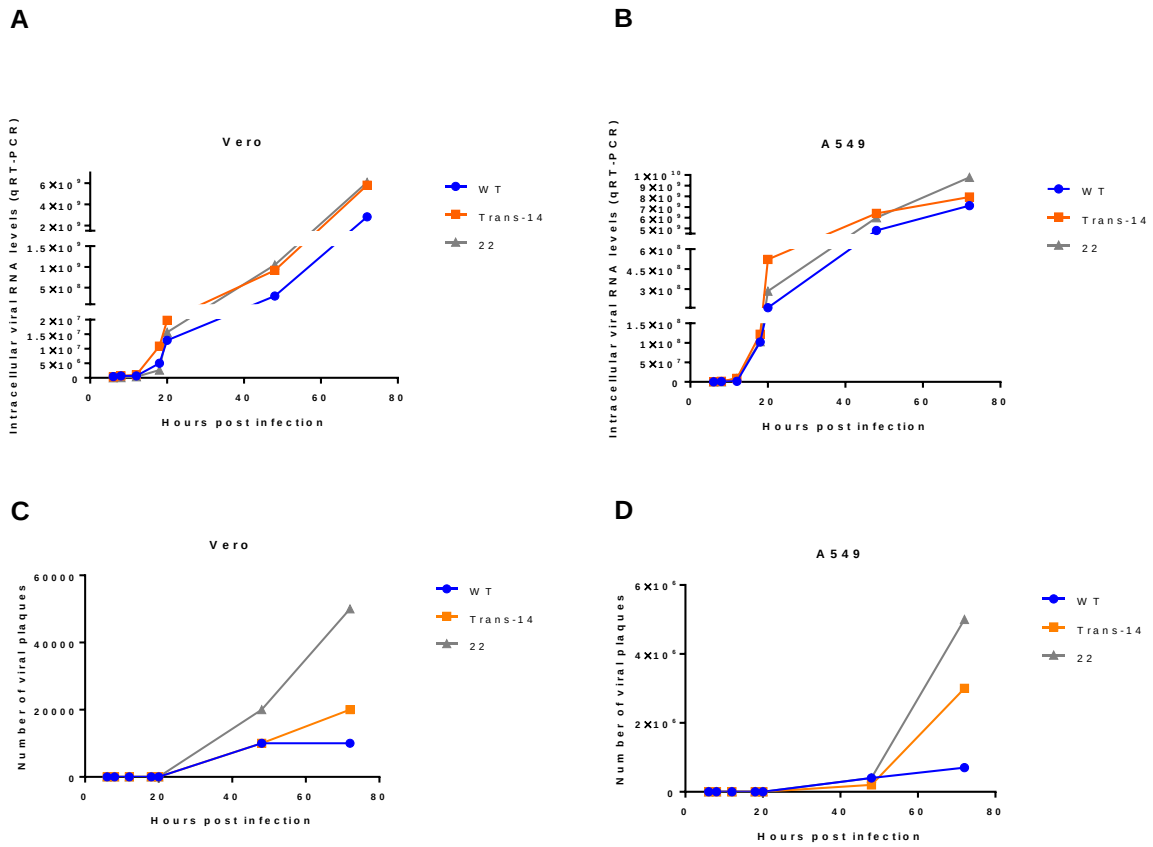
Fig 4B:


Number of viral plaques
25
20
15
10
5
0
ns
ns
Virus control [10-3]
22 [10-3]
Virus control [10-4]
22 [10-4]

Growth kinetics experiment

A simple growth kinetic experiment was conducted in both Vero and A549 cells, by incubating the P16 viral stocks (resistant and WT strains) at MOI 0.1 in the absence of any compound pressure at different time points (6h, 8h, 12h, 18h, 20h, 48h and 72h post infection). Specifically, we evaluated the intracellular viral RNA levels, by performing qRT-PCR. As observed by the Figure panels below (A and B), **22**-resistant mutant (grey) presented slightly higher levels of viral RNA, as compared to *trans*-**14** (orange) and WT (blue) in both cell lines. However, this is more obvious between 48h and 72h post infection. In Vero cells, both resistant mutants showed a similar tendency in the increase of viral load, as compared to the one for WT strain (slower rate of viral replication). On the other hand, **22**-resistant mutant seemed to have a faster replication rate, especially after 48h post infection, as compared to *trans*-**14** and WT strains in A549 cells. To conclude: vRNA $_{\mathbf{22}}$ > vRNA $_{\textit{trans}\text{-}\mathbf{14}}$ > vRNA $_{WT}$ in both tested cell lines.

Moreover, we assessed virus infectivity, by performing a plaque assay (using BHK cells). Unfortunately, we were not able to collect sufficient number of viral plaques before 20h post infection, probably due to the incomplete virus replication cycle. As observed in Figure panels C and D, virus infectivity of **22**-resistant mutant is higher, compared to *trans*- and WT virus strains in both Vero and A549 cells. These results confirm our assumption and show that **22**-resistant virus stock does indeed have an intrinsic replication advantage over the *trans*-**14** resistant and WT virus strains.



References

1. Amat M, Checa B, Llor N, Molins E, Bosch J. 2009. Enantioselective total synthesis of the indole alkaloid 16-episilicine. Chem Commun (Camb) 20:2935–7.

2. Amat M, Llor N, Checa B, Molins E, Bosch J. 2010. A synthetic approach to ervatamine-silicine alkaloids. Enantioselective total synthesis of (-)-16-episilicine. J Org Chem 75:178–89.

3. Amat M, Checa B, Llor N, Pérez M, Bosch J. 2011. Conjugate addition of 2-acetylindole enolates to unsaturated oxazolopiperidone lactams: Enantioselective access to the tetracyclic ring system of ervitsine. European J Org Chem 5:898–907.

4. Romo D, Meyers AI. 1991. Chiral non-racemic bicyclic lactams. Vehicles for the construction of natural and unnatural products containing quaternary carbon centers. Tetrahedron 47:9503–69.

5. Escolano C, Amat M, Bosch J. 2006. Chiral oxazolopiperidone lactams: Versatile intermediates for the enantioselective synthesis of piperidine-containing natural products. Chem - A Eur J 12:8198–207.

6. Amat M, Pérez M, Llor N, Bosch J, Lago E, Molins E. 2001. Conjugate addition of organocuprates to chiral bicyclic delta-lactams. Enantioselective synthesis of cis-3,4-disubstituted and 3,4,5-trisubstituted piperidines. Org Lett 3:611-4

7. Amat M, Pérez M, Llor N, Escolano C, Luque FJ, Molins E, Bosch J. 2004. Conjugate additions to phenylglycinol-derived unsaturated delta-lactams. Enantioselective synthesis of uleine alkaloids. J Org Chem 69:8681-93

8. Amat M, Checa B, Llor N, Molins E, Bosch J. 2009. Enantioselective total synthesis of the indole alkaloid 16-episilicine. Chem Commun (Camb) 20:2935-7

9. Wilm A, Aw Kim PP, Bertrand D, Ting Yeo GH, Ong SH, Wong CH, Khor CC, Petric R, Hibberd ML, Nagarajan N. 2012. LoFreq: a sequence-quality aware, ultra-sensitive variant caller for uncovering cell-population heterogeneity from high-throughput sequencing datasets. Nucleic Acids Res 40:11189-201

10. Lequime S, Fontaine A, Ar Gouilh M, Moltini-Conclois I, Lambrechts L. 2016. Genetic Drift, Purifying Selection and Vector Genotype Shape Dengue Virus Intra-host Genetic Diversity in Mosquitoes. PLOS Genetics 12:e1006111