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Discovery of (7*R*)-14-Cyclohexyl-7-{[2-(dimethylamino)ethyl](methyl) amino}-7,8-dihydro-6*H*-indolo[1,2-*e*][1,5]benzoxazocine-11-carboxylic Acid (MK-3281), a Potent and Orally Bioavailable Finger-Loop Inhibitor of the Hepatitis C Virus NS5B Polymerase†

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Infections caused by hepatitis C virus (HCV) are a significant world health problem for which novel therapies are in urgent demand. The polymerase of HCV is responsible for the replication of viral genome and has been a prime target for drug discovery efforts. Here, we report on the further development of tetracyclic indole inhibitors, binding to an allosteric site on the thumb domain. Structure−activity relationship (SAR) studies around an indolo-benzoxazocine scaffold led to the identification of compound **33** (MK-3281), an inhibitor with good potency in the HCV subgenomic replication assay and attractive molecular properties suitable for a clinical candidate. The compound caused a consistent decrease in viremia in vivo using the chimeric mouse model of HCV infection.

Introduction

It has been estimated that more than 170 million people worldwide are infected with the hepatitis C virus (HCV[a]).[1,2] Although the infection is typically slow progressing and often asymptomatic, it becomes chronic in a majority of patients and in a significant fraction of these can develop into chronic hepatitis, liver cirrhosis, and eventually hepatocellular carcinoma. Chronic HCV infection has become the leading indication for liver transplants in the developed world[3] and is the principal cause of death in HIV coinfected patients.[4] Although the number of new infections is diminishing, the mortality due to HCV infection is expected to increase in Europe and the USA.[5] The genetic heterogeneity of the virus, which has been classified into six different genotypes (gt), having as much as 35% nucleotide sequence difference among them, has been a major obstacle for the development of a vaccine.[6] Currently, the standard of care (SOC) is based upon a combination of subcutaneous pegylated α-interferon and oral ribavirin. It is poorly tolerated and associated with severe flu-like symptoms, depression, and anemia, resulting in poor patient compliance.[7] Moreover, duration of therapy and response rate are genotype specific. The SOC is efficient in about 80% of patients infected with the gt 2 and 3 of the virus but only in about 50% of patients infected with gt 1, which accounts for approximately 70% of infections in the Western world.[8] To improve treatment outcome for chronic hepatitis C infection with respect to efficacy, tolerability, and duration, research in the pharmaceutical industry and in academia has focused on the characterization of viral enzymes as targets for small molecule intervention.[9] Proof-of-concept in the clinic has been obtained for small molecule inhibitors which target viral proteins such as the NS3/4A protease, the NS5B polymerase, and most recently also NS5A.[10−14] The RNA-dependent RNA polymerase NS5B plays a key role in the life cycle of the virus since it is responsible for the replication of the viral genome.[15] Classical nucleoside analogues[16] as well as allosteric inhibitors, which were shown to bind to at least four distinct sites on the polymerase, have been described.[17]

Recently, we and other groups reported on the discovery of indole-based inhibitors of NS5B, also called thumb-pocket 1 or finger-loop inhibitors.[18−20] This class of molecules was shown to inhibit the initiation of RNA synthesis by binding to the upper part of the thumb domain, where they interrupt a key interaction between the λ1 loop, which extends from the fingertips, and the thumb domain, impeding a conformational change of the enzyme thought to be important for RNA synthesis.[21−23] Successive optimization of our indole series, exemplified by **1** (Figure 1),[24] led to conformationally constrained indoles, where the ortho position of the C2-aryl group is tethered to the indole nitrogen,[25] an approach that was also disclosed recently by a group of scientists at Japan Tobacco.[26] The tetracyclic benzodiazocine **2** had good intrinsic and cell-based

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[a] Abbreviations: 1,2-DCE, 1,2-dichloroethane; DCM, dichloromethane; DIPEA, diisopropylethylamine; DMP, Dess−Martin periodinane; FCS, fetal calf serum; gt, genotype; HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyl uronium hexafluorophosphate; HCV, hepatitis C Virus; NHS, normal human serum; SAR, structure−activity relationship; SD, standard deviation; SFC, supercritical fluid chromatography; SOC, standard of care; TBAT, tetrabutylammonium triphenyldifluorosilicate; TFA, trifluoroacetic acid.

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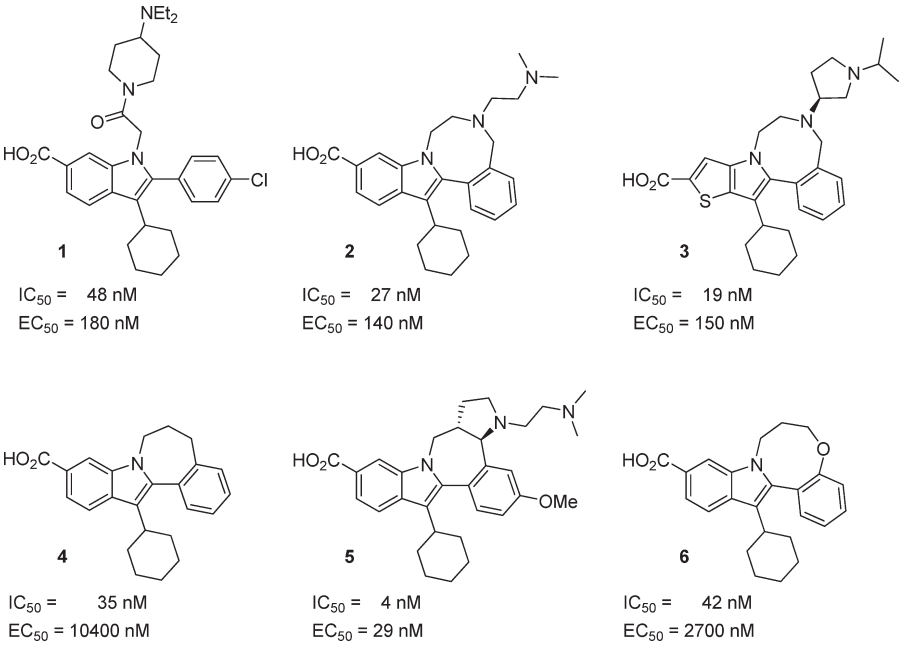


Figure 1

potency and a superior DMPK profile with respect to **1**.[27] Our efforts to improve the potency of this inhibitor class focused on replacement of the indole core and optimization of the tether moiety. Thieno[3,2-*b*]pyrroles were identified as the only viable replacements for the indole core.[28] Optimization led to tetracyclic thienopyrrole **3**, which had similar potency and pharmacokinetic properties with respect to **2**.[29] Since we were unable to improve potency of this series further, we turned our attention to the modification of the tether moiety in the indole series, which was more successful. SAR around **2** and related series had shown that 7- as well as 8-membered rings were equally well tolerated.[25,26] Starting from the tetracyclic benzoazepine **4**, novel pentacyclic compounds such as **5** with superior potency in the cell-based assay with respect to our previous compounds were discovered.[30] However, different from **2**, pentacycle **5** and related compounds lacked oral bioavailability in rodents.

In our search for alternative tetracyclic scaffolds which eventually could combine the potency of **5** with the good pharmacokinetic properties of **2**, we also had prepared the simple benzoxazocine **6**, which showed better cell-based activity compared to tetracyclic benzoazepine **4** and thus was deemed to provide a better starting point for optimization.[26b] In this paper, we describe the synthesis and structure−activity relationship within this class of compounds, leading to the identification of compound **33**, which combined improved potency with good oral bioavailability in preclinical animal models and which was evaluated in the SCID/*Alb-uPA* mouse model of HCV infection.[31]

Chemistry

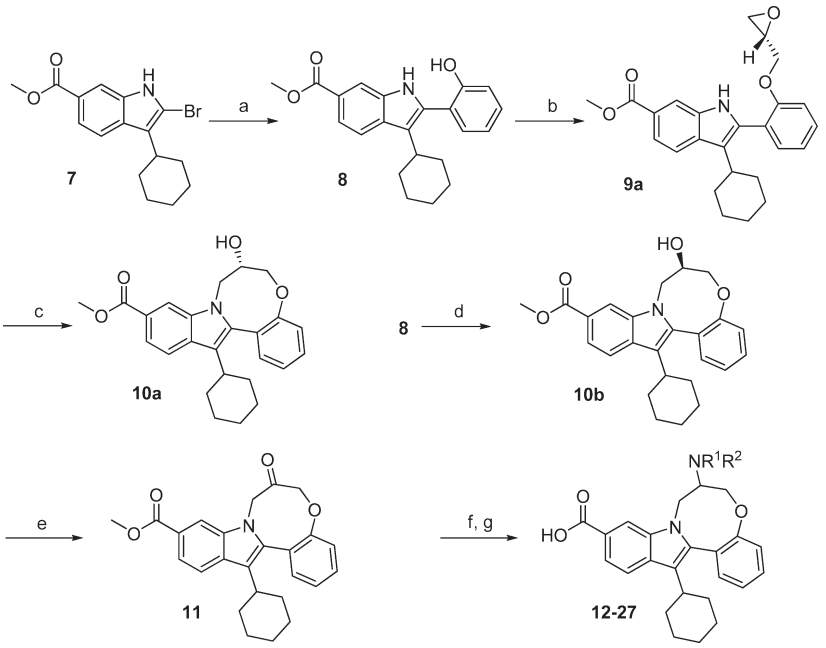
Benzoxazocine **6** was readily prepared from the phenol **8**, the Suzuki-coupling product obtained from bromide **7** and the corresponding boronic acid, by double alkylation with dibromopropane (not shown). To obtain a suitable handle for further modification of the tether, ketone **11** was targeted, but all attempts of alkylating **8** with dichloroacetone failed (Scheme 1). As an alternative, **8** was reacted with (*S*)-glycidyl nosylate under the influence of cesium fluoride to give (*S*)-epoxide **9a**.[32] Several base-catalyzed conditions, such as sodium hydride in DMF, LHMDS in THF, or cesium carbonate in DMF, induced cyclization to the benzoxazocine alcohol (*S*)-**10a**, with the latter giving the highest yield. Reaction of **8** with (*R*)-glycidyl nosylate led to epimeric alcohol **10b**. Oxidation of either alcohol gave ketone **11**, which was reacted with a variety of amines under reducing conditions to yield the racemic amino acids **12**−**27** after hydrolysis of the methyl ester. The compounds exist as mixtures of atropisomers, as was evident from their NMR spectra (see Experimental Section).

Yields were satisfactory for primary amines, but secondary amines such as dimethylamine or piperidine gave only low yields, racemic alcohol **10** being the major product. Better yields were obtained for compounds **21**−**27** by using a double reductive amination procedure, where **11** was first reacted with the primary amine and subsequently with the corresponding aldehyde to introduce the R^2-substituent.

The *N*-acylated derivatives **28** and **29** were obtained as shown in Scheme 2. For the preparation of compound **29**, the methyl ester needed to be hydrolyzed prior to amine acylation to avoid cleavage of the tertiary amide bond during the final ester deprotection.

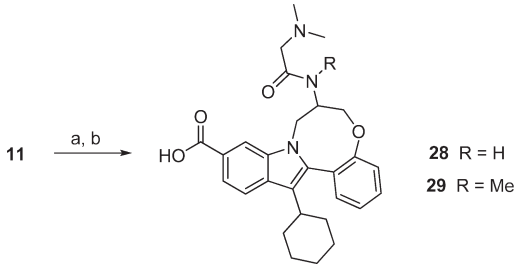
Compound **21** could be separated into the enantiomers **33** and **34**. These were also prepared independently from alcohols **10a** and **10b**. Tosylate **30a** was obtained from **10a**, and reaction with TMS-azide and TBAT as the fluoride source[33] (Scheme 3) gave azide **31a** with inversion of configuration in 84% yield and 99.3% enantiomeric excess (ee). This method was superior with respect to yield and enantiomeric purity to reaction of the tosylate with sodium azide in DMF (50% yield, 72% ee) or classical Mitsunobu conditions of alcohol **10a**, which gave low yields (31% yield, 99.5% ee). The high enantiomeric purity of **31a** also showed that epoxide **9a** was obtained by nearly exclusive attack of the phenol on the nosylate moiety of (*S*)-glycidyl 3-nitrobenzenesulfonate. This excludes a potential racemization at this step via epoxide opening and Payne rearrangement to give the enantiomer of **9a**.[32] Reduction of the azide provided the amino ester **32a**, which was converted to **33** in three more steps with an enantiomeric excess of 96%,

Scheme 1[a]



[a] Reagents and conditions: (a) (2-hydroxyphenyl)boronic acid, $PdCl_2[P(Ph)_3]_2$, Na_2CO_3, dioxane, 110 °C; (b) (*S*)-glycidyl 3-nitrobenzenesulfonate, CsF, DMF, RT; (c) Cs_2CO_3, dioxane, 100 °C; (d) (i) (*R*)-glycidyl 3-nitrobenzenesulfonate, CsF, DMF, RT, (ii) Cs_2CO_3, dioxane, 100 °C; (e) DMP, DCM (f) **12**−**20**: R^1R^2NH, $NaBH(OAc)_3$, AcOH, 1,2-DCE, rt; **21**−**27**: (i) R^1NH_2, $NaBH(OAc)_3$, AcOH, 1,2-DCE, (ii) R^2CHO, $NaBH(OAc)_3$; (g) KOH, MeOH/THF (1:1), reflux.

Scheme 2[a]



[a] Reagents and conditions: (a) NH_4OAc or $MeNH_2 \cdot HCl$ NaOAc; $NaBH_3CN$, MeOH, RT; (b) **28**: (i) *N*,*N*-dimethylglycine, HATU, DIPEA, DCM, RT, (ii) BBr_3, DCM; **29**: (i) KOH, 75 °C; (ii) *N*,*N*-dimethylglycine, HATU, DIPEA, DCM, RT.

as determined by separation on a chiral column. The same route led from **10b** to **34** having the (*S*)-configuration in 99% enantiomeric excess (Scheme 3).

Alkylation of alcohol **10a** with 2-chloro-*N*,*N*-dimethylethaneamine yielded, after hydrolysis of the ester, the aminoether **35** (Scheme 4). Enantiomer **36** was prepared in the same way starting from the (*R*)-configurated alcohol **10b**.

Spiroazetidines **38** and **39** could be accessed from the reaction of **8** with 5,5-bis(bromomethyl)-2,2-dimethyl-1,3-dioxane,[34] leading to spiroketal **37**. Hydrolysis, conversion of the alcohols into the corresponding triflates, and reaction with isopropylamine or *N*,*N*-dimethylethane-1,2-diamine gave the desired compounds after hydrolysis of the methyl ester (Scheme 5).

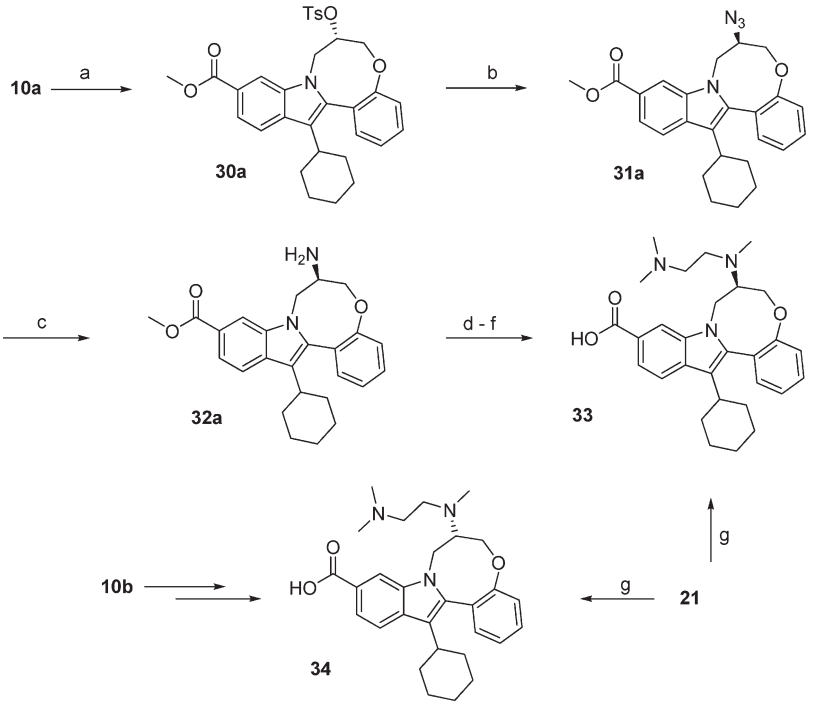
Results and Discussion

Optimization of Replicon Activity. Compounds were assayed in a polymerase enzyme inhibition assay (IC_{50}) on the HCV genotype 1b NS5B (ΔC21) polymerase.[21] The cellular activity (EC_{50}) was determined in the replicon system expressing genotype 1b in a Huh-7 hepatoma cell line in the presence of 10% fetal calf serum (FCS) or 50% normal human serum (NHS) for compounds with replicon EC_{50}s < 1 μM in 10% FCS.[24]

Introduction of a basic amine onto the indole carboxylic acids had usually proven the method of choice to gain cell-based potency for this series of compounds.[25] This was also the case for benzoxazocine **6** (Table 1). Methyl amine **12** was already 2-fold more active in the replicon assay in 10% FCS compared to **6**. Introduction of another methyl group increased cellular activity even further (**13**, EC_{50} 570 nM), although intrinsic potency of **12** and **13** did not change much with respect to **6**. Introduction of an ethane-1,2-diamine gave **14**, which displayed similar levels of cell-based activity with respect to **13**. Methylation of the distal amine yielded **15**, with a 3-fold improvement in EC_{50}. Elongation of the distance between the two amines, as in **16**, or incorporating the outer amine into a piperidine ring as in **17**, led to a slight loss in cell-based potency with respect to **15**. Testing compounds **13**−**17** in high serum conditions resulted in a moderate 4−5-fold shift in replicon potency.

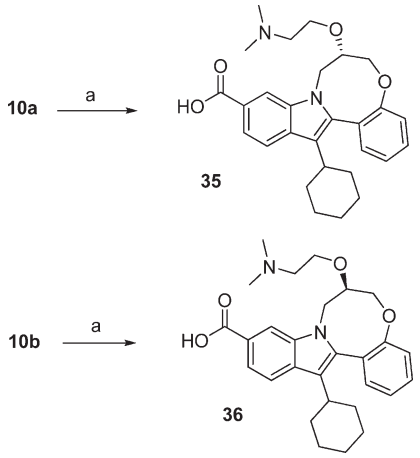
Compound **15** had the same level of potency when compared to **2** and was characterized further with respect to metabolic stability and rodent PK. The compound demonstrated an encouraging PK profile in Sprague−Dawley rats after single intravenous and oral doses. Following intravenous dosing, plasma clearance and half-lives were significantly improved with respect to **2**, consistent with the increased metabolic stability of **15** in rat liver microsomes (Table 2). After an oral dose of 3 mg/kg of **15**, bioavailability was determined to be 20%, slightly lower than that for **1** (31%). Elevated concentrations of **15** were found in rat liver tissue 6 h after oral dosing, corresponding to a liver tissue to plasma concentration ratio of 21 at this point in time (16 for compound **1**). The stability in dog and human liver microsome preparations was similar to rat, with the low intrinsic clearance rates observed in all three species.

Scheme 3[a]



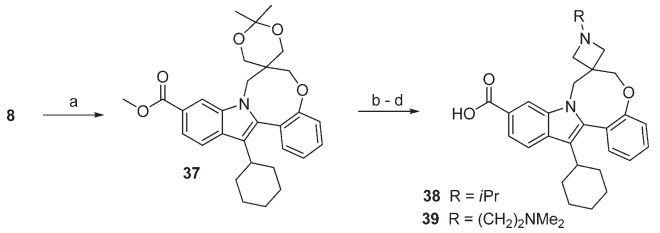
[a] Reagents and conditions: (a) TsCl, pyridine; (b) $TMSN_3$, TBAT, THF, 70 °C; (c) H_2, Pd/C, MeOH; (d) *N*-Boc-aminoacetaldehyde, $HC(OMe)_3$; $NaCNBH_3$, HOAc, MeOH; (e) DCM/TFA (1:1); HCHO, NaOAc, $NaCNBH_3$, DCM; (f) KOH, dioxane/H_2O (2:1); (g) separation by SFC.

Scheme 4[a]



[a] Reagents and conditions: (a) 2-chloro-*N*,*N*-dimethylethanamine, Bu_4NBr, toluene/30% NaOH, 60 °C.

Scheme 5[a]



[a] Reagents and conditions: (a) NaH, DMF, 70 °C; 5,5-bis(bromomethyl)-2,2-dimethyl-1,3-dioxane, 70 °C, 50%; (b) $TsOH \cdot H_2O$, MeOH/THF, RT, 89%; (c) Tf_2O, MeCN, DIPEA, 0 °C, then *i*$PrNH_2$ (**38**) or H_2N-$(CH_2)_2NMe_2$ (**39**), 70 °C; (d) KOH, dioxane, 75 °C.

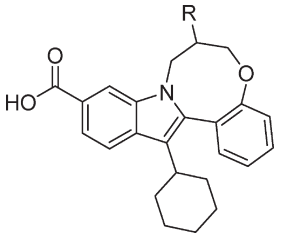
Encouraged by these results, we undertook further SAR around the ethane-diamine side chain of **15** and key results are summarized in Table 3. A distal basic amine was required for cell-based activity. Methylether **18** lost an order of magnitude in activity compared to **15**, and amide **19** was 4-fold less potent. In both cases, the loss in intrinsic potency was only about 2-fold. Conversely, the conversion of the inner amine to an amide as in **28** was tolerated and cell-based activity was further improved by methylation of the nitrogen, leading to tertiary amide **29** (EC_{50} 73 nM). Removal of the amide oxygen gave the fully methylated bis-amine **21**, which was equipotent to **29** in the standard replicon conditions. Compounds **21** and **29** were the first examples displaying an $EC_{50} < 100$ nM in the tetracyclic indole series. In the presence of 50% NHS, compound **29** showed a nearly 10-fold shift in potency, whereas **21** shifted only 4-fold under high serum conditions, reflecting the higher binding to human plasma protein of **29** (0.5% free) versus **21** (2.7% free). With respect to replicon potency under low and high serum conditions, the methyl substitution on the two amines proved to be optimal, although several analogues with modifications on the distal amine such compounds **22**−**25** displayed similar replicon potency under standard replicon conditions. For the most potent of these analogues, *N*-methylpiperazine **25** (EC_{50} 66 nM), a low free fraction in the presence of human plasma proteins (1.1% free), led to a 9-fold shift in the replicon assay. Exchanging the methyl group on the inner amine for an ethyl- and isopropyl group as in compounds **26** and **27** resulted in a 2-fold loss in cell-based activity and a more pronounced shift (7-fold) under high serum conditions.

To investigate the impact of the stereochemistry on the tether, compound **21** was separated into the enantiomers **33** and **34**. The activity resided mostly in the (*R*)-configurated compound **33**, which was 4-fold more potent intrinsically with respect to the (*S*)-enantiomer **34** (Table 4). Moreover, **33** was

about 10-fold more potent than **34** in the cell-based assay, with an EC_{50} of 38 nM and a moderate 3-fold shift under high serum conditions. The absolute configuration of **33** was established also by synthesis from **10a** and later confirmed by an X-ray structure of **33** bound to NS5B (vide infra).

Further SAR around the side chain established that the inner methylamine group could be replaced with oxygen, giving compounds **35** and **36**. The more active epimer **35** was 2-fold less potent in the replicon assay with respect to **33**, and the EC_{50} shifted 6-fold under high serum conditions. Different from what had been observed for bis-amines **33** and **34**, the activity in the resulting amino ethers **35** and **36** resided mostly in (*S*)-enantiomer **35**, having the opposite configuration at position 7 of the tether. We also briefly investigated the possibility to eliminate the stereogenic center at this position by introduction of a spirocycle. Geminal disubstitution was tolerated, as spiro-azetidines **38** and **39** show. Monoamine **38** had similar potency levels with respect to monoamine **13** (see Table 1), and bisamine **39** showed slightly improved intrinsic potency with respect to **33** but was 2-fold less active in the replicon assay, with a 5−6-fold shift in the presence of 50% NHS.

Table 1. Enzymatic and Cell-Based Activity of Compounds **6** and **12**−**16**



No.	R	IC_{50} (nM)[a]	EC_{50} (nM)[a] (10% FCS / 50% NHS)
6	H	42 ± 7	2740 ± 480 -
12		51 ± 17	1030 ± 512 4980
13		32 ± 4	570 ± 223 2113
14		12 ± 4	591 ± 51 1700 ± 350
15		32 ± 12	173 ± 22 856 ± 353
16		17 ± 8	258 1370
17		21 ± 10	224 1129

[a] Values are the mean ± SD of at least three independent measurements. Values without SD are the average of at two independent measurements.

On the basis of its potency in cell-based assay, compound **33** was characterized further.

Against a panel of chimeric replicons containing different NS5B genotypes, **33** was an equally potent inhibitor of gt 1a (EC_{50} = 28 nM), 1b BK (EC_{50} = 9 nM), and 3a (EC_{50} = 37 nM) replicons, whereas it resulted 2 orders of magnitude less active on 2a (EC_{50} = 2018 nM) and 2b (EC_{50} = 1833 nM) replicons.[35] The lower sensitivity of the finger-loop inhibitors against HCV genotype 2b is due to differences in the amino acid composition of the binding pocket, resulting in a different shape between the two genotypes.[36]

Crystal Structure of 33 Bound to NS5B. Compound **33** was successfully soaked into crystals of a NS5B ΔC_{55} genotype 1b construct (Figure 2). As had been previously observed with the related indole and thienopyrrole inhibitors, the tetracyclic compound binds in the allosteric pocket in the thumb domain, which in the apoenzyme is occupied by a short α-helix protruding from the finger domain and displays the same binding mode.[37] The cyclohexyl ring is tightly bound in a lipophilic pocket, and the indole scaffold itself is accommodated mainly in a lipophilic environment with its carboxylate forming a salt bridge to Arg503. The dihedral angle between the C2-phenyl ring and the indole in the complex structure is 46°, as predicted form our docking studies. The amine containing side chain is pointing out into solvent, preferring an orientation which brings the basic amine into proximity of the indole caboxylate. The structure confirms the (*R*)-configuration on the 7-position of the benzoxazocine ring. In this configuration, the 2-(dimethylamino)ethyl]-(methyl)amino moiety occupies the pseudoequatorial position on the benzoxazocine-ring, which is calculated to be 13 kJ/mol lower in energy with respect to the corresponding pseudoaxial conformation which would be required for binding of the (*S*)-configurated epimer **34**.[38]

In the enantiomorphic pseudoequatorial conformation of the *S*-epimer, the ring would adopt an orientation incompatible with binding because it clashes with the enzyme surface. This might be the reason for the difference in activity for the enantiomers. In the oxygen-linked series, the more active isomer, compound **35**, has the side chain in the pseudoaxial position. We hypothesize that the pseudoaxial arrangement is favored by a gauche effect between the exocyclic O-atom and the endocyclic nitrogen and oxygen atoms. Sterically the ether O-atom is less disfavoring for the pseudoaxial arrangement than the sterically more demanding N-Me group.

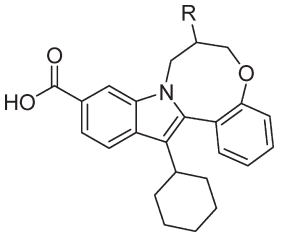
Animal Pharmacokinetics and Safety Assessment. The pharmacokinetic profile of compound **33** was evaluated in rats, dogs, and rhesus monkeys (Table 5). The compound displayed moderate plasma clearance in rats, dogs, and rhesus monkeys,

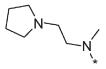
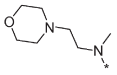
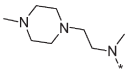
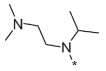
Table 2. Pharmacokinetic Profile for Compounds **2** and **15** Following Single Dose Administration to Rats[a] and Their Stability in Liver Microsome Preparations

	iv, 3 mg/kg			po, 3 mg/kg				
compd	Cl (mL/min/kg)	$t_{1/2}$ (h)	Vd (L/kg)	C_{max} (μM)	AUC (μM·h)	*F* (%)	rat [liver] 6 h (μM)	Cl_{int} r, d, h (μL/min/mg)
2	61	1.6	5.1	0.2	0.7	31	0.64	100, 26, 5
15	21	3.0	3.2	0.3	0.9	20	1.3	7, 7, 5

[a] Compounds **2** and **15** were dosed as their respective TFA salts to Sprague−Dawley rat, *n* = 3 (DMSO/PEG400/water 20%/60%/20%); po (PEG400).

Table 3. Enzymatic and Cell-Based Activity of Compounds **18**−**29**

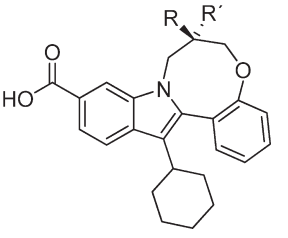


No.	R	IC_{50} (nM)[a]	EC_{50} (nM)[a]
15		32 ± 12	173 ± 22 856 ± 353
18		63 ± 26	2480 -
19		51 ± 29	766 ± 381 > 5000
28		47 ± 14	170 ± 63 1427 ± 73
29		31 ± 2	73 ± 12 743
21		15 ± 3	73 ± 24 313 ± 100
20		10 ± 6	120 ± 23 361 ± 95
22		9 ± 4	83 ± 25 550 ± 227
23		11 ± 3	99 ± 42 335 ± 122
24		10 ± 2	90 ± 37 312
25		8 ± 5	66 ± 23 581 ± 60
26		11 ± 4	153 1030
27		34 ± 11	138 1200

[a] Values are the mean ± SD of at least three independent measurements. Values without SD are the average of at two independent measurements.

with 22, 7, and 7 mL/min/kg, respectively. The volumes of distribution were 3.7 in rats, 2.6 in dogs, and 2.2 L/kg in monkeys. Terminal half-lives were 3.4 h in rats, 6.1 h in dogs, and 5.7 h in monkeys. The oral bioavailability of the amorphous bis-HCl salt was 49% in rats, 78% in dogs, and 35% in monkeys.

Table 4



No.	R	R'	IC_{50} (μM)[a]	EC_{50} (μM)[a]
33		H	6 ± 3	38 ± 13 110 ± 32
34	H		35 ± 7	338 1680
35	H		19 ± 4	78 ± 22 493 ± 149
36		H	72	432 1412
38			30 ± 19	338 ± 95 590
39			4 ± 1	80 ± 23 454 ± 254

[a] Values are the mean ± SD of at least three independent measurements. Values without SD are the average of at two independent measurements.

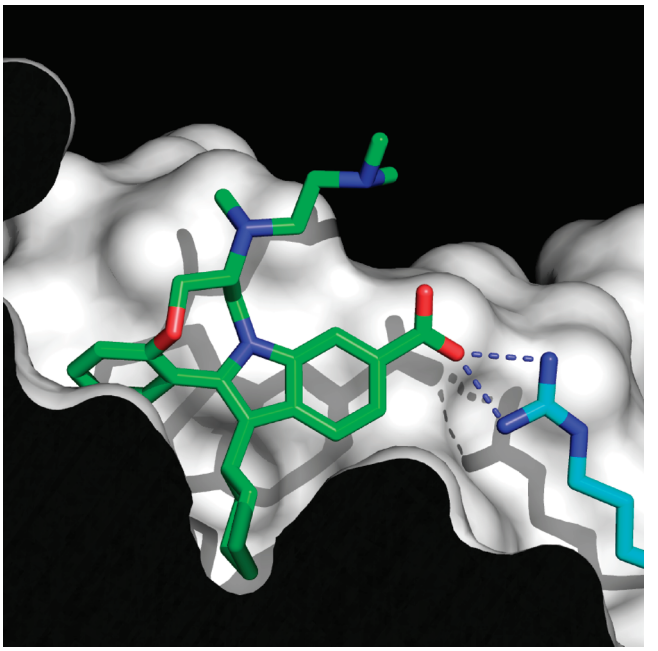


Figure 2. Crystal structure of HCV ΔC_{55} NS5B polymerase from genotype 1b in complex with compound **33**[37] (2.53 Å resolution, compound is in stick representation in green, with nitrogen in blue and oxygen in red). The lipophilic protein surface is in white and light gray, except for Arg503, shown in stick representation.

Table 5. Pharmacokinetic Profile of Compound **33** Following Single Dose Administration[a]

species	Cl (mL/min/kg)	Vd_{ss} (L/kg)	$t_{1/2}$ (h)	C_{max} (μM)	AUC_{po} (μM·h)	F (%)
rat	22 ± 3	3.7 ± 0.5	3.4 ± 0.1	0.5 ± 0.1	2.4 ± 0.1	49 ± 2
dog	7 ± 2	2.6 ± 0.4	6.1 ± 0.9	1.0	8.6	78
rhesus	7 ± 1.9	2.2 ± 0.6	5.7 ± 0.2	0.8 ± 0.5	7.4 ± 0.5	35 ± 12

[a] Compound **33** was dosed as the bis-HCl salt; rat, dog, and rhesus $n = 3$ (except dog po $n = 2$); iv 3, 1, and 2 mg/kg (DMSO/PEG400/water 20%/60%/20%); po 3, 2, and 4 mg/kg (PEG400 rat and dog, PEG400/water 70%/30% rhesus).

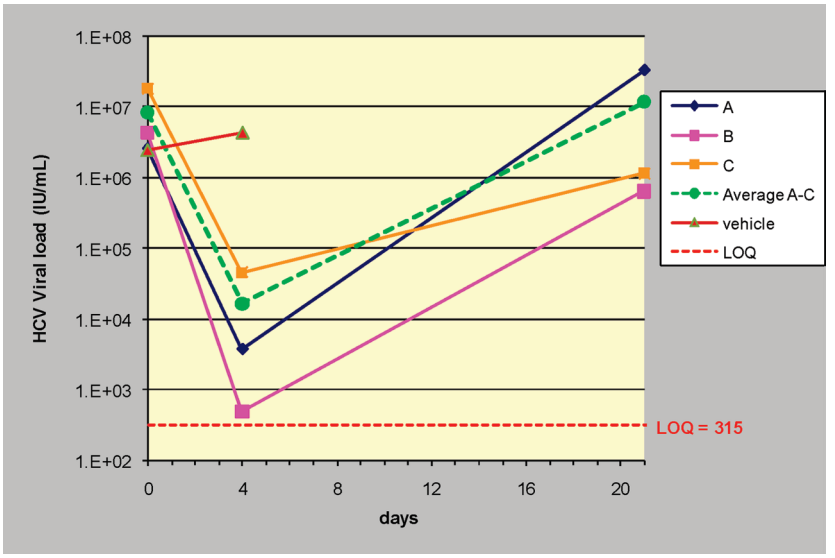


Figure 3. Antiviral response induced by treatment with compound **33** of humanized $uPA^{+/+}$-SCID mice (A, B, C) infected with HCV of genotype 1b. LOQ: lower limit of detection of HCV RNA (= 315 IU/mL). Vehicle = PEG400/water 30%/70%.

After an oral dose of 3 mg/kg to rat, liver concentrations 6 h after dosing of **33** were determined to be 0.42 ± 0.16 μM, which corresponds to a liver tissue to plasma concentration ratio of 5. The levels are 3-fold lower than those observed for compound **15** at the same dose, but they are 4-fold higher than the EC_{50} in 50% NHS, which compares favorably with compounds **2** and **15**, where this ratio is about 1 and 2, respectively.

Compound **33** displayed relatively high binding to rat (97%), dog (97.3%), monkey (92.6%), and human (96.9%) plasma proteins. NADPH-dependent metabolism was observed in liver microsomes, with low intrinsic clearance in dog, human, and rat, 3, 7, and 7 μL/min/mg, respectively. In liver microsomes supplemented with UDPGA, **33** exhibited very low turnover in all species, < 4 μL/min/mg.

The compound did not inhibit CYP3A4, CYP2D6, or CYP2C9 at concentrations up to 50 μM and was not a time-dependent inhibitor of human CYP3A4. In the PXR transactivation assay, **33** showed no potential for inducing CYP3A4.

In the PanLabs screen, only 2 off-target activities were seen for matrix metalloprotease 12 (IC_{50} = 8.3 μM) and protein kinase Cα (IC_{50} = 59 μM). Selectivity versus inhibition of NS5B is >1000-fold. The human DNA polymerases α, β, and γ were not inhibited at concentrations up to 50 μM.

Viral Response in HCV-Infected Chimeric Mice. The in vivo efficacy of **33** was demonstrated preclinically in a humanized mouse model for the study of viral hepatitis. T- and B-cell deficient mice carrying a tandem array of murine urokinase-type plasminogen activator transgenes under the control of the mouse albumin promoter (Alb/$uPA^{+/+}$-SCID) suffer from a severe liver disease and consequently are excellent recipients for primary human hepatocyte transplantation. Several weeks after transplantation, the liver of these mice can harbor up to 90% of well-organized, functional, and healthy human hepatocytes.[39] They can then be inoculated with infectious HCV particles of different origins and genotypes and support durable replication of the virus at levels equivalent to those seen in infected humans.[31a,40] This animal model has been validated with IFN-α, non-nucleoside inhibitors of the polymerase such as HCV-796 and pyrano-indole-based inhibitors, protease inhibitors such as BILN-2061, and entry inhibitors like anti-CD81 antibodies.[41]

In preparation of the experiment, SCID mice (n = 3) were dosed by intraperitoneal injection for five consecutive days twice daily with 50 mg/kg of compound **33**. The compound was well tolerated, and no gross changes in body or organ weight were observed. Twelve hours after administration of the last dose, plasma levels of compound were 3.6 μM. The same dose of 50 mg/kg of **33** was then administered by intraperitoneal injection to three humanized $uPA^{+/+}$-SCID mice infected with HCV of genotype 1b twice daily over the course of 4 days. Treatment caused a consistent decrease in the viremia in the three animals A−C (A, −2.84 $\log_{10}$; B, −3.94 $\log_{10}$; C, −2.60 $\log_{10}$), with a mean decrease of HCV plasma RNA levels of 3.13 ± 0.7 $\log_{10}$. The mean plasma trough concentration of **33** 12 h after administration of the last dose was similar in all three animals, 2.6 ± 0.2 μM. Viral titers rebounded after treatment withdrawal as measured on day 21 (Figure 3). Three mice were also dosed only with the vehicle (30% PEG400 + 70% water) for four days, and no decrease in viremia was observed (range 0−0.3 $\log_{10}$).

At a 5-fold lower dose of 10 mg/kg, the mean $C_{12\ h}$ of **33** was determined to be 0.67 ± 0.2 μM, and also at this dose an antiviral effect with a mean drop in HCV RNA of 1.4 ± 0.25 $\log_{10}$ was observed (data not shown).

Conclusion

In summary, a novel second-generation series of tetracyclic allosteric finger-loop inhibitors of the HCV NS5B polymerase

was identified. Exploration of the SAR around the amine side-chain on the benzoxazocine tether of compound **6** led to the identification of compound **33**, designated as MK-3281,[42] which combined excellent cell-based potency with good pharmacokinetic properties in preclinical species. The compound was efficacious in the chimeric mouse model of HCV infection, where at a twice daily dose of 10 mg/kg and 50 mg/kg bid a consistent drop in viremia of 1.4 $\log_{10}$ and 3.1 $\log_{10}$, respectively, was observed. On the basis of this promising profile, the compound was further characterized for efficacy in HCV-infected chimpanzee and advanced into phase I clinical trials.[43]

Experimental Section

Chemistry. Solvents and reagents were obtained from commercial suppliers and were used without further purification. Organic extracts were dried over sodium sulfate and were concentrated (after filtration of the drying agent) on rotary evaporators operating under reduced pressure. Flash chromatography purifications were performed on Merck silica gel (200−400 mesh) as the stationary phase or on commercial flash chromatography systems (Biotage Corporation and Jones Flashmaster) utilizing prepacked columns. Petroleum ether (PE) refers to the fraction boiling between 40 and 60 °C. ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 K (if not stated otherwise) in the indicated solvent on Bruker AMX spectrometers operating at the reported frequencies. Chemical shifts (δ) for signals corresponding to nonexchangeable protons (and exchangeable protons where visible) are recorded in parts per million (ppm) relative to tetramethylsilane or to the residual solvent peak. Signals are tabulated in the order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad, and combinations thereof); coupling constant(s) in hertz; number of protons. HPLC-MS data were obtained on an Acquity Waters UPLC operating in negative (ES^-) or positive (ES^+) ionization mode, and results are reported as the ratio of mass over charge (m/z) for the parent ion only. High resolving power accurate mass measurement electrospray (ES) mass spectral data were acquired by use of a Bruker Daltonics apex-Qe Fourier transform ion cyclotron resonance mass spetrometer (FT-ICR MS). External calibration was accomplished with oligomers of polypropylene. The purity of the final compounds was determined by analytical RP-HPLC with a Waters Alliance separation module 2695, using acetonitrile/water gradients (both modified with 0.1% TFA). Peaks were detected with a Waters 996 PDA detector using the Maxplot option (210−400 nM) contained in the PDA program. Method A: Waters Sunfire C18 column (2.1 mm × 50 mm, 3.5 μm), flow rate 0.5 mL/min; gradient 10% acetonitrile, 0.5 min isocratic, linear to 100% acetonitrile over 6 min, then isocratic. Method B: Waters Sunfire C18 column (4.6 mm × 150 mm, 5 μM), flow 1.0 mL/min; gradient 70% acetonitrile, 1 min isocratic, then linear to 70% acetonitrile over 10 min, then linear to 100% acetonitrile over 5 min and then isocratic. All compounds described in this article showed purities higher than 95% in both analytical methods, except for compound **14** (method A 99%, method B 91%) and **27** (method A 96.9%, method B 90.5%). A table with purity data and retention times can be found in the Supporting Information. Preparative scale HPLC separations were carried out on a Waters 2525 pump, equipped with a 2487 dual absorbance detector or on a mass-triggered automated Waters MassLynx purification system. Compounds were eluted with linear gradients of water and MeCN with water containing 0.1% TFA. Supercritical fluid chromatography (SFC) was performed on a Berger system equipped with a Berger ALS 719.

Methyl 3-Cyclohexyl-2-{2-[(2*S*)-oxiran-2-ylmethoxy]phenyl}-1*H*-indole-6-carboxylate (9a). To a solution of **8** (6.0 g, 17 mmol) in DMF (200 mL) were added cesium fluoride (7.74 g, 51 mmol) and (*S*)-glycidyl 3-nitrobenzenesulfonate (5.79 g, 22 mmol). The resulting mixture was stirred at RT overnight and then diluted with EtOAc and washed with water and brine. The crude product was purified by trituration with Et_2O to afford **9a** as a colorless foam (5.11 g, 74%); $[\alpha]_D^{20} = +22°$ (c = 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.14−1.40 (m, 3H), 1.62−1.80 (m, 5H), 1.81−1.96 (m, 2H), 2.58−2.68 (m, 1H), 2.59 (dd, J = 2.5, 5.0 Hz, 1H), 2.76 (t, J = 5.0 Hz, 1H), 3.19−3.25 (m, 1H), 3.85 (s, 3H), 3.91 (dd, J = 6.2, 11.6 Hz, 1H), 4.33 (dd, J = 2.7, 11.6 Hz, 1H), 7.11 (t, J = 7.4 Hz, 1H), 7.20 (d, J = 8.3 Hz, 1H), 7.32 (dd, J = 1.6, 7.4 Hz, 1H), 7.44 (dt, J = 1.6, 8.3 Hz, 1H), 7.58 (dd, J = 1.3, 8.4 Hz, 1H), 7.79 (d, J = 8.4 Hz, 1H), 7.98 (d, J = 1.2 Hz, 1H), 11.28 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.3, 27.07, 27.1, 33.0, 33.1, 36.6, 44.6, 50.3, 51.8, 68.4, 113.2, 113.5, 119.5, 120.0, 120.2, 121.7, 122.1, 122.9, 130.4, 129.6, 131.7, 133.8, 135.4, 156.2, 168.3. MS (ES^+) m/z 406.5 $(M + H)^+$.

Methyl (7*S*)-14-Cyclohexyl-7-hydroxy-7,8-dihydro-6*H*-indolo-[1,2-*e*][1,5]benzoxazocine-11-carboxylate (10a).[44] A solution of **9a** (4.36 g, 10.7 mmol) in dioxane (220 mL) was treated with Cs_2CO_3 (7.0 g, 21.5 mmol), and the resulting suspension heated to reflux for 48 h. The reaction was cooled to RT, diluted with EtOAc, and washed with water and brine. The crude product was purified by flash chromatography on silica gel (DCM/MeOH 98:2) to afford **10a** as a colorless powder (2.38 g, 55%); $[\alpha]_D^{20} = -28°$ (c = 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, DMSO-d_6, 2 diastereomers, 2:1*) δ 1.02−1.40 (m, 3H), 1.48−1.59 (m, 1H), 1.60−1.78 (m, 2H), 1.79−1.90 (m, 1H), 1.90−2.06 (m, 3H), 2.60−2.78 (m, 1H), 3.63 (dd, J = 10.6, 14.7 Hz, 1H), 3.68 (dd, J = 8.2, 12.0 Hz, 1H), 3.77* (d, J = 12.7 Hz, 1H), 3.80−3.83 (m, 1H), 3.86*, 3.89 (s, 3H), 3.90−4.06* (m, 3 H), 4.32 (dd, J = 4.4, 12.0 Hz, 1H), 4.40 (dd, J = 1.0, 14.7 Hz, 1H), 4.58* (dd, J = 1.3, 12.7 Hz, 1H), 5.24*, 5.66 (d, J = 3.0*, 4.8 Hz, 1H, OH), 7.15−7.33 (m, 3H), 7.47*, 7.52 (dt, J = 1.7*, 8.4*, 1.9, 8.5 Hz, 1H), 7.61*, 7.68 (d, J = 8.4*, 8.5 Hz, 1H), 7.84*, 7.89 (d, J = 8.4*, 8.5 Hz, 1H), 8.20, 8.22* (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.2, 26.9, 27.0, 33.0, 33.1, 33.3*, 33.4*, 36.6, 46.2, 46.5*, 51.9, 68.1*, 68.4, 72.7*, 78.5, 111.3*, 112.9, 119.4*, 119.9, 120.0*, 120.03, 120.3*, 120.4, 121.0, 122.3, 123.0*, 123.3*, 123.7, 130.1*, 130.4, 130.7*, 131.0, 132.2, 133.2*, 135.8, 136.9*, 137.4*, 138.0, 158.6*, 159.0, 168.2. MS (ES^+) m/z 406.6 $(M + H)^+$.

Methyl 14-Cyclohexyl-7-oxo-7,8-dihydro-6*H*-indolo[1,2-*e*]-[1,5]benzoxazocine-11-carboxylate (11). A solution of **10a** (3.59 g, 8.85 mmol) in DCM (85 mL) was treated with Dess−Martin periodinane (4.5 g, 10.6 mmol), and the mixture was stirred for 1 h at RT. At this point, another 0.45 g of DMP (1.06 mmol) were added and the reaction left overnight. The reaction mixture was diluted with EtOAc and washed with an aqueous solution of a 1:1 mixture containing sodium thiosulfate and sodium hydrogen carbonate (both saturated solutions) and then with water and brine. Drying over Na_2SO_4, filtration, and concentration in vacuo gave crude **11** (3.4 g, 95%), which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30−1.48 (m, 3H), 1.71−1.85 (m, 3H), 1.86−2.03 (m, 4H), 2.73−2.89 (m, 1H), 3.95 (s, 3H), 4.46 (d, J = 16.7 Hz, 1H), 4.62 (d, J = 17.2 Hz, 1H), 4.74 (d, J = 17.2 Hz, 1H), 4.84 (d, J = 16.7 Hz, 1H), 7.30 (d, J = 8.1 Hz, 1H), 7.34 (dt, J = 0.9, 7.6 Hz, 1H), 7.43 (dd, J = 0.8, 7.6 Hz, 1H), 7.53 (dt, J = 1.8, 8.1 Hz, 1H), 7.82 (dd, J = 1.2, 8.6 Hz, 1H), 7.87 (d, J = 8.6 Hz, 1H), 8.07 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.2, 26.9, 27.0, 33.3, 33.5, 36.6, 52.0, 53.7, 77.8, 111.4, 120.5, 120.7, 121.1, 121.4, 121.6, 123.8, 125.0, 130.5, 131.3, 131.5, 136.3, 137.0, 157.4, 167.9, 202.6. MS (ES^+) m/z 404.5 $(M + H)^+$.

General Procedure for the Reductive Amination of Ketone 11 and Ester Hydrolysis: 14-Cyclohexyl-7-(methylamino)-7,8-dihydro-6*H*-indolo[1,2-*e*][1,5]benzoxazocine-11-carboxylic Acid (12). To a suspension of **11** (40 mg, 0.1 mmol) in 1,2-dichloroethane (2 mL) was added acetic acid (0.008 mL, 0.15 mmol) and methylamine (0.14 mL, 1 M in THF). After stirring for 15 min at RT, sodium triacetoxyborohydride (32 mg, 0.15 mmol) was added in one portion. The resulting mixture was stirred at RT for 2 h. Sodium hydroxide was added (2 mL, 1N) and after stirring for 5 min the mixture was taken into EtOAc. The phases were separated, and the organic phase was washed with water and brine. The crude

methyl ester was used without further purification. The ester was dissolved in a mixture of THF and methanol (2 mL, 1:1 v/v) and treated with 1 N KOH (1 mL). The resulting solution was heated to 60 °C for 4 h. After cooling to RT, the reaction mixture was brought to pH 2 by the dropwise addition of hydrochloric acid (1 N) and then diluted with MeCN and purified by RP-HPLC to afford **12** as its TFA salt (31 mg, 58%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.07−1.28 (m, 1H), 1.30−1.46 (m, 2H), 1.56 (bd, J = 11.9 Hz, 1H), 1.65−1.79 (m, 2H), 1.80−2.09, m, 4H), 2.61−2.80 (m, 1H), 2.77 (s, 3H), 3.57−3.71 (m, 1H), 3.91 (bt, J = 12.8 Hz, 1H), 4.08−4.27 (m, 2H), 4.85 (d, J = 14.4 Hz, 1H), 7.22−7.35 (m, 3H), 7.53 (dt, J = 2.1, 7.9 Hz, 1H), 7.72 (d, J = 8.6 Hz, 1H), 7.91 (d, J = 8.6 Hz, 1H), 8.30 (s, 1H), 8.94 (bs, 1H), 12.68 (bs, 1H). ^{13}C NMR (75 MHz, DMSO-d_6, 320 K) δ 25.6, 26.6, 30.8, 32.6, 36.4, 41.3, 55.4, 67.8, 11.9, 119.1, 119.3, 120.1, 120.3, 121.3, 123.1, 124.2, 129.7, 131.1, 132.7, 135.4, 136.7, 157.9, 168.2. MS (ES$^+$) m/z 405.3 (M + H)$^+$. HRMS (M + H)$^+$ calcd for $C_{25}H_{29}N_2O_3$ 405.2178; found 405.218.

14-Cyclohexyl-7-{[2-(dimethylamino)ethyl]amino}-7,8-dihydro-6*H*-indolo[1,2-*e*][1,5]benzoxazocine-11-carboxylic Acid (15). Compound **15** was prepared following the procedure for the synthesis of **12** from **11** (100 mg, 0.25 mmol) and *N*,*N*-dimethylethane-1,2-diamine (0.033 mL, 0.30 mmol). HPLC analysis of the crude methyl ester (120 mg, quantitative) indicated a ratio of ester to racemic alcohol **10** of about 9:1; MS (ES$^+$) m/z 476 (M + H)$^+$. Hydrolysis and purification by RP-HPLC afforded **15** as its bis-TFA salt (109 mg). ^{1}H NMR (400 MHz, DMSO-d_6, 2 atropisomers 7:1*) δ 1.09−1.22 (m, 1H), 1.25−1.46 (m, 2H), 1.50−1.58 (m, 1H), 1.65−1.78 (m, 2H), 1.82−1.88 (m, 1H), 1.89−2.07 (m, 3H), 2.64−2.75 (m, 1H), 2.79*, 2.82 (s, 6H), 2.98−3.10 (m, 1H), 3.10−3.90 (m, 5H, together with water peak and probably the three exchangeable protons), 3.96−4.02 (m, 1H), 4.18, 4.32* (dd, J = 3.0, 12.6 Hz, 1H), 4.74, 4.84* (d, J = 11.9 Hz, 1H), 7.22−7.28 (m, 2H), 7.30 (dd, J = 1.9, 7.9 Hz, 1H), 7.52 (dt, J = 1.9, 8.5 Hz, 1H), 7.64*, 7.70 (dd, J = 1.0, 8.4 Hz, 1H), 7.86*, 7.99 (d, J = 8.5*, 8.4 Hz, 1H), 8.22, 8.35* (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6, 320 K, only data for the major atropisomer shown) δ 25.6, 26.7, 32.7, 36.4, 40.4, 42.9, 43.3, 54.4, 55.5, 70.3, 111.6, 118.9, 119.8, 120.06, 120.08, 121.3, 122.9, 124.0, 129.6, 131.1, 132.6, 135.4, 136.9, 158.3, 168.2. MS (ES$^+$) m/z 462.3 (M + H)$^+$. HRMS (M + H)$^+$ calcd for $C_{28}H_{36}N_3O_3$ 462.2757; found 462.2766.

14-Cyclohexyl-7-{[2-(dimethylamino)ethyl](methyl)amino}-7,8-dihydro-6*H*-indolo[1,2-*e*][1,5]benzoxazocine-11-carboxylic Acid (21). To a solution of crude methyl ester of compound **15** (100 mg, 0.21 mmol) in DCM (2 mL) were added aq formaldehyde (37%, 0.050 mL, 0.63 mmol) and acetic acid (0.018 mL, 0.32 mmol), followed after 5 min by solid sodium cyanoborohydride (20 mg, 0.32 mmol). After stirring at RT for 2 h, sodium hydroxide (4 mL, 1 N) was added and the resulting suspension stirred for another 5 min before being taken into EtOAc and washed with water and brine. Drying over sodium sulfate and concentration in vacuo gave the crude ester, which was used without any further purification. MS (ES$^+$) m/z 490 (M + H)$^+$. The ester was dissolved in a mixture of dioxane/water (2 mL, 2:1, v/v), and aq potassium hydroxide (1 mL, 1N) was added. The mixture was stirred at 70 °C for 6 h and then brought to pH 2 by the dropwise addition of hydrochloric acid (1 N) and then diluted with MeCN. After purification by RP-HPLC, **21** was obtained as its bis-TFA salt (66 mg, 45%). For proton and carbon spectra, see compound **33**. MS (ES$^+$) m/z 476.5 (M + H)$^+$. HRMS (M + H)$^+$ calcd for $C_{29}H_{38}N_3O_3$ 476.2913; found 476.2924.

14-Cyclohexyl-7-[(*N*,*N*-dimethylglycyl)(methyl)amino]-7,8-dihydro-6*H*-indolo[1,2-*e*][1,5]benzoxazocine-11-carboxylic Acid (29). To a solution of **12** (23 mg, 0.044 mmol) in DCM (0.5 mL) was added *N*,*N*-diisopropylethylamine (0.015 mL, 0.088 mmol), followed by a solution of *N*,*N*-dimethylglycine (18 mg, 0.18 mmol), HATU (67 mg, 0.18 mmol), and *N*,*N*-diisopropylethylamine (38 μL, 0.22 mmol) in dichloromethane (1 mL). The reaction mixture was concentrated in vacuo after 2 h and dissolved in acetonitrile (1 mL). Potassium hydroxide (1 N, 0.31 mL, 0.310 mmol) was added, and the mixture was stirred at 45 °C for three days to destroy the mixed anhydride. The reaction mixture was concentrated in vacuo, dissolved in DMSO, and purified by RP-HPLC to afford **29** as its TFA salt (16 mg, 58%). ^{1}H NMR (400 MHz, DMSO-d_6, two atropisomers 1:1) δ 1.09−1.44 (m, 3H), 1.47−1.59 (m, 1H), 1.63−2.03 (m, 6H), 2.26 (s, 1.5 H), 2.62−2.94 (m, 7H), 2.97 (s, 1.5 H), 3.81 (d, J = 17.0, 0.5H), 3.90 (d, J = 15.4, 0.5H), 4.13 (d, J = 17.0, 0.5H), 4.15−4.39 (m, 3.5H), 4.46 (dd, J = 14.0, 4.3, 0.5H), 4.56 (d, J = 14.6, 0.5H), 4.62−4.72 (m, 1H), 7.31−7.39 (m, 3H), 7.54−7.63 (m, 1H), 7.65−7.70 (m, 1H), 7.76 (bs, 0.5H), 7.87−7.92 (m, 1H), 8.10 (bs, 0.5H), 9.50−9.79 (bm, 1H), 12.51−12.82 (bs, 1H). ^{13}C NMR (100 MHz, CD_3OD) 27.3, 28.2, 31.3, 34.5, 34.7, 38.3, 44.2, 45.2, 54.3, 59.6, 78.1, 111.9, 121.1, 121.6, 123.8, 125.9, 127.1, 131.6, 132.7, 132.8, 138.3, 138.4, 161.2, 166.6, 171.2. MS (ES$^+$) m/z 490.4 (M + H)$^+$. HRMS (M + H)$^+$ calcd for $C_{29}H_{35}N_3O_4$ 490.2706; found 490.2717.

Methyl (7*S*)-14-Cyclohexyl-7-{[(4-methylphenyl)sulfonyl]oxy}-7,8-dihydro-6*H*-indolo[1,2-*e*][1,5]benzoxazocine-11-carboxylate (30a). To a solution of **10a** (325 mg, 0.802 mmol) in pyridine (10 mL), tosyl chloride (229 mg, 1.203 mmol) was added and the reaction was stirred at room temperature overnight. The reaction mixture was diluted with EtOAc and washed with hydrochloric acid (1 N), sodium hydrogen carbonate (saturated solution), and brine. The crude product was purified by flash chromatography on silica gel (PE/EtOAc 9:1) to afford **30a** as a pale-yellow oil (423 mg, 94%). ^{1}H NMR (400 MHz, $CDCl_3$, 2 atropisomers 7:3) δ 1.29−1.44 (m, 3H), 1.58−1.68 (m, 1H) 1.69−1.81 (m, 2H), 1.82−2.09 (m, 4H), 2.43*(s, 3H*), 2.46 (s, 3H), 2.68−2.80 (m, 1H), 3.92 (dd, J = 6.6, 12.8, 1H), 3.97* (s, 3H*), 4.01 (s, 3H), 4.03−4.17 (m, 2H), 4.26* (dd, J = 6.5, 12.8, 1H*), 4.51−4.66 (m, 2H), 4.73* (dd, J = 2.4, 16.0, 1H*), 4.98−5.55* (bs, 1H*), 7.05−7.28 (m, 3H, partially overlapped with $CHCl_3$ peak), 7.31* (d, J = 8.1, 2H*), 7.39 (d, J = 8.1, 2H), 7.37−7.47 (m, 1H), 7.75* (d, J = 8.3, 1H*), 7.79−7.87 (m, 2H + 1H*), 7.90 (d, J = 8.3, 2H), 8.18 (s, 1H), 8.21* (s, 1H*). MS (ES$^+$) m/z 560 (M + H)$^+$.

Methyl (7*R*)-7-Azido-14-cyclohexyl-7,8-dihydro-6*H*-indolo-[1,2-*e*][1,5]benzoxazocine-11-carboxylate (31a). To a solution of **30a** (423 mg, 0.757 mmol) in anhyd THF (12 mL), azidotrimethylsilane (0.26 mL, 2.06 mmol) and tetrabutylammonium triphenyldifluorosilicate (1.140 g, 2.112 mmol) were added at RT. The reaction was stirred for 20 h at 65 °C, and then more azidotrimethylsilane (0.26 mL, 2.06 mmol) and tetrabutylammonium triphenyldifluorosilicate (1.140 g, 2.112 mmol) were added. The mixture was stirred at 65 °C for another 36 h. The volatiles were evaporated in vacuo, and the residue was dissolved in EtOAc and washed with hydrochloric acid (1 N), sodium hydrogen carbonate (saturated solution) and brine. The crude product was purified by flash chromatography on silica gel (PE/EtOAc 95:5) to afford azide **31a** as white foam (210 mg, 62%). ^{1}H NMR (400 MHz, $CDCl_3$, atropisomers 6:4*) δ 1.14−1.31 (m, 1H), 1.32−1.45 (m, 2H), 1.62−1.72(m, 1H), 1.73−1.83 (m, 2H), 1.84−2.13 (m, 4H), 2.69−2.84 (m, 1H), 3.58−3.66* (m, 1H*), 3.67−3.76 (m, 1H), 3.93−4.03 (m, 2H), 3.99 (s, 3H), 4.06* (d, J = 11.8, 1H*), 4.19−4.27 (m, 1H), 4.58 (dd, J = 3.9, 15.1, 1H), 4.63* (dd, J = 3.5, 12.9, 1H*), 4.71* (dd, J = 4.6, 15.6, 1H*), 7.13−7.32 (m, 3H), 7.40−7.50 (m, 1H), 7.75−7.84 (m,1H), 7.89 (d, J = 8.6,1H), 8.13 (s, 1H), 8.29* (s, 1H*). MS (ES$^+$) m/z 431 (M + H)$^+$. SFC chromatography indicated an ee of 99.3% (Chiracel OJ-H 250 μm × 1.0, 5 μm; T_{col} 35 °C, P_{col} 100 bar, flow 9.99 mL/min, modifier MeOH, 0.2% diethylamine (A); gradient 0−1 min 20% A, 1−6.7 min to 60% A, then another 2 min isocratic.

Methyl (7*R*)-7-Amino-14-cyclohexyl-7,8-dihydro-6*H*-indolo-[1,2-*e*][1,5]benzoxazocine-11-carboxylate (32a). A solution of **31a** (210 mg, 0.520 mmol) in methanol (10 mL) containing Pd/C (10%, w/w, 30 mg) was stirred under hydrogen at atmospheric pressure for 4 h. The catalyst was filtered off, and the solution was concentrated to dryness under reduced pressure to afford **32a** (192 mg, 92%). ^{1}H NMR (400 MHz, $CDCl_3$, 2 atropisomers 6:4*) δ 1.14−1.30 (m,1H), 1.31−1.52 (m, 2H), 1.58−1.70 (m, 1H), 1.71−1.86 (m,

2H), 1.87−2.26 (m, 4H), 2.68−2.89 (m, 1H), 3.44* (s, 3H*), 3.68−3.84 (bs, 4H), 3.85−4.02* (bs, 1H*), 4.03−4.25 (m, 3H + 1H*), 4.31* (dd, J = 2.9, 13.6, 1H*), 4.89 (t, J = 12.9, 1H + 1H*), 5.02* (dd, J = 3.8, 16.3, 1H*), 7.00−7.12 (m, 1H + 2H*), 7.19 (dd, J = 1.4, 7.6, 1H), 7.22−7.31 (m, 2H, partialy overlapped with $CHCl_3$ peak), 7.34* (t, J = 7.5, 1H*), 7.43* (d, J = 8.6, 1H*), 7.47−7.55* (m, 1H*), 7.70−7.78 (m, 1H), 7.85 (d, J = 8.6, 1H), 8.08 (s, 1H), 8.27* (s, 1H*). MS (ES^+) m/z 405 $(M + H)^+$; $[\alpha]_D^{20}$ = +46.4 (c = 1.0, $CHCl_3$).

(7*R*)-14-Cyclohexyl-7-{[2-(dimethylamino)ethyl](methyl)amino}-7,8-dihydro-6*H*-indolo[1,2-*e*][1,5]benzoxazocine-11-carboxylic Acid (33). To a solution of **32a** (868 mg, 2.146 mmol) in anhyd trimethyl orthoformate (6 mL) was added *tert*-butyl *N*-(2-oxoethyl)-carbamate (359 mg, 2.25 mmol), and the mixture was stirred at room temperature overnight. The solution was concentrated to dryness under reduced pressure and the crude dissolved in dry methanol (9 mL). The resulting solution was treated with acetic acid (0.37 mL, 6.44 mmol) and sodium cyanoborohydride (202 mg, 3.22 mmol). The mixture was stirred at room temperature for 1 h. Sodium hydroxide (2 mL, 1 N) was added, and after stirring for 5 min the mixture was taken into EtOAc and washed with water and brine. The crude product was purified by flash chromatography on silica gel (1:4 EtOAc + 0.1% triethylamine/petroleum ether + 0.1%triethylamine; then 6/4) to afford methyl (7*R*)-7-({2-[(*tert*-butoxycarbonyl)amino]ethyl}amino)-14-cyclohexyl-7,8-dihydro-6*H*-indolo[1,2-*e*][1,5]benzoxazocine-11-carboxylate as colorless foam (524 mg, 46%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.05−1.50 (m, 12H), 1.51−1.62 (m, 1H), 1.64−1.78 (m, 2H), 1.79−1.88 (m, 1H), 1.89−2.07 (m, 3H), 2.73−2.77 (m, 1H), 3.08−3.40 (m, 5H), 3.65−3.84 (m, 1H), 3.89 (s, 3H), 3.84−4.03 (m, 1H), 4.11−4.29 (m, 2H), 4.91 (dd, J = 3.0, 14.8, 1H), 7.02−7.12 (bt, 1H), 7.15−7.36 (m, 3H), 7.48−7.58 (m, 1H), 7.72 (dd, J = 1.1, 8.3, 1H), 7.93 (d, J = 8.6, 1H), 8.33 (s, 1H). MS (ES^+) m/z 548 $(M + H)^+$. This compound (524 mg, 0.956 mmol) was dissolved in a mixture of dichloromethane/trifluoroacetic acid (3:1, v/v, 8 mL), and the mixture was stirred at room temperature. The solution was concentrated to dryness under reduced pressure. The residue was dissolved in dichloromethane and sodium acetate (470 mg, 5.736 mmol), and 40% aq solution of formaldehyde (0.46 mL, 5.74 mmol) and sodium cyanoborohydride (356 mg, 5.74 mmol) were added. After stirring at RT for 1 H, sodium hydroxide (2 mL, 1 N) was added, and after stirring for 5 min, the mixture was taken into EtOAc and washed with brine. Drying over sodium sulfate and concentration in vacuo gave methyl (7*R*)-14-cyclohexyl-7-{[2-(dimethylamino)ethyl](methyl)-amino}-7,8-dihydro-6*H*-indolo[1,2-*e*][1,5]benzoxazocine-11-carboxylate, which was used without any further purification. MS (ES^+) m/z 490 $(M + H)^+$. To a solution of the crude product (280 mg, 0.572 mmol) in dioxane (12 mL) 1N KOH (3 mL, 1N in water) was added and the mixture was stirred at 80 °C for 2 h. Evaporation of the volatiles gave a residue, which was purified by RP-HPLC (column Waters Symmetry prep. C18, 7 μm, 19 mm × 300 mm). Fractions containing the pure compound were combined and freeze-dried to afford the bis-TFA salt of **33** as a white powder (123 mg, 45% over two steps). ^{1}H NMR (400 MHz, DMSO-d_6, 2 atropisomers 94:6*) δ 1.09−1.25 (m, 1H), 1.26−1.43 (m, 2H), 1.54 (d, J = 12.2, 1H), 1.62−1.78 (m, 2H), 1.79−2.06 (m, 4H), 2.39 (s, 3H), 2.61*(s, 6H*), 2.65−2.77 (m, 1H), 2.85 (s, 6H), 2.94 (dt, J = 5.5, 13.6, 1H), 3.04−3.15 (m, 2H), 3.18 (dt, J = 5.6, 13.0, 1H), 3.23−3.34 (m, 1H), 3.74−3.80* (m, 1H* partially overlapped with the signal at 3.85 ppm), 3.85 (dd, J = 10.2, 14.4, 1 H), 4.07 (dd, J = 9.0, 12.1, 1H), 4.11−4.17* (m, 1H* partially overlapped with the signal at 4.07 ppm), 4.31 (dd, J = 4.3, 12.1, 1H), 4.64 (d, J = 14.4, 1H), 4.75* (d, J = 13.1, 1H*), 4.79−4.87* (m, 1H*), 7.24−7.37 (m, 3H), 7.50−7.59 (m, 1H), 7.63−7.66* (m, 1H* partially overlapped with the signal at 7.69), 7.69 (dd, J = 0.8, 8.3, 1H), 7.82−7.85* (m, 1H* partially overlapped with dd at 7.87 ppm), 7.87 (d, J = 8.3, 1H), 8.15, 8.32* (s, 1H). ^{13}C NMR (100 MHz; DMSO-d_6, 320 K, only data for the major atropisomer reported) δ 25.7, 26.4, 30.3, 32.4, 36.1, 37.5, 42.0, 47.6, 50.2, 61.1, 73.2, 111.7, 119.1, 119.3, 119.9, 120.1, 121.4, 123.2, 123.9, 129.4, 131.1, 132.4, 135.2, 136.4, 157.5, 168.4. MS (ES^+) m/z 476 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{29}H_{38}N_3O_3$ 476.2913; found 476.2922. Chromatography on Chiralpak AD column gave an ee of 96% (250 mm × 4.6 mm, 5 μm; flow 1.0 mL/min, isocratic 70:30; eluent A 0.2% TFA/*n*-hexane; eluent B 0.2% TFA in EtOH (containing 3% MeOH); 300 nm; R_t 5.37 min. $[\alpha]_D^{20}$ = +36.8 (c = 0.5, $CHCl_3$).

(7*S*)-14-Cyclohexyl-7-{[2-(dimethylamino)ethyl](methyl)amino}-7,8-dihydro-6*H*-indolo[1,2-*e*][1,5]benzoxazocine-11-carboxylic Acid (34). The compound was obtained as described for its enantiomer **33** starting from alcohol **10b**. HRMS $(M + H)^+$ calcd for $C_{29}H_{38}N_3O_3$ 476.2913; found 476.2918. Chromatography on Chiralpak AD column (conditions see compound **33**) gave an ee of 99%; RT 14.55 min. $[\alpha]_D^{20}$ = −40 (c = 0.1, $CHCl_3$).

(7*S*)-14-Cyclohexyl-7-[2-(dimethylamino)ethoxy]-7,8-dihydro-6*H*-indolo[1,2-*e*][1,5]benzoxazocine-11-carboxylic Acid (35). To a suspension of **10a** (49 mg, 0.12 mmol) in toluene (2.4 mL) was added 30% w/w aq NaOH (0.16 mL, 1.20 mmol), followed by tetrabutylammonium bromide (10 mg, 0.03 mmol). After stirring for 30 min, 2-chloro-*N*,*N*-dimethylethanaminium chloride (35 mg, 0.24 mmol) was added and the reaction mixture was stirred at 60 °C for 16 h. More 2-chloro-*N*,*N*-dimethylethanaminium chloride (17 mg, 0.12 mmol) was added, and the reaction mixture was stirred at 80 °C for a further 4 h. The reaction mixture was concentration in vacuo, dissolved in DMSO, and purified by RP-HPLC to afford the TFA salt of **35** as a white powder (33 mg, 47%). ^{1}H NMR (400 MHz, DMSO-d_6, two atropisomers 1:1) δ 1.08−1.23 (m, 1H), 1.24−1.44 (m, 2H), 1.49−1.61 (m, 1H), 1.64−1.77 (m, 2H), 1.80−1.88 (m, 1H), 1.89−2.07 (m, 3H), 2.56 (s, 1.5 H), 2.63−2.73 (m, 1H), 2.78 (s, 1.5H), 3.07 (m, 1H), 3.27 (m, 1H), 3.68 (dd, J = 10.7, 14.5, 0.5H), 3.75−3.97 (m, 3H), 3.98−4.14 (m, 1.5H), 4.24 (dd, J = 4.2, 13.2, 0.5H), 4.77 (dd, J = 2.7, 14.6, 0.5H), 4.93 (dd, J = 3.1, 15.6, 0.5H), 7.16−7.34 (m, 3H), 7.49 (d, J = 6.8, 0.5H), 7.50 (d, J = 6.8, 0.5H), 7.62 (d, J = 8.1, 0.5H), 7.69 (d, J = 8.3, 0.5H), 7.82 (d, J = 8.6, 0.5H), 7.89 (d, J = 8.3, 0.5H), 8.20 (s, 0.5H), 8.21 (s, 0.5H). ^{13}C NMR (150 MHz, CD_3OD, 300 K, only signals for major atropisomer reported) δ 26.9, 27.8, 34.1, 38.0, 43.3, 44.6, 57.8, 63.9, 72.8, 78.0, 113.9, 120.0, 120.4, 120.8, 122.5, 123.9, 124.0, 124.5, 130.9, 131.9, 133.4, 138.3, 159.6, 171.4. MS (ES^+) m/z 463 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{28}H_{35}N_2O_4$ 463.2597; found 463.2591; $[\alpha]_D^{20}$ = −41.2 (c = 0.31, CH_3OH).

Methyl 14′-Cyclohexyl-2,2-dimethylspiro[1,3-dioxane-5,7′-indolo[1,2-*e*][1,5]benzoxazocine]-11-carboxylate (37). NaH (151 mg, 6.30 mmol, 60% dispersion in mineral oil) was added to a degassed solution of **8** (440 mg, 1.26 mmol) in DMF (10 mL). The suspension was allowed to stir at RT for 20 min, and the resulting solution was placed in an oil bath preheated at 70 °C. A degassed solution of 5,5-bis(bromomethyl)-2,2-dimethyl-1,3-dioxane (0.57 g, 1.89 mmol), prepared as described in ref 34 in dry DMF (6 mL), was added and the mixture was stirred for at 70 °C for 1 h. Additional electrophile (0.57 g, 1.89 mmol) was added in the same way, and stirring was continued at 70 °C for another 3 h. After cooling to RT, the reaction was quenched with an aq satd NH_4Cl, acidified with 1N HCl and extracted with Et_2O. The crude material was purified by chromatography (PE/EtOAc 5:1) to afford the **37** (50%) as the first fraction (second fraction: recovered **8**, 44%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21−1.38 (m, 3H), 1.45 (s, 3H), 1.66 (s, 3H), 1.68−2.11 (m, 7H), 2.72−2.80 (m, 1H), 3.49 (d, J = 12.3 Hz, 1H), 3.59 (d, J = 11.8 Hz, 1H), 3.69 (d, J = 12.3 Hz, 1H), 3.72 (d, J = 15.3 Hz, 1H), 3.77 (d, J = 12.5 Hz, 1H), 3.83 (d, J = 11.8 Hz, 1H), 3.93 (s, 3H), 4.15 (d, J = 12.5 Hz, 1H), 4.79 (d, J = 15.3 Hz, 1H), 7.13−7.17 (m, 2H), 7.23 (dd, J = 7.8, 1.7 Hz, 1H), 7.41 (bt, J = 7.8 Hz, 1H), 7.74 (dd, J = 8.5, 1.3 Hz, 1H), 7.84 (d, J = 8.5 Hz, 1H), 8.44 (s, 1H). MS (ES^+) m/z 490 $(M + H)^+$.

14′-Cyclohexyl-1-[2-(dimethylamino)ethyl]spiro[azetidine-3,7′-indolo[1,2-*e*][1,5]benzoxazocine]-11′-carboxylic Acid (39). To a suspension of **37** (135 mg, 0.28 mmol) in a mixture of MeOH and

THF (1:2, v/v, 12 mL) was added TsOH·H_2O (6 mg, 0.03 mmol), and the solution was stirred at RT for 3 h. Filtration over a pad of neutral alumina using EtOAc as eluent afforded, after evaporation of the solvent, methyl 14-cyclohexyl-7,7-bis(hydroxymethyl)-7,8-dihydro-6*H*-indolo[1,2-*e*][1,5]benzoxazocine-11-carboxylate (110 mg, 89%). MS (ES^+) *m*/*z* 450.4 $(M + H)^+$. Triflic anhydride (220 mg, 0.78 mmol) was added at 0 °C to a solution of the diol (100 mg, 0.22 mmol) in anhyd MeCN (4 mL). DIPEA (0.16 mL, 0.89 mmol) was then added, and the mixture was stirred at 0 °C for 15 min. Another 0.16 mL of DIPEA were added, followed by *N*,*N*-dimethylethane-1,2-diamine (0.049 mL, 0.445 mmol). The mixture was stirred at 70 °C for 2 h. After removal of the solvent, EtOAc was added, and the organic phase was washed with water and brine. After drying and evaporation, the residue was dissolved in dioxane (2 mL) and 1N KOH (1 mL) was added. After heating to 75 °C for 2 h, the reaction was cooled to 0 °C, acidified with 1N HCl, and purified by RP-HPLC to afford the bis-TFA salt of **39** as a colorless powder (50 mg, 25%). ^{1}H NMR (300 MHz, CD_3OD) δ 1.18−1.27 (m, 1H), 1.43 (bt, J = 9.9 Hz, 2H), 1.67 (bd, J = 12.6 Hz, 1H), 1.74−1.85 (m, 2H), 1.86−2.22 (m, 4H), 2.67−2.81 (m, 1H), 2.81 (s, 6H), 3.27 (t, J = 6.0 Hz, 2H), 3.46 (t, J = 6.0 Hz, 2H), 3.57 (d, J = 9.8 Hz, 1H), 3.88 (d, J = 9.5 Hz, 1H), 3.99 (d, J = 9.8 Hz, 1H), 4.03 (d, J = 9.5 Hz, 1H), 4.08 (d, J = 15.4 Hz, 1H), 4.23 (d, J = 12.7 Hz, 1H), 4.42 (d, J = 12.7 Hz, 1H), 5.10 (d, J = 15.4 Hz, 1H), 7.28−7.33 (m, 3H), 7.52 (dt, J = 1.8, 7.6 Hz, 1H), 7.81 (d, J = 8.5 Hz, 1H), 7.92 (d, J = 8.5 Hz, 1H), 8.53 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ 27.6, 28.45, 28.50, 34.2, 34.6, 38.6, 41.5, 46.0, 48.6, 53.6, 60.5, 62.2, 77.0, 113.5, 121.4, 121.6, 121.9, 122.0, 122.9, 124.4, 125.1, 132.1, 138.4, 139.1, 132.6, 134.5, 160.1, 171.6. MS (ES^+) *m*/*z* 488.5 $(M + H)^+$. HRMS $(M + H)^+$ calcd for $C_{30}H_{38}N_3O_3$ 488.2913; found 488.2916.

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Supporting Information Available: Synthetic procedures and spectroscopic data for compounds **6**, **8**, **10b**, **13**, **14**, **16**−**20**, **22**−**28**, **36**, and **38**; table with purity data and retention times for all tested compounds; enzyme and cell-based assay protocols; soaking experiments and X-ray data collection. This material is available free of charge via the Internet at http://pubs.acs.org.

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