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Original article

Influence of 4- or 5-substituents on the pyrrolidine ring of 5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin derivatives on their inhibitory activities towards caspases-3 and -7

Panupun Limpachayaporn [a,b], Burkhard Riemann [c], Klaus Kopka [c], Otmar Schober [c], Michael Schäfers [d], Günter Haufe [a,d,*]

[a] Organisch-Chemisches Institut, Westfälische Wilhelms-Universität Münster, Corrensstraße 40, D-48149 Münster, Germany
[b] International NRW Graduate School of Chemistry, Westfälische Wilhelms-Universität Münster, Wilhelm-Klemm-Straße 10, D-48149 Münster, Germany
[c] Klinik für Nuklearmedizin, Universitätsklinikum Münster, Albert-Schweitzer-Campus 1, Gebäude A1, D-48149 Münster, Germany
[d] European Institute for Molecular Imaging (EIMI), Westfälische Wilhelms-Universität Münster, Mendelstraße 11, D-48149 Münster, Germany

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Dedicated to Professor Dr. Ernst-Ulrich Würthwein on the occasion of his 65th birthday.

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ABSTRACT

A series of new 4- or 5-substituted pyrrolidine derivatives of 5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin bearing additional *n*-butyl or 4-fluorobutyl groups at the isatin nitrogen were prepared and their inhibitory activities have been tested against caspases-3 and -7, which are known to participate in the execution of the programmed cell death, called apoptosis. Several analogues fluorinated at the 4-position of the pyrrolidine ring were also synthesized since such inhibitors might be developed as ^{18}F-radiotracers for molecular imaging of activated caspases *in vivo* by PET. Enantiomerically pure diastereomeric 4-fluoropyrrolidinyl derivatives inhibited the enzymes in the nanomolar scale, i.e.100–1000 times more efficient than the corresponding 4-methoxy analogues. The 4,4-difluorinated compound showed the best result with IC_{50} = 362 nM and 178 nM for the aforementioned caspases. In contrast, the 4-methoxy and 4-trifluoromethyl analogues exhibited less inhibition potencies for the enzymes in the μM scale, whereas all 4-OPEG$_4$ (PEG$_4$ = tetraethyleneglycol) and 5-methoxymethyl derivatives were inactive.

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1. Introduction

Apoptosis, the programmed type I cell death, leads to a regulated destruction of moribund cells. This natural mechanism strictly controls the number of cells by removing excess, potentially dangerous as well as damaged cells and there, by maintains homeostasis. It is triggered either by an extrinsic, specific protein/receptor interaction leading to an intracellular death signal (death receptor pathway), or by DNA damage followed by an intrinsic mitochondrial induction of the cell death program (mitochondrial pathway) finally resulting in the removal of cellular residues, called apoptotic bodies, without inflammatory response. The apoptotic pathway is executed by a set of enzymes called caspases (***c***ysteinyl ***asp***artate-specific prote***ases***). In humans, dysfunctional apoptosis – enhanced or reduced – can induce many different diseases, e.g. cancer, cardiovascular and autoimmune defects, as well as neurodegeneration including Alzheimer's, Parkinson's and Huntington's diseases [1]. Although more than a dozen of caspases have been identified in humans, inhibition of caspases-3 and -7 alone is sufficient to slow down or block the cell death program. Thus, there is a need for novel therapeutic and preventive drugs targeting the executioner caspases, such as caspases-3 and -7, for treatment and diagnosis of diseases associated with dysregulated apoptosis [2].

(*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]isatin and (*S*)-*N*-methyl-5-[1-(2-phenoxymethylpyrrolidinyl)sulfonyl]isatin have been identified as potent, non-peptide, selective inhibitors of caspases-3 and -7 [2–4]. The X-ray co-crystal structure of the complex of recombinant human caspase-3 with (*S*)-*N*-methyl-5-[1-(2-phenoxymethylpyrrolidinyl)sulfonyl]isatin demonstrated the embedding of the inhibitor in the enzyme's active site. The 3-carbonyl group is the cysteine-163 binding site resulting in a tetrahedral thiohemiketal intermediate. The pyrrolidine ring is accommodated in the S_2-pocket, while the phenoxymethyl group

* Corresponding author. Organisch-Chemisches Institut, Westfälische Wilhelms-Universität Münster, Corrensstraße 40, D-48149 Münster, Germany. Tel.: +49 (0) 251 83 33281; fax: +49 (0)251 83 39772.
E-mail addresses: haufe@uni-muenster.de, jfc@uni-muenster.de (G. Haufe).

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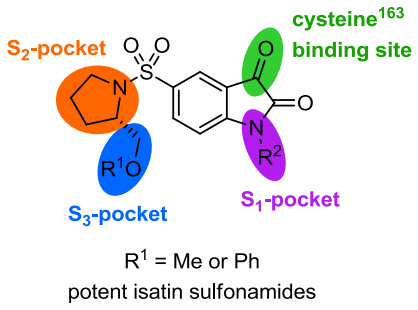


Fig. 1. The general structure of the isatin sulfonamide derivatives with the assignment of the interactions with the enzyme's subpockets [2].

of the pyrrolidine is in contact with the S_3-pocket and the isatin *N*-substituent putatively interacts with the S_1-pocket (Fig. 1) [2].

New *N*-substituted pyrrolidinyl sulfonyl isatin derivatives including fluorinated analogues were prepared and intensively investigated in the past decade. Substitution at this position resulted in significantly improved activities [3–6]. Moreover, the pyrrolidine moiety was replaced by several heterocyclic amines. These compounds showed moderate to excellent inhibitory activities towards caspases-3 and -7 [7,8].

Although it was reported that phenoxymethyl derivatives have higher inhibitory activities towards the enzymes *in vitro* compared to the methoxymethyl series [3], the latter showed superior inhibition efficiency in an *in vivo* cell culture model [4]. Complementary, most of the *N*-substituted isatin sulfonamides of the methoxymethyl series also possessed good to excellent enzyme inhibition *in vitro* [4–7]. Thus, (*S*)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**1**) was selected as lead compound. Recently, we and others have reported on the feasible application of *N*-fluoroalkylated isatin sulfonamides as new radiotracers which could be used for the noninvasive molecular imaging by positron emission tomography (PET). This putatively allows the visualization and therapy monitoring of activated caspases in corresponding diseases *in vivo* with high specificity [9–11]. However, our recent metabolism study using electrochemistry-liquid chromatography/mass spectrometry showed that the *N*-position of the isatin is labile for debenzylation and the methoxymethyl moiety tends to terminal demethylation during oxidative phase I metabolism [12]. Radiofluorination at those positions yielding corresponding PET-compatible radiotracers could result in a partial loss of ^{18}F-activity *in vivo* and hence in an insufficient deposit of the tracer in the tissue to be noninvasively examined. As a consequence, a weak PET signal will be observed *in vivo*. Therefore, fluorination at the pyrrolidine ring might be an alternative approach to overcome the aforementioned problems.

Herein we report on syntheses of various 4- or 5-substituted pyrrolidine derivatives of the lead compound. Additionally, we elucidate the influence of the chosen substitution pattern on the inhibition potencies for caspases-3 and -7. In this connection we explore the environment of the enzyme's S_2-pocket and suggest further modifications in order to improve the pharmacological properties of the isatin sulfonamides. Furthermore, fluorination of this moiety might allow the development of this potential inhibitor class towards new metabolically more stable radiotracers for the molecular imaging of apoptosis *in vivo*.

2. Results and discussion

2.1. Chemistry

The derivatives of (*S*)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**1**) and the lead compound itself were synthesized by coupling of the substituted *N*-protected pyrrolidine and 5-chlorosulfonyl isatin similar to procedures described earlier [3–6].

The substituted pyrrolidine ring has to be protected with a suitable protecting group (PG) depending on the reaction conditions. In this study, benzyl (Bn) and *tert*-butyloxycarbonyl (Boc) groups were applied. The pyrrolidine and isatin moieties were bridged with a sulfonyl group using 5-chlorosulfonyl isatin which was achieved in two steps starting from isatin, i.e. sulfonation using oleum and chlorination using $POCl_3$ [3]. Finally the corresponding isatin sulfonamides were modified with different substituents (Fig. 2). Since Podichetty et al. demonstrated that (*S*)-*N*-butyl- and (*S*)-*N*-(4-fluorobutyl)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatins were much more active inhibitors than the parent NH-compounds, we preferred to attach these groups to our new ligands [5].

2.1.1. (2S)-5-[1-(4-methoxy-2-methoxymethylpyrrolidinyl)sulfonyl] isatin derivatives

The absolute configuration of inhibitors is important for enzyme binding potencies. Therefore, two target diastereomers of *N*-Boc-pyrrolidines **4a** and **4b** were synthesized. Their preparations were smoothly performed by reduction of the *N*-Boc-protected 4-hydroxyproline esters **2a** or **2b** with $LiBH_4$ in THF followed by dimethylation of the formed alcohols **3a** or **3b** [13] using methyl iodide in the presence of NaH with good yields as illustrated in Scheme 1.

The *trans*-*N*-Boc- and *cis*-*N*-Boc-pyrrolidines **4a** and **4b** were deprotected under acidic conditions using TFA in DCM resulting in the corresponding free amines, which were coupled with 5-chlorosulfonyl isatin using DIPEA leading to (2*S*,4*R*)- and (2*S*,4*S*)-5-[1-(4-methoxy-2-methoxymethylpyrrolidinyl)sulfonyl]isatins (**20a**

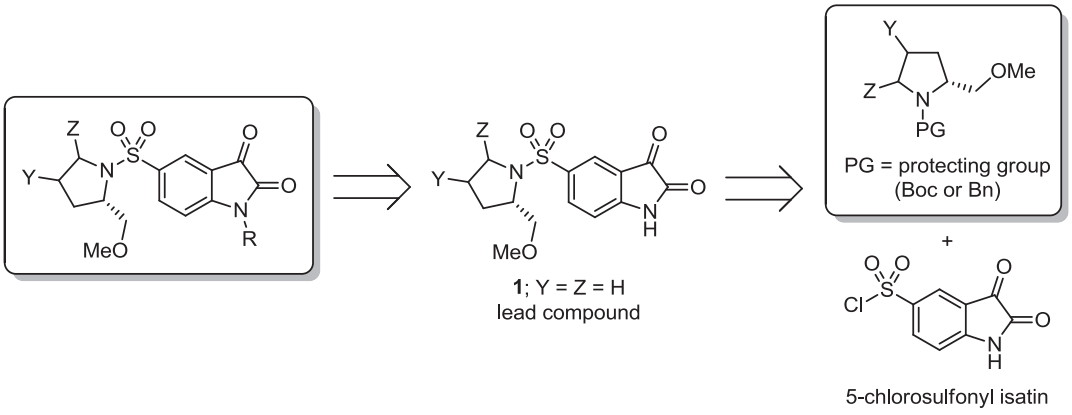
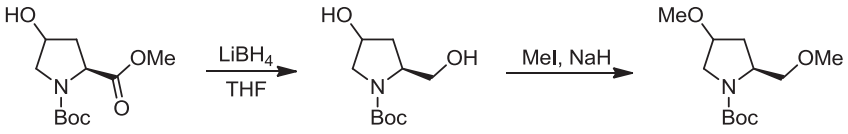


Fig. 2. Retrosynthetic analysis of 5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin derivatives.



(2*S*,4*R*)-**2a** (2*S*,4*S*)-**2b** (2*S*,4*R*)-**3a** 87% (2*S*,4*S*)-**3b** 88% (2*S*,4*R*)-**4a** 84% (2*S*,4*S*)-**4b** 82%

Scheme 1. Synthetic route to (2*S*,4*R*)- and (2*S*,4*S*)-*N*-*tert*-butyloxycarbonyl-4-methoxy-2-methoxymethylpyrrolidine (**4a** and **4b**).

and **20b**) with 62% and 64% yields, respectively. Subsequently, the obtained compounds were *N*-alkylated with 1-bromobutane and 1-bromo-4-fluorobutane using Cs_2CO_3 and microwave irradiation to furnish the corresponding inhibitors **21a**, **22a**, **21b** and **22b** in good to excellent yields (see Table 1).

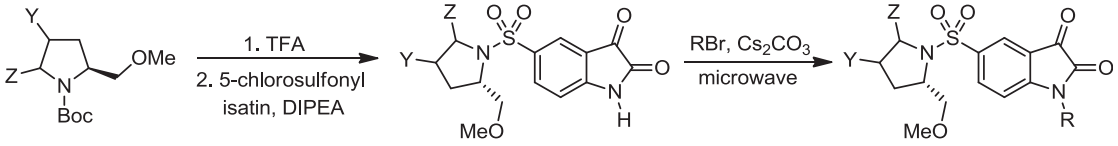
2.1.2. (2S)-5-[1-(2-methoxymethyl-4-PEG$_4$yloxypyrrolidinyl)sulfonyl] isatin derivatives

Metabolic resistance and delivery of drugs *in vivo* are an important topic intensively discussed in drug development today. One method, which helps to improve the metabolic stability and enhance the hydrophilicity of compounds, is the introduction of a polar polyethyleneglycol (PEG) unit to the structure. PEG$_4$ylated (PEG$_4$ = tetraethyleneglycol) compounds are more polar and may limit the accessibility for other enzymes and antibodies [14]. Thus, PEG$_4$ was attached to the 4-position of the pyrrolidine moiety.

The ω-tosylated tetraethyleneglycol monomethyl ether **5** was obtained by monomethylation and tosylation of tetraethyleneglycol. Nucleophilic substitution of the (2*S*,4*R*)- and (2*S*,4*S*)-*N*-Boc-4-hydroxyproline esters **2a** and **2b** with **5** was performed using NaH in THF leading to the PEG$_4$ylated hydroxyproline derivatives **6a** and **6b** in moderate yields. Similar to the synthesis of the 4-methoxypyrrolidine derivatives, after reduction and methylation using the conditions described above, the diastereomeric 4-OPEG$_4$ pyrrolidines **8a** and **8b** were obtained in good yields as depicted in Scheme 2.

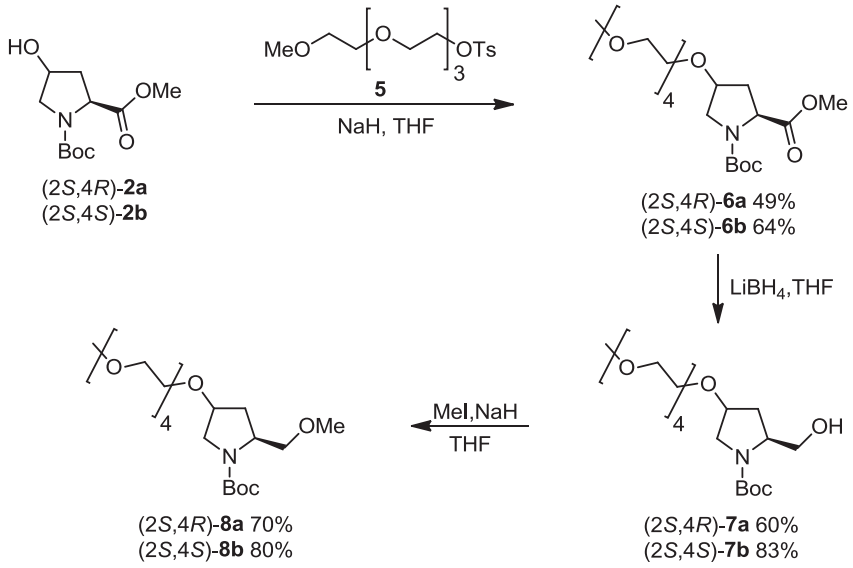
After Boc-deprotection the coupling reactions with 5-chlorosulfonyl isatin gave the target 4-OPEG$_4$ pyrrolidinyl sulfonyl isatins **23a** and **23b** in low yields (see Table 1). The corresponding (2*S*,4*S*)-*N*-butyl-5-[1-(2-methoxymethyl-4-PEG$_4$yloxypyrrolidinyl) sulfonyl]isatin (**24**) was obtained with 69% yield by microwave accelerated alkylation. Unfortunately, compound **24** was shown to

Table 1
Synthesis and caspase inhibition potencies of compounds **19**–**33** expressed as IC_{50} values [μM].



Comp	Y	Z	R	Yield[a] [%]	IC_{50} [μM]	
					Caspase-3	Caspase-7
1[4]	H	H	H	n.d.	0.0849 ± 0.0256	1.290 ± 0.466
20a	(*R*)-OMe	H	H	62	>500	>500
21a	(*R*)-OMe	H	$CH_2(CH_2)_2CH_3$	72	28.5 ± 2.0	26.4 ± 1.3
22a	(*R*)-OMe	H	$CH_2(CH_2)_2CH_2F$	72	33.4 ± 1.5	59.6 ± 13.3
20b	(*S*)-OMe	H	H	64	>500	>500
21b	(*S*)-OMe	H	$CH_2(CH_2)_2CH_3$	85	81.1 ± 6.2	>500
22b	(*S*)-OMe	H	$CH_2(CH_2)_2CH_2F$	91	42.6 ± 5.6	42.6 ± 104
23a	(*R*)-OPEG$_4$	H	H	20	>500	>500
23b	(*S*)-OPEG$_4$	H	H	40	>500	>500
24	(*S*)-OPEG$_4$	H	$CH_2(CH_2)_2CH_3$	69	>500	>500
25a	(*R*)-CF_3	H	H	27	>500	>500
26a	(*R*)-CF_3	H	$CH_2(CH_2)_2CH_3$	84	18.1 ± 3.9	52.7 ± 5.9
27a	(*R*)-CF_3	H	$CH_2(CH_2)_2CH_2F$	91	31.8 ± 3.0	67.7 ± 5.1
25b	(*S*)-CF_3	H	H	64	101 ± 9	n.d.[b]
26b	(*S*)-CF_3	H	$CH_2(CH_2)_2CH_3$	92	16.5 ± 1.1	78.3 ± 1.7
27b	(*S*)-CF_3	H	$CH_2(CH_2)_2CH_2F$	96	24.5 ± 0.6	147 ± 12
28a	(*R*)-F	H	H	51	2.39 ± 0.94	8.46 ± 0.84
29a	(*R*)-F	H	$CH_2(CH_2)_2CH_3$	75	0.795 ± 0.036	1.88 ± 0.09
28b	(*S*)-F	H	H	46	1.13 ± 0.57	12.1 ± 0.9
29b	(*S*)-F	H	$CH_2(CH_2)_2CH_3$	82	0.961 ± 0.013	1.25 ± 0.08
30	F,F	H	H	28	2.57 ± 0.07	14.9 ± 2.5
31	F,F	H	$CH_2(CH_2)_2CH_3$	86	0.362 ± 0.019	0.178 ± 0.049
19a	H	(*R*)-CH_2OMe	H	89	>500	>500
32a	H	(*R*)-CH_2OMe	$CH_2(CH_2)_2CH_3$	73	>500	>500
33a	H	(*R*)-CH_2OMe	$CH_2(CH_2)_2CH_2F$	77	>500	>500
19b	H	(*S*)-CH_2OMe	H	14	>500	>500
32b	H	(*S*)-CH_2OMe	$CH_2(CH_2)_2CH_3$	68	5.99 ± 0.19	11.9 ± 2.0
33b	H	(*S*)-CH_2OMe	$CH_2(CH_2)_2CH_2F$	54	8.84 ± 1.44	47.8 ± 3.1

[a] Yield of the last step.
[b] n.d. = not determined.



Scheme 2. Synthesis of the 4-OPEG$_4$ pyrrolidine derivatives **8a** and **8b**.

be biologically inactive (see Table 1). Thus, no further analogues of this class were prepared.

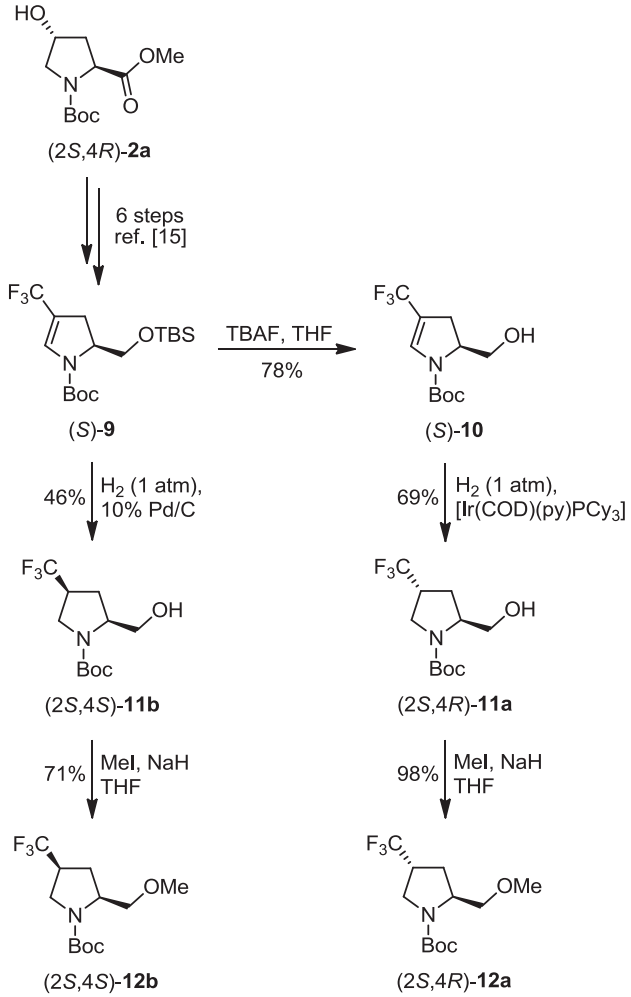
2.1.3. (2S)-5-[1-(4-trifluoromethyl-2-methoxymethylpyrrolidinyl) sulfonyl]isatin derivatives

Trifluoromethyl substituents are known to enhance or modify the biological activities in many cases. The stereoselective 4-trifluoromethylation of L-proline was reported by Del Valle and Goodman [15]. According to these authors, the key intermediate **9** was synthesized in 6 steps (Scheme 3) starting from (2*S*,4*R*)-*N*-Boc-4-hydroxyproline methyl ester (**2a**). Subsequently, compound **9** was transformed to the diastereomeric 4-trifluoromethylprolinols **11a** and **11b** depending on the catalyst used in the hydrogenation step. Under standard conditions using 10% Pd on activated charcoal, the hydrogen atoms were added from the sterically less hindered side of the ring resulting in the formation of the *cis*-trifluoromethyl alcohol **11b**. In contrast, after TBS deprotection, Crabtree's catalyst was applied in the hydrogenation of **10** to produce the desired *trans*-isomer **11a**. Methylation of both diastereomers led to the target (2*S*,4*R*)- and (2*S*,4*S*)-*N*-Boc-4-trifluoromethyl-2-methoxy methylpyrrolidines (**12a** and **12b**) with good to excellent yields.

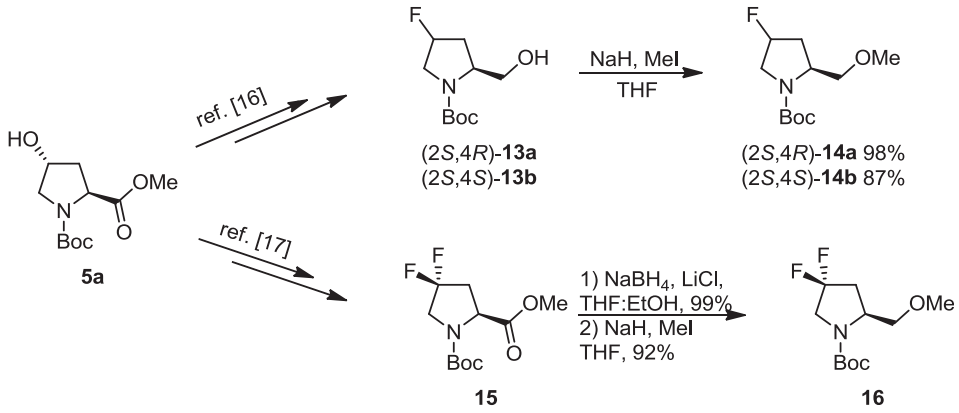
The succeeding coupling with 5-chlorosulfonyl isatin was performed to get the corresponding trifluoromethyl compounds **25a** and **25b** in 27% and 64% yields, respectively. Binding of *n*-butyl and 4-fluorobutyl substituents to the isatin nitrogen was achieved using Cs_2CO_3 and 1-bromobutane or 1-bromo-4-fluorobutane in dry DMF under microwave irradiation to obtain compounds **26a**, **27a**, **26b** and **27b** in very good yields (Table 1).

2.1.4. (2S)-5-[1-(4-fluoro-2-methoxymethylpyrrolidinyl)sulfonyl] isatin derivatives

The introduction of a single fluorine to a lead structure generally results in a modification of the chemical and physical properties of compounds and moreover, might allow to radiolabel the ligands with fluorine-18 for PET imaging. According to literature procedures [16], fluoroprolinols **13a** and **13b** were obtained smoothly. Subsequent methylation of fluoroalcohols **13a** and **13b** using MeI and NaH gave the target fluoropyrrolidines **14a** and **14b** in excellent yields. The *gem*-difluoropyrrolidinyl methyl ester **15** was prepared as described earlier [17] and was converted to the difluoroether **16**



Scheme 3. Synthetic route to prepare trifluoromethylpyrrolidine methyl ester **12a** and **12b**.



Scheme 4. Synthetic pathways to the fluorinated and difluorinated pyrrolidines **14a**, **14b** and **16**.

by reduction with $NaBH_4$ in the presence of LiCl followed by methylation (Scheme 4).

Subsequent coupling of all fluorinated pyrrolidines with the isatin fragment gave compounds **28a**, **28b** and **30** in moderate yields (Table 1). The *N*-alkylation with *n*-butylbromide was performed under microwave irradiation obtaining the corresponding fluorinated inhibitors **29a**, **29b** and **31** in good to excellent yields (Table 1).

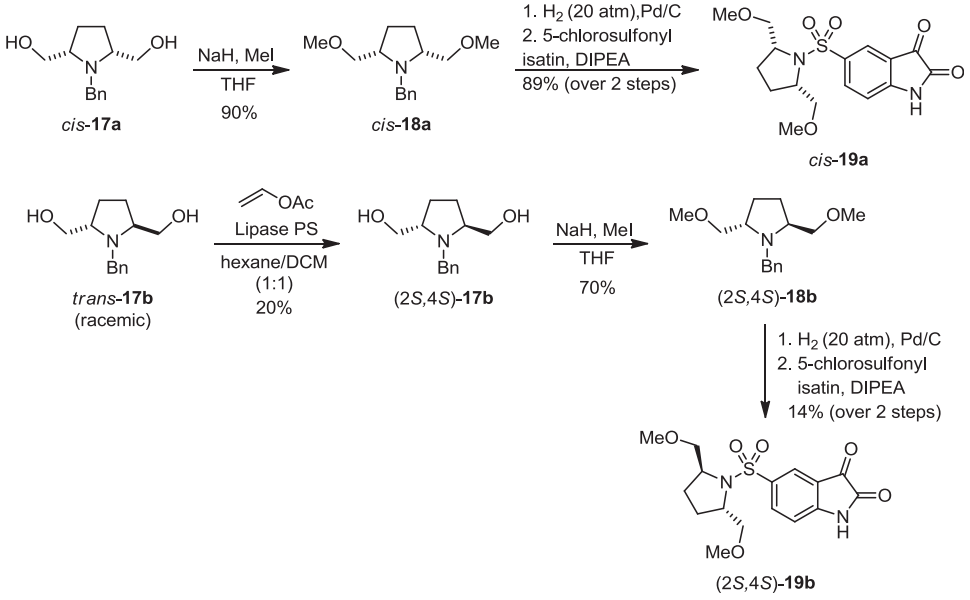
2.1.5. (2S)-5-{1-[2,5-bis(methoxymethyl)pyrrolidinyl]sulfonyl} isatin derivatives

The C_S-symmetric *cis*- and C_2-symmetric (2*S*,4*S*)-*N*-benzyl-2,5-bis(hydroxymethyl)pyrrolidines (**17a** and **17b**) were prepared starting from adipoyl chloride as reported by Sibi and Lu [18]. The *cis*-diol **17a** was directly dimethylated using MeI and NaH to produce the corresponding *N*-benzyl dimethylether **18a**, whereas the enantiopure (2*S*,4*S*)-diol **17b** (*trans*-configuration) was obtained by enzymatic acetylation using lipase PS and vinyl acetate in hexane/DCM [18]. Subsequent dimethylation smoothly led to the corresponding (2*S*,4*S*)-*N*-benzyl-2,5-bis(methoxymethyl)pyrrolidine (**18b**) (Scheme 5).

Prior to coupling, *N*-debenzylation was tried under different conditions. With 5% $Pd(OH)_2$ and 10% Pd on activated charcoal as catalysts under H_2 atmosphere (5 atm), no conversion was observed after overnight stirring at room temperature. Fortunately, the hydrogenolytic debenzylation was performed successfully by stirring the mixture with 10% Pd on activated charcoal under 20 atm of H_2 atmosphere for 6 h. Subsequent coupling of the unprotected pyrrolidines with 5-chlorosulfonyl isatin yielded the *cis*- and *trans*-bis(methoxymethyl)pyrrolidinylsulfonyl isatins **19a** and **19b** in 89% and 14% yields over two steps, respectively, as shown in Scheme 5. Finally the *N*-alkylation resulted in the desired analogues **32a**, **33a**, **32b** and **33b** in good to excellent yields (Table 1).

2.2. Study of in vitro biological activity

Various substituents were attached to the pyrrolidine ring of the lead structure **1** to evaluate the inhibition potencies for caspases-3 and -7 indicated as IC_{50} values. As a result, several of the *N*-unsubstituted compounds were active only in millimolar concentrations. Interestingly, the *N*-unsubstituted mono- and difluorinated



Scheme 5. Synthetic routes to the symmetric pyrrolidinylisatins **19a** and **19b**.

analogues exhibited caspase inhibition with IC_{50} in one-digit micromolar range.

The activities increased to μM or nM values by attachment of *n*-butyl and 4-fluorobutyl groups to the isatin nitrogen. In most of the cases the *N*-butyl group led to slightly higher activities than the *N*-(4-fluorobutyl) one. However, they are weaker inhibitors compared to the lead compound. 4-methoxy and 4-trifluormethylated analogues showed IC_{50} values in the μM range, whereas the 4-$OPEG_4$ and *cis*-5-methoxymethyl analogues were inactive. The *trans*-5-methoxymethylisatin derivatives showed affinities in the micromolar scale. Among the ligands substituted at the pyrrolidine ring, the *N*-butyl mono- and difluorinated ones exhibited high activity in the nM scale, which is 100–1000 times better than that of the 4-OMe and 4-CF_3 compounds. The 4,4-difluoropyrrolidinyl derivative **31** showed the best result with IC_{50} = 362 nM and 178 nM for caspases-3 and -7, respectively. In addition we observed that the inhibition potencies for (*R*)- and (*S*)-substituted ligands do not differ (Table 1).

3. Conclusion

4-Methoxy, 4-$OPEG_4$, 4-trifluoromethyl, 4-fluoro, 4,4-difluoro and 5-methoxymethylpyrrolidinylsulfonylisatin derivatives were synthesized and tested for their caspase inhibition potencies *in vitro*. Their inhibitory properties for the executioner caspases-3 and -7 are ranging in the micromolar scale. Altogether the here presented isatin sulfonamides are less active for both enzymes compared to the lead compound **1**. These findings confirm a dramatic decrease of the biological activity by any oxygen-containing substituents at the 4- and 5-position of the hydrophobic pyrrolidine moiety. However, as a main finding, we have proven that the 4-fluorinated and 4,4-difluorinated analogues indeed exhibited maintained caspase inhibition potencies in the nanomolar range, though they are generally less active compared to compounds bearing fluorinated groups in the 2-position [19] or at the isatin nitrogen [5,6,20].

4. Experimental section

4.1. Materials and methods

All starting materials, reagents and solvents for the syntheses were analytical grade and used without further purification. 1-bromo-4-fluorobutane was purchased from Apollo Scientific. Absolute DMF was obtained from Acros. For all moisture sensitive reactions, the glassware was heated under high vacuum prior use and the transformation was performed under argon atmosphere. ^1H NMR (300 MHz, 400 MHz, 500 MHz and 600 MHz), ^{13}C NMR (75 MHz, 100 MHz, 125 MHz and 150 MHz) and ^{19}F NMR (282 MHz) spectra were recorded using Bruker machines in $CDCl_3$ or CD_3OD with TMS for ^1H NMR, $CDCl_3$ or CD_3OD for ^{13}C NMR and $CFCl_3$ for ^{19}F NMR as internal standards. DEPT, COSY, HSQC and HMBC spectra were recorded to assign the signals in the complex structures. All chemical shifts were indicated in ppm. Exact mass analyses were recorded with a Bruker MicroTOF apparatus. All spectroscopic and analytical investigations were performed by staff members of the Organic Chemistry Institute, University of Münster. Thin layer chromatography (TLC) analyses were performed on silica coated aluminium foils (Silica gel 60 F_{254}) with 0.2 mm layer thickness from MERCK. Silica gel (60–120 mesh) was used for column chromatography. High pressure hydrogenation was performed in an autoclave apparatus. Reactions using microwave irradiation were performed in closed glass vessels using a CEM Discovery microwave machine.

4.2. General procedures

4.2.1. Methylation of N-protected prolinols

Under argon atmosphere, a stirred solution of the *N*-protected L-prolinol (1.0 mmol) in dry THF (6 mL) was cooled to −10 °C and treated with NaH (1.5 mmol, 60% in mineral oil). After stirring for 15 min, MeI (1.5 mmol) was added dropwise to the above suspension and the resulting reaction mixture was stirred at this temperature for 30 min, at 0 °C for 3 h and at r.t. for further 18 h. After this time, the reaction mixture was cooled to 0 °C and quenched with sat. aq. NH_4Cl until no more H_2 evolved. Subsequently, H_2O (30 mL) was added and the mixture was extracted with EtOAc (3 × 40 mL). The combined organic phases were washed with brine (1 × 40 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography to obtain the corresponding *N*-protected methoxymethylpyrrolidines.

4.2.2. Coupling of the pyrrolidines with 5-chlorosulfonyl isatin

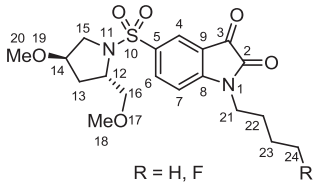
To a stirred solution of the *N*-Boc-protected pyrrolidine (1.0 mmol) in dry DCM (3 mL) at 0 °C under argon atmosphere, TFA (1.14 g, 10.0 mmol) was added dropwise. The obtained solution was stirred at 0 °C for 30 min followed by stirring at r.t. for 2 h. Subsequently, the solution was poured to cold 10% aq. NaOH (15 mL) and extracted with DCM (3 × 15 mL). The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure resulting in the corresponding unprotected pyrrolidine, which was used in the next step without further purification.

The free pyrrolidine (ca. 1 mmol) from the previous step was then taken up in $CHCl_3$ (2.2 mL) and DIPEA (2.0 mmol) was added. The solution was added dropwise to a stirred solution of 5-chlorosulfonyl isatin (1.5 mmol) in $CHCl_3$/THF (1:1, 15 mL) at room temperature. The resulting mixture was stirred further for 1 h and the solvent was removed under reduced pressure. After purification by flash column chromatography the target isatin sulfonamide was obtained.

4.2.3. N-Alkylation of the isatin moiety with alkyl bromides using microwave irradiation

In a thick-glass vessel for microwave reaction, a stirred solution of the *N*-unsubstituted isatin sulfonamide (0.2 mmol) in dry DMF (5.5 mL) under argon atmosphere was treated with Cs_2CO_3 (0.4 mmol) at room temperature. After stirring for 15 min, it turned purple and 1-bromobutane or 1-bromo-4-fluorobutane (2 mmol was added. The vessel was sealed with a PE-cap and the obtained mixture was stirred at 95 °C for 10 min under microwave irradiation. The precipitate was removed by filtration and the filtrate was concentrated under reduced pressure. The crude was purified by flash column chromatography to obtain the desired product.

4.3. (2S,4R)-5-[1-(4-methoxy-2-methoxymethylpyrrolidinyl)sulfonyl]isatins



4.3.1. (2S,4R)-N-tert-butyloxycarbonyl-4-hydroxy-2-hydroxymethyl pyrrolidine (**3a**)

A stirred solution of methyl (2*S*,4*R*)-*N*-*tert*-butyloxycarbonyl-4-hydroxypyrrolidine-2-carboxylate (**2a**) (500 mg, 2.04 mmol, 1.00 equiv.) in dry THF (7 mL) was cooled to 0 °C under argon atmosphere and treated dropwise with 2 M $LiBH_4$ in THF solution (2.0 mL, 4.08 mmol, 2.00 equiv.). The resulting solution was allowed to warm to r.t. and stirred for 18 h. After this time, the reaction mixture was quenched by very slow addition of water (5 mL) and the resulting clear solution was extracted with DCM (5 × 15 mL). The combined organic phases were washed with sat. aq. $NaHCO_3$ (1 × 10 mL), brine (1 × 10 mL) and dried over $MgSO_4$. After removal of the solvent, the crude product was purified by flash column chromatography (silica gel, 10% acetone in EtOAc) to obtain a colorless oil (384 mg, 1.77 mmol, 87%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.43–4.29 (m, 1H, 4-CH), 4.21–4.02 (m, 1H, 2-CH), 3.80 (br s, 2H, 4-COH, 10-COH), 3.74–3.48 (m, 2H, 5-CH_2), 3.57 (dd, $^2J_{H,H}$ = 11.4 Hz, $^3J_{H,H}$ = 6.5 Hz, 1H, 10-CH_a), 3.41 (dd, $^2J_{H,H}$ = 12.0 Hz, $^3J_{H,H}$ = 4.2 Hz, 1H, 10-CH_b), 2.14–1.55 (m, 2H, 3-CH_2), 1.47 (s, 9H, 9-CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.5 (6-CO), 80.6 (8-CO), 69.1 (4-CH), 60.5 (10-CH_2), 58.6 (2-CH), 55.7 (5-CH_2), 37.4 (3-CH_2), 28.4 (3C, 9-CH_3) ppm. HRMS (ESI+, MeOH): *m*/*z* = 240.1204 $[M + Na]^+$; calcd. 240.1206 for $C_{10}H_{19}NO_4$+Na.

4.3.2. (2S,4R)-N-tert-butyloxycarbonyl-4-methoxy-2-methoxymeth ylpyrrolidine (**4a**)

A stirred solution of (2*S*,4*R*)-*N*-*tert*-butyloxycarbonyl-4-hydroxy-2-hydroxymethylpyrrolidine (**3a**) (180 mg, 0.829 mmol, 1.00 equiv.) in dry THF (8 mL) under argon atmosphere was cooled to −10 °C and treated with 60% NaH (165 mg, 4.14 mmol, 5.00 equiv.). After stirring for 15 min, MeI (206 μL, 3.32 mmol, 4.00 equiv.) was added dropwise. The reaction mixture was stirred at this temperature for 30 min, 0 °C for 1 h and r.t. for 18 h. After this time, the mixture was cooled to 0 °C and quenched with a small amount of sat. aq. NH_4Cl until no more H_2 evolved. Subsequently, water (10 mL) was added and the obtained mixture was extracted with EtOAc (3 × 15 mL). The combined organic phases were washed with water (1 × 10 mL), brine (1 × 10 mL) and dried over $MgSO_4$. After removal of the solvent, the residue was purified by flash column chromatography (silica gel, 5% THF in DCM) to obtain a colorless oil (171 mg, 0.697 mmol, 84%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.13–3.96 (m, 1H, 2-CH), 4.12–3.81 (m, 1H, 4-CH), 3.69–3.23 (m, 2H, 5-CH_2), 3.55–3.45 (m, 2H, 10-CH_2), 3.34 (s, 3H, 12-CH_3), 3.31 (s, 3H, 14-CH_3), 2.18–1.95 (m, 2H, 3-CH_2), 1.47 (s, 9H, 9-CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 154.6 (6-CO), 79.5 (8-CO), 78.2 (4-CH), 73.3 (10-CH_2), 59.2 (12-CH_3), 56.7 (14-CH_3), 55.6 (2-CH), 51.1 (5-CH_2), 33.9 (3-CH_2), 28.5 (3C, 9-CH_3) ppm. HRMS (ESI+, MeOH): *m*/*z* = 268.1515 $[M + Na]^+$, 513.3142 $[2M + Na]^+$; calcd. 268.1519 for $C_{12}H_{23}NO_4$ + Na, 513.3146 for 2($C_{12}H_{23}NO_4$) + Na.

4.3.3. (2S,4R)-5-[1-(4-methoxy-2-methoxymethylpyrrolidinyl)sulfo nyl]isatin (**20a**)

(2*S*,4*R*)-*N*-*tert*-butyloxycarbonyl-4-methoxy-2-methoxymethylpyrrolidine (**4a**) (300 mg, 1.22 mmol, 1.00 equiv.) was converted to (2*S*,4*R*)-5-[1-(4-methoxy-2-methoxymethylpyrrolidinyl)sulfonyl] isatin (**20a**) using TFA followed by the reaction with 5-chlorosulfonyl isatin and DIPEA as described in the general procedure in Section 4.2.2. The crude product was purified by flash column chromatography (silica gel, gradient DCM, 10% THF in DCM and 20% THF in DCM) to obtain a yellow wax (267 mg, 0.753 mmol, 62%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.37 (br s, 1H, 1-NH), 8.02 (dd, $^3J_{H,H}$ = 8.8 Hz, $^4J_{H,H}$ = 1.9 Hz, 1H, 6-CH), 8.02 (d, $^4J_{H,H}$ = 1.9 Hz, 1H, 4-CH), 7.07 (d, $^3J_{H,H}$ = 8.8 Hz, 1H, 7-CH), 3.87–3.81 (m, 1H, 14-CH), 3.81–3.71 (m, 1H, 12-CH_3), 3.64 (dd, $^2J_{H,H}$ = 9.7 Hz, $^3J_{H,H}$ = 3.3 Hz, 1H, 16-CH_a), 3.56 (dd, $^2J_{H,H}$ = 9.7 Hz, $^3J_{H,H}$ = 5.9 Hz, 1H, 16-CH_b), 3.47 (d, $^3J_{H,H}$ = 3.1 Hz, 2H, 15-CH_2), 3.38 (s, 3H, 18-CH_3), 3.04 (s, 3H, 20-CH_3), 2.05–1.95 (m, 2H, 13-CH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 182.3 (3-CO), 159.1 (2-CO), 152.4 (8-CN), 138.0 (6-CH), 134.0 (5-CSO_2), 125.2 (4-CH), 117.5 (9-CCO), 112.8 (7-CH), 78.7 (14-CH), 74.9 (16-CH_2), 59.3 (18-CH_3), 58.6 (12-CH), 56.3 (20-CH_3), 54.0 (15-CH_2), 35.1 (13-CH_2) ppm. HRMS (ESI+, MeOH): *m*/*z* = 377.0785 $[M + Na]^+$, 409.1046 $[M + Na + MeOH]^+$; calcd. 377.0778 for $C_{15}H_{18}N_2O_6S$ + Na, 409.1040 for $C_{15}H_{18}N_2O_6S$ + Na + MeOH.

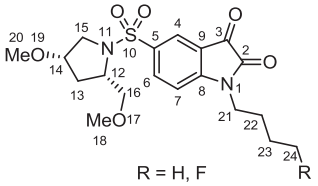
4.3.4. (2S,4R)-N-butyl-5-[1-(4-methoxy-2-methoxymethylpyrrolidi nyl)sulfonyl]isatin (**21a**)

(2*S*,4*R*)-5-[1-(4-methoxy-2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**20a**) (60 mg, 0.169 mmol, 1.00 equiv.) was converted to (2*S*,4*R*)-*N*-butyl-5-[1-(4-methoxy-2-methoxymethylpyrrolidinyl) sulfonyl]isatin (**21a**) using 1-bromobutane and Cs_2CO_3 as described in the general procedure in Section 4.2.3. The crude product was purified by flash column chromatography (silica gel, 50% EtOAc in cyclohexane) to obtain a yellow-orange solid (50 mg, 0.122 mmol, 72%). M.p. 116 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.07 (dd, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.9 Hz, 1H, 6-CH), 8.04 (d, $^4J_{H,H}$ = 1.6 Hz, 4-CH), 7.01 (d, $^3J_{H,H}$ = 8.3 Hz, 1H, 7-CH), 3.89–3.79 (m, 1H, 14-CH), 3.78 (t, $^3J_{H,H}$ = 7.6 Hz, 2H, 21-CH_2), 3.77–3.73 (m, 1H, 12-CH), 3.64 (dd, $^2J_{H,H}$ = 9.6 Hz, $^3J_{H,H}$ = 3.2 Hz, 1H, 16-CH_a), 3.55 (dd, $^2J_{H,H}$ = 9.6 Hz, $^3J_{H,H}$ = 6.1 Hz, 1H, 16-CH_b), 3.47 (d, $^3J_{H,H}$ = 3.2 Hz, 2H, 15-CH_2), 3.38 (s, 3H, 18-CH_3), 3.04 (s, 3H, 20-CH_3), 2.06–2.00 (m, 1H, 13-CH_a), 2.00–1.94 (m, 1H, 13-CH_b), 1.70 (quintet, $^3J_{H,H}$ = 7.6 Hz, 2H, 22-CH_2), 1.42 (sextet, $^3J_{H,H}$ = 7.4 Hz, 2H, 23-CH_2), 0.98 (t, $^3J_{H,H}$ = 7.4 Hz, 3H, 24-CH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 182.4 (3-CO), 158.0 (2-CO), 153.8 (8-CN), 137.9 (6-CH), 134.0 (5-CSO_2), 125.1 (4-CH), 117.3 (9-CCO), 110.2 (7-CH), 78.9 (14-CH), 75.1 (16-CH_2), 59.5 (18-CH_3), 58.7 (12-CH), 56.5 (20-CH_3), 54.1 (15-CH_2), 40.6 (21-CH_2), 35.3 (13-CH_2), 29.4 (22-CH_2), 20.3 (23-CH_2), 13.8 (24-CH_3) ppm. HRMS (ESI+, MeOH): *m*/*z* = 433.1407 $[M + Na]^+$, 465.1667 $[M + Na + MeOH]^+$; calcd. 433.1404 for $C_{19}H_{26}N_2O_6S$ + Na, 465.1666 for $C_{19}H_{26}N_2O_6S$ + Na + MeOH.

4.3.5. (2S,4R)-N-(4-fluorobutyl)-5-[1-(4-methoxy-2-methoxymethyl pyrrolidinyl)sulfonyl]isatin (**22a**)

(2*S*,4*R*)-5-[1-(4-methoxy-2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**20a**) (60 mg, 0.169 mmol, 1.00 equiv.) was converted to (2*S*,4*R*)-*N*-(4-fluorobutyl)-5-[1-(4-methoxy-2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**22a**) using 1-bromo-4-fluorobutane and Cs_2CO_3 as described in the general procedure in Section 4.2.3. The crude product was purified by flash column chromatography (silica gel, 75% EtOAc in cyclohexane) to obtain a yellow-orange solid (52 mg, 0.121 mmol, 72%). M.p. 120 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.07 (dd, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.9 Hz, 1H, 6-CH), 8.04 (d, $^4J_{H,H}$ = 1.9 Hz, 1H, 4-CH), 7.04 (d, $^3J_{H,H}$ = 8.3 Hz, 1H, 7-CH), 4.52 (dt, $^2J_{H,F}$ = 47.3 Hz, $^3J_{H,H}$ = 5.6 Hz, 2H, 24-CH_2), 3.87–3.82 (m, 1H, 14-CH), 3.84 (t, $^3J_{H,H}$ = 7.1 Hz, 2H, 21-CH_2), 3.78–3.74 (m, 1H, 12-CH), 3.64 (dd, $^2J_{H,H}$ = 9.6 Hz, $^3J_{H,H}$ = 3.2 Hz, 1H, 16-CH_a), 3.55 (dd, $^2J_{H,H}$ = 9.6 Hz, $^3J_{H,H}$ = 6.1 Hz, 1H, 16-CH_b), 3.47 (d, $^3J_{H,H}$ = 3.2 Hz, 2H, 15-CH_2), 3.38 (s, 3H, 18-CH_3), 3.04 (s, 3H, 20-CH_3), 2.06–1.96 (m, 2H, 13-CH_2), 1.89 (quintet, $^3J_{H,H}$ = 7.1 Hz, 2H, 22-CH_2), 1.88–1.73 (m, 2H, 23-CH_2) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 182.2 (3-CO), 158.1 (2-CO), 153.5 (8-CN), 138.0 (6-CH), 134.2 (5-CSO_2), 125.1 (4-CH), 117.3 (9-CCO), 110.2 (7-CH), 83.4 (d, $^1J_{C,F}$ = 165.5 Hz, 24-CH_2), 78.9 (14-CH), 75.1 (16-CH_2), 59.5 (18-CH_3), 58.7 (12-CH), 56.5 (20-CH_3), 54.1 (15-CH_2), 40.3 (21-CH_2), 35.2 (13-CH_2), 27.8 (d, $^2J_{C,F}$ = 20.1 Hz, 23-CH_2), 23.7 (d, $^3J_{C,F}$ = 4.0 Hz, 22-CH_2) ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = −219.9 (s, 1F, 24-CH_2F) ppm. HRMS (ESI+, MeOH): *m*/*z* = 451.1303 $[M + Na]^+$, 483.1569 $[M + Na + MeOH]^+$; calcd. 451.1310 for $C_{19}H_{25}FN_2O_6S$ + Na, 483.1572 for $C_{19}H_{25}FN_2O_6S$ + Na + MeOH.

4.4. (2S,4S)-5-[1-(4-methoxy-2-methoxymethylpyrrolidinyl)sulfonyl]isatins



4.4.1. (2S,4S)-N-tert-butyloxycarbonyl-4-hydroxy-2-hydroxymethyl pyrrolidine (**3b**)

A stirred solution of methyl (2*S*,4*S*)-*N*-*tert*-butyloxycarbonyl-4-hydroxypyrrolidine-2-carboxylate (**2b**) (400 mg, 1.63 mmol, 1.00 equiv.) in THF (6 mL) were cooled to 0 °C and treated with 2 M $LiBH_4$ in THF solution (1.63 mL, 3.25 mmol, 2.00 equiv.). The resulting mixture was stirred at r.t. for 18 h. After this time, the milky mixture was cooled to 0 °C, quenched with 10% aq. citric acid and adjusted to pH 4. The solvent was removed under reduced pressure and the residue was taken up in water (15 mL). It was then extracted with DCM (3 × 15 mL). The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure to obtain colorless oil (312 mg, 1.43 mmol, 88%). The crude product was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.84 (br s, 2H, 4-COH, 10-COH), 4.35–4.25 (m, 1H, 4-CH), 4.05–3.90 (m, 1H, 2-CH), 4.03 (dd, $^2J_{H,H}$ = 11.3 Hz, $^3J_{H,H}$ = 2.6 Hz, 1H, 10-CH_a), 3.63–3.46 (m, 1H, 10-CH_b), 3.56–3.35 (m, 2H, 5-CH_2), 2.41–2.24 (m, 1H, 3-CH_a), 1.99–1.79 (m, 1H, 3-CH_b), 1.46 (s, 9H, 9-CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.7 (6-CO, rotamer A), 154.9 (6-CO, rotamer B), 80.1 (8-CO, rotamer B), 80.0 (8-CO, rotamer A), 69.7 (4-CH, rotamer A), 68.9 (4-CH, rotamer B), 64.1 (10-CH_2, rotamer A), 63.5 (10-CH_2, rotamer B), 58.5 (2-CH, rotamer A), 58.3 (2-CH, rotamer B), 56.7 (5-CH_2), 38.1 (3-CH_2, rotamer B), 37.3 (3-CH_2, rotamer A), 28.5 (3C, 9-CH_3) ppm. HRMS (ESI+, MeOH): *m*/*z* = 240.1208 $[M + Na]^+$; calcd. 240.1206 for $C_{10}H_{19}NO_4$ + Na.

4.4.2. (2S,4S)-N-tert-butyloxycarbonyl-4-methoxy-2-methoxymethyl pyrrolidine (**4b**)

Under argon atmosphere, a stirred solution of **3b** (300 mg, 1.38 mmol, 1.00 equiv.) in dry THF (13 mL) was cooled to −10 °C and treated with 60% NaH (275 mg, 6.90 mmol, 5.00 equiv.). After stirring for 15 min, MeI (343 μL, 5.52 mmol, 4.00 equiv.) was added dropwise. The resulting mixture was stirred further at this temperature for 30 min, 0 °C for 1 h and r.t. for 24 h. After this time, the reaction mixture was cooled to 0 °C and quenched with a small amount of sat. aq. NH_4Cl until no more H_2 evolved. Water (20 mL) was added and the resulting mixture was extracted with EtOAc (3 × 20 mL). The combined organic phases were washed with water (10 mL), brine (10 mL) and dried over $MgSO_4$. After removal of the solvent, the residue was purified by flash column chromatography (silica gel, 5% THF in cyclohexane) to obtain a light yellow oil (277 mg, 1.13 mmol, 82%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.10–3.90 (m, 1H, 2-CH), 3.95–3.87 (m, 1H, 4-CH), 3.75–3.58 (m, 1H, 10-CH_a), 3.66–3.48 (m, 1H, 5-CH_a), 3.42–3.35 (m, 2H, 5-CH_b, 10-CH_b), 3.36 (s, 3H, 12-CH_3), 3.31 (s, 3H, 14-CH_3), 2.23–2.10 (m, 1H, 3-CH_a), 2.08–1.92 (m, 1H, 3-CH_b), 1.47 (s, 9H, 9-CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 154.5 (6-CO), 79.8 (4-CH, rotamer B), 79.5 (8-CO), 79.1 (4-CH, rotamer A), 73.9 (10-CH_2, rotamer A), 73.2 (10-CH_2, rotamer B), 58.9 (12-CH_3), 56.5 (14-CH_3), 55.6 (2-CH), 52.4 (5-CH_2, rotamer B), 51.7 (5-CH_2, rotamer A), 33.4 (3-CH_2, rotamer A), 32.2 (3-CH_2, rotamer B), 28.5 (3C, 9-CH_3) ppm. HRMS (ESI+, MeOH): *m*/*z* = 268.1514 $[M + Na]^+$, 513.3144 $[2M + Na]^+$; calcd. 268.1519 for $C_{12}H_{23}NO_4$ + Na, 513.3146 for 2($C_{12}H_{23}NO_4$) + Na.

4.4.3. (2S,4S)-5-[1-(4-methoxy-2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**20b**)

(2*S*,4*S*)-*N*-*tert*-butyloxycarbonyl-4-methoxy-2-methoxymethyl pyrrolidine (**4b**) (200 mg, 0.815 mmol, 1.00 equiv.) was converted to (2*S*,4*S*)-5-[1-(4-methoxy-2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**20b**) using TFA followed by the reaction with 5-chloro sulfonyl isatin and DIPEA as described in the general procedure in Section 4.2.2. The crude product was purified by flash column chromatography (silica gel, gradient DCM, 10% THF in DCM and then 20% THF in DCM) to obtain a yellow solid (185 mg, 0.522 mmol, 64%). M.p. 77–78 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.54 (s, 1H, 1-NH), 8.06 (dd, $^3J_{H,H}$ = 8.2 Hz, $^4J_{H,H}$ = 1.9 Hz, 1H, 6-CH), 8.03 (d, $^4J_{H,H}$ = 1.9 Hz, 1H, 4-CH), 7.20 (d, $^3J_{H,H}$ = 8.3 Hz, 1H, 7-CH), 3.91–3.83 (m, 1H, 12-CH), 3.88–3.81 (m, 1H, 14-CH), 3.66 (dd, $^2J_{H,H}$ = 9.1 Hz, $^3J_{H,H}$ = 5.3 Hz, 1H 16-CH_a), 3.50–3.44 (m, 1H, 15-CH_a), 3.48–3.41 (m, 1H, 16-CH_b), 3.37–3.31 (m, 1H, 15-CH_b), 3.34 (s, 3H, 18-CH_3), 3.28 (s, 3H, 20-CH_3), 2.14–2.05 (m, 1H, 13-CH_a), 1.87–1.76 (m, 1H, 13-CH_b) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 182.3 (3-CO), 159.0 (2-CO), 152.7 (8-CN), 137.7 (6-CH), 133.7 (5-CSO_2), 124.9 (4-CH), 117.7 (9-CCO), 113.4 (7-CH), 79.3 (14-CH), 75.0 (16-CH_2), 58.9 (18-CH_3), 58.5 (12-CH), 56.7 (20-CH_3), 53.7 (15-CH_2), 33.3 (13-CH_2) ppm. HRMS (ESI+, MeOH): *m*/*z* = 377.0777 $[M + Na]^+$, 409.1038 $[M + Na + MeOH]^+$; calcd. 377.0778 for $C_{15}H_{18}N_2O_6S$ + Na, 409.1040 for $C_{15}H_{18}N_2O_6S$ + Na + MeOH.

4.4.4. (2S,4S)-N-butyl-5-[1-(4-methoxy-2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**21b**)

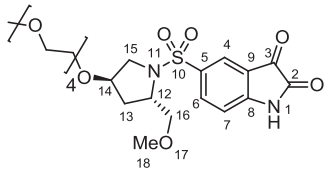
(2*S*,4*S*)-5-[1-(4-methoxy-2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**20b**) (50 mg, 0.141 mmol, 1.00 equiv.) was converted to (2*S*,4*S*)-*N*-butyl-5-[1-(4-methoxy-2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**21b**) using 1-bromobutane and Cs_2CO_3 as described in the general procedure in Section 4.2.3. The crude product was purified by flash column chromatography (silica gel, 60% EtOAc in cyclohexane) to obtain a yellow-orange solid (49 mg, 0.119 mmol, 85%). M.p. 119 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.09 (dd, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.9 Hz, 1H, 6-CH), 8.04 (d, $^4J_{H,H}$ = 1.9 Hz, 1H, 4-CH), 7.03 (d, $^3J_{H,H}$ = 8.3 Hz, 1H, 7-CH), 3.91–3.86 (m, 1H, 12-CH), 3.86–3.82 (m, 1H, 14-CH), 3.78 (t, $^3J_{H,H}$ = 7.4 Hz, 2H, 22-CH_2), 3.65 (dd, $^2J_{H,H}$ = 9.0 Hz, $^3J_{H,H}$ = 5.1 Hz, 1H, 16-CH_a), 3.47–3.42 (m, 1H, 15-CH_a), 3.50–3.40 (m, 1H, 16-CH_a), 3.36 (dd, $^2J_{H,H}$ = 11.0 Hz, $^3J_{H,H}$ = 5.2 Hz, 1H, 15-CH_b), 3.34 (s, 3H, 18-CH_3), 3.28 (s, 3H, 20-CH_3), 2.13–2.08 (m, 1H, 13-CH_a), 1.83 (ddd, $^2J_{H,H}$ = 14.0 Hz, $^3J_{H,H}$ = 8.7 Hz, $^3J_{H,H}$ = 5.5 Hz, 1H, 13-CH_b), 1.70 (quintet, $^3J_{H,H}$ = 7.5 Hz, 2H, 22-CH_2), 1.43 (sextet, $^3J_{H,H}$ = 7.4 Hz, 2H, 23-CH_2), 0.99 (t, $^3J_{H,H}$ = 7.4 Hz, 3H, 24-CH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 182.3 (3-CO), 157.9 (2-CO), 154.0 (8-CN), 137.6 (6-CH), 134.1 (5-CSO_2), 124.7 (4-CH), 117.5 (9-CCO), 110.6 (7-CH), 79.5 (14-CH), 75.1 (16-CH_2), 59.1 (18-CH_3), 58.6 (12-CH), 56.9 (20-CH_3), 53.7 (15-CH_2), 40.7 (21-CH_2), 33.6 (13-CH_2), 29.4 (22-CH_2), 20.3 (23-CH_2), 13.8 (24-CH_2) ppm. HRMS (ESI+, MeOH): *m*/*z* = 433.1400 $[M + Na]^+$, 465.1661 $[M + Na + MeOH]^+$; calcd. 433.1404 for $C_{19}H_{26}N_2O_6S$ + Na, 465.1666 for $C_{19}H_{26}N_2O_6S$ + Na + MeOH.

4.4.5. (2S,4S)-N-(4-fluorobutyl)-5-[1-(4-methoxy-2-methoxymethyl pyrrolidinyl)sulfonyl]isatin (**22b**)

(2*S*,4*S*)-5-[1-(4-methoxy-2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**20b**) (50 mg, 0.141 mmol, 1.00 equiv.) was converted to (2*S*,4*S*)-*N*-(4-fluorobutyl)-5-[1-(4-methoxy-2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**22b**) using 1-bromo-4-fluorobutane and Cs_2CO_3 as described in the general procedure in Section 4.2.3. The crude product was purified by flash column chromatography (silica gel, 75% EtOAc in cyclohexane) to obtain a yellow-orange solid (55 mg, 0.129 mmol, 91%). M.p. 114 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.09 (dd, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.9 Hz, 1H, 6-CH), 8.04 (d, $^4J_{H,H}$ = 1.9 Hz, 1H, 4-CH), 7.06 (d, $^3J_{H,H}$ = 8.3 Hz, 1H, 7-CH), 3.91–3.84

(m, 1H, 12-CH), 3.86–3.82 (m, 1H, 14-CH), 3.84 (t, $^3J_{H,H}$ = 7.0 Hz, 2H, 21-CH_2), 3.64 (dd, $^2J_{H,H}$ = 9.0 Hz, $^3J_{H,H}$ = 5.1 Hz, 1H, 16-CH_a), 4.52 (dt, $^2J_{H,F}$ = 47.4 Hz, $^3J_{H,H}$ = 5.6 Hz, 2H, 24-CH_2), 3.48–3.41 (m, 2H, 15-CH_a, 16-CH_b), 3.39–3.33 (m, 1H, 15-CH_b), 3.34 (s, 3H, 18-CH_3), 3.28 (s, 3H, 20-CH_3), 2.10 (m, 1H, 13-CH_a), 1.89 (quintet, $^3J_{H,H}$ = 7.5 Hz, 2H, 22-CH_2), 1.87–1.74 (m, 3H, 13-CH_b, 23-CH_2) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 182.1 (3-CO), 158.0 (2-CO), 153.7 (8-CN), 137.7 (6-CH), 134.2 (5-CSO_2), 124.8 (4-CH), 117.5 (9-CCO), 110.6 (7-CH), 83.4 (d, $^1J_{C,F}$ = 165.6 Hz, 24-CH_2), 79.5 (14-CH), 75.1 (16-CH_2), 59.1 (18-CH_3), 58.6 (12-CH), 56.9 (20-CH_3), 53.7 (15-CH_2), 40.4 (21-CH_2), 33.5 (13-CH_2), 27.8 (d, $^2J_{C,F}$ = 20.1 Hz, 23-CH_2), 23.7 (d, $^3J_{C,F}$ = 4.0 Hz, 22-CH_2) ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = −219.9 (s, 1F, 24-CH_2F) ppm. HRMS (ESI+, MeOH): m/z = 451.1307 $[M + Na]^+$, 483.1568 $[M + Na + MeOH]^+$; calcd. 451.1310 for $C_{19}H_{25}FN_2O_6S$ + Na, 483.1572 for $C_{19}H_{25}FN_2O_6S$ + Na + MeOH.

4.5. (2S,4R)-5-[1-(2-methoxymethyl-4-PEG$_4$yloxypyrrolidinyl) sulfonyl]isatins



4.5.1. Methyl (2S,4R)-N-tert-butyloxycarbonyl-4-PEG$_4$yloxypyrroli dine-2-carboxylate (**6a**)

Under argon atmosphere, a stirred solution of methyl (2*S*,4*R*)-*N*-*tert*-butyloxycarbonyl-4-hydroxypyrrolidine-2-carboxylate (**2a**) (1.00 g, 4.08 mmol, 1.00 equiv.) in dry THF (30 mL) was cooled to −10 °C, treated with 60% NaH (245 mg, 6.12 mmol, 1.50 equiv. in mineral oil). After stirring at −10 °C for 15 min, 2,5,8,11-tetraoxatridecan-13-yl-*p*-toluenesulfonate (1.97 g, 5.44 mmol, 1.33 equiv.) was added dropwise. The reaction mixture was stirred at this temperature for 30 min, 0 °C for 4 h and at r.t. for 24 h. After this time, the mixture was cooled to 0 °C and quenched by addition of sat. aq. NH_4Cl until no more H_2 evolved. Then H_2O (40 mL) was added and the mixture was extracted with EtOAc (3 × 40 mL). The combined organic phases were washed with brine (1 × 40 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, 3% MeOH in DCM) to obtain a colorless oil (871 mg, 2.00 mmol, 49%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.45–4.03 (m, 2H, 2-CH, 4-CH), 3.75–3.52 (m, 21H, 5-CH_2, 12-CH_3, 8 × OCH_2), 3.38 (s, 3H, OCH_3), 2.46–2.16 (m, 1H, 3-CH_a), 2.12–1.99 (m, 1H, 3-CH_b), 1.49–1.39 (m, 9H, 9-CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.6 (10-CO, rotamer A), 173.4 (10-CO, rotamer B), 154.3 (6-CO, rotamer B), 153.7 (6-CO, rotamer A), 80.1 (8-CO, rotamer A), 80.0 (8-CO, rotamer B), 77.2 (4-CH), 71.9 (OCH_2), 70.6 (2C, OCH_2), 70.5 (4C, OCH_2), 68.6 (OCH_2), 59.0 (OCH_3), 58.0 (2-CH, rotamer A), 57.6 (2-CH, rotamer B), 52.2 (5-CH_2, rotamer B), 52.0 (5-CH_2, rotamer A), 51.4 (12-CH_3, rotamer A), 51.2 (12-CH_3, rotamer B), 36.6 (3-CH_2), 28.4 (9-CH_3, rotamer B), 28.2 (9-CH_3, rotamer A) ppm. HRMS (ESI+, MeOH): m/z = 458.2360 $[M + Na]^+$; calcd. 458.2361 for $C_{20}H_{37}NO_9$ + Na.

4.5.2. (2S,4R)-N-tert-butyloxycarbonyl-2-hydroxymethyl-4-PEG$_4$ yloxypyrrolidine (**7a**)

Under argon atmosphere, a stirred solution of methyl (2*S*,4*R*)-*N*-*tert*-butyloxycarbonyl-4-PEG$_4$yloxypyrrolidine-2-carboxylate (**6a**) (697 mg, 1.60 mmol, 1.00 equiv.) in dry THF (8 mL) was cooled to 0 °C and treated with 4 M $LiBH_4$ in THF (800 μL, 3.20 mmol, 2.00 equiv.) dropwise. The resulting solution was stirred further at r.t. for 18 h. After this time the mixture was cooled to 0 °C, quenched with 10% aq. citric acid and adjusted to pH 4. The organic solvent was removed completely under reduced pressure. H_2O (10 mL) was added to the residue, which was subsequently extracted with DCM (3 × 20 mL). The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The crude was purified by flash column chromatography (silica gel, 3.5% MeOH in DCM) to obtain a colorless oil (390 mg, 0.957 mmol, 60%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.12–3.98 (m, 2H, 2-CH, 4-CH), 3.75–3.50 (m, 20H, 5-CH_2, 10-CH_2, 8 × OCH_2), 3.38 (s, 3H, OCH_3), 2.22–2.06 (m, 1H, 3-CH_a), 1.93–1.55 (m, 1H, 3-CH_b), 1.47 (s, 9H, 9-CH_3) ppm. HRMS (ESI+, MeOH): m/z = 430.2403 $[M + Na]^+$; calcd. 430.2411 for $C_{19}H_{37}NO_8$ + Na.

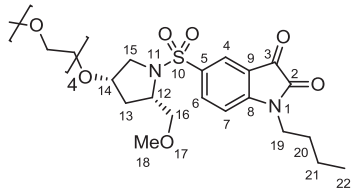
4.5.3. (2S,4R)-N-tert-butyloxycarbonyl-2-methoxymethyl-4-PEG$_4$ yloxypyrrolidine (**8a**)

(2*S*,4*R*)-*N*-*tert*-butyloxycarbonyl-2-hydroxymethyl-4-PEG$_4$yloxypyrrolidine (**7a**) (400 mg, 0.982 mmol, 1.00 equiv.) was converted to (2*S*,4*R*)-*N*-*tert*-butyloxycarbonyl-2-methoxymethyl-4-PEG$_4$yloxypyrrolidine (**8a**) using MeI and NaH as described in the general procedure in Section 4.2.1. The residue was purified by flash column chromatography (silica gel, 3.5% MeOH in DCM) to obtain a colorless oil (288 mg, 0.683 mmol, 70%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.19–3.86 (m, 2H, 2-CH, 4-CH), 3.70–3.37 (m, 20H, 5-CH_2, 10-CH_2, 8 × OCH_2), 3.38 (s, 3H, OCH_3), 3.36 (s, 12-CH_3, rotamer B), 3.34 (s, 12-CH_3, rotamer A), 2.24–1.93 (m, 2H, 3-CH_2), 1.46 (s, 9H, 9-CH_3) ppm. HRMS (ESI+, MeOH): m/z = 444.2564 $[M + Na]^+$; calcd. 444.2568 for $C_{20}H_{39}NO_8$ + Na.

4.5.4. (2S,4R)-5-[1-(2-methoxymethyl-4-PEG$_4$yloxypyrrolidinyl) sulfonyl]isatin (**23a**)

(2*S*,4*R*)-*N*-*tert*-butyloxycarbonyl-2-methoxymethyl-4-PEG$_4$yloxypyrrolidine (**8a**) (250 mg, 0.593 mmol, 1.00 equiv.) was converted to (2*S*,4*R*)-5-[1-(2-methoxymethyl-4-PEG$_4$yloxypyrrolidinyl)sulfonyl]isatin (**23a**) using TFA followed by reaction with 5-chlorosulfonyl isatin and DIPEA as described in the general procedure in Section 4.2.2. The residue was purified by flash chromatography (silica gel, 5% MeOH in DCM) to obtain a yellow oil (63 mg, 0.119 mmol, 20%). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 9.94 (s, 1H, 1-NH), 8.03 (dd, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.9 Hz, 1H, 6-CH), 8.00 (d, $^4J_{H,H}$ = 1.9 Hz, 1H, 4-CH), 7.19 (d, $^3J_{H,H}$ = 8.3 Hz, 1H, 7-CH), 3.84–3.81 (m, 1H, 14-CH), 3.80–3.65 (m, 6H, 3 × OCH_2), 3.79–3.75 (m, 1H, 16-CH_a), 3.72–3.66 (m, 1H, 12-CH), 3.63–3.57 (m, 1H, 16-CH_b), 3.61–3.52 (m, 4H, 2 × OCH_2), 3.56–3.51 (m, 1H, 15-CH_a), 3.49–3.44 (m, 1H, 15-CH_b), 3.46–3.38 (m, 2H, OCH_2), 3.41 (s, 3H, 18-CH_3), 3.31 (s, 3H, OCH_3), 3.26–3.20 (m, 2H, OCH_2), 3.18–3.13 (m, 1H, OCH_a), 3.06–3.02 (m, 1H, OCH_b), 2.13–2.03 (m, 1H, 13-CH_a), 1.91 (ddd, $^2J_{H,H}$ = 13.7 Hz, $^3J_{H,H}$ = 9.1 Hz, $^3J_{H,H}$ = 4.2 Hz, 1H, 13-CH_b) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 183.2 (3-CO), 158.8 (2-CO), 153.7 (8-CN), 137.8 (6-CH), 132.5 (5-CSO_2), 124.7 (4-CH), 117.1 (9-CCO), 113.6 (7-CH), 77.9 (14-CH), 75.2 (16-CH_2), 71.6 (OCH_2), 70.7 (2C, OCH_2), 70.5 (OCH_2), 70.4 (OCH_2), 70.3 (OCH_2), 70.1 (OCH_2), 67.7 (OCH_2), 59.4 (18-CH_3), 58.7 (OCH_3), 58.7 (12-CH), 54.9 (15-CH_2), 35.0 (13-CH_2) ppm. HRMS (ESI+, MeOH): m/z = 553.1822 $[M + Na]^+$, 585.2088 $[M + Na + MeOH]^+$; calcd. 553.1826 for $C_{23}H_{34}N_2O_{10}S$ + Na, 585.2089 for $C_{23}H_{34}N_2O_{10}S$ + Na + CH_3OH.

4.6. (2S,4S)-5-[1-(2-methoxymethyl-4-PEG$_4$yloxypyrrolidinyl)sulfonyl]isatins



4.6.1. Methyl (2S,4S)-N-tert-butyloxycarbonyl-4-PEG$_4$yloxypyrrolidine-2-carboxylate (**6b**)

The preparation of methyl (2*S*,4*S*)-*N*-*tert*-butyloxycarbonyl-4-PEG$_4$yloxypyrrolidine-2-carboxylate (**6b**) was achieved starting from (2*S*,4*S*)-*N*-*tert*-butyloxycarbonyl-4-hydroxypyrrolidine-2-carboxylate (**2b**) (1.00 g, 4.08 mmol, 1.00 equiv.) using dry THF (30 mL), 60% NaH (245 mg, 6.12 mmol, 1.50 equiv. in mineral oil) and 2,5,8,11-tetraoxatridecan-13-yl-*p*-toluenesulfonate (**5**) (1.97 g, 5.44 mmol, 1.33 equiv.) as described in Section 4.5.1. The crude product was purified by flash column chromatography (silica gel, 3% MeOH in DCM) to obtain a colorless oil (1.13 g, 2.59 mmol, 64%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.45–4.24 (m, 1H, 4-CH), 4.24–4.03 (m, 1H, 2-CH), 3.77–3.51 (m, 21H, 5-CH_2, 12-CH_3, 8 × OCH_2), 3.38 (s, 3H, OCH_3), 2.45–2.16 (m, 1H, 3-CH_a), 2.12–1.95 (m, 1H, 3-CH_b), 1.52–1.37 (m, 9H, 9-CH_3) ppm. HRMS (ESI+, MeOH): *m*/*z* = 458.2369 [M + Na]$^+$; calcd. 458.2361 for $C_{20}H_{37}NO_9$ + Na.

4.6.2. (2S,4S)-N-tert-butyloxycarbonyl-2-hydroxymethyl-4-PEG$_4$yloxypyrrolidine (**7b**)

The preparation of (2*S*,4*S*)-*N*-*tert*-butyloxycarbonyl-2-hydroxymethyl-4-PEG$_4$yloxypyrrolidine (**7b**) was achieved starting from methyl (2*S*,4*S*)-*N*-*tert*-butyloxycarbonyl-4-PEG$_4$yloxypyrrolidine-2-carboxylate (**6b**) (540 mg, 1.24 mmol, 1.00 equiv.) using dry THF (5 mL) for dissolving the ester **6b** and 4 M $LiBH_4$ in THF (620 μL, 2.48 mmol, 2.00 equiv.) as described in Section 4.5.2. The crude product was purified by flash column chromatography (silica gel, 3.5% MeOH in DCM) to obtain a colorless oil (417 mg, 1.02 mmol, 83%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.13–3.93 (m, 2H, 2-CH, 4-CH), 3.76–3.50 (m, 20H, 5-CH_2, 10-CH_2, 8 × OCH_2), 3.38 (s, 3H, OCH_3), 3.10 (br s, 1H, 10-COH), 2.21–2.08 (m, 1H, 3-CH_a), 1.96–1.56 (m, 1H, 3-CH_b), 1.47 (s, 9H, 9-CH_3) ppm. HRMS (ESI+, MeOH): *m*/*z* = 430.2402 [M + Na]$^+$; calcd. 430.2411 for $C_{19}H_{37}NO_8$ + Na.

4.6.3. (2S,4S)-N-tert-butyloxycarbonyl-2-methoxymethyl-4-PEG$_4$yloxypyrrolidine (**8b**)

(2*S*,4*S*)-*N*-*tert*-butyloxycarbonyl-2-hydroxymethyl-4-PEG**$_4$**yloxypyrrolidine (**7b**) (375 mg, 0.920 mmol, 1.00 equiv.) was converted to (2*S*,4*S*)-*N*-*tert*-butyloxycarbonyl-2-methoxymethyl-4-PEG$_4$yloxypyrrolidine (**8b**) using MeI and NaH as described in the general procedure in Section 4.2.1. The crude product was purified by flash column chromatography (silica gel, 5% MeOH in DCM) to obtain a colorless oil (312 mg, 0.740 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.17–3.88 (m, 2H, 2-CH, 4-CH), 3.69–3.32 (m, 20H, 5-CH_2, 10-CH_2, 8 × OCH_2), 3.38 (s, 3H, OCH_3), 3.35 (s, 12-CH_3, rotamer B), 3.34 (s, 12-CH_3, rotamer A), 2.23–1.93 (m, 2H, 3-CH_2), 1.46 (s, 9H, 9-CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 154.6 (6-CO), 79.4 (8-CO), 77.3 (4-CH), 74.1 (10-CH_2, rotamer A), 73.3 (10-CH_2, rotamer B), 71.9 (OCH_2), 70.6 (4C, OCH_2), 70.5 (2C, OCH_2), 68.5 (OCH_2), 59.1 (12-CH_3), 59.0 (OCH_3), 58.8 (2-CH), 55.6 (5-CH_2, rotamer A), 55.5 (5-CH_2, rotamer B), 35.4 (3-CH_2, rotamer B), 34.2 (3-CH_2, rotamer A), 28.5 (3C, 9-CH_3) ppm. HRMS (ESI+, MeOH): *m*/*z* = 444.2570 [M + Na]$^+$; calcd. 444.2568 for $C_{20}H_{39}NO_8$+Na.

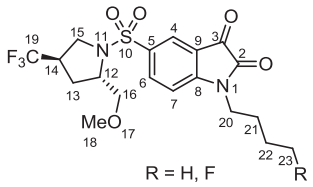
4.6.4. (2S,4S)-5-[1-(2-methoxymethyl-4-PEG$_4$yloxypyrrolidinyl)sulfonyl]isatin (**23b**)

(2*S*,4*S*)-*N*-*tert*-Butyloxycarbonyl-2-methoxymethyl-4-PEG$_4$yloxypyrrolidine (**8b**) (311 mg, 0.738 mmol, 1.00 equiv.) was converted to (2*S*,4*S*)-5-[1-(2-methoxymethyl-4-PEG$_4$yloxypyrrolidinyl)sulfonyl]isatin (**23b**) using TFA followed by reaction with 5-chloro sulfonyl isatin and DIPEA as described in the general procedure in Section 4.2.2. The residue was purified by flash chromatography (silica gel, 4% MeOH in DCM) to obtain a yellow oil (156 mg, 0.294 mmol, 40%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.96 (s, 1H, 1-NH), 8.03 (dd, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.9 Hz, 1H, 6-CH), 8.00 (d, $^4J_{H,H}$ = 1.9 Hz, 1H, 4-CH), 7.19 (d, $^3J_{H,H}$ = 8.3 Hz, 1H, 7-CH), 3.85–3.80 (m, 1H, 14-CH), 3.80–3.63 (m, 6H, OCH_2), 3.79–3.73 (m, 1H, 16-CH_a), 3.72–3.66 (m, 1H, 12-CH), 3.64–3.57 (m, 1H, 16-CH_b), 3.62–3.50 (m, 4H, OCH_2), 3.56–3.44 (m, 2H, 15-CH_2), 3.47–3.37 (m, 2H, OCH_2), 3.41 (s, 3H, 18-CH_3), 3.31 (s, 3H, OCH_3), 3.26–3.13 (m, 3H, OCH_2), 3.07–3.02 (m, 1H, OCH_2), 2.14–2.04 (m, 1H, 13-CH_a), 1.91 (ddd, $^2J_{H,H}$ = 13.7 Hz, $^3J_{H,H}$ = 9.0 Hz, $^3J_{H,H}$ = 4.2 Hz, 1H, 13-CH_b) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 183.2 (3-CO), 158.9 (2-CO), 153.7 (8-CN), 137.9 (6-CH), 132.5 (5-CSO_2), 124.7 (4-CH), 117.2 (9-CCO), 113.6 (7-CH), 77.9 (14-CH), 75.2 (16-CH_2), 71.6 (OCH_2), 70.7 (2C, OCH_2), 70.5 (OCH_2), 70.4 (OCH_2), 70.3 (OCH_2), 70.2 (OCH_2), 67.7 (OCH_2), 59.4 (18-CH_3), 58.8 (OCH_3), 58.7 (12-CH), 54.9 (15-CH_2), 35.0 (13-CH_2) ppm. HRMS (ESI+, MeOH): *m*/*z* = 553.1826 [M + Na]$^+$, 585.2084 [M + Na + MeOH]$^+$; calcd. 553.1826 for $C_{23}H_{34}N_2O_{10}S$ + Na, 585.2089 for $C_{23}H_{34}N_2O_{10}S$ + Na + MeOH.

4.6.5. N-butyl-(2S,4S)-5-[1-(2-methoxymethyl-4-PEG$_4$yloxypyrrolidinyl)sulfonyl]isatin (**24**)

(2*S*,4*S*)-5-[1-(2-Methoxymethyl-4-PEG$_4$yloxypyrrolidinyl)sulfonyl]isatin (**23b**) (50 mg, 94.2 μmol, 1.00 equiv.) was converted to *N*-butyl-(2*S*,4*S*)-5-[1-(2-methoxymethyl-4-PEG$_4$yloxypyrrolidinyl)sulfonyl]isatin (**24**) using 1-bromobutane and Cs_2CO_3 as described in the general procedure in Section 4.2.3. The resulting residue was purified by flash column chromatography (silica gel, 5% MeOH in DCM) to obtain a yellow oil (38 mg, 64.8 μmol, 69%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.08 (dd, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.9 Hz, 1H, 6-CH), 8.04 (d, $^4J_{H,H}$ = 1.9 Hz, 1H, 4-CH), 7.03 (d, $^3J_{H,H}$ = 8.3 Hz, 1H, 7-CH), 4.09–4.00 (m, 1H, 14-CH), 3.82–3.73 (m, 1H, 12-CH), 3.78 (t, $^3J_{H,H}$ = 7.4 Hz, 2H, 19-CH_2), 3.69–3.46 (m, 13H, OCH_2), 3.66–3.59 (m, 1H, 16-CH_a), 3.59–3.51 (m, 1H, 16-CH_b), 3.56–3.50 (m, 1H, 15-CH_a), 3.44–3.37 (m, 1H, 15-CH_b), 3.43–3.30 (m, 3H, OCH_2), 3.37 (s, 3H, 18-CH_3), 3.37 (s, 3H, OCH_3), 2.09–1.92 (m, 2H, 13-CH_2), 1.70 (quintet, $^3J_{H,H}$ = 7.4 Hz, 2H, 20-CH_2), 1.43 (sextet, $^3J_{H,H}$ = 7.4 Hz, 2H, 21-CH_2), 0.99 (t, $^3J_{H,H}$ = 7.4 Hz, 3H, 22-CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 182.2 (3-CO), 157.9 (2-CO), 153.6 (8-CN), 137.8 (6-CH), 133.8 (5-CSO_2), 124.9 (4-CH), 117.2 (9-CCO), 110.2 (7-CH), 77.4 (14-CH), 75.0 (16-CH_2), 71.9 (OCH_2), 70.6 (OCH_2), 70.5 (3C, OCH_2), 70.4 (OCH_2), 70.3 (OCH_2), 68.2 (OCH_2), 59.3 (18-CH_3), 59.0 (OCH_3), 58.5 (12-CH), 54.5 (15-CH_2), 40.4 (19-CH_2), 35.2 (13-CH_2), 29.3 (20-CH_2), 20.1 (21-CH_2), 13.6 (22-CH_3) ppm. HRMS (ESI+, MeOH): *m*/*z* = 609.2450 [M + Na]$^+$, 641.2707 [M + Na + MeOH]$^+$; calcd. 609.2452 for $C_{27}H_{42}N_2O_{10}S$ + Na, 641.2715 for $C_{27}H_{42}N_2O_{10}S$ + Na + MeOH.

4.7. (2S,4R)-5-[1-(4-trifluoromethyl-2-methoxymethylpyrrolidinyl) sulfonyl]isatins



4.7.1. (2S,4R)-N-tert-butyloxycarbonyl-4-trifluoromethyl-2-methoxy methyl pyrrolidine (**12a**)

(2*S*,4*R*)-*N*-*tert*-butyloxycarbonyl-4-trifluoromethyl-2-hydroxy methylpyrrolidine (**11a**) (180 mg, 0.668 mmol, 1.00 equiv.) was converted to (2*S*,4*R*)-*N*-*tert*-butyloxycarbonyl-4-trifluoromethyl-2-methoxymethyl pyrrolidine (**12a**) using MeI and NaH as described in the general procedure in Section 4.2.1. The crude was purified by flash column chromatography (silica gel, 20% EtOAc in cyclohexane) to obtain a colorless oil (186 mg, 0.657 mmol, 98%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.20–3.92 (m, 1H, 2-CH), 3.63–3.33 (m, 4H, 5-CH_2, 10-CH_2), 3.34 (s, 3H, 12-CH_3), 3.23–3.01 (m, 1H, 4-CH), 2.22–1.98 (m, 2H, 3-CH_2), 1.47 (s, 9H, 9-CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 154.0 (6-CH), 127.0 (q, $^1J_{C,F}$ = 276.8 Hz, 13-CF_3), 80.0 (8-CO), 74.1 (10-CH_2, rotamer B), 73.5 (10-CH_2, rotamer A), 59.2 (12-CH_3), 56.6 (2-CH), 46.0 (5-CH_2), 42.3–39.9 (m, 4-CH), 28.9 (3-CH_2, rotamer B), 28.5 (3C, 9-CH_3), 28.0 (3-CH_2, rotamer A) ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = −71.9 (s, 3F, 13-CF_3) ppm. HRMS (ESI+, MeOH): *m*/*z* = 306.1294 $[M + Na]^+$, 589.2689 $[2M + Na]^+$; calcd. 306.1287 for $C_{12}H_{20}F_3NO_3$ + Na, 589.2683 for 2($C_{12}H_{20}F_3NO_3$) + Na.

4.7.2. (2S,4R)-5-[1-(4-trifluoromethyl-2-methoxymethylpyrrolidinyl) sulfonyl]isatin (**25a**)

(2*S*,4*R*)-*N*-*tert*-butyloxycarbonyl-4-trifluoromethyl-2-methoxy methylpyrrolidine (**12a**) (190 mg, 0.670 mmol, 1.00 equiv.) was converted to (2*S*,4*R*)-5-[1-(4-trifluoromethyl-2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**25a**) using 5-chlorosulfonyl isatin and DIPEA as described in the general procedure in Section 4.2.2. The crude product was purified by flash column chromatography (silica gel, 50% EtOAc in cyclohexane) to obtain a sticky yellow wax (70 mg, 0.178 mmol, 27%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.77 (br s, 1H, 1-NH), 8.10–8.05 (m, 2H, 4-CH, 6-CH), 7.17–7.12 (m, 1H, 7-CH), 4.02–3.92 (m, 1H, 12-CH), 3.68 (dd, $^2J_{H,H}$ = 9.9 Hz, $^3J_{H,H}$ = 7.9 Hz, 1H, 15-CH_a), 3.56 (dd, $^2J_{H,H}$ = 9.8 Hz, $^3J_{H,H}$ = 4.8 Hz, 1H, 16-CH_a), 3.51 (dd, $^2J_{H,H}$ = 9.8 Hz, $^3J_{H,H}$ = 3.3 Hz, 1H, 16-CH_b), 3.36 (s, 3H, 18-CH_3), 3.32–3.23 (m, 1H, 15-CH_b), 3.26–3.09 (m, 1H, 14-CH), 2.14 (ddd, $^2J_{H,H}$ = 12.9 Hz, $^3J_{H,H}$ = 7.2 Hz, $^3J_{H,H}$ = 2.2 Hz, 1H, 13-CH_a), 1.87 (dt, $^2J_{H,H}$ = 12.8 Hz, $^3J_{H,H}$ = 9.2 Hz, 1H, 13-CH_b) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 181.5 (3-CO), 158.4 (2-CO), 152.0 (8-CN), 137.5 (6-CH), 134.0 (5-CSO_2), 124.9 (4-CH), 117.9 (9-CCO), 112.9 (7-CH), 75.1 (16-CH_2), 59.4 (18-CH_3), 59.3 (12-CH), 48.0 (q, $^3J_{C,F}$ = 2.8 Hz, 15-CH_2), 41.6 (q, $^2J_{C,F}$ = 29.1 Hz, 14-CH), 29.0 (q, $^3J_{C,F}$ = 2.1 Hz, 13-CH_2) ppm.* ^{19}F NMR (282 MHz, $CDCl_3$): δ = −71.7 (s, 3F, 19-CF_3) ppm. HRMS (ESI+, MeOH): *m*/*z* = 415.0544 $[M + Na]^+$, 447.0806 $[M + Na + MeOH]^+$; calcd. 415.0546 for $C_{15}H_{15}F_3N_2O_5S$ + Na, 447.0808 for $C_{15}H_{15}F_3N_2O_5S$ + Na + MeOH.

* The ^{13}C NMR signal of 19-CF_3 could not be observed because of sample dilution.

4.7.3. (2S,4R)-N-butyl-5-[1-(4-trifluoromethyl-2-methoxymethyl pyrrolidinyl)sulfonyl]isatin (**26a**)

(2*S*,4*R*)-5-[1-(4-trifluoromethyl-2-methoxymethylpyrrolidinyl) sulfonyl]isatin (**25a**) (25 mg, 63.7 μmol, 1.00 equiv.) was converted to (2*S*,4*R*)-*N*-butyl-5-[1-(4-trifluoromethyl-2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**26a**) using 1-bromobutane and Cs_2CO_3 as described in the general procedure in Section 4.2.3. The crude product was purified by flash column chromatography (silica gel, 60% EtOAc in cyclohexane) to obtain a yellow oil (24 mg, 53.5 μmol, 84%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.08 (dd, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 2.0 Hz, 1H, 6-CH), 8.04 (d, $^4J_{H,H}$ = 1.9 Hz, 1H, 4-CH), 7.05 (d, $^3J_{H,H}$ = 8.3 Hz, 1H, 7-CH), 4.01–3.92 (m, 1H, 12-CH), 3.79 (t, $^3J_{H,H}$ = 7.3 Hz, 2H, 20-CH), 3.67 (dd, $^2J_{H,H}$ = 9.7 Hz, $^3J_{H,H}$ = 7.8 Hz, 1H, 15-CH_a), 3.56 (dd, $^2J_{H,H}$ = 9.8 Hz, $^3J_{H,H}$ = 4.8 Hz, 1H, 16-CH_a), 3.51 (dd, $^2J_{H,H}$ = 9.8 Hz, $^3J_{H,H}$ = 3.3 Hz, 1H, 16-CH_b), 3.36 (s, 3H, 18-CH_3), 3.31–3.20 (m, 1H, 15-CH_b), 3.26–3.09 (m, 1H, 14-CH), 2.14 (ddd, $^2J_{H,H}$ = 12.9 Hz, $^3J_{H,H}$ = 7.1 Hz, $^3J_{H,H}$ = 2.2 Hz, 1H, 13-CH_a), 1.87 (dt, $^2J_{H,H}$ = 12.8 Hz, $^3J_{H,H}$ = 9.5 Hz, 1H, 13-CH_b), 1.71 (quintet, $^3J_{H,H}$ = 7.4 Hz, 2H, 21-CH_2), 1.43 (sextet, $^3J_{H,H}$ = 7.4 Hz, 2H, 22-CH_2), 0.99 (t, $^3J_{H,H}$ = 7.3 Hz, 3H, 23-CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 182.0 (3-CO), 157.7 (2-CO), 154.0 (8-CN), 137.3 (6-CH), 133.4 (5-CSO_2), 124.4 (4-CH), 117.4 (9-CCO), 110.5 (7-CH), 75.1 (16-CH_2), 59.3 (18-CH_3), 59.3 (12-CH), 47.9 (q, $^3J_{C,F}$ = 3.4 Hz, 15-CH_2), 41.6 (q, $^2J_{C,F}$ = 29.2 Hz, 14-CH), 40.5 (20-CH_2), 29.2 (21-CH_2), 29.0 (q, $^3J_{C,F}$ = 2.3 Hz, 13-CH_2), 20.1 (22-CH_2), 13.6 (23-CH_3) ppm.* ^{19}F NMR (282 MHz, $CDCl_3$): δ = −71.6 (s, 3F, 19-CF_3) ppm. HRMS (ESI+, MeOH): *m*/*z* = 471.1178 $[M + Na]^+$, 503.1441 $[M + Na + MeOH]^+$; calcd. 471.1172 for $C_{19}H_{23}F_3N_2O_5S$ + Na, 503.1434 for $C_{19}H_{23}F_3N_2O_5S$ + Na + MeOH.

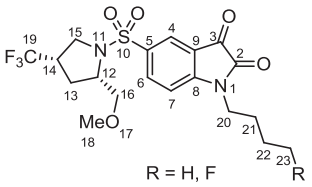
* The ^{13}C NMR signal of 19-CF_3 could not be observed because of sample dilution.

4.7.4. (2S,4R)-N-(4-fluorobutyl)-5-[1-(4-trifluoromethyl-2-methoxy methylpyrrolidinyl)-sulfonyl]isatin (**27a**)

(2*S*,4*R*)-5-[1-(4-trifluoromethyl-2-methoxymethylpyrrolidinyl) sulfonyl]isatin (**25a**) (25 mg, 63.7 μmol, 1.00 equiv.) was converted to (2*S*,4*R*)-*N*-(4-fluorobutyl)-5-[1-(4-trifluoromethyl-2-methoxy methylpyrrolidinyl)sulfonyl]isatin (**27a**) using 1-bromo-4-fluor obutane and Cs_2CO_3 as described in the general procedure in Section 4.2.3. The crude product was purified by flash column chromatography (silica gel, 50% EtOAc in cyclohexane) to obtain a yellow oil (27 mg, 57.9 μmol, 91%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.09 (dd, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.9 Hz, 1H, 6-CH), 8.05 (d, $^4J_{H,H}$ = 1.9 Hz, 1H, 4-CH), 7.08 (d, $^3J_{H,H}$ = 8.3 Hz, 1H, 7-CH), 4.53 (dt, $^2J_{H,F}$ = 47.4 Hz, $^3J_{H,H}$ = 5.4 Hz, 2H, 23-CH_2), 4.01–3.92 (m, 1H, 12-CH), 3.85 (t, $^3J_{H,H}$ = 7.1 Hz, 2H, 20-CH_2), 3.67 (dd, $^2J_{H,H}$ = 11.0 Hz, $^3J_{H,H}$ = 7.9 Hz, 1H, 15-CH_a), 3.56 (dd, $^2J_{H,H}$ = 9.8 Hz, $^3J_{H,H}$ = 4.8 Hz, 1H, 16-CH_a), 3.51 (dd, $^2J_{H,H}$ = 9.8 Hz, $^3J_{H,H}$ = 3.2 Hz, 1H, 16-CH_b), 3.36 (s, 3H, 18-CH_3), 3.31–3.23 (m, 1H, 15-CH_b), 3.26–3.10 (m, 1H, 14-CH), 2.14 (ddd, $^2J_{H,H}$ = 12.9 Hz, $^3J_{H,H}$ = 7.1 Hz, $^3J_{H,H}$ = 2.2 Hz, 1H, 13-CH_a), 1.98–1.70 (m, 5H, 13-CH_b, 21-CH_2, 22-CH_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 181.8 (3-CO), 157.7 (2-CO), 153.7 (8-CN), 137.4 (6-CH), 133.6 (5-CSO_2), 124.5 (4-CH), 117.4 (9-CCO), 110.5 (7-CH), 83.2 (d, $^1J_{C,F}$ = 165.7 Hz, 23-CH_2), 75.1 (16-CH_2), 59.3 (18-CH_3), 59.3 (12-CH), 47.9 (q, $^3J_{C,F}$ = 2.9 Hz, 15-CH_2), 41.6 (q, $^2J_{C,F}$ = 29.2 Hz, 14-CH), 40.3 (20-CH_2), 29.0 (q, $^3J_{C,F}$ = 2.7 Hz, 13-CH_2), 27.6 (d, $^2J_{C,F}$ = 20.1 Hz, 22-CH_2), 23.5 (d, $^3J_{C,F}$ = 4.0 Hz, 21-CH_2) ppm.* ^{19}F NMR (282 MHz, $CDCl_3$): δ = −71.6 (s, 3F, 19-CF_3), −220.0 (s, 1F, 23-CH_2F) ppm. HRMS (ESI+, MeOH): *m*/*z* = 489.1077 $[M + Na]^+$, 521.1339 $[M + Na + MeOH]^+$; calcd. 489.1078 for $C_{19}H_{22}F_4N_2O_5S$ + Na, 521.1340 for $C_{19}H_{22}F_4N_2O_5S$ + Na + MeOH.

* The ^{13}C NMR signal of 19-CF_3 could not be observed because of sample dilution.

4.8. (2S,4S)-5-[1-(4-trifluoromethyl-2-methoxymethylpyrrolidinyl) sulfonyl]isatins



*4.8.1. (2S,4S)-N-tert-butyloxycarbonyl-4-trifluoromethyl-2-methoxy methylpyrrolidine (**12b**)*

(2*S*,4*S*)-*N*-*tert*-butyloxycarbonyl-4-trifluoromethyl-2-hydroxy methylpyrrolidine (**11b**) (160 mg, 0.594 mmol, 1.00 equiv.) was converted to (2*S*,4*S*)-*N*-*tert*-butyloxycarbonyl-4-trifluoromethyl-2-methoxymethyl pyrrolidine (**12b**) using MeI and NaH as described in the general procedure in Section 4.2.1. The crude product was purified by flash column chromatography (silica gel, 15% EtOAc in cyclohexane) to obtain a colorless oil (119 mg, 0.420 mmol, 71%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 4.12-3.86$ (m, 1H, 2-CH), 3.97–3.73 (m, 1H, 5-CH_a), 3.70–3.31 (m, 2H, 10-CH_2), 3.36 (s, 3H, 12-CH_3), 3.25 (t, $^2J_{H,H} = 10.6$ Hz, 1H, 5-CH_b), 2.96–2.75 (m, 1H, 4-CH), 2.31 (dt, $^2J_{H,H} = 13.3$ Hz, $^3J_{H,H} = 8.1$ Hz, 1H, 3-CH_a), 2.15–2.00 (m, 1H, 3-CH_b), 1.47 (s, 9H, 9-CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 154.0$ (6-C), 126.4 (q, $^1J_{C,F} = 276.8$ Hz, 13-CF_3), 80.1 (8-CO), 72.8 (10-CH_2), 59.2 (12-CH_3), 56.2 (2-CH), 46.2 (5-CH_2), 41.4 (4-CH), 29.0 (3-CH_2), 28.4 (3C, 9-CH_3) ppm. ^{19}F NMR (282 MHz, $CDCl_3$): $\delta = -71.0$ (s, 3F, 13-CF_3) ppm. HRMS (ESI+, MeOH): $m/z = 306.1289$ $[M + Na]^+$; calcd. 306.1287 for $C_{12}H_{20}F_3NO_3 + Na$.

*4.8.2. (2S,4S)-5-[1-(4-trifluoromethyl-2-methoxymethylpyrrolidinyl) sulfonyl]isatin (**25b**)*

(2*S*,4*S*)-*N*-*tert*-butyloxycarbonyl-4-trifluoromethyl-2-methoxy methylpyrrolidine (**12b**) (90 mg, 0.318 mmol, 1.00 equiv.) was converted to (2*S*,4*S*)-5-[1-(4-trifluoromethyl-2-methoxymethylpy rrolidinyl)sulfonyl]isatin (**25b**) using TFA followed by reaction with 5-chlorosulfonyl isatin and DIPEA as described in the general procedure in Section 4.2.2. The crude product was purified by flash column chromatography (silica gel, 60% EtOAc in toluene) to obtain a sticky yellow wax (80 mg, 0.204 mmol, 64%) as the desired product. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 10.57$ (br s, 1H, 1-NH), 8.06–8.02 (m, 2H, 4-CH, 6-CH), 7.11–7.07 (m, 1H, 7-CH), 4.02–3.94 (m, 1H, 12-CH), 3.75 (dd, $^2J_{H,H} = 11.6$ Hz, $^3J_{H,H} = 7.9$ Hz, 1H, 15-CH_a), 3.60 (dd, $^2J_{H,H} = 9.7$ Hz, $^3J_{H,H} = 4.1$ Hz, 1H, 16-CH_a), 3.50 (dd, $^2J_{H,H} = 9.6$ Hz, $^3J_{H,H} = 6.0$ Hz, 1H, 16-CH_b), 3.38–3.33 (m, 1H, 15-CH_b). 3.35 (s, 3H, 18-CH_3), 2.69–2.53 (m, 1H, 14-CH), 2.26 (dt, $^2J_{H,H} = 13.5$ Hz, $^3J_{H,H} = 7.9$ Hz, 1H, 13-CH_a), 2.04 (ddd, $^2J_{H,H} = 13.4$ Hz, $^3J_{H,H} = 10.5$ Hz, $^3J_{H,H} = 7.7$ Hz, 1H, 13-CH_b) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 182.6$ (3-CO), 159.1 (2-CO), 153.4 (8-CN), 137.3 (6-CH), 134.1 (5-CSO_2), 124.8 (4-CH), 117.8 (9-CCO), 113.1 (7-CH), 74.0 (16-CH_2), 59.1 (18-CH_3), 59.1 (12-CH), 48.2 (q, $^3J_{C,F} = 3.2$ Hz, 15-CH_2), 42.0 (q, $^2J_{C,F} = 29.0$ Hz, 14-CH), 28.9 (q, $^3J_{C,F} = 2.3$ Hz, 13-CH_2) ppm.* ^{19}F NMR (282 MHz, $CDCl_3$): $\delta = -70.7$ (s, 3F, 19-CF_3) ppm. HRMS (ESI+, MeOH): $m/z = 415.0538$ $[M + Na]^+$, 447.0799 $[M + Na + MeOH]^+$; calcd. 415.0546 for $C_{15}H_{15}F_3N_2O_5S + Na$, 447.0808 for $C_{15}H_{15}F_3N_2O_5S + Na + MeOH$.

* The ^{13}C NMR signal of 19-CF_3 could not be observed because of sample dilution.

*4.8.3. (2S,4S)-N-butyl-5-[1-(4-trifluoromethyl-2-methoxymethyl pyrrolidinyl)sulfonyl]isatin (**26b**)*

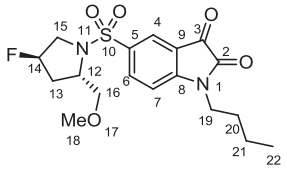
(2*S*,4*S*)-5-[1-(4-trifluoromethyl-2-methoxymethylpyrrolidinyl) sulfonyl]isatin (**25b**) (20 mg, 51.0 μmol, 1.00 equiv.) was converted to (2*S*,4*S*)-*N*-butyl-5-[1-(4-trifluoromethyl-2-methoxymethylpyrro lidinyl)sulfonyl]isatin (**26b**) using 1-bromobutane and Cs_2CO_3 as described in the general procedure in Section 4.2.3. The crude product was purified by flash column chromatography (silica gel, 40% EtOAc in cyclohexane) to obtain a yellow-orange solid (21 mg, 46.8 μmol, 92%) as the desired product. M.p. 86 °C. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.10$ (dd, $^3J_{H,H} = 8.3$ Hz, $^4J_{H,H} = 2.0$ Hz, 1H, 6-CH), 8.06 (d, $^4J_{H,H} = 2.0$ Hz, 1H, 4-CH), 7.05 (d, $^3J_{H,H} = 8.3$ Hz, 1H, 7-CH), 4.07–3.96 (m, 1H, 12-CH), 3.80–3.72 (m, 1H, 15-CH_a), 3.79 (t, $^3J_{H,H} = 7.5$ Hz, 2H, 20-CH_2), 3.59 (dd, $^2J_{H,H} = 9.7$ Hz, $^3J_{H,H} = 4.1$ Hz, 1H, 16-CH_a), 3.51 (dd, $^2J_{H,H} = 9.7$ Hz, $^3J_{H,H} = 5.9$ Hz, 1H, 16-CH_b), 3.37–3.28 (m, 1H, 15-CH_b), 3.34 (s, 3H, 18-CH_3), 2.71–2.58 (m, 1H, 14-CH), 2.28 (dt, $^2J_{H,H} = 13.4$ Hz, $^3J_{H,H} = 8.0$ Hz, 1H, 13-CH_a), 2.06 (ddd, $^2J_{H,H} = 13.2$ Hz, $^3J_{H,H} = 10.5$ Hz, $^3J_{H,H} = 7.8$ Hz, 1H, 13-CH_b), 1.71 (quintet, $^3J_{H,H} = 7.5$ Hz, 2H, 21-CH_2), 1.43 (sextet, $^3J_{H,H} = 7.5$ Hz, 2H, 22-CH_2), 0.99 (t, $^3J_{H,H} = 7.3$ Hz, 3H, 23-CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 182.0$ (3-CO), 157.7 (2-CO), 154.0 (8-CN), 137.3 (6-CH), 134.5 (5-CSO_2), 124.5 (4-CH), 117.4 (9-CCO), 110.5 (7-CH), 73.9 (16-CH_2), 59.1 (18-CH_3), 59.0 (12-CH), 48.1 (q, $^3J_{C,F} = 3.0$ Hz, 15-CH_2), 42.0 (q, $^2J_{C,F} = 29.1$ Hz, 14-CH), 40.5 (20-CH_2), 29.2 (21-CH_2), 28.8 (q, $^3J_{C,F} = 2.2$ Hz, 13-CH_2), 20.1 (22-CH_2), 13.6 (23-CH_3) ppm. * ^{19}F NMR (282 MHz, $CDCl_3$): $\delta = -70.7$ (s, 3F, 19-CF_3) ppm. HRMS (ESI+, MeOH): $m/z = 471.1176$ $[M + Na]^+$, 503.1437 $[M + Na + MeOH]^+$; calcd. 471.1172 for $C_{19}H_{23}F_3N_2O_5S + Na$, 503.1434 for $C_{19}H_{23}F_3N_2O_5S + Na + MeOH$.

* The ^{13}C NMR signal of 19-CF_3 could not be observed because of sample dilution.

*4.8.4. (2S,4S)-N-(4-fluorobutyl)-5-[1-(4-trifluoromethyl-2-methoxy methylpyrrolidinyl)sulfonyl]isatin (**27b**)*

(2*S*,4*S*)-5-[1-(4-trifluoromethyl-2-methoxymethylpyrrolidinyl) sulfonyl]isatin (**25b**) (21 mg, 53.5 μmol, 1.00 equiv.) was converted to (2*S*,4*S*)-*N*-(4-fluorobutyl)-5-[1-(4-trifluoromethyl-2-methoxymethyl pyrrolidinyl)sulfonyl]isatin (**27b**) using 1-bromo-4-fluorobutane and Cs_2CO_3 as described in the general procedure in Section 4.2.3. The crude product was purified by flash column chromatography (silica gel, 50% EtOAc in cyclohexane) to obtain a yellow solid (24 mg, 51.5 μmol, 96%) as the desired product. M.p. 123 °C. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.10$ (dd, $^3J_{H,H} = 8.3$ Hz, $^4J_{H,H} = 2.0$ Hz, 1H, 6-CH), 8.06 (d, $^4J_{H,H} = 2.0$ Hz, 1H, 4-CH), 7.08 (d, $^3J_{H,H} = 8.3$ Hz, 1H, 7-CH), 4.53 (dt, $^2J_{H,F} = 47.4$ Hz, $^3J_{H,H} = 5.4$ Hz, 2H, 23-CH_2), 4.01 (tdd, $^3J_{H,H} = 7.8$ Hz, $^3J_{H,H} = 5.9$ Hz, $^3J_{H,H} = 4.2$ Hz, 1H, 12-CH), 3.85 (t, $^3J_{H,H} = 7.1$ Hz, 2H, 20-CH_2), 3.76 (dd, $^2J_{H,H} = 11.4$ Hz, $^3J_{H,H} = 7.9$ Hz, 1H, 15-CH_a), 3.59 (dd, $^2J_{H,H} = 9.7$ Hz, $^3J_{H,H} = 4.1$ Hz, 1H, 16-CH_a), 3.51 (dd, $^2J_{H,H} = 9.7$ Hz, $^3J_{H,H} = 5.9$ Hz, 1H, 16-CH_b), 3.37–3.28 (m, 1H, 15-CH_b), 3.34 (s, 3H, 18-CH_3), 2.72–2.59 (m, 1H, 14-H), 2.28 (dt, $^2J_{H,H} = 13.4$ Hz, $^3J_{H,H} = 8.1$ Hz, 1H, 13-CH_a), 2.05 (ddd, $^2J_{H,H} = 13.3$ Hz, $^3J_{H,H} = 10.5$ Hz, $^3J_{H,H} = 7.8$ Hz, 1H, 13-CH_b), 1.96–1.70 (m, 4H, 21-CH_2, 22-CH_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 181.8$ (3-CO), 157.7 (2-CO), 153.7 (8-CN), 137.3 (6-CH), 134.6 (5-CSO_2), 125.6 (q, $^1J_{C,F} = 277.1$ Hz, 19-CF_3), 124.5 (4-CH), 117.4 (9-CCO), 110.5 (7-CH), 83.2 (d, $^1J_{C,F} = 165.5$ Hz, 23-CH_2), 73.9 (16-CH_2), 59.1 (18-CH_3), 59.1 (12-CH), 48.0 (q, $^3J_{C,F} = 2.8$ Hz, 15-CH_2), 42.0 (q, $^2J_{C,F} = 29.0$ Hz, 14-CH), 40.2 (20-CH_2), 28.8 (q, $^3J_{C,F} = 2.0$ Hz, 13-CH_2), 23.5 (d, $^3J_{C,F} = 3.9$ Hz, 21-CH_2), 27.6 (d, $^2J_{C,F} = 20.1$ Hz, 22-CH_2) ppm. ^{19}F NMR (282 MHz, $CDCl_3$): $\delta = -70.7$ (s, 3F, 19-CF_3), -219.9 (s, 1F, 23-CH_2F) ppm. HRMS (ESI+, MeOH): $m/z = 489.1077$ $[M + Na]^+$, 521.1342 $[M + Na + MeOH]^+$; calcd. 489.1078 for $C_{19}H_{22}F_4N_2O_5S + Na$, 521.1340 for $C_{19}H_{22}F_4N_2O_5S + Na + MeOH$.

4.9. (2S,4R)-5-[1-(4-fluoro-2-methoxymethylpyrrolidinyl)sulfonyl] isatins



*4.9.1. (2S,4R)-N-tert-butyloxycarbonyl-4-fluoro-2-hydroxymethyl pyrrolidine (**13a**)*

A stirred solution of methyl (2*S*,4*R*)-*N*-*tert*-butyloxycarbonyl-4-fluoropyrrolidine-2-carboxylate (600 mg, 2.43 mmol, 1.00 equiv.) in THF (10 mL) was treated with anhydrous LiCl (227 mg, 5.35 mmol, 2.20 equiv.) followed by $NaBH_4$ (230 mg, 6.08 mmol, 2.50 equiv.) at ambient temperature. The resulting mixture was cooled to 0 °C, before EtOH (20 mL) was added dropwise over 15 min. The reaction mixture was stirred further at this temperature for 1 h and at r.t. for 21 h. After this time, the milky mixture was cooled to 0 °C and acidified to pH 4 by addition of 10% aq. citric acid. The solvent was completely removed under reduced pressure. Subsequently, the residue was taken up in water (40 mL) and extracted with DCM (3 × 40 mL). The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure to yield a pale yellow oil (514 mg, 2.34 mmol, 96%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.30–4.98 (m, 1H, 4-CH), 4.21–4.05 (m, 1H, 2-CH), 3.95–3.70 (m, 2H, 5-CH_a, 10-CH_a), 3.60–3.28 (m, 2H, 5-CH_b, 10-CH_b), 2.44–2.23 (m, 1H, 3-CH_a), 1.89–1.59 (m, 1H, 3-CH_b), 1.48 (s, 9H, 9-CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.6 (6-CO), 91.2 (d, $^1J_{C,F}$ = 176.3 Hz, 4-CH), 80.9 (8-CO), 66.3 (10-CH_2), 58.8 (2-CH), 54.2 (d, $^2J_{C,F}$ = 23.0 Hz, 5-CH_2), 35.5 (d, $^2J_{C,F}$ = 22.0 Hz, 3-CH_2), 28.4 (3C, 9-CH_3) ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = −177.3 (4-CHF, rotamer A), −177.7 (4-CHF, rotamer B) ppm. HRMS (ESI+, MeOH): *m*/*z* = 242.1161 $[M + Na]^+$; calcd. 242.1163 for $C_{10}H_{18}FNO_3$ + Na.

*4.9.2. (2S,4R)-N-tert-butyloxycarbonyl-4-fluoro-2-methoxymethyl pyrrolidine (**14a**)*

(2*S*,4*R*)-*N*-*tert*-butyloxycarbonyl-4-fluoro-2-hydroxymethylpyrrolidine (**13a**) (450 mg, 2.05 mmol, 1.00 equiv.) was converted to (2*S*,4*R*)-*N*-*tert*-butyloxycarbonyl-4-fluoro-2-methoxymethylpyrrolidine (**14a**) using MeI and NaH as described in the general procedure in Section 4.2.1. The residue was purified by flash column chromatography (silica gel, 20% EtOAc in cyclohexane) to obtain a pale yellow oil (470 mg, 2.01 mmol, 98%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.16 (dt, $^2J_{H,F}$ = 53.6 Hz, $^3J_{H,H}$ = 2.2 Hz, 1H, 4-CH), 4.22–4.01 (m, 1H, 2-CH), 4.06–3.53 (m, 2H, 5-CH_2), 3.61–3.25 (m, 2H, 10-CH_2), 3.34 (s, 3H, 12-CH_3), 2.44–2.04 (m, 2H, 3-CH_2), 1.48 (s, 9H, 9-CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 154.5 (6-CO), 92.1 (d, $^1J_{C,F}$ = 176.5 Hz, 4-CH, rotamer A), 91.6 (d, $^1J_{C,F}$ = 176.5 Hz, 4-CH, rotamer B), 79.8 (8-CO), 73.7 (10-CH_2, rotamer B), 72.7 (10-CH_2, rotamer A), 59.2 (12-CH_3), 55.5 (2-CH), 53.7 (d, $^2J_{C,F}$ = 22.6 Hz, 5-CH_2, rotamer A), 53.3 (d, $^2J_{C,F}$ = 23.5 Hz, 5-CH_2, rotamer B), 36.2 (d, $^2J_{C,F}$ = 21.2 Hz, 3-CH_2), 35.1 (d, $^2J_{C,F}$ = 21.4 Hz, 3-CH_2), 28.5 (3C, 9-CH_3) ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = −177.0 (s, 4-CHF, rotamer A), −177.4 (s, 4-CHF, rotamer B) ppm. HRMS (ESI+, MeOH): *m*/*z* = 256.1333 $[M + Na]^+$; calcd. 256.1319 for $C_{11}H_{20}FNO_3$ + Na.

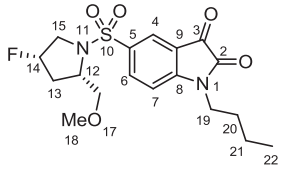
*4.9.3. (2S,4R)-5-[1-(4-fluoro-2-methoxymethylpyrrolidinyl)sulfonyl] isatin (**28a**)*

(2*S*,4*R*)-*N*-*tert*-butyloxycarbonyl-4-fluoro-2-methoxymethylpyrrolidine (**14a**) (300 mg, 1.37 mmol, 1.00 equiv.) was converted to (2*S*,4*R*)-5-[1-(4-fluoro-2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**28a**) using TFA followed by reaction with 5-chlorosulfonyl isatin and DIPEA as described in the general procedure in Section 4.2.2. The resulting residue was purified by flash column chromatography (silica gel, 60% EtOAc in toluene and then 80% EtOAc in toluene) to obtain an orange-yellow solid (241 mg, 0.704 mmol, 51%). M.p. 159 °C. ^{1}H NMR (300 MHz, CD_3CN): δ = 9.33 (s, 1H, 1-NH), 8.02 (dd, $^3J_{H,H}$ = 8.4 Hz, $^4J_{H,H}$ = 1.9 Hz, 1H, 6-CH), 7.92 (d, $^4J_{H,H}$ = 1.9 Hz, 1H, 4-CH), 7.10 (d, $^3J_{H,H}$ = 8.4 Hz, 1H, 7-CH), 5.19–4.94 (m, 1H, 14-CH), 3.91–3.79 (m, 1H, 12-CH), 3.83–3.68 (m, 1H, 15-CH_a), 3.62–3.37 (m, 3H, 15-CH_b, 16-CH_2), 3.31 (s, 3H, 18-CH_3), 2.19–1.92 (m, 2H, 13-CH_2) ppm. ^{13}C NMR (75 MHz, CD_3CN): δ = 183.9 (3-CO), 159.8 (2-CO), 154.2 (8-CN), 138.5 (6-CH), 133.9 (5-CSO_2), 125.1 (4-CH), 118.8 (9-CCO), 113.5 (7-CH), 93.3 (d, $^1J_{C,F}$ = 175.7 Hz, 14-CH), 75.0 (16-CH_2), 59.4 (18-CH_3), 59.3 (12-CH), 56.5 (d, $^2J_{C,F}$ = 21.8 Hz, 15-CH_2), 36.6 (d, $^2J_{C,F}$ = 21.3 Hz, 13-CH_2) ppm. ^{19}F NMR (282 MHz, CD_3CN): δ = −175.8 (s, 1F, 14-CHF) ppm. HRMS (ESI+, MeOH): *m*/*z* = 365.0581 $[M + Na]^+$, 397.0842 $[M + Na + MeOH]^+$; calcd. 365.0578 for $C_{14}H_{15}FN_2O_5S$ + Na, 397.0840 for $C_{14}H_{15}FN_2O_5S$ + Na + MeOH.

*4.9.4. (2S,4R)-N-butyl-5-[1-(4-fluoro-2-methoxymethylpyrrolidinyl) sulfonyl]isatin (**29a**)*

(2*S*,4*R*)-5-[1-(4-fluoro-2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**28a**) (40 mg, 0.117 mmol, 1.00 equiv.) was converted to (2*S*,4*R*)-*N*-butyl-5-[1-(4-fluoro-2-methoxymethylpyrrolidinyl) sulfonyl]isatin (**29a**) using 1-bromobutane and Cs_2CO_3 as described in the general procedure in Section 4.2.3. The obtained residue was purified by flash column chromatography (silica gel, 40% THF in cyclohexane) to yield a yellow solid (35 mg, 87.8 μmol, 75%). M.p. 85 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06 (dd, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 2.0 Hz, 1H, 6-CH), 8.03 (d, $^4J_{H,H}$ = 2.0 Hz, 1H, 4-CH), 7.02 (d, $^3J_{H,H}$ = 8.3 Hz, 1H, 7-CH), 5.06 (dm, $^2J_{H,F}$ = 52.5 Hz, 1H, 14-CH), 3.88–3.81 (m, 1H, 12-CH), 3.83–3.72 (m, 1H, 15-CH_a), 3.77 (t, $^3J_{H,H}$ = 7.4 Hz, 2H, 19-CH_2), 3.67–3.63 (m, 2H, 16-CH_2), 3.55 (ddd, $^3J_{H,F}$ = 39.1 Hz, $^2J_{H,H}$ = 13.6 Hz, $^3J_{H,H}$ = 2.8 Hz, 1H, 15-H_b), 3.39 (s, 3H, 18-CH_3), 2.30–2.04 (m, 2H, 13-CH_2), 1.76–1.62 (m, 2H, 20-CH_2), 1.49–1.33 (m, 2H, 21-CH_2), 0.98 (t, $^3J_{H,H}$ = 7.4 Hz, 3H, 22-CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 182.2 (3-CO), 157.8 (2-CO), 153.9 (8-CN), 137.6 (6-CH), 133.5 (5-CSO_2), 124.8 (4-CH), 117.1 (9-CCO), 110.3 (7-CH), 91.9 (d, $^1J_{C,F}$ = 178.3 Hz, 14-CH), 74.4 (16-CH_2), 59.3 (18-CH_3), 58.5 (12-CH), 55.9 (d, $^2J_{C,F}$ = 22.0 Hz, 15-CH_2), 40.5 (19-CH_2), 36.1 (d, $^2J_{C,F}$ = 21.4 Hz, 13-CH_2), 29.2 (20-CH_2), 20.1 (21-CH_2), 13.6 (22-CH_3) ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = −176.8 (s, 1F, 14-CHF) ppm. HRMS (ESI+, MeOH): *m*/*z* = 421.1203 $[M + Na]^+$, 453.1461 $[M + Na + MeOH]^+$; calcd. 421.1204 for $C_{18}H_{23}FN_2O_5S$ + Na, 453.1466 for $C_{18}H_{23}FN_2O_5S$ + Na + MeOH.

4.10. (2S,4S)-5-[1-(4-fluoro-2-methoxymethylpyrrolidinyl)sulfonyl] isatins



*4.10.1. (2S,4S)-N-tert-butyloxycarbonyl-4-fluoro-2-hydroxymethyl pyrrolidine (**13b**)*

A stirred solution of methyl (2*S*,4*S*)-*N*-*tert*-butyloxycarbonyl-4-fluoropyrrolidine-2-carboxylate (750 mg, 3.03 mmol, 1.00 equiv.) in THF (8.0 mL) was treated with anhydrous LiCl (283 mg, 6.67 mmol, 2.20 equiv.) followed by $NaBH_4$ (287 mg, 7.58 mmol, 2.50 equiv.) at ambient temperature. The mixture was cooled to 0 °C, before EtOH (16 mL) was added dropwise over 15 min. The resulting mixture was stirred at this temperature for 1 h and then at r.t. for 20 h. After this time the milky mixture was cooled down to 0 °C and acidified to pH 4 by addition of 10% aq. citric acid. Subsequently the solvent was completely removed under reduced pressure and the residue was taken up in water (30 mL). It was extracted with DCM (3 × 30 mL), the combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography (silica gel, 40% EtOAc in cyclohexane) to obtain a colorless oil (600 mg, 2.74 mmol, 90%) as the required product. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.32–4.99 (m, 1H, 4-CH),

4.23–4.09 (m, 1H, 2-CH), 3.91–3.80 (m, 1H, 10-CH_a), 3.75–3.46 (m, 2H, 5-CH_2), 3.66–3.55 (m, 1H, 10-CH_b), 2.37–1.95 (m, 2H, 3-CH_2), 1.48 (s, 9H, 9-CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 156.7 (6-CO), 92.1 (d, $^1J_{C,F}$ = 176.4 Hz, 4-CH), 80.9 (8-CO), 67.8 (10-CH_2), 59.2 (2-CH), 53.9 (d, $^2J_{C,F}$ = 23.7 Hz, 5-CH_2), 35.1 (d, $^2J_{C,F}$ = 21.1 Hz, 3-CH_2), 28.4 (3C, 9-CH_3) ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = −170.2 (s, 4-CHF, rotamer B), −171.4 (s, 4-CHF, rotamer A) ppm. HRMS (ESI+, MeOH): *m/z* = 242.1164 $[M + Na]^+$, 270.1109 $[M + Na + MeOH]^+$; calcd. 242.1163 for $C_{10}H_{18}FNO_3$ + Na, 270.1112 for $C_{10}H_{18}FNO_3$ + Na + MeOH.

4.10.2. (2*S*,4*S*)-*N*-tert-butyloxycarbonyl-4-fluoro-2-methoxymethyl pyrrolidine (**14b**)

(2*S*,4*S*)-*N*-*tert*-butyloxycarbonyl-4-fluoro-2-hydroxymethylpyrrolidine (**13b**) (573 mg, 2.61 mmol, 1.00 equiv.) was converted to (2*S*,4*S*)-*N*-*tert*-butyloxycarbonyl-4-fluoro-2-methoxymethylpyrrolidine (**14b**) using MeI and NaH as described in the general procedure in Section 4.2.1. The resulting residue was purified by flash column chromatography (silica gel, 30% EtOAc in cyclohexane) to obtain a pale yellow oil (527 mg, 2.26 mmol, 87%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.35–5.07 (m, 1H, 4-CH), 4.22–3.92 (m, 1H, 2-CH), 3.80–3.43 (m, 3H, 5-CH_a, 10-CH_2), 3.37 (s, 3H, 12-CH_3), 3.31 (ddd, $^3J_{H,F}$ = 10.3 Hz, $^2J_{H,H}$ = 8.8 Hz, $^3J_{H,H}$ = 1.8 Hz, 1H, 5-CH_b), 2.44–2.33 (m, 1H, 3-CH_a), 2.21–1.94 (m, 1H, 3-CH_b), 1.48 (s, 9H, 9-CH_3) ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = −170.8 (s, 4-CHF, rotamer A), −171.2 (s, 4-CHF, rotamer B) ppm. HRMS (ESI+, MeOH): *m/z* = 256.1319 $[M + Na]^+$, 489.2750 $[2M + Na]^+$; calcd. 256.1319 for $C_{11}H_{20}FNO_3$ + Na, 489.2747 for 2($C_{11}H_{20}FNO_3$) + Na.

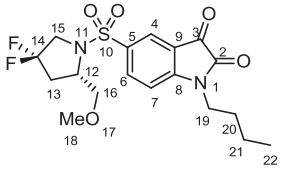
4.10.3. (2*S*,4*S*)-5-[1-(4-fluoro-2-methoxymethylpyrrolidinyl)sulfonyl] isatin (**28b**)

(2*S*,4*S*)-*N*-*tert*-butyloxycarbonyl-4-fluoro-2-methoxymethylpyrrolidine (**14b**) (500 mg, 2.14 mmol, 1.00 equiv.) was converted to (2*S*,4*S*)-5-[1-(4-fluoro-2-methoxymethylpyrrolidinyl)sulfonyl] isatin (**28b**) using TFA followed by reaction with 5-chlorosulfonyl isatin and DIPEA as described in the general procedure in Section 4.2.2. The crude was purified by flash column chromatography (silica gel, 60% and then 80% EtOAc in cyclohexane) to obtain a yellow solid (370 mg, 0.993 mmol, 46%). M.p. 169 °C. ^{1}H NMR (300 MHz, CD_3CN): δ = 9.37 (br s, 1H, 1-NH), 8.01 (dd, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.9 Hz, 1H, 6-CH), 7.91 (d, $^4J_{H,H}$ = 1.9 Hz, 1H, 4-CH), 7.13 (d, $^3J_{H,H}$ = 8.3 Hz, 1H, 7-CH), 5.13 (dt, $^2J_{H,F}$ = 53.1 Hz, $^3J_{H,H}$ = 4.2 Hz, 1H, 14-CH), 3.95–3.80 (tdd, $^3J_{H,H}$ = 9.1 Hz, $^3J_{H,H}$ = 5.2 Hz, $^3J_{H,H}$ = 1.5 Hz, 1H, 12-CH), 3.68–3.53 (m, 2H, 15-CH_a, 16-CH_a), 3.46–3.26 (m, 2H, 15-CH_b, 16-CH_b), 3.30 (s, 3H, 18-CH_3), 2.21–2.05 (m, 1H, 13-CH_a), 1.98–1.71 (m, 1H, 13-CH_b) ppm. ^{13}C NMR (75 MHz, CD_3CN): δ = 183.8 (3-CO), 159.7 (2-CO), 154.4 (8-CN), 138.4 (6-CH), 132.8 (5-CSO_2), 125.0 (4-CH), 119.0 (9-CCO), 114.0 (7-CH), 94.0 (d, $^1J_{C,F}$ = 176.0 Hz, 14-CH), 75.7 (16-CH), 59.2 (12-CH), 59.1 (18-CH_3), 56.0 (d, $^2J_{C,F}$ = 23.8 Hz, 15-CH_2), 35.3 (d, $^2J_{C,F}$ = 20.0 Hz, 13-CH_2) ppm. ^{19}F NMR (282 MHz, CD_3CN): δ = −171.9 (s, 1F, 14-CHF) ppm. HRMS (ESI+, MeOH): *m/z* = 365.0573 $[M + Na]^+$, 397.0837 $[M + Na + MeOH]^+$; calcd. 365.0578 for $C_{14}H_{15}FN_2O_5S$ + Na, 397.0840 for $C_{14}H_{15}FN_2O_5S$ + Na + MeOH.

4.10.4. (2*S*,4*S*)-*N*-butyl-5-[1-(4-fluoro-2-methoxymethylpyrrolidinyl) sulfonyl]isatin (**29b**)

(2*S*,4*S*)-5-[1-(4-fluoro-2-methoxymethylpyrrolidinyl)sulfonyl] isatin (**28b**) (50 mg, 0.146 mmol, 1.00 equiv.) was converted to (2*S*,4*S*)-*N*-butyl-5-[1-(4-fluoro-2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**29b**) using 1-bromobutane and Cs_2CO_3 as described in the general procedure in Section 4.2.3. The crude product was purified by flash column chromatography (silica gel, 35% THF in cyclohexane) to obtain a sticky yellow gum (48 mg, 0.121 mmol, 82%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06 (dd, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.9 Hz, 1H, 6-CH), 8.02 (dd, $^4J_{H,H}$ = 1.9 Hz, $^5J_{H,H}$ = 0.6 Hz, 1H, 4-CH), 7.01 (dd, $^3J_{H,H}$ = 8.3 Hz, $^5J_{H,H}$ = 0.6 Hz, 1H, 7-CH), 5.15 (dt, $^2J_{H,F}$ = 52.7 Hz, $^3J_{H,H}$ = 4.2 Hz, 1H, 14-CH), 4.01–3.92 (m, 1H, 12-CH), 3.74 (t, $^3J_{H,H}$ = 7.4 Hz, 2H, 19-CH_2), 3.73–3.61 (m, 1H, 15-CH_a), 3.69–3.61 (m, 1H, 16-CH_a), 3.48–3.34 (m, 1H, 15-CH_b), 3.39–3.32 (m, 1H, 16-CH_b), 3.32 (s, 3H, 18-CH_3), 2.36–2.22 (m, 1H, 13-CH_a), 1.98–1.77 (m, 1H, 13-CH_b), 1.67 (quintet, $^3J_{H,H}$ = 7.5 Hz, 2H, 20-CH_2), 1.39 (sextet, $^3J_{H,H}$ = 7.4 Hz, 2H, 21-CH_2), 0.96 (t, $^3J_{H,H}$ = 7.3 Hz, 3H, 22-CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 182.0 (3-CO), 157.7 (2-CO), 153.9 (8-CN), 137.5 (6-CH), 133.5 (5-CSO_2), 124.6 (4-CH), 117.4 (9-CCO), 110.5 (7-CH), 92.4 (d, $^1J_{C,F}$ = 179.6 Hz, 14-CH), 74.7 (16-CH_2), 58.9 (18-CH_3), 58.3 (12-CH), 55.0 (d, $^2J_{C,F}$ = 24.3 Hz, 15-CH_2), 40.5 (19-CH_2), 35.0 (d, $^2J_{C,F}$ = 20.4 Hz, 13-CH_2), 29.2 (20-CH_2), 20.1 (21-CH_2), 13.6 (22-CH_3) ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = −172.2 (s, 1F, 14-CHF) ppm. HRMS (ESI+, MeOH): *m/z* = 421.1197 $[M + Na]^+$, 453.1460 $[M + Na + MeOH]^+$; calcd. 421.1204 for $C_{18}H_{23}FN_2O_5S$ + Na, 453.1466 for $C_{18}H_{23}FN_2O_5S$ + Na + MeOH.

4.11. (*S*)-5-[1-(4,4-difluoro-2-methoxymethylpyrrolidinyl)sulfonyl] isatins



4.11.1. (*S*)-*N*-tert-butyloxycarbonyl-4,4-difluoro-2-hydroxymethyl pyrrolidine

A stirred solution of methyl (*S*)-*N*-*tert*-butyloxycarbonyl-4,4-difluoropyrrolidine-2-carboxylate (**15**) (790 mg, 2.98 mmol, 1.00 equiv.) in THF (10 mL) was treated with LiCl anhydrous (278 mg, 6.55 mmol, 2.20 equiv.) and followed by $NaBH_4$ (282 mg, 7.45 mmol, 2.50 equiv.) at ambient temperature. The resulting mixture was cooled to 0 °C, before EtOH (20 mL) was added dropwise over 15 min. Subsequently the mixture was stirred at this temperature for 1 h and at r.t. for 24 h. After this time, the milky mixture was cooled to 0 °C and acidified to pH 4 by addition of 10% aq. citric acid. The solvent was completely removed under reduced pressure and it was taken up in water (30 mL). The mixture was extracted with DCM (3 × 30 mL) and the combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography (silica gel, 40% EtOAc in cyclohexane) to obtain a colorless oil (700 mg, 2.95 mmol, 99%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.26–4.02 (m, 1H, 2-CH), 3.95–3.54 (m, 4H, 5-CH_2, 10-CH_2), 2.60–2.38 (m, 1H, 3-CH_a), 2.30–2.04 (m, 1H, 3-CH_b), 1.48 (s, 9H, 9-CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.7 (6-CO), 126.4 (t, $^1J_{C,F}$ = 246.3 Hz, 4-CF_2), 81.4 (8-CO), 65.1 (10-CH_2), 58.3 (2-CH), 54.0 (t, $^2J_{C,F}$ = 31.2 Hz, 5-CH_2), 36.5 (t, $^2J_{C,F}$ = 23.7 Hz, 3-CH_2), 28.3 (3C, 9-CH_3) ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = −99.8 (br s, 4-CF_2, rotamer B), −100.1 (d, $^2J_{F,F}$ = 232.2 Hz, 4-CF_2, rotamer A), −101.4 (d, $^2J_{F,F}$ = 233.2 Hz, 4-CF_2, rotamer A) ppm. HRMS (ESI+, MeOH): *m/z* = 260.1063 $[M + Na]^+$; calcd. 260.1069 for $C_{10}H_{17}F_2NO_3$ + Na.

4.11.2. (*S*)-*N*-tert-butyloxycarbonyl-4,4-difluoro-2-methoxymethyl pyrrolidine (**16**)

(*S*)-*N*-*tert*-butyloxycarbonyl-4,4-difluoro-2-hydroxymethylpyrrolidine (640 mg, 2.70 mmol, 1.00 equiv.) was converted to (*S*)-*N*-tert-butyloxycarbonyl-4,4-difluoro-2-methoxymethylpyrrolidine (**16**) using MeI and NaH as described in the general procedure in

Section 4.2.1. The residue was purified by flash column chromatography (silica gel, 15% EtOAc in cyclohexane) to yield a colorless oil (625 mg, 2.49 mmol, 92%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.27–4.02 (m, 1H, 2-CH), 3.95–3.70 (m, 1H, 5-CH_a), 3.69–3.23 (m, 3H, 5-CH_b, 10-CH_2), 3.36 (s, 3H, 12-CH_3), 2.55–2.33 (m, 2H, 3-CH_2), 1.47 (s, 9H, 9-CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 153.8 (6-CO), 127.2 (t, $^1J_{C,F}$ = 247.6 Hz, 4-CF_2), 80.5 (8-CO), 72.4 (10-CO), 59.1 (12-CH_3), 55.0 (2-CH), 53.7 (m, 5-CH_2), 36.6 (m, 3-CH_2), 28.4 (3C, 9-CH_3) ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = −98.4 (d, $^2J_{F,F}$ = 234.9 Hz, 4-CF_2, rotamer B), −99.1 (br s, 4-CF_2, rotamer A), −99.7 (d, $^2J_{F,F}$ = 234.7 Hz, 4-CF_2, rotamer B) ppm. HRMS (ESI+, MeOH): m/z = 274.1224 $[M + Na]^+$, 525.2561 $[2M + Na]^+$; calcd. 274.1225 for $C_{11}H_{19}F_2NO_3$ + Na, 525.2558 for 2($C_{11}H_{19}F_2NO_3$) + Na.

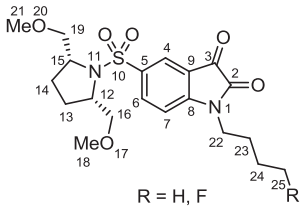
4.11.3. (S)-5-[1-(4,4-difluoro-2-methoxymethylpyrrolidinyl)sulfonyl] isatin (**30**)

(*S*)-*N*-*tert*-butyloxycarbonyl-4,4-difluoro-2-methoxymethylpyrrolidine (**16**) (600 mg, 2.39 mmol, 1.00 equiv.) was converted to (*S*)-5-[1-(4,4-difluoro-2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**30**) using TFA followed by reaction with 5-chlorosulfonyl isatin and DIPEA as described in the general procedure in Section 4.2.2. The crude product was purified by flash column chromatography (silica gel, 40% EtOAc in toluene) to obtain a yellow solid (245 mg, 0.680 mmol, 28%). M.p. 69–70 °C. ^{1}H NMR (300 MHz, CD_3CN): δ = 9.35 (br s, 1H, 1-NH), 8.03 (dd, $^3J_{H,H}$ = 8.4 Hz, $^4J_{H,H}$ = 2.0 Hz, 1H, 6-CH), 7.95 (d, $^4J_{H,H}$ = 2.0 Hz, 1H, 4-CH), 7.14 (d, $^3J_{H,H}$ = 8.4 Hz, 1H, 7-CH), 4.07–3.96 (m, 1H, 12-CH), 3.85–3.58 (m, 2H, 15-CH_2), 3.58–3.50 (m, 2H, 16-CH_2), 3.31 (s, 3H, 18-CH_3), 2.43–2.26 (m, 2H, 13-CH_2) ppm. ^{13}C NMR (75 MHz, CD_3CN): δ = 183.8 (3-CO), 159.7 (2-CO), 154.5 (8-CN), 138.4 (6-CH), 133.4 (5-CSO_2), 128.0 (dd, $^1J_{C,F}$ = 250.0 Hz, $^1J_{C,F}$ = 247.6 Hz, 14-CF_2), 125.1 (4-CH), 119.0 (9-CCO), 113.9 (7-CH), 74.5 (16-CH_2), 59.4 (18-CH_3), 58.8 (dd, $^3J_{C,F}$ = 4.5 Hz, $^3J_{C,F}$ = 2.5 Hz, 12-CH), 55.4 (dd, $^2J_{C,F}$ = 33.6 Hz, $^2J_{C,F}$ = 30.4 Hz, 15-CH_2), 37.3 (dd, $^2J_{C,F}$ = 25.0 Hz, $^2J_{C,F}$ = 23.2 Hz, 13-CH_2) ppm. ^{19}F NMR (282 MHz, CD_3CN): δ = −97.5 (d, $^2J_{F,F}$ = 230.5 Hz, 1F, 14-CF_2), −102.4 (d, $^2J_{F,F}$ = 230.5 Hz, 1F, 14-CF_2) ppm. HRMS (ESI+, MeOH): m/z = 383.0493 $[M + Na]^+$; calcd. 383.0484 for $C_{14}H_{14}F_2N_2O_5S$ + Na.

4.11.4. (S)-N-butyl-5-[1-(4,4-difluoro-2-methoxymethylpyrrolidinyl) sulfonyl]isatin (**31**)

(*S*)-5-[1-(4,4-difluoro-2-methoxymethylpyrrolidinyl)sulfonyl] isatin (**30**) (50 mg, 0.139 mmol, 1.00 equiv.) was converted to (*S*)-*N*-butyl-5-[1-(4,4-difluoro-2-methoxymethylpyrrolidinyl)sulfonyl] isatin (**31**) using 1-bromobutane and Cs_2CO_3 as described in the general procedure in Section 4.2.3. The residue was purified by flash column chromatography (silica gel, 30% THF in cyclohexane) to obtain a yellow wax (50 mg, 0.120 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06 (dd, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 2.0 Hz, 1H, 6-CH), 8.03 (d, $^4J_{H,H}$ = 2.0 Hz, 1H, 4-CH), 7.06 (d, $^3J_{H,H}$ = 8.3 Hz, 1H, 7-CH), 4.02–3.94 (m, 1H, 12-CH), 3.78 (t, $^3J_{H,H}$ = 7.4 Hz, 2H, 19-CH_2), 3.75–3.62 (m, 2H, 15-CH_2), 3.63–3.54 (m, 2H, 16-CH_2), 3.38 (s, 3H, 18-CH_3), 2.52–2.24 (m, 2H, 13-CH_2), 1.71 (quintet, $^3J_{H,H}$ = 7.5 Hz, 2H, 20-CH_2), 1.43 (sextet, $^3J_{H,H}$ = 7.4 Hz, 2H, 21-CH_2), 0.99 (t, $^3J_{H,H}$ = 7.4 Hz, 3H, 22-CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 182.0 (3-CO), 157.7 (2-CO), 154.2 (8-CN), 137.5 (6-CH), 133.3 (5-CSO_2), 126.3 (dd, $^1J_{C,F}$ = 252.2 Hz, $^1J_{C,F}$ = 248.2 Hz, 14-CF_2), 124.6 (4-CH), 117.4 (9-CCO), 110.6 (7-CH), 73.8 (16-CH_2), 59.2 (18-CH_3), 57.9 (dd, $^3J_{C,F}$ = 4.4 Hz, $^3J_{C,F}$ = 2.2 Hz, 12-CH), 54.7 (dd, $^2J_{C,F}$ = 33.3 Hz, $^2J_{C,F}$ = 30.3 Hz, 15-CH_2), 40.5 (19-CH_2), 36.8 (dd, $^2J_{C,F}$ = 24.8 Hz, $^2J_{C,F}$ = 23.1 Hz, 13-CH_2), 29.2 (20-CH_2), 20.1 (21-CH_2), 13.6 (22-CH_3) ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = −98.3 (d, $^2J_{F,F}$ = 232.1 Hz, 1F, 14-CF_2), −103.3 (d, $^2J_{F,F}$ = 232.0 Hz, 1F, 14-CF_2) ppm. HRMS (ESI+, MeOH): m/z = 439.1118 $[M + Na]^+$, 471.1374 $[M + Na + MeOH]^+$; calcd. 439.1110 for $C_{18}H_{22}F_2N_2O_5S$ + Na, 471.1372 for $C_{18}H_{22}F_2N_2O_5S$ + Na + MeOH.

4.12. cis-5-{1-[2,5-bis(methoxymethyl)pyrrolidinyl]sulfonyl}isatins



4.12.1. cis-N-benzyl-2,5-bis(methoxymethyl)pyrrolidine (**18a**)

A stirred suspension of 60% NaH in mineral oil (163 mg, 4.08 mmol, 3.00 equiv.) in dry THF (5 mL) under argon atmosphere was cooled to 0 °C and treated with a solution of *cis*-*N*-benzyl-2,5-bis(hydroxymethyl)pyrrolidine (**17a**) (300 mg, 1.36 mmol, 1.00 equiv.) in dry THF (3 mL) dropwise. The mixture was stirred at 0 °C for 15 min and at r.t. for 30 min, before it was cooled to 0 °C again and MeI (254 μL, 4.08 mmol, 3.00 equiv.) was added dropwise over 5–10 min. The reaction mixture was stirred at r.t. for 20 h. After this time, it was cooled to 0 °C, diluted with DCM (20 mL) and quenched by slow addition of sat. aq. NH_4Cl until no more H_2 evolved. H_2O (10 mL) was added and two phases were separated. The aq. phase was extracted with DCM (3 × 10 mL). The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography (silica gel, 25% EtOAc in cyclohexane) to obtain a colorless oil (302 mg, 1.21 mmol, 89%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37–7.20 (m, 5H, 2 × 8-CH, 2 × 9-CH, 10-CH), 3.89 (s, 2H, 6-CH_2), 3.35–3.08 (m, 4H, 11-CH_2, 14-CH_2), 3.24 (s, 6H, 13-CH_3, 16-CH_3), 3.05–2.90 (m, 2H, 2-CH, 5-CH), 1.94–1.76 (m, 2H, 3-CH_a, 4-CH_a), 1.70–1.52 (m, 2H, 3-CH_b, 4-CH_b) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 139.9 (7-CCH_2), 129.2 (2C, 2 × 8-CH), 128.0 (2C, 2 × 9-CH), 126.8 (10-CH), 76.8 (2C, 11-CH_2, 14-CH_2), 64.3 (2C, 2-CH, 5-CH), 59.0 (6-CH_2), 58.9 (2C, 13-CH_3, 16-CH_3), 27.7 (2C, 3-CH_2, 4-CH_2) ppm. HRMS (ESI+, MeOH): m/z = 250.1814 $[M + H]^+$; calcd. 250.1802 for $C_{15}H_{23}NO_2$ + H.

4.12.2. cis-5-{1-[2,5-bis(methoxymethyl)pyrrolidinyl]sulfonyl} isatin (**19a**)

A mixture of *cis*-*N*-benzyl-2,5-bis(methoxymethyl)pyrrolidine (**18a**) (620 mg, 2.49 mmol, 1.00 equiv.) and 10% Pd/C (62 mg, 10% w/w) in MeOH (12 mL) was stirred in an autoclave at ambient temperature under H_2 atmosphere (20 atm) for 6 h. After this time, the mixture was filtered through Celite® and washed again with MeOH. The solvent was removed under reduced pressure to obtain the corresponding unprotected bis(methoxymethyl)pyrrolidine as a light yellow oil. The solution of obtained free amine *cis*-2,5-bis(methoxymethyl)pyrrolidine and DIPEA (766 μL, 4.40 mmol, 2.00 equiv.) in $CHCl_3$ (5 mL) was added dropwise to a stirred solution of 5-chlorosulfonyl isatin (1.08 g, 4.40 mmol, 2.00 equiv.) in $CHCl_3$/THF (1:1, 50 mL) at room temperature. The resulting mixture was stirred further for 2 h and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, 90% EtOAc in cyclohexane) to yield a yellow solid (723 mg, 1.96 mmol, 89%). M.p. 168 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.46 (br s, 1H, 1-NH), 8.05 (dd, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 2.0 Hz, 1H, 6-CH), 7.80 (d, $^4J_{H,H}$ = 1.9 Hz, 1H, 4-CH), 7.09 (d, $^3J_{H,H}$ = 8.3 Hz, 1H, 7-CH), 3.73–3.58 (m, 2H, 12-CH, 15-CH), 3.52–3.20 (m, 4H, 16-CH_2, 19-CH_2), 3.37 (s, 6H, 18-CH_3, 21-CH_3), 1.78–1.62 (m, 2H, 13-CH_a, 14-CH_a), 1.57–1.37 (m, 2H, 13-CH_b, 14-CH_b) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 182.9 (3-CO), 159.4 (2-CO), 153.6 (8-CN), 136.7 (6-CH), 130.8 (5-CSO_2), 123.1 (4-CH), 118.1 (9-CCO), 112.7 (7-CH), 74.4 (2C, 16-CH_2, 19-CH_2), 59.9 (2C, 12-CH, 15-CH), 58.4 (2C, 18-CH_3, 21-CH_3), 26.9 (2C, 13-CH_2, 14-CH_2) ppm. HRMS (ESI+, MeOH): m/z = 391.0931

$[M + Na]^+$, 423.1194 $[M + Na + MeOH]^+$; calcd. 391.0934 for $C_{16}H_{20}N_2O_6S$ + Na, 423.1196 for $C_{16}H_{20}N_2O_6S$ + Na + MeOH.

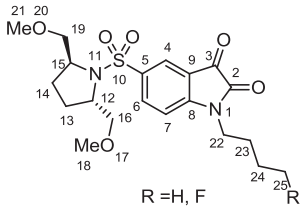
4.12.3. *cis*-*N*-butyl-5-{1-[2,5-bis(methoxymethyl)pyrrolidinyl] sulfonyl}isatin (**32a**)

cis-5-{1-[2,5-bis(methoxymethyl)pyrrolidinyl]sulfonyl}isatin (**19a**) (100 mg, 0.271 mmol, 1.00 equiv.) was converted to *cis*-*N*-butyl-5-{1-[2,5-bis(methoxymethyl)pyrrolidinyl]sulfonyl}isatin (**32a**) using 1-bromobutane and Cs_2CO_3 as described in the general procedure in Section 4.2.3. The residue was purified by flash column chromatography (silica gel, 35% EtOAc in toluene) to obtain a yellow solid (84 mg, 0.198 mmol, 73%). M.p. 115 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.09 (dd, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.9 Hz, 1H, 6-CH), 8.04 (d, $^4J_{H,H}$ = 1.9 Hz, 1H, 4-CH), 7.05 (d, $^3J_{H,H}$ = 8.3 Hz, 1H, 7-CH), 3.79–3.69 (m, 2H, 12-CH, 15-CH), 3.78 (t, $^3J_{H,H}$ = 7.4 Hz, 2H, 22-CH_2), 3.60 (dd, $^2J_{H,H}$ = 9.4 Hz, $^3J_{H,H}$ = 4.2 Hz, 2H, 16-CH_a, 19-CH_a), 3.40–3.34 (m, 2H, 16-CH_b, 19-CH_b), 3.37 (s, 6H, 18-CH_3, 21-CH_3), 1.95–1.79 (m, 2H, 13-CH_a, 14-CH_a), 1.75–1.66 (m, 2H, 23-CH_2), 1.72–1.58 (m, 2H, 13-CH_b, 14-CH_b), 1.43 (sextet, $^3J_{H,H}$ = 7.5 Hz, 2H, 24-CH_2), 0.99 (t, $^3J_{H,H}$ = 7.4 Hz, 3H, 25-CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 182.2 (3-CO), 157.8 (2-CO), 153.8 (8-CN), 137.5 (6-CH), 133.7 (CSO_2), 124.5 (4-CH), 117.3 (9-CCO), 110.5 (7-CH), 74.9 (2C, 16-CH_2, 19-CH_2), 60.7 (2C, 12-CH, 15-CH), 59.2 (18-CH_3), 40.5 (22-CH_2), 29.2 (23-CH_2), 27.6 (2C, 13-CH_2, 14-CH_2), 20.1 (24-CH_2), 13.6 (25-CH_3) ppm. HRMS (ESI+, MeOH): *m*/*z* = 447.1561 $[M + Na]^+$, 479.1820 $[M + Na + MeOH]^+$; calcd. 447.1560 for $C_{20}H_{28}N_2O_6S$ + Na, 479.1822 for $C_{20}H_{28}N_2O_6S$ + Na + MeOH.

4.12.4. *cis*-*N*-(4-fluorobutyl)-5-{1-[2,5-bis(methoxymethyl)pyrroli dinyl]sulfonyl}isatin (**33a**)

cis-5-{1-[2,5-bis(methoxymethyl)pyrrolidinyl]sulfonyl}isatin (**19a**) (100 mg, 0.271 mmol, 1.00 equiv.) was converted to *cis*-*N*-(4-fluorobutyl)-5-{1-[2,5-bis(methoxymethyl)pyrrolidinyl]sulfonyl} isatin (**33a**) using TFA followed by reaction with 5-chlorosulfonyl isatin and DIPEA as described in the general procedure in Section 4.2.3. The residue was purified by flash column chromatography (silica gel, 60% EtOAc in toluene) to obtain a yellow solid (93 mg, 0.210 mmol, 77%). M.p. 145 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.10 (dd, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.9 Hz, 1H, 6-CH), 8.05 (d, $^4J_{H,H}$ = 1.9 Hz, 1H, 4-CH), 7.07 (d, $^3J_{H,H}$ = 8.3 Hz, 1H, 7-CH), 4.52 (dt, $^2J_{H,F}$ = 47.5 Hz, $^3J_{H,H}$ = 5.4 Hz, 2H, 25-CH_2), 3.84 (t, $^3J_{H,H}$ = 7.1 Hz, 2H, 17-CH_2), 3.80–3.68 (m, 2H, 12-CH, 15-CH), 3.60 (dd, $^2J_{H,H}$ = 9.4 Hz, $^3J_{H,H}$ = 4.2 Hz, 2H, 16-CH_a, 19-CH_a), 3.42–3.32 (m, 2H, 16-CH_b, 19-CH_b), 3.36 (s, 6H, 18-CH_3, 21-CH_3), 1.96–1.79 (m, 2H, 13-CH_a, 14-CH_a), 1.91–1.71 (m, 4H, 23-CH_2, 24-CH_2), 1.71–1.57 (m, 2H, 13-CH_b, 14-CH_b) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 182.0 (3-CO), 157.8 (2-CO), 153.5 (8-CN), 137.6 (6-CH), 133.9 (5-CSO_2), 124.6 (4-CH), 117.4 (9-CCO), 110.4 (7-CH), 83.3 (d, $^1J_{C,F}$ = 165.5 Hz, 25-CH_2), 74.9 (2C, 16-CH_2, 19-CH_2), 60.7 (2C, 12-CH, 15-CH), 59.2 (2C, 18-CH_3, 21-CH_3), 40.2 (22-CH_2), 27.6 (d, $^2J_{C,F}$ = 20.1 Hz, 24-CH_2), 27.6 (2C, 13-CH_2, 14-CH_2), 23.5 (d, $^3J_{C,F}$ = 4.0 Hz, 23-CH_2) ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = −219.9 (s, 1F, 25-CH_2F) ppm. HRMS (ESI+, MeOH): *m*/*z* = 465.1461 $[M + Na]^+$, 497.1723 $[M + Na + MeOH]^+$; calcd. 465.1466 for $C_{20}H_{27}FN_2O_6S$ + Na, 497.1728 for $C_{20}H_{27}FN_2O_6S$ + Na + MeOH.

4.13. *trans*-5-{1-[2,5-bis(methoxymethyl)pyrrolidinyl]sulfonyl}isatins



4.13.1. *trans*-*N*-benzyl-2,5-bis(methoxymethyl)pyrrolidine (**18b**)

trans-*N*-benzyl-2,5-bis(hydroxymethyl)pyrrolidine (**17b**) (628 mg, 2.84 mmol, 1.00 equiv.) was converted to *trans*-*N*-benzyl-2,5-bis(methoxymethyl)pyrrolidine (**18b**) using the same procedure of the synthesis of **18a** (Section 4.12.1). The crude product was purified by flash column chromatography (silica gel, 15% EtOAc in cyclohexane) to obtain a colorless oil (493 mg, 1.98 mmol, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40–7.34 (m, 2H, 2 × 8-CH), 7.32–7.26 (m, 2H, 2 × 9-CH), 7.23–7.18 (m, 1H, 10-CH), 4.00 (d, $^2J_{H,H}$ = 14.3 Hz, 1H, 6-CH_a), 3.88 (d, $^2J_{H,H}$ = 14.3 Hz, 1H, 6-CH_b), 3.37–3.21 (m, 4H, 11-CH_2, 14-CH_2), 3.27 (s, 6H, 13-CH_3, 16-CH_3), 3.23–3.12 (m, 2H, 2-CH, 5-CH), 2.06–1.90 (m, 2H, 3-CH_a, 4-CH_a), 1.75–1.60 (m, 2H, 3-CH_b, 4-CH_b) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 140.8 (7-C), 128.2 (2C, 2 × 8-CH), 128.1 (2C, 2 × 9-CH), 126.5 (10-CH), 74.6 (2C, 11-CH_2, 14-CH_2), 60.4 (2C, 2-CH, 5-CH), 59.0 (2C, 13-CH_3, 16-CH_3), 52.7 (6-CH_2), 27.2 (2C, 3-CH_2, 4-CH_2) ppm. HRMS (ESI+, MeOH): *m*/*z* = 250.1810 $[M + H]^+$; calcd. 250.1802 for $C_{15}H_{23}NO_2$ + H.

4.13.2. *trans*-5-{1-[2,5-bis(methoxymethyl)pyrrolidinyl]sulfonyl} isatin (**19b**)

trans-*N*-benzyl-2,5-bis(methoxymethyl)pyrrolidine (**18b**) (491 mg, 1.97 mmol, 1.00 equiv.) was converted to *trans*-5-{1-[2,5-bis(methoxymethyl)pyrrolidinyl]sulfonyl} isatin (**19b**) with the same conditions as described in the synthesis of **19a** (Section 4.12.2). The residue was purified by flash column chromatography (silica gel, 80% EtOAc in cyclohexane) to furnish a yellow oil (104 mg, 0.282 mmol, 14%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.03 (br s, 1H, 1-NH), 8.10 (d, $^4J_{H,H}$ = 2.0 Hz, 1H, 4-CH), 8.10 (dd, $^3J_{H,H}$ = 8.8 Hz, $^4J_{H,H}$ = 2.0 Hz, 1H, 6-CH), 7.10 (d, $^3J_{H,H}$ = 8.8 Hz, 1H, 7-CH), 4.01–3.90 (m, 2H, 12-CH, 15-CH), 3.54–3.46 (m, 4H, 16-CH_2, 19-CH_2), 3.22 (s, 6H, 18-CH_3, 21-CH_3), 2.20–2.04 (m, 2H, 13-CH_a, 14-CH_a), 1.98–1.83 (m, 2H, 13-CH_b, 14-CH_b) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 181.9 (3-CO), 158.9 (2-CO), 151.7 (8-CN), 138.0 (5-CSO_2), 137.3 (6-CH), 124.6 (4-CH), 117.5 (9-CCO), 112.6 (7-CH), 73.5 (2C, 16-CH_2, 19-CH_2), 60.4 (2C, 12-CH, 15-CH), 58.8 (2C, 18-CH_3, 21-CH_3), 27.9 (2C, 13-CH_2, 14-CH_2) ppm. HRMS (ESI+, MeOH): *m*/*z* = 391.0927 $[M + Na]^+$, 423.1192 $[M + Na + MeOH]^+$; calcd. 391.0934 for $C_{16}H_{20}N_2O_6S$ + Na, 423.1196 for $C_{16}H_{20}N_2O_6S$ + Na + MeOH.

4.13.3. *trans*-*N*-butyl-5-{1-[2,5-bis(methoxymethyl)pyrrolidinyl] sulfonyl}isatin (**32b**)

trans-5-{1-[2,5-bis(methoxymethyl)pyrrolidinyl]sulfonyl}isatin (**19b**) (45 mg, 0.122 mmol, 1.00 equiv.) was converted to *trans*-*N*-butyl-5-{1-[2,5-bis(methoxymethyl)pyrrolidinyl]sulfonyl}isatin (**32b**) using 1-bromobutane and Cs_2CO_3 as described in the general procedure in Section 4.2.3. The crude product was purified by flash column chromatography (silica gel, 40% EtOAc in cyclohexane) to obtain a yellow oil (35 mg, 82.4 μmol, 68%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.11 (dd, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 2.0 Hz, 1H, 6-CH), 8.07 (d, $^4J_{H,H}$ = 2.0 Hz, 1H, 4-CH), 7.00 (d, $^3J_{H,H}$ = 8.3 Hz, 1H, 7-CH), 3.98–3.90 (m, 2H, 12-CH, 15-CH), 3.78 (t, $^3J_{H,H}$ = 7.3 Hz, 2H, 22-CH_2), 3.55–3.45 (m, 4H, 16-CH_2, 19-CH_2), 3.22 (s, 6H, 18-CH_3, 21-CH_3), 2.20–2.06 (m, 2H, 13-CH_a, 14-CH_a), 1.97–1.83 (m, 2H, 13-CH_b, 14-CH_b), 1.70 (quintet, $^3J_{H,H}$ = 7.4 Hz, 2H, 23-CH_2), 1.42 (sextet, $^3J_{H,H}$ = 7.4 Hz, 2H, 24-CH_2), 0.98 (t, $^3J_{H,H}$ = 7.3 Hz, 3H, 25-CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 182.2 (3-CO), 157.9 (2-CO), 153.3 (8-CN), 137.7 (5-CSO_2), 137.1 (6-CH), 124.2 (4-CH), 117.2 (9-CCO), 110.0 (7-CH), 73.5 (2C, 16-CH_2, 19-CH_2), 60.4 (2C, 12-CH, 15-CH), 58.8 (2C, 18-CH_3, 21-CH_3), 40.4 (22-CH_2), 29.2 (23-CH_2), 27.8 (2C, 13-CH_2, 14-CH_2), 20.1 (24-CH_2), 13.6 (25-CH_3) ppm. HRMS (ESI+, MeOH): *m*/*z* = 447.1557 $[M + Na]^+$, 479.1820 $[M + Na + MeOH]^+$; calcd. 447.1560 for $C_{20}H_{28}N_2O_6S$ + Na, 479.1822 for $C_{20}H_{28}N_2O_6S$ + Na + MeOH.

4.13.4. *trans*-N-(4-fluorobutyl)-5-{1-[2,5-bis(methoxymethyl) pyrrolidinyl]sulfonyl}isatin (**33b**)

trans-5-{1-[2,5-bis(methoxymethyl)pyrrolidinyl]sulfonyl}isatin (**19b**) (45 mg, 0.122 mmol, 1.00 equiv.) was converted to *trans*-N-(4-fluorobutyl)-5-{1-[2,5-bis(methoxymethyl)pyrrolidinyl]sulfonyl} isatin (**33b**) using 1-bromo-4-fluorobutane and Cs_2CO_3 as described in the general procedure in Section 4.2.3. The crude product was purified by flash column chromatography (silica gel, 50% EtOAc in cyclohexane) to obtain a yellow oil (29 mg, 65.5 μmol, 54%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.12 (dd, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 2.0 Hz, 1H, 6-CH), 8.08 (d, $^4J_{H,H}$ = 2.0 Hz, 1H, 4-CH), 7.02 (d, $^3J_{H,H}$ = 8.3 Hz, 1H, 7-CH), 4.52 (dt, $^2J_{H,F}$ = 47.4 Hz, $^3J_{H,H}$ = 5.5 Hz, 2H, 25-CH_2), 3.99–3.89 (m, 2H, 12-CH, 15-CH), 3.84 (t, $^3J_{H,H}$ = 7.1 Hz, 2H, 22-CH_2), 3.57–3.43 (m, 4H, 16-CH_2, 19-CH_2), 3.22 (s, 6H, 18-CH_3, 21-CH_3), 2.21–2.05 (m, 2H, 13-CH_a, 14-CH_a), 1.98–1.78 (m, 6H, 13-CH_b, 14-CH_b, 23-CH_2, 24-CH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 182.0 (3-CO), 157.9 (2-CO), 153.0 (8-CN), 137.9 (5-CSO_2), 137.2 (6-CH), 124.3 (4-CH), 117.2 (9-CCO), 110.0 (7-CH), 83.2 (d, $^1J_{C,F}$ = 165.6 Hz, 25-CH_2), 73.5 (2C, 16-CH_2, 19-CH_2), 60.4 (2C, 12-CH, 15-CH), 58.8 (2C, 18-CH_3, 21-CH_3), 40.2 (22-CH_2), 27.8 (2C, 13-CH_2, 14-CH_2), 27.6 (d, $^2J_{C,F}$ = 20.1 Hz, 24-CH_2), 23.5 (d, $^3J_{C,F}$ = 4.0 Hz, 23-CH_2) ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ = −220.0 (s, 1F, 25-CH_2F) ppm. HRMS (ESI+, MeOH): m/z = 465.1465 $[M + Na]^+$, 497.1731 $[M + Na + MeOH]^+$; calcd. 465.1466 for $C_{20}H_{27}FN_2O_6S$ + Na, 497.1728 for $C_{20}H_{27}FN_2O_6S$ + Na + MeOH.

4.14. In vitro assays

The caspase inhibition potencies of the target isatin sulfonamides were indicated as IC_{50} values. The recombinant human caspases-3 and -7 including their peptide-specific substrate Ac-DEVD-AMC (Ac-Asp-Glu-Val-Asp-AMC) were purchased from Alexis® Biochemicals (Switzerland). As already described [4,7], reaction rates showing inhibitory potencies of the inhibitors were assessed by measuring the accumulation of the cleaved fluorogenic product AMC (7-amino-4-methylcoumarin) with a Fusion universal microplate analyzer (PerkinElmer) at excitation and emission wavelengths of 360 and 460 nm, respectively. All assays were performed at a volume of 200 μL at 37 °C in reaction buffer [4,7]. Buffers contained the target compounds in DMSO in single doses (end concentrations 500 μM, 50 μM, 5 μM, 500 nM, 50 nM, 5 nM, 500 pM, or 50 pM). Recombinant caspases were diluted into the appropriate buffer to a concentration of 0.5 units per assay (= 500 pmol substrate conversion after 60 min). After 10 min incubation time, the peptide substrate (end concentration 10 μM) was added and reacted for further 10 min. The IC_{50} values were determined by nonlinear regression analysis using the XMGRACE program (Linux software).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.04.011.

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