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Supplementary Information for

Structure-based development of a subtype-selective orexin 1 receptor antagonist

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Supplementary Text

Organic Synthesis

The 5-chlorobenzoxazole **21** was synthesized in one step by treatment of 2-Amino-4-chlorophenol with triethyl orthoformate in ring closure reaction (Scheme S1) (1). The carboxylic acid **24** was synthesized in three steps starting from 1-bromo-4-methylbenzene. First, the bromine was substituted by triazole in a *Buchwald* reaction to obtain **22** (2, 3). *Ortho*-bromination using *N*-bromosuccinimide and $Pd(OAc)_2$ as catalyst gave compound **23**(2). The bromine was substituted by a nitrile by treatment with copper cyanide which was subsequently hydrolyzed with aqueous sodium hydroxide solution to obtain the carboxylic acid **24** (2). The central homopiperazine building blocks were either synthesized from the corresponding amino acid or if commercially available from hydrochloride salts of the corresponding amino acid methyl esters **25a-l**. Those amino acids which were not commercially available as methyl esters were converted to the corresponding methyl esters **25a-l** by treatment with a methanolic hydrochloride solution. A *Michael* addition of acrylonitrile to the amine followed by a Boc protection led to the nitrile compounds **26a-l**. Platinum catalyzed hydration of the nitriles gave the primary amines. The methyl esters were hydrolyzed under basic conditions. An intramolecular amide bond formation between the primary amines and the carboxylic acids using HATU as coupling reagent provided the lactam compounds **27a-l**. The carbonyl moiety was reduced using a borane-THF complex. The Boc protected homopiperazine compounds **28a-l** were coupled to **21** in a copper catalyzed oxidative coupling reaction under air oxygen to obtain compounds **29a-l** (1). Cleavage of the Boc protecting group under acidic conditions gave the secondary amines **30a-l** which were coupled to carboxylic acid **24** using HATU as coupling reagent. The final compounds **1-9** and **12-14** were obtained after purification by preparative HPLC or flash column chromatography.

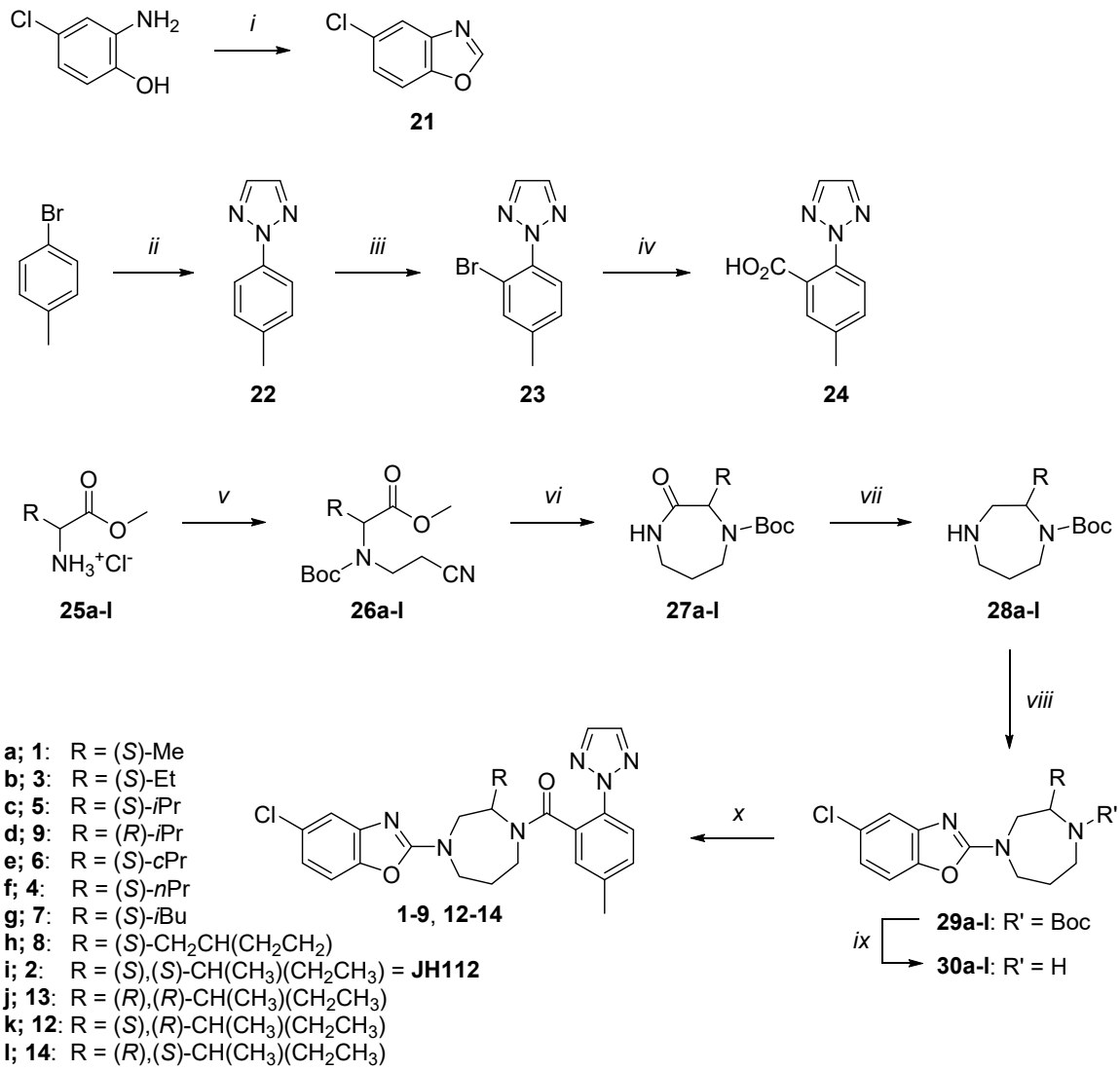
Compounds **10-11** were synthesized starting from the intermediates **28c-d** (Scheme S2) which were already synthetic intermediates for the ligands **5** and **9**. The secondary amines of compounds **28c-d** were coupled to the carboxylic acid **24** under standard amide coupling conditions using HATU as coupling reagent and DIPEA as non-nucleophilic base to obtain **31c-d**. Cleavage of the Boc protecting group under acidic conditions gave the secondary amines **32c-d** which were coupled to **21** in a copper catalyzed reaction under air oxygen. The final compounds **10-11** were obtained after purification using a preparative HPLC.

The first step of the synthesis of compounds **15-16** (Scheme S3) was the treatment of methyl *L*-phenylalaninate hydrochloride with acrylonitrile in a *Michael* addition to obtain a secondary amine as intermediate which was subsequently Boc protected to get compound **33**. Platinum catalyzed hydration reduced not only the nitrile to the primary amine but partially the aromatic system as well resulting in a mixture of compounds. Since the separation of the two compounds turned out to be difficult at this stage, the synthesis was proceeded with a mixture of two compounds. The methyl esters were hydrolyzed under basic conditions using lithium hydroxide. An intramolecular amide bond formation between the primary amines and the carboxylic acids using HATU as coupling reagent and DIPEA as non-nucleophilic base provided the lactam compounds **34m-n**. The carbonyl moiety was reduced with a borane-THF complex. The Boc protected homopiperazine compounds **35m-n** were coupled to **21** in a copper catalyzed reaction under air oxygen to obtain a compound mixture. Separation of the two compounds by flash column chromatography provided the pure compounds **36m-n**. Cleavage of the Boc protecting group under acidic conditions gave the secondary amines **37m-n** which were coupled to the carboxylic acid **24** using HATU as coupling reagent. The final compounds **15-16** were obtained after purification with a preparative HPLC.

Compounds **17-20** were synthesized after Scheme S4. The Boc protected diamine building blocks were coupled to **21** in copper-catalyzed reaction under air oxygen to obtain **38o-r**. Cleavage of the Boc

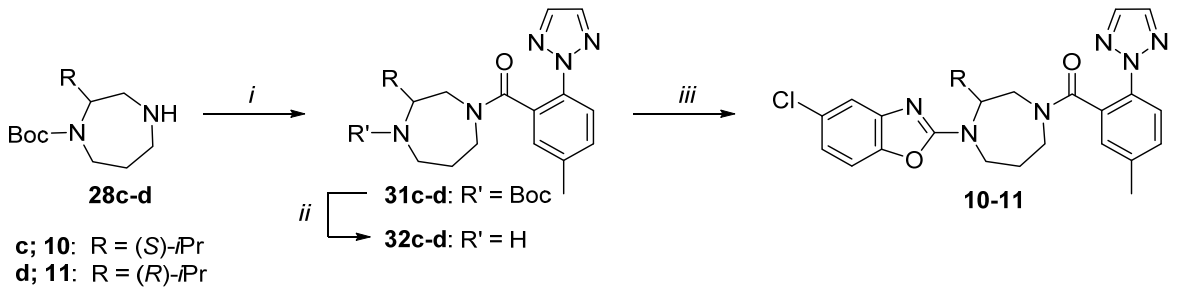
protecting group under acidic conditions gave the secondary amines **39o-r** which were coupled to **24** using HATU as coupling reagent. The final compounds **17-20** were obtained after purification with a preparative HPLC. Compound **19** was described before (4).

Synthesis Schemes

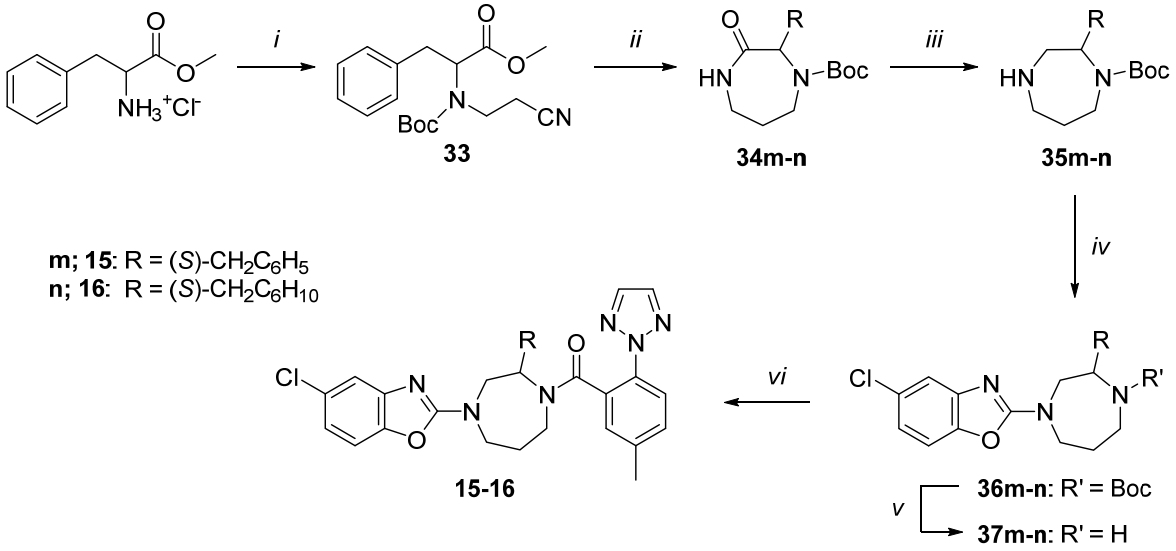


a; 1: R = (*S*)-Me
b; 3: R = (*S*)-Et
c; 5: R = (*S*)-*i*Pr
d; 9: R = (*R*)-*i*Pr
e; 6: R = (*S*)-*c*Pr
f; 4: R = (*S*)-*n*Pr
g; 7: R = (*S*)-*i*Bu
h; 8: R = (*S*)-$CH_2CH(CH_2CH_2)$
i; 2: R = (*S*),(*S*)-$CH(CH_3)(CH_2CH_3)$ = **JH112**
j; 13: R = (*R*),(*R*)-$CH(CH_3)(CH_2CH_3)$
k; 12: R = (*S*),(*R*)-$CH(CH_3)(CH_2CH_3)$
l; 14: R = (*R*),(*S*)-$CH(CH_3)(CH_2CH_3)$

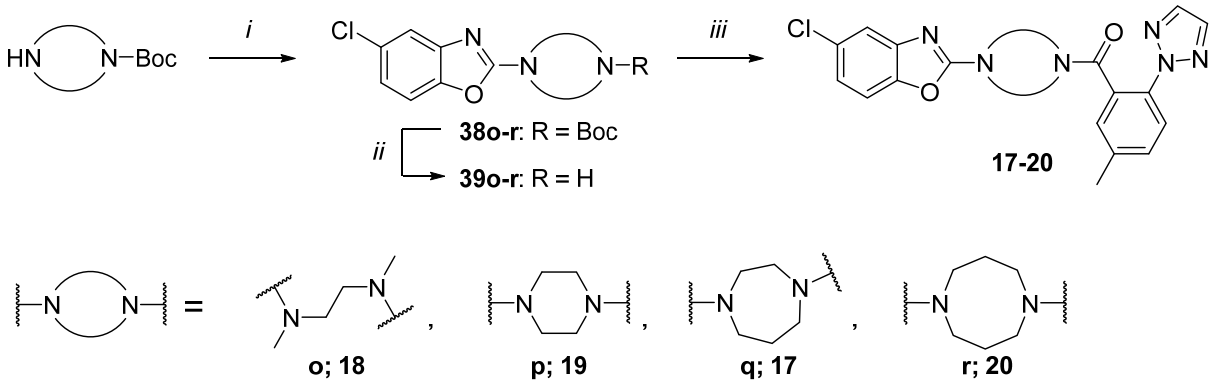
Scheme S1. Reagents and conditions: *i*: triethyl orthoformate, 160 °C, 1.5 h, 68%(1); *ii*: 2*H*-1,2,3-triazole, K_3PO_4, tris(dibenzylideneacetone)dipalladium(0), Me_4*t*BuXPhos, toluene, 120 °C, 16 h, 74%(2); *iii*: NBS, $Pd(OAc)_2$, CH_3CO_2H, 120 °C, 60 h, 82%(2); *iv*: 1. CuCN, DMF, 120 °C, 16 h; 2. NaOH (aq.), CH_3CH_2OH, 80 °C, 16 h, 69%(2); *v*: 1. for **25a,c,e-l**: acrylonitrile, DIPEA, CH_3OH, 0 °C to 75 °C, overnight; for **25b,d**: acrylonitrile, NaOH, CH_3OH, 0 °C to 75 °C, overnight; 2. for **25a-l**: Boc_2O, CH_3OH, 75 °C, overnight, 6 – 86%; *vi*: 1. PtO_2, H_2, CH_3CH_2OH, $CHCl_3$, rt, 16 h – 6 d; 2. LiOH (aq.), CH_3OH, THF, rt, 18 – 72 h; 3. HATU, with or without DIPEA, DMF, 16 – 41 h, 21 – 94%; *vii*: BH_3, THF, 0 °C to 75 °C, 4 – 72 h, 15 – 41%; *viii*: **21**, $Cu(OAc)_2$ x H_2O, CH_3CO_2H, CH_3CN, air, 80 °C, 18 – 45 h, 18 – 73%; *ix*: CF_3CO_2H, CH_2Cl_2, 2 – 27 h, 89 – 100%; *x*: **24**, HATU, DIPEA, DMF, 3 – 26 h, 1 – 70%.



Scheme S2. Reagents and conditions: *i*: **24**, HATU, DIPEA, DMF, 48 h, **31c**: not isolated, **31d**: 4%; *ii*: CF_3CO_2H, CH_2Cl_2, 3 – 5 h, **32c-d**: not isolated; *iii*: **21**, $Cu(OAc)_2$ x H_2O, CH_3CO_2H, CH_3CN, air, 80 °C, 16 – 48 h, 3 – 6%.



Scheme S3. Reagents and conditions: *i*: 1. acrylonitrile, DIPEA, CH_3OH, 0 °C to 75 °C, overnight; 2. Boc_2O, CH_3OH, 75 °C, overnight, 45%; *ii*: 1. PtO_2, H_2, CH_3CH_2OH, $CHCl_3$, rt, 4 d; 2. LiOH (aq.), CH_3OH, THF, rt, 24 h; 3. HATU, DIPEA, DMF, 18 h, not isolated; *iii*: BH_3, THF, 0 °C to 75 °C, 22 h, not isolated; *iv*: **21**, $Cu(OAc)_2$ x H_2O, CH_3CO_2H, CH_3CN, air, 80 °C, 22 h, 0.6 – 0.7% over four steps; *v*: CF_3CO_2H, CH_2Cl_2, 4 – 5 h, not isolated; *vi*: **24**, HATU, DIPEA, DMF, 48 h, 14 – 33%.



Scheme S4. Reagents and conditions: *i*: **21**, $Cu(OAc)_2$ x H_2O, CH_3CO_2H, CH_3CN, air, 80 °C, 2 – 69 h, 54 – 81%; *ii*: CF_3CO_2H, CH_2Cl_2, 3 – 16 h, 92% – quant.; *iii*: **24**, HATU, DIPEA, DMF, 2 – 43 h, 2 – 68%.

Figures S1-S15

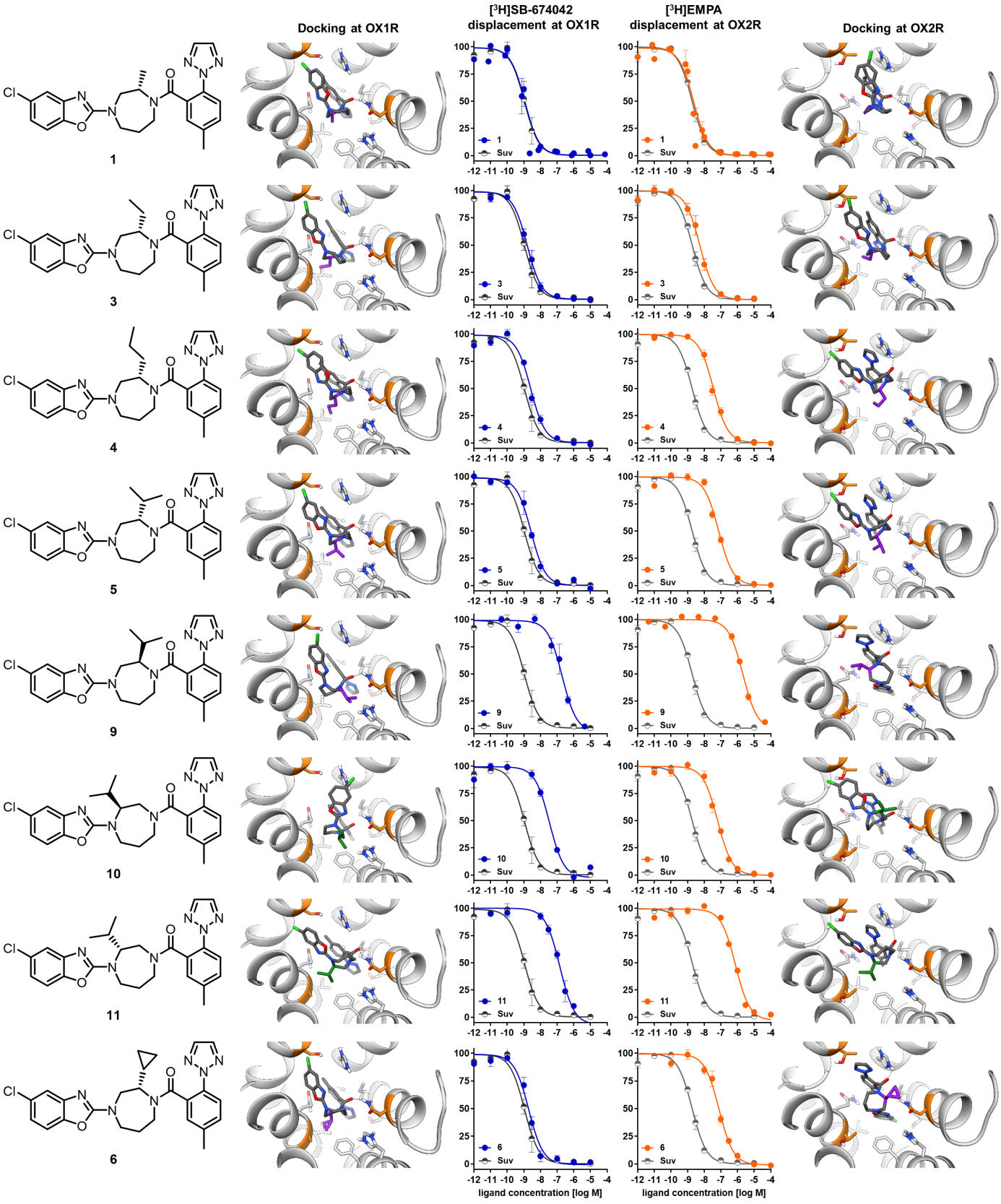


Figure S1. Chemical structures, receptor binding curves and docking poses of compounds 1-6 and 9-11 in OX1R (left) and OX2R (right). In each subpanel, the competition curve obtained with suvorexant is displayed as reference. Data from competition binding experiments were normalized to total (100%) and unspecific binding (0%) and are displayed as mean ± SEM from at least three independent experiments.

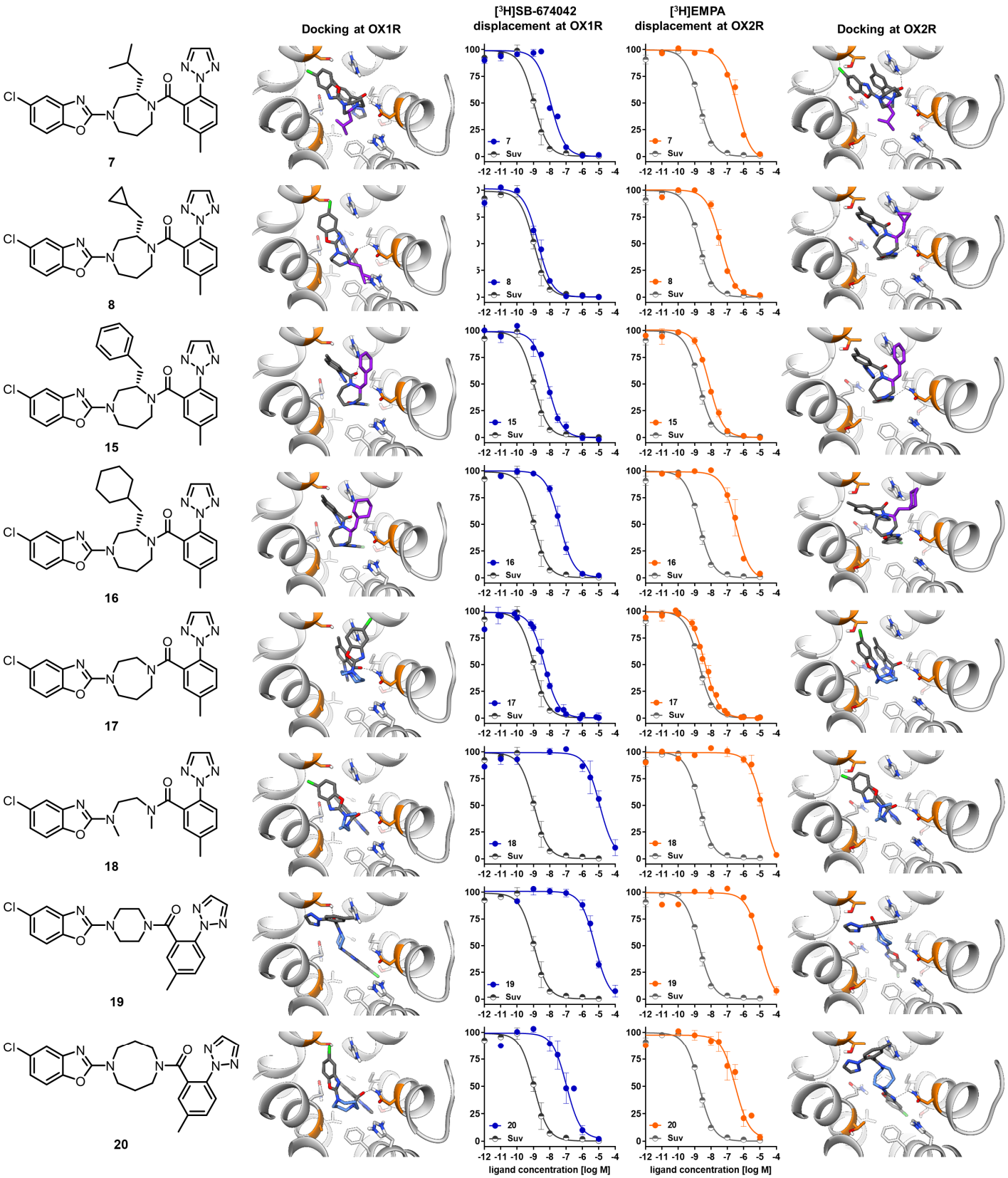


Figure S2. Chemical structures, receptor binding curves and docking poses of compounds 7, 8 and 15-20 in OX1R (left) and OX2R (right). In each subpanel, the competition curve obtained with suvorexant is displayed as reference. Data from competition binding experiments were normalized to total (100%) and unspecific binding (0%) and are displayed as mean ± SEM from at least three independent experiments.

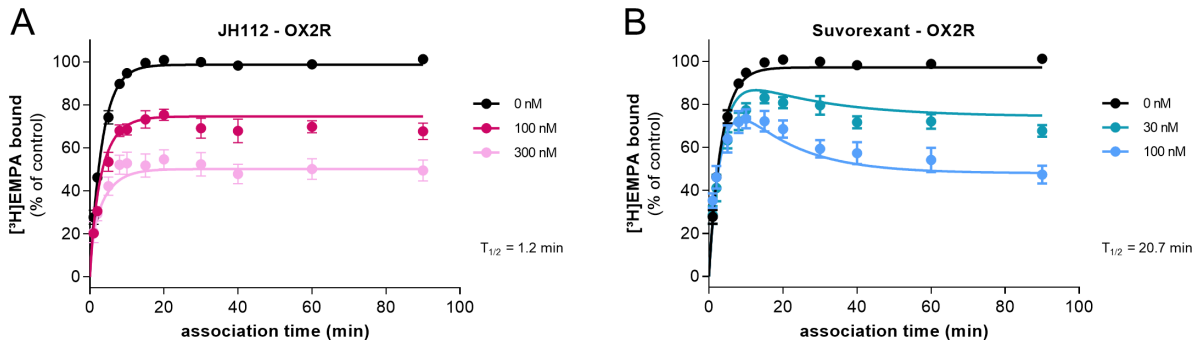


Figure S3. Ligand binding kinetics of JH112 and suvorexant at OX2R. Kinetic binding experiments with the radioligand [³H]EMPA and membranes from HEK293T cells expressing OX2R reveal a dissociation half-life of (**A**) 1.2 min for **JH112** and (**B**) 20.7 min for suvorexant. Data represent mean ± SEM and the global fit from five to eight individual experiments.

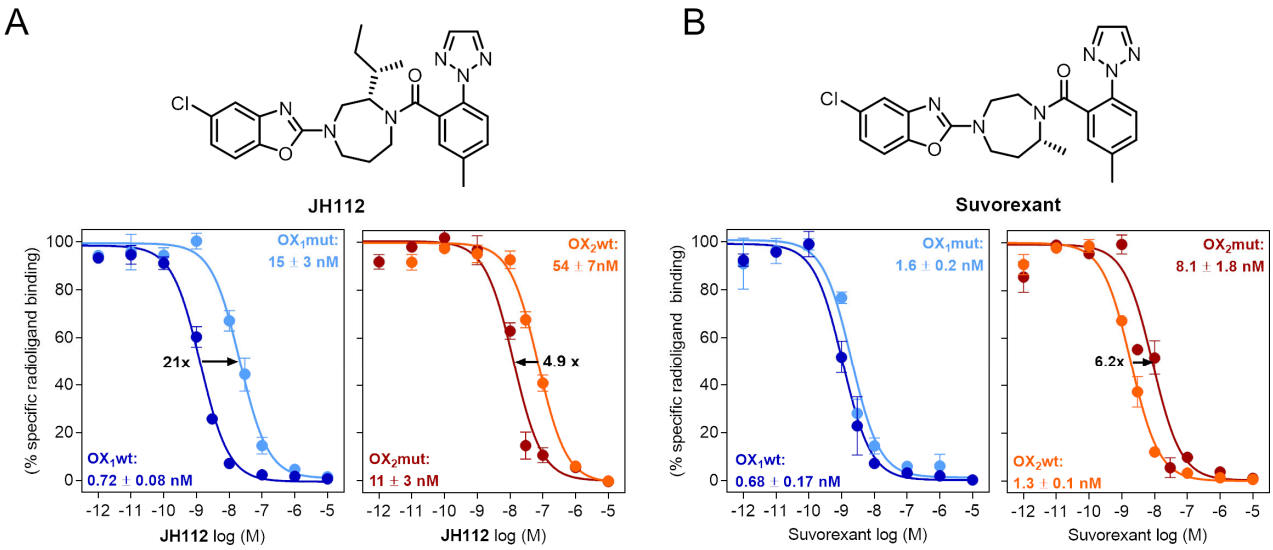


Figure S4. Mutational analysis of suvorexant and JH112 binding to OX1R and OX2R. The role of Ala/Thr$^{3.33}$ in OX1R/OX2R for receptor selectivity of (**A**) **JH112** in comparison to (**B**) the reference suvorexant was examined determining the binding affinity at the mutant OX1_A127T carrying the bulkier Thr at position 127 and its inverse mutant OX2_T135A. While suvorexant shows only minor sensitivity to the mutation for OX1R (2.4-fold, light and dark blue curves), a slight decrease (6.2-fold) of binding affinity is observed for the OX2R mutant (dark red curve) compared to the wildtype (orange). In contrast, the affinity of **JH112** is reduced by 21-fold for the OX1R mutant (dark blue) with the bulkier threonine residue. The binding affinity of **JH112** for OX2R_T135A slightly increases (4.9-fold), presumably because the smaller alanine provides the *sec*-butyl residue of **JH112** more space in the binding pocket. Data show the mean curve ± SEM from 5-6 independent experiments, each performed in triplicates. K_i values were calculated from the EC_{50} of the binding curves employing the equation of Cheng and Prusoff (5) and are indicated in the respective colors.

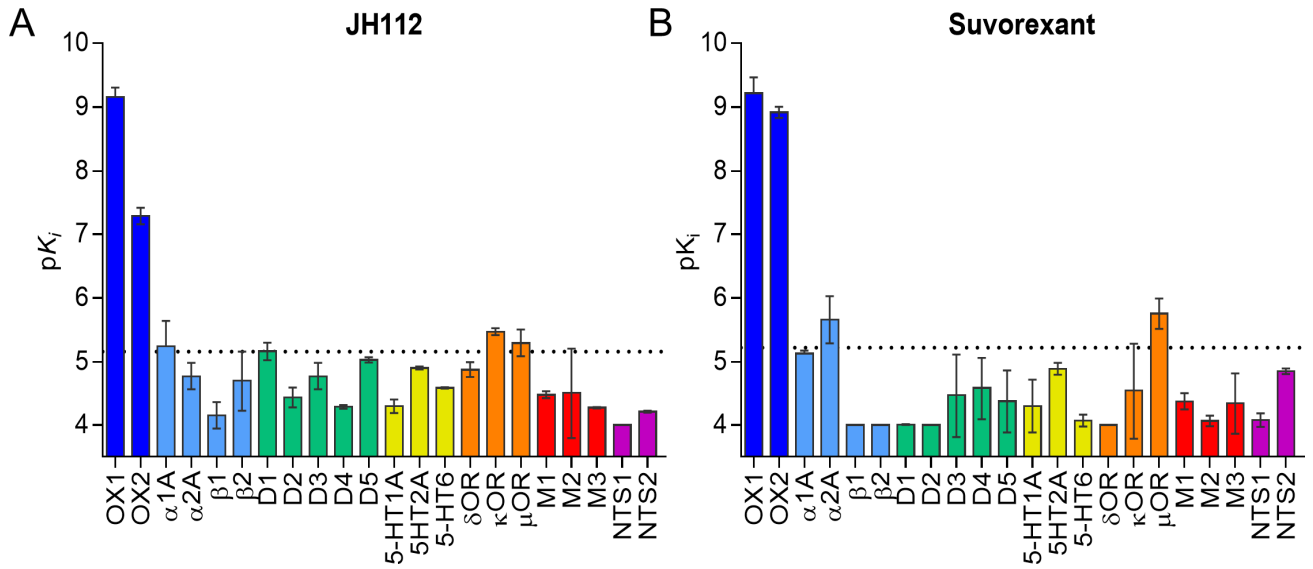


Figure S5. Selectivity over 20 aminergic and peptidergic GPCRs. pK_i values for 20 representative class A GPCRs including adrenergic (light blue), dopaminergic (green), serotonergic (yellow), opioid (orange), muscarinic acetylcholine (red) and neurotensin receptors (magenta) were determined by radioligand competition and compared to the affinity for OX1R and OX2R (dark blue) for (**A**) **JH112** and (**B**) suvorexant. The dashed line indicates a selectivity for OX1R of > than 10,000-fold. Bars represent the mean ± SD of two individual experiments, each performed in triplicate.

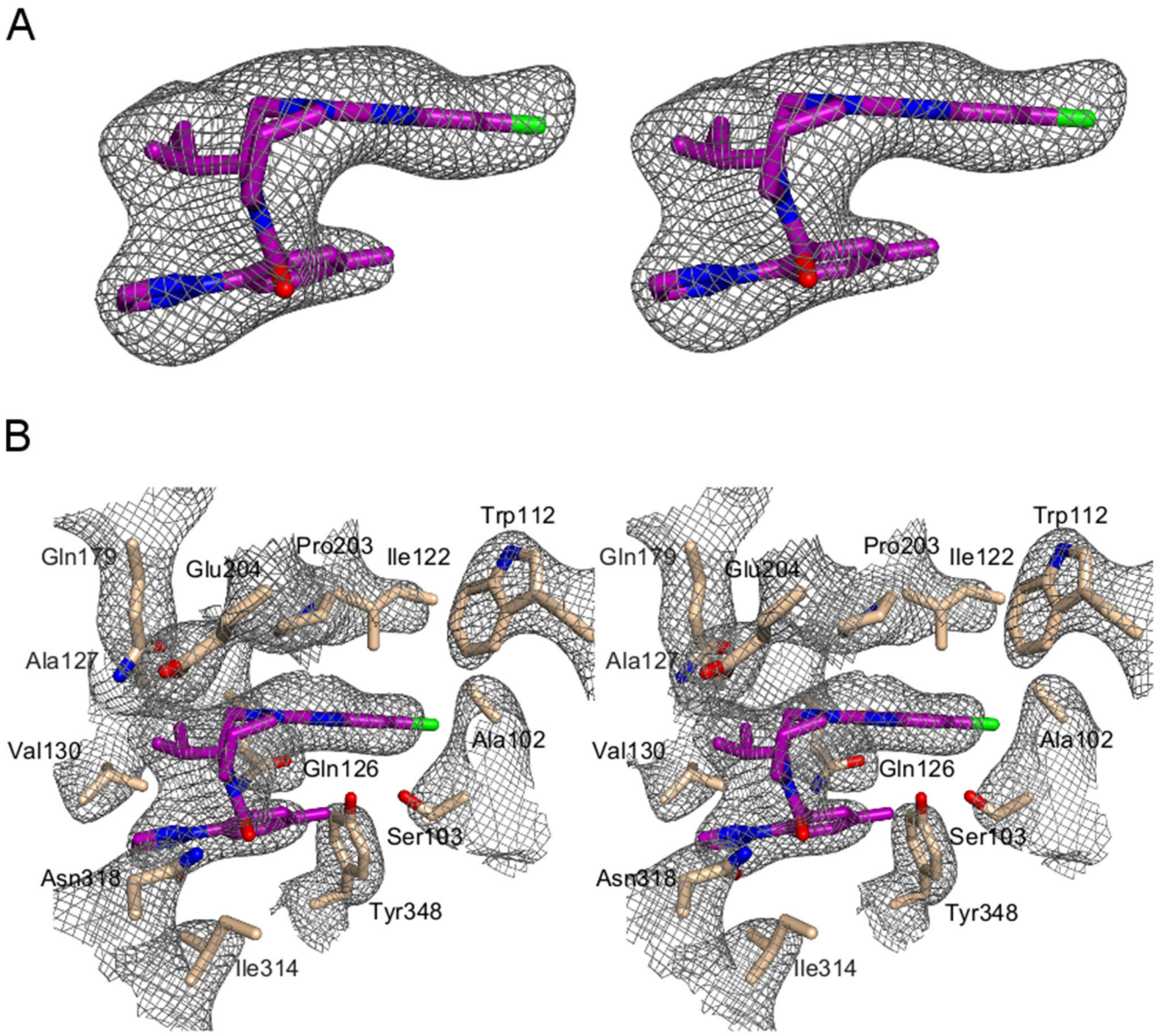


Figure S6. Electron density for the ligand and orthosteric binding pocket. (**A**) Stereoview of polder OMIT map for the ligand **JH112**, contoured at 3.5 σ. Ligand is shown as sticks with purple carbons. (**B**) Stereoview of 2Fo-Fc map for the orthosteric binding pocket region, contoured at 1 σ. Sidechains of the receptor are shown as sticks with beige carbons.

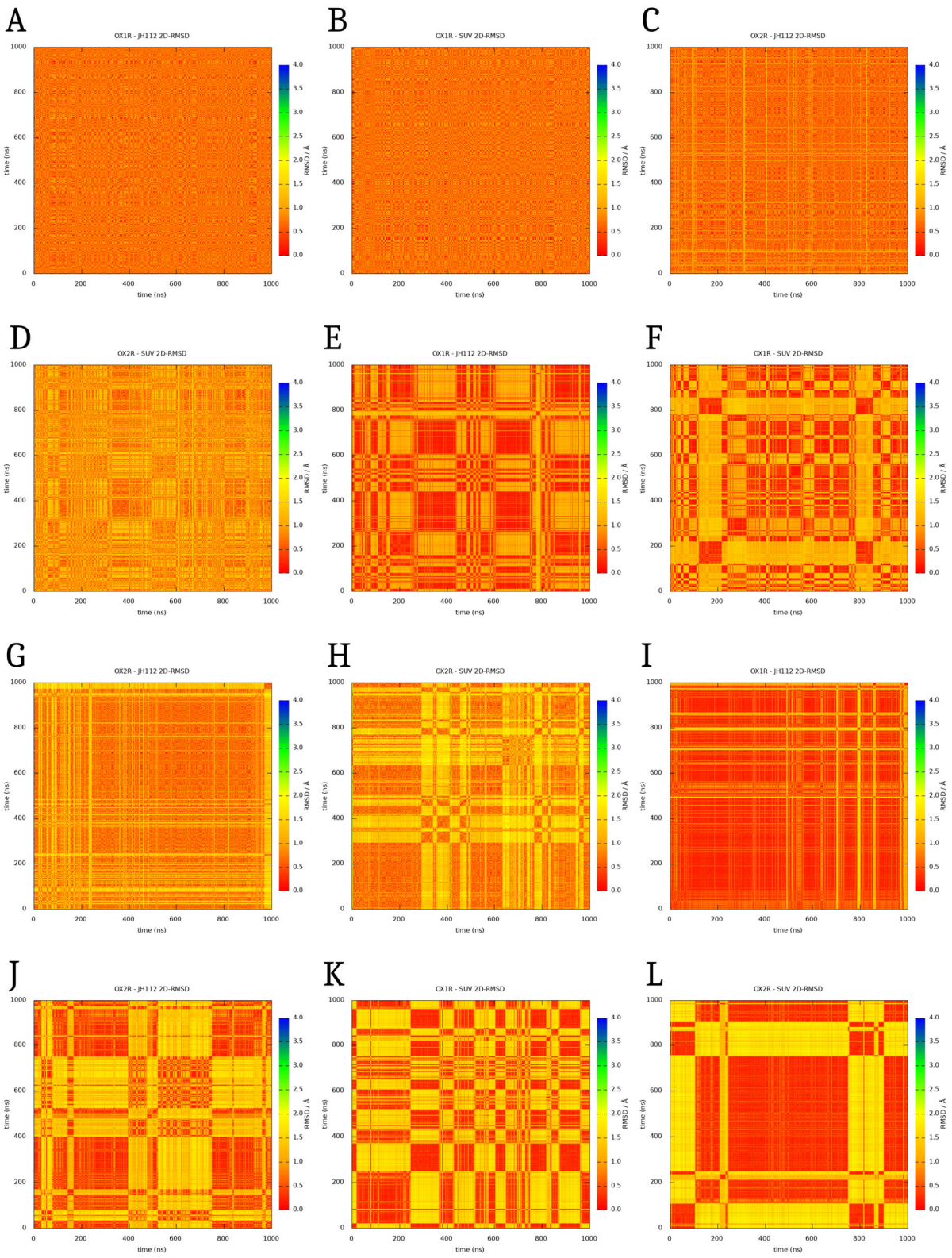


Figure S7. 2D-RMSD plots of $A^{3.33}$ from OX1R with **JH112** (**A**) and suvorexant (**B**), of $T^{3.33}$ from OX2R with **JH112** (**C**) and suvorexant (**D**), of $S^{2.61}$ from OX1R with **JH112** (**E**) and suvorexant (**F**), of $T^{2.61}$ from OX2R with **JH112** (**G**) and suvorexant (**H**) and of $N^{6.55}$ in OX1R with **JH112** (**I**), in OX2R with **JH112** (**J**), in OX1R with suvorexant (**K**) and OX2R with suvorexant (**L**).

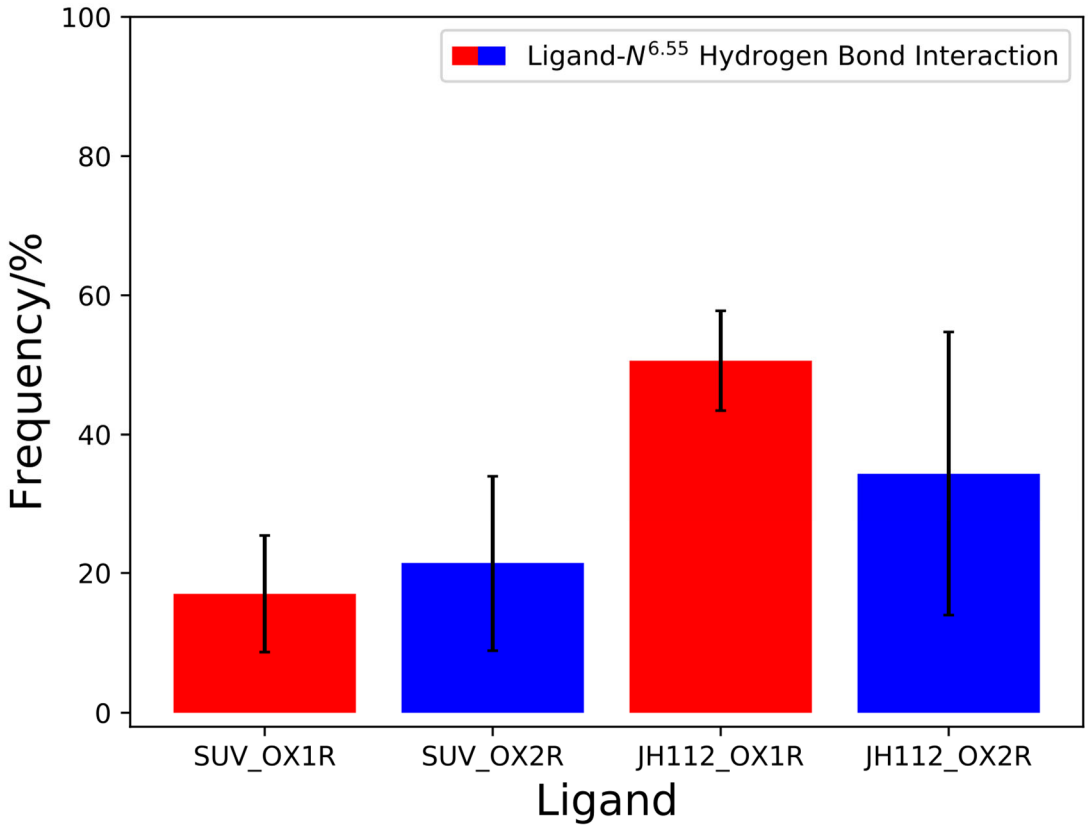


Figure S8. Hydrogen bond analysis of suvorexant in OX1R (red) and OX2R (blue) and of **JH112** in OX1R (red) and OX2R (blue).

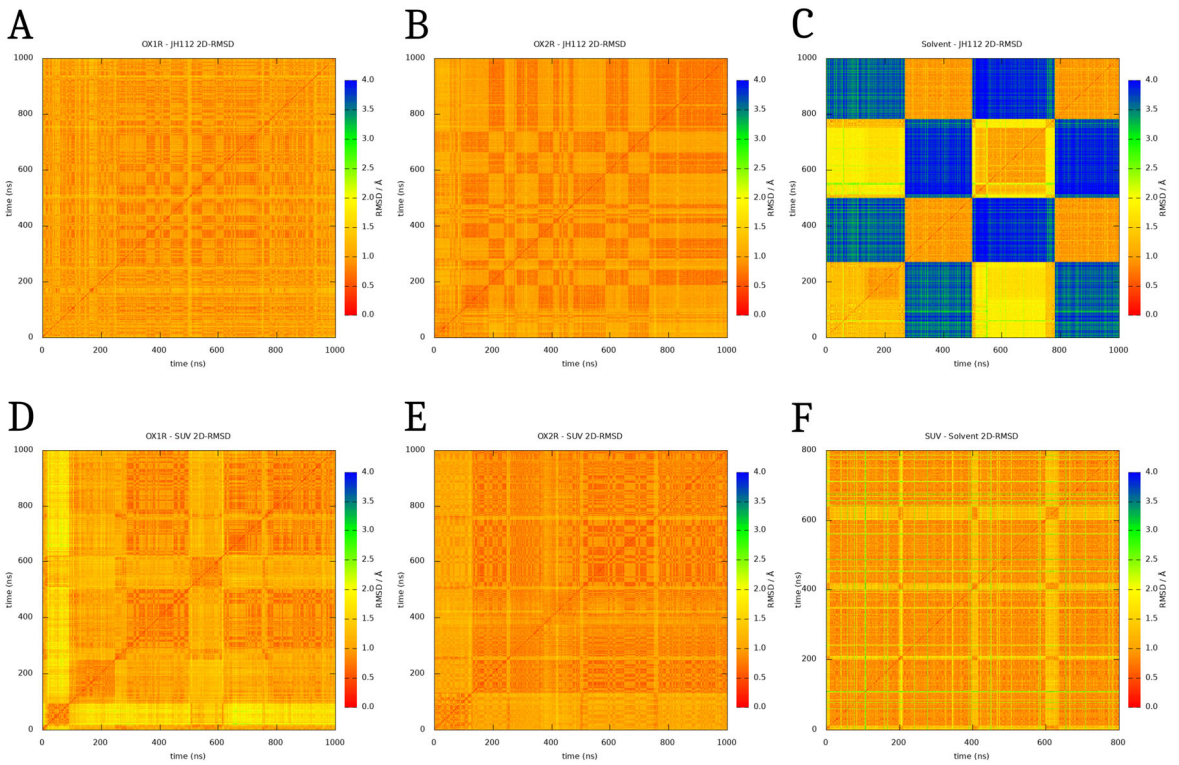


Figure S9. 2D-RMSD plots of **JH112** in OX1R (**A**), in OX2R (**B**) and in solution (**C**) and of suvorexant in OX1R (**D**), in OX2R (**E**) and in solution (**F**).

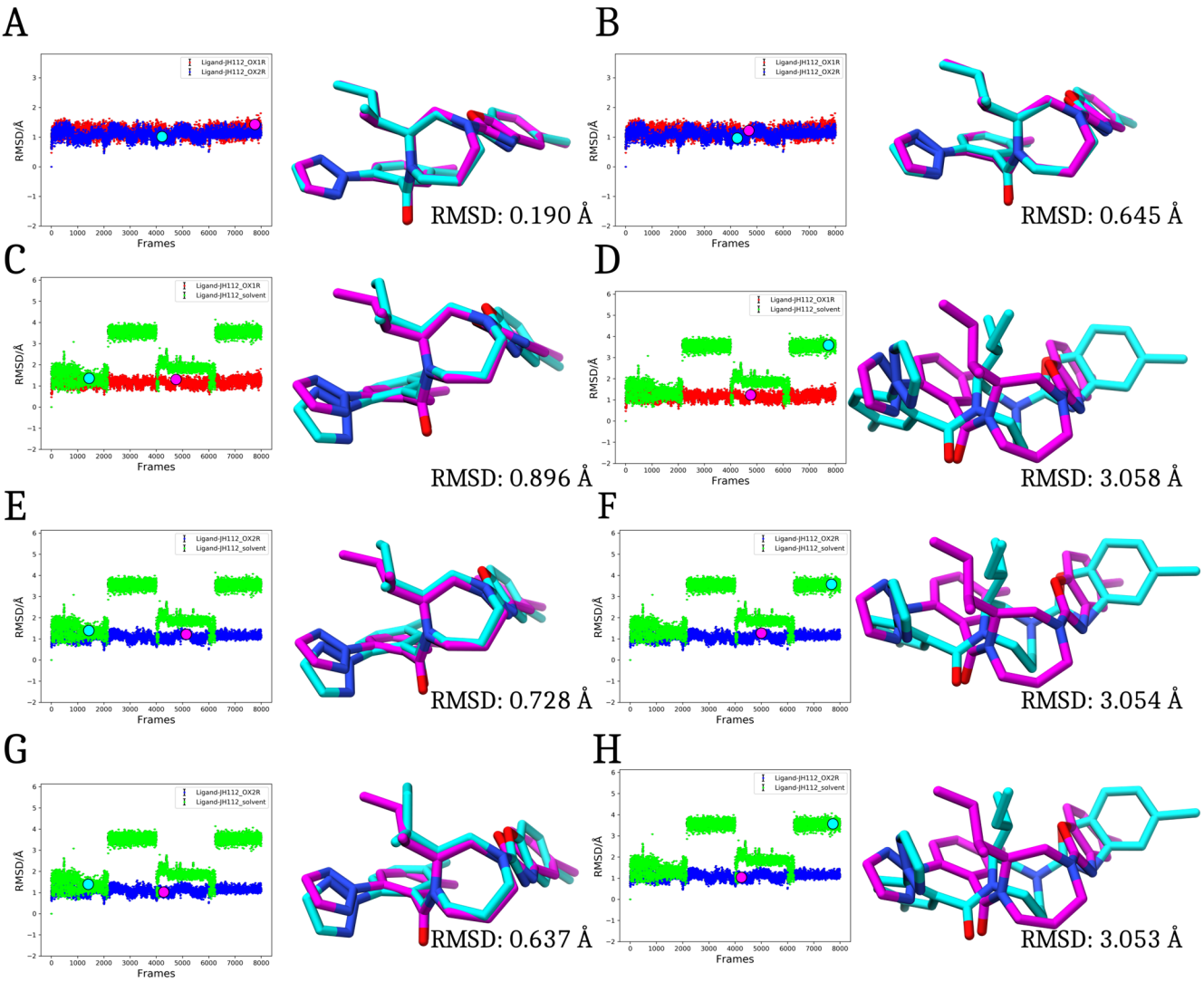


Figure S10. Comparison of conformations of JH112. (**A**) Comparison of the most frequent clusters of **JH112** in OX1R (red, magenta) and OX2R (blue, cyan), (**B**) comparison of the most frequent cluster of **JH112** in OX1R (red, magenta) and the second most frequent cluster of **JH112** in OX2R (blue, cyan), (**C**) comparison of the most frequent cluster of **JH112** in OX1R (red, magenta) and **JH112** in solution (green, cyan), (**D**) comparison of the most frequent cluster of **JH112** in OX1R (red, magenta) and the second most frequent cluster of **JH112** in solution (green, cyan), (**E**) comparison of the most frequent clusters of **JH112** in OX2R (blue, magenta) and **JH112** in solution (green, cyan), (**F**) comparison of the most frequent cluster of **JH112** in OX2R (blue, magenta) and the second most frequent cluster of **JH112** in solution (green, cyan), (**G**) comparison of the second most frequent cluster of **JH112** in OX2R (blue, magenta) and the most frequent cluster of **JH112** in solution (green, cyan) and (**H**) comparison of the second most frequent clusters of **JH112** in OX2R (blue, magenta) and **JH112** in solution (green, cyan).

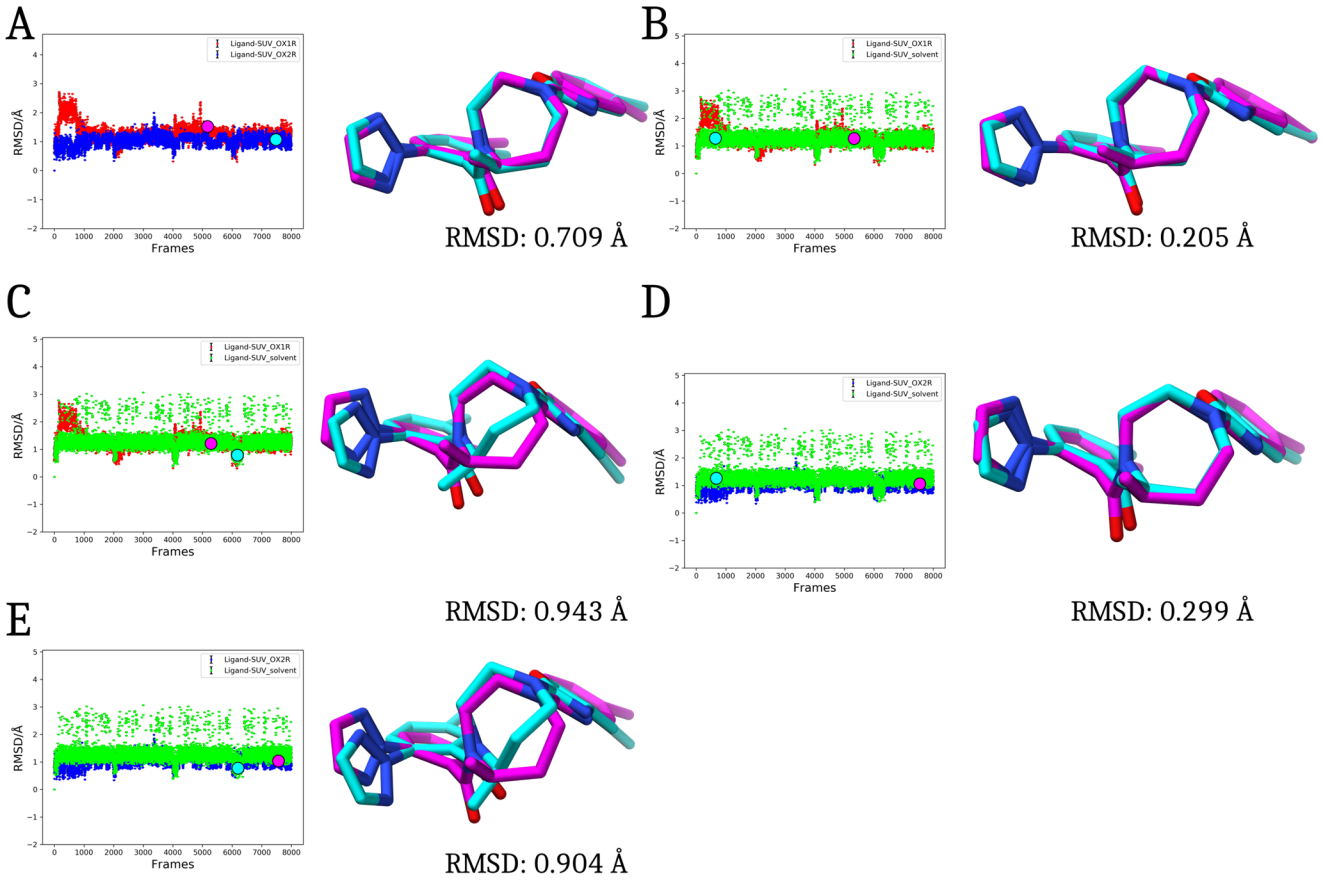


Figure S11. Comparison of conformations of suvorexant. (**A**) comparison of the most frequent clusters of suvorexant in OX1R (red, magenta) and suvorexant in OX2R (blue, cyan), (**B**) comparison of the most frequent clusters of suvorexant in OX1R (red, magenta) and suvorexant in solution (green, cyan), (**C**) comparison of the most frequent cluster of suvorexant in OX1R (red, magenta) and the second most frequent cluster of suvorexant in solution (green, cyan), (**D**) comparison of the most frequent clusters of suvorexant in OX2R (blue, magenta) and suvorexant in solution (green, cyan) and (**E**) comparison of the most frequent cluster of suvorexant in OX2R (blue, magenta) and the second most frequent cluster of suvorexant in solution (green, cyan).

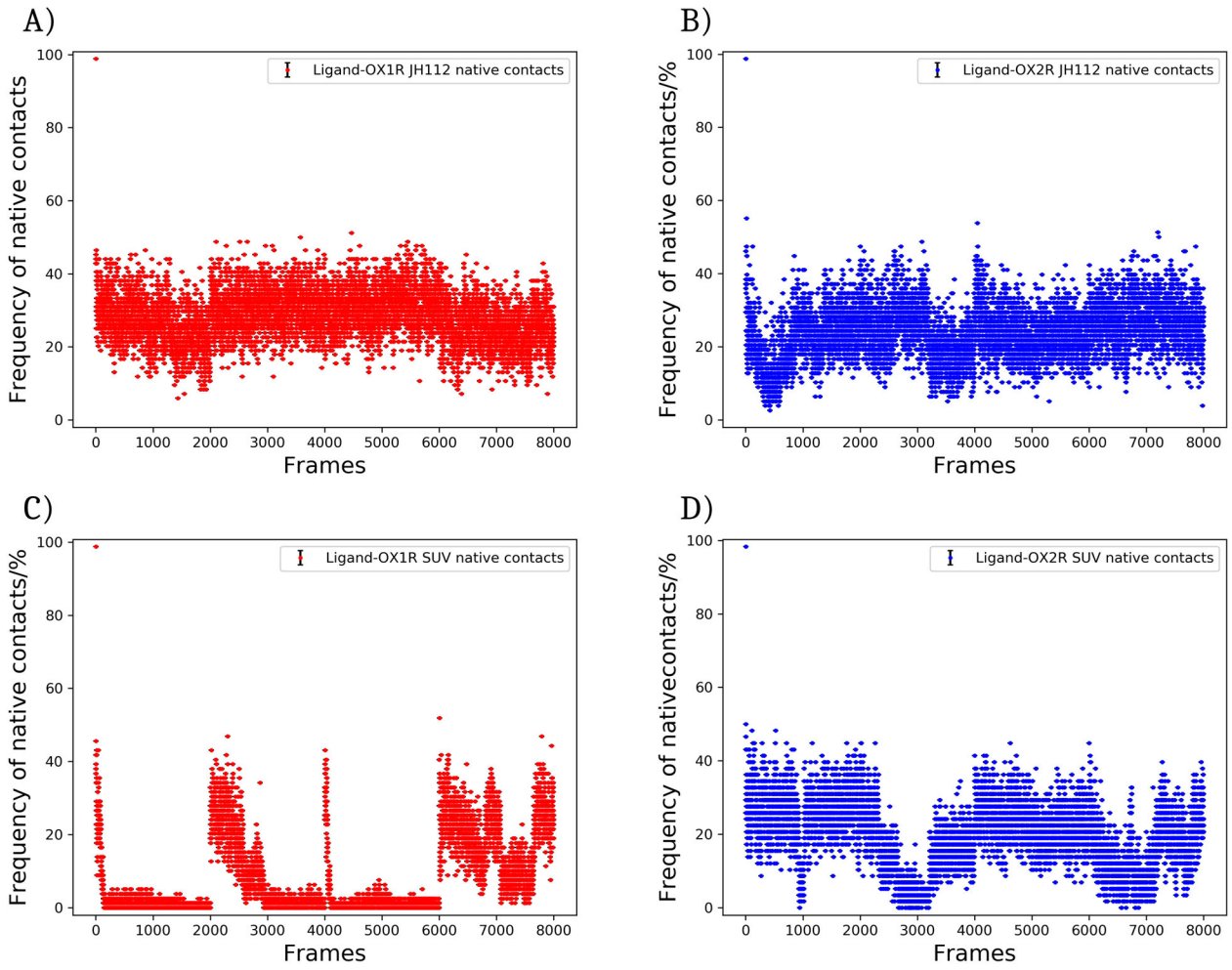


Figure S12. Native contact analysis of **JH112** in OX1R (**A**) and OX2R (**B**) and of suvorexant in OX1R (**C**) and OX2R (**D**).

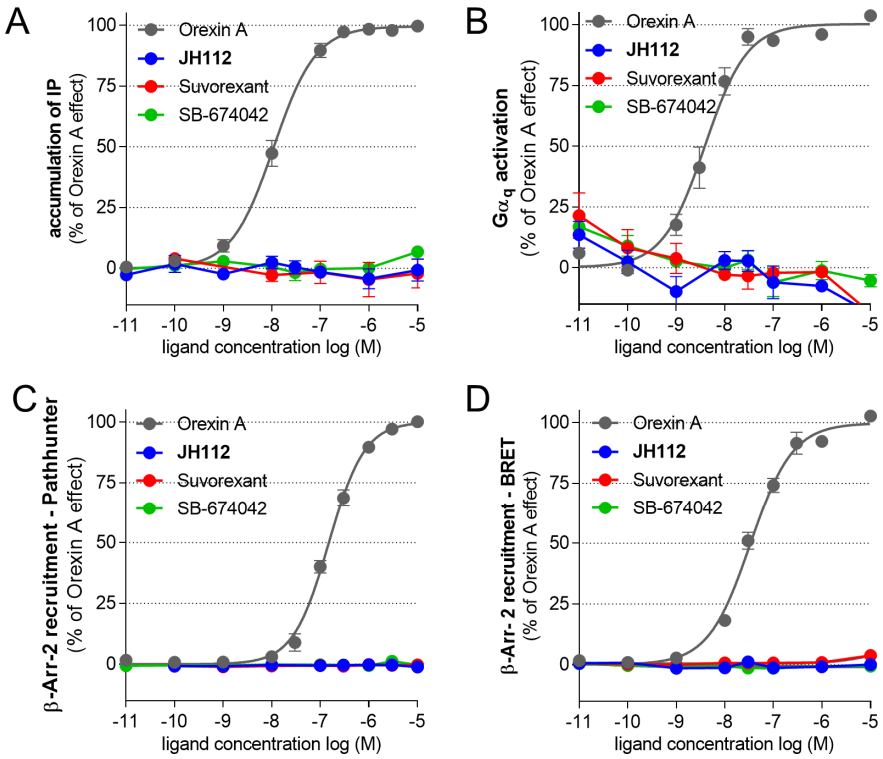


Figure S13. OX1R activation by orexin A compared to the effect of JH112, suvorexant and SB-674042. Activation of OX1R was monitored using different assay principles for canonical $G\alpha_q$ activation and recruitment of β-arrestin-2: (**A**) an IP_1 accumulation assay (Cisbio), (**B**) a $G\alpha_q$-RlucII/Gγ-GFP10 biosensor BRET assay, (**C**) a β-arrestin-2 recruitment assay based on enzyme fragment complementation (DiscoverX Pathhunter), (**D**) a BRET-assay for the recruitment of β-arrestin-2 to the plasma membrane. While orexin A leads to sigmoid concentration-response curves in each case, **JH112**, suvorexant and SB-674042 do not elicit detectable signals. Curves represent the mean ± SEM from 3-12 individual experiments, each performed in duplicates (**A** and **C**) or triplicates (**B** and **D**).

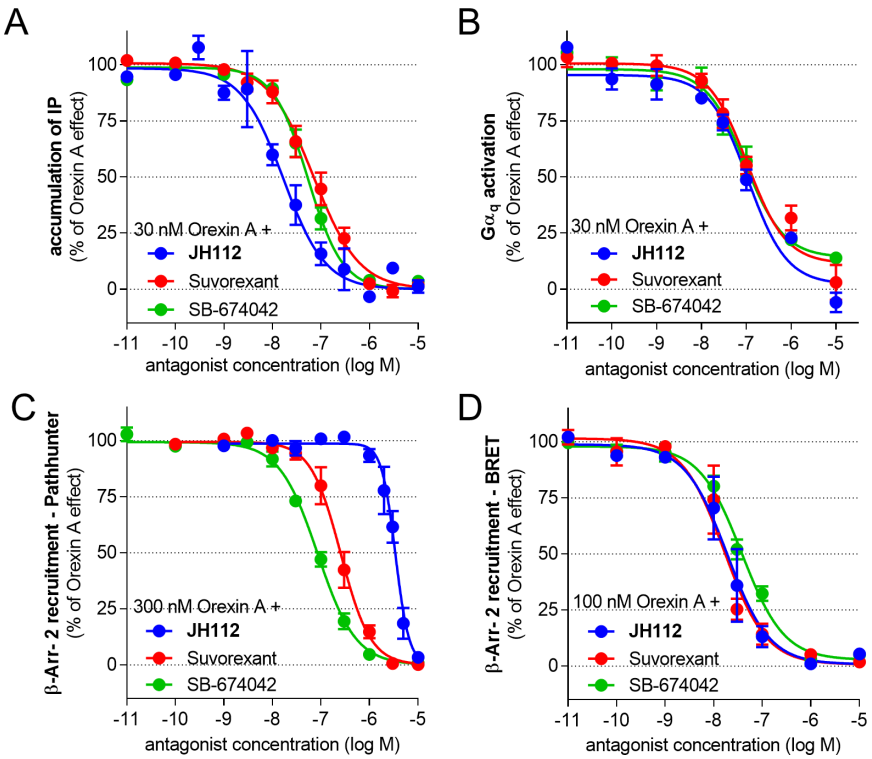


Figure S14. Antagonist-mediated inhibition of orexin A effect. Inhibition of the orexin A effect was determined in (**A**) an IP_1 accumulation assay (Cisbio), (**B**) a $G\alpha_q$-RlucII/$G\gamma$-GFP10 biosensor BRET assay, (**C**) a β-arrestin-2 recruitment assay based on enzyme fragment complementation (DiscoverX), (**D**) a BRET assay for the recruitment of β-arrestin-2 to the plasma membrane. In each case, cells are stimulated with an EC_{80} concentration of orexin A and concentration-response curves for variable concentrations of the antagonist are collected. Curves represent the mean ± SEM from 3-12 individual experiments, each performed in duplicates (**A** and **C**) or triplicates (**B** and **D**).

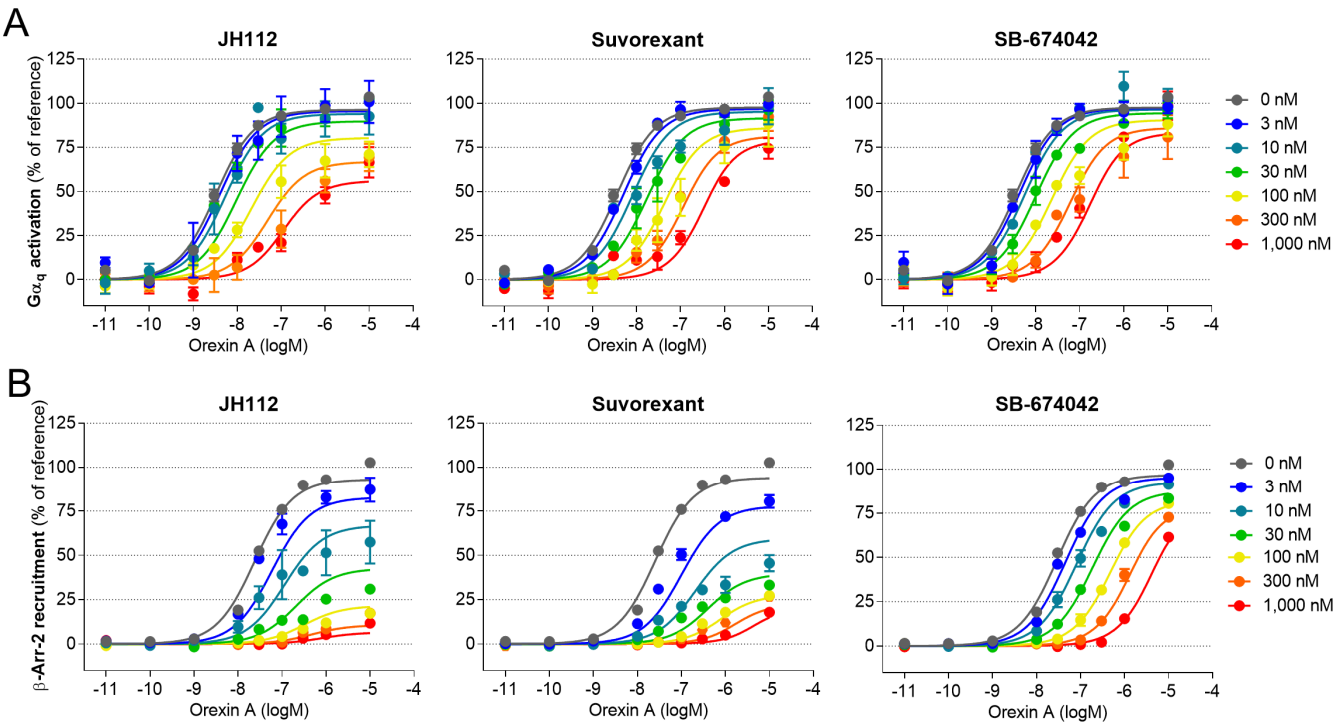


Figure S15. Hemi-equilibrium model analysis for the inhibition of OX1R activation. Effect of increasing concentrations of **JH112**, suvorexant and SB-674042 on orexin A stimulated (**A**) $G\alpha_q$ activation or (**B**) β-arrestin-2 recruitment in HEK293T cells transiently transfected with OX1R. Grouped data were fitted globally using an operational hemi-equilibrium model to derive ligand dissociation constants (as detailed in SI Appendix Methods).

Tables S1-S6

Table S1. Affinities of the test compounds for OX1R and OX2R determined by radioligand binding.

compound	OX1R [³H]SB-674042 K_i (nM)[a]	OX2R [³H]EMPA K_i (nM)[a]	selectivity OX2R-K_i / OX1R-K_i
Suvorexant	0.68 ± 0.17	1.3 ± 0.1	1.9
1	0.83 ± 0.18	2.0 ± 0.3	2.4
2 (**JH112**)	0.72 ± 0.08	54 ± 7	75
3	0.98 ± 0.31	4.1 ± 1.1	4.2
4	1.7 ± 0.3	28 ± 6	16
5	1.7 ± 0.6	58 ± 8	34
6	1.1 ± 0.2	57 ± 11	52
7	6.1 ± 0.7	240 ± 30	39
8	1.2 ± 0.2	31 ± 4	26
9	120 ± 30	1,500 ± 200	13
10	18 ± 3	49 ± 6	2.7
11	71 ± 14	520 ± 50	7.3
12	1.8 ± 0.4	51 ± 9	28
13	27 ± 4	360 ± 60	13
14	67 ± 10	970 ± 80	14
15	4.5 ± 1.2	5.0 ± 0.1	1.1
16	25 ± 7	190 ± 50	7.6
17	2.5 ± 0.2	3.2 ± 0.3	1.3
18	9,800 ± 3,000	9,100 ± 2,100	0.93
19	3,500 ± 900	6,500 ± 700	1.9
20	83 ± 37	210 ± 90	3.5

[a] K_i values are the mean ± SEM from three to seven separate experiments.

Table S2. Assessment of association and dissociation kinetics for suvorexant and **JH112** derived from kinetic binding experiments with membranes from HEK293T cells expressing OX2R and the radioligand [³H]EMPA.

ligand	association constant (k_{on}, $min^{-1} \cdot M^{-1}$)			dissociation constant (k_{off}, min^{-1})			half-life ($T_{1/2}$, min)
	mean	SEM	n	mean	SEM	n	
[³H]EMPA	17.64×10^6	3.123×10^6	8	0.2124	0.0112	5	2.3
suvorexant	0.485×10^6	0.062×10^6	5	0.0334	0.0065	5	20.7
JH112	2.543×10^6	0.804×10^6	5	0.5576	0.1835	5	1.2

Table S3. GPCR targets and experimental conditions for the investigation of receptor selectivity of **JH112** and suvorexant in radioligand competition experiments.

GPCR[a]	gene	radioligand		homogenate		
		radioligand[c]	conc RL [nM]	K_D [nM]	B_{max} [fmol/mg]	protein [µg/well]
α_{1A}	ADRA1A	[^{3}H]prazosin	0.20	0.080	1400	6
α_{2A}	ADRA2A	[^{3}H]RX821002	0.30	0.80	3000	4
β_1	ADRB1	[^{3}H]CGP12177	0.30	0.25	4000	3
β_2	ADRB2	[^{3}H]CGP12177	0.30	0.060	4000	3
D1	DRD1	[^{3}H]SCH23390	0.40	0.35	3000	4
$D2_{long}$[b]	DRD2	[^{3}H]spiperone	0.20	0.10	900	4
D3[b]	DRD3	[^{3}H]spiperone	0.30	0.25	3500	2
D4[b]	DRD4	[^{3}H]spiperone	0.40	0.40	1800	6
D5	DRD5	[^{3}H]SCH23390	0.50	0.50	1000	14
5-HT_{1A}	HTR1A	[^{3}H]WAY100635	0.20	0.10	3000	2
5-HT_{2A}	HTR2A	[^{3}H]ketanserin	0.30	0.35	3400	10
5-HT_6	HTR6	[^{3}H]LSD	0.70	2.10	2900	6
δOR	OPRD1	[^{3}H]diprenorphine	0.30	0.20	1300	10
κOR	OPRK1	[^{3}H]diprenorphine	0.30	0.13	6500	2
µOR	OPRM1	[^{3}H]diprenorphine	0.30	0.095	4000	4
M1	CHRM1	[^{3}H]NMS	0.30	0.075	5600	2
M2	CHRM2	[^{3}H]NMS	0.50	0.50	450	14
M3	CHRM3	[^{3}H]NMS	0.30	0.17	4700	2
NTS1	NTSR1	[^{3}H]NT8-13[d]	0.70	1.50	6500	2
NTS2	NTSR2	[^{3}H]NT8-13[d]	0.70	1.50	400	10

[a] Membranes were derived from HEK 293T cells, transiently transfected with the cDNAs coding for the individual receptors. [b] Membranes were derived from CHO cells stably expressing the different D2-like receptors. [c] All radioligands were purchased from standard suppliers for radiochemicals. [d] [^{3}H]NT8-13 was available as custom synthesis (GE Healthcare, Freiburg, Germany).

Table S4. Data collection and refinement statistics

	hOX1R-PGS/**JH112**[a]
Data collection	
Space group	P212121
Cell dimensions	
a, *b*, *c* (Å)	64.7, 66.3, 182.5
Resolution (Å)	44.9 (3.5)[b]
R_{sym} or R_{merge}[c]	0.20 (N/A)
$I/\sigma I$	5.6 (0.95)
Completeness (%)	89.2 (94.4)
Redundancy	2.7 (2.6)
$CC_{1/2}$ in the highest shell	0.29
Refinement	
Resolution (Å)	44.9-3.5 (3.85-3.50)
No. reflections	8146
R_{work}/ R_{free}	0.24/0.27 (0.29/0.30)
No. atoms	
Protein	4031
Ligand	35
Other (Lipid, ion and water)	48
B-factors	
Protein	64.0
Fusion protein	64.0
Ligand	55.9
Other (Lipid, ion and water)	69.3
R.m.s deviations	
Bond lengths (Å)	0.004
Bond angles (°)	0.679
Ramachandran plot statistics (%)	
Favored regions	95.6
Allowed regions	4.4
Disallowed regions	0

[a] Diffraction data from 8 crystals were merged into a complete data set. [b] High-resolution shell is shown in parenthesis. [c] R_{merge} higher than 1 is statistically meaningless, therefore Scalepack does not report it.

Table S5. Assessment of kinetic parameters for ligand-mediated antagonism of orexin A stimulated OX1R activation in the IPOne, $G\alpha_q$ activation and β-arrestin-2 recruitment assays according to an operational hemi-equilibrium model for competitive antagonism (6-8).

	IPOne[a]		$G\alpha_q$ activation			β-arrestin-2 recruitment (BRET)[b,c]		
	Suvorexant	**JH112**	SB-674042	Suvorexant	**JH112**	SB-674042	Suvorexant	**JH112**
LogK_A	-6.25 ± 0.14	-5.51 ± 0.13	-6.83 ± 0.15	-6.83 ± 0.14	-7.07 ± 0.10	-6.05 ± 0.07	-6.37 ± 0.05	-6.32 ± 0.02
LogK_B	-7.75 ± 0.05	-7.70 ± 0.06	-7.78 ± 0.06	-8.10 ± 0.05	-7.83 ± 0.07	-8.29 ± 0.03	-9.07 ± 0.04	-8.83 ± 0.03
Log_τ	1.71 ± 0.13	2.41 ± 0.13	1.60 ± 0.14	1.60 ± 0.13	1.41 ± 0.09	1.47 ± 0.06	1.19 ± 0.05	1.12 ± 0.05
E_m (%) [d]	= 100	= 100	= 100	= 100	= 100	= 100	= 100	= 100
k_{off} (min^{-1})	0.0014 ± 0.00034	0.000029 ± 0.00016	0.00097 ± 0.00051	0.00076 ± 0.00034	0.00031 ± 0.00011	0.00098 ± 0.00025	0.00014 ± 0.00003	= 0
$T_{1/2}$ (h) [e]	8	400	12	15	37	12	89	∞

Data represent parameter estimates ± fitted standard errors derived from the grouped analysis of 3 to 19 independent experiments. [a] Data for SB-674042 in the IPOne assay was not analyzed, as equilibrium was observed under the experimental conditions used, suggesting a short dissociation half-life. [b] For **JH112**, comparison of curve fits with k_{off} unconstrained and constrained to zero indicated preference for the latter, suggesting pseudo-irreversible behavior under these experimental conditions. [c] β-arrestin-2 recruitment determined by enzyme fragment complementation resulted in complete depression of the orexin A effect, preventing hemi-equilibrium analysis without the use of constraints. [d] The maximal system response was constrained to 100% consistent with orexin A being a full agonist. [e] Dissociation half-life was calculated as $\ln 2/k_{off}$.

Table S6. Assessment of association and dissociation kinetics for suvorexant and **JH112** derived from kinetic binding experiments with membranes from HEK293T cells expressing OX1R and the radioligand [³H]SB-674042.

ligand	association constant (k_{on}, $min^{-1} \cdot M^{-1}$)			dissociation constant (k_{off}, min^{-1})			half-life ($T_{1/2}$, min)	kinetic affinity (K_D, nM)
	mean	SEM	n	mean	SEM	n		
[³H]SB-674042	3.337×10^7	0.417×10^7	10	0.1383	0.0094	5	5.1	4.14
suvorexant	1.850×10^7	0.191×10^7	4	0.02752	0.00426	4	25	1.49
JH112	1.957×10^7	0.228×10^7	5	0.01915	0.00367	5	36	0.98

Supplementary Materials and Methods

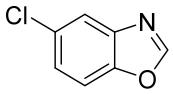
General materials and methods for organic synthesis

Dry solvents and reagents were of commercial quality and were used as purchased. ESI-TOF high mass accuracy and resolution experiments were performed on an AB Sciex Triple TOF660 Sciex, on a Bruker maXis MS or a Bruker timsTOF Pro. NMR spectra were obtained on a Bruker Avance 400 (^{1}H at 400 MHz, ^{13}C (DEPTQ) at 101 MHz) or a Bruker Avance 600 (^{1}H at 600 MHz, ^{13}C (DEPTQ) at 151 MHz) spectrometer at 298K using the solvents indicated. Chemical shifts are reported relative to TMS or to the residual solvent peak. Specific optical rotation measurement was performed in a JASCO P-2000 polarimeter in methanolic solutions. Path length: 100 mm, volume: 1.0 mL. IR spectra were performed on a Jasco FT/IR 4100 spectrometer (film on a NaCl crystal). Purification by flash chromatography was performed using Silica Gel 60 (40-63 µm mesh) from Merck as stationary phase; TLC analyses were performed using Merck 60 F254 aluminum sheets and the spots were visualized under UV light (254 nm) and with reagents such as $KMnO_4$ or ninhydrin solutions. Purification by preparative RP-HPLC was performed on AGILENT 1260 Preparative Series equipped with a VWD detector (230 nm; 254 nm) using a Zorbax Eclipse XDB-C8 21.2 x 150 mm column with 5 µm particles [C8], flow rate 10 mL/min, employing solvent systems as specified below. Analytical HPLC/MS was performed on a Thermo Scientific Dionex Ultimate 3000 HPLC system using DAD detection (230 nm; 254 nm) equipped with either a Kinetex 2.6u mesh C8 100A (2.1 x 75 mm, 2.6 µm) HPLC column or a Zorbax Eclipse XDB-C8 (4.6 x 150 mm, 5 µm) HPLC column, using mass detection on a BRUKER amaZon SL mass spectrometer with ESI or APCI ionization source. HPLC purity analyses were performed with an Agilent 1260 infinity binary gradient system using UV detection (λ = 220, 254 and 280 nm) in combination with ChemStation software. A Zorbax Eclipse XDB-C8 (4.6 mm x 150 mm, 5 µm) column was used with a flow rate of 0.5 mL/min in reversed phase mode (gradient 1: 5% for 3 min, 5% to 95% in 15 min, 95% for 6 min, 95% to 5% in 3 min, 5% for 3 min; eluent system 1: methanol / H_2O + 0.1% HCOOH; eluent system 2: acetonitrile / H_2O+0.1% HCOOH; eluent system 3: methanol / H_2O + 0.1% CF_3CO_2H; eluent system 4: acetonitrile / H_2O + 0.1% CF_3CO_2H); (gradient 2: 5% for 1 min, 5% to 95% in 6 min, 95% for 5 min, 95% to 5% in 2 min, 5% for 1 min; eluent system 5: methanol / H_2O + 0.1% CF_3CO_2H). Chiral HPLC analyses were performed with an Agilent 1100 series HPLC system using UV detection (λ = 220, 254 and 280 nm) in combination with ChemStation software. A chiralpak IC column (4.6 mm x 250 mm, 5 µm) was used as stationary phase with a flow rate of 1.0 mL/min in normal phase mode (isocratic eluent system 6: n-hexane / ethanol + 0.1% ethylene diamine = 9 : 1 for 60 min).

General experimental procedures

Unless otherwise noted, reactions were performed under nitrogen atmosphere. All reactions were carried out using a magnetic stirrer with optional aluminum heating block or ice bath for sealed microwave vials and oil or ice bath for round bottom flasks, respectively. Solvents were vaporized by a rotation evaporator with a membrane vacuum pump. Products purified by preparative HPLC using aqueous solvents were lyophilized.

Detailed synthesis procedures
5-Chlorobenzoxazole (21)



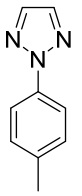
2-Amino-4-chlorophenol (4.07 g, 28.4 mmol) was dissolved in triethyl orthoformate (65 mL, 0.39 mol) and stirred at 160 °C for 1.5 h. The reaction mixture was slowly cooled to ambient temperature and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 2 to 5% ethyl acetate in *n*-hexane to obtain the pure product as pale yellow crystals (2.94 g, 19.2 mmol, 68%) (1).

ESI-MS: *m/z* 153.9 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 7.79 (d, *J* = 2.0 Hz, 1H), 7.52 (d, *J* = 8.7 Hz, 1H), 7.38 (dd, *J* = 8.7, 2.0 Hz, 1H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 153.7, 148.6, 141.2, 130.2, 126.1, 120.6, 111.8.

2-(*p*-Tolyl)-2*H*-1,2,3-triazole (22)



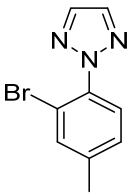
Tris(dibenzylideneacetone)dipalladium(0) (0.12 g, 0.13 mmol) and Me_4*t*BuXPhos (0.15 g, 0.31 mmol) were dissolved in a dried flask in toluene (dry, 16 mL) and stirred at 120 °C for 3 min until the color turned from purple to dark brown. In a second dried flask potassium phosphate (7.44 g, 35.1 mmol) was suspended in toluene (dry, 18 mL) and toluene solutions (dry, 3 mL respectively) of 2*H*-1,2,3-triazole (1.22 mL, 21.1 mmol) and 1-bromo-4-methylbenzene (2.16 mL, 17.5 mmol) were added by syringe. After addition of the hot catalyst solution, the reaction mixture was stirred at 120 °C for 16 h. The reaction mixture was slowly cooled to ambient temperature and diluted with ethyl acetate (200 mL). The organic layer was washed with brine (3 x 50 mL), dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 5 to 20% ethyl acetate in isohexane to obtain the product as pale yellow crystals (2.07 g, 13.0 mmol, 74%) (2).

ESI-MS: *m/z* 160.0 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$) δ 8.00 – 7.91 (m, 2H), 7.79 (s, 2H), 7.31 – 7.27 (m, 2H), 2.40 (s, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 136.7, 136.4, 134.2, 128.8, 117.8, 20.0.

2-(2-Bromo-4-methylphenyl)-2*H*-1,2,3-triazole (23)



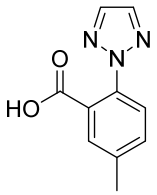
22 (2.00 g, 12.6 mmol), *N*-bromosuccinimide (2.46 g, 13.8 mmol) and $Pd(OAc)_2$ (0.14 g, 0.63 mmol) were dissolved in acetic acid (50 mL) and the solution was stirred at 120 °C for 60 h. After cooling to ambient temperature, the reaction mixture was diluted with EtOAc (200 mL) and the pH was adjusted to pH = 8 using aq. NaOH (2 N). The organic layer was washed with aq. NaOH (2 N, 2 x 50 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 3 to 10% ethyl acetate in isohexane to obtain the product as white crystals (2.44 g, 10.2 mmol, 82%) (2).

ESI-MS: *m/z* 237.9 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.92, 7.87 (s, 2H), 7.57 (dd, *J* = 1.6, 0.7 Hz), 7.50 (d, *J* = 0.7 Hz, 1H), 7.42 (d, *J* = 8.1 Hz, 1H), 7.24 (ddt, *J* = 8.1, 1.3, 0.7 Hz, 1H), 2.42 (s, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 140.2, 134.6, 134.4, 133.3, 131.8, 127.7, 126.7, 121.9, 117.5, 19.9.

5-Methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoic acid (24)



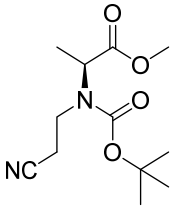
23 (2.37 g, 9.95 mmol) and CuCN (1.78 g, 19.9 mmol) were dissolved in DMF (40 mL) in a dried flask and stirred at 120°C for 16 h. The reaction mixture was slowly cooled to ambient temperature and diluted with water (200 mL), resulting in the formation of a white precipitate. The precipitate was extracted with DCM (10 x 50 mL) and the combined organic layers were washed with brine (100 mL), dried over sodium sulfate and concentrated under reduced pressure to obtain the crude nitrile intermediate. The intermediate was dissolved in ethanol (150 mL) and aq. NaOH (6 N, 150 mL) was added. The reaction mixture was stirred at 80 °C for 16 h. After cooling to ambient temperature the reaction mixture was acidified to a pH value of pH = 5 using aq. HCl (6 N) indicated by a color change from light blue to light green. The product was extracted with DCM (15 x 40 mL). The combined organic layers were washed with brine (100 mL), dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 3 to 6% methanol in dichloromethane with 0.5% acetic acid to obtain the product as pale yellow crystals (1.40 g, 6.87 mmol, 69%) (2).

ESI-MS: *m/z* 225.9 $[M+Na]^+$

^{1}H NMR: (400 MHz, dmso-d_6) δ 12.98 (s, 1H), 8.04 (s, 2H), 7.62 (d, *J* = 8.1 Hz, 1H), 7.58 (dd, *J* = 1.4, 0.5 Hz, 1H), 7.50 (ddd, *J* = 8.7, 1.9, 0.5 Hz, 1H), 2.42 (s, 3H).

^{13}C NMR: (101 MHz, dmso-d_6) δ 167.6, 138.7, 136.0, 135.4, 132.0, 129.8, 128.3, 124.3, 20.4.

Methyl *N*-(*tert*-butoxycarbonyl)-*N*-(2-cyanoethyl)-*L*-alaninate (26a)



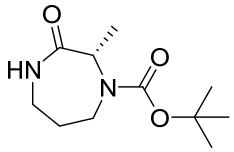
Methyl *L*-alaninate hydrochloride (7.17 g, 51.38 mmol) was dissolved in dry methanol (50 mL) in a dried flask. DIPEA (13.34 mL, 77.07 mmol) was added and the reaction mixture was stirred at ambient temperature for 10 minutes. Acrylonitrile (6.73 mL, 0.10 mol) was added dropwise over 30 minutes and the reaction mixture was stirred under reflux overnight. After cooling to ambient temperature, a solution of di-*tert*-butyldicarbonat (22.43 g, 0.10 mol) in methanol (30 mL) was added and the reaction mixture was stirred under reflux overnight. The solvent was evaporated. The residue was taken up in ethyl acetate and washed with water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 15% to 30% ethyl acetate in isohexane. The product was obtained as pale yellow oil (11.29 g, 44.06 mmol, 86%).

ESI-MS: *m/z* 278.9 $[M+Na]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 4.64, 4.23 (q, *J* = 7.2, 7.1 Hz, 1H), 3.74 (s, 3H), 3.70 – 3.51, 3.51 – 3.38 (m, 2H), 2.83 – 2.57 (m, 2H), 1.69 – 1.36 (m, 12H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 172.7, 172.4, 154.8, 154.6, 118.4, 118.0, 81.6, 81.4, 56.4, 54.6, 52.3, 43.2, 41.5, 28.3, 28.2, 18.2, 17.5, 16.2, 15.7.

Tert-butyl (_S_)-2-methyl-3-oxo-1,4-diazepane-1-carboxylate (27a)



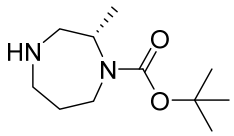
26a (11.28 g, 44.02 mmol) was dissolved in ethanol (50 mL) and chloroform (10 mL). Platinum dioxide (1.10 g, 4.84 mmol) was added and the reaction mixture was stirred at ambient temperature under hydrogen atmosphere for four days. The reaction mixture was filtered over celite and the volatiles were evaporated. The residue was dissolved in THF (20 mL) and methanol (60 mL). A 4 M aqueous solution of lithium hydroxide monohydrate (16.51 mL, 66.03 mmol) was added and the reaction mixture was stirred at ambient temperature for 50 h. The solvents were evaporated and the residue was taken up in a mixture of toluene and methanol (1 : 4, 20 mL). The volatiles were evaporated and the residue was dissolved in DMF (150 mL). DIPEA (15.17 mL, 87.70 mmol) and subsequently HATU (25.01 g, 65.77 mmol) were added to the solution. The reaction mixture was stirred at ambient temperature for 17 h. The reaction was quenched by addition of saturated aqueous solution of ammonium chloride (100 mL). The product was extracted with ethyl acetate and the combined organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 30% to 60% ethyl acetate in isohexane. The product was obtained as yellow oil (8.46 g, 37.06 mmol, 85%).

ESI-MS: *m/z* 228.9 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 6.50 – 6.06 (m, 1H), 5.05 – 4.55 (m, 1H), 4.02 – 3.73, 3.45 – 3.30 (m, 2H), 3.24 – 3.03 (m, 2H), 2.02 – 1.89, 1.86 – 1.70 (m, 2H), 1.48 (s, 9H), 1.45 – 1.38 (m, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 175.5, 175.3, 154.7, 154.5, 80.5, 80.3, 56.2, 55.6, 46.9, 46.8, 39.6, 38.8, 28.4, 27.3, 27.1, 16.3.

***Tert*-butyl (*S*)-2-methyl-1,4-diazepane-1-carboxylate (28a)**



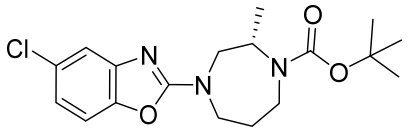
27a (2.14 g, 9.37 mmol) was dissolved in a dried flask in THF (50 mL). A 1 M solution of a borane-THF-complex in THF (45.0 mL, 45.0 mmol) was added dropwise under ice cooling. The reaction mixture was stirred under reflux at 75 °C for 19 h. After cooling to ambient temperature, the reaction was quenched by dropwise addition of methanol (10 mL) under ice cooling. The reaction mixture was diluted with ethyl acetate (150 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 70 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product purified by flash column chromatography applying a gradient of 1% to 5% methanol in dichloromethane. The product was obtained as pale yellow oil (0.29 g, 1.37 mmol, 15%).

ESI-MS: *m/z* 215.0 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 4.45 – 4.15, 4.08 – 3.98 (m, 1H), 3.70 – 3.62 (m, 1H), 3.55 – 3.45 (m, 1H), 3.45 – 3.35 (m, 1H), 2.98 – 2.81 (m, 1H), 2.71 – 2.52 (m, 2H), 1.91 – 1.74 (m, 2H), 1.48 (s, 9H), 1.06 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 154.8, 153.5, 80.6, 80.5, 61.0, 60.9, 58.0, 57.9, 49.9, 48.8, 39.4, 38.6, 29.3, 29.2, 28.4, 28.4, 16.9, 16.6.

***Tert*-butyl (*S*)-4-(5-chlorobenzoxazol-2-yl)-2-methyl-1,4-diazepane-1-carboxylate (29a)**



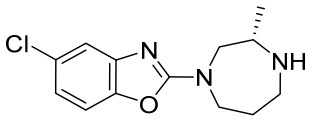
21 (0.17 g, 1.12 mmol), **28a** (0.25 g, 1.18 mmol) and copper(II) acetate hydrate (0.22 g, 1.12 mmol) were dissolved in acetonitrile (40 mL). Concentrated acetic acid (0.13 mL, 2.24 mmol) was added and the reaction mixture was stirred under air at 80 °C for 24 h. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate (100 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 50 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 2% to 5% methanol in dichloromethane. The product was obtained as brown oil (0.14 g, 0.39 mmol, 35%).

ESI-MS: *m/z* 366.1 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 7.30 (d, *J* = 8.2 Hz, 1H), 7.14 (d, *J* = 8.4 Hz, 1H), 6.96 (d, *J* = 8.3 Hz, 1H), 4.84 – 4.59, 4.33 – 4.24, 4.23 – 4.08, 4.06 – 3.89, 3.89 – 3.75, 3.70 – 3.60, 3.33 – 3.06, 3.06 – 2.85 (m, 7H), 2.13 – 1.96, 1.83 – 1.65 (m, 2H), 1.34, 1.22 (s, 9H), 1.18 – 1.07 (m, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 162.2, 162.2, 155.1, 155.0, 147.4, 129.4, 129.3, 120.3, 116.1, 109.3, 109.1, 79.74, 54.7, 54.34, 50.7, 50.4, 48.7, 48.5, 40.5, 40.1, 28.3, 28.1, 27.8, 16.5, 16.3.

(*S*)-5-chloro-2-(3-methyl-1,4-diazepan-1-yl)benzoxazole (30a)



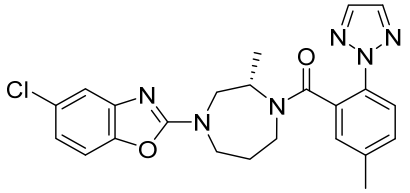
29a (0.14 g, 0.38 mmol) was dissolved in dichloromethane (3 mL). Trifluoroacetic acid (0.3 mL) was added and the reaction mixture was stirred at ambient temperature for 21 h. The reaction mixture was basified to pH 11 using 1 M aqueous NaOH solution. The product was extracted with dichloromethane (3x). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure to obtain the pure product as brown oil (90.4 mg, 0.34 mmol, 89%).

ESI-MS: *m/z* 265.9 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.30 (d, *J* = 2.1 Hz, 1H), 7.14 (d, *J* = 8.4 Hz, 1H), 6.95 (dd, *J* = 8.4, 2.1 Hz, 1H), 4.10 – 3.89 (m, 2H), 3.65 (ddd, *J* = 13.9, 7.5, 5.8 Hz, 1H), 3.19 (ddd, *J* = 15.7, 6.5, 2.9 Hz, 1H), 2.69 (ddd, *J* = 14.1, 10.9, 3.7 Hz, 1H), 2.12 – 2.02 (m, 2H), 1.99 – 1.85 (m, 3H), 1.19 (d, *J* = 6.0 Hz, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 163.1, 147.5, 144.9, 129.2, 120.0, 116.1, 109.1, 61.8, 56.9, 55.0, 47.3, 46.6, 29.7, 29.3, 19.5, 14.1.

(*S*)-[4-(5-chlorobenzoxazol-2-yl)-2-methyl-1,4-diazepan-1-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone (1)



30a (86.5 mg, 0.33 mmol), **24** (79.4 mg, 0.39 mmol) and HATU (0.25 g, 0.65 mmol) were dissolved in dimethylformamide (dry, 15 mL). DIPEA (0.28 mL, 1.63 mmol) was added and the reaction mixture was stirred at ambient temperature for 8 h. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution (3x) and brine (1x). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified using a preparative HPLC (gradient of 5% to 95% methanol in water with 0.1% formic acid). The product was obtained as white powder after lyophilization of the product containing fractions (97.5 mg, 0.22 mmol, 66%).

ESI-MS: *m/z* 451.1 $[M+H]^+$

HR-ESI-MS: *m/z* $[M+H]^+$ calcd. 451.1644 for $C_{23}H_{23}ClN_6O_2$, found 451.1644

HPLC: eluent system 2: λ = 254 nm, t_R = 18.7 and 19.0 min, rotamers, purity: 97.0%

eluent system 3: λ = 254 nm, t_R = 21.4 min, purity: 99.0%

eluent system 4: λ = 254 nm, t_R = 18.6 and 18.9 min, rotamers, purity: 99.3%

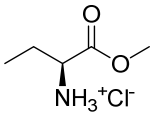
$[\alpha]_D^{24}$: + 94.6° (c = 0.15 in methanol)

^{1}H NMR: (600 MHz, $CDCl_3$, four sets of signals were observed, rotamers, assignments of signals analog to compound **2** (**JH112**)) δ 7.90 (d, *J* = 7.2 Hz, 0.1H), 7.88 (d, *J* = 8.4 Hz, 0.4H), 7.86 (d, *J* = 8.3 Hz, 0.2H), 7.83 (d, *J* = 8.3 Hz, 0.3H), 7.76 (s, 0.6H), 7.73 (s, 0.4H), 7.36

– 7.32 (m, 0.5H), 7.31 – 7.27 (m, 1.0H), 7.21 – 7.17 (m, 0.8H), 7.17 – 7.11 (m, 1.6H), 7.10 – 7.07 (m, 0.1H), 7.05 – 7.03 (m, 0.2H), 7.01 (td, *J* = 8.3, 2.0 Hz, 0.9H) 6.91 (dd, *J* = 8.4, 2.0 Hz, 0.3H), 6.29 (d, *J* = 0.9 Hz, 0.3H), 5.23 – 5.07 (m, 0.2H), 4.65 – 4.60 (m, 0.3H), 4.48 – 4.36 (m, 1.0H), 4.30 – 4.28 (m, 0.1H), 4.26 (d, *J* = 5.5 Hz, 0.2H), 4.24 (d, *J* = 5.6 Hz, 0.1H), 4.22 – 4.16 (m, 0.3H), 4.14 (d, *J* = 6.5 Hz, 0.2H), 4.12 (d, *J* = 5.8 Hz, 0.3H), 4.10 – 3.97 (m, 0.7H), 3.74 – 3.65 (m, 0.1H), 3.51 – 3.43 (m, 0.3H), 3.32 – 3.22 (m, 0.8H), 3.22 – 3.09 (m, 1.0H), 3.07 – 3.00 (m, 0.4H), 2.95 – 2.87 (m, 0.7H), 2.40 (s, 1.6H), 2.37 (s, 1.1H), 1.93 – 1.80 (m, 1.3H), 1.80 (s, 0.6H), 1.67 – 1.58 (m, 0.7H), 1.31 (d, *J* = 6.6 Hz, 0.3H), 1.25 (d, *J* = 6.5 Hz, 0.7H), 1.14 (d, *J* = 6.2 Hz, 1.1H), 0.53 (d, *J* = 6.4 Hz, 1.0H).

^{13}C NMR: (151 MHz, $CDCl_3$, four sets of signals were observed, rotamers, assignments of signals analog to compound **2** (**JH112**)) δ 169.7, 169.7, 169.4, 169.4, 162.3, 162.1, 162.1, 161.8, 147.5, 147.4, 147.1, 144.6, 138.4, 138.1, 137.4, 135.5, 135.4, 134.8, 133.8, 133.6, 133.5, 133.4, 130.4, 130.4, 130.3, 129.6, 129.5, 129.3, 128.8, 128.5, 128.3, 128.2, 128.1, 122.3, 121.9, 121.9, 121.6, 120.6, 120.5, 120.4, 120.2, 116.5, 116.3, 116.2, 115.9, 109.5, 109.4, 108.9, 54.9, 54.1, 53.5, 53.0, 51.8, 50.9, 50.8, 50.2, 50.0, 49.4, 48.6, 48.5, 43.5, 42.6, 39.3, 39.0, 28.8, 27.8, 27.2, 21.0, 20.9, 20.3, 17.5, 15.8, 15.4, 14.2.

Methyl (*S*)-2-aminobutanoate hydrochloride (25b)



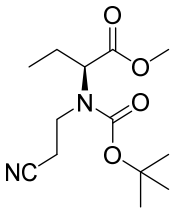
(*S*)-2-aminobutanoic acid (1.06 g, 10.28 mmol) was dissolved in methanolic hydrochloride solution (1.25 M, 82.23 mL, 0.10 mol) and stirred at ambient temperature for 24 h. The pure product was obtained as white powder after evaporation of the volatiles (1.57 g, 10.24 mmol, 100%).

ESI-MS: *m/z* 118.1 [M, cation]$^+$

^{1}H NMR: (400 MHz, $CDCl_3$) δ 8.80 (s, 3H), 4.13 (s, 1H), 3.83 (s, 3H), 2.26 – 1.99 (m, 2H), 1.13 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR: (151 MHz, $CDCl_3$) δ 169.8, 54.5, 53.1, 23.9, 9.7.

Methyl (*S*)-2-[(*tert*-butoxycarbonyl)(2-cyanoethyl)amino]butanoate (26b)



25b (2.30 g, 14.96 mmol) was dissolved in dry methanol (30 mL) in a dried flask. Sodium hydroxide (0.68 g, 16.88 mmol) was added and the reaction mixture was stirred at ambient temperature for 10 minutes. Acrylonitrile (2.16 mL, 32.91 mmol) was added dropwise over 30 minutes and the reaction mixture was stirred under reflux overnight. After cooling to ambient temperature, a solution of di-*tert*-butyldicarbonat (3.92 g, 17.98 mmol) in methanol (20 mL) was added and the reaction mixture was stirred under reflux overnight. The solvent was evaporated. The residue was taken up in ethyl acetate and washed with water and brine. The organic layer was dried over sodium sulfate, filtered and

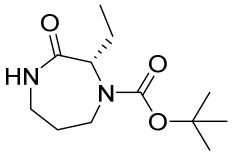
concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 20% to 40% ethyl acetate in *n*-hexane. The product was obtained as pale yellow oil (0.77 g, 2.83 mmol, 19%).

ESI-MS: *m/z* 293.1 $[M+Na]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 4.53 – 4.41 and 4.08 – 4.01 (m, 1H), 3.74 (s, 3H), 3.70 – 3.25 (m, 2H), 2.88 – 2.55 (m, 2H), 2.15 – 1.67 (m, 2H), 1.50 and 1.43 (s, 9H), 1.07 – 0.91 (m, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 172.4, 171.9, 155.1, 154.8, 118.3, 118.0, 81.4, 62.4, 52.2, 43.6, 41.7, 28.2, 23.5, 22.9, 17.9, 17.0, 11.1, 10.9.

***Tert*-butyl (*S*)-2-ethyl-3-oxo-1,4-diazepane-1-carboxylate (27b)**



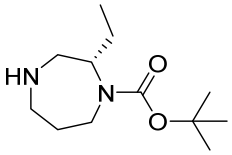
26b (0.80 g, 2.94 mmol) was dissolved in ethanol (40 mL) and chloroform (1 mL). Platinum dioxide (0.17 g, 0.74 mmol) was added and the reaction mixture was stirred at ambient temperature under hydrogen atmosphere for 23 h. The reaction mixture was filtered over celite and the volatiles were evaporated. The residue was dissolved in THF (20 mL) and methanol (10 mL). A 1 M aqueous solution of lithium hydroxide monohydrate (4.37 mL, 4.37 mmol) was added and the reaction mixture was stirred at ambient temperature for 24 h. The solvents were evaporated and the residue was taken up in a mixture of toluene and methanol (1 : 4, 10 mL). The volatiles were evaporated and the residue was dissolved in DMF (40 mL). DIPEA (1.02 mL, 5.83 mmol) and subsequently HATU (2.22 g, 5.83 mmol) were added to the solution. The reaction mixture was stirred at ambient temperature for 16 h. The reaction was quenched by addition of saturated aqueous solution of ammonium chloride (50 mL). The product was extracted with ethyl acetate and the combined organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 25% to 75% ethyl acetate in *n*-hexane. The product was obtained as yellow oil (0.50 g, 2.05 mmol, 70%).

ESI-MS: *m/z* 265.1 $[M+Na]^+$

^{1}H NMR: (600 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 6.20 – 5.72 (m, 1H), 4.87 – 4.71, 4.62 – 4.45 (m, 1H), 4.08 – 3.77, 3.41 – 2.96 (m, 4H), 2.14 – 1.82 (m, 4H), 1.47 (s, 9H), 0.95 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 175.3, 174.9, 155.4, 80.7, 61.8, 60.7, 40.0, 39.2, 32.5, 31.0, 28.4, 27.7, 27.5, 23.3, 23.1, 10.6, 10.3.

***Tert*-butyl (*S*)-2-ethyl-1,4-diazepane-1-carboxylate (28b)**



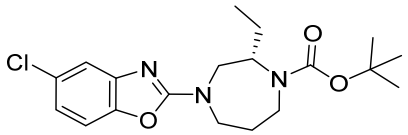
27b (0.26 g, 1.06 mmol) was dissolved in a dried flask in THF (20 mL). A 1 M solution of a borane-THF-complex in THF (5.32 mL, 5.32 mmol) was added dropwise under ice cooling. The reaction mixture was stirred under reflux at 75 °C for 24 h. After cooling to ambient temperature, the reaction was quenched by dropwise addition of methanol (2 mL) under ice cooling. The reaction mixture was diluted with ethyl acetate (70 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 40 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product purified by flash column chromatography applying a gradient of 20% to 50% ethyl acetate in *n*-hexane. The product was obtained as pale yellow oil (53.4 mg, 0.23 mmol, 22%).

ESI-MS: *m/z* 228.9 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 4.36 – 4.22, 4.17 – 4.06 (m, 1H), 4.05 – 3.95, 3.88 – 3.80 (m, 1H), 3.78 – 3.59 (m, 1H), 3.57 – 3.36 (m, 2H), 2.95 – 2.45 (m, 3H), 1.96 – 1.68 (m, 2H), 1.48 (s, *J* = 11.4 Hz, 9H), 1.46 – 1.37 (m, 2H), 0.95 – 0.85 (m, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 155.4, 155.0, 80.6, 80.3, 60.0, 59.8, 58.2, 58.0, 54.7, 53.7, 40.0, 39.2, 28.9, 28.6, 28.4, 28.4, 25.3, 25.2, 10.5, 10.2.

***Tert*-butyl (*S*)-4-(5-chlorobenzoxazol-2-yl)-2-ethyl-1,4-diazepane-1-carboxylate (29b)**



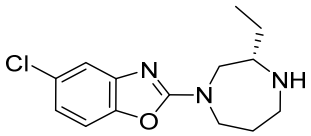
21 (16.4 mg, 0.11 mmol), **28b** (25.4 mg, 0.11 mmol) and copper(II) acetate hydrate (53.3 mg, 0.27 mmol) were dissolved in acetonitrile (2 mL). Concentrated acetic acid (12.0 µL, 0.21 mmol) was added and the reaction mixture was stirred under air at 80 °C for 18 h. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate (30 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 10 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 2% to 5% methanol in dichloromethane. The product was obtained as brown oil (29.6 mg, 77.9 µmol, 73%).

ESI-MS: *m/z* 380.1 $[M+H]^+$

^{1}H NMR: (600 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 7.29 (dd, *J* = 18.0, 1.8 Hz, 1H), 7.14 (d, *J* = 8.4 Hz, 1H), 6.95 (td, *J* = 8.3, 1.9 Hz, 1H), 4.68 – 3.70 (m, 4H), 3.37 – 2.77 (m, 3H), 2.27 – 1.88 (m, 1H), 1.79 – 1.61 (m, 1H), 1.53 – 1.38 (m, 2H), 1.32, 1.14 (s, 9H), 0.94 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 164.9, 162.3, 155.5, 147.5, 144.8, 129.2, 120.2, 116.2, 109.3, 109.1, 79.6, 54.2, 53.9, 54.0, 53.4, 51.0, 50.5, 40.8, 40.3, 28.3, 28.0, 26.9, 26.6, 24.4, 24.3, 10.7, 10.5.

(*S*)-5-chloro-2-(3-ethyl-1,4-diazepan-1-yl)benzoxazole (30b)



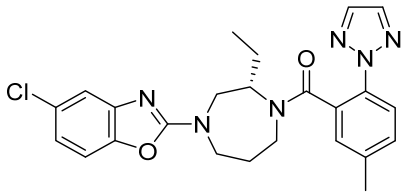
29b (29.6 mg, 77.9 µmol) was dissolved in dichloromethane (3 mL). Trifluoroacetic acid (0.3 mL) was added and the reaction mixture was stirred at ambient temperature for 2 h. The reaction mixture was basified to pH 11 using 1 M aqueous NaOH solution. The product was extracted with dichloromethane (3x). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure to obtain the pure product as brown oil (19.5 mg, 69.7 µmol, 89%).

ESI-MS: *m/z* 280.0 $[M+H]^+$

^{1}H NMR: (600 MHz, $CDCl_3$) δ 7.30 (d, *J* = 2.1 Hz, 1H), 7.13 (d, *J* = 8.4 Hz, 1H), 6.94 (dd, *J* = 8.4, 2.1 Hz, 1H), 4.02 (dd, *J* = 13.9, 3.2 Hz, 1H), 3.97 – 3.85 (m, 1H), 3.67 (ddd, *J* = 14.1, 7.3, 5.4 Hz, 1H), 3.25 – 3.10 (m, 2H), 2.86 – 2.74 (m, 1H), 2.66 (ddd, *J* = 14.0, 10.7, 3.7 Hz, 1H), 2.08 – 1.83 (m, 2H), 1.57 – 1.39 (m, 3H), 1.02 (t, *J* = 7.5 Hz, 3H).

^{13}C NMR: (151 MHz, $CDCl_3$) δ 163.2, 147.5, 145.1, 129.2, 119.8, 116.1, 109.0, 60.9, 55.7, 53.4, 46.9, 29.7, 26.8, 10.8.

(*S*)-[4-(5-chlorobenzoxazol-2-yl)-2-ethyl-1,4-diazepan-1-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone (3)



30b (43.4 mg, 0.16 mmol), **24** (37.8 mg, 0.19 mmol) and HATU (0.12 g, 0.31 mmol) were dissolved in dimethylformamide (dry, 4 mL). DIPEA (0.14 mL, 0.78 mmol) was added and the reaction mixture was stirred at ambient temperature for 5 h. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution (3x) and brine (1x). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified using a preparative HPLC (gradient of 5% to 95% methanol in water with 0.1% formic acid). The product was obtained as white powder after lyophilization of the product containing fractions (34.8 mg, 74.9 µmol, 48%).

ESI-MS: *m/z* 465.3 $[M+H]^+$

HR-ESI-MS: *m/z* $[M+H]^+$ calcd. 465.1800 for $C_{24}H_{25}ClN_6O_2$, found 465.1799

HPLC: eluent system 2: λ = 254 nm, t_R = 19.6 and 20.0 min, rotamers, purity: 97.9%

eluent system 3: λ = 254 nm, t_R = 21.9 min, purity: 95.9%

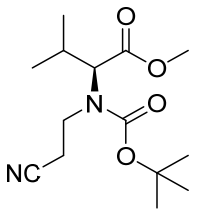
$[\alpha]_D^{24}$: + 51.6° (c = 0.07 in methanol)

^{1}H NMR: (400 MHz, $CDCl_3$, four sets of signals were observed, rotamers, assignments of signals analog to compound **2** (**JH112**)) δ 7.91 (d, *J* = 8.4 Hz, 0.2H), 7.88 (d, *J* = 8.4 Hz, 0.3H), 7.85 (d, *J* = 8.2 Hz, 0.2H), 7.82 (d, *J* = 8.3 Hz, 0.3H), 7.76 (s, 0.5H), 7.73 (s, 0.5H), 7.47 (s, 0.4H), 7.36 (s, 0.6H), 7.35 – 7.27 (m, 1.6H), 7.24 – 7.11 (m, 1.7H), 7.11 – 7.07 (m,

0.2H), 7.06 – 6.96 (m, 1.4H), 6.93 (dd, *J* = 8.4, 2.1 Hz, 0.4H), 6.39 (d, *J* = 1.1 Hz, 0.3H), 5.11 – 4.93 (m, 0.5H), 4.72 – 4.60 (m, 0.4H), 4.54 – 4.43 (m, 0.3H), 4.40 – 4.30 (m, 0.3H), 4.28 – 4.09 (m, 1.2H), 4.07 – 3.71 (m, 1.9H), 3.55 – 3.42 (m, 1.5H), 3.37 (ddd, *J* = 14.5, 10.8, 4.5 Hz, 0.3H), 3.29 – 3.18 (m, 0.4H), 3.18 – 3.04 (m, 0.7H), 3.03 – 2.87 (m, 0.7H), 2.41 (s, 1.7H), 2.37 (s, 1.0H), 2.36 – 2.27 (m, 0.4H), 2.03 – 1.93 (m, 0.5H), 1.78 – 1.66 (m, 1.2H), 1.07 (t, *J* = 7.5 Hz, 1.5H), 0.87 (t, *J* = 7.5 Hz, 1.3H), 0.84 – 0.70 (m, 0.4H), 0.62 (t, *J* = 7.5 Hz, 0.8H).

^{13}C NMR: (151 MHz, $CDCl_3$, four sets of signals were observed, rotamers, assignments of signals analog to compound **2** (**JH112**)) δ 170.0, 169.9, 169.8, 169.5, 162.7, 162.2, 159.6, 147.5, 144.8, 138.4, 138.4, 137.7, 135.5, 135.5, 135.5, 135.1, 130.4, 130.4, 130.4, 130.4, 129.6, 129.5, 129.4, 129.3, 128.8, 128.6, 128.4, 128.3, 122.5, 122.1, 122.1, 121.9, 120.5, 120.1, 116.5, 116.3, 116.1, 109.5, 109.4, 109.0, 57.7, 53.7, 53.2, 52.2, 52.0, 51.6, 50.9, 49.9, 49.5, 49.0, 43.8, 43.0, 40.4, 39.6, 28.0, 27.3, 27.0, 26.5, 25.2, 24.8, 23.5, 23.1, 21.0, 20.9, 20.4, 10.4, 10.1, 9.8.

Methyl *N*-(*tert*-butoxycarbonyl)-*N*-(2-cyanoethyl)-*L*-valinate (26c)



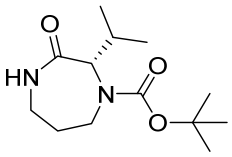
Methyl *L*-valinate hydrochloride (7.60 g, 45.35 mmol) was dissolved in dry methanol (50 mL) in a dried flask. DIPEA (11.77 mL, 68.02 mmol) was added and the reaction mixture was stirred at ambient temperature for 10 minutes. Acrylonitrile (5.94 mL, 90.70 mmol) was added dropwise over 30 minutes and the reaction mixture was stirred under reflux overnight. After cooling to ambient temperature, a solution of di-*tert*-butyldicarbonat (19.79 g, 90.70 mmol) in methanol (30 mL) was added and the reaction mixture was stirred under reflux overnight. The solvent was evaporated. The residue was taken up in ethyl acetate and washed with water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 15% to 30% ethyl acetate in isohexane. The product was obtained as pale yellow oil (4.45 g, 15.65 mmol, 35%).

APCI-MS: *m/z* 285.1 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 4.35, 3.91 (d, *J* = 10.2, 9.8 Hz, 1H), 3.74 (s, 3H), 3.71 – 3.43 (m, 2H), 2.82 – 2.54 (m, 2H), 2.33 – 2.10 (m, 1H), 1.49, 1.45 (s, 9H), 1.03 – 0.92 (m, 3H), 0.96 – 0.87 (m, 3H).

^{13}C NMR: (151 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 172.2, 171.4, 155.1, 154.8, 118.1, 117.8, 81.5, 65.9, 63.6, 52.0, 42.5, 40.8, 28.9, 28.6, 28.3, 20.4, 19.7, 19.3, 19.0, 17.7, 16.7.

***Tert*-butyl (*S*)-2-isopropyl-3-oxo-1,4-diazepane-1-carboxylate (27c)**



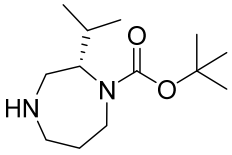
26c (4.45 g, 15.65 mmol) was dissolved in ethanol (15 mL) and chloroform (3 mL). Platinum dioxide (0.36 g, 1.56 mmol) was added and the reaction mixture was stirred at ambient temperature under hydrogen atmosphere for 25 h. The reaction mixture was filtered over celite and the volatiles were evaporated. The residue was dissolved in THF (10 mL) and methanol (30 mL). A 4 M aqueous solution of lithium hydroxide monohydrate (5.86 mL, 23.46 mmol) was added and the reaction mixture was stirred at ambient temperature for 50 h. The solvents were evaporated and the residue was taken up in a mixture of toluene and methanol (1 : 4, 20 mL). The volatiles were evaporated and the residue was dissolved in DMF (50 mL). DIPEA (5.37 mL, 31.05 mmol) and subsequently HATU (8.86 g, 23.29 mmol) were added to the solution. The reaction mixture was stirred at ambient temperature for 17 h. The reaction was quenched by addition of saturated aqueous solution of ammonium chloride (50 mL). The product was extracted with ethyl acetate and the combined organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 30% to 75% ethyl acetate in isohexane. The product was obtained as yellow oil (3.48 g, 13.58 mmol, 87%).

ESI-MS: *m/z* 279.0 $[M+Na]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 6.23 – 5.90 (m, 1H), 4.73 – 4.18 (m, 1H), 4.01 – 3.67, 3.39 – 3.17 (m, 4H), 2.57 – 2.40, 1.94 – 1.66 (m, 4H), 1.47 (s, 9H), 1.04 (d, *J* = 6.7 Hz, 3H), 0.92 (d, *J* = 6.6 Hz, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 174.7, 155.4, 80.7, 80.3, 67.1, 64.4, 40.7, 39.9, 38.6, 28.4, 28.1, 26.9, 20.1, 19.9, 19.1, 18.9.

***Tert*-butyl (*S*)-4-(5-chlorobenzoxazol-2-yl)-2-isopropyl-1,4-diazepane-1-carboxylate (28c)**



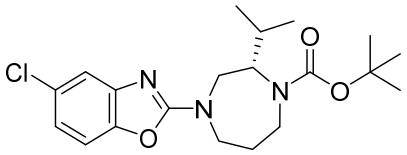
27c (3.40 g, 13.26 mmol) was dissolved in a dried flask in THF (50 mL). A 1 M solution of a borane-THF-complex in THF (66.3 mL, 66.32 mmol) was added dropwise under ice cooling. The reaction mixture was stirred under reflux at 75 °C for 46 h. After cooling to ambient temperature, the reaction was quenched by dropwise addition of methanol (15 mL) under ice cooling. The reaction mixture was diluted with ethyl acetate (150 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 70 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude mixture was purified by flash column chromatography applying a gradient of 10% to 50% ethyl acetate in isohexane to obtain the product as yellow oil (0.48 g, 1.99 mmol, 15%).

ESI-MS: *m/z* 243.1 $[M+H]^+$

^{1}H NMR: (600 MHz, dmso-d_6, two sets of signals were observed, rotamers) δ 3.86 – 3.58 (m, 2H), 3.09 (ddd, *J* = 35.5, 14.6, 5.7 Hz, 1H), 3.02 – 2.90 (m, 2H), 2.49 – 2.35 (m, 1H), 1.83 – 1.61 (m, 2H), 1.57 – 1.47 (m, 2H), 1.41, 1.36 (s, 9H), 0.88 – 0.72 (m, 6H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 155.7, 155.1, 80.8, 80.4, 59.0, 58.8, 58.5, 58.3, 57.5, 54.2, 40.7, 40.1, 34.9, 33.8, 28.7, 28.2, 25.4, 25.3, 25.0, 24.8, 13.9, 13.9.

***Tert*-butyl (*S*)-4-(5-chlorobenzoxazol-2-yl)-2-isopropyl-1,4-diazepane-1-carboxylate (29c)**



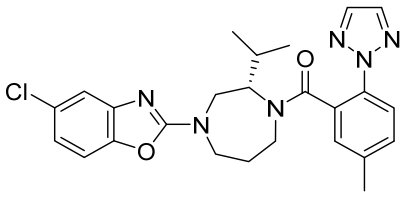
21 (0.28 g, 1.82 mmol), **28c** (0.46 g, 1.91 mmol) and copper(II) acetate hydrate (0.36 g, 1.82 mmol) were dissolved in acetonitrile (40 mL). Concentrated acetic acid (0.21 mL, 3.65 mmol) was added and the reaction mixture was stirred under air at 80 °C for 19 h. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate (100 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 50 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 2% to 5% methanol in dichloromethane. The product was obtained as brown oil (0.14 g, 0.37 mmol, 3%).

ESI-MS: *m/z* 394.1 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 7.33 – 7.27 (m, 1H), 7.15 (dd, *J* = 8.4, 3.9 Hz, 1H), 6.95 (ddd, *J* = 8.5, 6.4, 2.1 Hz, 1H), 4.52 – 4.07 (m, 2H), 4.00 – 3.90, 3.87 – 3.71, 3.69 – 3.62 (m, 2H), 3.30 – 3.16, 3.16 – 2.96, 2.96 – 2.75 (m, 3H), 2.31 – 2.08 (m, 1H), 1.49 – 1.31 (m, 2H), 1.27, 1.09 (s, 9H), 0.99 – 0.85 (m, 6H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 163.3, 162.2, 155.5, 155.4, 144.8, 144.6, 129.2, 129.0, 120.1, 116.2, 116.1, 109.3, 109.0, 79.5, 58.2, 58.0, 53.2, 52.5, 51.4, 51.1, 41.2, 41.2, 31.1, 30.5, 28.2, 27.9, 26.2, 26.0, 20.0, 19.7, 19.3, 19.3.

(*S*)-[4-(5-chlorobenzoxazol-2-yl)-2-isopropyl-1,4-diazepan-1-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone (5)



29c (0.14 g, 0.35 mmol) was dissolved in dichloromethane (3 mL). Trifluoroacetic acid (0.3 mL) was added and the reaction mixture was stirred at ambient temperature for 7 h. The reaction mixture was basified to pH 11 using 1 M aqueous NaOH solution. The product was extracted with dichloromethane (3x). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure to obtain the intermediate **30c** which was dissolved in DMF (4 mL). **24** (51.5 mg, 0.25 mmol), HATU (0.16 g, 0.42 mmol) and DIPEA (0.18 mL, 1.06 mmol) were added and the reaction mixture was stirred at ambient temperature for 23 h. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution (3x) and brine (1x). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified using a preparative HPLC (gradient of 5% to 95% methanol in water with 0.1% formic acid).

The product was obtained as white powder after lyophilization of the product containing fractions (50.9 mg, 0.11 mmol, 31%).

ESI-MS: *m/z* 294.1 [Intermediate **30c**+H]$^+$

m/z 479.2 [M+H]$^+$

HR-ESI-MS: *m/z* [M+H]$^+$ calcd. 479.1957 for $C_{25}H_{27}ClN_6O_2$, found 479.1955

HPLC: eluent system 1: λ = 254 nm, t_R = 22.0 and 22.2 min, rotamers, purity: 96.4%

eluent system 2: λ = 254 nm, t_R = 20.4 min, purity: 97.1%

eluent system 3: λ = 254 nm, t_R = 22.0 and 22.2 min, rotamers, purity: 98.2%

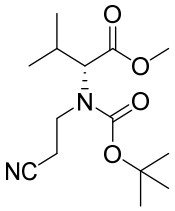
eluent system 4: λ = 254 nm, t_R = 20.3 min, purity: 98.3%

$[\alpha]_D^{24}$: + 139.7° (c = 0.21 in methanol)

^{1}H NMR: (600 MHz, $CDCl_3$, four sets of signals were observed, rotamers, assignments of signals analog to compound **2** (**JH112**)) δ 7.91 (d, *J* = 8.3 Hz, 0.3H), 7.88 (d, *J* = 8.3 Hz, 0.1H), 7.87 (d, *J* = 8.3 Hz, 0.2H), 7.81 (d, *J* = 8.3 Hz, 0.3H), 7.75 (s, 0.2H), 7.72 (s, 0.6H), 7.53 (s, 0.5H), 7.50 (s, 0.6H), 7.34 (dd, *J* = 4.8, 1.9 Hz, 0.7H), 7.33 – 7.30 (m, 0.6H), 7.29 – 7.27 (m, 0.4H), 7.25 (d, *J* = 1.9 Hz, 0.2H), 7.23 – 7.13 (m, 1.1H), 7.09 – 7.06 (m, 0.4H), 7.06 (s, 0.1H), 7.02 – 6.98 (m, 0.7H), 6.95 (dd, *J* = 8.4, 2.1 Hz, 0.2H), 6.92 (s, 0.3H), 6.48 (d, *J* = 0.7 Hz, 0.1H), 4.89 – 4.72 (m, 0.5H), 4.63 (dt, *J* = 13.9, 3.7 Hz, 0.2H), 4.59 – 4.52 (m, 0.1H), 4.34 – 4.26 (m, 0.1H), 4.20 (dd, *J* = 15.1, 5.6 Hz, 0.4H), 4.18 – 4.07 (m, 0.3H), 4.05 – 3.94 (m, 0.6H), 3.94 – 3.85 (m, 0.9H), 3.85 – 3.77 (m, 0.3H), 3.77 – 3.69 (m, 0.2H), 3.61 – 3.42 (m, 1.1H), 3.43 – 3.28 (m, 0.8H), 3.24 – 3.12 (m, 0.6H), 3.06 – 2.99 (m, 0.1H), 2.95 (ddd, *J* = 14.2, 11.6, 2.9 Hz, 0.2H), 2.42 (s, 0.7H), 2.41 (s, 0.9H), 2.36 (s, 1.0H), 2.35 – 2.30 (m, 0.2H), 2.24 – 2.15 (m, 0.3H), 2.13 – 2.01 (m, 0.6H), 2.01 – 1.93 (m, 0.3H), 1.91 (s, 0.4H), 1.89 – 1.78 (m, 0.5H), 1.74 – 1.64 (m, 0.7H), 1.64 – 1.54 (m, 1.0H), 1.55 – 1.44 (m, 0.2H), 1.16 (d, *J* = 6.6 Hz, 0.9H), 1.11 – 1.02 (m, 2.9H), 0.99 (d, *J* = 6.6 Hz, 0.8H), 0.87 (d, *J* = 6.9 Hz, 0.8H), 0.82 (d, *J* = 6.7 Hz, 0.4H), 0.51 (d, *J* = 7.0 Hz, 0.3H).

^{13}C NMR: (151 MHz, $CDCl_3$, four sets of signals were observed, rotamers, assignments of signals analog to compound **2** (**JH112**)) δ 170.2, 169.9, 169.9, 169.9, 162.9, 162.7, 162.4, 162.2, 147.6, 147.5, 147.4, 147.2, 144.7, 144.7, 144.5, 144.4, 138.4, 138.1, 138.1, 137.9, 135.6, 135.5, 135.4, 134.1, 133.8, 133.7, 133.3, 130.4, 130.3, 130.3, 129.8, 129.5, 129.5, 129.5, 129.4, 129.0, 129.0, 128.6, 128.4, 128.2, 128.1, 122.6, 122.3, 122.3, 122.2, 120.5, 120.4, 120.3, 120.2, 116.5, 116.3, 116.2, 109.5, 109.4, 109.0, 62.8, 61.0, 57.8, 57.8, 50.7, 50.3, 50.1, 49.9, 49.7, 49.7, 49.2, 49.0, 44.7, 44.0, 41.5, 40.1, 30.3, 29.1, 28.9, 28.6, 27.8, 27.0, 27.0, 25.7, 21.0, 21.0, 21.0, 20.5, 19.9, 19.8, 19.5, 19.4, 19.1, 18.9, 18.5, 17.7.

Methyl *N*-(*tert*-butoxycarbonyl)-*N*-(2-cyanoethyl)-*D*-valinate (26d)

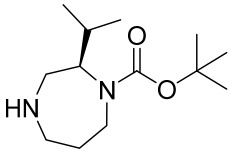


Methyl *D*-valinate hydrochloride (3.00 g, 17.90 mmol) was dissolved in dry methanol (30 mL) in a dried flask. Sodium hydroxide (0.72 g, 17.90 mmol) was added and the reaction mixture was stirred at

ambient temperature for 10 minutes. Acrylonitrile (1.88 mL, 28.70 mmol) was added dropwise over 30 minutes and the reaction mixture was stirred under reflux overnight. After cooling to ambient temperature, a solution of di-*tert*-butyldicarbonat (13.67 g, 62.64 mmol) in methanol (30 mL) was added and the reaction mixture was stirred under reflux overnight. The solvent was evaporated. The residue was taken up in ethyl acetate and washed with water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 15% to 30% ethyl acetate in *n*-hexane. The product was obtained as pale yellow oil (0.96 g, 3.36 mmol, 19%).

For analytical data see compound **26c**.

***Tert*-butyl (*R*)-4-(5-chlorobenzoxazol-2-yl)-2-isopropyl-1,4-diazepane-1-carboxylate (28d)**



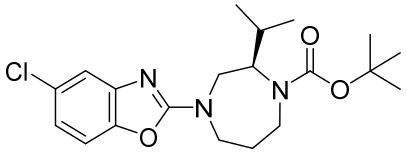
26d (0.96 g, 3.36 mmol) was dissolved in ethanol (20 mL) and chloroform (0.3 mL). Platinum dioxide (0.19 g, 0.84 mmol) was added and the reaction mixture was stirred at ambient temperature under hydrogen atmosphere for 16 h. The reaction mixture was filtered over celite and the volatiles were evaporated. The residue was dissolved in THF (4 mL) and methanol (2 mL). A 1 M aqueous solution of lithium hydroxide monohydrate (3.36 mL, 3.36 mmol) was added and the reaction mixture was stirred at ambient temperature for 16 h. The solvents were evaporated and the residue was taken up in a mixture of toluene and methanol (1 : 4, 10 mL). The volatiles were evaporated and the residue was dissolved in THF (10 mL). DIPEA (1.17 mL, 6.72 mmol) and subsequently HATU (2.56 g, 6.72 mmol) were added to the solution. The reaction mixture was stirred at ambient temperature for 17 h. The reaction was quenched by addition of saturated aqueous solution of ammonium chloride (20 mL). The product was extracted with ethyl acetate and the combined organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude intermediate (**27d**) was purified by flash column chromatography applying a gradient of 30% to 75% ethyl acetate in *n*-hexane. Intermediate **27d** was dissolved in a dried flask in THF (10 mL). A 1 M solution of a borane-THF-complex in THF (16.1 mL, 16.1 mmol) was added dropwise under ice cooling. The reaction mixture was stirred under reflux at 75 °C for 18 h. After cooling to ambient temperature, the reaction was quenched by dropwise addition of methanol (10 mL) under ice cooling. The reaction mixture was diluted with ethyl acetate (100 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 50 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude mixture was purified by flash column chromatography applying a gradient of 1% to 5% methanol in dichloromethane to obtain the product as yellow oil (98.5 mg, 0.41 mmol, 12%).

ESI-MS: *m/z* 256.9 [Intermediate **27d**+H]$^+$

m/z 243.0 [M+H]$^+$

For further analytical data see compound **28c.**

***Tert*-butyl (*R*)-4-(5-chlorobenzoxazol-2-yl)-2-isopropyl-1,4-diazepane-1-carboxylate (29d)**

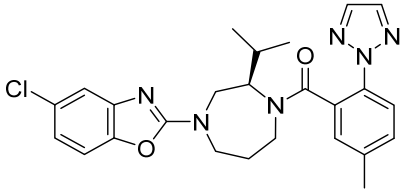


21 (12.0 mg, 78.4 µmol), **28d** (19.0 mg, 78.4 µmol) and copper(II) acetate hydrate (14.9 mg, 68.2 µmol) were dissolved in acetonitrile (2 mL). Concentrated acetic acid (9.0 µL, 0.16 mmol) was added and the reaction mixture was stirred under air at 80 °C for 16 h. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate (10 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 10 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 20% to 40% ethyl acetate in *n*-hexane. The product was obtained as brown oil (17.9 mg, 45.4 µmol, 58%).

ESI-MS: *m/z* 394.1 $[M+H]^+$

For further analytical data see compound **29c**.

(*R*)-[4-(5-chlorobenzoxazol-2-yl)-2-isopropyl-1,4-diazepan-1-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone (9)



29d (4.9 mg, 12.4 µmol) was dissolved in dichloromethane (1 mL). Trifluoroacetic acid (0.1 mL) was added and the reaction mixture was stirred at ambient temperature for 15 h. The reaction mixture was basified to pH 11 using 1 M aqueous NaOH solution. The product was extracted with dichloromethane (3x). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure to obtain the intermediate **30d** which was dissolved in DMF (1 mL). **24** (2.3 mg, 12.4 µmol), HATU (5.2 mg, 13.6 µmol) and DIPEA (10 µL, 57.8 µmol) were added and the reaction mixture was stirred at ambient temperature for 18 h. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution (3x) and brine (1x). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified using a preparative HPLC (gradient of 5% to 95% acetonitrile in water with 0.1% trifluoroacetic acid). The product was obtained as white powder after lyophilization of the product containing fractions (0.80 mg, 1.67 µmol, 13%).

ESI-MS: *m/z* 294.0 [Intermediate **30d**+H]$^+$

m/z 479.1 $[M+H]^+$

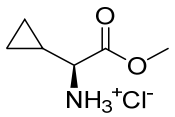
HR-ESI-MS: *m/z* $[M+H]^+$ calcd. 479.1957 for $C_{25}H_{27}ClN_6O_2$, found 479.1960

HPLC: eluent system 3: λ = 254 nm, t_R = 21.2 and 21.4 min, rotamers, purity: 97.4%

$[\alpha]_D^{25}$: - 100.9° (c = 0.11 in methanol)

For further analytical data see compound **5**.

Methyl (*S*)-2-amino-2-cyclopropylacetate hydrochloride (25e)



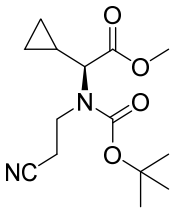
(*S*)-2-amino-2-cyclopropylacetic acid (0.90 g, 7.82 mmol) was dissolved in methanolic hydrochloride solution (1.25 M, 31.27 mL, 39.09 mmol) and stirred at ambient temperature for 22 h. The pure product was obtained as white powder after evaporation of the volatiles (1.29 g, 7.78 mmol, 100%).

ESI-MS: *m/z* 130.1 [M, cation]$^+$

^{1}H NMR: (600 MHz, dmso-d_6) δ 8.60 (s, 3H), 3.76 (s, 3H), 3.39 (dq, *J* = 10.7, 5.3 Hz, 1H), 1.19 – 0.99 (m, 1H), 0.76 – 0.47 (m, 4H).

^{13}C NMR: (101 MHz, dmso-d_6) δ 169.5, 56.1, 52.7, 11.8, 4.1, 4.0.

Methyl (*S*)-2-[(*tert*-butoxycarbonyl)(2-cyanoethyl)amino]-2-cyclopropylacetate (26e)



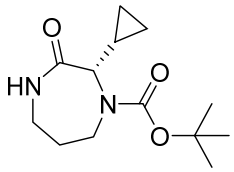
25e (1.29 g, 7.78 mmol) was dissolved in dry methanol (30 mL) in a dried flask. DIPEA (2.04 mL, 11.68 mmol) was added and the reaction mixture was stirred at ambient temperature for 10 minutes. Acrylonitrile (1.28 mL, 19.47 mmol) was added dropwise over 30 minutes and the reaction mixture was stirred under reflux overnight. After cooling to ambient temperature, a solution of di-*tert*-butyldicarbonat (3.40 g, 15.58 mmol) in methanol (20 mL) was added and the reaction mixture was stirred under reflux overnight. The solvent was evaporated. The residue was taken up in ethyl acetate and washed with water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 20% to 50% ethyl acetate in *n*-hexane. The product was obtained as pale yellow oil (0.81 g, 2.85 mmol, 37%).

ESI-MS: *m/z* 305.2 [M+Na]$^+$

^{1}H NMR: (600 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 3.85 – 3.77 (m, 1H), 3.75 (s, 3H), 3.71 – 3.57 (m, 1H), 3.46 (d, *J* = 10.0 Hz, 1H), 2.86 – 2.63 (m, 2H), 1.49, 1.41 (s, 9H), 1.22 – 1.05 (m, 1H), 0.86 – 0.77 (m, 1H), 0.75 – 0.53 (m, 2H), 0.42 – 0.35 (m, 1H).

^{13}C NMR: (151 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 172.4, 172.2, 155.2, 154.7, 118.3, 118.0, 81.6, 81.4, 65.3, 63.6, 52.3, 52.2, 41.6, 40.9, 28.3, 28.2, 18.3, 17.4, 11.7, 11.5, 6.2, 5.8, 4.3, 4.1.

***Tert*-butyl (*S*)-2-cyclopropyl-3-oxo-1,4-diazepane-1-carboxylate (27e)**



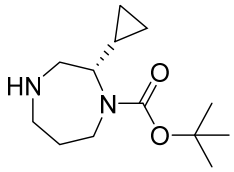
26e (0.81 g, 2.85 mmol) was dissolved in ethanol (40 mL). Platinum dioxide (0.19 g, 0.86 mmol) was added and the reaction mixture was stirred at ambient temperature under hydrogen atmosphere for 24 h. The reaction mixture was filtered over celite and the volatiles were evaporated. The residue was dissolved in THF (20 mL) and methanol (10 mL). A 2 M aqueous solution of lithium hydroxide monohydrate (1.70 mL, 3.39 mmol) was added and the reaction mixture was stirred at ambient temperature for 21 h. The solvents were evaporated and the residue was taken up in a mixture of toluene and methanol (1 : 4, 10 mL). The solvents were evaporated and the residue was dissolved in DMF (40 mL). DIPEA (0.99 mL, 5.66 mmol) and subsequently HATU (2.15 g, 5.66 mmol) were added to the solution. The reaction mixture was stirred at ambient temperature for 21 h. The reaction was quenched by addition of saturated aqueous solution of ammonium chloride (50 mL). The product was extracted with ethyl acetate and the combined organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 25% to 75% ethyl acetate in *n*-hexane. The product was obtained as yellow oil (0.31 g, 1.21 mmol, 43%).

ESI-MS: *m/z* 255.0 $[M+H]^+$

^{1}H NMR: (600 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 6.30 – 5.84 (m, 1H), 4.41 – 4.15, 4.09 – 3.74, 3.57 – 3.15 (m, 5H), 2.02 – 1.76 (m, 2H), 1.46 (s, 9H), 1.39 – 1.30 (m, 1H), 0.80 – 0.26 (m, 4H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 175.3, 155.4, 155.1, 80.7, 80.3, 65.7, 47.6, 47.5, 40.6, 39.8, 29.7, 28.2, 28.3, 11.6, 11.4, 5.9, 5.3, 2.8, 2.2.

***Tert*-butyl (*S*)-2-cyclopropyl-1,4-diazepane-1-carboxylate (28e)**



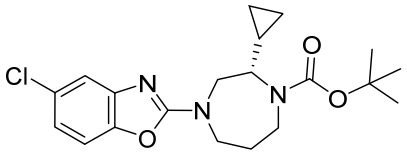
27e (0.29 g, 1.14 mmol) was dissolved in a dried flask in THF (20 mL). A 1 M solution of a borane-THF-complex in THF (5.70 mL, 5.70 mmol) was added dropwise under ice cooling. The reaction mixture was stirred under reflux at 75 °C for 3 d. After cooling to ambient temperature, the reaction was quenched by dropwise addition of methanol (2 mL) under ice cooling. The reaction mixture was diluted with ethyl acetate (70 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 40 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product purified by flash column chromatography applying a gradient of 20% to 50% ethyl acetate in *n*-hexane. The product was obtained as pale yellow oil (86.2 mg, 0.36 mmol, 31%).

ESI-MS: *m/z* 241.0 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 4.13 – 3.86 (m, 1H), 3.83 – 3.71, 3.62 – 3.34 (m, 4H), 3.09 – 2.73 (m, 2H), 1.92 – 1.71 (m, 2H), 1.48 (s, 9H), 0.77 – 0.67 (m, 1H), 0.61 – 0.32, 0.31 – 0.14 (m, 4H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 155.4, 154.8, 80.8, 80.4, 60.0, 59.7, 58.3, 58.1, 57.5, 56.5, 40.6, 39.9, 29.0, 28.8, 28.4, 13.1, 3.1, 2.8, 2.8, 2.3.

***Tert*-butyl (*S*)-4-(5-chlorobenzoxazol-2-yl)-2-cyclopropyl-1,4-diazepane-1-carboxylate (29e)**



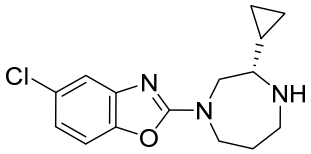
21 (49.1 mg, 0.32 mmol), **28e** and copper(II) acetate hydrate (63.8 mg, 0.32 mmol) were dissolved in acetonitrile (10 mL). Concentrated acetic acid (36.6 µL, 0.64 mmol) was added and the reaction mixture was stirred under air at 80 °C for 28 h. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate (50 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 30 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 25% to 50% ethyl acetate in *n*-hexane. The product was obtained as brown oil (52.5 mg, 0.13 mmol, 42%).

ESI-MS: *m/z* 392.2 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 7.33 – 7.27 (m, 1H), 7.13 (d, *J* = 8.4 Hz, 1H), 6.95 (d, *J* = 8.3 Hz, 1H), 4.34 – 4.20 (m, 1H), 4.05 – 3.72, 3.57 – 3.30, 3.30 – 2.87 (m, 6H), 2.23 – 2.06, 1.90 – 1.62 (m, 2H), 1.32, 1.14 (s, 9H), 0.93 – 0.77 (m, 1H), 0.65 – 0.55, 0.54 – 0.31 (m, 4H).

^{13}C NMR: (151 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 162.6, 162.3, 155.4, 155.1, 147.5, 144.9, 144.7, 129.2, 129.1, 120.1, 116.2, 109.3, 109.1, 79.6, 57.9, 57.8, 53.6, 53.3, 51.0, 50.4, 41.6, 41.3, 28.2, 28.0, 27.0, 12.6, 12.5, 4.0, 3.3, 3.3, 2.7.

(*S*)-5-chloro-2-(3-cyclopropyl-1,4-diazepan-1-yl)benzoxazole (30e)



29e (50.0 mg, 0.13 mmol) was dissolved in dichloromethane (5 mL). Trifluoroacetic acid (0.3 mL) was added and the reaction mixture was stirred at ambient temperature for 2 h. The reaction mixture was basified to pH 11 using 1 M aqueous NaOH solution. The product was extracted with dichloromethane (3x). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure to obtain the pure product as brown oil (37.2 mg, 0.13 mmol, 100%).

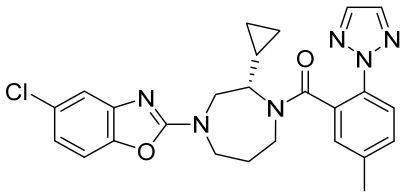
ESI-MS: *m/z* 292.0 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.29 (d, *J* = 2.1 Hz, 1H), 7.13 (d, *J* = 8.4 Hz, 1H), 6.94 (dd, *J* = 8.4, 2.1 Hz, 1H), 4.18 (dd, *J* = 13.9, 3.1 Hz, 1H), 3.92 (dt, *J* = 13.7, 6.2 Hz, 1H), 3.67 (ddd, *J* = 14.1, 7.4, 5.6 Hz, 1H), 3.32 (dd, *J* = 14.0, 10.2 Hz, 1H), 3.20 (ddd, *J* = 13.5, 4.8, 4.0

Hz, 1H), 2.57 (ddd, *J* = 13.6, 10.9, 3.8 Hz, 1H), 2.11 – 1.97 (m, 2H), 1.96 – 1.82 (m, 1H), 0.93 – 0.74 (m, 1H), 0.67 – 0.50 (m, 2H), 0.40 (td, *J* = 9.4, 5.0 Hz, 1H), 0.24 (td, *J* = 9.3, 5.1 Hz, 1H).

^{13}C NMR: (151 MHz, $CDCl_3$) δ 163.1, 147.5, 145.0, 129.2, 119.8, 116.1, 109.0, 65.6, 59.5, 55.6, 53.4, 47.4, 47.1, 29.7, 29.3, 14.9, 3.4, 3.3.

(*S*)-[4-(5-chlorobenzoxazol-2-yl)-2-cyclopropyl-1,4-diazepan-1-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone (6)



30e (33.9 mg, 0.12 mmol), **24** (29.0 mg, 0.14 mmol) and HATU (90.0 mg, 0.24 mmol) were dissolved in dimethylformamide (dry, 5 mL). DIPEA (0.10 mL, 0.58 mmol) was added and the reaction mixture was stirred at ambient temperature for 3 h. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution (3x) and brine (1x). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified using a preparative HPLC (gradient of 5% to 95% methanol in water with 0.1% formic acid). The product was obtained as white powder after lyophilization of the product containing fractions (38.7 mg, 81.1 µmol, 70%).

ESI-MS: *m/z* 477.2 $[M+H]^+$

HR-ESI-MS: *m/z* $[M+H]^+$ calcd. 477.1800 for $C_{25}H_{25}ClN_6O_2$, found 477.1804

HPLC: eluent system 2: λ = 254 nm, t_R = 20.2 min, purity: 99.1%

eluent system 3: λ = 254 nm, t_R = 22.2 min, purity: 99.7%

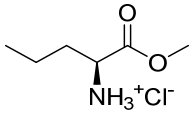
$[\alpha]_D^{26}$: + 30.0° (c = 0.09 in methanol)

^{1}H NMR: (600 MHz, $CDCl_3$, four sets of signals were observed, rotamers, assignments of signals analog to compound **2** (**JH112**)) δ 7.98 (d, *J* = 8.4 Hz, 0.2H), 7.90 (d, *J* = 8.3 Hz, 0.2H), 7.85 (d, *J* = 8.2 Hz, 0.2H), 7.83 (d, *J* = 8.1 Hz, 0.3H), 7.74 (s, 0.9H), 7.50 (s, 0.4H), 7.33 (d, *J* = 1.8 Hz, 0.8H), 7.31 – 7.28 (m, 0.1H), 7.25 (d, *J* = 1.0 Hz, 0.3H), 7.21 (t, *J* = 5.2, 1.6 Hz, 0.3H), 7.20 – 7.14 (m, 0.8H), 7.12 (s, 0.2H), 7.06 – 6.95 (m, 1.2H), 6.93 (dd, *J* = 8.4, 2.1 Hz, 0.2H), 6.33 (d, *J* = 0.8 Hz, 0.2H), 4.69 (dt, *J* = 14.0, 3.5 Hz, 0.2H), 4.58 (dd, *J* = 13.9, 3.4 Hz, 0.2H), 4.49 – 4.32 (m, 0.7H), 4.25 – 4.12 (m, 0.5H), 4.07 (dd, *J* = 14.9, 5.4 Hz, 0.3H), 4.04 – 3.90 (m, 0.8H), 3.88 – 3.73 (m, 1.0H), 3.65 – 3.55 (m, 0.8H), 3.55 – 3.38 (m, 1.0H), 3.31 – 3.20 (m, 0.9H), 3.19 – 3.11 (m, 0.5H), 2.42 (s, 0.7H), 2.41 (s, 0.9H), 2.38 (s, 1.0H), 2.37 – 2.33 (m, 0.2H), 2.05 – 1.97 (m, 0.3H), 1.90 (s, 0.6H), 1.89 – 1.76 (m, 1.2H), 1.73 – 1.65 (m, 0.9H), 1.07 – 0.79 (m, 1.1H), 0.76 – 0.60 (m, 1.3H), 0.60 – 0.44 (m, 1.6H), 0.34 (tt, *J* = 8.9, 5.6 Hz, 0.2H), 0.23 – 0.16 (m, 0.3H), 0.09 – 0.02 (m, 0.2H), 0.01 – -0.05 (m, 0.2H), -0.12 – -0.19 (m, 0.2H), -0.51 (dq, *J* = 10.9, 5.4 Hz, 0.2H).

^{13}C NMR: (151 MHz, $CDCl_3$, four sets of signals were observed, rotamers, assignments of signals analog to compound **2** (**JH112**)) δ 169.9, 169.7, 169.6, 162.9, 162.7, 162.7, 162.1, 147.5, 147.5, 147.5, 147.2, 144.9, 144.8, 144.7, 144.7, 138.4, 138.2, 137.7, 137.3, 135.5, 135.4, 135.1, 135.1, 134.1, 133.8, 133.6, 133.4, 130.4, 130.4, 130.4, 130.3, 130.1, 129.5, 129.4,

129.2, 128.9, 128.6, 128.4, 128.0, 127.4, 122.6, 122.1, 121.6, 121.1, 120.5, 120.4, 120.2, 120.1, 116.5, 116.3, 116.2, 116.1, 109.4, 109.4, 109.3, 109.0, 60.5, 59.8, 57.5, 57.1, 53.2, 52.8, 52.5, 51.8, 50.8, 49.6, 49.6, 48.7, 44.2, 42.8, 40.8, 40.1, 27.3, 27.3, 26.6, 21.0, 21.0, 20.9, 20.5, 13.0, 12.4, 11.9, 11.5, 4.9, 4.5, 3.9, 3.8, 3.6, 3.4, 3.3, 1.6.

Methyl (*S*)-2-aminopentanoate hydrochloride (25f)



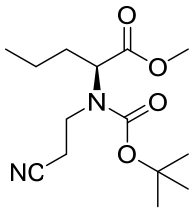
(*S*)-2-aminopentanoic acid (5.01 g, 42.77 mmol) was dissolved in methanolic hydrochloride solution (1.25 M, 170.93 mL, 0.21 mol) and stirred at ambient temperature for 26 h. The pure product was obtained as white powder after evaporation of the volatiles (7.15 g, 42.65 mmol, 100%).

ESI-MS: *m/z* 132.0 [M, cation]$^+$

^{1}H NMR: (400 MHz, $CDCl_3$) δ 8.61 (s, 3H), 4.28 – 4.05 (m, 1H), 3.83 (s, 3H), 2.12 – 1.89 (m, 2H), 1.68 – 1.38 (m, 2H), 0.98 (t, *J* = 6.9 Hz, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 170.0, 53.3, 50.5, 32.4, 18.5, 13.6.

Methyl (*S*)-2-[(*tert*-butoxycarbonyl)(2-cyanoethyl)amino]pentanoate (26f)



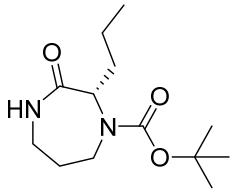
25f (7.00 g, 41.76 mmol) was dissolved in dry methanol (60 mL) in a dried flask. DIPEA (10.92 mL, 62.67 mmol) was added and the reaction mixture was stirred at ambient temperature for 10 minutes. Acrylonitrile (5.47 mL, 83.52 mmol) was added dropwise over 30 minutes and the reaction mixture was stirred under reflux overnight. After cooling to ambient temperature, a solution of di-*tert*-butyldicarbonat (18.23 g, 83.52 mmol) in methanol (40 mL) was added and the reaction mixture was stirred under reflux overnight. The solvent was evaporated. The residue was taken up in ethyl acetate and washed with water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 20% to 40% ethyl acetate in *n*-hexane. The product was obtained as pale yellow oil (3.01 g, 10.58 mmol, 25%).

ESI-MS: *m/z* 307.1 [M+Na]$^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 4.66 – 4.50, 4.26 – 4.16 (m, 1H), 3.74 (s, *J* = 8.3 Hz, 3H), 3.69 – 3.30 (m, 2H), 2.81 – 2.58 (m, 2H), 2.01 – 1.67 (m, 2H), 1.50, 1.43 (s, 9H), 1.40 – 1.29 (m, 4H), 0.97 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR: (151 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 172.6, 172.1, 155.1, 154.8, 118.3, 118.0, 81.5, 81.4, 60.4, 58.7, 52.2, 43.3, 41.6, 32.3, 31.7, 28.3, 28.2, 19.6, 17.9, 17.0, 13.7, 13.6.

***Tert*-butyl (*S*)- 3-oxo-2-propyl-1,4-diazepane-1-carboxylate (27f)**



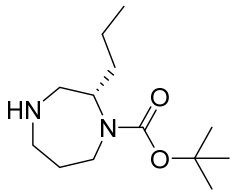
26f (3.01 g, 10.58 mmol) was dissolved in ethanol (30 mL). Platinum dioxide (0.23 g, 0.99 mmol) was added and the reaction mixture was stirred at ambient temperature under hydrogen atmosphere for 30 h. The reaction mixture was filtered over celite and the volatiles were evaporated. The residue was dissolved in THF (20 mL) and methanol (10 mL). A 4 M aqueous solution of lithium hydroxide monohydrate (3.51 mL, 14.04 mmol) was added and the reaction mixture was stirred at ambient temperature for 23 h. The solvents were evaporated and the residue was taken up in a mixture of toluene and methanol (1 : 4, 10 mL). The volatiles were evaporated and the residue was dissolved in DMF (120 mL). DIPEA (3.24 mL, 18.73 mmol) and subsequently HATU (4.35 g, 11.43 mmol) were added to the solution. The reaction mixture was stirred at ambient temperature for 22 h. The reaction was quenched by addition of saturated aqueous solution of ammonium chloride (100 mL). The product was extracted with ethyl acetate and the combined organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 25% to 75% ethyl acetate in *n*-hexane. The product was obtained as yellow oil (0.50 g, 1.94 mmol, 18%).

ESI-MS: *m/z* 279.0 $[M+Na]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 6.17 – 5.98 (m, 1H), 4.96 – 4.73, 4.73 – 4.48 (m, 1H), 4.05 – 3.76, 3.39 – 3.24, 3.23 – 3.01 (m, 4H), 2.10 – 1.88 (m, 2H), 1.87 – 1.69 (m, 2H), 1.47 (s, 9H), 1.42 – 1.27 (m, 2H), 0.95 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR: (151 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 175.7, 175.2, 155.4, 80.7, 80.2, 60.1, 59.5, 49.8, 47.3, 40.1, 39.2, 32.3, 31.7, 28.4, 27.8, 27.6, 19.1, 13.9, 13.7.

***Tert*-butyl (*S*)-2-propyl-1,4-diazepane-1-carboxylate (28f)**



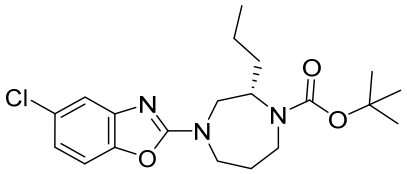
27f (0.49 g, 1.90 mmol) was dissolved in a dried flask in THF (25 mL). A 1 M solution of a borane-THF-complex in THF (9.52 mL, 9.52 mmol) was added dropwise under ice cooling. The reaction mixture was stirred under reflux at 75 °C for 44 h. After cooling to ambient temperature, the reaction was quenched by dropwise addition of methanol (3 mL) under ice cooling. The reaction mixture was diluted with ethyl acetate (70 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 40 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product purified by flash column chromatography applying a gradient of 20% to 50% ethyl acetate in *n*-hexane. The product was obtained as pale yellow oil (0.13 g, 0.52 mmol, 27%).

ESI-MS: *m/z* 243.0 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 4.40 – 4.27, 4.23 – 4.12 (m, 1H), 4.04 – 3.96, 3.90 – 3.77, 3.69 – 3.60, 3.56 – 3.36 (m, 4H), 2.93 – 2.76 (m, 1H), 2.71 – 2.48 (m, 2H), 1.89 – 1.59 (m, 4H), 1.48, 1.46 (s, 9H), 1.37 – 1.26 (m, 2H), 0.96 – 0.85 (m, 3H).

^{13}C NMR: (151 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 155.3, 154.9, 80.8, 80.4, 60.3, 60.1, 58.3, 58.1, 53.1, 52.2, 39.9, 39.2, 34.4, 34.3, 28.9, 28.6, 28.4, 28.4, 19.0, 11.5.

Tert-butyl (_S_)-4-(5-chlorobenzoxazol-2-yl)-2-propyl-1,4-diazepane-1-carboxylate (29f)



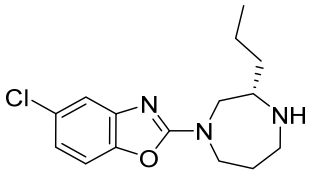
21 (73.1 mg, 0.48 mmol), **28f** (0.12 g, 0.50 mmol) and copper(II) acetate hydrate (0.10 g, 0.52 mmol) were dissolved in acetonitrile (20 mL). Concentrated acetic acid (54.5 µL, 0.95 mmol) was added and the reaction mixture was stirred under air at 80 °C for 44 h. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate (60 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 30 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 20% to 50% ethyl acetate in *n*-hexane. The product was obtained as brown oil (98.0 mg, 0.25 mmol, 52%).

ESI-MS: *m/z* 394.1 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 7.31, 7.28 (d, *J* = 2.0, 1.9 Hz, 1H), 7.14 (d, *J* = 8.4 Hz, 1H), 6.96, 6.94 (dd, *J* = 8.4, 2.1 Hz, 1H), 4.78 – 4.53 (m, 1H), 4.31 – 4.12, 4.12 – 4.05, 3.95 – 3.87 (m, 1H), 3.80 – 3.58 (m, 1H), 3.37 – 3.24, 3.24 – 3.05, 3.01 – 2.79, 2.24 – 2.10 (m, 3H), 1.79 – 1.56 (m, 2H), 1.46, 1.32, 1.13 (s, 9H), 1.44 – 1.32 (m, 2H), 0.98 – 0.89 (m), 0.88 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 162.5, 162.3, 155.4, 155.3, 147.5, 147.5, 144.8, 144.7, 129.2, 129.1, 120.2, 116.2, 109.3, 109.1, 79.8, 79.6, 54.2, 53.6, 52.6, 52.1, 51.0, 50.5, 40.8, 40.3, 33.5, 28.5, 28.3, 28.0, 26.9, 26.5, 19.3, 19.2, 11.5.

(_S_)-5-chloro-2-(3-propyl-1,4-diazepan-1-yl)benzoxazole (30f)



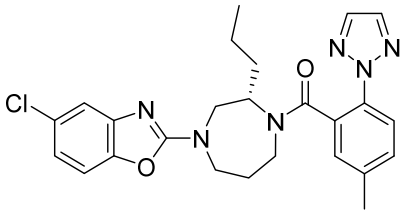
29f (90.0 mg, 0.23 mmol) was dissolved in dichloromethane (3 mL). Trifluoroacetic acid (0.3 mL) was added and the reaction mixture was stirred at ambient temperature for 3 h. The reaction mixture was basified to pH 11 using 1 M aqueous NaOH solution. The product was extracted with dichloromethane (3x). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure to obtain the pure product as brown oil (66.5 mg, 0.23 mmol, 99%).

ESI-MS: *m/z* 293.9 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.29 (d, *J* = 2.0 Hz, 1H), 7.13 (d, *J* = 8.4 Hz, 1H), 6.94 (dd, *J* = 8.4, 2.0 Hz, 1H), 4.06 – 3.86 (m, 2H), 3.71 – 3.60 (m, 1H), 3.22 – 3.08, 2.73 – 2.59 (m, 2H), 2.95 – 2.80 (m, 1H), 2.13 – 1.81 (m, 2H), 1.80 – 1.60 (m, 2H), 1.54 – 1.31 (m, 4H), 1.00 – 0.94 (m, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 163.2, 147.5, 145.0, 129.2, 119.8, 116.1, 109.0, 61.4, 59.3, 59.1, 55.9, 53.4, 47.5, 46.9, 36.0, 29.7, 19.5, 19.1, 11.4.

(*S*)-[4-(5-chlorobenzoxazol-2-yl)-2-propyl-1,4-diazepan-1-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone (4)



30f (60.0 mg, 0.20 mmol), **24** (49.8 mg, 0.25 mmol) and HATU (0.17 g, 0.46 mmol) were dissolved in dimethylformamide (dry, 4 mL). DIPEA (0.18 mL, 1.02 mmol) was added and the reaction mixture was stirred at ambient temperature for 3 h. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution (3x) and brine (1x). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified using a preparative HPLC (gradient of 5% to 95% methanol in water with 0.1% formic acid). The product was obtained as white powder after lyophilization of the product containing fractions (52.0 mg, 0.10 mmol, 53%).

ESI-MS: *m/z* 479.1 $[M+H]^+$

HR-ESI-MS: *m/z* $[M+H]^+$ calcd. 479.1957 for $C_{25}H_{27}ClN_6O_2$, found 479.1951

HPLC: eluent system 1: λ = 254 nm, t_R = 22.2 min, purity: 96.3%

eluent system 2: λ = 254 nm, t_R = 20.6 and 20.9 min, rotamers, purity: 96.4%

eluent system 3: λ = 254 nm, t_R = 22.2 min, purity: 96.6%

eluent system 4: λ = 254 nm, t_R = 20.5 and 20.7 min, rotamers, purity: 95.9%

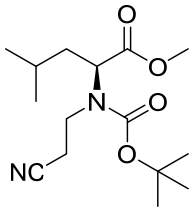
$[\alpha]_D^{25}$: + 42.5° (c = 0.14 in methanol)

^{1}H NMR: (600 MHz, $CDCl_3$, four sets of signals were observed, rotamers, assignments of signals analog to compound **2** (**JH112**)) δ 7.90 (d, *J* = 8.4 Hz, 0.2H), 7.88 (d, *J* = 8.3 Hz, 0.3H), 7.84 (d, *J* = 8.3 Hz, 0.3H), 7.82 (d, *J* = 8.4 Hz, 0.3H), 7.77 (s, 0.5H), 7.73 (s, 0.5H), 7.47 (s, 0.4H), 7.35 – 7.29 (m, 1.5H), 7.32 – 7.26 (m, 0.6H), 7.23 – 7.10 (m, 1.7H), 7.07 (d, *J* = 0.9 Hz, .2H), 7.00 (ddd, *J* = 8.4, 5.3, 2.1 Hz, 1.0H), 6.97 (d, *J* = 0.9 Hz, 0.3H), 6.92 (dd, *J* = 8.4, 2.1 Hz, 0.3H), 6.37 (d, *J* = 1.0 Hz, 0.3H), 5.19 – 5.11 (m, 0.2H), 5.06 (dq, *J* = 11.6, 5.9 Hz, 0.3H), 4.64 (dt, *J* = 14.0, 3.5 Hz, 0.3H), 4.48 (dd, *J* = 13.9, 3.3 Hz, 0.3H), 4.35 (dd, *J* = 14.3, 4.9 Hz, 0.3H), 4.24 – 4.09 (m, 1.2H), 4.00 (dd, *J* = 15.1, 4.4 Hz, 0.5H), 3.93 – 3.70 (m, 1.2H), 3.51 – 3.40 (m, 1.3H), 3.37 (ddd, *J* = 14.7, 10.8, 4.3 Hz, 0.3H), 3.20 (ddd, *J* = 14.4, 12.3, 4.0 Hz, 0.5H), 3.11 (ddd, *J* = 15.3, 11.5, 2.4 Hz, 0.3H), 3.05 (dd, *J* = 15.0, 9.6 Hz, 0.3H), 2.99 (ddd, *J* = 13.7, 11.6, 1.8 Hz, 0.3H), 2.92 (td, *J* = 13.8, 11.5, 1.7 Hz, 0.3H), 2.41 (s, 0.5H), 2.41 (s, 1.0H), 2.37 (s, 1.0H), 2.36 – 2.31 (m, 0.4H),

1.97 (dd, *J* = 13.4, 3.3 Hz, 0.3H), 1.83 (s, 0.4H), 1.80 – 1.66 (m, 0.5H), 1.64 – 1.60 (m, 0.5H), 1.57 – 1.43 (m, 1.5H), 1.36 – 1.12 (m, 1.3H), 1.08 – 1.02 (m, 0.3H), 1.00 (t, *J* = 7.2 Hz, 1.6H), 0.80 (t, *J* = 7.3 Hz, 1.0H), 0.63 (t, *J* = 7.3 Hz, 0.9H), 0.60 – 0.53 (m, 0.3H).

^{13}C NMR: (151 MHz, $CDCl_3$, four sets of signals were observed, rotamers, assignments of signals analog to compound **2** (**JH112**)) δ 169.9, 169.7, 169.7, 169.4, 162.8, 162.7, 162.5, 161.9, 147.6, 147.5, 147.5, 147.3, 144.9, 144.8, 144.7, 144.6, 138.5, 138.4, 138.1, 137.6, 135.5, 135.5, 135.1, 134.0, 133.7, 133.4, 133.3, 130.4, 130.4, 130.3, 130.3, 129.6, 129.5, 129.4, 129.4, 129.2, 129.0, 128.8, 128.6, 128.6, 128.4, 128.4, 128.3, 122.5, 122.0, 122.0, 121.8, 120.4, 120.4, 120.3, 120.0, 116.5, 116.3, 116.2, 116.1, 109.5, 109.4, 109.3, 109.0, 56.2, 55.3, 53.5, 52.3, 52.3, 52.2, 52.1, 51.9, 50.9, 49.9, 49.6, 49.0, 43.8, 42.9, 40.4, 39.6, 34.5, 34.1, 32.6, 32.4, 28.0, 27.3, 26.9, 26.6, 21.0, 21.0, 20.9, 20.4, 19.3, 19.1, 18.8, 18.6, 14.4, 14.2, 13.9, 13.9.

Methyl *N*-(*tert*-butoxycarbonyl)-*N*-(2-cyanoethyl)-*L*-leucinate (26g)



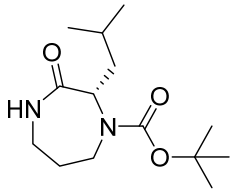
Methyl *L*-leucinate hydrochloride (3.00 g, 16.51 mmol) was dissolved in dry methanol (30 mL) in a dried flask. DIPEA (4.31 mL, 24.77 mmol) was added and the reaction mixture was stirred at ambient temperature for 10 minutes. Acrylonitrile (2.70 mL, 41.29 mmol) was added dropwise over 30 minutes and the reaction mixture was stirred under reflux overnight. After cooling to ambient temperature, a solution of di-*tert*-butyldicarbonat (7.21 g, 33.03 mmol) in methanol (20 mL) was added and the reaction mixture was stirred under reflux overnight. The solvent was evaporated. The residue was taken up in ethyl acetate and washed with water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 10% to 50% ethyl acetate in *n*-hexane. The product was obtained as pale yellow oil (1.95 g, 6.54 mmol, 40%).

ESI-MS: *m/z* 321.2 $[M+Na]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 4.84 – 4.56, 4.48 – 4.28 (m, 1H), 3.73, 3.72 (s, 3H), 3.67 – 3.57, 3.57 – 3.26 (m, 2H), 2.86 – 2.54 (m, 2H), 1.79 – 1.69 (m, 1H), 1.49 and1.44 (s, 9H), 0.97 (d, *J* = 6.3 Hz, 3H), 0.95 (d, *J* = 6.3 Hz, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 172.8, 172.4, 155.0, 154.8, 118.3, 118.0, 81.6, 81.4, 58.5, 56.9, 52.3, 42.7, 41.2, 39.1, 38.5, 28.2, 25.0, 24.8, 23.1, 21.6, 17.9, 16.9.

***Tert*-butyl (*S*)-2-isobutyl-3-oxo-1,4-diazepane-1-carboxylate (27g)**



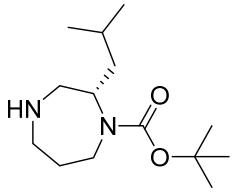
26g (1.94 g, 6.48 mmol) was dissolved in ethanol (20 mL). Platinum dioxide (0.27 g, 1.18 mmol) was added and the reaction mixture was stirred at ambient temperature under hydrogen atmosphere for 20 h. The reaction mixture was filtered over celite and the volatiles were evaporated. The residue was dissolved in THF (10 mL) and methanol (5 mL). A 4 M aqueous solution of lithium hydroxide monohydrate (1.94 mL, 7.78 mmol) was added and the reaction mixture was stirred at ambient temperature for 18 h. The solvents were evaporated and the residue was taken up in a mixture of toluene and methanol (1 : 4, 10 mL). The volatiles were evaporated and the residue was dissolved in DMF (25 mL). DIPEA (2.23 mL, 12.83 mmol) and subsequently HATU (4.88 g, 12.83 mmol) were added to the solution. The reaction mixture was stirred at ambient temperature for 20 h. The reaction was quenched by addition of saturated aqueous solution of ammonium chloride (50 mL). The product was extracted with ethyl acetate and the combined organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 25% to 75% ethyl acetate in *n*-hexane. The product was obtained as yellow oil (0.46 g, 1.70 mmol, 27%).

ESI-MS: *m/z* 293.1 $[M+Na]^+$

^{1}H NMR: (400 MHz, $CDCl_3$) δ 6.16 – 5.88 (m, 1H), 5.02 – 4.50 (m, 1H), 4.02 – 3.74, 3.37 – 3.02 (m, 4H), 2.08 – 1.67 (m, 4H), 1.67 – 1.54 (m, 1H), 1.47 (s, 9H), 0.96 (d, *J* = 6.6 Hz, 6H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 176.2, 155.4, 80.8, 58.6, 47.6, 40.3, 38.3, 28.4, 27.9, 24.4, 23.4, 21.3.

***Tert*-butyl (*S*)-2-isobutyl-1,4-diazepane-1-carboxylate (28g)**



27g (0.44 g, 1.63 mmol) was dissolved in a dried flask in THF (20 mL). A 1 M solution of a borane-THF-complex in THF (8.14 mL, 8.14 mmol) was added dropwise under ice cooling. The reaction mixture was stirred under reflux at 75 °C for 28 h. After cooling to ambient temperature, the reaction was quenched by dropwise addition of methanol (2 mL) under ice cooling. The reaction mixture was diluted with ethyl acetate (100 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 50 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 20% to 50% ethyl acetate in *n*-hexane. The product was obtained as pale yellow oil (0.17 g, 0.66 mmol, 41%).

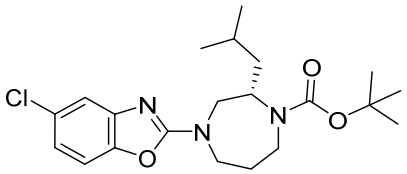
ESI-MS: *m/z* 257.0 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 4.44, 4.21 (dq, *J* = 12.8, 6.6 Hz, 1H), 4.03 – 3.92, 3.88 – 3.74 (m, 1H), 3.59 – 3.37 (m, 2H), 2.94 – 2.76 (m, 1H),

2.71 – 2.46 (m, 2H), 1.88 – 1.71 (m, 2H), 1.61 – 1.51, 1.36 – 1.27 (m, 2H), 1.49, 1.48 (s, 9H), 1.18 – 1.05 (m, 1H), 1.01 – 0.79 (m, 6H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 155.2, 154.8, 80.9, 80.4, 60.4, 60.3, 58.4, 58.2, 51.5, 50.5, 41.6, 41.2, 39.8, 39.1, 28.7, 28.4, 24.7, 24.5, 23.0, 22.8, 22.6, 22.6.

***Tert*-butyl (*S*)-2-isobutyl-4-(5-chlorobenzoxazol-2-yl)-1,4-diazepane-1-carboxylate (29g)**



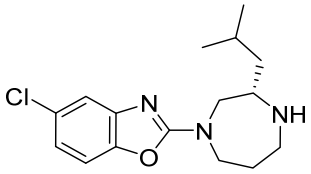
21 (92.5 mg, 0.60 mmol), **28g** (162.2 mg, 0.63 mmol) and copper(II) acetate hydrate (122.7 mg, 0.61 mmol) were dissolved in acetonitrile (20 mL). Concentrated acetic acid (0.07 mL, 1.20 mmol) was added and the reaction mixture was stirred under air at 80 °C for 41 h. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate (50 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 30 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 30% to 50% ethyl acetate in *n*-hexane. The product was obtained as brown oil (135.0 mg, 0.33 mmol, 55%).

ESI-MS: *m/z* 408.2 $[M+H]^+$

^{1}H NMR: (400 MHz, CDCl3, two sets of signals were observed, rotamers) δ 7.31, 7.28 (d, *J* = 2.0 Hz, 1H), 7.14 (d, *J* = 8.4 Hz, 1H), 6.96 (dd, *J* = 8.4, 2.0 Hz, 1H), 4.82 – 4.60 (m, 1H), 4.32 – 4.01 (m, 2H), 3.93 – 3.84, 3.76 – 3.66 (m, 1H), 3.38 – 3.26, 3.24 – 3.05 (m, 2H), 3.01 – 2.78 (m, 2H), 2.27 – 2.10 (m, 1H), 1.78 – 1.55 (m, 3H), 1.52 – 1.34, 1.18 – 1.13 (m, 2H), 1.31, 1.12 (s, 9H), 0.97, 0.94 (d, *J* = 6.6 Hz, 6H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 162.5, 162.3, 155.3, 155.2, 147.5, 144.9, 144.7, 129.3, 129.1, 120.1, 116.2, 116.1, 109.3, 109.1, 79.7, 79.6, 54.3, 53.8, 51.0, 50.7, 50.6, 50.5, 40.8, 40.6, 40.3, 28.2, 27.9, 26.6, 26.2, 25.0, 24.8, 23.4, 23.0, 22.6, 22.4.

(*S*)-5-chloro-2-(3-isobutyl-1,4-diazepan-1-yl)benzoxazole (30g)



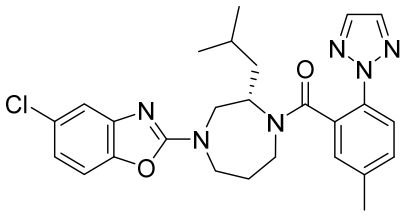
29g (135.0 mg, 0.33 mmol) was dissolved in dichloromethane (5 mL). Trifluoroacetic acid (0.3 mL) was added and the reaction mixture was stirred at ambient temperature for 7 h. The reaction mixture was basified to pH 11 using 1 M aqueous NaOH solution. The product was extracted with dichloromethane (3x). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The product was obtained as yellow oil (92.0 mg, 0.30 mmol, 90%) and used for the next step without purification.

ESI-MS: *m/z* 308.0 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.29 (d, *J* = 2.1 Hz, 1H), 7.13 (d, *J* = 8.4 Hz, 1H), 6.94 (dd, *J* = 8.4, 2.1 Hz, 1H), 3.98 (dd, *J* = 13.8, 3.1 Hz, 1H), 3.95 – 3.87 (m, 1H), 3.67 (ddd, *J* = 14.1, 7.3, 5.5 Hz, 1H), 3.24 – 3.05 (m, 2H), 2.92 (dt, *J* = 6.6, 3.3 Hz, 1H), 2.72 – 2.57 (m, 1H), 2.10 – 1.96 (m, 1H), 1.95 – 1.81 (m, 1H), 1.82 – 1.67 (m, 1H), 1.30 (t, *J* = 7.0 Hz, 2H), 0.97 (d, *J* = 6.6 Hz, 3H), 0.94 (d, *J* = 6.6 Hz, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 163.2, 147.5, 145.1, 129.2, 119.8, 116.0, 109.0, 57.3, 56.4, 47.5, 47.0, 43.0, 29.8, 24.9, 23.2, 22.3.

(*S*)-[4-(5-chlorobenzoxazol-2-yl)-2-isobutyl-1,4-diazepan-1-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone (7)



30g (82.3 mg, 0.27 mmol), **24** (66.6 mg, 0.33 mmol) and HATU (206.7 mg, 0.54 mmol) were dissolved in dimethylformamide (dry, 10 mL). DIPEA (0.23 mL, 1.34 mmol) was added and the reaction mixture was stirred at ambient temperature for 3 h. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution (3x) and brine (1x). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified using a preparative HPLC (gradient of 5% to 95% methanol in water with 0.1% formic acid). The product was obtained as white powder after lyophilization of the product containing fractions (68.4 mg, 0.14 mmol, 52%).

ESI-MS: *m/z* 493.2 $[M+H]^+$

HR-ESI-MS: *m/z* $[M+H]^+$ calcd. 493.2113 for $C_{26}H_{29}ClN_6O_2$, found 493.2107

HPLC: eluent system 2: λ = 254 nm, t_R = 21.1 and 21.4 min, rotamers, purity: 97.8%

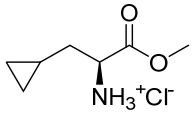
eluent system 3: λ = 254 nm, t_R = 22.6 min, purity: 98.7%

$[\alpha]_D^{25}$: + 41.6° (c = 0.11 in methanol)

^{1}H NMR: (600 MHz, $CDCl_3$, four sets of signals were observed, rotamers, assignments of signals analog to compound **2** (**JH112**)) δ 7.90 (d, *J* = 8.3 Hz, 0.2H), 7.89 (d, *J* = 8.3 Hz, 0.3H), 7.87 (d, *J* = 8.4 Hz, 0.3H), 7.84 (d, *J* = 8.3 Hz, 0.2H), 7.76 (s, 0.5H), 7.71 (s, 0.4H), 7.50 (s, 0.3H), 7.33 (d, *J* = 2.0 Hz, 0.4H), 7.34 – 7.30 (m, 0.5H), 7.30 (s, 0.7H), 7.31 – 7.26 (m, 0.5H), 7.23 – 7.14 (m, 1.3H), 7.13 (d, *J* = 8.4 Hz, 0.3H), 7.05 (d, *J* = 0.8 Hz, 0.2H), 7.04 – 6.97 (m, 1.2H), 6.93 (dd, *J* = 8.4, 2.1 Hz, 0.3H), 6.36 (d, *J* = 0.9 Hz, 0.2H), 5.17 (dt, *J* = 52.1, 6.3 Hz, 0.4H), 4.62 (dt, *J* = 14.0, 3.4 Hz, 0.4H), 4.47 (dt, *J* = 13.9, 3.6 Hz, 0.3H), 4.37 (dd, *J* = 14.3, 5.0 Hz, 0.3H), 4.27 – 4.12 (m, 0.9H), 4.10 – 4.03 (m, 0.5H), 4.00 (dd, *J* = 15.0, 4.3 Hz, 0.2H), 3.93 – 3.81 (m, 0.9H), 3.75 (dd, *J* = 15.2, 5.7 Hz, 0.4H), 3.55 – 3.34 (m, 1.6H), 3.25 – 3.16 (m, 0.5H), 3.09 (ddd, *J* = 15.1, 11.4, 2.9 Hz, 0.3H), 3.03 – 2.95 (m, 0.6H), 2.93 (ddd, *J* = 13.7, 11.8, 1.5 Hz, 0.3H), 2.41 (s, 0.6H), 2.40 (s, 1.1H), 2.37 (s, 0.7H), 2.36 – 2.31 (m, 0.4H), 2.00 – 1.94 (m, 0.4H), 1.83 (s, 0.5H), 1.82 – 1.60 (m, 2.0H), 1.50 (ddd, *J* = 10.7, 8.4, 5.5 Hz, 0.5H), 1.46 – 1.19 (m, 1.6H), 1.08 – 0.99 (m, 2.5H), 0.83 (d, *J* = 6.5 Hz, 1.1H), 0.64 (d, *J* = 6.5 Hz, 0.8H), 0.57 (d, *J* = 6.5 Hz, 1.1H), 0.49 (d, *J* = 6.5 Hz, 0.8H), 0.40 (ddd, *J* = 13.6, 10.6, 3.4 Hz, 0.3H).

^{13}C NMR: (151 MHz, $CDCl_3$, four sets of signals were observed, rotamers, assignments of signals analog to compound **2** (**JH112**)) δ 169.8, 169.6, 169.5, 169.4, 162.8, 162.6, 162.5, 161.9, 147.6, 147.5, 147.5, 147.3, 144.9, 144.8, 144.7, 138.4, 138.2, 138.1, 137.6, 135.5, 135.4, 135.4, 135.1, 134.1, 133.7, 133.4, 133.3, 130.4, 130.4, 130.3, 130.3, 129.6, 129.5, 129.4, 129.4, 129.2, 129.0, 128.7, 128.5, 128.3, 128.2, 128.1, 122.6, 122.0, 121.7, 121.7, 120.4, 120.4, 120.2, 120.0, 116.6, 116.3, 116.2, 116.1, 109.4, 109.3, 108.9, 54.9, 53.8, 53.5, 52.6, 52.1, 50.8, 50.7, 50.5, 49.7, 49.5, 48.9, 43.7, 42.6, 41.6, 40.7, 40.2, 39.5, 39.4, 39.2, 27.8, 27.3, 27.0, 26.7, 25.1, 24.7, 24.6, 24.2, 23.9, 23.9, 23.3, 23.0, 22.8, 22.6, 21.0, 21.0, 20.9, 20.8, 20.4, 20.4.

Methyl (*S*)-2-amino-3-cyclopropylpropanoate hydrochloride (25h)



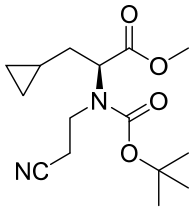
(*S*)-2-amino-3-cyclopropylpropanoic acid (4.97 g, 38.48 mmol) was dissolved in methanolic hydrochloride solution (1.25 M, 153.92 mL, 0.19 mol) and stirred at ambient temperature for 24 h. The pure product was obtained as white powder after evaporation of the volatiles (6.56 g, 36.54 mmol, 95%).

ESI-MS: *m/z* 144.0 [M, cation]$^+$

^{1}H NMR: (400 MHz, $CDCl_3$) δ 9.07 – 8.60 (m, 3H), 4.31 – 4.14 (m, 1H), 3.82 (s, 3H), 2.10 – 1.90 (m, 1H), 1.03 – 0.88 (m, 1H), 0.69 – 0.49 (m, 2H), 0.28 – 0.12 (m, 2H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 169.8, 53.8, 53.1, 35.0, 6.6, 4.7, 4.6.

Methyl (*S*)-2-[(*tert*-butoxycarbonyl)(2-cyanoethyl)amino]-3-cyclopropylpropanoate (26h)



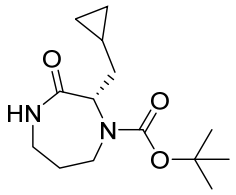
25h (6.54 g, 36.43 mmol) was dissolved in dry methanol (50 mL) in a dried flask. DIPEA (9.45 mL, 54.64 mmol) was added and the reaction mixture was stirred at ambient temperature for 10 minutes. Acrylonitrile (4.77 mL, 72.86 mmol) was added dropwise over 30 minutes and the reaction mixture was stirred under reflux overnight. After cooling to ambient temperature, a solution of di-*tert*-butyldicarbonat (15.90 g, 72.86 mmol) in methanol (40 mL) was added and the reaction mixture was stirred under reflux overnight. The solvent was evaporated. The residue was taken up in ethyl acetate and washed with water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 20% to 50% ethyl acetate in *n*-hexane. The product was obtained as pale yellow oil (5.51 g, 18.59 mmol, 51%).

ESI-MS: *m/z* 319.0 [M+Na]$^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 4.50 – 4.39, 4.17 – 4.06 (m, 1H), 3.73 (s, 3H), 3.83 – 3.34 (m, 2H), 2.86 – 2.69 (m, 2H), 1.99 – 1.63 (m, 2H), 1.50, 1.43 (s, 9H), 0.80 – 0.64 (m, 1H), 0.59 – 0.40 (m, 2H), 0.22 – 0.05 (m, 2H).

^{13}C NMR: (151 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 172.1, 171.7, 154.7, 154.5, 118.3, 118.0, 81.5, 81.4, 61.7, 60.2, 52.2, 44.6, 43.0, 35.3, 34.6, 28.3, 28.2, 17.8, 17.0, 8.2, 5.1, 4.9, 4.2.

***Tert*-butyl (*S*)-2-(cyclopropylmethyl)-3-oxo-1,4-diazepane-1-carboxylate (27h)**



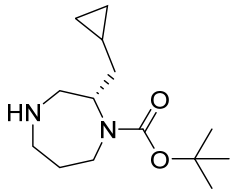
26h (5.49 g, 18.52 mmol) was dissolved in ethanol (50 mL). Platinum dioxide (0.32 g, 1.39 mmol) was added and the reaction mixture was stirred at ambient temperature under hydrogen atmosphere for 92 h. The reaction mixture was filtered over celite and the volatiles were evaporated. The residue was dissolved in THF (30 mL) and methanol (15 mL). A 4 M aqueous solution of lithium hydroxide monohydrate (6.93 mL, 27.71 mmol) was added and the reaction mixture was stirred at ambient temperature for 20 h. The solvents were evaporated and the residue was taken up in a mixture of toluene and methanol (1 : 4, 15 mL). The volatiles were evaporated and the residue was dissolved in DMF (130 mL). DIPEA (6.39 mL, 36.94 mmol) and subsequently HATU (8.43 g, 22.17 mmol) were added to the solution. The reaction mixture was stirred at ambient temperature for 19 h. The reaction was quenched by addition of saturated aqueous solution of ammonium chloride (100 mL). The product was extracted with ethyl acetate and the combined organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 25% to 75% ethyl acetate in *n*-hexane. The product was obtained as yellow oil (2.09 g, 7.79 mmol, 42%).

ESI-MS: *m/z* 269.0 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 6.21 (s, 1H), 5.07 – 4.51 (m, 1H), 4.03 – 3.74 (m, 1H), 3.45 – 3.29 (m, 1H), 3.29 – 3.03 (m, 2H), 2.12 – 1.64 (m, 4H), 1.47 (s, 9H), 0.82 – 0.67 (m, 1H), 0.56 – 0.35 (m, 2H), 0.20 – 0.05 (m, 2H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 175.1, 174.8, 155.2, 154.9, 80.6, 80.1, 60.9, 60.0, 47.1, 46.4, 39.7, 38.9, 38.6, 35.1, 28.4, 27.5, 27.2, 23.4, 8.4, 7.9, 4.8, 4.5, 4.3.

***Tert*-butyl (*S*)-2-(cyclopropylmethyl)-1,4-diazepane-1-carboxylate (28h)**



27h (1.00 g, 3.73 mmol) was dissolved in a dried flask in THF (40 mL). A 1 M solution of a borane-THF-complex in THF (18.63 mL, 18.63 mmol) was added dropwise under ice cooling. The reaction mixture was stirred under reflux at 75 °C for 25 h. After cooling to ambient temperature, the reaction was quenched by dropwise addition of methanol (2 mL) under ice cooling. The reaction mixture was diluted with ethyl acetate (70 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 40 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The

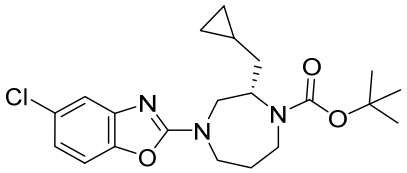
crude product purified by flash column chromatography applying a gradient of 20% to 50% ethyl acetate in *n*-hexane. The product was obtained as pale yellow oil (0.27 g, 1.06 mmol, 28%).

ESI-MS: *m/z* 255.0 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 4.50 – 4.38, 4.29 – 4.16 (m, 1H), 4.08 – 3.99, 3.89 – 3.79, 3.51 – 3.37 (m, 2H), 3.70 – 3.51 (m, 2H), 2.96 – 2.79, 2.73 – 2.57 (m, 2H), 1.89 – 1.72 (m, 2H), 1.48 (s, 9H), 1.44 – 1.29 (m, 2H), 0.69 – 0.57 (m, 1H), 0.54 – 0.42 (m, 2H), 0.10 – 0.02 (m, 2H).

^{13}C NMR: (151 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 155.1, 154.8, 80.6, 80.4, 59.9, 59.7, 58.3, 58.1, 54.2, 54.1, 40.2, 39.4, 37.3, 36.8, 29.1, 28.9, 28.5, 28.4, 7.4, 7.3, 4.9, 4.7, 4.4, 4.2.

Tert-butyl (*S*)-4-(5-chlorobenzoxazol-2-yl)-2-(cyclopropylmethyl)-1,4-diazepane-1-carboxylate (29h)



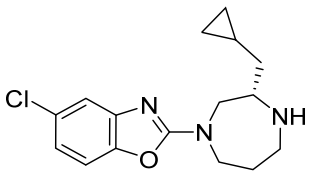
21 (0.15 mg, 0.97 mmol), **28h** (0.26 g, 1.02 mmol) and copper(II) acetate hydrate (0.25 g, 1.27 mmol) were dissolved in acetonitrile (20 mL). Concentrated acetic acid (0.11 mL, 1.95 mmol) was added and the reaction mixture was stirred under air at 80 °C for 23 h. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate (70 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 50 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 25% to 50% ethyl acetate in *n*-hexane. The product was obtained as brown oil (0.20 g, 0.50 mmol, 52%).

ESI-MS: *m/z* 406.1 $[M+H]^+$

^{1}H NMR: (600 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 7.35 – (m, 1H), 7.15 (d, *J* = 8.4 Hz, 1H), 6.99 – 6.92 (m, 1H), 4.85 – 4.64 (m, 1H), 4.32 – 4.14 (m, 2H), 4.00 – 3.91, 3.83 – 3.74, 3.32 – 3.20, 3.16 – 3.06 (m, 2H), 3.04 – 2.92, 2.92 – 2.81 (m, 2H), 2.21 – 2.05, 1.78 – 1.61 (m, 2H), 1.46 – 1.27 (m, 2H), 1.33, 1.15 (s, 9H), 0.78 – 0.64 (m, 1H), 0.55 – 0.40 (m, 2H), 0.15 – 0.03 (m, 2H).

^{13}C NMR: (151 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 162.5, 162.3, 155.3, 155.2, 147.6, 147.5, 144.9, 144.7, 129.2, 129.1, 120.2, 120.1, 116.2, 116.2, 109.3, 109.1, 79.6, 79.5, 53.9, 53.2, 53.4, 52.9, 51.1, 50.5, 41.4, 40.6, 36.4, 36.2, 28.3, 28.0, 27.3, 26.8, 7.9, 7.7, 4.7, 4.6, 4.5, 4.3.

(*S*)-5-chloro-2-[3-(cyclopropylmethyl)-1,4-diazepan-1-yl]benzoxazole (30h)



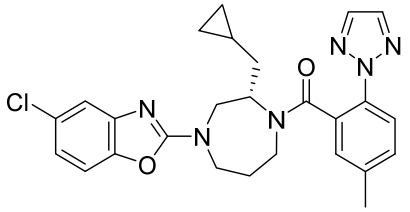
29h (0.19 g, 0.48 mmol) was dissolved in dichloromethane (3 mL). Trifluoroacetic acid (0.3 mL) was added and the reaction mixture was stirred at ambient temperature for 27 h. The reaction mixture was basified to pH 11 using 1 M aqueous NaOH solution. The product was extracted with dichloromethane (3x). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure to obtain the pure product as brown oil (0.14 g, 0.46 mmol, 96%).

ESI-MS: *m/z* 306.0 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.30 (d, *J* = 2.1 Hz, 1H), 7.13 (d, *J* = 8.4 Hz, 1H), 6.94 (dd, *J* = 8.4, 2.1 Hz, 1H), 4.08 (dd, *J* = 13.8, 3.0 Hz, 1H), 4.01 – 3.87 (m, 1H), 3.71 – 3.58 (m, 1H), 3.27 – 3.07 (m, 2H), 3.06 – 2.90 (m, 1H), 2.75 – 2.57 (m, 1H), 2.14 – 1.99 (m, 1H), 1.97 – 1.72 (m, 1H), 1.58 – 1.45 (m, 1H), 1.35 – 1.18 (m, 2H), 0.81 – 0.69 (m, 1H), 0.63 – 0.41 (m, 2H), 0.23 – 0.04 (m, 2H).

^{13}C NMR: (151 MHz, $CDCl_3$) δ 163.2, 147.5, 145.0, 129.2, 119.9, 116.1, 109.0, 60.4, 55.9, 47.5, 47.0, 38.5, 29.7, 29.4, 7.9, 5.1, 3.9.

(*S*)-[4-(5-chlorobenzoxazol-2-yl)-2-(cyclopropylmethyl)-1,4-diazepan-1-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone (8)



30h (0.13 g, 0.43 mmol), **24** (0.11 g, 0.52 mmol) and HATU (0.20 g, 0.52 mmol) were dissolved in dimethylformamide (dry, 5 mL). DIPEA (0.38 mL, 2.17 mmol) was added and the reaction mixture was stirred at ambient temperature for 17 h. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution (3x) and brine (1x). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified using a preparative HPLC (gradient of 5% to 95% methanol in water with 0.1% formic acid). The product was obtained as white powder after lyophilization of the product containing fractions (0.16 g, 0.32 mmol, 74%).

ESI-MS: *m/z* 491.2 $[M+H]^+$

HR-ESI-MS: *m/z* $[M+H]^+$ calcd. 491.1957 for $C_{26}H_{27}ClN_6O_2$, found 491.1950

HPLC: eluent system 1: λ = 254 nm, t_R = 22.2 min, purity: 97.1%

eluent system 2: λ = 254 nm, t_R = 20.4 and 20.7 min, rotamers, purity: 98.6%

eluent system 3: λ = 254 nm, t_R = 22.1 min, purity: 98.6%

eluent system 4: λ = 254 nm, t_R = 20.3 and 20.6 min, rotamers, purity: 98.7%

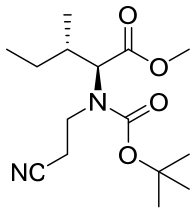
$[\alpha]_D^{21}$: + 29.9° (c = 0.21 in methanol)

^{1}H NMR: (600 MHz, $CDCl_3$, four sets of signals were observed, rotamers, assignments of signals analog to compound **2** (**JH112**)) δ 7.90 (d, *J* = 8.3 Hz, 0.2H), 7.89 (d, *J* = 8.3 Hz, 0.3H), 7.85 (d, *J* = 8.2 Hz, 0.2H), 7.83 (d, *J* = 8.2 Hz, 0.3H), 7.74 (s, 0.5H), 7.71 (s, 0.3H), 7.37 (s, 0.3H), 7.35 – 7.32 (m, 0.7H), 7.31 (s, 0.6H), 7.31 – 7.27 (m, 0.6H), 7.23 – 7.13 (m, 1.6H), 7.08 (d, *J* = 1.0 Hz, 0.2H), 7.05 – 6.98 (m, 1.1H), 6.93 (dd, *J* = 8.4, 2.1 Hz, 0.3H),

6.34 (d, *J* = 0.9 Hz, 0.3H), 5.26 – 5.14 (m, 0.1H), 5.08 (tt, *J* = 9.9, 5.2 Hz, 0.2H), 4.64 (dt, *J* = 14.0, 3.5 Hz, 0.3H), 4.48 (dt, *J* = 13.9, 3.4 Hz, 0.3H), 4.44 (dd, *J* = 15.2, 5.8 Hz, 0.2H), 4.40 (dd, *J* = 14.5, 5.1 Hz, 0.3H), 4.33 (dd, *J* = 15.1, 6.5 Hz, 0.3H), 4.27 (dt, *J* = 14.1, 4.2 Hz, 0.2H), 4.24 – 4.15 (m, 0.5H), 4.15 – 4.04 (m, 0.3H), 4.00 (dt, *J* = 14.1, 4.3 Hz, 0.2H), 3.97 – 3.89 (m, 0.7H), 3.78 (dt, *J* = 15.3, 3.8 Hz, 0.1H), 3.68 (dd, *J* = 15.1, 9.0 Hz, 0.1H), 3.59 – 3.41 (m, 1.1H), 3.37 – 3.23 (m, 0.5H), 3.23 – 3.10 (m, 0.7H), 3.10 – 2.93 (m, 0.6H), 2.40 (s, 1.6H), 2.37 (s, 0.9H), 2.36 – 2.29 (m, 0.3H), 2.01 – 1.93 (m, 0.4H), 1.93 – 1.84 (m, 0.3H), 1.82 (s, 0.7H), 1.75 (ddd, *J* = 13.7, 7.0, 4.5 Hz, 0.3H), 1.71 – 1.57 (m, 2.0H), 1.57 – 1.42 (m, 1.3H), 1.15 (ddd, *J* = 14.0, 9.6, 6.7 Hz, 0.3H), 0.92 – 0.80 (m, 0.4H), 0.66 – 0.53 (m, 1.1H), 0.53 – 0.46 (m, 0.6H), 0.46 – 0.27 (m, 1.2H), 0.26 – 0.19 (m, 0.3H), 0.19 – 0.11 (m, 0.3H), 0.06 – -0.01 (m, 0.4H), -0.18 (tt, *J* = 7.7, 4.7 Hz, 0.6H), -0.27 (dq, *J* = 9.6, 5.0 Hz, 0.3H).

^{13}C NMR: (151 MHz, $CDCl_3$, four sets of signals were observed, rotamers, assignments of signals analog to compound **2** (**JH112**)) δ 169.7, 169.5, 169.4, 162.6, 162.6, 162.4, 161.9, 147.6, 147.5, 147.5 147.3, 144.8, 144.8, 144.8, 144.6, 138.5, 138.4, 138.1, 137.5, 135.4, 135.1, 134.0, 133.7, 133.4, 133.4, 130.4, 130.4 130.3, 129.5, 129.5, 129.4, 129.4 129.2, 128.9, 128.8, 128.5, 128.4, 128.4, 128.3, 128.2, 122.5, 121.9, 121.9, 121.8, 120.4, 120.3, 120.0, 116.6, 116.3, 116.2, 116.1, 109.5, 109.4, 109.0, 57.0, 55.9, 53.4, 53.1, 52.5, 52.0, 51.8, 51.0, 50.0, 49.7, 49.3, 44.4, 43.6, 40.7, 39.8, 37.1, 36.4, 35.5, 34.7, 28.7, 27.6, 27.2, 26.6, 21.0, 21.0, 20.9, 20.4, 7.5, 7.4, 7.1, 6.7, 5.6, 5.5, 5.4, 4.9, 4.6, 4.5, 4.2, 3.8.

Methyl *N*-(*tert*-butoxycarbonyl)-*N*-(2-cyanoethyl)-*L*-isoleucinate (26i)



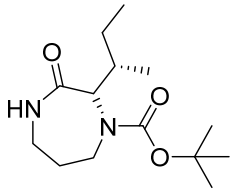
Methyl *L*-isoleucinate hydrochloride (14.30 g, 78.72 mmol) was dissolved in dry methanol (200 mL) in a dried flask. DIPEA (27.24 mL, 0.16 mol) was added and the reaction mixture was stirred at ambient temperature for 10 minutes. Acrylonitrile (6.19 mL, 94.46 mmol) was added dropwise over 30 minutes and the reaction mixture was stirred under reflux overnight. After cooling to ambient temperature, a solution of di-*tert*-butyldicarbonat (34.36 g, 0.16 mol) in methanol (100 mL) was added and the reaction mixture was stirred under reflux overnight. The solvent was evaporated. The residue was taken up in ethyl acetate and washed with saturated aqueous solution of citric acid and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 5% to 30% ethyl acetate in isohexane. The product was obtained as white needles (5.40 g, 18.10 mmol, 23%).

ESI-MS: *m/z* 321.1 $[M+Na]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 4.48, 4.08 (d, *J* = 10.2, 10.0 Hz, 1H), 3.73 (s, 3H), 3.67 – 3.43 (m, 2H), 2.81 – 2.51 (m, 2H), 2.09 – 1.82 (m, 1H), 1.49, 1.45 (s, 9H), 1.42 – 1.24 (m, 1H), 1.17 – 1.00 (m, 1H), 1.00 – 0.81 (m, 6H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 172.4, 171.6, 155.0, 154.8, 118.1, 117.9, 81.6, 81.5, 64.3, 62.1, 52.0, 41.8, 40.4, 34.8, 34.6, 28.2, 25.1, 17.6, 16.7, 16.2, 15.7, 10.8.

***Tert*-butyl (*S*)-2-[(*S*)-*sec*-butyl]-3-oxo-1,4-diazepane-1-carboxylate (27i)**



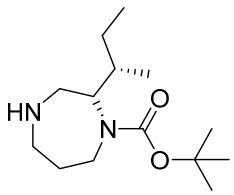
26i (26.64 g, 89.28 mmol) was dissolved in ethanol (300 mL) and chloroform (20 mL). Platinum dioxide (1.42 g, 6.25 mmol) was added and the reaction mixture was stirred at ambient temperature under hydrogen atmosphere for 28 h. The reaction mixture was filtered over celite and the volatiles were evaporated. The residue was dissolved in THF (100 mL) and methanol (70 mL). A 4 M aqueous solution of lithium hydroxide monohydrate (35.71 mL, 0.14 mol) was added and the reaction mixture was stirred at ambient temperature for 3 days. The solvents were evaporated and the residue was taken up in a mixture of toluene and methanol (1 : 4, 100 mL). The volatiles were evaporated and the residue was dissolved in DMF (500 mL). HATU (67.45 g, 0.18 mol) was dissolved in DMF (500 mL) and both solutions were added dropwise over 3 h two a 3-necked flask at ambient temperature. After complete addition, the reaction mixture was stirred at ambient temperature for 2 h. The reaction mixture was concentrated by the evaporation of the solvent, taken up in ethyl acetate and washed with saturated aqueous solution of citric acid (200 mL). The aqueous layer was extracted with ethyl acetate and the combined organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 25% to 75% ethyl acetate in isohexane. The product was obtained as colorless gum (18.15 g, 67.11 mmol, 76%).

ESI-MS: *m/z* 293.0 $[M+Na]^+$

^{1}H NMR: (600 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 6.26 – 5.76 (m, 1H), 4.80 – 3.66 (m, 1H), 3.39 – 3.18 (m, 3H), 2.35 – 2.17 (m, 1H), 1.90 – 1.66 (m, 3H), 1.56 – 1.48 (m, 1H), 1.46 (s, 9H), 1.15 – 1.06 (m, 1H), 1.01 (d, *J* = 6.7 Hz, 3H), 0.91 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 175.4, 155.3, 80.9, 80.4, 65.4, 60.4, 41.1, 40.3, 33.4, 32.0, 28.3, 28.3, 25.2, 25.0, 15.7, 14.2, 11.1, 10.3.

***Tert*-butyl (*S*)-2-[(*S*)-*sec*-butyl]-1,4-diazepane-1-carboxylate (28i)**

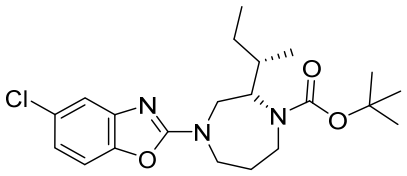


27i (13.64 g, 50.45 mmol) was dissolved in a dried flask in THF (250 mL). A 1 M solution of a borane-THF-complex in THF (0.25 L, 0.25 mol) was added dropwise under ice cooling. The reaction mixture was stirred under reflux at 75 °C for 7 h. After cooling to ambient temperature, the reaction was quenched by dropwise addition of 0.1 M HCl (400 mL) under ice cooling. The quenched reaction mixture was basified to pH = 10 with aqueous NaOH solution and extracted with MTBE (5 x 200 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure

(400 mbar at 40 °C, compound is volatile). The crude product was used for the next step without purification.

ESI-MS: *m/z* 257.0 $[M+H]^+$

Tert-butyl (_S_)-2-[(_S_)-_sec_-butyl]-4-(5-chlorobenzoxazol-2-yl)-1,4-diazepane-1-carboxylate (29i)



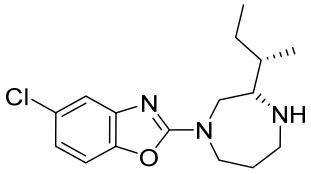
21 (7.37 g, 47.99 mmol), **28i** (12.92 g, 50.39 mmol) and copper(II) acetate hydrate (9.58 g, 47.99 mmol) were dissolved in acetonitrile (300 mL). Concentrated acetic acid (5.49 mL, 95.99 mmol) was added and the reaction mixture was stirred under air at 80 °C for 49 h. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate (500 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 300 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 20% to 50% ethyl acetate in isohexane. The product was obtained as brown gum (3.57 g, 8.76 mmol, 18% over two steps).

ESI-MS: *m/z* 408.1 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 7.33 – 7.27 (m, 1H), 7.15 (dd, *J* = 8.4, 3.4 Hz, 1H), 6.95 (ddd, *J* = 8.5, 6.5, 2.1 Hz, 1H), 4.50 – 4.15 (m, 3H), 4.00 – 3.86, 3.81 – 3.70 (m, 2H), 3.33 – 2.76 (m, 3H), 2.29 – 2.11 (m, 1H), 1.74 – 1.63, 1.60 – 1.41 (m, 4H), 1.27, 1.09 (s, 9H), 1.02 – 0.95 (m, 3H), 0.93 – 0.85 (m, 3H).

^{13}C NMR: (151 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 162.5, 162.3, 155.4, 155.3, 147.6, 147.6, 144.9, 144.7, 129.2, 129.0, 120.1, 116.2, 116.1, 109.3, 109.0, 79.6, 52.9, 52.3, 51.3, 51.0, 50.9, 42.0, 41.3, 37.3, 36.7, 28.2, 27.9, 26.3, 25.9, 25.8, 15.3, 15.2, 14.1, 14.1, 11.1, 10.9.

2-{(_S_)-3-[(_S_)-_sec_-butyl]-1,4-diazepan-1-yl}-5-chlorobenzoxazole (30i)



29i (2.37 g, 5.81 mmol) was dissolved in dichloromethane (120 mL). Trifluoroacetic acid (4.45 mL) was added and the reaction mixture was stirred at ambient temperature for 6 h. The reaction mixture was basified to pH 11 using 1 M aqueous NaOH solution. The product was extracted with dichloromethane (3x). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The product was obtained as red oil (1.79 g, 5.81 mmol, quant.) and used for the next step without purification.

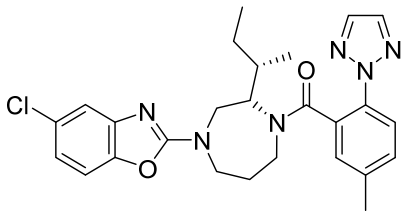
ESI-MS: *m/z* 308.0 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.29 (d, *J* = 2.1 Hz, 1H), 7.13 (d, *J* = 8.4 Hz, 1H), 6.94 (dd, *J* = 8.4, 2.1 Hz, 1H), 3.98 (dd, *J* = 13.9, 3.2 Hz, 1H), 3.86 (ddd, *J* = 13.4, 7.8, 5.2 Hz, 1H), 3.69

(ddd, *J* = 14.1, 6.5, 5.2 Hz, 1H), 3.36 – 3.16 (m, 2H), 2.75 (ddd, *J* = 10.4, 4.7, 3.3 Hz, 1H), 2.64 (ddd, *J* = 14.1, 10.7, 3.7 Hz, 1H), 2.08 – 1.95 (m, 1H), 1.95 – 1.81 (m, 1H), 1.61 – 1.48 (m, 2H), 1.36 – 1.27 (m, 1H), 1.00 (d, *J* = 6.9 Hz, 3H), 0.95 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR: (151 MHz, $CDCl_3$) δ 163.3, 147.5, 145.1, 129.1, 119.8, 116.0, 109.0, 66.8, 63.4, 52.8, 47.7, 38.9, 29.8, 25.9, 15.3, 11.8.

{(*S*)-2-[(*S*)-*sec*-butyl]-4-(5-chlorobenzoxazol-2-yl)-1,4-diazepan-1-yl} [5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone 2 (JH112)



30i (1.79 g, 5.81 mmol), **24** (1.42 g, 6.98 mmol) and HATU (2.65 g, 6.98 mmol) were dissolved in dimethylformamide (dry, 60 mL). DIPEA (5.03 mL, 29.08 mmol) was added and the reaction mixture was stirred at ambient temperature for 19 h. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution (3x) and brine (1x). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 20% to 50% ethyl acetate in isohexane. The product containing fractions were evaporated and taken up in 30% methanol in water and lyophilized to obtain the pure product as pale orange powder (1.73 g, 60%).

ESI-MS: *m/z* 493.1 $[M+H]^+$

HR-ESI-MS: *m/z* $[M+H]^+$ calcd. 493.2113 for $C_{26}H_{29}ClN_6O_2$, found 493.2103

HPLC: eluent system 1: λ = 254 nm, t_R = 22.3 and 22.5 min, rotamers, purity: 98.3%

eluent system 2: λ = 254 nm, t_R = 21.1 min, purity: 97.7%

eluent system 3: λ = 254 nm, t_R = 22.3 and 22.5 min, rotamers, purity: 98.7%

eluent system 4: λ = 254 nm, t_R = 21.0 min, purity: 97.6%

chiral HPLC: eluent system 6: λ = 254 nm, t_R = 20.8 and 45.2 min, rotamers, *ee:* 100%

$[\alpha]_D^{24}$: + 94.9° (c = 0.12 in methanol)

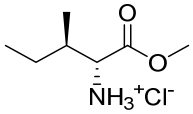
IR (NaCl): 2962, 2921, 2875, 2095, 1644, 1569, 1503, 1452, 1250, 961, 824 cm^{-1}

^{1}H NMR: (600 MHz, $CDCl_3$, four sets of signals were observed, rotamers, signals were assigned with the endorsement of 2D spectroscopy) δ {[7.90 (d, *J* = 8.3 Hz, 0.2H)], [7.89 (d, *J* = 8.3 Hz, 0.2H)], [7.88 (d, *J* = 8.3 Hz, 0.3H)], [7.81 (d, *J* = 8.3 Hz, 0.3H)], 1H (CH_toluene)}, {[7.80 (s, 0.1H)], [7.75 (s, 0.3H)], [7.73 (s, 0.6H)], [7.45 (s, 0.5H)], [7.24 (s, 0.5H)], 2H (CH_triazole)}, {[7.60 (d, *J* = 2.0 Hz, 0.0H)], [7.33 (d, *J* = 2.0 Hz, 0.2H)], [7.30 (dd, *J* = 8.4, 1.1 Hz, 0.4H)], [7.28 (dd, *J* = 7.8, 1.2 Hz, 0.4H)], 1H (CH_toluene)}, {[7.34 (d, *J* = 2.0 Hz, 0.6H)], [7.23 (d, *J* = 1.6 Hz, 0.3H)], 1H (CH_benzoxazole)}, {[7.19 (d, *J* = 8.4 Hz, 0.5H)], [7.16 (d, *J* = 8.3 Hz, 0.1H)], [7.14 (d, *J* = 8.4 Hz, 0.2H)], [7.02 (d, *J* = 8.4 Hz, 0.2H)], 1H (CH_benzoxazole)}, {[7.17 (d, *J* = 1.2 Hz, 0.2H)], [7.06 (d, *J* = 0.9 Hz, 0.2H)], [6.97 – 6.90 (m, 0.4H)], [6.33 (d, *J* = 1.2 Hz, 0.1H)], 1H (CH_toluene)}, {[7.03 – 6.97 (m, 0.4H)], [6.97 – 6.90 (m, 0.5H)], 1H (CH_benzoxazole)}, {[4.94 – 4.84 (m, 0.5H)], [4.17 – 4.07 (m,

0.2H)], [3.67 – 3.62 (m, 0.3H)] 1H (CH_homopiperazine)}, {[4.76 (dt, *J* = 14.0, 3.3 Hz, 0.3H)], [4.62 (dt, *J* = 14.2, 3.1 Hz, 0.2H)], [3.85 (dt, *J* = 15.5, 4.1 Hz, 0.3H)], [3.55 – 3.51 (m, 0.1H)], [3.24 – 3.15 (m, 0.5H)], [3.13 – 2.99 (m, 0.4H)], 2H (C*H*$_2$NCO_homopiperazine)}, {[4.39 (dd, *J* = 14.2, 4.8 Hz, 0.2H)], [4.28 – 4.18 (m, 0.9H)], [4.07 – 3.95 (m, 0.5H)], [3.90 (dd, *J* = 15.3, 7.9 Hz, 0.3H)], 2H (CHC*H*$_2$_homopiperazine)}, {[4.17 – 4.07 (m, 0.2H)], [3.74 – 3.67 (m, 0.2H)], [3.62 – 3.54 (m, 0.4H)], [3.53 – 3.43 (m, 0.5H)], [3.45 – 3.34 (m, 0.3H)], [3.33 – 3.22 (m, 0.5H)], 2H (benzoxazole-NC*H*$_2$CH$_2$_homopiperazine)}, {[2.41 (s, 0.7H)], [2.40 (s, 0.8H)], [2.36 (s, 1.0H)], [1.80 (s, 0.5H)], 3H (CH$_3$_toluene)}, {[2.36 – 2.24 (m, 0.2H)], [1.87 – 1.80 (m, 0.3H)], [1.75 – 1.68 (m, 0.4H)], [1.60 – 1.49 (m, 1.2H)], 2H (CH$_2$C*H*$_2$CH$_2$_homopiperazine)}, {[2.08 – 2.02 (m, 0.3H)], [2.01 – 1.96 (m, 0.2H)], [1.75 – 1.68 (m, 0.4H)], [1.08 – 1.03 (m, 0.2H)], 1H (CH_*sec*-butyl)}, {[1.96 – 1.90 (m, 0.2H)], [1.69 – 1.58 (m, 1.0H)], [1.36 – 1.19 (m, 1.2H)], 2H (CH$_2$_*sec*-butyl)}, {[1.10 (d, *J* = 6.7 Hz, 0.7H)], [1.05 – 0.98 (m, 0.7H)], [0.98 (d, *J* = 6.8 Hz, 0.9H)], [0.78 (d, *J* = 6.7 Hz, 0.5H)], 3H (CHC*H*$_3$_*sec*-butyl)}, {[1.05 – 0.98 (m, 1.9H)], [0.67 (t, *J* = 7.4 Hz, 0.7H)], [0.54 (t, *J* = 7.2 Hz, 0.4H)], 3H (CH2C*H*$_3$_*sec*-butyl)}.

^{13}C NMR: (151 MHz, CDCl$_3$, four sets of signals were observed, rotamers, signals were assigned with the endorsement of 2D spectroscopy) δ (170.3, 170.1, 170.0, 169.8, N*C*=O), (162.7, 162.5, 162.4, 162.0, N=*C*-O_benzoxazole), (147.6, 147.5, 147.5, 147.3, N-*C*=CH_benzoxazole), (144.8, 144.8, 144.6, O-*C*=CH_benzoxazole), (138.4, 138.1, 138.1, 137.5, *C*CH$_3$_toluene), (135.5, 135.4, 135.3, 135.1, *C*H-*C*H_triazole), (134.1, 133.6, 133.4, 133.2, O=C-*C*_toluene), (130.4, 130.3, 130.3, 130.2, CH$_3$-C-*C*H-CH_toluene), (129.9, 129.0, 128.2, 128.1, *C*-N_toluene), (129.6, 128.9, 128.3, 128.2, C-*C*H-C-CH$_3$_toluene), (129.5, 129.4, 129.4, 129.3, *C*-Cl_benzoxazole), (122.6, 122.2, 121.9, 121.5, N-C-*C*H_toluene), (120.4, 120.4, 120.2, 120.0, Cl-C-*C*H-CH_benzoxazole), (116.6, 116.3, 116.2, 116.1, C-*C*H-C-Cl_benzoxazole), (109.5, 109.3, 109.3, 109.0, C-*C*H-CH-C-Cl_benzoxazole), (70.5, 70.5, 69.3, 68.8, 50.1, 49.6, 48.6, 48.1, benzoxazole-N-*C*H$_2$-CH$_2$_homopiperazine), (67.4, 67.3, 64.5, 63.0, 50.7, 49.3, 49.2, 49.0, benzoxazole-N-*C*H$_2$-CH_homopiperazine), (59.6, 58.2, 56.6, 56.5, *C*H_homopiperazine), (45.3, 44.4, 42.2, 41.3, O=C-N-*C*H2_homopiperazine), (37.4, 37.4, 35.4, 34.7, *C*H_*sec*-butyl), (28.4, 27.8, 27.4, 26.8, CH$_2$-*C*H$_2$-CH$_2$_homopiperazine), (26.6, 26.4, 26.2, 25.9, *C*H$_2$_*sec*-butyl), (21.0, 21.0, 20.9, 20.3, *C*H$_3$_toluene), (15.1, 14.7, 14.6, 14.1, CH-*C*H$_3$_*sec*-butyl), (11.7, 11.7, 11.4, 11.2, CH$_2$-*C*H$_3$_*sec*-butyl).

Methyl *D*-isoleucinate hydrochloride (25j)



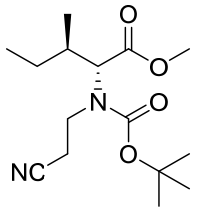
D-isoleucine (2.16 g, 16.46 mmol) was dissolved in methanolic hydrochloride solution (3.0 M, 27.43 mL, 82.28 mmol) and stirred at ambient temperature for 20 h. The pure product was obtained as white powder after evaporation of the volatiles (2.97 g, 16.46 mmol, 99%).

ESI-MS: *m/z* 145.9 [M, cation]$^+$

^{1}H NMR: (400 MHz, dmso-d_6, two sets of signals were observed, rotamers) δ 8.82 – 8.40 (m, 3H), 3.97 – 3.86 (m, 1H), 3.75 (s, 3H), 1.96 (ddt, *J* = 9.2, 7.0, 4.9 Hz, 1H), 1.63 – 1.48, 1.19 – 1.03 (m, 2H), 0.94 (d, *J* = 7.0 Hz, 3H), 0.87 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR: (101 MHz, dmso-d_6, two sets of signals were observed, rotamers) δ 170.4, 169.4, 56.0, 55.9, 52.5, 35.6, 35.3, 24.4, 24.2, 14.5, 14.3, 11.4, 11.3.

Methyl *N*-(*tert*-butoxycarbonyl)-*N*-(2-cyanoethyl)-*D*-isoleucinate (26j)



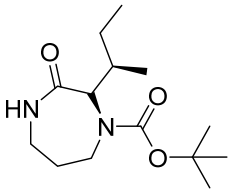
25j (2.97 g, 16.35 mmol) was dissolved in dry methanol (50 mL) in a dried flask. DIPEA (4.24 mL, 24.52 mmol) was added and the reaction mixture was stirred at ambient temperature for 10 minutes. Acrylonitrile (2.14 mL, 32.70 mmol) was added dropwise over 30 minutes and the reaction mixture was stirred under reflux overnight. After cooling to ambient temperature, a solution of di-*tert*-butyldicarbonat (7.14 g, 32.70 mmol) in methanol (20 mL) was added and the reaction mixture was stirred under reflux overnight. The solvent was evaporated. The residue was taken up in ethyl acetate and washed with water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 1% to 5% methanol in dichloromethane. The product was obtained as pale yellow oil (1.30 g, 4.36 mmol, 27%).

ESI-MS: *m/z* 321.1 $[M+Na]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 4.49 – 4.41, 4.10 – 3.99 (m, 1H), 3.75, 3.74 (s, 3H), 3.68 – 3.44 (m, 2H), 3.24 – 3.16, 3.11 – 2.99 (m, 1H), 2.75 – 2.60, 2.58 – 2.47 (m, 2H), 2.10 – 1.71, 1.33 – 1.07 (m, 2H), 1.49, 1.45 (s, 9H), 0.97 – 0.83 (m, 6H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 172.3, 171.5, 155.1, 154.8, 118.4, 118.1, 81.6, 81.5, 64.1, 62.1, 52.1, 51.9, 42.2, 40.8, 35.1, 34.8, 28.3, 28.2, 26.7, 26.3, 17.6, 16.7, 15.1, 14.9, 11.7, 11.1.

***Tert*-butyl (*R*)-2-[(*R*)-*sec*-butyl]-3-oxo-1,4-diazepane-1-carboxylate (27j)**



26j (0.52 g, 1.74 mmol) was dissolved in ethanol (10 mL). Platinum dioxide (39.6 mg, 0.17 mmol) was added and the reaction mixture was stirred at ambient temperature under hydrogen atmosphere for six days. The reaction mixture was filtered over celite and the volatiles were evaporated. The residue was dissolved in THF (10 mL) and methanol (5 mL). A 4 M aqueous solution of lithium hydroxide monohydrate (0.65 mL, 2.61 mmol) was added and the reaction mixture was stirred at ambient temperature for 22 h. The solvents were evaporated and the residue was taken up in a mixture of toluene and methanol (1 : 4, 10 mL). The volatiles were evaporated and the residue was dissolved in DMF (60 mL). DIPEA (0.60 mL, 3.48 mmol) and subsequently HATU (1.32 g, 3.48 mmol) were added to the solution. The reaction mixture was stirred at ambient temperature for 21 h. The reaction was quenched

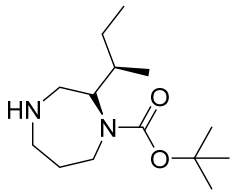
by addition of saturated aqueous solution of ammonium chloride (80 mL). The product was extracted with ethyl acetate and the combined organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 25% to 75% ethyl acetate in isohexane. The product was obtained as yellow oil (0.15 g, 0.56 mmol, 32%).

ESI-MS: *m/z* 293.1 $[M+Na]^+$

^{1}H NMR: (400 MHz, dmso-d_6, two sets of signals were observed, rotamers) δ 7.64 – 7.43 (m, 1H), 4.49 – 4.14 (m, 1H), 3.96 – 3.42 (m, 2H), 3.19 – 2.94 (m, 2H), 2.31 – 2.16 (m, 1H), 1.65 – 1.53 (m, 2H), 1.50 – 1.44, 1.13 – 1.00 (m, 2H), 1.42 – 1.35 (m, 9H), 0.89 (t, *J* = 7.4 Hz, 3H), 0.80 – 0.74 (m, 3H).

^{13}C NMR: (101 MHz, dmso-d_6, two sets of signals were observed, rotamers) δ 172.9, 154.5, 79.2, 72.6, 48.1, 48.0, 45.0, 45.0, 32.4, 32.4, 28.6, 28.1, 27.9, 18.5, 14.0, 13.5, 11.4, 11.2.

Tert-butyl (_R_)-2-[(_R_)-_sec_-butyl]-1,4-diazepane-1-carboxylate (28j)

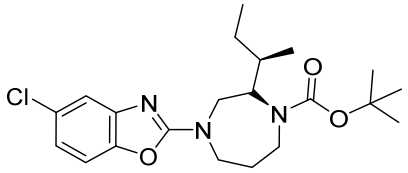


27j (0.13 g, 0.47 mmol) was dissolved in a dried flask in THF (15 mL). A 1 M solution of a borane-THF-complex in THF (2.37 mL, 2.37 mmol) was added dropwise under ice cooling. The reaction mixture was stirred under reflux at 75 °C for 2 h. After cooling to ambient temperature, the reaction was quenched by dropwise addition of methanol (2.0 mL) under ice cooling. The reaction mixture was diluted with ethyl acetate (100 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 50 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was used for the next reaction step without purification.

ESI-MS: *m/z* 257.1 $[M+H]^+$

For further analytical data see compound **28i**.

Tert-butyl (_R_)-2-[(_R_)-_sec_-butyl]-4-(5-chlorobenzoxazol-2-yl)-1,4-diazepane-1-carboxylate (29j)

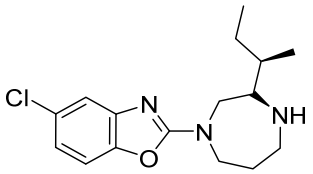


21 (20.3 mg, 0.13 mmol), **28j** (35.5 mg, 0.14 mmol) and copper(II) acetate hydrate (26.4 mg, 0.13 mmol) were dissolved in acetonitrile (4 mL). Concentrated acetic acid (15.1 µL, 0.26 mmol) was added and the reaction mixture was stirred under air at 80 °C for 64 h. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate (20 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 20 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography using a mixture of isohexane : ethyl acetate of 2 : 1 as eluent to obtain the product as yellow oil (17.7 mg, 43.4 µmol, 33%).

ESI-MS: *m/z* 408.2 $[M+H]^+$

For further analytical data see compound **29i**.

2-{(*R*)-3-[(*R*)-*sec*-butyl]-1,4-diazepan-1-yl}-5-chlorobenzoxazole (30j)

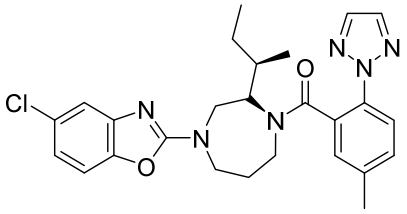


29j (17.7 mg, 43.4 µmol) was dissolved in dichloromethane (3 mL). Trifluoroacetic acid (0.3 mL) was added and the reaction mixture was stirred at ambient temperature for 3 h. The reaction mixture was basified to pH 11 using 1 M aqueous NaOH solution. The product was extracted with dichloromethane (3x). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The product was obtained as brown oil (12.9 mg, 41.9 µmol, 97%) and used for the next step without purification.

ESI-MS: *m/z* 308.1 $[M+H]^+$

For further analytical data see compound **30i**.

{(*R*)-2-[(*R*)-*sec*-butyl]-4-(5-chlorobenzoxazol-2-yl)-1,4-diazepan-1-yl} [5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone (13)



30j (12.9 mg, 41.9 µmol), **24** (9.4 mg, 46.1 µmol) and HATU (23.9 mg, 62.9 µmol) were dissolved in dimethylformamide (dry, 10 mL). DIPEA (36.3 µL, 0.21 mmol) was added and the reaction mixture was stirred at ambient temperature for 6 d. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution (3x) and brine (1x). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified using a preparative HPLC (gradient of 5% to 95% methanol in water with 0.1% formic acid). The product was obtained as pale brown powder after lyophilization of the product containing fractions (6.0 mg, 12.2 µmol, 29%).

ESI-MS: *m/z* 493.2 $[M+H]^+$

HR-ESI-MS: *m/z* $[M+H]^+$ calcd. 493.2113 for $C_{26}H_{29}ClN_6O_2$, found 493.2115

HPLC: eluent system 1: λ = 254 nm, t_R = 22.1 and 22.4 min, rotamers, purity: 94.8%

eluent system 2: λ = 254 nm, t_R = 20.4 and 20.7 min, rotamers, purity: 96.0%

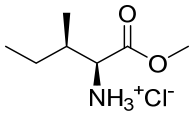
eluent system 3: λ = 254 nm, t_R = 22.1 and 22.4 min, rotamers, purity: 94.6%

chiral HPLC: eluent system 6: λ = 254 nm, t_R = 31.3 and 35.1 min, rotamers, *ee:* 80.8%

$[\alpha]_D^{23}$: - 86.0° (c = 0.07 in methanol)

For further analytical data see compound **2** (**JH112**).

Methyl *L*-alloisoleucinate hydrochloride (25k)



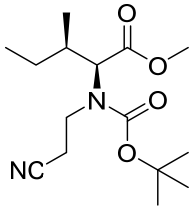
L-alloisoleucine (2.21 g, 16.88 mmol) was dissolved in methanolic hydrochloride solution (3.0 M, 28.1 mL, 84.39 mmol) and stirred at ambient temperature for 21 h. The pure product was obtained as white powder after evaporation of the volatiles (3.07 g, 16.88 mmol, 100%).

APCI-MS: *m/z* 145.9 $[M, cation]^+$

^{1}H NMR: (400 MHz, dmso-d_6, two sets of signals were observed, rotamers) δ 8.91 – 8.42 (m, 3H), 4.02 – 3.86 (m, 1H), 3.75 (s, 3H), 2.03 – 1.86 (m, 1H), 1.63 – 1.45, 1.20 – 1.03 (m, 2H), 0.93 (d, *J* = 7.0 Hz, 3H), 0.87 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR: (101 MHz, dmso-d_6, two sets of signals were observed, rotamers) δ 170.5, 169.4, 56.0, 55.9, 52.6, 35.6, 35.3, 24.4, 24.2, 14.4, 14.3, 11.4, 11.3.

Methyl *N*-(*tert*-butoxycarbonyl)-*N*-(2-cyanoethyl)-*L*-alloisoleucinate (26k)



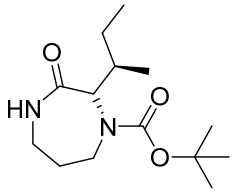
25k (3.07 g, 16.88 mmol) was dissolved in dry methanol (40 mL) in a dried flask. DIPEA (4.39 mL, 25.35 mmol) was added and the reaction mixture was stirred at ambient temperature for 10 minutes. Acrylonitrile (2.21 mL, 16.88 mmol) was added dropwise over 30 minutes and the reaction mixture was stirred under reflux overnight. After cooling to ambient temperature, a solution of di-*tert*-butyldicarbonat (7.38 g, 33.80 mmol) in methanol (20 mL) was added and the reaction mixture was stirred under reflux overnight. The solvent was evaporated. The residue was taken up in ethyl acetate and washed with water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 1% to 5% methanol in dichloromethane. The product was obtained as pale yellow oil (1.12 g, 3.75 mmol, 22%).

ESI-MS: *m/z* 321.1 $[M+Na]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 4.50 – 4.32, 4.08 – 4.00 (m, 1H), 3.74 (s, 3H), 3.65 – 3.46, 3.09 – 2.99 (m, 2H), 2.76 – 2.44 (m, 2H), 2.11 – 1.81 (m, 1H), 1.80 – 1.61, 1.33 – 1.04 (m, 2H), 1.53 – 1.41 (m, 9H), 0.97 – 0.82 (m, 6H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 175.3, 173.3, 155.7, 154.8, 118.6, 118.1, 81.6, 81.5, 64.9, 52.1, 51.8, 44.3, 38.0, 37.7, 28.3, 26.4, 26.2, 19.1, 18.8, 14.7, 14.5, 11.7, 11.1.

***Tert*-butyl (*S*)-2-[(*R*)-*sec*-butyl]-3-oxo-1,4-diazepane-1-carboxylate (27k)**



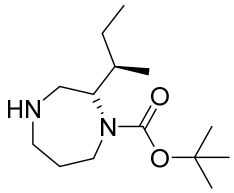
26k (1.11 g, 3.72 mmol) was dissolved in ethanol (20 mL). Platinum dioxide (84.4 mg, 0.37 mmol) was added and the reaction mixture was stirred at ambient temperature under hydrogen atmosphere for four days. The reaction mixture was filtered over celite and the volatiles were evaporated. The residue was dissolved in THF (10 mL) and methanol (5 mL). A 4 M aqueous solution of lithium hydroxide monohydrate (1.26 mL, 5.06 mmol) was added and the reaction mixture was stirred at ambient temperature for 30 h. The solvents were evaporated and the residue was taken up in a mixture of toluene and methanol (1 : 4, 10 mL). The volatiles were evaporated and the residue was dissolved in DMF (30 mL). DIPEA (1.17 mL, 6.74 mmol) and subsequently HATU (2.56 g, 6.74 mmol) were added to the solution and the reaction mixture was stirred at ambient temperature for 41 h. The reaction was quenched by addition of saturated aqueous solution of ammonium chloride (60 mL). The product was extracted with ethyl acetate and the combined organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 25% to 75% ethyl acetate in isohexane. The product was obtained as yellow oil (0.35 g, 1.28 mmol, 35%).

ESI-MS: *m/z* 293.1 $[M+Na]^+$

^{1}H NMR: (400 MHz, dmso-d_6) δ 4.49 – 4.13 (m, 1H), 3.93 – 3.53 (m, 2H), 3.25 – 2.90 (m, 2H), 2.31 – 2.20 (m, 1H), 1.67 – 1.52 (m, 2H), 1.50 – 1.44, 1.13 – 0.97 (m, 2H), 1.44 – 1.32 (m, 9H), 0.88 (t, *J* = 7.3 Hz, 3H), 0.82 – 0.73 (m, 3H).

^{13}C NMR: (101 MHz, dmso-d_6) δ 173.4, 155.0, 79.6, 79.4, 78.0, 63.8, 44.6, 32.8, 29.0, 28.5, 28.3, 26.1, 15.5, 11.6.

***Tert*-butyl (*S*)-2-[(*R*)-*sec*-butyl]-1,4-diazepane-1-carboxylate (28k)**

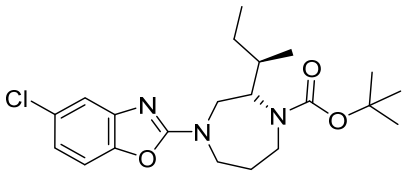


27k (0.33 g, 1.20 mmol) was dissolved in a dried flask in THF (20 mL). A 1 M solution of a borane-THF-complex in THF (6.01 mL, 6.01 mmol) was added dropwise under ice cooling. The reaction mixture was stirred under reflux at 75 °C for 4 h. After cooling to ambient temperature, the reaction was quenched by dropwise addition of methanol (2 mL) under ice cooling. The reaction mixture was diluted with ethyl acetate (100 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 50 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 20% to 50% ethyl acetate in isohexane. The product was obtained as pale yellow oil (87.0 mg, 0.34 mmol, 28%).

ESI-MS: *m/z* 257.1 $[M+H]^+$

For further analytical data see compound **28l**.

***Tert*-butyl (*S*)-2-[(*R*)-*sec*-butyl]-4-(5-chlorobenzoxazol-2-yl)-1,4-diazepane-1-carboxylate (29k)**

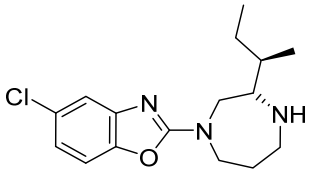


21 (8.0 mg, 0.052 mmol), **28k** (14.0 mg, 0.055 mmol) and copper(II) acetate hydrate (10.4 mg, 0.052 mmol) were dissolved in acetonitrile (2 mL). Concentrated acetic acid (6.0 µL, 0.10 mmol) was added and the reaction mixture was stirred under air at 80 °C for 40 h. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate (20 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 20 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 2% to 5% methanol in dichloromethane. The product was obtained as brown oil (11.5 mg, 0.028 mmol, 54%).

ESI-MS: *m/z* 408.2 $[M+H]^+$

For further analytical data see compound **29l.**

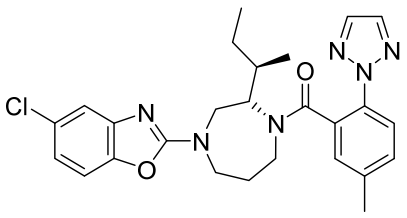
2-{(*S*)-3-[(*R*)-*sec*-butyl]-1,4-diazepan-1-yl}-5-chlorobenzoxazole (30k)



29k (11.5 mg, 0.028 mmol) was dissolved in dichloromethane (2 mL). Trifluoroacetic acid (0.2 mL) was added and the reaction mixture was stirred at ambient temperature for 21 h. The reaction mixture was basified to pH 11 using 1 M aqueous NaOH solution. The product was extracted with dichloromethane (3x). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The product was obtained as yellow oil (8.7 mg, 0.028 mmol) and used for the next step without purification.

ESI-MS: *m/z* 308.1 $[M+H]^+$

{(*S*)-2-[(*R*)-*sec*-butyl]-4-(5-chlorobenzoxazol-2-yl)-1,4-diazepan-1-yl} [5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone (12)



30k (8.7 mg, 0.028 mmol), **24** (6.9 mg, 0.034 mmol) and HATU (21.4 mg, 0.056 mmol) were dissolved in dimethylformamide (dry, 2 mL). DIPEA (24.4 µL, 0.14 mmol) was added and the reaction mixture was stirred at ambient temperature for 1 d. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution (3x) and brine (1x). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified

using a preparative HPLC (gradient of 5% to 95% methanol in water with 0.1% formic acid). The product was obtained as white powder after lyophilization of the product containing fractions (7.0 mg, 0.014 mmol, 50%).

ESI-MS: *m/z* 493.2 $[M+H]^+$

HR-ESI-MS: *m/z* $[M+H]^+$ calcd. 493.2113 for $C_{26}H_{29}ClN_6O_2$, found 493.2113

HPLC: eluent system 2: λ = 254 nm, t_R = 21.0 and 21.3 min, rotamers, purity: 95.3%

eluent system 3: λ = 254 nm, t_R = 22.3 and 22.5 min, rotamers, purity: 97.9%

eluent system 4: λ = 254 nm, t_R = 20.9 and 21.2 min, rotamers, purity: 96.2%

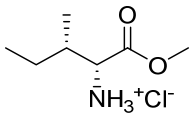
chiral HPLC: eluent system 6: λ = 254 nm, t_R = 23.3 and 37.9 min, rotamers, *ee:* 100%

$[\alpha]_D^{25}$: + 97.0° (c = 0.12 in methanol)

^{1}H NMR: (600 MHz, $CDCl_3$, four sets of signals were observed, rotamers, assignments of signals analog to compound **2** (**JH112**)) δ 7.92 (d, *J* = 8.4 Hz, 0.3H), 7.89 (d, *J* = 8.4 Hz, 0.1H), 7.86 (d, *J* = 8.3 Hz, 0.2H), 7.81 (d, *J* = 8.3 Hz, 0.3H), 7.75 (s, 0.2H), 7.72 (s, 0.5H), 7.59 (s, 0.5H), 7.52 (s, 0.6H), 7.45 – 7.40 (m, 0.6H), 7.35 (d, *J* = 2.0 Hz, 0.2H), 7.32 (d, *J* = 1.9 Hz, 0.5H), 7.33 – 7.29 (m, 0.3H), 7.29 (d, *J* = 8.1 Hz, 0.4H), 7.24 – 7.14 (m, 1.1H), 7.10 – 7.05 (m, 0.6H), 7.05 (dd, *J* = 8.5, 2.1 Hz, 0.5H), 7.02 (dd, *J* = 8.4, 2.0 Hz, 0.2H), 6.98 (dd, *J* = 8.5, 1.9 Hz, 0.2H), 6.94 (s, 0.3H), 6.46 (s, 0.1H), 5.00 – 4.75 (m, 0.7H), 4.68 – 4.54 (m, 0.4H), 4.29 (dt, *J* = 13.9, 4.0 Hz, 0.2H), 4.21 (dd, *J* = 14.9, 5.4 Hz, 0.5H), 4.19 – 4.08 (m, 0.2H), 4.05 (dd, *J* = 14.7, 7.1 Hz, 0.4H), 4.01 – 3.87 (m, 1.3H), 3.84 (dt, *J* = 10.0, 4.7 Hz, 0.6H), 3.68 – 3.57 (m, 0.9H), 3.56 – 3.51 (m, 0.5H), 3.49 (s, 0.6H), 3.47 – 3.26 (m, 0.9H), 3.20 (ddd, *J* = 14.6, 11.0, 3.1 Hz, 0.6H), 3.06 – 2.98 (m, 0.2H), 2.92 (ddd, *J* = 14.2, 11.7, 3.0 Hz, 0.3H), 2.41 (s, 1.7H), 2.38 (s, 1.1H), 2.12 – 1.41 (m, 1.7H), 1.04 (d, *J* = 6.7 Hz, 1.2H), 0.99 (tt, *J* = 11.3, 5.0 Hz, 3.1H), 0.87 (d, *J* = 6.8 Hz, 1.4H), 0.83 (t, *J* = 7.3 Hz, 0.9H), 0.67 (t, *J* = 7.3 Hz, 0.3H), 0.57 (t, *J* = 7.4 Hz, 0.1H), 0.49 (d, *J* = 7.0 Hz, 0.3H).

^{13}C NMR: (151 MHz, $CDCl_3$, four sets of signals were observed, rotamers, assignments of signals analog to compound **2** (**JH112**)) δ 170.4, 170.0, 169.8, 169.8, 162.9, 162.8, 161.6, 160.8, 147.8, 147.0, 144.9, 144.9, 144.1, 138.6, 138.2, 138.1, 138.0, 135.6, 135.5, 135.4, 134.2, 134.2, 134.0, 130.6, 130.5, 130.3, 129.7, 129.5, 129.0, 128.3, 128.2, 122.8, 122.5, 122.4, 122.2, 116.5, 116.0, 115.9, 115.9, 109.9, 109.7, 109.4, 109.3, 61.3, 57.8, 56.1, 50.9, 50.7, 50.5, 50.4, 50.3, 50.2, 44.6, 44.6, 41.9, 40.2, 36.9, 35.7, 35.2, 35.0, 31.9, 31.9, 30.9, 29.7, 29.4, 27.8, 26.8, 25.9, 25.4, 25.3, 24.9, 21.1, 21.0, 21.0, 20.5, 15.7, 15.7, 15.3, 15.0, 11.8, 11.4, 11.4.

Methyl *D*-alloisoleucinate hydrochloride (25l)



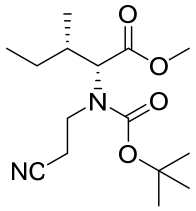
D-alloisoleucine (4.00 g, 30.49 mmol) was dissolved in methanolic hydrochloride solution (3.0 M, 50.8 mL, 152.47 mmol) and stirred at ambient temperature for 21 h. The pure product was obtained as white powder after evaporation of the volatiles (5.54 g, 30.49 mmol, 100%).

ESI-MS: *m/z* 145.9 $[M+H]^+$

^{1}H NMR: (400 MHz, dmso-d_6, two sets of signals were observed, rotamers) δ 8.84 – 8.30 (m, 3H), 3.98 – 3.86 (m, 1H), 3.75, 3.16 (s, 3H), 2.05 – 1.86 (m, 1H), 1.63 – 1.45, 1.25 – 1.00 (m, 2H), 0.94 (d, *J* = 7.0 Hz, 3H), 0.88 (td, *J* = 7.3, 4.5 Hz, 3H).

^{13}C NMR: (101 MHz, dmso-d_6, two sets of signals were observed, rotamers) δ 170.4, 169.4, 56.0, 55.9, 52.6, 35.6, 35.3, 24.4, 24.2, 14.5, 14.3, 11.4, 11.3.

Methyl *N*-(*tert*-butoxycarbonyl)-*N*-(2-cyanoethyl)-*D*-alloisoleucinate (26l)



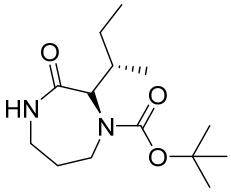
25l (5.54 g, 30.49 mmol) was dissolved in dry methanol (60 mL) in a dried flask. DIPEA (7.91 mL, 45.74 mmol) was added and the reaction mixture was stirred at ambient temperature for 10 minutes. Acrylonitrile (4.00 mL, 60.99 mmol) was added dropwise over 30 minutes and the reaction mixture was stirred under reflux overnight. After cooling to ambient temperature, a solution of di-*tert*-butyldicarbonat (13.31 g, 60.99 mmol) in methanol (30 mL) was added and the reaction mixture was stirred under reflux overnight. The solvent was evaporated. The residue was taken up in ethyl acetate and washed with water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 0.5% to 5% methanol in dichloromethane. The product was obtained as pale yellow oil (0.52 g, 1.73 mmol, 6%).

ESI-MS: *m/z* 321.1 $[M+Na]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 4.52 – 4.32, 4.10 – 3.98 (m, 1H), 3.74 (s, 3H), 3.70 – 3.42 (m, 2H), 2.77 – 2.57 (m, 2H), 2.13 – 1.83 (m, 1H), 1.50, 1.45 (s, 9H), 1.65 – 1.33, 1.31 – 1.04 (m, 2H), 0.94 (td, *J* = 7.4, 3.8 Hz, 3H), 0.89 – 0.79 (m, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 172.3, 171.5, 155.1, 154.8, 118.1, 117.8, 81.6, 81.5, 64.1, 62.1, 52.1, 42.2, 40.8, 35.1, 34.8, 28.3, 28.2, 26.7, 26.2, 17.6, 16.7, 15.2, 14.9, 11.7, 11.1.

***Tert*-butyl (*R*)-2-[(*S*)-*sec*-butyl]-3-oxo-1,4-diazepane-1-carboxylate (27l)**



26l (1.00 g, 3.35 mmol) was dissolved in ethanol (20 mL). Platinum dioxide (76.1 mg, 0.34 mmol) was added and the reaction mixture was stirred at ambient temperature under hydrogen atmosphere for two days. The reaction mixture was filtered over celite and the volatiles were evaporated. The residue was dissolved in THF (10 mL) and methanol (5 mL). A 4 M aqueous solution of lithium hydroxide monohydrate (1.89 mL, 7.57 mmol) was added and the reaction mixture was stirred at ambient temperature for 19 h. The solvents were evaporated and the residue was taken up in a mixture of toluene

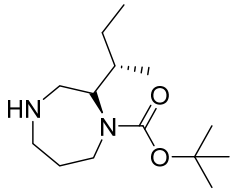
and methanol (1 : 4, 10 mL). The volatiles were evaporated and the residue was dissolved in DMF (40 mL). DIPEA (1.75 mL, 10.13 mmol) and subsequently HATU (3.85 g, 10.13 mmol) were added to the solution. The reaction mixture was stirred at ambient temperature for 17 h. The reaction was quenched by addition of saturated aqueous solution of ammonium chloride (80 mL). The product was extracted with ethyl acetate and the combined organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 25% to 75% ethyl acetate in isohexane. The product was obtained as yellow oil (0.41 g, 1.52 mmol, 45%).

ESI-MS: *m/z* 293.1 $[M+Na]^+$

^{1}H NMR: (400 MHz, dmso-d_6) δ 7.60 – 7.36 (m, 1H), 4.64 – 4.15 (m, 1H), 3.92 – 3.38, 3.22 – 2.93 (m, 4H), 2.32 – 2.16 (m, 1H), 1.68 – 1.50 (m, 2H), 1.49 – 1.42, 1.13 – 1.00 (m, 2H), 1.41 – 1.34 (m, 9H), 0.88 (t, *J* = 7.3 Hz, 3H), 0.81 – 0.73 (m, 3H).

^{13}C NMR: (101 MHz, dmso-d_6) δ 173.5, 155.1, 79.7, 79.5, 73.1, 64.0, 46.3, 33.0, 32.8, 29.2, 28.4, 26.3, 26.2, 15.7, 11.8.

***Tert*-butyl (*R*)-2-[(*S*)-*sec*-butyl]-1,4-diazepane-1-carboxylate (28l)**



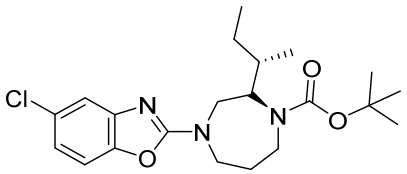
27l (0.39 g, 1.44 mmol) was dissolved in a dried flask in THF (20 mL). A 1 M solution of a borane-THF-complex in THF (7.21 mL, 7.21 mmol) was added dropwise under ice cooling. The reaction mixture was stirred under reflux at 75 °C for 16 h. After cooling to ambient temperature, the reaction was quenched by dropwise addition of methanol (2 mL) under ice cooling. The reaction mixture was diluted with ethyl acetate (100 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 50 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 20% to 50% ethyl acetate in isohexane. The product was obtained as pale yellow oil (0.14 g, 0.56 mmol, 39%).

ESI-MS: *m/z* 257.2 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 4.27 – 3.79 (m, 2H), 3.79 – 3.54 (m, 1H), 3.51 – 3.27 (m, 1H), 3.21 – 2.92 (m, 1H), 2.93 – 2.72 (m, 1H), 2.72 – 2.39 (m, 2H), 1.97 – 1.64 (m, 2H), 1.64 – 1.51, 1.21 – 1.11 (m, 2H), 1.50 – 1.44 (m, 9H), 0.97 – 0.81 (m, 6H).

^{13}C NMR: (151 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 155.7, 155.1, 80.8, 80.4, 56.2, 48.4, 48.3, 42.4, 42.2, 40.9, 40.3, 38.0, 37.9, 28.7, 28.2, 28.6, 28.4, 25.6, 25.3, 15.5, 15.2, 11.3, 11.0.

***Tert*-butyl (*R*)-2-[(*S*)-*sec*-butyl]-4-(5-chlorobenzoxazol-2-yl)-1,4-diazepane-1-carboxylate (29l)**



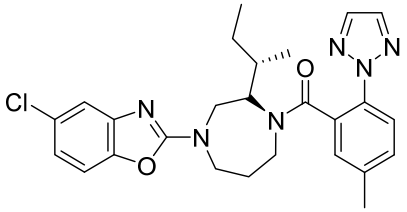
21 (78.0 mg, 0.51 mmol), **28l** (0.14 g, 0.53 mmol) and copper(II) acetate hydrate (0.10 g, 0.51 mmol) were dissolved in acetonitrile (20 mL). Concentrated acetic acid (58.1 µL, 1.02 mmol) was added and the reaction mixture was stirred under air at 80 °C for 45 h. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate (60 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 40 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 2% to 5% methanol in dichloromethane. The product was obtained as brown oil (0.13 g, 0.33 mmol, 64%).

ESI-MS: *m/z* 408.2 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 7.31, 7.28 (d, *J* = 2.0 Hz, 1H), 7.15, 7.14 (d, *J* = 8.4 Hz, 1H), 6.99 – 6.92 (m, 1H), 4.56 – 4.12 (m, 3H), 4.04 – 3.86, 3.85 – 3.68 (m, 2H), 3.36 – 2.73 (m, 3H), 2.30 – 2.08 (m, 1H), 1.74 – 1.63, 1.60 – 1.41 (m, 4H), 1.28, 1.09 (s, 9H), 0.97 (t, *J* = 7.3 Hz, 3H), 0.91 – 0.85 (m, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 162.2, 161.2, 155.6, 155.4, 147.5, 147.5, 144.7, 144.5, 129.3, 129.2, 120.6, 120.2, 116.2, 116.0, 109.4, 109.1, 79.6, 79.5, 56.4, 56.2, 53.5, 53.3, 52.6, 51.4, 41.3, 37.5, 36.8, 29.7, 28.2, 27.9, 26.2, 25.6, 15.6, 15.4, 11.2, 11.1.

{(*R*)-2-[(*S*)-*sec*-butyl]-4-(5-chlorobenzoxazol-2-yl)-1,4-diazepan-1-yl} [5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone (14)



29l (56.2 mg, 0.14 mmol) was dissolved in dichloromethane (3 mL). Trifluoroacetic acid (0.3 mL) was added and the reaction mixture was stirred at ambient temperature for 26 h. The reaction mixture was basified to pH 11 using 1 M aqueous NaOH solution. The product was extracted with dichloromethane (3x). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure to obtain the crude secondary amine intermediate **30l** which was dissolved in DMF (3 mL). **24** (28.5 mg, 0.14 mmol), HATU (88.9 mg, 0.23 mmol) and DIPEA (0.10 mL, 0.58 mmol) were added and the reaction mixture was stirred at ambient temperature for 26 h. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution (3x) and brine (1x). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified using a preparative HPLC (gradient of 5% to 95% methanol in water with 0.1% formic acid). The product was obtained as white powder after lyophilization of the product containing fractions (35.5 mg, 0.072 mmol, 51%).

ESI-MS: *m/z* 308.1 [Intermediate **30l**+H]$^+$

m/z 493.2 $[M+H]^+$

HR-ESI-MS: *m/z* $[M+H]^+$ calcd. 493.2113 for $C_{26}H_{29}ClN_6O_2$, found 493.2112

HPLC: eluent system 1: λ = 254 nm, t_R = 22.3 and 22.5 min, rotamers, purity: 98.2%

eluent system 2: λ = 254 nm, t_R = 21.0 and 21.3 min, rotamers, purity: 97.4%

eluent system 3: λ = 254 nm, t_R = 22.3 and 22.6 min, rotamers, purity: 97.5%

eluent system 4: λ = 254 nm, t_R = 20.9 and 21.2 min, rotamers, purity: 98.2%

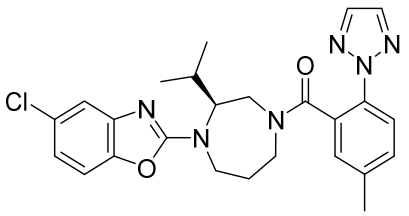
chiral HPLC: eluent system 6: λ = 254 nm, t_R = 32.9 and 36.3 min, rotamers, *ee:* 100%

$[\alpha]_D^{24}$: - 104.7° (c = 0.32 in methanol)

^{1}H NMR: (600 MHz, $CDCl_3$, four sets of signals were observed, rotamers, assignments of signals analog to compound **2** (**JH112**)) δ 7.91 (d, *J* = 8.4 Hz, 0.3H), 7.89 (d, *J* = 8.4 Hz, 0.1H), 7.86 (d, *J* = 8.3 Hz, 0.3H), 7.80 (d, *J* = 8.3 Hz, 0.3H), 7.75 (s, 0.3H), 7.72 (s, 0.5H), 7.59 (s, 0.5H), 7.52 (s, 0.6H), 7.36 (s, 0.6H), 7.34 (d, *J* = 2.0 Hz, 0.1H), 7.31 (d, *J* = 8.3 Hz, 0.6H), 7.29 (d, *J* = 1.1 Hz, 0.2H), 7.28 (d, *J* = 1.1 Hz, 0.4H), 7.22 (d, *J* = 1.3 Hz, 0.1H), 7.22 – 7.13 (m, 1.0H), 7.10 – 7.04 (m, 0.6H), 7.02 (dt, *J* = 8.4, 2.1 Hz, 0.7H), 6.96 (dd, *J* = 8.4, 2.1 Hz, 0.3H), 6.94 (s, 0.3H), 6.47 (d, *J* = 0.8 Hz, 0.1H), 4.97 – 4.88 (m, 0.3H), 4.88 – 4.76 (m, 0.2H), 4.59 (dt, *J* = 13.7, 3.6 Hz, 0.3H), 4.56 (d, *J* = 3.3 Hz, 0.1H), 4.28 (dt, *J* = 14.0, 3.8 Hz, 0.1H), 4.19 (dd, *J* = 15.0, 5.5 Hz, 0.4H), 4.18 – 4.09 (m, 0.2H), 4.04 (dd, *J* = 15.1, 6.9 Hz, 0.3H), 3.94 (dd, *J* = 15.2, 4.1 Hz, 0.7H), 3.93 – 3.86 (m, 0.3H), 3.86 – 3.77 (m, 0.8H), 3.67 – 3.53 (m, 0.6H), 3.50 (ddd, *J* = 11.3, 7.6, 3.1 Hz, 0.6H), 3.40 (dd, *J* = 14.9, 9.4 Hz, 0.4H), 3.37 – 3.30 (m, 0.1H), 3.29 (dd, *J* = 14.9, 3.8 Hz, 0.3H), 3.19 (ddd, *J* = 14.7, 10.9, 3.3 Hz, 0.6H), 3.03 (ddd, *J* = 14.0, 11.8, 2.1 Hz, 0.1H), 2.92 (ddd, *J* = 14.3, 11.6, 3.1 Hz, 0.2H), 2.41 (s, 1.6H), 2.37 (s, 0.9H), 2.36 – 2.32 (m, 0.2H), 2.12 – 2.01 (m, 0.3H), 1.97 – 1.87 (m, 0.7H), 1.87 – 1.75 (m, 1.1H), 1.76 – 1.54 (m, 2.4H), 1.54 – 1.40 (m, 0.4H), 1.40 – 1.20 (m, 1.1H), 1.03 (d, *J* = 6.8 Hz, 1.2H), 0.99 (td, *J* = 7.3, 4.0 Hz, 2.9H), 0.96 – 0.86 (m, 0.2H), 0.85 (d, *J* = 6.9 Hz, 0.8H), 0.82 (t, *J* = 7.3 Hz, 0.8H), 0.67 (t, *J* = 7.3 Hz, 0.3H), 0.49 (d, *J* = 7.0 Hz, 0.3H).

^{13}C NMR: (151 MHz, $CDCl_3$, four sets of signals were observed, rotamers, assignments of signals analog to compound **2** (**JH112**)) δ 170.3, 170.0, 170.0, 169.8, 162.6, 162.5, 162.1, 161.9, 147.4, 147.3, 147.1, 147.0, 138.5, 138.1, 138.1, 138.0, 135.6, 135.5, 135.5, 135.4, 134.2, 133.9, 133.7, 133.3, 130.5, 130.4, 130.3, 129.8, 129.7, 129.6, 129.6, 129.5, 129.0, 128.9, 128.7, 128.3, 128.2, 128.1, 122.7, 122.4, 122.3, 120.9, 120.8, 120.5, 120.5, 116.5, 116.1, 116.0, 116.0, 109.6, 109.5, 109.4, 109.1, 61.5, 60.6, 60.6, 56.3, 50.7, 50.5, 50.2, 50.1, 50.1, 50.1, 49.4, 49.0, 44.6, 44.1, 44.1, 41.9, 40.1, 36.9, 35.8, 35.0, 35.0, 30.9, 29.7, 29.7, 27.8, 27.1, 26.9, 25.9, 25.5, 25.4, 24.9, 23.9, 21.0, 21.0, 21.0, 20.5, 15.7, 15.3, 15.0, 11.9, 11.5, 11.4, 11.3.

(*S*)-[4-(5-Chlorobenzoxazol-2-yl)-3-isopropyl-1,4-diazepan-1-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone (10)



28c (23.5 mg, 97.0 µmol), **24** (19.7 mg, 97.0 µmol) and HATU (73.7 mg, 0.19 mmol) were dissolved in dimethylformamide (dry, 4 mL). DIPEA (84.0 µL, 0.48 mmol) was added and the reaction mixture was stirred at ambient temperature for two days. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution (3x) and brine (1x). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to obtain the crude intermediate **31c** which was dissolved in dichloromethane (2 mL). Trifluoroacetic acid (0.2 mL) was added and the reaction mixture was stirred at ambient temperature for 5 h. The reaction mixture was basified to pH 11 using 1 M aqueous NaOH solution. The product was extracted with dichloromethane (3x). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure to obtain the crude intermediate **32c** which was dissolved in acetonitrile (2 mL). **21** (3.0 mg, 19.6 µmol), copper(II) acetate hydrate (3.4 mg, 17.0 µmol) and subsequently concentrated acetic acid (10 µL, 17.5 µmol) were added and the reaction mixture was stirred under air at 80 °C for 16 h. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate (20 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 20 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified using a preparative HPLC (gradient of 5% to 95% acetonitrile in water with 0.1%trifluoroacetic acid). The product was obtained as white powder after lyophilization of the product containing fractions (2.9 mg, 6.1 µmol, 6%).

ESI-MS: *m/z* 428.2 [Intermediate **31c**+H]$^+$

m/z 328.1 [Intermediate **32c**+H]$^+$

m/z 479.1 [M+H]$^+$

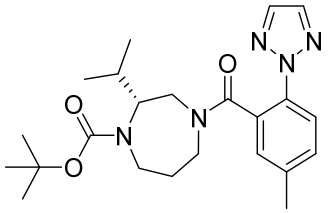
HR-ESI-MS: *m/z* [M+H]$^+$ calcd. 479.1957 for $C_{25}H_{27}ClN_6O_2$, found 479.1955

HPLC: eluent system 4: λ = 254 nm, t_R = 20.5 min, purity: 99.5%

$[\alpha]_D^{25}$: + 39.6° (c = 0.02 in methanol)

^{1}H NMR: (600 MHz, $CDCl_3$, four sets of signals were observed, rotamers, assignments of signals analog to compound **2** (**JH112**)) δ 7.88 (d, *J* = 8.3 Hz, 0.2H), 7.81 (d, *J* = 8.3 Hz, 0.4H), 7.77 (s, 1.1H), 7.75 (d, *J* = 8.3 Hz, 0.4H), 7.37 (d, *J* = 2.0 Hz, 0.8H), 7.36 – 7.27 (m, 0.9H), 7.21 (dd, *J* = 8.4, 3.8 Hz, 1.2H), 7.16 (d, *J* = 8.4 Hz, 0.3H), 7.14 – 7.11 (m, 0.3H), 7.03 (dd, *J* = 8.4, 2.0 Hz, 0.4H), 7.00 (dd, *J* = 8.4, 2.1 Hz, 0.6H), 6.97 – 6.89 (m, 0.6H), 6.44 – 6.37 (m, 0.4H), 4.82 (dd, *J* = 14.0, 6.0 Hz, 0.6H), 4.58 – 4.43 (m, 0.8H), 4.30 – 4.12 (m, 1.0H), 3.77 (dd, *J* = 15.6, 6.7 Hz, 0.2H), 3.64 (d, *J* = 13.0 Hz, 0.4H), 3.49 (dd, *J* = 14.6, 5.4 Hz, 0.7H), 3.41 – 3.31 (m, 0.8H), 3.31 – 3.10 (m, 0.6H), 3.09 – 3.00 (m, 0.4H), 2.99 – 2.83 (m, 1.0H), 2.74 (td, *J* = 13.1, 3.3 Hz, 0.2H), 2.50 (s, 0.5H), 2.40 (s, 1.0H), 2.30 – 2.06 (m, 0.9H), 2.01 (s, 1.5H), 1.97 – 1.70 (m, 1.5H), 1.55 – 1.39 (m, 1.6H), 1.11 (d, *J* = 6.7 Hz, 1.3H), 1.09 (d, *J* = 6.8 Hz, 1.0H), 1.05 (d, *J* = 6.9 Hz, 1.0H), 1.03 (d, *J* = 6.7 Hz, 1.4H), 0.80 (d, *J* = 6.7 Hz, 0.5H), 0.74 (d, *J* = 6.4 Hz, 0.2H), 0.70 (d, *J* = 6.6 Hz, 0.5H) , 0.66 (d, *J* = 6.4 Hz, 0.2H).

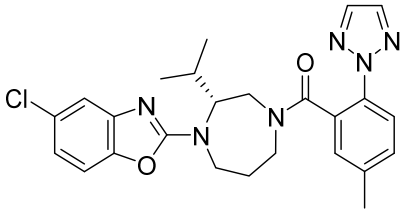
***Tert*-butyl (*R*)-2-isopropyl-4-[5-methyl-2-(2*H*-1,2,3-triazol-2-yl)benzoyl]-1,4-diazepane-1-carboxylate (31d)**



28d (98.5 mg, 0.41 mmol), **24** (82.6 mg, 0.41 mmol) and HATU (0.31 g, 0.81 mmol) were dissolved in dimethylformamide (dry, 5 mL). DIPEA (0.36 mL, 2.05 mmol) was added and the reaction mixture was stirred at ambient temperature for two days. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution (3x) and brine (1x). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified using a preparative HPLC (gradient of 5% to 95% acetonitrile in water with 0.1% trifluoroacetic acid). The product was obtained as white powder after lyophilization of the product containing fractions (6.3 mg, 14.7 µmol, 4%).

ESI-MS: *m/z* 450.2 $[M+Na]^+$

(*R*)-[4-(5-Chlorobenzoxazol-2-yl)-3-isopropyl-1,4-diazepan-1-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone (11)



31d (6.3 mg, 14.7 µmol) was dissolved in dichloromethane (3 mL). Trifluoroacetic acid (0.3 mL) was added and the reaction mixture was stirred at ambient temperature for 3 h. The reaction mixture was basified to pH 11 using 1 M aqueous NaOH solution. The product was extracted with dichloromethane (3x). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure to obtain the crude intermediate **32d** which was dissolved in acetonitrile (2 mL). **21** (1.6 mg, 10.4 µmol), copper(II) acetate hydrate (2.0 mg, 9.03 µmol) and subsequently concentrated acetic acid (1.2 µL, 20.8 µmol) were added and the reaction mixture was stirred under air at 80 °C for two days. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate (20 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 20 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified using a preparative HPLC (gradient of 5% to 95% acetonitrile in water with 0.1%trifluoroacetic acid). The product was obtained as white powder after lyophilization of the product containing fractions (0.23 mg, 0.48 µmol, 3%).

ESI-MS: *m/z* 328.1 [Intermediate **32d**+H]$^+$

m/z 479.1 $[M+H]^+$

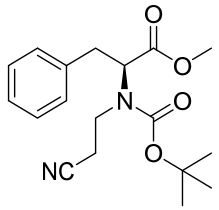
HR-ESI-MS: *m/z* $[M+H]^+$ calcd. 479.1957 for $C_{25}H_{27}ClN_6O_2$, found 479.1958

HPLC: eluent system 4: λ = 254 nm, t_R = 20.5 min, purity: 98.8%

$[\alpha]_D^{25}$: - 15.7° (c = 0.02 in methanol)

For further analytical data see compound **10**.

Methyl *N*-(*tert*-butoxycarbonyl)-*N*-(2-isocyanoethyl)-*L*-phenylalaninate (33)



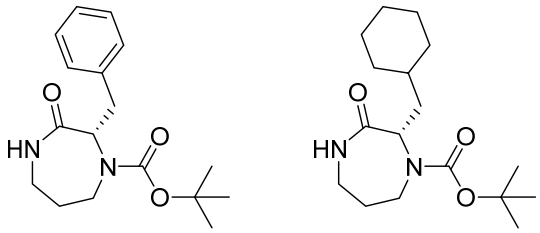
Methyl *L*-phenylalaninate hydrochloride (5.00 g, 23.18 mmol) was dissolved in dry methanol (30 mL) in a dried flask. DIPEA (6.06 mL, 34.77 mmol) was added and the reaction mixture was stirred at ambient temperature for 10 minutes. Acrylonitrile (3.04 mL, 46.37 mmol) was added dropwise over 30 minutes and the reaction mixture was stirred under reflux overnight. After cooling to ambient temperature, a solution of di-*tert*-butyldicarbonat (10.12 g, 46.37 mmol) in methanol (30 mL) was added and the reaction mixture was stirred under reflux overnight. The solvent was evaporated. The residue was taken up in ethyl acetate and washed with water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 15% to 30% ethyl acetate in isohexane. The product was obtained as pale yellow oil (3.50 g, 10.54 mmol, 45%).

ESI-MS: *m/z* 333.2 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 7.38 – 7.20 (m, 3H), 7.20 – 7.11 (m, 2H), 4.45 – 4.26, 4.19 – 4.04 (m, 1H), 3.78, 3.76 (s, 3H), 3.47 – 3.27, 3.26 – 3.01, 3.01 – 2.81 (m, 4H), 2.54 – 2.36, 2.36 – 2.17 (m, 2H), 1.44, 1.43 (s, 9H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 171.3, 171.0, 154.3, 154.1, 137.5, 129.1, 129.1, 128.8, 128.7, 127.0, 126.9, 118.1, 117.9, 81.6, 81.5, 63.8, 62.5, 52.5, 45.4, 44.3, 36.2, 35.3, 28.2, 17.1, 16.4.

***Tert*-butyl (*S*)-2-benzyl-3-oxo-1,4-diazepane-1-carboxylate (34m), t*ert*-butyl (*S*)-2-(cyclohexylmethyl)-3-oxo-1,4-diazepane-1-carboxylate (34n)**



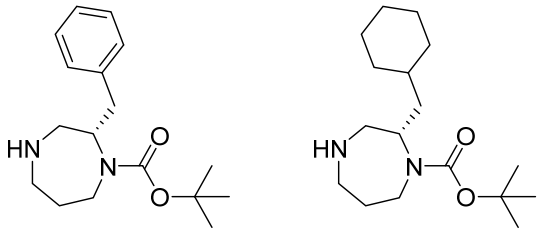
33 (3.49 g, 10.50 mmol) was dissolved in ethanol (30 mL) and chloroform (10 mL). Platinum dioxide (0.48 g, 2.10 mmol) was added and the reaction mixture was stirred at ambient temperature under hydrogen atmosphere for four days. The reaction mixture was filtered over celite, the volatiles were evaporated and the residue was dissolved in THF (6 mL) and methanol (3 mL). A 4 M aqueous solution of lithium hydroxide monohydrate (1.68 mL, 6.73 mmol) was added and the reaction mixture was stirred at ambient temperature for 24 h. The solvents were evaporated and the residue was taken up in a mixture of toluene and methanol (1 : 4, 20 mL). The volatiles were evaporated and the residue was dissolved in DMF (60 mL). DIPEA (1.57 mL, 8.99 mmol) and subsequently HATU (3.42 g, 8.99 mmol) were added to the solution. The reaction mixture was stirred at ambient temperature for 18 h. The

reaction was quenched by addition of saturated aqueous solution of ammonium chloride (60 mL). The product was extracted with ethyl acetate and the combined organic layers were washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product mixture was purified by flash column chromatography applying a gradient of 20% to 60% ethyl acetate in isohexane. The product mixture was obtained as yellow oil (0.75 g).

ESI-MS: *m/z* 327.2 [**34m**+Na]$^+$

m/z 333.2 [**34n**+Na]$^+$

***Tert*-butyl (*S*)-2-benzyl-1,4-diazepane-1-carboxylate (35m), *tert*-butyl (*S*)-2-(cyclohexyl-methyl)-1,4-diazepane-1-carboxylate (35n)**

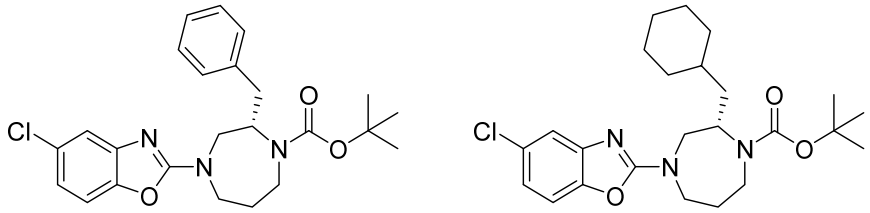


The mixture of **34m** and **34n** (0.75 g) was dissolved in a dried flask in THF (20 mL). A 1 M solution of a borane-THF-complex in THF (12.2 mL, 12.2 mmol) was added dropwise under ice cooling. The reaction mixture was stirred under reflux at 75 °C for 22 h. After cooling to ambient temperature, the reaction was quenched by dropwise addition of methanol (10 mL) under ice cooling. The reaction mixture was diluted with ethyl acetate (100 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 50 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product purified by flash column chromatography applying a gradient of 15% to 30% ethyl acetate in isohexane. The product mixture was obtained as pale yellow oil (0.12 g).

ESI-MS: *m/z* 291.2 [**35m**+H]$^+$

m/z 297.2 [**35n**+H]$^+$

***Tert*-butyl (*S*)-2-benzyl-4-(5-chlorobenzoxazol-2-yl)-1,4-diazepane-1-carboxylate (36m), *tert*-butyl (*S*)-4-(5-chlorobenzoxazol-2-yl)-2-(cyclohexylmethyl)-1,4-diazepane-1-carboxylate (36n)**



21 (58.7 mg, 0.38 mmol), a mixture of **35m** and **35n** (0.12 g) and copper(II) acetate hydrate (76.3 mg, 0.38 mmol) were dissolved in acetonitrile (5 mL). Concentrated acetic acid (43.7 µL, 0.76 mmol) was added and the reaction mixture was stirred under air at 80 °C for 22 h. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate (20 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 10 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 20% to 50% ethyl acetate in isohexane. The products were obtained as brown oils (**36m**: 30.4 mg, 68.8 µmol, 0.7% over four steps, **36n**: 28.4 mg, 63.4 µmol, 0.6% over four steps).

ESI-MS: *m/z* 442.1 [**36m**+H]$^+$

m/z 448.2 [**36n**+H]$^+$

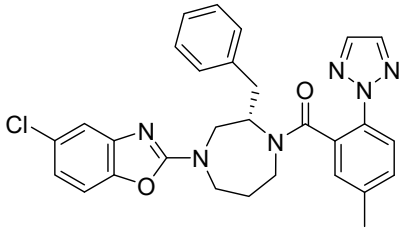
^{1}H NMR: (**36m**: 400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 7.53 (dd, *J* = 8.6, 0.7 Hz, 0.3H), 7.39 – 7.34 (m, 0.3H), 7.34 – 7.27 (m, 2.4H), 7.25 – 7.18 (m, 1.5H), 7.18 – 7.11 (m, 2.5H), 6.96 (dd, *J* = 8.5, 2.1 Hz, 1.0H), 5.03 – 4.73 (m, 1H), 4.41 – 3.93 (m, 2.5H), 3.80 – 3.42 (m, 1.5H), 3.37 – 3.00 (m, 1.5H), 2.99 – 2.71 (m, 2H), 2.71 – 2.59 (m, 0.5H), 1.80 – 1.51 (m, 2H), 1.33 and 1.30 1.28 (s, 9H).

(**36n**: 400 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 7.33 (d, *J* = 1.8 Hz, 1H), 7.16 (d, *J* = 8.4 Hz, 1H), 6.98 (dd, *J* = 8.5, 2.1 Hz, 1H), 4.92 – 4.68 (m, 1H), 4.32 – 4.04 (m, 2H), 3.98 – 3.65 (m, 2H), 3.17 – 3.05 (m, 1H), 2.99 – 2.78 (m, 1H), 1.77 – 1.52, 1.35 – 1.20, 1.20 – 1.04 (m, 24H).

^{13}C NMR: (**36m**: 151 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 162.1, 155.0, 154.8, 147.6, 147.3, 147.1, 138.5, 138.5, 137.7, 137.3, 129.4, 129.3, 128.4, 128.4, 126.5, 126.5, 124.5, 124.0, 120.6, 120.4, 116.1, 116.1, 109.4, 109.2, 79.9, 79.4, 54.0, 53.9, 52.7, 51.2, 50.4, 41.8, 41.4, 29.7, 29.7, 27.6, 22.7, 22.7.

(**36n**: 151 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 166.9, 155.2, 147.3, 147.1, 129.5, 120.5, 119.1, 116.0, 115.8, 109.6, 109.2, 79.7, 54.5, 54.1, 51.3, 50.1, 49.3, 39.0, 38.8, 34.2, 33.8, 33.2, 33.0, 31.6, 31.4, 30.9, 30.2, 29.7, 29.7, 28.2, 27.9, 26.5, 26.4, 26.4, 26.2.

[(*S*)-2-benzyl-4-(5-chlorobenzoxazol-2-yl)-1,4-diazepan-1-yl] [5-methyl-2-(2*H*-1,2,3-triazol-2-yl)-phenyl]methanone (15)



36m (25.6 mg, 57.9 µmol) was dissolved in dichloromethane (3 mL). Trifluoroacetic acid (0.3 mL) was added and the reaction mixture was stirred at ambient temperature for 4 h. The reaction mixture was basified to pH 11 using 1 M aqueous NaOH solution. The product was extracted with dichloromethane (3x). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure to obtain the intermediate **37m** as yellow oil which was dissolved in DMF (10 mL). **24** (12.1 mg, 59.7 µmol), HATU (22.7 mg, 59.7 µmol) and DIPEA (43.0 µL, 0.25 mmol) were added and the reaction mixture was stirred at ambient temperature for 48 h. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution (3x) and brine (1x). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified using a preparative HPLC (gradient of 5% to 95% methanol in water with 0.1% formic acid). The product was obtained as white powder after lyophilization of the product containing fractions (4.2 mg, 8.0 µmol, 14%).

ESI-MS: *m/z* 342.1 [Intermediate **37m**+H]$^+$

m/z 527.2 [M+H]$^+$

HR-ESI-MS: *m/z* [M+H]$^+$ calcd. 527.1957 for $C_{29}H_{27}ClN_6O_2$, found 527.1977

HPLC: eluent system 2: λ = 254 nm, t_R = 21.0 min, purity: 94.2%

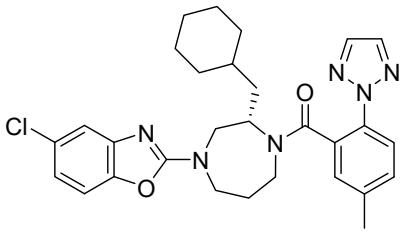
eluent system 3: λ = 254 nm, t_R = 22.4 min, purity: 90.5%

$[\alpha]_D^{23}$: - 33.4° (c = 0.09 in methanol)

^{1}H NMR: (600 MHz, $CDCl_3$, four sets of signals were observed, rotamers, assignments of signals analog to compound **2** (**JH112**)) δ 7.98 – 7.83 (m, 1.1H), 7.80 (s, 0.4H), 7.71 (s, 0.4H), 7.45 – 7.34 (m, 2.9H), 7.34 – 7.27 (m, 3.4H), 7.25 – 7.07 (m, 1.3H), 7.06 – 6.91 (m, 2.7H), 6.60 – 6.55 (m, 0.2H), 6.53 – 6.45 (m, 0.4H), 6.36 – 6.34 (m, 0.2H), 5.55 – 5.42 (m, 0.2H), 5.27 – 5.09 (m, 0.1H), 4.68 (d, *J* = 14.3 Hz, 0.4H), 4.50 (d, *J* = 14.1 Hz, 0.2H), 4.41 – 4.27 (m, 0.5H), 4.23 – 4.10 (m, 0.9H), 4.10 – 4.02 (m, 0.4H), 4.02 – 3.94 (m, 0.5H), 3.85 – 3.70 (m, 0.6H), 3.69 – 3.50 (m, 1.3H), 3.48 – 3.20 (m, 0.9H), 3.13 (dd, *J* = 14.9, 9.6 Hz, 0.6H), 3.08 – 2.91 (m, 1.3H), 2.91 – 2.75 (m, 0.7H), 2.70 (dd, *J* = 13.1, 10.1 Hz, 0.2H), 2.53 – 2.43 (m, 0.3H), 2.38 (s, 1.0H), 2.33 (s, 0.7H), 2.28 (s, 1.3H), 2.03 – 1.84 (m, 0.9H), 1.77 – 1.65 (m, 1.1H).

^{13}C NMR: (151 MHz, $CDCl_3$, four sets of signals were observed, rotamers, assignments of signals analog to compound **2** (**JH112**)) δ 170.0, 169.9, 169.7, 169.6, 161.7, 161.7, 160.4, 160.4, 147.4, 147.1, 144.6, 144.5, 138.6, 138.5, 138.0, 137.8, 137.6, 137.0, 136.5, 136.1, 135.7, 135.5, 135.4, 135.2, 134.0, 133.7, 133.6, 133.6, 130.5, 130.5, 130.4, 129.5, 129.4, 128.8, 128.7, 128.7, 128.5, 128.4, 128.3, 128.3, 128.2, 128.0, 127.1, 126.9, 126.8, 126.8, 122.3, 122.1, 122.0, 121.9, 120.8, 120.5, 116.6, 116.1, 115.9, 109.7, 109.6, 109.4, 109.3, 57.9, 56.2, 52.9, 52.8, 52.6, 51.8, 51.6, 50.9, 49.7, 49.7, 49.4, 44.1, 40.8, 39.7, 37.9, 37.8, 36.5, 36.1, 28.7, 27.4, 27.4, 26.3, 21.0, 21.0, 20.9, 20.3.

(*S*)-[4-(5-chlorobenzoxazol-2-yl)-2-(cyclohexylmethyl)-1,4-diazepan-1-yl] [5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]methanone (16)



36n (24.5 mg, 54.7 µmol) was dissolved in dichloromethane (3 mL). Trifluoroacetic acid (0.3 mL) was added and the reaction mixture was stirred at ambient temperature for 5 h. The reaction mixture was basified to pH 11 using 1 M aqueous NaOH solution. The product was extracted with dichloromethane (3x). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure to obtain the intermediate **37n** as yellow oil which was dissolved in DMF (10 mL). **24** (9.5 mg, 46.9 µmol), HATU (17.8 mg, 46.9 µmol) and DIPEA (33.8 µL, 0.20 mmol) were added and the reaction mixture was stirred at ambient temperature for 48 h. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution (3x) and brine (1x). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified using a preparative HPLC (gradient of 5% to 95% methanol in water with 0.1% formic acid). The product was obtained as white powder after lyophilization of the product containing fractions (9.5 mg, 17.8 µmol, 33%).

ESI-MS: *m/z* 348.2 [Intermediate **37n**+H]$^+$

m/z 533.1 [M+H]$^+$

HR-ESI-MS: *m/z* $[M+H]^+$ calcd. 533.2426 for $C_{29}H_{33}ClN_6O_2$, found 533.2442

HPLC: eluent system 2: λ = 254 nm, t_R = 22.9 min, purity: 97.1%

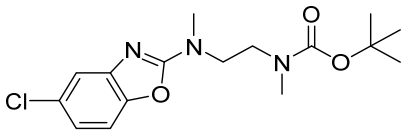
eluent system 3: λ = 254 nm, t_R = 23.3 min, purity: 97.3%

$[\alpha]_D^{23}$: + 21.2° (c = 0.11 in methanol)

^{1}H NMR: (600 MHz, $CDCl_3$, four sets of signals were observed, rotamers, assignments of signals analog to compound **2** (**JH112**)) δ 8.00 – 7.97 (m, 0.0H), 7.93 – 7.87 (m, 0.7H), 7.83 (d, *J* = 8.3 Hz, 0.2H), 7.76 (s, 0.5H), 7.72 (s, 0.3H), 7.50 (s, 0.3H), 7.39 (s, 0.7H), 7.37 – 7.27 (m, 1.4H), 7.25 – 7.21 (m, 0.4H), 7.21 – 7.17 (m, 0.5H), 7.17 – 7.11 (m, 0.7H), 7.06 – 6.98 (m, 1.0H), 6.98 – 6.95 (m, 0.2H), 6.93 (dd, *J* = 8.4, 2.1 Hz, 0.3H), 6.40 – 6.32 (m, 0.2H), 5.34 – 5.07 (m, 0.2H), 4.61 (dt, *J* = 13.9, 3.4 Hz, 0.3H), 4.46 (d, *J* = 13.9 Hz, 0.2H), 4.37 (dd, *J* = 14.3, 4.8 Hz, 0.2H), 4.27 – 4.03 (m, 1.1H), 3.97 (dd, *J* = 15.0, 4.2 Hz, 0.2H), 3.94 – 3.83 (m, 0.5H), 3.83 – 3.74 (m, 0.2H), 3.71 (dd, *J* = 15.1, 4.5 Hz, 0.4H), 3.69 – 3.60 (m, 0.1H), 3.60 – 3.50 (m, 0.5H), 3.46 – 3.33 (m, 0.6H), 3.26 – 3.15 (m, 0.3H), 3.15 – 3.05 (m, 0.1H), 3.05 – 2.88 (m, 0.7H), 2.41 (s, 1.6H), 2.37 (s, 0.6H), 2.36 – 2.26 (m, 0.5H), 2.04 – 1.96 (m, 0.3H), 1.93 – 1.80 (m, 1.7H), 1.80 – 1.71 (m, 0.8H), 1.70 – 1.56 (m, 3.5H), 1.55 – 1.41 (m, 2.8H), 1.40 – 1.35 (m, 0.6H), 1.34 – 1.20 (m, 3.3H), 1.20 – 1.09 (m, 1.8H), 1.09 – 0.79 (m, 2.8H), 0.65 – 0.49 (m, 0.8H), 0.47 – 0.37 (m, 0.2H).

^{13}C NMR: (151 MHz, $CDCl_3$, four sets of signals were observed, rotamers, assignments of signals analog to compound **2** (**JH112**)) δ 169.7, 169.5, 169.4, 169.4, 162.8, 162.7, 162.5, 162.0, 147.6, 147.5, 147.4, 147.3, 144.8, 144.7, 144.6, 138.4, 138.1, 138.1, 137.7, 135.5, 135.3, 135.2, 134.2, 133.8, 133.4, 133.4, 130.4, 130.3, 130.3, 129.6, 129.5, 129.4, 129.4, 129.3, 129.0, 128.9, 128.7, 128.5, 128.4, 128.2, 128.0, 122.6, 122.0, 121.9, 121.6, 120.4, 120.4, 120.3, 120.1, 116.6, 116.3, 116.2, 116.1, 109.5, 109.4, 109.3, 109.0, 54.5, 53.8, 53.1, 52.5, 52.2, 52.1, 50.9, 50.9, 49.9, 49.8, 49.4, 48.9, 43.7, 42.8, 39.4, 39.2, 38.1, 37.8, 34.6, 34.5, 34.0, 34.0, 33.9, 33.6, 33.4, 33.4, 33.1, 32.3, 31.4, 31.2, 30.2, 29.7, 29.7, 29.4, 27.9, 27.2, 27.0, 26.6, 26.5, 26.4, 26.4, 26.4, 26.2, 26.2, 26.0, 26.0, 25.9, 21.0, 21.0, 20.8, 20.4.

Tert-butyl {2-[(5-chlorobenzoxazol-2-yl)(methyl)amino]ethyl} (methyl)carbamate (38o)



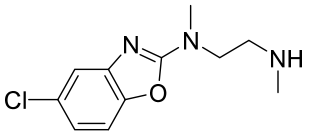
21 (27.0 mg, 0.18 mmol), *tert*-butyl methyl[2-(methylamino)ethyl]carbamate (39.7 mg, 0.21 mmol) and copper(II) acetate hydrate (33.5 mg, 0.17 mmol) were dissolved in acetonitrile (10 mL). Concentrated acetic acid (20.1 µL, 0.35 mmol) was added and the reaction mixture was stirred under air at 80 °C for 19 h. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate (30 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 30 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 3% to 6% methanol in dichloromethane. The product was obtained as brown oil (48.5 mg, 0.14 mmol, 81%).

ESI-MS: *m/z* 340.1 $[M+H]^+$

^{1}H NMR: (600 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 7.29 (d, *J* = 1.3 Hz, 1H), 7.12 (d, *J* = 8.4 Hz, 1H), 6.95 (d, *J* = 8.2 Hz, 1H), 3.67 (dt, *J* = 30.6, 5.8 Hz, 2H), 3.51 (t, *J* = 6.1 Hz, 2H), 3.22 (s, 3H), 2.91, 2.88 (s, 3H), 1.38, 1.36 (s, 9H).

^{13}C NMR: (101 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 163.5, 163.1, 155.8, 155.4, 145.0, 144.9, 129.3, 129.2, 120.2, 120.0, 116.2, 116.2, 109.1, 79.9, 79.7, 48.6, 48.2, 46.8, 46.3, 36.8, 36.1, 35.3, 34.7, 28.3.

_N_-(5-chlorobenzoxazol-2-yl)-_N_,_N'_-dimethylethane-1,2-diamine (39o)



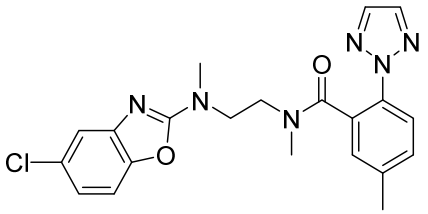
38o (30.4 mg, 89.5 µmol) was dissolved in dichloromethane (3 mL). Trifluoroacetic acid (0.3 mL) was added and the reaction mixture was stirred at ambient temperature for 3 h. The reaction mixture was basified to pH 11 using 1 M aqueous NaOH solution. The product was extracted with dichloromethane (3x). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The product was obtained as yellow oil and used for the next step without purification (20.9 mg, 87.2 µmol, 97%).

ESI-MS: *m/z* 239.9 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$) δ 6.78 – 6.74 (m, 2H), 6.72 (dd, *J* = 8.3, 2.4 Hz, 1H), 3.38 (s, 4H), 2.73 (s, 6H).

^{13}C NMR: (151 MHz, $CDCl_3$) δ 158.6, 147.9, 136.8, 123.7, 120.6, 120.2, 113.6, 48.5, 35.2.

_N_-{2-[(5-chlorobenzoxazol-2-yl)(methyl)amino]ethyl}-_N_,5-dimethyl-2-(2_H_-1,2,3-triazol-2-yl)benzamide (18)



39o (29.3 mg, 0.12 mmol), **24** (30.2 mg, 0.15 mmol) and HATU (94.3 mg, 0.25 mmol) were dissolved in DMF (dry, 2 mL). DIPEA (0.10 mL, 0.61 mmol) was added and the reaction mixture was stirred at ambient temperature for 3 h. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution (3x) and brine (1x). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified using a preparative HPLC (gradient of 5% to 95% methanol in water with 0.1% formic acid). The product was obtained as white powder after lyophilization of the product containing fractions (2.2mg, 5.18 µmol, 2%).

ESI-MS: *m/z* 425.1 $[M+H]^+$

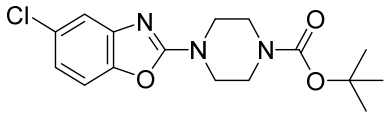
HR-ESI-MS: *m/z* $[M+H]^+$ calcd. 425.1487 for $C_{21}H_{21}ClN_6O_2$, found 425.1489

HPLC: eluent system 1: λ = 254 nm, t_R = 16.4 min, purity: 95.7%

^{1}H NMR: (600 MHz, $CDCl_3$) δ 7.82 (d, *J* = 1.4 Hz, 1H), 7.81 (s, 2H), 7.70 (d, *J* = 8.2 Hz, 1H), 7.45 (dd, *J* = 8.2, 1.4 Hz, 1H), 7.00 (d, *J* = 8.5 Hz, 1H), 6.92 (d, *J* = 2.5 Hz, 1H), 6.79 (dd, *J* = 8.5, 2.5 Hz, 1H), 3.17 (s, 4H), 2.61 (s, 6H), 2.47 (s, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 164.4, 156.5, 143.4, 141.3, 138.6, 136.4, 135.6, 132.6, 131.0, 130.9, 126.6, 124.6, 123.4, 122.9, 119.8, 48.3, 34.8, 21.1.

***Tert*-butyl 4-(5-chlorobenzoxazol-2-yl)piperazine-1-carboxylate (38p)**



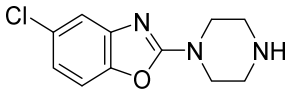
21 (0.20 g, 1.33 mmol), *tert*-butyl piperazine-1-carboxylate (0.30 g, 1.61 mmol) and copper(II) acetate hydrate (0.24 g, 1.19 mmol) were dissolved in acetonitrile (10 mL). Concentrated acetic acid (0.16 mL, 2.66 mmol) was added and the reaction mixture was stirred under air at 80 °C for 2 h. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate (30 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 30 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 20% to 40% ethyl acetate in n-hexane. The product was obtained as brown oil (0.24 g, 0.71 mmol, 54%).

ESI-MS: *m/z* 338.1 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.31 (d, *J* = 2.0 Hz, 1H), 7.15 (d, *J* = 8.5 Hz, 1H), 6.99 (dd, *J* = 8.5, 2.1 Hz, 1H), 3.67 (dd, *J* = 6.3, 3.9 Hz, 4H), 3.56 (dd, *J* = 6.3, 3.9 Hz, 4H), 1.49 (s, 9H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 162.7, 154.5, 147.4, 144.3, 129.4, 120.8, 116.5, 109.3, 80.5, 45.4, 28.4.

5-Chloro-2-(piperazin-1-yl)benzoxazole (39p)



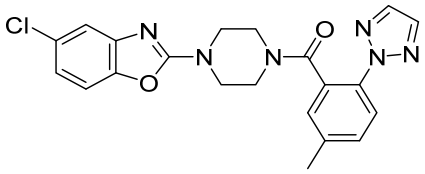
38p (48.8 mg, 0.15 mmol) was dissolved in dichloromethane (3 mL). Trifluoroacetic acid (0.3 mL) was added and the reaction mixture was stirred at ambient temperature for 3 h. The reaction mixture was basified to pH 11 using 1 M aqueous NaOH solution. The product was extracted with dichloromethane (3x). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The product was obtained as yellow oil (34.3 mg, 0.14 mmol, quant.) and used for the next step without purification.

ESI-MS: *m/z* 238.0 $[M+H]^+$

^{1}H NMR: (600 MHz, $CDCl_3$) δ 7.30 (d, *J* = 2.1 Hz, 1H), 7.13 (d, *J* = 8.4 Hz, 1H), 6.97 (dd, *J* = 8.4, 2.1 Hz, 1H), 3.69 – 3.64 (m, 4H), 3.02 – 2.94 (m, 4H), 1.70 (s, 1H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 163.0, 147.3, 144.5, 129.3, 120.4, 116.3, 109.2, 46.5, 45.4.

[4-(5-Chlorobenzoxazol-2-yl)piperazin-1-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl]-methanone (19)



39p (25.0 mg, 0.11 mmol), **24** (23.0 mg, 0.11 mmol) and HATU (81.0 mg, 0.21 mmol) were dissolved in dimethylformamide (dry, 2 mL). DIPEA (90.0 µL, 0.56 mmol) was added and the reaction mixture was stirred at ambient temperature for 2 h. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution (3x) and brine (1x). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified using a preparative HPLC (gradient of 5% to 95% methanol in water with 0.1% formic acid). The product was obtained as white powder after lyophilization of the product containing fractions (21.2 mg, 50.0 µmol, 38%) (4).

ESI-MS: *m/z* 423.2 $[M+H]^+$

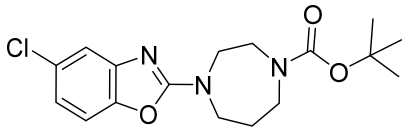
HR-ESI-MS: *m/z* $[M+H]^+$ calcd. 423.1331 for $C_{21}H_{19}ClN_6O_2$, found 423.1326

HPLC: eluent system 1: λ = 254 nm, t_R = 20.7 min, purity: 98.4%

^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.90 (d, *J* = 8.3 Hz, 1H), 7.78 (s, 2H), 7.37 (dd, *J* = 8.4, 1.3 Hz, 1H), 7.30 (d, *J* = 2.0 Hz, 1H), 7.22 (d, *J* = 1.3 Hz, 1H), 7.15 (d, *J* = 8.5 Hz, 1H), 7.00 (dd, *J* = 8.5, 2.1 Hz, 1H), 4.03 – 3.73 (m, 4H), 3.58 – 3.48 (m, 1H), 3.46 – 3.29 (m, 2H), 3.25 – 3.13 (m, 1H), 2.44 (s, 3H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 168.6, 162.5, 147.4, 144.2, 138.7, 135.8, 134.1, 131.0, 129.5, 128.4, 128.1, 122.3, 120.9, 116.6, 109.4, 46.0, 44.9, 44.8, 41.1, 21.0.

***Tert*-butyl 4-(5-chlorobenzoxazol-2-yl)-1,4-diazepane-1-carboxylate (38q)**



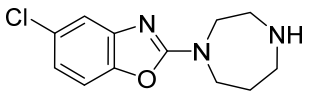
21 (0.30 g, 1.98 mmol), *tert*-butyl 1,4-diazepane-1-carboxylate (0.44 g, 2.18 mmol) and copper(II) acetate hydrate (0.40 g, 1.98 mmol) were dissolved in acetonitrile (20 mL). Concentrated acetic acid (0.23 mL, 3.96 mmol) was added and the reaction mixture was stirred under air at 80 °C for 69 h. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate (100 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 50 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 30% to 50% ethyl acetate in isohexane. The product was obtained as brown oil (0.54 g, 1.54 mmol, 78%).

ESI-MS: *m/z* 352.1 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.32 (d, *J* = 2.0 Hz, 1H), 7.15 (d, *J* = 8.4 Hz, 1H), 6.98 (dd, *J* = 8.4, 2.1 Hz, 1H), 3.81 (t, *J* = 6.0 Hz, 2H), 3.73 (t, *J* = 6.0 Hz, 2H), 3.63 (t, *J* = 5.0 Hz, 2H), 3.46, 3.39 (t, *J* = 5.9, 6.1 Hz, 2H), 2.10 – 1.97 (m, 2H), 1.43 (s, 9H).

^{13}C NMR: (151 MHz, $CDCl_3$) δ 162.4, 162.3, 155.1, 154.8, 147.3, 129.5, 120.5, 116.1, 109.3, 109.3, 80.1, 80.0, 49.7, 49.5, 47.8, 47.7, 47.7, 47.5, 46.5, 46.0, 28.3, 26.7, 26.5.

5-Chloro-2-(1,4-diazepan-1-yl)benzoxazole (39q)



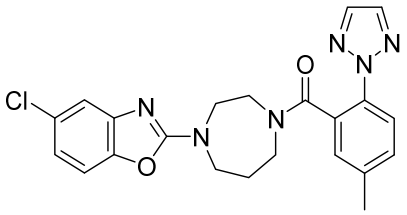
38q (0.53 g, 1.51 mmol) was dissolved in dichloromethane (10 mL). Trifluoroacetic acid (1 mL) was added and the reaction mixture was stirred at ambient temperature for 16 h. The reaction mixture was basified to pH 11 using 1 M aqueous NaOH solution. The product was extracted with dichloromethane (3x). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The product was obtained as yellow oil (0.38 g, 1.50 mmol, 99%) and used for the next step without purification.

ESI-MS: *m/z* 251.98 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.30 (dd, *J* = 2.1, 0.2 Hz, 1H), 7.13 (dd, *J* = 8.5, 0.2 Hz, 1H), 6.95 (dd, *J* = 8.4, 2.1 Hz, 1H), 3.86 – 3.73 (m, 4H), 3.11 – 3.07 (m, 2H), 2.98 – 2.90 (m, 2H), 2.33 – 2.25 (m, 1H), 2.01 – 1.94 (m, 2H).

^{13}C NMR: (151 MHz, $CDCl_3$) δ 163.1, 147.5, 144.9, 129.2, 120.0, 116.1, 109.1, 50.5, 48.9, 48.3, 47.2, 29.7.

[4-(5-chlorobenzoxazol-2-yl)-1,4-diazepan-1-yl][5-methyl-2-(2*H*-1,2,3-triazol-2-yl)phenyl)]-methanone (17)



39q (12.4 mg, 49.3 µmol), **24** (10.0 mg, 49.3 µmol) and HATU (37.5 mg, 98.5 µmol) were dissolved in dimethylformamide (dry, 3 mL). DIPEA (42.3 µL, 0.25 mmol) was added and the reaction mixture was stirred at ambient temperature for 6 h. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution (3x) and brine (1x). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified using a preparative HPLC (gradient of 50% to 95% acetonitrile in water with 0.1% formic acid). The product was obtained as white powder after lyophilization of the product containing fractions (9.9 mg, 22.9 µmol, 46%).

ESI-MS: *m/z* 437.0 $[M+H]^+$

HR-ESI-MS: *m/z* $[M+H]^+$ calcd. 437.1487 for $C_{22}H_{21}ClN_6O_2$, found 437.1490

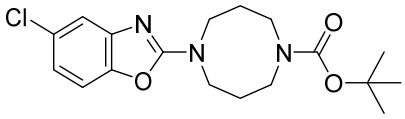
HPLC: eluent system 5: λ = 254 nm, t_R = 12.0 min, purity: 100%

^{1}H NMR: (600 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 7.91 – 7.83 (m, 1H), 7.64, 7.58 (s, 2H), 7.37 – 7.27 (m, 2H), 7.19, 7.10 (d, *J* = 8.4 Hz, 1H), 7.13 – 7.12, 6.99 – 6.97 (m, 1H), 7.01, 6.97 (dd, *J* = 8.4, 1.9 Hz, 1H), 4.13 – 4.07 4.00 – 3.87, 3.87 – 3.74,

3.75 – 3.67, 3.61 – 3.50, 3.45 – 3.33, 3.32 – 3.23 (m, 8H), 2.39, 2.31 (s, 3H), 1.89 – 1.79 (m, 2H).

^{13}C NMR: (151 MHz, $CDCl_3$, two sets of signals were observed, rotamers) δ 169.5, 169.4, 147.4, 144.4, 138.5, 138.4, 135.6, 135.5, 133.9, 130.7, 130.7, 129.5, 129.5, 128.7, 128.6, 128.4, 128.3, 122.4, 122.2, 120.5, 120.4, 116.3, 109.4, 109.2, 49.0, 48.8, 48.7, 48.6, 47.9, 46.5, 45.6, 44.0, 27.9, 26.6, 21.0, 20.9.

Tert-butyl 5-(5-chlorobenzoxazol-2-yl)-1,5-diazocane-1-carboxylate (38r)



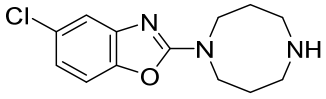
21 (78.0 mg, 0.51 mmol), *tert*-butyl 1,5-diazocane-1-carboxylate (0.12 g, 0.56 mmol) and copper(II) acetate hydrate (93.0 mg, 0.47 mmol) were dissolved in acetonitrile (20 mL). Concentrated acetic acid (59.0 µL, 1.02 mmol) was added and the reaction mixture was stirred under air at 80 °C for 43 h. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate (50 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 50 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 3% to 7% methanol in dichloromethane. The product was obtained as brown oil (0.16 g, 0.44 mmol, 68%).

ESI-MS: *m/z* 366.1 $[M+H]^+$

^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.30 (dd, *J* = 2.1, 0.2 Hz, 1H), 7.13 (dd, *J* = 8.4, 0.3 Hz, 1H), 6.94 (dd, *J* = 8.4, 2.1 Hz, 1H), 3.73 – 3.63 (m, 4H), 3.48 – 3.32 (m, 4H), 2.12 – 1.98 (m, 4H), 1.30 (s, 9H).

^{13}C NMR: (151 MHz, $CDCl_3$) δ 162.3, 154.9, 147.5, 144.9, 129.1, 119.9, 116.1, 109.1, 79.6, 50.0, 48.3, 28.3, 26.5.

5-chloro-2-(1,5-diazocan-1-yl)benzoxazole (39r)



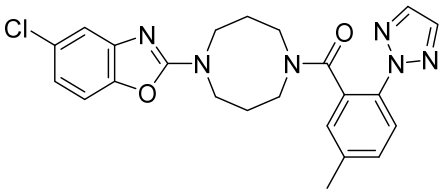
38r (0.16 g, 0.43 mmol) was dissolved in dichloromethane (3 mL). Trifluoroacetic acid (0.3 mL) was added and the reaction mixture was stirred at ambient temperature for 4 h. The reaction mixture was basified to pH 11 using 1 M aqueous NaOH solution. The product was extracted with dichloromethane (3x). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography applying a gradient of 5% to 10% methanol in dichloromethane with 0.5% ammonia. The product was obtained as yellow solid (0.10 g, 0.39 mmol, 92%).

ESI-MS: *m/z* 265.9 $[M+H]^+$

^{1}H NMR: (600 MHz, CD_3OD) δ 7.30 (d, *J* = 8.5 Hz, 1H), 7.26 (d, *J* = 2.1 Hz, 1H), 7.03 (dd, *J* = 8.5, 2.1 Hz, 1H), 3.86 – 3.77 (m, 4H), 3.23 – 3.11 (m, 4H), 2.16 – 2.03 (m, 4H).

^{13}C NMR: (101 MHz, CD_3OD) δ 164.4, 148.9, 145.5, 130.6, 121.6, 116.5, 110.8, 49.2, 48.0, 27.2.

[5-(5-chlorobenzoxazol-2-yl)-1,5-diazocan-1-yl][5-methyl-2-(2H-1,2,3-triazol-2-yl)phenyl]methanone (20)



39r (0.10 g, 0.38 mmol), **24** (91.8 mg, 0.45 mmol) and HATU (0.29 g, 0.75 mmol) were dissolved in dimethylformamide (dry, 5 mL). DIPEA (0.33 mL, 1.88 mmol) was added and the reaction mixture was stirred at ambient temperature for 4 h. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution (3x) and brine (1x). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified using a preparative HPLC (gradient of 5% to 95% methanol in water with 0.1% formic acid). The product was obtained as white powder after lyophilization of the product containing fractions (68.3 mg, 0.15 mmol, 40%).

ESI-MS: *m/z* 451.1 $[M+H]^+$

HR-ESI-MS: *m/z* $[M+H]^+$ calcd. 451.1644 for $C_{23}H_{23}ClN_6O_2$, found 451.1643

HPLC: eluent system 1: λ = 254 nm, t_R = 20.5 min, purity: 99.8%

^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.87 (d, *J* = 8.3 Hz, 1H), 7.70 (s, 2H), 7.34 (d, *J* = 2.0 Hz, 1H), 7.30 – 7.24 (m, 1H), 7.17 (d, *J* = 8.4 Hz, 1H), 6.99 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.84 (d, *J* = 1.0 Hz, 1H), 4.05 – 3.78 (m, 3H), 3.69 – 3.48 (m, 2H), 3.44 – 3.24 (m, 2H), 3.19 – 3.02 (m, 1H), 2.55 – 2.36 (m, 1H), 2.29 (s, 3H), 2.08 – 1.89 (m, 2H), 1.83 – 1.69 (m, 1H).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 169.8, 162.4, 147.6, 144.7, 138.3, 135.5, 133.5, 130.4, 129.3, 129.0, 128.8, 121.8, 120.1, 116.2, 109.4, 49.8, 49.2, 48.8, 46.2, 26.9, 26.1, 20.9.

Ligands

Orexin A was purchased from Bachem (Bubendorf, Switzerland) and stock solutions were prepared in H_2O at a concentration of 1 mM. Suvorexant was purchased from Carbosynth (Compton, UK), SB-674042 and EMPA were purchased from Sigma Aldrich (Darmstadt, Germany). Stock solutions of suvorexant, SB-674042, EMPA and all test ligands were prepared in DMSO at a concentration of 10 mM. Serial dilutions of the compounds were prepared in the buffers indicated for each assay.

Site-directed mutagenesis

Mammalian expression vectors coding for the human isoforms of OX1R and OX2R were obtained from the cDNA Resource Center (HCRTR1, www.cDNA.org) or Genescript (NM_005126), respectively. Mutant receptors OX1_A127T and OX2_T135A were generated using one-step site directed mutagenesis protocols (9) and confirmed by DNA sequencing.

Radioligand binding studies

Affinities of the test ligands for the human orexin receptors OX1R and OX2R (Table S1) were determined by radioligand competition in analogy to previously described protocols (10). In brief, HEK 293T (ATCC accession number CRL-11268) cells were transiently transfected with the cDNAs coding for OX1R or OX2R, respectively, using the Mirus TransIT-293 transfection reagent (PeqLab, Erlangen, Germany) or a solution of polyethylenimine in PBS (PEI, linear, 25 kDa, Polysciences) as transfection reagent at a 3:1 PEI to cDNA ratio (11, 12). After preparation of membranes (12), radioligand displacement assays were run in binding buffer (50 mM Tris-HCl, 1 mM EDTA, 5 mM $MgCl_2$, 100 µg/mL bacitracin, 5 µg/mL soybean trypsin inhibitor, pH 7.4) with the radioligands [^{3}H]SB-674042 (specific activity = 45 Ci/mmol, Novandi, Södertälje, Sweden) at a final concentration of 0.50-0.70 nM for OX1R, or [^{3}H]EMPA (specific activity = 81 Ci/mmol, Novandi, Södertälje, Sweden) at a final concentration of 0.50-0.75 nM for OX2R, respectively. The assays were carried out in 96-well format at a protein concentration of 2-4 µg/assay tube, with a B_{max} value of 5,700 ± 900 fmol/mg, and a K_D value of 0.85 ± 0.08 nM for OX1R, and a B_{max} of 4,900 ± 1,100 fmol/mg, K_D of 1.18 ± 0.13 nM for OX2R, respectively. Unspecific binding was determined in the presence of 10 µM of the unlabeled ligands SB-674042 or EMPA, respectively. Protein concentrations were determined employing the method of Lowry with bovine serum albumin as standard (13). The resulting competition curves of the receptor binding experiments were analyzed by nonlinear regression using the algorithms in PRISM 6.0 (GraphPad Software, San Diego, USA). The data were initially fit using a sigmoid model to provide IC_{50} values which were subsequently transformed to K_i values according to the equation of Cheng and Prusoff (5). Three to seven experiments per compound were performed, with each concentration in triplicate. Binding assays with the mutant receptors OX1_A127T and OX2_T135A were carried out in an analogous manner, with a protein concentration of 10 µg/assay tube, [^{3}H]SB-674042 at a final concentration of 0.7 nM, B_{max} 1400 fmol/mg, K_D 1.7nM for OX1_A127T and a protein concentration of 20 µg/test tube, [^{3}H]EMPA at a final concentration of 0.7 nM, B_{max} 1000 fmol/mg, K_D 1.7 nM for OX2_T135A, respectively.

The dissociation rate constant for [^{3}H]SB-674042 was determined by incubation of OX1R expressing cell membranes (5 µg protein/test tube) with 0.66 – 0.80 nM [^{3}H]SB-674042 in binding buffer for 1 h at 37°C, before an equal volume of unlabeled SB-674042 was added (final concentration 10 µM) to prevent re-association of [^{3}H]SB-674042. Membranes were harvested after an additional incubation period at 37°C for 1-120 min. Unspecific binding was determined in the presence of unlabeled SB-674042 (10 µM). Specific binding at the different time points was analyzed using a one-phase exponential decay curve in GraphPad Prism to determine the dissociation rate constant (k_{off}, $T_{1/2}$). The association binding

rate of [³H]SB-674042 was determined by incubation of OX1R-expressing membranes with the radioligand at a final concentration of 2.0 to 5.4 nM for 1-120 min at 37°C. Membranes were harvested and specific binding at the different time points was analyzed employing the association kinetics analysis in GraphPad Prism to determine the association rate constant (k_{on}) from the observed association kinetics (k_{obs}). Association and dissociation rate constants for suvorexant and **JH112** were determined indirectly using the kinetic binding assay described by Guo et al. (14) with [³H]SB-674042 at a final concentration of 3.0 nM and an incubation time of 1-120 min. For each compound, 3 different concentrations were used and the resulting binding curves were analyzed globally using the algorithms implemented in GraphPad Prism with the association and dissociation rates of [³H]SB-674042 as determined above.

The dissociation rate constant for [³H]EMPA from OX2R was determined as described for OX1R using OX2R expressing membranes (4 µg protein/test tube) and 1.0 nM [³H]EMPA for 40 min at 37°C. Unlabeled EMPA at a final concentration of 10 µM was added and membranes were harvested after an additional incubation period at 37°C for 1-60 min. Unspecific binding was determined in the presence of unlabeled EMPA (10 µM). Analysis of specific binding at different time points resulted in the dissociation rate constant (k_{off}, $T_{1/2}$). Association binding of [³H]EMPA was determined by incubation of OX2R-expressing membranes with the radioligand at a final concentration of 5.0 nM for 1-90 min at 37°C. The association rate constant (k_{on}) was determined from the observed association kinetics (k_{obs}). Association and dissociation rate constants for suvorexant and **JH112** were determined indirectly using the kinetic binding assay described by Guo et al. (14) with [³H]EMPA at a final concentration of 5.0 nM and an incubation time of 1-90 min. For each compound, two different concentrations were used and the resulting binding curves were analyzed globally using the algorithms implemented in GraphPad Prism with the association and dissociation rates of [³H]EMPA as determined above.

Determination of receptor specificity

To investigate receptor specificity, we determined the binding affinities of **JH112** for 20 non-orexin GPCRs and compared them to the reference antagonist suvorexant (Fig. S5). Thus, we measured the ability of **JH112** and suvorexant to displace a radioligand with membranes from cells transiently or stably expressing the appropriate receptor with the conditions listed in Table S3 in analogy to the protocol described above. K_i values were determined in their logarithmic form (pK_i) by non-linear regression employing the algorithms for one-site binding implemented in GraphPad Prism 6.0 (GraphPad Software, USA). Per compound and receptor, two experiments were performed with each concentration in triplicates.

IP_1 accumulation assay

OX1R-mediated activation of $G\alpha_q$ was determined applying the IP-One HTRF® assay (Cisbio, Codolet, France) according to the manufacturer's protocol and in analogy to previously described protocols (15). In brief, HEK 293T cells were grown to a confluence of approx. 70% and transiently transfected with the cDNA coding for OX1R in pcDNA 3.1 (cDNA Resource Center, cdna.org) applying the Mirus TransIT-293 transfection reagent. After one day, cells were detached from the culture dish with Versene (Life Technologies, Darmstadt, Germany), seeded into black 384-well plates (10,000 cells/well, Greiner Bio-One, Frickenhausen, Germany) and maintained for 24 h at 37 °C. On the day of the experiment, cells were incubated first with the test compounds dissolved in stimulation buffer for 30 min at 37°C, before the endogenous agonist orexin A (final concentration 10 pM to 30 µM) was added and the incubation was continued for 90 min at 37 °C. Incubation was stopped by addition of the detection reagents (IP_1-d2 conjugate and Anti-IP_1cryptate TB conjugate each dissolved in lysis buffer) and incubation for further

60 min at room temperature. Time resolved fluorescence resonance energy transfer was measured using the Clariostar plate reader (BMG, Ortenberg, Germany) equipped with the appropriate filter set. The obtained FRET-signals were normalized to the maximum effect of a saturating concentration of orexin A (100%) and vehicle (0%) in the absence of antagonist. Concentration-response curves were analyzed using the algorithms for four parameter non-linear regression implemented in PRISM 6.0 (GraphPad Software, USA) to derive EC_{50} and E_{max} values. Three to twelve experiments per compound were performed, with each concentration in duplicate.

Recruitment of β-arrestin-2 (Pathhunter)

Orexin A stimulated recruitment of β-arrestin-2 to OX1R was investigated employing the Pathhunter assay (DiscoverX, Birmingham, U.K.) which is based on enzyme fragment complementation in analogy to previously described protocols (12, 16). OX1R was fused in frame with the C-terminal PK1-tag using overlapping PCR and Gibson assembly (17) and transiently transfected in HEK 293 cells stably expressing the enzyme acceptor-β-arrestin-2 using Mirus TransIT-293 as the transfection reagent. Cells were maintained in DMEM/F12 medium supplemented with 10% FBS (Life Technologies, Darmstadt, Germany) at 37°C and 5% of CO_2 for 24 h, before they were detached with Versene and transferred into white 384-well plates (Greiner Bio-One, Frickenhausen, Germany) at a density of 5000 cells/well in CP7 Reagent (DiscoverX). On the day of the experiment, cells were incubated first with the test compounds dissolved in PBS for 30 min at 37°C, followed by the addition of the endogenous agonist orexin A (final concentration 10 pM to 30 µM). After further incubation for 90 min at 37 °C, the detection mix was added and incubation was continued at room temperature for 60 min. Chemoluminescence was determined on a Clariostar plate reader (BMG) in luminescence mode. Three to sixteen experiments per condition were performed, with each concentration in duplicate. All responses were normalized to the effect of a saturating concentration of orexin A (100%) and buffer conditions (0%). Concentration-response curves were analyzed by four parameter non-linear regression in PRISM 6.0 (GraphPad Software, USA) to derive EC_{50} and E_{max} values.

Bioluminescence resonance energy transfer-based functional assays

OX1R-mediated activation of $G\alpha_q$ proteins was determined employing a BRET²-biosensor based on the separation of RLucII-$G\alpha_q$ and Gβγ-GFP10 in analogy to previously described protocols (18-20). Recruitment of RLucII-β-arrestin-2 was determined employing enhanced bystander BRET with CAAX-rGFP (21). Briefly, HEK 293T cells were seeded into 12-well plates (1.2 mL/2.0 x 10^5 cells/mL) in DMEM/F12 medium supplemented with 10% FBS, 100 µg/mL penicillin, 100 µg/mL streptomycin and transfected using a solution of polyethylenimine in PBS (PEI, linear 25 kDa, Polysciences, 1 mg/mL) as transfection reagent at a 3:1 PEI to cDNA ratio. For each combination of receptor and biosensors, BRET titration experiments were performed to determine optimal donor/acceptor cDNA ratios (19). Cells were simultaneously transfected with plasmids (pcDNA3.1) encoding the cDNAs of wildtype OX1R, RLucII-$G\alpha_{iq}$, $G\beta_1$ and $G\gamma_2$-GFP10 (ratio 4:1:1:3.5), or wildtype OX1R, RLucII- β-arrestin-2 and CAAX-rGFP (1:0.1:2), respectively. The total amount of cDNA was kept at 1 µg cDNA/well by addition of single stranded salmon sperm DNA (Sigma Aldrich). Transfected cells were transferred to white 96-well plates (100 µL/well, Greiner, Frickenhausen, Germany) and incubated at 37°C, 5% CO_2 for 48h. On the day of the experiment, the medium was replaced by 70 µL dPBS (+Ca^{2+}/Mg^{2+}, Invitrogen) and the cells were kept at 37°C for 30 min. All ligand dilutions were prepared in dPBS (+Ca^{2+}/Mg^{2+}). If not stated otherwise, cells were incubated with the agonist orexin A (10 µL) for 30 minutes at 37°C, before antagonists (10 µL) were added and the incubation was continued for further 120 min. 10 µL Coelenterazine 400a (2.5 µM final concentration), was added 5 min before the BRET measurement. BRET readings were obtained

on a Clariostar (BMG Labtech, Ortenberg, Germany) microplate reader with the respective filter set (donor 410-80 nm and acceptor 515-30 nm) as ratio of the light emitted by the acceptor (GFP10 or rGFP) divided by signal from the light emitted by the donor (RLucII). Ligand induced changes in BRET signal (ΔBRET) were calculated as difference between the BRET ratio obtained under vehicle conditions and the BRET ratio obtained with addition of ligands. Data was analyzed using the algorithms for three parameter non-linear regression implemented in PRISM 6.0 (GraphPad Software, USA). Responses were normalized to the maximum effect of a saturating concentration of orexin A (100%) and vehicle (0%). In the inhibitory mode, results were normalized to the orexin A prestimulated effect (EC_{80}, 30 nM for $G\alpha_q$, 100 nM for β-arrestin-2, set to 100%) and the signal obtained in the absence of any ligand (0%). Three to nineteen experiments per compound were performed, with each concentration in triplicate.

Operational model of hemi-equilibrium competitive antagonism

Since increasing concentrations of **JH112**, suvorexant and SB-674042 led to insurmountable antagonism of the orexin A stimulated activation of OX1R in $G\alpha_q$ activation and β-arrestin-2 recruitment assays, we analyzed the data employing an hemi-equilibrium model as previously described for OX2R (6). Thus, our grouped functional data from IPOne accumulation, BRET-$G\alpha_q$ and BRET-β-arrestin-2 recruitment assays were globally fitted using the equations below in PRISM 6.0. Concentration-response curves from the Pathhunter assay were not analyzed, since there **JH112** and suvorexant resulted in a complete depression of the orexin A response. In the IPOne assay, data for SB-674042 were excluded from the analysis as equilibrium was observed. Thus, for each ligand and assay, data were analyzed using

$$Y = \frac{[A]/K_A(1 - (\alpha \cdot (1 - e^{-k_{off} \cdot \gamma \cdot t}) + \cdot \beta \cdot e^{-k_{off} \cdot \gamma \cdot t})) \cdot \tau \cdot E_m}{[A]/K_A((1 - (\alpha \cdot (1 - e^{-k_{off} \cdot \gamma \cdot t}) + \cdot \beta \cdot e^{-k_{off} \cdot \gamma \cdot t})) \cdot \tau + 1) + 1}$$

with

$$\alpha = \frac{[B]/K_B}{([B]/K_B + [A]/K_A + 1)}$$

$$\beta = \frac{[B]/K_B}{([B]/K_B + 1)}$$

$$\gamma = \frac{([B]/K_B + [A]/K_A + 1)}{([A]/K_A + 1)}$$

where [A] is the concentrations of the agonist orexin A and [B] represents the concentration of SB-674042, suvorexant or **JH112**, respectively; K_A and K_B represent the respective equilibrium dissociation constants, k_{off} is the dissociation rate constant for the antagonist (min^{-1}), t is the assay incubation time (min), τ is the operational efficacy of orexin A (accounting for cell-, assay- and agonist-dependent properties) and E_m is the maximal system response. All parameters were shared across the data sets except E_m and t, which were fixed to 100% and the assay incubation time (90 min for IPOne, 120 min for $G\alpha_q$ and β-arrestin-2), respectively. Subsequently, the dissociation constant was transformed into the dissociation half-life for each ligand and assay.

Pharmacokinetics of JH112

Studies were performed by Preclinical Pharmacology Core at the University of Texas Southwestern Medical Center. 21 female CD-1 mice were dosed intraperitoneal with 10 mg/kg **JH112** (0.2 mL/mouse

formulated with 5% DMSO, 5 % Tween 80, 90% D5W (5% Dextrose in water)). At each time point, the whole blood was collected from three mice in a syringe coated with ACD. Plasma concentration of **JH112** was determined by liquid chromatography – mass spectrometry (LC/MS). The brains of the mice were also collected and homogenized for determination of the **JH112** concentrations by LC/MS.

Metabolism of JH112

Metabolism experiments were performed as described previously (22-24). In brief, the incubation mixture contained **JH112** (20 µM) or positive controls (suvorexant, imipramine and rotigotine), pooled microsomes from male rat liver (Sprague Dawley, Sigma Aldrich, 0.5 mg of microsomal protein/mL of incubation mixture) in Tris-$MgCl_2$ buffer (50 mM Tris, 5.0 mM $MgCl_2$, pH 7.4). The final incubation volume was 0.5 mL. Microsomal reactions were initiated by addition of 50 µL of enzyme cofactor solution NADPH (Carl Roth, final concentration of 1 mM). At 0, 15, 30 and 60 min aliquots of 100 µL were taken and the enzymatic reactions were terminated by addition of 100 µL of ice-cold acetonitrile (containing 5 µM internal standard **16**), and precipitated protein was removed by centrifugation (15,000 rcf for 3 min). The supernatant was analyzed by HPLC/MS (column; agilent XDB-C8, 3.5 µm, 3.0 x 100 mm, binary solvent system: eluent methanol in 0.1% aqueous formic acid, 10−80% methanol in 17 min, 80−100% methanol in 3 min, 100% methanol for 5 min, flow rate of 0.4 mL/min). Per compound, three independent experiments were performed. Control incubations were conducted in the absence of cofactor solution to determine unspecific binding to matrix. Substrate remaining and metabolite formation was calculated as a mean ± SEM of three independent experiments by comparing AUC of metabolites and substrate after predetermined incubation time to AUC of substrate at time 0 min, estimating a similar ionization rate, corrected by a factor calculated from the AUC of internal standard at each time point.

Cloning, expression, purification, and crystallographic structure determination

Crystallization of hOX1R with **JH112** was carried out as previously reported (25), aside from incorporation of a thermostabilizing mutant Ala133$^{3.39}$Lys (26). The resulting construct was transfected into Sf9 cells to produce a recombinant baculovirus with the Bac-to-Bac system (Invitrogen). Sf9 cells at a density of 3 × 10^6 ml^{-1} were infected with high titer viruses and 1 µM of **JH112** was added into media during growth. After 48 hours, cells were collected and stored at -80 °C for future use.

For purification, Sf9 cell pellets were resuspended and lysed in hypotonic buffer containing 10 mM Tris-HCl pH 7.4, 1 mM EDTA, 160 µg/ml benzamidine, 100 µg/ml leupeptin, 2 mg/ml iodoacetimide and 1 µM **JH112** for 30 min at 4 °C. Lysed cells were spun down and harvested membranes were dounce homogenized in solubilization buffer containing 50 mM Tris-HCl pH 7.4, 500 mM NaCl, 1% (w/v) n-dodecyl-β-D-maltopyranoside (DDM, Anatrace), 0.2% sodium cholate, 0.2% cholesteryl hemi-succinate (CHS), 10% glycerol, 2 mg/ml iodoacetamide and 5 µM **JH112**. Solubilization proceeded for 1 hour at 4 °C, followed by ultracentrifuge at 35,000 rpm for 30 min at 4 °C. The supernatant was incubated with Ni-NTA agarose beads (GE Healthcare) in batch-binding mode for 4 hours at 4 °C in the presence of 20 mM imidazole. After binding, the Ni-NTA beads were spun down and transferred into a glass column. Then beads were washed with 10 volumes of wash buffer: 50 mM Tris-HCl pH 7.4, 500 mM NaCl, 0.05% DDM, 0.01% sodium cholate, 0.01% CHS, 5% glycerol, 50 mM imidazole, 5 µM **JH112**. Receptor was eluted with 5 volumes of elution buffer: 50 mM Tris-HCl pH 7.4, 500 mM NaCl, 0.05% DDM, 0.01% sodium cholate, 0.01% CHS, 5% glycerol, 200 mM imidazole, 5 µM ligand. The eluate from Ni affinity chromatography was supplemented with 2 mM calcium chloride and tandemly applied onto M1 anti-Flag affinity beads (Sigma). On the M1 beads, DDM was incrementally exchanged to 0.05% lauryl maltose

neopentyl glycol (LMNG, Anatrace). **JH112**-bound hOX1R was eluted from M1 beads with buffer containing 200 µg/ml FLAG peptide plus 5 mM EDTA. To remove *N*-linked glycans from the receptor, PNGaseF (NEB) was incubated with the receptor at 4 °C for 8 hours. Finally, the receptor was concentrated and applied onto Superdex 200 size exclusion column (GE Healthcare). A single peak corresponding to monomeric receptor was collected, concentrated using a 100 kDa cutoff Vivaspin concentrator (Sartorius), and used in crystallization.

For setting in meso crystallization, concentrated receptor (30-50 mg/ml) was reconstituted into a lipid mixture consisting of 90% (w/w) monoolein and 10% (w/w) cholesterol (Sigma) at mass ratio 2:3. Crystallization experiments were carried out in 96-well glass sandwich plates (Molecular Dimensions) with a Gryphon LCP crystallization robot (Art Robbins Instruments). Setups consisted of 40 nl protein cubic phase mixture overlaid with 800 nl precipitant solution. Plates were incubated at 20 °C. Crystals typically appeared in three days and reached their full size in one week. Optimized crystals grew in 100 mM sodium citrate pH 5.1, 31% PEG400, 200 mM ammonium formate. Crystals were cryo-protected in the crystallization mother liquid, harvested with 100 micron loops (MiTeGen) and immediately flash frozen in liquid nitrogen.

Diffraction data was collected at the 23ID-D (GM/CA-CAT) beamline, Advanced Photon Source, Argonne National Laboratory. Data was collected using a Pilatus3 6M detector. All data was acquired using a 20 µm collimated mini beam at the wavelength 1.0330 Å. For each crystal, 25 frames were collected with 0.4° oscillation and 1s exposure without attenuation of the beam. A full data set was attained by merging data from 8 crystals. HKL3000(27) was employed to index, integrate, scale data and perform anisotropic analysis and resolution cutoff. The correction for anisotropy was applied along a*, b* and c* during scaling, with cutoffs at 3.7 Å, 4.0 Å, and 3.5 Å, respectively.

The structure of hOX1R-PGS was solved by molecular replacement with Phaser (28) in Phenix (29). The PGS (PDB 2BFW) and hOX1R structure (PDB 4ZJ8) were used as independent search models. The resulting solution contained one receptor fusion protein per asymmetric unit. The model was manually built in Coot (30) and refinement was performed with Phenix. Translation-libration-screw (TLS) refinement was employed to model atomic displacement factors, with TLS groups generated by Phenix. Initial coordinates and refinement parameters for the ligand were prepared with the PRODRG web server (31). MolProbity (32) was used to evaluate the final structures. The MolProbity score was 1.46 with a 100% percentile rank in the resolution range of 3.25-3.75 Å. In the Ramachandran plot, 95.6% and 4.4% of residues were in favored and allowed regions, respectively. The statistics for data collection and refinement are included in Table S4. The 2Fo-Fc electron density map for the binding pocket region and the polder OMIT map (33) for the ligand **JH112** are shown in Fig. S6. Atomic coordinates have been deposited in the PDB under accession code 6V9S.

Conformation generation & docking

Conformations were generated using OMEGA (34). For each SMILES, a maximum of 100 (400 for the cyclopropyl derivative **6** and the ethyl derivative **3**) conformations were generated, with the rms value of each conformation differing between 0-0.5. The energy window was set to 10. All conformations were docked with OpenEye's FRED tool (35, 36) to both OX1R (PDB: 4ZJ8) and OX2R (PDB: 4S0V) crystal structures and the docking poses were evaluated manually.

In the FRED docking calculations, ten poses were generated for each molecule and the docking resolution was set to „high". After docking, the sidechains of residues within 5 Å of the docking molecule as well as the docked molecule itself were minimized using OpenEye's SZYBKI tool (37-40) (36-39).

NAMD Simulations

Molecular dynamics simulations were carried out in the NPT conditions. Four replicas of 200 ns each were simulated with the temperature set to 303.15 K. The respective crystal structures of OX1R (PDB: 4ZJ8) and OX2R (PDB: 4S0V) with suvorexant and OX1R with **JH112** were used for the simulations. The starting structure of **JH112** in OX2R was derived from the docked pose. Receptor structures were prepared for docking by complementing valences with hydrogens, with $H^{7.39}$ doubly protonated, and minimizing all hydrogen atoms. The ICL3-residues were connected to close the loop. The disulfide bridge between $C^{3.25}$ and C202 was established as well.

The membrane builder CHARMM (41-46) was used to generate the complete system for the simulation. CHARMM topology and parameter files were calculated for the CHARMM general force field for the ligand. The N-terminus was acetylated (ACE) and the C-terminus was amidated (CT2).

The receptor was aligned to the z-axis. Water thickness was set to 20.0 and 2 x 67 POPC lipids were placed next to the receptor as a membrane bilayer. The system is rectangular and it was built by using the replacement method. For charge equilibration, 0.15 M NaCl were added with the Monte-Carlo method. The equilibration and production simulations were run with default parameters from the CHARMM-GUI (45, 47) setup.

MD-Simulation Evaluation

Trajectories were concatenated in VMD (48). Frames where wrapped and aligned to the first frame of the simulation, and lipids were removed. Trajectories were analyzed with cpptraj (49), using the *hbond* function for the hydrogen bond analysis. The 2d-RMSD plots were made with the function *2drms* and the fractional native contact analysis were made using *nativecontacts*. For this analysis, residues within 5 Å of the ligand **JH112** were considered in the native contact mask and the cutoff of interaction was set to 3 Å. Five clusters with equal average distances between the clusters were calculated for each ligand conformation. Every representative conformation from a cluster with >9 % in frequency was used to compare conformations from different systems. Cluster analysis was conducted using cpptraj and the *cluster* function.

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