Drug Design

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Rational Design and Asymmetric Synthesis of Potent and Neurotrophic Ligands for FK506-Binding Proteins (FKBPs) **

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Abstract: *To create highly efficient inhibitors for FK506-binding proteins, a new asymmetric synthesis for pro-(S)-C^5-branched [4.3.1] aza-amide bicycles was developed. The key step of the synthesis is an HF-driven N-acyliminium cyclization. Functionalization of the C^5 moiety resulted in novel protein contacts with the psychiatric risk factor FKBP51, which led to a more than 280-fold enhancement in affinity. The most potent ligands facilitated the differentiation of N2a neuroblastoma cells with low nanomolar potency.*

FK506-binding proteins (FKBPs) are part of the immunophillin family and best known for their immunosuppressive activity as complexes with the natural products FK506 and rapamycin.[1] In addition, FKBPs are prominently expressed in the central nervous system and non-immunosuppressive FKBP ligands have repeatedly displayed neuroprotective and neurotrophic effects.[2] Brain-specific deletion of FKBP12 was shown to enhance contextual memory, whereas FKBP52 controls neuronal growth cone guidance.[3] Among the human FKBPs, FKBP51 has gained particular interest as a regulator of stress-coping behavior and as a risk factor for stress-related psychiatric disorders, thus suggesting FKBP51 inhibition as a potential therapeutic approach for these indications.[4] However, the development of potent drug-like inhibitors for FKBPs in general and for FKBP51 in particular is challenging owing to the shallow FK506 binding site.[5] Inspired by work from Agouron,[6c] we previously identified a [4.3.1] bicyclic scaffold as a preferred FKBP binding motif, which is preorganized to mimic the active conformation of known FKBP ligands (Figure 1).[6a,b] The [4.3.1] cyclization strategy repeatedly enhanced ligand efficiency compared to previous monocyclic scaffolds, although the actual affinities for the large FKBPs remained moderate. We hypothesized that the [4.3.1] bicycles might also serve as a rigid framework to attach further substituents in a conformationally defined manner. Herein, we report a synthesis for C^5-branched [4.3.1] aza-amide bicycles, which resulted in the first low nanomolar and highly ligand-efficient FKBP inhibitors with neurotrophic activity.

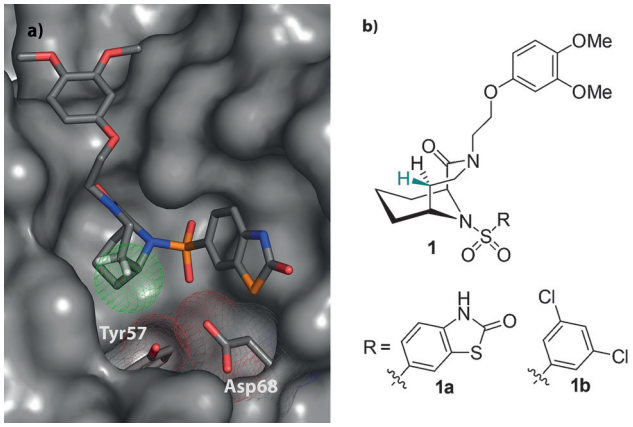

Figure 1. a) Cocrystal structure of **1a** in complex with the FK506-binding domain of FKBP51 (PDB code: 4JFK, from Ref. [6a]). The free space for a pro-(*S*)-C^5 substituent is shown as green wire mesh. Residues in close proximity to a potential pro-(*S*)-C^5 substituent (Tyr57 and Asp68) are shown as sticks and wire mesh. b) Examples of C^5-unsubstituted [4.3.1] bicyclic FKBP ligands.[6] The pro-(*S*)-C^5 position is indicated in green.

Analysis of the cocrystal structure of bicyclic [4.3.1] aza-amide ligands[6a] with FKBP51 indicated that a substituent in the pro-(*S*)-C^5 position could fit into the binding pocket and enable additional interactions with the protein (Figure 1a).

Since no examples of C^5-derivatized 3,10-diazabicyclo-[4.3.1]decan-2-one systems were found in the literature,[7] the development of a new synthesis was required to investigate this new class of FKBP ligands. Inspired by work on aza-bridged bicyclic ring systems,[8] we envisioned a TMS-facilitated N-acyliminium cyclization for the construction of the C^5-branched bicyclic core fragment (Scheme 1).

The synthesis commenced with the alkylation of allylamine with alkylbromide **2**,[9] a precursor for a preferred moiety at the N^3-position of FKBP ligands (Scheme 2).[6a] For

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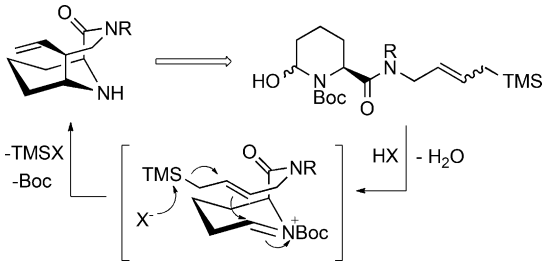
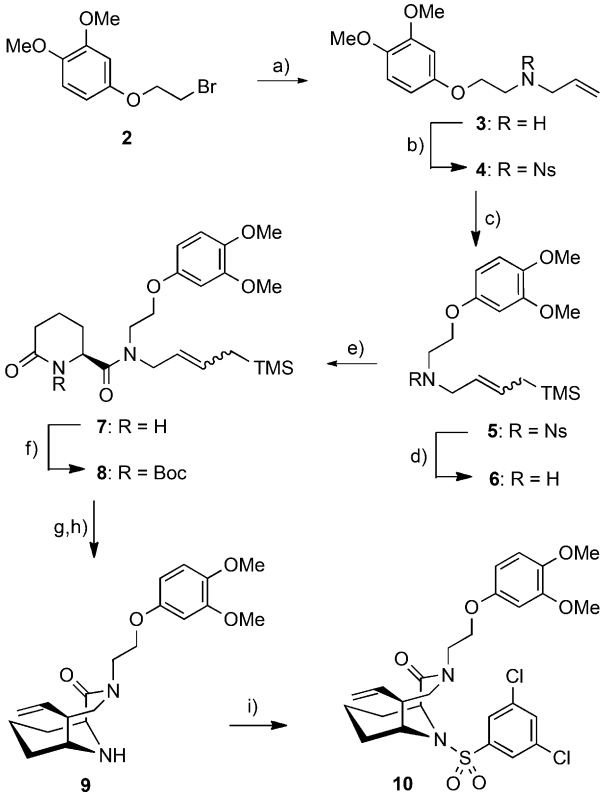
Supporting information for this article (including experimental details) is available on the WWW under http://dx.doi.org/10.1002/anie.201408776.



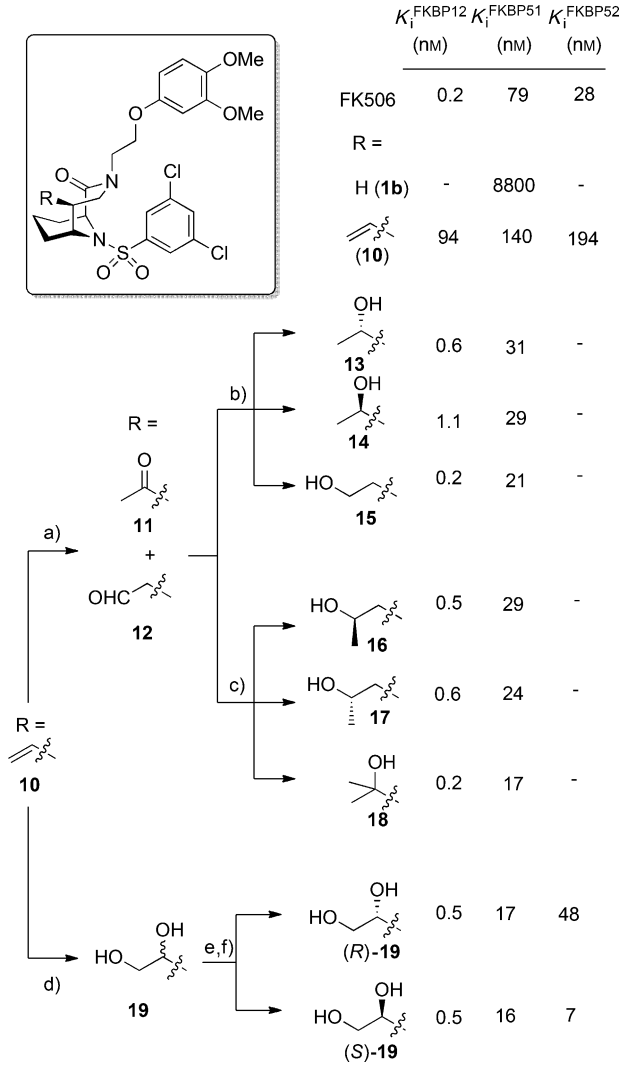
Scheme 1. Mechanism of the TMA-facilitated N-acyliminium cyclization. Boc = *tert*-butyloxycarbonyl.



Scheme 2. Reagents and conditions: a) Allylamine, CH_2Cl_2, 50 °C, 24 h, 85 %. b) NsCl, TEA, CH_2Cl_2, RT, 1 h, 95 %. c) AllylTMS, *p*-benzoquinone, Grubbs-Hoveyda II gen, CH_2Cl_2, 60 °C, 2 h, 95 %. d) PhSH, K_2CO_3, DMF, RT, 2 h, 65 %. e) (*S*)-6-Oxo-2-piperidinecarboxylic acid, HATU, DIPEA, DMF, RT, 2 h, 72 %. f) Boc_2O, TEA, DMAP, CH_2Cl_2, RT, 3 h, 86 %. g) DIBAL-H, THF, −78 °C. h) HF·Pyridine, CH_2Cl_2, −78 → 0 °C, 1 h, 55 % (2 steps). i) 3,5-Dichlorobenzenesulfonylchloride, DMAP, DIPEA, CH_2Cl_2, RT, 24 h, 55 %. DIBAL-H = diisobutylaluminum hydride, DIPEA = *N*,*N*-diisopropylethylamine, DMAP = 4-dimethylaminopyridine, HATU = O-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, NsCl = 2-nitrobenzenesulfonyl chloride, TEA = triethylamine, THF = tetrahydrofuran, TMS = trimethylsilane.

cross-metathesis of the resulting compound **3** with allyl trimethylsilane, it was necessary to temporarily mask the secondary amine to avoid inactivation of the ruthenium catalysts. Nosyl protection was the protection group of choice and allowed *p*-benzoquinone-assisted cross-metathesis[10] to give **5** with excellent yields. After removal of the nosyl group, amide bond formation between **6** and commercially available (*S*)-6-oxo-2-piperidinecarboxylic acid furnished **7** in a good yield of 72 %. Subsequent Boc protection of the amide activated the C^6-carbonyl, thereby setting the stage for the critical chemoselective reduction/N-acyliminium cyclization sequence. Thus, reduction of **8** with DIBAL-H gave an unstable hydroxy carbamate, which after aqueous work-up was subjected to various cyclization conditions. At temperatures between −78 °C and 0 °C at different dilutions, a variety of Brønsted or Lewis acids produced only dimers, trimers, or decomposition products (Table S1 in the Supporting Information). Analysis of the reaction mixtures revealed that despite the strongly acidic conditions, the trimethylsilane group was still present in the dimeric/trimeric products. Therefore, we decided to explore simultaneous activation of the hydroxy carbamate and the TMS leaving group. Gratifyingly, treatment of the reduction product of **8** with HF·pyridine smoothly yielded the cyclization product **9** as the only diastereomer in good yield (55 % over 2 steps). Reaction with 3,5-dichlorosulfonylchloride as an exemplary N^{10}-substituent then afforded the key intermediate **10** with 9 % overall yield from **2**. A cocrystal structure of compound **10** with FKBP51 validated the correct absolute stereochemistry of **10** and showed that the desired binding mode is possible for C^5-substituted [4.3.1] bicycles (Figure S1 in the Supporting Information). In a fluorescence polarization assay,[11] compound **10** bound to FKBP12, FKBP51, and FKBP52 with K_i values of 94 nM, 140 nM, and 194 nM, respectively, thus providing a first proof of concept for C^5-substituents as affinity-enhancing modifications to FKBP ligands.

An appealing aspect of the synthetic route chosen in Scheme 2 was that the newly installed C^5-vinyl group represents a unique reactivity for subsequent derivatization. In the hope of increasing the affinity even further, we decided to introduce polar groups at the C^5-vinyl substituent to explore possible contacts with the polar Tyr57 or Asp68 residue (Figure 1 a). Wacker oxidation of **10** provided an equimolar mixture of methylketone **11** and aldehyde **12**, which after reduction with $NaBH_4$ afforded the separable alcohols **13**, **14**, and **15** (Scheme 3).[12] Likewise, addition of MeMgBr to **11**/**12** yielded the secondary or tertiary alcohols **16**, **17**, and **18** (Scheme 3). Treatment of **10** with AD-Mix-α or AD-Mix-β both resulted in inseparable, equimolar mixtures of diastereomers (*R*)-**19** and (*S*)-**19**. However, temporary bis-TBDMS protection of the diol enabled the separation of the two diastereomers by preparative HPLC to yield the two diastereomerically pure diols (*R*)-**19** and (*S*)-**19** after deprotection with TBAF. Alcohols **13**–**18** and diols (*R*)-**19** and (*S*)-**19** all bound to FKBP51 with low nanomolar affinities (Scheme 3), up to five-fold more potently than the prototypic FKBP ligand FK506. Like FK506 and most synthetic derivatives thereof, the C^5-derivatized bicycles are pan-selective FKBP ligands with a slight preference for the small FKBP12. Compound **18**, tested as a representative example, also tightly bound FKBP12.6 (K_i = 6 nM) and FKBP13 (K_i = 70 nM). Direct comparison with compound **1b**,[7a] a direct analogue of compounds **13**–**19** that lacks the C^5 substituent, showed that polar C^5 substituents contributed more than 14 kJ mol^{-1} of additional binding energy, thereby leading to a more than 280-fold enhanced affinity.



Scheme 3. Reagents and conditions: a) $PdCl_2$, CuCl, O_2, DMF/H_2O, RT, 24 h. b) 1. $NaBH_4$, EtOH/CH_2Cl_2, RT, 1 h; **13:** 16%, **14:** 9%; **15:** 23% (over 2 steps each). c) MeMgBr, THF, −78 °C, 1 h; **16:** 12%; **17:** 9%; **18:** 34% (over 2 steps each). d) AD-Mix-β, *t*BuOH/H_2O, RT, 24 h, 75%. e) TBDMSOTf, 2,6-Lutidine, CH_2Cl_2, 1 h, preparative HPLC. f) TBAF, THF, RT, 30 min. Binding to FKBP12 or the FK506-binding domain of FKBP51 or FKBP52 was determined by a competitive fluorescence polarization assay with 40-fluorescein-Gly-rapamycin as a tracer.[11] DMF = *N*,*N*-dimethylformamide, TBAF = tetra-n-butylammonium fluoride, TBDMS = *tert*-butyldimethylsilyl, Tf = trifluoromethanesulfonyl.

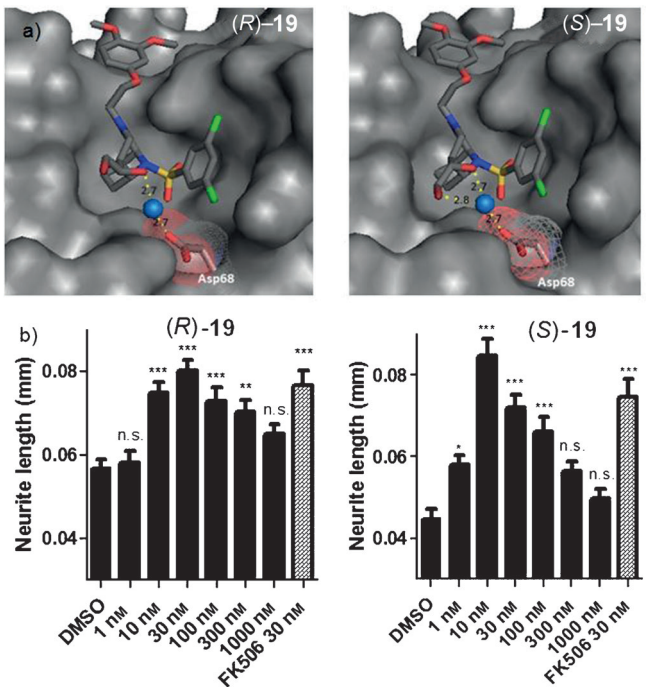


Figure 2. a) Cocrystal structures of (*R*)-**19** (PDB: 4W9O) and (*S*)-**19** (PDB: 4W9P) in complex with the FK506-binding domain of FKBP51. Hydrogen bonds are indicated by yellow dotted lines with distances in Å. The conserved water molecule is shown as a blue sphere and the van der Waals surface of the interacting Asp68 is shown as wire mesh. b) Stimulation of neurite outgrowth by (*R*)-**19** and (*S*)-**19**. N2a cells were transfected with a plasmid encoding a myristoylated yellow fluorescent protein, deprived of serum, and treated with the compounds for 24 h, followed by fluorescence imaging and morphological analysis. FK506 at its most effective concentration (30 nM) was included as a positive control. Data represent averages of more than 35 cells. Error bars represent ± standard error. ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, n.s.: not significant.

To investigate the structural basis for the observed affinity gain, we solved the cocrystal structures of (*R*)-**19** and (*S*)-**19** with the FK506-binding domain of FKBP51.[13] Both ligands form a water-mediated hydrogen bond between the secondary hydroxyl group and Asp68, which directs two different orientations of the terminal hydroxymethylene for (*R*)-**19** and (*S*)-**19** (Figure 2a). For (S)-**19**, the C^5-diol can also adopt a different conformer to form a direct hydrogen bond to Asp68 (Figure S2).

The potency of the novel ligands allowed their application in more advanced biological systems. FKBP51 is known to inhibit neurite outgrowth of N2a neuroblastoma cells,[14] a model for the early steps of neuronal differentiation. In these cells, (*R*)-**19** and (*S*)-**19** both increased neurite outgrowth at low nanomolar concentrations (Figure 2b and Figure S3). However, we also observed that both compounds lost their neurite-outgrowth-enhancing activity at higher concentrations. A similar bell-shaped dose–response curve has previously been observed with FK506 and close analogues thereof in SH-SY5Y neuroblastoma cells[15]. Notably, the immunosuppressive FKBP ligand rapamycin potently and completely blocked neurite outgrowth (Figure S4), likely through inhibition of mTOR, which is the main effect of rapamycin.[16] The fact that structurally different ligands like (*R*)-**19** and (*S*)-**19** fully replicate the bell-shaped neurite outgrowth effect of FK506 strongly suggests that inhibition of an FKBP is responsible for the initial neurite outgrowth stimulation and for the reduction at higher concentrations. Considering the reported anti- and pro-neuritotrophic functions of FKBP51 and FKBP52, respectively,[14] we suggest that inhibition of FKBP51, and at higher concentrations, of FKBP52, are responsible for the rising and declining arms of the bell-shaped dose–response curve.

Taken together, C^5-branched 3,10-diazabicyclo-[4.3.1]decan-2-ones are a highly efficient class of FKBP ligands that are suitable for probing the cellular role of FKBPs. Polar substituents in the C^5 position robustly increase affinity independently of their exact position and orientation. C^5 substitution within the [4.3.1] bicyclic scaffold is thus a general strategy to enhance affinity for FKBPs, while leaving flexibility for modifications to fine-tune additional properties such as solubility, lipophilicity, or selectivity. Structure–activity relationship studies to address this will be reported in due course.

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