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Fragment-Based Synthesis and SAR of Modified FKBP Ligands: Influence of Different Linking on Binding Affinity

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The viability of the fragment-based approach for lead discovery depends on reliable fragment-screening methods combined with straightforward fragment-linking—or fragment-growing—chemistry. In the present study we sought a flexible synthetic approach that would allow efficient synthesis of a variety of linkers that can subsequently be tested for biological activity. We applied this approach to fragments known to bind to FKBP12 (FK506 binding protein), a peptidyl-prolyl isomerase involved in immunosuppression and neural functioning. In our set of linked FKBP ligands, ester and thioester linkages resulted in high-affinity ligands, whereas an amide linkage decreased affinity remarkably; oxime and triazole linkages were not tolerated by the target protein's binding pocket, rendering these ligands ineffective. By investigating corresponding derivatized non-linked fragments and docking studies of linked fragments, we were able to evaluate the effect of the linker region on ligand binding affinity.

Introduction

In recent years the fragment-based approach to drug discovery, generating high-affinity ligands from weak binding fragments, has emerged as an alternative to high-throughput screening.[1–4] Important for this approach is that by covalently linking low-affinity fragments, an additive effect of binding energies is attained.[5–7] Several successful examples of the fragment-based approach have been reported over the last couple of years.[8–12] A requirement for this approach is a fragment-screening process that allows the detection of weak binders, and NMR spectroscopy is well suited for this purpose.[13,14] We have developed a new NMR-based method, called target-immobilized NMR screening (TINS), which makes use of an immobilized target.[15,16] Immobilization allows screening of targets that have poor solution characteristics or that are insoluble, such as membrane proteins, for which 3D structural information may be incomplete or entirely absent. We are aiming to develop an integrated process from screening functionalized fragments to lead discovery, by applying versatile linking chemistry to hits obtained using any target protein. As fragment screening is most useful if the fragments can be readily linked together to produce high-affinity effector molecules, both the chemistry to accomplish this and the effect of the nature of the linker on the binding affinities have to be investigated. As a model target protein to probe a flexible linking strategy, we chose the well-known immunophilin FK506-binding protein (FKBP). FK506 is a natural macrolide immunosuppressant isolated from *Streptomyces tsukubaensis*, and is clinically used in blocking immune responses in organ transplantation.[17] The first reports of the neuroregenerative and neuroprotective properties of FK506[18,19] raised the possibility of applying FKBP ligands toward novel therapeutic strategies in the treatment of nerve injuries and neurodegenerative disorders such as Alzheimer's and Parkinson's disease.[20–22] The structure of FKBP has been solved by NMR spectroscopy[23,24] and X-ray crystallography,[25] with and without ligands.[26–32] These structures indicate a unique FK506 binding pocket, which can be subdivided into two sites (Figure 1). Site 1 is the binding location for the pipecolinic acid ligands, whereas site 2 lies about 7 Å away. Selective fragments with a range of affinities for sites 1 or 2 have been reported, some of which have been linked to generate high-affinity ligands (Figure 1).[5,33] Whereas the dependence of binding affinity on linker length has been elucidated,[5,7] this has not been investigated for the chemical nature of the linker. Herein we report the efficient synthesis of suitably functionalized site 1 and site 2 fragments that can be readily ligated to each other following a flexible linking strategy. The products thereby obtained are designed in such a way that various linkers of fixed length are produced so that their binding affinities can be compared by using a fluorescence-based binding assay.

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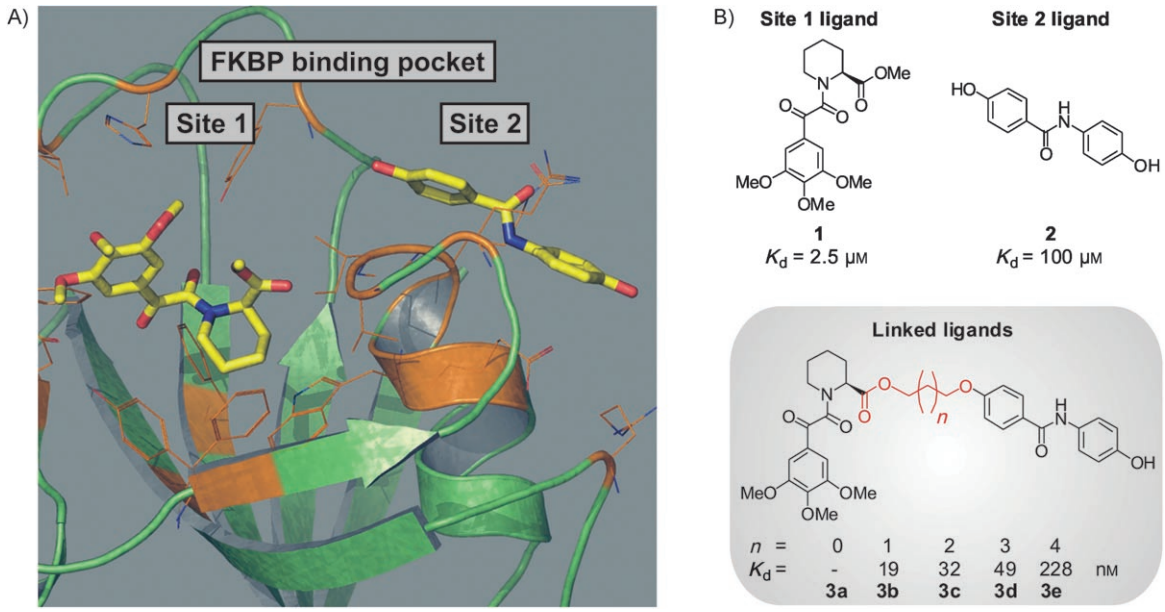
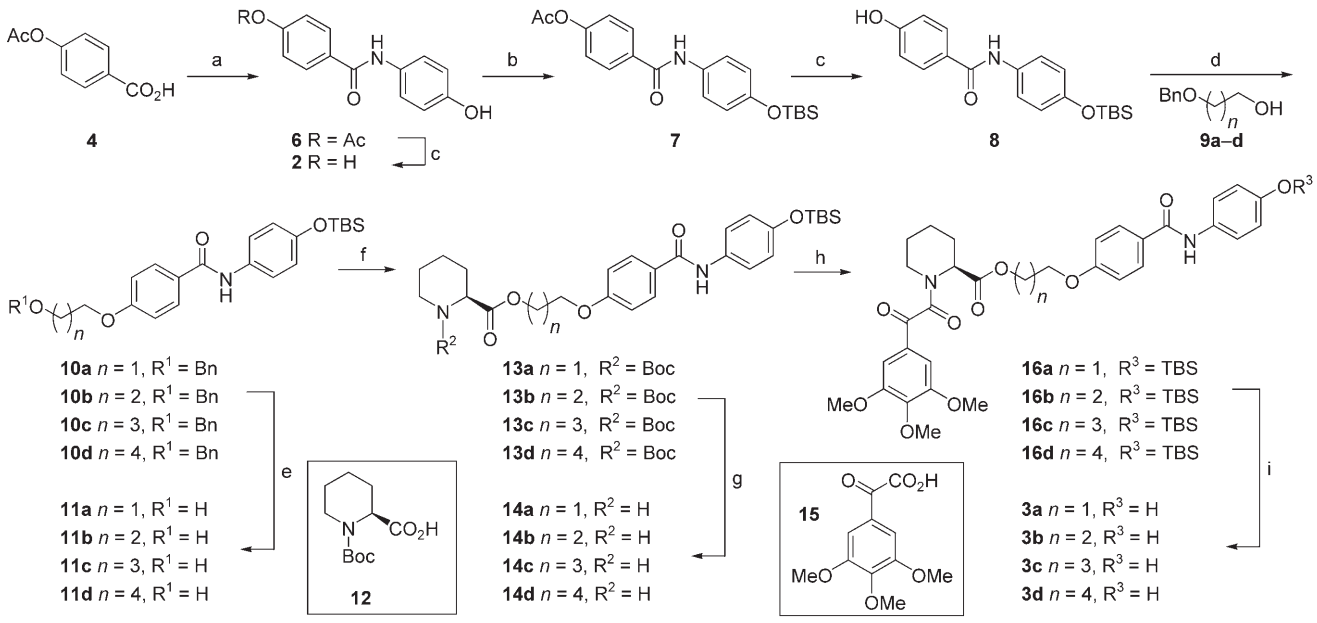


Figure 1. A) View inside the binding pocket of FKBP with two site-selective ligands bound (NMR structure);[34] B) reported site-selective ligands and successful fragment-linking approach.[5]

Chemistry

We chose a fluorescence quenching assay as a simple method to quantify the binding of ligands to FKBP.[35,36] Because it is well known that such assays are acutely sensitive to small variations in conditions, it was important to compare our assay with previously reported results.[5] Therefore, we commenced our synthetic efforts with the synthesis of the known pipecolinic acid-derived ester-linked ligands **3b**–**3d**. In addition, the new compound **3a** (shortest linker) was synthesized to complete the series. To enable a flexible synthetic approach, we envisioned introduction of the linker moiety of the site 2 fragment by a Mitsunobu reaction. To this end, commercially available *p*-acetoxybenzoic acid (**4**) was converted into its acid chloride and condensed with excess *p*-aminophenol (**5**) to give compound **6** in 85% yield (Scheme 1). The coupling was found to be selective for the amino functionality under the conditions applied. With *tert*-butyldimethylsilyl chloride (TBSCl) and

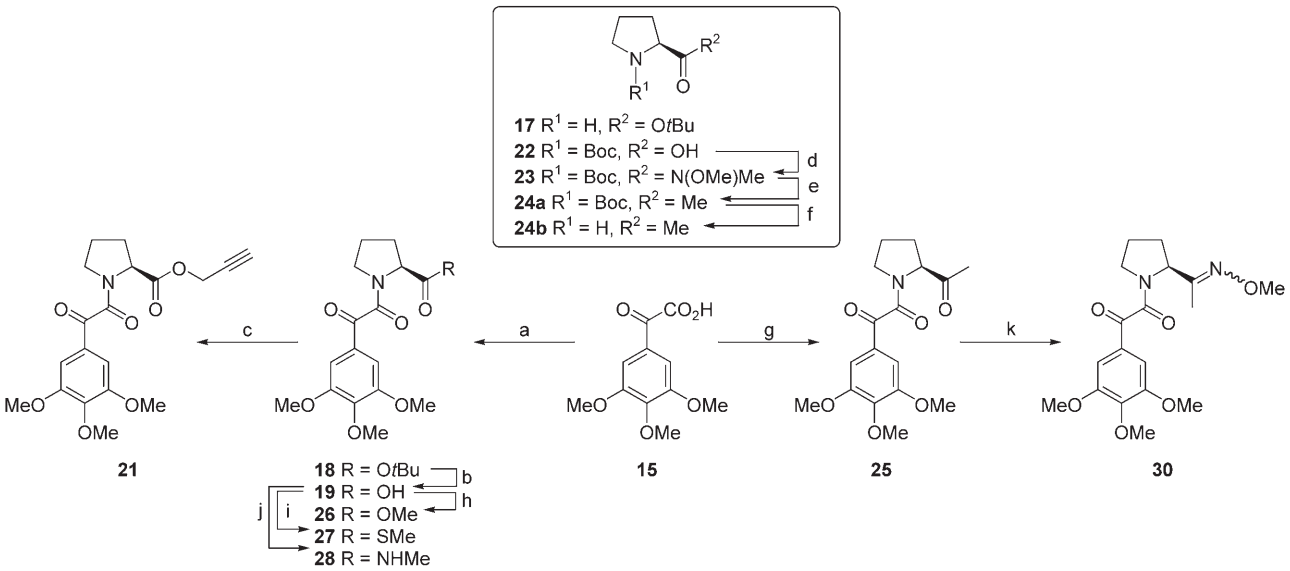


Scheme 1. Synthesis of compounds **3a**–**d**. Reagents and conditions: a) 1) $SOCl_2$, cat. DMF, reflux, 2) *p*-aminophenol (**5**), cat. DMAP, DMF, 0 °C (85%); b) TBSCl, imidazole, DMF (97%); c) NaOEt, EtOH (90%); d) **9a**–**d**, PPh_3, diethylazodicarboxylate (DEAD), THF, 16 h (87–94%); e) H_2, Pd/C, EtOH/EtOAc (97–99%); f) **12**, EDC·HCl, DIPEA, HOBt, cat. DMAP, CH_2Cl_2, 16 h (91–99%); g) TFA, CH_2Cl_2, 2 h (64–94%); h) **15**, EDC·HCl, HOBt, DIPEA, cat. DMAP, CH_2Cl_2 (71–95%); i) TBAF, THF (80–91%).

imidazole in slight excess, the phenolic hydroxy group could be protected in excellent yields (**6**→**7**, 97%), without undesired cleavage of the acetyl group.[37] After basic removal of the acetyl group with sodium ethoxide, phenol **8** was obtained in 90% yield after a straightforward purification step. Next, phenol **8** was subjected to dehydrative Mitsunobu conditions employing an excess of the appropriate commercially available monobenzyloxyalkyl alcohols of varying length (compounds **9a**–**d**) to give excellent yields of the coupled products **10a**–**d** (87–94%). After hydrogenolytic benzyl deprotection, the alcohols obtained, **11a**–**d** (yields 97–99%), were coupled with commercially available Boc-protected pipecolinic acid **12** with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) as coupling reagent, generating **13a**–**d** in very good yields (91–99%). Subsequent acidic removal of the Boc group (**13a**–**d**→**14a**–**d**, yields 64–94%), followed by EDC-mediated coupling with α-ketoacid **15**[32] (**14a**–**d**→**16a**–**d**, yields 71–95%) and removal of the TBS group with tetra-*n*-butylammonium fluoride (TBAF), provided target compounds **3a**–**d** in good yields (80–91%).

With compounds **3a**–**d** in hand, we focused our attention on the synthesis of suitably derivatized fragments to allow various linking chemistry. We decided to employ proline-derived, as opposed to pipecolinic acid-based site 1 fragments for further investigation, as these have been shown previously to exhibit increased binding affinity to FKBP.[38–40] Other studies have shown the activities of proline- and pipecolinic acid-derived ligands to be similar.[32,41–43] The synthesis of site 1 ligands equipped with suitable functionalities was started by coupling α-ketoacid **15** with commercially available *t*Bu-protected proline **17** to yield compound **18** (Scheme 2, 80%). After acidic deprotection, proline derivative **19** was coupled with propargylic alcohol **20** to afford ester **21**, amenable to installation of a triazole-linked site 2 fragment via "click chemistry".[44] To enable installation of an oxime linkage, Boc-protected proline **22** was converted into Weinreb amide **23**, which in turn was subjected to a Grignard reaction with methyl magnesium bromide to furnish methyl ketone **24**.[38] After acidic removal of the Boc group followed by EDC coupling of the resulting secondary amine with compound **15**, ketone **25** was obtained in moderate yield (55% over both steps). Several derivatives of compound **19** were synthesized to investigate the effect of derivatization of the carboxyl functionality on the binding affinity of non-linked site 1 ligands. To this end, methyl ester **26**, methyl thioester **27** and methyl amide **28** were prepared by EDC couplings with methanol, methanethiol and methyl amine, respectively. In addition, ketone **25** was treated with methoxyamine (**29**) under acidic conditions to furnish oxime **30** (Scheme 2).

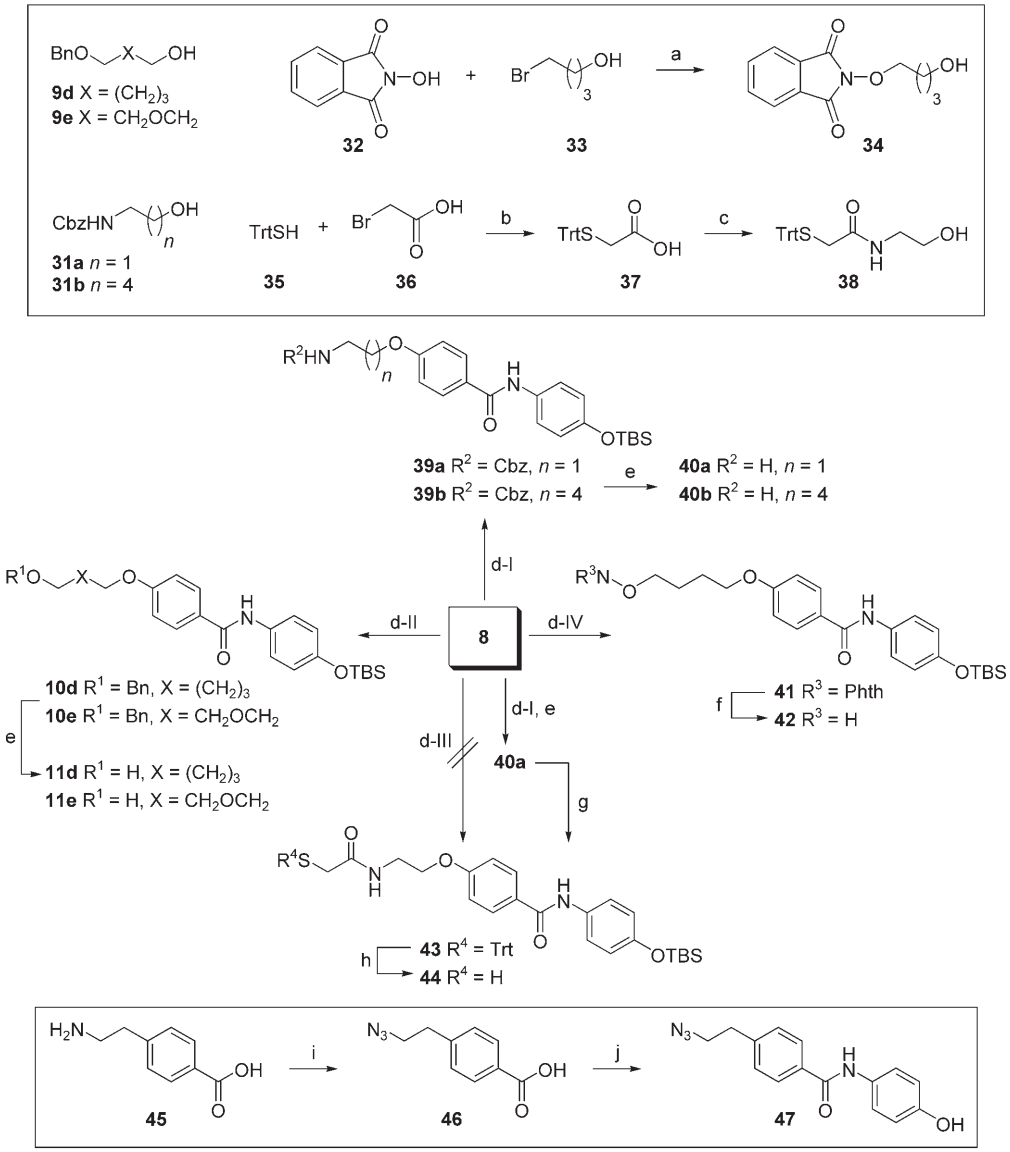
Next, with the goal of enabling a readily applicable and flexible linking approach, we investigated the Mitsunobu reaction for installation of a set of various linkers on the site 2 ligand **8**.[45] To do so, linkers bearing a free hydroxy group on one end and a masked second functionality on the other end were required. The length was chosen to be two atoms longer than the reported optimum[5] (five instead of three atoms) to allow a greater variety in the chemical nature of the linkers. In addition to the commercially available linkers (**9d**, **9e**, **31a**, and **31b**), two more linkers were synthesized. First, a linker bearing a phthalimide-masked hydroxyamine (compound **34**) was generated by alkylation of hydroxyphthalimide (**32**) with 4-bromobutanol (**33**), and second, a linker bearing a thioether-linked trityl group (compound **38**) was produced by reaction of triphenylmethanethiol (**35**) with α-bromoacetic acid (**36**) to furnish **37**; subsequent coupling with β-aminoethanol yielded **38**.



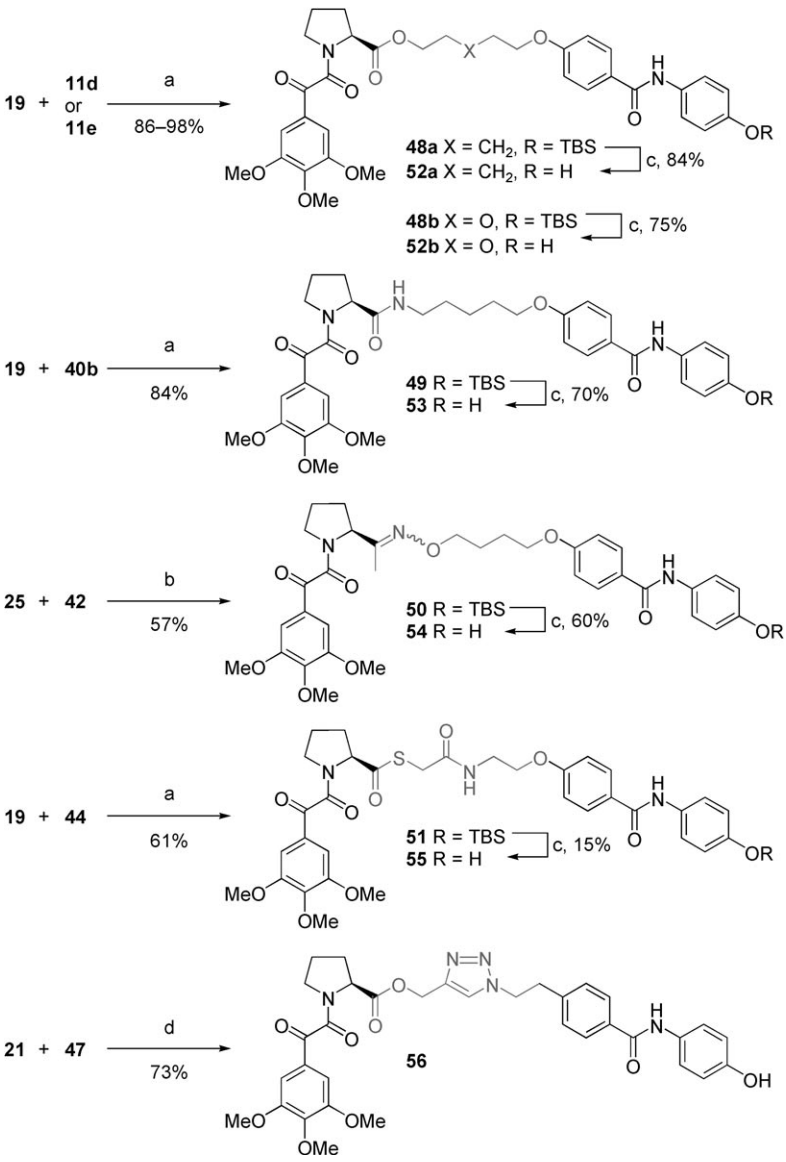
Scheme 2. Synthesis of suitably functionalized site 1 ligands. Reagents and conditions: a) L-proline *tert*-butyl ester **17**, EDC·HCl, HOBt, DIPEA, cat. DMAP, 0 °C (80%); b) TFA, CH_2Cl_2, (98%); c) propargylic alcohol **20**, EDC·HCl, HOBt, DIPEA, cat. DMAP, 0 °C (97%); d) HN(OMe)Me, EDC·HCl, HOBt, DIPEA, cat. DMAP, 0 °C, DMF (99%); e) MeMgCl, THF (95%); f) TFA, CH_2Cl_2; g) **24b**, EDC·HCl, HOBt, DIPEA, cat. DMAP, 0 °C (55%); h) MeOH, EDC·HCl, HOBt, DIPEA, cat. DMAP, 0 °C (90%); i) 1) MeSSMe, $LiAlH_4$, Et_2O, 1 h, 2) **19**, EDC·HCl, 0 °C, Et_2O, 16 h (70%); j) methylamine, EDC·HCl, HOBt, DIPEA, cat. DMAP, 0 °C (40%); k) methoxyamine·HCl **29**, AcOH, EtOAc (40%).

With these various linkers in hand, we performed Mitsunobu couplings using phenol **8** as the substrate. We were pleased to find that by using conditions described earlier, a whole set of linkers (**9d**, **9e**, **31a**, **31b**, and **34**) could be introduced in good yields (giving **10d**, **10e**, **39a**, **39b**, and **41**), to provide access to compounds **11d**, **11e**, **40a**, **40b**, and **42** after standard deprotection (Scheme 3).[46] Unfortunately, the Mitsunobu reaction failed to form **43** directly from the thioester-enabling linker **38**; aziridine formation was observed instead. Therefore, amine **40a** was coupled with trityl-protected α-thioacetic acid **37** to yield **43**. The trityl group of **43** was removed selectively in the presence of the TBS group to furnish thiol **44** in very good yield (91%). Furthermore, to enable linkage formation by click chemistry,[44] commercially available *p*-(aminoethyl)benzoic acid (**45**) was subjected to diazo transfer[47] (yielding **46** quantitatively), and was subsequently coupled to *p*-aminophenol (**5**) to afford azide **47** exclusively (yield 69%).

Coupling of site 1 and site 2 ligands was performed as shown in Scheme 4. Ester, amide, and thioester bond formation was performed under nearly identical conditions using EDC coupling, leading to linked esters **48a** (86%) and **48b** (98%), amide **49** (84%), and thioester **51** (61%). Reaction of ketone **25** and hydroxyamine **42** was found to occur preferably at the methyl ketone position under the applied conditions; oxime **50** was obtained in 52% yield (the compound originating from reaction at the phenyl ketone position was isolated as a minor by-product). Next, the TBS group of compounds **48a**, **48b**, **49**, **50**, and **51** was removed under identical conditions in each case, employing TBAF in wet tetrahydrofuran (THF). Compounds **52a** (84%), **52b** (75%), **53** (70%), and **54**



Scheme 3. Installation of linkers on the site 2 ligand. Reagents and conditions: a) NEt_3, DMF, 85 °C, 16 h (25%); b) DIPEA, DMF, 4 h (84%); c) ethanolamine, EDC·HCl, HOBt, CH_2Cl_2/THF; d) PPh_3, DEAD, THF, 0 °C, 16 h, 1) **31a** or **31b**, 2) **9d** or **9e**, 3) **38**, 4) **34**; e) H_2, Pd/C, EtOAc/EtOH; f) $H_4N_2{\cdot}H_2O$, EtOAc, reflux, 5 h; g) **37**, EDC·HCl, HOBt, DIPEA, cat. DMAP, 0 °C, CH_2Cl_2, 16 h (83%); h) TFA, TES, CH_2Cl_2, 8 h (91%); i) TfN_3, K_2CO_3, $CuSO_4{\cdot}5H_2O$, H_2O/MeOH/CH_2Cl_2, 16 h (99%); j) 1) $SOCl_2$, reflux, 3 h, 2) **5**, CH_2Cl_2 (69%).



Scheme 4. Linking of fragments and final deprotection. Reagents and conditions: a) EDC·HCl, HOBt, DIPEA, cat. DMAP, 0 °C, CH_2Cl_2, 16 h; b) AcOH, EtOAc, 16 h (57%); c) TBAF (0.5 equiv), THF; d) sodium ascorbate, $CuSO_4 \cdot 5H_2O$, H_2O/*t*BuOH, 27 h (73%).

(60%) were isolated in good to moderate yields. However, these conditions gave only poor yields for thioester **55** (15%). Nevertheless, sufficient material for the binding assay was isolated. Finally, click chemistry[44] with acetylide **21** and azide **47** under standard conditions afforded triazole **56** in 73% yield.

Results and Discussion

The binding affinity of each FKBP ligand synthesized was determined by using a fluorescence quenching assay.[31,35,36] The measured values for the non-linked ligands are summarized in Figure 2. The binding affinities of site 1 ligand **1** and site 2 ligand **2** were determined to be in good agreement with reported data ($K_d = 3.5$ μM versus 2 μM for **1** and $K_d = 94$ μM versus 100 μM for **2**).[5] The proline derivatives **26** and **27** were found to bind in the same affinity range as pipecolinic ester **1** (with $K_d = 5$ μM for **26** and 3.5 μM for **27**), whereas amide **28** was clearly less potent ($K_d =$ 16 μM). This could indicate an acceptor role for the ester atom (oxygen or sulfur) in a hydrogen bond with the target protein. This idea is supported by the clear loss of binding affinity of methyl ketone **22** ($K_d = 147$ μM), which further decreases with the oxime **30** ($K_d = 380$ μM). This is further discussed in the docking studies of their corresponding linked fragments (see below). The azide-containing site 2 ligand **47** bound FKBP with a K_d value approximately fivefold lower than that of ligand **2** (500 μM versus 94 μM).

The binding affinities of the linked compounds (Figure 3) were compared with the reported pipecolinic acid-derived compounds **3b**–**3d**[5] (**3b**: $K_d =$ 19 nM versus 60 nM, **3c**: $K_d = 32$ nM versus 68 nM, and **3d** $K_d = 49$ nM versus 101 nM). In addition, we determined the K_d value of the shortest-linked ligand **3a**, which had not been reported previously, and found it less potent than the most potent ligand **3b** (**3a**: $K_d = 88$ nM versus **3b**: $K_d = 60$ nM). This trend of activities, with the three-methylene spacer imparting greatest activity, is in agreement with the aforementioned data.[5] Moreover, a structurally related study by Armistead et al.[29] reported the same trend for the linker length employing a simple phenyl ring as site 2 ligand. In the set of linked proline-derived compounds that were all designed to contain a five-atom linker, the esters **52a** ($K_d = 80$ nM), **52b** ($K_d = 113$ nM), and the thioester **55** ($K_d = 87$ nM) were potent binders. The amide **53** was less potent ($K_d = 357$ nM), and the oxime- and triazole-linked compounds **54** ($K_d = 15$ μM) and **56** ($K_d = 10$ μM), respectively, were poor binders. Hence, the replacement of the pipecolinic acid by the proline moiety did not have a negative effect on binding affinity (**52a**: $K_d = 80$ nM versus **3d**: $K_d =$ 101 nM).

To properly judge the effect of the linker moiety on ligand binding, we took the respective non-linked site 1 and site 2 ligands (Figure 2) into account. According to the literature, optimal fragment linking can be estimated by multiplication of the respective binding affinities of the non-linked fragments.[7] If the length, geometry, and chemical properties of the linker are chosen properly, it may be possible to come close to this estimated value.[48] In Figure 3 we thus included the theoretical linker factor (f_L), defined as the ratio of measured K_d to theoretical (calcd) K_d. For example, an f_L value of 1 indicates optimal linking of the fragments, whereas values >1 indicate that the linker does not align the ligands in an optimal direction and distance, or that the linker itself experiences unfavorable interactions. The best linker factor was obtained for the alkyl ester compound **52a**, which is 170-fold less potent than a "theoretical" optimally linked ligand ($f_L = 170$). The presence of an oxygen atom within the linker (compound

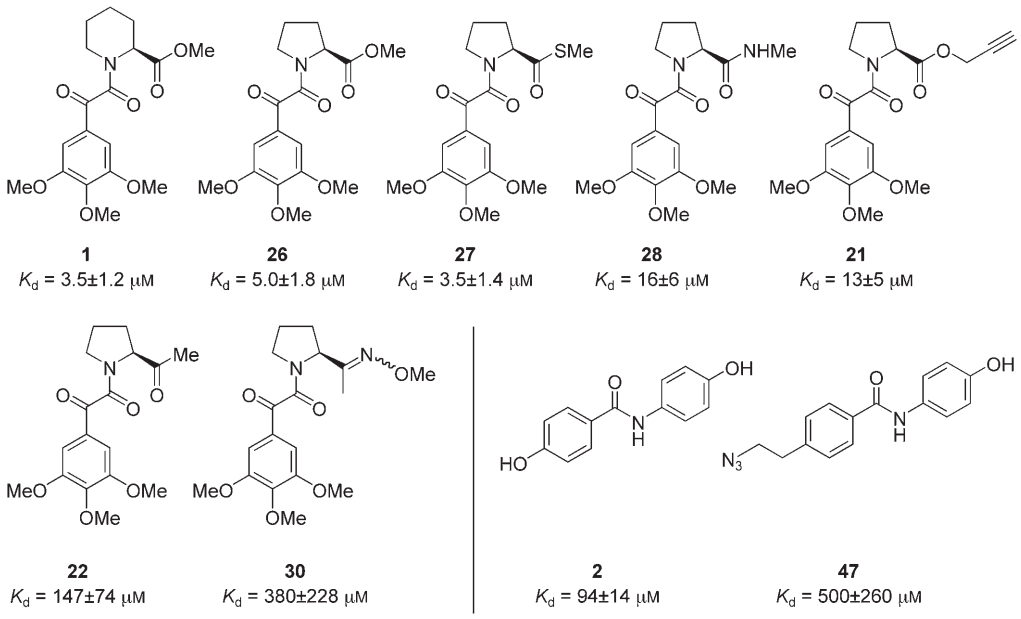


Figure 2. Binding affinities of non-linked, site-selective FKBP ligands used in this study as determined by fluorescence quenching. Compounds **2** and **47** are site 2 ligands; all others are site 1 ligands.

52b) increases this factor ($f_L = 240$), indicating that a hydrophilic linker is less favorable. For the amide **53**, also with a higher linker factor than the ester **52a** ($f_L = 238$ versus 170), we assume that the more rigid amide bond prevents optimal spatial alignment of the ligands. A much stronger deviation from the best linker factor is found for the oxime **54** ($f_L = 417$), which may be explained best by the fact that oxime **54** was measured as mixture of *E*/*Z* isomers, presumably having at least one of the isomers in a geometry that interferes with productive interaction with FKBP.

The linker amide of thioester **55**, in turn, appears to have a strong negative effect on binding ($f_L = 264$) which is consistent with the idea that the linker should be more hydrophobic for optimal binding to FKBP. However, the most dramatic linker effect was found for the triazole-linked ligand **56** ($f_L = 1538$), which strongly indicates that the triazole ring is sterically too demanding.

Next, to obtain more detailed insight into the binding mode of the reported ligands, selected linked fragments were used in docking studies on FKBP using the program Surflex (Figure 4).[49] Due to the presence of two pairs of hydrogen bond acceptors showing the same geometrical features in all these molecules, restraints were introduced to maintain the H bonds with Tyr 82 and Ile 56, as shown in the experimental structures of both pipecolinic acid and a pipecolinic acid-derived ligand in complex with FKBP (see Experimental Section below). Figure 4A shows the docked conformation of the high-affinity ligand **52a** in complex with the protein. The overall binding mode of compound **52a** is in agreement with related compounds binding to the protein as investigated by NMR[34] and X-ray crystallography.[26–32] Part of the proline ring is deeply buried in the hydrophobic binding pocket referred to as site 1. The aromatic amide moiety is directed toward the shallow groove referred to as site 2, as anticipated. Several hydrogen bonding interactions contribute to the positioning of the ligand, including the OH group of Tyr 82 with the C=O function of the proline amide, Ile 56 NH with C=O of the proline ester (as expected), but also Tyr 82 OH with the O atom of the proline ester, Gln 53 C=O with the NH group of the aromatic amide moiety, and Arg 57 guanidine C=NH with the phenolic OH group of **52a**. Interestingly, the linker is flexible enough to adopt a folded conformation, thereby bringing the two binding regions within relatively close proximity, in agreement with the findings of Hajduk and co-workers.[5]

Compound **53**, which has an amide linkage rather than an ester moiety and a binding affinity toward FKBP that is about fourfold lower than that of **52a** (Figure 3), shows basically the same overall binding mode. The decrease in affinity may be ascribed to the loss of one H bond with Tyr 82 because of the replacement of the H-bond-accepting oxygen atom of the proline ester with the H-bond-donating NH group of the amide in compound **53**. Apparently, this change is too subtle with regard to our docking parameters, thus providing the limitations of our docking study.

Both isomers of oxime **54** (shown separately in Figures 4C and D) were found to have severe disruptions of the site 1 ligand–FKBP interaction, thereby providing a rationale for the poor binding properties. Apparently the geometry of the oxime linkage puts a constraint on the linker which does not allow the proper positioning of the two binding moieties as described above. As a consequence of this constraint, the proline ring is forced out of the site 1 binding pocket.

Interestingly, compound **55**, despite having the conformationally restricted amide functionality within the linker, has a high affinity for FKBP and binds in a similar manner as compounds **52a** and **53**. The docking study as shown in Figure 4E

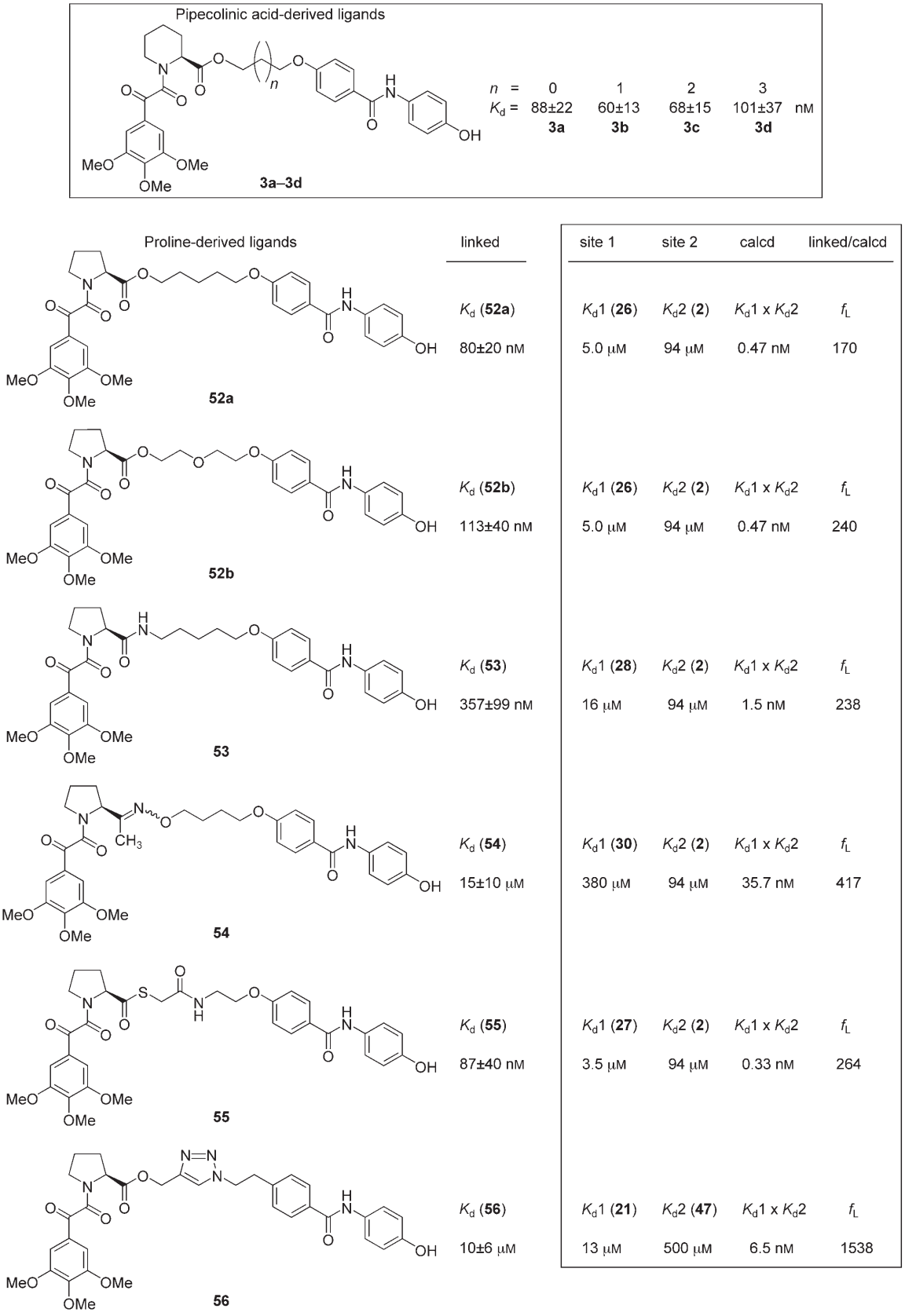


Figure 3. Binding affinities of linked FKBP ligands and calculation of a virtual "linker factor".

provides a plausible explanation for this unexpected affinity by suggesting an additional H-bonding interaction between the linker amide and the backbone C=O of Glu 54. In addition, the location of the site 2 ligand of **55** more closely resembles that

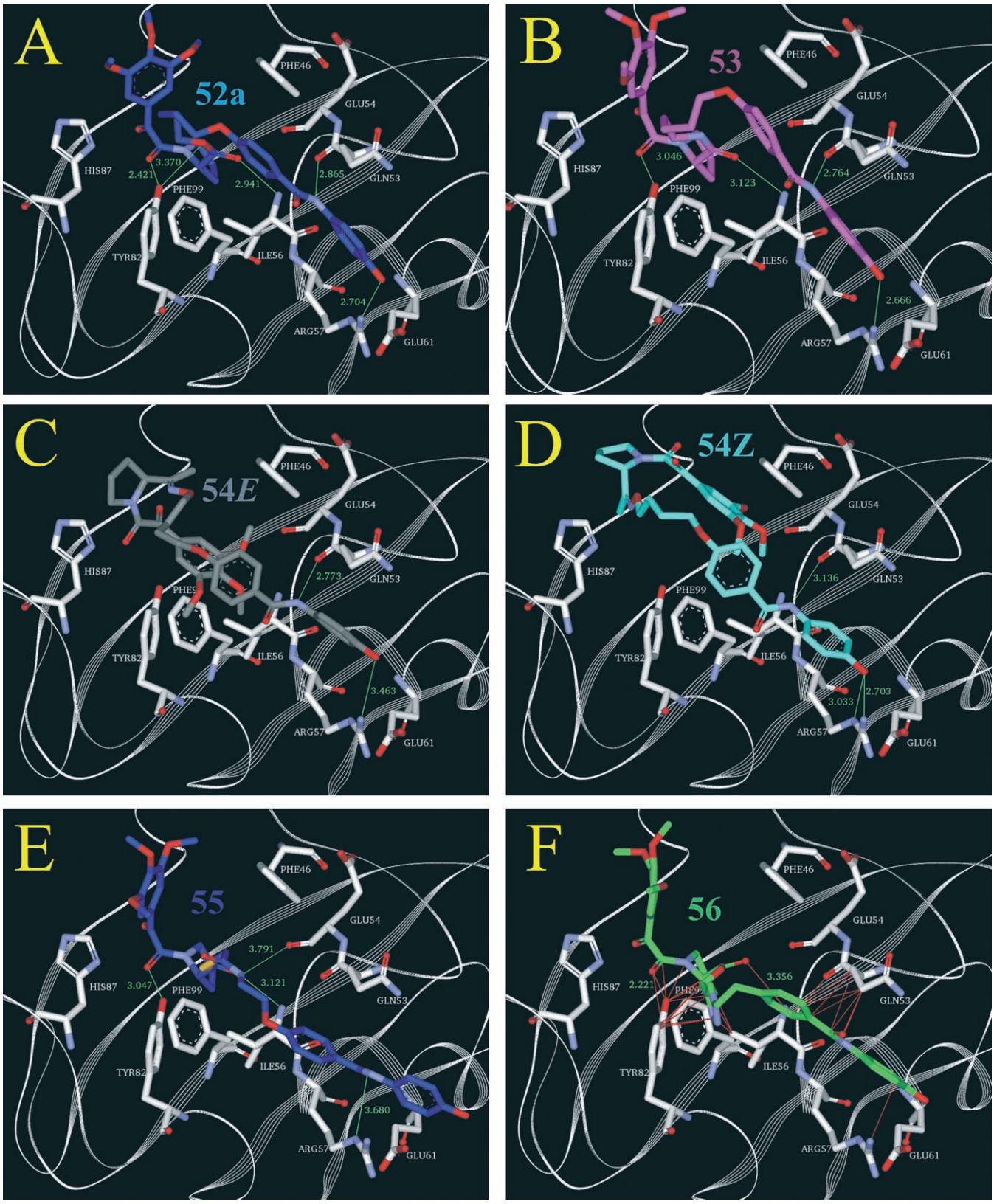


Figure 4. Docking studies on FKBP with selected ligands: A) **52a**, B) **53**, C) (*E*)-**54**, D) (*Z*)-**54**, E) **55**, F) **56**. After docking using the program Surflex,[49] the images were generated with the software DS Visualizer from Accelrys.

of the structure of the unlinked ligand. Finally, the triazole-containing linker of compound **56** results in a strong steric clash within the hydrophobic site 1 binding pocket, explaining its poor binding affinity.

Conclusion

We have synthesized new and potent FKBP ligands (**3a**, **52a**, **52b**, and **55**) by a fragment-based and flexible linking approach. In this regard, the usefulness and broad scope of the Mitsunobu reaction was demonstrated once again.[45] Moreover, we investigated the detailed effects of the linker on binding affinity by comparing the binding data of the non-linked with the linked ligands. This work highlights the significant effect that the chemical nature of the linker can have on the binding affinity of linked fragments. Even if the fragments and the length of the linker are held constant, binding can vary over nearly three orders of magnitude. Based on these results, we emphasize that besides length, the nature of the linking moiety is of critical importance in fragment-based drug discovery. In our ongoing research on the design of potential leads based on fragment screening of target proteins that lack structural information, it is vital to be able to rapidly link compounds with a variety of linker lengths and chemistries in order to evolve the low-affinity hits in an efficient manner. The installation of a set of diverse linker moieties using a flexible synthetic approach such as the one reported herein will allow us to generate ligands of sufficient affinity for a wide range of target proteins. These targets, in turn, can be studied in biochemical and cell-based assays, thus enabling the entire toolbox of traditional medicinal chemistry approaches.

Experimental Section

General methods. All reagents were purchased from Aldrich, Acros, or Novabiochem, and were used as supplied. MS data were recorded on a PerkinElmer Sciex API 165 instrument equipped with a custom-made electrospray ionization (ESI) interface, and HRMS (SIM mode) data were recorded on a TSQ Quantum (Thermo Finnigan) instrument fitted with an accurate mass option, interpolating between PEG calibration peaks. For LC–MS analysis, a Jasco HPLC system (detection simultaneously at $\lambda = 214$ and 254 nm) equipped with an analytical Alltima C_{18} column (Alltech, 4.0 mm (∅) × 250 mm (*l*), 5 μm particle size) was used in combination with buffers A: H_2O, B: MeCN, and C: TFA (aq, 1.0%). ^{1}H and ^{13}C APT NMR spectra were recorded on a Bruker AC-200 MHz (200/50.2) or a Bruker AV-500 MHz (500/125.8) spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane (TMS) as an internal standard (^{1}H NMR) or to the peak of the solvent used (^{13}C NMR). Fluorescence spectra were recorded on a PerkinElmer LS 50 B luminescence spectrometer.

4-Acetoxy-*N*-[4-(hydroxy)phenyl]benzamide (6). A catalytic amount (a few drops) of *N*,*N*-dimethylformamide (DMF) was added to a solution of *p*-acetoxybenzoic acid (9.5 g, 52.7 mmol) in thionyl chloride (19.4 mL, 263.7 mmol), and the solution was heated at reflux for 90 min. The mixture was cooled to room temperature, and excess thionyl chloride was removed under reduced pressure to furnish the acid chloride as a light-yellow oil. The acid chloride was dissolved in DMF (50 mL) and added slowly at a temperature of 0 °C to a solution of *p*-aminophenol (17.25 g, 158.1 mmol) and a catalytic amount of 4-dimethylaminopyridine (DMAP) in DMF (100 mL). Stirring was continued for 1 h, after which the solution was transferred to a 2-L separatory funnel with EtOAc (1 L). The mixture was washed three times with 2 N HCl (3 × 400 mL), once with 0.5 N $NaHCO_3$ (400 mL), and once with brine (400 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. Compound **6** was obtained as a white solid (12.2 g, 44.8 mmol, 85%). ^{1}H NMR (200 MHz, [D$_6$]DMSO): $\delta = 2.29$ (s, 3H), 6.73 (d, 2H, $J =$ 9.1 Hz), 7.26 (d, 2H, $J = 8.8$ Hz), 7.51 (d, 2H, $J = 9.1$ Hz), 7.96 (d, 2H, $J = 8.8$ Hz), 9.24 (s, 1H), 10.02 ppm (s, 1H); ^{13}C NMR (50.2 MHz, [D$_6$]DMSO): $\delta = 20.9$, 115.0, 121.7, 122.2, 129.0, 130.6, 132.7, 152.7, 153.7, 164.2, 169.0 ppm; HRMS: calcd for $C_{15}H_{14}NO_4$ $[M+H]^+$ 272.0917, found 272.0913; MS (ESI): *m/z* 272.1 $[M+H]^+$; FTIR (thin film): 1747, 1647, 1609, 1514, 1435, 1367, 1323, 922, 824 cm^{-1}; TLC: $R_f = 0.27$ (80% toluene/acetone).

4-Acetoxy-*N*-[4-(*tert*-butyldimethylsilyloxy)phenyl]benzamide (7). *tert*-Butylchlorodimethylsilane (6.5 g, 43 mmol) and imidazole (2.93 g, 43 mmol) were added at room temperature to a solution of compound **6** (7.8 g, 29 mmol) in DMF (60 mL), and the mixture was stirred until TLC (90% toluene/acetone) indicated completion of the reaction (~2 h). The mixture was transferred with ethyl acetate (300 mL) to a separatory funnel and was washed twice with 2 N HCl (2 × 100 mL) and once with 1 N $NaHCO_3$ (100 mL). After washing with brine (100 mL), the organic layer was dried over $MgSO_4$ and concentrated in vacuo. Compound **7** was obtained as a white solid (10.86 g, 28.2 mmol, 97%). ^{1}H NMR (200 MHz, [D$_6$]DMSO): $\delta = 0.18$ (s, 6H), 0.95 (s, 9H), 2.29 (s, 3H), 6.83 (d, 2H, $J = 9.1$ Hz), 7.27 (d, 2H, $J = 8.8$ Hz), 7.63 (d, 2H, $J = 9.1$ Hz), 7.97 (d, 2H, $J = 8.8$ Hz), 10.13 ppm (s, 1H); ^{13}C NMR (50.2 MHz, [D$_6$]DMSO): $\delta = -4.6$, 17.9, 20.8, 25.5, 119.6, 121.7, 121.9, 129.0, 132.6, 132.9, 151.2, 152.7, 164.3, 168.9 ppm; HRMS: calcd for $C_{21}H_{28}NO_4Si$ $[M+H]^+$ 386.1782, found 386.1771; MS (ESI): *m/z* 386.2 $[M+H]^+$, 770.8 $[2M+H]^+$; FTIR (thin film): 1753, 1641, 1605, 1508, 1248, 914, 843 cm^{-1}; TLC: $R_f = 0.44$ (90% toluene/acetone).

4-Hydroxy-*N*-[4-(*tert*-butyldimethylsilyloxy)phenyl]benzamide (8). NaOEt (1.88 g, 27.7 mmol) was added at room temperature to a solution of **7** (10.7 g, 27.7 mmol) in ethanol (100 mL), and the solution was stirred until TLC (80% toluene/acetone) indicated complete removal of the acetyl group (~1 h). The solution was neutralized by addition of 2 N HCl (14 mL, 28 mmol), and the ethanol was removed under reduced pressure. The resulting suspension was dissolved in EtOAc (400 mL) and water (100 mL) and transferred to a separatory funnel. The organic layer was separated and washed once with 2 N HCl (50 mL), twice with 1 N $NaHCO_3$ (50 mL), and once with brine (50 mL). The organic layer was dried over $MgSO_4$, concentrated in vacuo, and the remaining residue was purified by flash chromatography (toluene/ethyl acetate/ethanol 80:13:7 *v/v/v*) to afford compound **8** as a white solid (8.6 g, 25 mmol, 90%). ^{1}H NMR (200 MHz, [D$_6$]DMSO): $\delta = 0.17$ (s, 6H), 0.94 (s, 9H), 6.82 (m, 4H), 7.59 (d, 2H, $J = 8.8$ Hz), 7.81 (d, 2H, $J = 8.8$ Hz), 9.86 (s, 1H), 10.05 ppm (br s, 1H); ^{13}C NMR (50.2 MHz, [D$_6$]DMSO): $\delta = -4.6$, 18.1, 25.6, 114.8, 119.6, 121.9, 125.6, 129.5, 133.3, 150.9, 160.4 ppm; HRMS: calcd for $C_{19}H_{26}NO_3Si$ $[M+H]^+$ 344.1677, found 344.1665; MS (ESI): *m/z* 344.3 $[M+H]^+$, 386.1 $[M+Na]^+$, 686.8 $[2M+H]^+$; FTIR (thin film): 1650, 1610, 1506, 1252, 1221, 1169, 912, 837 cm^{-1}; TLC: $R_f = 0.47$ (80% toluene/acetone).

General procedure A (Mitsunobu). Diethyl azodicarboxylate (2.71 mL, 5.82 mmol, 2 equiv, 40% in toluene) was added over a period of 10 min at 0 °C to a solution of phenol **8** (1.00 g, 2.91 mmol, 1 equiv), the linker alcohol (2 equiv), and PPh_3 (1.52 g, 5.82 mmol, 2 equiv) in absolute THF (15 mL). After 30 min the cooling bath was removed, and the solution was stirred at room temperature for 16 h. The solvent was concentrated in vacuo, and the remaining residue was purified by flash chromatography.

4-(2-(Benzyloxy)ethoxy)-*N*-[4-(*tert*-butyldimethylsilyloxy)phenyl]-benzamide (10a). Starting from **8** (0.89 g, 2.6 mmol) and 2-benzyloxyethanol (0.79 g, 5.21 mmol), **10a** was synthesized according to general procedure A. Flash chromatography (98→90% toluene/acetone) afforded **10a** as a white solid (1.08 g, 2.26 mmol, 87%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.20 (s, 6H), 0.99 (s, 9H), 3.85 (t, 2H, J = 4.8 Hz), 4.19 (t, 2H, J = 4.8 Hz), 4.64 (s, 2H), 6.82 (d, 2H, J = 8.8 Hz), 6.95 (d, 2H, J = 9.1 Hz), 7.31–7.38 (m, 5H), 7.47 (d, 2H, J = 8.8 Hz), 7.80 ppm (d, 3H, J = 8.8 Hz, 2H_{arom}, N*H*); ^{13}C NMR (50.2 MHz, $CDCl_3$): δ = −4.6, 18.1, 25.6, 67.4, 68.2, 73.3, 114.2, 120.1, 121.9, 127.3, 127.6, 128.3, 128.8, 131.8, 137.7, 152.2, 161.3, 165.3 ppm; HRMS: calcd for $C_{28}H_{36}NO_4Si$ [M+H]$^+$ 477.2335, $C_{28}H_{35}NO_4SiNa$ [M+Na]$^+$ 500.2228, found 478.2393, 500.2225; MS (ESI): m/z 478.3 [M+H]$^+$, 977.0 [2M+Na]$^+$; FTIR (thin film): 1638, 1605, 1504, 1250, 1180, 1103, 910, 841, 780, 741, 694 cm^{-1}; TLC: R_f = 0.38 (98% toluene/acetone).

4-(3-(Benzyloxy)propyloxy)-*N*-[4-(*tert*-butyldimethylsilyloxy)phenyl]benzamide (10b). Starting from **8** (0.81 g, 2.36 mmol) and 3-benzyloxy-1-propanol (0.78 g, 4.72 mmol), **10b** was synthesized according to general procedure A. Flash chromatography (98→90% toluene/acetone) afforded **10b** as a white solid (1.09 g, 2.22 mmol, 94%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.20 (s, 6H), 0.99 (s, 9H), 2.11 (quint, 2H, J = 6.2 Hz), 3.67 (t, 2H, J = 6.2 Hz), 4.14 (t, 2H, J = 6.2 Hz), 4.53 (s, 2H), 6.83 (d, 2H, J = 8.8 Hz), 6.93 (d, 2H, J = 8.8 Hz), 7.32 (s, 5H), 7.47 (d, 2H, J = 8.8 Hz), 7.74 (s, 1H), 7.80 ppm (d, 2H, J = 8.8 Hz). ^{13}C NMR (50.2 MHz, $CDCl_3$): δ = −4.5, 18.1, 25.6, 29.5, 64.9, 66.4, 72.9, 114.2, 120.2, 121.9, 127.0, 127.5, 128.3, 128.8, 131.8, 138.2, 152.2, 161.6, 165.3 ppm; HRMS: calcd for $C_{29}H_{38}NO_4Si$ [M+H]$^+$ 492.2565, found 492.2550; MS (ESI): m/z 492.3 [M+H]$^+$, 982.9 [2M+H]$^+$; FTIR (thin film): 1648, 1605, 1504, 1250, 1180, 1103, 1018, 910, 779, 733, 694 cm^{-1}; TLC: R_f = 0.40 (98% toluene/acetone).

4-(4-(Benzyloxy)butyloxy)-*N*-[4-(*tert*-butyldimethylsilyloxy)phenyl]benzamide (10c). Starting from **8** (0.74 g, 2.16 mmol) and 4-benzyloxy-1-butanol (0.78 g, 4.32 mmol), **10c** was synthesized according to general procedure A. Flash chromatography (98→90% toluene/acetone) afforded **10c** as a white solid (0.98 g, 1.94 mmol, 90%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.20 (s, 6H), 1.00 (s, 9H), 1.77–1.95 (m, 4H), 3.56 (t, 2H, J = 6.0 Hz), 4.02 (t, 2H, J = 6.0 Hz), 4.53 (s, 2H), 6.82 (d, 2H, J = 8.8 Hz), 6.90 (d, 2H, J = 8.8 Hz), 7.34 (s, 5H), 7.47 (d, 2H, J = 8.8 Hz), 7.79 (d, 2H, J = 8.8 Hz), 7.84 ppm (br s, 1H); ^{13}C NMR (50.2 MHz, $CDCl_3$): δ = −4.5, 18.1, 25.6, 25.9, 26.2, 67.7, 69.7, 72.8, 114.1, 120.1, 121.9, 126.9, 127.5, 128.3, 128.8, 131.8, 138.4, 152.2, 161.6, 165.3 ppm; HRMS: calcd for $C_{30}H_{40}NO_4Si$ [M+H]$^+$ 506.22721, $C_{30}H_{39}NO_4SiNa$ [M+Na]$^+$ 528.2541, found 506.2709, 528.2534; MS (ESI): m/z 506.3 [M+H]$^+$, 1010.9 [2M+H]$^+$; FTIR (thin film): 1648, 1612, 1504, 1250, 1180, 1111, 918, 841, 772, 741, 687 cm^{-1}; TLC: R_f = 0.42 (98% toluene/acetone).

4-(5-(Benzyloxy)pentyloxy)-*N*-[4-(*tert*-butyldimethylsilyloxy)phenyl]benzamide (10d). Starting from **8** (0.8 g, 2.33 mmol) and 5-benzyloxy-1-pentanol (0.91 g, 4.66 mmol), **10d** was synthesized according to general procedure A. Flash chromatography (98→90% toluene/acetone) afforded **10d** as a white solid (1.13 g, 2.17 mmol, 93%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.19 (s, 6H), 0.99 (s, 9H), 1.50–1.90 (m, 6H), 3.51 (t, 2H, J = 6.2 Hz), 4.01 (t, 2H, J = 6.2 Hz), 4.52 (s, 2H), 6.83 (d, 2H, J = 8.8 Hz), 6.93 (d, 2H, J = 8.8 Hz), 7.29–7.35 (m, 5H), 7.46 (d, 2H, J = 8.8 Hz), 7.72 (s, 1H), 7.80 ppm (d, 2H, J = 8.4 Hz). ^{13}C NMR (50.2 MHz, $CDCl_3$): δ = −4.6, 18.1, 22.7, 25.6, 28.8, 29.4, 67.9, 70.1, 72.8, 114.1, 120.2, 121.9, 126.9, 127.4, 127.5, 128.2, 128.8, 131.9, 138.4, 152.2, 161.7, 165.3 ppm; HRMS: calcd for $C_{31}H_{42}NO_4Si$ [M+H]$^+$ 520.2878, found 520.2865; MS (ESI): m/z 520.3 [M+H]$^+$, 1039.0 [2M+H]$^+$, 1061.0 [2M+Na]$^+$; FTIR (thin film): 1648, 1605, 1504, 1250, 1180, 1103, 1018, 910, 841, 779, 694, 733 cm^{-1}; TLC: R_f = 0.44 (98% toluene/acetone).

4-(2-(2-(Benzyloxy)ethoxy)ethoxy)-*N*-[4-(*tert*-butyldimethylsilyloxy)phenyl]benzamide (10e). Starting from **8** (0.5 g, 1.46 mmol) and di(ethylene glycol) monobenzyl ether (1.71 g, 8.7 mmol), **10e** was synthesized according to general procedure A. Flash chromatography (91% toluene/acetone) provided **10e** as a white solid (1.49 g, 2.87 mmol, 99%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.20 (s, 6H), 0.99 (s, 9H), 3.63–3.69 (m, 2H), 3.72–3.78 (m, 2H), 3.87 (t, 2H, J = 4.8 Hz), 4.15 (t, 2H, J = 4.8 Hz), 4.56 (s, 2H), 6.81 (d, 2H, J = 9.1 Hz), 6.91 (d, 2H, J = 8.8 Hz), 7.30–7.34 (m, 5H), 7.49 (d, 2H, J = 8.8 Hz), 7.78 (d, 2H, 8.8 Hz), 7.97 ppm (s, 1H); ^{13}C NMR (50.2 MHz, $CDCl_3$): δ = −4.5, 18.1, 25.6, 67.5, 69.4, 69.5, 70.9, 73.2, 114.4, 120.2, 121.9, 127.4, 127.6, 127.7, 128.3, 128.8, 131.8, 138.1, 152.3, 161.4, 165.2 ppm; HRMS: calcd for $C_{30}H_{40}NO_5Si$ [M+H]$^+$ 522.2670, found 522.2672; MS (ESI): m/z 522.3 [M+H]$^+$, 544.1 [M+Na]$^+$; FTIR (thin film): 1636, 1609, 1508, 1250, 1101, 916, 839 cm^{-1}; TLC: R_f = 0.73 (80% toluene/acetone).

General procedure B (hydrogenolytic deprotection). The compound to be deprotected (2.0 mmol) was dissolved in a mixture of EtOAc and EtOH (20 mL, 1:1), and the solution was degassed by bubbling an argon stream through it for 2 min. Then Pd/C (106 mg, 5 mol% Pd, 10 wt% Pd on activated carbon) was added, the flask was equipped with a double-mantled hydrogen balloon, and the suspension was stirred for 16 h at room temperature. The catalyst was removed by filtering through Hyflo Super Cel, and the mixture was concentrated in vacuo to afford the deprotected compounds in high purity.

4-(2-(Hydroxy)ethoxy)-*N*-[4-(*tert*-butyldimethylsilyloxy)phenyl]-benzamide (11a). Starting from **10a** (0.841 g, 1.76 mmol), **11a** was synthesized according to general procedure B. **11a** was obtained as a white foam (0.66 g, 1.71 mmol, 97%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.18 (s, 6H), 0.98 (s, 9H), 2.61 (br s, 1H), 3.95 (d, 2H, J = 4.5 Hz), 4.05 (d, 2H, J = 4.6 Hz), 6.80 (d, 2H, J = 8.8 Hz), 6.86 (d, 2H, J = 8.4 Hz), 7.47 (d, 2H, J = 8.8 Hz), 7.76 (d, 2H, J = 8.4 Hz), 8.02 ppm (br s, 1H); ^{13}C NMR (50.2 MHz, $CDCl_3$): δ = −4.6, 18.1, 25.6, 60.8, 69.2, 114.1, 120.1, 122.1, 127.2, 128.9, 131.8, 152.2, 161.2, 165.7 ppm; HRMS: calcd for $C_{21}H_{30}NO_4Si$ [M+H]$^+$ 388.1939, found 388.1925; MS (ESI): m/z 388.0 [M+H]$^+$, 410.0 [M+Na]$^+$, 775.3 [2M+H]$^+$, 797.3 [2M+Na]$^+$; FTIR (thin film): 1636, 1605, 1504, 1250, 1173, 1080, 1042, 903, 833, 779 cm^{-1}; TLC: R_f = 0.22 (83% toluene/acetone).

4-(3-(Hydroxy)propyloxy)-*N*-[4-(*tert*-butyldimethylsilyloxy)phenyl]benzamide (11b). Starting from **10b** (0.822 g, 1.67 mmol), **11b** was synthesized according to general procedure B. **11b** was obtained as a white foam (0.67 g, 1.65 mmol, 99%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.18 (s, 6H), 0.98 (s, 9H), 2.02 (quint, 2H, J = 5.9 Hz), 2.21 (br s, 1H), 3.83 (t, 2H, J = 5.9 Hz), 4.11 (t, 2H, J = 5.9 Hz), 6.80 (d, 2H, J = 8.8 Hz), 6.87 (d, 2H, J = 8.8 Hz), 7.46 (d, 2H, J = 8.8 Hz), 7.76 (d, 2H, J = 8.8 Hz), 7.96 ppm (s, 1H); ^{13}C NMR (50.2 MHz, $CDCl_3$): δ = −4.6, 18.1, 25.6, 31.8, 59.3, 65.2, 114.1, 120.1, 122.0, 127.0, 128.9, 131.8, 152.2, 161.4, 165.6 ppm; HRMS: calcd for $C_{22}H_{32}NO_4Si$ [M+H]$^+$ 402.2095, found 402.2087; MS (ESI): m/z 402.1 [M+H]$^+$, 424.0 [M+Na]$^+$, 803.4 [2M+H]$^+$, 825.3 [2M+Na]$^+$; FTIR (thin film): 1643, 1605, 1504, 1250, 1180, 1111, 1065, 910, 833, 779 cm^{-1}; TLC: R_f = 0.24 (83% toluene/acetone).

4-(4-(Hydroxy)butyloxy)-*N*-[4-(*tert*-butyldimethylsilyloxy)phenyl]-benzamide (11c). Starting from **10c** (0.854 g, 1.69 mmol), **11c** was synthesized according to general procedure B. **11c** was obtained as a white foam (0.68 g, 1.64 mmol, 97%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.19 (s, 6H), 0.98 (s, 9H), 1.61 (br s, 1H), 1.68–1.98 (m,

4H), 3.73 (t, 2H, $J=6.2$ Hz), 4.05 (t, 2H, $J=6.2$ Hz), 6.83 (d, 2H, $J=8.8$ Hz), 6.93 (d, 2H, $J=8.8$ Hz), 7.46 (d, 2H, $J=8.8$ Hz), 7.71 (br s, 1H), 7.80 ppm (d, 2H, $J=8.8$ Hz). ^{13}C NMR (50.2 MHz, $CDCl_3$): $\delta=-4.5$, 18.2, 25.6, 29.2, 62.3, 67.9, 114.3, 120.3, 121.9, 127.1, 128.8, 131.8, 152.3, 161.6, 165.3 ppm; HRMS: calcd for $C_{23}H_{34}NO_4Si$ $[M+H]^+$ 416.2252, found 416.2245; MS (ESI): m/z 416.3 $[M+H]^+$, 438.2 $[M+Na]^+$, 831.5 $[2M+H]^+$, 853.4 $[2M+Na]^+$; FTIR (thin film): 1643, 1605, 1504, 1404, 1319, 1250, 1173, 1042, 1011, 903, 833, 679, 625 cm^{-1}; TLC: $R_f=0.26$ (83% toluene/acetone).

4-(5-(Hydroxy)pentyloxy)-*N*-[4-(*tert*-butyldimethylsilyloxy)phenyl]benzamide (11d). Starting from **10d** (581 mg, 1.12 mmol), **11d** was synthesized according to general procedure B. **11d** was obtained as a white foam (0.48 g, 1.11 mmol, 99%). ^{1}H NMR (200 MHz, $CDCl_3$): $\delta=0.19$ (s, 6H), 0.98 (s, 9H), 1.50–1.70 (m, 5H), 1.81–1.89 (m, 2H), 3.69 (t, 2H, $J=6.2$ Hz), 4.02 (t, 2H, $J=6.2$ Hz), 6.83 (d, 2H, $J=8.8$ Hz), 6.93 (d, 2H, $J=9.0$ Hz), 7.46 (d, 2H, $J=8.8$ Hz), 7.70 (br s, 1H), 7.80 ppm (d, 2H, $J=8.8$ Hz). ^{13}C NMR (50.2 MHz, $CDCl_3$): $\delta=-4.5$, 18.2, 22.3, 25.6, 28.9, 32.3, 62.5, 68.0, 114.2, 120.3, 121.9, 127.0, 128.8, 131.8, 152.3, 161.7, 165.3 ppm; HRMS: calcd for $C_{24}H_{36}NO_4Si$ $[M+H]^+$ 430.2408, $C_{24}H_{35}NO_4SiNa$ $[M+Na]^+$ 452.2228, found 430.2409, 452.2219; MS (ESI): m/z 430.3 $[M+H]^+$; FTIR (thin film): 1643, 1605, 1504, 1466, 1404, 1250, 1173, 1111, 1018, 910, 833, 779, 694 cm^{-1}; TLC: $R_f=0.28$ (83% toluene/acetone).

4-(2-(2-(Hydroxy)ethoxy)ethoxy)-*N*-[4-(*tert*-butyldimethylsilyloxy)phenyl]benzamide (11e). Starting from **10e** (225 mg, 0.43 mmol), **11e** was synthesized according to general procedure B. Flash chromatography (80% toluene/acetone) provided **11e** as a white foam (155 mg, 0.36 mmol, 84%). ^{1}H NMR (200 MHz, $CDCl_3$): $\delta=0.17$ (s, 6H), 0.97 (s, 9H), 2.59 (br s, 1H), 3.60–3.65 (m, 2H), 3.70–3.75 (m, 2H), 3.80–3.85 (m, 2H), 4.08–4.13 (m, 2H), 6.78 (d, 2H, $J=8.8$ Hz), 6.85 (d, 2H, $J=8.8$ Hz), 7.47 (d, 2H, $J=8.8$ Hz), 7.76 (d, 2H, $J=8.8$ Hz), 8.17 ppm (s, 1H); ^{13}C NMR (50.2 MHz, $CDCl_3$): $\delta=-4.5$, 18.1, 25.6, 61.6, 67.4, 69.4, 72.6, 114.3, 120.2, 121.9, 127.5, 128.9, 131.8, 152.2, 161.2, 165.3 ppm; HRMS: calcd for $C_{23}H_{34}NO_5Si$ $[M+H]^+$ 432.2201, $C_{23}H_{33}NO_5SiNa$ $[M+Na]^+$ 454.2020, found 432.2198, 454.2022; MS (ESI): m/z 432.2 $[M+H]^+$, 454.1 $[M+Na]^+$, 863.4 $[2M+H]^+$, 885.4 $[2M+Na]^+$; FTIR (thin film): 1663, 1609, 1506, 1248, 1219, 1117, 1055, 916, 837, 758 cm^{-1}; TLC: $R_f=0.21$ (80% toluene/acetone).

General procedure C (coupling of a carboxylic acid with a primary alcohol). The alcohol (1 mmol) was added at a temperature of 0 °C to a solution of the acid (1.2 mmol), EDC·HCl (1.2 mmol), 1-hydroxybenzotriazole (HOBt, 1.5 mmol), *N*,*N*-diisopropylethylamine (DIPEA, 1.2 mmol), and a catalytic amount of DMAP in absolute dichloromethane (20 mL). After 30 min, the mixture was allowed to warm to room temperature, and stirring was continued for 16 h. The mixture was concentrated in vacuo and purified by flash chromatography.

(*S*)-Ethoxy-2-(*N*′-[4-(*tert*-butyldimethylsilyloxy)phenyl]benzamidyl)-*N*-(*tert*-butoxycarbonyl)-2-piperidine carboxylate (13a). Starting from **11a** (0.22 g, 0.57 mmol) and **12** (0.16 g, 0.68 mmol), **13a** was synthesized according to general procedure C. Flash chromatography (92% toluene/acetone) provided **13a** as a white foam (0.31 g, 0.52 mmol, 91%). ^{1}H NMR (200 MHz, $CDCl_3$, 3:2 mixture of trans–cis amide rotamers, data for major rotamer): $\delta=0.18$ (s, 6H), 0.98 (s, 9H), 1.42 (s, 9H), 1.35–1.52 (m, 1H), 1.58–1.70 (m, 4H), 2.10–2.26 (m, 1H), 2.80–3.10 (m, 1H), 3.90–4.00 (m, 1H), 4.20–4.30 (m, 2H), 4.45–4.55 (m, 2H), 4.75–4.90 (m, 1H), 6.81 (d, 2H, $J=8.8$ Hz), 6.92 (d, 2H, $J=8.8$ Hz), 7.47 (d, 2H, $J=8.8$ Hz), 7.80 (d, 2H, $J=8.8$ Hz), 7.90 ppm (br s, 1H); ^{13}C NMR (50.2 MHz, $CDCl_3$, 3:2 mixture of trans–cis amide rotamers, data for major rotamer): $\delta=-4.5$, 18.2, 20.7, 24.7, 25.7, 26.8, 28.3, 41.0, 42.2, 53.8, 54.9, 62.9, 65.9, 80.0, 114.4, 120.3, 121.8, 127.8, 128.9, 131.8, 152.3, 155.7, 161.0, 165.1, 171.9 ppm; HRMS: calcd for $C_{32}H_{47}N_2O_7Si$ $[M+H]^+$ 599.3147, $C_{32}H_{46}N_2O_7SiNa$ $[M+Na]^+$ 621.2967, found 599.3139, 621.2951; MS (ESI): m/z 499.3 $[M-Boc+H]^+$, 599.4 $[M+H]^+$, 1197.8 $[2M+H]^+$; FTIR (thin film): 1745, 1690, 1605, 1504, 1404, 1250, 1157, 1042, 910, 833, 756 cm^{-1}; $[\alpha]_D^{20}=-23.6°$ ($c=10$ mg mL^{-1}, $CHCl_3$); TLC: $R_f=0.44$ (91% toluene/acetone).

(*S*)-Propyloxy-3-(*N*′-[4-(*tert*-butyldimethylsilyloxy)phenyl]benzamidyl)-*N*-(*tert*-butoxycarbonyl)-2-piperidine carboxylate (13b). Starting from **11b** (0.24 g, 0.61 mmol) and **12** (0.17 g, 0.73 mmol), **13b** was synthesized according to general procedure C. Flash chromatography (92% toluene/acetone) provided **13b** as a white foam (0.37 g, 0.60 mmol, 99%). ^{1}H NMR (200 MHz, $CDCl_3$, 3:2 mixture of trans–cis amide rotamers, data for major rotamer): $\delta=0.17$ (s, 6H), 0.97 (s, 9H), 1.41 (s, 9H), 1.42–1.70 (m, 5H), 2.08–2.20 (m, 3H), 2.70–3.00 (m, 1H), 3.89–3.99 (m, 1H), 4.05 (t, 2H, $J=6.0$ Hz), 4.29–4.33 (m, 2H), 4.69–4.90 (m, 1H), 6.79 (d, 2H, $J=8.8$ Hz), 6.88 (d, 2H, $J=8.8$ Hz), 7.47 (d, 2H, $J=8.8$ Hz), 7.80 (d, 2H, $J=8.8$ Hz), 8.04 ppm (s, 1H); ^{13}C NMR (50.2 MHz, $CDCl_3$, 3:2 mixture of trans–cis amide rotamers, data for major rotamer): $\delta=-4.7$, 17.9, 20.4, 24.3, 25.4, 26.5, 28.1, 40.8, 41.9, 53.6, 54.7, 61.4, 64.0, 79.7, 113.8, 119.9, 121.9, 127.2, 128.9, 132.0, 151.9, 155.7, 161.0, 165.2, 171.6 ppm; HRMS: calcd for $C_{33}H_{49}N_2O_7Si$ $[M+H]^+$ 613.3304, $C_{33}H_{48}N_2O_7SiNa$ $[M+Na]^+$ 635.3123, found 613.3291, 635.3112; MS (ESI): m/z 513.2 $[M-Boc+H]^+$, 613.3 $[M+H]^+$, 635.4 $[M+Na]^+$, 1225.8 $[2M+H]^+$; FTIR (thin film): 1745, 1690, 1605, 1504, 1404, 1250, 1157, 1042, 910, 833, 756 cm^{-1}; $[\alpha]_D^{20}=-22.0°$ ($c=10$ mg mL^{-1}, $CHCl_3$); TLC: $R_f=0.49$ (91% toluene/acetone).

(*S*)-Butyloxy-4-(*N*′-[4-(*tert*-butyldimethylsilyloxy)phenyl]benzamidyl)-*N*-(*tert*-butoxycarbonyl)-2-piperidine carboxylate (13c). Starting from **11c** (0.25 g, 0.59 mmol) and **12** (0.16 g, 0.71 mmol), **13c** was synthesized according to general procedure C. Flash chromatography (92% toluene/acetone) provided **13c** as a white foam (0.37 g, 0.58 mmol, 99%). ^{1}H NMR (200 MHz, $CDCl_3$, 3:2 mixture of trans–cis amide rotamers, data for major rotamer): $\delta=0.18$ (s, 6H), 0.97 (s, 9H), 1.44 (s, 9H), 1.45–1.75 (m, 5H), 1.46–1.92 (m, 4H), 2.10–2.28 (m, 1H), 2.75–3.05 (m, 1H), 3.85–4.10 (m, 3H), 4.14–4.30 (m, 2H), 4.70–4.90 (m, 1H), 6.80 (d, 2H, $J=8.8$ Hz), 6.90 (d, 2H, $J=8.8$ Hz), 7.47 (d, 2H, $J=8.8$ Hz), 7.80 (d, 2H, $J=8.8$ Hz), 7.94 ppm (s, 1H); ^{13}C NMR (50.2 MHz, $CDCl_3$, 3:2 mixture of trans–cis amide rotamers, data for major rotamer): $\delta=-4.7$, 17.9, 20.5, 24.5, 25.5, 26.5, 28.1, 40.9, 41.8, 53.6, 54.7, 64.3, 67.1, 79.7, 113.9, 119.9, 121.9, 127.0, 128.9, 132.0, 151.9, 155.8, 161.2, 165.3, 171.8 ppm; HRMS: calcd for $C_{34}H_{51}N_2O_7Si$ $[M+H]^+$ 627.3460, $C_{34}H_{50}N_2O_7SiNa$ $[M+Na]^+$ 649.3280, found 627.3449, 649.3265; MS (ESI): m/z 527.2 $[M-Boc+H]^+$, 627.5 $[M+H]^+$, 649.4 $[M+Na]^+$, 1253.8 $[2M+H]^+$, 1276.0 $[2M+Na]^+$; FTIR (thin film): 1745, 1690, 1605, 1504, 1396, 1242, 1157, 1042, 910, 833, 779 cm^{-1}; $[\alpha]_D^{20}=-21.8°$ ($c=10$ mg mL^{-1}, $CHCl_3$); TLC: $R_f=0.51$ (91% toluene/acetone).

(*S*)-Pentyloxy-5-(*N*′-[4-(*tert*-butyldimethylsilyloxy)phenyl]benzamidyl)-*N*-(*tert*-butoxycarbonyl)-2-piperidine carboxylate (13d). Starting from **11d** (0.17 g, 0.41 mmol) and **12** (0.11 g, 0.49 mmol), **13d** was synthesized by general procedure C. Flash chromatography (92% toluene/acetone) provided **13d** as white a foam (0.24 g, 0.37 mmol, 92%). ^{1}H NMR (200 MHz, $CDCl_3$, 3:2 mixture of trans–cis amide rotamers, data for major rotamer): $\delta=0.17$ (s, 6H), 0.97 (s, 9H), 1.43 (s, 9H), 1.44–1.90 (m, 11H), 2.10–2.25 (m, 1H), 2.80–3.00 (m, 1H), 3.85–4.05 (m, 3H), 4.07–4.20 (m, 2H), 4.65–4.91 (m, 1H), 6.80 (d, 2H, $J=8.8$), 6.90 (d, 2H, $J=8.8$ Hz), 7.46 (d, 2H, $J=8.8$ Hz), 7.80 (d, 2H, $J=8.8$ Hz), 7.92 ppm (s, 1H); ^{13}C NMR (50.2 MHz, $CDCl_3$,

3:2 mixture of trans–cis amide rotamers, data for major rotamer): $\delta = -4.7$, 17.9, 20.5, 22.3, 24.5, 25.5, 26.5, 28.1, 28.4, 40.9, 41.7, 53.6, 54.7, 60.7, 64.5, 67.5, 79.6, 113.8, 119.9, 121.9, 126.9, 128.9, 132.0, 151.9, 156.0, 161.3, 165.2, 171.7 ppm; HRMS: calcd for $C_{35}H_{52}N_2O_7Si$ $[M+H]^+$ 641.3617, found 641.3600; MS (ESI): *m/z* 541.3 $[M-Boc+H]^+$, 641.5 $[M+H]^+$, 1282.0 $[2M+H]^+$; FTIR (thin film): 1745, 1690, 1605, 1504, 1396, 1242, 1157, 1042, 910, 833, 756 cm^{-1}; $[\alpha]_D^{20} = -23.0^\circ$ ($c = 10$ mg mL^{-1}, $CHCl_3$). TLC $R_f = 0.56$ (91% toluene/acetone).

General procedure D (Boc removal). TFA (10 mmol) was added at room temperature to a solution of the compound to be deprotected (0.50 mmol) in dichloromethane (5 mL), and the solution was stirred for 2 h (TLC control). $NaHCO_3$ (1 N, 20 mL) was added slowly over a period of 10 min at room temperature. After the gas formation had stopped, the mixture was transferred with EtOAc (150 mL) to a separatory funnel. The organic layer was extracted once with 1 N $NaHCO_3$ (50 mL), and dried over $MgSO_4$. The organic layer was concentrated in vacuo. The desired compounds were purified by flash chromatography.

(*S*)-Ethoxy-2-(*N*-[4-(*tert*-butyldimethylsilyloxy)phenyl]benzamidyl)-2-piperidine carboxylate (14a). Starting from **13a** (0.3 g, 0.50 mmol), **14a** was synthesized according to general procedure D. Flash chromatography (toluene/acetone/methanol 57:38:5→45:45:10 *v/v/v*) provided **14a** as a light-yellow oil (0.16 g, 0.32 mmol, 64%). ^1H NMR (200 MHz, $CDCl_3$): $\delta = 0.17$ (s, 6H), 0.97 (s, 9H), 1.39–1.61 (m, 4H), 1.70–1.82 (m, 1H), 1.90–2.08 (m, 1H), 2.58–2.72 (m, 1H), 2.97 (br s, 1H), 2.98–3.15 (m, 1H), 3.43 (dd, 1H, $J = 3.1$, 9.7 Hz), 4.19 (t, 2H, $J = 4.6$ Hz), 4.47 (t, 2H, $J = 4.6$ Hz), 6.79 (d, 2H, $J = 8.8$ Hz), 6.89 (d, 2H, $J = 8.8$ Hz), 7.47 (d, 2H, $J = 8.8$ Hz), 7.79 (d, 2H, $J = 8.8$ Hz), 8.04 ppm (s, 1H); ^{13}C NMR (50.2 MHz, $CDCl_3$): $\delta = -4.6$, 18.0, 23.6, 25.2, 25.6, 28.7, 45.3, 58.1, 62.7, 65.7, 114.2, 120.1, 121.9, 127.7, 128.9, 131.8, 152.2, 160.8, 165.2, 172.9 ppm; HRMS: calcd for $C_{27}H_{41}N_2O_6Si$ $[M+H_2O+H]^+$ 517.2728, $C_{28}H_{43}N_2O_6Si$ $[M+MeOH+H]^+$ 531.2885, found 517.2720, 531.2877; MS (ESI): *m/z* 531.3 $[M+MeOH+H]^+$; FTIR (thin film): 2932, 1736, 1643, 1605, 1504, 1404, 1250, 1173, 1119, 1049, 910, 833, 779 cm^{-1}; $[\alpha]_D^{20} = -7.2^\circ$ ($c = 10$ mg mL^{-1}, $CHCl_3$); TLC: $R_f = 0.28$ (50% toluene/acetone).

General procedure E (coupling of a carboxylic acid and a secondary amine). The amine (0.5 mmol) was added at 0 °C to a solution of the carboxylic acid (0.75 mmol), EDC·HCl (0.75 mmol), HOBt (1 mmol), DIPEA (1.5 mmol), and a catalytic amount of DMAP in absolute dichloromethane (30 mL). After 30 min, the mixture was allowed to warm to room temperature, and stirring was continued for 16 h. The mixture was concentrated in vacuo, and the remaining residue was purified by flash chromatography.

(*S*)-Ethoxy-2-(*N*′-[4-(*tert*-butyldimethylsilyloxy)phenyl]benzamidyl)-*N*-[2-oxo-2-(3,4,5-trimethoxyphenyl)acetyl)]-2-piperidine carboxylate (16a). Starting from **14a** (103 mg, 0.21 mmol) and **15** (76 mg, 0.32 mmol), **16a** was synthesized according to general procedure E. Flash chromatography (91→80% toluene/acetone) provided **16a** as a colorless oil (141 mg, 0.20 mmol, 95%). ^1H NMR (200 MHz, $CDCl_3$, 3:1 mixture of trans–cis amide rotamers, data for major rotamer): $\delta = 0.17$ (s, 6H), 0.97 (s, 9H), 1.40–1.85 (m, 5H), 2.25–2.40 (m, 1H), 3.10–3.31 (m, 1H), 3.40–3.55 (m, 1H), 3.90 (s, 6H), 3.93 (s, 3H), 4.25 (t, 2H, $J = 5.1$ Hz), 4.55 (t, 2H, $J = 5.1$ Hz), 5.30–5.40 (m, 1H), 6.79 (d, 2H, $J = 8.8$ Hz), 6.89 (d, 2H, $J = 8.8$ Hz), 7.32 (s, 2H), 7.47 (d, 2H, $J = 8.8$ Hz), 7.79 (d, 2H, $J = 8.8$ Hz), 8.05 ppm (s, 1H); ^{13}C NMR (50.2 MHz, $CDCl_3$, 3:1 mixture of trans–cis amide rotamers, data for major rotamer): $\delta = -4.6$, 18.0, 20.9, 24.6, 25.5, 26.1, 29.5, 44.2, 51.6, 56.2, 60.8, 63.4, 65.6, 106.8, 114.1, 120.0, 121.7, 127.8, 129.0, 131.9, 143.9, 152.1, 153.4, 160.7, 165.1, 167.8, 170.1, 190.6 ppm; HRMS: calcd for $C_{38}H_{49}N_2O_{10}Si$ $[M+H]^+$ 721.3151, found 721.3134; MS (ESI): *m/z* 721.5 $[M+H]^+$, 743.4 $[M+Na]^+$, 1442.7 $[2M+H]^+$; FTIR (thin film): 2940, 1736, 1643, 1504, 1458, 1412, 1327, 1250, 1126, 1003, 910, 841, 779 cm^{-1}; $[\alpha]_D^{20} = -10.0^\circ$ ($c = 10$ mg mL^{-1}, $CHCl_3$); TLC: $R_f = 0.5$ (80% toluene/acetone).

General procedure F (TBS removal). TBAF (200 μL, 1 M in THF, 0.2 mmol) was added slowly at room temperature to a solution of the compound to be deprotected (0.4 mmol) in wet THF (10 mL). After 1 h, TLC (75% toluene/acetone) indicated completion of the reaction. Water (10 mL) was added, and the mixture was transferred to a separatory funnel with EtOAc (60 mL). After addition of brine (10 mL), the layers were separated. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The desired compound was purified by flash chromatography.

(*S*)-Ethoxy-2-(*N*′-[4-(hydroxy)phenyl]benzamidyl)-*N*-[2-oxo-2-(3,4,5-trimethoxyphenyl)acetyl)]-2-piperidine carboxylate (3a). Starting from **16a** (125 mg, 0.17 mmol), **3a** was synthesized according to general procedure F. Flash chromatography (75% toluene/acetone) provided **3a** as a colorless oil (94 mg, 0.15 mmol, 91%). ^1H NMR (500 MHz, $CDCl_3$, 5:1 mixture of trans–cis amide rotamers, data for major rotamer): $\delta = 1.20$–1.39 (m, 1H), 1.42–1.52 (m, 1H), 1.59–1.63 (m, 1H), 1.70–1.80 (m, 2H), 2.02 (br s, 1H), 2.30–2.35 (m, 1H), 3.25 (dt, 1H, $J = 11.5$, 3.1 Hz), 3.44–3.49 (m, 1H), 3.79 (s, 6H), 3.92 (s, 3H), 4.15–4.22 (m, 2H), 4.49–4.53 (m, 2H), 5.30–5.35 (m, 1H), 6.72 (d, 2H, $J = 8.8$ Hz), 6.82 (d, 2H, $J = 8.8$ Hz), 7.29–7.37 (m, 4H), 7.76 (d, 2H, $J = 8.8$ Hz), 8.30 ppm (s, 1H); ^{13}C NMR (50.2 MHz, $CDCl_3$, 5:1 mixture of trans–cis amide rotamers, data for major rotamer): $\delta = 20.9$, 24.6, 26.2, 44.3, 51.8, 56.3, 60.9, 63.5, 65.6, 106.9, 114.2, 115.6, 123.0, 127.4, 127.8, 129.1, 130.1, 144.0, 153.4, 153.6, 160.9, 165.9, 168.0, 170.2, 190.7 ppm; HRMS: calcd for $C_{32}H_{35}N_2O_{10}$ $[M+H]^+$ 607.2286, $C_{32}H_{34}N_2O_{10}Na$ $[M+Na]^+$ 629.2106, found 607.2279, 629.2084; MS (ESI): *m/z* 607.3 $[M+H]^+$, 629.4 $[M+Na]^+$, 1214.0 $[2M+H]^+$; FTIR (thin film): 1736, 1636, 1504, 1450, 1327, 1242, 1119, 995, 918, 833, 756 cm^{-1}; $[\alpha]_D^{20} = -5.2^\circ$ ($c = 10$ mg mL^{-1}, $CHCl_3$); TLC: $R_f = 0.25$ (75% toluene/acetone).

(*S*)-*tert*-Butyl-*N*-(2-oxo-2-(3,4,5-trimethoxyphenyl)acetyl)proline (18). Starting from L-proline *tert*-butyl ester (3.2 g, 18.7 mmol) and **15** (6.75 g, 28.1 mmol), **18** was synthesized according to general procedure E. Flash chromatography (75% petrol ether/ethyl acetate) provided **18** as a light-yellow oil (5.89 g, 14.96 mmol, 80%). ^1H NMR (200 MHz, $[D_6]DMSO$, 6:1 mixture of trans–cis amide rotamers, data for major rotamer): $\delta = 1.44$ (s, 9H), 1.82–2.00 (m, 3H), 2.20–2.35 (m, 1H), 3.30–3.40 (m, 2H), 3.78 (s, 3H), 3.86 (s, 6H), 4.40–4.50 (m, 1H), 7.34 ppm (s, 2H); ^{13}C NMR (50.2 MHz, $[D_6]DMSO$, 6:1 mixture of trans–cis amide rotamers, data for major rotamer): $\delta = 24.2$, 27.6, 28.6, 46.8, 56.1, 58.5, 60.3, 81.3, 106.9, 127.4, 153.2, 164.8, 170.7, 190.3 ppm; HRMS: calcd for $C_{20}H_{28}NO_7$ $[M+H]^+$ 394.1860, $C_{20}H_{27}NO_7Na$ $[M+Na]^+$ 416.1680, found 394.1859, 416.1668; MS (ESI): *m/z* 394.2 $[M+H]^+$, 787.7 $[2M+H]^+$; FTIR (thin film): 1728, 1643, 1582, 1504, 1412, 1327, 1227, 1119, 995, 918, 833, 756, 718, 640 cm^{-1}; $[\alpha]_D^{20} = +17.1^\circ$ ($c = 10$ mg mL^{-1}, $CHCl_3$); TLC: $R_f = 0.53$ low running rotamer, 0.63 high running rotamer (80% toluene/acetone).

***N*-(2-Oxo-2-(3,4,5-trimethoxyphenyl)acetyl)proline (19)**. TFA (8.14 g, 5.5 mL, 71.4 mmol) was added at room temperature to a solution of **18** (2.4 g, 6.1 mmol) in dichloromethane (20 mL). The solution was stirred until TLC (75% toluene/acetone) indicated complete removal of the *tert*-butyl group (~8 h). $NaHCO_3$ (1 N, 100 mL) was added slowly over a period of 10 min at room tem-

perature. After gas formation had stopped, the mixture was transferred with EtOAc (50 mL) to a separatory funnel. The organic layer was discarded, and the aqueous layer was acidified carefully with 2 N HCl. The aqueous layer was extracted twice with EtOAc (500 mL), and the organic layer was subsequently dried over $MgSO_4$ and concentrated in vacuo. Flash chromatography (toluene/acetone/methanol 80:20:0→50:25:25 *v/v/v*) provided **19** as white foam (2.02 g, 5.98 mmol, 98%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.95–2.10 (m, 2H), 2.15–2.36 (m, 2H), 3.45–3.65 (m, 2H), 3.89 (s, 6H), 3.93 (s, 3H), 4.70 (dd, 1H, J = 4.0, 8.8 Hz), 7.35 (s, 2H), 10.35 ppm (br s, 1H); ^{13}C NMR (50.2 MHz, $CDCl_3$): δ = 24.5, 28.9, 47.3, 56.2, 58.1, 60.8, 107.1, 127.5, 144.0, 153.3, 165.7, 175.3, 189.8 ppm; HRMS: calcd for $C_{16}H_{20}NO_7$ $[M+H]^+$ 338.1234, $C_{16}H_{19}NO_7Na$ $[M+Na]^+$ 360.1054, found 338.1234, 360.1044; MS (ESI): *m/z* 338.0 $[M+H]^+$; FTIR (thin film): 1733, 1670, 1635, 1578, 1456, 1416, 1327, 1119, 995, 864, 748 cm^{-1}; $[\alpha]_D^{20}$ = +13.8° (*c* = 10 mg mL^{-1}, $CHCl_3$); TLC: R_f = 0.0–0.3 (75% toluene/acetone).

(*S*)-Prop-2-ynyl-1-(2-oxo-2-(3,4,5-trimethoxyphenyl)acetyl)pyrrolidine-2-carboxylate (21). Starting from **19** (500 mg, 1.48 mmol) and propargyl alcohol (260 μL, 4.5 mmol), **21** was synthesized according to general procedure C. Flash chromatography (99→70% toluene/acetone) provided **21** as an off-white solid (538 mg, 1.45 mmol, 97%). ^{1}H NMR (200 MHz, $CDCl_3$, 5:1 mixture of trans–cis amide rotamers, data for major rotamer): δ = 1.98–2.40 (m, 6H), 2.51 (t, 1H, J = 2.5 Hz), 3.51–3.63 (m, 2H), 3.94 (s, 9H), 4.53–4.92 (m, H), 7.39 ppm (s, 2H); ^{13}C NMR (50.2 MHz, $CDCl_3$, 5:1 mixture of trans–cis amide rotamers, data for major rotamer): δ = 22.0, 28.6, 46.9, 52.5, 56.1, 57.8, 59.0, 60.6, 75.2, 107.0, 127.4, 153.1, 165.3, 170.4, 189.9 ppm; HRMS: calcd for $C_{19}H_{22}NO_7$ $[M+H]^+$ 376.11391, $C_{19}H_{21}NO_7Na$ $[M+Na]^+$ 398.1210, found 376.1383, 398.1200; MS (ESI): *m/z* 376.0 $[M+H]^+$, 398.0 $[M+Na]^+$, 751.3 $[2M+H]^+$, 773.3 $[2M+Na]^+$; FTIR (thin film): 1744, 1628, 1582, 1504, 1450, 1412, 1327, 1165, 1042, 1003, 918, 872, 718 cm^{-1}; $[\alpha]_D^{20}$ = +12.6° (*c* = 10 mg mL^{-1}, $CHCl_3$); TLC: R_f = 0.44 (86% toluene/acetone).

(*S*)-1-(2-Acetylpyrrolidin-1-yl)-2-(3,4,5-trimethoxyphenyl)ethane-1,2-dione (25). TFA (6 mL) was added to a solution of **24**[38] (0.85 g, 4 mmol) in dichloromethane (10 mL), and the reaction was stirred at room temperature until TLC (90% toluene/acetone) indicated complete removal of the Boc group (~2 h). The mixture was concentrated in vacuo to afford the TFA salt (the free amine is volatile and tends to polymerize), which was subsequently redissolved in dry dichloromethane (50 mL). **15** (2.88 g, 12 mmol) and EDC·HCl (1.53 g, 8 mmol) were added, and the mixture was stirred at room temperature for 16 h. The mixture was transferred with dichloromethane (50 mL) to a separatory funnel and washed twice with 1 N $NaHCO_3$, once with water (50 mL), once with 10% citric acid (50 mL), and once with brine (50 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. After flash chromatography (80% toluene/acetone) **25** was obtained as a colorless oil (0.74 g, 2.2 mmol, 55%). ^{1}H NMR (500 MHz, $CDCl_3$, 5:1 mixture of trans–cis amide rotamers, data for major rotamer): δ = 1.90–2.03 (m, 4H), 2.31 (s, 3H), 3.45–3.49 (m, 1H), 3.55–3.59 (m, 1H), 3.95 (s, 3H), 3.98 (s, 6H), 4.78–4.81 (m, 1H), 7.42 ppm (s, 2H); ^{13}C NMR (50.2 MHz, $CDCl_3$, 5:1 mixture of trans–cis amide rotamers, data for major rotamer): δ = 24.4, 27.3, 27.7, 47.2, 56.4, 61.0, 64.6, 107.3, 127.8, 143.9, 153.5, 165.4, 190.6, 204.6 ppm; HRMS: calcd for $C_{17}H_{22}NO_6$ $[M+H]^+$ 336.1442, found 336.1435; MS (ESI): *m/z* 336.2 $[M+H]^+$, 357.9 $[M+Na]^+$, 671.2 $[2M+H]^+$, 693.2 $[2M+Na]^+$; FTIR (thin film): 1720, 1634, 1582, 1412, 1327, 1119, 995, 748, 710 cm^{-1}; $[\alpha]_D^{20}$ = +44.1° (*c* = 10 mg mL^{-1}, $CHCl_3$); TLC: R_f = 0.27 (50% toluene/ethyl acetate).

4-(*N*-Benzyloxycarbonyl-2-aminoethyl-1-oxy)-*N*-[4-(*tert*-butyldimethylsilyloxy)phenyl]benzamide (39a). Starting from *N*-benzyloxycarbonyl-2-aminoethan-1-ol (3.32 g, 17 mmol) and **8** (2.92 g, 8.5 mmol), **39a** was synthesized according to general procedure A. After flash chromatography (91% toluene/acetone) and crystallization from EtOAc/petroleum ether at a temperature of −20 °C for 16 h, **39a** was obtained as white crystals (2.73 g, 5.24 mmol, 62%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.19 (s, 6H), 0.99 (s, 9H), 3.52–3.65 (m, 2H), 4.04 (t, 2H, J = 5.1 Hz), 5.11 (s, 2H), 5.34 (br s, 1H), 6.75–6.91 (m, 4H), 7.27 (br s, 5H), 7.48 (d, 2H, J = 8.8 Hz), 7.79 (d, 2H, J = 8.5 Hz), 7.94 ppm (s, 1H); ^{13}C NMR (50.2 MHz, $CDCl_3$): δ = −4.5, 14.3, 18.1, 25.7, 40.4, 67.0, 114.2, 120.3, 121.9, 127.7, 128.1, 128.2, 128.5, 128.9, 131.8, 136.3, 152.3, 156.4, 161.1, 165.1 ppm; HRMS: calcd for $C_{29}H_{37}N_2O_5Si$ $[M+H]^+$ 521.2466, found 521.2453; MS (ESI): *m/z* 521.3 $[M+H]^+$, 1040.9 $[2M+H]^+$; FTIR (thin film): 1699, 1647, 1614, 1541, 1506, 1246, 1219, 914, 833, 777 cm^{-1}; TLC: R_f = 0.43 (90% toluene/acetone).

4-(*N*-Benzyloxycarbonyl-5-aminopent-1-oxy)-*N*-[4-(*tert*-butyldimethylsilyloxy)phenyl]benzamide (39b). Starting from *N*-benzyloxycarbonyl-5-aminopentan-1-ol (1.38 g, 5.8 mmol) and **8** (1.0 g, 2.9 mmol), **39b** was synthesized according to general procedure A. Flash chromatography (91% toluene/acetone) provided **39b** as a white foam (0.9 g, 1.6 mmol, 55%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.19 (s, 6H), 0.99 (s, 9H), 1.40–1.60 (m, 4H), 1.70–1.90 (m, 2H), 3.15–3.30 (m, 2H), 3.96 (t, 2H, J = 6.2 Hz), 4.93 (br s, 1H), 5.09 (s, 2H), 6.78–6.91 (m, 4H), 7.34 (s, 5H), 7.48 (d, 2H, J = 8.8 Hz), 7.79 (d, 2H, J = 8.8 Hz), 7.97 ppm (s, 1H); ^{13}C NMR (50.2 MHz, $CDCl_3$): δ = −4.5, 18.3, 23.2, 25.7, 28.7, 29.7, 40.9, 66.6, 67.8, 114.3, 120.3, 121.9, 127.1, 128.0, 128.5, 128.9, 131.9, 152.3, 156.5, 161.7, 165.2 ppm; HRMS: calcd for $C_{32}H_{43}N_2O_5Si$ $[M+H]^+$ 563.2936, found 563.2921; MS (ESI): *m/z* 563.4 $[M+H]^+$; FTIR (thin film): 1686, 1649, 1609, 1508, 1252, 1227, 1178, 1138, 1109, 916, 831, 779, 762 cm^{-1}; TLC: R_f = 0.36 (90% toluene/acetone).

4-(2-Aminoethoxy)-*N*-[4-(*tert*-butyldimethylsilyloxy)phenyl]benzamide (40a). Starting from **39a** (2.5 g, 4.80 mmol), **40a** was synthesized according to general procedure B. **40a** was obtained as a white solid (1.84 g, 4.75 mmol, 99%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.17 (s, 3H), 0.96 (s, 9H), 1.81 (br s, 2H), 3.00–3.10 (m, 2H), 3.96 (t, 2H, J = 4.9 Hz), 6.78 (d, 2H, J = 8.8 Hz), 6.85 (d, 2H, J = 8.8 Hz), 7.46 (d, 2H, J = 8.8 Hz), 7.77 (d, 2H, J = 8.8 Hz), 8.22 ppm (s, 1H); ^{13}C NMR (50.2 MHz, $CDCl_3$): δ = −4.7, 17.9, 25.4, 41.0, 69.7, 113.9, 119.9, 122.0, 127.2, 128.9, 131.9, 152.0, 161.2, 165.5 ppm; HRMS: calcd for $C_{21}H_{31}N_2O_3Si$ $[M+H]^+$ 387.2099, found 387.2084; MS (ESI): *m/z* 387.1 $[M+H]^+$, 773.4 $[2M+H]^+$; FTIR (thin film): 2970, 2932, 2160, 1643, 1605, 1504, 1250, 1180, 1049, 903, 833, 779 cm^{-1}; TLC: R_f = 0.09 (67% toluene/acetone).

4-(5-Aminopentoxy)-*N*-[4-(*tert*-butyldimethylsilyloxy)phenyl]benzamide (40b). Starting from **39b** (390 mg, 0.69 mmol), **40b** was synthesized according to general procedure B. **40b** was obtained as an oil (281 mg, 0.66 mmol, 95%) and was directly converted into **49**.

4-(4-(Phthalimidoxy)butoxy)-*N*-[4-(*tert*-butyldimethylsilyloxy)-phenyl]benzamide (41). Starting from **8** (0.34 g, 1.0 mmol) and *N*-(hydroxybutoxy)phthalimide (0.59 g, 2.5 mmol), **41** was synthesized according to general procedure A. Flash chromatography (96→80% toluene/acetone) provided **41** as a white waxy solid (0.39 g, 0.7 mmol, 70%). ^{1}H NMR (200 MHz, [D$_6$]DMSO): δ = 0.17 (s, 6H), 0.94 (s, 9H), 1.70–1.92 (m, 4H), 4.12 (t, 2H, J = 5.9 Hz), 4.21 (t, 2H, J = 5.9 Hz), 6.81 (d, 2H, J = 8.8 Hz), 7.05 (d, 2H, J = 8.8 Hz), 7.60 (d, 2H, J = 8.8 Hz), 7.86 (s, 5H), 7.91 (d, 2H, J = 8.8 Hz), 9.96 ppm (s, 1H); ^{13}C NMR (50.2 MHz, [D$_6$]DMSO): δ = −4.5, 19.8, 24.4, 25.6, 67.2,

77.3, 114.0, 119.6, 121.9, 123.2, 127.0, 129.4, 133.1, 134.8, 139.1, 150.9, 159.9, 163.3, 168.2 ppm; HRMS: calcd for $C_{31}H_{36}N_2O_6Si$ $[M+H]^+$ 561.2421, found 561.2399; MS (ESI): *m/z* 561.27 $[M+H]^+$, 583.33 $[M+Na]^+$, 1121.12 $[2M+H]^+$, 1143.13 $[2M+Na]^+$; FTIR (thin film): 1726, 1655, 1607, 1508, 1474, 1404, 1252, 1225, 1184, 1034, 914, 835, 779 cm^{-1}; TLC: $R_f=0.75$ (50% toluene/ethyl acetate).

4-[4-Aminooxybutoxy]-*N*-[4-(*tert*-butyldimethylsilyloxy)phenyl]-benzamide (42). A mixture of **41** (330 mg, 0.59 mmol) and hydrazine hydrate (23.1 mg, 0.71 mmol) was heated at reflux for 5 h in EtOAc (8 mL). The precipitate was removed by filtration, and the clear filtrate was added directly to **25** in the next reaction step (see synthesis of **50**); TLC: $R_f=0.16$ (50% toluene/ethyl acetate).

4-[2-(2-(Tritylthio)acetamido)ethoxy)]-*N*-[4-(*tert*-butyldimethylsilyloxy)phenyl]benzamide (43). Starting from **40a** (334 mg, 1 mmol) and **37** (503 mg, 1.3 mmol), **43** was synthesized according to general procedure E. Flash chromatography (66% petroleum ether/ethyl acetate) provided **43** as a white solid (580 mg, 0.83 mmol, 83%). ^{1}H NMR (200 MHz, $CDCl_3$): $\delta=0.20$ (s, 6H), 0.99 (s, 9H), 3.15 (s, 2H), 3.34–3.42 (m, 2H), 3.90 (t, 2H, $J=4.9$ Hz), 6.46 (br s, 1H), 6.81–6.93 (m, 4H), 7.20–7.50 (m, 17H), 7.68 (s, 1H), 7.82 ppm (d, 2H, $J=8.8$ Hz). ^{13}C NMR (50.2 MHz, $CDCl_3$): $\delta=-4.5$, 18.1, 25.6, 35.8, 38.9, 66.4, 67.8, 114.2, 120.3, 121.8, 127.0, 127.7, 128.1, 128.9, 129.4, 131.8, 143.9, 152.3, 161.0, 165.0, 168.3 ppm; HRMS: calcd for $C_{42}H_{46}N_2O_4SSi$ $[M+H]^+$ 703.3026, found 703.3006; MS (ESI): *m/z* 725.33 $[M+Na]^+$, 1427.93 $[2M+Na]^+$; FTIR (thin film): 1648, 1627, 1600, 1506, 1277, 1252, 1178, 924, 908, 835, 746, 702 cm^{-1}; TLC: $R_f=0.75$ (66% toluene/acetone).

4-[2-(2-(Thio)acetamido)ethoxy)]-*N*-[4-(*tert*-butyldimethylsilyloxy)phenyl]benzamide (44). TFA (1.2 mL, 1.78 g, 15.6 mmol) was added under argon over a period of 10 min at room temperature to a mixture of **43** (1.70 g, 2.42 mmol) and triethylsilane (0.31 g, 2.66 mmol) in dichloromethane (40 mL). After 8 h, TLC (66% toluene/acetone) indicated complete removal of the trityl group, and 1 N $NaHCO_3$ (20 mL) was added carefully to the solution. The mixture was transferred with dichloromethane (60 mL) to a separatory funnel, and water (20 mL) was added to improve phase separation. The organic layer was separated and washed with brine (30 mL). After drying over Na_2SO_4 the mixture was concentrated in vacuo, and **44** was obtained by crystallization for 3 h at a temperature of $-20\,^\circ$C from EtOAc/petroleum ether as white crystals (1.01 g, 2.20 mmol, 91%). ^{1}H NMR (200 MHz, [D$_6$]DMSO): $\delta=0.17$ (s, 6H), 0.94 (s, 9H), 2.74 (t, 1H, $J=7.7$ Hz), 3.13 (d, 2H, $J=7.7$ Hz), 3.46 (q, 2H, $J=5.5$ Hz), 4.08 (t, 2H, $J=5.5$ Hz), 6.81 (d, 2H, $J=8.8$ Hz), 7.05 (d, 2H, $J=8.8$ Hz), 7.61 (d, 2H, $J=8.8$ Hz), 7.93 (d, 2H, $J=8.8$ Hz), 8.30 (t, 1H, $J=5.5$ Hz), 9.96 ppm (s, 1H); ^{13}C NMR (50.2 MHz, [D$_6$]DMSO): $\delta=-4.6$, 18.1, 25.6, 27.0, 38.5, 66.5, 114.1, 119.6, 121.9, 127.2, 129.5, 133.1, 151.0, 160.9, 164.4, 170.0 ppm; HRMS: calcd for $C_{23}H_{33}N_2O_4SSi$ $[M+H]^+$ 461.1925, found 461.1913; MS (ESI): *m/z* 461.1 $[M+H]^+$, 482.9 $[M+Na]^+$, 921.6 $[2M+H]^+$; FTIR (thin film): 1647, 1506, 1252, 1167, 1121, 907, 839 cm^{-1}; TLC: $R_f=0.37$ (66% toluene/acetone).

4-(2-Azidoethyl)-*N*-(4-hydroxyphenyl)benzamide (47). A suspension of **46** (500 mg, 2.6 mmol) in $SOCl_2$ (2 mL, 26.2 mmol) was heated at reflux for 3 h. Subsequently, the mixture was concentrated in vacuo, and the remaining residue was redissolved in dry dichloromethane (5 mL) and added dropwise at 0 °C to a suspension of *p*-aminophenol (572 mg, 5.2 mmol) in dry dichloromethane (10 mL). After 20 min, the mixture was allowed to warm to room temperature, and stirring was continued for 18 h. The mixture was transferred to a separatory funnel with EtOAc (200 mL) and was washed once with 1 N $NaHCO_3$ (50 mL), once with 2 N HCl (50 mL), and once with brine (50 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The remaining residue was purified by flash chromatography (83% toluene/acetone) to afford **47** as a yellow solid (514 mg, 1.79 mmol, 69%). ^{1}H NMR (200 MHz, [D$_6$]acetone): $\delta=2.99$ (t, 2H, $J=6.9$ Hz), 3.64 (t, 2H, $J=6.9$ Hz), 6.83 (d, 2H, $J=8.8$ Hz), 7.43 (d, 2H, $J=8.0$ Hz), 7.65 (d, 2H, $J=8.8$ Hz), 7.94 (d, 2H, $J=8.0$ Hz), 8.21 (br s, 1H), 9.33 ppm (br s, 1H); ^{13}C NMR (50.2 MHz, [D$_6$]acetone): $\delta=35.6$, 52.7, 115.8, 122.7, 128.4, 129.7, 132.4, 134.9, 143.1, 154.6, 164.4 ppm; HRMS: calcd for $C_{15}H_{14}N_4O_2$ $[M+H]^+$ 283.1190, found 283.1185; MS (ESI): *m/z* 283.13 $[M+H]^+$; FTIR (thin film): 2098, 1636, 1605, 1512, 1435, 1188, 1096, 1049, 903, 826 cm^{-1}; TLC: $R_f=0.29$ (80% toluene/acetone).

(*S*)-Pentyloxy-5-(*N'*-[4-(*tert*-butyldimethylsilyloxy)phenyl]benzamidyl)-*N*-[2-oxo-2-(3,4,5-trimethoxyphenyl)acetyl)]proline (48a). Starting from **11d** (174 mg, 0.41 mmol) and **19** (176 mg, 0.52 mmol), **48a** was synthesized according to general procedure C. Flash chromatography (91% toluene/acetone) provided **48a** as a colorless oil (300 mg, 0.40 mmol, 98%). ^{1}H NMR (200 MHz, $CDCl_3$, 7:1 mixture of trans–cis amide rotamers, data for major rotamer): $\delta=0.13$ (s, 6H), 0.92 (s, 9H), 1.40–2.05 (m, 9H), 2.19–2.30 (m, 1H), 3.40–3.52 (m, 2H), 3.85 (s, 6H), 3.87 (s, 3H), 3.86–3.94 (m, 2H), 4.08–4.22 (m, 2H), 4.50–4.58 (m, 1H), 6.70–6.80 (m, 4H), 7.33 (s, 2H), 7.46 (d, 2H, $J=8.8$ Hz), 7.73 (d, 2H, $J=8.8$ Hz), 8.38 ppm (s, 1H); ^{13}C NMR (50.2 MHz, $CDCl_3$, 7:1 mixture of trans–cis amide rotamers, data for major rotamer): $\delta=-4.7$, 22.1, 24.4, 25.4, 28.1, 28.4, 28.9, 46.9, 56.1, 58.1, 60.7, 65.0, 67.5, 107.1, 113.9, 119.9, 121.7, 126.9, 127.6, 128.8, 132.0, 143.7, 151.9, 153.2, 161.4, 165.2, 165.5, 171.2, 190.2 ppm; HRMS: calcd for $C_{40}H_{53}N_2O_{10}Si$ $[M+H]^+$ 749.3464, found 749.3457; MS (ESI): *m/z* 749.3 $[M+H]^+$, 771.2 $[M+Na]^+$; FTIR (thin film): 2939, 1736, 1643, 1504, 1458, 1412, 1327, 1250, 1119, 1003, 910, 833, 779 cm^{-1}; $[\alpha]_D^{20}=+11.9^\circ$ ($c=4.4$ mg mL^{-1}, $CHCl_3$); TLC: $R_f=0.56$ (80% toluene/acetone).

(*S*)-2'-(Ethoxy)ethoxy-2''-(*N'*-[4-(*tert*-butyldimethylsilyloxy)phenyl]benzamidyl)-*N*-[2-oxo-2-(3,4,5-trimethoxyphenyl)acetyl)]proline (48b). Starting from **11e** (153 mg, 0.35 mmol) and **19** (148 mg, 0.44 mmol), **48b** was synthesized according to general procedure C. Flash chromatography (91% toluene/acetone) provided **48b** as a colorless oil (225 mg, 0.30 mmol, 86%). ^{1}H NMR (200 MHz, $CDCl_3$, 5:1 mixture of trans–cis amide rotamers, data for major rotamer): $\delta=0.15$ (s, 3H), 0.95 (s, 9H), 1.90–2.25 (m, 4H), 3.40–3.51 (m, 2H), 3.70–3.90 (m, 4H), 3.87 (s, 6H), 3.89 (s, 3H), 4.09–4.15 (m, 2H), 4.29–4.36 (m, 2H), 4.50–4.68 (m, 1H), 6.76 (d, 2H, $J=8.8$ Hz), 6.85 (d, 2H, $J=8.8$ Hz), 7.34 (s, 2H), 7.46 (d, 2H, $J=8.8$ Hz), 7.75 (d, 2H, $J=8.8$ Hz), 8.20 ppm (s, 1H); ^{13}C NMR (50.2 MHz, $CDCl_3$, 5:1 mixture of trans–cis amide rotamers, data for major rotamer): $\delta=-4.6$, 18.0, 24.4, 25.6, 29.0, 47.1, 56.2, 58.2, 60.8, 64.2, 67.4, 69.0, 69.5, 107.1, 114.2, 120.1, 121.8, 127.4, 127.6, 128.8, 131.9, 143.8, 152.1, 152.8, 153.3, 161.2, 165.2, 165.6, 171.3, 190.3 ppm; HRMS: calcd for $C_{39}H_{51}N_2O_{11}Si$ $[M+H]^+$ 751.3257, found 751.3232; MS (ESI): *m/z* 751.4 $[M+H]^+$, 773.3 $[M+Na]^+$; FTIR (thin film): 1736, 1636, 1506, 1456, 1416, 1327, 1248, 1123, 1040, 910, 837 cm^{-1}; $[\alpha]_D^{20}=+12.0$ ($c=2$ mg mL^{-1}, $CHCl_3$); TLC: $R_f=0.52$ (80% toluene/acetone).

(*S*)-Pentyloxy-5-(*N'*-[4-(*tert*-butyldimethylsilyloxy)phenyl]benzamidyl)-*N*-[2-oxo-2-(3,4,5-trimethoxyphenyl)acetyl)]proline amide (49). Starting from **19** (209 mg, 0.62 mmol) and **40b** (291 mg, 0.68 mmol), **49** was synthesized according to general procedure C. Flash chromatography (91→75% toluene/acetone) provided **49** as a colorless oil (388 mg, 0.52 mmol, 84%). ^{1}H NMR (200 MHz, $CDCl_3$, 6:1 mixture of trans–cis amide rotamers, data for major rotamer): $\delta=0.18$ (s, 6H), 0.97 (s, 9H), 1.49–1.65 (m, 4H), 1.70–1.85 (m, 2H), 2.05–2.30 (m, 4H), 3.23–3.32 (m, 2H), 3.42–3.54

(m, 2H), 3.82–4.00 (m, 2H), 3.89 (s, 6H), 3.91 (s, 3H), 4.50–4.56 (m, 1H), 6.60 (brt, 1H, $J=5.5$ Hz), 6.79–6.86 (m, 4H), 7.30 (s, 2H), 7.48 (d, 2H, $J=8.8$ Hz), 7.73 (d, 2H, $J=8.8$ Hz), 7.94 ppm (s, 1H); ^{13}C NMR (50.2 MHz, $CDCl_3$, 6:1 mixture of trans–cis amide rotamers, data for major rotamer): $\delta=-4.5$, 23.2, 24.8, 25.6, 26.2, 28.6, 29.2, 39.4, 47.6, 56.3, 59.9, 61.0, 67.8, 107.2, 114.2, 120.3, 121.8, 127.0, 127.6, 128.7, 131.9, 152.3, 153.4, 161.7, 165.2, 165.5, 170.7, 190.2 ppm; HRMS: calcd for $C_{40}H_{54}N_3O_9Si$ $[M+H]^+$ 748.3624, $C_{40}H_{53}N_3O_9SiNa$ $[M+Na]^+$ 770.3443, found 748.3613, 770.3427; MS (ESI): m/z 748.3 $[M+H]^+$, 770.4 $[M+Na]^+$; FTIR (thin film): 2939, 1736, 1643, 1504, 1458, 1412, 1327, 1250, 1119, 1003, 910, 833, 779 cm^{-1}; $[\alpha]_D^{20}=-12.5^\circ$ ($c=2$ mg mL^{-1}, $CHCl_3$); TLC: $R_f=0.63$ (60% toluene/acetone).

(*S*,*E*/*Z*)-*N*-(4-(*tert*-butyldimethylsilyloxy)phenyl)-4-(4-(1-(1-(2-oxo-2-(3,4,5-trimethoxyphenyl)acetyl)pyrrolidin-2-yl)ethylideneaminooxy)butoxy)benzamide (50). A freshly prepared solution of **42** (254 mg, 0.59 mmol) in ethyl acetate (10 mL) was directly filtered at room temperature into a flask containing **25** (296 mg, 0.88 mmol), and subsequently acetic acid (5 mL) was added. Stirring was continued at room temperature for 16 h. The solution was transferred to a separatory funnel, extracted three times with 1 N $NaHCO_3$ (3×20 mL) and washed with brine (30 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The remaining residue was purified by flash chromatography (66% toluene/ethyl acetate) to afford **50** as a white foam (250 mg, 0.33 mmol, 57%) being an inseparable mixture of rotamers and *E*/*Z* isomers. ^{1}H NMR (500 MHz, $CDCl_3$, data for major isomer): $\delta=0.19$ (s, 6H), 0.98 (s, 9H), 1.7–2.3 (m, 11H), 3.4–3.72 (m, 2H), 3.86 (s, 6H), 3.93 (s, 3H), 3.99–4.07 (m, 2H), 4.12–4.19 (m, 2H), 4.66–4.78 (m, 1H), 6.80–6.83 (m, 2H), 6.85–6.90 (m, 2H), 7.28 (s, 2H), 7.47–7.50 (m, 2H), 7.78–7.83 (m, 2H), 7.99 ppm (brs, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$, data for major isomer): $\delta=-4.5$, 11.8, 18.1, 22.2, 25.6, 25.7, 29.2, 47.4, 56.2, 60.2, 60.7, 67.7, 73.2, 107.7, 114.2, 120.2, 121.8, 127.0, 128.3, 128.8, 131.9, 149.0, 152.1, 153.3, 155.5, 161.6, 165.1, 189.0 ppm; HRMS: calcd for $C_{40}H_{54}N_3O_9Si$ $[M+H]^+$ 748.3624, $C_{40}H_{53}N_3O_9SiNa$ $[M+Na]^+$ 770.3443, found 748.3620, 770.3422; MS (ESI): m/z 748.2 $[M+H]^+$, 770.3 $[M+Na]^+$, 1517.9 $[2M+Na]^+$; FTIR (thin film): 1636, 1504, 1458, 1412, 1327, 1234, 1165, 1049, 910, 833, 752 cm^{-1}; $[\alpha]_D^{20}=-42.0^\circ$ ($c=10$ mg mL^{-1}, $CHCl_3$); TLC: $R_f=0.55$ (50% toluene/ethyl acetate).

(*S*)-*S*-2-(2-(4-(4-(*tert*-butyldimethylsilyloxy)phenylcarbamoyl)phenoxy)ethylamino)-2-oxoethyl-1-(2-oxo-2-(3,4,5-trimethoxyphenyl)acetyl)pyrrolidine-2-carbothioate (51). Starting from **44** (880 mg, 1.91 mmol) and **19** (810 mg, 2.4 mmol), **51** was synthesized according to general procedure C. Flash chromatography (75% toluene/acetone) provided **51** as a colorless foam (975 mg, 1.25 mmol, 65%). ^{1}H NMR (500 MHz, $CDCl_3$, 5:4 mixture of trans–cis amide rotamers, data for major rotamer): $\delta=0.20$ (s, 6H), 0.99 (s, 9H), 1.95–2.25 (m, 4H), 3.48–3.74 (m, 4H), 3.76–3.83 (m, 2H), 3.84–3.95 (m, 9H), 3.96–4.13 (m, 2H), 4.58–4.61 (m, 1H), 6.75–6.90 (m, 4H), 6.94 (brs, 1H), 7.33 (s, 2H), 7.48–7.52 (m, 2H), 7.70 (d, 2H, $J=8.8$ Hz), 7.92 ppm (s, 1H); ^{13}C NMR (50.2 MHz, $CDCl_3$, 5:4 mixture of trans–cis amide rotamers, data for major rotamer): $\delta=-4.7$, 18.0, 24.5, 25.5, 29.5, 32.0, 38.7, 47.4, 56.1, 60.8, 65.4, 107.1, 113.9, 120.0, 121.7, 127.3, 127.5, 128.9, 132.0, 143.8, 152.0, 153.3, 160.8, 165.4, 167.3, 171.0, 190.3, 198.0 ppm; HRMS: calcd for $C_{39}H_{50}N_3O_{10}SSi$ $[M+H]^+$ 780.2981, found 780.2964; MS (ESI): m/z 780.3 $[M+H]^+$, 802.3 $[M+Na]^+$; FTIR (thin film): 1638, 1508, 1458, 1414, 1327, 1229, 1167, 1124, 997, 908, 841 cm^{-1}; $[\alpha]_D^{20}=+10.2^\circ$ ($c=10$ mg mL^{-1}, $CHCl_3$); TLC: $R_f=0.67$ (60% toluene/acetone).

(*S*)-Pentyloxy-5-(*N*′-[4-(hydroxy)phenyl]benzamidyl)-*N*-[2-oxo-2-(3,4,5-trimethoxyphenyl)acetyl)]proline (52a). Starting from **48a** (260 mg, 0.35 mmol), **52a** was synthesized according to general procedure F. Flash chromatography (80→75% toluene/acetone 4:1) provided **52s** as a colorless oil (190 mg, 0.30 mmol, 86%). ^{1}H NMR (500 MHz, $CDCl_3$, 5:1 mixture of trans–cis amide rotamers, data for major rotamer): $\delta=1.54$–1.60 (m, 2H), 1.73–1.84 (m, 4H), 1.97–2.09 (m, 3H), 2.29–2.33 (m, 1H), 3.51–3.58 (m, 2H), 3.91 (s, 6H), 3.93 (s, 3H), 3.97 (t, 2H, $J=6.4$ Hz), 4.13–4.16 (m, 1H), 4.27–4.31 (m, 1H), 4.64 (dd, 1H, $J=3.9$, 8.9 Hz), 6.77 (d, 2H, $J=8.8$ Hz), 6.86 (d, 2H, $J=8.8$ Hz), 7.38 (d, 2H, $J=8.8$ Hz), 7.39 (s, 2H), 7.75 (d, 2H, $J=8.8$ Hz), 7.94 ppm (s, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$, 5:1 mixture of trans–cis amide rotamers, data for major rotamer): $\delta=$ 22.4, 24.6, 28.3, 28.6, 29.1, 47.2, 56.4, 58.4, 61.0, 65.3, 67.8, 107.3, 114.3, 115.7, 122.7, 126.8, 127.7, 128.9, 130.5, 147.5, 153.3, 153.4, 161.8, 165.8, 171.5, 190.3 ppm; HRMS: calcd for $C_{34}H_{39}N_2O_{10}$ $[M+H]^+$ 635.2599, $C_{34}H_{38}N_2O_{10}Na$ $[M+Na]^+$ 657.2419, found 635.2588, 657.2383; MS (ESI): m/z found: 635.2 $[M+H]^+$, 657.3 $[M+Na]^+$, 1291.0 $[2M+H]^+$; FTIR (thin film): 1736, 1636, 1504, 1450, 1327, 1242, 1119, 1049, 1003, 833, 748 cm^{-1}; $[\alpha]_D^{20}=+12.5^\circ$ ($c=$ 1.67 mg mL^{-1}, $CHCl_3$); TLC: $R_f=0.33$ (75% toluene/acetone).

(*S*)-2′-(Ethoxy)ethoxy-2′′-(*N*′-[4-(hydroxy)phenyl]benzamidyl)-*N*-[2-oxo-2-(3,4,5-trimethoxyphenyl)acetyl)]proline (52b). Starting from **48b** (120 mg, 0.16 mmol), **52b** was synthesized according to general procedure F. Flash chromatography (66% toluene/acetone) provided **52b** as a colorless oil (76 mg, 0.12 mmol, 75%). ^{1}H NMR (500 MHz, $CDCl_3$, 5:1 mixture of trans–cis amide rotamers, data for major rotamer): $\delta=1.93$–2.00 (m, 2H), 2.04–2.08 (m, 1H), 2.20–2.29 (m, 1H), 3.44–3.55 (m, 2H), 3.76–3.78 (m, 2H), 3.83–3.85 (m, 2H), 3.89 (s, 6H), 3.92 (s, 3H), 4.08–4.10 (m, 2H), 4.29–4.39 (m, 2H), 4.61–4.65 (m, 1H), 6.73 (d, 2H, $J=8.8$ Hz), 6.82 (d, 2H, $J=8.8$ Hz), 7.28–7.36 (m, 4H), 7.42 (brs, 1H), 7.74 (d, 2H, $J=8.8$ Hz), 8.31 ppm (s, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$, 5:1 mixture of trans–cis amide rotamers, data for major rotamer): $\delta=24.5$, 29.0, 47.2, 56.3, 58.3, 60.9, 64.3, 67.4, 69.1, 69.5, 107.2, 114.3, 115.6, 122.9, 127.1, 127.6, 128.9, 130.3, 143.9, 152.9, 153.4, 153.5, 161.3, 165.8, 171.3, 190.3 ppm; HRMS: calcd for $C_{33}H_{37}N_2O_{11}$ $[M+H]^+$ 637.2392, found 637.2374; MS (ESI): m/z 637.3 $[M+H]^+$, 659.4 $[M+Na]^+$; FTIR (thin film): 1736, 1636, 1504, 1450, 1327, 1242, 1119, 1049, 1003, 833, 748 cm^{-1}; $[\alpha]_D^{20}=+13.1^\circ$ ($c=10$ mg mL^{-1}, $CHCl_3$); TLC: $R_f=0.32$ (66% toluene/acetone).

(*S*)-Pentyloxy-5-(*N*′-[4-(hydroxy)phenyl]benzamidyl)-*N*-[2-oxo-2-(3,4,5-trimethoxyphenyl)acetyl)]proline amide (53). Starting from **49** (148 mg, 0.2 mmol), **53** was synthesized according to general procedure F. Flash chromatography (60% toluene/acetone) provided **53** as a colorless oil (89 mg, 0.14 mmol, 70%). ^{1}H NMR (500 MHz, $CDCl_3$, 7:1 mixture of trans–cis amide rotamers, data for major rotamer): $\delta=1.30$–1.55 (m, H), 1.56–1.70 (m, 2H), 1.80–1.90 (m, 1H), 2.00–2.15 (m, 2H), 3.12–3.30 (m, 2H), 3.44–3.55 (m, 2H), 3.68–3.80 (m, 2H), 3.86 (s, 6H), 3.89 (s, 3H), 4.44–4.54 (m, 1H), 6.65 (d, 2H, $J=8.8$ Hz), 6.72 (s, 2H, $J=8.8$ Hz), 6.96 (s, 1H), 7.22–7.40 (m, 4H), 7.66 (d, 2H, $J=8.8$ Hz), 8.18 (brs, 1H), 8.55 ppm (s, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$, 7:1 mixture of trans–cis amide rotamers, data for major rotamer): $\delta=23.1$, 24.8, 28.5, 29.0, 29.2, 39.5, 47.7, 56.3, 59.9, 60.9, 67.7, 107.3, 114.1, 115.5, 123.0, 126.4, 127.4, 129.0, 130.3, 144.1, 153.4, 153.7, 161.6, 166.1, 171.1, 190.3 ppm; HRMS: calcd for $C_{34}H_{40}N_3O_9$ $[M+H]^+$ 634.2759, found 634.2757; MS (ESI): m/z 634.4 $[M+H]^+$, 656.4 $[M+Na]^+$; FTIR (thin film): 1736, 1636, 1506, 1456, 1418, 1327, 1242, 1123, 999, 910, 837 cm^{-1}; $[\alpha]_D^{20}=-13.1^\circ$ ($c=10$ mg mL^{-1}, $CHCl_3$); TLC: $R_f=0.32$ (67% toluene/acetone).

(*S*,*E*/*Z*)-*N*-(4-hydroxyphenyl)-4-(4-(1-(1-(2-oxo-2-(3,4,5-trimethoxyphenyl)acetyl)pyrrolidin-2-yl)ethylideneaminooxy)butoxy)benzamide (54). Starting from **50** (150 mg, 0.2 mmol), **54** was synthe-

sized according to general procedure F. Flash chromatography (66% toluene/acetone) provided **54** as a colorless foam (76 mg, 0.12 mmol, 60%) being an inseparable mixture of rotamers and *E*/*Z* isomers. ^{1}H NMR (500 MHz, $CDCl_3$, data for major isomer): $\delta = 1.75$–2.3 (m, 8H), 1.88 (s, 3H), 3.40–3.60 (m, 2H), 3.85 (s, 6H), 3.92 (m, H), 3.95–4.02 (m, 2H), 4.11–4.17 (m, 2H), 4.69–4.77 (m, 1H), 6.76–6.79 (m, 2H), 6.83–6.89 (m, 2H), 7.27 (s, 2H), 7.36–7.40 (m, 2H), 7.75–7.80 ppm (m, 2H), 8.05 (br s, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$, data for major isomer): $\delta = 11.8$, 24.1, 25.5, 30.9, 47.5, 56.3, 60.4, 60.8, 67.1, 73.2, 107.8, 114.3, 115.7, 122.8, 126.7, 127.7, 128.9, 130.4, 152.9, 153.3, 155.3, 161.8, 165.1, 165.2, 165.7, 189.9 ppm; HRMS: calcd for $C_{34}H_{40}N_3O_9$ $[M+H]^+$ 634.2759, found 634.2750; MS (ESI): *m*/*z* 634.4 $[M+H]^+$, 656.3 $[M+Na]^+$; FTIR (thin film): 1605, 1504, 1412, 1327, 1234, 1165, 1096, 1049, 995, 83, 748 cm^{-1}; $[\alpha]_D^{20} = -47.8^\circ$ ($c = 4.0$ mg mL^{-1}, $CHCl_3$); TLC: $R_f = 0.33$ (33% toluene/ethyl acetate).

(*S*)-*S*-2-(2-(4-(4-hydroxyphenylcarbamoyl)phenoxy)ethylamino)-2-oxoethyl-1-(2-oxo-2-(3,4,5-trimethoxyphenyl)acetyl)pyrrolidine-2-carbothioate (55). Starting from **51** (250 mg, 0.32 mmol) and employing a substoichiometric amount of TBAF (64 μL, 1 M in THF, 0.064 mmol), **55** was synthesized according to general procedure F. Flash chromatography (60% toluene/acetone) provided **55** as a white solid (32 mg, 0.048 mmol, 15%). ^{1}H NMR (500 MHz, $CDCl_3$, 5:1 mixture of trans–cis amide rotamers, data for major rotamer): $\delta = 1.80$–2.20 (m, 4H), 3.45–3.85 (m, 8H), 3.87 (s, 6H), 3.90 (s, 3H), 4.54 (m, 1H), 6.61 (d, 2H, $J = 8.8$ Hz), 6.67 (d, 2H, $J = 8.8$ Hz), 7.15 (m, 1H), 7.26 (s, 2H), 7.35 (m, 2H), 7.62 (d, 2H, $J = 8.8$ Hz), 8.52 ppm (br s, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$, 5:1 mixture of trans–cis amide rotamers, data for major rotamer): $\delta = 24.8$, 29.3, 39.0, 46.4, 47.8, 56.4, 59.9, 61.0, 66.4, 107.3, 114.2, 115.5, 123.2, 127.0, 127.5, 129.1, 130.3, 144.1, 153.5, 153.6, 161.1, 166.2, 171.3, 190.3, 191.8 ppm; FTIR (thin film): 1636, 1605, 1508, 1416, 1327, 1234, 1161, 1096, 1053, 829 cm^{-1}; $[\alpha]_D^{20} = -4.4^\circ$ ($c = 1.5$ mg mL^{-1}, $CHCl_3$); TLC: $R_f = 0.39$ (60% toluene/acetone).

(*S*)-(1-(4-(*p*-Hydroxyphenylcarbamoyl)phenethyl)-1*H*-1′,2′,3′-triazol-4′-yl)methyl-*N*-(2″-(3,4,5-trimethoxyphenyl)-2″-oxoacetyl)pyrrolidine-2‴-carboxylate (56). Sodium ascorbate (40 mg, 0.2 mmol) and $CuSO_4 \cdot 5H_2O$ (5 mg, 0.02 mmol) were added to a suspension of **21** (133 mg, 0.35 mmol) and **47** (100 mg, 0.35 mmol) in H_2O/*t*BuOH (2 mL, 2:1 *v*/*v*). The heterogeneous mixture was stirred vigorously for 27 h until TLC (83% toluene/acetone) indicated complete consumption of the starting materials. The reaction mixture was transferred to a separatory funnel with EtOAc and water and was extracted twice with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The remaining residue was purified by column chromatography (83→50% toluene/acetone) to afford **56** as a white foam (168.6 mg, 0.26 mmol, 73%). ^{1}H NMR (200 MHz, $CDCl_3$, 5:1 mixture of trans–cis amide rotamers, data for major rotamer): $\delta = 1.93$–2.04 (m, 6H), 3.15 (t, 2H, $J = 6.6$ Hz), 3.49 (t, 2H, $J = 6.6$ Hz), 3.86 (s, 6H), 3.91 (s, 3H), 4.53–4.60 (m, 2H), 5.22 (ddd, 1H, $J = 11.9$, 11.9, 12.1 Hz), 6.68 (d, 2H, $J = 8.4$ Hz), 6.97 (d, 2H, $J = 8.0$ Hz), 7.15–7.32 (m, 4H), 7.47 (s, 1H), 7.69 ppm (d, 2H, $J = 8.0$ Hz, 8.72 (br s, 1H); ^{13}C NMR (50.2 MHz, $CDCl_3$, 5:1 mixture of trans–cis amide rotamers, data for major rotamer): $\delta = 24.4$, 28.7, 35.9, 47.1, 50.9, 56.1, 58.2, 58.4, 60.7, 107.0, 115.3, 122.8, 127.2, 127.5, 128.5, 129.9, 133.2, 140.4, 142.0, 153.2, 153.6, 165.6, 165.8, 170.9, 189.9 ppm; HRMS: calcd for $C_{34}H_{36}N_5O_9$ $[M+H]^+$ 658.2508, $C_{34}H_{35}N_5O_9Na$ $[M+Na]^+$ 680.2327, found 658.2493, 680.2310; MS (ESI): *m*/*z* 658.4 $[M+H]^+$, 680.4 $[M+Na]^+$; FTIR (thin film): 1739, 1643, 1512, 1416, 1327, 1234, 1157, 1092, 1045, 829, 748 cm^{-1}; $[\alpha]_D^{20} = +18.0^\circ$ ($c = 4.2$ mg mL^{-1}, $CHCl_3$); TLC: $R_f = 0.38$ (50% toluene/acetone).

Binding assay. The fluorescence quenching assay was mainly performed and analyzed as described,[31,35,36] with the following details and modifications: FKBP was obtained by overexpression in *E. coli* strain BL21 pLysS containing the encoding expression vector pET20bFKBP and was purified to homogeneity by subsequent His-tag-mediated, Ni-affinity chromatography and gel filtration. The concentration in gel filtration buffer (25 mM HEPES pH 7.5, 50 mM NaCl, 1 mM DTT) was determined by UV/Vis ($\varepsilon_{280} = 9970$ M^{-1} cm^{-1}), and adequate concentrations for the binding assay were prepared by dilution with standard phosphate buffered saline. The fluorescence measurement (300–400 nm, $\lambda_{ex} = 280$ nm, excitation slit width: 10 nm, emission slit width: 15 nm) was performed on a PerkinElmer LS 50B luminescence spectrometer employing a 60-μL fluorescence cell (Hellma GmbH & Co. KG, Müllheim, Germany). The FKBP concentration used for the measurement of linked ligands (**3a**–**3d**, **52a**, **52b**, and **53**–**56**) was 300 nM; measurements were performed at 0 °C to increase the fluorescence signal. The FKBP concentration for non-linked ligands (**1**, **2**, **21**, **22**, **26**–**28**, **30**, and **47**) was 2 μM, and measurements were carried out at 16 °C. Ten different ligand concentrations (ranging from 50 mM to 50 nM) were measured by adding from an adequate dilution series, each to a final constant DMSO concentration of 0.1%. Analysis was performed with readout of the fluorescence maximum at $\lambda = 330$ nm using the GraphPad software package.[50] Estimated errors are based on GraphPad calculation and multiplied by a factor of 2 to account for additional error sources not exclusively ascertainable by statistics, and were found to vary between 20–40% of absolute values.

Docking studies. Docking studies were performed using the program Surflex[49] (v.2.11), which generates a pseudo-binding site from a hydrogen-containing protein mol2 file, hereafter referred to as *protomol*. Subsequently, each individual ligand is fragmented into pieces and these are aligned to the protomol to obtain poses that maximize molecular and chemical complementarity with the binding site. The full molecule is regenerated from the best-aligned fragments using an incremental construction process, and scored using an empirically derived function including charged and hydrogen bond polar terms, solvation, entropic, and hydrophobic complementarity terms. Best poses are subjected to gradient-based optimization and returned along with their scores. Version 2.11 of Surflex allows the introduction of constraints during the docking process. This is achieved by the definition of one or several fragments placed in the binding site and which guide the alignment aspect of docking, whereas the conformational search is still guided by the protomol and scoring function. The definition of the protomol is a crucial step, as the docking performance depends on the area considered to form the binding site. To this end, we used the option *proto* with a pseudo-ligand created by linking site 1 ligand **1** and site 2 ligand **2** and leaving the other parameters on default. We defined a pharmacophore from a cycle and the two H bond acceptors involved in the H bonds with Tyr82 and Ile56 that is conserved in experimentally determined structures of FKBP in complex with site 1 ligands.[26–32] Because all ligands of our study present this particular kind of pharmacophore twice within their structure, a constraint had to be introduced during the docking process, thereby obtaining these two H bond acceptors at relevant positions in the binding site. The proline α-ketoamide sub-fragment was used to define this constraint. The docking of each ligand into the FKBP structure[51] was done using the "whole molecule" approach and the options *−fcoarse* and *−pgeom*. Moreover, a penalty of 300 for deviating from the placed fragment was added using the option *−cpen*. Finally, the best pose among the

20 generated was optimized using the option *opt*. The figures were generated using the software DS Visualizer from Accelrys.

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[1] E. R. Zartler, M. J. Shapiro, *Curr. Opin. Chem. Biol.* **2005**, *9*, 366 – 370.
[2] D. Fattori, *Drug Discovery Today* **2004**, *9*, 229 – 238.
[3] D. C. Rees, M. Congreve, C. W. Murray, R. Carr, *Nat. Rev. Drug Discovery* **2004**, *3*, 660 – 672.
[4] D. A. Erlanson, R. S. McDowell, T. O'Brien, *J. Med. Chem.* **2004**, *47*, 3463 – 3482.
[5] S. B. Shuker, P. J. Hajduk, R. P. Meadows, S. W. Fesik, *Science* **1996**, *274*, 1531 – 1534.
[6] P. J. Hajduk, R. P. Meadows, S. W. Fesik, *Science* **1997**, *278*, 497 – 499.
[7] E. T. Olejniczak, P. J. Hajduk, P. A. Marcotte, D. G. Nettesheim, R. P. Meadows, R. Edalji, T. F. Holzman, S. W. Fesik, *J. Am. Chem. Soc.* **1997**, *119*, 5828 – 5832.
[8] M. R. Arkin, M. Randal, W. L. DeLano, J. Hyde, T. N. Luong, J. D. Oslob, D. R. Raphael, L. Taylor, J. Wang, R. S. McDowell, J. A. Wells, A. C. Braisted, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 1603 – 1608.
[9] A. C. Braisted, J. D. Oslob, W. L. Delano, J. Hyde, R. S. McDowell, N. Waal, C. Yu, M. R. Arkin, B. C. Raimundo, *J. Am. Chem. Soc.* **2003**, *125*, 3714 – 3715.
[10] B. G. Szczepankiewicz, G. Liu, P. J. Hajduk, C. Abad-Zapatero, Z. Pei, Z. Xin, T. H. Lubben, J. M. Trevillyan, M. A. Stashko, S. J. Ballaron, H. Liang, F. Huang, C. W. Hutchins, S. W. Fesik, M. R. Jirousek, *J. Am. Chem. Soc.* **2003**, *125*, 4087 – 4096.
[11] G. Liu, Z. Xin, Z. Pei, P. J. Hajduk, C. Abad-Zapatero, C. W. Hutchins, H. Zhao, T. H. Lubben, S. J. Ballaron, D. L. Haasch, W. Kaszubska, C. M. Rondinone, J. M. Trevillyan, M. R. Jirousek, *J. Med. Chem.* **2003**, *46*, 4232 – 4235.
[12] P. J. Hajduk, G. Sheppard, D. G. Nettesheim, E. T. Olejniczak, S. B. Shuker, R. P. Meadows, D. H. Steinman, G. M. Carrera, Jr., P. A. Marcotte, J. Severin, K. Walter, H. Smith, E. Gubbins, R. Simmer, T. F. Holzman, D. W. Morgan, S. K. Davidsen, J. B. Summers, S. W. Fesik, *J. Am. Chem. Soc.* **1997**, *119*, 5818 – 5827.
[13] J. Klages, M. Coles, H. Kessler, *Mol. BioSyst.* **2006**, *2*, 318 – 332.
[14] A. R. Leach, M. M. Hann, J. N. Burrows, E. J. Griffen, *Mol. BioSyst.* **2006**, *2*, 429 – 446.
[15] S. Vanwetswinkel, R. J. Heetebrij, J. van Duynhoven, J. G. Hollander, D. V. Filippov, P. J. Hajduk, G. Siegal, *Chem. Biol.* **2005**, *12*, 207 – 216.
[16] T. Marquardsen, M. Hofmann, J. G. Hollander, C. M. Loch, S. R. Kiihne, F. Engelke, G. Siegal, *J. Magn. Reson.* **2006**, *182*, 55 – 65.
[17] S. L. Schreiber, *Science* **1991**, *251*, 283 – 287.
[18] W. E. Lyons, E. B. George, T. M. Dawson, J. P. Steiner, S. H. Snyder, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 3191 – 3195.
[19] J. Sharkey, S. P. Butcher, *Nature* **1994**, *371*, 336 – 339.
[20] L. Zhao, H. Liu, L. Wang, S. Li, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4385 – 4390.
[21] B. G. Gold, *Expert Opin. Invest. Drugs* **2000**, *9*, 2331 – 2342.
[22] G. S. Hamilton, J. P. Steiner, *J. Med. Chem.* **1998**, *41*, 5119 – 5143.
[23] S. W. Michnick, M. K. Rosen, T. J. Wandless, M. Karplus, S. L. Schreiber, *Science* **1991**, *252*, 836 – 839.
[24] J. M. Moore, D. A. Peattie, M. J. Fitzgibbon, J. A. Thomson, *Nature* **1991**, *351*, 248 – 250.
[25] G. D. Van Duyne, R. F. Standaert, P. A. Karplus, S. L. Schreiber, J. Clardy, *Science* **1991**, *252*, 839 – 842.
[26] G. D. Van Duyne, R. F. Standaert, P. A. Karplus, S. L. Schreiber, J. Clardy, *J. Mol. Biol.* **1993**, *229*, 105 – 124.
[27] D. A. Holt, J. I. Luengo, D. S. Yamashita, H. J. Oh, A. L. Konialian, H. K. Yen, L. W. Rozamus, M. Brandt, M. J. Bossard, M. A. Levy, D. S. Eggleston, J. Liang, L. W. Schultz, T. J. Stout, J. Clardy, *J. Am. Chem. Soc.* **1993**, *115*, 9925 – 9938.
[28] K. P. Wilson, M. M. Yamashita, M. D. Sintchak, S. H. Rotstein, M. A. Murcko, J. Boger, J. A. Thomson, M. J. Fitzgibbon, J. R. Black, M. A. Navia, *Acta Crystallogr. Sect. D* **1995**, *51*, 511 – 521.
[29] D. M. Armistead, M. C. Badia, D. D. Deininger, J. P. Duffy, J. O. Saunders, R. D. Tung, J. A. Thomson, M. T. DeCenzo, O. Futer, D. J. Livingston, M. A. Murcko, M. M. Yamashita, M. A. Navia, *Acta Crystallogr. Sect. D* **1995**, *51*, 522 – 528.
[30] C. Sich, S. Improta, D. J. Cowley, C. Guenet, J. P. Merly, M. Teufel, V. Saudek, *Eur. J. Biochem.* **2000**, *267*, 5342 – 5355.
[31] P. Burkhard, P. Taylor, M. D. Walkinshaw, *J. Mol. Biol.* **2000**, *295*, 953 – 962.
[32] G. M. Dubowchik, V. M. Vrudhula, B. Dasgupta, J. Ditta, T. Chen, S. Sheriff, K. Sipman, M. Witmer, J. Tredup, D. M. Vyas, T. A. Verdoorn, S. Bollini, A. Vinitsky, *Org. Lett.* **2001**, *3*, 3987 – 3990.
[33] I. Muegge, Y. C. Martin, P. J. Hajduk, S. W. Fesik, *J. Med. Chem.* **1999**, *42*, 2498 – 2503.
[34] The graphic was generated with the program PyMol, W. L. DeLano, **2003** (http://pymol.sourceforge.net/).
[35] S. T. Park, R. A. Aldape, O. Futer, M. T. DeCenzo, D. J. Livingston, *J. Biol. Chem.* **1992**, *267*, 3316 – 3324.
[36] X. Xia, J. T. Lin, R. K. Kinne, *Biochemistry* **2003**, *42*, 6115 – 6120.
[37] We also investigated benzyl protection of the second phenolic hydroxy group. However, the poor solubility of the resulting compounds in most organic solvents, giving rise to tedious purifications, prompted us to investigate silyl protection.
[38] Y. Q. Wu, D. E. Wilkinson, D. Limburg, J. H. Li, H. Sauer, D. Ross, S. Liang, D. Spicer, H. Valentine, M. Fuller, H. Guo, P. Howorth, R. Soni, Y. Chen, J. P. Steiner, G. S. Hamilton, *J. Med. Chem.* **2002**, *45*, 3558 – 3568.
[39] M. L. Lamb, J. Tirado-Rives, W. L. Jorgensen, *Bioorg. Med. Chem.* **1999**, *7*, 851 – 860.
[40] G. S. Hamilton, Y. Q. Wu, D. C. Limburg, D. E. Wilkinson, M. J. Vaal, J. H. Li, C. Thomas, W. Huang, H. Sauer, D. T. Ross, R. Soni, Y. Chen, H. Guo, P. Howorth, H. Valentine, S. Liang, D. Spicer, M. Fuller, J. P. Steiner, *J. Med. Chem.* **2002**, *45*, 3549 – 3557.
[41] R. E. Babine, S. L. Bender, *Chem. Rev.* **1997**, *97*, 1359 – 1472.
[42] D. S. Yamashita, H.-J. Oh, H.-K. Yen, M. J. Bossard, M. Brandt, M. A. Levy, T. Newman-Tarr, A. Badger, J. I. Luengo, D. A. Holt, *Bioorg. Med. Chem. Lett.* **1994**, *4*, 325 – 328.
[43] L. Wei, Y. Wu, D. E. Wilkinson, Y. Chen, R. Soni, C. Scott, D. T. Ross, H. Guo, P. Howorth, H. Valentine, S. Liang, D. Spicer, M. Fuller, J. Steiner, G. S. Hamilton, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1429 – 1433.
[44] H. C. Kolb, K. B. Sharpless, *Drug Discovery Today* **2003**, *8*, 1128 – 1137.
[45] O. Mitsunobu, *Synthesis* **1981**, 1 – 28.
[46] To probe if the two phenolic hydroxy groups of benzamide **2** show different reactivities, we also performed the Mitsunobu reaction with **2** as substrate, but obtained a mixture of products, indicating simultaneous reaction of both hydroxy groups.
[47] P. T. Nyffeler, C.-H. Liang, K. M. Koeller, C.-H. Wong, *J. Am. Chem. Soc.* **2002**, *124*, 10773 – 10778.
[48] In rare cases, if the linker enables additional positive interactions with the target protein, even super-additive effects can be obtained. See for an example reference [7].
[49] A. N. Jain, *J. Med. Chem.* **2003**, *46*, 499 – 511.
[50] GraphPad Prism, GraphPad Software Inc., San Diego, CA 92130 (USA).
[51] The FKBP PDB files were generously provided by Philip J. Hajduk.

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