Solid Phase Synthesis of Vancomycin Mimics

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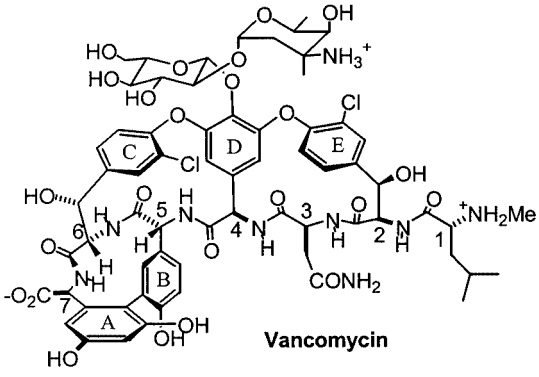
A solid phase synthesis of a model system of the DE ring system of vancomycin is described. The synthesis involved biocatalytic resolutions of unnatural amino acids, is compatible with conventional solid phase peptide synthesis and contains as the key step: an on-bead S_NAr cyclization. Binding of a cyclic peptide to the carboxylate of N-Ac-D-Ala was demonstrated.

Introduction

Vancomycin is an important antibiotic due to its efficacy against Gram-positive bacteria, including many methicillin resistant strains.[1,2] Unfortunately, also vancomycin-resistant bacteria have emerged. Their dominant resistance mechanism is based on the replacement of the natural acylated D-Ala-D-Ala sequence of the growing cell wall by acylated D-Ala-D-Lac.[3] Vancomycin binds the latter sequence 1000-fold less strong due to electrostatic repulsion in its complex.[4] For this reason it is an important goal to find high affinity compounds for the altered sequence. Besides its clinical importance, vancomycin is also a remarkable compound from a molecular recognition point of view. It is a relatively small molecule that binds its small, polar and flexible target in water by a combination of hydrogen bonds and hydrophobic effects. Many carboxylate receptors have been synthesized[5] and many efforts to design and synthesize vancomycin mimics de novo, have led to a great deal of innovative and creative chemistry but not thus far to very effective systems.[6−10]

The structure of vancomycin clearly carries subtle features that make it effective. One way to gain insights into these features is to simplify the structure and see what binding power remains. We[11] and others[12,13] have previously made simplified versions of the carboxylate-binding pocket. In these simplifications two rings, the AB and the CD rings, of the vancomycin skeleton were deleted, leaving only the DE ring framework, i.e. the carboxylate binding pocket, intact. With our system we observed tight binding of relevant carboxylates in organic solvents.[11] Ellman and co-workers[13] have taken the intact native binding pocket and varied amino acids at the *C*-terminal end and concluded from ITC binding studies in unbuffered water that binding to relevant carboxylates took place with selected receptors. Our interest is to vary the carboxylate-binding pocket itself, i.e. amino acid 3[14] in order to see what modifications are tolerated and what the effects of alternative residues would be. In the complex of vancomycin with its ligands the primary amide group of amino acid 3, an asparagine in vancomycin, is in close proximity to the ligand's carboxylate oxygen atoms, and in fact has been implicated in hydrogen bonding to it.[15] Despite the fact that X-ray structures did not confirm the hydrogen bond, proximity to the side chain of amino acid 3 is certain[16,17] and its replacement with e.g. a positively charged residue may have a sizeable effect. Varying amino acid 1 (Figure 1), which is D-leucine in van-



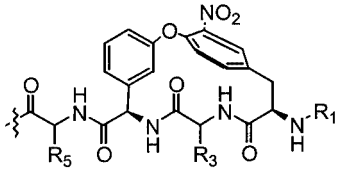


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Figure 1. Structure of vancomycin and a generic structure of a simplified mimic

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comycin, is also of interest for the proximity of its *N*-terminus and its side chain to the bound carboxylate. These issues led to the following conditions of our synthetic work: 1) The synthesis would have to allow variation of both amino acids 1 and 3; and 2) be compatible with common solid phase peptide chemistry. Thus vancomycin mimics of general structure **1** were targeted by application of the S_NAr cyclization reaction.[18−23] This reaction was used successfully by Zhu and co-workers in the synthesis of vancomycin model compounds and other biaryl ether containing compounds of medicinal interest.[18] By using this reaction amino acids 3 (and, 1) can be chosen at will and by performing it on-bead it proved compatible with solid phase Fmoc-peptide chemistry and should be suitable for the future generation of libraries.

Medicinal relevance of compounds like **1** is also present in arenas other than the glycopeptide antibiotics as is exemplified by the compounds shown in Figure 2. **K-13** is an inhibitor of angiotensin I converting enzyme;[24] the compounds **OF4949 I-IV** show anti-tumor activity,[25−29] and (+)-piperazinomycin is an antibiotic.[30] In fact, a few members in the vancomycin-class of antibiotics, namely chloropeptin and complestatin have shown activity against HIV-1 integrase (IC50 = 0.3−0.5 μM).[31] The structural similarity of all these molecules is noticeable despite the distinct medicinal targets of these compounds. In general, cyclic peptides are currently popular targets for medicinal purposes due to their increased in vivo stability, reduced conformational mobility and better-defined geometry.[32] Natural cyclic peptides are often good starting points in structure-based drug design.[33] Furthermore cyclic peptides are often used to mimic the important recognition units: the turns in proteins.[23,34] To explore these and other advantages, a cyclization method that is compatible with common peptide synthesis, as described here, represents a powerful tool to the medicinal chemist.

Results and Discussion

Preparation of Unnatural Amino Acid Starting Materials

The synthesis of the vancomycin mimic started with the preparation of the two unnatural amino acids **7** and **17,** a substituted phenylalanine derivative and a phenylglycine derivative, respectively. Fluoro(nitro)phenylalanine derivative **7** (Scheme 1) was prepared from 4-fluorobenzaldehyde (**2**) following known,[35] but slightly modified procedures. Starting with a nitration reaction, and subsequent $NaBH_4$ reduction of the aldehyde group the resulting benzylic alcohol was converted into **4** with PBr_3. According to work of Zhu et al.[36] **4** was used to alkylate acetamidomalonate; the formed adduct was decarboxylated in concd. HCl and **(+/-)-6** was obtained after esterification of the carboxyl group with MeOH and trifluoroacetylation of the amino group. Compound **(+/-)-6** was resolved in a kinetic resolution using the protease type VIII-A from *Bacillus Licheniformus* which selectively hydrolyzed the methyl ester of the L-enantiomer and the required D-enantiomer remained fully protected. After separation by extraction, it was found to be enantiomerically pure as determined by optical rotation. After acidic hydrolysis and *N*-terminus protection with a Boc group, **7** was obtained in 40% yield from the protected racemic amino acid **(+/-)-6**, (maximum yield 50%) and could be run on a multigram scale.

Phenylglycine derivative **17** was also prepared from a racemic amino acid involving an alternative enzymatic kinetic resolution (Scheme 2). To this end 3-hydroxybenzaldehyde (**8**) was converted into the corresponding hydantoin **9** using the Bucherer−Bergs reaction.[37] The hydantoin **9** was sub-

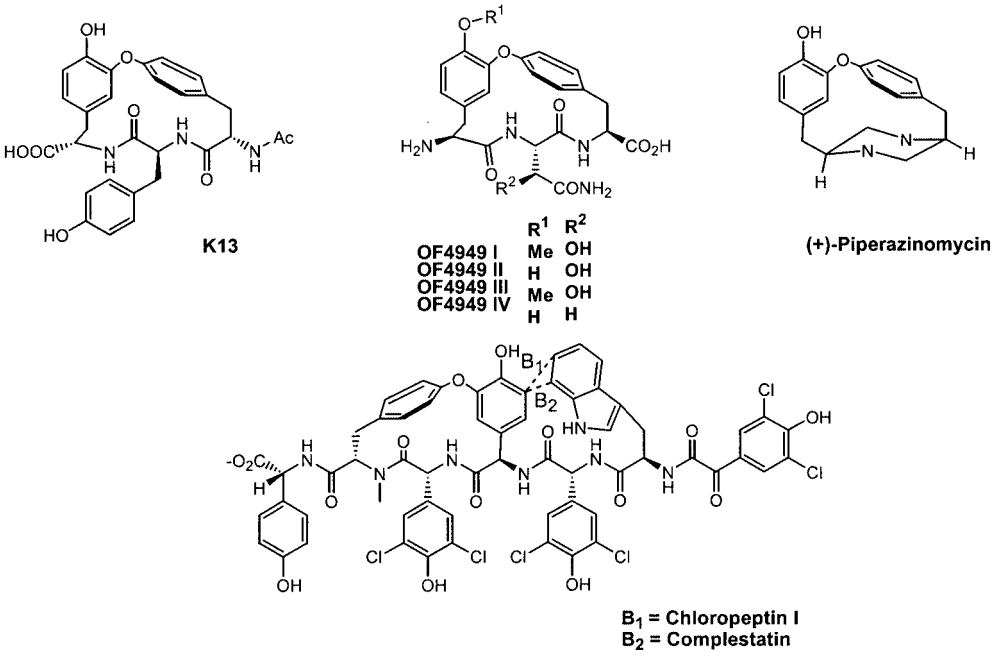
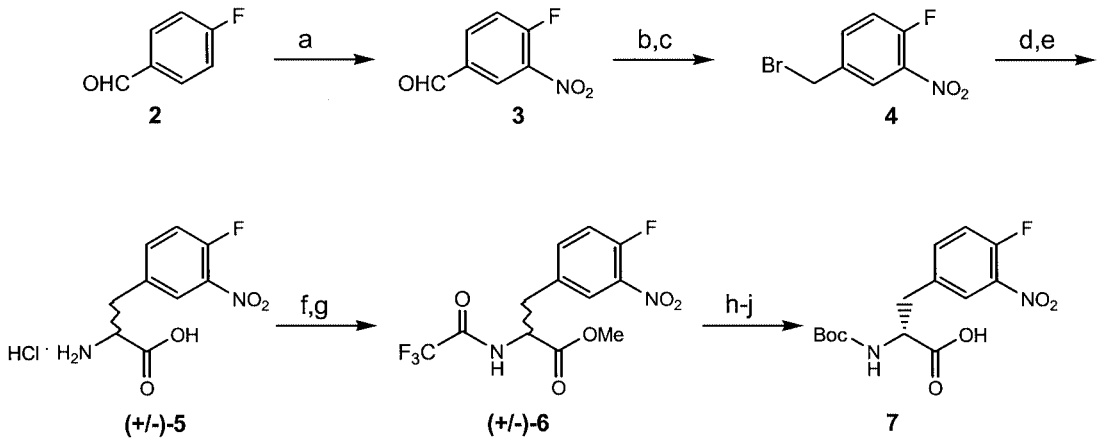


Figure 2. Biologically active compounds with structural features related to vancomycin (mimics)

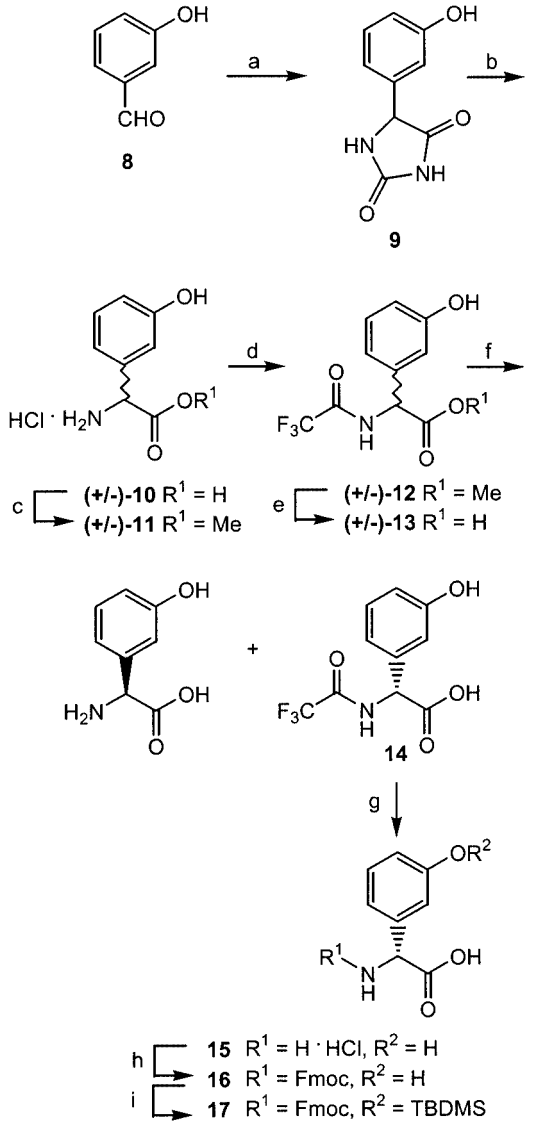


Scheme 1. Reagents and conditions: a) HNO_3, H_2SO_4, −5 °C, 60%; b) $NaBH_4$, room temp. then c) PBr_3; d) $CH_3CONCH(CO_2Et)_2$, NaH; e) 6 M HCl, 65 °C; f) $SOCl_2$, MeOH; g) $(CF_3CO)_2O$, TEA; h) protease type VIII-A from *Bacillus licheniformus*, pH 7.5, 37 °C; i) 6 M HCl, AcOH, 65 °C; j) Boc_2O, $NaHCO_3$ Steps d−j according to ref.[36].

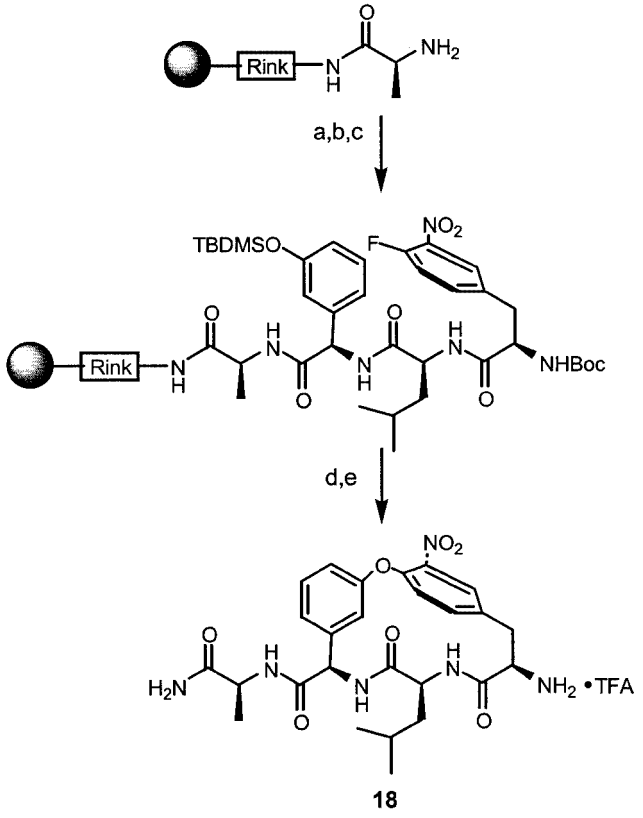
sequently hydrolyzed under acidic conditions to obtain the free amino acid hydrochloride salt. Its carboxylic acid group was converted into a methyl ester using thionyl chloride in methanol and the amino function was subsequently outfitted with a trifluoroacetate group to yield **(+/-)-12**. This protected (D,L)-phenylglycine derivative was first subjected to protease VIII-A from *Bacillus licheniformus* as was done with **(+/-)-6,** under several conditions in order to obtain the D-enantiomer but with limited success. The solvent mixtures, temperature, pH, and phenolic protecting groups were all factors that were varied. The methyl ester was therefore hydrolyzed with an aqueous LiOH/dioxane solution to obtain the free racemic carboxylic acid and was subsequently treated with the acylase AMANO 30000.[21,38,39] The non-deacylated and resolved compound **14** was obtained in 45% yield with an *ee* of 82%. Its trifluoroacetate group was removed under acidic conditions in nearly quantitative yield to give the unprotected amino acid **15**. The amino group of compound **15** was first protected with an Fmoc group using Fmoc-OSu at pH 8.5 in a 1:1 mixture of acetonitrile and water. The phenolic hydroxy group of the resulting compound **16** was then silylated with TBDMS-Cl to yield the amino acid derivative **17**, ready for solid phase synthesis, in 76% yield after lyophilization.

Solid-Phase Synthesis

The solid-phase synthesis was performed as shown in Scheme 3. Argogel® OH resin outfitted with a Rink linker was chosen to test the validity of the on-bead S_NAr cyclization to be performed. Fmoc chemistry therefore was used for amino acid chain elongation with BOP as the amino acid coupling reagent. Normally, 4 equivalents of the amino acid are used relative to the loading of the precursor on the solid phase. However, with the more precious amino acids **7** and **17**, 2.5 equivalents were used combined with longer reaction times. The tetra peptide **18** was synthesized in this fashion and cyclization was realized by treatment of the uncyclized receptor on the solid phase with a 0.1 M solution of TBAF in DMF. It proved necessary for the mixture to react for 40 h to complete the reaction. In our previous paper,[11] CsF in DMF was used as the fluoride source for deprotection and cyclization. However, when used for receptors on the solid phase, mass spectrometry results showed incomplete cyclization. This is likely be due to the poor solubility of CsF in DMF. The fluoride ion in this reaction has a dual purpose since it first has to act as a nucleophile to deprotect the silyl phenyl ether and then acts as a base to facilitate the S_NAr cyclization. After treatment with TFA in the presence of triisopropylsilane and water amide **18** was obtained. HPLC analysis of the crude reaction mixture showed the result of an efficient synthesis[40] with a major peak flanked by a minor peak in a 9:1 ratio. Since the enantiomeric purity of the substituted phenylglycine used was 82% these two peaks were likely due to the formation of diastereoisomers (Figure 3, a). A sample was purified and the major peak was separated using preparative HPLC and was isolated in 30% yield (Figure 3, b). Based on ^1H NMR spectra obtained from this peak, it was concluded that it consisted of a mixture of two compounds. The ratio was based on the characteristic signals derived from the proton on the phenylglycine benzene ring, located between the ether linkage and the link to the α-carbon atom. These signals were located at around $\delta = 6$ ppm and had moved upfield upon ring closing due to the shielding effect of the phenylalanine ring. Since epimerization was not expected to occur under the conditions used,[41,42] the two compounds (ratio 4:1) were presumed to be two atropisomers. The atropisomeric ratio is not expected to change based on the work of Boger and co-workers where they showed that under ambient conditions, no rotation is seen about the biaryl ether linkage once ring closing is achieved.[43] Additional experiments confirmed our interpretation about compound **18**. The single peak could indeed be resolved into two peaks (atropisomers) using a different HPLC column. A resynthesis of compound **18** using the racemic building block **17**, yielded material which



Scheme 2. Reaction conditions: a) $(NH_4)_2CO_3$, KCN, then HCl, 40%; b) 6 M HCl, 120 °C, quant.; c) $SOCl_2$, MeOH, 80%; d) $(CF_3CO)_2O$, NEt_3, 82%; e) LiOH(aq.)/dioxane, quant.; f) AMANO 30000, pH 7.5, 5 d, 25 °C, 45%; g) 6 M HCl/AcOH (1:1), 65 °C, quant.; h) Fmoc-OSu, NEt_3, pH 8.5, 96%; i) TBDMS-Cl, pyridine, 65 °C, 76%.



Scheme 3. Synthesis of receptor on solid phase. Reaction conditions: a) Coupling − 2.5 equiv. **17**, 2.5 equiv. BOP, 5 equiv. DIPEA, then deprotection − 20% piperidine in NMP, (3 × 8 min), and washing − NMP, CH_2Cl_2, (3 × 2 min); b) Coupling − 4 equiv. Fmoc-Leu-OH, 4 equiv. BOP, 8 equiv. DIPEA, deprotection, washing; c) Coupling − 2.5 equiv. **7**, 2.5 equiv. BOP, 5 equiv. DIPEA; d) 7 equiv. (0.1 M) TBAF in DMF, 40 h; e) TFA/TIS/H_2O, 95:2.5:2.5 v:v:v

showed four peaks under these conditions (see Supporting Information, for Supporting Information see also the footnote on the first page), strongly indicating that the diastereomeric integrity can be maintained during the synthesis. To assess the versatility of applying this methodology for a future parallel library synthesis, 2 additional receptors were synthesized with a Lys and Tyr incorporated at position 3. As before, mass spectrometric analysis indicated a clean and efficient synthesis of these compounds (see Supporting Information).

Another consideration that should be addressed is the possibility of covalent dimerization upon ring closing. Analysis of the electrospray mass spectra obtained for compound **18** indicated a small peak at m/z = 2M (singly charged particle) that could indicate a dimer of **18**. High resolution mass spectra was therefore obtained and upon analysis of the isotope patterns, as well as the technique of tandem mass spectrometry (MS/MS), unambiguously confirmed structure of monomeric **18**.

As in our previous paper,[11] ^{1}H NMR titration studies were performed on compound **18** as an indication of binding of carboxylate ions. Addition of the tetra-*n*-butyl ammonium salt of NAc-D-Ala (0.75−42 mM) to a solution of receptor **18** in CD_3CN (1 mM) resulted in downfield shifts of at least two of the NH resonances to δ = 9.02 and 10.52 ppm. These shifts are even further downfield than that of our previously reported receptor molecule[11] where the maximum shift was to δ = 9.00 ppm (Figure 4). This suggests that the hydrogen bonding in compound **18** to NAc-D-Ala is taking place in a similar fashion but differences are also apparent.

UV spectroscopy was previously employed in our lab to measure binding affinities and directly observe interaction of the receptor-ligand complex. Titrations with vancomycin were performed as well in aqueous solution and consistently gave binding constants that agreed with previously reported values.[44−46] A UV titration is compatible with the biaryl ether-type receptors since the aryl systems have an absorbance in the UV region where the ligands do not. In the

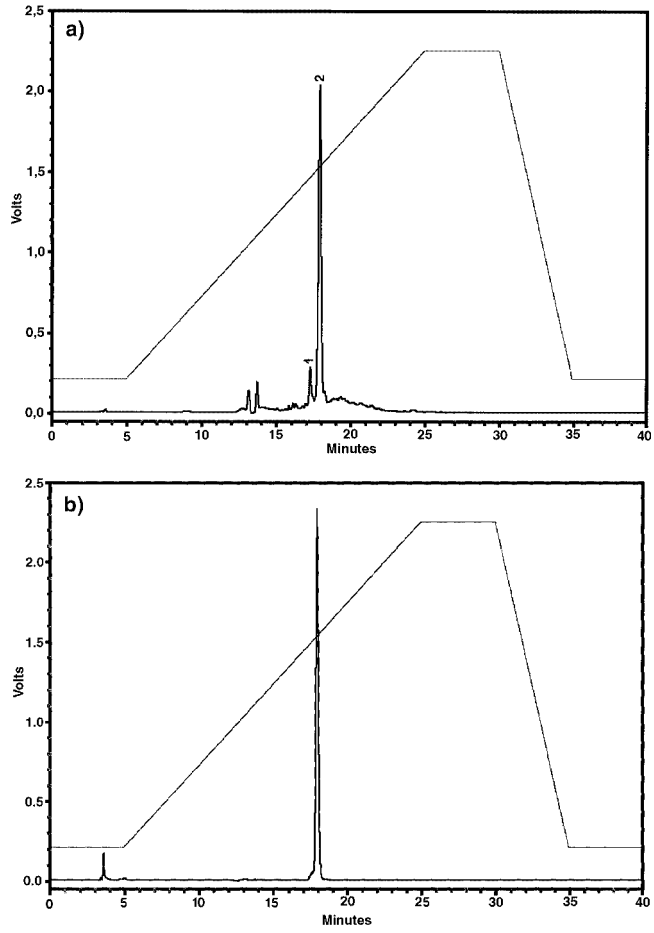


Figure 3. HPLC traces of a) crude, and b) purified **18**

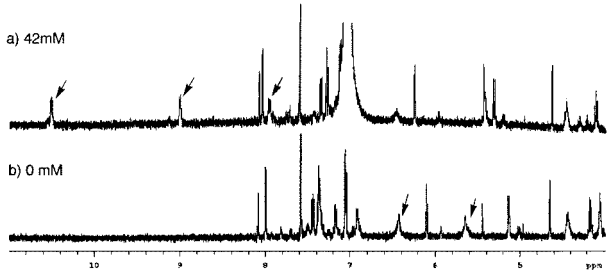


Figure 4. NMR spectra of a 1 mM solution of **18** in CD_3CN with the addition of a) a saturating amount of N-Ac-D-Ala-$O^{-}{}^{+}NBu_4$ (42 mM) and b) with no ligand added

same way[4], titration of a dilute solution of **18** (1×10^{-4} M in CH_3CN) with a solution of the tetra-*n*-butyl ammonium salt of N-Ac-D-Ala (while maintaining a constant receptor concentration), resulted in an increased absorption around 270−280 nm. This increase was fitted to a 1:1 binding isotherm and indicated an association constant of 7.3×10^3 M^{-1} or 5.2 kcal mol^{-1} of binding free energy. This value is actually about 4−5 times weaker than our related receptor **19**, where alanine is present in the middle of the carboxylate binding pocket.[11] The lower association constant and also the NMR chemical shift positions mentioned above, might be an indication that the small variation of the amino acid in the center of the carboxylate binding pocket can have an impact on binding and varying this position may further alter the delicate hydrogen bonding system of the vancomycin(mimic)-carboxylate complexes.

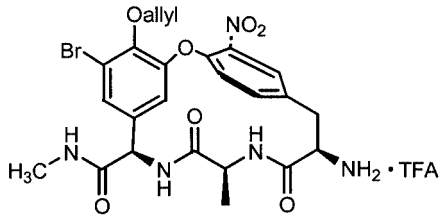


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Figure 5. Structure of receptor **19**

Conclusion

Methodology to build vancomycin-like mimics on the solid phase was developed. Using biocatalysis it was possible to prepare the components needed for the solid phase peptide chemistry that was used to assemble the system. An on-bead S_NAr reaction was successfully applied to effect the necessary cyclization of two peptide side chains. Variation of the amino acid in the middle of the carboxylate binding pocket seems to have an effect on binding to small carboxylates and can possibly disturb or enhance the delicate hydrogen bonding system. Parallel libraries will be prepared to determine this. The synthetic strategy used here in the preparation of cyclic peptides may also be useful in preparing other new compounds with e.g. anti-viral properties or enzyme-inhibitory properties.

Experimental Section

General Remarks: Chemicals and resins were obtained from commercial sources and used without further purification unless stated otherwise. The solvents DMF, NMP, CH_2Cl_2, MeOH and dioxane were purchased from Biosolve, the Netherlands. The solvents CH_2Cl_2, DMF, NMP, and Et_2O were kept dry with molecular sieves (4 Å); for MeOH 3-Å molecular sieves was used. The base iPr_2NEt was distilled from ninhydrin and KOH. Column chromatography was performed on ICN Silica 32−63, 60 Å. Thin layer chromatography (TLC) was performed on Merck precoated Silica 60 plates. Visualization was accomplished with UV light and staining with ninhydrin. Protease type VIII-A from *Bacillus licheniformus* was obtained from Sigma. Acylase "Amano 30000" was purchased from the Amano Pharmaceutical Co., Ltd. Nagoya, Japan. Amino acids were purchased from Multisyntech. Rotations were measured with a Jasco P-1010 polarimeter. ^1H NMR spectra were recorded with a Varian G-300 spectrometer (300 MHz); NMR titrations and spectra for **18** were obtained with a Varian INOVA spectrometer. (500 MHz). ^{13}C NMR spectra were recorded with a Varian G-300 spectrometer at 75.4 MHz. All spectra were referenced to the solvent signal: CD_3CN (^1H: δ = 1.93 ppm, ^{13}C: δ = 1.3 ppm), $CD_3OD(H)$ (^1H: δ = 3.3 ppm, ^{13}C: δ = 49.0 ppm), $CDCl_3$ (^1H: δ = 7.24 ppm, ^{13}C: δ = 77.0 ppm) and [D_6]DMSO (δ = 2.49, 39.5 ppm). ^{13}C NMR spectra were recorded using the attached proton test (APT) sequence where indicated. Electrospray ionization (ESI) mass spectrometry was carried out using a Shim-

adzu LCMS QP-8000 single quadrupole bench top mass spectrometer (m/z range < 2000), coupled with a QP-8000 data system, except for compounds **13**, **14** and **18** which were carried out using electrospray MS (nano ESI-TOF-MS) run on a Micromass LC-TOF mass spectrometer by spraying a CH_3CN/H_2O solution from a gold-coated glass capillary in a Z-spray nanospray ionization source (cone voltage 30−40). Elemental Analysis was measured by the Firma H. Kolbe, Mikroanalytisches Laboratorium, Mülheim a.d. Ruhr, Germany. UV measurements were carried out with a Helios-β Spectrophotometer. Analytical HPLC was performed with a Shimadzu Class-VP automated HPLC using an analytical reversed-phase column (Alltech Adsorbosphere C18, 300 Å, 5μm, 250 × 4.6 mm) and a UV detector operating at 220 nm and 254 nm. Preparative HPLC was performed with a Gilson automated HPLC using a preparative reversed-phase column (Alltech Adsorbosphere C18, 10 μm, 250 × 22 mm) and a UV detector operating at 220 nm and 254 nm. Elution was effected using an appropriate gradient from 0.1% TFA in water to 0.085% TFA in acetonitrile/water (95:5, v/v) using a flow rate of 1 mL·min^{-1} (analytical) or 11.5 mL·min^{-1} (preparative). Separation of the enantiomers of **17** was performed by HPLC (Gilson) using a Chirobiotic T column from Advanced Separation Technologies Inc. Elution was effected using 50% MeOH and 50% (1% TEAA in water) using a flow rate of 1 mL·min^{-1}.

4-Fluoro-3-nitrobenzaldehyde (3): 4-Fluorobenzaldehyde (**2**, 15.6 mL, 18 g, 0.145 mol) was slowly added dropwise to a solution of H_2SO_4 (72 mL), and HNO_3 (10 mL) at −5 °C. The temperature was kept below 5 °C. The solution was warmed to room temp. (1 h), and after pouring into ice, (400 g) the precipitate was filtered and washed with H_2O. The aqueous phase was extracted with CH_2Cl_2 and combined with the precipitate. The solution was washed with $NaHCO_3$, brine, and dried with Na_2SO_4. The solvent was removed in vacuo and the residue was recrystallized from diethyl ether to yield 14.5 g (60%) of large clear white crystals. R_f = 0.48 (CH_2Cl_2/hexane, 2:1). ^{1}H NMR ($CDCl_3$): δ = 7.50 (t, J = 9 Hz, 1 H, ArH), 8.20 (oct, J = 2 Hz, 1 H, ArH), 8.60 (dd, J = 7, J = 2 Hz, 1 H, ArH), 10.04 (s, 1 H, CHO) ppm. $C_7H_4FNO_3$ (169.11): calcd. C 49.72, H 2.38, N 8.28; found C 49.68, H 2.36, N 8.22.

4-Fluoro-3-nitrobenzyl Bromide (4): In a round-bottom flask equipped with an addition funnel 4-fluoro-3-nitrobenzaldehyde (**3**, 14.5 g, 0.085 mol) was dissolved in methanol, (90 mL). $NaBH_4$ (12.3 g, 0.325 mol) in water (22 mL) was added dropwise, while keeping the reaction mixture at room temp. After the addition was complete, a small test portion of the reaction mixture was tested in dilute H_2SO_4. H_2(g) was formed and indicated a finished reaction. The methanol was removed in vacuo and water was added (200 mL). The aqueous phase was extracted with diethyl ether (5 × 150 mL) and the diethyl ether layer was washed with water, brine, and dried with $MgSO_4$. The solvent was removed in vacuo to give an oil (14.6 g) and without further purification redissolved in diethyl ether (275 mL). To the stirred solution was added dropwise PBr_3 (8.2 mL, 23.37 g, 0.086 mol). After 1 hour water (400 mL) was carefully added. The aqueous phase was extracted with diethyl ether and the combined organic fractions were washed with $NaHCO_3$, brine, and dried with Na_2SO_4. The solvents were removed in vacuo and the product purified by recrystallization in hexane for a yield of 10 g over 2 steps. (50% sparkling yellow solid). R_f = 0.33 (hexane/EtOAc, 5:1). ^{1}H NMR ($CDCl_3$): δ = 4.49 (s, 2 H, CH_2Br), 7.29 (t, J = 7 Hz, 1 H, ArH), 7.67 (oct, J = 2 Hz, 1 H, ArH), 8.10 (dd, J = 7, J = 2 Hz, 1 H, ArH) ppm. $C_7H_5BrFNO_2$ (234.02): calcd. C 35.93, H 2.15, N 5.99; found C 35.81, H 2.17, N 5.86.

(*RS*)-4-Fluoro-3-nitrophenylalanine [(+/-)-5], (*RS*)-4-Fluoro-3-nitro-*N*-(trifluoroacetyl)phenylalanine Methyl Ester [(+/-)-6] and **(*R*)-*N*-Boc-4-Fluoro-3-nitro-phenylalanine (7):** These compounds were prepared from 4-fluoro-3-nitrobenzyl bromide (**4**) according to ref.[36].

(3-Hydroxy)phenylhydantoin (9): 3-Hydroxybenzaldehyde (**8**, 6.1 g, 0.05 mol) was dissolved in ethanol (80 mL) and added to a solution of ammonium carbonate (24 g, 0.25 mol) and KCN (3.9 g, 0.06 mol) in water (80 mL). The flask was fitted with a condenser and heated to 60 °C for 150 min. After cooling in ice, the solution was acidified in a well-ventilated fumehood to pH 2 with 6 M HCl while bubbling N_2 through the mixture. After acidification, the mixture was degassed for an additional 30 min to ensure complete removal of HCN. The solution was poured into a beaker and dried by evaporation. The solid was dissolved in dry MeOH and solution decanted from the salts (4 ×). The MeOH fractions were combined and the solvents evaporated in vacuo and the residue dissolved in 100 mL MeOH/DCM (5:1) and filtered to remove remaining salt content to yield a crude solid (6 g). Column chromatography was used to purify the hydantoin using a gradient of solvents (DCM/MeOH, 8:1 to 1:3) to yield 3.87 g (40%) as a light beige powder, m.p. 204−205 °C, (ref.[47] 212 °C). R_f = 0.5 (DCM/MeOH, 6:1). ^{1}H NMR (CD_3OD): δ = 5.05 (s, 1 H, H_α), 6.79 (m, 3 H, ArH), 7.20 (t, 1 H, ArH) ppm. ^{1}H NMR ([D$_6$]DMSO): δ = 5.04 (s, 1 H, H_α), 6.73 (m, 3 H, ArH), 7.17 (t, 1 H, ArH), 8.36 (s, 1 H, NH), 9.54 (s, 1 H, NH), 10.75 (s, 1 H, OH) ppm. ^{13}C NMR (CD_3OD): δ = 63.5, 114.5, 116.7, 118.8, 131.0, 138.0, 159.1, 160.1, 176.4 ppm.

(*RS*)-(3-Hydroxy)phenylglycine (10): In a round-bottom flask fitted with a condenser (3-hydroxy)phenylhydantoin (5 g, 0.026 mol) was heated to 120 °C in 6 M HCl for 5 d. This solution was evaporated to dryness and used without further purification. ^{1}H NMR (D_2O): δ = 4.81 (s, 1 H, H_α), 6.76−6.84 (m, 3 H, ArH), 7.19 (t, J = 8 Hz, 1 H, ArH) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 55.7, 115.3, 116.5, 118.7, 130.1, 134.5, 158.0, 169.9 ppm.

(*RS*)-(3-Hydroxy)phenylglycine Methyl Ester (11): This compound was prepared according to ref.[48] Spectroscopic data: ^{1}H NMR (CD_3OD): δ = 3.66 (s, 3 H, OCH_3), 4.47 (s, 1 H, H_α), 6.70 (dd, J = 8, J = 1.5 Hz, 1 H, ArH), 6.80 (m, 2 H, ArH), 7.15 (t, J = 8 Hz, 1 H, ArH) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 52.3, 58.6, 114.3, 114.9, 118.1, 129.9, 142.9, 157.9, 175.1 ppm.

(*RS*)-(3-Hydroxy)phenyl-*N*-trifluoroacetylglycine Methyl Ester [(+/−)-12]: (+/−)-(3-hydroxy)phenylglycine methyl ester **(11,** 3.3 g, 15 mmol) was dissolved in DCM (100 mL) and TEA (3 mL) and trifluoroacetic anhydride (3 mL, 21.3 mmol) were added dropwise. The solution was stirred for 4 h and kept at a basic pH with TEA. The solvent was removed in vacuo and DCM (100 mL) was added. The mixture was washed with $NaHCO_3$ (2 × 75 mL), dilute aqueous HCl (2 × 75 mL), brine (2 × 75 mL) and dried with Na_2SO_4. The solvent was removed in vacuo and **(+/-)-12** was purified by recrystallization (EtOAc/hexane) to yield 2.91 g (70%). R_f = 0.5 (CH_2Cl_2/MeOH, 7:1). ^{1}H NMR ($CDCl_3$): δ = 3.73 (s, 3 H, CH_3), 5.49 (d, J = 7 Hz, 1 H, H_α), 6.74 (br. s, 1 H, OH), 6.79−6.88 (3 H, ArH), 7.20 (t, J = 8 Hz, 1 H, ArH), 7.70 (d, J = 7 Hz, 1 H, NH) ppm. ^{13}C NMR APT ($CDCl_3$): δ = 53.4, 56.6, 114.3, 116.5, 119.1, 130.5, 115.4 (q, $J_{C,F}$ = 287 Hz), 135.5, 156.5, 156.9 (q, J_{CCF} = 39 Hz), 170.0 ppm. $C_{11}H_{10}F_3NO_4$ (277.20): calcd. C 47.66, H 3.64, N 5.05; found C 47.73, H 3.67, N 4.97.

(*RS*)-(3-Hydroxy)phenyl-*N*-trifluoroacetylglycine [(+/-)-13]: (+/-)-12 (1.84 g, 6.6 mmol) was dissolved in *t*BuOH (100 mL) and water (50 mL) and LiOH·H_2O (0.545 g, 13 mmol) was added . The mixture was stirred at room temp. for 100 min and then diluted with water (100 mL). The mixture was acidified with 1 M $KHSO_4$, the

volitile components were removed in vacuo and the remaining aqueous phase was extracted with EtOAc (3 × 80 mL). The organic fractions were combined and extracted with 5% $NaHCO_3$ (3 × 100 mL), acidified with concd. HCl (15 mL), and extracted with EtOAc (3 × 100 mL). The organic fractions were washed with brine, dried with Na_2SO_4, and the solvents were removed in vacuo to yield pure **(+/-)-13** in quantitative yield. (1.74 g). All data matched **14** (below).

Enzymatic Resolution of (14): (+/−)-13 (0.41 g, 1.56 mmol) was dissolved in phosphate buffer (40 mL, pH 7) and $CoCl_2 \cdot 6H_2O$ (0.5 mg 2.1 μmol) and NaN_3 (0.8 mg, 13 μmol) were added to it. After addition of "Amano 30000" (0.144 g) the reaction was stirred for 5 d at 25 °C. The mixture was then acidified to pH 2−3 with a citric acid solution and extracted with EtOAc (3 × 100 mL). The organic layer was filtered through celite and washed with brine (2 × 150 mL). The organic solvents were removed in vacuo and co-evaperated with $CHCl_3$ (2 ×) to yield a white solid. The solid was then dissolved in H_2O (4 mL) and lyophilized giving **14** in 45% yield (185 mg) with an *ee* of 82% (based on rotation of **15**, see below) $[\alpha]_D = -161$ ($c = 0.39$, MeOH). $R_f = 0.31$ (hexane/EtOAc/AcOH, 1:1:0.05). ^{1}H NMR (CD_3OD): δ = 5.43 (s, 1 H, H_α), 6.78 (d, J = 8 Hz, 1 H, ArH), 6.90 (m, 2 H, ArH), 7.19 (t, J = 8 Hz, 1 H, ArH) ppm. ^{13}C NMR (CD_3OD): δ = 58.3, 116.0, 116.7, 120.1, 130.9, 117.4 (q, $J_{C,F}$ = 286 Hz), 137.9, 159.0, 158.6 (q, J_{CCF} = 45 Hz), 172.3 ppm. $C_{10}H_8F_3NO_4$ (263.17): calcd. C 45.64, H 3.06, N 5.32; found C 45.38, H 3.11, N 5.26.

(3-Hydroxy)phenylglycine·HCl (15): To **14** (0.1 g, 0.4 mmol) was added aqueous HCl (6 M, 4 mL) and acetic acid (4 mL) in a round bottom flask fitted with a condenser. The mixture was heated to 65 °C overnight and then evaporated to dryness to quantitatively yield the free amino acid hydrochloride salt (0.08 g) after lyophilization. $[\alpha]_D = -128$ ($c = 0.13$, 6 M HCl), 82% *ee*, (ref.[49] $[\alpha]_D = -151$ (97% *ee*, $c = 0.13$, 6 M HCl).

Fmoc-(3-Hydroxy)phenylglycine (16): (3-Hydroxy)phenylglycine·HCl (**15**, 0.245 g, 1.2 mmol) was dissolved in water (4 mL) and the pH was adjusted to 9 with NEt_3 (20 drops). Fmoc-OSu (0.410 g, 1.2 mmol) was dissolved in acetonitrile (4 mL) and added all at once to the stirred solution. The reaction was stirred at room temperature for 30 min and the pH was kept at 8.5 with NEt_3. The clear light-beige reaction mixture was neutralized with HCl and the acetonitrile was removed in vacuo. 1 M $KHSO_4$ (50 mL) was added and then extracted with EtOAc (3 × 100 mL). The organic solvents were then combined, washed with brine, and dried with Na_2SO_4. The solvent was removed in vacuo and purified using column chromatography (eluent: CH_2Cl_2/MeOH/AcOH, 10:1:0.1) yielding 0.448 g (96%) of **16** as a white powder, m.p. 173−174 °C. $R_f = 0.49$ (CH_2Cl_2/MeOH/AcOH, 5:1:0.1). ^{1}H NMR (CD_3CN): δ = 4.22 (m, 1 H, C*H*-Fmoc), 4.32 (d, 2 H, CH_2-Fmoc), 5.17 (d, 1 H, NH), 6.48 (d, 1 H, αCH), 6.79 (dd, 1 H, ArH), 6.75 (m, 2 H, ArH), 7.21 (t, 1 H, ArH), 7.28−7.43 (m, 4 H, Ar*H*-Fmoc), 7.66 (d, 2 H, Ar*H*-Fmoc), 7.81 (d, 2 H, Ar*H*-Fmoc) ppm. ^{13}C NMR (CD_3CN): δ = 47.9, 58.8, 67.5, 115.4, 116.3, 119.9, 121.0, 126.2, 128.4, 131.0, 142.1, 144.9, 158.1, 172.3 ppm. MS: m/z = 390.1 $[M + H]^+$, 412.1 $[M + Na]^+$. $C_{23}H_{19}NO_5$ (389.13): calcd. C 70.94, H 4.92, N 3.60; found C 71.04, H 4.86, N 3.51.

Fmoc-(3-TBDMSO)phenylglycine (17): 16 (390 mg, 1.0 mmol) was dissolved in pyridine (1.5 mL) and then a solution of TBDMS-Cl (380 mg, 2.5 mmol) in pyridine (2 mL) was added. The solution was fitted with a condenser, and stirred overnight at 70 °C. The solvent was removed in vacuo, then 1 M $KHSO_4$ (30 mL) and EtOAc (80 mL) were added to the residue. The organic phase was then washed with water, brine, and dried with Na_2SO_4. The solvent was removed in vacuo and the residue was purified with column chromatography (eluent: CH_2Cl_2/MeOH/AcOH, 20:0:0.1 gradient to 20:1:0.1). Lyophilization gave 382 mg (76%) of compound **17** as a white powder, m.p. 85 °C (dec.). $R_f = 0.6$ (CH_2Cl_2/MeOH, 3:1). $[\alpha]_D = -48.3$ ($c = 0.18$, MeOH). Chiral HPLC analysis: 80% *ee* R_t: 5.4 min (*S*), R_t: 18.6 min (*R*). ^{1}H NMR (CD_3OD): δ = 0.19 [s, 6 H, $Si(CH_3)_2$], 0.98 [s, 9 H, $SiC(CH_3)_3$], 4.22 (t, J = 6 Hz, 1 H, C*H*-Fmoc), 4.30, 4.32 (2 H, CH_2-Fmoc), 5.17 (s, 1 H, H_α), 6.78 (dd, J = 8.5, J = 1.5 Hz, 1 H, ArH), 6.94 (s, 1 H, ArH), 7.02 (d, J = 8 Hz, 1 H, ArH), 7.21 (t, J = 8 Hz, 1 H, ArH), 7.29 (t, J = 6.6 Hz, 2 H, ArH-Fmoc), 7.38 (t, J = 6.6 Hz, 2 H, ArH-Fmoc), 7.65 (d, J = 7.5 Hz, 1 H, ArH-Fmoc), 7.78 (d, J = 7.5 Hz, 2 H, ArH-Fmoc) ppm. ^{13}C NMR (CD_3OD): δ = −4.5, 18.1, 25.6, 46.8, 47.0, 57.7, 58.1, 67.3, 68.3, 99.9, 118.8, 119.1, 119.9, 120.4, 124.9, 125.0, 127.1, 127.7, 129.7, 130.0, 137.1, 138.7, 141.1, 141.2, 143.3, 143.5, 143.6, 143.7, 155.5, 156.0, 157.2, 173.2, 175.1 ppm. ES-MS: m/z = 504.4 $[M + H]^+$, 526.3 $[M + Na]^+$. $C_{29}H_{33}NO_5Si$ (503.21): calcd. C 69.16, H 6.60, N 2.78; found C 69.28, H 6.53, N 2.85.

Synthesis of Receptor 18: Synthesis was performed on Fmoc-Rink-Argogel® with a loading of 0.335 mmol/g**.** 0.5 g Resin was deprotected (see *Fmoc Deprotection)* and then coupled (see *Amino Acid Coupling*) with sequentially: Fmoc-Ala-OH, **17,** Fmoc-Leu-OH, and **7**. After cyclization, deprotection, and cleavage (see below), the crude material was purified using preparative HPLC to obtain a white solid in 30% yield (27 mg). R_t: 18.0 min. ^{1}H NMR (CD_3OH): δ = 0.94 [d, J = 5.5 Hz, 6 H, Leu$(CH_3)_2$), 1.30 (d_3, J = 7.0 Hz, 3 H, AlaCH), 1.60 (m, 2 H, LeuβCH$_2$), 1.69 (m, 1 H, LeuγCH), 2.14 (s, 2 H, NH_2), 3.10 (dd, J = 14.5, J = 5.5 Hz, 1 H, PheβCH), 3.53 (dd, J = 14.5, J = 4.0 Hz, 1 H, PheβCH), 4.24 (m, 3 H, 3NH), 5.10 (d, J = 5.5 Hz, 1 H, PhgαH), 6.06 (s, 1 H, PhgC^{2}H], 7.02, 7.56 (s, 2 H, NH_2), 7.07 (d, J = 7.0 Hz, 1 H, ArH), 7.13 (d, J = 8.5 Hz, 1 H, ArH), 7.19 (d, J = 7.5 Hz, 1 H, ArH), 7.37 (t, J = 7.5 Hz, 1 H, ArH), 7.57 (s, 1 H, ArH), 7.99 (s, 1 H, ArH), 8.24 (d, J = 6.5 Hz, 1 H, NH), 8.31 (d, J = 4.5 Hz, 1 H, NH) ppm. ES-MS: m/z = 541.3 $[M + H]^+$, 563.3 $[M + Na]^+$.

Fmoc Deprotection: To the resin was added 20% piperidine in NMP (3 × 8 min) and agitated by bubbling nitrogen through the solution. The resin was washed with NMP (3 × 2 min) and CH_2Cl_2 (3 × 2 min). The "Kaiser test"[50] was performed on a small portion of beads for elucidation of the free amine terminus.

Amino Acid Coupling: To the reaction vessel containing the deprotected resin (500 mg) was added sequentially the appropriate amino acid (4 equivalents, 0.67 mmol) in NMP (3 mL), BOP (296 mg, 0.67 mmol) in NMP (3 mL) and DIPEA (233 μL, 1.34 mmol). The reaction mixture was agitated by bubbling nitrogen through the solution for 75 min. For amino acids **7** and **17**, 2.5 equivalents were used with reaction times of 150 min. The beads were then washed with NMP (3 × 2 min) and CH_2Cl_2 (3 × 2 min) and the "Kaiser test" was performed on a small portion of beads. The negative test provided evidence for successful coupling.

On-bead Cyclization: TBAF·3H_2O (1.29 g, 4.1 mmol) was dissolved in DMF (45 mL) and dried with molecular sieves (4 Å, 3 h). The TBAF solution in DMF (11 mL) was then added to the resin and shaken (40 h). The resin was washed exhaustively with NMP, CH_2Cl_2, NMP, CH_2Cl_2, 3% AcOH in dioxane, Et_2O, NMP, and CH_2Cl_2. (each 2 × 2 min).

Deprotection and Cleavage from the Resin: The resin was treated with (TFA/water/TIS, 95:2.5:2.5) for 3 h. The resin was washed with TFA (1 mL, 2 × 2 min) and the organic solvents were removed

in vacuo. After coevaporation with ethanol (2 × 10 mL), the solution was lyophilized from a 1:1 mixture of acetonitrile and water.

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