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Synthesis of a Radioiodinated Park Nucleotide Analog: A New Tool for Antibacterial Screen Development

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SUMMARY

The Park nucleotide is an important biological building block used in the construction of bacterial cell walls. Herein, we describe the synthesis of a radiolabeled Park nucleotide analog, *p*-iodophenoxyacyl-Ala-(D)-*iso*-Glu-Lys-(D)-Ala-(D)-Ala-OH-[^{125}I], using electrophilic destannylation. Anti-Park nucleotide antibody binding assays using a scintillation proximity assay (SPA) system showed good recognition of the radiolabeled surrogate. This methodology could be used for establishing a screen to identify inhibitors of peptidoglycan biosynthesis.

KEYWORDS: Park nucleotide, radioiodinated, screen, peptidoglycan

INTRODUCTION

The emergence of bacterial strains resistant to glycopeptide and ß-lactam antibiotics has prompted research toward the discovery of new, novel antibiotics that inhibit earlier steps in the peptidoglycan pathway peptidoglycan. [1,2] As part of this effort, biological screens were developed which focused on the MURC, -D, -E and -F genes that

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sequentially add amino acids to uridine diphosphate-*N*-acetyl-muramic acid (UDP-MurNAc) and complete the synthesis of the Park nucleotide, UDP-MurNAc-Ala-(D)-*iso*-Glu-R^3-(D)-Ala-(D)-Ala-OH. [3] The R^3 residue is species specific and can be (L)-lysine, *meso*-diaminopimelic acid, (L)-ornithine, (L)-diaminobutyric acid, (L)-homoserine or (L)-alanine (Figure 1).

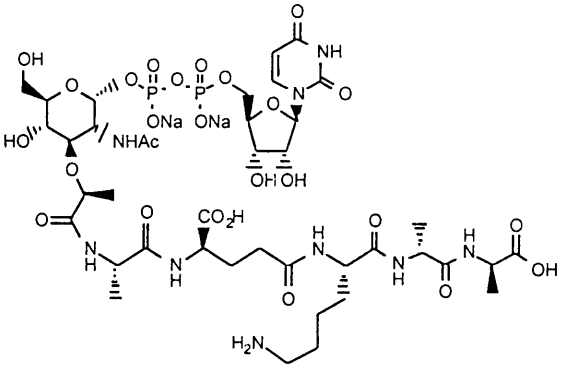

Figure 1. The Park nucleotide with (L)-lysine at R^3.

This paper describes the synthesis and binding properties of a radioiodinated analog of the Park nucleotide wherein the uridine diphosphate-*N*-acetyl-muramyl moiety has been replaced with a simple [^{125}I]-aryl isostere. This radiolabeled analog could compete with unlabeled Park nucleotide for a Park nucleotide specific antibody (anti-PN) in a scintilation proximity assay.

RESULTS

Preparation of an anti-Park nucleotide antibody was acheived through Keyhole Limpet Hemocyanin (KLH) conjugate methodology.[4] The Park nucleotide most commonly encountered in Gram positive cocci contains (L)-lysine at the R^3 residue. Therefore, the KLH conjugated immutagen was randomly coupled to the pentapeptide, H-Ala-(D)-*iso*-Glu-Lys-(D)-Ala-(D)-Ala-OH using glutaraldehyde cross linking. The solicited antibody had a high degree of recognition for the corresponding Park nucleotide and was further purified by affinity chromatography using immobilized Park nucleotide.

The anti-PN antibody specifity was probed with a variety of peptides.[5] Those results suggested that a simplified Park nucleotide analog with a radiolabeled aryl group located at

the pentapeptide's N-terminus could be employed in an SPA format. The pentapeptide that was used in the generation of the anti-PN antibody could not be selectively functionalized at the N-terminal due to the presence of the unprotected lysine ε-amino residue. To overcome this problem, we synthesized a differentially protected pentapeptide that allowed for selective N-terminal modification. We chose to synthesize the *p*-[^{125}I]-phenoxyacetyl containing analog (**1**) because we could employ mild electrophilic destannylation as the last synthetic step.[6]

The synthesis of **1** required an orthogonal protection strategy that employed methyl ester, Cbz, Boc, and trifluoroacetamide groups and is outlined in Figure 2. We also explored other protection stratagies that were to avoid proto-destannylation during the peptide deprotecton. For example, we synthesized the allyl ester, alloc amide analog of intermediate **8** but were unable to deprotect the alloc amide without forcing proto-destannylation.

The synthesis of **1** proceeded from commercially available Cbz-(D)-Ala-(D)-Ala-OMe, Boc-Lys-OH, Boc-Ala-OH and Boc-(D)-Glu-OMe through standard protection/deprotection strategies to provide the fully protected pentapeptide Boc-Ala-(D)-*iso*-Glu(OMe)-Lys(N$^{\varepsilon}$-$COCF_3$)-(D)-Ala-(D)-Ala-OMe (**6**) in good overall yield. Selective N-terminal deprotection of **6** with 50% TFA in dichloromethane at room temperature gave **7**.[7]

p-(trimethyltin)phenoxy acetic acid was synthesized according to a previously published procedure[8] and was used for the subsequent conversion of **7** into crude stannylated product **8**. This was purified by trituration with ethyl acetate followed by flash chromatography on deactivated silica gel.[9] Removal of the peptide protecting groups was accomplished with three equivalents of LiOH in THF/H_2O to provide the dilithium salt **9**. The product was kept as the carboxylate salt so as to avoid protodestannylation. Finally, established iododestannylation protocols using chloramine-T and cold NaI provided **1**.[10] The reactions were monitored by reverse phase HPLC using pH 7.0 triethylammonium phosphate buffer.

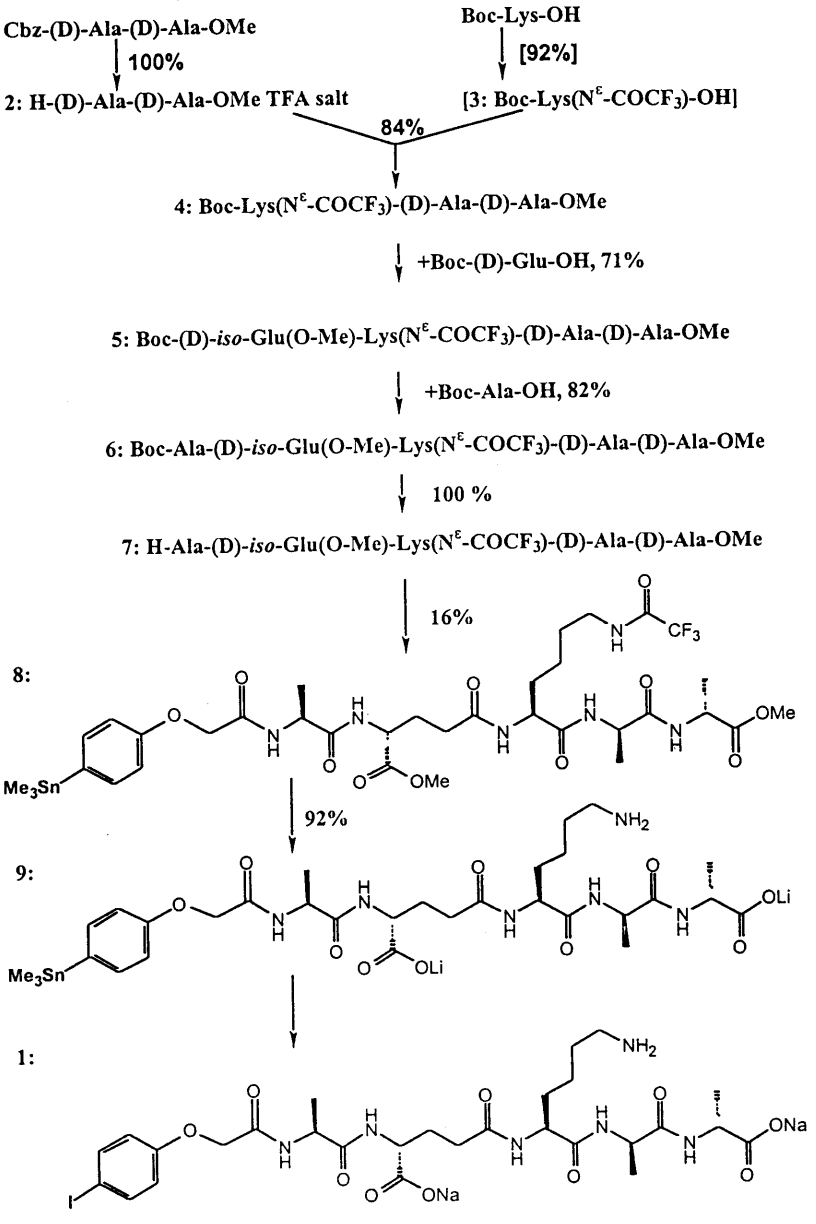

Figure 2. Synthesis of *p*-Iodo V capped pentapeptide.

Competition between ^{125}I-pentapeptide and cold pentapeptide 1 for binding to the anti-Park nucleotide antibody

Anti-Park nucleotide antibody coated scintillation proximity assay beads were obtained by conjugating the purified antibody with anti-rabbit SPA beads (from Amersham) at the ratio of 10 μg antibody/mL per bead. Various concentrations of cold pentapeptide **1** in 50 μL phosphate buffer were mixed with 15 μL of anti-PN coated SPA beads in an OptiPlate from Packard. A 250 μL aliquot of the diluted ^{125}I-pentapeptide (**1**) solution (a 100,000 fold dilution from the radioiodination preparation in 10 mM phosphate buffer pH 7.0) was added to each well and allowed to settle overnight before being counted. Results shown in Figure 3 indicate that ^{125}I-pentapeptide (**1**) is an effective competitor of cold pentapeptide **1**. The detection sensitivity in the current experimental condition is very good. Pentapeptide concentrations of approximately 0.01 μM can be measured by this method.

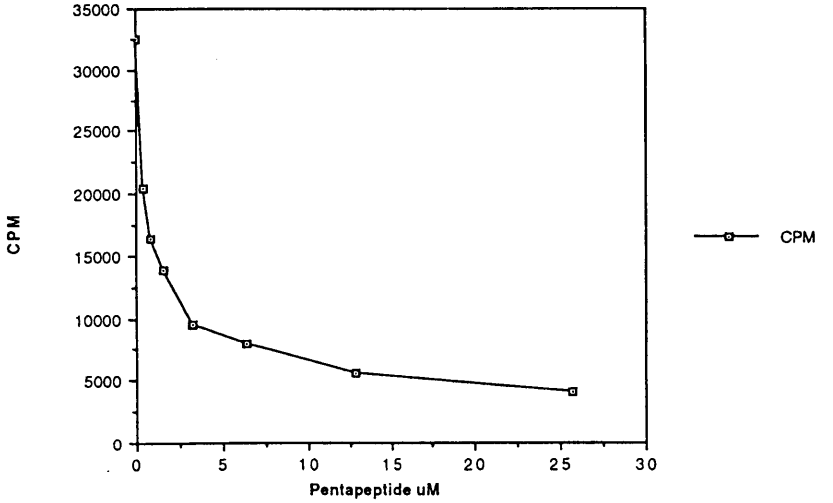

Figure 3: I-125 Pentapeptide (2.5 μM) binding to antibody: competition with cold pentapeptide

Detection of Park nucleotide using ^{125}I-pentapeptide (1) in an SPA system

Park nucleotide was the product of a MURE and MURF enzyme coupled reaction containing the following reagents: 20 mM $MgCl_2$, 1 mM ATP, 50 μM UDPMurNAc-Ala-(D)-Glu-OH, 2 mM H-Lys-OH, 0.2 mM H-(D)-Ala-(D)-Ala-OH, and 1 μg/ml each of MurE and MurF enzyme in a 50 mM Tris buffer at pH 8.0. The reaction was carried out at room temperature for 2 h. A parallel reaction without the added enzymes was tested in order to

determine the interference effect of the reagents on SPA signal. In the detection assay, samples containing a 50 μL aliquot of diluted reaction solution, 50 μL of anti-PN coated SPA beads, 10 μL of 0.36 μM ^{125}I-pentapeptide (**1**), and 150 μL of 50 mM phosphate buffer pH 7.0 were kept at room temperature overnight and then counted. Results summarized in Table 1 indicate that there was a slight quenching effect by the reagents. However, this effect was greatly diminished after a 1/120 dilution. In the normal reaction mixture, competition due to Park nucleotide could still be detected after a 1/120 dilution. Using the cold pentapeptide **1** competition curve, the Park nucleotide produced in this reaction was detected at approximately 3.6 μM.

Table1: Competition between ^{125}I-pentapeptide and MurE + MurF reaction mixture in an SPA system

	Rx agents - MURE - MURF					Rx agents + MURE + MURF			
Dilution	1/15	1/30	1/60	1/120	1/240	1/30	1/60	1/120	1/240
CPM	3249	3420	3501	3810	3748	945	1130	1486	1833

Cold pentapeptide **1** (mM)	0	0.037	0.075	0.15	0.3	1
CPM	3847	1346	1010	853	753	628

CONCLUSION

We successfully synthesized a radiolabeled Park nucleotide analog and demonstrated it to be a good competitor for binding to an anti-Park nucleotide antibody in a SPA format. The assay format is amenable to large scale screen development directed toward identification of inhibitors of bacterial peptidoglycan biosynthesis.

EXPERIMENTAL

General. The following abbreviations are used: Boc, *tert*-butyloxycarbonyl; DCC, *N,N'*-dicyclohexylcarbodiimide; Bzl, benzyl; DIEA, diisopropylethylamine; Cbz, carbobenzyloxy; HOBt, 1-hydroxybenzeotriazole; HCl, hydrochloric acid; EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide; Et_2O, diethyl ether; EtOAc, ethylacetate; Et_3N, triethylamine; TFA,

trifluoroacetic acid; THF, tetrahydrofuran; $CHCl_3$, chloroform; MeOH, methanol; CH_2Cl_2, dichloromethane; Glu, glutamic acid; Ala, alanine; Lys, lysine. Unless otherwise indicated, all amino acids were of the natural (L) configuration. Other abbreviations used were those recommended by the IUPAC-IUB Commission (Biochem. J. 219: 345 (1984)).

Physical Methods. NMR spectra were recorded on a General Electric QE 300 spectrometer and are reported in ppm relative to TMS. Coupling constants (*J*) are listed in Hz. Optical rotations were recorded on a Perkin-Elmer Model 241 polarimeter. Mass spectra were taken on VG ZAB-3 or Varian-MAT Model 731 mass spectrometers. Elemental analyses were performed by the physical chemistry department of The Lilly Research Laboratories. Satisfactory microanalyses were obtained for all compounds: C ± 0.28, H ± 0.26, N ± 0.14. Thin-layer chromatography (TLC) experiments were performed on Merck silica gel 60 F_{254} plates. All reactions were run under an argon atmosphere in anhydrous solvents unless otherwise stated.

H-(D)-Ala-(D)-Ala-OMe (**2**):

To a suspension of Cbz-(D)-Ala-(D)-Ala-OMe (2.36 g, 7.7 mmol) and $Pd(OH)_2/C$ (1.58 g) in MeOH (25 mL) was added TFA (0.68 mL, 8.8 mmol). The reaction vessel was alternately evacuated and filled with hydrogen (3 X) and then blanketed with hydrogen (balloon pressure) at 23°C for 3 h. The solution was filtered through talc and solvent removed under reduced pressure to yield **2** (2.20 g, 100%). This was used without further purification. ^{1}H NMR (MeOH-d_4): δ 1.47 (d, 3 H, J = 7.3 Hz, Ala-C**H**$_3$), 1.58 (d, 3 H, J = 7.0 Hz, Ala-C**H**$_3$), 3.78 (s, 3 H, -OC**H**$_3$), 4.02 (q, 1 H, J = 7.0 Hz, Chiral H), 4.52 (m, 1 H, Chiral H). FDMS (70 eV) *m/z* (relative intensity): 175 (M^+ + 1, 100), 350 ($2M^+$ + 2, 10).

Boc-Lys-(N^ε-$COCF_3$)-OH (**3**):

To a suspension of Boc-Lys-OH (2.75 g, 11.2 mmol) in MeOH (30 mL) was added Et_3N (1.6 mL, 11.5 mmol) and ethyl trifluoroacetate (1.6 mL, 13.4 mmol). The clear yellow solution was stirred at 23°C for 3 h. The solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2. The yellow solution was washed with 0.1 N HCl and water and dried over $MgSO_4$. The solution was filtered and the solvent removed under

reduced pressure to yield **3** (2.62 g, 68%). This compound was hygroscopic and was used immediately without further purifcation. ^{1}H NMR (CDCl3): δ 1.43 (bs, 11 H, -C(CH_3)$_3$ + -CH_2-), 1.63 (m, 2 H, -CH_2-), 1.72 (m, 1 H, -CH_2-), 1.86 (m, 1 H, -CH_2-), 3.36 (m, 2 H, -CH_2NHCOCF_3), 4.26 (m, 1 H, chiral H), 5.23 (d, 1 H, J = 7.5 Hz, -NHC(O)OC(CH_3)$_3$), 7.02 (bm, 1 H, NHCOCF_3).

Boc-Lys-(N$^\varepsilon$-COCF_3)-(D)-Ala-(D)-Ala-OMe (**4**):

A solution of **2** (2.62 g, 7.7 mmol), **3** (2.30 g, 8.0 mmol), and HOBT (1.31 g, 8.6 mmol) in THF (20 mL) was stirred at 0°C. DCC (1.75 g, 8.5 mmol) was added and the solution was stirred at 0°C for 5 h. The solution was filtered and condensed under reduced pressure. The residue was dissolved in EtOAc (50 mL) and washed with 0.1 N $NaHCO_3$ (3 X 50 mL), 0.1 N HCl (3 X 50 mL), and water (3 X 50 mL). The solution was dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure to yield a yellow oil. Flash column chromatography (75% EtOAc / 25%hexanes) yielded **4** (2.58 g, 67%). ^{1}H NMR ($CDCl_3$): δ 1.38 (d, 3 H, J = 7.0 Hz, Ala-CH_3), 1.40 (d, 3 H, J = 7.1 Hz, Ala-CH_3), 1.42 (s, 11 H, -C(CH_3)$_3$ + -CH_2-), 1.63 (m, 3 H, -CH_2-), 1.82 (m, 1 H, -CH_2-), 3.36 (m, 2 H, -CH_2NHCOCF_3), 3.73 (s, 3 H, -OCH_3), 4.10 (m, 1 H, Lys chiral H), 4.51 (m, 2 H, Ala chiral H's), 5.27 (bd, 1 H, -NHC(O)OC(CH_3)$_3$), 6.88 (d, 1 H, J = 7.4 Hz, -NH-), 7.01 (bm, 2 H, -NH-). FDMS (70 eV) *m/z* (relative intensity): 499 (M^+ + 1, 100). Anal. Calcd. for $C_{20}H_{33}N_4O_7F_3$: C, 48.19; H, 6.67; N, 11.24. Found: C, 48.43; H, 6.64; N, 11.19.

Boc-(D)-*iso*-Glu-(OMe)-Lys-(N$^\varepsilon$-COCF_3)-(D)-Ala-(D)-Ala-OMe (**5**):

A solution of **4** (0.74 g, 1.48 mmol) in 20 mL ice cold 20% TFA in CH_2Cl_2 was stirred at 0°C for 1 h. The solvent was removed under reduced pressure to provide a colorless oil. In a separate reaction vessel Boc-(D)-Glu-OMe (0.45 g, 1.7 mmol) and *N*-hydroxysuccinimide (0.24 g, 2.1 mmol) were dissolved in THF (20 mL) at 0°C. DCC (0.39 g, 1.9 mmol) was added, and the solution was stirred at 0°C for 1 h, then at 23°C for 20 h. This solution was filtered directly into the vessel containing the tripeptide TFA salt and Et_3N (0.46 mL, 3.3 mmol) was added. The solution was stirred at 23°C for 24 h. The solvent was removed and the residue was partitioned between CH_2Cl_2 (20 mL) and water (20 mL). The organic layer was washed with water (3 X 20 mL) and brine (1 X 20 mL) and dried over $MgSO_4$. Filtration and removal of solvent under reduced pressure yielded a white

residue. Flash column chromatography (95% $CHCl_3$ / 5% MeOH) yielded **5** (0.57 g, 60%). ^1H NMR (MeOH-d_4): δ 1.34 (d, 3 H,J = 7.1, Ala-CH_3), 1.39 (d, 3 H,J = 7.5, Ala-CH_3), 1.42 (s, 11 H, -$C(CH_3)_3$ + -CH_2-), 1.57 (m, 2 H, -CH_2-), 1.72 (m, 2 H, -CH_2-), 1.87 (m, 1 H, -CH_2-), 2.07 (m, 1 H, -CH_2-), 2.32 (t, 2 H, J = 7.4 Hz, -CH_2-), 3.26 (m, 2 H, -$CH_2NHCOCF_3$), 3.67 (s, 3 H, -OCH_3), 3.69 (s, 3 H, -OCH_3), 4.09 (m, 1 H, Lys chiral H), 4.17 (t, 1 H, J = 7.0 Hz, Glu chiral H), 4.35 (m, 2 H, Ala chiral H's). $[a]^{20}_D$ = 0.159 (c = 1, MeOH). FDMS (70 eV) *m/z* (relative intensity): 511 (20), 642 (M^+ + 1, 100). Anal. Calcd. for $C_{26}H_{42}N_5O_{10}F_3$: C, 48.67; H, 6.60; N, 10.91. Found: C, 48.47; H, 6.50; N, 10.74.

Boc-Ala-(D)-*iso*-Glu-(OMe)-Lys-($N^ε$-$COCF_3$)-(D)-Ala-(D)-Ala-OMe (**6**):

A solution of **5** (1.75 g, 2.7 mmol) in 20 mL ice cold 20% TFA in CH_2Cl_2 was stirred at 0°C for 1 h. The solvent was removed under reduced pressure to yield a colorless oil. In a separate reaction vessel Boc-Ala-OH (0.62 g, 3.3 mmol) and *N*-hydroxysuccinimide (0.46 g, 4.0 mmol) were dissolved in DMF (20 mL) at 0°C. DCC (0.81 g, 3.9 mmol) was added, and the solution was stirred at 0°C for 1 h, then at 23°C for 20 h. This solution was filtered directly into the vessel containing the deprotected tetrapeptide TFA salt and Et_3N (1.4 mL, 10 mmol) was added. The solution was stirred at 23°C for 24 h, after which the solvent was removed under reduced pressure to yield a brown oil. Flash column chromatography of this oil (95% $CHCl_3$ / 5% MeOH) yielded **6** (1.15 g, 60%). ^1H NMR (MeOH-d_4): δ 1.39 (d, 3 H, J = 7.2 Hz, Ala-CH_3), 1.43 (d, 3 H, J = 7.2 Hz, Ala-CH_3), 1.47 (d, 3 H, J = 7.4 Hz, Ala-CH_3), 1.52 (bs, 11 H, -$C(CH_3)_3$ + -CH_2-), 1.64 (m, 2 H, -CH_2-), 1.79 (m, 3 H, -CH_2-), 2.07 (m, 1 H, -CH_2-), 2.35 (m, 2H, -CH_2-), 3.37 (m, 2 H, -$CH_2NHCOCF_3$), 3.75 (s, 3 H, -OCH_3), 3.77 (s, 3 H, -OCH_3), 4.14 (m, 1 H, Lys Chiral H), 4.25 (t, 1 H, J = 6.9 Hz, Glu Chiral H), 4.45 (m, 3 H, Ala Chiral H's). $[a]^{20}_D$ = 0.099 (c = 1, MeOH). FDMS (70 eV) *m/z* (relative intensity): 582 (32), 713 (M^+ + 1, 100). Anal. Calcd. for $C_{29}H_{47}N_6O_{11}F_3$: C, 48.87; H, 6.65; N, 11.79. Found: C, 49.04; H, 6.57; N, 11.80.

H-Ala-(D)-*iso*-Glu-(OMe)-Lys-($N^ε$-$COCF_3$)-(D)-Ala-(D)-Ala-OMe (**7**):

Compound **7** was deprotected as described for **4**. ^1H NMR (MeOH-d_4): δ 1.38 (d, 3H, J = 7.3 Hz), 1.43 (d, 3H, J = 7.02 Hz), 1.43 (d, 3H, J = 7.02 Hz), 1.58 (m, 2H), 1.69 (m, 1H), 1.92 (m, 1H), 2.28 (m, 1H), 2.36 (m, 2H), 3.31 (m, 2H), 3.71 (s, 3H), 3.75 (s, 3H), 4.02 (q, 1H, J = 7.02 Hz), 4.27 (dd, 1H, J = 5.80, 8.55 Hz), 4.40 (q, 1H, J = 7.02 Hz), 4.46 (q,

1H, J = 7.32 Hz), 4.52 (dd, 1H, J = 4.88, 9.77 Hz). FDMS (70 eV)) *m/z* (relative intensity): 613 (M^+, 100). Anal. Calcd. for $C_{24}H_{39}N_6O_9F_3/C_2HF_3O_2$: C, 42.98; H, 5.55; N, 11.56. Found: C, 43.72; H, 5.45; N, 11.01.

p-[Trimethylstannyl]phenoxyacetyl-Ala-(D)-*iso*-Glu-(OMe)-Lys-(N^{ε}-$COCF_3$)-(D)-Ala-(D)-Ala-OMe (**8**):

A solution of *p*-[trimethylstannyl]phenoxyacetic acid, tetrabutyl ammonium salt (348 mg, 0.65 mmol) and 2-chloro-4,6-dimethoxytriazine (169 mg, 0.96 mmol) in CH_2Cl_2 (20 mL) was stirred at room temperature for 5 hours. The solution was cooled to 0°C and *N*-methylmorpholine (150 µl, 1.36 mmol) was added. This solution was transferred to a reaction vessel containing **7** (233 mg, 0.32 mmol) and stirred at 23°C for 24 h. The solvent was removed under reduced pressure and the residue purified by flash column chromatography (EtOAc) to yield 231 mg of a white solid. Tritration of this solid with EtOAc yielded **8** (97 mg, 16%). ^{1}H NMR (MeOH-d_4): δ 0.31 (s, 9 H, -Sn(CH_3)$_3$), 1.42 (d, 3 H, J = 7.3 Hz, Ala-CH_3), 1.46 (d, 3 H, J = 7.5 Hz, Ala-CH_3), 1.49 (d, 3 H, J = 7.4 Hz, Ala-CH_3), 1.64 (m, 2 H, -CH_2-), 1.78 (m, 4H, -CH_2-), 2.00 (m, 1 H, -CH_2-), 2.28 (m, 1 H, -CH_2-), 2.36 (m, 2H, -CH_2-), 3.36 (m, 2 H, -$CH_2NHCOCF_3$), 3.73 (s, 3 H, -OCH_3), 3.77 (s, 3 H, -OCH_3), 4.26 (m, 1 H, chiral H), 4.44 (m, 3 H, Ala Chiral H's), 4.56 (m, 1 H, Chiral H), 4.65 (s, 2 H, -C(O)CH_2Ar), 7.06 (d, 2 H, J = 8.1 Hz, Ar), 7.47 (d, 2 H, J = 8.6 Hz, Ar). FABMS (70 eV) *m/z* (relative intensity): 911 (M^+, 100). Exact mass Calcd. for $C_{35}H_{54}N_6O_{11}F_3Sn$: 911.2825. Found: 911.2837.

p-[Trimethylstannyl]phenoxyacetyl-Ala-(D)-*iso*-Glu-Lys-(D)-Ala-(D)-Ala-OH, di-lithium salt (**9**):

To a solution of **8** (43 mg, 0.05 mmol) in 2 mL 1:1 THF/water was added 1.0 M LiOH (0.16 mL, 0.16 mmol), and the solution was stirred at 23°C for 24 h. The solvent was removed under reduced pressure to yield **9** (35 mg, 92%). ^{1}H NMR (MeOH-d_4): δ 0.30 (bs, 9 H, -Sn(CH_3)$_3$), 1.47 (bm, 9 H, Ala-CH_3), 1.70 - 2.05 (bm, 10 H), 2.23 - 2.38 (m, 4 H), 4.24 (m, 1 H), 4.46 (m, 3 H), 4.56 (m, 1 H), 4.68 (bs, 2 H, -C(O)CH_2Ar), 7.06 (d, 2 H, J = 8.1 Hz), 7.47 (d, 2 H, J = 8.6 Hz).

p-Iodophenoxyacetyl-Ala-(D)-*iso*-Glu-Lys-(D)-Ala-(D)-Ala-OH-[^{125}I] (**1**):

A 4 μL aliquot from a solution of trimethylstannyl-pentapeptide **9** (4 mg/ml) in 0.2 M phosphate buffer at pH 7.0 was diluted with 22.2 μL of buffer and combined with 7.6 μL of Na^{125}I (103.89 mCi/mL, 17.4 Ci/mg from DuPont NEN). A 4 μL aliquot of freshly prepared chloramine-T (2 mg/mL in buffer) was added. After 5 min. in ice, a 2 μL portion of NaI (2 mg/mL in water) was added and the reaction mixture allowed to stand at 0°C for an additional 15 min. Finally the reaction was terminated by the addition of 4 μL of sodium meta-bisulfite solution (2 mg/mL in water). The solution thus obtained was stored at 4°C and diluted as necessary for the competition experiments.

p-Iodophenoxyacetyl-Ala-(D)-*iso*-Glu-Lys-(D)-Ala-(D)-Ala-OH (**1**):

This compound was synthesized quantitatively in the same fashion as hot [^{125}I]-(**1**) shown above, with the exception that cold NaI was used. ^{1}H NMR (DMSO-d_6, 50°C): δ 1.39 (d, 7.2 Hz, 3 H), 1.43 (d, 7.2 Hz, 3 H), 1.47 (d, 7.2 Hz, 3 H), 1.40-1.43 (m, 2 H), 1.44-1.60 (m, 3 H), 1.62-1.75 (m, 2 H), 1.80-2.01 (m, 1 H), 2.10-2.24 (m, 2 H), 3.82 (t, J = 7.2 Hz, 1 H), 3.83-3.93 (m, 1 H), 4.12 (t, J = 7.2 Hz, 1 H), 4.16-4.22 (m, 1 H), 4.42-4.52 (m, 3 H), 7.45 (bd, 1 H), 7.70 (bd, 3 H), 7.82 (d, J = 2.9 Hz, 2 H), 8.04 (bd, 1 H), 8.20 (bd, 1 H), 8.71 (bd, 1 H). FABMS (70 eV) *m/z* (relative intensity): 606 (M^+, 100).

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7. Intermediate **7** was deprotected with 3 eq. of NaOH in dioxane and converted to the TFA salt. This material compared favorably to the TFA salt of commercially available pentapeptide (Bachem) and to the reported literature optical rotation (Goles, D. et al.- Croat. Chem. Acta 48: 365 (1976)). ^{1}H NMR (DMSO-d_6): δ 1.19 (d, J = 7.2 Hz, 3 H), 1.27 (d, J = 7.2 Hz, 3 H), 1.37 (d, J = 7.2 Hz, 3 H), 1.45-1.55 (m, 3 H), 1.56-1.65 (m, 1 H), 1.73-1.83 (m, 1 H), 1.95-2.05 (m, 1 H), 2.12-2.25 (m, 2 H), 2.70-2.80 (m, 2 H), 4.13-4.35 (m, 4 H), 7.82 (bs, 3 H), 8.08 (bd, 1 H), 8.12 (bd 1 H), 8.15 (bd, 4 H), 8.72 (bd, 1 H). $[a]^{20}_D$ = 16.18 (c = 1, H_2O). Exact mass Calcd. for $C_{20}H_{37}N_6O_8$: 489.2673 Found: 489.2679.
8. (a) Blaszczak L. C., Halligan N. G., Seitz D. E.-J. Labelled Compd. Radiopharmacol. 27: 401 (1989); (b) Preston D. A., Wu C. Y. E., Blaszczak L. C., Seitz D. E., Halligan N. G.-Antimic. Agents Chem. 34: 718 (1990)
9. Normal phase silica gel was deactivated by stirring in acetone followed by removing solvent under vacuum at room temperature.
10. Nonradioactive **1** was also synthesized utilizing solid support chemistry on Wang resin. The intermediate H-Ala-(D)-*iso*-Glu(O-*t*-Bu)-Lys(N_ε-Boc)-(D)-Ala-(D)-Ala-O-Wang resin was capped with *p*-Iodophenoxyacetic acid using EDCI/HOBT and cleaved with 95%TFA:2.5%H_2O:2.5%Et_3Si to provide **1.**