Examination of Acylated 4-Aminopiperidine-4-carboxylic Acid Residues in the Phosphotyrosyl+1 Position of Grb2 SH2 Domain-Binding Tripeptides

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A 4-aminopiperidine-4-carboxylic acid residue was placed in the pTyr+1 position of a Grb2 SH2 domain-binding peptide to form a general platform, which was then acylated with a variety of groups to yield a library of compounds designed to explore potential binding interactions, with protein features lying below the βD strand. The highest affinities were obtained using phenylethyl carbamate and phenylbutyrylamide functionalities.

Introduction

Significant research has been devoted to enhancing binding affinity, cellular potency, and metabolic stability of Grb2 SH2 domain binding antagonists.[1] These efforts have included the use of bend-inducing 1-aminocyclohexanecarboxylic acid (Ac_6c[a]) residues in the pTyr+1 position of "pTyr−Xxx−Asn"-containing peptides.[2] However, little has been reported regarding potential binding interactions that might result by extending functionality outward from the Ac_6c side chain ring.[3] Residues Val99, Phe101, Gln106, Phe108, Ser141, Asn143, and Gln144 of the Grb2 SH2 domain lie along and below the protein βD strand (nomenclature based on structural homology among SH2 domains).[4] These residues form a region proximal to the pTyr+1 position that could potentially interact with functionality derived from suitably modified Ac_6c residues. Starting from a previously reported Ac_6c-containing tripeptide inhibitor,[5] replacement of the Ac_6c residue with 4-aminopiperidine-4-carboxylic acid (Apc)[6] and elaboration at the piperidinyl 1-position would yield analogues of type **8** (Scheme 1) that could potentially interact with this region of the protein.

To explore these potential interactions, preparation of a small library of Apc-containing final products (**8**) was accomplished using the known intermediates **1**,[7] **2**,[8] and **4**,[5] as shown in Scheme 1. Of note, coupling **3** with the protected pTyr mimetic **4** required HOAt (1-hydroxy-7-azabenzotriazole) active ester methodology due to steric hindrance.[5] Hydrogenolytic cleavage of the Apc 1-amino-Cbz group in **5** gave the free piperidino-containing analogue **6**, which upon acylation, yielded the series of analogues **7**. The products **7a**−**d** and **7j** were prepared using chloroformates, while HOBT active ester coupling was employed for compound **7e**, and acid chlorides were used for **7f**, **7g**, and **7i**. The synthesis of urea **7h** employed an isocyanate. Treatment of the *tert*-butyl-protected products **7** with TFA gave the final products **8a**−**j**, which were purified by HPLC.

Evaluation of Inhibitors in a Grb2 SH2 Domain-Binding System. Kinetic binding data for peptides **8a**−**j** were obtained by surface plasmon resonance experiments that measured the direct interaction of synthetic ligands with surface-bound Grb2 SH2 domain protein (Table 1).[9] The pTyr+1 Ac_6c-containing peptide has a reported Grb2 SH2 domain-binding affinity of 24 nM.[3] Methyl carbamate **8a** represents the simplest analogue examined in the current study and its binding affinity (K_D = 106 nM) serves as a reference for comparison with the remainder of the series. Elaboration of the methyl carbamate to a 2-methoxyethyl carbamate (**8b**, K_D = 202 nM) resulted in a loss of affinity, and introduction of an aromatic group resulted in further loss of affinity when a one-methylene spacer was employed (benzyl carbamate **8c**, K_D = 320 nM). However, adding an additional methylene group to **8c** increased affinity 10-fold (phenylethyl carbamate **8d**, K_D = 35.7 nM). Molecular modeling simulations were performed on compounds **8a**, **8c**, and **8d** bound to the Grb2 SH2 domain protein with the Insight II2000.0/Discover 97.0 modeling package (Molecular Simulations, Inc., San Diego, CA) using the cff91 force field. When the crystal structure of mAZ-pY-(αMe)pY-N−NH_2 bound to the Grb2 SH2 domain (1JYQ.pdb) was used, the peptide ligand was modified using 3D-sketcher tools to yield inhibitors **8a**, **8c**, **8d**, and **8j**, and these were subjected to energy minimization and molecular dynamics simulations (see the Supporting Information). It was found that **8d** exhibited the best extension within the target region below the βD strand (Figure 1).

Replacement of the carbamoyl oxygen of **8d** with a methylene yielded the corresponding phenylbutyrylamide **8e** (K_D = 27 nM). Addition of one methylene to **8e** gave the phenylvaleramide **8f**, which suffered a significant loss of binding affinity (K_D = 2180 nM). This indicated that a chain extension of 3 units beyond the carbonyl was preferable to 4 units. Accordingly, the indole-containing analogue **8g** was prepared to present a 3-unit extension between the phenyl ring and the amide carbonyl (the indolyl nitrogen is considered as part of the extending chain). Likewise, analogue **8h** was prepared as a urea variant of carbamate **8d**. The affinities of **8g** (K_D = 458 nM) and **8h** (K_D = 272 nM) were less than **8d**, as was the affinity of sulfonamide **8i** (K_D = 2140 nM).

The analogue **8j** contains a 4-carboxybenzyl carbamate moiety. Previous work has shown that pTyr or pTyr mimetics placed at the pTyr+1 position can promote high affinity binding by interacting with the Grb2 SH2 domain Arg142 residue.[3,10]

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[a] Abbreviations: pTyr, phosphotyrosyl; Ac_6c, aminocyclohexanecarboxylic acid; Apc, 4-aminopiperidine-4-carboxylic acid; HOAt, 1-hydroxy-7-azabenzotriazole.

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Scheme 1. Synthesis of Analogues Used in the Current Study

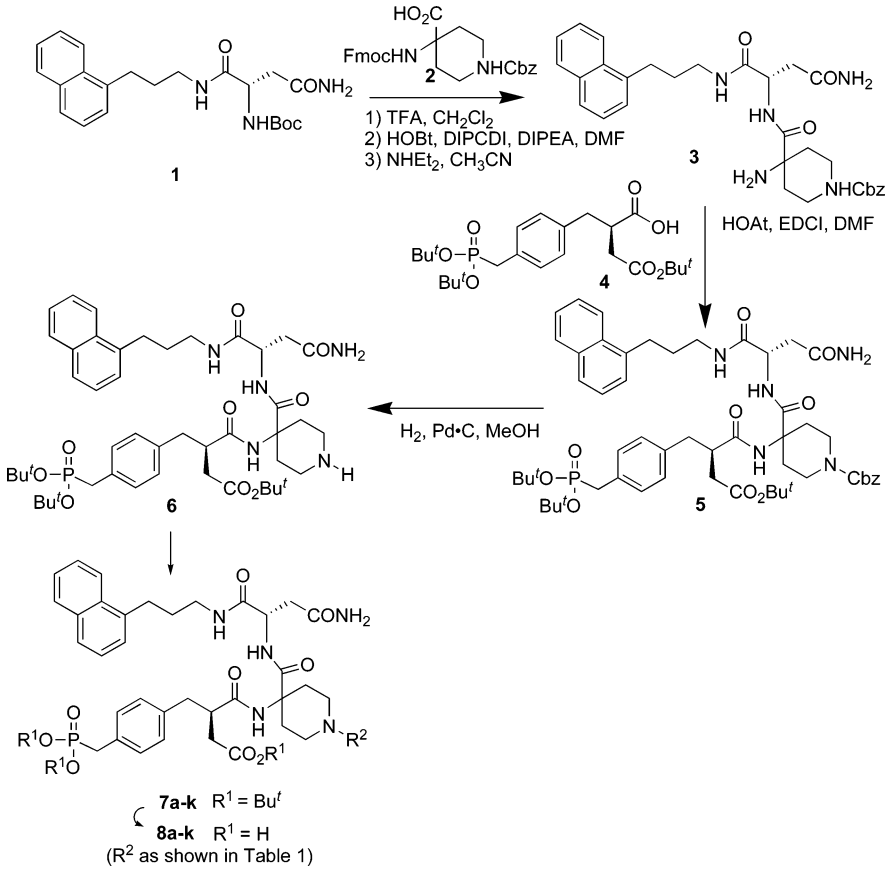
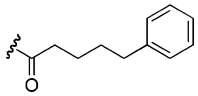
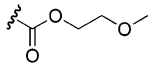
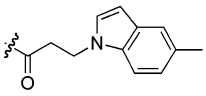
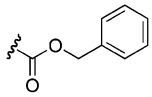
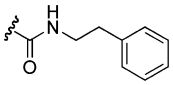
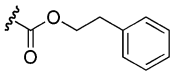
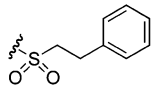
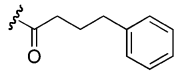
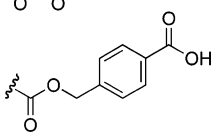


Table 1. Grb2 SH2 Domain-Binding Affinities of Synthetic Inhibitors Determined by Surface Plasmon Resonance

No.	R^2 [a]	K_D (nM) [b]	No.	R^2 [a]	K_D (nM) [b]
8a		106	**8f**		2180
8b		202	**8g**		458
8c		320	**8h**		272
8d		36	**8i**		2140
8e		27	**8j**		871

[a] Placement of R groups is as shown in Scheme 1. [b] Data was obtained by surface plasmon resonance experiments as described in the Supporting Information.[9]

Although the structure of the 4-carboxybenzyl carbamoyl moiety represents a departure from the more traditional pTyr mimetics used in these prior studies, modeling studies showed that interaction of the 4-carboxybenzyl group with the Arg142 residue was possible (Figure 1D). However, the poor affinity of **8j** (K_D = 871 nM) indicated that the desired interaction of the 4-carboxybenzyl group with the Arg142 residue was not achieved.

Conformationally-Constrained Amide Surrogates. The phenylbutyrylamide side chain of the most potent analogue **8e** could potentially bind in two distinct conformations due to rotational restriction about the amide C(═O)—N bond. This type of rotamer would not be expected for the carbamoyl linkage in the homologous structure **8d**. As one approach toward examining whether rotamers about the phenylbutyrylamide bond at the Apc 4-position might contribute differently to the affinity

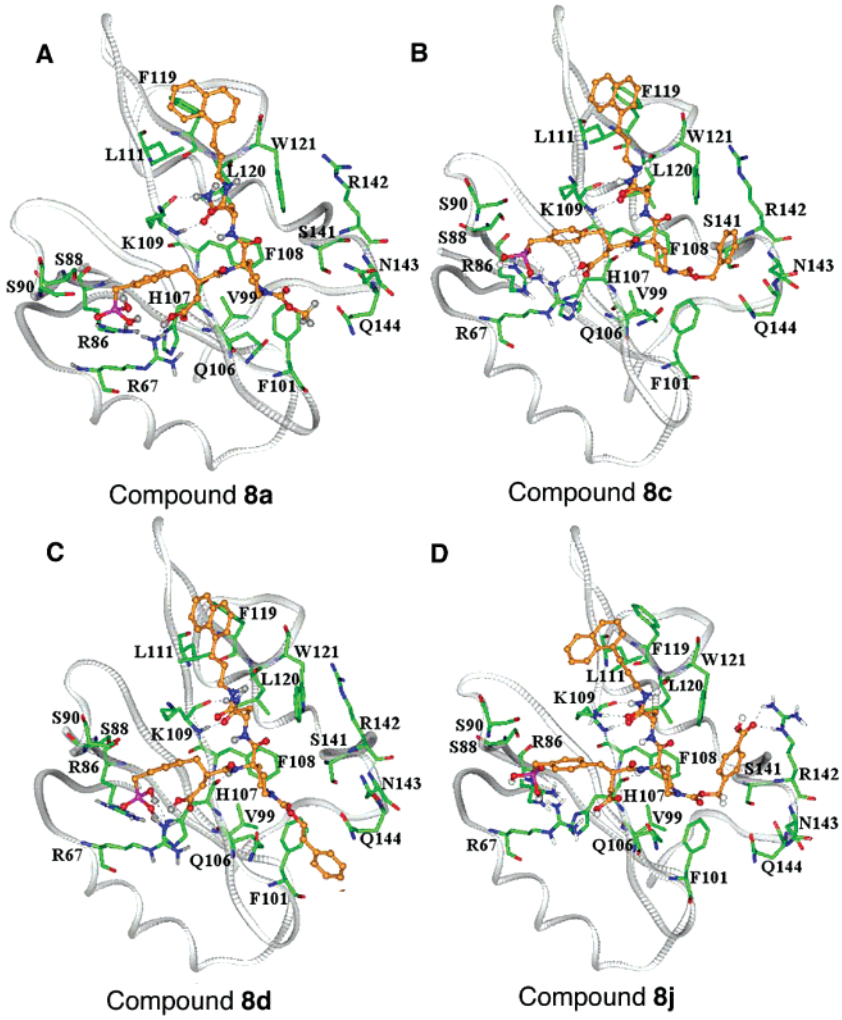


Figure 1. Structures of analogues **8a**, **8c**, **8d**, and **8j** (in gold) docked into the Grb2 SH2 domain. The protein is shown in ribbon depiction, with important side chain groups shown in ball and stick rendering.

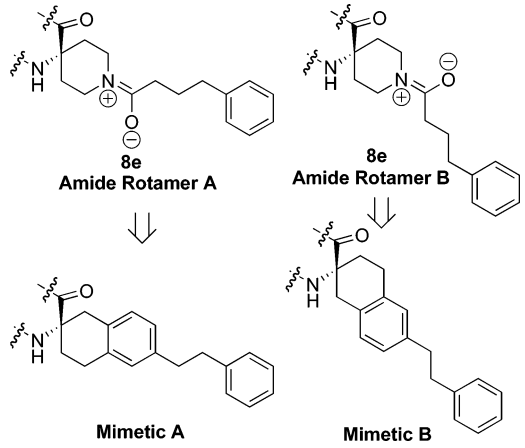


Figure 2. Use of pTyr+1 2-amino-2-carboxytetralin-based residues as conformationally constrained mimetics of amide rotamers arising from the phenylbutyrylamide of **8e**.

of compound **8e**, the tetralin-based analogues "mimetic A" and "mimetic B" were designed as conformationally constrained amide replacements (Figure 2).[11] However, the low affinity of both mimetic A and mimetic B (K_D values > 10 μM) indicated that the tetralin platforms lacked critical functionality needed to function as conformationally constrained amide bond surrogates.

Binding affinities alone may not reflect subtle differences in binding kinetics, because similar K_D values may arise from markedly different rates of association and dissociation. Interaction kinetic maps that plot association rates (k_a) versus dissociation rates (k_d), with an overlay of K_D isovalues, can allow visual discrimination of classes of compounds based on similar binding behaviors.[12] Plotting **8a**−**j** in this fashion indicated three groups of compounds (Figure 3): Group A compounds (**8d** and **8e**) display slow dissociate rates and relatively higher association rates. Although conversion of the carbamate functionality of **8d** into the amide group of **8e** introduced significant changes in rotational flexibility and hydrogen-bonding potential, little difference was observed in the actual binding affinities of **8d** and **8e**. Instead, the structural change resulted in a marked decrease in the rate of dissociation for **8e**. This may mean that **8e** is a more desirable inhibitor than **8d**, because low dissociation rates have been hypothesized to enhance the efficacy of drug-target interactions.[12] The Group B compound (**8a**) exhibits a higher association rate, but this is also accompanied by a more rapid dissociation rate. Group C compounds (**8b**, **8c**, and **8f**−**j**) exhibit slower association rates as well as moderate to rapid dissociation rates.

Conclusions

When a 4-piperidinyl variant of the known pTyr+1 residue Ac_6c was used, the current study investigated the effects of extending functionality outward from the Ac_6c residue to take

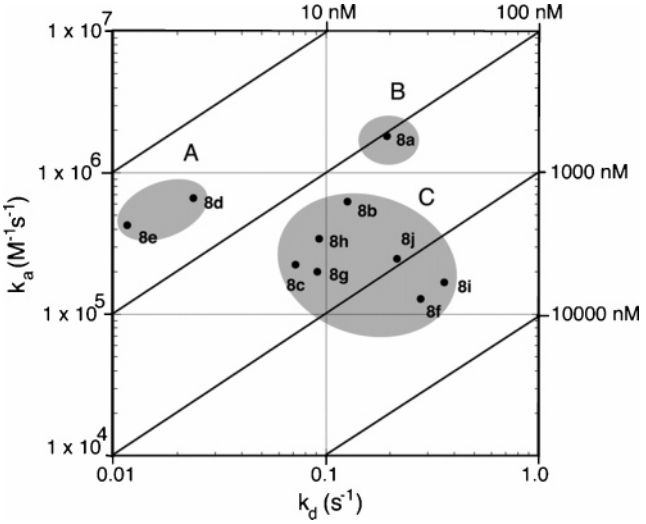


Figure 3. Interaction kinetic maps for Grb2 SH2 domain-binding inhibitors **8a**−**e** plotting association rate (k_a) versus dissociation rate (k_d) with an overlay of K_D isovalues. Data was organized into three groups, A, B, and C, depending on a combination of these factors.

advantage of potential interactions with an unexplored region of the Grb2 protein lying beneath the βD strand. Carbamoyl, amido, urea, and sulfonamido linkages to the 4-piperidinyl nitrogen were prepared. Tethering phenyl rings at varying distances from the piperidinyl nitrogen indicated that the best affinities were provided by a three-unit chain, with a phenylbutyryl amide group provided the highest affinity. Because SH2 domains share a high degree of structural homology, the current approach may be useful in preparing binding inhibitors directed at other SH2 domains.

Experimental Section

Synthesis of Dipeptide 3. Reacting **1**[7] and commercially available N^{α}-Fmoc-(4-*N*-Cbz-piperidinyl)carboxylic acid[8] according to protocols similar to those reported for the synthesis of the corresponding Ac_6c-containing congener, the dipeptide **3** was obtained in 89% yield as a white solid (mp 169−171 °C). ^{1}H NMR (DMSO-d_6) δ 8.43 (1H, s), 8.06 (1H, d, J = 8.4 Hz), 7.90 (1H, m), 7.83 (1H, t, J = 5.5 Hz), 7.75 (1H, d, J = 7.8 Hz), 7.56−7.47 (2H, m), 7.43−7.29 (8H, m), 6.87 (1H, s), 5.07 (2H, s), 4.46 (1H, m), 3.75 (2H, m), 3.21−3.10 (4H, m), 3.02 (2H, t, J = 7.6 Hz), 2.57−2.42 (2H, m), 1.91−1.73 (4H, m), 1.33 (2H, t, J = 14.3 Hz). FAB-MS (+VE) m/z 560 (MH^+).

Synthesis of Protected Tripeptide Mimetic 5. Similar to a previously reported procedure,[5] to a solution of dipetide **3** (784 mg, 1.40 mmol) in DMF (2 mL) was added an active ester solution formed by reacting **4**[5] (988 mg, 2.10 mmol), HOAt (0.5 M in DMF, 4.20 mL, 2.10 mmol), and EDCI·HCl (425 mg, 3.36 mmol) in DMF (3 mL; 15 min at room temperature), and the mixture was stirred at 40 °C (21 h). Solvent was removed by vacuum distillation, and the remaining residue was purified by silica gel column chromatography to provide **5** as a white solid (835 mg, 59% yield), mp 153−155 °C. ^{1}H NMR ($CDCl_3$) δ 8.06 (1H, d, J = 8.0 Hz), 7.88 (1H, d, J = 7.8 Hz), 7.82 (1H, m), 7.68 (1H, dd, J = 3.1 and 6.4 Hz), 7.49 −7.40 (3H, m), 7.37−7.29 (6H, m), 7.19−7.09 (3H, m), 6.99 (2H, d, J = 7.8 Hz), 6.92 (1H, s), 6.46 (1H, s), 5.49 (1H, s), 5.10 (2H, s), 4.60 (1H, s), 3.92 (2H, m), 3.35 (2H, m), 3.12 (2H, m), 3.04−2.88 (7H, m), 2.66 (1H, m), 2.49 (2H, m), 2.25 (1H, m), 2.13−1.80 (6H, m), 1.41 (9H, s), 1.39 (9H, s), 1.34 (9H, s). FAB-MS (+VE) m/z 1012 (MH^+).

General Procedures for the Synthesis of Final Products 8a−**8j.** Protected tripeptide mimetic **5** in MeOH was hydrogenated over 10% Pd·C (50% by weight of **5**) using a balloon filled with H_2 gas (overnight). The reaction mixture was filtered and taken to dryness then dissolved in CH_2Cl_2 to provide a 0.1 M stock solution of free amine **6**. Aliquots of the stock solution were reacted with appropriate acylating agents in the presence of diisopropylethylamine to yield protected intermediates **7a**−**j**. Yields and spectral data are provided as Supporting Information. For intermediates **7a**−**d** and **7j**, acylations were performed using chloroformates, while HOBT active ester coupling was employed for intermediate **7e**, and acid chlorides were used for **7f**, **7g**, and **7i**. The synthesis of urea **7h** employed the appropriate isocyanate. Treatment of the *tert*-butyl-protected products **7** with a mixture of TFA/H_2O/ethanedithiol (3:0.1:0.1) gave the globally deprotected final products **8a**−**j**. Physical data and combined yields from **6** following HPLC purification are provided below.

Final Product 8a: 64% yield; ^{1}H NMR (DMSO-d_6) δ 8.62 (1H, s), 8.11 (1H, m), 7.99 (1H, d, J = 8.2 Hz), 7.92 (1H, m), 7.77 (1H, t, J = 4.7 Hz), 7.53−7.49 (2H, m), 7.46−7.39 (4H, m), 7.13−7.11 (2H, m), 6.96−6.93 (3H, m), 4.35 (1H, m), 3.68 (2H, m), 3.59 (3H, s), 3.25−3.02 (7H, m), 2.89 (2H, d, J = 21.3 Hz), 2.71 (1H, dd, J = 6.4 and 14.6 Hz), 2.53−2.42 (2H, m), 2.33 (1H, m), 2.01−1.66 (8H, m). FAB-MS (−VE) m/z 766 (M − H). HRMALDI-MS (+VE) calcd for $C_{37}H_{46}N_5O_{11}NaP$ [M+Na], 790.2824; found, 790.2787.

Final Product 8b: 52% yield; ^{1}H NMR (DMSO-d_6) δ 8.63 (1H, s), 8.11 (1H, d, J = 7.6 Hz), 8.00 (1H, d, J = 8.2 Hz), 7.92 (1H, m), 7.78 (1H, t, J = 4.7 Hz), 7.54−7.41 (6H, m), 7.12 (2H, m), 6.95 (3H, m), 4.36 (1H, m), 4.11 (2H, m), 3.69 (2H, m), 3.51 (2H, t, J = 4.5 Hz), 3.26 (3H, s), 3.26−3.00 (7H, m), 2.89 (2H, d, J = 21.5 Hz), 2.71 (1H, dd, J = 6.4 and 15.8 Hz), 2.54−2.43 (2H, m), 2.33 (1H, m), 2.01−1.70 (8H, m). FAB-MS (−VE) m/z 810 (M − H). HRMALDI-MS (+VE) calcd for $C_{39}H_{50}N_5O_{12}NaP$ [M + Na], 834.3121; found, 834.3086.

Final Product 8c: 76% yield; ^{1}H NMR (DMSO-d_6) δ 8.64 (1H, s), 8.11 (1H, m), 8.00 (1H, d, J = 8.2 Hz), 7.91 (1H, m), 7.77 (1H, t, J = 4.9 Hz), 7.54−7.30 (11H, m), 7.12 (2H, m), 6.94 (3H, m), 5.08 (2H, s), 4.35 (1H, m), 3.73 (2H, m), 3.24−2.91 (7H, m), 2.89 (2H, d, J = 21.3 Hz), 2.70 (1H, dd, J = 6.8 and 15.8 Hz), 2.53−2.43 (2H, m), 2.33 (1H, m), 2.01−1.70 (8H, m). FAB-MS (−VE) m/z 842 (M − H). HRMALDI-MS (+VE) calcd for $C_{43}H_{20}N_5O_{11}$-NaP [M + Na], 866.3137; found, 866.3101.

Final Product 8d: 65% yield; ^{1}H NMR (DMSO-d_6) δ 8.61 (1H, s), 8.11 (1H, m), 7.99 (1H, d, J = 8.0 Hz), 7.92 (1H, m), 7.77 (1H, t, J = 4.5 Hz), 7.53−7.48 (2H, m), 7.46−7.39 (4H, m), 7.31−7.19 (5H, m), 7.11 (2H, m), 6.95 (3H, m), 4.35 (1H, m), 4.17 (2H, m), 3.66 (2H, m), 3.25−3.00 (7H, m), 2.91−2.86 (4H, m), 2.71 (1H, dd, J = 6.3 and 15.6 Hz), 2.54−2.42 (2H, m), 2.33 (1H, m), 1.99 (1H, m), 1.92−1.78 (6H, m), 1.67 (1H, m). FAB-MS (−VE) m/z 856 (M − H). HRMALDI-MS (+VE) calcd for $C_{44}H_{52}N_5O_{11}NaP$ [M + Na], 880.3293; found, 880.3261.

Final Product 8e: 65% yield; ^{1}H NMR (DMSO-d_6) δ 8.65 (1H, d, J = 13.3 Hz), 8.11 (1H, m), 8.00 (1H, t, J = 8.8 Hz), 7.92 (1H, m), 7.78 (1H, t, J = 4.9 Hz), 7.55−7.39 (6H, m), 7.28 (2H, m), 7.19−7.11 (5H, m), 6.98−6.92 (3H, m), 4.35 (1H, m), 4.05 (1H, m), 3.56 (1H, m), 3.27−2.68 (11H, m), 2.60−2.24 (4H, m), 2.40−2.22 (3H, m), 2.05−1.61 (9H, m). HRMALDI-MS (+VE) calcd for $C_{45}H_{54}N_5O_{10}NaP$ [M + Na], 878.3501; found, 878.3513.

Final Product 8f: 60% yield; ^{1}H NMR (DMSO-d_6) δ 8.64 (1H, d, J = 8.2 Hz), 8.11 (1H, d, J = 8.0 Hz), 8.00 (1H, t, J = 7.0 Hz), 7.91 (1H, m), 7.77 (1H, t, J = 4.9 Hz), 7.55−7.39 (6H, m), 7.27 (2H, m), 7.16 (5H, m), 6.96 (3H, m), 4.35 (1H, m), 4.03 (1H, m), 3.61 (1H, m), 3.27−2.97 (7H, m), 2.89 (2H, d, J = 21.5 Hz), 2.82−2.68 (2H, m), 2.60−2.29 (8H, m), 2.04−1.75 (6H, m), 1.65−1.46 (4H, m). FAB-MS (−VE) m/z 868 (M − H). HRMALDI-MS (+VE) calcd for $C_{46}H_{56}N_5O_{10}NaP$ [M + Na], 892.3657; found, 892.3697.

Final Product 8g: 57% yield; ^{1}H NMR (DMSO-d_6) δ 8.64 (1H, s), 8.10 (1H, d, J = 8.2 Hz), 7.97 (1H, d, J = 7.4 Hz), 7.90 (1H, m), 7.77 (1H, m), 7.54−7.29 (9H, m), 7.11 (2H, m), 6.94 (4H, m), 6.28 (1H, d, J = 2.5 Hz), 4.35 (3H, m), 4.05 (1H, m), 3.53 (1H, m), 3.24−2.97 (7H, m), 2.92−2.68 (5H, m), 2.53−2.43 (2H, m), 2.36 (3H, s), 2.36−2.29 (1H, m), 2.00−1.64 (8H, m). FAB-MS (−VE) m/z 893 (M − H). HRMALDI-MS (+VE) calcd for $C_{47}H_{55}N_6O_{10}NaP$ [M + Na], 917.3610; found, 917.3636.

Final Product 8h: 67% yield; ^{1}H NMR (DMSO-d_6) δ 8.58 (1H, s), 8.12 (1H, d, J = 7.8 Hz), 7.97−7.91 (2H, m), 7.78 (1H, m), 7.55−7.47 (3H, m), 7.44−7.39 (3H, m), 7.30−7.27 (2H, m), 7.18 (3H, m), 7.12 (2H, m), 6.97−6.92 (3H, m), 6.60 (1H, br s), 4.35 (1H, m), 3.64 (2H, m), 3.25−2.90 (8H, m), 2.91 (2H, d, J = 21.1 Hz), 2.79−2.69 (4H, m), 2.55−2.45 (2H, m), 2.35 (1H, dd, J = 10.0 and 13.7 Hz), 2.03−1.63 (8H, m). FAB-MS (−VE) *m*/*z* 855 (M − H). HRMALDI-MS (+VE) calcd for $C_{44}H_{53}N_6O_{10}NaP$ [M + Na], 879.3453; found, 879.3416.

Final Product 8i: 32% yield; ^{1}H NMR (DMSO-d_6) δ 8.61 (1H, s), 8.11 (1H, d, J = 7.7 Hz), 8.02 (1H, d, J = 8.1 Hz), 7.91 (1H, m), 7.77 (1H, t, J = 5.0 Hz), 7.55−7.20 (11H, m), 7.10 (2H, m), 6.92 (3H, m), 4.36 (1H, m), 3.43 (2H, m), 3.30 (2H, m), 3.19 (2H, m), 3.07 (5H, m), 2.94 (2H, m), 2.88 (2H, d, J = 21.5 Hz), 2.71 (1H, dd, J = 6.6 and 15.8 Hz), 2.54−2.42 (2H, m), 2.31 (1H, m), 2.01−1.79 (8H, m). FAB-MS (−VE) *m*/*z* 876 (M − H). HRMALDI-MS (+VE) calcd for $C_{43}H_{52}N_5O_{11}NaP$ [M + Na], 900.3014; found, 900.3039.

Final Product 8j: 55% yield; ^{1}H NMR (DMSO-d_6) δ 8.65 (1H, s), 8.11 (1H, m), 8.01 (1H, d, J = 8.4 Hz), 7.96−7.90 (3H, m), 7.77 (1H, t, J = 5.1 Hz), 7.57−7.40 (8H, m), 7.12 (2H, m), 6.97−6.93 (3H, m), 5.16 (2H, s), 4.36 (1H, m), 3.75 (2H, m), 3.25−3.01 (7H, m), 2.89 (2H, d, J = 21.3 Hz), 2.71 (1H, dd, J = 6.3 and 15.8 Hz), 2.54−2.43 (2H, m), 2.33 (1H, m), 2.02−1.72 (8H, m). FAB-MS (−VE) *m*/*z* 886 (M − H). HRMALDI-MS (+VE) calcd for $C_{44}H_{50}N_5O_{13}NaP$ [M + Na], 910.3035; found, 910.3006.

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Supporting Information Available: Spectroscopic data for compounds **7a**−**j**, synthetic experimental procedures for the acylating species used to synthesize compounds **7j**, and synthetic procedures for mimetics A and B, as well as details of the docking protocol used to generate Figure 1 and procedures for Biacore binding analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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