The Structure of PA1221, a Non-Ribosomal Peptide Synthetase containing Adenylation and Peptidyl Carrier Protein Domains

Carter A. Mitchell,[1] Ce Shi,[2]

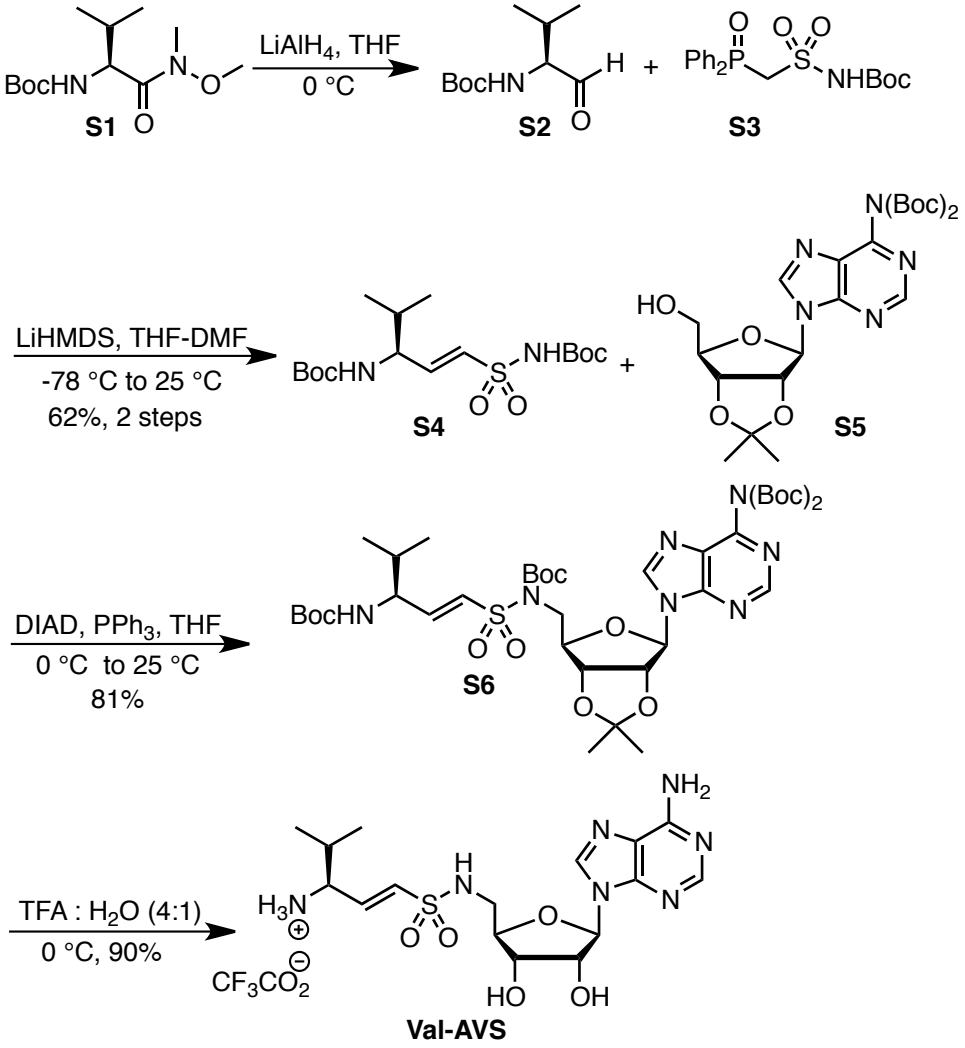
Courtney C. Aldrich,[2] and Andrew M. Gulick*[1]

Supporting Information

Synthesis of the Valine-adenosine vinylsulfonamide (Val-AVS).

Figure S1. Analysis of the valine binding pocket of PA1221

Scheme S1. Synthesis of **Val-AVS**.



General Methods: All reactions were performed under an inert atmosphere of dry Ar in oven-dried (150 °C) glassware. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 600 MHz spectrometer. Proton chemical shifts are reported in ppm from an internal standard of residual chloroform (7.26 ppm) or methanol (3.31 ppm), and carbon chemical shifts are reported using an internal standard of residual chloroform (77.3 ppm) or methanol (49.1 ppm). Proton chemical data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, br = broad), integration, coupling constant. High resolution mass spectra were obtained on an Agilent TOF II TOF/MS instrument equipped with either an ESI or APCI interface. TLC analyses

were performed on TLC silica gel 60F254 from EMD Chemical Inc., and were visualized with UV light or 10% PMA solution. Purifications were performed by flash chromatography on silica gel (Dynamic Adsorbents, 60A).

Materials: Chemicals, reagents and solvents were purchased from Sigma Aldrich Company, Chem-Impex or Acros Organic Fischer Company, and were used as received. An anhydrous solvent dispensing system (J. C. Meyer) using 2 packed columns of neutral alumina was used for drying THF, Et_2O, while 2 packed columns of molecular sieves were used to dry DMF and the solvents were dispensed under argon. Compound **1** was purchased from Chem-Impex and used as received. Compound **S3**[1] and **S5**[2] were synthesized according to the reported procedures.

(*S,E*)-*tert*-Butyl 3-(*tert*-butoxycarbonylamino)-4-methylpent-1-enylsulfonylcarbamate (S4).

To a solution of Weinreb amide **S1** (130 mg, 0.5 mmol, 1.0 equiv) in THF (5 mL) at 0 ^oC, was added $LiAlH_4$ (28 mg, 0.75 mmol, 1.5 equiv) in 2 portions. The reaction was quenched carefully with MeOH (0.5 mL) and diluted with EtOAc (20 mL) and potassium sodium tartrate aqueous solution (1N, 20 mL). The mixture was warmed up to 25 °C and stirred for 30 min. The aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layer was washed with H_2O (20 mL), saturated NaCl solution (20 mL), dried ($MgSO_4$) and concentrated to afford the crude *N*-*tert*-butoxycarbonyl-L-valinal **S2**, which was used directly in the next reaction.

To a solution of *tert*-butyl (diphenylphosphoryl)methylsulfonylcarbamate **S3** (395 mg, 1.0 mmol, 2.0 equiv) in 1:3 DMF/THF (4 mL) at -78 °C, was added LiHMDS in THF solution (1 N, 2.0 mL, 4.0 equiv) dropwise over 15 min. The solution was then stirred at -78 °C for 15 min, then *N*-*tert*-butoxycarbonyl-L-valinal **S2** prepared above in THF (1 mL) was added to the reaction over 15 min. The solution was gradually warmed to 25 °C and stirred for 15 h. The solvent was removed in vacuo and the mixture was re-dissolved in H_2O (30 mL). The pH was then adjusted to 3–4 using 1 N aqueous HCl. The resulting suspension was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with water (30 mL) and brine (30 mL), dried over $MgSO_4$, and concentrated in vacuo. Purification by flash chromatography (10% EtOAc in hexanes to 50% EtOAc in hexanes) afforded the title compound as a colorless oil (115 mg, 62%): R_f 0.50 (50:50,

EtOAc/hexanes); $[\alpha]_D^{23}$ +0.5 (*c* 0.02, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.91 (d, *J* = 6.6 Hz, 3H), 0.93 (d, *J* = 6.6 Hz, 3H), 1.42 (s, 9H), 1.45 (s, 9H), 1.86–1.89 (m, 1H), 4.70 (br d, *J* = 8.4 Hz, 1H), 6.48 (br s, 1H), 6.54 (d, *J* = 15 Hz, 1H), 6.87 (dd, *J* = 15, 4.8 Hz, 1H), 8.10 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1, 18.7, 27.8, 28.2, 41.1, 60.4, 80.0, 83.8, 127.8, 147.4, 149.3, 155.1; HRMS (ESI$^-$): calcd for $C_{16}H_{29}N_2O_6S$ $[M - H]^-$ 377.1725, found 377.1737 (error 3.1 ppm).

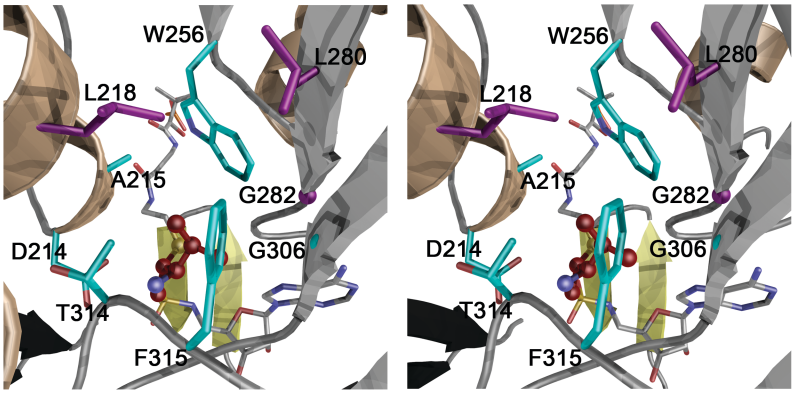
(*E*)-*N*^{6},*N*^{6}-bis(*tert*-Butoxycarbonyl)-5′-deoxy-5′-*N*-*tert*-butoxycarbonyl-5′-*N*-[(*S,E*)-3-(*tert*-butoxycarbonylamino)-4-methylpent-1-enylsulfonyl]amino-2′,3′-*O*-isopropylideneadeno-sine (S6).

To a solution of *N*^{6}-bis(*tert*-butoxycarbonyl)-2',3'-*O*-isopropylideneadenosine **S5** (73 mg, 0.14 mmol, 1.1 equiv), vinylsulfonamide **S4** (50 mg, 0.13 mmol, 1.0 equiv) and PPh_3 (56 mg, mmol, 0.21 mmol, 1.7 equiv) in THF (1 mL) at 0 °C, was added a solution of DIAD (42 µL, 0.21 mmol, 1.7 equiv) in THF (1 mL) over 1 h using a syringe pump. The solution was gradually warmed up to 23 °C and stirred 16 h. The mixture was then concentrated in vacuo and purified by flash chromatography (20% EtOAc/hexanes to 50% EtOAc/hexanes) to afford the title compound as a colorless foam (93 mg, 81%): R_f 0.45 (50:50, EtOAc/hexanes); $[\alpha]_D^{23}$ +30.5 (*c* 0.05, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (d, *J* = 6.8 Hz, 3H), 0.89 (d, *J* = 6.8 Hz, 3H), 1.38 (s, 3H), 1.42 (s, 9H), 1.44 (s, 18H), 1.45 (s, 9H), 1.60 (s, 3H), 3.89–3.95 (m, 2H), 4.09–4.15 (m, 1H), 4.41–4.46 (m, 1H), 4.55 (d, *J* = 9.2 Hz, 1H), 5.09–5.12 (m, 1H), 5.42 (dd, *J* = 6.0, 1.6 Hz, 1H), 6.13 (d, *J* = 1.6 Hz, 1H), 6.54 (br s, 2H), 8.17 (br s, 1H), 8.84 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 18.9, 25.3, 27.2, 27.7, 27.9, 28.3, 32.0, 47.4, 60.3, 79.9, 82.3, 83.8, 84.4, 84.9, 85.4, 90.5, 114.6, 128.6, 129.5, 144.1, 146.2, 150.4, 150.6, 150.8, 152.1, 152.4, 155.1; HRMS (APCI+): calcd for $C_{39}H_{62}N_7O_{13}S$ $[M + H]^+$ 868.4121, found 868.4156 (error 4.0 ppm).

(*E*)-5′-Deoxy-5′-*N*-[(*S,E*)-3-amino-4-methylpent-1-enylsulfonyl]aminoadenosine trifluoroacetic acid salt (Val-AVS).

To a solution of **S6** (50 mg, 0.06 mmol) was added 80% aq TFA (1 mL) at 0 °C. The solution was stirred for 6 h at 0 °C then concentrated. Recrystallization from MeOH/Et_2O (5 mL, 1/20) afforded the title compound (28 mg, 90%) as off white solid: mp 148–150 °C; $[\alpha]_D^{23}$ –14.3 (*c* 0.1, MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 0.94 (d, *J* = 7.2 Hz, 3H),

1.00 (d, *J* = 7.2 Hz, 3H), 2.02–2.05 (m, 1H), 3.32–3.34 (m, 2H), 3.79 (t, *J* = 7.8 Hz, 1H), 4.23–4.26 (m, 1H), 4.35–4.37 (m, 1H), 4.79 (t, *J* = 5.4 Hz, 1H), 5.95 (d, *J* = 6.0 Hz, 1H), 6.60 (dd, *J* = 15, 7.2 Hz, 1H), 6.77 (d, *J* = 15 Hz, 1H), 8.28 (s, 1H), 8.32 (s, 1H); ^{13}C NMR (125 MHz, CD_3OD) *δ* 18.0, 19.0, 32.3, 45.8, 58.0, 72.9, 74.8, 85.7, 91.6, 121.3, 135.6, 137.3, 143.3, 149.9, 150.9, 153.1; HRMS (ESI+) calcd for $C_{16}H_{26}N_7O_5S$ $[M + H]^+$ 428.1711, found 428.1726 (error 3.5 ppm).



The Selectivity-Conferring Code of NRPS A Domains.

Substrate	Position* 235	236	239	278	299	301	322	330	331	517	Protein
Phe	D	A	W	T	I	A	A	V	C	K	PheA (GrsA)
Val(1)	D	A	F	W	I	G	G	T	F	K	GrsB, SrfAB, TycC
Val(2)	D	F	E	S	T	A	A	V	Y	K	AcvA
Val(3)	D	A	W	M	F	A	A	V	L	K	CssA
Val	D	A	L	W	L	G	G	T	F	K	PA1221

*Position number defined by PheA (GrsA)

Figure S1. Stereo representation of *holo*-PA1221 active site. The phosphopantetheine is rendered as sticks with grey carbon atoms. The valine moiety of Val-AVS is shown with dark red spheres. The residues predicted in prior analyses that interact with the substrate are shown in teal and those that are too far from the valine substrate and do not interact with the substrate are purple. Below the figure are the predicted residues based on sequence alignment with the structure of the PheA domain, the first NRPS adenylation domain structure, adapted from Stachelhaus.

Supporting Information References

1) Reuter, D.C.; McIntosh, J.E.; Guinn, A.C. & Madera, A.M. Synthesis of vinyl sulfonamides using the Horner reaction. *Synthesis*, **2003**, 2321-2324 (2003).
2) Ikeuchi, H.; Meyer, M.E.; Ding, Y.; Hiratake, J. & Richards, N.G.J. A critical electrostatic interaction mediates inhibitor recognition by human asparagine synthetase. *Bioorg. Med. Chem.* **17**, 6641–6650 (2009).