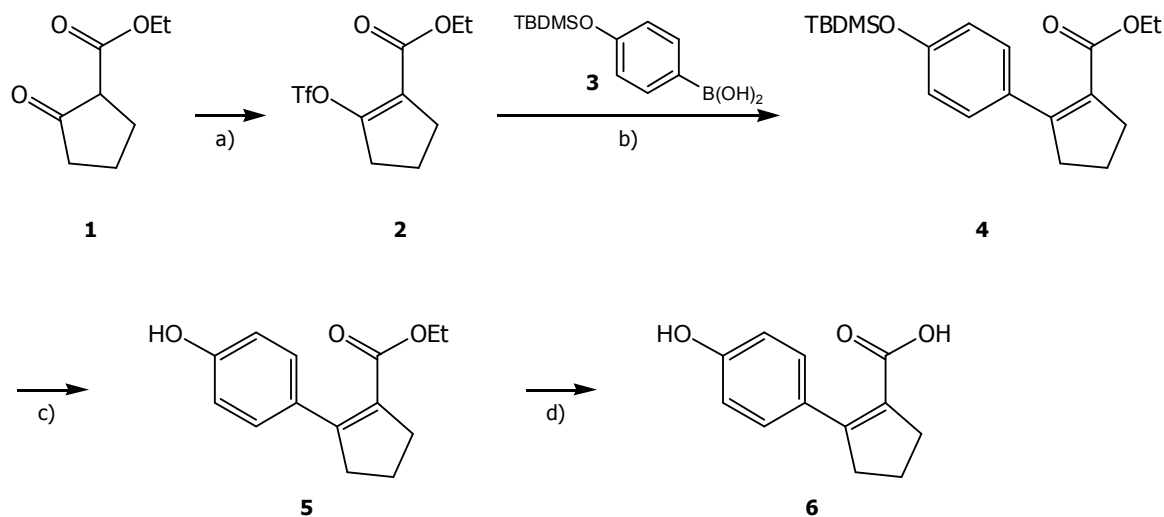


Supplemental material

Locked chromophore analogs reveal that photoactive yellow protein regulates biofilm formation in the deep sea bacterium *Idiomarina loihiensis*

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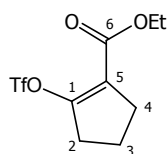
Synthetic route



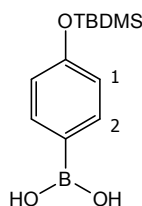
Scheme 1. Reagents: (a) DIPEA, Tf_2O , CH_2Cl_2 , rt, 62%; (b) **3**¹, K_3PO_4 , $\text{Pd}(\text{PPh}_3)_4$ (cat), dioxane, 85°C , 71%;² (c) TBAF, THF, 0°C , 95%; (d) Tesser's base 60°C , 90%.

Experimental

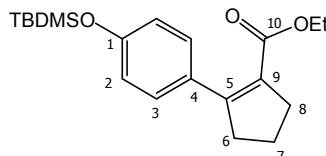
General: Reagents were purchased at the highest available commercial quality and used without further purification unless stated otherwise. Chromatographic purification refers to flash chromatography using the indicated solvent (mixture) and ACROS silica gel (particle size 35–70 μm). Tesser's base is a solution of dioxane, methanol and 4N NaOH (16:4:1 v/v/v)³. Infrared spectra were recorded using a Bruker IFS 28 spectrophotometer and absorptions are reported in units of cm^{-1} . Nuclear magnetic resonance (NMR) spectra were obtained using a Bruker ARX 400 (400 MHz) spectrometer. Spectra are reported in units of ppm on the δ scale relative to an internal standard of residual chloroform (7.27 ppm). Accurate mass determination data are reported as m/z (relative intensity) and were performed on a JEOL JMS SX/SX102A four-sector mass spectrometer, coupled to a JEOL MS-MP7000 data system. Electrospray (ESI) spectra were recorded on a Finnigan TSQ7000 mass spectrometer using Xcaliber 1.2 software for acquisition and data processing. The samples were introduced into the ESI-source by means of the flow injection technique in a solvent flow of 200 μl containing $\text{H}_2\text{O}/\text{MeOH}$ 1:1 with 1% acetic acid.



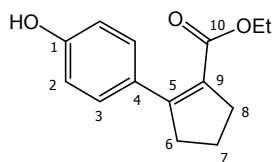
2-trifluoromethanesulfonyloxy-cyclopent-1-enecarboxylic acid ethyl ester (2): a solution of ethyl-2-oxocyclopentane carboxylate (**1**) (5.00 g, 32 mmol) and DIPEA (27.9 ml, 160 mmol) in CH_2Cl_2 (50 ml) was cooled to -78°C . TiF_2O (10.8 g, 38.4 mmol) was added and the reaction mixture was slowly warmed to room temperature and stirred for 16 hours. Then the reaction mixture was washed with cold 10% citric acid (2 x 50 ml), sat. NaHCO_3 (50 ml), dried over MgSO_4 and concentrated *in vacuo*. After distillation (110 $^\circ\text{C}$, 0.8 mbar) **2** was obtained as a light green oil (5.71 g, 19.8 mmol, 62%). $^1\text{H-NMR}$ δ = 4.26 (q, J = 7.1 Hz, OCH_2CH_3), 2.77-2.68 (m, 4H, H2 and H4), 2.0 (quintet, J = 7.7 Hz, 2H, H3), 1.32 (t, J = 7.1 Hz, 3H, OCH_2CH_3); $^{13}\text{C-NMR}$ δ = 162.1 (C6), 153.2 (SO_2CF_3), 123.21 (C1), 116.54 (C5), 60.9 (OCH_2CH_3), 32.5 (C2), 29.1 (C4), 18.6 (C3), 13.8 (OCH_2CH_3);



4-(tert-butyl-dimethyl-silanyloxy)-phenyl-boric acid (3)¹: to a solution of (4-bromophenoxy)-tert-butyl dimethylsilane (2.51 g, 8.73 mmol) in dry THF (12 ml) at -78°C was added *n*-butyllithium (1.6 M in hexanes, 6.0 ml, 9.6 mmol) over a 30 min period and the reaction mixture was stirred an additional 15 min. Triisopropyl borate (4.92 g, 26.1 mmol) was added and the mixture was stirred for 10 min at -78°C and then allowed to warm to room temperature. The solution was acidified with a 5% HCl solution (~6 ml) until the aqueous layer became acidic. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to provide **3** as an off-white solid (1.95 g, 7.71 mmol, 89%). $^1\text{H-NMR}$ δ = 8.11 (d, J = 8.1 Hz, 2H, H2), 6.95 (d, J = 8.1 Hz, 2H, H1), 1.01 (s, 9H, $\text{Si-C}(\text{CH}_3)_3$), 0.25 (s, 6H, $\text{Si}(\text{CH}_3)_2$);

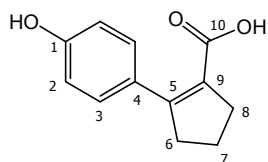


2-[4-(tert-butyl-dimethyl-silanyloxy)-phenyl]-cyclopent-1-enecarboxylic acid ethyl ester (4)²: to a solution of **2** (1.88 g, 6.52 mmol) in dioxane (30 ml) was added **3** (1.81 g, 7.19 mmol), K_3PO_4 (2.08 g, 9.80 mmol) and $\text{Pd}(\text{PPh}_3)_4$ (189 mg, 0.163 mmol). The resulting mixture was heated at reflux for 5 hours. The reaction mixture was diluted with benzene (50 ml) and treated with aqueous NaOH (3M, 3 ml) and H_2O_2 (35%, 2.5 ml) and the mixture was stirred for 30 min. The reaction mixture was extracted with hexane (40 ml) and the organic layer was washed with brine (2 x 40 ml), dried over Na_2SO_4 and concentrated *in vacuo*. Purification by flash column chromatography (Et_2O /pentane 1:8) yielded **4** as a yellow/green oil (1.61 g, 4.66 mmol, 71%). $^1\text{H-NMR}$ δ = 7.25 (d, J = 8.7 Hz, 2H, H3), 6.78 (d, J = 8.6 Hz, 2H, H2), 4.10 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 2.85-2.79 (m, 4H, H6 and H8), 1.95 (quintet, J = 7.6 Hz, 2H, H7), 1.15 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 0.98 (s, 9H, $\text{Si-C}(\text{CH}_3)_3$), 0.2 (s, 6H, $\text{Si}(\text{CH}_3)_2$); $^{13}\text{C-NMR}$ δ = 166.9 (C10), 155.8 (C1), 152.6 (C5), 130.0 (C4), 129.5 (C3), 128.2 (C9), 119.4 (C2), 60.1 (OCH_2CH_3), 40.1 (C6), 35.5 (C8), 25.9 ($\text{OSi}(\text{CH}_3)_2\text{C}(\text{CH}_3)_3$), 22.1 (C7), 18.4 (OCH_2CH_3), -4.2 ($\text{OSi}(\text{CH}_3)_2\text{C}(\text{CH}_3)_3$);



2-(4-Hydroxy-phenyl)-cyclopent-1-enecarboxylic acid ethyl ester (5): a solution of **4** (865 mg, 2.50 mmol) in dry THF (25 ml) was cooled to 0°C and TBAF (3.25 ml, 1.0 M in THF, 3.25 mmol) was added. After stirring for 2 hours at room temperature water (40 ml) was added. The reaction mixture was extracted with EtOAc (2 x 50 ml) and the organic layer was washed with brine (40 ml), dried over Na_2SO_4 and concentrated *in vacuo*. Purification by flash column chromatography (Et_2O /pentane 1:1) yielded **5** as an off-white solid (550 mg, 2.36 mmol,

95 %). $^1\text{H-NMR}$ δ = 7.26 (d, J = 8.6 Hz, 2H, H3), 6.76 (d, J = 8.6 Hz, 2H, H2), 5.04 (s, 1H, PhOH), 4.12 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 2.85 – 2.79 (m, 4H, H6 and H8), 1.96 (quintet, J = 7.6 Hz, 2H, H7), 1.18 (t, J = 7.1, 3H, OCH_2CH_3); $^{13}\text{C-NMR}$ δ = 167.0 (C10), 155.9 (C1), 154.1 (C5), 129.2 (C3), 128.4 (C4), 127.1 (C9), 114.6 (C2), 60.1 (OCH_2CH_3), 39.9 (C6), 34.9 (C8), 21.6 (C7), 13.8 (OCH_2CH_3);



2-(4-Hydroxy-phenyl)-cyclopent-1-enecarboxylic acid (6): **5** (542 mg, 2.33 mmol) was dissolved in Tesser's base (120 ml) and the solution was heated to 60 °C and stirred for 5 hours. The mixture was acidified with 1M KHSO_4 (50 ml) and extracted with CH_2Cl_2 (3 x 100ml). The organic layers were combined and washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. After flash column chromatography **6** was obtained as a white solid (429 mg, 2.10 mmol, 90%). $^1\text{H-NMR}$ (MeOH-d_4) δ = 7.24 (dt, J = 8.7, 2.4 Hz, 2H, H3), 6.72 (dt, J = 8.7, 2.5 Hz, 2H, H2), 4.85 (s, PhOH, COOH and H_2O), 2.85-2.75 (m, 4H, H6 and H8), 1.95 (quintet, J = 7.6 Hz, 2H, H7); $^{13}\text{C-NMR}$ (MeOH-d_4) δ = 170.6 (C10), 158.7 (C1), 155.3 (C5), 130.7 (C3), 129.3 (C4), 128.5 (C9), 115.62 (C2), 40.9 (C6), 36.7 (C8), 23.0 (C7); IR ν = 3591, 3290, 1701, 1609, 1513, 1267, 1174; mp 147.0 – 147.5

Supplemental References

- 1) Hajduk, P.J.; Sheppard, G.; Nettesheim, D.G.; Olejniczak, E.T.; Shuker, S.B.; Meadows, R.P.; Steinman, D.H.; Carrera, G.M. Jr.; Marcotte, P.A.; Severin, J.; Walter, K.; Smith, H.; Gubbins, E.; Simmer, R.; Holzman, T.F.; Morgan, D.W.; Davidsen, S.K.; Summers, J.B.; Fesik, S.W. *J. Am. Chem. Soc.* **1997**, 119, 5818-5827.
- 2) Oh-e, T.; Miyaura, N.; Suzuki, A. *J. Org. Chem.* **1993**, 58, 2201-2208.
- 3) Tesser, G. I.; Balvert-Geers, I. C. *Int. J. Peptide Protein Res.* **1975**, 7, 295-305.
- 4) Hou, S.; Saw, J. H.; Lee, K. S.; Freitas, T. A.; Belisle, C.; Kawarabayasi, Y.; Donachie, S. P.; Pikina, A.; Galperin, M. Y.; Koonin, E. V.; Makarova, K. S.; Omelchenko, M. V.; Sorokin, A.; Wolf, Y. I.; Li, Q. X.; Keum, Y. S.; Campbell, S.; Denery, J.; Aizawa, S.; Shibata, S.; Malahoff, A.; Alam, M. *Proc. Natl. Acad. Sci. USA* **2004**, 101, 18036-18041.

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Rspa : -----MLAMSPKFAVELDRHIDLDQAHAVASGGARIVLAPPARDRCRASEARLGAVIREARHVYGLTTGFGPLANRLISGENVRTL
Rcap : MLDATIGRKRMTLQSQTAKDCLALDGALTLVQCEAIATHRSRISVTPALRERCARAHARLEHATAEQRHIYGITTTGFGPLANRLIGADQGAEL
Hhal : -----MAEVDLAGSLSAADIEAIGYGHRTATVSPGWKRLRSAAEYLQRLVDERRQVYGVTTGYGPLATSRIDPSASRTL
IL2450: -----MSHFELQPGQLQLSELRDWIFYQHQTLLKLSDEAKDNIATSAKTVADVLEQGRVYVYGINTTGFGLLANTRIPPERLTDL
IL0135: -----MTTSIIAFGGRPLTKDVEITANGKAQIALSDAPEFVEKVDAGVRLDELLEQDGVYVYGVTTGYGDSCTVSVPENLVNEL
                                     @ @

Rspa : QANLVH-HLASGVGFVLDWTTARAMVLARLVSIAGGASGASEGTIARLIDLLNSELAPAVPSRGTVGASGDLTPLAHMVLCLQGRGDFLDRDG
Rcap : QQNLII-HLATGVGPKLSWAEARALMLARLNSILQGASGASPETIDRIVAVLNAGFAVEVPAQGTVGASGDLTPLAHMVLALQGRGRMIDPSG
Hhal : QRNLVY-HLCSGVGEPLSRCHTRATLGARIASVTRGHSGVTPAVVERLLAWLEHDVVPEVPAIGTVGASGDLTPLAHVARALMGEGRVCINGG
IL2450: QRRIVLSHAA-GTGDLMEDSVVRLMLLLKINSLSRGFSQVRQVLVDALIKLLNAEVYPCPEKGSVGASGDLAPLAHMVLPVCEG-TVRHNG
IL0135: PIHLTRFHGC-GLGDTFDEQETRAILATRLSSLAQGYSGVSWELLERLVVMLNENMLPLIPKEGSVGASGDLTPLSYIAGALIGERDVRFRN-
                                     @ @ @ @

Rspa : TRLDGAEGLRGRQLPLDLSHRDALALVNGTSAMTGIALVNAHACRHLGNVAVALTALLAECLRGRTAEAWAALSRLRPHPGQKDAARLRAR
Rcap : RVQAGAVMDRLCGGPLTLAARDGLALVNGTSAMTAIAALTGVEAARADAAALRHSAVLMEVLSGHAEAWHPAFALRPHPGQLRATERIAQA
Hhal : EWEPADAAQRRLLGWEPWTLTGKDAIALVNGTSMTAGICAVNGAGAERAAGVCAVLGMVYAEELGGHAEAFQPAIGAVRPHPGQMRHAWLTAL
IL2450: KVLNAEEGLKIAGIEPFELAPKEGLALLNGTQASTALALAGLFRIERNFHAAIVVGATSVEAAMGSRAPFDERVHAVRGQPGQIKAAEMLRHV
IL0135: KVMNSAEAFELLGMSVHKLRPKEGLAIMNGTAVMTALACLAWDRAEYLRSLSSRITSLASIALEGNSNHFFDDLFAVKPHKGQQQVASWIIQDD

Rspa : VDGSARVVRHVIAERRLDAG--DIGTEPEAGQDAYSLRCAQVQLGAGFTTAWHDRVLTIELNAVTDNPFVFPDGSVPALHGGNFMGQHVALT
Rcap : LDGAGRVCRTLTAARRLTAA--DLRPEDHPAQDAYSLRVVPQVLGAVWTLTDWHDRVVTCELNSVTDNPIFPEGCAVPALHGGNFMGVHVALA
Hhal : AEDSQRLQPWTGTPPRLTEGQEAFLPDQPLPQDPYSIRCLPQALGAVLDSITFHNQTVASELDAASDNPLLPD-EGRVLHGGNFMGQHLAFA
IL2450: LADSSEIAKDHEN-----CEKVQDPYSLRCQPVQMGAVLDQIEHASGILVREANGVTDNPLVFSE-EQDIISGGNFHAEFPVAMA
IL0135: LNHVEHPR-----NSSRLQDRYSIRCAHVICVVKDLSLPWFKETIENELNSANDNPIIDGL-GEVHLHGGHFGYGGHIAMV
                                     @ @

Rspa : SDALATAVTVLAGLAERQIARLTDERLNRGLPPFLHRGPA---GLNSGFMGQAQVTATALLAEMRATG-PASIHHSISTNAANC-DVVSGLGTIAAR
Rcap : SDALNAALVTLAGLVERQIARLTDEKLNKGLPAFLHGGQA---GLQSGFMGAQVTATALLAEMRANATFVSQSLSTNGANQ-DVVSMTGTIAAR
Hhal : ADALNNAVQLALHSERRISRITDSTR-SGFPAFMQPRQT---GLHSGFMGAQVTASALVAEMRTGAHPASIQSIPTNADNQ-DIVPMSTRAAR
IL2450: ADILAIASEIGALSERRSALLIDSHLSKLPALFLVNDG----GVNSGFMLAQVTAALASENKTLAHPASVDSLPTSANQEDHVSMTAFAAR
IL0135: MDSMKTAVANLADLHDMALLMDTKMNHGLPSNLSAAEGERKSIHNGEKAVQIGCSAWTAEALKLTMPASVFSRSTECHNQ-DKVSMTGTIAAR
                                     @ @

Rspa : LCREKIDRWAEILAILALCLAAELRCG--SGLDGVSPAGKKLVQALREQFPPLETDRPLGQETIAALATHLLQQSPV-----
Rcap : RRAQLPLSQIQAILALALAAQAMDLLDDP-EGQAGWSLTARDLRIRAVSPGLRADREPLAGHIEAVAQGLRHPSAAADPPA-----
Hhal : QAATNLHDLQRILAIEALVLAQGLELADG-----VGFSSARRTLGVVRELAPPLEDDRPLAEIARVAAALATPYQAHRLVAGLPGAPPGPAS
IL2450: RLTDIAKNVSDIIAIEWLEAAQGLDFRRP-----LGAAAVETAFCNLRQVAYYAEDRFFAPDIKAASDLIQNGELAAVVKLPHILSEV----
IL0135: DCIRILELTEQVVVATLLAAYQGVELRKRTDKFPVNPSAGVLEMLRSLADQFELLTETRQLEPELRYWLTQLRLKVWNLYQEP-----

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Supplemental Figure 1. Multiple sequence alignment of the TAL from *Rhodobacter capsulatus*, which has been studied biochemically, with the TAL from *Rb. sphaeroides*, for which the crystal structure has been determined, and the two TAL candidates from *Il. loihiensis*. The functionally important residues discussed in the text are indicated with a @; the Ala-Ser-Gly sequence that undergoes spontaneous cyclization is underlined, and the His side chain that hydrogen bonds with the phenolic group in the Tyr substrate is indicated by an arrow. Light gray residues are moderately conserved, dark gray residues are strongly conserved.

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Hhal: -----MQGLNADEVLRLLRSLIPGELAEGRGHRNDPPEGTDICADTRLD--HTPIRAD-----
Rcap: ----MPEVRRAGSGALSPPAPGPDGLGAVLPQAPDAAMVRRLISLIR---AEARRGRNQILPEAAFTGDPRID--EEGLGFDS-----
Idio: -----MTDSLQQHLIVTVIGDLIADELARMRPAESEYWKRRQWHEDDTLVAKNKSTKDNGEDDVVDS-----
Alca: MQTVNEMLRRAATRAPDHCA LAVPARGRLRTHAELRARVEAVAARLHADGLRPQQRVAVVAPNSADVVIALLALHRLGAVPALLNPRKSAEL

Hhal: -----LDRLHLASALNRLFCLHETGVEDRLLTVRRIGDIAELIAE-----GSQHTSGLSFSTSGSTGTPQSHHHSWSALTQEAELA
Rcap: -----LARLDLIGAVRDFDLSRTGIEDYVYVEPTLQGWIDRIMQHFDLLAARSETAQAVERTSGSTGTPKPIPHWPFKLMREAASMA
Idio: -----LERLAGR VVQFFHMGDSGVEDYLLRRNSLAEWAEEVVLK-----SRQVHTQNLTVTTSGSTGQPKACEHSWSALVEEVREFV
Alca: AELIKRGEMTAAVIAVGRQVADAI FQSGSGARIIFLGDLVRDGEPIYSYGPPIEDPQREPAQPAFI FYTSGTTGLPKAAIIPQRAESRVLFMS

Hhal: AALG-HHRR---VIAWLPLHHLYGFVFGVALPRTL GSTVVESHEAP-AALFRNPAPD--DLIASVFARWRYLLDSD-HRFPGGTGVSS----
Rcap: RDQG-LVPAPGGAVIGLVPAHHLFGCLFTALLPELAGAALRDLTAAAPPASALRTAQPG--DLIIATFHLWAHLGAAG-AFPPLRGVSS----
Idio: RIFDNDYELSPVRIVALVPSHHIYGFLFTVLLPHLVDAPVLRGFKAYSHVRNGGLRAG--DAVVGFEELLTQLSSEMPPLPGVLFISS----
Alac: TQVG-LRHGRHNVLGLMPLYHVVGFFAVLVAALALDGTYYVVEEFRPVDALQLVQQEQVTSLFATETHLDALAAAAAHAGSSLKLDLSLRHVT
      @@

Hhal: --TAPLEAACRHGLPRAGLDALVEVYGATETGGIGLRWAPAE DYRLLP-----YWQCNADGNLRR
Rcap: --GAPMPDALWHSLLAAGLEDL TEVYGASETGGIGLRRAPGAFTLLP-----FLSR SADDGIS-
Idio: --AGPCPASTVHQLYAIGAARAVEIYGSSETAGMAYRSKPENNYRLS-----RWRKNTENHQQ L
Alca: FAGATMPDAVLETVHQHLPGEKVNIXGTT EAMNSLYMRQPKTGTEMAPGFFSEVRIVRIGGGVDEIVANGEEGELIVAASDSAFVGYLNQPQA
      @ @

Hhal: ALPEGSAVTITPLDRLEWLDERVFRPRGRIDDIIQIGGVNVSPQHVARFESHEA VAACATRSHGEGSQRR LKAFIVPAHPETDPEELRQALE
Rcap: -----
Idio: IDRQTKVIYEIPDN-TQWHTED EFQITGRVDKAVSIRGINVFPAHIAKCLRQHPAVADATVRPMRSDEGYGLKAFIVLQEN- ISETVTEQSVQ
Alca: TAEKLQDGWYRTSDVAVWTP EGTVRILGRVDDMIISGGENIHPSEIERVLGTAPGVTEVVIG-LADQRWGQSVTACVVPRLG ETL SADALDT

Hhal: TWAWEHLPAVERPTDLRIGPELPRNAMGKLQDWD-----
Rcap: -----
Idio: TWLSDNLCAAEIPERISFGEQLPINSMGKAQDWSIDNSPTGKPLN
Alca: FCRSSELADFKRPKRYFILDQLPKNALNKVLRRLQVLVQQVSS----

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Supplemental Figure 2. Multiple sequence alignment of the *pCL* from *Rhodobacter bacter capsulatus*, which has been studied biochemically, with the *pCL* from *H. halophila* and the 4-chlorobenzoate:CoA ligase from *Alcaligenes* sp. AL3007, for which the crystal structure has been determined, with the *pCL* candidate from *I. loihiensis*. The functionally important residues discussed in the text are indicated with a @. Light gray residues are moderately conserved, dark gray residues are strongly conserved.