# SUPPORTING INFORMATION

# Specific inhibitors explore a terminal electron transfer step in the Na<sup>+</sup>-pumping NADH-ubiquinone oxidoreductase from *Vibrio cholerae*

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Proposed mechanism of the nucleophilic addition of carboxylic group to the carboxy-nitrile imine followed by the  $O \rightarrow N$  acyl shift to generate the specific photo-adduct (ref. 33).



natural korormicin A ( $IC_{50} = 5.0 \text{ nM}$ )





5-dealkyl derivative ( $IC_{50} = 2700 \text{ nM}$ )



9'-deepoxy derivative ( $IC_{50} = 23 \text{ nM}$ )

The structure-activity relationship of korormicin analogs that were synthesized in our laboratory.





The NADH-UQ oxidoreductase activity with isolated V. cholerae  $Na^+$ -NQR (0.90 nM). UQ<sub>1</sub> or UQ<sub>2</sub> was used as a substrate quinone.



Double-reciprocal plots of initial velocity vs. UQ concentration at fixed concentrations of korormicin A (0.25, 0.50, and 1.0 nM). The concentration of isolated V. cholerae  $Na^+$ -NQR was 0.90 nM. Data are representative of two independent experiments.

	<b>D9</b>	10	) 20	30	40	D49-	<b>5</b> 0 r	_ <u>D52</u>	60
			1	1	1	<u> </u>	↓ ↓	,	1
V. cholerae serotype O1	MGLKKFI		HHFEPGGKHEKWFALY	EAAATLFYT	PGLVTK	RSSHVR	DSVD	LKRI <mark>M</mark> I	M <mark>V</mark>
V. alainolyticus	MALKKFI	EDI	HHFEPGGKHEKWFALY	EAVATVFYT.	PGIVTN	KSSHVR	DSVD	LKRI <mark>M</mark> I	MV
V. parahaemolyticus	MALKKFI		HHFEPGGKHEKWFALY	EAVATVFYT	PGLVTK	KSSHVR	DSVD	LKRI <mark>M</mark> I	MV
V. campbellii	-MLKKFI		HHFEPGGKHEKWFALY	EAAATLFYT.	PGLVTK	RSSHVR	DSVD	LKRI <mark>M</mark> I	MV
V. vulnificus	MGLKKFI	E <mark>DI</mark>	HHFEPGGKHEKWFALY	EAAATLFYT.	PGLVTK	KS <mark>S</mark> HVR	DSVD	LKRI <mark>M</mark> I	MV
V. anguillarum	MGLKKFI	EDI	HHFEPGGKHEKWFALY	EAAATLFYT.	PGLVTK	RS <mark>S</mark> HVR	DSVD	LKRI <mark>M</mark> I	MV
H. ducreyi	MGLKNLE	E KM	PAFHKGGKYEKWYTLF	EATYTILYT.	PGTVTK	KDSHVR	DALD	SKRMMI	LV
P. multocida	MGLKHFI	EKI	PAFLPGGKYEKWYALF	<mark>E</mark> ATATFLYT	PGTVTH	KASHVR	DALD	SKRMMV	Γ <mark>Λ</mark>
H. influenzae	MGLKNLE	E <mark>K</mark> ME	PAFLPGGKYSKLYPIF	ESIYTLLYT.	PGTVTH	KNTHVR	DALD	SKRMMI	TV
N. meningitidis serogroup A	MGLKHFI	E <mark>KI</mark>	PHFLPGGKHEKWYALY	EAAATIFYT.	SGAVTR	KAAHVR	DALD	SKRMMI	LV
N. meningitidis serogroup B	MGLKHFI	E <mark>KI</mark>	PHFLPGGKHEKWYALY	<mark>E</mark> AAATIFYT	SGAVTR	KAAHVR	DALD	SKRMMI	LV
K. pneumoniae subsp.	MGLKHLI	E <mark>K</mark> L	PHFTHGGKLEKYYPLY	<mark>E</mark> AAATIFYT	PGQVTR	GAAHVR	DAID	LKRMMI	LV
Y. pestis	MGLKNFI	E <mark>KI</mark>	HHFEAGGKLEKYYPLY	EAAATIFYT	QGKVTP	GA <mark>S</mark> HVR	DAID	LKRM <mark>M</mark> I	LV
P. atlantica	MGLKAYI	E <mark>KI</mark>	PNFEAGGKYEKWYALY	<mark>E</mark> AAATIFYT	PGKVNK	AGTHVR	DSID	LKRI <mark>M</mark> I	MV
P. stutzeri	MGIRAFI	D <mark>KI</mark>	HHFEKGGKYEKWYALY	<mark>e</mark> aadtflyr	PGSVTK	TTAHVR	DGID	LKRM <mark>M</mark> I	TV
P. mendocina	MGIRAFI	D <mark>KI</mark>	HNFEKGGKYEKWYALY	<mark>E</mark> AIDTFFYR	PGSVTK	TTAHVR	DGID	LKRM <mark>M</mark> I	Τ <mark>V</mark>
P. aeruginosa (strain PA7)	MGLRNLI	D <mark>K</mark> V	HHFEKGGRYEKWYPLY	<mark>E</mark> AVDTFLYR	PGSVTR	TTAHVR	DGID	LKRMMI	VI
P. aeruginosa (strain UCBPP-PA14)	MGLRNLI	D <mark>K</mark> V	HHFEKGGRYEKWYPLY	<mark>E</mark> AVDTFLYR	PGSVTR	TTAHVR	DGID	LKRMMI	VI
C. salexigens	MGIRNTI	D <mark>K</mark> L	PHFHQGGKYEKFYALY	<mark>E</mark> AVDTIFYS	PPSVTK	STAHVR	DGID	LKRI <mark>M</mark> I	Τ <mark>V</mark>
C. trachomatis	-MLEKLV	/ <mark>D</mark> SL	KICRKS-KFQHMTPIA	DAVDTFCFE.	PLHTPS	SPPFVR	DAVD	VKRW <b>M</b> M	Γ <mark>Λ</mark>
C. muridarum	-MLEKEV	/ <mark>D</mark> SL	KFCRKS-KFQYMTPVA	DAVDSFCFE.	PLHTPS	SPPFVR	DAVD	VKRW <mark>M</mark> M	Γ <mark>Λ</mark>
C. felis	-MLKRFV	/ <mark>NSI</mark>	EICQKD-KFQRFTPVA	<mark>D</mark> AIDTFCYE	PIHKSS	SPPFIR	DAVD	VKRW <mark>M</mark> I	Γ <mark>Λ</mark>
C. abortus	-MLKRFV	/ <mark>N</mark> SI	EICQKD-KFQRFTPVA	<mark>D</mark> AIDTFCYE	PIHQAS	SPPFIR	DAVD	ikrw <b>m</b> m	LV
C. caviae	-MLKRFV	/ <mark>NSI</mark>	EICQKD-KFQRFTPVA	<mark>D</mark> AIDTFCYE	PIHQPS	SPPFIR	DAVD	VKRW <mark>M</mark> M	LV
C. pneumoniae	-MLKKF1	I <mark>NS</mark> L	KLCQQD-KYQRFTPIV	<mark>D</mark> AIDTFCYE	PIETPS	KPPFIR	DSVD	VKRWMM	Γ <mark>Λ</mark>
	D90-								
			D	90					
			D	90-	1.0.0				100
			<b>D</b> 70 80	90 90	100	)	110	)	120
V shalarga saratupa 01			<b>D</b> 70 80 I I	90 90	100 	)	110 	)	120 
V. cholerae serotype 01	WLAVF	PAMF	D 70 80 I I WGMYNAGGQAIAALNHI	90 90 LYSGDQLAAI	100 I IVAGNWH	) HYWLTEN	110   4LGG1	) IMSS <b>D</b> AG	120   G-WG
V. cholerae serotype O1 V. alginolyticus V. parabaemolyticus	WLAVF WFAVF	PAMF	D 70 80 I I WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI	90 90 LYSGDQLAAI 4YAGDQLATV	100   IVAGNWH /ISGNWH	) HYWLTEN HYWLTEN	110   4LGG1 4LGG1	) TMSSDAG	120   G-WG G-VG
V. cholerae serotype O1 V. alginolyticus V. parahaemolyticus V. compaliii	WLAVF WFAVF WFAVF	PAMF PAMF PAMF	D 70 80 I I WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHH WGMYNAGGQAIAALNHH	90 90 LYSGDQLAAI MYAGDQLATV MYAGDQLATV	100 IVAGNWH /ISGNWH /IAGNWH	) HYWLTEN HYWLTEN HYWLTEN	110 I ALGGJ ALGGS ALGGS	) MSSDAG MSSDAG SISADAG	120   5-WG 5-VG 5-VG
V. cholerae serotype O1 V. alginolyticus V. parahaemolyticus V. campbellii V. wulnifuus	WLAVF WFAVF WFAVF WFAVF	PAMF PAMF PAMF PAMF	D 70 80 1 1 WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHN WGMYNAGGQAIAALNHN WGMYNAGGQAIAALNHN	90 90 LYSGDQLAAI MYAGDQLATV MYAGDQLATV MYAGDQLATV	100 I VAGNWF VISGNWF VIAGNWF VIAGNWF	) HYWLTEN HYWLTEN HYWLTEN HYWLTEN	110   4LGG1 4LGG3 4LGG3 4LGG3	) MSSDAG MSSDAG MSSDAG MSSDAG	120   G-WG G-VG G-VG G-VG
V. cholerae serotype O1 V. alginolyticus V. parahaemolyticus V. campbellii V vulnificus	WLAVF WFAVF WFAVF WFAVF WFAVF	PAMF PAMF PAMF PAMF PAMF	D 70 80 I I WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI	90 90 LYSGDQLAAI MYAGDQLATV MYAGDQLATV MYAGDQLATV MYAGDQLATV	100 I IVAGNWH /ISGNWH /IAGNWH /IAGNWH IIAGNWH	) HYWLTEN HYWLTEN HYWLTEN HYWLTEN HYWLTEN	110   4LGG1 4LGG3 4LGG3 4LGG1 4LGG1	MSSDAG TIAADAG TISADAG TIGAEAG TISADAS	120   5-WG 5-VG 5-VG 5-VG 5-VG 5-WG
V. cholerae serotype O1 V. alginolyticus V. parahaemolyticus V. campbellii V vulnificus V anguillarum H. ducrevi	WLAVF WFAVF WFAVF WFAVF WFAVF WLAVF	PAMF PAMF PAMF PAMF PAMF PAMF	D 70 80 I I WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI WGMYNVGHQSITALNHI	90 90 1 LYSGDQLAAI 4YAGDQLAAI 4YAGDQLAAI 4YAGDQLAAI LYSGAELAAI 	100 IVAGNWH ISGNWH IIAGNWH IIAGNWH IIAGNWH IISGNWH	) HYWLTEN HYWLTEN HYWLTEN HYWLTEN HYWLTEN	110   4LGG1 4LGG3 4LGG3 4LGG1 4LGG1 4LGG1	MSSDAG TIAADAG TISADAG TIGAEAG TISADAS TISADAS	120   G-WG G-VG G-VG G-VG G-WG G-WA
V. cholerae serotype O1 V. alginolyticus V. parahaemolyticus V. campbellii V vulnificus V anguillarum H. ducreyi P. multocida	WLAVF WFAVF WFAVF WFAVF WLAVF WLALF WLALF	PAMF PAMF PAMF PAMF PAMF PAMF	D 70 80 I I WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI WGMYNVGQQAIAALNHI WGMYNVGQQAILATSHI WGMYNVGQQALLATSHI	90 90 1 LYSGDQLAAI MYAGDQLATV MYAGDQLATV MYAGDQLATV MYAGDQLAQI LYSGAELATV GLTDQ GLTDQ	100 I VAGNWF VISGNWF VIAGNWF VIAGNWF VISGNWF SIANNWF	) HYWLTEN HYWLTEN HYWLTEN HYWLTEN HYWLTEN HYAFSEA	110 I ALGGI ALGGI ALGGI ALGGI ALGGI AVGAI	MSSDAG TIAADAG TIAADAG TISADAG TISADAS TISTQAG TITADAG	120   G-WG G-VG G-VG G-VG G-WG G-WA G-WG
V. cholerae serotype O1 V. alginolyticus V. parahaemolyticus V. campbellii V vulnificus V anguillarum H. ducreyi P. multocida H. influenzae	WLAVF WFAVF WFAVF WFAVF WLAVF WLALF WLSLF FLALF	PAMF PAMF PAMF PAMF PAMF PAMF PAMF	D 70 80 I I WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHN WGMYNAGGQAIAALNHN WGMYNAGGQAIAALNHN WGMYNAGGQAIAALNHI WGMYNVGHQSITALNHI WGMYNVGQQAILATBHI YGMYNVGQQAILATDAI	90 90 1 YSGDQLAAI MYAGDQLATV MYAGDQLATV MYAGDQLAQI YSGAELATV GSLTDS GTLQQA	100 IVAGNWF VISGNWF VIAGNWF VIAGNWF VISGNWF VISGNWF VISGNWF VISGNWF VISGNWF VISGNWF VISGNWF	) HYWLTEN HYWLTEN HYWLTEN HYWLTEN HYWLTEN HYAFSEA QYALSHA VALACS	110 I ALGGI ALGGI ALGGI ALGGI ALGGI AVGAI ALGAI	) TASSDAG TIAADAG TISADAG TISADAS TISADAS TISADAS TISADAS TITADAG DITISAG DITISAG	120   G-WG G-VG G-VG G-WG G-WA G-WG G-WA
V. cholerae serotype O1 V. alginolyticus V. parahaemolyticus V. campbellii V vulnificus V vangiillarum H. ducreyi P. multocida H. influenzae N. meninaitidis seroaroup A	WLAVF WFAVF WFAVF WFAVF WLAVF WLALF FLALF WLALF	PAMF PAMF PAMF PAMF PAMF PAMF PAMF PAMF	D 70 80 I I WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI WGMYNAGQAIAALNHI WGMYNVGQAIAALNHI WGMYNVGQQAILATDAI YGMYNVGQQAILATDAI YGMYNVGQQAILATDAI YGMYNVGQAALAALATDAI YGMYNVGQAALAALAT	90 90 1 YSGDQLAAI MYAGDQLATV MYAGDQLATV MYAGDQLATV MYAGDQLAQI YSGAELATV GSLTDS GTLQQA GNLDQI	100     IVAGNWH /IAGNWH /IAGNWH /IAGNWH /ISGNWH /ISGNWG LIANDWH SIANNWH	) IYWLTEN IYWLTEN IYWLTEN IYWLTEN IYWLTEN IYAFSEA IYALSHA IYALASS IYALASS	110 ILGGI ILGGI ILGGI ILGGI ILGGI AVGAI ALGAI SLGLI	) TASDAG SISADAG TISADAG TISADAG TISADAG CLTADAG DLTLSAG DLTLSAG DLTANAT	120   G-WG G-VG G-VG G-WG G-WA G-WA G-WA C-WG G-VL
V. cholerae serotype O1 V. alginolyticus V. parahaemolyticus V. campbellii V vulnificus V anguillarum H. ducreyi P. multocida H. influenzae N. meningitidis serogroup A N. meningitidis serogroup B	WLAVF WFAVF WFAVF WFAVF WLAVF WLALF WLALF WLALF WLALF	PAMF PAMF PAMF PAMF PAMF PAMF PAMF PAIF PAMF	D 70 80 1 1 WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI WGMYNVGQQAIAALNHI WGMYNVGQQAILATSHI YGMYNVGQQAILATDAI YGMYNVGQQAILATDAI YGMYNVGQQAILATDAI YGMYNVGQQAILATDAI YGMYNVGQQAILATDAI	90 90 1 YSGDQLAAI MYAGDQLATV MYAGDQLATV MYAGDQLATV MYAGDQLATV SCAELA	100     IVAGNWH /IAGNWH /IAGNWH /IAGNWH /ISGNWH /IAGNWH /IANDWH JIANDWH JIANDWH	) IYWLTEN IYWLTEN IYWLTEN IYWLTEN IYWLTEN IYWLTEN IYAFSEJ IYALASS IYALASS IYALANJ	110   4LGG1 4LGG3 4LGG3 4LGG3 4LGG3 4LGG3 4LGG1 4LGA1 4LGA1	) TASDAG TIAADAG TISADAG TISADAS TISADAS TISADAG DITISAG DITISAG MISSEAG	120   G-WG G-VG G-VG G-VG G-WG G-WG G-WG G-VL
V. cholerae serotype O1 V. alginolyticus V. parahaemolyticus V. campbellii V vulnificus V anguillarum H. ducreyi P. multocida H. influenzae N. meningitidis serogroup A N. meningitidis serogroup B K. pneumoniae subsp.	WLAVF WFAVF WFAVF WFAVF WLAVF WLALF FLALF WLALF WLALF	PAMF PAMF PAMF PAMF PAMF PAMF PAMF PAMF	D 70 80 1 1 WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI WGMYNVGQQAIAALNHI WGMYNVGQQAIAALNHI YGMYNVGQQAILATSHI YGMYNVGQQAILATSHI YGMYNVGQQAILATDAI YGMYNVGQQAILATDAI YGMYNVGQQAFGALTP- YGMYNVGQQAFGALTP-	90 90 1 YSGDQLAAI MYAGDQLATV MYAGDQLATV MYAGDQLATV MYAGDQLATV MYAGDQLATV MYAGDQLATV MYAGDQLATV MYAGDQLATV SCHOLODA SCHOLDAN	100 I VAGNWF VISGNWF VIAGNWF VIAGNWF VISGNWF VISGNWF VISGNWF VIANDWF VIANDWF VIANDWF VIANDWF	) HYWLTEN HYWLTEN HYWLTEN HYWLTEN HYWLTEN HYAFSEA 2YALSHA HYALANA HYALANA HYAFANA	110   4LGG3 4LGG3 4LGG3 4LGG3 4LGG3 4LGG3 4LGG4 4LGA1 4LGA1 4LG1 4LG1 4LG1 4LG1	) TASSDAG TIAADAG TISADAG TISADAS TISADAS TISADAG TITADAG DITISAG TITANAT MSSEAG MSSEAG	120   G-WG G-VG G-VG G-VG G-WG G-WA G-WG G-VL G-VS G-VL
V. cholerae serotype O1 V. alginolyticus V. parahaemolyticus V. campbellii V vulnificus V anguillarum H. ducreyi P. multocida H. influenzae N. meningitidis serogroup A N. meningitidis serogroup B K. pneumoniae subsp. Y. cestis	WLAVF WFAVF WFAVF WFAVF WLAVF WLALF FLALF WLALF WLALF WFAVF	PAMF PAMF PAMF PAMF PAMF PAMF PAMF PAMF	D 70 80 I I WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI WGMYNVGQAIAALNHI WGMYNVGQAILATSHI YGMYNVGQAILATDAI YGMYNVGAQAILATDAI YGMYNVGAQAIPALNQI YGMYNVGAQAFGALTP- YGMYNVGAQAFGALTP- YGMYNVGQAIPALNQI	90 90 1 JYSGDQLAAI MYAGDQLATV MYAGDQLATV MYAGDQLATV MYAGDQLATV MYAGDQLATV MYAGDQLATV MYAGDQLATV JYSGAELATV JGLLQQS JDLLQQS JYSGAELADV YSGAELADV	100 I VAGNWF VISGNWF VIAGNWF VIAGNWF VISGNWF SIANNWF VIANDWF VIANDWF AIANNWF VIAGNWF	) HYWLTEN HYWLTEN HYWLTEN HYWLTEN HYWLTEN HYAFSEZ DYALSHZ HYALANZ HYAFANZ HYAFANZ HYSVAQU	110   4LGG3 4LGG3 4LGG3 4LGG3 4LGG3 4LGG3 4LGA1 4LG1 4LG1 4LG1 4LG1 4LG1 4LG1 4LG1 4LG	) TMSSDAG TIAADAG TIAADAG TIAADAG TIADAG TIADAG TITADAG TITADAG TITADAG TITADAG TITADAG TITADAG TITADAG TITADAG TIATAG TI	120   G-WG G-VG G-VG G-VG G-WG G-WG G-WG G-VL G-VL G-VS G-WL
V. cholerae serotype O1 V. alginolyticus V. parahaemolyticus V. campbellii V vulnificus V anguillarum H. ducreyi P. multocida H. influenzae N. meningitidis serogroup A N. meningitidis serogroup B K. pneumoniae subsp. Y. pestis P. atlantica	WLAVF WFAVF WFAVF WFAVF WLAVF WLALF FLALF WLALF WLALF WFAVF WFAVF	PAMF PAMF PAMF PAMF PAMF PAMF PAMF PAMF	D 70 80 I I WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI WGMYNVGQQAIAALNHI WGMYNVGQQAILATSHI YGMYNVGQQAILATSHI YGMYNVGQQAILATDAI YGMYNVGQQAIPALNQI YGMYNVGQQAFGALTP- YGMYNVGQQAFGALTP- WGMYNVGQQAFGALTP- WGMYNVGQQAFGALTP- WGMYNVGQQAFGALTP-	90 90 1 YSGDQLAAI MYAGDQLATV MYAGDQLATV MYAGDQLATV MYAGDQLAQI YSGAELATV GSLTD2 GLQQA DLLQQA YSGAELQQV YSGAELQQV GASL	100 I VAGNWH VISGNWH VIAGNWH VIAGNWH VISGNWH SIANNWH VISGNWH SIANDWH VIADWH VIADWH VIADWH VIAGDWH IANNWH	) HYWLTEN HYWLTEN HYWLTEN HYWLTEN HYWLTEN HYWLTEN HYAFSEA JYALANA HYAFANA HYAFANA HYSVAQU HYRLAQN	110   ALGGJ ALGGJ ALGGJ ALGGJ ALGGJ ALGGI ALGGI ALGJ ALGJ ALGGS ALGGS	) TMSSDAG TIAATAG TIAA	120   G-WG G-VG G-VG G-VG G-WA G-WA C-WG G-VL G-VS G-WL G-WA F-GGL
V. cholerae serotype O1 V. alginolyticus V. parahaemolyticus V. campbellii V vulnificus V anguillarum H. ducreyi P. multocida H. influenzae N. meningitidis serogroup A N. meningitidis serogroup B K. pneumoniae subsp. Y. pestis P. atlantica P. stutzeri	WLAVF WFAVF WFAVF WLAVF WLAVF WLALF WLALF WLALF WLALF WFAVF WFAVF WFAVF	PAMF PAMF PAMF PAMF PAMF PAMF PAMF PAMF	D 70 80 I I WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHN WGMYNAGGQAIAALNHN WGMYNAGGQAIAALNHN WGMYNAGQAIAALNHN WGMYNVGAQAILATDAI YGMYNVGAQAILATDAI YGMYNVGAQAILATDAI YGMYNVGAQAFGALTP- YGMYNVGAQAFGALTP- WGMYNVGQQAFGALTP- WGMYNVGQQAFGALTP- WGMYNVGQQAFGALTP- GMYNVGQQAFDALTPI WGMYNVGQQAFDALTPI YGMYNVGQQAFDALTPI YGMYNVGQQAFDALTPI YGMYNVGQQAFDALTPI	90 90 1 YSGDQLAAI 4YAGDQLAT 4YAGDQLAT 4YAGDQLAT 4YAGDQLAT 4YAGDQLAT 5 5 5 5 5 5 5 5 5 5 5 5 5	100 IVAGNWH VISGNWH VISGNWH VIAGNWH VISGNWH SIANDWH SIANDWH SIANDWH AIANNWH VIAGDWH AIANNWH VIAGDWH ADAOGWWH	) iywlten i iywlten i i i i i i i i i i i i i i i i i i	110 I MLGGT MLGGST MLGGT MLGGT MLGGT AVGAT ALGAL ALGIN MLGVS MLGVS MLGQS ALGGQ AFAG-	) TMSSDAG TIAADAG TIAADAG TIAADAG TIAADAG DITAAAG DITAAAG MSSEAG SFSADAG SFSADAG STSADAG SITPDAG 21GLEST FFDPNG	120 
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V. cholerae serotype O1 V. alginolyticus V. parahaemolyticus V. campbellii V vulnificus V anguillarum H. ducreyi P. multocida H. influenzae N. meningitidis serogroup A N. meningitidis serogroup B K. pneumoniae subsp. Y. pestis P. atlantica P. stutzeri P. mendocina P. stutzeri P. mendocina P. aeruginosa (strain PA7) P. aeruginosa (strain VCBPP-PA14) C. salexigens C. trachomatis C. muridarum C. felis C. abortus	WLAVF WFAVF WFAVF WLAVF WLAVF WLALF WLALF WLALF WLALF WLALF WLCTF WLCTF WLCTF WLCTF WLCTF VLALM VVALF VVALF	PAMF PAMF PAMF PAMF PAMF PAMF PAMF PAMF	D 70 80 1 1 WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI WGMYNAGGQAIAALNHI WGMYNAGQQAIAALNHI WGMYNVGQQAILATAINHI WGMYNVGQQAILATAINHI WGMYNVGQQAILATAINI YGMYNVGQQAILATAINI YGMYNVGQQAILATDAI YGMYNVGQQAILATDAI YGMYNVGQQAILATDAI YGMYNVGQQAILATAI YGMYNVGQQAILATAI YGMYNVGQQAILATAI YGMYNVGQQAILATAI YGMYNVGQQAILATAI YGMYNVGQQAILATAI YGMYNVGQQAILATAI YGMYNVGQAILATAI YGMYNVGQAILAI YGMYNVGQAILIFAQS FGMNNGQQANLIFAQS FGMYNVGQANLIFAQS FGMYNAGLQANMAIGDC AAVWNSGLQALVYQSS- VAIWNSGLQALVYQSS- SAIWNSGQALVYGSG-	90 90 90 1 90 1 90 1 90 1 90 1 90 1 90 1 1 90 1 1 1 1 1 1 1 1 1 1 1 1 1	100 I VAGNWF VI SGNWF VI SGNWF VI SGNWF VI SGNWF VI SGNWF SI ANNWF SI ANNWF VI SGHWG VI ANDWF VI ADWF VI AGDWF SAQDGWF SAQDGWF SAQDGWF SALGGWF MEAFLF MEAFLF	) HYWLTEN	1110   HLGG7 HLGG7 HLGG7 HLGG7 HLGG7 AVGA7 ALGA8 HLGG7 HLGA8 ALG1N HLG48 ALG4 ALG4 ALG4 ALG4 ALAG5 SYF58 SYF58 SYF58 SYF58 SYF58 SYF58 SYF58 SYF58	CMSSDAG CIAADAG CIAADAG CISADA	120   
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Alignment of the N-terminal region of the NqrB subunit in many bacterial species.





*Upper panel*: All cysteines in *V. cholerae* Na<sup>+</sup>-NQR are indicated by *red spheres*. *Lower panel*: Close up of the NqrB subunit.





*Upper panel*: NqrB-G141 and G140 in *V. cholerae* Na<sup>+</sup>-NQR are indicated by *red spheres*. *Lower panel*: Close up of the NqrB subunit.



*Upper panel*: Phe<sup>185</sup> and Phe<sup>211</sup> in TMH 4 and 5 of NqrB, respectively, and Pro<sup>185</sup>, Leu<sup>190</sup>, and Phe<sup>193</sup> in TMH 6 of NqrD in *V. cholerae* Na<sup>+</sup>-NQR are indicated.

Lower panel: Close up of the membrane domain.



The competition tests between [<sup>125</sup>I]PAD-3 and PAD-3 in the presence of UQ<sub>1</sub> (50  $\mu$ M), which partially suppresses the labeling by [<sup>125</sup>I]PAD-3 (see Figure 4). Data are representative of two independent experiments.

### **General procedures**

All moisture- and air-sensitive reactions were performed in oven-dried glassware under N<sub>2</sub> (or Ar) atmosphere with dry solvents using standard syringe septum techniques. <sup>1</sup>H-NMR spectra were recorded at 400 MHz or 500 MHz with Bruker AVANCE III 400 or AVANCE III 500 spectrometers, respectively, using tetramethylsilane (TMS) as an internal standard. <sup>13</sup>C-NMR spectra were recorded at 100 MHz or 125 MHz. Chemical shifts ( $\delta$ ) are given in ppm relative to TMS with coupling constants (*J*) in Hz. Thin-layer chromatography (TLC) was performed on Merk TLC plate silica-gel 60F<sup>254</sup>, and the spot was detected by iodine, anis, phosphomolybdic acid or UV absorbance. Dry solvents were used either as purchased or freshly distilled using common practices where appropriate. HPLC purification was carried out with a Shimadzu LC-20 AD system, and elution profiles were detected with a Shimadzu SPD-10A.

#### Abbreviations

Ac<sub>2</sub>O, acetic anhydride; DCC, *N*,*N*'-dicyclohexylcarbodiimide; DIBAL-H, diisobutylaluminium hydride; DMAP, 4-dimethylaminopyridine; DME, 1,2-dimethoxyethane; DMF, dimethylformamide; HATU, hexafluorophosphate azabenzotriazole tetramethyl uronium; HMPA, hexamethylphosphoric triamide; HOAt, 1-hydroxy-7-azabenzotriazole; NaHMDS, sodium hexamethylenedisilane; NBS, *N*-bromosuccinimide; PCC, pyridinium chlorochromate; PPTS, pyridinium *p*-toluenesulfonate; rt, rt; TBAF, tetrabutylammonium fluoride; TBS, *tert*-butyldimethylsilyl; THF, tetrahydrofuran; TMS, trimethylsilyl; Tr, trityl.

# Outline of the syntheses of PAD-3 and [125]PAD-3

**PAD-3** and [<sup>125</sup>I]**PAD-3** were synthesized according to the previous methods [*14*, *34*, *48*], as outlined in Scheme S1. The reaction of **10** with prenyl bromide **9**, followed by the reductive cyclization with Fe dust gave the key intermediate **12**. The amidation of **13** with appropriate carboxylic acids provided **PAD-3** and **18**. [<sup>125</sup>I]**PAD-3** was prepared from a stanyl precursor **18** using chloramine T and [<sup>125</sup>I]NaI as an oxidant and a radioisotope donor, respectively.

Scheme S1.



*Reagents and conditions*: (a) NBS, THF/H<sub>2</sub>O, rt, 1.5 h, 64%; (b) NaH, THF, rt, 21 h, 80%; (c) NaIO<sub>4</sub>, H<sub>5</sub>IO<sub>6</sub>, THF/H<sub>2</sub>O, rt, 3 h, 83%; (d) NaBH<sub>4</sub>, MeOH, -10 °C, 2 h, 85%; (e) MsCl, Et<sub>3</sub>N, THF, 0 °C, 3 h, 95%; (f) NaN<sub>3</sub>, DMF, 40 °C, 5 h, 87%; (g) H<sub>2</sub>, Boc<sub>2</sub>O, Pb-poisoned 5% Pd on CaCO<sub>3</sub>, MeOH, rt, 23 h, 62%; (h) K<sub>2</sub>CO<sub>3</sub>, MeOH,

rt, 1 h, quant.; (i) Et<sub>3</sub>N, MsCl, LiBr, THF, 0 °C, 1.5 h, 79%; (j) *i*-Pr<sub>2</sub>NH, *n*-BuLi, acetone, THF, -78 °C to rt, 21 h, 35%; (k) **9**, NaH, THF, 0 °C to 60 °C, 5 h, crude; (l) 6.0 M HCl aq., Fe dust, EtOH, reflux, 24 h, 31% (2 steps); (m) conc. HCl, MeOH, rt, 24 h, quant.; (n) 1) NaNO<sub>2</sub>, conc. HCl, EtOH, 0 °C, 10 min, 2) ethyl glyoxylate, benzenesulfonyl hydrazide, pyridine, -10 °C, 3.5 h, 71%; (o) 2.0 M NaOH aq., EtOH, rt, 1 h, quant.; (p) **13**, Et<sub>3</sub>N, HATU, HOAt, DMF, 80 °C, 3 h, 19%; (q) bis(tributyltin), Pd(PPh<sub>3</sub>)<sub>4</sub>, dioxane, 100 °C, 6 h, 23%; (r) 2.0 M NaOH aq., EtOH, rt, 10 min, quant.; (s) **13**, Et<sub>3</sub>N, HATU, HOAt, DMF, 50 °C, 3 h, 35%; (t) [<sup>125</sup>I]NaI, chloramine T, water, rt, 10 min.

### Synthesis of 1

To an ice-cooled solution of *trans*, *trans*-farnesyl acetate (5.0 g, 18 mmol) in a mixture of THF/water (75 mL/37 mL), NBS (4.2 g, 23 mmol) was added, and the mixture was stirred for 1.5 h at rt. The reaction mixture was extracted with Et<sub>2</sub>O and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 15% EtOAc/*n*-hexane) afforded **1** as a colorless oil (4.2 g, 12 mmol, 64%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.32 (t, *J* = 7.0 Hz, 1H), 5.16 (t, *J* = 3.8 Hz, 1H), 4.57 (d, *J* = 7.0 Hz, 2H), 3.94 (dd, *J* = 11.4, 1.9 Hz, 1H), 2.30 (m, 2H), 2.11-2.02 (m, 6H), 2.03 (s, 3H), 1.68 (s, 3H), 1.57 (s, 3H), 1.31 (d, *J* = 4.4 Hz, 6H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  171.39, 142.18, 133.78, 125.60, 118.70, 72.67, 70.99, 61.63, 39.59, 38.35, 32.27, 26.81, 26.36, 26.08, 21.29, 16.69, 16.07; ESI-MS (*m/z*) 161.1 [M+H]<sup>+</sup>.

#### Synthesis of 2

To an ice-cooled suspension of NaH (50% in mineral oil, 1.1 g, 23 mmol) in THF (77 mL), **1** (4.2 g, 12 mmol) was added under N<sub>2</sub> atmosphere, and the mixture was stirred for 21 h at rt. The reaction was quenched with saturated aqueous NH<sub>4</sub>Cl, extracted with Et<sub>2</sub>O, washed with brine, and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 15% EtOAc/*n*-hexane) afforded **2** as a colorless oil (2.6 g, 9.2 mmol, 80%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.34 (t, *J* = 7.1 Hz, 1H), 5.15 (t, *J* = 3.9 Hz, 1H), 4.58 (d, *J* = 7.1 Hz, 2H), 2.70 (t, *J* = 6.2 Hz, 1H), 2.14-2.05 (m, 6H), 2.05 (s, 3H), 1.70 (s, 3H), 1.63-1.62 (m, 2H), 1.28 (d, *J* = 15.9 Hz, 6H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  171.34, 142.31, 134.82, 124.47, 118.58, 64.37, 61.59, 58.52, 39.65, 36.52, 27.67, 26.39, 25.11, 21.28, 18.97, 16.68, 16.23; ESI-MS (*m/z*) 303.2 [M+Na]<sup>+</sup>.

### Synthesis of 3

To an ice-cooled solution of 2 (2.6 g, 9.2 mmol) in THF (60 mL), a solution of NaIO<sub>4</sub> (3.9 g, 18

mmol) in water (10 mL) and a solution of H<sub>5</sub>IO<sub>6</sub> (1.1 g, 4.6 mmol) in THF (43 mL) were added carefully. Then, the mixture was allowed to warm to rt over 1.5 h, followed by further stirring for 1.5 h. The reaction mixture was quenched with 1.0 M aqueous HCl, extracted with EtOAc, washed with saturated aqueous NaHCO<sub>3</sub> and water, and dried over anhydrous MgSO<sub>4</sub>. The purification by silicagel column chromatography (Wako gel<sup>®</sup> C-200, 15% EtOAc/*n*-hexane) afforded **3** as a colorless oil (1.8 g, 7.6 mmol, 83%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  9.72 (t, *J* = 1.9 Hz, 1H), 5.29 (t, *J* = 6.5 Hz, 1H), 5.10 (t, *J* = 6.0 Hz, 1H), 4.55 (d, *J* = 7.1 Hz, 2H), 2.48 (m, 2H), 2.29 (t, *J* = 7.4 Hz, 2H), 2.08 (m, 2H), 2.03 (m, 5H), 1.67 (s, 3H), 1.59 (s, 3H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  202.76, 171.32, 142.07, 133.68, 124.97, 118.74, 61.56, 42.32, 39.49, 32.02, 26.26, 21.26, 16.63, 16.32.

### Synthesis of 4

To a cooled solution of **3** (1.8 g, 7.6 mmol) in MeOH (76 mL), NaBH<sub>4</sub> (340 mg, 9.1 mmol) was slowly added in small portions at -10 °C. After stirring for 2 h at -10 °C, the mixture was quenched with ice-cold water. After the removal of MeOH under reduced pressure, the residue was saturated with NaCl, extracted with Et<sub>2</sub>O and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 30% EtOAc/*n*-hexane) afforded **4** as a colorless oil (1.6 g, 6.5 mmol, 85%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.34 (t, *J* = 6.4 Hz, 1H), 5.13 (t, *J* = 6.6 Hz, 1H), 4.59 (d, *J* = 7.0 Hz, 2H), 3.62 (t, *J* = 6.5 Hz, 2H), 2.12 (m, 2H), 2.08-2.04 (m, 4H), 2.06 (s, 3H), 1.70 (s, 3H), 1.69-1.65 (m, 2H), 1.61 (s, 3H), 1.50 (s, 1H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  171.38, 142.22, 135.32, 124.34, 118.68, 62.87, 61.62, 39.62, 36.09, 30.86, 26.22, 21.26, 16.60, 16.09; ESI-MS (*m/z*) 263.2 [M+Na]<sup>+</sup>.

### Synthesis of 5

To an ice-cooled solution of **4** (400 mg, 1.7 mmol) in THF (17 mL), MsCl (380 mg, 3.3 mmol) and Et<sub>3</sub>N (670 mg, 6.6 mmol) were added under N<sub>2</sub> atmosphere, and the mixture was stirred for 3 h at 0 °C. The reaction was quenched with brine, extracted with Et<sub>2</sub>O and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 30% EtOAc/*n*-hexane) afforded **5** as a colorless oil (500 mg, 1.6 mmol, 95%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.33 (t, *J* = 3.2 Hz, 1H), 5.14 (t, *J* = 6.6 Hz, 1H), 4.59 (d, *J* = 7.1 Hz, 2H), 4.20 (t, *J* = 6.5 Hz, 2H), 3.00 (s, 3H), 2.16-2.04 (m, 6H), 2.05 (s, 3H), 1.87-1.83 (m, 2H), 1.70 (s, 3H), 1.61 (s, 3H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  171.21, 142.03, 133.58, 125.26, 118.63, 69.75, 61.46, 39.45, 37.47, 35.28, 27.37, 26.21, 21.17, 16.55, 15.95.

#### Synthesis of 6

To an ice-cooled solution of **5** (500 mg, 1.6 mmol) in DMF (16 mL), NaN<sub>3</sub> (200 mg, 3.1 mmol) was added under N<sub>2</sub> atmosphere. Then, the mixture was allowed to warm to 40 °C, followed by further stirring for 5 h. The reaction mixture was quenched with ice-cold water, extracted with Et<sub>2</sub>O and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 10% EtOAc/*n*-hexane) afforded **6** as a colorless oil (360 mg, 1.4 mmol, 87%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.31 (t, *J* = 6.5 Hz, 1H), 5.10 (t, *J* = 6.6 Hz, 1H), 4.56 (d, *J* = 7.1 Hz, 2H), 3.20 (t, *J* = 6.9 Hz, 2H), 2.13-2.01 (m, 6H), 2.03 (s, 3H), 1.68 (s, 3H), 1.70-1.61 (m, 2H), 1.57 (s, 3H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  171.30, 142.17, 134.08, 125.09, 118.65, 61.56, 51.06, 39.57, 36.66, 27.15, 26.29, 21.23, 16.62, 16.00; ESI-MS (*m/z*) 268.2 [M+Na]<sup>+</sup>.

# Synthesis of 7

To a solution of **6** (360 mg, 1.4 mmol) in MeOH (7 mL), Boc<sub>2</sub>O (360 mg, 1.6 mmol) and 5% Pd/CaCO<sub>3</sub> poisoned with Pb (36 mg) was added carefully. Then, and the suspension was stirred for 23 h at rt under H<sub>2</sub> atmosphere. The mixture was filtered through a celite pad and purified by silicagel column chromatography (Wako gel<sup>®</sup> C-200, 10% EtOAc/*n*-hexane) to provide **7** as a colorless oil (284 mg, 0.84 mmol, 62%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.30 (t, *J* = 6.5 Hz, 1H), 5.06 (t, *J* = 3.9 Hz, 1H), 4.54 (d, *J* = 7.1 Hz, 2H), 3.04 (d, *J* = 6.4 Hz, 2H), 2.07 (t, *J* = 5.9 Hz, 2H), 2.03 (m, 2H), 2.01 (s, 3H), 1.96 (t, *J* = 7.6 Hz, 2H), 1.66 (s, 3H), 1.55 (s, 3H), 1.57-1.50 (m, 2H), 1.40 (s, 9H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  171.30, 156.13, 142.26, 134.91, 124.41, 118.59, 79.18, 61.56, 40.49, 39.60, 37.03, 28.61, 28.30, 26.26, 21.22, 16.60, 16.03; ESI-MS (*m*/z) 340.2 [M+H]<sup>+</sup>, 362.2 [M+Na]<sup>+</sup>.

# Synthesis of 8

To a solution of **7** (390 mg, 1.2 mmol) in MeOH (7.7 mL), K<sub>2</sub>CO<sub>3</sub> (80 mg, 0.58 mmol) was added, and the mixture was stirred for 1 h at rt. The reaction was quenched with brine, extracted with EtOAc, and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 35% EtOAc/*n*-hexane) afforded **8** as a colorless oil (370 mg, 1.2 mmol, quant.): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.37 (t, *J* = 7.4 Hz, 1H), 5.08 (t, *J* = 6.32 Hz, 1H), 4.52 (s, 1H), 4.12 (d, *J* = 6.8 Hz, 2H), 3.04 (d, *J* = 6.5 Hz, 2H), 2.13-2.07 (m, 2H), 2.03-1.95 (m, 4H), 1.64 (s, 3H), 1.56 (s, 3H), 1.58-1.51 (m, 2H), 1.41 (s, 9H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  139.10, 134.59, 124.82, 124.14, 79.32, 59.56, 40.40, 39.54, 36.96, 28.63, 28.00, 26.17, 16.37, 15.99, 14.40; ESI-MS (*m/z*) 298.2 [M+H]<sup>+</sup>, 320.2 [M+Na]<sup>+</sup>, 332.2 [M+Cl]<sup>-</sup>.

#### Synthesis of 9

To a solution of **8** (370 mg, 1.2 mmol) and Et<sub>3</sub>N (630 µL, 6.3 mmol) in THF (9 mL), MsCl (430 mg, 3.8 mmol) was added under N<sub>2</sub> atmosphere, and the mixture was stirred for 1 h at -10 °C. After the addition of LiBr (1.09 g, 12.5 mmol), the mixture was allowed to warm to 0 °C over 1.5 h. The reaction was quenched with saturated aqueous NaHCO<sub>3</sub>, extracted with Et<sub>2</sub>O, washed with brine, and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 15% EtOAc/*n*-hexane) afforded **9** as a colorless oil (360 mg, 1.0 mmol, 79%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.50 (t, *J* = 8.5 Hz, 1H), 5.08-5.05 (m, 1H), 4.50 (s, 1H), 4.00 (d, *J* = 8.4 Hz, 2H), 3.05 (d, *J* = 6.4 Hz, 2H), 2.10-2.05 (m, 4H), 1.97 (t, *J* = 7.4 Hz, 2H), 1.70 (d, *J* = 1.2 Hz, 3H), 1.58-1.53 (m, 2H), 1.57 (s, 3H), 1.42 (s, 9H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  156.14, 143.65, 135.09, 124.22, 120.90, 40.51, 39.63, 37.04, 29.84, 28.65, 28.31, 26.20, 16.14, 16.10.

#### Synthesis of 10

To an ice-cooled solution of *i*Pr<sub>2</sub>NH (2.0 mL, 14 mmol) in THF (17.6 mL), *n*-BuLi (5.8 mL, 2.6 M in *n*-hexane, 15 mmol) was added under N<sub>2</sub> atmosphere, and the mixture was stirred for 30 min at 0 °C, followed by the addition of acetone (627 mg, 11 mmol), and the mixture was stirred for 1 h at -78 °C. To the reaction mixture, 2-nitrobenzoyl chloride (2.0 g, 11 mmol) in THF (4.0 mL) was added carefully, and the mixture was gradually warmed to rt and stirred for 21 h. The reaction mixture was quenched with saturated aqueous NH<sub>4</sub>Cl, extracted with Et<sub>2</sub>O and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 15-60% EtOAc/*n*-hexane and subsequent CHCl<sub>3</sub>) afforded **10** as an orange oil (783 mg, 3.8 mmol, 35%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.90 (dd, *J* = 7.9, 1.2 Hz, 1H), 7.64 (td, *J* = 7.5, 1.3 Hz, 1H), 7.58 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.54 (td, *J* = 5.2, 1.4 Hz, 1H), 5.78 (s, 1H), 2.14 (s, 3H); ESI-MS (*m*/*z*) 208.1 [M+H]<sup>+</sup>, 230.1 [M+Na]<sup>+</sup>, 206.0 [M-H]<sup>-</sup>.

#### Synthesis of 11

To an ice-cooled suspension of NaH (50% in mineral oil, 44 mg, 0.91 mmol) and HMPA (327 mg, 1.8 mmol) in THF (2.0 mL) under Ar atmosphere, **10** (170 mg, 0.83 mmol) in THF (2.0 mL) was slowly added and the mixture was stirred for 15 min at 0 °C. Then, prenyl bromide **9** (360 mg, 0.99 mmol) in THF (2.0 mL) was added to the mixture, and the reaction was warmed to rt over 1 h, followed by heating at 60 °C for 4 h. The reaction mixture was poured onto saturated aqueous NH<sub>4</sub>Cl, extracted with Et<sub>2</sub>O, washed with brine, dried over anhydrous MgSO<sub>4</sub>, and purified by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 20–30% EtOAc/*n*-hexane) to provide a keto-enol

tautomeric mixture of **11** as an orange oil (330 mg, crude): ESI-MS (m/z) 487.3 [M+H]<sup>+</sup>, 509.3 [M+Na]<sup>+</sup>, 485.3 [M-H]<sup>-</sup>, 521.3 [M+Cl]<sup>-</sup>.

### Synthesis of 12

To a solution of **11** (330 mg, crude) in EtOH (33 mL), 6.0 M aqueous HCl (570  $\mu$ L) and Fe dust (280 mg, 5.0 mmol) were added, and the mixture was stirred for 24 h at 90 °C. Additional 6.0 M aqueous HCl (570  $\mu$ L) and Fe dust (5.0 mmol) were added to the mixture until TLC indicated the completion of the reaction. The mixture was diluted with EtOAc and water, filtered through celite, extracted with EtOAc, washed with brine, dried over anhydrous MgSO<sub>4</sub>, and purified by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 60% EtOAc/*n*-hexane) to provide **12** as an yellow solid (110 mg, 0.26 mmol, 31%, 2 steps from **10**): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.37 (d, *J* = 7.9 Hz, 1H), 7.55-7.49 (m, 2H), 7.26 (t, *J* = 7.4 Hz, 1H), 5.10 (t, *J* = 6.9 Hz, 1H), 4.96-4.92 (m, 1H), 3.41 (d, *J* = 6.7 Hz, 2H), 2.99 (q, *J* = 6.7 Hz, 2H), 2.41 (s, 3H), 2.05-1.94 (m, 4H), 1.83 (t, *J* = 8.4 Hz, 2H), 1.71 (s, 3H), 1.50 (s, 3H), 1.46 (s, 9H), 1.44 (m, 2H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  177.13, 156.61, 147.33, 139.61, 134.36, 134.12, 131.15, 126.10, 124.49, 124.41, 123.50, 123.12, 119.82, 117.85, 60.61, 40.81, 39.48, 36.93, 28.68, 25.89, 24.17, 18.58, 16.21, 16.12, 14.39; ESI-MS (*m*/*z*) 439.3 [M+H]<sup>+</sup>, 437.4 [M-H]<sup>-</sup>, 473.3 [M+Cl]<sup>-</sup>.

#### Synthesis of 13

To a solution of **12** (40 mg, 0.091 mmol) in EtOAc (0.9 mL), concentrated HCl (12 drops) was added, and the mixture was stirred for 24 h at rt. Then, the reaction mixture was basified with 6.0 M aqueous NaOH, extracted with EtOAc, dried over anhydrous MgSO<sub>4</sub>. The organic layer was concentrated under reduced pressure to afford **13** as a red oil (31 mg, quant.): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.29 (d, *J* = 7.8 Hz, 1H), 7.57 (d, *J* = 8.1 Hz, 1H), 7.44 (t, *J* = 7.2 Hz, 1H), 7.21 (t, *J* = 7.6 Hz, 1H), 5.06-4.98 (m, 2H), 3.32 (d, *J* = 6.5 Hz, 2H), 2.67 (m, 2H), 2.39 (s, 3H), 2.05-2.00 (m, 2H), 1.95-1.92 (m, 4H), 1.68 (s, 3H), 1.53-1.48 (m, 2H), 1.51 (s, 3H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  176.98, 147.88, 139.61, 134.62, 134.46, 131.10, 125.77, 124.78, 124.24, 123.21, 123.16, 119.61, 118.06, 58.39, 39.62, 36.98, 26.18, 24.26, 18.64, 18.46, 16.17, 16.11; ESI-MS (*m*/*z*) 339.2 [M+H]<sup>+</sup>, 361.3 [M+Na]<sup>+</sup>, 337.3 [M-H]<sup>-</sup>, 373.2 [M+Cl]<sup>-</sup>.

# Synthesis of 14

To a solution of ethyl 2-oxoacetate (47% in toluene, 700 mg, 3.2 mmol) in EtOH (13 mL), benzenesulfonyl hydrazide (430 mg, 2.5 mmol) was added, and the mixture was stirred for 1 h at rt.

The solvent was removed under reduced pressure, and the residue was dissolved in pyridine (13 mL). Separately, to an ice-cooled suspension of 3-iodoaniline (500 mg, 2.3 mmol) and concentrated HCl (0.9 mL) in H<sub>2</sub>O (1.7 mL) and EtOH (2.1 mL), NaNO<sub>2</sub> (160 mg, 2.3 mmol) was added. After 10-minute stirring, this mixture was added dropwise to the previously prepared pyridine solution at -10 °C. The mixture was warmed up to rt over 3.5 h with stirring. The reaction mixture was quenched with ice-cooled water, extracted with CH<sub>2</sub>Cl<sub>2</sub>, washed with 2.0 M aqueous HCl and brine, and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 10% EtOAc/*n*-hexane) afforded **14** as an orange solid (550 mg, 1.6 mmol, 71%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.58 (t, *J* = 1.8 Hz, 1H), 8.20 (m, 1H), 7.89 (m, 1H), 7.32 (t, *J* = 8.1 Hz, 1H), 4.58 (q, *J* = 7.2 Hz, 2H), 1.48 (t, *J* = 20.5 Hz, 3H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  157.79, 139.88, 131.44, 130.67, 129.48, 129.20, 119.68, 94.50, 63.17, 14.41; ESI-MS (*m*/*z*) 345.0 [M+H]<sup>+</sup>, 380.8 [M+Cl]<sup>-</sup>.

#### Synthesis of 15

To a solution of **14** (60 mg, 0.17 mmol) in EtOH (1.5 mL), 2.0 M aqueous NaOH (3 mL) was added, and the mixture was stirred for 1 h at rt. Then, the reaction mixture was acidified with 1.0 M aqueous HCl, extracted with EtOAc, and dried over anhydrous MgSO<sub>4</sub>. The organic layer was concentrated under reduced pressure to afford **15** as a red oil (60 mg, crude): <sup>1</sup>H-NMR (400 MHz, MeOD)  $\delta$  8.52 (t, *J* = 1.8 Hz, 1H), 8.19 (ddd, *J* = 0.96, 1.16, 0.96 Hz, 1H), 7.96 (ddd, *J* = 0.92, 0.64, 0.96 Hz, 1H), 7.40 (t, *J* = 8.1 Hz, 1H); <sup>13</sup>C-NMR (100 MHz, MeOD)  $\delta$  141.04, 138.64, 136.54, 132.83, 130.24, 120.85, 95.16; ESI-MS (*m/z*) 317.0 [M+H]<sup>+</sup>, 315.0 [M-H]<sup>-</sup>.

# Synthesis of PAD-3

To a solution of **15** (21 mg, 0.066 mmol) in DMF (0.45 mL), HATU (20 mg, 0.053 mmol), HOAt (7 mg, 0.053 mmol), Et<sub>3</sub>N (13 mg, 0.13 mmol), and a solution of **13** (15 mg, 0.044 mmol) in DMF (0.45 mL) were added under N<sub>2</sub> atmosphere, and the mixture was stirred for 3 h at 80 °C. The reaction was quenched with brine, extracted with CH<sub>2</sub>Cl<sub>2</sub>, washed with saturated aqueous NaHCO<sub>3</sub>, dried over anhydrous MgSO<sub>4</sub>, and purified by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 60% EtOAc/*n*-hexane) to provide **PAD-3** as a white solid (5.2 mg, 0.0082 mmol, 19%): <sup>1</sup>H-NMR (400 MHz, MeOD)  $\delta$  8.52 (t, *J* = 1.8 Hz, 1H), 8.31 (m, 1H), 8.21 (dd, *J* = 8.2, 1.0 Hz, 1H), 8.14 (dt, *J* = 8.2, 0.9 Hz, 1H), 7.78 (dt, *J* = 7.9, 1.0 Hz, 1H), 7.50 (ddd, *J* = 8.4, 6.9, 1.5 Hz, 1H), 7.41 (m, 1H), 7.31 (t, *J* = 8.1 Hz, 1H), 7.24 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H), 5.10-5.05 (m, 2H), 3.39-3.34 (m, 4H), 2.40 (s, 3H), 2.09-2.05 (m, 2H), 2.00 (m, 4H), 1.75 (s, 3H), 1.68 (quin, *J* = 7.6 Hz, 2H), 1.56 (s, 3H); <sup>13</sup>C-NMR (100 MHz, MeOD)  $\delta$  177.52, 160.33, 157.33, 151.05, 148.30, 139.96, 139.44, 137.32,

135.03, 134.28, 131.69, 129.23, 125.81, 125.38, 124.18, 123.66, 123.01, 120.00, 119.69, 117.79, 94.55, 39.80, 38.81, 37.25, 27.67, 26.50, 24.22, 18.14, 16.26, 16.08; ESI-MS (*m/z*) 637.1 [M+H]<sup>+</sup>, 635.1 [M-H]<sup>-</sup>, 671.1 [M+Cl]<sup>-</sup>.

#### Synthesis of 16

A solution of **14** (85 mg, 0.25 mmol), Pd(PPh<sub>3</sub>)<sub>4</sub> (29 mg, 0.025 mmol), and bis(tributyltin) (440 mg, 0.75 mmol) in dioxane (2.5 mL) was stirred for 6 h at 100 °C under Ar atmosphere. The reaction mixture was concentrated under reduced pressure, and the residue was purified by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 5% EtOAc/*n*-hexane) to provide **16** as an yellow oil (29 mg, 0.057 mmol, 23%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.23 (d, *J* = 2.3 Hz, 1H), 8.08 (m, 1H), 7.63 (d, *J* = 7.2 Hz, 1H), 7.51 (t, *J* = 7.9 Hz, 1H), 4.58 (q, *J* = 7.2 Hz, 2H), 1.58 (m, 12H), 1.50 (t, *J* = 7.1 Hz, 3H), 1.35 (m, 6H), 0.92 (t, *J* = 7.3 Hz, 9H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  158.18, 145.61, 138.90, 137.52, 136.24, 129.13, 127.68, 120.26, 63.00, 29.22, 27.52, 16.64, 14.43, 13.86; ESI-MS (*m/z*) 509.2 [M+H]<sup>+</sup>.

#### Synthesis of 17

To a solution of **16** (29 mg, 0.057 mmol) in EtOH (0.60 mL), 2.0 M aqueous NaOH (1.2 mL) was added, and the mixture was stirred for 10 min at rt. Then, the reaction mixture was neutralized with 1.0 M aqueous HCl, and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was concentrated under reduced pressure to afford **17** as a white solid (30 mg, quant.): <sup>1</sup>H-NMR (400 MHz, MeOD)  $\delta$  8.24 (d, J = 2.3 Hz, 1H), 8.09 (ddd, J = 1.24, 1.04, 1.28 Hz, 1H), 7.65 (m, 1H), 7.58 (m, 1H), 1.60 (m, 6H), 1.39-1.34 (m, 6H), 1.17 (m, 6H), 0.90 (t, J = 7.3 Hz, 9H); <sup>13</sup>C-NMR (100 MHz, MeOD)  $\delta$  146.17, 139.41, 138.00, 130.39, 128.32, 121.08, 30.34, 28.49, 14.14, 10.76.

#### Synthesis of 18

To a solution of **17** (40 mg, 0.084 mmol) in DMF (0.3 mL), HATU (27 mg, 0.071 mmol), HOAt (10 mg, 0.071 mmol), Et<sub>3</sub>N (18 mg, 0.18 mmol), and a solution of **13** (20 mg, 0.059 mmol) in DMF (0.3 mL) were added under N<sub>2</sub> atmosphere, and the mixture was stirred for 3 h at 50 °C. The reaction was quenched with brine, extracted with CH<sub>2</sub>Cl<sub>2</sub>, washed with saturated aqueous NaHCO<sub>3</sub>, dried over anhydrous MgSO<sub>4</sub>, and purified by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 50% EtOAc/*n*-hexane) to provide **18** as an yellow oil (17 mg, 0.021 mmol, 35%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  9.43 (s, 1H), 8.34 (d, *J* = 7.9 Hz, 1H), 8.22 (d, *J* = 2.4 Hz, 1H), 8.06 (dq, *J* = 8.1, 1.2 Hz, 1H), 7.61 (d, *J* = 7.1 Hz, 1H), 7.52-7.42 (m, 4H), 7.22 (m, 1H), 5.08 (t, *J* = 6.5 Hz, 1H), 4.99 (t, *J* =

6.0 Hz, 1H), 3.38 (m, 2H), 2.36 (s, 3H), 2.10-2.02 (m, 4H), 1.91 (t, J = 7.3 Hz, 2H), 1.72 (m, 5H), 1.63-1.51 (m, 9H), 1.35-1.24 (m, 8H), 1.13-1.09 (m, 6H), 0.87 (t, J = 7.3 Hz, 9H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  177.33, 157.29, 159.95, 145.71, 139.32, 138.90, 136.45, 133.86, 131.22, 129.17, 127.55, 126.44, 124.92, 124.44, 123.74, 123.18, 120.15, 120.07, 117.48, 39.34, 38.84, 37.00, 29.84, 29.21, 28.06, 27.51, 25.58, 24.07, 18.86, 16.05, 13.86, 10.06; ESI-MS (*m/z*) 801.4 [M+H]<sup>+</sup>.

# Synthesis of [125]PAD-3

To a mixture of a stanyl-precursor **18** (1.0 mM in EtOH, 20  $\mu$ L) and chloramine T (2.0 mM in 1.0 M KPi buffer (pH 7.4), 5  $\mu$ L) in screw-capped 1.5 mL plastic-tube was added [<sup>125</sup>I]NaI (PerkinElmer, NEZ 033A, 1 mCi, 2,000 Ci/mmol, 10  $\mu$ L). The mixture was incubated at rt for 10 min. The reaction was terminated with 5% (w/v) aqueous NaHSO<sub>3</sub> (50  $\mu$ L), and the resulting mixture was carefully extracted with CHCl<sub>3</sub> (3 × 100  $\mu$ L). The combined organic layer was concentrated using vacuum-centrifugal evaporator. The crude product was dissolved in MeOH (30  $\mu$ L), and subjected to HPLC purification (Shimadzu LC-10AS, Kyoto, Japan) using an ODS column (COSMOSIL 5C<sub>18</sub>-MS-II, 4.6 x 150 mm, Nacalai Tesque, Kyoto, Japan) at a flow rate of 0.8 mL/min with isocratic 80% MeOH/water system as an eluent. The fraction was collected every 30 s (400  $\mu$ L). To check the elution pattern of the radioactivities and their radiochemical purity, each fraction was measured by  $\gamma$ -counting system and radio-TLC analysis. The strong radioactive fractions, corresponding to the retention time of **PAD-3**, were combined and the solvent was evaporated by a vacuum-centrifugal evaporator. [<sup>125</sup>I]PAD-3 from the initial [<sup>125</sup>I]NaI was 6.7%.

# Outline of the syntheses of PAD-4 and [<sup>125</sup>I]PAD-4

The syntheses of **PAD-4** and [<sup>125</sup>**I**]**PAD-4** were outlined in Scheme S2. **PAD-4** and [<sup>125</sup>**I**]**PAD-4** were synthesized according to the same procedures as those used for the preparation of **PAD-3** and [<sup>125</sup>**I**]**PAD-3**, respectively, using geranyl acetate as a starting material.

Scheme S2.



*Reagents and conditions*: (a) NBS, THF/H<sub>2</sub>O, rt, 40 min, 81%; (b) NaH, THF, rt, 22 h, 98%; (c) NaIO<sub>4</sub>, H<sub>3</sub>IO<sub>6</sub>, THF/H<sub>2</sub>O, rt, 3 h, 47%; (d) NaBH<sub>4</sub>, MeOH, -10 °C, 3 h, 63%; (e) MsCl, Et<sub>3</sub>N, THF, 0 °C, 1 h, quant.; (f) NaN<sub>3</sub>, DMF, 40 °C, 22 h, 80%; (g) H<sub>2</sub>, Boc<sub>2</sub>O, Pb-poisoned 5% Pd on CaCO<sub>3</sub>, MeOH, rt, 5 h, 23%; (h) K<sub>2</sub>CO<sub>3</sub>, MeOH, rt, 1 h, 80%; (i) Et<sub>3</sub>N, MsCl, LiBr, THF, 0 °C, 30 min, 63%; (j) **27**, NaH, THF, 0 °C to 60 °C, 5.5 h, crude; (k) 6.0 M HCl aq., Fe dust, EtOH, reflux, 22 h, 22% (2 steps); (l) conc. HCl, MeOH, rt, 26 h, quant.; (m) **15**, Et<sub>3</sub>N, HATU, HOAt, DMF, 70 °C, 3 h, 63%; (n) **17**, Et<sub>3</sub>N, HATU, HOAt, DMF, 40 °C, 4 h, 32%; (o) [<sup>125</sup>I]NaI, chloramine T, water, rt, 10 min.

#### Synthesis of 19

To an ice-cooled solution of geranyl acetate (5.0 g, 25 mmol) in a mixture of THF/water (100 mL/50 mL), NBS (5.7 g, 33 mmol) was added, and the mixture was stirred for 40 min at rt. The reaction mixture was extracted with Et<sub>2</sub>O and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 20-40% EtOAc/*n*-hexane) afforded **19** as a colorless oil (5.9 g, 20 mmol, 81%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.38 (t, *J* = 7.0 Hz, 1H), 4.56 (d, *J* = 7.0 Hz, 2H), 3.93 (dd, *J* = 11.4, 1.9 Hz, 1H), 2.39 (m, 1H), 2.15-2.10 (m, 2H), 2.04-1.97 (m, 5H), 1.68 (s, 3H), 1.32 (d, *J* = 5.1 Hz, 6H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  171.29, 140.57, 119.90, 72.68, 70.48, 61.42, 38.32, 31.99, 26.82, 26.19, 16.63.

#### Synthesis of 20

To an ice-cooled suspension of NaH (50% in mineral oil, 1.9 g, 40 mmol) in THF (130 mL), **19** (5.9 g, 20 mmol) was added under N<sub>2</sub> atmosphere, and the mixture was stirred for 22 h at rt. The reaction was quenched with saturated aqueous NH<sub>4</sub>Cl, extracted with Et<sub>2</sub>O, washed with brine, and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 20% EtOAc/*n*-hexane) afforded **20** as a colorless oil (4.1 g, 19.5 mmol, 98%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.36 (tq, *J* = 7.1, 1.3 Hz, 1H), 4.57 (d, *J* = 7.2 Hz, 2H), 2.68 (t, *J* = 6.2 Hz, 1H), 2.21-2.11 (m, 2H), 2.03 (s, 3H), 1.70 (s, 3H), 1.67-1.63 (m, 2H), 1.28 (s, 3H), 1.24 (s, 3H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  171.29, 141.48, 119.13, 64.13, 61.47, 58.59, 36.40, 27.29, 25.04, 21.24, 18.96, 16.68; ESI-MS (*m/z*) 235.2 [M+Na]<sup>+</sup>.

# Synthesis of 21

To an ice-cooled solution of **20** (4.10 g, 19.5 mmol) in THF (120 mL), a solution of NaIO<sub>4</sub> (8.3 g, 39 mmol) in water (20 mL) and a solution of H<sub>5</sub>IO<sub>6</sub> (2.2 g, 9.8 mmol) in THF (22 mL) were added carefully. Then, the mixture was allowed to warm to rt over 1.5 h, followed by further stirring for 1.5 h. The reaction mixture was quenched with 1.0 M aqueous HCl, extracted with EtOAc, washed with saturated aqueous NaHCO<sub>3</sub> and water, and dried over anhydrous MgSO<sub>4</sub>. The purification by silicagel column chromatography (Wako gel<sup>®</sup> C-200, 20% EtOAc/*n*-hexane) afforded **21** as an yellow oil (1.6 g, 9.2 mmol, 47%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  9.75 (s, 1H), 5.33 (tq, *J* = 7.0, 1.4 Hz, 1H), 4.55 (d, *J* = 7.0 Hz, 2H), 2.55 (m, 2H), 2.35 (t, *J* = 7.5 Hz, 2H), 2.02 (s, 3H), 1.70 (s, 3H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  201.86, 171.23, 140.19, 119.55, 61.29, 41.93, 31.64, 21.20, 16.79.

### Synthesis of 22

To a cooled solution of **21** (1.6 g, 9.2 mmol) in MeOH (92 mL), NaBH<sub>4</sub> (420 mg, 11.0 mmol) was slowly added in small portions at -10 °C. The mixture was stirred for 3 h at -10 °C, and quenched with ice-cold water. After the removal of MeOH under reduced pressure, the residue was saturated with NaCl, extracted with Et<sub>2</sub>O and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 30-50% EtOAc/*n*-hexane) afforded **22** as a colorless oil (1.0 g, 5.8 mmol, 63%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.35 (t, *J* = 7.2 Hz, 1H), 4.56 (d, *J* = 7.0 Hz, 2H), 3.62 (t, *J* = 6.3 Hz, 2H), 2.11 (t, *J* = 7.2 Hz, 2H), 2.03 (s, 3H), 1.70-1.66 (m, 5H), 1.33 (s, 1H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  142.06, 118.91, 62.76, 61.52, 35.98, 30.69, 21.24, 16.58, 14.41.

### Synthesis of 23

To an ice-cooled solution of **22** (1.0 g, 5.8 mmol) in THF (58 mL), MsCl (1.3 g, 12 mmol) and Et<sub>3</sub>N (2.3 g, 23 mmol) were added under N<sub>2</sub> atmosphere, and the mixture was stirred for 1 h at 0°C. The reaction was quenched with brine, extracted with Et<sub>2</sub>O and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 40% EtOAc/*n*-hexane) afforded **23** as a colorless oil (1.5 g, 5.8 mmol, quant.): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.34 (m, 1H), 4.55 (d, J = 7.0 Hz, 2H), 4.18 (t, J = 6.4 Hz, 2H), 2.98 (s, 3H), 2.13 (t, J = 7.2 Hz, 2H), 2.02 (s, 3H), 1.90-1.83 (m, 2H), 1.68 (s, 3H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  171.22, 140.19, 119.95, 69.45, 61.31, 37.55, 35.25, 27.11, 21.18, 16.48, 14.38.

#### Synthesis of 24

To an ice-cooled solution of **23** (1.5 g, 5.8 mmol) in DMF (58 mL), NaN<sub>3</sub> (750 mg, 12 mmol) was added under N<sub>2</sub> atmosphere. Then, the mixture was allowed to warm to 40 °C, followed by further stirring for 22 h. The reaction mixture was quenched with ice-cold water, extracted with Et<sub>2</sub>O and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 15% EtOAc/*n*-hexane) afforded **24** as a colorless oil (910 mg, 4.6 mmol, 80%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.35 (t, *J* = 6.5 Hz, 1H), 4.56 (dd, *J* = 7.1, 0.4 Hz, 2H), 3.24 (t, *J* = 6.8 Hz, 2H), 2.10 (t, *J* = 7.3 Hz, 2H), 2.03 (s, 3H), 1.74-1.67 (m, 5H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  171.27, 140.79, 119.64, 61.40, 51.07, 30.56, 26.93, 21.22, 16.51.

### Synthesis of 25

To a solution of **24** (910 mg, 4.6 mmol) in MeOH (23 mL),  $Boc_2O$  (1.5 g, 6.9 mmol) and 5% Pd/CaCO<sub>3</sub> poisoned with Pb (91 mg) was added carefully. Then, the suspension was stirred for 5 h at

rt under H<sub>2</sub> atmosphere. The mixture was filtered through celite and purified by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 15-30% EtOAc/*n*-hexane) to provide **25** as a colorless oil (290 mg, 1.1 mmol, 23%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.32 (t, *J* = 7.1 Hz, 1H), 4.55 (d, *J* = 7.1 Hz, 2H), 3.07 (d, *J* = 6.5 Hz, 2H), 2.02 (m, 5H), 1.67 (s, 3H), 1.59 (q, *J* = 8.1 Hz, 2H), 1.42 (s, 9H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  171.29, 156.14, 141.64, 119.02, 61.46, 40.46, 36.89, 28.63, 28.16, 21.24, 16.54, 14.40; ESI-MS (*m/z*) 272.2 [M+H]<sup>+</sup>, 294.2 [M+Na]<sup>+</sup>.

### Synthesis of 26

To a solution of **25** (740 mg, 2.7 mmol) in MeOH (18 mL), K<sub>2</sub>CO<sub>3</sub> (190 mg, 1.4 mmol) was added, and the mixture was stirred for 1 h at rt. The reaction was quenched with brine, extracted with EtOAc, and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 40% EtOAc/*n*-hexane) afforded **26** as a colorless oil (500 mg, 2.2 mmol, 80%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.40 (t, *J* = 6.9 Hz, 1H), 4.52 (s, 1H), 4.12 (m, 2H), 3.08 (d, *J* = 6.5 Hz, 2H), 2.02 (m, 2H), 1.65 (s, 3H), 1.59 (quin, *J* = 7.4 Hz, 2H), 1.42 (s, 9H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  156.15, 138.96, 124.32, 59.47, 40.27, 36.81, 28.64, 28.03, 21.25, 16.27, 14.40; ESI-MS (*m/z*) 252.2 [M+Na]<sup>+</sup>.

#### Synthesis of 27

To a solution of **26** (500 mg, 2.2 mmol) and Et<sub>3</sub>N (1.10 g, 11 mmol) in THF (15 mL), MsCl (750 mg, 6.5 mmol) was added under N<sub>2</sub> atmosphere, and the mixture was stirred for 1 h at -10 °C. After the addition of LiBr (1.9 g, 22 mmol), the mixture was allowed to warm to 0 °C over 30 min. The reaction was quenched with saturated aqueous NaHCO<sub>3</sub>, extracted with Et<sub>2</sub>O, washed with brine, and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 15% EtOAc/*n*-hexane) afforded **27** as a colorless oil (400 mg, 1.4 mmol, 63%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.52 (tq, *J* = 8.4, 1.2 Hz, 1H), 3.98 (d, *J* = 8.4 Hz, 2H), 3.07 (t, *J* = 7.0 Hz, 2H), 2.08-2.02 (m, 2H), 1.70 (s, 3H), 1.60 (quin, *J* = 7.3 Hz, 2H), 1.42 (m, 9H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  142.97, 121.23, 111.37, 48.36, 36.93, 29.51, 28.75, 28.65, 28.10, 16.05.

# Synthesis of 28

To an ice-cooled suspension of NaH (50% in mineral oil, 60 mg, 1.3 mmol) and HMPA (450 mg, 2.5 mmol) in THF (2.9 mL) under Ar atmosphere, **10** (240 mg, 1.1 mmol) in THF (2.9 mL) was slowly added and the mixture was stirred for 15 min at 0 °C. Then, prenyl bromide **27** (400 mg, 1.4 mmol) in THF (1.8 mL) was added to the mixture, and the reaction was warmed to rt over 1 h, followed by heating at 60 °C for 4.5 h. The reaction mixture was poured onto saturated aqueous

NH<sub>4</sub>Cl, extracted with Et<sub>2</sub>O, washed with brine, dried over anhydrous MgSO<sub>4</sub>, and purified by silicagel column chromatography (Wako gel<sup>®</sup> C-200, 30–50% EtOAc/*n*-hexane) to provide a keto-enol tautomeric mixture of **28** as a red oil (420 mg, crude): ESI-MS (m/z) 441.2 [M+Na]<sup>+</sup>.

# Synthesis of 29

To a solution of **28** (420 mg) in EtOH (50 mL), 6.0 M aqueous HCl (850 µL) and Fe dust (420 mg, 7.5 mmol) were added, and the mixture was stirred for 22 h at 90 °C. Additional 6.0 M aqueous HCl (850 µL) and Fe dust (7.5 mmol) were added to the mixture until TLC indicated the completion of the reaction. The mixture was diluted with EtOAc and water, filtered through a celite pad, extracted with EtOAc, washed with brine, dried over anhydrous MgSO<sub>4</sub>, and purified by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 70% EtOAc/*n*-hexane) to provide **29** as a white powder (93 mg, 0.25 mmol, 22%, 2 steps from **10**): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  11.21 (s, 1H), 8.36 (d, *J* = 8.0 Hz, 1H), 7.53-7.48 (m, 2H), 7.28-7.24 (m, 1H), 5.07 (s, 1H), 4.68 (s, 1H), 3.40 (d, *J* = 6.4 Hz, 2H), 2.99 (d, *J* = 6.5 Hz, 2H), 2.44 (s, 3H), 1.86 (t, *J* = 7.0 Hz, 2H), 1.68 (s, 3H), 1.41 (m, 11H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  177.29, 156.28, 147.42, 139.60, 134.59, 131.30, 125.97, 124.37, 123.29, 123.17, 119.73, 117.96, 60.62, 40.55, 36.92, 28.63, 24.42, 18.68, 16.34, 14.40; ESI-MS (*m*/*z*) 371.2 [M+H]<sup>+</sup>, 393.2 [M+Na]<sup>+</sup>, 369.2 [M-H]<sup>-</sup>, 405.2 [M+Cl]<sup>-</sup>.

#### Synthesis of 30

To a solution of **29** (30 mg, 0.081 mmol) in MeOH (0.90 mL), concentrated HCl (3 drops) was added, and the mixture was stirred for 26 h at rt. Then, the reaction mixture was basified with 6.0 M aqueous NaOH, extracted with EtOAc, and dried over anhydrous MgSO<sub>4</sub>. The organic layer was concentrated under reduced pressure to afford **30** as a red oil (27 mg, quant.): <sup>1</sup>H-NMR (400 MHz, MeOD)  $\delta$  8.22 (m, 1H), 7.61 (ddd, *J* = 8.4, 6.9, 1.4 Hz, 1H), 7.50 (d, *J* = 8.0 Hz, 1H), 7.33 (ddd, *J* = 8.2, 7.0, 1.1 Hz, 1H), 5.13 (ddd, *J* = 8.0, 5.6, 1.2 Hz, 1H), 3.39 (d, *J* = 6.6 Hz, 2H), 2.58 (t, *J* = 7.9 Hz, 2H), 2.46 (s, 3H), 2.03 (m, 2H), 1.81 (s, 3H), 1.57 (quin, *J* = 7.6 Hz, 2H); <sup>13</sup>C-NMR (100 MHz, MeOD)  $\delta$  178.62, 149.90, 140.71, 135.97, 132.76, 126.44, 125.16, 124.65, 124.12, 120.80, 118.83, 61.67, 42.23, 38.11, 24.96, 18.38, 16.39; ESI-MS (*m/z*) 271.2 [M+H]<sup>+</sup>, 293.2 [M+Na]<sup>+</sup>, 269.2 [M-H]<sup>-</sup>.

# Synthesis of PAD-4

To a solution of **15** (32 mg, 0.10 mmol) in DMF (0.8 mL), HATU (36 mg, 0.094 mmol), HOAt (13 mg, 0.094 mmol),  $Et_3N$  (24 mg, 0.23 mmol), and a solution of **30** (21 mg, 0.078 mmol) in DMF (0.8 mL) were added under N<sub>2</sub> atmosphere, and the mixture was stirred for 3 h at 70 °C. The reaction

was quenched with brine, extracted with CH<sub>2</sub>Cl<sub>2</sub>, washed with saturated aqueous NaHCO<sub>3</sub>, dried over anhydrous MgSO<sub>4</sub>, and purified by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 5% MeOH/CHCl<sub>3</sub>) to provide **PAD-4** as an orange powder (28 mg, 0.049 mmol, 63%): <sup>1</sup>H-NMR (400 MHz, MeOD)  $\delta$  8.53 (t, *J* = 1.8 Hz, 1H), 8.25 (dd, *J* = 8.2, 0.9 Hz, 1H), 8.15 (dt, *J* = 8.2, 1.2 Hz, 1H), 7.90 (dd, *J* = 7.8, 0.9 Hz, 1H), 7.56 (td, *J* = 7.0, 1.4 Hz, 1H), 7.45 (m, 1H), 7.36-7.32 (m, 2H), 5.18 (t, *J* = 6.8 Hz, 1H), 3.48-3.40 (m, 2H), 2.47 (s, 3H), 2.11 (t, *J* = 7.4 Hz, 2H), 1.83 (s, 3H), 1.82-1.76 (m, 2H), 1.27 (s, 2H), 0.89 (s, 1H); <sup>13</sup>C-NMR (100 MHz, MeOD)  $\delta$  203.56, 193.48, 178.49, 160.92, 157.89, 148.88, 140.30, 139.66, 137.79, 134.79, 132.07, 129.60, 126.02, 124.67, 124.01, 120.06, 118.16, 100.28, 94.77, 40.13, 37.48, 27.80, 24.67, 18.40, 16.42; ESI-MS (*m/z*) 569.1 [M+H]<sup>+</sup>, 591.1 [M+Na]<sup>+</sup>, 567.1 [M-H]<sup>-</sup>, 603.0 [M+C]]<sup>-</sup>.

# Synthesis of 31

To a solution of **17** (26 mg, 0.054 mmol) in DMF (0.4 mL), HATU (37 mg, 0.097 mmol), HOAt (13 mg, 0.097 mmol), Et<sub>3</sub>N (25 mg, 0.24 mmol), and a solution of **30** (27 mg, 0.081 mmol) in DMF (0.4 mL) were added under N<sub>2</sub> atmosphere, and the mixture was stirred for 4 h at 40 °C. The reaction was quenched with brine, extracted with CH<sub>2</sub>Cl<sub>2</sub>, washed with saturated aqueous NaHCO<sub>3</sub>, dried over anhydrous MgSO<sub>4</sub>, and purified by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 60% EtOAc/*n*-hexane) to provide **31** as an orange oil (19 mg, 0.026 mmol, 32%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  10.6 (s, 1H), 8.35 (d, *J* = 8.0 Hz, 1H), 8.21 (m, 1H), 8.01 (dq, *J* = 8.0, 0.9 Hz, 1H), 7.59 (d, *J* = 7.1 Hz, 1H), 7.49-7.43 (m, 4H), 7.24 (m, 1H), 5.14 (t, *J* = 6.1 Hz, 1H), 3.44 (m, 2H), 2.46 (s, 3H), 1.99 (t, *J* = 7.3 Hz, 2H), 1.73 (s, 3H), 1.67 (t, *J* = 7.2 Hz, 2H), 1.58-1.50 (m, 6H), 1.37-1.28 (m, 8H), 1.13-1.09 (m, 6H), 0.88 (t, *J* = 7.3 Hz, 9H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  177.38, 160.02, 156.95, 146.91, 145.50, 139.48, 138.70, 136.27, 134.13, 131.30, 129.09, 127.50, 126.13, 124.40, 123.80, 123.24, 120.10, 119.72, 117.74, 39.71, 37.01, 29.19, 27.48, 24.45, 18.79, 16.34, 13.84, 10.02, 8.31; ESI-MS (*m*/*z*) 733.3 [M+H]<sup>+</sup>, 755.3 [M+Na]<sup>+</sup>, 731.1 [M-H]<sup>-</sup>, 767.2 [M+Cl]<sup>-</sup>.

# Synthesis of [125]PAD-4

To a mixture of a stanyl-precursor **31** (1.0 mM in EtOH, 20  $\mu$ L) and chloramine T (2.0 mM in 1.0 M KPi buffer (pH 7.4), 5  $\mu$ L) in screw-capped 1.5 mL plastic-tube, [<sup>125</sup>I]NaI (PerkinElmer, NEZ 033A, 1 mCi, 2,000 Ci/mmol, 10  $\mu$ L) was added. The mixture was incubated at rt for 10 min. The reaction was terminated with 5% (w/v) aqueous NaHSO<sub>3</sub> (50  $\mu$ L), and the resulting mixture was carefully extracted with CHCl<sub>3</sub> (3 × 100  $\mu$ L). The combined organic layer was concentrated using vacuum-centrifugal evaporator. The crude product was dissolved in MeOH (30  $\mu$ L), and subjected to

HPLC purification (Shimadzu LC-10AS, Kyoto, Japan) using an ODS column (COSMOSIL  $5C_{18}$ -MS-II, 4.6 x 150 mm, Nacalai Tesque, Kyoto, Japan) at a flow rate of 0.8 mL/min with isocratic 70% MeOH/water system as an eluent. The fraction was collected every 30 s (400 µL). To check the elution pattern of the radioactivities and their radiochemical purity, each fraction was measured by  $\gamma$ -counting system and radio-TLC analysis. The strong radioactive fractions, corresponding to the retention time of cold-type **PAD-4**, were combined and the solvent was evaporated by a vacuum-centrifugal evaporator. [<sup>125</sup>I]PAD-4 was stored as an ethanolic solution (0.10 µM) at 4 °C. The radiochemical yield of [<sup>125</sup>I]PAD-4 from the initial [<sup>125</sup>I]NaI was 1.6%.

# Outline of the syntheses of PKRD-1 and |<sup>125</sup>I|PKRD-1

The synthetic procedure of [<sup>125</sup>I]PKRD-1 was outlined in Scheme S3. The key intermediates A and B were synthesized according to the method of Kobayashi *et al* and Uehara *et al* [49, 50]. Mono-TBS ether protection of 1,6-hexanediol gave 32. After oxidation of 32, Horner-Wadsworth-Emmons reaction provided 34, which was treated with DIBAL-H, followed by halogenation with Appel reaction. Sharpless asymmetric dihydroxylation with AD-mix- $\alpha$  of 36 gave 37. After epoxidation of 37, mesylation of hydroxy group gave 39. The epoxide of 39 was opened by lithium trimethylsilylacetylide to afford 40. After deprotection of TMS group and subsequent ring closure reaction, acetyl protection of hydroxy group gave 42. Hydroboration and subsequent transesterification provided 44. Suzuki-Miyaura cross coupling of C and 44 gave 45. After deprotection of acetyl group and mesylation of hydroxy group, S<sub>N</sub>2 reaction with potassium salt of phthalimide gave 48. Deprotection of TBS group and phthalimide group provided 50. The amidation of 50 with appropriate carboxylic acids provided PKRD-1 and 51. [<sup>125</sup>I]PKRD-1 was prepared from a stanyl precursor 51 using chloramine T and [<sup>125</sup>I]NaI as an oxidant and a radioisotope donor, respectively.

Scheme S3.



*Reagents and conditions*: (a) DCC, DMAP, PPTS, CH<sub>2</sub>Cl<sub>2</sub>, rt, 12 h, 54%; (b) TBSCl, NaH, THF, rt, 20 °C, 61%; (c) PCC, NaOAc, celite, CH<sub>2</sub>Cl<sub>2</sub>, rt, 1 h, 73%; (d) triethyl phosphonoacetate, NaH, THF, rt, 30 min, 50%; (e) DIBAL-H, CH<sub>2</sub>Cl<sub>2</sub>, -78 °C, 1 h, 80%; (f) MsCl, LiCl, 2,4,6-trimethylpyridine, DMF, 0 °C, 3 h, 81%; (g) ADmix- $\alpha$ , MeSO<sub>2</sub>NH<sub>2</sub>, *t*-BuOH/H<sub>2</sub>O, 4 °C, 24 h; (h) NaOH, THF, rt, 1 h, 78% (2 steps); (i) MsCl, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 40 min; (j) TMS acetylene, *n*-BuLi, BF<sub>3</sub>·Et<sub>2</sub>O, THF, -78 °C, 1 h; (k) K<sub>2</sub>CO<sub>3</sub>, MeOH, rt, 10 h, 38% (3 steps); (l) Ac<sub>2</sub>O, Et<sub>3</sub>N, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, rt, 4 h, 88%; (m) 1)  $\alpha$ -(-)-pinene, BH<sub>3</sub>·SMe<sub>2</sub>, THF, 0 °C, 2 h, 2) acetaldehyde, THF, reflux, 19 h; (n) 2,2-dimethyl-1,3-propanediol, THF, rt, 5 h, 42% (2 steps); (o) C, Pd(PPh<sub>3</sub>)<sub>4</sub>, Na<sub>2</sub>CO<sub>3</sub>, DME/H<sub>2</sub>O, 90 °C, 1 h, 42%; (p) K<sub>2</sub>CO<sub>3</sub>, MeOH/H<sub>2</sub>O, rt, 3 h, 67%; (q) MsCl, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 1 h; (r) potassium phthalimide, DMF, rt, 3 days, 69% (2 steps); (s) TBAF, THF, rt, 30 min, quant.; (t) MeNH<sub>2</sub>, THF/MeOH, 0 °C, 4 h, 49%; (u) **15**, HATU, HOAt, Et<sub>3</sub>N, DMF, rt, 24 h, 59%; (v) **17**, HATU, HOAt, Et<sub>3</sub>N, DMF, rt, 24 h, 21%; (w) [<sup>125</sup>I]NaI, chloramine T, water, rt, 10 min.

### Synthesis of A

This compound was synthesized according to the procedure described in ref 4: <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.83 (s, 1H), 3.72 (br, 2H), 1.66-1.83 (m, 2H), 1.42 (s, 3H), 0.86 (t, *J* = 7.4 Hz, 3H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  170.80, 132.67, 119.81, 86.60, 32.75, 25.14, 8.42.

### Synthesis of **B**

This compound was synthesized according to the procedure described in ref 5: <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  6.28-6.33 (m, 2H), 4.81-4.86 (m, 1H), 2.54-2.56 (m, 2H), 0.86 (s, 9H), 0.11 and 0.07 (s, 6H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  176.23, 142.89, 81.57, 72.95, 41.71, 25.88 (3C), 18.15, -4.17, -4.79.

# Synthesis of C

To a solution of **A** (29 mg, 0.20 mmol) and **B** (70 mg, 0.20 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1.5 mL), DMAP (6 mg, 0.04 mmol), PPTS (16 mg, 0.06 mmol), and DCC (46 mg, 0.22 mmol) were added under Ar atmosphere at rt. After stirring for 12 h at rt, the reaction mixture was quenched with water, extracted with CH<sub>2</sub>Cl<sub>2</sub> and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 10-20% EtOAc/*n*-hexane) afforded **C** as a yellow oil (52 mg, 0.11 mmol, 54%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.22 (s, 1H), 7.35 (s, 1H), 6.36-6.29 (m, 2H), 4.80 (td, *J* = 7.2, 3.8 Hz, 1H), 2.61 (dd, *J* = 14.4, 3.8 Hz, 1H), 2.52 (dd, *J* = 14.4, 7.0 Hz, 1H), 1.90-1.70 (m, 2H), 1.47 (s, 3H), 0.87 (s, 9H), 0.86 (t, *J* = 7.4 Hz, 3H), 0.09 (s, 6H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  169.14, 168.95, 142.05, 133.84, 124.67, 88.28, 81.98, 73.28, 43.78, 32.06, 25.71 (3C), 24.32, 18.00, 8.15, -4.45, -4.99; ESI-MS (*m*/*z*) 480.2 [M+H]<sup>+</sup>, 518.2 [M+K]<sup>+</sup>.

#### Synthesis of 32

To an ice-cooled suspension of NaH (60% in mineral oil, 3.38 g, 85 mmol) under N<sub>2</sub> atmosphere, 1,6-hexanediol (10.0 g, 85 mmol) in THF (340 mL) was slowly added and the mixture was stirred for 30 min at 0 °C. After stirring for 2 h at rt, TBSCl (12.8 g, 84.6 mmol) was added to the mixture, and the reaction mixture was stirred at rt for 20 h. The reaction mixture was quenched with H<sub>2</sub>O, extracted with Et<sub>2</sub>O and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 20% EtOAc/*n*-hexane) afforded **32** as a colorless oil (12.0 g, 52 mmol, 61%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  3.64 (t, *J* = 6.8 Hz, 2H), 3.60 (t, *J* = 6.4 Hz, 2H), 1.58-1.35 (m, 8H), 0.89 (s, 9H), 0.05 (s, 6H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  68.19, 63.38, 33.00 (2C), 26.19 (3C), 25.84, 25.75, 18.59, -5.05 (2C).

#### Synthesis of 33

To a suspension of silica gel (5.0 g), NaOAc (1.88 g, 23 mmol) and PCC (4.92 g, 23 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (75 mL), **32** (3.54 g,15 mmol) was added under N<sub>2</sub> atmosphere, and the mixture was stirred for 1 h at rt. The reaction mixture was diluted with Et<sub>2</sub>O, filtered, and purified by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 15% EtOAc/*n*-hexane) to provide **33** as a colorless oil (2.54 g, 11 mmol, 73%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  9.76 (s, 1H), 3.61 (t, *J* = 6.4 Hz, 2H), 2.43 (dt, *J* = 7.4, 1.8 Hz, 2 H), 1.51-1.65 (m, 6 H), 0.89 (s, 9H), 0.04 (s, 6H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  202.96, 63.09, 44.13, 32.75, 26.17 (3C), 25.69, 22.12, 18.57, -5.08 (2C).

### Synthesis of 34

To an ice-cooled suspension of NaH (60% in mineral oil, 521 mg, 13 mmol) in THF (12 mL) under N<sub>2</sub> atmosphere, triethyl phosphonoacetate (4.0 g, 18 mmol) was added and the mixture was stirred for 5 min at 0 °C. After stirring for 30 min at rt, **2** (2.50 g, 11 mmol) in a small amount of THF (2 mL) was slowly added to the mixture, and then the reaction mixture was stirred at rt for 30 min. The reaction mixture was quenched with ice-cooled H<sub>2</sub>O, extracted with *n*-hexane and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 10% EtOAc/*n*-hexane) afforded **33** as a colorless oil (1.65 g, 5.5 mmol, 50%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  6.95 (dt, *J* = 15.6, 7.0 Hz, 1 H), 5.80 (dt, *J* = 15.6, 5.8 Hz, 1H), 4.18 (q, *J* = 7.1 Hz, 2 H), 3.60 (t, *J* = 6.5 Hz, 3H), 2.20 (q, *J* = 8.7 Hz, 2H), 1.39-1.59 (m, 6H), 0.89 (s, 9H), 0.04 (s, 6 H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  167.01, 149.46, 121.56, 63.23, 60.34, 32.78, 32.39, 30.06, 28.03, 26.19 (3C), 18.58, 14.50, -5.06 (2C).

#### Synthesis of 35

To a solution of **34** (1.50 g, 5.0 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL), DIBAL-H (1.0 M in toluene, 10.0 mL, 10 mmol) was slowly added under N<sub>2</sub> atmosphere at -78 °C. After stirring for 1 h at -78 °C, the reaction mixture was quenched with saturated aqueous potassium sodium tartrate, and stirred for 2 h at 0 °C. The mixture was filtered through celite, and extracted with Et<sub>2</sub>O and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 15% EtOAc/*n*-hexane) afforded **35** as a colorless oil (1.04 g, 4.0 mmol, 80%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.66 (m, 2H), 4.11 (m, 1H), 3.60 (t, *J* = 6.6 Hz, 2H), 2.05 (m, 2H), 1.24-1.61 (m, 6H), 0.89 (s, 9H), 0.05 (s, 6H); <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  133.60, 129.02, 64.07, 63.40, 32.91, 32.40, 29.13, 26.20 (3C), 25.58, 18.59, -5.04 (2C).

#### Synthesis of 36

To a solution of **35** (658 mg, 2.6 mmol) in DMF (42 mL) under N<sub>2</sub> atmosphere, LiCl (1.08 g, 26 mmol) and 2,4,6-trimethyl pyridine (3.15 g, 26 mmol) were added. After stirring for 5 min at 0 °C, MsCl (871 mg, 7.6 mmol) was added to the mixture, and then the reaction mixture was stirred for 3 h at 0 °C. The reaction mixture was quenched with ice-cooled H<sub>2</sub>O, extracted with *n*-hexane, washed with 1.0 M aqueous HCl (3 times) and saturated aqueous NaHCO<sub>3</sub> (3 times), and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 5% EtOAc/*n*-hexane) afforded **36** as a colorless oil (575 mg, 2.1 mmol, 81%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.76 (m, 1H), 5.60 (m, 1H), 4.04 (dd, *J* = 7.0, 0.8 Hz, 2H), 3.60 (t, *J* = 6.6 Hz, 2H), 2.06 (dt, *J* = 7.0, 6.8 Hz, 2H), 1.31-1.55 (m, 6H), 0.89 (s, 9H), 0.04 (s, 6H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  136.32, 126.18, 63.35, 45.70, 32.86, 32.25, 28.85, 26.20 (3C), 25.55, 18.58, -5.05 (2C).

# Synthesis of 37

To a solution of AD-mix- $\alpha$  (14.3 g), MeSO<sub>2</sub>NH<sub>2</sub> (948 mg, 10 mmol) in *t*-BuOH (50 mL) and H<sub>2</sub>O (50 mL), **36** (2.67 g, 10 mmol) was slowly added under N<sub>2</sub> atmosphere at 0 °C. After stirring for 24 h at 0 °C, the reaction mixture was quenched with saturated aqueous NaHCO<sub>3</sub>, extracted with Et<sub>2</sub>O, washed with 1.0 M aqueous KOH, and dried over anhydrous MgSO<sub>4</sub>. The resulting mixture was filtered and concentrated *in vacuo* to afford crude **37** as a light-yellow oil (2.11 g, <10 mmol), which was used for the next step without further purification.

### Synthesis of 38

To a solution of **37** (2.11 g, <10 mmol, crude) in THF (22 mL) under N<sub>2</sub> atmosphere, NaOH (4.0 g, 100 mmol) was added. After stirring for 1 h at rt, the reaction mixture was diluted with Et<sub>2</sub>O, quenched with brine, extracted with Et<sub>2</sub>O, and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 30% EtOAc/*n*-hexane) afforded **38** as a colorless oil (2.14 g, 7.8 mmol, 78%, 2 steps): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  3.61 (t, *J* = 6.5 Hz, 2H), 3.44 (m, 1H), 2.98 (m, 1H), 2.82 (m, 1H), 2.71 (m, 1H), 1.39-1.65(m, 8H), 0.89 (s, 9H), 0.04 (s, 6H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  71.81, 63.34, 60.60, 55.53, 45.37, 34.69, 32.95, 26.20 (3C), 26.07, 18.59, -5.05 (2C).

#### Synthesis of 39

To a solution of 38 (2.41 g, 7.8 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) and Et<sub>3</sub>N (2.5 g, 25 mmol) under N<sub>2</sub>

atmosphere, MsCl (0.9 mL, 12 mmol) was added at 0 °C. After stirring for 1 h at 0 °C, the reaction mixture was diluted with  $Et_2O$ , quenched with saturated aqueous NaHCO<sub>3</sub>, extracted with  $Et_2O$ , and dried over anhydrous MgSO<sub>4</sub>. The resulting mixture was filtered and concentrated *in vacuo* to afford crude **39** as a light yellow oil (2.69 mg, <7.8 mmol), which was used for the next step without further purification.

#### Synthesis of 40

To a solution of TMS acetylene (314 mg, 3.2 mmol) in THF (5 mL), *n*-BuLi (2.7 M in *n*-hexane, 1.0 mL, 2.65 mmol) was added at -78 °C under N<sub>2</sub> atmosphere. After stirring for 30 min at -78 °C, BF<sub>3</sub>·Et<sub>2</sub>O (383 mg, 2.7 mmol) was added. After stirring for 10 min at -78 °C, **39** (560 mg, <1.6 mmol, crude) in THF (2.5 mL) was added. After stirring for 1 h at -78 °C, the mixture was allowed to warm to 0°C. The reaction mixture was quenched with saturated aqueous NH<sub>4</sub>Cl, extracted with Et<sub>2</sub>O and dried over anhydrous MgSO<sub>4</sub>. The resulting mixture was filtered and concentrated *in vacuo* to afford crude **40** as a light yellow oil (638 mg, < 1.6 mmol, crude), which was used for the next step without further purification.

### Synthesis of 41

To a solution of **40** (1.50 g, <3.33 mmol) in MeOH (14 mL), K<sub>2</sub>CO<sub>3</sub> (1.80 g, 13 mmol) was added at rt. After stirring for 10 h at rt, the reaction mixture was diluted with Et<sub>2</sub>O, quenched with saturated aqueous NH<sub>4</sub>Cl, extracted with Et<sub>2</sub>O, and dried over anhydrous MgSO<sub>4</sub>. The purification by silicagel column chromatography (Wako gel<sup>®</sup> C-200, 10% EtOAc/*n*-hexane) afforded **41** as a colorless oil (282 mg, 1.7 mmol, 38%, 3 steps): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  3.66 (t, *J* = 6.4 Hz, 2H), 3.16 (m, 1H), 2.97 (m, 1H), 2.60 (ddd, *J* = 17.2, 5.5, 2.5 Hz, 1H), 2.28 (ddd, *J* = 17.2, 7.3, 2.8 Hz, 1H), 2.05 (s, 1H), 1.39-1.64 (m, 8H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  79.61, 70.59, 63.02, 57.07, 54.96, 32.82, 27.69, 26.51, 25.82, 18.73.

# Synthesis of 42

To an ice-cooled solution of **41** (300 mg, 1.8 mmol) in  $CH_2Cl_2$  (4 mL), DMAP (11.0 mg, 0.09 mmol), Ac<sub>2</sub>O (449 mg, 4.4 mmol), and Et<sub>3</sub>N (233 mg, 2.3 mmol) were added under N<sub>2</sub> atmosphere at 0 °C. After stirring for 90 min at 0 °C and further 4 h at rt, the reaction mixture was quenched with saturated aqueous NH<sub>4</sub>Cl, extracted with Et<sub>2</sub>O, washed with saturated aqueous NaHCO<sub>3</sub>, and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 50% EtOAc/*n*-hexane) afforded **42** as a colorless oil (329 mg, 1.6 mmol, 88%): <sup>1</sup>H-NMR (400 MHz,

CDCl<sub>3</sub>)  $\delta$  4.07 (t, J = 6.7 Hz, 2H), 3.16 (m, 1H), 2.97 (m, 1H), 2.61 (ddd, J = 17.2, 5.5, 2.5 Hz, 1H), 2.28 (ddd, J = 17.2, 7.3, 2.1Hz, 1H), 2.06 (s, 1H), 2.05 (s, 3H), 1.43-1.70 (m, 8H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  171.40, 70.64, 64.56, 57.00, 54.93, 28.73, 27.64, 26.38, 26.02, 21.22, 18.73.

### Synthesis of 43

To a solution of BH<sub>3</sub>·SMe<sub>2</sub> (152 mg, 2.0 mmol) in THF (0.8 mL), (-)- $\alpha$ -pinene (682 mg, 5.0 mmol) was added under N<sub>2</sub> atmosphere at 0 °C. After stirring for 1 h at 0 °C and 2 h at rt, the reaction mixture was cooled to -30 °C. After addition of **42** (100 mg, 0.48 mmol), the reaction mixture was stirred for 10 min at -30 °C and 2 h at 0 °C. After addition of acetaldehyde (1.15 g, 26 mmol), the reaction mixture was stirred for 19 h at 40 °C. The resulting mixture was concentrated *in vacuo* to afford crude **43** as a light yellow oil (<1.6 mmol, crude), which was used for the next step without further purification.

### Synthesis of 44

To a solution of **43** (<1.6 mmol, crude) in THF (0.8 mL), 2,2-dimethyl-1,3-propanediol (197 mg, 1.9 mmol) was added under N<sub>2</sub> atmosphere at rt. After stirring for 5 h at rt, the resulting mixture was concentrated *in vacuo*. The crude product was purified by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 10% EtOAc/*n*-hexane) to afford **44** as a colorless oil (61 mg, 0.20 mmol, 42%, 2 steps): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  6.53 (dt, *J* = 17.9, 5.3 Hz, 1H), 5.50 (dt, *J* = 17.9, 1.6 Hz, 1H), 4.05 (t, *J* = 4.0 Hz, 2H), 3.64 (s, 4H), 3.03 (m, 1H), 2.94 (m, 1H), 2.48 (m, 1H), 2.26 (m, 1H), 2.04 (s, 3H), 1.37-1.66 (m, 8H), 0.93 (s, 6H); <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  171.18, 145.57, 125.91 (br), 72.08, 64.41, 56.89, 55.54, 33.96, 31.78, 30.37, 28.54, 27.63, 25.87, 21.85, 21.00.

#### Synthesis of 45

To a solution of **C** (51 mg, 0.11 mmol) and Pd(PPh<sub>3</sub>)<sub>4</sub> (6.2 mg, 0.0055 mmol) in DME (1.5 mL), **44** (42 mg, 0.13 mmol) and aqueous Na<sub>2</sub>CO<sub>3</sub> (12 mg, 0.11 mmol in 0.8 mL H<sub>2</sub>O) were added. After stirring for 1 h at 90 °C, the reaction mixture was cooled to rt, and quenched with brine, extracted with EtOAc and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 20-30% EtOAc/*n*-hexane) afforded **45** as a yellow oil (26 mg, 0.046 mmol, 42%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.15 (s, 1H), 7.34 (s, 1H), 6.40 (ddd, *J* = 15.0, 11.5, 0.8 Hz, 1H), 5.98 (t, *J* = 11.2 Hz, 1H), 5.78 (dt, *J* = 15.0, 6.9 Hz, 1H), 5.36 (dd, *J* = 10.7, 9.0 Hz, 1H), 5.01 (td, *J* = 8.0, 3.4 Hz, 1H), 4.07 (t, *J* = 6.6 Hz, 1H), 3.02-2.90 (m, 2H), 2.57 (dd, *J* = 14.2, 3.8 Hz, 1H), 2.49 (dd, *J* = 14.3, 7.7 Hz, 1H), 2.41-2.22 (m, 2H), 2.05 (s, 3H), 1.90-1.72 (m, 2H), 1.69-1.40 (m, 11H), 0.890.80 (m, 12H), 0.07 (s, 3H), 0.06 (s, 3H).

#### Synthesis of 46

To a solution of **45** (26 mg, 0.046 mmol) in MeOH (1.8 mL), aqueous K<sub>2</sub>CO<sub>3</sub> (19 mg, 0.14 mmol in 0.6 mL H<sub>2</sub>O) was added. After stirring for 3 h at rt, the reaction mixture was quenched with saturated aqueous NH<sub>4</sub>Cl, extracted with EtOAc and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 40-50% EtOAc/*n*-hexane) afforded **46** as a yellow oil (16 mg, 0.031 mmol, 67%): <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.19 (s, 1H), 7.34 (s, 1H), 6.39 (ddd, *J* = 14.9, 11.2, 1.0 Hz, 1H), 5.98 (t, *J* = 11.1 Hz, 1H), 5.78 (dt, *J* = 15.0, 7.0 Hz, 1H), 5.36 (dd, *J* = 10.9, 8.8 Hz, 1H), 5.01 (td, *J* = 7.8, 3.8 Hz, 1H), 3.66 (t, *J* = 6.5 Hz, 1H), 3.02-2.94 (m, 2H), 2.56 (dd, *J* = 14.1, 3.8 Hz, 1H), 2.52-2.41 (m, 2H), 2.30 (dt, *J* = 15.3, 6.9 Hz, 1H), 1.91-1.72 (m, 2H), 1.68-1.42 (m, 11H), 0.89-0.78 (m, 12H), 0.06 (s, 3H), 0.05 (s, 3H).

### Synthesis of 47

To an ice-cooled solution of **46** (5.4 mg, 10.3  $\mu$ mol) in CH<sub>2</sub>Cl<sub>2</sub> (0.5 mL), Et<sub>3</sub>N (6  $\mu$ L, 43  $\mu$ mol) and MsCl (3.3  $\mu$ L, 43  $\mu$ mol) were added under N<sub>2</sub> atmosphere at 0 °C. After stirring for 1 h at 0 °C, Et<sub>3</sub>N (3  $\mu$ L, 22  $\mu$ mol), MsCl (1.6  $\mu$ L, 21  $\mu$ mol) were added to the reaction mixture. After stirring for 1 h at 0 °C, the reaction mixture was quenched with saturated aqueous NH<sub>4</sub>Cl, extracted with EtOAc and dried over anhydrous MgSO<sub>4</sub>. The resulting mixture was concentrated *in vacuo* to afford crude **47** (<10.3  $\mu$ mol, crude, yellow oil), which was used for the next step without further purification.

### Synthesis of 48

To a solution of **47** (<10.3 µmol, crude) in DMF (0.5 mL), potassium phthalimide (5.3 mg, 0.029 mmol) was added under N<sub>2</sub> atmosphere at 0 °C. After stirring for 3 days at rt, additional potassium phthalimide (2.6 mg, 0.015 mmol) was added to the reaction mixture. After stirring for 12 h at rt, the reaction mixture was quenched with saturated aqueous NH<sub>4</sub>Cl, extracted with EtOAc and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 30% EtOAc/*n*-hexane) afforded **48** as a colorless oil (4.3 mg, 6.6 µmol, 64%, 2 steps): <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  8.15 (s, 1H), 7.84 (dd, *J* = 5.4, 3.0 Hz, 2H), 7.71 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.33 (s, 1H), 6.39 (dd, *J* = 14.5, 11.8 Hz, 1H), 5.98 (t, *J* = 11.1 Hz, 1H), 5.77 (dt, *J* = 15.0, 6.9 Hz, 1H), 5.35 (dd, *J* = 10.6, 9.1 Hz, 1H), 5.01 (td, *J* = 8.0, 3.6 Hz, 1H), 3.70 (t, *J* = 7.3 Hz, 2H), 2.99-2.93 (m, 2H), 2.57 (dd, *J* = 14.2, 3.7 Hz, 1H), 2.49 (dd, *J* = 14.2, 7.6 Hz, 1H), 2.39 (dt, *J* = 15.6, 6.3 Hz, 2H), 2.31 (dt, *J* = 15.4, 6.1 Hz, 1H), 1.89-1.75 (m, 2H), 1.71 (quin, *J* = 7.4 Hz, 2H), 1.57-1.51 (m, 4H), 1.49-1.40 (m,

5H), 0.90-0.84 (m, 12H), 0.06 (s, 3H), 0.05 (s, 3H); <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>) *δ* 169.91, 169.28, 168.63 (2C), 134.48, 134.07 (2C), 132.40 (2C), 132.29, 131.38, 129.02, 127.08, 124.95, 123.39 (2C), 88.36, 66.82, 57.05, 56.06, 46.14, 38.05, 32.28, 31.67, 28.70, 27.84, 26.91, 26.37, 25.94 (3C), 24.64, 18.24, 8.33, -4.15, -4.83; ESI-MS (*m*/*z*) 649.3 [M-H]<sup>-</sup>.

# Synthesis of 49

To a solution of **48** (9.4 mg, 0.014 mmol) in THF (0.8 mL), TBAF (1.0 M in THF, 0.030 mL, 0.028 mmol) was added under N<sub>2</sub> atmosphere. After stirring for 30 min at rt, the reaction mixture was quenched with saturated aqueous NH<sub>4</sub>Cl, extracted with EtOAc and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 60% EtOAc/*n*-hexane) afforded **49** as a colorless oil (7.6 mg, 0.014 mmol, quant.): <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  8.45 (s, 1H), 7.85 (dd, *J* = 5.4, 3.0 Hz, 2H), 7.16 (dd, *J* = 5.4, 3.0 Hz, 2H), 7.36 (s, 1H), 6.46 (dd, *J* = 15.0, 11.8 Hz, 1H), 6.08 (t, *J* = 11.0 Hz, 1H), 5.82 (dt, *J* = 15.1, 6.7 Hz, 1H), 5.40 (dd, *J* = 10.6, 9.0 Hz, 1H), 5.06 (td, *J* = 8.0, 3.0 Hz, 1H), 3.68 (t, *J* = 7.3 Hz, 2H), 3.01 (td, *J* = 6.2, 4.2 Hz, 1H), 2.96 (td, *J* = 6.2, 4.3 Hz, 1H), 2.65 (dd, *J* = 15.8, 8.2 Hz, 1H), 2.60 (dd, *J* = 15.8, 3.7 Hz, 1H), 2.40 (dt, *J* = 15.8, 6.7 Hz, 1H), 1.49 (s, 3H), 1.42 (quin, *J* = 7.2 Hz, 2H), 0.89 (t, *J* = 7.4 Hz, 3H); <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  170.58, 169.39, 168.75 (2C), 134.33, 134.15 (2C), 132.80 (2C), 132.39, 131.00, 129.92, 126.97, 124.90, 123.46 (2C), 88.59, 64.98, 57.14, 55.88, 43.64, 38.06, 32.32, 31.58, 28.64, 27.77, 26.88, 26.26, 24.49, 8.43; ESI-MS (*m*/z) 559.2 [M+Na]<sup>+</sup>, 535.2 [M-H]<sup>-</sup>.

### Synthesis of 50

To a solution of **49** (7.6 mg, 0.014 mmol) in THF/MeOH (2:10, 0.6 mL), 40% MeNH<sub>2</sub> (0.153 mL, 1.74 mmol) was added under N<sub>2</sub> atmosphere. After stirring for 4 h at 0 °C, the reaction mixture was concentrated *in vacuo* and purified by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, MeOH) afforded **50** as a colorless oil (2.8 mg, 6.9 µmol, 49%): <sup>1</sup>H-NMR (500 MHz, MeOD)  $\delta$  7.40 (s, 1H), 6.56 (dd, *J* = 14.5, 11.7 Hz, 1H), 6.05 (t, *J* = 11.0 Hz, 1H), 5.79 (dt, *J* = 15.1, 6.9 Hz, 1H), 5.37 (t, *J* = 9.9 Hz, 1H), 5.02 (td, *J* = 8.2, 5.4 Hz, 1H), 3.04-2.97 (m, 2H), 2.72 (t, *J* = 7.2 Hz, 2H), 2.66 (dd, *J* = 14.5, 8.1 Hz, 1H), 2.50 (dd, *J* = 14.6, 5.0 Hz, 1H), 2.38-2.32 (m, 2H), 1.89-1.76 (m, 2H), 1.65-1.47 (m, 6H), 1.47-1.40 (m, 5H), 0.85 (t, *J* = 7.4 Hz, 3H); <sup>13</sup>C-NMR (125 MHz, MeOD)  $\delta$  172.54, 170.73, 136.36, 132.67, 132.35, 131.00, 128.89, 126.74, 89.30, 65.89, 58.55, 57.48, 45.24, 42.13, 33.03, 32.44, 28.75, 27.81, 27.57, 24.73, 8.51; ESI-MS (*m*/*z*) 405.2 [M-H]<sup>-</sup>, 407.2 [M+H]<sup>+</sup>, 429.2 [M+Na]<sup>+</sup>.

#### Synthesis of PKRD-1

To a solution of **50** (2.8 mg, 6.9 µmol) and **15** (3.3 mg, 0.010 mmol) in DMF (1 mL), Et<sub>3</sub>N (3.0 µL, 0.021 mmol), HATU (3.2 mg, 8.3 µmol), and HOAt (1.1 mg, 8.3 µmol) were added under N<sub>2</sub> atmosphere. After stirring for 24 h at rt, the reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub>, quenched with brine, extracted with CH<sub>2</sub>Cl<sub>2</sub>, washed with saturated aqueous NaHCO<sub>3</sub> and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 40-60% EtOAc/*n*-hexane) afforded **PKRD-1** as a colorless oil (2.9 mg, 4.1 µmol, 59%): <sup>1</sup>H-NMR (500 MHz, MeOD)  $\delta$  8.56 (t, *J* = 1.8 Hz, 1H), 8.21 (ddd, *J* = 8.2, 2.0, 0.8 Hz, 1H), 7.97 (d, *J* = 7.9 Hz, 1H), 7.42 (t, *J* = 8.1 Hz, 1H), 7.39 (s, 1H), 6.56 (dd, *J* = 15.0, 12.0 Hz, 1H), 6.05 (t, *J* = 11.0 Hz, 1H), 5.79 (dt, *J* = 15.1, 6.9 Hz, 1H), 5.36 (t, *J* = 10.0 Hz, 1H), 5.03 (td, *J* = 8.2, 5.2 Hz, 1H), 3.48 (t, *J* = 7.1 Hz, 2H), 3.03-2.97 (m, 2H), 2.66 (dd, *J* = 17.2 Hz, 2H), 1.63-1.47 (m, 5H), 1.44 (s, 3H), 0.83 (t, *J* = 7.4 Hz, 3H); <sup>13</sup>C-NMR (125 MHz, MeOD)  $\delta$  177.58, 172.52, 170.71, 140.96, 138.71, 136.33, 132.81, 132.66, 132.35, 131.00, 130.28, 128.93, 126.73, 120.83, 95.11, 89.29, 65.88, 58.58, 57.49, 45.24, 40.75, 33.03, 32.47, 30.34, 28.75, 27.92, 27.40, 24.72, 8.52; ESI-MS (*m*/*z*) 703.0 [M-H]<sup>-</sup>, 705.3 [M+H]<sup>+</sup>, 727.1 [M+Na]<sup>+</sup>.

# Synthesis of 51

To a solution of **50** (3.7 mg, 9.1 µmol) and **17** (6.55 mg, 13.7 µmol) in DMF (1.5 mL), Et<sub>3</sub>N (3.8 µL, 0.027 mmol), HATU (4.2 mg, 10.9 µmol), and HOAt (1.7 mg, 10.9 µmol) were added under N<sub>2</sub> atmosphere. After stirring for 24 h at rt, the reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub>, quenched with brine, extracted with CH<sub>2</sub>Cl<sub>2</sub>, washed with saturated aqueous NaHCO<sub>3</sub> and dried over anhydrous MgSO<sub>4</sub>. The purification by silica-gel column chromatography (Wako gel<sup>®</sup> C-200, 10-20% EtOAc/*n*-hexane) afforded **51** as a yellow oil (1.7 mg, 1.9 µmol, 21%): <sup>1</sup>H-NMR (500 MHz, MeOD)  $\delta$  8.24 (t, J = 2.3 Hz, 1H), 8.10 (dt, J = 8.1, 1.1 Hz, 1H), 7.69 (d, J = 2.2 Hz, 1H), 7.60 (t, J = 7.5 Hz, 1H), 7.39 (s, 1H), 6.56 (dd, J = 15.1, 6.7 Hz, 1H), 6.04 (t, J = 11.0 Hz, 1H), 5.79 (dt, J = 15.1, 6.9 Hz, 1H), 5.36 (t, J = 9.9 Hz, 1H), 5.03 (td, J = 8.3, 5.2 Hz, 1H), 3.48 (t, J = 7.1 Hz, 2H), 3.03-2.97 (m, 2H), 2.66 (dd, J = 14.5, 8.1 Hz, 1H), 2.50 (dd, J = 14.6, 5.1 Hz, 1H), 2.38-2.33 (m, 2H), 1.86-1.75 (m, 2H), 1.71 (quin, J = 7.1 Hz, 2H), 1.64-1.45 (m, 12H), 1.44 (s, 3H), 1.37 (tt, J = 7.5, 7.5 Hz, 6H), 1.18 (t, J = 7.9 Hz, 6H), 0.90 (t, J = 7.3 Hz, 9H), 0.83 (t, J = 7.4 Hz, 3H); <sup>13</sup>C-NMR (125 MHz, MeOD)  $\delta$  172.52, 161.49, 159.18, 139.88, 137.78, 136.32, 132.66, 131.00, 130.49, 129.41, 128.93, 128.47, 126.72, 121.26, 89.28, 65.88, 58.59, 57.50, 45.24, 40.74, 33.03, 32.47, 30.37, 30.32 (3C), 28.76, 28.46 (3C), 27.95, 27.42, 24.73, 14.10, 10.78, 8.52; ESI-MS (*m*/*z*) 869.3 [M+H]<sup>+</sup>, 891.3 [M+Na]<sup>+</sup>.

# Synthesis of [125]PKRD-1

To a mixture of a stanyl-precursor **51** (1.0 mM in EtOH, 20  $\mu$ L) and chloramine T (2.0 mM in 1.0 M KPi buffer (pH 7.4), 5  $\mu$ L) in screw-capped 1.5 mL plastic-tube was added [<sup>125</sup>I]NaI (PerkinElmer, NEZ 033A, 1 mCi, 2,000 Ci/mmol, 10  $\mu$ L). The mixture was incubated at rt for 10 min. The reaction was terminated with 5% (w/v) aqueous NaHSO<sub>3</sub> (50  $\mu$ L), and the resulting mixture was carefully extracted with CHCl<sub>3</sub> (3 × 100  $\mu$ L). The combined organic layer was concentrated using vacuum-centrifugal evaporator. The crude product was dissolved in MeOH (30  $\mu$ L), and subjected to HPLC purification (Shimadzu LC-10AS, Kyoto, Japan) using an ODS column (COSMOSIL 5C<sub>18</sub>-MS-II, 4.6 x 150 mm, Nacalai Tesque, Kyoto, Japan) at a flow rate of 0.8 mL/min with isocratic 75% MeOH/water system as an eluent. The fraction was collected every 30 s (400  $\mu$ L). To check the elution pattern of the radioactivities and their radiochemical purity, each fraction was measured by  $\gamma$ -counting system and radio-TLC analysis. The strong radioactive fractions, corresponding to the retention time of cold-type **PKRD-1** was stored as an ethanolic solution (0.20  $\mu$ M) at 4 °C. The radiochemical yield of [<sup>125</sup>I]**PKRD-1** from the initial [<sup>125</sup>I]NaI was 4.6%.