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ABSTRACT

Targeted protein degradation using small molecules is a novel strategy for drug development. We have developed hybrid molecules named Specific and Non-genetic inhibitor of apoptosis protein [IAP]-dependent Protein Erasers (SNIPERs) that recruit IAP ubiquitin ligases to degrade target proteins. Here, we show novel SNIPERs capable of inducing proteasomal degradation of the androgen receptor (AR). Through derivatization of the SNIPER(AR) molecule at AR ligand, IAP ligand and linker, we developed **42a** (SNIPER(AR)-51), which shows effective protein knockdown activity against AR. Consistent with the degradation of the AR protein, **42a** inhibits AR-mediated gene expression and proliferation of androgen-dependent prostate cancer cells. In addition, **42a** efficiently induces caspase activation and apoptosis in prostate cancer cells, which was not observed in the cells treated with AR antagonists. These results suggest that SNIPER(AR)s could be leads for an anti-cancer drug against prostate cancers that exhibit AR-dependent proliferation.

INTRODUCTION

Prostate cancer (PC) is the second-leading cause of cancer deaths, after lung cancer, in American males.[1] Since the progression of PC is primarily stimulated by androgens, inhibition of the secretion and/or action of androgens is highly effective in controlling the growth of PC.[2-4] Besides surgical castration (orchiectomy) or androgen synthesis inhibitors, androgen receptor (AR) antagonists are widely used to treat PC. These antagonists suppress androgen signaling by inhibiting the binding of androgens to AR. Currently, AR antagonists such as flutamide, bicalutamide and enzalutamide are used clinically.[5] In combination, AR antagonists and androgen synthesis inhibitors show significant synergistic effects by blocking androgen signaling as well as by suppressing transient increase in testosterone levels. Although such therapy is initially effective, a considerable population of patients develops castration-resistant prostate cancer (CRPC) after the prolonged use of an AR antagonist, primarily because of an increased level of AR expression in clinical situations.[6-8] Therefore, down-regulation of the AR level is regarded as a promising strategy to treat

castration-resistant PCs.

Down-regulation of the AR protein can be achieved by genetic knockdown technologies, such as antisense oligonucleotides, RNA interference and genome editing. Despite its great therapeutic potential, genetic knockdown remains clinically challenging,[9, 10] because oligonucleotides are not efficiently taken up into cells and delivery to the desired target tissues is technically difficult. An alternative approach is protein knockdown, which induces the degradation of target proteins via the ubiquitin proteasome system (UPS). Based on this approach, molecules named Specific and Non-genetic inhibitor of apoptosis protein [IAP]-dependent Protein Erasers (SNIPERs)[11-22] and Proteolysis Targeting Chimeras (PROTACs)[23-32] have been developed. Both classes represent hybrid molecules composed of two different ligands connected by a linker; one ligand is for the protein of interest (POI) and the other is for E3 ubiquitin ligases. Accordingly, these molecules are expected to crosslink the POIs and E3 ubiquitin ligases in cells, resulting in the ubiquitylation and subsequent degradation of the POIs *via* the UPS. To date, we have developed several SNIPERs by connecting POI ligands to **79** (bestatin)[33] a ligand for

cellular IAP 1 (cIAP1), which induces the degradation of the POIs comprising cellular retinoic acid binding protein-II,[13, 21] estrogen receptor,[16] transforming acidic coiled-coil-3[17] and BCR-ABL.[19]

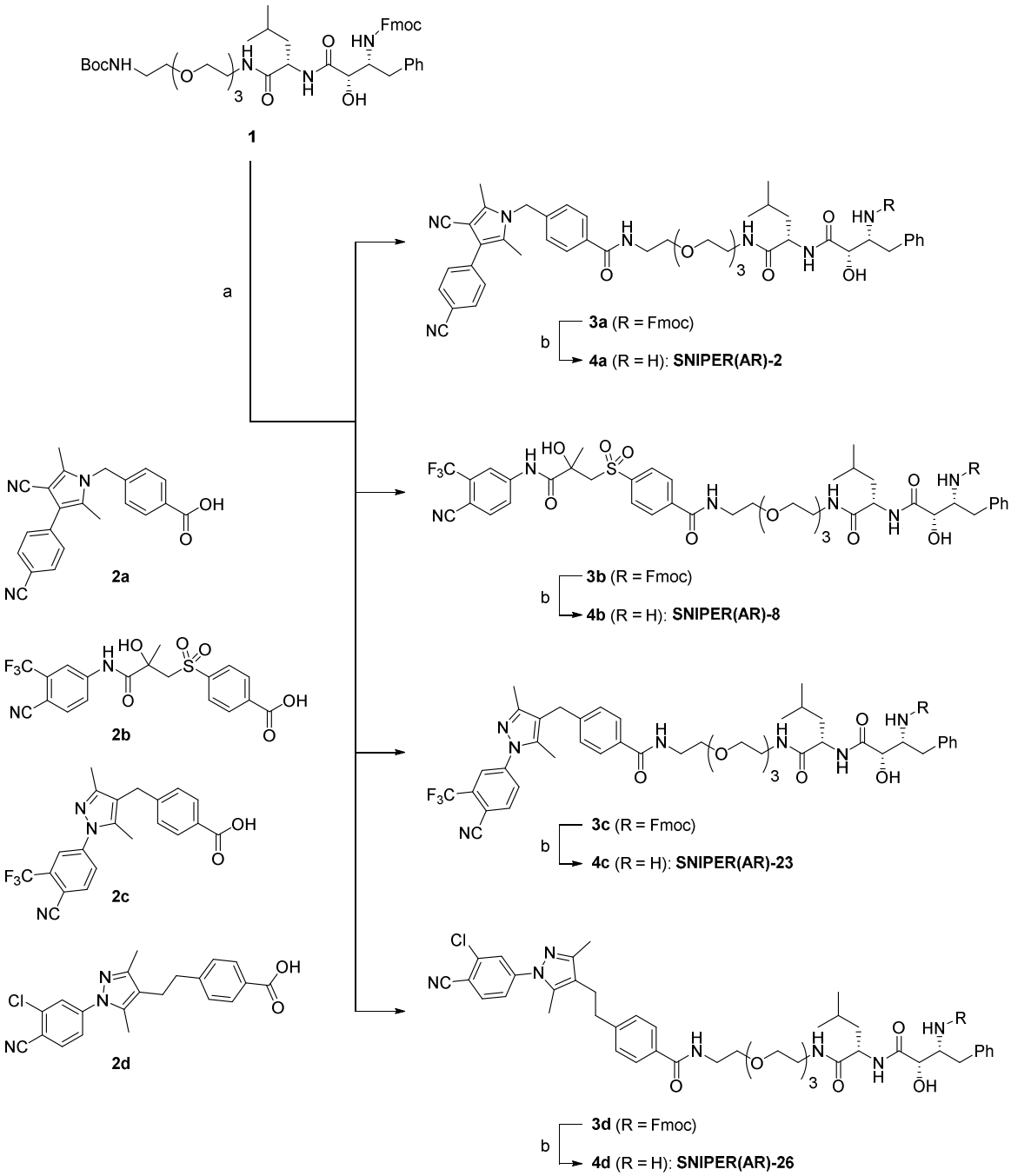
In this study, we designed and synthesized SNIPER(AR)s that induce proteasomal degradation of the AR protein by conjugating an AR antagonist to an IAP ligand, **24** (LCL-161[34] derivative). We also showed that SNIPER(AR)s not only inhibit the growth but also induce the cell death of PC cells, which exhibit AR-dependent proliferation.

RESULTS AND DISCUSSION

CHEMISTRY

The syntheses of **4a-d** (SNIPER(AR)-2, 8, 23 and 26, respectively) were performed according to Scheme 1. Removal of the Boc group of bestatin analog **1**, followed by condensation with the AR ligand part **2a-d** afforded the corresponding coupling products **3a-d**, which were treated with dimethylamine to give the bestatin-based SNIPER(AR)s **4a-d**.

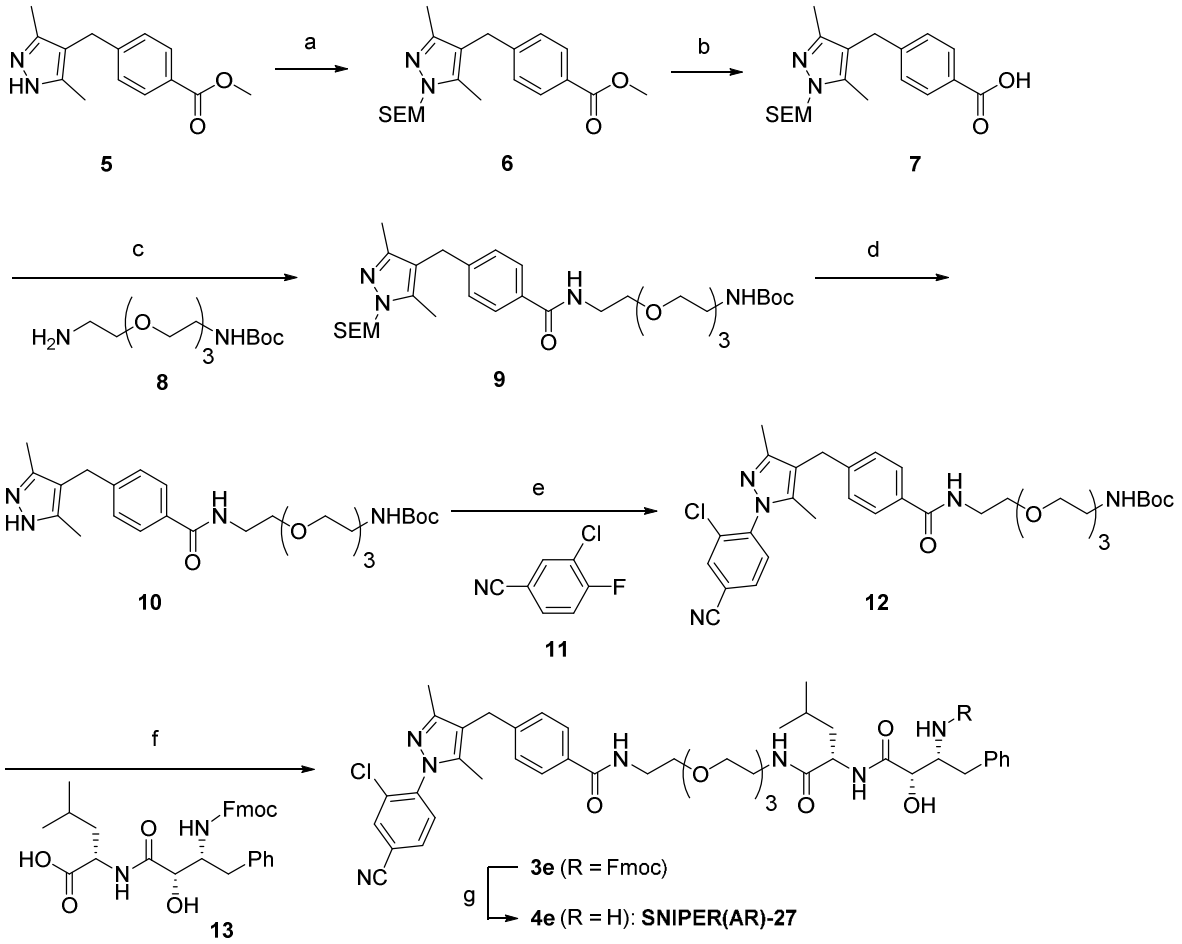
Scheme 1. Synthesis of 4a-d[a]



[a]Reagents and conditions: (a) (1) aq. HCl, THF, rt; (2) **2a**, **2b**, **2c**, or **2d**, EDC, HOBt·H_2O, DIPEA, THF, rt, 41–76%; (b) Me_2NH, MeOH, rt, 68–98%.

Compound **4e** (SNIPER(AR)-27) having a different pyrazole-based AR ligand was prepared by the route shown in Scheme 2. Protection of the pyrazole nitrogen atom of **5** with 2-(trimethylsilyl)ethoxymethyl (SEM) group and subsequent alkaline hydrolysis of the ester group yielded the benzoic acid derivative **7**, which was condensed with the Boc-protected diamine **8** to give **9**. Treatment of **9** with hydrochloride–cyclopropyl methyl ether (CPME) provided the fully-deprotected intermediate, which was reacted with excess di-*tert*-butyl dicarbonate under basic conditions, and subsequently treated with sodium methoxide to furnish the pyrazole analog **10**. Nucleophilic substitution of the fluorine atom of **11** with **10** gave **12**. Boc-deprotection of **12** followed by condensation with **13** produced **3e**, which was converted to the target compound **4e** by Fmoc-deprotection.

Scheme 2. Synthesis of 4e[a]

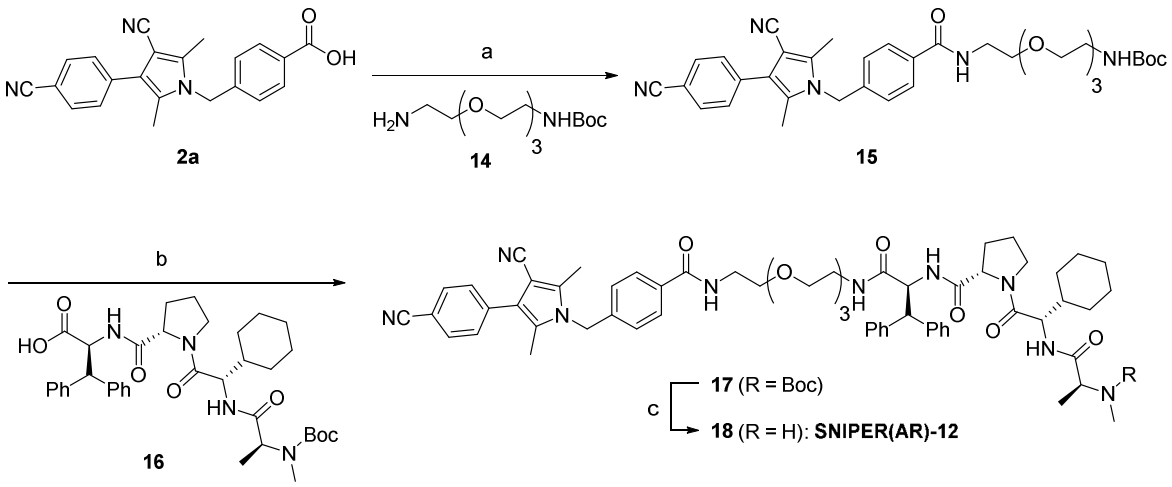


[a]Reagents and conditions: (a) SEMCl, NaH, DMF, 0 °C to rt, 89%; (b) aq. NaOH, THF, MeOH, rt, 84%; (c) **8**, EDC, HOBt, DMF, rt, 97%; (d) (1) 4 M HCl in CPME, MeOH, THF, reflux; (2) Boc_2O, aq. $NaHCO_3$, THF, rt, then NaOMe, MeOH, rt, 77%; (e) **11**, NaH, DMF, 0 °C to rt, 97%; (f) (1) 4M HCl in CPME, THF, dioxane, rt; (2) **13**, EDC, HOBt, DIPEA, DMF, rt, 73%; (g) Me_2NH, MeOH, rt, 81%.

Compound **18** (SNIPER(AR)-12) was synthesized by the route depicted in Scheme 3. The carboxylate **2a** was condensed with the Boc-protected diamine **14** to produce **15**. Removal of the Boc group of **15** and subsequent condensation of the obtained

amine with **16** yielded the coupling product **17**. Final Boc-deprotection of **17** afforded the desired compound **18**.

Scheme 3. Synthesis of 18[a]

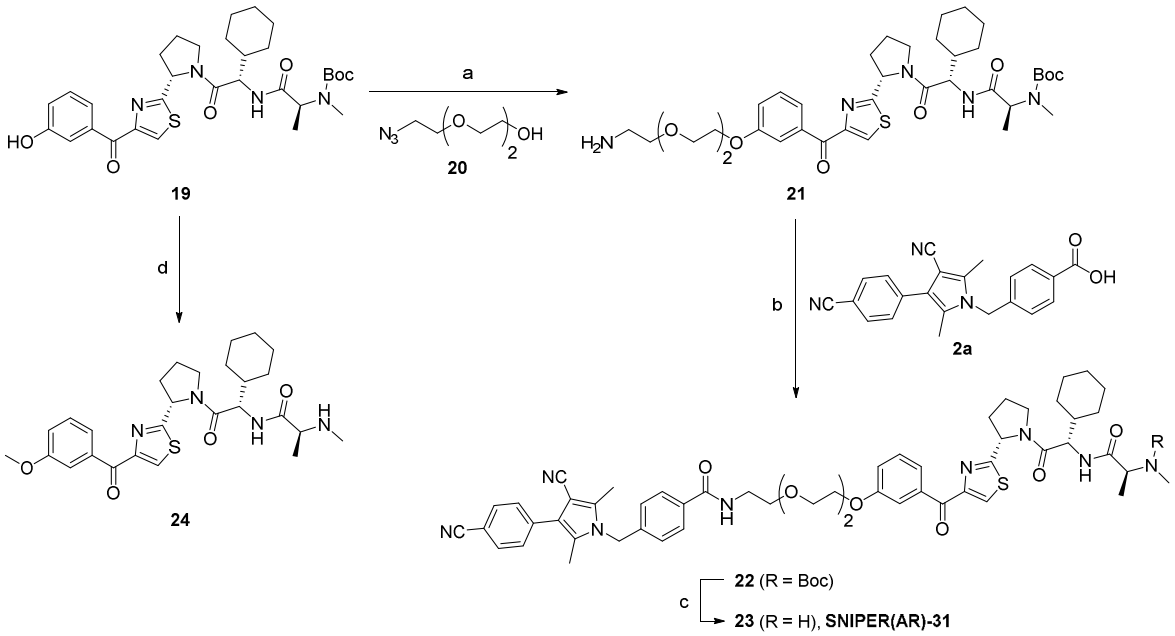


[a] Reagents and conditions: (a) **14**, EDC, HOBt, THF, rt, 98%; (b) (1) 2 M HCl in CPME, THF, rt; (2) **16**, HATU, DIPEA, DMF, 0 °C, 95%; (c) 4 M HCl in CPME, THF, rt, 84%.

The synthetic route to **23** (SNIPER(AR)-31) is presented in Scheme 4. Mitsunobu reaction of the compound **19** with azido-alcohol **20** gave the corresponding coupling product, which was subjected to Staudinger reduction to provide the primary amine **21**. Coupling of **21** with the carboxylic acid **2a** afforded **22**, which was converted to the target

compound **23** by Boc-deprotection. The reference compound **24** was obtained from **19** under Mitsunobu reaction conditions.

Scheme 4. Synthesis of 23 and 24[a]

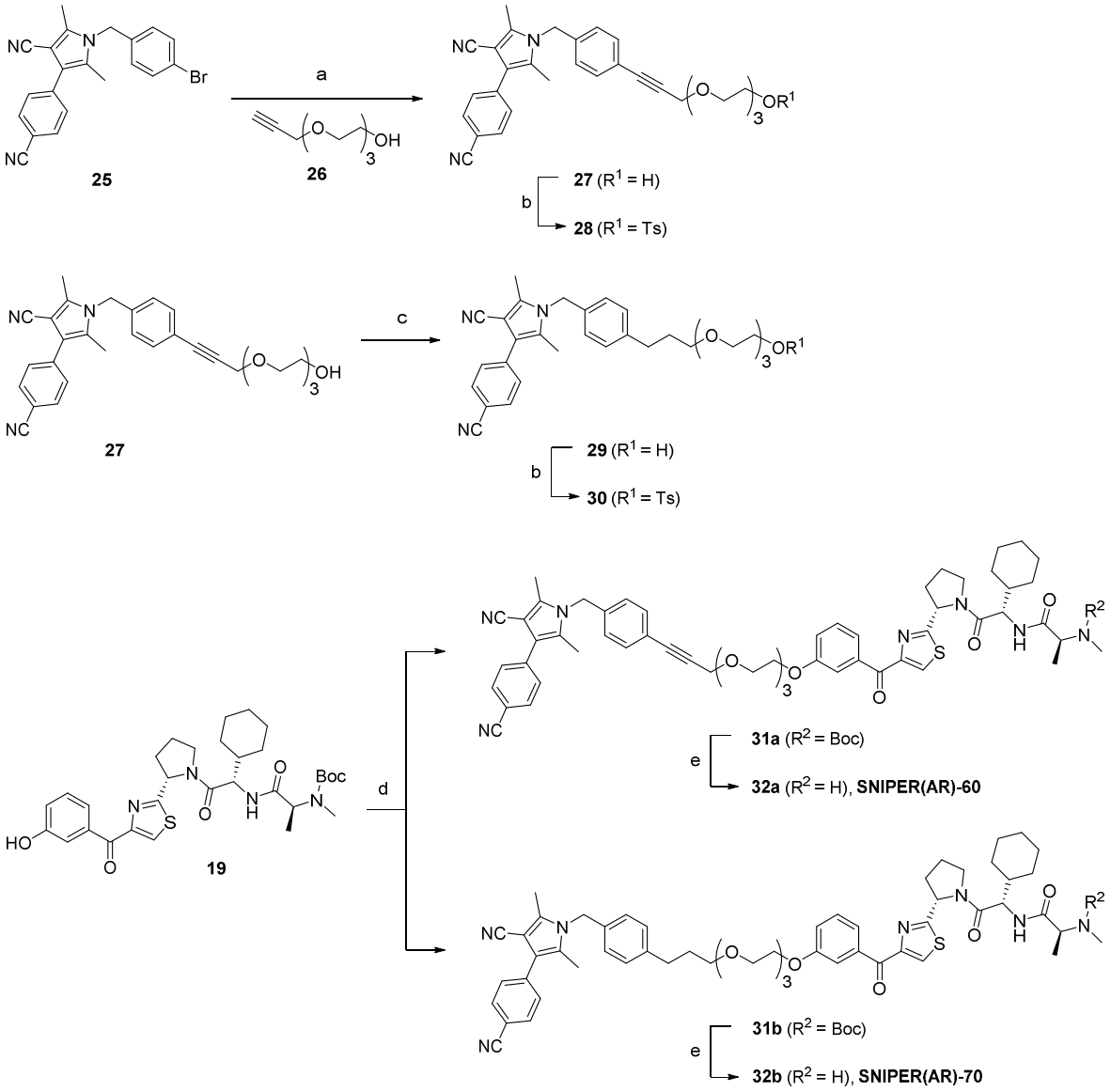


[a] Reagents and conditions: (a) (1) **20**, DEAD, Ph_3P, THF, 0 °C to rt; (2) Ph_3P, H_2O, quant.; (b) **2a**, EDC, HOBt, THF, DMF, rt, 37%; (c) 4 M HCl in CPME, THF, rt, 74%; (d) (1) MeOH, DEAD, Ph_3P, THF, 0 °C to rt; (2) 4 M HCl in CPME, rt, 42%.

Compounds **32a** (SNIPER(AR)-60) and **32b** (SNIPER(AR)-70) having different linker moieties were synthesized according to Scheme 5. Sonogashira coupling of the

bromobenzene analog **25** with the acetylene **26** produced **27**, which was sulfonylated with *p*-toluenesulfonyl chloride to give **28**. Reaction of **28** with **19** under basic conditions, followed by removal of the Boc group of **31a** furnished the desired product **32a**. Palladium catalyzed hydrogenation of the alkyne derivative **27** produced **29**, which was converted to the target compound **32b** via the three-step procedure, i.e. tosylation, substitution with **19**, and final deprotection.

Scheme 5. Synthesis of 32a and 32b[a]

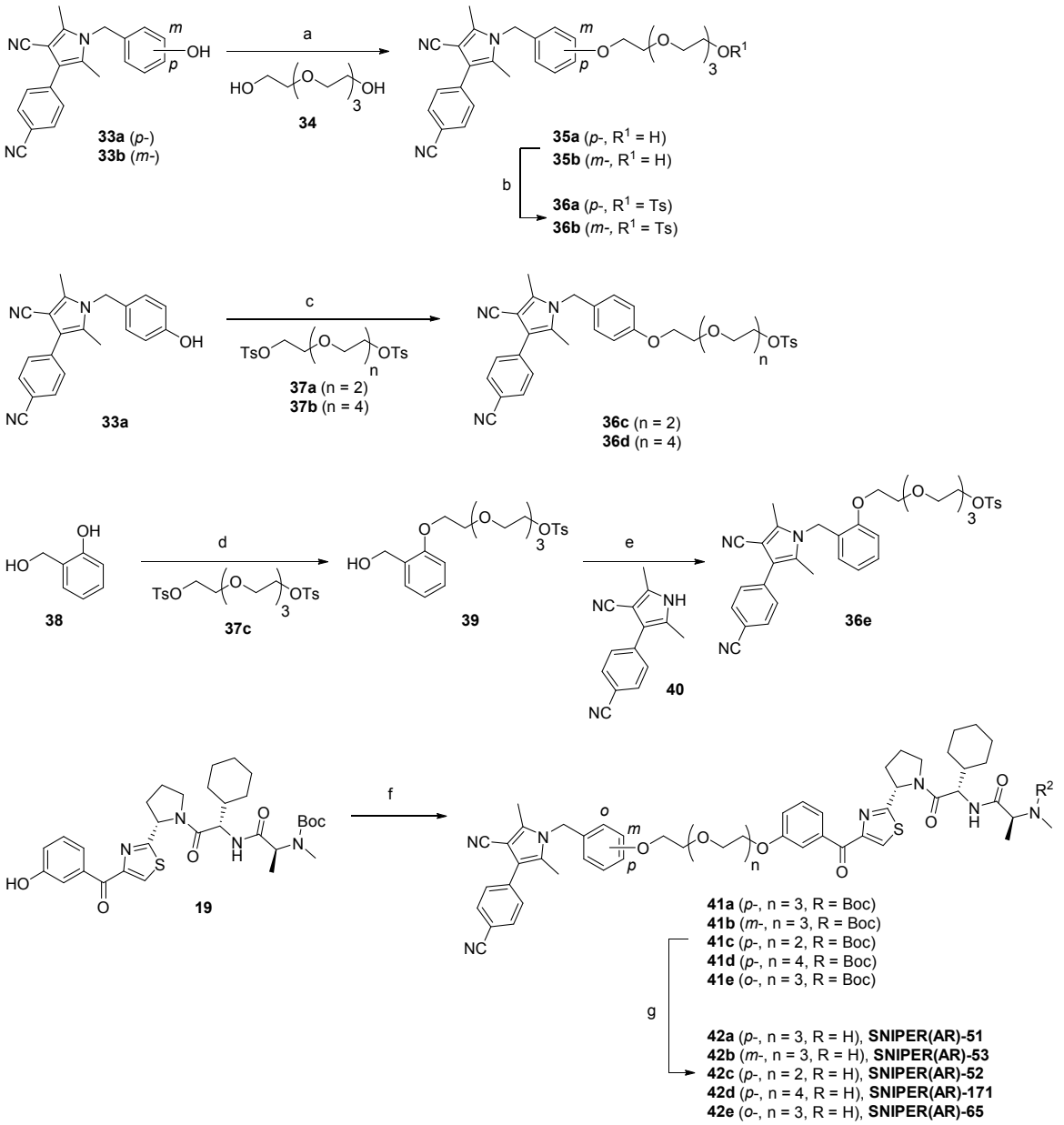


[a]Reagents and conditions: (a) **26**, $Pd(PPh_3)_4$, CuI, Et_3N, DMF, 60 °C, 49%; (b) TsCl, pyridine, 0 °C to rt, 72–83%; (c) H_2, 10% Pd–C, EtOAc, rt, 84%; (d) **28** or **30**, K_2CO_3, DMF, 50 °C, 67–72%; (e) TFA, rt, 78–86%.

The syntheses of **42a–e** (SNIPER(AR)-51, -53, -52, -171, and -65, respectively) having

different substitution pattern and linker length were carried out by the procedure depicted in Scheme 6. Various tosylated precursors (**36a–e**) were prepared by the three protocols: (i) reaction of the phenol analogs **33a,b** with the diol **34** under Mitsunobu reaction conditions, followed by tosylation; (ii) coupling of **33a** with the di-tosylated diol **37a** or **37b**; (iii) coupling of **38** with **37c**, mesylation of the benzyl alcohol **39**, and subsequent reaction with the pyrrole **40**. The tosylates **36a–e** were converted to the desired products **42a–e** in a similar procedure described for the preparation of **32** (Scheme 5).

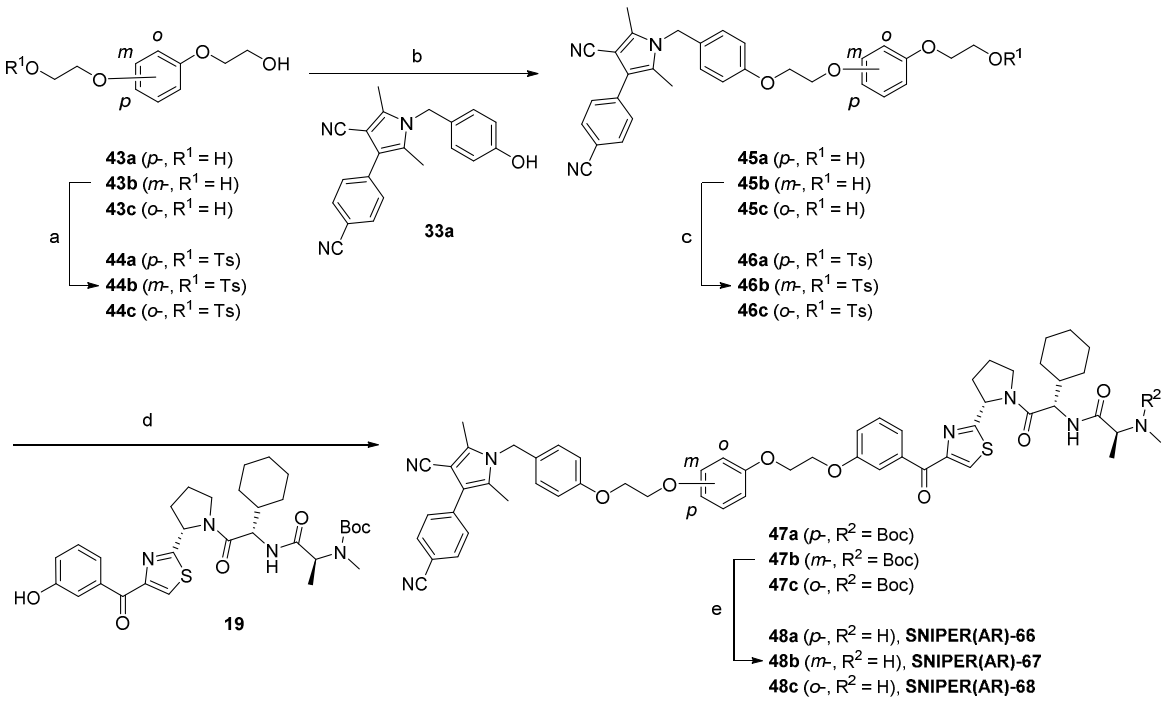
Scheme 6. Synthesis of 42a–e[a]



[a]Reagents and conditions: (a) **34**, DEAD, Ph_3P, THF, 0 °C to rt, 73–94%; (b) TsCl, pyridine, 0 °C to rt, 68–75%; (c) **37a** or **37b**, K_2CO_3, DMF, 50 °C, 46–51%; (d) **37c**, K_2CO_3, DMF, 40 °C, 46%; (e) (1) MsCl, Et_3N, THF, 0 °C; (2) **40**, NaH, DMF, 0 °C, 83%; (f) **36a**, **36b**, **36c**, **36d**, or **36e**, K_2CO_3, DMF, rt to 60 °C, 67–81%; (g) TFA, rt, 70–90%.

Scheme 7 shows the procedure used for the preparation of **48a–c** (SNIPER(AR)-66, -67, and -68, respectively) having a benzene ring embedded in the linker moiety. Mono-tosylation of the diol **43a–c**, followed by reaction with **33a** under basic conditions yielded **45a–c**, which were tosylated to give **46a–c**. Reaction of **46a–c** with **19** and final Boc-deprotection of **47a–c** produced the desired products **48a–c**.

Scheme 7. Synthesis of 48a–c[a]



[a]Reagents and conditions: (a) TsCl, pyridine, 0 °C, 52–56%; (b) **33a**, K_2CO_3, DMF, 60 °C, 82–99%; (c) TsCl, pyridine, 0 °C to rt, 83–87%; (d) **19**, K_2CO_3, DMF, rt to 50 °C, 74–82%; (e) TFA, rt, 85–89%.

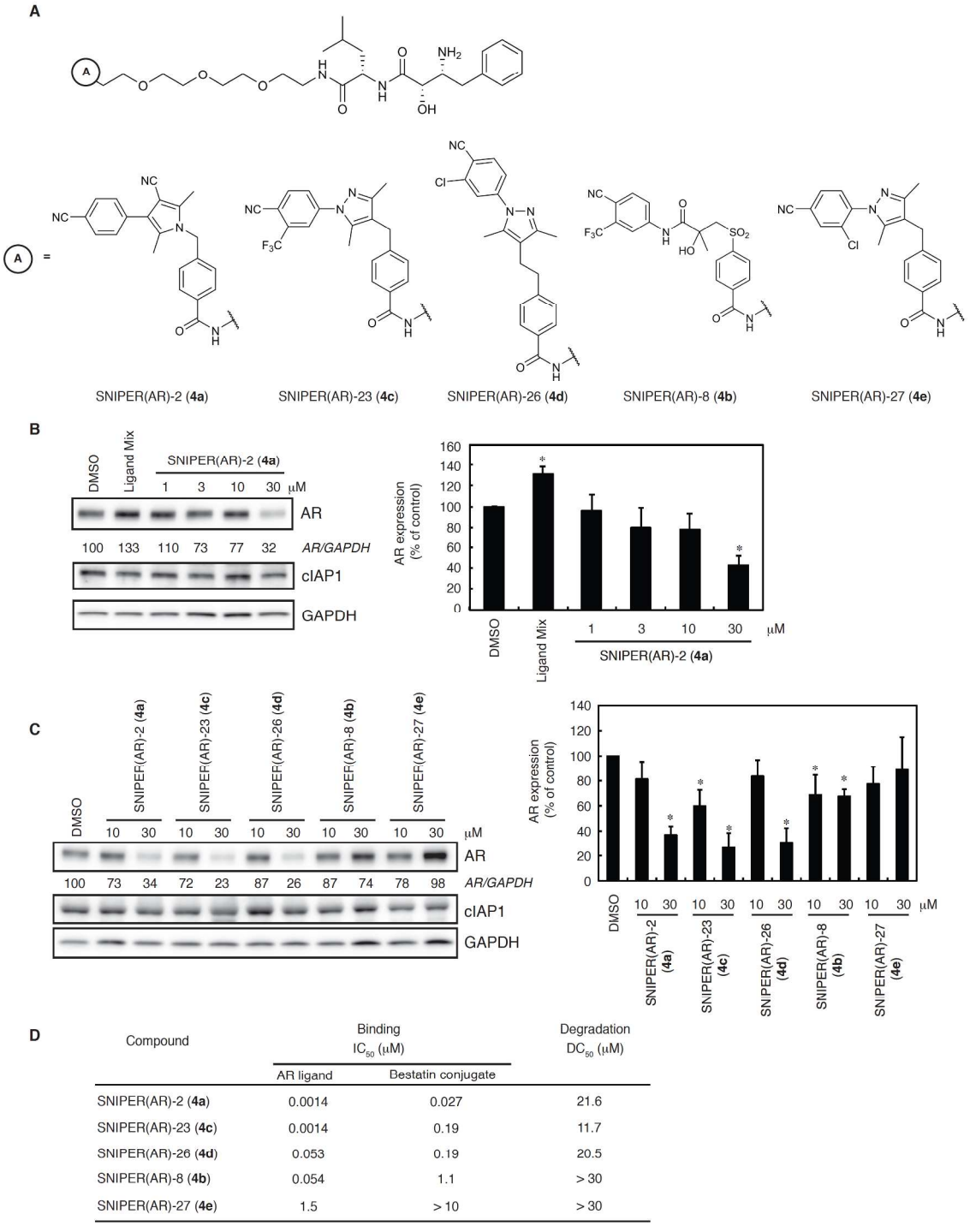
Induction of AR protein degradation by SNIPER(AR)

We designed a hybrid molecule **4a**, composed of **79** and **76 (**AR antagonist-1), which possesses strong binding affinity to AR.[35] Based on the co-crystal structure of AR ligand-binding domain (W741L mutant) complexed with bicalutamide (Protein Data Bank (PDB) ID: 1Z95),[36] the fluorophenyl moiety of bicalutamide is located at the AR surface. Therefore, the PEG (polyethylene glycol) linker was ligated to the corresponding benzyl moiety of **76** at one end, and **79** at the other end, as shown in Figure 1A.

To examine whether **4a** reduces the AR protein level, human PC 22Rv1 cells were treated with graded concentrations of **4a**, and the whole-cell lysates were analyzed by western blot. The AR protein level was significantly decreased by treatment with **4a** at 30 μM for 6 h (Figure 1B). Combination treatment with 30 μM **76** and 30 μM **80** (methyl

bestatin)[33] did not decrease the AR protein level, indicating that linking the two ligands into a single molecule is essential for the reduction of the AR protein (Figure 1B).

We next conjugated **79** to various AR ligands,[35, 37] whose binding affinities to AR ranged from 10^{-9} to 10^{-6} M. Compounds **4a, 4c** and **4d**, which contain AR ligands with the binding affinity of 0.0014, 0.0014 and 0.053 μM, showed IC_{50} values of 0.027, 0.19, and 0.19 μM in binding affinity to AR, and DC_{50} values of 21.6, 11.7 and 20.5 μM in the reduction of AR protein expression, respectively (Figure 1C, D). However, **4b** and **4e**, which contain AR ligands with IC_{50} values of 0.054 and 1.5 μM, had IC_{50} values of 1.1 and >10 μM, and DC_{50} values of >30 and >30 μM, respectively. These results suggest that incorporation of an AR ligand with a higher binding affinity is preferable to develop a SNIPER(AR) with effective protein knockdown activity.



D

Compound	Binding IC_{50} (μM)		Degradation DC_{50} (μM)
	AR ligand	Bestatin conjugate	
SNIPER(AR)-2 (**4a**)	0.0014	0.027	21.6
SNIPER(AR)-23 (**4c**)	0.0014	0.19	11.7
SNIPER(AR)-26 (**4d**)	0.053	0.19	20.5
SNIPER(AR)-8 (**4b**)	0.054	1.1	> 30
SNIPER(AR)-27 (**4e**)	1.5	> 10	> 30

Figure 1 Reduction of AR protein levels by bestatin-conjugated SNIPER(AR)s.

(A) Chemical structures of the SNIPER(AR)s. (B) Reduction of AR protein by **4a**

(SNIPER(AR)-2). (C) Reduction of AR protein by SNIPER(AR)s containing various AR ligands. 22Rv1 cells were incubated with the indicated concentrations of SNIPER(AR)s or a mixture of the two chemically separate ligands (**76** and **80**, 30 μM each) for 6 h. Numbers below the AR panel represent AR/GAPDH ratio normalized by vehicle control as 100. Data in the graphs are means ± standard deviation (n = 3). *, $P<0.05$ compared with vehicle control (B, C). (D) Binding affinity and protein knockdown activity of the bestatin-conjugated SNIPER(AR)s. IC_{50} values and DC_{50} values (concentrations of the SNIPER(AR)s required to reduce the protein expression of AR by 50%) of SNIPER(AR)s were obtained from three separate experiments. The binding affinities of the AR ligands were cited from References 34 and 36.

Optimization of SNIPER(AR)

We selected **4a** as a lead, and derivatized it at several locations in turn – the E3 ligase ligand, the connection point (joint) between the AR ligand and linker, and finally the linker itself – to improve the protein knockdown efficacy against AR. First, we replaced bestatin-moiety

with two other specific IAP antagonists, methyl (2S)-2-(((2S)-1-((2S)-2-cyclohexyl-2-(((2S)-2-(methylamino)propanoyl)amino)acetyl)pyrrolidine-2-carbonyl)amino)-3,3-diphenylpropanoate (MV-1)[38] and **24**, to generate **18** and **23**, respectively (Figure 2A), since **79** is not a specific ligand for cIAP1.[39, 40] For **4a** and **18**, the minimum concentrations necessary to reduce the AR protein levels were 30 and 20 μM, respectively, while **23** was effective at 3 μM and above. Thus, among the three tested IAP ligands, incorporation of **24** conferred the greatest AR protein degradation activity on the corresponding SNIPER(AR).

Compound **23** was further derivatized by modification of the joint (the functional group connecting **76** and the linker), i.e., amide (**23**), ether (**42a**), acetylene (**32a**) or alkyl groups (**32b**). These compounds all showed comparable abilities to reduce the AR protein level (Figure 2B). From **42a**, we further derivatized the molecule by varying the position at which the linker was connected to **76**: either the *o*-, *m*- or *p*-position on the benzyl moiety. The *m*-analog (**42b**) showed the same ability to reduce the AR protein level as did the *p*-analog (**42a**) (Figure 2C). However, the *o*-analog (**42e**) showed no activity against the AR

protein at concentrations of 1 or 3 μM (Figure 2C). Since the benzyl moiety of **76** is likely to be located on the surface of the AR protein, conjugation of the linker at the *o*-position may have hindered the binding of **42e** to the AR protein.

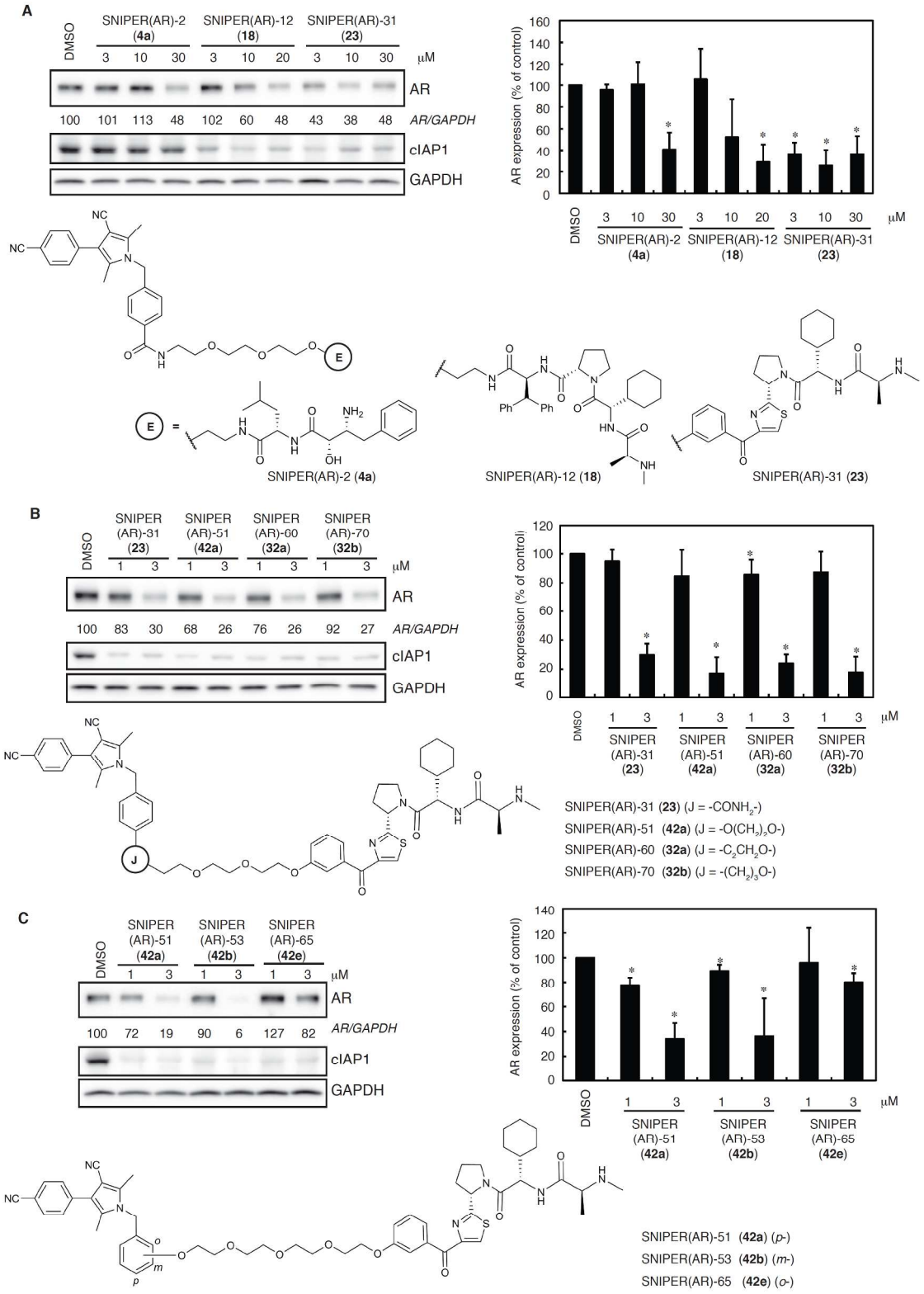

A
DMSO
SNIPER(AR)-2 (4a)
SNIPER(AR)-12 (18)
SNIPER(AR)-31 (23)
3 10 30 3 10 20 3 10 30 μM
AR
100 101 113 48 102 60 48 43 38 48 AR/GAPDH
cIAP1
GAPDH
AR expression (% of control)
SNIPER(AR)-2 (4a)
SNIPER(AR)-12 (18)
SNIPER(AR)-31 (23)
B
SNIPER (AR)-31 (23)
SNIPER (AR)-51 (42a)
SNIPER (AR)-60 (32a)
SNIPER (AR)-70 (32b)
1 3 μM
100 83 30 68 26 76 26 92 27 AR/GAPDH
SNIPER(AR)-31 (23) (J = -CONH2-)
SNIPER(AR)-51 (42a) (J = -O(CH2)3O-)
SNIPER(AR)-60 (32a) (J = -C2CH2O-)
SNIPER(AR)-70 (32b) (J = -(CH2)3O-)
C
SNIPER (AR)-51 (42a)
SNIPER (AR)-53 (42b)
SNIPER (AR)-65 (42e)
100 72 19 90 6 127 82 AR/GAPDH
SNIPER(AR)-51 (42a) (p-)
SNIPER(AR)-53 (42b) (m-)
SNIPER(AR)-65 (42e) (o-)

Figure 2 Derivatization of SNIPER(AR) by changing the cIAP1 ligand (A), joint (functional group between **76** and linker) (B), and position of joint (C). 22Rv1 cells were incubated with the indicated concentrations of SNIPER(AR)s for 6 h. Numbers below the AR panel represent AR/GAPDH ratio normalized by vehicle control as 100. Data in the graphs are means ± standard deviation (n = 3). *, $P<0.05$ compared with vehicle control.

From **42a**, we further synthesized analogs with different linker lengths. SNIPER(AR)s with different lengths of PEG linker chain (n = 3, 4, 5) substantially reduced the AR protein levels at 3 μM, while combination treatment with 3 μM **76** and 3 μM **24** did not affect the AR protein level (Figure 3A). Next, we examined the effect of linker flexibility on the protein knockdown activity. In contrast to **42a**, which contains a flexible PEG linker, the series **48a–c**, with rigid phenyl linkers, showed no activity against AR protein (Figure 3B), suggesting that flexibility of the linker is important for the reduction of AR protein levels by SNIPER(AR)s. On the basis of these results, we chose **42a** for further biological study.

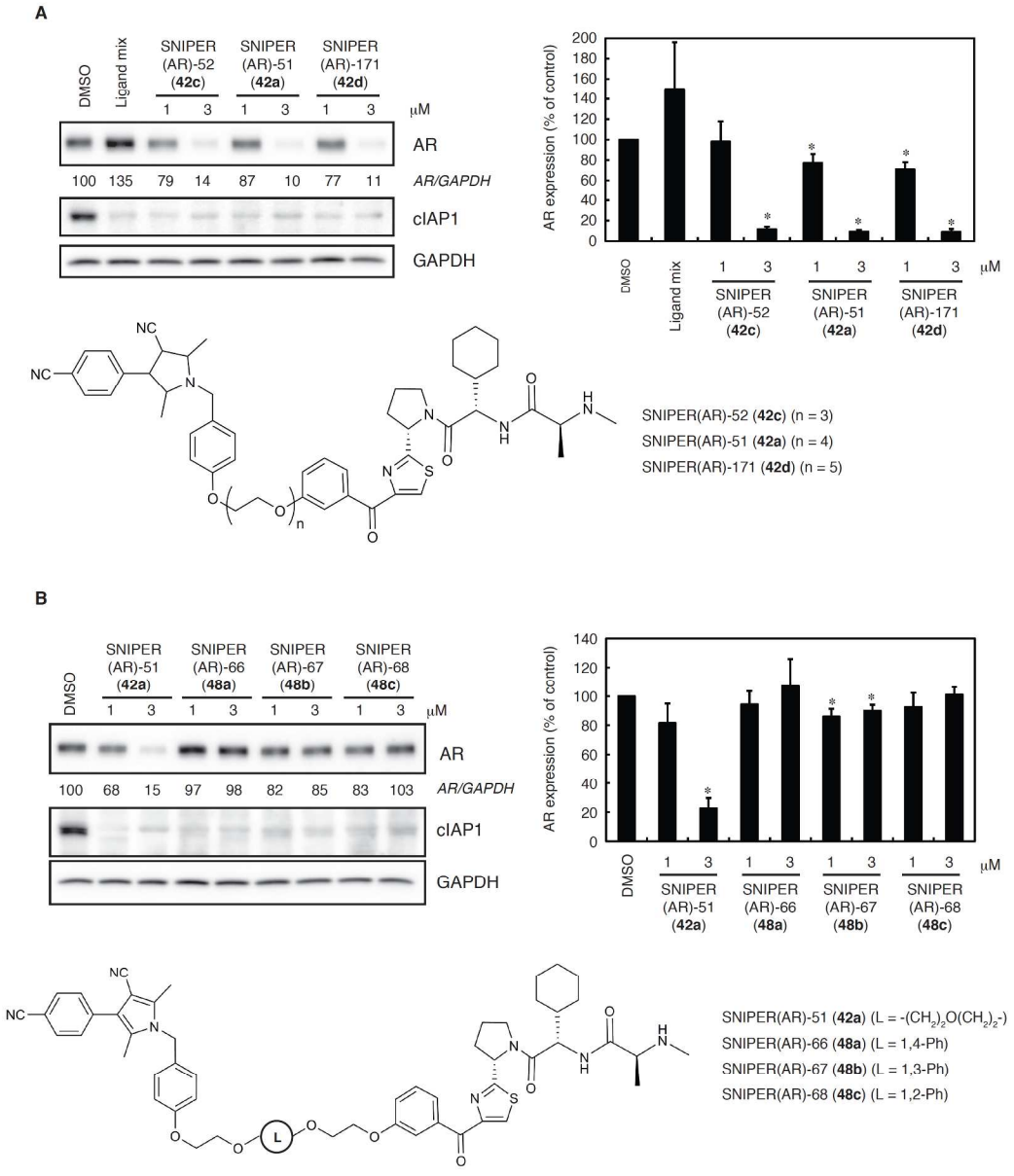


Figure 3 Derivatization of SNIPER(AR) by changing linker length (A) and linker molecules (B). 22Rv1 cells were incubated with the indicated concentrations of SNIPER(AR)s or 3 μM of the two chemically separate ligands (**76** and **24**) for 6 h. Numbers below the AR panel represent AR/GAPDH ratio normalized by vehicle control as

100. Data in the graphs are means ± standard deviation (n = 3). *, $P<0.05$ compared with vehicle control.

Biological Evaluation

SNIPER(AR)s are presumed to induce the ubiquitylation and subsequent degradation of AR via the UPS. Treatment of 22Rv1 cells with **42a** at 3 μM reduced the AR protein level in a time-dependent manner, while this reduction of AR by **42a** was abrogated by a proteasome inhibitor, benzyl *N*-((2S)-4-methyl-1-(((2*S*)-4-methyl-1-(((2*S*)-4-methyl-1-oxopentan-2-yl)amino)-1-oxopentan-2-yl)amino)-1-oxopentan-2-yl)carbamate **77** (MG132)[41] (Figure 4A). These results indicate that **42a** induces the proteasomal degradation of AR. The proteasome-dependent reduction of AR protein by **42a** was similarly observed in another PC cell line, VCaP, which has an endogenous AR gene amplification and exhibits androgen-dependent proliferation[42] (Figure 4B).

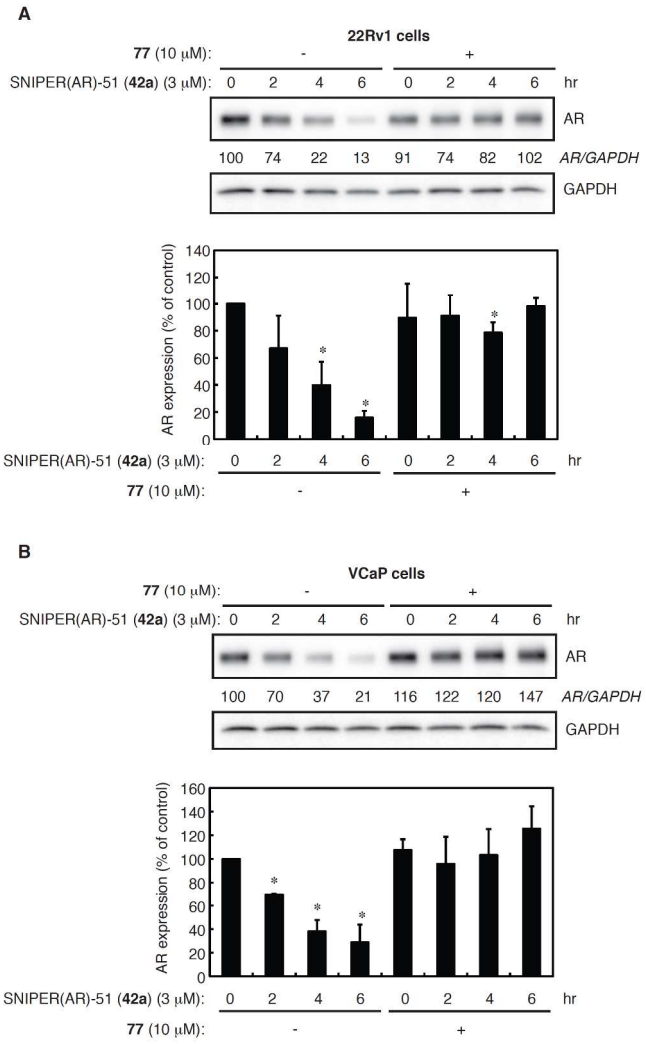


Figure 4 SNIPER(AR) induces the proteasomal degradation of AR protein. **(**A, B) Effect of proteasome inhibitor, **77**, on protein knockdown activity of **42a** in 22Rv1 (A) and VCaP (B) cells. Cells were incubated with 3 μM **42a** with or without 10 μM **77** for the indicated time. Numbers below the AR panel represent AR/GAPDH ratio normalized by

vehicle control as 100. Data in the graphs are means ± standard deviation (n = 3). *, $P<0.05$ compared with vehicle control.

Next, we tested the effect of **42a** on AR-mediated gene expression and proliferation in androgen-dependent PC VCaP cells. When cells were treated with 1 nM dihydrotestosterone (DHT), the expressions of AR-regulated genes (PSA, TMPRSS2, KLK2 and NKX3.1)[43-45] were up-regulated. Compound **42a** at 3 μM suppressed the expression of these AR-regulated genes (Figure 5A), which is consistent with the reduction of AR protein. In line with this, **42a** at 3 μM dramatically inhibited the growth of VCaP cells (Figure 5B). A similar growth-inhibitory effect of **42a** was also observed in another PC cell line, LNCaP (Figure 5C), which expresses the AR protein and exhibits androgen-dependent proliferation. However, **42a** did not inhibit cell growth in the androgen-independent cell lines PC-3, A549 or HT1080 under the same conditions (Figure 5C). Treating the cells with **42a** at 100 μM for 6 h seriously reduced the viability of all the cell lines, suggesting the toxicity of this compound. These data indicate that AR

degradation by **42a** results in the inhibition of (i) AR-mediated gene expression and (ii) androgen-dependent PC cell proliferation.

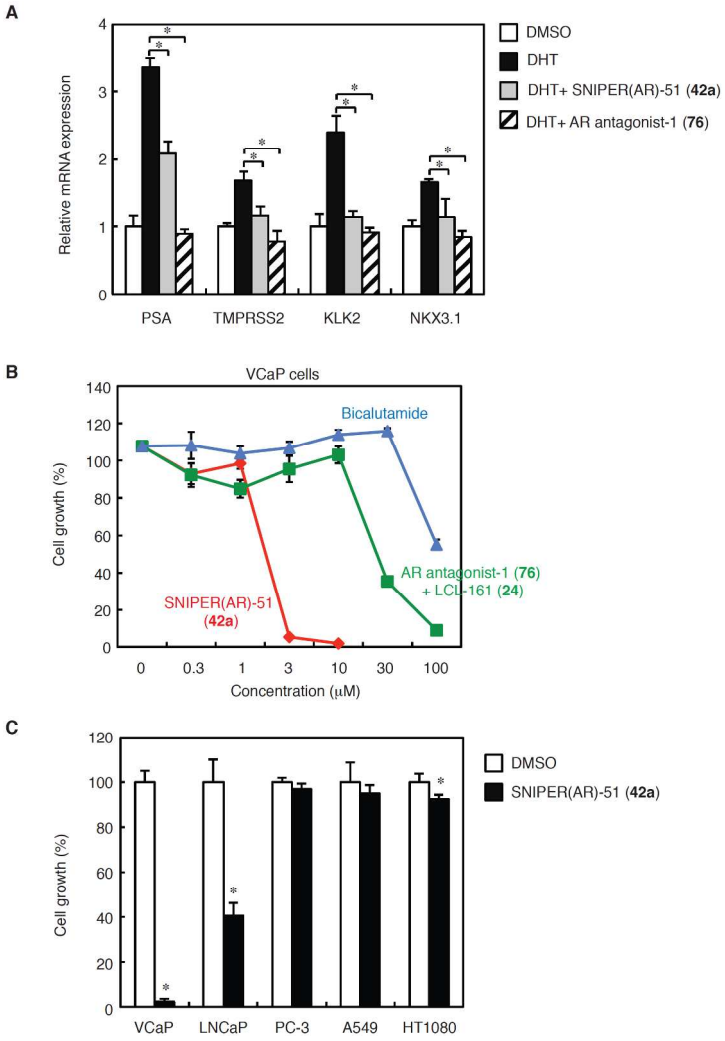


Figure 5 SNIPER(AR) inhibits AR-mediated gene expression and proliferation of androgen-dependent PC cells. (A) Expression of androgen-responsive genes in VCaP cells. Cells were incubated with 1 nM DHT in the absence or presence of 3 μM **42a** or **76**

for 24 h. Expression levels are relative to vehicle treatment, which was arbitrarily set to 1. *, $P<0.05$. (B, C) Effect of **42a** on the growth of various cells. VCaP cells were incubated with the indicated concentrations of **42a** (red line), bicalutamide (blue line) or combination of **76** and **24** (green line) for 48 h (B). The indicated cells were incubated with 3 μM **42a** for 48 h (C). Data in the graphs are means ± standard deviation (n = 3). *, $P<0.05$ compared with vehicle control.

By microscopy, we observed that **42a** exhibited a pronounced effect on the cell morphology of VCaP cells, appearing to induce apoptosis (Figure 6A). Therefore, we examined whether **42a** indeed induced apoptosis in the VCaP cells. Figure 6B shows that **42a** at 3 μM, but not **76** or bicalutamide, induced caspase-3 activation and poly (ADP-ribose) polymerase (PARP) cleavage in VCaP cells. These effects of **42a** were abrogated by a pan-caspase inhibitor, **78** (zVAD-FMK)[46] (Figure 6C). In line with this, **42a** treatment increased the count of Annexin V-positive apoptotic cells, which was suppressed by **78** (Figure 6D). The induction of apoptosis by **42a** was prevented by **77** (Figure 6E).

These results suggest that the degradation of the AR protein by SNIPER(AR)s hampers androgen signaling, resulting in the inhibition of cell proliferation and induction of apoptosis in the AR-dependent PC cells.

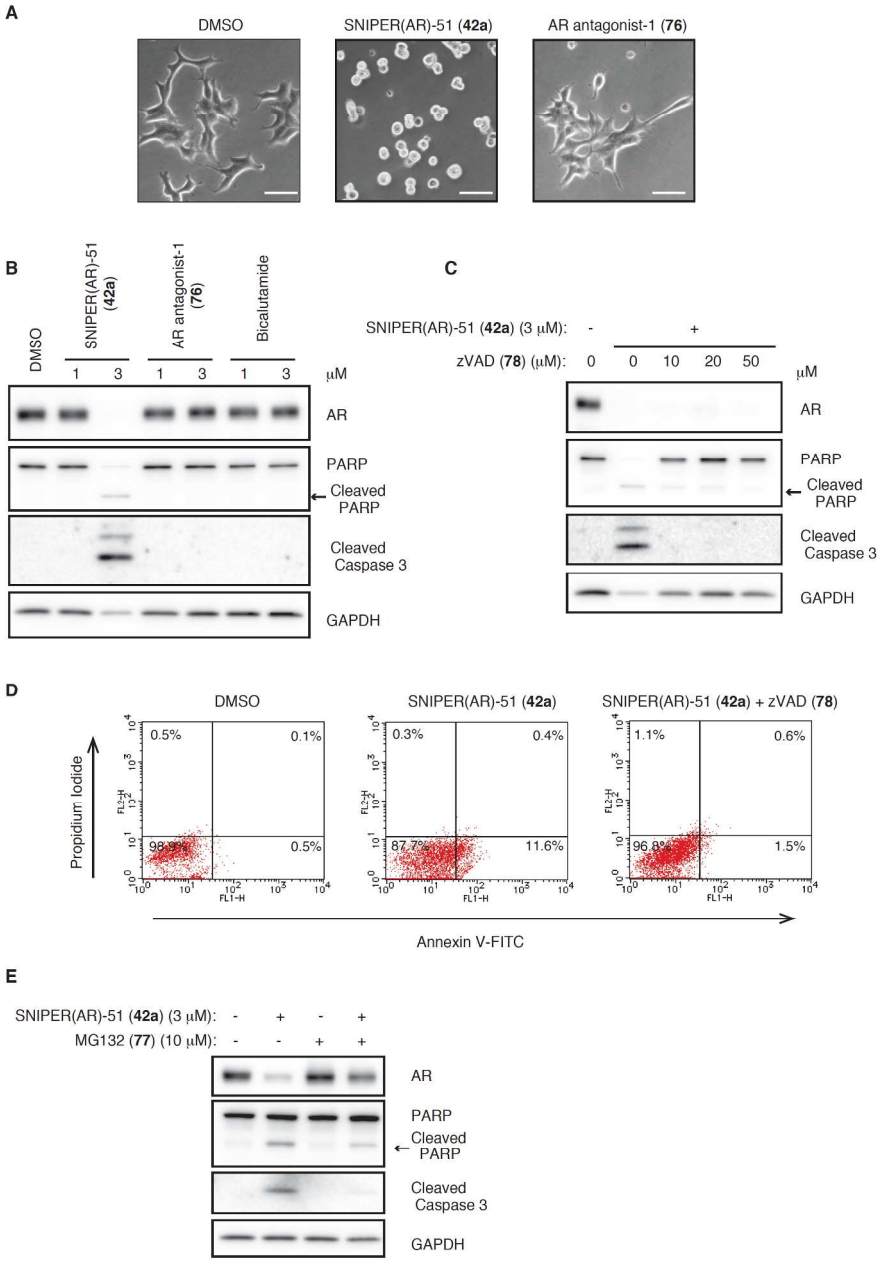


Figure 6 SNIPER(AR) induces apoptosis. (A) VCaP cells were treated with 3 μM **42a** or **76** for 24 h. Bar: 50 μm. **(**B, C) Effect of **42a** on caspase activation in VCaP cells. Cells were incubated with the indicated concentrations of compounds (B), or 3 μM **42a** with or without the indicated concentrations of **78** (C) for 24 h. (D) Cell death by **42a** was determined using Annexin V and PI (propidium iodide) staining. VCaP cells were incubated with 3 μM **42a** with or without 50 μM **78** for 24 h. Double negative cells represent living cells (lower left), whereas Annexin V-positive cells represent apoptotic cells (lower right). PI-positive (upper left) and double positive (upper right) cells represent necrotic cells. (E) Proteasome inhibitor prevents apoptosis induced by **42a** in VCaP cells. Cells were incubated with 3 μM **42a** and/or 10 μM **77** for 9 h.

CONCLUSION

Here, we reported the development of SNIPER(AR)s, which induce proteasomal degradation of the AR protein. Compound **42a** inhibits androgen signaling and cell proliferation, and induces apoptosis in androgen-dependent PC cells. Previously, Crews et al. reported PROTACs against AR that induce AR protein degradation by connecting an AR ligand to ligands for von Hippel–Lindau (VHL) and Mdm2 E3 ubiquitin ligases.[24, 26, 27] Thus, ligands for three E3 ubiquitin ligases, VHL, Mdm2 and cIAP1, can be used to produce small-molecule-based AR degradation inducers. We have shown that the ligand for the target protein, ligand for E3 ligase, linker composition and linker length are all important factors that affect the efficacy of target-protein degradation by hybrid molecules such as SNIPERs and PROTACs. Since protein knockdown is a promising strategy for the treatment of CRPC, further optimization is necessary to develop more efficient AR degradation inducers for clinical use in future.

Compared with PROTACs, SNIPERs induce the degradation of both POIs and cIAP1, which may limit the full potential and duration of knockdown activity by SNIPERs.

However, cIAP1 is an anti-apoptotic protein frequently overexpressed in human tumor cells, which is believed to be involved in the resistance to tumor therapy.[47, 48] Therefore, degradation of cIAP1 simultaneously with POIs by SNIPERs could be beneficial to effectively kill cancer cells.

EXPERIMENTAL SECTION

1. Chemistry.

1-1. General Procedures, Materials, and Information.

All solvents and chemicals were used as purchased without further purification. The progress of all reactions was monitored by thin-layer chromatography (TLC) analysis on silica gel 60 F_{254} plates (Merck) or NH TLC plates (Fuji Silysia Chemical Ltd.). Column chromatography was performed with a silica gel column [(Merck Kieselgel 60, 70–230 mesh, Merck) or (Chromatorex® NH-DM1020, 100–200 mesh, Fuji Silysia Chemical Ltd.)], or with prepacked Purif-Pack columns [silica gel or NH (3-aminopropyl-functionalized) silica gel, particle size: 60 μm, Fuji Silysia Chemical Ltd.]. The proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker AVANCE II (300 or 400 MHz) spectrometer. Chemical shifts for ^{1}H NMR were reported in parts per million (ppm) downfield from tetramethylsilane (δ) as the internal standard in deuterated solvent and coupling constants (*J*) are in Hertz (Hz). The following abbreviations are used for spin

multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, dd = doublet of doublets, dt = doublet of triplets, qd = quartet of doublets, dquin = doublet of quintets, m = multiplet, and br s = broad singlet. Low-resolution mass spectra (MS) were acquired using an Agilent LC/MS system (Agilent1200SL/Agilent6130MS, Agilent1200SL/Agilent1956MS or Agilent1200SL/Agilent6110MS), Shimadzu UFLC/MS (Shimadzu LC-20AD/LCMS-2020) operating in electron spray ionization mode (ESI+). The column used was an L-column 2 ODS (3.0 × 50 mm I.D., 3 μm, CERI, Japan) with a temperature of 40 °C and a flow rate of 1.2 or 1.5 mL/min. Condition 1: Mobile phases A and B under acidic conditions were 0.05% TFA in water and 0.05% TFA in MeCN, respectively. The ratio of mobile phase B was increased linearly from 5 to 90% over 0.9 min, and held at 90% over the next 1.1 min. Condition 2: Mobile phases A and B under neutral conditions were a mixture of 5 mmol/L $AcONH_4$ and MeCN (9:1, v/v) and a mixture of 5 mmol/L $AcONH_4$ and MeCN (1:9, v/v), respectively. The ratio of mobile phase B was increased linearly from 5 to 90% over 0.9 min, and held at 90% over the next 1.1 min. High-resolution mass spectrometry (HRMS) were carried out by Takeda

Analytical Research Laboratories, Sumika Chemical Analysis Service, Ltd., or measured by Bruker Autoflex Speed (MALDI-TOF Mass). Elemental analyses were carried out by Takeda Analytical Laboratories Ltd. The purities of all compounds tested in biological systems were assessed as being >95% using analytical HPLC. Analytical HPLC was performed with NQAD (Nano Quantity Analyte Detector) or Corona CAD (Charged Aerosol Detector). The column was an L-column 2 ODS (30 × 2.1 mm I.D., CERI, Japan) or a Capcell Pak C18AQ (50 mm × 3.0 mm I.D., Shiseido, Japan) with a temperature of 50 °C and a flow rate of 0.5 mL/min. Mobile phases A and B under neutral conditions were a mixture of 50 mmol/L ammonium acetate, water and acetonitrile (1:8:1, v/v/v) and a mixture of 50 mmol/L ammonium acetate and MeCN (1:9, v/v), respectively. The ratio of mobile phase B was increased linearly from 5 to 95% over 3 min, and held at 95% over the next 1 min. Optical rotation analyses were carried out by Sumika Chemical Analysis Service, Ltd. Specific rotations $[\alpha]_D^{25}$ are given in units of deg cm^3 g^{-1} dm^{-1}.

tert-Butyl

((5*R*,6*S*,9*S*)-5-benzyl-1-(9*H*-fluoren-9-yl)-6-hydroxy-9-isobutyl-3,7,10-trioxo-2,14,17,20-te

traoxa-4,8,11-triazadocosan-22-yl)carbamate **1** was synthesized according to the procedures of Ohoka.[17] Methyl 4-((3,5-dimethyl-1*H*-pyrazol-4-yl)methyl)benzoate **5**, 4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carbonitrile **49**, 2-chloro-4-(4-iodo-3,5-dimethyl-1*H*-pyrazol-1-yl)benzonitrile **56**, and the tested 1-benzyl-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carbonitrile **76** were synthesized according to the procedures of Yamamoto.[35, 37] *N*-(4-Cyano-3-(trifluoromethyl)phenyl)-2-methyloxirane-2-carboxamide **52** and bicalutamide were synthesized according to the procedures of Tucker .[49] 4-((1-(4-Cyano-3-(trifluoromethyl)phenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)methyl)benzoic acid and *tert*-butyl (2*S*)-2-(4-(methoxy(methyl)carbamoyl)-1,3-thiazol-2-yl)pyrrolidine-1-carboxylate **72** were synthesized according to the procedures described in patent applications.[34, 50] Compound **79** **(**(2*S*)-2-(((2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutanoyl)amino)-4-methylpentanoate) was purchased from OChem Inc. and **80** (methyl (2*S*)-2-(((2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutanoyl)amino)-4-methylpentanoate) was

synthesized as described previously.[33] Schemes and experimental procedures for synthesis of AR and IAP ligand parts are provided in the Supporting Information.

1-2.

***N*-(13-(4-((3-Cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenyl)-13-oxo-3,6,9-trioxa-12-azatridec-1-yl)-*N*^{2}-((2*S*,3*R*)-3-(((9*H*-fluoren-9-ylmethoxy)carbonyl)amino)-2-hydroxy-4-phenylbutanoyl)-L-leucinamide (3a).**

To a solution of **1** (300 mg, 0.373 mmol) in THF (5 mL) was added 4 M aq. HCl (3.7 mL, 15 mmol) at room temperature. The mixture was stirred at room temperature for 5 h. The mixture was concentrated to give a residual oil (276 mg); MS *m/z* 705 $(M+H)^+$.

To a solution of the obtained oil (276 mg), HOBt·H_2O (72 mg, 0.47 mmol), DIPEA (0.34 mL, 2.0 mmol), and **2a** (140 mg, 0.394 mmol) in THF (5 mL) was added EDC (82 μL, 0.47 mmol) at room temperature. The mixture was stirred at room temperature overnight. The mixture was poured into water at room temperature and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated *in*

vacuo. The residue was purified by column chromatography on silica gel (eluted with 0–15% MeOH in EtOAc) to give the title compound (274 mg, 67%) as a colorless amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.73–0.90 (6H, m), 1.47–1.71 (3H, m), 2.14 (3H, s), 2.32 (3H, s), 2.83–3.06 (2H, m), 3.18–3.32 (1H, m), 3.32–3.72 (16H, m), 4.04–4.34 (5H, m), 4.40–4.56 (1H, m), 5.09 (2H, s), 5.45 (1H, d, *J* = 8.4 Hz), 6.88–7.00 (3H, m), 7.14–7.33 (8H, m), 7.36 –7.43 (2H, m), 7.45–7.55 (4H, m), 7.69 (2H, d, *J* = 8.4 Hz), 7.75 (2H, d, *J* = 7.5 Hz), 7.82 (2H, d, *J* = 8.4 Hz); MS *m/z* 1042.9 $(M+H)^+$.

1-3. 9*H*-Fluoren-9-ylmethyl ((2*R*,3*S*,6*S*)-21-(4-((3-((4-cyano-3-(trifluoromethyl)phenyl)amino)-2-hydroxy-2-methyl-3-oxopropyl)sulfonyl)phenyl)-3-hydroxy-6-isobutyl-4,7,21-trioxo-1-phenyl-11,14,17-trioxa-5,8,20-triazahenicosan-2-yl)carbamate (3b).

Compound **3b** was prepared from **1** and **2b** in a similar manner to that described for compound **3a** and obtained as a colorless amorphous solid (76%). ^{1}H NMR (300 MHz, CD_3OD) δ 0.72–0.88 (6H, m), 1.41–1.67 (6H, m), 2.79–3.01 (2H, m), 3.17–3.41 (2H, m),

3.42–3.72 (15H, m), 3.93–4.14 (4H, m), 4.21–4.37 (2H, m), 4.38–4.59 (1H, m), 7.07–7.43 (9H, m), 7.48–7.64 (2H, m), 7.70–7.82 (2H, m), 7.83–8.06 (6H, m), 8.26 (1H, d, J = 1.6 Hz); MS m/z 1143 $(M+H)^+$.

1-4.

***N*-(13-(4-((1-(4-Cyano-3-(trifluoromethyl)phenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)methyl)phenyl)-13-oxo-3,6,9-trioxa-12-azatridec-1-yl)-*N*^{2}-((2*S*,3*R*)-3-(((9*H*-fluoren-9-ylmethoxy)carbonyl)amino)-2-hydroxy-4-phenylbutanoyl)-L-*l*eucinamide (3c).**

Compound **3c** was prepared from **1** and **2c** in a similar manner to that described for compound **3a** and obtained as a colorless amorphous solid (49%); MS m/z 1086.8 $(M+H)^+$.

1-5.

***N*-(13-(4-(2-(1-(3-Chloro-4-cyanophenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)ethyl)phenyl)-13-oxo-3,6,9-trioxa-12-azatridec-1-yl)-*N*^{2}-((2*S*,3*R*)-3-(((9*H*-fluoren-9-ylmethoxy)carbonyl)amino)-2-hydroxy-4-phenylbutanoyl)-L-leucinamide (3d).**

Compound **3d** was prepared from **1** and **2d** in a similar manner to that described for compound **3a** and obtained as a colorless amorphous solid (41%); MS *m/z* 1066.8 $(M+H)^+$.

1-6.

***N*-(13-(4-((1-(2-Chloro-4-cyanophenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)methyl)phenyl)-13-oxo-3,6,9-trioxa-12-azatridec-1-yl)-N^2-((2*S*,3*R*)-3-(((9*H*-fluoren-9-ylmethoxy)carbonyl)amino)-2-hydroxy-4-phenylbutanoyl)-L-leucinamide (3e).**

To a solution of **12** (261 mg, 0.408 mmol) in THF (1 mL) was added 4 M HCl in CPME (2.0 mL, 8.2 mmol). Dioxane (5 mL) was added to the mixture, and the whole mixture was stirred at room temperature for 2 h, then concentrated. The residue was diluted with toluene, and concentrated *in vacuo*, then dissolved in DMF (4 mL). To the solution were added DIPEA (0.356 mL, 2.04 mmol), **13** (260 mg, 0.490 mmol), HOBt (83 mg, 0.61 mmol), and EDC (0.11 mL, 0.61 mmol). The mixture was stirred at room temperature for 4 h. The mixture was poured into sat. aq. $NaHCO_3$, and extracted with EtOAc. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The

residue was purified by column chromatography on silica gel (eluted with 0–5% MeOH in EtOAc) and preparative HPLC. The desired fraction was neutralized with sat. aq. $NaHCO_3$, and extracted with EtOAc. The organic layer was separated, dried over $MgSO_4$, and concentrated *in vacuo* to give the title compound (315 mg, 73%) as a colorless amorphous solid. MS *m/z* 1052.8 $(M+H)^+$.

1-7. Purification conditions of preparative HPLC: Instrument: Waters 2525 separations module; Column: L-column 2 ODS (20 × 150 mm I.D., 5 μm); Mobile phase: A: H_2O/TFA (1000:1, v/v); B: MeCN/TFA (1000:1, v/v); Gradient: B from 60% to 95% in 7 min; Flow rate: 20 mL/min; Column temperature: room temperature; Wavelength: 220 nm.

1-8.

N^2-((2*S*,3*R*)-3-Amino-2-hydroxy-4-phenylbutanoyl)-*N*-(13-(4-((3-cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenyl)-13-oxo-3,6,9-trioxa-12-azatridec-1-yl)-L-leucinamide (4a).

To a solution of **3a** (100 mg, 0.0959 mmol) in MeOH (1 mL) was added 2 M Me_2NH in MeOH (4.8 mL, 9.6 mmol) at room temperature. The mixture was stirred at room temperature overnight. The mixture was concentrated and purified by column chromatography on NH silica gel (eluted with 0–15% MeOH in EtOAc) to give the title compound (77 mg, 98%) as a colorless amorphous solid. ^{1}H NMR (300 MHz, CD_3OD) δ 0.89–1.01 (6H, m), 1.51–1.73 (3H, m), 2.22 (3H, s), 2.38 (3H, s), 2.66 (1H, dd, *J* = 13.4, 7.8 Hz), 2.90 (1H, dd, *J* = 13.4, 6.9 Hz), 3.24–3.40 (3H, m), 3.44–3.69 (14H, m), 3.94 (1H, d, *J* = 3.0 Hz), 4.38–4.48 (1H, m), 5.33 (2H, s), 7.10 (2H, d, *J* = 8.3 Hz), 7.17–7.34 (5H, m), 7.61 (2H, d, *J* = 8.5 Hz), 7.76–7.89 (4H, m); MS *m/z* 820.5 $(M+H)^+$; HRMS *m/z* $(M+H)^+$ Calcd for $C_{46}H_{58}N_7O_7$: 820.4398, Found: 820.4373; Purity: 100% (HPLC); $[\alpha]_D^{25}$ −16.9 (*c* 0.200, methanol).

1-9.

***N*^{2}-((2*S*,3*R*)-3-Amino-2-hydroxy-4-phenylbutanoyl)-*N*-(13-(4-((3-((4-cyano-3-(trifluoromethyl)phenyl)amino)-2-hydroxy-2-methyl-3-oxopropyl)sulfonyl)phenyl)-13-oxo-3,6,9-trioxa-12-azatridec-1-yl)-L-leucinamide (4b).**

Compound **4b** was prepared from **3b** in a similar manner to that described for compound **4a** and obtained as a colorless amorphous solid (89%). ^{1}H NMR (300 MHz, CD_3OD) δ 0.84–1.02 (6H, m), 1.49 (3H, s), 1.56–1.74 (3H, m), 2.65 (1H, dd, *J* = 13.4, 7.8 Hz), 2.89 (1H, dd, *J* = 13.4, 6.9 Hz), 3.19–3.40 (3H, m), 3.44–3.72 (15H, m), 3.92 (1H, d, *J* = 3.0 Hz), 4.08 (1H, d, *J* = 14.7 Hz), 4.36–4.47 (1H, m), 7.13–7.34 (5H, m), 7.83–8.08 (6H, m), 8.17–8.37 (1H, m); MS *m/z* 921.3 $(M+H)^+$; HRMS *m/z* $(M+H)^+$ Calcd for $C_{43}H_{56}F_3N_6O_{11}S$: 921.3680, Found: 921.3629; Purity: 98.1% (HPLC); $[\alpha]_D^{25}$ −16.7 (*c* 0.200, methanol).

1-10.

N^2-((2*S*,3*R*)-3-Amino-2-hydroxy-4-phenylbutanoyl)-*N*-(13-(4-((1-(4-cyano-3-(trifluoromethyl)phenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)methyl)phenyl)-13-oxo-3,6,9-trioxa-12-azatridec-1-yl)-L-leucinamide (4c).

Compound **4c** was prepared from **3c** in a similar manner to that described for compound **4a** and obtained as a colorless solid (68%). ^{1}H NMR (300 MHz, CD_3OD) δ 0.88–0.97 (6H, m), 1.54–1.72 (3H, m), 2.15 (3H, s), 2.38 (3H, s), 2.61–2.72 (1H, m), 2.86–2.95 (1H, m), 3.25–

3.39 (3H, m), 3.42–3.68 (14H, m), 3.85–3.96 (3H, m), 4.37–4.46 (1H, m), 7.15–7.32 (7H, m), 7.76 (2H, d, J = 8.3 Hz), 7.91–7.98 (1H, m), 8.06–8.13 (2H, m); MS *m/z* 864.5 $(M+H)^+$; HRMS *m/z* $(M+H)^+$ Calcd for $C_{45}H_{57}F_3N_7O_7$: 864.4272, Found: 864.4228; Purity: 97.6% (HPLC); $[\alpha]_D^{25}$ −13.6 (*c* 0.278, methanol).

1-11.

N^2-((2*S*,3*R*)-3-Amino-2-hydroxy-4-phenylbutanoyl)-*N*-(13-(4-(2-(1-(3-chloro-4-cyanophenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)ethyl)phenyl)-13-oxo-3,6,9-trioxa-12-azatridec-1-yl)-L-leucinamide (4d).

Compound **4d** was prepared from **3d** in a similar manner to that described for compound **4a** and obtained as a colorless amorphous solid (86%). ^{1}H NMR (300 MHz, CD_3OD) δ 0.93 (6H, t, J = 6.0 Hz), 1.54–1.71 (3H, m), 2.08 (6H, s), 2.60–2.93 (6H, m), 3.26–3.35 (3H, m), 3.43–3.68 (14H, m), 3.93 (1H, d, J = 3.0 Hz), 4.37–4.47 (1H, m), 7.14–7.32 (7H, m), 7.54 (1H, d, J = 8.5 Hz), 7.69–7.77 (3H, m), 7.88 (1H, dd, J = 8.5, 2.0 Hz); MS *m/z* 844.5

(M+H)$^+$; HRMS *m*/*z* (M+H)$^+$ Calcd for $C_{45}H_{59}ClN_7O_7$: 844.4165, Found: 844.4133; Purity: 96.4% (HPLC); $[\alpha]_D^{25}$ −13.7 (*c* 0.503, methanol).

1-12.

N^2-((2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutanoyl)-*N*-(13-(4-((1-(2-chloro-4-cyanophenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)methyl)phenyl)-13-oxo-3,6,9-trioxa-12-azatridec-1-yl)-L-leucinamide (4e).

A mixture of **3e** (0.34 g, 0.32 mmol) and 2 M Me_2NH in MeOH (4.0 mL, 8.0 mmol) was stirred at room temperature for 3.5 h, then concentrated. The residue was purified by column chromatography on NH silica gel (eluted with 0–5% EtOAc in hexane) to give the title compound (216 mg, 81%) as a colorless amorphous solid. ^{1}H NMR (300 MHz, CD_3OD) δ 0.93 (6H, t, *J* = 5.9 Hz), 1.56–1.71 (3H, m), 2.05 (3H, s), 2.13 (3H, s), 2.60–2.69 (1H, m), 2.83–2.94 (1H, m), 3.27–3.35 (3H, m), 3.43–3.68 (14H, m), 3.87–3.95 (3H, m), 4.38–4.46 (1H, m), 7.14–7.33 (7H, m), 7.65 (1H, d, *J* = 8.2 Hz), 7.77 (2H, d, *J* = 8.2 Hz), 7.87 (1H, dd, *J* = 8.2, 1.5 Hz), 8.08 (1H, d, *J* = 1.5 Hz); MS *m/z* 830.5 (M+H)$^+$; HRMS *m*/*z*

$(M+H)^+$ Calcd for $C_{44}H_{57}ClN_7O_7$: 830.4008, Found: 830.3980; Purity: 99.4% (HPLC); $[\alpha]_D^{25}$ −13.3 (*c* 0.514, methanol).

1-13. Methyl 4-((3,5-dimethyl-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrazol-4-yl)methyl)benzoate (6).

To a solution of **5** (305 mg, 1.25 mmol) in DMF (12 mL) was added NaH (60% w/w, 54.9 mg, 1.37 mmol) at 0 °C. The mixture was stirred at 0 °C for 30 min. SEMCl (0.26 mL, 1.5 mmol) was added to the mixture, and the whole mixture was stirred at room temperature for 1.5 h. The mixture was poured into iced water and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (eluted with 0–25% EtOAc in hexane) to give the title compound (415 mg, 89%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.03 (9H, s), 0.84–0.92 (2H, m), 2.09 (3H, s), 2.20 (3H, s), 3.52–

3.60 (2H, m), 3.78 (2H, s), 3.90 (3H, s), 5.34 (2H, s), 7.16 (2H, d, *J* = 8.2 Hz), 7.92 (2H, d, *J* = 8.2 Hz); MS *m/z* 375.3 $(M+H)^+$.

1-14.

4-((3,5-Dimethyl-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrazol-4-yl)methyl)benzoic acid (7).

To a solution of **6** (252 mg, 0.673 mmol) in THF (2 mL) and MeOH (2 mL) was added 2 M aq. NaOH (2.0 mL, 4.0 mmol). The mixture was stirred at room temperature overnight. The mixture was acidified with 2 M aq. HCl at 0 °C, and extracted with EtOAc. The organic layer was separated, dried over $MgSO_4$, and concentrated in *vacuo* to give the title compound (203 mg, 84%) as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ −0.07 (9H, s), 0.78 (2H, t, *J* = 7.9 Hz), 1.98 (3H, s), 2.19 (3H, s), 3.48 (2H, t, *J* = 7.9 Hz), 3.76 (2H, s), 5.28 (2H, s), 7.21 (2H, d, *J* = 8.2 Hz), 7.83 (2H, d, *J* = 8.2 Hz), 12.79 (1H, br s); MS *m/z* 361.3 $(M+H)^+$.

1-15. *tert*-Butyl (13-(4-((3,5-dimethyl-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrazol-4-yl)methyl)phenyl)-13-oxo-3,6,9-trioxa-12-azatridec-1-yl)carbamate (9).

A mixture of **7** (200 mg, 0.555 mmol), **8** (197 mg, 0.674 mmol), EDC (0.15 mL, 0.84 mmol), and HOBt (114 mg, 0.844 mmol) in DMF (6 mL) was stirred at room temperature for 2 days. The mixture was poured into sat. aq. $NaHCO_3$, and extracted with EtOAc. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (eluted with 30–100% EtOAc in hexane) to give the title compound (345 mg, 97%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.04–−0.01 (9H, m), 0.82–0.93 (2H, m), 1.43 (9H, s), 2.09 (3H, s), 2.20 (3H, s), 3.22–3.32 (2H, m), 3.45–3.70 (16H, m), 3.76 (2H, s), 4.97–5.08 (1H, m), 5.34 (2H, s), 6.70 (1H, br s), 7.14 (2H, d, J = 8.1 Hz), 7.69 (2H, d, J = 8.1 Hz); MS *m/z* 635.6 $(M+H)^+$.

1-16. *tert*-Butyl (13-(4-((3,5-dimethyl-1*H*-pyrazol-4-yl)methyl)phenyl)-13-oxo-3,6,9-trioxa-12-azatridec-1-yl)carbamate (10).

To a solution of **9** (345 mg, 0.543 mmol) in MeOH (1 mL) was added 4 M HCl in CPME (1.5 mL, 6.0 mmol) at room temperature. The mixture was refluxed for 1 h. To the mixture was added THF (1 mL), and the whole mixture was refluxed for 7 h, then concentrated. The residue was suspended with THF (4 mL) and neutralized with sat. aq. $NaHCO_3$ at 0 °C. To the mixture was added Boc_2O (0.14 mL, 0.60 mmol), and the whole mixture was stirred at room temperature overnight. Additional Boc_2O (0.139 mL, 0.60 mmol) was added to the mixture, and the whole mixture was stirred at room temperature for 5 h. The mixture was poured into water and extracted with EtOAc. The organic layer was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (eluted with 0–10% MeOH in EtOAc). The residue was dissolved in MeOH (3.5 mL). To the solution was added sodium methoxide in MeOH (28% w/w, 21 mg, 0.11 mmol). The mixture was stirred at room temperature for 1 h. The mixture was poured into

water and extracted with EtOAc. The organic layer was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (eluted with 0–10% EtOAc in hexane) to give the title compound (213 mg, 77%) as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.36 (9H, s), 2.04 (6H, br s), 3.04 (2H, q, J = 5.7 Hz), 3.34–3.56 (14H, m), 3.70 (2H, s), 6.74 (1H, t, J = 5.5 Hz), 7.17 (2H, d, J = 8.1 Hz), 7.73 (2H, d, J = 8.1 Hz), 8.39 (1H, t, J = 5.3 Hz), 11.99 (1H, br s); MS *m/z* 505.4 $(M+H)^+$.

1-17. *tert*-Butyl (13-(4-((1-(2-chloro-4-cyanophenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)methyl)phenyl)-13-oxo-3,6,9-trioxa-12-azatridec-1-yl)carbamate (12).

To a solution of **10** (213 mg, 0.422 mmol) in DMF (4 mL) was added NaH (60% w/w, 20 mg, 0.51 mmol) at 0 °C. The mixture was stirred at 0 °C for 30 min. To the mixture was added **11** (131 mg, 0.842 mmol), and the whole mixture was stirred at room temperature overnight. The mixture was poured into sat. aq. $NaHCO_3$, and extracted with EtOAc. The

organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (eluted with 0–100% EtOAc in hexane) to give the title compound (261 mg, 97%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (9H, s), 2.03 (3H, s), 2.18 (3H, s), 3.22–3.32 (2H, m), 3.48–3.53 (2H, m), 3.59–3.71 (12H, m), 3.84 (2H, s), 5.02 (1H, br s), 6.74 (1H, br s), 7.20 (2H, d, J = 8.1 Hz), 7.55 (1H, d, J = 8.1 Hz), 7.66–7.78 (3H, m), 7.84 (1H, d, J = 1.7 Hz); MS m/z 640.5 $(M+H)^+$.

1-18. *tert*-Butyl (13-(4-((3-cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenyl)-13-oxo-3,6,9-trioxa-12-azatridec-1-yl)carbamate (15).

A mixture of **2a** (110 mg, 0.310 mmol), **14** (110 mg, 0.376 mmol), HOBt (57 mg, 0.42 mmol), and EDC (74 µl, 0.42 mmol) in THF (3 mL) was stirred at room temperature overnight. The mixture was diluted with EtOAc, washed with 5% aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column

chromatography on silica gel (eluted with 0–20% MeOH in EtOAc) to give the title compound (185 mg, 98%) as a colorless amorphous powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (9H, s), 2.19 (3H, s), 2.37 (3H, s), 3.26 (2H, q, *J* = 5.0 Hz), 3.50 (2H, t, *J* = 5.2 Hz), 3.56–3.74 (12H, m), 5.04 (1H, br s), 5.15 (2H, s), 6.92 (1H, br s), 6.99 (2H, d, *J* = 8.0 Hz), 7.48–7.59 (2H, m), 7.66–7.77 (2H, m), 7.83 (2H, d, *J* = 7.4 Hz); MS *m/z* 530.5 (M+H–Boc)$^+$.

1-19.

1-((2*S*)-2-((*N*-(*tert*-Butoxycarbonyl)-*N*-methyl-L-alanyl)amino)-2-cyclohexylacetyl)-L-prolyl-*N*-(13-(4-((3-cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenyl)-13-oxo-3,6,9-trioxa-12-azatridec-1-yl)-β-phenyl-L-phenylalaninamide (17).

To a mixture of **15** (185 mg, 0.290 mmol) in THF (0.75 mL) was added 2 M HCl in CPME (4.5 mL, 9.0 mmol). The mixture was stirred at room temperature for 1.5 h. The mixture was diluted with toluene (5 mL), and concentrated *in vacuo* to give a colorless amorphous powder (181 mg). To a mixture of the obtained amorphous powder (164 mg, 0.290 mmol),

16 (192 mg, 0.290 mmol), and DIPEA (0.15 mL, 0.87 mmol) in DMF (4.5 mL) was added HATU (165 mg, 0.430 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h. The mixture was diluted with EtOAc, washed with 5% aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (eluted with 0–30% MeOH in EtOAc), then on NH silica gel (eluted with 0–20% MeOH in EtOAc) to give the title compound (322 mg, 95%) as a pale yellow solid. ^{1}H NMR (300 MHz, CD_3OD) δ 0.95–1.42 (9H, m), 1.42–1.55 (9H, m), 1.56–1.88 (9H, m), 2.17 (3H, s), 2.34 (3H, s), 2.76–3.22 (7H, m), 3.32–3.47 (2H, m), 3.49–3.83 (12H, m), 4.23–4.64 (4H, m), 5.08–5.21 (1H, m), 5.29 (2H, s), 7.00–7.47 (12H, m), 7.57 (2H, d, *J* = 8.5 Hz), 7.71–7.94 (4H, m); MS *m/z* 1175.0 $(M+H)^+$.

1-20.

1-((2*S*)-2-Cyclohexyl-2-((*N*-methyl-L-alanyl)amino)acetyl)-L-prolyl-*N*-(13-(4-((3-cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenyl)-13-oxo-3,6,9-trioxa-12-azatridec-1-yl)-beta-phenyl-L-phenylalaninamide (18).

A mixture of **17** (308 mg, 0.260 mmol) and 4 M HCl in CPME (3.0 mL, 12 mmol) in THF (1.5 mL) was stirred at room temperature for 3 h. The mixture was diluted with toluene (5 mL) and concentrated *in vacuo*. The residue was diluted with sat. aq. $NaHCO_3$, and extracted with EtOAc–THF (3:1). The organic layer was separated, washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on NH silica gel (eluted with 0–10% MeOH in EtOAc) to give the title compound (237 mg, 84%) as a colorless solid. ^{1}H NMR (300 MHz, CD_3OD) δ 0.95–1.48 (9H, m), 1.56–2.06 (9H, m), 2.17 (3H, s), 2.24–2.43 (6H, m), 2.79–3.04 (2H, m), 3.04–3.27 (3H, m), 3.33–3.45 (2H, m), 3.47–3.93 (12H, m), 4.19–4.52 (3H, m), 5.07–5.22 (1H, m), 5.25–5.41 (2H, m), 7.01–7.48 (12H, m), 7.52–7.62 (2H, m), 7.72–7.87 (4H, m); MS *m/z* 1074.7 $(M+H)^+$; HRMS *m/z* $(M+H)^+$ Calcd for $C_{62}H_{76}N_9O_8$: 1074.5817, Found: 1074.5761; Purity: 99.8% (HPLC); $[\alpha]_D^{25}$ −13.8 (*c* 0.202, methanol).

1-21. *tert*-Butyl ((2*S*)-1-(((1*S*)-2-((2*S*)-2-(4-(3-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)benzoyl)-1,3-thiazol-

2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)methylcarbamate (21).

To a mixture of Ph_3P (425 mg, 1.62 mmol) in THF (1.5 mL) was added 40% DEAD in toluene (0.71 g, 1.6 mmol) at 0 °C. After the mixture was stirred at 0 °C for 10 min, a solution of **19** (194 mg, 0.320 mmol) and **20** (0.5 M MTBE solution: 3.3 mL, 1.7 mmol) in THF (3 mL) was added to the mixture. After the mixture was stirred at room temperature for 5 h, water (0.4 mL) and Ph_3P (850 mg, 3.24 mmol) were added to the mixture. The mixture was stirred at room temperature overnight. The mixture was diluted with EtOAc, washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on NH silica gel (eluted with 0–20% MeOH in EtOAc) to give the title compound (308 mg, quant.) as a colorless oil. ^{1}H NMR (300 MHz, CD_3OD) δ 0.97–1.39 (8H, m), 1.41–1.52 (9H, m), 1.52–1.94 (6H, m), 2.07–2.53 (4H, m), 2.67–2.95 (5H, m), 3.45–4.30 (12H, m), 4.42–4.70 (2H, m), 5.38–5.75 (1H, m), 7.24 (1H, dd, J = 7.8, 1.9 Hz), 7.44 (1H, t, J = 8.2 Hz), 7.67–7.84 (2H, m), 8.32 (1H, s); MS *m/z* 730.6 $(M+H)^+$.

1-22. *tert*-Butyl ((2*S*)-1-(((1*S*)-2-((2*S*)-2-(4-(3-(2-(2-(2-((4-((3-cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)benzoyl)amino)ethoxy)ethoxy)ethoxy)benzoyl)-1,3-thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)methylcarbamate (22).

A mixture of **21** (234 mg, 0.321 mmol), **2a** (341 mg, 0.960 mmol), HOBt (130 mg, 0.962 mmol), and EDC (0.17 mL, 0.97 mmol) in THF (3 mL) and DMF (1 mL) was stirred at room temperature for 3 h. The mixture was diluted with EtOAc, washed with 5% aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on NH silica gel (eluted with 50–100% EtOAc in hexane then 0–10% MeOH in EtOAc) and by column chromatography on silica gel (eluted with 0–10% MeOH in EtOAc) to give the title compound (125 mg, 37%) as a colorless solid. ^{1}H NMR (300 MHz, CD_3OD) δ 1.04–1.39 (8H, m), 1.40–1.49 (9H, m), 1.49–1.85 (6H, m), 2.06–2.37 (10H, m), 2.66–2.90 (3H, m), 3.50–4.02 (13H, m), 4.11–4.18 (2H, m), 4.43–4.66 (2H, m), 5.27 (2H, s), 5.41–5.67 (1H, m), 7.03 (2H, d, J = 8.3 Hz), 7.17 (1H, dd,

J = 7.9, 2.1 Hz), 7.39 (1H, t, *J* = 8.0 Hz), 7.56 (2H, d, *J* = 8.6 Hz), 7.63–7.86 (6H, m), 8.26–8.33 (1H, m); MS *m/z* 1067.9 $(M+H)^+$.

1-23.

4-((3-Cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)-*N*-(2-(2-(2-(3-((2-((2*S*)-1-((2*S*)-2-cyclohexyl-2-((*N*-methyl-L-alanyl)amino)acetyl)pyrrolidin-2-yl)-1,3-thiazol-4-yl)carbonyl)phenoxy)ethoxy)ethoxy)ethyl)benzamide (23).

A mixture of **21** (120 mg, 0.112 mmol) in THF (3 mL) and 4 M HCl in CPME (3.0 mL, 12 mmol) was stirred at room temperature for 4 h. The mixture was diluted with toluene (5 mL), and concentrated *in vacuo*. The residue was dissolved in EtOAc–THF(1:1), washed with sat. aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on NH silica gel (eluted with 0–10% MeOH in EtOAc) to give the title compound (81 mg, 74%) as a colorless solid. ^{1}H NMR (300 MHz, CD_3OD) δ 0.95–1.35 (8H, m), 1.52–1.83 (6H, m), 2.07–2.42 (13H, m), 2.86–3.19 (1H, m), 3.50–4.04 (12H, m), 4.13 (2H, t, *J* = 4.5 Hz), 4.47–4.62 (1H, m), 5.26 (2H, s), 5.41–5.70

(1H, m), 7.03 (2H, d, *J* = 8.4 Hz), 7.16 (1H, dd, *J* = 8.3, 1.7 Hz), 7.38 (1H, t, *J* = 8.0 Hz), 7.50–7.86 (8H, m), 8.25–8.32 (1H, m); MS *m/z* 967.6 $(M+H)^+$; HRMS *m/z* $(M+H)^+$ Calcd for $C_{54}H_{63}N_8O_7S$: 967.4540, Found: 967.4543; Purity: 96.5% (HPLC); $[\alpha]_D^{25}$ −71.0 (*c* 0.202, methanol).

1-24.

***N*-((1*S*)-1-Cyclohexyl-2-((2*S*)-2-(4-(3-methoxybenzoyl)-1,3-thiazol-2-yl)pyrrolidin-1-yl)-2-oxoethyl)-*N*^{2}-methyl-L-alaninamide (24).**

To a mixture of **19** (120 mg, 0.200 mmol), Ph_3P (158 mg, 0.602 mmol), and MeOH (24 μL, 0.59 mmol) in THF (3 mL) was added 40% DEAD in toluene (0.28 g, 0.64 mmol) at 0 °C. The mixture was stirred at room temperature overnight, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (eluted with 50–100% EtOAc in hexane) to give the title compound as a colorless gum. A mixture of the obtained gum and 4 M HCl in CPME (3.0 mL, 12 mmol) was stirred at room temperature for 1 h. The resultant solid precipitate was collected by filtration and washed with EtOAc. The solid was

neutralized with saturated aq. $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on NH silica gel (eluted with 50–100% EtOAc in hexane), and crystallized from EtOAc–hexane to give the title compound (43 mg, 42%) as colorless crystals. ^{1}H NMR (300 MHz, CD_3OD) δ 0.96–1.30 (8H, m), 1.54–1.86 (6H, m), 2.06–2.50 (7H, m), 2.89–3.22 (1H, m), 3.83–4.09 (5H, m), 4.49–4.61 (1H, m), 5.42–5.71 (1H, m), 7.21 (1H, ddd, J = 8.3, 2.6, 0.9 Hz), 7.43 (1H, t, J = 7.8 Hz), 7.65–7.77 (2H, m), 8.27–8.36 (1H, m); MS *m/z* 513.4 $(M+H)^+$; HRMS *m/z* $(M+H)^+$ Calcd for $C_{27}H_{37}N_4O_4S$: 513.2536, Found: 513.2508; Purity: 97.8% (HPLC); $[\alpha]_D^{25}$ −146.9 (*c* 0.103, methanol).

1-25.

4-(4-Cyanophenyl)-1-(4-(3-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)prop-1-yn-1-yl)benzyl)-2,5-dimethyl-1*H*-pyrrole-3-carbonitrile (27).

A mixture of **25** (585 mg, 1.50 mmol), **26** (565 mg, 3.00 mmol), Et_3N (0.84 mL, 6.0 mmol), copper(I) iodide (29 mg, 0.15 mmol), and $Pd(Ph_3P)_4$ (173 mg, 0.150 mmol) in DMF (4.5

mL) was stirred at 60 °C under Ar overnight. After cooling, the mixture was diluted with EtOAc, washed with sat. aq. NH_4Cl and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on NH silica gel (eluted with 0–20% MeOH in EtOAc) to give the title compound (365 mg, 49%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.18 (3H, s), 2.36 (3H, s), 2.48 (1H, t, J = 6.2 Hz), 3.56–3.84 (12H, m), 4.42 (2H, s), 5.10 (2H, s), 6.87 (2H, d, J = 8.4 Hz), 7.43 (2H, d, J = 8.3 Hz), 7.49–7.58 (2H, m), 7.66–7.76 (2H, m); MS *m/z* 498.4 $(M+H)^+$.

1-26.

2-(2-(2-((3-(4-((3-Cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenyl)prop-2-yn-1-yl)oxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (28).

To a mixture of **27** (320 mg, 0.643 mmol) in pyridine (4.5 mL) was added TsCl (0.18 g, 0.96 mmol) at 0 °C. The mixture was stirred at room temperature for 4 h. To the mixture was then added TsCl (0.18 g, 0.96 mmol), and the mixture was stirred at room temperature for 1 h. The mixture was diluted with EtOAc, washed with 1 M HCl, sat. aq. $NaHCO_3$, and

brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (eluted with 50–100% EtOAc in hexane) to give the title compound (302 mg, 72%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.19 (3H, s), 2.36 (3H, s), 2.44 (3H, s), 3.61 (4H, s), 3.64–3.78 (6H, m), 4.12–4.19 (2H, m), 4.41 (2H, s), 5.10 (2H, s), 6.88 (2H, d, J = 8.3 Hz), 7.34 (2H, d, J = 8.1 Hz), 7.43 (2H, d, J = 8.2 Hz), 7.53 (2H, d, J = 8.4 Hz), 7.71 (2H, d, J = 8.4 Hz), 7.79 (2H, d, J = 8.3 Hz); MS *m/z* 652.5 $(M+H)^+$.

1-27.

4-(4-Cyanophenyl)-1-(4-(3-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)propyl)benzyl)-2,5-dimethyl-1*H*-pyrrole-3-carbonitrile (29).

A mixture of **27** (365 mg, 0.730 mmol) and 10% Pd–C (73 mg) in EtOAc (3 mL) was stirred under a hydrogen atmosphere at room temperature for 2 h. The mixture was filtered, and the filtrate was concentrated *in vacuo.* The residue was purified by column chromatography on silica gel (eluted with EtOAc) to give the title compound (310 mg,

84%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.81–1.96 (2H, m), 2.20 (3H, s), 2.37 (3H, s), 2.52–2.73 (3H, m), 3.47 (2H, t, *J* = 6.4 Hz), 3.55–3.77 (12H, m), 5.07 (2H, s), 6.84 (2H, d, *J* = 8.2 Hz), 7.18 (2H, d, *J* = 8.1 Hz), 7.49–7.58 (2H, m), 7.67–7.75 (2H, m); MS *m/z* 502.4 $(M+H)^+$.

1-28.

2-(2-(2-(3-(4-((3-Cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenyl)propoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (30).

To a mixture of **29** (310 mg, 0.618 mmol) in pyridine (4.5 mL) was added TsCl (353 mg, 1.85 mmol) at 0 °C. The mixture was stirred at room temperature for 3 h. The mixture was diluted with EtOAc, washed with 1 M HCl, sat. aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (eluted with 40–90% EtOAc in hexane) to give the title compound (338 mg, 83%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.81–1.96 (2H, m), 2.20 (3H, s), 2.37 (3H, s), 2.44 (3H, s), 2.61–2.74 (2H, m), 3.46 (2H, t, *J* = 6.4 Hz), 3.53–3.73 (10H, m), 4.12–4.19

(2H, m), 5.07 (2H, s), 6.84 (2H, d, *J* = 8.1 Hz), 7.17 (2H, d, *J* = 8.1 Hz), 7.33 (2H, d, *J* = 8.0 Hz), 7.49–7.57 (2H, m), 7.65–7.74 (2H, m), 7.75–7.84 (2H, m); MS *m/z* 656.5 $(M+H)^+$.

1-29. *tert*-Butyl ((2*S*)-1-(((1*S*)-2-((2*S*)-2-(4-(3-(2-(2-(2-((3-(4-((3-cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenyl)prop-2-yn-1-yl)oxy)ethoxy)ethoxy)ethoxy)benzoyl)-1,3-thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)methyl carbamate (31a).

A mixture of **28** (302 mg, 0.460 mmol), **19** (277 mg, 0.460 mmol), and K_2CO_3 (77 mg, 0.56 mmol) in DMF (2 mL) was stirred at room temperature overnight, and then heated at 50 °C for 24 h. After cooling, the mixture was diluted with EtOAc, washed with 5% aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (eluted with 50–100% EtOAc in hexane) and then NH silica gel (eluted with 50–100% EtOAc in hexane) to give the title compound (334 mg, 67%) as a colorless amorphous solid. ^{1}H NMR (300 MHz, CD_3OD) δ 0.94–1.38 (8H, m),

1.40–1.49 (9H, m), 1.50–1.83 (6H, m), 2.04–2.42 (10H, m), 2.63–2.90 (3H, m), 3.62–3.77 (8H, m), 3.80–4.03 (4H, m), 4.14–4.21 (2H, m), 4.38 (2H, s), 4.45–4.68 (2H, m), 5.23 (2H, s), 5.41–5.67 (1H, m), 6.94 (2H, d, J = 8.3 Hz), 7.20 (1H, dd, J = 8.4, 1.7 Hz), 7.34–7.45 (3H, m), 7.57 (2H, d, J = 8.5 Hz), 7.65–7.73 (2H, m), 7.77 (2H, d, J = 8.5 Hz), 8.26–8.33 (1H, m); MS *m/z* 1078.8 $(M+H)^+$.

1-30. *tert*-Butyl ((2*S*)-1-(((1*S*)-2-((2*S*)-2-(4-(3-(2-(2-(2-(3-(4-((3-cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenyl)propoxy)ethoxy)ethoxy)benzoyl)-1,3-thiazol-2-yl) pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)methylcarbamate (31b).

Compound **31b** was prepared from **19** and **30** in a similar manner to that described for compound **31a** and obtained as a colorless amorphous powder (72%). ^{1}H NMR (300 MHz, CD_3OD) δ 0.90–1.37 (8H, m), 1.39–1.50 (9H, m), 1.51–1.88 (8H, m), 2.06–2.43 (10H, m), 2.64 (2H, t, J = 7.6 Hz), 2.68–2.90 (3H, m), 3.37–3.47 (2H, m), 3.50–3.75 (8H, m), 3.79–

4.02 (4H, m), 4.17 (2H, dd, *J* = 5.4, 3.9 Hz), 4.44–4.69 (2H, m), 5.17 (2H, s), 5.42–5.66 (1H, m), 6.87 (2H, d, *J* = 8.2 Hz), 7.11–7.23 (3H, m), 7.39 (1H, t, *J* = 8.1 Hz), 7.52–7.61 (2H, m), 7.66–7.80 (4H, m), 8.23–8.33 (1H, m); MS *m/z* 1082.9 $(M+H)^+$.

1-31.

_N_-((1_S_)-2-((2_S_)-2-(4-(3-(2-(2-(2-((3-(4-((3-Cyano-4-(4-cyanophenyl)-2,5-dimethyl-1_H_-pyrrol-1-yl)methyl)phenyl)prop-2-yn-1-yl)oxy)ethoxy)ethoxy)ethoxy)benzoyl)-1,3-thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)-N^2-methyl-L-alaninamide (32a).

A mixture of **31a** (334 mg, 0.310 mmol) in TFA (3 mL) was stirred at room temperature for 10 min. The mixture was diluted with toluene (5 mL), and concentrated *in vacuo*. The residue was dissolved in EtOAc, washed with sat. aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on NH silica gel (eluted with 0–10% MeOH in EtOAc) to give the title compound (237 mg, 78%) as a colorless solid. ^{1}H NMR (300 MHz, CD_3OD) δ 0.96–1.35 (8H, m), 1.49–1.84 (6H, m), 2.04–2.45 (13H, m), 2.87–3.20 (1H, m), 3.63–3.76 (8H, m), 3.83–4.04 (4H, m),

4.14–4.21 (2H, m), 4.38 (2H, s), 4.49–4.62 (1H, m), 5.23 (2H, s), 5.43–5.67 (1H, m), 6.94 (2H, d, J = 8.2 Hz), 7.16–7.24 (1H, m), 7.34–7.45 (3H, m), 7.53–7.61 (2H, m), 7.65–7.73 (2H, m), 7.77 (2H, d, J = 8.5 Hz), 8.25–8.31 (1H, m); MS *m/z* 978.4 $(M+H)^+$; HRMS *m/z* $(M+H)^+$ Calcd for $C_{56}H_{64}N_7O_7S$: 978.4588, Found: 978.4528; Purity: 99.2% (HPLC); $[\alpha]_D^{25}$ −77.6 (*c* 0.201, methanol).

1-32.

***N*-((1*S*)-2-((2*S*)-2-(4-(3-(2-(2-(2-(3-(4-((3-Cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenyl)propoxy)ethoxy)ethoxy)benzoyl)-1,3-thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)-*N*^{2}-methyl-L-alaninamide (32b).**

Compound **32b** was prepared from **31b** in a similar manner to that described for compound **32b** and obtained as a colorless solid (86%). ^{1}H NMR (300 MHz, CD_3OD) δ 0.96–1.39 (8H, m), 1.50–1.93 (8H, m), 2.02–2.42 (13H, m), 2.64 (2H, t, J = 7.6 Hz), 3.14 (1H, q, J = 6.9 Hz), 3.42 (2H, t, J = 6.3 Hz), 3.50–3.76 (8H, m), 3.81–4.03 (4H, m), 4.17 (2H, dd, J = 5.5, 3.9 Hz), 4.47–4.64 (1H, m), 5.17 (2H, s), 5.42–5.68 (1H, m), 6.87 (2H, d, J = 8.2 Hz),

7.10–7.23 (3H, m), 7.33–7.44 (1H, m), 7.52–7.60 (2H, m), 7.66–7.81 (4H, m), 8.24–8.31 (1H, m); MS *m/z* 982.4 $(M+H)^+$; HRMS *m/z* $(M+H)^+$ Calcd for $C_{56}H_{68}N_7O_7S$: 982.4901, Found: 982.4825; Purity: 99.8% (HPLC); $[\alpha]_D^{25}$ −77.6 (*c* 0.200, methanol).

1-33.

4-(4-Cyanophenyl)-1-(4-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethoxy)benzyl)-2,5-dimethyl-1*H*-pyrrole-3-carbonitrile (35a).

To a mixture of **33a** (392 mg, 1.20 mmol), **34** (1.28 mL, 7.41 mmol), and Ph_3P (960 mg, 3.66 mmol) in THF (12 mL) was dropwise added 40% DEAD in toluene (3.20 g, 7.35 mmol) at 0 °C. The mixture was stirred at room temperature overnight. The mixture was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (eluted with 0–20% MeOH in EtOAc) to give the title compound (440 mg, 73%) as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$) δ 2.20 (3H, s), 2.37 (3H, s), 2.64 (1H, br s), 3.56–3.78 (12H, m),

3.81–3.90 (2H, m), 4.06–4.24 (2H, m), 5.04 (2H, s), 6.79–6.95 (4H, m), 7.48–7.58 (2H, m), 7.65–7.76 (2H, m); MS *m/z* 504.4 $(M+H)^+$.

1-34.

4-(4-Cyanophenyl)-1-(3-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethoxy)benzyl)-2,5-dimethyl-1*H*-pyrrole-3-carbonitrile (35b).

Compound **35b** was prepared from **33b** in a similar manner to that described for compound **35a** and obtained as a colorless oil (94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.20 (3H, s), 2.37 (3H, s), 2.63 (1H, br s), 3.52–3.79 (12H, m), 3.80–3.90 (2H, m), 4.05–4.13 (2H, m), 5.07 (2H, s), 6.44–6.57 (2H, m), 6.84 (1H, dd, *J* = 8.1, 1.7 Hz), 7.21–7.31 (1H, m), 7.53 (2H, d, *J* = 8.3 Hz), 7.71 (2H, d, *J* = 8.3 Hz); MS *m/z* 504.4 $(M+H)^+$.

1-35.

2-(2-(2-(2-(4-((3-Cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (36a).

To a mixture of **35a** (220 mg, 0.437 mmol) in pyridine (3 mL) was added TsCl (125 mg, 0.656 mmol) at 0 °C. The mixture was stirred at room temperature for 3 h. The mixture was diluted with EtOAc, washed with 1 M HCl, sat. aq. $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (eluted with 50–100% EtOAc in hexane) to give the title compound (216 mg, 75%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.20 (3H, s), 2.37 (3H, s), 2.44 (3H, s), 3.54–3.76 (10H, m), 3.81–3.89 (2H, m), 4.07–4.22 (4H, m), 5.04 (2H, s), 6.79–6.95 (4H, m), 7.33 (2H, d, J = 8.0 Hz), 7.52 (2H, d, J = 8.5 Hz), 7.70 (2H, d, J = 8.4 Hz), 7.79 (2H, d, J = 8.3 Hz); MS *m/z* 658.5 $(M+H)^+$.

1-36.

2-(2-(2-(2-(3-((3-Cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenoxy)ethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (36b).

Compound **36b** was prepared from **35b** in a similar manner to that described for compound **36a** and obtained as a colorless oil (68%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.19 (3H, s), 2.37

(3H, s), 2.44 (3H, s), 3.59 (4H, s), 3.62–3.75 (6H, m), 3.80–3.90 (2H, m), 4.06–4.19 (4H, m), 5.06 (2H, s), 6.45–6.56 (2H, m), 6.78–6.88 (1H, m), 7.21–7.30 (1H, m), 7.33 (2H, d, *J* = 8.1 Hz), 7.53 (2H, d, *J* = 8.4 Hz), 7.65–7.74 (2H, m), 7.79 (2H, d, *J* = 8.3 Hz); MS *m/z* 658.5 $(M+H)^+$.

1-37.

2-(2-(2-(4-((3-Cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenoxy) ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (36c).

A mixture of **70a** (197 mg, 0.602 mmol), **74** (0.33 g, 0.73 mmol), and K_2CO_3 (0.13 g, 0.92 mmol) in DMF (5 mL) was stirred at 50 °C for 10 h. The mixture was poured into water and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (eluted with 0–75% EtOAc in hexane) to give the title compound (190 mg, 51%) as a colorless gum. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.17–2.22 (3H, m), 2.35–2.39 (3H, m), 2.41–2.46 (3H, m), 3.58–3.73 (6H, m), 3.83 (2H, dd, *J* = 5.4,

4.1 Hz), 4.06–4.18 (4H, m), 5.04 (2H, s), 6.82–6.94 (4H, m), 7.33 (2H, d, *J* = 7.9 Hz), 7.49–7.56 (2H, m), 7.66–7.73 (2H, m), 7.76–7.82 (2H, m); MS *m/z* 614.4 $(M+H)^+$.

1-38.

14-(4-((3-Cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenoxy)-3,6,9,12-tetraoxatetradec-1-yl 4-methylbenzenesulfonate (36d).

Compound **36d** was prepared from **33a** and **37b** in a similar manner to that described for compound **36c** and obtained as a colorless amorphous powder (46%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.20 (3H, s), 2.37 (3H, s), 2.44 (3H, s), 3.55–3.75 (14H, m), 3.81–3.88 (2H, m), 4.07–4.18 (4H, m), 5.04 (2H, s), 6.81–6.92 (4H, m), 7.33 (2H, d, *J* = 8.0 Hz), 7.49–7.56 (2H, m), 7.67–7.73 (2H, m), 7.79 (2H, d, *J* = 8.3 Hz); MS *m/z* 702.4 $(M+H)^+$.

1-39.

2-(2-(2-(2-(2-((3-Cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenoxy)ethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (36e).

To a mixture of **39** (1.02 g, 2.24 mmol) and Et_3N (0.63 mL, 4.5 mmol) in THF (6 mL) was added MsCl (0.21 mL, 2.7 mmol) at 0 °C. The mixture was stirred at 0 °C for 30 min. The mixture was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo* to give a colorless oil. A solution of the obtained oil in DMF (3 mL) was added to a mixture of **40** (332 mg, 1.50 mmol) and NaH (60% w/w, 72 mg, 1.8 mmol) in DMF (3 mL) at 0 °C. The mixture was stirred at 0 °C for 1.5 h and was poured into 5% aq. $NaHCO_3$.The mixture was extracted with EtOAc, washed with 5% aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (eluted with 40–90% EtOAc in hexane) to give the title compound (0.82 g, 83%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.17 (3H, s), 2.33 (3H, s), 2.44 (3H, s), 3.53–3.78 (10H, m), 3.85–3.96 (2H, m), 4.10–4.29 (4H, m), 5.10 (2H, s), 6.34 (1H, dd, J = 7.4, 0.8 Hz), 6.83–6.96 (2H, m), 7.22–7.30 (1H, m), 7.33 (2H, d, J = 8.1 Hz), 7.54 (2H, d, J = 8.3 Hz), 7.66–7.74 (2H, m), 7.78 (2H, d, J = 8.3 Hz); MS *m/z* 658.5 $(M+H)^+$.

1-40. 2-(2-(2-(2-(2-(Hydroxymethyl)phenoxy)ethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (39).

A mixture of **38** (620 mg, 4.99 mmol), **37c** (5.03 g, 10.0 mmol), and K_2CO_3 (0.83 g, 6.0 mmol) in DMF (10 mL) was stirred at 40 °C overnight. After cooling, the mixture was diluted with EtOAc, washed with 5% aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (eluted with 50–100% EtOAc in hexane) to give the title compound (1.05 g, 46%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.43 (3H, s), 3.19 (1H, t, *J* = 6.8 Hz), 3.57 (4H, s), 3.60–3.74 (6H, m), 3.85 (2H, dd, *J* = 5.5, 3.7 Hz), 4.14 (2H, dd, *J* = 5.4, 4.2 Hz), 4.20 (2H, dd, *J* = 5.4, 3.7 Hz), 4.65 (2H, d, *J* = 6.4 Hz), 6.84–6.98 (2H, m), 7.19–7.28 (2H, m), 7.33 (2H, d, *J* = 8.1 Hz), 7.79 (2H, d, *J* = 8.3 Hz).

1-41. *tert*-Butyl ((2*S*)-1-(((1*S*)-2-((2*S*)-2-(4-(3-(2-(2-(2-(2-(4-((3-cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenoxy)ethoxy)ethoxy)ethoxy)ethoxy)benzoyl)-1,3-thiazol-2-yl)

pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)methylcarbamate (41a).

A mixture of **36a** (210 mg, 0.319 mmol), **19** (190 mg, 0.317 mmol), and K_2CO_3 (49 mg, 0.35 mmol) in DMF (1.5 mL) was stirred at room temperature overnight, and then heated at 60 °C for 4 h. After cooling, the mixture was diluted with EtOAc, washed with 5% aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (eluted with 50–100% EtOAc in hexane, then 0–10% MeOH in EtOAc), and then on NH silica gel (eluted with 50–100% EtOAc in hexane) to give the title compound (231 mg, 67%) as a colorless amorphous powder. ^{1}H NMR (300 MHz, CD_3OD) δ 0.94–1.37 (8H, m), 1.38–1.49 (9H, m), 1.49–1.88 (6H, m), 2.02–2.44 (10H, m), 2.65–2.91 (3H, m), 3.50–4.26 (18H, m), 4.43–4.69 (2H, m), 5.13 (2H, s), 5.41–5.67 (1H, m), 6.85–6.93 (4H, m), 7.15–7.24 (1H, m), 7.40 (1H, t, J = 8.2 Hz), 7.56 (2H, d, J = 8.5 Hz), 7.66–7.73 (2H, m), 7.76 (2H, d, J = 8.5 Hz), 8.26–8.31 (1H, m); MS *m/z* 1084.9 $(M+H)^+$.

1-42. *tert*-Butyl ((2*S*)-1-(((1*S*)-2-((2*S*)-2-(4-(3-(2-(2-(2-(2-(3-((3-cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenoxy)ethoxy)ethoxy)ethoxy)ethoxy)benzoyl)-1,3-thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)methylcarbamate (41b).

Compound **41b** was prepared from **36b** and **19** in a similar manner to that described for compound **41a** as a colorless amorphous powder (81%). ^{1}H NMR (300 MHz, CD_3OD) δ 0.94–1.39 (8H, m), 1.40–1.49 (9H, m), 1.52–1.85 (6H, m), 2.03–2.41 (10H, m), 2.66–2.91 (3H, m), 3.56–4.21 (18H, m), 4.42–4.68 (2H, m), 5.17 (2H, s), 5.41–5.66 (1H, m), 6.46–6.58 (2H, m), 6.83 (1H, dd, *J* = 8.2, 2.3 Hz), 7.13–7.29 (2H, m), 7.39 (1H, t, *J* = 8.2 Hz), 7.56 (2H, d, *J* = 8.4 Hz), 7.65–7.72 (2H, m), 7.76 (2H, d, *J* = 8.4 Hz), 8.23–8.33 (1H, m); MS *m/z* 1084.9 $(M+H)^+$.

1-43. *tert*-Butyl ((2*S*)-1-(((1*S*)-2-((2*S*)-2-(4-(3-(2-(2-(2-(4-((3-cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-

pyrrol-1-yl)methyl)phenoxy)ethoxy)ethoxy)ethoxy)benzoyl)-1,3-thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)methylcarbamate (41c).

Compound **41c** was prepared from **36c** and **19** in a similar manner to that described for compound **41a** and obtained as a colorless amorphous powder (75%). ^{1}H NMR (300 MHz, CD_3OD) δ 0.94–1.37 (8H, m), 1.39–1.49 (9H, m), 1.51–1.84 (6H, m), 2.03–2.38 (10H, m), 2.65–2.90 (3H, m), 3.66–4.24 (14H, m), 4.43–4.71 (2H, m), 5.13 (2H, s), 5.40–5.65 (1H, m), 6.85–6.93 (4H, m), 7.19 (1H, dd, *J* = 8.3, 1.7 Hz), 7.39 (1H, t, *J* = 7.9 Hz), 7.56 (2H, d, *J* = 8.4 Hz), 7.66–7.73 (2H, m), 7.76 (2H, d, *J* = 8.4 Hz), 8.26–8.33 (1H, m); MS *m/z* 1040.8 $(M+H)^+$.

1-44. *tert*-Butyl ((2*S*)-1-(((1*S*)-2-((2*S*)-2-(4-(3-((14-(4-((3-cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenoxy)-3,6,9,12-tetraoxatetradec-1-yl)oxy)benzoyl)-1,3-thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)methylcarbamate (41d).

Compound **41d** was prepared from **36d** and **19** in a similar manner to that described for compound **41a** and obtained as a colorless amorphous powder (67%). ^{1}H NMR (300 MHz, CD_3OD) δ 0.95–1.20 (5H, m), 1.27–1.49 (12H, m), 1.52–1.83 (6H, m), 2.07–2.42 (10H, m), 2.67–2.89 (3H, m), 3.58–3.71 (12H, m), 3.75–4.01 (6H, m), 4.04–4.10 (2H, m), 4.13–4.21 (2H, m), 4.45–4.66 (2H, m), 5.14 (2H, s), 5.42–5.65 (1H, m), 6.85–6.96 (4H, m), 7.20 (1H, dt, *J* = 8.2, 1.3 Hz), 7.35–7.45 (1H, m), 7.52–7.60 (2H, m), 7.66–7.80 (4H, m), 8.26–8.31 (1H, m); MS *m/z* 1128.7 $(M+H)^+$.

1-45. *tert*-Butyl ((2*S*)-1-(((1*S*)-2-((2*S*)-2-(4-(3-(2-(2-(2-(2-(2-((3-cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenoxy)ethoxy)ethoxy)ethoxy)ethoxy)benzoyl)-1,3-thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)methylcarbamate (41e).

Compound **41e** was prepared from **36e** and **19** in a similar manner to that described for compound **41a** and obtained as a colorless amorphous powder (76%). ^{1}H NMR (300 MHz,

CD_3OD) δ 0.94–1.38 (8H, m), 1.40–1.49 (9H, m), 1.51–1.84 (6H, m), 2.04–2.43 (10H, m), 2.65–2.90 (3H, m), 3.60–3.74 (8H, m), 3.78–4.01 (6H, m), 4.05–4.24 (5H, m), 4.42–4.68 (2H, m), 5.17 (2H, s), 5.42–5.66 (1H, m), 6.42 (1H, dd, *J* = 7.5, 0.9 Hz), 6.87 (1H, t, *J* = 7.4 Hz), 7.00 (1H, d, *J* = 8.2 Hz), 7.17 (1H, dd, *J* = 7.8, 2.1 Hz), 7.20–7.30 (1H, m), 7.38 (1H, t, *J* = 7.9 Hz), 7.50–7.59 (2H, m), 7.64–7.79 (4H, m); MS *m/z* 1084.9 $(M+H)^+$.

1-46.

***N*-((1*S*)-2-((2*S*)-2-(4-(3-(2-(2-(2-(2-(4-((3-Cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenoxy)ethoxy)ethoxy)ethoxy)ethoxy)benzoyl)-1,3-thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)-*N*^{2}-methyl-L-alaninamide (42a).**

A mixture of **41a** (225 mg, 0.208 mmol) in TFA (3 mL) was stirred at room temperature for 10 min. The mixture was diluted with toluene (5 mL), and concentrated *in vacuo*. The residue was dissolved in EtOAc washed with sat. aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on NH silica gel (eluted with 0–10% MeOH in EtOAc) to give the title compound (143 mg,

70%) as a colorless solid. ^{1}H NMR (300 MHz, CD_3OD) δ 0.98–1.38 (8H, m), 1.49–1.84 (6H, m), 2.03–2.42 (13H, m), 3.14 (1H, q, *J* = 6.8 Hz), 3.59–3.73 (8H, m), 3.75–4.02 (6H, m), 4.02–4.08 (2H, m), 4.15 (2H, dd, *J* = 5.4, 3.8 Hz), 4.45–4.61 (1H, m), 5.12 (2H, s), 5.41–5.67 (1H, m), 6.83–6.92 (4H, m), 7.19 (1H, ddd, *J* = 8.2, 2.5, 0.9 Hz), 7.34–7.44 (1H, m), 7.51–7.59 (2H, m), 7.65–7.72 (2H, m), 7.73–7.82 (2H, m), 8.25–8.30 (1H, m); MS *m/z* 984.6 $(M+H)^+$; HRMS *m/z* $(M+H)^+$ Calcd for $C_{55}H_{66}N_7O_8S$: 984.4694, Found: 984.4657; Purity: 98.3% (HPLC); $[\alpha]_D^{25}$ −77.5 (*c* 0.115, methanol).

1-47.

***N*-((1*S*)-2-((2*S*)-2-(4-(3-(2-(2-(2-(2-(3-((3-Cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenoxy)ethoxy)ethoxy)ethoxy)benzoyl)-1,3-thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)-*N*^{2}-methyl-L-alaninamide (42b).**

Compound **42b** was prepared from **41b** in a similar manner to that described for compound **42a** and obtained as a colorless amorphous powder (85%). ^{1}H NMR (300 MHz, CD_3OD) δ 0.96–1.40 (8H, m), 1.50–1.86 (6H, m), 2.03–2.43 (13H, m), 2.88–3.21 (1H, m), 3.57–4.21

(18H, m), 4.46–4.64 (1H, m), 5.17 (2H, s), 5.41–5.67 (1H, m), 6.46–6.58 (2H, m), 6.83 (1H, dd, *J* = 8.1, 2.1 Hz), 7.14–7.31 (2H, m), 7.34–7.45 (1H, m), 7.56 (2H, d, *J* = 8.5 Hz), 7.65–7.73 (2H, m), 7.76 (2H, d, *J* = 8.5 Hz), 8.23–8.35 (1H, m); MS *m/z* 984.4 $(M+H)^+$; HRMS *m/z* $(M+H)^+$ Calcd for $C_{55}H_{66}N_7O_8S$: 984.4694, Found: 984.4625; Purity: 100% (HPLC); $[\alpha]_D^{25}$ −75.3 (*c* 0.202, methanol).

1-48.

***N*-((1*S*)-2-((2*S*)-2-(4-(3-(2-(2-(2-(4-((3-Cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenoxy)ethoxy)ethoxy)ethoxy)benzoyl)-1,3-thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)-*N*^{2}-methyl-L-alaninamide (42c).**

Compound **42c** was prepared from **41c** in a similar manner to that described for compound **42a** and obtained as a colorless amorphous powder (85%). ^{1}H NMR (300 MHz, CD_3OD) δ 0.97–1.39 (8H, m), 1.46–1.86 (6H, m), 2.02–2.41 (13H, m), 3.14 (1H, q, *J* = 6.8 Hz), 3.71 (4H, s), 3.76–4.22 (10H, m), 4.47–4.61 (1H, m), 5.12 (2H, s), 5.41–5.67 (1H, m), 6.84–6.93 (4H, m), 7.15–7.23 (1H, m), 7.39 (1H, t, *J* = 8.0 Hz), 7.55 (2H, d, *J* = 8.4 Hz), 7.66–7.73

(2H, m), 7.76 (2H, d, *J* = 8.5 Hz), 8.23–8.32 (1H, m); MS *m/z* 940.6 $(M+H)^+$; HRMS *m/z* $(M+H)^+$ Calcd for $C_{53}H_{62}N_7O_7S$: 940.4431, Found: 940.4383; Purity: 100% (HPLC); $[\alpha]_D^{25}$ −73.8 (*c* 0.200, methanol).

1-49.

***N*-((1*S*)-2-((2*S*)-2-(4-(3-((14-(4-((3-Cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenoxy)-3,6,9,12-tetraoxatetradec-1-yl)oxy)benzoyl)-1,3-thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)-*N*^{2}-methyl-L-alaninamide (42d).**

Compound **42d** was prepared from **41d** in a similar manner to that described for compound **42a** and obtained as a colorless amorphous powder (90%). ^{1}H NMR (300 MHz, CD_3OD) δ 0.99–1.26 (8H, m), 1.54–1.82 (6H, m), 2.06–2.41 (13H, m), 3.14 (1H, q, *J* = 6.8 Hz), 3.59–3.71 (12H, m), 3.76–4.03 (6H, m), 4.07 (2H, dd, *J* = 5.5, 3.7 Hz), 4.17 (2H, dd, *J* = 5.4, 3.8 Hz), 4.49–4.59 (1H, m), 5.14 (2H, s), 5.43–5.65 (1H, m), 6.88–6.93 (4H, m), 7.17–7.23 (1H, m), 7.37–7.44 (1H, m), 7.53–7.59 (2H, m), 7.67–7.73 (2H, m), 7.73–7.80 (2H, m), 8.27–8.31 (1H, m). NH protons were omitted. MS *m/z* 1028.3 $(M+H)^+$; HRMS *m/z* $(M+H)^+$

Calcd for $C_{57}H_{70}N_7O_9S$: 1028.4956, Found: 1028.4945; Purity: 98.2% (HPLC); $[\alpha]_D^{25}$ −73.7 (*c* 0.200, methanol).

1-50.

***N*-((1*S*)-2-((2*S*)-2-(4-(3-(2-(2-(2-(2-(2-((3-Cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenoxy)ethoxy)ethoxy)ethoxy)ethoxy)benzoyl)-1,3-thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)-*N*^{2}-methyl-L-alaninamide (42e).**

Compound **42e** was prepared from **41e** in a similar manner to that described for compound **42a** and obtained as a colorless amorphous powder (87%). ^{1}H NMR (300 MHz, CD_3OD) δ 0.96–1.38 (8H, m), 1.51–1.84 (6H, m), 2.02–2.40 (13H, m), 2.85–3.20 (1H, m), 3.60–4.03 (14H, m), 4.09–4.25 (4H, m), 4.45–4.60 (1H, m), 5.17 (2H, s), 5.42–5.67 (1H, m), 6.42 (1H, dd, *J* = 7.4, 0.9 Hz), 6.87 (1H, t, *J* = 7.5 Hz), 7.00 (1H, d, *J* = 8.1 Hz), 7.17 (1H, dd, *J* = 8.0, 2.1 Hz), 7.24 (1H, td, *J* = 7.8, 1.4 Hz), 7.33–7.44 (1H, m), 7.49–7.58 (2H, m), 7.64–7.79 (4H, m), 8.24–8.30 (1H, m); MS *m/z* 984.4 $(M+H)^+$; HRMS *m/z* $(M+H)^+$ Calcd for

$C_{55}H_{66}N_7O_8S$: 984.4694, Found: 984.4626; Purity: 100% (HPLC); $[\alpha]_D^{25}$ −74.8 (*c* 0.205, methanol).

1-51. 2-(4-(2-Hydroxyethoxy)phenoxy)ethyl 4-methylbenzenesulfonate (44a).

To a mixture of **43a** (4.00 g, 20.2 mmol) in pyridine (50 mL) was added TsCl (3.85 g, 20.2 mmol) at 0 °C. The mixture was stirred at 0 °C for 2 h. The mixture was diluted with EtOAc, neutralized with 6 M HCl at 0 °C, and extracted with EtOAc. The organic layer was separated, washed with 6 M HCl, 5% aq. $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (eluted with 50–100% EtOAc in hexane) to give the title compound (3.68 g, 52%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.98–2.05 (1H, m), 2.45 (3H, s), 3.88–4.13 (6H, m), 4.34 (2H, dd, *J* = 5.6, 4.0 Hz), 6.67–6.89 (4H, m), 7.34 (2H, d, *J* = 8.2 Hz), 7.82 (2H, d, *J* = 8.3 Hz).

1-52. 2-(3-(2-Hydroxyethoxy)phenoxy)ethyl 4-methylbenzenesulfonate (44b).

Compound **44b** was prepared from **43b** in a similar manner to that described for compound **44a** and obtained as a colorless oil (56%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.97–2.03 (1H, m), 2.45 (3H, s), 3.89–4.20 (6H, m), 4.36 (2H, dd, *J* = 5.6, 3.9 Hz), 6.34–6.46 (2H, m), 6.49–6.59 (1H, m), 7.15 (1H, t, *J* = 8.1 Hz), 7.34 (2H, d, *J* = 8.1 Hz), 7.82 (2H, d, *J* = 8.3 Hz); MS *m/z* 353.2 $(M+H)^+$.

1-53. 2-(2-(2-Hydroxyethoxy)phenoxy)ethyl 4-methylbenzenesulfonate (44c).

Compound **44c** was prepared from **43c** in a similar manner to that described for compound **44a** and obtained as a colorless amorphous solid (55%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.44 (3H, s), 2.66 (1H, t, *J* = 6.5 Hz), 3.86–3.97 (2H, m), 4.05–4.12 (2H, m), 4.22 (2H, dd, *J* = 5.6, 3.7 Hz), 4.39 (2H, dd, *J* = 5.7, 3.8 Hz), 6.78–7.01 (4H, m), 7.34 (2H, d, *J* = 8.0 Hz), 7.82 (2H, d, *J* = 8.4 Hz); MS *m/z* 353.2 $(M+H)^+$.

1-54.

4-(4-Cyanophenyl)-1-(4-(2-(4-(2-hydroxyethoxy)phenoxy)ethoxy)benzyl)-2,5-dimethyl-1*H*-pyrrole-3-carbonitrile (45a).

A mixture of **33a** (327 mg, 1.00 mmol), **44a** (423 mg, 1.20 mmol), and K_2CO_3 (193 mg, 1.40 mmol) in DMF (4 mL) was stirred at 60 °C overnight. After cooling, the mixture was diluted with EtOAc, washed with 5% aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (eluted with 50–100% EtOAc in hexane) to give the title compound (418 mg, 82%) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.04 (1H, t, *J* = 6.0 Hz), 2.20 (3H, s), 2.38 (3H, s), 3.88–4.08 (4H, m), 4.28 (4H, s), 5.05 (2H, s), 6.79–6.99 (8H, m), 7.52 (2H, d, *J* = 8.4 Hz), 7.70 (2H, d, *J* = 8.4 Hz); MS *m/z* 508.3 $(M+H)^+$.

1-55.

4-(4-Cyanophenyl)-1-(4-(2-(3-(2-hydroxyethoxy)phenoxy)ethoxy)benzyl)-2,5-dimethyl-1*H*-pyrrole-3-carbonitrile (45b).

Compound **45b** was prepared from **44b** and **33a** in a similar manner to that described for compound **45a** and obtained as a colorless amorphous solid (90%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.01 (1H, t, *J* = 6.1 Hz), 2.20 (3H, s), 2.38 (3H, s), 3.89–4.00 (2H, m), 4.02–4.10

(2H, m), 4.30 (4H, s), 5.05 (2H, s), 6.49–6.61 (3H, m), 6.83–6.99 (4H, m), 7.14–7.24 (1H, m), 7.52 (2H, d, J = 8.3 Hz), 7.70 (2H, d, J = 8.4 Hz); MS *m/z* 508.3 $(M+H)^+$.

1-56.

4-(4-Cyanophenyl)-1-(4-(2-(2-(2-hydroxyethoxy)phenoxy)ethoxy)benzyl)-2,5-dimethyl-1*H*-pyrrole-3-carbonitrile (45c).

Compound **45c** was prepared from **44c** and **33a** in a similar manner to that described for compound **45a** and obtained as a colorless amorphous solid (99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.20 (3H, s), 2.38 (3H, s), 2.79 (1H, t, J = 6.6 Hz), 3.85 (2H, dt, J = 6.3, 4.6 Hz), 4.07–4.17 (2H, m), 4.28–4.41 (4H, m), 5.05 (2H, s), 6.82–7.02 (8H, m), 7.49–7.57 (2H, m), 7.67–7.74 (2H, m); MS *m/z* 508.4 $(M+H)^+$.

1-57.

2-(4-(2-(4-((3-Cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenoxy)ethoxy)phenoxy)ethyl 4-methylbenzenesulfonate (46a).

To a mixture of **45a** (0.42 g, 0.82 mmol) in pyridine (6 mL) was added TsCl (471 mg, 2.47 mmol) at 0 °C. The mixture was stirred at room temperature for 2 h. The mixture was poured into 5% aq. $NaHCO_3$ and extracted with EtOAc. The organic layer was separated, washed with 5% aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (eluted with 30–80% EtOAc in hexane) to give the title compound (450 mg, 83%) as a colorless amorphous powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.20 (3H, s), 2.38 (3H, s), 2.45 (3H, s), 4.11 (2H, dd, *J* = 5.6, 3.9 Hz), 4.20–4.30 (4H, m), 4.33 (2H, dd, *J* = 5.6, 3.9 Hz), 5.05 (2H, s), 6.68–6.98 (8H, m), 7.34 (2H, d, *J* = 8.2 Hz), 7.52 (2H, d, *J* = 8.3 Hz), 7.66–7.74 (2H, m), 7.81 (2H, d, *J* = 8.3 Hz); MS *m/z* 662.4 $(M+H)^+$.

1-58.

2-(3-(2-(4-((3-Cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenoxy) ethoxy)phenoxy)ethyl 4-methylbenzenesulfonate (46b).

Compound **46b** was prepared from **45b** in a similar manner to that described for compound **46a** and obtained as a colorless amorphous solid (87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.20 (3H, s), 2.38 (3H, s), 2.44 (3H, s), 4.09–4.15 (2H, m), 4.29 (4H, s), 4.35 (2H, dd, *J* = 5.6, 3.9 Hz), 5.05 (2H, s), 6.37–6.46 (2H, m), 6.51–6.59 (1H, m), 6.81–6.99 (4H, m), 7.08–7.22 (1H, m), 7.34 (2H, d, *J* = 8.1 Hz), 7.52 (2H, d, *J* = 8.2 Hz), 7.70 (2H, d, *J* = 8.2 Hz), 7.81 (2H, d, *J* = 8.2 Hz); MS *m/z* 662.4 $(M+H)^+$.

1-59.

2-(2-(2-(4-((3-Cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenoxy) ethoxy)phenoxy)ethyl 4-methylbenzenesulfonate (46c).

Compound **46c** was prepared from **45c** in a similar manner to that described for compound **46a** and obtained as a colorless amorphous solid (85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.21 (3H, s), 2.38 (3H, s), 2.41 (3H, s), 4.17–4.24 (2H, m), 4.27–4.38 (6H, m), 5.06 (2H, s), 6.78–7.02 (8H, m), 7.29 (2H, d, *J* = 7.9 Hz), 7.49–7.56 (2H, m), 7.65–7.73 (2H, m), 7.74–7.82 (2H, m); MS *m/z* 662.5 $(M+H)^+$.

1-60. *tert*-Butyl ((2*S*)-1-(((1*S*)-2-((2*S*)-2-(4-(3-(2-(4-(2-(4-((3-cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenoxy)ethoxy)phenoxy)ethoxy)benzoyl)-1,3-thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)methylcarbamate (47a).

A mixture of **46a** (0.45 g, 0.68 mmol), **19** (0.41 g, 0.68 mmol), and K_2CO_3 (113 mg, 0.818 mmol) in DMF (3 mL) was stirred at room temperature overnight and then heated at 50 °C for 24 h. After cooling, the mixture was diluted with EtOAc, washed with 5% aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (eluted with 50–100% EtOAc in hexane), and then on NH silica gel (eluted with 50–100% EtOAc in hexane) to give the title compound (546 mg, 74%) as a colorless solid. ^{1}H NMR (300 MHz, CD_3OD) δ 0.96–1.37 (8H, m), 1.38–1.48 (9H, m), 1.49–1.83 (6H, m), 2.03–2.46 (10H, m), 2.62–2.93 (3H, m), 3.75–4.42 (10H, m), 4.43–4.73 (2H, m), 5.17 (2H, s), 5.38–5.68 (1H, m), 6.80–7.05 (8H, m), 7.25 (1H, dd, J = 8.3, 1.5 Hz), 7.44 (1H, t, J = 8.0 Hz), 7.57 (2H, d, J = 8.2 Hz), 7.66–7.88 (4H, m), 8.31 (1H, s); MS *m/z* 1088.8 $(M+H)^+$.

1-61. *tert*-Butyl ((2*S*)-1-(((1*S*)-2-((2*S*)-2-(4-(3-(2-(3-(2-(4-((3-cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenoxy)ethoxy)phenoxy)ethoxy)benzoyl)-1,3-thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)methylcarbamate (47b).

Compound **47b** was prepared from **46b** and **19** in a similar manner to that described for compound **47a** and obtained as a colorless amorphous solid (82%). ^{1}H NMR (300 MHz, CD_3OD) δ 0.94–1.38 (8H, m), 1.39–1.48 (9H, m), 1.49–1.82 (6H, m), 2.03–2.41 (10H, m), 2.63–2.91 (3H, m), 3.74–4.01 (2H, m), 4.21–4.66 (10H, m), 5.16 (2H, s), 5.39–5.66 (1H, m), 6.51–6.63 (3H, m), 6.87–7.00 (4H, m), 7.12–7.21 (1H, m), 7.25 (1H, dd, *J* = 8.3, 2.4 Hz), 7.39–7.49 (1H, m), 7.57 (2H, d, *J* = 8.4 Hz), 7.67–7.86 (4H, m), 8.31 (1H, s); MS *m/z* 1088.8 $(M+H)^+$.

1-62. *tert*-Butyl ((2*S*)-1-(((1*S*)-2-((2*S*)-2-(4-(3-(2-(2-(2-(4-((3-cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-

pyrrol-1-yl)methyl)phenoxy)ethoxy)phenoxy)ethoxy)benzoyl)-1,3-thiazol-2-yl)pyrrolid in-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)methylcarbamate (47c).

Compound **47c** was prepared from **46c** and **19** in a similar manner to that described for compound **47a** and obtained as a colorless amorphous solid (79%). ^{1}H NMR (300 MHz, CD_3OD) δ 0.96–1.37 (8H, m), 1.38–1.48 (9H, m), 1.49–1.82 (6H, m), 2.03–2.38 (10H, m), 2.60–2.90 (3H, m), 3.71–4.00 (2H, m), 4.20–4.40 (8H, m), 4.43–4.68 (2H, m), 5.10 (2H, s), 5.38–5.65 (1H, m), 6.79–7.10 (8H, m), 7.19 (1H, dd, *J* = 8.0, 2.2 Hz), 7.36 (1H, t, *J* = 7.9 Hz), 7.50–7.60 (2H, m), 7.63–7.82 (4H, m), 8.23–8.29 (1H, m); MS *m/z* 1088.9 $(M+H)^+$.

1-63.

***N*-((1*S*)-2-((2*S*)-2-(4-(3-(2-(4-(2-(4-((3-Cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrr ol-1-yl)methyl)phenoxy)ethoxy)phenoxy)ethoxy)benzoyl)-1,3-thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)-*N*^{2}-methyl-L-alaninamide (48a).**

Compound **48a** was prepared from **47a** in a similar manner to that described for compound **42a** and obtained as a colorless amorphous powder (85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ

0.93–1.38 (8H, m), 1.51–1.89 (7H, m), 2.05–2.54 (13H, m), 2.83–3.12 (1H, m), 3.72–4.01 (2H, m), 4.25–4.42 (8H, m), 4.63 (1H, dd, J = 9.3, 6.2 Hz), 5.05 (2H, s), 5.55 (1H, dd, J = 7.7, 2.7 Hz), 6.82–6.99 (8H, m), 7.19 (1H, ddd, J = 8.3, 2.6, 0.9 Hz), 7.40 (1H, t, J = 8.0 Hz), 7.49–7.56 (2H, m), 7.66–7.73 (3H, m), 7.77 (1H, dd, J = 2.5, 1.5 Hz), 7.83 (1H, dt, J = 7.7, 1.1 Hz), 8.03–8.17 (1H, m); MS *m/z* 988.4 $(M+H)^+$; HRMS *m/z* $(M+H)^+$ Calcd for $C_{57}H_{62}N_7O_7S$: 988.4431, Found: 988.4403; Purity: 99.8% (HPLC); $[\alpha]_D^{25}$ −68.2 (*c* 0.201, methanol).

1-64.

***N*-((1*S*)-2-((2*S*)-2-(4-(3-(2-(3-(2-(4-((3-Cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenoxy)ethoxy)phenoxy)ethoxy)benzoyl)-1,3-thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)-*N*^{2}-methyl-L-alaninamide (48b).**

Compound **48b** was prepared from **47b** in a similar manner to that described for compound **42a** and obtained as a colorless amorphous solid (86%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92–1.40 (8H, m), 1.48–1.87 (7H, m), 2.06–2.54 (13H, m), 2.81–3.14 (1H, m), 3.71–4.01

(2H, m), 4.25–4.44 (8H, m), 4.62 (1H, dd, *J* = 9.2, 6.3 Hz), 5.05 (2H, s), 5.55 (1H, dd, *J* = 7.7, 2.7 Hz), 6.51–6.64 (3H, m), 6.81–6.98 (4H, m), 7.13–7.25 (2H, m), 7.40 (1H, t, *J* = 7.9 Hz), 7.48–7.56 (2H, m), 7.65–7.73 (3H, m), 7.76 (1H, dd, *J* = 2.4, 1.5 Hz), 7.84 (1H, dt, *J* = 7.7, 1.0 Hz), 8.04–8.17 (1H, m); MS *m/z* 988.4 $(M+H)^+$; HRMS *m/z* $(M+H)^+$ Calcd for $C_{57}H_{62}N_7O_7S$: 988.4431, Found: 988.4432; Purity: 97.8% (HPLC); $[\alpha]_D^{25}$ −71.0 (*c* 0.200, methanol).

1-65.

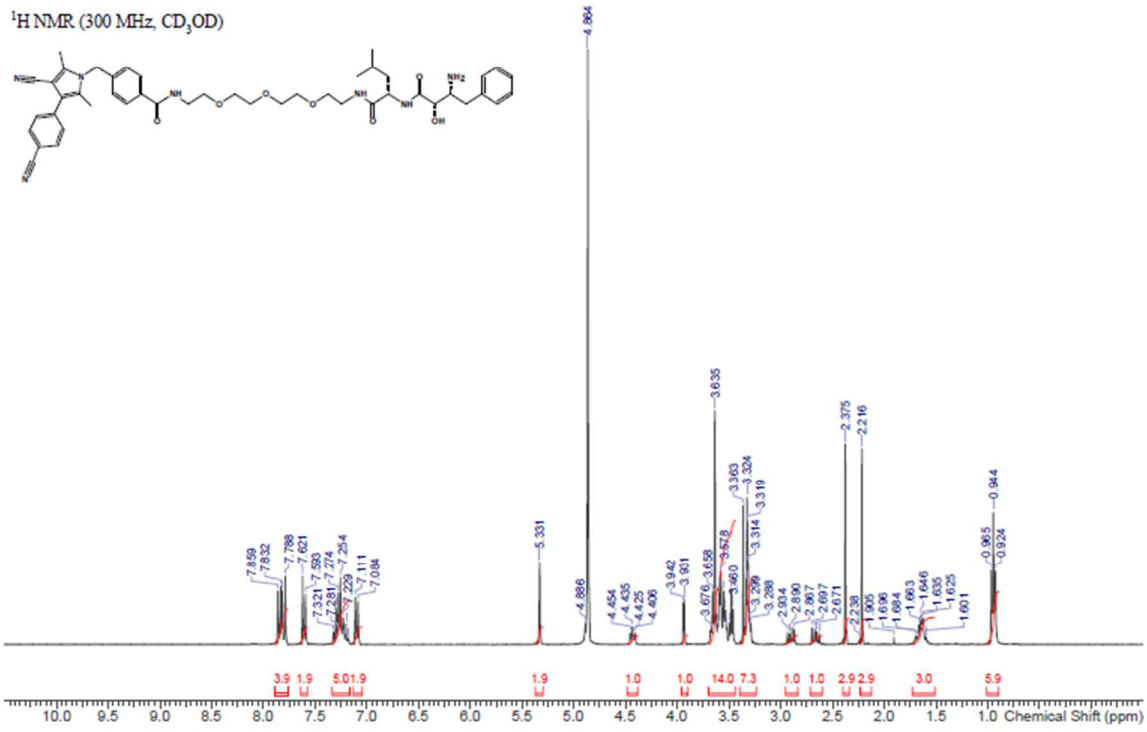
***N*-((1*S*)-2-((2*S*)-2-(4-(3-(2-(2-(2-(4-((3-Cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl)methyl)phenoxy)ethoxy)phenoxy)ethoxy)benzoyl)-1,3-thiazol-2-yl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)-*N*^{2}-methyl-L-alaninamide (48c).**

Compound **48c** was prepared from **47c** in a similar manner to that described for compound **42a** and obtained as a colorless amorphous solid (89%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93–1.39 (8H, m), 1.52–1.87 (7H, m), 2.06–2.54 (13H, m), 2.81–3.13 (1H, m), 3.70–4.02 (2H, m), 4.24–4.46 (8H, m), 4.63 (1H, dd, *J* = 9.3, 6.2 Hz), 5.01 (2H, s), 5.54 (1H, dd, *J* =

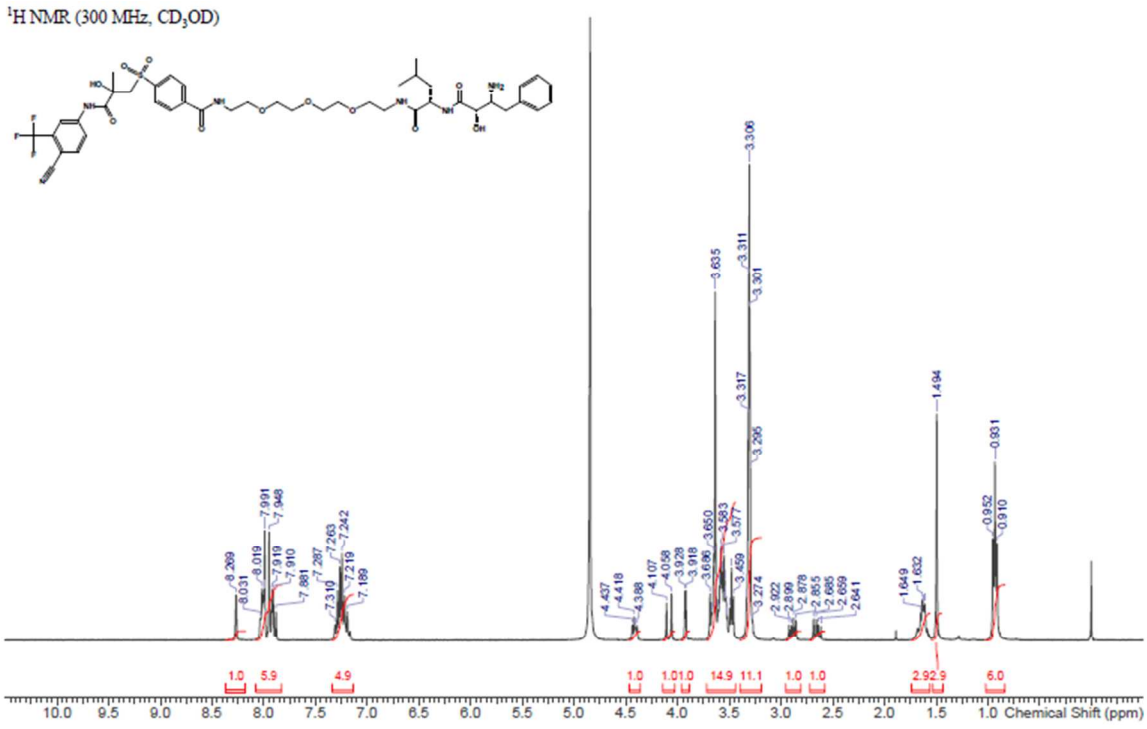
7.7, 2.6 Hz), 6.75–7.06 (8H, m), 7.16 (1H, ddd, J = 8.2, 2.6, 0.8 Hz), 7.36 (1H, t, J = 7.9 Hz), 7.47–7.57 (2H, m), 7.64–7.86 (5H, m), 8.02–8.15 (1H, m); MS m/z 988.4 $(M+H)^+$; HRMS m/z $(M+H)^+$ Calcd for $C_{57}H_{62}N_7O_7S$: 988.4431, Found: 988.4393; Purity: 99.6% (HPLC); $[\alpha]_D^{25}$ −72.7 (c 0.202, methanol).

1-66. NMR SPECTRA

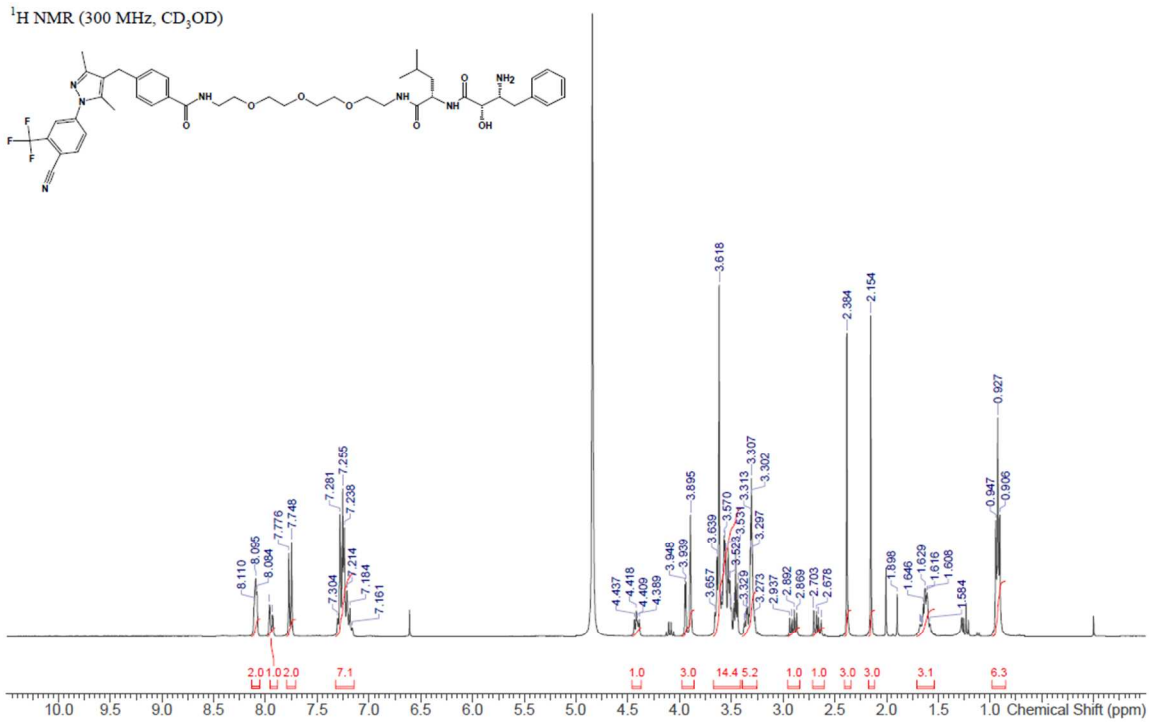
Compound 4a



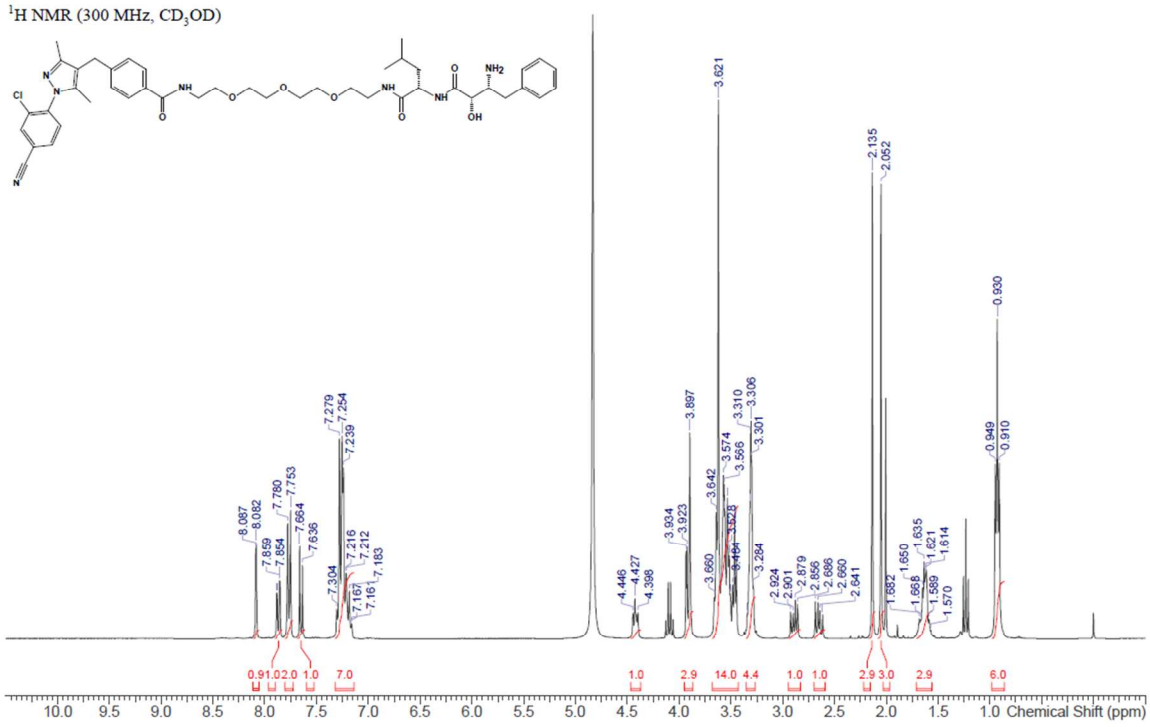
Compound 4b


^{1}H NMR (300 MHz, CD_3OD)
8.269
8.031
8.019
7.991
7.948
7.919
7.910
7.881
7.287
7.263
7.242
7.219
7.189
4.437
4.418
4.388
4.107
4.058
3.928
3.918
3.690
3.650
3.635
3.583
3.577
3.459
3.306
3.311
3.301
3.317
3.295
3.274
2.922
2.899
2.878
2.855
2.685
2.659
2.641
1.649
1.632
1.494
0.952
0.931
0.910
1.0 5.9 4.9 1.0 1.01.0 14.9 11.1 1.0 1.0 2.92.9 6.0
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 Chemical Shift (ppm)

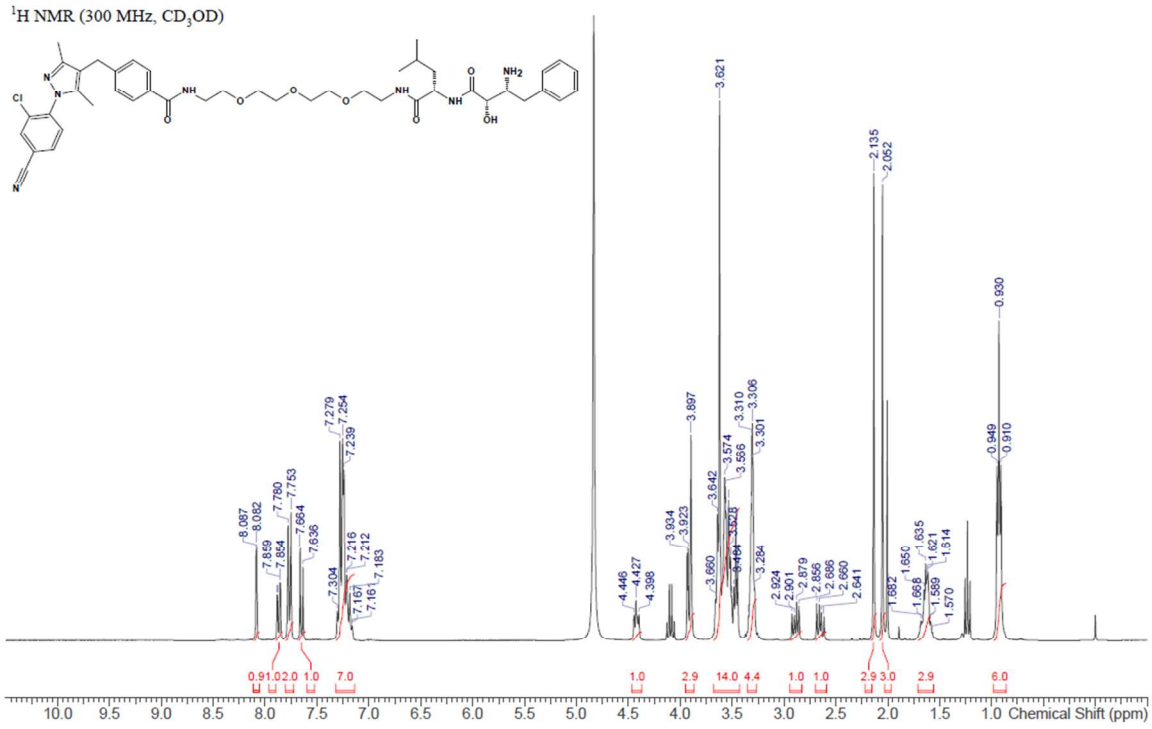
Compound 4c


^{1}H NMR (300 MHz, CD$_3$OD)
8.110
8.095
8.084
7.776
7.748
7.304
7.281
7.255
7.238
7.214
7.184
7.161
4.437
4.418
4.409
4.389
3.946
3.939
3.895
3.657
3.639
3.618
3.570
3.531
3.523
3.329
3.313
3.307
3.302
3.297
3.273
2.937
2.892
2.869
2.703
2.678
2.384
2.154
1.996
1.646
1.629
1.616
1.608
1.584
0.947
0.927
0.906
2.0 1.0 2.0 7.1 1.0 3.0 14.4 5.2 1.0 1.0 3.0 3.0 3.1 6.3
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 Chemical Shift (ppm)

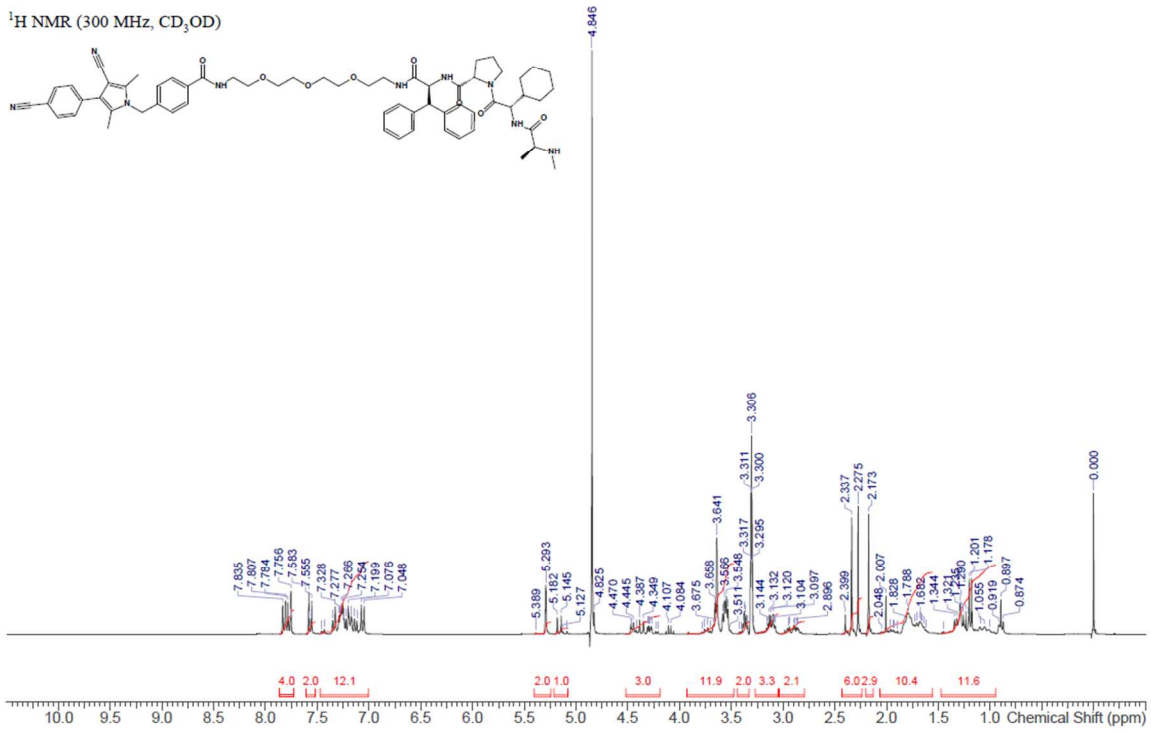
Compound 4d


^{1}H NMR (300 MHz, CD_3OD)
8.067
8.082
7.859
7.854
7.780
7.753
7.664
7.636
7.304
7.279
7.254
7.239
7.216
7.212
7.183
7.167
7.161
3.934
3.923
3.897
3.660
3.642
3.621
3.574
3.568
3.310
3.306
3.301
3.284
4.446
4.427
4.398
2.924
2.901
2.879
2.856
2.686
2.660
2.641
2.135
2.052
1.682
1.650
1.635
1.621
1.614
1.668
1.589
1.570
0.949
0.930
0.910
0.91 1.02.0 1.0 7.0
1.0 2.9 14.0 4.4 1.0 1.0 2.9 3.0 2.9 6.0
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 Chemical Shift (ppm)

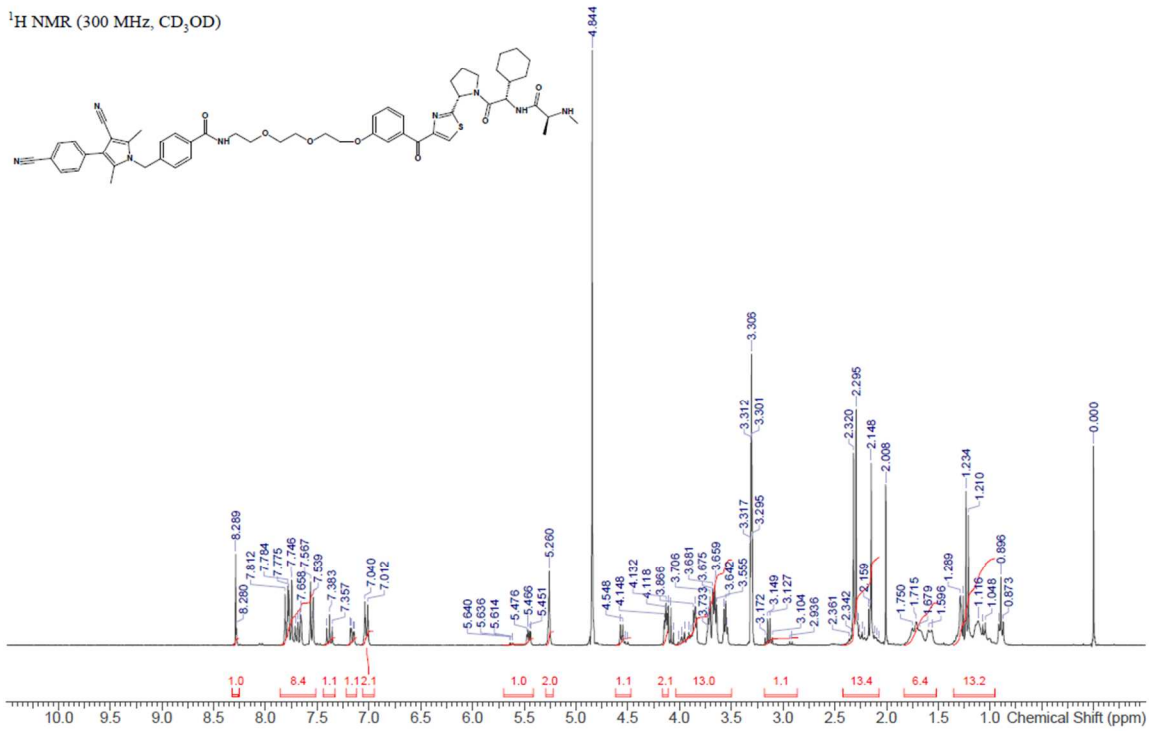
Compound 4e



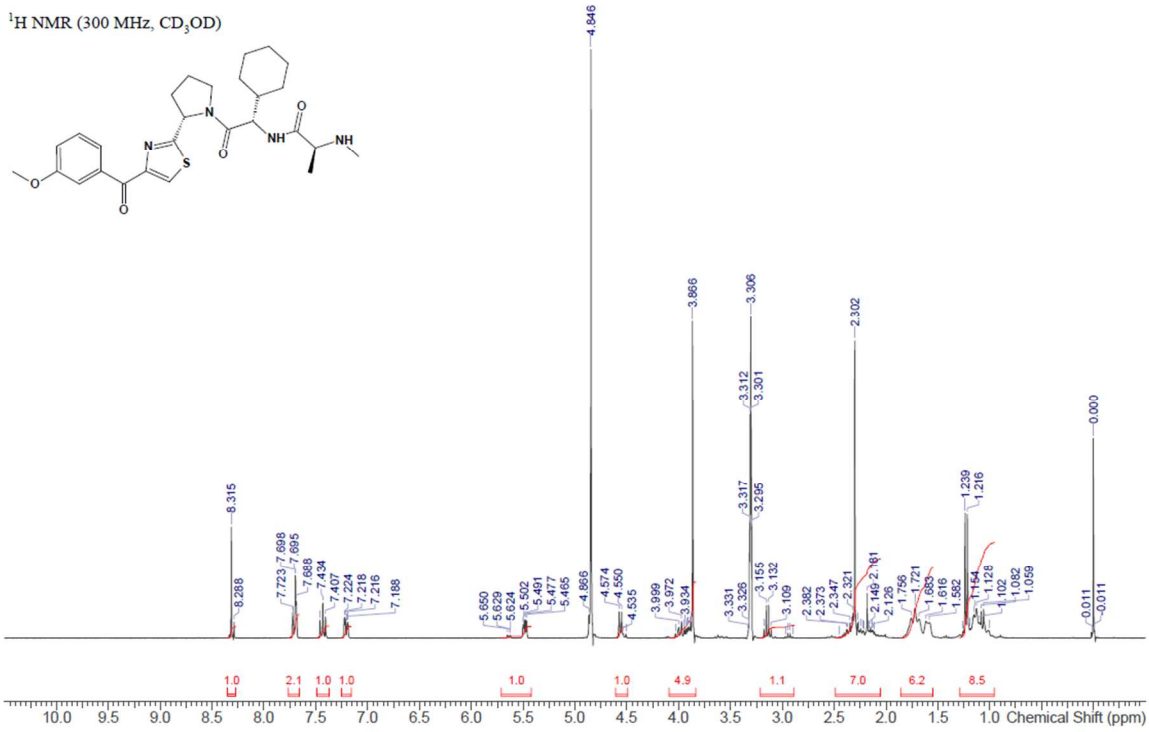
Compound 18


^{1}H NMR (300 MHz, CD$_3$OD)
4.846
3.306
3.311
3.300
3.641
3.317
3.295
2.337
2.275
2.173
0.000
7.835
7.807
7.784
7.756
7.583
7.565
7.328
7.277
7.266
7.254
7.199
7.076
7.046
5.389
5.293
5.182
5.145
5.127
4.825
4.470
4.445
4.397
4.349
4.107
4.084
3.675
3.658
3.566
3.548
3.511
3.144
3.132
3.120
3.104
3.097
2.896
2.399
2.048
2.007
1.828
1.788
1.682
1.344
1.201
1.178
1.055
0.919
0.897
0.874
4.0 2.0 12.1 2.0 1.0 3.0 11.9 2.0 3.3 2.1 6.0 2.9 10.4 11.6
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 Chemical Shift (ppm)

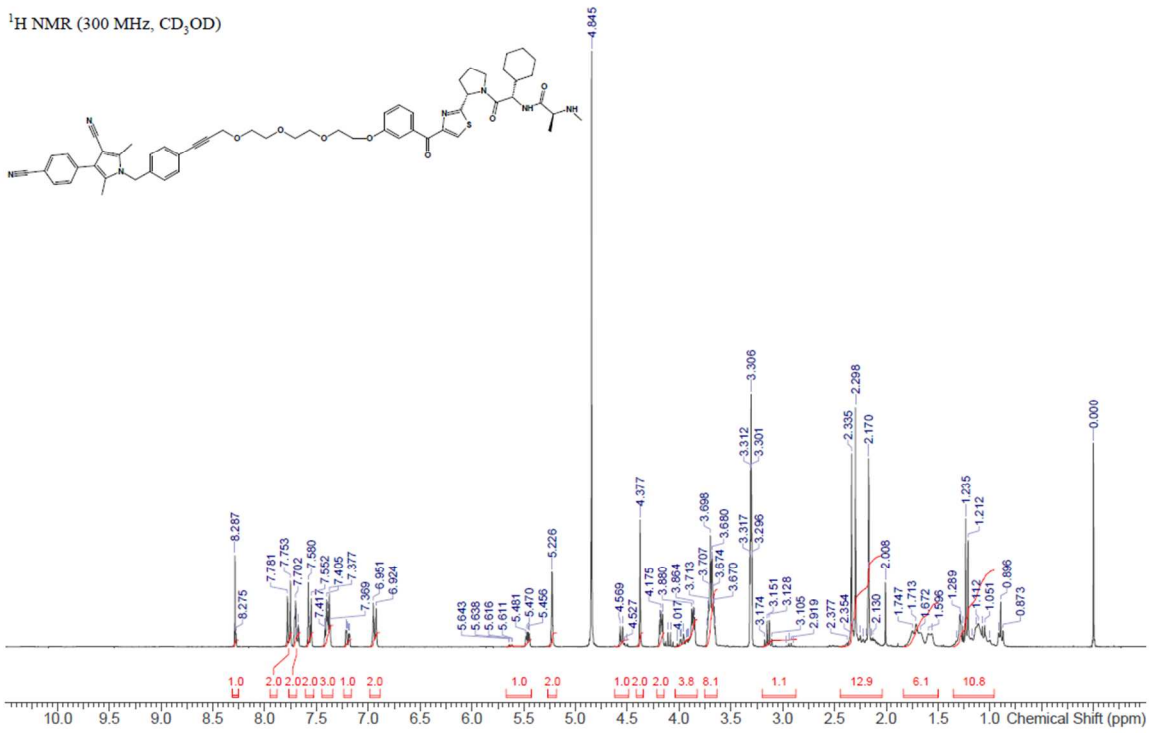
Compound 23



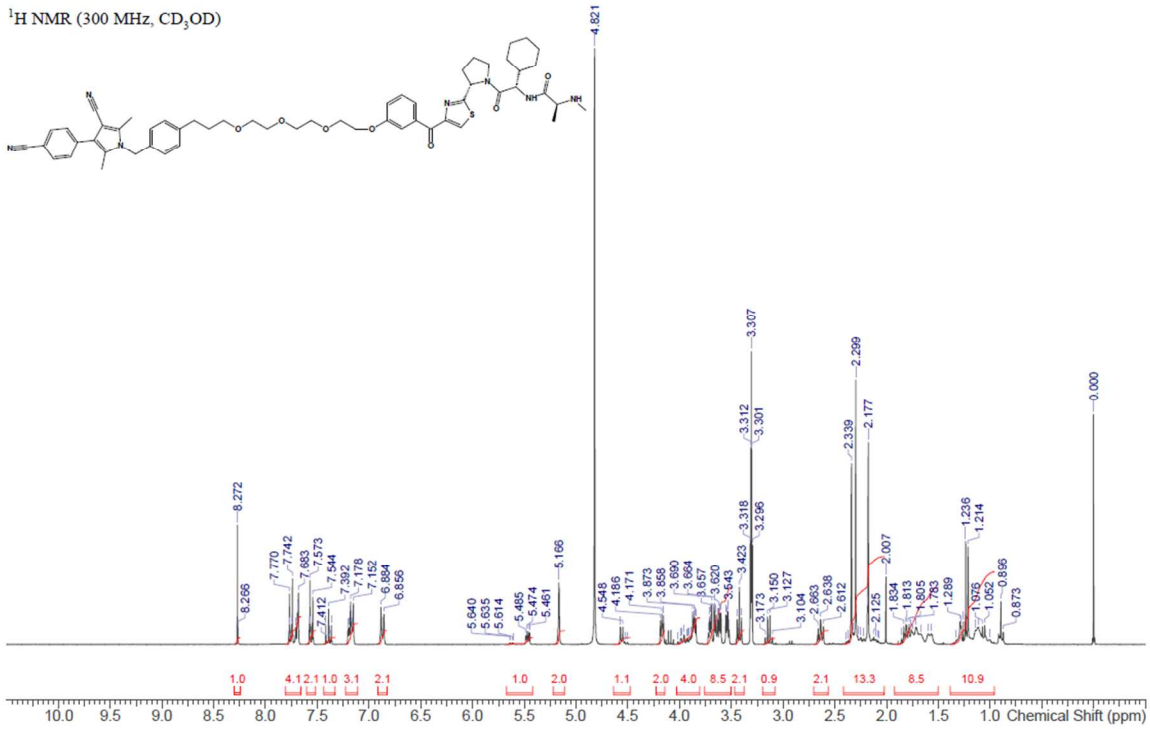
Compound 24



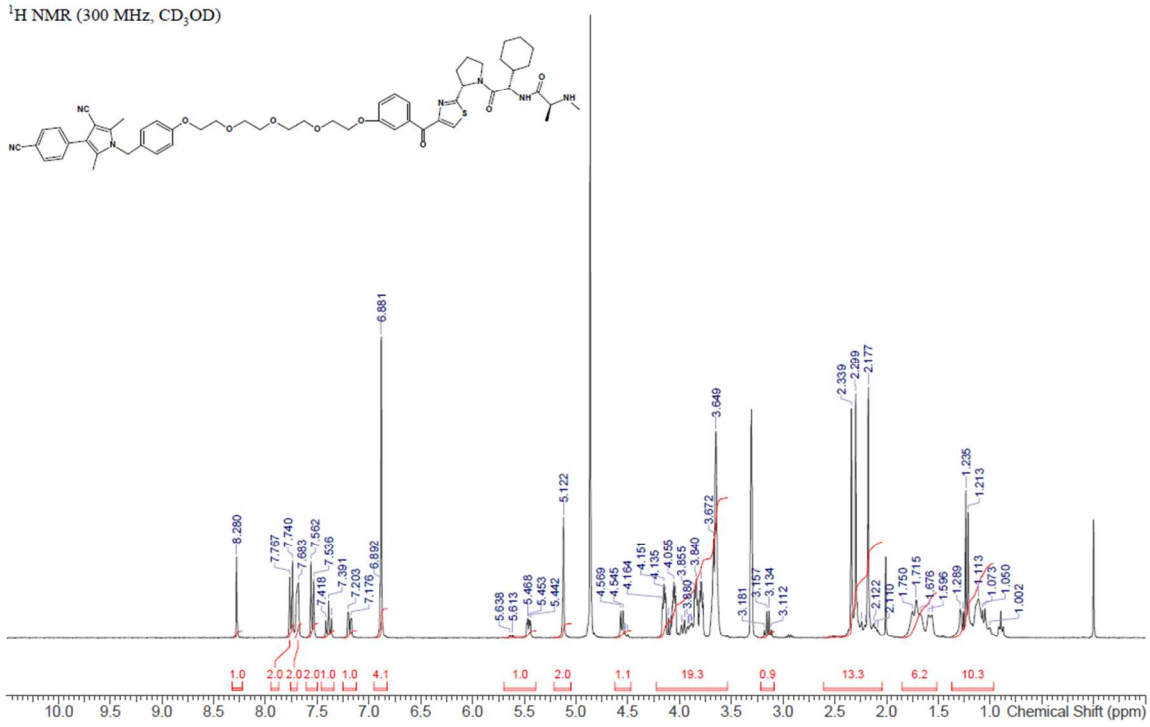
Compound 32a



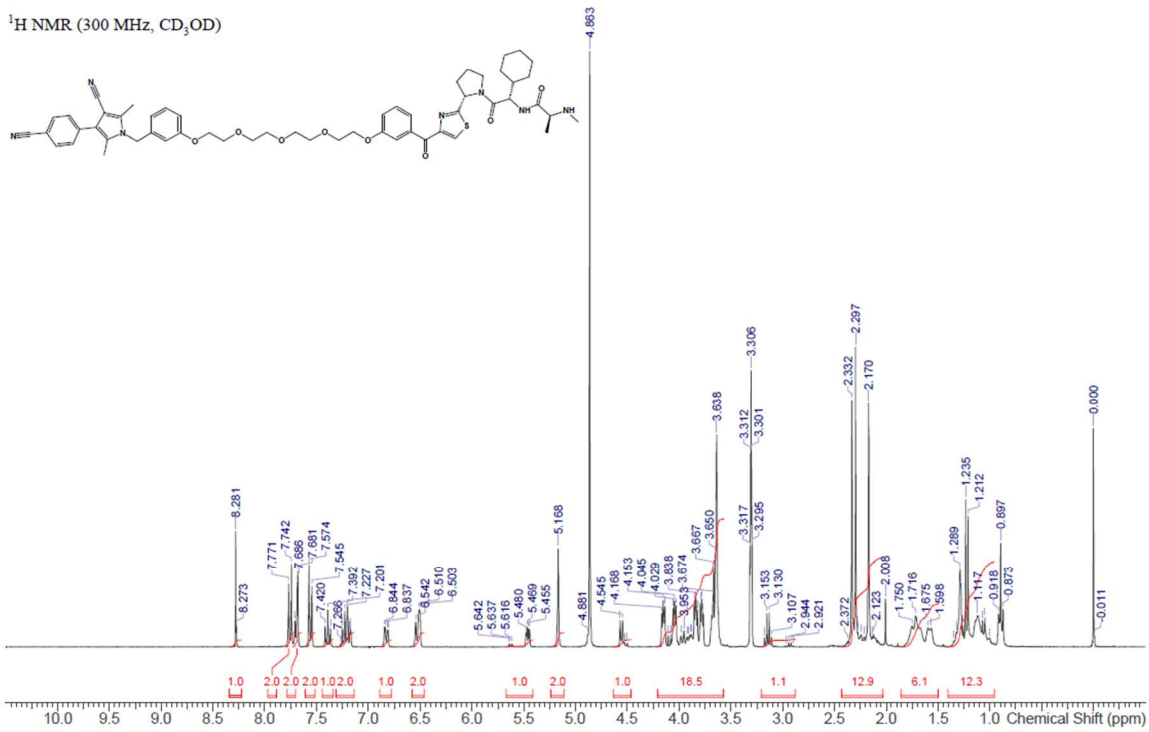
Compound 32b



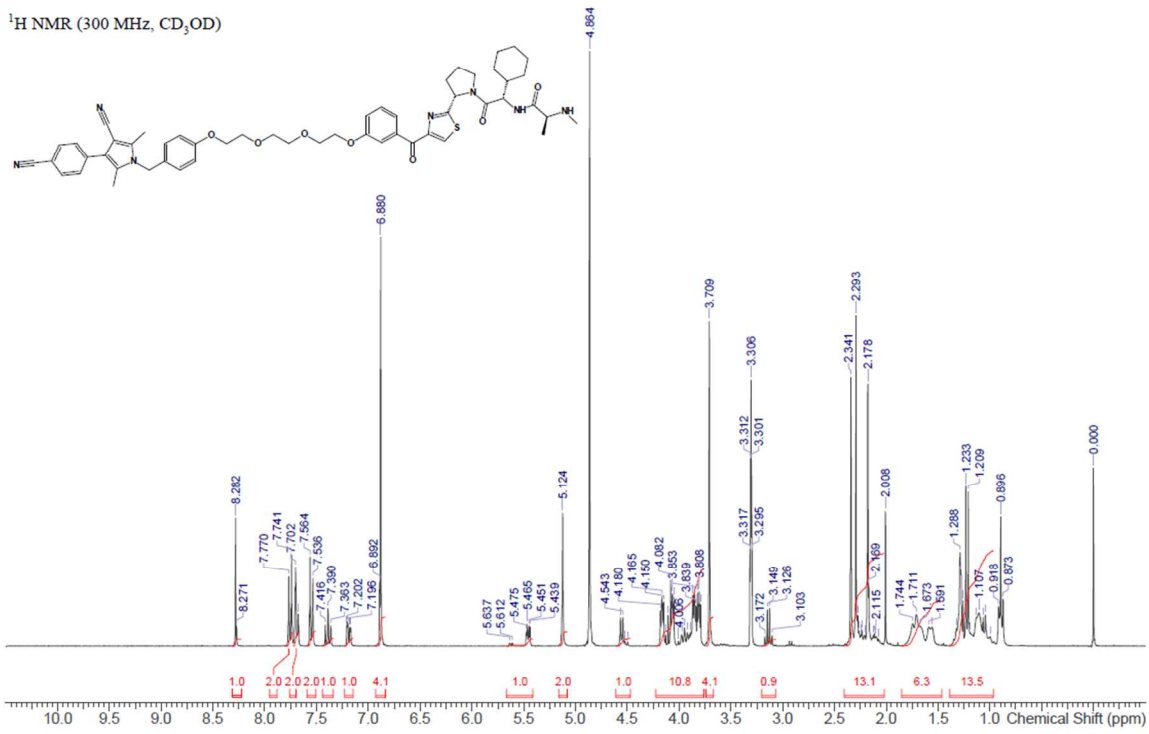
Compound 42a



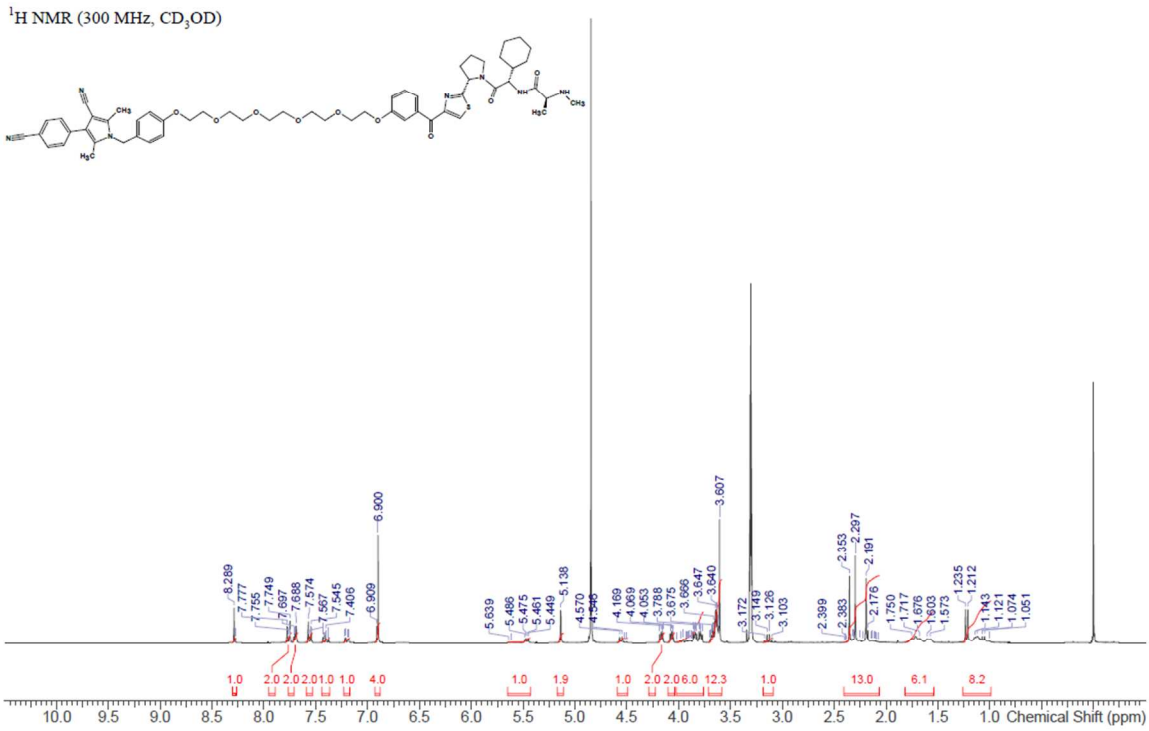
Compound 42b



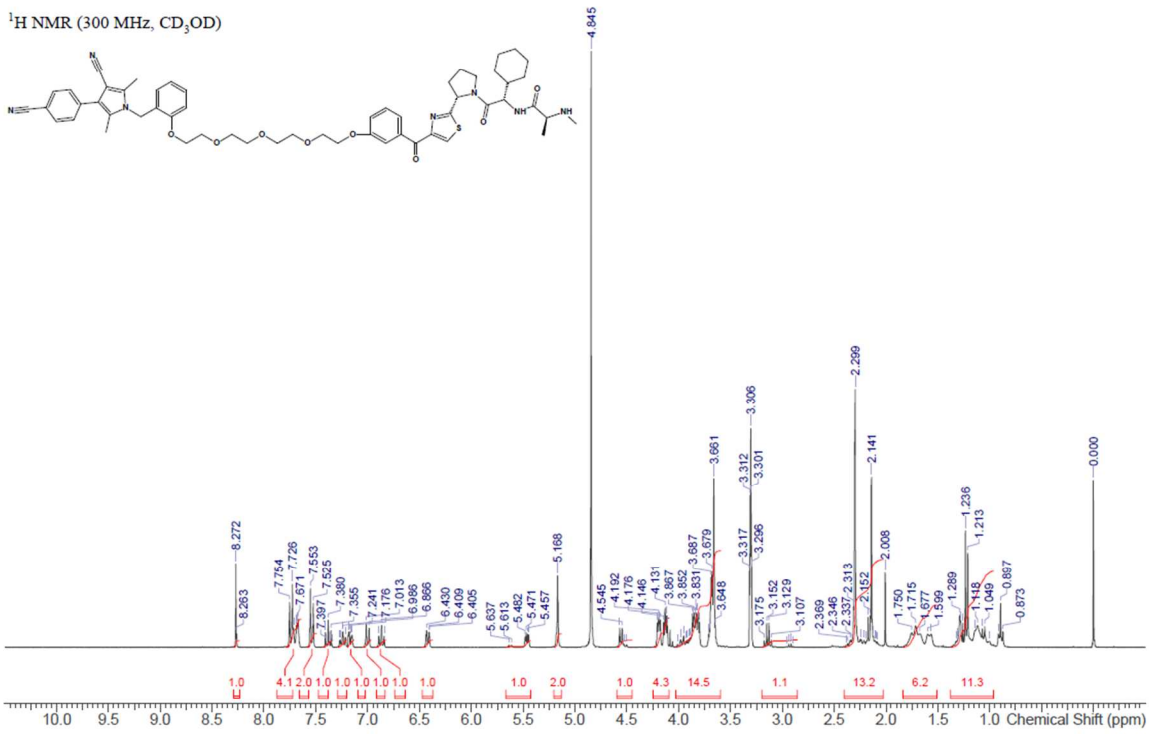
Compound 42c



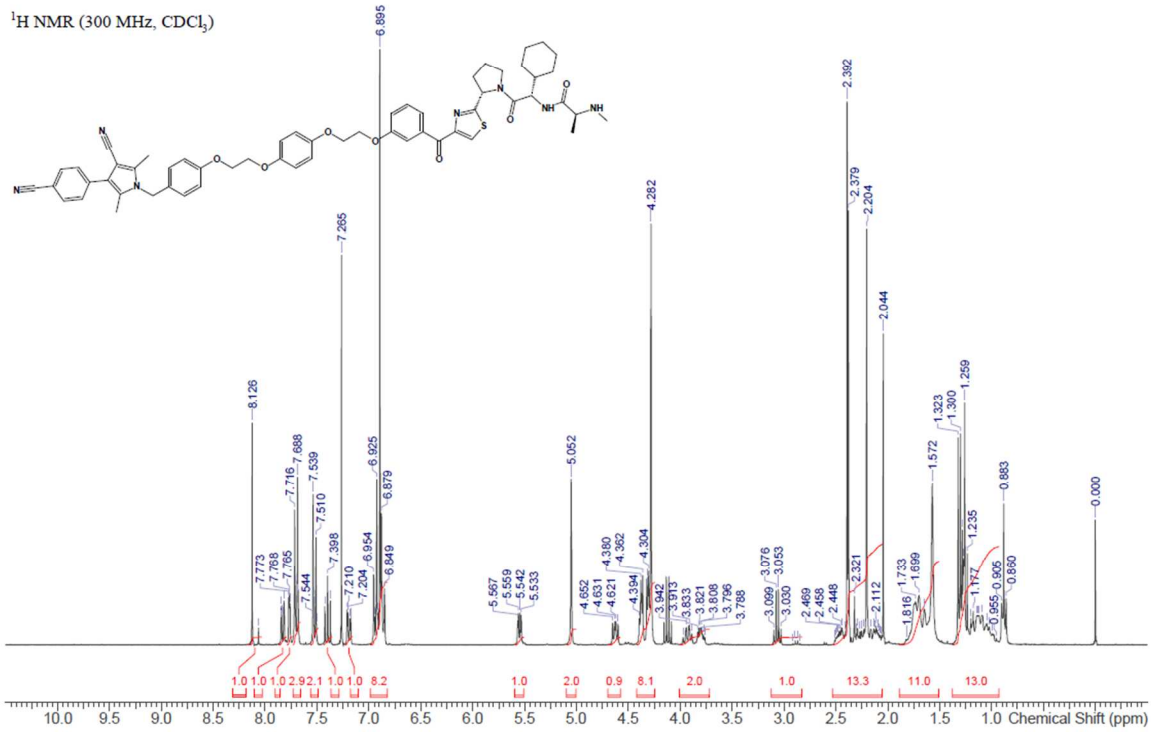
Compound 42d



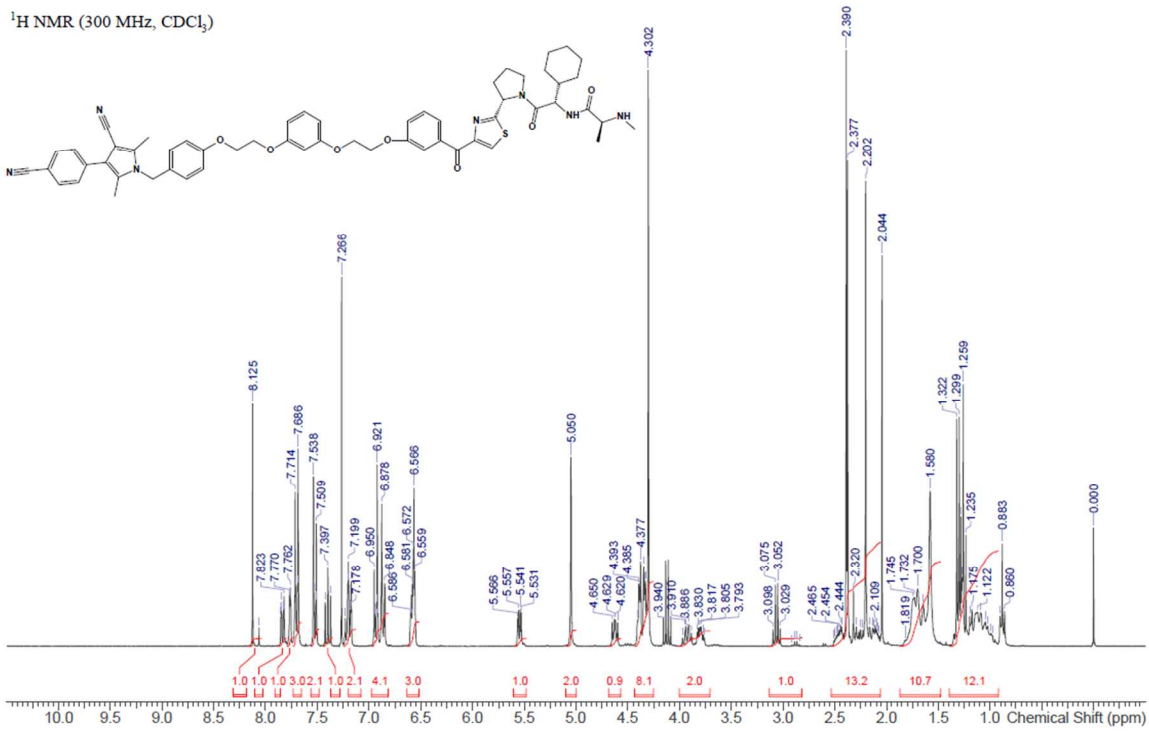
Compound 42e



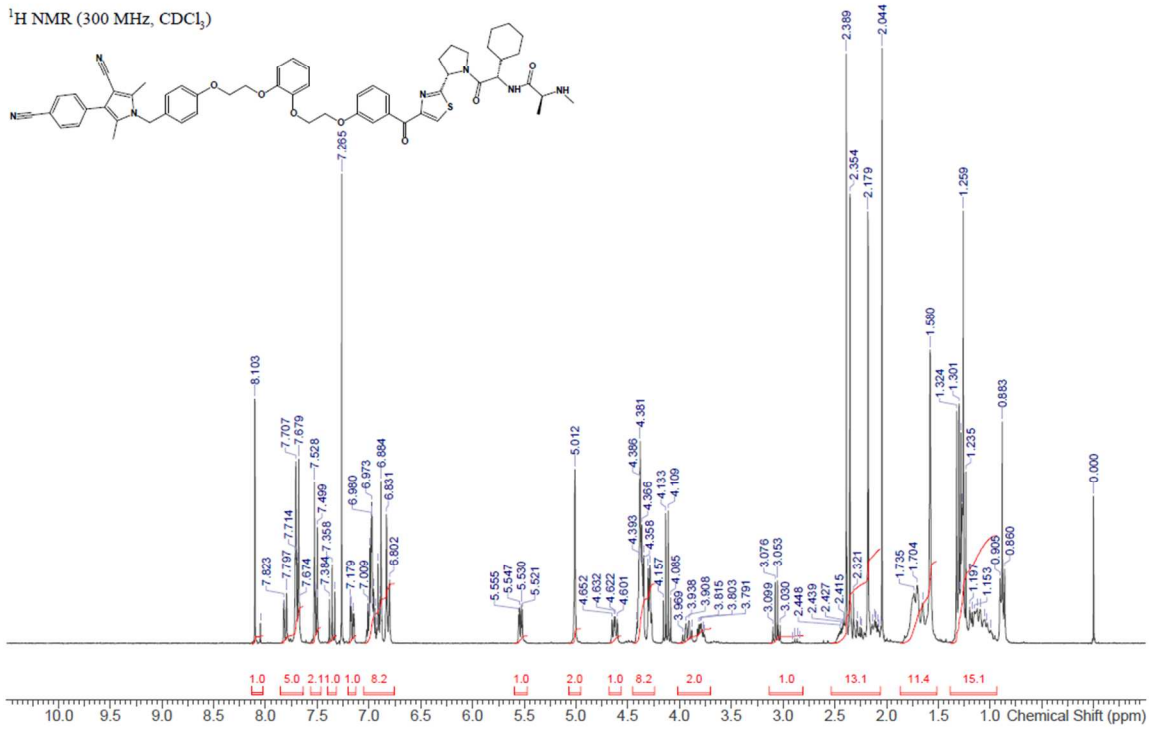
Compound 48a



Compound 48b

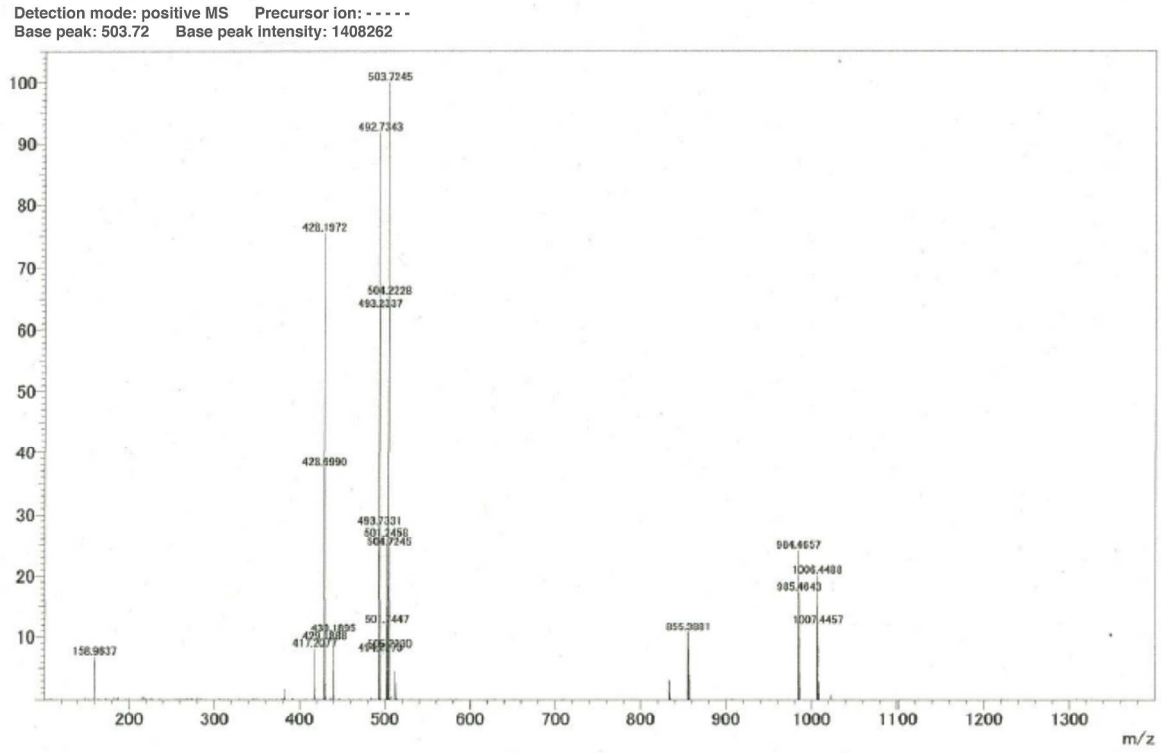


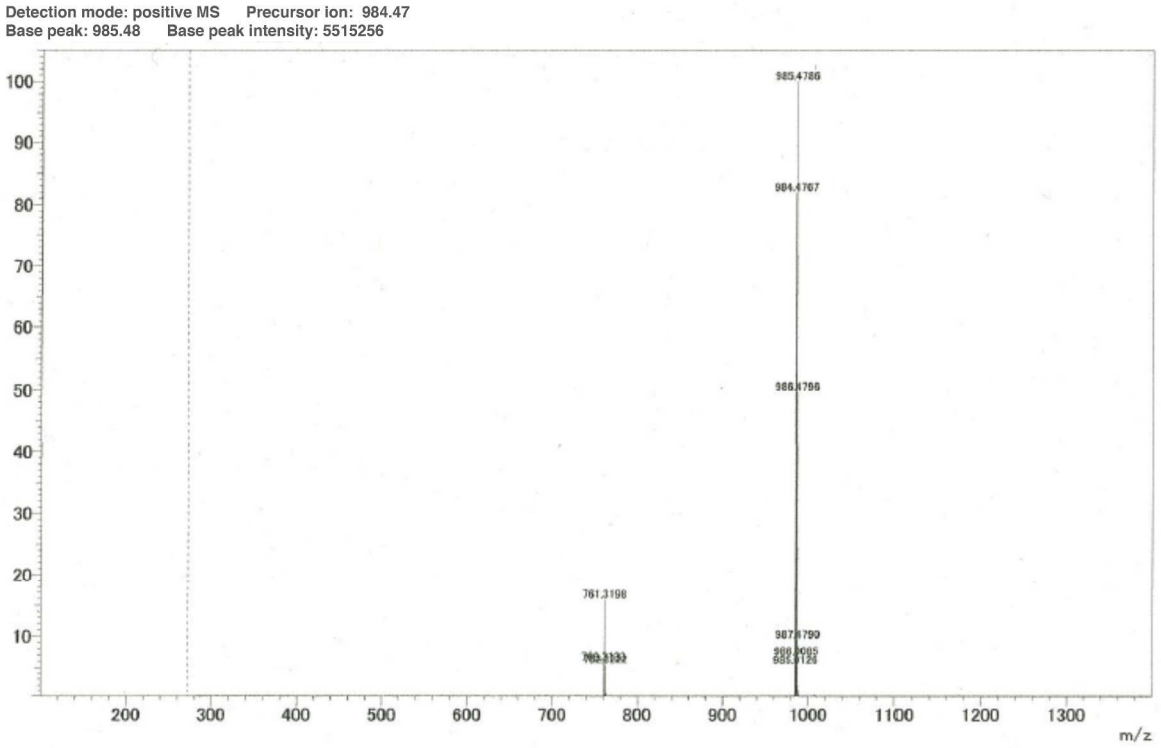
Compound 48c



1-67. HRMS CHART

Compound 42a


Detection mode: positive MS Precursor ion: - - - - -
Base peak: 503.72 Base peak intensity: 1408262
503.7245
492.7343
428.1972
504.2228
493.2337
428.6990
493.7331
984.4657
1006.4488
985.4643
1007.4457
855.3981
158.9837
m/z


Detection mode: positive MS Precursor ion: 984.47
Base peak: 985.48 Base peak intensity: 5515256
985.4786
984.4767
986.4796
761.3198
987.4799
m/z

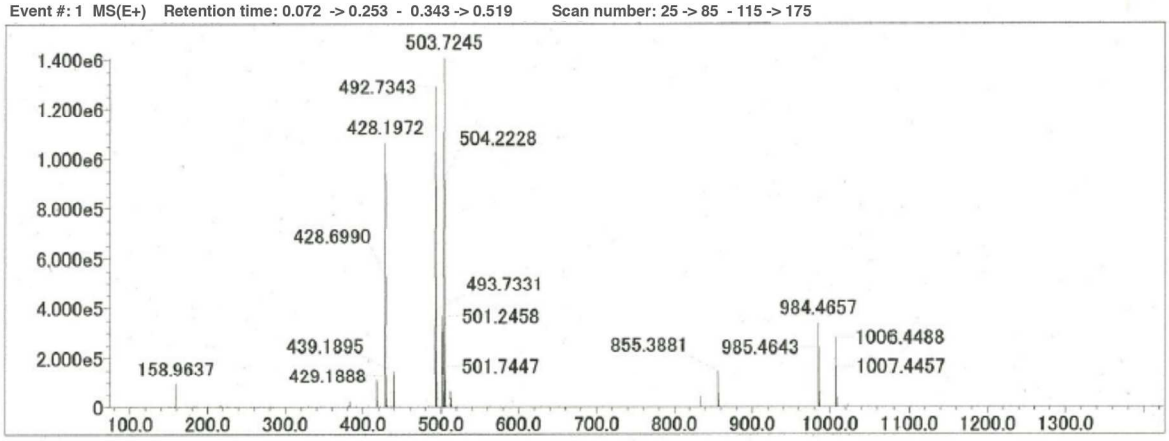
Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	0	66	N	3	0	7	P	3	0	0	Br	1	0	0	H
B	3	0	0	O	2	0	8	S	2	0	1	Ru	2	0	0	K
C	4	0	55	F	1	0	0	Cl	1	0	0					

Error Margin (mDa): 10.0
HC Ratio: unlimited
Max Isotopes: all
MSn Iso RI (%): 75.00

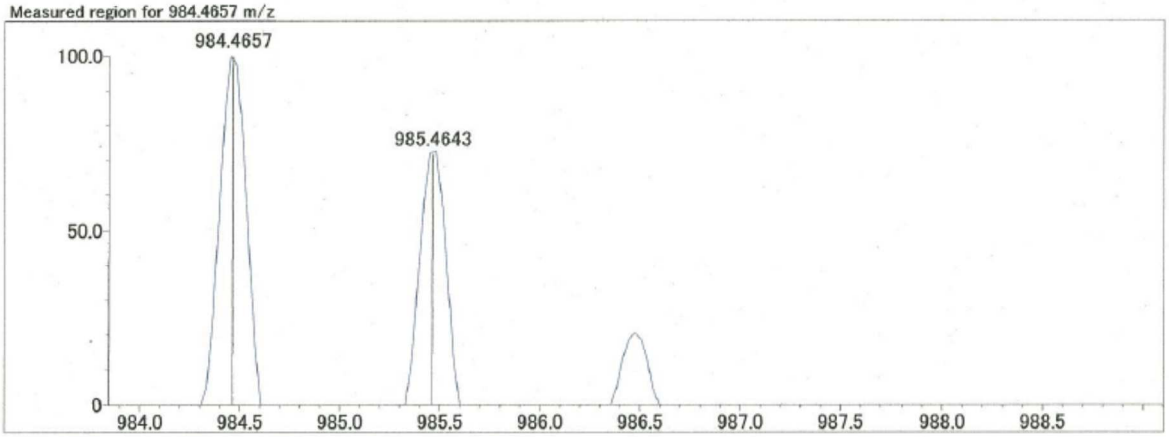
DBE Range: not fixed
Apply N Rule: no
Isotope RI (%): 1.00
MSn Logic Mode: AND

Electron Ions: odd
Use MSn Info: no
Isotope Res: 10000
Max Results: 100

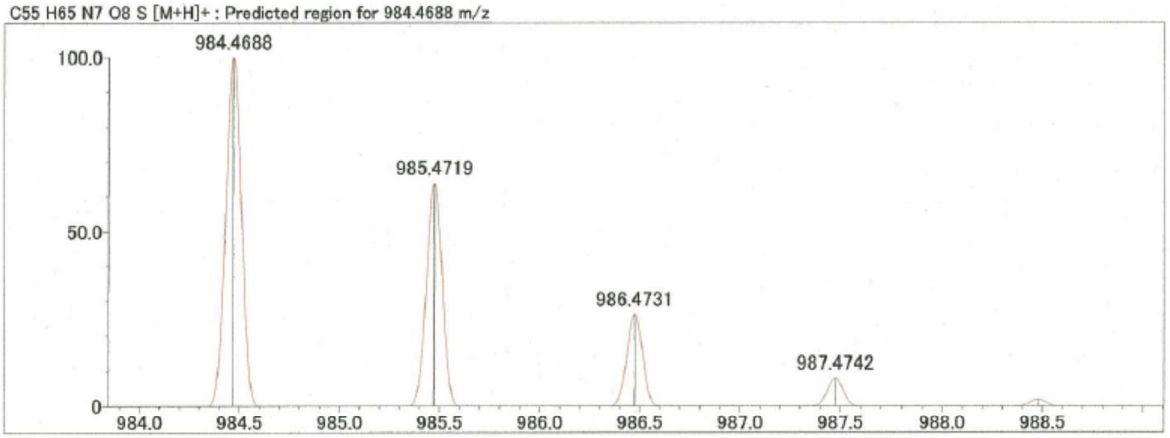
Event #: 1 MS(E+) Retention time: 0.072 -> 0.253 - 0.343 -> 0.519 Scan number: 25 -> 85 - 115 -> 175



Measured region for 984.4657 m/z



C55 H65 N7 O8 S [M+H]+ : Predicted region for 984.4688 m/z

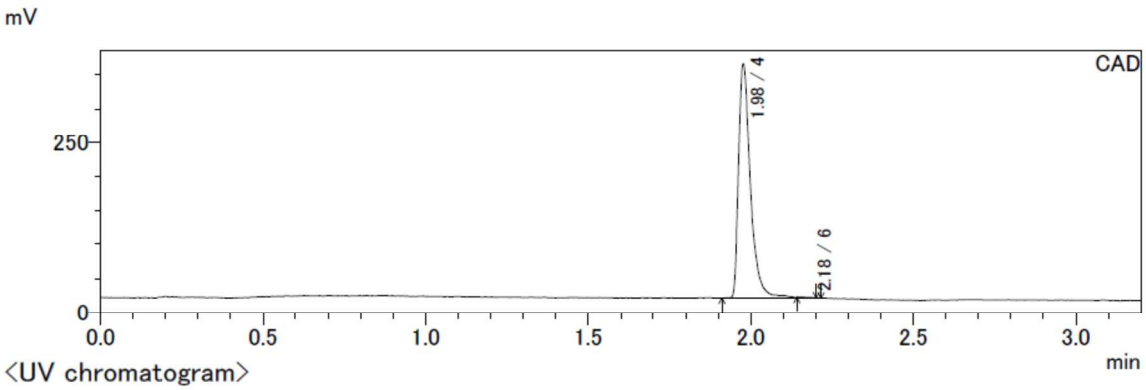


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	0.00	C55 H65 N7 O8 S	[M+H]+	984.4657	984.4688	-3.1	-3.15	0.00	27.0

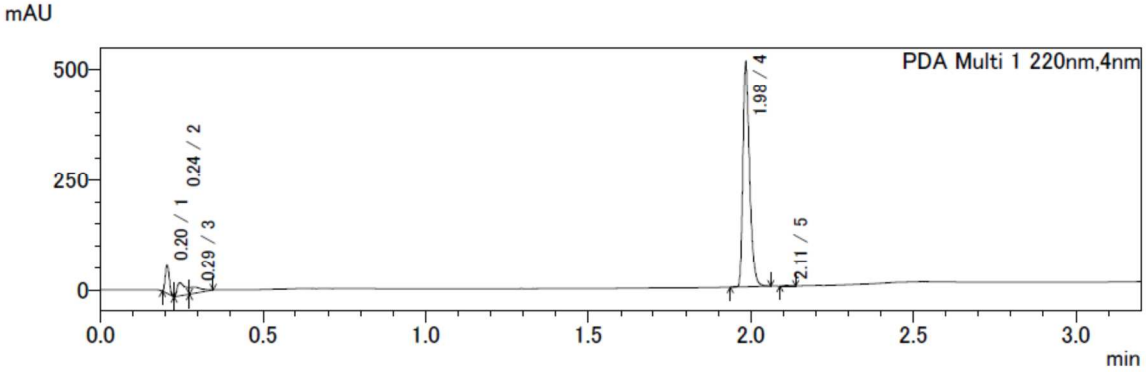
1-68. HPLC CHART

Compound 42a

<CAD chromatogram>



<UV chromatogram>



<Peak table>

CAD peak table

CAD

Peak #	Retention time	Area	Height	Area%
4	1.98	875135	341302	99.84
6	2.18	1373	700	0.16

UV peak table

PDA Ch1 220nm

Peak #	Retention time	Area	Height	Area%
1	0.20	54005	63281	6.24
2	0.24	52264	29031	6.04
3	0.29	33556	13859	3.88
4	1.98	723471	510470	83.56
5	2.11	2543	1918	0.29

2. Reagents.

Tissue culture plastics were purchased from Greiner Bio-One (Tokyo, Japan). Compound **77** and methyl (3*S*)-5-fluoro-3-(((2*S*)-2-(((2*S*)-3-methyl-2-(phenylmethoxycarbonylamino)butanoyl)amino)propanoyl)amino)-4-oxopentanoate **78** were purchased from Peptide Institute (Osaka, Japan). 5α-Dihydrotestosterone (DHT) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).

3. Cell culture.

Human PC cell lines (22Rv1, VCaP, LNCaP and PC-3) and human lung adenocarcinoma cell line A549 were maintained in Roswell Park Memorial Institute (RPMI)-1640 medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum (Gibco) and 50 μg/mL of kanamycin (Sigma-Aldrich). Human fibrosarcoma cell line HT1080 was maintained in Dulbecco's modified Eagle's medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum and 50 μg/mL of kanamycin. To examine the effects of androgen on VCaP cells, cells were cultured in phenol red-free RPMI-1640 medium (Gibco) containing 5%

charcoal-stripped fetal bovine serum (HyClone Laboratories, Logan, UT, USA) for 2 days, and then DHT was added to the medium.

4. Western blotting.

Cells were lysed with SDS lysis buffer (0.1 M Tris-HCl at pH 8.0, 10% glycerol, 1% SDS). Protein concentration was measured by the BCA method (Pierce) and an equal amount of protein lysate was separated by SDS-PAGE, transferred to PVDF membranes (Millipore), and analyzed by Western blot using the appropriate antibodies. The immunoreactive proteins were visualized using Clarity Western ECL substrate (Bio-Rad, Hercules, CA, USA), and their light emission was quantified with a LAS-3000 lumino-image analyzer (Fuji, Tokyo, Japan). The following antibodies were used: anti-AR (5153), anti-PARP (9532), anti-cleaved Caspase-3 (9664) (Cell Signaling Technology), anti-cIAP1 (AF8181) (R&D systems), anti-GAPDH (sc-25778 HRP) (Santa Cruz).

5. Measurement of binding affinity.

After FreeStyle293F (Invitrogen) cells were transfected with pcDNA3.1 containing an AR gene (wild-type AR) by using 293fectin transfection reagent (Invitrogen), these cells were

seeded into Erlenmeyer flask (Corning, 1L, 430518) at 1.1 × 10^6 cells/mL in FreeStyle293 Expression Medium (Invitrogen). After a 48 h shaking incubation (125 rpm) at 37 °C in an 8% CO_2 atmosphere, these cells were washed with TEG Buffer (10 mM Tris-HCl (pH 7.2), 50 mM EDTA, 10% Glycerol), and suspended with TEGM Buffer (10 mM Tris-HCl (pH 7.2), 1 mM EDTA, 10% glycerol, 10 mM Na_2MoO_4, 1 mM DTT, 1 mM 2-ME, 1 × Complete protease inhibitor tablet (Roche)). After freezing and thawing to lyse cells, lysate was centrifuged at 228,000 × g at 4 °C for 20 min. The supernatant was stored at -80 °C as AR cell lysate. To cell lysate solution containing an AR were added [17-α-methyl-^{3}H] mibolerone (final 3 nM, PerkinElmer NET-919) and a compound, and the mixture was incubated at 4 °C for 3 h. B (Bound)/ F (Free) were separated by the dextran/charcoal method.[51] The label count of B was measured, and the inhibitory rate of the compound was calculated.

6. RNA isolation and quantitative PCR.

Total RNA was prepared from cells with RNeasy (Qiagen). First-strand cDNA was synthesized from 1 μg total RNA with an oligo-dT primer using the SuperScript

First-Strand Synthesis System (Invitrogen). Quantitative real-time PCR was performed with an ABI Prism 7300 sequence detection system (Applied Biosystems, Foster City, CA) using SYBR GreenER (Invitrogen) with gene-specific primers. Human 36B4 mRNA was used as an invariant control. The following PCR primers were used (5′ to 3′): *36b4*, GGC CCG AGA AGA CCT CCT T and CCA GTC TTG ATC AGC TGC ACA; *Psa*, CAG TCT GCG GCG GTG TTC and TGC CGA CCC AGC AAG ATC; *Tmprss2*, GAC CAG GAG TGT ACG GGA ATG T and AGG ACG AAG ACC ATG TGG ATT AG; *Klk2*, GCT GCC CAT TGC CTA AAG AA and TGG GAA GCT GTG GCT GAC A; *Nkx3-1*, GGA GAG AGG GAA AAT CAA GTG GTA and GTG ACA CAG GAG GAT GGA GTT G. The relative amounts of each mRNA were calculated using the comparative Ct method.[52]

7. Cell viability assay.

Cell viability was determined using water-soluble tetrazolium WST-8 (4-[3-(2-methoxy-4-nitrophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfonate) for the spectrophotometric assay according to the manufacturer's instructions (Dojindo, Tokyo, Japan). Cells were seeded at a concentration of 5×10^3 cells per well in a

96-well culture plate. After 24 h, the cells were treated with the indicated compounds for 48 h. The WST-8 reagent was added and the cells were incubated for 0.5 h at 37 °C in a humidified atmosphere of 5% CO_2. The absorbance at 450 nm of the medium was measured using an EnVision Multilabel Plate Reader (PerkinElmer).

8. Microscopy.

After treatment with the tested compounds, phase-contrast images of the cells were obtained by a BZ-9000 (Keyence, Osaka, Japan).

9. Measurement of apoptosis by flow cytometry.

Apoptosis was analyzed with an Annexin V-FITC Apoptosis Detection Kit (BioVision, Milpitas, CA, USA), as previously described.[17, 53] Briefly, after being treated with the tested compounds, the cells were harvested and resuspended in binding buffer. The cells were stained with annexin V-FITC and propidium iodide (PI) at room temperature for 5 min in the dark, according to the manufacturer's instructions, and analyzed in a FACScan flow cytometer (Becton Dickinson, Braintree, MA, USA).

10. Statistical analysis

Student's *t*-test was used to determine the significance of differences among the experimental groups. Values of $P<0.05$ were considered significant.

ASSOCIATED CONTENT

Supporting Information

Schemes and experimental procedures for synthesis of AR and IAP ligand parts.

Molecular formula strings.

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Notes

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ABBREVIATIONS USED

SNIPERs, Specific and Non-genetic IAP-dependent Protein Erasers; cIAP, cellular inhibitor of apoptosis protein; AR, androgen receptor; PC, prostate cancer; CRPC, castration-resistant prostate cancer; UPS, ubiquitin proteasome system; PROTACs, Proteolysis Targeting

Chimeras; POI, protein of interest; PEG, polyethylene glycol; DHT, 5α-dihydrotestosterone; VHL, von Hippel–Lindau. MeOH, methanol; CD_3OD, deuterated methanol; EtOH, ethanol; $CDCl_3$, deuterated chloroform; DMSO-d_6, deuterated dimethyl sulfoxide; MeCN, acetonitrile; CPME, cyclopentyl methyl ether; EtOAc, ethyl acetate; *m*-CPBA, *m*-chloroperbenzoic acid; DIPEA, *N*,*N*′-diisopropylethylamine; EDC, 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide; Et_3N, triethylamine; HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; HOBt·H_2O, 1-hydroxy-1*H*-benzotriazole hydrate; SEMCl, 2-(trimethylsilyl)ethoxymethyl chloride; MsCl, methanesulfonyl chloride; TsCl, *p*-toluenesulfonyl chloride; TrCl, triphenylmethyl chloride; TsOH·H_2O, *p*-toluenesulfonic acid monohydrate; IPE, diisopropyl ether; TBAC, tetrabutylammonium chloride; Me_2NH, dimethylamine; Pd–C, palladium on carbon; $Pd(OAc)_2$, palladium(II) acetate; $Pd(Ph_3P)_4$, tetrakis(triphenylphosphine)palladium(0); Ph_3P, triphenylphosphine; Boc_2O, di-*tert*-butyl dicarbonate.

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