

# A General Strategy for the Preparation of Thalidomide-Conjugate Linkers

J. W. Papatzimas<sup>a#</sup>  
 E. Gorobets<sup>a#</sup>  
 D.K. Brownsey<sup>a</sup>  
 R. Maity<sup>b</sup>  
 N. J. Bahlis<sup>b</sup>  
 D. J. Derksen<sup>a\*</sup>

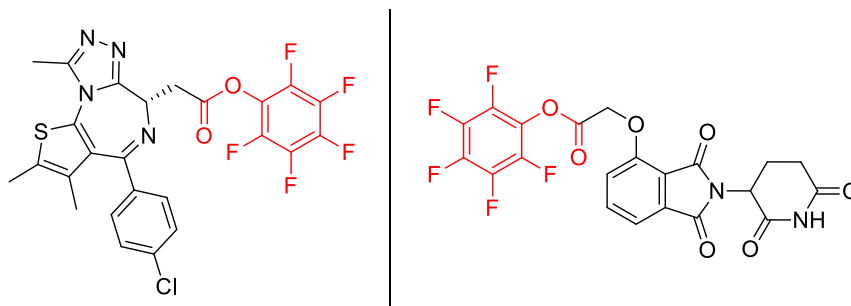
<sup>a</sup> Department of Chemistry, University of Calgary, 2500 University Drive NW, Calgary, Alberta, Canada, T2N 1N4.

<sup>b</sup> Department of Hematology and Oncology, University of Calgary, Calgary, Alberta, Canada, T2N 4N1.

\* indicates the main/corresponding author, # these authors contributed equally.

dderksen@ucalgary.ca

[Click here to insert a dedication.](#)



Received:  
 Accepted:  
 Published online:  
 DOI:

**Abstract** The synthesis of small molecule linkers for installation of thalidomide based conjugates is described. Linker properties have been recognized as vital to conjugate success in drug discovery and delivery systems. These small molecule tethers act as linkages between molecules, can also aid in cell permeability, and act as solubilizing agents. This work shows our progress in synthesizing conjugates with a variety of linker characteristics. The adaptability and manipulation of these and other linkers holds potential in improving synthetic control of chemical connectivities toward therapeutic development.

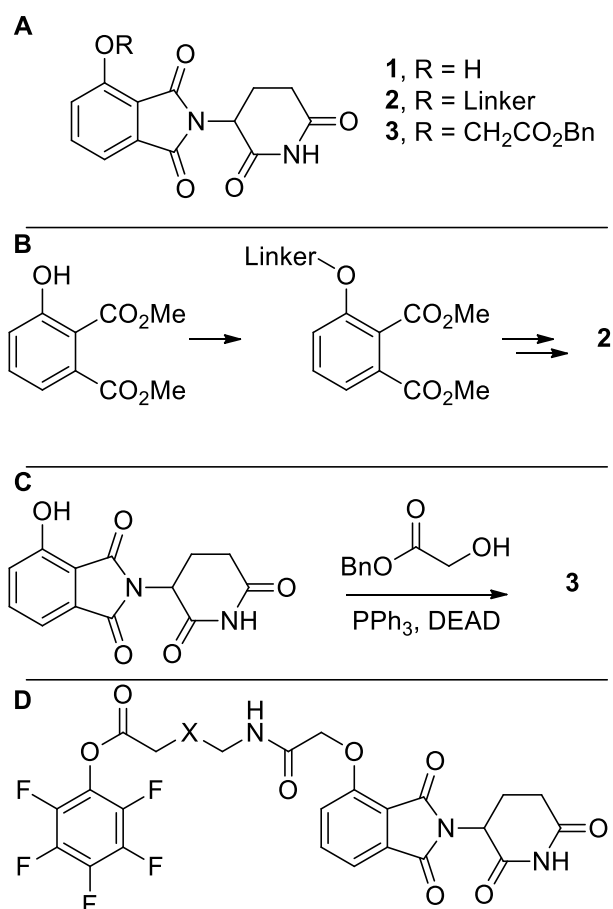
**Key words** PROTAC, Thalidomide, Conjugates, Linker, Pentafluorophenyl Ester

Since the seminal reports by Bradner<sup>1</sup> and Crews<sup>2</sup> on the use of thalidomide conjugation for *in vivo* protein degradation, our group and others have become interested in the synthetic preparation of small-molecule conjugates incorporating this moiety (Scheme 1, A).<sup>3</sup> As both linker length and composition have been shown to be essential for preparing functional conjugates,<sup>1-5</sup> we have worked to develop a sufficiently convergent synthetic strategy to prepare multiple linkers for structure-activity relationship studies.

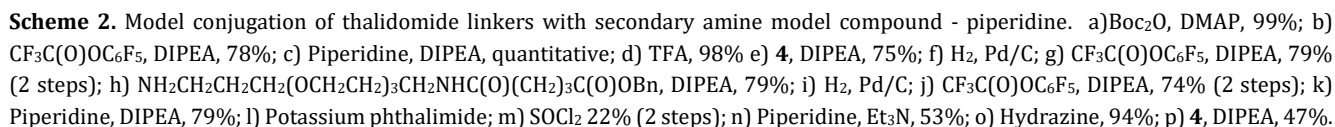
A recent paradigm shift in proximity directed protein degradation has seen the implementation of Proteolysis Targeting Chimeras (PROTACs) as a means of inducing protein degradation.<sup>4,6,7,8</sup> PROTACs are small molecule conjugates which enhance proximity between proteins of interest (POIs) through bifunctional targeting conjugates to initiate protein degradation.<sup>4</sup> Thalidomide derivatives have been successfully used as targeting ligands in previously published PROTAC work.<sup>12,3b,9</sup> Due to the importance of proximity enhancement for PROTACs, linker length between targeting molecules becomes vital to effective recruitment and positioning of POIs.

Our initial approach to thalidomide conjugates relied on a highly linear synthesis from literature where the linker moiety needed to be introduced prior to glutarimide introduction (Scheme 1, B).<sup>10</sup> Our own attempts to improve the synthesis found that direct phenol alkylation of 4-hydroxythalidomide (**1**) led to competing N-alkylation of the glutarimide moiety. We were

working to make this synthesis more convergent when elegant work from Miller showed that the phenolic site of hydroxyl-thalidomide could be functionalized directly using modified Mitsunobu conditions (Scheme 1, C).<sup>11</sup> This advancement allowed us to synthesize our desired compounds in a much shorter time frame by making the synthesis much more convergent.



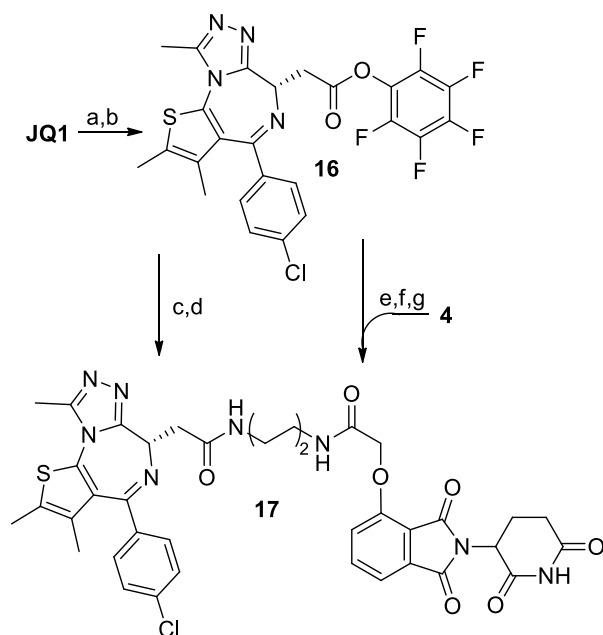
**Scheme 1.** 4-hydroxythalidomide derivative syntheses. X = CH<sub>2</sub> or (CH<sub>2</sub>)<sub>2</sub>C(O)NH(CH<sub>2</sub>)<sub>3</sub>(O CH<sub>2</sub>CH<sub>2</sub>)<sub>3</sub>.



using TLC and complete conversion was observed after only one hour. The free acid was then converted to the active Pfp ester **4** using pentafluorophenyl trifluoroacetate, the products of which can be easily purified via trituration with diethyl ether.<sup>15</sup> Pfp ester **4** was found to be stable for prolonged storage and was then available for conjugation with a variety of amine containing compounds such as our model piperidine.<sup>16</sup>

First generation linkers consisted of four carbon linkages following a system which had shown promising results in recently published work.<sup>1</sup> The primary amine of the simple building block,  $\gamma$ -aminobutyric acid (**5**) was Boc protected (Scheme 2). The remaining free acid was then transformed to reactive Pfp ester **6** using pentafluorophenyl trifluoroacetate. Compound **6** was then coupled to piperidine under basic conditions to afford compound **7**. Intermediate **7** was exposed to trifluoroacetic acid (TFA) to liberate the terminal amine which was then exposed to **4**,<sup>17</sup> affording **9** in 2 hours at ambient temperature.<sup>18</sup>

Our second generation of linkers was designed to investigate the synthetic differences in employing longer linkers with more polar functionalities, inspired by recent publications.<sup>1, 2, 3b, 19</sup> We looked to derivatize the polyethylene glycol (PEG) diamine 4,7,10-trioxa-1,13-tridecanediamine. While this linker was considerably longer than the four carbon alkyl linker, it was inherently much more water soluble.. Succinic anhydride was opened using benzyl alcohol and NaH to afford a mono-protected diacid. The remaining acid was then converted to an acid chloride *in situ*. The PEG diamine was first mono-Boc protected and then exposed to the activated acid to afford the desired intermediate consisting of two different protected terminal functionalities. The amine pole was then



**Scheme 3.** Syntheses of known PROTAC 17. a)  $\text{HCO}_2\text{H}$ , quantitative;<sup>20</sup> b)  $\text{CF}_3\text{C}(\text{O})\text{OC}_6\text{F}_5$ , DIPEA, 56%;<sup>21</sup> c) 1,4-diaminobutane, DIPEA; d) **4**, DIPEA, 48%;<sup>22</sup> e) **4**,  $\text{H}_2\text{N}(\text{CH}_2)_4\text{NHC}(\text{O})\text{OC}(\text{CH}_3)_3$ , DIPEA, 81%; f) TFA, quantitative;<sup>11</sup> g) DIPEA, **16**, 81%.<sup>23</sup>

deprotected using TFA, and the newly formed primary amine was reacted with **4** to form intermediate **10** which could also be easily purified by trituration with diethyl ether. The remaining benzyl ester was hydrogenated over Pd/C in two hours to afford the free acid which was exposed to pentafluorophenyl trifluoroacetate to form the respective Pfp ester **11** in one pot (Scheme 2). Intermediate **11** was reacted with piperidine to afford conjugate **12** in a 79% yield.<sup>24</sup>

To expand the scope of linkages beyond simple amide bonds, sulfonamides were also explored. A Gabriel synthesis of 1,4-butane sultone (**13**) was performed, opening the ring to produce a straight chain linker with already established sulfonate and protected amine terminal motifs. The sulfonate salt was then converted to sulfonyl chloride **14**, which proved to be stable to flash chromatography (Scheme 2). Previous attempts to open the sultone ring were successfully performed using  $\text{NH}_4\text{OH}$  to yield a straight chain linker with a free primary amine which was Boc protected. However, attempts at forming the sulfonyl chloride afforded low yields (6%), due to rapid intramolecular recyclization of the mono-protected amine. The Gabriel synthesis was employed as a means of introducing a doubly protected terminal amine in one step. Sulfonyl chloride **14** was then exposed to the model reaction conditions with piperidine to afford the desired sulfonamide in moderate yield. The phthalimide ring was then cleaved with hydrazine monohydrate to liberate the free terminal amine, which was reacted with **4** to yield conjugate **15**.<sup>25</sup>

In order to showcase the versatility of **4** in our synthetic strategy we employed this approach to synthesize a PROTAC reported by the Bradner group (Scheme 3).<sup>1</sup> We developed two novel synthetic routes for forming **17** via the Pfp ester of JQ1 (**16**). As this approach minimizes the amount of byproduct formation, this strategy allows purification by flash chromatography instead of HPLC as previously reported.<sup>1</sup>

Conjugates **9**, **12**, **15**, and **17** were successfully formed through a synthetic route with clear advantages over previous thalidomide-containing syntheses. One of the most beneficial characteristics of this linker strategy is the use of pentafluorophenyl trifluoroacetate in order to form respective Pfp esters. The ease of purification associated with simple trituration eliminates issues associated with isolation of highly polar compounds. In addition to the ease of synthesis and purification, this linker strategy can easily be tailored to accommodate a broad scope of targeting molecules. The adaptability of this synthetic strategy lends itself to the diverse linkers required for PROTAC synthesis. By varying the length, hydrophobic properties, as well as linkage identity we have developed a strategy for conjugating a broad scope of target molecules through a variety of functional groups. *In vitro* structure activity relationship studies are currently underway.

### Funding Information

This work was funded by the Multiple Myeloma Research Foundation, NSERC, the University of Calgary, Alberta Children's Hospital Foundation and Research Institute, and the Charbonneau Cancer Institute.

### Supporting Information

YES

### Primary Data

NO

### References and Notes

- (1) Winter, G.E.; Buckley, D.L.; Paulk, J.; Roberts, J.M.; Souza, A.; Dhe-Paganon, S.; Bradner, J.E. *Science* **2015**, *348*, 1376.
- (2) Lu, J.; Qian, Y.; Altieri, M.; Dong, H.; Wang, J.; Raina, K.; Hines, J.; Winkler, J.D.; Crew, A.P.; Coleman, K.; Crews, C.M. *Chem. Biol.* **2015**, *22*, 755.
- (3) (a) Fischer, E.S.; Bohm, K.; Lydeard, J.R.; Yang, H.; Stadler, M.B.; Cavadini, S.; Nagel, J.; Serluca, F.; Acker, V.; Lingaraju, G.M.; Tichkule, R.B.; Schebesta, M.; Forrester, W.C.; Schirle, M.; Hassiepen, U.; Ottl, J.; Hild, M.; Beckwith, R.E.J.; Harper, J.W.; Jenkins, J.L.; Thoma, N.H. *Nature* **2014**, *512*, 49. (b) Lebraud, H.; Wright, D.J.; Johnson, C.N.; Heightman, T.D. *ACS Cent. Sci.* **2016**, *2*, 927.
- (4) Long, M.J.C.; Poganik, J.R.; Aye, Y. *J. Am. Chem. Soc.* **2016**, *138*, 3610.
- (5) Srinivasarao, M.; Galliford, C.V.; Low, P.S. *Nat. Rev. Drug Discov.* **2015**, *14*, 203.
- (6) DeRose, R.; Miyamoto, T.; Inoue, T. *Pfluegers Arch.* **2013**, *465*, 409.
- (7) Corson, T.W.; Aberle, N.; Crews, C.M.; *ACS Chem. Biol.* **2008**, *3*, 677.
- (8) Lai, A.C.; Crews, C.M. *Nat. Rev. Drug Discov.* **2017**, *16*, 101.
- (9) (a) Remillard, D.; Buckley, D.L.; Paulk, J.; Brien, G.L.; Sonnett, M.; Seo, H.-S.; Dastjerdi, S.; Wühr, M.; Dhe-Paganon, S.; Armstrong, S.A.; Bradner, J.E. *Angew. Chem. Int. Ed.* **2017**, *56*, 5738. (b) Schiedel, M.; Herp, D.; Hammelmann, S.; Swyter, S.; Lehotzky, A.; Robaa, D.; Oláh, J.; Ovádi, J.; Sippl, W.; Jung, M. *J. Med. Chem.* [Online early access]. DOI: 10.1021/acs.jmedchem.6b01872. Published Online: Apr 5, **2017**. (c) Lai A.C.; Toure, M.; Hellerschmied, D.; Salami, J.; Jaime-Figueroa, S.; Ko, E.; Hines, J.; Crews, C.M. *Angew. Chem. Int. Ed.* **2016**, *55*, 807.
- (10) Gladysz, J. A.; Lee, S. J.; Tomasello, J. A. V.; Yu, Y. S. *J. Org. Chem.* **1977**, *42*, 4170.
- (11) Lohbeck, J.; Miller, A.K. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 5260.
- (12) Montalbetti, C. A. G. N.; Falque, V. *Tetrahedron* **2005**, *61*, 10827.
- (13) Wurz, R.P.; Dellamaggiore, K.; Dou, H.; Javier, N.; Lo M.-C.; McCarter, J.D.; Mohl, D.; Sastri, C.; Lipford, J.R.; Cee, V.J. *J. Med. Chem.* [Online early access]. DOI: 10.1021/acs.jmedchem.6b01781. Published Online: Apr 5, **2017**.

- (14) Orchin, M.; Macomber, R.S.; Pinhas, A.R.; Wilson, R.M. *The Vocabulary and Concepts of Organic Chemistry*, 2<sup>nd</sup> Ed.; John Wiley & Sons: Hoboken, 2005.
- (15) **General Procedure for the Synthesis of 4**  
A solution of **3** (0.691 g, 1.64 mmol) in DMF (15 mL) was stirred with Pd/C (0.04 g, 10 mol%) under hydrogen for 1.5 h, filtered and concentrated *in vacuo*. The crude product (0.148 g, 0.45 mmol) was then redissolved in DMF (6 mL), and DIPEA (0.118 g, 0.16 mL, 0.92 mmol) was added with stirring and the solution was cooled to 0 °C. Pentafluorophenyl trifluoroacetate (0.187 g, 0.12 mL, 0.67 mmol) was then added with stirring. The reaction mixture was allowed to come to ambient temperature for 2 h. The mixture was concentrated *in vacuo*, and purified by trituration with diethyl ether to afford 0.175 g (79 %) of **4**.  
<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.99 (s, 1H), 7.76 (dd, *J* = 8.4, 7.3 Hz, 1H), 7.63 (dd, *J* = 7.4, 0.7 Hz, 1H), 7.26 (dd, *J* = 8.5, 0.8 Hz, 1H), 5.34 (d, *J* = 1.4 Hz, 2H), 5.04 – 4.97 (m, 1H), 2.98 – 2.74 (m, 3H), 2.22 – 2.14 (m, 1H). HRMS (ESI) *m/z* calc. for [C<sub>21</sub>H<sub>11</sub>F<sub>5</sub>N<sub>2</sub>O<sub>7</sub> + Na]<sup>+</sup> = 521.0379, found 521.0368.
- (16) No decomposition observed after 5 weeks at -20 °C.
- (17) **General Procedure for Coupling to 4**  
To a solution of corresponding free amine (1 equiv.) in DMF, was added DIPEA (3 equiv.) under stirring. A solution of **4** (1.1 equiv.) in DMF was added to the reaction mixture at ambient temperature. After 2 h the mixture was concentrated *in vacuo* and purified by silica gel flash chromatography to afford the title compound.
- (18) **2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)oxy)-N-(4-oxo-4-(piperidin-1-yl)butyl)acetamide (9)**  
Purified by silica gel flash chromatography (5% MeOH in CHCl<sub>3</sub>) to afford 0.119 g (75%) as a colourless oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.70 (s, 1H), 7.75 (dd, *J* = 8.4, 7.3 Hz, 1H), 7.71 (t, *J* = 5.6 Hz, 1H), 7.57 (dd, *J* = 7.4, 0.7 Hz, 1H), 7.23 (dd, *J* = 8.5, 0.7 Hz, 1H), 5.00 – 4.92 (m, 1H), 4.73 – 4.60 (m, 2H), 3.58 – 3.52 (m, 2H), 3.45 (ddd, *J* = 14.7, 7.4, 6.0 Hz, 1H), 3.41 – 3.33 (m, 3H), 2.98 – 2.70 (m, 3H), 2.42 – 2.36 (m, 2H), 2.22 – 2.15 (m, 1H), 1.97 – 1.88 (m, 2H), 1.66 – 1.60 (m, 2H), 1.59 – 1.49 (m, 4H). <sup>13</sup>C NMR (400 MHz, CDCl<sub>3</sub>) δ 170.80, 170.42, 168.04, 166.89, 166.54, 154.97, 137.01, 133.61, 120.51, 118.56, 117.68, 77.20, 69.00, 49.42, 46.61, 42.78, 39.12, 31.34, 30.66, 26.48, 25.55, 24.84, 24.54, 22.73. HRMS (ESI) *m/z* calc. for [C<sub>24</sub>H<sub>28</sub>N<sub>4</sub>O<sub>7</sub> + H]<sup>+</sup> = 485.2031, found 485.2047.
- (19) Lu, J.; Qian, Y.; Altieri, M.; Dong, H.; Wang, J.; Raina, K.; Hines, J.; Winkler, J.D.; Crew, A.P.; Coleman, K.; Crews, C.M.; *Chem. Biol.* **2015**, *22*, 755.
- (20) **2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetic acid (JQ1-Acid)**  
JQ1 (0.05g, 0.1099 mmol) was dissolved in formic acid (4.5 mL) and stirred for 4 days. The solvent was removed *in vacuo* to afford a fine yellow powder. The product was used without purification.
- (21) **perfluorophenyl 2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetate (16)**  
To a solution of JQ1-Acid (0.055 g, 0.136 mmol) in DMF (2 mL) was added DIPEA (0.17 mL, 0.123 g, 0.952 mmol) and pentafluorophenyl trifluoroacetate (0.05 mL, 0.076 g, 0.272 mmol). The solution was stirred for 1 hour and solvent was removed *in vacuo*. The residue was purified by silica gel flash chromatography (1:1 EtOAc:Hexanes:THF) to afford 0.0432 g (56%) as a yellow oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.49 – 7.34 (m, 4H), 4.71 (dd, *J* = 9.5, 4.6 Hz, 1H), 4.08 (dd, *J* = 16.9, 9.5 Hz, 1H), 3.91 (dd, *J* = 16.9, 4.6 Hz, 1H), 2.73 (s, 3H), 2.45 (d, *J* = 0.9 Hz, 3H), 1.73 (d, *J* = 0.9 Hz, 3H). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ 167.62, 164.38, 154.65, 150.13, 141.90, 140.26, 138.70, 137.03, 136.30, 132.22, 131.00, 130.97, 130.26, 129.83, 128.76, 53.57, 36.39, 14.40, 13.11, 11.85. HRMS (MALDI) *m/z* calc. for [C<sub>25</sub>H<sub>16</sub>ClF<sub>5</sub>N<sub>4</sub>O<sub>2</sub>S + H]<sup>+</sup> = 567.0675, found 567.0771.
- (22) **2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)oxy)acetamido)butyl)acetamide (17)**  
To a solution of 1,4-diaminobutane (0.03 g, 0.371 mmol) in DMF (2 mL) was added DIPEA (0.065 mL, 0.048 g, 0.371 mmol) and **16** (0.021 g, 0.0371 mmol) and was stirred for 1.5 hours. The mixture was concentrated *in vacuo* and re-dissolved in DMF (1 mL). DIPEA (0.026 mL, 0.019 g, 0.0148 mmol) and **4** (0.028 g, 0.0557 mmol) was added and stirred overnight. The mixture was concentrated *in vacuo* and purified by silica gel flash chromatography (5% to 10% MeOH in CHCl<sub>3</sub>) to afford 0.014 g (48%) of the title compound as a mixture of diastereomers. <sup>1</sup>H NMR (600 MHz, MeOD) δ 8.34 – 8.29 (\*m, 1H), 8.12 (\*q, *J* = 5.7 Hz, 1H), 7.80 (dd, *J* = 8.4, 7.3 Hz, 1H), 7.52 (d, *J* = 7.3 Hz, 1H), 7.45 – 7.38 (m, 5H), 5.10 (ddd, *J* = 12.4, 5.5, 3.1 Hz, 1H), 4.77 (d, *J* = 1.7 Hz, 2H), 4.63 (ddd, *J* = 9.1, 5.4, 1.1 Hz, 1H), 3.44 – 3.32 (m, 4H), 3.30 – 3.25 (m, 2H), 2.86 – 2.77 (m, 1H), 2.73 – 2.65 (m, 5H), 2.43 (d, *J* = 2.5 Hz, 3H), 2.10 (dddd, *J* = 10.7, 8.0, 4.9, 2.6 Hz, 1H), 1.70 – 1.60 (m, 7H). \* exchangeable protons. <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ 174.4, 172.77, 172.68, 171.30, 171.27, 169.91, 168.24, 167.79, 166.24, 166.21, 157.00, 156.29, 152.19, 138.23, 138.09, 137.95, 134.87, 133.53, 133.18, 132.04, 132.02, 131.96, 131.31, 129.78, 121.90, 121.87, 119.37, 118.00, 69.57, 69.55, 55.23, 50.55, 50.54, 40.21, 40.19, 40.09, 40.06, 39.84, 38.88, 38.86, 32.14, 32.12, 27.77, 27.66, 27.64, 23.63, 23.61, 14.41, 12.92, 11.62. HRMS (MALDI) *m/z* calc. for [C<sub>38</sub>H<sub>37</sub>ClN<sub>6</sub>O<sub>7</sub>S + H]<sup>+</sup> = 785.2267, found 785.2232.
- (23) **2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(4-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)oxy)acetamido)butyl)acetamide (17)**  
To a solution of the free amine **4**-linker (0.021 g, 0.052 mmol) in DMF (1.5 mL) was added DIPEA (0.09 mL, 0.067 g, 0.5 mmol) and **16** (0.022 g, 0.039 mmol) and stirred overnight. The reaction mixture was stirred with potassium carbonate (0.021 g) for 30 minutes, filtered, concentrated *in vacuo* and purified by silica gel flash chromatography (5% to 10% MeOH in CHCl<sub>3</sub>) to afford 0.025 g (81%) of the title compound as a mixture of diastereomers. Data identical to reference 22.
- (24) **N-(1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)oxy)-2-oxo-7,10,13-trioxo-3-azahexadecan-16-yl)-4-oxo-4-(piperidin-1-yl)butanamide (12)**  
Purified by flash chromatography (5% MeOH in CHCl<sub>3</sub>) to afford 0.011 g (79%) as a colourless oil. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 9.43 (s, 1H), 7.74 (dd, *J* = 8.4, 7.4 Hz, 1H), 7.60 (t, *J* = 5.8 Hz, 1H), 7.55 (dd, *J* = 7.5, 0.7 Hz, 1H), 7.21 (dd, *J* = 8.4, 0.6 Hz, 1H), 6.66 (t, *J* = 5.6 Hz, 1H), 5.03 – 4.96 (m, 1H), 4.65 (d, *J* = 2.7 Hz, 2H), 3.67 – 3.64 (m, 4H), 3.64 – 3.61 (m, 2H), 3.58 (tt, *J* = 6.5, 2.7 Hz, 3H), 3.53 (td, *J* = 5.7, 1.8 Hz, 4H), 3.50 – 3.44 (m, 2H), 3.43 – 3.39 (m, 2H), 3.31 (qd, *J* = 6.4, 4.9 Hz, 2H), 2.91 – 2.75 (m, 3H), 2.66 (td, *J* = 6.7, 1.3 Hz, 2H), 2.49 (t, *J* = 6.8 Hz, 2H), 2.18 – 2.13 (m, 1H), 1.87 (p, *J* = 6.4 Hz, 2H), 1.82 (s, 1H), 1.75 (q, *J* = 6.3 Hz, 2H), 1.62 (td, *J* = 6.9, 4.9 Hz, 2H), 1.58 – 1.48 (m, 4H). <sup>13</sup>C NMR (600 MHz, CDCl<sub>3</sub>) δ 172.57, 171.34, 170.13, 168.33, 166.69, 166.62, 154.61, 136.93, 133.62, 119.68, 117.33, 70.41, 70.40, 70.15, 70.06, 69.39, 68.73, 68.22, 49.35, 46.40, 42.90, 37.31, 36.49, 31.59, 31.46, 29.23, 28.97, 28.80, 26.30, 25.54, 24.48, 22.69. HRMS (ESI) *m/z* calc. for [C<sub>34</sub>H<sub>47</sub>N<sub>5</sub>O<sub>11</sub> + Na]<sup>+</sup> = 724.3163, found 724.3168.
- (25) **2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)oxy)-N-(4-(piperidin-1-ylsulfonyl)butyl)acetamide (15)**  
Purified by flash chromatography (5% MeOH in CHCl<sub>3</sub>) to afford 0.016 g (47%) as a white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.08 (s, 1H), 7.75 (dd, *J* = 8.4, 7.4 Hz, 1H), 7.67 (s, 1H), 7.56 (dd, *J* = 7.3, 0.7 Hz, 1H), 7.20 (dd, *J* = 8.4, 0.7 Hz, 1H), 5.05 – 4.98 (m, 1H), 4.65 (d, *J* = 2.3 Hz, 2H), 3.43 (sept, *J* = 6.9 Hz, 2H), 3.29 – 3.12 (m, 4H), 2.97 – 2.89 (m, 3H), 2.89 – 2.71 (m, 2H), 2.23 – 2.14 (m, 1H), 1.99 – 1.87 (m, 2H), 1.74 (dt, *J* = 14.4, 6.9 Hz, 2H), 1.67 – 1.53 (m, 6H). <sup>13</sup>C NMR (400 MHz, CDCl<sub>3</sub>) δ 170.82, 168.00, 166.95, 166.55, 154.65, 137.10, 133.55, 120.08, 118.46, 117.64, 77.21, 68.49, 49.38, 48.51, 46.64, 38.44, 31.40, 28.27, 25.67, 23.81, 22.57, 20.55. HRMS (ESI) *m/z* calc. for [C<sub>24</sub>H<sub>30</sub>N<sub>4</sub>O<sub>8</sub>S + H]<sup>+</sup> = 535.1857, found 535.1853.

