Scalable and Cost-Effective Synthesis of a Linker for Bioconjugation with a Peptide and a Monoclonal Antibody

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Abstract: An efficient, scalable, and cost-effective synthesis of a linker employed in a bioconjugation process with a peptide and a monoclonal antibody is presented. Several routes were investigated that resulted in the identification of a short synthesis to a key acid intermediate from inexpensive and readily available starting materials. The final coupling of this acid with an aniline to afford the desired linker has been optimized to produce multi-gram quantities of material for clinical studies. The very limited purifications needed for both intermediates and final product make this route amenable to scale.

Key words: linker, bioconjugation, peptide, monoclonal antibody, imide, azetidinone

Biotechnology-based pharmaceuticals or biopharmaceuticals have received considerable attention over the past few years. The term 'bioconjugation' is applied to the covalent derivatization of biomolecules, a process in which two or more molecules come together to afford a complex that showcases the combined properties of each one of its individual components.[1] This process has found applications for the minimization of the immunogenicity of polypeptides, the protection of substances prone to enzymatic degradation, and the stabilization of substances in blood, among others.[2] Bioconjugation has also been employed in drug delivery systems for some oncology treatments, in which the active ingredient is connected to the carrier through a linker or spacer. This complex can then bind to its specific receptor contained on the cancer cell and the net result is the delivery of the therapeutic molecule to the desired site of action.[3] Examples of carrier molecules are monoclonal antibodies (mAb)[4] and synthetic polymers.[5]

During the implementation of a project in our laboratories, we were requested to prepare multi-gram quantities of linker **1** (Figure 1).

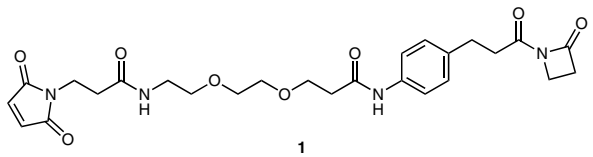

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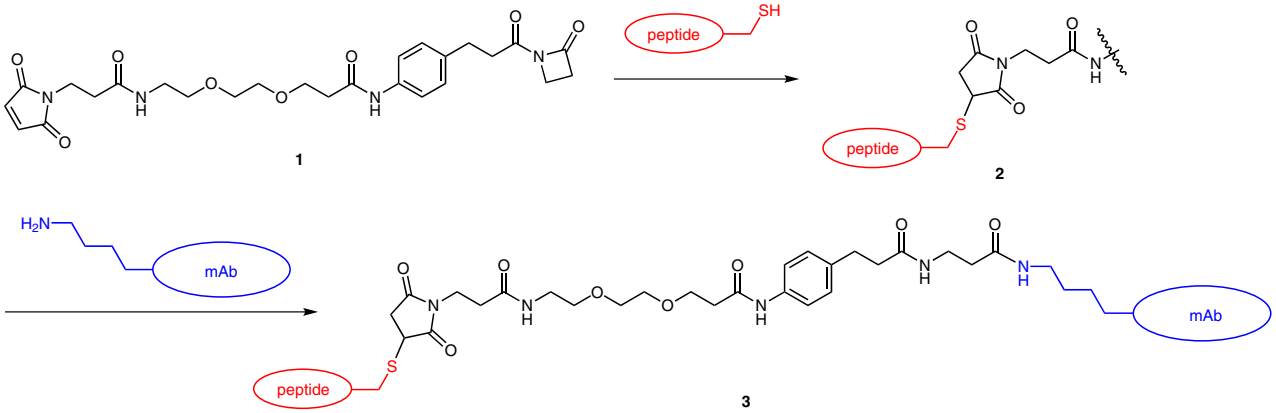
Figure 1 Structure of linker **1**

This material is employed to tether a peptide and an mAb to provide bioconjugate drug substance **3** in a process de-



Scheme 1 Conjugation process between linker **1**, a peptide, and a monoclonal antibody (mAb) to provide bioconjugate drug substance **3**

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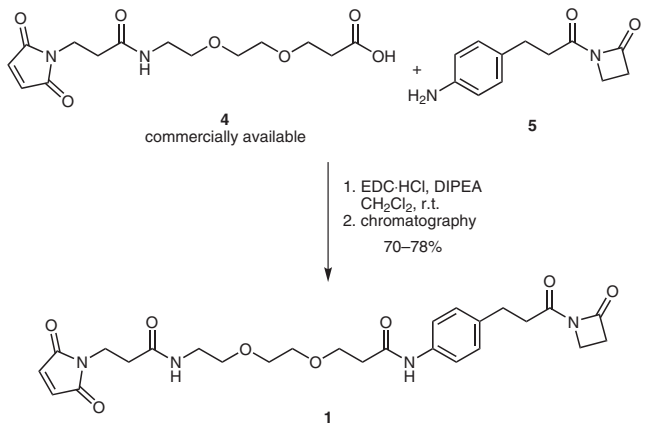
picted in Scheme 1. A thiol group of a cysteine residue on the peptide undergoes Michael addition on the maleimide moiety of **1** to form a new carbon–sulfur bond and provide intermediate **2**. In a second step, the amino group of a lysine residue on the mAb reacts with the azetidinone functionality on **2** to generate a new amide bond and afford bioconjugate **3**.

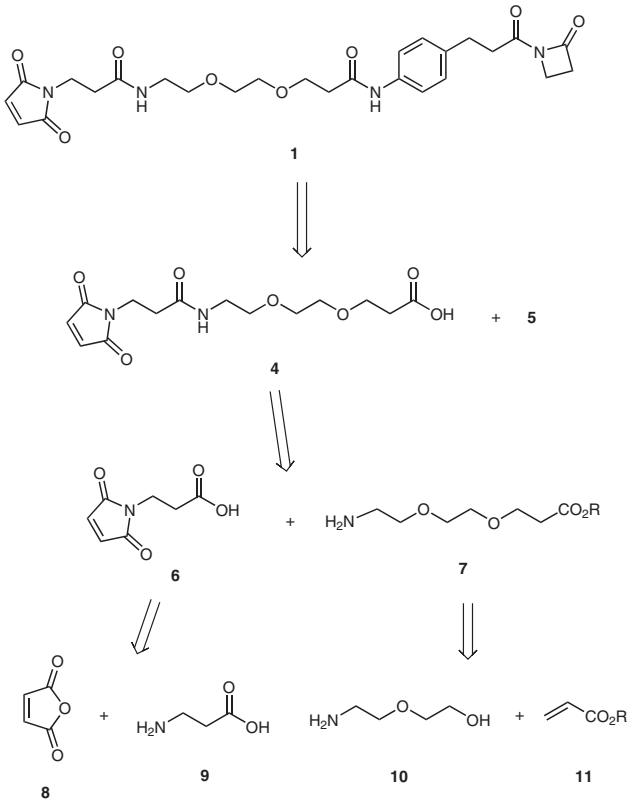
During the early stages of the project, our medicinal chemistry group had prepared small amounts of **1**[6] through the reaction of commercially available acid **4**[6–8] and aniline **5** in the presence of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl) as coupling agent followed by chromatography in 70–78% yield (Scheme 2).[9] After further project progression, larger quantities of **1** were required to support additional clinical studies and the cost associated with the purchase of acid **4** made this route prohibitively expensive. Therefore, our group decided to investigate the in-house preparation of **4** and optimize the reaction conditions of the last amide bond formation step to generate linker **1**.[10]



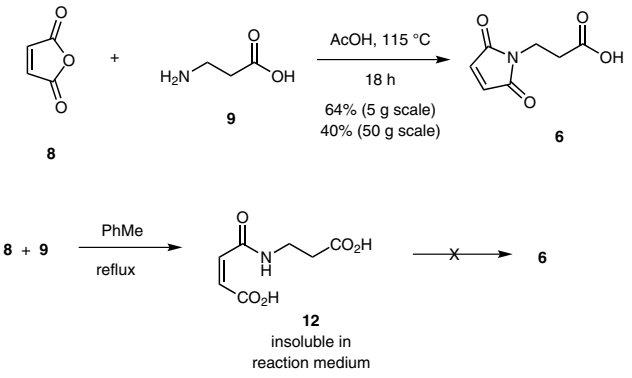
Scheme 2 Medicinal chemistry preparation of linker **1** via amide bond formation between acid **4** and aniline **5**

The retrosynthetic analysis for **1** (Scheme 3) shows that the most logical initial disconnections are the two amide bonds. Thus, after generating fragments **4** and **5**,[11] acid **4** can be further broken down into acid **6** and amino ester **7**. Acid **6** then comes from the reaction between maleic anhydride (**8**) and β-alanine (**9**) whereas **7** can be produced from the Michael addition of amino alcohol **10** to acrylate **11**.

Acid **6** was prepared starting from maleic anhydride (**8**) and β-alanine (**9**) following a known literature procedure (Scheme 4).[12] Even though a fair yield (64%) was obtained on small scale, when the reaction was scaled up to 50 grams, the need to remove residual acetic acid by slurrying in ethyl acetate caused the yield to drop considerably (40%). Despite the modest yield, this approach allowed us to generate enough material to meet our immediate needs. An alternative protocol carried out in toluene at reflux stalled upon generating intermediate **12** due to its low solubility in the reaction medium.

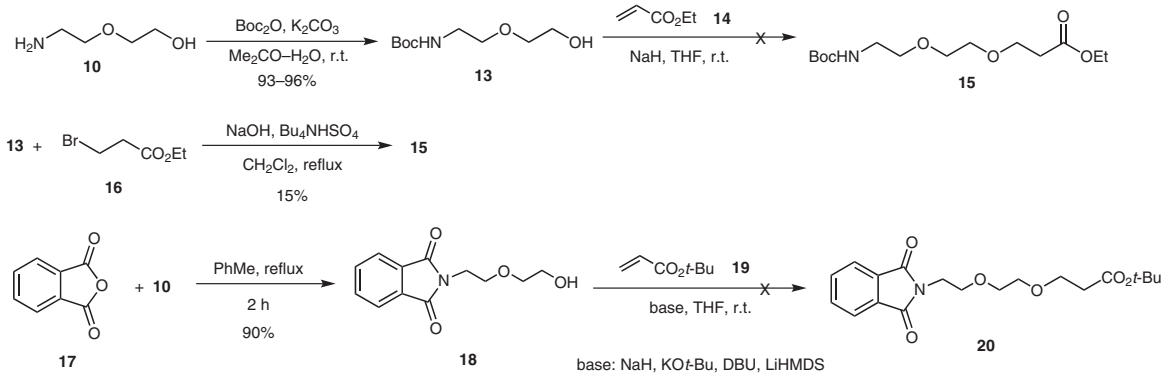


Scheme 3 Retrosynthetic analysis for linker **1**



Scheme 4 Initial attempts to prepare acid **6**

With acid **6** on hand, we turned our attention to the preparation of the second coupling partner, amino ester **7**. Several approaches were tried, which are described in Scheme 5. 2-(2-Aminoethoxy)ethanol (**10**), which already possesses the desired amino functionality found in **1**, was treated with Boc_2O to afford the protected intermediate **13** in excellent yield.[13] The subsequent Michael addition with ethyl acrylate (**14**) could not be reproduced after following the same reaction conditions that have been employed by other groups for very similar substrates.[14] Even though the desired product could be detected by mass spectrometry analysis, the reaction did not proceed to completion and some decomposition was observed in the presence of catalytic sodium hydride or Na metal and a large excess of acrylate.

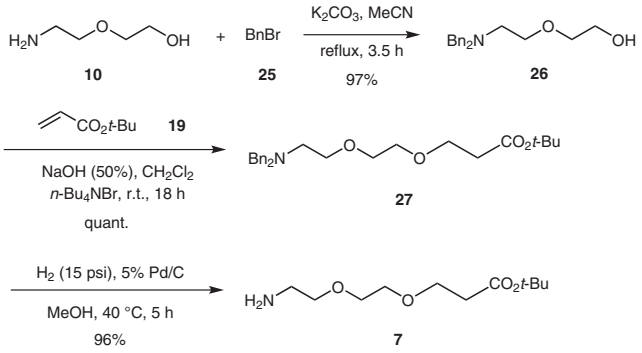


Scheme 5 Failed attempts to generate amino ester **7**

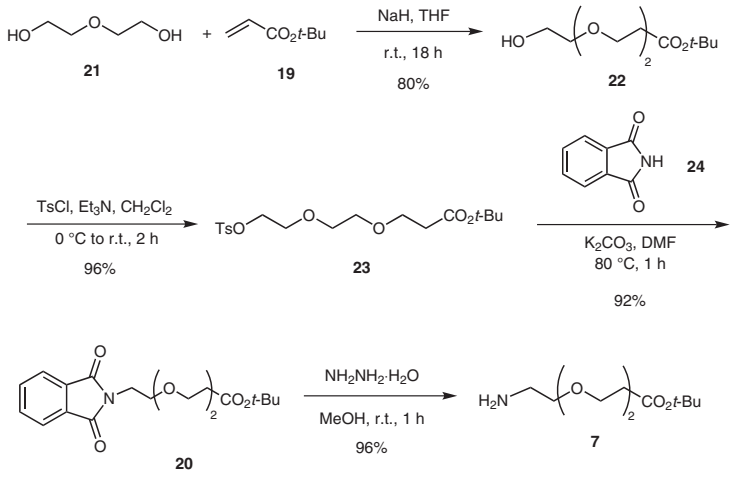
A second approach involved the reaction between alcohol **13** and ethyl 3-bromopropionate (**16**) to give ester **15**. A screen of conditions was carried out (Et_3N, 70 °C,[15] K_2CO_3 in acetone, 70 °C,[16] K_2CO_3 in DMF, r.t. or 70 °C,[17] K_2CO_3 in MeCN, 70 °C, KO*t*-Bu in *t*-BuOH, r.t.,[18] NaOH in *n*-Bu_4NHSO_4 in CH_2Cl_2, r.t. or reflux,[19] DIPEA in CH_2Cl_2, reflux,[20] NaOH and *n*-Bu_4NBr in toluene, r.t., NaOH and *n*-Bu_4NBr in MTBE, r.t.) but only the combination of sodium hydroxide and tetrabutylammonium bisulfate in dichloromethane at reflux gave the desired product albeit in very low yield (15%) after chromatography. When sodium hydride in tetrahydrofuran was employed,[21] the desired product was also detected by mass spectroscopy analysis but, due to the low conversion, it was not isolated.

Finally, a third approach was pursued that involved the Michael addition of phthalimido alcohol **18**[22] to *tert*-butyl acrylate (**19**), but all the conditions that were tried led to complex mixtures.

In view of the lack of success of the previous approaches, a new synthetic route was devised that started from diethylene glycol (**21**, Scheme 6). As has been previously reported,[21] the Michael addition of **21** to *tert*-butyl acrylate (**19**) in the presence of catalytic sodium hydride gave hydroxy ester **22** in 80% yield. Tosylation of **22**[23] followed by displacement of the tosylate group with phthalimide (**24**)[24] provided intermediate **20**. The cleavage of the phthalimido group was carried out with hydrazine hydrate[25] to afford amine **7** in almost quantitative yield. Methyl hydrazine and methyl amine could also be used for this transformation, but lower purities were obtained with these two reagents. On the other hand, the use of concentrated ammonium hydroxide in a sealed reactor led to



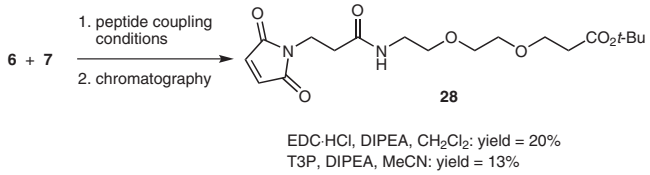
Scheme 7 Concise, final approach to amine **7** from (2-aminoethoxy)ethanol (**10**)



Scheme 6 First successful route to amine **7**

decomposition.[22b] Even though all the intermediates in this route are oils, no chromatographic purification was required for any of them and the crude intermediates could be used without further purification.

In addition to this route from diethylene glycol (**21**), our synthetic efforts eventually translated into a successful and shorter route from 2-(2-aminoethoxy)ethanol (**10**) that became the route of choice (Scheme 7). Thus, the amino group in **10** was protected with benzyl bromide (**25**) to provide intermediate **26** in excellent yield.[26] A screen was then performed to determine the optimal conditions for the Michael addition of **26** to *tert*-butyl acrylate (**19**). A base screen in tetrahydrofuran as solvent gave mostly unreacted alcohol (LiHMDS, DBU) or decomposition (KO*t*-Bu). Based on some published work on similar substrates,[27] our attention was focused on phase-transfer catalysis. Several catalysts were tested (*n*-Bu_4NBr, *n*-Bu_4NOH, *n*-Bu_4HSO_4, $BnMe_3NOH$, $BnEt_3NCl$, $MeBu_3NCl$, 0.1 equiv) with 50% sodium hydroxide (5 equiv) and *tert*-butyl acrylate (1.5 equiv) in dichloromethane at room temperature. Even though all the catalysts generated the desired product to a great extent, tetrabutylammonium bromide gave the cleanest impurity profile with less than 6% of unreacted alcohol. Further optimization of the reactions conditions led to the use of 5 equivalents of *tert*-butyl acrylate (**19**), 0.2 equivalent of tetrabutylammonium bromide, and 5 equivalents of 50% sodium hydroxide in 10 volumes of dichloromethane, which resulted in complete conversion to Michael adduct **27** in quantitative yield and excellent purity (≥95%, area%). Therefore, the choice of the right protection for the amino group was found to be critical for the successful outcome of the subsequent Michael addition step. The synthesis of amine **7** was completed by hydrogenolysis of dibenzylamine **27** in the presence of 5% Pd/C.[24]

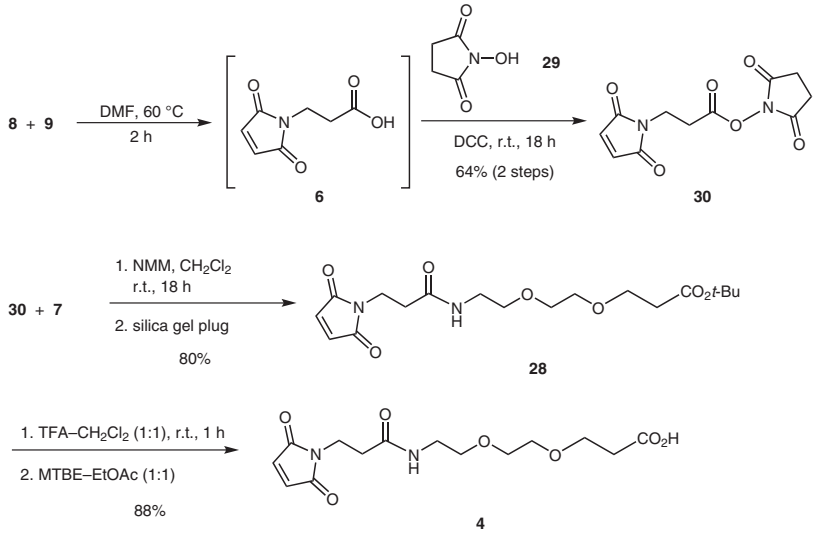


Scheme 8 Initial amide bond formation conditions for the coupling of acid **6** and amine **7**

With the syntheses of both acid **6** and amine **7** accomplished, an investigation of the reaction conditions to couple them was performed. Only EDC and propylphosphonic anhydride (T3P) provided the desired product **28**, even though in very low yield after chromatography (Scheme 8). Other coupling reagents such as (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP), *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU), and *N*,*N*′-diisopropylcarbodiimide (DIC) gave decomposition. Interestingly, when 1,1′-carbonyldiimidazole (CDI) was employed, the product resulting from the Michael addition of imidazole to **28** was obtained.

A literature search revealed that this type of coupling is usually implemented by utilizing a preactivated acid such as **30** (Scheme 9).

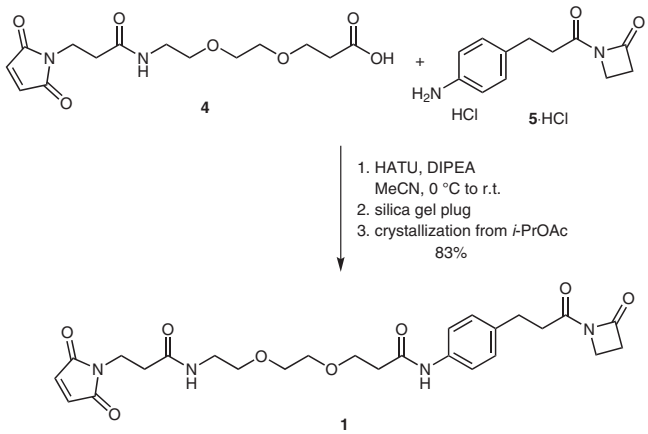
We therefore prepared this material according to a modified literature procedure in a two-step, one-pot process via the reaction between maleic anhydride (**8**) and β-alanine (**9**) in *N*,*N*-dimethylformamide at 60 °C to give acid **6** which, without isolation, underwent reaction with *N*-hydroxysuccinimide in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) as coupling reagent.[28] An ethanol slurry provided material of excellent purity in 64% yield. The subsequent coupling with amine **7** was carried out in dichloromethane with 4-methylmorpholine (NMM) as base to afford *tert*-butyl ester **28** in 80% yield after a silica gel plug to remove polar impurities.[29] Other base and solvent combinations (Et_3N–DMF,[30] aq $NaHCO_3$ in MeOH[31]) also provided the desired product but in lower purities. The synthesis of **4** was completed after *tert*-butyl



Scheme 9 Final coupling conditions between acid **6** and amine **7** and *tert*-butyl ester cleavage to give acid **4**

ester cleavage with 1:1 trifluoroacetic acid–dichloromethane, which gave a much cleaner reaction than anhydrous 4 M hydrochloric acid in 1,4-dioxane. After removing the solvent under vacuum, the addition of 1:1 MTBE–EtOAc caused acid **4** to crystallize in 88% yield and ≥95% purity (area%).

With the synthetic route to acid **4** developed, the optimization of the reaction conditions for the final coupling with aniline **5** was investigated. In our hands, the amide bond formation in the presence of EDC·HCl also required chromatography and the impurity profile was not always reproducible. In addition, some of the impurities were difficult to remove by recrystallization or trituration from a number of solvents. As a consequence, alternative coupling reagents such as *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU), PyBOP, and DIC were investigated. HATU in *N*,*N*-dimethylformamide gave complete and very clean reaction after only 30 minutes, whereas PyBOP and DIC in this same solvent gave a small amount of the desired product as part of complex mixtures by HPLC. The coupling with HATU in acetonitrile was also tried and it performed equally well than in *N*,*N*-dimethylformamide. Since residual *N*,*N*-dimethylformamide was found as a contaminant after workup, we opted for running the reaction in acetonitrile. The final conditions for this coupling are shown in Scheme 10.



Scheme 10 Optimized coupling conditions between acid **4** and aniline hydrochloride **5** to produce linker **1**

After the reaction was complete and an aqueous workup was performed to remove water-soluble by-products from the coupling reagent, the crude material was passed through a silica gel plug to remove baseline impurities. The addition of *i*-PrOAc to the resulting oily product caused the crystallization of linker **1** in 83% yield and >98% purity (area%).

In conclusion, a short and efficient synthetic approach for the preparation of linker **1** has been developed. An optimized procedure for the preparation of acid **4** afforded this material in five steps and 65% yield for the longest synthetic branch from inexpensive and readily available starting materials. Optimized conditions have also been found for the coupling between acid **4** and aniline hydrochloride **5** with HATU as coupling agent. Due to the lack of chromatographic purifications, this route has been easily scaled up to produce multi-hundred grams of non-GMP material for preliminary testing and over 60 g under cGMP conditions for clinical studies.

All reagents were obtained from commercial sources and used as received. Reaction completion and product purity were evaluated by HPLC or GC using the following conditions: a) HPLC: column: Zorbax SB-C18 4.6 × 50 mm, 1.8 μm; wavelength: 215 nm; column temperature: 25 °C; injection volume: 5 μL; eluent: A) H_2O (0.2% $HClO_4$), B) MeCN; gradient: (0 min) A) 90%, B) 10%; (5 min) A) 5%, B) 95%; (10 min) A) 5%, B) 95%; b) GC: column: Agilent HP-1, 0.2 mm × 12 m, 0.33 μm; initial temperature: 35 °C; final temperature: 290 °C; rate: 30 °C/min. ^{1}H NMR and ^{13}C NMR spectra were recorded on a 400 MHz spectrometer in either $CDCl_3$ or DMSO-d_6 as both solvent and internal standard. Nominal and accurate HRMS experiments were performed using Thermo Orbitrap FTMS spectrometer in the positive and negative mode.

2,5-Dioxopyrrolidin-1-yl 3-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)propanoate (30)

To a solution of maleic anhydride (**8**; 86.0 g, 877 mmol) in DMF (1.03 L) was added β-alanine (**9**; 78.14 g, 877 mmol). The resulting suspension was heated to 60 °C and, after 1 h, a solution was obtained. After 2 h, the mixture was cooled to 0–5 °C and *N*-hydroxysuccinimide (**29**; 126.17 g, 1.09 mol) was added followed by DCC (361.9 g, 1.75 mol) over 30 min in several portions while the internal temperature was held below 22 °C. The thick, white slurry was then stirred at 20 °C for 18 h. The slurry was filtered and the solid (dicyclohexyl urea) was washed with H_2O (1 L) and CH_2Cl_2 (1 L) and discarded. To the filtrates was added CH_2Cl_2 (1 L) and the phases were separated. The aqueous layer was extracted with CH_2Cl_2 (2 × 300 mL) and the combined organic extracts were washed with H_2O [500 mL; a small amount of brine (50 mL) was added to facilitate phase separation], sat. aq $NaHCO_3$ (2 × 300 mL), and dried ($MgSO_4$). The solvent was removed to give an oily, light tan solid that was slurried in EtOH (520 mL) for 2 h at 20 °C. The solid was filtered, washed with EtOH (2 × 75 mL) and dried under vacuum at 30 °C for 72 h to give 186.35 g (64%) of NHS ester **30** as a white solid;[32] HPLC purity: 96.6% (area%); mp 169–171 °C.

IR (ATR cell): 3088, 2954, 1825, 1783, 1705, 1583, 1446, 1433, 1382, 1325, 1298, 1250, 1211, 1149, 1070, 1049, 998, 956, 901, 836, 813, 786, 767, 756, 696, 651, 634, 596, 561, 544, 528 cm^{-1}.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.80 (s, 4 H), 3.05 (t, J = 6.90 Hz, 2 H), 3.75 (t, J = 6.78 Hz, 2 H), 7.05 (s, 2 H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 25.38, 29.00, 32.67, 134.63, 166.72, 169.93, 170.52.

HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{11}H_{11}N_2O_6$: 267.06116; found: 267.06147.

2-[2-(Dibenzylamino)ethoxy]ethanol (26)

To a solution of 2-(2-aminoethoxy)ethanol (**10**; 50.0 g, 475 mmol) in MeCN (1.25 L) was added benzyl bromide (**25**; 162.68 g, 951 mmol) followed by K_2CO_3 (157.74 g, 1.14 mol). The resulting mixture was heated to 50–55 °C for 3.5 h. The mixture was cooled to 20 °C, the solids were filtered off, washed with MeCN (2 × 100 mL), and the filtrates were concentrated to an oil. The flask was placed in an ice water bath and aq 1 M HCl (500 mL) was slowly added to dissolve the oil while the internal temperature was held below 25 °C. The aqueous phase was washed with EtOAc (1 × 200 mL, 1 × 100 mL) and the organic extracts were discarded. The acidic aqueous phase was cooled in an ice water bath and aq 50% NaOH was slowly added to bring the pH up to 10–11 (30 mL) while the internal

temperature was held below 20 °C. The aqueous phase was extracted with CH_2Cl_2 (4 × 250 mL) and the combined organic extracts were dried ($MgSO_4$). The solvent was removed under vacuum to give 136.68 g (97%) of alcohol **26** as a yellow oil that was used in the next step without further purification; HPLC purity: 97% (area%).

IR (ATR cell): 3410, 3061, 3026, 2869, 1601, 1494, 1452, 1367, 1246, 1118, 1058, 1027, 976, 888, 806, 732, 697, 621, 565, 530 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 2.63 (br s, 1 H), 2.74 (t, *J* = 5.90 Hz, 2 H), 3.50–3.55 (m, 2 H), 3.63 (t, *J* = 6.02 Hz, 2 H), 3.68–3.74 (m, 6 H), 7.26–7.32 (m, 2 H), 7.37 (t, *J* = 7.53 Hz, 4 H), 7.40–7.45 (m, 4 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 53.01, 59.02, 61.57, 69.62, 72.12, 127.02, 128.28, 128.94, 139.38.

HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{18}H_{24}NO_2$: 286.18016; found: 286.17984.

tert-Butyl 3-{2-[2-(Dibenzylamino)ethoxy]ethoxy}propanoate (27)

To a solution of alcohol **26** (26.0 g, 91 mmol) and *tert*-butyl acrylate (**19**; 58.38 g, 455 mmol) in CH_2Cl_2 (235 mL) was added *n*-Bu_4NBr (5.87 g, 18 mmol) and aq 50% NaOH (36.44 g, 455 mmol) as a small stream. The mixture was stirred at 20 °C for 3 h and H_2O (200 mL) was added. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic extracts were washed with H_2O (50 mL) and dried ($MgSO_4$). The solvent was removed under vacuum to give a yellow oil that partially solidified due to the presence of residual phase-transfer catalyst. Heptane (100 mL) was added to the residue and the suspension was stirred at 20 °C for 1 h. The mixture was filtered through Celite and the Celite pad was washed with heptane (2 × 25 mL). The filtrates were concentrated under vacuum to give 36.74 g (97%) of *tert*-butyl ester **27** as a pale yellow oil that was used in the next step without further purification; HPLC purity: 95.9% (area%).

IR (ATR cell): 3027, 2869, 1728, 1601, 1494, 1453, 1392, 1366, 1328, 1251, 1155, 1113, 1074, 1027, 847, 745, 733, 698, 606, 541, 534 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 1.46 (s, 9 H), 2.49 (t, *J* = 6.60 Hz, 2 H), 2.71 (t, *J* = 6.20 Hz, 2 H), 3.51–3.55 (m, 2 H), 3.55–3.62 (m, 4 H), 3.66 (s, 4 H), 3.70 (t, *J* = 6.60 Hz, 2 H), 7.20–7.26 (m, 2 H), 7.28–7.34 (m, 4 H), 7.36–7.41 (m, 4 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 34.70, 34.86, 34.88, 39.48, 66.44, 69.82, 70.05, 70.32, 134.45, 170.55, 170.86, 174.80.

HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{25}H_{36}NO_4$: 414.26389; found: 414.26337.

tert-Butyl 3-[2-(2-Aminoethoxy)ethoxy]propanoate (7)

To a solution of dibenzylamine **27** (35.0 g, 85 mmol) in MeOH (420 mL) was added 5% Pd/C (JM US 2, 14 g) and the mixture was hydrogenated in an Atlantis reactor at 40 °C and 15 psi. After 2 h, the catalyst was filtered off and the filtrates were concentrated to give 18.90 g (96%) of amine **7** as a hazy oil that was used in the next step without any further purification.

IR (ATR cell): 2977, 2868, 1727, 1456, 1393, 1367, 1254, 1157, 1113, 847, 755, 589, 564, 547, 536 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 1.30 (s, 9 H), 2.36 (t, *J* = 6.43 Hz, 2 H), 2.71 (t, *J* = 5.19 Hz, 2 H), 3.36 (t, *J* = 5.19 Hz, 2 H), 3.43–3.50 (m, 4 H), 3.57 (t, *J* = 6.64 Hz, 2 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.18, 36.36, 41.92, 66.98, 70.32, 70.44, 73.58, 80.52, 170.92.

HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{11}H_{24}NO_4$: 234.16998; found: 234.17009.

tert-Butyl 3-(2-{2-[3-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)propanamido]ethoxy}ethoxy)propanoate (28)

To a solution of amine **7** (18.83 g, 81 mmol) in CH_2Cl_2 (180 mL) was added *N*-methylmorpholine (10.20 g, 101 mmol). The flask was cooled in a water bath at 10 °C and NHS ester **30** (17.90 g, 67 mmol) was added in small portions while the internal temperature was held below 20 °C. The cooling bath was removed and the resulting mixture was stirred at 20 °C for 18 h. The cloudy, orange mixture was cooled in an ice water bath and, when the internal temperature was below 15 °C, 10% aq citric acid (220 mL) was added to bring the pH down to 3. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent was removed to give 32 g of a pale brown oil that was passed through a plug of silica gel (EtOAc as mobile phase) to give 21.25 g (80%) of maleimido *tert*-butyl ester **28** as a very thick, colorless oil; HPLC purity: 95.8% (area%).

IR (ATR cell): 3307, 2872, 1704, 1652, 1542, 1445, 1407, 1367, 1251, 1112, 956, 828, 755, 695, 613, 544, 536, 528 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 1.36 (s, 9 H), 2.38–2.49 (m, 4 H), 3.29–3.36 (m, 2 H), 3.41–3.47 (m, 2 H), 3.51 (s, 4 H), 3.64 (t, *J* = 6.22 Hz, 2 H), 3.75 (t, *J* = 7.26 Hz, 2 H), 6.45 (br s, 1 H), 6.63 (s, 2 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.25, 34.50, 34.68, 36.42, 39.37, 67.00, 69.82, 70.34, 70.38, 80.82, 134.37, 170.00, 170.65, 171.20.

HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{18}H_{28}N_2O_7$ + Na: 407.17887; found: 407.17844.

3-(2-{2-[3-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)propanamido]ethoxy}ethoxy)propanoic Acid (4)

A solution of *tert*-butyl ester **28** (14.88 g, 39 mmol) in CH_2Cl_2 (148 mL) was cooled in a water bath at 10 °C and trifluoroacetic acid (148 mL, 1.97 mol) was added as a small stream. The cooling bath was removed and the mixture was allowed to warm to 20 °C and stirred for 1 h. The solvent was removed under vacuum and the oily residue was coevaporated with toluene (2 × 150 mL). To the resulting oil was added 1:1 EtOAc–MTBE (100 mL) and some seeds of acid **4**. The suspension was stirred at 20 °C for 1 h and the solid was filtered, washed with 1:1 EtOAc–MTBE (10 mL) and dried under vacuum at 35 °C for 1 h to give 9.66 g of acid **4** as a white solid; HPLC purity: 99.0% (area%). The filtrates were concentrated and slurried in 1:1 EtOAc–MTBE (40 mL) at 20 °C for 72 h. The resulting solid was filtered, washed with 1:1 EtOAc–MTBE (10 mL) and dried as for the previous batch to give an additional 1.51 g of acid **4** as a white solid for a combined yield of 88%; mp 72–74 °C; HPLC purity: 99.0% (area%).

IR (ATR cell): 3294, 3088, 2913, 1691, 1633, 1557, 1496, 1481, 1449, 1415, 1373, 1328, 1315, 1240, 1218, 1128, 987, 948, 922, 841, 762, 697, 644, 618, 549, 536 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 2.56 (t, *J* = 7.50 Hz, 2 H), 2.63 (t, *J* = 5.81 Hz, 2 H), 3.38–3.45 (m, 2 H), 3.50–3.56 (m, 2 H), 3.57–3.66 (m, 4 H), 3.77 (t, *J* = 5.81 Hz, 2 H), 3.85 (t, *J* = 7.50 Hz, 2 H), 6.53 (br s, 1 H), 6.70 (s, 2 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 34.70, 34.86, 34.88, 39.48, 66.44, 69.82, 70.05, 70.32, 134.45, 170.55, 170.86, 174.80.

HRMS (ESI): *m*/*z* $[M - H]^+$ calcd for $C_{14}H_{19}N_2O_7$: 327.11970; found: 327.11871.

3-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-*N*-{2-[2-(3-oxo-3-{4-[3-oxo-3-(2-oxoazetidin-1-yl)propyl]phenylamino}propoxy)ethoxy]ethyl}propanamide (1)

Acid **4** (1.00 g, 3.02 mmol) and aniline hydrochloride **5** (0.70 g, 2.75 mmol) were suspended in MeCN (14 mL). The suspension was cooled in an ice water bath and HATU (1.25 g, 3.30 mmol) and DIPEA (1.44 mL, 8.24 mmol) were added. The cooling bath was removed and the resulting yellow solution was allowed to warm to 20 °C. After 1 h, the solvent was removed under vacuum to give a yel-

low oil that was dissolved in CH_2Cl_2 (40 mL). The organic phase was washed with H_2O (2 × 10 mL), aq 1 M HCl (5 mL), H_2O (5 mL), and dried ($MgSO_4$) with a small amount of solid $NaHCO_3$. The solvent was removed to give 2.9 g of a yellow oil that was passed through a pad of silica gel (MeCN as mobile phase) to give 1.74 g of a clear oil. To this oil was added *i*-PrOAc (40 mL), which caused the oil to crystallize. The suspension was stirred for 2 h at 20 °C and the solid was filtered, washed with *i*-PrOAc (2 × 10 mL) and dried under vacuum at 40 °C for 18 h to give 1.22 g (84%) of **1** as a white solid; HPLC purity: 98.6% (area%); mp 98–99 °C.

IR (ATR cell): 3248, 3081, 2898, 2864, 2162, 1980, 1784, 1698, 1652, 1633, 1604, 1570, 1544, 1516, 1489, 1445, 1411, 1379, 1318, 1265, 1247, 1212, 1140, 1066, 1043, 1028, 1009, 985, 963, 923, 835, 827, 760, 723, 697, 617, 595, 570, 552, 529 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 2.38 (t, *J* = 7.23 Hz, 2 H), 2.58 (t, *J* = 5.68 Hz, 2 H), 2.84–2.96 (m, 4 H), 2.98 (t, *J* = 5.31 Hz, 2 H), 3.30 (q, *J* = 5.31 Hz, 2 H), 3.47 (t, *J* = 5.10 Hz, 1 H), 3.51 (t, *J* = 5.31 Hz, 2 H), 3.54–3.67 (m, 4 H), 3.72 (t, *J* = 7.23 Hz, 2 H), 3.78 (t, *J* = 5.77 Hz, 2 H), 6.32 (t, *J* = 5.22 Hz, 1 H), 6.63 (s, 2 H), 7.10 (d, *J* = 8.42 Hz, 2 H), 7.39 (d, *J* = 8.42 Hz, 2 H), 8.56 (s, 1 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 29.55, 34.47, 34.68, 36.08, 36.75, 37.97, 38.28, 39.32, 67.15, 69.86, 70.20, 70.34, 120.32, 129.15, 134.40, 136.37, 136.67, 165.30, 170.02, 170.11, 170.32, 170.75.

HRMS (ESI): *m/z* $[M + Na]^+$ calcd for $C_{26}H_{32}N_4O_8$ + Na: 551.21233; found: 551.21190.

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Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synthesis.

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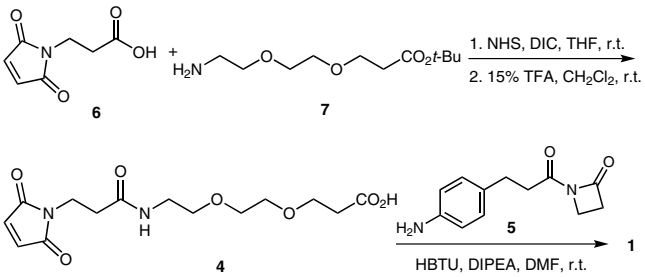
(10) During the preparation of this manuscript, a synthesis of linker **1** has been reported from acid **6** and amine **7** (Scheme 11). However, no experimental details, yields, or information on reaction scale were included. See: Palanki, M. S. S.; Bhat, A.; Lappe, R. W.; Liu, B.; Oates, B.; Rizzo, J.; Stankovic, N.; Bradshaw, C. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4249.



Scheme 11 Synthesis of linker **1** from acid **6** and amine **7**

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