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Photoprotected Peptide α-Ketoacids and Hydroxylamines for Iterative and One-Pot KAHA Ligations: Synthesis of NEDD8

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Dedication ((optional))

The convergent synthesis of proteins by multiple ligations requires segments protected at the N- and/or C-terminus with masking groups that are orthogonal to the acid and base labile protecting groups used in Fmoc-SPPS. They must be stable to solid phase peptide synthesis, HPLC purification, and ligation conditions and easily removed in the presence of unprotected side chains. In this report, we document photolabile protecting groups for both α-ketoacids and hydroxylamines, the key functional groups employed in the α-ketoacid–hydroxylamine (KAHA) ligation. The novel photoprotected α-ketoacid is easily installed onto numerous different C-terminal peptide α-ketoacids and removed by UV light under aqueous conditions. These advances were applied to the one-pot synthesis of NEDD8, an important modifier protein, by three different convergent routes. These new protecting groups provide greater flexibility in the order of fragment assembly and reduce the number of reaction and purification steps needed for protein synthesis with the KAHA ligation.

Keywords: Ligation • Protecting groups • Photochemistry • Modifier Proteins • Peptides

Introduction

The total chemical synthesis of proteins allows direct access to molecules not available by biological methods. In particular, synthesis allows the incorporation of dyes, tags, post-translational modifications, unnatural amino acids, and D-amino acids. The most widely practiced method relies on the remarkable native chemical ligation of C-terminal peptide thioesters and segments with N-terminal cysteine residues, as first report by Kent and co-workers.[i] More recently, alternative ligation methods including the Ser/Thr ligation (STL)[ii] and the α-ketoacid–hydroxylamine (KAHA)[iii,iv] ligations have emerged and compliment NCL by operating at different ligation sites and under acidic, rather than basic, ligation conditions.

One of the remaining challenges in chemical protein synthesis is the union of multiple segments by sequential ligations to form larger structures.[v] In the case of NCL, kinetically controlled ligations of peptide thioesters of different reactivity can sometimes be used to join three segments in a one-pot fashion.[vi] For larger targets, either the N-terminal Cys or the C-terminal thioester must be masked during one of the ligation reactions. The most common approach is protection of the N-terminal Cys as thiazolidine (Thz) group, introduced by Bang and Kent in 2004 for one-pot ligations.[vii] Using the same strategy, Liu identified a new protecting group for terminal cysteines, which can be removed by H_2O_2 under ligation conditions.[viii] This is effective, but these groups can be difficult to remove, although recent work using $PdCl_2$ induced deprotection is a promising advance.[ix] The C-terminal thioesters are more challenging to mask, but surrogates including peptide hydrazides – which can be oxidized and converted to thioesters post-ligation – and the *N*-sulfanylethylanilide linker (SEAlide), which acts as a masked thioester.[x] More recently, photoprotecting groups on the SEAlide or cysteine, make possible various C to N sequential ligations and convergent strategies.[xi] Dong and collaborators also recently described a ligation forming – after desulfurization – a native proline at the ligation site using an internal activation protocol of a prolyl thioester.[xii] Despite these advances, NCL is not ideal for the synthesis of some proteins due to necessity for either correctly placed Cys residues or additional desulfurization steps.

The KAHA ligation involves the chemoselective reaction between a peptide bearing a C-terminal α-ketoacid and a peptide with a N-terminal hydroxylamine.[xiii] During the development of KAHA ligation for protein synthesis, we successfully prepared several proteins by multi-segment ligation.[xiv-xix] Masking of the hydroxylamine – most commonly (*S*)-5-oxaproline – was achieved with an Fmoc group that could be removed from the ligation products under basic conditions. For masking the C-terminal peptide α-ketoacids, we employed cyanosulfurylides that were oxidized to the α-ketoacids with aqueous Oxone. The cyanosulfurylides are ideal in terms of stability and ease of preparation, but the oxidation step is not compatible with peptides containing unprotected Cys, Met, and Trp residues. Fmoc-protection of the hydroxylamine works well but the deprotection cannot be directly combined with KAHA ligation, as it requires highly diluted basic conditions with the addition of scavengers.

In this report, we introduce photoprotected α-ketoacids and hydroxylamines for sequential and one-pot KAHA ligations for protein synthesis. These masking groups are stable to all reagents and conditions required for Fmoc-SPPS, peptide purification, and KAHA ligation. They are easily removed by irradiation with a standard UV lamp under aqueous conditions, including one-pot ligation/deprotection protocols. The identification of stable, readily handled and removed masking groups for both the hydroxylamine and α-ketoacid reaction partners greatly facililitates protein synthesis from multiple fragments by enabling multiple different segment assembly strategies or one-pot ligations of three or more segments. We demonstrate the utlity of these new protecting groups by the synthesis of the important modifier protein NEDD8 by three different approaches.

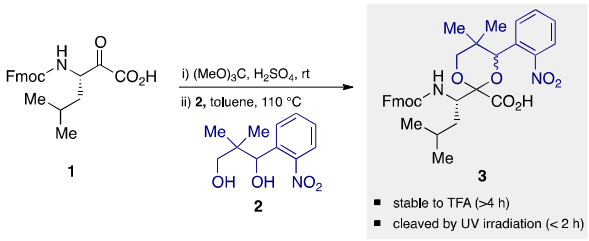
Results and Discussion

Development of Photoprotected α-ketoacids. At the outset of our studies, we established several criteria for the development of an orthogonally protected peptide α-ketoacid: 1) the protecting group must be compatible with Fmoc-SPPS; 2) the protected α-ketoacid must survive the strongly acidic conditions of resin cleavage and side-chain deprotection; 3) the protecting group must be stable to the aqueous, acidic conditions of KAHA ligation and reverse phase HPLC; 4) the deprotection should occur under homogeneous aqueous conditions in the presence of unprotected side chains; and 5) the deprotection should ideally have no added reagents that can interfere with base-labile or nucleophilic functional groups. Although we initially introduced an orthogonal protecting group onto the aromatic ring of the masking group – resulting in the formation of a phenol upon deprotection – complete removal of the protecting group required treatment with concentrated TFA and was not suitable for in-situ deprotection. As an alternative, we sought a ketone protecting group that would be directly and cleanly removed under photochemical conditions.

Photolabile protecting groups for ketones include cyclic acetals bearing a 2-nitro group,[xx,xxi] coumarine derivative used for 1- or 2-photon deprotection,[xxii] thiochromone-type photolabile protecting group[xxiii] and 5-methoxy salicylic alcohol derivative.[xxiv] During the course of our development of acid-labile protecting groups for peptide α-ketoacids, we identified 6-membered ring acetals bearing a gem-dimethyl group as the best choices in terms of introduction of the protecting group, stability to Fmoc-SPPS, and epimerization-free deprotection. More recently, we developed a new protocol for preparing the protected α-ketoacids by transacetalization of a

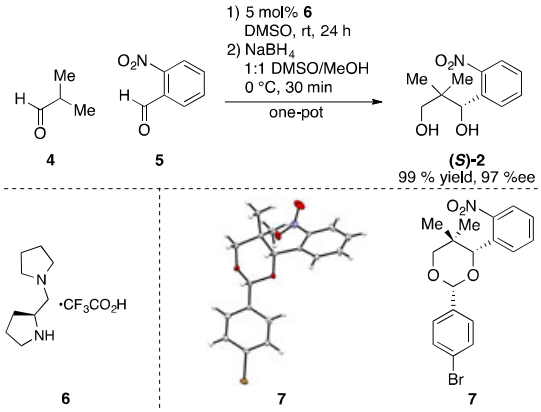
dimethylacetal intermediate under almost neutral conditions (Scheme 1). These conditions allowed us to expand the scope of the protected amino acids to most of the canonical residues.[xxv]

Based on this work, we selected diol **2** as a potential protecting group. The requisite, racemic starting material could be easily prepared in one step from the corresponding aldehydes. We were pleased to find that transacetalization of the bis-dimethylacetal of Fmoc-Leu-α-ketoacid worked well under thermal conditions to give the stable adduct as cyclic acetal **3**. Importantly, preliminary tests demonstrated that the protected α-ketoacids were stable to treatment with neat TFA. Irradiation of a solution of **3** in dilute aqueous CH_3CN at 365 nm with a handheld UV lamp for two hours cleanly returned the α-ketoacid. One of our main concerns about this strategy was possible epimerization during photorelease of the α-ketoacid. Satisfyingly, no epimerization was observed during the deprotection using our previously reported assay.[xiv]



Scheme 1. Protection of α-ketoacids as photolabile cyclic acetals.

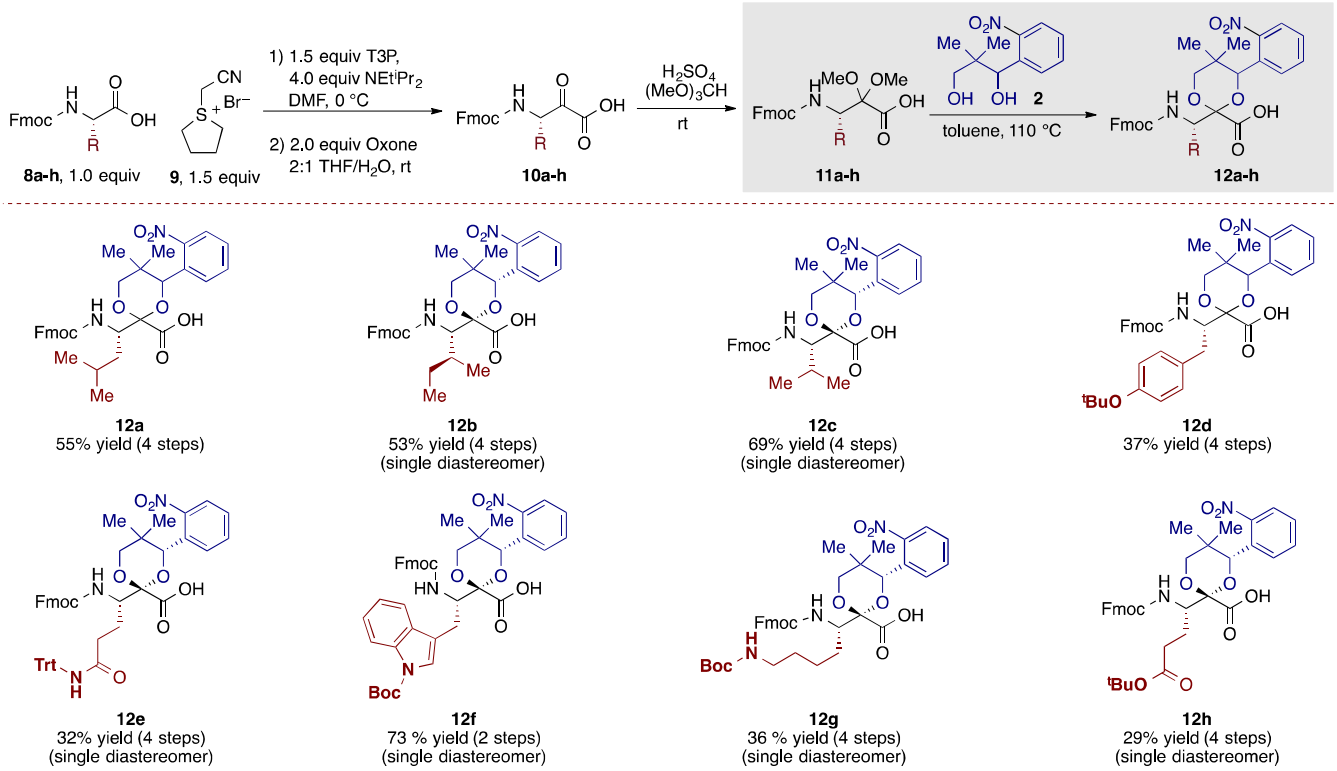
The photoprotected α-ketoacids are formed as a mixture of diastereomers. Although this does not present any obstacles for its use as a masking group, the resulting diastereomeric peptides sometimes show different retention times during HPLC analysis (for example, see **17** in Scheme 4b). Previous studies in our group have shown that only two out of four possible diastereomers are formed, and we postulated that an enantiopure protecting group would result in the formation of a single diastereoisomer of the protected α-ketoacids. Based on work by Barbas III,[xxvi] we identified an organocatalytic approach to prepare diol **2** in two steps from inexpensive starting materials in almost quantitative yield with 97% ee without chromatographic purification (Scheme 2). Attempts to obtain single crystals of enantioenriched diol **2** were unsuccessful. The absolute configuration of **7** was determined to be (*S*) by derivatization to acetal **7**, which could be crystallized and analyzed by single-crystal x-ray diffraction.



Scheme 2. Synthesis of enantiomerically enriched protecting group **2** and determination of the absolute configuration of acetal derivative **7** by single-crystal x-ray diffraction (thermal ellipsoids shown in the ORTEP model are set at a 50% propability level).

Although leucine is both by far the most abundant natural amino acid and performs well in the KAHA ligation, we wished to establish a more general approach to the orthogonal protection of α-ketoacids. To this end, we applied these protection conditions to a variety of α-ketoacids derived from Fmoc-amino acids, using either the racemic or enantioenriched diol. The starting α-ketoacids were prepared by our previously reported route via the cyanosulfurylides and dimethylacetal formation. In all cases, the transacetalization with **2** performed well to give the protected α-ketoacids in good yield (Scheme 3). In the case of isoleucine, valine, glutamine, tryptophan, lysine

and glutamic acid we used the enantiopure diol **(*S*)-2,** resulting in the formation of a single diastereomer of the protected product. The configuration of the newly formed acetal stereocenter was determined by ROESY to be (S) after derivatization of **12f** to the corresponding methyl ester **13**. Relevant ROESY correlations are shown in Figure 1.



Scheme 3. Preparation of a library of photoprotected α-ketoacids.

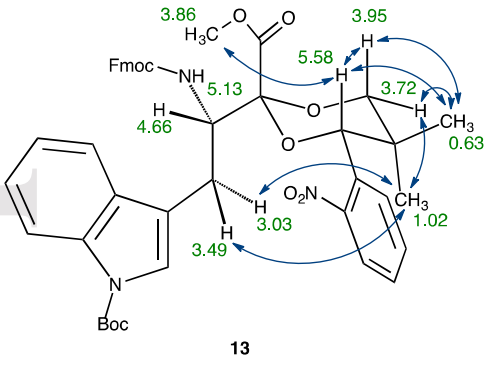
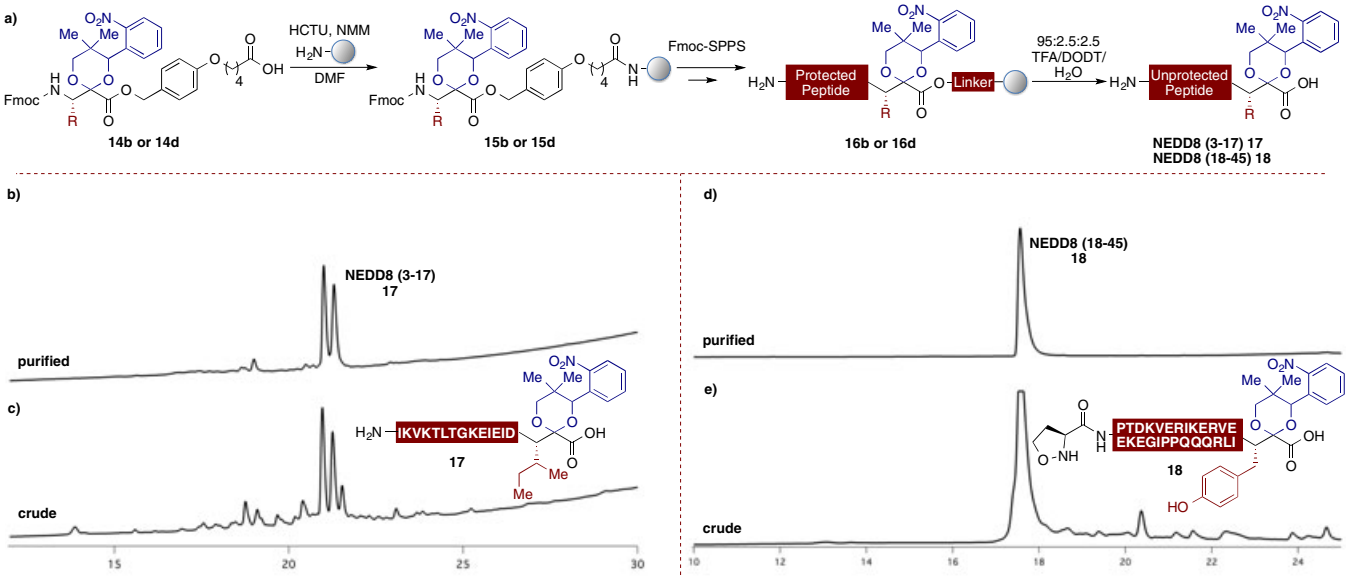


Figure 1: Relevant ROESY correlations (blue arrows) and NMR shifts (green) of **13**.

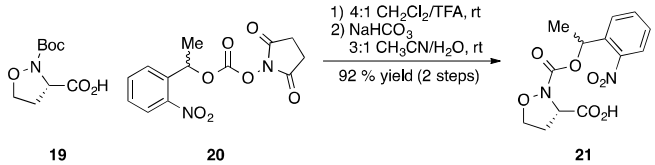
Fmoc-SPPS of Photoprotected C-Terminal Peptide α-ketoacids. In order to utilize the photoprotected α-ketoacids for iterative segment ligations, we applied them to the Fmoc-SPPS of C-terminal protected peptides. Using the same linker we previously reported for traceless preparation of C-terminal peptide α-ketoacids, we loaded the photoprotected α-ketoacids onto a suitable solid support (Scheme 4). This resulting resin was suitable for standard, Fmoc-based SPPS without any deviation from the standard procedures. Upon completion of the peptide synthesis, resin cleavage and side chain deprotection afforded the masked α-ketoacid peptides in good yield. The segments were purified by preparative reverse phase HPLC. Despite the presence of the photolabile group, the linkers and protected peptides could be handled without any special precautions other than protecting them from light during prolonged storage. Using this

approach we prepared several masked peptide α-ketoacids for our synthesis of NEDD8 (vide infra). Preliminary studies on the light-promoted unmasking of the side-chain unprotected peptide α-ketoacids cleanly returned to the desired products.



Scheme 4. Fmoc-SPPS using photoprotected α-ketoacid building blocks (a) and analytical HPLC traces (220 nm) of crude (c and e) and purified (b and d) peptide segments with photoprotected α-ketoacids. In the case of NEDD8 (3-17) **17** (b and c) the two diastereomeric peptides resulting from the racemic protecting group **rac-2** elute as separate peaks.

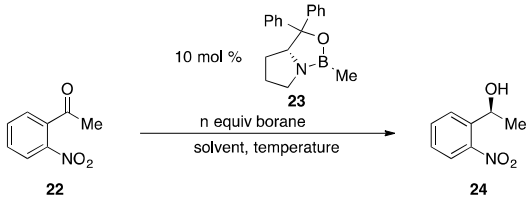
Synthesis of Photoprotected (*S*)-5-Oxaproline. With the goal of establishing one-pot, convergent KAHA ligations for protein synthesis, we also sought to prepare photoprotected hydroxylamines for introduction at the N-terminus of a peptide segment. Among photolabile carbamate protecting groups, 2-nitrophenyl derivatives have emerged as the protecting group of choice in terms of ease of deprotection, minimization of side products, and stability towards standard organic manipulations. Boc-(*S*)-5-Oxaproline **19** was converted to the photolabile carbamate **21** by treatment with TFA followed by coupling with succinimidyl carbonate **20**. Although the use of the racemic protecting group led to the formation of diastereomers, we have so far found that the presence the racemic stereocenter does not usually lead to distinct peaks for the peptide segments during purification or analysis. Importantly, this protected hydroxylamine was completely stable during introduction at the N-terminus of a solid supported peptide segment, peptide cleavage with TFA, and purification by preparative HPLC. The photoprotected (*S*)-5-oxaproline **21** could also be unmasked under mild conditions by irradiation at 365 nm in CH_3CN/H_2O containing 0.1% TFA.



Scheme 5. Synthesis of photoprotected (*S*)-5-Oxaproline **21**.

The enantioenriched protecting group can be prepared by enantioselective Corey-Bakashi-Shibata (CBS) reduction[xxvii] of 2-nitroacetophenone (**22**) using catalytic amounts of chiral oxazaborolidine **23** and catecholborane as the stoichiometric reducing agent. Optimization of the reaction conditions allows access to the enantiopure alcohol in >99.9:0.1 er after recrystallization. The reaction was conveniently scaled up to 8 grams of product. The resulting alcohol can be transformed to the corresponding chloroformate or succinimidyl carbonate and coupled to (*S*)-5-oxaproline by the same procedure as shown in Scheme 5.

Table 1. Optimization of the CBS reduction for the synthesis of the enantioenriched alcohol **24**.

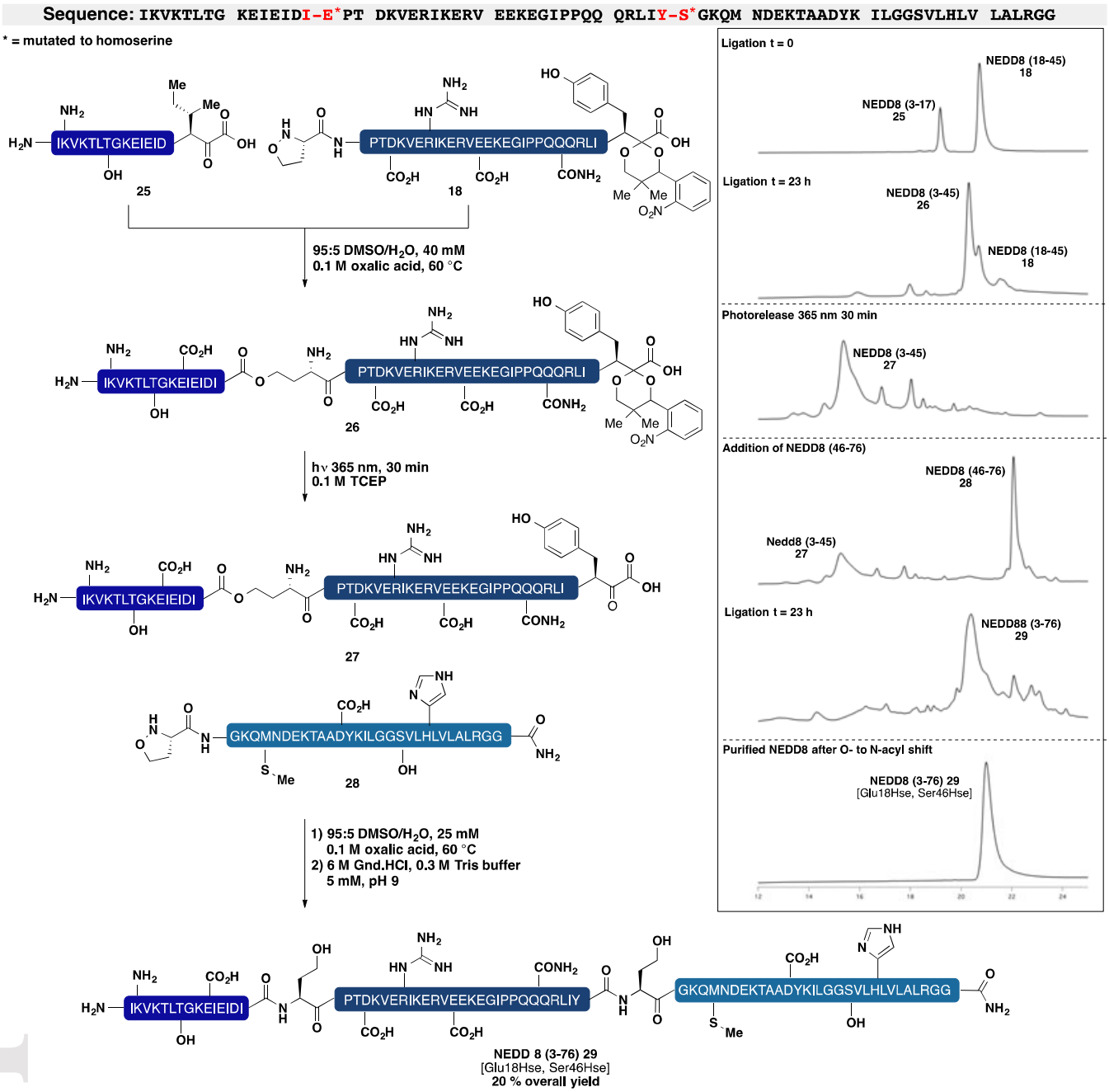


entry	borane	equiv borane	solvent	temperature	er[a]
1	BH_3⋅SMe_2	0.66	THF	0 °C	62.1 : 37.9
2	CatBH	1.50	toluene	0 °C	83.5 : 16.5
3	CatBH	1.50	toluene	23 °C	78.7 : 21.3
4	CatBH	1.50	toluene	-78 °C	92.7 : 7.3
5	CatBH	1.50	CH_2Cl_2	0 °C	87.8 : 12.2
6	CatBH	1.50	CH_2Cl_2	-78 °C	94.1 : 5.9
7[b]	CatBH	1.50	CH_2Cl_2	-78 °C	>99.9 : 0.1

a) determined by chiral SFC (column Chiralpak ADH) with detection at 220 nm. b) after recrystallization from 25:1 hexanes/EtOAc (74% recrystallization yield). CatBH = catecholborane

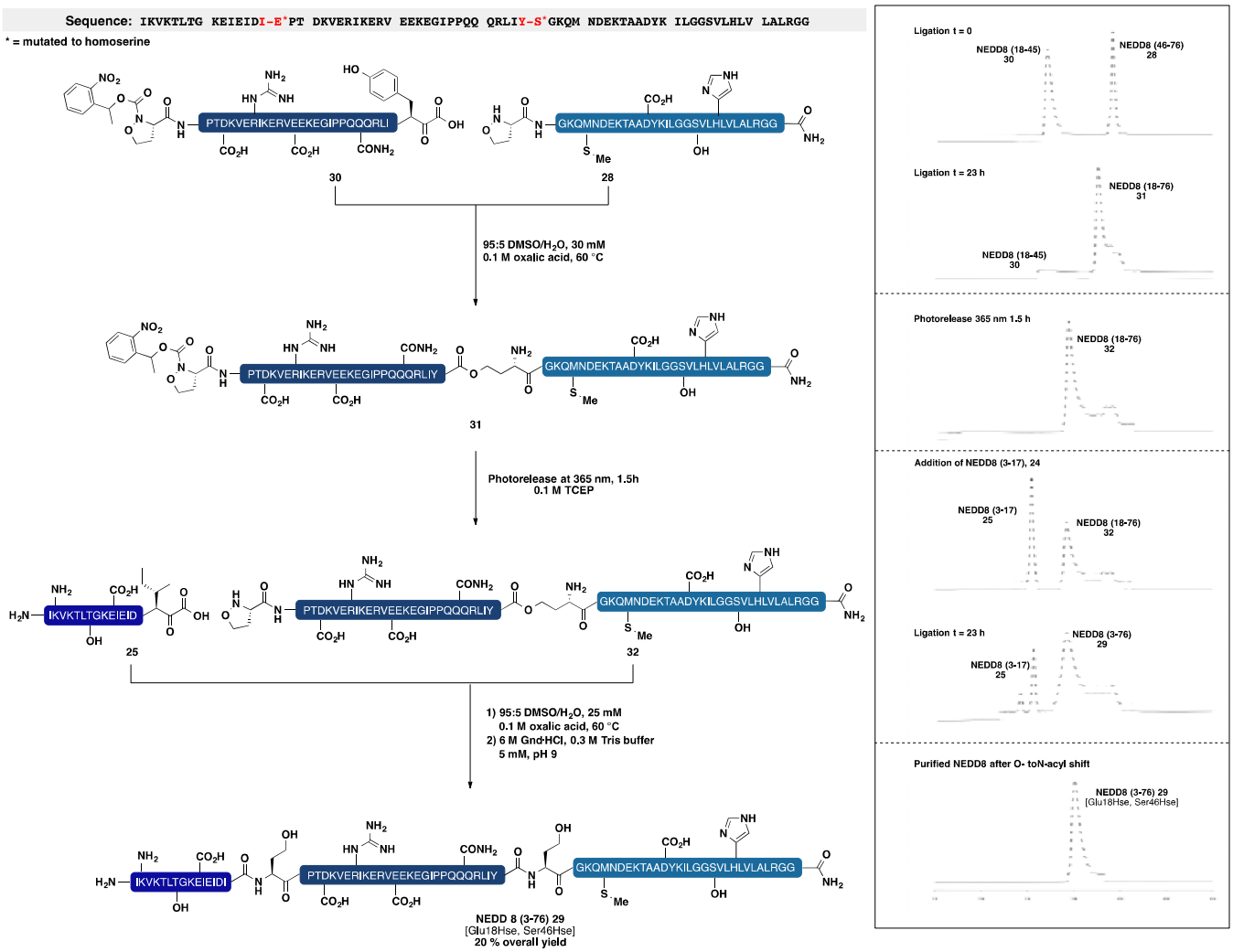
Convergent and One-Pot Ligations of NEDD8. NEDD8 (neural precursor cell expressed, developmentally down-regulated 8) is a small ubiquitin like protein involved in the activation of the cullin-RING ligases, a major family of E3-ligases. Proteins of the cullin family are the most prominent and well-understood examples of NEDD8 substrates but many other unrelated targets[xxviii], such as the Von Hippel–Lindau (VHL) tumor supresor[xxix] or the epidermal growth factor receptor[xxx] (EGFR), have been identified and it is likely that the number of NEDD8 substrates will keep increasing. In a process similar to ubiquitination, NEDD8 is attached to a lysine on its protein substrate in four steps: release of the mature NEDD8, ATP dependent activation, transfer to an E2 enzyme and finally ligation to the target protein.[xxxi,xxxii] In order to further study the neddylation process, which is hampered by the very low expression feasibility, an efficient strategy to easily prepare variants of NEDD8 is required. In order to assemble NEDD8, we envisioned using multiple strategies: a 3 segments process in the N-to C-direction using the photoprotected tyrosine α-ketoacid, a three segments strategy in the C- to N-direction using the photorpotected oxaproline and a convergent four segments strategy using both monomers.

All peptides were prepared by standard Fmoc SPPS. NEDD8 (3-17) (**25**) was prepared from protected isoleucine α-ketoacid and assembled until Thr7 after which Fmoc deprotection appeared to be difficult. From this point on, the deprotection was achieved using 20% piperidine in DMF with 3% of DBU, preceded by a LiCl (0.8 M in DMF) wash. Two variants of the NEDD8 (18-45) segments were prepared, one (**18**) with the photoprotected tyrosine α-ketoacid and (*S*)-Boc-5-oxaproline and the other one (**30**) with a tyrosine α-ketoacid and photoprotected (*S*)-5-oxaproline. For the three segment strategies, NEDD8 (46-76) **28** was synthesized on Rink amide resin. For the convergent synthesis, NEDD8 (46-62) **33** was prepared with a N-terminal photoprotected (*S*)-5-oxaproline and NEDD8 (63-76) **34** was synthesized on Rink amide resin with a final coupling of (*S*)-Boc-5-Oxaproline.



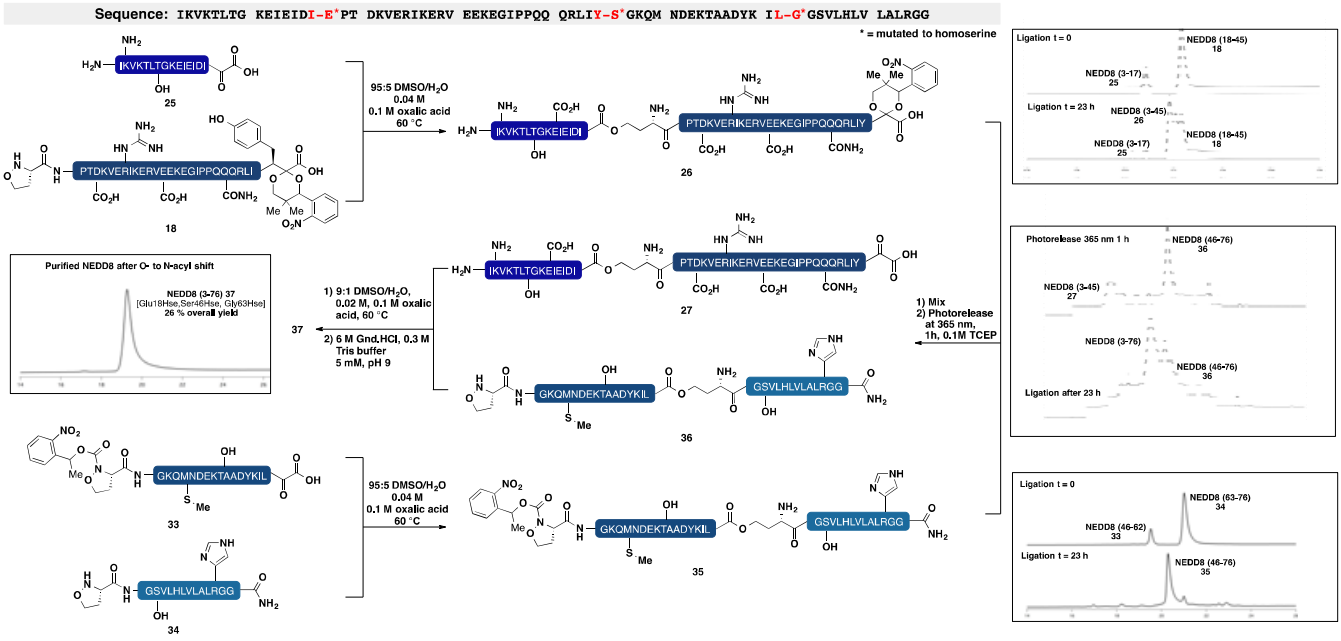
Scheme 6: One-pot synthesis of Nedd8 in the N- to C-direction using a photoprotected tyrosine α-ketoacid.

With all of the required segments in hand, we started the synthesis of NEDD8 with the first strategy involving sequential ligations from N- to C-terminus (Scheme 6). The first ligation between **18** and **25** was performed at 60 °C in a mixture of DMSO/H_2O containing 0.1 M oxalic acid over 23 h. The reaction stopped at about 80% conversion, possibly due to steric hindrance of the isoleucine α-ketoacid in **25**. We noticed that the same ligation with leucine α-ketoacid was achieved with better conversion. The ligated product **26** appeared as a broad peak on the HPLC due to the formation of diastereoisomers induced by the racemic photoprotecting group. Tris(2-carboxyethyl)phosphin (TCEP) was added (final concentration of TCEP: 0.1 M) to avoid side reactions and the ligation mixture was directly irradiated at 365 nm for 30 min. HPLC and MALDI-MS confirmed complete deprotection and the free α-ketoacid peptide **27** appeared as a broad peak typical for peptides containing α-ketoacids. The C-terminal segment **28** was added and the mixture diluted to 25 mM before being stirred for 22 h at 60 °C. Finally, the ligation mixture was diluted to 5 mM with 6 M guanidinium hydrochloride at pH 9 in order to achieve the O-to N-shift. The crude product was directly purified by preparative HPLC to give the synthetic NEDD8 **29** in 20% yield over two ligations (four steps in total).



Scheme 7: One-pot synthesis of NEDD8 in C- to N-direction using photoprotected (*S*)-5-oxaproline.

NEDD8 was also assembled from the C-terminal to the N-terminal in a one-pot fashion using the photoprotected (S)-5-Oxaproline (Scheme 7). The first ligation between **30** and **28** went to completion after 20 h of reaction at 60 °C in a mixture of DMSO/H_2O containing 0.1 M oxalic acid. The mixture was immediately irradiated after addition of TCEP (0.1 M) and the deprotection was complete after 1.5 h as determined by HPLC. The third segment **32** was added and the second ligation was performed at a concentration of 25 mM. Again, this ligation did not go to completion, possibly due to a diminished reactivity of the isoleucine α-ketoacid. The O- to N-shift was achieved at pH 9 as described above. In this case, the purification appeared to be more difficult because of overlap of the final NEDD8 and the intermediate ligated product. After optimization of the purification, the final protein was isolated in 20% yield.



Scheme 8: One-pot convergent synthesis of NEDD8 from four segments.

Finally, we sought to prepare NEDD8 using a convergent synthesis, which could be useful for the preparation of larger proteins (Scheme 8). As in the other strategies, the ligation involving the isoleucine α-ketoacid containing segment **25**, did not go to completion. The other ligation, between smaller segments **33** and **34**, occurred cleanly and went to completion. These two ligation mixtures were directly combined and TCEP was added before the concomitant photorelease of the α-ketoacid and the (*S*)-5-oxaproline at 365 nm. Complete deprotection of both fragments was observed after 1.5 h and the mixture was heated to 60 °C to finish the synthesis of NEDD8. After 24 h, the mixture was diluted with 6 M guanidinium chloride and adjusted to pH 9 to induce O- to N-shifts to give final product **37**. The reaction mixture was purified by preparative HPLC and the synthetic protein **37** was isolated in 26% yield over three ligations. Although the final HPLC traces of the ligation/deprotection mixture were not as clean as ligations on the isolated segments, the final product could be obtained in good purity and acceptable yield without intermediate handling or isolation steps. Such protocols will be useful for preparing libraries of peptides or proteins for chemical biology applications.

Conclusions

We have developed new photoprotected α-ketoacids and (*S*)-5-oxaproline which enabled one-pot multiple KAHA ligations. The photoprotected α-ketoacids and (*S*)-5-oxaproline could be cleanly unmasked under mild conditions by irradiation at 365 nm, without degradation or epimerization. These new protected α-ketoacids and oxaproline were applied to the one-pot synthesis of NEDD8 using three distinct strategies. A convergent synthesis involving three ligations was feasible and the final synthetic NEDD8 was obtained with only one HPLC purification. These new masking groups will facilitate the convergent synthesis of larger proteins and libraries of protein derivatives by expanding the possible combinations of the protein segments and reducing the number of intermediate steps and purifications.

Experimental Section

General

Reagents and solvents: Fmoc-amino acids with suitable side chain protecting groups and HCTU (*O*-(1H-6-Chlorobenzotriazol-1-yl)-N,N,N',N'-tetramethyluroniumhexafluorophosphate) were purchased from Peptides International (Louisville, KY, USA) and ChemImpex (Wood Dale, IL, USA). Solvents for flash chromatography (hexanes, cyclohexane, EtOAc, MeOH, MTBE, CH_2Cl_2) were of technical grade

and distilled prior to use. CH_3CN, THF, DMF and CH_2Cl_2 were dried by passage over two columns of anhydrous neutral A-2 alumina under an atmosphere of N_2. Dry DMSO was purchased from Acros. H_2O used for preparing ligation solutions was obtained from a Millipore purification system. HPLC grade CH_3CN from Sigma-Aldrich was used for analytical and preparative HPLC purification. Other commercially available reagents and solvents were purchased from Sigma-Aldrich (Buchs, Switzerland), Acros Organics (Geel, Belgium), ABCR (Karlsruhe, Germany) and TCI Europe (Zwijndrecht, Belgium). All other starting materials were used as supplied by commercial vendors or prepared by the method described in the corresponding reference.

Characterization: ^{1}H and ^{13}C NMR spectra were recorded on Bruker AVIII400, Bruker AVIII300 and Bruker AVIII600 spectrometers. Chemical shifts for ^{1}H NMR (300, 400 and 600 MHz) and ^{13}C NMR (101 and 150 MHz) are expressed in parts per million and are referred to residual undeuterated solvent signals. Coupling constants are reported in Hertz (Hz) and corresponding splitting patterns are indicated as follows: s, singlet; d, doublet; dd, doublet of doublet; td, triplet of doublet; t, triplet; m, multiplet. High-resolution mass spectra were recorded by the Mass Service of the Laboratory of Organic Chemistry at ETH Zurich either with a Bruker maXis instrument (ESI-QTOF MS) equipped with an ESI source and a Qq-TOF detector or with a Bruker solariX instrument (MALDI FTMS) with a FT-ICR detector using 4-hydroxy-α-cyanocinnamic acid as matrix.

Reactions and purification: Unless otherwise noted, all reactions were carried out in oven-dried glassware sealed with rubber septa under an atmosphere of dry N_2 and were stirred with Teflon-coated magnetic stir bars. Reactions and fractions from flash chromatography were monitored by thin layer chromatography using precoated glass plates (Merck, silica 60 F254) and visualized by staining with basic $KMnO_4$ solution, ninhydrine solution or CAM (cerium-ammonium-molybdate) solution. Flash chromatography was performed on Silicycle SiO_2 type F60 (230-400 mesh) using a forced flow of air at 1.0 bar. Peptides and protein fragments were analyzed and purified by reversed phase high performance liquid chromatography (RP-HPLC) on Jasco analytical and preparative instruments equipped with dual pumps, mixer and in-line degasser, a variable wavelength UV detector (simultaneous monitoring of the eluent at 220 nm, 254 nm and 301 nm) and a Rheodyne injector fitted with a 20 or 1000 µl injection loop or on a Gilson preparative instrument. The mobile phase for RP-HPLC were Milipore-H_2O containing 0.1% (v/v) TFA and HPLC grade CH_3CN containing 0.1% (v/v) TFA. Analytical HPLC was performed on Shiseido Capcell Pak C18 MG II (5 µm, 4.6 mm I.D. x 250 mm) columns at a flow rate of 1 mL/min. Preparative HPLC was performed on a Shiseido Capcell Pak MGII (5 µm, 20 mm I.D. x 250 mm) column at a flow rate of 10 mL/min. Optical rotations were measured on a Jasco P-1010 operating at the sodium D line with a 100 mm path length cell. **SFC** (Supercritical Fluid Chromatography) on chiral stationary phase were performed on *JASCO* liquid chromatography units using *Daicel* Chiralpak columns (0.46 x 25 cm).

Synthesis of photocleavable α-ketoacid protecting group

(*RS*)-2,2-dimethyl-1-(2-nitrophenyl)propane-1,3-diol *rac*-2: KOH (1.86 g, 33.1 mmol, 1.0 equiv) was dissolved in cooled EtOH (80 ml) and a mixture of 2-nitrobenzaldehyde (5 g, 33.1 mmol, 1.0 equiv) and isobutyraldehyde (6.04 ml, 66.2 mmol, 2 equiv) was added over 30 min, so that the reaction temperature does not exceed 30 °C. The yellow solution was heated to 55 °C overnight. After cooling to rt, 200 ml H_2O were added and the mixture extracted with CH_2Cl_2 (3 x 200 ml). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The crude was purified by flash chromatography by flash chromatography (SiO_2, hexane/AcOEt 3/1 to 2/1) to give the product (4.84 g, 65 %) as brown solid. The analytical data are in accordance with reported data[xxxiii] and with the analytical data reported below for the enantioenriched diol.

(S)-2,2-dimethyl-1-(2-nitrophenyl)propane-1,3-diol (*S*)-2: 2-Nitrobenzaldehyde (10.0 g, 66.2 mmol, 1.00 equiv) was dissolved in dry DMSO (33 mL) in a flame-dried flask under nitrogen atmosphere. Isobutyraldehyde (9.54 g, 12.1 mL, 132 mmol, 2.00 equiv) was added at rt. A solution of (*S*)-(+)-1-(2-pyrrolidinylmethyl)pyrrolidine (510 mg, 3.31 mmol, 0.05 equiv) and trifluoroacetic acid (377 mg, 255 µL, 3.31 mmol, 0.05 equiv) in dry DMSO (3.3 mL) was added and stirred for 20 h at rt. CH_3OH (33 mL) was added and the mixture was cooled to 0°C. $NaBH_4$ (2.50 g, 66.2 mmol, 1.00 equiv) was added portionwise over a period of 30 min keeping the internal temperature below 20°C. The mixture was stirred for 30 min at 15°C. H_2O (60 mL) and 1 M HCl (60 mL) were added slowly keeping the internal temperature below 20°C. The mixture was diluted with H_2O (180 mL) and EtOAc (60 mL) was added. The layers were separated and the aqueous layer was extracted with EtOAc (3 x 60 mL). The combined organic layers were washed with H_2O (2 x 60 mL) and brine (30 mL), dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure and the residue was dried at high vacuum for 6 h to yield an orange oil (14.6 g, 64.9 mmol, 98 %) The crude product is essentially pure and is be used for the next step without purification. **^{1}H NMR** (400 MHz,

$CDCl_3$) δ 7.84 (dd, *J* = 7.9, 1.5 Hz, 1H, CH), 7.76 (dd, *J* = 8.1, 1.3 Hz, 1H, CH), 7.66 – 7.57 (m, 1H, CH), 7.41 (ddd, *J* = 8.1, 7.3, 1.5 Hz, 1H, CH), 5.63 (s, 1H, CH), 3.57 (d, *J* = 10.8 Hz, 1H, CH_2), 3.53 (d, *J* = 10.8 Hz, 1H, CH_2), 0.81 (s, 3H, CH_3), 0.80 (s, 3H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 149.5 (C-NO_2), 135.8 (CH) , 132.3 (CH), 130.0 (CH), 128.3 (CH), 124.0 (CH), 74.4 (CH), 72.5 (CH_2), 40.2 (C), 22.1 (CH_3), 18.6 (CH_3) **$[\alpha]_D^{26}$** = +407°(c = 0.810, $CHCl_3$). **IR** (υ/cm^{-1}, thin film): 3356, 2965, 2876, 1525, 1474, 1353, 1038, 855, 787, 736, 689. **HRMS** (ESI): calculated for $C_{11}H_{15}NNaO_4$ $[M+Na]^+$: 248.0893, found: 248.0894. **SFC** column: Daicel Chiralpak ADH (4.6 × 250 mm); gradient: 5 % *i*PrOH in CO_2 to 50 % *i*PrOH in CO_2 over 10 min; flow: 3.0 mL/min; detection: 220 nm. Retention time: t_R = 6.21 min (major), 6.51 (minor). Enantiomeric excess: 97%ee.

(2*R*,4*S*)-2-(4-bromophenyl)-5,5-dimethyl-4-(2-nitrophenyl)-1,3-dioxane 7: 4-Bromobenzaldehyde (1.00 equiv, 616 mg, 3.30 mmol), (S)-2,2-dimethyl-1-(2-nitrophenyl)propane-1,3-diol (1.10 equiv, 825 mg, 3.66 mmol) and p-toluenesulfonic acid (0.10 equiv, 63.3 mg, 333 µmol) were dissolved in toluene and refluxed with azeotropic removal of water for 18 h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (SiO_2, hexanes/diethyl ether 4:1) to obtain the product (850 mg, 2.17 mmol, 65 %) as yellow oil which solidified at room temperature upon standing. To obtain single crystals, the repurified product was recrystallized from petrol ether (60/90). A saturated solution of the repurified product in petrol ether (60/90) at room temperature was slowly cooled to 4 °C to obtain single-crystals suitable for single-crystal x-ray diffraction. A suitable needle-shaped crystal was selected and mounted on a Bruker/Nonius Kappa Apex2 diffractometer. The crystal was kept at 100.0(2) K during data collection. Using Olex2[xxxiv], the structure was solved with the ShelXS structure solution program using Direct Methods and refined with the XL[xxxv] refinement package using Least Squares minimisation. The crystal structure has been deposited at the Cambridge Crystallographic Data Centre (CCDC 1498367). **Crystal Data**: monoclinic, space group $P2_1$ (no. 4), *a* = 10.311(3) Å, *b* = 8.042(2) Å, *c* = 10.718(3) Å, *β* = 105.399(9)°, *V* = 856.8(4) $Å^3$, *Z* = 2, *T* = 100.0(2) K, μ(MoKα) = 2.420 mm^{-1}, *Dcalc* = 1.520 g/cm^3, 5610 reflections measured (6.398° ≤ 2Θ ≤ 50.044°), 2488 unique (R_{int} = 0.0318, R_{sigma} = 0.0632) which were used in all calculations. The final R_1 was 0.0265 (I > 2σ(I)) and wR_2 was 0.0534 (all data). **NMR** (400 MHz, $CDCl_3$) δ [ppm] = 7.86 (dd, *J* = 8.2, 1.2 Hz, 1H, CH), 7.80 (dd, *J* = 8.0, 1.4 Hz, 1H, CH), 7.64 (td, *J* = 7.6, 1.1 Hz, 1H, CH), 7.58 – 7.54 (m, 2H, CH), 7.51 – 7.45 (m, 3H, CH), 5.76 (s, 1H, CH), 5.73 (s, 1H, CH), 3.90 – 3.82 (m, 2H, CH_2), 1.00 (s, 3H, CH_3), 0.70 (s, 3H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 148.8 (C-NO_2), 137.3 (C), 132.4 (CH), 132.0 (C), 131.6 (CH), 130.5 (CH), 128.6 (CH), 128.2 (CH), 124.2 (CH), 123.2 (C), 101.6 (CH), 80.3 (CH), 78.9 (CH_2), 35.4 (C), 20.9 (CH_3), 19.2 (CH_3). **IR** (υ/cm^{-1}, thin film): 2963, 1528, 1491, 1469, 1389, 1353, 1109, 1099, 1070, 1025, 1011, 985, 856, 821, 808, 785, 756, 736, 720, 698. **HRMS** (ESI): calculated for $C_{18}H_{19}BrNO_4$ $[M+H]^+$: 382.0492, found: 382.0493. **$[\ ]_D^{23}$** = +212°(c = 1.00, $CHCl_3$). m.p. (petrol ether 60/90) = 118 °C.

Fmoc-(*S*)-Trp photoprotected α-ketoacid methyl ester 13 (single diastereomer): Fmoc-(*S*)-Trp photoprotected α-ketoacid **12f** (1.00 equiv, 100 mg, 131 µmol) was dissolved in 1:3 methanol/toluene (1.3 ml). TMS-diazomethane (2 M in diethyl ether, 1.30 equiv, 85.3 µl) was added dropwise at room temperature and the mixture was stirred for 10 min. The solvents were removed under reduced pressure and the residue was purified by flash chromatography (SiO_2, hexanes/EtOAc 4:1) to obtain the product (86.3 mg, 111 µmol, 85 %) as a white foam. The NMR spectra show two conformational isomers (ratio 7.1:1). By 1D-NOE experiments with selective pulses at 5.58 ppm and 5.13 ppm it was shown that the two isomers are equilibrating conformers and not diastereomers.[xxxvi] See the Supporting Information for details. Chemical Shifts are reported for the major conformer. **^{1}H NMR** (600 MHz, $CDCl_3$) δ [ppm] = 8.14 (bs, 1H, CH), 7.79 (d, *J* = 8.1 Hz, 1H, CH), 7.72 (d, *J* = 7.6 Hz, 2H, CH), 7.64 (d, *J* = 7.8 Hz, 2H, CH), 7.59 – 7.54 (m, 2H, CH), 7.46 (d, *J* = 7.5 Hz, 1H, CH), 7.43 (t, *J* = 7.7 Hz, 1H, CH), 7.40 (d, *J* = 7.5 Hz, 1H, CH), 7.37 – 7.33 (m, 2H, CH), 7.31 (t, *J* = 7.7 Hz, 1H, CH), 7.27 – 7.24 (m, 1H, CH), 7.24 – 7.20 (m, 2H, CH), 5.58 (s, 1H, CH), 5.13 (d, *J* = 10.6 Hz, 1H, NH), 4.66 (td, *J* = 10.5, 3.6 Hz, 1H, CH), 4.21 – 4.09 (m, 3H, CH, CH_2), 3.95 (d, *J* = 11.6 Hz, 1H, CH_2), 3.86 (s, 3H, OCH_3), 3.72 (d, *J* = 11.6 Hz, 1H, CH_2), 3.49 (dd, *J* = 15.4, 3.3 Hz, 1H, CH_2), 3.03 (dd, *J* = 15.4, 10.6 Hz, 1H, CH_2), 1.58 (s, 9H, CH_3), 1.02 (s, 3H, CH_3), 0.63 (s, 3H, CH_3).**^{13}C NMR** (150 MHz, $CDCl_3$) δ [ppm] = 168.9 (CO), 156.0 (CO), 149.7 (C-NO_2), 149.1 (CO), 144.0* (C), 143.9* (C), 141.3 (C), 135.7 (C), 132.3 (CH), 130.9 (C), 130.8 (C), 130.1 (CH), 128.9 (CH), 127.69* (CH), 127.68* (CH), 127.1 (CH), 125.32* (CH), 125.28* (CH), 124.6 (CH), 124.2 (CH), 123.9 (CH), 122.7 (CH), 119.9 (CH), 118.9 (CH), 116.5 (C), 115.5 (CH), 101.5 (C), 83.5 (C), 76.9 (CH), 74.9 (CH_2), 67.3 (CH_2), 55.9 (CH), 53.2 (OCH_3), 47.1 (CH), 35.0 (C), 28.3 (CH_3), 25.0 (CH_2), 20.9 (CH_3), 19.1 (CH_3), (*Signals of rotamers). **IR** (υ/cm^{-1}, thin film): 2973, 1731, 1530, 1474, 1452, 1370, 1308, 1256, 1225, 1160, 1091, 1071, 1055, 1023, 852, 784, 759, 739, 706, 667. **HRMS** (ESI): calculated for $C_{44}H_{46}N_3O_{10}$ $[M+H]^+$: 776.3178, found: 776.3161.

Synthesis of photoprotected α-ketoacids

Protected α-ketoacids with linker were prepared by our recently published method.[xxv] See the supporting information for details. All new intermediates and final products are reported herein.

General procedure Fmoc-(*S*)-AA cyanosulfurylide: Fmoc amino acid (1.00 equiv) was dissolved in dry CH_2Cl_2 (0.1 M) and cooled to 0 °C. NEt^iPr_2 (3.00 equiv) and T3P (50 % w/w in EtOAc; 1.00 equiv) were successively added and the mixture was stirred for 5 min before the addition of 1-cyanomethyl-tetrahydrothiophenium bromide (1.00 equiv). The reaction was stirred for 1 h at 0 °C. The mixture was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ (3x150 mL), water (3x100 mL) and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give the desired product, which was used without further purification in the next step.

Fmoc-(*S*)-Trp cyanosulfurylide 38f: prepared following the general procedure using Fmoc-(*S*)-Trp(Boc)-OH **8f** (1.00 equiv, 15.0 g, 28.5 mmol), T3P (1.50 equiv, 25.4 ml, 42.7 mmol), NEt^iPr_2 (4.00 equiv, 19.9 ml, 114 mmol) and 1-cyanomethyl-tetrahydrothiophenium bromide **9** (1.50 equiv, 8.89 g, 42.7 mmol) in dry CH_2Cl_2. The reaction mixture was stirred for 1 h at 0 °C. The crude product (18.1 g) was used without purification in the next step. For analytical purpose, a sample was purified by flash chromatography (SiO_2, EtOAc to EtOAc/MeOH 4:1). **^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 8.12 (d, *J* = 8.2 Hz, 1H, CH), 7.75 (d, *J* = 7.5 Hz, 2H, CH), 7.66 – 7.54 (m, 3H, CH), 7.51 (s, 1H, CH), 7.43 – 7.35 (m, 2H, CH), 7.34 – 7.25 (m, 3H, CH), 7.19 (t, *J* = 7.5 Hz, 1H, CH), 5.79 (d, *J* = 7.8 Hz, 1H, NH), 4.95 (appq, *J* = 6.9 Hz, 1H, CH), 4.38 (dd, *J* = 10.5, 7.4 Hz, 1H, CH_2), 4.32 (dd, *J* = 10.5, 7.1 Hz, 1H, CH_2), 4.21 (appt, *J* = 7.3 Hz, 1H, CH), 3.36 – 2.97 (m, 5H, CH_2), 2.79 (bs, 1H, CH_2), 2.49 – 2.26 (m, 2H, CH_2), 2.02 – 1.82 (m, 2H, CH_2), 1.64 (s, 9H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 188.2 (CO), 155.6 (CO), 149.8 (CO), 144.14* (C), 144.08* (C), 141.4 (C), 135.4 (C), 131.2 (C), 127.7 (CH), 127.2 (CH), 125.4* (CH), 125.3* (CH), 124.9 (CH), 124.3 (CH), 122.5 (CH), 120.0 (CH), 119.3 (CH), 118.9 (CN), 115.43 (C), 115.35 (CH), 83.7 (C), 67.1 (CH_2), 56.3 (CH), 55.1 (CS), 47.3 (CH), 44.9 (CH_2), 44.8 (CH_2), 29.0 (CH_2), 28.6 (CH_2), 28.4 (CH_2), 28.3 (CH_3). **IR** (υ/cm^{-1}, thin film): 2977, 2170, 1591, 1451, 1368, 1326, 1308, 1254, 1156, 1084, 1051. **HRMS** (ESI): calculated for $C_{37}H_{38}N_3O_5S$ $[M+H]^+$: 636.2527, found: 636.2523.

Fmoc-(*S*)-Glu cyanosulfurylide 38h: prepared following the general procedure using Fmoc-(*S*)-Glu(O^{t}Bu)-OH **8h** (1.00 equiv, 14.2 g, 32.0 mmol), T3P (1.50 equiv, 28.6 ml, 48.1 mmol), NEt^iPr_2 (4.00 equiv, 22.3 ml, 128 mmol) and 1-cyanomethyl-tetrahydrothiophenium bromide **9** (1.95 equiv, 13.0 g, 62.5 mmol) in dry CH_2Cl_2. The reaction mixture was stirred for 3 h at 0 °C. The crude product (18.8 g) was used without purification in the next step. For analytical purpose, a sample was purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH 95/5 to 9/1). **^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 7.76 (d, *J* = 7.5 Hz, 2H, CH), 7.67 – 7.57 (m, 2H, CH), 7.43 – 7.36 (m, 2H, CH), 7.35 – 7.27 (m, 2H, CH), 5.71 (d, *J* = 8.0 Hz, 1H, NH), 4.67 (td, *J* = 8.0, 4.6 Hz, 1H, CH), 4.35 (d, *J* = 7.3 Hz, 2H, CH_2), 4.21 (t, *J* = 7.2 Hz, 1H, CH), 3.50 – 3.23 (m, 4H, CH_2), 2.67 – 2.46 (m, 2H, CH_2), 2.46 – 2.19 (m, 2H, CH_2), 2.19 – 2.00 (m, 3H, CH_2), 2.00 – 1.83 (m, 1H, CH_2), 1.44 (s, 9H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 189.0 (CO), 172.5 (CO), 155.9 (CO), 144.2* (C), 144.0* (C), 141.41* (C), 141.39* (CO), 127.8 (CH), 127.2 (CH), 125.4 (CH), 120.1 (CH), 119.1 (CN), 80.6 (C), 67.0 (CH_2), 55.6 (CH), 54.4 (CS), 47.4 (CH), 45.8 (CH_2), 45.2 (CH_2), 31.6 (CH_2), 28.9 (CH_2), 28.70 (CH_2), 28.65 (CH_2), 28.2 (CH_3) **IR** (υ/cm^{-1}, thin film): 3294, 2976, 2170, 1719, 1592, 1497, 1449, 1366, 1249, 1151, 1048, 846, 759, 741, 665. **HRMS** (ESI): calculated for $C_{30}H_{35}N_2O_5S$ $[M+H]^+$: 535.2261, found: 535.2262.

General procedure Fmoc-(*S*)-AA α-ketoacid: Fmoc-(*S*)-AA cyanosulfurylide (1.00 equiv) was dissolved in THF/H_2O (2:1, 40.0 mM) and Oxone (1.50 equiv) was added in one portion. The suspension was stirred until LC-MS analysis showed complete consumption of starting material. The mixture was diluted with EtOAc and separated. The organic layer was washed with saturated aqueous NH_4Cl (100 mL). The aqueous phase was extracted with EtOAc (3x50 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was used in the next step without further purification.

Fmoc-(*S*)-Trp α-ketoacid 1of: prepared following the general procedure using crude Fmoc-(*S*)-Trp cyanosulfurylide **38f** (1.00 equiv) from the previous step and Oxone (2.00 equiv, 35.1 g, 57.0 mmol) in THF/H_2O (2:1, 530 ml). The reaction mixture was stirred for 1 h at rt. The crude product (15.8 g) was used without purification in the next step. For analytical purpose, a sample was purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH 95/5 to 9/1). **^{1}H NMR** (400 MHz, $(CD_3)_2SO$) δ [ppm] = 8.03 (d, *J* = 8.4 Hz, 1H, CH), 7.90 – 7.83 (m, 2H, CH), 7.83 – 7.73 (m, 2H, CH, NH), 7.67 – 7.53 (m, 3H, CH), 7.45 – 7.17 (m, 6H, CH), 4.97 (ddd, *J* = 11.2, 8.3, 3.2 Hz, 1H, CH), 4.27 – 4.06 (m, 3H, CH, CH_2), 3.33 (dd, *J* = 14.8, 3.4 Hz, 1H, CH_2), 2.87 (dd, *J* = 14.8, 10.6 Hz, 1H, CH_2), 1.56 (s, 9H, CH_3). **^{13}C NMR** (101 MHz, $(CD_3)_2SO$) δ [ppm] = 198.6 (CO), 165.0 (CO), 155.9 (CO), 149.0 (CO), 143.7* (C), 143.6* (C), 140.7* (C), 140.6* (C), 134.7 (C), 130.1 (C), 127.6 (CH), 127.01* (CH), 126.96* (CH), 125.22* (CH), 125.17* (CH), 124.4 (CH), 123.9 (CH), 122.6 (CH), 120.1 (CH), 119.3 (CH), 116.7 (C), 114.7 (CH), 83.5 (C), 65.8

(CH_2), 56.9 (CH), 46.5 (CH), 27.6 (CH_3), 24.9 (CH_2), (*Signals of rotamers). **IR** (υ/cm^{-1}, thin film): 2978, 1729, 1519, 1451, 1370, 1339, 1309, 1256, 1227, 1157, 1085, 1034, 1017, 855. **HRMS** (ESI): calculated for $C_{32}H_{34}N_3O_7$ $[M+NH_4]^+$: 572.2391, found: 572.2400.

Fmoc-(*S*)-Glu α-ketoacid 10h: prepared following the general procedure using crude Fmoc-(*S*)-Glu cyanosulfurylide **38h** (1.00 equiv) from the previous step and Oxone (1.00 equiv, 39.6 g, 64.1 mmol) in THF/H_2O (2:1, 230 ml). The reaction mixture was stirred for 1 h at rt. The crude product (15.9 g) was used without purification in the next step. For analytical purpose, a sample was purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH 95/5 to 9/1).**^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 7.80 – 7.71(m, 2H, CH), 7.63 – 7.50 (m, 2H, CH), 7.44 – 7.36 (m, 2H), 7.31 (td, *J* = 7.5, 1.2 Hz, 2H, CH), 5.77 (d, *J* = 7.5 Hz, 1H, NH), 5.44 (bs, 1H, COOH), 5.05 (appq, *J* = 6.9 Hz, 1H, CH), 4.49 – 4.31 (m, 2H, CH_2), 4.20 (appt, *J* = 6.8 Hz, 1H, CH), 2.48 – 2.15 (m, 3H, CH_2), 2.11 – 1.98 (m, 1H, CH_2), 1.43 (s, 9H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 192.9 (CO), 173.1 (CO), 159.8 (CO), 156.3 (CO), 143.8* (C), 143.6* (C), 141.5 (C), 127.9 (CH), 127.3 (CH), 125.2 (CH), 120.2 (CH), 81.9 (C), 67.5 (CH_2), 56.4 (CH), 47.2 (CH), 30.9 (CH_2), 28.2 (CH_3), 26.1 (CH_2) **IR** (υ/cm^{-1}, thin film): 2978, 1725, 1522, 1450, 1368, 1331, 1252, 1153, 1032, 845, 759, 741, 669. **HRMS** (ESI): calculated for $C_{25}H_{27}NNaO_7$ $[M+Na]^+$: 476.1680, found: 476.1680.

General procedure Fmoc-(*S*)-AA α-ketoacid dimethyl acetal: Fmoc-(*S*)-AA α-ketoacid (1.00 equiv) was dissolved in trimethyl orthoformate (20.0 equiv) and H_2SO_4 (0.25 equiv) was added dropwise in trimethyl orthoformate (2.00 mL). The mixture was stirred at rt until LC-MS showed complete conversion. The reaction was diluted with EtOAc and quenched with 1 M HCl solution. The aqueous phase was extracted with EtOAc (3 x 100 mL) and the organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. Depending on the amino acid, the dimethyl acetal can be directly used in the next step or purified by flash chromatography.

Fmoc-(*S*)-Trp α-ketoacid dimethyl acetal 11f: prepared following the general procedure using crude Fmoc-(*S*)-Trp α-ketoacid **10f** (1.00 equiv) from the previous step in trimethyl orthoformate (16.0 equiv, 49.4 ml, 456 mmol), MeOH (0.80 equiv, 925 µmol, 22.8 mmol) and H_2SO_4 (0.80 equiv, 1.22 ml, 22.8 mmol). The reaction mixture was stirred for 18 h at rt. The crude mixture was purified by flash chromatography (CH_2Cl_2 to CH_2Cl_2/EtOAc 4:1) to obtain the desired product (8.80 g, 14.7 mmol, 51% over 3 steps) as a white foam. **^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 8.12 (d, *J* = 8.0 Hz, 1H, CH), 7.75 (d, *J* = 7.6 Hz, 2H, CH), 7.59 (d, *J* = 7.7 Hz, 1H, CH), 7.52 (bs, 2H, CH), 7.46 – 7.21 (m, 7H, CH), 5.31 (d, *J* = 10.0 Hz, 1H, NH), 4.67 – 4.52 (m, 1H, CH), 4.34 – 4.12 (m, 3H, CH, CH_2), 3.44 (s, 6H, CH_3), 3.24 (ddd, *J* = 15.5, 3.5, 1.2 Hz, 1H, CH_2), 2.97 – 2.85 (m, 1H, CH_2), 1.67 – 1.51 (m, 9H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 168.8 (CO), 156.6 (CO), 149.8 (CO), 144.0* (C), 143.9* (C), 141.4* (C), 141.3* (C), 135.6 (C), 130.7 (C), 127.7 (CH), 127.2* (CH), 127.1* (CH), 125.2* (CH), 125.1* (CH), 124.6 (CH), 123.6 (CH), 122.7 CH), 120.0 (CH), 118.8 (CH), 116.5 (C), 115.5 (CH), 102.4 (C), 83.6 (C), 67.2 (CH_2), 52.2 (CH), 51.1 (CH_3), 47.2 (CH), 28.3 (CH_3), 26.1 (CH_2), (*Signals of rotamers). **IR** (υ/cm^{-1}, thin film): 2925, 2851, 1728, 1524, 1478, 1451, 1370, 1256, 1155, 1086, 855, 758, 741, 668. **HRMS** (ESI): calculated for $C_{34}H_{37}N_2O_8$ $[M+H]^+$: 601.2544, found: 601.2538.

Fmoc-(*S*)-Glu α-ketoacid dimethyl acetal 11h: prepared following the general procedure using crude Fmoc-(*S*)-Glu α-ketoacid **10h** (1.00 equiv) from the previous step in trimethyl orthoformate (20.0 equiv, 21.1 ml, 195 mmol) and H_2SO_4 (1.25 equiv, 651 µl, 12.2 mmol, in two portions: 1x1.00 equiv, 1x0.25 equiv after 3 h). The reaction mixture was stirred for 18 h at rt. The crude mixture was purified by flash chromatography (SiO_2, hexanes/EtOAc 4:1 to hexanes/EtOAc/MeOH 5:4:1) to obtain the product (2.24 g, 4.48 mmol, 46 %) as white foam.

^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm] = 7.88 – 7.69 (m, 2H, CH), 7.69 – 7.53 (m, 2H, CH), 7.46 – 7.35 (m, 2H, CH), 7.35 – 7.28 (m, 2H, CH), 5.43 (bs, 1H, NH), 4.54 – 4.11 (m, 4H, CH_2, CH), 3.44 (s, 3H, CH_3), 3.32 (s, 3H, CH_3), 2.31 (t, *J* = 7.2 Hz, 2H, CH_2), 2.12 – 1.91 (m, 1H, CH_2), 1.60 – 1.48 (m, 1H), 1.43 (s, 9H). COOH not observed. **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 172.7 (CO), 157.0 (CO), 144.2* (C), 143.9* (C), 141.5* (C), 141.4* (C), 127.8 (CH), 127.2 (CH), 125.4* (CH), 125.2* (CH), 120.1 (CH), 102.0 (C), 80.9 (C), 67.1 (CH_2), 52.2 (CH), 51.4 (CH_3), 50.4 (CH_3), 47.4 (CH), 31.7 (CH_2), 28.2 (CH_3), 25.2 (CH_2), (*Signals of rotamers), one carbonyl carbon signal was not resolved. **IR** (υ/cm^{-1}, thin film): 2977, 1724, 1513, 1450, 1367, 1335, 1248, 1153, 1117, 1052, 1002, 846, 741, 759, 666 cm^{-1}. **HRMS** (ESI): calculated for $C_{27}H_{33}NNaO_8$ $[M+Na]^+$: 522.2098, found: 522.2097.

General procedure Fmoc-(*S*)-AA photoblabile protected α-ketoacid: In a flame-dried flask, Fmoc-(*S*)-AA α-ketoacid dimethyl acetal (1.00 equiv) was dissolved in dry toluene (300 mM). Diol **2** (1.20 equiv) was added and the mixture was stirred under reflux for 1 h. The mixture was allowed to cool to rt and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography.

Fmoc-(*S*)-Leu photoprotected α-ketoacid 12a (mixture of diastereomers): following the general procedure using Fmoc-(*S*)-Leu α-ketoacid dimethyl acetal **11a** (1.00 equiv, 1.30 g, 3.05 mmol) and racemic diol ***rac*-2** (1.50 equiv, 1.03 g, 4.56 mmol) in dry toluene. The

reaction mixture was stirred for 2 h at reflux. The crude mixture was purified by flash chromatography (SiO_2, hexanes/AcOEt 3/1 to hexanes/AcOEt/MeOH 4/2/1 + 0.1% AcOH) to give the desired product as white foam (984 mg, 1.67 mmol, 55%) over 4 steps starting from Fmoc-(*S*)-Leu-OH. The analytical data is given for an inseparable mixture of diastereomers and rotamers. ^{1}H NMR signals are generally described as multiplets. All observed diastereomeric ^{13}C NMR signals are listed. **^{1}H NMR** (400 MHz, CD_3OD) δ [ppm] = 7.86 – 7.57 (m, 6H), 7.48 – 7.31 (m, 4H), 7.29 – 7.21 (m, 2H), 5.71 (s, 0.5H), 5.68 (s, 0.5H), 4.48 – 4.29 (m, 2H, CH_2), 4.28 – 4.08 (m, 2H, CH), 3.82 (m, 1H, CH_2), 3.50 (m, 1H, CH_2), 1.74 – 1.37 (m, 3H, CH_2+CH), 0.99 – 0.88 (m, 6H, CH_3), 0.86 (m, 3H, CH_3), 0.52 (m, 3H, CH_3). **^{13}C NMR** (101 MHz, CD_3OD) δ [ppm] = 175.2, 175.1, 159.3, 159.1, 150.3, 150.2, 145.4, 145.39, 145.3, 145.1, 142.6, 142.5, 133.4, 133.2, 133.1, 133.0, 131.7, 131.6, 129.6, 129.4, 128.8, 128.7, 128.6, 128.2, 128.1, 128.0, 126.4, 126.35, 126.3, 126.2, 124.9, 124.8, 120.9, 120.88, 120.8, 103.7, 77.1, 76.7, 75.5, 75.4, 68.1, 68.0, 56.3, 56.2, 49.4, 48.5, 48.4, 38.8, 35.8, 25.9, 24.4, 24.3, 21.9, 21.8, 21.4, 19.5, 19.3. **IR** (υ/cm^{-1}, thin film): 2957, 2871, 1703, 1530, 1352, 1225, 1189, 1056, 852, 737, 668. **HRMS** (ESI): calculated for $C_{33}H_{36}N_2O_8$ [M+H]$^+$: 589.2544, found: 589.2532.

Fmoc-(*S*)-Ile photoprotected α-ketoacid 12b (single diastereomer): prepared following the general procedure using Fmoc-(*S*)-Ile α-ketoacid dimethyl acetal **11b** (1.00 equiv, 1.30 g, 3.00 mmol) and enantioenriched diol **(*S*)-2** (1.30 equiv, 890 mg, 3.95 mmol) in dry toluene. The reaction mixture was stirred for 2 h at reflux. The crude mixture was purified by flash chromatography (SiO_2, hexanes/AcOEt 3/1 to hexanes/AcOEt/MeOH 4/2/1 + 0.1% AcOH) to give the desired product as white foam (940 mg, 1.60 mmol, 53%) over 4 steps starting from Fmoc-(*S*)-Ile-OH. **^{1}H NMR** (400 MHz, CD_3OD) δ [ppm] = 7.85 (m, 1H, CH), 7.78 (m, 3H, CH), 7.68 – 7.58 (m, 3H, CH), 7.44 (m, 1H, CH), 7.36 (m, 2H, CH), 7.26 (m, 2H, CH), 5.75 (s, 1H, CH), 4.42 – 4.29 (m, 2H, CH_2), 4.21 (t, *J* = 6.9 Hz, 1H, CH), 4.00 (d, *J* = 3.2 Hz, 1H, CH), 3.85 (d, *J* = 11.4 Hz, 1H, CH_2), 3.48 (d, *J* = 11.4 Hz, 1H, CH_2), 2.04 (m, 1H, CH), 1.96 (m, 1H, CH_2), 1.00 (d, *J* = 6.8 Hz, 4H, CH_2+CH_3), 0.89 (t, *J* = 7.5 Hz, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.52 (s, 3H, CH_3). **^{13}C NMR** (101 MHz, CD_3OD) δ [ppm] = 175.3 (CO), 159.6 (CO), 150.3 (C), 145.4* (C), 145.1* (C), 142.6* (C), 142.5* (C), 133.3* (C), 133.2* (C), 131.5 (CH), 129.6 (CH), 128.8* (CH), 128.7* (CH), 128.2* (CH), 128.1* (CH), 126.3* (CH), 126.2* (CH), 125.0 (CH), 120.9 (CH), 104.2 (C), 77.1 (CH), 75.4 (CH_2), 68.1 (CH_2), 62.4 (CH), 48.4 (CH), 36.0 (CH_2), 35.7 (C), 23.7 (CH_2), 21.4 (CH_3), 19.4 (CH_3), 18.7 (CH_3), 12.4 (CH_3), (*Signals of rotamers). **IR** (υ/cm^{-1}, thin film): 3374, 2960, 2873, 1699, 1607, 1530, 1451, 1354, 1183, 1032, 737. **HRMS** (ESI): calculated for $C_{33}H_{36}N_2O_8$ [M+H]$^+$: 589.2544, found: 589.2530.

Fmoc-(*S*)-Val photoprotected α-ketoacid 12c (single diastereomer): prepared following the general procedure using Fmoc-(*S*)-Val α-ketoacid dimethyl acetal **11c** (1.00 equiv, 1.50 g, 3.63 mmol) and enantioenriched diol **(*S*)-2** (1.25 equiv, 1.02 g, 4.54 mmol) in dry toluene. The reaction mixture was stirred for 3 h at 100 °C. The crude mixture was purified by flash chromatography (SiO_2, hexanes/AcOEt 9:1 to AcOEt + 0.1% AcOH) to give the desired product (1.45 g, 2.49 mmol, 69 %) as white foam. **^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 7.79 – 7.68 (m, 4H, CH), 7.62 – 7.53 (m, 3H, CH), 7.44 – 7.24 (m, 8H, CH), 5.69 (s, 1H, CH), 5.25 (d, *J* = 10.9 Hz, 1H, NH), 4.44 (dd, *J* = 10.7, 6.9 Hz, 1H, CH_2), 4.35 (dd, *J* = 10.7, 7.3 Hz, 1H, CH_2), 4.21(appt, *J* = 7.0 Hz, 1H, CH), 4.14 (dd, *J* = 10.9, 2.6 Hz, 1H, CH), 3.88 (d, *J* = 11.7 Hz, 2H, CH_2), 3.60 (d, *J* = 11.7 Hz, 1H, CH_2), 2.51 – 2.38 (m, 1H, CH), 1.03 (d, *J* = 6.8 Hz, 3H, CH_3), 1.02 (d, *J* = 6.7 Hz, 3H, CH_3), 0.91 (s, 3H, CH_3), 0.58 (s, 3H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 171.1 (CO), 157.4 (CO), 148.9 (C-NO_2), 144.2* (C), 143.8* (C), 141.4 (C), 132.3 (CH), 131.3 (C), 130.0 (CH), 128.7 (CH), 127.73* (CH), 127.67* (CH), 127.2* (CH), 127.1* (CH), 125.4* (CH), 125.3* (CH), 124.3 (CH), 120.0 (CH), 101.7 (C), 76.5 (CH), 74.8 (CH2), 67.3 (CH_2), 60.1 (CH), 47.3 (CH), 34.9 (C), 27.6 (CH), 22.3 (CH_3), 20.9 (CH_3), 19.1 (CH_3), 17.3 (CH_3), (*Signals of rotamers). **IR** (υ/cm^{-1}, thin film): 2963, 2876, 1722, 1529, 1470, 1449, 1350, 1219, 1183, 1091, 1019, 852, 758, 738, 666. **HRMS** (ESI): calculated for $C_{32}H_{35}N_2O_8$ [M+H]$^+$: 575.2388, found: 575.2383.

Fmoc-(*S*)-Tyr photoprotected α-ketoacid 12d (mixture of diastereomers): prepared following the general procedure using Fmoc-(*S*)-Tyrosine α-ketoacid dimethyl acetal **11d** (1.00 equiv, 3.00 g, 5.60 mmol) and racemic diol ***rac*-2** (1.50 equiv, 1.90 g, 8.40 mmol) in dry toluene. The reaction mixture was stirred for 2 h at reflux. The crude mixture was purified by flash chromatography (SiO_2, hexanes/AcOEt 3/1 to hexanes/AcOEt/MeOH 4/2/1 + 0.1% AcOH) to give the desired product as white foam (1.43 g, 2.06 mmol, 37%) over 4 steps starting from Fmoc-(*S*)-Tyr(*t*Bu)-OH. The analytical data is given for an inseparable mixture of diastereomers and rotamers. ^{1}H NMR signals are generally described as multiplets. All observed diastereomeric ^{13}C NMR signals are listed.**^{1}H NMR** (400 MHz, CD_3OD) δ [ppm] = 7.84 – 7.70 (m, 4H), 7.71 – 7.52 (m, 3H), 7.51 – 7.40 (m, 1H), 7.40 – 7.30 (m, 2H), 7.30 – 7.15 (m, 4H), 6.83 – 6.74 (m, 2H), 5.83 (s, 0.5H), 5.76 (s, 0.5H), 4.35 – 4.16 (m, 2H), 4.08 – 3.87 (m, 4H), 3.56 (dd, *J* = 11.3, 6.5 Hz, 1H), 3.28 – 3.10 (m, 1H), 2.80 (m, 1H), 1.14 (s, 9H), 0.92 (s, 3H), 0.56 (s, 3H).**^{13}C NMR** (101 MHz, CD_3OD) δ [ppm] = 174.6, 174.4, 157.7, 157.5, 153.4, 153.3, 149.0, 148.9, 144.1, 143.9, 143.6, 141.1, 141.09, 141.06, 141.03, 134.0, 133.9, 132.1, 131.8, 131.7, 131.6, 130.3, 129.5, 128.2, 128.0, 127.4, 127.3, 126.8, 126.7, 125.2, 125.1, 124.9, 124.8, 123.7, 123.6, 123.58, 123.5, 119.5, 119.4, 102.3, 102.2, 77.9, 76.0, 75.4, 74.1, 74.0, 67.0, 66.9, 58.3, 46.8, 46.7, 34.4, 34.2, 34.1, 27.7, 20.0, 18.2, 18.0.**IR**

(υ/cm^{-1}, thin film): 3418, 2973, 1684, 1530, 1448, 1364, 1179, 1057, 900, 735. **HRMS** (ESI): calculated for $C_{40}H_{42}N_2O_9$ [M+Na]$^+$: 717.2783, found: 717.2769.

Fmoc-(*S*)-Gln photoprotected α-ketoacid 12e (single diastereomer): prepared following the general procedure using Fmoc-(*S*)-Gln α-ketoacid dimethyl acetal **11e** (1.00 equiv, 400 mg, 0.58 mmol) and enantioenriched diol **(*S*)-2** (1.20 equiv, 158 mg, 0.70 mmol) in dry toluene. The reaction mixture was stirred for 2 h at reflux. The crude mixture was purified by flash chromatography (SiO_2, hexanes/AcOEt 3/1 to hexanes/AcOEt/MeOH 4/2/1 + 0.1% AcOH) to give the desired product as white foam (160 mg, 0.29 mmol, 32%) over 4 steps starting from Fmoc-(*S*)-Gln(Trt)-OH. **^{1}H NMR** (400 MHz, CD_3OD) δ [ppm] = 7.78 (m, 4H, CH), 7.65 (m, 2H, CH), 7.55 (m, 1H, CH), 7.45 (m, 1H, CH), 7.35 (m, 2H, CH), 7.29 – 7.19 (m, 11H, CH), 7.17 (m, 2H, CH), 5.62 (s, 1H, CH), 4.40 (m, 1H, CH_2), 4.30 (m, 1H, CH_2), 4.24 – 4.15 (m, 2H, CH), 3.80 (d, *J* = 11.5 Hz, 1H, CH_2), 3.58 (d, *J* = 11.5 Hz, 1H, CH_2), 2.41 – 2.17 (m, 3H, CH_2), 1.84 (m, 1H, CH_2), 0.87 (s, 3H), 0.54 (s, 3H). **^{13}C NMR** (101 MHz, CD_3OD) δ [ppm] = 174.8 (CO), 171.7 (CO), 159.1 (CO), 150.4 (C), 146.0 (C), 145.4 (C), 145.1 (C), 142.6* (C), 142.5* (C), 133.3 (C), 132.21 (CH), 131.6 (CH), 130.0 (CH), 129.8 (CH), 128.8 (CH), 128.7 (CH), 128.2* (CH), 128.1* (CH), 127.7 (CH), 126.4* (CH), 126.3* (CH), 124.9 (CH), 120.9 (CH), 102.3 (C), 77.5 (CH), 75.6 (CH_2), 71.7 (C), 67.9 (CH_2), 57.4 (CH), 48.5 (CH), 35.7 (CH_2), 34.6 (C), 26.4 (CH_2), 21.2 (CH_3), 19.2 (CH_3), (*Signals of rotamers). **IR** (υ/cm^{-1}, thin film): 3404, 3059, 2926, 2854, 1708, 1529, 1448, 1182, 1060, 849, 740, 701. **HRMS** (ESI): calculated for $C_{51}H_{47}N_3O_9$ [M+Na]$^+$: 868.3205, found: 868.3190.

Fmoc-(*S*)-Trp photoprotected α-ketoacid 12f (single diastereomer): prepared following the general procedure using Fmoc-(*S*)-Trp α-ketoacid dimethyl acetal **11f** (1.00 equiv, 8.80 g, 14.7 mmol) and diol **(*S*)-2** (1.25 equiv, 4.14 g, 18.4 mmol) in dry toluene. The reaction mixture was stirred for 2 h at reflux. The crude product (12.9 g) was used without purification in the next step. For analytical purpose, a sample was purified by flash chromatography (SiO_2, hexanes/EtOAc 7:3 to hexanes/EtOAc/MeOH 210:90:9 with 0.1% AcOH). **^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 8.17 – 8.05 (m, 1H, CH), 7.76 (d, *J* = 8.1 Hz, 1H, CH), 7.70 – 7.58 (m, 4H, CH), 7.57 – 7.49 (m, 2H, CH), 7.44 – 7.34 (m, 2H, CH), 7.33 – 7.26 (m, 4H, CH), 7.23 – 7.17 (m, 1H, CH), 7.16 – 7.06 (m, 2H, CH), 5.76 (s, 1H, CH), 5.29 (d, *J* = 10.6 Hz, 1H, NH), 4.79 – 4.66 (m, 1H, CH), 4.17 (d, *J* = 7.5 Hz, 2H, CH_2), 4.07 (t, *J* = 7.3 Hz, 1H, CH), 4.00 (d, *J* = 11.7 Hz, 1H, CH_2), 3.69 (d, *J* = 11.7 Hz, 1H, CH_2), 3.49 (dd, *J* = 15.1, 2.2 Hz, 1H, CH_2), 3.04 (dd, *J* = 15.1, 11.6 Hz, 1H, CH_2), 1.56 (s, 9H, CH_3), 0.98 (s, 3H, CH_3), 0.63 (s, 3H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 170.5 (CO), 156.7 (CO), 149.8 (CO), 148.9 (C-NO_2), 143.9* (C), 143.8* (C), 141.2 (C), 135.7 (C), 132.3 (CH), 131.1 (C), 130.8 (C), 130.1 (CH), 128.8 (CH), 127.6 (CH), 127.1 (CH), 125.3* (CH), 125.2* (CH), 124.6 (CH), 124.3 (CH), 123.9 (CH), 122.7 (CH), 119.8 (CH), 118.9 (CH), 116.5 (C), 115.4 (CH), 100.9 (C), 83.6 (C), 76.9 (CH), 74.9 (CH_2), 67.5 (CH_2), 56.0 (CH), 47.0 (CH), 35.1 (C), 28.2 (CH_3), 24.9 (CH_2), 20.9 (CH_3), 19.1 (CH_3), (*Signals of rotamers). **IR** (υ/cm^{-1}, thin film): 2973, 1730, 1530, 1474, 1451, 1370, 1308, 1256, 1223, 1159, 1089, 1071, 1019, 851, 757, 740, 698, 666. **HRMS** (ESI): calculated for $C_{43}H_{44}N_3O_{10}$ [M+H]$^+$: 762.3021, found: 762.3019.

Fmoc-(*S*)-Lys photoprotected α-ketoacid 12g (single diastereomer): prepared following the general procedure using Fmoc-(*S*)-Lys α-ketoacid dimethyl acetal **11g** (1.00 equiv, 330 mg, 0.61 mmol) and enantioenriched diol **(*S*)-2** (1.20 equiv, 164 mg, 0.73 mmol) in dry toluene. The reaction mixture was stirred for 2 h at reflux. The crude mixture was purified by flash chromatography (SiO_2, hexanes/AcOEt 3/1 to hexanes/AcOEt/MeOH 4/2/1 + 0.1% AcOH) to give the desired product as white foam (155 mg, 0.22 mmol, 36%). over 4 steps starting from Fmoc-(*S*)-Lys(Boc)-OH. **^{1}H NMR** (400 MHz, CD_3OD) δ [ppm] = 7.90 – 7.84 (m, 1H, CH), 7.82 – 7.74 (m, 3H, CH), 7.70 – 7.59 (m, 3H, CH), 7.49 – 7.41 (m, 1H, CH), 7.36 (m, 2H, CH), 7.31 – 7.24 (m, 2H, CH), 5.65 (s, 1H, CH), 4.46 – 4.33 (m, 1H, CH_2), 4.24 (m, 2H, CH_2+ CH), 4.07 (m, 1H, CH), 3.74 (d, *J* = 11.5 Hz, 1H, CH_2), 3.58 (d, *J* = 11.5 Hz, 1H, CH_2), 3.04 (dd, *J* = 6.8, 2.9 Hz, 2H, CH_2), 1.90 – 1.74 (m, 1H, CH_2), 1.41 (d, *J* = 3.7 Hz, 14H, CH_2+3xCH_3), 0.89 (s, 3H, CH_3), 0.54 (s, 3H, CH_3). **^{13}C NMR** (101 MHz, CD_3OD) δ [ppm] = 171.9 (CO), 159.1 (CO), 150.4 (CO), 145.4 (C), 145.1 (C), 142.6* (C), 142.5* (C), 133.2 (CH), 132.4 (C), 131.6 (CH), 129.8 (CH), 128.8* (CH), 128.7* (CH), 128.2* (CH), 128.1* (CH), 126.6 (CH), 126.5 (CH), 124.9 (CH), 120.84* (CH), 120.8* (CH), 102.7 (C), 77.5 (C), 75.6 (CH_2), 68.0 (CH_2), 57.7 (CH), 48.4 (CH), 41.2 (CH_2), 35.7 (C), 29.2 (CH_2), 28.8 (3xCH_3), 24.3 (CH_2), 21.1 (CH_2), 19.1 (2xCH_3), (*Signals of rotamers). **IR** (υ/cm^{-1}, thin film): 3337, 3066, 2967, 2872, 1714, 1530, 1450, 1352, 1250, 1171, 1063, 738. **HRMS** (ESI): calculated for $C_{38}H_{45}N_3O_{10}$ [M+H]$^+$: 704.3178, found: 704.3176.

Fmoc-(*S*)-Glu photoprotected α-ketoacid 12h (single diastereomer): prepared following the general procedure using Fmoc-(*S*)-Glu α-ketoacid dimethyl acetal **11h** (1.00 equiv, 2.16 g, 4.33 mmol) and enantioenriched diol **(*S*)-2** (1.15 equiv, 1.12 g, 4.98 mmol) in dry toluene (15 ml). The reaction mixture was stirred for 2 h at reflux. The crude mixture was purified by flash chromatography (SiO_2, hexanes/EtOAc 4:1 to hexanes/EtOAc/MeOH 5:5:1) to give the desired product (1.82 g, 2.75 mmol, 64%) as a white foam. **^{1}H NMR** (400 MHz, CD_3OD) δ [ppm] = 7.88 (dd, *J* = 8.0, 1.4 Hz, 1H, CH), 7.81 – 7.75 (m, 3H, CH), 7.69 – 7.60 (m, 3H, CH), 7.48 – 7.43 (m, 1H, CH), 7.39 – 7.33 (m, 2H, CH), 7.31 – 7.25 (m, 2H, CH), 5.68 (s, 1H, CH), 4.40 – 4.27 (m, 2H, CH_2), 4.21 (appt, *J* = 6.9 Hz, 1H, CH), 4.09 (dd, *J* = 11.6, 2.9 Hz, 1H, CH), 3.81 (d,

J = 11.4 Hz, 1H, CH_2), 3.54 (d, *J* = 11.4 Hz, 1H, CH_2), 2.40 – 2.14 (m, 3H, CH_2), 1.82 (dddd, *J* = 13.8, 11.7, 7.9, 5.5 Hz, 1H, CH_2), 1.45 (s, 9H, CH_3), 0.88 (s, 3H, CH_3), 0.54 (s, 3H, CH_3). **^{13}C NMR** (101 MHz, CD_3OD) δ [ppm] = 174.4 (CO), 159.1 (CO), 150.3 (C-NO_2), 145.4* (C), 145.1* (C), 142.6* (C), 142.5* (C), 133.2 (CH), 132.8 (C), 131.6 (CH), 129.7 (CH), 128.8* (CH), 128.7* (CH), 128.2* (CH), 128.1* (CH), 126.4* (CH), 126.3* (CH), 124.9 (CH), 120.9 (CH), 120.8 (CH), 102.9 (C), 81.6 (C), 77.3 (CH_2), 75.5 (CH_2), 68.0 (CH_2), 57.4 (CH), 48.4 (CH), 35.8 (C), 33.1 (CH_2), 28.4 (CH_3), 25.4 (CH_2), 21.3 (CH_3), 19.2 (CH_3), (*Signals of rotamers). **IR** (υ/cm^{-1}, thin film): 2974, 1725, 1530, 1449, 1392, 1351, 1254, 1155, 1060, 850, 758, 737, 665. **HRMS** (ESI): calculated for $C_{36}H_{41}N_2O_{10}$ $[M+H]^+$: 661.2756, found: 661.2748.

General procedure Fmoc-(*S*)-AA photoprotected α-ketoacid with protected linker: In a flame-dried flask, K_2CO_3 (0.50 equiv) and KI (0.25 equiv) were introduced and dried under vacuum. Fmoc-(*S*)-AA photoprotected α-ketoacid (1.00 equiv) and 5-(4-chloromethyl)phenoxy)pentanoate (1.20 equiv) were added and dissolved in dry acetone (1.00 M). The mixture was stirred at rt until complete consumption of the starting material. The reaction was diluted with EtOAc and quenched with 1 M HCl solution. The aqueous phase was extracted with EtOAc (3x100 mL) and the combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography (SiO_2, hexanes/EtOAc 4:1 to 3:1) to give the product as a white foam.

Fmoc-(*S*)-Leu photoprotected α-ketoacid with protected linker 39a (mixture of diastereomers): prepared following the general procedure using Fmoc-(*S*)-Leu photoprotected α-ketoacid **12a** (1.00 equiv, 1.50 g, 2.55 mmol), allyl 5-(4-chloromethyl)phenoxy)pentanoate (1.20 eq, 864 mg, 3.06 mmol), K_2CO_3 (0.50 eq, 176 mg, 1.27 mmol) and KI (0.25 eq, 106 mg, 0.64 mmol) in dry acetone. The reaction mixture was stirred for 12 h at rt. The crude mixture was purified by flash chromatography (SiO_2, hexanes/AcOEt 4/1 to 3/1) to give the desired product as white foam (1.35 g, 1.62 mmol, 63%). The analytical data is given for an inseparable mixture of diastereomers and rotamers. ^{1}H NMR signals are generally described as multiplets. All observed diastereomeric ^{13}C NMR signals are listed. **^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 7.75 (m, 3H, CH), 7.67 – 7.49 (m, 3H, CH), 7.45 – 7.20 (m, 8H, CH), 6.80 (m, 2H, CH), 5.99 – 5.83 (m, 1H, CH), 5.63 (s, 0.5H, CH), 5.57 (s, 0.5H, CH), 5.39 – 5.08 (m, 4H, CH_2), 4.96 – 4.82 (m, 1H, CH), 4.58 (m, 2H, CH_2), 4.48 – 4.33 (m, 2H, CH_2), 4.32 – 4.15 (m, 3H, CH+CH_2), 3.95 – 3.84 (m, 2H, CH_2), 3.80 (m, 1H, CH_2), 3.56 (m, 1H, CH_2), 2.39 (m, 2H, CH_2), 1.79 (m, 4H, CH_2), 1.62 (m, 1H, CH), 1.54 – 1.32 (m, 1.5H, CH_2), 1.24 – 1.13 (m, 0.5H, CH_2), 0.93 – 0.84 (m, 6H), 0.80 (d, *J* = 6.5 Hz, 3H), 0.55 (d, *J* = 1.9 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 173.2, 168.8, 159.4, 159.3, 156.5, 156.4, 149.0, 148.9, 144.2, 144.1, 144.0, 143.9, 141.4, 141.3, 132.4, 132.3, 132.2, 131.5, 131.2, 131.2, 131.0, 130.2, 130.0, 128.7, 128.6, 127.82, 127.80, 127.7, 127.6, 127.34, 127.32, 127.2, 127.18, 127.14, 127.09, 125.4, 125.3, 125.28, 125.12, 124.16, 124.1, 120.1, 120.0, 119.98, 119.97, 118.4, 114.6, 101.7, 101.5, 76.6, 75.9, 74.7, 74.69, 67.9, 67.8, 67.5, 67.4, 67.1, 67.0, 65.2, 55.0, 54.8, 47.5, 47.3, 38.1, 38.0, 35.0, 34.0, 28.7, 24.6, 24.5, 24.0, 23.9, 21.7, 21.5, 21.2, 20.94, 20.91, 19.00, 18.98. **IR** (υ/cm^{-1}, thin film): 2956, 2871, 1736, 1529, 1247, 1172, 1058, 738, 667. **HRMS** (ESI): calculated for $C_{48}H_{54}N_2O_{11}$ $[M+Na]^+$: 857.3620, found: 857.3608.

Fmoc-(*S*)-Ile photoprotected α-ketoacid with protected linker 39b (single diastereomer): prepared following the general procedure using Fmoc-(*S*)-Ile photoprotected α-ketoacid **12b** (1.00 equiv, 500 mg, 0.85 mmol), allyl 5-(4-chloromethyl)phenoxy)pentanoate (1.20 equiv, 288 mg, 1.02 mmol), K_2CO_3 (0.50 eq, 59.0 mg, 0.42 mmol) and KI (0.25 eq, 35.0 mg, 0.21 mmol) in dry acetone. The reaction mixture was stirred for 12 h at rt. The crude mixture was purified by flash chromatography (SiO_2, hexanes/AcOEt 4/1 to 3/1) to give the desired product as white foam (472 mg, 0.57 mmol, 67%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 7.76 (m, 3H, CH), 7.66 (m, 1H, CH), 7.56 (m, 3H, CH), 7.38 (m, 3H, CH), 7.34 – 7.23 (m, 4H, CH), 6.77 (d, *J* = 8.7 Hz, 2H, CH), 5.91 (m, 1H, CH), 5.57 (s, 1H, CH), 5.31 (m, 1H, CH_2), 5.27 – 5.19 (m, 2H, CH_2), 5.11 (m, 2H, CH_2+NH), 4.57 (dt, *J* = 5.7, 1.4 Hz, 2H, CH_2), 4.37 (m, 1H, CH_2), 4.28 (m, 1H, CH_2), 4.20 (t, *J* = 7.2 Hz, 1H, CH), 4.09 (dd, *J* = 10.9, 2.9 Hz, 1H, CH), 3.87 (m, 2H, CH_2), 3.79 (d, *J* = 11.7 Hz, 1H, CH_2), 3.58 (d, *J* = 11.7 Hz, 1H, CH_2), 2.39 (m, 2H, CH_2), 1.95 (m, 1H, CH_2), 1.88 – 1.69 (m, 5H, CH_2), 1.00 (m, 4H, CH_2+CH_3), 0.92 – 0.83 (m, 6H, CH_3), 0.55 (s, 3H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 173.2 (CO), 168.8 (CO), 159.3 (CO), 156.7 (C), 148.9 (C), 144.2 (C), 144.0 (C), 141.4* (C), 141.3* (C), 132.4* (CH), 132.3* (CH), 131.26 (CH), 130.9 (CH), 130.1 (CH), 128.7 (CH), 127.8* (CH), 127.7* (CH), 127.2* (CH), 127.1* (CH), 125.4* (CH), 125.3* (CH), 124.2 (CH), 120.0 (CH), 118.4 (CH_2), 114.6 (CH), 102.2 (C), 76.5 (CH), 74.7 (CH_2), 68.0 (CH_2), 67.4 (CH_2), 67.1 (CH_2), 65.2 (CH_2), 60.6 (CH), 47.3 (CH), 34.9 (C), 34.7 (CH), 34.0 (CH_2), 28.7 (CH_2), 23.9 (CH_2), 21.7(CH_2), 21.0 (CH_3), 19.0 (CH_3), 18.2 (CH_3), 12.3 (CH_3), (*Signals of rotamers). **IR** (υ/cm^{-1}, thin film): 2962, 2875, 1737, 1529, 1450, 1352, 1244, 1173, 1087, 1061, 827, 738. **HRMS** (ESI): calculated for $C_{48}H_{54}N_2O_{11}$ $[M+Na]^+$: 857.3620, found: 857.3612.

Fmoc-(*S*)-Val photoprotected α-ketoacid with protected linker 39c (single diastereomer): was prepared following the general procedure using Fmoc-(*S*)-Val photoprotected α-ketoacid **12c** (1.00 equiv, 1.34 g, 2.33 mmol), allyl 5-(4-chloromethyl)phenoxy)pentanoate (1.25 equiv, 821 mg, 2.91 mmol), K_2CO_3 (0.50 equiv, 162 mg, 1.17 mmol) and KI (0.25 equiv, 96.7 mg, 583 µmol) in dry acetone. The reaction mixture was stirred for 12 h at rt. The crude mixture was purified by flash chromatography (SiO_2, hexanes/AcOEt 17:3 to 7:3) to give the desired product (1.29 g, 1.57 mmol, 67 %) as white foam. **^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 7.81 – 7.72 (m, 3H, CH), 7.71 – 7.64 (m, 1H, CH), 7.64 – 7.52 (m, 3H, CH), 7.43 – 7.34 (m, 3H, CH), 7.34 – 7.25 (m, 4H, CH), 6.80 – 6.73 (m, 2H, CH), 5.91 (ddt, *J* = 17.3, 10.4, 5.7 Hz, 1H, CH), 5.58 (s, 1H, CH), 5.31 (ddd, *J* = 17.3, 3.1, 1.5 Hz, 1H, CH_2), 5.27 – 5.18 (m, 2H, CH_2), 5.13 (d, *J* = 11.7 Hz, 1H, CH_2), 5.11 (d, *J* = 10.9 Hz, 1H, NH), 4.57 (d appt, *J* = 5.7, 1.4 Hz, 2H, CH_2), 4.38 (dd, *J* = 10.4, 7.0 Hz, 1H, CH_2), 4.28 (dd, *J* = 10.4, 7.4 Hz, 1H, CH_2), 4.22 (appt, *J* = 7.1 Hz, 1H, CH), 4.09 (dd, *J* = 10.9, 2.6 Hz, 1H, CH), 3.92 – 3.82 (m, 2H, CH_2), 3.80 (d, *J* = 11.6 Hz, 1H, CH_2), 3.58 (d, *J* = 11.6 Hz, 1H, CH_2), 2.43 – 2.34 (m, 2H, CH_2), 2.31 (sep d, *J* = 6.8, 2.6 Hz, 1H, CH), 1.84 – 1.72 (m, 4H, CH_2), 0.99 (d, *J* = 6.9 Hz, 3H, CH_3), 0.97 (d, *J* = 6.8 Hz, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.55 (s, 3H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 173.2 (CO), 168.8 (CO), 159.3 (C), 156.7 (CO), 149.0 (C-NO_2), 144.2* (C), 144.0* (C), 141.41* (C), 141.36* (CH), 132.4 (CH), 132.3 (CH), 131.2 (C), 130.9 (CH), 130.2 (CH), 128.7 (CH), 127.8* (CH), 127.7* (CH), 127.18* (CH, C), 127.15* (CH), 125.4* (CH), 125.3* (CH), 124.2 (CH), 120.0 (CH), 118.4 (CH_2), 114.6 (CH), 102.2 (C), 76.4 (CH), 74.7 (CH_2), 68.0 (CH_2), 67.4 (CH_2), 67.2 (CH_2), 65.2 (CH_2), 60.1 (CH), 47.3 (CH), 34.9 (C), 34.0 (CH_2), 28.7 (CH_2), 27.6 (CH_2), 22.3 (CH_3), 21.7 (CH_2), 21.0 (CH_3), 19.1 (CH_3), 17.2 (CH_3), (*Signals of rotamers). **IR** (υ/cm^{-1}, thin film): 2960, 1735, 1528, 1514, 1472, 1351, 1301, 1219, 1173, 1087, 1059, 1013, 759, 740. **HRMS** (ESI): calculated for $C_{47}H_{53}N_2O_{11}$ $[M+H]^+$: 821.3644, found: 821.3637.

Fmoc-(*S*)-Tyr photoprotected α-ketoacid with protected linker 39d (mixture of diastereomers): prepared following the general procedure using Fmoc-(*S*)-Tyrosine photoprotected α-ketoacid **12d** (1.00 equiv, 1.40 g, 2.02 mmol), allyl 5-(4-chloromethyl)phenoxy)pentanoate (1.20 equiv, 684 mg, 2.40 mmol), K_2CO_3 (0.50 eq, 139 mg, 1.00 mmol) and KI (0.25 equiv, 84.0 mg, 0.50 mmol) in dry acetone. The reaction mixture was stirred for 12 h at rt. The crude mixture was purified by flash chromatography (SiO_2, hexanes/AcOEt 4/1 to 3/1) to give the desired product as white foam (872 mg, 0.93 mmol, 46%). The analytical data is given for an inseparable mixture of diastereomers and rotamers. ^{1}H NMR signals are generally described as multiplets. All observed diastereomeric ^{13}C NMR signals are listed. **^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 7.85 – 7.68 (m, 3H), 7.69 – 7.53 (m, 2H), 7.49 (m, 2H), 7.44 – 7.21 (m, 7H), 7.09 (m, 2H), 6.89 – 6.72 (m, 4H), 5.98 – 5.85 (m, 1H), 5.75 (s, 0.5H), 5.59 (s, 0.5H), 5.31 (dt, *J* = 17.2, 1.4 Hz, 1H), 5.26 – 5.16 (m, 2H), 5.12 – 4.93 (m, 1H), 4.57 (m, 2H), 4.54-4.32 (m, 1H), 4.08 (m, 2H), 3.99-3.80 (m, 3H), 3.63 (t, *J* = 12.1 Hz, 1H), 3.22 (m, 1H), 2.71 (m, 1H), 2.38 (m, 2H), 1.90 – 1.68 (m, 4H), 1.21 (s, 9H), 0.94 (m, 3H), 0.59 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 173.0, 168.4, 159.2, 159.1, 155.7, 155.6, 153.9, 148.9, 148.8, 144.1, 143.9, 143.88, 143.82, 141.2, 141.1, 132.2, 132.19, 132.16, 132.12, 131.2, 130.9, 130.8, 130.7, 130.4, 130.1, 129.9, 129.8, 129.7, 129.6, 128.7, 128.6, 127.7, 127.63, 127.60, 127.6, 127.5, 127.2, 127.1, 127.0, 127.01, 126.9, 125.3, 125.22, 125.20, 125.1, 124.1, 124.06, 124.03, 123.9, 119.9, 119.8, 118.2, 114.4, 101.2, 101.1, 78.2, 77.2, 76.0, 74.7, 74.6, 67.9, 67.8, 67.3, 67.2, 67.1, 67.0, 65.0, 57.4, 57.1, 47.0, 46.9, 34.9, 34.86, 34.81, 34.6, 33.8, 28.9, 28.8, 28.7, 28.6, 28.5, 21.6, 20.9, 20.8, 18.9. **IR** (υ/cm^{-1}, thin film): 3356, 2972, 2874, 1737, 1611, 1529, 1449, 1242, 1173, 1065, 909, 737. **HRMS** (ESI): calculated for $C_{55}H_{60}N_2O_{12}$ $[M+Na]^+$: 963.4038, found: 963.4031.

Fmoc-(*S*)-Gln photoprotected α-ketoacid with protected linker 39e (single diastereomer): was prepared following the general procedure using Fmoc-(*S*)-Gln photoprotected α-ketoacid **12e** (1.00 equiv, 120 mg, 0.14 mmol), allyl 5-(4-chloromethyl)phenoxy)pentanoate (1.20 equiv, 48.0 mg, 0.17 mmol), K_2CO_3 (0.50 equiv, 10.0 mg, 0.07 mmol) and KI (0.25 equiv, 6.0 mg, 0.035 mmol) in dry acetone. The reaction mixture was stirred for 12 h at rt. The crude mixture was purified by flash chromatography (SiO_2, hexanes/AcOEt 4/1 to 3/1) to give the desired product as white foam (125 mg, 0.11 mmol, 81%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 7.77 (m, 3H, CH), 7.62 (m, 1H, CH), 7.58 – 7.48 (m, 3H, CH), 7.43 – 7.34 (m, 3H, CH), 7.29 (m, 2H, CH), 7.23 – 7.07 (m, 17H), 6.57 (d, *J* = 8.7 Hz, 2H, CH), 5.90 (m, 1H, CH), 5.51 (s, 1H, CH), 5.31 (m, 1H, CH_2), 5.27 – 5.19 (m, 2H, CH_2), 5.08 (d, *J* = 10.3 Hz, 1H, NH), 5.02 (d, *J* = 11.0 Hz, 1H, CH_2), 4.57 (m, 2H, CH_2), 4.34 (m, 1H, CH_2), 4.22 (m, 2H, CH_2+CH), 4.12 (m, 1H, CH), 3.91 (d, *J* = 11.5 Hz, 1H, CH_2), 3.78 – 3.61 (m, 2H, CH_2), 3.56 (d, *J* = 11.5 Hz, 1H, CH_2), 2.33 (m, 4H, CH_2), 1.85 (m, 1H, CH_2), 1.78 – 1.58 (m, 3H, CH_2), 0.87 (s, 3H, CH_3), 0.55 (s, 3H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 173.2 (CO), 171.6 (CO), 168.5 (CO), 159.1 (CO), 156.7 (C), 148.9 (C), 144.9 (C), 144.2 (C), 143.7 (C), 141.5* (C), 141.4* (C), 132.7 (C), 132.4 (C), 130.8 (C), 130.6 (CH), 130.5 (C), 128.9 (CH), 128.7 (CH), 127.9 (CH), 127.86* (CH), 127.81* (CH), 127.22* (CH), 127.18* (CH), 127.0 (CH), 126.9 (CH), 125.4* (CH), 125.3* (CH), 124.0 (CH), 120.1* (CH), 120.0* (CH), 118.7 (CH), 114.5 (CH), 100.9 (C), 76.7 (CH), 74.7 (CH_2), 70.8 (C), 67.9 (CH_2), 67.3 (CH_2), 67.0 (CH_2), 65.2 (CH_2), 56.1 (CH), 47.3 (CH), 35.0 (CH_2), 34.1 (C), 34.0 (CH_2), 28.7 (CH_2), 26.1 (CH_2), 21.7 (CH_2), 20.9 (CH_3), 18.9 (CH_3), (*Signals of rotamers). **IR** (υ/cm^{-1}, thin film): 3338, 3058, 2946, 2874, 1735, 1528, 1246, 1174, 1058, 738, 701. **HRMS** (ESI): calculated for $C_{66}H_{65}N_3O_{12}$ $[M+H]^+$: 1092.4641, found: 1092.4636.

Fmoc-(*S*)-Trp photoprotected α-ketoacid with protected linker 39f (single diastereomer): prepared following the general procedure using crude Fmoc-(*S*)-Trp photoprotected α-ketoacid **12f** (1.00 equiv) from the previous step, allyl 5-(4-chloromethyl)phenoxy)pentanoate (1.35 equiv, 5.60 g, 19.8 mmol), K_2CO_3 (0.50 equiv, 1.02 g, 7.35 mmol) and KI (0.25 equiv, 610 mg, 3.68 mmol) in dry acetone (20 ml). The reaction mixture was stirred for 12 h at rt. The crude mixture was purified by flash chromatography (SiO_2, hexanes/EtOAc 4:1 to 3:2) to give the desired product (10.9 g, 10.8 mmol, 73 % over 2 steps) as a light yellow foam. **^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 8.17 – 8.06 (m, 1H, CH), 7.82 – 7.66 (m, 3H, CH), 7.65 – 7.58 (m, 1H, CH), 7.56 – 7.45 (m, 3H, CH), 7.45 – 7.27 (m, 8H, CH), 7.24 – 7.11 (m, 3H, CH), 6.79 – 6.74 (m, 2H, CH), 5.98 – 5.86 (m, 1H, CH), 5.63 (s, 1H, CH), 5.37 – 5.21 (m, 3H, CH_2), 5.17 (d, *J* = 11.7 Hz, 1H, CH_2), 5.07 (d, *J* = 10.7 Hz, 1H, NH), 4.64 (dd, *J* = 10.7, 3.6 Hz, 1H, CH), 4.60 – 4.56 (m, 2H, CH_2), 4.16 – 4.05 (m, 3H, CH, CH_2), 3.93 (d, *J* = 11.7 Hz, 1H, CH_2), 3.88 – 3.74 (m, 2H, CH_2), 3.68 (d, *J* = 11.7 Hz, 1H, CH_2), 3.35 (dd, *J* = 15.4, 2.5 Hz, 1H, CH_2), 2.89 (dd, *J* = 15.4, 10.7 Hz, 1H, CH_2), 2.48 – 2.31 (m, 2H, CH_2), 1.89 – 1.67 (m, 4H, CH_2), 1.58 (s, 9H, CH_3), 0.98 (s, 3H, CH_3), 0.61 (s, 3H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 173.2 (CO), 168.5 (CO), 159.3 (C), 156.0 (CO), 149.7 (CO), 148.9 (C-NO_2), 144.02* (C), 143.97* (C), 141.3* (C), 141.2* (C), 135.6 (C), 132.4 (CH), 132.3 (CH), 131.1 (C), 130.9 (CH), 130.4 (C), 130.2 (CH), 128.8 (CH), 127.7 (CH), 127.2 (C), 127.1 (CH), 125.35* (CH), 125.33 (CH), 124.5 (CH), 124.2 (CH), 123.9 (CH), 122.6 (CH), 119.9 (CH), 118.9 (CH), 118.3 (CH_2), 116.6 (C), 115.4 (CH), 114.6 (CH), 101.4 (C), 83.4 (C), 76.8 (CH), 74.8 (CH_2), 68.1 (CH_2), 67.4 (CH_2), 67.2 (CH_2), 65.2 (CH_2), 56.2 (CH), 47.1 (CH), 35.1 (C), 34.0 (CH_2), 28.7 (CH_2), 28.3 (CH_3), 25.0 (CH_2), 21.7 (CH_2), 21.0 (CH_3), 19.1 (CH_3), (*Signals of rotamers). **IR** (υ/cm^{-1}, thin film): 2965, 1732, 1612, 1529, 1515, 1473, 1451, 1371, 1308, 1249, 1161, 1088, 1069, 935, 851, 826, 759, 741. **HRMS** (ESI): calculated for $C_{58}H_{65}N_4O_{13}$ $[M+NH_4]^+$: 1025.4543, found: 1025.4524.

Fmoc-(*S*)-Lys photoprotected α-ketoacid with protected linker 39g (single diastereomer): was prepared following the general procedure using Fmoc-(*S*)-Lys photoprotected α-ketoacid **12g** (1.00 equiv, 200 mg, 0.28 mmol), allyl 5-(4-chloromethyl)phenoxy)pentanoate (1.20 equiv, 96.0 mg, 0.34 mmol), K_2CO_3 (0.50 equiv, 20.0 mg, 0.14 mmol) and KI (0.25 equiv, 12 mg, 0.07 mmol) in dry acetone. The reaction mixture was stirred for 12 h at rt. The crude mixture was purified by flash chromatography (SiO_2, hexanes/AcOEt 4/1 to 3/1) to give the desired product as white foam (137 mg, 0.14 mmol, 51%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 7.75 (m, 3H, CH), 7.63 (m, 1H, CH), 7.56 (m, 3H, CH), 7.42 – 7.34 (m, 3H, CH), 7.33 – 7.26 (m, 4H, CH), 6.77 (d, *J* = 8.6 Hz, 2H, CH), 5.91 (m, 1H, CH), 5.54 (s, 1H, CH), 5.31 (m, 1H, CH_2), 5.26 – 5.18 (m, 2H, CH_2), 5.15 (d, *J* = 11.7 Hz, 1H, CH_2), 4.94 (d, *J* = 10.6 Hz, 1H), 4.57 (m, 2H, CH_2), 4.39 (m, 1H, CH_2), 4.28 – 4.16 (m, 2H, CH+ CH_2), 4.13 (m, 1H, CH), 3.91-3.78 (m, 3H, CH_2), 3.57 (d, *J* = 11.6 Hz, 1H, CH_2), 3.08 (m, 2H, CH_2), 2.44 – 2.33 (m, 2H, CH_2), 1.76 (m, 4H, 2xCH_2), 1.43 (m, 13H, 3x CH_3+2xCH_2), 0.90 (s, 3H, CH_3), 0.55 (s, 3H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 173.2 (CO), 168.7 (CO), 159.3 (CO), 156.5 (CO), 156.1 (C), 148.9 (C), 144.2 (C), 143.9 (C), 141.4* (C), 141.3* (C), 132.4 (CH), 132.3 (CH), 131.1 (C), 130.9 (CH), 130.2 (CH), 128.7 (C), 127.8* (CH), 127.7* (CH), 127.3 (CH), 127.2* (CH), 127.1* (CH), 125.4 (CH), 125.3 (CH), 124.1 (CH), 120.0 (CH), 118.4 (CH_2), 114.6 (CH), 101.3 (C), 77.4 (C), 76.6 (CH), 74.8 (CH_2), 68.0 (CH_2), 67.4 (CH_2), 67.1 (CH_2), 65.2 (CH_2), 56.4 (CH), 47.3 (CH), 40.5 (CH_2), 35.0 (C), 34.0 (CH_2), 28.7 (CH_2), 28.6 (3xCH_3), 23.1 (CH_2), 21.7 (CH_2), 20.9 (CH_3), 18.9 (CH_3), (*Signals of rotamers). **IR** (υ/cm^{-1}, thin film): 3393, 2939, 2871, 1735, 1529, 1450, 1364, 1246, 1173, 1061, 934, 739. **HRMS** (ESI): calculated for $C_{53}H_{63}N_3O_{13}$ $[M+H]^+$: 950.4434, found: 950.4424.

Fmoc-(*S*)-Glu photoprotected α-ketoacid with protected linker 39h (single diastereomer): prepared following the general procedure using Fmoc-(*S*)-Glu photoprotected α-ketoacid **12h** (1.00 equiv, 1.52 g, 2.30 mmol), allyl 5-(4-chloromethyl)phenoxy)pentanoate (1.25 equiv, 811 mg, 2.88 mmol), K_2CO_3 (0.50 equiv, 159 mg, 1.15 mmol) and KI (0.25 equiv, 95.5 mg, 575 µmol) in dry acetone (7.5 mL). The reaction mixture was stirred for 2 d at rt. The crude mixture was purified by flash chromatography (SiO_2, hexanes/EtOAc 4:1 to 2:1) to give the desired product (983 mg, 1.08 mmol, 47%) as a white foam. **^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 7.82 – 7.73 (m, 3H, CH), 7.71 – 7.64 (m, 1H, CH), 7.62 – 7.51 (m, 3H, CH), 7.43 – 7.34 (m, 3H, CH), 7.34 – 7.25 (m, 4H, CH), 6.81 – 6.73 (m, 2H, CH), 5.91 (dddd, J = 16.1, 10.4, 6.8, 4.7 Hz, 1H, CH), 5.53 (s, 1H, CH), 5.31 (dq, *J* = 17.2, 1.6 Hz, 1H, CH_2), 5.26 – 5.20 (m, 2H, CH_2), 5.11 (d, *J* = 11.7 Hz, 1H, CH_2), 5.00 (d, J = 10.6 Hz, 1H, NH), 4.57 (dt, *J* = 5.6, 1.4 Hz, 2H, CH_2), 4.39 (dd, *J* = 9.7, 6.1 Hz, 1H, CH_2), 4.24 – 4.13 (m, 3H, CH, CH_2), 3.91 – 3.79 (m, 3H, CH_2), 3.58 (d, J = 11.6 Hz, 1H, CH_2), 2.46 – 2.16 (m, 5H, CH_2), 1.92 – 1.70 (m, 5H, CH_2), 1.44 (s, 9H, CH_3), 0.91 (s, 3H, CH_3), 0.55 (s, 3H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 173.2 (CO), 172.7 (CO), 168.6 (CO), 159.2 (C), 156.3 (CO), 148.9 (C-NO_2), 144.2* (C), 143.8* (C), 141.4* (C), 141.3* (C), 132.37 (CH), 132.35 (CH) , 131.1 (C), 130.8 (CH), 130.3 (CH), 128.7 (CH), 127.8* (CH), 127.7* (CH), 127.20* (CH, C), 127.16* (CH), 125.4* (CH), 125.3* (CH), 124.1 (CH), 120.02* (CH), 120.00* (CH), 118.4 (CH_2), 114.6 (CH), 101.1 (C), 80.5 (C), 76.6 (CH), 74.7 (CH_2), 68.0 (CH_2), 67.4 (CH_2), 67.1 (CH_2), 65.2 (CH_2), 56.2 (CH), 47.2 (CH), 35.0 (C), 34.0 (CH_2), 32.1 (CH_2), 28.7 (CH_2), 28.3 (CH_3), 24.3 (CH_2), 21.7 (CH_2), 20.9 (CH_3), 19.0 (CH_3), (*Signals of rotamers). **IR** (υ/cm^{-1}, thin film): 2964, 1730, 1612, 1529, 1515, 1450, 1366, 1352, 1246, 1158, 1093, 986, 935, 849, 828, 759, 740, 666. **HRMS** (ESI): calculated for $C_{51}H_{59}N_2O_{13}$ $[M+H]^+$: 907.4012, found: 907.4011.

General procedure Fmoc-(*S*)-AA photoprotected α-ketoacid with free linker: Fmoc-(*S*)-AA photoprotected α-ketoacid with protected linker (1.00 equiv) was dissolved in dry CH_2Cl_2 (200 mM) and cooled to 0 °C. Morpholine (1.10 equiv) was added and the mixture was degassed by evacuating the flask until the solvent boiled and refilling the flask with N_2 (repeated two times). $Pd(PPh_3)_4$ (0.05 equiv) was added and the mixture was stirred 1 h at 0 °C. Saturated aqueous NH_4Cl was added and the aqueous phase was extracted with CH_2Cl_2 (3x50 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography (SiO_2, hexanes/EtOAc 3:1 followed by hexanes/EtOAc 2:1 + 10 to 20 % MeOH) to give the product as an off-white foam.

Fmoc-(*S*)-Leu photoprotected α-ketoacid with free linker 14a (mixture of diastereomers): prepared following the general procedure using Fmoc-(*S*)-Leu photoprotected α-ketoacid with protected linker **39a** (1.00 equiv, 1.30 g, 1.56 mmol), $Pd(PPh_3)_4$ (0.05 equiv, 90.0 mg, 0.078 mmol) and morpholine (1.10 equiv, 148 µl, 1.71 mmol) in dry CH_2Cl_2. The reaction mixture was stirred for 1 h at 0 °C. The crude mixture was purified by flash chromatography (SiO_2, hexanes/AcOEt 3/1 to hexanes/AcOEt/MeOH 4/2/1 + 0.1% AcOH) to give the desired product as white foam (1.32 g, 1.67 mmol, 99%). The analytical data is given for an inseparable mixture of diastereomers and rotamers. ^{1}H NMR signals are generally described as multiplets. All observed diastereomeric ^{13}C NMR signals are listed. **^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 7.76 (m, 3H, CH), 7.67 – 7.50 (m, 3H, CH), 7.48 – 7.22 (m, 8H), 6.84 – 6.74 (m, 2H, CH), 5.64 (s, 0.5H), 5.57 (s, 0.5H), 5.43 – 5.03 (m, 2H, CH_2), 4.95 (m, 1H, NH), 4.49 – 4.34 (m, 2H, CH_2), 4.31 – 4.15 (m, 2H, CH), 3.94 – 3.85 (m, 2H, CH_2), 3.79 (m, 1H, CH_2), 3.56 (m, 1H, CH_2), 2.48 – 2.35 (m, 2H, CH_2), 1.79 (m, 4H, CH_2), 1.63 (m, 1H, CH), 1.55 – 1.31 (m, 1.5H, CH_2), 1.29 – 1.15 (m, 0.5H, CH_2), 0.95 – 0.84 (m, 6H, CH_3), 0.80 (m, 3H, CH_3), 0.55 (m, 3H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 178.5, 168.8, 159.3, 159.29, 156.5, 156.4, 149.0, 148.9, 144.2, 144.1, 144.09, 143.9, 141.4, 141.3, 132.4, 132.22, 132.20, 131.4, 131.2, 131.1, 131.0, 130.2, 130.0, 128.7, 128.7, 128.6, 128.5, 127.82, 127.80, 127.74, 127.68, 127.4, 127.3, 127.2, 127.18, 127.14, 127.1, 125.4, 125.3, 125.1, 124.2, 124.1, 120.1, 120.0, 119.98, 119.97, 114.7, 114.6, 101.7, 101.5, 77.4, 76.6, 75.9, 74.75, 74.70, 67.9, 67.8, 67.44, 67.4, 67.1, 67.0, 55.0, 54.9, 47.4, 47.3, 38.1, 38.0, 34.9, 33.6, 28.6, 24.6, 24.5, 24.0, 23.9, 21.6, 21.5, 21.2, 20.94, 20.91, 19.0, 18.9. **IR** (υ/cm^{-1}, thin film): 2958, 2872, 1714, 1530, 1247, 1174, 1059, 827, 737. **HRMS** (ESI): calculated for $C_{45}H_{50}N_2O_{11}$ $[M+H]^+$: 795.3487, found: 795.3483.

Fmoc-(*S*)-Ile photoprotected α-ketoacid with free linker 14b (single diastereomer): prepared following the general procedure using Fmoc-(*S*)-Ile photoprotected α-ketoacid with protected linker **39b** (1.00 equiv, 1.30 g, 1.56 mmol), $Pd(PPh_3)_4$ (0.05 equiv, 90.0 mg, 0.078 mmol) and morpholine (1.50 equiv, 148 µl, 1.71 mmol) in dry CH_2Cl_2. The reaction mixture was stirred for 1 h at 0 °C. The crude mixture was purified by flash chromatography (SiO_2, hexanes/AcOEt 3/1 to hexanes/AcOEt/MeOH 4/2/1 + 0.1% AcOH) to give the desired product as white foam (1.22 g, 1.54 mmol, 99%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 7.75 (m, 3H, CH), 7.66 (m, 1H, CH), 7.57 (m, 3H, CH), 7.38 (m, 3H, CH), 7.29 (m, 4H, CH), 6.77 (d, *J* = 8.6 Hz, 2H, CH), 5.57 (s, 1H, CH), 5.24 (m, 1H, CH_2), 5.13 (m, 2H, NH+CH_2), 4.36 (m, 1H, CH_2), 4.28 (m, 1H, CH_2), 4.20 (t, *J* = 7.2 Hz, 1H, CH), 4.08 (dd, *J* = 10.9, 2.9 Hz, 1H, CH), 3.86 (m, 2H, CH_2), 3.79 (d, *J* = 11.6 Hz, 1H, CH_2), 3.58 (d, *J* = 11.6 Hz, 1H, CH_2), 2.40 (m, 2H, CH_2), 1.97 (m, 1H, CH_2), 1.86 – 1.71 (m, 5H, CH_2), 1.00 (m, 3H, CH_3), 0.98 – 0.81 (m, 7H, CH+CH_3), 0.54 (s, 3H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 178.6 (CO), 168.9 (CO), 159.3 (CO), 156.8 (C), 148.9 (C), 144.2 (C), 144.0 (C), 141.4 (C), 132.3 (C), 131.2 (CH), 131.0 (CH), 130.9 (CH), 130.1 (CH), 128.7 (CH), 127.8 (CH), 127.7 (CH), 127.21 (CH), 127.18 (CH), 127.14 (CH), 125.4 (CH), 125.3 (CH), 124.2 (CH), 120.0 (CH), 114.6 (CH), 102.24 (C), 76.5 (CH), 74.7 (CH_2), 68.0 (CH_2), 67.4 (CH_2), 67.2 (CH_2), 60.6 (CH), 47.3 (CH), 34.9 (C), 34.7 (CH), 33.6 (CH_2), 28.6 (CH_2), 23.9 (CH_2), 21.5 (CH_2), 21.0 (CH_3), 19.0 (CH_3), 18.2 (CH_3), 12.2 (CH_3). **IR** (υ/cm^{-1}, thin film): 2961, 2874, 1734, 1529, 1449, 1352, 1244, 1174, 1087, 1029, 741. **HRMS** (ESI): calculated for $C_{45}H_{50}N_2O_{11}$ $[M+H]^+$: 795.3487, found: 795.3478.

Fmoc-(*S*)-Val photoprotected α-ketoacid with free linker 14c (single diasteromer): prepared following the general procedure using Fmoc-(*S*)-Val photoprotected α-ketoacid with protected linker **39c** (1.00 equiv, 1.25 g, 1.60 mmol), $Pd(PPh_3)_4$ (0.015 equiv, 25.3 mg, 240 µmol) and morpholine (1.50 equiv, 280 µl, 3.20 mmol) in dry CH_2Cl_2. The reaction mixture was stirred for 1 h at 0 °C. The crude mixture was purified by flash chromatography (SiO_2, hexanes/AcOEt 17:3 to hexanes/AcOEt 7:3 + 0.1% AcOH) to give the desired product (1.18 g, 1.51 mmol, 94 %) as white foam. **^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 7.79 – 7.73 (m, 3H, CH), 7.70 – 7.65 (m, 1H, CH), 7.62 – 7.54 (m, 3H, CH), 7.42 – 7.35 (m, 3H, CH), 7.32 – 7.26 (m, 4H, CH), 6.78 – 6.74 (m, 2H, CH), 5.58 (s, 1H, CH), 5.23 (d, *J* = 11.7 Hz, 1H, CH_2), 5.14 (d, *J* = 11.0 Hz, 1H, NH), 5.13 (d, *J* = 11.7 Hz, 1H, CH_2), 4.37 (dd, *J* = 10.4, 7.1 Hz, 1H, CH_2), 4.28 (dd, *J* = 10.4, 7.3 Hz, 1H, CH_2), 4.21 (appt, *J* = 7.2 Hz, 1H, CH), 4.09 (dd, *J* = 11.0, 2.6 Hz, 1H, CH), 3.91 – 3.84 (m, 2H, CH_2), 3.80 (d, *J* = 11.6 Hz, 1H, CH_2), 3.58 (d, *J* = 11.6 Hz, 1H, CH_2), 2.45 – 2.37 (m, 2H, CH_2), 2.36 – 2.27 (m, 1H, CH), 1.84 – 1.73 (m, 4H, CH_2), 0.99 (d, *J* = 6.9 Hz, 3H, CH_3), 0.97 (d, *J* = 6.9 Hz, 3H, CH_3), 0.91 (s, 3H, CH_3), 0.55 (s, 3H, CH_3), COO*H* not observed. **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 178.3 (CO), 168.8 (CO), 159.2 (C), 156.7 (CO), 149.0 (C-NO_2), 144.2* (C), 144.0* (C), 141.42* (C), 141.37* (CH), 132.3 (CH), 131.2 (C), 130.9 (CH), 130.2 (CH), 128.7 (CH), 127.8* (CH), 127.7* (CH),

127.22 (C), 127.19* (CH), 127.15* (CH), 125.4* (CH), 125.3* (CH), 124.2 (CH), 120.0 (CH), 114.6 (CH), 102.2 (C), 76.5 (CH), 74.7 (CH_2), 68.0 (CH_2), 67.4 (CH_2), 67.2 (CH_2), 60.1 (CH), 47.3 (CH), 34.9 (C), 33.5 (CH_2), 28.6 (CH_2), 27.6 (CH_2), 22.3 (CH_3), 21.5 (CH_2), 21.0 (CH_3), 19.1 (CH_3), 17.2 (CH_3), (*Signals of rotamers). **IR** (υ/cm^{-1}, thin film): 2960, 1737, 1613, 1514, 1468, 1450, 1302, 1248, 1175, 1091, 1034, 834, 758, 741. **HRMS** (ESI): calculated for $C_{44}H_{49}N_2O_{11}$ $[M+H]^+$: 781.3331, found: 781.3317.

Fmoc-(*S*)-Tyr photoprotected α-ketoacid with free linker 14d (mixture of diastereomers): prepared following the general procedure using Fmoc-(*S*)-Tyr photoprotected α-ketoacid with protected linker **39d** (1.00 equiv, 850 mg, 0.90 mmol), $Pd(PPh_3)_4$ (0.05 eq, 52.0 mg, 0.045 mmol) and morpholine (1.10 eq, 86.0 µl, 0.99 mmol) in dry CH_2Cl_2. The reaction mixture was stirred for 1 h at 0 °C. The crude mixture was purified by flash chromatography (SiO_2, hexanes/AcOEt 3/1 to hexanes/AcOEt/MeOH 4/2/1 + 0.1% AcOH) to give the desired product as white foam (795 mg, 0.87 mmol, 98%). The analytical data is given for an inseparable mixture of diastereomers and rotamers. ^{1}H NMR signals are generally described as multiplets. All observed diastereomeric ^{13}C NMR signals are listed. **^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 7.84 – 7.59 (m, 5H), 7.59 – 7.51 (m, 1H), 7.51 – 7.44 (m, 3H), 7.44 – 7.27 (m, 5H), 7.11 (m, 2H), 6.92 – 6.69 (m, 4H), 5.59 (s, 1H), 5.33 – 5.13 (m, 2H), 5.00 (d, *J* = 10.7 Hz, 1H), 4.16 – 4.01 (m, 2H), 3.96 – 3.81 (m, 3H), 3.77 (m, 2H), 3.65 (d, *J* = 11.6 Hz, 1H), 3.31 (m, 1H), 2.72 (m, 1H), 2.38 (m, 2H), 1.76 (m, 4H), 1.20 (s, 9H), 0.95 (s, 3H), 0.59 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 177.9 (CO), 168.56 (CO), 159.3 (C), 155.8 (CO), 154.0 (C), 148.9 (C), 144.1* (C), 144.0* (C), 141.2 (C), 132.3 (CH), 132.2 (C), 131.0 (C), 130.9 (CH), 130.3 (CH), 129.8 (CH), 128.8 (CH), 128.7 (CH), 128.6 (C), 127.7 (CH), 127.3 (CH), 127.1 (CH), 125.4* (CH), 125.3* (CH), 124.2 (CH), 124.2 (CH), 119.9 (CH), 114.60 (CH), 101.3 (C), 78.4 (C), 76.8 (CH), 74.8 (CH_2), 68.1 (CH_2), 67.42 (CH_2), 67.2 (CH_2), 66.2 (CH_2), 57.2 (CH), 47.1 (CH), 35.0 (C), 34.70 (CH_2), 33.79 (CH_2), 28.86 (CH_3), 28.67 (CH_2), 21.59 (CH_2), 20.98(CH_3), 19.05 (CH_3), (*Signals of rotamers). **IR** (υ/cm^{-1}, thin film): 2972, 2872, 1734, 1612, 1530, 1449, 1363, 1242, 1174, 1062, 740. **HRMS** (ESI): calculated for $C_{52}H_{56}N_2O_{12}$ $[M+H]^+$: 901.3906, found: 901.3900.

Fmoc-(*S*)-Gln photoprotected α-ketoacid with free linker 14e (single diastereomer): prepared following the general procedure using Fmoc-(*S*)-Gln photoprotected α-ketoacid with protected linker **39e** (1.00 equiv, 100 mg, 91.6 µmol), $Pd(PPh_3)_4$ (0.05 equiv, 5.0 mg, 4.3 µmol) and morpholine (1.10 equiv, 9.0 µl, 0.10 mmol) in dry CH_2Cl_2. The reaction mixture was stirred for 1 h at 0 °C. The crude mixture was purified by flash chromatography (SiO_2, hexanes/AcOEt 3/1 to hexanes/AcOEt/MeOH 4/2/1 + 0.1% AcOH) to give the desired product as white foam (89.0 mg, 84.6 µmol, 92%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 7.88-7.71 (m, 3H, CH), 7.62 – 7.46 (m, 4H, CH), 7.41 (m, 3H, CH), 7.37 – 7.25 (m, 3H, CH), 7.23 – 7.04 (m, 14H, CH), 7.01 (m, 2H, CH), 6.47 (m, 2H, CH), 5.49 (s, 1H, CH), 5.26 (d, *J* = 11.7 Hz, 1H, CH_2), 5.18 (d, *J* = 10.1 Hz, 1H, NH), 4.96 (d, *J* = 11.7 Hz, 1H, CH_2), 4.33 – 4.14 (m, 3H, CH_2+CH), 4.07 (t, *J* = 6.9 Hz, 1H, CH), 3.93 (d, *J* = 11.6 Hz, 1H, CH_2), 3.66 (m, 1H, CH_2), 3.62 – 3.52 (m, 2H, CH_2), 2.57 – 2.16 (m, 4H, CH_2), 1.84 (m, 1H, CH_2), 1.65 (m, 3H, CH_2), 0.86 (s, 3H, CH_3), 0.55 (s, 3H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 178.3 (CO), 174.1 (CO), 168.5 (CO), 159.0 (CO), 157.1 (CO), 148.9 (C), 144.2 (C), 144.0 (C), 143.6 (C), 141.5* (C), 141.4* (C), 132.8 (C), 132.4* (C), 132.3* (C), 130.64 (CH), 130.58 (CH), 128.9 (CH), 128.8 (CH), 128.0 (CH), 127.93 (CH), 127.90 (CH), 127.3* (CH), 127.2* (CH), 127.0 (CH), 126.9 (CH), 125.34* (CH), 125.32* (CH), 123.9 (CH), 120.12* (CH), 120.10* (CH), 114.5 (CH), 100.7 (C), 76.7 (CH), 74.7 (CH_2), 71.5 (C), 67.9 (CH_2), 67.2 (CH_2), 67.1 (CH_2), 55.6 (CH), 47.3 (CH), 35.0 (CH_2), 33.6 (C), 33.5 (CH_2), 28.5 (CH_2), 26.7 (CH_2), 21.4 (CH_2), 20.9 (CH_3), 18.8 (CH_3), (*Signals of rotamers). **IR** (υ/cm^{-1}, thin film): 3309, 3059, 2955, 2875, 1737, 1528, 1448, 1247, 1173, 1058, 909, 733, 701. **HRMS** (ESI): calculated for $C_{63}H_{61}N_3O_{12}$ $[M+H]^+$: 1052.4328, found: 1052.4321.

Fmoc-(*S*)-Trp photoprotected α-ketoacid with free linker 14f (single diastereomer): was prepared following the general procedure using Fmoc-(*S*)-Trp photoprotected α-ketoacid with protected linker **39f** (1.00 equiv, 9.97 g, 9.89 mmol), $Pd(PPh_3)_4$ (0.015 equiv, 158 mg, 150 µmol) and morpholine (2.00 equiv, 1.73 ml, 19.8 mmol) in dry CH_2Cl_2 (25 ml). The reaction mixture was stirred for 1 h at 0 °C. The crude mixture was purified by flash chromatography (SiO_2, hexanes/EtOAc 4:1 to hexanes/EtOAc/MeOH 4:2:1 + 0.1% AcOH) to give the desired product (8.51 g, 8.79 mmol, 89 %) as a white foam. **^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 8.11 (d, *J* = 7.8 Hz, 1H, CH), 7.86 – 7.69 (m, 3H, CH), 7.68 – 7.60 (m, 1H, CH), 7.58 – 7.47 (m, 3H, CH), 7.47 – 7.28 (m, 8H, CH), 7.24 – 7.09 (m, 3H, CH), 6.82 – 6.72 (m, 2H, CH), 5.63 (s, 1H, CH), 5.27 (d, *J* = 11.7 Hz, 1H, CH_2), 5.17 (d, *J* = 11.7 Hz, 1H, CH_2), 5.09 (d, *J* = 10.7 Hz, 1H, NH), 4.62 (td, *J* = 10.7, 3.6 Hz, 1H, CH), 4.18 – 4.01 (m, 3H, CH, CH_2), 3.93 (d, *J* = 11.6 Hz, 1H, CH_2), 3.89 – 3.74 (m, 2H, CH_2), 3.68 (d, *J* = 11.6 Hz, 1H, CH_2), 3.41 – 3.32 (m, 1H, CH_2), 2.89 (dd, *J* = 15.3, 10.7 Hz, 1H, CH_2), 2.52 – 2.34 (m, 2H, CH_2), 1.87 – 1.71 (m, 4H, CH_2), 1.57 (s, 9H, CH_3), 0.98 (s, 3H, CH_3), 0.60 (s, 3H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 178.5 (CO), 168.5 (CO), 159.3 (C), 156.0 (CO), 149.8 (CO), 148.9 (C-NO_2), 144.02* (C), 143.96* (C), 141.26* (C), 141.25* (C), 135.6 (C), 132.3 (CH) , 131.1 (C), 130.9 (CH), 130.4 (C), 130.2 (C), 128.8 (CH), 127.7 (CH), 127.3 (C), 127.1 (CH), 125.4* (CH), 125.3* (CH), 124.5 (CH), 124.2 (CH), 123.9 (CH), 122.6 (CH), 119.9 (CH), 118.9 (CH), 116.6 (C), 115.4 (CH), 114.6 (CH), 101.4 (C), 83.5 (C), 76.8 (CH), 74.8 (CH_2), 68.1 (CH_2), 67.3 (CH_2), 67.2 (CH_2), 56.2 (CH), 47.1 (CH), 35.1 (C), 33.6 (CH_2), 28.6 (CH_2), 28.3 (CH_3), 25.0 (CH_2),

21.5 (CH_2), 21.0 (CH_3), 19.1 (CH_3), (*Signals of rotamers). **IR** (υ/cm^{-1}, thin film): 2967, 1732, 1612, 1529, 1515, 1473, 1451, 1370, 1308, 1252, 1088, 1068, 851, 829, 758, 741, 668. **HRMS** (ESI): calculated for $C_{55}H_{58}N_3O_{13}$ $[M+H]^+$: 968.3964, found: 968.3948.

Fmoc-(*S*)-Lys photoprotected α-ketoacid with free linker 14g (single diastereomer): prepared following the general procedure using Fmoc-(*S*)-Lys photoprotected α-ketoacid with protected linker **39g** (1.00 equiv, 100 mg, 0.11 mmol), $Pd(PPh_3)_4$ (0.05 equiv, 6.00 mg, 0.005 mmol) and morpholine (1.10 equiv, 10.0 µl, 0.12 mmol) in dry CH_2Cl_2. The reaction mixture was stirred for 1 h at 0 °C. The crude mixture was purified by flash chromatography (SiO_2, hexanes/AcOEt 3/1 to hexanes/AcOEt/MeOH 4/2/1 + 0.1% AcOH) to give the desired product as white foam (87 mg, 0.10 mmol, 91%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 7.75 (m, 2H, CH), 7.63 (m, 1H, CH), 7.58 (m, 1H, CH), 7.53 (m, 2H, CH), 7.42 – 7.34 (m, 4H, CH), 7.32 – 7.27 (m, 4H, CH), 6.75 (d, *J* = 8.6 Hz, 2H, CH), 5.55 (s, 1H, CH), 5.24 (m, 1H, CH_2), 5.15 (m, 1H, CH_2), 4.99 (d, *J* = 10.7 Hz, NH), 4.39 (m, 1H, CH_2), 4.28 – 4.05 (m, 3H, CH+CH_2), 3.89 – 3.77 (m, 3H, CH_2), 3.57 (d, *J* = 11.5 Hz, 1H, CH_2), 3.06 (s, 3H), 2.39 (m, 2H, CH_2), 1.75 (m, 5H), 1.43 (s, 15H), 0.90 (s, 3H, CH_3), 0.56 (s, 3H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 178.0 (CO), 168.7 (CO), 159.3 (CO), 156.7 (CO), 149.0 (C), 144.1 (C), 143.9 (C), 141.4* (C), 141.3* (C), 132.3 (CH), 131.0 (C), 130.9 (CH), 130.2 (CH), 128.8 (C), 127.8* (CH), 127.7* (CH), 127.3 (C), 127.2* (CH), 127.1* (CH), 125.4* (CH), 125.3* (CH), 124.1 (CH), 120.0 (CH), 114.6 (CH), 101.3 (C), 77.4 (C), 76.7 (CH), 74,8 (CH_2), 68.0 (CH_2), 67.4 (CH_2), 67.2 (CH_2), 56.4 (CH), 47.2 (CH), 40.6 (CH_2), 35.0 (C), 33.5 (CH_2), 29.6 (CH_2), 28.7 (CH_2), 28.6 (CH_2), 28.5 (3xCH_3), 23.0 (CH_2), 21.4 (CH_2), 20.9 (CH_3), 18.9 (CH_3), (*Signals of rotamers). **IR** (υ/cm^{-1}, thin film): 3340, 3067, 2964, 2873, 1711, 1529, 1450, 1247, 1173, 1061, 910, 736. **HRMS** (ESI): calculated for $C_{50}H_{59}N_3O_{13}$ $[M+H]^+$: 910.4121, found: 910.4109.

Fmoc-(*S*)-Glu photoprotected α-ketoacid with free linker 14h (single diastereomer): prepared following the general procedure using Fmoc-(*S*)-Glu photoprotected α-ketoacid with protected linker **39h** (1.00 equiv, 844 mg, 931 µmol), $Pd(PPh_3)_4$ (0.015 equiv, 14.7 mg, 14.0 µmol) and morpholine (2.00 equiv, 163 µl, 1.86 mmol) in dry CH_2Cl_2 (2.5 ml). The reaction mixture was stirred for 1 h at 0 °C. The crude mixture was purified by flash chromatography (SiO_2, hexanes/EtOAc 4:1 to hexanes/EtOAc 1:1 + 0.1% AcOH) to give the desired product (799 mg, 922 µmol, 99%) as a white foam. **^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 7.80 – 7.73 (m, 3H, CH), 7.68 (dd, *J* = 8.0, 1.4 Hz, 1H, CH), 7.62 – 7.51 (m, 3H, CH), 7.43 – 7.34 (m, 3H, CH), 7.33 – 7.26 (m, 4H, CH), 6.80 – 6.71 (m, 2H, CH), 5.53 (s, 1H, CH), 5.23 (d, *J* = 11.7 Hz, 1H, CH_2), 5.11 (d, *J* = 11.7 Hz, 1H, CH_2), 5.02 (d, *J* = 10.7 Hz, 1H, NH), 4.38 (dd, *J* = 9.8, 6.3 Hz, 1H, CH_2), 4.26 – 4.09 (m, 3H, CH, CH_2), 3.94 – 3.77 (m, 3H, CH_2), 3.58 (d, *J* = 11.6 Hz, 1H, CH_2), 2.47 – 2.18 (m, 5H, CH_2), 1.91 – 1.71 (m, 5H, CH_2), 1.44 (s, 9H, CH_3), 0.91 (s, 3H, CH_3), 0.55 (s, 3H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 178.4 (CO), 172.8 (CO), 168.6 (CO), 159.2 (C), 156.3 (CO), 148.9 (C-NO_2), 144.2* (C), 143.9* (C), 141.4* (C), 141.3* (C), 132.4 (CH), 131.1 (C), 130.8 (CH), 130.3 (CH), 128.7 (CH), 127.8* (CH), 127.7* (CH), 127.23 (C), 127.20* (CH), 127.17* (CH), 125.4* (CH), 125.3* (CH), 124.1 (CH), 120.02* (CH), 120.00* (CH), 114.6 (CH), 101.1 (C), 80.6 (C), 76.6 (CH), 74.7 (CH_2), 68.0 (CH_2), 67.3 (CH_2), 67.2 (CH_2), 56.2 (CH), 47.2 (CH), 35.0 (C), 33.6 (CH_2), 32.1 (CH_2), 28.6 (CH_2), 28.3 (CH_3), 24.3 (CH_2), 21.5 (CH_2), 20.9 (CH_3), 19.0 (CH_3) ,(*Signals of rotamers). **IR** (υ/cm^{-1}, thin film): 2967, 1726, 1612, 1515, 1529, 1450, 1351, 1246, 1173, 1092, 1059, 829, 785, 759, 740, 667. **HRMS** (ESI): calculated for $C_{48}H_{55}N_2O_{13}$ $[M+H]^+$: 867.3699, found: 867.3690.

Synthesis of photoprotected oxaproline

(*RS*)-1-(2-nitrophenyl)ethan-1-ol *rac*-24: 2-nitrobenzaldehyde (1.00 equiv, 3.50 g, 23.2 mmol) was dissolved in dry CH_2Cl_2 (115 ml) and cooled to 0 °C. $AlMe_3$ (2 M in toluene, 1.50 equiv, 17.4 ml, 34.7 mmol) was added dropwise. After addition, the mixture was slowly warmed to rt and stirred for 2 h (completion monitored by TLC). The mixture was cooled to 0 °C, diluted with CH_2Cl_2 and slowly quenched with water. The organic layer was separated and washed with a saturated aqueous solution of NH_4Cl, brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography (SiO_2, hexane/AcOEt). **^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 7.90 (dd, *J* = 8.1, 1.3 Hz, 1H, CH), 7.84 (m, 1H), 7.65 (m, 1H, CH), 7.42 (m, 1H, CH), 5.42 (q, *J* = 6.4 Hz, 1H, CH), 2.31 (s, 1H, OH), 1.58 (d, *J* = 6.4 Hz, 3H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 148.0, 141.0, 133.76, 128.3, 127.7, 124.5, 65.7, 24.3. **IR** (υ/cm^{-1}, thin film): 3359, 2978, 2931, 1521, 1346, 1191, 1105, 1071, 1007, 899, 855, 788, 746. **HRMS** (ESI): calculated for $C_8H_9NNaO_3$ $[M+Na]^+$: 190.0475, found: 190.0474

(*RS*)-N-succinimidyl-(1-(2-nitrophenyl)ethyl) carbonate *rac*-20: Alcohol ***rac*-24** (1.00 equiv, 240 mg, 1.44 mmol) was dissolved in dry acetonitrile (5 ml) and cooled to 0 °C. Et_3N (2.00 equiv, 400 µl, 2.87 mmol) was added dropwise followed by the addition of disuccinimide carbonate (1.20 equiv, 441 mg, 1.72 mmol). The mixture was warmed to room temperature and stirred for 4 h (completion monitored by TLC). After dilution with AcOEt, the mixture was washed with a saturated aqueous solution of NH_4Cl. The aqueous layer was extracted with AcOEt and the combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography (SiO_2, hexane/AcOEt). **^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 8.02 (d, *J* 8.0 Hz, 1H),

7.73 (m, 2H), 7.50 (m, 1H), 6.39 (q, *J* 6.4 Hz, 1H), 2.79 (s, 4H), 1.79 (d, *J* = 6.4 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 168.6 (CO), 150.8 (CO), 147.4 (C), 136.0 (C), 134.5 (CH), 129.4 (CH), 127.1 (CH), 124.9 (CH), 76.1 (CH), 25.6 (CH_2), 22.3 (CH_2). **IR** (υ/cm^{-1}, thin film): 3514, 2991, 2943, 1812, 1789, 1742, 1526, 1361, 1257, 1234, 1202, 1058, 992, 788, 748. **HRMS** (ESI): calculated for $C_{13}H_{12}N_2NaO_7$ [M+Na]$^+$: 331.05375, found: 331.0541.

Photoprotected (*S*)-5-Oxaproline 21 (mixture of diastereomers): (*S*)-Boc-5-Oxaproline (1.00 equiv, 1.10 g, 5.06 mmol) was deprotected by treatment with a mixture of TFA/CH_2Cl_2 (1:4, 20 ml) for 1 h at rt. Solvent was removed under reduced pressure. The residue was dissolved in CH_3CN/H_2O (3:1, 0.5 M) and $NaHCO_3$ (3.00 equiv, 1.26 g, 15.1 mmol) was added at rt followed by the addition of racemic succinimidyl carbonate ***rac*-20** (1.10 equiv, 1.53 g, 5.54 mmol). The mixture was vigorously stirred for 2 h (completion monitored by TLC) before dilution with AcOEt and addition of NH_4Cl saturated solution. The aqueous layer was extracted with AcOEt. The combined organic layers were washed with brine, dried over MgSO4 and concentrated under reduced pressure to yield the product (1.44g, .4.66 mmol, 92%) as light brown oil. **^{1}H NMR** (400 MHz, $CDCl_3$) [ppm] = 7.93 (dt, *J* = 8.2, 1.2 Hz, 1H, CH), 7.73 – 7.58 (m, 2H, CH), 7.48 – 7.38 (m, 1H, CH), 6.34 (dd, *J* = 7.5, 6.2 Hz, 1H, CH), 4.76 – 4.62 (m, 1H, CH), 4.15 (m, 1H, CH_2), 3.82 (m, 1H, CH_2), 2.63 (m, 1H, CH_2), 2.53 (m, 1H, CH_2), 1.68 (m, 3H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) [ppm] = 174.6, 156.2, 156.1, 147.6, 147.5, 137.44, 137.38, 134.1, 134.0, 129.2, 128.78, 128.76, 128.4, 127.45, 127.39, 125.4, 124.64, 124.62, 71.2, 71.1, 69.4, 69.2, 59.8, 59.7, 32.97, 32.92, 22.2, 22.1. **IR** (/cm^{-1}, thin film): 3502, 3080, 2986, 2888, 1715, 1524, 1352, 1200, 1081, 1060, 856, 748. **HRMS** (ESI): calculated for $C_{13}H_{14}N_2NaO_7$ [M+Na]$^+$: 333.0693, found: 333.0693.

(*S*)-1-(2-nitrophenyl)ethan-1-ol (S)-24: (*R*)-(+)-2-Methyl-CBS-oxazaborolidine (0.077 equiv, 1.16 g, 4.19 mmol) was dissolved in anhydrous CH_2Cl_2 (20 mL) under nitrogen atmosphere. Catecholborane (1.50 equiv, 9.80 g, 81.7 mmol) were added at room temperature and the mixture was stirred for 5 min. The mixture was cooled to -78 °C and a solution of 2-Nitroacetaldehyde (1.00 equiv, 9.00 g, 54.5 mmol) in anhydrous CH_2Cl_2 (20 mL) was added within 30 min. The mixture was stirred for 1 d at -78 °C. The mixture was allowed to warm to room temperature, diluted with CH_2Cl_2 (100 mL), washed with 1 M HCl (15 mL) and brine (15 mL), dried over $MgSO_4$, filtered and the solution was concentrated under reduced pressure. The crude product was purified by flash chromatography (SiO_2, CH_2Cl_2) to obtain the product (8.09 g, 48.4 mmol, 89 %) as light-yellow oil which solidified upon storage for 1 d at 4 °C. The enantiomeric excess was determined to be 88 %ee by normal-phase HPLC. The product can be recrystallized (25 mL hexanes, 1 mL EtOAc, 1.40 g of purified product, vigorous stirring and cooling to 4°C) to obtain the product (1.04 g, 74 %) as crystalline off-white solid with >99 %ee (determined by normal-phase HPLC). The analytical data matched the above reported data for the racemic alcohol. $[\alpha]_D^{28}$ = +32.7°(c = 1.05, $CHCl_3$) (before recrystallization). $[\alpha]_D^{28}$ = +38.1°(c = 0.95, $CHCl_3$) (after recrystallization). Literature[xxxvii]: $[\alpha]_D^{27}$ = +54.2°(c = 0.98, $CHCl_3$) (for (*S*)-1-(2-nitrophenyl)ethan-1-ol). **HPLC** column: Daicel Chiralpak ADH (4.6 x 250 mm); eluent: 9:1 hexanes/isopropanol: flow: 1 ml/min; detection: 220 nm; retention time: t_R = 8.31 min (major), 9.01 min (minor). Area major/minor: 84.1:5.9 (88 %ee) before recrystallization. Area major/minor >99.9:0.1 (>99.9 %ee) after recrystallization.

(*S*)-1-(2-nitrophenyl)ethyl chloroformate 40: Triphosgene (1.00 equiv, 879 mg, 2.99 mmol) and K_2CO_3 (1.00 equiv, 413 mg, 2.99 mmol) were suspended in THF (12.5 mL) and cooled to 0 °C. (*S*)-1-(2-nitrophenyl)ethan-1-ol (1.00 equiv, 500 mg, 2.99 mmol) and pyridine (0.10 equiv, 23.7 mg, 299 µmol) were added and the mixture was allowed to warm to room temperature and was stirred overnight. The mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was dissolved in EtOAc (25 mL) and washed with 10 % aqueous citric acid (5 mL), saturated aqueous $NaHCO_3$ (5 mL) and brine (5 mL). The solution was dried over Na_2SO_4, filtered and concentrated under reduced pressure to obtain the product (623 mg, 2.71 mmol, 91 %) as light-brown oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 8.03 (ddd, *J* = 8.2, 1.1, 0.6 Hz, 1H, CH), 7.74 – 7.68 (m, 2H, CH), 7.52 (ddd, *J* = 8.2, 6.3, 2.5 Hz, 1H, CH), 6.46 (q, *J* = 6.4 Hz, 1H, CH), 1.77 (d, *J* = 6.5 Hz, 3H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 149.9 (CO), 147.5 (C-NO_2), 135.8 (C), 134.3 (CH), 129.4 (CH), 127.1 (CH), 125.0 (CH), 76.5 (CH), 22.0 (CH_3). **IR** (υ/cm^{-1}, thin film): 2988, 2939, 1773, 1612, 1579, 1525, 1446, 1355, 1161, 1145, 1055, 991, 846, 788, 747, 694, 671. **HRMS** (EI$^+$): molecular ion not observed. Calculated for $C_8H_5ClNO_4$ [M-CH_3]$^+$: 213.9919, found: 213.9907; calculated for $C_9H_8ClO_2$ [M-NO_2]$^+$: 183.0213, found: 183.0219; calculated for $C_8H_8NO_2$ [M-$CClO_2$]$^+$: 150.0555, found: 150.0551. $[\alpha]_D^{25}$ = +273°(c = 1.20, $CHCl_3$).

Photoprotected (*S*)-5-oxaproline 21 (single diastereomer): Boc-(*S*)-5-oxaproline (1.00 equiv, 543 mg, 2.50 mmol) was dissolved in CH_2Cl_2 (10 mL) and trifluoroacetic acid (10 mL) was added at 0°C. The mixture was allowed to warm to room temperature and was stirred for 1 h. The mixture was concentrated under reduced pressure. The residue was dissolved in toluene and the solvent was reduced under reduced pressure to remove residual trifluoroacetic acid. This step was repeated twice. The residue was dissolved in CH_2Cl_2 (11 mL) and cooled to 0 °C. Triethylamine (2.50 equiv, 632 mg, 6.25 mmol) and (*S*)-1-(2-nitrophenyl)ethyl chloroformate **40** (1.05 equiv, 603 mg, 2.63 mmol) were added. The mixture was allowed to warm to room temperature and was stirred overnight. The mixture was diluted with

CH_2Cl_2 (20 mL) and washed with 1 M HCl (10 mL), saturated aqueous $NaHCO_3$ (10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude material was purified by flash chromatography (SiO_2, CH_2Cl_2 to CH_2Cl_2/MeOH 9:1) to obtain the product (741 mg, 2.39 mmol, 96 %) as light-brown oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ [ppm] = 7.95 (dd, *J* = 8.2, 0.9 Hz, 1H, CH), 7.89 (bs, 1H, CH), 7.70 – 7.60 (m, 2H, CH), 7.45 (ddd, *J* = 8.2, 6.9, 1.9 Hz, 1H, CH), 6.38 (q, *J* = 6.5 Hz, 1H, CH), 4.77 (dd, *J* = 9.4, 5.3 Hz, 1H, CH), 4.19 (td, *J* = 8.1, 4.5 Hz, 1H, CH_2), 3.83 (td, *J* = 8.1, 7.1 Hz, 1H, CH_2), 2.74 – 2.63 (m, 1H, CH_2), 2.62 – 2.52 (m, 1H, CH_2), 1.72 (d, *J* = 6.5 Hz, 3H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ [ppm] = 174.2 (CO), 156.1 (CO), 147.7 (C-NO_2), 137.3 (C), 133.9 (CH), 128.8 (CH), 127.3 (CH), 124.7 (CH), 71.3 (CH), 69.4 (CH_2), 59.4 (CH), 32.9 (CH_2), 22.1 (CH_3). **IR** (υ/cm^{-1}, thin film): 2984, 1717, 1611, 1578, 1524, 1447, 1351, 1299, 1200, 1082, 1061, 998, 856, 789, 747, 707, 673. **HRMS** (ESI): calculated for $C_{13}H_{14}N_2NaO_7$ $[M+Na]^+$: 333.0693, found: 333.0702. $[\alpha]_D^{28}$ = +127°(c = 0.99, $CHCl_3$).

NEDD8 Ligations

N-to C-terminus one-pot ligation (Scheme 6): NEDD8 (3–17)-α-ketoacid **25** (1.7 mg, 0.98 µmol, 1.00 equiv) and NEDD8 (18–45)-photolabile-Tyr-α-ketoacid **18** (3.6 mg, 0.98 µmol, 1.00 equiv) were dissolved in 95:5 DMSO:H_2O with 0.1 M oxalic acid (25 µL, 40 mM) and warmed to 60 °C. The progress of the ligation was monitored by analytical HPLC using a Shiseido Capcell Pak MGII C18 column (4.6 x 250 mm) with a gradient of 30 to 60% CH_3CN with 0.1% TFA in 22 min. An aliquot of the ligation mixture (0.5 µL) was taken at various time point, diluted to 20 µL with 1:1 CH_3CN:H_2O and injected on HPLC. After 24 h of ligation, TCEP was added (0.1 M), the mixture was diluted to 20 mM and the reaction mixture was irradiated for 30 min. The deprotection was monitored by analytical HPLC using a Shiseido Capcell Pak MGII C18 column (4.6 x 250 mm) with a gradient of 30 to 60% CH_3CN with 0.1% TFA in 22 min. An aliquot of the ligation mixture (0.5 µL) was taken at various time point, diluted to 20 µL with 1:1 CH_3CN:H_2O and injected on HPLC. NEDD8 (46–76) **28** (4.2 mg, 1.28 µmol, 1.30 equiv) was added and the mixture was warmed to 60 °C. The progress of the ligation was monitored by analytical HPLC using a Shiseido Capcell Pak MGII C18 column (4.6 x 250 mm) with a gradient of 30 to 60% CH_3CN with 0.1% TFA in 22 min. An aliquot of the ligation mixture (0.5 µL) was taken at various time point, diluted to 20 µL with 1:1 CH_3CN:H_2O and injected on HPLC. After 24 h of ligation, the reaction was diluted with phosphate buffer pH 9 to 0.05 mM and stirred for 6 h at rt. Finally the reaction was diluted to1 mL with 1:1 CH_3CN:H_2O and purified by preparative HPLC using a Shiseido Capcell Pak C18 column (20 x 250 mm) with a gradient of 30 to 55% CH_3CN with 0.1% TFA in 27 min. The product peak eluting at t_R = 20.7 min was pooled and lyophilized to give pure NEDD8 (3–76, Glu18Hse, Ser46Hse) **29** (1.6 mg, 0.19 µmol, 20% yield for ligation, deprotection and purification steps). Analytical HPLC and ESI-MS were used to confirm the purity and identity of **29.** m/z calculated for **29** $C_{367}H_{622}N_{103}O_{112}S$ $[M+H]^+$: 8296.5858; measured 8296.6430.

C- to N-terminus one-pot ligation (Scheme 7): Photoprotected-Oxaproline-NEDD8 (18–45) **30** (2.0 mg, 0.56 µmol, 1.00 equiv) and NEDD8 (46–76) **28** (1.8 mg, 0.56 µmol, 1.00 equiv) were dissolved in 95:5 DMSO:H_2O with 0.1 M oxalic acid (19 µL, 30 mM) and warmed to 60 °C. The progress of the ligation was monitored by analytical HPLC using a Shiseido Capcell Pak MGII C18 column (4.6 x 250 mm) with a gradient of 30 to 60% CH_3CN with 0.1% TFA in 22 min. An aliquot of the ligation mixture (0.5 µL) was taken at various time point, diluted to 20 µL with 1:1 CH_3CN:H_2O and injected on HPLC. After 24 h of ligation, TCEP was added (0.1 M), the mixture was diluted to 20 mM and the reaction mixture was irradiated for 30 min. The deprotection was monitored by analytical HPLC using a Shiseido Capcell Pak MGII C18 column (4.6 x 250 mm) with a gradient of 30 to 60% CH_3CN with 0.1% TFA in 22 min. An aliquot of the ligation mixture (0.5 µL) was taken at various time point, diluted to 20 µL with 1:1 CH_3CN:H_2O and injected on HPLC. NEDD8 (3–17)-α-ketoacid **25** (1.9 mg, 1.11 µmol, 2.00 equiv) was added and the mixture was warmed to 60 °C. The progress of the ligation was monitored by analytical HPLC using a Shiseido Capcell Pak MGII C18 column (4.6 x 250 mm) with a gradient of 25 to 65% CH_3CN with 0.1% TFA in 22 min. An aliquot of the ligation mixture (0.5 µL) was taken at various time point, diluted to 20 µL with 1:1 CH_3CN:H_2O and injected on HPLC. After 24 h of ligation, the reaction was diluted with phosphate buffer pH 9 to 0.05 mM and stirred for 6 h at rt. Finally the reaction was diluted to1 mL with 1:1 CH_3CN:H_2O and purified by preparative HPLC using a Shiseido Capcell Pak C18 column (20 x 250 mm) with a gradient of 30 to 55% CH_3CN with 0.1% TFA in 27 min. The product peak eluting at t_R = 21.0 min was pooled and lyophilized to give pure NEDD8 (3–76, Glu18Hse, Ser46Hse) **29** (0.9 mg, 0.11 µmol, 20% yield for ligation, deprotection and purification steps). Analytical HPLC and ESI-MS were used to confirm the purity and identity of **29.** m/z calculated for **29** $C_{367}H_{622}N_{103}O_{112S}$ $[M+H]^+$: 8296.5858; measured 8296.6117.

One-pot convergent ligation (Scheme 8): NEDD8 (3–17)-α-ketoacid **25** (1.7 mg, 0.98 µmol, 1.00 equiv) and NEDD8 (18–45)-photolabile-Tyr-α-ketoacid **18** (3.6 mg, 0.98 µmol, 1.00 equiv) were dissolved in 95:5 DMSO:H_2O with 0.1 M oxalic acid (24 µL, 40 mM) and warmed to 60 °C. The progress of the ligation was monitored by analytical HPLC using a Shiseido Capcell Pak MGII C18 column (4.6 x 250 mm) with a gradient of 30 to 60% CH_3CN with 0.1% TFA in 22 min. An aliquot of the ligation mixture (0.5 µL) was taken at various time point, diluted

to 20 µL with 1:1 $CH_3CN:H_2O$ and injected on HPLC. In parallel, photoprotected-Oxaproline-NEDD8 (46–62) **33** (2.1 mg, 0.98 µmol, 1.00 equiv) and NEDD8 (63–76) **34** (1.5 mg, 1.07 µmol, 1.10 equiv) were dissolved in 95:5 DMSO:H_2O with 0.1 M oxalic acid (24 µL, 40 mM) and warmed to 60 °C. The progress of the ligation was monitored by analytical HPLC using a Shiseido Capcell Pak MGII C18 column (4.6 x 250 mm) with a gradient of 30 to 60% CH_3CN with 0.1% TFA in 22 min. An aliquot of the ligation mixture (0.5 µL) was taken at various time point, diluted to 20 µL with 1:1 $CH_3CN:H_2O$ and injected on HPLC. After completion, both ligation mixture were mixed together and TCEP was added (0.1M). The mixture was irradiated for 30 min. The deprotection was monitored by analytical HPLC using a Shiseido Capcell Pak MGII C18 column (4.6 x 250 mm) with a gradient of 30 to 60% CH_3CN with 0.1% TFA in 22 min. An aliquot of the ligation mixture (0.5 µL) was taken at various time point, diluted to 20 µL with 1:1 $CH_3CN:H_2O$ and injected on HPLC. After 24 h of ligation, the reaction was diluted with phosphate buffer pH 9 to 0.05 mM and stirred for 6 h at rt. Finally the reaction was diluted to1 mL with 1:1 $CH_3CN:H_2O$ and purified by preparative HPLC using a Shiseido Capcell Pak C18 column (20 x 250 mm) with a gradient of 30 to 60% CH_3CN with 0.1% TFA in 27 min. The product peak eluting at t_R = 20.7 min was pooled and lyophilized to give pure NEDD8 (3-76, Glu18Hse, Ser46Hse, Gly63Hse) **37** (2.1 mg, 0.25 µmol, 26%). Analytical HPLC and ESI-MS were used to confirm the purity and identity of **37.** m/z calculated for **37** $C_{369}H_{626}N_{103}O_{113}S$ $[M+H]^+$: 8340.6120; measured 8340.6656.

Supplementary

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/MS-number.

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Author Contribution Statement

F.T., F.R., and J.W.B. designed the studies. F.T., F.R., and A.Z. performed the experiments. F.R., F.T., and J.W.B. wrote the manuscript.

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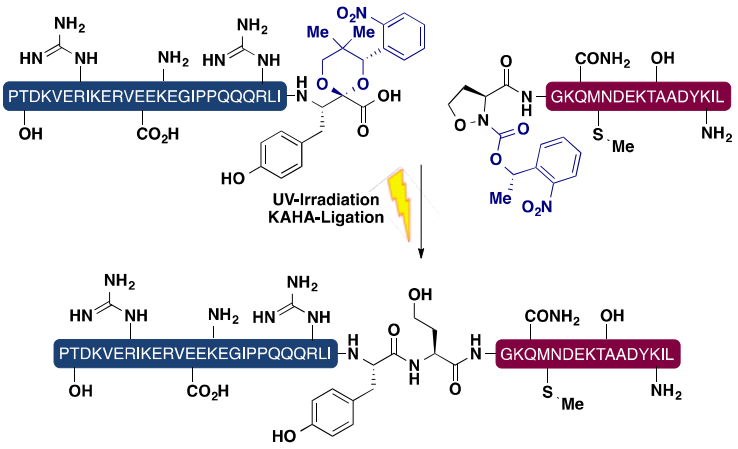

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