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Access to analogues within the highly cytotoxic natural product family of tubulysins has previously required lengthy routes involving multiple functional group manipulations that is costly and time intensive on scale. A concise and modular total synthesis of the highly potent N¹⁴-desacetoxytubulysin H has been accomplished in a short sequence from commercially available building blocks. Our work highlights the complexity augmenting and route shortening power of multicomponent reaction (MCR) as well as the role of catalysts in controlling diastereoselectivity. Our operationally simple and step economical total synthesis can be easily performed on gram scale without compromising the yield.

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Concise total synthesis of N^{14} -desacetoxytubulysin H

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Supporting Information Placeholder

ABSTRACT: Access to analogues within the highly cytotoxic natural product family of tubulysins has previously required lengthy routes involving multiple functional group manipulations that is costly and time-intensive on scale. A concise and modular total synthesis of the highly potent N^{14} -desacetoxytubulysin H has been accomplished in a short sequence from commercially available building blocks. Our work highlights the complexity-augmenting and route-shortening power of multicomponent reaction (MCR) as well as the role of catalysts in controlling diastereoselectivity. Our operationally simple and step-economical total synthesis can be easily performed on gram scale without compromising the yield.

The Ugi four-component (U-4CR)¹ and the Passerini three-component (P-3CR)² are the lynchpin in modern organic chemistry. As “*mix and go*” multicomponent reactions multiple bonds are formed in one-pot with minimal waste, *ecce* a blueprint of green chemistry.³ These truly versatile reactions have found extensive applications in drug discovery, natural products, pharmaceuticals, polymers and complex macrocycles.⁴ Despite the massive benefits of U-4CR and P-3CR, they normally gave racemic products *via* the newly formed stereo center which results in tedious separations of the active enantiopure product for medicinal chemistry applications.⁵ To overcome the stereochemical limitation of multicomponent reactions, control is necessary.⁶ Lewis acids⁷ and chiral phosphoric acid catalysts⁸ recently emerged as promising catalysts to control the new chiral center formed in these multicomponent reactions. Enantioselective multicomponent reactions thus open an exciting opportunity for the synthetic and medicinal chemists to easily access molecular complexity and diversity.⁹

The natural product family of Tubulysins since their discovery by Höfle in 2000 from myxobacterial fermentation broth has experienced impressive progress with respect to understanding biology and drug development.¹⁰

Figure 1. Retrosynthesis of tubulysins

Tubulysins exhibit extraordinary potent cytotoxicities against various cancer cells exerted through tubulin binding.¹¹ Strikingly, tubulysins are 20 to 1000-fold more potent than the epothilones, vinblastine and taxol as cell growth inhibitors and thus they appeared as promising lead compounds for the development of new anticancer drugs.¹² It rapidly turned out that the therapeutic window for single agent use is too small for any human application. Recently, tubulysins as payloads, folic acid conjugates or antibody drug conjugates (ADCs) showed high clinical promiss.¹³ However, the fermentation of tubulysins is still a poorly solved challenge. Thus they are exciting targets for total synthesis in several laboratories around the world.¹⁴ Our laboratory¹⁵ and the Zanda group¹⁶ disclosed the first total synthesis of tubulysin U and V, Ellman described the first total synthesis of Tubulysin D^{17a} and later N-methyl tubulysins.^{17b,c} Later onwards several modifications on tubulysins and their total synthesis have been well documented.¹⁸

All the classical total synthesis of the non-ribosomal peptidic structure of tubulysins involves multistep approach and proceeds through sequential coupling of four amino acid fragments such as D-N-methyl pipecolic acid

(Mep), isoleucine (Ile), tubuvaline (Tuv) and the tubu-phenylalanine (Tup) as logical precursors (Figure 1).¹⁹ Most of the current synthetic routes suffers from several drawbacks. First, the synthesis of Tuv and Tup through extensive functional group manipulations and chiral separations. Next, sequential difficult coupling of sterically hindered Mep, Ile and Tuv amino acids. These costly and labor-intensive routes have hampered large scale synthesis. We have previously shown the multicomponent reactions tactic in our first total synthesis of tubulysin U and V,¹⁵ and also the Wessjohann group extended the scope of the multicomponent reactions for the synthesis of Tubugis through the combination of P-3CR and U-4CR.²⁰

Entry ^[b]	Ligand	Yield of 5a [%] ^b	dr 5a:5b ^[c, d]
1	none	82	80:20
2	L1	71	92:8
3	L2	68	70:30
4	L3	60	85:15
5	L4	63	76:24
6	L5	70	72:28
7	L6	55	69:39

[a] Screening of solvents, concentration and other catalysts have been studied (please see supporting information). [b] Equimolar amounts of all three components were added to a solution containing ZnBr₂ and corresponding ligand in CH₂Cl₂ at 0 °C. [c] Yield was determined after chromatographic purification. [d] Diastereomeric ratio was determined by HPLC analysis of the crude reaction mixture. [d] Absolute configuration of the major diastereomer was determined by employing to the total synthesis of **1**.

Scheme 1. Diastereoselective Passerini three component reaction (P-3CR).^[a]

From the activity point of view, N¹⁴-desacetoxytubulysin H was found to be highly potent among all the tubulysinanalogs with increased hydrolytical stability.²¹ Based on these findings, the Ellman,^{17c} Nicolaou,^{21a} and Wipf groups^{21b} have developed synthetic routes to access **1** and its analogs. While total synthesis landmark achievements, most reported approaches suffer in general from

high synthetic step-count and overall low yields. For example, the tubulysin-linker part of the ADC MEDI4276 was reportedly synthesized by a more than 60 step synthesis and thus the gram prize compared to the one of platinum.²² To attenuate the current demand for tubulysins in ADC therapies,²³ we targeted a general and stereoselective synthetic method to access tubulysins in just few steps and overall high yield. This communication documents our high yielding short synthesis of **1** in 19 steps from commercially available very simple building blocks. For the first time, a diastereoselective P-3CR has been applied to the natural product synthesis of tubulysins. Based on our retrosynthetic analysis, we envisioned that the intermediates **2**, **3** and **4** bearing orthogonal protecting groups could serve as advanced intermediates for our divergent multicomponent reaction synthesis of **1**. We thus initially designed efficient routes to synthesize the building blocks on a multigram scale (see Supporting Information (SI) for the preparation of the building blocks **2** - **4**).²⁴

We initiated our studies by probing various P-3CR parameters such as solvent, temperature and concentration to obtain the desired diastereomer **5a** (see SI) at best yield. The first optimization revealed that excellent yield (90%) of the P-3CR product was obtained as a 1:1 mixture of diastereomers **5a** and **5b** at 1 M concentration in at room temperature for 48 h. At 0.1 M concentration we observed a slight improvement in the diastereoselectivity, however, with reduced yield of 50%. These studies showed that temperature, solvent and concentration have great impact on the P-3CR. However, complete stereocontrol of the newly formed asymmetric carbon is challenging to tune based on those reaction parameters. Taking advantage of the chiral and bulky nature of our designed components, we hypothesized that the judicious choice of a catalyst or additive might help to improve the diastereoselectivity. Inspired by the work of Tan on chiral phosphoric acid (CPA) catalyzed enantioselective MCRs,^{8a} we initially examined various promising chiral phosphoric acid catalysts. After screening readily available catalysts, CPA 5 (see SI) provided excellent 95:5 dr, albeit in very low yields (38%). This might be due to the fact that highly acidic phosphoric acids considerably cleave the side chain trityl group resulting in a series of side reactions (see SI). We then speculated that basic ligands could protect the cleavage of trityl group. Thus, the P-3CR was conducted in the presence of 20 mol% pyridine and 10 mol% of CPA 2. However, in this case we obtained mostly hydroxylated product (P-2CR) with a 95:5 diastereomeric ratio (70%, see SI). Banfi and coworkers showed that chiral components yields diastereoselective P-3CR in presence of achiral Lewis acids.^{7d,e}

Reagents and conditions: (a) step 1: 1M TiCl_4 in CH_2Cl_2 (3.0 eq.), 0 °C, 48 h; step 2: activated MnO_2 , (10 eq.) 70 °C, 3h. (b) step 1: diethylamine (30 eq.), CH_3CN , 0 °C, 2 h; step 2: CH_2Cl_2 , sat. NaHCO_3 , 50 °C, 24 h. (c) 2M LiOH , THF : H_2O : MeOH , 6 h, rt. (d) step 1: DIC, pentafluorophenol, 0 °C, CH_2Cl_2 , step 2: **6** (3.0 eq.), DMF , DIPEA , rt, 12 h. (e) Ac_2O , pyridine, CH_2Cl_2 , rt, 24 h. (f) H_2 , Pd/C , HCHO , MeOH , rt, 24h.

Scheme 3. End game of the total synthesis of **1**

We initiated our studies by probing various P-3CR parameters such as solvent, temperature and concentration to obtain the desired diastereomer **5a** (see SI) at best yield. The first optimization revealed that excellent yield (90%) of the P-3CR product was obtained as a 1:1 mixture of diastereomers **5a** and **5b** at 1 M concentration in at room temperature for 48 h. At 0.1 M concentration we observed a slight improvement in the diastereoselectivity, however, with reduced yield of 50%. These studies showed that temperature, solvent and concentration have great impact on the P-3CR. However, complete stereocontrol of the newly formed asymmetric carbon is challenging to tune based on those reaction parameters. Taking advantage of the chiral and bulky nature of our designed components, we hypothesized that the judicious choice of a catalyst or additive might help to improve the diastereoselectivity. Inspired by the work of Tan on chiral phosphoric acid (CPA) catalyzed enantioselective MCRs,^{8a} we initially examined various promising chiral phosphoric acid catalysts. After screening readily available catalysts, CPA 5 (see SI) provided excellent 95:5 dr, albeit in very low yields (38%). This might be due to the fact that highly acidic phosphoric acids considerably cleave the side chain trityl group resulting in a series of side reactions (see SI). We then speculated that basic ligands could protect the cleavage of trityl group. Thus, the P-3CR was conducted in the presence of 20 mol% pyridine and 10 mol% of CPA 2. However, in this case we obtained mostly hydroxylated product (P-2CR) with a 95:5 diastereomeric ratio (70%, see SI). Banfi and coworkers showed that chiral components yields diastereoselective P-3CR in presence of achiral Lewis acids.^{7d,e} Thus, we extensively screened various Lewis acids in P-3CR (see SI) and we were delighted to find that 10 mol% of ZnBr_2 gave moderate diastereoselectivity (80:20) without diminishing the yield (86%). Our previous studies on screening of Lewis

acids along with ligands in enantioselective P-3CR revealed that, Lewis acids along with supporting bulky chiral ligands may completely lock the other phase of the isocyanide attack thereby leading to high diastereoselectivity.^{7a,25} Based on this hypothesis, we examined several chiral ligands in association with ZnBr_2 and the results are summarized in scheme 1. Interestingly, when ligand **L1** was used as the chiral ligand together with ZnBr_2 , the P-3CR pathway afforded **5a** as the major product formation with high diastereoselectivity (92:8) in acceptable yield (70%). Other tested ligands gave moderate yields and diastereoselectivities (Scheme 1 and SI).

Reaction conditions: [a] AcCl (3 eq.), 100 °C, 2h. [b] CuI (10m mol%), tBuXPhos (20 mol%), 2M PhCH_2MgBr in THF , THF (0.2M), -78 °C, 5h. [c] H_2 (55 bar), NH_4Cl (3.0 eq.), $\text{Ru}(\text{OAc})_2$, R-dm-SEGPHOS , Trifluoroethanol (TFE), 12 h. [d] Absolute configuration of the major diastereomer was determined.

Scheme 2. Diastereoselective synthesis of **Tup 9**

Next, we turned our attention to develop an innovative synthesis of **Tup** (Scheme 2). We figured that commercially available and affordable *S*-($-$)-methyl succinic acid **6** would provide a versatile component *via* its anhydride **7**. Dehydration of **6** with acetyl chloride yields anhydride **7** as stable crystalline solid. We set out to investigate the

regioselective ring opening of **7** by a Grignard reagent.²² However, the direct addition of Grignard reagent to the anhydride **7** gave a mixture of regioisomers **8a** and **8b** in equal proportion (SI). We observed an important solvent and temperature effect on the yield and regioselectivity. The use of THF as the primary solvent for Grignard addition at -78 °C was beneficial to the yield and regioselectivity (See SI). To improve the selective formation of **8a**, various copper catalysts were examined (SI).²⁵ To our delight, bulky *t*-BuXPhos along with CuI resulted in exclusive formation of **8a** in > 99:1 regioselectivity in 70% yield. The regioselective outcome can be rationalized by the steric encumbrance imparted by the bulky copper complex effectively shielding the carbonyl group next to the methyl group of **7**. The enantiopure keto acid **8a** was then subjected to a known Ruthenium catalyzed reductive amination protocol with slight modifications²⁶ to afford **9** in good yield (78%) and excellent diastereoselectivity (98:2). A one-pot synthesis of **7** to **9** was also conducted, however, in this case yield obtained was quite low (43%).

With the key building blocks in our hand, we focused on the final assembly of **1**. We first installed the thiazole *via* post-translational modification of **5a** through one-pot two step strategy. TiCl₄ mediated cyclodehydration of Cys(Trt) amide affords thiazoline and the crude thiazoline was subjected to a MnO₂ oxidation to afford **10** in excellent yield without racemization (SI).²⁷ The resulting thiazole **10** was then subjected to Fmoc-deprotection followed by acyl migration under mild conditions to give hydroxy tetra peptide analogue **11** in excellent 90% yield. Such Passerini reaction-amine deprotection-acyl migration (PADAM) was first described by Banfi and independently by Semple and coworkers.²⁸ However, its practical utility has never been disclosed in the sterically hindered amide formation. The resulting tetrapeptide **11** was then hydrolyzed to afford 98% of **12**. Synthesis of **13** was accomplished by activation of **12** as the Pentafluorophenyl ester mediated coupling of **9** and **12** gave **13** in 83% overall yield. Further, acetylation of hydroxyl group was produced **14** in 86% yield. Finally, the late stage *N*-methylation of the *N*-terminus was installed by the catalytic hydrogenation with paraformaldehyde affording **1** in good yield (90%). The advantage of Cbz group lays in late stage modifications either ¹³C labelling or to introduce any kind of alkyl groups.²⁹ The synthesized analog **1** is equivalent to reported **1** in all spectroscopic aspects. Our synthetic **1** was also highly cytotoxic in HCT116 and HEC293 cell lines (SI).

In conclusion, the total synthesis of *N*¹⁴-desacetoxy-tubulysin H was accomplished in less than 19 steps with an overall yield of 28%. Key features of this strategy included (a) a one-pot unprecedented highly diastereoselective P-3CR, and (b) a two-step diastereoselective synthesis of Tup. We anticipate that the practicality, scalability and conciseness of our strategy should have implications to access a variety of other analogs of tubulysins which is under progress.

ASSOCIATED CONTENT

The Supporting Information is available

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Concise total synthesis of *N*¹⁴-desacetytubulysin H

Tubu_chem rivx_tmv_AD.pdf (619.08 KiB)

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Concise total Synthesis of **M¹⁴-desacetoxytubulysin H**

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General Information

All reactions involving moisture-sensitive reagents were conducted in oven-dried glassware under a nitrogen or argon atmosphere. All chemicals and catalysts were purchased in Sigma Aldrich and used as it is. Analytical thin-layer chromatography (TLC) was performed on SiO₂ 60 F-254 plates. Visualization was accomplished by UV irradiation at 254 nm or by staining with any one of the following reagents: iodine, 5% phosphomolybdic acid hydrate in ethanol, ninhydrin (0.3% w/v in glacial acetic acid/n-butyl alcohol 3:97), Vaughn's reagent (4.8 g of (NH₄)₆Mo₇O₂₄·4H₂O, and 0.2 g of Ce(SO₄)₂·4H₂O in 10 mL of conc. H₂SO₄ and 90 mL of H₂O), or anisaldehyde (7.5 mL of p-anisaldehyde, 25 mL of conc. H₂SO₄, and 7.5 mL of acetic acid in 675 mL of 95% ethanol). Column chromatography was performed using SiO₂ 60 (particle size 0.040–0.055 mm, 230–400 mesh). Flash chromatography was conducted on a silica gel (230–400 mesh) using a Teledyne ISCO CombiFlash® Rf or Grace Reveleris X2. Hydrogenation reactions were performed on an H-cube. Melting points were obtained on a capillary melting point apparatus fitted with a digital thermometer and are not corrected. Optical rotation was obtained on Jasco P-1020 instrument at the designated concentration and temperature using a 1 dm cell. Proton and carbon NMR spectra were obtained on 400, 500, and 600 MHz NMR spectrometers. Chemical shifts are reported as δ values in parts per million (ppm) as referenced to residual solvent. ¹H NMR spectra are tabulated as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet), number of protons, and coupling constant(s). HRMS was obtained on Shimadzu LCMS-IT-TOF. RP-HPLC analyses were carried out on a Chromaster system equipped with a Hitachi 5160 pump, a Hitachi 5260 auto sampler and a Hitachi 5430 diode array detector. SFC-HPLC chromatograms were Supercritical Fluid Chromatograph (SFC) with a 3100 MS Detector (ESI) using a solvent system of methanol or isopropanol and CO₂ on a Viridis silica gel column (4.6 × 250 mm, 5 μ m particle size) or Ethyl pyridine column and reported as (m/z). Conditions and columns used for RP-HPLC or SFC-HPLC analysis are given in the individual compounds.

Previous methods for the synthesis of Tubuvaline

Previous MCR approaches

Previous methods for the synthesis of Tubuphenylalanine

For Review See: U. Kazmaier, A. Ullrich, J. Hoffmann, *The Open Natural Products Journal* **2013**, 6, 12. And reference cited therein.

Recent works by Nicolaou see:

K. C. Nicolaou, R. D. Erande, J. Yin, D. Vourloumis, M. Aujay, J. Sandoval, S. Munneke, J. Gavriluk, *J. Am. Chem. Soc.* **2018**, 140, 3690.

Recent work by Wipf see:

R. Colombo, Z. Wang, J. Han, R. Balachandran, H. N. Daghestani, D. P. Camarco, A. Vogt, B. W. Day, D. Mendel, P. Wipf, *J. Org. Chem.* **2016**, 81, 10302

Synthesis of building blocks for the present studies

Diastereoselective synthesis of 5a

Table 1. Solvent Studies

Entry	Solvent	Yield (%) ^a (mixture of 5a and 5b)	dr ^b (5a : 5b)	Yield (%) ^a 5c
1	CH ₃ CN	52	1:1	25
2	THF	57	1:1	24
3	CHCl ₃	72	1:1	20
4	CH ₂ Cl ₂	88	1:1	05
^a Isolated yields are given; ^b dr are measured by integrating at 4.1 and 4.3 min				

Under argon, a dry Schlenk tube (10 mL) was charged with anhydrous MgSO₄ (100 mg), aldehyde **2** (35.12 mg, 0.1 mmol), acid **4** (37.62 mg, 0.1 mmol), and dry solvents (0.1 mL). The resulting solution was stirred for 10 min at room temperature, and then isocyanide **3** (38.72 mg, 0.1 mmol) was added to the mixture in one portion under argon. After the mixture was stirred at room temperature for 48 h. The solvent was evaporated and diluted with EtOAc (5 mL) and washed with water. The combined organic layer was washed with brine, dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure. The crude residue was directly analysed through HPLC. Yields were determined after flash column chromatography on silica using *n*-hexane/EtOAc (90:10 to 70:30 over 30 min)

RP-HPLC: Chromolith High Resolution RP-18e (150 Å, 10 × 4.6 mm, 3 mL/min flow rate)
Gradient: 80 - 90% CH₃CN/H₂O +0.1%TFA over 8 min.
Absorbance: 214 nm

Compound	Retention time (min)
4	2.2
2	4.8
3	6.3
5a	4.1
5b	4.3
5c	3.0 and 3.1

Table 2. Concentration

Entry	Concentration	Yield (%) ^a (mixture of 5a and 5b)	dr ^b (5a:5b)	Yield (%) ^a 5c
1	1 M	88	1:1	05
2	0.5 M	75	60:40	22
3	0.2 M	75	65:35	8

4	0.1 M	50	68:32	25
5	0.01 M	15	70:30	50
^a Isolated yields are given; ^b dr ratios are measured by integrating at 4.1 and 4.3 min

Under argon, a dry Schlenk tube (10 mL) was charged with anhydrous MgSO_4 (100 mg), aldehyde **2** (35.12 mg, 0.1 mmol), acid **4** (37.62 mg, 0.1 mmol), and dry CH_2Cl_2 with indicated concentrations in the table. The resulting solution was stirred for 10 min at room temperature, and then isocyanide **3** (38.72 mg, 0.1 mmol) was added to the mixture in one portion under argon. After the mixture was stirred at room temperature for 48 h, the solvent was evaporated and diluted with EtOAc (5 mL) and washed with water. The combined organic layer was washed with brine, dried over anhydrous MgSO_4 , filtered, and concentrated under reduced pressure. The crude residue was directly analyzed through HPLC. Yields were determined after flash column chromatography on silica using *n*-hexane/EtOAc (90:10 to 70:30 over 30 min)

RP-HPLC: Chromolith High Resolution RP-18e (150 Å, 10 × 4.6 mm, 3 mL/min flow rate)

Gradient: 80 - 90% CH₃CN/H₂O + 0.1%TFA over 8 min.

Absorbance: 214 nm

Compound	Retention time (min)
4	2.2
2	4.8
3	6.3
5a	4.1
5b	4.3
5c	3.0 and 3.1

Table 3. Screening of Temperature

Entry	Temperature	Yield (%) ^a (mixture of 5a and 5b)	dr ^b (5a:5b)	Yield (%) ^a 5c
1	20 °C	75	65:35	8
2	0 °C	55	65:35	40
3	-20 °C	12	63:37	25
4	-78 °C	5	68:32	8
^a Isolated yields are given; ^b dr ratios are measured by integrating at 4.1 and 4.3 min				

Under argon, a dry Schlenk tube (10 mL) was charged with anhydrous MgSO_4 (100 mg), aldehyde **2** (35.12 mg, 0.1 mmol), acid **4** (37.62 mg, 0.1 mmol), and dry CH_2Cl_2 (0.8 mL) were cooled to indicated temperature in the table. The resulting solution was stirred for 10 min at indicated temperature, and then isocyanide **3** (38.72 mg, 0.1 mmol) was added to the mixture in one portion under argon. After the mixture was stirred at same temperature for 48 h, the solvent was evaporated and diluted with EtOAc (5 mL) and washed with water. The combined organic layer was washed with brine, dried over anhydrous MgSO_4 , filtered, and concentrated under reduced pressure. The crude residue was directly analyzed through HPLC. Yields were determined after flash column chromatography on silica using *n*-hexane/EtOAc (90:10 to 70:30 over 30 min)

RP-HPLC: Chromolith High Resolution RP-18e (150 Å, 10 × 4.6 mm, 3 mL/min flow rate)

Gradient: 80 - 90% CH₃CN/H₂O +0.1%TFA over 8 min.

Absorbance: 214 nm

Compound	Retention time (min)
4	2.2
2	4.8
3	6.3
5a	4.1
5b	4.3
5c	3.0 and 3.1

Table 4. Screening of chiral phosphoric acid catalysts

Entry	Catalyst (10 mol%)	Additive (20 mol%)	Yield (%) ^{a,b} (mixture of 5a and 5b)	dr ^c (5a : 5b)	Yield (%) ^d 5c
1	CP1	-	20 (8)	80:20	20
2	CP2	-	18 (12)	85:15	16
3	CP3	-	15 (10)	88:12	18
4	CP4	-	22 (18)	92:8	12
5	CP5	-	38 (31)	95:5	10
6	CP5	Py (20)	minor ^e	-	70
7	CP4	Py (20)	minor ^e	-	72

^aIsolated yields of **5a** and **5b** is are given; ^b Isolated yields of **5a** is given in the parenthesis; ^c dr are measured by integrating at 4.1 and 4.3 min; ^d isolated yields of **5c** is given; ^e not isolated

Under argon, a dry Schlenk tube (10 mL) was charged with anhydrous MgSO_4 (100 mg), aldehyde **2** (35.12 mg, 0.1 mmol), acid **4** (37.62 mg, 0.1 mmol), chiral phosphoric acid (0.01 mmol) were dissolved in dry CH_2Cl_2 (800 μL). The resulting solution was stirred for 10 min at room temperature, and then isocyanide **3** (38.72 mg, 0.1 mmol) was added to the mixture in one portion under argon. After the mixture was stirred at room temperature 48 h, the solvent was evaporated and diluted with EtOAc (5 mL) and washed with water. The combined organic layer was washed with brine, dried over anhydrous MgSO_4 , filtered, and concentrated under reduced pressure. The crude residue was directly

analyzed through HPLC. Yields were determined after flash column chromatography on silica using *n*-hexane/EtOAc (90:10 to 70:30 over 30 min). The combined fractions of **5a** and **5b** were again purified to obtain pure **5a**.

RP-HPLC: Chromolith High Resolution RP-18e (150 Å, 10 × 4.6 mm, 3 mL/min flow rate)

Gradient: 80 - 90% CH₃CN/H₂O +0.1%TFA over 8 min.

Absorbance: 214 nm

Compound	Retention time (min)
4	2.2
2	4.8
3	6.3
5a	4.1
5b	4.3
5c	3.0 and 3.1

Table 5. Screening of Lewis acids

Entry	Lewis acid (20 mol%)	Yield (%) ^{a,b} (mixture of 5a and 5b)	dr ^c (5a : 5b)	Yield (%) ^d 5c
1	ZnBr ₂	86 (61)	80:20	5
2	AgOTf	65 (35)	70:30	10
3	CuOTf	63 (28)	66:34	5
4	Zn(OTf) ₂	50 (20)	75:25	12
5	Ti(OiPr) ₄	55 (22)	73:27	18

^aIsolated yields of **5a** and **5b** are given; ^bIsolated yields of **5a** are given in the parenthesis; ^cdr are measured by integrating at 4.1 and 4.3 min; ^disolated yields of **5c** are given

Under argon, a dry Schlenk tube (10 mL) was charged with anhydrous MgSO_4 (100 mg), aldehyde **2** (35.12 mg, 0.1 mmol), acid **4** (37.62 mg, 0.1 mmol), Lewis acid (0.02 mmol) were dissolved in dry CH_2Cl_2 (800 μL). The resulting solution was stirred for 10 min at room temperature, and then isocyanide **3** (38.72 mg, 0.1 mmol) was added to the mixture in one portion under argon. After the mixture was stirred at room temperature 48 h, the solvent was evaporated and diluted with EtOAc (5 mL) and washed with water and brine. The organic layer was dried over anhydrous MgSO_4 , filtered, and concentrated under reduced pressure. The crude residue was directly analysed through HPLC. The crude residue was directly analysed through HPLC. Yields were determined after flash column chromatography on silica using *n*-hexane/EtOAc (90:10 to 70:30 over 30 min). The combined fractions of **5a** and **5b** were again purified to obtain pure **5a**.

RP-HPLC: Chromolith High Resolution RP-18e (150 Å, 10 × 4.6 mm, 3 mL/min flow rate)
Gradient: 80 - 90% CH₃CN/H₂O + 0.1%TFA over 8 min.
Absorbance: 214 nm

Compound	Retention time (min)
4	2.2
2	4.8
3	6.3
5a	4.1
5b	4.3
5c	3.0 and 3.1

Table 5. Screening of Lewis acids and chiral ligands

Entry	Ligand (30 mol%)	Yield (%) ^{a,b} (mixture of 5a and 5b)	dr ^c 5a:5b	Yield (%) ^d 5c
1	L1	76 (70)	92:8	5
2	L2	68 (51)	70:30	8
3	L3	60 (49)	85:15	10
4	L4	56 (33)	76:24	28
5	L5	70 (45)	72:28	15
6	L6	55 (28)	69:39	10

^aIsolated yields of **5a** and **5b** is are given; ^b Isolated yields of **5a** is given in the parenthesis; ^cdr are measured by integrating at 4.1 and 4.3 min; ^d isolated yields of **5c** is given

Under argon, a dry Schlenk tube (10 mL) was charged with anhydrous MgSO_4 (100 mg), aldehyde **2** (35.12 mg, 0.1 mmol), acid **4** (37.62 mg, 0.1 mmol), Lewis acid (0.02 mmol) and Ligand (0.03 mmol) were dissolved in dry CH_2Cl_2 (800 μL). The resulting solution was stirred for 10 min at room temperature, and then isocyanide **3** (38.72 mg, 0.1 mmol) was added to the mixture in one portion under argon. After the mixture was stirred at room temperature 48 h, the solvent was evaporated and diluted with EtOAc (5 mL) and washed with water and brine. The organic layer was dried over anhydrous MgSO_4 , filtered, and concentrated under reduced pressure. The crude residue was directly analyzed through HPLC. The crude residue was directly analyzed through HPLC. Yields were determined after flash column chromatography on silica using *n*-hexane/EtOAc (90:10 to 70:30 over 30 min). The combined fractions of **5a** and **5b** were again purified to obtain pure **5a**.

RP-HPLC: Chromolith High Resolution RP-18e (150 Å, 10 × 4.6 mm, 3 mL/min flow rate)

Gradient: 80 - 90% CH₃CN/H₂O + 0.1%TFA over 8 min.

Absorbance: 214 nm

Compound	Retention time (min)
4	2.2
2	4.8
3	6.3
5a	4.1
5b	4.3
5c	3.0 and 3.1

Regioselective ring opening of **7**

Entry	Temperature	Catalyst	Yield (%) ^a 6/ 8a/ 8b
1	0 °C	-	8/ 45/ 43
2	-78 °C	-	3/ 55/ 40
3	-78 °C	CuI (10 mol%)	10/ 58/ 33
4	-78 °C	CuI (10mol%) / PPh ₃ (20 mol%)	20/ 65/ 20
5	-78 °C	CuI (10mol%) / Xphos (20 mol%)	17/ 72/10
6	-78 °C	CuI (10mol%)+ ^t BuXPhos (20 mol%)	20/ 70/0

XPhos **tBuXPhos**

^a HPLC yields are given by integrating the retention times at 7.7, 9.1 and 9.3 min

To a solution of anhydride **7** (114 mg, 1.0 mmol) in anhydrous THF (10 mL), the respective catalyst was added. The round bottomed flask was degassed and filled with Nitrogen. The solution was then cooled to desired temperature as shown in table. Benzyl magnesium bromide (2.0 M in THF, 0.9 mmol) was slowly added to the reaction mixture in 30 mins *via* syringe pump (a purple colour forms, but it disappears after the addition is finished). The reaction mixture was stirred at same temperature for additional 6h and then warm to room temperature for 1h. H₂O (20 mL) and 1N HCl (20 mL) were added and stirred for 5 mins. The reaction mixture diluted with was EtOAc (2 X 20 mL) and filtered through pad of Cellite. The organic layer was separated, dried over MgSO₄ and evaporated under reduced pressure. The crude product was analysed through RP-HPLC analysis. The major desired regioisomer was purified through flash column chromatography on silica (n-hexane/EtOAc, 100:0 to 90:10) confirmed through ¹H NMR analysis.

RP-HPLC: Chromolith High Resolution RP-18e (150 Å, 10 × 4.6 mm, 3 mL/min flow rate)
Gradient: 10 - 90% CH₃CN/H₂O + 0.1%TFA over 14 min.
Absorbance: 211 nm

Compound	Retention time (min)
8a	9.3
8b	9.1
6 (methyl succinic acid)	7.7
Benzyl alcohol and phenolic by-products	1-4 broad peaks

Diastereoselective synthesis of Tubuphenylalanine (**9**)

To a 100-mL stainless steel autoclave equipped with a glass inner lining and a Teflon coated stirrer bar was placed $\text{Ru}(\text{OAc})_2$ ((*R*)-dm-segphos) (4.7 mg, 0.005 mmol), **18** (103.0 mg, 0.5 mmol) and ammonium acetate (192.5 mg, 2.5 mmol). The atmosphere was replaced with nitrogen gas, followed by addition of Trifluoroethanol (2 mL). Hydrogen was initially introduced into the autoclave at a pressure of 145 psi, before being reduced to 14.5 psi by carefully releasing the stop valve. After this procedure was repeated three times, hydrogen was introduced at 435 psi. The autoclave was placed in a pre-warmed oil-bath set at 80 °C, and the mixture was stirred for 12 h. The solution was cooled to 20 °C then hydrogen gas was carefully released. Hydrochloric acid (1 mL, 1M) was added to the reaction mixture and the aqueous phase was washed with diethyl ether for three times and basified with sodium hydroxide (0.5 mL, 5 M), followed by extraction with ethyl ether (2 mL x 3). The combined organic phase was dried over sodium sulfate, filtered and evaporated under reduced pressure. The resulting free amine was then dissolved in acetonitrile (1 mL) was cooled to 0 °C then HCl in EtOH (1M, 1mL) was added dropwise with stirring. The resulting hydrochloride salt of **9** was isolated by filtration and washing with cold acetonitrile (3 X 1 mL) and dried under vacuum desiccator. The white powder obtained was directly subjected to NMR analysis, measurement of optical rotation and HRMS.

To facilitate the measurement of chiral SFC, the **9** obtained was derived into its Boc-protected **9a**.

Chiral SFC analysis of **9** as its derivative **9a**

9 (0.1 mg, 0.0004 mmol) was dissolved in Dioxane:H₂O (1:1, 0.5 mL) followed by addition of NaOH (0.032 mg, 0.0008 mmol) at 0 °C. After stirring for 1min (Boc)₂O (0.113 mg, 0.00052 mmol) was added. The reaction mixture was stirred at same temperate for 5 h. The solvent was evaporated and dilute with H₂O (2 mL) and washed with diethyl ether (2 X 2 mL) and the aqueous layer was acidified to pH 2 using citric acid solution and extract with EtOAc (2 X 2 mL). The organic phase was separated, dried and evaporated under reduced pressure. The corresponding **9a** obtained were used for chiral SFC-HPLC test to evaluate the de value.

Chiral SFC HPLC analysis:

Method: Reprosil Chiral-AM column (4.6 × 250 mm, 5μm) with 5 - 30% *i*-PrOH in CO₂ for 9 min; γ = 214 nm.

Indicated 98:2 er: *t_R* (major) = 4.09 min, *t_R* (minor) = 5.90 min.

Chiral SFC HPLC chromatogram of **9a**

Chiral SFC analysis of diastereomeric **9** as derivative of **9b**

Step 1: To a 50-mL stainless steel autoclave equipped with a glass inner lining and a Teflon coated stirrer bar was placed **18** (50.0 mg, 0.25 mmol), MeOH (0.5 mL) and ammonia solution (7N in MeOH, 0.2 mL, 1.25 mmol). Hydrogen was initially introduced into the autoclave at a pressure of 145 psi, before being reduced to 14.5 psi by carefully releasing the stop valve. After this procedure was repeated three times, hydrogen was introduced at 500 psi. The autoclave was placed in a pre-warmed oil-bath set at 80 °C, and the mixture was stirred for 12 h. The solution was cooled to 20 °C then hydrogen gas was carefully released. Hydrochloric acid (1 mL, 1M) was added to the reaction mixture and the aqueous phase was washed with diethyl ether for three times and basified with sodium hydroxide (0.5 mL, 5 M), followed by extraction with ethyl ether (2 mL x 3). The combined organic phase was dried over MgSO₄, filtered and evaporated under reduced pressure.

Step 2: The resulting free amine was then protected with Boc by using the protocol as described for the synthesis of **9a**. The resulting racemic **9b** were used for chiral SFC-HPLC test.

Chiral SFC HPLC analysis:

Method: Reprosil Chiral-AM column (4.6 × 250 mm, 5μm) with 5 - 30% *i*-PrOH in CO₂ for 9 min; γ = 214 nm.

t_R = 4.21 min and 5.96 min.

Chiral SFC HPLC chromatogram of racemic **9b**

Synthesis of methyl S-trityl-L-cysteine (16)

To a solution of triphenyl methanol (95.98 g, 369 mmol, 1.0 eq.) in 400 mL of trifluoroacetic acid L-cysteine methyl ester hydrochloride **15** (50 g, 369.0 mmol). The resulting red-orange solution was left to stir at room temperature for 3 h before it was concentrated to orange oil, which was partitioned between 1000 mL of CH_2Cl_2 and 1000 mL of H_2O , resulting in bleaching of the orange color. Portions of K_2CO_3 were then slowly added to the aqueous layer until a basic pH was sustained for up to 1 h. The organic layer was then separated, washed with saturated NaHCO_3 (3 X 200 mL) and brine, dried over MgSO_4 and concentrated to a pale oil which solidified after overnight stirring in hexanes. The white solids thus obtained were isolated *via* vacuum filtration.

Yield: 97% (135 g)

TLC: 0.41 (Petroleum ether/EtOAc, 50:50)

White solid

Mp: 55 – 57 °C

$[\alpha]_{\text{D}}^{25} = +40.2$ (c 1, CHCl_3)

HRMS (ESI) m/z calculated for $\text{C}_{23}\text{H}_{23}\text{NO}_2\text{S}$ $[\text{M}+\text{H}]^+$: 378.1527, found 378.1521

^1H NMR (500 MHz, CDCl_3) δ 7.58 – 7.45 (m, 6H), 7.37 – 7.30 (m, 6H), 7.30 – 7.18 (m, 3H), 3.69 (s, 3H), 3.24 (dd, $J = 7.8, 4.8$ Hz, 1H), 2.64 (dd, $J = 12.5, 4.9$ Hz, 1H), 2.52 (dd, $J = 12.5, 7.8$ Hz, 1H).

^{13}C NMR (126 MHz, CDCl_3): δ 174.1, 144.5, 130.0, 129.5, 128.3, 127.9, 126.8, 66.8, 53.7, 52.2, 36.9.

Synthesis of methyl N-formyl-S-trityl-L-cysteine (17)

Amine **16** (40.0 g, 106 mmol) was dissolved in methyl formate (700 mL, solvent) and the assembly was allowed to reflux at 60 °C until TLC showed complete consumption of starting material (usually 24 h). The solvent was evaporated under reduced pressure. Diethyl ether was added to the crude reaction mixture and stirred at 0 °C until the product gets precipitated (1 h). The solid product was collected by filtration and washed

thoroughly with cold ether (3 X 200 mL). The filtrate is stored at 0 °C overnight and the remaining solid were filtered off. The white solid **17** was used without further purification.

Yield: 95% (40.8 g)

TLC: 0.50 (Petroleum ether/EtOAc, 50:50)

White solid

Mp = 130 – 132 °C

$[\alpha]_D^{25} = +28.2$ (c 1, CHCl₃)

HRMS (ESI) m/z calculated for C₂₀H₂₈N₂O₅ [M+H]⁺: 406.1476, found 406.1471

¹H NMR (600 MHz, CDCl₃) δ 7.91 (s, 1H), 7.38 – 7.31 (m, 5H), 7.27 – 7.22 (m, 5H), 7.22 – 7.20 (m, 3H), 7.19 – 7.16 (m, 2H), 5.78 (d, *J* = 8.1 Hz, 1H), 4.63 – 4.57 (m, 1H), 3.66 (s, 3H), 2.72 (dd, *J* = 12.7, 5.6 Hz, 1H), 2.55 (dd, *J* = 14.3, 7.8 Hz, 1H).

¹³C NMR (151 MHz, CDCl₃) δ 170.3, 160.2, 144.2, 128.1, 128.0, 127.0, 126.9, 67.1, 52.8, 33.6.

28g Synthesis of methyl (*R*)-2-isocyano-3-(tritylthio)propanoate (**3**)

A solution of *N*-formyl Cys(Trt)-methyl ester **17** (35 g, 86.3 mmol) in CH₂Cl₂ (450.0 mL), was cooled to -78 °C. *N*-methylmorpholine (19.0 mL, 172.6 mmol, 2 eq.) was added. After 5 min triphosgene (10.2 g, 34.52 mmol, 0.40 eq.) in CH₂Cl₂ (150 mL) was added drop wise over 30 min. After addition was complete the reaction mixture was stirred for 7h at -78 °C (TLC analysis). Saturated NaHCO₃ solution (200 mL) was added at same temperature and then allowed to warm to room temperature. The organic layer was separated and the aqueous phase was washed CH₂Cl₂ (3 X 100 mL). The combined organic extracts were separated, dried over anhydrous MgSO₄, filtered, and evaporated under reduced pressure. The crude product was dissolved in diethyl ether (300 mL) and stored -15 °C for 5 h resulted in pure solid of isocyanide **3** which was collected by filtration. The isocyanide **3** was used further without purification.

TLC: 0.42 (Petroleum ether/EtOAc, 9:1)

Yield: 86% (28.7 g)

White solid

Mp = 97 – 99 °C

$[\alpha]_D^{25} = +38.4$ (c 1, CHCl₃)

HRMS (ESI) m/z calculated for C₂₄H₂₁NO₂S [M+H]⁺: 388.1365, found 388.1366

¹H NMR (600 MHz, CDCl₃) δ 7.40 – 7.13 (m, 15H), 3.65 (s, 3H), 3.27 (dd, *J* = 8.0, 5.8 Hz, 1H), 2.75 – 2.70 (m, 1H), 2.68 (dd, *J* = 13.7, 8.0 Hz, 1H).

¹³C NMR (151 MHz, CDCl₃) δ 165.6, 160.9, 146.8, 143.9, 129.4, 128.2, 127.9, 127.2, 127.1, 67.5, 53.4, 34.2.

40g Synthesis of (S)-(9H-fluoren-9-yl)methyl (1-diazo-4-methyl-2-oxopentan-3-yl)carbamate (**19**)

Under an argon atmosphere *N*-methyl morpholine (20.28 mL, 184.5 mmol, 1.5 eq.) and isobutylchloroformate (20.7 mL, 159.9 mmol, 1.30 eq) were carefully added at -20 °C to the Fmoc-Val-OH **18** (43.0 g, 123.0 mmol) dissolved in dry THF (550 mL). The reaction mixture was stirred at -20 °C for 5 hr and then under exclusion of light diazomethane in diethyl ether (3.0 eq. generated from the *N*-nitroso *N*-methyl urea using Diazard kit), was added and it was stirred at rt for 12 h. Afterwards, the excess of diazomethane was quenched by addition of glacial acetic acid (10 mL) and the mixture was taken up in 10% NaHCO₃ (3 X 200 mL) and extracted with EtOAc (3 X 300 mL). The combined organic phases were washed with saturated NH₄Cl (3 X 200 mL), saturated NaCl, dried over MgSO₄ and the solvent was removed under reduced pressure. The product (yellow solid) was recrystallized using CH₂Cl₂: n-pentane (70:135 mL) and pure **19** collected by filtration and dried.

Yield: 88% (39.3 g)

TLC: 0.52 (Petroleum ether/EtOAc, 7:3)

Pale yellow solid

mp 122 – 124 °C

$[\alpha]_{\text{D}}^{25} = +16.3$ (c 1, CHCl₃)

HRMS (ESI) *m/z* calculated for C₂₁H₂₁N₃O₃ [M+H]⁺: 364.1661, found 363.1655

¹H NMR (500 MHz, CDCl₃) δ 7.79 (d, *J* = 7.6 Hz, 2H), 7.66 – 7.55 (m, 2H), 7.46 – 7.30 (m, 4H), 5.51 (d, *J* = 9.0 Hz, 1H), 5.36 (br, s, 1H), 4.46 (d, *J* = 7.2 Hz, 2H), 4.24 (t, *J* = 6.7 Hz, 1H), 4.18 – 4.12 (m, 1H), 2.18 – 2.08 (m, 1H), 1.01 (d, *J* = 6.8 Hz, 3H), 0.93 (d, *J* = 6.9 Hz, 3H).

¹³C NMR (121 MHz, CDCl₃) δ 193.3, 156.3, 143.8, 141.3, 127.7, 127.7, 127.1, 127.0, 125.1, 125.0, 120.0, 66.8, 47.3, 31.0, 25.5, 19.4, 17.4.

26g Synthesis of (R)-3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-methylpentanoic acid (**20**)

The diazo ketone **19** (30 g, 82.6 mmol) was dissolved in Dioxane/H₂O (9:1, 495 mL) and under exclusion of light silver benzoate (1.8 g, 8.26 mmol, 0.1 eq.) was added at 0 °C and the reaction mixture was stirred at same temperature for 30 min. Then the reaction

mixture was refluxed at 70 °C for 5 h. After completion of the reaction (TLC analysis), the mixture was cooled down to room temperature and diluted with H₂O (330 mL), adjusted to pH = 2-3 by the addition of 1.0 M HCl and extracted with EtOAc (3 X 300 mL). The combined organic phases were dried over MgSO₄ and the solvent was removed under reduced pressure. The crude product was taken up in DCM (128 mL) and was cooled to -22 °C, cold pentane (78 mL) was added dropwise. The obtained precipitate was filtered off, washed with cold pentane (2 X 50 mL) and dried overnight under reduced pressure to yield **20**.

Yield: 95% (26.3 g)

TLC: 0.42 (CHCl₃/ MeOH/ AcOH, 40:2:1)

White solid

mp 153 – 155 °C

$[\alpha]_D^{25} = -37.5$ (c 1, CHCl₃)

HRMS (ESI) *m/z* calculated for C₂₁H₂₃NO₄ [M+Na]⁺: 354.1705, found 354.1700

¹H NMR (400 MHz, CDCl₃) δ 10.85 (s, 1H), 7.78 (d, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 7.4 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.33 (td, *J* = 7.5, 1.2 Hz, 2H), 5.17 (d, *J* = 3.8 Hz, 1H), 4.43 (d, *J* = 7.0 Hz, 2H), 4.24 (t, *J* = 6.8 Hz, 1H), 3.84 (td, *J* = 12.2, 9.8, 6.0 Hz, 1H), 2.66 – 2.53 (m, 2H), 1.89 (dt, *J* = 13.7, 6.9 Hz, 1H), 0.96 (d, *J* = 6.8 Hz, 3H), 0.84 (d, *J* = 7.2 Hz, 3H).

¹³C NMR (126 MHz, CDCl₃) δ 176.9, 156.1, 143.8, 141.3, 127.6, 127.1, 127.0, 125.1, 125.0, 119.9, 66.6, 54.0, 47.3, 36.8, 31.6, 19.3, 18.6.

20g Synthesis of (*R*)-(9*H*-fluoren-9-yl)methyl 4-isopropyl-6-oxo-1,3-oxazinane-3-carboxylate (**21**)

The Fmoc-β-Val-OH **20** (22 g, 66 mmol), paraformaldehyde (12.4 g) and *p*-toluene sulfonic acid (1.2 g, 0.1 eq.) were suspended in toluene (800 mL). The mixture was refluxed at 95 °C in a Dean–Stark setup until no more starting material could be detected by TLC analysis (6 h). The solution was cooled, washed with saturated NaHCO₃ (3 X 200 mL) and the aqueous phase was extracted with EtOAc (3 X 200 mL). The combined organic layer was then washed with brine and dried over anhydrous MgSO₄. The solvent was evaporated *in vacuo* gave the crude product, which was purified by silica gel column chromatography with ethyl acetate–*n*-hexane (3:7).

Yield: 82% (19.7 g)

TLC: 0.45 (Petroleum ether/EtOAc, 7:3)

Gummy solid

$[\alpha]_{\text{D}}^{25} = +93.1$ (c 1, CHCl_3)

HRMS (ESI) m/z calculated for $\text{C}_{22}\text{H}_{23}\text{NO}_4$ $[\text{M}+\text{H}]^+$: 366.1705, found 366.1695

^1H NMR (600 MHz, CDCl_3) (major rotamer) δ 7.64 – 7.18 (m, 8H), 5.65 (d, $J = 22.4$ Hz, 1H), 5.62 (d, $J = 20.6$ Hz, 1H), 4.43 (d, $J = 10.6$ Hz, 2H), 4.11 (t, $J = 16.6$ Hz, 1H), 4.09 – 4.03 (m, 1H), 2.65 (s, 3H), 2.56 (dd, $J = 15.1, 4.3$ Hz, 1H), 2.69 – 2.57 (m, 1H), 2.18 – 2.10 (m, 2H), 0.84 (d, $J = 6.5$ Hz, 3H), 0.75 (d, $J = 6.4$ Hz, 3H).

^{13}C NMR (126 MHz, CDCl_3) (major rotamer) δ 170.1, 154.9, 143.2, 141.1, 127.7, 127.1, 124.4, 119.8, 72.8, 67.0, 46.9, 31.6, 25.6, 18.1.

^1H NMR (600 MHz, CDCl_3) (minor rotamer) δ 7.64 – 7.18 (m, 5H), 5.34 (d, $J = 20.4$ Hz, 0.55 H), 5.27 (d, $J = 18.6$ Hz, 0.55H), 4.60 (d, $J = 10.6$ Hz, 1.4H), 4.09 (t, $J = 14.4$ Hz, 0.5H), 4.04 – 4.00 (m, 0.7H), 2.62 – 2.03 (m, 1.5 H), 0.52 (d, $J = 6.6$ Hz, 2H), 0.39 (d, $J = 6.4$ Hz, 2H).

^{13}C NMR (126 MHz, CDCl_3) (minor rotamer) δ 170.9, 154.7, 143.3, 141.2, 127.6, 127.0, 124.3, 119.8, 71.6, 67.8, 49.2, 31.0, 23.7, 20.7.

9g Synthesis of (*R*)-3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)(methyl)amino)-4-methylpentanoic acid (**22**)

The oxazolidinone **21** (10 g, 27.3 mmol) was dissolved in 300 mL of CHCl_3 , and 300 mL of trifluoroacetic acid and triethylsilane (13.0 mL, 82.0 mmol) were added. The solution was stirred at room temperature for 48 h followed by concentration in vacuo to an oil. The oil was dissolved in CH_2Cl_2 and re-concentrated several times with CH_2Cl_2 . The resultant oil was purified on silica gel flash column chromatography (PE/ EA 100:0 to 20:80 then to 0:100).

Yield: 90% (9.01 g)

TLC: 0.34 (Petroleum ether/ EtOAc/ AcOH, 5:5:0.2)

$[\alpha]_{\text{D}}^{25} = -23.6$ (c 1, CHCl_3)

HRMS (ESI) m/z calculated for $\text{C}_{22}\text{H}_{25}\text{NO}_4$ $[\text{M}+\text{Na}]^+$: 390.1681, 390.1680

^1H NMR (600 MHz, CDCl_3) (major rotamer) δ 8.86 (s, 1H), 7.65 (dt, $J = 15.9, 7.4$ Hz, 2H), 7.57 – 7.46 (m, 2H), 7.28 (q, $J = 7.5$ Hz, 2H), 7.20 (q, $J = 7.4, 6.4$ Hz, 2H), 4.37 (d, $J = 22.4$, 2H), 4.29 (t, $J = 10.6$, 1H), 4.03 – 3.93 (m, 1H), 2.65 (s, 3H), 2.56 (dd, $J = 15.1, 4.3$ Hz, 1H), 2.46 (dd, $J = 15.2, 10.5$ Hz, 1H), 2.21 (d, $J = 14.6$ Hz, 1H), 2.05 (s, 1H), 1.80 – 1.70 (m, 1H), 0.85 (d, $J = 6.6$ Hz, 3H), 0.75 (d, $J = 6.6$ Hz, 3H).

^{13}C NMR (151 MHz, CDCl_3) (major rotamer) δ 176.9, 156.7, 144.2, 141.3, 127.6, 127.5, 127.0, 125.1, 124.7, 119.9, 67.3, 60.2, 47.3, 37.6, 35.8, 30.5, 19.8, 19.5.

^1H NMR (600 MHz, CDCl_3) (minor rotamer) δ 7.65 – 7.20 (m, 5H), 4.52 (d, J = 10.9, 0.7H), 4.13 (t, J = 6.9 Hz, 0.5H), 4.03 – 3.93 (m, 0.3H), 2.68 (s, 1.7H), 2.56 (dd, J = 15.1, 4.3 Hz, 2H), 2.46 (dd, J = 15.2, 10.5 Hz, 2H), 2.21 (dd, J = 14.6, 7.4 Hz, 0.5H), 2.05 (dd, J = 12.6, 8.2 Hz, 0.5H), 1.80 – 1.70 (m, 2H), 0.62 (d, J = 6.6 Hz, 1.7H), 0.49 (d, J = 6.6 Hz, 1.8H).

^{13}C NMR (151 MHz, CDCl_3) (minor rotamer) δ 176.5, 156.3, 144.1, 141.3, 127.6, 127.1, 127.0, 125.0, 119.8, 67.1, 47.3, 37.6, 35.6, 30.5, 19.8, 19.2.

Synthesis of (*R*)-(9*H*-fluoren-9-yl)methyl (1-hydroxy-4-methylpentan-3-yl)(methyl)carbamate (**23**)

A solution of **23** (3.67 g, 10 mmol) in THF (50 mL) was cooled to -15 °C (ice/ salt bath) under a nitrogen atmosphere. NMM (1.62 mL, 15 mmol, 1.5 eq.) and isobutyl chlorformate (IBCF, 1.55 mL, 12 mmol, 1.2 eq.) were added successively in a dropwise manner. After 3 h, the reaction mixture was filtered. The filtrate was cooled to -15 °C (ice/ salt bath), and a solution of NaBH_4 (1.6 g, 40 mmol, 4.0 eq.) in H_2O (2.5 mL) was added in one portion. After complete reduction (TLC analysis), the suspension was diluted with EtOAc and H_2O (1:1). The organic layer was separated and the aqueous layer was extracted with EtOAc (3 X 50 mL) and the combined organic layer was dried over MgSO_4 and evaporated under vacuum. The crude product was purified on silica gel flash column chromatography (Pet ether / EtOAc, 100:0 to 20:80).

Yield: 96% (3.39 g)

TLC: 0.25 (Petroleum ether/EtOAc, 5:5)

$[\alpha]_{\text{D}}^{25} = -10.4$ (c 1, CHCl_3)

HRMS (ESI) m/z calculated for $\text{C}_{22}\text{H}_{27}\text{NO}_3$ $[\text{M}+\text{H}]^+$: 354.2069, found 354.2060

^1H NMR (600 MHz, CDCl_3) (major rotamer) δ 7.82 – 7.34 (m, 8H), 4.53 – 4.50 (m, 2H), 4.26 (t, J = 4.3 Hz, 1H), 3.86 (ddd, J = 12.1, 10.4, 3.2 Hz, 1H), 3.54 (ddd, J = 11.7, 5.6, 2.9 Hz, 1H), 3.34 – 3.26 (m, 1H), 2.61 (s, 3H), 1.95 (dddd, J = 14.1, 10.7, 5.5, 3.2 Hz, 1H), 1.70 (dp, J = 10.4, 6.6 Hz, 1H), 1.47 – 1.35 (m, 1H), 0.98 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H).

^{13}C NMR (151 MHz, CDCl_3) (major rotamer) δ 157.2, 143.3, 140.7, 126.9, 126.8, 126.3, 126.2, 124.1, 123.6, 119.1, 66.3, 58.1, 52.6, 46.7, 31.1, 29.3, 26.8, 19.3, 18.5.

^1H NMR (600 MHz, CDCl_3) (minor rotamer) (selected signals) δ 7.82 – 7.34 (m, 3H), 4.68 (dd, J = 10.7, 4.5 Hz, 0.6H), 4.22 (t, J = 4.7 Hz, 0.3H), 3.86 (ddd, J = 12.1, 10.4, 3.2 Hz,

3H), 3.54 (ddd, $J = 11.7, 5.6, 2.9$ Hz, 3H), 3.34 – 3.26 (m, 0.3H), 2.63 (s, 0.7H), 1.47 – 1.35 (m, 0.3H), 0.73 (d, $J = 6.8$ Hz, 1H), 0.49 (d, $J = 6.6$ Hz, 1H).

^{13}C NMR (151 MHz, CDCl_3) (minor rotamer) δ 155.9, 143.0, 140.5, 126.8, 126.3, 126.2, 124.0, 123.6, 119.1, 65.6, 57.6, 52.2, 46.57, 30.8, 29.1, 19.2, 19.1.

Synthesis of (*R*)-(9*H*-fluoren-9-yl)methyl methyl(4-methyl-1-oxopentan-3-yl)carbamate (**3**)

Alcohol **23** (3.0 g, 8.4 mmol) was dissolved in CH_3CN (50 mL) and freshly prepared IBX (4.7 g, 16.8 mmol, 2.0 eq.) was added. The resulting suspension was immersed in an oil bath set to 80 °C and stirred vigorously in an open atmosphere. After 2 h (TLC monitoring), the reaction was cooled to 5 °C and filtered through a medium glass frit. The filter cake was washed with cold ethyl acetate (3 X 10 mL), and the combined filtrates were concentrated. The crude aldehyde **3** was pure enough for further reactions. For NMR and SFC analysis small amount was purified by flash column chromatography (PE/EA 100:0 to 70:30) to yield aldehyde **3** as a yellow gum.

Yield: 96% (2.4 g)

TLC: 0.51 (Petroleum ether/EtOAc, 7:3)

$[\alpha]_{\text{D}}^{25} = -92.6$ (c 1, CHCl_3)

HRMS (ESI) m/z calculated for $\text{C}_{22}\text{H}_{25}\text{NO}_3$ $[\text{M}+\text{H}]^+$: 352.1912, found 352.1906

^1H NMR (500 MHz, CDCl_3) (major rotamer) δ 9.66 (s, 1H), 7.79 – 7.30 (m, 8H), 4.72 – 4.63 (m, 1H), 4.47 (d, $J = 6.8$ Hz, 2H), 4.34 – 4.22 (m, 1H), 2.73 (s, 3H), 2.70 (dd, $J = 4.3, 1.2$ Hz, 1H), 2.56 (dd, $J = 10.8, 4.2$ Hz, 1H), 1.86 – 1.81 (m, 1H), 0.98 (d, $J = 6.6$ Hz, 3H), 0.88 (d, $J = 6.6$ Hz, 3H).

^{13}C NMR (126 MHz, CDCl_3) (major rotamer) δ 200.9, 156.4, 144.1, 143.8, 141.4, 127.5, 127.1, 126.9, 124.9, 124.8, 124.3, 119.8, 67.0, 57.6, 47.3, 44.7, 31.8, 29.6, 19.8, 19.2, 18.8.

^1H NMR (500 MHz, CDCl_3) (minor rotamer) (selected signals) δ 9.04 (s, 0.7H), 7.79 – 7.30 (m, 5.6H), 4.72 – 4.63 (m, 1H), 4.34 – 4.22 (m, 2H), 2.66 (s, 2H), 2.25 (dd, $J = 16.4, 4.5$ Hz, 1H), 2.15 (dd, $J = 9.9, 3.5$ Hz, 1H), 1.36 – 1.34 (m, 0.8H), 0.66 (d, $J = 6.6$ Hz, 2H), 0.50 (d, $J = 6.6$ Hz, 2H).

^{13}C NMR (126 MHz, CDCl_3) (minor rotamer) δ 200.5, 155.9, 143.9, 141.2, 127.1, 126.9, 124.8, 124.3, 119.7, 66.4, 57.8, 47.2, 44.6, 30.2, 29.2, 19.7, 18.8.

26g Synthesis of (*R*)-benzyl 2-(((2*S*,3*R*)-1-methoxy-3-methyl-1-oxopentan-2-yl)carbamoyl)piperidine-1-carboxylate (26**)**

Acid **24** (20.0 g, 76.0 mmol), amine **25** (12.1 g, 83.6 mmol, 1.1 eq.), pyridine (20.7 mL, 266 mmol, 1 volume), and EtOAc (42 mL, 2 volumes) were charged in an 500 mL flask with constant stirring. The heterogeneous mixture was cooled between -20 °C and -10 °C as 91.4 mL of T3P solution (50 wt% in EtOAc, 152 mmol, 2.0 eq.) were added *via* addition funnel at a rate to maintain an internal temperature below 0 °C. The resulting homogeneous, golden-yellow solution was stirred at 0 °C for 20 h, at which time the coupling was complete and amide **26** was formed. The reaction mixture was again cooled between -20 °C and -10 °C as 60 mL (3 volumes) of 0.5 M aq HCl solution were added (exothermic) *via* addition funnel at a rate to maintain an internal temperature below 5 °C. The mixture was diluted with 300 mL of EtOAc and the phases were separated. The organic layer was washed with 100 mL of 0.5 M aq HCl, 100 mL of sat. NaCl solution, and concentrated. The pure product was isolated by flash column chromatography (Pet Ether / EtOAc, 100:0 to 70:30).

Yield: 89% (26.4 g)

TLC: 0.35 (Petroleum ether/EtOAc, 8:2)

Colour less gum

$[\alpha]_{\text{D}}^{25} = +35.5$ (c 1, CHCl₃)

HRMS (ESI) *m/z* calculated for C₂₁H₃₀N₂O₅ [M+Na]⁺: 413.2052, found 413.2051

¹H NMR (600 MHz, MeOD) δ 7.41 – 7.27 (m, 5H), 5.16 (s, 2H), 4.87 (dd, *J* = 6.2, 2.3 Hz, 1H), 4.44 (d, *J* = 6.2 Hz, 1H), 4.09 – 4.03 (m, 1H), 3.69 (s, 3H), 3.26 (dt, *J* = 13.2, 6.5 Hz, 1H), 2.19 – 2.12 (m, 1H), 1.91 (dtd, *J* = 8.8, 6.5, 4.2 Hz, 1H), 1.75 – 1.71 (m, 1H), 1.64 (td, *J* = 7.1, 3.5 Hz, 1H), 1.48 – 1.41 (m, 4H), 1.24 – 1.14 (m, 1H), 0.94 – 0.88 (m, 6H).

¹³C NMR (151 MHz, MeOD) δ 172.6, 172.0, 156.8, 136.6, 128.2, 128.0, 127.7, 127.4, 126.8, 67.1, 63.8, 56.7, 51.1, 41.9, 36.9, 26.8, 24.9, 24.3, 19.7, 14.8, 10.3.

18g Synthesis of (2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-3-methylpentanoic acid (**4**)

To a solution of the **26** (20 g, 51.2 mmol) in THF: H₂O: MeOH (1:0.5:1, 100 mL), lithium hydroxide (2.10 g, 76.8 mmol) was added at 0 °C. The mixture was stirred for 3 h at R.T. and then acidified to pH 2.0 with 1N HCl. The aqueous layer was extracted with ethyl acetate (3 X 150 mL). The combined organic extracts were dried over anhydrous MgSO₄ and evaporated in vacuum to yield **4** and used without further purification.

Yield: 95% (18.3 g)

TLC: 0.35 (CHCl₃/ MeOH/ AcOH, 40:2:1)

Colour less gum

[α]_D²⁵ = +42.3 (c 1, CHCl₃)

HRMS (ESI) m/z calculated for C₂₀H₂₈N₂O₅ [M+H]⁺: 377.2076, found 377.2069

¹H NMR (600 MHz, MeOD) δ 7.36 (d, *J* = 5.8 Hz, 3H), 7.30 (d, *J* = 8.6 Hz, 2H), 5.16 (s, 2H), 4.87 (dd, *J* = 6.2, 2.3 Hz, 1H), 4.46 – 4.40 (m, 1H), 4.09 – 4.05 (m, 2H), 3.24 (td, *J* = 12.9, 3.1 Hz, 1H), 2.16 (t, *J* = 18.2 Hz, 1H), 1.93 (tdd, *J* = 12.4, 8.4, 5.3 Hz, 1H), 1.71 – 1.64 (m, 2H), 1.48 (tdd, *J* = 15.1, 11.9, 7.7 Hz, 3H), 1.18 (ddt, *J* = 14.2, 8.8, 7.2 Hz, 1H), 0.96 – 0.90 (m, 6H).

¹³C NMR (151 MHz, MeOD) δ 173.1, 171.5, 156.2, 136.5, 128.1, 127.6, 127.4, 67.1, 60.1, 56.5, 41.9, 36.9, 24.7, 19.4, 19.3, 14.7, 13.0, 10.3.

Synthesis of (S)-3-methyldihydrofuran-2,5-dione (**7**)

(S)-3-methyldihydrofuran-2,5-dione (1.32 g, 10.0 mmol) and acetyl chloride (2.83 mL, 30.0 mmol) were placed in a single neck round bottomed flask and the assembly was refluxed (75 °C) on the steam bath for 3 h. The solution is allowed to cool undisturbed and is finally chilled in an ice bath. The succinic anhydride **7**, which separates in beautiful crystals, and is collected on a Büchner funnel, washed with cold ether (2 X 75 mL), and dried in a vacuum desiccator.

Yield: 98% (1.11 g)

TLC: 0.54 (Petroleum ether/EtOAc, 6:4)

$[\alpha]_{\text{D}}^{25} = -11.0$ (c 1, CHCl_3)
HRMS (ESI) m/z calculated for $\text{C}_5\text{H}_6\text{O}_3$ $[\text{M}+\text{Na}]^+$: 137.0214, found 137.100

^1H NMR (500 MHz, CDCl_3) δ 3.27 – 3.14 (m, 2H), 2.70 – 2.59 (m, 1H), 1.45 (d, $J = 6.1$ Hz, 3H).

^{13}C NMR (126 MHz, CDCl_3) δ 174.4, 170.0, 36.0, 35.0, 16.1.

Synthesis of (S)-2-methyl-4-oxo-5-phenylpentanoic acid (8a)

To a solution of anhydride **17** (1.14 g, 10 mmol), CuI (190 mg, 1 mmol, 0.1 eq.) and $t\text{BuXPhos}$ (849.2 mg, 0.2 mmol, 0.2 eq.) in dry THF (100 mL) was cooled to -78°C . benzyl magnesium bromide (2.0 M in THF, 0.9 mmol) slowly added to the reaction mixture over 30 min (A purple color forms, but it disappear after the addition is finished). The reaction mixture was stirred at same temperature for additional 6h and then room temperature for 1h. H_2O (50 mL) and 1N HCl (50 mL) were added and stirred for 5 mins. The reaction mixture diluted with was EtOAc (2 X 200 mL) and filtered through pad of Cellite. The organic layer was separated, dried over MgSO_4 and evaporated under reduced pressure. The crude product was purified though column chromatography. No other regioisomer was observed through HPLC analysis.

Yield: 70 % (798 mg)

TLC: 0.51 (Petroleum ether/ EtOAc /TFA, 7:3:0.1)

$[\alpha]_{\text{D}}^{25} = -25.8$ (c 1, CHCl_3)

HRMS (ESI) m/z calculated for $\text{C}_{12}\text{H}_{14}\text{O}_3$ $[\text{M}+\text{Na}]^+$: 229.0840, found 229.0837

^1H NMR (500 MHz, CDCl_3) δ 10.54 (s, 1H), 7.40 – 7.34 (m, 5H), 5.17 (s, 2H), 3.07 – 2.96 (m, 1H), 2.53 (dd, $J = 6.9, 3.0$ Hz, 1H), 2.50 (dd, $J = 12.5, 6.0$ Hz, 1H), 1.29 (d, $J = 7.2$ Hz, 3H).

^{13}C NMR (126 MHz, CDCl_3) δ 201.8, 175.0, 135.7, 128., 128.2, 128.0, 125.7, 66.5, 44.5, 37.3, 16.8.

Synthesis of (2S,4R)-4-(14-azaneyl)-2-methyl-5-phenylpentanoic acid (Tup,9)

To a 100-mL stainless steel autoclave equipped with a glass inner lining and a Teflon coated stirrer bar was placed Ru(OAc)₂((*R*)-dm-segphos) (4.7 mg, 0.005 mmol), **18** (103.0 mg, 0.5 mmol) and ammonium acetate (192.5 mg, 2.5 mmol). The atmosphere was replaced with nitrogen gas, followed by addition of Trifluoroethanol (2 mL). Hydrogen was initially introduced into the autoclave at a pressure of 145 psi, before being reduced to 14.5 psi by carefully releasing the stop valve. After this procedure was repeated three times, hydrogen was introduced at 435 psi. The autoclave was placed in a pre-warmed oil-bath set at 80 °C, and the mixture was stirred for 12 h. The solution was cooled to 20 °C then hydrogen gas was carefully released. Hydrochloric acid (1 mL, 1M) was added to the reaction mixture and the aqueous phase was washed with diethyl ether for three times and basified with sodium hydroxide (0.5 mL, 5 M), followed by extraction with ethyl ether (2 mL x 3). The combined organic phase was dried over sodium sulfate, filtered and evaporated under reduced pressure. The resulting free amine was then dissolved in acetonitrile (1 mL) was cooled to 0 °C then HCl in EtOH (1M, 1mL) was added dropwise with stirring. The resulting hydrochloride salt of **9** was isolated by filtration and washing with cold acetonitrile (3 X 1 mL) and dried under vacuum desiccator. The white powder obtained was directly subjected to NMR analysis, measurement of optical rotation and HRMS.

Yield: 81% (98.4 mg)

TLC: 0.31 (CH₂Cl₂/ MeOH/ NH₄OH, 9:1:0.2)

$[\alpha]_{\text{D}}^{25} = +11.2$ (*c* 1, MeOH)

HRMS (ESI) *m/z* calculated for C₁₂H₁₇NO₂ [M+H]⁺: 208.1332, found 208.1330

¹H NMR (600 MHz, D₂O) δ 7.30 (d, *J* = 7.5 Hz, 2H), 7.27 (t, *J* = 7.3 Hz, 1H), 7.22 – 7.14 (m, 2H), 3.54 – 3.42 (m, 1H), 2.88 (dd, *J* = 14.1, 6.8 Hz, 1H), 2.83 – 2.78 (m, 1H), 2.64 – 2.54 (m, 1H), 1.94 – 1.86 (m, 1H), 1.67 – 1.56 (m, 1H), 1.05 (d, *J* = 7.2 Hz, 3H).

¹³C NMR (151 MHz, D₂O) δ 179.7, 135.4, 129.4, 129.2, 129.1, 127.5, 126.1, 52.5, 38.3, 35.8, 35.7, 16.7.

Synthesis of benzyl (*R*)-2-(((2*S*,3*R*)-1-(((5*R*,7*R*,10*R*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-10-(methoxycarbonyl)-4-methyl-3,8-dioxo-13,13,13-triphenyl-2-oxa-12-thia-4,9-diazatridecan-7-yl)oxy)-3-methyl-1-oxopentane-2-yl)carbamoyl)piperidine-1-carboxylate (5a**)**

Under argon, a dry Schlenk tube (10 mL) was charged with anhydrous MgSO_4 (100 mg), aldehyde **2** (351.2 mg, 1.0 mmol), acid **4** (376.2 mg, 1.0 mmol), ZnBr_2 (45.04 mg, 0.2 mmol, 0.2 eq.) and Ligand **L1** (61.2 mg, 0.3 mmol, 0.3 eq.) were dissolved in dry CH_2Cl_2 (4.5 mL). The resulting solution was stirred for 10 min at room temperature, and then isocyanide **3** (38.72 mg, 1.0 mmol) was added to the mixture in one portion under argon. After the mixture was stirred at room temperature 48 h, the solvent was evaporated and diluted with EtOAc (15 mL) and washed with water and brine. The organic layer was dried over anhydrous MgSO_4 , filtered, and concentrated under reduced pressure. The crude residue was directly analysed through HPLC. The crude residue was directly analysed through HPLC. Yields were determined after flash column chromatography on silica using *n*-hexane/EtOAc (90:10 to 70:30 over 30 min). The combined fractions of **5a** and **5b** were again purified to obtain pure **5a**.

Major Diastereomer (**5a**): TLC: 0.31 (Petroleum ether/EtOAc, 7.5:2.5)

Minor Diastereomer(**5b**): TLC: 0.34 (Petroleum ether/EtOAc, 7.5:2.5)

Yield: 71% (790 mg)

TLC: 0.34 (Petroleum ether/EtOAc, 7:3)

$[\alpha]_{\text{D}}^{25} = -96.5$ (c 1, CHCl_3)

HRMS (ESI) m/z calculated for $\text{C}_{66}\text{H}_{74}\text{N}_4\text{O}_{10}\text{S}$ $[\text{M}+\text{H}]^+$: 1115.5198, found 1115.5182.

^1H NMR (600 MHz, CDCl_3) (major rotamer) δ 7.77 (br, s, 1H), 7.66 (t, $J = 7.7$ Hz, 2H), 7.52 (d, $J = 7.5$ Hz, 1H), 7.44 (d, $J = 7.5$ Hz, 1H), 7.35 – 6.96 (m, 22H), 6.55 (br, s, 1H), 4.90 – 4.69 (m, 2H), 4.55 (d, $J = 8.1$ Hz, 2H), 4.28 (t, $J = 6.5$ Hz, 1H), 3.50 (s, 3H), 2.67 – 2.66 (m, 1H), 2.63 (s, 3H), 2.62 – 2.58 (m, 2H), 2.58 – 2.53 (m, 2H), 2.50 (d, $J = 11.1$ Hz, 1H), 2.16 – 2.01 (m, 2H), 2.00 – 1.88 (m, 4H), 1.85 (dd, $J = 5.5, 1.9$ Hz, 2H), 1.60 (dt, $J = 9.8, 6.4$ Hz, 2H), 1.56 – 1.43 (m, 2H), 1.43 – 1.23 (m, 3H), 0.90 (d, $J = 6.8$ Hz, 3H), 0.84 (t, $J = 6.6$ Hz, 3H), 0.80 (d, $J = 11.7, 6.7$ Hz, 3H), 0.72 (d, $J = 6.7, 2.5$ Hz, 3H).

^{13}C NMR (151 MHz, CDCl_3) (major rotamer) δ 169.9, 168.8, 168.6, 167.9, 156.1, 154.8, 142.6, 142.3, 142.1, 139.5, 134.5, 127.8, 127.6, 126.7, 126.2, 125.9, 125.7, 125.6, 125.2, 125.1, 124.9, 123.3, 123.1, 118.1, 118.0, 117.9, 114.6, 70.0, 65.8, 65.8, 65.3, 65.1, 56.1,

54.9, 50.7, 50.4, 49.2, 45.6, 45.5, 35.5, 34.2, 31.7, 31.1, 29.9, 29.8, 28.5, 27.8, 23.1, 22.8, 18.5, 18.33, 18.0, 17.9, 17.4, 13.8, 13.7, 9.8.

¹H NMR (600 MHz, CDCl₃) (minor rotamer) (selected signals) δ 7.48 (d, *J* = 7.5 Hz, 1H), 7.47 – 6.96 (m, 12), 6.25 (br, s, 0.5H), 5.05 (s, 0.8H), 4.65 – 4.59 (m, 1H), 4.38 (dd, *J* = 10.6, 6.9 Hz, 1H), 4.20 – 4.13 (m, 2H), 4.11 (q, *J* = 7.5, 6.3 Hz, 2H), 3.98 (s, 1H), 3.54 (s, 1.1H), 3.51 (s, 1H), 2.75 (s, 1.2H), 2.29 – 2.16 (m, 2H), 1.73 – 2.16 (s, 2H), 1.03 (d, *J* = 6.5 Hz, 1.2H), 1.00 – 0.64 (m, 4H).

¹³C NMR (151 MHz, CDCl₃) (minor rotamer) δ 168.8, 168.82, 168.7, 168.4, 167.0, 155.1, 142.6, 142.2, 142.1, 139.4, 127.7, 127.6, 126.7, 126.3, 126.1, 125.8, 125.6, 125.2, 124.9, 123.2, 123.1, 118.1, 117.9, 69.4, 65.8, 65.4, 65.3, 56.5, 56.0, 54.6, 50.6, 50.1, 45.6, 45.5, 35.5, 33.93, 31.3, 30.0, 28.7, 28.6, 28.1, 27.8, 23.1, 23.0, 18.5, 18.2, 17.9, 13.8, 13.7, 13.6, 9.7.

Synthesis of methyl 2-((5*R*,7*R*,10*S*)-12-((*R*)-1-((benzyloxy)carbonyl)piperidin-2-yl)-10-((*R*)-sec-butyl)-1-(9H-fluoren-9-yl)-5-isopropyl-4-methyl-3,9,12-trioxo-2,8-dioxo-4,11-diazadodecan-7-yl)thiazole-4-carboxylate (10**)**

A solution of **5a** (1.0 mmol) in dry CH₂Cl₂ (10 mL) was treated with TiCl₄ (1.0 M solution in CH₂Cl₂, 3.0 mmol) and stirred at 0 °C for 18 h. The reaction mixture was stirred vigorously with cold saturated aqueous NaHCO₃ (20 mL) for 30 min. The aqueous layer was extracted with CH₂Cl₂ (2 X 10 mL), and the combined organic layers were dried over MgSO₄, filtered, and concentrated. The resultant crude product was pass with pad of silica and washed with EtOAc and evaporated to dryness. The crude thiazoline was dissolved in CHCl₃ (5 mL), activated MnO₂ (< 10 micron, 10 mmol) was added. The reaction mixture was refluxed at 70 °C for 5 h, then filtered through a short silica gel and celite column and washed with CHCl₃. The organic solution was concentrated. The resulting crude product was purified by flash chromatography (EtOAc/hexane, 0:100 to 30:70) to afford compound **10** as a white foam.

Yield: 71% (790 mg)

TLC: 0.30 (Petroleum ether/EtOAc, 7:3)

[α]_D²⁵ = +12.5 (c 1, CHCl₃)

HRMS (ESI) *m/z* calculated for C₄₇H₅₆N₄O₉S [M+H]⁺: 853.3840, found 853.3837

¹H NMR (600 MHz, CDCl₃) (major rotamer) δ 8.00 (s, 1H), 7.66 (d, *J* = 7.6, 2H), 7.51 (d, *J* = 7.4 Hz, 2H), 7.33 – 7.16 (m, 9H), 6.43 (br, s, 1H), 5.91 d, *J* = 6.5 Hz, 1H), 5.11 (s, 2H), 4.79 (d, *J* = 6.5 Hz, 1H), 4.59 (dd, *J* = 8.9, 4.6 Hz, 1H), 4.32 – 4.30 (m, 2H), 4.13 (t, *J* = 23.8, 7.0 Hz, 1H), 3.84 (s, 3H), 3.77 – 3.67 (m, 1H), 3.66 – 3.52 (m, 1H), 2.83 (td, *J* = 13.0, 3.0 Hz, 1H), 2.64 (s, 3H), 2.47 – 2.43 (m, 1H), 2.25 – 2.11 (m, 5H), 1.98 – 1.91 (m, 2H), 1.91 – 1.88 (m, 2H), 1.57 – 1.50 (m, 2H), 1.47 – 1.42 (m, 2H), 1.32 (dddd, *J* = 16.3, 12.2, 6.2, 2.8 Hz, 2H), 0.90 (d, *J* = 6.6 Hz, 3H), 0.87 – 0.83 (m, 6H), 0.75 (d, *J* = 6.6 Hz, 3H).

¹H NMR (600 MHz, CDCl₃) (minor rotamer) (selected signals) δ 7.68 (s, 0.5 H), 7.63 (d, *J* = 7.4 Hz, 0.5H), 7.45 (d, *J* = 7.2 Hz, 0.5H), 7.33 – 7.16 (m, 8H), 6.03 (br, d, 0.5H), 5.10 (s, 1H), 4.73 (br, s, 0.5H), 4.53 (dd, *J* = 8.6, 4.4 Hz, 0.5H), 4.28 – 4.25 (m, 1H), 4.10 (t, *J* = 25.8, 7.0 Hz, 0.5H), 3.83 (s, 1.5H), 3.77 – 3.67 (m, 0.5H), 3.66 – 3.52 (m, 0.5H), 2.81 (td, *J* = 13.0, 3.0 Hz, 0.5H), 2.61 (s, 1.5H), 2.54 – 2.53 (m, 0.5H), 2.32 – 2.30 (m, 1H), *J* = 16.4, 4.6 Hz, 1H), 2.33 – 2.30 (m, 1H), 2.26 – 2.16 (m, 4H), 1.69 – 1.66 (m, 1H), 1.57 – 1.50 (m, 1H), 1.47 – 1.42 (m, 1H),), 1.33 – 1.26 (m, 1H), 0.87 (d, *J* = 6.4 Hz, 1.5H), 0.77 – 0.79 (m, 3H), 0.67 (d, *J* = 6.6 Hz, 1.5H).

¹³C NMR (151 MHz, CDCl₃) (major rotamer) δ 171.4, 170.7, 170.1, 161.6, 157.0, 155.5, 146.6, 144.3, 144.0, 141.3, 141.3, 136.3, 128.5, 128.4, 128.0, 127.7, 127.6, 127.5, 127.1, 127.0, 126.9, 125.1, 124.7, 119.9, 119.8, 71.1, 67.6, 67.2, 61.4, 56.4, 52.3, 47.3, 42.1, 37.1, 31.9, 30.3, 29.7, 24.8, 24.6, 22.7, 20.3, 20.1, 19.4, 15.6, 11.6.

¹³C NMR (151 MHz, CDCl₃) (minor rotamer) δ 169.6, 161.3, 156.8, 156.7, 146.6, 144.20, 144.1, 141.3, 141.3, 136.6, 128.5, 128.2, 128.1, 127.8, 127.6, 127.5, 127.0, 126.9, 125.1, 124.7, 119.9, 119.9, 71.5, 67.6, 67.3, 61.7, 56.5, 52.4, 47.4, 42.2, 37.2, 34.9, 30.3, 29.6, 24.7, 24.5, 22.7, 20.4, 20.0, 19.7, 15.7, 11.5.

Synthesis of methyl 2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylate (11**)**

To a solution of **10** (852 mg, 1.0 mmol) in CH₃CN (12 mL) at 0 °C under Ar was added Et₂NH (8 mL). The solution was stirred at room temperature for 1 h. After complete deprotection of Fmoc group (TLC analysis) the mixture was evaporated and co-evaporated with CH₂Cl₂ to remove DEA. The crude product which was dissolved in CH₂Cl₂ (10 mL) and DIPEA (0.52 mL) was added and the resulting mixture was stirred at 50 °C for 18 h. The crude hydroxyl derivative **11** was purified by column chromatography. The non-acylated amine was isolated and again stirred in presence of CH₂Cl₂/DIPEA for overnight.

Yield: 75% (472 mg)

TLC: 0.32 (Petroleum ether/ EtOAc/ AcOH, 3:7)

$[\alpha]_D^{25} = +68.2$ (c 1, MeOH)

HRMS (ESI) m/z calculated for $C_{32}H_{46}N_4O_7S$ $[M+H]^+$: 631.3160, found 631.3158

1H NMR (600 MHz, MeOD) δ 8.50 (s, 1H), 7.64 (d, $J = 13.2$ Hz, 1H), 7.33 (dt, $J = 21.4$, 5.5 Hz, 5H), 5.13 (s, 2H), 4.79 (d, $J = 5.7$ Hz, 1H), 4.73 (t, $J = 8.8$ Hz, 1H), 4.33 (br, s, 1H), 4.06 (d, $J = 12.1$ Hz, 1H), 3.94 (s, 3H), 3.13 (s, 3H), 3.12 (dt, $J = 12.6$, 5.6, 3.9 Hz, 1H), 3.05 – 3.02 (m, 2H), 2.37 (d, $J = 14.1$ Hz, 1H), 2.19 (d, $J = 13.8$ Hz, 1H), 2.06 – 1.94 (m, 1H), 1.94 – 1.83 (m, 1H), 1.70 – 1.59 (m, 4H), 1.56 – 1.52 (m, 1H), 1.49 – 1.39 (m, 2H), 1.38 – 1.30 (m, 1H), 1.10 (d, $J = 6.5$ Hz, 3H), 0.95 (d, $J = 6.6$ Hz, 3H), 0.88 (d, $J = 6.8$ Hz, 3H), 0.72 (d, $J = 6.9$ Hz, 3H).

^{13}C NMR (151 MHz, MeOD) δ 174.6, 173.1, 172.0, 158.2, 157.6, 138.1, 129.7, 129.2, 129.2, 128.9, 72.8, 68.7, 62.8, 62.5, 55.3, 52.8, 43.5, 38.5, 37.7, 31.9, 25.7, 25.6, 21.4, 20.6, 20.1, 16.3, 11.2.

Synthesis of 2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylic acid (12)

LiOH (35.95 mg, 1.5 mmol, 3.0 eq.) was added to a solution of **11** (315 mg, 0.5 mmol) in a mixture of THF: MeOH: H₂O (1:1:0.5, 0.5 M), and the reaction mixture was stirred at room temperature for 3 h. The mixture was concentrated under reduced pressure. The crude product was purified through silica gel column chromatography using CH₂Cl₂/MeOH.

Yield: 99% (305 mg)

TLC: 0.25 (CH₂Cl₂/ MeOH/ AcOH, 9:1:0.2)

$[\alpha]_D^{25} = +38.4$ (c 1, MeOH)

HRMS (ESI) m/z calculated for $C_{31}H_{44}N_4O_7S$ $[M+Na]^+$: 639.2828, found 639.2820

1H NMR (600 MHz, MeOD) δ 8.31 (s, 1H), 7.68 (d, $J = 26.3$ Hz, 1H), 7.36 (t, $J = 6.6$ Hz, 4H), 7.31 (dt, $J = 17.0$, 5.6 Hz, 1H), 5.15 (s, 2H), 4.85 (t, $J = 6.5$ Hz, 1H), 4.82 – 4.79 (m, 1H), 4.75 (t, $J = 8.7$ Hz, 1H), 4.36 (br, m, 1H), 4.07 (d, $J = 13.5$ Hz, 1H), 3.13 (dd, $J = 13.1$, 2.9 Hz, 1H), 3.09 (s, 3H), 3.06 – 3.04 (m, 2H), 2.33 (dd, $J = 6.1$, 3.6 Hz, 1H), 2.31 (dd, $J = 6.4$, 3.8 Hz, 1H), 2.27 – 2.11 (m, 1H), 2.07 – 1.99 (m, 1H), 1.90 (ddt, $J = 12.0$, 6.9, 2.7 Hz, 1H), 1.66 (d, $J = 13.9$ Hz, 2H), 1.56 – 1.53 (m, 1H), 1.48 – 1.41 (m, 2H), 1.39 – 1.34 (m, 1H), 1.13 (d, $J = 6.5$ Hz, 3H), 0.96 (d, $J = 7.0$ Hz, 3H), 0.89 (d, $J = 6.8$ Hz, 3H), 0.75 (d, $J = 4.5$ Hz, 3H).

^{13}C NMR (151 MHz, MeOD) δ 177.6, 173.2, 171.6, 162.8, 155.9, 146.8, 136.5, 128.1, 127.7, 127.7, 127.6, 127.4, 69.8, 67.1, 54.7, 53.8, 48.0, 47.8, 47.7, 47.6, 47.4, 47.3, 47.1, 41.9, 37.4, 36.1, 30.4, 24.3, 24.2, 24.1, 19.8, 19.1, 18.6, 14.7, 9.6.

Synthesis of (2*S*,4*R*)-4-(2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (13**)**

Acid **12** (50.0 mg, 0.0811 mmol) was added to a 0.2 M solution of pentafluorophenol (23.0 mg, 0.125 mmol) and DIC (14.4 μL , 0.0919 mmol) in CH_2Cl_2 at 0 $^\circ\text{C}$. The reaction mixture was warmed to rt, stirred for 24 h, and concentrated under reduced pressure. EtOAc (10 mL) was added, and the crude product was filtered, with rinsing of the reaction vessel with EtOAc. The filtrate was concentrated under reduced pressure, and the crude material was used without further purification. DMF (0.335 mL, 0.25 M) was added to the crude product, followed by the hydrochloride salt of tubuphenylalanine (**9**) (61.0 mg, 0.250 mmol) and diisopropylethylamine (73.0 μL , 0.419 mmol). The reaction mixture was stirred for 24 h at rt, and DMF was removed under vacuum. Column purification (100:0 to 90:10 CH_2Cl_2 :MeOH) afforded **13** as white foamy solid.

Yield: 90% (58.5 mg)

TLC: 0.28 (CH_2Cl_2 /MeOH/AcOH, 9:1:0.1)

$[\alpha]_{\text{D}}^{25} = +11.5$ (*c* 1, MeOH)

HRMS (ESI) m/z calculated for $\text{C}_{43}\text{H}_{59}\text{N}_5\text{O}_8\text{S}$ $[\text{M}+\text{Na}]^+$: 828.3982, found 828.3975

^1H NMR (600 MHz, MeOD) δ 8.59 (d, $J = 9.3$ Hz, 1H), 8.01 (s, 1H), 7.35 – 7.29 (m, 5H), 7.26 – 7.20 (m, 5H), 7.17 (qd, $J = 6.5, 5.5, 3.8$ Hz, 1H), 5.15 (s, 2H), 4.84 – 4.81 (m, 1H), 4.72 (d, $J = 3.7$ Hz, 1H), 4.70 (d, $J = 3.8$ Hz, 1H), 4.42 – 4.29 (m, 1H), 4.13 (dt, $J = 15.5, 9.5$ Hz, 1H), 3.70 (dd, $J = 18.1, 12.3$ Hz, 1H), 3.59 (dd, $J = 18.0, 12.2$ Hz, 1H), 3.38 – 3.34 (m, 1H), 3.14 (s, 3H), 2.96 (dd, $J = 13.7, 7.7$ Hz, 2H), 2.88 – 2.85 (m, 1H), 2.61 (ddd, $J = 10.5, 7.2, 4.3$ Hz, 1H), 2.27 (d, $J = 8.8$ Hz, 1H), 2.26 (d, $J = 8.5$ Hz, 1H), 2.26 – 2.23 (m, 1H), 2.12 – 2.10 (m, 1H), 2.07 (d, $J = 3.3$ Hz, 1H), 2.03 (d, $J = 3.4$ Hz, 1H), 1.90 (ddt, $J = 11.9, 6.6, 2.8$ Hz, 2H), 1.68 (d, $J = 12.8$ Hz, 3H), 1.56 – 1.52 (m, 1H), 1.51 – 1.42 (m, 2H), 1.41 – 1.27 (m, 2H), 1.16 (d, $J = 7.1$ Hz, 3H), 0.95 – 0.91 (m, 3H), 0.87 (d, $J = 6.5$ Hz, 3H), 0.83 (d, $J = 6.4$ Hz, 3H).

^{13}C NMR (151 MHz, MeOD) δ 178.4, 176.0, 173.1, 161.8, 161.0, 149.2, 138.4, 136.4, 128.9, 128.2, 127.8, 127.8, 127.4, 125.9, 122.3, 72.0, 68.6, 67.3, 60.8, 53.8, 53.7, 42.1, 41.5, 38.0, 37.8, 37.0, 36.6, 30.6, 29.2, 24.7, 24.2, 19.9, 19.1, 18.8, 17.5, 14.3, 9.7.

Synthesis of (2*S*,4*R*)-4-(2-((1*R*,3*R*)-1-acetoxy-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (14**)**

A 0.1 M solution of **13** (40 mg, 0.049 mmol) in pyridine (0.50 mL) was cooled to 0 °C, and acetic anhydride (34.0 μL , 0.366 mmol) was added. The reaction mixture was allowed to warm to rt over 2 h and was stirred at rt for 24 h. The reaction mixture was then cooled to 0 °C, and a 1:1 mixture of dioxane/water (1.80 mL) was added. The mixture was allowed to warm to rt, and was stirred for 6 h at rt. The solvent was removed under reduced pressure. Column chromatography (100% CH_2Cl_2 to 10% MeOH/ CH_2Cl_2) afforded **14** as an amorphous solid.

Yield: 95% (39.4 mg)

TLC: 0.42 ($\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{AcOH}$, 9:1:0.1)

$[\alpha]_{\text{D}}^{25} = +26.5$ (c 1, MeOH)

HRMS (ESI) m/z calculated for $\text{C}_{45}\text{H}_{61}\text{N}_5\text{O}_9\text{S}$ $[\text{M}+\text{H}]^+$: 848.4268, found 848.4261

^1H NMR (600 MHz, MeOD) δ 8.47 (d, $J = 9.3$ Hz, 1H), 8.09 (s, 1H), 7.46 (br, s, 1H), 7.33 (s, 4H), 7.28 – 7.20 (m, 4H), 7.16 (h, $J = 4.4$ Hz, 1H), 5.86 (dd, $J = 10.9, 3.6$ Hz, 1H), 5.13 (s, 2H), 4.84 (d, $J = 9.4$ Hz, 2H), 4.68 – 4.61 (m, 1H), 4.36 (d, $J = 4.8$ Hz, 1H), 4.13 (d, $J = 8.5$ Hz, 1H), 3.38 – 3.33 (m, 2H), 3.16 (s, 3H), 3.11 – 3.09 (m, 2H), 2.95 (dd, $J = 13.7, 7.7$ Hz, 1H), 2.89 – 2.86 (m, 1H), 2.61 (t, $J = 9.8$ Hz, 1H), 2.26 – 2.24 (m, 1H), 2.22 (d, $J = 26.6$ Hz, 2H), 2.18 (s, 3H), 2.05 (d, $J = 7.2$ Hz, 2H), 1.93 (d, $J = 7.4$ Hz, 1H), 1.85 (s, 1H), 1.67 (d, $J = 13.1$ Hz, 3H), 1.60 – 1.51 (m, 1H), 1.50 – 1.44 (m, 1H), 1.41 – 1.33 (m, 1H), 1.16 (d, $J = 7.1$ Hz, 3H), 0.98 – 0.95 (m, 3H), 0.89 (d, $J = 6.4$ Hz, 3H), 0.80 (d, $J = 52.4$ Hz, 3H).

^{13}C NMR (151 MHz, MeOD) δ 179.9, 174.8, 173.1, 171.3, 170.3, 162.9, 151.0, 146.0, 139.8, 138.0, 130.5, 129.7, 129.4, 129.3, 129.0, 127.5, 124.7, 71.9, 68.8, 57.1, 55.4, 55.3, 49.7, 43.0, 39.4, 38.4, 38.1, 36.3, 31.8, 30.9, 30.6, 25.8, 25.7, 21.4, 20.9, 20.6, 20.2, 18.9, 16.0, 11.2.

Synthesis of (2S,4R)-4-(2-((1R,3R)-1-acetoxy-3-((2S,3R)-N,3-dimethyl-2-((R)-1-methylpiperidine-2-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (1**)**

The compound **14** (20 mg, 0.023 mmol) was dissolved in a mixture of MeOH (3 mL). Paraformaldehyde (5 mg) and 20% Pd/C (0.09 mg, 0.0023 mmol) were added. The reaction mixture was stirred under hydrogen atmosphere for 24 h and afterwards filtered through Cellite. The solvent was then removed under reduced pressure. The product was purified through column chromatography (100% CH₂Cl₂ to 10% MeOH/CH₂Cl₂) to afford **1** as a pale-yellow film that was further purified by RP chromatography (12-g C18 cartridge, 0.1% TFA/H₂O/CH₃CN 90:10 – 0:60). Lyophilization afforded **1** as a colorless fluffy solid:

Yield: 86% (14.3 mg)

TLC: 0.25 (CH₂Cl₂/ MeOH/ AcOH; 9:1:0.1).

$[\alpha]_{\text{D}}^{25} = +19.2$ (c 1.0, MeOH).

HRMS (ESI) *m/z* calculated for C₃₈H₅₇N₅O₇S [M+H]⁺: 728.4056, found 728.4050

¹H NMR (600 MHz, MeOD) δ 8.36 (d, *J* = 9.2 Hz, 1H), 8.12 (s, 1H), 7.28 – 7.23 (m, 4H), 7.19 – 7.17 (m, 1H), 5.89 (d, *J* = 3.5 Hz, 1H), 4.73 (d, *J* = 8.2 Hz, 1H), 4.37 (ddd, *J* = 12.0, 5.7, 3.7 Hz, 2H), 3.77 (dd, *J* = 10.4, 1.8 Hz, 1H), 3.52 (d, *J* = 11.6 Hz, 1H), 3.22 (td, *J* = 13.6, 3.9 Hz, 1H), 3.14 (s, 3H), 3.00 (dd, *J* = 13.7, 7.8 Hz, 1H), 2.93 (dd, *J* = 13.7, 6.1 Hz, 1H), 2.77 (s, 3H), 2.63 (ddd, *J* = 9.5, 7.0, 4.4 Hz, 1H), 2.37 (ddd, *J* = 13.9, 9.5, 3.3 Hz, 1H), 2.33 – 2.30 (m, 1H), 2.26 – 2.19 (m, 4H), 2.06 – 2.04 (m, 1H), 1.91 – 1.84 (m, 4H), 1.84 – 1.77 (m, 2H), 1.66 (ddd, *J* = 13.8, 10.7, 3.1 Hz, 1H), 1.65 – 1.59 (m, 2H), 1.30 – 1.22 (m, 4H), 1.20 (d, *J* = 7.1 Hz, 3H), 1.06 (d, *J* = 6.8 Hz, 3H), 0.94 (t, *J* = 4.2 Hz, 3H), 0.82 (d, *J* = 6.6 Hz, 3H).

¹³C NMR (151 MHz, MeOD) δ 178.9, 173.3, 170.3, 170.0, 166.8, 161.5, 149.9, 138.0, 129.0, 127.5, 126.4, 124.7, 69.9, 66.6, 57.1, 54.8, 54.4, 48.6, 41.7, 40.3, 37.2, 36.3, 35.9, 34.0, 29.7, 28.1, 24.2, 23.0, 21.4, 20.2, 19.5, 18.9, 17.5, 14.3, 10.0.

¹H NMR of methyl S-trityl-L-cysteine (16)

¹³C NMR of methyl S-trityl-L-cysteine (16)

HRMS of methyl S-trityl-L-cysteine (16)

SFC-HPLC of methyl *S*-trityl-L-cysteine (16)

SFC-HPLC: (Viridis silica gel column (4.6 × 250 mm, 5 μm, gradient: 5 – 30% MeOH in CO₂ over 6 min, flow rate: 1.0 mL/min), Absorbance: 214 nm, t_R = 2.91 min

^1H NMR of methyl *N*-formyl-*S*-trityl-*L*-cysteine (17)

¹³C NMR of methyl *N*-formyl-*S*-trityl-L-cysteine (17)

HRMS of methyl *N*-formyl-*S*-trityl-*L*-cysteine (17)

SFC-HPLC of methyl *N*-formyl-*S*-trityl-*L*-cysteine (17)

SFC-HPLC: (Viridis silica gel column (4.6 × 250 mm, 5 μm, gradient: 5 – 30% MeOH in CO₂ over 6 min, flow rate: 1.0 mL/min), Absorbance: 214 nm, *t_R* = 2.88 min)

¹H NMR of methyl (*R*)-2-isocyano-3-(tritylthio)propanoate (3)

¹³C NMR of methyl (*R*)-2-isocyano-3-(tritylthio)propanoate (3)

HRMS of methyl (*R*)-2-isocyano-3-(tritylthio)propanoate (3)

SFC-HPLC of methyl (*R*)-2-isocyano-3-(tritylthio)propanoate (3)

SFC-HPLC: (Viridis silica gel column (4.6 × 250 mm, 5 μm, gradient: 5 – 30% MeOH in CO₂ over 6 min, flow rate: 1.0 mL/min), Absorbance: 214 nm.

t_R = 3.02 min

Chiral SFC–HPLC chromatogram of enantiopure methyl (*R*)-2-isocyano-3-(tritylthio)propanoate (3)

Method: Reprosil Chiral-AM column (4.6 X 250 mm, 5 μ m) with 5 - 30% *i*PrOH in CO₂ for 9 min; Absorbance = 230 nm, flow rate: 0.8 mL/min, t_R = 2.88 min ee: >98%.

¹H NMR of (S)-(9H-fluoren-9-yl)methyl (1-diazo-4-methyl-2-oxopentan-3-yl)carbamate (19)

¹³C NMR of (S)-(9H-fluoren-9-yl)methyl (1-diazo-4-methyl-2-oxopentan-3-yl)carbamate (19)

HRMS of (S)-(9H-fluoren-9-yl)methyl (1-diazo-4-methyl-2-oxopentan-3-yl)carbamate (19)

SFC-HPLC of (S)-(9H-fluoren-9-yl)methyl (1-diazo-4-methyl-2-oxopentan-3-yl)carbamate (19)

SFC-HPLC: (Ethyl pyridine column (4.6 × 250 mm, 5 μm, gradient: 5 – 30% MeOH in CO₂ over 7 min, flow rate: 1.0 mL/min), Absorbance: 214 nm.

t_R = 4.18 min

¹H NMR of (R)-3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-methylpentanoic acid (20)

¹³C NMR of (R)-3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-methylpentanoic acid (20)

HRMS (*R*)-3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-methylpentanoic acid (20)

SFC-HPLC of (*R*)-3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-methylpentanoic acid (20)

SFC-HPLC: (Ethyl pyridine column (4.6 × 250 mm, 5 μm, gradient: 5 – 40% MeOH in CO₂ over 6 min, flow rate: 1.0 mL/min), Absorbance: 214 nm.

t_R = 3.02 min

¹H NMR of (R)-(9H-fluoren-9-yl)methyl 4-isopropyl-6-oxo-1,3-oxazinane-3-carboxylate (21)

¹³C NMR of (R)-(9H-fluoren-9-yl)methyl 4-isopropyl-6-oxo-1,3-oxazinane-3-carboxylate (21)

HRMS of (*R*)-(9*H*-fluoren-9-yl)methyl 4-isopropyl-6-oxo-1,3-oxazinane-3-carboxylate (21)

SFC-HPLC of (*R*)-(9*H*-fluoren-9-yl)methyl 4-isopropyl-6-oxo-1,3-oxazinane-3-carboxylate (21)

SFC-HPLC: Ethyl pyridine column (4.6 × 250 mm, 5 μm, gradient: 10 – 30% MeOH in CO₂ over 6 min, flow rate: 1.0 mL/min), Absorbance: 214 nm.

t_R = 4.69 min

¹H NMR of (*R*)-3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)(methyl)amino)-4-methylpentanoic acid (22)

¹³C NMR of (R)-3-((((9H-fluoren-9-yl)methoxy)carbonyl)(methyl)amino)-4-methylpentanoic acid (22)

HRMS of (*R*)-3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)(methyl)amino)-4-methylpentanoic acid (22)

SFC-HPLC of (*R*)-3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)(methyl)amino)-4-methylpentanoic acid (22)

SFC-HPLC: Ethyl pyridine column (4.6 × 250 mm, 5 μm, gradient: 5 – 40% MeOH in CO₂ over 8 min, flow rate: 1.0 mL/min), Absorbance: 214 nm.

t_R = 3.41 min

¹³C NMR of HRMS of (*R*)-(9*H*-fluoren-9-yl)methyl (1-hydroxy-4-methylpentan-3-yl)(methyl)carbamate (23)

HRMS of (*R*)-(9*H*-fluoren-9-yl)methyl (1-hydroxy-4-methylpentan-3-yl)(methyl)carbamate (23)

SFC-HPLC of (*R*)-(9*H*-fluoren-9-yl)methyl (1-hydroxy-4-methylpentan-3-yl)(methyl)carbamate (23)

SFC-HPLC: Ethyl pyridine column (4.6 × 250 mm, 5 μm, gradient: 5 – 30% MeOH in CO₂ over 6 min, flow rate: 1.0 mL/min), Absorbance: 214 nm.

t_R = 3.82 min

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¹³C NMR of (R)-(9H-fluoren-9-yl)methyl methyl(4-methyl-1-oxopentan-3-yl)carbamate (2)

HRMS of (R)-(9H-fluoren-9-yl)methyl methyl(4-methyl-1-oxopentan-3-yl)carbamate (2)

SFC-HPLC of (*R*)-(9*H*-fluoren-9-yl)methyl methyl(4-methyl-1-oxopentan-3-yl)carbamate (2)

SFC-HPLC: Viridis silica gel column (4.6 × 250 mm, 5 μm, gradient: 5 – 30% MeOH in CO₂ over 7 min, flow rate: 1.0 mL/min), Absorbance: 214 nm.

t_R = 4.50 min

Chiral SFC–HPLC chromatogram of (*R*)-(9*H*-fluoren-9-yl)methyl methyl(4-methyl-1-oxopentan-3-yl)carbamate (2**)**

Method: Reprosil Chiral-AM column (4.6 × 250 mm, 5μm, with 2 -40% *i*PrOH in CO₂ for 9 min; γ = 254 nm, flow rate: 0.8 mL/ min). *t_R* = 2.87 min; ee >98%

^1H NMR of (*R*)-benzyl 2-(((2*S*,3*R*)-1-methoxy-3-methyl-1-oxopentan-2-yl)carbamoyl)piperidine-1-carboxylate (26)

¹³C NMR of (R)-benzyl 2-(((2S,3R)-1-methoxy-3-methyl-1-oxopentan-2-yl)carbamoyl)piperidine-1-carboxylate (26)

HRMS of (*R*)-benzyl 2-(((2*S*,3*R*)-1-methoxy-3-methyl-1-oxopentan-2-yl)carbamoyl)piperidine-1-carboxylate (26)

SFC-HPLC of (*R*)-benzyl 2-(((2*S*,3*R*)-1-methoxy-3-methyl-1-oxopentan-2-yl)carbamoyl)piperidine-1-carboxylate (26)

SFC-HPLC: Ethyl pyridine column (4.6 × 250 mm, 5 μm, gradient: 5 – 30% MeOH in CO₂ over 6 min, flow rate: 1.0 mL/min), Absorbance: 214 nm.
t_R = 5.12 min

¹H NMR of (2S,3R)-2-((R)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-3-methylpentanoic acid (4)

¹³C NMR of (2S,3R)-2-((R)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-3-methylpentanoic acid (4)

HRMS of (2S,3R)-2-((R)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-3-methylpentanoic acid (4)

SFC-HPLC of (2S,3R)-2-((R)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-3-methylpentanoic acid (4)

SFC-HPLC: Ethyl pyridine column (4.6 × 250 mm, 5 µm, gradient: 5 – 30% MeOH in CO₂ over 6 min, flow rate: 1.0 mL/min), Absorbance: 214 nm.

t_R = 3.30 min

Chiral SFC-HPLC chromatogram of (2S,3R)-2-((R)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-3-methylpentanoic acid (4)

Method: Reprosil Chiral-AM column (4.6 × 250 mm, 5μm, with 3 - 30% *i*PrOH in CO₂ for 9 min; γ = 214 nm, flow rate: 0.6 mL/ min). *t_R* = 3.99 min; ee >98%

¹H NMR of (S)-3-methyldihydrofuran-2,5-dione (7)

^{13}C NMR of (S)-3-methyldihydrofuran-2,5-dione (7)

HRMS of (S)-3-methyldihydrofuran-2,5-dione (7)

SFC-HPLC of (S)-3-methyldihydrofuran-2,5-dione (7)

SFC-HPLC: Viridis silica gel column (4.6 × 250 mm, 5 μm, gradient: 5 – 30% MeOH in CO₂ over 6 min, flow rate: 1.0 mL/min), Absorbance: 214 nm.

t_R = 2.57 min

¹H NMR of (S)-2-methyl-4-oxo-5-phenylpentanoic acid (8a)

¹³C NMR of (S)-2-methyl-4-oxo-5-phenylpentanoic acid (8a)

HRMS of (S)-2-methyl-4-oxo-5-phenylpentanoic acid (8a)

SFC-HPLC: Viridis silica gel column (4.6 × 250 mm, 5 µm, gradient: 5 – 40% MeOH in CO₂ over 7 min, flow rate: 1.0 mL/min), Absorbance: 214 nm.
 $t_R = 3.21$ min

¹H NMR of (2S,4R)-4-(14-azaneyl)-2-methyl-5-phenylpentanoic acid (Tup,9)

¹³C NMR of (2S,4R)-4-(14-azaneyl)-2-methyl-5-phenylpentanoic acid (Tup,9)

HRMS of (2S,4R)-4-(14-azaneyl)-2-methyl-5-phenylpentanoic acid (Tup,9)

¹H NMR of benzyl (*R*)-2-(((2*S*,3*R*)-1-(((5*R*,7*R*,10*R*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-10-(methoxycarbonyl)-4-methyl-3,8-dioxo-13,13,13-triphenyl-2-oxa-12-thia-4,9-diazatridecan-7-yl)oxy)-3-methyl-1-oxopentane-2-yl)carbamoyl)piperidine-1-carboxylate (5a)

¹³C NMR of benzyl (*R*)-2-(((2*S*,3*R*)-1-(((5*R*,7*R*,10*R*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-10-(methoxycarbonyl)-4-methyl-3,8-dioxo-13,13,13-triphenyl-2-oxa-12-thia-4,9-diazatridecan-7-yl)oxy)-3-methyl-1-oxopentane-2-yl)carbamoyl)piperidine-1-carboxylate (5a)

HRMS of benzyl (*R*)-2-(((2*S*,3*R*)-1-(((5*R*,7*R*,10*R*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-10-(methoxycarbonyl)-4-methyl-3,8-dioxo-13,13,13-triphenyl-2-oxa-12-thia-4,9-diazatridecan-7-yl)oxy)-3-methyl-1-oxopentane-2-yl)carbamoyl)piperidine-1-carboxylate (5a)

RP-HPLC of benzyl (*R*)-2-(((2*S*,3*R*)-1-(((5*R*,7*R*,10*R*)-1-(9*H*-fluoren-9-yl)-5-isopropyl-10-(methoxycarbonyl)-4-methyl-3,8-dioxo-13,13,13-triphenyl-2-oxa-12-thia-4,9-diazatridecan-7-yl)oxy)-3-methyl-1-oxopentane-2-yl)carbamoyl)piperidine-1-carboxylate (5a)

Analytical RP-HPLC: (Agilent Zorbax SB C₁₈ column: 2.1 x 150 mm, 5 μm, gradient: 50 - 90% CH₃CN/H₂O + 0.1%TFA over 35 min, flow rate: 0.8 mL/min), Absorbance: 254 nm, *t_R* = 25.7 min

¹H NMR of methyl 2-((5*R*,7*R*,10*S*)-12-((*R*)-1-((benzyloxy)carbonyl)piperidin-2-yl)-10-((*R*)-sec-butyl)-1-(9*H*-fluoren-9-yl)-5-isopropyl-4-methyl-3,9,12-trioxo-2,8-dioxo-4,11-diazadodecan-7-yl)thiazole-4-carboxylate (10)

¹³C NMR of methyl 2-((5*R*,7*R*,10*S*)-12-((*R*)-1-((benzyloxy)carbonyl)piperidin-2-yl)-10-((*R*)-sec-butyl)-1-(9H-fluoren-9-yl)-5-isopropyl-4-methyl-3,9,12-trioxo-2,8-dioxo-4,11-diazadodecan-7-yl)thiazole-4-carboxylate (10)

HRMS of methyl 2-((5*R*,7*R*,10*S*)-12-((*R*)-1-((benzyloxy)carbonyl)piperidin-2-yl)-10-((*R*)-sec-butyl)-1-(9*H*-fluoren-9-yl)-5-isopropyl-4-methyl-3,9,12-trioxo-2,8-dioxo-4,11-diazadodecan-7-yl)thiazole-4-carboxylate (10)

RP-HPLC of methyl 2-((5*R*,7*R*,10*S*)-12-((*R*)-1-((benzyloxy)carbonyl)piperidin-2-yl)-10-((*R*)-sec-butyl)-1-(9*H*-fluoren-9-yl)-5-isopropyl-4-methyl-3,9,12-trioxo-2,8-dioxo-4,11-diazadodecan-7-yl)thiazole-4-carboxylate (10)

Analytical RP-HPLC: (Agilent Zorbax SB C₁₈ column: 2.1 x 150 mm, 5 μm, gradient: 50 - 90% CH₃CN/H₂O + 0.1%TFA over 35 min, flow rate: 0.8 mL/min), **Absorbance:** 254 nm, *t_R* = 23.2 min

¹H NMR of methyl 2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylate (11)

^{13}C NMR of methyl 2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylate (11)

HRMS of methyl 2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylate (11)

RP-HPLC of methyl 2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylate (11)

Analytical RP-HPLC: (Agilent Zorbax SB C₁₈ column: 2.1 x 150 mm, 5 μ m, gradient: 50 – 70 % CH₃CN/H₂O + 0.1%TFA over 35 min, flow rate: 0.8 mL/min), **Absorbance:** 214 nm,
 $t_R = 20.1$ min

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^{13}C NMR of 2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylic acid (12)

HRMS of 2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylic acid (12)

RP-HPLC of 2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxylic acid (12)

Analytical RP-HPLC: (Agilent Zorbax SB C₁₈ column: 2.1 x 150 mm, 5 μm, gradient: 50 - 70% CH₃CN/H₂O + 0.1%TFA over 35 min, flow rate: 0.8 mL/min), **Absorbance:** 214 nm,
t_R = 18.0 min

¹H NMR of (2S,4R)-4-(2-(((1R,3R)-3-(((2S,3R)-2-((R)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-N,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (13)

^{13}C NMR of (2*S*,4*R*)-4-(2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (13)

HRMS of (2*S*,4*R*)-4-(2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (13)

RP-HPLC of (2*S*,4*R*)-4-(2-((1*R*,3*R*)-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-1-hydroxy-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (13)

Analytical RP-HPLC: (Agilent Zorbax SB C₁₈ column: 2.1 x 150 mm, 5 μm, gradient: 30 – 60 % CH₃CN/H₂O + 0.1%TFA over 35 min, flow rate: 0.8 mL/min), Absorbance: 214 nm,
t_R = 20.0 min

¹H NMR of (2*S*,4*R*)-4-(2-((1*R*,3*R*)-1-acetoxy-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (14)

¹³C NMR of (2*S*,4*R*)-4-(2-((1*R*,3*R*)-1-acetoxy-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (14)

HRMS of **(2*S*,4*R*)-4-(2-((1*R*,3*R*)-1-acetoxy-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (14)**

RP-HPLC of **(2*S*,4*R*)-4-(2-((1*R*,3*R*)-1-acetoxy-3-((2*S*,3*R*)-2-((*R*)-1-((benzyloxy)carbonyl)piperidine-2-carboxamido)-*N*,3-dimethylpentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (14)**

Analytical RP-HPLC: (Agilent Zorbax SB C₁₈ column: 2.1 x 150 mm, 5 μm , gradient: 20 – 90 % CH₃CN/H₂O + 0.1%TFA over 35 min, flow rate: 0.8 mL/min), **Absorbance:** 214 nm,
 $t_R = 22.8 \text{ min}$

¹H NMR of (2S,4R)-4-(2-((1R,3R)-1-acetoxy-3-((2S,3R)-N,3-dimethyl-2-((R)-1-methylpiperidine-2-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (1)

¹³C NMR of (2S,4R)-4-(2-((1R,3R)-1-acetoxy-3-((2S,3R)-N,3-dimethyl-2-((R)-1-methylpiperidine-2-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (1)

HRMS of (2S,4R)-4-(2-((1R,3R)-1-acetoxy-3-((2S,3R)-N,3-dimethyl-2-((R)-1-methylpiperidine-2-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (1)

HPLC trace of (2S,4R)-4-(2-((1R,3R)-1-acetoxy-3-((2S,3R)-N,3-dimethyl-2-((R)-1-methylpiperidine-2-carboxamido)pentanamido)-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoic acid (1)

Analytical RP-HPLC: (Agilent Zorbax SB C₁₈ column: 2.1 x 150 mm, 5 μm, gradient: 20 - 80% CH₃CN/H₂O + 0.1%TFA over 35 min, flow rate: 0.8 mL/min), Absorbance: 214 nm, t_R = 14.8 min

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