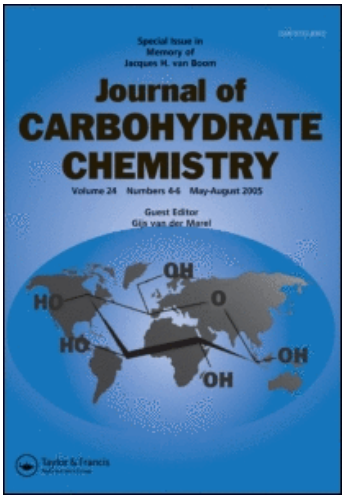

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Transformation of Glucose into a Novel Carbasugar Amino Acid Dipeptide Isostere

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The synthesis of a novel carbasugar amino acid (**15**), starting from D-glucose and using the Ferrier rearrangement as a key step, is reported. Compound **15** is implemented as dipeptide isostere in the synthesis of a Leu-enkephalin analog.

Keywords: Sugar amino acid, Peptidomimetic, Carbasugar, Leu-enkephalin, Mukaiyama-Michael addition

INTRODUCTION

Sugar amino acids (SAAs) are defined as carbohydrate-based compounds that feature an amine and a carboxylate.[1] As such, SAAs are widely spread in nature, and neuraminic acid and *N*-acetyl muramic acid constitute important structural elements in many oligo(poly)saccharides and glycoconjugates. Interestingly, SAAs in nature are almost exclusively linked through interglycosidic bonds, not through amide bonds. The full potential of SAAs[2] as carbohydrate-peptide hybrids was recognized first by Kessler and coworkers,[3] who disclosed an efficient synthesis of D-glucose-derived SAAs and their incorporation in a series of linear and cyclic oligopeptide structures. Inspired by this work, many researchers have become actively involved in SAA-related research. SAA homooligomers have been generated with the aim to attain oligosaccharide mimics that have the interglycosidic linkages replaced by amide bonds.[4] Cyclic SAA homooligomers have been prepared[5,6] with the ultimate goal to

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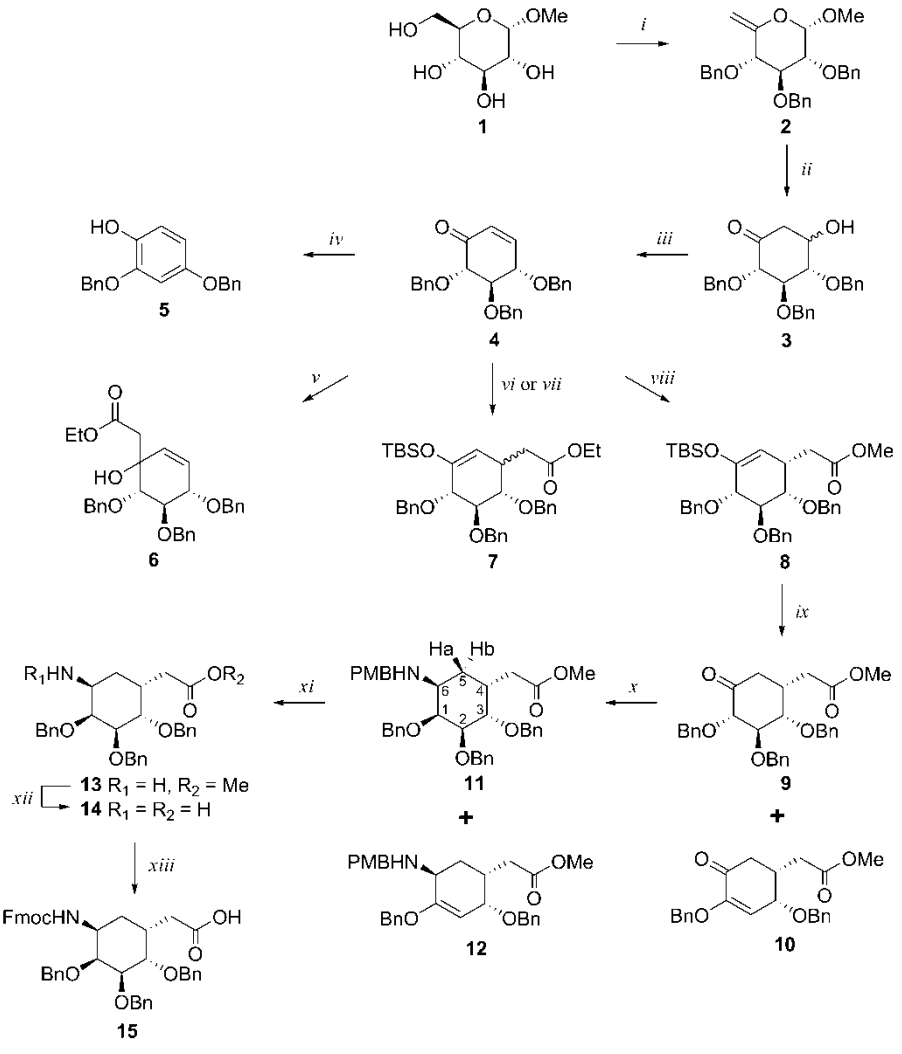
develop cyclodextrin analogous receptor molecules.[7] The relevance of the hybrid nature of SAAs comes to the fore in the multitude of reported applications in which selected amino acid residues are replaced in biologically relevant oligopeptides. Here the aim is twofold: The nature of the parent carbohydrate (the furan or pyran ring) in combination with the positioning of the amine and carboxylate may impart a desired secondary structure on the target oligopeptide, whereas the residual functionalities on the furan/pyran core may be used to introduce additional desirable properties to the peptide.[8]

In conjunction with the growing interest in the application of SAAs, recent years have witnessed numerous reports describing synthetic strategies toward new SAAs.[9] Next to aiming for control over the relative positioning of the amine- and carboxylate functionalities (as in α, β, γ, δ, and ϵ SAAs), research objectives in these studies include the development of constrained SAAs, for instance, through annulation of a second ring system,[10] but also the preparation of linear SAA derivatives.[11]

Given the extensive research efforts involving SAAs, reported examples of SAA building blocks in which the ring oxygen is replaced by carbon[12] are scarce. This is surprising, especially when considering the numerous strategies available for transforming a carbohydrate into carbasugars.[13] We here describe the application of the Ferrier rearrangement[14] of a carbohydrate-derived enopyranoside and its transformation into carbasugar amino acid **15** (CSAA). The applicability of dipeptide isostere CSAA **15** in standard solid phase peptide synthesis is demonstrated by the synthesis of Leu-enkephalin derivative **21**.

RESULTS AND DISCUSSION

Our synthetic approach commenced with the five-step conversion of commercially available methyl α-D-glucopyranoside **1** into the enopyranoside **2** in 61% yield following a literature procedure (Sch. 1).[15] Submitting compound **2** to Ferrier rearrangement conditions (mercury(II)chloride in aqueous media at elevated temperature) cleanly provided the known functionalized cyclohexanone **3** in 80% yield. Ketone **3** was converted into the intermediate enone **4**. At this stage, it was envisioned that the required carboxymethyl substituent could be introduced via a Michael addition. In a first attempt, treatment of compound **4** with the diethylmalonate anion, generated under the agency of sodium hydride or sodium methoxide, did not result in the expected 1,4-addition. Instead, elimination of benzyl alcohol followed by tautomerization led to the quantitative formation of phenol derivative **5**. Because of the apparent instability of enone **4** toward basic conditions, attention was focused on the Lewis acid-mediated Mukaiyama-Michael addition. Treatment of compound **4** with 1-(*tert*-butyldimethylsilyloxy)-1-ethoxyethene[16] and titanium(IV)chloride led to 1,2-addition giving allylic alcohol **6** as a single diastereoisomer in 78%



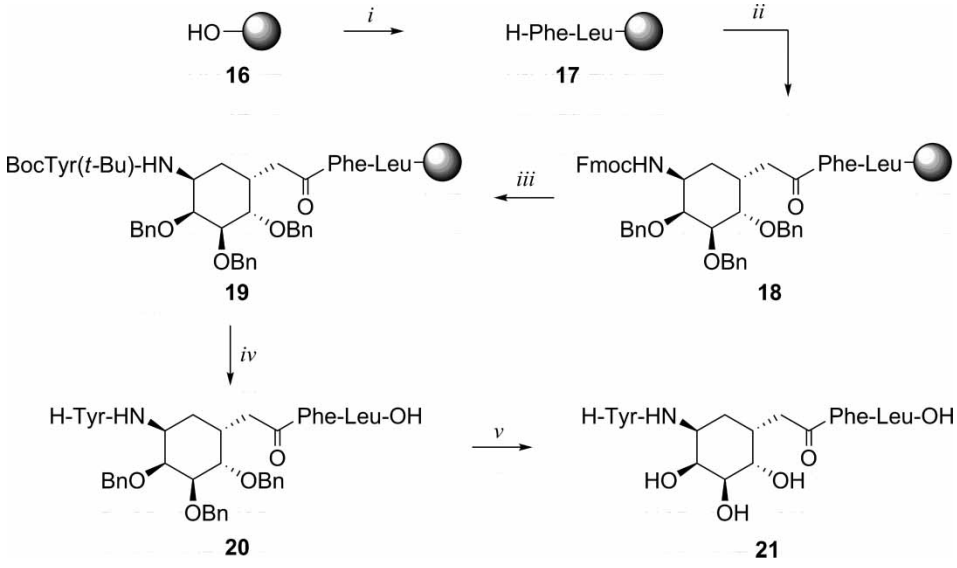
Scheme 1: *Conditions*: *i)* reference 15 *ii)* $HgCl_2$ (1.1 equiv.), acetone/H_2O (2:1), reflux, 2 h, 80%. *iii)* MsCl (2.7 equiv.), DMAP (cat.), pyr., rt, 2 h, 87%. *iv)* diethylmalonate (1.5 equiv.), NaH or NaOEt (1.5 equiv.), THF, rt, 45 min, 99%. *v)* TBSOC(OEt)=CH_2 (1.5 equiv.), $TiCl_4$ (1.5 equiv.), DCM, −78°C, 30 min, 78%. *vi)* TBSOC(OEt)=CH_2 (1.5 equiv.), $SnCl_4$ (1.5 equiv.), DCM, −78°C, 20 min, 55%. *vii)* TBSOC(OEt)=CH_2 (1.5 equiv.), $LiClO_4$ (5.0 equiv.), Et_2O, rt, 48 h, 73%. *viii)* TBSOC(OMe)=CH_2 (3.0 equiv.), $LiClO_4$ (10.0 equiv.), Et_2O, rt, 30 min, 98%. *ix)* HF·pyr., THF/pyr. (4:1), rt, 30 min, 99%. *x)* *p*-MeOBnNH_2 (2.0 equiv.), Na(OAc)$_3$BH (1.5 equiv.), HOAc (1.0 equiv.), 1,2-DCE, rt, 1 h, **11**: 51%, **12**: 15% (2 steps). *xi)* CAN (2.5 equiv.), H_2O/MeCN (1:2), rt, 24 h, 56%. *xii)* LiOH, H_2O/dioxane (1:4), rt, 3 h, quant. *xiii)* FmocOSu, sat. aq. $NaHCO_3$/dioxane (4:1), 17 h, 92%.

yield. Despite the good yield and selectivity, this approach was rendered ineffective as the tertiary allylic hydroxyl functionality proved to be inert toward further transformations. Upon application of tin(IV)chloride as Lewis acid in dichloromethane, 1,4-addition was achieved resulting in the formation of

ester **7** in 55% as an inseparable mixture of isomers. The yield of this reaction could be further optimized by switching to lithium perchlorate in Et_2O,[17] affording after 48 h reaction time ester **7** in 73% yield, again as a mixture of diastereoisomers (4:1). By changing the alkylating species to (*tert*-butyldimethylsilyloxy)-1-methoxyethene and using a larger excess of reagents, a spectacular change in the outcome of the reaction was observed. Using this reagent, enone **4** was consumed in only 30 min, resulting in Michael adduct **8** as a single diastereoisomer in 98% yield. The newly created stereocenter was unambiguously assigned using NMR spectroscopy.

In continuation of our synthetic studies, silyl enol ether **8** was transformed into ketone **9** amenable for ensuing reductive amination. Initially, the conversion of compound **9** from **8** was hampered by the formation of the elimination product **10** in yields varying from 70% to 80%, under several desilylating conditions. Fortunately, it was found that elimination could be suppressed completely by switching to HF · pyridine as the desilylating agent. Under these conditions, ketone **9** was isolated as the sole product and in quantitative yield. Importantly, the reaction with HF · pyridine was complete within 30 min. Prolonged reaction times led to the formation of enone **10** through elimination of benzyl alcohol. It was found that this transformation also occurs, over time, upon storage of **9**, dictating the necessity to proceed with the next step directly. Thus, after hydrolysis and workup, immediate reductive amination employing 4-methoxybenzylamine, acetic acid, and sodium triacetoxyborohydride afforded CSAA **11** in 51% over two steps. Rather surprisingly, complete epimerization at C1 occurred under the conditions applied. The absolute configuration of **11** was firmly established by NOE difference experiments, with key NOEs between H5b-H1 and H5a-H3. In addition, the *trans*-disposition of the newly introduced amine- and carboxymethyl substituent was assigned. The epimerization of the stereocenter at C1 is most likely the result of tautomerization of the intermediate imine, resulting in the formation of amine **11** as the more stable isomer. In a competing process, benzyl alcohol proved to be prone to elimination, leading to the formation of enol ether **12** as major side product, in a yield of 15% over the last two steps. Removal of the *N*-methoxybenzyl group of **11**, using ceric ammonium nitrate (CAN), liberated the free amine to give compound **13**. Saponification of the methyl ester under the agency of LiOH furnished amino acid **14** in 56% yield over two steps. Protection of the amine function of **14** was accomplished using Fmoc-OSu in saturated aqueous $NaHCO_3$/dioxane, to give CSAA **15** in a yield of 92%.

CSAA **15** was applied in a standard Fmoc-based solid phase peptide synthesis protocol toward the synthesis of a novel Leu-enkephalin derivative (Sch. 2). Thus, Wang resin **16** was transformed in a standard way to give H-Phe-Leu-Wang resin **17**. Condensation of acid **15** with **17** using HCTU as coupling agent proceeded smoothly to give immobilized tripeptide **18**. After removal of the Fmoc group, further elongation with a Boc-protected tyrosine



Scheme 2: *Conditions*: *i*) a) DIC, DMAP, Fmoc-Leu-OH, CH_2Cl_2; b) 20% piperidine in DMF, 3 × 10 min; c) Fmoc-Phe-OH, HCTU, DiPEA, DMF, 3 × 1 h; d) 20% piperidine in DMF, 3 × 10 min. *ii*) **15** (2.0 equiv.), HATU (0.95 equiv.), DiPEA, DMF (2.5 equiv.), 2 × 10 min. *iii*) a) 20% piperidine in DMF, 3 × 10 min; b) Boc-Tyr(*t*-Bu)-OH, HCTU, DiPEA, DMF, 2 h. *iv*) TFA/TIS/H_2O (95:2.5:2.5), 15 min; 95% *v*) H_2, 10% Pd/C (cat.), HOAc (1.0 equiv.), *t*-BuOH/H_2O (1:1), 17 h, rt, quant.

(**19**) and subsequent acidic cleavage from the resin, along with removal of the acid labile protective groups, gave partially deprotected peptide **20**. Finally, hydrogenolysis of the benzyl ethers followed by reverse-phase HPLC purification afforded Leu-enkephalin analog **21** in 95% overall yield.

In summary, a synthetic route to novel CSAA **15** was developed having a high-yielding and stereoselective lithium perchlorate assisted Mukaiyama-Michael 1,4-addition on enone **4** as a key step. In addition, dipeptide isostere **15** was successfully applied in a solid phase protocol toward Leu-enkephalin analog **21**.

EXPERIMENTAL SECTION

General Methods and Materials

Acetone, 1,2-dichloroethane (1,2 DCE), dichloromethane (DCM), dimethyl formamide (DMF), 1,4-dioxane, ethanol, *n*-hexane, pyridine (pyr.), and toluene (Biosolve) were stored over molecular sieves (4Å). Acetonitrile and methanol (HPLC grade) (Biosolve) were stored over molecular sieves (3Å). Diethyl ether and tetrahydrofuran (THF) (Biosolve) were distilled from $LiAlH_4$ prior to use. Eluents ethyl acetate, petroleum ether (40–60), and toluene (Riedel-de Haën) were of technical grade and distilled prior to use. All other chemicals were used as received. Abbreviations of chemicals used in solid phase peptide

synthesis: Boc: *tert*-butyloxycarbonyl; DIC: diisopropylcarbodiimide; DiPEA: *N,N*-diisopropylethylamine; DMAP: 4-(dimethylamino)pyridine: Fmoc: 9-fluorenylmethyloxycarbonyl; Glu: L-glutamic acid; Gly: glycine; HATU: 2-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HCTU: 2-(6-chloro-1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; Leu: L-leucine; Phe: L-phenylalanine; Su: succinimide; TFA; trifluoroacetic acid; TIS: triisopropylsilane; Tyr: L-tyrosine. All reactions were performed under an inert atmosphere and at ambient temperature unless stated otherwise. Prior to reactions that require anhydrous conditions, traces of water from starting material and reagents were removed by coevaporation with toluene or 1,2-dichloroethane. All solvents were removed by evaporation under reduced pressure. Reactions were monitored by TLC analysis using DC-fertigfolien (Schleicher & Schuell, F1500, LS254) or HPTLC aluminum sheets (Merck, silica gel 60, F254). Compounds were visualized by UV absorbtion (254 nm) where applicable and by spraying with 20% H_2SO_4 in ethanol followed by charring at $\sim$150°C or by spraying with a solution of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4 \cdot 2H_2O$ (10 g/L) in 10% sulfuric acid followed by charring at ˜150°C. Olefins were visualized by spraying with a solution of $KMnO_4$ (2%) and K_2CO_3 (1%) in water. Column chromatography was performed on silica gel (Merck, 40–60 μm). Optical rotations ($[\alpha]_D^{20}$) were measured on a Propol automatic polarimeter (sodium D line, $\lambda = 589$ nm). ^1H and ^{13}C APT NMR spectra were recorded on a Jeol JNM-FX-200 (200/50.1 MHz), a Bruker 300 WM-300 (330/75 MHz), a Bruker AV 400 (400/100 MHz), or a Bruker DMX-600 (600/150 MHz) spectrometer. Chemical shifts (δ) are given in ppm relative to tetramethylsilane as internal standard. Coupling constants (J) are given Hz. Where indicated, NMR-peak assignments were made using COSY and NOESY experiments. Infrared spectra were recorded on a Shimadzu FTIR-8300 and data are reported in cm^{-1}. Mass spectra were recorded on a PE/Sciex API 165 instrument with an ion spray interface. High-resolution mass spectra and MS/MS were recorded on a Finnigan LTQ-FT (Thermo electron). LC-MS analysis was conducted on a Jasco system (detection simultaneously at 214 nm and 254 nm) equipped with an Alltima C-18 analytical column (Alltech, 4.6 mm × 150 mm, 5-μm particle size). Preparative HPLC was performed on a BioCad Vision (Applied Biosystems, Inc.) using a Alltima C-18 column (Alltech, 10.0 mm × 250 mm, 5 μm particle size).

(2*S*,3*R*,4*S*,5*R*/*S*)-2,3,4-Tris-benzyloxy-5-hydroxycyclohexanone (3)

Compound **2** (5.96 g, 13.36 mmol) was dissolved in a mixture of acetone/water (2:1). $HgCl_2$ (3.99 g, 14.69 mmol, 1.1 equiv.) was added and the mixture was heated under reflux. After 2 h the mixture was cooled to rt and

concentrated. The resulting white solid was dissolved in DCM, washed with water (2×) and brine (2×). The organic layer was separated, dried ($MgSO_4$), filtered, and concentrated. Purification by silica gel column chromatography(EtOAc/PE 1:3 to 2:3) gave **3** as a mixture of hydroxyketones in a combined overall yield (4.64 g, 10.73 mmol) of 80%. Major isomer **3*S***: ^{13}C NMR (50 MHz, $CDCl_3$): δ 204.0 (C-1), 138.3, 137.6 (3 × C_q Bn), 128.6, 128.4, 128.2, 127.9, 127.8, 127.6, 127.5, 127.1 (CH_{arom}), 85.2, 81.6, 81.4 (C-2, C-3, C-4), 75.7, 73.3, 72.9 (3 × CH_2 Bn), 66.3 (C-5), 42.6 (C-6). Minor isomer **3*R***: ^{13}C NMR (50 MHz, $CDCl_3$): δ 203.2 (C-1), 138.0, 137.9, 137.4 (3 × C_q Bn), 128.6, 128.4, 128.1, 128.0, 127.9 (CH_{arom}), 85.9, 84.6, 81.9 (C-2, C-3, C-4), 75.6, 75.3, 73.5 (3 × CH_2 Bn), 67.9 (C-5), 44.1 (C-6).

(4*S*,5*R*,6*S*)-4,5,6-Tris-benzyloxycyclohex-2-enone (4)

To a mixture of alcohol **3** (0.432 g, 1.00 mmol) and mesylchloride (0.209 mL, 2.7 mmol, 2.7 equiv.) dissolved in pyridine (10 mL) was added a catalytic amount of DMAP. After 2 h the reaction was complete according to TLC analysis (EtOAc/PE 1:1). Ice was added and the mixture was extracted with Et_2O. The organic layer was separated, washed with brine, dried ($MgSO_4$), and concentrated. After purification by column chromatography (EtOAc/PE 1:3 to 1:1), unsaturated ketone **4** was obtained (0.361 g, 0.87 mmol) in a yield of 87%. ^1H NMR (200 MHz, $CDCl_3$): δ 7.46–7.26 (m, 15H, CH_{arom}), 6.81 (dd, 1H, $J_{3,2} = 10.2$ Hz, $J_{3,4} = 2.2$ Hz, H-3), 6.39 (dd, 1H, $J_{2,3} = 10.2$ Hz, $J_{2,4} = 2.2$ Hz, H-2), 5.11–4.70 (m, 6H, 3 × CH_2 Bn), 4.35 (dt, 1H, $J_{4,2} = J_{4,3} = 2.2$ Hz, $J_{4,5} = 7.3$ Hz, H-4), 4.04 (d, 1H, $J_{6,5} = 10.2$ Hz, H-6), 3.97 (dd, 1H, $J_{5,4} = 7.3$ HZ, $J_{5,6} = 10.2$ Hz, H-5). ^{13}C NMR (50 MHz, $CDCl_3$): δ 197.3 (C-1), 148.0 (C-3), 138.1, 137.7, 137.5 (3 × C_q Bn), 128.5 (C-2), 128.3–127.7 (CH_{arom}), 84.6, 83.7, 78.9 (C-4, C-5, C-6), 75.6, 74.4, 73.5 (3 × CH_2).

2,4-Bis(benzyloxy)phenol (5)

To a mixture of NaH (6 mg, 0.15 mmol 60% dispersion in mineral oil, 1.5 equiv.) or NaOEt (0.15 mL 1.0 M, prepared from 103 mg Na in 45.0 mL EtOH, 1.5 equiv.) in freshly distilled THF (2.5 mL) was added diethylmalonate (23 μL, 0.15 mmol, 1.5 equiv.) at 0°C. After 15 min of stirring a solution of unsaturated ketone **4** (42 mg, 0.10 mmol) in THF (0.5 mL) was added dropwise and the mixture was allowed to reach rt. After 45 min TLC analysis (EtOAc/PE 1:4) revealed complete consumption of enone **4** and the reaction was quenched by addition of water and diluted with EtOAc. The aqueous layer was separated and extracted once more with EtOAc. The combined organic layers were washed with water and brine, dried ($MgSO_4$), and concentrated. Purification of the residue by column chromatography (PE to EtOAc/PE 1:19) gave phenol derivative **5** (30 mg, 0.10 mmol) in a quantitative yield. ^1H NMR (200 MHz, $CDCl_3$): δ 7.42–7.31 (m, 10H, CH_{arom} Bn), 6.84 (d, 1H, $J_{6,5} = 8.8$ Hz, H-6),

6.63 (d, 1H, $J_{3,5} = 2.9$ Hz, H-3), 6.48 (dd, 1H, $J_{5,3} = 2.9$ Hz, $J_{5,6} = 8.8$ Hz, H-5), 5.28 (s, 1H, OH), 5.04 (s, 2H, CH_2 Bn), 4.97 (s, 2H, CH_2 Bn). ^{13}C NMR (50 MHz, $CDCl_3$): δ 146.2, 140.2, 140.2, 137.1, 136.1 (5× C_q), 128.6, 128.5, 128.3, 127.8, 127.5, 126.9 (CH Bn), 114.3, 106.1, 101.7 (C-3, C-5, C-6), 71.0, 70.7 (2 × CH_2 Bn). MS (ESI): $m/z = 307.1$ $[M + H]^+$, 329.2 $[M + Na]^+$, 635.4 $[2M + Na]^+$.

(4*S*,5*R*,6*S*)-4,5,6-Tris(benzyloxy)-1-ethoxycarbonylmethylcyclohex-2-enol (6)

α,β-Unsatured ketone **4** (0.124 g, 0.30 mmol) was dissolved in freshly distilled DCM (2.5 mL) under an argon atmosphere. 1-(*tert*-Butyldimethylsilyloxy)-1-ethoxyethene[16] (91.0 mg, 0.45 mmol, 1.5 equiv.) was added and the mixture was cooled to −78°C. After dropwise addition of $TiCl_4$ (49.6 μL, 0.45 mmol, 1.5 equiv.), the mixture turned dark red. After 30 min TLC analysis (EtOAc/PE 1:3) showed complete disappearance of starting material along with the formation of a lower running spot. The reaction was quenched by addition of water (1.0 mL) and warmed to rt, after which the red color disappeared. The mixture was diluted with Et_2O and the separated organic layer was collected, washed with brine, dried ($MgSO_4$), and concentrated. Purification of the residue by silica gel column chromatography (EtOAc/PE 1:19 to 1:9) gave 1,2-adduct **6** (0.117 g, 0.23 mmol, 78%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35–7.24 (m, 15H, CH_{arom}), 5.80 (2× dd, 2H, $J = 1.8$ Hz, $J = 10.2$ Hz, H-2, H-3), 5.09 (d, 1H, $J = 11.2$ Hz, CH Bn), 4.91 (d, 1H, $J = 11.0$ Hz, CH Bn), 4.85 (d, 1H, $J = 11.0$ Hz, CH Bn), 4.69 (s, 2H, CH_2 Bn), 4.69 (d, 1H, $J = 11.2$ Hz, CH Bn), 4.16 (dt, 1H, $J = 1.8$ Hz, $J_{4,5} = 7.8$ Hz, H-4), 4.09 (dd, 1H, $J_{5,4} = 7.8$ Hz, $J_{5,6} = 9.8$ Hz, H-5), 4.01 (q, 2H, $J = 7.1$ Hz, CH_2 Et), 3.66 (d, 1H, $J_{6,5} = 9.8$ Hz, H-6), 3.36 (s, 1H, OH), 2.57 (dd, 2H, $J = 14.3$ Hz, CH_2CO), 1.18 (t, 3H, $J = 7.1$ Hz, CH_3 Et). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5 (C═O), 138.6, 138.4, 138.1 (3 × C_q Bn), 129.8, 129.7 (C-2, C-3), 128.3–127.5 (CH_{arom}), 81.3 (C-5), 80.4 (C-6), 80.1 (C-4), 75.5, 75.2 (2 × CH_2 Bn), 72.2 (C-1), 71.8 (CH_2 Bn), 60.6 (CH_2 Et), 43.4 (*C*H_2CO), 14.1 (CH_3 Et). IR (thin film): 3030, 1728, 1497, 1454, 1367, 1302, 1209, 1177, 1067, 1026, 734, 696 cm^{-1}. MS (ESI): $m/z = 503.4$ $[M + H]^+$, 520.3 $[M + NH_4]^+$, 1005.7 $[2M + H]^+$, 1022.7 $[2M + NH_4]^+$.

(3*R*/*S*,4*S*,5*R*,6*S*)-4,5,6-Tris-benzyloxy-1-(*tert*-butyldimethylsilyloxy)-3- ethoxycarbonylmethylcyclohexene (7)

$SnCl_4$ mediated Mukaiyama-Michael addition: To a solution of unsaturated ketone **4** (57 mg, 0.14 mmol) in freshly distilled DCM (1.0 mL) was added a solution of silylketen acetal (50 mg, 0.25 mmol, 1.8 equiv.) in DCM (1.0 mL) under an argon atmosphere. The mixture was cooled to −78°C and two drops of $SnCl_4$ were added. After 20 min TLC analysis (EtOAc/PE 1:4) revealed complete consumption of starting material, water (0.5 mL) was

added, and the mixture was warmed to rt. The mixture was diluted with Et_2O and the organic layer was separated. After extraction of the aqueous layer with Et_2O, all organic layers were combined, dried ($MgSO_4$), and concentrated. Purification of the residue by column chromatography (EtOAc/PE 1:19) gave ester **7** as a colorless oil as a mixture of diastereoisomers (47 mg, 0.076 mmol, 55%).

$LiClO_4$ mediated Mukaiyama-Michael addition: To a solution of unsaturated ketone **4** (83 mg, 0.20 mmol) in freshly distilled Et_2O (2.0 mL) was added a solution of silylketene acetal (61 mg, 0.30 mmol, 1.5 equiv.) in Et_2O (0.3 mL) at rt. Next, a 1.0 M solution of $LiClO_4$ in Et_2O (1.0 mL, 0.106 g, 5.0 equiv.) was added. After 48 h, TLC analysis (EtOAc/PE 1:4) showed all starting material was converted into a higher running spot. The reaction was quenched by addition of sat. aq. $NaHCO_3$ and the organic layer was separated, washed with brine, dried ($MgSO_4$), and concentrated. Purification of the residue as described above resulted in 1,4-addition product **7** (90 mg, 0.15 mmol, 73%) as an inseparable mixture of diastereoisomers. ^{1}H NMR did not provide useful data. ^{13}C NMR (200 MHz, $CDCl_3$): δ 173.0, 172.2 (C=O), 149.6, 149.2 (C-1), 128.2, 127.9, 127.8, 127.6, 127.4 (CH_{arom}), 106.3, 106.1 (C-2), 84.7, 82.0, 81.2, 81.0, 80.0, 78.2 (C-4, C-5, C-6), 75.0, 74.8, 74.6, 74.0, 73.7, 72.1 (CH_2 Bn), 60.3 (CH_2 Et), 37.4, 36.1 (CH_2C=O), 37.0, 33.0 (C-1), 25.8 (C-3), 18.2, 16.2 (C_q TBS), 14.2 (CH_3 Et). MS (ESI): $m/z = 617.5\ [M + H]^+, 639.4\ [M + Na]^+$.

(3*S*,4*S*,5*R*,6*S*)-4,5,6-Tris-benzyloxy-1-(*tert*-butyldimethylsilyloxy)-3- methoxycarbonylmethylcyclohexene (8)

To a 1.0 M solution of $LiClO_4$ in Et_2O (110 mL) was added compound **4** (4.575 g, 11.04 mmol) at rt. This mixture was stirred until the ketone dissolved completely, followed by the addition of (*tert*-butyldimethylsilyloxy)-1-methoxyethene (7.23 mL, 33.11 mmol, 3.0 equiv.). Stirring was continued for 30 min after which TLC analysis (EtOAc/toluene 1:19) indicated complete disappearance of starting material together with the formation of a higher running spot. After quenching the reaction, by addition of sat. aq. $NaHCO_3$, the organic layer was separated and the aqueous layer was extracted once more with Et_2O. All ether layers were combined, dried ($MgSO_4$), filtered, and concentrated. Purification of the residue by silica gel column chromatography (EtOAc/toluene 1:49) gave methylester **8** (6.53 g, 10.83 mmol, 98%) as a single stereoisomer. $[\alpha]_D^{20}$ −18.9 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42–7.21 (m, 15H, CH_{arom}), 4.94 (d, 1H, $J_{2,3} = 5.8$ Hz, H-2), 4.85 (d, 1H, $J = 11.0$ Hz, CH Bn), 4.82 (d, 1H, $J = 11.0$ Hz, CH Bn), 4.71 (d, 1H, $J = 11.0$ Hz, CH Bn), 4.70 (d, 1H, $J = 11.0$ Hz, CH Bn), 4.63 (s, 2H, CH_2 Bn), 4.00 (d, 1H, $J_{6,5} = 6.2$ Hz, H-6), 3.83 (dd, 1H, $J_{5,4} = 9.8$ Hz, $J_{5,6} = 6.2$ Hz, H-5), 3.72 (dd, 1H, $J_{4,3} = 5.6$ Hz, $J_{4,5} = 9.8$ Hz, H-4), 3.59 (s, 3H, CH_3 OMe), 3.10 (ddt, 1H, $J_{3,CHH} = J_{3,4} = 5.6$ Hz, $J_{3,CHH} = 8.9$ Hz, $J_{3,2} = 5.8$ Hz, H-3), 2.79 (dd, 1H,

$J_{CHH,CHH} = 15.8$ Hz, $J_{CHH,3} = 5.6$ Hz, C*H*H), 2.26 (dd, 1H, $J_{CHH,CHH} = 15.8$ Hz, $J_{CHH,3} = 8.9$ Hz, CH*H*), 0.92 (s, 9H, 3× CH_3 *t*-Bu), 0.16 (s, 3H, CH_3 TBS), 0.15 (s, 3H, CH_3 TBS). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.6 (C═O), 149.3 (C-1), 138.4, 138.1 (3 × C_q Bn), 128.6, 128.2, 128.1, 127.8, 127.7, 127.4, 127.3, 127.2, 127.0 (CH_{arom}), 106.0 (C-2), 80.6 (C-6), 79.4 (C-5), 77.8 (C-4), 74.0, 73.5, 71.1 (3× CH_2 Bn), 50.8 (CH_3 OMe), 35.5 (*C*H_2CO), 32.7 (C-3), 25.4 (CH_3 *t*-BuSi), 17.8 (C_q *t*-BuSi), −4.7, −4.9 (2 × CH_3 SiMe). IR (thin film): 3032, 2928, 2858, 2359, 1734, 1661, 1454, 1205, 1094, 839, 696 cm^{-1}. MS (ESI): $m/z = 603.4$ $[M + H]^+$, 625.4 $[M + Na]^+$. HRMS (ESI): calcd for $[C_{36}H_{46}O_6Si + NH_4]^+$ 620.3407. Found 620.3439.

(2*S*,3*R*,4*S*,5*R*)-2,3,4-Tris-benzyloxy-5-methoxycarbonylmethylcyclohexanone (9)

To a solution of enol ether **8** (0.301 g, 0.50 mmol) dissolved in THF (2.0 mL) and pyridine (0.5 mL) was added HF·pyridine (70/30 v/v, 0.25 mL). After 30 min water and Et_2O were added to the reaction mixture. The organic phase was separated and the aqueous phase was extracted once more with Et_2O. All organic layers were combined, dried ($MgSO_4$), filtered, and concentrated. Purification of the residue by column chromatography (EtOAc/PE 1:9 to 1:4) gave ketone **9** (0.242 g, 0.495 mmol) in a yield of 99%. $[\alpha]_D^{20}$ −60.6 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39–7.7.22 (m, 15H, CH_{arom}), 4.86 (d, 1H, $J = 11.8$ Hz, CH Bn), 4.75 (d, 1H, $J = 11.1$ Hz, CH Bn), 4.71 (d, 1H, $J = 11.1$ Hz, CH Bn), 4.60 (d, 1H, $J = 11.4$ Hz, CH Bn), 4.50 (d, 1H, $J = 11.8$ Hz, CH Bn), 4.49 (d, 1H, $J = 11.4$ Hz, CH Bn), 4.09 (d, 1H, $J = 6.9$ Hz, H-2), 3.80–3.75 (m, 2H, H-3, H-4), 3.59 (s, 3H, CH_3 OMe), 2.95–2.87 (m, 1H, H-5), 2.55 (dd, 1H, $J = 5.5$ Hz, $J = 16.2$ Hz, C*H*H), 2.46 (dd, 1H, $J = 6.0$ Hz, $J = 15.4$ Hz, H-6a), 2.40 (dd, 1H, $J = 6.7$ Hz, $J = 15.4$ Hz, H-6b), 2.15 (dd, 1H, $J = 8.9$ Hz, $J = 16.2$ Hz, CH*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 205.7 (C-1), 172.3 (C═O CO_2Me), 137.9, 137.6, 137.5 (3 × C_q Bn), 128.2–127.6 (CH_{arom}), 85.0, 81.6, 79.0 (C-2, C-3, C-4), 74.1, 73.0, 71.8 (3 × CH_2 Bn), 51.6 (CH_3 OMe), 40.2 (C-6), 33.7 (*C*H_2CO), 32.0 (C-5). IR (thin film): 1730, 1497, 1454, 1437, 1352, 1205, 1092, 1074, 1051, 1026, 908, 731, 696, 611 cm^{-1}. MS (ESI): $m/z = 489.2$ $[M + H]^+$, 511.5 $[M + Na]^+$. HRMS (ESI): calcd for $[C_{30}H_{32}O_6 + NH4]^+$ 506.2543. Found 506.2543.

(4*S*,5*R*)-2,4-Bis-benzyloxy-5-methoxycarbonylmethylcyclohex-2-enone (10)

Ketone **9** degraded into unsaturated ketone **10** upon storage at room temperature: ^{1}HNMR (200 MHz, $CDCl_3$): δ 7.38–7.22 (m, 15H, CH_{arom}), 5.86 (d, 1H, $J_{3,4} = 5.1$ Hz, H-3), 4.89 (d, 1H, $J = 13.2$ Hz, CH Bn), 4.82 (d, 1H, $J = 13.2$ Hz, CH Bn), 4.48 (d, 1H, $J = 11.7$ Hz, CH Bn), 4.41 (d, 1H, $J = 11.7$ Hz, CH Bn), 4.24

(dd, 1H, $J_{4,5} = 2.9$ Hz, $J_{4,3} = 5.1$ Hz, H-4), 3.65 (s, 3H, CH_3 OMe), 2.81-2.29 (m, 5H, H-5, H-6a, H-6b, C*H*H, CH*H*). ^{13}C NMR (50 MHz, $CDCl_3$): δ 192.4 (C-1), 172.1 (C=O CO_2Me), 150.3 (C-2), 137.6, 135.3 (2 × C_q Bn), 128.2, 127.9, 127.8, 127.6, 127.3, 126.8, 126.4 (CH_{arom}), 115.1 (C-3), 71.5 (C-4), 70.3, 69.1 (2 × CH_2 Bn), 51.2 (CH_3 OMe), 39.3 (C-6), 35.2 (C-5), 34.3 (*C*H_2CO_2Me). IR (thin film): 2363, 2343, 1734, 1697, 1624, 1497, 1456, 1261, 1204, 1140, 1067, 1028, 999, 739, 698, 623 cm^{-1}. MS (ESI): $m/z = 403.1$ $[M + Na]^+$.

(1*S*,2*R*,3*S*,4*R*,6*S*)-1,2,3-Tris-benzyloxy-6-(*para*-methoxybenzylamino)-4- methoxycarbonyl-methylcyclohexane (11)

Enol ether **8** (0.602 g, 1.00 mmol) was cleaved using HF · pyridine, following the procedure described above for the preparation of **9**. After workup, without further purification, intermediate **9** was dissolved in 1,2-DCE (10 mL), followed by addition of $Na(OAc)_3BH$ (0.318 g, 1.50 mmol, 1.5 equiv.). Next, *p*-MeOBnNH_2 (0.261 mL, 2.0 mmol, 2.0 equiv.) and acetic acid (57.7 μL, 1.0 mmol, 1.0 equiv.) were added. The reaction was stirred for 1 h at rt, after which TLC analysis (EtOAc/PE 3:7) indicated the complete disappearance of starting material along with the formation of three lower running spots. The reaction was quenched by the addition of sat. aq. $NaHCO_3$ and diluted with DCM. The organic phase was collected, dried ($MgSO_4$), concentrated, and purified by column chromatography (MeOH/DCM 1:99) resulting in protected CSAA **11** (0.311 g, 0.51 mmol) in a yield of 51% over two steps. $[\alpha]_D^{20}$ +17.0 (*c* 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, MeOD, T = 333K): δ 7.36–7.23 (m, 15H, CH_{arom} Bn), 7.20–7.15 (m, 2H, CH_{arom} PMB), 6.84–6.80 (m, 2H, CH_{arom} PMB), 4.68 (s, 2H, CH_2 Bn), 4.66 (d, 1H, $J = 11.9$ Hz, CH Bn), 4.55 (d, 1H, $J = 11.9$ Hz, CH Bn), 4.47 (s, 2H, CH_2 Bn), 3.89–3.86 (m, 1H, H-1), 3.85–3.82 (m, 1H, H-3), 3.78–3.75 (m, 1H, H-2), 3.75 (s, 3H, CH_3 OMe PMB), 3.61 (s, 3H, CH_3 OMe), 3.02–2.96 (m, 1H, H-6), 2.69–2.60 (m, 1H, H-4), 2.53 (dd, 1H, $J = 7.2$ Hz, $J_{CHH,CHH} = 15.4$ Hz, *CH*H), 2.20 (dd, 1H, $J = 7.4$ Hz, $J_{CHH,CH}$-H = CH*H*), 1.72 (ddd, 1H, $J = 4.1$ Hz, $J = 6.8$ Hz, $J_{5a,5b} = 13.5$ Hz, H-5a), 1.58 (ddd, 1H, $J = 3.8$ Hz, $J = 9.0$ Hz, $J_{5b,5a} = 13.5$ Hz, H-5b). IR (thin film): 2872, 1732, 1611, 1510, 1454, 1246, 1099, 737, 698 cm^{-1}. MS (ESI): $m/z = 610.4$ $[M + H]^+$, 1220.0 $[2M + H]^+$. HRMS (ESI): calcd for $[C_{38}H_{43}NO_6 + H]^+$ 610.3169. Found 610.3213.

(3*S*,4*R*,6*S*)-1,3-Bis-benzyloxy-6-(para-methoxybenzyl-amino)-4- methoxycarbonylmethylcyclohex-1-ene (12)

Enamine **12** (73.0 mg, 0.15 mmol, 15% over two steps) was formed as a minor product in the procedure described above, going from compound **8** to amine **11**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37–7.22 (m, 10H, CH_{arom} Bn),

7.20–7.16 (m, 2H, CH_{arom} PMB), 6.84–6.78 (m, 2H, CH_{arom} PMB), 5.09 (d, 1H, $J_{2,3} = 5.4$ Hz, H-2), 4.75 (s, 2H, CH_2 Bn), 4.61 (d, 1H, $J = 11.8$ Hz, CH Bn), 4.40 (d, 1H, $J = 11.8$ Hz, CH Bn), 3.97 (dd, 1H, $J_{3,4} = 3.3$ Hz, $J_{3,2} = 5.4$ Hz, H-3), 3.77–3.67 (m, 2H, CH_2 PMB), 3.76 (s, 3H, CH_3 OMe PMB), 3.64 (s, 3H, CH_3 OMe), 3.47 (dd, 1H, $J = 6.4$ Hz, $J = 10.1$ Hz, H-6), 2.65 (dd, 1H, $J = 7.4$ Hz, $J_{CHH,CHH} = 16.0$ Hz, *CH*H), 2.49 (dd, 1H, $J = 7.0$ Hz, $J_{CHH,CHH} = 16.0$ Hz, CH*H*), 2.24–2.15 (m, 1H, H-4), 2.00–1.88 (m, 1H, H-5a), 1.86–1.79 (m, 1H, H-5b). ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.5 (C=O), 158.8 (C_q OMe PMB), 158.4 (C-1), 139.1, 136.6 (2 × C_q Bn), 132.3 (C_q PMB), 129.3 (CH_{arom} PMB), 128.4. 128.1. 127.9, 127.5, 127.2 (CH_{arom} Bn), 113.6 (CH_{arom} PMB), 96.6 (C-3), 72.0 (C-4), 69.8, 69.1 (2× CH_2 Bn), 55.1 (CH_3 OMe PMB), 54.9 (C-6), 51.3 (CH_3 OMe), 49.3 (CH_2 PMB), 36.4 (*C*H_2CO_2), 34.9 (C-4), 29.5 (C-5). MS (ESI): $m/z = 502.3$ $[M + H]^+$, 1003.6 $[2M + H]^+$.

(1*S*,2*R*,3*S*,4*R*,6*S*)-6-Amino-1,2,3-tris-benzyloxy-4-methoxycarbonylmethylcyclohexane (13)

To a solution of compound **11** (0.476 g, 0.781 mmol) in a mixture of acetonitrile (6 mL) and water (3 mL) was added CAN (1.07 g, 1.95 mmol, 2.5 equiv.). This orange two-phase system was vigorously stirred for 24 h. After addition of sat. aq. $NaHCO_3$ followed by dilution with EtOAc, the aqueous phase was separated and washed twice with EtOAc. All organic layers were combined, dried ($MgSO_4$), and concentrated. Purification of the residue by silica gel chromatography (MeOH/DCM 1:19) gave title compound **13** (0.214 g, 0.437 mmol, 56%). ^{1}H NMR (400 MHz, MeOD): δ 7.39–7.22 (m, 15H, CH_{arom}), 4.67 (d, 1H, $J = 12.3$ Hz, CH Bn), 4.62 (d, 1H, $J = 12.3$ Hz, CH Bn), 4.59 (s, 2H, CH_2 Bn), 4.54 (d, 1H, $J = 12.1$ Hz, CH Bn), 4.48 (d, 1H, $J = 12.1$ Hz, CH Bn), 3.88 (ddd, 1H, $J = 1.2$ Hz, $J = 3.1$ Hz, $J_{2,1} = 4.3$ Hz, H-2), 3.78 (dd, 1H, $J_{1,2} = 3.1$ Hz, $J = 4.4$ Hz, H-1), 3.71 (m, 1H, H-3), 3.65–3.55 (m, 1H, H-6), 3.63 (s, 3H, CH_3 OMe), 2.52 (m, 1H, H-4), 2.45 (dd, $J_{CHH,4} = 7.7$ Hz, $J_{CHH,CHH} = 15.8$ Hz, C*H*H), 2.27 (dd, 1H, $J_{CHH,4} = 6.5$ Hz, $J_{CHH,CHH} = 15.8$ Hz, CH*H*), 1.80 (m, 2H, H-5a, H-5b). MS (ESI): $m/z = 490.3$ $[M + H]^+$, 979.7 $[2M + H]^+$.

(1*S*,2*R*,3*S*,4*R*,6*S*)-6-Amino-1,2,3-tris-benzyloxy-4-carboxymethylcyclohexane (14)

To a solution of ester **13** (95 mg, 0.194 mmol) in 1,4-dioxane (2.0 mL), was added an aq. solution of LiOH (0.5 mL, 1.0 M). After 3 h, TLC analysis (MeOH/DCM 15:85) showed complete conversion of starting material into a lower running spot. The mixture was neutralized with 1.0 M aq. HCl to pH 7 and extracted thoroughly with EtOAc (three times). The combined organic phases were dried ($MgSO_4$) and concentrated to give amino acid **14** (92 mg,

0.194 mmol) in quantitative yield. ^{1}H NMR (400 MHz, MeOD): δ 7.33–7.26 (m, 15H, CH_{arom}), 4.63 (d, 1H, J =12.0 Hz, CH Bn), 4.57 (d, 1H, J = 12.0 Hz, CH Bn), 4.53 (d, 1H, J = 11.9 Hz, CH Bn), 4.43 (d, 1H, J = 11.9 Hz, CH Bn), 4.40 (d, 1H, J = 11.8 Hz, CH Bn), 4.27 (d, 1H, J = 11.8 Hz, CH Bn), 3.85–3.82 (ddd, 1H, J = 1.3 Hz, J = 2.9 Hz, $J_{2,1}$ = 4.3 Hz, H-2), 3.76 (dd, 1H, J = 3.1 Hz, $J_{1,2}$ = 4.3 Hz, H-1), 3.76 (m, 1H, H-3), 3.70 (dd, 1H, J = 3.6 Hz, J = 7.3 Hz, H-6), 2.51 (m, 1H, H-4), 2.42 (dd, 1H, $J_{CH\mathrm{H},4}$ = 7.2 Hz, $J_{C\mathit{H}\mathrm{H},C\mathrm{H}\mathit{H}}$ = 16.0 Hz, *CH*H), 2.29 (1H, dd, $J_{\mathrm{CH}\mathit{H},4}$ = 6.6 Hz, $J_{\mathrm{CH}\mathit{H},C\mathit{H}\mathrm{H}}$ = 16.0 Hz, CH*H*), 1.99-1.92 (dddd, 1H, J = 1.2 Hz, J = 3.1 Hz, J = 4.1 Hz, $J_{5a,5b}$ = 14.8 Hz, H-5a), 1.86–1.77 (dd, 1H, J = 3.8 Hz, $J_{5b,5a}$ = 14.8 Hz, H-5b). MS (ESI): m/z = 476.2 $[M + H]^+$.

(1*S*,2*R*,3*S*,4*R*,6*S*)-*N*-(9-Fluorenylmethoxycarbonyl)-6-amino-1,2,3-tris-benzyloxy-4-carboxymethyl-cyclohexane (15)

To a suspension of amino acid **14** (0.194 mmol) in dioxane (0.5 mL) and sat. aq. $NaHCO_3$ (2.0 mL) was added Fmoc-OSu (85 mg, 0.252 mmol, 1.3 equiv.). After stirring for 17 h, TLC analysis (MeOH/DCM 1:19) revealed complete consumption of starting material into a higher running spot. Water and dioxane were added to the suspension and the resulting solution was acidified with 1.0 M aq. HCl to pH 5. The mixture was diluted with EtOAc, the organic layer was separated, and the aqueous layer was extracted twice with EtOAc. All organic layers were combined, dried ($MgSO_4$), filtered, and concentrated. After purification by silica gel column chromatography (EtOAc/PE 1:1 + 1.0% HOAc), carbamate **15** was obtained (0.124 g, 0.178 mmol, 92%). $[\alpha]_D^{20}$ + 25.5 (*c* 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, C_6D_6): δ 7.56–7.54 (m, 4H, CH_{arom} Fmoc), 7.37–7.30 (m, 4H, CH_{arom} Fmoc), 7.22–7.06 (m, 15H, CH_{arom} Bn), 6.79 (bs, 1H, NH), 4.64 (d, 1H, J = 11.8 Hz, CH Bn), 4.60 (d, 1H, J = 11.8 Hz, CH Bn), 4.58 (m, 1H, H-6), 4.54 (d, 1H, J = 11.8 Hz, CH Bn), 4.50 (dd, 1H, J = 10.6 Hz, J = 7.3 Hz, C*H*H, CH_2Fmoc), 4.27 (dd, 1H, J = 10.6 Hz, J = 7.3 Hz, CH*H* CH_2Fmoc), 4.22 (m, 1H, CH Bn), 4.16 (m, 3H, CH_2 Bn, CH Fmoc), 3.84 (m, 1H, H-2), 3.74 (m, 1H, H-3), 3.71 (m, 1H, H-1), 2.69 (m, 1H, H-4), 2.40 (m, 1H, C*H*H CH_2CO_2H), 2.11 (m, 1H, CH*H* CH_2CO_2H), 1.92 (m, 1H, H-5a), 1.60 (m, 1H, H-5b). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 177.6 (C=O CO_2H), 156.5 (C=O Fmoc), 144.1, 141.2 (2 × C_q Fmoc), 138.3, 137.9, 137.8 (3 × C_q Bn), 129.0, 128.5, 128.4, 128.4, 127.9, 127.8, 127.6, 127.5, 127.0 (CH_{arom} Bn), 125.3, 119.8 (CH_{arom} Fmoc), 77.3 77.0, 76.7 (C-1, C-2, C-3), 73.8, 72.6, 70.4 (3 × CH_2 Bn), 66.8 (CH_2 Fmoc), 47.8 (C-6), 47.2 (CH Fmoc), 30.7 (CH_2CO_2), 29.7 (C-4), 28.2 (C-5). IR (thin film): 3032, 2924, 2870, 2363, 2341, 1705, 1514, 1452, 1248, 1055, 739, 698 cm^{-1}. MS (ESI): m/z = 698.5 $[M + H]^+$, 1395.6 $[2M + H]^+$. HRMS (ESI): calcd for $[C_{44}H_{43}NO_7 + NH_4]^+$ 715.3383. Found 715.3370.

H-Phe-Leu-Wang resin (17)

Loading of resin: Commercially available Wang resin **16** (0.96 mmol/g, 1.79 g) was allowed to swell in DCM (40 mL). A solution was prepared of DIC (1.07 mL, 0.87 g, 6.88 mmol, 4.0 equiv.), Fmoc-Leu-OH (2.43 g, 6.88 mmol, 4.0 equiv.), and DMAP (cat.) in DCM. The mixture was left for 16 h with occasional shaking. The resin was filtered, subsequently washed with DMF and DCM, and dried (air). The loading was determined by treatment of the dried resin (2.3 mg) with a solution of 20% piperidine/DMF (1.0 mL). After stirring for 10 min followed by dilution to 10.00 mL with EtOH, the absorption of the solution was measured at 300 nm. The loading was calculated to be 0.49 mmol/g, using the formula: Loading (mmol/g) = $[A_{300}]^*10/[7.8^*m]$.

Peptide coupling: Fmoc-Leu-Wang-resin (1.00 g, 0.49 mmol) was treated with 10 mL 20% piperidine/DMF (3 × 10 min) to effect Fmoc cleavage. The resin was filtered, washed (DMF and DCM), and swollen in DMF. The resin was treated with a solution of Fmoc-Phe-OH (0.75 g, 1.94 mmol, 4.0 equiv.), HCTU (0.80 g, 1.94 mmol, 4.0 equiv.), and DiPEA (0.64 mL, 3.88 mmol, 8.0 equiv.) in DMF (5.0 mL). After shaking the mixture for 1 h, the resin was filtered and rinsed with DMF. This procedure was repeated twice to ensure complete coupling indicated by a negative Kaiser test. Any unreacted amines were capped using a solution of 0.5 M Ac_2O and 0.125 M DiPEA in DMF (50 mL, 5 min). After filtration and washing with DMF and DCM, the resin was filtered and dried by an air flow. The Fmoc was removed with 20% piperidine/DMF solution as described above.

Fmoc-CSAA-$(OBn)_3$-Phe-Leu-Wang resin (18)

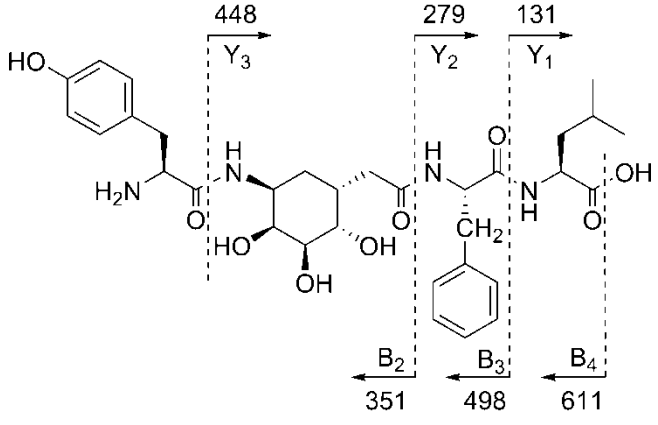
Resin **17** (70.0 mg, 34.3 μmol) was swollen in DMF. CSAA **15** (47.9 mg, 68.7 μmol, 2.0 equiv.) in DMF (700 μL) was preactivated for 30 sec with HATU (12.0 mg, 32.3 μmol, 0.95 equiv.) and DiPEA (15.0 μL, 84.9 μmol, 2.5 equiv) and the mixture was subsequently added to the resin and shaken for 10 min. After filtration of the resin, followed by rinsing with DMF, this coupling procedure was repeated once more. After a negative Kaiser test revealed complete consumption of free amines, the resin was washed with DMF and DCM and dried (air flow).

Boc-Tyr(t-Bu)-CSAA-$(OBn)_3$-Phe-Leu-Wang resin (19)

Resin **18** was swollen in DMF followed by Fmoc cleavage using a 20% piperidine/DMF (3 × 10 min), washed with DMF and DCM, filtered, and dried (air). Boc-Tyr(*t*-OBu)-OH (50.6 mg, 0.15 mmol, 4.4 equiv.) was coupled using HCTU (62.1 mg, 0.15 mmol, 4.4 equiv.) and DiPEA (49.6 μL, 0.300 mmol, 8.8 equiv.) in DMF and shaken for 2 h. A negative Kaiser test indicated a complete coupling of the free amines. The resin was filtered, washed with DMF and DCM, and air dried.

H-Tyr-CSAA-Phe-Leu-OH (21)

Cleavage from the resin along with the removal of the *t*-Bu and Boc group was effected transferring immobilized peptide **19** into a glass tube followed by the addition of a mixture of TFA/TIS/water (95:2.5:2.5, 1.0 mL). After shaking for 15 min, the mixture was filtered, washed with DMF and DCM, and dried. Purification by silica gel chromatography (MeOH/DCM 1:9) gave compound **20** (29.4 mg, 32.7 μmol, 95%). LC/MS (50% to 90% acetonitrile/water), R_t 10.02 min. MS (ESI): $m/z = 899.9$ $[M+H]^+$, 1799.4 $[2M+H]^+$. Compound **20** (16.2 mg, 18.0 μmol) was dissolved in *t*-BuOH (1.0 mL) and water (1.0 mL), and the resulting solution was degassed. A catalytic amount of Pd/C was added and after degassing the solution was stirred under a hydrogen atmosphere for a second time. After 15 h, TLC analysis (MeOH/DCM 15:85) indicated complete conversion of starting material into a lower running spot. The reaction mixture was filtered over Celite and the filtrate was concentrated to give Leu-enkephalin analog **21** in quantitative yield (11.3 mg, 18.0 μmol). After HPLC purification (18% to 30% acetonitrile/water), an analytical sample was obtained. LC/MS (10–40% acetonitrile/water), R_t 14.02 min. MS (ESI): $m/z = 629.5$ $[M+H]^+$, 651.4 $[M+Na]^+$, 1257.8 $[2M+H]^+$. ^{1}H NMR (600 MHz, DMSO d_6, T = 313K): δ 9.25 (s, 1H), 8.09 (d, 1H, $J = 7.2$ Hz), 7.99 (bs, 1H), 7.84 (d, 1H, $J = 7.2$ Hz), 7.32–6.91 (m, 9H), 6.71–6.68 (m, 2H), 4.62 (m, 1H), 4.48 (m, 1H), 4.20 (m, 1H), 4.04–3.93 (m, 2H), 3.69 (m, 1H), 3.59 (m, 1H), 3.35–3.21 (m, 2H), 3.08–3.00 (m, 2H), 2.81 (m, 1H), 2.78 (m, 1H), 2.64 (m, 1H), 2.31 (m, 1H), 2.23 (m, 1H), 1.95 (m, 1H), 1.77–1.74 (m, 2H), 1.75 (m, 1H), 1.71–1.66 (m, 2H), 0.89 (m, 6H). IR (thin film): 3275, 2963, 2361, 2341, 1678, 1015 cm^{-1}. HRMS (ESI): calcd for $[C_{32}H_{44}N_4O_9+H]^+$ 629.3181. Found 629.3178. The following sequence ions were detected after recording a MS/MS spectrum:



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