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Controlled Synthesis of (*S*,*S*)-2,7-Diaminosuberic Acid: A Method for Regioselective Construction of Dicarba Analogues of Multicystine-Containing Peptides

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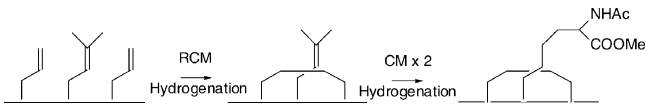
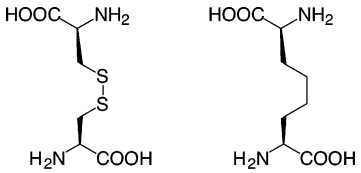


A method to facilitate regioselective formation of multiple dicarba isosteres of cystine is described. A sequence of ruthenium-catalyzed cross metathesis and rhodium-catalyzed hydrogenation of nonproteinaceous allylglycine derivatives has been developed to achieve high-yielding and unambiguous formation of diaminosuberic acid derivatives. Allylglycine derivatives readily undergo ruthenium-catalyzed metathesis and hydrogenation to yield diaminosuberic acid derivatives in near quantitative yield. Under the same experimental conditions, prenylglycine was found to be inert to both Grubbs' and Wilkinson's catalyzed metathesis and hydrogenation, respectively, but was readily activated for metathesis via cross metathesis with *Z*-butene. Subsequent cross metathesis of the metathesis-formed crotylglycine derivative, followed by hydrogenation, yielded the second diaminosuberic acid derivative in excellent yield.

Introduction

Cystine bridges are common structural motifs found in peptides and proteins. In some cases, the cystine bridge constitutes part of a peptide binding domain or active site where reduction results in the release of metal-chelating thiol groups.[1] In many other peptides, however, the cystine bridge serves only a structural role to maintain secondary and tertiary structure. Here, cystine may be replaced with isosteric units, such as a nonreducible all-carbon $-(CH_2)_4-$ bridge, without significantly affecting biological activity. Replacement of cystine (**1**) by (*S*,*S*)-2,7-diaminosuberic acid (**2**, (*S*,*S*)-2,7-diaminooctanedioic acid)[2] in several naturally occurring and synthetic cyclic peptides, such as vasopressin,[3] natriuretic β-ANP,[4] octreotide,[5] oxytocin,[6] calcitocin,[7] bradykinin antagonists,[8] and hematoregulatory peptides,[9] has been reported. These dicarba analogues were shown to be biologically active and many showed improved pharmacokinetic properties relative to their cognate peptides.



Until recently, synthesis and incorporation of (*S*,*S*)-2,7-diaminosuberic acid into a peptide was challenging. Several elegant approaches have recently been reported, which include Schöllkopf bislactam ether methodology,[10] Kolbe coupling of

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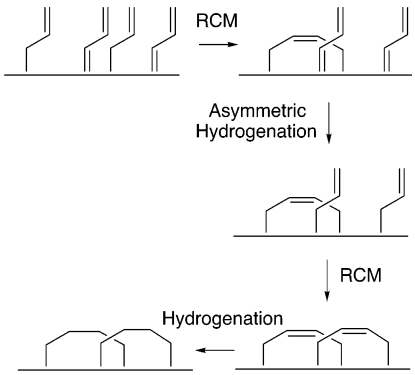
SCHEME 1



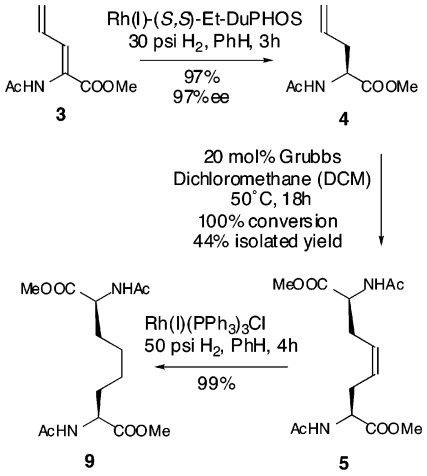
glutamic acid derivatives,[11] and the use of chiral auxiliaries.[12] The use of ruthenium-catalyzed metathesis has also emerged as a powerful tool for the preparation of this cystine isostere. Commercially available allylglycine residues (Hag) are used in place of cysteine and cyclized under mild experimental conditions to yield carbocyclic structures.[5,6b,13] The initial product of the metathesis is an unsaturated C4-bridge, where both *E*- and *Z*-isomers are possible, and hydrogenation is readily achieved to give the dicarba analogue. This provides scope for the probing of conformational preferences around the replaced cystine bridge and is also useful for library generation. Furthermore, all of the synthesis, both linear peptide construction, cyclization, and reduction, can be accomplished on a solid support.

Many peptides, such as conotoxins[14] and cyclotides,[15] contain multiple cystine bridges within a single peptide chain. Challenges associated with selective cystine formation and the facile reduction of disulfide bonds to reactive thiol groups have led us to investigate the use of homogeneous catalysis for the regioselective construction of dicarba analogues of multicystine-containing peptides.

Results and Discussion

Our initial strategy planned to capitalize on the use of α-*N*-acyldienamide (**3**), a masked precursor to allylglycine derivatives.[16] We devised a strategy involving a double metathesis−hydrogenation sequence (Scheme 1). This requires a selective ring closing metathesis (RCM) of allylglycine units in the presence of dienamide functionalities. Grubbs and co-workers have previously reported that selective cross metathesis can be accomplished with olefins of varying reactivity.[17] Terminal olefins (e.g., allylglycine) undergo rapid homodimerization with

SCHEME 2



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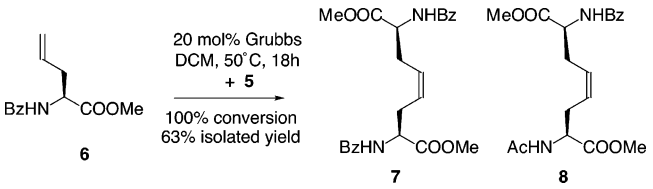

both Grubbs' catalyst ($(PCy_3)_2(Cl)_2Ru{=}CHPh$)[18] and second generation Grubbs' catalyst ($(IMesH_2)(PCy_3)(Cl)_2Ru{=}CHPh$),[19] whereas the electron-deficient α-*N*-acyldienamide (**3**) should be considerably less reactive. Subsequent asymmetric hydrogenation of the dienamide moieties would lead to reactive allylglycine units and a subsequent ring closing metathesis reaction would then produce the second carbocycle. The last step involves hydrogenation of the unsaturated carbocycles, if required, to afford the saturated cystine isosteres.

To validate the proposed strategy, we needed to show that (i) the dienamide would not react under conditions required for the ring closing metathesis of allylglycine residues, (ii) asymmetric hydrogenation of the dienamide would proceed in a highly regioselective and stereoselective manner, (iii) ring closing metathesis of the resulting allylglycine units would proceed in the presence of an unsaturated carbocycle (without resulting in mixed cross metathesis products), and (iv) the unsaturated carbocycles could be reduced to afford saturated dicarba bridges. We therefore conducted a series of independent experiments that would serve as a model to the peptide system.

The allylglycine derivative **4** was prepared via Rh(I)-DuPHOS[20] catalyzed asymmetric hydrogenation of the dienamide **3**[16] with excellent stereoselectivity (95% ee) and yield (97%) and readily cross metathesized using a ruthenium benzylidene catalyst, Grubbs' catalyst, to produce dimer **5** (Scheme 2). Importantly, the dienamide **3** did not react under these cross metathesis conditions. Cross metathesis of allyl-

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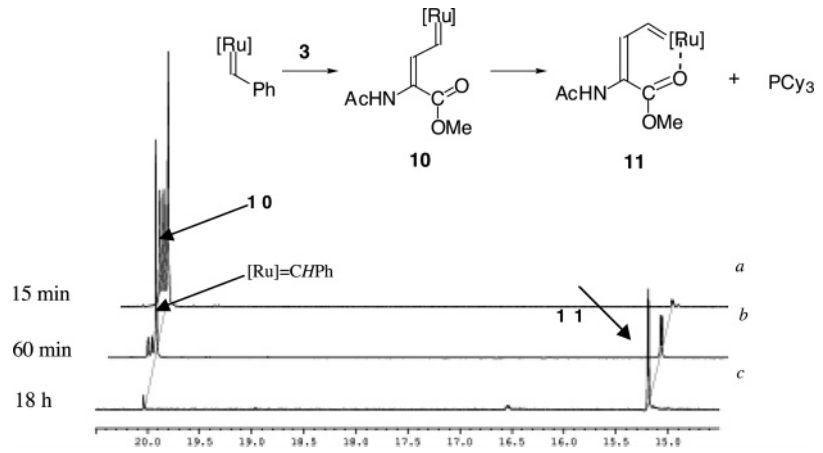

FIGURE 1. ^{1}H NMR binding studies between Grubbs' catalyst and dienamide **3**.

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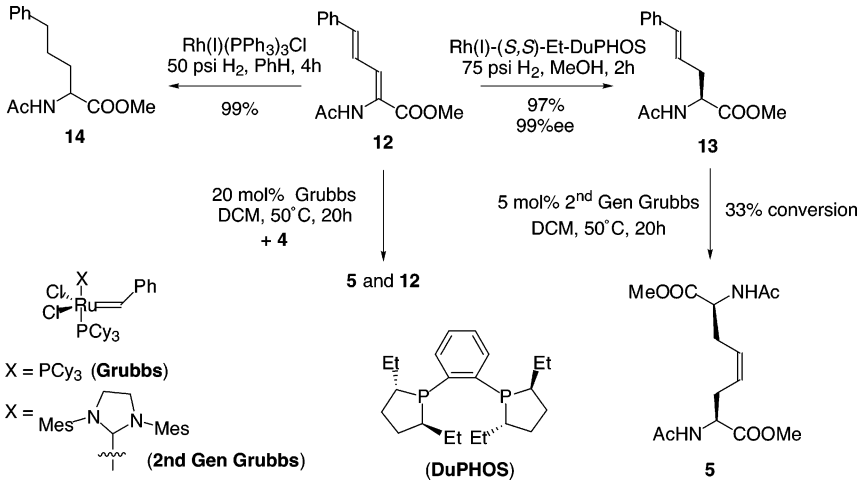
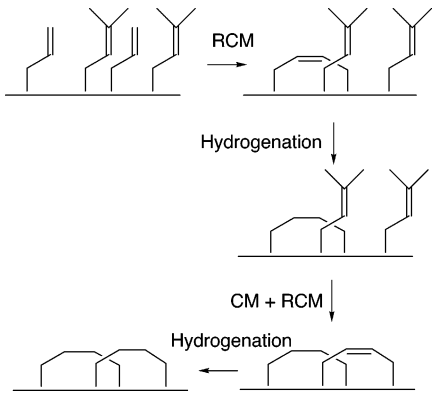


SCHEME 5



glycine derivative **6** in the presence of unsaturated dimer **5** proceeded with Grubbs' catalyst to afford dimer **7** (Scheme 3). No mixed cross metathesis product **8** was observed. Use of second generation Grubbs' catalyst did, however, lead to a mixture of cross metathesis products **7** and **8**. Homogeneous hydrogenation of dimer **5** with Wilkinson's catalyst, Rh(I)-$(PPh_3)_3Cl$, under mild experimental conditions, gave the saturated dicarba analogue **9** in quantitative yield (Scheme 2).

These results looked very promising; however, our attempts to dimerize allylglycine **4** *in the presence of* dienamide **3** were unsuccessful. ^{1}H NMR binding studies between the catalyst, dienamide **3**, and allylglycine **4** (ratio of 1:1:1) showed that the dienamide rapidly and preferentially coordinated to the ruthenium center forming a [Ru]-vinylalkylidene complex **10** (Figure 1, spectrum a), which slowly transformed into a more stable ruthenium species **11** (spectrum b) with the elimination of tricyclohexylphosphine. This transformation was complete after 2 h and the resulting complex was unreactive over an 18 h period (spectrum c).[21] Unfortunately, attempts to isolate this complex were unsuccessful. One possible explanation for these results is that the first formed [Ru]-vinylalkylidene slowly coordinates to the ester carbonyl group leading to the formation of the cyclic compound **11**.

Furthermore, attempts to regenerate the dienamide (**3**) from the Ru-carbonyl chelate (**11**), via reaction with ethyl vinyl ether and formation of the Fischer-type carbene complex,[22] failed due to conjugate addition of liberated tricyclohexylphosphine to the dienamide substrate (**3**). This highlighted the sensitivity of **3** to *N*- and *P*-based nucleophiles and potential problems arising

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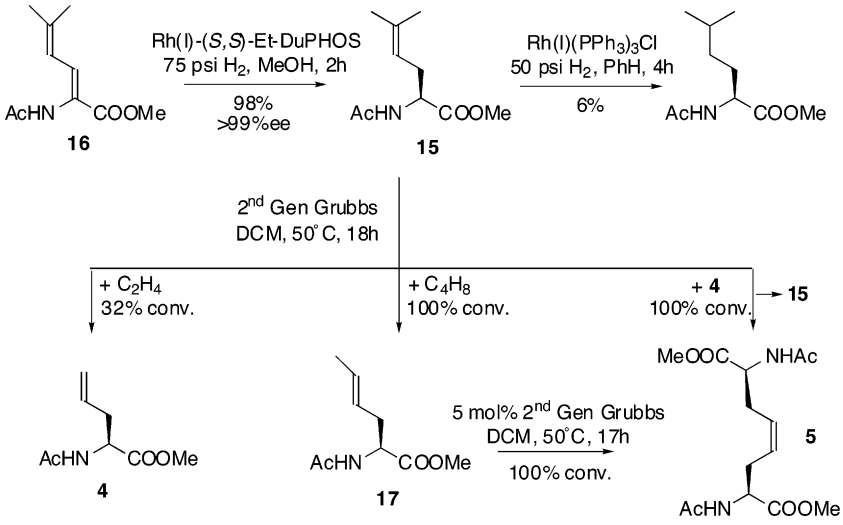
SCHEME 6



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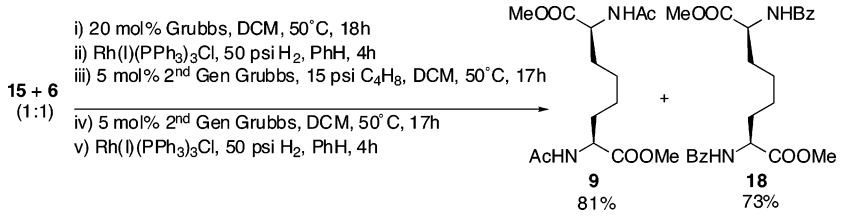

during peptide synthesis, where piperidine is routinely used to facilitate FMOC-cleavage from residues prior to coupling.

A revised strategy was then investigated centering on the use of nonproteinaceous, terminally functionalized allylglycine units. This alternate route involved (i) metathesis of allylglycine units in the presence of the phenyl-substituted dienamide **12** and (ii) subsequent hydrogenation of the dienamide **12** to yield a more reactive olefin **13** for the second ring closing metathesis (Scheme 4). We postulated that the presence of a phenyl substituent at the olefin terminus might impede binding of the metathesis catalyst. Significantly, ^{1}H NMR binding studies of 1:1 mixtures of Ru-benzylidene catalyst and dienamide **12** showed *no* alkylidene formation. Hence, the poor chelating properties of the modified dienamide **12** to Grubbs' catalyst now facilitated dimerization (28%) of allylglycine **4** to dimer **5** (Scheme 4). Rh(I)-DuPHOS catalyzed asymmetric hydrogenation of dienamide **12** under mild conditions (75 psi H_2) gave **13** in 97% yield and 99% ee. Disappointingly, cross metathesis of **13** with Grubbs' catalyst was unsuccessful. After 13 h, ^{1}H NMR spectroscopy showed no conversion to the desired dimer. Conditions to facilitate CM were found by using the more reactive second generation Grubbs' catalyst. After treatment of **13** with a 5 mol % solution of second generation Grubbs' catalyst over 20 h, 33% conversion to **5** was achieved. This reaction was not optimized, however, because use of this catalyst makes the previously formed unsaturated carbocycle vulnerable to further CM and would result in mixed cross metathesis products.

Reduction of the first-formed unsaturated carbocycle *prior* to the second metathesis reaction would, however, eliminate the chance of mixed cross metathesis (Scheme 5). We therefore subjected the phenyl-substituted olefins **12** and **13** to the hydrogenation conditions previously developed for the hydrogenation of the unsaturated dimer **5** (Scheme 4). Unfortunately, these conditions resulted in reduction of *all* C═C double bonds to give **14** (Scheme 4). This disappointing observation was perhaps not unexpected. The rate of olefin reduction by Wilkinson's catalyst is profoundly influenced by steric hindrance about the C═C double bond, but related reductions involving styrene have previously shown that electronic effects override these steric effects and that the aromatic substituent enhances the rate of reduction.[23]

This led to a final revision of our strategy which would enable the hydrogenation of an unsaturated carbocycle in a peptide while maintaining a reactive olefin for metathesis (Scheme 5). We decided to capitalize on the slow reactivity of trisubstituted olefins to Wilkinson's hydrogenation and their reduced reactivity to metathesis. 1,1-Disubstituted olefins, for example, do *not* undergo homodimerization and only react with more reactive olefins.[17] This differential reactivity would therefore facilitate the CM of allylglycine units and subsequent hydrogenation *without* interference from the 1,1-disubstituted olefin residues. A simple transformation can then render the trisubstituted olefin more reactive to metathesis and complete the second cyclization.

The prenyl olefin **15** was prepared via asymmetric hydrogenation of the corresponding dienamide **16** in quantitative yield and excellent enantioselectivity (Scheme 6). This enamide **15** was subjected to the hydrogenation conditions that quantitatively reduce the dimer **5** to **9** and, encouragingly, 94% of the starting enamide **15** was recovered (Scheme 6). This was a very promising result that prompted us to further investigate cross metathesis reactions involving this substrate **15**.

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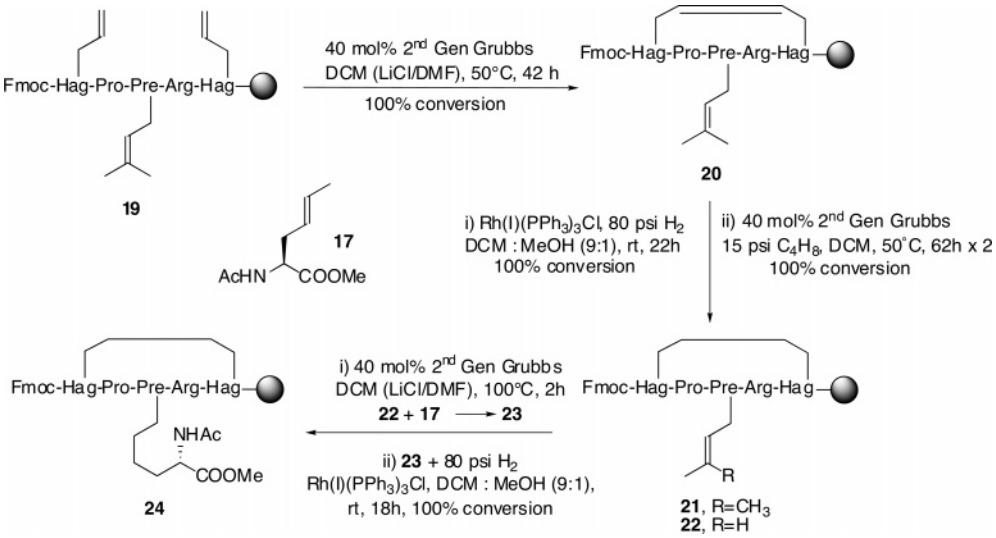
SCHEME 8



Cross metathesis of allylglycine unit **4** to dimer **5** *in the presence* of the prenyl enamide **15** proceeded smoothly in excellent yield (Scheme 6); the starting prenyl enamide **15** was recovered unchanged. The prenyl compound **15** was subjected to ethenolysis to convert it to the more reactive allylglycine derivative **4** (Scheme 6). Exposure of **15** to 20 mol % of second generation Grubbs' catalyst under an atmosphere of ethylene (15 psi) resulted in only poor conversions to **4** (<32%). We postulated that this result may be due to the unstable nature of the in situ generated ruthenium-methylidene intermediate at elevated temperature[24] or unfavorable competition between the rising concentration of terminal olefins and **15** for binding to the ruthenium catalyst.[25] To circumvent this problem, the prenyl enamide **15** was instead exposed to an atmosphere of *cis*-2-butene (15 psi) thereby facilitating the catalysis via the more stable [Ru]-ethylidene complex. Butenolysis of **15** in the presence of 5 mol % second generation Grubbs' catalyst gave the expected crotylglycine derivative **17** in quantitative yield. This olefin was then readily cross metathesized to the expected dimer **5** with 5 mol % of second generation Grubbs' catalyst (Scheme 6).

Finally, a one-pot equimolar mixture of olefins **15** and **6** was exposed to a tandem sequence of the five homogeneous catalytic reactions described above (Scheme 7). Quantitative conversion of the reactive substrate occurred in each step and ultimately yielded **9** and **18** as the *only* products in 81% and 73% isolated yields, respectively. No intermediate isolation was conducted during this catalytic sequence and no reaction between **15** and **6** was observed.

The above-described methodology was last applied to a simple resin-bound pentapeptide sequence **19**. Standard solid-phase peptide synthesis, using HATU-NMM activation and Fmoc-protected amino acids, was used to construct the linear peptide sequence **19** on Wang resin (Scheme 8). This sequence possesses three nonproteinaceous residues, two L-allylglycine (Hag) residues and one L-prenylglycine (Pre) residue, to facilitate construction of two dicarba bridges. Linear peptide intermediates were carried through without purification or characterization up to the pentapeptide **19**. A sample of the linear peptide was obtained by cleavage from the resin and determined to be of >95% purity by reverse-phase analytical chromatography. Mass spectral analysis showed the required molecular ion peak for **19** at *m*/*z* 813.5 [M + H^+].

The first catalytic step involved selective ring closing metathesis of the allylglycine residues in the presence of the less reactive prenyl side chain. RCM of the resin-tethered pentapeptide was performed with 40 mol % second generation Grubbs' catalyst in dichloromethane and 10% lithium chloride in dimethylformamide and, as expected, incorporation of prenylglycine did not hinder cyclization (Scheme 8). Mass spectral analysis of a cleaved aliquot of peptide confirmed formation of the *intramolecular* dicarba bond and unsaturated carbocycle **20** with the appearance of a molecular ion peak at *m*/*z* 785.4 $(M + H)^+$. Importantly, the prenylglycine residue remained inert to these metathesis conditions and no mixed cross metathesis products were observed. Attempts to decrease reaction time and catalyst loading led to incomplete reaction; however, decreasing peptide loading on the resin (from 0.9 to 0.3 mmol g^{-1}) enabled complete RCM with 10 mol % of second generation Grubbs' catalyst. Selective hydrogenation of the resin-bound unsaturated carbocycle **20** was performed under 80 psi of H_2 with homogeneous Wilkinson's catalyst, Rh(I)P-$(PPh_3)_3Cl$, in a mixture of dichloromethane: methanol (9:1). After 22 h, a small aliquot of peptide was cleaved and analyzed by mass spectrometry. The appearance of a peak at *m*/*z* 787.3 $(M + H)^+$ was consistent with formation of the saturated carbocycle **21**. Importantly, the prenyl group remained stable to these reducing conditions and was consistent with the observed reactivity of prenylglycine in the solution phase model studies.

Activation of the prenyl group was achieved via butenolysis of the resin-bound pentapeptide **21**. The peptide was exposed to an atmosphere of *cis*-2-butene (15 psi) and 40 mol % second generation Grubbs' catalyst in dichloromethane for 62 h. This led to a mixture of the desired product **22** and the starting peptide **21**. This activation step was unexpectedly and inexplicably slow. The recovered resin-peptide was re-subjected to the butenolysis conditions to achieve complete conversion to the target crotyl-

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glycine-containing peptide **22**. Mass spectral analysis of a cleaved aliquot displayed the product molecular ion peak at *m*/*z* 773.2 $(M + H)^+$. A final cross metathesis reaction between the activated-resin bound peptide **22** and crotylglycine derivative **17**[26] was then performed. Microwave irradiation of a mixture of resin-tethered peptide **22** with 40 mol % second generation Grubbs' catalyst, excess crotylglycine derivative **17** (~50 equiv) in dichloromethane, and 10% lithium chloride in dimethylformamide resulted in formation of the second dicarba bond, an *intermolecular* linkage, and unsaturated peptide **23** accompanied by dimer **5**. Mass spectrometry confirmed product formation of **23** with the appearance of a molecular ion peak at *m*/*z* 902.3 $(M + H)^+$. Wilkinson's hydrogenation of the unsaturated intermolecular bridge was achieved under conditions previously established (80 psi of H_2, dichloromethane:methanol (9:1), room temperature, 18 h) to give the target peptide **24** containing two selectively constructed dicarba bridges.

Conclusions

In conclusion, these model studies demonstrate that through the combination of homogeneous catalysis and judicious selection of nonproteinaceous allylglyine residues of varying reactivity, a highly efficient, unambiguous, and regioselective synthesis of dicarba analogues of multicystine-containing peptides is achievable. The high selectivity, efficiency, and generic nature of this catalytic sequence, coupled with the accessibility of both allyl-[27]and prenylglycine,[28] is likely to ensure the widespread applicability of this approach to peptide synthesis. Furthermore, the disparate reactivity of the allyl and prenyl groups toward Ru- and Rh-catalyzed metathesis and hydrogenation could be further exploited in syntheses involving nonpeptidic substrates. We are currently applying this methodology to the synthesis of natural products and dicarba mimics of cystine-containing peptides and exploring tandem catalytic sequences for achieving the same end.

Experimental Section

Preparation of Dienamides. Dienamides **3** and **12** were prepared as described by Teoh et al.[16] and Burk et al.,[29] respectively.

(2*Z*)-Methyl 2*N*-acetylamino-5-methylhexa-2,4-dienoate (16): Dienamide **16** was prepared according to modified literature procedures.[16,29] Tetramethylguanidine (0.78 mL, 6.22 mmol) was added to a solution of methyl 2*N*-acetylamino-2-(dimethoxyphosphinyl)acetate (1.11 g, 4.64 mmol) in distilled tetrahydrofuran (50 mL) at −78 °C. After 15 min, 3-methyl-2-butenal (0.54 mL, 5.60 mmol) was added and the mixture was stirred for 2 h at −78 °C. The mixture was warmed to 25 °C with a warm water bath and stirred at this temperature for an additional 18 h. The reaction mixture was then diluted with dichloromethane (100 mL) and washed with dilute hydrochloric acid solution (1 M, 2 × 75 mL), copper sulfate solution (1 M, 2 × 75 mL), saturated sodium bicarbonate solution (2 × 75 mL), and saturated sodium chloride solution (1 × 75 mL). The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure to give an oil (0.79 g). Purification by flash chromatography (SiO_2, dichloromethane:ethyl acetate:light petroleum, 2:1:1) gave dienoate **16** (0.61 g, 67%) as a pale brown solid, mp 115−116 °C. t_R = 6.3 min (GC column 30QC5/BPX5, 150 °C for 1 min, 10 °C min^{-1} to 280 °C for 6 min). ν_{max} (neat): 3258 m, 3009 w, 2956 w, 1729 s, 1663 s, 1610 m, 1560 w, 1522 s, 1440 m, 1374 m, 1338 m, 1286 s, 1255 s, 1208 s, 1156 m, 1123 s, 1041 w, 1016 m, 687 m, 896 w, 868 m, 768 s, 716 m, 658 w, 603 m, 581 w, 603 m, 561 w cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 1.89 (s, 6H), 2.13 (s, 3H), 3.77 (s, 3H), 5.95 (d, J = 11.8 Hz), 6.97 (br s, 1H), 7.34 (br d, J = 11.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.3, 23.6, 27.1, 52.4, 120.8, 121.1, 130.5, 166.0, 168.0. HRMS (ESI^+, MeOH): *m*/*z* calcd for $C_{10}H_{15}NO_3Na$ $[(M + Na)^+]$ 220.0950, found 220.0947.

General Asymmetric Hydrogenation Procedure. In a drybox, a Fischer−Porter tube was charged with catalyst (1−3 mg), deoxygenated solvent (~5 mL), and substrate (28−108 mg). Three vacuum/argon cycles to purge the gas line of any oxygen followed by three vacuum/argon cycles of the vessel were carried out before the tube was pressurized with hydrogen to the specified pressure (psi). The reaction was then stirred at room temperature for the specified period of time. The pressure in the vessel was then released, and the contents were evaporated under reduced pressure. The crude product was passed through a short plug of silica (eluent = ethyl acetate) prior to spectroscopic and chromatographic analysis. Hydrogenation experiments are described by using the following format: substrate, solvent, catalyst, hydrogen pressure, reaction time, isolated yield, enantiomeric excess (assigned configuration), retention time (GC conditions).

(2*S*)-Methyl 2*N*-acetylaminopent-4-enoate (4):[30] (2*Z*)-Methyl 2*N*-acetylaminopenta-2,4-dienoate (**3**) (108.0 mg, 0.64 mmol), benzene (7 mL), [(COD)Rh(*S*,*S*)-Et-DuPHOS]OTf (3 mg), 30 psi H_2, 3 h, 97% yield, 97% ee (2*S*-**4**), t_R = 16.5 min (*S*) (GC chiral column Model C-024, 100 °C for 1 min, 5 °C min^{-1} to 280 °C for 9 min). $[\alpha]^{22}_D$ +45.0° (*c* 0.76, $CHCl_3$). Spectral data were consistent with literature data.[30]

General Metathesis Procedure. A Schlenk flask was charged with catalyst (5−20 mol %), deoxygenated solvent (~5 mL), and substrate (10−60 mg). The reaction mixture was left to stir at 50 °C for the specified period of time. Metathesis reactions were terminated upon exposure to oxygen and volatile species removed under reduced pressure. The crude product was purified by flash chromatography. Metathesis experiments are described by using the following format: substrate, solvent, catalyst, reaction time, reaction temperature, percent conversion. Chromatographic purification conditions (isolated yield).

(2*S*,7*S*)-Dimethyl 2,7-*N*,*N*′-diacetylamino-oct-4-enedioate (5):[32] (2*S*)-Methyl 2*N*-acetylaminopent-4-enoate (**4**) (14.0 mg, 0.08 mmol), dichloromethane (4 mL), Grubbs' catalyst (13.5 mg, 0.02 mmol, 20 mol %), 24 h, 50 °C, 100% conversion. Purification by flash chromatography (SiO_2, dichloromethane:light petroleum:ethyl acetate, 1:1:1) furnished pure dimer **5** (11.4 mg, 44%) as a brown oil, t_R(*E*/*Z*) = 12.2, 12.7 min (GC column 30QC5/BPX5, 150 °C for 1 min, 10 °C min^{-1} to 280 °C for 6 min). $[\alpha]^{22}_D$ +92.0 (*c* 0.004, $CHCl_3$). ν_{max} (neat): 3286 br m, 2956 m, 2931 m, 2856 w, 1742 s, 1659 s, 1542 m, 1438 m, 1375 m, 1267 m, 1220 m, 1138 w, 1017 w cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 2.04 (s, 6H), 2.40−2.50 (m, 4H), 3.74 (s, 6H), 4.64−4.70 (m, 2H), 5.36−5.40 (m, 2H), 6.34 (br d, J = 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 23.1, 35.1, 51.7, 52.6, 128.8, 170.3, 172.6. HRMS (ESI^+, MeOH): *m*/*z* calcd for $C_{14}H_{22}N_2O_6Na$ $[(M + Na)^+]$ 337.1376, found 337.1375. Spectral data were consistent with literature data.[32]

Competitive Cross Metathesis Studies: Cross Metathesis of (2*S*)-Methyl 2*N*-Benzoylaminopent-4-enoate (6) in the Presence

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of (2*S*,7*S*)-Dimethyl-2,7-*N*,*N*′-diacetylamino-oct-4-enedioate (5). (2*S*)-Methyl 2*N*-benzoylaminopent-4-enoate(**6**) (37.0 mg, 0.16 mmol), (2*S*,7*S*)-dimethyl 2,7-*N*,*N*′-diacetylamino-oct-4-enedioate (**5**) (30.0 mg, 0.09 mmol), dichloromethane (4 mL), Grubbs' catalyst (26.1 mg, 0.03 mmol, 20 mol %), 18 h, 50 °C, 100% conversion of **6** into dimer **7**. Purification by flash chromatography (SiO_2, dichloromethane:ethyl acetate:light petroleum, 2:2:1) gave pure dimers **5** (30.0 mg, 100% recovery) and **7** (43.8 mg, 63% yield) as brown oils. Spectral data were in agreement with those previously obtained.

NMR Studies of Cross Metathesis Reactions. (a) NMR Study of Grubbs' Catalyst with Dienamide 3. In a drybox, a Teflon-sealed NMR tube was charged with (2*S*)-methyl 2*N*-acetylamino-penta-2,4-dienoate (**3**) (21.0 mg, 0.12 mmol), Grubbs' catalyst (102 mg, 0.12 mmol), and degassed deuterated dichloromethane (0.8 mL) at room temperature. The NMR tube was shaken gently and reaction progress was monitored by ^{1}H and ^{31}P NMR spectroscopy. Compounds were identified by the following diagnostic resonances. ^{1}H NMR (300 MHz, CD_2Cl_2): After 15 min: Grubbs' catalyst, δ 8.61 (d, J = 7.6 Hz, 2H), 20.05 (s, 1H); ruthenium-dienamide complex **10**, δ 7.96 (d, J = 11.0 Hz, 1H), 20.11 (d, J = 11.0 Hz, 1H); ruthenium-dienamide chelate **11** (trace amount), δ 15.20 (d, J = 4.2 Hz, 1H). Ratio of ruthenium complexes [Ru]═CHPh:**10**:**11** = 1:1:0.08. After 60 min: Grubbs' catalyst, δ 8.45 (d, J = 7.6 Hz, 2H), 20.04 (s, 1H); ruthenium-dienamide complex **10**, δ 7.96 (d, J = 11.0 Hz, 1H), 20.10 (d, J = 11.0 Hz, 1H); ruthenium-dienamide chelate **11**, δ 6.73 (d, J = 3.0 Hz, 1H) (*peak obscured by liberated styrene)*, 15.19 (d, J = 4.2 Hz, 1H). Ratio of ruthenium complexes [Ru]═CHPh:**10**:**11** = 3:1:1. After 120 min: ruthenium-dienamide chelate **11**: δ 6.71 (d, J = 3.0 Hz, 1H) (*peak obscured by liberated styrene)*, 15.19 (d, J = 4.0 Hz, 1H). ^{31}P NMR (300 MHz, $CDCl_3$): δ 35.0, **11**; 37.0, Grubbs' catalyst; 38.8, **10**.

(b) NMR Study of Grubbs' Catalyst with Dienamide 12. In a drybox, a Teflon-sealed NMR tube was charged with (2*S*)-methyl 2*N*-acetylamino-5-phenylpenta-2,4-dienoate (**12**) (10.0 mg, 0.04 mmol), Grubbs' catalyst (33.6 mg, 0.04 mmol), and degassed deuterated dichloromethane (0.8 mL) at room temperature. The NMR tube was shaken gently and reaction progress was monitored by ^{1}H NMR spectroscopy. After 4 h, no alkylidene formation was evident and only peaks corresponding to Grubbs' catalyst and the starting dienamide **12** were present.

Homogeneous Hydrogenation Reactions. (a) Wilkinson's Hydrogenation of Dimer 5: (2*S*,7*S*)-Dimethyl 2,7-*N*,*N*′-Diacetyl-aminooctanedioate (9). (2*S*,7*S*)-Dimethyl-2,7-*N*,*N*′-diacetylamino-oct-4-enedioate (**5**) (25.0 mg, 0.08 mmol), benzene (5 mL), Wilkinson's catalyst (2 mg), 50 psi H_2, 4 h, 99% yield of **9** as a brown oil. t_R = 14.4 min (GC column 30QC5/BPX5, 150 °C for 1 min, 10 °C min^{-1} to 280 °C for 6 min). ν_{max} (neat): 3426 br m, 3055 w, 2932 m, 2857 w, 2360 w, 1741 s, 1666 s, 1543 w, 1438 m, 1375 w, 1266 s, 1177 w, 1120 w, 896 w, 738 w, 702 w cm^{-1}. ^{1}H NMR. (300 MHz, $CDCl_3$): δ 1.30−1.40 (m, 4H), 1.82−1.90 (m, 4H), 2.02 (s, 6H), 3.74 (s, 3H), 4.56−4.63 (m, 2H), 6.16 (bd, J = 7.5 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 23.3, 24.7, 32.3, 52.0, 52.5, 170.0, 173.1. HRMS (ESI$^+$, MeOH): *m*/*z* calcd for $C_{14}H_{24}N_2O_6Na$ [(M + Na)$^+$] 339.1532, found 339.1531.

(b) Ethenolysis of (2*S*)-Methyl 2*N*-Acetylamino-5-methylhex-4-enoate (15). (2*S*)-Methyl 2*N*-acetylamino-5-methylhex-4-enoate (**15**) (24.3 mg, 0.12 mmol), dichloromethane (5 mL), second generation Grubbs' catalyst (31.1 mg, 0.04 mmol, 30 mol %), 60 psi ethylene, 38 h, 50 °C, 32% conversion into **4**.

(c) Butenolysis of (2*S*)-Methyl 2*N*-Acetylamino-5-methylhex-4-enoate (15). (2*S*)-Methyl 2*N*-acetylamino-5-methylhex-4-enoate (**15**) (16.2 mg, 0.08 mmol), dichloromethane (5 mL), second generation Grubbs' catalyst (3.5 mg, 0.004 mmol, 5 mol %), 15 psi *cis*-2-butene, 15 h, 100% conversion into (2*S*)-methyl 2*N*-acetylaminohex-4-enoate (**17**). Purification by flash chromatography (SiO_2, dichloromethane:ethyl acetate:light petroleum:methanol, 1:2:1:0.2) gave the crotylglycine derivative **17**[16,26] (20.3 mg, 84% yield) as a brown oil. t_R(*E*/*Z*) = 4.2 min, 4.4 min (GC column 30QC5/BPX5, 150 °C for 1 min, 10 °C min^{-1} to 280 °C for 6 min). ν_{max} (neat): 3284 s, 2966 w, 2954 m, 2856 w, 1747 s, 1658 s, 1547 s, 1437 s, 1375 s, 1217 m, 1142 m, 1072 w, 1016 w, 968 m, 848 m cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.60 (dd, J = 6.3, 1.2 Hz, 3H), 1.95 (s, 3H), 2.36−2.44 (m, 2H), 3.67 (s, 3H), 4.55 (dt, J = 7.8 Hz, 5.9 Hz, 1H), 5.16−5.31 (m, 1H), 5.40−5.57 (m, 1H), 6.17 (br d, J = 6.4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.1, 23.3, 35.4, 52.1, 52.4, 124.6, 130.2, 169.7, 172.6. Mass spectrum (ESI$^+$, MeOH): *m*/*z* 208.1 $C_9H_{15}NO_3Na$ [(M + Na)$^+$]. Spectroscopic data were consistent with literature data.[16,29]

Pentapeptide Transformations. (a) Cyclic Pentapeptide: Fmoc-c[Hag-Pro-Pre-Arg-Hag]-OH (20). The resin-bound peptide **19** was subjected to the conventional RCM procedure under the following conditions: Resin-peptide **19** (70.0 mg, 0.06 mmol), dichloromethane (DCM) (5 mL), LiCl/DMF (0.4 M, 0.5 mL), second generation Grubbs' catalyst (21.6 mg, 0.03 mmol, 40 mol %), 50 °C, 42 h, 100% conversion to **20**. At the end of the reaction period, a small aliquot of peptidyl-resin was subjected to the standard TFA-mediated cleavage procedure. Mass spectral analysis of the isolated residue confirmed formation of the cyclic peptide **20**. Mass spectrum (ESI$^+$, MeCN/H_2O): *m*/*z* 785.4 (M + H)$^+$, $C_{41}H_{53}N_8O_8$ requires 785.4; *m*/*z* 803.3 (M + H_2O + H)$^+$, $C_{41}H_{55}N_8O_9$ requires 803.4; *m*/*z* 899.4 (M + TFA + H)$^+$, $C_{43}H_{54}F_3N_8O_{10}$ requires 899.4.

(b) Reduced Cyclic Pentapeptide: Fmoc-rc[Hag-Pro-Pre-Arg-Hag]-OH (21). The resin-bound peptide **20** was subjected to the general Wilkinson's hydrogenation procedure under the following conditions: Resin-peptide **20** (350 mg, 0.32 mmol), DCM:MeOH (9:1, 8 mL), Wilkinson's catalyst, 80 psi of H_2, 22 °C, 22 h, 100% conversion to **21**. At the end of the reaction period, a small aliquot of peptidyl-resin was subjected to the standard TFA-mediated cleavage procedure. Mass spectral analysis of the isolated residue confirmed formation of the reduced cyclic pentapeptide **21**. Mass spectrum (ESI$^+$, MeCN/H_2O): *m*/*z* 787.2 (M + H)$^+$, $C_{41}H_{55}N_8O_8$ requires 787.4; *m*/*z* 805.2 (M + H_2O + H)$^+$, $C_{41}H_{57}N_8O_9$ requires 803.4; *m*/*z* 901.3 (M + TFA + H)$^+$, $C_{43}H_{56}F_3N_8O_{10}$ requires 901.4.

(c) Reduced Cyclic Pentapeptide−Olefin Activation: Fmoc-rc[Hag-Pro-Crt-Arg-Hag]-OH (22). The resin-bound peptide **21** was subjected to the general conditions for CM with *cis*-but-2-ene under the following conditions: Resin-peptide **21** (212 mg, 0.19 mmol), DCM (8 mL), second generation Grubbs' catalyst (82 mg, 9.7 μmol, 50 mol %), *cis*-but-2-ene (15 psi), 50 °C, 42 h. At the end of the reaction period, a small aliquot of peptidyl-resin was subjected to the cleavage procedure. Mass spectral analysis of the isolated residue indicated the presence of the starting peptide and the desired butenolysis product. The recovered resin-peptide was subjected to the same butenolysis conditions in order to drive the reaction to completion. After 42 h, a small aliquot of peptidyl-resin was subjected to the cleavage. Mass spectral analysis of the isolated residue confirmed quantitative conversion to the activated peptide **22**. Mass spectrum (ESI$^+$, MeCN/H_2O): *m*/*z* 773.2 (M + H)$^+$, $C_{40}H_{53}N_8O_8$ requires 773.4.

(d) Reduced Cyclic Pentapeptide−CM of Activated Olefin: Fmoc-rc[Hag-Pro-(N-Ac-Dehydro-Sub-OMe)-Arg-Hag]-OH (23). The resin-bound peptide **22** was subjected to the general microwave-accelerated CM under the following conditions: Resin-peptide **22** (20.0 mg, 18 μmol), DCM (4 mL), LiCl/DMF (0.4 M, 0.4 mL), second generation Grubbs' catalyst (6.2 mg, 7.3 μmol, 40 mol %), (2*S*)-methyl 2-*N*-acetylaminohex-4-enoate (**17**) (70.0 mg, 0.38 mmol), 100 °C, 2 h. At the end of the reaction period, a small aliquot of peptidyl-resin was subjected to the TFA-mediated cleavage procedure. Mass spectral analysis of the isolated residue confirmed formation of the CM product, peptide **23**. Mass spectrum (ESI$^+$, MeCN/H_2O): *m*/*z* 902.4 (M + H)$^+$, $C_{45}H_{60}N_9O_{11}$ requires 902.4.

(e) Reduced Cyclic Pentapeptide−Wilkinson's Hydrogenation: Fmoc-rc[Hag-Pro-(N-Ac-Sub-OMe)-Arg-Hag]-OH (24).

The resin-bound peptide **23** was subjected to the general Wilkinson's hydrogenation procedure under the following conditions: Resin-peptide **23** (15.0 mg, 14 μmol), DCM:MeOH (9:1, 5 mL), Wilkinson's catalyst, 80 psi of H_2, 22 °C, 22 h, 100% conversion to **24**. At the end of the reaction period, a small aliquot of peptidyl-resin was subjected to the TFA-mediated cleavage. Mass spectral analysis of the isolated residue confirmed formation of the reduced cyclic pentapeptide **24**. Mass spectrum (ESI^+, $MeCN/H_2O$): *m*/*z* 904.4 $(M + H)^+$, $C_{45}H_{62}N_9O_{11}$ requires 904.5.

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Supporting Information Available: Experimental procedures and spectral data for compounds **3**−**7**, **9**, and **12**−**19**. This material is available free of charge via the Internet at http://pubs.acs.org.

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