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Constrained peptidomimetics: building bicyclic analogs of pyrazoline derivatives

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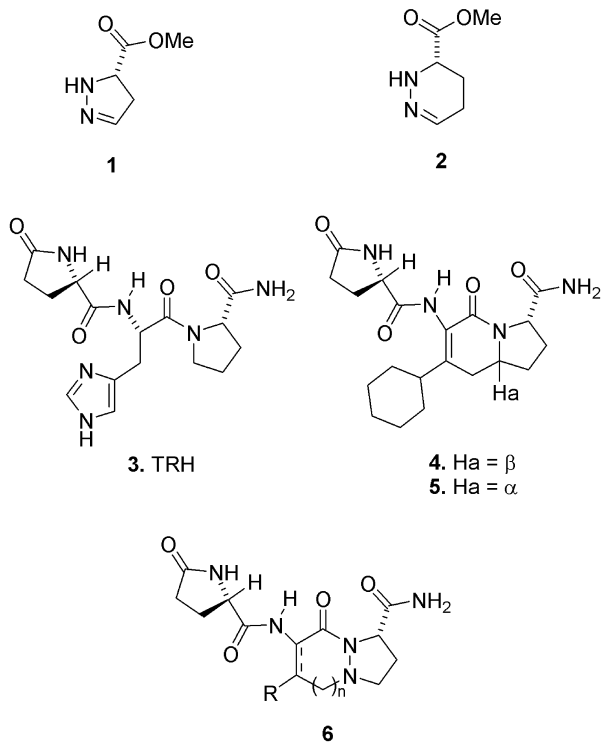
Abstract—A synthetic route has been developed for incorporating pyrazoline derivatives as proline surrogates in constrained X-Pro peptidomimetics. The route allows for the synthesis of dipeptide building blocks having either a six or seven-membered-ring annulated onto the pyrazoline moiety, as well as for the asymmetric synthesis of analogs having substituents on N-terminal side of the building block.

1. Introduction

In 1997, Carreira and co-workers first reported a new class of proline surrogates having the overall structure of **1** (Scheme 1).[1] These analogs were analogous to the previously reported six-membered ring pyridazine-3-carboxylic acid derivatives **2**,[2] and in principle could be used in a similar fashion to synthesize a variety of intriguing new peptidomimetics.[3] For example, efforts to utilize bicyclic peptidomimetics to 'map' the three dimensional requirements for the binding of thyrotropin releasing hormone (TRH/**3**) to its endocrine receptor (TRH-R_1)[4,5] found that the success of the bicyclic analogs was strongly dependent upon the stereochemistry at the bridgehead position of the bicyclic peptide derivative (Scheme 1). Analog **4** having an R-configuration at the bridgehead carbon was over 400 times more active than the directly analogous analog **5** with the opposite bridgehead stereochemistry.[5c] Hence, it was clear that the construction of future analogs would require the bridgehead configuration found in **4** and not **5**. This was a worrisome observation because the bridgehead carbon in **4** had an R-configuration and therefore possessed *cis*-stereochemistry about the proline ring. In general, 5-substituted proline rings having *cis*-stereochemistry can be difficult to synthesize and often require the formation and subsequent separation of nearly 1:1 mixtures of both the *cis*- and *trans*-isomers.[6] For the initial synthesis of TRH analog **4**, this was not a problem because isomer **5** was also needed for probing questions about bridgehead stereochemistry. But now that this question was answered, synthesizing an analog like **4** meant synthesizing both analogs and then discarding the one with the wrong bridgehead stereochemistry.

It was tempting to suggest that these synthetic problems could be avoided by incorporating a proline surrogate like **1** into the bicyclic peptidomimetic. Energy calculations[7] suggest that the barrier for inversion of the bridgehead nitrogen in analogs like **6** would range from 3 to 13 kcal/mol



Scheme 1.

Keywords: proline surrogates; pyrazoline; bicyclic analogs.
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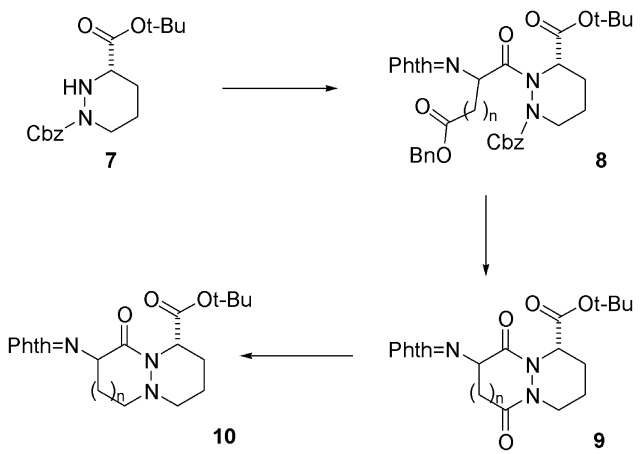


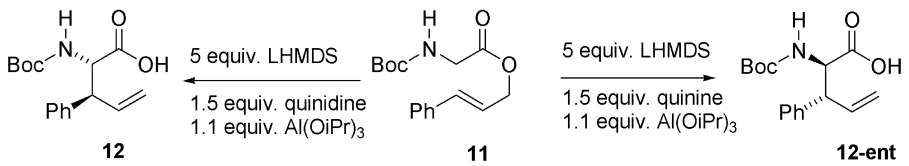
Scheme 2.

(3.2 kcal/mol for n=1 and 12.8 kcal/mol for n=2).[8] Since a barrier of approximately 20–25 kcal/mol is typically needed for nitrogen isomers to be isolated at room temperature,[9] it would appear that the bridgehead amine in analogs like **6** readily invert. Even at physiological pH where the amine would be largely protonated, enough of the free amine should be present to allow for this inversion. Hence, the use of a bicyclic peptidomimetic like **6** would allow for inversion of the bridgehead stereochemistry, and thereby allow all of the molecules synthesized a chance to populate the conformation with the correct bridgehead stereochemistry for binding the receptor. But is such a scenario really feasible, and if so how would the introduction of this bridgehead nitrogen alter the biological activity of a constrained, bicyclic TRH analog? In order to begin addressing these questions, we first needed a convenient strategy for placing proline surrogates like **1** into bicyclic peptide analogs like **6**. We report herein a synthetic approach for accomplishing this initial objective.

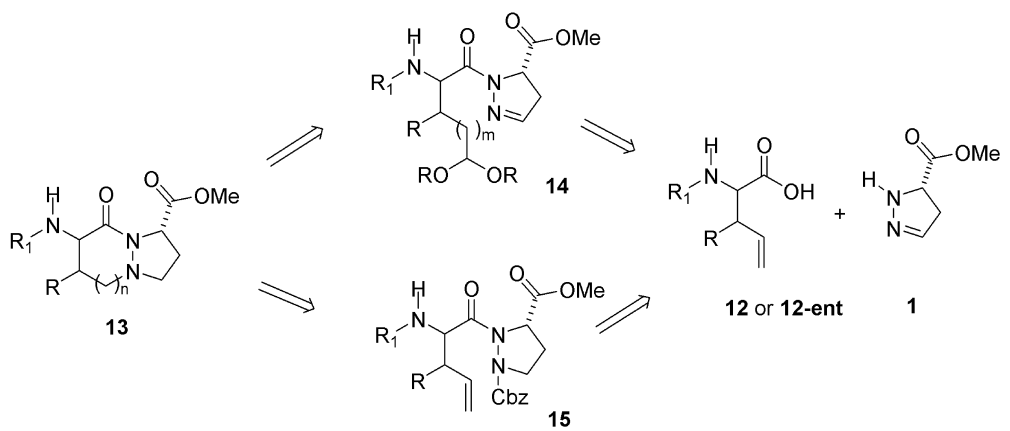
2. Initial studies

Six-membered ring aza pipecolic acid surrogates like **2** have already been incorporated into bicyclic building blocks.[10] In this case, the amino acid derivative was reduced and N_6 (pipecolic acid numbering) of the ring system protected as a benzyl group. The amino acid derivative was then transformed into the bicyclic compound using the chemistry outlined in Scheme 2. While this strategy was effective and suggested a route for constructing the analogous proline based bicyclic lactam derivative, our desire to use the analogs to probe the binding of TRH to the TRH-R endocrine receptor required that the lactam ring bear a functional group on the carbon beta to the amide carbonyl. This requirement forced us to consider alternative starting points for the N-terminal side of the building block. To this end, the chemistry developed by Kazmaier and co-workers appeared ideal.[11] By utilizing chiral amines to influence the stereochemical outcome of ester enolate Claisen rearrangements, Kazmaier and co-workers developed a strategy for rapidly constructing 3-substituted-2-amino-4-pentenoic acid derivatives **12** having either absolute stereochemistry (Scheme 3). If the stereochemistry of the olefin in **11** was changed, then the relative stereochemistry of the substituents in the amino acid derivatives could also be varied. In earlier efforts, these pentenoic acid derivatives proved to be an excellent starting point for synthesizing the N-terminal portion of bicyclic analogs such as **6**.[5,6] For this reason, we also sought to take advantage of the Kazmaier chemistry in the current synthetic efforts.

Two possible strategies were readily apparent (Scheme 4). In the first, the bicyclic derivative was envisioned as arising from a reductive amination reaction starting from **14**. This plan was initially favored because of the relative ease with which pyrazoline derivative **1** underwent reductive amination and the fact that this route would avoid having to



Scheme 3.

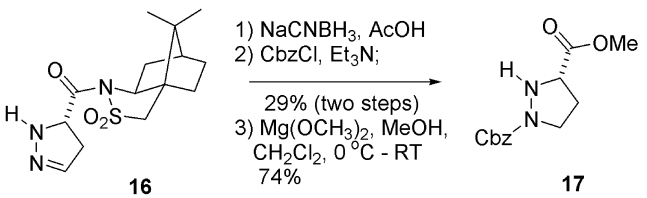


Scheme 4.

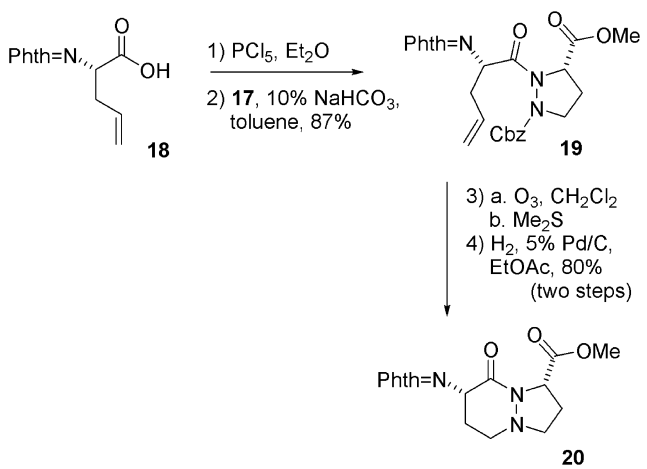
protect and then deprotect the N_5 nitrogen (proline numbering). Unfortunately, this initial strategy was problematic. When the olefin in **12** was cleaved and the resulting aldehyde protected as a dimethoxy acetal prior to coupling with **1** (Scheme 3), the coupling reaction did not proceed well. The dimethoxy acetal group was not stable to any of the reaction conditions attempted. When a more robust cyclic acetal was used, the coupling reaction proceeded well. However, the subsequent reduction of the imine in the pyrazoline ring was not successful.

Because of these complications, the second approach outlined in Scheme 4 quickly arose as the method of choice. In analogy to the earlier syntheses of **10**, this plan called for first reducing the pyrazoline ring and then protecting N_5 with a Cbz group. The reduced proline surrogate would then be coupled to the pentenoic acid derivative to form **15** and the aldehyde needed for the reductive amination reaction released with the use of an ozonolysis reaction.

The reduced pyrazoline **17** (Scheme 5) needed for this sequence was synthesized as outlined by Carriera.[1a] Using this procedure, **16** was treated with sodium cyanoborohydride, the N_5 nitrogen protected, and the chiral auxiliary used to synthesize the pyrazoline removed. With **17** in hand, we initiated efforts to synthesize a bicyclic analog having a 1,6-diazabicyclo[4.3.0]nonane ring skeleton (Scheme 6). This effort began with the coupling of the reduced pyrazoline **17** to a phthalimide-protected vinylalanine (**18**). The olefin in the resulting product **19** was cleaved using an ozonolysis reaction and then the Cbz protecting group removed and the intramolecular reductive amination completed using hydrogen over palladium on carbon. The ozonolysis-deprotection, reductive amination strategy afforded the bicyclic product **20** in an 80% combined yield.



Scheme 5.

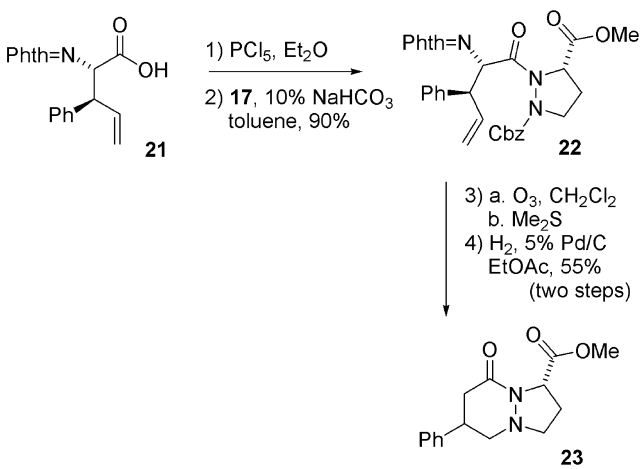


Scheme 6.

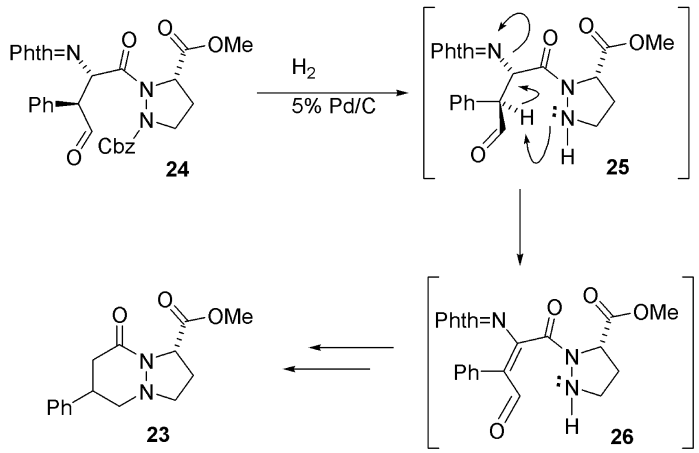
3. Building a functionalized TRH analog

With these initial studies completed, our interest in building constrained TRH analogs focused attention on the synthesis of analogs having either a phenyl or cyclohexyl substituent beta to the carbonyl in the lactam ring. While an analog of the natural TRH (*p*-GluHisPro-NH_2) would require an imidazole substituent on the lactam ring, the constrained analogs studied to date have replaced this imidazole group with either a phenyl or cyclohexyl ring.[5c] This was done in order to simplify the synthesis of the analogs. The change did significantly reduce the affinity of the analogs for the TRH endocrine receptor, however the constrained analogs synthesized did still bind the endocrine receptor and are fully potent at high concentrations.[5b] In the time since these initial analogs were studied, the methodology needed to build bicyclic analogs having an imidazole ring substituent has now been developed.[12] Nevertheless, for the current studies the phenyl and cyclohexyl substituents were still targeted for study so that the newly synthesized TRH analogs could be directly compared with the previously synthesized conformational probes **4** and **5**, as well as previously synthesized seven-membered ring lactam based probes.[6]

For the first analog, a phenyl substituent was selected (Scheme 7). This effort began by synthesizing amino acid derivative **21** from the known *t*-Boc protected 2-amino-3-phenylpentenoic acid.[11] The coupling of **21** to the reduced pyrazoline **17** proceeded well. However, subsequent cleavage of the olefin, removal of the Cbz group, and reductive amination did not afford the desired product. Instead, product **23** was obtained. Product **23** apparently arose from an elimination of the pthalimide group followed by reduction of the resulting olefin. An identical elimination reaction was observed when a trifluoroacetate protecting group was used in place of the pthalimide group in **21** and **22**. A possible mechanism for this transformation is suggested in Scheme 8. The key to this sequence is that removal of the Cbz group leads to the formation of an internal base. An intramolecular deprotonation reaction can then trigger the β-elimination reaction that forms a styrene derivative that is in turn reduced under the hydrogenation conditions. Since the elimination reaction was not observed



Scheme 7.



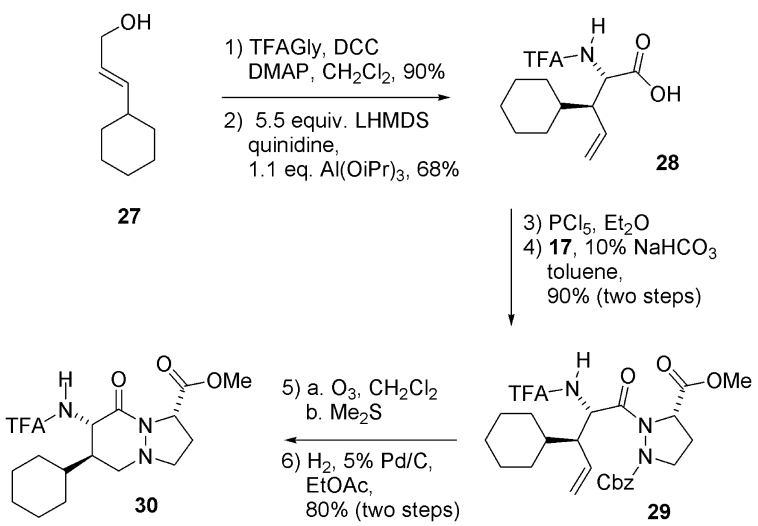
Scheme 8.

when the substrate without the phenyl ring (**19**/Scheme 6) was utilized for the sequence, nor when the reduced pyrazoline was replaced with proline, it would appear that both the presence of the phenyl ring and the internal base were required for the elimination reaction.

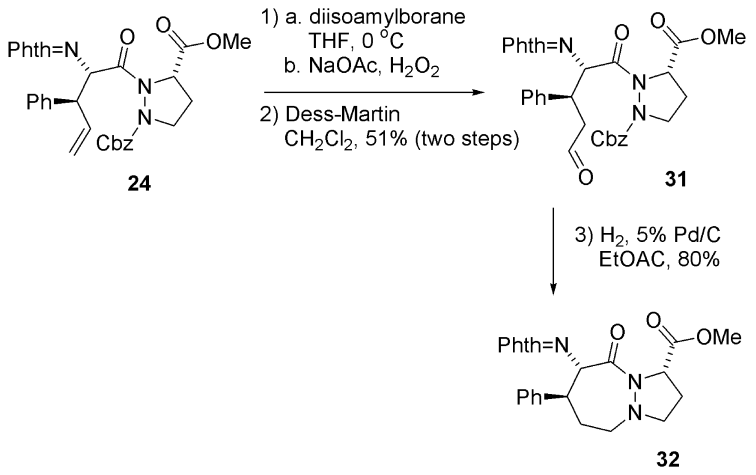
At this point, it was not clear if the elimination would remain a problem if a different substituent were employed beta to the amide carbonyl. We suspected that the elimination observed in Scheme 7 was aided by the presence of the aromatic ring and the formation of the styrene product. With this in mind, a TRH substrate having a cyclohexyl substituent beta to the amide carbonyl was targeted for synthesis. This effort began by constructing the required 3-substituted cyclohexyl derivative **28** (Scheme 9) by first coupling the known allylic alcohol **27**[5c] to a trifluoroacetic acid protected glycine using DCC and DMAP and then utilizing the chemistry developed by Kazmaier to accomplish an asymmetric enolate Claisen reaction.[11] The absolute stereochemistry of the product was assumed to be directly analogous to that obtained for the rearrangement utilizing a phenyl substituent in place of the cyclohexyl. The degree of enantiomeric excess was not determined at this point, but instead the product was immediately coupled to the reduced pyrazoline **17**. The resulting amide product **29** was isolated in a 90% yield as a single diastereomer. Compound **29** was subjected to the ozonolysis–reduction sequence described above in order to form an 80% isolated yield of the constrained TRH building block. Clearly, the use of the cyclohexyl substituent circumvented the earlier elimination problems encountered with the phenyl-substituted substrate and allowed for construction of the desired bicyclic building block.

4. Building seven-membered lactam ring analogs

Having established a rapid route for the synthesis of the bicyclic building block, we sought to extend the work to include the synthesis of building blocks having a 1,7-diazabicyclo[5.3.0]decane ring skeleton (Scheme 10). The synthesis started with the previously prepared intermediate **24**. In this case, the olefin was converted into an aldehyde using a hydroboration–oxidation sequence instead of the previously utilized ozonolysis. The result was an aldehyde one carbon longer that was perfectly set up for deprotection of the Cbz group and then intramolecular reductive amination to make the desired **32**. Because of the extra carbon in the chain, the elimination reaction that interfered in the six-membered ring lactam case was not an issue, and



Scheme 9.



Scheme 10.

the synthesis was perfectly compatible with the presence of the phenyl substituent beta to the amide carbonyl.

5. Conclusions

A synthetic route has been developed for rapidly incorporating pyrazoline derivatives into substituted bicyclic lactam peptidomimetics. The route, which is analogous to earlier efforts to synthesize related pipecolic acid based substrates, capitalizes on the availability of chiral 3-substituted-2-amino-4-pentenoic acid derivatives for constructing the N-terminal portion of the peptidomimetics. Bicyclic building blocks having either a six- or seven-membered ring lactam constraining the N-terminal side of the building block can by synthesized. In addition, the approach taken allows for the asymmetric synthesis of analogs that are substituted beta to the amide carbonyl. For the six-membered ring lactam cases, a competitive elimination reaction interfered with the final reductive amination reaction in the synthesis of analogs having a phenyl substituent beta to the lactam carbonyl. The elimination was not a problem for either substrates having a cyclohexyl substituent beta to the carbonyl or substrates leading to seven-membered ring lactams.

6. Experimental

6.1. Data for compounds

6.1.1. 4-{[(3*S*)-1-Benzylpyrazolidin-3-yl] carbonyl}-10,10-dimethyl-4-azatricyclo [5.2.1.0^{1,5}]-decane. To a solution of $^{2}\Delta$-pyrazoline **16** (830 mg, 2.67 mmol) was added $NaCNBH_3$ (419 mg, 6.67 mmol). The reaction mixture was stirred at room temperature for 1 h at which time it was diluted with EtOAc and quenched with the addition of a saturated aqueous solution of potassium carbonate. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed using a rotary evaporator and the resulting product dissolved in CH_2Cl_2 (10 mL). To this solution was added CbzCl (460 μL, 3.2 mmol) and triethylamine (408 μL, 2.9 mmol). The reaction was stirred at room temperature for 2 h and then the reaction mixture quenched by the addition of water. The aqueous layer was extracted with dichloromethane and the combined organic layers were washed with brine and dried over Na_2SO_4. The solvent was removed in vacuo and the residue submitted to purification by flash chromatography through silica gel (3:1 hexane/EtOAc) to afford the carbamate product (350 mg, 29% over two steps) as an oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.42–7.29m, 5H), 5.26 (a of ab, *J*=12.6 Hz, 1H), 5.15 (b of ab, *J*=12.6 Hz, 1H), 4.39 (t, *J*=7.5 Hz, 1H), 3.89 (dd, *J*=7.5, 5.4 Hz, 1H), 3.74–3.58 (m, 2H), 3.54 (a of ab, *J*=14.1 Hz, 1H), 3.45 (b of ab, *J*=14.1 Hz, 1H), 2.61– 2.57 (m, 1H), 2.19–2.06 (m, 3H), 1.94–1.88 (m, 3H), 1.43–1.22 (m, 2H), 1.45 (s, 3H), 0.98 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.0, 156.0, 136.5, 128.4, 128.3, 128.0, 127.9, 127.8, 67.4, 65.4, 59.9, 53.0, 48.9, 47.8, 46.8, 44.5, 38.1, 32.7, 32.5, 26.4, 20.7, 19.9; IR (neat/NaCl): 2960, 2885, 1697, 1455, 1411, 1335, 1136 cm^{-1}; LRMS (FAB) *m/e* (rel. intensity 454.1 (M+Li$^+$, 8), 313.1 (20), 160.1 (100); HRMS (FAB) calculated for $C_{22}H_{29}O_5N_3SLi$ (M+Li$^+$) 454.1988, found 454.1980.

6.1.2. 1-Benzyl 3-methyl (3*S*)-pyrazolidine-1,3-dicarboxylate (17). To a solution of the carbamates synthesized above (100 mg, 0.22 mmol) in CH_2Cl_2/MeOH (1:1, 3 mL) was added a 10% solution of $Mg(OCH_3)_2$ in MeOH (390 μL) at 0°C. The reaction mixture was stirred at same temperature for 1 h and monitored by TLC until the starting material disappeared. At the end of the reaction, CH_2Cl_2 (5 mL) was added followed by a saturated NH_4Cl solution (5 mL). The aqueous phase was extracted with CH_2Cl_2 several times and the combined organic phases were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified using flash chromatography through silica gel (hexane/EtOAc, 1:1) to afford compound **17** (44 mg, 74%) as a foamy solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.42–7.26 (m, 5H), 5.26 (a of ab, *J*=12.0 Hz, 1H), 5.15 (b of ab, *J*=12.0 Hz, 1H), 3.90 (t, *J*=7.5, 1H), 3.77–3.71 (m, 1H), 3.75 (s, 3H), 3.59–3.50 (m, 1H), 2.46–2.39 (m, 1H), 2.24–2.13 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.7, 155.9, 136.4, 128.3, 128.0, 127.9, 67.3, 59.6, 52.4, 45.8, 31.8; IR (Neat/ NaCl): 3256,

2959, 1737, 1696, 1437, 1400, 1348, 1214, 1118 cm^{-1}; LRMS (FAB) *m/e* (rel. intensity) 271 ($M+Li^+$, 100), 160 (90); HRMS (FAB) calculated for $C_{13}H_{16}O_4N_2Li$ 271.1270, found 272.1263.

6.1.3. Methyl 2-[2-(1,3-dihydro-2*H*-isoindol-2-yl) pent-4-enoyl]-1-benzylpyrazolidine-3-carboxylate (19). To a solution of **18** (110 mg, 0.45 mmol) in anhydrous ether (1 mL) was added PCl_5 (93.6 mg, 0.45 mmol) at 0°C. The reaction mixture was stirred at 0°C for 1 h until all of the solid dissolved. The solvent was then removed using a rotary evaporator and the residue placed on a vacuum line for 1 h in order to afford the acid chloride. The acid chloride was taken up in toluene (1.5 mL) and then added to a mixture of **17** (80 mg, 0.30 mmol) in toluene (1 mL) and 10% aqueous solution of $NaHCO_3$ (1 mL). The resulting reaction mixture was stirred overnight at room temperature. When the reaction was complete, EtOAc (20 mL) was added to the mixture and the organic phase washed with saturated $NaHCO_3$ and brine. The combined organic layers were then dried over Na_2SO_4, filtered, and concentrated in vacuo in order to provide a crude product that was purified by chromatography through silica gel (hexane/EtOAc, 1:1) to afford the pure coupled product **19** (130 mg, 87%) as an oil.

^1H NMR (300 MHz, $CDCl_3$): δ 7.87–7.70 (m, 4H), 7.45–7.26 (m, 5H), 5.70–5.59 (m, 1H), 5.28–5.18 (m, 3H), 5.02–4.91 (m, 3H), 4.02–3.94 (m, 1H), 3.62 (s, 3H), 2.84–2.80 (m, 2H), 2.65 (q, J=8.1 Hz), 2.31– 2.11 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ?170.6, 169.1, 167.2, 134.5, 134.2, 133.4, 131.3, 128.6, 128.5, 128.4, 123.7, 123.5, 118.6, 69.2, 64.9, 57.8, 52.5, 50.7, 47.1, 33.5, 29.4, 25.5; IR (Neat/NaCl): 2953, 1772, 1716, 1387, 1200 cm^{-1}; LRMS (FAB) *m/e* (rel. intensity) 498 ($M+Li^+$, 100), 313 (10), 160 (65); HRMS (FAB) calculated for $C_{26}H_{25}O_7N_3Li$ 498.1853, found 498.1834.

6.1.4. Methyl (1*S*,7*S*)-7-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-8-oxohexahydro-1*H*-pyrazolo[1,2-*a*]pyridazine-1-carboxylate (20). Ozone was bubbled through to a solution of **19** (120 mg, 0.25 mmol) in CH_2Cl_2 (5 mL) at −78°C until a purple blue color persisted. Oxygen was then passed through the solution to remove the excess ozone. After the color of the solution disappeared, dimethyl sulfide (180 μL, 2.5 mmol) was added at −78°C, the reaction mixture allowed to warm to room temperature, and the mixture stirred overnight. The solvent was then removed using a rotary evaporator and the residue passed through a short plug of silica gel in order to afford the aldehyde. The aldehyde was carried on without further purification.

To a solution of the aldehyde (70 mg, 0.14 mmol) in EtOAc (50 mL) was added 5% Pd/C (70 mg). The reaction mixture was stirred under H_2 atmosphere until no starting material was observed by TLC. The catalyst was removed by filtration through a short plug of silica gel and then the silica gel was washed with EtOAc. Removal of the solvents yielded the crude product. The crude product was chromatographed through silica gel (hexane/EtOAc/MeOH, 3:2:1) to afford product **20** (80% over two steps) as a foamy solid.

^1H NMR (300 MHz, $CDCl_3$): δ 7.84–7.80 (m, 2H), 7.71–7.66 (m, 2H), 4.94 (dd, J=10.5, 7.8 Hz, 1H), 4.63 (dd, J=9.3, 6 Hz, 1H), 3.77 (s, 3H), 3.50–3.45 (m, 1H), 3.33–3.28 (m, 1H), 2.94–2.80 (m, 2H), 2.78–2.69 (m, 1H), 2.55–2.48 (m, 1H), 2.32–2.20 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.8, 167.7, 167.5, 161.6, 134.6, 134.2, 132.3, 131.9, 123.9, 123.8, 57.3, 55.4, 52.3, 47.1, 28.6, 26.6; IR (neat/NaCl): 3472, 2953, 2848, 1716, 1643, 1393, 1175 cm^{-1}; GC–MS (EI) *m/e* (rel. intensity) 343 (M^+, 20), 229 (5), 186 (5), 143 (10), 83 (100).

6.1.5. 2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-3-phenylpent-4-enoic acid (21). The titled compound was made from the corresponding *t*-Boc protected amino acid derivative. The *t*-Boc protected amino acid[11] (1.27 g, 4.36 mmol) was dissolved in 3 M HCl–EtOAc (10 mL) and stirred at room temperature for 2 h. The solvents were removed under reduced pressure and the residue was again dissolved in a 5% Na_2CO_3 solution (20 mL). *N*-Ethoxycarbonylphthalimide (1.0 g, 4.6 mmol) was then added and the resulting solution was stirred at room temperature for 2 h. At the end of the reaction, the reaction mixture was washed with ether. The pH of the solution was then adjusted to 1 by the addition of 6N HCl and the aqueous phase was extracted three times with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4 and concentrated in vacuo to provide phthalimide **21** (1.0 g, 76%) as an oil. The purity of the product thus obtained was confirmed by NMR analysis.

^1H NMR ($CDCl_3$, 300 MHz): δ 7.71–7.60 (m, 4H), 7.14–7.12 (m, 4H), 7.10–7.01 (m, 1H), 6.20 (ddd, J=17.4, 9.9, 8.1 Hz, 1H), 5.27–5.18 (m, 2H), 4.59 (dd, J=10.8, 7.5 Hz); ^{13}C ($CDCl_3$, 75 MHz): δ 173.8, 167.1, 139.1, 137.9, 134.0, 131.0, 128.4, 127.9, 127.0, 123.4, 117.3, 54.9, 48.6; IR (neat/NaCl): 3084, 3063, 1832, 1775, 1720, 1386, 1068, 719 cm^{-1}; LRMS (FAB) *m/e* (rel. intensity) 322.1 ($M+H^+$, 20), 154 (100), 136 (90), 107 (45), 89 (90); HRMS (FAB) calculated for $C_{19}H_{16}O_4N$ ($M+H^+$) 322.1079, found 322.1078.

6.1.6. Methyl 1-benzyl-2-[2-(1,3-di-oxo-1,3-dihydro-2*H*-isoindol-2-yl)-3-phenylpent-4-enoyl] pyrazolidine-3-carboxylate (22). To a solution of **21** (282 mg, 0.88 mmol) in anhydrous ether (3 mL) at 0°C was added PCl_5 (183 mg, 0.88 mmol). The reaction mixture was stirred at 0°C for 1 h until the entire solid dissolved. The solvent was then removed using a rotary evaporator and the residue put on a vacuum line for another 1 h to afford the acid chloride. The acid chloride was taken up in toluene (3 mL) and then added to a mixture of **17** (120 mg, 0.45 mmol) in toluene (1 mL) and 10% aqueous $NaHCO_3$ (2 mL). The resulting reaction mixture was stirred overnight at room temperature. At the end of the reaction, EtOAc (30 mL) was added to reaction mixture and the organic phase washed with saturated $NaHCO_3$ and brine. The organic layer was then dried over Na_2SO_4, filtered, and concentrated in vacuo in order to provide the crude product. The crude product was purified by chromatography through silica gel (hexane/EtOAc, 1:1) to afford the coupled product **22** (230 mg, 90%) as an oil.

^1H NMR (300 MHz, $CDCl_3$): δ 7.66–7.02 (m, 9H), 5.92–5.89 (m, 1H), 5.46 (dd, J=10.5, 10.5 Hz, 2H), 5.20 (d, J=5.4 Hz, 1H), 5.10–4.95 (m, 3H), 4.60 (dd, J=11.1, 6.6 Hz, 1H), 4.00–3.91 (m, 1H), 3.65 (s, 3H), 2.47–2.41

(m, 1H), 2.20–2.10 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.6, 167.9, 166.6, 157.2, 138.8, 137.9, 137.4, 135.2, 134.1, 130.8, 128.8, 128.6, 128.5, 128.4, 128.1, 126.8, 123.2, 122.8, 116.5, 69.2, 57.5, 53.0, 52.5, 47.2, 46.4, 29.8; IR (neat/NaCl): 2953, 1719, 1384, 1199 cm^{-1}; LRMS (FAB) *m/e* (rel. intensity) 574 (M+Li$^+$, 100), 313 (10), 160 (60); HRMS (FAB) calculated for $C_{32}H_{29}O_7N_3Li$ 574.2166, found 574.2142.

6.1.7. Methyl 8-oxo-6-phenylhexahydro-1*H*-pyrazolo[1,2-*a*]pyridazine-1-carboxylate (23). Ozone was bubbled through a solution of **22** (200 mg, 0.35 mmol) in CH_2Cl_2 (15 mL) at −78°C until a purple blue color persisted. Oxygen was then passed through the solution to remove the excess ozone. After the color of the solution disappeared, dimethyl sulfide (256 μL, 3.5 mmol) was added to the −78°C reaction mixture. The reaction was then allowed to warm to room temperature and stirred overnight. The solvent was then removed using a rotary evaporator and the resulting residue passed through a short silica gel pad and washed with Et_2O to afford the aldehyde. The aldehyde was carried on without further purification.

To a solution of aldehyde synthesized above in EtOAc (60 mL) was added 5% Pd/C (100 mg). The reaction mixture was stirred under an atmosphere of hydrogen until TLC analysis no longer indicated the presence of starting material. The catalyst was removed by filtration through a short silica gel pad and was washed with EtOAc. Removal of the solvent and purification by flash chromatography through silica gel (Hexane/EtOAc, 1:3) yielded product **23** (53 mg, 55%) as a foamy solid.

^{1}H ($CDCl_3$, 300 MHz): δ 7.40–7.25 (m, 5H), 4.68 (dd, *J*=11.1, 2.2 Hz, 1H), 4.8 (s, 3H), 3.42–3.38 (m, 2H), 3.25–3.18 (m, 1H), 3.05–2.96 (m, 2H), 2.78 (a of abx, *J*=15.0, 8.1 Hz, 1H), 2.62 (b of abx, *J*=15.0, 8.1 Hz, 1H), 2.54–2.42 (m, 1H), 2.18–2.12 (m, 1H); ^{13}C ($CDCl_3$, 75 MHz): δ 171.2, 164.0, 141.7, 128.8, 127.2, 127.1, 58.6, 56.4, 54.1, 52.6, 38.9, 36.2, 28.0; IR (neat/NaCl): 2952, 1746, 1647, 1197 cm^{-1}; GC–MS (EI): *m/e* (rel. intensity) 274 (M$^+$, 80), 215 (20), 187 (25), 143 (100), 83 (70).

6.1.8. (2*E*)-3-Cyclohexylprop-2-enyl [(trifluoroacetyl) amino] acetate. To a solution of allyl alcohol **27** (4.0 g, 28.6 mmol) and TFA protected glycine (4.88 g, 28.6 mmol) in CH_2Cl_2 (120 mL) was added DCC (6.48 g, 31.5 mmol) and DMAP (1.74 g, 14.3 mmol). The reaction mixture was stirred at room temperature for 20 h. A white solid was formed that was then removed by filtration. The filtrate was washed with 1 M $KHSO_4$, 5% $NaHCO_3$, and brine. The organic phase was then dried over Na_2SO_4, and concentrated. The residue was purified by chromatography through silica gel (hexane/EtOAc, 3:1) to afford the ester product (8.0 g, 95%) as a pale yellow oil.

^{1}H ($CDCl_3$, 300 MHz): δ 7.00 (br s, 1H), 5.75 (dd, *J*=15.7, 6.3 Hz, 1H), 5.24 (td, *J*=15.7, 5.4 Hz, 1H), 4.62 (d, *J*=6.6 Hz, 1H), 4.12 (d, *J*=5.4 Hz, 1H), 2.02–1.92 (m, 1H), 1.88–1.62 (m, 5H), 1.35–1.01 (m, 5H); ^{13}C ($CDCl_3$, 75 MHz) 168.0, 143.7, 120.0, 67.6, 41.4, 40.3, 42.4, 26.0, 25.8; IR (neat/NaCl): 3337, 2927, 2853, 1716, 1555, 1450, 1182 cm^{-1}; LRMS (FAB) *m/e* (rel. intensity) 593.2 (2M+Li$^+$, 5), 300.1 (M+Li$^+$, 30), 231.2 (15), 178.0 (100); HRMS (FAB) calculated for $C1_3H_{18}F_3O_3NLi$ (M+Li$^+$) 300.1399, found 300.1405.

6.1.9. (2*S*,3*S*)-3-Cyclohexyl-2-[(trifluoroacetyl)amino]-pent-4-enoic acid (28). To a slurry solution of the ester synthesized in the preceding experiment (2.93 g, 10 mmol), quinidine (6.48 g, 20 mmol), and Al(O*i*Pr)$_3$ (2.25 g, 11 mmol) in THF (20 mL) at −40°C was added slowly LiHMDS (55 mmol, prepared by adding 22 mL of 2.5 M *n*BuLi in hexane to 13.7 mL of HMDS at −20°C in 20 mL of THF). The resulting solution was stirred at −40°C for an additional 1 h before it was allowed to warm to room temperature and stirred overnight. At the end of the reaction, 1N $KHSO_4$ was added to quench the reaction and the aqueous phase extracted with ether four times. The combined organic phase was dried over Na_2SO_4 and concentrated in vacuo in order to provide a crude product that was purified by recrystallization with *R*-methylbenzylamine to afford amino acid **28** (2.0 g, 68%) as a foamy solid.

^{1}H ($CDCl_3$, 300 MHz): δ 6.81 (br d, *J*=7.8 Hz, 1H), 5.52 (td, *J*=16.8, 10.2 Hz, 1H), 5.26 (dd, *J*=10.2, 2.1 Hz, 1H), 5.11 (dd, *J*=17.1, 1.8 Hz, 1H), 4.88 (dd, *J*=8.7, 5.1 Hz, 1H), 2.16 (ddd, *J*=10.2, 7.8, 2.1 Hz, 1H), 2.03 (br d, *J*=8.6 Hz, 1H), 1.8–0.8 (m, 10H); ^{13}C ($CDCl_3$, 75 MHz): δ 173.9, 134.8, 120.5, 53.7, 51.9, 37.2, 31.1, 30.8, 26.2, 25.9; IR (neat/NaCl): 3283, 2928, 2854, 1713, 1548, 1170 cm^{-1}; LRMS (FAB) *m/e* (rel. intensity) 294 (M+H$^+$, 50), 248 (20), 198 (100), 166 (50), 154 (30), 123 (30); HRMS (FAB) calculated for $C_{13}H_{18}O_3F_3N$ 294.1318, found 294.1314.

6.1.10. Methyl (3*S*)-2-{(2*S*,3*S*)-3-cyclohexyl-2-[(trifluoroacetyl) amino] pent-4-enoyl}-1-benzylpyrazolidine-3-carboxylate (29). To a solution of **28** (282 mg, 0.88 mmol) in anhydrous ether (3 mL) at 0°C was added PCl_5 (183 mg, 0.88 mmol). The reaction mixture was stirred at 0°C until the all of the solid dissolved. The solvent was then removed using a rotary evaporator and the resulting residue placed on a vacuum line for another 1 h to afford the acid chloride. The acid chloride was taken up in toluene (3 mL) and then added to a mixture of **17** (120 mg, 0.45 mmol) in toluene (1 mL) and 10% $NaHCO_3$ in H_2O (2 mL). The resulting reaction mixture was stirred overnight at room temperature. At the end of the reaction, EtOAc was added to the mixture and the organic phase washed with saturated $NaHCO_3$ and brine. The organic layer was then dried over Na_2SO_4, filtered, and concentrated in vacuo in order to provide the crude product. The crude product was purified by chromatography through silica gel (hexane/EtOAc, 1:1) to afford the product **29** (230 mg, 90%) as an oil.

^{1}H ($CDCl_3$, 300 MHz): δ 6.91 (br d, *J*=9.6 Hz, 1H), 5.62 (td, *J*=17.1, 6.9 Hz, 1H), 5.42 (d, *J*=12.0 Hz, 1H), 5.28 (t, *J*=10.2, 1H), 5.08–4.94 (m, 3H), 4.64 (dd, *J*=6.3, 6.0 Hz, 1H), 4.36–4.28 (m, 1H), 3.47 (s, 3H), 3.20 (q, *J*=10.2 Hz, 1H), 2.38–1.98 (m, 3H), 1.82–1.58 (m, 5H), 1.42–0.92 (m, 6H); ^{13}C ($CDCl_3$, 75 MHz): δ 171.6, 169.9, 157.9, 134.8, 134.0, 128.3, 128.1, 128.0, 119.4, 69.4, 57.3, 54.9, 52.5, 50.7, 47.4, 37.4, 32.0, 29.9, 27.6, 26.6, 26.3; IR (neat/NaCl): 2924, 2851, 1734, 1661, 1208, 1174 cm^{-1}; LRMS (FAB) *m/e* (rel. intensity) 546.2 (M+Li$^+$, 65), 412 (10), 313 (30),

160 (100); HRMS (FAB) calculated for $C_{26}H_{32}O_6F_3N_3Li$ (M+Li^+) 546.2403, found 546.2376.

6.1.11. Methyl (1*S*,6*R*,7*S*)-6-cyclohexyl-8-oxo-7-[(trifluoroacetyl) amino] hexahydro-1*H*-pyrazolo [1,2-*a*] pyridazine-1-carboxylate (30). Ozone was bubbled through a solution of **29** (200 mg, 0.37 mmol) in CH_2Cl_2 (15 mL) at −78°C until a purple blue color persisted. Oxygen was then passed through the solution to remove the excess ozone. After the color of the solution disappeared, dimethyl sulfide (270 μL, 3.7 mmol) was added to the reaction mixture. The resulting solution was then allowed to warm up to room temperature and stirred overnight. The solvent was removed using a rotary evaporator and the resulting residue passed through a short plug of silica gel to afford the aldehyde. The aldehyde was carried on to the next step without further purification.

To a solution of the aldehyde synthesized above in EtOAc (70 mL) was added 5% Pd/C (80 mg). The reaction mixture was stirred under an atmosphere of hydrogen until no starting material remained evident by TLC. The catalyst was removed by filtration through a short plug of silica gel and the silica gel then washed with EtOAc. The resulting solution was concentrated in vacuo and the resulting residue purified by chromatography through silica gel (hexane/EtOAc, 2:3) to afford **30** (80%) as an oil.

^{1}H ($CDCl_3$, 300 MHz):δ 7.45 (br s, 1H), 4.66 (dd, *J*=9, 1.8 Hz, 1H), 4.56 (dd, *J*=8.7, 5.7 Hz, 1H), 3.78 (s, 3H), 3.42 (dt, *J*=6.9, 2.4 Hz, 1H), 3.25 (t, *J*=10.2 Hz, 1H), 3.11 (dq, *J*=9.9, 3.3 Hz, 1H), 2.94–2.83 (m, 2H), 2.56–2.42 (m, 1H), 2.37–2.29 (m, 1H), 1.73–0.92 (m, 11H); ^{13}C ($CDCl_3$, 75 MHz): δ 170.4, 164.2, 56.5, 54.8, 53.5, 52.8, 51.3, 40.6, 37.1, 30.7, 29.8, 26.6, 26.2, 26.0; IR (neat/NaCl): 3248, 2924, 2851, 1745, 1720, 1653, 1448, 1211, 1180, 1155 cm^{-1}; GC–MS (EI) *m/e* (rel. intensity): 391 (M^+, 30), 332 ($M^+-COOCH_3$, 10), 308 ($M^+-C_6H_{11}$, 20), 248 (25), 143 (25), 83 (100); HRMS (FAB) calculated for $C_{17}H_{24}O_4N_3F_3Li$ (M+Li^+) 398.1879, found 398.1874.

6.1.12. Methyl (3*S*)-2-[(2*S*)-2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-3-phenyl-5-oxopentanoyl]-1-benzylpyrazolidine-3-carboxylate (31). To a solution of BH_3.MeS (100 μL, 10 M, 1 mmol) in THF (1 mL) at 0°C was slowly added 2-methyl-2-butene (212 μL, 2 mmol). The resulting solution was stirred at 0°C for 1 h after which the vinylphenylalanine derivative **24** (227 mg, 0.4 mmol) in THF (1 mL) was added via a cannula. The resulting solution was allowed to warm to room temperature and stirred for 4 h. The reaction was diluted with THF (5 mL) and saturated NaOAc (2 mL) and then H_2O_2 (2 mL) was added. The resulting mixture was stirred at room temperature for 8 h. Ether was then added to dilute the reaction, the layers separated, and the aqueous phase exacted three times with ether. The combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The resulting residue was purified using flash chromatography through silica gel (hexane/ether, 1:2) in order to afford the alcohol.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.63–7.01 (m, 9 aromatic H′s), 5.48 (d, *J*=11.7 Hz, 1H), 5.30–5.22 (m, 2H), 4.94 (dd, *J*=8.5, 5.7 Hz, 1H), 4.02–3.95 (m, 2H), 3.71 (s, 3H), 3.37 (t, *J*=5.7 Hz, 2H), 2.44–2.38 (m, 1H), 2.22–2.08 (m, 2H). The compound was carried onto the next step without further characterization.

To the above alcohol in CH_2Cl_2 (3 mL) was added Dess–Martin reagent (CH_2Cl_2 solution, 15% by weight) and the resulting solution stirred at room temperature for 4 h. 10% $NaHCO_3$ and saturated $Na_2S_2O_3$ were then added until both the aqueous and organic phases became clear. The organic phase was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash chromatography through silica gel (Hexane/Et_2O, 3:1) to afford the aldehyde **31** (115 mg, 51 % over two steps) as an oil.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.47 (s, 1H), 5.47 (d, t=12.0 Hz, 1H), 5.28–5.5.14 (m, 2H), 4.92 (dd, *J*=8.4, 5.7 Hz, 1H), 4.42 (dt, *J*=10.5, 3.3 Hz, 1H), 4.00–3.92 (m, 1H), 3.70 (s, 3H), 2.87–2.80 (m, 1H), 2.42–2.10 (m, 4H); ^{13}C ($CDCl_3$, 75 MHz): δ 200.0, 170.5, 167.5, 166.5, 156.4, 138.2, 135.2, 134.1, 128.9, 128.8, 128.7, 128.3, 128.2, 127.2, 123.3, 69.4, 57.6, 53.7, 52.6, 47.2, 46.6, 38.6, 29.7; IR (neat/NaCl): 2947, 2913, 2846, 1720, 1381, 1200 cm^{-1}; LRMS (FAB) *m/e* (rel. intensity) 590 (M+Li^+, 20), 269 (20), 160 (100); HRMS (FAB) calculated for $C_{32}H_{29}O_8N_3Li$ (M+Li^+) 590.2115, found 590.2124.

6.1.13. Methyl (1*S*,8*S*)-8-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-9-oxo-7-phenylhexahydro-1*H*,5*H*-pyrazolo [1,2-*a*][1,2]diazepine-1-carboxylate (32). To a solution of aldehyde **31** (60 mg, 0.10 mmol) in EtOAc (70 mL) was added 5% Pd/C (60 mg). The reaction mixture was stirred under an atmosphere of hydrogen until TLC analysis indicated that no starting material remained. The catalyst was removed by filtration through a short plug of silica gel and the silica gel washed with EtOAc. Removal of the solvents and purification by chromatography through silica gel (hexane/EtOAc, 2:3) afforded **32** (35 mg, 80%).

^{1}H ($CDCl_3$, 300 MHz): δ 7.67–7.63 (m, 2H), 7.58–7.52 (m, 2H), 7.27–7.01 (m, 5H), 5.86 (d, *J*=7.5 Hz, 1H), 4.64 (t, *J*=7.8 Hz, 1H), 3.99 (dt, *J*=9.9 Hz, 1H), 3.80 (s, 3H), 3.50–3.41 (m, 1H), 3.37–3.33 (m, 1H), 3.18–3.11 (m, 1H), 2.94–2.87 (m, 1H), 2.50–2.15 (m, 4H); ^{13}C ($CDCl_3$, 75 MHz): δ 171.8, 167.8, 166.2, 142.8, 133.5, 128.3, 127.6, 126.5, 123.0, 58.5, 56.5, 55.3, 54.9, 52.7, 42.8, 38.0, 29.8; IR (neat/NaCl): 3030, 2952, 1745, 1720, 1667, 1437, 1382, 1200 cm^{-1}; GC–MS (EI) *m/e* (rel. intensity): 433 (M^+, 25), 405 (M^+-CO, 20), 372 (20), 346 (50), 317 (30), 284 ($M^+-Phth{=}N$, 100), 207 (45), 185 (50), 143 (90), 104 (80), 83 (100); HRMS (FAB) calculated for $C_{24}H_{23}O_5N_3Li$ 440.1798, found 440.1789.

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