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Concise Total Synthesis of (±)-Salinosporamide A, (±)-Cinnabaramide A, and Derivatives via a Bis-Cyclization Process: Implications for a Biosynthetic Pathway?

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Abstract

4-Alkylidene-β-lactones (hetero ketene dimers) and α-amino acids are useful precursors for total syntheses of the β-lactone containing proteasome inhibitors, salinosporamide A, cinnabaramide A, and derivatives. A key step is a nucleophile-promoted, bis-cyclization of keto acids that simultaneously generates the γ-lactam-and β-lactone of these natural products. This reaction sequence may have implications for the biosynthesis of these natural products.

Omuralide (**1**), derived from lactacystin (**2**)[1] the salinosporamides e.g. salinosporamide A (**3**),[2] and the recently disclosed cinnabaramides e.g. cinnabaramide A (**4**)[3] are unique bicyclic β-lactone-containing natural products of bacterial origin that have recently attracted intense interest from both the synthetic and biological perspectives (Figure 1). Several synthetic efforts including numerous total syntheses of omuralide[1] and three syntheses of salinosporamide A[4] attest to the interest in these novel proteasome inhibitors due to their highly functionalized [3.2.0] bicyclic core and due to the validation of this therapeutic target for cancer.[5] Recent crystallographic studies have elucidated fascinating details regarding inhibition of the 20S proteasome by salinosporamide A involving acylation of the active site threonine by the β-lactone with concomitant cyclization of the incipient alkoxide with the C13 chloro substituent leading to a tetrahydrofuran.[6] Salinosporamide is currently in phase I human clinical studies for multiple myeloma.

We previously reported a catalytic, asymmetric intramolecular, nucleophile catalyzed aldol-lactonization (NCAL) process employing aldehyde acids that allows access to carbocycle-fused-β-lactones[7] and this process was recently extended to keto acid substrates.[8] This methodology was initially inspired by omuralide which contains such a bicyclic β-lactone core. Regarding the biosynthesis of these metabolites, one could speculate the joining of an appropriate amino acid **5** with an activated β-keto ester **6** followed by either an aldol-lactonization sequence[9] or a [2+2] cycloaddition via a ketene intermediate, a mechanism commonly invoked for related bis-cyclizations (Figure 1).[10]

Building on our work with carbocycle-fused-β-lactones, we envisioned a concise synthetic strategy to the bicyclic core of these natural products by simultaneous formation of the C-C and C-O bonds from a keto acid precursor **10** via an intramolecular bis-cyclization process (Figure 2, 10→**9**). Attachment of the cyclohexenyl moiety, or other side-chains, would rely on the strategy of Corey developed in the course of their salinosporamide synthesis on simpler aldehyde γ-lactam precursors[11] This would entail addition of a cyclohexenyl zinc reagent to

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the aldehyde derived from benzyl ether **9**, however the success of this process and subsequent manipulations was not guaranteed given the presence of the β-lactone.[12] The keto acid substrate **10** could be derived from coupling of an α-amino acid **11** and a ketene dimer **12**, the latter serving as a suitable latent equivalent for a β-ketoester.

Ultimately, we sought the development of an asymmetric strategy. However, one difficulty to be overcome was the potential for enolization of the substrate *β*-ketoamide rendering the ketone non-electrophilic and most importantly, the possibility of rapid racemization at C2. However, due to the known conformationally controlled acidity of β-ketoamides owing to $A^{1,3}$ strain,[13] retention of optical activity appeared plausible. Herein we describe the implementation of the first goal of this strategy; namely, the bis-cyclization process which has led to concise total syntheses of *rac*-salinosporamide A (**3**), *rac*-cinnabaramide A (**4**), and derivatives.

We began our studies with simple C2 unsubstituted substrates which were readily prepared by coupling of racemic ketene homodimer **14a**[14] with *N*-PMB-glycine benzyl ester (**13a**) by the method of Calter,[15] which proceeded efficiently to provide keto acid substrate **17a** following hydrogenolysis. We were pleased to find that bis-cyclization employing conditions similar to those developed for carbocycles,[8] using 4-pyrrolidinopyridine (4-PPY) as nucleophilic promoter, proceeded efficiently to give bicyclic-β-lactones **19a–d** (Table 1). However, the C2-unsubstituted ketoamide **17d** gave only 25% yield (entry 4). Without the C2-substitutent, facile enolization of the ketoamide likely leads to diminished rates of the initial aldol step. Interestingly, increased diastereoselectivity is obtained during bis-cyclization if the reaction is performed at 25 °C for extended times (1.5 d) by selective degradation of the minor diastereomer (confirmed by ^{1}H NMR reaction monitoring).

A likely mechanistic pathway for this bis-cyclization building on our related work with carbocycle-fused-β-lactones[8] involves initial activation of the carboxylic acid as pyridone ester **20** with modified Mukaiyama reagent **18**. Following transacylation with 4-PPY, deprotonation by Hünig's base leads to ammonium enolate **21**. Subsequent net aldol-lactonization via aldolate **22** then provides the *γ*-lactam-fused-β-lactone **19a** with concomitant regeneration of the nucleophilic promoter, 4-PPY. However, a [2+2] cycloaddition mechanism via an intermediate ketene has not been excluded at this time.[16] The relative configuration of the major diastereomeric β-lactone **19a** was confirmed by X-ray analysis following cleavage of the PMB group with ceric ammonium nitrate (CAN) to provide amide **23a** (Scheme 1). Importantly, the relative configuration corresponds to that found in the salinosporamides and the cinnabaramides.

We next studied the impact of a C2 substituent during the bis-cyclization by targeting the synthesis of cinnabaramide A (**4**). The synthesis commenced by reductive amination of commercially available *O*-benzyl-*L*-serine with *p*-anisaldehyde (Scheme 2). Subsequent esterification provided the protected serine derivative **11a** in 58% overall yield (2 steps). The required unsymmetrical ketene dimer **12a** was obtained by heterodimerization of acetyl and octanoyl chlorides.[14a] Coupling of ketene dimer **12a** with *(L)-N*-PMB-serine **11a** gave diastereomeric esters **25** (dr, 1:1) and subsequent Sn-mediated hydrolysis[17] provided acids **26** in good overall yield. The key bis-cyclization provided diastereomeric β-lactones **27**/**28** in 45% yield with moderate diastereoselectivity (dr 3.3:1), however the major diastereomer corresponded to that found in the natural product as verified by nOe analysis.[18] Deprotection of the benzyl ether enabled separation of the major alcohol diastereomer **29** (79%, dr >19:1) and this was followed by Parikh-Doering oxidation[19] to give an intermediate aldehyde which was used directly in the next step. Applying the method developed by Corey with zinc reagent **30**[4a] gave alcohols **31** in 57% yield (2 steps, dr 4.7:1). Finally oxidative cleavage of the PMB group gave *rac*-cinnabaramide A (**4**) which could be isolated diastereomerically pure in 48%

yield. Spectral data for the synthetic material correlated with the published data.[3] Further verification of relative configuration was accomplished by X-ray analysis.[18]

To further validate the mildness of this strategy, we targeted the synthesis of salinosporamide A bearing the required chloro-substituent in the keto acid substrate. In this case, *N*-PMB serine allyl ester **11b**, available in 2 steps from serine, was utilized to enable mild ester deprotection since cyclopropane formation was observed during attempted saponification of the corresponding keto methylester (not shown, *cf*. **25**) (Scheme 3). The amine **11b** was coupled with heteroketene dimer **12b**, readily available in gram quantities from heterodimerization of acetyl chloride and commercially available 4-chlorobutanoyl chloride,[18] to provide keto acids **3 3** following Pd-mediated ester deprotection. Bis-cyclization provided bicyclic-*β*-lactones **34**/**35** in 25-35% yield (dr 2-3:1) favoring the relative configuration found in salinosporamide A.[20] Deprotection of the benzyl ether enabled enrichment of the major diastereomer to 6-10:1 upon purification. Modified Moffatt[21] oxidation using 1-(1,3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDCI)[22] and dichloroacetic acid[23] followed by addition of zinc reagent **30** gave predominantly two diastereomeric alcohols **37** (dr 3.5:1) in 33% yield (2 steps).[24] Final deprotection of the PMB group enabled isolation of diastereomerically pure *rac*-salinosporamide A, which correlated with the published data and the relative configuration was further confirmed by X-ray analysis.[18]

In summary, we have developed concise synthetic routes to *rac*-salinosporamide A, *rac*-cinnabaramide A, and simplified derivatives. This strategy is unique in enabling simultaneous construction of both the γ-lactam and fused-β-lactone found in these metabolites via a bis-cyclization process. The β-lactone in these systems and the chloro-substituent in salinosporamide precursors is shown to be tolerant to several transformations which contributes to the brevity of the sequence.[1c] The described bis-cyclization process points to a logical biosynthetic origin for these intriguing natural products and raises the interesting question of whether such a bis-cyclization might be involved in the biosynthesis of these natural products.[9] Further optimization of this process including mechanistic studies and extension to an enantioselective strategy premised on $A^{1,3}$-strain in keto acid precursors, *e.g.* ketoacids **25** and **33**, constitute our ongoing efforts in this area.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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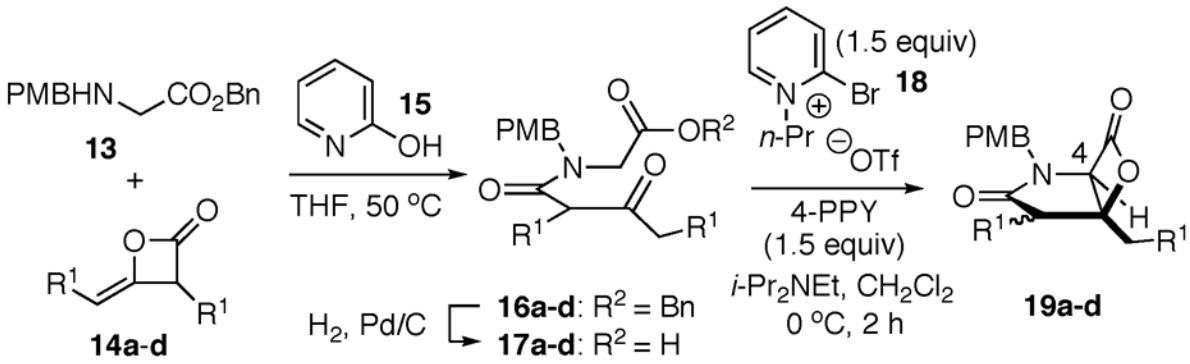
20. Lower yields in this bis-cyclization and that leading to cinnabaramide A (26→27, 28) in comparison with C4-unsubstituted substrates (Table 1) are clearly a result of increased steric issues during the bis-cyclization. While no starting material is recovered, two major byproducts have been identified, A and B (20–30% combined yield).



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24. Yields in this 2 step process are lower due to incomplete oxidation and inability to purify the aldehyde due to some sensitivity of this intermediate. Diastereoselectivity is considerably lower than that reported previously (see refs. 4a–c) however this appears to be highly substrate dependent given that Danishefsky observed reduced diastereoselectivity with *N*-unprotected substrates (ref. 4c).

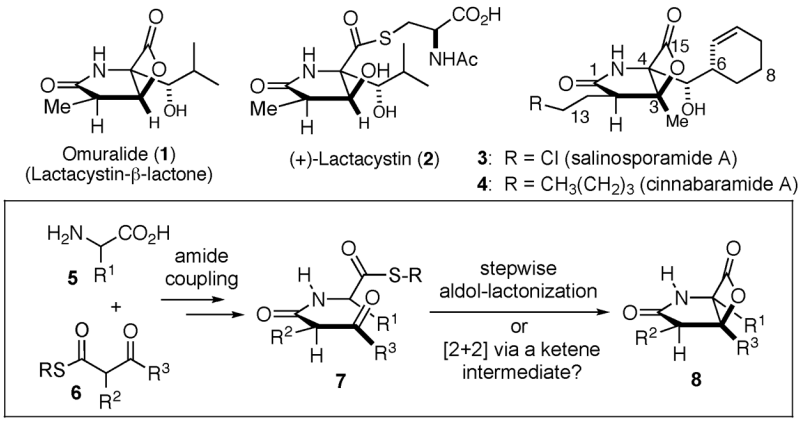
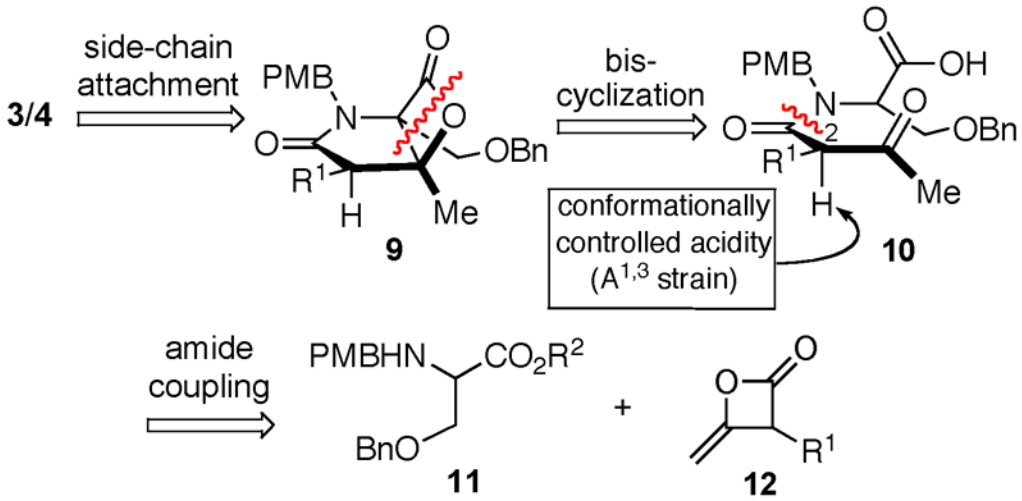
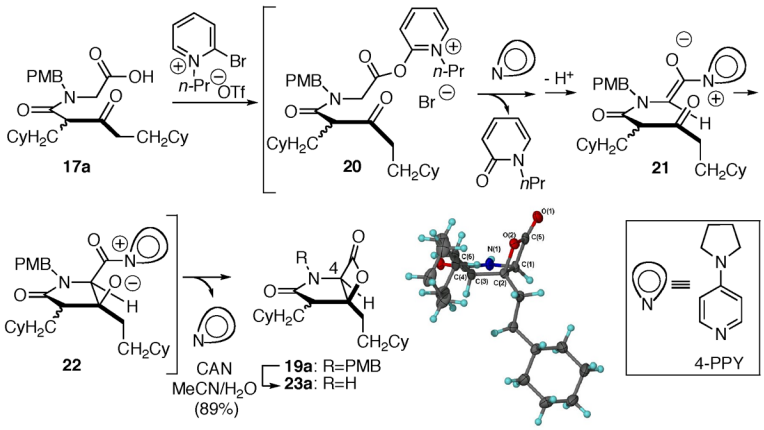


Figure 1.
Structures of proteasome inhibitors and a possible biosynthetic origin for the γ-lactam-fused-β-lactone core.

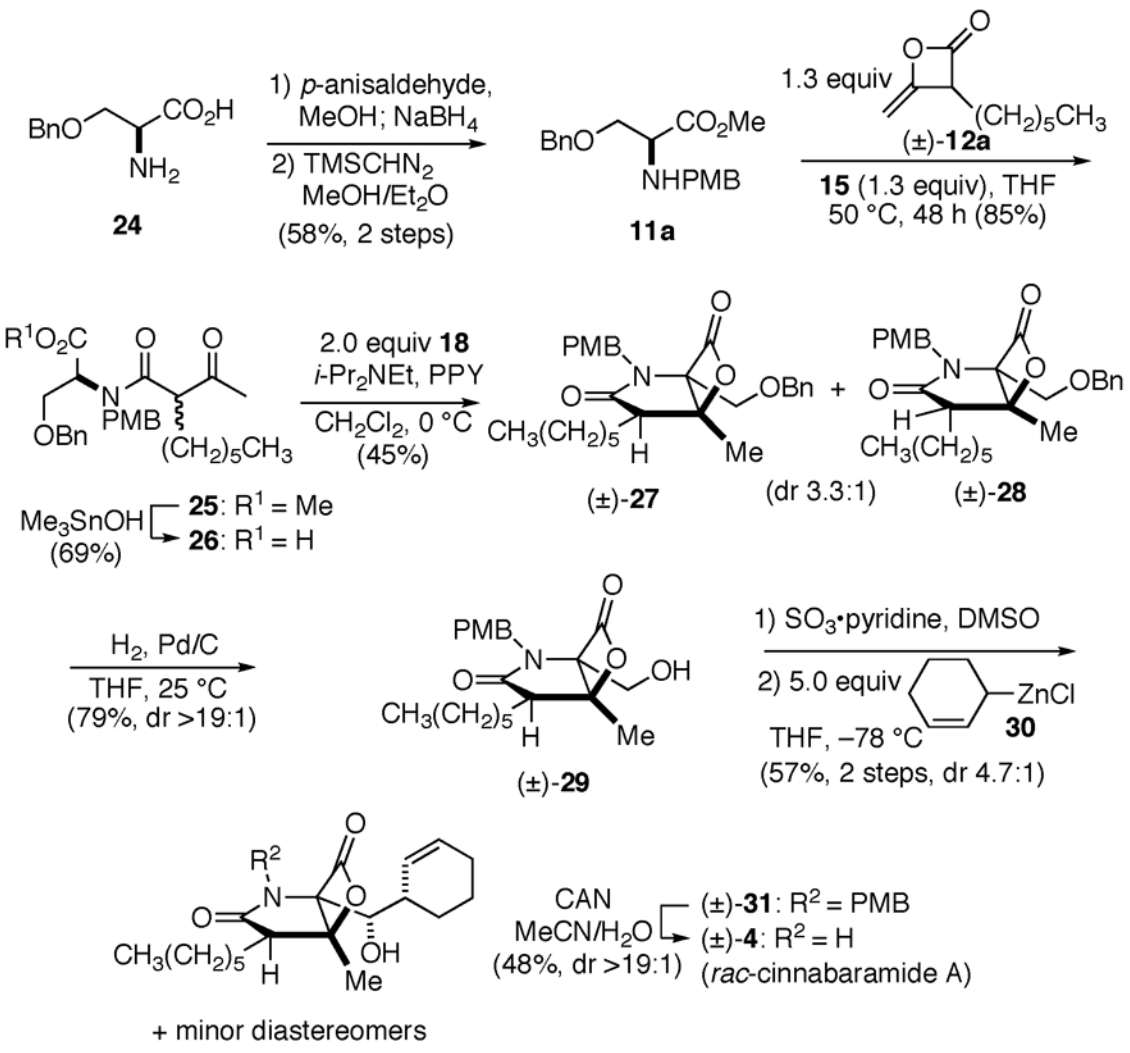


cinnabaramide A, and derivatives.

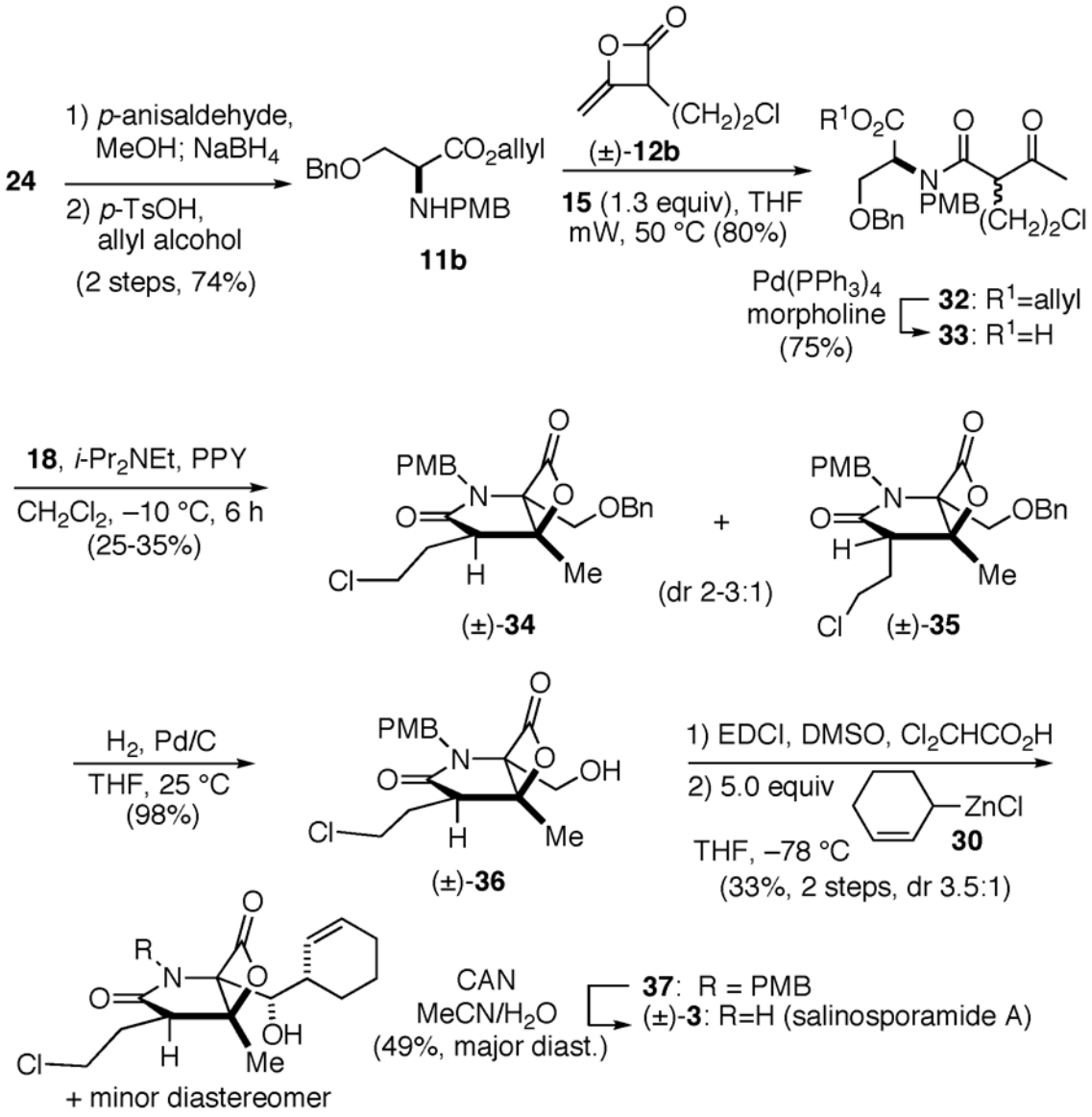
Figure 2.
Retrosynthetic analysis of salinosporamide A, cinnabaramide A, and derivatives.



Scheme 1.
Proposed mechanism for the bis-cyclization and X-ray structure of *β*-lactone **23a**



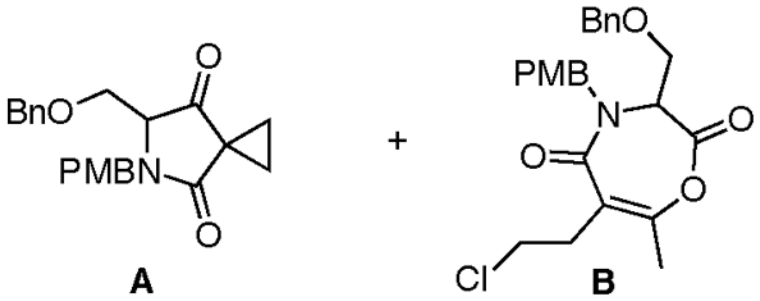
Scheme 2.
Total synthesis of *rac*-cinnabaramide A (**4**)



Scheme 3.
Total synthesis of *rac*-salinosporamide A (**3**)

Table 1

Synthesis of simplified, C4-unsubstituted salinosporamide/cinnabaramide derivatives **19a–d**



Entry	R^1	% yield (17)[a,b]	% yield (19)[b]	dr[c]
1	$CyCH_2$	84 (**17a**)	93 (**19a**)	2.2:1
2	*n*Hexyl	80 (**17b**)	90 (**19b**)	2.2:1
3	$PhCH_2$	72 (**17c**)	85 (**19c**)	2.5:1 (>19:1)[d]
4	H	77 (**17d**)	25 (**19d**)	-

[a] Yield is for 2 steps.

[b] Yields refer to isolated, purified (SiO_2) product.

[c] Determined by ^1H NMR analysis of crude reaction mixtures.

[d] Observed diastereomeric ratio (dr) if reaction is allowed to proceed at 25 °C for 1.5 d (54% yield). PMB = *p*-methoxybenzyl, 4-PPY = 4-pyrrolidinopyridine, Cy = cyclohexyl.