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An Efficient RCM-Based Synthesis of Orthogonally Protected *meso*-DAP and FK565[†]

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Received August 16, 2004

Abstract: A condensation−ring-close−ring-open sequence was employed for the synthesis of orthogonally protected *meso*-2,6-diaminopimelic acid, starting from easily accessible chiral synthons. Condensation of suitably protected L-allylglycine and D-vinylglycinol derivatives was followed by Grubbs' ring-closing metathesis to generate the key lactam intermediate. This strategy has been applied to a concise total synthesis of the potent immunostimulatory peptide FK565.

Peptidoglycan is a key structural component in the cell walls of most pathogenic bacteria.[1] The structural integrity of the rigid peptidoglycan layer is highly dependent on *meso*-2,6-diaminopimelic acid (*meso*-DAP), which acts as a cross-linking agent between glycan strands.[2] Presumably, therapeutics that inhibit the diaminopimelate pathway will serve to disrupt the biopolymerization process necessary for peptidoglycan formation. Because mammals do not produce DAP, such inhibitors are not likely to exhibit toxicity in humans, making them attractive drug targets in the search for novel antibacterial agents.[2] In addition, a number of peptidoglycan fragments featuring the DAP residue exhibit antitumor, immunostimulant, and sleep-inducing biological activity.[3] DAP is thus a versatile building block with a number of potential medicinal applications.

To obtain DAP derivatives of practical synthetic value, a preparative strategy should allow for functional group differentiation and be amenable to analogue synthesis. Although stereospecificity is a key objective in the synthesis of DAP, the goal of orthogonal protection is generally considered to be equally important. Recently, a number of reports have appeared describing the stereospecific synthesis of differentially protected DAP derivatives.[4,5] Most of these syntheses involve setting one of the two chiral centers by stereoselective susbtrate-directed reactions or the use of chiral reagents.

The structure of DAP suggests that ring-closing metathesis (RCM) can be an effective tool for construction of the seven-membered carbon chain.[6] Toward this end, we envisioned the condensation of two olefin building blocks, providing a suitable nine-carbon RCM substrate. Following ring closure, hydrolysis of the cyclic intermediate at the original point of attachment would then provide a linear carbon-bridged product. Here, we present an efficient preparation of orthogonally protected *meso*-DAP, and of the potent immunostimulatory DAP-containing peptide FK565, based on this condensation−ring-close−ring-open sequence.

In planning our synthesis, D-vinylglycine and L-allylglycine (**1**) were initially selected as appropriate chiral synthons, but the known tendency for vinylglycine to isomerize[5e] prompted us to turn to D-vinylglycinol (**2**) as an alternative four-carbon building block. Vinylglycinol is also advantageous in providing an additional site for condensation, via the hydroxyl group. Thus, two viable retrosynthetic routes are depicted in Scheme 1. The cyclic olefins represent key lactone and lactam intermediates resulting from the RCM of linear ester and amide precursors, respectively.

Our synthesis of the desired ester RCM substrate, from Boc-D-vinylglycinol[7] and Cbz-L-allylglycine (**3**), is depicted in Scheme 2. Condensation of these building blocks proceeded in good yield under Mitsunobu conditions to give compound **4**.

With the diene ester in hand, we turned our attention to the corresponding amide intermediates for the lactam-based strategy (Scheme 3). Although the lactam and lactone routes are conceptually similar, the introduction of a Boc group onto the amide nitrogen would be necessary to allow for eventual hydrolysis.[8] To avoid concomitant protection of the carbamate nitrogen during this

[†] Dedicated to the memory of Professor Murray Goodman, for a lifetime of achievements in bioorganic chemistry.

[‡] Deceased June 1, 2004.

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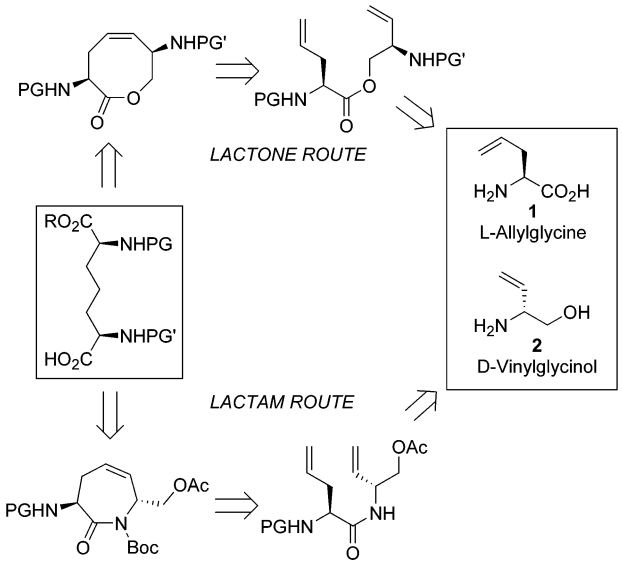
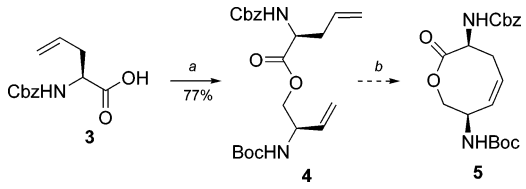
10.1021/jo0485738 CCC: $27.50
Published on Web 11/09/2004

SCHEME 1. Retrosynthesis of Orthogonally Protected *meso*-DAP

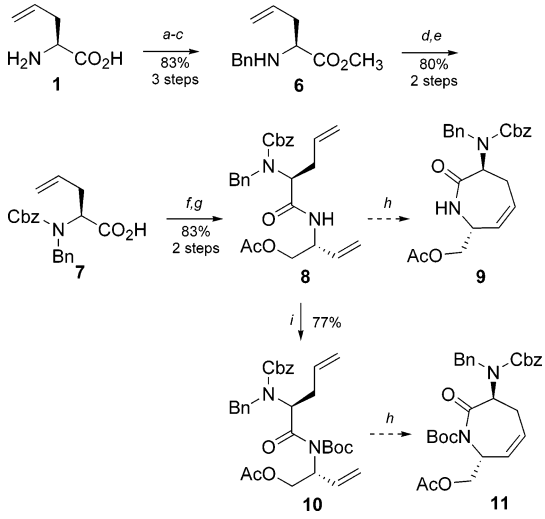


SCHEME 2. Synthesis of Ester Diene 4[a]



[a] Reagents and conditions: (a) (*N*-Boc)-D-vinylglycinol, PPh_3, DIAD, THF; (b) RCM (see Table 1).

SCHEME 3. Synthesis of Amide Dienes 8 and 10[a]



[a] Reagents and conditions: (a) $SOCl_2$, MeOH; (b) PhCHO, TEA, MeOH; (c) $NaBH_4$, MeOH; (d) CbzCl, 10% aq Na_2CO_3, EtOAc; (e) 2 M aq NaOH, MeOH; (f) D-vinylglycinol (**2**), PyBOP, TEA, DCM; (g) Ac_2O, pyridine, DCM; (h) RCM (see Table 1); (i) Boc_2O, DMAP, THF.

process, N,N-diprotected derivative **7** was synthesized for condensation with D-vinylglycinol (**2**). Amide bond formation mediated by PyBOP was followed by hydroxyl acetylation to give **8** in 83% yield. Because Boc protection of the resulting amide could theoretically be carried out before or after RCM, diene **8** was deemed a suitable substrate for ring closing. Alternatively, compound **10** was prepared by treatment of **8** with excess Boc_2O and catalytic DMAP.

TABLE 1. RCM of Diene Substrates 4, 8, and 10[a]

diene	catalyst[b]	mol %	time (h)	product[c]	yield[d] (%)
4	A	5	20	**5**	NR
4	A	30	28	**5**	NR
4	B	5	18	**5**	8
8	A	5	18	**9**	NR
8	A	50	48	**9**	NR[e]
10	A	5	48	**11**	19
10	A	30	20	**11**	40
10	B	2	2	**11**	99
10	B	2	48	**11**	64[f]

[a] All reactions, except those noted, were carried out at 0.01 M in DCM at reflux. [b] A = Grubbs' first-generation catalyst, B = Grubbs' second-generation catalyst. [c] Desired cyclic product. [d] Isolated yield after silica gel column. NR = no reaction. [e] Reaction concentration was 0.05 M. [f] Reaction was carried out at room temperature.

Dienes **4**, **8**, and **10** were subjected to a variety of olefin metathesis conditions along with Grubbs' first- and second-generation catalysts (Table 1).[9] As expected, we encountered difficulty in forming the eight-membered lactone **5**. Successful examples of eight-membered ring formation by Grubbs' RCM are relatively scarce and generally rely on a conformational bias to position the olefins within a reactive proximity.[6b,10] While no reaction was observed employing Grubbs' first-generation catalyst (**A**), utilization of the second-generation catalyst (**B**) resulted in a complex, largely inseparable mixture of products.

Amide **8** surprisingly failed to give any of the desired lactam product (**9**) when treated with catalyst **A**, even after extended reaction times at reflux. We were encouraged to see that *N*-Boc amide **10**, when subjected to the same conditions, underwent ring closure to give lactam **11** in 19% yield. Although the presence of a Boc group in **10** most likely increases the population of the reactive cis amide rotamer, high catalyst loading and extended reaction times failed to push this reaction to completion. By employing 2 mol % of catalyst **B**, however, we found that quantitative formation of **11** was complete within 2 h at reflux. We have prepared lactam **11** on up to a 20 mmol scale without any compromise in yield. It is interesting to note that the same reaction carried out at room temperature was still incomplete after 2 days.

The synthesis of orthogonally protected *meso*-DAP from intermediate **10** was completed as shown in Scheme 4. Beginning with the linear amide, we have developed an efficient five-step sequence which requires only minimal purification en route to the final product. After olefin metathesis, as described above, the crude lactam was treated with LiOH in THF/water to effect simultaneous hydrolysis of the amide and acetate ester bonds. The crude hydroxy acid was then esterified by alkylation, and

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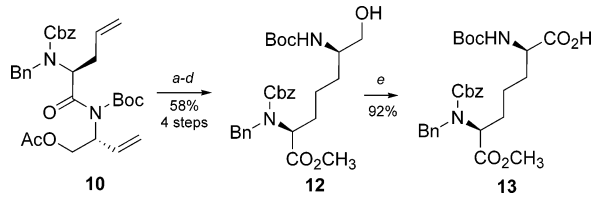
SCHEME 4. Synthesis of Orthogonally Protected DAP Derivative 13 from Diene 10[a]



[a] Reagents and conditions: (a) 2 mol % Grubbs' second-generation catalyst, DCM, reflux, 2 h; (b) 2 M aq LiOH, THF; (c) MeI, K_2CO_3, DMF; (d) 2 mol % [Ir(COD)PyPCy$_3$]PF_6, H_2, DCM; (e) NaClO, $NaClO_2$, TEMPO, MeCN/H_2O.

the cis olefin was selectively hydrogenated using 2 mol % of the Crabtree catalyst. Alcohol **12** was isolated in 58% overall yield from **10** after rapid filtration through a pad of silica gel. Finally, oxidation with sodium chlorite, bleach, and TEMPO[11] gave orthogonally protected DAP derivative **13** in good yield.

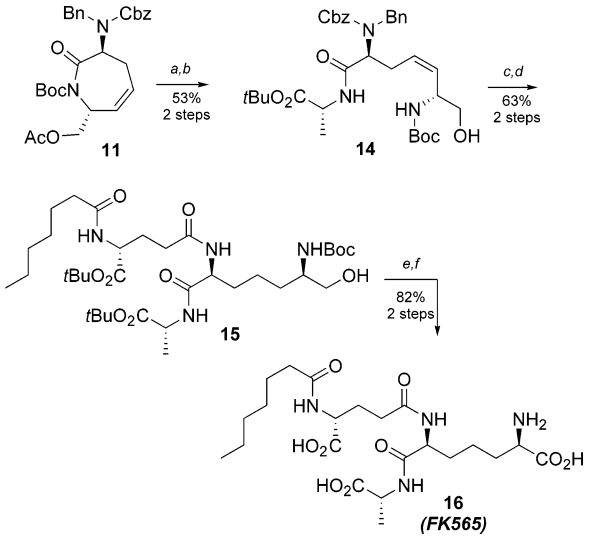
Although the ^{1}H NMR spectrum of **13** taken in $CDCl_3$ was complicated by the presence of multiple rotamers, the ^{13}C NMR spectrum of **13** and a ^{1}H NMR spectrum taken in DMSO-d_6 both exhibit only one set of resonances. After treatment of an analytical sample of **13** with 50% TFA/DCM (for Boc group removal), a RP-HPLC spectrum also showed a single compound, supporting the purity of the product.[12]

In an effort to extend our strategy to the preparation of biologically active DAP-containing structures, we undertook the stereoselective synthesis of the potent immunostimulatory peptide FK565 (**16**).[3d,g] A close analogue of the natural product FK156, FK565 was discovered at Fujisawa Pharmaceutical Co. and was found to exhibit various biological activities including tumoricidal and antimicrobial effectiveness in animal models.[13] Alone, and in combination with zidovudene (AZT), FK565 also inhibits retroviral infection by the Friend leukemia virus in mice, indicating its potential for use in the treatment of AIDS.[14]

Conceptually, acyl tripeptide **16** should be easily accessible from an appropriately protected *meso*-DAP derivative. Although the synthesis of FK565 has been described previously, both routes rely on enzymatic hydrolysis to obtain the key DAP building block.[13d,15] By employing lactam intermediate **11**, we envisioned a more direct route to the target structure.

As shown in Scheme 5, hydrolysis of lactam **11** afforded the crude hydroxy acid, which was used directly in peptide coupling. Condensation with H-Ala-O*t*Bu, mediated by DEPBT, provided the dipeptide alcohol (**14**) in

SCHEME 5. Synthesis of FK565 (16) from Lactam 11[a]



[a] Reagents and conditions: (a) 2 M aq LiOH, THF; (b) D-Ala-O*t*Bu, DEPBT, TEA, DCM; (c) 20% $Pd(OH)_2$/C, H_2, MeOH; (d) α-*tert*-butyl-(*N*-heptanoyl)-D-glutamate, PyBOP, TEA, DCM; (e) NaClO, $NaClO_2$, TEMPO, MeCN/H_2O; (f) 95% TFA/DCM.

53% overall yield. None of the eight-membered lactone byproduct was observed despite the possibility for intramolecular ring closure. Compound **14** was hydrogenated in the presence of 20% $Pd(OH)_2$/C to effect concomitant removal of the Cbz and benzyl protecting groups and reduction of the olefin. The crude hydroxyamine was then coupled with α-*tert*-butyl-(*N*-heptanoyl)-D-glutamate[16] to give tripeptide alcohol **15**.

Mild oxidation of **15** and treatment of the product with 95% TFA/DCM afforded **16** in 82% overall yield. The RP-HPLC spectrum of **16** indicated >95% purity without the need for further purification. High-resolution mass spectrometry confirmed the chemical composition of **16**, and the ^{1}H NMR spectrum we obtained was in good agreement with that reported in the literature for FK565.[15]

In summary, a novel route toward orthogonally protected *meso*-DAP and FK565 has been described. The starting materials for our synthesis are easily accessible chiral synthons, obviating the need for stereoselective reactions. This strategy features a Grubbs' ring-closing metathesis reaction as the key carbon−carbon bond-forming step and is complementary to recent reports utilizing asymmetric reduction or alkylation. These results should find application in the concise synthesis of various biologically relevant DAP-containing compounds as well as close structural analogues of DAP.

Acknowledgment. We thank Joseph Taulane for helpful discussions and the National Institutes of Health for support of this research (NIH DA 05539).

Supporting Information Available: Experimental procedures and spectral data for selected compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0485738

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