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A Study of the Paterson Boron Aldol Reaction as Used in the Large-Scale Total Synthesis of the Anticancer Marine Natural Product (+)-Discodermolide

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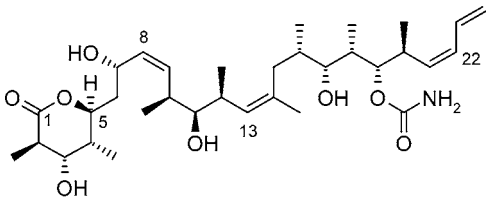
Abstract:

This note discusses an optimisation study of a key reagent-controlled enantioselective boron enolate aldol reaction forming the C_6-C_7 bond and the C_7 hydroxyl-bearing stereocenter in (+)-discodermolide. Conditions were found which increased the yield, decreased the excess of enolate necessary, and increased product stability with respect to the published procedure.



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Introduction

A small but structurally diverse collection of naturally occurring non-taxane microtubule stabilizing agents (MTS) has been discovered over the past decade. These include the epothilones (EPO), eleutherobin, laulimalide, and discodermolide. (+)-Discodermolide (**1**) is a novel polyketide natural product first isolated from extracts of the marine sponge *Discodermia dissoluta* by researchers at Harbor Branch Oceanographic Institution (HBOI).[1] Discodermolide stabilizes microtubules faster and more potently than any of the other known MTS agents, is a potent inhibitor of tumor cell growth in vitro including paclitaxel (PTX)- and EPO-resistant cells.[2] Discodermolide also demonstrates significant human tumor growth inhibition in hollow fiber and xenograft mouse models (including paclitaxel-resistant tumors).[3]

Structurally, discodermolide consists of a linear polypropionate chain containing thirteen stereocentres, six of which are hydroxyl-bearing, with one of these esterified as a δ-lactone (C_5) and another as a carbamate (C_{19}). It also features seven methyl-bearing stereocentres and three *Z*-configured alkenes, one of these being part of the terminal diene unit. Also present in the structure is a common stereotriad (methyl, hydroxyl, and methyl) that is repeated three times. The Schreiber group has synthesized both antipodes, thus establishing the absolute configuration of **1**.[4] Since the publications of Schreiber's synthesis, several total syntheses[5–8] and preparations of various discodermolide fragments[9] have appeared in the literature. A useful review of the available synthetic approaches has recently been published.[10]

The compound supply for development cannot be met through the isolation and purification of discodermolide from *Discodermia sp.* (which must be harvested using manned submersibles). Attempts to reproducibly isolate a discodermolide-producing microorganism for fermentation have not

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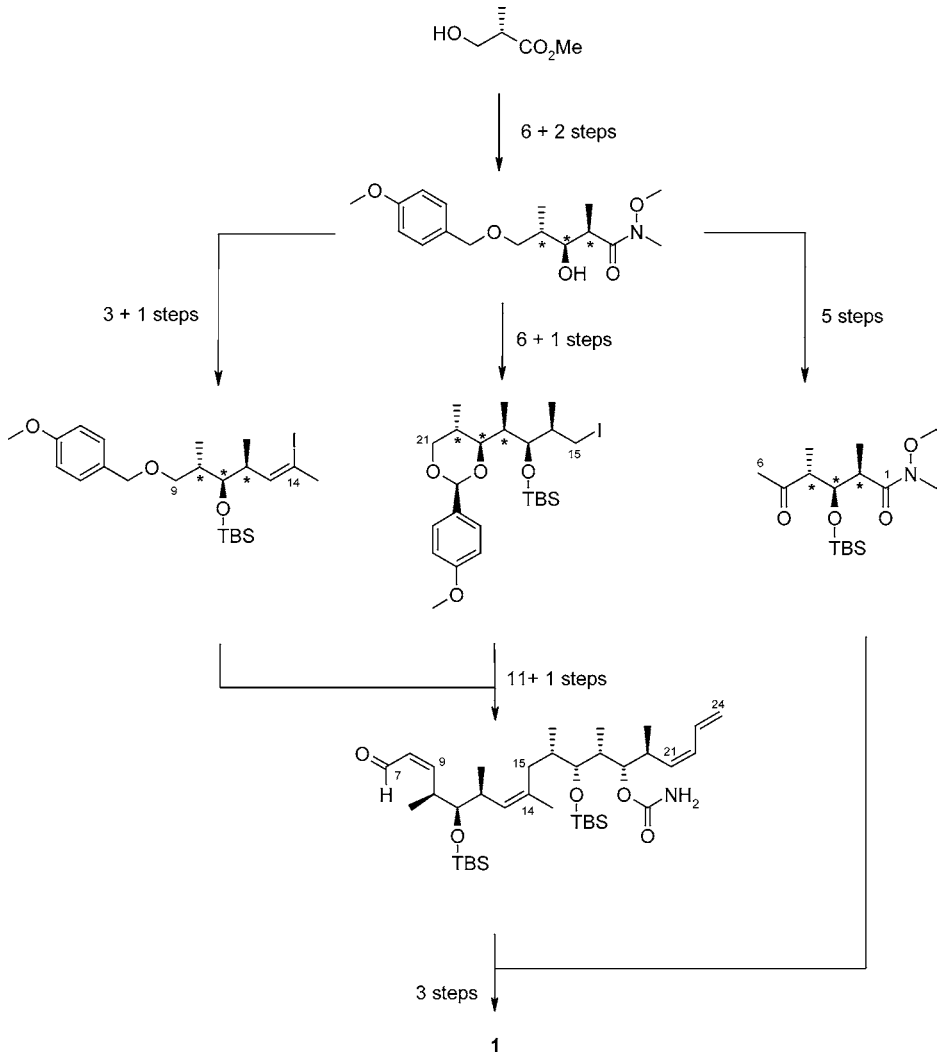

Figure 1. **Novartis synthetic route to (+)-discodermolide.**

been successful to date. Therefore, all discodermolide used for late preclinical research and development activities has been supplied by total synthesis.[11]

The synthetic route used for the preparation of multigram amounts of discodermolide (Figure 1) was envisaged as a hybrid synthesis which advantageously incorporated the best features of the published syntheses by Smith and Paterson (vide infra). Selection of these two syntheses was made after a detailed analysis of every publication on discodermolide and related syntheses.

Embedded within this synthetic sequence are three aldol reactions (Figure 2), two Evans *syn*-aldol reactions for the assembly of the $C_{10}-C_{11}$ and $C_{16}-C_{17}$ bonds, respectively, and one Paterson aldol which constructs the C_6-C_7 bond and sets the stereochemistry of the C_7-hydroxyl group.

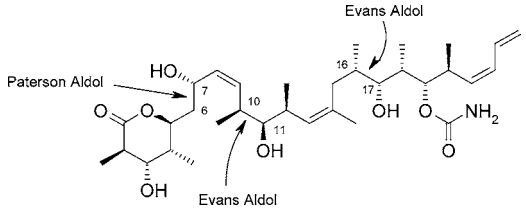

Figure 2. **Key aldol bond-forming reactions used in the Novartis synthetic route to (+)-discodermolide.**

Complex Aldol Coupling. The key Paterson aldol coupling as utilised by us[11e] is shown in Scheme 1. This reaction is a crucial element in the synthesis as it completes

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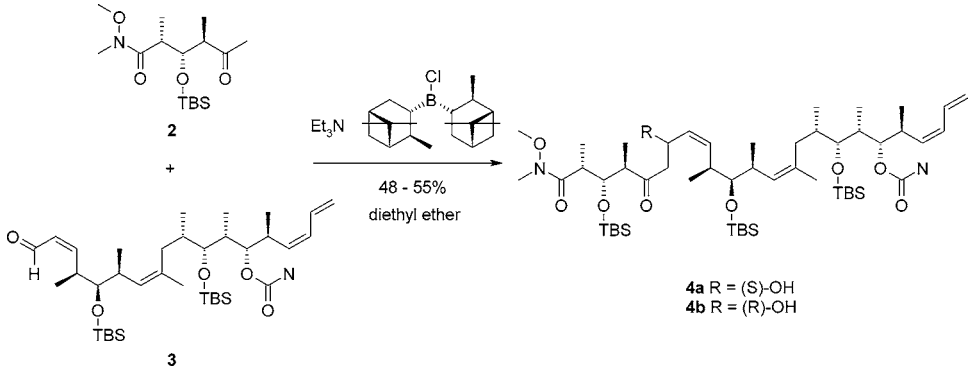
Scheme 1. **Paterson aldol reaction**



the construction of the discodermolide skeleton and establishes the correct (*S*) stereochemistry of the C_7 hydroxy group. Paterson has extensively studied this type of system[12] and has found that the natural stereochemical bias of the substrate (i.e. *re* face attack of the (*Z*)-enal **3**) is completely overturned by the chiral boron reagent to give reasonably high levels of 1,4-stereoinduction in the direction required for (+)-discodermolide.[8]

We found this process to be suboptimal[11e] for the following reasons:

(1) A large excess of enolate (5.4 equiv, based on aldehyde) is required to push the reaction to completion, complicating workup and purification.

(2) A solvent change is necessary before beginning the oxidative workup.

(3) Aqueous quench and evaporation of the reaction mixture (before oxidative workup) leads to significant decomposition of the product **4a** and its epimer **4b**.

(4) Oxidative workup leads to significant decomposition of the product and its epimer.

(5) Under certain reaction conditions the *cis*-enal **3** isomerises almost quantitatively to the corresponding *trans*-enal **6** which then undergoes the aldol reaction producing the trans isomers **5a** and **5b** (Figure 3).

(6) The product is not crystalline.

(7) Tedious reverse-phase silica gel chromatography requires large quantities of solvent.

This technical note describes our attempts to address some of these problems and discusses the optimisation of this key reaction in the (+)-discodermolide synthesis.

Results and Discussion

Analysis of Reaction Products. Compounds **2** and **3** were prepared according to the literature procedure.[11] In addition to the desired product, **4a**, the other products isolated during the optimisation of this process are shown in Figure 3. These compounds were all isolated and identified (NMR, HPLC) by comparison with authentic samples.[11e] An HPLC method (see Experimental Section) was devised which allowed the detection of all of these compounds in the reaction mixture (Table 1). Furthermore the method allowed us to quantify the amount of **4a** formed during the reaction by comparison of peak intensities with that of a reference sample. The results were then expressed as % chemical yield.

Initial Reaction Conditions. The conditions previously described,[11e] already an improvement to the original conditions where 10 equiv of the enolate were required to run this reaction successfully,[8c] were initially employed for this investigation, briefly: A solution of 6.6 equiv of ketone **2** in diethyl ether (2.1 M solution) was added to 5.4 equiv of

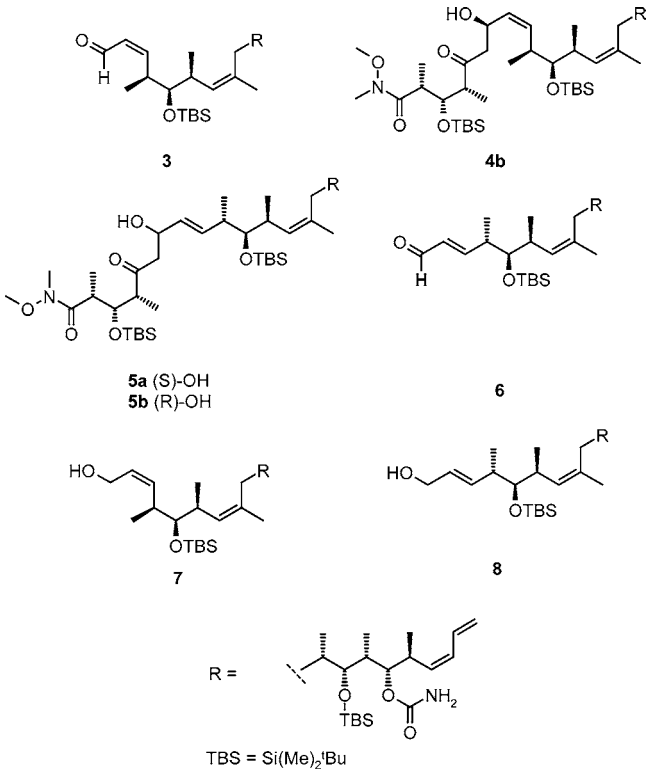


Figure 3. **Isolated reaction products.**

Table 1. **HPLC retention times of reaction products**

cmpd	retention time (min)	cmpd	retention time (min)
3	4.59	**5b**	7.93
4a	7.26	**6**	4.97
4b	8.27	**7**	4.14
5a	7.71	**8**	3.59

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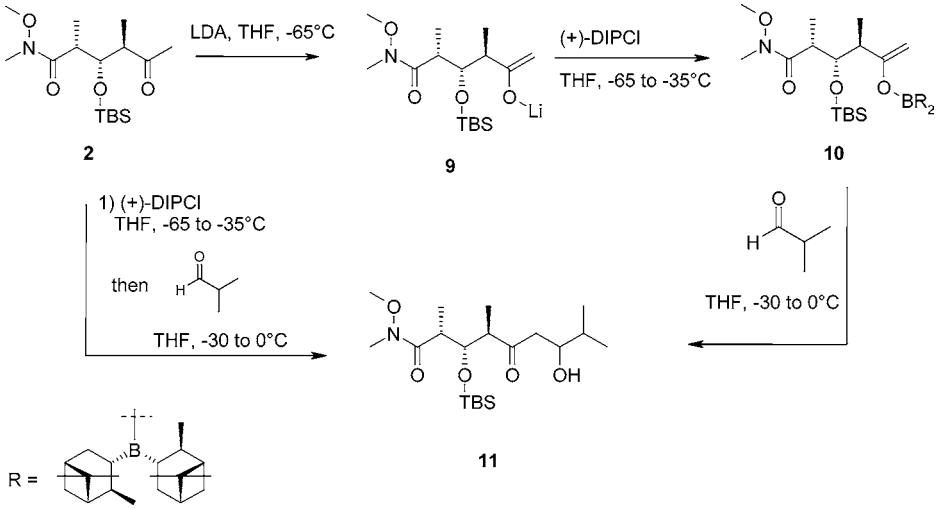
Scheme 2. **Model system for trans metallation**



(+)-B-chlorodiisopinocampheylborane ((+)-DIP-Cl) in diethyl ether (1.34 M solution) at 0 °C. Triethylamine (6.6 equiv) was added and the mixture stirred for 2 h at 0 °C. The enolate solution was cooled to −78 °C, and a solution of 1.0 equiv of aldehyde **3** in diethyl ether (0.45 M solution) was added. After warming slowly to −30 °C over a period of 6 h the reaction mixture was quenched and directly applied to a reverse-phase silica gel column which was then eluted with acetonitrile/TBME/water mixtures to deliver pure **4a** in 48−55% yield.

The workup in the above process is unconventional. This is required because, as previously noted, the product is apparently not stable to either the large excesses of reagent or the oxidative workup conditions. We investigated the effect of adding additives to the reaction mixture before quench to try and trap the "reactive components" which were assumed to cause the decomposition.

Additives. The nature of the "reactive components" causing decomposition is unknown, but it may be assumed that they are some sort of boron species. If this is the case, they may well cause the product losses by a hydroboration/elimination or a reductive pathway. Therefore, with this in mind, we selected several additives to attempt to trap out such species. We added propionaldehyde, 1,3-butadiene, 1,3-cyclohexadiene, ethanolamine, *N*-methylglucosamine, a boron-specific ion-exchange resin (IRA 743), methanol, and pivalic acid. None of these demonstrated any beneficial effect on the product stability and the yields after workup were all ≤40%.

Solvent. Diethyl ether is a problematic solvent when employed on a large scale therefore it was desirable to investigate the solvent effect in this reaction. Employing standard conditions two alternative solvents were examined, dichloromethane and *tert*-butylmethyl ether. In both cases the isomer ratio was slightly worse, around 2/1 in favour of **4a**. Therefore we remained with diethyl ether as reaction solvent.

Alternative Enolisation Method. An alternative method of enolate generation may result in a reduction of the excess of enolate required. Consequently, we examined first generating the lithium enolate of **2** followed by a lithium−boron exchange reaction in the model system shown in Scheme 2.

This process can be conveniently monitored by FT-IR. The two carbonyl groups in **2** absorb at 1714 and 1660 cm^{-1} corresponding to the keto and the amide carbonyls, respectively. During the reaction of 1 equiv of **2** with 1.2 equiv of LDA at −60 °C, the signal at 1714 cm^{-1} is rapidly replaced by a new one appearing at 1632 cm^{-1}, presumably due to the formation of the lithium enolate. The addition of 1.05 equiv of (+)-DIP-Cl at −60 °C caused this signal to partially disappear, and a new signal at 1668 cm^{-1} was observed. Warming this sample to −30 °C resulted in complete disappearance of the peak at 1632 cm^{-1}. This new signal at 1668 cm^{-1} is presumably due to the boron enolate. Quenching the reaction mixture with methanol regenerated **2** (Figure 4). This experiment demonstrates that the trans-metalation requires higher temperatures to run to completion.

Generation of the boron enolate under these conditions and subsequent reaction with excess isobutyraldehyde followed by an aqueous workup produced the aldol product **11**, in good yield, as a 4/1 mixture of diastereoisomers (determined by ^1H NMR) which were not separated. Direct formation of the boron enolate with (+)-DIP-Cl/Et_3N at −30 °C followed by an isobutyraldehyde quench delivered the same product(s) in a slightly better ratio.

Disappointingly, when this experimental protocol was applied to the real system, a 1/1 mixture of **4a** and **4b** resulted. Conversion was also incomplete, with some 62% of **3** remaining after the standard reaction time, extension of which did not improve the conversion. We therefore abandoned this approach and turned our attention to reducing the excess of enolate.

Enolate Excess. The use of excess reagents is wasteful and inefficient and in this case complicates the workup and purification of the reaction mixture. Therefore, an investigation into the optimum stoichiometry required was undertaken (Table 2). We systematically reduced the excesses of ketone **2**, (+)-DIP-Cl, and triethylamine with respect to aldehyde **3** and observed the effect on the yield of **4a**, the isomer ratio **4a**/**4b**, as well as the side-product formation.

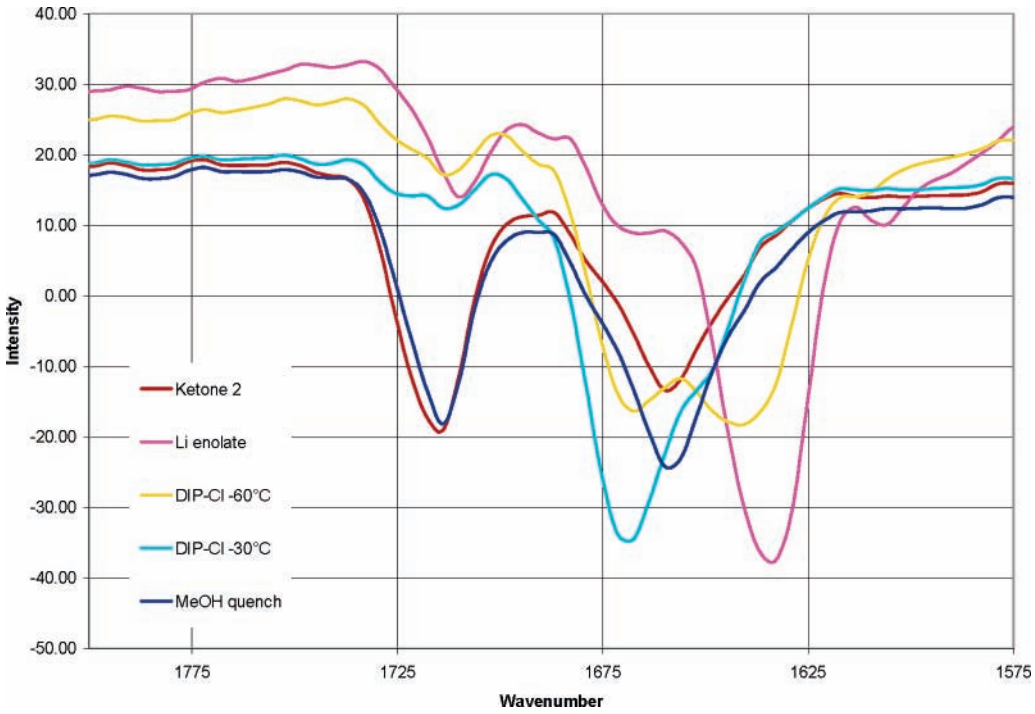


Figure 4. **FT-IR curves of lithium−boron exchange reaction.**

Table 2. **Optimisation of excess of enolate**

entry	**2** (equiv)	DIP-Cl (equiv)	Et_3N (equiv)	yield of **4a** (%)	ratios **4a**/**4b**	ratios **4a**/**5a**	ratios **4a**/**3**	comments
1	6.6	5.4	6.6	55	3.9/1	25/1	28/1	standard conditions
2	4.0	3.0	4.0	56	3.9/1	30/1	30/1	
3	3.3	2.7	3.3	48	4/1	28/1	35/1	
4	**3.3**	**2.5**	**3.3**	**68**	**3/1**	**22/1**	**22/1**	
5	2.4	2.0	2.4	49	3.6/1	28/1	5/1	slow reaction, 24 h, −20 °C
6	1.5	1.2	1.5	34	3.6/1	64/1	1/1	slow reaction, 48 h, −20 °C
7	1.5	1.2	1.5	31	3.4/1	60/1	1/1	**3** added in three portions, then 48 h −20 °C
8	3.3	2.5	3.3	0				trace of water; isomerisation of **3** to **6**, 1/1 after standard reaction time

The observed yields of 56% for entry 2 and 48% for entry 3 correspond to that observed under the previous standard set of reaction conditions. This low yield can be attributed to the instability of the product to the reagent excess and the workup conditions, as previously described. Entries 5 and 6 indicate that when the enolate excess falls below a certain value the reaction becomes very slow, and incomplete conversion is observed, even after extended reaction times. An attempt to increase the concentration by portionwise addition of **3** did not improve matters (entry 6). Remarkable was the effect of small quantities of moisture (entry 8). In this case the (+)-DIP-Cl was weighed in a closed system and exposed to atmospheric moisture for a period of 5 s, by removing the stopper, before carrying out the aldol reaction. No aldol product was formed, and a 1/1 mixture of **3** and its trans isomer **6** was observed. This dramatic effect of traces of water remains unexplained.

Thus, the conditions of entry 4 appear to be optimal. However, this reaction is very intolerant towards slight manipulative variations, and very narrow limits between slow reaction and decomposition of product during workup exist. These conditions brought a further benefit; the product formed was found to be indefinitely stable to the reagents and workup system, which is in direct contrast to the standard procedure. Thus, quenching and complete removal of the solvent produced the crude material as an oil. Stability tests indicated no change in quantity and quality of **4a** over a period of several weeks at room temperature. Stressing this sample at 40 °C also induced no change. Removal of the boron by oxidation of the crude material with hydrogen peroxide followed by reduction of the excess peroxide also had no effect on the stability of the crude material, the chemical yield of **4a** remaining constant at 66−68% throughout. This was confirmed by isolation of the product by reverse-phase chromatography which delivered pure **4a** in 68.6% yield. The control experiment i.e., **no** oxidation, and direct purification of the reaction mixture by chromatography delivered **4a** in 51% yield, the rest of the material was present in mixed fractions where we were unable to effect an efficient separation. However, in total around 67% chemical yield of **4a** was present. This suboptimal separation is very likely due to the presence of various boron residues.

Chromatography. The conditions described here are optimal for the oxidised reaction mixture after extractive

Table 3. **HPLC method for quantification of 4a/4b[a] and side products**

Conditions	
column	symmetric shield RP-18 3.5 μM, 75 mm × 4.6 mm
operating temperature	50 °C
flow rate	1.5 mL/min
detection	215 nM
injection volume	5 μL
solvent A	water
solvent B	acetonitrile/ *tert*-butylmethyl ether 85/15

Gradient		
time (min)	solvent A (%)	solvent B (%)
0	25	75
1	15	85
7	10	90
8	0	100
9	0	100
10	25	75

[a] Reference: A solution of 111 mg/mL of a mixture of **4a** and **4b** in acetonitrile. Figure 5 shows a representative HPLC.

workup. Direct chromatography of the reaction mixture under these conditions affords a poorer separation. This is presumably due to reaction components adversely affecting the separation. Apart from increasing the column loading from 1/20 to 1/10 we were unable to significantly reduce the large solvent quantities required for a successful purification. This remains an unsatisfactory aspect of this process.

Summary. A careful optimisation of a complex aldol reaction led to a resolution of previously noted stability problems of the product. The large excess of reagents has been reduced and as a result the process became more robust, and the product was obtained in higher yield. The overall yield of this aldol reaction (sum of **4a** and **4b**) is now >90%.

Experimental Section

Reagents and solvents were obtained from commercial sources and used as received. Proton NMR data were recorded on a Brukker SP 400 instrument at 400.1 MHz, respectively. FTIR experiments were recorded with the ReactIR 1000 System of Mettler-Toledo AG, Switzerland, IR-sensor: ZnSe.

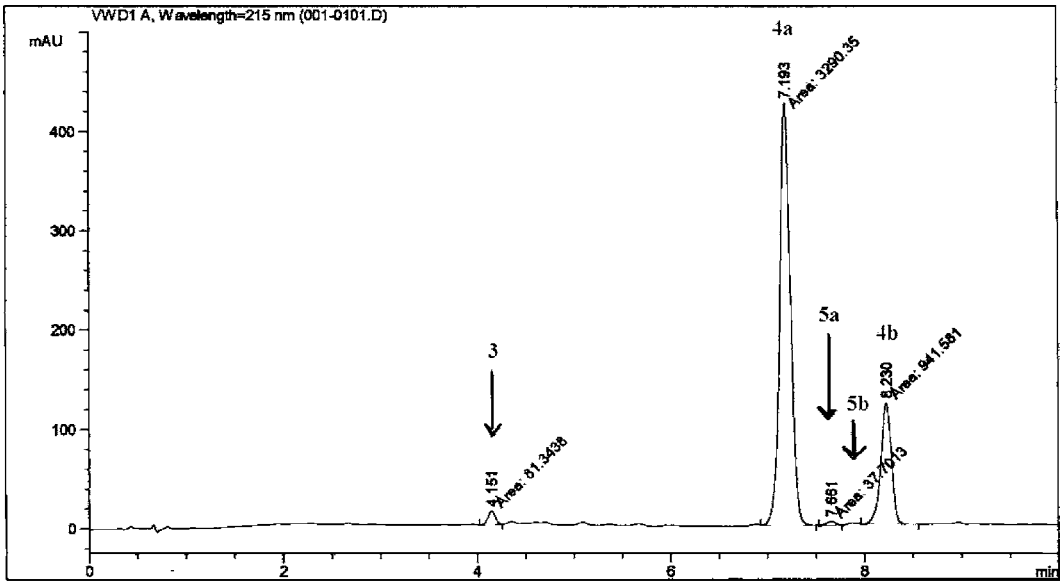
MPLC was carried out on a semipreperative column (stainless steel, diameter 4 cm, length 45 cm) filled with 100 g of TLC silica gel Merck 60 H 15 μ (Art. No. 11695) and operated at a pressure of 35 bar; eluent was dichloromethane/methanol, 98:2 (v/v). TLC eluent for identification of product fractions was dichloromethane; visualisation used 10% aqueous phosphoric−molybdenic acid.

HPLC data was accumulated with a Hewlett-Packard series 1100 instrument (see Table 3 and Figure 5.

(2*S*,3*S*,4*R*)-3-(*tert*-Butyldimethylsilanyloxy)-7-hydroxy-2,4,8-trimethyl-5-oxo-nonanoic Acid Methoxymethyl Amide 11. (A) By Direct Reaction with (+)-DIP-Cl. A solution of (+)-DIP-Cl (1.374 g of a 70.4% solution in hexane, 3.016 mmol) in 1.7 mL of diethyl ether was cooled to an internal temperature of 0 °C, and triethylamine (0.303 g, 3.02 mmol) was added within 5 min. A solution of **2** (0.5 g, 1.51 mmol) in diethyl ether (0.9 mL) was added the reaction mixture stirred for 10 min at 0 °C and cooled to −35 °C. Isobutyraldehyde (0.13 g, 1.81 mmol) in diethyl ether (0.9 mL) was added and the mixture warmed to 0 °C over a period of 20 min. A further 0.13 g (1.81 mmol) of isobutyraldehyde was added and the reaction stirred for 25 min at 0 °C. Ammonium chloride (1.0 mL of a saturated solution) was added and the two-phase system stirred for 10 min at room temperature. The mixture was diluted with water (5 mL) and washed with diethyl ether (5 mL). The organic phase was removed and the aqueous phase re-extracted with diethyl ether (10 mL) The combined organic phases were washed with water (10 mL) and dried over anhydrous sodium sulphate; the solvent was removed to give an oil. This oil was dissolved in methanol (6 mL) and treated with 1 g of a 30% solution of hydrogen peroxide. The mixture was stirred for 2 h at room temperature and the solvent removed in a vacuum. Water was added and the mixture extracted with *tert*-butylmethyl ether. The organic phase was dried over sodium sulphate and the solvent removed to give 1.08 g of an oil which was purified by MPLC to give pure **11**, 0.36 g, 70% as an oily mixture of diastereoisomers. ^{1}HNMR ($CDCl_3$) δ 4.33 (m, 1H), 3.91−3.68 (m, 5H), 3.11 (br s, 3H), 2.80 (m, 2H), 2.4 5(m, 1H), 1.69 (m, 2H, CH + OH), 1.15 (d, J = 10.0 Hz, 3H), 1.07 (d, J = 10.0 Hz, 3H), 0.92(s, 9H), 0.12 (m, 6H). [M^+ + H] 404.

(B) By Lithium−Boron Exchange. To a solution of LDA (1.81 mmol) in tetrahydrofuran (1.8 mL) at −60 °C was added a solution of **2** (0.505 g, 1.52 mmol) in tetrahydrofuran (0.9 mL) within 5 min. The resulting mixture was stirred for 20 min at −60 °C and treated with a solution of (+)-DIP-Cl in hexane (0.763 g, 1.68 mmol) of a 70.4% hexane solution). The addition was exothermic, and the internal temperature was allowed to reach −35 °C. After the mixture stirred for 10 min, a solution of isobutyraldehyde (0.131 g, 1.83 mmol) in tetrahydrofuran (0.9 mL) was added over a period of 20 min. The reaction mixture was warmed to 0 °C and quenched with a 10% solution of ammonium chloride (1.0 mL), diluted with diethyl ether, and extracted. The organic phase was dried and the solvent removed to give the same compound as obtained by direct reaction with (+)-DIP-Cl.

Carbamic Acid (6*Z*,11*E*)-(1*S*,2*R*,3*R*,4*S*,8*S*,9*S*,10*S*,13*R*,-16*R*,17*S*,18*R*)-3,9,17-Tris-(*tert*-butyldimethylsilanyloxy)-13-hydroxy-18-(methoxymethylcarbamoyl)-2,4,6,8,10,16-hexamethyl-1-((*Z*)-(*S*)-1-methylpenta-2,4-dienyl)-15-oxo-nonadeca-6,11-dienyl ester 4a by Lithium−Boron Exchange. A solution of diisopropylamine (0.034 mL, 0.24 mmol) in 0.8 mL of diethyl ether was cooled to 0 °C, and 0.15 mL of a 1.6 M solution of butyllithium in hexane was added. The solution was stirred for 10 min at 0 °C and cooled to −55 °C. At this temperature a solution of **2** (73 mg, 0.22 mmol) in diethyl ether (1 mL) was added. The reaction was stirred for 10 min at −55 °C and treated with 123 mg of (+)-DIP-Cl (60.7% solution in hexane, 0.23 mmol) dissolved in diethyl ether (1.0 mL). The mixture was warmed to −30 °C and stirred for 20 min, and a solution of 133 mg of **3** (0.2 mmol) in 1.0 mL of diethyl ether was added. The

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================================================================================
                          Area Percent Report
================================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: VWD1 A, Wavelength=215 nm

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]  mAU   *s   [mAU   ]      %
----|-------|----|-------|----------|----------|--------|
   1   4.151 MM    0.0936   81.34378   14.48619   1.8696
   2   7.193 MM    0.1289 3290.34595  425.44324  75.6232
   3   7.661 MM    0.1331   37.70134    4.72011   0.8665
   4   8.230 MM    0.1276  941.58112  122.93926  21.6407

Totals :                  4350.97219  567.58880
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Figure 5. **HPLC of 4a reference material.**

reaction was stirred for 90 min at −30 °C. HPLC area analysis indicated approximately 32% of a 1/1 mixture of **4a** and **4b**, together with 62% of **3**, to be present, the remainder being traces of the other components. The reaction products were not further examined.

Improved Method for the Preparation of Carbamic Acid (6*Z*,11*E*)-(1*S*,2*R*,3*R*,4*S*,8*S*,9*S*,10*S*,13*R*,16*R*,17*S*,18*R*)-3,9,17-Tris-(*tert*-butyldimethylsilanyloxy)-13-hydroxy-18-(methoxymethylcarbamoyl)-2,4,6,8,10,16-hexamethyl-1-((*Z*)-(*S*)-1-methylpenta-2,4-dienyl)-15-oxo-nonadeca-6,11-dienyl ester 4a. A solution of (+)-DIP-Cl (9.33 g of a 60.7% solution in hexane, 17.66 mmol) in 20 mL of diethyl ether was cooled to an internal temperature of 0 °C, and triethylamine (3.25 mL) was added within 5 min. To the slightly turbid solution was added a 15.44 g of a 50% solution of **2** in diethyl ether (7.72 g, 23.30 mmol of **2**) within 20 min. The resulting white suspension was stirred for 2 h at 0°C and cooled to an internal temperature of −78 °C; a solution of **3** (4.69 g, 7.06 mmol) in diethyl ether (20 mL) was added within 20 min. The reaction mixture was stirred at an internal temperature of −78 °C for 60 min and warmed to an internal temperature of −50 °C within 15 min. Stirring was continued at −50 °C for 2 h, and then the reaction mixture was warmed to an internal temperature of −35 °C within 15 min. The mixture was stirred for 2 h at −35° to −30 °C. The cooling bath was removed, and water (75 mL) was added, allowing the temperature to warm to 0 °C. The mixture was diluted with 12 mL of *tert*-butylmethyl ether and the organic layer separated. The aqueous layer was diluted with saturated aqueous sodium chloride solution (25 mL) and re-extracted with 50 mL of *tert*-butylmethyl ether. The combined organic layers were washed with a mixture of water/saturated aqueous sodium chloride solution (75 mL/25 mL). Water (4.6 mL) was added to the organic phase and the mixture concentrated in a vacuum to a final weight of 30 g. The residue was redissolved in 40 mL of a mixture of acetonitrile/*tert*-butylmethyl ether/water (60/28/12) to give 78.65 g of a clear solution. HPLC analysis at this point indicated a chemical yield of **4a** of 69%.

This was divided into two parts: one used for oxidation and chromatography, the other for direct chromatography.

Part 1: Oxidation and Chromatography. The acetonitrile/*tert*-butylmethyl ether/water (39.3 g) was concentrated in a vacuum to a final weight of 15 g and partitioned between water (50 mL) and dichloromethane (50 mL). The aqueous phase was re-extracted with dichloromethane (50 mL), and the combined organic layers were diluted with pH 7 phosphate buffer (80 mL) and cooled to an internal temper-

ature of 0 °C. Hydrogen peroxide (3.43 g of a 35% solution) was added within 30 min and the resulting two-phase system stirred for a further 60 min at 0 °C. A solution of sodium sulphite (18.4 g of a 50% aqueous solution) was added within 60 min, maintaining the internal temperature at 0 °C. After the addition was complete, the mixture was warmed to room temperature, and the phases were separated. The aqueous phase was washed with dichloromethane (50 mL), and the combined organic phases were re-extracted with water (80 mL) and evaporated to a volume of around 20 mL. The residue was diluted with acetonitrile (60 mL) and water (5 mL), followed by *tert*-butylmethyl ether (20 mL). HPLC analysis at this point indicated a chemical yield of 66.6%.

This solution was chromatographed over LiChroprep RP-18 silica gel 25−40 μM (312 g) which had been conditioned with acetonitrile/*tert*-butylmethyl ether/water 60/28/12. The column was eluted with acetonitrile/*tert*-butylmethyl ether/water 60/28/12, collecting fractions of around 70 mL. The product-containing fractions were combined and concentrated in a vacuum at 35 °C to a volume of around 100 mL. The residue was partitioned between water (50 mL) and dichloromethane (50 mL). The organic layer was removed and the aqueous layer re-extracted with dichloromethane (30 mL). The combined organic phases were evaporated to dryness in a vacuum at 40 °C to give **4a** as a nonhydroscopic foam, 2.38 g, 68.6%).

Part 2: Direct Chromatography. The acetonitrile/*tert*-butylmethyl ether/water (39.3 g) reaction mixture solution was chromatographed over LiChroprep RP-18 silica gel 25−40 μM (312 g) which had been conditioned with acetonitrile/*tert*-butylmethyl ether/water 60/28/12. The column was eluted with acetonitrile/*tert*-butylmethyl ether/water 60/28/12, collecting fractions of around 70 mL. The fractions containing pure **4a** were combined, and the solvent was removed in a vacuum at 40 °C. The residue was extracted with a mixture of water (50 mL) and dichloromethane (50 mL) and the organic layer separated. The aqueous layer was re-extracted with dichloromethane (50 mL), and the combined organic layers were dried over sodium sulphate; the solvent was removed to give 1.82 g, 51.8%, of **4a** as a nonhydroscopic foam.

Stability Test. A sample of the acetonitrile/*tert*-butylmethyl ether/water (60/28/12) solution of the crude reaction mixture was stored at 4 °C. The content of **4a** was monitored by HPLC. After 4 weeks the content of **4a** was found to correspond to a chemical yield of 66.6%, i.e. no change over the original measurement.

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