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MODEL STUDIES FOR THE TOTAL SYNTHESIS OF THE MAYTANSINOIDS BASED ON THE INTRAMOLECULAR NITRILE OXIDE–OLEFIN [3+2] CYCLOADDITION REACTION†

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Abstract—The macrocyclization of the olefinic nitrile oxide **30** to the ansa-macrolide skeleton **37**, a model for a novel approach to the maytansinoids, is described.

The maytansinoids are a group of structurally related ansa-macrolides isolated originally by Kupchan *et al.*[1] in 1972 from the East African plant species *Maytenus serrata*. Recently, maytansine (**1**) and several cogeners have also been obtained from the fermentation broth of a *Norcardia* species.[2] The most thoroughly studied representative of this class of compounds is maytansine itself, which exhibits potent antitumor activity against various cell lines. Although the primary binding site for maytansine is believed to be the microtubule proteins,[3] recent studies of the mechanism of action against murine leukemia cells indicates substantial inhibition of DNA synthesis may also be involved, suggesting the nucleic acids as additional target sites.[4]

Due to these important biological properties, maytansine has been the target of numerous synthetic efforts.[5] The molecule possesses unique structural features highlighted by its challenging ansa-macrolide framework. Three independent total syntheses have been reported to date, employing an amide formation, a Wittig reaction, or an aldol condensation to achieve the macrocyclic ring closure.[6,5a–e] As part of our ongoing program utilizing [3+2] cycloaddition chemistry for the total synthesis of natural products, we have investigated a novel macrocyclization route, involving an intramolecular nitrile oxide–olefin cycloaddition reaction on the key step (structure **5**), for a new approach to the total synthesis of maytansine (Scheme 1). This cycloaddition would yield an isoxazoline moiety imbedded in the resulting ring **4** which presumably could be converted to the corresponding β-hydroxy ketone **3** and subsequently to the carbinol carbamate unit of maytansine, which is often suggested as the active site of maytansine.[7]

Intermolecular nitrile oxide–olefin cyclizations (*model studies*)

To investigate the feasibility of our [3+2] cycloaddition strategy, we decided to verify that the isoxazoline, product of the key reaction, could in fact be further elaborated into the carbinol carbamate. The active-site analog **14**, which has the same absolute configuration as C(10) of maytansine, was chosen as the first target molecule for a model study. It was hoped that the phenyl moiety in this structure would mimic in biological systems, the diene system attached to C(10) of maytansine itself. To this end, D-(−)-mandelic acid (**6**) was converted to methyl O-methylmandelate (**8**) by refluxing for 2.5 hr in methanol containing conc H_2SO_4 followed by treatment with methyl iodide and Ag_2O in DMF for 22 hr at room temperature (Scheme 2). Conversion of the ester **8** to the optically active oxime **9** $\{[\alpha]_D = 12.2°(CHCl_3, c = 0.62)$ as a mixture of *E* and *Z* isomers$\}$ was accomplished by reduction to the corresponding aldehyde using 1.1 equiv of DiBAL-H in CH_2Cl_2 at −78 to −30° for 2 hr and treatment with hydroxylamine at pH 7 for 1.5 hr at room temperature. The overall yield for the four steps was 72%. The key nitrile–olefin cycloaddition occurred smoothly when the oxime **9** was reacted with Chlorox® in a two-phase system in the presence of allylbenzene at 0° to afford the desired isozaloline **11** regiospecifically as a mixture of two diastereomers. No detectable amount of regioisomers was formed in the reaction. Hydrolytic reduction of the isoxazoline **11** with Raney Ni under H_2 in the presence of acetic acid afforded the corresponding β-hydroxy ketone **12** in 43% overall yield from the oxime **9**.[8] Conversion of **12** to the carbinol carbamate **14** was effected by first treatment with *p*-nitrophenyl chloroformate and pyridine at 0° followed by ammonia at −78 to −20°. A small amount (~15%) of uncyclized product **15** could also be isolated by chromatography. This surprisingly stable molecule, which would not cyclize to the closed form **14** under the reaction conditions, could be converted to **14** by treatment with either DBU in THF or 1 N HCl in CH_3CN. This "open form" material presumably arises from attack of ammonia at the formate derived carbonyl of the intermediate **13** leading directly to **15**, whereas reaction at the ketone site of **12** to form a hemiaminal followed by cyclization accounts for the formation of **14**. The highly simplified carbinol carbamate **14** shows activity against human colon tumor cells (HCT-15) at an effective dose of 2.05 $\mu g\ ml^{-1}$.[9] This biological test result supports the assignment of the carbinol carbamate moiety as one of the important active sites of maytansine.

Macrocyclization by intramolecular [3+2] *cycloaddition reaction—preparation of a model ansa-macrolide* 37

We next turned our attention to the central issue of macrocyclization. Using the method described by Krishnamurthy,[10] N-methyl *m*-toluidine (**17**) was prepared from *m*-toluidine (**16**) (Scheme 3). The

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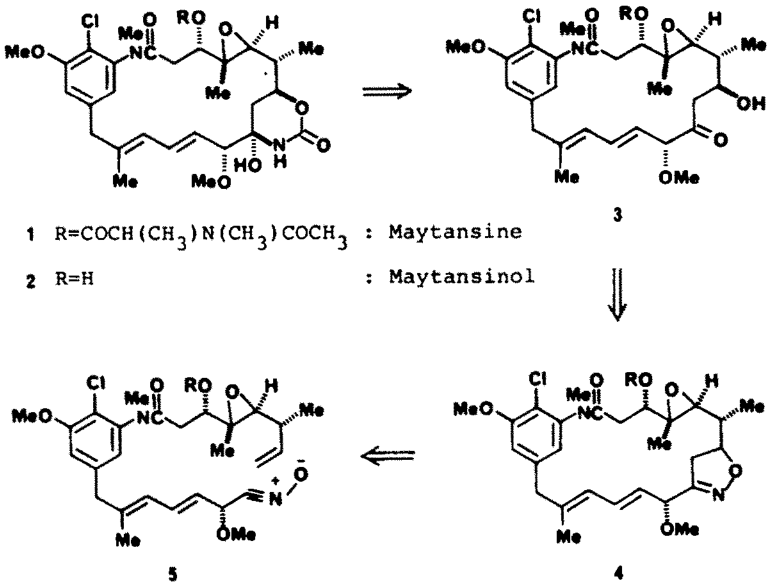
† For preliminary communication see P. N. Confalone and S. S. Ko, *Tetrahedron Lett.* **25**, 947 (1984).

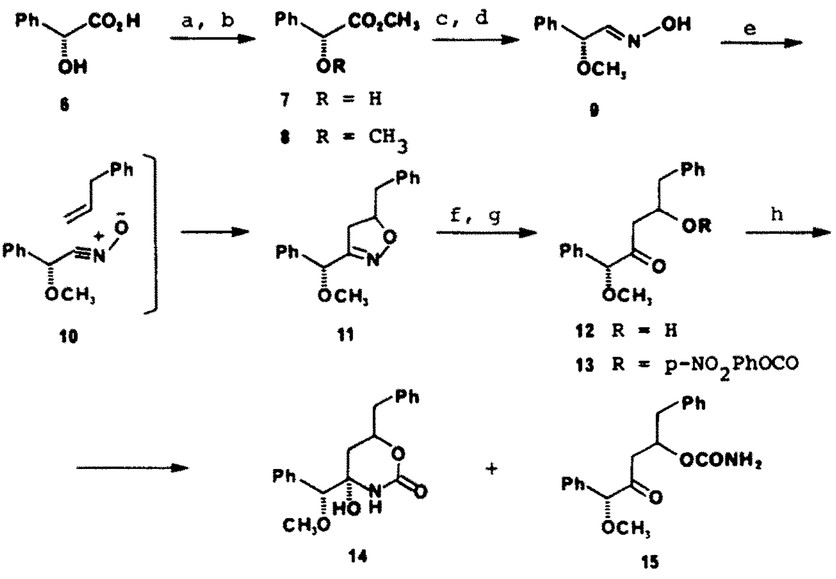


1 R=COCH(CH_3)N(CH_3)$COCH_3$: Maytansine

2 R=H : Maytansinol

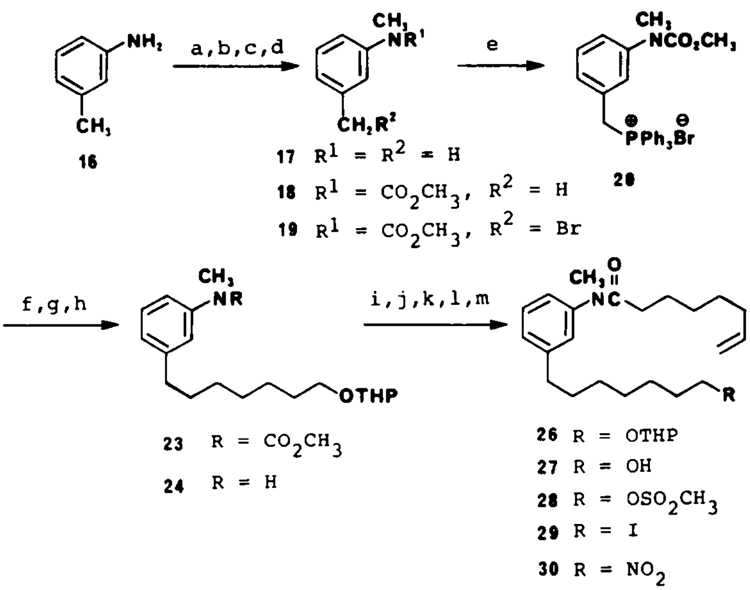
Scheme 1.

resulting secondary amine was then protected as the carbamate **18** by treatment with methyl chloroformate and pyridine at 0° and allylicly brominated with N-bromosuccinimide to provide the corresponding bromide **19**. This was treated with triphenylphosphine in refluxing toluene to afford the desired phosphonium salt **20**. A Wittig reaction between the THP ether of 6-hydroxyhexanal **21** and the ylid, generated from **20** by treatment with potassium t-butoxide, provided the styrene derivative **22** as a mixture of *cis* and *trans* isomers in over 90% yield. Catalytic hydrogenation of **22** with 10% Pd/C in methanol in the presence of



a) C-H_2SO_4, CH_3OH/reflux, 2.5 h; b) CH_3I, Ag_2O/DMF/RT, 22 h; c) DIBAL-H/CH_2Cl_2/-78° ∿ -30°C, 2 h; d) NH_2OH/EtOH/RT, 15 h; e) allylbenzene, Chlorox®, NEt_3/CH_2Cl_2/RT, 1 h; f) H_2, Raney Ni/CH_3OH-H_2O-HOAc/RT, 16 h; g) p-nitrophenyl chloroformate, pyridine/CH_2Cl_2/0°C, 0.5 h; h) NH_3/CH_3OH-CH_2Cl_2/-78°C ∿ -20°C, 1 h.

Scheme 2.



a) CH_3CO_2COH/THF/RT, 2 h; b) BH_3-Me_2S/THF/reflux, 3 h; c) $ClCO_2CH_3$, pyridine/CH_2Cl_2/0°C, 1h; d) NBS/CCl_4/reflux, 6 h; e) Ph_3P/toluene/reflux, 30 h; f) ^tBuOK/THF/0°C, 0.5 h, then $OHC(CH_2)_4CH_2OTHP$ (21)/0°C $\sim$ RT, 4 h; g) H_2, 10% Pd-C, $NaHCO_3$/CH_3OH/RT, overnight; h) 12N KOH/EtOH/reflux overnight; i) 7-Octenyl chloride (25), pyridine/CH_2Cl_2/0°C, 2 h, RT, 3 h; j) Dowex-50X8-400®/CH_3OH/50°X, 20 h; k) $CH_3SO_2Cl_2$, NEt_3/CH_2Cl_2/0°C, 1 h; l) LiI/DMF/90°C, 3.5 h; m) $AgNO_2$/Et_2O/RT, 12 days

Scheme 3.

$NaHCO_3$, followed by hydrolysis of surprisingly stable carbamate with 12 N KOH in refluxing ethanol for 18 hr yielded the required N-methylaniline derivative **24** in 84% yield. When the hydrogenation was attempted without $NaHCO_3$ substantial amounts of free hydroxy compound were obtained, owing to acidic impurities in the catalyst. The free amine **24** was then treated with 7-octenoyl chloride (**25**), itself prepared from 7-octenoic acid[11] and thionyl chloride at 40°, to provide the amide **26** in 83% yield based on recovered starting material. After acid-catalyzed methanolysis of the THP protecting group of **26**, the resulting alcohol **27** was treated with methanesulfonyl chloride and triethylamine at 0°, followed by LiI treatment in DMF at 90° to yield the iodide **29**. Treatment of the iodide **29** with $AgNO_2$ in Et_2O afforded the desired nitro-olefin **30** in 72% overall yield based on the recovered iodide **29** (82% conversion after 12 days at room temperature).[12]

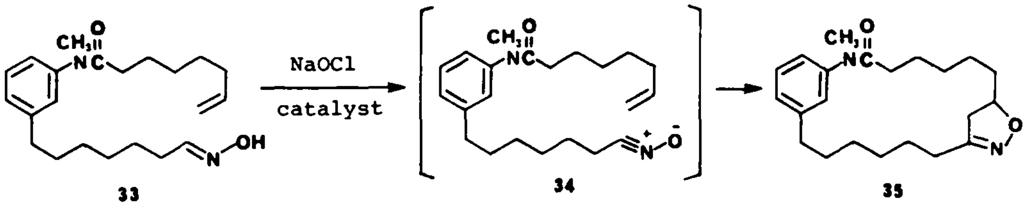
Similarly the corresponding oxime **33**, an alternative precursor of nitrile oxide, was prepared from the intermediate **20** via the olefin-acetal **32** in the sequence of reactions depicted in Scheme 4.

Treatment of the oxime-olefin **33** with Chlorox® and triethylamine at 0°, the conditions employed for intermolecular cyclization (e.g. **9** → **11**), yielded no appreciable amount of the desired product and only unreacted starting material was recovered. However, by changing the catalyst and the reaction temperature, we were able to raise the yield of the 19-membered cycloadduct **35** to 51% (Table 1).

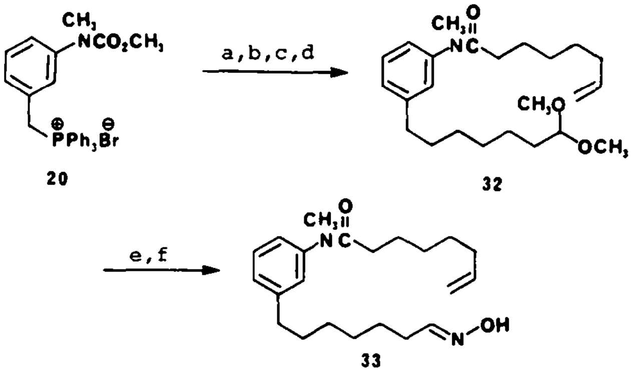
The nitro-olefin **30**, on the other hand, was found to be an excellent precursor for the macrocyclization. In a highly diluted toluene solution (0.005 mol l^{-1}) treatment of **30** with *p*-chlorophenylisocyanate and triethylamine at 80° afforded regiospecifically the desired macrocycloadduct **35** in 82% isolated yield! No detectable amount of dimeric or polymeric products were formed from the reaction in spite of the predicted difficulty in the closure of the 19-membered ansa-macrolide ring (Scheme 5). Hydrolytic reduction of **35** with Raney Ni in aqueous methanol containing acetic acid provided the β-hydroxy ketone **36** in 77% yield. Final transformation to the targeted maytansine analog **37** was achieved by treatment with *p*-nitrophenyl chloroformate and pyridine at 0°, followed by NH_3 at −78 to 0°. As expected, **37**, embodying the "carbon backbone" of maytansine, also exhibited *in vitro* antitumor activity against HCT-15 (ED_{50} = 2.12 μg ml^{-1}).

One additional model study was required before a final assault on maytansine employing this methodology could be justified. Our concern was directed toward the presence of the diene system spanning C(11) to C(14) in the natural product and its possible complications to the key nitrile oxide–olefin cycloaddition step. Therefore, the highly functionalized

Table 1.

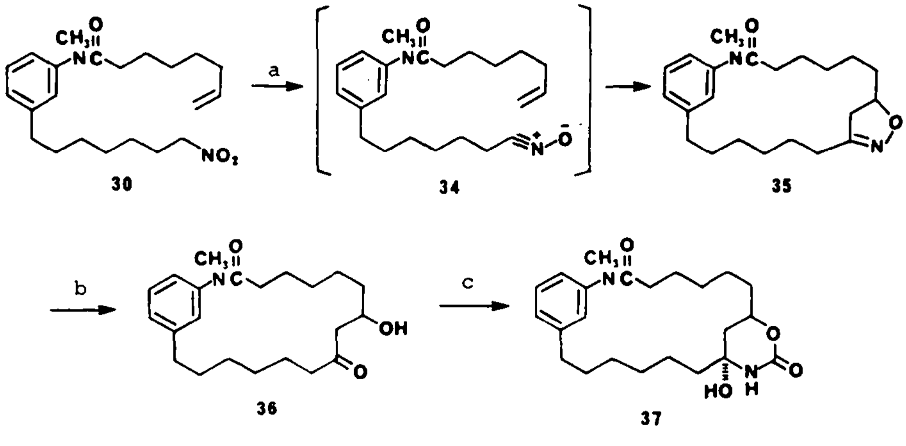


Catalyst	Temperature	Time	Concentration (mol l^{-1})	Yield (%)
NEt_3	0°	28 hr	0.004	0
NEt_3	RT	3 days	0.07	19.4
n-Bu_4NBr	RT	40 hr	0.002	39.5
n-Bu_4NOH	RT	3 days	0.002	51



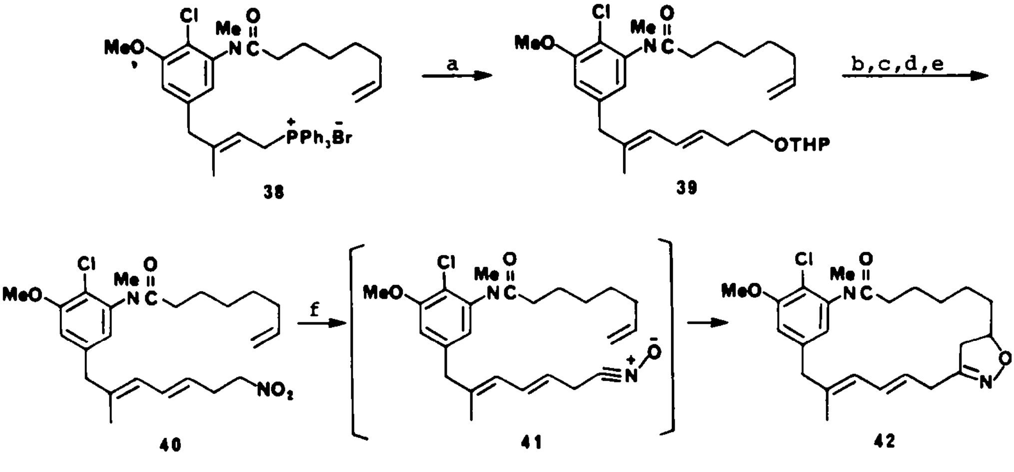
a) ^{t}BuOK/THF/0°C, 0.5 h, then $OHC(CH_2)_4CH(OCH_3)_2$ (31)[13] 0°C ~ RT, 4 h; b) H_2, 10% Pd-C, $NaHCO_3/CH_3OH$/RT, 5 h; c) 12N KOH/EtOH/ reflux, 16 h; d) 7-octenoyl chloride (25), pyridine/CH_2Cl_2/ 0°C ∿ RT, 5 h; e) 75% aq HOAc/50°C, 1h; f) NH_2OH/Et_2OH-H_2O/RT, 1 h

Scheme 4.



a) p-chlorophenylisocyanate, NEt_3/toluene/80°C, 48 h; b) H_2, Raney-Ni/$CH_3OH-H_2O-HOAc-CH_2Cl_2$/RT, 5 h; c) p-nitrophenyl chloroformate, pyridine/CH_2Cl_2/0°C, 25 min, then $NH_3/CH_3OH-CH_2Cl_2$/ -78° ∿ 0°C, 1 h

Scheme 5.



a) $LiN(SiMe_3)_2$/THF/−78°C, 5 min, then $OHC(CH_2)_2OTHP$/−78°C ∿ 20°C, 1 h, 42%; b) Dowex-50X8-400 /CH_3OH/RT, 24 h; c) CH_3SO_2Cl, NEt_3/ CH_2Cl_2/0°C, 0.5 h; d) LiI/DMF/80°C, 15 h; e) $NaNO_2$, catechol/DMSO/ RT, 16 h, 40% overall; f) p-chlorophenylisocyanate, NEt_3/toluene/ 80°C, 36 h

Scheme 6.

nitrotriene **40** was synthesized by a Wittig reaction between the phosphonium salt **38**[14] and THP ether of 3-hydroxypropanal to the *E,E* diene **39**.[15] This was followed by a similar sequence to that previously outlined for the transformation **26** → **30** to afford the nitrotriene **40** (Scheme 6). This substance smoothly cyclized to the desired macrocyclic isoxazoline **42** without any untoward effects arising from the diene functionality. This satisfying transformation proceeded in about 50% yield (unoptimized) and augurs well for the completion of the total synthesis of maytansine by this route. Efforts in this regard are in progress.

EXPERIMENTAL

General methods. All the reactions were performed under N_2 unless otherwise stated. THF and diethyl ether were distilled from sodium benzophenone ketyl prior to use. CH_2Cl_2 was freshly distilled from P_2O_5 and toluene was freshly distilled from CaH_2. DMF and DMSO were dried over 4 Å molecular sieves. Pyridine was distilled from KOH and stored over KOH and Et_3N was distilled from CaH_2 and stored over 4 Å molecular sieves. M.ps were measured on a Thomas–Hoover capillary m.p. apparatus and are uncorrected. B.ps are also uncorrected. ^1H-NMR spectra were recorded on IBM NR/80, Varian EM-390, Nicolet QE-300 or Nicolet 360 NT. IR spectra were obtained on Perkin–Elmer 1420 infrared spectrometer. High resolution mass spectra were taken on VG MM-7070.

O-*Methylmandelaldehyde oxime* (**9**)

To a stirred soln of **8** (14.7 g, 0.08 mol) in 500 ml of dry CH_2Cl_2 at −78° was added 90 ml of DiBAL-H in hexane (1 M l^{-1}) dropwise over 40 min and the mixture was stirred for 2 hr at 78 to −30°. The reaction was quenched by adding 6.5 ml of MeOH dropwise and stirring for 10 min at −30°. Then 11 ml of H_2O was also added and the mixture was stirred for 1 hr at room temp. After filtration through Celite the filtrate was evaporated to give O-methylmandelaldehyde as an oil. To a soln of hydroxylamine hydrochloride (8.5 g, 0.122 mol) in 30 ml of EtOH in a separate vessel was added NaOH aq (4.9 g, 0.122 mol, in 25 ml of H_2O) dropwise at 0° and it was stirred for 5 min at 0°. The pH of the soln was adjusted to 7. The hydroxylamine soln was added to a soln of O-methylmandelaldehyde in 200 ml of EtOH and the mixture was stirred for 1.5 hr at 0°. After concentration to *ca* 50 ml under reduced pressure, the product was extracted with CH_2Cl_2 (2×). The combined CH_2Cl_2 extracts were washed with water, dried over $MgSO_4$ and evaporated to give **9** as a 3.2 : 1 mixture of *E* and *Z* isomers (12.2 g, 91% yield). This material was routinely used for cycloaddition reaction without purification. $[\alpha]_D = 12.2°$ ($CHCl_3$, $c = 0.62$). NMR ($CDCl_3$) *E* isomer, δ 8.05 (brs, 1H), 7.46 (d, J = 7.2 Hz, 1H), 7.38 (s, 5H), 4.80 (d, J = 7.2 Hz, 1H), 3.4 (s, 3H); *Z* isomer, δ 8.30 (brs, 1H), 7.38 (s, 5H), 6.93 (d, J = 6.3 Hz, 1H), 5.55 (d, J = 6.3 Hz, 1H), 3.38 (s, 3H). IR (neat) 3340, 3080, 3070, 3024, 2985, 2935, 2825, 1735, 1493, 1453, 1087 cm^{-1}.

5 - *Benzyl* - 4,5 - *dihydro* - 3 - (α - *methoxy* - *benzyl*) - *isoxazole* (**11**)

To a stirred soln of **9** (3.3 g, 0.02 mol), Et_3N (0.28 ml, 0.002 mol) and allylbenzene (2.84 g, 0.024 mol) in 90 ml of CH_2Cl_2 at 0° was added 5.25% Chlorox® (57.2 ml, 0.04 mol) dropwise and the two-phase soln was stirred vigorously for 2 hr at the same temp. At the end of the stirring the CH_2Cl_2 layer was separated and the aqueous layer was extracted with CH_2Cl_2 once more. The combined CH_2Cl_2 extracts were washed with water twice, dried ($MgSO_4$) and evaporated to give the desired **11** (5.34 g) as an oil. This material was transformed to the corresponding ketone **12** without purification.

Diastereomer **A**: NMR ($CDCl_3$) δ 7.33–7.18 (m, 10H), 5.11 (s, 1H), 4.76 (m, 1H), 3.26 (s, 3H), 3.00 (dd, J = 13.84, 5.62 Hz, 1H, A of AB), 2.83 (dd, J = 13.84, 6.91 Hz, 1H, B of AB), 2.67 (d, J = 7.8 Hz, 1H, A of AB), 2.65 (d, J = 9.8 Hz, 1H, B of AB). IR (neat) 3060, 3030, 2935, 2830, 1607, 1497, 1455, 1334, 1190, 1095 cm^{-1}.

Diastereomer **B**: NMR ($CDCl_3$) δ 7.38–7.00 (m, 10H), 5.15 (s, 1H), 4.81 (m, 1H), 3.39 (s, 3H), 2.90 (dd, J = 17.08, 10.2 Hz, 1H, A of AB), 2.89 (dd, J = 13.7, 5.53 Hz, 1H, A of AB), 2.61 (dd, J = 13.7, 7.17 Hz, 1H, B of AB), 2.38 (dd, J = 17.08, 7.49 Hz, 1H, B of AB). Ir (neat) 3060, 3030, 2935, 2830, 1607, 1497, 1455, 1335, 1190, 1095 cm^{-1}.

1,5-*Diphenyl-4-hydroxy-1-methoxy-2-pentanone* (**12**)

To a soln of **11** (5.34 g, crude) in 100 ml of MeOH, 20 ml of water and 4.5 ml of HOAc was introduced about 2 g of Raney Ni and it was stirred under H_2 (atm pressure) overnight. After removal of the catalyst by filtering through Celite the filtrate was concentrated to about 50 ml and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with sat $NaHCO_3$ and water, dried ($MgSO_4$) and evaporated to give an oily residue. Two diastereomers were separated by column chromatography on silica gel with elution by 1 : 1 EtOAc–hexane to afford pure samples (diastereomer A, 1.39 g : diastereomer B, 1.06 g) in 43% overall yield for two steps. Diastereomer A $[\alpha]_D = -1.5°$ ($CHCl_3$, $c = 1.04$). NMR ($CDCl_3$) δ 7.33 (s, 5H), 7.19 (m, 5H), 4.63 (s, 1H), 4.19 (qn, J = 6.1 Hz, 1H), 3.37 (s, 3H), 2.76–2.65 (m, 4H). IR (neat) 3460, 3055, 3023, 2930, 2825, 1720, 1602, 1493, 1452, 1197, 1090, 1070 cm^{-1}. HRMS calc for $C_{18}H_{20}O_3$ 284.1412, found 284.1449. Diastereomer B $[\alpha]_D = -10.1°$ ($CHCl_3$, $c = 1.01$). NMR ($CDCl_3$) δ 7.34 (s, 5H), 7.20 (m, 5H), 4.65 (s, 1H), 4.20 (m, 1H), 3.33 (s, 3H), 2.72 (d, J = 3.5 Hz, 2H) 2.68 (d, J = 3 Hz, 1H), 2.62 (d, J = 3 Hz, 1H). IR (neat) 3465, 3055, 3023, 2930, 2825, 1720, 1602, 1493, 1452, 1197, 1090 cm^{-1}. HMRS calc for $C_{18}H_{20}O_3$ 284.1413, found 284.1386.

Preparation of carbinol carbamate **14**

To a stirred soln of **12** (1.35 g, 5.75 mmol) and dry pyridine (1.92 ml, 23.75 mmol) in 25 ml of dry CH_2Cl_2 was added a soln of *p*-nitrophenyl chloroformate (2.4 g, 11.88 mmol) in 6 ml of CH_2Cl_2 dropwise at 0° and this was stirred for 30 min at the same temp. The mixture was then cooled down to −78° and 15 ml of NH_3–sat MeOH was added at once. After stirring for 1 hr at −78 to 20°, it was diluted with 100 ml of ether and the ether soln was washed with water, sat $NaHCO_3$ and brine, dried (Na_2SO_4) and evaporated to give an oily residue. It was column chromatographed on silica gel with elution by 1 : 1 EtOAc–hexane to afford pure **14**, m.p. 144–147°, decomposed (Et_2O and hexane). NMR ($CDCl_3$) δ 7.4–7.1 (m, 10H), 6.07 (s, 1H), 4.61 (s, 1H), 4.18 (m, 1H), 4.11 (s, 1H), 3.26 (s, 3H), 3.09 (dd, J = 14.04, 6.4 Hz, 1H), 2.81 (dd, J = 14.04, 7.63 Hz, 1H), 2.03 (dd, J = 14.52, 2.87 Hz, 1H), 1.61 (dd, J = 14.52, 9.79 Hz, 1H). IR (film) 3320, 3055, 3022, 2925, 2820, 1695, 1490, 1450, 1310, 1090 cm^{-1}.

Diastereomer **B** was synthesized by the same method as described above from diastereomer **B** of the hydroxy ketone, m.p. 128.5–129° (Et_2O and hexane). NMR ($CDCl_3$) δ 7.38–7.15 (m, 10H), 6.33 (s, 1H), 4.76 (m, 1H), 4.09 (s, 1H), 3.27 (s, 3H), 3.24 (s, 1H), 2.95 (dd, J = 14.14, 6.5 Hz, 1H, A of AB), 2.85 (dd, J = 14.14, 5.5 Hz, 1H, B of AB), 1.57 (t, J = 12 Hz, 1H), 1.42 (d, J = 13.56 Hz, 1H). IR (KBr) 3420, 3063, 3031, 2960, 2830, 1695, 1460, 1420, 1320, 1090 cm^{-1}.

N-*Carbomethoxy*-N-*methyl-m-toluidine* (**18**)

To a stirred soln of **17** (28.5 g, 0.236 mol) and pyridine (57.2 ml, 0.708 mol) in 200 ml of CH_2Cl_2 was added methyl chloroformate (54.6 ml, 0.708 mol) dropwise at 0° and the mixture was stirred for 1 hr at the same temp. After slow addition of water (50 ml), the CH_2Cl_2 layer was separated and the aqueous layer was extracted with CH_2Cl_2 once more. The combined extracts were washed with water, dried ($MgSO_4$) and evaporated to give a liquid residue. It was distilled under vacuum to obtain 26 g of the carbamate **18** as an oil (87–90°/0.4 mmHg). NMR ($CDCl_3$) δ 7.4–6.9 (m, 4H), 3.7 (s, 3H), 3.27 (s, 3H), 2.35 (s, 3H). IR (neat) 3020, 2953, 2920, 1720, 1708, 1607, 1588, 1490, 1450, 1357, 1155 cm^{-1}.

α-Bromo-N-*carbomethoxy*-N-*methyl-m-toluidine* (**19**)

A soln of **18** (25.9 g, 0.144 mol), N-bromosuccinimide (28.2 g, 0.158 mol) and AIBN (0.5 g) in 400 ml of CCl_4 was refluxed under N_2 for 3 hr and cooled down to room temp. The insoluble succinimide was removed by filtration and evaporation of the solvent afforded an oily residue. It was chromatographed on silica gel with elution by 2 : 8 EtOAc–hexane to give the bromide **19** as an oil (26.8 g). NMR ($CDCl_3$) δ 7.26 (m, 4H), 4.46 (s, 2H), 3.70 (s, 3H), 3.28 (s, 3H). IR (neat) 2950, 1712, 1700, 1607, 1590, 1490, 1448, 1353, 1155 cm^{-1}. HRMS calc for $C_{10}H_{12}NO_2Br$ 257.0050, found 257.0041.

Preparation of phosphonium salt **20**

A soln of **19** (26.8 g, 0.104 mol) and triphenylphosphine (32.7 g, 0.125 mol) in 350 ml of toluene was refluxed under N_2 for 1.5 days. The desired **20** was precipitated out during the reflux and collected by filtration after cooling (34 g), m.p. 220–221°. NMR ($CDCl_3$) δ 7.95–7.60 (m, 15H), 7.2–7.0 (m, 5H), 5.42 (d, J = 14.1 Hz, 2H), 3.6 (s, 3H), 3.07 (s, 3H).

Preparation of olefin **22**

To a stirred soln of **20** (32 g, 0.062 mol) in 500 ml of dry THF at 0° was added t-BuOK (6.9 g, 0.062 mol) in small portions and the resulting red–orange soln was stirred for 30 min at the same temp. Then a soln of the THP ether of **21** (12.3 g, 0.062 mol) in 100 ml of dry THF was added dropwise over 20 min and the mixture was stirred for 4 hr at 0°. After addition of sat NH_4Cl (100 ml) it was extracted with Et_2O twice and the combined extracts were washed with brine, dried (Na_2SO_4) and evaporated to give a solid residue. It was chromatographed on silica gel with elution by 25 : 75 EtOAc–hexane to obtain 22 g of pure **22** (mixture of *cis* and *trans* isomers) as an oil. The mixture of the two isomers was directly subjected to the next reaction.

Hydrogenation of the olefin **22**

A soln of **22** (21 g, 0.058 mol), $NaHCO_3$ (10.5 g) and 10% Pd/C (2.1 g) in 500 ml of MeOH was stirred overnight under H_2 (atm pressure). The catalyst was filtered off and the solvent was removed by evaporation. The resulting residue was dissolved in ether (250 ml) and the insoluble $NaHCO_3$ was removed by filtration. Evaporation of the solvent afforded the desired **23** as an oil in near quantitative yield. NMR ($CDCl_3$) δ 7.24 (t, J = 7.74 Hz, 1H), 7.02 (brd, 3H), 4.56 (s, 1H), 3.86 (m, 1H), 3.74 (m, 1H), 3.69 (s, 3H), 3.49 (m, 1H), 3.38 (dt, J = 9.48, 6.62 Hz, 1H), 3.28 (s, 3H), 2.59 (t, J = 7.84, Hz, 2H), 1.83–1.35 (m, 16H). IR (neat) 2930, 2855, 1722, 1712, 1605, 1588, 1490, 1450, 1350, 1155, 1032 cm^{-1}.

Preparation of the free amine **24**

To a soln of **23** (20 g, 0.055 mol) in 200 ml of abs EtOH was added 23 ml of 12 N KOH (5 equiv) and the mixture was refluxed gently overnight. Most of the EtOH was removed by evaporation and about 100 ml of water was added to the residue. It was extracted with Et_2O three times and the combined extracts were washed with water and brine, dried (Na_2SO_4) and evaporated to give an oily residue. It was chromatographed on silica gel with elution by 3 : 7 EtOAc–hexane to afford 14.2 g of pure **24** as an oil. NMR ($CDCl_3$) δ 7.08 (t, J = 7.73 Hz, 1H), 6.53 (d, J = 7.42 Hz, 1H), 6.42 (brs, 2H), 4.56 (s, 1H), 3.86 (m, 1H), 3.70 (dt, J = 9.52, 6.9 Hz, 1H), 3.45 (m, 1H), 3.37 (dt, J = 9.52, 6.68 Hz, 1H), 2.81 (s, 3H), 2.52 (t, J = 7.82 Hz, 2H), 1.8–1.34 (m, 16H). IR (neat) 3395, 2925, 2855, 1605, 1588, 1510, 1490, 1032 cm^{-1}.

7-*Octenoyl chloride* (**25**)

To 5.59 g of 7-octenoic acid[11] (0.039 mol) under N_2 was added $SOCl_2$ (4.3 ml, 0.059 mol) dropwise and the mixture was stirred overnight at room temp and for 2 hr at 40°. The excess $SOCl_2$ was evaporated off under reduced pressure and the residue of the desired **25**, weighing 6.4 g, was used for the next reaction without purification.

Preparation of the amide **26**

To a stirred soln of **24** (12 g, 0.039 mol) and pyridine (9.6 ml, 0.117 mol) in 100 ml of dry CH_2Cl_2 at 0° was added a soln of **25** (0.039 mol) in 50 ml of dry CH_2Cl_2 dropwise and the mixture was stirred for 2 hr at 0° and for 3 hr at room temp. After addition of 300 ml of ether it was washed with water and brine, dried (Na_2SO_4) and evaporated to give an oily residue. It was chromatographed on silica gel with elution by 1:3 EtOAc–hexane to obtain 11.3 g of the desired **26** along with 2 g of the starting **24** (83% yield based on the recovered starting material). NMR ($CDCl_3$) δ 7.30 (t, J = 8 Hz, 1H), 7.14 (d, J = 7.6 Hz, 1H), 6.96 (br, 2H), 5.75 (ddt, J = 17, 10.3, 6.7 Hz, 1H), 4.94 (dd, J = 17, 1.8 Hz, 1H), 4.90 (dd, J = 10.3, 1 Hz, 1H), 4.56 (dd, J = 2.9, 2.7 Hz, 1H), 3.87 (ddd, J = 11, 7.4, 3.6 Hz, 1H), 3.73 (dt, J = 9.6, 6.9 Hz, 1H), 3.49 (m, 1H), 3.38 (dt, J = 9.6, 6.6 Hz, 1H), 3.25 (s, 3H), 2.62 (t, J = 7.5 Hz, 2H), 2.06 (t, J = 7.4 Hz, 2H), 1.98 (q, J = 7 Hz, 2H) 1.86–1.15 (m, 22H). IR (neat) 3070, 2920, 2850, 1660, 1585, 1120, 1030 cm^{-1}. HRMS calc for $C_{27}H_{43}NO_3$ 429.3245, found 429.3214.

Preparation of the alcohol 27

A soln of **26** (11.2 g, 0.024 mol) and 5 g of DOWEX-50X8-400® in 200 ml of MeOH was stirred for 20 hr at 50° and the resin was filtered off after cooling. The filtrate was evaporated under reduced pressure to give the desired **27** as an oil in quantitative yield. NMR ($CDCl_3$) δ 7.3 (t, J = 7.8 Hz, 1H), 7.14 (d, J = 7.8 Hz, 1H), 6.97 (d, J = 7.8 Hz, 1H), 6.96 (s, 1H), 5.75 (ddt, J = 17.2, 10.2, 6.68 Hz, 1H), 4.94 (dd, J = 17.2, 1.76 Hz, 1H), 4.90 (dd, J = 10.2, 1 Hz, 1H), 3.64 (t, J = 6.55 Hz, 2H), 3.25 (s, 3H), 2.62 (t, J = 7.54 Hz, 2H), 2.10 (t, J = 7.33 Hz, 2H), 1.98 (q, J = 6.86 Hz, 2H), 1.84 (br, 1H), 1.67–1.15 (m, 16 Hz). IR (neat) 3440, 3070, 2920, 2850, 1660, 1640, 1060 cm^{-1}. HRMS calc for $C_{22}H_{35}NO_2$ 345.2668, found 345.2666.

Preparation of methanesulfonate 28

To a stirred soln of **27** (8.14 g, 0.024 mol) and Et_3N (1.02 ml, 0.072 mol) in 100 ml of dry CH_2Cl_2 at 0° was added methanesulfonyl chloride (2.83 ml, 0.036 mol) dropwise over 10 min and the mixture was stirred for 1 hr at 0°. After dilution with ether (400 ml) the soln was washed with water, 1 M HCl, sat $NaHCO_3$, water and brine, dried (Na_2SO_4) and evaporated to give chromatographically pure **28** as an oil in near quantitative yield. NMR ($CDCl_3$) δ 7.31 (t, J = 8 Hz, 1H), 7.14 (d, J = 7.63 Hz, 1H), 6.98 (d, J = 7 Hz, 1H), 6.97 (s, 1H), 5.75 (ddt, J = 17, 10.3, 6.7 Hz, 1H), 4.94 (dd, J = 19.3 Hz, 1H), 4.22 (t, J = 6.55 Hz, 2H), 3.25 (s, 3H), 3.0 (s, 3H), 2.62 (t, J = 7.56 Hz, 2H), 2.06 (t, J = 7.26 Hz, 2H), 1.98 (q, J = 7.03 Hz, 2H) 1.8–1.15 (m, 16H). IR (neat) 3070, 2930, 2850, 1655, 1600, 1585, 1485, 1350, 1175 cm^{-1}. HRMS calc for $C_{23}H_{37}NO_4S$ 423.2443, found 423.2463.

Preparation of iodide 29

To a soln of **28** (10 g, 0.024 mol) in 100 ml of dry DMF was added anhyd LiI (6.53 g, 0.049 mol) at once and the mixture was stirred for 3.5 hr at 90°. After cooling to room temp about 500 ml of ether was added and the soln was washed with water (3 ×) and brine, dried (Na_2SO_4) and evaporated to give an oily residue. It was passed through a bed of silica gel with elution by 3:7 EtOAc–hexane to remove any trace of DMF and polar impurities. Evaporation of the solvent afforded a colorless oil of the desired **29** (10.6 g). NMR ($CDCl_3$) δ 7.31 (t, J = 8 Hz, 1H), 7.14 (d, J = 7.66 Hz, 1H) 6.98 (d, J = 7 Hz, 1H), 6.97 (s, 1H), 5.75 (ddt, J = 17, 10.3, 6.7 Hz, 1H), 4.94 (dd, J = 17, 1.8 Hz, 1H), 4.90 (dd, J = 10.3, 1 Hz, 1H), 3.25 (s, 3H), 3.18 (t, J = 6.99, 1 Hz, 1H), 3.25 (s, 3H), 3.18 (t, J = 6.99 Hz, 2H), 2.62 (t, J = 7.5 Hz, 2H), 2.62 (t, J = 7.5 Hz, 2H), 1.98 (q, J = 7.01 Hz, 2H), 1.81 (qn, J = 6.94 Hz, 2H), 1.67–1.15 (m, 14H). IR (neat) 3070, 2920, 2850, 1660, 1600, 1585, 1484 cm^{-1}. HRMS calc for $C_{22}H_{34}NOI$ 455.1686, found 455.1687.

Preparation of nitro-olefin 30

A mixture of **29** (10.6 g, 0.024 mol) and $AgNO_2$ (11.26 g, 0.073 mol) in 125 ml of Et_2O was stirred for 1 day at 0° and for 12 days at room temp in the dark. The Ag salts were then removed by filtration through Celite and the solvent was evaporated off to give an oily residue (9.98 g). It was purified by chromatography on silica gel with elution by 3:7 EtOAc–hexane to obtain the recovered **29** (R_f 0.38, 1.87 g), the desired **30** (R_f 0.25, 5.44 g, 72% yield based on the recovered starting material) and the corresponding **27** (R_f 0.05, 0.96 g) which arose from nitrite ester, a side product. NMR ($CDCl_3$) δ 7.31 (t, J = 8 Hz, 1H), 7.13 (d, J = 7.5 Hz, 1H), 6.98 (d, J = 7 Hz, 1H), 6.97 (s, 1H), 5.74 (ddt, J = 17.1, 10.2, 6.7 Hz, 1H), 4.94 (d, J = 17.1 Hz, 1H), 4.89 (d, J = 10.2 Hz, 1H), 4.37 (t, J = 7 Hz, 2H), 3.24 (s, 3H), 2.62 (t, J = 7.7 Hz, 2H), 2.0 (m, 6H), 1.6 (m, 4H), 1.4–1.12 (m, 10H). IR (neat) 3060, 2920, 2850, 1650, 1600, 1582, 1540, 1485, 1435, 1376, 1110, 905 cm^{-1}.

Preparation of ansa-macrocyclic isoxazoline 35

Macrocyclization by intramolecular nitrile oxide cycloaddition reaction. To a stirred soln of *p*-chlorophenylisocyanate (15.4 g, 0.1 mol) and dry Et_3N (14 ml, 0.1 mol) in 1.5 l of dry toluene at 80° was added a soln of **30** (4.0 g, 0.0107 mol) in 500 ml of dry toluene dropwise over 26 hr and the mixture was stirred for 48 hr at 80°. After addition of 10 ml of MeOH the solvent was evaporated to give a solid residue, which was purified by column chromatography on silica gel with elution by 6:4 EtOAc–hexane. The desired **35** was obtained as a solid residue (3.12 g, 82% yield) after evaporation of the solvent and it was crystallized from ether and hexane, m.p. 95–96°. NMR ($CDCl_3$) δ 7.31 (t, J = 8 Hz, 1H), 7.12 (d, J = 8 Hz, 1H), 7.00 (d, J = 8 Hz, 1H), 6.99 (s, 1H), 4.60 (m, 1H), 3.24 (s, 3H), 2.84 (dd, J = 17, 10 Hz, 1H), 2.63 (m, 2H), 2.54 (dd, J = 17, 6 Hz, 1H), 2.48 (dt, J = 9, 5 Hz), 2.2 (dt, J = 14, 5 Hz, 1H), 2.07 (m, 2H), 1.65–1.15 (m, 16H). IR (film) 3020, 2920, 2845, 1655, 1645, 1598, 1580, 1485, 1378 cm^{-1}. HRMS calc for $C_{22}H_{32}N_2O_2$ 356.2464, found 356.2487.

Preparation of hydroxy ketone 36

A soln of **35** (0.7 mg, 1.96 mol) and Raney Ni (0.2 g) in 20 ml of CH_2Cl_2, 10 ml of MeOH, 2 ml of H_2O and 0.54 ml of HOAc was stirred under H_2 (atm pressure) for 5 hr at room temp and the catalyst was filtered off. The filtrate was washed with water, sat $NaHCO_3$ and water, dried ($MgSO_4$) and evaporated to give an oily residue. Then it was purified by column chromatography on silica gel with elution by 7:3 EtOAc–hexane to afford 544 mg of pure hydroxy ketone **36** as a solid (77% yield). It was crystallized from CH_2Cl_2, ether and hexane, m.p. 109–110°. NMR ($CDCl_3$) δ 7.34 (t, J = 7.56 Hz, 1H), 7.15 (d, J = 7.42 Hz, 1H), 7.01 (d, J = 7.92 Hz, 1H), 7.0 (s, 1H), 3.88 (m, 1H), 3.24 (s, 3H), 3.05 (d, J = 4.4 Hz, 1H), 2.62 (m, 3H), 2.43–2.30 (m, 3H), 2.05 (t, J = 7.44 Hz, 2H), 1.73–1.14 (m, 16H). IR (film) 3420, 2920, 2850, 1700, 1653, 1635, 1597, 1580, 1485, 1380 cm^{-1}.

Preparation of carbinol carbamate 37

To a stirred soln of **36** (300 mg, 0.83 mmol) and pyridine (0.34 ml, 4.15 mmol) in 8 ml of dry CH_2Cl_2 was added a soln of *p*-nitrophenyl chloroformate (336 mg, 1.66 mmol) in 2 ml of CH_2Cl_2 dropwise at 0° and it was stirred for 25 min at the same temp. After cooling to −78°, about 3 ml of NH_3 sat MeOH was introduced to the mixture and NH_3 was bubbled through the soln for a few min. It was stirred for 1 hr at −78 to 0° and partitioned between CH_2Cl_2 and water. The CH_2Cl_2 soln was washed with sat $NaHCO_3$ and water, dried ($MgSO_4$) and evaporated to give an oily residue. It was then purified by column chromatography on silica gel with elution by 1:1 THF–hexane to obtain 135 mg of the desired **37** as a solid which was crystallized from CH_2Cl_2 and hexane, m.p. 119–120°. NMR ($CDCl_3$) δ 7.33 (t, J = 8 Hz, 1H), 7.15 (d, J = 8 Hz, 1H), 6.99 (m, 2H), 5.04 (m, 1H), 4.60 (brs, 2H), 3.25 (s, 3H), 2.65 (m, 4H), 2.36 (m, 2H), 2.05 (brt, J = 7 Hz, 2H), 1.70–1.15 (16H). IR (KBr) 3430, 2930, 2860, 1709, 1697, 1660, 1608, 1590, 1492, 1388, 1340, 1065 cm^{-1}. HRMS calc for $C_{22}H_{31}NO_2$ ($M^+ - H_2O - HNCO$) 341.2355, found 341.2372.

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REFERENCES AND NOTES

[1] S. M. Kupchan, Y. Komoda, W. A. Court, G. J. Thomas, R. M. Smith, A. Karim, C. J. Gilmore, R. C. Haltiwanger and R. F. Bryan, *J. Am. Chem. Soc.* **94**, 1354 (1972).

[2a] S. Tanida, T. Hasegawa, K. Hatano, E. Higashide and M. Yoneda, *J. Antibiotics* **33**, 192 (1980); [b] A. Asai, E. Mijuta, M. Izawa, K. Haibara and T. Kishi, *Tetrahedron* **35**, 1079 (1979).

[3] Y. Komoda and T. Kishi, *Anticancer Agents Based on Natural Product Models* (Edited by J. M. Cassady and J. D. Douros), pp. 385–388. Academic Press, New York (1980).

[4a] M. K. Wolpert-DeFilippes, R. H. Adamson, R. L. Cysyk and D. G. Johns, *Biochem. Pharmacol.* **24**, 751 (1975); [b] M. K. Wolpert-DeFilippes, V. H. Bono, R. L. Dion and D. G. Johns, *Ibid.* **24**, 1735 (1975).

[5a] E. J. Corey, L. O. Weigel, D. Floyd and M. G. Bock, *J. Am. Chem. Soc.* **100**, 2916 (1978); [b] E. J. Corey, L. O. Weigel, A. R. Chamberlin and B. Lipshutz, *Ibid.* **102**, 1439 (1980); [c] A. I. Meyers, D. M. Roland, D. L. Comins, R. Henning, M. P. Fleming and K. Shimizu, *Ibid.* **101**, 4732 (1979); [d] A. I. Meyers, D. L. Comins, D. M. Roland, R. Henning and K. Shimizu, *Ibid.* **101**, 7104 (1979); [e] A. I. Meyers, K. A. Babiak, A. L. Campbell, D. L. Comins, M. P. Fleming, R. Henning, M. Heuschman, J. P. Hudspeth, J. M. Kane, P. J. Reider, D. M. Roland, K. Shimizu, K. Tomioka and R. D. Walkup, *Ibid.* **105**, 5015 (1983); [f] D. H. Barton, S. D. Gero and C. D. Maycock, *J. Chem. Soc. Perkin Trans. I* 1541 (1982); [g] E. Gotschi, F. Schneider, H. Wagner and K. Bernauer, *Org. Prep. Proc.* **13**, 23 (1981); [h] P.-T. Ho, *Can. J. Chem.* **58**, 861 (1980); [i] P.-T. Ho, *Ibid.* **58**, 858 (1980); [j] G. Gormley, Y. Y. Chan and J. Fried, *J. Org. Chem.* **45**, 1447 (1980); [k] P.-T. Ho, *Can. J. Chem.* **55**, 371 (1977); [l] R. Bonjouklian and B. Ganem, *Tetrahedron Lett.* 2835 (1977); [m] M. Samson, P. Declerq, H. DeWilde and M. Vandewalle, *Ibid.* 3195 (1977); [n] W. J. Elliot and J. Fried, *J. Org. Chem.* **41**, 2469 (1976).

[6a] E. J. Corey, L. O. Weigel, R. Chamberlin, H. Cho and D. H. Hua, *J. Am. Chem. Soc.* **102**, 6613 (1980); [b] A. I. Meyers, P. J. Reider and A. L. Campbell, *Ibid.* **102**, 6597 (1980); [c] M. Isobe, M. Kitamura and T. Gato, *Ibid.* **104**, 4997 (1982); [d] M. Kitamura, M. Isobe, Y. Ichikawa and T. Goto, *Ibid.* **106**, 3252 (1984).

[7] For a successful application of an intramolecular nitrile oxide–olefin cycloaddition strategy for macrocyclization see M. Asaoka, M. Abe, T. Mukuta and H. Takei, *Chem. Lett.* 215 (1982).

[8a] D. P. Curran, *J. Am. Chem. Soc.* **104**, 4024 (1982); [b] A. P. Kozikowski and M. Adamczyk, *Tetrahedron Lett.* **23**, 3123 (1982).

[9] The effective dose (ED_{50}) of maytansine is $1.4 \times 10^{-3}\,g\,ml^{-1}$ against L-1210 leukemia.

[10] S. Krishnamurthy, *Tetrahedron Lett.* **23**, 3315 (1982).

[11] G. M. Rubotom, R. Marrero, D. S. Krueger and J. L. Schreiner, *Tetrahedron Lett.* 4013 (1977).

[12] The alcohol **26**, which arose from hydrolysis of nitrite ester, the side product, was also isolated in 14% yield from this reaction.

[13] S. L. Schreiber, R. E. Claus and J. Reagan, *Tetrahedron Lett.* **23**, 3867 (1982).

[14] The phosphonium salt **38** was synthesized from 1-[4-chloro-3-methoxy-5-(methylamino)phenyl]-2-propanone[5g] in six steps: (i) $Ph_3P{=}CHCO_2CH_3$/toluene/reflux, 36 hr; (ii) DiBAL-H/CH_2Cl_2/0°, 1.5 hr; (iii) 7-octenoyl chloride (**25**), pyridine/CH_2Cl_2/0°, 1 hr; (iv) 10% aq K_2CO_3/MeOH/RT, 5 hr; (v) PBr_3, LiBr, collidine/Et_2O–THF/0°, 25 min; (vi) Ph_3P/CH_3CN/RT, 2 hr.

[15] The ratio between *E*, *E* and *E*, *Z* dienes was about 1 : 1.