to contain the unusual dolapyrrolidone (Dpy, **2**), 2-hydroxyisovaleric acid (Hiva, **3**), dolavaline (Dov[3e]), proline, valine, and *N*-methylvaline units. The paucity of natural product prevented us from determining the absolute configuration of dolastatin 15. On the assumption that the dolastatin 15 amino acids most probably possessed the common L configuration (*S* used in the sequel) found in dolastatin 10,[2d] the total synthesis was initiated on that basis.

Synthesis of the key Dpy (**2**) intermediate **6** was achieved as summarized in Scheme I. The hydroxyisovaleric acid (Hiva) component was obtained from (*S*)-Val via a diazotization sequence.[4] Condensation of unprotected (*S*)-Hiva with (*S*)-Phe-OMe, employing diethyl phosphorocyanidate, proceeded well to afford (60%) (*S*)-Hiva-(*S*)-Phe-OMe (**4a**) as needles from toluene–hexane, melting at 70 °C: $[\alpha]_D$ −45° (*c*, 0.002). Protection (**4a** → **4b**, oil, 87% yield)[5] employing *tert*-butyldimethylsilyl chloride, saponification (**4b** → **4c**), and reaction of the resulting carboxylic acid (**4c**, mp 87–8 °C from hexane, 90% yield) with pentafluorophenyl trifluoroacetate followed by Meldrum's ester[6] gave pyrrolidone **5a** as a viscous oil. Methylation with dimethyl sulfate provided (21% overall from **4c**) the corresponding methyl ether (**5b**). Cleavage of the silyl group with pyridinium polyhydrogen fluoride gave (90%) (*S*)-Hiva-(*S*)-Dpy (**6**) as an oil: $[\alpha]_D$ +285° (*c*, 0.002). Esterification[7] of alcohol **6** with Boc-(*S*)-Pro gave (74% yield) depsipeptide **7** [(mp 157–158 °C, $[\alpha]_D$ +96.2° (*c*, 1.85, $CHCl_3$)]. In order to unequivocally establish the absolute configuration of depsipeptide **7**, a specimen crystallized from acetone–hexane was subjected to X-ray crystal structure analysis (see the supplementary material).[8-11] Absolute stereochemical assignments (Figure 1, supplementary material) to the three chiral centers were made, based upon the known stereochemical configuration of (*S*)-Pro. The stereochemical designations for the three chiral centers were determined to be C-4(*S*), C-7(*S*), and C-10(*S*).

Tetrapeptide **10** was prepared by starting with dipeptide **8** [(77%, needles from ethyl acetate–hexane, mp 100–2 °C, $[\alpha]_D$ −144.6° (*c*, 2.04, $CHCl_3$)] using the procedure outlined in Scheme I. Hydrogenolysis followed by a pivaloyl mixed anhydride peptide bond forming step gave tripeptide **9** [83%, oil $[\alpha]_D$ −145° (*c*, 2.56, $CHCl_3$)]. Subsequent hydrogenolysis and in situ reaction with (*S*)-Dov-OPfp led to tetrapeptide **10** [(84%, amorphous powder from acetone–hexane, mp 137–40 °C, $[\alpha]_D$ −180° (*c*, 2.08, $CHCl_3$)]. Finally, the carboxylic acid obtained by saponification of methyl ester **10** was condensed with the amine derivative of proline **7** to give, following separation on Sephadex LH-20 (2:2.5:7.5, hexane–methylene chloride–methanol), natural (−)-dolastatin 15 (**1**, 68% yield as an amorphous powder from acetone–hexane), mp 112–4 °C, $[\alpha]_D$ −48.2° (*c*, 0.11, CH_3OH), identical by TLC and ^1H and ^{13}C NMR with an authentic sample. Synthetic dolastatin 15 gave the same (ED_{50} 10^{-3} μg/mL) potent activity against the P388 lymphocytic leukemia routinely obtained with the natural product.

The first total synthesis of natural (−)-dolastatin 15 herein summarized has firmly established the overall absolute configuration (all chiral centers *S*). Synthesis of potentially useful structural and chiral modifications of (−)-dolastatin 15 (**1**) and a broad evaluation of biological properties are in progress. Presently (−)-dolastatin 15 is undergoing preclinical development as a potential anticancer drug.

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Registry No. 1, 123884-00-4; **3**, 17407-55-5; **4a**, 134816-19-6; **4b**, 134816-26-5; **4c**, 134816-27-6; **5b**, 134816-20-9; **6**, 134816-21-0; **7**, 134816-22-1; **8**, 134816-23-2; **9**, 134816-24-3; **10**, 134816-25-4; Val, 72-18-4; Phe-OMe·HCl, 7524-50-7; Boc-Pro, 15761-39-4; Pro-OMe·HCl, 2133-40-6; (Z)-MeVal, 42417-65-2; MeVal-Pro-OMe·HCl, 134816-28-7; (Z)-Val, 1149-26-4; Dov-OC_6F_5, 97800-78-7; Dov-Val-MeVal-Pro-OH, 134816-29-8; Meldrum's acid, 2033-24-1.

Supplementary Material Available: Experimental section for synthesis of dolastatin 15 and crystal structure details, tables of bond distances and angles, and positional parameters for Dpy derivative **7** (22 pages). Ordering information is given on any current masthead page.

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Antineoplastic Agents. 224. Isolation and Structure of Neristatin 1[1a]

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The marine bryozoan *Bugula neritina* has been found to contain a unique series of closely related macrocyclic (22-membered) lactones now known as the bryostatins.[2,3] Because of their very selective antineoplastic and cytostatic activity, potent influence on protein kinase C biochemical pathways, antitumor promoter effects, and stimulation of bone marrow progenitor cells to form colonies (GM-CSF activity), bryostatin 1 (**1**)[4] has been selected for clinical evaluation. Discovery and study of bryostatin biosynthetic precursors or degradation products has been considered necessary to gain further mechanistic and structure/activity insights. We now report the isolation and structural elucidation of the first such example, herein designated neristatin 1 (**2**).

A 1000-kg (approximate damp weight) re-collection (1986, Gulf of Mexico coast of Florida) of *B. neritina* Linnaeus was extracted with 2-propanol. Initial solvent partitioning and steric exclusion chromatographic procedures were conducted as previously described for the closely related bryozoan *Amathia convoluta*.[5] Separation was guided by bioassay employing the P388 lymphocytic leukemia cell line with a combination of gel permeation (and partition on Sephadex LH-20) and partition (silica gel)

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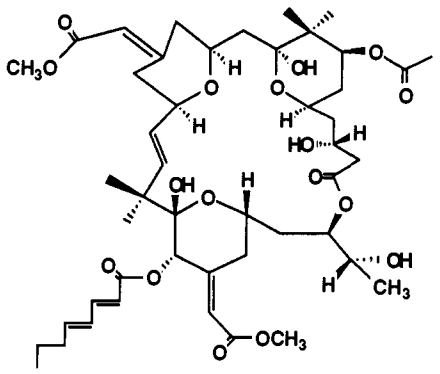
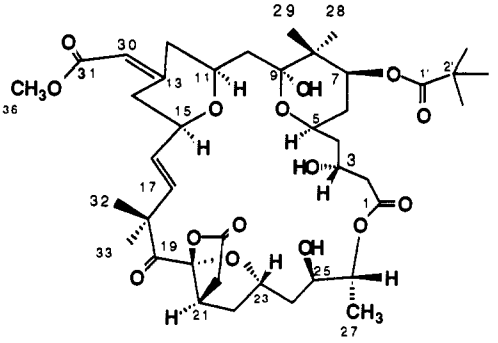


1, BRYOSTATIN 1



2, NERISTATIN 1

chromatography, high-speed countercurrent distribution,[6] and HPLC[7] techniques to afford 8.0 mg (8.0×10^{-7}% yield) of colorless neristatin 1 (**2**): mp 214–216 °C; $[\alpha]_D = +98°$ (CH_2Cl_2, c = 0.26); R_f 0.62 (silica gel, toluene–ethyl acetate–methanol, 3:1:1); HPLC retention times, 11 min (RP C-8 column, 10 mm × 25 cm, 80% methanol–water, 0.8 mL/min) and 9 min (normal-phase column, 10.0 mm × 25 cm, hexane–methylene chloride–methanol, 14:8:1, with UV detection at λ = 254 nm); HRFABMS (with LiI) found 799.4090, calcd for $C_{41}H_{60}O_{15}Li$ 799.4092; EIMS 70 eV, m/z, M^+ 792 (33%) calcd 792 for $C_{41}H_{60}O_{15}$, 774 (100%) $[M - H_2O]^+$ and 756 (8%) $[774 - H_2O]^+$. The NMR data have been entered in ref 8.

The possible relationship of neristatin 1 to the bryostatins was first suggested by the characteristic color it displayed on a thin layer chromatographic plate.[4] However, a detailed 2D NMR study rapidly uncovered major structural differences. Only one methoxy signal appeared in the ^1H NMR spectrum, and signals assigned by using ^1H–^1H COSY and ^1H–^{13}C correlated spectra suggested that neristatin 1 did not possess a bryopyran ring.[8] In the ^1H NMR spectrum, the H-26 signal was shifted downfield at δ 4.87 and the H-25 signal shifted upfield at δ 3.47 compared with those of the bryostatins. More importantly, a doublet signal at δ 4.92 (J = 6.3 Hz) was found coupled with the H-25 signal. The former disappeared and the latter was simplified upon addition of D_2O. Such evidence suggested that a free hydroxy group was present at C-25 and lactonization involved the C-26 position. The presence of four ^{13}C ester carbonyl signals at δ 169.13, 166.87, 174.19, and 178.00 combined with results of heteronuclear multiple-bond correlation (HMBC) experiments also supported the presence of a γ-lactone. The two signals at δ 2.87 and 2.40 correlated with a carbonyl signal at δ 174.19 (C-35) and a signal at δ 40.41 (C-21). Interestingly the C-20–C-23 region of neristatin 1 proved refractory to rigorous (and convincing) 2D NMR interpretation until the crystal structure analysis was in hand.

Unlike the very stable bryostatin series (except for bryostatin 3),[9] neristatin 1 proved sensitive to recrystallization attempts, and a number of small samples rapidly degraded in various solvents until it recrystallized unchanged from acetone–hexane. While only relatively poor quality (cracked, vapor pockets and other imperfections) crystals were available for the crystal structure determination (see supplementary material),[10–12] we were able to surmount this difficulty and obtain an unequivocal structure for neristatin 1 (**2**). The absolute stereochemical configuration assigned to neristatin 1 is based upon the known absolute stereochemistry of the related bryostatins.[9] Stereochemical assignments for the 10 chiral centers of neristatin 1 are as follows: 3(*R*), 5(*R*), 7(*S*), 9(*S*), 11(*S*), 15(*R*), 20(*S*), 21(*S*), 23(*S*), 25(*R*), 26(*R*).

The binding affinity of neristatin 1 for protein kinase C was determined by competition for [26-^{3}H]bryostatin 4 binding, under the conditions previously developed for measurement of highly potent bryostatins.[13] The K_i for neristatin was found to be 124 ± 10 nM (mean ± SEM, four experiments), compared to 1.3 nM for bryostatin 4[13] and 12.3 nM for the typical phorbol 12,13-dibutyrate.[13] Because of the relatively low affinity of neristatin, it was again assayed under phorbol ester binding conditions.[14] Here, the K_i of neristatin 1 was 21.2 ± 1.3 nM (mean ± SEM, three experiments), compared to 0.53 nM for phorbol 12,13-dibutyrate and K_d = 13 nM for bryostatin 4.[15] Thus, neristatin 1 retains appreciable affinity for protein kinase C, albeit 1 order of magnitude less than that of phorbol 12,13-dibutyrate and at least 2 less than that of bryostatin 4.

Consistent with the binding results, neristatin was only weakly active (ED_{50} = 10 μg/mL) against the P388 cell line. The reduced but still significant potency of neristatin 1 compared to the bryostatins was considered very important for further defining structure/activity relationships and biochemical mechanisms among this remarkable series of biosynthetic products.

Acknowledgment. Financial support was provided by Outstanding Investigator Grant CA44344-01A1 and Grant CA-16049-10-12 awarded by the Division of Cancer Treatment, NCI,

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Registry No. 2, 135004-30-7; protein kinase, 9026-43-1.

Supplementary Material Available: Crystal structure determination data for neristatin 1 including coordinates, bond lengths, bond angles, and coefficients (9 pages). Ordering information is given on any current masthead page.

A Molecular Clip with Allosteric Binding Properties

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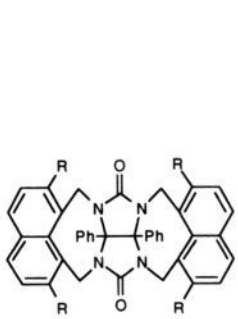
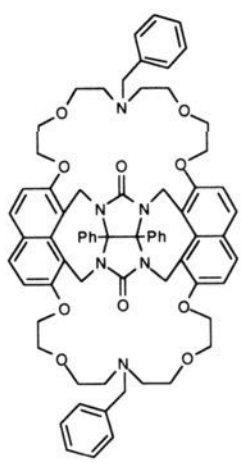
In allosteric receptors the binding of one substrate is influenced by the binding of a second substrate at a remote site. Allosteric effects play an important role in regulating biological processes like oxygen transport and enzyme activity.[1] There are only a few reports in literature on synthetic receptors in which binding is regulated by the allosteric effect. Most of these reports concern hosts with binding sites for metal ions.[2] Very recently, Schneider and Ruf have described a synthetic receptor which shows enhanced binding of aromatic substrates in the presence of Cu^{2+} or Zn^{2+}, due to an allosteric effect.[3] We report herein a molecular receptor (**1**), that can exist in different conformations, one of which is able to bind 1,3-dinitrobenzene by $\pi-\pi$ interactions. Moreover, we describe a bis-crown ether analogue of this receptor (**2**) in which a dinitrobenzene-binding conformation can be induced by addition of a metal salt (Figure 1a).



1a R = H
1b R = OAc
1c R = OMe



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Compound **1b** exists in $CDCl_3$ solution as a mixture of conformers, that interconvert slowly on the NMR time scale. This process could be monitored with a two-dimensional ^1H NMR exchange experiment.[4] Inspection of CPK models and molecular mechanics calculations[5] on **1a** suggest that compounds **1** can exist in three conformations of similar energy, designated as *ss* (syn syn), *sa* (syn anti), and *aa* (anti anti), respectively (Figure 1b). This feature is in accordance with the presence of four AB patterns in the ^1H NMR spectrum for the methylene protons of **1b**, one AB pattern for each of the *aa* and *ss* conformers, and two for the less symmetric *as* conformer.[6] The NMR data show that at 25 °C, 91% of molecules **1b** are in the *as* conformation, 4.7% in the *ss* conformation, and 4.3% in the *aa* conformation. Compound **1c**, of which an even lower proportion of the molecules is present in the *aa* conformation, forms a complex with 1,3-dinitrobenzene (DNB) in $CHCl_3$, as can be concluded from the development of a yellow color upon addition of this substrate. In the ^1H NMR spectrum of **1c**, the intensity of the signals of the *aa* conformer shift and increase in intensity when DNB is added, whereas the signals of the other conformers decrease in intensity.[7] Therefore this receptor has a higher affinity for DNB in its *aa* conformation, leading to an induced fit type of binding mechanism. From a UV titration in $CHCl_3$, the association constant for DNB was calculated to be $K_a = 4.2 \pm 0.4\ M^{-1}$.

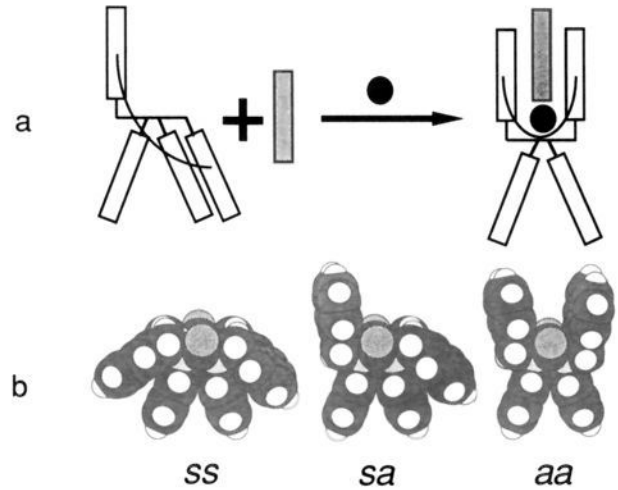

Figure 1. (a) Induced binding of 1,3-dinitrobenzene in **2** by addition of metal ions and (b) modeled structures of the three conformations of **1a**.

Compound **2** is an analogue of **1**, in which the naphthalene moieties are bridged by two aza crown ether rings. Compounds similar to **2** are strong binders of alkali-metal ions.[8] Like **1b** and **1c**, **2** exists predominantly in the *sa* conformation. In this conformation as well as in the *ss* form, the crown ether parts of the molecule cannot effectively bind an alkali-metal ion due to interference by the glycoluril part of the molecule. CPK models show, however, that in the *aa* conformation each of the crown ether moieties can wrap nicely around an ion. Addition of a potassium salt to a solution of **2** leads to an increase in intensity of the ^1H NMR signals of the *aa* conformation. When a solution of **2** in $CDCl_3$/DMSO-d_6 (3:1 v/v) was titrated with potassium picrate, initially 2.08 potassium ions were required to bring one molecule of **2** in the *aa* conformer. This indicates that **2** binds potassium ions in a 1:2 stoechiometry.

Since the *aa* conformer of **2** is expected to display the highest affinity for 1,3-dinitrobenzene, we were tempted to assess the ability of metal ions to induce substrate binding by an allosteric

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(5) Molecular mechanics calculations with the MMP2(85) force field parameters yield energies for the three conformers that are within 1.6 kcal of each other.
(6) Peaks in the spectrum could be assigned on the basis of the fact that in the *ss* and *sa* conformers the protons of the phenyl groups undergo an upfield shift due to the proximity of the naphthalene rings and on the fact that the signals from the nonequivalent sides of the *sa* conformer have the same intensity.
(7) From the observed induced shifts on host and guest protons we conclude that DNB is bound with its NO_2 groups pointing away from the cavity.
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