Evolving Trends in the Dereplication of Natural Product Extracts: New Methodology for Rapid, Small-Scale Investigation of Natural Product Extracts

Gerhard Lang,[†] Nor Ainy Mayhudin,[†,‡] Maya I. Mitova,[†] Lin Sun,[†] Sonia van der Sar,[†,§] John W. Blunt,[†] Anthony L. J. Cole,[‡] Gill Ellis,[†] Hartmut Laatsch,[⊥] and Murray H. G. Munro*[,†]

Department of Chemistry and School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch, New Zealand, and Department of Organic and Biomolecular Chemistry, University of Göttingen, Göttingen, Germany

Received April 7, 2008

The use of an HPLC bioactivity profiling/microtiter plate technique in conjunction with capillary probe NMR instrumentation and access to appropriate databases effectively short-circuits conventional dereplication procedures, necessarily based on multimilligram extracts, to a single, more rapid submilligram operation. This approach to dereplication is illustrated using fungal or bacterial extracts that contain known compounds. In each case the dereplication steps were carried out on microgram quantities of extract and demonstrate the discriminating power of ^{1}H NMR spectroscopy as a definitive dereplication tool.

Natural products continue to play an important role in the discovery and development of new chemical entities as drug-lead compounds. Considerable effort is being expended by many groups worldwide on the isolation of natural products from terrestrial and marine macro- and microorganisms. These efforts are often combined with the application of bioassays to crude extracts to identify those that might contain bioactive compounds that can then be isolated by a bioassay-guided fractionation process. Conventionally, this process involves the extraction of significant amounts of source organism, followed by extensive chromatography leading to pure compounds, which are then characterized by instrumental techniques including mass spectrometry and UV, IR, and NMR spectroscopies (Supporting Information, Figure 1a). The commitments are becoming increasingly expensive in terms of manpower, collection costs, and technology. Increasingly, the disappointing outcome of this extended approach to the dereplication of crude extracts is the isolation of previously characterized compounds. (Dereplication is the process of differentiating those natural product extracts that contain nuisance compounds, or known secondary metabolites, from those that contain novel compounds that are of interest.)

There are numerous approaches to dereplication based on hyphenated techniques, and each has its own advantages in sensitivity, resolution, or scale (mg vs μg). The most common approaches are LC-UV, LC-MS, LC-MS/MS, and LC-NMR, or combinations thereof, and the increasing use of capillary and cryo NMR probes.[1−9]

The dereplication methods based on the use of LC-MS and LC-MS/MS are very sensitive and provide structural information (low- or high-resolution molecular mass, molecular formula), which is searchable in most of the commercial databases (AntiBase,[10] Dictionary of Natural Products,[11] MarinLit,[12] and, with limitations, in SciFinder Scholar or STN International (CAPlus)). The disadvantages of these methods, which can lead to false compound identification, are the uncertainty regarding the apparent pseudomolecular ion, which could not be easily attributed to certain adduct ions (MH^+, MNa^+, MK^+, MNH_4^+, MCH_4CN^+, etc.),[3] or the presence of interfering ions from minor components that ionize more readily than the component of interest.

Access to 1D ^{1}H NMR data at the initial steps of dereplication of crude extracts can significantly accelerate the whole process. The presence or absence of easily recognized functional groups in a compound of interest is an extremely discriminating approach to dereplication. The genesis of the NMR approach taken in MarinLit and subsequently in AntiMarin dates back to a conference presentation in 2000,[13] while the use of NMR functional groups was initially commented on in the literature by Bradshaw et al. in 2001.[14] They were able to demonstrate that a combination of the numbers of methyl, methylene, and methine groups together with mass data was an excellent discriminator and, furthermore, unlike the MS/MS approach was sensitive to stereochemical differences if chemical shift data were considered. A similar approach was used by Bitzer et al.[15] However, the general applicability of these two approaches to dereplication (Bradshaw and Bitzer) is not possible, as the databases used are not available.

Herein we present a method of extract evaluation using HPLC profiling with biological evaluation[16] followed by capillary probe NMR spectroscopy/ESMS/UV combined with NMR database (AntiMarin) evaluation, which reduces the crude extract requirement for dereplication to submilligram quantities.[17] Furthermore, for compounds of molecular mass less than ~600 Da, it is possible to acquire high-quality 1D and 2D NMR data over a reasonable time frame, allowing full characterization from the material collected by HPLC into a single microtiter plate well. This effectively telescopes crude extract evaluation, isolation, dereplication, and characterization into just one process (Supporting Information, Figure 1b).[17]

Description of the Method. Typically, fungal or bacterial isolates were grown on agar slopes (4 mL), on a single Petri dish, or as small liquid cultures (10 mL) using a variety of media and temperatures, followed by extraction with EtOAc. The crude extracts (typically in the range 0.5−2 mg) were evaluated for cytotoxic, antimicrobial, and antifungal properties.[18] The dereplication process for bioactive extracts was initiated by HPLC analysis of 250−750 μg of crude extract using an analytical reversed-phase column and elution with a standardized acetonitrile/water gradient.[16]

In the first pass of the dereplication process, the UV spectra and retention times recorded for each bioactive peak during the HPLC separation are searched in an in-house library of previously isolated and known fungal and bacterial metabolites. This database has been built within the HPLC operating system (Dionex). Particular attention has been paid to ensure that all HPLC parameters have been maintained constant over the years so that it is possible to realistically include the retention time along with a direct com-

* To whom correspondence should be addressed. Tel: +64-3-3642434. Fax: +64-3-3642110. E-mail: murray.munro@canterbury.ac.nz.
[†] Department of Chemistry, University of Canterbury.
[‡] School of Biological Sciences, University of Canterbury.
[§] Current address: Kekulé Institute of Organic Chemistry and Biochemistry, University of Bonn, Gerhard-Domagk-Str. 1, 53121 Bonn, Germany.
[⊥] University of Göttingen.

10.1021/np8002222 CCC: $40.75 © 2008 American Chemical Society and American Society of Pharmacognosy
Published on Web 08/19/2008

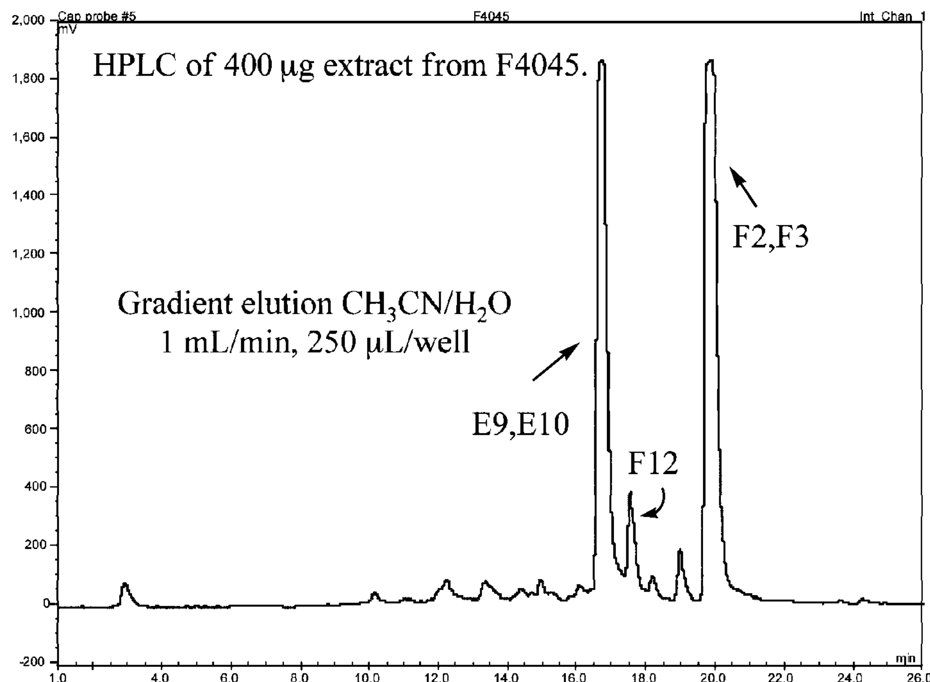


Figure 1. HPLC chromatogram, with ELSD detection, of 400 μg of extract from a nonsporulating endophyte, F4045, isolated from a New Zealand *Muehlenbeckia* sp.

parison of the UV data. As necessary, the retention time comparison can be omitted. If a match of data with a known compound is found, then no further effort is expended on the isolation of that component.

If no match is found in the in-house UV library, then the ESMS and/or UV data can be entered as search components into databases such as MarinLit[12] or AntiBase.[10] In our experience searches using UV and/or ESMS data in MarinLit or AntiBase may not always produce a definitive answer. This can arise from the lack of characteristic UV maxima and uncertainty over the apparent molecular ion from the ESMS. The use of ESMS/MS can circumvent this problem. At the University of Göttingen, an in-house ESIMS/MS database with more than 1000 entries has been established. However, the availability of a sufficient number of authentic compounds and difficulties to transfer and run the database on other spectrometers remain a problem (*vide infra*).

The approach that makes this dereplication process very discriminating is the inclusion in the databases of ^{1}H NMR structural information in searchable numeric fields. Such structural information can readily be obtained from a very preliminary interpretation of a ^{1}H NMR spectrum. For example, signals arising from methyl groups are usually very easy to recognize, and additionally, these can be characterized as singlets, doublets, or triplets, or as being attached to oxygen or nitrogen. Variously substituted benzene rings can often be established from the coupling patterns of the aromatic proton signals. To implement this approach, the compound data contained in the MarinLit and AntiBase databases have been merged to form AntiMarin,[19] which has data for ~47 000 unique compounds. For every compound in AntiMarin, the number and type of methyl groups, the number of sp^3-hybridized methylene or methine protons, the full range of benzenoid, pyridine, and alkene substitution patterns, and carbinol, acetal, ether, amide, and formyl groups have been coded into the database as numeric searchable features. These data can be searched independently or in combination with all the other usual searchable features (UV, mass, bibliographic information, etc.) as well as substructure searching.

From the scale of the HPLC separation described above, it is expected that each well in the microtiter plate might contain 2−40 μg of bioactive compound. Until recently it has not been possible to obtain meaningful spectra on such small amounts. However, by using a capillary NMR probe (CapNMR) (cell volume of ~6 μL

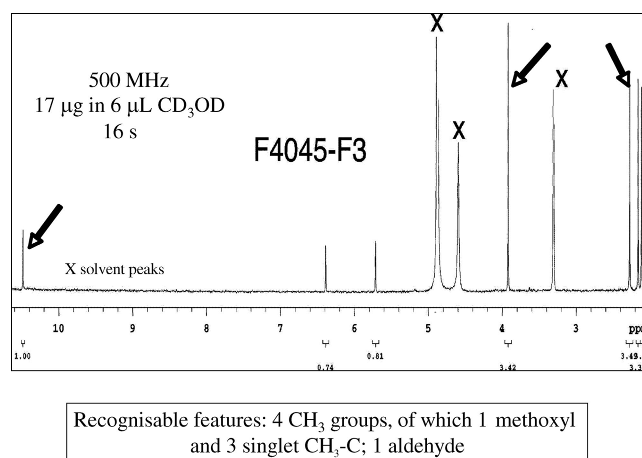


Figure 2. ^{1}H NMR spectrum of F3 from HPLC/bioactivity profiling of 400 μg of extract from the nonsporulating endophytic fungus F4045, isolated from a *Muehelenbeckia* sp.

in a 500 MHz NMR instrument), it is now possible to obtain good spectra in a few minutes on as little as 2 μg of compound. These spectra can then be examined for characteristic features that are then searched against the AntiMarin database for dereplication purposes.

As an example we investigated the crude extract of an unidentified nonsporulating endophyte (strain F4045). HPLC on an aliquot (400 μg) of this extract gave a simple HPLC trace showing two major (wells E9/E10, **1** and F2/F3) and one minor (F12; **2**) bioactive compound (Figure 1). Well F3 (17 μg; see Experimental Section for quantitation method) gave a simple ^{1}H NMR spectrum that was acquired in 16 s. The ^{1}H NMR spectrum (Figure 2) revealed that compound **1** contained a total of four singlet methyl peaks, one of which was from a methoxyl group (δ_H 3.8) and one formyl group (δ_H 10.5). Using this profile, a search in AntiMarin returned 23 hits from ~47 000 compounds (Supporting Information, Figure 2). The search profile could be refined further by using the *absence* of certain functional groups, in this case, the lack of any proton signals from sp^3-hybridized methylene or methine groups. When the search

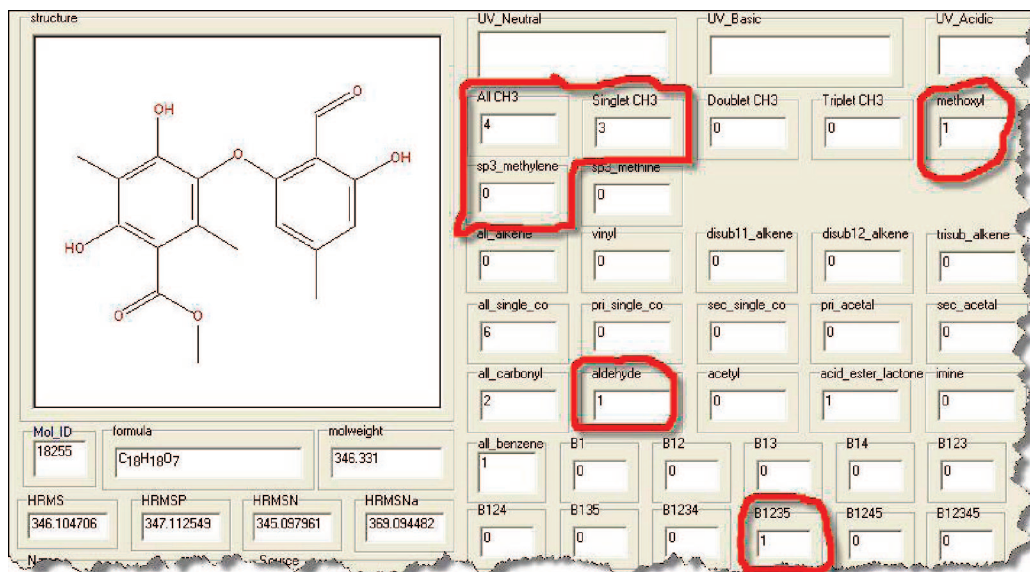
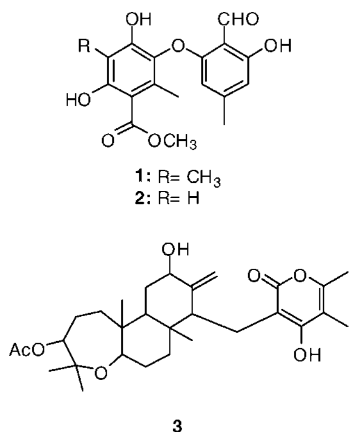


Figure 3. Screen shot from AntiMarin showing profile used to identify phomosine A (**1**).

was repeated with the addition of zero sp^3-hybridized methylene protons, the number of hits was reduced to only 11 (Supporting Information, Figure 3). Alternatively, the search profile could be tightened by using additional data: two slightly broadened (*meta*-coupled) one-proton aromatic singlets were observed (δ_H 6.40 and 5.71), characteristic of a 1,2,3,5-tetrasubstituted benzene ring. If this was included in the search profile, only one hit, that for phomosine A, was returned (Figure 3). Another approach, which returned the same answer, was to include data from the positive ESMS spectrum (346 Da; searched on 345−347 Da) (Supporting Information, Figure 4). When the ^{1}H NMR data for the compound **1** were compared with the literature data for phomosine A, they were found to be identical.[20]



In order to demonstrate the sensitivity of this approach, the minor component **2** was also examined. In the ^{1}H NMR spectrum of **2** (2 μg) three singlet methyl groups, one of which was a methoxyl, and a formyl group were observed (Supporting Information, Figure 5). Searching AntiMarin with these data and a molecular mass of 332 Da (from ESMS) gave only one hit, corresponding to phomosine C (Supporting Information, Figure 6).[20] The remaining component in the extract (wells E9/E10; 14 μg) was also a phomosine derivative.

The second example is based on a bioactive extract from a Malaysian endophytic *Aspergillus* sp. The HPLC profile of the crude extract contained in excess of 15 peaks (Figure 4). HPLC/bioactivity profiling established that just one of the peaks, a relatively minor one, was bioactive. ^{1}H NMR data for compound **3** (Figure 5), located in well F9, very clearly showed seven singlet methyl groups, one of which arises from an acetyl group (δ_H 2.17). Searching AntiMarin on seven singlet methyls gave 327 hits, which were reduced to 34 if one of the methyl groups was assigned as an acetyl group and to just five hits if two vinyl methyl groups were included in the search profile (based on their chemical shifts; δ_H 1.85 and 2.05) (Supporting Information, Figure 7). The use of the molecular mass of **3** (502 Da) together with the NMR data resulted in only one hit. The compound was identified as NF-00659-A3, isolated previously from an *Aspergillus* sp. (no stereochemistry had been assigned).[21] It is pertinent to note that if the mass data alone had been used in the search profile (502 Da; searching on 502.5−502.7), 52 hits were returned. This situation can be improved, however, if HRMS data are accessible. Even an accuracy of only two significant places (503.30 for MH^+) is sufficient to reduce the 47 000 entries of AntiMarin to just five possible compounds, all having the formula $C_{29}H_{42}O_7$.

Results and Discussion

Using the techniques described above, extracts from many different sources and differing genera have been dereplicated in a minimal time frame and a wide range of known compounds have been identified. For any compound that does not show ^{1}H NMR features that can be matched in the AntiMarin database, the problem becomes one of structure elucidation. For simpler molecules, this can be achieved by using the same sample that was used for the dereplication process, and 2D NMR spectra could be obtained using the CapNMR probe. For larger molecules this approach becomes more problematical.

Our particular approach to dereplication has several advantages: cost, throughput, scale, access, and integration, and each aspect has significant implications for the effectiveness and applicability of any dereplication effort.

Cost. Items not routinely found in, or available to, a natural products isolation laboratory are the capillary NMR probe and possibly the databases. However, running costs for a capillary NMR probe are low, as no tubes are required and only 50−70 μL of deuterated solvent are required per sample, including recovery. The cost for either the MarinLit[12] or AntiMarin[19] databases is modest.

Throughput. The HPLC/microtiter plate analysis time is 40 min/sample. Preparation of daughter plates for bioassay and MS studies can be grouped but take only minutes per sample. Routine acquisition of ^{1}H NMR spectra from a CapNMR probe takes less

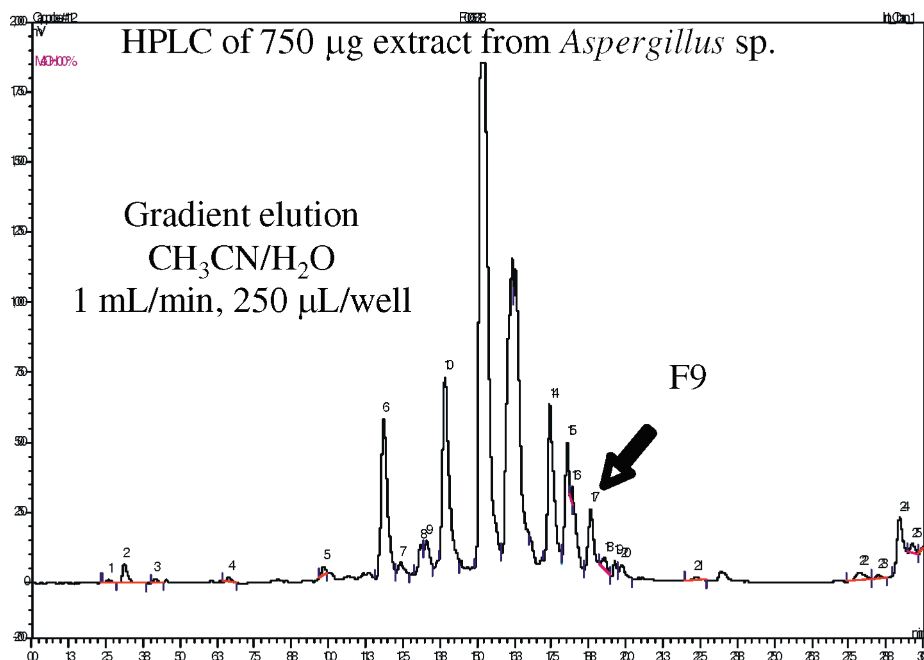


Figure 4. HPLC chromatogram with ELSD detection of 750 μg of extract from an *Aspergillus* sp., F6878, isolated from the Malaysian tree Kenondong Koot.

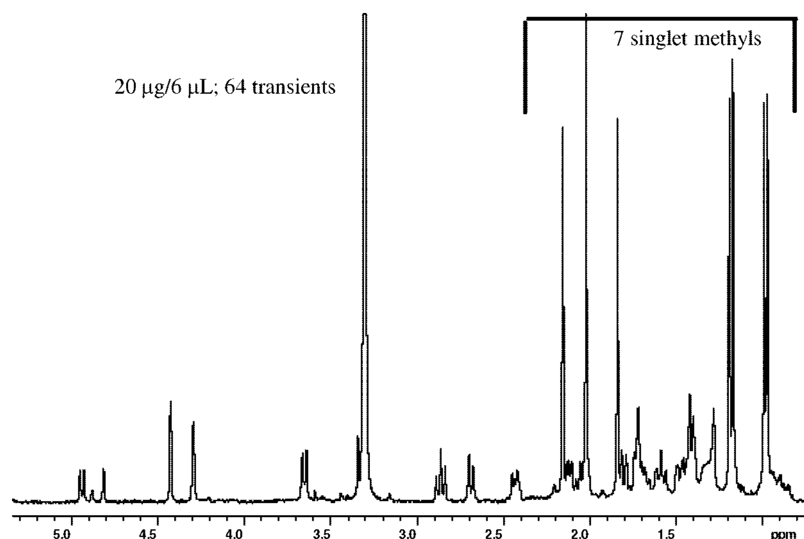


Figure 5. ^{1}H NMR spectrum of F9 from HPLC/bioactivity profiling of 750 μg of extract from an *Aspergillus* sp., F6878, isolated from the Malaysian tree Kenondong Koot.

than 10 min/sample. However, for those samples for which 2D homonuclear and heteronuclear data are required, the time requirements rise quite rapidly, but that is normally a problem only if a new compound is suspected (*vide supra*).

Scale. It is now possible to operate routinely using just a single injection of <700 μg of crude extract onto an analytical HPLC column with collection of the eluent into a 96-well mictrotiter plate.

Access. For this approach to dereplication the only databases that contain structural information as searchable fields and available commercially are MarinLit[12] and AntiMarin.[19] For comparison of UV data the Marine Group at the University of Canterbury has generated a library of ~1000 known and unknown UV profiles, primarily of fungal origin, accumulated from samples we have examined. This library has been developed using HPLC operating software[22] and is available on application.[23] In addition, the next update of AntiMarin[19] (and AntiBase[10]) will contain an additional 12 000 UV data sets from Berdy's database of natural products.[24]

Integration. The final aspect of our approach to dereplication is the tight integration of the data. The UV data for searching UV libraries are acquired during the HPLC run and can be directly correlated with specific wells on the microtiter plate. The balance of the data, biological, ESMS, and NMR, are acquired directly from specific wells. This tight integration of the data is of great advantage when correlating results, as this can be done on a well-by-well basis.

We believe that this combination of cost-effectiveness, potential for throughput, the microgram scale of the approach, the ready availability of appropriate databases, and the tight integration of the results represents a practicable, new, inexpensive, and innovative approach to sample dereplication. An example of dealing with a more complex new metabolite is dealt with in Evolving Trends in

the Dereplication of Natural Product Extracts: Part 2.[25] We are now exploring the extension of this approach to include a database of *all* natural products (>145 000), each one coded with the numbers of ^{1}H NMR recognizable features.

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a Varian INOVA AS-500 spectrometer (500 and 125 MHz for ^{1}H and ^{13}C NMR, respectively), using the signals of the residual solvent protons and the solvent carbons as internal references (δ_H 3.3 and δ_C 49.3 ppm for CD_3OD). A Protasis CapNMR capillary probe was used for the microplate dereplication studies. ESIMS were acquired using a Micromass LCT TOF mass spectrometer. Solvents used for extraction and isolation were distilled prior to use. Bioactivity assays were measured using standard protocols.[16,18]

Isolation and Cultivation of the Fungal Strains. The nonsporulating endophyte F4045 was isolated from a New Zealand native plant *Muehlenbeckia* sp. A twig was placed on MYE medium and incubated for 30 day at 26 °C.

The endophytic *Aspergillus* sp. was isolated from the Malaysian native plant Kenondong Koot using PDA medium and inoculation for 30 days at 26 °C.

Voucher specimens of F4045 and the endophytic *Aspergillus* sp. have been deposited in the University of Canterbury and the Universiti Teknologi Mara fungal collections, respectively.

For chemical studies F4045 was grown in liquid culture (10 mL of MYE medium) for 28 days (14 days shaking followed by 14 days static). The fermentation broth was macerated with EtOAc and centrifuged (4000*g*), and the EtOAc was removed and concentrated under reduced pressure to give the crude extract (3.2 mg). The endophytic *Aspergillus* sp. was grown to maturity on PDA medium (15 plates; 20 mL). The combined agar was macerated with EtOAc and centrifuged (4000*g*), and the EtOAc was removed and concentrated under reduced pressure to give the crude extract (45.3 mg).

Evaluation of Extracts. An aliquot of extract (up to 750 μg) was analyzed by HPLC (RP-18, solvents: (A) H_2O + 0.05% TFA, (B) MeCN; gradient: 0 min 10% B, 2 min 10% B, 14 min 75% B, 24 min 75% B, 26 min 100% B; flow: 1 mL/min; 40 °C). The eluent from the DAD was split in a 1:10 ratio between an ELSD and a fraction collector configured to collect into a 96-well microtiter plate (15 s/well). A total of 88 wells were collected (2.5−24.5 min). A daughter plate was prepared by transferring an aliquot (5 or 50 μL) from each well of the master plate, which was then dried *in vacuo* at room temperature. After complete evaporation of the solvent in the daughter plate the wells were analyzed for activity against P388 murine leukemia cells as described previously.[16,18] For screening against microorganisms such as *Bacillus subtilis* it was necessary to make duplicate master plates, as much higher concentrations are required to reveal the bioactive wells. The assay serves to correlate cytotoxicity and antimicrobial activity against the HPLC-ELSD-UV trace.

Those wells in the dried master plate containing compounds of interest were then analyzed using capillary probe NMR spectroscopy. The contents of each well to be examined were each dissolved in CD_3OD (7 μL) and transferred into the Protasis CapNMR capillary probe. The quantity of the compound in each well was estimated according to the formula: (MW/$^{\#}$H) × (total integral for $^{\#}$H)/(integral for CHD_2OD) × CF, where MW is the actual molecular weight of the compound (ESMS) or an estimated value, $^{\#}$H is the number of protons included in the integration of the ^{1}H NMR spectrum, and CF is the calibration factor that had previously been determined from a standard solution containing quinine (30 μg in 6 μL) in the same CD_3OD solvent.

Acknowledgment. This research was supported by a Postdoctoral Fellowship from the University of Canterbury to M.I.M. and by a Fellowship within the Postdoctoral Program of the German Academic Exchange Service (DAAD) for G.L. We thank M. R. Boyd, J. H. Cardellina II, and K. R. Gustafson (formerly LDDRD now MTDP, NCI) for discussions and access to facilities during the early stages of the development of the approaches described in this paper.

Supporting Information Available: HPLC traces, 1D and 2D NMR spectra, and screen shot of the searches in the AntiMarin database for compounds **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Koehn, F. E. In *Progress in Drug Research*; Petersen, F., Amstutz, R., Eds.; Birkhauser Verlag: Basel, Switzerland, 2008; Vol. 65, pp 177−210.
(2) Wolfender, J.-L.; Queiroz, E. F.; Hostettmann, K. In *Bioactive Natural Products: Detection, Isolation, and Structural Determination*, 2nd ed.; Colegate, S. M., Molyneux, R. J., Eds.; CRC Press: Boca Raton, 2007; pp 143−190.
(3) Nielsen, K. F.; Smedsgaard, J. *J. Chromatogr. A* **2003**, *1002*, 111–136.
(4) Wolf, D.; Siems, K. *Chimia* **2007**, *61*, 339–345.
(5) Fredenhagen, A.; Derrien, C.; Gassmann, E. *J. Nat. Prod.* **2005**, *68*, 385–391.
(6) Konishi, Y.; Kiyota, T.; Draghici, C.; Gao, J.-M.; Yeboah, F.; Acoca, S.; Jarussophon, S.; Purisima, E. *Anal. Chem.* **2007**, *79*, 1187–97.
(7) Lambert, M.; Wolfender, J.-L.; Staerk, D.; Christensen, S. B.; Hostettmann, K.; Jaroszewski, J. W. *Anal. Chem.* **2007**, *79*, 727–735.
(8) Larsen, T. O.; Petersen, B. O.; Duus, J. O.; Sorensen, D.; Frisvad, J. C.; Hansen, M. E. *J. Nat. Prod.* **2005**, *68*, 871–874.
(9) Hu, J.-F.; Garo, E.; Yoo, H.-D.; Cremin, P. A.; Zeemg, I.; Goering, M. G.; O'Neil-Johnson, M.; Eldridge, G. R. *Phytochem. Anal.* **2005**, *16*, 127–133.
(10) Laatsch, H. *AntiBase, A Data Base for Rapid Dereplication and Structure Determination of Microbial Natural Products*; Wiley-VCH: Weinheim, Germany, 2007; http://wwwuser.gwdg.de/~ucoc/laatsch/AntiBase.htm.
(11) *Dictionary of Natural Products on CD-ROM*; Chapman & Hall Chemical Database, 2006.
(12) *MarinLit* database; Department of Chemistry, University of Canterbury: http://www.chem.canterbury.ac.nz/marinlit/marinlit.shtml.
(13) MarinLit−2000 and Beyond. Blunt, J. W.; Munro, M. H. G. Pacifichem 2000, International Chemical Congress of Pacific Basin Societies, Honolulu, HI, December, 2000.
(14) Bradshaw, J.; Butina, D.; Dunn, A. J.; Green, R. H.; Hajek, M.; Jones, M. M.; Lindon, J. C.; Sidebottom, P. J. *J. Nat. Prod.* **2001**, *64*, 1541–1544.
(15) Bitzer, J.; Köpcke, B.; Stadler, M.; Hellwig, V.; Ju, Y.-M.; Seip, S.; Henkel, T. *Chimia* **2007**, *61*, 332–338.
(16) Lang, G.; Mitova, M.; Ellis, G.; van der Sar, S.; Phipps, R. K.; Blunt, J. W.; Cummings, N. J.; Cole, A. L. J.; Munro, M. H. G. *J. Nat. Prod.* **2006**, *69*, 621–624.
(17) (a) Blunt, J. W.; Cole, A. L. J.; de Silva, D.; Lang, G.; Mitova, M.; MacLean, W.; van der Sar, S. 4th European Conference on Marine Natural Products, Paris, September 12−16, 2005. (b) Blunt, J. W.; Cole, A. L. J.; de Silva, D.; Lang, G.; Mitova, M.; MacLean, W.; van der Sar, S. Pacifichem 2005, International Chemical Congress of Pacific Basin Societies, Honolulu, HI, December 14−19, 2005. (c) Lang, G.; Mitova, M.; van der Sar, S.; Mahyudin, N. A.; Cummings, N. J.; Abdullah, N.; Blunt, J. W.; Cole, A. L. J.; Din, L. B.; Vikneswary, S.; Munro, M. H. G. ICOB-5 & ISCNP-25 IUPAC International Conference on Biodiversity and Natural Products, Kyoto, Japan, July 23−28, 2006. (d) Blunt, J. W. 47th Annual Meeting of the American Society of Pharmacognosy, Arlington, VA, August 5−9, 2006. (e) Blunt, J. W.; Munro, M. H. G. 5th European Conference on Marine Natural Products, Ischia, September 16−21, 2007. (f) Blunt, J. W.; Munro, M. H. G. 2nd CMDD Symposium on Marine Natural Products, Seoul, Korea, November 1−4, 2007. (g) Blunt, J. W.; Chamyuang, S.; Cole, A. L. J.; Hickford, S. J.; Lang, G.; Mahyudin, N.; Mitova, M.; Munro, M. H. G.; van der Sar, S.; Sun, L.; Yusof, M. T. Asian Mycology Congress (2007) and Xth International Marine and Freshwater Symposium, Penang, Malaysia, December 2−6, 2007.
(18) Mitova, M. I.; Stuart, B. G.; Cao, G. H.; Blunt, J. W.; Cole, A. L. J.; Munro, M. H. G. *J. Nat. Prod.* **2006**, *69*, 1481–1484.
(19) *AntiMarin* Database; Blunt, J. W., Munro, M. H. G., Laatsch, H., Eds.; University of Canterbury: Christchurch, New Zealand, and University of Göttingen, Göttingen, Germany, 2007. john.blunt@canterbury.ac.nz.
(20) Krohn, K.; Michel, A.; Roemer, E.; Floerke, U.; Aust, H.-J.; Draeger, S.; Schultz, B.; Wray, V. *Nat. Prod. Lett.* **1995**, *6*, 309–314.
(21) Suzuki, K.; Kuwahara, A.; Nishikiori, T.; Nakagawa, T. *J. Antibiot.* **1997**, *50*, 318–324.
(22) Chromeleon software as part of the Dionex operating system (www.dionex.com).
(23) E-mail: murray.munro@canterbury.ac.nz.
(24) Berdy, J. Bioactive Natural Products Database and DEREP data. *CRC Handbook of Bioactive Microbial Metabolites*; CRC Press, 2000, CD-ROM.
(25) Mitova, M.; Murphy, A. C.; Lang, G.; Blunt, J. W.; Cole, A. L. J.; Ellis, G.; Munro, M. H. G. Submitted to *J. Nat. Prod*. 1600−1603.

NP8002222