

Biochemical, Organic, Physical, Analytical, and Inorganic Mass Spectrometry

Results from Prior NSF Funding

Award Information. "Computer Aided Molecular Design," T. W. Shattuck, B. P. Mundy, J. T. Millard, D. W. King, and D. Bourgaize, Award number 9512457; funded at \$89,121 with a Colby match of \$89,121 from August 1, 1995 to July 1, 1996.

Summary of the Results of the Completed Work. Computer Aided Molecular Design, CAMD, is a combination of computational chemistry and information technology tools that mines 2D- and 3D-structure databases to suggest new compounds that show promise for a given purpose. CAMD also uses theoretical calculations to predict the properties and activities of these new compounds. We feel CAMD is a unifying concept that will help students to understand the power and utility of chemistry to solve some of the challenges faced in our world. Chemistry is increasingly multi-disciplinary, drawing upon areas in molecular and cell biology. Molecular design integrates considerations of biological activity into the framework of chemical design.

We used CAMD to find guests for the small molecule host, nizatidine. We studied the formation of vicinal dianions, which are involved in synthesis of natural products. We used 2D-databases to find compounds related to a newly isolated natural product from lichens, and have discovered a new compound. The resources made possible by this grant allowed us to receive funding for a chemical reactions database, which allowed us to completely reorganize our Organic courses. The facilities have also been used by the Biology and Physics departments in course work and research. We have also compiled 760 DFT-level molecular orbital calculations of small molecules and ions at "<http://www.colby.edu/chemistry/webmo/mointro.html>".

Publications and presentations resulting from the NSF award. Four publications and presentations under this grant have included student authors, see Appendix II references 25, 49, 50, and 51. Additionally, 21 presentations at American Chemical Society National Meetings have included student authors, please see Appendix II, references 2, 3, 8, 12, 13, 15, 20, 24, 26, 31, 35, 42, 43, 45, 46, 52, 54, 57, 58, 60, 62.

Project Overview

We wish to purchase a mass spectrometer with electrospray and atmospheric pressure chemical ionization interfaces. Major advances in the fields of combinatorial chemistry [1], natural products [2], biochemistry [3], and environmental chemistry [4] have resulted from the new applications of mass spectrometry. In addition, high performance liquid chromatography/mass spectrometry (LC/MS) has become a routine tool in many biochemistry, organic chemistry, and analytical chemistry labs [5]. Several of our graduates that have gone on to work in the pharmaceutical industry report that LC/MS has almost completely supplanted thin layer chromatography for day-to-day reaction monitoring in organic synthesis. Electrospray (ESI) and atmospheric pressure chemical ionization (APCI) interfaces have greatly extended the utility of LC/MS. LC/MS has become an important enabling technology along with Computer Aided Molecular Design (CAMD) in the rapid development of solutions to complex problems.

High throughput screening is one of the most important advances in chemistry in the last decade [1, 6, 7]. Combinatorial chemistry in particular has brought about a distinct change in the philosophy of chemistry [8]. We have many challenges facing us, for example, broad-spectrum antibiotic resistance of bacteria, parasites, new diseases like AIDS, and agricultural pest control. Combinatorial methods were developed to meet these challenges. We do our students a disservice if we don't introduce these exciting and useful new concepts in our courses [9].

Computational approaches to chemistry and facile use of information technology are necessary for students to become productive scientists. Mass spectrometry is the ideal experimental tool to show the interrelationships among computational chemistry, information technology, and experimentation. MS rapidly provides the information necessary to use bioinformatics tools.

Independent student research plays a very important role in undergraduate education. Teaching through research is an important principle in our curriculum, so we structure our courses to provide the tools and expertise for students to become proficient in research. The boundaries between student research and the teaching laboratory should fade as students progress through the curriculum. We introduce research quality instrumentation and tools early in the curriculum and repeat their use often. LC/MS will be an important part of the core of our curriculum. We

will adapt MS experiments for General, Organic, Physical, Instrumental, Inorganic and Biochemistry courses that teach necessary skills and prepare students for productive independent research. These experiments will be available the first semester the instrument is installed.

A central component of this proposal is to implement a program in combinatorial chemistry that links our Organic, Physical, Instrumental and Biochemistry courses. To ensure that the new instrument is put to use immediately in our curriculum, we will arrange combinatorial experiments so that, in year one, the components in different courses are already prepared. By year two, students will carry their samples with them from course to course.

We have assembled an active, vibrant, and creative faculty. Profs. Shari and Steve Dunham have completed their second year, and along with recently tenured associate professor Prof. Julie Millard, provide depth to our biochemistry program. Prof. Dasan Thamattoor has completed his first year as our physical organic chemist, and along with Prof. Brad Mundy, provide breadth in our organic program. Prof. Rebecca Conry will start in our tenure track inorganic position this summer. Prof. Whitney King, chair of the department, is a central figure in the analytical and environmental program. Prof. Tom Shattuck teaches our Physical Chemistry course and has guided our computational chemistry program.

The Biochemistry major at Colby has been offered by the Chemistry Department for 23 years. We also have an interdisciplinary program with the Biology Department in Cell and Molecular Biology/Biochemistry. The Biology Department complements the Chemistry offerings with Molecular Biology, and Cellular Biochemistry. All four courses have required laboratories and will benefit from the instrument.

Two years ago the Science Division at Colby was recognized by the National Science Foundation in its Awards for the Integration of Research and Education at Baccalaureate Institutions (AIRE) program for our integration of teaching and research in improving the quality of teaching effectiveness and student learning. We have also received two grants from the NSF for computational chemistry, which have allowed us to build one of the best computational chemistry curricula in undergraduate institutions. Our instrumentation is also strong, Appendix I.

All chemistry majors are required to do research projects and their work has resulted in 64 publications or presentations at regional or national meetings over the last five years, Appendix II. Our current sophomore, junior, and senior classes have 20, 20, and 18 majors respectively.

Goals and Objectives

We are committed to teaching students to speak and write well, think critically, solve problems, formulate questions, become inquisitive, and be active learners. To become independent and creative, our students need to learn modern skills and techniques, before they move into challenging positions in industry and some of the best Ph.D. programs, Appendix III.

Our new combinatorial chemistry project will involve cooperation across the curriculum. As students progress through their courses they will see the interrelationships of the different areas of chemistry and they will learn a radical new way of thinking about solving problems. They will also learn about ion sources, fragmentation processes, ion-molecule reactions and kinetics. The central importance of mass spectrometry in chemistry and the needs of our curriculum and student research program make the addition of an LC/MS essential.

Chemistry Curriculum

ESI-MS (without LC) is a powerful technique in inorganic chemistry [10], including transition metal complexes, cluster complexes, and bio-inorganic systems [11]. We currently do the Cr(III) acetylacetonate complex microscale synthesis in our Honors General (32 students) and General Chemistry(130-150 students) labs [12]. The characterization of this complex, however, is simply by melting point. A definitive characterization of the complex using ESI/MS will add greatly to the learning objectives for this lab. Cr(acac)₃ readily forms cation adducts [13] and the electron ionization (EI) mass spectrum has also been reported [14]. Therefore, the stoichiometry of the complex will be easily and rapidly determined by infusion using either ESI or APCI. In addition to using the molecular weight and fragmentation, our students will calculate the isotope pattern to verify the formula [15]. This experiment will be extended in our senior Inorganic Chemistry lab (5 students) for the ESI observation of trinuclear Fe complexes [16].

Our courses are planned so that by the end of the second year our students have used all the major instruments in the department. For example, the first experiment in our General Chemistry

courses uses GC/MS. We will use LC/MS in our Organic course (80 students) for natural products isolation. The last portion of the Organic lab is a "special projects" experience, for which we will adapt the analysis of green and black tea for catechins [17].

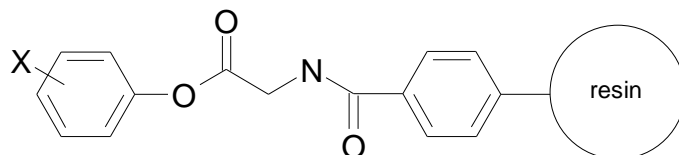
Our Instrumental Analysis course (5-8 students) is a natural place for learning modern MS techniques. The power of LC/MS will allow our curriculum to include more attention to bioanalytical experiments, for example the combinatorial project discussed below. Applications in environmental chemistry are also important including direct mixture analysis by MS/MS [18]. We will discuss the ionization and mass analysis processes and ion-molecule interactions through collision induced dissociation (CID), which we don't now cover.

Computer Aided Molecular Design (CAMD) uses information technology and computational chemistry tools to design molecules with desired activity. CAMD is used throughout the Physical Chemistry course to provide a broad perspective on why PChem is important and also as an organizing focus. One consistent theme that emerges is the importance of aqueous solvation. We try to pair computational experiments with wet-laboratory experiments. One pair on solvation is the determination of the Henry's Law constant and the Free Energy perturbation calculation of the Gibbs Free Energy of solvation of the same compound [19]. We also use the Free Energy of Solvation as a descriptor in QSAR. To expand our treatment of solvation in the Physical Chemistry laboratory (19 students), we will use MS/MS to determine gas phase proton affinities [20] in conjunction with gas phase and continuum solvation molecular orbital calculations [21].

Our biochemistry courses (28-34 students) will be greatly enhanced by the availability of LC/MS [3]. MS is the method of choice for determinations of molecular weights and sequences of proteins and short oligonucleotides [5, 22-24]. LC/MS is also used to determine post-translational glycosylation and phosphorylation of proteins and for enzyme- and immuno- assays [25, 26]. Our Biochemistry students will determine the mass spectrum of myoglobin and cytochrome C and their tryptic digests [27]. The molecular weight and partial sequence information will be used with on-line bioinformatics databases [28-30]. We will adapt exercises using MS-Fit, MS-Tag, and MS-Edman [31], and other genetic and protein databases [32-38].

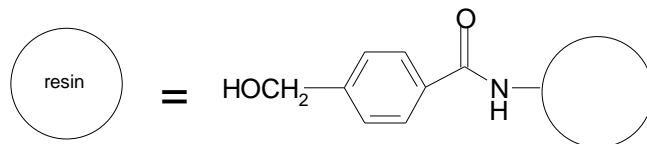
Combinatorial Chemistry. Mass spectrometry is one of the critical enabling technologies that has allowed combinatorial chemistry to thrive [39]. LC/MS is used in combinatorial assays, in deconvolution, and in rapid structural determination of compounds in mixtures [40]. We plan to make LC/MS/MS a central component of our implementation of combinatorial chemistry. Combinatorial style experiments are beginning to appear in the chemical education literature [9], yet realistic exercises for students are hampered by the lack of suitable high-throughput screening instrumentation such as MS.

Extensive QSAR studies have been published that map the active site of the protease papain [41]. A series of glycine based esters have protease hydrolysis rates that differ by a factor of 5000. We will do solid phase supported synthesis of mixtures of these esters in our organic lab:



In Biochemistry, we will use these resin-bound substrates in a kinetic assay with papain with analysis by ESI/MS. The results of this assay will be used in the Physical Chemistry course in QSAR analysis and receptor modeling. Determining their own activity data will engage our students in the QSAR process much more than our current exercises that use literature data.

The synthesis of these resin bound glycine esters uses important Fmoc and t-BOC chemistry [42, 43]. Solid phase supported peptide synthesis is an important technique that is being increasingly incorporated into sophomore organic laboratories [44-46]. Incorporation of this type of chemistry will be a valuable addition to our organic lab and will strengthen the ties between our organic and biochemistry courses. The enzyme assay is particularly simple and robust [42] and provides direct deconvolution [47]. Active substrates will be rapidly cleaved from the resin. The supernatant will be filtered to remove protein and then directly infused into the mass spectrometer. Papain's active site cleft is on the surface of the enzyme, which will allow the direct interaction with the resin bound substrate [48]. Polyethylene glycol grafted resins (e.g. ArgoGel) allow access of the bead bound substrate to proteins [47].



Even though these resins are expensive (\$45/gram), each student needs use only a few beads, because of the excellent sensitivity of ESI/MS. This type of resin has the advantage that NH₃ is used as the cleavage reagent, instead of fluorinated acids. The search for inhibitors will be done by cleaving the substrates from the resin and using ESI/MS to directly look for complexes with the enzyme. Extended mass range will be important for this part of the assay. The cleaved substrates will also be used in Instrumental Analysis with MS/MS to verify the structures.

Supporting Women Faculty. Prof. Jennifer Shosa, a geochemist, will be joining Colby's Geology department this fall. Geochemistry is a new area for our Geology department, and a LC/MS will help provide the infrastructure to build her courses. She will use the LC/MS in her petroleum geology course to "fingerprint" oils by determining the types and concentrations of specific biomarkers present in the high-end hydrocarbons in oil [49]. Prof. Shosha and Prof. Conry (Inorganic) are new faculty, and Prof. Dunham (Biochemistry) will begin her third year at Colby next fall. Together with tenured Prof. Millard, we are proud to have developed a core of women scientists to act as mentors to all our students. In the last three years, on average, half of our majors have been women.

Student/Faculty Research Summary

Natural Product Synthesis, Isolation and Identification: Prof. Bradford Mundy's students study plants used by the Maritime Indians as traditional herbal medicines using bioactivity-directed searches[50]. They are active users of our GC/MS, but they have become increasingly frustrated because their bioactive compounds are not volatile. These problems will be eased by switching to LC/MS based separations and structural determinations aided by MS/MS [51, 52]. Our short experience with APCI and ESI/MS has convinced us that these new methods are revolutionizing natural products chemistry. In a one hour demonstration at PE/Biosystems, we generated more useful information for Martha Healy's ('99) senior thesis than she was able to obtain in a year of

research. This work is in collaboration with Prof. Frank Fekete, a microbiologist in our Biology Department, and his students.

Prof. Mundy's group has a long history in the development of synthetic strategies for natural products. Using bicyclic ketal protocols developed in this lab, Mundy's group is developing a strategy for ring-opening to gain unique entry into seven-membered ring heterocycles. Of particular interest is zoapatanol, a bioactive oxepane found in the Mexican zoapatle plant [53-55]. His group has also developed a novel protocol for the conversion of 1,2-diols to the corresponding 1,2-dimethyl derivatives, using dimethyltitanium dichloride [56]. They are now working towards extending this method to mastigophorenes [57]. Soft-ionization and MS/MS will make the new LC/MS the most used instrument in the department.

Sequence and Structure of a Chromium-Binding Peptide: Prof. Shari Dunham's students study metal-protein and metal-DNA interactions. One focus is a chromium-binding peptide isolated from mammalian tissue [58]. They will use LC/MS/MS [59] and 2D-NMR spectroscopy to determine the amino acid sequence and the three dimensional solution structure. The goal of this research is to understand the ability of this Cr-containing peptide to affect glucose metabolism [60, 61]. Just 20 minutes during a LC/MS demonstration at PE/Biosystems has changed the approach to the purification of these proteins and has shown that the true molecular weight may be half the expected value. Dunham's group also studies tin-DNA interactions that might be useful in cancer chemotherapy [62].

Structural Implications of Incorporating 6-thioguanine into Deoxyribonucleotides: Prof. Stephen Dunham's group studies nucleic acid structure. An initial goal of this research is to determine the structure of a DNA duplex using 2D-NMR when the oxygen atom of guanine (G) is substituted with sulfur to form 6-thioguanine (6-thioG). Treatment with 6-thioG is part of the clinical regime for acute leukemia, and a pro-drug of 6-thioG is believed to play a role in cancers developed by organ transplant patients [63, 64]. Another application of this work is in telomers, which are four-stranded structures at the ends of chromosomes believed to be important in cancer transformations and aging. Recent advances have made ESI/MS/MS a powerful tool for oligonucleotides [65-67]. High-resolution LC/MS/MS will be used to verify the identity [68] and

determine isotopic substitution patterns of the oligonucleotides. One of the major advances in mass spectrometry has been the discovery that ESI and APCI LC/MS can be used to study non-covalently bound protein and DNA complexes [69-72]. This ability will be especially useful in studying the formation of telomeric structures from multiple DNA strands.

Characterization and Structure Elucidation of DNA Cross-linking Agents Isolated from Fungi:

Prof. Julie Millard studies the interaction of DNA with cross-linking agents. Her students have previously determined the DNA targets for several interstrand cross-linkers (mitomycin C, nitrogen mustards, and several diepoxyalkanes) to determine the chemical mechanisms by which antitumor agents and carcinogens recognize DNA [73, 74]. The cross-linking agents that Prof. Millard's group currently isolates and studies from Maine fungi includes the diepoxide repandiol [75]. Prof. Millard's group makes extensive use of gel electrophoresis. Their work will be accelerated by the availability of quantitative determinations using LC/MS to assess purity and perhaps directly determine cross-linking sites using MS/MS [76].

Mechanisms and Utility of Carbene Addition in Organic Synthesis: Prof. Dasan Thamattoor's

research is on the mechanisms and utility of carbene addition in organic synthesis. One project is to devise a widely applicable procedure for the construction of heterocyclobutenes fused to pyrimidines using carbene insertion into an adjacent amino, hydroxy, or mercapto group [77]. One goal is to develop folate-based anti-tumor agents [78], for example the azetinopyrimidine analog of folic acid [77].

Aquatic Photochemistry of Fe(II) and Fe(III): Prof. Whitney King's group studies organic ligand binding to Fe(II) and Fe(III) and the photochemistry of those complexes. He has also been doing extensive mechanistic studies on free-radical reactions of peroxide, hydroxyl radical, and superoxide with small organics, especially pesticides.

Computer Aided Molecular Design and Guest-Host Chemistry: Prof. Tom Shattuck's group

studies small molecule guest-host chemistry and molecular recognition. LC/MS will allow parallel determinations with many guests. Binding constants for a large number of guests are necessary to understand enthalpy-entropy compensation [79, 80]. Hydrogen bonded complexes

with hosts such as crown ethers have been studied using ESI/MS [81]. ESI/MS will allow the efficient study of larger host systems than now possible.

Experience and Capabilities of the Principal Investigators

Prof. Shattuck is doing a sabbatical at Los Alamos National Laboratory on extending ESI/MS for biomolecular guest-host chemistry. He will also have time for curriculum development both at Los Alamos and at Colby during the year. Prof. Shattuck has been responsible for much of our program in computational chemistry and CAMD, which has catalyzed our interest in combinatorial chemistry, bioinformatics, and in strengthening the links between physical and biochemistry. The combinatorial portion of our development has the commitment of the faculty in Organic (Thamattoor, Mundy), Physical (Shattuck), Instrumental (Shari Dunham, King), and Biochemistry (Dunham, Dunham, Millard) courses. Our General Chemistry (Mundy, King, Millard), and Honors General Chemistry (Shattuck) courses will be greatly strengthened.

Bradford Mundy, Miselis Professor of Chemistry, specializes in natural products synthesis and isolation. He is the author of two well-respected undergraduate texts and two monographs [82, 83]. Shari Dunham is a bioanalytical chemist whose analytical specialty is HPLC separations of proteins and nucleic acids. Stephen Dunham worked at EPIX Medical Inc. (Cambridge, MA) and helped develop combinatorial strategies for rational drug design. Whitney King's expertise in environmental chemistry will accelerate the use of the instrument in our curriculum for our environmental concentrators. Frank Fekete has developed biological assays for sulfanilamide drugs for our Organic Chemistry sequence and will develop assays for natural products screening.

Dissemination

Research by Colby chemistry majors has resulted in 64 publications or presentations at regional or national meetings over the last five years. We have taken 42 students to spring national ACS meetings since 1995. We are committed to continuing this tradition. Our faculty have also presented the results of previous NSF grants for our computational chemistry programs at national ACS meetings, at regional ACS meetings, at a Council on Undergraduate Research meeting, at New England Consortium for Undergraduate Science Education (NECUSE)

meetings, at joint Colby Bates and Bowdoin conferences on Information Technology sponsored by the Mellon Foundation, and two Gordon Conferences. Prof. Shattuck has consulted at Bowdoin and Vassar colleges on computational chemistry issues. We will make every effort to continue to present the results of our curriculum development activities and student/faculty research. We will also publish our lab experiments in the Journal of Chemical Education (Appendix II 49,51), and provide Web access to the lab write-ups.

Our Web site makes available tutorials on molecular mechanics and CAMD, which will be expanded to include combinatorial chemistry using LC/MS. Our Web site has the highest external "hit rate" of Colby's academic Web pages. Our Web site also includes tools for NMR, IR, and MS spectral analysis [84-86]. We will extend these tools to be useful for ESI and APCI.

Assessment

Our LC/MS program will be incorporated into our continuing assessment efforts, which include alumni surveys, the Colby Overseers program, a focus group, and course specific evaluations. We will use an outside reviewer for our curriculum materials.

We are committed to yearly alumni surveys for the Science Division for our NSF AIRE and our Howard Hughes Medical Institute (HHMI) grants, which includes all science alumni over the last ten years. We will include questions on this survey that address the perceptions of our students of their preparation for their careers and the strengths and weaknesses of our curriculum in preparation for their current activities. We have reports that our students have an advantage over other applicants because of their active use of 2D-NMR, computational chemistry, and CAMD. We will include a question that assesses the advantages of the use of advanced instrumentation in their job or graduate school interviews and acceptance.

Each department at Colby is reviewed every five years by a group of Colby Overseers, who include alumni, overseers designated by the Board of Trustees, and an outside expert. Our overseers will address the effectiveness of our use of new techniques and technologies in our curriculum and determine if the LC/MS is being used extensively throughout our curriculum.

Another part of the evaluation effort for our NSF AIRE grant is a focus group to review our approach to education through research. This group is comprised of overseers and alumni who

had the opportunity to conduct research while at Colby. We will also include evaluation of the effects of using advanced instrumentation on the strength of our student's training and on attracting students into our majors. Also as part of the AIRE project, we will send surveys to the chairs of graduate and professional school programs at the end of the first year after the matriculation of our students and ask how our students compare to their peers.

Every course at Colby is evaluated at the end of each semester using an all-college form. These forms are each read multiple times and provide useful information on the effectiveness of each course. We also use course and lab specific forms. We will include the new LC/MS based labs in these surveys to gain immediate feedback on the quality of our student's experience. We also use the ACS General Chemistry test to assess the quality of our General Chemistry courses.

For this proposal, we will use an outside reviewer for our curriculum materials. Dr. Hugh R. Gregg, Colby '77, is a staff scientist at Lawrence Livermore National Laboratory in the Chemistry and Materials Sciences-Analytical Services Division. He specializes in MS, and did his Ph.D. on MS/MS. Dr. Gregg has already been invaluable in providing advice for the preparation of this proposal.

Instrumentation

All our major instruments are actively used by students in course work and research. Ease of use by students, flexibility, and the ability to give the most structural information are the principal issues for our LC/MS/MS system. MS/MS capability will also allow greater sample throughput in teaching laboratory settings, because HPLC separation can often be bypassed.

Of the available ionization methods, both ESI and APCI are needed to meet the wide range of our needs across the curriculum. ESI will be used by most projects, especially protein and nucleic acid work. APCI will be critical for our natural products and environmental work and for Physical Chemistry. MALDI has been unsuccessful for our natural products and protein work. Our oligonucleotides are small enough that our needs can be met by ESI using extended mass range and MS/MS techniques [40, 65, 68, 87]. There is no one mass analyzer, however, that can meet all our needs. The choice of analyzer is necessarily a compromise, and we are selecting the currently most popular analyzer for our use. Our first choice is the Finnigan LCQ-Deca. This

instrument was introduced this past year. The important capabilities of the instruments we have evaluated are given in the table below.

Analyzer	Manufacturers	Accuracy	MS/MS Precursor Scan Resolution	MS/MS Daughter Scan Resolution	Mass limit
Ion trap	TSP/Finnigan	0.2 amu	$\cong 2000$	$\cong 2000$	20,000
TOF	PE/Biosystems Micromass	10 ppm	-	-	20,000
Magnetic Sector	JEOL	10 ppm	300	1000	8,000
Triple Quadrupole	PE/Sciex Micromass	0.2 amu	$\cong 2000$	$\cong 2000$	4,000

Time-of-Flight ESI-TOF (\$211,500) is a robust high-resolution technique. The calibration for high-mass accuracy is more stable than a sector instrument and the sensitivity at high resolution is better than a sector instrument. TOF is the fastest analyzer, which complements high-speed chromatography. However, orthogonal acceleration TOF cannot do any form of MS/MS or detect metastable ions.

Triple-Quadrupole The most common MS for analytical, pharmaceutical, and physical use is the triple-quadrupole. Triple-quads are probably the most flexible analyzers because CID is done in a collision cell where the conditions can be carefully controlled, as opposed to in-source CID or CID in ion-traps. Triple-quadrupole analyzers are more expensive (\$272,500) than ES-TOF and ion traps and they are limited to mass resolution of about 0.2 Da under usual conditions. Both triple quadrupole and ion trap instruments can circumvent the necessity of high resolution for molecular formula determination by MSⁿ on M+1 isotope peaks [88, 89].

Magnetic Sector Double-focussing sector instruments (\$240,00) have been the workhorse instruments for high resolution and for MS/MS analysis using linked-scans for decades. The advantage of sector instruments is that both high resolution and MS/MS are available. Both in-source CID and a collision cell that operates at high-energy are available. High-energy CID is more reproducible than low-energy CID on triple quadrupole and ion-trap instruments and has a better fragmentation efficiency for stable ring compounds. The probability of rearrangements is also less than an ion trap. On the negative side, resolution on desk-top instruments for daughter

ion and neutral-loss scans is 1000, and linked scan MS/MS has a much lower precursor resolution than a dedicated MS/MS. The mass stability is also not as good as TOF.

Quadrupole Ion-Trap Protein labs use a combination of ESI-quadrupole ion traps and MALDI-TOF almost universally. Quadrupole ion traps (Deca LCQ \$185,000) are excellent at protein sequencing, but offer only 0.2 amu resolution. Zoom scan gets around this limitation and makes possible the determination of charge states. The weak point for the Finnigan LCQ was its source. The source of the LCQ-Deca has been greatly improved and has ten times better detection limits. Since much of our work involves guest-host chemistry and oligonucleotides, where only a few charge states dominate, the extended mass range of the LCQ-Deca will also allow the use of the instrument in a much wider range of studies. In choosing an ion trap, our students will receive extensive training on the instrument that they are most likely to find after leaving college.

Summary Electrospray instruments had the reputation of needing the dedicated support of a mass spectrometrists. The newest advances in desk-top design have overcome these limitations, and we feel that a ESI ion trap instrument provides the flexibility that is needed in our extremely heterogeneous environment. Phone interviews with four current users of the LCQ have shown these labs to be very enthusiastic about the ease of use of these instruments in educational settings. The improved source design and availability of extended mass range of the LCQ-Deca coupled with chromatographic separations as a routine tool along with the ability to do daughter and neutral loss scans are needed for our projects and will enhance the education of our students.

Equipment on Hand for the Project

The chemistry department has an excellent collection of research quality instrumentation, Appendix I. Laboratory courses take priority for instrument use. Otherwise, our students have free and open access to all instruments in the department for their research projects. Students will gain after-hours access to our LC/MS by obtaining a “driver’s license.” Our 400 MHz NMR, GC/MS, FT-IR, elemental analyzer, Chromatotron, capillary GC, two isocratic HPLCs, and three gradient HPLCs provide the necessary complementary instruments needed for an excellent curriculum and a productive research program. The HPLC that will be used with the MS was donated to Colby by Pfizer Pharmaceuticals and includes a Gilson autosampler for unattended

overnight use. The experiments using the LC/MS will complement binding studies using our titration calorimeter, UV/Visible diode array spectrophotometers, and our spectrofluorimeter. The microplate spectrophotometer, nucleic acid synthesizer, high speed centrifuges, and gel electrophoresis instrumentation will allow great flexibility in biochemical studies.

Implementation and Maintenance

Implementation. The cost includes a training course. Prof. Shattuck will use part of his sabbatical at Los Alamos and at Colby for curriculum development. The installation of the instrument will be late second semester or early summer, so use of an instrument at Los Alamos will shorten our development time. We will also have a teaching assistant, Tina Beachy (Colby '93, MS Biochem at Penn State '99), who will help develop the LC/MS, CAMD, and bioinformatics exercises for our Organic and Biochemistry labs.

Past Maintenance History. Each of our major instruments is overseen by one of our faculty members, who handle training, scheduling of routine maintenance, and identifying problems. Our full-time Instrument Maintenance Technician employed by the Science Division at Colby, handles troubleshooting and the actual repairs.

Since its purchase in 1988, the department has not held a service contract on our JEOL NMR. Without exception, problems have been solved with the aid of phone support. Over its long history of use, this instrument has had only a handful of days of downtime. This expedient and thrifty repair record is due, in part, to our excellent Instrument Maintenance Technician, and the substantial maintenance and repair budget of the Department of Chemistry of \$19,000/yr. Our Hewlett Packard GC/MS is the most heavily used instrument in our department. At 14 years of age, the instrument is reliable and is used by all our General and Organic Chemistry students.

LC/MS Maintenance. Prof. Shattuck will be the faculty member in charge of oversight for the instrument. He has extensive experience in instrument design and has been in charge of oversight of our NMR system. HPLC and MS hardware maintenance will be with on-site troubleshooting by our Divisional Instrument Maintenance Technician. Support for the computer system will be with a college-funded service contract. In addition, the science division has a full-time, dedicated Workstation Administrator who will be available to assist with a variety of computing issues.