Imaging Probe Development Center: a National Institutes of Health core synthesis resource for imaging probes

1 Introduction

Molecular imaging was identified as a key component of the National Institutes of Health (NIH) “Roadmap” for medical research in the 21st century, under the “New Pathways to Discovery” theme, during original Roadmap initiation meetings held in May 2002. This molecular imaging initiative was highlighted at the last Inter-Institute Workshop on Optical Imaging from Bench to Bedside, held in 2004 and approached realization with a new director and facility in place. As stated in 2004, the initiative was created to enhance the availability of known and new reagents for molecular imaging, with the ultimate aim of enabling profiling of cells at their most basic molecular level, and even developing individualized approaches to diagnosing and treating disease. These broad aims are very ambitious and may further encompass combinatorial library derived probes, enhancement of probe characteristics, affinity and avidity studies on potential probes, the use of probes in the development of more effective therapies, the monitoring of biological behavior, and the development of new probes through molecular modeling or screening methods.

Abstract. The Imaging Probe Development Center (IPDC) has been set up under the auspices of the National Institutes of Health (NIH) Roadmap as part of the molecular libraries and imaging initiatives. It comprises a core synthesis facility dedicated to the preparation of imaging probes, initially for intramural NIH scientists, and later, for the extramural scientific community. The facility opened fully in late 2006, in refurbished laboratories in Rockville, Maryland, and a staff of around a dozen was recruited into place by early 2007; the director was hired in late 2005. The IPDC provides a mechanism for the production of sensitive probes for use by imaging scientists who cannot obtain such probes commercially. The probes to be made will encompass all major imaging modalities including radionuclide, magnetic resonance, and optical. The operation of the IPDC is outlined, together with the results of interim achievements while the IPDC maintained a small temporary laboratory in Bethesda. As of December 2006, a total of eleven probe compositions had been made, and several of these are described with particular mention of those probes intended for use in optical applications. © 2007 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.2778702]

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The Imaging Probe Development Center (IPDC) operates as a trans-NIH initiative [administered by the National Heart, Lung, and Blood Institute (NHLBI)] under a steering committee with more than a dozen members who share an interest in, and knowledge of, imaging sciences and are recruited from various NIH institutes and centers. The scientific process for probe acceptance by IPDC through probe production can basically involve some or all of the following steps: (1) need identification, from initial receipt of a proposal from a scientist; (2) developmental planning, where IPDC scientists decide on strategies to prepare probes; (3) molecular modeling, if indicated, to determine the shape of possible probe designs; (4) synthesis of the probe; (5) characterization, up to and including receptor and/or probe target binding studies; (6) scale-up, stability testing, and ongoing quality control on multiple batch preparations; (7) storage, with cataloging into imaging probe libraries, and dissemination of information to enable future use of any particular probe by other requesting scientists.

As a core synthesis facility devoted to imaging probes, the IPDC will first offer its services to NIH intramural scientists, which it has begun to do already as outlined below, but later will offer broader services to the extramural scientific community. Regarding known probes already described in the scientific literature, IPDC services are likely to focus on those that are not commercially available or not available from any...
other source. For instance, we can address the custom syntheses of probes that may be too expensive or specialized to be of general interest to a commercial organization. Some of our projects may just involve a “one-shot” synthesis, or be directed to a complex probe where there are few expert companies. In some production situations, the cost per unit can be prohibitively expensive, particularly if only small amounts of material are needed by the requestor. For difficult syntheses, commercial outfits will often ask for up-front funds amounting to up to 50% of final cost on a nonreimbursable basis with no guarantee of producing the final product, comprising a substantial and risky gamble for the requesting scientist. These and other important niches can be addressed by IPDC.

We will also prepare probes originally described by academic scientists in peer-reviewed literature, who then do not have the wherewithal to provide such agents to other scientists on a regular basis; who, in turn, would like to expand on and develop the originally described work. In fact, most academic scientists are somewhat precluded from such a commitment by the requirement to publish original articles and then move on to fresh pastures, if only for support renewal reasons, often leaving follow-up supply of interesting probes unaddressed. Also, not addressable by many academicians are quality assurance aspects very familiar to commercial suppliers, related to ongoing quality control programs, production, process development, shelf-life stability and storage issues; the installation of standard operating procedures to ensure lot-to-lot and operator-to-operator reproducibility; and the setting of acceptable purity criteria. These and similar aspects may be looked on as mundane by some academic scientists primarily devoted to publishing original articles, but are crucial in establishing certitude with regard to underlying chemistry, without which later expensive biological studies can be rendered futile, or worse, misleading.

The commercial custom synthesis option is also less attractive, or even not practicable if, in a particular project, variants of the originally described probe are going to be needed on an ongoing basis, or if multiple agents need to be studied in parallel. Again, academic scientists are not well placed to provide such services. Finally, many of the projects taken on by the IPDC will be very specialized in nature and may sometimes involve the capability to produce probes with multiple detectable moieties. These include highly specialized probes involving at least two selected from optical, magnetic resonance imaging (MRI), and/or radiolabeled species, further limiting potential commercial and standard academic interests and capabilities.

Over a range of applications from the intracellular level to the in vivo level, covering every imaging modality, and the entire breadth of chemical technology, the realization of the IPDC’s promise offers tremendous potential for extraordinary gains in imaging sciences in the coming years. Against the above background of need, and within the context of the current explosion in new imaging applications, due both to rapid technological advancements and the pressing need to track reagents, drugs, and surrogates more accurately, the IPDC may be seen as a resource whose time has come.

2 Results and Discussion

When the first solicitation for project proposals was distributed to principal investigators within the NIH community in 2006, it was met with an enthusiastic response in Bethesda and at other campuses around the country. However, since the IPDC facility was not built at that time and had to operate in a restricted space and with limited personnel, strict priorities were placed on proposals that were accepted for IPDC attention. Proposals were prioritized by the steering committee according to the following elements listed in decreasing order of importance: (1) feasibility of synthesis, (2) scientific significance, (3) lack of alternative supply of the imaging probe, (4) potential benefits to the wider community, (5) ability of the submitting scientist(s) to perform their subsequent planned studies, (6) overall cost to the IPDC.

As anticipated, a wide range of technologies were represented in these first submissions, and IPDC scientists began work on six selected projects, some requiring preparation of more than one composition. Radiolabeled probes were put on hold at that time due to the difficulties inherent in performing such work without dedicated and specifically designed facilities, meaning that the works undertaken focused on MRI and optical probes.

In the MRI arena, dendrimer-based probes using gadolinium (Gd) were requested by colleagues at four separate institutes encompassing studies on microvasculature, brain tumor studies, renal cancer, and the study of molecular size in ocular drug discovery. The dendrimers were those previously prepared by Kobayashi et al.,4 which were based on the polyamidoamine (PAMAM) backbone,5 and were of increasing molecular size (Fig. 1). Second- (G2), fourth-(G4), and sixth- (G6) generation dendrimers were employed, nominally containing 16, 64, and 256 terminal amino groups, respectively. The bifunctional chelating agent used to stably bind the Gd metal to the dendrimers was the methyldiethylenetriamine pentaacetic acid (Mx-DTPA) derivative developed by Brechbiel and Gansow,6 synthesized in approximately seven steps starting from p-nitrophenylalanine (Fig. 2). In this iteration, the chelate was coupled to the dendrimer backbones using either an isothiocyanate or a glutaric acid coupling linkage, resulting in six distinct compositions. Chelate-dendrimer conjugates were then complexed with Gd using Gd acetate, purified by extensive dialysis, and stored after lyophilization. Complexes were verified by size exclusion–high-performance liquid chromatography (SE-HPLC), matrix-assisted laser desorption/ionization–time-of-flight (MALDI-TOF), and elemental analyses, with prepared amounts in the 200- to 1100-mg range. Saturation rates [how many of the dendrimers available terminal amino groups were in fact substituted with a chelating group (~40 to 60%)] and how many of those bore a Gd ion (>95%)] were comparable to the compositional standards available from Dr. Brechbiel’s laboratory.

Among colorimetric detectable agents, IPDC prepared a reduced, essentially colorless, form of the well-known dye Coomassie Brilliant Blue. Figure 3 shows the structures of the original dye (a) and the novel derivative (b) produced via sodium cyanoborohydride reduction, along with the absorbance trace from the UV scan indicating the removal of the absorbance peak near 600 nm in going from (a) to (b). Coomassie dyes are ubiquitous in the analysis of proteins, including proteins separated by polyacrylamide gel electrophoresis (PAGE), where they not only function to highlight the charge-separated protein bands due to their intense blue color, but importantly, also assist in efficient protein separation due to...
their strong hydrophobic and/or charge interactions with various protein amino acids. This addition of charged groups to proteins enables them to run in distinct bands rather than streaking on PAGE. However, the intense color imparted to the proteins by Coomassie precludes further colorimetric analyses. We realized that a minimal change to Coomassie structure to eliminate its color while retaining the same protein binding properties would be most useful and achieved this by controlled reduction of the central triphenylmethenyl group, breaking the extended chain of unsaturated groups in the original dye. The reduced dye is being tested in a new analytical procedure termed ghost native (GN) electrophoresis, which will allow further probing of protein interactions following GN-PAGE, the details of which will be described fully in a separate publication. Promisingly, use of the new GN-PAGE may extend and enhance the utility of clear native (CN) PAGE, which is performed without a dye and therefore loses the desirable modified protein in-gel properties seen with (blue native) BN-PAGE.

IPDC was called on to produce the interesting trifunctional agent termed NBzM, whose synthesis we accomplished as described in the literature and is outlined below and in Fig. 4. The synthesis involves six steps beginning from a Wang resin-bound differentially protected lysine backbone and proceeds to the final product in a reasonable 24% overall yield. The NBzM trifunctionality includes a nitrilotriacetic acid (NTA) subunit designed to bind to histidine (His) tags on expressed recombinant proteins, a benzophenone subunit capable of photolytic coupling reactions, and a maleimide group for subsequent reaction with free thiol groups on other moieties. When NBzM is added to His tag-containing proteins, the NTA subunit associates specifically with the His tag found on the C terminus, whereupon photolytic reaction at 365 nm
creates a regiospecific covalent chemical bond between the benzophenone subunit and the protein surface. The process has the effect of placing a thiol-specific reactive group—a maleimide—specifically at the recombinant protein’s C terminus, and away from the protein’s active or binding sites. Because most recombinant proteins are made with His tags to enable ready purification from culture media, the tagging method should be widely applicable and enable multiple agents to be site-specifically attached at the maleimido group. This strategic approach also offers interesting possibilities of a general nature from a conjugation chemistry point of view.

In a different arena, a NIH colleague requested that we produce three fluorescent agents used for the tagging of RNA and DNA. The compositions were adenosine and guanosine analogs in which the natural bases were replaced by pteridine nucleoside analogs, capable of acting as hybridization probes for the study of strand interactions.11–13 These syntheses, of probes termed 6-MAP, 3-MI, and 6-MI, are long and require up to 15 reaction steps before reaching the final product. As of December 2006, two of the three have been completed. The shortest synthesis, that of 6-MAP, is outlined in Fig. 5 and is shown by way of example. The pteridine ring is constructed sequentially from the triaminopyrimidine while overcoming significant solubility problems at a key step that improved the reaction yield from 4 to 43% from that described in the literature procedure. Separately, the protected and activated glycosidic unit is made and then coupled to the pteridine ring derivative by displacement of the 2-chloro group on the ribose ring. Again, a significant improvement in yield was achieved as we obtained 63% of the correct diastereoisomer product, versus only 5% claimed in the literature, again due mainly to solving reactant solubility issues.

Figure 6 shows the activation of the 6-MAP to the corresponding six-protected phosphoramidate derivative, which is
suitable for use in place of a normal derivatized nucleoside in automated DNA and/or RNA synthesis machines. This project is illustrative of the fact that preparing the composition of matter is only part of the project. In our original scheme, only milligram amounts of the 6-MAP material were produced, but this has since been scaled up to produce multimgram amounts, which is vital if these analogs are to be used in automated oligonucleotide syntheses, which generally call for vials containing around 500 mg of protected monomers to be used on the machines. The 6-MAP phosphoramidite must also be made reproducibly, be supplied in a rugged yet easy-to-use format, in a manner the end user is familiar with, show little or no lot-to-lot variability, be handled under proper storage conditions, have shelf-life data acquired, be supplied with instructions for use, and shown to be workable and practicable during routine automated syntheses. When all these issues and more are addressed and solved, this promising approach can then be widely and generally accepted and studied and can then develop its true potential.

Every project handled by the IPDC may involve issues such as those described for the 6-MAP analog above, to a greater or lesser extent. It will be important to solve these issues, in transitioning often poorly described, nonoptimized, low-scale methods into fully workable processes that can be developed for routine use by others. An extension of this is to ensure that the IPDC’s message, even its existence, is well circulated within the scientific community. To achieve this, apart from presences at leading scientific meetings, IPDC data will be placed on publicly accessible databases, first on the IPDC website. It is important to recognize the potential improvements in imaging agents (optical, MRI, and radionuclide) that can be achieved by the judicious application of selected chemistries to solve biological and imaging problems. To these ends, IPDC will work to improve imaging agents’ inherent properties such as improved quantum yields, photobleach-

Fig. 6 Activation of the 6-MAP analog to the corresponding phosphoramidate for use in automated coupling reactions during oligonucleotide syntheses.