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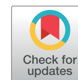

Production of diverse PET probes with limited resources: 24 ^{18}F-labeled compounds prepared with a single radiosynthesizer

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New radiolabeled probes for positron-emission tomography (PET) are providing an ever-increasing ability to answer diverse research and clinical questions and to facilitate the discovery, development, and clinical use of drugs in patient care. Despite the high equipment and facility costs to produce PET probes, many radiopharmacies and radiochemistry laboratories use a dedicated radiosynthesizer to produce each probe, even if the equipment is idle much of the time, to avoid the challenges of reconfiguring the system fluidics to switch from one probe to another. To meet growing demand, more cost-efficient approaches are being developed, such as radiosynthesizers based on disposable "cassettes," that do not require reconfiguration to switch among probes. However, most cassette-based systems make sacrifices in synthesis complexity or tolerated reaction conditions, and some do not support custom programming, thereby limiting their generality. In contrast, the design of the ELIXYS FLEX/CHEM cassette-based synthesizer supports higher temperatures and pressures than other systems while also facilitating flexible synthesis development. In this paper, the syntheses of 24 known PET probes are adapted to this system to explore the possibility of using a single radiosynthesizer and hot cell for production of a diverse array of compounds with wide-ranging synthesis requirements, alongside synthesis development efforts. Most probes were produced with yields and synthesis times comparable to literature reports, and because hardware modification was unnecessary, it was convenient to frequently switch among probes based on demand. Although our facility supplies probes for preclinical imaging, the same workflow would be applicable in a clinical setting.

radiosynthesis module | positron-emission tomography | resource efficiency | radiochemistry | PET tracer

New probes for the molecular imaging modality positron-emission tomography (PET), and the in vivo assays they provide (1), are enabling an ever-increasing insight into a variety of biological, biochemical, and pharmacological processes. The use of PET for the in vivo investigation of these processes in basic science and clinical research (2–4) and in the discovery, development, and clinical use of drugs in patient care (5) is leading to a growing demand for availability of diverse probes (6, 7) to interrogate the in vivo biology of disease. This in turn is placing increasing demands on radiopharmacies and radiochemistry laboratories to provide a convenient supply of these probes for basic science research and clinical trials of imaging probes and for the use of imaging probes to facilitate and increase the efficiency of drug discovery and clinical trials (8).

The production of radiopharmaceuticals requires expensive shielding infrastructure (hot cells) and equipment (radiosynthesizers) that typically have low duty cycles in their use. Due to the need for probe-specific plumbing modifications on many radiosynthesizers, these systems, and the hot cells in which they are housed, are often dedicated to the production of a single probe. Short-term reconfiguration to accommodate the synthesis of a different probe is often avoided as it imposes added complexities because of the risk of introducing errors and the extensive tests that must be performed each time to validate the new configuration, even if the probe has previously been produced on the system.

While dedicated synthesis modules are suitable for situations where a single probe (or small number of probes) is produced nearly every day, this production model is not cost effective when more numerous probes are supplied at lower frequency (e.g., once per week or month), because it would require significant laboratory space and would leave expensive equipment and hot cells idle most of the time. This becomes an increasing issue as the number of probes to be produced at each site grows and even more so when producing diverse probes at multiple sites in a

Significance

Molecular imaging with PET can provide a dynamic, whole-body picture of the rate of biological processes or distribution of biological targets by tracking the distribution of radiolabeled molecules or particles in the body over time. Continual efforts to develop new PET probes are expanding the variety of processes and targets that can be visualized, facilitating basic research, drug development, and patient care. However, access to these probes at all stages of their development is hindered by high costs arising, in large part, from the significant resources that are typically dedicated to production of a single probe. Emerging technologies with increased synthesis flexibility are allowing increased probe diversity with fewer resources and could significantly increase access to new molecular imaging agents.

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consistent manner under the same cGMP requirements of the Food and Drug Administration (FDA).

The emergence of disposable-cassette–based systems has helped facilities deal with these constraints by supporting the production of more than one probe on a single synthesizer within a single hot cell in a convenient manner (9, 10). One can purchase different cassettes with the accompanying reagents kits, each cassette preplumbed with the necessary fluid path configuration for synthesizing that probe. In this concept, changing only the cassette allows for the synthesis of different probes, while the base system does not change. Examples of recent systems include the Eckert & Ziegler PharmTracer (11), Scintomics GRP (12), Trasis AllInOne (13), GE TracerLab MX, GE FastLab 2 (14), IBA Synthera (15), ORA Neptis (16), and Sofie Biosciences ELIXYS FLEX/CHEM (17), each with different degrees of chemical flexibility and number of probes offered. Cassettes also offer additional advantages for routine clinical production, such as a simplified setup workflow and the elimination of time-consuming cleaning processes or cleaning validation studies. For each of these cassette-based systems, there is literature showing that multiple probes can be implemented with a suitable set of reagents, automation program, and cassette flow path. For example, a recent report demonstrated that the Trasis AllInOne could be used for the automated production of six different probes on a single synthesizer in a clinical setting (18). The ELIXYS differs significantly from other cassette-based systems in that its cassette is "universal"; the cassette itself, including its fluidic paths and reagent vial sizes, does not change from probe to probe. This allows a large number of probes to be produced in a do-it-yourself fashion by changing only the reagents used, without requiring any modification of the cassette fluid path.

In addition to performing the radiochemistry, several cassette-based systems also include integrated or modular capabilities for HPLC purification and formulation (e.g., Trasis AllInOne, IBA Synthera, ORA Neptis, ELIXYS FLEX/CHEM), while other systems are limited to cartridge purification and formulation or require connection to a third-party radio-HPLC system. Furthermore, some systems enable multiple batches of probes to be made in a single day. For example, the IBA Synthera comprises an automatic cassette ejector that can dispose the used cassette into a shielded waste bucket. Some systems (e.g., IBA Synthera, Eckert & Ziegler PharmTracer, etc.) are small enough that throughput could be enhanced by placing multiple units within the same hot cell, some are designed specifically for multiple productions (e.g., ORA Neptis Nx3), and some can be configured for multiple sequential runs (e.g., Sofie Biosciences ELIXYS FLEX/CHEM) (19). In such cases, setup for all batches is performed at the same time before the radioisotope is introduced into the hot cell, and then the batches are produced sequentially throughout the day.

Despite the advantages of cassette-based systems, most have a limited range of temperatures and pressures that are tolerated due to the types of fittings, valves, and materials used in the cassette construction. In addition, most of these systems have only a single reaction vessel, which prohibits the automated synthesis of probes that require multipot protocols. These limitations affect the diversity of probes that can be produced without significant chemistry development efforts to simplify the synthesis protocol or make reaction conditions less harsh. In addition, most cassette-based systems are not convenient platforms for synthesis development due to the need for cassette or software modifications and due to restricted access to the reaction mixture at intermediate steps. In contrast, the ELIXYS system has a unique design that provides a robust environment for carrying out reactions under more challenging conditions (20) and can perform synthesis protocols under conditions that lead to leaks or damage in other synthesis modules (21). Furthermore, it has features that facilitate synthesis development, including access to the reaction vessel contents for intermediate measurements and a drag-and-drop programming interface based on intuitive "unit operations" (22). These features allow the use of a single system and hot cell for both synthesis development and routine production, including production of probes for clinical use, enabling further increases in the duty cycle of expensive radiochemistry equipment and facilities.

We describe in this paper our experience with the ELIXYS FLEX/CHEM radiosynthesizer at the University of California, Los Angeles (UCLA) Crump Institute for Molecular Imaging. Our radiochemistry facilities are shared among several members of the radiochemistry research faculty and only one hot cell and one synthesizer are dedicated to the production of probes for our preclinical imaging facility. In response to the requests of preclinical imaging center clients, we have used these limited resources both to adapt the syntheses of 24 known PET probes (Fig. 1) into "routine" protocols [9 of which were previously reported (19, 23)] and to perform production for imaging as needed. This is part of our goal to build a diverse library of probes (and the assays they provide) to expand research in the biology of disease and pharmacologic interventions in vivo in animal models and to facilitate their translation to clinical research and ultimately to molecular imaging diagnostics in patient care. While these probes are being produced for preclinical imaging, because there is no

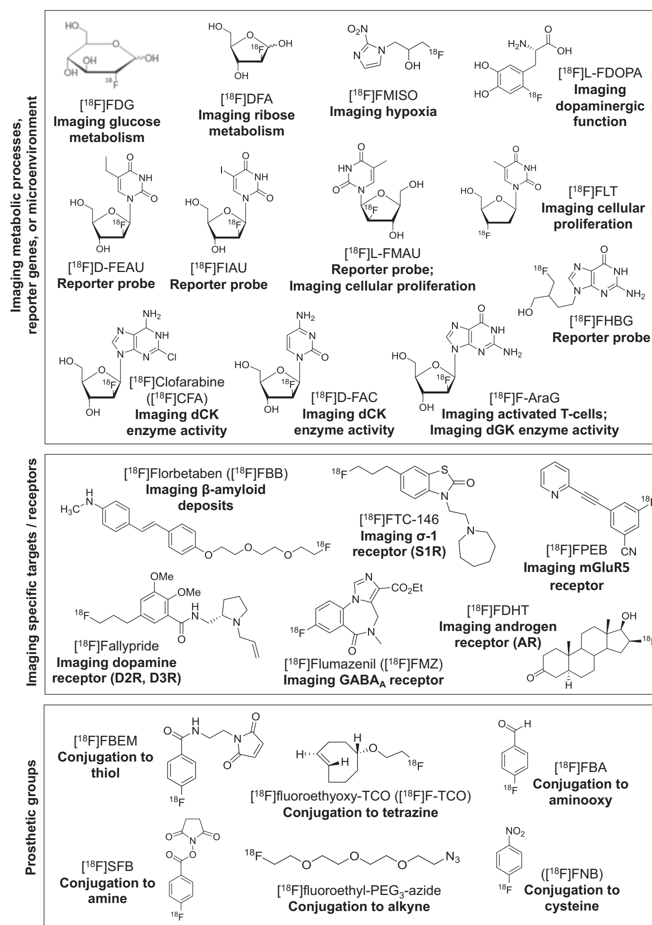

Fig. 1. Probes synthesized in our laboratory using the ELIXYS FLEX/CHEM radiosynthesizer. This set of 24 ^{18}F-labeled PET compounds represents significant synthesis diversity, requiring from one to three reaction vessels, temperatures up to 200 °C, and the use of challenging solvents and reagents including HBr, toluene, dichloromethane, and 1,2-dichloroethane. The probes are used for imaging a wide range of biological targets and biological processes or act as prosthetic groups for conjugation of fluorine-18 to fragile biomolecules (function indicated in boldface type).

need for hardware reconfiguration to switch from one probe to another, the same pattern of usage could be adopted in a clinical setting. In fact, several of these protocols are being transferred to the UCLA Ahmanson Biomedical Cyclotron Facility for implementation in clinical research trials of imaging probes, as imaging probes in drug trials, or for imaging in patient care. Using ELIXYS to produce probes validated for human use has also been reported by Ravert et al. (24).

Results and Discussion

Production of Multiple Probes. The 24 known PET probes described here were provided to our preclinical imaging center on an as-needed basis as we expanded the set of available probes (and the assays they provide) to meet increasing demands from basic science research into the biology of disease and the development of pharmacologic interventions. Most of the protocols were developed after a need was identified, using published synthesis details as a guideline. Creation of an ELIXYS program for each probe took no more than 1–2 h, including an abbreviated dry run to ensure its correctness. Many probes were successfully synthesized on the first attempt. Although the reaction vessel volume was a limitation and required modification of a few synthesis protocols, in general, the flexibility of the system allowed protocols to be carried out as published, without having to change conditions to reduce temperatures or pressures.

The probes were produced as frequently as once per day, generally with different probes on subsequent days from a single ELIXYS radiosynthesizer and hot cell. Although not performed in this study, the possibility to perform multiple syntheses per day has been demonstrated previously (19). In general, the system was left to decay after the synthesis, and cleaning was performed the morning of the next production. Cleaning of cassettes, reagent preparation, and setup of the system took ∼15–20 min. The cleaning step could be eliminated by using cassettes in a disposable manner as they are intended. Alternatively, by having a backup set of cassettes, the timing of cleaning could be decoupled from synthesis and performed at an alternate convenient time.

Performance of Syntheses. Each of the probes was produced with yields and synthesis times that were, for the most part, comparable to literature values (Table 1), despite not being extensively optimized for the ELIXYS.

The radiochemical purity after purification and formulation for all probes was between 95% and 99%, except for [^{18}F]fluoroethoxy-*trans*-cyclooctene (TCO) (2-[^{18}F]-(E)-5-(2-fluoroethoxy)cyclooct-1-ene). Examples of semiprep HPLC purification chromatograms and radio-TLC chromatograms are shown in *SI Appendix*, section 7. The purity of [^{18}F]fluoroethoxy-TCO was slightly lower due to its radiolytic instability. Keliher et al. (25) reported the synthesis of 0.3 ± 0.1 GBq (8 ± 3 mCi) [^{18}F]fluoroethoxy-TCO formulated in 600 μL of dichloromethane with a radiochemical purity of >93%. However, to use [^{18}F]fluoroethoxy-TCO as a prosthetic group for ^{18}F labeling of antibody fragments, we needed to formulate the probe in significantly higher activity concentrations and observed rapid radiolysis (i.e., isomerization of the highly strained *trans*-cyclooctene product to the unreactive *cis*-cyclooctene compound). Fortunately, the undesirable isomerization could be significantly hindered with the addition of a radical scavenger (26) (sodium ascorbate) to the reaction mixture following synthesis and throughout the purification and formulation steps. This adaptation enabled activities up to 2.5 GBq (67 mCi) and formulated activity concentrations of up to 6.7 GBq/mL (180 mCi/mL). Radiochemical purities ranged from 87% to 94% and the probe was successfully used in subsequent experiments, but further optimization is needed to increase stability of [^{18}F]fluoroethoxy-TCO in high-activity concentrations and ensure purities >95%.

Radiochemical yields (RCYs) were in general comparable with literature reports. In a small number of cases {[^{18}F]L-FDOPA (3,4-dihydroxy-6-[^{18}F]fluoro-L-phenylalanine) (DiMagno method), [^{18}F]FHBG (9-(4-[^{18}F]fluoro-3-[hydroxymethyl]butyl)guanine), [^{18}F]FMZ ([^{18}F]flumazenil)}, the RCYs were significantly lower than reported in the literature, but potentially can be improved via optimization. Typically, measurements of radioactivity at different stages, combined with analysis of samples via radio-TLC and radio-HPLC, enable us to prioritize such optimization efforts. In contrast, for several probes, the observed yields were significantly higher than literature reports, including [^{18}F]CFA ([^{18}F]clofarabine), [^{18}F]FBB ({4-[2-(4-{2-2[2-(2-[^{18}F]fluoro-ethoxy)-ethoxyl]-ethoxy}-phenyl)-vinyl]-phenyl}-methylamine), [^{18}F]FPEB (3-[^{18}F]fluoro-5-[(pyridin-3-yl)ethynyl] benzonitrile), and [^{18}F]FTC-146 (6-(3-[^{18}F]fluoropropyl)-3-(2-(azepan-1-yl)ethyl)benzo[d]thiazol-2(3H)-one). In the case of [^{18}F]FTC-146, the ability to reach higher fluorination temperature than other systems was a significant benefit: At 185 °C and 200 °C, the RCYs were 12.3 ± 1.8% ($n = 4$) and 15.5 ± 0.7% ($n = 2$), respectively, compared with the 3.7 ± 1.9% ($n = 13$) obtained at 150 °C on the GE TRACERlab FX_{FN} (27). For the other probes, some small differences in reaction conditions (e.g., precursor amount, reaction temperature, reaction time), or in heating efficiency of the ELIXYS system compared with other systems, may contribute to improvements. For example, in the synthesis of [^{18}F]FPEB we used 2 mg of precursor compared with the 1 mg reported in the literature.

In terms of synthesis time, most probes were synthesized in a timeframe similar to literature reports. In a few cases, significantly shorter times were observed, including [^{18}F]CFA, [^{18}F]FBB, [^{18}F]L-FDOPA (DiMagno method), and [^{18}F]FPEB. For [^{18}F]CFA and [^{18}F]L-FDOPA, the syntheses reported in the literature appear to be performed manually, perhaps explaining the longer synthesis times. For [^{18}F]FBB, our reported synthesis time is shorter because formulation was not performed after HPLC purification. On the other hand, in a few cases, the synthesis time was somewhat longer than literature reports, i.e., [^{18}F]FEAU (2′-[^{18}F]fluoro-5-ethyl-1-β-D-arabinofuranosyluracil) (1-pot), [^{18}F]FHBG, [^{18}F]FMISO (1H-1-(3-[^{18}F]fluoro-2-hydroxypropyl)-2-nitroimidazole), [^{18}F]fluoroethoxy-TCO, and [^{18}F]SFB (*N*-succinimidyl-4-[^{18}F]fluorobenzoate). The reported synthesis of [^{18}F]FEAU (1-pot) used a microwave reaction step, possibly explaining the shorter synthesis time. For [^{18}F]FMISO and [^{18}F]fluoroethoxy-TCO, we performed numerous intermediate measurements which adversely impacted the synthesis time. Our synthesis of [^{18}F]SFB was slightly longer than reported in the literature because of the use of semipreparative HPLC for product purification instead of solid-phase extraction.

Molar activities obtained were in the range of 37–260 GBq/μmol (1–7 Ci/μmol). Lower values were observed in a few cases, i.e., [^{18}F]Fallypride, [^{18}F]FEAU (3-pot), [^{18}F]FEAU (1-pot), [^{18}F]FMZ, and [^{18}F]FNB (1-[^{18}F]fluoro-4-nitrobenzene). These probes were synthesized using only relatively low amounts of radioactivity, and it is well known that molar activity is affected by the amount of starting activity used (28). Higher molar activities would thus be expected as the starting amount of [^{18}F]fluoride is increased.

Most syntheses could be performed on the ELIXYS system without the need to modify the hardware configuration. Only the program, the set of reagents and purification cartridges, and a small number of connections between cassettes needed to be changed between productions of different probes. Syntheses involving cartridge purification of an intermediate often require dilution with a large volume of water before loading onto the solid-phase extraction cartridge. With [^{18}F]FPEB, we showed that a sufficient degree of dilution can be obtained directly within the reaction vessel (5 mL capacity) by scaling down the amount of organic solvent in the fluorination reaction. In the case of [^{18}F]FTC-146, a simple addition of an external reservoir to perform the dilution was used. It is likely that reduction in fluorination volume

CHEMISTRY

Table 1. Summary of synthesis performance data and comparison with literature methods performed in other vial-based radiosynthesizers

				Literature comparison		
^{18}F probe	RCY d.c., %	Duration, min	A_m, GBq/μmol (Ci/μmol)	RCY d.c., %	Duration, min	Refs.
This work						
CFA	22 ± 5 (*n* = 3)	90*	N.M.	10–15 (*n* = ?)	210	31, 32
DFA	51 ± 5 (*n* = 4)	74	N.M.	?	?	33
F-AraG	5 ± 4 (*n* = 5)	165	56–270 (1.5–7.3)	7–10 (*n* = 10)	140–160	34
FBA	64 ± 12 (*n* = 3)	45*	N.M.	72 (*n* = 1)†	45†	35
				50–55 (*n* = ?)	30	36
FBB	60 ± 9 (*n* = 3)	44*,‡	N.M.	27–34 (*n* = 5)	50	37
				18	75	38
FBEM	35 ± 8 (*n* = 7)	98	N.M.	36 ± 15 (*n* = 21)	110–115	39
				24–31 (*n* = 10)	70	40
L-FDOPA (DiMagno method)	4.5 ± 1.3 (*n* = 3)	71	30–33 (0.8–0.9)	14 ± 4 (*n* = ?)	117 ± 4	41
FIAU (1-pot)	13.9 ± 1.7 (*n* = 3)	134	67 (1.8)	9.9 ± 0.4 (*n* = 4)	150	42
				10 ± 6 (*n* = 5)	110	43
Fluoroethoxy-TCO	44 ± 9 (*n* = 10)	71*	N.M.	45 ± 8 (*n* = 16)	41 ± ? (*n* = 16)	25
Fluoroethyl-PEG$_3$-azide [mesylate (OMs) precursor]	39 ± 11 (*n* = 9)	55	N.M.	37 ± 8 (*n* = 16) [tosylate (OTs) precursor]	?	44
FMISO	50 ± 4 (*n* = 3)	96*,‡	N.M.	58.5 ± 3.5 (*n* = 10)	60.0 ± 5.2 (*n* = 10)	45
FMZ (nitro precursor)	7 ± 2 (*n* = 3)	90	7.4–30 (0.2–0.8)§	~30	75–80	46
				20–23 (*n* = 3)	~155	47
FNB	69 ± 13 (*n* = 3)	N.M.	11–15 (0.3–0.4)§	71 ± 4.5 (*n* = 5)	?	48
				35–75	45	49
FPEB (nitro precursor)	31 ± 3 (*n* = 4)	66	37–152 (1.0–4.1)	12.4 ± 3.7 (*n* = ?)¶ (TracerLab method)	90¶	50
FTC-146	12.3 ± 1.8 (*n* = 4)	75	N.M.	3.7 ± 1.9 (*n* = 13)	75	27
Previous work						
D-FAC (3-pot)	31 ± 4 (*n* = 11)	163‡	37–44 (1.0–1.2)	39 ± 5 (*n* = 13)	~240	51
Fallypride	66 ± 8 (*n* = 6)	56‡	15–78 (0.4–2.1)§	68 ± 2 (*n* = 42)	51 ± 1	52
FDG	70 ± 9 (*n* = 3)	38	N.M.	70 ± 6 (*n* > 100)	22	53
				63 ± 3 (*n* = 40)	38	54
FDHT	29 ± 5 (*n* = 7)	90‡	78–170 (2.7–4.6)	31–48 (*n* = ?)	90	55
				25–33 (*n* = 11)	90	56
FEAU (3-pot)	39 ± 4 (*n* = 3)	180‡	3.8–5.1 (0.1–0.14)§	37 ± 9 (*n* = 2)	240	51
FEAU (1-pot)	28 ± 4 (*n* = 3)	140‡	7.4–20 (0.2–0.53)§	25 ± 2 (*n* = 3) (microwave)	120	57
FHBG	11 ± 2 (*n* = 3)	87‡	92–189 (2.5–5.1)	32.0 ± 1.2 (*n* = 5)	38 ± 2	58
				33.8 ± 4.2 (*n* = 10)	60	59
FLT	69 ± 3 (*n* = 5)	65‡	67–481 (1.8–13)	60.2 ± 5.2 (*n* = 10)	71 ± 11	60
				45–60 (*n* = 4)	52	61
L-FMAU (3-pot)	49 ± 7 (*n* = 6)	170‡	100–170 (2.7–4.6)	42.1 ± 12.1 (*n* = 9)	~180	62
				35 ± 6 (*n* = 10)	~240	51
SFB	69 ± 8 (*n* = 6)	78‡	63 (1.7)	80 ± 5 (*n* = 22)	58	63

RCY d.c. is the decay-corrected radiochemical yield and is listed as average ± SD (*n* = no. of experiments) when data are available and otherwise as a range of values. Synthesis duration includes synthesis, purification, and formulation except as noted. A_m is the molar activity, reported as a range of values at the end of synthesis. Question mark denotes that the data were not reported in the literature. D-FAC, 1-(2′-deoxy-2′-[^{18}F]fluoro-β-D-arabinofuranosyl)-cytosine; Fallypride, (*S*)-*N*-((1-allyl-2-pyrrolidinyl)methyl)-5-(3-[^{18}F]fluoropropyl)-2,3-dimethoxybenzamide; FBEM, *N*-[2-(4-[^{18}F]fluorobenzamido)ethyl]maleimide; FDG, 2-[^{18}F]fluoro-2-deoxy-D-glucose; FDHT, 16β-[^{18}F]fluoro-5α-dihydrotestosterone; FIAU, 2′-[^{18}F]fluoro-2′-deoxy-1-β-D-arabinofuranosyl-5-iodouracil; FLT, 3′-deoxy-3′-[^{18}F]fluorothymidine; L-FMAU, 2′-deoxy-2′-[^{18}F]fluoro-5-methyl-1-β-L-arabinofuranosyluracil; N.M., not yet measured.

*The synthesis was performed with many stops for intermediate measurements. Actual time is shorter.

†Full synthesis time not reported (i.e., omits purification). Decay correction is based on partial synthesis time and underestimates the actual decay-corrected yield.

‡Synthesis time does not include formulation. Typically only a few additional minutes were needed.

§Experiments used low starting activity and higher A_m is expected when using more radioactivity.

¶Synthesis time not reported, but assumed to be the same as Lim et al. (64), i.e., 90 min, for the purpose of decay correction.

would be possible as an alternative approach, which would eliminate the need for manual intervention, but this was not pursued.

With the exception of this dilution step in [^{18}F]FTC-146, and the intermediate HPLC purification step in [^{18}F]F-AraG (2′-deoxy-2′-[^{18}F]fluoro-9-β-D-arabinofuranosylguanine) (which would pose a problem on every radiosynthesizer), all probes could be synthesized automatically without operator intervention up to the final purification step. Although the ELIXYS system did not support integrated HPLC purification and formulation at the time most of this work was performed, the purification and formulation steps can be automated using the ELIXYS PURE/FORM module (29), as was done for [^{18}F]FMISO. For most probes, purification methods were adopted directly from previous literature reports and the product was collected by monitoring the output of HPLC detectors (gamma and UV absorbance) and activating an electronic fraction valve when the

product peak was observed. Finally, the purified fraction was formulated, either by evaporation of the mobile phase followed by resuspension of the probe in saline or by solid-phase extraction to exchange the mobile phase with an ~10% ethanol–saline mixture. Two of the probes ([^{18}F]CFA and [^{18}F]FHBG) have been validated by the UCLA Ahmanson Translational Imaging Division for use in the clinic. While several batches of different probes have undergone quality-control testing, further study and possibly optimization would be needed to produce the described probes for human use.

Conclusions

A single ELIXYS FLEX/CHEM radiosynthesizer and hot cell has been used in our institute to support the development of protocols for 24 different probes and to produce these as needed for imaging studies for a wide array of biological scientists. The radiochemical yields and synthesis times were generally comparable to those in the literature from which the protocols were adapted. Because hardware modification was not needed, it was convenient to switch from one probe to another, and thus it would be possible to provide a similarly diverse collection of probes in a clinical setting using the same resources. The ability to provide this number of probes with a single system presents a significant improvement in terms of space, cost, and resource utilization over existing practices in many radiochemistry facilities of dedicated hot cells and synthesizers for each probe. Even further improvement could be realized by operating the ELIXYS FLEX/CHEM in a minicell instead of a hot cell (*SI Appendix*, section 9).

The use of a single synthesizer for multiple probes could also help to standardize and simplify the preparation of regulatory documents and compliance with FDA requirements in a consistent, efficient, and low-cost manner. The emergence of electronic platforms such as the Sofie Probe Network (SPN) (30), for sharing ELIXYS synthesis protocols, documentation, and other information, could further extend this advantage across multiple sites. Such information sharing and consistency of documentation could accelerate and streamline probe discovery, fundamental research into the biology of disease, clinical trials for probes, clinical use of probes as biomarkers in drug trials, and the transfer of probes into clinical practice.

Materials and Methods

Materials. No-carrier–added [^{18}F]fluoride was produced by the (p, n) reaction of [^{18}O]H_2O (84% isotopic purity; Zevacor Pharma) in an RDS-112 cyclotron (Siemens) at 11 MeV, using a 1-mL tantalum target with havar foil. Starting radioactivity ranged from 1.9 GBq to 37 GBq (50 mCi to 1 Ci) and averaged around 15 GBq (400 mCi). Solvents, cartridges, and most precursors and reference standards were obtained from commercial sources and used without further purification. For a few probes, the precursors and reference standards were synthesized in-house. Complete details are provided in *SI Appendix*, section 2.

Radiosynthesizer Programming. Automated programs ("sequences") were created and tested for each probe, using the standard ELIXYS software. Programs are constructed by assembling a short sequence of intuitive "unit operations" (macros), using a drag-and-drop interface, and specifying appropriate parameters for each (22). Unless otherwise specified, REACT and EVAPORATE operations were set to perform cooling of the reaction vessel to 35 °C after completion of heating, all REACT steps were performed in a sealed environment with stirring, all EVAPORATE steps were performed with stirring under continuous vacuum and with inert gas supply of 7 psi, and reagent addition ("ADD") operations were performed at 5 psi for 15 s. Transfer of the crude reaction mixture to the HPLC loop, if applicable, was set to manual mode and was performed remotely by using pressure/vacuum via a 12-mL syringe (located outside the hot cell).

Radiosynthesizer Setup. The number of cassettes needed depended on the probes being synthesized. The cassettes were reused and cleaned before each experiment (*SI Appendix*, section 3). The cleaned cassettes were loaded into the ELIXYS and locked into position. Clean reactor vials (W986259NG; Wheaton), each with a magnetic stir bar (14-513-65; Fisher Scientific), were placed in the ELIXYS reactor positions. Reagent vials (62413P-2; Voigt) were filled with the appropriate reagents for the probe being synthesized (*SI Appendix*, Tables S2 and S3) and then sealed with a septum (224100-072; Wheaton) and crimp cap (224177-01; Wheaton). Once sealed, the reagents vials were inverted and loaded into the appropriate cassette reagent positions. Preconditioned cartridges were installed on the cassettes using Luer fittings, paying attention to the proper flow orientation. Details of cartridges and preconditioning methods are described in *SI Appendix*, Table S4. If needed, the HPLC injection loop input line (previously cleaned with 5 mL of water and then dried with N_2) was connected to the appropriate cassette for loading the contents of the final reaction vessel onto the HPLC loop. (Detailed interconnections among cassettes, cartridges, and other system components are shown in *SI Appendix*, Fig. S1.) Finally, the vacuum trap was emptied and a mixture of MeOH (20–30 mL) and dry ice was added to the vacuum trap dewar.

Chromatography and Analytical Methods. All probes [except [^{18}F]DFA (2′-deoxy-2′-[^{18}F]fluoroarabinose), [^{18}F]FBA (4-[^{18}F]fluorobenzaldehyde), [^{18}F]FNB] were purified by semipreparative HPLC. Injection was performed using the ELIXYS HPLC injection valve connected to a 5-mL loop. Radioanalytical HPLC was used to confirm the identity and radiochemical purity and to determine RCY and molar activity (A_m). [^{18}F]DFA, [^{18}F]FBA, and [^{18}F]FNB were analyzed via radio-TLC. Complete details are given in *SI Appendix*, section 5.

Synthesis of Probes. After radiosynthesizer setup as described above, [^{18}F]fluoride was delivered into the system by connecting a septum-sealed source vial to the cassette 1 inlet line and to a N_2 pressure needle. The program corresponding to the desired probe was then loaded and run to completion. The detailed descriptions and ELIXYS programs for synthesizing all probes are included in *SI Appendix*, sections 6 and 8, and the programs are also available online to members of the Sofie Probe Network (30).

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