Supplemental Methods

Detailed three reactor syntheses of D-[^{18}F]FAC, L-[^{18}F]FMAU, and D-[^{18}F]FEAU

The D-sugar or L-sugar solution (10 mg in 1 mL MeCN, 17 mM) was added to the activated [^{18}F]fluoride residue in reaction vessel one, and reacted at 160°C for 15 min with stirring. The crude fluorinated intermediate solution was then passed through a silica cartridge (WAT020520) to trap the intermediate, which was subsequently eluted with ethyl acetate (2 mL) into reaction vessel two. The ethyl acetate solution in reaction vessel two was evaporated at 80°C until dry (2.5 min). One additional elution and evaporation step was performed. HBr solution (0.15 mL) was added to reaction vessel 2 via one of the external reagent lines connected to cassette 2 (Figure 1). This step was immediately followed by the addition of DCE (0.4 mL), using the same reagent delivery line. The bromination reaction was carried out at 80°C for 10 min. The crude mixture was then partially evaporated at 80°C for 1.5 min. Toluene (0.9 mL) was added to reaction vessel two, and the solution was evaporated to dryness at 90°C (2.5 min). FAC precursor (30 mg in 1 mL DCE, 117 mM), FMAU precursor (100 mg in 1 mL DCE, 370 mM), or FEAU precursor (50 mg in 1 mL DCE, 176 mM) was added to reaction vessel two and reacted at 165°C for 30 min. The resulting solution was then trapped on a silica cartridge (WAT043400) and subsequently eluted with 10% MeOH in DCM (2 mL) to reaction vessel three. The MeOH in DCM solution in reaction vessel three was evaporated to dryness at 80°C (2 min). Two additional elutions and evaporations were performed. 0.5 M NaOMe in MeOH (0.8 mL) was added to reaction vessel three and reacted at 105°C for 5 min.1 N HCl (0.4 mL) was used to quench the reaction. This solution was partially evaporated at 80°C for 1.5 min to remove the MeOH. D-[^{18}F]FAC HPLC mobile phase (1 mL), L-[^{18}F]FMAU HPLC mobile phase (1 mL), or D-[^{18}F]FEAU HPLC mobile phase (1 mL) (Table 2) was added to reaction vessel three prior to being injected into the semi-preparative HPLC for purification.

Supplemental Tables

SUPPLEMENTAL TABLE 1
Reagent positions on the disposable cassettes for each synthesis

Tracer	Cassette #	Reagent vial positions* 1	2	3	4	5	6	7	8
D-[^{18}F]FAC/ L-[^{18}F]FMAU/ D-[^{18}F]FEAU (three-reactor)	1	Base solution 1[†]	MeCN	MeCN	L or D-sugar	ethyl acetate	ethyl acetate		
	2	HBr solution	DCE	toluene	FAC, FMAU, or FEAU precursor	MeOH in DCM	MeOH in DCM	MeOH in DCM	
	3	0.5 M NaOMe	1 N HCl	HPLC mobile phase[#]					
D-[^{18}F]FEAU (one-reactor)	1	Base solution 2[‡]	MeCN	MeCN	D-sugar	5-ethyluracil solution	2.5 M NaOMe	6 N HCl	water
[^{18}F]FDG	1	Base solution 1[†]	MeCN	MeCN	Mannose Triflate	2 N NaOH	water	water	water …**
[^{18}F]FLT	1	Base solution 3[§]	MeCN	MeCN	FLT precursor	1 N HCl	2 N NaOH	HPLC mobile phase[#]	
[^{18}F]Fallypride	1	Base solution 3[§]	MeCN	MeCN	Tosyl-Fallypride	HPLC mobile phase[#]			
[^{18}F]FHBG	1	Base solution 4[‖]	MeCN	MeCN	Tosyl-FHBG	1 N HCl	2 N NaOH	HPLC mobile phase[#]	
[^{18}F]SFB	1	Base solution 5[¶]	MeCN	MeCN	FB-precursor	1 M TMAOH	2 mL MeCN	TSTU	HPLC mobile phase[#]

Base solutions were each prepared in 0.3 mL water with 0.5 mL MeCN, with the exception of [^{18}F]FLT and [^{18}F]Fallypride that were in 0.2 mL water and 0.6 mL MeCN and the one-reactor D-[^{18}F]FEAU that was in 0.1 mL water and 1 mL of MeCN. Specific values for each reagent can be found in the Materials and Methods section.

*Only 8 of the 12 possible reagent positions were used in each cassette with the exception of [^{18}F]FDG

[†]1 mg (7 μmol) K_2CO_3 and 10 mg (27 μmol) Kryptofix K_{222}

[‡]13 mg (43 μmol) $TBAHCO_3$

[§]4.6 mg (15 μmol) $TBAHCO_3$

[‖]5 mg (35 μmol) K_2CO_3 and 22 mg (59 μmol) Kryptofix K_{222}

[¶]4.25 mg (30 μmol) K_2CO_3 and 25 mg (68 μmol) Kryptofix K_{222}

[#]Specific mobile phase composition for each tracer is found in Table 2.

**Reagent vials in positions 6 – 12 were each filled with 2 mL of water for [^{18}F]FDG

SUPPLEMENTAL TABLE 2
HPLC conditions for the various tracers

Tracer	Semi-preparative HPLC			Analytical HPLC		
	Mobile phase (*v/v*)	Flow rate (mL/min)	Retention time (min)	Mobile phase (*v/v*)	Flow rate (mL/min)	Retention time (min)
D-[^{18}F]FAC*	1% EtOH in 10 mM $NH_4H_2PO_4$	5.0	15	10% EtOH in 50 mM NH_4OAc	1.0	4.5
L-[^{18}F]FMAU*	4% MeCN in 50 mM NH_4OAc	5.0	20	5% MeCN in 50 mM NH_4OAc	1.0	12
D-[^{18}F]FEAU	7% MeCN in water	5.0	18	15% MeCN in water	1.0	7.0
[^{18}F]FDG†	N/A	N/A	N/A	N/A	N/A	N/A
[^{18}F]FLT	8% EtOH in 20 mM KH_2PO_4	5.0	20	10% EtOH in water	1.0	12
[^{18}F]Fallypride	60% MeCN in 25 mM ammonium formate w/ 1%TEA	5.0	12	60% MeCN in 25 mM ammonium formate w/ 1%TEA	1.0	12
[^{18}F]FHBG	5% MeCN in 50 mM NH_4OAc	6.0	17	10% MeCN in 50 mM NH_4OAc	1.0	8.5
[^{18}F]SFB	50% MeCN in water with 0.1% TFA	5.0	13	50% MeCN in water with 0.1% TFA	1.0	7.5

*Denotes tracers purified with the Gemini-NX semi-preparative column (see section 2.3). Other tracers were purified with the Luna column.

†[^{18}F]FDG was purified via solid-phase extraction cartridges and verified by radio-TLC (95% MeCN in water (*v/v*), *Rf* = 0.56).