ACS OMEGA

Article

Cite This: ACS Omega 2017, 2, 8649−8659

[^{18}F]Fluorophenylazocarboxylates: Design and Synthesis of Potential Radioligands for Dopamine D3 and μ-Opioid Receptor

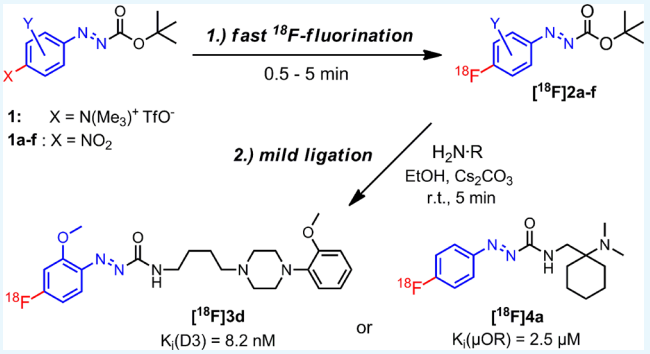
Natascha Nebel,[†] Brigitte Strauch,[†] Simone Maschauer,[†] Roman Lasch,[‡] Hannelore Rampp,[‡,§] Stefanie K. Fehler,[‡,∥] Leonard R. Bock,[‡] Harald Hübner,[‡] Peter Gmeiner,[‡] Markus R. Heinrich,*[,‡] and Olaf Prante*[,†]

[†]Department of Nuclear Medicine, Molecular Imaging and Radiochemistry, Friedrich Alexander University Erlangen-Nürnberg (FAU), Schwabachanlage 6, Erlangen D-91054, Germany

[‡]Department of Chemistry and Pharmacy, Medicinal Chemistry, Emil Fischer Center, Friedrich Alexander University Erlangen-Nürnberg (FAU), Schuhstrasse 19, Erlangen D-91052, Germany

Supporting Information

ABSTRACT: ^{18}F-Labeled building blocks from the type of [^{18}F]fluorophenylazocarboxylic-*tert*-butyl esters offer a rapid, mild, and reliable method for the ^{18}F-fluoroarylation of biomolecules. Two series of azocarboxamides were synthesized as potential radioligands for dopamine D3 and the μ-opioid receptor, revealing compounds **3d** and **3e** with single-digit and sub-nanomolar affinity for the D3 receptor and compound **4c** with only micromolar affinity for the μ-opioid receptor, but enhanced selectivity for the μ-subtype in comparison to the lead compound AH-7921. A "minimalist procedure" without the use of a cryptand and base for the preparation of 4-[^{18}F]fluorophenylazocarboxylic-*tert*-butyl ester [^{18}F]**2a** was established, together with the radiosynthesis of methyl-, methoxy-, and phenyl-substituted derivatives ([^{18}F]**2b−f**). With the substituted [^{18}F]fluorophenylazocarbylates in hand, two prototype azocarboxylates radioligands were synthesized by ^{18}F-fluoroarylation, namely the methoxy azocarboxamide [^{18}F]**3d** as the D3 receptor radioligand and [^{18}F]**4a** as a prototype structure of the μ-opioid receptor radioligand. By introducing the new series of [^{18}F]fluorophenylazocarboxylic-*tert*-butyl esters, the method of ^{18}F-fluoroarylation was significantly expanded, thereby demonstrating the versatility of ^{18}F-labeled phenylazocarboxylates for the design of potential radiotracers for positron emission tomography .



INTRODUCTION

Positron emission tomography (PET) has emerged as a powerful and highly sensitive imaging technique for the diagnosis and monitoring of treatment of diseases in routine nuclear medicine and in preclinical radiopharmaceutical sciences. The most frequently used positron-emitting radioisotope is fluorine-18 because of its favorable physical properties and a chemistry-compatible half-life of 110 min. Offering a wide range of compounds that are amenable to ^{18}F-labeling would be essential for broadening the scope of PET in future applications.[1] Therefore, highly effective radiochemical labeling methods for the introduction of fluorine-18 into molecules are needed.

The aromatic noncarrier-added (n.c.a.) ^{18}F-nucleophilic substitutions are demanding and feasible only if activated aromatic systems are employed. Electron-withdrawing substituents such as nitro, cyano, aldehyde, or carboxylic functions in the ortho or para position to the leaving group increase the reactivity toward nucleophilic aromatic ^{18}F-fluorination.[2−4] Onium salts, nitro, or halides are typical leaving groups, and moreover, significant advances have been made in broadening the scope of nucleophilic aromatic radiofluorination using triarylsulfonium,[5,6] iodonium ylide precursors,[7,8] and transition-metal-mediated fluorination.[9−12] In addition, the use of aromatic boronic acids[13] or aryl stannane precursors[14,15] or methods for the in situ formation of iodonium salts of electron-rich arenes[16] that allow the effective Cu-mediated direct ^{18}F-fluorination obtaining the ^{18}F-labeled target compound in a single reaction step has been reported recently. However, especially large molecules, oligo-nuclides, and peptides are frequently not amenable to fluorination through direct nucleophilic substitution because of the harsh reaction conditions that lead to the formation of side products or when reactive functional groups such as free amino-, carboxylic acid-, or other CH-acidic-functionalities are present.[17]

Received: September 14, 2017
Accepted: November 16, 2017
Published: December 6, 2017

DOI: 10.1021/acsomega.7b01374
ACS Omega 2017, 2, 8649−8659

In the past, ^{18}F-fluoroarylation required harsh reaction conditions and laborious processing, which led to low radiochemical yields (RCYs) of the product.[18,19] The procedure described by Höfling et al. demonstrated a first attempt to improve the RCY employing the radical ^{18}F-fluoroarylation by the use of ^{18}F-labeled arenediazonium chloride for the example of a potential D3 radioligand of the cinnamoyl amide type.[20] Recently, we have developed an efficient strategy for the ^{18}F-fluoroarylation of biomolecules by the use of 4-[^{18}F]-fluorophenylazocarboxylic-*tert*-butyl ester [^{18}F]**2a**, which is obtained by fluorination of trimethylammonium triflate precursor **1** with fluorine-18 in only 30 s in acetonitrile at 85 °C in nearly quantitative yields.[21,22] The discovery of [^{18}F]**2a**-enabling efficient ^{18}F-fluoroarylation offered structurally very similar azocarboxamide D3 radioligands, such as [^{18}F]**3a** (Figure 1A), obtainable in only two steps under far more convenient reaction conditions (see Scheme 1).[21] After separating **1** from the ^{18}F-labeled building block [^{18}F]**2a** by simple solid-phase extraction, the ^{18}F-fluoroarylation reaction could be performed with a primary amine at room temperature (rt) in ethanol (Scheme 1).[21,22] A further exceptional advantage of [^{18}F]**2a** is its capability to selectively undergo different types of reaction pathways besides nucleophilic substitution, such as radical reactions, benzylation, and allylation in a Barbier-type reaction as well as cycloadditions.[21,23−26]

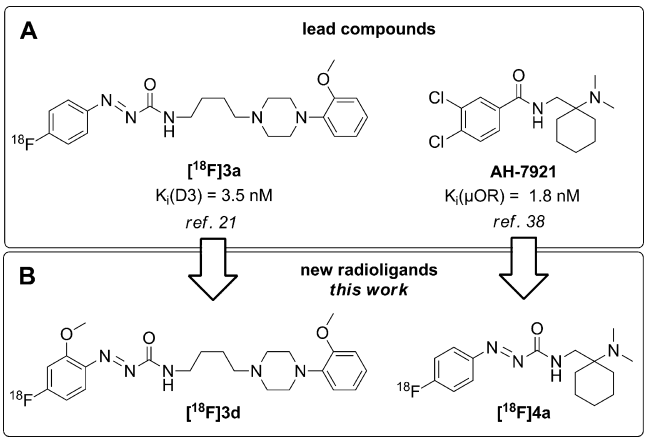
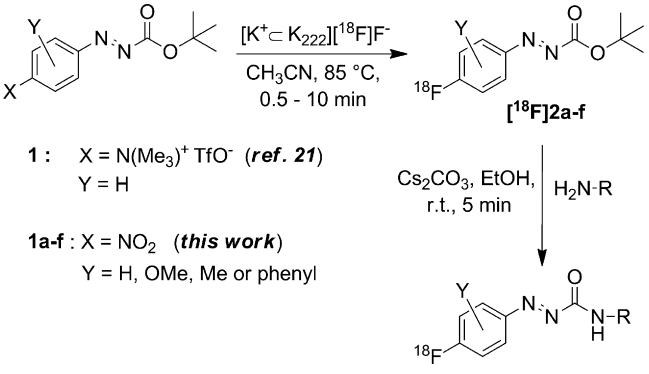


Figure 1. Structures of lead compounds (A) and newly derived ^{18}F-labeled azocarboxamide radioligands (B) for the dopamine D3 receptor (D3) and μ-opioid receptor (μOR).

Scheme 1. General Scheme for the Radiosyntheses of [^{18}F]Fluorophenylazocarboxylic-*tert*-butyl Esters [^{18}F]2a−f and the ^{18}F-Fluoroarylation of Amines to Afford New ^{18}F-Labeled Ligands of the Azocarboxamide Type



The azocarboxamide [^{18}F]**3a** belongs to the class of phenylpiperazinyl benzamides as D3 subtype selective ligands (Figure 1A).[27,28] Several efforts have been made to develop a subtype selective D3 PET ligand for in vivo use, however, a suitable radioligand is still missing.[29] In our previous work, we conducted detailed in vitro and in vivo studies with PET ligands from the azocarboxamide type of compounds,[30−33] demonstrating that [^{18}F]**3a** was suitable for the selective detection of the dopamine D3 receptor distribution in vitro in the rat brain, but failed to image the dopamine D3 receptor in vivo by PET-imaging studies.[21,34] In addition, the modifications of the substitution pattern of the phenyl moiety at the piperazine ring of [^{18}F]**3a** or the addition of a hydroxyl function at the butyl linker to reduce lipophilicity have been made but did not lead to any major improvement in the D3 selectivity profile or the biodistribution of the respective D3 radioligand.[34,35]

Therefore, substituted [^{18}F]fluorophenylazocarboxylic-*tert*-butyl esters could offer the possibility of studying the effects on D3 receptor selectivity and affinity that variations on the aromatic moiety of the azo unit have. Moreover, [^{18}F]-fluorophenylazocarboxylic-*tert*-butyl esters could also be useful to obtain a series of potential μ-opioid receptor-selective radioligands based on the structure of AH-7921 (Figure 1A).[36−38]

The aim of this study was to further explore and improve the advantages of using[^{18}F]fluorophenylazocarboxylic-*tert*-butyl esters for ^{18}F-fluoroarylation reactions. First, we aimed at improving the overall RCY of ^{18}F-fluoroarylation with [^{18}F]-fluorophenylazocarboxylic-*tert*-butyl ester through applying and optimizing the so-called "minimalist procedure"[39] that makes use of ammonium triflate precursors, such as **1** (Scheme 1) that are able to elute [^{18}F]fluoride from an anion-exchange cartridge, thereby shortening the reaction time of the ^{18}F-synthesis in an automated process significantly. Second, we aimed at enhancing the diversity of [^{18}F]-uorophenylazo-carboxylic-*tert*-butyl esters as ^{18}F-labeled building blocks by additional substitution of the phenyl ring in the ortho and meta position to fluorine (Scheme 1 and Figure 2D). Third, we demonstrated ^{18}F-fluoroarylation using unsubstituted [^{18}F]-fluorophenylazocarboxylic-*tert*-butyl ester [^{18}F]**2a** achieved by the "minimalist procedure" for the radiosynthesis of azocarboxamide [^{18}F]**4a** as a potential μ-opioid receptor radioligand (Figure 1B), and we successfully applied the methoxy-substituted [^{18}F]fluorophenylazocarboxylic-*tert*-butyl ester [^{18}F]**2d** to the radiosynthesis of the D3 radioligand candidate [^{18}F]**3d** (Figure 1B). Both ^{18}F-labeled compounds are interesting PET tracers for future in vivo characterization by small animal PET.

RESULTS AND DISCUSSION

The synthesis of precursor compounds **1a−f** for radiolabeling and ^{19}F-fluorinated reference compounds **2a−f**, **3a−e**, and **4a−e** started either from commercially available 4-fluoronitrobenzenes and 4-fluoroanilines or, if available, from the respective arylhydrazines (Scheme 2). As 4-fluoro- and 4-nitrophenylhydrazine were the only commercially available arylhydrazines, only phenylazocarboxylates **1a** and **2a** could be prepared via a short two-step sequence including treatment of the 4-fluoro- or 4-nitrophenylhydrazine with di-*tert*-butyl dicarbonate, followed by oxidation with manganese dioxide [Scheme 2, conditions (c)

DOI: 10.1021/acsomega.7b01374
ACS Omega 2017, 2, 8649−8659

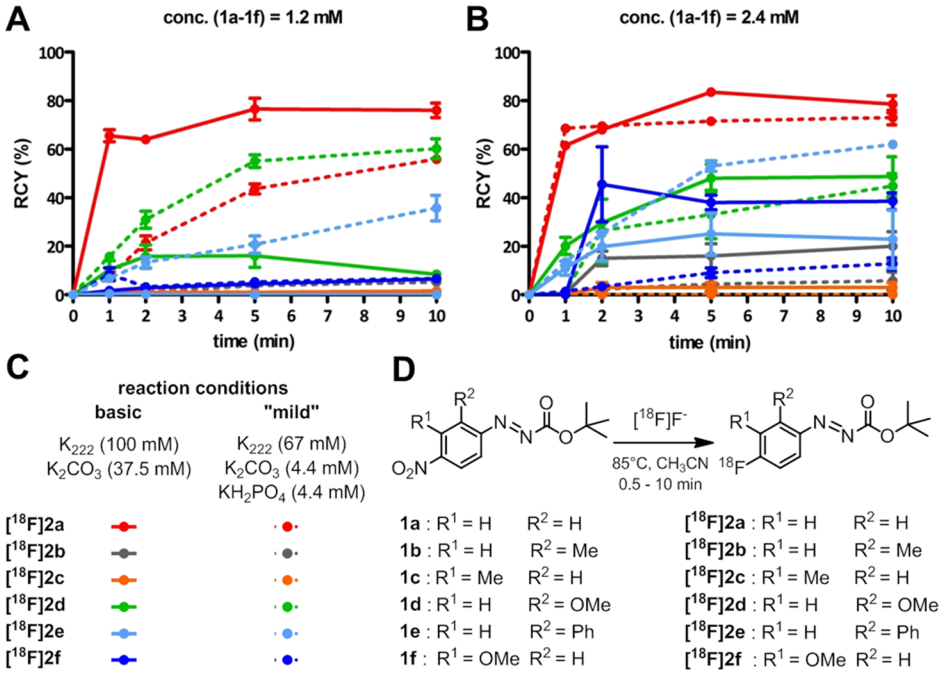
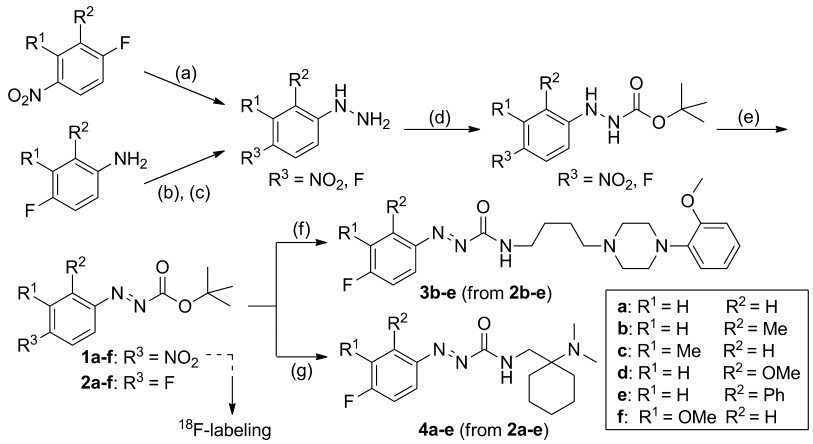


Figure 2. Structures of differently substituted nitrophenylazocarboxylic-*tert*-butyl esters **1a**–**f** as labeling precursors for nucleophilic ^{18}F-fluorination to give phenylazocarboxylic-*tert*-butyl esters [^{18}**F**]**2a**–**f**. (A,B) Comparison of the RCY of [^{18}**F**]**2a**–**f** using a precursor concentration of **1a**–**f** of 1.2 mM (A) or 2.4 mM (B). Data points are given as mean ± SEM for n = 2–4 independent experiments. (C) Legend assigning the curves of (A) and (B) to the corresponding structure and the respective basic or "mild"[40] reaction conditions. (D) General scheme for the radiosynthesis of phenylazocarboxylic-*tert*-butyl esters [^{18}**F**]**2a**–**f**.

Scheme 2. Synthesis of the Precursors and Reference Compounds[a]



[a]Reaction conditions: (a) *N*-Methyl-2-pyrrolidone, hydrazine monohydrate, 65 °C, 7 h. (b) Hydrochloric acid, sodium nitrite. (c) Tin(II) chloride dihydrate, 0 °C, 3 h. (d) CH_3CN, di-*tert*-butyl dicarbonate, rt. (e) CH_2Cl_2, MnO_2, rt. (f) 4-Amino-1-(4-(2-methoxyphenyl)piperazine-1-yl)butane, EtOH, 40 °C, 24 h. (g) *N*-[1-(Aminomethyl)cyclohexyl]-*N*,*N*-dimethylamine, EtOH, 40 °C, 24 h. If no reaction time is given, the consumption of reactants was monitored by thin-layer chromatography (TLC).

and (d)]. In this way, **1a** and **2a** were prepared in yields of 73% (**1a**) and 94% (**2a**) (over two steps), which is consistent with the previously reported data.[21,25,26] The synthesis of nitro-substituted azocarboxylates **1b**–**f** required previous preparation of the corresponding 4-nitrophenylhydrazines through nucleophilic substitution of suitably substituted 4-fluoronitrobenzenes by hydrazine [Scheme 2, condition (a)], so that three synthetic steps were necessary in total [Scheme 2, conditions (a), (d), and (e)]. Fluoro-substituted phenylazocarboxylates **2b**–**f** were prepared from the respective 4-fluoroanilines over four steps [Scheme 2, conditions (b), (c), (d), and (e)] and were obtained in lower overall yields of 10–30%, whereas the particular low yield for **2f** did not permit further conversion of this compound to ligands of types **3** and **4**.

Starting from azocarboxylates **2a**–**e**, 4-fluorophenylazocarboxamides **3b**–**e** and **4a**–**e** were synthesized. These syntheses were performed through nucleophilic substitution at the carbonyl moieties of **2a**–**e** mediated by Cs_2CO_3 as the base and using ethanol as the solvent. The potential dopamine D3 receptor ligands **3b**–**e** were obtained through a reaction with the primary amine 4-amino-1-(4-(2-methoxyphenyl)piperazine-1-yl)butane in yields of approximately 30%. The potential μ-opioid receptor ligands **4a**–**e** were provided by the reaction of

DOI: 10.1021/acsomega.7b01374
ACS Omega 2017, 2, 8649–8659

Table 1. Receptor-Binding Affinities of Compounds 3a−e and 4a−e[a]

	K_i values (nM ± SD)[b]								
	D1	D5	$D2_{long}$	$D2_{short}$	D3	D4	5-HT_{1A}	5-HT_{2A}	α_{1A}
3a[c]	1300 ± 320	2800 ± 570	38 ± 4.6	36 ± 3.0	3.5 ± 0.38	94 ± 13	6.4 ± 1.1	540 ± 75	5.2 ± 0.38
3b	360 ± 130	2900 ± 2500	52 ± 23	15 ± 4.2	2.0 ± 0	62 ± 0.71	13 ± 11	76 ± 34	2.8 ± 1.8
3c	1400 ± 1400	2400 ± 990	29 ± 11	7.5 ± 1.6	1.8 ± 0	36 ± 5.7	21 ± 17	99 ± 45	2.1 ± 1.3
3d	1400 ± 570	6800 ± 4500	81 ± 13	34 ± 0	8.2 ± 4.0	460 ± 120	24 ± 21	98 ± 59	5.0 ± 1.6
3e	69 ± 44	510 ± 320	48 ± 2.8	21 ± 7.8	0.40 ± 0.03	20 ± 6.4	17 ± 9.2	32 ± 5.0	2.7 ± 0.57

	K_i values (μM ± SD)[b]		
	δOR	κOR	μOR
AH-7921	0.75 ± 0.49	0.26 ± 0.05	0.11 ± 0.03
4a	12 ± 3.4	3.1 ± 0.78	2.5 ± 1.1
4b	12 ± 7.3	6.1 ± 1.8	4.9 ± 0.35
4c	30 ± 11	4.8 ± 1.8	1.1 ± 0.35
4d	38 ± 38	7.2 ± 1.8	7.4 ± 5.2
4e	25 ± 0.71	4.8 ± 1.3	2.1 ± 0.57

[a]Receptor-binding affinities derived from radio ligand competition-binding experiments using the human G-protein coupled receptors, which were stably transfected in CHO cells (for $D2_{long}$, $D2_{short}$, D3, and D4) or transiently transfected in HEK 293T cells (for D1, D5, 5-HT_{1A}, 5-HT_{2A}, α_1, δOR, κOR, and μOR). [b]K_i values represent the mean of two independent experiments ± SD, each done in triplicate. [c]K_i values ±standard error of the mean (SEM) taken from ref 21.

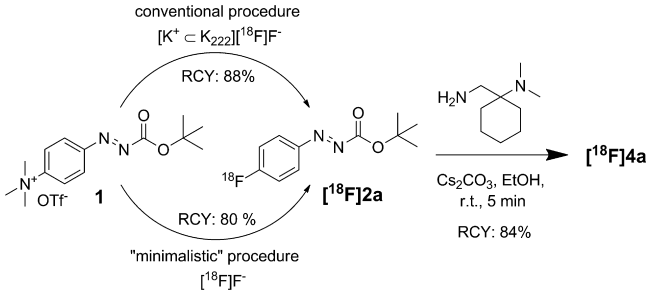
2a−e with the primary amine *N*-[1-(aminomethyl)cyclohexyl]-*N*,*N*-dimethylamine in slightly higher yields of about 40%. The coupling of *tert*-butyl 4-fluorophenylazocarboxylates with primary amines under basic conditions has been previously reported[21,35] using K_2CO_3 as the base in the solvent ethyl acetate, which results in a suspension as the reaction mixture and thus to a heterogenic reaction. Now, using the reaction conditions as optimized previously for ^{18}F-radiochemistry,[22] applying Cs_2CO_3 as the base and ethanol as the solvent, the yields of target compounds **3b−e** and **4a−e** could be increased by about 10%.

The receptor-binding studies determined the affinity of compounds **3b−e** to subtypes of the dopamine receptors and to the related biogenic amine receptors 5-HT_{1A}, 5-HT_2, and α_1. The binding affinity of **4a−e** was investigated toward the δ, κ, and μ-opioid receptor subtypes (Table 1). The affinity of candidates **3b−e** for binding at the D3 receptor was in the same nanomolar range as for reference compound **3a** (K_i(D3) = 3.5 nM). The selectivity of **3b**, **c**, and **d** concerning the binding at the other dopamine subtypes and the related amine receptors was also comparable to reference compound **3a**. An improvement in D3 selectivity over 5-HT_{1A} could be seen for **3b−f** in comparison with **3a** as the reference [K_i(5-HT_{1A}/D3) = 1.8], especially for phenyl compound **3e** (K_i(5-HT_{1A}/D3) = 43). The selectivity of reference compound **3a** (K_i(D2/D3) = 10) has been shown to be sufficient to selectively address and quantify the D3 receptor in vitro, without interfering the D2 binding.[34] Concerning the affinity for binding at the D_3 receptor, the position of the substituent in the ortho or meta position to fluorine did not reveal any major effect on the D3 affinity. One exception is the phenyl substituent of **3e**, leading to an increased D3 affinity by 10-fold (K_i = 0.4 nM) compared to ortho- and meta-unsubstituted reference compound **3a**.

Opioid receptor candidates **4a−e** show more than 10-fold less affinity for binding at the μ-opioid receptor than the reference compound AH-7921, which exhibits a K_i(μOR) of 0.11 μM. The highest affinity for binding at the μOR-subtype was determined with methyl-substituted compound **4c** [K_i(μOR) = 1.1 μM]. Ortho-methoxy compound **4d** did not show any selectivity between the κ and μ opioid receptor subtypes, similar to **4a**, **b**, **d**, and **e**. Interestingly, the subtype selectivity of compound **4c** [K_i(κOR/μOR) = 4.4; K_i(δOR/μOR) = 27] was doubled compared to the reference compound AH-7921 [K_i(κOR/μOR) = 2.4; K_i(δOR/μOR) = 6.8].

As the starting point for the "minimalist procedure" according to the Neumaier group,[39] the precursor concentration of **1** of 1.2 mM was used to elute fluorine-18 from the cartridge (Scheme 3). The recovery value of 100% of fluorine-18 from the cartridge was defined by comparison with the elution of fluorine-18 from the anion exchange QMA cartridge using cryptate 222 and K_2CO_3 as the eluent. Under these conditions, an RCY of 88% for the subsequent labeling of [^{18}F]**2a** was achieved (Scheme 3), which is in accordance with the literature.[21]

Scheme 3. Radiosynthesis of [^{18}F]4a by ^{18}F-Fluoroarylation with [^{18}F]2a Produced by the Conventional Procedure and "Minimalist Procedure"



The results of the [^{18}F]fluoride recovery for the elution of fluoride-18 by ammonium triflate precursor **1** using different solvent systems is shown in Figure S1A, demonstrating that acetonitrile is not suitable and that methanol and ethanol revealed recovery values of around 80% (Figure S1A). The precursor concentration of **1** needed to be raised to 2.4 mM, when the eluent for [^{18}F]fluoride had to be changed to a mixture of ethanol and acetonitrile, whereby the optimal ratio of the two solvents was determined. It turned out that a ratio of EtOH/CH_3CN = 4:1 was optimal for the recovery of fluorine-18 of nearly 100% (Figure S1A). The subsequent labeling proceeded after the evaporation of the solvent system and

DOI: 10.1021/acsomega.7b01374
ACS Omega 2017, 2, 8649−8659

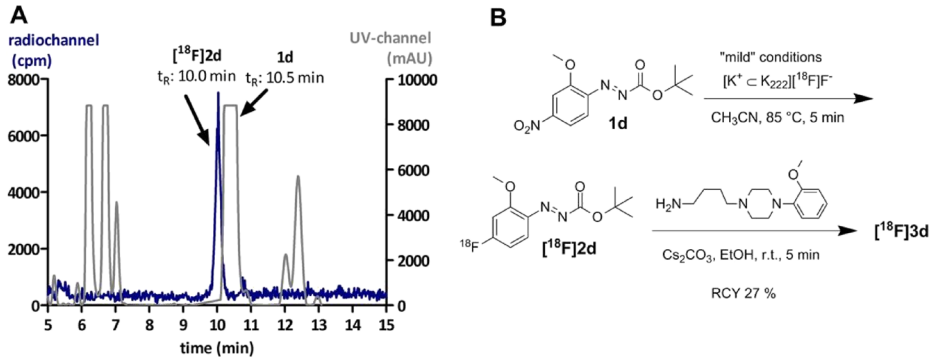


Figure 3. (A) Isolation of [^{18}F]**2d** from radioactive and nonradioactive components, including precursor **1d**, by the use of semipreparative HPLC. (B) Reaction scheme of ^{18}F-fluoroarylation with [^{18}F]**2d** to obtain [^{18}F]**3d**.

addition of dry acetonitrile with an RCY of about 75% after 30 s at 85 °C. These findings were in accordance with the literature,[39] concerning the elution of [^{18}F]fluoride in alcoholic solvents and the subsequent necessity of evaporating the solvent and redissolving in appropriate reaction solvents using a trimethylammonium triflate-bearing precursor.

Further increasing the precursor amount to 5.0 mM offered a slightly higher RCY of 80% coming close to the 88% RCY, which was achieved under conventional conditions (Scheme 3). The labeling to yield the versatile prosthetic group [^{18}F]**2a** is therefore possible under the conditions of the "minimalist procedure." The "minimalist procedure" though requires a higher precursor amount of twice or thrice the concentration necessary for the conventional approach to enable labeling with fluorine-18. Precursor **1** is though very easily separated from [^{18}F]**2a** through solid-phase extraction (tC18, Waters), and the higher excess of the unreacted precursor did not influence the subsequent coupling of [^{18}F]**2a** to give the potential opioid receptor PET ligand [^{18}F]**4a** (Scheme 3). The ^{18}F-fluoroarylation with [^{18}F]**2a** obtained under conventional conditions offered [^{18}F]**4a** in an RCY of 84 ± 1% (n = 2). [^{18}F]**2a** obtained under the conditions of the "minimalist procedure" offered [^{18}F]**4a** in an RCY of 78 ± 2% (n = 2). The time of evaporation and redissolving step (≈1 min) is very short compared to azeotropic drying (10−15 min); therefore, both approaches can be considered reasonable in manual radiosyntheses, depending on which factor is more crucial, the time advantage or the precursor amount. However, most importantly, the "minimalist procedure" could be of exceptional importance for automated processes in ^{18}F-synthesis modules because of the provided time benefit.

Besides the optimization of the "minimalist procedure" using trimethylammonium triflate precursor **1**, we aimed at the variation of the substitution pattern at the aromatic ring of [^{18}F]**2a**. This could be achieved by using the series of nitro precursors **1a−f** that turned out to be more easily accessible than the corresponding trimethylammonium triflates. The nucleophilic ^{18}F-fluorination of **1a−f** was performed using the conventional ^{18}F-labeling conditions of **1** as a starting point, as depicted in Scheme 1. To identify the influence of the substitution at the phenyl ring on the RCY of [^{18}F]**2b−f**, the RCY of [^{18}F]**2a**, prepared from nitro precursor **1a**, served as a reference (red color, Figure 2). The RCY of [^{18}F]**2a** starting from both **1** and **1a** was nearly the same, the labeling of the nitro precursor though required a longer reaction time of 5 min (Figure 2B).

The results of the ^{18}F-labeling experiments showed that it was not possible to radiolabel methyl-substituted precursors **1b** and **1c** using the concentration of 1.2 mM (Figure 2A). The concentration was therefore increased to 2.4 mM (Figure 2B), so that [^{18}F]**2b** and [^{18}F]**2c** were obtained in a low RCY of 18 and 3%, respectively. It is tempting to speculate that the low RCY could be ascribed to the presence of the benzylic proton that reacts with [^{18}F]fluoride to give hydrogen fluoride, thereby reducing the RCY of the nucleophilic fluorination significantly. The labeling of ortho- and meta-methoxy substituted compounds **1d** and **1f** was more successful, when changing the reaction conditions from classical conditions (37.5 mM K_2CO_3) to the so-called "mild" conditions,[40] applying K_2CO_3 and KH_2PO_4. Applying the precursor concentrations of **1d** and **1f** of 1.2 mM, an RCY of almost 60% could be achieved for [^{18}F]**2d** under "mild" reaction conditions (Figure 2A). An increase of the precursor concentration to 2.4 mM revealed that the RCY of both [^{18}F]**2d** and [^{18}F]**2f** reached 40−50% after 10 min reaction time (Figure 2A,B). The decreased RCY compared to the unsubstituted azocarboxylate [^{18}F]**2a** can be explained by the +M effect of the methoxy group, increasing the electron density at the aromatic core. The effect is thereby independent of the position of the methoxy group because ortho substitution (relative to the nitro group), as in **1f**, can destabilize the negatively charged cyclohexadienyl intermediate resulting from the attack of the ^{18}F-fluoride, and meta substitution will reduce the electron-withdrawing properties of the azocarbonyl unit through the formation of a push−pull system. The activating effect of the azocarbonyl moiety is, however, crucial for the successful incorporation of ^{18}F-fluoride into the azocarboxylate. Consequently, the biphenyl compound [^{18}F]**2e**, in which the additional phenyl group does not exert a strong electron-donating effect, was obtained in a higher RCY of up to 60% under mild reaction conditions (Figure 2B).

Finally, as a proof of concept for the capability of ^{18}F-labeled building blocks [^{18}F]**2a−f** to react with primary amines of biological relevance, the ^{18}F-fluoroarylation step was performed with [^{18}F]**2d** that was separated from nitro precursor **1d** by high-performance liquid chromatography (HPLC) (Figure 3A). Coupling of [^{18}F]**2d** to (2-methoxyphenyl)piperazinyl amine provided the D3 radioligand candidate [^{18}F]**3d** in 27% RCY in ethanol in the presence of Cs_2CO_3 after 5 min at rt (Figure 3B).

CONCLUSIONS

In conclusion, the fluorine-substituted azocarboxamide building blocks **2a−f** were applied to the synthesis of a series of

DOI: 10.1021/acsomega.7b01374
ACS Omega 2017, 2, 8649−8659

potential D3 ligands (**3a−e**) and opioid ligands (**4a−e**), from which **3e** turned out to be a highly subtype selective D3 ligand with a subnanomolar receptor affinity, demonstrating and confirming that the azocarboxamide substructure is well-tolerated by the D3 receptor. However, in the case of the series of opioid ligands, the azocarboxamides are far less potent than the carboxamide reference AH-7921, suggesting that the azo unit is not well-tolerated by the opioid receptor for the series of compounds under study.

The preparation of [^{18}F]fluorophenylazocarboxylic-*tert*-butyl ester [^{18}F]**2a** was successfully optimized by applying the "minimalist procedure" that is of utmost importance for rapid automated radiosyntheses of PET tracers. Moreover, applying the nitro precursors **1a−f**, the radiochemical synthesis of a series of methyl-, methoxy-, and phenyl-substituted [^{18}F]-fluorophenylazocarboxylic-*tert*-butyl esters [^{18}F]**2a−f** was studied, revealing that the methoxy- and phenyl-substituted compounds ([^{18}F]**2d−f**) showed an RCY of 40−60% in the presence of KH_2PO_4. Providing evidence for the applicability of the new series of ^{18}F-labeled building blocks, the subsequent exemplary labeling of the azocyclohexyl amide [^{18}F]**4a** as an opioid receptor radioligand was successfully accomplished, and the ^{18}F-labeled D3 radioligand [^{18}F]**3d** was prepared by ^{18}F-fluoroarylation with [^{18}F]**2d** in 27% RCY.

By introducing the new series of substituted ^{18}F-labeled phenylazocarboxylic esters as ^{18}F-labeled building blocks, the method of ^{18}F-fluoroarylation was significantly expanded, thereby demonstrating the versatility of ^{18}F-labeled phenylazocarboxylates for the design of ^{18}F-labeled biomolecules as radiotracers for PET.

EXPERIMENTAL SECTION

General. Solvents and reagents were obtained from commercial sources and used as received. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 600 (^{1}H: 600 MHz, ^{13}C: 151 MHz) and a Bruker Avance 360 spectrometer (^{1}H: 360 MHz, ^{13}C: 91 MHz). $CDCl_3$ was used as the solvent referenced to tetramethylsilane (δ = 0.00 ppm). Chemical shifts are reported in parts per million (ppm). Coupling constants are in hertz (*J*, Hz). The following abbreviations are used for the description of signals: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and brs (broad singlet). Mass spectra were recorded on a JEOL GC mate II GC−MS-system using electron impact, on a LC−MS 1100 Agilent/API 2000 system using electronspray ionization (ESI), and on a Bruker maXis UHR-TOF system using ESI.

Analytical TLC was carried out on Merck silica gel plates using short-wave (254 nm) ultraviolet (UV) light, $KMnO_4$ [3.0 g $KMnO_4$, 20 g potassium carbonate, 5.0 mL aqueous sodium hydroxide (5 w/w %) in 300 mL H_2O], and ninhydrine (200 mg ninhydrine in 100 mL ethanol) to visualize the components. For column chromatography, silica gel (silica gel 60, grain size 40−63 μm, Merck) was used. All reactions were carried out following common organic chemistry laboratory procedures. Special emphasis was on anhydrous solvents for ^{18}F-radiosyntheses.

The identity and purity of compounds was ensured with NMR spectroscopy. The purity of **1a−f** was additionally confirmed with HPLC method 2. The identity of compounds **3b−e** and **4a−e** was additionally confirmed with high-resolution mass spectra. The purity of compounds **3b−e** and **4a−e** (1 mg/mL) was additionally confirmed by two different HPLC methods (method 1 or 2 and method 3). The HPLC system (Series 1100, Agilent) was equipped with a VWD UV-lamp (detection at λ = 214 and λ = 254 nm), and for radiochemistry, it was additionally connected to a radio detector (500 TR Series, Packard). The conditions used for analytical HPLC are described in methods 1−3. Method 1: Chromolith RP-18e, 100 × 4.6 mm, flow rate: 4 mL/min, solvent A: water [0.1% trifluoroacetic acid (TFA)], solvent B: acetonitrile (0.1% TFA), gradient A/B: 90:10 to 50:50 in 5 min, λ = 214 nm. Method 2: Luna C18(2) 5 μm, 150 × 4.6 mm, flow rate: 1.5 mL/min, solvent: A: water (0.1% TFA), solvent B: acetonitrile (0.1% TFA), gradient A/B: 85:15 to 10:90 in 30 min, λ = 254 nm. Method 3: Chromolith RP-18e, 100 × 4.6 mm, flow rate: 4 mL/min, solvent A: water (0.1% TFA), solvent B: acetonitrile (0.1% TFA), gradient A/B: 90:10 to 100% B in 5 min, λ = 214 nm.

Fluorine-18 was provided by Advanced Accelerator Applications Germany GmbH (Erlangen, Germany) and delivered as n.c.a. [^{18}F]fluoride in solution with an average activity range of 0.6−1.0 GBq. The identity of radioligands was determined using the reference compounds bearing the natural fluorine isotope. The reference compound was coinjected with the radioligand for the HPLC analysis, and the identity was confirmed by overlapping the retention times using HPLC methods 1 or 2 and 3. When using radio-TLC (silica gel 60, F_{254}, Merck) to determine the RCY, the R_f-value of the reference compounds was used as identification.

General Procedure and Analytical Data for the Preparation of Substituted *tert*-Butyl Phenylazocarboxylates 1a−f and 2a−f. The syntheses of 4-nitro- and 4-fluorophenylazocarboxylates **1a** and **2a** were carried out as previously described.[25]

*Preparation of Substituted 4-Nitrophenylhydrazines from 4-Fluoronitrobenzenes for the Synthesis of **1b−f**.* A solution of fluoronitrobenzene (13.8 mmol) and hydrazine hydrate (69.0 mmol, 5.58 g) in ethanol (20 mL) was heated under reflux for 6 h. After cooling to rt, the precipitated solid was collected by filtration, and the filter cake was washed with cold ethanol. Drying under reduced pressure provided crude 4-nitrophenylhydrazine, which was used for the next step without further purification.

*Preparation of Substituted 4-Fluorophenylhydrazines from 4-Fluoroanilines for the Synthesis of **2b−f**.* A solution of the substituted 4-fluoroaniline (10.0 mmol) in glacial acetic acid (5.0 mL) was treated with concentrated hydrochloric acid (25 mL) and cooled to 0 °C. A solution of sodium nitrite (10.0 mmol, 690 mg) in water (2.0 mL) was added over a period of 20 min, and the reaction was stirred for 1 h at 0 °C. A precooled solution of tin chloride dihydrate (22.2 mmol, 5.00 g) in concentrated hydrochloric acid (5.0 mL) was added dropwise over a period of 45 min. After stirring for 1 h at 0 °C, the precipitate was collected by filtration and was subsequently treated with sodium hydroxide solution (0.05 M). The resulting mixture was extracted with diethyl ether. The combined organic phases were washed with a saturated aqueous solution of sodium chloride and dried over sodium sulfate. Removal of the solvent under reduced pressure provided 4-fluorophenylhydrazine, which was used for the next step without further purification.

*Preparation of the Phenylazocarboxylates **1b−f** and **2b−f** from the Corresponding 4-Nitro- and 4-Fluorophenylhydrazines.*[25] The crude phenylhydrazine (ca. 1 mmol; for preparation, see above) was dissolved in dry acetonitrile (15 mL) and treated with di-*tert*-butyl dicarbonate (1.50 mmol, 327

DOI: 10.1021/acsomega.7b01374
ACS Omega 2017, 2, 8649−8659

mg) under an argon atmosphere. After the mixture was stirred at an ambient temperature overnight, the solvent was removed under reduced pressure. The crude mixture was then redissolved in dichloromethane (10 mL), and manganese dioxide (5.40 mmol, 469 mg) was added under an argon atmosphere. After the complete consumption of the reactant, as monitored by TLC (*n*-hexane/ethyl acetate = 5:1), the mixture was filtered over celite. The filtrate was concentrated under reduced pressure, and the obtained residue was subjected to column chromatography (silica gel, hexane/ethyl acetate = 5:1) to give compounds **1b−f** and **2b−f** in overall yields of 10−30% as light to dark orange solids.

tert-Butyl 2-(4-Nitrophenyl)azocarboxylate ***1a***.[25] The synthesis of **1a** was performed as described previously, and all analytical data were in accordance to the literature.[25] HPLC: method 2: t_R = 21.05 min (99%).

tert-Butyl 2-(2-Methyl-4-nitrophenyl)azocarboxylate ***1b***. ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 1.67 (s, 9H), 2.76 (s, 3H), 7.60 (d, J = 8.9 Hz, 1H), 8.08−8.10 (m, 1H), 8.23 (d, J = 2.5 Hz, 1H). ^{13}C NMR (91 MHz, $CDCl_3$): δ (ppm) 17.5 (CH_3), 27.9 (3 × CH_3), 85.9 (C_q), 116.8 (CH), 121.8 (CH), 126.6 (CH), 140.6 (C_q), 149.9 (C_q), 152.9 (C_q), 160.7 (C_q). HPLC: method 2: t_R = 23.10 min (99%).

tert-Butyl 2-(3-Methyl-4-nitrophenyl)azocarboxylate ***1c***. ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 1.67 (s, 9H), 2.67 (s, 3H), 7.81−7.84 (m, 1H), 7.85−7.86 (m, 1H), 8.08 (d, J = 8.6 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 20.3 (CH_3), 27.9 (3 × CH_3), 86.0 (C_q), 121.3 (CH), 125.8 (CH), 127.6 (CH), 135.0 (C_q), 151.4 (C_q), 152.9 (C_q), 160.6 (C_q). HPLC: method 2: t_R = 22.90 min (99%).

tert-Butyl 2-(2-Methoxy-4-nitrophenyl)azocarboxylate ***1d***. ^{1}H NMR (360 MHz, $CDCl_3$): δ (ppm) 1.67 (s, 9H), 4.11 (s, 3H), 7.63 (d, J = 8.8 Hz, 1H), 7.85 (dd, J = 2.3, 8.8 Hz, 1H), 7.95 (d, J = 2.3 Hz, 1H). ^{13}C NMR (91 MHz, $CDCl_3$): δ (ppm) 27.9 (3 × CH_3), 56.9 (CH_3), 85.9 (C_q), 108.3 (C_q), 115.7 (CH), 117.9 (CH), 144.2 (CH), 151.4 (C_q), 157.7 (C_q), 160.4 (C_q). HPLC: method 2: t_R = 20.59 min (99%).

tert-Butyl 2-(4-Nitrobiphen-2-yl)azocarboxylate ***1e***. ^{1}H NMR (360 MHz, $CDCl_3$): δ (ppm) 1.62 (s, 9H), 7.40−7.44 (m, 2H), 7.44−7.49 (m, 3H), 7.66 (d, J = 8.8 Hz, 1H), 8.27 (dd, J = 2.5, 8.8 Hz, 1H), 8.49 (d, J = 2.3 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 27.8 (3 × CH_3), 85.8 (C_q), 117.5 (CH), 123.0 (C_q), 126.1 (CH), 128.2 (CH), 128.8 (2 × CH), 130.8 (2 × CH), 135.5 (C_q), 142.5 (C_q), 149.7 (CH), 152.0 (C_q), 160.5 (C_q). HPLC: method 2: t_R = 25.62 min (99%).

tert-Butyl 2-(3-Methoxy-4-nitrophenyl)azocarboxylate ***1f***. ^{1}H NMR (360 MHz, $CDCl_3$): δ (ppm) 1.68 (s, 9H), 4.03 (s, 3H), 7.57 (d, J = 1.9 Hz, 1H), 7.59 (d, J = 1.9, 8.5 Hz, 1H), 7.96 (d, J = 8.5 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 27.8 (3 × CH_3), 56.8 (CH_3), 86.1 (C_q), 106.9 (CH), 116.5 (CH), 126.3 (CH), 142.0 (C_q), 153.4 (C_q), 154.0 (C_q), 160.5 (C_q). HPLC: method 2: t_R = 20.95 min (99%).

tert-Butyl 2-(4-Fluoro-2-methylphenyl)azocarboxylate ***2b***. ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 1.66 (s, 9H), 2.69 (s, 3H), 6.90−6.93 (m, 1H), 7.04 (dd, J_{HF} = 2.6 Hz, J = 9.0 Hz, 1H), 7.62 (dd, J_{HF} = 5.7 Hz, J = 9.0 Hz, 1H). ^{13}C NMR (91 MHz, $CDCl_3$): δ (ppm) 17.5 (CH_3), 27.9 (3 × CH_3), 84.7 (C_q), 113.8 (d, J_{CF} = 23.2 Hz, CH), 117.7 (d, J_{CF} = 1.8 Hz, C_q), 117.8 (d, J_{CF} = 10.5 Hz, CH), 143.5 (d, J_{CF} = 9.2 Hz, CH), 146.4 (d, J_{CF} = 2.9 Hz, C_q), 161.3 (C_q), 165.8 (d, J_{CF} = 254.8 Hz, C_q).

tert-Butyl 2-(4-Fluoro-3-methylphenyl)azocarboxylate ***2c***. ^{1}H NMR (360 MHz, $CDCl_3$): δ (ppm) 1.66 (s, 9H), 2.34 (d, J_{HF} = 2.2 Hz, 3H), 7.13 (t, J_{HF} = 9.2 Hz, J = 9.2 Hz, 1H), 7.76−7.80 (m, 2H). ^{13}C NMR (91 MHz, $CDCl_3$): δ (ppm) 14.6 (d, J_{CF} = 3.4 Hz, CH_3), 27.7 (3 × CH_3), 84.9 (C_q), 115.9 (d, J_{CF} = 24.2 Hz, CH), 124.2 (d, J_{CF} = 9.4 Hz, CH), 126.2 (d, J_{CF} = 19.1 Hz, C_q), 126.5 (d, J_{CF} = 6.6 Hz, CH), 147.9 (d, J_{CF} = 3.2 Hz, C_q), 161.0 (C_q), 164.5 (d, J_{CF} = 254.7 Hz, C_q).

tert-Butyl 2-(4-Fluoro-2-methoxyphenyl)azocarboxylate ***2d***. ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 1.65 (s, 9H), 3.99 (3H), 6.67 (ddd, J = 2.6, 7.7 Hz, J_{HF} = 9.1 Hz, 1H), 6.79 (dd, J_{HF} = 2.5, 10.5 Hz, 1H), 7.67 (dd, J = 6.5 Hz, J_{HF} = 9.1 Hz, 1H). ^{13}C NMR (91 MHz, $CDCl_3$): δ (ppm) 27.8 (3 × CH_3), 56.5 (CH_3), 84.6 (C_q), 100.7 (d, J_{CF} = 25.7 Hz, CH), 107.7 (d, J_{CF} = 24.6 Hz, CH), 118.7 (d, J_{CF} = 18.4 Hz, CH), 137.7 (d, J_{CF} = 3.3 Hz, C_q), 160.1 (C_q), 160.7 (d, J_{CF} = 11.0 Hz, C_q), 167.4 (d, J_{CF} = 254.5 Hz, C_q).

tert-Butyl 2-(4-Fluorobiphen-2-yl)azocarboxylate ***2e***. ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 1.60 (s, 9H), 7.11 (ddd, J = 2.8, 7.7, 9.0 Hz, 2H), 7.29 (dd, J = 2.8, 9.2 Hz, 2H), 7.40−7.44 (m, 2H), 7.70 (dd, J_{HF} = 9.0 Hz, J = 5.6 Hz, 2H). ^{13}C NMR (91 MHz, $CDCl_3$): δ (ppm) 27.9 (3 × CH_3), 84.8 (C_q), 115.2 (d, J_{CF} = 23.1 Hz, CH), 117.5 (d, J_{CF} = 22.9 Hz, CH), 118.1 (d, J_{CF} = 9.7.4 Hz, CH), 127.9 (CH), 128.2 (CH), 130.9 (CH), 136.7 (CH), 145.3 (d, J_{CF} = 2.8 Hz, C_q), 145.4 (d, J_{CF} = 8.9 Hz, C_q), 161.1 (C_q), 165.3 (d, J_{CF} = 254.7 Hz, C_q).

tert-Butyl 2-(4-Fluoro-3-methoxyphenyl)azocarboxylate ***2f***. ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 1.66 (s, 9H), 3.93 (s, 3H), 7.22 (dd, J_{HF} = 8.5 Hz, J = 10.4 Hz, 1H), 7.48 (dd, J_{HF} = 2.3 Hz, J = 7.9 Hz, 1H), 7.62 (ddd, J = 2.3, 4.4 Hz, J_{HF} = 8.5 Hz, 1H). ^{13}C NMR (91 MHz, $CDCl_3$): δ (ppm) 26.8 (3 × CH_3), 55.2 (CH_3), 84.0 (C_q), 103.7 (d, J_{CF} = 3.4 Hz, CH), 115.3 (d, J_{CF} = 19.3 Hz, CH), 120.0 (d, J_{CF} = 7.4 Hz, CH), 147.2 (d, J_{CF} = 3.1 Hz, CH), 147.5 (C_q), 159.9 (C_q), 164.7 (d, J_{CF} = 257.6 Hz, C_q).

General Procedure for the Preparation of Azocarboxamides ***3b−3e*** *and* ***4a−4e***. Under an argon atmosphere, a solution of **2a−e** (0.55 mmol) in EtOH (3 mL) was stirred together with Cs_2CO_3 (40 mg, 0.13 mmol), and commercially available 4-amino-1-(4-(2-methoxyphenyl)piperazine-1-yl)-butane (0.5 mmol; for the syntheses of **3b−e**) or *N*-[1-(aminomethyl)cyclohexyl]-*N*,*N*-dimethylamine (0.5 mmol; for the syntheses of **4a−e**) was added. The mixture was stirred at 40 °C overnight. Afterward, the mixture was diluted with H_2O, and the product was extracted with EtOAc. The combined organic phases were washed with a saturated saline solution and dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was first purified by column chromatography ($MeOH/CH_2Cl_2$ 1:9, R_f: 0.5−0.7) and then by semipreparative HPLC [Luna C18(2) 5 μm, 250 × 10 mm, flow rate: 4 mL/min., solvent A: water 0.1% TFA, solvent B: acetonitrile 0.1% TFA, gradient A/B: 70:30 to 40:60 in 25 min], λ = 254 nm. Method 3, t_R = 15.00−19.00 min to give compounds **3b−e** and **4a−e** after lyophilization of the product fraction as orange to red solids in yields of 30−40%.

2-(4-Fluoro-2-methylphenyl)-N-(4-(4-(2-methoxyphenyl)-piperazine-1-yl)but-1-yl)azocarboxamide ***3b***. ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 1.75−1.83 (m, 2H), 1.90−1.99 (m, 2H), 2.70 (s, 3H), 3.01−3.29 (m, 6H), 3.45−3.56 (m, 4H), 3.61−3.68 (m, 2H), 3.88 (s, 3H), 6.90−6.95 (m, 3H), 6.97−7.02 (m, 1H), 7.02−7.09 (m, 2H), 7.67 (dd, J_{HF} = 5.6 Hz, J = 9.0 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 17.6 (CH_3), 20.7 (CH_2), 26.3 (CH_2), 39.1 (CH_2), 47.5 (CH_2), 52.2

DOI: 10.1021/acsomega.7b01374
ACS Omega 2017, 2, 8649−8659

(2 × CH_2), 55.4 (2 × CH_2), 56.5 (CH_3), 111.3 (CH), 113.9 (d, J_{CF} = 23.2, CH), 117.8 (J_{CF} = 22.1 Hz, CH), 118.2 (d, J_{CF} = 10.0 Hz, CH), 118.8 (CH), 121.2 (CH), 124.4 (CH), 138.8 (C_q), 143.7 (d, J_{CF} = 9.2 Hz, C_q), 145.9 (d, J_{CF} = 2.8 Hz, C_q), 152.06 (C_q), 161.53 (C_q), 165.92 (d, J_{CF} = 255.6 Hz, C_q). HRMS: $[M]^+$ calcd: 427.2384; found: 428.2456 ($[M + H]^+$). HPLC: method 1: t_R = 3.73 min (99%), method 2: t_R = 10.77 min (99%).

2-(4-Fluoro-3-methylphenyl)-N-(4-(4-(2-methoxyphenyl)-piperazine-1-yl)but-1-yl)azocarboxamide ***3c***. ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 1.83−1.77 (m, 2H), 2.00−1.92 (m, 2H), 2.35 (s, 3H) 3.07 (t, J = 11.8 Hz, 2H), 3.12−3.18 (m, 2H), 3.24 (t, J = 12.4 Hz, 2H), 3.48−3.58 (m, 4H), 3.65 (d, J = 11.5 Hz, 2H), 3.88 (s, 3H), 6.89 (d, J = 8.8 Hz, 1H), 6.93−6.94 (m, 2H), 7.06−7.10 (m, 2H), 7.15 (t, J = 8.7 Hz, 1H), 7.80−7.87 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 14.5 (CH_3), 20.7 (CH_2), 26.4 (CH_2), 39.1 (CH_2), 47.5 (CH_2), 52.2 (2 × CH_2), 55.4 (2 × CH_2), 56.4 (CH_3), 111.3 (CH), 115.9 (d, J_{CF} = 24.2, CH), 118.9 (CH), 121.2 (CH), 124.5 (CH), 124.7 (d, J_{CF} = 9.4 Hz, CH), 126.3 (d, J_{CF} = 19.0 Hz, CH), 126.7 (d, J_{CF} = 6.7 Hz, CH), 138.8 (C_q), 147.4 (d, J_{CF} = 3.1 Hz, C_q), 152.05 (C_q), 161.03 (C_q), 164.66 (d, J_{CF} = 255.4 Hz, C_q). HRMS: $[M]^+$ calcd: 427.2384; found: 428.2456 ($[M + H]^+$). HPLC: method 1: t_R = 3.64 min (99%), method 2: t_R = 10.45 min (99%).

2-(4-Fluoro-2-methoxyphenyl)-N-(4-(4-(2-methoxyphenyl)piperazine-1-yl)but-1-yl)azocarboxamide ***3d***. ^{1}H NMR (360 MHz, $CDCl_3$): δ (ppm) 1.65−1.75 (m, 2H), 1.83−1.95 (m, 2H), 3.13−3.47 (m, 6H), 3.56−3.74 (m, 6H), 3.88 (s, 3H), 3.93 (s, 3H), 5.35 (br s, 1H), 5.96 (s, 1H), 6.43 (dd, J_{HF} = 1.8 Hz, J = 9.9 Hz, 1H), 6.88−7.06 (m, 4H), 7.14 (dd, J = 2.3 Hz, J_{HF} = 7.9 Hz, 1H). ^{13}C NMR (96 MHz, $CDCl_3$): δ (ppm) 20.6 (CH_2), 26.9 (CH_2), 38.7 (CH_2), 47.8 (2 × CH_2), 52.1 (2 × CH_2), 55.5 (CH_3), 56.3 (CH_3), 56.9 (CH_2), 106.4 (CH), 111.6 (2 × CH), 114.2 (C_q), 117.0 (C_q), 119.4 (d, J_{CF} = 20.4, CH), 121.4 (J_{CF} = 10.81, CH), 125.6 (d, J_{CF} = 24.8 Hz, CH), 128.0 (CH), 137.2 (C_q), 137.5 (d, J_{CF} = 3.3 Hz, C_q), 153.4 (C_q), 161.4 (C_q), 165.4 (d, J_{CF} = 260.7 Hz, C_q). HRMS: $[M]^+$ calcd: 443.2333; found: 444.2405 ($[M + H]^+$). HPLC: method 1: t_R = 1.65 min (99%), method 2: t_R = 6.40 min (99%).

2-(4-Fluorobiphen-2-yl)-N-(4-(4-(3-methoxyphenyl)-piperazine-1-yl)but-1-yl)azocarboxamide ***3e***. ^{1}H NMR (360 MHz, $CDCl_3$): δ (ppm) 1.59−1.70 (m, 2H), 1.79−1.86 (m, 2H), 3.03 (t, J = 11.7 Hz, 2H), 3.07−3.11 (m, 2H), 3.24 (t, J = 12.3 Hz, 2H), 3.36 (q, J = 6.6 Hz, 2H), 3.54 (dd, J = 62.5, 12.3 Hz, 4H), 3.87 (s, 3H), 6.87−6.91 (m, 1H), 6.92−6.94 (m, 2H), 6.96 (dd, J_{HF} = 9.1, J = 2.7 Hz, 1H), 7.04 (d, J_{HF} = 2.7 Hz, 1H), 7.06−7.10 (m, 1H), 7.39−7.45 (m, 5H), 7.91 (d, J_{HF} = 9.1 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 20.5 (CH_2), 26.7 (CH_2), 38.9 (CH_2), 47.5 (2 × CH_2), 52.1 (2 × CH_2), 55.4 (CH_3), 56.5 (CH_2), 111.3 (CH), 115.0 (CH), 115.5 (d, J_{CF} = 23.1 Hz, CH), 118.2 (d, J_{CF} = 23.2 Hz, CH), 118.9 (J_{CF} = 10.2 Hz, CH), 121.2 (CH), 124.4 (CH), 127.8 (2 × CH), 128.0 (CH), 130.5 (2 × CH), 138.2 (C_q), 138.8 (C_q), 141.6 (d, J_{CF} = 2.9 Hz, C_q), 146.7 (d, J_{CF} = 9.1 Hz, C_q), 152.1 (C_q), 161.2 (C_q), 163.3 (J_{CF} = 254.5 Hz,C_q). HRMS: $[M]^+$ calcd: 489.2540; found: 490.2613 ($[M + H]^+$). HPLC: method 1: t_R = 5.05 min (99%), method 2: t_R = 14.41 min (99%), method 3: t_R = 2.91 min (99%).

(E)-N-((1-(Dimethylamino)cyclohexyl)methyl)-2-(4-fluorophenyl)diazene-1-carboxamide ***4a***. ^{1}H NMR (360 MHz, $CDCl_3$): δ (ppm) 1.46−1.59 (m, 5H), 1.62−1.71 (m, 5H), 2.30 (s, 6H), 3.61 (d, J = 4.3 Hz, 2H), 7.17−7.24 (m, 2H), 7.98−8.06 (m, 2H). ^{13}C NMR (91 MHz, $CDCl_3$): δ (ppm) 22.2 (2 × CH_2), 25.8 (CH_2), 28.3 (2 × CH_2), 37.5 (2 × CH_3), 41.4 (CH_2), 43.45 (C_q), 116.4 (d, J_{CF} = 23.0 Hz, 2 × CH), 126.3 (d, J_{CF} = 9.4 Hz, 2 × CH), 147.9 (d, J_{CF} = 3.0 Hz, C_q), 160.9 (C_q), 165.8 (d, J_{CF} = 256.0 Hz, C_q). HRMS: $[M]^+$ calcd: 306.1856; found: 307.1929 ($[M + H]^+$). HPLC: method 1: t_R = 2.30 min (99%), method 2: t_R = 6.44 min (99%).

(E)-N-((1-(Dimethylamino)cyclohexyl)methyl)-2-(4-fluoro-2-methylphenyl)diazene-1-carboxamide ***4b***. ^{1}H NMR (360 MHz, $CDCl_3$): δ (ppm) 1.61−1.79 (m, 5H), 1.80−2.09 (m, 5H), 2.68 (s, 3H), 2.85 (s, 6H), 3.98 (d, J = 6.3 Hz, 2H), 6.74 (dd J = 2.8 Hz, J_{HF} = 9.1 Hz, 1H), 6.80−6.81 (m, 1H), 7.74 (d, J_{HF} = 9.0 Hz, 1H). ^{13}C NMR (91 MHz, $CDCl_3$): δ (ppm) 14.6 (CH_3), 22.2 (2 × CH_2), 24.4 (CH_2), 28.3 (2 × CH_2), 37.6 (2 × CH_3), 39.5 (CH_2), 63.9 (C_q), 113.2 (J_{CF} = 22.9 Hz, CH), 115.9 (CH), 118.3 (J_{CF} = 10.5 Hz, CH), 144.3 (d, J_{CF} = 10.0 Hz, CH), 144.83 (C_q), 162.7 (C_q), 164.0 (d, J_{CF} = 256.3 Hz, C_q). HRMS: $[M]^+$ calcd: 320.2012; found: 321.2085 ($[M + H]^+$). HPLC: method 1: t_R = 3.43 min (98%), method 2: t_R = 7.81 min (99%).

(E)-N-((1-(Dimethylamino)cyclohexyl)methyl)-2-(4-fluoro-3-methylphenyl)diazene-1-carboxamide ***4c***. ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 1.69−1.71 (m, 2H), 1.83−1.90 (m, 6H), 2.00−2.05 (m, 2H), 2.33 (s, 3H), 2.85 (s, 6H), 4.04 (d, J = 6.1 Hz, 2H), 7.13 (t, J = 8.7 Hz, 1H), 7.83−7.89 (m, 2H). ^{13}C NMR (91 MHz, $CDCl_3$): δ (ppm) 14.4 (CH_3), 22.0 (2 × CH_2), 24.6 (CH_2), 27.6 (2 × CH_2), 37.6 (2 × CH_3), 39.9 (CH_2), 67.6 (C_q), 115.8 (d, J_{CF} = 24.2 Hz, CH), 125.3 (d, J_{CF} = 9.6 Hz, CH), 126.4 (d, J_{CF} = 6.7 Hz, CH), 147.7 (C_q), 162.1 (C_q), 162.7 (C_q) 164.7 (d, J_{CF} = 255.0 Hz, C_q). HRMS: $[M]^+$ calcd: 320.2012; found: 321.2085 ($[M + H]^+$). HPLC: method 1: t_R = 2.92 min (99%), method 2: t_R = 8.10 min (99%).

(E)-N-((1-(Dimethylamino)cyclohexyl)methyl)-2-(4-fluoro-2-methoxyphenyl)diazene-1-carboxamide ***4d***. ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 1.63−1.75 (m, 5H), 1.80−2.06 (m, 5H), 2.85 (s, 6H), 3.99 (s, 3H), 4.00 (d, J = 6.2 Hz, 2H), 6.64−6.71 (m, 1H), 6.78 (dd, J = 2.5 Hz, J_{HF} = 10.5 Hz, 1H), 7.75 (dd, J = 6.5 Hz, J_{HF} = 9.1 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 22.1 (2 × CH_2), 24.5 (CH_2), 27.8 (2 × CH_2), 37.5 (2 × CH_3), 39.7 (CH_2), 56.3 (CH_3), 63.9 (C_q), 100.5 (d, J_{CF} = 26.4, CH), 108.0 (J_{CF} = 23.2, CH), 119.1 (d, J_{CF} = 11.5 Hz, CH), 137.4 (C_q), 160.5 (d, J_{CF} = 11.1 Hz, C_q), 162.3 (C_q), 167.8 (d, J_{CF} = 256.6 Hz, C_q). HRMS: $[M]^+$ calcd: 336.1962; found: 337.2034 ($[M + H]^+$). HPLC: method 1: t_R = 2.58 min (98%), method 2: t_R = 6.73 min (99%).

(E)-N-((1-(Dimethylamino)cyclohexyl)methyl)-2-(4-fluorobiphen-2-yl)diazene-1-carboxamide ***4e***. ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 1.58−1.92 (m, 10 H), 2.75 (s, 6H), 3.86 (d, J = 6.4 Hz, 2H), 7.12−7.17 (m, 1H), 7.38−7.44 (m, 5H), 7.81 (br s, 1H), 7.85 (dd, J = 5.5 Hz, J_{HF} = 9.0 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 22.1 (2 × CH_2), 24.3 (CH_2), 28.3 (2 × CH_2), 37.5 (2 × CH_3), 39.1 (CH_2), 66.8 (C_q), 115.6 (d, J_{CF} = 23.1 Hz, CH), 117.5 (d, J_{CF} = 22.8 Hz, CH), 118.6 (d, J_{CF} = 10.0 Hz, CH), 137.2 (C_q), 127.9 (2 × CH_2), 128.1 (CH_2), 130.6 (2 × CH_2), 144.6 (C_q), 146.6 (C_q), 162.0 (d, J_{CF} = 16.0 Hz, C_q), 165.8 (d, J_{CF} = 257.1 Hz, C_q). HRMS: $[M]^+$ calcd: 382.2242; found: 383.2242 ($[M + H]^+$). HPLC: method 1: t_R = 4.14 min (98%), method 2: t_R = 11.51 min (99%).

Radiolabeling of Differently Substituted Phenylazocarboxylic-tert-Butyl Esters Using Basic and "Mild" Reaction Conditions by ^{18}F-for-Nitro Nucleophilic Substitution (***[^{18}F]2a−f***). Fluorine-18 in target water was diluted with

DOI: 10.1021/acsomega.7b01374
ACS Omega 2017, 2, 8649−8659

water to 10−20 mL. Thereafter, portions of 1 mL (50−200 MBq) were fixed on QMA cartrigdes (carbonate form, 46 mg, Waters) and eluted either with a solution of Kryptofix 2.2.2 (15 mg, 39.8 μmol) and K_2CO_3 (1.0 M, 15 μL) in CH_3CN (900 μL) or with Kryptofix 2.2.2 (10 mg, 26.5 μmol), K_2CO_3 (0.1 M, 17.5 μL), and KH_2PO_4 (0.1 M, 17.5 μL) in CH_3CN (900 μL) and water (165 μL). The water was removed by evaporation of CH_3CN (3 × 500 μL) under a stream of nitrogen at 85 °C. The respective precursors **1a**−**e** (1.2 or 2.4 mM) dissolved in 500 μL of CH_3CN were added to the dried K^+/Kryptofix 2.2.2/^{18}F complex, and the solution was stirred at 85 °C in CH_3CN. Aliquots (20 μL) were drawn from the reactor after 0.5, 2, and 5 min and quenched in CH_3CN/H_2O (0.1% TFA) 1:1 (500 μL). The RCY was determined by radio-TLC (hexane/EtOAc = 5:1, R_f ([^{18}F]**2a**−**f**) = 0.4−0.6). The experiments were performed in duplicate or triplicate.

2-(4-[^{18}F]Fluoro-2-methoxyphenyl)-N-(4-(4-(2-methoxyphenyl)piperazine1-yl)but-1-yl)azocarboxamide [^{18}F]3d. [^{18}F]Fluoride was eluted from the QMA cartridge with a solution of Kryptofix 2.2.2 (10 mg, 26.5 μmol), K_2CO_3 (0.1 M, 17.5 μL), and KH_2PO_4 (0.1 M, 17.5 μL) in CH_3CN (900 μL) and water (165 μL). The water was removed by evaporation of CH_3CN (3 × 500 μL) under a stream of nitrogen at 85 °C. Precursor **1d** (1.2 mM) dissolved in 500 μL of CH_3CN was added to the dried K^+/Kryptofix 2.2.2/^{18}F complex, and the solution was stirred at 85 °C for 10 min. Afterward, the solution was diluted with water (0.1% TFA, 1 mL) and subjected to semipreparative HPLC [Luna(C18), 250 × 8 mm, flow rate: 4 mL/min, solvent: A: H_2O (0.1% TFA), solvent B: CH_3CN (0.1% TFA), isocrat. A/B: 70:30, t_R([^{18}F]**2d**) = 10.0 min; t_R(**1d**) = 10.5 min] in three portions of 500 μL to achieve the purification of [^{18}F]**2d**. The intermediate [^{18}F]**2d** was isolated from the product fraction by solid-phase extraction on a cartridge (Sep Pak C18, Waters), from where it was then eluted with ethanol (1 mL) into a reaction vial containing 4-amino-1-(4-(2-methoxyphenyl)-piperazine-1-yl)butane (15 mg, 54 μmol) and Cs_2CO_3 (7.5 mg, 23 μmol). After the solution was stirred for 5 min at an ambient temperature, the RCY was determined by radio-TLC from a sample withdrawn from the reaction mixture.

(E)-N-((1-(Dimethylamino)cyclohexyl)methyl)-2-(4-[^{18}F]-fluorophenyl)diazene-1-carboxamide [^{18}F]4a. The radiosynthesis of [^{18}F]**4a** was performed through ^{18}F-fluoroarylation with [^{18}F]**2a**, which was obtained from the labeling of **1** with fluorine-18 under the conventional procedure as described previously[21] and under the conditions of the "minimalist procedure" (see the Supporting Information). *tert*-Butyl 2-(4-[^{18}F]fluorophenyl)azocarboxylate [^{18}F]**2a** was eluted with EtOH (1 mL) from the cartridge (tC18, Waters) into a reactor containing commercially available *N*-[1-(aminomethyl)-cyclohexyl]-*N*,*N*-dimethylamine (10 mg, 54 μmol) and Cs_2CO_3 (7.5 mg, 23 μmol). After the solution was stirred for 5 min at rt, an aliquot (20 μL) was drawn from the reactor and quenched in CH_3CN/H_2O (0.1% TFA) 1:1 (500 μL), and the RCY was determined with radio-TLC and double-checked with HPLC (method 1). The identity of the title compound was confirmed through coinjection with reference compound **4a** using HPLC methods 1 and 2. The ^{18}F-fluoroarylation was performed in duplicates each for [^{18}F]**2a** produced under conventional procedure and "minimalist procedure" (see the Supporting Information).

Receptor-Binding Studies. Affinities of the test compounds toward human D_1R, $D_{2L}R$, $D_{2S}R$, D_3R, D_4R, D_5R, 5-$HT_{1A}R$, 5-$HT_{2A}R$, and α_{1A}-AR as well as the human opioid receptors δOR, κOR, and μOR were determined as described previously.[41] In brief, membranes containing human $D_{2L}R$, $D_{2S}R$, D_3R, or D_4R obtained from CHO cells stably expressing the corresponding receptors were used together with the radioligand [^{3}H]spiperone (specific activity of 73 Ci/mmol, PerkinElmer, Rodgau, Germany) at a final concentration of 0.20 nM for $D_{2L}R$, $D_{2S}R$, and D_3R, and 0.30 nM for D_4R. The parameters for the homogenates were K_D 0.10 nM, B_{max} 1500 fmol/mg, protein concentration 4 μg/test tube for $D_{2L}R$, K_D 0.050 nM, B_{max} 2000 fmol/mg, protein concentration 2 μg/test tube for $D_{2S}R$, K_D 0.060 nM, B_{max} 1800 fmol/mg, protein concentration 4 μg/test tube for D_3R, and K_D 0.30 nM, B_{max} 750 fmol/mg, protein concentration 10 μg/test tube for D_4R. Competition-binding experiments with human D_1R, D_5R, 5-$HT_{1A}R$, 5-$HT_{2A}R$, and α_{1A}-AR as well as δOR, κOR, and μOR were performed in an analogous manner with membranes from HEK293T cells transiently transfected with the receptor of interest using the Mirus TransIT-293 transfection reagent (peqlab, Erlangen, Germany) or a solution of linear polyethyleneimine in phosphate-buffered saline (PBS), as described previously.[42] The cells were incubated together with [^{3}H]SCH23390 (80 Ci/mmol, Biotrend, Cologne, Germany, final concentration 0.40 nM, K_D 0.27 nM, B_{max} 4900 fmol/mg, protein concentration 1.5 μg/test tube for D_1R and 0.50 nM, K_D 0.30 nM, B_{max} 500 fmol/mg, 10 μg/test tube for D_5R), with [^{3}H]WAY100635 (80 Ci/mmol, Biotrend, 0.20 nM final concentration, K_D 0.10 nM, B_{max} 3400 fmol/mg, protein concentration 2 μg/test tube for 5-$HT_{1A}R$), with [^{3}H]ketanserin (47 Ci/mmol, PerkinElmer, final concentration 0.20 nM, K_D 0.075 nM, B_{max} 500 fmol/mg, protein concentration 10 μg/test tube for 5-$HT_{2A}R$) or with [^{3}H]-prazosin (84 Ci/mmol, PerkinElmer, 0.20 nM final concentration, K_D 0.095 nM, B_{max} 7500 fmol/mg, protein concentration 1.5 μg/test tube for α_{1A}-AR). Binding affinities to the human opioid receptors were determined using the radioligand [^{3}H]diprenorphine (37 Ci/mmol, Biotrend and 0.20 nM final concentration, K_D 0.085 nM, B_{max} 1700 fmol/mg, protein concentration 6 μg/test tube for δOR, 0.30 nM final concentration, K_D 0.070 nM, B_{max} 4500 fmol/mg, protein concentration 3 μg/test tube for κOR, and 0.20 nM final concentration, K_D 0.070 nM, B_{max} 5000 fmol/mg, protein concentration 2 μg/test tube for μOR, respectively). Nonspecific binding was determined in the presence of haloperidol (10 μM for D_1R−D_5R), WAY100635 (10 μM for 5-$HT_{1A}R$), ketanserin (10 μM for 5-$HT_{2A}R$), prazosin (10 μM for $\alpha_{1A}R$), or naloxone (10 μM for δOR, κOR, μOR). Test compounds were dissolved in dimethylsulfoxide at a concentration of 10 mM and diluted in the binding buffer[41] to final concentrations in the range of 0.001 nM to 100 μM. Competition tests were generally performed in 96-well plates at a final volume of 200 μM. The test compound and radioligand were incubated together with the appropriate receptor homogenates at 37 °C for 60 min before separating the bound and free radioligands by filtration through GF/B glass fiber mats. Protein concentration was established by the method of Lowry using bovine serum albumin as the standard.[43] Data analysis of the competition-binding curves from the radioligand displacement experiments was done by the nonlinear regression analysis when applying the algorithms of the program PRISM6.0 (GraphPad Software, San Diego, CA). EC_{50} values were derived from each dose response curve and were subsequently transformed into the corresponding K_i values by applying the equation of Cheng and

DOI: 10.1021/acsomega.7b01374
ACS Omega 2017, 2, 8649−8659

Prusoff.[44] Mean K_i values are the result of two individual experiments, each done in triplicate.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b01374.

HPL chromatograms of compounds **1a**, **1b**, **1d**, **1e**, **1f** and reference compounds **3b**, **3c**, **3d**, **3e** and **4a**, **4b**, **4c**, **4d**, **4e**, experimental protocol for the "minimalist procedure" for the radiosynthesis of [^{18}F]**2a**, and comparison of the conventional procedure with the "minimalist procedure" (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: markus.heinrich@fau.de (M.R.H.).
*E-mail: olaf.prante@uk-erlangen.de (O.P.).

ORCID

Peter Gmeiner: 0000-0002-4127-197X
Markus R. Heinrich: 0000-0001-7113-2025
Olaf Prante: 0000-0003-0247-3656

Present Addresses

§ITG Isotope Technologies Garching GmbH, Lichtenbergstraße 1, D-85748 Garching, Germany.
‖4SC AG, Fraunhoferstrasse 22, D-82152 Planegg-Martinsried, Germany.

Author Contributions

All authors have given approval to the final version of the manuscript.

Funding

This work was supported by the Deutsche Forschungsgemeinschaft (DFG, grants PR 677/6-3, HE5413/3-3, GRK1910/B3).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors gratefully acknowledge Waqas Rafique and Patrick Riss (University of Oslo, Norway) for providing a sample of AH-7921 and Christian Huck for his contribution to the synthesis of some of the compounds **1a–f** and **2a–f**. We acknowledge support by Deutsche Forschungsgemeinschaft and Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU) within the funding program Open Access Publishing.

■ REFERENCES

(1) van der Born, D.; Pees, A.; Poot, A. J.; Orru, R. V. A.; Windhorst, A. D.; Vugts, D. J. Fluorine-18 labelled building blocks for PET tracer synthesis. *Chem. Soc. Rev.* **2017**, *46*, 4709–4773.

(2) Cai, L.; Lu, S.; Pike, V. W. Chemistry with [^{18}F]fluoride ion. *Eur. J. Org. Chem.* **2008**, *2008*, 2853–2873.

(3) Ametamey, S. M.; Honer, M.; Schubiger, P. A. Molecular imaging with PET. *Chem. Rev.* **2008**, *108*, 1501–1516.

(4) Jacobson, O.; Kiesewetter, D. O.; Chen, X. Fluorine-18 radiochemistry, labeling strategies and synthetic routes. *Bioconjugate Chem.* **2015**, *26*, 1–18.

(5) Mu, L.; Fischer, C. R.; Holland, J. P.; Becaud, J.; Schubiger, P. A.; Schibli, R.; Ametamey, S. M.; Graham, K.; Stellfeld, T.; Dinkelborg, L. M.; Lehmann, L. ^{18}F-Radiolabeling of Aromatic Compounds Using Triarylsulfonium Salts. *Eur. J. Org. Chem.* **2012**, *2012*, 889–892.

(6) Sander, K.; Gendron, T.; Yiannaki, E.; Cybulska, K.; Kalber, T. L.; Lythgoe, M. F.; Årstad, E. Sulfonium salts as leaving groups for aromatic labelling of drug-like small molecules with fluorine-18. *Sci. Rep.* **2015**, *5*, 9941.

(7) Cardinale, J.; Ermert, J.; Humpert, S.; Coenen, H. H. Iodonium ylides for one-step, no-carrier-added radiofluorination of electron rich arenes, exemplified with 4-(([^{18}F]fluorophenoxy)-phenylmethyl)-piperidine NET and SERT ligands. *RSC Adv.* **2014**, *4*, 17293–17299.

(8) Rotstein, B. H.; Stephenson, N. A.; Vasdev, N.; Liang, S. H. Spirocyclic hypervalent iodine(III)-mediated radiofluorination of non-activated and hindered aromatics. *Nat. Commun.* **2014**, *5*, 4365.

(9) Ichiishi, N.; Brooks, A. F.; Topczewski, J. J.; Rodnick, M. E.; Sanford, M. S.; Scott, P. J. H. Copper-Catalyzed [^{18}F]Fluorination of (Mesityl)(aryl)iodonium Salts. *Org. Lett.* **2014**, *16*, 3224–3227.

(10) Lee, E.; Hooker, J. M.; Ritter, T. Nickel-mediated oxidative fluorination for PET with aqueous [^{18}F]fluoride. *J. Am. Chem. Soc.* **2012**, *134*, 17456–17458.

(11) Lee, E.; Kamlet, A. S.; Powers, D. C.; Neumann, C. N.; Boursalian, G. B.; Furuya, T.; Choi, D. C.; Hooker, J. M.; Ritter, T. A fluoride-derived electrophilic late-stage fluorination reagent for PET imaging. *Science* **2011**, *334*, 639–642.

(12) Tredwell, M.; Preshlock, S. M.; Taylor, N. J.; Gruber, S.; Huiban, M.; Passchier, J.; Mercier, J.; Génicot, C.; Gouverneur, V. A general copper-mediated nucleophilic ^{18}F fluorination of arenes. *Angew. Chem., Int. Ed.* **2014**, *53*, 7751–7755.

(13) Mossine, A. V.; Brooks, A. F.; Makaravage, K. J.; Miller, J. M.; Ichiishi, N.; Sanford, M. S.; Scott, P. J. H. Synthesis of [^{18}F]Arenes via the Copper-Mediated [^{18}F]Fluorination of Boronic Acids. *Org. Lett.* **2015**, *17*, 5780–5783.

(14) Gamache, R. F.; Waldmann, C.; Murphy, J. M. Copper-Mediated Oxidative Fluorination of Aryl Stannanes with Fluoride. *Org. Lett.* **2016**, *18*, 4522–4525.

(15) Makaravage, K. J.; Brooks, A. F.; Mossine, A. V.; Sanford, M. S.; Scott, P. J. H. Copper-Mediated Radiofluorination of Arylstannanes with [^{18}F]KF. *Org. Lett.* **2016**, *18*, 5440–5443.

(16) McCammant, M. S.; Thompson, S.; Brooks, A. F.; Krska, S. W.; Scott, P. J. H.; Sanford, M. S. Cu-Mediated C–H ^{18}F-Fluorination of Electron-Rich (Hetero)arenes. *Org. Lett.* **2017**, *19*, 3939–3942.

(17) Cole, E.; Stewart, M.; Littich, R.; Hoareau, R.; Scott, P. J. H. Radiosyntheses using fluorine-18: the art and science of late stage fluorination. *Curr. Top. Med. Chem.* **2014**, *14*, 875–900.

(18) Feliu, A. L. Synthetic Studies with [^{18}F]P-Fluorobenzenediazonium Chloride Application to the Synthesis of a Radiolabelled Glucocorticoid: [^{18}F]Win 44577. *J. Labelled Compd. Radiopharm.* **1988**, *25*, 1245–1254.

(19) Patt, J. T.; Patt, M. Reaction of [^{18}F]4-fluorobenzenediazonium cations with cysteine or the cysteinyl group: preparation of ^{18}F-labeled S-aryl-cysteine and a radiolabeled peptide. *J. Labelled Compd. Radiopharm.* **2002**, *45*, 1229–1238.

(20) Höfling, S. B.; Maschauer, S.; Hübner, H.; Gmeiner, P.; Wester, H.-J.; Prante, O.; Heinrich, M. R. Synthesis, biological evaluation and radiolabelling by ^{18}F-fluoroarylation of a dopamine D3-selective ligand as prospective imaging probe for PET. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6933–6937.

(21) Fehler, S. K.; Maschauer, S.; Höfling, S. B.; Bartuschat, A. L.; Tschammer, N.; Hübner, H.; Gmeiner, P.; Prante, O.; Heinrich, M. R. Fast and Efficient ^{18}F-Labeling by [^{18}F]Fluorophenylazocarboxylic Esters. *Chem.—Eur. J.* **2014**, *20*, 370–375.

(22) Nebel, N.; Maschauer, S.; Hocke, C.; Hübner, H.; Gmeiner, P.; Prante, O. Optimization and synthesis of an ^{18}F-labeled dopamine D3 receptor ligand using [^{18}F]fluorophenylazocarboxylic tert-butylester. *J. Labelled Compd. Radiopharm.* **2016**, *59*, 48–53.

(23) Lasch, R.; Heinrich, M. R. Zinc-Mediated Allylation and Benzylation of Phenylazocarboxylic Esters. *J. Org. Chem.* **2015**, *80*, 10412–10420.

(24) Fehler, S. K.; Pratsch, G.; Heinrich, M. R. The trapping of phenyldiazenes in cycloaddition reactions. *Angew. Chem., Int. Ed. Engl.* **2014**, *53*, 11361–11365.

(25) Jasch, H.; Höfling, S. B.; Heinrich, M. R. Nucleophilic substitutions and radical reactions of phenylazocarboxylates. *J. Org. Chem.* **2012**, *77*, 1520–1532.

DOI: 10.1021/acsomega.7b01374
ACS Omega 2017, 2, 8649–8659

(26) Höfling, S. B.; Bartuschat, A. L.; Heinrich, M. R. 4-Substituted *tert*-Butyl Phenylazocarboxylates-Synthetic Equivalents for the para-Phenyl Radical Cation. *Angew. Chem., Int. Ed.* **2010**, *49*, 9769−9772.
(27) Pilla, M.; Perachon, S.; Sautel, F.; Garrido, F.; Mann, A.; Wermuth, C. G.; Schwartz, J.-C.; Everitt, B. J.; Sokoloff, P. Selective inhibition of cocaine-seeking behaviour by a partial dopamine D3 receptor agonist. *Nature* **1999**, *400*, 371−375.
(28) Garcia-Ladona, F. J.; Cox, B. F. BP 897, a selective dopamine D3 receptor ligand with therapeutic potential for the treatment of cocaine-addiction. *CNS Drug Rev.* **2003**, *9*, 141−158.
(29) Mach, R. H.; Luedtke, R. R. Challenges in the development of dopamine D2- and D3-selective radiotracers for PET imaging studies. *J. Labelled Compd. Radiopharm.* **2017**, DOI: 10.1002/jlcr.3558.
(30) Hocke, C.; Cumming, P.; Maschauer, S.; Kuwert, T.; Gmeiner, P.; Prante, O. Biodistribution studies of two ^{18}F-labeled pyridinyl-phenyl amides as subtype selective radioligands for the dopamine D3 receptor. *Nucl. Med. Biol.* **2014**, *41*, 223−228.
(31) Hocke, C.; Maschauer, S.; Hübner, H.; Löber, S.; Utz, W.; Kuwert, T.; Gmeiner, P.; Prante, O. A series of ^{18}F-labelled pyridinylphenyl amides as subtype-selective radioligands for the dopamine D3 receptor. *ChemMedChem* **2010**, *5*, 941−948.
(32) Hocke, C.; Prante, O.; Löber, S.; Hübner, H.; Gmeiner, P.; Kuwert, T. Synthesis and evaluation of ^{18}F-labeled dopamine D3 receptor ligands as potential PET imaging agents. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4819−4823.
(33) Hocke, C.; Prante, O.; Salama, I.; Hübner, H.; Löber, S.; Kuwert, T.; Gmeiner, P. ^{18}F-Labeled FAUC 346 and BP 897 derivatives as subtype-selective potential PET radioligands for the dopamine D3 receptor. *ChemMedChem* **2008**, *3*, 788−793.
(34) Nebel, N.; Maschauer, S.; Kuwert, T.; Hocke, C.; Prante, O. In Vitro and In Vivo Characterization of Selected Fluorine-18 Labeled Radioligands for PET Imaging of the Dopamine D3 Receptor. *Molecules* **2016**, *21*, 1144.
(35) Nebel, N.; Maschauer, S.; Bartuschat, A. L.; Fehler, S. K.; Hübner, H.; Gmeiner, P.; Kuwert, T.; Heinrich, M. R.; Prante, O.; Hocke, C. Synthesis and evaluation of fluoro substituted pyridinylcarboxamides and their phenylazo analogues for potential dopamine D3 receptor PET imaging. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 5399−5403.
(36) Brittain, R. T.; Kellett, D. N.; Neat, M. L.; Stables, R. Proceedings: Anti-nociceptive effects in N-substituted cyclohexylmethylbenzamides. *Br. J. Pharmacol.* **1973**, *49*, 158P−159P.
(37) Wohlfarth, A.; Scheidweiler, K. B.; Pang, S.; Zhu, M.; Castaneto, M.; Kronstrand, R.; Huestis, M. A. Metabolic characterization of AH-7921, a synthetic opioid designer drug: in vitro metabolic stability assessment and metabolite identification, evaluation of in silico prediction, and in vivo confirmation. *Drug Test. Anal.* **2016**, *8*, 779−791.
(38) Rafique, W.; Khanapur, S.; Spilhaug, M. M.; Riss, P. J. Reaching out for Sensitive Evaluation of the Mu Opioid Receptor in Vivo: Positron Emission Tomography Imaging of the Agonist [^{11}C]AH7921. *ACS Chem. Neurosci.* **2017**, *8*, 1847−1852.
(39) Richarz, R.; Krapf, P.; Zarrad, F.; Urusova, E. A.; Neumaier, B.; Zlatopolskiy, B. D. Neither azeotropic drying, nor base nor other additives: a minimalist approach to ^{18}F-labeling. *Org. Biomol. Chem.* **2014**, *12*, 8094−8099.
(40) Maschauer, S.; Prante, O. A series of 2-O-trifluoromethylsulfonyl-D-mannopyranosides as precursors for concomitant ^{18}F-labeling and glycosylation by click chemistry. *Carbohydr. Res.* **2009**, *344*, 753−761.
(41) Hübner, H.; Haubmann, C.; Utz, W.; Gmeiner, P. Conjugated enynes as nonaromatic catechol bioisosteres: synthesis, binding experiments, and computational studies of novel dopamine receptor agonists recognizing preferentially the D_3 subtype. *J. Med. Chem.* **2000**, *43*, 756−762.
(42) Männel, B.; Dengler, D.; Shonberg, J.; Hübner, H.; Möller, D.; Gmeiner, P. Hydroxy-Substituted Heteroarylpiperazines: Novel Scaffolds for β-Arrestin-Biased D_2R Agonists. *J. Med. Chem.* **2017**, *60*, 4693−4713.
(43) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.
(44) Cheng, Y.-C.; Prusoff, W. H. Relationship between the inhibition constant (KI) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.

DOI: 10.1021/acsomega.7b01374
ACS Omega 2017, 2, 8649−8659