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Iodinated Choline Transport-Targeted Tracers

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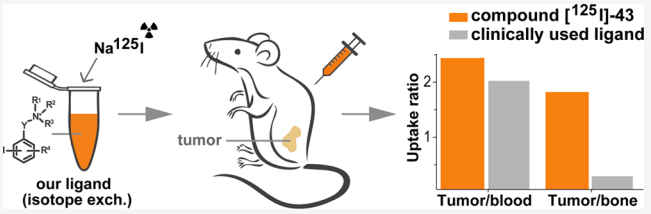
ABSTRACT: We present a novel series of radioiodinated tracers and potential theranostics for diseases accompanied by pathological function of proteins involved in choline transport. Unlike choline analogues labeled with ^{11}C or ^{18}F that are currently used in the clinic, the iodinated compounds described herein are applicable in positron emission tomography, single-photon emission computed tomography, and potentially in therapy, depending on the iodine isotope selection. Moreover, favorable half-lives of iodine isotopes result in much less challenging synthesis by isotope exchange reaction. Six of the described compounds were nanomolar ligands, and the best compound possessed an affinity 100-fold greater than that of choline. Biodistribution data of ^{125}I-labeled ligands in human prostate carcinoma bearing (PC-3) mice revealed two compounds with a biodistribution profile superior to that of [^{18}F]fluorocholine.



INTRODUCTION

Radionuclide imaging methods, such as planar scintigraphy, positron emission tomography (PET), and single-photon emission computed tomography (SPECT), have become invaluable because of their ability to distinguish biochemical changes in tissues (functional imaging) and are routinely used for staging, prognosing, and predicting the therapeutic outcome of many diseases, especially cancer.[1] Plain film radiography, X-ray computed tomography (CT), and magnetic resonance imaging (MRI) can be used to evaluate the anatomical extent of diseases with sub-millimeter resolution. PET and SPECT have lower spatial resolution,[2] but they are able to visualize *in vivo* biological processes in real time and detect the concentrations of specific biomolecules in the picomolar range.[3] Both PET and SPECT rely on radiolabeled pharmaceuticals, which are used as imaging agents.

Choline is an essential precursor for many signaling and constitutive biomolecules[4] (*e.g.*, acetylcholine and phospholipids), and alterations in choline metabolism in cancer cells have been recognized as hallmarks of malignancy. These alterations in choline metabolism are frequently associated with activation of major oncogenic drivers in the Ras and PI3K pathways.[5−7] Numerous studies employing mainly PET[8−13] and magnetic resonance spectroscopy[14−17] have been conducted in this regard. There is evidence that the increased uptake of choline is at least partially caused by the overexpression of choline transporter-like proteins (CTL) by certain types of tumor cells, particularly in prostate carcinomas,[6] gliomas,[6,18] and small cell lung carcinomas.[19] Thus, proteins responsible for choline transport across the plasma membrane represent targetable molecular structures for diagnostic and therapeutic radiopharmaceuticals. Three radiolabeled choline derivatives are currently employed in tumor imaging (Figure 1).[20]

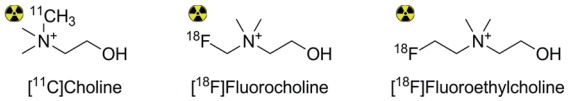


Figure 1. Structures of choline derivatives clinically used as tracers.

However, currently used choline-based imaging agents have significant limitations. First, the ^{11}C and ^{18}F nuclides can be used only for PET imaging. This might be a barrier in the massive use of prostate cancer imaging employing choline-based tracers because the overall cost of PET examinations is still higher compared to SPECT. The short half-lives of both ^{11}C ($t_{1/2}$ = 20 min) and ^{18}F ($t_{1/2}$ = 110 min) impose strict requirements on the synthesis and logistics of these radiopharmaceuticals. Furthermore, for the visualization of prostate carcinoma with [^{18}F]fluorocholine ([^{18}F]FCh), the short isotope half-life combined with rapid renal absorption and excretion causes problems.[21] The image quality is significantly decreased by background activity because of

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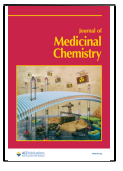

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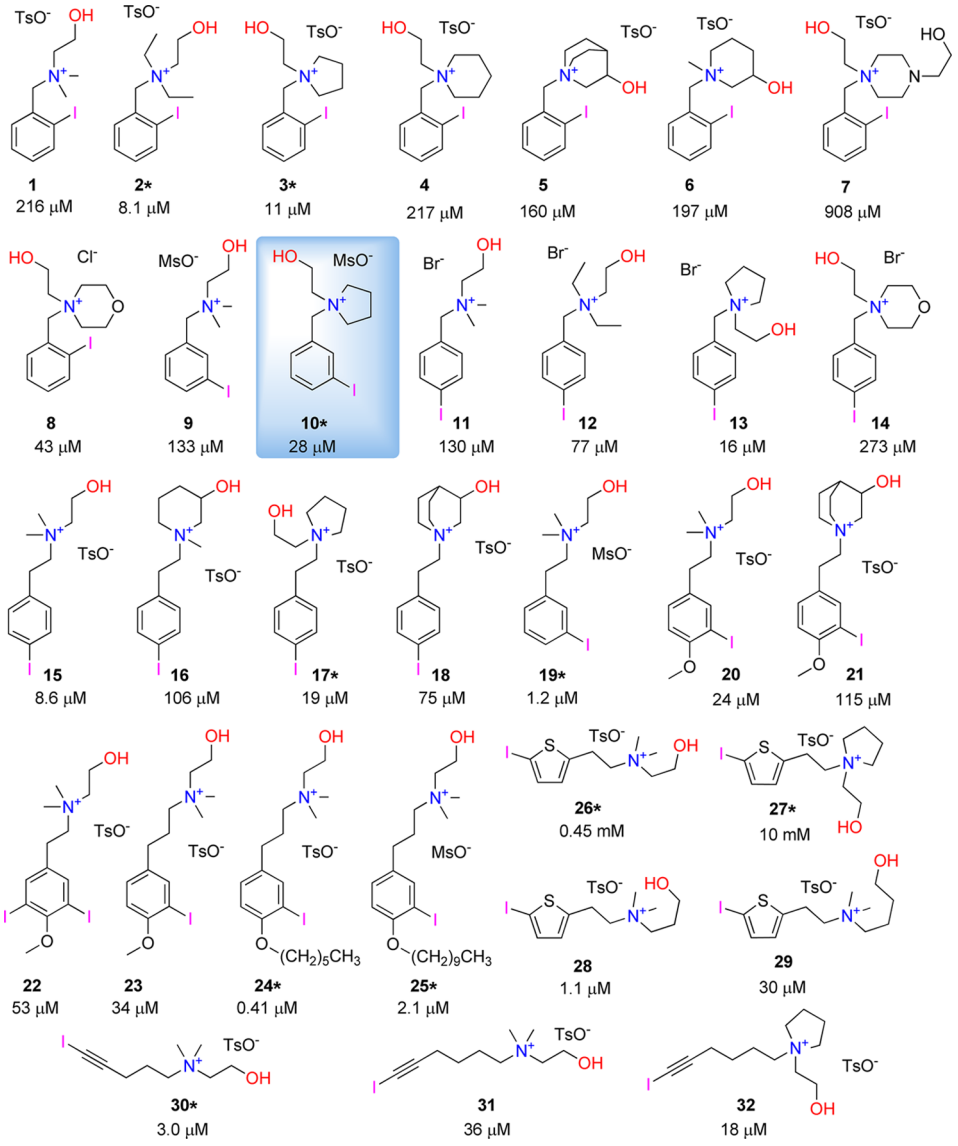

Figure 2. Choline analogues **1**–**32** and their IC_{50} values for [^{3}H]choline uptake by PC-3 cells (n = 3, for standard error see Table 1); compounds selected for *in vivo* evaluation are marked with an asterisk.

accumulation in the urinary bladder and kidney. Moreover, kidney and liver are the highly exposed dose-critical organs limiting the administration levels of [^{18}F]FCh.[22,23]

Theranostic approach in principle enables a significantly more accurate monitoring of the dosage, distribution, and treatment.[24] In the ideal case, theranostic agents would involve chemically and, therefore, biologically identical compounds.[25] The best-known examples which meet this ideal are radioiodide (^{123}I$^-$/^{124}I$^-$ and ^{131}I$^-$ for imaging/treatment of thyroid carcinoma[26,27]) and metaiodobenzylguanidine (MIBG, imaging/treatment of pheochromocytoma[28,29]).

Unfortunately, even a subtle structural change such as the substitution of trivalent diagnostic radiometals (^{111}In and ^{68}Ga) for their therapeutic counterparts (^{90}Y and ^{177}Lu) can lead to significant changes in structure and biological properties.[30] Nevertheless, a diagnostic molecule which is not chemically or biologically identical but has a sufficiently similar biodistribution can adequately predict the biodistribution of its therapeutic analogue. ^{68}Ga-labeled prostate-specific membrane antigen (PSMA)-11 and ^{177}Lu-PSMA-617 are an example of a very promising theranostic pair for patients with PMSA-positive prostate cancer.[31]

In this feasibility study, we developed a new class of iodinated choline-based tracers with both diagnostic and potential therapeutic (theranostics) applications, for example, in prostate cancer, which is the most common type of cancer in males worldwide and one of the major causes of mortality.[32] The main advantage of iodinated tracers is that there are four medicinally relevant isotopes with suitable half-lives; thus, a single compound can be labeled with different iodine radioisotopes suitable either for SPECT (^{123}I, $t_{1/2}$ = 13.2 h), PET (^{124}I, $t_{1/2}$ = 4.2 d), therapy (^{124}I, ^{131}I, $t_{1/2}$ = 8.0 d), or for *in vitro* analysis and preclinical development (^{125}I, $t_{1/2}$ = 60 d).[33]

The cellular uptake of choline has both facilitative and nonfacilitative components and there are many different choline transporters (ChTs), for example, high affinity ChT, CTLs (CTL1–CTL5), and organic cation transporters.[34] Unfortunately, only limited data are available on choline transport into tumor cells.[35] There is also a lack of knowledge

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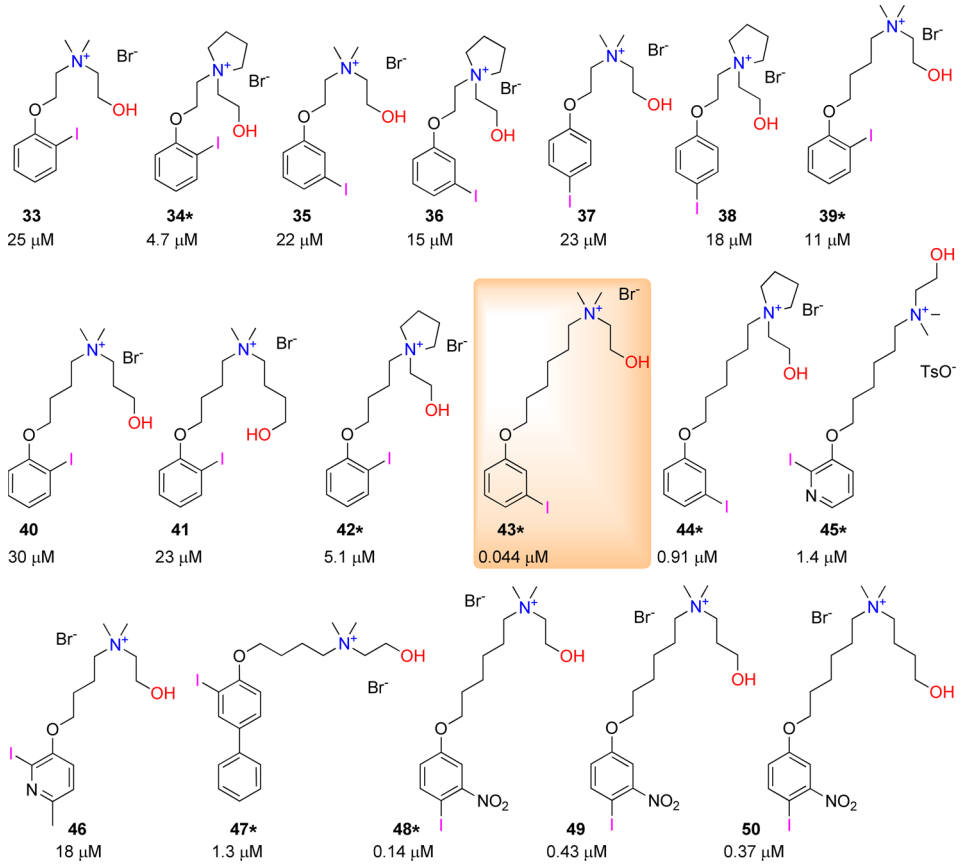


Figure 3. Choline analogues **33**–**50** and their IC_{50} values for [^{3}H]choline uptake by PC-3 cells (n = 3, for standard errors see Table 1); compounds selected for *in vivo* evaluation are marked with an asterisk.

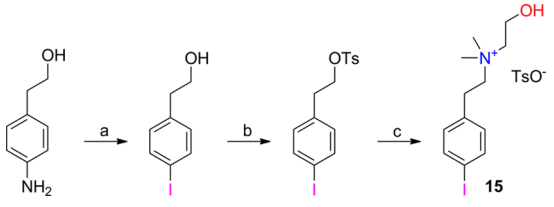
regarding the three-dimensional (3D) structure of these transporters, which hampers the rational design of radiotracers.

RESULTS AND DISCUSSION

Ligand Design and Synthesis. We focused on the synthesis of compounds with an iodine atom attached to an aromatic/heteroaromatic ring or a terminal alkyne to prevent undesirable radioiodide loss by nucleophilic substitution (Figures 2, 3). In cases where suitable iodinated starting compounds were not commercially available, the general synthetic strategy consisted of the following three fundamental steps: (i) the introduction of the iodine atom onto an appropriately hydroxyalkyl-substituted aromatic/heteroaromatic ring or terminal alkyne; (ii) substrate conversion into a sufficiently reactive alkylating agent; and (iii) quaternization of a tertiary amine (Schemes 1–3).

Less electron-rich iodinated aromatics were synthesized from the corresponding substituted anilines by a Sandmeyer reaction (Scheme 1, final compounds **15**–**19** and **48**–**50**).

Scheme 1. Synthesis of Compound 15: (a) $NaNO_2/H_2SO_4/H_2O$; NaI/H_2O; (b) $TsCl/Et_3N$//DCM; (c) DMAE



The iodination of electron-rich phenols was carried out using Barluenga's reagent[36,37] in the presence of trifluoroacetic acid (TFA, Scheme 2, compounds **20**–**25**), and the free phenol moiety was then alkylated with an appropriate alkyl iodide or methyl tosylate. The activated phenolic compounds were somewhat prone to multiple iodination with Barluenga's reagent, even when an excess of the substrate and a decreased reaction temperature were used.

The iodination of 2-thiopheneethanol with Barluenga's reagent led to more complex mixtures of products, which were challenging to separate. Therefore, we introduced the iodine atom onto the thiophene ring by ortho-lithiation, followed by iododemetallation (Scheme 2, final compounds **26**–**29**). An analogous procedure was also used for the synthesis of derivatives with iodinated triple bond (final compounds **30**–**32**). In accordance with a previously published study,[38] the intended direct transformation of the resulting iodinated *tert*-butyldimethylsilyl (TBS)-protected alcohols into tosylates by reaction with a 4-methylbenzene-1-sulfonyl fluoride (TsF) and a 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) catalyst did not occur at room temperature (rt). However, at reflux, contrary to the previously published results using a similar substrate,[38] the formed tosylate directly alkylated the DBU catalyst and hence could not be isolated (Scheme 2, step f).

Aryloxyalkyl and heteroaryloxyalkyl derivatives (final compounds **33**–**50**) of choline were prepared by a reaction of iodinated phenols with dibromoalkane yielding the desired alkylating agent for amine quaternization (Scheme 3).

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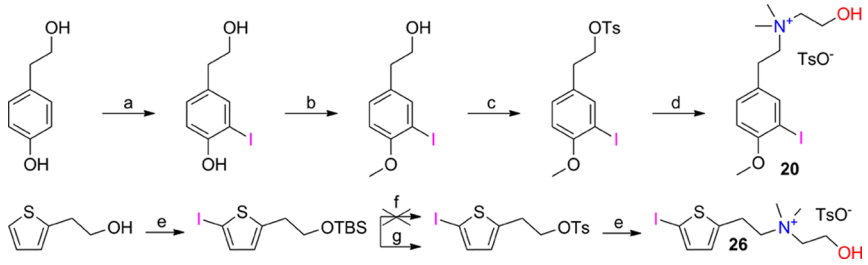
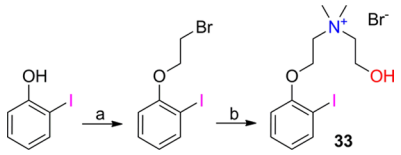
Scheme 2. Synthesis of Compounds 20 and 26: (a) $[I(py)_2][BF_4]$/TFA/ACN; (b) MeOTs/K_2CO_3/DMF; (c) TsCl/Et_3N/DMAP/DCM; (d) DMAE/DMF; (e) TBSCl/Imidazole/DMAP/DCM and *n*-BuLi/THF/I_2; (f) TsF/DBU/ACN; (g) TBAF/THF and TsCl/Et_3N/DMAP/DCM; and (h) DMAE/DMF



Scheme 3. Synthesis of Compound 33: (a) $Br(CH_2)_2Br$/K_2CO_3/DMF; (b) DMAE



After the successful conversion of iodinated intermediates into alkylating agents such as iodides, bromides, tosylates or mesylates, these agents were further used for the quaternization of corresponding tertiary amines. The quaternization reaction with bulky amines was occasionally complicated by an undesirable elimination side reaction, especially in the case of alkylating agents where the formation of a conjugated double bond by elimination was possible.

Affinity toward Choline Transporters. We evaluated the affinity of the individual derivatives toward choline transport systems by analyzing the ability of each compound to compete with the cellular uptake of [^{3}H]choline (IC_{50} value). The assay was performed *in vitro* on the PC-3 cell line (derived from human prostate carcinoma), which overexpresses choline transporters.[34] The IC_{50} values were determined for the 50 analogues of choline (Figures 2, 3) that were synthesized in this study and for hemicholinium-3 (HCh-3, ChT inhibitor, 36 ± 4 μM), FCh ([^{18}F]FCh used as tracer, 20 ± 5 μM) and choline (natural ligand, 4.7 ± 1.3 μM). The IC_{50} values we obtained for choline and HCh-3 were consistent with the previously published data.[39]

Analysis of the assay results of compounds **1**–**25** (Figure 2, Table 1) revealed that the binding constant of choline derivatives is typically improved by the attachment of more lipophilic substituents. This trend was noticeable, for example, according to the comparison of the IC_{50} values for compounds **20** and **23**–**25**. Compound **24**, which contained a hexyloxy substituent on the benzene ring, was the first nanomolar ligand prepared in this work and had an affinity 2 orders of magnitude higher than its methoxy analogue **23**. However, this effect could be assigned to the increased lipophilicity of compound **24** (Clog *P*,[40] see Table 2) resulting in similar values of lipophilic efficiency (for LipE and LipE plots of all analogues, see Table S4 and Figures S2 and S3, Supporting Information).

Table 2. Calculated log *P*[40] Values of Choline Analogues

cmpd.	log *P*	cmpd.	log *P*	cmpd.	log *P*	cmpd.	log *P*
1	−1.74	**14**	−1.93	**27**	−0.61	**40**	−0.37
2	−0.93	**15**	−1.31	**28**	−0.78	**41**	0.09
3	−1.11	**16**	−0.67	**29**	−0.33	**42**	−0.19
4	−0.77	**17**	−0.68	**30**	−1.54	**43**	0.09
5	−0.97	**18**	−0.54	**31**	−1.09	**44**	0.71
6	−1.10	**19**	−1.31	**32**	−0.46	**45**	−0.82
7	−2.34	**20**	−1.38	**33**	−1.73	**46**	−1.33
8	−1.93	**21**	−0.61	**34**	−1.10	**47**	0.84
9	−1.74	**22**	−0.94	**35**	−1.73	**48**	−0.84
10	−1.11	**23**	−0.92	**36**	−1.10	**49**	−0.38
11	−1.74	**24**	1.30	**37**	−1.73	**50**	0.07
12	−0.93	**25**	3.12	**38**	−1.10		
13	−1.11	**26**	−1.24	**39**	−0.82		

Table 1. IC_{50} ± SE (n = 3) of Choline Analogues for [^{3}H]Choline Uptake by PC-3 Cells

cmpd.	PC-3 [μM]	cmpd.	PC-3 [μM]	cmpd.	PC-3 [μM]	cmpd.	PC-3 [μM]
HCh-3	36 ± 4	**12**	77 ± 17	**26**	0.45 ± 0.12	**40**	30 ± 8
FCh	20 ± 5	**13**	16 ± 4	**27**	10 ± 2	**41**	23 ± 3
Ch	4.7 ± 1.3	**14**	273 ± 43	**28**	1.1 ± 0.3	**42**	5.1 ± 1.1
1	216 ± 38	**15**	8.6 ± 1.2	**29**	30 ± 8	**43**	0.044 ± 0.01
2	8.1 ± 0.4	**16**	106 ± 16	**30**	3.0 ± 0.3	**44**	0.91 ± 0.09
3	11 ± 5	**17**	19 ± 2	**31**	36 ± 4	**45**	1.4 ± 0.1
4	217 ± 25	**18**	75 ± 19	**32**	18 ± 6	**46**	18 ± 7
5	160 ± 22	**19**	1.2 ± 0.3	**33**	25 ± 6	**47**	1.3 ± 0.3
6	197 ± 32	**20**	24 ± 3	**34**	4.7 ± 2.1	**48**	0.14 ± 0.05
7	908 ± 251	**21**	115 ± 32	**35**	22 ± 4	**49**	1.1 ± 0.3
8	43 ± 5	**22**	53 ± 31	**36**	15 ± 2	**50**	0.37 ± 0.09
9	133 ± 20	**23**	34 ± 17	**37**	23 ± 3		
10	28 ± 5	**24**	0.41 ± 0.08	**38**	18 ± 7		
11	130 ± 16	**25**	2.1 ± 0.3	**39**	11 ± 2		

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Table 3. $IC_{50} \pm SE$ ($n = 3$) of Choline Analogues for [^{3}H]Choline Uptake by U87-MG Cells

cmpd.	U-87 [μM]	cmpd.	U-87 [μM]	cmpd.	U-87 [μM]	cmpd.	U-87 [μM]
2	4.3 ± 0.6	**10**	8.8 ± 2.6	**13**	23 ± 3	**24**	2.1 ± 0.8
3	3.3 ± 0.5	**11**	129 ± 28	**17**	29 ± 3	**25**	1.7 ± 1.1
9	25 ± 5	**12**	77 ± 23	**19**	0.78 ± 0.13		

Interestingly, the IC_{50} value of the compound **19** with a structure similar to compound **24** was comparable but its lipophilicity is significantly lower. We also observed that excessive steric hindrance and substituent rigidity at the quaternary nitrogen atom had a strong negative impact on affinity (*e.g.*, **2** and **3** *vs* **4**–**6**). Therefore, we proceeded with the synthesis of compounds derived only from 2-(dimethylamino)ethanol and 1-(2-hydroxyethyl)pyrrolidine.

We also prepared and analyzed compounds with bioisosteric iodinated thiophene rings (**26**–**29**), with less bulky iodinated triple bonds (**30**–**32**), and compounds with longer aliphatic spacers between the aromatic moiety and quaternary nitrogen. This synthetic effort yielded several other low-micromolar (**28**, **30**, **34**, **42**, **45**, **47**, **49**) and nanomolar ligands (**26**, **43**, **44**, **48**, **50**). Thiophene derivative **26** exhibited both excellent affinity as well as lipophilic efficiency. A surprising disparity in IC_{50} values was observed in the case of iodoalkyne choline analogues **30** and **31**, which differ only in one methylene group. The affinity of the shorter choline derivative **30** was 1 order of magnitude higher than in the case of compound **31**.

The optimal spacer length between the aromatic ring and quaternary nitrogen in the case of iodo(hetero)aryloxyalkyl derivatives (**33**–**50**) was approximately 7 atoms (Figures 2, 3). The use of a hexyloxy spacer between the choline moiety and the aromatic ring resulted in improved binding affinities as well as significantly higher lipophilic efficiencies compared to hexyloxy substituted compound **24** (compare **24** *vs* **43** and **48**). The highest affinity of all analyzed choline analogues was observed with compound **43**. An increase in chain length between the quaternary nitrogen and the hydroxyl group led to decreased binding affinities (**26** *vs* **28**–**29** and **48** *vs* **49**–**50**).

Twenty-eight iodinated derivatives exhibited better IC_{50} values than FCh, and 14 analogues had IC_{50} values that were better than the IC_{50} value determined for choline. The best achieved IC_{50} value (**43**) was 2 orders of magnitude less than that of choline.

To further support the idea of a more general applicability of our compounds, we performed the same cellular assay using a selection of choline derivatives on a glioblastoma cell line (U-87 MG), which is also known to overexpress choline transporters.[41] Indeed, the IC_{50} values obtained in the U-87 MG cells were similar to those obtained in the PC-3 cells (Tables 1 and 3).

Because the cellular uptake of [^{3}H]choline can also be influenced by the presence of toxic compounds, all derivatives were investigated for cytotoxicity. The determined cytotoxic concentrations of our choline analogues were all greater than 50 μM, except for compounds **24**, **25**, and **47**. However, for compounds **24**, **25**, and **47**, their corresponding IC_{50} values for [^{3}H]choline uptake remained well below the cytotoxic concentrations (Table S5, Supporting Information).

Pharmacokinetic Properties (Absorption, Distribution, Metabolism, and Excretion, ADME). The introduction of an iodine atom can potentially have a negative impact on the pharmacokinetic properties of small organic molecules because of increased molecular weight and lipophilicity. However, the increase in lipophilicity is typically moderate, and other parameters such as size, volume, or molecular surface area do not change dramatically.[42] To evaluate the *in vitro* pharmacokinetic properties of all investigated iodinated choline analogues, the stability in human plasma, binding to plasma proteins, microsomal stability, and permeability through artificial membrane (parallel artificial membrane permeability assay, PAMPA) were analyzed (Table S5, Supporting Information). Most of the iodinated analogues of choline demonstrated sufficient stability in human plasma after incubation for 2 h (70–90% of the original quantity of the substance remaining) and a quite high stability in hepatic microsomes (typically 70–90% of the original quantity of the substance remained after incubation for 1 h). Binding to plasma proteins primarily influences the distribution of the drug in the organism. Plasma protein binding (PPB) has shown intermediate values for most of the investigated analogues of choline (4–82% bound to plasmatic proteins). With respect to their ionic nature, the investigated compounds exhibited a low or medium ability to pass across the artificial membrane by passive diffusion (PAMPA). This is a desirable property that enables a specific distribution of these substances and further supports their potential use as radiopharmaceuticals (choline transporters are localized on the plasma membrane of cells). In general, the substances showed appropriate pharmacokinetic properties for both diagnostic and therapeutic applications (for plasma and microsomal stability of the compounds selected for *in vivo* experiments see Table 4).

Table 4. Plasma Stability (Pl.; % of Compound Remaining after 120 min Incubation) and Microsomal Stability (Mic.; % of Compound Remaining after 60 min Incubation) of Choline Analogues Selected for *In Vivo* Experiments[a]

cmpd.	Pl.	Mic.	cmpd.	Pl.	Mic.	cmpd.	Pl.	Mic.
2	85	100	**25**	85	82	**42**	91	70
3	81	74	**26**	89	86	**43**	83	85
10	66	90	**27**	86	86	**44**	91	79
17	98	96	**30**	89	95	**45**	86	86
19	80	100	**34**	81	78	**47**	90	89
24	60	93	**39**	91	54	**48**	89	92

[a]For complete data, see Table S5, Supporting Information.

Isotope Exchange Labeling. The introduction of an iodine radioisotope onto an aromatic ring can be accomplished by several routes.[33] A reliable, reproducible, and facile (if possible) labeling protocol is crucial for successful *in vivo* biodistribution experiments and for the prospective application of the intended radiopharmaceuticals in clinical practice. The two most common options employed in radioiodination are iododestannylation and isotope exchange labeling. The high inherent polarity of the choline moiety, unfortunately, renders normal-phase chromatography of stannylated choline precursors unfeasible, and employment of a reverse-phase high-performance liquid chromatography (HPLC) in acidic

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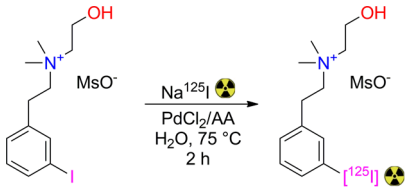
aqueous mobile phase would lead to *ipso* substitution of the stannyl group with a hydrogen atom.[43] Considering that we previously prepared a set of nonradioactive iodinated choline analogues containing natural iodine (^{127}I), the isotope exchange reaction seemed to be the most straightforward synthetic option. The disadvantage of this approach is a lower molar activity of the obtained radiolabeled product, which could potentially lead to a less optimal biodistribution profile.

Initially, we examined the feasibility of the isotope exchange reaction on model compound **23** at rt with the following four different catalysts: CuI, Pd/C, [$Pd(PPh_3)_4$], and a $PdCl_2$/ascorbic acid (AA) mixture (*in situ* formation of Pd-black). Interestingly, for compound **23**, there was almost no detectable radioiodinated product in the reaction mixtures except for the $PdCl_2$/AA catalytic system, where significant conversion was observed.

After further optimization of the isotope exchange reaction with the $PdCl_2$/AA catalyst, we achieved up to 75% radiochemical yield in short reaction time depending on the labeled substrate. To eliminate the possible competitive interference of halide counterions in quaternary ammonium salts during the radioiodination process, all compounds initially prepared as halide salts were first transformed into corresponding tosylate salts prior to the isotope exchange reaction; compounds prepared as mesylates were used directly. The achieved molar activities of the labeled products were approximately 2 PBq·mol^{-1} (2 GBq·μmol^{-1}), and the radiochemical purity was ≥95%.

The optimized labeling protocol (Scheme 4) requires relatively short reaction times, takes place in aqueous

Scheme 4. Isotope Exchange Reaction with 19 and Na^{125}I



19

[^{125}I]-19
Radiochem. yield 75 %
Mol. act. ≈ 2 GBq · µmol^{-1}

environment under mild conditions, and is applicable to almost all compounds presented in this study. Purification of the radiolabeled product can be performed by the simple removal of free radioiodide by an ion-exchange resin and silver metal. This facile labeling protocol could enable the future manufacturing of radiopharmaceutical kits for the convenient synthesis of radiopharmaceuticals in the clinic immediately prior to their administration to a patient. The achieved molar activity of the compounds obtained by isotope exchange is approximately 1 order of magnitude higher than that in the case of the standard [^{123}I]MIBG used in the clinics but 1 order of magnitude lower than that in the case of the newer high-specific-activity [^{123}I]MIBG-HSA[44] or [^{18}F]FCh.[45,46]

The ligands that could not be labeled using the $PdCl_2$/AA catalyst include thiophene or pyridine derivatives (Pd-poisoning), nitro-derivatives (substrate reduction), or iodinated terminal alkynes (decomposition observed). The labeling of these compounds was performed with $CuCl_2$ as a catalyst at 90 °C in *N*,*N*-dimethylformamide (DMF). However, the radiochemical yields achieved with the $CuCl_2$ catalyst were rather low, and the reaction time could not be further increased because of the formation of radioactive side products.

Biodistribution. To verify the potential of the iodinated choline analogues as imaging agents, we performed an initial study of the *ex vivo* biodistribution of 18 compounds in human prostate carcinoma-bearing (PC-3) xenograft mice. The ligands were radiolabeled with ^{125}I using the abovementioned protocol, and 100 kBq of the labeled compound was intravenously administered to each mouse in a group of six mice for each ligand. The commercially available [^{18}F]FCh served as a control. The biodistribution was determined at 1 h postinjection (p.i.) in three animals and at 3 h p.i. in three other animals for each compound as well as for the control group (n = 3) (Figure 4A,B, for the complete data, see Tables S6−S26 and Figures S4−S22, Supporting Information).

With the exception of compounds **26**, **27**, **30**, and **48**, the radioactivity accumulated in the thyroid was below 1% of the injected dose for all analyzed iodinated choline analogues (see Table S26, Supporting Information). This indicates a negligible release of free radioiodide and sufficient stabilities *in vivo*. None of the ^{125}I-labeled compounds were able to achieve a better tumor-to-blood uptake ratio than [^{18}F]FCh at 1 h p.i. However, compounds **10** and **43** had similar or higher tumor-to-blood and tumor-to-muscle uptake ratios than [^{18}F]FCh at 3 h p.i. and substantially superior tumor-to-bone uptake ratios (Figure 4C). The absolute value of uptake of **43** in the tumor was slightly lower compared with [^{18}F]FCh, but the tumor-to-blood, tumor-to-muscle, and tumor-to-bone ratios should allow a sufficient image contrast to visualize the tumor.

The compounds **10** and **43** also manifested very low kidney uptake compared with [^{18}F]FCh (Figure 4D). Thus, our results are encouraging because the main disadvantages of the [^{18}F]FCh imaging agent lie in its high accumulation in the bladder and kidneys, which results in diminished image quality and unfavorable lesion detection sensitivity in close proximity to the genitourinary system, and the high radiation dose absorbed by kidneys increases the stochastic risk of such examinations.[22] Because of the propensity of prostate cancer cells to migrate into the axial skeleton,[47] early detection of bone metastases is very important. The increased tumor-to-bone uptake ratios with the reported iodinated choline analogues compared with [^{18}F]FCh could lead to improved sensitivity and specificity for the detection of bone metastases in prostate cancer patients.

In the cases of compounds that generally did not perform favorably in comparison with [^{18}F]FCh, we observed a significant decrease in tissue uptake between 1 and 3 h p.i. This phenomenon could be caused by a gradual washout of the nonspecifically bound fraction of the tracer. Interestingly, even the analogues with a poor biodistribution all exhibited better tumor-to-bone uptake ratios than [^{18}F]FCh.

***In Vivo* Imaging.** To provide further evidence that our compounds could serve as a better alternative to [^{18}F]FCh, we performed a set of *in vivo* imaging experiments with prostate carcinoma-bearing (PC-3) xenograft mice. The ligands **10** and **43** were radiolabeled with ^{123}I using the herein described isotope exchange protocol, and 15−20 MBq (molar activity ≥ 2.5 GBq·μmol^{-1}) of the corresponding labeled compound was administered intravenously. The SPECT images were acquired at 3 h p.i. (see Figure S23A,B, Supporting Information). Unfortunately, neither of the two compounds [^{123}I]-**10** and [^{123}I]-**43** was able to visualize the tumor. Initially, we ascribed the decreased tumor accumulation of compounds [^{123}I]-**10**

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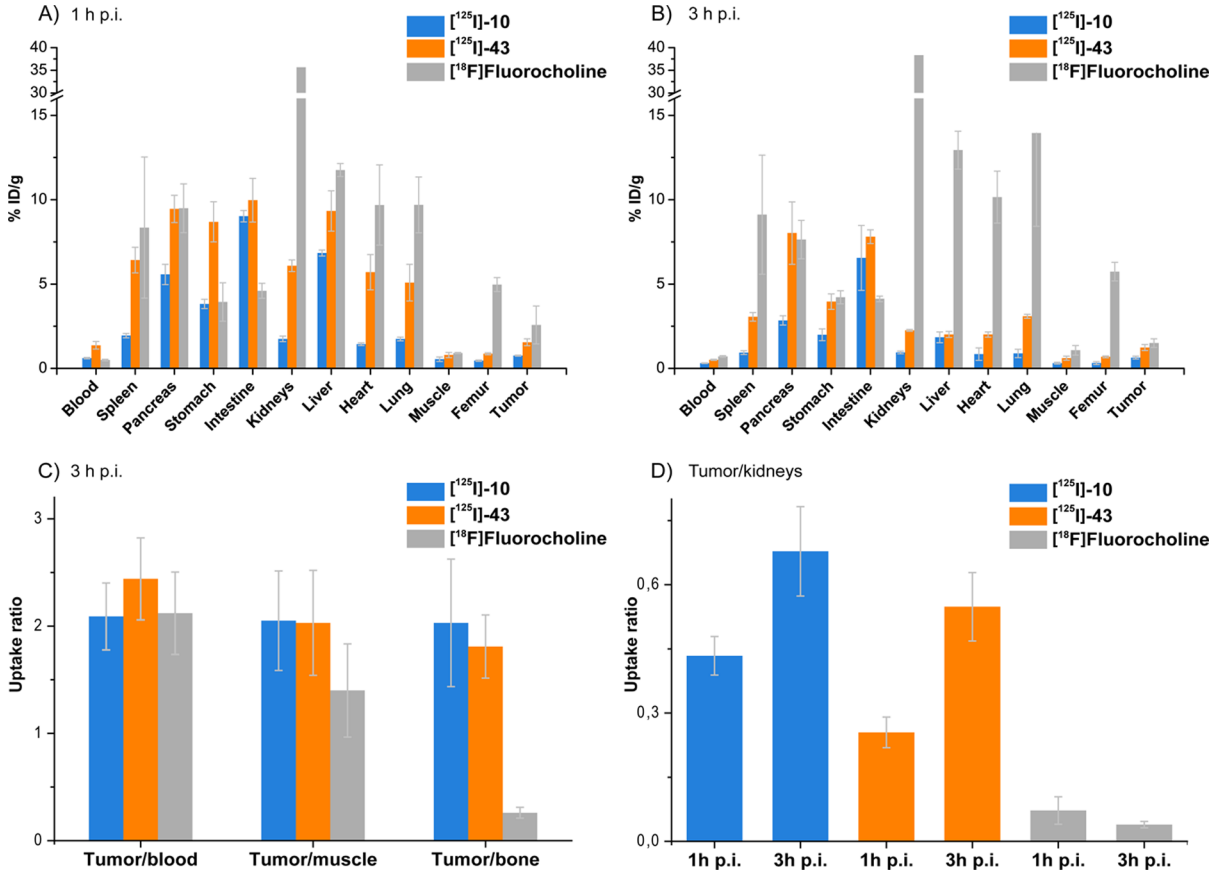
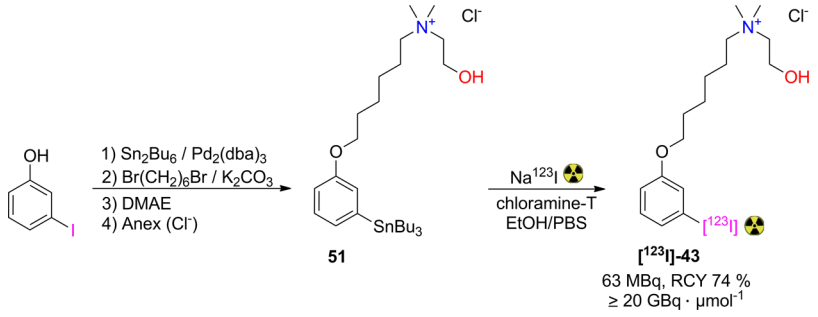


Figure 4. *Ex vivo* biodistribution at 1 (A) and 3 h p.i. (B) and the corresponding tumor-to-blood, tumor-to-muscle, and tumor-to-bone uptake ratios at 3 h p.i. (C); tumor-to-kidney uptake ratios at 1 and 3 h p.i. (D) for ^{125}I-labeled compound **10**, compound **43**, and [^{18}F]FCh in PC-3 tumor-bearing mice.

Scheme 5. Synthesis of the Stannylated Precursor 51 and Its Labeling with ^{123}I by Iododestannylation Yielding High-Molar-Activity [^{123}I]-43-HSA: (1) $Sn_2Bu_6/Pd_2(dba)_3$/DIPEA/DMF/1,4-Dioxane; (2) $Br(CH_2)_2Br/K_2CO_3$/DMF; (3) DMAE/DMF; (4) Amberlite IRA-900 (Chloride Form)/MeOH



and [^{123}I]-**43** in our SPECT experiments to the lower molar activity of [^{123}I]-**10** and [^{123}I]-**43** prepared by isotopic exchange labeling. Mediocre molar activity of [^{123}I]-**10** and [^{123}I]-**43** in combination with the necessity to administer 2 orders of magnitude higher dose of the tracer than in the case of *ex vivo* biodistribution experiments (100 kBq of ^{125}I-labeled *vs* 15−20 MBq of ^{123}I-labeled compounds possessing almost the same molar activity) could potentially lead to low tumor uptake.

For this reason, we have decided to synthesize high-molar-activity (high-specific-activity) [^{123}I]-**43-HSA** (Scheme 5) with a comparable molar activity as the clinically used [^{18}F]FCh. Nucleophilic [^{18}F]F$^-$ used for the synthesis of [^{18}F]FCh is produced with molar activity in the 10^2 GBq·μmol^{-1} range,[48,49] and the specific activity of [^{18}F]FCh used in the clinics falls between 10^1 and 10^2 GBq·μmol^{-1}.[45,46] The [^{123}I]-**43-HSA** prepared by iododestannylation possessed molar activity ≥20 GBq·μmol^{-1}. However, to our surprise, not even [^{123}I]-**43-HSA** was able to visualize the tumor in PC-3 xenograft mice at 3 h p.i. (Figure S23C, Supporting Information), showing high accumulation of activity only in the gastrointestinal tract and liver. Immediately after the SPECT imaging with [^{123}I]-**43-HSA**, the mice were sacrificed, and we performed additional determination of the tracer biodistribution *ex vivo*. The biodistribution data were similar to our previous results obtained after injecting 100 kBq of [^{125}I]-**43** with the

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significant difference that the activity accumulated in the tumor was 54% lower and the activity accumulated in the gastrointestinal tract was higher.

These findings led us to a hypothesis that the tumor uptake of iodinated choline derivatives in the PC-3 murine model becomes saturated at doses necessary for imaging even with high-molar-activity compounds, resulting in poor accumulation of activity in the tumor. Therefore, we performed also PET imaging in PC-3 xenograft mice using commercially available radiopharmaceutical-grade [^{18}F]FCh. The mice were injected with [^{18}F]FCh at a dose of 5−7 MBq per animal and the acquisition of PET scans was performed both at 1 and 3 h p.i. as [^{18}F]FCh exhibited better tumor-to-organ ratios at 1 h p.i. in our previous *ex vivo* biodistribution experiments. Surprisingly, we could not achieve tumor visualization even with [^{18}F]FCh, and we observed significant signals only in the kidneys and liver (Figure S23D,E, Supporting Information).

The [^{18}F]FCh tracer is routinely used in the clinics as a successful tool for cancer imaging. However, to the best of our knowledge, despite copious amount of *in vitro* data in PC-3 cells and *ex vivo* biodistribution studies, there is no report in the literature of a satisfactory tumor visualization in murine PC-3 xenograft model using [^{18}F]FCh.[50−52] This strongly suggests, that in murine PC-3 xenograft model, the choline transport mechanisms become saturated at doses necessary for imaging even with high-molar-activity tracers routinely used in humans. The imaging data in the literature as well as our SPECT and PET experiments lead us to a conclusion that a bigger animal model is necessary for successful *in vivo* imaging with tracers targeting choline transport proteins. Murine PC-3 xenograft was originally employed for *ex vivo* studies in the development of the [^{18}F]FCh and similar tracers. Despite our failed imaging attempts with both commercial [^{18}F]FCh and the new iodinated choline analogues using this model, the presented *ex vivo* data indicate that iodinated choline derivatives could present a superior alternative to [^{18}F]FCh.

CONCLUSIONS

In summary, 28 iodinated derivatives from this study exhibited better IC_{50} values than FCh and 14 derivatives had IC_{50} values that were even better than the IC_{50} value determined for the natural ligand (choline). Six compounds from this study are nanomolar inhibitors, and the best analogue (compound **43**) surpassed the affinity of choline by 2 orders of magnitude.

We successfully developed a straightforward radiolabeling protocol that can be used for the preparation of SPECT and PET tracers and potentially theranostic radiopharmaceuticals depending on the selection of the particular iodine isotope. In contrast to [^{18}F]FCh, radiopharmaceuticals based on our compounds could be distributed from a central production site to more-remote medical facilities (longer half-lives). Furthermore, our radiolabeling procedure also allows for the manufacture of kits for the synthesis of these radiopharmaceuticals directly in the clinic. The molar activity of iodinated compounds prepared by isotope exchange labeling was on the order of ≈2 GBq·μmol^{-1}, which is significantly lower than the molar activity of ^{18}F-based tracers prepared using nucleophilic substitution with [^{18}F]F$^-$. However, the molar activity we achieved with isotope exchange labeling is at least 1 order of magnitude higher than that in the case of tracers prepared by electrophilic fluorination with [^{18}F]F_2 (*e.g.*, [^{18}F]FDOPA) used in the clinics.[53,54] We have also demonstrated that synthesis of high-molar-activity labeled choline derivatives (≥20 GBq·μmol^{-1}) by iododestannylation is possible.

The *ex vivo* biodistribution study in human prostate carcinoma-bearing mice with 18 radiolabeled derivatives revealed two promising compounds with a biodistribution profile superior to that of [^{18}F]FCh, which is routinely used for imaging in patients with prostate carcinoma. However, it is important to note that using microPET/microSPECT imaging techniques neither the commercial [^{18}F]FCh nor [^{123}**I**]**-43-HSA** could visualize the tumor in the murine PC-3 xenograft. This xenograft model originally employed in *ex vivo* studies during the development of [^{18}F]FCh and similar tracers appears to be unsuitable for imaging experiments. Therefore, the potential of iodinated choline analogues as tracers should be further validated in a bigger animal model.

EXPERIMENTAL SECTION

Materials and General Procedures. Bis(pyridine)iodonium tetrafluoroborate was prepared according to a described procedure.[37] Solvents were purchased from Lach-Ner Ltd. (Neratovice, Czech Republic) and were of analytical grade. All chemicals and dry solvents were purchased from Sigma-Aldrich Ltd. (Prague, Czech Republic). The radioactive sodium iodides Na^{125}I and Na^{123}I were purchased from MGP Ltd. (Zlín, Czech Republic).

The reactions were monitored by thin-layer chromatography (TLC) unless stated otherwise. Column chromatography was carried out using silica gel (70−230 mesh). Flash chromatography was performed using a PuriFlash XS 420 (Interchim, France) system and spherical silica gel (40−75 μm). TLC plates (silica gel 60 F254 or silica gel-RP18 60 F254) were visualized under UV and/or with $KMnO_4$/Na_2CO_3. The purity of all final compounds (**1**−**50**) was >95% as determined by HPLC-UV with detection at 225 nm (for retention times and purity, see also Table S1, Supporting Information).

Synthesis of Choline Derivatives (1−50). *General Procedure for the Synthesis of Iodinated Choline Analogues. Procedure A.* A pear-shaped flask was charged with a magnetic stir bar, the corresponding alkylating agent, DMF, and the corresponding tertiary amine. The flask was closed with a rubber septum, flushed with argon, immersed into a heated oil bath, and the reaction mixture was stirred. The reaction progress was checked by a TLC analysis. When either conversion of the alkylating agent was complete or formation of elimination product became apparent, the heating was stopped, and the solution was concentrated *in vacuo* (oily products optionally coevaporated with toluene in order to remove residual DMF). The residue was redissolved in a minimum amount of MeOH, transferred into a centrifugation tube, precipitated with 1:1 diethyl ether/hexane mixture (45 mL), and the suspension was centrifuged and decanted. Then, the residue was redissolved in MeOH, precipitated with diethyl ether (40 mL), centrifuged, and decanted. The solid product was transferred with a spatula into a glass vial and dried under high vacuum. Some of the products were further subjected to anion exchange in order to remove halogenide counterions, which could interfere with isotope exchange radioiodination. This was done by passing the MeOH solution of the compound through a column containing Amberlite IRA-900 anion-exchange resin in TsO$^-$ cycle (15 mL of resin per 1 mmol of choline analogue).

(2-Hydroxyethyl)-(2-iodobenzyl)-dimethylammonium 4-Methylbenzenesulfonate (1). General procedure A was used. 2-Iodobenzyl iodide (400 mg, 1.16 mmol, 1 equiv), DMF (5.0 mL), and 2-(dimethylamino)ethanol (351 μL, 3.49 mmol, 3 equiv) were used for the synthesis of compound **1**. The reaction time was 2 h at a temperature of 50 °C. The reaction mixture was concentrated *in vacuo* and coevaporated with toluene (3×). The crude product was precipitated from a MeOH solution once with a mixture of Et_2O and hexane (2:1 v/v, 45 mL) and once with pure Et_2O (45 mL). The product was converted to 4-methylbenzenesulfonate salt by ion exchange, and (2-hydroxyethyl)-(2-iodobenzyl)-dimethylammonium

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4-methylbenzenesulfonate (**1**) was obtained as a colorless viscous liquid (500 mg, 1.05 mmol, 90%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.08 (dd, J = 8.0, 1.2 Hz, 1H), 7.72 (dd, J = 7.7, 1.6 Hz, 1H), 7.55 (ddd, J = 7.5, 7.5, 1.3 Hz, 1H), 7.48 (d, J = 8.0 Hz, 2H), 7.27 (ddd, J = 7.7, 7.7, 1.6 Hz, 1H), 7.11 (d, J = 7.8 Hz, 2H), 5.41 (br s, 1H), 4.72 (s, 2H), 3.97−3.91 (m, 2H), 3.56−3.53 (m, 2H), 3.09 (s, 6H), 2.28 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 145.71, 140.97, 137.64, 134.94, 132.28, 131.16, 128.82, 128.09, 125.50, 105.11, 70.48, 66.54, 55.15, 50.31, 20.82. HRMS (ESI) m/z: [M$^+$] ($C_{11}H_{17}INO^+$) calcd, 306.034934; found, 306.03521. HPLC-UV purity 98.5%.

*Diethyl-(2-hydroxyethyl)-(2-iodobenzyl)ammonium 4-Methylbenzenesulfonate (**2**).* General procedure A was used. 2-Iodobenzyl iodide (400 mg, 1.16 mmol, 1 equiv), DMF (5.0 mL), and 2-(diethylamino)ethanol (463 μL, 3.49 mmol, 3 equiv) were used for the synthesis of compound **2**. The reaction time was 2 h at a temperature of 50 °C. The reaction mixture was concentrated *in vacuo* and coevaporated with toluene (3×). The crude product was precipitated from a MeOH solution once with a mixture of Et_2O and hexane (2:1 v/v, 45 mL) and once with pure Et_2O (45 mL). The product was converted to 4-methylbenzenesulfonate salt by ion exchange. Compound **2** was obtained as a colorless oil, which crystallized upon cooling (477 mg, 0.94 mmol, 81%). mp 103−105 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.07 (dd, J = 7.9, 1.3 Hz, 1H), 7.67 (dd, J = 7.8, 1.4 Hz, 1H), 7.55 (ddd, J = 7.6, 7.6, 1.3 Hz, 1H), 7.48 (d, J = 8.0 Hz, 2H), 7.26 (ddd, J = 7.7, 7.7, 1.6 Hz, 1H), 7.11 (d, J = 7.9 Hz, 2H), 5.44 (br s, 1H), 4.72 (s, 2H), 3.87 (br s, 2H), 3.47−3.37 (m, 6H), 2.28 (s, 3H), 1.28 (t, J = 7.1 Hz, 6H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 145.71, 140.92, 137.63, 134.11, 132.17, 131.26, 129.07, 128.08, 125.49, 105.51, 65.31, 59.38, 54.76, 54.32, 20.81, 8.18. HRMS (ESI) m/z: [M$^+$] ($C_{13}H_{21}INO^+$) calcd, 334.066234; found, 334.06648. Elem. Anal. $C_{20}H_{28}INO_4S$, Calcd: C (47.53), H (5.58), N (2.77), I (25.11), S (6.34). Found: C (47.42), H (5.60), N (2.74), I (25.01), S (6.51). HPLC-UV purity 99.2%.

*1-(2-Hydroxyethyl)-1-(4-iodobenzyl)pyrrolidin-1-ium 4-Methylbenzenesulfonate (**3**).* General procedure A was used. 2-Iodobenzyl iodide (400 mg, 1.16 mmol, 1 equiv), DMF (5.0 mL), and 2-(diethylamino)ethanol (463 μL, 3.49 mmol, 3 equiv) were used for the synthesis of compound **3**. The reaction time was 2 h at a temperature of 50 °C. The reaction mixture was concentrated *in vacuo* and coevaporated with toluene (3×). The crude product was precipitated from a MeOH solution once with a mixture of Et_2O and hexane (2:1 v/v, 45 mL) and once with pure Et_2O (45 mL). The product was converted to 4-methylbenzenesulfonate salt by ion exchange. Compound **3** was obtained as a pale-yellow oil, which crystallized upon cooling (453 mg, 0.90 mmol, 78%). mp 97−99 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.07 (dd, J = 8.0, 1.2 Hz, 1H), 7.74 (dd, J = 7.8, 1.6 Hz, 1H), 7.54 (ddd, J = 7.5, 7.5, 1.3 Hz, 1H), 7.48 (d, J = 7.9 Hz, 2H), 7.26 (ddd, J = 7.7, 7.7, 1.7 Hz, 1H), 7.11 (d, J = 7.8 Hz, 2H), 5.47 (t, J = 4.4 Hz, 1H), 4.80 (s, 2H), 3.99−3.93 (m, 2H), 3.83−3.63 (m, 2H), 3.54−3.45 (m, 2H), 3.44−3.29 (m, 2H), 2.28 (s, 3H), 2.14−1.87 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 145.69, 140.81, 137.63, 134.80, 132.16, 131.57, 128.94, 128.07, 125.48, 105.48, 66.43, 62.17, 60.26, 55.70, 22.41, 20.80. HRMS (ESI) m/z: [M$^+$] ($C_{13}H_{19}INO^+$) calcd, 332.050584; found, 332.05093. Elem. Anal. $C_{20}H_{26}INO_4S$, Calcd: C (47.72), H (5.21), N (2.78), S (6.37). Found: C (47.68), H (5.21), N (2.78), S (6.31). HPLC-UV purity 98.8%.

*1-(2-Hydroxyethyl)-1-(2-iodobenzyl)piperidin-1-ium 4-Methylbenzenesulfonate (**4**).* 2-Iodobenzyl chloride (600 mg, 2.38 mmol, 1 equiv) and 1-(2-hydroxyethyl)piperidine (4 mL) were stirred under argon in a pear-shaped flask for 16 h at a temperature of 80 °C. The reaction mixture was concentrated *in vacuo* and coevaporated with toluene (3×). The crude product was dissolved in water (50 mL), and excess amine was removed by extraction with EtOAc (3 × 40 mL). The product was converted to 4-methylbenzenesulfonate salt by ion exchange. Compound **4** was obtained as a brown viscous liquid, which crystallized upon cooling (938 mg, 1.81 mmol, 76%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.08 (dd, J = 8.0, 1.3 Hz, 1H), 7.68 (dd, J = 7.8, 1.6 Hz, 1H), 7.54 (ddd, J = 7.5, 7.5, 1.3 Hz, 1H), 7.49 (d, J = 8.1 Hz, 2H), 7.26 (ddd, J = 7.8, 7.8, 1.5 Hz, 1H), 7.11 (d, J = 7.9 Hz, 2H), 5.48 (t, J = 4.7 Hz, 1H), 4.80 (s, 2H), 4.08−3.87 (m, 2H), 3.73−3.58 (m, 4H), 3.19−3.07 (m, 2H), 2.28 (s, 3H), 2.05−1.83 (m, 2H), 1.82−1.67 (m, 2H), 1.65−1.47 (m, 1H), 1.40−1.17 (m, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 145.66, 140.99, 137.65, 135.25, 132.16, 130.87, 128.79, 128.07, 125.48, 105.67, 69.34, 58.65, 57.77, 54.94, 20.80, 20.09, 19.29. HRMS (ESI) m/z: [M$^+$] ($C_{14}H_{21}INO^+$) calcd, 346.066234; found, 346.06628. Elem. Anal. $C_{21}H_{28}INO_4S$, Calcd: C (48.75), H (5.45), N (2.71), S (6.20). Found: C (49.07), H (5.58), N (2.62), S (6.28). HPLC-UV purity 99.7%.

*3-Hydroxy-1-(2-iodobenzyl)quinuclidin-1-ium 4-Methylbenzenesulfonate (**5**).* General procedure A was used. 2-Iodobenzyl iodide (400 mg, 1.16 mmol, 1 equiv), DMF (5.0 mL), and 3-quinuclidinol (444 mg, 3.49 mmol, 3 equiv) were used for the synthesis of compound **5**. The reaction time was 2 h at a temperature of 50 °C. The reaction mixture was concentrated *in vacuo* and coevaporated with toluene (3×). The crude product was precipitated from a MeOH solution once with a mixture of Et_2O and hexane (2:1 v/v, 45 mL) and once with pure Et_2O (45 mL). The product was converted to 4-methylbenzenesulfonate salt by ion exchange. Compound **5** was obtained as a white solid (496 mg, 0.96 mmol, 83%). mp 154−156 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.07 (dd, J = 8.0, 1.2 Hz, 1H), 7.66 (dd, J = 7.7, 1.6 Hz, 1H), 7.54 (ddd, J = 7.5, 7.5, 1.3 Hz, 1H), 7.48 (d, J = 8.1 Hz, 2H), 7.25 (ddd, J = 7.7, 7.7, 1.6 Hz, 1H), 7.11 (d, J = 7.9 Hz, 2H), 5.60 (s, 1H), 4.59 (d, J = 13.5 Hz, 1H), 4.54 (d, J = 13.5 Hz, 1H), 4.08−4.02 (m, 1H), 3.74 (ddd, J = 12.7, 8.3, 3.0 Hz, 1H), 3.51 (tt, J = 11.2, 3.8 Hz, 1H), 3.47−3.32 (m, 3H), 3.14 (dt, J = 12.7, 2.8 Hz, 1H), 2.28 (s, 3H), 2.15−2.07 (m, 1H), 2.03−1.99 (m, 1H), 1.94−1.87 (m, 1H), 1.80−1.70 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 145.73, 140.90, 137.63, 135.04, 132.17, 130.73, 128.78, 128.08, 125.50, 105.03, 69.40, 63.31, 63.21, 54.68, 53.51, 26.39, 20.97, 20.82, 17.47. HRMS (ESI) m/z: [M$^+$] ($C_{14}H_{19}INO^+$) calcd, 344.050584; found, 344.05065. Elem. Anal. $C_{21}H_{26}INO_4S$, Calcd: C (48.94), H (5.08), N (2.72), I (24.62), S (6.22). Found: C (48.64), H (5.15), N (2.59), I (24.21), S (6.11). HPLC-UV purity 98.0%.

*3-Hydroxy-1-(2-iodobenzyl)-1-methylpiperidin-1-ium 4-Methylbenzenesulfonate (**6**).* General procedure A was used. 2-Iodobenzyl iodide (400 mg, 1.16 mmol, 1 equiv), DMF (5.0 mL), and 3-hydroxy-1-methylpiperidine (402 μL, 3.49 mmol, 3 equiv) were used for the synthesis of compound **6**. The reaction time was 2 h at a temperature of 50 °C. The reaction mixture was concentrated *in vacuo* and coevaporated with toluene (3×). The crude product was precipitated from a MeOH solution once with a mixture of Et_2O and hexane (2:1 v/v, 45 mL) and once with pure Et_2O (45 mL). The product was converted to 4-methylbenzenesulfonate salt by ion exchange. Compound **6** was obtained as a mixture of two diastereomers (2:1) in the form of an off-white hygroscopic solid (424 mg, 0.84 mmol, 73%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.08 (dd, J = 8.0, 1.3 Hz, 1H), 7.72 (dd, J = 7.8, 1.7 Hz, 0.67H), 7.65 (dd, J = 7.7, 1.7 Hz, 0.33H), 7.58−7.52 (m, 1H), 7.49 (d, J = 8.0 Hz, 2H), 7.27 (ddd, J = 7.7, 7.7, 1.7 Hz, 1H), 7.11 (d, J = 7.9 Hz, 2H), 5.50 (s, 1H), 4.84−4.58 (m, 2H), 4.22−4.14 (m, 0.33H), 4.05−3.93 (m, 0.67H), 3.57−3.01 (m, 4H), 3.14 (s, 1H), 3.04 (s, 2H), 2.29 (s, 3H), 2.11−1.69 (m, 3H), 1.68−1.52 (m, 0.33H), 1.41−1.22 (m, 0.67H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 145.71, 141.03, 141.03, 137.64, 135.17, 134.86, 132.27, 132.25, 130.77, 130.72, 128.79, 128.74, 128.07, 125.49, 105.15, 105.11, 72.20, 70.77, 63.78, 63.55, 61.95, 61.00, 60.46, 59.78, 49.24, 46.66, 30.51, 28.51, 20.79, 17.77, 16.31. HRMS (ESI) m/z: [M$^+$] ($C_{13}H_{19}INO^+$) calcd, 332.050584; found, 332.05079. Elem. Anal. $C_{20}H_{26}INO_4S{\cdot}0.5H_2O$, Calcd: C (46.88), H (5.31), N (2.73), I (24.77), S (6.26). Found: C (47.13), H (5.36), N (2.78), I (24.67). HPLC-UV purity 98.0%.

*1,4-Bis(2-hydroxyethyl)-1-(2-iodobenzyl)piperazin-1-ium 4-Methylbenzenesulfonate (**7**).* General procedure A was used. 2-Iodobenzyl iodide (400 mg, 1.16 mmol, 1 equiv), DMF (5.0 mL), and 1,4-bis(2-hydroxyethyl)piperazine (608 mg, 3.49 mmol, 3 equiv) were used for the synthesis of compound **7**. The reaction time was 2 h at a temperature of 50 °C. The reaction mixture was concentrated *in vacuo* and coevaporated with toluene (3×). The crude product was precipitated from a MeOH solution once with a mixture of Et_2O and hexane (2:1 v/v, 45 mL) and once with pure Et_2O (45 mL). The

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product was converted to 4-methylbenzenesulfonate salt by ion exchange. Compound **7** was obtained as a white amorphous hygroscopic solid (502 mg, 0.89 mmol, 77%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.09 (dd, J = 8.0, 1.2 Hz, 1H), 7.71 (dd, J = 7.7, 1.6 Hz, 1H), 7.56 (ddd, J = 7.6, 7.6, 1.2 Hz, 1H), 7.49 (d, J = 8.0 Hz, 2H), 7.28 (ddd, J = 7.7, 7.7, 1.6 Hz, 1H), 7.12 (d, J = 7.9 Hz, 2H), 5.52 (t, J = 4.8 Hz, 1H), 4.86 (s, 2H), 4.49 (br s, 1H), 4.03−3.99 (m, 2H), 3.77−3.65 (m, 4H), 3.44 (q, J = 5.7 Hz, 2H), 3.24 (t, J = 10.7 Hz, 2H), 2.96−2.86 (m, 2H), 2.82−2.65 (m, 2H), 2.46−2.41 (m, 2H), 2.28 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 145.64, 141.06, 137.70, 135.55, 132.33, 130.64, 128.85, 128.11, 125.50, 105.87, 69.36, 58.71, 58.47, 58.27, 56.81, 54.94, 46.05, 20.82. HRMS (ESI) m/z: [M$^+$] ($C_{15}H_{24}IN_2O_2^+$) calcd, 391.087697; found, 391.08771. HPLC-UV purity 98.0%.

*4-(2-Hydroxyethyl)-4-(2-iodobenzyl)morpholin-4-ium Bromide (**8**).* 2-Iodobenzyl bromide (400 mg, 1.35 mmol, 1 equiv) and 4-(2-hydroxyethyl)morpholine (2.0 mL, 16.5 mmol, 13.9 equiv) were stirred in a pear-shaped flask at 75 °C for 4 h. The reaction mixture was diluted with MeOH, transferred into a centrifugation tube, and mixed with Et_2O/hexane (1:1, 40 mL) (oily product formation). The tube was centrifuged, and the solvent was decanted from the product. The product was then dissolved in MeOH (5 mL) and precipitated with Et_2O (40 mL). This was repeated two times. The product **8** was dried under high vacuum yielding an off-white solid (509 mg, 1.19 mmol, 88%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.10 (dd, J = 8.0, 1.3 Hz, 1H), 7.74 (dd, J = 7.8, 1.7 Hz, 1H), 7.57 (ddd, J = 7.8, 7.4, 1.3 Hz, 1H), 7.29 (ddd, J = 8.0, 7.4, 1.7 Hz, 1H), 5.50 (t, J = 4.8 Hz, 1H), 4.94 (s, 2H), 4.09−3.99 (m, 4H), 3.95−3.88 (m, 2H), 3.88−3.83 (m, 2H) 3.74−3.65 (m, 2H), 3.39−3.30 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 141.03, 135.51, 132.34, 130.42, 128.80, 106.10, 69.76, 59.91, 58.67, 56.50, 55.10. HPLC-UV purity 99.0%.

*(2-Hydroxyethyl)-(3-iodobenzyl)-dimethylammonium Methanesulfonate (**9**).* General procedure A was used. The alkylating agent **A** (200 mg, 0.67 mmol, 1 equiv, see the Supporting Information), DMF (1.5 mL), and 2-(dimethylamino)ethanol (134 μL, 1.33 mmol, 2 equiv) were used for the synthesis of compound **9**. The reaction time was 1 h at a temperature of 80 °C. The product was precipitated once from a dichloromethane (DCM) solution with a mixture of Et_2O and hexane (2:1 v/v, 45 mL) and once from MeOH solution by a mixture of Et_2O and hexane (2:1 v/v, 45 mL). The product was obtained in as a colorless viscous liquid (259 mg, 0.67 mmol, 100%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.99 (dd, J = 1.8, 1.7 Hz, 1H), 7.91 (ddd, J = 7.9, 1.8, 1.1 Hz, 1H), 7.60 (ddd, J = 7.7, 1.7, 1.1 Hz, 1H), 7.32 (dd, J = 7.9, 7.7 Hz, 1H), 5.36 (t, J = 4.9 Hz, 1H), 4.53 (s, 2H), 3.95−3.87 (m, 2H), 3.41−3.34 (m, 2H), 3.01 (s, 6H), 2.30 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 141.32, 138.91, 132.53, 130.89, 130.59, 95.17, 66.43, 65.16, 54.92, 49.89, 39.78, 39.52. HRMS (ESI) m/z: [M$^+$] ($C_{11}H_{17}INO^+$) calcd, 306.03493; found, 306.03526. HPLC-UV purity 97.4%.

*1-(2-Hydroxyethyl)-1-(3-iodobenzyl)pyrrolidin-1-ium Methanesulfonate (**10**).* General procedure A was used. The alkylating agent **A** (400 mg, 1.27 mmol, 1 equiv, see the Supporting Information), DMF (2.0 mL), and 2-(pyrrolidin-1-yl)ethanol (223 μL, 1.91 mmol, 1.5 equiv) were used for the synthesis of compound **10**. The reaction time was 1 h at a temperature of 75 °C. The product was precipitated from a MeOH solution once with a mixture of Et_2O and hexane (2:1 v/v, 45 mL) and once with pure Et_2O (45 mL). The product was obtained as a yellowish viscous liquid (500 mg, 1.16 mmol, 91%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.02 (t, J = 1.7 Hz, 1H), 7.89 (dt, J = 7.9, 1.1 Hz, 1H), 7.65 (dt, J = 7.7, 1.2 Hz, 1H), 7.31 (t, J = 7.8 Hz, 1H), 5.51 (t, J = 4.8 Hz, 1H), 4.61 (s, 2H), 3.94 (dt, J = 5.0, 4.8 Hz, 2H), 3.64−3.44 (m, 4H), 3.32−3.25 (m, 2H), 2.34 (s, 3H), 2.11−2.03 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 141.04, 138.83, 132.29, 131.34, 130.95, 95.30, 61.67, 60.97, 59.94, 55.40, 54.94, 20.84. HRMS (ESI) m/z: [M$^+$] ($C_{13}H_{19}INO^+$) calcd, 332.0506; found, 332.0495. HPLC-UV purity 96.9%.

*(2-Hydroxyethyl)-(4-iodobenzyl)-dimethylammonium Bromide (**11**).* General procedure A was used. 4-Iodobenzyl bromide (400 mg, 1.35 mmol, 1 equiv), DMF (2 mL), and 2-(dimethylamino)-ethanol (303 μL, 3.01 mmol, 2.2 equiv) were used for the synthesis of compound **11**. The reaction time was 30 min at a temperature of 75 °C. The product was precipitated from a MeOH solution once with a mixture of Et_2O and hexane (3:1 v/v, 40 mL) and once with pure Et_2O (40 mL). The product **11** was obtained as a white solid (472 mg, 1.22 mmol, 91%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.88 (d, J = 8.2 Hz, 2H), 7.39 (d, J = 8.2 Hz, 2H), 5.34 (t, J = 5.0 Hz, 1H), 4.63 (s, 2H), 3.94−3.86 (m, 2H), 3.43−3.37 (m, 2H), 3.03 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 137.67, 135.10, 127.75, 98.01, 66.51, 64.88, 54.83, 49.78. HRMS (ESI) m/z: [M$^+$] ($C_{11}H_{17}INO^+$) calcd, 306.0349; found, 306.0341. HPLC-UV purity 98.7%.

*Diethyl-(2-hydroxyethyl)-(4-iodobenzyl)ammonium Bromide (**12**).* General procedure A was used. 4-Iodobenzyl bromide (400 mg, 1.35 mmol, 1 equiv), DMF (2 mL), and 2-(diethylamino)ethanol (268 μL, 2.02 mmol, 1.5 equiv) were used for the synthesis of compound **12**. The reaction time was 30 min at a temperature of 75 °C. The precipitation was performed from a MeOH solution once with the mixture of Et_2O and hexane (3:1 v/v, 40 mL) and once with pure Et_2O (40 mL). The product **12** was obtained as a white solid (459 mg, 1.11 mmol, 82%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.87 (d, J = 8.3 Hz, 2H), 7.36 (d, J = 8.3 Hz, 2H), 5.38 (t, J = 5.1 Hz, 1H), 4.58 (s, 2H), 3.88 (dt, J = 5.2, 5.1 Hz, 1H), 3.30−3.20 (m, 6H), 1.31 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 137.76, 134.87, 127.48, 97.87, 60.30, 58.06, 54.45, 53.25, 7.79. HRMS (ESI) m/z: [M$^+$] ($C_{13}H_{21}INO^+$) calcd, 334.0662; found, 334.0650. HPLC-UV purity 97.7%.

*1-(2-Hydroxyethyl)-1-(4-iodobenzyl)pyrrolidin-1-ium Bromide (**13**).* General procedure A was used. 4-Iodobenzyl bromide (400 mg, 1.35 mmol, 1 equiv), DMF (2 mL), and 2-(pyrrolidin-1-yl)ethanol (236 μL, 2.02 mmol, 1.5 equiv) were used for the synthesis of compound **13**. The reaction time was 30 min at a temperature of 75 °C. The product was precipitated from a MeOH solution once with a mixture of Et_2O and hexane (3:1 v/v, 40 mL) and once with pure Et_2O (40 mL). The product **13** was obtained as a white solid (484 mg, 1.17 mmol, 87%).

^{1}H NMR (300 MHz, DMSO-d_6): δ 7.87 (d, J = 8.3 Hz, 2H), 7.42 (d, J = 8.3 Hz, 2H), 5.43 (t, J = 4.9 Hz, 1H), 4.63 (s, 2H), 3.93 (dt, J = 4.9, 4.9 Hz, 2H), 3.64−3.45 (m, 4H), 3.31−3.23 (m, 2H), 2.14−1.99 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 137.75, 134.86, 128.42, 97.83, 61.71, 60.90, 59.75, 55.31, 20.78. HRMS (ESI) m/z: [M$^+$] ($C_{13}H_{19}INO^+$) calcd, 332.0506; found, 332.0494. HPLC-UV purity 98.9%.

*4-(2-Hydroxyethyl)-4-(4-iodobenzyl)morpholin-4-ium Bromide (**14**).* 4-Iodobenzyl bromide (300 mg, 1.01 mmol, 1 equiv) and 4-(2-hydroxyethyl)morpholine (4.00 mL, 33,0 mmol, 32.7 equiv) were stirred in a pear-shaped flask overnight at 72 °C. The reaction mixture was diluted with MeOH, transferred into a centrifugation tube, and mixed with Et_2O/hexane (1:1, 40 mL) (oily product formation). The tube was centrifuged, and the solvent was decanted from the oily product. The product was then dissolved in MeOH (5 mL) and triturated with Et_2O (40 mL). This was repeated two times. The oily product **14** was dried under high vacuum yielding an amorphous pink solid (418 mg, 0.98 mmol, 97%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.89 (d, J = 8.3 Hz, 2H), 7.39 (d, J = 8.3 Hz, 2H), 5.49 (t, J = 4.7 Hz, 1H), 4.81 (s, 2H), 4.03−3.90 (m, 6H), 3.54−3.40 (m, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 137.79, 135.48, 126.83, 98.15, 64.11, 59.90, 57.01, 56.67, 54.51. HRMS (ESI) m/z: [M$^+$] ($C_{13}H_{19}INO_2^+$) calcd, 348.04550; found, 348.04523. HPLC-UV purity 99.0%.

*(2-Hydroxyethyl)-[2-(4-iodophenyl)ethyl]-dimethylammonium 4-Methylbenzenesulfonate (**15**).* General procedure A was used. The alkylating agent **B3** (400 mg, 0.99 mmol, 1 equiv, see the Supporting Information), DMF (5 mL), and 2-(dimethylamino)ethanol (300 μL, 2.98 mmol, 3 equiv) were used for the synthesis of compound **15**. The reaction time was 2 h at a temperature of 60 °C and 2 h at a temperature of 80 °C. The precipitation was done once using the mixture of Et_2O and hexane (1:1 v/v, 45 mL) and subsequently twice with pure Et_2O (40 mL). The product **15** was obtained as a white solid (270 mg, 0.55 mmol, 56%). mp 204−206 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.70 (d, J = 8.2 Hz, 2H), 7.48 (d, J = 8.0 Hz, 2H), 7.13 (d, J = 8.2 Hz, 2H), 7.11 (d, J = 8.0 Hz, 2H), 5.35 (t, J = 5.0 Hz, 1H), 3.88−3.83 (m, 2H), 3.55−3.50 (m, 2H), 3.49−3.44 (m,

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2H), 3.13 (s, 6H), 3.03−2.97 (m, 2H), 2.28 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 145.66, 137.67, 137.38, 136.25, 131.48, 128.10, 125.48, 92.88, 64.78, 64.27, 55.04, 50.90, 27.73, 20.82. HRMS (ESI) m/z: [M$^+$] ($C_{12}H_{19}INO^+$) calcd, 320.050584; found, 320.05048. Elem. Anal. $C_{19}H_{26}INO_4S$, Calcd: C (46.44), H (5.33), N (2.85), I (25.83), S (6.53). Found: C (46.65), H (5.32), N (2.64), I (25.56), S (6.55). HPLC-UV purity 99.3%.

*3-Hydroxy-1-[2-(4-iodophenyl)ethyl]-1-methylpiperidin-1-ium 4-Methylbenzenesulfonate (**16**).* General procedure A was used. The alkylating agent **B3** (400 mg, 0.99 mmol, 1 equiv, see the Supporting Information), DMF (5 mL), and 1-methylpiperidine-3-ol (344 μL, 2.98 mmol, 3 equiv) were used for the synthesis of compound **16**. The reaction time was 2 h at a temperature of 60 °C, 2 h at a temperature of 80 °C, and 2 h at a temperature of 90 °C. The precipitation was done three times using a mixture of Et_2O and hexane (1:1 v/v, 45 mL). The product **16** was obtained as a mixture of two diastereomers (1:1 ratio according to the NMR analysis) as a pale-yellow solid (269 mg, 0.52 mmol, 53%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.71 (d, J = 8.3 Hz, 1H, diastereomer 1), 7.70 (d, J = 8.3 Hz, 1H, diastereomer 2), 7.48 (d, J = 8.2 Hz, 2H, 4-methylbenzenesulfonate), 7.16 (d, J = 8.2 Hz, 1H), 7.13 (d, J = 8.2 Hz, 1H), 7.11 (d, J = 8.2 Hz, 2H, 4-methylbenzenesulfonate), 5.52 (d, J = 4.5 Hz, 1H), 5.44 (d, J = 4.2 Hz, 1H), 4.06−3.99 (m, 2H), 3.72−3.66 (m, 1H), 3.58−3.49 (m, 2H), 3.48−3.30 (m, 6H), 3.21 (s, 3H), 3.16 (d, J = 7.0 Hz, 1H), 3.14 (d, J = 6.9 Hz, 1H), 3.10 (s, 3H), 3.07−2.94 (m, 4H), 2.28 (s, 6H), 1.98−1.87 (m, 2H), 1.87−1.75 (m, 4H), 1.52−1.40 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 145.70, 137.64, 137.41, 137.34, 136.37, 136.19, 131.58, 131.50, 128.09, 125.49, 92.89, 92.88, 65.02, 63.55, 63.03, 62.78, 61.45, 61.31, 60.02, 59.84, 49.93, 47.83, 30.00, 29.38, 27.24, 26.93, 20.82, 17.06, 16.70. HRMS (ESI) m/z: [M$^+$] ($C_{14}H_{21}INO^+$) calcd, 346.066234; found, 346.06600, Elem. Anal. $C_{21}H_{28}INO_4S$, Calcd: C (48.75), H (5.45), N (2.71), I (24.53), S (6.20). Found: C (48.91), H (5.48), N (2.59), I (24.25), S (6.25). HPLC-UV purity 99.4%.

*1-(2-Hydroxyethyl)-1-[2-(4-iodophenyl)ethyl]pyrrolidin-1-ium 4-Methylbenzenesulfonate (**17**).* General procedure A was used. The alkylating agent **B3** (400 mg, 0.99 mmol, 1 equiv, see the Supporting Information), DMF (5 mL), and 2-(pyrrolidin-1-yl)ethanol (350 μL, 2.99 mmol, 3 equiv) were used for the synthesis of compound **17**. The reaction time was 2 h at a temperature of 60 °C, 2 h at a temperature of 80 °C, and 2 h at a temperature of 90 °C. The precipitation was done three times using a mixture of Et_2O and hexane (1:2 v/v, 45 mL). The product **17** was obtained in as a pale-yellow solid (165 mg, 0.32 mmol, 32%). mp 152−154 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.69 (d, J = 7.9 Hz, 2H), 7.48 (d, J = 7.8 Hz, 2H), 7.15 (d, J = 7.9 Hz, 2H), 7.11 (d, J = 7.8 Hz, 2H), 5.40 (t, J = 5.0 Hz, 1H), 3.88−3.83 (m, 2H), 3.66−3.54 (m, 4H), 3.52−3.46 (m, 4H), 3.04−2.99 (m, 2H), 2.28 (s, 3H), 2.10−2.02 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 145.67, 137.66, 137.32, 136.36, 131.51, 128.09, 125.48, 92.84, 62.78, 60.27, 60.08, 55.73, 28.36, 21.21, 20.82. HRMS (ESI) m/z: [M$^+$] ($C_{14}H_{21}INO^+$) calcd, 346.066234; found, 346.06629, Elem. Anal. $C_{21}H_{28}INO_4S$, Calcd: C (48.75), H (5.45), N (2.71), I (24.53), S (6.20). Found: C (48.93), H (5.46), N (2.53), I (24.07), S (6.30). HPLC-UV purity 96.5%.

*3-Hydroxy-1-[2-(4-iodophenyl)ethyl]quinuclidin-1-ium 4-Methylbenzenesulfonate (**18**).* General procedure A was used. The alkylating agent **B3** (400 mg, 0.99 mmol, 1 equiv, see the Supporting Information), DMF (5 mL), and quinuclidin-3-ol (380 mg, 2.99 mmol, 3 equiv) were used for the synthesis of compound **18**. The reaction time was 3 h at a temperature of 60 °C. The precipitation was done once using a mixture of Et_2O and hexane (1:1 v/v, 45 mL) and twice with pure Et_2O (40 mL). The product **18** was obtained as an off-white solid (425 mg, 0.80 mmol, 80%). mp 217−218 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.70 (d, J = 8.2 Hz, 2H), 7.48 (d, J = 8.0 Hz, 2H), 7.14−7.10 (m, 4H), 5.60 (d, J = 3.5 Hz, 1H), 4.10−4.06 (m, 1H), 3.69 (ddd, J = 12.7, 8.3, 3.0 Hz, 1H), 3.48−3.42 (m, 1H), 3.41−3.28 (m, 5H), 3.09 (dt, J = 12.9, 2.8 Hz, 1H), 2.99−2.90 (m, 2H), 2.28 (s, 3H), 2.17−2.09 (m, 1H), 2.06−2.01 (m, 1H), 1.95−1.88 (m, 1H), 1.81−1.70 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 145.69, 137.65, 137.35, 136.43, 131.46, 128.09, 125.49, 92.83, 63.44, 63.02, 62.67, 54.04, 52.74, 27.06, 26.36, 20.90, 20.82, 17.39. HRMS (ESI) m/z: [M$^+$] ($C_{15}H_{21}INO^+$) calcd, 358.066234; found, 358.06608, Elem. Anal. $C_{22}H_{28}INO_4S$, Calcd: C (49.91), H (5.33), N (2.65), I (23.97), S (6.06). Found: C (50.12), H (5.37), N (2.46), I (23.73), S (6.21). HPLC-UV purity 98.8%.

*(2-Hydroxyethyl)-[2-(3-iodophenyl)ethyl]-dimethylammonium Methanesulfonate (**19**).* General procedure A was used. The alkylating agent **C3** (250 mg, 0.767 mmol, 1 equiv, see the Supporting Information), DMF (1.2 mL), and 2-(dimethylamino)ethanol (463 μL, 4.60 mmol, 6 equiv) were used for the synthesis of compound **19**. The reaction time was 3 h at a temperature of 70 °C and 2 h at a temperature of 80 °C. The precipitation was done from MeOH (4 mL) solution once using the mixture of Et_2O and hexane (1:1 v/v, 45 mL). The product was dissolved in chloroform (4 mL) and precipitated with neat E_2O (20 mL). Compound **19** was obtained as a yellow viscous liquid (253 mg, 0.609 mmol, 79%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.75 (dd, J = 1.6, 1.6 Hz, 1H), 7.65 (ddd, J = 7.8, 1.6, 1.1 Hz, 1H), 7.34 (ddd, J = 7.7, 1.6, 1.1 Hz, 1H), 7.16 (dd, J = 7.8, 7.7 Hz, 1H), 5.35 (t, J = 4.9 Hz, 1H), 3.91−3.81 (m, 2H), 3.59−3.50 (m, 2H), 3.50−3.43 (m, 2H), 3.13 (s, 6H), 3.07−2.98 (m, 2H), 2.30 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 139.15, 137.57, 135.65, 130.77, 128.62, 95.08, 64.85, 64.35, 55.01, 50.89, 39.77, 27.64. HRMS (ESI) m/z: [M$^+$] ($C_{12}H_{19}INO^+$) calcd, 320.05058; found, 320.05090. HPLC-UV purity 98.0%.

*(2-Hydroxyethyl)-[2-(3-iodo-4-methoxyphenyl)ethyl]-dimethylammonium 4-Methylbenzenesulfonate (**20**).* General procedure A was used. The alkylating agent **D3** (200 mg, 0.463 mmol, 1 equiv, see the Supporting Information), DMF (2 mL), and 2-(dimethylamino)-ethanol (140 μL, 1.39 mmol, 3 equiv) were used for the synthesis of compound **20**. The reaction time was 3 h at a temperature of 80 °C. The precipitation was done twice using a mixture of Et_2O and hexane (1:1 v/v, 45 mL). The product **20** was obtained as a white solid (137 mg, 0.263 mmol, 57%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.75 (d, J = 1.9 Hz, 1H), 7.48 (d, J = 8.0 Hz, 2H), 7.30 (dd, J = 8.4, 1.8 Hz, 1H), 7.11 (d, J = 7.9 Hz, 2H), 6.97 (d, J = 8.4 Hz, 1H), 5.34 (t, J = 5.0 Hz, 1H), 3.88−3.83 (m, 2H), 3.80 (s, 3H), 3.52−3.47 (m, 2H), 3.48−3.44 (m, 2H), 3.13 (s, 6H), 2.98−2.94 (m, 2H), 2.28 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 156.76, 145.69, 139.27, 137.65, 130.47, 130.43, 128.09, 125.49, 111.62, 86.24, 64.82, 64.68, 56.42, 55.04, 50.87, 26.73, 20.82. HRMS (ESI) m/z: [M$^+$] ($C_{13}H_{21}INO_2^+$) calcd, 350.061148; found, 350.06124. Elem. Anal. $C_{21}H_{28}INO_4S$, Calcd: C (46.07), H (5.41), N (2.69), S (6.15). Found: C (46.02), H (5.48), N (2.58), S (6.24). HPLC-UV purity 97.7%.

*3-Hydroxy-1-[2-(3-iodo-4-methoxyphenyl)ethyl]quinuclidin-1-ium 4-Methylbenzenesulfonate (**21**).* General procedure A was used. The alkylating agent **D3** (200 mg, 0.463 mmol, 1 equiv, see the Supporting Information), DMF (2 mL), and quinuclidin-3-ol (178 mg, 1.40 mmol, 3 equiv) were used for the synthesis of compound **21**. The reaction time was 3 h at a temperature of 80 °C. The precipitation was done first using a mixture of Et_2O and hexane (1:1 v/v, 45 mL), and subsequently with pure Et_2O (45 mL). The product **21** was obtained as an off-white solid (238 mg, 0.425 mmol, 92%). mp 173−176 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.75 (d, J = 2.2 Hz, 1H), 7.48 (d, J = 8.0 Hz, 2H), 7.29 (dd, J = 8.4, 2.2 Hz, 1H), 7.11 (d, J = 7.9 Hz, 2H), 6.97 (d, J = 8.5 Hz, 1H), 5.60 (d, J = 3.5 Hz, 1H), 4.11−4.06 (m, 1H), 3.80 (s, 3H), 3.68 (ddd, J = 12.7, 8.2, 2.9 Hz, 1H), 3.47−3.41 (m, 1H), 3.40−3.27 (m, 5H), 3.09 (dt, J = 12.8, 2.8 Hz, 1H), 2.96−2.86 (m, 2H), 2.28 (s, 3H), 2.16−2.10 (m, 1H), 2.06−2.02 (m, 1H), 1.96−1.89 (m, 1H), 1.81−1.71 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 156.73, 145.73, 139.24, 137.62, 130.61, 130.43, 128.08, 125.49, 111.58, 86.22, 63.44, 62.65, 56.42, 54.03, 52.71, 26.36, 26.05, 20.90, 20.81, 17.39. HRMS (ESI) m/z: [M$^+$] ($C_{16}H_{23}INO_2^+$) calcd, 388.076798; found, 388.07672. Elem. Anal. $C_{23}H_{30}INO_5S$, Calcd: C (49.38), H (5.40), N (2.50), I (22.68), S (5.73). Found: C (49.51) H (5.35), N (2.24), I (22.40), S (5.76). HPLC-UV purity 95.5%.

*(2-Hydroxyethyl)-[2-(3,5-diiodo-4-methoxyphenyl)ethyl]-dimethylammonium 4-Methylbenzenesulfonate (**22**).* General procedure A was used. The alkylating agent **E3** (350 mg, 0.627 mmol, 1 equiv, see the Supporting Information), DMF (3 mL), and 2-

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(dimethylamino)ethanol (190 μL, 1.888 mmol, 3 equiv) were used for the synthesis of compound **22**. The reaction time was 4 h at a temperature of 85 °C. The precipitation was done once using a mixture of Et_2O and hexane (1:1 v/v, 45 mL) and subsequently with pure Et_2O (40 mL). The product **22** was obtained as a white solid (258 mg, 0.399 mmol, 64%). mp 178−180 °C, ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.82 (s, 2H), 7.48 (d, J = 8.1 Hz, 2H), 7.11 (d, J = 7.9 Hz, 2H), 5.34 (t, J = 5.0 Hz, 1H), 3.88−3.83 (m, 2H), 3.73 (s, 3H), 3.52−3.48 (m, 2H), 3.46−3.43 (m, 2H), 3.11 (s, 6H), 3.00−2.93 (m, 2H), 2.28 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 157.38, 145.67, 140.12, 137.64, 136.74, 128.08, 125.48, 91.52, 64.94, 64.21, 60.29, 55.00, 50.90, 26.26, 20.82. HRMS (ESI) m/z: [M$^+$] ($C_{13}H_{20}I_2NO_2^+$) calcd, 475.957791; found, 475.95731, Elem. Anal. $C_{20}H_{27}I_2NO_5S$, Calcd: C (37.11), H (4.20), N (2.16), I (39.21), S (4.95). Found: C (37.14), H (4.27), N (1.98), I (39.23), S (5.17). HPLC-UV purity 99.4%.

*(2-Hydroxyethyl)-[3-(3-iodo-4-methoxyphenyl)propyl]-dimethylammonium 4-Methylbenzenesulfonate (**23**).* General procedure A was used. The alkylating agent **F3a** (342 mg, 0.766 mmol, 1 equiv, see the Supporting Information), DMF (3 mL), and 2-(dimethylamino)-ethanol (190 μL, 1.888 mmol, 3 equiv) were used for the synthesis of compound **23**. The reaction time was 2 h at a temperature of 80 °C. The product was purified by chromatography on a C_{18}-silica gel (eluent 4:6 MeOH/water v/v). Compound **23** was obtained as a white solid (354 mg, 0.661 mmol, 86%). mp 120−122 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.67 (d, J = 2.2 Hz, 1H), 7.49 (d, J = 8.0 Hz, 2H), 7.23 (dd, J = 8.4, 2.2 Hz, 1H), 7.12 (d, J = 7.9 Hz, 2H), 6.93 (d, J = 8.5 Hz, 1H), 5.28 (br s, 1H), 3.82−3.78 (m, 2H), 3.79 (s, 3H), 3.40−3.36 (m, 2H), 3.34−3.30 (m, 2H), 3.05 (s, 6H), 2.49 (t, J = 8.0 Hz, 2H), 2.28 (s, 3H), 1.98−1.92 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 156.22, 145.70, 138.53, 137.66, 134.67, 129.62, 128.10, 125.49, 111.44, 86.14, 64.63, 63.60, 56.37, 54.97, 50.91, 30.23, 23.86, 20.82. HRMS (ESI) m/z: [M$^+$] ($C_{14}H_{23}INO_2^+$) calcd, 364.076798; found, 364.07690. Elem. Anal. $C_{21}H_{30}INO_5S$, Calcd: C (47.11), H (5.65), N (2.62), S (5.99). Found: C (47.18), H (5.63), N (2.47), S (6.04). HPLC-UV purity 97.6%.

*[3-(4-Hexyloxy-3-iodophenyl)propyl]-(2-hydroxyethyl)-dimethylammonium 4-Methylbenzenesulfonate (**24**).* General procedure A was used. The alkylating agent **F3b** (338 mg, 0.655 mmol, 1 equiv, see the Supporting Information), DMF (0.8 mL), and 2-(dimethylamino)ethanol (350 μL, 3.48 mmol, 5.3 equiv) were used for the synthesis of compound **24**. The reaction time was 2 h at a temperature of 75 °C. Precipitation was performed from a DCM solution with hexane (45 mL), and the product was then redissolved in a mixture of DCM and MeOH (2 mL, 9:1 v/v) and precipitated with a mixture of Et_2O and hexane. The solid was then once more redissolved in pure DCM and precipitated with pure hexane (45 mL). Compound **24** was obtained as a white solid (391 mg, 0.65 mmol, 99%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.66 (d, J = 2.1 Hz, 1H), 7.49 (d, J = 8.0 Hz, 2H), 7.21 (dd, J = 8.4, 2.1 Hz, 1H), 7.11 (d, J = 7.9 Hz, 2H), 6.91 (d, J = 8.4 Hz, 1H), 5.30 (t, J = 5.0 Hz, 1H), 3.98 (t, J = 6.2 Hz, 2H), 3.81 (br s, 2H), 3.42−3.28 (m, 4H), 3.06 (s, 6H), 2.49 (t, J = 7.4 Hz, 2H), 2.28 (s, 3H), 2.02−1.88 (m, 2H), 1.77−1.65 (m, 2H), 1.53−1.40 (m, 2H), 1.37−1.24 (m, 4H), 0.93−0.84 (m, 3H), ^{13}C NMR (75 MHz, DMSO-d_6): δ 155.62, 145.74, 138.43, 137.61, 134.57, 129.56, 128.06, 125.48, 112.43, 86.81, 68.63, 64.63, 63.63, 54.95, 50.90, 30.88, 30.25, 28.53, 25.22, 23.84, 22.08, 20.78, 13.91. HRMS (ESI) m/z: [M$^+$] ($C_{19}H_{33}INO_2^+$) calcd, 434.1551; found, 434.1535. HPLC-UV purity 98.8%.

*[3-(4-Decyloxy-3-iodophenyl)propyl]-(2-hydroxyethyl)-dimethylammonium Methanesulfonate (**25**).* General procedure A was used. The alkylating agent **F3c** (250 mg, 0.504 mmol, 1 equiv, see the Supporting Information), DMF (0.7 mL), and 2-(dimethylamino)-ethanol (200 μL, 1.99 mmol, 3.9 equiv) were used for the synthesis of compound **25**. The reaction was run 1 h at a temperature of 65 °C, and the temperature was then increased to 75 °C for 45 min. However, as the conversion was still not complete, the temperature was increased to 82 °C for additional 30 min. The product was purified by precipitation from a MeOH solution using a mixture of Et_2O and hexane (1:4 v/v). The compound **25** was obtained as a white waxy solid (186 mg, 0.318 mmol, 63%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.67 (s, 1H), 7.21 (d, J = 8.4 Hz, 1H), 6.92 (d, J = 8.4 Hz, 1H), 5.28 (t, J = 4.9 Hz, 1H), 3.98 (t, J = 6.1 Hz, 2H), 3.81 (br s, 2H), 3.40−3.37 (m, 2H), 3.35−3.30 (m, 2H), 3.06 (s, 6H), 2.49 (t, J = 7.5 Hz, 2H), 2.30 (s, 3H), 2.00−1.92 (m, 2H), 1.75−1.67 (m, 2H), 1.49−1.42 (m, 2H), 1.36−1.20 (m, 12H), 0.86 (t, J = 6.6 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 156.57, 139.05, 133.56, 129.55, 112.29, 87.02, 69.49, 65.78, 65.27, 56.24, 51.95, 39.76, 32.02, 30.87, 29.69, 29.67, 29.44 (2C), 29.22, 26.20, 24.80, 22.80, 14.26. HRMS (ESI) m/z: [M$^+$] ($C_{23}H_{41}INO_2^+$) calcd, 490.2176; found, 490.2158. HPLC-UV purity 97.4%.

*(2-Hydroxyethyl)-[2-(5-iodothiophen-2-yl)ethyl]-dimethylammonium 4-Methylbenzenesulfonate (**26**).* General procedure A was used. The alkylating agent **G4** (250 mg, 0.612 mmol, 1 equiv, see the Supporting Information), DMF (1 mL), and 2-(dimethylamino)-ethanol (370 μL, 3.67 mmol, 6.0 equiv) were used for the synthesis of compound **26**. The reaction time was 2 h at a temperature of 65 °C. The product **26** was obtained as a white solid (273 mg, 0.55 mmol, 90%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.48 (d, J = 8.0 Hz, 2H), 7.22 (d, J = 3.6 Hz, 1H), 7.11 (d, J = 8.0 Hz, 2H), 6.74 (d, J = 3.6 Hz, 1H), 5.33 (t, J = 4.9 Hz, 1H), 3.89−3.80 (m, 2H), 3.63−3.53 (m, 2H), 3.50−3.43 (m, 2H), 3.35−3.26 (m, 2H), 3.12 (s, 3H), 2.29 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 145.71, 144.08, 137.63, 136.81, 128.67, 128.07, 125.48, 74.32, 64.80, 63.82, 55.01, 50.95, 22.83, 20.80. HRMS (ESI) m/z: [M$^+$] ($C_{10}H_{17}INOS^+$) calcd, 326.00700; found, 326.00725. HPLC-UV purity 98.4%.

*1-(2-Hydroxyethyl)-1-[2-(5-iodothiophen-2-yl)ethyl]pyrrolidin-1-ium 4-Methylbenzenesulfonate (**27**).* General procedure A was used. The alkylating agent **G4** (250 mg, 0.612 mmol, 1 equiv, see the Supporting Information), DMF (1 mL), and 2-(pyrrolidin-1-yl)ethanol (358 μL, 3.06 mmol, 5.0 equiv) were used for the synthesis of compound **27**. The reaction time was 2 h at a temperature of 65 °C. The product **27** was obtained as a brown solid (120 mg, 0.23 mmol, 37%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.47 (d, J = 8.0 Hz, 2H), 7.22 (d, J = 3.6 Hz, 1H), 7.11 (d, J = 8.0 Hz, 1H), 6.75 (d, J = 3.6 Hz, 1H), 5.36 (t, J = 4.8 Hz, 1H), 3.90−3.76 (m, 2H), 3.70−3.51 (m, 6H), 3.51−3.43 (m, 2H), 3.34−3.27 (m, 2H), 2.29 (s, 3H), 2.13−2.00 (m, 4H). HRMS (ESI) m/z: [M$^+$] ($C_{12}H_{19}INO_2S^+$) calcd, 352.02265; found, 352.02235. HPLC-UV purity 98.0%.

*(3-Hydroxypropyl)-[2-(5-iodothiophen-2-yl)ethyl]-dimethylammonium 4-Methylbenzenesulfonate (**28**).* General procedure A was used. The alkylating agent **G4** (150 mg, 0.367 mmol, 1 equiv, see the Supporting Information) and 3-(dimethylamino)propanol (1.5 mL) were used for the synthesis of compound **28**. The reaction time was 1 h at a temperature of 70 °C. The product **28** was obtained as a pale-yellow solid (165 mg, 0.323 mmol, 88%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.47 (d, J = 8.0 Hz, 2H), 7.23 (d, J = 3.6 Hz, 1H), 7.11 (d, J = 8.0 Hz, 2H), 6.76 (d, J = 3.6 Hz, 1H), 4.81 (t, J = 5.0 Hz, 1H), 3.55−3.50 (m, 2H), 3.50−3.46 (m, 2H), 3.40−3.36 (m, 2H), 3.30−3.26 (m, 2H), 3.07 (s, 6H), 2.29 (s, 3H), 1.86−1.81 (m, 2H). ^{13}C (126 MHz, DMSO-d_6): δ 145.77, 143.99, 137.59, 136.85, 128.68, 128.05, 125.48, 74.24, 62.70, 61.42, 57.62, 50.15, 25.34, 22.60, 20.78. HRMS (ESI) m/z: [M$^+$] ($C_{11}H_{19}INOS^+$) calcd, 340.02265; found, 340.02307. HPLC-UV purity 99.0%.

*(4-Hydroxybutyl)-[2-(5-iodothiophen-2-yl)ethyl]-dimethylammonium 4-Methylbenzenesulfonate (**29**).* General procedure A was used. The alkylating agent **G4** (150 mg, 0.367 mmol, 1 equiv, see the Supporting Information), 4-(dimethylamino)butanol (172 μL, 1.29 mmol, 3.5 equiv), and DMF (1.5 mL) were used for the synthesis of compound **29**. The reaction time was 2 h at a temperature of 70 °C. The product **29** was obtained as a brown viscous liquid (100 mg, 0.190 mmol, 52%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.47 (d, J = 8.0 Hz, 2H), 7.23 (d, J = 3.6 Hz, 1H), 7.11 (d, J = 8.0 Hz, 3H), 6.76 (d, J = 3.6 Hz, 1H), 4.58 (t, J = 5.0 Hz, 1H), 3.55−3.40 (m, 4H), 3.32−3.23 (m, 4H), 3.05 (s, 6H), 2.29 (s, 3H), 1.78−1.63 (m, 2H), 1.48−1.36 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 145.74, 144.08, 137.62, 136.84, 128.70, 128.06, 125.49, 74.25, 63.23, 62.78, 59.84, 50.01, 29.00, 22.63, 20.78, 18.73. HRMS (ESI) m/z: [M$^+$]

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($C_{12}H_{21}INOS^+$) calcd, 354.03830; found, 354.003824. HPLC-UV purity 99.3%.

*(2-Hydroxyethyl)-(5-iodopent-4-ynyl)-dimethylammonium 4-Methylbenzenesulfonate (**30**).* General procedure A was used. The alkylating agent **H4** (369 mg, 1.00 mmol, 1 equiv, see the Supporting Information), 4-(dimethylamino)ethanol (604 μL, 6.00 mmol, 6 equiv), and DMF (1 mL) were used for the synthesis of compound **30**. The reaction time was 1 h at a temperature of 65 °C. The product **30** was obtained as a white solid (432 mg, 0.953 mmol, 95%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.48 (d, J = 8.0 Hz, 2H), 7.12 (d, J = 8.0 Hz, 2H), 5.27 (t, J = 5.0 Hz, 1H), 3.80 (br s, 2H), 3.43−3.28 (m, 4H), 3.06 (s, 6H), 2.38 (t, J = 7.1 Hz, 2H), 2.29 (s, 3H) 1.94−1.79 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 145.65, 137.69, 128.11, 125.49, 91.29, 64.69, 62.97, 54.94, 50.98, 21.41, 20.81, 17.28, 5.28. HRMS (ESI) m/z: [M$^+$] ($C_9H_{17}INO^+$) calcd, 282.03493; found, 282.03483. HPLC-UV purity 95.7%.

*(2-Hydroxyethyl)-(6-iodohex-5-ynyl)-dimethylammonium 4-Methylbenzenesulfonate (**31**).* General procedure A was used. The alkylating agent **I4** (378 mg, 1.00 mmol, 1 equiv, see the Supporting Information), 4-(dimethylamino)ethanol (604 μL, 6.00 mmol, 6 equiv), and DMF (1 mL) were used for the synthesis of compound **31**. The reaction time was 1 h at a temperature of 65 °C. The product **31** was obtained as an off-white solid (456 mg, 0.976 mmol, 98%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.48 (d, J = 8.0 Hz, 2H), 7.12 (d, J = 8.0 Hz, 2H), 5.26 (t, J = 4.9 Hz, 1H), 3.81 (br s, 2H), 3.39−3.27 (m, 4H), 3.04 (s, 6H), 2.38 (t, J = 7.1 Hz, 2H), 2.29 (s, 3H), 1.79−1.64 (m, 2H), 1.43 (tt, J = 7.1, 7.1 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 145.72, 137.63, 128.07, 125.48, 92.45, 64.66, 63.41, 54.92, 50.80, 25.02, 21.10, 20.80, 19.53, 4.20. HRMS (ESI) m/z: [M$^+$] ($C_{10}H_{19}INO^+$) calcd, 296.05058; found, 296.05053. HPLC-UV purity 96.3%.

*1-(2-Hydroxyethyl)-1-(6-iodohex-5-ynyl)-pyrrolidin-1-ium 4-Methylbenzenesulfonate (**32**).* General procedure A was used. The alkylating agent **I4** (369 mg, 1.00 mmol, 1 equiv, see the Supporting Information), 2-(pyrrolidin-1-yl)ethanol (585 μL, 5.00 mmol, 5 equiv), and DMF (1 mL) were used for the synthesis of compound **32**. The reaction time was 1 h at a temperature of 65 °C. The product **32** was obtained as a yellow oil, which crystallized upon cooling (279 mg, 0.565 mmol, 57%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.48 (d, J = 8.0 Hz, 2H), 7.12 (d, J = 8.0 Hz, 2H), 5.27 (t, J = 5.0 Hz, 1H), 3.83−3.74 (m, 2H), 3.64−3.52 (m, 2H), 3.52−3.40 (m,2H), 3.39−3.33 (m, 2H), 3.32−3.25 (m, 2H), 2.38 (t, J = 7.1 Hz, 2H), 2.29 (s, 3H), 2.10−1.98 (m, 4H), 1.77−1.64 (m, 2H), 1.45 (tt, J = 7.2, 7.1 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 145.68, 137.65, 128.08, 125.48, 92.48, 62.68, 60.05, 58.68, 55.42, 24.98, 21.82, 21.11, 20.79, 19.48, 4.14. HRMS (ESI) m/z: [M$^+$] ($C_{12}H_{21}INO^+$) calcd, 322.06623; found, 322.06602. HPLC-UV purity 96.9%.

*(2-Hydroxyethyl)-[2-(2-iodophenoxy)ethyl]-dimethylammonium Bromide (**33**).* General procedure A was used. The alkylating agent **J** (350 mg, 1.07 mmol, 1 equiv, see the Supporting Information), DMF (1 mL), and 2-(dimethylamino)ethanol (646 μL, 6.42 mmol, 6.0 equiv) were used for the synthesis of compound **33**. The reaction time was 1 h at a temperature of 67 °C. The product **33** was obtained as a white solid (398 mg, 0.96 mmol, 90%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.80 (dd, J = 7.7, 1.5 Hz, 1H), 7.42−7.38 (m, 1H), 7.09 (dd, J = 8.2, 0.8 Hz, 1H), 6.81 (ddd, J = 7.7, 7.7, 1.1 Hz, 1H), 5.35 (t, J = 4.9 Hz, 1H), 4.55−4.50 (m, 2H), 3.95−3.88 (m, 4H), 3.64−3.60 (m, 2H), 3.28 (s, 6H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 156.30, 139.11, 129.75, 123.34, 112.79, 86.25, 65.88, 63.09, 62.49, 55.11, 51.89. HRMS (ESI) m/z: [M$^+$] ($C_{12}H_{19}INO_2^+$) calcd, 336.0455; found, 336.0441. HPLC-UV purity 98.1%.

*1-(2-Hydroxyethyl)-1-[2-(2-iodophenoxy)ethyl]pyrrolidin-1-ium 4-Methylbenzenesulfonate (**34**).* General procedure A was used. The alkylating agent **J** (350 mg, 1.07 mmol, 1 equiv, see the Supporting Information), DMF (1 mL), and 2-(pyrrolidin-1-yl)ethanol (376 μL, 3.21 mmol, 3.0 equiv) were used for the synthesis of compound **34**. The reaction time was 90 min at a temperature of 77 °C. The product **34** was obtained as a pale-yellow viscous liquid (331 mg, 0.75 mmol, 70%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.81 (dd, J = 7.7, 1.6 Hz, 1H), 7.49−7.46 (m, 2H), 7.40 (ddd, J = 8.4, 7.4, 1.6 Hz, 1H), 7.13−7.09 (m, 2H), 7.06 (dd, J = 8.3, 1.2 Hz, 1H), 6.81 (ddd, J = 7.6, 7.6, 1.3 Hz, 1H), 5.34 (t, J = 4.8 Hz, 1H), 4.51−4.47 (m, 2H), 3.93−3.87 (m, 4H), 3.80−3.68 (m, 4H), 3.61−3.57 (m, 2H), 2.28 (s, 3H), 2.20−2.07 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 156.31, 145.78, 139.17, 137.58, 129.78, 128.05, 125.49, 123.38, 112.71, 85.98, 63.54, 63.04, 60.92, 57.89, 55.75, 20.97, 20.77. HRMS (ESI) m/z: [M$^+$] ($C_{14}H_{21}INO_2^+$) calcd, 362.0612; found, 362.0597. HPLC-UV purity 96.9%.

*(2-Hydroxyethyl)-[2-(3-iodophenoxy)ethyl]-dimethylammonium Bromide (**35**).* General procedure A was used. The alkylating agent **K** (300 mg, 0.918 mmol, 1 equiv, see the Supporting Information), DMF (1 mL), and 2-(dimethylamino)ethanol (554 μL, 5.51 mmol, 6.0 equiv) were used for the synthesis of compound **35**. The reaction time was 2 h at a temperature of 70 °C. The product **35** was obtained as a white solid (368 mg, 0.88 mmol, 96%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.40−7.34 (m, 2H), 7.15−7.08 (m, 1H), 7.05−7.00 (m, 1H), 5.32 (t, J = 5.0 Hz, 1H), 4.51−4.42 (m, 2H), 3.92−3.81 (m, 4H), 3.58−3.51 (m, 2H), 3.20 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 158.10, 131.37, 130.13, 123.14, 114.72, 95.25, 65.66, 62.82, 61.75, 54.95, 51.63. HRMS (ESI) m/z: [M$^+$] ($C_{12}H_{19}INO_2^+$) calcd, 336.0455; found, 336.0439. HPLC-UV purity 99.4%.

*1-(2-Hydroxyethyl)-1-[2-(3-iodophenoxy)ethyl]pyrrolidin-1-ium Bromide (**36**).* General procedure A was used. The alkylating agent **K** (300 mg, 0.918 mmol, 1 equiv, see the Supporting Information), DMF (1 mL), and 2-(pyrrolidin-1-yl)ethanol (322 μL, 2.75 mmol, 3.0 equiv) were used for the synthesis of compound **36**. The reaction time was 2 h at a temperature of 70 °C. The product **36** was obtained as a yellow viscous liquid, which crystallized upon cooling (380 mg, 0.86 mmol, 80%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.40−7.33 (m, 2H), 7.15−7.07 (m, 1H), 7.05−6.98 (m, 1H), 5.32 (t, J = 5.0 Hz, 1H), 4.45 (t, J = 4.7 Hz, 2H), 3.92−3.79 (m, 4H), 3.78−3.58 (m, 4H), 3.56−3.47 (m, 2H), 2.17−2.02 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 158.09, 131.36, 130.16, 123.12, 114.66, 95.21, 63.35, 62.34, 60.66, 58.03, 55.44, 20.91. HRMS (ESI) m/z: [M$^+$] ($C_{14}H_{21}INO_2^+$) calcd, 362.0612; found, 362.0597. HPLC-UV purity 95.2%.

*(2-Hydroxyethyl)-[2-(4-iodophenoxy)ethyl]-dimethylammonium Bromide (**37**).* General procedure A was used. The alkylating agent **L** (350 mg, 1.07 mmol, 1 equiv, see the Supporting Information), DMF (1 mL), and 2-(dimethylamino)ethanol (646 μL, 6.42 mmol, 6.0 equiv) were used for the synthesis of compound **37**. The reaction time was 3 h at a temperature of 70 °C. The product **37** was obtained as a white solid (384 mg, 0.92 mmol, 86%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.67−7.61 (m, 2H), 6.90−6.83 (m, 2H), 5.31 (t, J = 4.9 Hz, 1H), 4.51−4.39 (m, 2H), 3.92−3.80 (m, 4H), 3.58−3.50 (m, 2H), 3.20 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 157.30, 138.02, 117.53, 84.25, 65.69, 62.81, 61.65, 54.94, 51.62. HRMS (ESI) m/z: [M$^+$] ($C_{12}H_{19}INO_2^+$) calcd, 336.0455; found, 336.0439. HPLC-UV purity 96.8%.

*1-(2-Hydroxyethyl)-1-[2-(4-iodophenoxy)ethyl]pyrrolidin-1-ium Bromide (**38**).* General procedure A was used. The alkylating agent **L** (350 mg, 1.07 mmol, 1 equiv, see the Supporting Information), DMF (1 mL), and 2-(pyrrolidin-1-yl)ethanol (376 μL, 3.21 mmol, 3.0 equiv) were used for the synthesis of compound **38**. The reaction time was 3 h at a temperature of 70 °C. The product **38** was obtained as a pale-yellow solid (380 mg, 0.86 mmol, 80%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.64 (d, J = 8.8 Hz, 2H), 6.86 (d, J = 8.8 Hz, 2H), 5.33 (t, J = 4.9 Hz, 1H), 4.43 (t, J = 4.5 Hz, 2H), 3.88−3.83 (m, 4H), 3.74−3.67 (m, 2H), 3.67−3.61 (m, 2H), 3.53−3.49 (m, 2H), 2.12−2.05 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 157.31, 138.05, 117.49, 84.30, 63.34, 62.24, 60.68, 58.02, 55.45, 20.92. HRMS (ESI) m/z: [M$^+$] ($C_{14}H_{21}INO_2^+$) calcd, 362.0612; found, 362.0597. HPLC-UV purity 98.1%.

*(2-Hydroxyethyl)-[4-(2-iodophenoxy)butyl]-dimethylammonium Bromide (**39**).* General procedure A was used. The alkylating agent **M** (355 mg, 1.00 mmol, 1 equiv, see the Supporting Information), DMF (1 mL), and 2-(dimethylamino)ethanol (604 μL, 6.00 mmol, 6.0 equiv) were used for the synthesis of compound **39**. The reaction time was 2.5 h at a temperature of 70 °C. The product **39** was obtained as a colorless viscous liquid (418 mg, 0.94 mmol, 94%). ^{1}H

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NMR (300 MHz, DMSO-d_6): δ 7.77 (dd, J = 7.7, 1.6 Hz, 1H), 7.36 (ddd, J = 8.2, 7.4, 1.6 Hz, 1H), 7.01 (dd, J = 8.3, 1.3 Hz, 1H), 6.79−6.72 (m, 1H), 5.28 (t, J = 5.0 Hz, 1H) 4.08 (t, J = 6.4 Hz, 2H), 3.90−3.79 (m, 2H), 3.52−3.37 (m, 4H), 3.10 (s, 6H), 2.01−1.86 (m, 2H), 1.86−1.71 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 156.89, 138.90, 129.79, 122.79, 112.77, 86.94, 67.81, 64.64, 63.76, 54.92, 50.92, 25.58, 19.05. HRMS (ESI) m/z: [M$^+$] ($C_{14}H_{23}INO_2^+$) calcd, 364.0768; found, 364.0752. HPLC-UV purity 97.7%.

*(3-Hydroxypropyl)-[4-(2-iodophenoxy)butyl]-dimethylammonium Bromide (**40**).* General procedure A was used. The alkylating agent **M** (300 mg, 0.845 mmol, 1 equiv, see the Supporting Information) and 3-(dimethylamino)propanol (2 mL) were used for the synthesis of compound **40**. The reaction time was 1.5 h at a temperature of 75 °C. The product **40** was obtained as a white solid (343 mg, 0.749 mmol, 89%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.77 (dd, J = 7.7, 1.6 Hz, 1H), 7.36 (ddd, J = 8.3, 7.4, 1.6 Hz, 1H), 7.01 (dd, J = 8.3, 1.3 Hz, 1H), 6.76 (ddd, J = 7.7, 7.4, 1.3 Hz, 1H), 4.79 (t, J = 5.0 Hz, 1H), 4.08 (t, J = 5.8 Hz, 2H), 3.52−3.30 (m, 6H), 3.04 (s, 6H), 1.98−1.72 (m, 6H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 156.87, 138.88, 129.77, 122.77, 112.76, 86.88, 67.79, 62.60, 61.18, 57.64, 50.21, 25.53, 25.31, 18.92. HRMS (ESI) m/z: [M$^+$] ($C_{15}H_{25}INO_2^+$) calcd, 378.09245; found, 378.09221. HPLC-UV purity 97.4%.

*(4-Hydroxybutyl)-[4-(2-iodophenoxy)butyl]-dimethylammonium Bromide (**41**).* General procedure A was used. The alkylating agent **M** (300 mg, 0.845 mmol, 1 equiv, see the Supporting Information), 4-(dimethylamino)butanol (396 μL, 2.96 mmol, 3.5 equiv), and DMF (1.5 mL) were used for the synthesis of compound **41**. The reaction time was 2 h at a temperature of 70 °C. The product **41** was obtained as a white amorphous solid (295 mg, 0.625 mmol, 74%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.77 (dd, J = 7.7, 1.6 Hz, 1H), 7.36 (ddd, J = 8.3, 7.4, 1.6 Hz, 1H), 7.01 (dd, J = 8.3, 1.3 Hz, 1H), 6.76 (ddd, J = 7.7, 7.4, 1.3 Hz, 1H), 4.57 (t, J = 5.0 Hz, 1H), 4.08 (t, J = 5.7 Hz, 2H), 3.47−3.24 (m, 6H), 3.02 (s, 6H), 1.97−1.84 (m, 2H), 1.84−1.64 (m, 4H), 1.49−1.37 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 156.86, 138.88, 129.79, 122.77, 112.73, 86.81, 67.73, 62.91, 62.52, 59.87, 50.11, 29.09, 25.53, 18.92, 18.78. HRMS (ESI) m/z: [M$^+$] ($C_{16}H_{27}INO_2^+$) calcd, 392.10810; found, 392.10791. HPLC-UV purity 96.2%.

*1-(2-Hydroxyethyl)-1-[4-(2-iodophenoxy)butyl]pyrrolidin-1-ium Bromide (**42**).* General procedure A was used. The alkylating agent **M** (355 mg, 1.00 mmol, 1 equiv, see the Supporting Information), DMF (1 mL), and 2-(pyrrolidin-1-yl)ethanol (468 μL, 4.00 mmol, 4.0 equiv) were used for the synthesis of compound **42**. The reaction time was 2.5 h at a temperature of 70 °C. The product **42** was obtained as a yellow viscous liquid (388 mg, 0.83 mmol, 83%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.76 (dd, J = 7.7, 1.6 Hz, 1H), 7.35 (ddd, J = 8.3, 7.4, 1.6 Hz, 1H), 7.01 (dd, J = 8.3, 1.3 Hz, 1H), 6.78−6.72 (m, 1H), 5.28 (t, J = 5.1 Hz, 1H), 4.07 (t, J = 5.9 Hz, 2H), 3.86−3.77 (m, 2H), 3.72−3.59 (m, 2H), 3.59−3.38 (m, 6H), 2.14−2.00 (m, 4H), 1.99−1.85 (m, 2H) 1.85−1.73 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 156.89, 138.88, 129.78, 122.75, 112.73, 86.94, 67.82, 62.80, 60.10, 59.03, 55.44, 25.59, 21.19, 19.81. HRMS (ESI) m/z: [M$^+$] ($C_{16}H_{25}INO_2^+$) calcd, 390.09245; found, 390.09230. HPLC-UV purity 97.4%.

*(2-Hydroxyethyl)-[6-(3-iodophenoxy)hexyl]-dimethylammonium Bromide (**43**).* General procedure A was used. The alkylating agent **N** (350 mg, 0.914 mmol, 1 equiv, see the Supporting Information), DMF (1 mL), and 2-(dimethylamino)ethanol (552 μL, 5.48 mmol, 6.0 equiv) were used for the synthesis of compound **43**. The reaction time was 2 h at a temperature of 70 °C. The product **43** was obtained as a white solid (395 mg, 0.84 mmol, 92%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.30−7.24 (m, 2H), 7.10−7.03 (m, 1H), 6.98−6.91 (m, 1H), 5.27 (t, J = 5.0 Hz, 1H), 3.96 (t, J = 6.4 Hz, 2H), 3.87−3.76 (m, 2H), 3.46−3.28 (m, 4H), 3.07 (s, 6H), 1.78−1.62 (m, 4H), 1.51−1.39 (m, 2H), 1.39−1.24 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 159.37, 131.34, 129.25, 122.87, 114.33, 95.20, 67.51, 64.53, 63.96, 54.89, 50.81, 28.26, 25.45, 24.97, 21.72. HRMS (ESI) m/z: [M$^+$] ($C_{16}H_{27}INO_2^+$) calcd, 392.1081; found, 392.1066. HPLC-UV purity 96.5%.

*1-(2-Hydroxyethyl)-1-[6-(3-iodophenoxy)hexyl]pyrrolidin-1-ium Bromide (**44**).* General procedure A was used. The alkylating agent **N** (350 mg, 0.914 mmol, 1 equiv, see the Supporting Information), DMF (1 mL), and 2-(pyrrolidin-1-yl)ethanol (320 μL, 2.74 mmol, 3.0 equiv) were used for the synthesis of compound **44**. The reaction time was 2 h at a temperature of 70 °C. The product **44** was obtained as a pale-yellow solid (399 mg, 0.88 mmol, 80%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.30−7.24 (m, 2H), 7.10−7.02 (m, 1H), 6.98−6.91 (m, 1H), 5.28 (t, J = 5.1 Hz, 1H), 3.96 (t, J = 6.4 Hz, 2H), 3.85−3.73 (m, 2H), 3.65−3.44 (m, 4H), 3.42−3.26 (m, 4H), 2.11−1.97 (m, 4H), 1.78−1.62 (m, 4H), 1.51−1.39 (m, 2H), 1.39−1.25 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 159.37, 131.33, 129.24, 122.87, 114.33, 95.19, 67.51, 62.62, 60.00, 59.30, 55.43, 28.29, 25.51, 24.95, 22.50, 21.11. HRMS (ESI) m/z: [M$^+$] ($C_{18}H_{29}INO_2^+$) calcd, 418.1237; found, 418.1227. HPLC-UV purity 97.8%.

*(2-Hydroxyethyl)-{6-[(2-iodopyridin-3-yl)oxy]hexyl}-dimethylammonium 4-Methylbenzenesulfonate (**45**).* The alkylating agent **O** (200 mg, 0.521 mmol, 1 equiv, see the Supporting Information) and 2-(dimethylamino)ethanol (2.00 mL, 19.9 mmol, 38.2 equiv) were stirred at a temperature of 75 °C for 40 min. The product was converted to 4-methylbenzenesulfonate salt by anion-exchange resin. Compound **45** was obtained as a colorless viscous liquid (240 mg, 0.51 mmol, 97%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.95 (ddd, J = 3.4, 2.6, 0.8 Hz, 1H), 7.52−7.44 (m, 2H), 7.34−7.33 (m, 2H), 7.12−7.10 (m, 2H), 5.27 (t, J = 4.9 Hz, 1H), 4.08 (t, J = 6.2 Hz, 2H), 3.81 (br s, 2H), 3.39−3.36 (m, 2H), 3.34−3.29 (m, 2H), 3.05 (s, 6H), 2.28 (s, 3H), 1.80−1.74 (m, 2H), 1.73−1.67 (m, 2H), 1.56−1.49 (m, 2H), 1.38−1.30 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 154.24, 145.76, 142.62, 137.60, 128.05, 125.49, 124.15, 119.02, 112.28, 68.72, 64.63, 64.09, 54.96, 50.80, 28.03, 25.41, 25.09, 21.78, 20.77. HRMS (ESI) m/z: [M$^+$] ($C_{15}H_{26}IN_2O_2^+$) calcd, 393.10335; found, 393.10294. HPLC-UV purity 99.96%.

*(2-Hydroxyethyl)-{4-[(2-iodo-6-methylpyridin-3-yl)oxy]butyl}-dimethylammonium Bromide (**46**).* The alkylating agent **P** (200 mg, 0.540 mmol, 1 equiv, see the Supporting Information) and 2-(dimethylamino)ethanol (2.00 mL, 19.9 mmol, 36.9 equiv) were stirred at a temperature of 75 °C for 40 min. Compound **46** was obtained as a colorless viscous liquid (246 mg, 0.540 mmol, 99%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.26 (d, J = 8.2 Hz, 1H), 7.19 (d, J = 8.2 Hz, 1H), 5.29 (t, J = 4.9 Hz, 1H), 4.08 (t, J = 6.3 Hz, 2H), 3.90−3.76 (m, 2H), 3.52−3.32 (m, 4H), 3.10 (s, 6H), 2.36 (s, 3H), 1.97−1.84 (m, 2H), 1.82−1.69 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 151.99, 151.27, 123.16, 119.81, 111.23, 68.12, 64.54, 63.56, 54.82, 50.84, 25.32, 22.30, 18.88. HRMS (ESI) m/z: [M$^+$] ($C_{14}H_{24}IN_2O_2^+$) calcd, 379.08770; found, 379.08744. HPLC-UV purity 99.7%.

*(2-Hydroxyethyl)-{4-[(3-iodo-[1,1′-biphenyl]-4-yl)oxy]butyl}-dimethylammonium Bromide (**47**).* The alkylating agent **Q** (200 mg, 0.464 mmol, 1 equiv, see the Supporting Information) and 2-(dimethylamino)ethanol (2.00 mL, 19.9 mmol, 44.2 equiv) were used for the synthesis of compound **47**. The reaction time was 40 min at a temperature of 75 °C. The product **47** was obtained as a colorless viscous liquid, which crystallized upon cooling (238 mg, 0.457 mmol, 99%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.04 (d, J = 2.3 Hz, 1H), 7.66 (dd, J = 8.5, 2.3 Hz, 1H), 7.64−7.57 (m, J = 7.3, 1.3 Hz, 2H), 7.47−7.39 (m, 2H), 7.36−7.30 (m, 1H), 7.09 (d, J = 8.5 Hz, 1H), 5.30 (t, J = 5.0 Hz, 1H), 4.13 (t, J = 5.9 Hz, 2H), 3.90−3.81 (m, 2H), 3.55−3.39 (m, 4H), 3.12 (s, 6H), 2.03−1.88 (m, 2H), 1.87−1.70 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 156.56, 138.36, 136.72, 134.76, 128.96, 127.98, 127.23, 126.33, 112.93, 87.80, 68.06, 64.64, 63.76, 54.93, 50.94, 25.57, 19.05. HRMS (ESI) m/z: [M$^+$] ($C_{20}H_{27}INO_2^+$) calcd, 440.10777; found, 440.10810. HPLC-UV purity 98.8%.

*(2-Hydroxyethyl)-[6-(4-iodo-3-nitrophenoxy)hexyl]-dimethylammonium Bromide (**48**).* The alkylating agent **R2** (300 mg, 0.700 mmol, 1 equiv, see the Supporting Information) and 2-(dimethylamino)ethanol (2.00 mL, 19.9 mmol, 28.4 equiv) were used for the synthesis of compound **48**. The reaction time was 40 min at a temperature of 75 °C. The product **48** was obtained as a bright yellow solid (362 mg, 0.70 mmol, 100%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.92 (d, J = 8.7 Hz, 1H), 7.52 (d, J = 2.9 Hz, 1H), 7.04

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(dd, J = 8.7, 2.9 Hz, 1H), 5.27 (t, J = 5.0 Hz, 1H), 4.04 (t, J = 6.4 Hz, 2H), 3.87−3.76 (m, 2H), 3.46−3.29 (m, 4H), 3.08 (s, 6H), 1.80−1.63 (m, 4H), 1.51−1.38 (m, 2H), 1.38−1.24 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 158.97, 154.15, 141.32, 120.68, 110.87, 76.49, 68.38, 64.52, 63.94, 54.88, 50.82, 28.02, 25.41, 24.86, 21.70. HRMS (ESI) m/z: [M$^+$] ($C_{16}H_{26}IN_2O_4^+$) calcd, 437.09318; found, 437.09299. HPLC-UV purity 98.3%.

*(3-Hydroxypropyl)-[6-(4-iodo-3-nitrophenoxy)hexyl]-dimethylammonium Bromide (**49**).* The alkylating agent **R2** (150 mg, 0.350 mmol, 1 equiv, see the Supporting Information) and 3-(dimethylamino)propanol (2 mL) were used for the synthesis of compound **49**. The reaction time was 1.5 h at a temperature of 75 °C. The product **49** was obtained as a yellow solid (183 mg, 0.344 mmol, 98%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.93 (d, J = 8.8 Hz, 1H), 7.54 (d, J = 2.9 Hz, 1H), 7.04 (dd, J = 8.8, 2.9 Hz, 1H), 4.79 (t, J = 5.0 Hz, 1H), 4.05 (t, J = 6.4 Hz, 2H), 3.52−3.43 (m, 2H), 3.33−3.19 (m, 4H), 3.00 (s, 6H), 1.88−1.59 (m, 6H), 1.53−1.39 (m, 2H), 1.39−1.25 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 158.97, 154.14, 141.32, 120.66, 110.87, 76.28, 68.38, 62.79, 61.07, 57.59, 50.09, 28.02, 25.39, 25.30, 24.86, 21.58. HRMS (ESI) m/z: [M$^+$] ($C_{17}H_{28}IN_2O_4^+$) calcd, 451.10883; found, 451.10872. HPLC-UV purity 97.9%.

*(4-Hydroxybutyl)-[6-(4-iodo-3-nitrophenoxy)hexyl]-dimethylammonium Bromide (**50**).* The alkylating agent **R2** (150 mg, 0.350 mmol, 1 equiv, see the Supporting Information), 4-(dimethylamino)-butanol (164 μL, 1.23 mmol, 3.5 equiv), and DMF (1.5 mL) were used for the synthesis of compound **50**. The reaction time was 2 h at a temperature of 70 °C. The product **50** was obtained as a yellow solid (173 mg, 0.318 mmol, 91%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.93 (d, J = 8.8 Hz, 1H), 7.54 (d, J = 2.9 Hz, 1H), 7.04 (dd, J = 8.8, 2.9 Hz, 1H), 4.57 (t, J = 5.0 Hz, 1H), 4.05 (t, J = 6.4 Hz, 2H), 3.49−3.39 (m, 2H), 3.29−3.18 (m, 4H), 2.99 (s, 6H), 1.79−1.60 (m, 6H), 1.51−1.26 (m, 6H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 158.97, 154.14, 141.31, 120.66, 110.87, 76.34, 68.37, 62.88, 62.82, 59.80, 49.92, 29.04, 28.02, 25.41, 24.85, 21.60, 18.71. HRMS (ESI) m/z: [M$^+$] ($C_{18}H_{30}IN_2O_4^+$) calcd, 465.12448; found, 465.12405. HPLC-UV purity 98.4%.

*(2-Hydroxyethyl)-[6-(3-tributylstannylphenoxy)hexyl]-dimethylammonium Bromide (**51**).* (A) 3-Iodophenol (1.50 g, 6.82 mmol, 1 equiv) and $Pd_2(dba)_3$ (156 mg, 0.171 mmol, 2.5 mol %) were put into a flask equipped with a magnetic stir bar, and the flask was closed with a rubber septum and was put under argon atmosphere *via* four cycles of vacuum−argon. DMF (15 mL), 1,4-dioxane (5 mL), and *N*,*N*-diisopropylethylamine (DIPEA) (3.00 mL, 17.2 mmol, 2.5 equiv) were added through the septum, finally followed by Sn_2Bu_6 (3.79 mL, 7.50 mmol, 1.1 equiv). The reaction mixture was stirred at rt for 3 h after which a TLC was run (10% EtOAc in hexane) showing approx. 50% conversion of the starting material. The reaction temperature was increased to 55 °C for another 2 h. Subsequently, the reaction mixture was evaporated *in vacuo*, and the 3-(tributylstannyl)phenol was isolated by flash chromatography on silica (gradient 0−30% EtOAc in cyclohexane) as a colorless oil (990 mg, 2.58 mmol, 38%).

(B) 3-(Tributylstannyl)phenol (384 mg, 1.00 mmol, 1 equiv) was reacted with 1,6-dibromohexane (920 μL, 1.46 g, 6 equiv) and K_2CO_3 (415 mg, 3.00 mmol, 3 equiv) in DMF (1.5 mL) under an argon atmosphere at rt overnight. The reaction mixture was then diluted with Et_2O and filtered through a syringe filter, and the solvents were removed *in vacuo*. The desired product {3-[(6-bromohexyl)oxy]-phenyl}tributylstannane could not be purified from the excess 1,6-dibromohexane even after 2 consecutive runs of flash chromatography (silica, gradient 0−15% EtOAc/cyclohexane) and was used as a mixture for the final step.

(C) The mixture of {3-[(6-bromohexyl)oxy]phenyl}-tributylstannane and 1,6-dibromohexane obtained at step (B) was reacted with excess 2-(dimethylamino)ethanol (1.5 mL) in DMF (5 mL) at 75 °C for 90 min. After that, all volatiles were exhaustively removed under reduced pressure, the remaining white solid was washed with DCM, and the resulting DCM suspension was filtered through a syringe filter to remove the insoluble dicationic product of the reaction between 1,6-dibromohexane and 2-(dimethylamino)-ethanol. The DCM solution was evaporated, and compound **51** (318 mg) was isolated as a colorless viscous liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.23 (dd, J = 8.2, 7.1 Hz, 1H), 7.01 (d, J = 7.1 Hz, 1H), 6.97 (d, J = 2.7 Hz, 1H), 6.80 (ddd, J = 8.2, 2.7, 0.9 Hz, 1H), 4.17−4.11 (m, 2H), 3.94 (t, J = 6.2 Hz, 2H), 3.77−3.72 (m, 2H), 3.59−3.53 (m, 2H), 3.35 (s, 6H), 1.85−1.75 (m, 4H), 1.61−1.42 (m, 10H), 1.37−1.24 (m, 6H), 1.07−1.00 (m, 6H), 0.87 (t, J = 7.3 Hz, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 158.44, 143.66, 128.94, 128.86, 122.62, 113.75, 67.30, 66.24, 65.89, 56.09, 52.32, 29.25, 29.20, 27.50, 26.19, 25.92, 22.98, 13.83, 9.73. MS (ESI) m/z: [M$^+$] ($C_{28}H_{54}NO_2Sn^+$) calcd, 556.32; found, 556.33.

Synthesis of Radiolabeled Choline Derivatives. *General Procedure for Isotopic Exchange Labeling Using Pd Catalyst.* Solution A: To a 2 mL microcentrifuge tube were added $PdCl_2$ (1 mg) and acetonitrile (ACN) (1 mL), and the suspension was sonicated in an ultrasonic bath for 15 min. The suspension was then centrifuged, and the solution was decanted from undissolved $PdCl_2$. Solution B: AA (30 mg) in deionized water (1 mL). Solution C: Corresponding iodinated choline derivative (1 mg) in deionized water (100 μL). A 0.5 mL microcentrifuge tube was charged with the solutions A (4 μL), B (3 μL), and C (1 μL). The mixture was gently shaken, left at rt for 15 min, and centrifuged. Then, a solution of $Na^{125}I$ (14 μL, approx. 60 MBq, no-carrier-added) was added, and the reaction mixture was gently shaken. The tube was sealed with parafilm, centrifuged, and immersed into a water bath heated to 75 °C. After 1 h, the reaction mixture was centrifuged, and was again immersed into a water bath heated to 75 °C. After another 1 h, the reaction mixture was cooled to rt and diluted with water (100 μL), and five beads of Amberlite IRA-900 anion-exchange resin in TsO^- cycle and a piece of silver wire were added in order to remove free $Na^{125}I$ and other inorganic iodine species from the radiolabeled product. The tube was then placed in a shaker for 5 min. Subsequently, the tube was centrifuged, beads and silver wire were decanted, the liquid was removed and transferred into a different microcentrifuge tube, the beads were washed with deionized water (2 × 80 μL), and the tube containing beads was centrifuged and another portion of radiolabeled product solution was collected. The washing solutions were added to the primary solution, yielding radiolabeled product (radiochemical yield was typically 40−80%, molar activity ≈ 1−3 GBq·μmol^{-1}) in radiochemical purity ≥95% according to radio-HPLC analyses using a C_{18} silica reverse-phase column (see the Methods section).

*Synthesis of High-Molar-Activity [**^{123}I**]-**43**-HSA by Iododestannylation.* Stannylated precursor **51** (5.0 mg, 7.87 μmol) was dissolved in MeOH (5 mL) and passed through a glass column containing a strong anion-exchange resin in Cl^- cycle (Amberlite IRA 900-Cl, 1.5 mL, 1.5 mequiv), and the methanolic solution of **51**-chloride was collected and immediately used for labeling. A 0.5 mL Eppendorf tube was loaded with the MeOH solution of **51**-chloride (2 μL, 3.15 nmol), phosphate-buffered saline (PBS) (10 μL), and EtOH (10 μL). Subsequently, $Na^{123}I$ (2 μL, 85 MBq, no-carrier-added) was added, immediately followed by chloramine-T solution in PBS (10 μL, 5 mg·mL^{-1}). The reaction mixture was left at rt for 15 min after which a solution of AA in PBS was added (10 μL, 50 mg·mL^{-1}). After another 10 min, six beads of Amberlite IRA 900-Cl were added and the Eppendorf tube was placed into a shaker (removal of free $^{123}I^-$). After 10 min, the suspension was centrifuged, the solution containing the labeled compound was collected using a pipette, the anion-exchange resin was washed with deionized water (35 μL), and the solutions containing the radiolabeled product were combined [**^{123}I**]-**43-HSA** (62.7 MBq, radiochemical yield 74%, molar activity ≥ 20 GBq·μmol^{-1}, radiochemical purity > 95% according to radio-HPLC analyses using C_{18} silica reverse-phase column) (see the Methods section, for radiochromatogram see the Supporting Information).

METHODS

^{1}H and ^{13}C NMR. ^{1}H and ^{13}C NMR spectra were acquired using a Bruker Avance III 600 spectrometer operating at 600.2 MHz (150.9 MHz), an Avance III HD 400 spectrometer operating at 400.1 MHz

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(100.6 MHz), an Avance DPX 300 spectrometer operating at 300.1 MHz (75.5 MHz), and JEOL ECZ 500R at 500.2 MHz (125.8 MHz).

High-Resolution MS Spectra. High-resolution MS (HRMS) spectra were recorded on an LTQ Orbitrap XL using electrospray ionization (ESI).

IR Spectrometry. Infrared (IR) spectra were measured by the attenuated total reflection technique on a Fourier transform IR spectrometer Nexus Nicolet 870 with a diamond prism.

HPLC Analyses. Analyses were performed on an HPLC chromatograph (Dionex UltiMate 3000, Dionex, USA) using a reverse-phase column (Chromolith Performance RP-18 endcapped 100 × 4.6 mm column equipped with Chromolith RP-18 endcapped 5 × 4.6 mm guard cartridge, Merck, Germany) and UV detection at 225 nm. A FlowStar LB 513 radio flow detector (Berthold Technologies, Germany) was used for the in-line radioactivity measurements. The following elution conditions were used for chemical purity determination of compounds **1**−**50**: phase A: 95% water, 5% ACN + 0.1% TFA; phase B: 5% water, 95% ACN + 0.1% TFA. Flow rate 0.00−0.25 min: 0.40−3.00 mL/min; 0.40−7.00 min: 3.00 mL/min. Mobile phase composition—0.00−0.25 min: 9% B; 0.25−5.50 min: linear gradient 9−70% B; 5.50−6.50 min: linear gradient 70−100% B; 7.00 min: 100% B.

Elemental Analyses. Elemental analyses for C/H/N were performed on a PE 2400 Series II analyzer (Perkin Elmer, USA). Elemental analyses for sulfur and iodine were performed on a X-ray fluorescence spectrometer SPECTRO iQ II (SPECTRO Analytical Instruments, Germany).

***In Vitro* Pharmacokinetic Properties (ADME).** The analysis of all samples in ADME assays was performed using a Agilent RapidFire 300 high-throughput mass spectrometry system (RF-MS, Agilent, Wakefield, MA) with subsequent detection in the mass spectrometer QTRAP 5500 (AB Sciex, Concord, Canada).

Chemical Stability Assay. The tested compounds from stock solution (10 mM in DMSO) were diluted using PBS (pH 7.4) for final incubation concentration 2 μM. The time points of incubation were 15, 30, 60, and 120 min at 37 °C. The incubation was stopped by addition of cold methanol in the corresponding time points. The samples were then vortexed and centrifuged (4000 rpm, 9 min, 4 °C), and the supernatant was freeze-dried. After freeze-drying the supernatant, the samples were dissolved in water with internal standard and were analyzed by RF-MS analysis.[55]

***In Vitro* Stability in Human Plasma.** The studied compounds (final incubation concentration 2 μM; DMSO concentration did not exceed 0.1%) were incubated with human plasma (Transfusion Department, University Hospital Olomouc, Olomouc, Czech Republic) at five different time points (0, 15, 30, 60, and 120 min) at 37 °C. The reactions were terminated by the addition of ACN−methanol mixture (2:1). The samples were left at −80 °C overnight, followed by centrifugation, freeze-drying, and analysis by RF-MS.[55] The RapidFire RF300 system (Agilent Technologies) was interfaced with the QTRAP 5500 (AB Sciex) mass spectrometer fitted with an ESI source and was run in the multiple reaction monitoring mode. The freeze-dried samples were redissolved in solvent A (95% water/5% ACN/0.01% formic acid) and aspirated into a 10 μL sample loop and passed through a C4 cartridge (Agilent Technologies) with solvent A (95% water/5% ACN/0.01% formic acid) at a flow rate of 1.5 mL·min^{-1} for 3 s. After the desalting step, the analyte retained on the cartridge was eluted with solvent B (95% ACN/5% water/0.01% formic acid) to the mass spectrometer at a flow rate of 0.4 mL·min^{-1} for 7 s. MS was carried out using ESI in the positive ion mode. Daughter ion peaks were identified using a multiple reaction monitoring protocol.

Microsomal Stability Assay. Reaction mixtures included the tested compound (2 μM), human liver microsomes (Thermo Fisher Scientific, 0.5 mg·mL^{-1}), and nicotinamide adenine dinucleotide phosphate-generating system consisting of $NADP^+$, isocitrate dehydrogenase, isocitric acid, and $MgSO_4$ in 0.1 M K_3PO_4 buffer. The mixture was incubated at 37 °C, and samples were collected in the following time points: 0, 15, 30, and 60 min. The microsomal activity was terminated using the ACN−methanol (2:1) mixture, followed by centrifugation (3000 rpm, 6 min, 4 °C) and the supernatant was freeze-dried for subsequent analysis. The freeze-dried samples were dissolved in water containing internal standard and were analyzed by RF-MS.[55] The intrinsic clearance was calculated using the formula: $CL_{int} = V\cdot(0.693/t_{1/2})$, where V is the volume of incubation in microliter related to the weight of microsomal protein in mg per reaction. Half-life values ($t_{1/2}$) were calculated using the equation $t_{1/2} = 0.693/k$, where k is the slope of the ln of the percent compound remaining versus time curve.[55]

Parallel Artificial Membrane Permeability Assay. The PAMPA was performed with the Millipore MultiScreen filter MultiScreen-IP Durapore 0.45 μm plates and receiver plates (Merck Millipore). The assay was performed according to the Merck Millipore protocol (PC040EN00). The tested compounds from stock solution (10 mM in DMSO) were diluted using PBS to a concentration of 20 μM (initiator solution). Briefly, the initiator solution was added to the donor wells. The filter membrane was coated with 10% lecithin (Sigma-Aldrich) in dodecane, and the acceptor wells were filled with PBS (pH 7.4). The acceptor filter plate was carefully placed on the donor plate to form a "sandwich".[56] Time of incubation was 18 h at rt. After incubation, 120 μL aliquots of acceptor and donor solutions were removed to the 96-well plate and freeze-dried, and the residues were dissolved in 200 μL of water prior the RF-MS analysis. The relative permeability log Pe was calculated by using the equation: $\log Pe = \log\{C \times -\ln(1 - \text{drugA}/\text{drugE})\}$, where $C = (V_A \times V_D)/\{(V_D + V_A) \times A \times T\}$. V_D and V_A are the volumes of the donor and acceptor solutions, A is the active surface area in cm^2, T is time of the incubation in seconds, and drugA and drugE are the mass of the compound in the acceptor solution and in the solution in theoretical equilibrium (as if the donor and acceptor were combined), respectively.

Plasma Protein Binding. Determination of PPB was carried out using the rapid equilibrium dialysis (Thermo Scientific, Rockford, USA) test, which is based on dialysis.[57] Inserts for this assay consist of two chambers (red and white) separated by a semipermeable membrane. Into the chamber R was added 10 μM solution of the tested substance in 10% plasma, and into the chamber W was added PBS buffer. The substance in the chambers was incubated at 37 °C (shaking) for 4 h. After incubation, equal volumes of solution were transferred into microtubes. To the PBS buffer was added the same amount of 10% plasma, and to the solution of the tested substance was added the PBS buffer to normalize their composition. The reaction was terminated by the addition of ACN−methanol (2:1). Following centrifugation (3000 rpm, 6 min, 4 °C), the supernatant was freeze-dried, diluted with water, and analyzed by RF-MS.

Determination of IC_{50} Values *In Vitro*. We employed human prostate cancer cell line PC-3 and human glioblastoma multiforme cell line U-87 MG, both purchased from ATCC (Manassas, Virginia, USA) as model cell lines. The PC-3 cell line was cultured in F12 Ham medium supplemented with 10% fetal bovine serum, 0.25% sodium bicarbonate, streptomycin, and penicillin. All mentioned components were from Sigma-Aldrich (St. Louis, Missouri, USA). U-87 MG cells were cultivated in Dulbecco's modified Eagle medium (Sigma-Aldrich, Missouri, USA) supplemented with 10% of fetal bovine serum, 0.1 mM nonessential amino acids, and 1.0 mM sodium pyruvate (all Sigma-Aldrich). The cell cultures were propagated at 37 °C in a humidified 5% carbon dioxide atmosphere. The cells were subcultured and used for experiments in confluence of 70−90%. The competition assay was performed in 24-well plates. The assay itself employed special "uptake buffer" instead of standard cell culture media to exclude the effect of choline contained in the culture media. This uptake buffer consisted of 25 mM Tris-HCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5 mM glucose, and 140 mM NaCl in H_2O, pH = 7.4.[39]

The cells in the 24-well plates were washed with PBS buffer and then the [^{3}H]choline (American Radiolabeled Chemicals, St. Louis, Missouri, USA) in uptake buffer was added as substrate for choline uptake, followed by 15 min incubation at 37 °C. Then, the studied choline derivative was added (triplicate wells) in 10 different concentrations to cover concentration range from 1 × 10^{-8} to 5 ×

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10^3 mol·L^{-1}. The cells were then incubated for 60 min at 37 °C after which the buffer was removed. The cells were rinsed with ice-cold PBS buffer and lysed with 0.1 M NaOH. The cell samples were collected for radioactivity and protein content measurement. The protein content was determined using a standard bicinchoninic acid assay (BCA) protein assay (Pierce BCA protein assay kit, Thermo Fisher Scientific). Radioactivity of the cell samples was measured in Tri-Carb liquid scintillation counter (PerkinElmer, Waltham, Massachusetts, USA).

The cellular uptake of [^{3}H]choline was calculated as the percentage of applied dose per gram of cell protein content. These uptake values were used to plot classical sigmoidal dose−response curve in the GraphPad Prism (GraphPad Software, San Diego, California, USA). The IC_{50} values were determined from this curve using fitting analysis by the above-mentioned software.

***Ex Vivo* Biodistribution Studies.** All experiments with animals were performed in accordance with the appropriate legal norms (Act no. 359/2012), with approval of Ministry of Education, Youth and Sports (MSMT-18724/2016-2) and approval of Ethical Committee of Faculty of Medicine and Dentistry, Palacky University in Olomouc. SCID male mice (Harlan, Indianapolis, Indiana, USA) 6−8 weeks old were used in this study. The mice were housed in specific-pathogen-free animal facility during the whole period of the study. For establishing the prostate tumor model, 5 × 10^6 of PC-3 cells resuspended in culture media were injected subcutaneously into the right flank of the mice. The tumor growth was continuously monitored using calipers. When tumor volume reached 100−300 mm^3, that is, 6−8 weeks after the cell inoculation, the mice were included into the *ex vivo* biodistribution study.

The employed choline derivatives (derivatives with IC_{50} comparable to [^{18}F]fluorocholine or better) were radiolabeled with ^{125}I by isotope exchange. The solutions of radioiodinated choline derivatives in distilled water were diluted with sterile saline to achieve the optimal concentration of radioactivity and were applied retro-orbitally.[58] The applied dose varied between 100 and 110 kBq per animal. The mice were anesthetized with isoflurane (4%) 1 h resp. 3 h post injection of the radiotracer and were sacrificed by cervical dislocation. Blood samples were collected from the carotid artery. In addition, the following organs were collected during the autopsy: spleen, pancreas, stomach, intestine, kidneys, liver, heart, lungs, muscle sample, bone sample (femur), brain, thyroid, and tumor. The organ samples were weighed, and their radioactivity was determined by automatic gamma counter Wizard (PerkinElmer, Waltham, Massachusetts, USA). The accumulation of the radiotracer in studied organs was expressed as the percentage of the injected dose per gram of organ or percent of injected dose in the case of the thyroid gland.

***In Vivo* SPECT Imaging.** The SPECT/CT imaging was performed with a small animal PET/SPECT/CT imaging system (Albira, Bruker Biospin Corporation, Woodbridge, CT, USA). During the scans, the mice were anesthetized with 2% isoflurane. Static whole-body SPECT scans of 30 min duration were performed at 1, 3, and 24 h after injection of [^{123}I]-**10** or [^{123}I]-**43** (15−20 MBq/animal, molar activity ≥ 2.5 GBq·μmol^{-1}). In the case of high-molar-activity [^{123}I]-**43-HSA** (15−20 MBq/animal, molar activity ≥ 20 GBq·μmol^{-1}), only 1 and 3 h p.i. scans were performed after which the animals were sacrificed and tracer biodistribution was determined *ex vivo*. The SPECT scans were followed by a double CT of 20 min (axial FOV 2 × 65 mm, 45 kVp, 400 μA, at 400 projections). Reconstruction of the acquired data was done by the Albira software (Bruker Biospin Corporation, Woodbridge, CT, USA) using the ordered subset expectation maximization and filtered backprojection (FBP) algorithms. All two-dimensional (2D) images were prepared using PMOD software, v. 3.307 (PMOD Technologies Ltd., Zurich, Switzerland), and all 3D images are from VolView software (Kitware, Clifton Park, NY, USA).

***In Vivo* PET Imaging.** The studied animals were imaged by μPET/SPECT/CT system Albira (Bruker, Billerica, Massachusetts, USA). Briefly, the mice were retro-orbitally injected with [^{18}F]-fluorocholine (UJV, Rez-Husinec, Czech Republic) at a dose of 5−7 MBq per animal. The animals under inhalation anaesthesia were placed in a prone position in the Albira system. The PET/CT images were acquired over a 30 min period starting 1, 3 h after the injection of the radiopharmaceutical. Shortly, 10 min PET scan (axial FOV 148 mm) was performed, followed by a double CT scan (axial FOV 2 × 65 mm, 45 kVp, 400 μA, at 400 projections). Scan data were reconstructed with the Albira software (Bruker Biospin Corporation, Woodbridge, CT, USA) using the maximum likelihood expectation maximization and FBP algorithms. The PMOD software (PMOD Technologies, Zurich, Switzerland) was used for 2D image output, while 3D images were created in VolView software (Kitware, Clifton Park, NY, USA).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c01710.

Experimental procedures, complete ADME and cytotoxicity data for choline analogues, figures and tables with the complete *ex vivo* biodistribution data, copies of ^{1}H and ^{13}C NMR spectra, and HPLC traces (PDF)

Molecular formula strings with IC_{50} values (CSV)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare the following competing financial interest(s): P.Š., Z.N., J.K., M.P., O.S., M. Hajdúch, and M. Hrubý are inventors of a patent application covering the title compounds.

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■ ABBREVIATIONS

AA, ascorbic acid; ACN, acetonitrile; ADME, absorption, distribution, metabolism, and excretion; BCA, bicinchoninic acid assay; br s, broad singlet; Ch, choline; ChT, choline transporter; log *P*, logarithm of water−octanol partition coefficient; CT, computed tomography; CTL, choline transporter-like protein; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DCM, dichloromethane; DMAE, 2-(dimethylamino)ethanol; DMAP, 4-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethylsulfoxide; ESI, electrospray ionization; FBP, filtered backprojection; FCh, fluorocholine, *N*-(fluoromethyl)-2-hydroxy-*N*,*N*-dimethylethan-1-aminium; HCh-3, hemicholinium-3; HRMS, high-resolution mass spectrometry; IC_{50}, half-maximum inhibitory concentration; LipE, lipophilic efficiency; mp, melting point; MeOTs, methyl tosylate; MRI, magnetic resonance imaging; *n*-BuLi, *n*-butyllithium; NMR, nuclear magnetic resonance; OCT, organic cation transporter; PAMPA, parallel artificial membrane permeability assay; PBS, phosphate-buffered saline; PET, positron-emission tomography; PI3K, phosphoinositide 3-kinase; PPB, plasma protein binding; RED, rapid equilibrium dialysis; RF-MS, RapidFire mass spectrometry; SCID, severe combined immunodeficiency; SPECT, single-photon emission computed tomography; TBAF, tetra-*n*-butylammonium fluoride; TBSCl, *tert*-butyldimethylsilyl chloride; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin-layer chromatography; TsCl, 4-methylbenzene-1-sulfonyl chloride; TsF, 4-methylbenzene-1-sulfonyl fluoride

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