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Abstracts

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Selected Abstracts

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NOVEL ORTHOESTERS IN ORGANIC SYNTHESIS

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A large number of natural products contain, embedded in their complex framework, a fused- or a spiro-bicyclic substructure. The ubiquitous presence of this fragment has stimulated a wealth of research and numerous methodologies have been devised to assemble such subunits.

We have found that easily available, functionalised orthoesters can be particularly well-suited annulating agents, that allow us to assemble readily and efficiently a range of fused and spirobicyclic skeletons, according to the approach depicted in Figure 1.[1(b),1(c)]

This novel methodology has been subsequently employed to prepare several natural products, such as erythrodiene and spirojatamol (Figure 2).[1(a)]

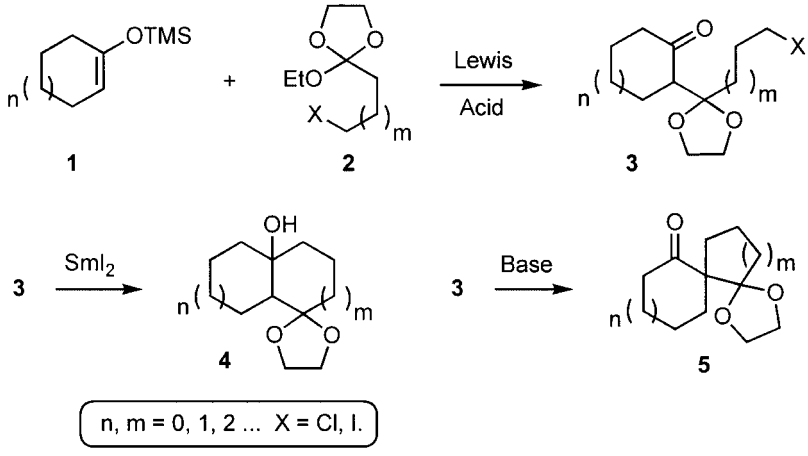

Figure 1.

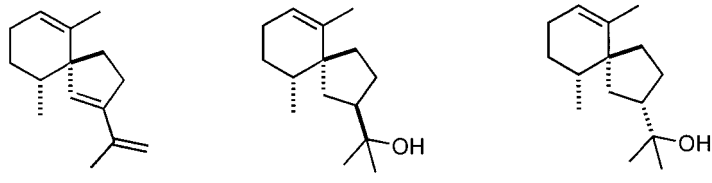

Figure 2.

Reference

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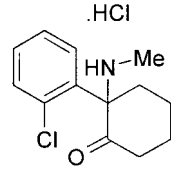
THE SYNTHESIS OF STABLE LABELLED KETAMINE

Simon J. Harwood

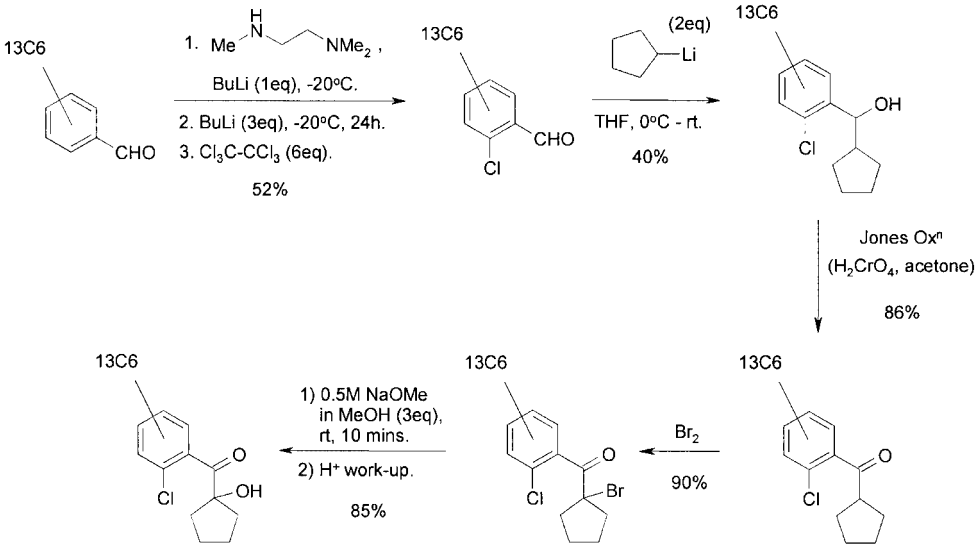
GlaxoSmithKline, UK

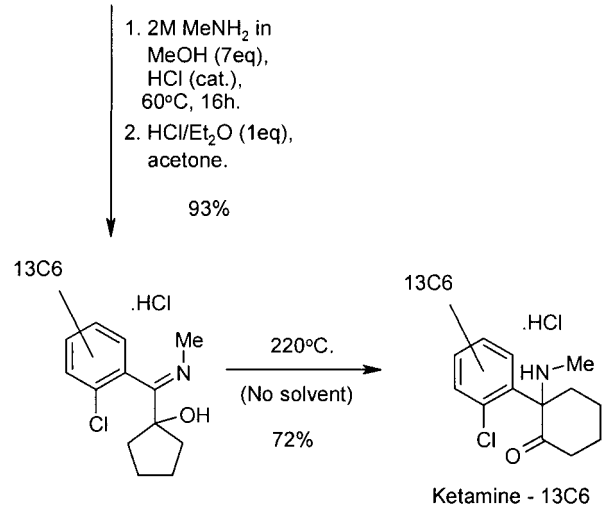
Ketamine hydrochloride (**1**) is a non-barbiturate, rapid-acting disassociative anaesthetic used on both animals and humans; it has also been used in experimental psychotherapy.



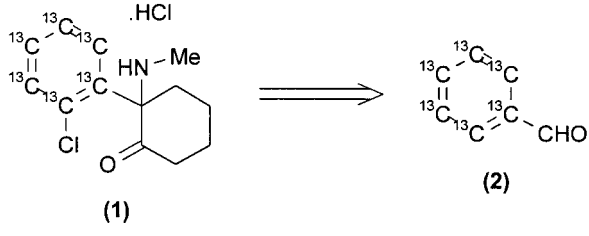
(1)

Stable labelled ketamine hydrochloride was required as an internal standard for use in LCMS assays. The preparation of [$^{13}C_6$]ketamine hydrochloride from commercially available [$^{13}C_6$]benzaldehyde was presented, and the significant synthetic challenges encountered in this work was discussed.





Ketamine hydrochloride is a non-barbiturate, rapid-acting disassociative anesthetic used on both animals and humans; it has also been used in experimental psychotherapy. Stable labelled ketamine hydrochloride was required as an internal standard for use in LCMS assays. The preparation of [$^{13}C_6$]ketamine hydrochloride (**1**) from commercially available [$^{13}C_6$]benzaldehyde (**2**) will be presented, and the synthetic challenges encountered in this work will be discussed.



C-13, C-14, N-15 LABELLING OF THE 4-CYANOBENZYL BROMIDE – A VERSATILE LABELLING BUILDING BLOCK

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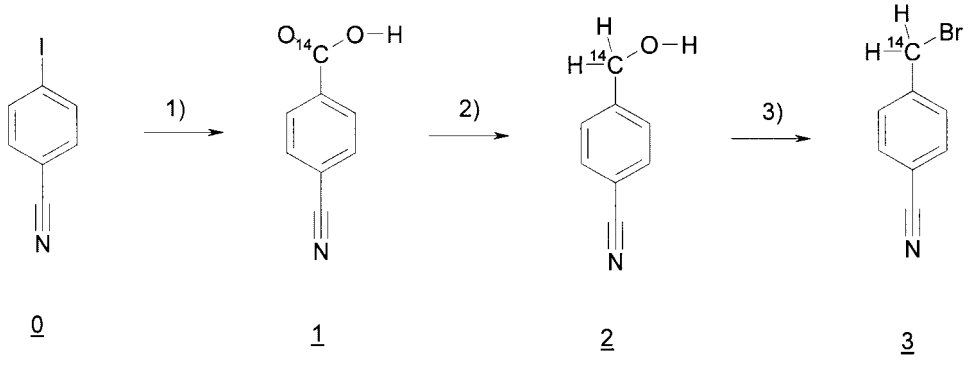
Introduction: For drug development radioactive and stable labelled 4-cyanobenzyl bromide was required as a highly suitable building block. Since ADME-studies excluded the C-14 labelling of the cyano-group and bioanalytical studies required stable labelled drug substance with a minimum of seven mass units, the following isotopoanalogues were prepared (3, 11). The following schemes illustrate our final synthetic strategies.

Results:

C-14 Labelling: Key-step of the labelling sequence is the iodo-magnesium exchange with isopropyl-magnesium chloride.[1] Carboxylation of the intermediate cyano substituted Grignard with $[^{14}C]CO_2$ provides 4-cyano-[^{14}C]benzoic acid 1. When activated with BOP-reagent, reduction to the alcohol 2 is achieved with $NaBH_4$.[2] Final bromination produces the [^{14}C]-4 cyanobenzyl bromide 3, which is shown in the following figure.

Reaction conditions: (1) (a) isopropylmagnesium chloride (2.0 M solution in THF), THF, −40°C, 1 h; (b) $[^{14}C]CO_2$, −78°C, 1 h, −40°C, 2 h, HCl, 67%; (2) benzotriazol-lyloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP-reagent), THF, *N,N*-diisopropylethylamine, $NaBH_4$, RT, 15 h, 100%; (3) N-bromosuccinimide, CH_2Cl_2, triphenylphosphine, −20°C, 20 min, RT, 30 min, flash chromatography, 80%.

C-13, N-15 Labelling: From a number of possible approaches the following strategy was selected due to its technical advantages namely avoiding formation of highly volatile low-molecular weight intermediates.



0 1 2 3

Figure. Synthesis of C-14-labelled 4-Cyano benzylbromide

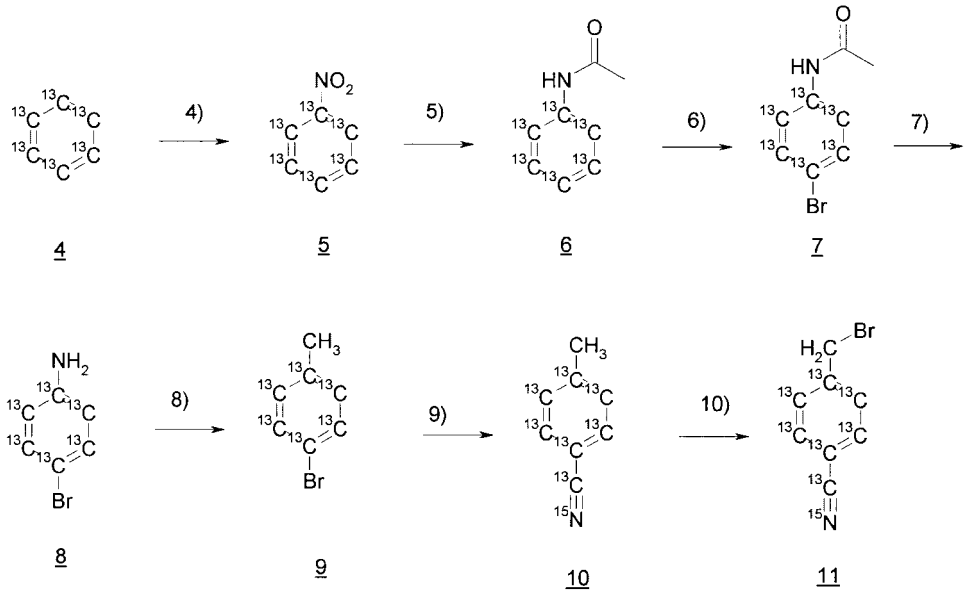


Figure. Synthesis of C-13, N-15 labelled 4-Cyano benzylbromide

Standard procedures provided [$^{13}C_6$] 4-bromoaniline 8, which after conversion into its arenediazonium salt is subject to a Stille-type C–C coupling.[3] In the presence of palladium(II) acetate tetramethyl tin allows highly efficient methyl transfer to form 4-bromo-toluene 9. Pd-catalyzed bromo–cyano exchange using Zn($^{13}C^{15}N)_2$ and radical-assisted benzylic bromination produces the title compound 11 in 23% overall yield as shown in the figure.

Reaction conditions: (4) $NaNO_3$, TFA, 5 h, RT, 81%; (5) 10% Pd/C, Ac_2O, H_2, RT, 16 h, 91%; (6) AcOH, Br_2, 10°C, 20 min, 98%; (7) 6N H_2SO_4, reflux, 5 h, 87%; (8) (a) $NaNO_2$, HPF_6, H_2O, 0°C; (b) CH_3CN, $Pd(OAc)_2$, $Sn(CH_3)_4$, 2 h, RT, 69%; (9) Zn($^{13}C^{15}N)_2$, $Pd(Ph_3)_4$, DMF, reflux, 24 h, 81%; (10) [bmim]PF_6, AIBN, NBS, 65°C.

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SYNTHESIS AND STEREOCONTROL WITH LITHIATED AMIDES

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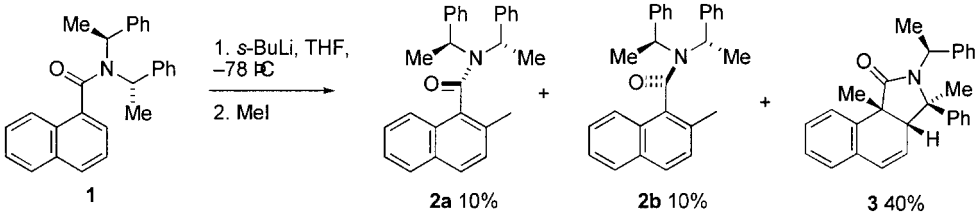
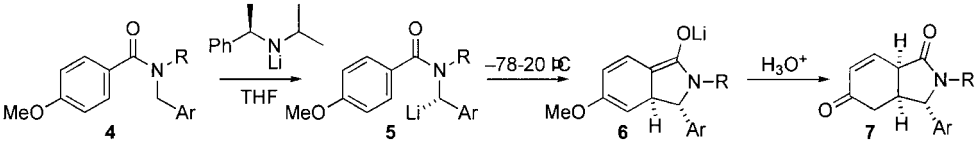
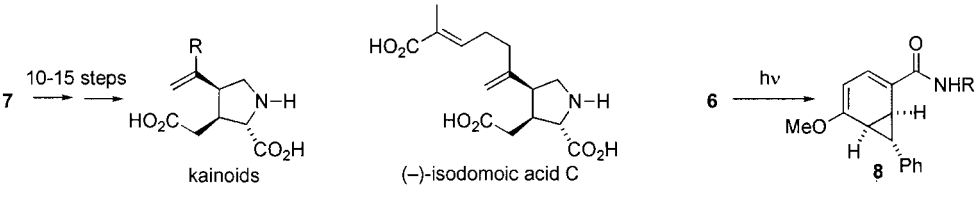
As part of a research program investigating the potential of rotationally restricted amides in synthesis, we carried out an attempted lithiation of **1** aiming to obtain atropisomers of **2**. The major product, however, was the unexpectedly dearomatised tricyclic lactam **3**.[1]



The reaction turns out to be a general route to 6,5-bicyclic lactams,[2] and the use of a chiral lithium amide as the deprotonating agent allows the dearomatising cyclisation to be carried out enantioselectively[3]: **4** gives **7** in up to 84% ee via the configurationally stable intermediate **5**.



The fact that organolithiums cyclise into aromatic rings – even relatively electron-rich ones – has allowed us to develop efficient routes to compounds such as kainoid amino acids[4–7] and tetrahydronaphthalene lignans.[8] For example, a synthesis of the algal metabolite (–)-isodomoic acid C, a neuroactive amino acid implicated in the occurrence of Amnesic Shellfish Poisoning, has recently been completed.[9]



Recent developments in the area of dearomatising cyclisation include the dearomatisation of 5- and 6-membered heterocycles[10,11] and the stereospecific photochemical rearrangement of the enolate products of the cyclisation to yield norcaradiene and cycloheptadienone ring systems.[12] We have also used

deuterium labelling to study mechanisms and control regioselectivity in lithiation reaction via an abnormally large kinetic isotope effect.[13]

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ISOTOPIC LABELLING OF STI571 AND ITS METABOLITES IN THE DEVELOPMENT OF GLEEVEC®

R. Salter, K. Bordeaux, P. Burtscher, Y. Metz, Th. Moenius, I. Rodriguez, R. Ruetsch, R. Voges and C. Zueger

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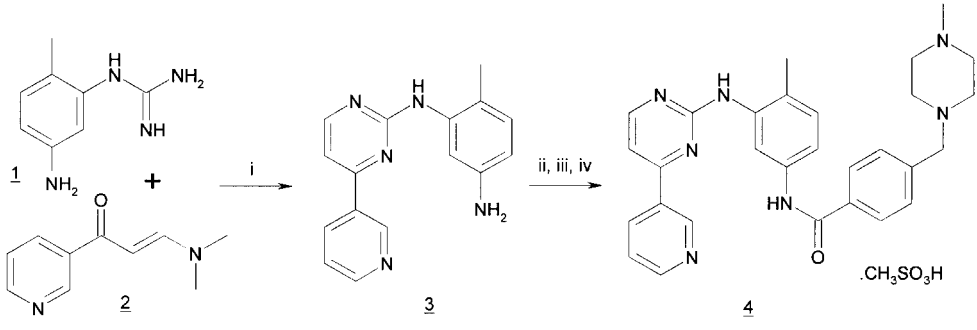
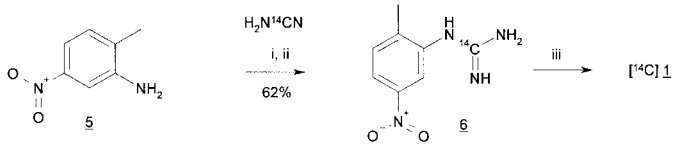
Imatinib mesylate 4 (Gleevec®, Glivec® formerly STI571) has demonstrated unprecedented efficacy for the treatment of chronic myelogenous leukaemia in all phases and is also indicated for metastatic and unresectable malignant gastrointestinal stromal tumours (GIST).[1] In response to development requirements, we describe a number of strategies leading to either isotopically labelled STI571 7, or its subsequent derivatives.

The unlabelled synthesis proceeds according to the following scheme:



(i) *n*-BuOH, 130°C, 3.5 h; (ii) 4-(chloromethyl)-benzoyl chloride, THF, RT, 16 h; (iii) 1-methylpiperazine, EtOH, 45°C, 5 h; (iv) $MeSO_3H$, MeOH.

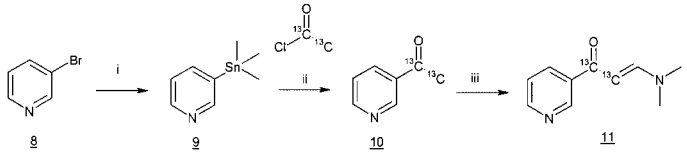
The key to the carbon-14 labelling is the accessability of the [^{14}C]guanidine 1 starting from [^{14}C]labelled cyanamide (in turn from $Ba^{14}CO_3$) and 2-methyl-4-nitroaniline 5, below. The first step in the production route was unsuitable for a radiolabelled synthesis because the yield of 5 from 2.1 equivalents cyanamide was poor (18% due to the deactivating influence of the meta-nitro group) and accompanied by a major by-product urea. To improve yield and circumvent urea formation, we employed anhydrous conditions and converted the aniline to the corresponding hydrochloride salt. In a protic solvent, this activates [^{14}C]cyanamide toward nucleophilic attack by the deactivated aniline 5 leading to an improved yield (65%) of 1 from a single equivalent of [^{14}C]cyanamide. Subsequent reduction of the nitro group gave the required intermediate 1.



(i) HCl (g), dioxane; (ii) *n*-BuOH, 100°C, 6 h; (iii) 10% Pd/C, H_2, *n*-BuOH.

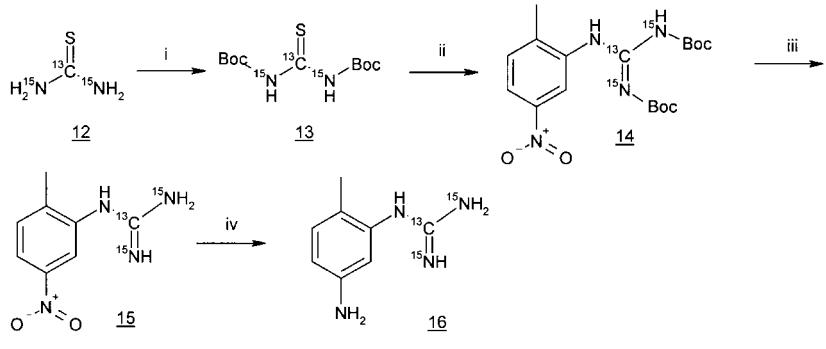
Several radiolabelled metabolites of Gleevec were also synthesized from the parent compound [^{14}C]STI571 by either selective cleavage or selective *N*-oxidation.

Since previously synthesized stable labelled [D_8]STI571 (prepared by coupling of 1-methyl[D_8]piperazine) did not allow quantification of metabolite 3, a corresponding isotopologue labelled in the pyrimidine moeity with a minimum of four mass units was required. The most efficient strategy utilized the following convergent synthesis: stannylation of 3-bromopyridine 8 followed by Pd II catalysed acetylation with commercially available [$^{13}C_2$]acetyl chloride afforded l-pyridine-3-yl-[$^{13}C_2$]ethanone 10 in good yields (60–70%). Subsequent coupling with DMF-dimethylacetal gave the target propenone intermediate 11.



(i) Et_2O, *n*-BuLi, *n*-Bu_3SnCl, −78°C-RT; (ii) $PdCl_2(PPh_3)_2$, C_6H_6, 85°C; (iii) *N*,*N*-DMF-dimethyl acetal, 105°C, 16 h.

The second half of the molecule was constructed from commercially available [^{13}C, $^{15}N_2$] thiourea. Prior activation was necessary by bis-bocylation followed by mercury(II) assisted nucleophilic displacement with the deactivated aniline 5 resulting in excellent yield of the M + 2 Boc-protected guanidine 14.



(i) NaH, THF, Boc_2O, 0°C to RT, 1.5 h; (ii) 2-methyl-4-nitroaniline, $HgCl_2$, DMF, Et_3N, 0°C to RT, 15 min; (iii) CH_2Cl_2, TFA, RT, 1 h; (iv) *n*-BuOH, 10% Pd/C, H_2, RT, 16 h.

Subsequent deprotection gave 15 which on reduction afforded the desired M + 3 fragment 16. Finally, heating 11 and 16 as described in the first scheme, furnished the target metabolite.

This approach shows that formation of guanidines from an electron deficient aniline can be achieved in good yield with economic use of labelled reagents by appropriate choice of reaction conditions and reagents. Furthermore, introduction of the label into the 3-position of pyridine can be efficiently achieved by Pd(II) catalysed coupling with the stannylated precursor.

Our efforts to support the PET study will be reported elsewhere.

Reference

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SYNTHESIS OF LABELLED CARBOHYDRATES

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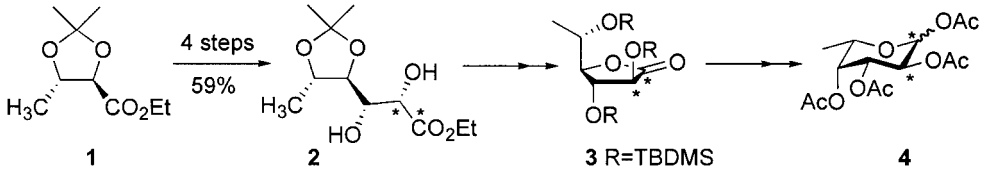
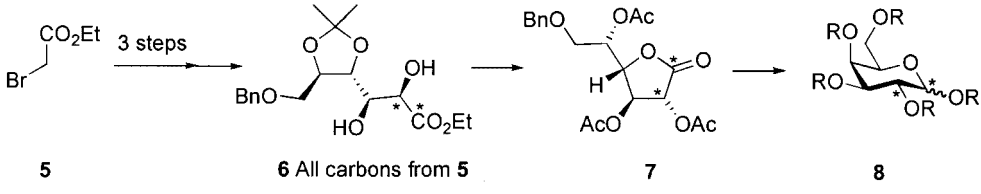
Synthetic routes towards enabling stereoselective synthesis of fucose and galactose with labelling versatility will be described, where a common synthetic route can be applied to synthesis of different ^{13}C labelled targets (e.g. regioisomerically labelled, multi-labelled targets). In this way, a catalogue of ^{13}C isotopomers is viable without a variety of different synthetic routes. The heterogeneity of potential sugar linkage isomers makes availability of a diversity of partly labelled monosaccharides important. Alternatives to the known iterative homologations of lower sugars are needed (which are rather label-target specific and require diastereomer separations and/or specific label isomerizations though possible only for C1–C2 transpositions).

We undertook a synthesis towards L-fucose labelled at C1 and C2, starting from **1**. Wittig reaction enabled introduction of labels, and asymmetric dihydroxylation introduces the remaining L-fucose stereocentres with very high diasterecontrol (matching) giving **2**. Removal of the acetal protecting group with concurrent lactonization could be directed exclusively to the L-fucono-γ-lactone furanone (**3**, after silylation). The structure has been unambiguously confirmed by X-ray analysis of the 1,2-$^{13}C_2$-labelled L-fucono-γ-lactol α-anomer. Reduction to the L-fucono-γ-lactol leads towards the 1.2-$^{13}C_2$-L-fucopyranose **4** *via* desilylation and isomerization (Scheme 1). This chemistry is trivially applicable to synthesis of either 1-^{13}C or 2-^{13}C-L-fucose by employing the available monolabelled ethylbromoacetate isotopomers. The intermediate L-fucono-γ-lactone **3** also relates closely to the enantiomer of the glycone of the antitumour gilvocarcins, and has been employed in other target syntheses.



Scheme 1.

The stereochemical analogy between D-Gal and L-Fuc (L-Fuc being 6-deoxy-L-Gal) allows for a similar tactic to be employed from the oxygenated variant of **1**, prepared in a labelling-versatile manner via **5**, using bromoethylacetate as the ultimate source of all 4 carbons. Since this is available as either mono-^{13}C-labelled isotopomer, or dilabelled, the single route *de facto* provides access to any possible number and location of ^{13}C labels in these 4 carbons (Scheme 2). The intermediate **6** (after protection as its isopropylidine acetal) can then be elaborated towards Gal using chemistry directly analogous to that employed towards L-Fucose from **1**. This approach is shown applied to 1,2-$^{13}C_2$-Gal in Scheme 2.



Scheme 2.

The synthesis of **6** from **5** also enables the introduction of specific deuteration, and intermediates are readily provided which combine regiospecific ^{13}C and ^{2}H labelling.

HOMOGENEOUS CATALYSIS – A POWERFUL TOOL FOR THE SYNTHESIS OF PHARMACEUTICALS

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More than 80% of all products of the chemical industry are made via catalysis. In this regard catalysis is a key factor for achieving a sustainable production of chemicals and pharmaceuticals today and in the future. In the talk it will be shown that homogeneous catalysis enables organic chemists to perform their synthesis more selectively and with improved economics. Examples from our own research in recent years which demonstrate the superiority of catalytic processes compared to more traditional stoichiometric reactions include palladium-catalyzed coupling reactions of aryl chlorides and bromides such as arylation, carbonylation, cyanation, olefination, and amination reactions.[1]

In addition, the importance of catalysis for the discovery and refinement of biologically active compounds will be shown. Areas, which will be discussed here are new catalytic syntheses of amines[2] and new multi component coupling reactions.[3]

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PREPARATION OF ORTHO-RADIOHALOGENATED PHENYLAZIDES AND TRIAZENES, VERSATILE BUILDING BLOCKS FOR INDIRECT LABELLING

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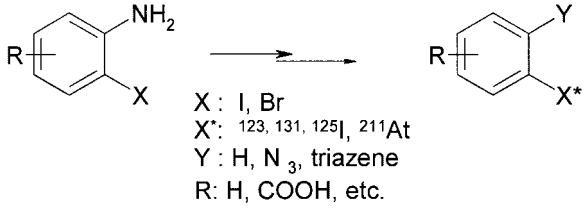
Radiohalogenated derivatives of aromatic azides and triazenes are recognised to be useful building stones for indirect labelling for various applications.[1] The azide group could be easily activated by UV light irradiation and the triazene group, actually protected diazonium salt, by gentle acidification. Classic Wallach method for radiohalogen label incorporation into small molecule consists in the replacement of such nitrogen functional group, followed by binding to desired compound.[2,3] Our approach was to try to invert this strategy – to keep the nitrogen functional group moiety to serve as a connector for conjugation, and to incorporate the label by halogen exchange reaction, especially in the *ortho*-position to the nitrogen moiety (Scheme 1).

In some cases, the so-called *ortho*-effect[4] of these groups probably facilitated the halogen exchange reaction, so that high radiochemical yields were obtained in relatively short reaction period and at mild conditions. With respect to the radiochemical yield, both methods are suitable for preparation of radioiodinated conjugates, but the astatination was achieved only by the triazene displacement.



Scheme 1.

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CONTROLLED POLYDEUTERATION OF ESTROGENIC COMPOUNDS

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Deuterated derivatives are valuable as reference standards in the quantitation of natural compounds. For hydroxylic compounds, often analyzed by GC/MS as their silylated derivatives, the requirement is that several D atoms must be present in the standard to overcome an overlap of the M^+ peak of the standard with the M + l, M + 2, etc. peaks of the analyte arising from the natural heavier C and Si isotope contribution. Another obvious requirement is that the D atoms in the standard must not back-exchange under the analytical conditions.

We have developed efficient techniques for the introduction of several D atoms in estrogenic compounds such as steroids, and polyphenolic phytoestrogens such as flavonoids, isoflavonoids, lignans and resorcinol derivatives. We have also established the circumstances and structural requirements under which the D atoms do not back-exchange, and elucidated precise sites of D incorporation by spectroscopic means. Whenever possible, another consideration has been the employment of environmentally benign methods of deuteration.

CATALYST STABILITY IN IRIDIUM-MEDIATED ISOTOPE EXCHANGE

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In many cases, iridium-mediated deuterium exchange appears not to be limited by the inherent activity of the catalyst as much as by the extent to which exchange can proceed before the catalyst is inactivated. Heys showed, from crossover experiments, that $IrH_2(Me_2CO)_2(PPh_3)_2BF_4$ retained activity as an exchange catalyst for at least 4 days at 2% catalyst loading.[1] However, catalyst inactivation is well known from the work of Crabtree[2]; this principally involves the formation of inactive hydride-bridged dimers, although ligand dissociation process may also be implicated.[3] At lower catalyst loadings, active catalyst may be stabilised by excess substrate binding to the metal and thereby preventing dimerisation. However, when labelling drug candidates it is often necessary to add one or more equivalents of pre-catalyst to overcome futile binding, and so catalyst inactivation becomes an important issue.

Reaction kinetics suggest that there is a stability difference between different types of catalyst. Exchange in the presence of iridium bis(phosphine) systems typically stops within hours but, with $Ir(cod)(PPh_3)_3BF_4$, exchange continues for 48 h and results in complete *ortho*-deuteration.[4] An even more stable system is $Ir(cod)(Ph_2AsCH_2CH_2AsPh_2)BF_4$, where complete exchange can take up to 5 days, after which time the catalyst remains active. The difference in activity between the 'aged' systems is particularly apparent if crossover experiments are performed (Table 1), where it is clear that systems using $Ir(cod)(PPh_3)_2BF_4$ or $Ir(cod)Py(PCy_3)PF_6$ lose much of their activity within a short time, whereas $Ir(cod)(Ph_2AsCH_2CH_2AsPh_2)BF_4$ is very much more stable.

Table 1. Crossover deuteration experiments with selected iridium catalysts[a]

Pre-catalyst	t_1 (h)	Exchange into **1** (%)	t_2 (h)	Exchange into **2** (%)	Expected exchange into **2** (%)[b]
$Ir(cod)(PPh_3)_2BF_4$	6	95	18	5	90
$Ir(cod)(PPh_3)_3BF_4$	6	98	18	90	98
$Ir(cod)Py(PCy_3)PF_6$	6	95	18	30	80
$Ir(cod)(Ph_2AsCH_2CH_2AsPh_2)BF_4$	112	99	48	95	95

[a]Method: 4-methylacetophenone (**1**) exchanged with D_2 and 100% catalyst during t_1; acetophenone (**2**) added, and exchanged with D_2 during t_2.
[b]Exchange after t_2 when fresh catalyst is used.

The effect of the substrate on catalyst stability is apparent if substrate concentrations are changed: with substrates that bind efficiently to the metal, such as acetophenone or 1-phenylpyrazole, varying the concentration between 2.5 and 20 mM has little effect on the extent of exchange. However, with ethyl benzoate, exchange is much reduced at lower concentrations, even if the exposure time is increased to compensate for a lower initial rate. For poorer substrates, it is therefore desirable to carry out exchange at a concentration of at least 10 mM.

Prospects are better for improving exchange, using complexes with bidentate ligands,[5] and particularly the very stable bis(arsine) complexes, $Ir(cod)[Ph_2As(CH_2)_nAsPh_2]BF_4$; these have good activity for exchange in ketones ($n = 2$) and in amides ($n = 3$). Some complexes of bidentate PN ligands have useful activity also, amongst which $Ir(cod)(QUINAP)BF_4$ and $Ir(cod)(Phox)BF_4$ are promising lead catalysts (Table 2).

Table 2. Deuterium exchange of representative substrates using iridium bis(arsine) and PN complexes[a]

	PhCOMe	BnCOMe	PhCOOEt	$PhCONMe_2$	$BnCONMe_2$
$Ir(cod)(Ph_2AsCH_2CH_2AsPh_2)BF_4$	95	85	0	70	50
$Ir(cod)[Ph_2As(CH_2)_3AsPh_2)BF_4$	90	40	20	80	70
$Ir(cod)(Phox)BF_4$[b]	75	5	20	70	80
$Ir(cod)(QUINAP)BF_4$	10	10	5	85	80

[a]Figures are the percent of theoretical *ortho*-exchange from deuterium gas after 112–120 h in DCM.
[b]Phox is 2-(2-diphenylphosphinophenyl)-4-phenyloxazoline.

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PD/C CATALYSED HYDROGENOLYTIC H/T EXCHANGE IN SOLVENTS. EFFECTS ON SOLVENTS AND PRODUCT IN A BROMINE–TRITIUM EXCHANGE REACTION

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Selective 10% palladium-on-carbon catalysed bromine-tritium exchange is a common and important way to introduce tritium into aromatic compounds. It is generally accepted, but never challenged, knowledge that carrier-free tritium gas can be diluted with hydrogen due to isotopic exchange with hydrogen atoms of the solvent or with exchangeable hydrogens of the compound itself. This would account for large amounts of radioactivity in the lyophilised reaction solvent and for a (much) lesser than maximum specific activity in the final product. Moreover, it would also counteradvise against the re-use of possibly recoverable tritium gas.

For all that, literature search revealed only one study[1] concerning tritium uptake in some solvents and with a limited number of catalysts, but palladium on carbon was avoided. Solvent impurities were thought to be tritiated, but unclear results and avoidance of Pd/C in the mentioned study plus improved solvent quality over the last 30 years justified further research. Results were expected to lead to a more efficient use of tritium gas and reduction of radioactive waste with a concomitant cost reduction.

Palladium on carbon induced tritium incorporation into pure solvents: The tritium uptake was studied with dry tetrahydrofuran using (3400 $\times$ hydrogen diluted) tritium gas. Adsorption to the glass of the reaction vessel and uptake in the solvent without use of catalyst (Wilzbach conditions)[1] were excluded. It was proportional to pressure and to volume increase of the tritium gas. Surprisingly use of 0.04% of thiophene as a catalyst poison only led to a meagre 50% reduction of radioactivity in the solvent. Radioactivity in the form of dissolved tritium gas was excluded.

Incorporation of tritium into the solvent could happen either by H–T exchange or by reaction leading to another compound. In case of exchange also T–H re-exchange might be forced, irrespective of isotope effects. After stirring the tritiated solvent with catalyst under hydrogen atmosphere for 18 h, the radioactive concentration dropped a factor 2. Continuing for a prolonged time period (72 h) gave no further change in radioactive concentration. These results hint towards partly incorporation via exchange and partly via reaction with the solvent.

The study was extended to other (predried) solvents, which were treated under standard conditions (100 µl of solvent, 0.5 mg of Pd/C 10%, 60 mbar tritium gas pressure, constant gas volume, 40 min reaction time) and comprised methanol (100), water (89), diisopropyl ether (83), ethyl acetate (75), 1,2-dimethoxyethane (71), ethanol (61), dioxane (59), tetrahydrofuran (54), *N,N*-dimethylformamide (42), *N,N*-dimethylacetamide (32), toluene (32) and benzene (5). The figures between brackets indicate the amount of tritium uptake with respect to methanol and might well indicate a solvent depending rate of reaction. Surprisingly, protic solvents like methanol and water had not even incorporated double the amount of tritium as had THF and ethanol had incorporated radioactivity in the same order as had THF.

If in the reaction mixture radioactivity was introduced in other compounds than the original solvent, or if radioactive reactants were formed, then adsorption of such compounds to, e.g. molecular sieves, would immediately be reflected by a lowered radioactive concentration. This was indeed, and substantially (60–95% drop in radioactive concentration) the case for all solvents with the exception of the protic solvents water, methanol and ethanol and also for toluene.

It was then decided to take tritium NMR spectra for the most important solvents used in catalytic Br–T exchange reactions, viz. THF, dioxane, ethyl acetate, DMA and DMF. Surprisingly the spectra showed the presence of only tritiated water; no trace of other radioactive material was spotted.

Analysis of the catalyst indicated a presence of 6.7% of water. Even if all this water was transformed to HTO (at the used tritium gas dilution), which is unlikely, then the balance would still leave amounts of tritiated water in the solvents which cannot be accounted for.

Triple-fold interaction of catalyst - aryl bromide - solvent: A pragmatic approach for studying the triple-fold interaction between catalyst, solvent and aryl bromide under the exchange conditions was chosen for. Standard conditions used carrier-free tritium gas at 120 mbar (2.3 Ci), dry THF with *N,N*-diisopropylethyl amine (DIPEA, 1%), catalyst (1.000 mg) and aryl bromide (1.000 mg).

Reactions with compound 1 (sulphide – secondary amides): Pure solvent with added DIPEA, gave 25% less incorporation than pure solvent alone, confirming the catalyst poisoning effect of nitrogen containing bases.[2] The selected aryl bromide (**1**) featured a diphenyl sulphide moiety and contained also a bis-aryl and an aryl-alkyl secondary amide (exchangeable hydrogens). The amounts of residual product after lyophilisation (18–20 mCi) and the products' specific activity (26–27 Ci/mmol) were independent of tritium gas pressure. Surprisingly also the radioactivity in the lyophilized solvent was independent of the employed pressure and was approximately 3.5 × the

radioactivity in the residue. Increase of reaction time gave a much less than proportional increase in solvent waste and product and an almost negligible drop in specific activity. Pre-treatment of the catalyst (1 h, 200°C, continuous high vacuum) in order to heat out the water present in the catalyst surprisingly led to a much higher radioactivity in the solvent waste, and to less product at a much lower s.a. (18 Ci/mmol).

Modification of compound 1 to sulphones and/or methyl amides 2–4: Modification of **1** to its sulphone (**3**) would lift the catalyst poisoning effect of the compounds' sulphide function. Replacement of the exchangeable hydrogens by methyl groups (**2**) was thought to avoid deposition of those hydrogens on the catalyst, which might lead to a positive effect on the specific activity. A combination of sulphone and methyl amides (**4**) would benefit both effects. Surprisingly all three compounds, including the sulphide (**2**) doubled the amount of radiochemical residue compared to **1** with complete conversion (for compound **1**: 67% of reactant and 33% of product was measured) and 100% product purity in the residue. For the methylated compounds no significant change in s.a. was noticed.

Solvent waste: The tritium ending up in the solvent waste may have different origins, e.g. exchange on the catalyst with the present water, exchange on the catalyst with labile hydrogens in the molecule, formation of the TBr ammonium salt (equals amount of radioactive residue = product + side product impurities) or other factors. Of these, the exchanged water dissolved in the solvent is lyophilised with the first lyophilisate. The molecule with possible N–T bonds and the TBr salt will remain as a residue together with the product. Contact with methanol will exchange those labile tritiums and will lyophilise in the second fraction. Individually captured lyophilisates might possibly pinpoint these origins. Reaction without bromide indicated 20% of labile tritium on the catalyst. Standard reactions performed for 0.5, 2 and 17 h showed 28% of labile tritium, equalling the amount of radioactivity found in the residue. The latter observation strongly pointed to radioactivity coming from the TBr salt (unavoidable part of the reaction). Contrasting are the results from the reaction with the pretreated catalyst (1 h, 200°C, vacuum, then reaction). Here labile radioactivity in the second fraction was 35% of the total waste, but strongly in contrast with the much lower amount of residue (3.5 ×). With compounds **1**, **2**, **3** and **4** under standard conditions the total amount of radioactivity in the waste was 3.5 × the amount of radioactivity found in the residue. For the secondary amides (**1** and **3**) the labile tritium fraction equalled the amount of radioactivity in the residue, and was in line with above observations. However for the tertiary amides the amount of radioactivity dropped to half this value, suggesting that the 1 equiv. of obligatory formed TBr was not preserved as such. Hence, in the reactions with the secondary amides, at least part of this

second-fraction radioactivity must have stemmed from some interaction with the N–H hydrogens.

Conclusion: Although a solid insight into the detailed reaction process is not obtained, some over-all remarks can be made:

— There is no solvent H–T exchange.
— In pure solvent, tritiated water is found as the only source of radioactivity.
— There may be H–T exchange with part of the water from the catalyst, this does not explain all of the radioactivity found in the lyophilised fractions.
— An exchange with N–H or O–H hydrogens is likely.
— TBr, captured as the DIPEA salt, is responsible for 1 equivalent of radioactivity. Exchange with the present water is possible.
— Lowered T_2 pressure has negligible impact on the specific activity or yield.
— Reaction with tritium gas at 120 mbar allows for an 8-fold increase in efficiency and for proportionally less waste per product.
— Re-use of T_2-gas is possible, with minor impact on the compounds' s.a.
— Under standard conditions 20–50 mCi of the desired products are obtained and with specific activities of approximately 25 Ci/mmol or higher.

Acknowledgement: Dr Albert Zeller, RC TRITEC AG, for kindly offering a discussion forum in the biannual RC TRITEC's workshop. Prof. Dr Bill Lockley for measuring the NMR spectra of the tritiated solvents. Dr Willem Meuldermans for continuous support. Herman Lenoir, Jos Thijssen, Bjorn Verreet and Steve Van Hoegaerden for the stimulating discussions in our laboratory.

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A NEW MODULE-ASSISTED SYNTHESIS OF THE VERSATILE, BIFUNCTIONAL LABELLING AGENT [^{18}F]SFB: FROM RADIOCHEMISTRY TO APPLICATIONS

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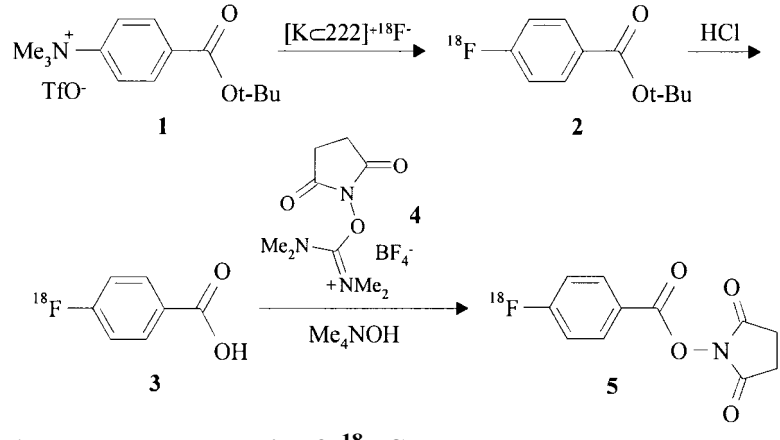
Complex biomolecules such as peptides and proteins cannot be labelled directly in single step by nucleophilic substitution with [^{18}F]fluoride. The reasons are obvious: denaturation of sensitive organic substrates due to the harsh reaction conditions, and the presence of functional groups such as free amino and carboxyl groups or other H-acidic moieties. The mild and specific introduction of ^{18}F in biomacromolecules requires 'fluorinating agents' also referred to as bifunctional labelling agents capable of being linked to functional groups (e.g. amino groups) typically found in such biomolecules.

The activated ester *N*-succinimidyl 4-[^{18}F]fluorobenzoate ([^{18}F]SFB, **5**) was shown to be a suitable acylation agent for radiolabelling of peptides, proteins and antibodies.[1–4] Such [^{18}F]fluorobenzoylated bioactive compounds may serve as useful radiotracers for *in vivo* studies of physiological processes by positron emission tomography (PET).

[^{18}F]SFB synthesis involves a laborious three-step procedure, which has to be performed manually so far. This implies a substantial radiation exposure to the radiochemist. Therefore, the frequent utilisation of [^{18}F]SFB requires a routine procedure aiming at a reliable, remotely controlled operating synthesis of [^{18}F]SFB.

Thus, we used the simplified [^{18}F]SFB synthesis[3] according to Scheme 1 to adapt this three-step two-pot procedure to a modified remotely controlled synthesis module 'TRACERlab Fx_{FDG}' (GE Medical Systems. Münster, Germany).[5]



Scheme 1. Three-step synthesis of [^{18}F]SFB

Starting from *tert*-butylester derivative **1**, the synthesis of 4-[^{18}F]fluorobenzoic acid (**3**) was described using microwave activation (2 min for the [^{18}F]fluorination step in DMSO, 30 s for the saponification step using trifluoroacetic acid).[3,6] As the use of the microwave is not possible in the synthesis module, the reaction vessels had to be heated conventionally. Therefore, the synthesis of **3** was investigated and optimised in the automated synthesis module first. The first reaction step (conversion of **1** into **2**) was tested in several solvents, such as DMSO, DMF and MeCN, employing several temperatures and reaction times. MeCN was found to be the solvent of choice when the reaction was carried out at 90°C for 10 min. Furthermore, diluted HCl was tested for the saponification step (conversion of **2** into **3**) to avoid the use of the corrosive and harmful trifluoroacetic acid. It was found that 1 M HCl was also suitable to hydrolyse the *tert*.-butyl ester group of **2** by heating at 100°C for 5 min. After diluting the acidic reaction mixture with water, **3** was purified by solid phase extraction at a polystyrene-based cartridge and eluted with MeCN. The radiochemical yields were up to 67% and the radiochemical purity was 97–98%.

Then, **3** was treated with Me_4NOH to form the corresponding tetramethylammonium salt, which was dried. Addition of the activating agent *O*-(*N*-succinimidyl)-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate (TSTU; **4**) in MeCN and 2 min heating at 90°C yielded the desired [^{18}F]SFB (**5**). After purification using a polystyrene-based SPE cartridge, compound **5** was eluted with MeCN in reproducible radiochemical yields of 34–38% (related to [^{18}F]fluoride; $n = 12$). The radiochemical purity was in the range of 93–96%. A typical experiment was started with 12 GBq [^{18}F]fluoride and yielded about 2.8 GBq [^{18}F]SFB within 68 min. [^{18}F]SFB could be obtained in radiochemical and chemical purity suitable for labelling amino acids, peptides and proteins containing free amino groups. Such ^{18}F-labelled compounds were subjected to a comprehensive radiopharmacological evaluation, such as biodistribution and dynamic small animal PET studies.

[^{18}F]SFB was successfully used for radiolabelling of the following compounds of interest:

1. Amino acid derivatives:
   - — Fructoselysine,[7] N^{ε}-carboxymethyllysine and N^{ε}-carboxyethyllysine as important 'advanced glycation end products' (AGEs).[8]
2. Peptides:
   - — Neurotensin(8–13) and three stabilised analogues (to investigate their potential to image tumours overexpressing neurotensin receptor 1 by PET).[9]

— The isopeptide N^{ε}-(γ-glutamyl)-L-lysine[3] (for understanding the potential link between the ingestion of isopeptides in food items and their biological pathways)

3. Proteins:
   — Annexin-V ([^{18}F]Fluorobenzoyl-annexin-V seems to be a suited PET-tracer for apoptosis imaging).[4,10]
4. Apolipoproteins:
   Oxidative modification of low-density lipoproteins (LDL) is regarded as a crucial event in atherogenesis. ^{18}F-Labelling of LDL apolipoprotein B-100 using [^{18}F]SFB[11] could prove to be a promising approach for studying the kinetics of oxidized LDL *in vivo*.
5. HSA microspheres:
   [^{18}F]Fluorobenzoylated HSA microspheres as tracers to investigate perfusion of organs.

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SYNTHESES AND FIRST BIOLOGICAL EVALUATION OF ^{18}F LABELLED BICYCLIC ANALOGUES OF VESAMICOL AS POTENTIAL VACHT IMAGING AGENTS

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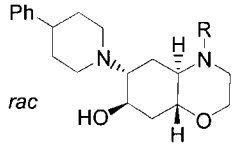
Introduction: Application of specific ^{18}F labelled ligands binding to the vesicular acetylcholine transporter (*VAChT*) is expected to establish a new imaging method to determine the loss of cholinergic terminals in human brain *in vivo* by using Positron-Emission-Tomography (PET).[1] The subject of our work[2] is to develop a new group of ^{18}F labelled vesamicol derivatives containing a cyclohexane-fused morpholine ring (octahydro-benzo[1,4]oxazine) with suitable lipophilicity, high binding affinity and selectivity to the VAChT.

6-(4-Phenyl-piperidin-1-yl)-octahydro-benzo[1,4]oxazin-7-ol derivatives



R = H (**I**)
[^{18}F]F-acetyl- (**Ia**),
4-[^{18}F]F-benzoyl- (**Ib**),
4-[^{18}F]F-benzyl- (**Ic**)

Methods: Studies with nonradioactive reference compounds were carried out *in vitro* (homogenate assays to determine affinity and selectivity, metabolic stability, and affinity to plasma proteins). In order to determine lipophilicity, partition coefficients of ^{18}F labelled derivatives were determined in an n-octanol-phosphate buffer-system. During first animal studies in rats biodistribution and tracer kinetic were investigated. First *in vivo* data on biodistribution and tracer kinetics were obtained in rats.

Results and discussion:

Labelling: Using a standard procedure (K_2CO_3, Kryptofix K222, acetonitrile, reflux. He-flow), an aqueous solution of [^{18}F]HF was converted into the reactive form needed for n.c.a. displacement reactions. The [^{18}F]F-acetyl derivative (**Ia**) was obtained from the corresponding bromoacetyl precursor by nucleophilic substitution (DMF, 145°C, 15 min) followed by solid-phase

extraction (SPE. Sep-Pak C18 cartridge plus) and semi-preparative HPLC (RP-18 column. 35% MeCN + 20 nM ammonium acetate). At present, yields up to 25% (decay corrected, 75 min) were obtained. An automatic procedure using a commercially available module (Tracerlab FX_{FN}, GE Medical System^{TM}, GE Healthcare) has been developed. The 4-[^{18}F]F-benzoyl derivative (**Ib**) was exclusively obtained from a 4-nitrobenzoyl-precursor using a microwave-assisted synthesis (DMSO, 145°C, 15 min). This precursor appears to be a good VAChT ligand too. However, the essential separation from the final product by HPLC was not successful. Therefore, the excess of unreacted nitro derivative has to be destroyed (e.g. by reduction with Na_2S, or $SnCl_2$ in DMF, or Zn/NH_4Cl in MeOH/EtOH, respectively). A purification procedure analogous to compound **Ia** (SPE and HPLC) was established. At present, yields up to 15% (decay corrected, 105 min) were achieved.

Due to some defluorination and the formation of several by-products, the 4-[^{18}F]F-benzyl derivative (**Ic**) could not be obtained in a good manner until now by reduction of ^{18}F labelled **Ib** (BH_3*THF, yield $<8\%$). Therefore, we prepared both 4-[^{18}F]fluorobenzaldehyde and 4-[^{18}F]fluorobenzylbromide by standard methods in order to use these electrophiles for coupling reactions with the secondary amino group of octahydro-benzo[1,4]oxazin-7-ol (**I**). The radiochemical purity of each final product has been proven to be $>95\%$, the chemical purity was $>93\%$ and the specific radioactivity was >3.7 GBq/μmol, starting from >5 GBq of [^{18}F]fluoride.

Biological evaluation: ^{18}F labelled vesamicol derivatives (**Ia**, **Ib**, **Ic**) appear to have somewhat improved biological properties, however, their high binding to plasma proteins resulted in a low uptake into the rat brain.

Conclusions: Reaction parameters were elaborated; stable yields are achievable. First results in biological evaluations were obtained from *ex vivo* and *in vivo* studies and show promising properties of the morpholine containing vesamicol derivatives. These investigations will be continued.

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